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(71) Applicant: **CALITHERA BIOSCIENCES, INC.** [US/US]; 343 Oyster Point Blvd., Suite 200, South San Francisco, CA 94080 (US).

(72) Inventors; and

(71) Applicants *(for US only)*: **BENNETT, Mark, K.** [US/US]; 9 Fieldbrook Pl., Moraga, CA 94556 (US). **GROSS, Matthew, I.** [US/US]; 38 Maybeck Street, Novato, CA 94949 (US). **BROMLEY, Susan, D.** [US/US]; 241 Downey Street, San Francisco, CA 94117 (US). **LI, Jim** [US/US]; 56 Otsego Avenue, San Francisco, CA 94112 (US). **CHEN, Lijing** [US/US]; 19500 Pruneridge Avenue, Apt. 3211, Cupertino, CA 95014 (US). **GOYAL, Bindu** [US/US]; 2189 Annapolis Drive, Fremont, CA 94539 (US). **LAIDIG, Guy** [US/US]; 585 Woodside Drive, Woodside, CA 94062 (US). **STANTON, Timothy, Friend** [US/US]; 24 Alta Vista Way, Daly City, CA 94014 (US). **SJOGREN, Eric, Brian** [US/US]; 442 Dell Avenue, Mountain View, CA 94043 (US).

(74) Agents: **HALSTEAD, David, P.** et al.; FOLEY HOAG LLP, Seaport West, 155 Seaport Boulevard, Boston, Massachusetts 02210-2600 (US).

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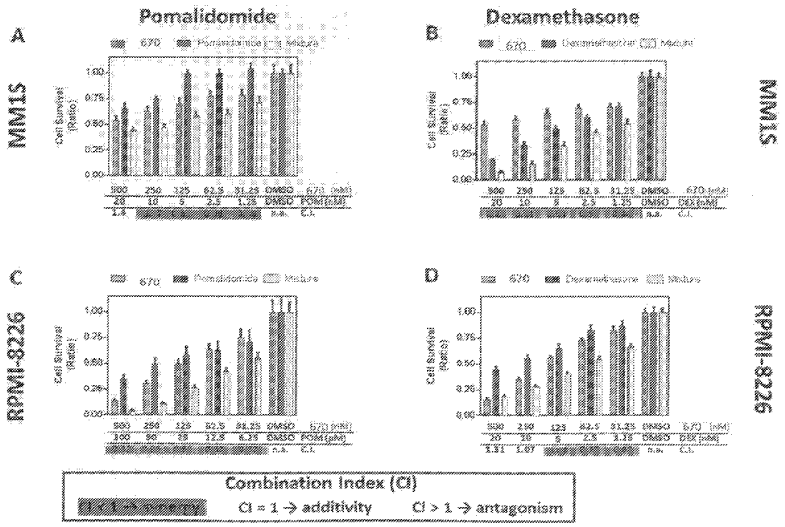
[Continued on next page]

(54) Title: TREATMENT OF CANCER WITH HETEROCYCLIC INHIBITORS OF GLUTAMINASE

Figure 18



(57) Abstract: The invention relates to novel heterocyclic compounds and pharmaceutical preparations thereof and the methods of treating or preventing cancer using the compounds of the invention. Other aspects relate to methods of identifying a cancer patient that may benefit from treatment with a glutaminase inhibitor comprising determining the ratio of glutamate to glutamine, the ratio of glutaminase enzyme to glutamine synthetase or glutaminase activity in cancer cells of the patient.

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MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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Treatment of Cancer with Heterocyclic Inhibitors of Glutaminase

Related Applications

This application claims the benefit of priority to U.S. Provisional Patent Application No. 61/732,755, filed December 3, 2012, U.S. Provisional Patent Application No. 61/749,016, filed January 4, 2013, U.S. Provisional Patent Application No. 61/784,984, filed March 14, 2013, U.S. Provisional Patent Application No. 61/809,795, filed April 8, 2013, and U.S. Provisional Patent Application No. 61/824,513, filed May 17, 2013, which applications are hereby incorporated by reference in their entirety.

Background

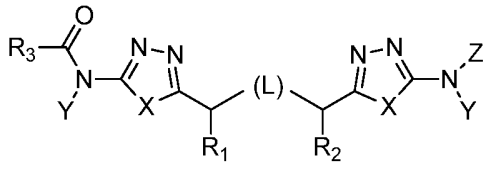
Glutamine supports cell survival, growth and proliferation through metabolic and non-metabolic mechanisms. In actively proliferating cells, the metabolism of glutamine to lactate, also referred to as "glutaminolysis" is a major source of energy in the form of NADPH. The first step in glutaminolysis is the deamination of glutamine to form glutamate and ammonia, which is catalyzed by the glutaminase enzyme (GLS). Thus, deamination via glutaminase is a control point for glutamine metabolism.

Ever since Warburg's observation that ascites tumor cells exhibited high rates of glucose consumption and lactate secretion in the presence of oxygen (Warburg, 1956), researchers have been exploring how cancer cells utilize metabolic pathways to be able to continue actively proliferating. Several reports have demonstrated how glutamine metabolism supports macromolecular synthesis necessary for cells to replicate (Curthoys, 1995; DeBardinis, 2008).

Thus, glutaminase has been theorized to be a potential therapeutic target for the treatment of diseases characterized by actively proliferating cells, such as cancer. The lack of suitable glutaminase inhibitors has made validation of this target impossible. Therefore, the creation of glutaminase inhibitors that are specific and capable of being formulated for in vivo use could lead to a new class of therapeutics.

Summary of Invention

The present invention provides a method of treating or preventing cancer comprising administering a compound of formula I,



(I),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or , preferably CH_2CH_2, wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X, independently for each occurrence, represents S, O or CH=CH, preferably S or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, arylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy;

R_3, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or

heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H.

In certain embodiments, the cancer is selected from breast cancer, colorectal cancer, endocrine cancer, melanoma, renal cancer and B cell malignancy. In certain such embodiments wherein the cancer is breast cancer, the breast cancer comprises basal-type breast cancer cells, triple-negative breast cancer cells or claudin-low breast cancer cells. In certain embodiments wherein the cancer is endocrine cancer, the endocrine cancer is selected from adrenal cortex adenoma, adrenal cortex carcicnoma, adrenal gland pheochromocytoma and parathyroid gland adenoma. In certain embodiments wherein the cancer is a B cell malignancy, the B cell malignancy is selected from multiple myeloma, leukemia, such as acute lymphoblastic leukemia or chronic lymphoblastic leukemia, and lymphoma, such as Burkitt's lymphoma, Diffuse large B cell lymphoma, follicular lymphoma or Hodgkin's lymphoma.

In certain embodiments, the present invention provides a pharmaceutical preparation suitable for use in a human patient in the treatment or prevention of cancer, such as breast cancer, colorectal cancer, endocrine cancer, melanoma, renal cancer or B cell malignancy, comprising an effective amount of any of the compounds described herein (e.g., a compound of the invention, such as a compound of formula

I), and one or more pharmaceutically acceptable excipients. In certain embodiments, the pharmaceutical preparations may be for use in treating or preventing a condition or disease as described herein. In certain embodiments, the pharmaceutical preparations have a low enough pyrogen activity to be suitable for intravenous use in a human patient.

Detailed Description of the Drawings

Figure 1 shows the correlation between glutamine-dependence and antiproliferative effect of compound 670 for a panel of breast tumor cell lines.

Figure 2 shows the differential expression of glutaminase and glutamine synthetase in triple-negative breast cancer subtype.

Figure 3 shows single-agent compound 402 treatment of MDA-MB-231 orthotopic xenograft model.

Figure 4 shows a combination study with compound 389 and paclitaxel in MDA-MB-231 orthotopic xenograft model.

Figure 5 shows results of the median glutaminase:glutamine synthetase expression ratio in various cancer types, including colorectal cancer, renal cancer, lymphoma, melanoma and myeloma.

Figure 6 shows that the glutaminase:glutamine synthetase expression ratio varies by subtypes in endocrine cancers.

Figure 7 depicts the median glutaminase:glutamine synthetase expression ratio in acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL).

Figure 8 shows the glutaminase:glutamine synthetase expression ratio for several subtypes of lymphomas within the B cell malignancy category.

Figure 9 shows the correlation between the antiproliferative effect of compound 670 and the glutamate:glutamine concentration ratios for a panel of breast tumor cell lines.

Figure 10 shows the correlation between the glutamate:glutamine concentration ratios to glutaminase:glutamine synthetase expression ratios and to glutaminase specific activity in a variety of primary tumor xenografts.

Figure 11 shows that intraperitoneal administration of compound 188 to mice results in reduced tumor size in a HCT116 colon carcinoma xenograft model.

Figure 12 shows that oral administration of compound 670 to mice results in reduced tumor size in a H2122 lung adenocarcinoma xenograft model.

Figure 13 shows the mRNA expression levels of GLS (KGA or GAC), GS, and the ratio of KGA:GS and GAC:GS in TNBC vs. HR+ or Her2+ cell lines. The "box" depicts the 2nd and 3rd quartiles with the median corresponding to the horizontal line; "whiskers" span the 10th and 90th percentile with data outside this range shown as individual data points.

Figure 14 shows correlation between the sensitivity to Compound 670 and mRNA expression levels of GLS, GS, or expression ratios. For each bivariate graph, the Compound 670 sensitivity is plotted on the x-axis and the expression parameter is plotted on the y-axis with each point representing an individual cell line.

Figure 15 shows western analysis of KGA, GAC and GS in breast cancer cell lines. Blots were probed with antibodies recognizing KGA, GAC and GS. The CAG antibody also recognizes KGA and the two are distinguishable on the blot by their molecular weight differences. Blots were stripped and re-probed with GAPDH as a loading control.

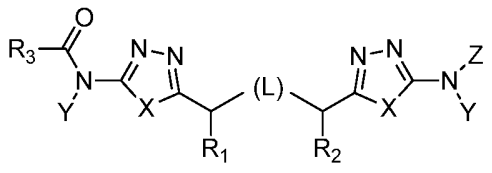
Figure 16 shows the correlation between the glutamate:glutamine concentration ratios to sensitivity to glutaminase inhibitor compound 670.

Figure 17 shows that oral administration of compound 670 to mice results in reduced tumor size in a RPMI-8226 multiple myeloma xenograft model.

Figure 18 shows that compound 670 synergizes with pomalidomide or dexamethasone to produce an anti-tumor effect in multiple myeloma cells.

Detailed Description of the Invention

The present invention provides a method of treating or preventing cancer comprising administering a compound of formula I,



(I),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or , preferably CH_2CH_2, wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X, independently for each occurrence, represents S, O or CH=CH, preferably S or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, arylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy;

R_3, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H.

In certain embodiments wherein alkyl, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl are substituted, they are substituted with one or more substituents selected from substituted or unsubstituted alkyl, such as perfluoroalkyl (e.g., trifluoromethyl), alkenyl, alkoxy, alkoxyalkyl, aryl, aralkyl, arylalkoxy, aryloxy, aryloxyalkyl, hydroxyl, halo, alkoxy, such as perfluoroalkoxy (e.g., trifluoromethoxy), alkoxyalkoxy, hydroxyalkyl, hydroxyalkylamino, hydroxyalkoxy, amino, aminoalkyl, alkylamino, aminoalkylalkoxy, aminoalkoxy, acylamino, acylaminoalkyl, such as perfluoro acylaminoalkyl (e.g., trifluoromethylacylaminoalkyl), acyloxy, cycloalkyl, cycloalkylalkyl, cycloalkylalkoxy, heterocyclyl, heterocyclylalkyl, heterocyclyloxy, heterocyclylalkoxy, heteroaryl, heteroarylalkyl, heteroarylalkoxy, heteroaryloxy, heteroaryloxyalkyl, heterocyclylaminoalkyl, heterocyclylaminoalkoxy, amido, amidoalkyl, amidine, imine, oxo, carbonyl (such as carboxyl, alkoxycarbonyl, formyl, or acyl, including perfluoroacyl (e.g., $C(O)CF_3$)), carbonylalkyl (such as carboxyalkyl, alkoxycarbonylalkyl, formylalkyl, or acylalkyl, including perfluoroacylalkyl (e.g., -alkyl$C(O)CF_3$)), carbamate, carbamatealkyl, urea, ureaalkyl, sulfate, sulfonate, sulfamoyl, sulfone, sulfonamide, sulfonamidealkyl, cyano, nitro, azido, sulfhydryl, alkylthio, thiocarbonyl (such as thioester, thioacetate, or thioformate), phosphoryl, phosphate, phosphonate or phosphinate.

In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, or CH_2NHCH_2, wherein any hydrogen atom of a CH_2 unit may be replaced by alkyl or alkoxy, and any hydrogen atom of a CH_2 unit of CH_2CH_2,

$CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxyl. In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2. In certain embodiments, L represents CH_2CH_2. In certain embodiments, L is not CH_2SCH_2.

In certain embodiments, Y represents H.

In certain embodiments, X represents S or CH=CH. In certain embodiments, one or both X represents CH=CH. In certain embodiments, each X represents S. In certain embodiments, one X represents S and the other X represents CH=CH.

In certain embodiments, Z represents $R_3(CO)$. In certain embodiments wherein Z is $R_3(CO)$, each occurrence of R_3 is not identical (e.g., the compound of formula I is not symmetrical).

In certain embodiments, R_1 and R_2 each represent H.

In certain embodiments, R_3 represents arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl. In certain embodiments, R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents aryl, arylalkyl, heteroaryl or heteroaralkyl, such as aryl, arylalkyl or heteroaryl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl, such as hydroxy, hydroxyalkyl or alkoxy.

In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, such as CH_2CH_2, CH_2S or SCH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and each R_3 represents arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl. In certain such embodiments, each occurrence of R_3 is identical.

In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and each R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents aryl, arylalkyl, heteroaryl or heteroaralkyl, such as aryl, arylalkyl or heteroaryl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl, such as hydroxy, hydroxyalkyl or alkoxy. In certain such embodiments, each occurrence of R_3 is identical.

In certain embodiments, L represents CH_2CH_2, Y represents H, X represents S or CH=CH, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and each R_3 represents substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl. In certain such embodiments, each X represents S. In other embodiments, one or both occurrences of X represents CH=CH, such as one occurrence of X represents S and the other occurrence of X represents CH=CH. In certain embodiments of the

foregoing, each occurrence of R_3 is identical. In other embodiments of the foregoing wherein one occurrence of X represents S and the other occurrence of X represents CH=CH, the two occurrences of R_3 are not identical.

In certain embodiments, L represents CH_2CH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and each R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents aryl, arylalkyl or heteroaryl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl or alkoxy. In certain such embodiments, R_8 represents aryl and R_{10} represents hydroxyalkyl. In certain such embodiments, each occurrence of R_3 is identical.

In certain embodiments wherein L represents CH_2, $CH_2CH_2CH_2$ or CH_2CH_2, X represents O, and Z represents $R_3(CO)$, both R_3 groups are not alkyl, such as methyl, or $C(R_8)(R_9)(R_{10})$, wherein R_8, R_9 and R_{10} are each independently hydrogen or alkyl.

In certain embodiments wherein L represents CH_2CH_2, X represents S, and Z represents $R_3(CO)$, both R_3 groups are not phenyl or heteroaryl, such as 2-furyl.

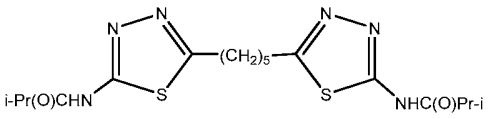
In certain embodiments wherein L represents CH_2CH_2, X represents O, and Z represents $R_3(CO)$, both R_3 groups are not $N(R_4)(R_5)$ wherein R_4 is aryl, such as phenyl, and R_5 is H.

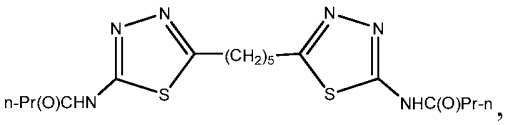
In certain embodiments wherein L represents CH_2SCH_2, X represents S, and Z represents $R_3(CO)$, both R_3 groups are not aryl, such as optionally substituted phenyl, aralkyl, such as benzyl, heteroaryl, such as 2-furyl, 2-thienyl or 1,2,4-trizole, substituted or unsubstituted alkyl, such as methyl, chloromethyl, dichloromethyl, n-propyl, n-butyl, t-butyl or hexyl, heterocyclyl, such as pyrimidine-2,4(1H,3H)-dione, or alkoxy, such as methoxy, pentyloxy or ethoxy.

In certain embodiments wherein L represents CH_2SCH_2, X represents S, and Z represents $R_3(CO)$, both R_3 groups are not $N(R_4)(R_5)$ wherein R_4 is aryl, such as substituted or unsubstituted phenyl (e.g., phenyl, 3-tolyl, 4-tolyl, 4-bromophenyl or 4-nitrophenyl), and R_5 is H.

In certain embodiments wherein L represents $CH_2CH_2CH_2$, X represents S, and Z represents $R_3(CO)$, both R_3 groups are not alkyl, such as methyl, ethyl, or propyl, cycloalkyl, such as cyclohexyl, or $C(R_8)(R_9)(R_{10})$, wherein any of R_8, R_9 and R_{10} together with the C to which they are attached, form any of the foregoing.

In certain embodiments, the compound is not one of the following:


i-Pr(O)CHN
(CH2)5
NHC(O)Pr-i
,


n-Pr(O)CHN
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NHC(O)Pr-n
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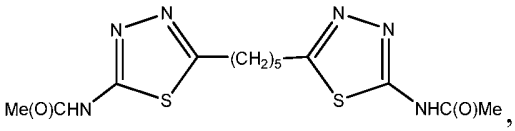
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(CH2)5
NHC(O)Me
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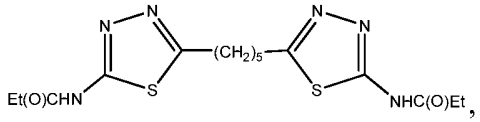
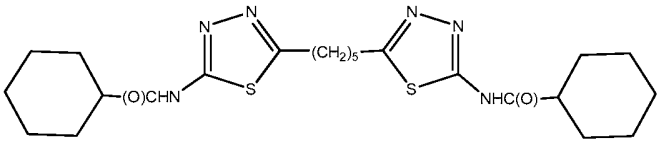
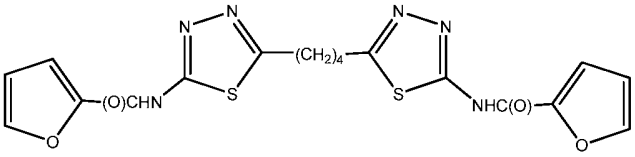
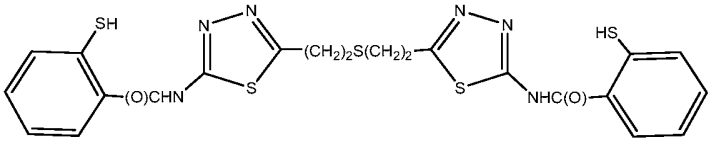
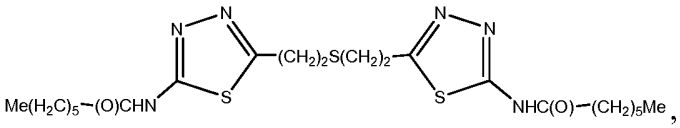
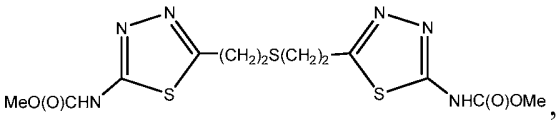

Et(O)CHN
(CH2)5
NHC(O)Et
,

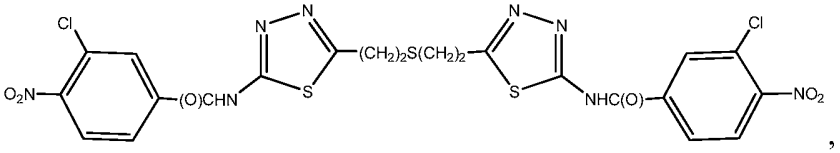

(O)CHN
(CH2)5
NHC(O)
,

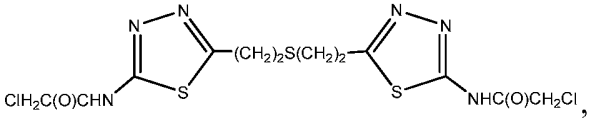

(O)CHN
(CH2)4
NHC(O)
,

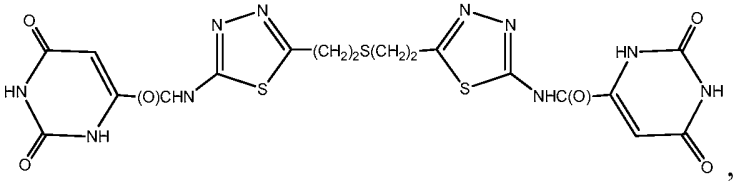

SH
(O)CHN
(CH2)2S(CH2)2
HS
NHC(O)
,

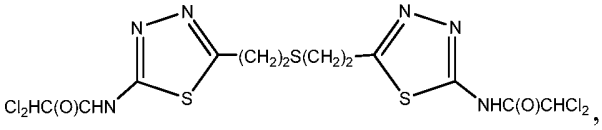

Me(H2C)5
(O)CHN
(CH2)2S(CH2)2
NHC(O)
(CH2)5Me
,

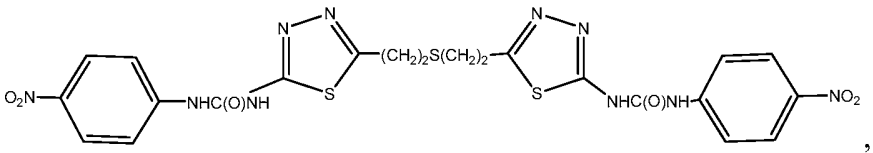

MeO(O)CHN
(CH2)2S(CH2)2
NHC(O)OMe
,

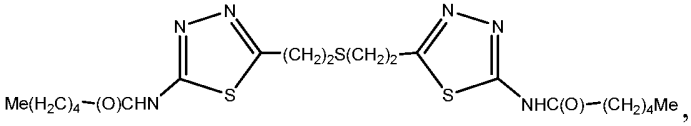

Cl
O2N
(O)CHN
(CH2)2S(CH2)2
NHC(O)
Cl
NO2
,

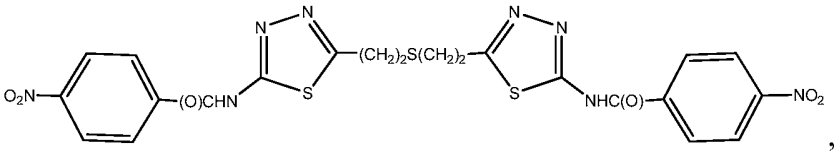

ClH2C(O)CHN
(CH2)2S(CH2)2
NHC(O)CH2Cl
,

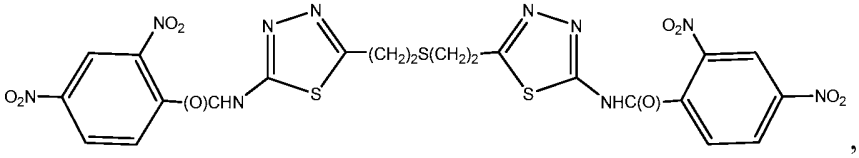

HN
O
NH
O
(O)CHN
(CH2)2S(CH2)2
NHC(O)
HN
O
NH
O
,

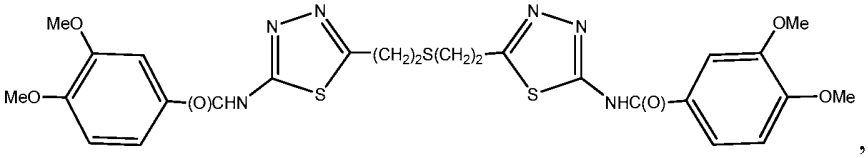

Cl2HC(O)CHN
(CH2)2S(CH2)2
NHC(O)CHCl2
,

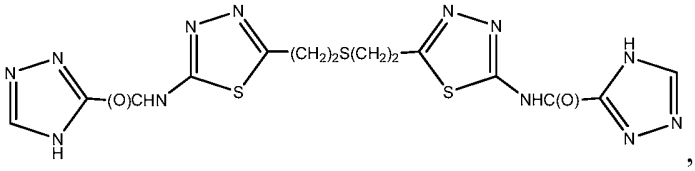


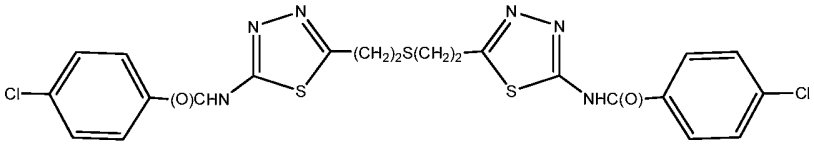


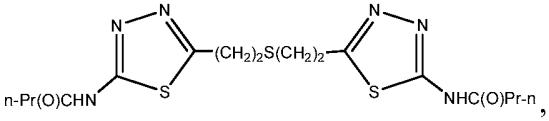


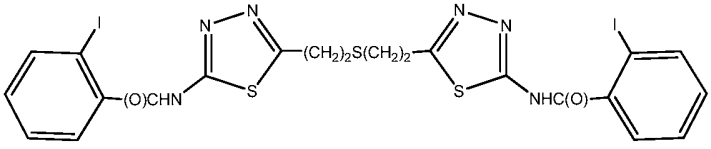


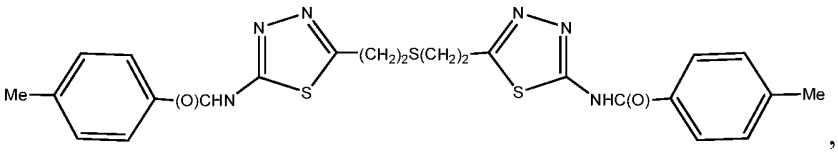


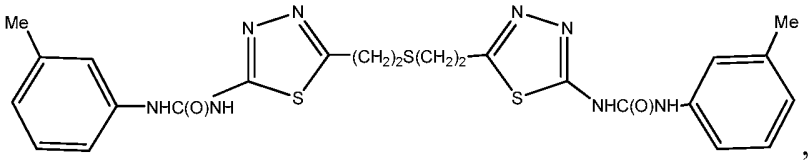












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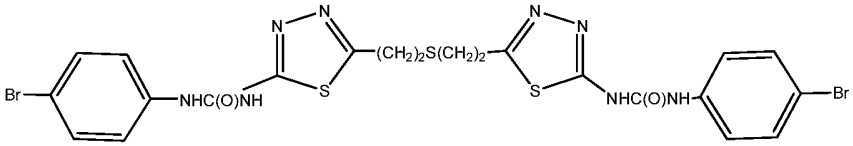

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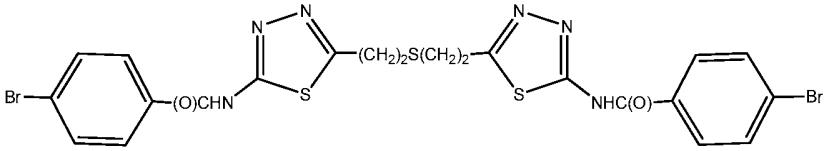

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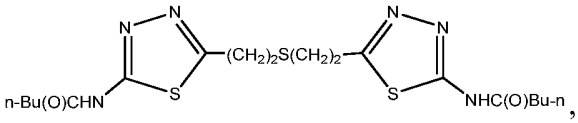

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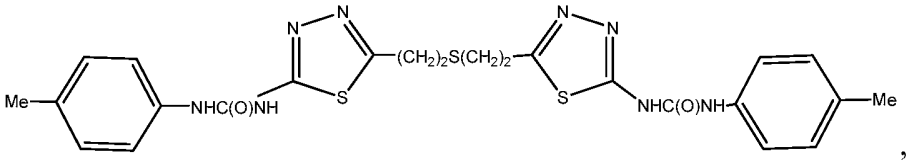

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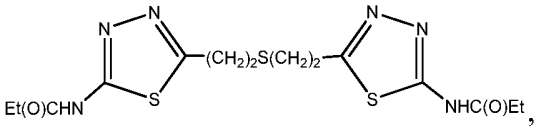

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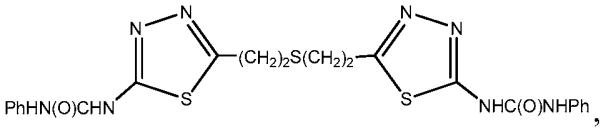

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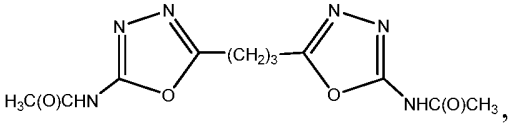

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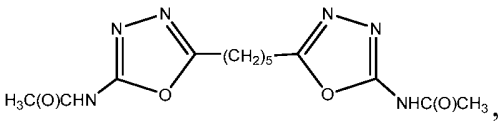
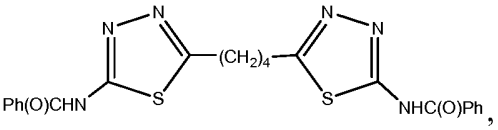


, 

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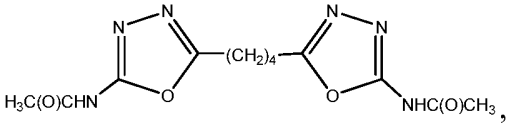
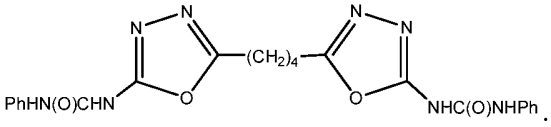


, or



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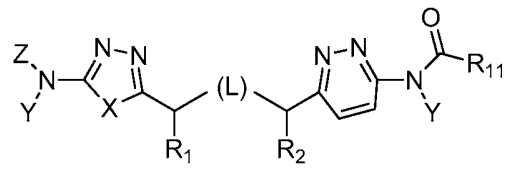
The present invention further provides a method of treating or preventing cancer comprising administering a compound of formula Ia,



(Ia),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or , preferably CH_2CH_2, wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X represents S, O or CH=CH, preferably S or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, arylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy, preferably H;

R_3 represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl,

heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H;

R_{11} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or $C(R_{12})(R_{13})(R_{14})$, $N(R_4)(R_{14})$ or OR_{14}, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_{12} and R_{13} each independently respresent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein both of R_{12} and R_{13} are not H; and

R_{14} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl.

In certain embodiments wherein alkyl, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl are substituted, they are substituted with one or more substituents selected from substituted or unsubstituted alkyl, such as perfluoroalkyl (e.g., trifluoromethyl), alkenyl, alkoxy, alkoxyalkyl, aryl, aralkyl, arylalkoxy, aryloxy, aryloxyalkyl, hydroxyl, halo, alkoxy, such as perfluoroalkoxy (e.g., trifluoromethylalkoxy), alkoxyalkoxy, hydroxyalkyl,

hydroxyalkylamino, hydroxyalkoxy, amino, aminoalkyl, alkylamino, aminoalkylalkoxy, aminoalkoxy, acylamino, acylaminoalkyl, such as perfluoro acylaminoalkyl (e.g., trifluoromethylacylaminoalkyl), acyloxy, cycloalkyl, cycloalkylalkyl, cycloalkylalkoxy, heterocyclyl, heterocyclylalkyl, heterocyclyloxy, heterocyclylalkoxy, heteroaryl, heteroarylalkyl, heteroarylalkoxy, heteroaryloxy, heteroaryloxyalkyl, heterocyclylaminoalkyl, heterocyclylaminoalkoxy, amido, amidoalkyl, amidine, imine, oxo, carbonyl (such as carboxyl, alkoxycarbonyl, formyl, or acyl, including perfluoroacyl (e.g., $C(O)CF_3$)), carbonylalkyl (such as carboxyalkyl, alkoxycarbonylalkyl, formylalkyl, or acylalkyl, including perfluoroacylalkyl (e.g., -alkyl$C(O)CF_3$)), carbamate, carbamatealkyl, urea, ureaalkyl, sulfate, sulfonate, sulfamoyl, sulfone, sulfonamide, sulfonamidealkyl, cyano, nitro, azido, sulfhydryl, alkylthio, thiocarbonyl (such as thioester, thioacetate, or thioformate), phosphoryl, phosphate, phosphonate or phosphinate.

In certain embodiments, R_{11} represents substituted or unsubstituted arylalkyl, such as substituted or unsubstituted benzyl.

In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, or CH_2NHCH_2, wherein any hydrogen atom of a CH_2 unit may be replaced by alkyl or alkoxy, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxyl. In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, preferably CH_2CH_2. In certain embodiments, L is not CH_2SCH_2.

In certain embodiments, each Y represents H. In other embodiments, at least one Y is $CH_2O(CO)R_7$.

In certain embodiments, X represents S or CH=CH. In certain embodiments, X represents S.

In certain embodiments, R_1 and R_2 each represent H.

In certain embodiments, Z represents $R_3(CO)$. In certain embodiments wherein Z is $R_3(CO)$, R_3 and R_{11} are not identical (e.g., the compound of formula I is not symmetrical).

In certain embodiments, Z represents $R_3(CO)$ and R_3 represents arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl. In certain embodiments, Z represents $R_3(CO)$ and R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents aryl, arylalkyl, heteroaryl or heteroaralkyl, such as aryl, arylalkyl or heteroaryl, R_9 represents H, and

R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl, such as hydroxy, hydroxyalkyl or alkoxy. In certain embodiments, Z represents $R_3(CO)$ and R_3 represents heteroarylalkyl.

In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, such as CH_2CH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, R_3 represents arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl, and R_{11} represents arylalkyl. In certain such embodiments, R_3 represents heteroarylalkyl.

In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, such as CH_2CH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents aryl, arylalkyl, heteroaryl or heteroaralkyl, such as aryl, arylalkyl or heteroaryl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl, such as hydroxy, hydroxyalkyl or alkoxy, and R_{11} represents arylalkyl. In certain such embodiments, R_8 represents heteroaryl.

In certain embodiments, L represents CH_2CH_2, Y represents H, X represents S or CH=CH, such as S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, R_3 represents substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl, and R_{11} represents arylalkyl. In certain such embodiments, R_3 represents heteroarylalkyl.

In certain embodiments, L represents CH_2CH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents aryl, arylalkyl or heteroaryl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl or alkoxy, and R_{11} represents arylalkyl. In certain such embodiments, R_8 represents aryl and R_{10} represents hydroxyalkyl. In certain other embodiments, R_8 represents heteroaryl.

In certain embodiments, the cancer is selected from breast cancer, colorectal cancer, endocrine cancer, melanoma, renal cancer and B cell malignancy. In certain such embodiments wherein the cancer is breast cancer, the breast cancer comprises basal-type breast cancer cells, triple-negative breast cancer cells or claudin-low breast cancer cells. In certain embodiments wherein the cancer is endocrine cancer, the endocrine cancer is selected from adrenal cortex adenoma, adrenal cortex carcicnoma, adrenal gland pheochromocytoma and parathyroid gland adenoma. In certain

embodiments wherein the cancer is a B cell malignancy, the B cell malignancy is selected from multiple myeloma, leukemia, such as acute lymphoblastic leukemia or chronic lymphoblastic leukemia, and lymphoma, such as Burkitt's lymphoma, Diffuse large B cell lymphoma, follicular lymphoma or Hodgkin's lymphoma.

In certain embodiments, the compound is selected from any one of the compounds disclosed in Table 3. Preferably, the compound is selected from compound 1, 2, 6, 7, 8, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 35, 36, 38, 39, 40, 41, 43, 44, 47, 48, 50, 51, 52, 54, 55, 58, 63, 64, 65, 67, 68, 69, 70, 71, 72, 73, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 92, 93, 94, 95, 97, 99, 100, 102, 105, 107, 111, 112, 114, 115, 116, 117, 118, 120, 121, 122, 123, 126, 127, 133, 135, 136, 138, 140, 141, 143, 146, 147, 148, 152, 153, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 168, 169, 170, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 185, 186, 187, 188, 189, 190, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 208, 210, 211, 213, 214, 216, 217, 219, 220, 226, 227, 228, 229, 231, 232, 234, 235, 236, 237, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 273, 274, 275, 276, 278, 279, 280, 281, 282, 283, 285, 286, 287, 288, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 302, 304, 1038, 306, 307, 308, 309, 310, 311, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 327, 329, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 527, 347, 348, 349, 350, 351, 352, 353, 354, 355, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569,

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In certain embodiments, compounds of the invention may be prodrugs of the compounds of formula I or Ia, e.g., wherein a hydroxyl in the parent compound is presented as an ester or a carbonate, or carboxylic acid present in the parent compound is presented as an ester. In certain such embodiments, the prodrug is metabolized to the active parent compound in vivo (e.g., the ester is hydrolyzed to the corresponding hydroxyl, or carboxylic acid).

In certain embodiments, compounds of the invention may be racemic. In certain embodiments, compounds of the invention may be enriched in one enantiomer. For example, a compound of the invention may have greater than 30% ee, 40% ee, 50% ee, 60% ee, 70% ee, 80% ee, 90% ee, or even 95% or greater ee. In certain embodiments, compounds of the invention may have more than one stereocenter. In certain such embodiments, compounds of the invention may be enriched in one or more diastereomer. For example, a compound of the invention may have greater than 30% de, 40% de, 50% de, 60% de, 70% de, 80% de, 90% de, or even 95% or greater de.

In certain embodiments, the present invention relates to methods of treating or preventing cancer, such as breast cancer, colorectal cancer, endocrine cancer, melanoma, renal cancer or B cell malignancy, with a compound of formula I or Ia, or a pharmaceutically acceptable salt thereof. In certain embodiments, the therapeutic preparation may be enriched to provide predominantly one enantiomer of a compound (e.g., of formula I or Ia). An enantiomerically enriched mixture may comprise, for example, at least 60 mol percent of one enantiomer, or more preferably at least 75, 90, 95, or even 99 mol percent. In certain embodiments, the compound enriched in one enantiomer is substantially free of the other enantiomer, wherein substantially free

means that the substance in question makes up less than 10%, or less than 5%, or less than 4%, or less than 3%, or less than 2%, or less than 1% as compared to the amount of the other enantiomer, *e.g.*, in the composition or compound mixture. For example, if a composition or compound mixture contains 98 grams of a first enantiomer and 2 grams of a second enantiomer, it would be said to contain 98 mol percent of the first enantiomer and only 2% of the second enantiomer.

In certain embodiments, the therapeutic preparation may be enriched to provide predominantly one diastereomer of a compound (e.g., of formula I or Ia). A diastereomerically enriched mixture may comprise, for example, at least 60 mol percent of one diastereomer, or more preferably at least 75, 90, 95, or even 99 mol percent.

In certain embodiments, the present invention provides a pharmaceutical preparation suitable for use in a human patient, comprising any of the compounds shown above (e.g., a compound of the invention, such as a compound of formula I or Ia), and one or more pharmaceutically acceptable excipients. In certain embodiments, the pharmaceutical preparations may be for use in treating or preventing a condition or disease as described herein. In certain embodiments, the pharmaceutical preparations have a low enough pyrogen activity to be suitable for use in a human patient.

Compounds of any of the above structures may be used in the manufacture of medicaments for the treatment of any diseases or conditions disclosed herein.

Uses of enzyme inhibitors

Glutamine plays an important role as a carrier of nitrogen, carbon, and energy. It is used for hepatic urea synthesis, for renal ammoniagenesis, for gluconeogenesis, and as respiratory fuel for many cells. Cells get their glutamine by either synthesizing it internally via an enzyme called glutamine synthetase (GS) or exogenously from the environment.

The conversion of glutamine into glutamate is initiated by the mitochondrial enzyme, glutaminase. There are two major forms of the enzyme, K-type and L-type, which are distinguished by their Km values for glutamine and response to glutamate, wherein the Km value, or Michaelis constant, is the concentration of substrate required to reach half the maximal velocity. The L-type, also known as "liver-type" or GLS2, has a high Km for glutamine and is glutamate resistant. The K-type, also

known as "kidney-type" or GLS1 or "KGA", has a low Km for glutamine and is inhibited by glutamate. An alternative splice form of GLS1, referred to as glutaminase C or "GAC", has recently been identified.

In addition to serving as the basic building blocks of protein synthesis, amino acids have been shown to contribute to many processes critical for growing and dividing cells, and this is particularly true for cancer cells. Nearly all definitions of cancer include reference to dysregulated proliferation. Numerous studies on glutamine metabolism in cancer indicate that many tumors are avid glutamine consumers (Souba, Ann. Surg., 1993; Collins et al., J. Cell. Physiol., 1998; Medina, J. Nutr., 2001; Shanware et al., J. Mol. Med., 2011), and this includes, but not limited to breast cancer. Certain embodiments of the invention relate to the use of the compounds described herein for the treatment of breast cancer.

While many cancer cells depend on exogenous glutamine for survival, the degree of glutamine dependence among tumor cell subtypes may make a population of cells more susceptible to the reduction of glutamine. As an example, gene expression analysis of breast cancers has identified five intrinsic subtypes (luminal A, luminal B, basal, HER2+, and normal-like) (Sorlie et al., Proc Natl Acad Sci USA, 2001). Although glutamine deprivation has an impact on cell growth and viability, basal-like cells appear to be more sensitive to the reduction of exogenous glutamine (Kung et al., PLoS Genetics, 2011). This supports the concept that glutamine is a very important energy source in basal-like breast cancer cell lines, and suggests that inhibition of the glutaminase enzyme would be beneficial in the treatment of breast cancers comprised of basal-like cells. Figure 1 further supports the correlation that cells dependent on exogenous glutamine are susceptible to the presence of a glutaminase inhibitor. Certain embodiments of the present invention relate to the method of treating basal-like breast cancer cells comprising administering a glutaminase inhibitor of the present application.

Enzyme expression levels can be determined in multiple manners, and quantitation is relative, based on a specific standard for each assay. The results can be used to provide a genetic profile, where the levels of certain genes, mRNAs or resulting expression products form a signature pattern that can used to characterize cell types. Kung et al, demonstrated that the basal-like breast cancer cells that showed glutamine dependency exhibited a genetic profile in which GLS expression was

relatively high and GS expression was relatively low. Furthermore, the expression level of GLS2 was relatively low. Analysis of primary breast tumors mRNA expression dataset (The Cancer Genome Atlas; N=756) support that basal-type cells generally have high GLS expression relative to GS expression.

Triple-negative breast cancer (TNBC) is characterized by a lack of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) expression. It has a higher rate of relapse following chemotherapy, and a poorer prognosis than with the other breast cancer subtypes (Dent et al., Clin Cancer Res, 2007). Interestingly, there appears to be significant similarities in metabolic profiling between TNBC cells and basal-like breast cancer cells. In particular, TNBC cells appear to have a similar genetic signature of high GLS expression and low GS expression (Figure 2). A more specific analysis of GLS expression in breast cancer cell lines revealed that TNBC cells express higher levels of both splice variants of GLS1, KGA and GAC, as well as significantly lower levels of GS, when compared to hormone receptor (HR)-positive, or Her2-positive cell lines (Figures 13 and 15). An aspect of the present invention provides a method for treating breast cancer comprising TNBC cells comprising administering a glutaminase inhibitor of the present application.

More recently, another breast cancer cell type has been identified, called claudin-low (Prat et al., Breast Cancer Res, 2010). The genetic profile of this cell type also exhibits relatively high GLS expression and low GS expression. Analysis of several claudin-low breast cancer cell lines revealed that these cells were generally dependent on exogenous glutamine and also susceptible to glutaminase inhibition. An aspect of the present invention provides a method for treating breast cancer comprising claudin-low cells comprising administering a glutaminase inhibitor of the present application.

Another aspect of the invention is the use of the compounds described herein for the treatment of breast cancer comprising cells selected from basal-type breast cancer cells, triple-negative breast cancer cells, and claudin-low breast cancer cells.

This led to the hypothesis that the high GLS expression and low GS expression profile may serve as a genetic signature to identify other cancers that may be particularly dependent on exogenous glutamine, and therefore susceptible to glutaminase inhibition. Upon analysis of a vast number of primary human cancers

from a commercial database, several cancers exhibited high GLS to low GS expression patterns. In addition to the breast cancers previously noted, colorectal cancer, endocrine cancers, lung cancer, melanoma, mesothelioma, renal cancer and B cell malignancies had notably high GLS/GS ratios (Figures 5 and 10). Certain embodiments of the invention relate to the use of the compounds described herein for the treatment of cancers selected from colorectal cancer, endocrine cancer, lung cancer, melanoma, mesothelioma, renal cancer and B cell malignancies.

As with breast cancer, certain subtypes of some of these cancers appear to have a more prevalent GLS/GS expression ratio. For example, of the endocrine cancers, adrenal cortex adenoma, adrenal cortex carcinoma, adrenal gland pheochromocytoma and parathyroid gland adenoma had ratios greater than three times that of endometrial entometrioid adenocarcinoma (Figure 6).

Within the data set, B cell malignancies included such cancers as multiple myeloma, leukemia (including acute lymphoblastic leukemia (ALL) and chronic lymphoblastic leukemia (CLL)) and lymphoma (including Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma and Hodgkin's lymphoma). All these cancers displayed a genetic profile comprising high GLS/GS expression level ratios, further suggesting that these cancers would be susceptible to glutaminase inhibition (Figures 7 and 8). Figure 17 demonstrates that administration of glutaminase inhibitor compound reduced tumor size in a multiple myeloma xenograft model, further supporting this concept. Certain embodiments of the invention relate to the use of the compounds described herein for the treatment of multiple myeloma, leukemia and lymphoma.

In some embodiments, the method of treating or preventing cancer, such as breast cancer, colorectal cancer, endocrine cancer, melanoma, renal cancer or B cell malignancy, may comprise administering a compound of the invention conjointly with one or more other chemotherapeutic agent(s). Chemotherapeutic agents that may be conjointly administered with compounds of the invention include: ABT-263, aminoglutethimide, amsacrine, anastrozole, asparaginase, bcg, bicalutamide, bleomycin, bortezomib, buserelin, busulfan, campothecin, capecitabine, carboplatin, carfilzomib, carmustine, chlorambucil, chloroquine, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, demethoxyviridin, dexamethasone, dichloroacetate, dienestrol,

diethylstilbestrol, docetaxel, doxorubicin, epirubicin, estradiol, estramustine, etoposide, everolimus, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil and 5-fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, ironotecan, lenalidomide, letrozole, leucovorin, leuprolide, levamisole, lomustine, lonidamine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, metformin, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide, oxaliplatin, paclitaxel, pamidronate, pentostatin, perifosine, PF-04691502, plicamycin, pomalidomide, porfimer, procarbazine, raltitrexed, rituximab, romidepsin, sorafenib, streptozocin, sunitinib, suramin, tamoxifen, temozolomide, temsirolimus, teniposide, testosterone, thalidomide, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, vinorelbine, and vorinostat (SAHA). For example, chemotherapeutic agents that may be conjointly administered with compounds of the invention include: aminoglutethimide, amsacrine, anastrozole, asparaginase, bcg, bicalutamide, bleomycin, bortezomib, buserelin, busulfan, campothecin, capecitabine, carboplatin, carfilzomib, carmustine, chlorambucil, chloroquine, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, demethoxyviridin, dichloroacetate, dienestrol, diethylstilbestrol, docetaxel, doxorubicin, epirubicin, estradiol, estramustine, etoposide, everolimus, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, ironotecan, lenalidomide, letrozole, leucovorin, leuprolide, levamisole, lomustine, lonidamine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, metformin, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide, oxaliplatin, paclitaxel, pamidronate, pentostatin, perifosine, plicamycin, pomalidomide, porfimer, procarbazine, raltitrexed, rituximab, sorafenib, streptozocin, sunitinib, suramin, tamoxifen, temozolomide, temsirolimus, teniposide, testosterone, thalidomide, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, and vinorelbine. In other embodiments, chemotherapeutic agents that may be conjointly administered with compounds of the invention include:

ABT-263, dexamethasone, 5-fluorouracil, PF-04691502, romidepsin, and vorinostat (SAHA). In certain embodiments of the methods of the invention described herein, the chemotherapeutic agent conjointly administered with compounds of the invention is a taxane chemotherapeutic agent, such as paclitaxel or docetaxel. In certain embodiments of the methods of the invention described herein, the chemotherapeutic agent conjointly administered with compounds of the invention is doxorubicin. In certain embodiments of the methods of the invention described herein, a compound of the invention is administered conjointly with a taxane chemotherapeutic agent (e.g., paclitaxel) and doxorubicin.

Many combination therapies have been developed for the treatment of cancer. In certain embodiments, compounds of the invention may be conjointly administered with a combination therapy. Examples of combination therapies with which compounds of the invention may be conjointly administered are included in Table 1.

Table 1: Exemplary combinatorial therapies for the treatment of cancer.

Name	**Therapeutic agents**
ABV	Doxorubicin, Bleomycin, Vinblastine
ABVD	Doxorubicin, Bleomycin, Vinblastine, Dacarbazine
AC (Breast)	Doxorubicin, Cyclophosphamide
AC (Sarcoma)	Doxorubicin, Cisplatin
AC (Neuroblastoma)	Cyclophosphamide, Doxorubicin
ACE	Cyclophosphamide, Doxorubicin, Etoposide
ACe	Cyclophosphamide, Doxorubicin
AD	Doxorubicin, Dacarbazine
AP	Doxorubicin, Cisplatin
ARAC-DNR	Cytarabine, Daunorubicin
B-CAVe	Bleomycin, Lomustine, Doxorubicin, Vinblastine
BCVPP	Carmustine, Cyclophosphamide, Vinblastine, Procarbazine, Prednisone
BEACOPP	Bleomycin, Etoposide, Doxorubicin, Cyclophosphamide, Vincristine, Procarbazine, Prednisone, Filgrastim
BEP	Bleomycin, Etoposide, Cisplatin
BIP	Bleomycin, Cisplatin, Ifosfamide, Mesna

Name	Therapeutic agents
BOMP	Bleomycin, Vincristine, Cisplatin, Mitomycin
CA	Cytarabine, Asparaginase
CABO	Cisplatin, Methotrexate, Bleomycin, Vincristine
CAF	Cyclophosphamide, Doxorubicin, Fluorouracil
CAL-G	Cyclophosphamide, Daunorubicin, Vincristine, Prednisone, Asparaginase
CAMP	Cyclophosphamide, Doxorubicin, Methotrexate, Procarbazine
CAP	Cyclophosphamide, Doxorubicin, Cisplatin
CaT	Carboplatin, Paclitaxel
CAV	Cyclophosphamide, Doxorubicin, Vincristine
CAVE ADD	CAV and Etoposide
CA-VP16	Cyclophosphamide, Doxorubicin, Etoposide
CC	Cyclophosphamide, Carboplatin
CDDP/VP-16	Cisplatin, Etoposide
CEF	Cyclophosphamide, Epirubicin, Fluorouracil
CEPP(B)	Cyclophosphamide, Etoposide, Prednisone, with or without/ Bleomycin
CEV	Cyclophosphamide, Etoposide, Vincristine
CF	Cisplatin, Fluorouracil or Carboplatin Fluorouracil
CHAP	Cyclophosphamide or Cyclophosphamide, Altretamine, Doxorubicin, Cisplatin
ChlVPP	Chlorambucil, Vinblastine, Procarbazine, Prednisone
CHOP	Cyclophosphamide, Doxorubicin, Vincristine, Prednisone
CHOP-BLEO	Add Bleomycin to CHOP
CISCA	Cyclophosphamide, Doxorubicin, Cisplatin
CLD-BOMP	Bleomycin, Cisplatin, Vincristine, Mitomycin
CMF	Methotrexate, Fluorouracil, Cyclophosphamide
CMFP	Cyclophosphamide, Methotrexate, Fluorouracil, Prednisone
CMFVP	Cyclophosphamide, Methotrexate, Fluorouracil,

Name	Therapeutic agents
	Vincristine, Prednisone
CMV	Cisplatin, Methotrexate, Vinblastine
CNF	Cyclophosphamide, Mitoxantrone, Fluorouracil
CNOP	Cyclophosphamide, Mitoxantrone, Vincristine, Prednisone
COB	Cisplatin, Vincristine, Bleomycin
CODE	Cisplatin, Vincristine, Doxorubicin, Etoposide
COMLA	Cyclophosphamide, Vincristine, Methotrexate, Leucovorin, Cytarabine
COMP	Cyclophosphamide, Vincristine, Methotrexate, Prednisone
Cooper Regimen	Cyclophosphamide, Methotrexate, Fluorouracil, Vincristine, Prednisone
COP	Cyclophosphamide, Vincristine, Prednisone
COPE	Cyclophosphamide, Vincristine, Cisplatin, Etoposide
COPP	Cyclophosphamide, Vincristine, Procarbazine, Prednisone
CP(Chronic lymphocytic leukemia)	Chlorambucil, Prednisone
CP (Ovarian Cancer)	Cyclophosphamide, Cisplatin
CT	Cisplatin, Paclitaxel
CVD	Cisplatin, Vinblastine, Dacarbazine
CVI	Carboplatin, Etoposide, Ifosfamide, Mesna
CVP	Cyclophosphamide, Vincristine, Prednisome
CVPP	Lomustine, Procarbazine, Prednisone
CYVADIC	Cyclophosphamide, Vincristine, Doxorubicin, Dacarbazine
DA	Daunorubicin, Cytarabine
DAT	Daunorubicin, Cytarabine, Thioguanine
DAV	Daunorubicin, Cytarabine, Etoposide
DCT	Daunorubicin, Cytarabine, Thioguanine
DHAP	Cisplatin, Cytarabine, Dexamethasone
DI	Doxorubicin, Ifosfamide
DTIC/Tamoxifen	Dacarbazine, Tamoxifen

Name	Therapeutic agents
DVP	Daunorubicin, Vincristine, Prednisone
EAP	Etoposide, Doxorubicin, Cisplatin
EC	Etoposide, Carboplatin
EFP	Etoposie, Fluorouracil, Cisplatin
ELF	Etoposide, Leucovorin, Fluorouracil
EMA 86	Mitoxantrone, Etoposide, Cytarabine
EP	Etoposide, Cisplatin
EVA	Etoposide, Vinblastine
FAC	Fluorouracil, Doxorubicin, Cyclophosphamide
FAM	Fluorouracil, Doxorubicin, Mitomycin
FAMTX	Methotrexate, Leucovorin, Doxorubicin
FAP	Fluorouracil, Doxorubicin, Cisplatin
F-CL	Fluorouracil, Leucovorin
FEC	Fluorouracil, Cyclophosphamide, Epirubicin
FED	Fluorouracil, Etoposide, Cisplatin
FL	Flutamide, Leuprolide
FZ	Flutamide, Goserelin acetate implant
HDMTX	Methotrexate, Leucovorin
Hexa-CAF	Altretamine, Cyclophosphamide, Methotrexate, Fluorouracil
ICE-T	Ifosfamide, Carboplatin, Etoposide, Paclitaxel, Mesna
IDMTX/6-MP	Methotrexate, Mercaptopurine, Leucovorin
IE	Ifosfamide, Etoposie, Mesna
IfoVP	Ifosfamide, Etoposide, Mesna
IPA	Ifosfamide, Cisplatin, Doxorubicin
M-2	Vincristine, Carmustine, Cyclophosphamide, Prednisone, Melphalan
MAC-III	Methotrexate, Leucovorin, Dactinomycin, Cyclophosphamide
MACC	Methotrexate, Doxorubicin, Cyclophosphamide, Lomustine

Name	Therapeutic agents
MACOP-B	Methotrexate, Leucovorin, Doxorubicin, Cyclophosphamide, Vincristine, Bleomycin, Prednisone
MAID	Mesna, Doxorubicin, Ifosfamide, Dacarbazine
m-BACOD	Bleomycin, Doxorubicin, Cyclophosphamide, Vincristine, Dexamethasone, Methotrexate, Leucovorin
MBC	Methotrexate, Bleomycin, Cisplatin
MC	Mitoxantrone, Cytarabine
MF	Methotrexate, Fluorouracil, Leucovorin
MICE	Ifosfamide, Carboplatin, Etoposide, Mesna
MINE	Mesna, Ifosfamide, Mitoxantrone, Etoposide
mini-BEAM	Carmustine, Etoposide, Cytarabine, Melphalan
MOBP	Bleomycin, Vincristine, Cisplatin, Mitomycin
MOP	Mechlorethamine, Vincristine, Procarbazine
MOPP	Mechlorethamine, Vincristine, Procarbazine, Prednisone
MOPP/ABV	Mechlorethamine, Vincristine, Procarbazine, Prednisone, Doxorubicin, Bleomycin, Vinblastine
MP (multiple myeloma)	Melphalan, Prednisone
MP (prostate cancer)	Mitoxantrone, Prednisone
MTX/6-MO	Methotrexate, Mercaptopurine
MTX/6-MP/VP	Methotrexate, Mercaptopurine, Vincristine, Prednisone
MTX-CDDPAdr	Methotrexate, Leucovorin, Cisplatin, Doxorubicin
MV (breast cancer)	Mitomycin, Vinblastine
MV (acute myelocytic leukemia)	Mitoxantrone, Etoposide
M-VAC Methotrexate	Vinblastine, Doxorubicin, Cisplatin
MVP Mitomycin	Vinblastine, Cisplatin
MVPP	Mechlorethamine, Vinblastine, Procarbazine, Prednisone
NFL	Mitoxantrone, Fluorouracil, Leucovorin
NOVP	Mitoxantrone, Vinblastine, Vincristine
OPA	Vincristine, Prednisone, Doxorubicin

Name	Therapeutic agents
OPPA	Add Procarbazine to OPA.
PAC	Cisplatin, Doxorubicin
PAC-I	Cisplatin, Doxorubicin, Cyclophosphamide
PA-CI	Cisplatin, Doxorubicin
PC	Paclitaxel, Carboplatin or Paclitaxel, Cisplatin
PCV	Lomustine, Procarbazine, Vincristine
PE	Paclitaxel, Estramustine
PFL	Cisplatin, Fluorouracil, Leucovorin
POC	Prednisone, Vincristine, Lomustine
ProMACE	Prednisone, Methotrexate, Leucovorin, Doxorubicin, Cyclophosphamide, Etoposide
ProMACE/cytaBOM	Prednisone, Doxorubicin, Cyclophosphamide, Etoposide, Cytarabine, Bleomycin, Vincristine, Methotrexate, Leucovorin, Cotrimoxazole
PRoMACE/MOPP	Prednisone, Doxorubicin, Cyclophosphamide, Etoposide, Mechlorethamine, Vincristine, Procarbazine, Methotrexate, Leucovorin
Pt/VM	Cisplatin, Teniposide
PVA	Prednisone, Vincristine, Asparaginase
PVB	Cisplatin, Vinblastine, Bleomycin
PVDA	Prednisone, Vincristine, Daunorubicin, Asparaginase
SMF	Streptozocin, Mitomycin, Fluorouracil
TAD	Mechlorethamine, Doxorubicin, Vinblastine, Vincristine, Bleomycin, Etoposide, Prednisone
TCF	Paclitaxel, Cisplatin, Fluorouracil
TIP	Paclitaxel, Ifosfamide, Mesna, Cisplatin
TTT	Methotrexate, Cytarabine, Hydrocortisone
Topo/CTX	Cyclophosphamide, Topotecan, Mesna
VAB-6	Cyclophosphamide, Dactinomycin, Vinblastine, Cisplatin, Bleomycin
VAC	Vincristine, Dactinomycin, Cyclophosphamide

Name	Therapeutic agents
VACAdr	Vincristine, Cyclophosphamide, Doxorubicin, Dactinomycin, Vincristine
VAD	Vincristine, Doxorubicin, Dexamethasone
VATH	Vinblastine, Doxorubicin, Thiotepa, Flouxymesterone
VBAP	Vincristine, Carmustine, Doxorubicin, Prednisone
VBCMP	Vincristine, Carmustine, Melphalan, Cyclophosphamide, Prednisone
VC	Vinorelbine, Cisplatin
VCAP	Vincristine, Cyclophosphamide, Doxorubicin, Prednisone
VD	Vinorelbine, Doxorubicin
VelP	Vinblastine, Cisplatin, Ifosfamide, Mesna
VIP	Etoposide, Cisplatin, Ifosfamide, Mesna
VM	Mitomycin, Vinblastine
VMCP	Vincristine, Melphalan, Cyclophosphamide, Prednisone
VP	Etoposide, Cisplatin
V-TAD	Etoposide, Thioguanine, Daunorubicin, Cytarabine
5 + 2	Cytarabine, Daunorubicin, Mitoxantrone
7 + 3	Cytarabine with/, Daunorubicin or Idarubicin or Mitoxantrone
"8 in 1"	Methylprednisolone, Vincristine, Lomustine, Procarbazine, Hydroxyurea, Cisplatin, Cytarabine, Dacarbazine

The proliferation of cancer cells requires lipid synthesis. Normally, acetyl-coA used for lipid synthesis is formed from a mitochondrial pool of pyruvate that is derived from glycolysis. Yet under hypoxic conditions, such as those normally found in a tumor environment, the conversion of pyruvate to acetyl-coA within the mitochondria is downregulated. Recent studies from Metallo et al. (2011) and Mullen et al. (2011) revealed that under such hypoxic conditions, cells instead largely switch to using a pathway involving the reductive carboxylation of alpha-ketoglutarate to make acetyl-coA for lipid synthesis. The first step in this pathway involves converting glutamine to glutamate via glutaminase enzymes. Subsequently, glutamate is

converting to alpha-ketoglutarate, and the resulting alpha-ketoglutarate is converted to isocitrate in a reductive carboxylation step mediated by the isocitrate dehydrogenase enzymes. A switch to this reductive carboxylation pathway also occurs in some renal carcinoma cell lines that contain either impaired mitochondria or an impaired signal for induction of the enzyme responsible for converting glycolytic pyruvate to acetyl-coA (Mullen et al 2011). A similar switch occurs in cells exposed to mitochondrial respiratory chain inhibitors such as metformin, rotenone, and antimycin (Mullen at al. 2011). Therefore, in some embodiments of this invention, we propose using combinations of mitochondrial respiratory chain inhibitors and glutaminase inhibitors to simultaneously increase cancer cells' dependence on glutaminase-dependent pathways for lipid synthesis while inhibiting those very pathways.

The increased dependence on glycolysis in tumor cells is likely because the hypoxic tumor environment impairs mitochondrial respiration. Furthermore, depletion of glucose induces apoptosis in cells transformed with the MYC oncogene. These findings suggest that inhibiting glycolysis would have a therapeutic value in preventing cancer cell proliferation. There are currently many documented glycolytic inhibitors (Pelicano et al. 2006). However, as pointed out by Zhao et al. (2012), "available glycolytic inhibitors are generally not very potent, and high doses are required, which may cause high levels of systemic toxicity." Since cancer cells typically use both glucose and glutamine at higher levels than normal cells, impairing utilization of each of those metabolites will likely have a synergistic effect. Therefore, in some embodiments of this invention, we propose using combinations of glycolytic pathway inhibitors and glutaminase inhibitors. Such glycolytic inhibitors include 2-deoxyglucose, lonidamine, 3-bromopyruvate, imatinib, oxythiamine, rapamycin, and their pharmacological equivalents. Glycolysis can be inhibited indirectly by depleting NAD+ via DNA damage induced by DNA alkylating agents through a pathway activated by poly(ADP-ribose) polymerase (Zong et al. 2004). Therefore, in some embodiments of this invention, we propose using a combination of DNA alkylating agents and glutaminase inhibitors. Cancer cells use the pentose phosphate pathway along with the glycolytic pathway to create metabolic intermediates derived from glucose. Therefore, in some embodiments of this

invention, we propose using a combination of pentose phosphate inhibitors such as 6-aminonicotinamide along with glutaminase inhibitors.

In certain embodiments, a compound of the invention may be conjointly administered with non-chemical methods of cancer treatment. In certain embodiments, a compound of the invention may be conjointly administered with radiation therapy. In certain embodiments, a compound of the invention may be conjointly administered with surgery, with thermoablation, with focused ultrasound therapy, with cryotherapy, or with any combination of these.

In certain embodiments, different compounds of the invention may be conjointly administered with one or more other compounds of the invention. Moreover, such combinations may be conjointly administered with other therapeutic agents, such as other agents suitable for the treatment of cancer, immunological or neurological diseases, such as the agents identified above. In certain embodiments, conjointly administering one or more additional chemotherapeutic agents with a compound of the invention provides a synergistic effect, such as shown in Figure 18. In certain embodiments, conjointly administering one or more additional chemotherapeutics agents provides an additive effect.

In certain embodiments, the present invention provides a kit comprising: a) one or more single dosage forms of a compound of the invention; b) one or more single dosage forms of a chemotherapeutic agent as mentioned above; and c) instructions for the administration of the compound of the invention and the chemotherapeutic agent for the treatment of cancer, wherein the cancer is selected from breast cancer, colorectal cancer, endocrine cancer, lung cancer, melanoma, mesothelioma, renal cancer and B cell malignancy.

The present invention provides a kit comprising:

a) a pharmaceutical formulation (e.g., one or more single dosage forms) comprising a compound of the invention; and

b) instructions for the administration of the pharmaceutical formulation, e.g., for treating or preventing cancer, such as breast cancer, colorectal cancer, endocrine cancer, lung cancer, melanoma, mesothelioma, renal cancer or B cell malignancy.

The present invention provides a kit comprising:

a) a pharmaceutical formulation (e.g., one or more single dosage forms) comprising a compound of the invention; and

b) instructions for the administration of the pharmaceutical formulation, e.g., for treating or preventing breast cancer, wherein the breast cancer comprises basal-type breast cancer cells, triple-negative breast cancer cells, or claudin-low breast cancer cells .

In certain embodiments, the kit further comprises instructions for the administration of the pharmaceutical formulation comprising a compound of the invention conjointly with a chemotherapeutic agent as mentioned above. In certain embodiments, the kit further comprises a second pharmaceutical formulation (e.g., as one or more single dosage forms) comprising a chemotherapeutic agent as mentioned above.

Both dependency on exogenous glutamine and the expression profile of high glutaminase (GLS) and low glutamine synthetase (GS) levels have been shown to correlate with a cancer cell's sensitivity to glutaminase inhibition. Utilizing this information, one may theorize that the amount of metabolic metabolites within a cancer cell may be used as a way to predict its sensitivity to glutaminase inhibition. Testing out this theory, glutamate and glutamine levels were determined in TNBC cells previously shown to be glutamine dependent and sensitive to glutaminase inhibition (Figure 9). Concentrations of glutamate and glutamine were determined by liquid chromatography tandem spectrometry (LC-MS/MS); however, any method of determining metabolite concentrations could be utilized. The cells with glutamate:glutamine ratios greater than or equal to 1.5 did appear to be sensitive to glutaminase inhibition. The correlation was even stronger when the glutamate:glutamine ratio was greater than or equal to 2. This result provides a means to identify cancer patients that may benefit from treatment with a glutaminase inhibitor.

Analysis of several primary tumor xenografts show that expression and metabolite correlation extends to other tumor types, such as lung and mesothelioma, in addition to those cancers previously discussed (Figure 10). Xenograft studies using HCT116 colon carcinoma cells (Figure 11) and H2122 lung adenocarcinoma cells (Figure 12) show that treatment with a glutaminase inhibitor described herein resulted in reduced tumor size.

In certain embodiments, the invention provides a method of identifying a cancer patient that may benefit from treatment with a glutaminase inhibitor comprising determining the ratio of glutamate to glutamine in cancer cells of the cancer patient, wherein a ratio greater than or equal to 1.5, such as greater than or equal to 1.6, greater than or equal to 1.7, greater than or equal to 1.8, greater than or equal to 1.9, or greater than or equal to 2.0, indicates the patient may benefit from treatment with a glutaminase inhibitor. In certain such embodiments, the method of determining the ratio includes measuring the amounts of glutamate and glutamine in the cancer cells of the cancer patient. In certain embodiments, the ratio is greater than or equal to 2.0. In certain embodiments of the foregoing, the glutaminase inhibitor is a compound described herein (e.g., a compound of formula I or Ia). In certain embodiments, the cancer is selected from B cell malignancy, breast cancer, colorectal cancer, endocrine cancer, lung cancer, melanoma, mesothelioma and renal cancer.

In certain embodiments, the invention provides a method of treating a cancer patient comprising 1) determining the ratio of glutamate to glutamine in cancer cells of the cancer patient; and 2) if the ratio of glutamate to glutamine is greater than or equal to 1.5, such as greater than or equal to 1.6, greater than or equal to 1.7, greater than or equal to 1.8, greater than or equal to 1.9, or greater than or equal to 2.0, treating the patient with a compound of formula I or Ia. In certain such embodiments, the method of determining the ratio includes measuring the amounts of glutamate and glutamine in the cancer cells of the cancer patient. In certain embodiments, the ratio of glutamate to glutamine is greater than or equal to 2.0. In certain embodiments, the cancer is selected from B cell malignancy, breast cancer, colorectal cancer, endocrine cancer, lung cancer, melanoma, mesothelioma and renal cancer.

As mentioned above, high glutaminase (GLS) and low glutamine synthetase (GS) expression levels have been shown to correlate with a cancer cell's sensitivity to glutaminase inhibition. One may therefore theorize that the levels of GLS and GS within a cancer cell could be used as a way to predict its sensitivity to glutaminase inhibition. Testing out this theory, GLS (both KGA and GAC) levels and GS levels were determined in TNBC cells, which are known to be more sensitive to glutaminase inhibition, and in HR+ or Her2+ cells, which known to be less sensitive to glutaminase inhibition (Figure 14 and Table 7).

Correlations were observed between glutaminase inhibitor sensitivity and expression of the GAC isoform of GLS. Cells expressing GAC appeared to be more sensitive to glutaminase inhibition. Thus, a cell with a detectable level of GAC would be sensitive to a glutaminase inhibitor such as the compounds described herein. A correlation was also observed for cells expressing a level of GAC that is equal or higher than KGA. Accordingly, in certain embodiments, the invention provides a method of identifying a cancer patient that may benefit from treatment with a glutaminase inhibitor, comprising determining the level of GAC and KGA expression in a cancer cell of the cancer patient, wherein an expression level of GAC is greater than, or equal to the expression level of KGA, indicates that the patient may benefit from treatment with a glutaminase inhibitor.

Significant correlations were observed between glutaminase inhibitor sensitivity and the ratio of GAC:GS. Cells with a GAC:GS ratios greater than or equal to 0.05 appeared to be sensitive to glutaminase inhibition. The correlation was even stronger for cells having a GAC:GS ratio greater than or equal to 1. This result provides a means to identify cancer patients that may benefit from treatment with a glutaminase inhibitor.

In certain embodiments, the invention provides a method of identifying a cancer patient that may benefit from treatment with a glutaminase inhibitor, comprising determining the ratio of glutaminase to glutamine synthetase in cancer cells of the cancer patient, wherein a ratio greater than or equal to 0.05, such as greater than or equal to 0.06, greater than or equal to 0.07, greater than or equal to 0.08, greater than or equal to 0.9, or greater than or equal to 1.0, indicates the patient may benefit from treatment with a glutaminase inhibitor. In certain such embodiments, the method of determining the ratio includes measuring the levels of glutaminase and glutamine synthetase in the cancer cells of the cancer patient. In certain embodiments, the ratio is greater than or equal to 1. In certain embodiments of the foregoing, the glutaminase inhibitor is a compound described herein (e.g., a compound of formula I or Ia). In certain embodiments, the glutaminase is both KGA and GAC. In certain embodiments, the glutaminase is KGA. In prefered embodiments, the glutaminase is GAC.

In certain embodiments, the invention provides a method of treating a cancer patient comprising 1) determining the ratio of glutaminase to glutamine synthetase in

cancer cells of the cancer patient; and 2) if the ratio of glutaminase to glutamine synthetase is greater than or equal to 0.05, such as greater than or equal to 0.06, greater than or equal to 0.07, greater than or equal to 0.08, greater than or equal to 0.9, or greater than or equal to 1.0, indicates the patient may benefit from treatment with a glutaminase inhibitor. In certain such embodiments, the method of determining the ratio includes measuring the amounts of glutaminase and glutamine synthetase in the cancer cells of the cancer patient. In certain embodiments, the ratio is greater than or equal to 1. In certain embodiments, the glutaminase is both KGA and GAC. In certain embodiments, the glutaminase is KGA. In prefered embodiments, the glutaminase is GAC.

In certain embodiments, the cancer is selected from B cell malignancy, breast cancer, colorectal cancer, endocrine cancer, lung cancer, melanoma, mesothelioma and renal cancer.

The level of a GLS (e.g., KGA and/or GAC) and GS can be measured using any suitable method. Some methods involve measuring protein levels, and others involve measuring levels of mRNA.

Protein amounts can be measured using antibodies. Antibodies suitable for use in the methods disclosed herein are commercially available, or can be prepared routinely. Methods for preparing and using antibodies in assays for proteins of interest are conventional, and are described in, for example, Green et al., Production of Polyclonal Antisera, in Immunochemical Protocols (Manson, ed.), (Humana Press 1992); Coligan et al., in Current Protocols in Immunology, Sec. 2.4.1 (1992); Kohler & Milstein (1975), Nature 256, 495; Coligan et al., sections 2.5.1-2.6.7; and Harlow et al., Antibodies: A Laboratory Manual, page 726 (Cold Spring Harbor Laboratory Pub. 1988).

Any of a variety of antibodies can be used in methods of the invention. Such antibodies include, for example, polyclonal, monoclonal (mAbs), recombinant, humanized or partially humanized, single chain, Fab, and fragments thereof. The antibodies can be of any isotype, e.g., IgM, various IgG isotypes such as IgG1, IgG2a, etc., and they can be from any animal species that produces antibodies, including goat, rabbit, mouse, chicken or the like. The term "an antibody specific for" a protein means that the antibody recognizes a defined sequence of amino acids, or epitope, in the protein, and binds selectively to the protein and not generally to proteins

unintended for binding to the antibody. The parameters required to achieve specific binding can be determined routinely, using conventional methods in the art.

In some embodiments of the invention, antibodies specific for KGA, GAC and/or GS are immobilized on a surface (e.g., are reactive elements on an array, such as a microarray, or are on another surface, such as used for surface plasmon resonance (SPR)-based technology, such as Biacore), and proteins in the sample are detected by virtue of their ability to bind specifically to the antibodies. Alternatively, proteins in the sample can be immobilized on a surface, and detected by virtue of their ability to bind specifically to the antibodies. Methods of preparing the surfaces and performing the analyses, including conditions effective for specific binding, are conventional and well-known in the art.

Among the many types of suitable immunoassays are immunohistochemical staining, ELISA, Western blot (immunoblot), immunoprecipitation, radioimmunoassay (RIA), fluorescence-activated cell sorting (FACS), etc. Assays used in methods of the invention can be based on colorimetric readouts, fluorescent readouts, mass spectroscopy, visual inspection, etc.

As mentioned above, expression levels of GLS (KGA and/or GAC) and GS can be measured by measuring mRNA amounts. The amount of an mRNA encoding a KGA, GAC and/or GS can be measured using any suitable method. Examples of such methods include, for example, reverse transcriptase-polymerase chain reaction (RT-PCR), including real time PCR, microarray analysis, nanostring, Northern blot analysis, differential hybridization, and ribonuclease protection assay. Such methods are well-known in the art and are described in, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, current edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., and Ausubel et al., Current Protocols in Molecular Biology, John Wiley & sons, New York, N.Y.

In some embodiments of the invention, a histological sample is obtained from a subject (e.g., from a tumor biopsy), using any method known in the art, and include, but are not limited to, tissue section, needle biopsy, and the like. Frequently the sample will be a "clinical sample", which is a sample derived from a patient, including sections of tissues such as frozen sections or paraffin sections taken for histological purposes. The sample can also be derived from supernatants (of cells) or the cells themselves from cell cultures, cells from tissue culture and other media.

Protein or mRNA is then obtained brom the sample, and used to quantitate the amounts of GLS (KGA and/or GAC) and GS.

An alternative way of viewing the correlation between glutaminase activity and sensitivity to glutaminase inhibitor is shown in Figure 16, wherein a glutaminase activity of 0.005 μmol/min/mg of protein predicts sensitivity to glutaminase inhibitor. In certain embodiments, the invention provides a method of identifying a cancer patient that may benefit from treatment with a glutaminase inhibitor comprising determining glutaminase activity in cancer cells of the cancer patient, wherein an activity greater than or equal to 0.005 μmol/min/mg of protein, such as greater than or equal to 0.006 μmol/min/mg of protein, greater than or equal to 0.007 μmol/min/mg of protein, greater than or equal to 0.008 μmol/min/mg of protein, greater than or equal to 0.009 μmol/min/mg of protein, or greater than or equal to 0.010 μmol/min/mg of protein, indicates the patient may benefit from treatment with a glutaminase inhibitor. In certain such embodiments, the method of determining the glutaminase activity includes measuring the glutaminase activity in the cancer cells of the cancer patient. In certain embodiments, the glutaminase activity is greater than or equal to 0.010. In certain embodiments of the foregoing, the glutaminase inhibitor is a compound described herein (e.g., a compound of formula I or Ia). In certain embodiments, the cancer is selected from B cell malignancy, breast cancer, colorectal cancer, endocrine cancer, lung, melanoma, mesothelioma and renal cancer.

In certain embodiments, the invention provides a method of treating a cancer patient comprising 1) determining glutaminase activity in cancer cells of the cancer patient; and 2) and wherein an activity greater than or equal to 0.005 μmol/min/mg of protein, such as greater than or equal to 0.006 μmol/min/mg of protein, greater than or equal to 0.007 μmol/min/mg of protein, greater than or equal to 0.008 μmol/min/mg of protein, greater than or equal to 0.009 μmol/min/mg of protein, or greater than or equal to 0.010 μmol/min/mg of protein, treating the patient with a compound of formula I or Ia. In certain such embodiments, the method of determining determining glutaminase activity in the cancer cells of the cancer patient. In certain embodiments, the ratio of glutamate to glutamine is greater than or equal to 2.0. In certain embodiments, the cancer is selected from B cell malignancy, breast cancer, colorectal cancer, endocrine cancer, lung, melanoma, mesothelioma and renal cancer.

The disclosure also provides kits for detecting whether a subject having a cancer is likely to be responsive to glutaminase inhibitors. The kit may include one or more agents for detecting the amount of expression of a protein of the invention [e.g., the amount of the protein, and/or the amount of a nucleic acid (e.g., an mRNA) encoding the protein]. The agents in the kit can encompass, for example, antibodies specific for the proteins, or probes specific for the mRNA that can be used to hybridize to the RNA (or to a cDNA generated from it) or to perform RT-PCR. The kit may also include additional agents suitable for detecting, measuring and/or quantitating the amount of protein or nucleic acid. Among other uses, kits of the invention can be used in experimental applications. A skilled worker will recognize components of kits suitable for carrying out a method of the invention.

Optionally, a kit of the invention may comprise instructions for performing the method. Optional elements of a kit of the invention include suitable buffers, containers, or packaging materials. The reagents of the kit may be in containers in which the reagents are stable, e.g., in lyophilized form or stabilized liquids. The reagents may also be in single use form, e.g., for the performance of an assay for a single subject.

Definitions

The term “acyl” is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)-, preferably alkylC(O)-.

The term “acylamino” is art-recognized and refers to an amino group substituted with an acyl group and may be represented, for example, by the formula hydrocarbylC(O)NH-.

The term “acyloxy” is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)O-, preferably alkylC(O)O-.

The term “alkoxy” refers to an alkyl group, preferably a lower alkyl group, having an oxygen attached thereto. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like.

The term “alkoxyalkyl” refers to an alkyl group substituted with an alkoxy group and may be represented by the general formula alkyl-O-alkyl.

The term “alkenyl”, as used herein, refers to an aliphatic group containing at least one double bond and is intended to include both "unsubstituted alkenyls" and "substituted alkenyls", the latter of which refers to alkenyl moieties having

substituents replacing a hydrogen on one or more carbons of the alkenyl group. Such substituents may occur on one or more carbons that are included or not included in one or more double bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed below, except where stability is prohibitive. For example, substitution of alkenyl groups by one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated.

An "alkyl" group or "alkane" is a straight chained or branched non-aromatic hydrocarbon which is completely saturated. Typically, a straight chained or branched alkyl group has from 1 to about 20 carbon atoms, preferably from 1 to about 10 unless otherwise defined. Examples of straight chained and branched alkyl groups include methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, pentyl, hexyl, pentyl and octyl. A C_1-C_6 straight chained or branched alkyl group is also referred to as a "lower alkyl" group.

Moreover, the term "alkyl" (or "lower alkyl") as used throughout the specification, examples, and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents, if not otherwise specified, can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), $-CF_3$, -CN and the like. Exemplary substituted alkyls are described below. Cycloalkyls can be further substituted with alkyls, alkenyls, alkoxys, alkylthios, aminoalkyls, carbonyl-substituted alkyls, $-CF_3$, -CN, and the like.

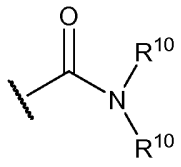
The term "C_{x-y}" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups that contain from x to y carbons in the chain. For example, the term "C_{x-y}alkyl" refers to substituted or unsubstituted saturated hydrocarbon groups, including straight-chain alkyl and branched-chain alkyl groups that contain from x to y carbons in the chain, including haloalkyl groups such as trifluoromethyl and 2,2,2-tirfluoroethyl, etc. C_0 alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. The terms "C_{2-y}alkenyl" and "C_{2-y}alkynyl" refer to substituted or unsubstituted unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The term "alkylamino", as used herein, refers to an amino group substituted with at least one alkyl group.

The term "alkylthio", as used herein, refers to a thiol group substituted with an alkyl group and may be represented by the general formula alkylS-.

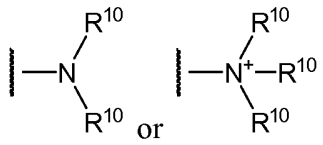
The term "alkynyl", as used herein, refers to an aliphatic group containing at least one triple bond and is intended to include both "unsubstituted alkynyls" and "substituted alkynyls", the latter of which refers to alkynyl moieties having substituents replacing a hydrogen on one or more carbons of the alkynyl group. Such substituents may occur on one or more carbons that are included or not included in one or more triple bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed above, except where stability is prohibitive. For example, substitution of alkynyl groups by one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated.

The term "amide", as used herein, refers to a group



wherein each R^{10} independently represent a hydrogen or hydrocarbyl group, or two R^{10} are taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines and salts thereof, e.g., a moiety that can be represented by



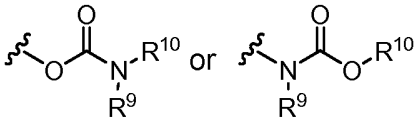
wherein each R^{10} independently represents a hydrogen or a hydrocarbyl group, or two R^{10} are taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term "aminoalkyl", as used herein, refers to an alkyl group substituted with an amino group.

The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group.

The term "aryl" as used herein include substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. Preferably the ring is a 5- to 7-membered ring, more preferably a 6-membered ring. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Aryl groups include benzene, naphthalene, phenanthrene, phenol, aniline, and the like.

The term "carbamate" is art-recognized and refers to a group



wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl group, such as an alkyl group, or R^9 and R^{10} taken together with the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

The terms "carbocycle", and "carbocyclic", as used herein, refers to a saturated or unsaturated ring in which each atom of the ring is carbon. The term carbocycle includes both aromatic carbocycles and non-aromatic carbocycles. Non-aromatic carbocycles include both cycloalkane rings, in which all carbon atoms are saturated, and cycloalkene rings, which contain at least one double bond. "Carbocycle" includes 5-7 membered monocyclic and 8-12 membered bicyclic rings. Each ring of a bicyclic carbocycle may be selected from saturated, unsaturated and aromatic rings. Carbocycle includes bicyclic molecules in which one, two or three or

more atoms are shared between the two rings. The term "fused carbocycle" refers to a bicyclic carbocycle in which each of the rings shares two adjacent atoms with the other ring. Each ring of a fused carbocycle may be selected from saturated, unsaturated and aromatic rings. In an exemplary embodiment, an aromatic ring, e.g., phenyl, may be fused to a saturated or unsaturated ring, e.g., cyclohexane, cyclopentane, or cyclohexene. Any combination of saturated, unsaturated and aromatic bicyclic rings, as valence permits, is included in the definition of carbocyclic. Exemplary "carbocycles" include cyclopentane, cyclohexane, bicyclo[2.2.1]heptane, 1,5-cyclooctadiene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]oct-3-ene, naphthalene and adamantane. Exemplary fused carbocycles include decalin, naphthalene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]octane, 4,5,6,7-tetrahydro-1H-indene and bicyclo[4.1.0]hept-3-ene. "Carbocycles" may be susbstituted at any one or more positions capable of bearing a hydrogen atom.

A "cycloalkyl" group is a cyclic hydrocarbon which is completely saturated. "Cycloalkyl" includes monocyclic and bicyclic rings. Typically, a monocyclic cycloalkyl group has from 3 to about 10 carbon atoms, more typically 3 to 8 carbon atoms unless otherwise defined. The second ring of a bicyclic cycloalkyl may be selected from saturated, unsaturated and aromatic rings. Cycloalkyl includes bicyclic molecules in which one, two or three or more atoms are shared between the two rings. The term "fused cycloalkyl" refers to a bicyclic cycloalkyl in which each of the rings shares two adjacent atoms with the other ring. The second ring of a fused bicyclic cycloalkyl may be selected from saturated, unsaturated and aromatic rings. A "cycloalkenyl" group is a cyclic hydrocarbon containing one or more double bonds.

The term "carbocyclylalkyl", as used herein, refers to an alkyl group substituted with a carbocycle group.

The term "carbonate" is art-recognized and refers to a group $-OCO_2-R^{10}$, wherein R^{10} represents a hydrocarbyl group.

The term "carboxy", as used herein, refers to a group represented by the formula $-CO_2H$.

The term "ester", as used herein, refers to a group $-C(O)OR^{10}$ wherein R^{10} represents a hydrocarbyl group.

The term "ether", as used herein, refers to a hydrocarbyl group linked through an oxygen to another hydrocarbyl group. Accordingly, an ether substituent of a

hydrocarbyl group may be hydrocarbyl-O-. Ethers may be either symmetrical or unsymmetrical. Examples of ethers include, but are not limited to, heterocycle-O-heterocycle and aryl-O-heterocycle. Ethers include "alkoxyalkyl" groups, which may be represented by the general formula alkyl-O-alkyl.

The terms "halo" and "halogen" as used herein means halogen and includes chloro, fluoro, bromo, and iodo.

The terms "hetaralkyl" and "heteroaralkyl", as used herein, refers to an alkyl group substituted with a hetaryl group.

The term "heteroalkyl", as used herein, refers to a saturated or unsaturated chain of carbon atoms and at least one heteroatom, wherein no two heteroatoms are adjacent.

The terms "heteroaryl" and "hetaryl" include substituted or unsubstituted aromatic single ring structures, preferably 5- to 7-membered rings, more preferably 5- to 6-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms "heteroaryl" and "hetaryl" also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, and pyrimidine, and the like.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, and sulfur.

The terms "heterocyclyl", "heterocycle", and "heterocyclic" refer to substituted or unsubstituted non-aromatic ring structures, preferably 3- to 10-membered rings, more preferably 3- to 7-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms "heterocyclyl" and "heterocyclic" also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heterocyclic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heterocyclyl groups include,

for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like.

The term "heterocyclylalkyl", as used herein, refers to an alkyl group substituted with a heterocycle group.

The term "hydrocarbyl", as used herein, refers to a group that is bonded through a carbon atom that does not have a =O or =S substituent, and typically has at least one carbon-hydrogen bond and a primarily carbon backbone, but may optionally include heteroatoms. Thus, groups like methyl, ethoxyethyl, 2-pyridyl, and trifluoromethyl are considered to be hydrocarbyl for the purposes of this application, but substituents such as acetyl (which has a =O substituent on the linking carbon) and ethoxy (which is linked through oxygen, not carbon) are not. Hydrocarbyl groups include, but are not limited to aryl, heteroaryl, carbocycle, heterocyclyl, alkyl, alkenyl, alkynyl, and combinations thereof.

The term "hydroxyalkyl", as used herein, refers to an alkyl group substituted with a hydroxy group.

The term "lower" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups where there are ten or fewer non-hydrogen atoms in the substituent, preferably six or fewer. A "lower alkyl", for example, refers to an alkyl group that contains ten or fewer carbon atoms, preferably six or fewer. In certain embodiments, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy substituents defined herein are respectively lower acyl, lower acyloxy, lower alkyl, lower alkenyl, lower alkynyl, or lower alkoxy, whether they appear alone or in combination with other substituents, such as in the recitations hydroxyalkyl and aralkyl (in which case, for example, the atoms within the aryl group are not counted when counting the carbon atoms in the alkyl substituent).

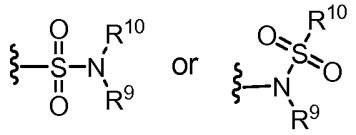
The terms "polycyclyl", "polycycle", and "polycyclic" refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls) in which two or more atoms are common to two adjoining rings, e.g., the rings are "fused rings". Each of the rings of the polycycle can be substituted or unsubstituted. In certain embodiments, each ring of the polycycle contains from 3 to 10 atoms in the ring, preferably from 5 to 7.

The term "silyl" refers to a silicon moiety with three hydrocarbyl moieties attached thereto.

The term "substituted" refers to moieties having substituents replacing a hydrogen on one or more carbons of the backbone. It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. Substituents can include any substituents described herein, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that substituents can themselves be substituted, if appropriate. Unless specifically stated as "unsubstituted," references to chemical moieties herein are understood to include substituted variants. For example, reference to an "aryl" group or moiety implicitly includes both substituted and unsubstituted variants.

The term "sulfate" is art-recognized and refers to the group $-OSO_3H$, or a pharmaceutically acceptable salt thereof.

The term "sulfonamide" is art-recognized and refers to the group represented by the general formulae



wherein R^9 and R^{10} independently represents hydrogen or hydrocarbyl, such as alkyl, or R^9 and R^{10} taken together with the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term "sulfoxide" is art-recognized and refers to the group -S(O)-R^{10}, wherein R^{10} represents a hydrocarbyl.

The term "sulfonate" is art-recognized and refers to the group SO_3H, or a pharmaceutically acceptable salt thereof.

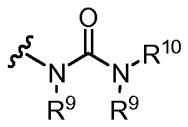
The term "sulfone" is art-recognized and refers to the group -$S(O)_2$-R^{10}, wherein R^{10} represents a hydrocarbyl.

The term "thioalkyl", as used herein, refers to an alkyl group substituted with a thiol group.

The term "thioester", as used herein, refers to a group -C(O)SR^{10} or -SC(O)R^{10} wherein R^{10} represents a hydrocarbyl.

The term "thioether", as used herein, is equivalent to an ether, wherein the oxygen is replaced with a sulfur.

The term "urea" is art-recognized and may be represented by the general formula



wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl, such as alkyl, or either occurrence of R^9 taken together with R^{10} and the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

"Protecting group" refers to a group of atoms that, when attached to a reactive functional group in a molecule, mask, reduce or prevent the reactivity of the functional group. Typically, a protecting group may be selectively removed as desired during the course of a synthesis. Examples of protecting groups can be found in Greene and Wuts, *Protective Groups in Organic Chemistry,* 3rd Ed., 1999, John Wiley & Sons, NY and Harrison et al., *Compendium of Synthetic Organic Methods*, Vols. 1-8, 1971-1996, John Wiley & Sons, NY. Representative nitrogen protecting groups include, but are not limited to, formyl, acetyl, trifluoroacetyl, benzyl, benzyloxycarbonyl ("CBZ"), tert-butoxycarbonyl ("Boc"), trimethylsilyl ("TMS"), 2-trimethylsilyl-ethanesulfonyl ("TES"), trityl and substituted trityl groups,

allyloxycarbonyl, 9-fluorenylmethyloxycarbonyl ("FMOC"), nitro-veratryloxycarbonyl ("NVOC") and the like. Representative hydroxylprotecting groups include, but are not limited to, those where the hydroxyl group is either acylated (esterified) or alkylated such as benzyl and trityl ethers, as well as alkyl ethers, tetrahydropyranyl ethers, trialkylsilyl ethers (e.g., TMS or TIPS groups), glycol ethers, such as ethylene glycol and propylene glycol derivatives and allyl ethers.

The term "healthcare providers" refers to individuals or organizations that provide healthcare services to a person, community, etc. Examples of "healthcare providers" include doctors, hospitals, continuing care retirement communities, skilled nursing facilities, subacute care facilities, clinics, multispecialty clinics, freestanding ambulatory centers, home health agencies, and HMO's.

As used herein, a therapeutic that "prevents" a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample.

The term "treating" includes prophylactic and/or therapeutic treatments. The term "prophylactic or therapeutic" treatment is art-recognized and includes administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) then the treatment is prophylactic (i.e., it protects the host against developing the unwanted condition), whereas if it is administered after manifestation of the unwanted condition, the treatment is therapeutic, (i.e., it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

The term "prodrug" is intended to encompass compounds which, under physiologic conditions, are converted into the therapeutically active agents of the present invention (e.g., a compound of formula I). A common method for making a prodrug is to include one or more selected moieties which are hydrolyzed under physiologic conditions to reveal the desired molecule. In other embodiments, the prodrug is converted by an enzymatic activity of the host animal. For example, esters or carbonates (e.g., esters or carbonates of alcohols or carboxylic acids) are preferred

prodrugs of the present invention. In certain embodiments, some or all of the compounds of formula I in a formulation represented above can be replaced with the corresponding suitable prodrug, e.g., wherein a hydroxyl in the parent compound is presented as an ester or a carbonate or carboxylic acid present in the parent compound is presented as an ester.

Pharmaceutical Compositions

The compositions and methods of the present invention may be utilized to treat an individual in need thereof. In certain embodiments, the individual is a mammal such as a human, or a non-human mammal. When administered to an animal, such as a human, the composition or the compound is preferably administered as a pharmaceutical composition comprising, for example, a compound of the invention and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known in the art and include, for example, aqueous solutions such as water or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil, or injectable organic esters. In a preferred embodiment, when such pharmaceutical compositions are for human administration, particularly for invasive routes of administration (i.e., routes, such as injection or implantation, that circumvent transport or diffusion through an epithelial barrier), the aqueous solution is pyrogen-free, or substantially pyrogen-free. The excipients can be chosen, for example, to effect delayed release of an agent or to selectively target one or more cells, tissues or organs. The pharmaceutical composition can be in dosage unit form such as tablet, capsule (including sprinkle capsule and gelatin capsule), granule, lyophile for reconstitution, powder, solution, syrup, suppository, injection or the like. The composition can also be present in a transdermal delivery system, e.g., a skin patch. The composition can also be present in a solution suitable for topical administration, such as an eye drop.

A pharmaceutically acceptable carrier can contain physiologically acceptable agents that act, for example, to stabilize, increase solubility or to increase the absorption of a compound such as a compound of the invention. Such physiologically acceptable agents include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. The choice of a pharmaceutically acceptable carrier, including a physiologically acceptable agent,

depends, for example, on the route of administration of the composition. The preparation or pharmaceutical composition can be a selfemulsifying drug delivery system or a selfmicroemulsifying drug delivery system. The pharmaceutical composition (preparation) also can be a liposome or other polymer matrix, which can have incorporated therein, for example, a compound of the invention. Liposomes, for example, which comprise phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

A pharmaceutical composition (preparation) can be administered to a subject by any of a number of routes of administration including, for example, orally (for example, drenches as in aqueous or non-aqueous solutions or suspensions, tablets,

capsules (including sprinkle capsules and gelatin capsules), boluses, powders, granules, pastes for application to the tongue); absorption through the oral mucosa (e.g., sublingually); anally, rectally or vaginally (for example, as a pessary, cream or foam); parenterally (including intramuscularly, intravenously, subcutaneously or intrathecally as, for example, a sterile solution or suspension); nasally; intraperitoneally; subcutaneously; transdermally (for example as a patch applied to the skin); and topically (for example, as a cream, ointment or spray applied to the skin, or as an eye drop). The compound may also be formulated for inhalation. In certain embodiments, a compound may be simply dissolved or suspended in sterile water. Details of appropriate routes of administration and compositions suitable for same can be found in, for example, U.S. Pat. Nos. 6,110,973, 5,763,493, 5,731,000, 5,541,231, 5,427,798, 5,358,970 and 4,172,896, as well as in patents cited therein.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 1 percent to about ninety-nine percent of active ingredient, preferably from about 5 percent to about 70 percent, most preferably from about 10 percent to about 30 percent.

Methods of preparing these formulations or compositions include the step of bringing into association an active compound, such as a compound of the invention, with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules (including sprinkle capsules and gelatin capsules), cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), lyophile, powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or

syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. Compositions or compounds may also be administered as a bolus, electuary or paste.

To prepare solid dosage forms for oral administration (capsules (including sprinkle capsules and gelatin capsules), tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; (10) complexing agents, such as, modified and unmodified cyclodextrins; and (11) coloring agents. In the case of capsules (including sprinkle capsules and gelatin capsules), tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions, such as dragees, capsules (including sprinkle capsules and gelatin capsules), pills and

granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions that can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms useful for oral administration include pharmaceutically acceptable emulsions, lyophiles for reconstitution, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, cyclodextrins and derivatives thereof, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions for rectal, vaginal, or urethral administration may be presented as a suppository, which may be prepared by mixing one or more active compounds with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations of the pharmaceutical compositions for administration to the mouth may be presented as a mouthwash, or an oral spray, or an oral ointment.

Alternatively or additionally, compositions can be formulated for delivery via a catheter, stent, wire, or other intraluminal device. Delivery via such devices may be especially useful for delivery to the bladder, urethra, ureter, rectum, or intestine.

Formulations which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to an active compound, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the active compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across

the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention. Exemplary ophthalmic formulations are described in U.S. Publication Nos. 2005/0080056, 2005/0059744, 2005/0031697 and 2005/004074 and U.S. Patent No. 6,583,124, the contents of which are incorporated herein by reference. If desired, liquid ophthalmic formulations have properties similar to that of lacrimal fluids, aqueous humor or vitreous humor or are compatable with such fluids. A preferred route of administration is local administration (*e.g.*, topical administration, such as eye drops, or administration via an implant).

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

Pharmaceutical compositions suitable for parenteral administration comprise one or more active compounds in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of

microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents that delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution, which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsulated matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions that are compatible with body tissue.

For use in the methods of this invention, active compounds can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

Methods of introduction may also be provided by rechargeable or biodegradable devices. Various slow release polymeric devices have been developed and tested *in vivo* in recent years for the controlled delivery of drugs, including proteinacious biopharmaceuticals. A variety of biocompatible polymers (including hydrogels), including both biodegradable and non-degradable polymers, can be used to form an implant for the sustained release of a compound at a particular target site.

Actual dosage levels of the active ingredients in the pharmaceutical compositions may be varied so as to obtain an amount of the active ingredient that is

effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound or combination of compounds employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound(s) being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound(s) employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the therapeutically effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the pharmaceutical composition or compound at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. By "therapeutically effective amount" is meant the concentration of a compound that is sufficient to elicit the desired therapeutic effect. It is generally understood that the effective amount of the compound will vary according to the weight, sex, age, and medical history of the subject. Other factors which influence the effective amount may include, but are not limited to, the severity of the patient's condition, the disorder being treated, the stability of the compound, and, if desired, another type of therapeutic agent being administered with the compound of the invention. A larger total dose can be delivered by multiple administrations of the agent. Methods to determine efficacy and dosage are known to those skilled in the art (Isselbacher *et al.* (1996) Harrison's Principles of Internal Medicine 13 ed., 1814-1882, herein incorporated by reference).

In general, a suitable daily dose of an active compound used in the compositions and methods of the invention will be that amount of the compound that is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above.

If desired, the effective daily dose of the active compound may be administered as one, two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage

forms. In certain embodiments of the present invention, the active compound may be administered two or three times daily. In preferred embodiments, the active compound will be administered once daily.

The patient receiving this treatment is any animal in need, including primates, in particular humans, and other mammals such as equines, cattle, swine and sheep; and poultry and pets in general.

In certain embodiments, compounds of the invention may be used alone or conjointly administered with another type of therapeutic agent. As used herein, the phrase "conjoint administration" refers to any form of administration of two or more different therapeutic compounds such that the second compound is administered while the previously administered therapeutic compound is still effective in the body (*e.g.*, the two compounds are simultaneously effective in the patient, which may include synergistic effects of the two compounds). For example, the different therapeutic compounds can be administered either in the same formulation or in a separate formulation, either concomitantly or sequentially. In certain embodiments, the different therapeutic compounds can be administered within one hour, 12 hours, 24 hours, 36 hours, 48 hours, 72 hours, or a week of one another. Thus, an individual who receives such treatment can benefit from a combined effect of different therapeutic compounds.

In certain embodiments, conjoint administration of compounds of the invention with one or more additional therapeutic agent(s) (e.g., one or more additional chemotherapeutic agent(s)) provides improved efficacy relative to each individual administration of the compound of the invention (e.g., compound of formula I or Ia) or the one or more additional therapeutic agent(s). In certain such embodiments, the conjoint administration provides an additive effect, wherein an additive effect refers to the sum of each of the effects of individual administration of the compound of the invention and the one or more additional therapeutic agent(s).

This invention includes the use of pharmaceutically acceptable salts of compounds of the invention in the compositions and methods of the present invention. In certain embodiments, contemplated salts of the invention include, but are not limited to, alkyl, dialkyl, trialkyl or tetra-alkyl ammonium salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, L-arginine, benenthamine, benzathine, betaine, calcium hydroxide, choline, deanol,

diethanolamine, diethylamine, 2-(diethylamino)ethanol, ethanolamine, ethylenediamine, N-methylglucamine, hydrabamine, 1H-imidazole, lithium, L-lysine, magnesium, 4-(2-hydroxyethyl)morpholine, piperazine, potassium, 1-(2-hydroxyethyl)pyrrolidine, sodium, triethanolamine, tromethamine, and zinc salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, Na, Ca, K, Mg, Zn or other metal salts.

The pharmaceutically acceptable acid addition salts can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, and the like. Mixtures of such solvates can also be prepared. The source of such solvate can be from the solvent of crystallization, inherent in the solvent of preparation or crystallization, or adventitious to such solvent.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: (1) water-soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal-chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

In certain embodiments, the invention relates to a method for conducting a pharmaceutical business, by manufacturing a formulation of a compound of the invention, or a kit as described herein, and marketing to healthcare providers the benefits of using the formulation or kit for treating or preventing any of the diseases or conditions as described herein.

In certain embodiments, the invention relates to a method for conducting a pharmaceutical business, by providing a distribution network for selling a formulation of a compound of the invention, or kit as described herein, and providing instruction material to patients or physicians for using the formulation for treating or preventing any of the diseases or conditions as described herein.

In certain embodiments, the invention comprises a method for conducting a pharmaceutical business, by determining an appropriate formulation and dosage of a compound of the invention for treating or preventing any of the diseases or conditions as described herein, conducting therapeutic profiling of identified formulations for efficacy and toxicity in animals, and providing a distribution network for selling an identified preparation as having an acceptable therapeutic profile. In certain embodiments, the method further includes providing a sales group for marketing the preparation to healthcare providers.

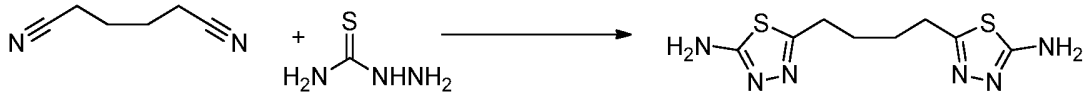
In certain embodiments, the invention relates to a method for conducting a pharmaceutical business by determining an appropriate formulation and dosage of a compound of the invention for treating or preventing any of the disease or conditions as described herein, and licensing, to a third party, the rights for further development and sale of the formulation.

Examples

Example 1: Synthetic Protocols

Synthesis of linker cores:

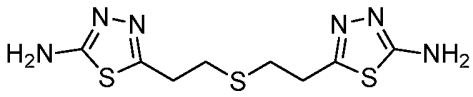
5,5'-(butane-1,4-diyl)-bis(1,3,4-thiadiazol-2-amine) (**1001**)



1001

A mixture of adiponitrile (8.00 g, 73.98 mmol) and thiosemicarbazide (13.48 g, 147.96 mmol) in trifluoroacetic acid (TFA) (75 mL) was heated at 80 °C for 17 hours. The reaction was cooled to room temperature and poured into a mixture of ice and water. Sodium hydroxide pellets were added to the mixture until it was basic (pH 14). The white precipitate was collected by suction filtration, rinsed with water and dried to provide 5,5'-(butane-1,4-diyl)-bis(1,3,4-thiadiazol-2-amine) (**1001**, 13.07 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.00 (s, 4H), 2.84 (bs, 4H), 1.68 (bs, 4H).

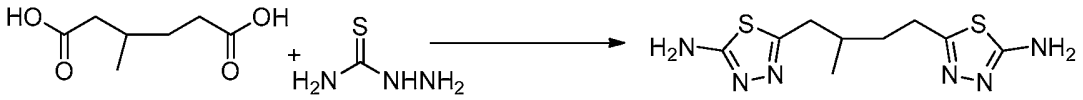
Synthesis of 5,5'-(thiobis(ethane-2,1-diyl))bis(1,3,4-thiadiazol-2-amine) **(1002)**



1002

Compound 1002 was prepared as described in US/2002/0115698 A1

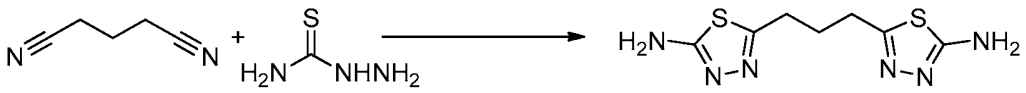
5,5'-(2-methylbutane-1,4-diyl)-bis(1,3,4-thiadiazol-2-amine) (**1003**)



1003

A mixture of 3-methyl adipic acid (5.00 g, 31.22 mmol) and thiosemicarbazide (5.69 g, 62.43 mmol) in $POCl_3$ (45 mL) was heated at 90 °C for 4 h. The reaction was cooled to room temperature and poured into a mixture of ice and water. Sodium hydroxide pellets were added to the mixture until it was basic (pH 14). The white precipitate was collected by suction filtration, rinsed with water and dried to provide 5,5'-(2-methylbutane-1,4-diyl)-bis(1,3,4-thiadiazol-2-amine) (**1003**, 8.97 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.00 (s, 4H), 2.89–2.81 (m, 3H), 2.89–2.81 (m, 3H), 2.69 (dd, J = 7.6, 7.6 Hz, 1H), 1.89–1.46 (m, 3H), 0.94 (d, J = 6.6 Hz, 3H).

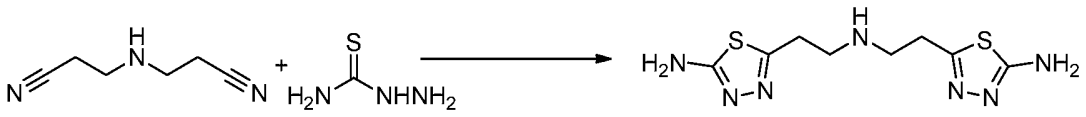
5,5'-(propane-1,3-diyl)-bis(1,3,4-thiadiazol-2-amine) (**1004**)



1004

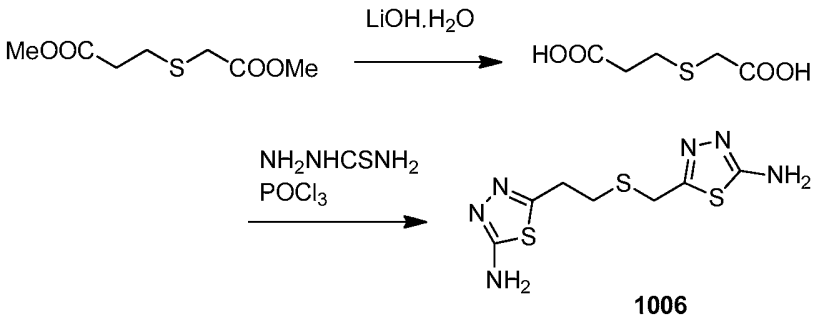
A mixture of glutaronitrile (5.00 g, 53.13 mmol) and thiosemicarbazide (9.68 g, 106.26 mmol) in TFA (50 mL) was heated at 85 °C for 4 h. The reaction was cooled to room temperature and poured into a mixture of ice and water. Sodium hydroxide pellets were added to the mixture until it was basic (pH 14).The white precipitate was collected by suction filtration, rinsed with water and dried to provide 5,5'-(propane-1,3-diyl)-bis(1,3,4-thiadiazol-2-amine) (**1004**, 13.72 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.06–7.03 (s, 4H), 2.87 (t, J = 7.5 Hz, 4H), 2.02 – 1.95 (m, 2H).

5-(2-((2-(5-amino-1,3,4-thiadiazol-2-yl)ethyl)amino)ethyl)-1,3,4-thiadiazol-2-amine (**1005**)



1005

A mixture of 3,3'-iminodipropionitrile (1.50 g, 12.18 mmol) and thiosemicarbazide (2.22 g, 24.36 mmol) in TFA (10 mL) was heated at 85 for 4.5 h. The reaction was cooled to room temperature and poured into a mixture of ice and water. Sodium hydroxide pellets were added to the mixture until it was basic (pH 14). The white precipitate was collected by suction filtration, rinsed with water and dried to provide 5-(2-((2-(5-amino-1,3,4-thiadiazol-2-yl)ethyl)amino)ethyl)-1,3,4-thiadiazol-2-amine (**1005**, 1.47 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.95 (s, 4H), 2.90 (d, J = 6.0 Hz, 4H), 2.83 (d, J = 6.3 Hz, 4H).

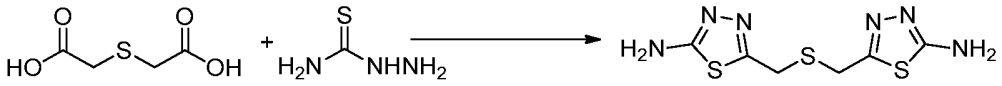


1006

To a solution of methyl 3-((2-methoxy-2-oxoethyl)thio)propanoate (5.0 g, 26 mmol) in THF/MeOH/water (60mL, 4:1:1) was added lithium hydroxide monohydrate (4.375 g, 101 mmol). The resulting mixture was stirred at room temperature overnight before it was concentrated under reduced pressure. The residue obtained was diluted with water (~100mL) and the resulting solution was acidified with 6N HCl. The mixture was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated to afford 3-((carboxymethyl)thio)propanoic acid (3.64g, 85%) as a white solid. ^{1}H NMR (300MHz, DMSO-d6) δ ppm 2.55-2.57 (t, 2H) 2.75-2.79 (t, 2H) 3.27 (s, 2H) 12.41 (s, 2H)

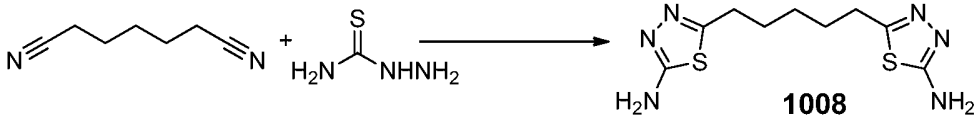
To a mixture of 3-((carboxymethyl)thio)propanoic acid (3.64g, 22.2 mmol) and thiosemicarbazide (4.1g, 45 mmol) was added phosphorus oxychloride (25mL) slowly. The resulting mixture was stirred at 90°C for 3hr before it was poured over crushed ice slowly. The solid separated was filtered and the filtrate was basified to

pH~13 by solid sodium hydroxide. The solid separated was filtered, washed with water and dried at 45°C under vacuum overnight to afford **1006** (~3g, 50%) as a tan solid. ^{1}H NMR (300MHz, DMSO-d6) δ ppm 2.79-2.83 (t, 2H) 3.06-3.10 (t, 2H) 3.99 (s, 2H) 7.04 (s, 2H) 7.16 (s, 2H)



1007

A mixture of 2,2'-Thiodiacetic acid (5.00 g, 33.3 mmol) and thiosemicarbazide (6.07 g, 66.6 mmol) in $POCl_3$ (40 mL) was heated at 90 °C for 5 h. The reaction was cooled to room temperature and carefully poured it onto a mixture of ice and water. Sodium hydroxide pellets were added to the mixture until it was basic (pH 14). The white precipitate was collected by suction filtration, rinsed with water and dried to afford **1007**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.18 (s, 4H), 3.96 (s, 4H).



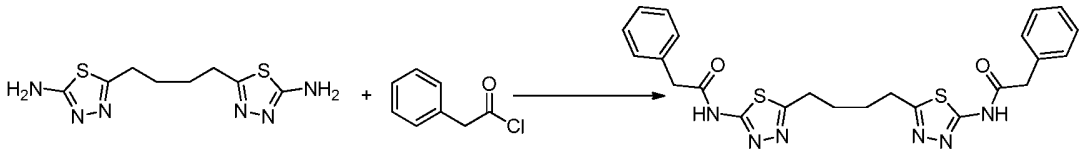
1008

A mixture of 1,5-dicyanopentane (1.00 g, 8.19 mmol) and thiosemicarbazide (1.5 g, 16.40 mmol) in TFA (3 mL) was heated at 85 ^{0}C for 5 h. The reaction was cooled to room temperature and poured into a mixture of ice and water. Sodium hydroxide pellets were added to the mixture until it was basic (pH 14).The white precipitate was collected by suction filtration, rinsed with water and dried to afford **1008**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.98 (s, 4H), 2.81 (t, 4H), 1.67 (m, 4H), 1.20 (m, 2H).

Acylation of diamino core:

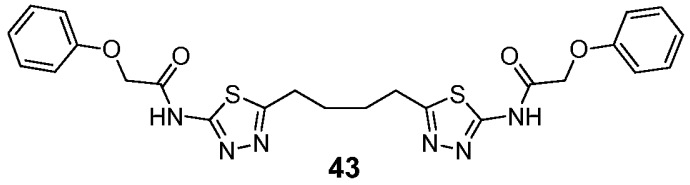
Method A: via acid chloride

N,N'-[5,5'-(butane-1,4-diyl)-bis(1,3,4-thiadiazole-5,2-diyl)]-bis(2-phenylacetamide) (**21**)



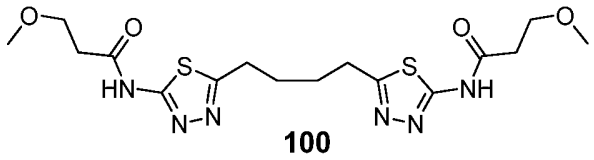
1001 21

To a suspension of **1001** (8.00 g, 31.21 mmol) in 1-Methyl-2-pyrrolidinone (NMP) 100 mL) at 0 °C was added phenylacetyl chloride (10.25 mL, 77.54 mmol) dropwise. The resulting mixture was stirred at 0 °C for 1 h before it was quenched by addition of water (~200 mL). The white precipitate was collected by suction filtration, rinsed with water and dried to provide *N*,*N*'-[5,5'-(butane-1,4-diyl)-bis(1,3,4-thiadiazole-5,2-diyl)]-bis(2-phenylacetamide) (**21**, 14.02 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66 (s, 2H), 7.34 (m, 10H), 3.81 (s, 4H), 3.01 (bs, 4H), 1.76 (bs, 4H).



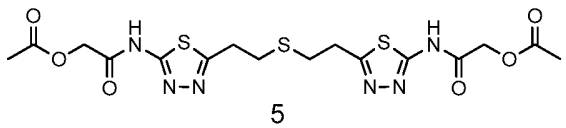
43

Compound **43** was prepared following Method A using phenoxyacetyl chloride. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.68 (s, 2H), 7.35–7.30 (m, 4H), 6.99–6.97 (m, 6H), 4.90 (s, 4H), 3.05 (bs, 4H), 1.79 (bs, 4H).



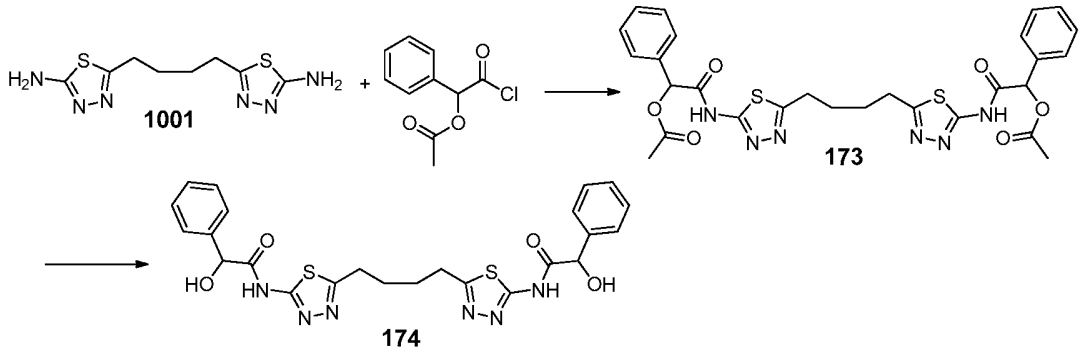
100

Compound **100** was prepared following Method A. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.42 (s, 2H), 3.64 (t, *J* = 5.6 Hz, 4H), 3.24 (s, 6H), 3.01 (bs, 4H), 2.72 (t, *J* = 6.2 Hz, 4H), 1.79 (bs, 4H).



5

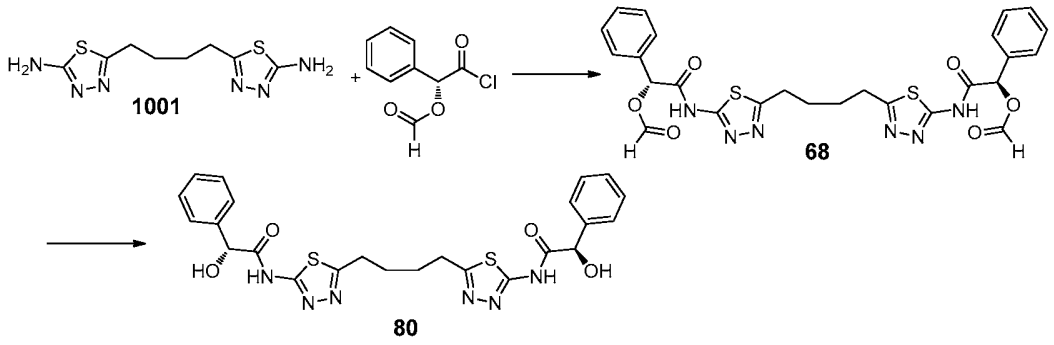
Compound **5** was prepared according to Method A: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66(s, 4H), 3.27(t, *J*=6.99 Hz, 4H), 2.95(t, *J*=7.02 Hz, 4H), 2.12(s, 6H).



To a suspension of **1001** (200 mg, 0.78 mmol) in NMP (2 mL) at 0 °C was added O-acetylmandelic acid chloride (0.44 mL, 1.95 mmol) dropwise. The resulting mixture was stirred at 0 °C for 1.5 h before it was quenched by addition of water (~10 mL). The white precipitate was collected by suction filtration, rinsed with more water and dried. The crude material was purified by recrystallization with a mixture of DMSO and MeOH to afford **173**.

A flask was charged with **173** and 2N ammonia in MeOH (3 ml) and the resulting mixture was stirred at room temperature for 6 h. The solvent was removed and the resulting material was dried in the oven to afford **174.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.42 (s, 2H), 7.53-7.31 (m, 10H), 6.35 (s, 2H), 5.34 (d, J = 1.14 Hz, 2H), 3.01 (bs, 4H), 1.76 (bs, 4H).

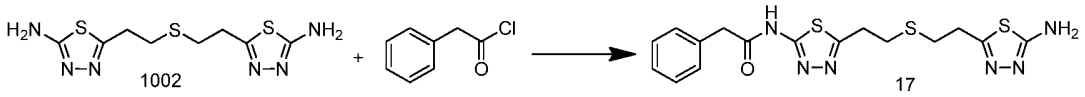
Compound **306** was prepared according to the procedure for compound **174** above.



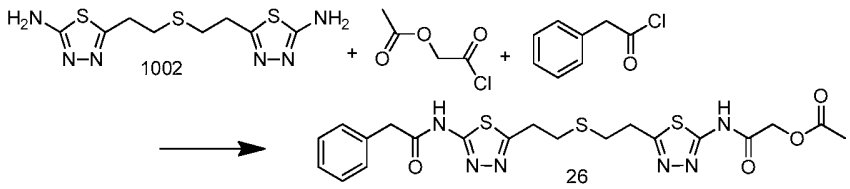
To a suspension of **1001** (400 mg, 1.56 mmol) in NMP (4 mL) at 0 °C was added (R)-(-)-O-formylmandeloyl chloride (0.61 mL, 3.90 mmol) dropwise. The resulting mixture was stirred at 0 °C for 1.5 h before it was quenched by addition of water (~10 mL). The white precipitate was collected by suction filtration, rinsed with more water

and dried. The crude material was purified by recrystallization with a mixture of DMSO and MeOH to afford **68**.

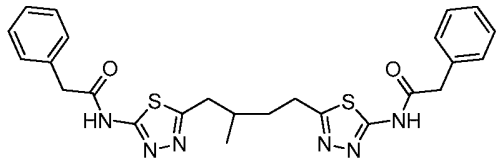
A flask was charged with **68** and 2N ammonia in MeOH (5 ml) and the resulting mixture was stirred at room temperature for 2 h. The solvent was removed and the resulting material was dried in the oven to afford **80.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.53-7.31 (m, 10H), 6.34 (s, 2H), 5.33 (s, 2H), 3.01 (bs, 4H), 1.75 (bs, 4H).



To a suspension of **1002** (544 mg, 1.89 mmol) in NMP (13 mL) at -15°C was added phenylacetyl chloride (0.249 mL, 1.89 mmol) dropwise. The resulting mixture was stirred at 0°C for 1 h and quenched by the addition of water (54 mL). The white precipitate was collected by suction filtration, rinsed with water (27 mL) and ethyl acetate (3x27 mL). The filtrate was basified to pH 11 using 2.5M NaOH. The layers were separated and the aqueous layer extracted with dichloromethane (3x54 mL). The combined organic layers were dried over magnesium sulfate and concentrated to afford N-(5-(2-((2-(5-amino-1,3,4-thiadiazol-2-yl)ethyl)thio)ethyl)-1,3,4-thiadiazol-2-yl)-2-phenylacetamide (**17**, 56 mg) ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71(s, 1H), 7.32(s, 5H), 3.81(s, 2H), 3.25(t, *J*=7.61 Hz, 2H) 3.06(t, *J*=7.25 Hz, 2H), 2.92(t, *J*=6.90 Hz, 2H), 2.85(t, *J*=6.86 Hz, 2H)

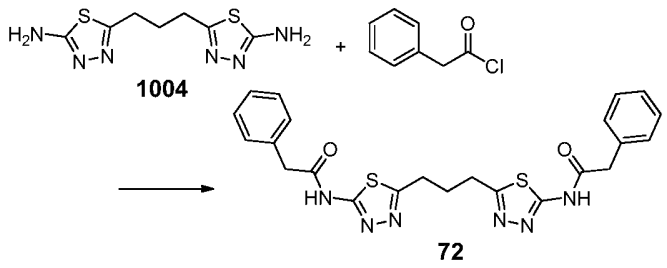


Phenylacetyl chloride (0.134 mL, 1.01 mmol) and acetoxyacetyl chloride (0.109 mL, 1.01 mmol) were mixed together in NMP (0.5 mL). This mixture was slowly added to a suspension of **1002** (292 mg, 1.01 mmol) in NMP (7 mL) at RT. The resulting mixture was stirred at RT for 1 h and quenched by the addition of water (20 mL). The white precipitate was collected by suction filtration, rinsed with water and dried under high vacuum. The crude material was purified by preparative HPLC. Compound 26: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.69(s, 2H), 7.34(3, 5H), 4.81(s, 2H), 3.82(s, 2H), 2.96(bs, 4H), 2.14(s, 3H).



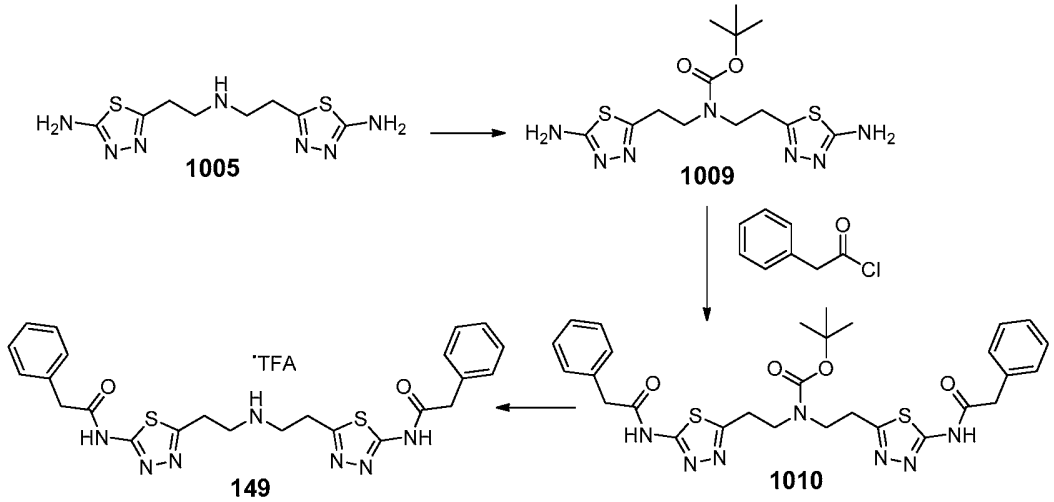
44

Compound **44** was prepared following the procedure for compound **21** described previously. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66 (s, 2H), 7.34–7.28 (m, 10H), 3.81 (s, 4H), 3.05–3.00 (m, 3H), 2.87 (dd, J = 7.9, 8.2 Hz, 1H), 1.95–1.77 (m, 3H), 0.94 (d, J = 6.5 Hz, 3H).



1004 72

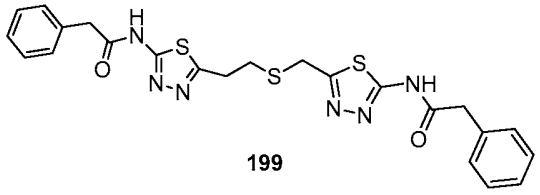
Compound **72** was prepared following the procedure for compound **21** described previously. To a suspension of diamine **1004** (0.70 g, 3.07 mmol) in NMP (15 mL) at 0 °C was added phenylacetyl chloride (811 μL, 6.13 mmol) dropwise. The resulting mixture was stirred at 0 °C for 1 h before it was quenched by addition of water. The white precipitate was collected by suction filtration, rinsed with water and dried to provide *N*,*N*'-[5,5'-(propane-1,3-diyl)-bis(1,3,4-thiadiazole-5,2-diyl)]-bis(2-phenylacetamide) (**72**, 1.37 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.68 (s, 2H), 7.38–7.27 (m, 10H), 3.82 (s, 4H), 3.06 (t, J = 7.2 Hz, 4H), 2.17–2.12 (m, 2H).



1005 1009 1010 149

To a suspension of compound **1005** (100 mg, 0.37 mmol) in DMF (12 mL) at room temperature was added a solution of $(t\text{-Boc})_2O$ (88 mg, 0.41 mmol) in DMF (2 mL). The mixture was stirred at room temperature for 24 h. To this reaction mixture was added NMP (2 mL) and followed by addition of phenylacetyl chloride (97 μL, 0.74 mmol). The reaction was stirred for 1 h before it was poured into a mixture of ice-water. The solid was collected by suction filtration, rinsed with water and dried to provide **1010** (180 mg).

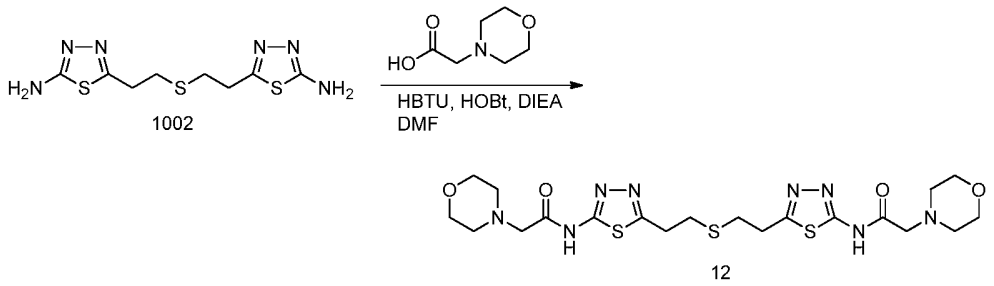
The above product **1010** (160 mg, 0.26 mmol) in a mixture of TFA (1.5 mL) and CH_2CH_2 (10 mL) was stirred at room temperature for 4 h before it was concentrated. The residue was re-taken up in CH_2Cl_2 (3×) and concentrated to provide *N*,*N*'-(5,5'-(azanediyl-bis(ethane-2,1-diyl))-bis(1,3,4-thiadiazole-5,2-diyl))-bis(2-phenylacetamide) trifluoroacetic acid (**149**, 122 mg). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.81 (s, 2H), 8.75 (bs, 2H), 7.38–7.27 (m, 10H), 3.84 (s, 4H), 3.45 (d, J = 2.9 Hz, 4H), 3.39 (d, J = 6.0 Hz, 4H).



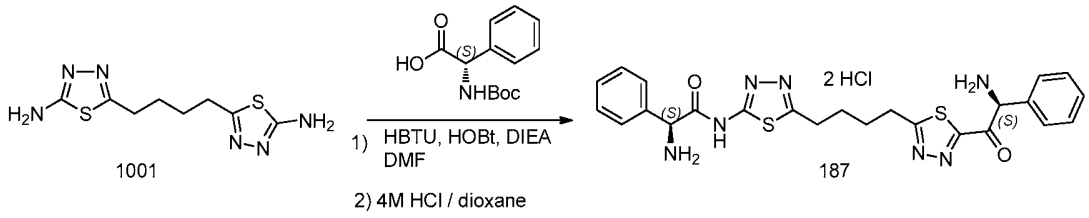
199

To a suspension of **1006** (0.274g, 1mmol) in NMP (5mL) was added phenyl acetyl chloride (0.263mL, 2mmol) dropwise. The mixture was stirred at room temperature for 1hr and afterwards it was diluted with water. Solid separated was filtered, washed with more water and dried. The crude material was purified by prep HPLC to afford **199** as a white solid. ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 2.87-2.91 (t, 2H) 3.25-3.29 (t, 2H) 3.82 (s, 4H) 4.19 (s, 2H) 7.26-7.33 (m, 10H) 12.71-12.72 (br s, 2H).

Method B: via acid using peptide coupling reagents

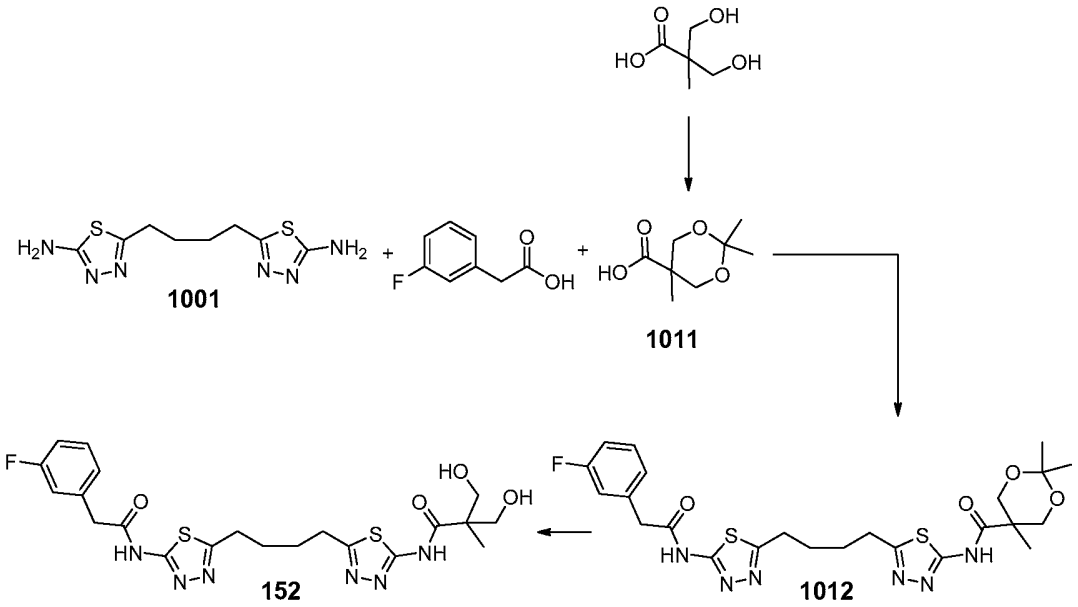


To a flask containing 5,5'-(thiobis(ethane-2,1-diyl))bis(1,3,4-thiadiazol-2-amine) (1002) (0.69 mmol, 0.20 g, 1.0 equiv.) was added 2-morpholinoacetic acid (1.52 mmol, 0.22 g, 2.2 equiv.), O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU) (2.20 mmol, 0.83 g, 3.2 equiv.), 1-Hydroxybenzotriazole (HOBT) (2.2 mmol, 0.29 g, 3.2 equiv.) 5 mL of DMF followed by N,N-Diisopropylethylamine (DIEA) (5.52 mmol, 0.71 g, 0.960 mL, 8.0 equiv.). The mixture was stirred overnight at room temperature and then diluted with 15 mL water. The mixture was extracted with EtOAc and the organic layers combined, washed with water, brine and dried over Na_2SO_4. The Na_2SO_4 was removed by filtration and the volatiles removed under reduced pressure to give 0.04 g of compound 12. ^{1}HNMR (300 MHz, $CDCl_3$) Compound 12: δ 3.80 (broad multiplet, 4H), 3.34 (dd, 4H, J = 7.2 Hz), 3.28 (s, 4 H), 3.00 (dd, 4H, J= 7.1 Hz), 2.63 (broad multiplet, 4H).



To a flask containing 5,5'-(butane-1,4-diyl)bis(1,3,4-thiadiazol-2-amine) (1101) (3.9 mmol, 1.0 g, 1.0 equiv.) was added (S)-2-((tert-butoxycarbonyl)amino)-2-phenylacetic acid (8.58 mmol, 2.15 g, 2.2 equiv.), HBTU (12.48 mmol, 4.73 g, 3.2 equiv.), HOBt (12.48 mmol, 1.69 g, 3.2 equiv.) 25 mL of DMF followed by DIEA (31.2 mmol, 4.03 g, 5.43 mL, 8.0 equiv.). The mixture was stirred overnight and poured into 150 mL water. The white solids that formed were collected by vacuum filtration, washed with water and dried under vacuum giving 2.47 g of the bis-Boc protected intermediate.

To a slurry of the bis-Boc protected intermediate (2.76 mmol, 2.0 g, 1.0 equiv.) in 20 mL of dichloromethane (DCM) was added 4 M HCl in dioxane (40 mmol, 10 mL) with vigorous stirring. The mixture briefly became clear and homogeneous then a white precipitate formed. The mixture was stirred overnight and diluted with 20 mL diethyl ether. The solids were collected by vacuum filtration washed with additional diethyl ether and dried under vacuum giving 0.9 g 187. ^{1}HNMR (300 MHz, DMSO, d_6) Compound 187: δ 9.13 (s, 4H), 7.61 (m, 4H), 7.48 (m, 6H), 6.2 (broad singlet, 4H), 5.32 (s, 2H), 3.04 (broad multiplet, 4H), 1.77 (broad multiplet, 4H).

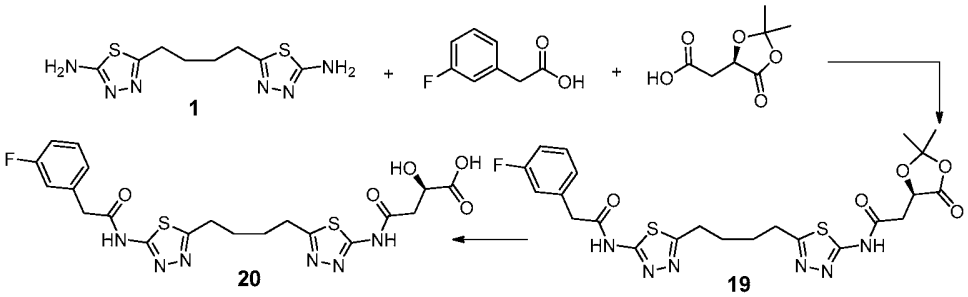


To a solution of 2,2-bis(hydroxymethyl)propionic acid (5.00 g, 37.28 mmol) in acetone (80 mL) at room temperature was added 2,2-dimethoxypropane (6.88 mL, 55.92 mmol) and *p*-TsOH·H_2O (0.36 g, 1.86 mmol). The reaction was stirred for 2 h before it was quenched with Et_3N (0.30 mL). The organic volatile was removed under reduced pressure. The residue was partitioned between EtOAc and water. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated to provide the desired product **1011** (5.17 g) as a white solid.

To a suspension of diamine **1001** (500 mg, 1.95 mmol), 3-fluorophenylacetic acid (361 mg, 2.34 mmol) and acid **1011** (442 mg, 2.54 mmol) in DMF (20 mL) at 0 °C was added HOBt (791 mg, 5.85 mmol) and followed by N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) (1.12 g, 5.85 mmol). The mixture was stirred from 0 °C to room temperature over 18 h before it was diluted with water. The

precipitate was collected by suction filtration, washed with water and dried. The crude product was purified by silica gel chromatography eluting with 1–10% MeOH in CH_2Cl_2 to provide *N*-(5-(4-(5-(2-(3-fluorophenyl)acetamido)-1,3,4-thiadiazol-2-yl)butyl)-1,3,4-thiadiazol-2-yl)-2,2,5-trimethyl-1,3-dioxane-5-carboxamide (**1012**, 208 mg).

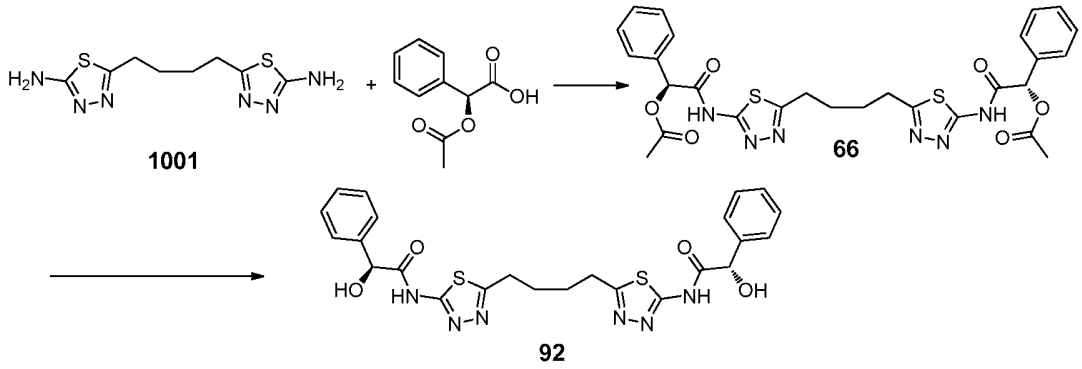
The above product **1012** (87 mg, 0.16 mmol) and TFA (2 mL) in a mixture of THF (8 mL) and water (2 mL) was heated at 50 °C for 5 h before it was concentrated under reduced pressure. The crude residue was purified by HPLC to provide *N*,*N*'-(5-(4-(5-(2-(3-fluorophenyl)acetamido)-1,3,4-thiadiazol-2-yl)butyl)-1,3,4-thiadiazol-2-yl)-3-hydroxy-2-(hydroxymethyl)-2-methylpropanamide (**152**). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.68 (s, 1H), 11.77 (s, 1H), 7.04–7.38 (m, 1H), 7.18–7.09 (m, 4H), 4.98 (s, 2H), 3.86 (s, 2H), 3.62 (dd, *J* = 10.7, 29.0 Hz, 4H), 3.03 (bs, 4H), 1.77 (bs, 4H), 1.14 (s, 3H).



To a suspension of diamine **1001** (400 mg, 1.56 mmol), 3-fluorophenylacetic acid (313 mg, 2.03 mmol), (*R*)-(–)-2,2-dimethyl-5-oxo-1,3-dioxolane-4-acetic acid (353 mg, 2.03 mmol) and Et_3N (200 μL) in DMF (20 mL) at 0 °C was added HOBt (633 mg, 4.68 mmol) and followed by EDC (897 mg, 4.68 mmol). The mixture was stirred from 0 °C to room temperature over 18 h before it was diluted with water. The precipitate was collected by suction filtration and washed with water. The solid was further rinsed with a mixture of hot MeOH-THF. The combined filtrate was concentrated and purified by silica gel chromatography eluting with 1–10% MeOH in CH_2Cl_2 to provide (*R*)-*N*-(5-(4-(5-(2-(3-fluorophenyl)acetamido)-1,3,4-thiadiazol-2-yl)butyl)-1,3,4-thiadiazol-2-yl)-3,4-dihydroxybutanamide (**1013**, 93 mg).

The above product **1013** (87 mg, 0.16 mmol) and TFA (2 mL) in a mixture of THF (8 mL) and water (2 mL) was heated at 50 °C for 5 h before it was concentrated under

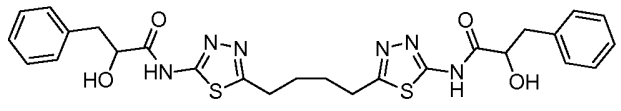
reduced pressure. The crude residue was purified by HPLC to provide (*R*)-*N*-(5-(4-(5-(2-(3-fluorophenyl)acetamido)-1,3,4-thiadiazol-2-yl)butyl)-1,3,4-thiadiazol-2-yl)-3,4-dihydroxybutanamide (**153**). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.67 (s, 1H), 12.43 (s, 1H), 7.41–7.38 (m, 1H), 7.20–7.12 (m, 4H), 4.45–4.40 (m, 1H), 3.86 (s, 2H), 3.03 (bs, 4H), 2.85–2.77 (m, 2H), 1.78 (bs, 4H).



1001 **66** **92**

To a suspension of (S)-(+)-O-acetylmandelic acid (666 mg, 3.43 mmol) and O-(7-Azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HATU) (1.47 g, 3.86 mmol) in DMF (4 mL) was added DIEA (0.672 ml, 3.86 mmol) followed by **1001** (400 mg, 1.56 mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by addition of water (~10 mL). The white precipitate was collected by suction filtration, rinsed with more water and dried. The crude material was purified by recrystallization with a mixture of DMSO and MeOH to afford **66**.

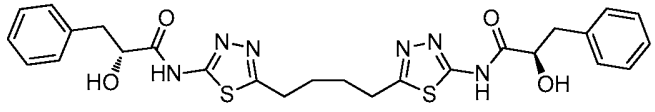
A flask was charged with **66** and 2N ammonia in MeOH (5 ml) and the resulting mixture was stirred at room temperature for 6 h. The solvent was removed and the resulting material was dried in the oven to afford **92.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.42 (s, 2H), 7.53-7.31 (m, 10H), 6.35 (s, 2H), 5.33 (s, 2H), 3.01 (bs, 4H), 1.76 (bs, 4H).



69

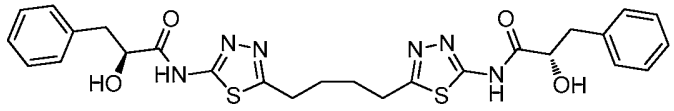
A flask was charged with **1001** (200 mg, 0.78 mmol), DL-3-phenyllactic acid (285 mg, 1.716 mmol), and HOBT (527 mg, 3.9 mmol) in DMF (3 ml) was added EDC

(897 mg, 4.68 mmol) followed by triethylamine (0.87 ml, 6.24 mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by addition of water (~5 mL). The mixture was partitioned between water and EtOAc. The organic extract was washed with water, dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in CH_2Cl_2 to afford **69.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20 (s, 2H), 7.24 (m, 10H), 5.75 (d, J = 6.87 Hz, 2H), 4.43 (m, 2H), 3.10 (m, 6H), 2.89-2.81 (m, 2H), 1.80 (bs, 4H).



169

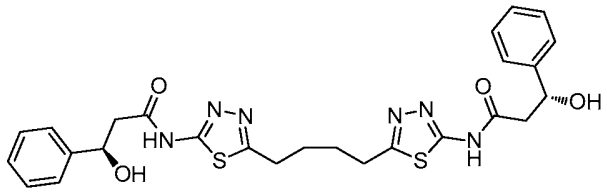
A flask was charged with **1001** (200 mg, 0.78 mmol), D-(+)-3-phenyllactic acid (285 mg, 1.716 mmol), and HOBt (464 mg, 3.43 mmol) in DMF (3 ml) was added EDC (822 mg, 4.28 mmol) followed by triethylamine (0.718 ml, 5.15 mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by addition of water (~5 mL). The mixture was partitioned between water and EtOAc. The organic extract was washed with water, dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in CH_2Cl_2 to afford **169.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20 (s, 2H), 7.24 (m, 10H), 5.75 (d, J = 6.87 Hz, 2H), 4.43 (m, 2H), 3.03 (m, 6H), 2.89-2.81 (m, 2H), 1.80 (bs, 4H).



146

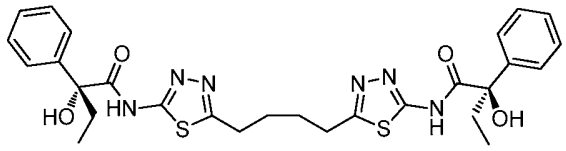
A flask was charged with **1001** (200 mg, 0.78 mmol), L-(-)-3-phenyllactic acid (285 mg, 1.716 mmol), and HOBt (464 mg, 3.43 mmol) in DMF (3 ml) was added EDC (822 mg, 4.28 mmol) followed by triethylamine (0.718 ml, 5.15 mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by addition of water (~5 mL). The mixture was partitioned between water and EtOAc. The organic extract was washed with more water, dried over sodium sulfate, filtered and

evaporated. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in CH_2Cl_2 to afford **146.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.27 (s, 2H), 7.31 (m, 10H), 5.78 (m, 2H), 4.44 (m, 2H), 3.05 (m, 6H), 2.87 (m, 2H), 1.79 (bs, 4H).



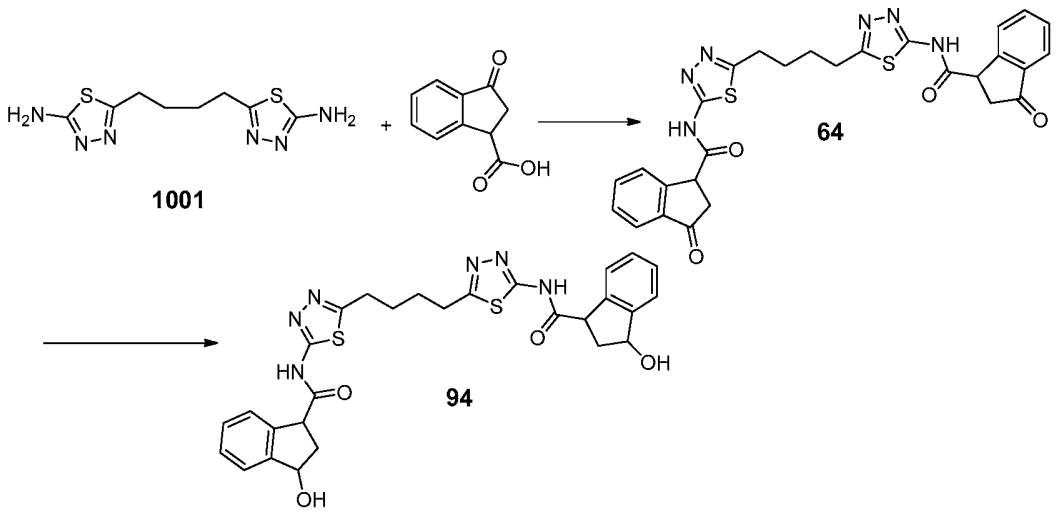
127

To a suspension of (R)-(+)-3-hydroxy-3-phenylpropionic acid (285 mg, 1.72 mmol) and HATU (719 mg, 1.89 mmol) in DMF (3 mL) was added DIEA (0.329 ml, 1.89 mmol) followed by **1001** (200 mg, 0.78 mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by addition of water (~10 mL). The white precipitate was collected by suction filtration, rinsed with more water and dried. The crude material was purified by recrystallization with DMSO and MeOH to afford **127**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.38 (s, 2H), 7.34 (m, 10H), 5.56 (m, 2H), 5.10 (m, 2H), 3.04 (bs, 4H), 2.80 (m, 4H), 1.80 (bs, 4H).



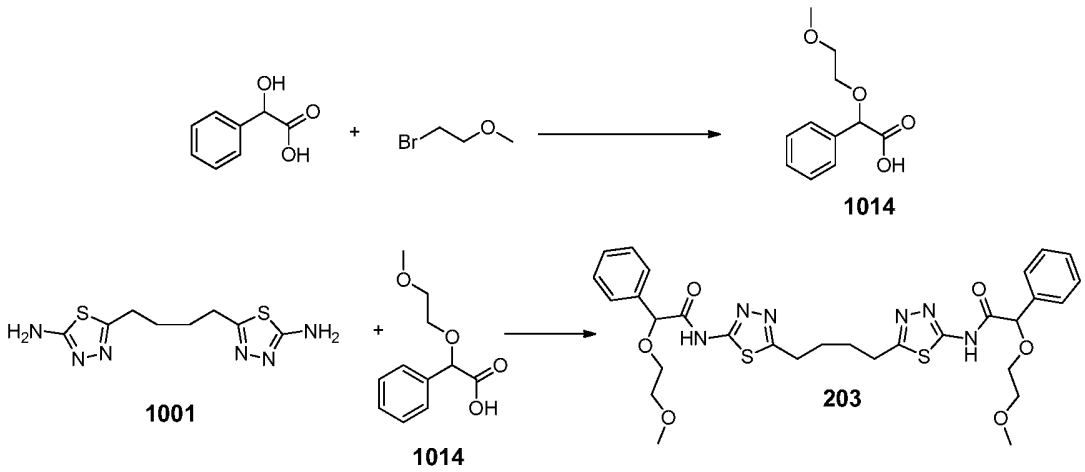
143

To a suspension of (R)-2-hydroxy-2-phenylbutyric acid (310 mg, 1.72 mmol) and HATU (719 mg, 1.89 mmol) in DMF (3 mL) was added DIEA (0.329 ml, 1.89 mmol) followed by **1001** (200 mg, 0.78 mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by addition of water (~10 mL). The crude material was purified by HPLC to afford **143**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.61 (d, J = 7.65 Hz, 4H), 7.34 (m, 6H), 2.99 (bs, 4H), 2.26 (m, 2H), 2.10 (m, 2H) 1.74 (bs, 4H), 0.80 (t, 6H).



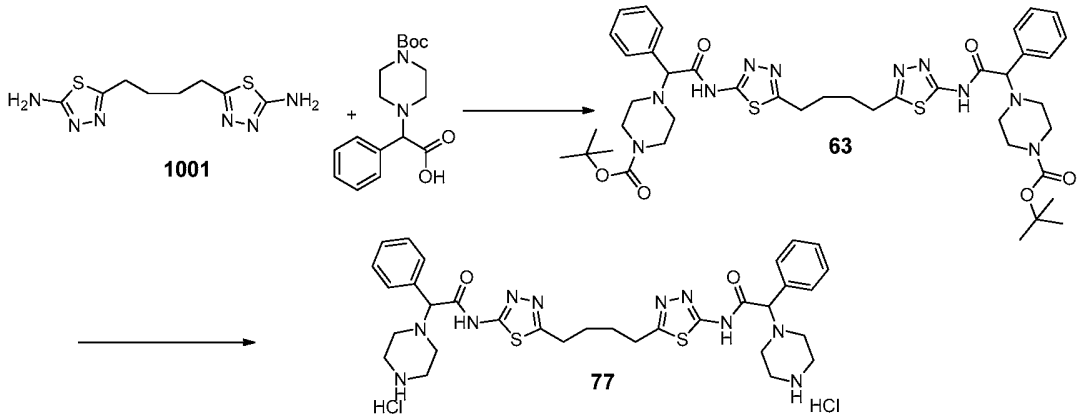
To a suspension of 3-Oxo-1-indancarboxylic acid (604 mg, 3.43 mmol) and HATU (1.47g, 3.86 mmol) in DMF (5 mL) was added DIEA (0.672 ml, 3.86 mmol) followed by **1001** (400 mg, 1.56 mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by addition of water (~10 mL). The light brown precipitate was collected by suction filtration, rinsed with water and dried. The crude material was purified by recrystallization with a mixture of DMSO and MeOH to afford **64**.

To a suspension of **64** (100 mg, 0.175 mmol) in EtOH (20 ml) at 0 ^{0}C was added $NaBH_4$ (15 mg, 0.384 mmol) and the resulting mixture was stirred for 1 h before it was quenched by 1N HCl. The mixture was partitioned between 1N HCl and EtOAc, the organic extract was dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in CH_2Cl_2 and further purified by recrystallization with a mixture of DMSO and MeOH to afford **94.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.81 (s, 2H), 7.34 (m, 8H), 5.56 (m, 2H), 5.11 (t, 2H), 4.15 (t, 2H), 3.05 (bs, 4H), 2.70 (m, 2H), 2.15 (m, 2H), 1.80 (bs, 4H).



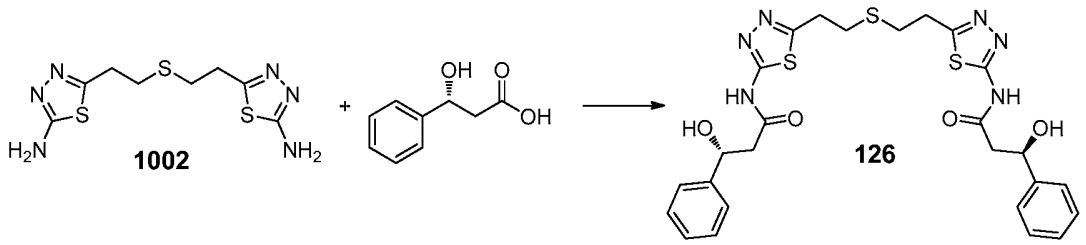
To a solution of DL-mandelic acid (1 g, 6.57 mmol) in DMF (10 ml) at 0 ^{0}C was added NaH (700 mg, 19.7 mmol) and allowed the mixture to stir for 20 minutes before 2-bromoethyl methyl ether (1.24 ml, 13.1 mmol) was added dropwise. The resulting mixture was stirred at 0 ^{0}C and slowly warmed up to room temperature overnight before it was quenched by 1N HCl. The mixture was partitioned between 1N HCl and EtOAc, the organic extract was washed with water, dried over sodium sulfate, filtered and evaporated to afford **1014**.

To a suspension of **1014** (500 mg, 2.37 mmol) and HATU (995 mg, 2.62 mmol) in DMF (3 mL) was added DIEA (0.456 ml, 2.62 mmol) followed by **1001** (277 mg, 1.08 mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by addition of water (~6 mL). The mixture was partitioned between water and EtOAc. The organic extract was washed with water, dried over sodium sulfate, filtered and evaporated. The crude material was purified by HPLC to afford **203.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.58 (s, 2H), 7.49-7.37 (m, 10H), 5.22 (s, 2H), 3.66-3.54 (m, 8H), 3.27 (s, 6H), 3.01 (bs, 4H), 1.75 (bs, 4H).

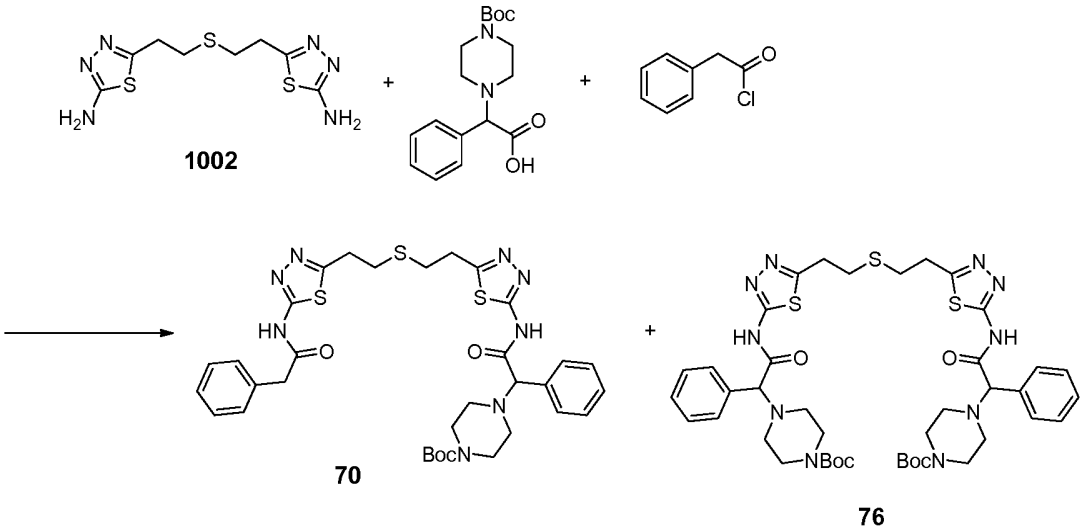


To a suspension of 2-(4-Boc-piperazinyl)-2-phenylacetic acid (1.1 g, 3.43 mmol) and HATU (1.47g, 3.86 mmol) in DMF (5 mL) was added DIEA (0.672 ml, 3.86 mmol) followed by **1001** (400 mg, 1.56 mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by addition of water (~10 mL). The white precipitate was collected by suction filtration, rinsed with water and dried. The crude material was purified by recrystallization with DMSO and MeOH to afford **63**.

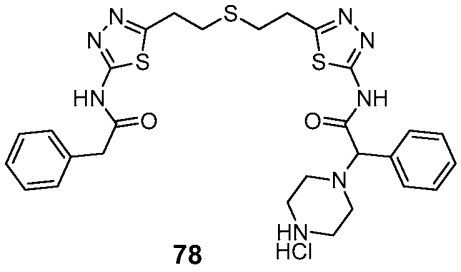
A flask was charged with **63** and 4N HCl in 1,4-dioxane (6 ml) and the resulting mixture was stirred at room temperature for 3 h. The precipitation was collected by filtration, rinse with EtOAc/CH_2Cl_2 and dried to afford **77.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.10 (bs, 4H), 7.51-7.41 (m, 10H), 4.90 (bs, 2H), 4.62 (s, 2H), 3.15 (bs, 8H), 3.03 (bs, 4H), 2.73 (bs, 8H), 1.76 (bs, 4H).



To a suspension of (R)-(+)-3-hydroxy-3-phenylpropionic acid (254 mg, 1.53 mmol) and HATU (640 mg, 1.68 mmol) in DMF (3 mL) was added DIEA (0.292 ml, 1.68 mmol) followed by **1002** (200 mg, 0.693 mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by addition of water (~10 mL). The white precipitate was collected by suction filtration, rinsed with water and dried. The crude material was purified by recrystallization with a mixture of DMSO and MeOH to afford **126**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.40 (s, 2H), 7.38 (m, 10H), 5.55 (m, 2H), 5.09 (m, 2H), 3.27 (t, 4H), 2.95 (t, 4H), 2.82 (m, 4H).

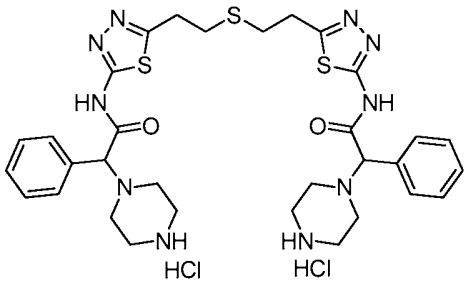


A flask was charged with **1002** (200 mg, 0.693 mmol), 2-(4-Boc-piperazinyl)-2-phenylacetic acid (244 mg, 0.763 mmol), and HOBt (187 mg, 1.39 mmol) in DMF (3 ml) was added EDC (332 mg, 1.73 mmol) followed by triethylamine (0.290 ml, 2.08 mmol). The resulting mixture was stirred at room temperature overnight before phenylacetyl chloride (0.037 ml, 0.277 mmol) was added dropwise at 0 ^{0}C and stirred for 1 h before it was quenched by addition of water (~10 mL). The white precipitate was collected by suction filtration, rinsed with water and dried. The crude material was purified by HPLC to afford **70** and **76.**



78

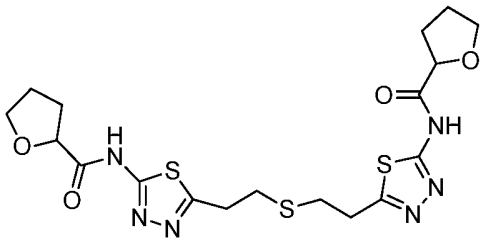
A flask was charged with **70** and 4N HCl in 1,4-dioxane (6 ml) and the resulting mixture was stirred at room temperature for 3 h. The precipitation was collected by filtration, rinse with EtOAc/CH_2Cl_2 and dried to afford **78.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.70 (s, 2H), 8.97 (bs, 2H), 7.50-7.29 (m, 10H), 4.72 (bs, 1H), 4.59 (s, 1H), 3.82 (s, 2H), 3.27 (t, 4H), 3.15 (bs, 4H), 2.92 (t, 4H), 2.70 (bs, 4H).



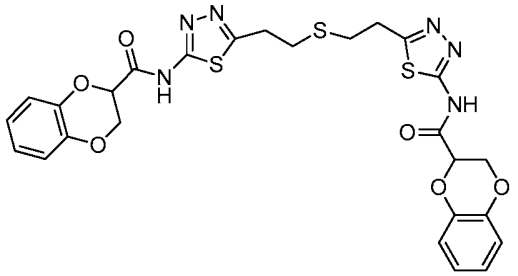
79

A flask was charged with **76** and 4N HCl in 1,4-dioxane (6 ml) and the resulting mixture was stirred at room temperature for 3 h. The precipitation was collected by filtration, rinse with EtOAc/CH_2Cl_2 and dried to afford **79.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.87 (s, 2H), 9.03 (bs, 4H), 7.50-7.40 (m, 10H), 4.67 (bs, 2H), 4.59 (s, 2H), 3.28 (t, 4H), 3.14 (bs, 8H), 2.97 (t, 4H), 2.71 (bs, 8H).

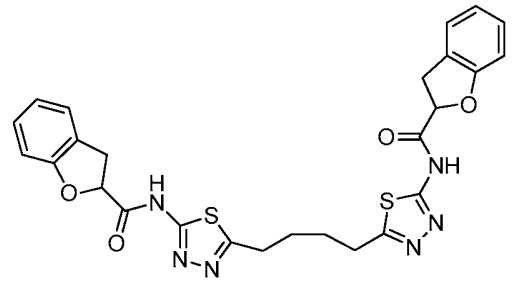
Amide Coupling General Procedure (used for following examples): To a 0.2 molar concentration suspension of carboxylic acid (2 equivalents) in DMF was added HATU (2 equivalents) and stirred till reaction mixture is clear followed by the addition of an amine (1 equivalent) and DIPEA(4 equivalents). The resulting mixture was stirred at room temperature overnight before it was quenched by the addition of water. The solid separated was filtered, washed with water and dried.



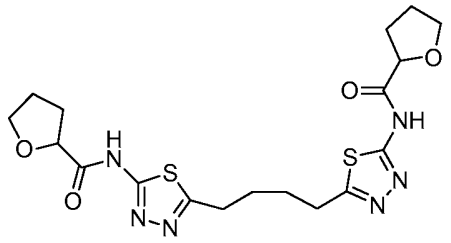
39: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.89-2.01 (m, 6H) 2.18-2.29 (m, 2H) 2.95-3 (m, 4H) 3.79-3.86 (m, 2H) 3.94-4.02 (m, 2H) 4.55-4.6 (m, 2H) 12.29 (brs, 2H).



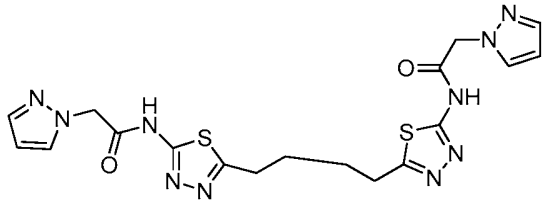
41: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 2.93-2.98 (m, 4H) 3.27-3.32 (m, 4H), 4.46 (s, 4H), 5.18-5.2 (br s, 2H) 6.88-7.03 (m, 8H) 12.87-12.92 (br s, 2H).



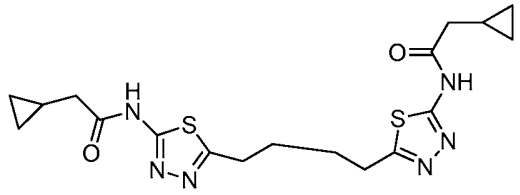
51: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.78 (br s, 4H) 3.05-3.06 (br s, 4H), 3.38-3.40 (m, 2H) 3.54-3.63 (m, 2H) 5.44-5.50 (m, 2H) 6.92-7.26 (m, 8H) 12.78 (br s, 2H).



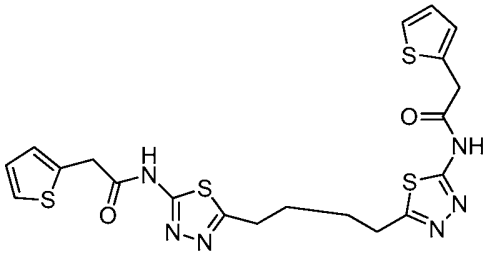
54: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.92-2.03 (m, 10H) 2.17-2.28 (m, 2H) 3.05 (br s, 4H) 3.79-3.85 (m, 2H) 3.94-4.01 (m, 2H) 4.55-4.59 (m, 2H) 12.27(br s, 2H).



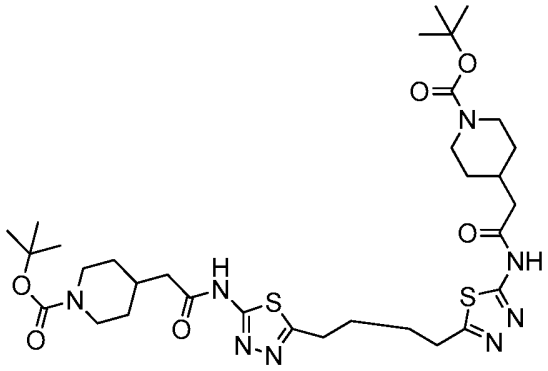
60: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.77 (br s, 4H) 3.04 (br s, 4H) 5.20 (s, 4H) 6.31 (br s, 2H) 7.49 (br s, 2H) 7.79 (br s, 2H) 12.80 (br s, 2H).



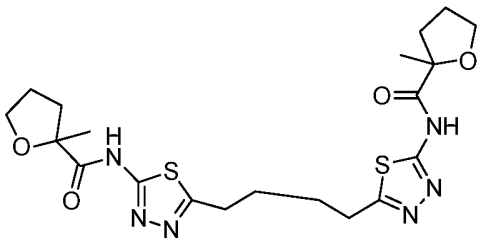
85: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 0.20-0.21 (br s, 4H) 0.48-0.50 (br s, 4H) 1.79 (br s, 4H) 2.35-2.38 (br s, 4H) 3.04 (br s, 4H) 12.32 (br s, 2H).



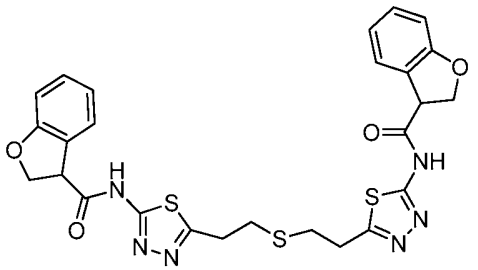
87: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.78 (br s, 4H) 3.03 (br s, 4H) 4.05 (s, 4H) 6.99 (br s, 4H) 7.42-7.44 (m, 2H) 12.68 (br s, 2H).



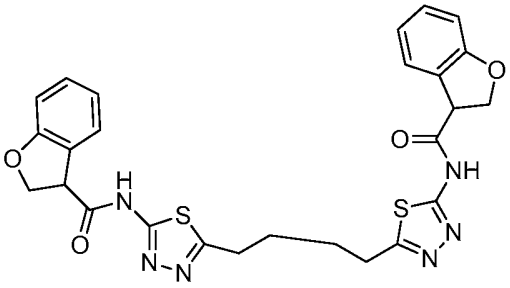
114: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.01-1.12 (m, 4H) 1,40 (s, 18H) 1.61-1.65 (m, 4H) 1.78 (br s, 4H) 1.95 (br s, 2H) 3.84 (m, 4H) 2.65-2.75 (m, 4H) 3.03 (br s, 4H) 3.89-3.93 (m, 4H) 12.39 (br s, 2H).



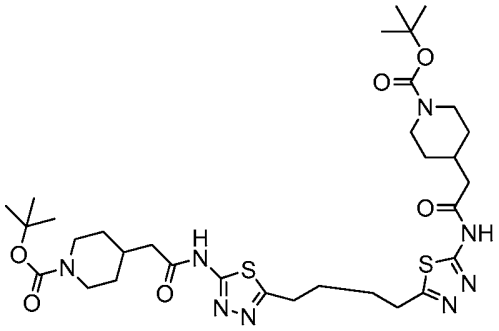
123: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.43 (s, 6H) 1.79-1.94 (m, 10H) 2.22-2.31 (m, 2H) 3.05 (br s, 4H) 3.85-4.01 (m, 4H) 11.85 (br s, 2H).



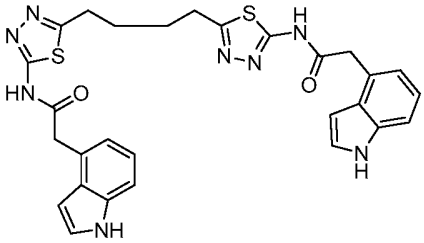
133: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 2.92-2.97 (m, 4H) 3.26-3.30 (m, 4H) 4.61-4.87 (m, 6H) 6.83-6.89 (m, 4H) 7.16-7.21 (m, 2H) 7.36-7.38 (m, 2H) 12.95 (br s, 2H).



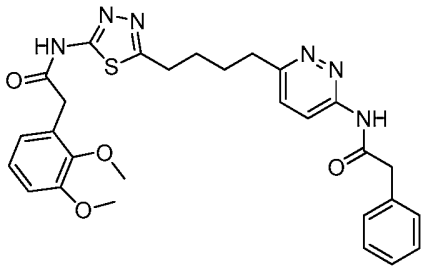
135: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.77 (br s, 4H) 3.03 (br s, 4H) 4.60-4.87 (m, 6H) 6.83-6.89 (m, 4H) 7.16-7.22 (m, 2H) 7.36-7.38 (m, 2H) 12.92 (br s, 2H).



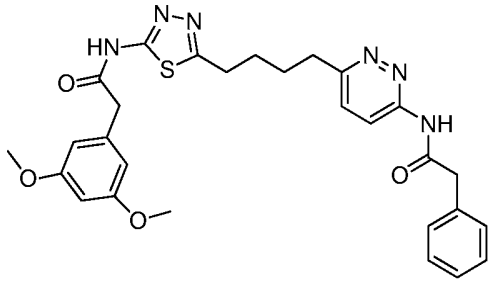
114: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.01-1.12 (m, 4H) 1,40 (s, 18H) 1.61-1.65 (m, 4H) 1.78 (br s, 4H) 1.95 (br s, 2H) 3.84 (m, 4H) 2.65-2.75 (m, 4H) 3.03 (br s, 4H) 3.89-3.93 (m, 4H) 12.39 (br s, 2H).



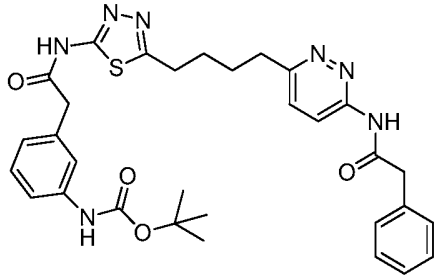
323: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.76 (brs, 4H) 3.01(brs, 4H) 4.02 (s, 4H) 6.56 (s, 2H) 6.94-7.05 (m, 4H) 7.31-7.33 (m, 4H) 11.12 (brs, 2H) 12.69 (s, 2H).



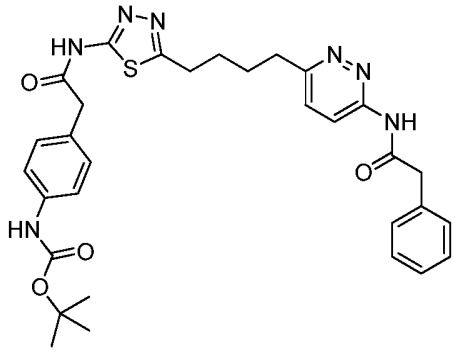
397: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.75 (brs, 4H) 2.90 (brs, 2H) 3.02 (brs, 2H) 3.67-3.82 (m, 10H) 6.85-7.03 (m, 4H) 7.26-7.36 (m, 5H) 7.55-7.58 (d, 1H) 8.18-8.21 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).



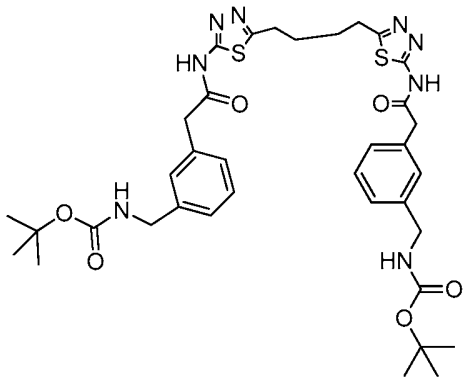
398: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm ppm 1.75 (brs, 4H) 2.90 (brs, 2H) 3.02 (brs, 2H) 3.72-3.78 (m, 10H) 6.42-6.51 (m, 4H) 7.36 (m, 5H) 7.54-7.58 (d, 1H) 8.18-8.21 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).



399: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.48 (s, 9H) 1.75 (brs, 4H) 2.90 (brs, 2H) 3.02 (brs, 2H) 3.74-3.78 (m, 4H) 6.92-6.94 (m,1H) 7.20-7.36 (m, 7H) 7.51-7.58 (m, 2H) 8.18-8.21 (d, 1H) 9.34 (s, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).

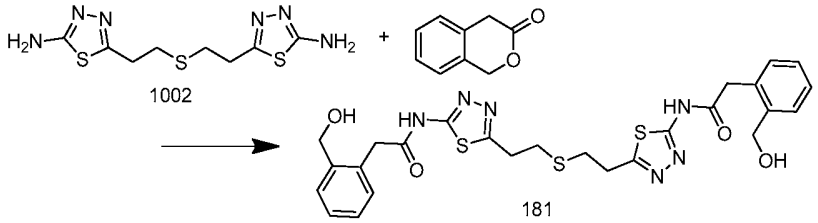


400: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.48 (s, 9H) 1.75 (brs, 4H) 2.90 (brs, 2H) 3.02 (brs, 2H) 3.71-3.78 (m, 4H) 7.18-7.42 (m, 9H) 7.54-7.58 (m, 2H) 8.18-8.21 (d, 1H) 9.34 (s, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).



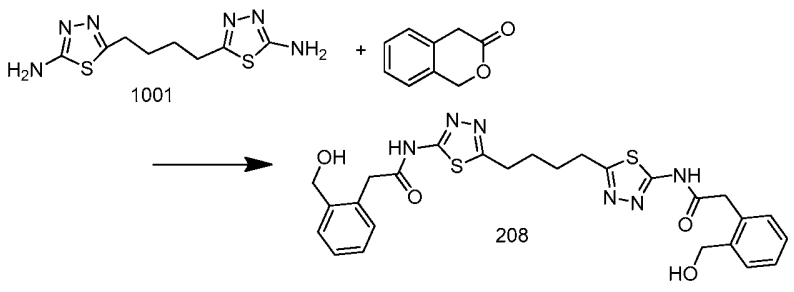
324: ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.39 (s, 18H) 1.76 (brs, 4H) 3.01(brs, 4H) 3.79 (s, 4H) 4.11-4.13 (brs, 4H) 7.13-7.38 (m, 8H) 12.65 (s, 2H).

Method C: via aluminum amide coupling with esters/lactones



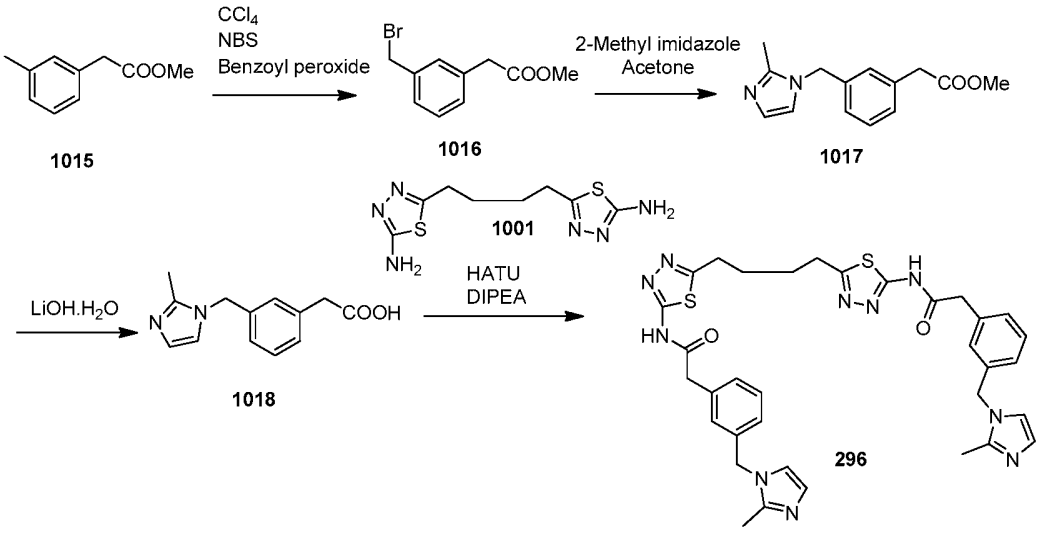
1002 181

To a suspension of **1002** (288 mg, 1.00 mmol) in toluene (9 mL) was added 3-isochromanone (311 mg, 2.10 mmol) followed by trimethyl aluminum (2M in toluene, 1.0 mL, 2.00 mmol). The resulting mixture was stirred at 75°C for 15 h, cooled to room temperature and diluted with ethyl acetate (50 mL). The organic layer was washed with water (3x20 mL), 10% sodium chloride solution (10 mL), dried (magnesium sulfate) and concentrated under reduced pressure. The crude product was purified by HPLC to afford N,N'-(5,5'-(thiobis(ethane-2,1-diyl))bis(1,3,4-thiadiazole-5,2-diyl))bis(2-(2-(hydroxymethyl)phenyl)acetamide) (**181**, 78 mg). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.42(d, *J*=6.84 Hz, 2H), 7.26(bs, 6H), 4.57(s, 4H), 3.90(s, 4H), 3.27(t, *J* =6.62 Hz, 4H), 2.94(t, *J* =6.44 Hz, 4H)



1001 208

To a suspension of **1001** (256 mg, 1.00 mmol) in toluene (8 mL) was added 3-isochromanone (311 mg, 2.10 mmol) followed by trimethyl aluminum (2M in toluene, 1.0 mL, 2.00 mmol). The resulting mixture was stirred at 75°C 15 h, cooled to room temperature and diluted with ethyl acetate (50 mL). The organic layer was washed with water (3x20 mL), 10% sodium chloride solution (10 mL), dried (magnsesium sulfate) and concentrated under reduced pressure. The crude product was purified by HPLC to afford N,N'-(5,5'-(thiobis(ethane-2,1-diyl))bis(1,3,4-thiadiazole-5,2-diyl))bis(2-(2-(hydroxymethyl)phenyl)acetamide) (**208**, 62 mg). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.41(s, 2H), 7.26(s, 6H), 4.56(s, 4H), 3.01(bs, 4H), 1.76(bs, 4H)



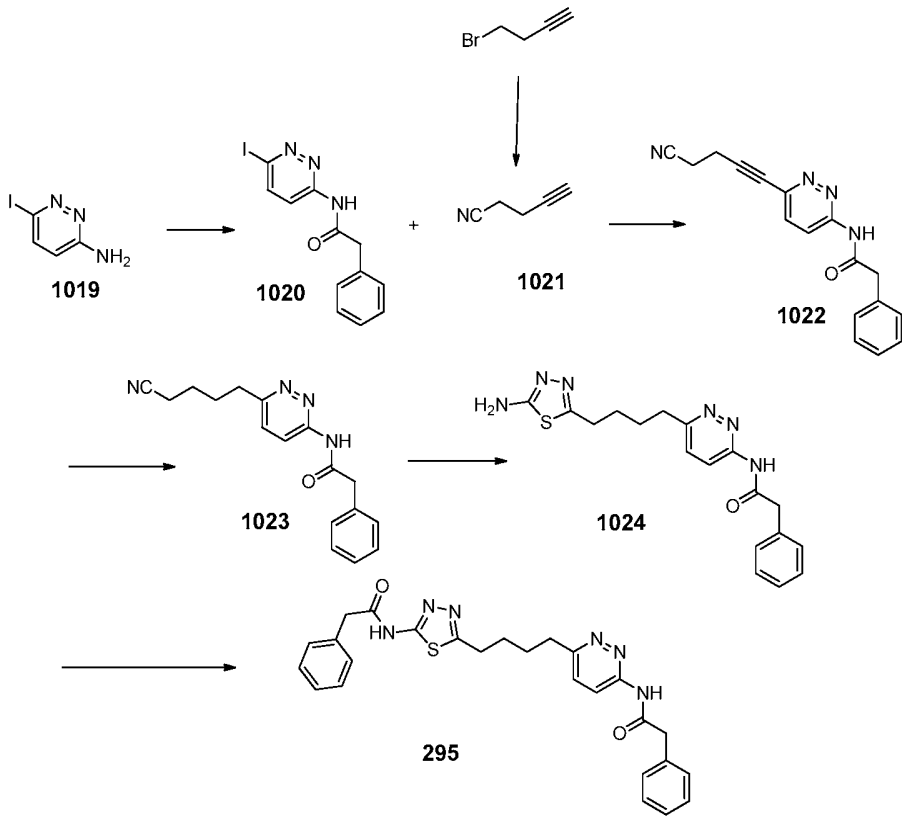
To a solution of **1015** (3.2g, 19.5mmol) in carbon tetrachloride (150mL) was added N-bromosuccinimide (3.47g, 19.6mmol) and benzoyl peroxide (10mg, catalytic). The resulting mixture was refluxed overnight before it was filtered hot. The filtrate was concentrated under reduced pressure and the residue obtained was purified by silica gel chromatography eluting with 20% ethylacetate/hexane to afford **1016** (2g, 42% yield) as an oil. ^{1}H NMR (300MHz, Chloroform-d) δ ppm 3.66 (s, 2H) 3.74 (s, 3H) 4.51(s, 2H) 7.35 (m, 4H).

To a solution of **1016** (0.243g, 1mmol) in acetone (10mL) was added 2-methyl imidazole (0.41g, 5mmol). The resulting mixture was refluxed overnight before it was concentrated under reduced pressure and the residue obtained was diluted with water (~100mL). The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium

sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with MeOH/dichloromethane to afford **1017** (0.17g, 69% yield) as an oil. ^{1}H NMR (300MHz, Chloroform-d) δ ppm 2.37 (s, 3H) 3.63 (s, 2H) 3.72 (s, 3H) 5.07 (s, 2H) 6.87 (s, 1H) 6.96-7.02 9m, 2H) 7.23-7.33 (m, 3H)

To a solution of **1017** (0.17g, 0.69mmol) in THF/MeOH/Water (10mL, 2mL, 2mL) was added lithium hydroxide monohydrate (0.06g, 1.42mmol). The resulting mixture was stirred at room temperature overnight before it was concentrated under reduced pressure. The residue obtained was diluted with water (~20mL) and the resulting solution was acidified with acetic acid. The aqueous layer was concentrated and the product was isolated by prep HPLC. The residue obtained was dissolved in water (5 mL) and concentrated hydrochloric acid (83 μL) was added to it before it was concentrated and dried to afford **1018** (0.15gm) as a hydrochloride salt.

To a suspension of carboxylic acid **1018** (105mg, 0.39mmol) in DMF (3mL) was added HATU (150mg, 0.39mmol) and stirred till reaction mixture is clear followed by the addition of an amine **1001** (50.5mg, 0.197mmol) and DIPEA (0.14mL, 0.8mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by the addition of water. The solid separated was filtered, washed with water and dried to afford **296** (112mg, 83%). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.76 (brs, 4H) 2.38 (s, 6H) 3.01(brs, 4H) 3.82 (s, 4H) 5.25 (s, 4H) 7.09-7.38 (m, 12H) 12.64-12.67 (brs, 2H).



To a suspension of **1019** (1.5 g, 6.8 mmol) in CH_2Cl_2 (15 mL) at 0 °C was added Et_3N (1.9 ml, 13.6 mmol) dropwise followed by phenyl acetyl chloride (1.07 ml, 8.1 mmol) dropwise. The resulting mixture was stirred at 0 °C and then slowly warmed up to room temperature for 2 days. The crude material was purified by silica gel chromatography eluting with 0-25% EtOAc in hexane to afford **1020**.

To a solution of 4-bromo-1-butyne (7 g, 53 mmol) in DMSO (30 ml) at 0 °C was added NaI (7.94 g, 53 mmol). The mixture was stirred at room temperature for 2 h before it was cooled to 0 °C and followed by addition of NaCN (5.2 g, 106 mmol). The resulting mixture was heated at 80 °C for 2.5 h and then stirred at room temperature overnight. The mixture was partitioned between water and EtOAc. The organic extract was washed with water, dried over sodium sulfate, filtered and evaporated to afford **1021.**

To a mixture of **1020** (400 mg, 1.18 mmol), $PdCl_2(PPh_3)_2$ (41 mg, 0.059 mmol) and CuI (11 mg, 0.059 mmol) in Et_3N (3 ml) and THF (6 ml) under argon atmosphere was added **1021** (187 mg, 2.36 mmol), then heated at 60 °C overnight. After removal of

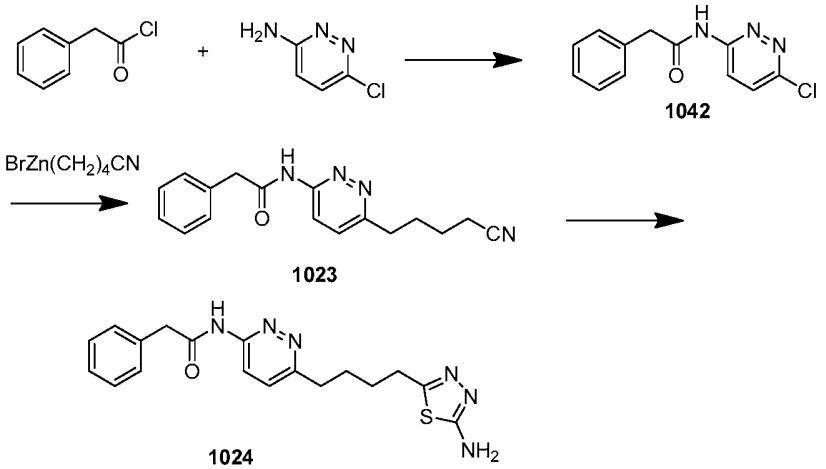
the solvent, the residue was purified by silica gel chromatography eluting with 0-60% EtOAc in Hexane to afford **1022**.

To a solution of **1022** (118 mg, 0.406 mmol) in the mixture of EtOAc (60 ml) and EtOH (15 ml) was added $Pd(OH)_2/C$ (50 mg, 0.356 mmol). Hydrogen was bubbled through the resulting mixture and stirred for 1 h. The Pd catalyst was filterd off and the filtrate was concentrated to afford **1023**.

A mixture of **1023** (127 mg, 0.431 mmol) and thiosemicarbazide (51 mg, 0.561 mmol) in TFA (3 mL) was heated at 85 ^{0}C for 5 h. The reaction was cooled to room temperature and poured onto a mixture of ice-water. The mixture was basified with NaOH pellets (pH 10). The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in CH_2Cl_2 to afford **1024**.

To a solution of **1024** (38.4 mg, 0.104 mmol) in NMP (1 mL) at 0 °C was added phenyl acetyl chloride (0.017 mL, 0.125 mmol) dropwise. The resulting mixture was stirred at 0 °C for 1.5 h before it was quenched by addition of water (~10 mL). The mixture was partitioned between water and EtOAc. The organic extract was washed with water, dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in CH_2Cl_2 to afford **295**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 8.82 Hz, 1H), 7.58-7.54 (d, J = 9.72 Hz, 1H), 7.36-7.28 (m, 10H), 3.81-3.78 (d, J = 8.43 Hz, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).

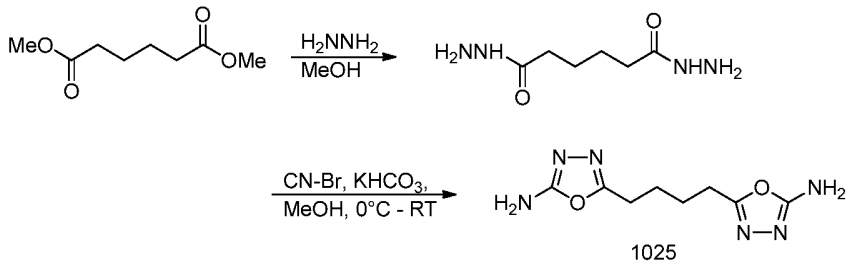
Compound **1024** can also be prepared according to the following procedure:



To a solution of 3-amino-6-chloropyridazine (11.14 g, 86.0 mmol) in NMP (279 mL) at 19°C was added phenylacetyl chloride (18.2 mL, 137.6 mmol) dropwise over 5 minutes with the internal temperature of the solution maintained $T_i \leq 28$ °C. The resulting mixture was stirred at 19°C for 90 minutes and poured into ice water (557 mL). The white precipitate was collected by suction filtration, rinsed with water (2x110 mL) and diethyl ether (110 mL). The product was dried overnight under high vacuum to afford N-(6-chloropyridazin-3-yl)-2-phenylacetamide (**xxx**, 18.8 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.57(s, 1H), 8.40(d, *J*=9.636 Hz, 1H), 7.90(d, *J*=9.516 Hz, 1H), 7.36(m, 5H) 3.82(s, 2H)

A 1000 mL three-neck flask fitted with internal temperature probe and addition funnel was flushed with $Ar_{(g)}$. Under positive Argon pressure 4-cyanobutylzinc bromide (0.5M in THF, 500mL, 250 mmol) was charged into the addition funnel then added to the reaction vessel at room temperature. Solid N-(6-chloropyridazin-3-yl)-2-phenylacetamide (20.6 g, 83.3 mmol) was added to the stirred solution at RT under $Ar_{(g)}$ flow, followed by the addition of $NiCl_2$(dppp) (4.52 g, 8.33 mmol). The resulting mixture was stirred at 19°C for 240 minutes and then quenched with ethanol (120 mL). Water (380mL) added to the stirred red solution, giving a thick precipitate. Ethyl acetate (760 mL) added and stirred well for 30 minutes. The solids were removed by filtration through a pad of celite. The mother liquor was then transferred to a separatory funnel and the organic layer was washed with H_2O (380mL), 0.5% ethylenediaminetetraacetic acid solution (380 mL) and again with H_2O (380mL). The organic layer was concentrated by rotoevaporation. Resulting red oil was redissolved in EtOAc (200 mL) and 1M HCl (380 mL) was added to the well stirred flask. After 30 minutes the mixture was transferred to separatory funnel and the aqueous layer collected. The organic layer was extracted with 1M HCl (2x380mL). The aqueous layer's pH was then adjusted to ~7 using 7.5% sodium bicarbonate solution and the pale yellow precipitate was collected by suction filtration, rinsed with water (200 mL) and diethyl ether (2x200mL). The solid was dried overnight under high vacuum to afford N-(6-(4-cyanobutyl)pyridazin-3-yl)- 2-phenylacetamide (**1023**, 14.76 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.29(s, 1H), 8.23(d, *J*=9.036 Hz, 1H), 7.59(d, *J*=9.246 Hz, 1H), 7.32(m, 5H), 3.79(s, 2H), 2.90(t, *J*= 7.357 Hz, 2H), 2.56(t, *J*= 7.038 Hz, 2H), 1.79(t, *J*= 7.311 Hz, 2H), 1.63(t, *J*= 7.01 Hz, 2H)

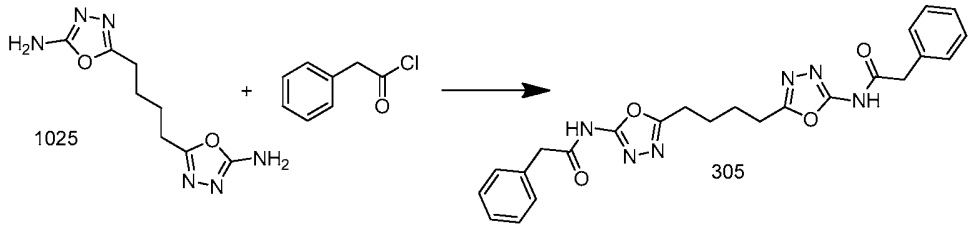
N-(6-(4-cyanobutyl)pyridazin-3-yl)-2-phenylacetamide (14.7 g, 50.2 mmol) was charged into a 250 mL round bottom flask fitted with an open top reflux condenser. To the flask was added thiosemicarbazide (5.03 g, 55.2 mmol) and trifluoroacetic acid (88 mL). The reaction slurry was heated in a 65°C bath for 2 h. After cooling to RT, H_2O (150 mL) was added and stirred for 30 minutes. The mixture was then slowly transferred to a stirred 7.5% sodium bicarbonate solution (1400mL) cooled in a 0°C bath. The precipitate was collected by suction filtration, rinsed with water (2x200 mL), diethyl ether (2x200mL) and dried under high vacuum overnight. The off-white solid was slurried in DMSO (200 mL) and heated in an 80°C bath until the internal temperature reached 65°C. DMSO (105 mL) was used to rinse sides of flask. H_2O (120 mL) was slowly added until the solution became slightly cloudy and then the mixture was removed from heat bath and allowed to cool to ambient temperature while stirring. The pale green precipitate was collected by suction filtration, rinsed with water (200 mL) and diethyl ether (2x200mL). The solid was dried overnight under high vacuum to provide N-(6-(4-(5-amino-1,3,4-thiadiazol-2-yl)butyl)pyridazin-3-yl)-2-phenylacetamide (**1024**, 15.01 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.28(s, 1H), 8.23(d, *J*=8.916 Hz, 1H), 7.59(d, *J*=8.826 Hz, 1H), 7.36(m, 5H), 7.07(s, 2H), 3.78(s, 2H), 2.87(t, *J*= 6.799 Hz, 4H), 1.69(bm, 4H)



1025

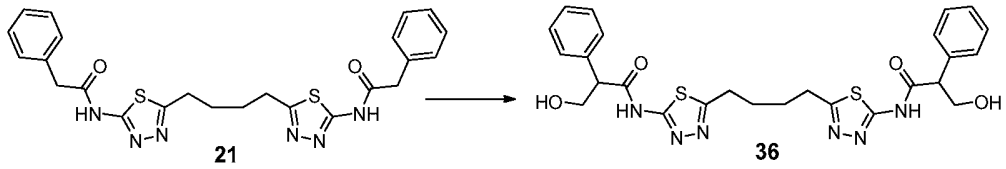
To a solution of dimethyl adipate (28.7 mmol, 5.0 g, 4.7 mL, 1.0 equiv.) in 20 mL of MeOH was added anhydrous hydrazine (229.6 mmol, 7.36 g, 7.51 mL, 8.0 equiv.) and the mixture heated to 50°C, giving a white precipitate. The mixture was heated for one hour and then allowed to cool to room temperature. The white solid was collected by filtration and washed with additional MeOH then dried under high vacuum giving 4.6 g of adipohydrizide. ^{1}HNMR (300 MHz, DMSO-d_6) δ 8.91 (s, 2H), 4.14 (s, 4H), 2.00 (br s, 4H), 1.46 (br s, 4H).

To a 0°C cooled slurry of adipohydrizide (12.49 mmol, 4.0 g, 1.0 equiv.), potassium bicarbonate (15.61 mmol, 1.56 g, 1.25 equiv.) in 25 mL of MeOH was added solid cyanogen bromide (13.74 mmol, 1.44 g, 1.1 equiv.) in one portion. This mixture was stirred at 0°C and allowed to warm to RT over one hour and then stirred overnight. The volatiles were removed under reduced pressure and the solids diluted with water. The pH was adjusted to 12 with 2.5 N NaOH and the solids collected by filtration. The white solid was washed with water and dried under high vacuum to give 1.73 g of oxadiazole **1025**. ^{1}HNMR (300 MHz, DMSO-d_6) δ 6.85 (s, 4H), 2.68 (s, 4H), 1.68 (s, 4H).



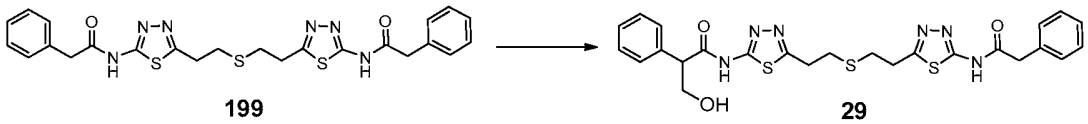
To a suspension of oxadiazole **1025** (181 mg, 0.81 mmol) in NMP (9 mL) was added triethylamine (0.564 mL, 4.05 mmol) and the mixture warmed to 70°C. The mixture was allowed to stir for 30 minutes followed by the addition of phenylacetyl chloride (0.234 mL, 1.77 mmol). The reaction temperature was held at 70°C for 15 hours then allowed to cool to room temperature. The crude reaction mixture was purified by reverse phase HPLC giving **305** (0.015 g). ^{1}HNMR (300 MHz, DMSO-d_6) δ 11.74(s, 2H), 7.33(s, 10H), 3.74(s, 4H), 2.85(s, 4H), 1.76(s, 4H).

Functionalization of diacylated cores:



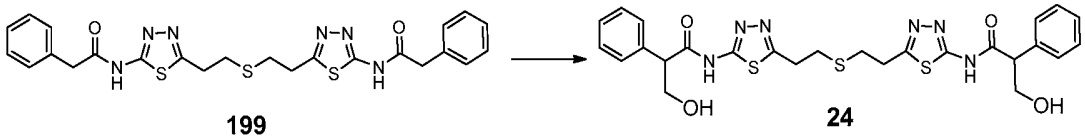
To a suspension of **21** (2.25 g, 4.57 mmol) in a mixture of THF (250 mL) and H_2O (20 mL) at room temperature was added NaOH (1.83 g, 45.67 mmol) and formaldehyde solution (37% in water, 14.83 mL, 182.70 mmol). The resulting mixture was heated at 60 °C for 7 h before it was cooled to 0 °C and acidified to pH 7 with aq. HCl solution. The white precipitate was collected by suction filtration, rinsed

with water and dried to provide *N*,*N*'-[5,5'-(butane-1,4-diyl)-bis(1,3,4-thiadiazole-5,2-diyl)]-bis(3-hydroxy-2-phenylpropanamide) (**36**, 624 mg). The 2nd precipitation from the filtrate provided additional product (1.29 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (bs, 2H), 7.35–7.30 (m, 10H), 5.09 (bs, 2H), 4.10–4.02 (m, 4H), 3.61 (d, J = 8.1 Hz, 2H), 3.02 (bs, 4H), 1.76 (bs, 4H).



199 **29**

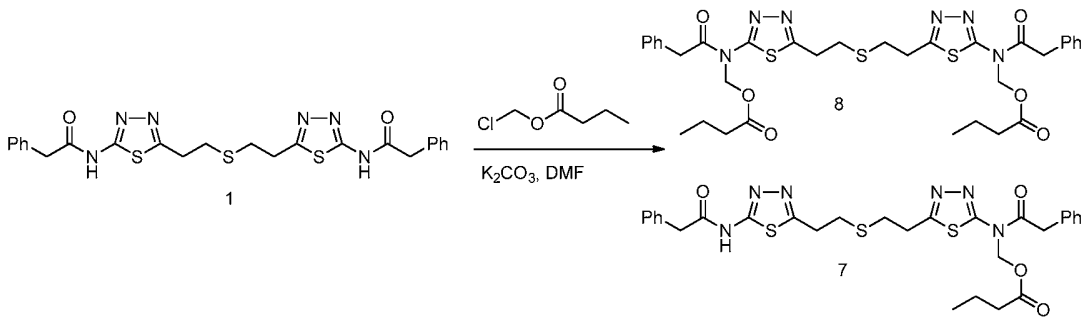
To a suspension of **199** (300 mg, 0.572 mmol) in a mixture of THF (50 mL) and MeOH (5 ml) was added potassium carbonate (158 mg, 1.144 mmol) and formaldehyde solution (37% in water, 2 mL). The resulting mixture was stirred at room temperature for 48 h before it was cooled to 0 °C and acidified to pH 7 with aq. HCl solution. The white precipitate was collected by suction filtration, rinsed with water and dried. The crude material was purified by HPLC to afford **29.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.34–7.26 (m, 10H), 4.13-4.02 (m, 2H), 3.81 (s, 2H), 3.62 (m, 2H), 3.24 (t, 4H), 2.93 (t, 4H).



199 **24**

To a suspension of **199** (2.0 g, 3.81 mmol) in a mixture of THF (250 mL) and MeOH (20 ml)H_2O (20 mL) at room temperature was added 1N NaOH (20 ml) and formaldehyde solution (37% in water, 15 mL). The resulting mixture was heated at 50 °C overnight before it was cooled to 0 °C and acidified to pH 7 with aq. HCl solution. The white precipitate was collected by suction filtration, rinsed with water and dried. The crude material was purified by HPLC to afford **24.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.67 (bs, 2H), 7.36–7.30 (m, 10H), 5.10 (bs, 2H), 4.10–4.02 (m, 4H), 3.61 (d, 2H), 3.27 (t, 4H), 2.95 (t, 4H).

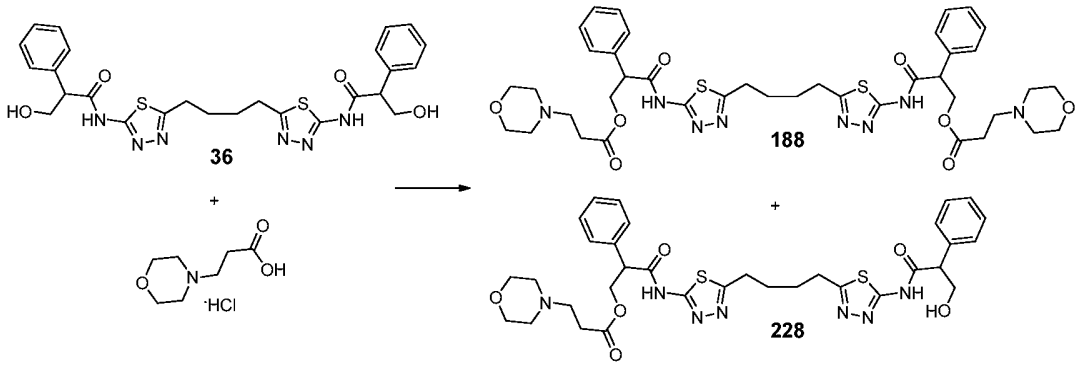
Prodrugs:



To a flask containing N,N'-(5,5'-(thiobis(ethane-2,1-diyl))bis(1,3,4-thiadiazole-5,2-diyl))bis(2-phenylacetamide) (1) (9.4 mmol, 5.0 g, 1.0 equiv.) was added 100 mL DMF, K_2CO_3 (20.98 mmol, 2.89 g, 2.2 equiv.), and chloromethyl butyrate (20.98 mmol, 2.86 g, 2.62 mL, 2.2 equiv.). The mixture stirred at room temperature for 15 hours then diluted with 200 mL water and 200 mL EtOAc. The layers were separated and the aqueous layer extracted with EtOAc (2 x 100 mL) and the organic layers combined, washed with water, brine and dried over Na_2SO_4. The Na_2SO_4 was removed by filtration and the volatiles removed under reduced pressure. The compounds were purified by reverse phase chromatography (MeCN, H_2O) giving 0.235 g of compound 8 and 0.126 g of compound 7.

^{1}HNMR (300 MHz, DMSO, d_6) Compound 8: δ 7.31 (m, 10H), 6.18 (s, 4H), 3.82 (s, 4H), 3.17 (dd, 2H, *J* =6.8 Hz), 2.92 (dd, 2H, *J* =6.8 Hz), 2.93 (m, 4H), 2.32 (dd, 2H, *J*=7.2 Hz), 1.54 (dt, 2H, *J* =7.2, 7.4 Hz), 0.87 (t, 3H, *J* = 7.4Hz).

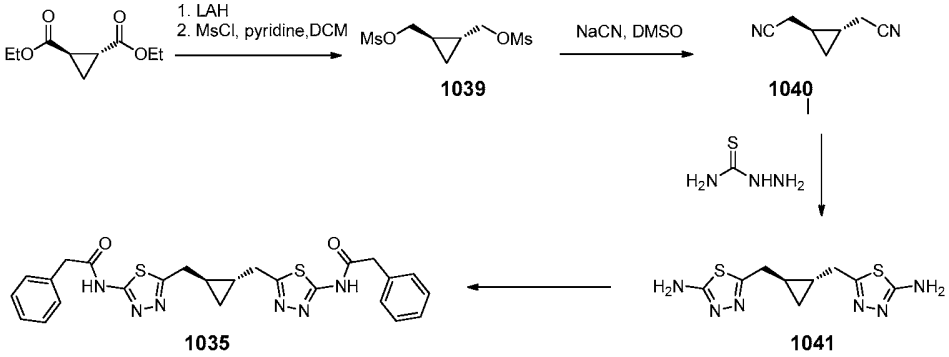
^{1}HNMR (300 MHz, DMSO, d_6) Compound 7: δ 12.68 (s, 1H), 7.32 (m, 10H), 6.18 (s, 2H), 3.82 (s, 4H), 3.26 (dd, 2H, *J* =7.0 Hz), 3.17 (dd, 2H, *J* =6.8 Hz), 2.93 (m, 4H), 2.32 (dd, 2H, *J* =7.2 Hz), 1.54 (dt, 2H, *J* =7.2, 7.4 Hz), 0.87 (t, 3H, *J* = 7.4Hz).



To a suspension of 3-morpholin-4-yl-propionic acid hydrochloride (500 mg, 2.56

mmol) in DMF (20 mL) at 0 °C was added *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (534 mg, 2.79 mmol). The resulting mixture was stirred at 0 °C for 40 min and followed by addition of diol **36** (642 mg, 1.16 mmol) and 4-DMAP (454 mg, 3.72 mmol). The resulting mixture was stirred from 0 °C to room temperature over a period of 3.5 h before it was diluted with EtOAc and cold water. The organic layer was separated and washed with water (3×50 mL), brine, dried ($MgSO_4$) and concentrated. The crude product was purified by silica gel chromatography eluting with 10–25% MeOH in EtOAc to provide {[5,5'-(butane-1,4-diyl)-bis(1,3,4-thiadiazole-5,2-diyl)]-bis(azanediyl)}-bis(3-oxo-2-phenylpropane-3,1-diyl)-bis(3-morpholinopropanoate) (**188**, 340 mg) and a less polar product, 3-((5-{4-[5-(3-hydroxy-2-phenylpropanamido)-1,3,4-thiadiazol-2-yl]butyl}-1,3,4-thiadiazol-2-yl)amino)-3-oxo-2-phenylpropyl 3-morpholinopropanoate (**228**, 103 mg). **188**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.80 (s, 2H), 7.39 (m, 10H), 4.62 (t, J = 9.6 Hz, 2H), 4.33–4.27 (m, 4H), 3.48 (bs, 8H), 3.02 (bs, 4H), 2.45 (bs, 8H), 2.25 (bs, 8H), 1.76 (bs, 4H).

228: ^{1}H NMR (300 MHz, MeOD-d_4) δ 7.43–7.37 (m, 10H), 4.71 (t, J = 10.5 Hz, 1H), 4.41 (m, 1H), 4.30–4.24 (m, 2H), 4.06–4.03 (m, 1H), 3.80–3.76 (m, 1H), 3.62 (bs, 4H), 3.11 (bs, 4H), 2.63–2.52 (m, 4H), 2.40 (bs, 4H), 1.90 (bs, 4H).



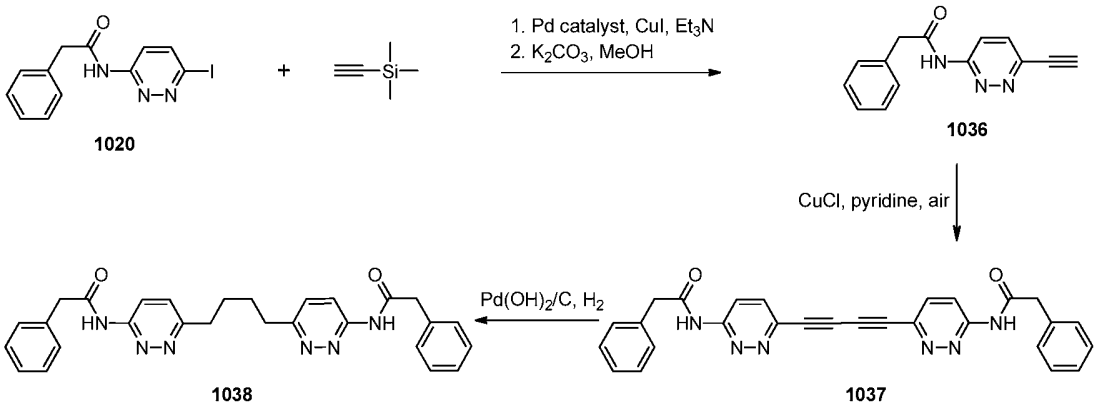
To a solution of diethyl *trans*-1,2-cyclopropanedicarboxylate (5.00 g, 26.85 mmol) in THF (20 mL) at 0 °C was added a solution of LAH (67.13 mL, 1.0 M in THF, 67.13 mmol) dropwise. The resulting mixture was stirred at 0 °C for 1.5 h before it was quenched with H_2O (20 mL), 2N aq. NaOH (20 mL) and H_2O (20 mL). The mixture was stirred vigorously for 1 h at room temperature before it was filtered through a

plug of celite. The filtrate was dried ($MgSO_4$) and concentrated to provide the desired diol (2.73 g) as a colorless oil.

A mixture of the diol (2.00 g, 19.58 mmol) in CH_2Cl_2 (75 mL) at 0 °C was added pyridine (6.34 mL, 78.33 mmol) and followed by MsCl (3.33 mL, 43.08 mmol) dropwise. The resulting mixture was stirred 0 °C for 1 h before it was warmed up to room temperature. The reaction was quenched with H_2O and diluted with ether. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated to provide **1039**. This crude product was dissolved in DMSO (75 mL), and added NaCN (2.88 g, 58.75 mmol) and NaI (294 mg, 1.96 mmol). The resulting mixture was heated at 45 °C for 8 h before it was allowed to cool to room temperature and diluted with EtOAc and H_2O. The organic layer was separated, washed with brine, dried ($MgSO_4$) and concentrated to provide the crude product **1040** which was used in the following step without purification.

A mixture of **1040** and thiosemicarbazide (3.75 g, 41.12 mmol) in trifluoroacetic acid (TFA) (20 mL) was heated at 80 °C for 5 h. The reaction was cooled to room temperature and poured into a mixture of ice and water. Sodium hydroxide pellets were added to the mixture until it was basic (pH 14). The white precipitate was collected by suction filtration, rinsed with water, ether and dried to provide **1041** (472 mg).

To a suspension of **1041** (70 mg, 0.26 mmol) in 1-Methyl-2-pyrrolidinone (NMP) (5 mL) at 0 °C was added phenylacetyl chloride (72 μL, 0.55 mmol) dropwise. The resulting mixture was stirred at 0 °C for 1 h before it was quenched by addition of water (~3 mL). The white precipitate was collected by suction filtration, rinsed with water and dried to provide **1035** (37 mg). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 2H), 7.34–7.27 (m, 10H), 3.82 (s, 4H), 3.04 – 2.75 (m, 4H), 1.14–1.12 (m, 2H), 0.63–0.59 (m, 2H).



1020 1036 1038 1037

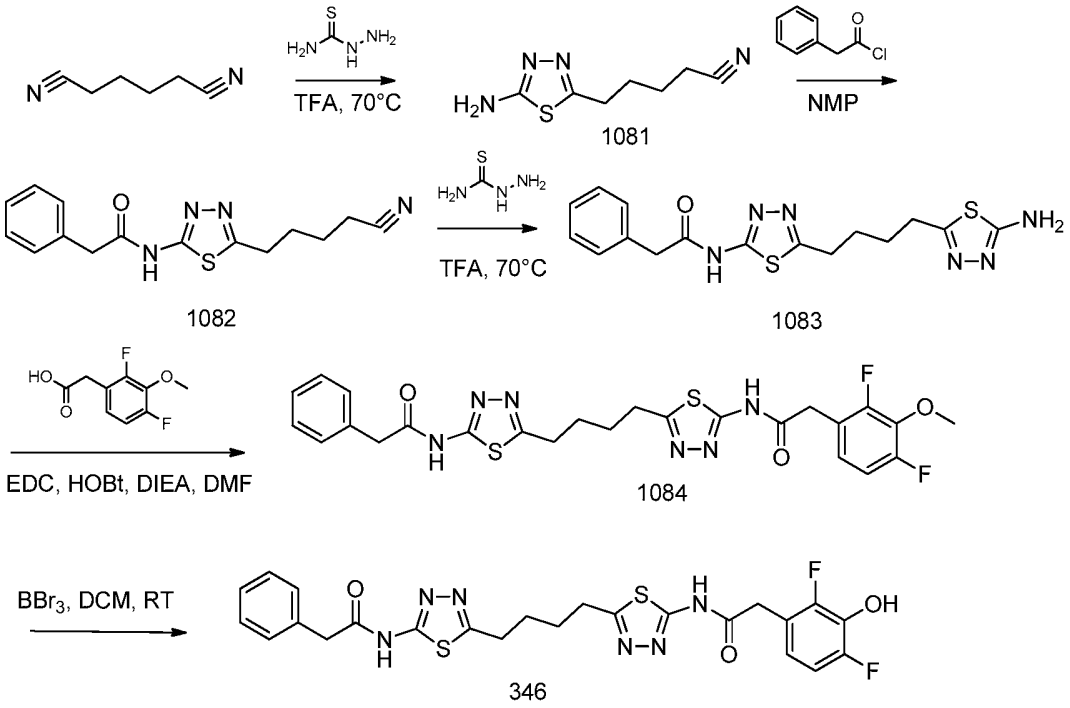
To a solution of **1020** (1.50 g, 4.42 mmol), ethynyltrimethylsilane (813 uL, 5.75 mmol), $PdCl_2(PPh_3)_2$ (310 mg, 0.44 mmol) and CuI (59 mg, 0.31 mmol) in THF (20 mL) under argon atmosphere at room temperature was added Et_3N (6.16 mL, 44.23 mmol). The resulting mixture was heated at 50 °C for 5 h before it was allowed to cool to room temperature and filtered through a plug of celite. The filtrate was concentrated and the crude residue was purified by flash column chromatography over silica gel eluting with 10–50% EtOAc in hexanes to provide the desired product (1.21 g) as a solid.

A mixture of the foregoing intermediate (1.07 g, 3.48 mmol) and K_2CO_3 (0.40 g, 2.90 mmol) in MeOH (100 mL) was stirred at room temperature for 5 h before it was concentrated under reduced pressure. The residue was re-dissolved in a mixture of EtOAc and H_2O, and was neutralized with 1N aq. HCl solution to pH 7. The organic layer was separated, washed with brine, dried ($MgSO_4$) and concentrated. The crude residue was purified by flash column chromatography over silica gel eluting with 10–50% EtOAc in hexanes to provide the desired alkyne **1036** (0.48 g) as a white solid.

To a solution of alkyne **1036** (52 mg, 0.22 mmol) in pyridine (5 mL) at room temperature was added CuCl (4.3 mg, 0.04 mmol). The resulting mixture was stirred under a stream of air for 40 min as all of the starting material was consumed. The reaction mixture was diluted with saturated aq. NH_4Cl solution (~2 mL). The off-white precipitate was collected by suction filtration, washed with H_2O and dried. This crude bis-acetylene product **1037** (52 mg) was used in the following step without further purification.

A mixture of **1037** (52 mg) and $Pd(OH)_2/C$ (100 mg) in a mixture of DMF (5 mL) and THF (10 mL) was stirred at room temperature under 1 atmosphere of H_2 for 3 h as all

of the starting material was consumed. The palladium catalyst was filtered off and the filtrate was concentrated. The crude residue was purified by column chromatography over silica gel eluting with 1–10% MeOH in CH_2Cl_2 to provide the desired product **1038** (18 mg) as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.26 (s, 2H), 8.20 (d, J = 8.97 Hz, 2H), 7.56 (d, J = 8.77 Hz, 2H), 7.36–7.24 (m, 10H), 3.78 (s, 4H), 2.90 (bs, 4H), 1.73 (bs, 4H).



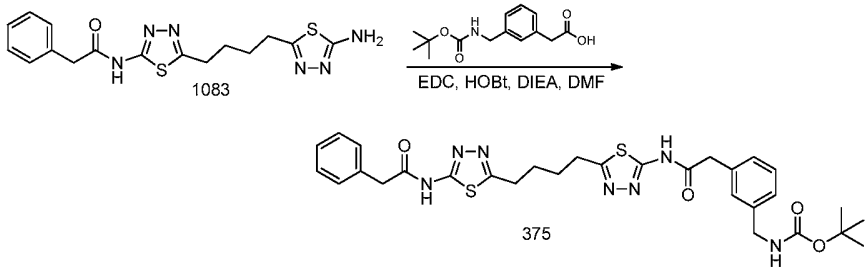
To a solution of adiponitrile (19.02 g, 175.8 mmol) in TFA (50 mL) was added thiosemicarbazide (16.02 g, 175.8 mmol) and the mixture heated to 70°C for 4 hours under an atmosphere of Argon. The mixture was allowed to cool to room temperature and the volatiles removed under reduced pressure. The residue was diluted with water (200 mL) and the pH adjusted to 7 with solid NaOH giving a white precipitate that was collected by filtration and washed with water. The solids were dried under high vacuum giving 9.22 g of **1081**. ^{1}HNMR (DMSO, d_6): δ 7.02 (br s, 2H) 2.84 (m, 2H), 2.55 (m, 2H), 1.67 (m, 4H).

To a solution of **1081** (0.625 g, 2.87 mmol) in NMP (12.5 mL) was added phenylacetyl chloride (0.487 g, 0.42 mL, 3.15 mmol) dropwise and the mixture stirred at room temperature for one hour under an atmosphere of Argon. The mixture was poured into water (100 mL) and the solids collected by filtration. The solids were washed with water and dried under high vacuum to give 0.805 g of **1082**. ^{1}HNMR

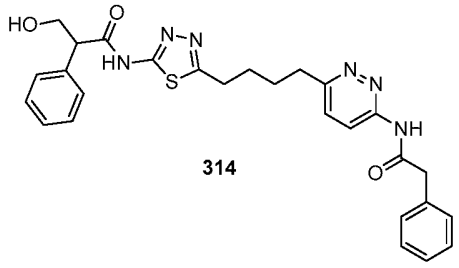
(DMSO, d_6): δ 12.65 (s, 1H) 7.31 (m, 5H), 3.80 (s, 2H), 3.00 (t, 2H, *J*= 7.3 Hz), 2.53 (t, 2H, *J*= 7.1 Hz), 1.78 (dq, 2H, *J*= 7.3, 7.1 Hz), 1.61 (dq, 2H, J= 7.3, 7.1 Hz).

To a solution of **1082** (0.49 g, 1.33 mmol) in TFA (10 mL) was added thiosemicarbazide (0.23 g, 1.46 mmol) and the mixture heated at 70°C overnight under an atmosphere of Argon. The mixture was allowed to cool to room temperature and the volatiles removed under reduced pressure. The residue was diluted with water (50 mL) and the pH adjusted to 7 with solid NaOH giving a white precipitate that was collected by filtration and washed with water. The solids were dried under high vacuum giving 0.367 g of **1083**. ^{1}HNMR (DMSO, d_6): δ 12.70 (s, 1H) 7.34 (br s, 5H), 7.16 (s, 2H), 3.82 (s, 2H), 3.01 (s, 2H), 2.84 (S, 2H), 1.71 (br s, 4H).

To a solution of **1083** (0.10 g, 0.267 mmol), 2,4-difluoro-3-methoxyphenylacetic acid (0.058 g, 0.267 mmol), EDC (0.127 g, 0.667 mmol), HOBt (0.090 g, 0.667 mmol) in DMF (4 mL) was added DIEA (0.171 g, 0.231 mL, 1.335 mmol) and the mixture stirred overnight under an atmosphere of Argon. The mixture was poured into water (20 mL) and the solids formed were collected by filtration, washed with water and dried under high vacuum. The crude **1084** was used in the following step without purification. To a solution of **1084** (0.050 g, 0.091 mmol) in dichloromethane (1 mL) was added BBr_3 (1.0 mL, 1 mmol, 1.0 M in dichloromethane) and the mixture stirred for 4 hours at room temperature under an atmosphere of Argon. The volatiles were removed under reduced pressure and the residue diluted with dichloromethane (5 mL). The volatiles were removed under reduced pressure and the residue diluted with water (15 mL) and the pH adjusted to 12. The aqueous layer was washed with dichloromethane (4 x 5 mL) and the pH adjusted to 4. The solids were collected by filtration, washed with water and dried under high vacuum giving 0.029 g of **346**. ^{1}HNMR (DMSO, d_6): δ 12.66 (s, 2H), 10.12 (s, 1H), 7.33 (s, 5H), 7.00 (m, 1H), 6.80 (m, 1H), 3.84 (s, 2H), 3.81 (s, 2H), 3.02 (br s, 4H), 1.76 (br s, 4H).

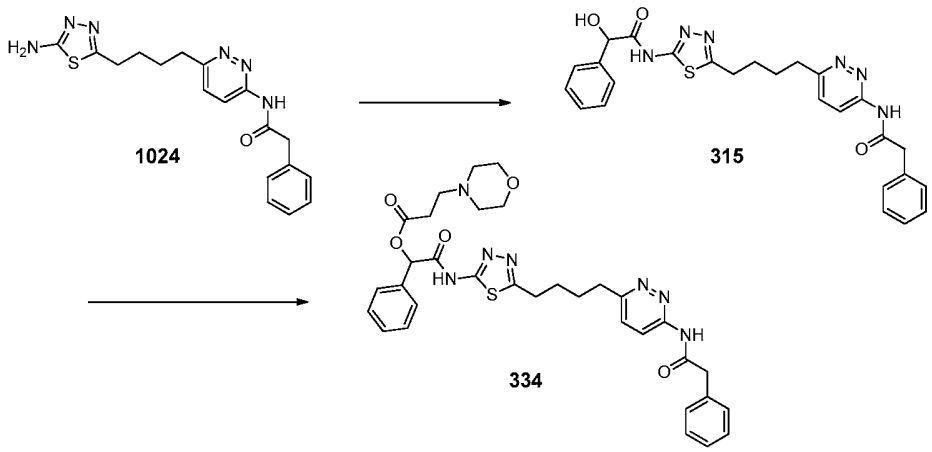


To a solution of **1083** (0.05 g, 0.133 mmol), Boc-3-aminomethyl-phenylacetic acid (0.035 g, 0.133 mmol), EDC (0.064 g, 0.332 mmol), HOBt (0.045 g, 0.332 mmol) in DMF (8 mL) was added DIEA (0.086 g, 0.115 mL, 0.665 mmol) and the mixture stirred overnight under an atmosphere of Argon. The mixture was poured into water (20 mL) and the solids formed were collected by filtration, washed with water and dried under high vacuum to give 0.023 g of **375**. ^{1}HNMR (DMSO, d_6): δ 12.66 (s, 2H), 7.27 (m, 10H), 4.11 (br s, 2H), 3.81 (s, 2H), 3.79 (s, 2H), 3.01(br s, 4H), 1.76 (br s, 4H), 1.39 (s, 9H).



314

A flask was charged with **1024** (100 mg, 0.27 mmol), tropic acid (54 mg, 0.326 mmol) in DMF (2 ml) at 0 ^{0}C was added HOBT (88 mg, 0.652 mmol) followed by EDCI (156 mg, 0.815 mmol). The resulting mixture was slowly warmed up to room temperature and stirred for 3 h before it was quenched by addition of water (~10 mL). The white precipitate was collected by suction filtration, rinsed with more water and dried to afford **314**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 8.82 Hz, 1H), 7.58-7.54 (d, J = 9.72 Hz, 1H), 7.36-7.28 (m, 10H), 4.10-4.05 (m, 2H), 3.78 (s, 3H), 3.65 (s, 1H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).

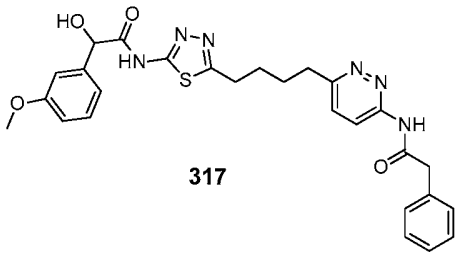


1024 **315** **334**

A flask was charged with **1024** (500 mg, 1.36 mmol), DL-mandelic acid (248 mg, 1.63 mmol) in DMF (10 ml) at 0 ^{0}C was added HOBT (441 mg, 3.26 mmol) followed

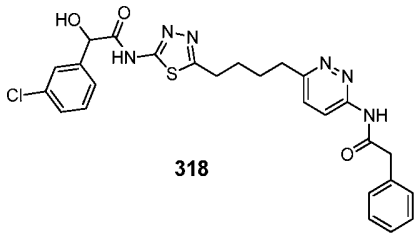
by EDCI (781 mg, 4.08 mmol). The resulting mixture was stirred at 0 ^{0}C for 10 minutes then warmed up to room temperature and stirred for 10 minutes before it was quenched by addition of water (~50 mL) at 0 ^{0}C. The white precipitate was collected by suction filtration, rinsed with more water and dried to afford **315**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 8.82 Hz, 1H), 7.58-7.50 (m, 3H), 7.36-7.28 (m, 8H), 6.35 (s, 1H), 5.32 (s, 1H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).

To a suspension of 3-morpholin-4-yl-propionic acid hydrochloride (209 mg, 1.07 mmol) in DMF (10 ml) was added EDCI (308 mg, 1.61 mmol). The resulting mixture was stirred at 0 ^{0}C for 1 hour and followed by addition of **315** (447 mg, 0.889 mmol) and 4-DMAP (261 mg, 2.14 mmol). The resulting mixture was stirred from 0 ^{o}C to room temperature over a period of 6 h before it was quenched by addition of ice water (~50mL). The white precipitate was collected by suction filtration, rinsed with more water. The crude material was purified by silica gel chromatography eluting with 0–6% MeOH in EtOAc to afford **334**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.95 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 9.45 Hz, 1H), 7.58-7.26 (m, 11H), 6.14 (s, 1H), 3.78 (s, 2H), 3.54 (bs, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.63 (bs, 4H), 2.38 (bs, 4H), 1.73 (bs, 4H).



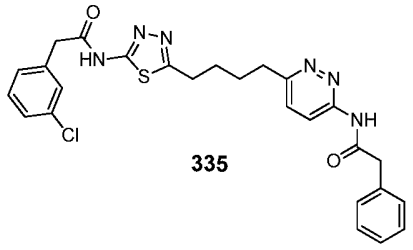
317

Compound **317** was prepared according to the procedure above for compound **315**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.40 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 9.03 Hz, 1H), 7.58-7.54 (d, J = 9.72 Hz, 1H), 7.36-6.87 (m, 9H), 6.35 (bs, 1H), 5.30 (s, 1H), 3.78 (m, 5H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



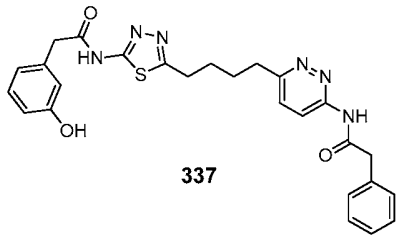
318

Compound **318** was prepared according to the procedure above for compound **315**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 9.43 Hz, 1H), 7.60-7.27 (m, 10H), 6.51 (bs, 1H), 5.35 (s, 1H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



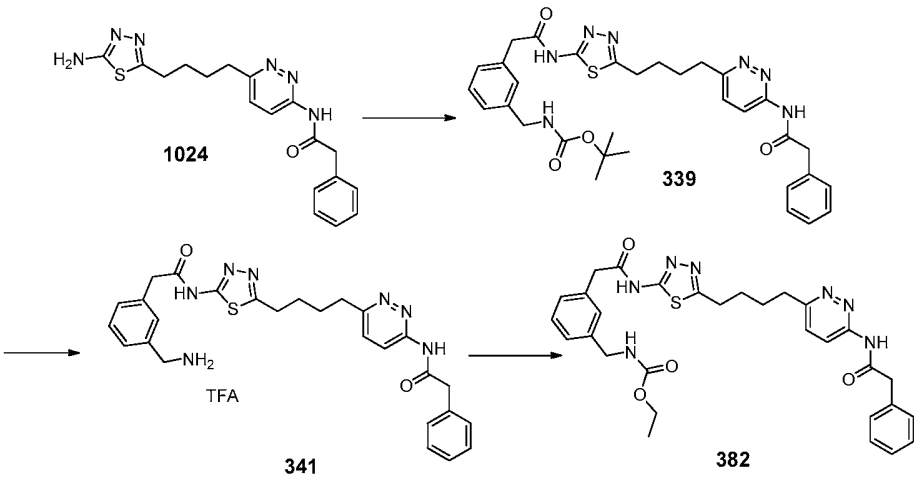
335

A flask was charged with **1024** (50 mg, 0.135 mmol), 3-chlorophenylacetic acid (28 mg, 0.163 mmol) in DMF (1 ml) at 0 ^{0}C was added HOBT (44 mg, 0.326 mmol) followed by EDCI (78 mg, 0.408 mmol). The resulting mixture was slowly warmed up to room temperature and stirred for 1 h before it was quenched by addition of water (~5 mL). The white precipitate was collected by suction filtration, rinsed with more water and ether then dried to afford **335**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 8.82 Hz, 1H), 7.58-7.54 (d, J = 9.72 Hz, 1H), 7.36-7.28 (m, 9H), 3.84 (s, 2H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



337

Compound **337** was prepared according to the procedure above for compound **335.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 9.38 (s, 1H), 8.22-8.19 (d, J = 8.37 Hz, 1H), 7.58-7.54 (d, J = 9.63 Hz, 1H), 7.36-7.09 (m, 6H), 6.75-6.65 (m, 3H), 3.78 (s, 2H), 3.70 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).

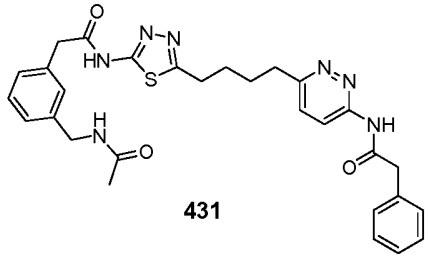


339, 341, 382: A flask was charged with **1024** (100 mg, 0.27 mmol), Boc-3-aminomethyl-phenylacetic acid (86 mg, 0.325 mmol) in DMF (2 ml) at 0 ^{0}C was added HOBT (88 mg, 0.65 mmol) followed by EDCI (156 mg, 0.812 mmol). The resulting mixture was stirred at 0 ^{0}C for 5 minutes then warmed up to room temperature and stirred for 1.5 h before it was quenched by addition of water (~10 mL) at 0 ^{0}C. The white precipitate was collected by suction filtration, rinsed with more water and ether then dried to afford **339**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 8.82 Hz, 1H), 7.58-7.54 (d, J = 9.42 Hz, 1H), 7.36-7.13 (m, 9H), 4.13-4.11 (d, J = 10.62, 2H), 3.78 (s, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H), 1.38 (s, 9H).

To a suspension of **339** (50 mg, 0.081 mmol) in dichloromethane (2 ml) was added TFA (2 ml) at 0 ^{0}C. The resulting mixture was stirred at room temperature for 20 minutes before it was evaporated under vacuo to dryness. Ether was added and the white precipitate was collected by suction filtration, rinsed with more ether and dichloromethane then dried to afford **341**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 8.82 Hz, 1H), 8.14-8.11 (bs, 2H), 7.58-7.54 (d, J = 9.42 Hz, 1H), 7.36-7.13 (m, 9H), 4.06-4.03 (m, 2H), 3.84 (s, 2H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).

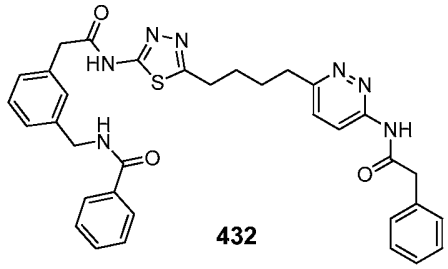
To a solution of **341** (10 mg, 0.0159mmol) in DMF (1 ml) at 0 ^{0}C was added triethylamine (4.4 ul, 0.0317 mmol) drop wise followed by ethyl chloroformate (1.8 ul, 0.0191 mmol) drop wise. The resulting mixture was slowly warmed up to room temperature and stirred for 30 minutes before it was quenched by addition of water

(~1 mL) at 0 ^{0}C. The mixture was partitioned between water and EtOAc. The organic extract was washed with water, dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in CH_2Cl_2 to afford **382**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 8.82 Hz, 1H), 7.67-7.58 (bs, 1H), 7.58-7.54 (d, J = 9.42 Hz, 1H), 7.36-7.13 (m, 9H), 4.18-4.16 (m, 2H), 4.06-4.0 (q, 2H), 3.78 (s, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H), 1.19-1.13 (t, 3H).



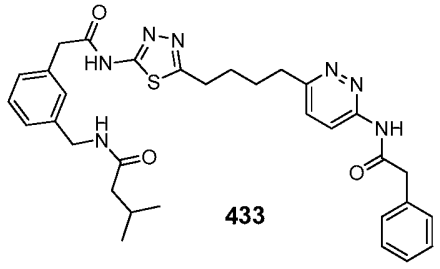
431

Compound **431** was prepared according to the procedure above for compound **382** with the appropriate reagents. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 8.35 (s, 1H), 8.22-8.19 (d, J = 8.88 Hz, 1H), 7.57-7.54 (d, J = 9.51 Hz, 1H), 7.38-7.15 (m, 9H), 4.25-4.24 (d, J = 5.64 Hz, 2H), 3.76 (s, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.87 (s, 3H), 1.73 (bs, 4H).



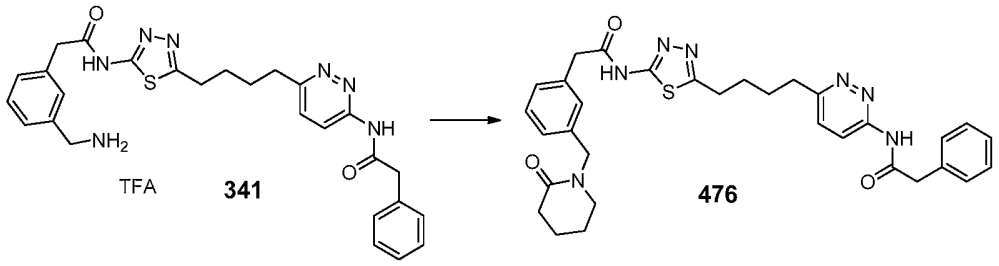
432

Compound **432** was prepared according to the procedure above for compound **382** with the appropriate reagents. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.63 (s, 1H), 11.26 (s, 1H), 9.04-9.01 (m, 1H), 8.22-8.19 (d, J = 8.91 Hz, 1H), 7.93-7.89 (d, J = 9.51 Hz, 2H), 7.58-7.25 (m, 13H), 4.50-4.48 (d, J = 5.91 Hz, 2H), 3.78 (s, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).

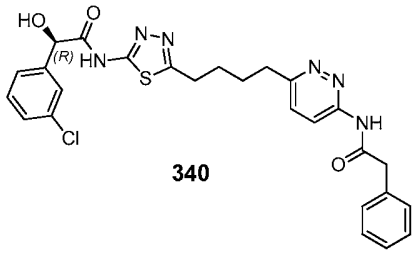


433

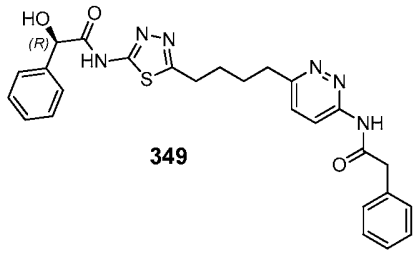
Compound **433** was prepared according to the procedure above for compound **382** with the appropriate reagents. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.63 (s, 1H), 11.26 (s, 1H), 8.31-8.21 (m, 1H), 8.20-8.19 (d, J = 9.57 Hz, 1H), 7.57-7.54 (d, J = 8.73 Hz, 1H), 7.35-7.13 (m, 9H), 4.26-4.24 (d, J = 5.52 Hz, 2H), 3.78 (s, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.0 (s, 3H), 1.73 (bs, 4H), 0.86-0.85 (d, J = 3.99 Hz, 6H).



To a solution of **341** (70 mg, 0.111mmol) in DMF (1 ml) at 0 ^{0}C was added triethylamine (31 ul, 0.22 mmol) drop wise followed by 5-bromovaleryl chloride (12 ul, 0.122 mmol) drop wise. The resulting mixture was slowly warmed up to room temperature and stirred for 1h. Potassium *tert*-butoxide (50 mg, 0.445 mmol) was then added to the reaction mixture at 0 ^{0}C. The resulting mixture was slowly warmed up to room temperature and stirred for overnight before it was quenched by addition of water (~2 mL) at 0 ^{0}C. The mixture was partitioned between water and EtOAc. The organic extract was washed with water, dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in CH_2Cl_2 to afford **476**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 8.82 Hz, 1H), 7.58-7.54 (d, J = 9.42 Hz, 1H), 7.36-7.13 (m, 9H), 4.50 (s, 2H), 3.78 (s, 4H), 3.35 (bs, 2H), 3.20 (bs, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.30 (bs, 2H), 1.68-1.80 (d, 6H).

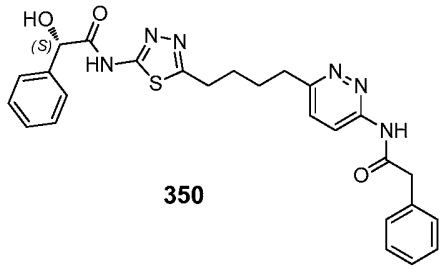


Compound **340** was prepared according to the procedure above for compound **315** with the appropriate reagents. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 9.24 Hz, 1H), 7.60-7.27 (m, 10H), 6.51 (bs, 1H), 5.35 (s, 1H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



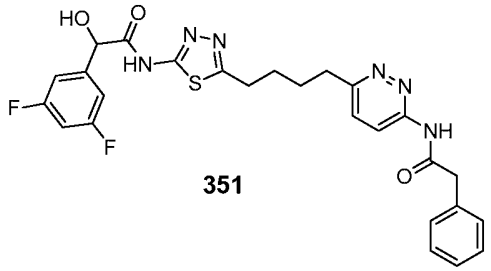
349

Compound **349** was prepared according to the procedure above for compound **315** with the appropriate reagents. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.41 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 8.76 Hz, 1H), 7.58-7.27 (m, 11H), 6.36 (s, 1H), 5.34 (s, 1H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



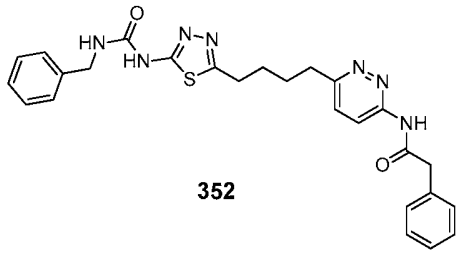
350

Compound **350** was prepared according to the procedure above for compound **315** with the appropriate reagents. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.41 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 8.67 Hz, 1H), 7.58-7.27 (m, 11H), 6.34 (s, 1H), 5.34 (s, 1H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



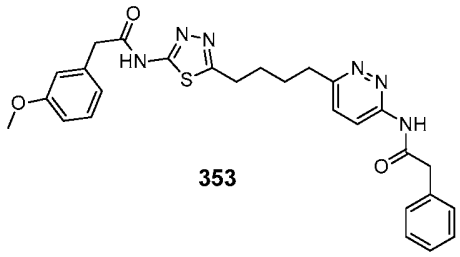
351

Compound **351** was prepared according to the procedure above for compound **315** with the appropriate reagents. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (s, 1H), 11.26 (s, 1H), 8.21-8.18 (d, J = 8.67 Hz, 1H), 7.58-7.54 (d, J = 9.72 Hz, 1H), 7.36-7.23 (m, 8H), 6.67 (s, 1H), 5.40 (s, 1H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



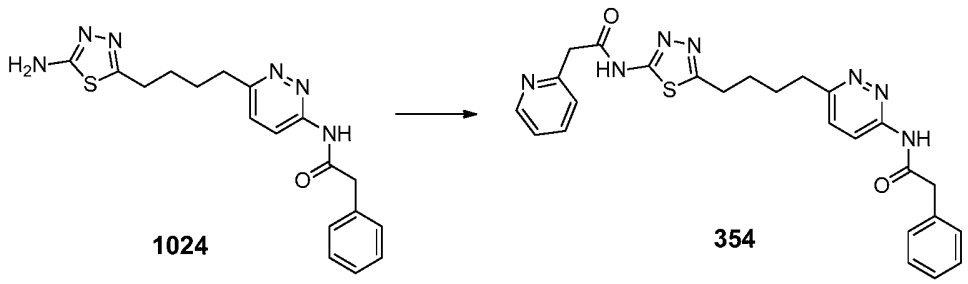
352

To a solution of **1024** (50 mg, 0.136 mmol) in DMF (1 ml) at 0 ^{0}C was added triethylamine (38 ul, 0.271 mmol) drop wise followed by benzyl isocyanate (20 ul, 0.163 mmol) drop wise. The resulting mixture was slowly warmed up to room temperature and stirred for 40 minutes before it was quenched by addition of water (~5 mL) at 0 ^{0}C. The white precipitate was collected by suction filtration, rinsed with more water. The crude material was purified by silica gel chromatography eluting with 0–6% MeOH in CH_2Cl_2 to afford **352**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.26 (s, 1H), 10.82 (s, 1H), 8.22-8.19 (d, J = 9.42 Hz, 1H), 7.58-7.54 (d, J = 8.79 Hz, 1H), 7.36-7.31 (m, 10H), 7.06 (bs, 1H), 4.37-4.35 (d, J = 5.22 Hz, 2H), 3.78 (s, 2H), 2.99-2.90 (m, 4H), 1.73 (bs, 4H).



353

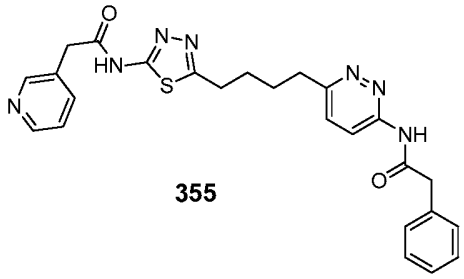
Compound **353** was prepared according to the procedure above for the preparation of compound **335**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.57 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 9.45 Hz, 1H), 7.57-7.54 (d, J = 9.48 Hz, 1H), 7.36-7.25 (m, 6H), 6.91-6.84 (m, 3H), 3.76 (m, 7H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



1024 354

A flask was charged with **1024** (50 mg, 0.135 mmol), 2-pyridine acetic acid hydrochloride (27 mg, 0.156 mmol) in DMF (1 ml) at 0 ^{0}C was added propylphosphonic anhydride solution (91 ul) followed by triethylamine (54 ul, 0.39 mmol). The resulting mixture was slowly warmed up to room temperature and stirred

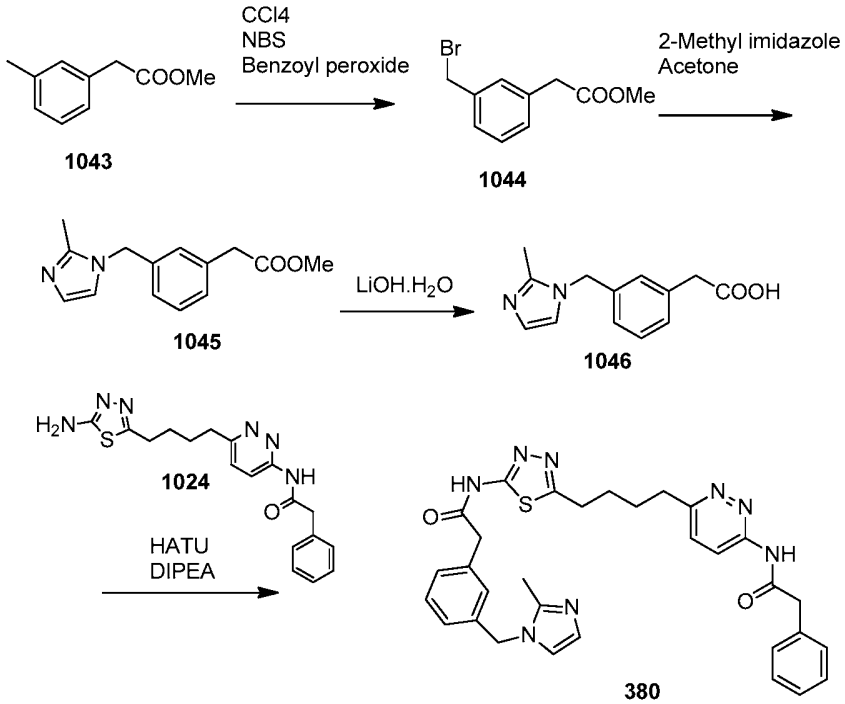
for 1 h before it was quenched by addition of water (~5 mL). The white precipitate was collected by suction filtration, rinsed with more water and ether then dried to afford **354**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 8.51 (s, 1H), 8.22-8.19 (d, J = 8.97 Hz, 1H), 7.81-7.76 (m, 1H), 7.58-7.54 (d, J = 9.06 Hz, 1H), 7.42-7.26 (m, 7H), 4.02 (s, 2H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



355

Compound **355** was prepared according to the procedure above for the preparation of compound **354**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.70 (s, 1H), 11.26 (s, 1H), 8.53-8.49 (m, 1H), 8.22-8.19 (d, J = 9.0 Hz, 1H), 7.77-7.73 (d, J = 8.46 Hz, 1H), 7.58-7.54 (d, J = 9.48 Hz, 1H), 7.38-7.26 (m, 7H), 3.88 (s, 2H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).

Compounds **309** and **310** were prepared according to the procedure above for the preparation of compound **354**.



1043 **1044** **1045** **1046** **1024** **380**

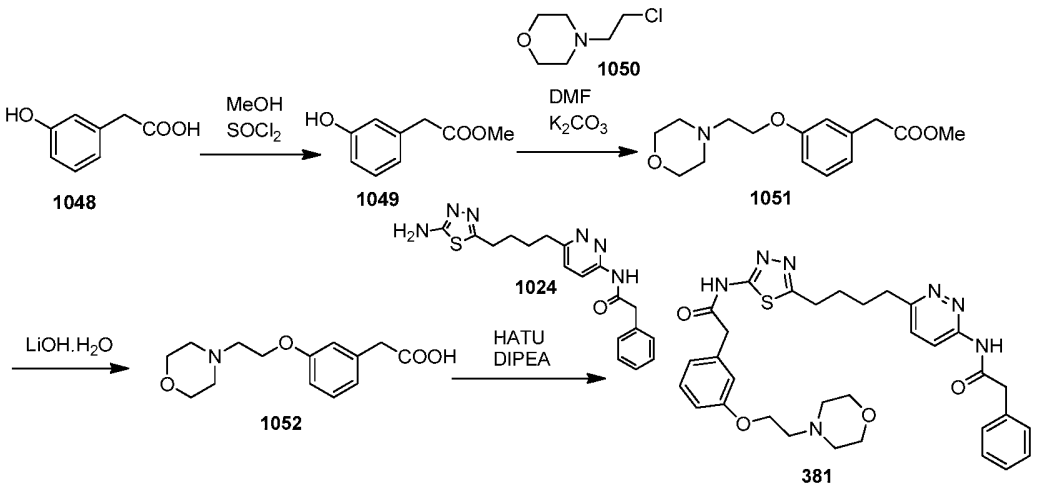
To a solution of **1043** (3.2g, 19.5mmol) in carbon tetrachloride (150mL) was added N-bromosuccinimide (3.47g, 19.6mmol) and benzoyl peroxide (10mg, catalytic). The resulting mixture was refluxed overnight before it was filtered hot. The filtrate was concentrated under reduced pressure and the residue obtained was purified by silica gel chromatography eluting with 20% ethylacetate/hexane to afford **1044** (2g, 42% yield) as an oil. ^{1}H NMR (300MHz, Chloroform-d) δ ppm 3.66 (s, 2H) 3.74 (s, 3H) 4.51(s, 2H) 7.35 (m, 4H)

To a solution of **1044** (0.243g, 1mmol) in acetone (10mL) was added 2-methyl imidazole (0.41g, 5mmol). The resulting mixture was refluxed overnight before it was concentrated under reduced pressure and the residue obtained was diluted with water (~100mL). The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with MeOH/dichloromethane to afford **1045** (0.17g, 69% yield) as an oil. ^{1}H NMR (300MHz, Chloroform-d) δ ppm 2.37 (s, 3H) 3.63 (s, 2H) 3.72 (s, 3H) 5.07 (s, 2H) 6.87 (s, 1H) 6.96-7.02 9m, 2H) 7.23-7.33 (m, 3H)

To a solution of **1045** (0.17g, 0.69mmol) in THF/MeOH/Water (10mL, 2mL, 2mL) was added lithium hydroxide monohydrate (0.06g, 1.42mmol). The resulting mixture was stirred at room temperature overnight before it was concentrated under reduced pressure. The residue obtained was diluted with water (~20mL) and the resulting solution was acidified with acetic acid. The aqueous layer was concentrated and the product was isolated by prep HPLC. The residue obtained was dissolved in water (mL) and concentrated hydrochloric acid (mL) was added to it before it was concentrated and dried to afford **1046** (0.15gm) as a hydrochloride salt.

To a suspension of carboxylic acid **1046** (41.8mg, 0.157mmol) in DMF (3mL) was added HATU (61.3mg, 0.161mmol) and stirred till reaction mixture is clear followed by the addition of an amine **1024** (52.5mg, 0.142mmol) and DIPEA (50ul, 0.29mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by the addition of water. The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained

was triturated with ether. The solid separated was filtered, washed with ether and dried to afford **380** (40mg, 48%). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.74 (brs, 4H) 2.91-3.02 (brs, 4H) 3.78-3.83 (m, 4H) 5.34 (s, 2H) 7.16-7.57 (m, 12H) 8.19-8.22 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H)



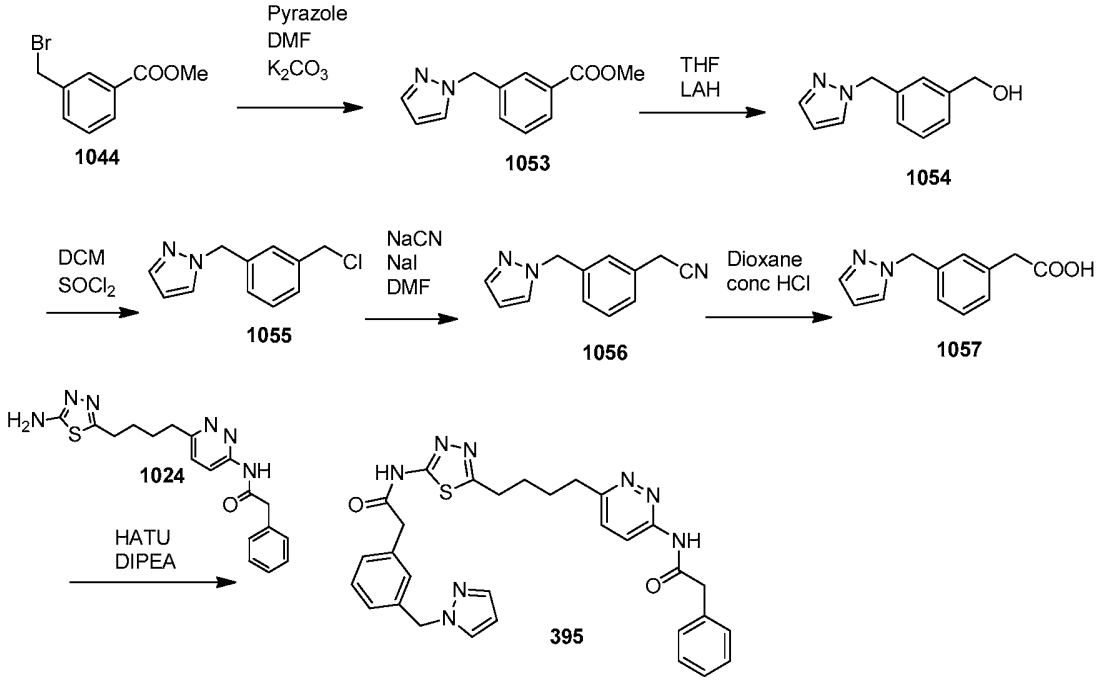
To an ice cold solution of **1048**(5g, 0.033mol) in methanol (50mL) was added thionyl chloride (0.2mL) and the resulting mixture was stirred at room temperature overnight before it was concentrated under reduced pressure. The residue obtained was dried at high vacuum overnight to afford **1049** (5gm) as an oil and was used as such for the next step. ^{1}H NMR (300MHz, Chloroform-d) δ ppm 3.62 (s, 2H) 3.74 (s, 3H) 6.76-6.87 (m, 3H) 7.18-7.21(m, 1H).

To a solution of **1049** (1g, 6mmol) in DMF (20mL) was added potassium carbonate (2.08g, 15mmol), **1050** (1.225g, 6.62mmol) and sodium iodide (10mg). The resulting mixture was stirred at 80 ^{0}C overnight before it was diluted with water (~100mL). The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with MeOH/dichloromethane to afford **1051** (1g, 60% yield) as an oil. ^{1}H NMR (300MHz, Chloroform-d) δ ppm 2.61 (s, 4H) 2.83 (t, 2H) 3.62 (s, 2H) 3.63 (s, 3H) 3.73-3.77 (m, 4H) 4.14 (t, 2H) 6.88-6.91 (m, 3H) 7.26-7.29 (m, 1H)

To a solution of **1051** (1g, 3.57mmol) in THF/MeOH/Water (30mL, 5mL, 5mL) was added lithium hydroxide monohydrate (0.3g, 7.14mmol). The resulting mixture was

stirred at room temperature overnight before it was concentrated under reduced pressure. The residue obtained was diluted with water (~50mL) and the resulting solution was acidified with 1N hydrochloric acid. The aqueous layer was concentrated and the product was isolated by prep HPLC. The residue obtained was dissolved in water (mL) and concentrated hydrochloric acid (mL) was added to it before it was concentrated and dried to afford **1052** as a hydrochloride salt.

To a suspension of carboxylic acid **1052** (47.4mg, 0.157mmol) in DMF (3mL) was added HATU (61.3mg, 0.161mmol) and stirred till reaction mixture is clear followed by the addition of an amine **1024** (52.5mg, 0.142mmol) and DIPEA (50ul, 0.29mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by the addition of water. The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with MeOH/dichloromethane to afford **381** (40mg, 46% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.74 (brs, 4H) 2.72 (t, 2H) 2.89-2.9 (m, 4H) 3.02 (brs, 4H) 3.336 (m, 2H) 3.76-3.78 (m,2H) 4.09 (m, 2H) 6.88-6.93 (m, 3H) 7.24-7.36 (m, 6H) 7.54-7.58 (d, 1H) 8.18-8.21 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).



To a solution of **1044** (2.29g, 0.01mol) in DMF (100mL) was added potassium

carbonate (1.38g, 0.01mmol) and pyrazole (0.68g, 0.01mol). The resulting mixture was stirred at 70 ^{0}C for 5hr before it was diluted with water (~100mL). The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1053** (1g, 50% yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 3.94 (s, 3H) 5.40 (s, 2H) 6.33 (s, 1H) 7.42-7.48 (m, 3H) 7.58 (s, 1H) 7.95 (s, 1H) 8.00-8.02 (m, 1H)

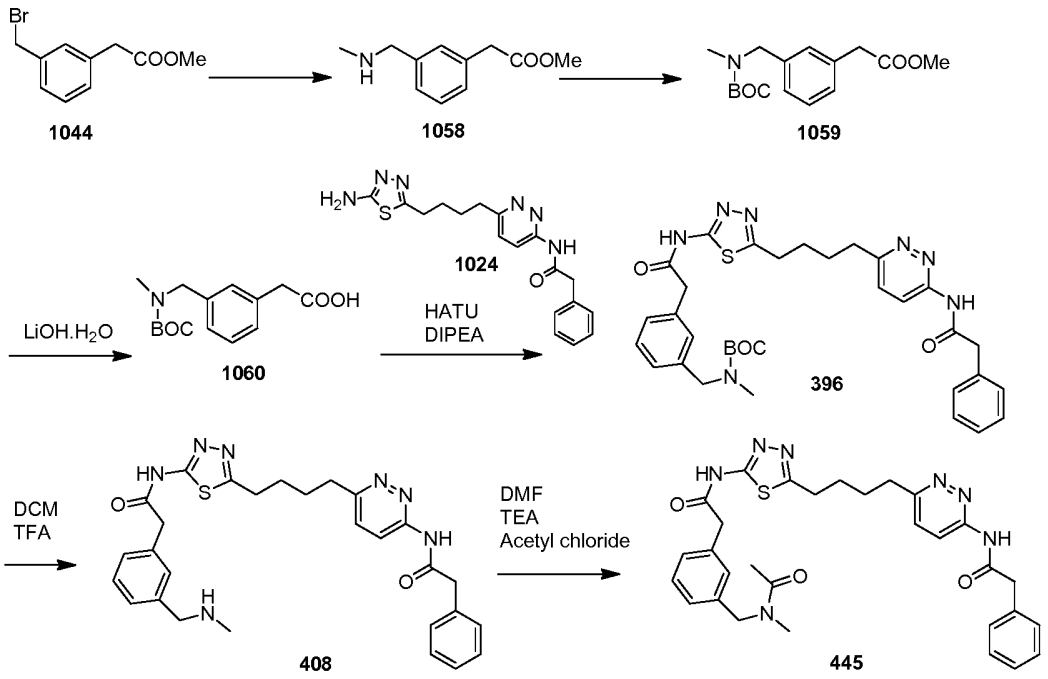
To an ice cold solution of **1053** (1g, 4.62mmol) in THF (20mL) was added lithium aluminum hydride (2.5mL, 2M/THF) drop wise and the resulting reaction mixture was stirred at 0 ^{0}C for 5hr before it was quenched with saturated Rochelle salt solution. The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated to afford **1054** (0.8g, 92% yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 4.71 (s, 2H) 5.35 (s, 2H) 6.30 (s, 1H) 7.15-7.43 (m, 5H) 7.58 (s, 1H)

To a solution of **1054** (0.8g, 4.2mmol) in dichloromethane (20mL) was added thionyl chloride and the resulting mixture was stirred at room temperature for 5hr before it was concentrated under the reduced pressure. The residue obtained was dried at high vacuum overnight to afford **1055** (1g, 97% yield) as a HCl salt. ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 4.75 (s, 2H) 5.38 (s, 2H) 6.30 (s, 1H) 7.19-7.50 (m, 5H) 7.86 (s, 1H) 11.49-11.60 (brs, 1H)

To a solution of **1055** (1g, 4.1mmol) in DMF (20mL) was added sodium cyanide (0.625g, 12.7mmol) and sodium iodide (20mg) and the resulting reaction mixture was stirred at 70 ^{0}C for 2hr before it was diluted with water. The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1056** (0.664g, 83% yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 3.76 (s, 2H) 5.38 (s, 2H) 6.35 (s, 1H) 7.19-7.46 (m, 5H) 7.61 (s, 1H)

To a solution of **1056** (0.664g, 3.3mmol) in dioxane (5mL) was added concentrated hydrochloric acid (5mL) and the resulting reaction mixture was stirred at 90 ^{0}C overnight before it was concentrated under the reduced pressure. The residue obtained was purified through prep HPLC and was converted to HCl salt to afford **1057** (0.5g, 40% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 3.55 (s, 2H) 5.33 (s, 2H) 6.29 (s, 1H) 7.14-7.20 (m, 4H) 7.48 (s, 1H) 7.84 (s, 1H) 11.97-11.99 (brs, 1H)

To a suspension of carboxylic acid **1057** (19.8mg, 0.0785mmol) in DMF (2mL) was added HATU (30.6mg, 0.08mmol) and stirred till reaction mixture is clear followed by the addition of an amine **1024** (26.25mg, 0.07mmol) and DIPEA (25ul, 0.15mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by the addition of water. The solid separated was filtered, washed with water and dried to afford **395** (18mg, 45%yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.74 (brs, 4H) 2.89-3.04 (m, 4H) 3.78 (s, 4H) 5.33 (s, 2H) 6.27-6.28 (s, 1H) 7.09-7.58 (m, 11H) 7.82 (s, 1H) 8.19-8.21 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H)



To a solution of **1044** (1g, 4.1mmol) in THF(5mL) was added 2M/THF methyl amine solution (2mL) and the resulting reaction mixture was stirred at room temperature overnight before it was concentrated under the reduced pressure. The residue obtained

was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with MeOH/dichloromethane to afford **1058** (0.26g, 33% yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 2.49 (s, 3H) 3.66 (s, 2H) 3.73 (s, 3H) 3.79 (s, 2H) 7.2-7.33 (m, 4H).

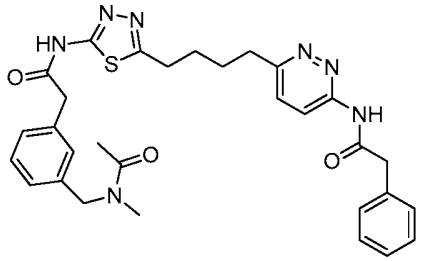
To a solution of **1058** (0.26g, 1.35mmol) in dichloromethane (5mL) was added boc anhydride (0.293g, 1.35mmol) and the resulting reaction mixture was stirred at room temperature for 4hr before it was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1059** (0.3g, 77% yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 1.5 (s, 9H) 2.84 (s, 3H) 3.66 (s, 2H) 3.73 (s, 3H) 4.44 (s, 2H) 7.17-7.32 (m, 4H).

To an ice cold solution of **1059** (0.3g, 1.02mmol) in dioxane (3mL) and water (2mL) was added lithium hydroxide monohydrate (0.086g, 2.04mmol) and the resulting reaction mixture was stirred at 0 ^{0}C for 3hr before it was acidified with 1N HCl. The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was dried at high vacuum overnight to afford **1060** (0.2g, 70%yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 1.5 (s, 9H) 2.84 (s, 3H) 3.66 (s, 2H) 4.43 (s, 2H) 7.17-7.32 (m, 4H)

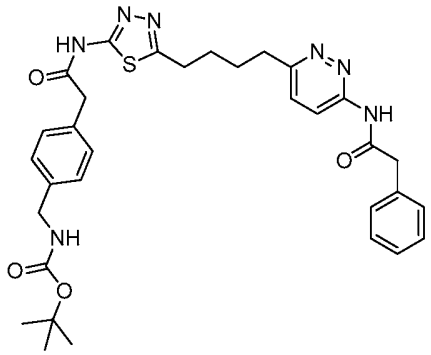
To a suspension of carboxylic acid **1060** (51.1mg, 0.183mmol) in DMF (3mL) was added HATU (69.7mg, 0.183mmol) and stirred till reaction mixture is clear followed by the addition of an amine **1024** (61.3mg, 0.166mmol) and DIPEA (58ul, 0.33mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by the addition of water. The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with MeOH/dichloromethane to afford **445** (0.06g, 57% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.37-1.38 (s, 9H) 1.74 (brs, 4H) 2.76 (s,3H) 2.89 (brs, 2H) 3.02 (brs, 2H)3.78-3.80

(m, 4H) 4.36 (s, 2H) 7.11-7.36 (m, 9H) 7.54-7.57 (d, 1H) 8.18-8.21 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).

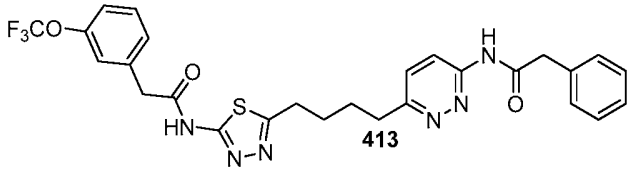
Prep of 445 via 396 deprotection to 408 and re-acylation:



To an ice cold solution of **408** (26mg, 0.04mmol) in DMF (1mL) was added triethylamine (12.3uL, 0.088mmol) and acetyl chloride (3.16uL, 0.044mmol). The resulting mixture was stirred at room temperature for 2hr before it was diluted with water. The solid separated was filtered, washed with water and dried at high vacuum overnight to afford **445** (10mg, 48% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.74 (brs, 4H) 2.05 (m, 3H) 2.91-3.02 (m,7H) 3.78-3.82 (m, 4H) 4.49-4.56 (m, 2H) 7.18-7.36 (m, 9H) 7.55-7.58 (d, 1H) 8.18-8.21 (d, 1H) 8.75-8.7 (brs, 2H) 11.26 (s, 1H) 12.65 (brs, 1H).



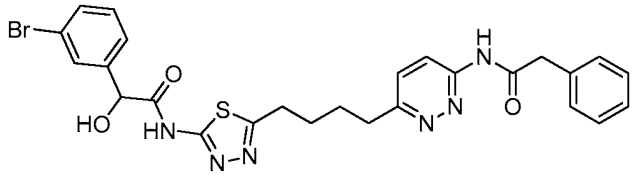
Compound **401** was prepared according to the procedure above for the preparation of compound **339**. ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.40 (s, 9H) 1.75 (brs, 4H) 2.87 (brs, 2H) 2.89 (brs, 2H) 3.78 (s, 4H) 4.09-4.11 (brs, 2H) 7.18-7.36 (m, 9H) 7.54-7.58 (d, 1H) 8.18-8.21 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H)



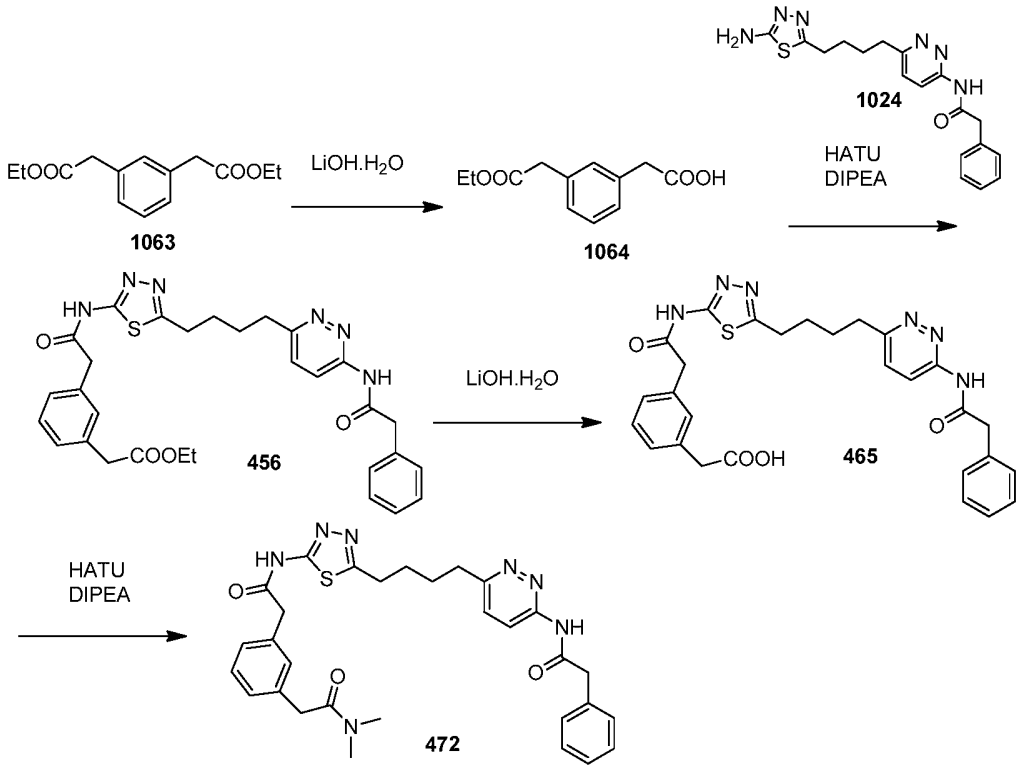
413

Compound **413** was prepared according to the procedure above for the preparation of compound **315**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.68 (bs, 1H), 11.26 (s, 1H), 8.20

(d, J = 9.46 Hz, 1H), 7.58–7.26 (m, 10H), 3.90 (s, 2H), 3.78 (s, 2H), 3.02 (bs, 2H), 2.90 (bs, 2H), 1.74 (bs, 4H).



Compound **415** was prepared according to the procedure above for the preparation of compound **315**.: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.48 (s, 1H), 11.26 (s, 1H), 8.20 (d, J = 8.95 Hz, 1H), 7.75 (s, 1H), 7.58–7.26 (m, 9H), 6.52 (m, 1H), 5.35 (m, 1H), 3.78 (s, 2H), 3.02 (m, 2H), 2.90 (m, 2H), 1.74 (bs, 4H).

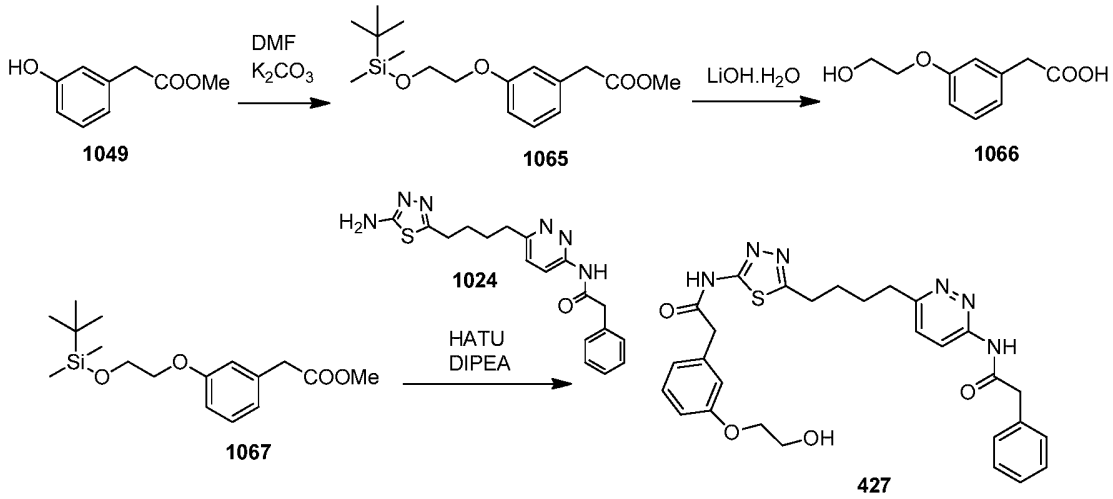


To a solution of **1063** (6.31g, 24.9mmol) in ethanol was added lithium hydroxide monohydrate (1.048g, 24.9mmol) and the resulting reaction mixture was stirred at room temperature for 3hr before it was concentrated under the reduced pressure. The residue obtained was diluted with water and was acidified with 6N HCl. The solution was extracted with ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/hexane to afford **1064** (3g, 53% yield).

To a suspension of carboxylic acid **1064** (0.1g, 0.44mmol) in DMF (2mL) was added HATU (0.17g, 0.44mmol) and stirred till reaction mixture is clear followed by the addition of an amine **1024** (0.15g, 0.4mmol) and DIPEA (0.14mL, 0.8mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by the addition of water. The solid separated was filtered, washed with water and dried to afford **456** (0.2, 86%yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.18 (t, 3H) 1.74 (brs, 4H) 2.88-2.90 (m,2H) 3.01-3.04 (m, 2H) 3.66 (s, 2H) 3.78 (s, 4H) 4.05-4.12 (q, 2H) 7.19-7.36 (m, 9H) 7.55-7.58 (m, 1H) 8.18-8.21 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).

To a solution of **456** (0.205g, 0.358mmol) in Dioxane/Water (20mL/ 6mL) was added lithium hydroxide monohydrate (0.06g, 1.42mmol). The resulting mixture was stirred at room temperature for 3hr before it was acidified with acetic acid. The solution was concentrated under reduced pressure and the residue obtained was diluted with water. The solid separated was filtered, washed with water and dried at high vacuum overnight. The residue obtained was purified by silica gel chromatography eluting with MeOH/dichloromethane to afford **465** (0.15g, 77% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.74 (brs, 4H) 2.90 (brs, 2H) 3.01 (brs, 2H) 3.5 (s, 2H) 3.78 (s, 4H) 7.19-7.36 (m, 9H) 7.55-7.58 (m, 1H) 8.18-8.21 (d, 1H) 11.26 (s, 1H) 12.32 (brs, 1H) 12.65 (s, 1H).

To a suspension of carboxylic acid **465** (25mg, 0.046mmol) in DMF (1mL) was added HATU (19.2mg, 0.05mmol) and stirred till reaction mixture is clear followed by the addition of an N,N-dimethylamine (2M/THF, 30uL, 0.05mmol) and DIPEA (16uL, 0.092mmol). The resulting mixture was stirred at room temperature for 3hr before it was quenched by the addition of water. The solid separated was filtered, washed with water and dried to afford **472** (19mg, 73%yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.74 (brs, 4H) 2.83-2.90 (brs, 6H) 3.01 (brs, 4H) 3.68 (s, 2H) 3.78 (s, 4H) 7.14-7.36 (m, 9H) 7.55-7.58 (d, 1H) 8.18-8.21 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).

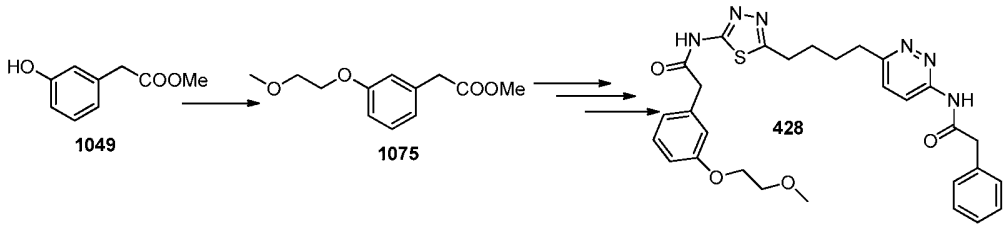


To a solution of **1049** (1g, 6mmol) in DMF (20mL) was added potassium carbonate (1.662g, 12mmol) and (2.16g, 9mmol). The resulting mixture was stirred at 70 ^{0}C overnight before it was diluted with water (~100mL). The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1065** (1.78g, 91% yield) as an oil. ^{1}H NMR (300MHz, Chloroform-d) δ ppm 0.13 (s, 6H) 0.95 (s, 9H) 3.63 (s, 2H) 3.73 (s, 2H) 3.99-4.06 (m, 4H) 6.87 (m, 3H) 7.3 (m, 1H).

To a solution of **1065** (1.78g, 5.5mmol) in THF/MeOH/Water (30mL, 3mL, 3mL) was added lithium hydroxide monohydrate (0.46g, 10.9mmol). The resulting mixture was stirred at room temperature overnight before it was concentrated under reduced pressure. The residue obtained was diluted with water (~20mL) and the resulting solution was acidified with 6N hydrochloric acid. The solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1065** and **1066**. ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 3.54 (s, 2H) 3.72 (brs, 2H) 3.96-3.98 (brs, 2H) 4.85 (brs, 1H) 6.82-6.85 (m, 3H) 7.0-7.22 (m, 1H) 12.3 (brs, 1H).

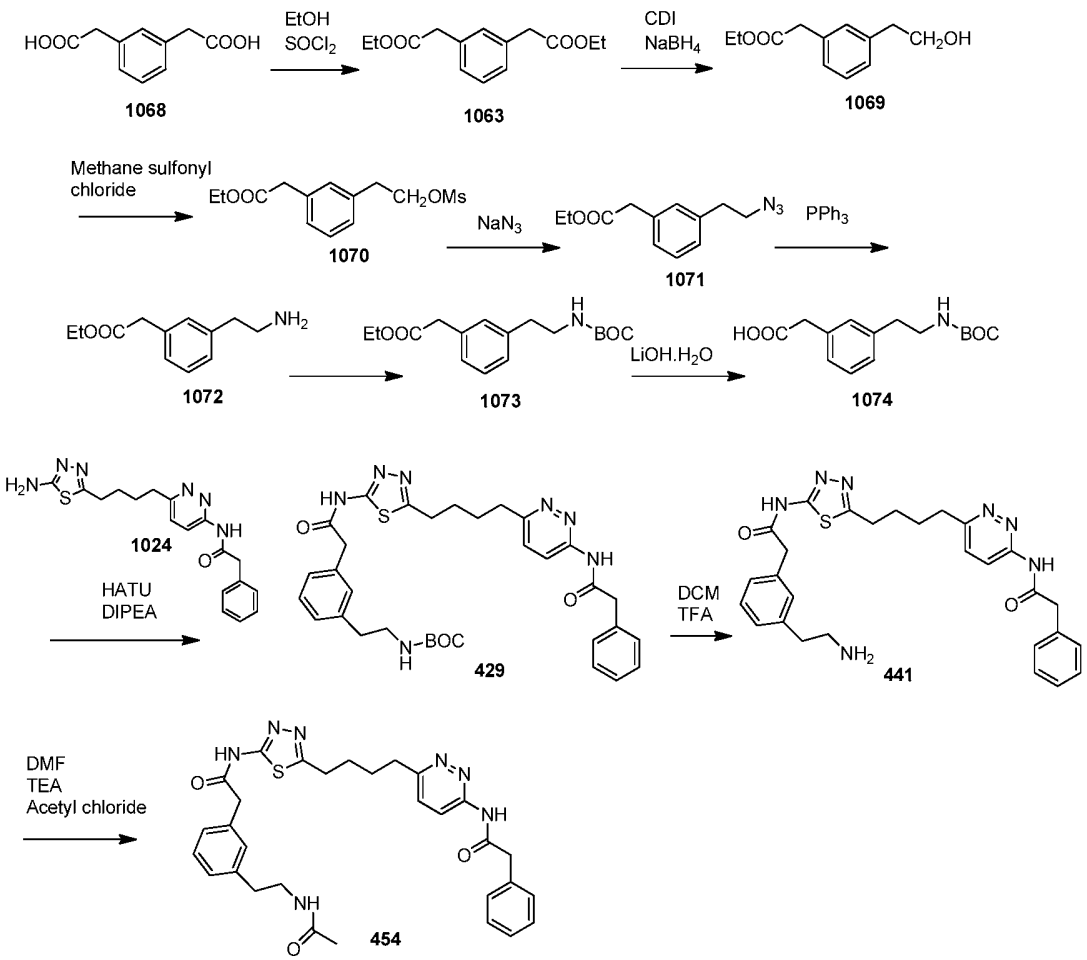
To a suspension of carboxylic acid **1065** (27mg, 0.137mmol) in DMF (2mL) was added HATU (52.2mg, 0.137mmol) and stirred till reaction mixture is clear followed

by the addition of an amine **1024** (46mg, 0.125mmol) and DIPEA (44ul, 0.25mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by the addition of water. The solid separated was filtered, washed with water and dried. The solid obtained was purified by prep HPLC to afford **427** (16mg, 23%yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.75 (brs, 4H) 2.90 (brs, 2H) 3.02 (brs, 2H) 3.71-3.78 (m, 6H) 3.98-3.99 (brs, 2H) 4.84-4.87 (brs, 1H) 6.83-6.92 (m,3H) 7.21-7.36 (m, 6H) 7.54-7.58 (d, 1H) 8.2-8.23 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).



To a solution of 1049 (1g, 6mmol) in acetone (50mL) was added cesium carbonate (2.545g, 7.83mmol), 2- bromoethyl methyl ether(0.92g, 6.62mmol) and sodium iodide(10mg). The resulting mixture was stirred at 50 ^{0}C overnight before it was filtered. The filtrate was evaporated and the residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1075** (0.97g, 72% yield) as oil. ^{1}H NMR (300MHz, Chloroform-d) δ ppm 3.48 (s, 3H) 3.63 (s, 2H) 3.72(brs, 2H) 4.14-4.15 (t, 2H) 6.86-6.9 (m, 3H) 7.26-7.29 (m, 1H).

The remainder of the preparation for compound **428** followed the procedure above for compound **427. 428:** ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.75 (brs, 4H) 2.90 (brs, 2H) 3.02 (brs, 2H) 3.32 (s, 3H) 3.66 (brs,2H) 3.78 (brs, 4H) 4.08 (brs, 2H) 6.88-6.92 (m,3H) 7.25-7.27 (m, 6H) 7.54-7.58 (d, 1H) 8.2-8.23 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).



To an ice cold solution of **1068** (6g, 30.9mmoL) in ethanol (50mL) was added thionyl chloride (2mL) and the resulting reaction mixture was stirred at room temperature overnight before it was concentrated under the reduced pressure. The residue obtained was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated to afford **1063** (6gm).

To a stirred solution of **1063** (3.35g, 13.4mmol) in THF (50mL) was added CDI (2.44g, 15mmol)and the resulting mixture was stirred for 2hr followed by the addition of water (13mL). The reaction mixture was cooled to 0 ^{0}C and sodium borohydride (2.87g, 76mmol) was added portionwise. The stirring was continued at room temperature for 3hr before it was diluted with ethyl acetate and acidifed with 6N HCl. The organic layer was separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1069** (0.563g, 20% yield) as an oil. ^{1}H NMR (300MHz,

Chloroform-d) δ ppm 1.27-1.31 (q, 3H) 2.87-2.92 (d, 2H) 3.63 (s, 2H) 3.87-3.92 (t, 2H) 4.18-4.2 (q, 2H) 7.19-7.31 (m, 4H).

To an ice cold solution of **1069** (0.563g, 2.7mmol) in dichloromethane (40mL) and triethylamine (0.47mL, 3.3mmol) was added methane sulfonylchloride (0.23mL, 3.3mmol) and the resulting mixture was stirred at 0 ^{0}C for 2hr and at room temperature for 1hr before it was diluted with saturated aqueous sodium bicarbonate solution. The solution was extracted with ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated to afford **1070** (0.78g, 100%yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 1.27-1.31 (q, 3H) 2.87 (s, 3H) 3.08 (t, 2H) 3.63 (s, 2H) 4.18-4.2 (t, 2H) 4.45 (q, 2H) 7.19-7.31 (m, 4H).

To a solution of **1070** (0.787g, 2.7mmol) in DMF (6mL) was added sodium azide (0.358g, 5.5mmol) and the resulting reaction mixture was stirred at 60 ^{0}C for 3hr before it was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1071** (0.5g, 78% yield) as an oil. ^{1}H NMR (300MHz, Chloroform-d) δ ppm 1.27-1.31 (q, 3H) 2.92 (t, 2H) 3.54 (t, 2H) 3.63 (s, 2H) 4.18-4.2 (q, 2H) 7.19-7.29 (m, 4H).

To a solution of **1071** (0.5g, 2.1mmol) in THF (25mL) was added triphenylphosphine (0.787g, 3mmol) and the reaction mixture was stirred at room temperature under argon for overnight before it was diluted with 1mL of water. The reaction was continued at 50 ^{0}C for 1hr before it was concentrated under the reduced pressure. The residue was partitioned between saturated sodium bicarbonate solution and dichloromethane. The organic layer was separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with MeOH/dichloromethane to afford **1072** (0.43g, 100% yield) as an oil. ^{1}H NMR (300MHz, Chloroform-d) δ ppm 1.27-1.31 (q, 3H) 2.75-2.79 (t, 2H) 2.98-3.02 (t, 2H) 3.63 (s, 2H) 4.18-4.2 (q, 2H) 7.13-7.29 (m, 4H).

To a solution of **1072** (0.427g, 2mmol) in dichloromethane (30mL) was added di-tert-butyl dicarbonate (0.447g, 2mmol) and the reaction mixture was stirred at room

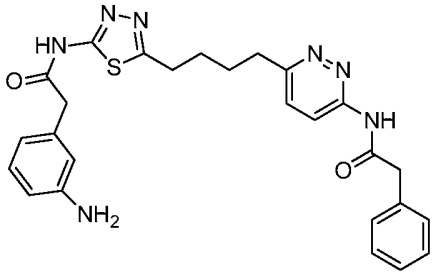
temperature for 5hr before it was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1073** (0.577g, 91% yield) as an oil. ^{1}H NMR (300MHz, Chloroform-d) δ ppm 1.27-1.31 (q, 3H) 1.59 (s, 9H) 2.82 (t, 2H) 3.4 (m, 2H) 3.63 (s, 2H) 4.18 (q, 2H) 7.13-7.29 (m, 4H).

To a solution of **1073** (0.577g, 1.8mmol) in Dioxane/Water (10mL/ 3mL) was added lithium hydroxide monohydrate (0.158g, 3.6mmol). The resulting mixture was stirred at room temperature overnight before it was concentrated under reduced pressure. The residue obtained was diluted with water (~20mL) and the resulting solution was acidified with 1N hydrochloric acid. The solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated to afford **1074** (0.35g, 67%yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 2.82 (m, 2H) 3.4 (m, 2H) 3.63 (s, 2H) 4.6 (brs, 1H) 7.13-7.29 (m, 4H).

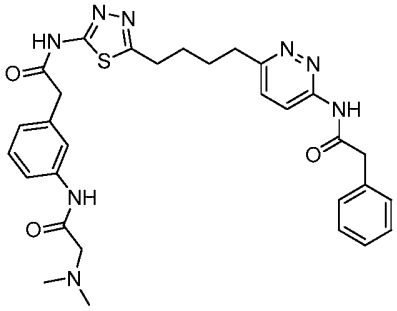
To a suspension of carboxylic acid **1074** (43.8mg, 0.157mmol) in DMF (2mL) was added HATU (61.3mg, 0.161mmol) and stirred till reaction mixture is clear followed by the addition of an amine **1024** (52.5mg, 0.142mmol) and DIPEA (50ul, 0.287mmol). The resulting mixture was stirred at room temperature overnight before it was quenched by the addition of water. The solid separated was filtered, washed with water and dried to afford **429** (60mg, 67%yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.37-1.38 (s, 9H) 1.74 (brs, 4H) 2.69-2.71 (m,2H) 2.87-2.88 (m, 2H) 2.9-3.15 (m, 4H) 3.78 (s, 4H) 7.09 (brs, 1H) 7.12-7.36 (m, 9H) 7.54-7.57 (d, 1H) 8.18-8.21 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).

To a suspension of **429** (50mg, 79.5mmol) in dichloromethane (5mL) was added TFA (1mL) and the reaction mixture was stirred at room temperature for overnight before it was concentrated under the reduced pressure. The residue obtained was triturated with ether. The solid separated was filtered, washed with ether and dried at high vacuum overnight to afford **441** (45mg, 88%yield) as a TFA salt. ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.74 (brs, 4H) 2.86-3.02 (m, 8H) 3.78-3.80 (s, 4H) 7.12-7.36 (m, 8H) 7.58 (d, 1H) 7.78 (brs, 3H) 8.18-8.21 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).

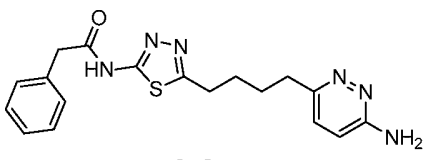
To an ice cold solution of **441** (23mg, 0.035mmol) in DMF (1mL) was added triethylamine (11uL, 0.079mmol) and acetyl chloride (2.8uL, 0.038mmol). The resulting mixture was stirred at room temperature for 2hr before it was diluted with water. The solid separated was filtered, washed with water and dried at high vacuum overnight to afford **454** (10mg, 50% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.75-1.79 (m, 7H) 2.67-2.70 (m, 2H) 2.9 (brs, 2H) 3.00-3.02 (m, 2H) 3.21-3.26 (m, 2H) 3.78 (s, 4H) 7.12-7.36 (m, 9H) 7.58 (d, 1H) 7.9 (brs, 1H) 8.18-8.21 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).



Compound **409** was prepared via TFA deprotection of compound **399** according to the procedure above for the preparation of compound **441**. ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.75 (brs, 4H) 2.90 (brs, 2H) 3.02 (brs, 2H) 3.78 (brs, 4H) 6.89-6.98 (m,4H) 7.25-7.36 (m, 7H) 7.51-7.58 (d, 1H) 8.2-8.23 (d, 1H) 9.34 (s, 1H) 11.26 (s, 1H) 12.65 (brs, 1H).

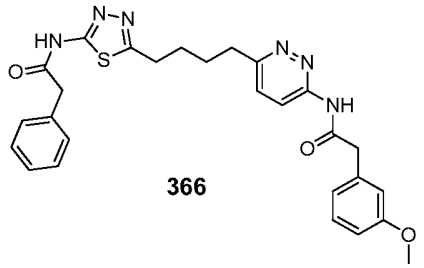


Compound **457** was prepared by acylation of **409** according to the amide coupling procedure above for the preparation of compound **39**. ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.74 (brs, 4H) 2.32 (s, 6H) 2.89 (m, 2H) 3.02 (m, 2H) 3.13 (s, 2H) 3.78 (s, 4H) 7.01-7.04 (m, 1H) 7.25-7.38 (m, 6H) 7.54-7.58 (m, 3H) 8.18-8.21 (d, 1H) 9.77 (s, 1H) 11.26 (s, 1H) 12.65 (brs, 1H)



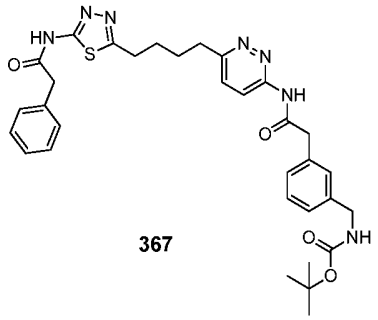
348

To a suspension of **295** (30 mg, 0.0617 mmol) in MeOH (2 ml) at 0 ^{0}C was added 2N NaOH (2 ml) solution. The resulting mixture was stirred at room temperature overnight. The solvent was evaporated under vacuo and the mixture was acidified with 1N HCl to pH 6. The white precipitate was collected by suction filtration, rinsed with more water and dried to afford **348**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.32-7.24 (m, 5H), 7.15-7.12 (d, J = 9.57 Hz, 1H), 6.72-6.69 (d, J = 9.15 Hz, 1H), 6.09 (s, 2H), 3.77 (s, 2H), 2.99-2.96 (bs, 2H), 2.76-2.70 (bs, 2H), 1.70 (bs, 4H).



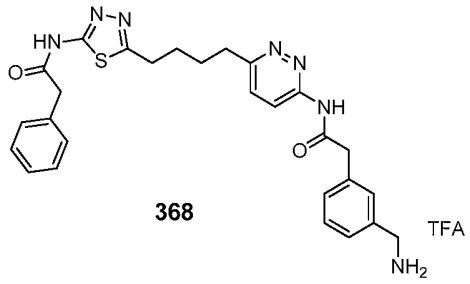
366

366: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 8.82 Hz, 1H), 7.58-7.54 (d, J = 9.32 Hz, 1H), 7.33-7.25 (m, 6H), 6.95-6.82 (m, 3H), 3.81 (s. 3H), 3.75 (s, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



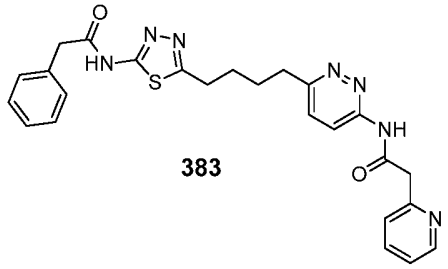
367

367: A flask was charged with **348** (100 mg, 0.27 mmol), Boc-3-aminomethyl-phenylacetic acid (86 mg, 0.325 mmol) in DMF (2 ml) at 0 ^{0}C was added HOBT (88 mg, 0.65 mmol) followed by EDCI (156 mg, 0.812 mmol). The resulting mixture was stirred at 0 ^{0}C for 5 minutes then warmed up to room temperature overnight before it was quenched by addition of water (~10 mL) at 0 ^{0}C. The white precipitate was collected by suction filtration, rinsed with more water. The crude material was purified by silica gel chromatography eluting with 0–6% MeOH in CH_2Cl_2 to afford **367**.



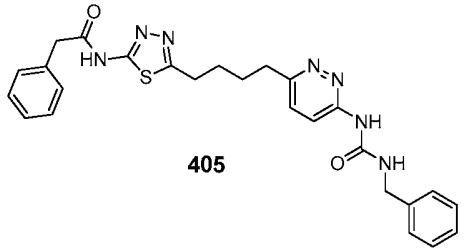
368

Compound **368** was prepared via the deprotection of compound 367 according to the procedure above for compound **341**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 8.22-8.16 (m, 3H), 7.58-7.54 (d, J = 9.27 Hz, 1H), 7.40-7.28 (m, 9H), 4.04 (s, 2H), 3.81 (s. 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



383

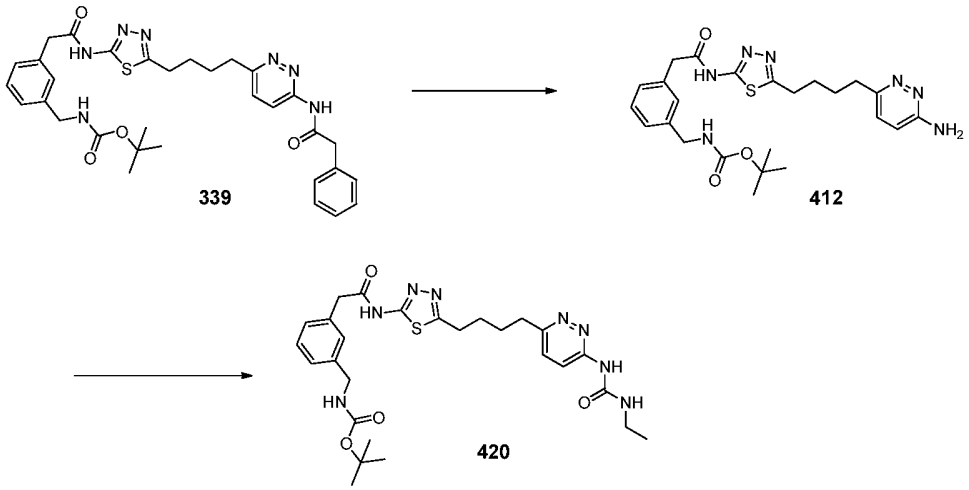
Compound **383** was prepared from compound **348** according to the procedure above for the preparation of compound **354**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.26 (s, 1H), 8.51 (s, 1H), 8.22-8.19 (d, J = 9.09 Hz, 1H), 7.81-7.76 (m, 1H), 7.58-7.54 (d, J = 9.12 Hz, 1H), 7.42-7.26 (m, 7H), 4.0 (s, 2H), 3.81 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



405

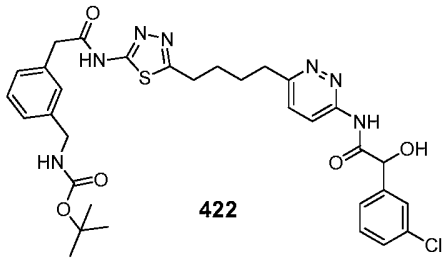
To a solution of **348** (56.5 mg, 0.153 mmol) in DMF (1 ml) at 0 ^{0}C was added triethylamine (43 ul, 0.306 mmol) drop wise followed by benzyl isocyanate (23 ul, 0.184 mmol) drop wise. The resulting mixture was slowly warmed up to room temperature and stirred for 6 h before it was quenched by addition of water (~5 mL) at 0 ^{0}C. The white precipitate was collected by suction filtration, rinsed with more water and ether and dichloromethane then dried to afford **405**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 9.57 (s, 1H), 8.25 (bs, 1H), 7.74-7.71 (d, J = 8.61 Hz, 1H), 7.50-

7.47 (d, *J* = 9.42 Hz, 1H), 7.34-7.27 (m, 10H), 4.42-4.40 (d, J = 5.46 Hz, 2H), 3.80 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



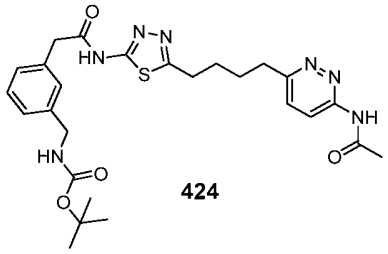
To a suspension of **339** (1 g, 1.62 mmol) in MeOH (10 ml) at 0 ^{0}C was added 2N NaOH (10 ml) solution. The resulting mixture was stirred at room temperature overnight. The solvent was evaporated under vacuo and the mixture was acidified with 6N HCl to pH 6 at 0 ^{0}C. The mixture was triturated with EtOAc and the white precipitate was collected by suction filtration, rinsed with more EtOAc and dried to afford **412**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66 (s, 1H), 7.29–7.22 (m, 2H), 7.19–7.13 (m, 4H), 6.72 (d, *J* = 8.86 Hz, 1H), 6.12 (bs, 2H), 4.12 (d, *J* = 6.09 Hz, 2H), 3.79 (s, 2H), 3.01 (m, 2H), 2.71 (m, 2H), 1.70 (bs, 4H), 1.39 (s, 9H).

To a solution of **412** (60 mg, 0.121 mmol) in DMF (1 ml) at 0 ^{0}C was added triethylamine (34 ul, 0.242 mmol) drop wise followed by ethyl isocyanate (11 ul, 0.145 mmol) drop wise. The resulting mixture was slowly warmed up to room temperature and stirred for 6 h before it was quenched by addition of water (~5 mL) at 0 ^{0}C. The white precipitate was collected by suction filtration. The crude material was purified by silica gel chromatography eluting with 0–6% MeOH in CH_2Cl_2 to afford **420**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.27 (s, 1H), 9.42 (s, 1H), 8.22-8.19 (d, *J* = 8.61 Hz, 1H), 7.77-7.13 (m, 5H), 6.56-6.53 (bs, 1H), 4.12-4.11 (d, 2H), 3.78 (s, 2H), 3.23-3.16 (m, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H), 1.38 (s, 9H), 1.10-1.07 (t, 3H).



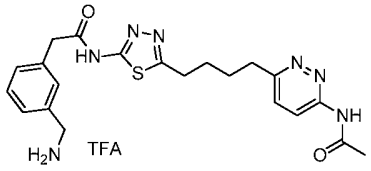
422

422: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 10.74 (s, 1H), 8.18-8.15 (d, J = 9.51 Hz, 1H), 7.61-7.12 (m, 9H), 6.62 (s, 1H), 5.33 (s, 1H), 4.13-4.11 (d, J = 5.58 Hz, 2H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H), 1.38 (s, 9H).



424

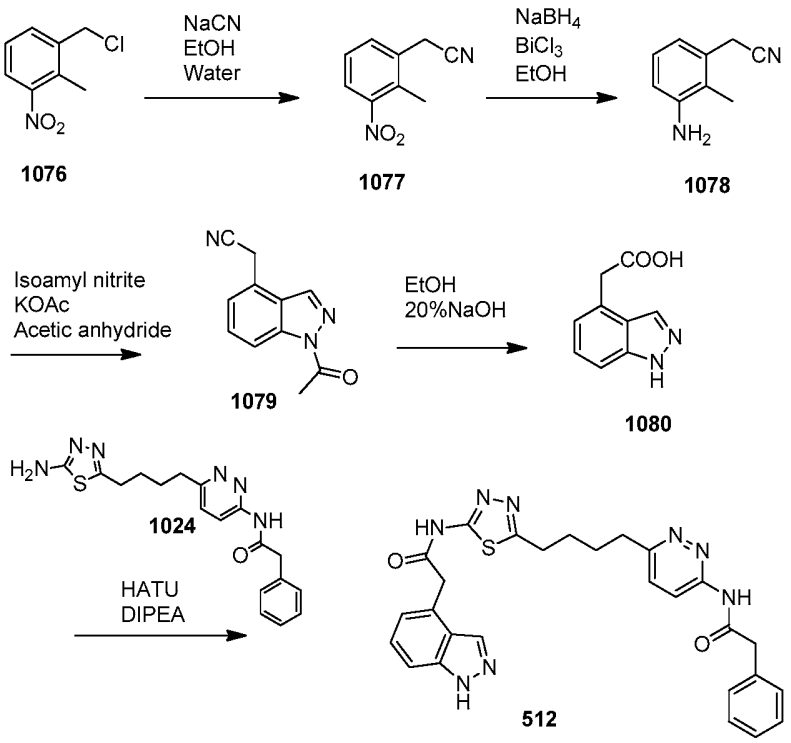
To a solution of **412** (40 mg, 0.0804 mmol) in DMF (1 ml) at 0 ^{0}C was added triethylamine (17 ul, 0.121 mmol) drop wise followed by acetic anhydride (8 ul, 0.0844 mmol) drop wise. The resulting mixture was slowly warmed up to room temperature and stirred overnight before it was quenched by addition of water (~5 mL) at 0 ^{0}C. The mixture was partitioned between water and EtOAc. The organic extract was washed with water, dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in CH_2Cl_2 to afford **424**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (s, 1H), 11.01 (s, 1H), 8.23-8.20 (d, J = 8.61 Hz, 1H), 7.57-7.55 (d, J = 8.16 Hz, 1H), 7.38-7.12 (m, 4H), 4.13-4.11 (d, J = 5.76 Hz, 2H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.14 (s, 3H), 1.75 (bs, 4H), 1.39 (s, 9H).



425

To a suspension of **424** (10 mg, 0.018 mmol) in dichloromethane (1 ml) was added TFA (1 ml) at 0 ^{0}C. The resulting mixture was stirred at room temperature for 1 h before it was evaporated under vacuo to dryness. Ether was added and the white precipitate was collected by suction filtration, rinsed with more ether and dried to afford **425**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.70 (s, 1H), 11.0 (s, 1H), 8.22-8.19

(d, J = 8.82 Hz, 1H), 8.16-8.08 (bs, 2H), 7.58-7.54 (d, J = 9.42 Hz, 1H), 7.39-7.30 (m, 4H), 4.06-4.03 (m, 2H), 3.84 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.14 (s, 3H), 1.75 (bs, 4H).



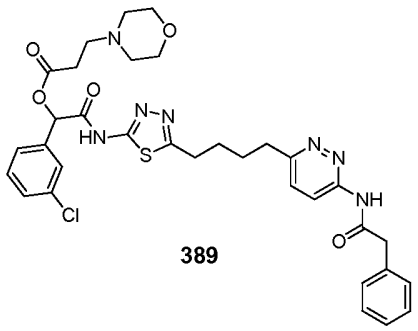
To a solution of **1076**(1.8g, 10mmmol) in ethanol/water (40mL/20mL) was added sodium cyanide (0.98g, 20mmol). The resulting mixture was stirred at 90 ^{0}C for 4hr before it was cooled to 0 ^{0}C. Solid separated was filtered, washed with water and dried at high vacuum overnight to afford **1077**(1.5g, 85% yield).

To an ice cold solution of **1077**(1g, 5.68mmmol) in ethanol (50mL) was added sodium borohydride (0.86g, 22.72mmol) followed by the addition of bismuth chloride (2g, 6.248mmol) portionwise. The resulting mixture was stirred at room temperature for 3hr before it was filtered through the celite pad. Filtrate was concentrated and the residue obtained was partitioned between aq sodium bicarbonate solution and ethyl acetate. The organic extract was separated, dried over sodium sulfate, filtered and evaporated to afford **1078** (0.82g, 100% yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 2.17(s, 3H) 3.69-3.71 (brs, 4H) 6.71-6.74 (d, 1H) 6.80-6.83(d, 1H) 7.04-7.09 (m, 1H).

To a solution of **1078** (0.3g, 2mmmol) in toluene (10mL) was added potassium acetate (0.2g, 2.04mmol) and acetic anhydride (0.55mL, 5.83mmol). The resulting mixture was stirred at 80 ^{0}C for 1hr followed by the addition of isoamyl nitrite (0.4mL, 3mmol). Stirring was continued at 80 ^{0}C overnight before it was cooled to room temperature. The solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1079** (0.22g, 54% yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 2.85(s, 3H) 4.09 (s, 2H) 7.39-7.41 (d, 1H) 7.58-7.63(m, 1H) 8.28 (s, 1H) 8.48-8.51(d, 1H)

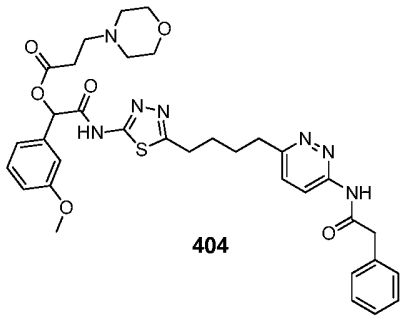
To a solution of **1079** (0.44g, 2.21mmmol) in ethanol (5mL) was added 20% aqueous sodium hydroxide (5mL). The resulting mixture was stirred at 90 ^{o} overnight before it was concentrated. The residue obtained was diluted with water, acidified with acetic acid and extracted with ethyl acetate. The organic extract was separated, dried over sodium sulfate, filtered and evaporated to afford **1080** (0.1g, 51% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 3.89 (s, 2H) 6.98-7.0 (d, 1H) 7.27-7.32(m, 1H) 7.43-7.46 (d, 1H) 8.10(s, 1H) 12.3-13.2(broad doublet, 2H)

To a suspension of carboxylic acid **1080** (60mg, 0.34mmol) in DMF (2mL) was added HATU (130mg, 0.34mmol) and stirred till reaction mixture is clear followed by the addition of an amine **1024** (114mg, 0.31mmol) and DIPEA (108uL, 0.62mmol). The resulting mixture was stirred at room temperature for 3hr before it was quenched by the addition of water. The solid separated was filtered, washed with water and dried. The residue obtained was purified by silica gel chromatography eluting with MeOH/dichloromethane to afford **512** (14mg, 9%yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.74 (brs, 4H) 2.89 (brs, 2H) 2.91 (brs, 2H) 3.78 (s, 2H) 4.13 (s, 2H) 7.05-7.08 (m, 1H) 7.27-7.57 (m, 8H) 8.19 (d, 2H) 11.26 (s, 1H) 12.76-12.80 (brs, 1H) 13.11 (s, 1H).



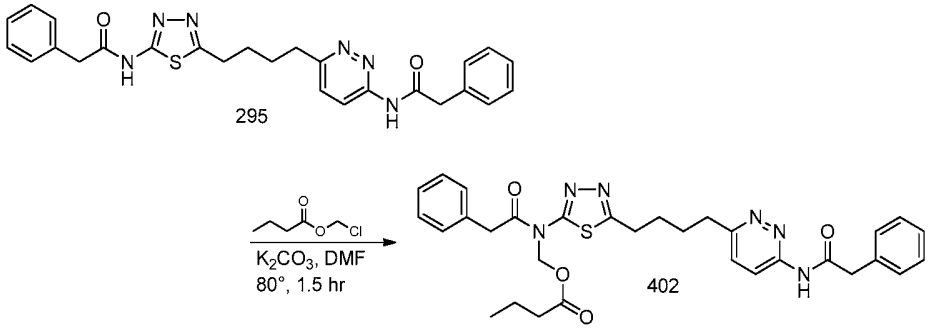
389

Compound **389** was prepared according to the procedure above for the preparation of compound **334**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.95 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 8.91 Hz, 1H), 7.61-7.26 (m, 10H), 6.17 (s, 1H), 3.78 (s, 2H), 3.54 (bs, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.67-2.62 (m, 4H), 2.38 (bs, 4H), 1.73 (bs, 4H).



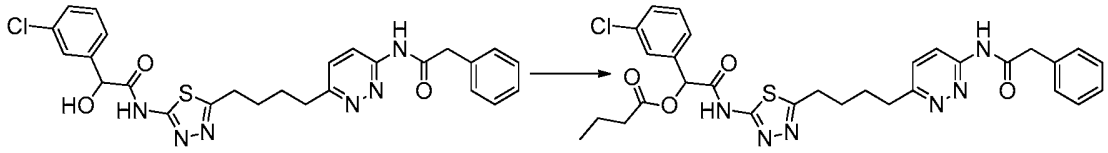
404

Compound **404** was prepared according to the procedure above for the preparation of compound **334**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.95 (s, 1H), 11.26 (s, 1H), 8.22-8.19 (d, J = 9.60 Hz, 1H), 7.58-7.54 (d, J = 9.03 Hz, 1H),7.39-7.26 (m, 6H), 7.12 (s, 2H), 7.01-6.98 (m, 1H), 6.10 (s, 1H), 3.78 (s, 5H), 3.54 (bs, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.64 (bs, 4H), 2.38 (bs, 4H), 1.74 (bs, 4H).



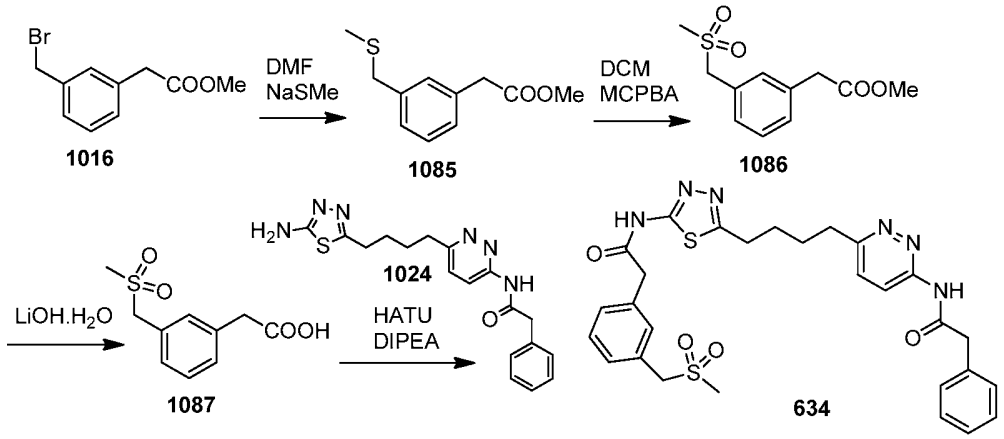
To a flask was added K_2CO_3 (0.28 g, 2.06 mmol), compound **295** (0.5 g, 1.03 mmol) followed by 25 mL of DMF. The mixture was stirred for 15 minutes and chloromethyl butyrate (0.17 g, 1.23 mmol) was added and the reaction placed under an atmosphere of argon. The mixture was heated to 80°C for 1.5 hours, allowed to cool to room temperature and poured into 200 ml water. The mixture was transferred to a

separatory funnel, extracted with EtOAc (3x100 mL), the organic layers separated and washed with water (3x50 mL), brine (2x50 ml) and dried over Na_2SO_4. The Na_2SO_4 was removed by filtration and the volatiles removed under reduced pressure. The crude material was purified by reverse-phase chromatography giving 0.15 g of compound **402**.



318 **439**

To a solution of **318** (100 mg, 0.19 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added pyridine (300 μL) and followed by addition of a solution of butyryl chloride (43 mL, 0.41 mmol) in CH_2Cl_2 (5 mL) dropwise. The resulting mixture was stirred at 0 °C for 1 h before it was partitioned between EtOAc and H_2O. The organic layer was separated, dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography over silica gel eluting with 1–10% MeOH in CH_2Cl_2 to provide the desired product **439** (117 mg). ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.01 (bs, 1H), 10.12 (s, 1H), 8.49 (d, J = 9.64 Hz, 1H), 7.77 (s, 1H), 7.57 (d, J = 7.11 Hz, 1H), 7.40–7.30 (m, 8H), 6.57 (s, 1H), 3.97 (s, 2H), 3.09 (bs, 2H), 3.00 (bs, 2H), 2.48 (m, 2H), 1.91 (bs, 4H), 1.85–1.62 (m, 2H), 0.98 (t, J = 7.07 Hz, 3H).



1016 **1085** **1086**

1024

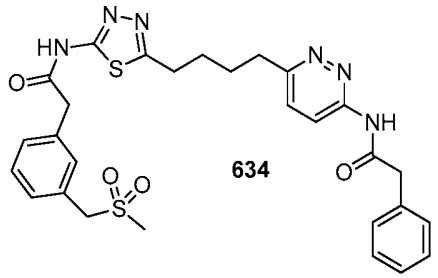
1087 **634**

To a solution of sodium thiomethoxide (0.266g, 3.8mmol) in DMF(10mL) was added a solution of **1016** (0.657g, 2.7mmol) in DMF and the resulting mixture was stirred at room temperature for overnight. The solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1085** (0.41g, 72% yield).

^{1}H NMR (300MHz, Chloroform-d) δ ppm 2.03-2.04(s, 3H) 3.66-3.73(m, 7H) 7.21-7.32(m, 4H).

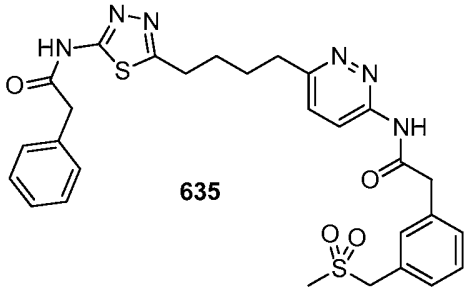
To a solution of **1085** (0.503g, 2.39mmol) in dichloromethane was added MCPBA (1.338g, 7.78mmol) and the resulting mixture was stirred at room temperature for 4hr before it was diluted with aq. Sodium thiosulfate solution. Organic layer was separated, washed with saturated aq. Sodium bicarbonate solution and water, dried over sodium sulfate, filtered and concentrated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1086** (0.5g, 86% yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 2.8(s, 3H) 3.7-3.74(m, 5H) 4.27(s, 2H) 7.30-7.4(m, 4H).

To an ice cold solution of **1086** (0.5g, 2.06mmol) in dioxane (10mL) and water (10mL) was added lithium hydroxide monohydrate (0.26g, 6.19mmol) and the resulting reaction mixture was stirred at room temperature for overnight before it was concentrated. The residue obtained was diluted with water and was acidified with acetic acid. The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was triturated with ether. The solid separated was filtered, washed with ether and dried at high vacuum overnight to afford **1087** (0.3g, 64%yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 2.92(s, 3H) 3.61(s, 2H) 4.48(s, 2H) 7.31-7.35(m, 4H) 12.37(s, 1H).



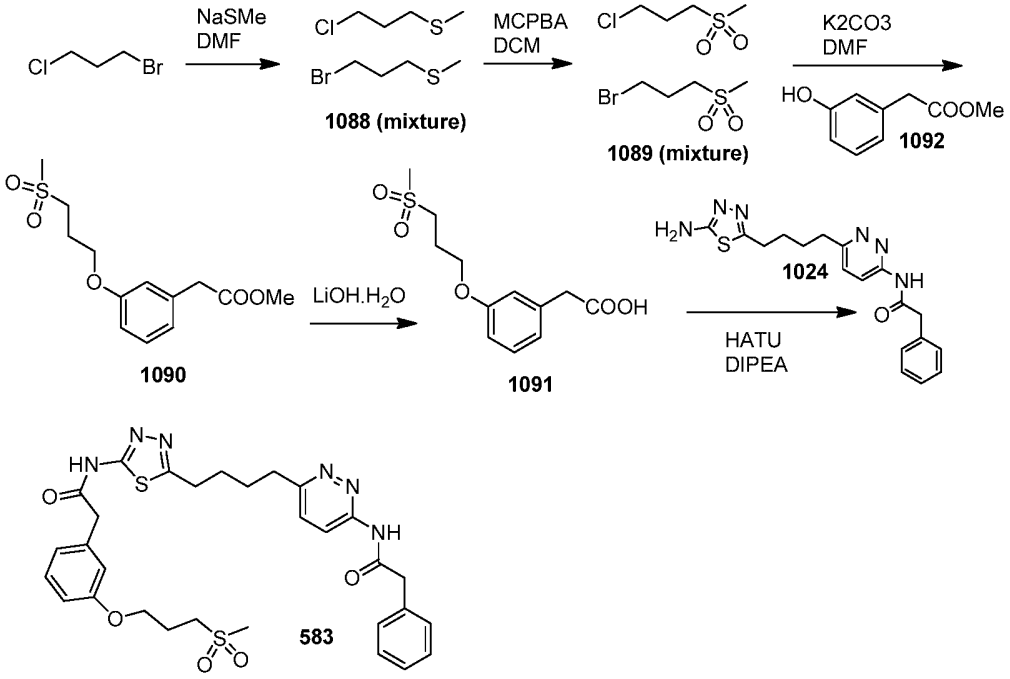
634

Compound **634** was prepared using procedures analogous to those above. ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.74 (brs, 4H) 2.91 (brs, 5H) 3.03(brs, 2H) 3.78 (s, 2H) 3.85 (s, 2H) 4.49 (s, 2H) 7.32-7.40 (m, 9H) 7.55-7.58 (d, 1H) 8.19 (d, 1H) 11.26 (s, 1H) 12.69 (s, 1H).



635

Compound **635** was prepared using procedures analogous to those above. ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.75 (brs, 4H) 2.91 (brs, 5H) 3.03(brs, 2H) 3.82 (s, 4H) 4.49 (s, 2H) 7.32-7.40 (m, 9H) 7.55-7.58 (d, 1H) 8.19 (d, 1H) 11.26 (s, 1H) 12.69 (s, 1H).



583

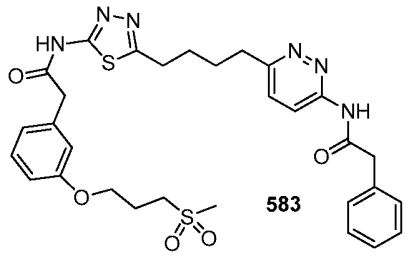
To a solution of 1,3-bromo chloropropane (1.57g, 10mmol) in DMF (10mL) was added sodium thiomethoxide (0.63g, 9mmol) and the resulting reaction mixture was stirred at room temperature overnight and at 70 ^{0}C for another day. The solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated to afford **1088** (1.3gm) which is used for the next step without purification.

To a solution of **1088** (1.3g, 7.7mmol) in dichloromethane (100mL) was added MCPBA(5.15g, 23.34mmol) and the resulting mixture was stirred at room temperature for overnight before it was diluted with aq. Sodium thiosulfate solution.

Organic layer was separated, washed with saturated aq. Sodium bicarbonate solution and water, dried over sodium sulfate, filtered and concentrated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1089** (0.3gm). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 2.38-2.49(m, 2H) 2.99(s, 3H) 3.22-3.27(m, 2H) 3.57-3.77(m, 2H).

To a solution of **1092** (0.525g, 3.16mmol) in DMF (15mL) was added potassium carbonate (0.873g, 6.32mmol), **1089** (0.74g, 4.74mmol) and sodium iodide (10mg). The resulting mixture was stirred at 70 °C overnight before it was diluted with water (~100mL). The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1090** (0.53g, 59% yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 2.35-2.40(m, 2H) 2.99(s, 3H) 3.26-3.31(m, 2H) 3.63(s, 2H) 3.73(s, 3H) 4.16(t, 2H) 6.81-6.93(m, 3H) 7.25(m, 1H).

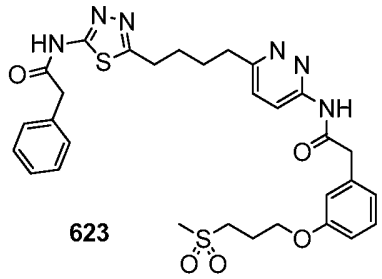
To a solution of **1090** (0.53g, 1.85mmol) in dioxane (8mL) and water (4mL) was added lithium hydroxide monohydrate (0.156g, 3.71mmol) and the resulting reaction mixture was stirred at room temperature for 5hr before it was acidified with acetic acid. The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was triturated with ether. The solid separated was filtered, washed with ether and dried at high vacuum overnight to afford **1091** (0.2g, 40%yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 2.32-2.42(m, 2H) 2.99(s, 3H) 3.26-3.31(m, 2H) 3.66(s, 2H) 4.12-4.16(t, 2H) 6.83-6.94(m, 3H) 7.26-7.31(m, 1H).



583

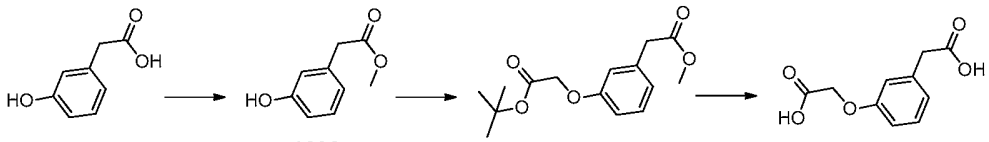
Compound **583** was prepared by coupling of **1091** with **1024** using procedure described for Amide Coupling General Procedure. ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.74 (brs, 4H) 2.15-2.19(m, 2H) 2.90-3.03(m, 7H) 3.27-

3.39 (m, 2H) 3.78(s, 4H) 4.07-4.11 (t, 2H) 6.90-6.93 (m, 3H) 7.24-7.37 (m, 6H) 7.55-7.58(d, 1H) 8.19 (d, 1H) 11.26 (s, 1H) 12.69 (s, 1H).



623

Compound **623** was prepared by coupling of 11 with **348** using procedure described for Amide Coupling General Procedure. ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.74 (brs, 4H) 2.15-2.19(m, 2H) 2.90-3.03(m, 7H) 3.27-3.39 (m, 2H) 3.75-3.78(m, 4H) 4.07-4.11 (t, 2H) 6.90-6.97 (m, 3H) 7.26-7.34 (m, 6H) 7.58(d, 1H) 8.19 (d, 1H) 11.26 (s, 1H) 12.69 (s, 1H).

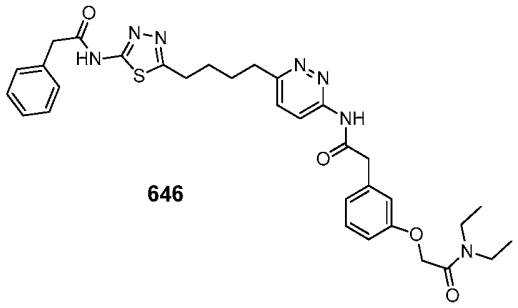


1093 1094 1095

To a solution of 3-hydroxyphenylacetic acid (1 g, 0.00657 mol) in MeOH (10 ml) at 0 ^{0}C was added (Trimethylsilyl) diazomethane solution (2 M in hexanes, 20 ml) dropwise. The resulting mixture was stirred at room temperature for 30 minutes before it was evaporated to dryness. The crude material was purified by silica gel chromatography eluting with 0-25% EtOAc in Hexanes to afford **1093**.

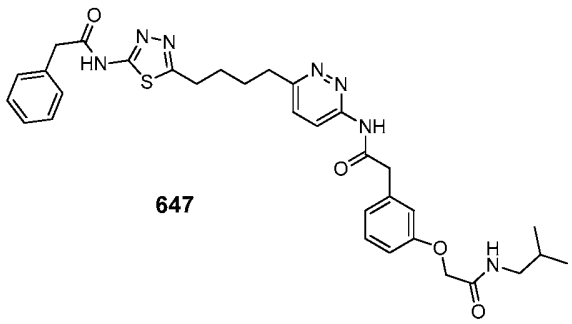
1094 was made using procedure described for compound **1119.**

1095 was made using procedure described for compound **1102.**



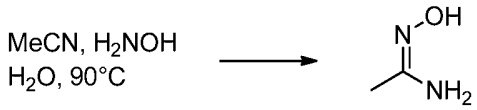
646

646 was made using procedure described for compound **666**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.32 (s, 1H), 8.50-8.47 (d, J = 8.52 Hz, 1H), 7.90-7.70 (m, 1H), 7.40-7.36 (m, 6H), 7.03-6.86 (m, 3H), 4.72 (s, 2H), 4.02 (s, 2H), 3.90 (s, 2H), 3.44-3.39 (m, 4H), 3.09-2.96 (d, 4H), 1.87 (bs, 4H), 1.24-1.16 (m, 6H).



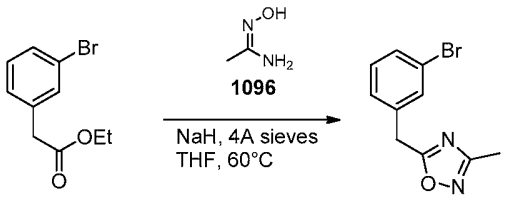
647

647 was made using procedure described for compound **666**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.61 (s, 1H), 11.22 (s, 1H), 8.22-8.19 (d, J = 9.18 Hz, 1H), 8.02-8.10 (t, 1H), 7.58-7.55 (d, J = 9.12 Hz, 1H), 7.36-7.24 (m, 5H), 6.99-6.84 (m, 3H), 4.48 (s, 2H), 3.82 (s, 2H), 3.75 (s, 2H), 3.50 (s, 2H), 3.01-2.90 (m, 5H), 1.73 (bs, 4H), 0.82-0.80 (d, J = 6.69 Hz, 6H).



1096

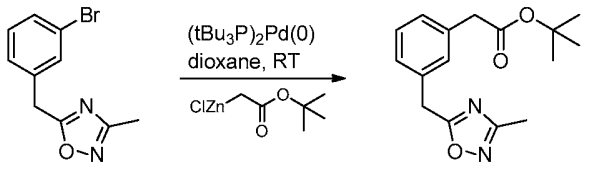
A solution of hydroxylamine (50% in water, 7.4 mL) was added to acetonitrile (60 mL) and the mixture heated to 90°C for 16 hours. The mixture was cooled to room temperature then cooled in a wet-ice bath giving a precipitate. The solids were collected by filtration and rinsed with cold acetonitrile (10 mL) and dried under high vacuum giving 4.47 g of N'-hydroxyacetimidamide **1096**. See Zemolka, S. *et al* PCT Int Appl 2009118174. ^{1}H NMR 300 MHz $CDCl_3$: δ 4.57 (br s, 2H), 1.89 (s, 3H).



1097 **1098**

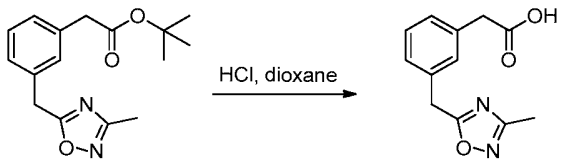
A flask was charged with N'-hydroxyacetimidamide **1096** (0.45 g, 6.17 mmol) followed by THF (25 mL), NaH (60% in oil, 0.246 g, 6.17 mmol), 4A molecular sieves (4.5 g) and the mixture heated to 60°C under an atmosphere of argon for 1 hour. A solution of ethyl 2-(3-bromophenyl)acetate **1097** (1.5 g, 6.17 mmol) in THF (12.5 mL) was added to the N'-hydroxyacetimidamide mixture and heated at 60°C for 16 hours. The mixture was diluted with water (100 mL) and extracted with EtOAc (2 x 25 mL). The organic layers were combined, washed with water (25 mL), brine (2 x

25 mL) and dried over Na_2SO_4. The Na_2SO_4 was removed by filtration and the volatiles removed under reduced pressure. The crude material was purified by normal phase chromatography 0-30% EtOAc / hexanes giving 0.56 g of 5-(3-bromobenzyl)-3-methyl-1,2,4-oxadiazole **1098**. ^{1}H NMR 300 MHz $CDCl_3$: δ 7.48-7.42 (m, 2H), 7.26-7.24 (m, 2H), 4.15 (s, 2H), 2.38 (s, 3H).



1098 **1099**

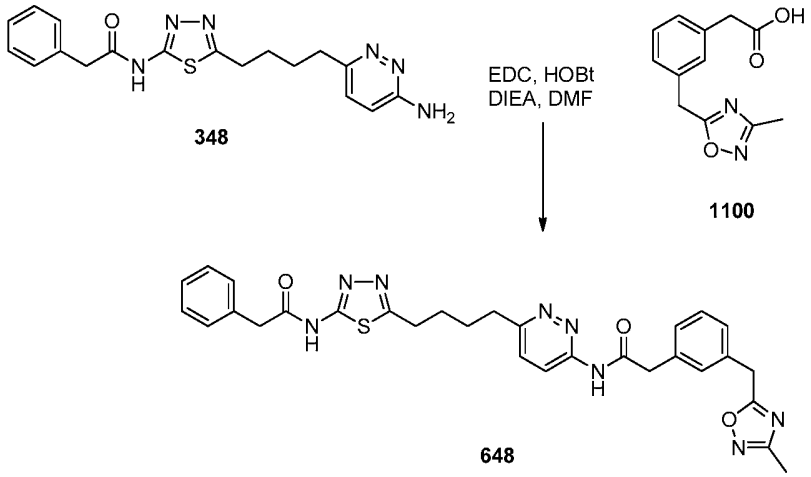
To a solution of 5-(3-bromobenzyl)-3-methyl-1,2,4-oxadiazole **1098** (0.50 g, 1.97 mmol) in dioxane (1 mL), under an atmosphere of Argon, was added Bis(tri-t-butylphosphine)palladium(0) (0.15 g, 0.295 mmol) followed by the addition of 2-tert-butoxy-2-oxoethylzinc chloride (0.5 M in diethyl ether, 4.92 mmol, 9.84 mL). The mixture was allowed to stir under argon for 20 hours and the volatiles were removed under reduced pressure. The residue was taken up in EtOAc (10 mL) and washed with water (2 x 5 mL), brine (2 x 5 mL) and dried over Na_2SO_4. The Na_2SO_4 was removed by filtration and the volatiles removed under reduced pressure. The crude material was purified by normal phase chromatography 0-50% EtOAc / Hexanes to give 0.300 g tert-butyl 2-(3-((3-methyl-1,2,4-oxadiazol-5-yl)methyl)phenyl)acetate **1099**. ^{1}H NMR 300 MHz $CDCl_3$: δ 7.40-7.18 (m, 4H), 4.17 (s, 2H), 3.51 (s, 2H), 2.36 (s, 3H), 1.43 (s, 9H).



1099 **1100**

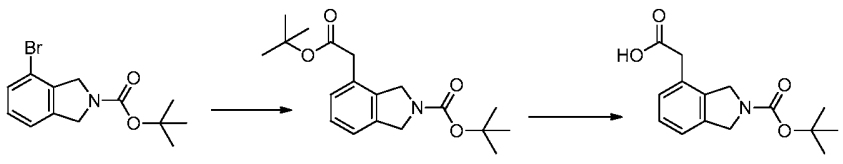
To a mixture of tert-butyl 2-(3-((3-methyl-1,2,4-oxadiazol-5-yl)methyl)phenyl)acetate **1099** (0.127 g, 0.44 mmol) in dioxane (3 mL) was added 4N HCl in dioxane (1 mL) and stirred under an atmosphere of argon for 2 hours. The volatiles were removed under reduced pressure and the residue diluted with water (5 mL) and the pH adjusted to 12 with 2.5 N NaOH. The mixture was washed with dichloromethane (4 x 2 mL) and the pH adjusted to 6 with 1 N HCl. The mixture was extracted with EtOAc (3 x 2

mL) and the organic layers combined, washed with brine and dried over Na_2SO_4. The Na_2SO_4 was removed by filtration and the volatiles removed under reduced pressure to give 0.041 g of 2-(3-((3-methyl-1,2,4-oxadiazol-5-yl)methyl)phenyl)acetic acid **1100**. ^{1}H NMR 300 MHz $CDCl_3$: δ 7.40-7.18 (m, 4H), 4.18 (s, 2H), 3.63 (s, 2H), 2.36 (s, 3H).



348 1100 648

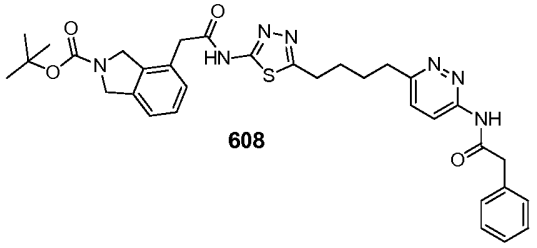
To a solution of N-(5-(4-(6-aminopyridazin-3-yl)butyl)-1,3,4-thiadiazol-2-yl)-2-phenylacetamide **348** (0.061 g, 0.0165 mmol), 2-(3-((3-methyl-1,2,4-oxadiazol-5-yl)methyl)phenyl)acetic acid **1100** (0.040 g, 0.18 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (0.078 g, 0.41 mmol), 1-hydroxybenzotriazole (0.055 g, 0.41 mmol) in DMF (3 mL) was added DIEA (0.085 g, 0.115 mL, 0.66 mmol) and the mixture stirred for 16 hours. The mixture was diluted with water (20 mL) and extracted with EtOAc (3 x 20 mL).The organic layers were combined, washed with water (3 x 20 mL), brine (2 x 20 mL) and dried over Na_2SO_4. The Na_2SO_4 was removed by filtration and the volatiles removed under reduced pressure. The crude material was purified by normal phase chromatography 0-5% MeOH / dichloromethane giving 0.003 g of 2-(3-((3-methyl-1,2,4-oxadiazol-5-yl)methyl)phenyl)-N-(6-(4-(5-(2-phenylacetamido)-1,3,4-thiadiazol-2-yl)butyl)pyridazin-3-yl)acetamide **648**. ^{1}H NMR 300 MHz $CDCl_3$: δ 12.59 (s, 1H), 10.53 (s, 1H), 8.45 (d, 1H, *J*= 12.2 Hz), 7.4-7.1 (m, 10H), 4.15 (s, 2H), 4.03 (s, 2H), 3.94 (s, 2H), 3.02 (m, 2H), 2.94 (m, 2H), 2.33 (s, 3H), 1.85 (m, 4H).



1101 1102

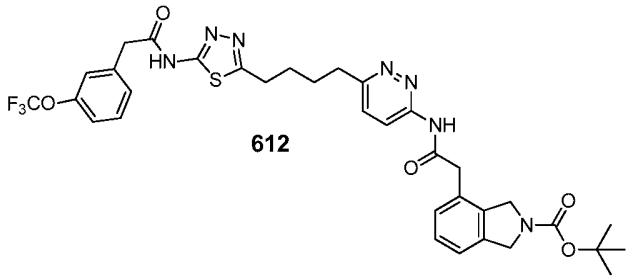
1101 was made using procedure described for compound **1119.**

To a solution of **1101** (470 mg, 1.41 mmol) in MeOH (5 ml) and H_2O (5 ml) at 0 ^{0}C was added lithium hydroxide monohydrate (296 mg, 7.05 mmol). The resulting mixture was stirred at room temperature for 3 days before it was evaporated to dryness. The mixture was then acidified with 1N HCl (pH 4), and it was partitioned between water and EtOAc. The organic extract was washed with water, dried over sodium sulfate, filtered and evaporated to afford **1102**.



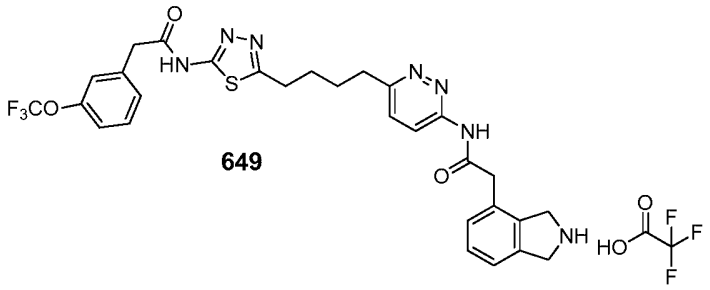
608

608 was made using procedure described for compound **664**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71 (s, 1H), 11.32 (s, 1H), 8.22-8.19 (d, J = 9.15 Hz, 1H), 7.58-7.54 (d, J = 9.27 Hz, 1H), 7.38-7.28 (m, 8H), 4.63 (bs, 4H), 3.82 (s, 2H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H), 1.48-1.44 (d, J = 5.93 Hz, 9H).



612

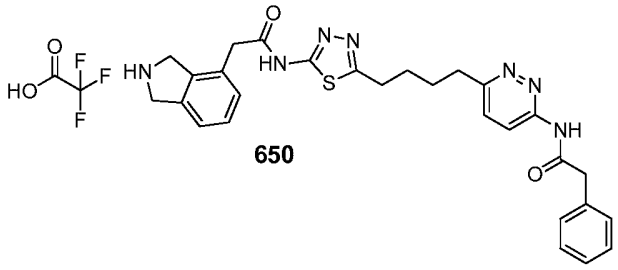
612 was made using procedure described for compound **666**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.32 (s, 1H), 8.22-8.19 (d, J = 9.78 Hz, 1H), 7.58-7.54 (d, J = 9.72 Hz, 1H), 7.48-7.28 (m, 7H), 4.67-4.61 (m, 4H), 3.88 (s, 2H), 3.80 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H), 1.48-1.44 (d, J = 9.93 Hz, 9H).



649

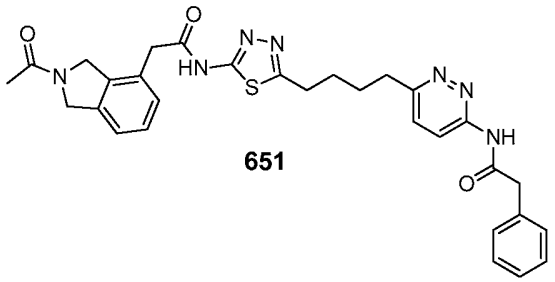
649 was made using procedure described for compound **695.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.36 (s, 1H), 8.20-8.17 (d, J = 9.78 Hz, 1H), 7.60-7.57 (d, J = 8.92 Hz,

1H), 7.52-7.32 (m, 7H), 4.61-4.56 (d, J = 16.99 Hz, 4H), 3.91 (s, 2H), 3.87 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



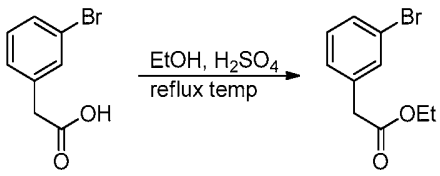
650

650 was made using procedure described for compound **695**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71 (s, 1H), 11.32 (s, 1H), 9.40 (bs, 1H), 8.22-8.19 (d, J = 9.09 Hz, 1H), 7.58-7.54 (d, J = 9.36 Hz, 1H), 7.38-7.28 (m, 8H), 4.63 (bs, 4H), 3.82 (s, 2H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



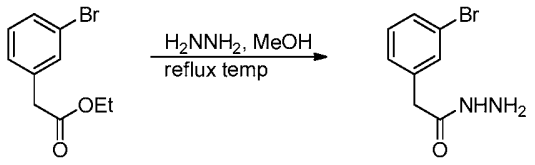
651

To a solution of **650** (30 mg, 0.0468 mmol) in DMF (1 ml) at 0 ^{0}C was added triethylamine (13 ul, 0.0936 mmol) dropwise followed by acetic anhydride (4.64 ul, 0.0491 mmol) dropwise. The resulting mixture was stirred at 0 ^{0}C for 20 minutes before it was quenched by addition of ice water (~5 mL). The white precipitate was collected by suction filtration, rinsed with more water. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in CH_2Cl_2 to afford **651**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71 (s, 1H), 11.32 (s, 1H), 8.22-8.19 (d, J = 9.27 Hz, 1H), 7.58-7.54 (d, J = 9.00 Hz, 1H), 7.38-7.28 (m, 8H), 4.88 (bs, 2H), 4.67 (bs, 2H), 3.82 (s, 2H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.11 (s, 3H), 1.73 (bs, 4H).



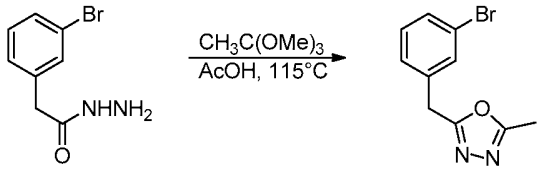
1103 1097

To a solution of 2-(3-bromophenyl)acetic acid **1103** (10.0 g, 46.5 mmol) in 100 mL EtOH was added conc. H_2SO_4 (10 drops) and the mixture heated to relux temperature for 3 hours. The mixture was allowed to cool to room temperature and the volatiles were removed under reduced pressure. The residue was taken up in EtOAc (100 mL) and washed with water (2 x 50 mL), saturated $NaHCO_3$ (1 x 25 mL), brine (2 x 25 mL) and dried over Na_2SO_4. The Na_2SO_4 was removed by filtration and the volatiles removed under reduced pressure to give ethyl 2-(3-bromophenyl)acetate **1097** (11.1 grams) as a liquid). ^{1}H NMR 300 MHz $CDCl_3$: δ 7.41 (m, 2 H), 7.20 (m, 2H), 4.14 (q, 2H, *J*= 9.5 Hz), 3.57 (s, 2H), 1.25 (t, 3H, *J*= 9.5 Hz).



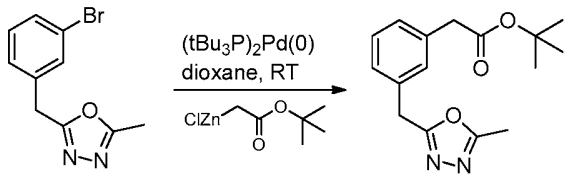
1097 **1104**

To a solution of ethyl 2-(3-bromophenyl)acetate **1097** (1.5 g, 6.17 mmol) in MeOH (20 mL) was added hydrazine (0.79 g, 24.7 mmol) and the mixture heated to reflux temperature for 4 hours. The mixture was allowed to cool to room temperature giving rise to a white precipitate which was collected by filtration and rinsed with MeOH (10 mL). After drying under reduced pressure 1.4 grams of 2-(3-bromophenyl)acetohydrazide **1104** was isolated. ^{1}H NMR 300 MHz $CDCl_3$: δ 7.42 (s, 2H), 7.20 (s, 2H), 6.73 (br s, 1H), 3.51 (s, 2H), 1.81 (br s, 2H).



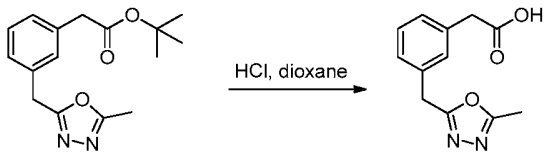
1104 **1105**

To a solution of 2-(3-bromophenyl)acetohydrazide **1104** (1.0 g, 4.37 mmol) in AcOH (10 mL) was added trimethylorthoacetate (2.62 g, 21.83 mmol) and the mixture heated to 115°C for 18 hours. The volatiles were removed under reduced pressure and the residue purified by reverse phase chromatography to give 0.59 g of 2-(3-bromobenzyl)-5-methyl-1,3,4-oxadiazole **1105**. ^{1}H NMR 300 MHz $CDCl_3$: δ 7.45 (m, 2H), 7.23 (m, 2H), 4.12 (s, 2H), 2.49 (s, 3H).



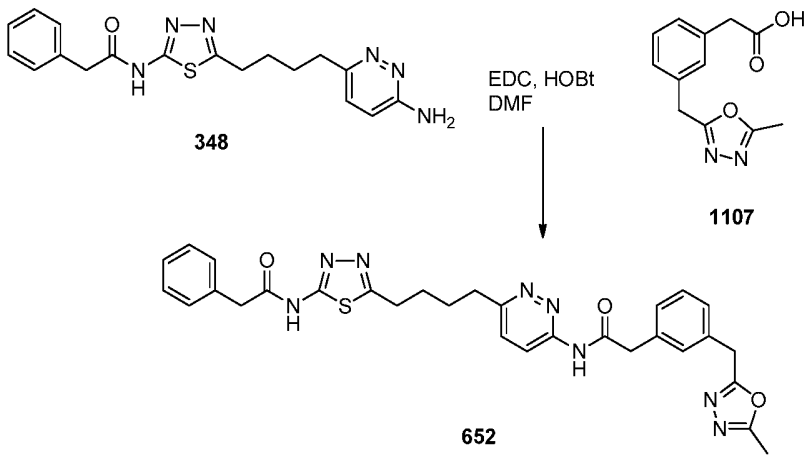
1105 1106

To a solution of 2-(3-bromobenzyl)-5-methyl-1,3,4-oxadiazole **1105** (0.50 g, 1.97 mmol) in dioxane (1 mL), under an atmosphere of Argon, was added Bis(tri-t-butylphosphine)palladium(0) (0.15 g, 0.295 mmol) followed by the addition of 2-tert-butoxy-2-oxoethylzinc chloride (0.5 M in diethyl ether, 4.92 mmol, 9.84 mL). The mixture was allowed to stir under Argon for 20 hours and the volatiles were removed under reduced pressure. The residue was taken up in EtOAc (10 mL) and washed with water (2 x 5 mL), brine (2 x 5 mL) and dried over Na_2SO_4. The Na_2SO_4 was removed by filtration and the volatiles removed under reduced pressure. The crude material was purified by normal phase chromatography 0-50% EtOAc / Hexanes to give 0.338 g of tert-butyl 2-(3-((5-methyl-1,3,4-oxadiazol-2-yl)methyl)phenyl)acetate **1106**. ^1H NMR 300 MHz $CDCl_3$: δ 7.24 (m, 4H), 4.12 (s, 2H), 3.51 (s, 2H), 2.46 (s, 3H), 1.43 (s, 9H).

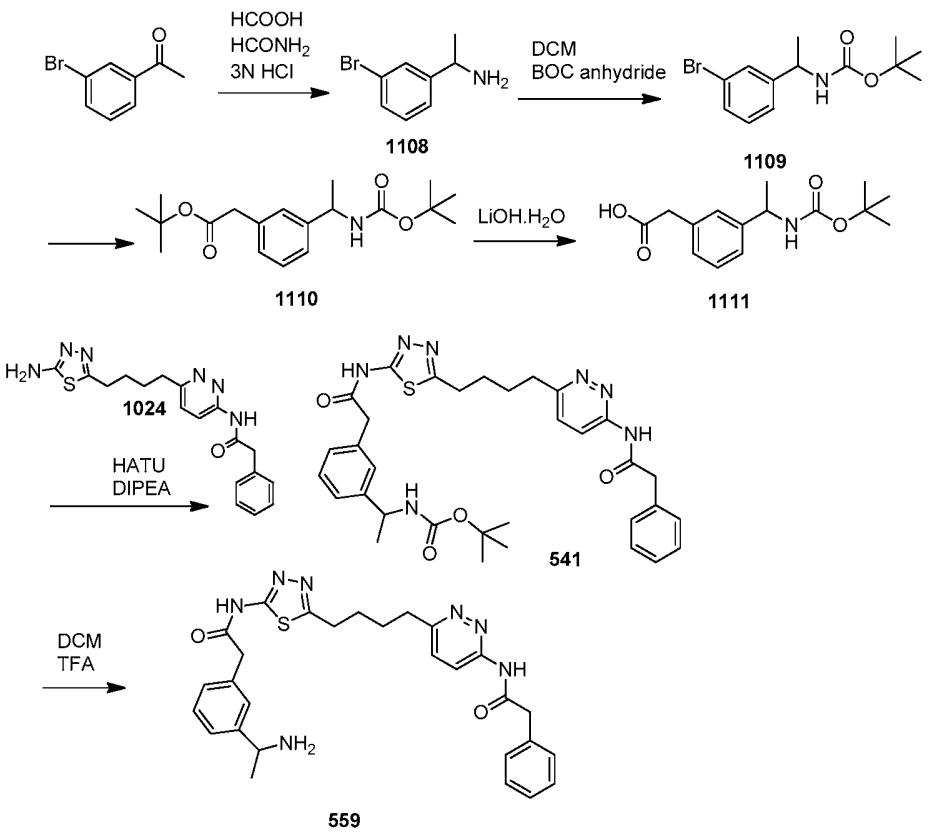


1106 1107

To a mixture of tert-butyl 2-(3-((5-methyl-1,3,4-oxadiazol-2-yl)methyl)phenyl)acetate **1106** (0.127 g, 0.44 mmol) in dioxane (3 mL) was added 4N HCl in dioxane (1 mL) and stirred under an atmosphere of Argon for 2 hours. The volatiles were removed under reduced pressure and the residue diluted with water (5 mL) and the pH adjusted to 12 with 2.5 N NaOH. The mixture was washed with dichloromethane (4 x 2 mL) and the pH adjusted to 6 with 1 N HCl. The mixture was extracted with EtOAc (3 x 2 mL) and the organic layers combined, washed with brine and dried over Na_2SO_4. The Na_2SO_4 was removed by filtration and the volatiles removed under reduced pressure to give 0.023 g of 2-(3-((5-methyl-1,3,4-oxadiazol-2-yl)methyl)phenyl)acetic acid **1107**.



A solution of N-(5-(4-(6-aminopyridazin-3-yl)butyl)-1,3,4-thiadiazol-2-yl)-2-phenylacetamide **348** (0.035 g, 0.094 mmol), 2-(3-((5-methyl-1,3,4-oxadiazol-2-yl)methyl)phenyl)acetic acid **1107** (0.023 g, 0.094 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (0.045 g, 0.235 mmol), 1-hydroxybenzotriazole (0.032 g, 0.235 mmol) in DMF (1.75 mL) was stirred for 16 hours and diluted with water (20 mL). The mixture was extracted with EtOAc (3 x 20 mL) the organic layers combined, washed with water (3 x 20 mL), brine (2 x 20 mL) and dried over Na_2SO_4. The Na_2SO_4 was removed by filtration and the volatiles removed under reduced pressure. The crude material was purified by reverse phase chromatography giving 0.004g of 2-(3-((5-methyl-1,3,4-oxadiazol-2-yl)methyl)phenyl)-N-(6-(4-(5-(2-phenylacetamido)-1,3,4-thiadiazol-2-yl)butyl)pyridazin-3-yl)acetamide **652**. ^{1}H NMR 300 MHz DMSO-d6: δ 12.62 (s, 1H), 11.24 (s, 1H), 8.16 (d, 1H, *J*=12.2 Hz), 7.54 (d, 1H, *J*= 12.2 Hz), 7.3-7.1 (m, 9H), 4.20 (s, 2H), 3.78 (s, 2H), 3.74 (s, 2H), 2.99 (m, 2H), 2.87 (m, 2H), 2.41 (s, 3H), 1.72 (m, 4H).



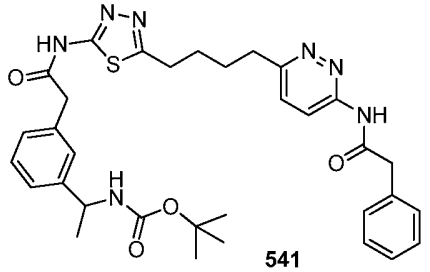
A mixture of 3-bromoacetophenone (5g, 25.1mmol) in formic acid (6gm) and formamide (25mL) was heated to 170 ^{0}C for overnight before it was extracted with toluene. Organic layer was separated and concentrated. The residue obtained was diluted with 3N HCl and the resulting mixture was refluxed overnight before it was cooled to room temperature. The solution was extracted with ether. Aqueous layer was separated, basified with aq. Sodium hydroxide solution and extracted with ether. Organic layer was separated, dried over sodium sulfate, filtered and concentrated to afford **1108** (3g, 60% yield). ^{1}H NMR (300MHz, Chloroform-d) δ ppm 1.22-1.25(d, 3H) 3.97-3.99(q, 1H) 7.23-7.4(m, 3H) 7.6(s, 1H).

To a solution of **1108** (2.945g, 14.7mmol) in dichloromethane (100mL) was added boc anhydride (3.21g, 14.7mmol) and the reaction mixture was stirred at room temperature overnight before it was concentrated and purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1109** (3g, 68% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.29-1.31(d, 3H) 1.38(s, 9H) 4.61-4.63(q, 1H) 7.3(brs, 2H) 7.41-7.5(m, 3H).

To a degassed solution of **1109** (0.5g, 1.66mmol) and bis(tri-tert-butylphosphine)palladium(0) (0.085g, 0.166mmol) in dioxane(3mL) was added 2-

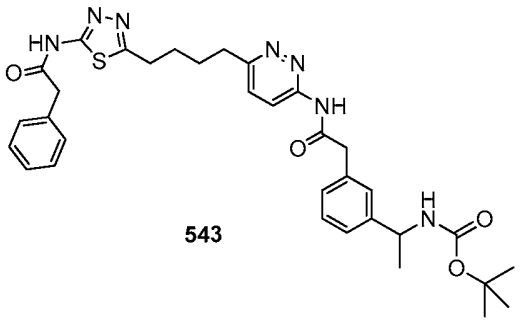
tert-Butoxy-2-oxoethylzinc chloride (8.5mL, 4.15mmol) under Argon and the resulting reaction mixture was stirred at room temperature for 4hr before it was quenched with saturated aqueous ammonium chloride solution. The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1110** (0.35g, 62% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.29-1.31(d, 3H) 1.388-1.42(brs, 18H) 3.53(s, 2H) 4.59-4.63(q, 1H) 7.09 (brs, 1H) 7.12-7.20(brs, 2H) 7.25-7.27(m, 1H) 7.27-7.30(m, 1H).

To a solution of **1110** (0.44g, 1.3mmol) in methanol (40mL) and water (10mL) was added lithium hydroxide monohydrate (0.4gm) and the resulting reaction mixture was stirred at room temperature for 2days before it was concentrated. The residue obtained was diluted with ice cold water and acidified with acetic acid. The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1111** (0.316g, 86% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.22-1.39(m, 12H) 3.55(s, 2H) 4.58-4.63(q, 1H) 7.11-7.38(m, 5H) 12.29(s, 1H).



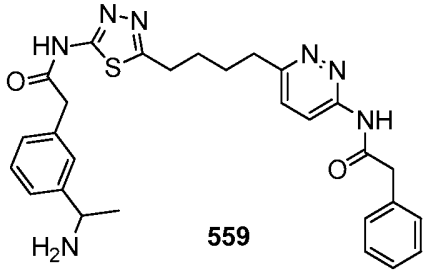
541

^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.43 (m, 12H) 1.89 (brs, 4H) 2.97-3.08 (m, 4H) 3.95-4.03 (m, 4H) 4.71-4.77 (q, 1H) 7.24-7.43 (m, 11H) 8.45-8.48 (d, 1H) 10.99 (s, 1H) 12.4 (brs, 1H).



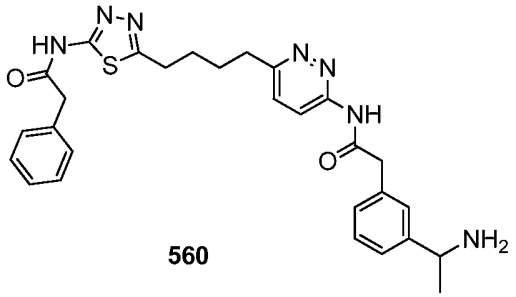
543

^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.43 (m, 12H) 1.89 (brs, 4H) 2.97-3.08 (m, 4H) 3.95-4.03 (m, 4H) 4.71-4.77 (q, 1H) 7.24-7.43 (m, 11H) 8.45-8.48 (d, 1H) 10.22 (brs, 1H) 12.4 (brs, 1H).



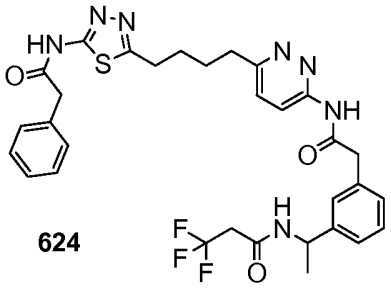
559

^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.5-1.52 (d, 3H) 1.75 (brs, 4H) 2.88-2.93 (m, 2H) 3.03-3.05 (m, 2H) 3.79(s, 2H) 3.86(s, 2H) 4.38-4.44 (q, 1H) 7.27-7.59 (m, 10H) 8.20-8.23 (m, 4H) 11.27 (s, 1H) 12.71 (s, 1H).



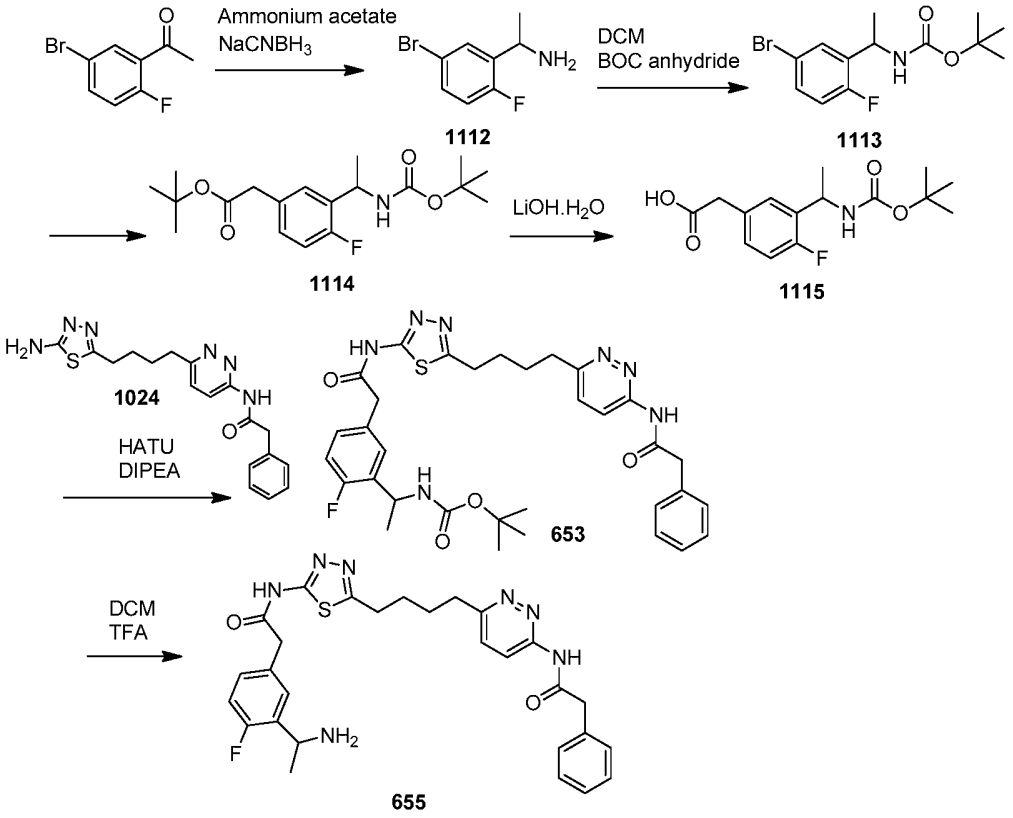
560

^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.5-1.52 (d, 3H) 1.75 (brs, 4H) 2.88-2.93 (m, 2H) 3.03-3.05 (m, 2H) 3.86(s, 4H) 4.38-4.44 (q, 1H) 7.27-7.59 (m, 10H) 8.20-8.23 (m, 4H) 11.27 (s, 1H) 12.71 (s, 1H).



624

^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.5-1.52 (d, 3H) 1.75 (brs, 4H) 2.88-2.93 (m, 2H) 3.03-3.05 (m, 2H) 3.78(s, 2H) 3.82(s, 2H) 4.91-4.96 (q, 1H) 7.20-7.35 (m, 9H) 7.55-7.58(d, 1H) 8.20-8.23(d, 1H) 8.68-8.71 (m, 1H) 11.27 (s, 1H) 12.71 (s, 1H).



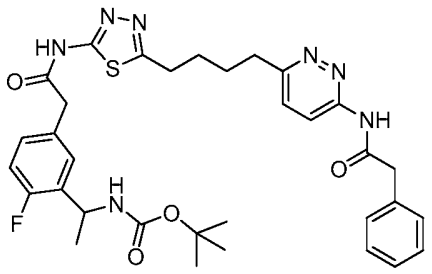
To an ice cold solution of 1-(5-bromo-2-fluorophenyl)ethanone (4.5g, 20.7mmol) in methanol(100mL) was added ammonium acetate(32g, 414.7mmol) and sodium cyanoborohydride(6.15g, 28.98mmol). The reaction mixture was stirred at room temperatue over the weekend before it was concentrated. The residue obtained was diluted with water, basified to pH~13 wih 1N NaOH and extracted with dichloromethane. The organic extract was separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1112** (1.8g, 40% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.24-1.26(d, 3H) 4.22-4.24(q, 1H) 7.1-7.16(t, 1H) 7.41-7.46(m, 1H) 7.76(m, 1H).

To a solution of **1112** (1.97g, 9mmol) in dichloromethane (100mL) was added boc anhydride (1.97g, 9mmol) and the reaction mixture was stirred at room temperature overnight before it was concentrated and purified by silica gel chromatography

eluting with EtOAc/Hexane to afford **1113** (2.4g, 83% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.29-1.32(d, 3H) 1.39(s, 9H) 4.87(q, 1H) 7.14-7.21(t, 1H) 7.46-7.58(m, 3H).

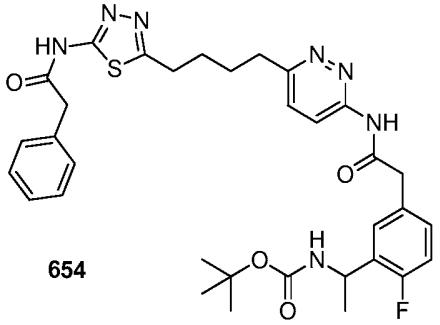
To a degassed solution of **1113** (2.4g, 7.54mmol) and bis(tri-tert-butylphosphine)palladium(0) (0.77g, 1.508mmol) in dioxane(12mL) was added 2-tert-Butoxy-2-oxoethylzinc chloride (38mL, 18.85mmol) under Argon and the resulting reaction mixture was stirred at room temperature for 4hr before it was quenched with saturated aqueous ammonium chloride solution. The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1114** (2g, 75% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.29-1.32(d, 3H) 1.38-1.41(m, 18H) 3.53(s, 2H) 4.87(q, 1H) 7.05-7.16(m, 2H) 7.26-7.29(m, 1H) 7.48(m, 1H).

To a solution of **1114** (2g, 5.66mmol) in methanol (100mL) and water (25mL) was added lithium hydroxide monohydrate (2gm) and the resulting reaction mixture was stirred at room temperature for 2days before it was concentrated. The residue obtained was diluted with ice cold water and acidified with acetic acid. The resulting solution was partitioned between water and ethyl acetate. The organic extract was washed with more water, separated, dried over sodium sulfate, filtered and evaporated. The residue obtained was purified by silica gel chromatography eluting with EtOAc/Hexane to afford **1115** (1.5g, 89% yield). ^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.29-1.31(d, 3H) 1.38 (s, 9H) 3.53(s, 2H) 4.87(q, 1H) 7.05-7.19(m, 2H) 7.26-7.29(m, 1H) 7.45- 7.48(m, 1H) 12.32(s, 1H).



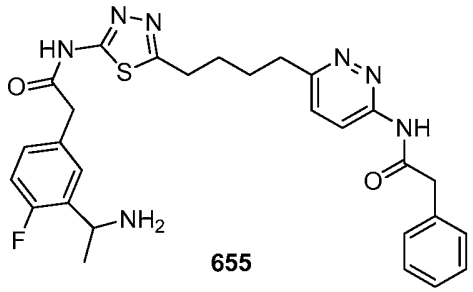
653

^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.30-1.33 (m, 12H) 1.74 (brs, 4H) 2.89(m, 2H) 3.02 (m, 2H) 3.78 (s, 4H) 4.85 (q, 1H) 7.10-7.57 (m, 11H) 8.19-8.22 (d, 1H) 11.26 (s, 1H) 12.64 (s, 1H).



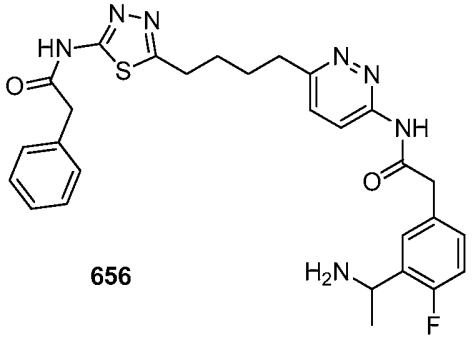
654

^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.28-1.32 (m, 12H) 1.73-1.75 (brs, 4H) 2.87(m, 2H) 2.89 (m, 2H) 3.75 (s, 2H) 3.81(s, 2H) 4.85 (q, 1H) 7.06-7.57 (m, 11H) 8.18-8.21(d, 1H) 11.26 (s, 1H) 12.64 (s, 1H).



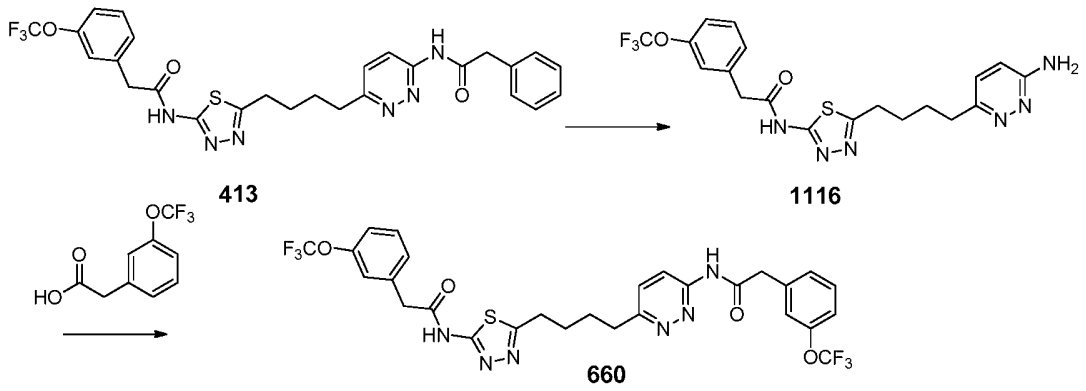
655

^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.51-1.53 (m, 3H) 1.75 (brs, 4H) 2.90(m, 2H) 3.02 (m, 2H) 3.78 (s, 2H) 3.85(s, 2H) 4.65 (q, 1H) 7.25-7.61 (m, 10H) 8.21-8.25 (d, 1H) 8.33-8.35(brs, 3H) 11.29 (s, 1H) 12.68 (s, 1H).

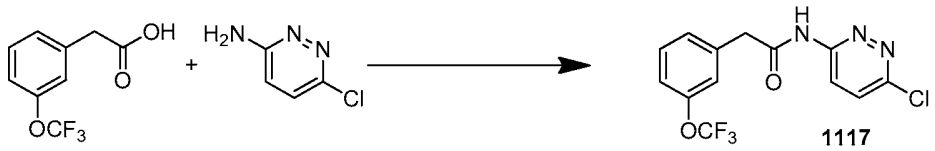


656

^{1}H NMR (300MHz, Dimethylsulfoxide-d6) δ ppm 1.54 (d, 3H) 1.75-1.76 (brs, 4H) 2.91(m, 2H) 3.02 (m, 2H) 3.81-3.83(m, 4H) 4.65 (q, 1H) 7.24-7.63 (m, 10H) 8.22-8.25 (d, 1H) 8.36(brs, 3H) 11.35 (s, 1H) 12.66 (s, 1H).

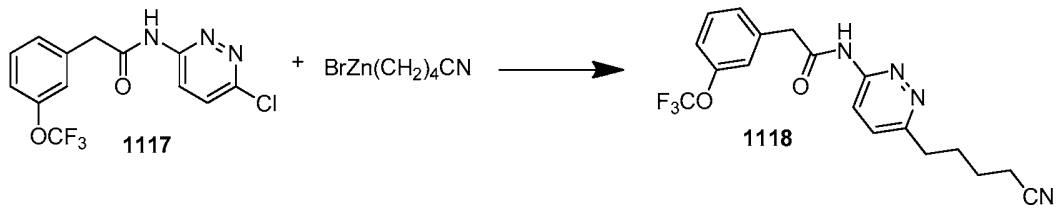


To a mixture of **413** (1.62 g) in MeOH (25 mL), THF (10 mL) and H_2O (10 mL) at room temperature was added 1N aq. NaOH (8 mL). This mixture was stirred for 24 h before the organic volatile was removed under reduced pressure. The residue was neutralized to pH 7 with 1N aq. HCl solution and extracted with EtOAc (2×20 mL). The combined extract was dried ($MgSO_4$) and concentrated. The crude was purified by silica gel chromatography eluting with 1–15% MeOH in dichloromethane to afford amine **1116**. The resulting amine **1116** was converted to **660** as described for **335**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.68 (bs, 1H), 11.31 (s, 1H), 8.20 (d, J = 9.2 Hz, 1H), 7.57 (d, J = 8.8 Hz, 1H), 7.52–7.21 (m, 8H), 3.90 (s, 2H), 3.87 (s, 2H), 3.06–2.86 (m, 4H), 1.77–1.72 (m, 4H).

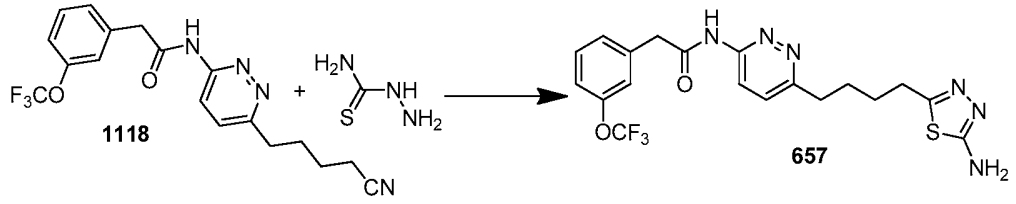


3-Amino-6-chloropyridazine (55.5 g, 0.428 mol) and 3-(Trifluoromethoxy)phenylacetic acid (1.1 equiv., 0.471 mol, 104 g) were dissolved in DMF (30.0 vol., 1.66 L) in a 3000 mL three neck round-bottom flask. Addition of DIEA (1.1 equiv., 0.471 mol, 82 mL) via addition funnel was done over 5 minutes. Propylphosphonic anhydride solution (300 mL of a 50% solution in DMF, 1.1 equiv., 0.471 mol,) was charged into a 500 mL addition funnel and added dropwise to reaction solution (keeping reaction temperature ≤ +30 °C). The reaction usually goes to completion after 3 hours (TLC: 6:4 hexanes-ethyl acetate). Reaction mixture was then poured into 7.5% sodium bicarbonate (80.0 vol., 4.4 L) which was chilled in an ice bath. Off-white crystalline powder was filtered through a Büchner funnel, rinsed with water (20.0 vol., 1.1 L). Dried in a 50 °C vacuum to a constant weight to afford N-(6-chloropyridazin-3-yl)-2-(3-(trifluoromethoxy)phenyl)acetamide **1117**: yield of

119.6 g (77%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.63 (s, 1H), 8.38(d, *J*=9.4 Hz, 1H), 7.88(d, *J*=9.4 Hz, 1H), 7.52 – 7.27(m, 4H), 3.90(s, 2H).

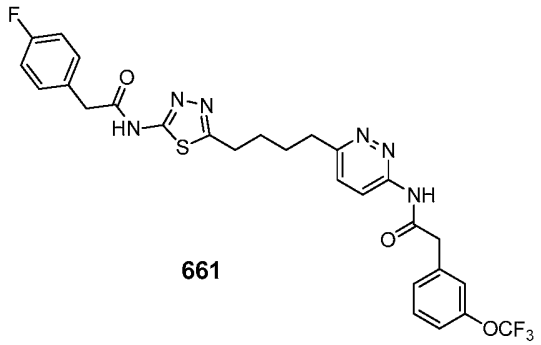


4-Cyanobutylzinc bromide solution (3.0 equiv., 0.50 mol, 1.0 L) was charged into an argon gas purged 5000 mL 3 neck round bottom flask. Argon$_{(g)}$ purge for 5 minutes followed by the addition of **1117** (1.0 equiv., 0.167 mol, 55.3 g) and $NiCl_2$(dppp) (0.15 equiv., 0.0251 mol, 13.6 g) under a blanket of argon$_{(g)}$. The reaction usually goes to completion after 4 hours (TLC: 1:1 hexanes-ethyl acetate). EtOAc (15 vol., 832 mL) added to deep red solution. Water (15 vol., 832 mL) was added, thick slurry formed. 1N HCl added until slurry breaks to pale blue layer (~6 vol., 333 mL). Transferred to separatory funnel and organic layer was washed with 1N HCl (2x500 mL), dried ($MgSO_4$) and concentrated by rotary evaporation (bath ≤ 30 °C) to a solid reddish oil. Oil dissolved in dichloromethane (15 vol., 832 mL), silica gel (100g) was slurried into red solution, this was concentrated by rotary evaporation (bath ≤ 30 °C) to a solid reddish powder. Loaded onto a bed of silica gel (5 cm x 11 cm), flushed with 25% hexanes in ethyl acetate (3 L), combined organics concentrated by rotary evaporation (bath ≤ 30 °C). Dried under high vacuum to a constant weight to afford N-(6-(4-cyanobutyl)pyridazin-3-yl)-2-(3-(trifluoromethoxy)phenyl)acetamide **1118**: yield of 58.2 g (92%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.41 (s, 1H), 8.28(d, *J*=9.2 Hz, 1H), 7.65(d, *J*=9.2 Hz, 1H), 7.52 – 7.27(m, 4H), 3.89(s, 2H), 2.92(t, *J*=7.5 Hz, 2H), 2.56(t, *J*=7.0 Hz, 2H), 1.80 (m, 2H), 1.61 (m, 2H).



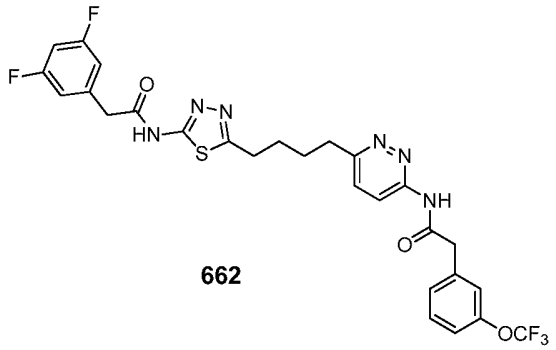
1118 (1.0 equiv., 0.154 mol, 58.2 g) was charged into a 500 mL round bottom flask along with thiosemicarbazide (1.2 equiv., 0.184 mol, 16.8 g). TFA (5 vol., 291 mL) slowly added to reaction vessel while stirring. The reaction slurry was heated in a 65°C bath with an open top reflux condenser. The reaction usually goes to completion after 5 hours (determined by LC/MS). Toluene (10 vol., 582 mL) added

to deep red solution, azeotroped by rotary evaporation (bath ≤ 30 °C) to a red oil. Slowly transferred oil to a well stirred 6000 mL Erlenmeyer flask containing 7.5% sodium bicarbonate solution (69 vol., 4.0 L) cooled in a 0°C bath. The crystals were filtered through a Büchner funnel and rinsed twice with diethyl ether (5 vol., 2x250 mL). Dried under high vacuum to a constant weight to afford N-(6-(4-(5-amino-1,3,4-thiadiazol-2-yl)butyl)pyridazin-3-yl)-2-(3-(trifluoromethoxy)phenyl)acetamide **657**; yield of 55.7 g (80%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.33 (s, 1H), 8.21(d, *J*=9.2 Hz, 1H), 7.58(d, *J*=9.2 Hz, 1H), 7.51 – 7.26(m, 4H), 6.99(s, 2H), 3.88(s, 2H), 2.87(m, 4H), 1.71 (m, 4H).



661

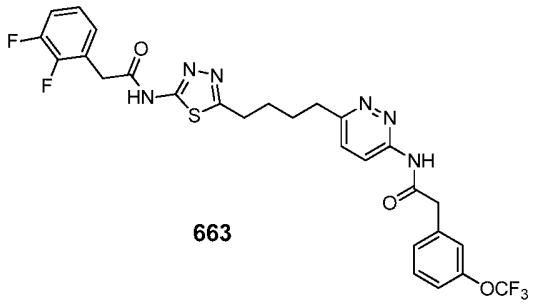
To a solution of **657** (50 mg, 0.11 mmol) in DMF (3 mL) at 0 °C was added 4-fluorophenyl acetic acid (22 mg, 0.14 mmol), HOBt (30 mg, 0.22 mmol) and EDCI (42 mg, 0.22 mmol). The resulting mixture was stirred at room temperature for 1.5 h before it was cooled to 0 °C and quenched with H_2O. The precipitate was collected by suction filtration and further purified by silica gel chromatography eluting with 1–10% MeOH in dichloromethane to afford **661**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.65 (bs, 1H), 11.31 (s, 1H), 8.20 (d, *J* = 9.1 Hz, 1H), 7.57 (d, *J* = 9.4 Hz, 1H), 7.49–7.14 (m, 8H), 3.87 (s, 2H), 3.81 (s, 2H), 3.06–2.86 (m, 4H), 1.77–1.72 (m, 4H).



662

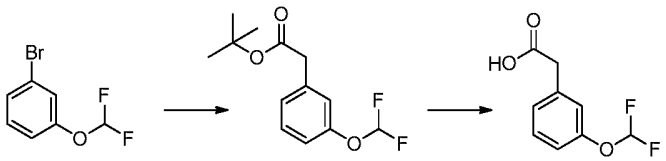
662 was prepared by the procedure as described for compound **661**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.67 (bs, 1H), 11.31 (s, 1H), 8.20 (d, *J* = 9.1 Hz, 1H), 7.57 (d, *J*

= 9.1 Hz, 1H), 7.51–7.07 (m, 7H), 3.89 (s, 2H), 3.87 (s, 2H), 3.06–2.86 (m, 4H), 1.77–1.72 (m, 4H).



663

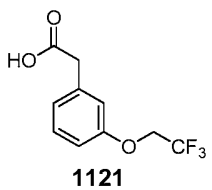
663 was prepared by the procedure as described for compound **661**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.74 (bs, 1H), 11.31 (s, 1H), 8.20 (d, J = 9.2 Hz, 1H), 7.57 (d, J = 9.2 Hz, 1H), 7.51–7.19 (m, 7H), 3.97 (s, 2H), 3.87 (s, 2H), 3.06–2.86 (m, 4H), 1.77–1.72 (m, 4H).



1119 1120

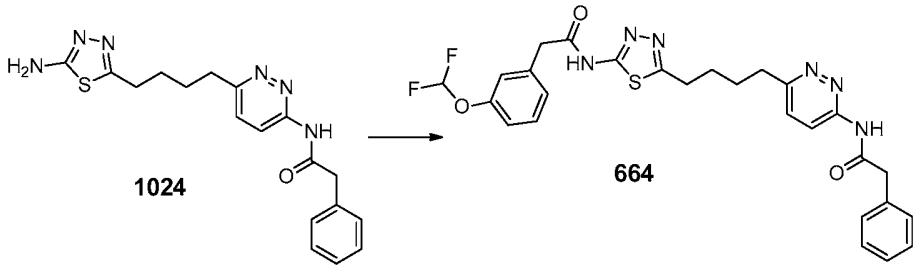
To a mixture of 1-bromo-3-(difluoromethoxy) benzene (1 g, 4.5 mmol), bis(tri-tert-butylphosphine) palladium(0) (460 mg, 0.9 mmol) in 1,4-dioxane (30 ml) under argon atmosphere was added 0.5 M of 2-tert-butoxy-2-oxoethyl zinc chloride in ether (22.5 ml). The resulting mixture was stirred at room temperature overnight. The mixture was partitioned between saturated NH_4Cl and EtOAc. The organic extract was washed with brine, dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel chromatography eluting with 0-10% EtOAc in Hexane to afford **1119**.

To a solution of **1119** (300 mg, 1.16 mmol) in dichloromethane (5 ml) at 0 ^{0}C was added TFA (3 ml) dropwise. The resulting mixture was stirred at room temperature overnight before it was evaporated to dryness then triturated the residue with ether to afford **1120**.

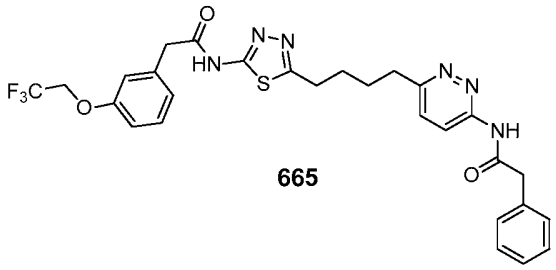


1121

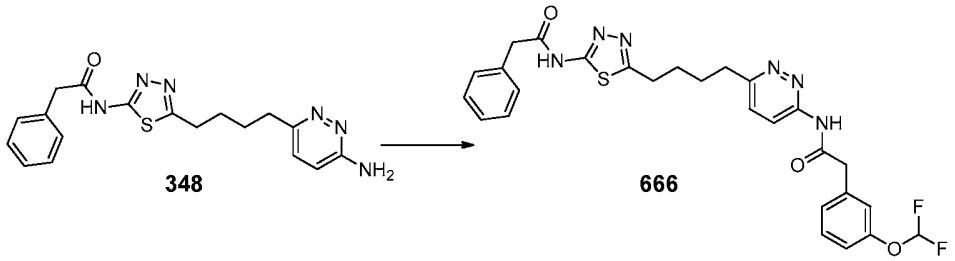
1121 was made using procedure described for compound **1120** from 1-Bromo-3-(2,2,2-trifluoroethoxy)benzene.



A flask was charged with **1024** (50 mg, 0.135 mmol), **1120** (28 mg, 0.142 mmol) in DMF (1 ml) at 0 ^{0}C was added HOBT (39 mg, 0.285 mmol) followed by EDCI (68 mg, 0.356 mmol). The resulting mixture was slowly warmed up to room temperature and stirred for 2 h before it was quenched by addition of ice water (~5 mL). The white precipitate was collected by suction filtration, rinsed with more water to afford **664**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71 (s, 1H), 11.32 (s, 1H), 8.22-8.19 (d, J = 9.12 Hz, 1H), 7.58-7.54 (d, J = 9.03 Hz, 1H), 7.48-6.99 (m, 10H), 3.85 (s, 2H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).

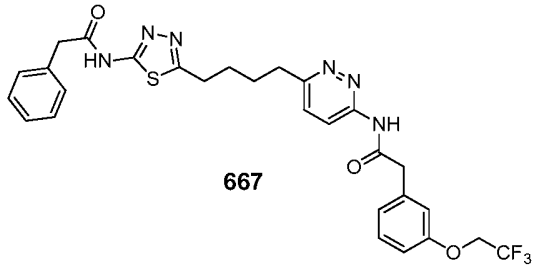


665 was made using procedure described for compound **664**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71 (s, 1H), 11.32 (s, 1H), 8.22-8.19 (d, J = 9.12 Hz, 1H), 7.58-7.54 (d, J = 9.03 Hz, 1H), 7.38-7.28 (m, 6H), 7.03-6.97 (m, 3H), 4.77-4.74 (q, 2H), 3.80-3.78 (d, J = 5.82 Hz, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



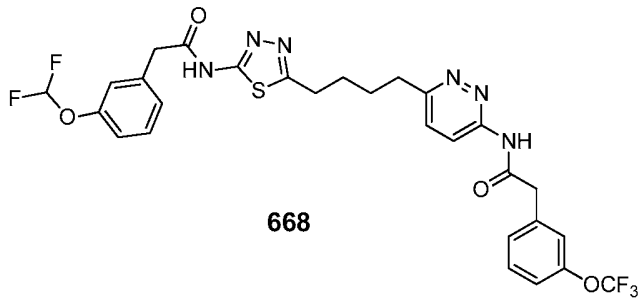
A flask was charged with **348** (50 mg, 0.135 mmol), **1120** (28 mg, 0.142 mmol) in DMF (1 ml) at 0 ^{0}C was added HOBT (39 mg, 0.285 mmol) followed by EDCI (68 mg, 0.356 mmol). The resulting mixture was slowly warmed up to room temperature

and stirred overnight before it was quenched by addition of ice water (~5 mL). The white precipitate was collected by suction filtration, rinsed with more water. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in dichloromethane to afford **666**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71 (s, 1H), 11.32 (s, 1H), 8.22-8.19 (d, J = 9.12 Hz, 1H), 7.58-7.54 (d, J = 9.03 Hz, 1H), 7.48-6.98 (m, 10H), 3.81 (bs, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



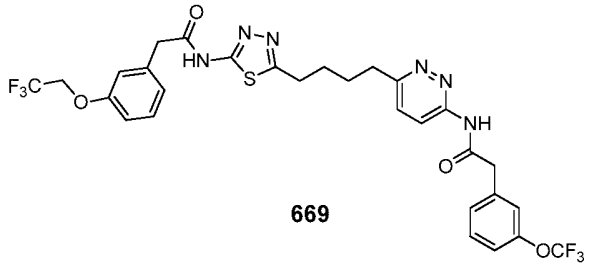
667

667 was made using procedure described for compound **666**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71 (s, 1H), 11.32 (s, 1H), 8.22-8.19 (d, J = 9.12 Hz, 1H), 7.58-7.54 (d, J = 8.97 Hz, 1H), 7.35-7.28 (m, 6H), 7.03-6.97 (m, 3H), 4.77-4.74 (q, 2H), 3.87 (s, 2H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



668

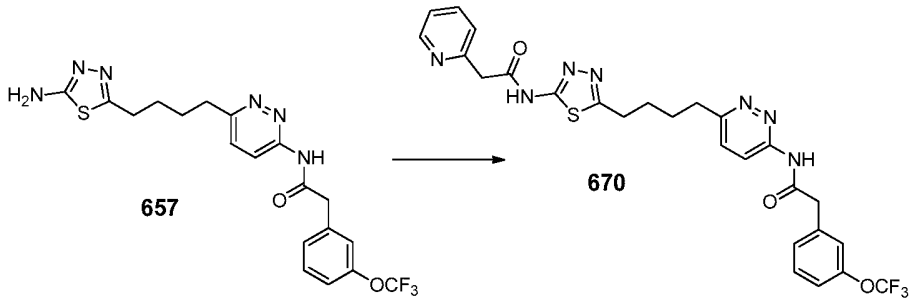
668 was made using procedure described for compound **675**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71 (s, 1H), 11.32 (s, 1H), 8.22-8.19 (d, J = 9.15 Hz, 1H), 7.58-6.99 (m, 10H), 3.87-3.84 (d, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



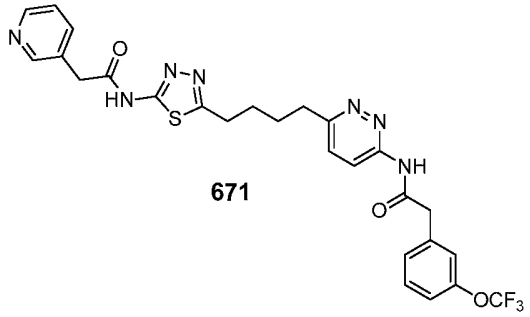
669

669 was made using procedure described for compound **675**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71 (s, 1H), 11.32 (s, 1H), 8.22-8.19 (d, J = 9.09 Hz, 1H), 7.58-7.54

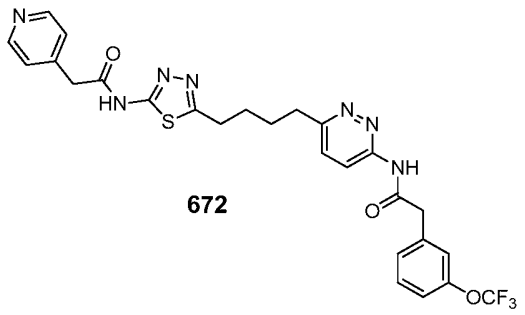
(d, J = 9.37 Hz, 1H), 7.48-7.28 (m, 6H), 7.03-6.97 (m, 2H), 4.77-4.74 (q, 2H), 3.87 (s, 2H), 3.78 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



A flask was charged with **657** (50 mg, 0.111 mmol), 2-pyridine acetic acid hydrochloride (20 mg, 0.116 mmol) in DMF (1 ml) at 0 ^{0}C was treated with propylphosphonic anhydride solution (91 ul) followed by triethylamine (40 ul, 0.29 mmol). The resulting mixture was slowly warmed up to room temperature and stirred for 1 h before it was quenched by addition of ice water (~5 mL). The yellow precipitate was collected by suction filtration, rinsed with more water. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in dichloromethane to afford **670**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.67 (s, 1H), 11.32 (s, 1H), 8.53-8.49 (m, 1H), 8.22-8.19 (d, J = 9.12 Hz, 1H), 7.78-7.76 (t, 1H), 7.58-7.26 (m, 7H), 4.01 (s, 2H), 3.87 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).

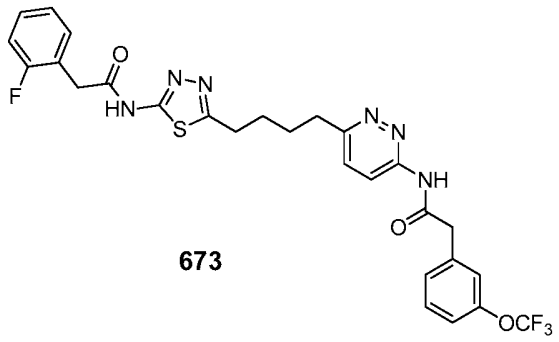


671 was made using procedure described for compound **670**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.70 (s, 1H), 11.32 (s, 1H), 8.53-8.48 (m, 2H), 8.22-8.19 (d, J = 9.12 Hz, 1H), 7.76-7.26 (m, 7H), 3.87 (s, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



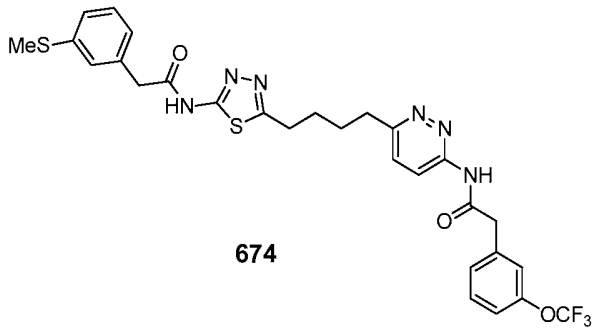
672

672 was made using procedure described for compound **670**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.32 (s, 1H), 8.53-8.52 (bs, 2H), 8.22-8.19 (d, J = 9.12 Hz, 1H), 7.58-7.26 (m, 7H), 3.87 (s, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



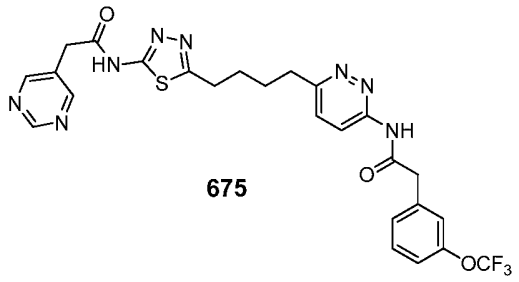
673

673 was prepared by the procedure as described for compound **661**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.69 (bs, 1H), 11.31 (s, 1H), 8.20 (d, J = 9.1 Hz, 1H), 7.57 (d, J = 9.1 Hz, 1H), 7.51–7.21 (m, 8H), 3.90 (s, 2H), 3.87 (s, 2H), 3.06–2.86 (m, 4H), 1.77–1.72 (m, 4H).



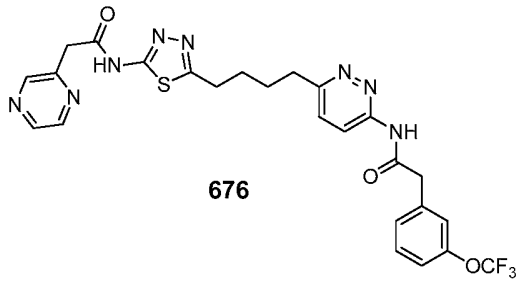
674

674 was prepared by the procedure as described for compound **661**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.63 (bs, 1H), 11.32 (s, 1H), 8.20 (d, J = 9.2 Hz, 1H), 7.57 (d, J = 9.2 Hz, 1H), 7.51–7.38 (m, 3H), 7.33–7.09 (m, 5H), 3.87 (s, 2H), 3.79 (s, 2H), 3.06–2.86 (m, 4H), 2.48 (s, 3H), 1.77–1.72 (m, 4H).



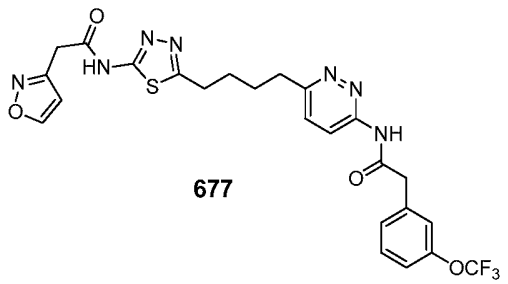
675

A flask was charged with **657** (70 mg, 0.155 mmol), 5-pyrimidineacetic acid (22 mg, 0.162 mmol) in DMF (1 ml) at 0 ^{0}C was added HOBT (44 mg, 0.326 mmol) followed by EDCI (78 mg, 0.408 mmol). The resulting mixture was slowly warmed up to room temperature and stirred for overnight before it was quenched by addition of ice water (~5 mL). The white precipitate was collected by suction filtration, rinsed with more water. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in dichloromethane to afford **675**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.75 (s, 1H), 11.32 (s, 1H), 9.11 (s, 1H), 8.76 (s, 1H), 8.22-8.19 (d, J = 9.12 Hz, 1H), 7.59-7.26 (m, 6H), 3.94 (s, 2H), 3.87 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



676

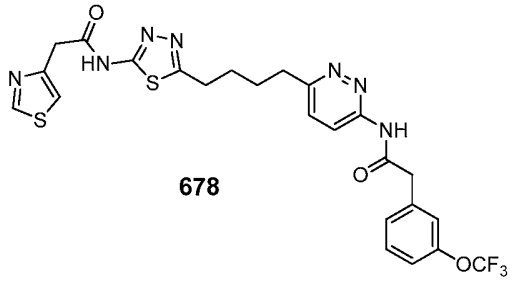
676 was made using procedure described for compound **675**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.75 (s, 1H), 11.32 (s, 1H), 8.70 (s, 1H), 8.61-8.57 (m, 2H), 8.22-8.19 (d, J = 9.36 Hz, 1H), 7.59-7.26 (m, 5H), 4.11 (s, 2H), 3.87 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



677

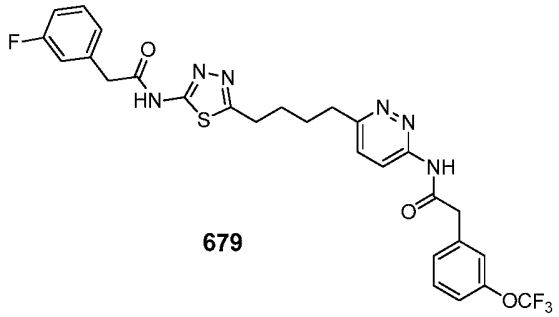
677 was made using procedure described for compound **675**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.75 (s, 1H), 11.32 (s, 1H), 8.89 (s, 1H), 8.22-8.19 (d, J = 9.15 Hz, 1H),

7.59-7.26 (m, 5H), 6.62 (s, 1H), 3.99 (s, 2H), 3.87 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



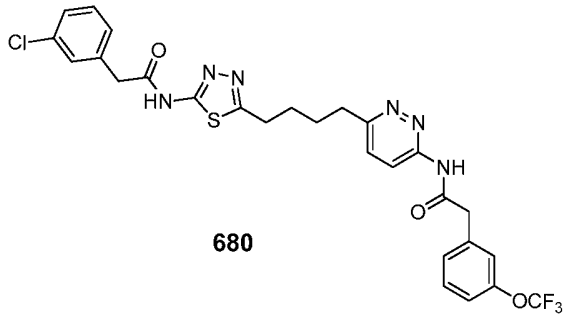
678

678 was made using procedure described for compound **675**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.75 (s, 1H), 11.32 (s, 1H), 9.06 (s, 1H), 8.22-8.19 (d, J = 9.21 Hz, 1H), 7.59-7.26 (m, 6H), 4.03 (s, 2H), 3.87 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



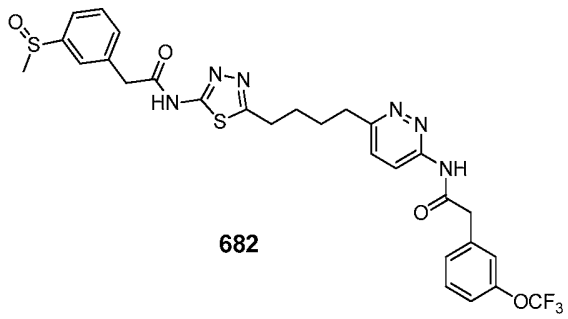
679

679 was prepared by the procedure as described for compound **661**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.67 (bs, 1H), 11.31 (s, 1H), 8.20 (d, J = 9.2 Hz, 1H), 7.57 (d, J = 9.2 Hz, 1H), 7.51–7.36 (m, 4H), 7.29–7.12 (m, 4H), 3.87 (s, 2H), 3.85 (s, 2H), 3.06–2.86 (m, 4H), 1.77–1.72 (m, 4H).



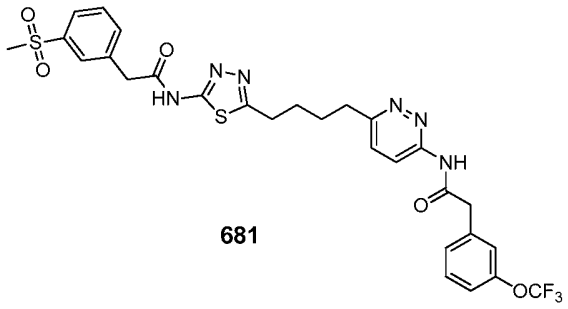
680

680 was prepared by the procedure as described for compound **661**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.67 (bs, 1H), 11.31 (s, 1H), 8.20 (d, J = 9.3 Hz, 1H), 7.57 (d, J = 9.0 Hz, 1H), 7.51–7.28 (m, 8H), 3.87 (s, 2H), 3.84 (s, 2H), 3.06–2.86 (m, 4H), 1.77–1.72 (m, 4H).



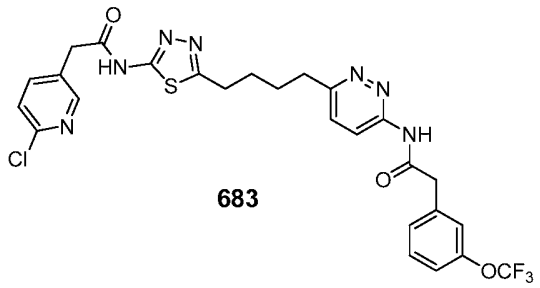
682

To a solution of **674** (100 mg, 0.16 mmol) in dichloromethane at –78 °C was added *m*-CPBA (60 mg, 0.24 mmol) in 4 portions. The resulting mixture was stirred at that temperature for 1 h before it was slowly warmed up to –10 °C and quenched with 25% aq. $Na_2S_2O_3$ solution. The reaction was diluted with EtOAc, washed with saturated aq. $NaHCO_3$ (3×10 mL). The combined organic layer was separated, washed with brine, dried ($MgSO_4$) and concentrated. The crude was purified by HPLC to afford **682**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.72 (bs, 1H), 11.31 (s, 1H), 8.20 (d, J = 9.0 Hz, 1H), 7.68 (m, 1H), 7.60–7.26 (m, 8H), 3.91 (s, 2H), 3.87 (s, 2H), 3.06–2.86 (m, 4H), 2.76 (s, 3H), 1.77–1.72 (m, 4H).



681

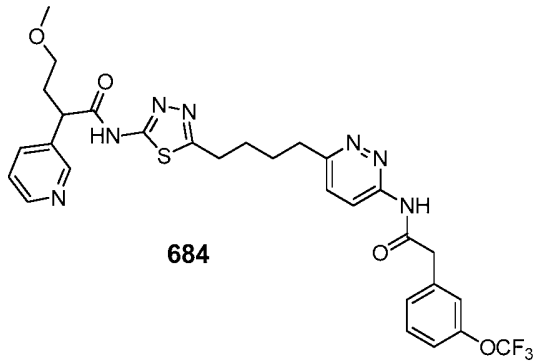
681 was prepared from **657** and 3-methylsulphonylphenyl acetic acid by the procedure as described for compound **661**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.72 (bs, 1H), 11.31 (s, 1H), 8.20 (d, J = 9.0 Hz, 1H), 7.92 – 7.83 (m, 2H), 7.70–7.26 (m, 7H), 3.93 (s, 2H), 3.87 (s, 2H), 3.23 (s, 3H), 3.06–2.86 (m, 4H), 1.77–1.72 (m, 4H).



683

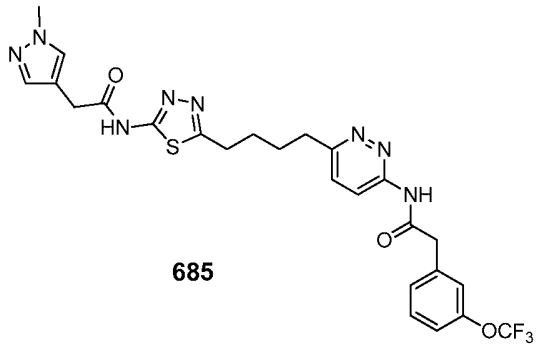
683 was made using procedure described for compound **675**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.75 (s, 1H), 11.32 (s, 1H), 8.36 (s, 1H), 8.21-8.18 (d, J = 9.18 Hz, 1H),

7.84-7.80 (d, J = 9.36 Hz, 1H), 7.59-7.26 (m, 6H), 3.90-3.87 (d, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



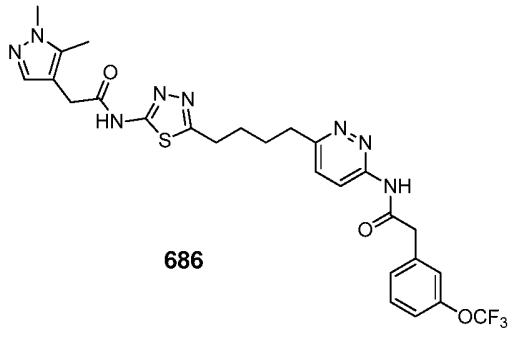
684

684 was made using procedure described for compound **675**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.75 (s, 1H), 11.32 (s, 1H), 8.57 (s, 1H), 8.51-8.49 (d, J = 9.18 Hz, 1H), 8.21-8.18 (d, J = 9.06 Hz, 1H), 7.79-7.75 (d, J = 9.36 Hz, 1H), 7.59-7.26 (m, 6H), 4.07 (t, 2H), 3.87 (s, 2H), 3.30-3.28 (m, 1H), 3.19 (s, 3H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.3-2.5 (m, 1H), 1.99-1.96 (m, 1H), 1.73 (bs, 4H).



685

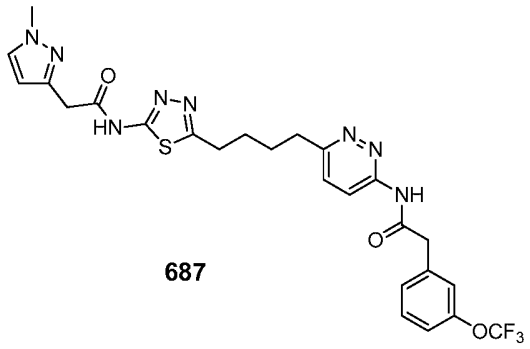
685 was prepared by the procedure as described for compound **661**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.52 (bs, 1H), 11.31 (s, 1H), 8.20 (d, J = 9.1 Hz, 1H), 7.61–7.25 (m, 7H), 3.87 (s, 2H), 3.80 (s, 3H), 3.62 (s, 2H), 3.06–2.86 (m, 4H), 1.77–1.72 (m, 4H).



686

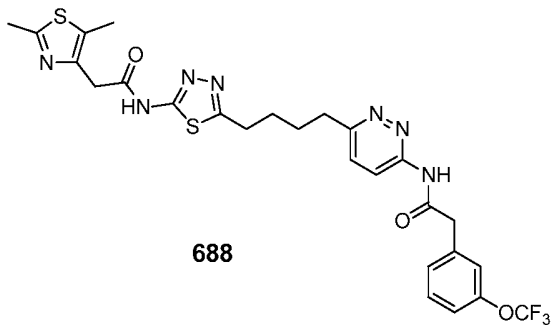
686 was prepared by the procedure as described for compound **661**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.53 (bs, 1H), 11.32 (s, 1H), 8.20 (d, J = 9.1 Hz, 1H), 7.58 (d, J

= 9.2 Hz, 1H), 7.52–7.26 (m, 4H), 5.96 (s, 1H), 3.87 (s, 2H), 3.67 (s, 2H), 3.64 (s, 3H), 3.06–2.86 (m, 4H), 2.21 (s, 3H), 1.77–1.72 (m, 4H).



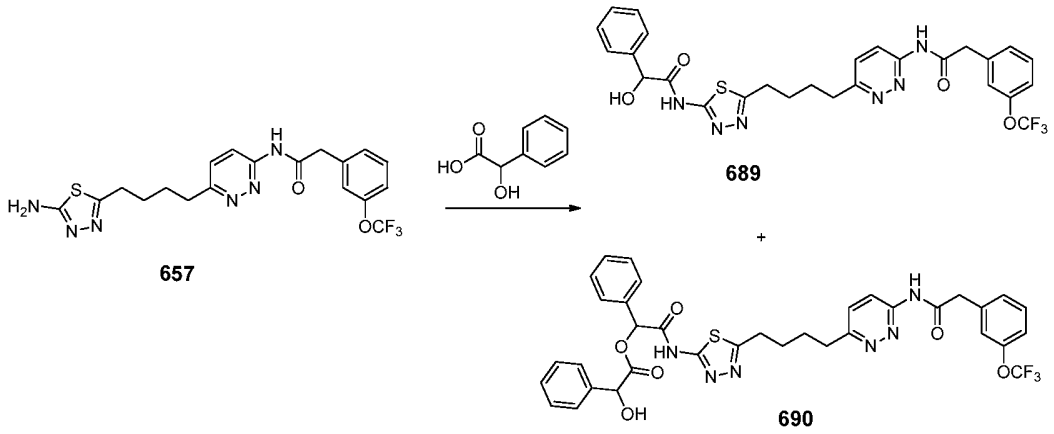
687

687 was prepared by the procedure as described for compound **661**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.56 (bs, 1H), 11.32 (s, 1H), 8.20 (d, J = 9.3 Hz, 1H), 7.61–7.38 (m, 6H), 6.17 (d, J = 2.2 Hz, 1H), 3.87 (s, 2H), 3.79 (s, 3H), 3.75 (s, 2H), 3.03–2.90 (m, 4H), 1.7 –1.72 (m, 4H).



688

688 was prepared by the procedure as described for compound **661**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.61 (bs, 1H), 11.32 (s, 1H), 8.20 (d, J = 9.3 Hz, 1H), 7.58 (d, J = 9.3 Hz, 1H), 7.51–7.26 (m, 4H), 3.87 (s, 2H), 3.84 (s, 2H), 3.07–2.86 (m, 4H), 1.77–1.72 (m, 4H).

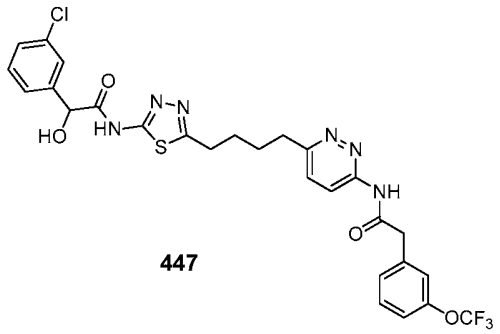


657

689

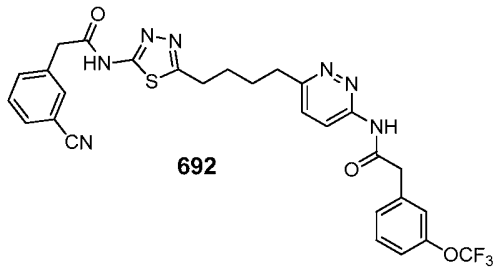
690

To a solution of **657** (200 mg, 0.44 mmol) in DMF (4 mL) at 0 °C was added mandelic acid (124 mg, 0.66 mmol), HOBt (119 mg, 0.88 mmol) and EDCI (170 mg, 0.88 mmol). The resulting mixture was stirred at room temperature for 1.5 h before it was cooled to 0 °C and quenched with H_2O. The precipitate was collected by suction filtration and further purified by silica gel chromatography eluting with 1–10% MeOH in dichloromethane to afford **690** and a more polar **689**. **689**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.42 (bs, 1H), 11.31 (s, 1H), 8.20 (d, J = 9.2 Hz, 1H), 7.58–7.27 (m, 10H), 6.35 (d, J = 4.4 Hz, 1H), 5.34 (d, J = 4.3 Hz, 1H), 3.87 (s, 2H), 3.03–2.89 (m, 4H), 1.77–1.73 (m, 4H). **690**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.05 (bs, 1H), 11.31 (s, 1H), 8.20 (d, J = 9.0 Hz, 1H), 7.59–7.26 (m, 15H), 6.26 (d, J = 5.5 Hz, 1H), 6.11 (s, 1H), 5.38 (d, J = 5.3 Hz, 1H), 3.87 (s, 2H), 3.03–2.88 (m, 4H), 1.76–1.73 (m, 4H).



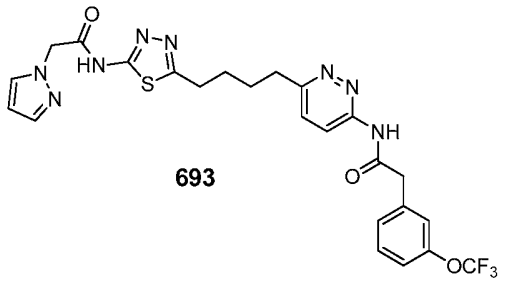
447

447 was prepared from **657** and 3-chloromandelic acid by the procedure as described for compound **689**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.48 (bs, 1H), 11.31 (s, 1H), 8.20 (d, J = 9.2 Hz, 1H), 7.59–7.26 (m, 9H), 6.53 (m, 1H), 5.36 (t, J = 0.7 Hz, 1H), 3.87 (s, 2H), 3.03–2.90 (m, 4H), 1.75–1.71 (m, 4H).



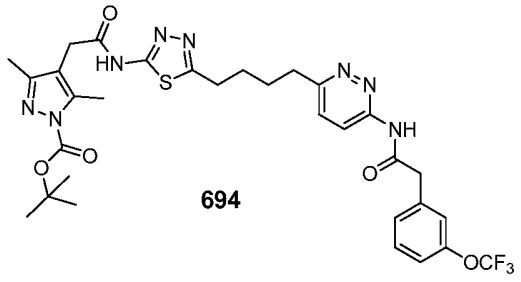
692

692 was made using procedure described for compound **675**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.75 (s, 1H), 11.32 (s, 1H), 8.21-8.18 (d, J = 9.18 Hz, 1H), 7.80-7.26 (m, 9H), 3.92 (s, 2H), 3.87 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



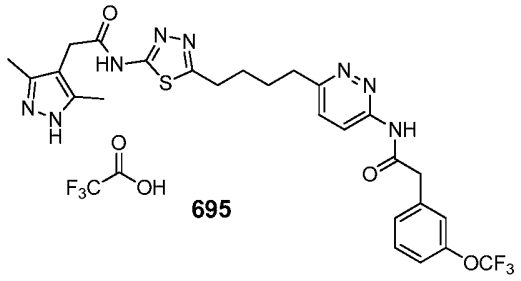
693

693 was made using procedure described for compound **675**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.75 (s, 1H), 11.32 (s, 1H), 8.22-8.19 (d, J = 9.06 Hz, 1H), 7.79 (s, 1H), 7.59-7.26 (m, 6H), 6.31 (s, 1H), 5.20 (s, 2H), 3.87 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



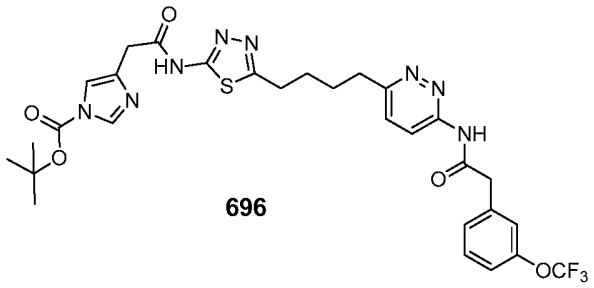
694

694 was made using procedure described for compound **675**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71 (s, 1H), 11.32 (s, 1H), 8.22-8.18 (d, J = 9.15 Hz, 1H), 7.58-7.54 (d, J = 9.18 Hz, 1H), 7.48-7.26 (m, 4H), 3.87 (s, 2H), 3.63 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.39 (s, 3H), 2.13 (s, 3H), 1.73 (bs, 4H), 1.57 (s, 9H).



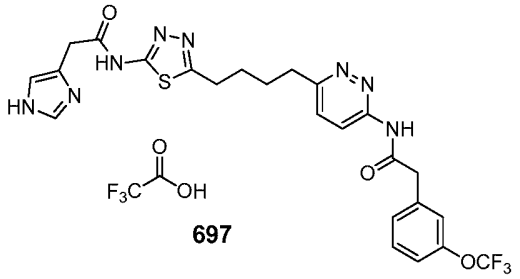
695

To a solution of **694** (50 mg, 0.081 mmol) in dichloromethane (2 ml) was added TFA (2 ml) at 0 ^{0}C. The resulting mixture was stirred at room temperature for 1 h before it was evaporated under vacuo to dryness. Ether was added and the white precipitate was collected by suction filtration, rinsed with more ether to afford **695**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71 (s, 1H), 11.32 (s, 1H), 8.22-8.19 (d, J = 9.36 Hz, 1H), 7.60-7.57 (d, J = 9.27 Hz, 1H), 7.51-7.28 (m, 4H), 3.88 (s, 2H), 3.57 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.45 (s, 3H), 2.15 (s, 3H), 1.73 (bs, 4H).



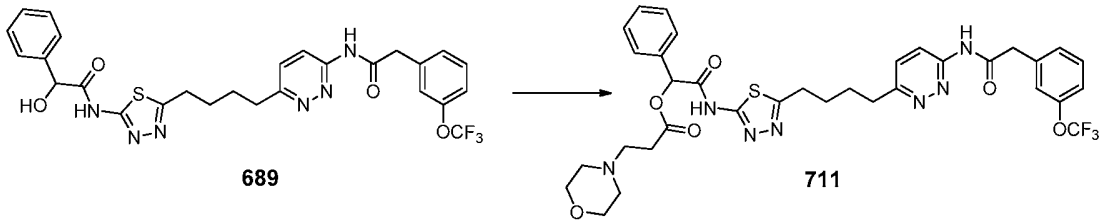
696

696 was made using procedure described for compound **695**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71 (s, 1H), 11.32 (s, 1H), 8.22-8.19 (d, J = 9.30 Hz, 1H), 8.15 (s, 1H), 7.58-7.54 (d, J = 9.30 Hz, 1H), 7.48-7.28 (m, 5H), 3.87 (s, 2H), 3.76 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H), 1.59 (s, 9H).



697

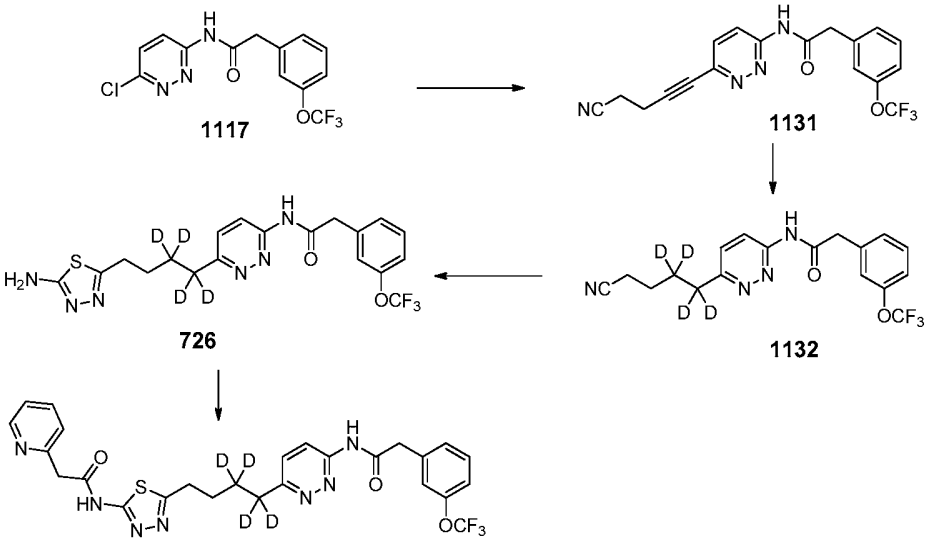
697 was made using procedure described for compound **695**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 14.22 (s, 1H), 12.71 (s, 1H), 11.32 (s, 1H), 9.01 (s, 1H), 8.22-8.19 (d, J = 9.15 Hz, 1H), 7.59-7.26 (m, 6H), 4.04 (s, 2H), 3.87 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.73 (bs, 4H).



689 711

To a suspension of 3-morpholin-4-yl-propionic acid hydrochloride (113 mg, 0.58 mmol) in DMF (8 mL) at 0 °C was added *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (130 mg, 0.67 mmol). The resulting mixture was stirred at 0 °C for 40 min and followed by addition of **689** (300 mg, 0.48 mmol) and 4-DMAP (165 mg, 1.35 mmol). The resulting mixture was stirred from 0 °C to room temperature over a period of 3.5 h before it was diluted with EtOAc and cold water. The organic layer was separated and washed with water (3×15 mL), brine, dried ($MgSO_4$) and concentrated. The crude product was purified by silica gel chromatography eluting with 0–15% MeOH in CH_2Cl_2 to provide **711** (297 mg) as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.75 (bs, 1H), 8.49 (d, J = 9.0 Hz, 1H),

7.64 (s, 1H), 7.50–7.26 (m, 7H), 7.16–7.15 (m, 1H), 6.51 (s, 1H), 4.04 (s, 2H), 3.80–3.72 (m, 4H), 3.88–2.81 (m, 8H), 2.75–2.71 (m, 5H), 1.89 (m, 4H).

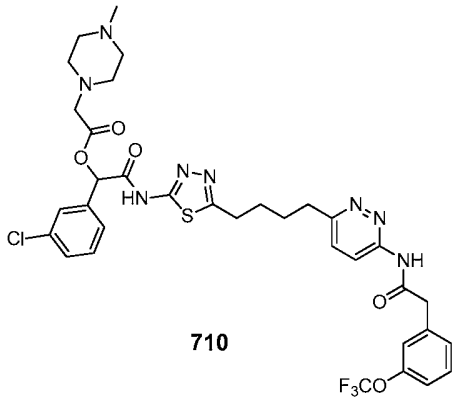


A mixture of **1117** (4.00 g, 12.06 mmol), 4-pentynenitrile (2.11 mL, 24.12 mmol), $PdCl_2(PPh_3)_2$ (847 mg, 1.21 mmol), CuI (184 mg, 0.96 mmol) and Et_3N (13.44 mL, 96.48 mmoL) in DMF (18 mL) was heated at 55 °C for 5 h. The reaction was cooled to room temperature and poured into a mixture of ice-water. The precipitate was collected by suction filtration and air dried. The crude product was further recrystallized from a mixture of i-PrOH-H_2O first and then from i-PrOH to provide alkyne **1131**.

A mixture of alkyne **1131** (6.00 g) and $Pd(OH)_2/C$ (1.00 g) in a mixture of EtOAc (150 mL), THF (75 mL) and MeOH (75 mL) was stirred under 1 atm of D_2 at room temperature for 3 h before the catalyst was filtered off a short plug of SiO_2 and rinsed with EtOAc. The filtrate was concentrated to provide the crude product which was further recrystallized from a mixture of EtOAc and ether to give the desired alkane **1132** as off-white solid (6.01 g)

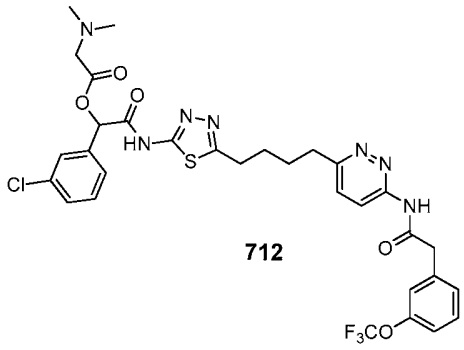
A mixture of nitrile **1132** (5.20 g, 13.61 mmol) and thiosemicarbazide (1.61 g, 17.69 mmol) in TFA (75 mL) was heated at 80 °C for 4 h. The reaction was cooled to room temperature and poured into a mixture of ice-water. The mixture was basified with NaOH pellets (pH 14). The white precipitate was collected by suction filtration, rinsed with water and dried to provide **726** (5.87 g).

To a solution of **726** (1.40 g, 3.07 mmol) and 2-pyridylacetic acid HCl salt (1.49 g, 8.59 mmol) in DMF (20 mL) at 0 °C was added Et_3N (1.50 mL, 10.73 mmol) and followed by 1-propanephosphonic anhydride (2.73 mL, 50% in DMF, 4.29 mmol). This mixture was stirred for 2.5 h at room temperature before it was cooled back to 0 °C and quenched with ice-H_2O. The precipitate was collected by suction filtration and air dried. This crude product was further purified by silica gel chromatography eluting with 0–15% MeOH in DCM to afford **727** (0.97 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.67 (s, 1H), 11.31 (s, 1H), 8.52–8.50 (m, 1H), 8.20 (d, J = 9.2 Hz, 1H), 7.78 (dt, J = 1.8, 7.6 Hz, 1H), 7.58 (d, J = 9.1 Hz, 1H), 7.51–7.26 (m, 6H), 4.02 (s, 2H), 3.87 (s, 2H), 3.03 (t, J = 7.4 Hz, 2H), 1.73 (t, J = 7.4 Hz, 2H).



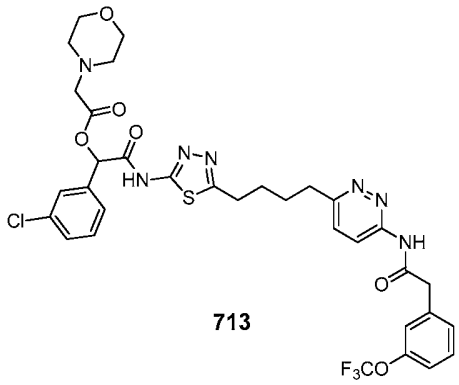
710

Compound **710** was prepared from compound **447** using a procedure analogous to that employed for the preparation of compound **711**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.32 (s, 1H), 8.21-8.18 (d, J = 9.06 Hz, 1H), 7.62-7.26 (m, 9H), 6.16 (s, 1H), 3.87 (s, 2H), 3.52-3.50 (d, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.80-2.71(m, 11H), 1.73 (bs, 4H).



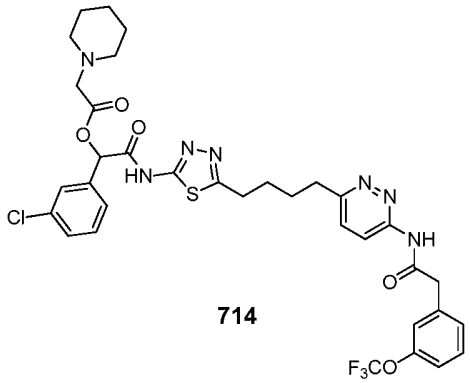
712

Compound **712** was prepared from compound **447** using a procedure analogous to that employed for the preparation of compound **711**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.32 (s, 1H), 8.21-8.18 (d, J = 9.06 Hz, 1H), 7.62-7.26 (m, 9H), 6.16 (s, 1H), 3.87 (s, 2H), 3.38-3.36 (d, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.29 (s, 6H), 1.73 (bs, 4H).



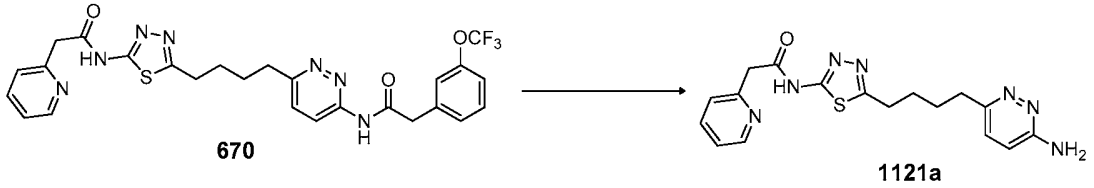
713

Compound **713** was prepared from compound **447** using a procedure analogous to that employed for the preparation of compound **711**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.11 (bs, 1H), 11.32 (s, 1H), 8.21-8.18 (d, J = 9.06 Hz, 1H), 7.62-7.26 (m, 9H), 6.16 (s, 1H), 3.87 (s, 2H), 3.60-3.57 (m, 4H), 3.44-3.42 (d, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.55-2.51 (m, 4H), 1.73 (bs, 4H).



714

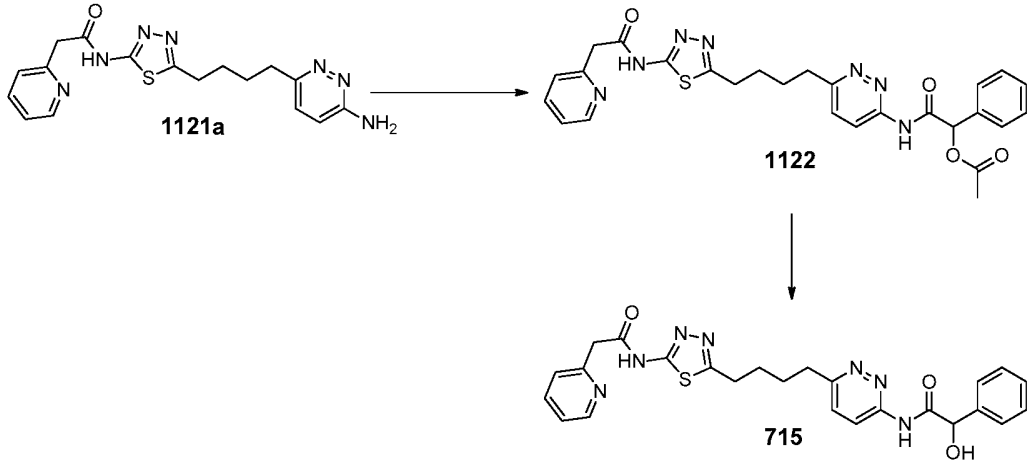
Compound **714** was prepared from compound **447** using a procedure analogous to that employed for the preparation of compound **711**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.32 (s, 1H), 8.21-8.18 (d, J = 9.06 Hz, 1H), 7.62-7.26 (m, 9H), 6.16 (s, 1H), 3.87 (s, 2H), 3.38-3.31 (d, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.49-2.47 (m, 4H), 1.93 (bs, 4H), 1.73 (bs, 4H), 1.72 (bs, 2H).



670 1121a

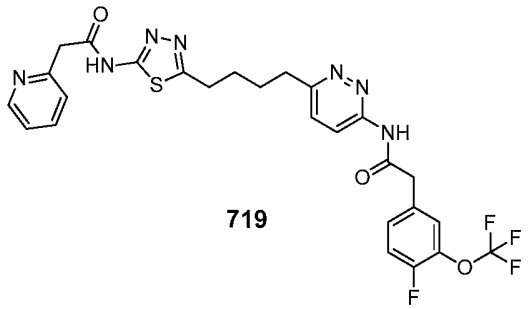
To a suspension of **670** (3 g, 5.24 mmol) in MeOH (50 ml) at 0 ^{0}C was added 2N NaOH (20 ml) solution. The resulting mixture was stirred at room temperature overnight. The solvent was evaporated under vacuo and the mixture was acidified

with 1N HCl to pH 6. The white precipitate was collected by suction filtration, rinsed with more water and dried to afford **1121a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66 (s, 1H), 8.51-8.50 (m, 1H), 7.81-7.76 (m, 1H), 7.42-7.28 (m, 2H), 7.16-7.13 (d, 1H), 6.73-6.70 (d, 1H), 6.10 (s, 2H), 4.0 (s, 2H), 3.01 (bs, 2H), 2.71 (bs, 2H), 1.70 (bs, 4H).



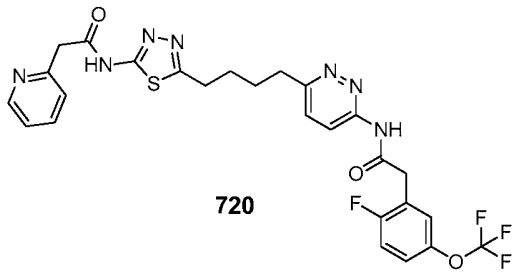
To a solution of **1121a** (20 mg, 0.054 mmol) in DMF (1 ml) at 0 ^{0}C was added triethylamine (11 ul, 0.081 mmol) drop wise followed by o-acetylmandelic acid chloride (15 ul, 0.065 mmol) drop wise. The resulting mixture was slowly warmed up to room temperature and stirred for 1 h before it was quenched by addition of water (~3 mL) at 0 ^{0}C. The mixture was partitioned between water and EtOAc. The organic extract was washed with brine, dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel chromatography eluting with 0-5% MeOH in DCM to afford **1122**.

A flask was charged with **1122** (20 mg, 0.037 mmol) and 2N ammonia in MeOH (5 ml). The mixture was stirred at room temperature for 2 hours. The solvent was evaporated under vacuo and the mixture was triturated with ether. The white precipitate was collected by suction filtration, rinsed with ether and dried to afford **715.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66 (s, 1H), 10.61 (s, 1H), 8.51-8.50 (m, 1H), 8.21-8.18 (d, J = 9.06 Hz, 1H), 7.81-7.76 (m, 1H), 7.61-7.53 (m, 3H), 7.42-7.28 (m, 5H), 6.49-6.47 (d, 1H), 5.30-5.28 (d, 1H), 4.0 (s, 2H), 3.02 (bs, 2H), 2.91 (bs, 2H), 1.75 (bs, 4H).



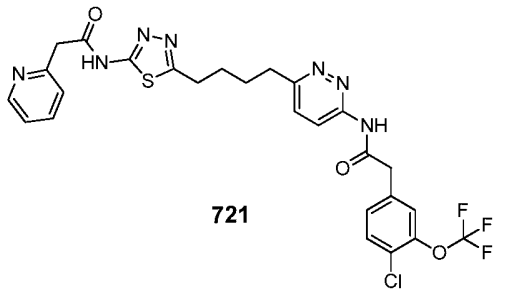
719

Compound **719** was prepared using a procedure analogous to that employed for the preparation of compound **670**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66 (s, 1H), 11.32 (s, 1H), 8.51-8.50 (m, 1H), 8.21-8.18 (d, J = 9.06 Hz, 1H), 7.79-7.76 (m, 1H), 7.59-7.30 (m, 6H), 4.0 (s, 2H), 3.87 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.75 (bs, 4H).



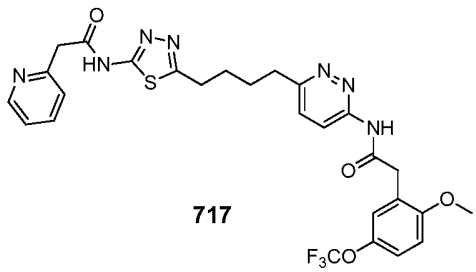
720

Compound **720** was prepared using a procedure analogous to that employed for the preparation of compound **670**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66 (s, 1H), 11.32 (s, 1H), 8.51-8.50 (m, 1H), 8.19-8.16 (d, J = 9.06 Hz, 1H), 7.79-7.76 (m, 1H), 7.59-7.30 (m, 6H), 4.01 (s, 2H), 3.95 (s, 2H), 3.03 (bs, 2H), 2.91 (bs, 2H), 1.76 (bs, 4H).



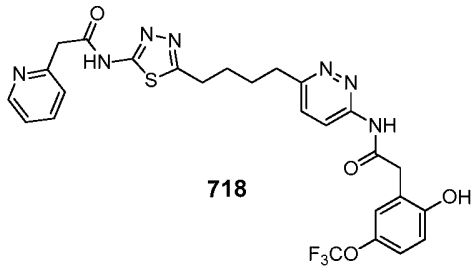
721

Compound **721** was prepared using a procedure analogous to that employed for the preparation of compound **670**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66 (s, 1H), 11.32 (s, 1H), 8.51-8.50 (m, 1H), 8.21-8.16 (d, J = 9.06 Hz, 1H), 7.81-7.28 (m, 7H), 4.01 (s, 2H), 3.89 (s, 2H), 3.03 (bs, 2H), 2.91 (bs, 2H), 1.76 (bs, 4H).



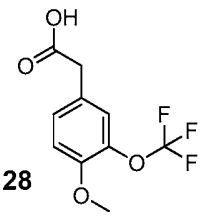
717

Compound **717** was prepared using a procedure analogous to that employed for the preparation of compound **670**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66 (s, 1H), 11.17 (s, 1H), 8.52-8.50 (m, 1H), 8.19-8.16 (d, J = 9.06 Hz, 1H), 7.81-7.76 (m, 1H), 7.58-7.55 (d, 1H), 7.42-7.09 (m, 4H), 7.08-7.06 (d, 1H), 4.01 (s, 2H), 3.83 (s, 2H), 3.79 (s, 3H), 3.03 (bs, 2H), 2.91 (bs, 2H), 1.76 (bs, 4H).



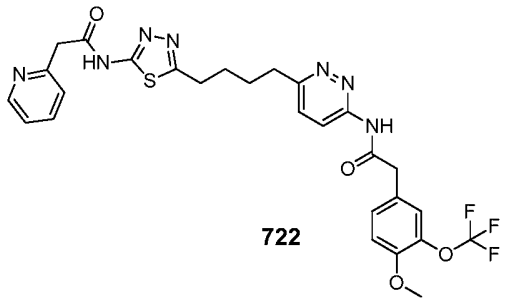
718

To a solution of **717** (10 mg, 0.017 mmol) in DCM (3 ml) at 0 ^{0}C was added boron tribromide solution (1N in DCM) (2 ml) drop wise. The resulting mixture was slowly warmed up to room temperature and stirred for 4.5 h before it was quenched by addition of water (~3 mL). The mixture was then basified with 1N NaOH to pH 8. The mixture was partitioned between water and DCM. The organic extract was washed with brine, dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel chromatography eluting with 0-10% MeOH in DCM to afford **718**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.17 (s, 1H), 8.52-8.50 (m, 1H), 8.21-8.18 (d, J = 9.06 Hz, 1H), 7.81-7.76 (m, 1H), 7.58-7.55 (d, 1H), 7.51-7.09 (m, 4H), 6.88-6.85 (d, 1H), 4.0 (s, 2H), 3.79 (s, 2H), 3.03 (bs, 2H), 2.91 (bs, 2H), 1.76 (bs, 4H).



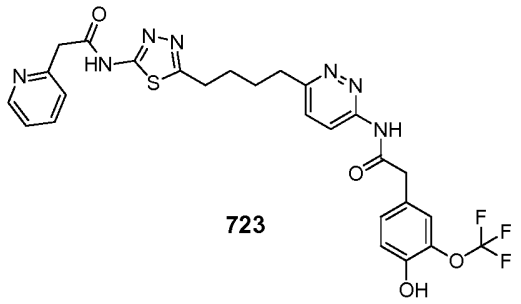
1128

Compound **1128** was prepared from 4-bromo-2-trifluoromethoxyanisole using a procedure analogous to that for compound **1124** below.



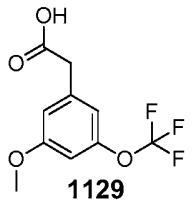
722

Compound **722** was prepared using compound **1128** with a procedure analogous to that for compound **670**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66 (s, 1H), 11.17 (s, 1H), 8.52-8.50 (m, 1H), 8.21-8.18 (d, J = 9.06 Hz, 1H), 7.81-7.76 (m, 1H), 7.58-7.55 (d, 1H), 7.42-7.19 (m, 5H), 4.0 (s, 2H), 3.85 (s, 3H), 3.79 (s, 2H), 3.03 (bs, 2H), 2.91 (bs, 2H), 1.76 (bs, 4H).



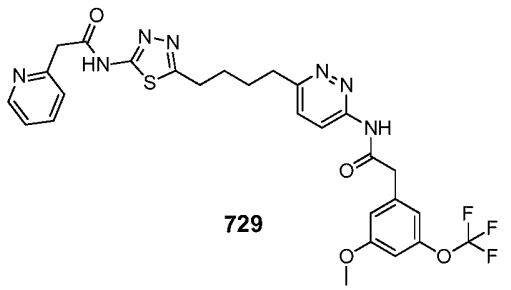
723

Compound **723** was prepared from compound **722** using a procedure analogous to that for the preparation of compound **718** above. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66 (s, 1H), 11.17 (s, 1H), 10.06 (s, 1H), 8.52-8.50 (m, 1H), 8.21-8.18 (d, J = 9.06 Hz, 1H), 7.81-7.76 (m, 1H), 7.58-7.55 (d, 1H), 7.42-7.19 (m, 4H), 6.99-6.96 (d, 1H), 4.0 (s, 2H), 3.70 (s, 2H), 3.03 (bs, 2H), 2.91 (bs, 2H), 1.76 (bs, 4H).



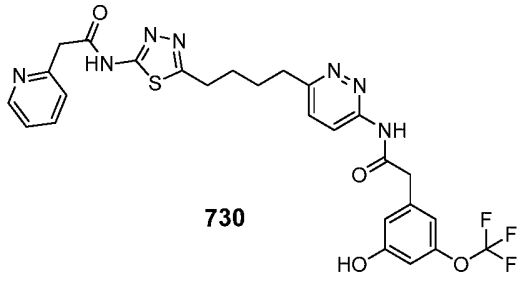
1129

Compound **1129** was prepared from 3-bromo-5-trifluoromethoxyanisole using a procedure analogous to that for compound **1126** below.



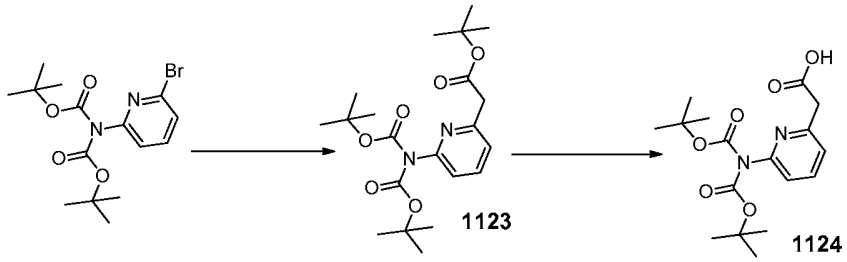
729

Compound **729** was prepared using compound **1129** with a procedure analogous to that for compound **670**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66 (s, 1H), 11.28 (s, 1H), 8.52-8.50 (m, 1H), 8.21-8.18 (d, J = 9.06 Hz, 1H), 7.81-7.76 (m, 1H), 7.58-7.55 (d, 1H), 7.42-7.29 (m, 2H), 6.99-6.95 (m, 2H), 6.84 (s, 1H), 4.0 (s, 2H), 3.80 (m, 5H), 3.03 (bs, 2H), 2.91 (bs, 2H), 1.76 (bs, 4H).



730

Compound **730** was prepared from compound **729** using a procedure analogous to that for the preparation of compound **718** above. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.66 (s, 1H), 11.28 (s, 1H), 10.04 (s, 1H), 8.52-8.50 (m, 1H), 8.21-8.18 (d, J = 9.06 Hz, 1H), 7.81-7.76 (m, 1H), 7.58-7.55 (d, 1H), 7.42-7.29 (m, 2H), 6.81-6.78 (m, 2H), 6.61 (s, 1H), 4.0 (s, 2H), 3.74 (m, 2H), 3.03 (bs, 2H), 2.91 (bs, 2H), 1.76 (bs, 4H).

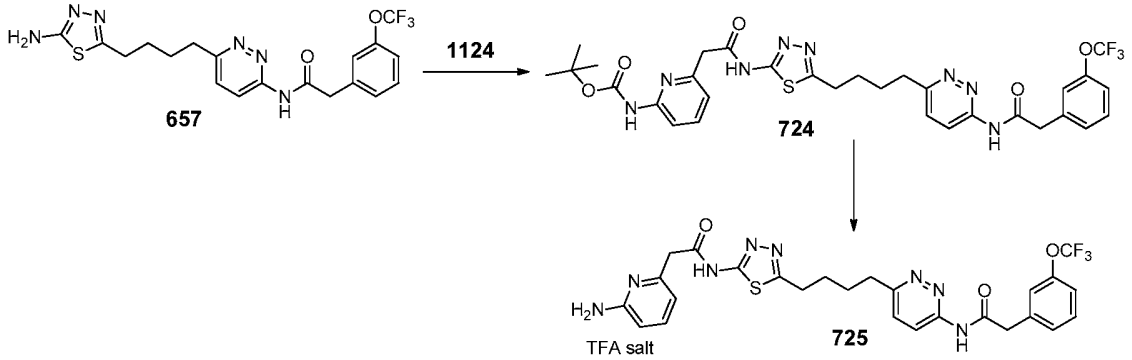


1123 1124

To a mixture of 6-(di-Boc-amino)-2-bromopyridine (1 g, 2.9 mmol), bis(tri-tert-butylphosphine) palladium(0) (300 mg, 0.59 mmol) in 1,4-dioxane (30 ml) under argon atmosphere was added 0.5 M of 2-tert-butoxy-2-oxoethyl zinc chloride in ether (15 ml). The resulting mixture was stirred at room temperature overnight. The mixture was partitioned between saturated NH_4Cl and EtOAc. The organic extract was washed with brine, dried over sodium sulfate, filtered and evaporated. The crude

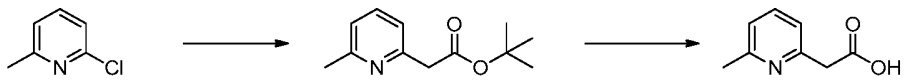
material was purified by silica gel chromatography eluting with 0-20% EtOAc in Hexane to afford **1123**.

To a solution of **1123** (150 mg, 0.37 mmol) in MeOH (6 ml) and water (2 ml) at 0 ^{0}C was added Lithium hydroxide monohydrate (100 mg, 2.38 mmol). The resulting mixture was stirred at room temperature for 2 days before it was evaporated to dryness. The mixture was then acidified with 1N HCl (pH 4), and it was partitioned between water and EtOAc. The organic extract was washed with water, dried over sodium sulfate, filtered and evaporated to afford **1124**.



A flask was charged with **657** (105 mg, 0.232 mmol), **1124** (90 mg, 0.255 mmol) in DMF (1 ml) at 0 ^{0}C was added propylphosphonic anhydride solution (300 ul) followed by triethylamine (89 ul, 0.64 mmol). The resulting mixture was slowly warmed up to room temperature and stirred for 3 h before it was quenched by addition of ice water (~5 mL). The precipitate was collected by suction filtration, rinsed with more water. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in DCM to afford **724**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.67 (s, 1H), 11.32 (s, 1H), 9.69 (s, 1H), 8.22-8.19 (d, J = 9.12 Hz, 1H), 7.72-7.01 (m, 8H), 3.91-3.87 (d, 4H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.75 (bs, 4H) 1.47 (s, 9H).

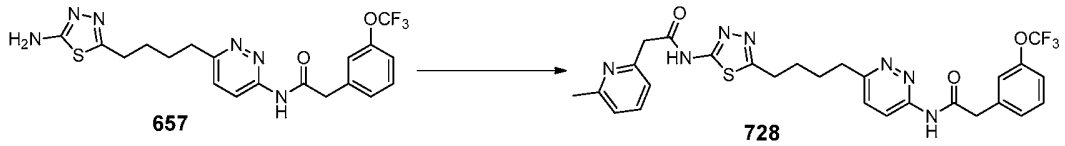
To a solution of **724** (50 mg, 0.07 mmol) in DCM (3 ml) at 0 ^{0}C was added TFA (3 ml) dropwise. The resulting mixture was stirred at room temperature for 3 h before it was evaporated to dryness then triturated the residue with ether to afford **725**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.67 (s, 1H), 11.32 (s, 1H), 8.22-8.19 (d, J = 9.12 Hz, 1H), 7.88-7.77 (m, 3H), 7.59-7.26 (m, 5H), 6.90-6.80 (m, 2H), 4.05 (s, 2H), 3.87 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.75 (bs, 4H).



1125 1126

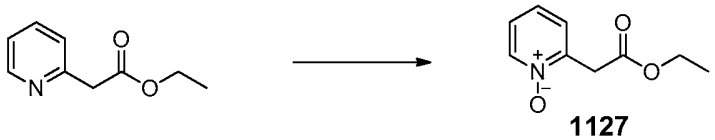
To a stirred solution of tert-butyl acetate (789 ul, 5.88 mmol), 2-chloro-6-methylpyridine (428 ul, 3.92 mmol), chloro(2-di-t-butylphosphino-2',4',6'-tri-1-propyl-1,1'-bi-phenyl)[2-(2-aminoethyl)phenyl]palladium(II) (27 mg, 0.039 mmol) in toluene (10 ml) at 0 ^{0}C under argon was added a solution of LHMDS (1M in toluene) (12 ml, 12 mmol) pre-cooled to 0 ^{0}C. The resulting mixture was stirred for 1 h. The mixture was partitioned between saturated NH_4Cl and EtOAc. The organic extract was washed with brine, dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel chromatography eluting with 0-15% EtOAc in Hexane to afford **1125**.

To a solution of **1125** (267 mg, 1.29 mmol) in DCM (3 ml) at 0 ^{0}C was added TFA (1.5 ml) dropwise. The resulting mixture was stirred at room temperature overnight before it was evaporated to dryness then triturated the residue with ether to afford **1126**.



657 728

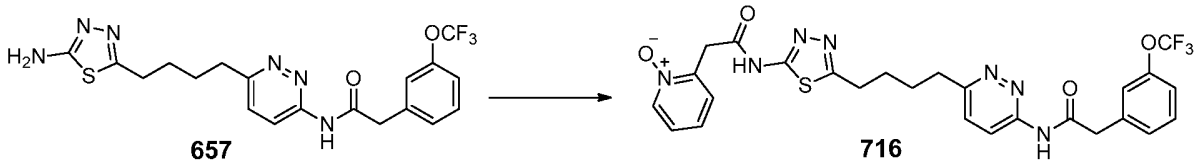
A flask was charged with **657** (50 mg, 0.111 mmol), **1126** (35 mg, 0.133 mmol) in DMF (1 ml) at 0 ^{0}C was added propylphosphonic anhydride solution (155 ul) followed by triethylamine (57 ul, 0.4 mmol). The resulting mixture was slowly warmed up to room temperature and stirred for 3 h before it was quenched by addition of ice water (~5 mL). The precipitate was collected by suction filtration, rinsed with more water. The crude material was purified by silica gel chromatography eluting with 0-6% MeOH in DCM to afford **728**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.67 (s, 1H), 11.32 (s, 1H), 8.22-8.19 (d, J = 9.12 Hz, 1H), 7.69-7.15 (m, 8H), 3.96 (s, 2H), 3.87 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 2.52 (s, 3H), 1.75 (bs, 4H).



1127

To a solution of ethyl 2-pyridyl acetate (1 g, 6.05 mmol) in DCM (20 ml) at 0 ^{0}C was added MCPBA (77% max) (1.77 g, 10.2 mmol). The resulting mixture was warmed up to room temperature for 3 h before it was partitioned between saturated sodium

bicarbonate and DCM. The organic extract was washed with brine, dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel chromatography eluting with 0-12% MeOH in EtOAc to afford **1127**.



657 **716**

To a suspension of **657** (331 mg, 0.73 mmol) in toluene was added **1127** (278 mg, 1.53 mmol) followed by trimethylaluminum (2M in toluene) (732 ul, 1.46 mmol). The resulting mixture was stirred at 60 ^{0}C overnight. The reaction mixture was partitioned between water and DCM. The organic extract was washed with brine, dried over sodium sulfate, filtered and evaporated. The crude material was purified by silica gel chromatography eluting with 0-5% MeOH in DCM then 0-15% MeOH in EtOAc to afford **716**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.67 (s, 1H), 11.32 (s, 1H), 8.29-8.27 (m, 1H), 8.21-8.19 (d, J = 9.12 Hz, 1H), 7.61-7.26 (m, 8H), 4.03 (s, 2H), 3.87 (s, 2H), 3.01 (bs, 2H), 2.90 (bs, 2H), 1.75 (bs, 4H).

Preparative HPLC Purification

All reverse phase preparative HPLC purifications were performed using a Shimadzu Prominence Preparative Liquid Chromatograph with the column at ambient temperature. Mobile phases A and B consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. Crude product mixtures were dissolved in DMF, DMSO or mixtures thereof at concentrations of approximately 100 mg/mL and chromatographed according to the methods described in Table 2. Appropriate chromatographic fractions were then evaporated under high vacuum at 45° C using a Savant Speed Vac Plus Model SC210A to yield purified products.

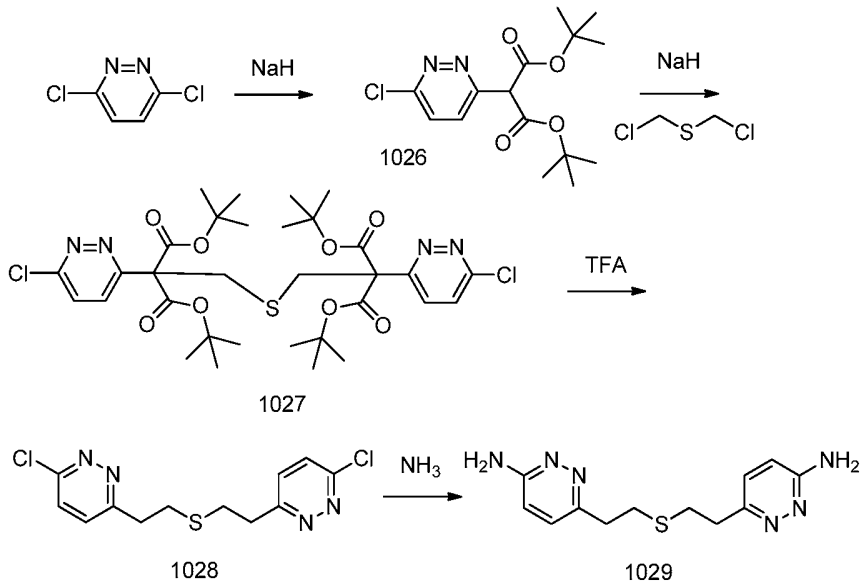
TABLE 2: Preparative HPLC Method Descriptions

Compound ID	Column	Time (min)	%MPB	Flow Rate (mL/min)	Product Retention Time (min)
7	1	0	20	2	7.4

		1	20	2	
		2	20	5	
		3	70	5	
		14	100	5	
8	1	0	20	2	11.5
		1	20	2	
		2	20	5	
		3	70	5	
		14	100	5	
26	1	0	40	1	6
		1	40	2	
		3.5	40	4	
		4	40	4.73	
		10	90	4.73	
29	2	0	40	2	7.7
		1	40	3	
		2	40	18.9	
		13	50	18.9	
36	2	0	32	3	12.1
		0.5	32	5	
		1	32	18.9	
		13	35	18.9	
143	2	0	50	3	9.1
		1	50	3	
		2	50	18.9	
		5	50	18.9	
		15	80	18.9	
153	2	0	35	3	6.2
		1	35	3	
		2	35	18.9	
		4	35	18.9	
		14	75	18.9	

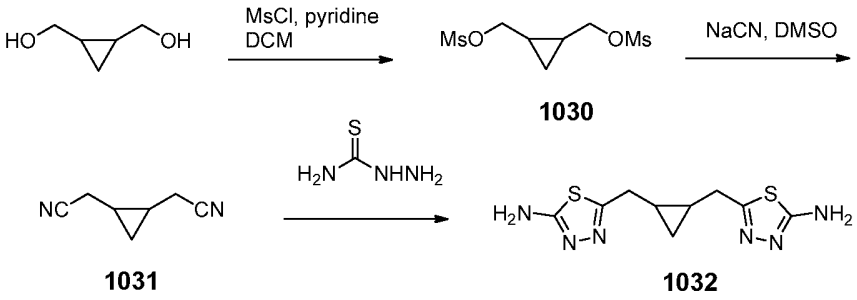
199	2	0	45	3	8.3
		1	45	3	
		2	45	18.9	
		3	45	18.9	
		13	65	18.9	
203	2	0	50	3	9.6
		1	50	3	
		2	50	18.9	
		5	50	18.9	
		15	60	18.9	
208	2	0	35	3	7.6
		1	35	3	
		2	35	18.9	
		4	35	18.9	
		14	50	18.9	

The following representative synthetic protocols may also be used for producing compounds of the invention.

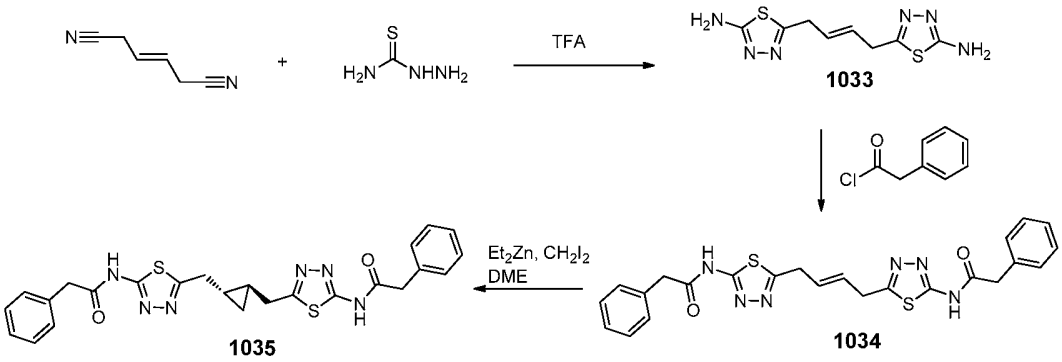


3,6-Dichloropyridazine is treated with di-*tert*butyl malonate and sodium hydride in THF or DMF to give 1026. Intermediate 1026 is then treated with sodium hydride in THF or DMF followed by bis-(chloromethyl)sulfide to give 1027. Intermediate 1027

is treated with TFA in dichloromethane to give 1028. Intermediate 1028 is treated with ammonia to give 1029. Intermediate 1028 is also converted to 1029 by sequential treatment with 2, 4-dimethoxybenzyl amine and TFA. The bis-amino intermediate 1029 may be converted to acylated products analogous to those described in Table 3 using the methods described in Synthetic Protocols section above for acylation of **1001-1008**.



Both *trans*- and *cis*-cyclopropane-1,2-diyldimethanols are converted into the corresponding bis-nitrile 1031 via bis-mesylated intermediate 1030. The bismesylate intermediate 1030 is prepared by treating the diol with methanesulfonyl chloride in the presence of pyridine or triethylamine in dichloromethane. The bisnitrile 1031 is prepared by treating 1030 with sodium cyanide in DMSO or ethanol/water. Using a procedure similar to that described for the preparation **1001**, bis-nitrile 1031 undergoes cyclization with thiosemicarbazide in TFA to provide bis-amino intermediate 1032**.** The bis-amino intermediate 1032 may be converted to acylated products analogous to those described in Table 3 using the methods described in Synthetic Protocols section above for acylation of **1001-1008**.



The alkene analog 1033 is prepared from *trans*-3-hexenedinitrile using a procedure similar to that described for the preparation **1001**. The bis-amino intermediate 1033

may be converted to acylated products analogous to those described in Table 3 (for example, 1034) using the methods described in Synthetic Protocols section above for acylation of **1001-1008**. The products may be further converted to cyclopropyl analogs (exemplified by 1035) under the Simmons-Smith conditions (Et_2Zn, CH_2I_2,1,2-dimethoxyethane).

Example 2: Compound Assays

Compounds were assayed in both an in vitro biochemical assay and a cell proliferation assay as follows. The IC50 results are provided in Table 3.

Recombinant Enzyme assay

Compounds were assessed for their ability to inhibit the enzymatic activity of a recombinant form of Glutaminase 1 (GAC) using a biochemical assay that couples the production of glutamate (liberated by GAC) to glutamate dehydrogenase (GDH) and measuring the change in absorbance for the reduction of NAD^+ to NADH. Substrate solution was prepared (50 mM Tris-HCl pH 8.0, 0.2 mM EDTA, 150 mM K_2HPO_4, 0.1 mg/ml BSA, 1 mM DTT, 20mM L-glutamine, 2 mM NAD^+, and 10 ppm antifoam) and 50 μL added to a 96-well half area clear plate (Corning #3695). Compound (2 μL) was added to give a final DMSO concentration of 2% at 2X the desired concentration of compound. Enzymatic reaction was started with the addition of 50 μL of enzyme solution (50 mM Tris-HCl pH 8.0, 0.2 mM EDTA, 150 mM K_2HPO_4, 0.1 mg/ml BSA, 1 mM DTT, 10 ppm antifoam, 4 units/ml GDH, 4 mM adenosine diphosphate, and 4 nM GAC) and read in a Molecular Devices M5 plate reader at 20°C. The plate reader was configured to read absorbance (λ=340 nm) in kinetic mode for 15 minutes. Data was recorded as milli-absorbance units per minute and slopes were compared to a control compound and a DMSO-only control on the same plate. Compounds with slopes less than the DMSO control were considered inhibitors and plate variability was assessed using the control compound.

Results from this assay for several compounds of the invention are shown in Tables 3a and 3b, expressed as IC50, or half maximal inhibitory concentration, wherein IC50 is a quantitative measure indicating how much compound is needed to inhibit a given biological activity by half.

Recombinant Enzyme assay – Time Dependence

Compounds were assessed for their ability to inhibit the enzymatic activity of a recombinant form of Glutaminase 1 (GAC) using a biochemical assay that couples the production of glutamate (liberated by GAC) to glutamate dehydrogenase (GDH) and measuring the change in absorbance for the reduction of NAD^+ to NADH. Enzyme solution was prepared (50 mM Tris-HCl pH 8.0, 0.2 mM EDTA, 150 mM K_2HPO_4, 0.1 mg/ml BSA, 1 mM DTT, 10 ppm antifoam, 4 units/ml GDH, 4 mM adenosine diphosphate, and 4 nM GAC) and 50 μL added to a 96-well half area clear plate (Corning #3695). Compound (2 μL) was added to give a final DMSO concentration of 2% at 2X the desired concentration of compound. The enzyme/compound mix was sealed with sealing foil (USA Scientific) and allowed to incubate, with mild agitation, for 60 minutes at 20°C. Enzymatic reaction was started with the addition of 50 μL of substrate solution (50 mM Tris-HCl pH 8.0, 0.2 mM EDTA, 150 mM K_2HPO_4, 0.1 mg/ml BSA, 1 mM DTT, 20mM L-glutamine, 2 mM NAD^+, and 10 ppm antifoam) and read in a Molecular Devices M5 plate reader at 20°C. The plate reader was configured to read absorbance (λ=340 nm) in kinetic mode for 15 minutes. Data was recorded as milli-absorbance units per minute and slopes were compared to a control compound and a DMSO-only control on the same plate. Compounds with slopes less than the DMSO control were considered inhibitors and plate variability was assessed using the control compound.

Results from this assay for several compounds of the invention are shown in Tables 3a and 3b, expressed as IC50, or half maximal inhibitory concentration, wherein IC50 is a quantitative measure indicating how much compound is needed to inhibit a given biological activity by half.

Cell proliferation assay

P493-6 (myc "on") cells were maintained in growth media (RPMI-1640, 10%FBS, 2mM glutamine, 100 units/ml Penicillin and 100μg/ml streptomycin) at 37°C with 5% CO_2. For compound assay, P493-6 cells were plated in 96-well V-bottom plates on the day of compound addition in 50 μl of growth media at a cell density of 200,000 cells/ml (10,000 cells/well). Compounds were serially diluted in 100% DMSO at 200-times the final concentration. Compounds were diluted 100-fold into growth media and then 50 μl of this mixture was added to cell plates making the final concentration of DMSO 0.5%. Cells were incubated with compound for 72 hrs at 37°C with 5% CO_2 and analyzed for antiproliferative effects either by Cell Titer

Glo (Promega) or FACS analysis using the Viacount (Millipore) kit on the Guava instrument.

Results from this assay for several compounds of the invention are shown in Tables 3a and 3b, expressed as IC50, or half maximal inhibitory concentration, wherein IC50 is a quantitative measure indicating how much compound is needed to inhibit a given biological activity by half.

Modified Recombinant Enzyme assay – Time Dependence

Compounds were assessed for their ability to inhibit the enzymatic activity of a recombinant form of glutaminase using a biochemical assay that couples the production of Glu (liberated by glutaminase) to GDH and measures the increase in fluorescence due to the reduction of NADP+ to NADPH.

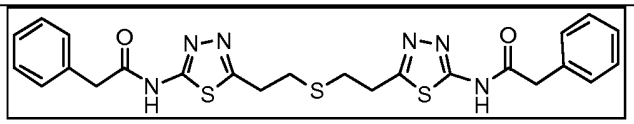
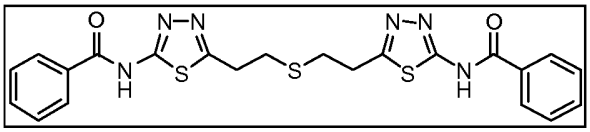
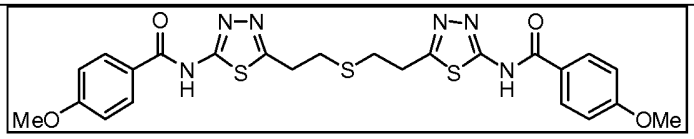
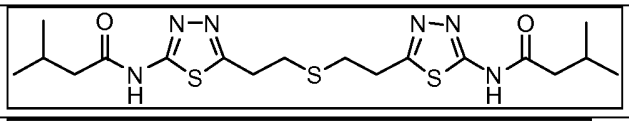
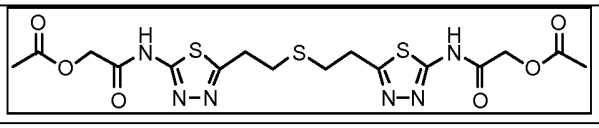
Assay Set-up: Glutaminase reaction buffer was prepared [50 mM Tris-HCl pH 8.8, 150 mM K2HPO4, 0.25 mM EDTA, 0.1 mg/ml BSA (Calbiochem no. 2960), 1 mM DTT, 2 mM NADP+ (Sigma Aldrich no. N5755), and 0.01% TX-100] and used to make 3x-enzyme-containing solution, 3x-substrate-containing solution, and 3x-inhibitor-containing solution (see below). Inhibitor-containing solution was made by diluting DMSO stocks of compounds into the glutaminase reaction buffer to create a 3x inhibitor solution containing 6% DMSO. 3x-enzyme-containing solution was made by diluting recombinant glutaminase and GDH from Proteus species (Sigma Aldrich no. G4387) into glutaminase buffer to create a 6 nM glutaminase plus 18 units/mL GDH solution. A 3x substrate solution containing either Gln, Glu, or NADPH was made by diluting a stock of Gln (Sigma Aldrich no. 49419), Glu (Sigma Aldrich no. 49449), or NADPH (Sigma Aldrich no. N1630) into glutaminase reaction buffer to create a 3x-substrate solution. Reactions were assembled in a 384-well low-volume black microtiter plates (Molecular Devices no. 0200-5202) by mixing 5 μL of inhibitor-containing solution with 5 μL of substrate-containing solution followed by 5 μL of enzyme-containing solution when no preincubation was required. When time-dependent effects of compound inhibition were tested, enzyme-containing solution was treated with inhibitor-containing solution for the indicated time prior to addition of substrate-containing solution.

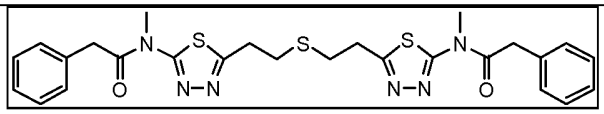
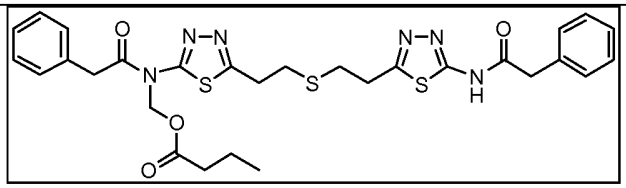
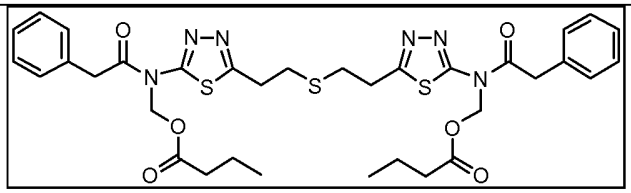
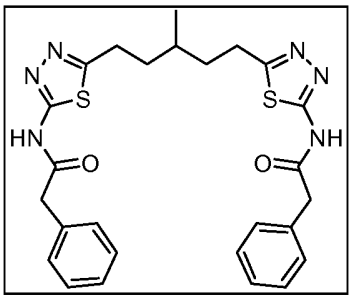
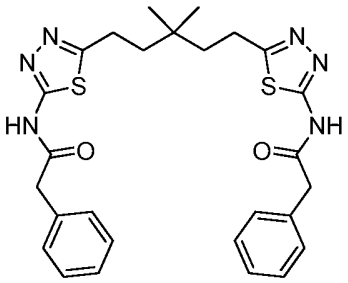
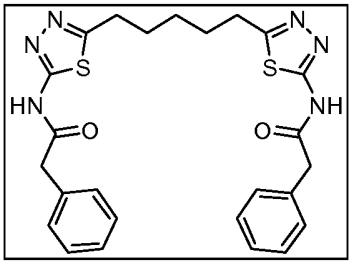
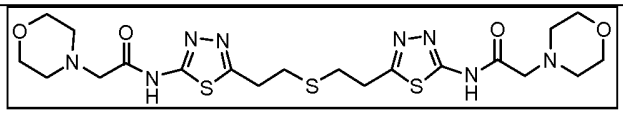
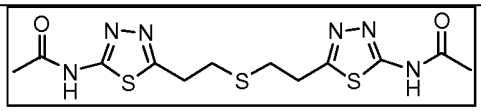
Measurement of glutaminase activity: Following the mixture of all three components, fluorescence increase (Ex: 340 nM, Em:460 nm) was recorded for 15 min at room temperature using the Spectromax M5e (Molecular Devices).

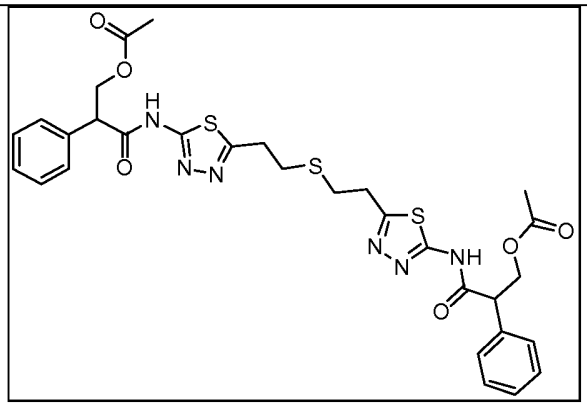
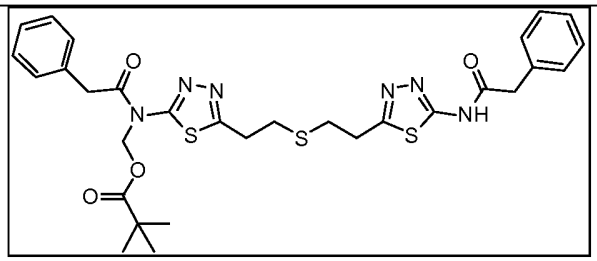
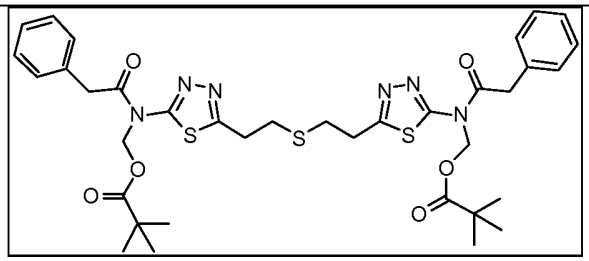
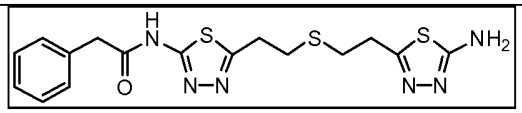
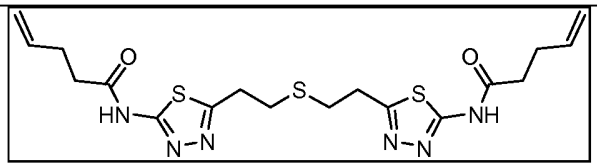
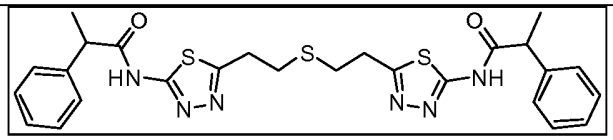
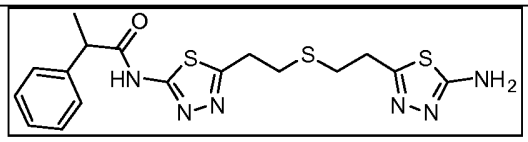
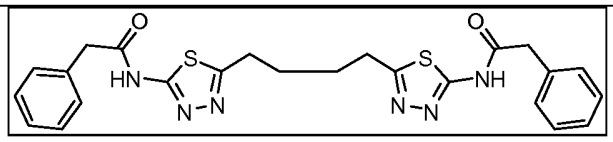
IC50 Determination: The initial velocities of each progress curve were calculated using a straight line equation (Y=Yintercept + (slope) * X). Initial velocity values were plotted against compound concentration and fit to a four parameter dose response equation (% activity =Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope))) to calculate an IC50 value.

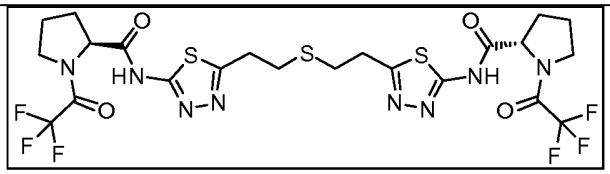
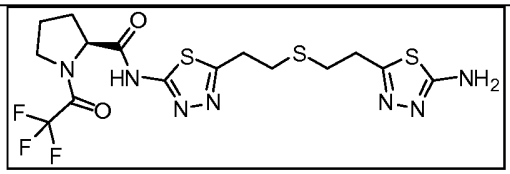
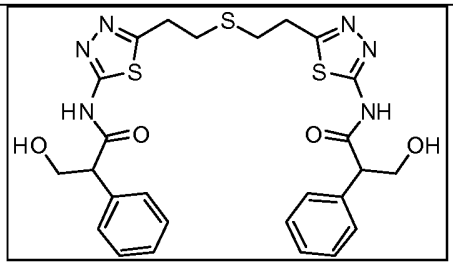
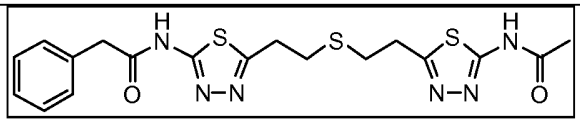
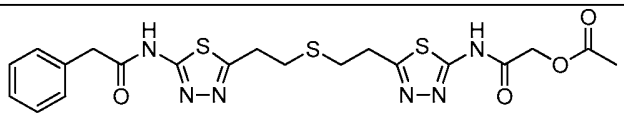
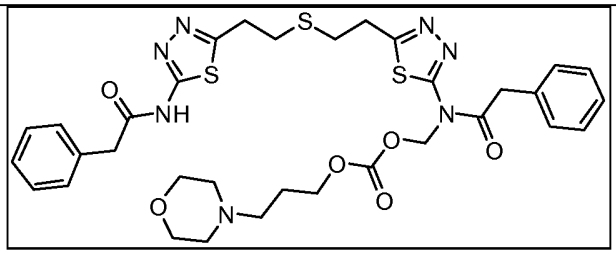
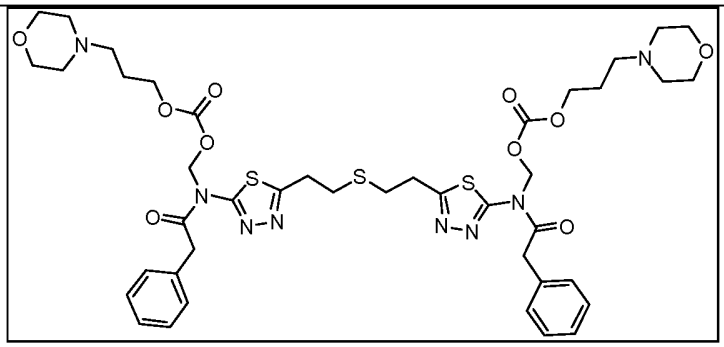
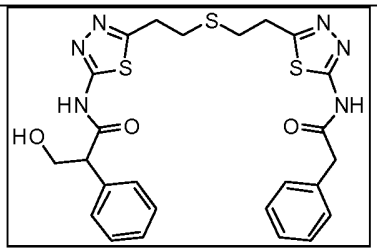
Results from this assay for several compounds are shown in Tables 3a and 3b, expressed as IC50, or half maximal inhibitory concentration, wherein IC50 is a quantitative measure indicating how much compound is needed to inhibit a given biological activity by half.

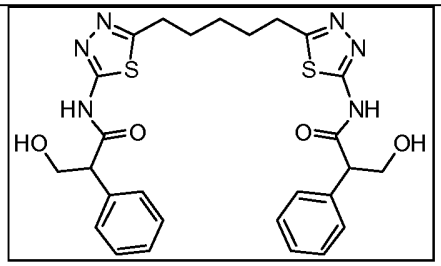
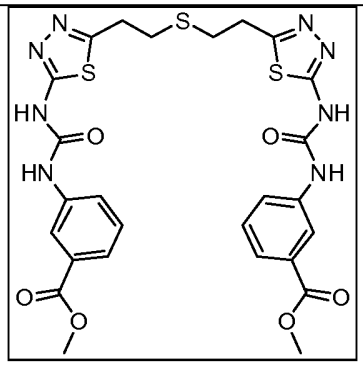
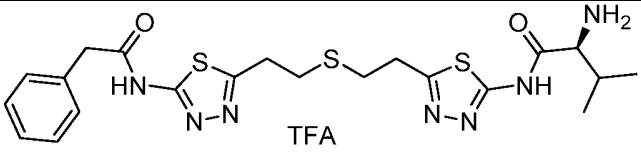
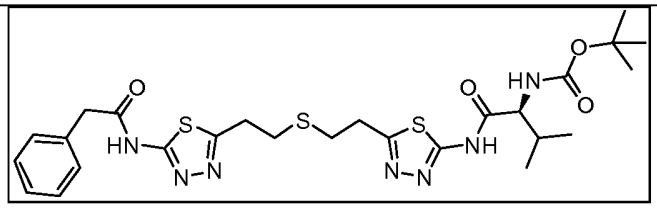
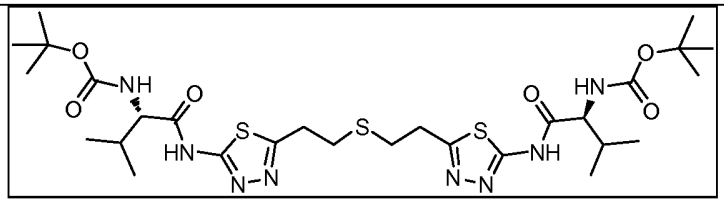
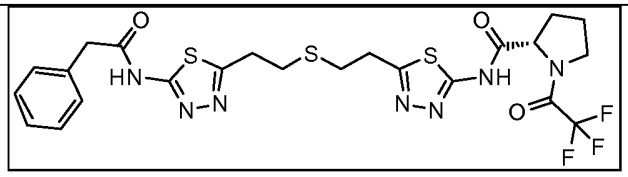
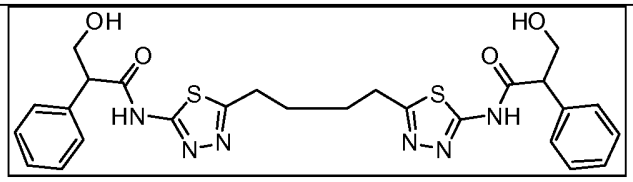
Table 3a:

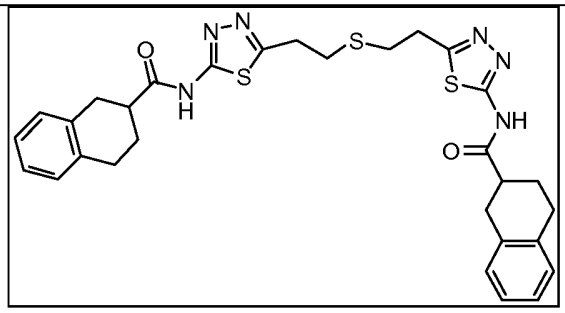
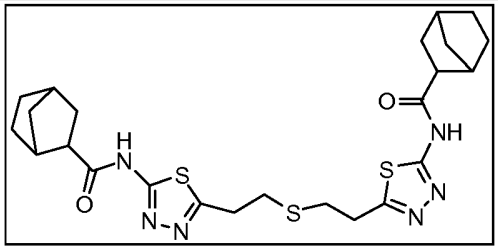
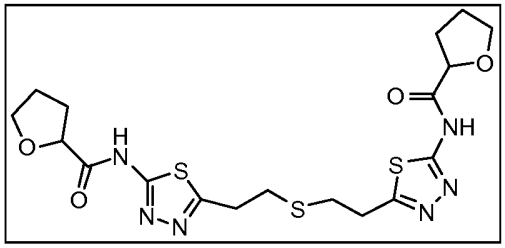
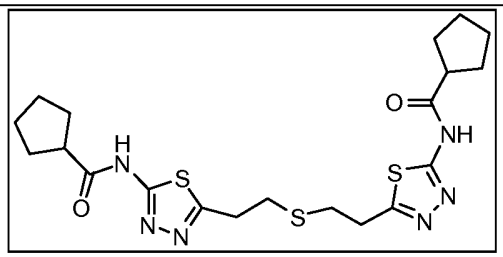
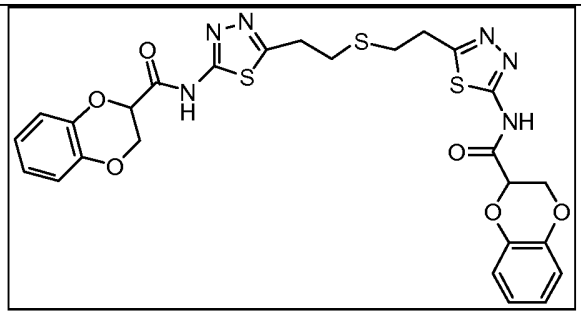
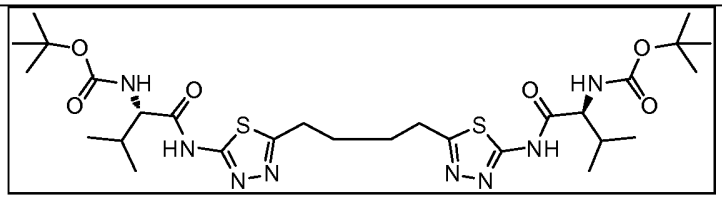
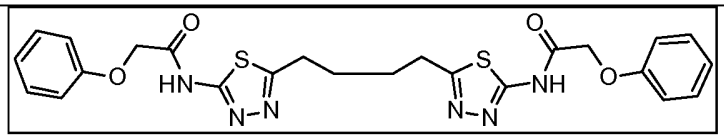
Cmpd ID	Structure	GAC Delta N2 IC50 60 min preinc (μM)	GAC Delta N2 IC50 no preinc (μM)	Cell prolif P493 72h IC50 (μM)
1		0.10	0.20	0.47
2			4.1	0.63
3			>50	>50
4			13	>50
5			>50	>50

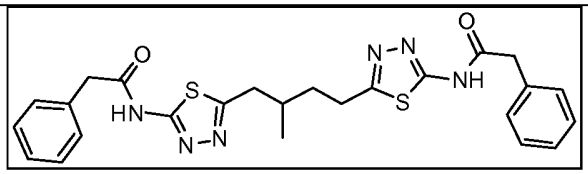
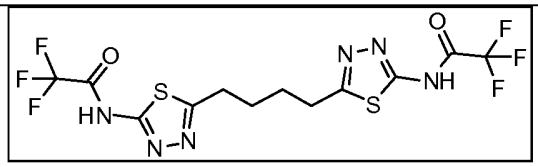
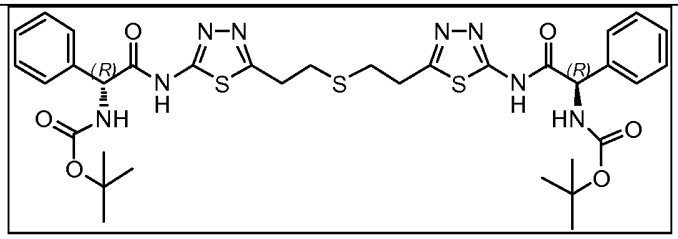
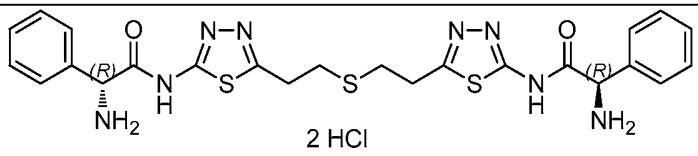
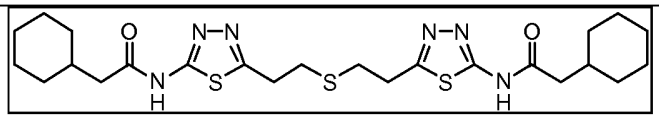
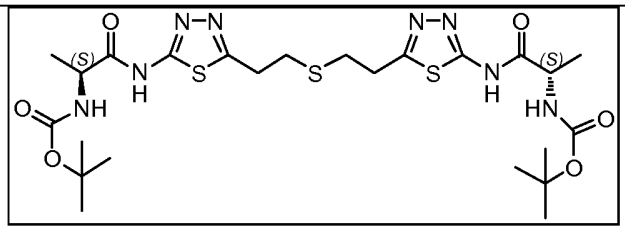
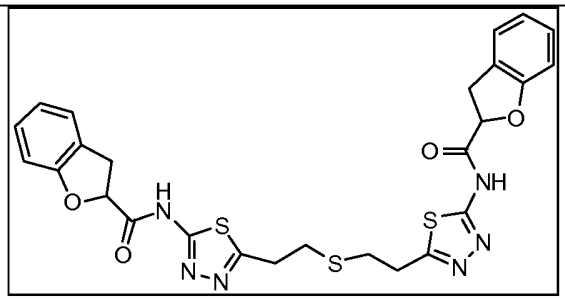
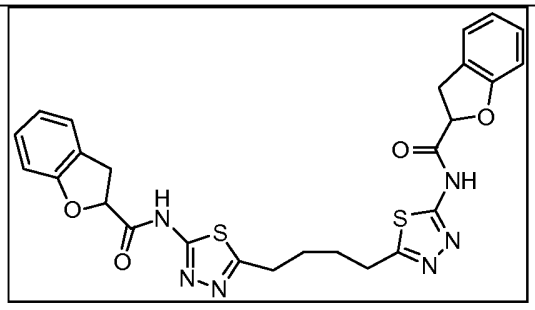
6			>50	2.7
7			>50	1.0
8			>50	1.6
9			>50	>50
10			>50	>50
11			1.4	0.89
12			>50	36
13			7.7	12

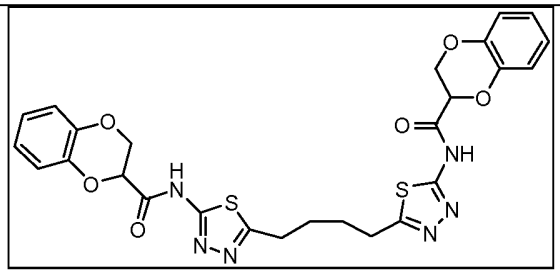
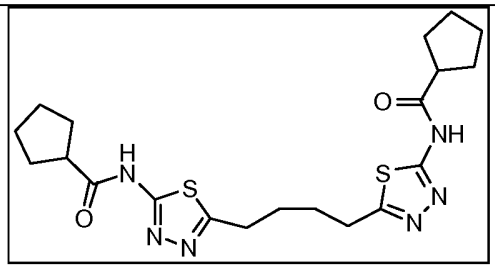
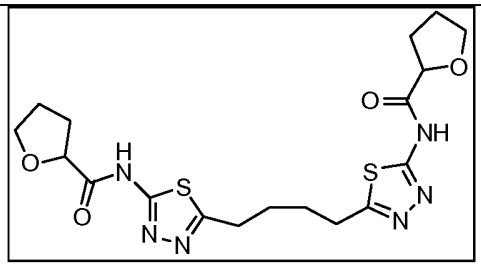
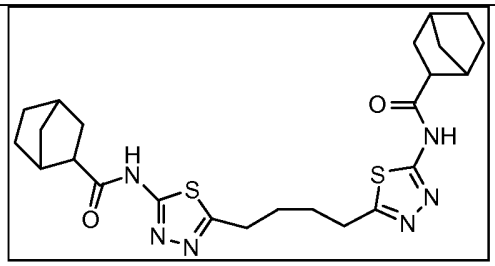
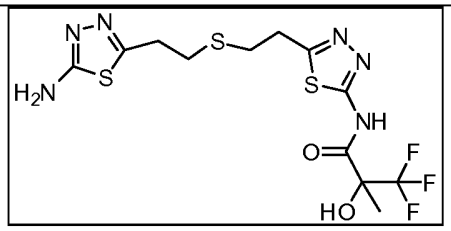
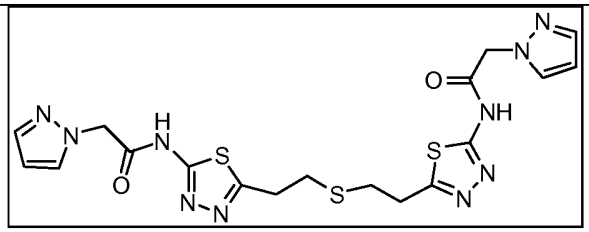
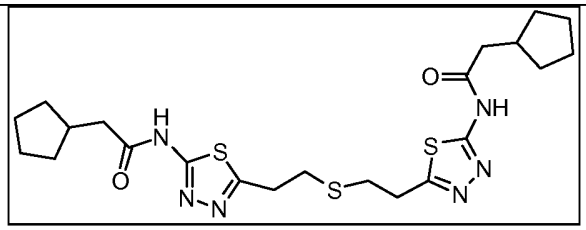
14			2.8	1.8
15			>50	1.2
16			>50	0.80
17			15	4.2
18			4.5	8.2
19			11	1.7
20			6.6	2.6
21			0.16	0.02

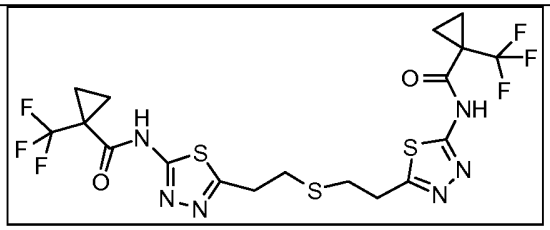
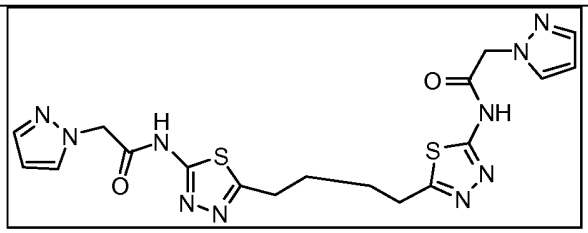
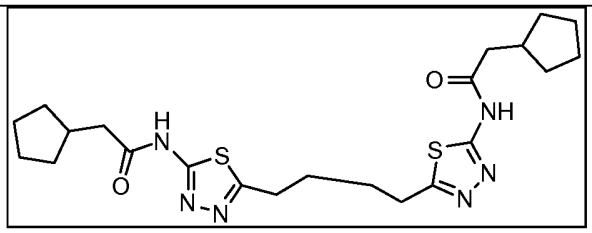
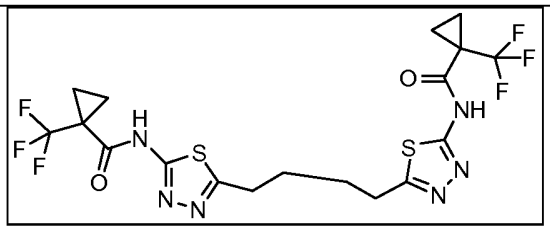
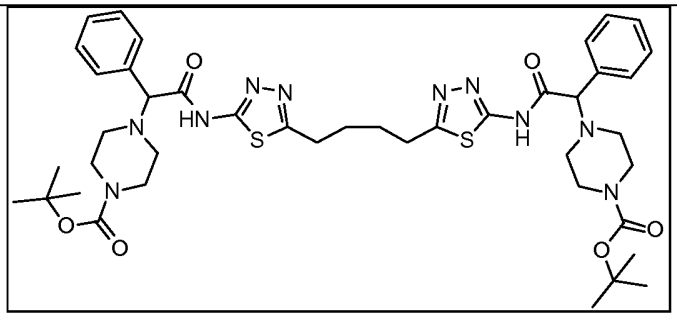
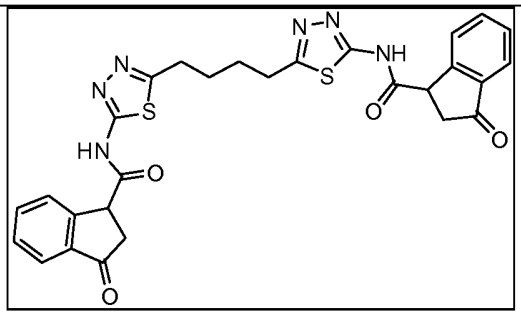
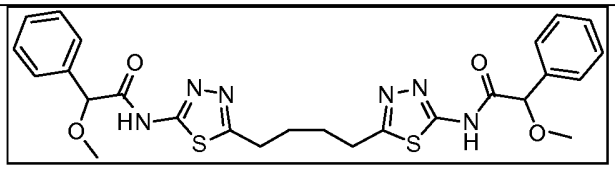
22			>50	>50
23			>50	>50
24			0.51	2.3
25			1.2	1.5
26			5.6	0.70
27			>50	0.47
28			>50	1.0
29			0.56	4.1

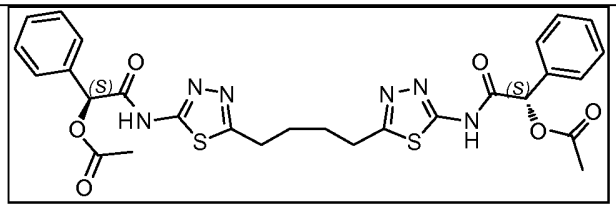
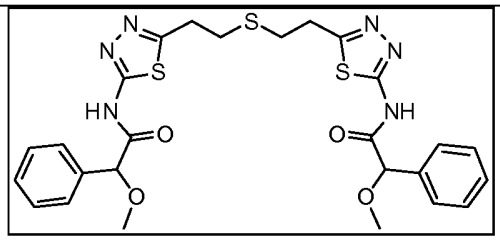
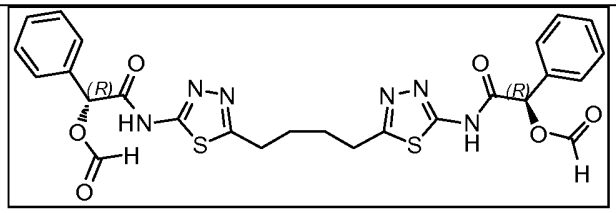
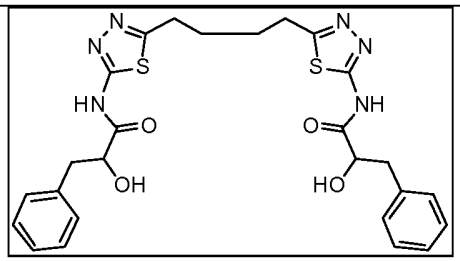
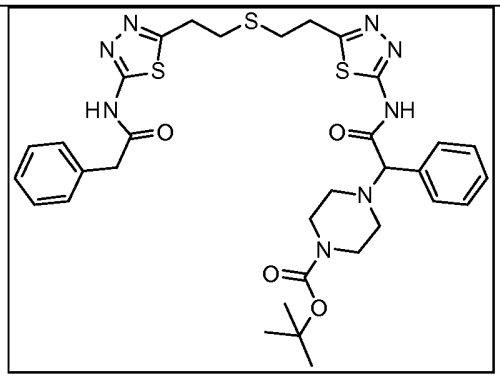
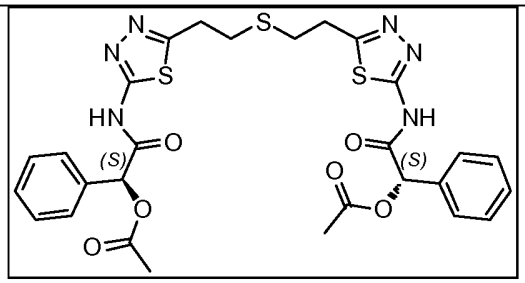
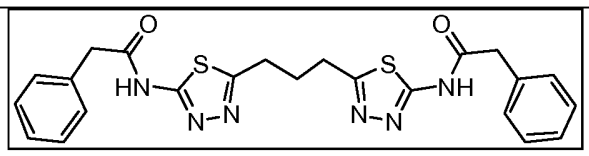
30			1.2	2.5
31			>50	4.3
32			7.0	11
33			13	5.3
34			>50	>50
35			18	3.8
36		0.04	0.22	0.16

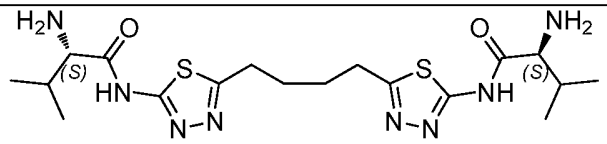
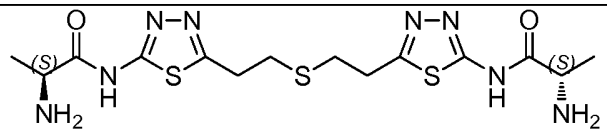
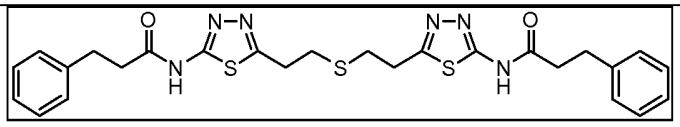
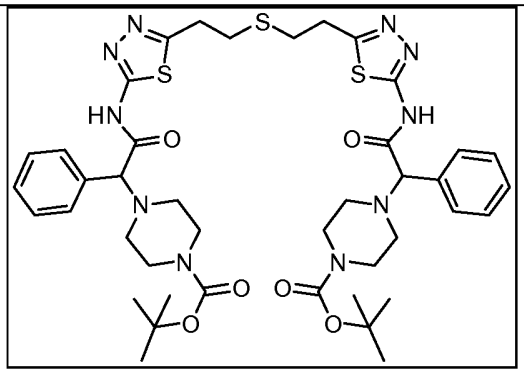
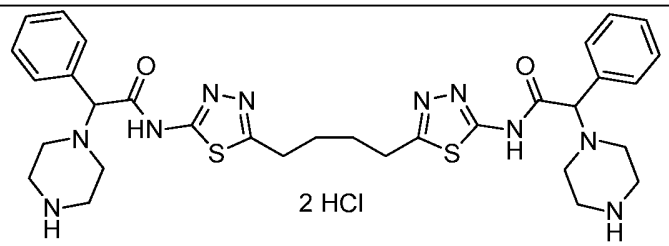
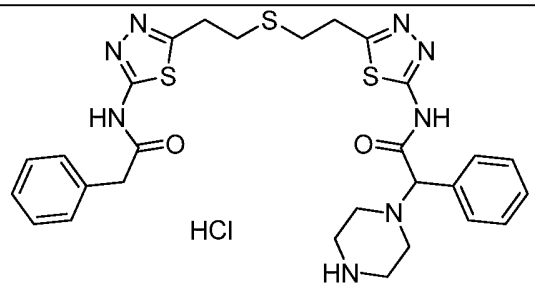
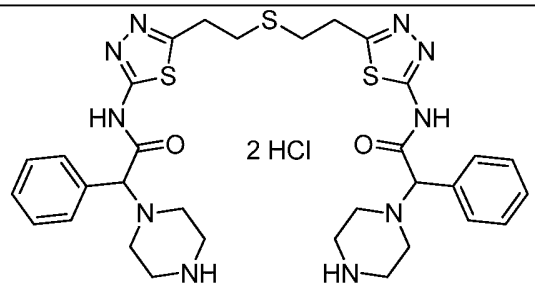
37			>50	>50
38			>50	3.2
39			26	4.5
40			3.7	0.56
41			7.9	33
42			>50	>50
43			2.3	>50

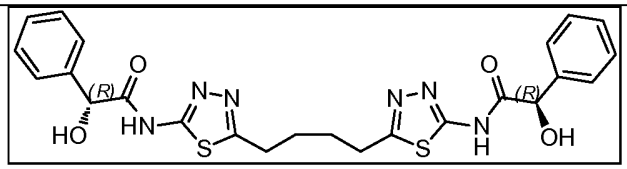
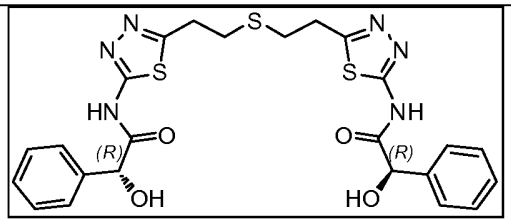
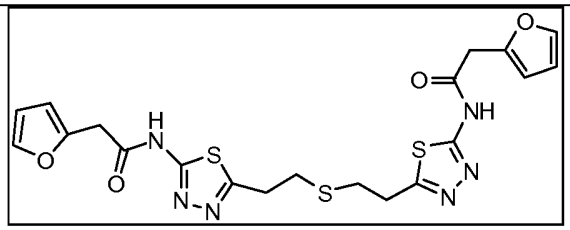
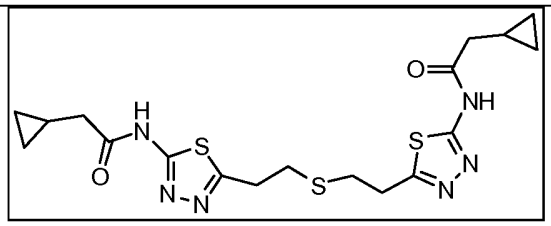
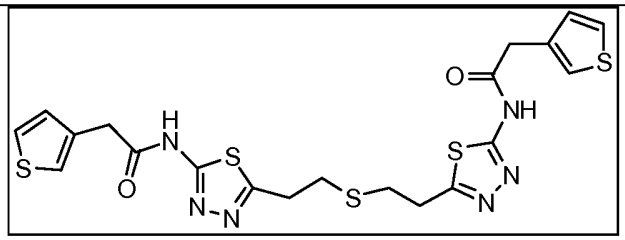
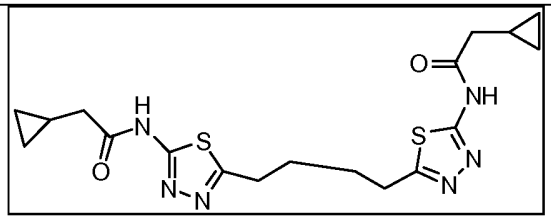
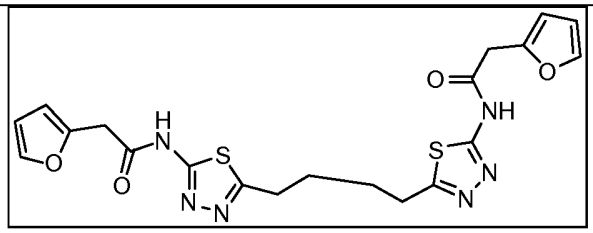
44			4.9	2.6
45			>50	>50
46			>50	16
47		2 HCl	8.3	35
48			>50	0.42
49			36	17
50			2.5	8.2
51			1.2	1.3

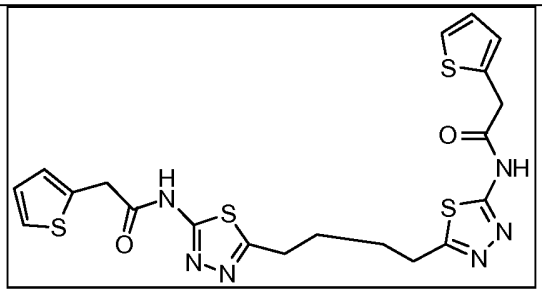
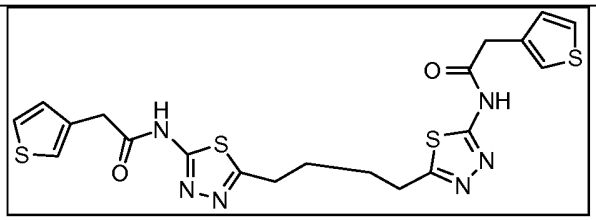
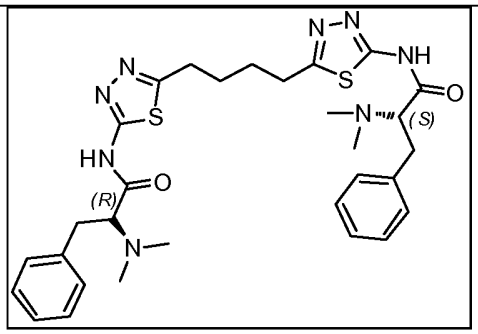
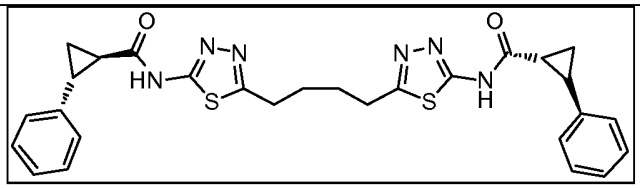
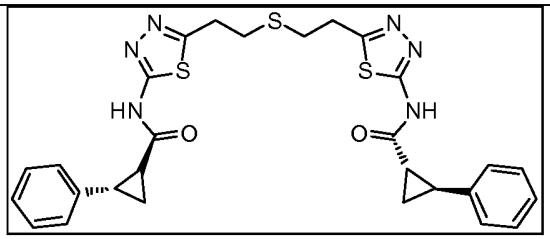
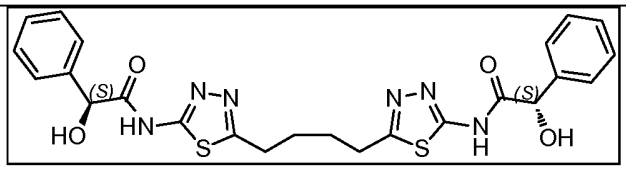
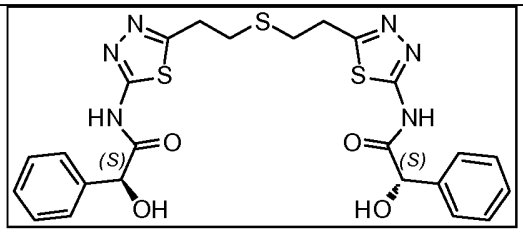
52			8.3	30
53			>50	34
54			9.2	1.6
55			>50	3.9
56			>50	
57			40	
58			>50	3.7

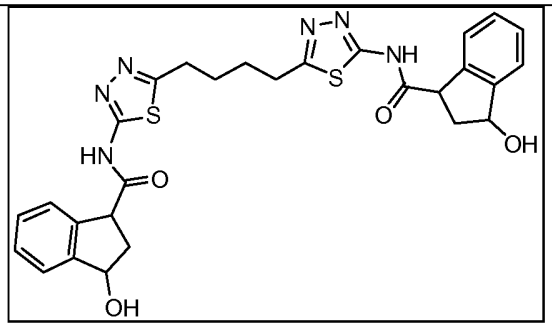
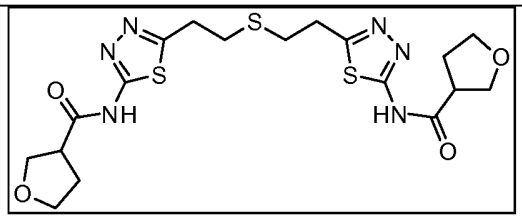
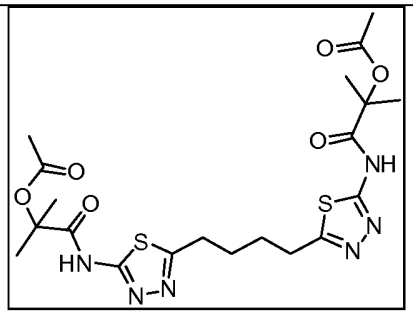
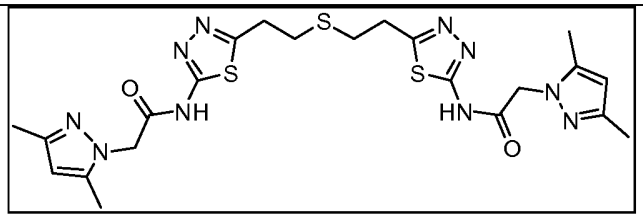
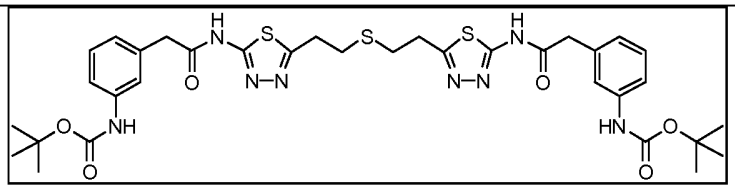
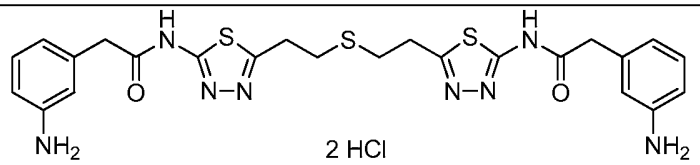
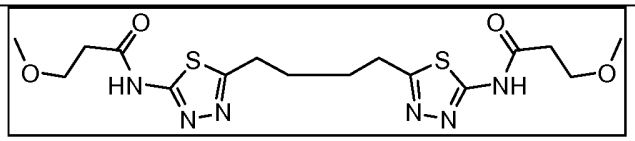
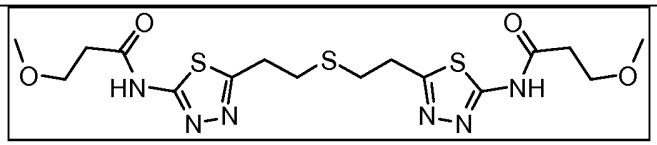
59			>50	
60			24	14
61			>50	
62			>50	19
63			25	2.6
64			1.3	0.23
65			1.3	0.52

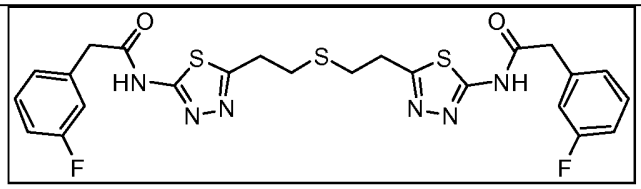
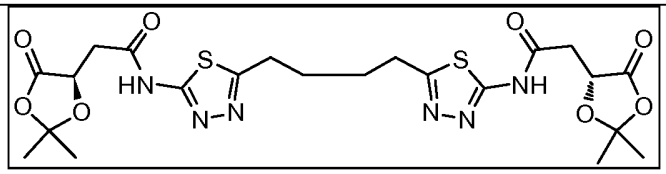
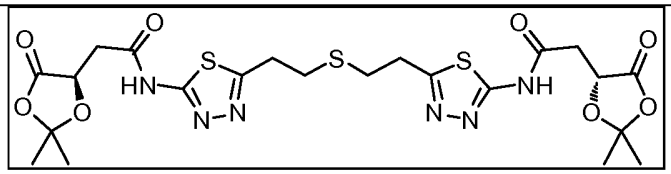
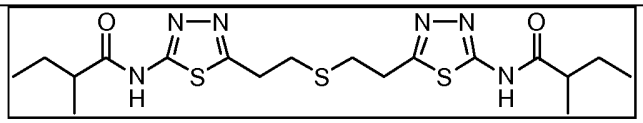
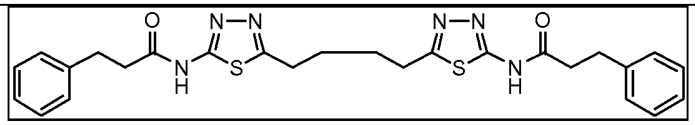
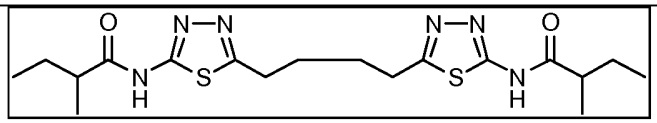
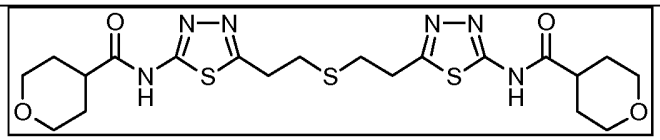
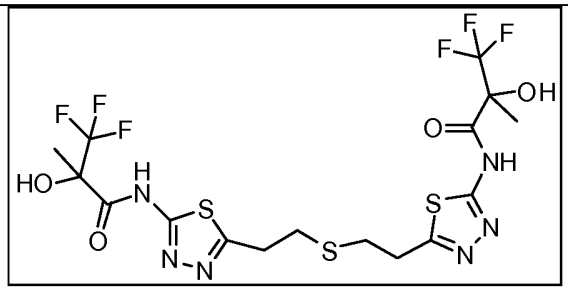
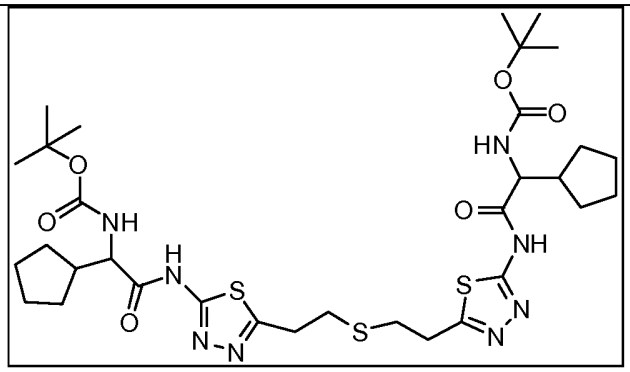
66			20	
67			3.0	1.8
68			4.9	0.34
69			0.69	0.33
70			3.4	3.4
71			>50	6.9
72			0.59	0.47

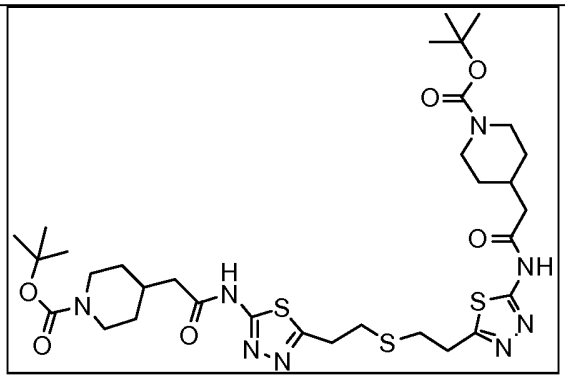
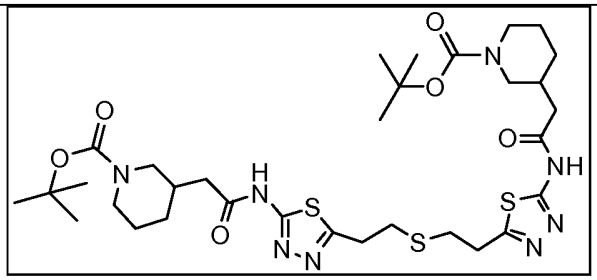
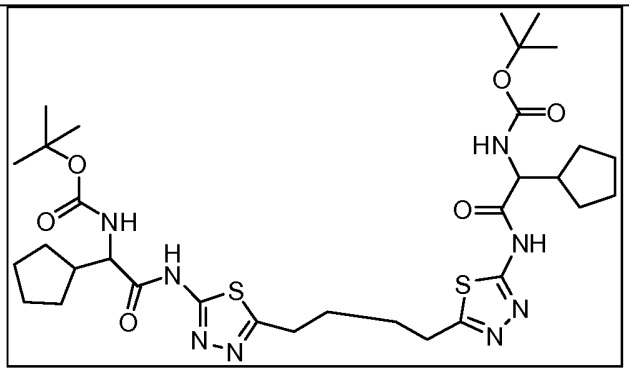
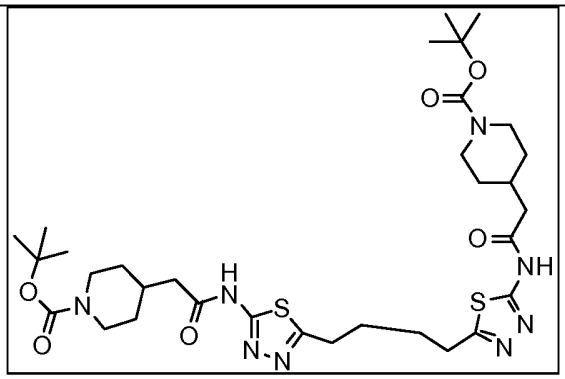
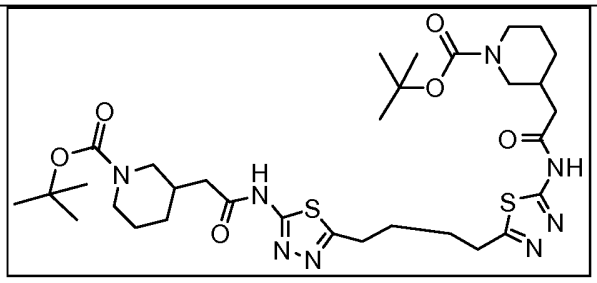
73	 2 TFA		>50	
74	 2 HCl		>50	
75			>50	
76			>50	
77	 2 HCl		6.1	34
78	 HCl		0.84	10
79	 2 HCl		2.0	20

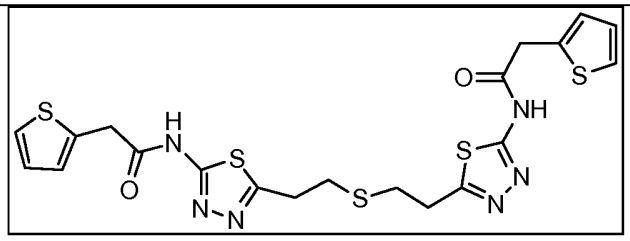
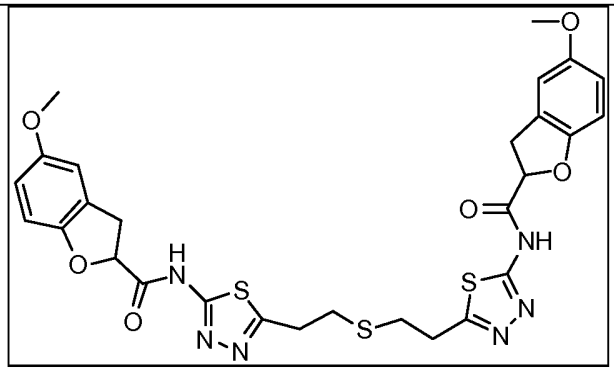
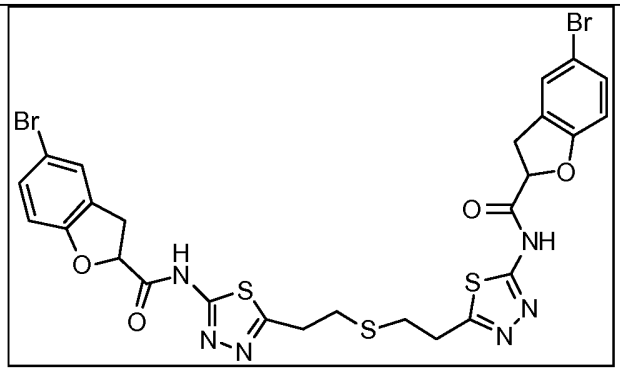
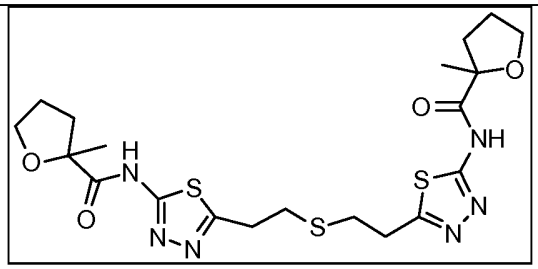
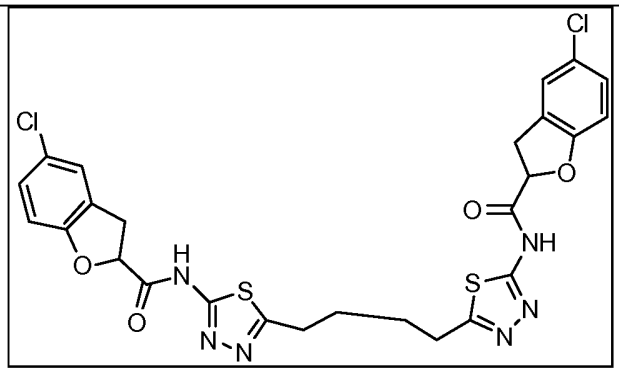
80			1.8	1.3
81			10	7.6
82			0.80	1.3
83			3.9	1.4
84			0.23	0.89
85			1.5	1.8
86			0.32	0.52

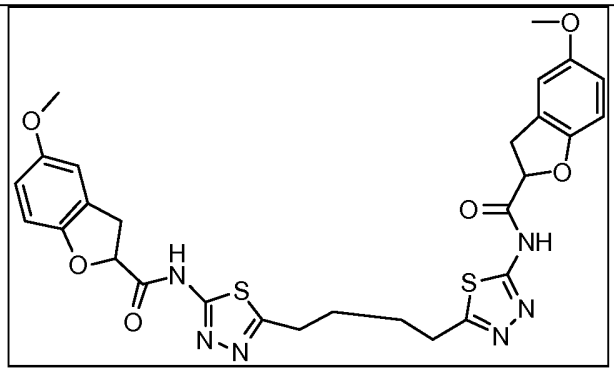
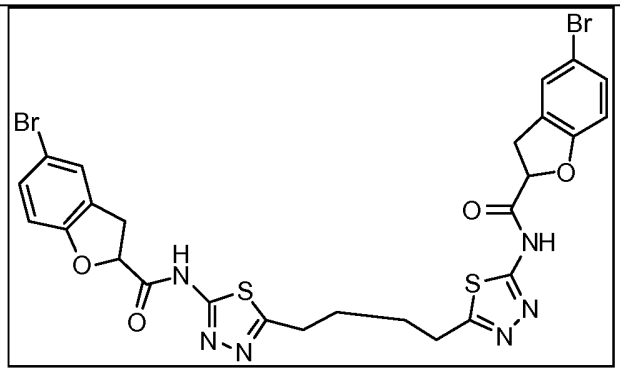
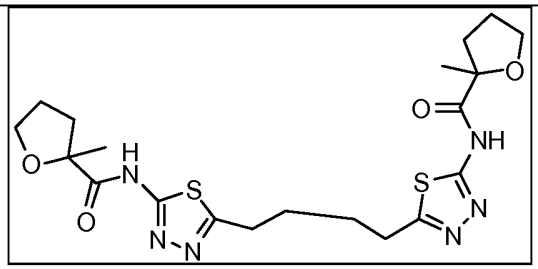
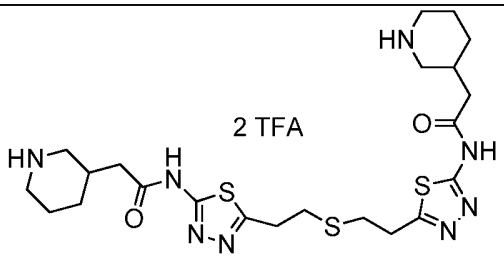
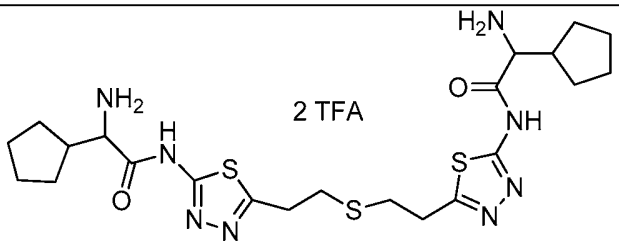
87			0.18	0.06
88			0.20	0.12
89			>20	
90			>20	
91			>20	
92		0.14	0.38	0.47
93			0.90	2.0

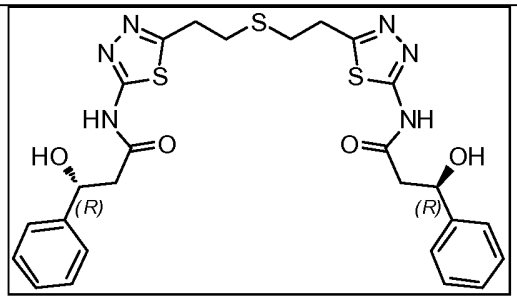
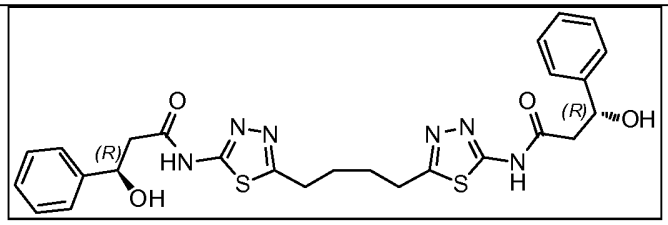
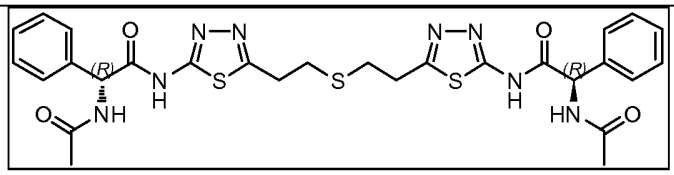
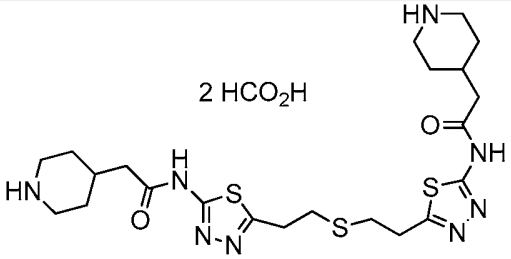
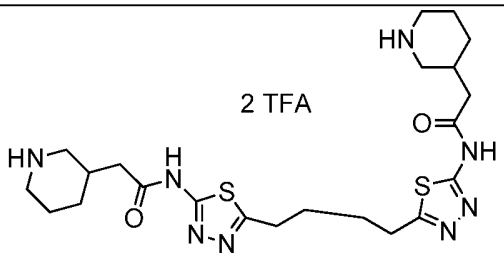
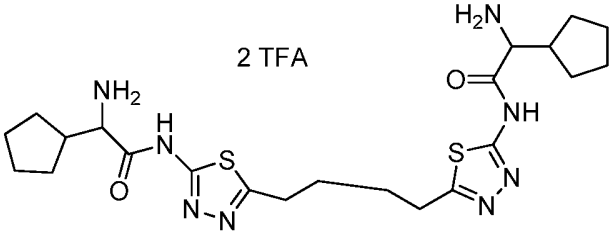
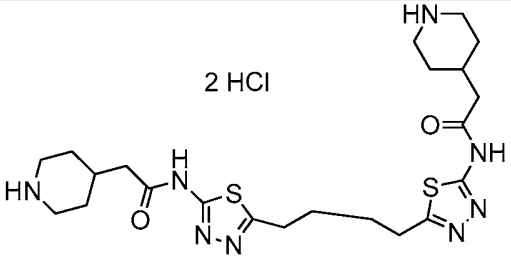
94			0.28	0.47
95			2.9	45
96			>20	
97			0.56	17
98			>20	3.9
99			2.7	1.0
100			8.1	9.0
101			24	17

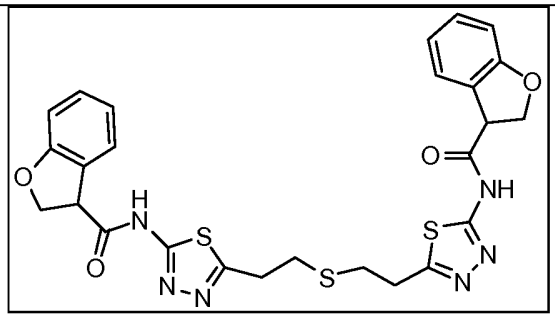
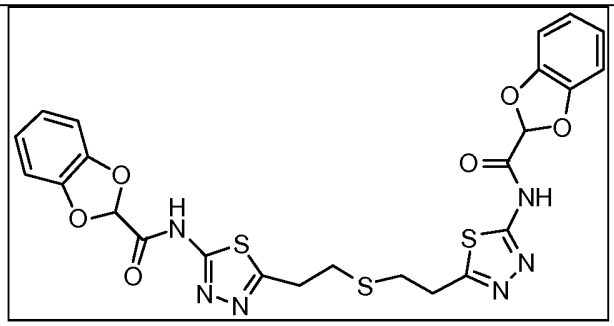
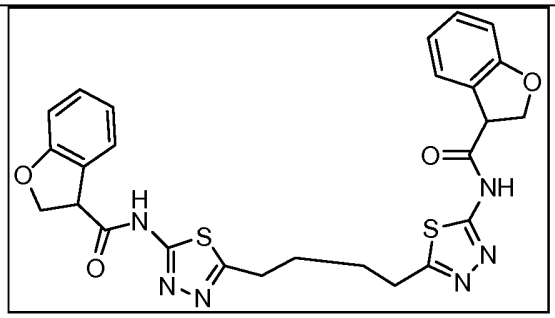
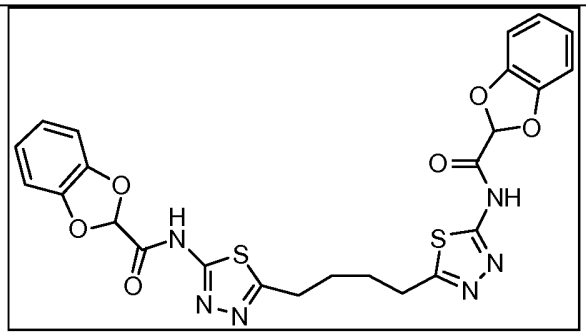
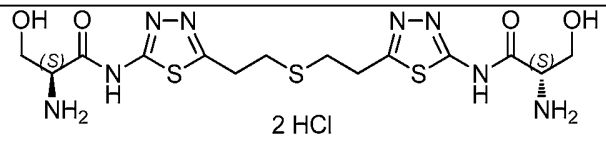
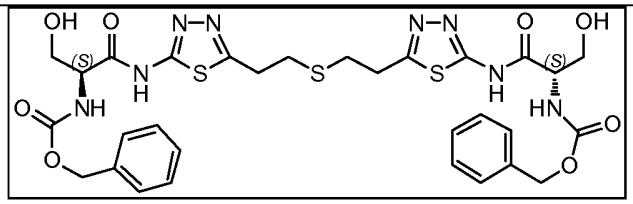
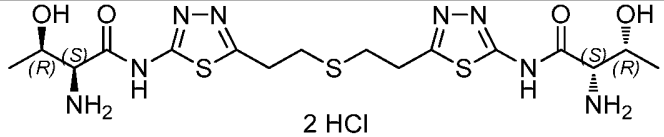
102			0.24	1.4
103			19	>50
104			>20	
105			9.9	119
106			>20	
107			4.3	1.2
108			>20	
109			>20	
110			>20	

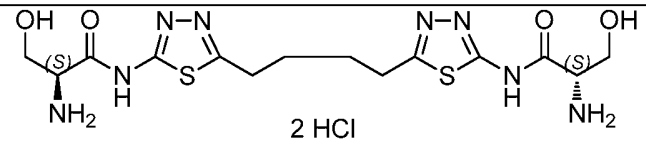
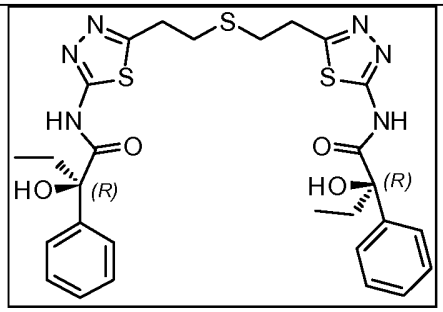
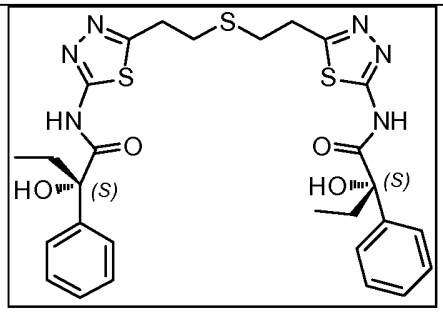
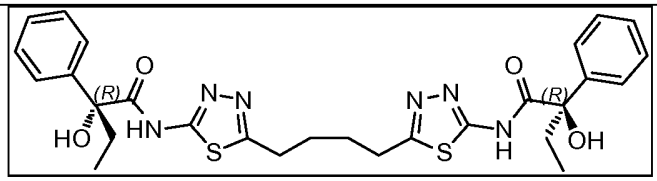
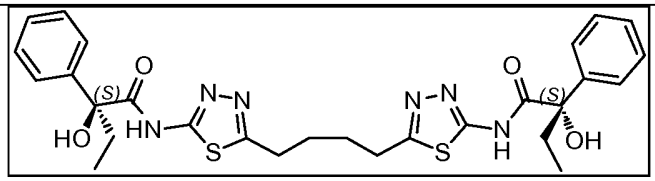
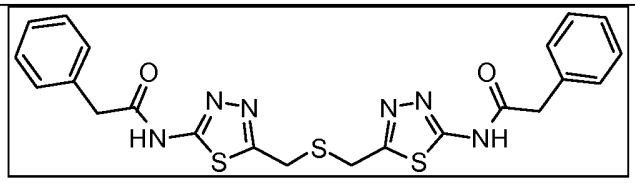
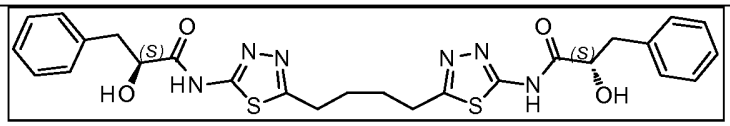
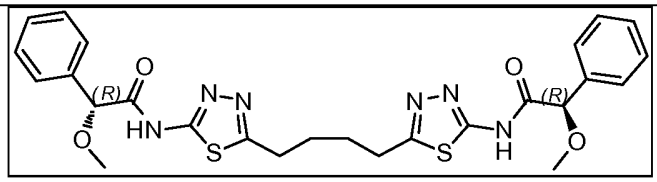
111			0.95	0.88
112			0.51	0.89
113			>20	
114			0.60	0.56
115			0.62	1.1

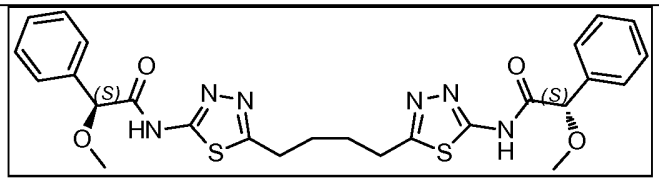
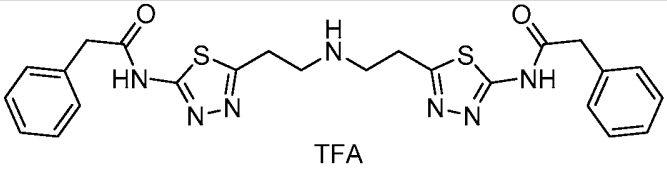
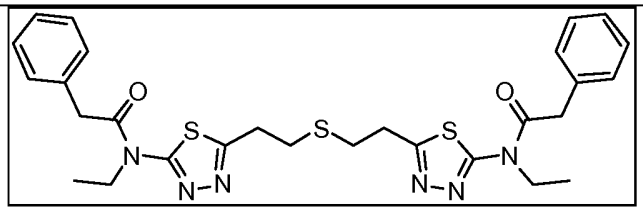
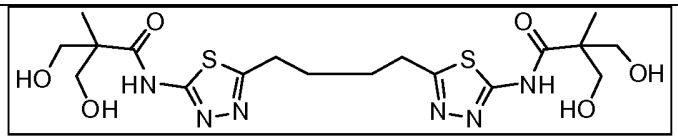
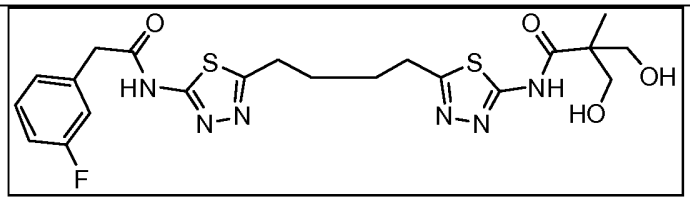
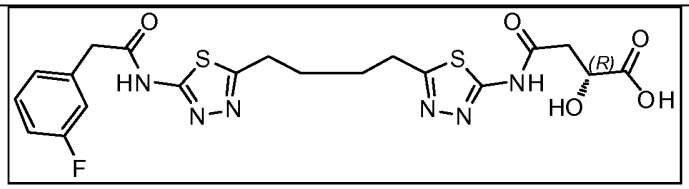
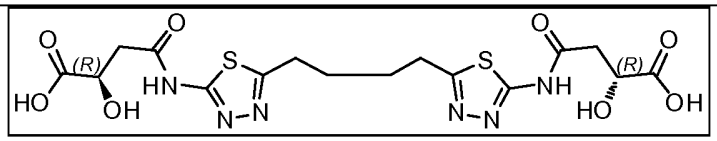
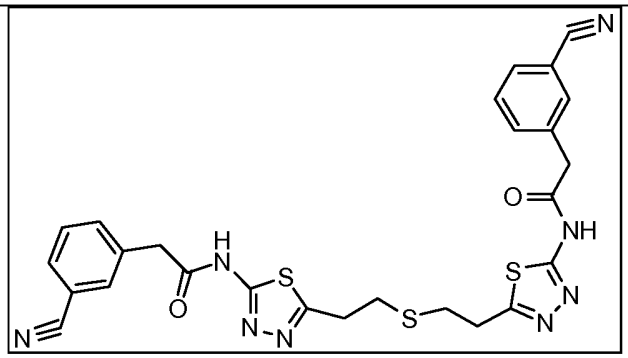
116			0.24	0.72
117			2.4	6.2
118			5.0	36
119			>20	13
120			1.8	38

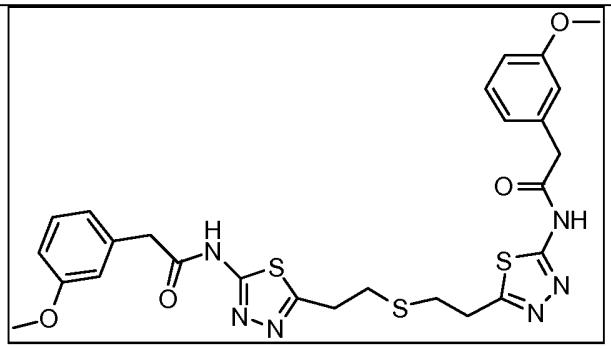
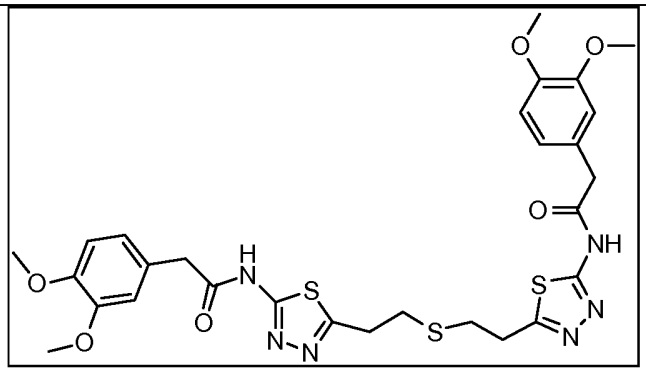
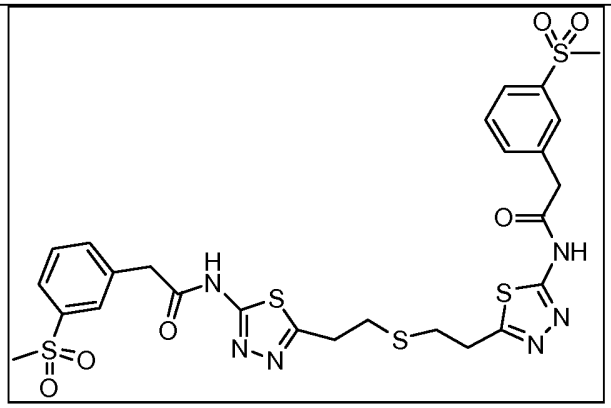
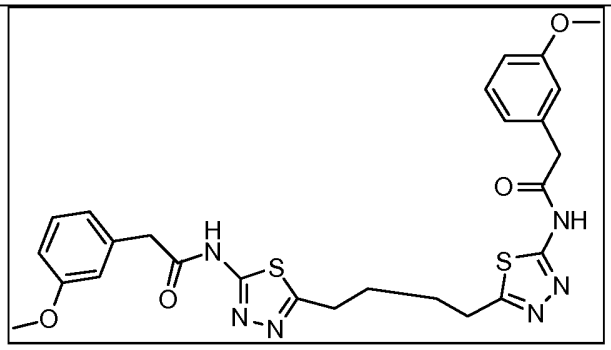
121			1.7	3.5
122			3.5	43
123			12	6.6
124			>20	
125			>20	

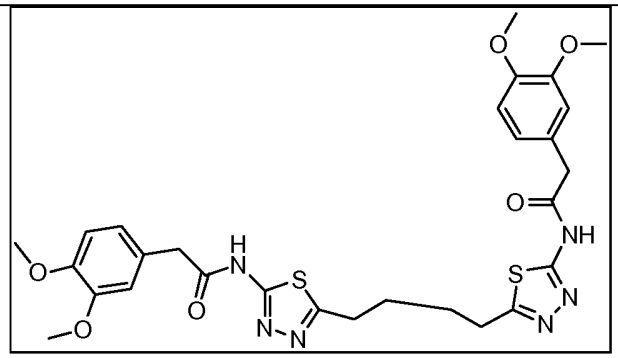
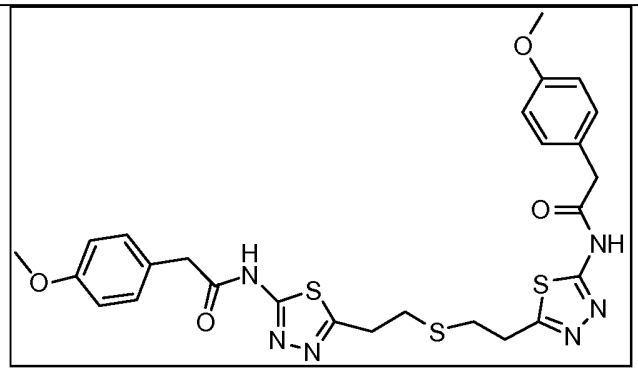
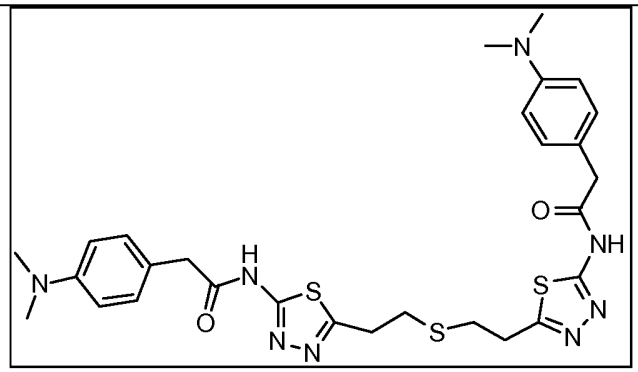
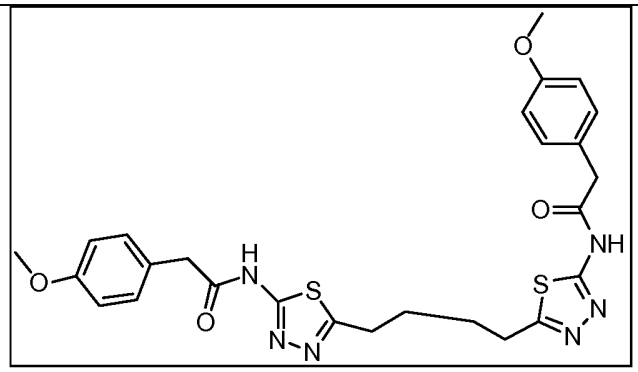
126			5.8	12
127			1.8	0.45
128			32	>50
129			>20	>50
130			>20	
131			19	
132			>20	

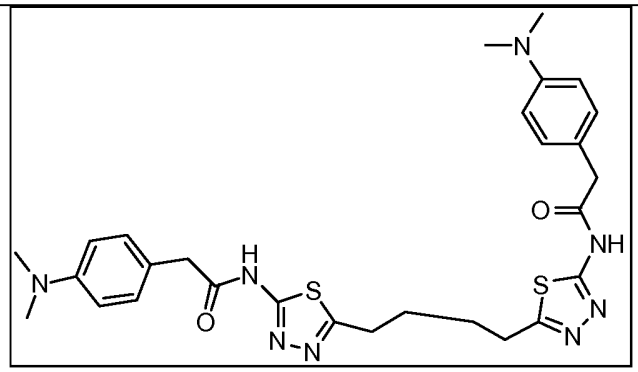
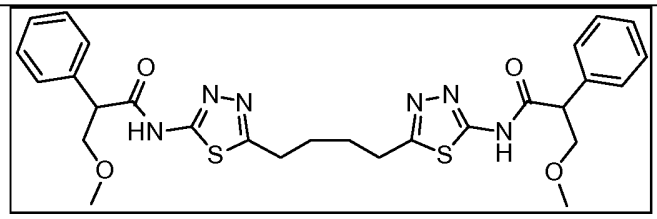
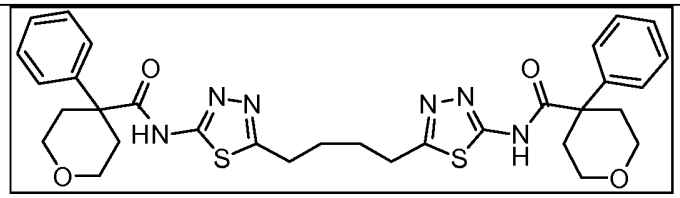
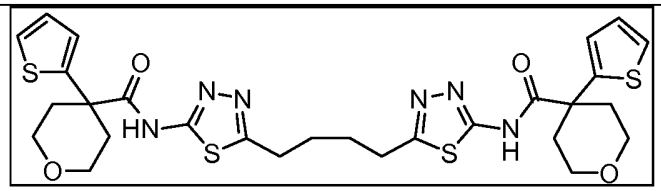
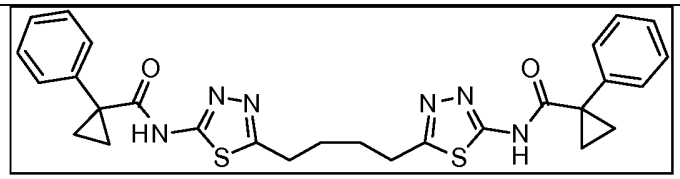
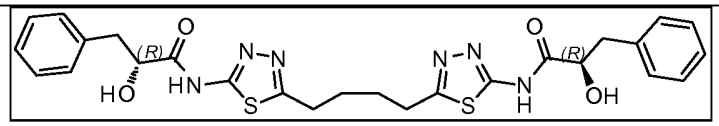
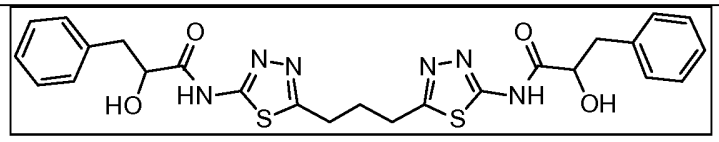
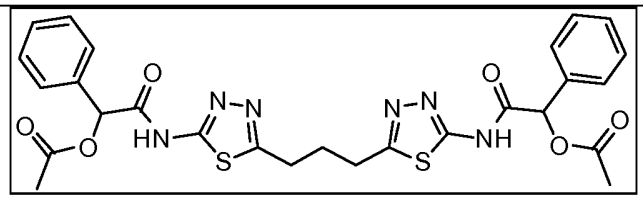
133			0.51	0.15
134			14	28
135			0.30	0.49
136			7.0	4.7
137	 2 HCl		>20	
138			0.75	2.7
139	 2 HCl		>20	

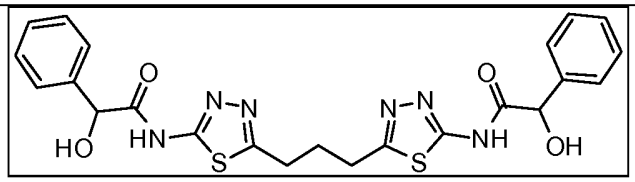
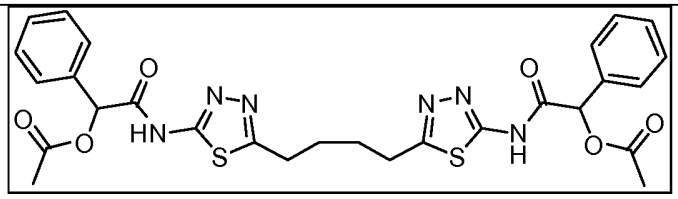
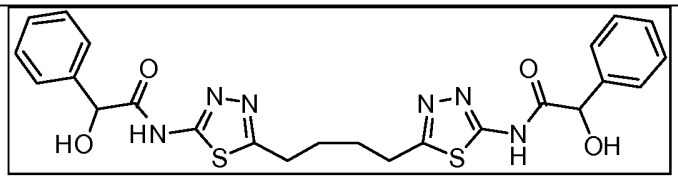
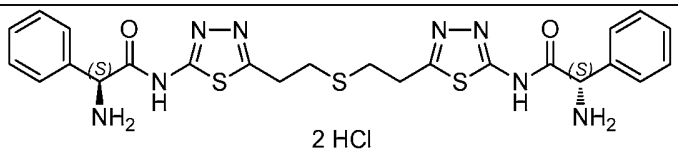
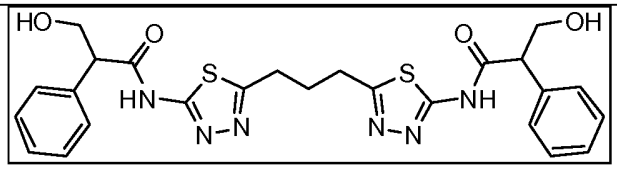
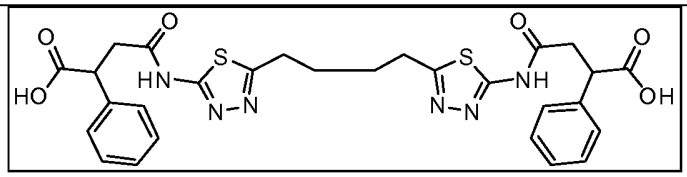
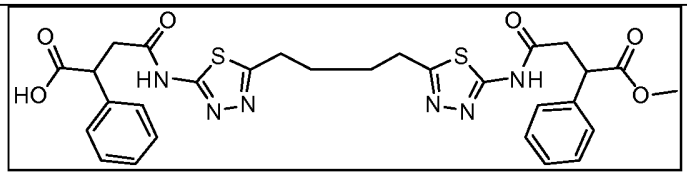
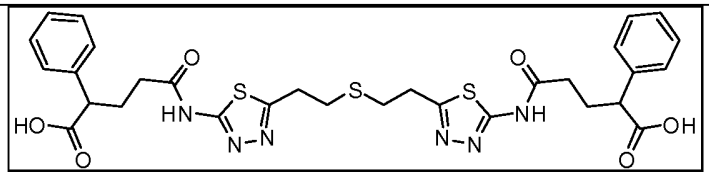
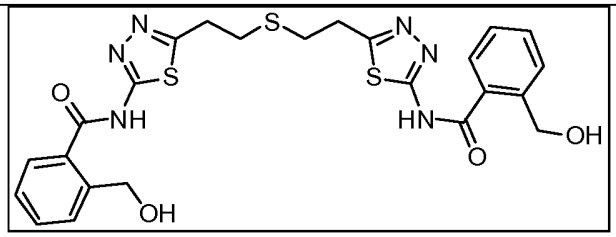
140	 2 HCl		3.4	>50
141			1.7	4.3
142			>20	
143			0.57	2.2
144			>20	
145			>20	
146			0.43	0.46
147			0.62	0.37

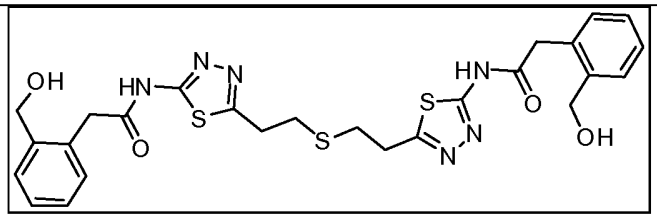
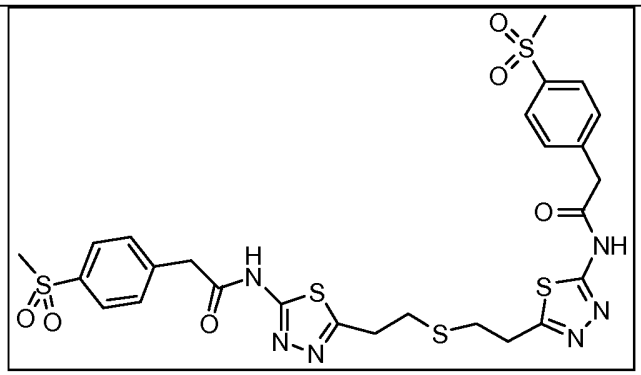
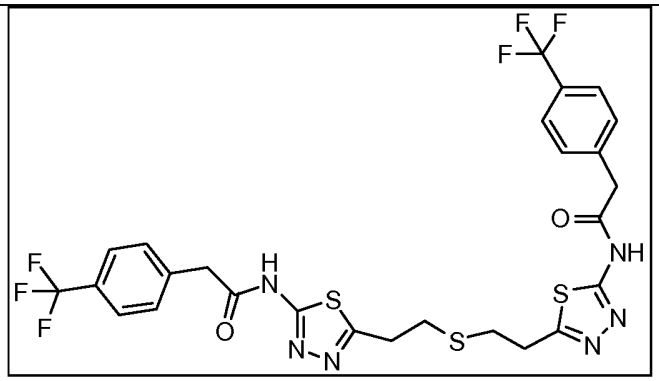
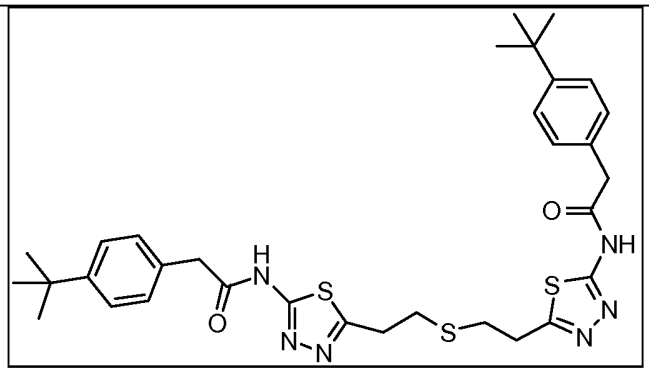
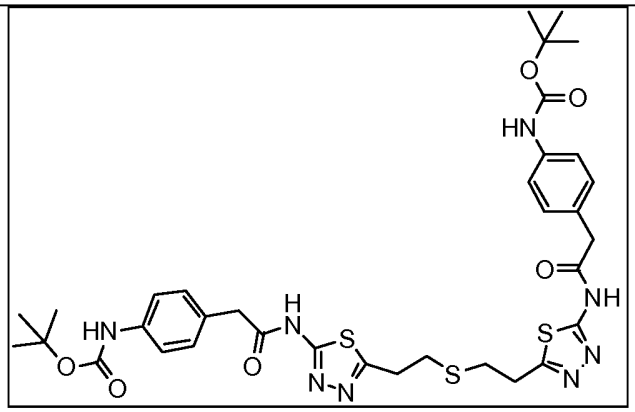
148			0.59	0.39
149	 TFA		15	
150			>20	
151			14	>50
152			0.73	1.1
153			1.0	>50
154			19	>50
155			0.27	1.9

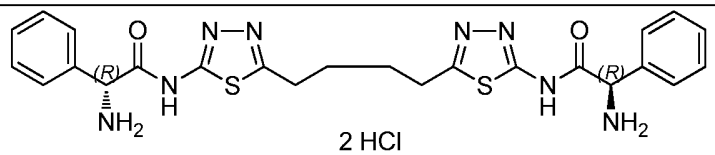
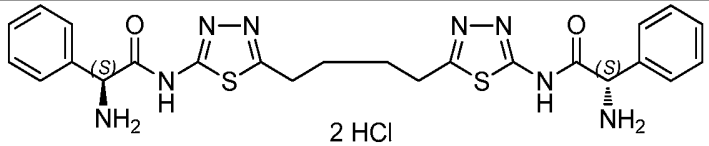
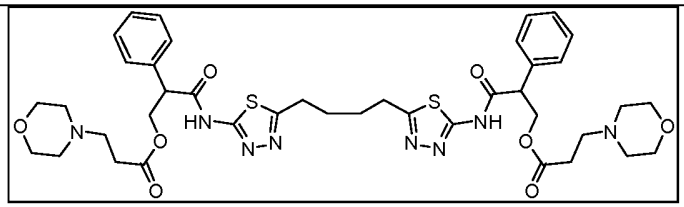
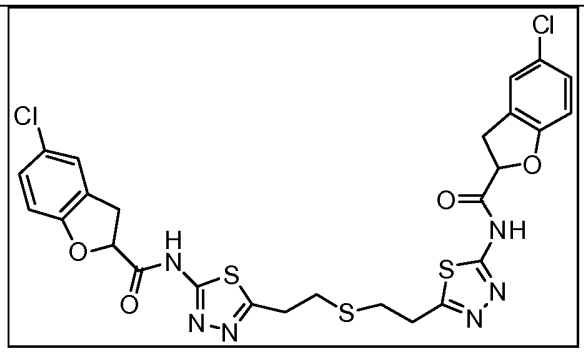
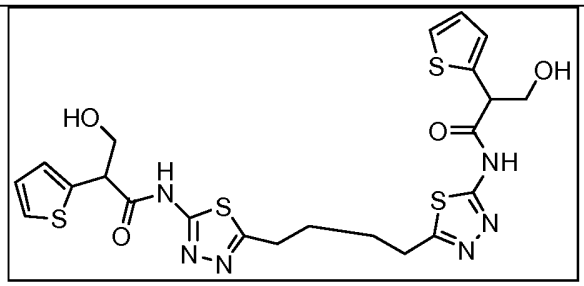
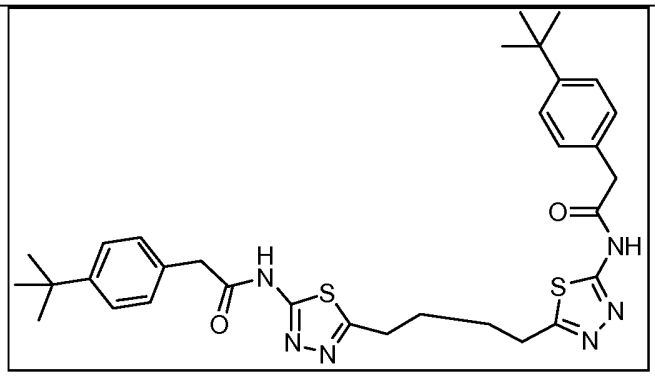
156			0.12	0.63
157			0.34	0.18
158			0.22	8.1
159			0.11	0.05

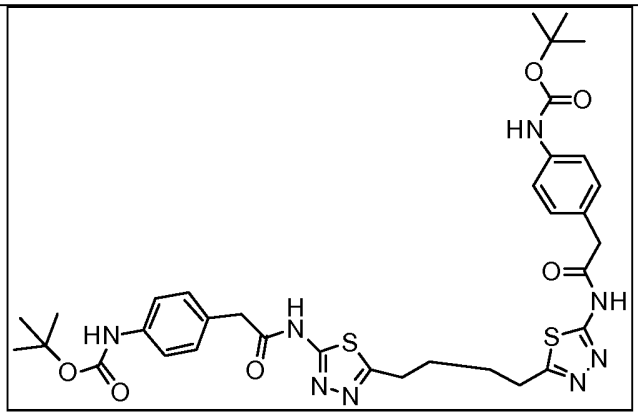
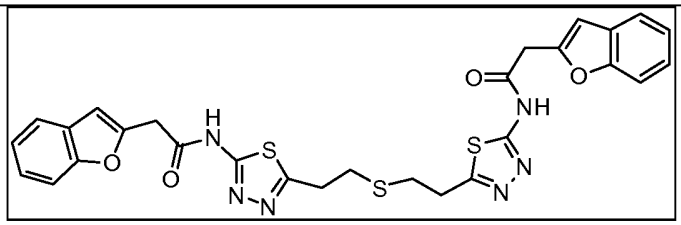
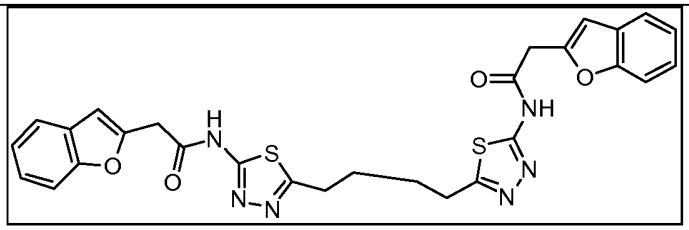
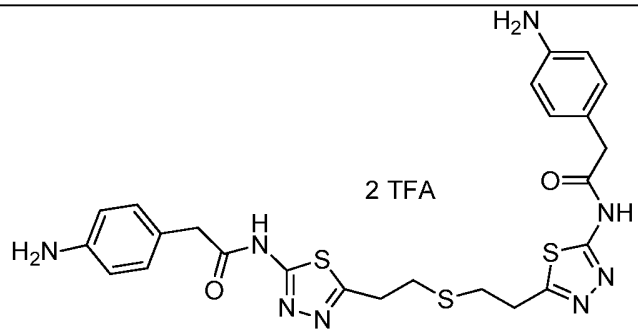
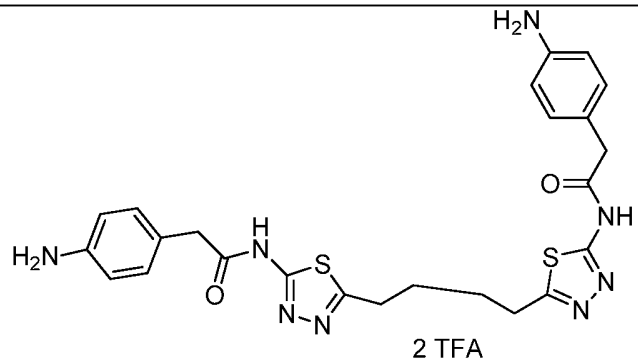
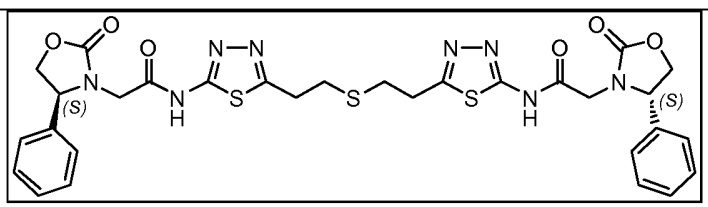
160			0.16	>50
161			0.15	1.4
162			0.23	0.15
163			0.13	>50

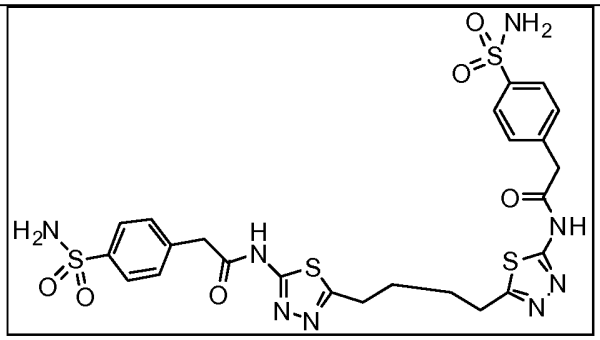
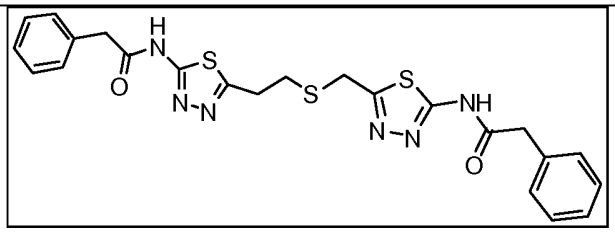
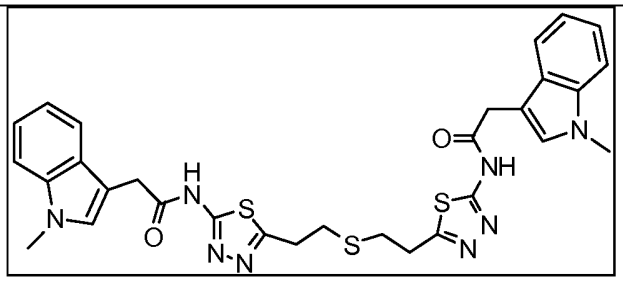
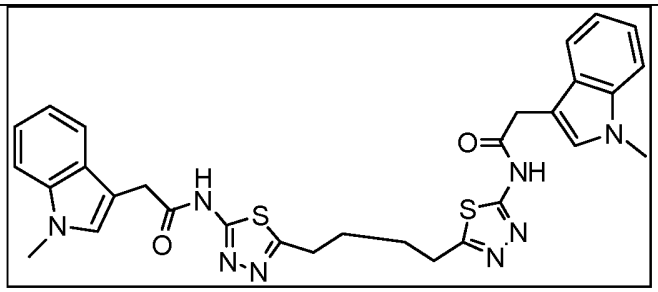
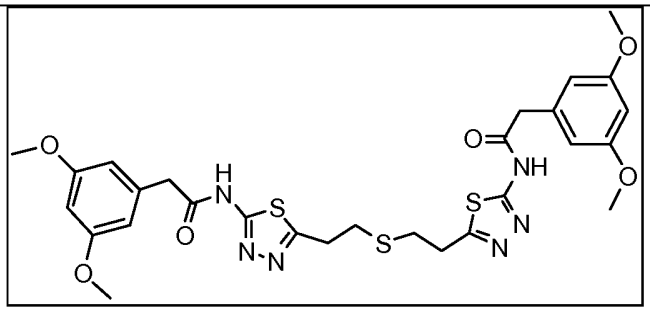
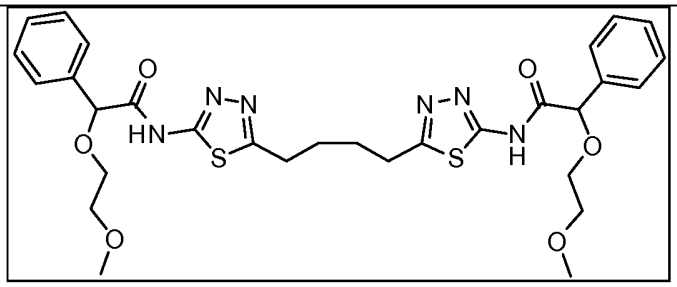
164			0.24	0.13
165			0.51	33
166			7.4	6.8
167			11	34
168			1.3	>50
169			0.71	3.4
170			7.4	9.3
171			>20	

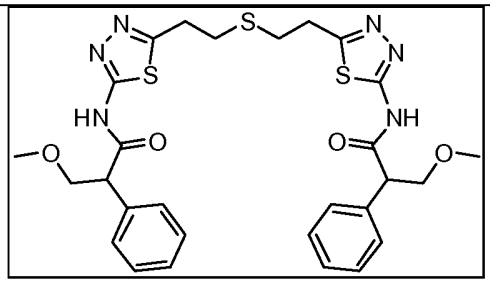
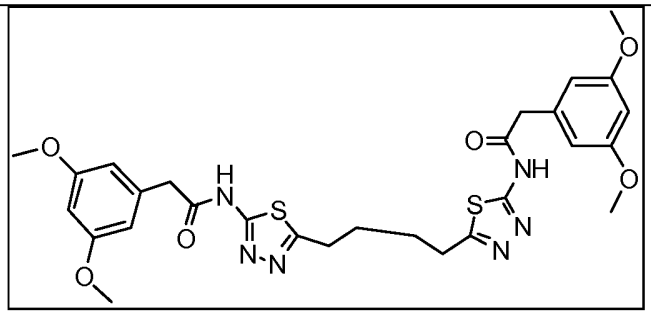
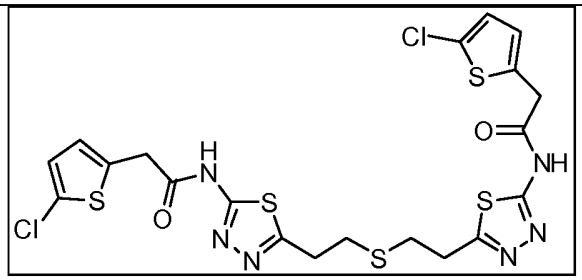
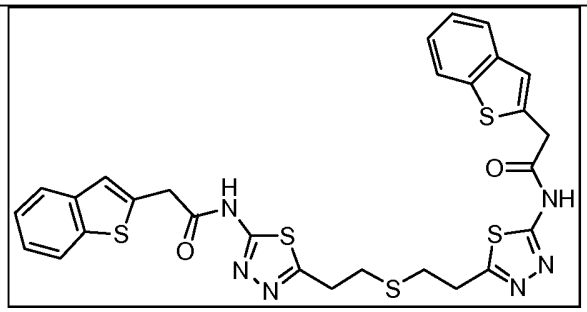
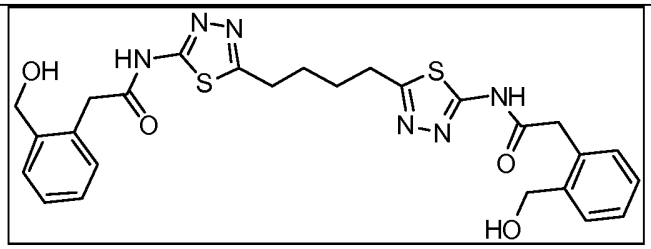
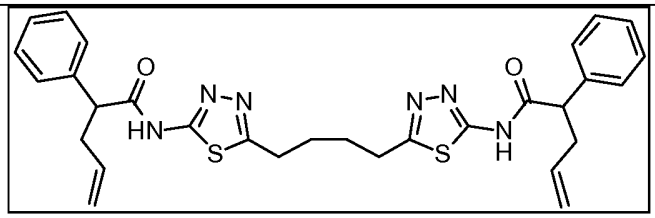
172			1.7	3.7
173			24	0.76
174			0.29	0.44
175	 2 HCl		6.3	23
176			0.57	1.5
177			1.1	>50
178			1.5	>50
179			3.1	>50
180			8.8	>50

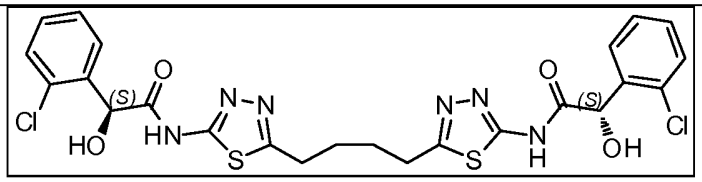
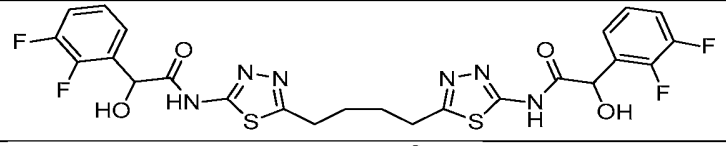
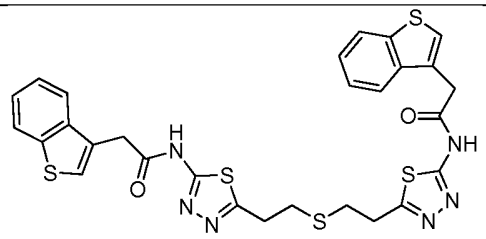
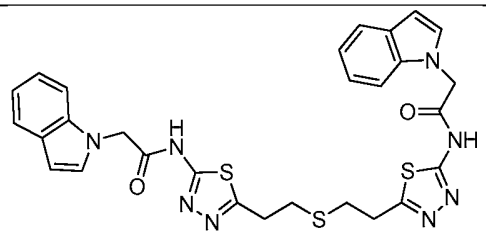
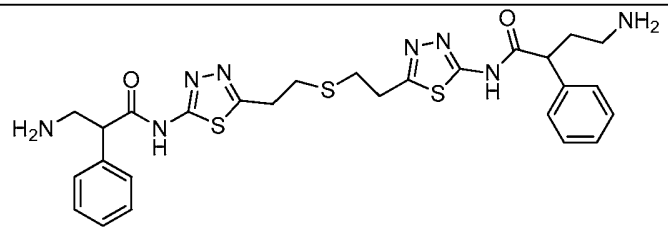
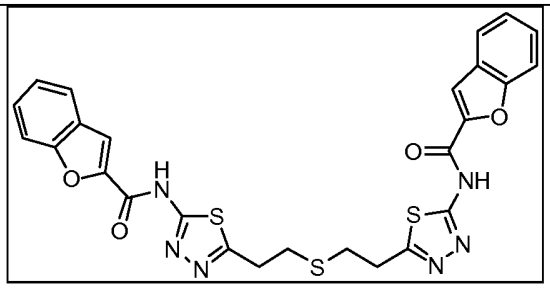
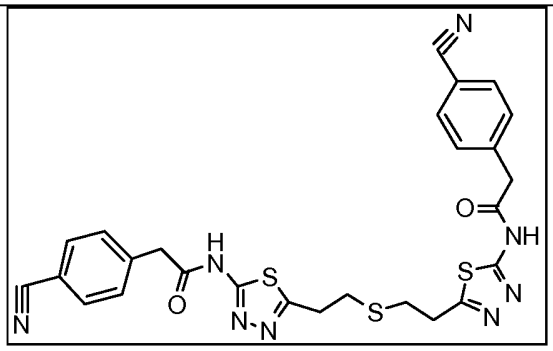
181			0.33	30
182			0.58	>50
183			>20	
184			>20	
185			>20	0.09

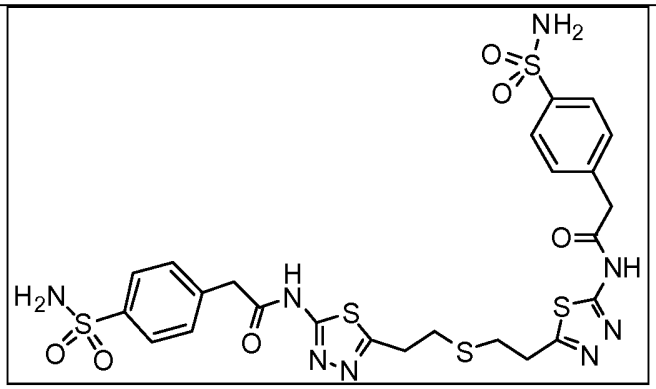
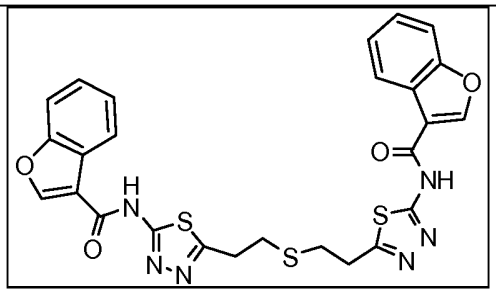
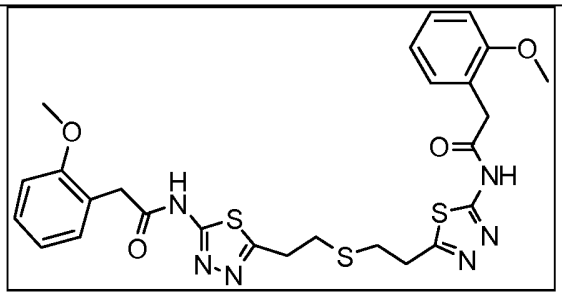
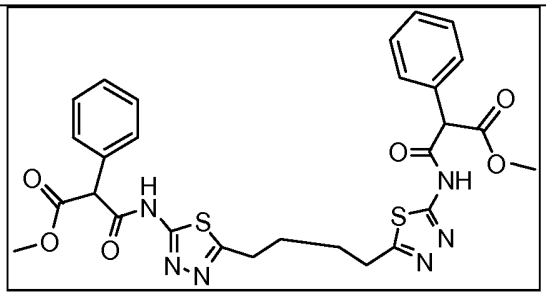
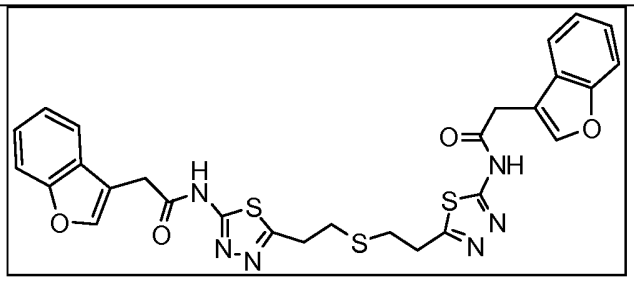
186	 2 HCl		3.1	13
187	 2 HCl		2.8	21
188			2.0	0.46
189			4.4	
190			0.25	0.49
191			>20	

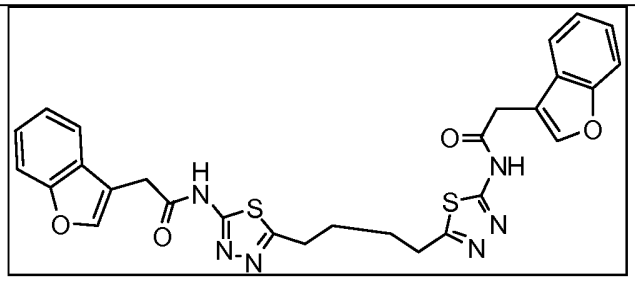
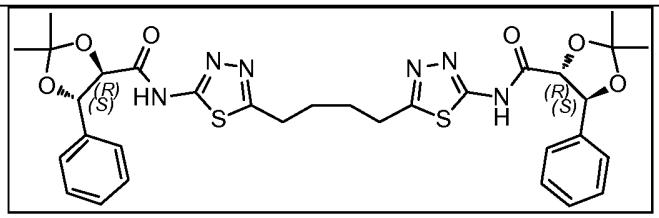
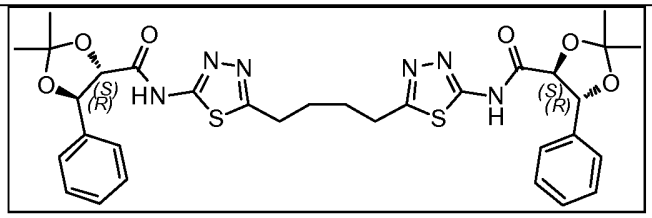
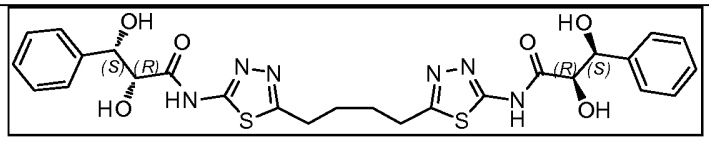
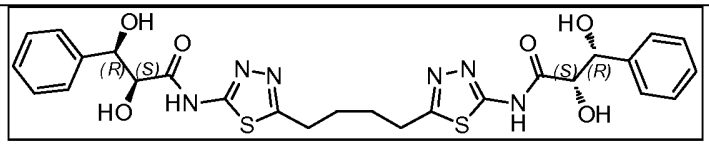
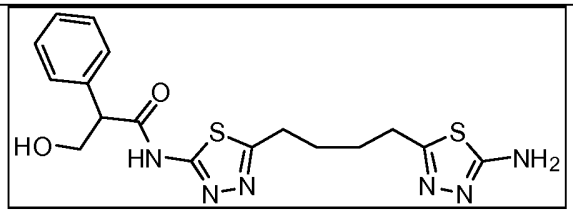
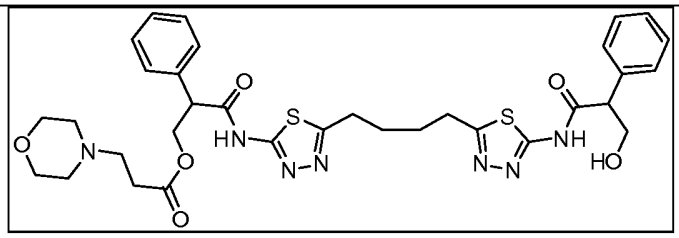
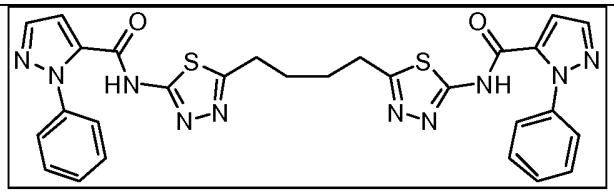
192			>20	0.03
193			3.4	
194			10	
195			0.30	1.3
196			0.19	0.61
197			6.9	

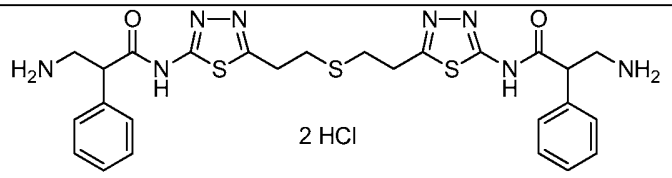
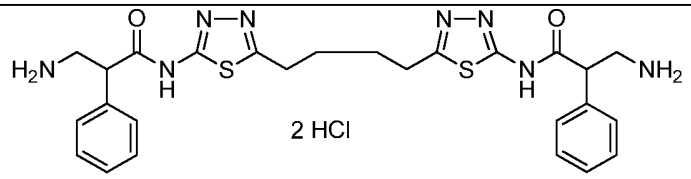
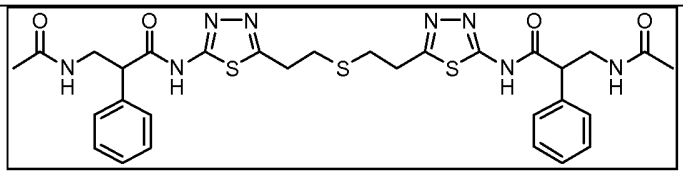
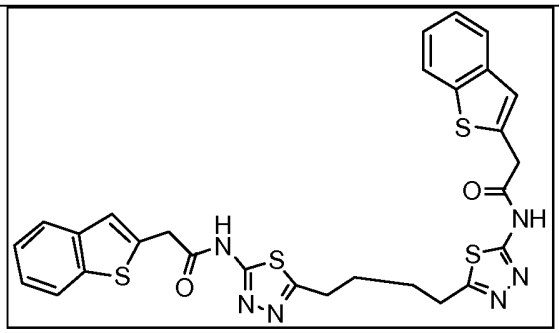
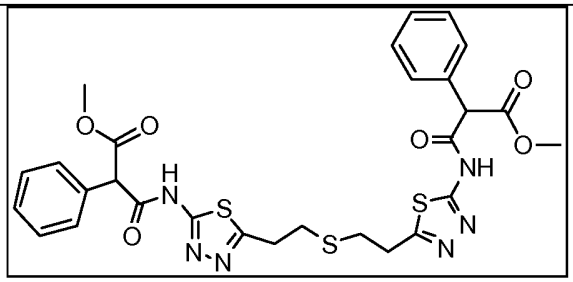
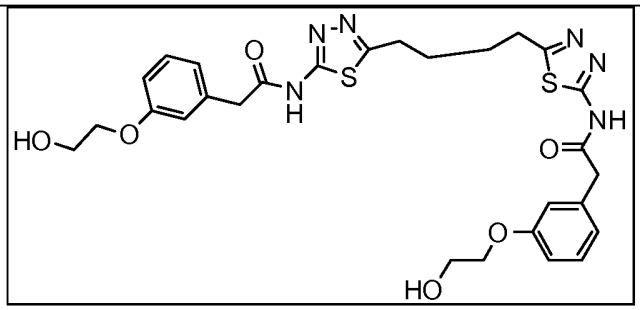
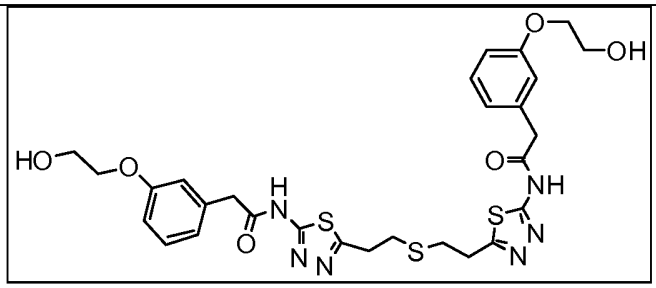
198			0.18	>50
199			0.12	0.17
200			0.61	
201			2.7	
202			0.18	0.14
203			1.7	1.7

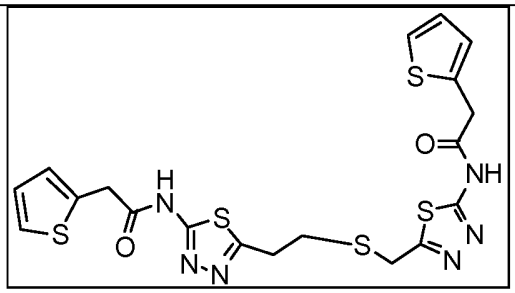
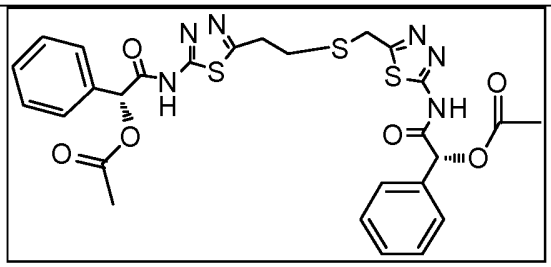
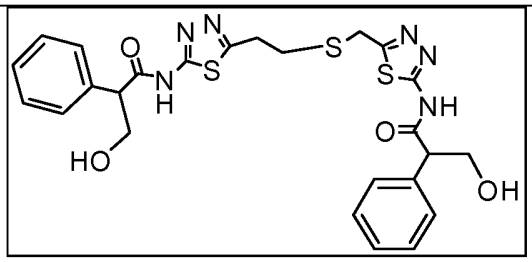
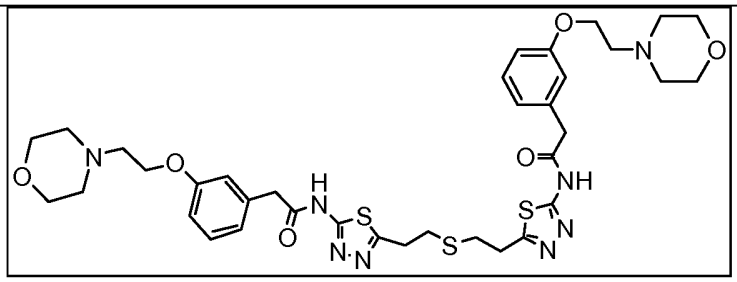
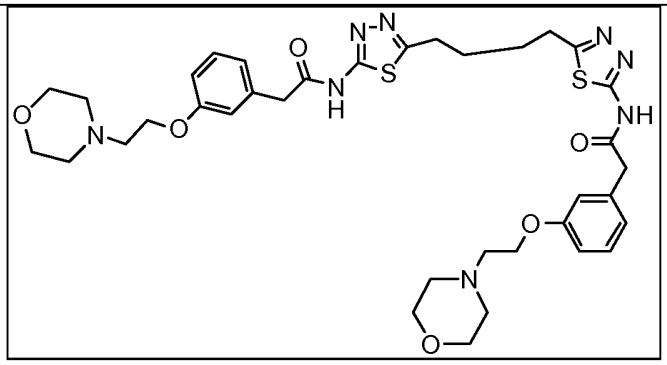
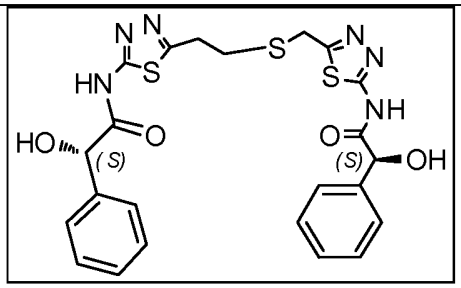
204			0.92	2.4
205			0.38	4.1
206			>20	
207			13	
208			0.17	9.0
209			>20	22

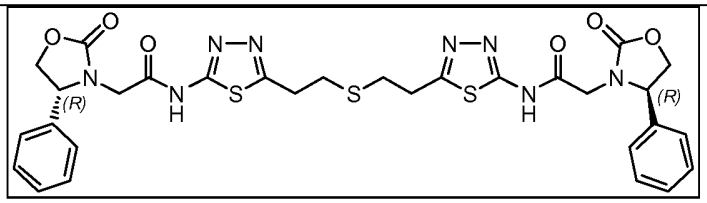
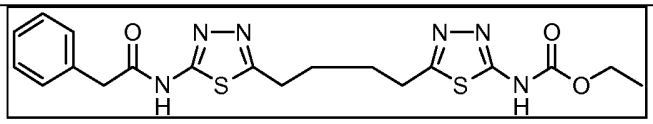
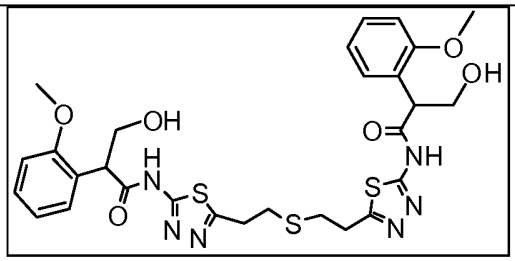
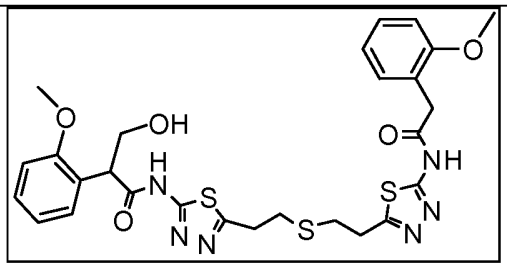
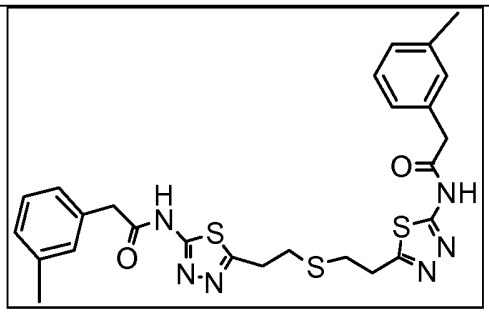
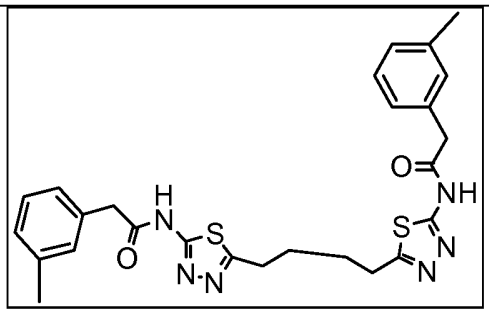
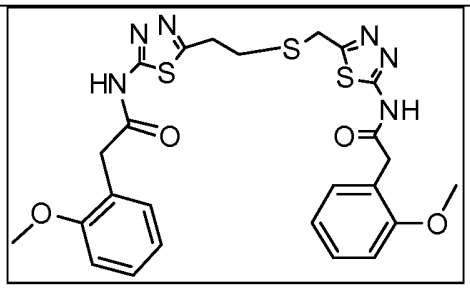
210			0.38	0.42
211			1.2	1.0
212			>20	
213			2.5	4.4
214			0.82	1.2
215			16	
216			0.89	>50

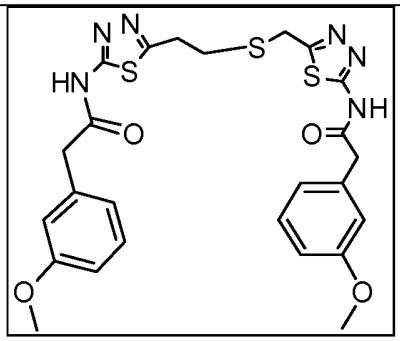
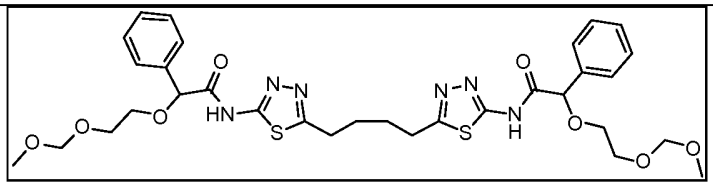
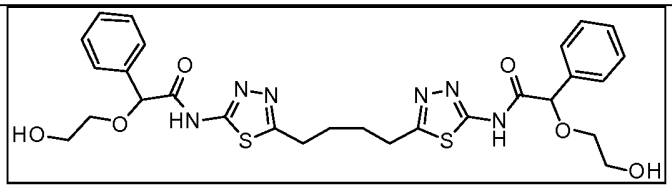
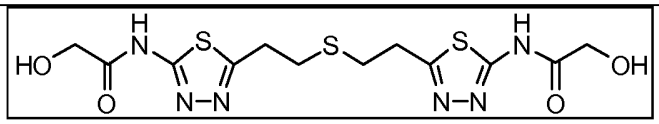
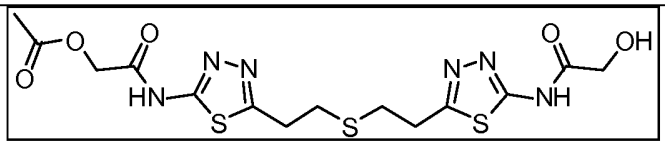
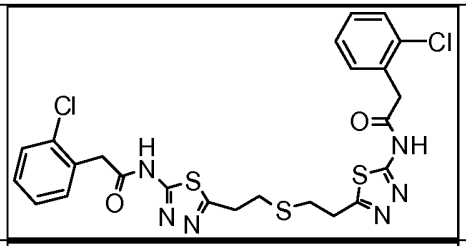
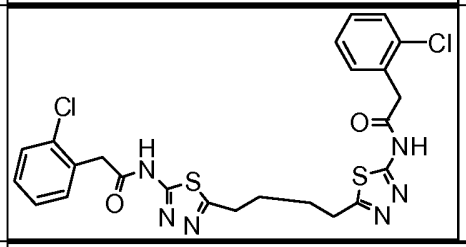
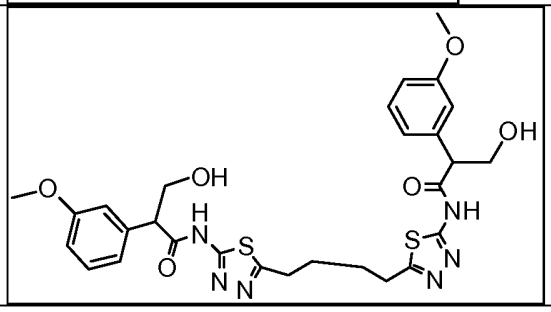
217			0.24	>50
218			>20	
219			0.17	0.57
220			1.6	0.31
221			>20	

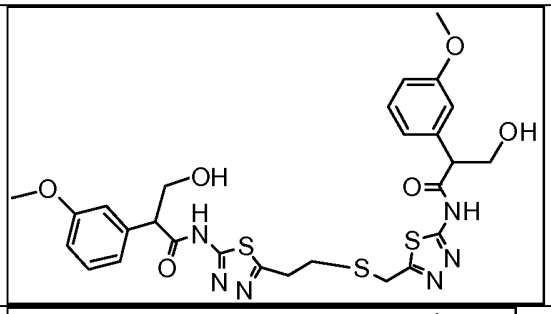
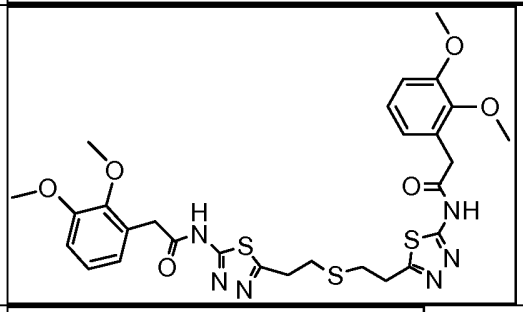
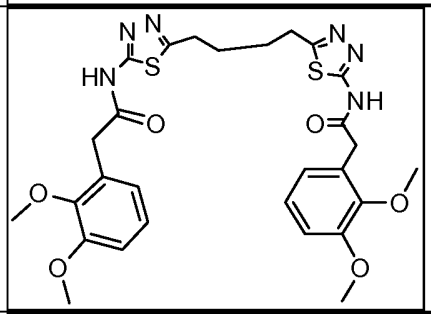
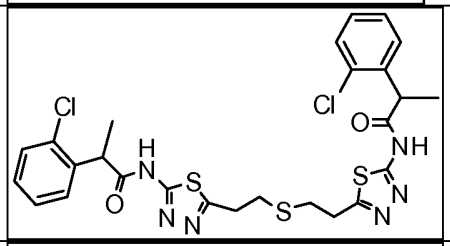
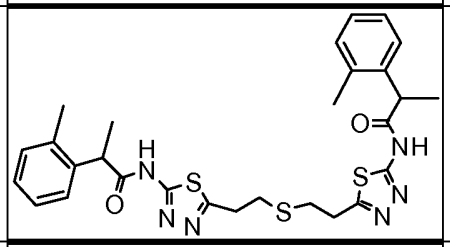
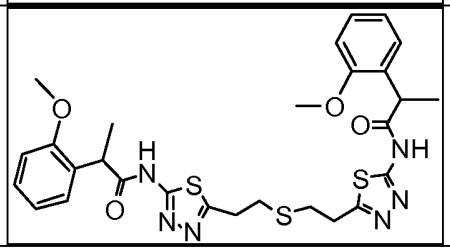
222			>20	
223			>20	
224			>20	
225			>20	
226			2.3	>50
227			9.9	3.3
228			0.57	0.13
229			3.9	

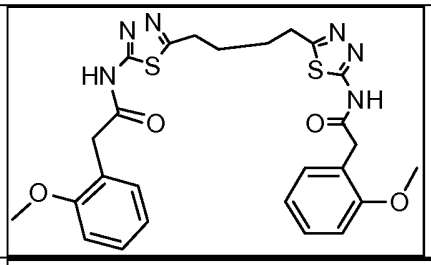
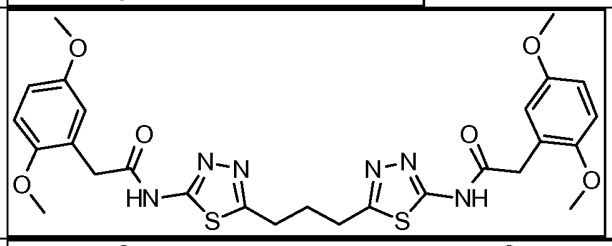
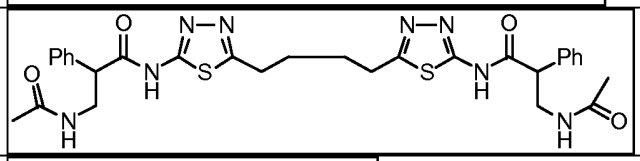
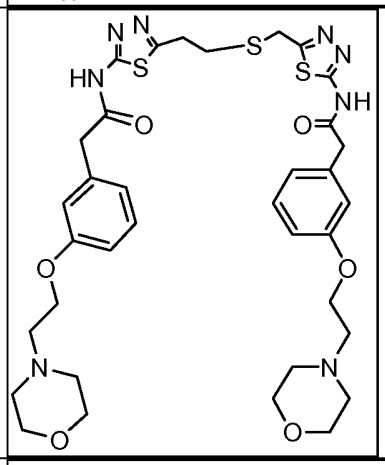
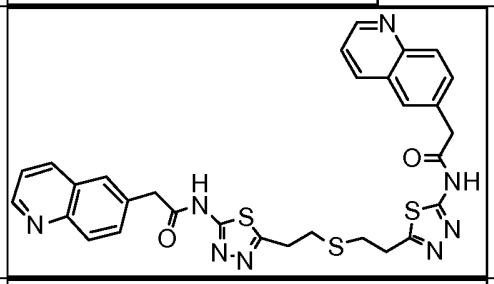
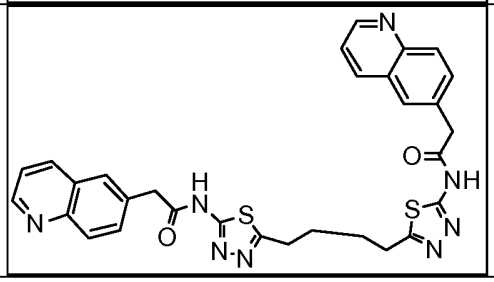
230			12	
231			7.4	
232			9.8	
233			15	
234			2.0	2.5
235			0.11	0.21
236			0.20	1.4

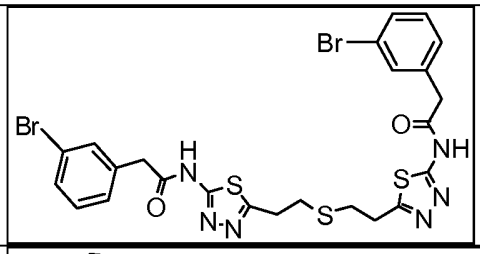
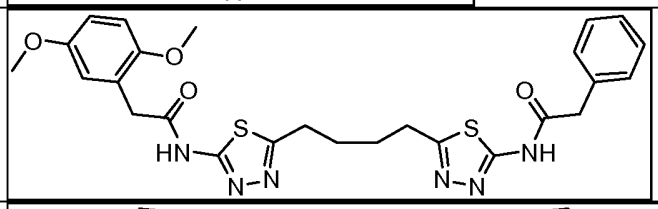
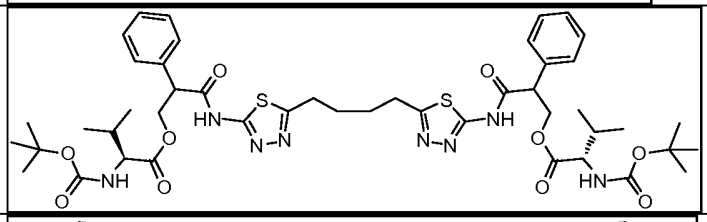
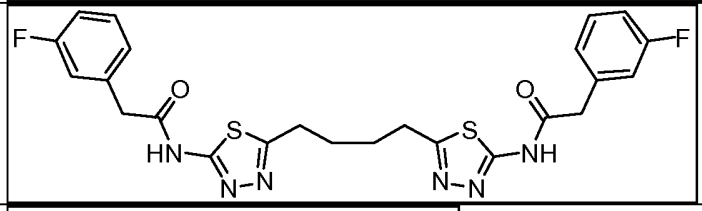
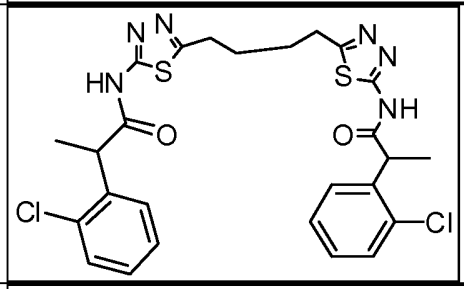
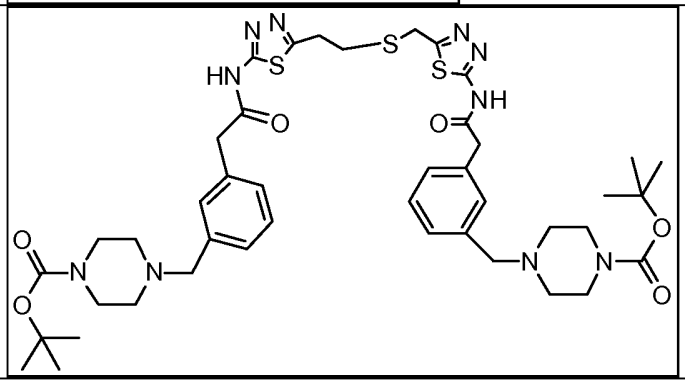
237			0.20	0.25
238			13	
239			0.30	0.30
240			0.54	1.3
241			0.38	0.87
242			0.36	0.22

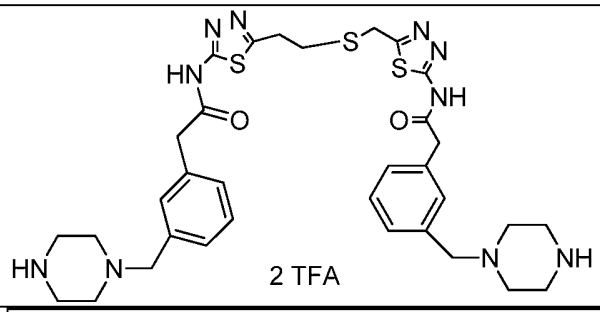
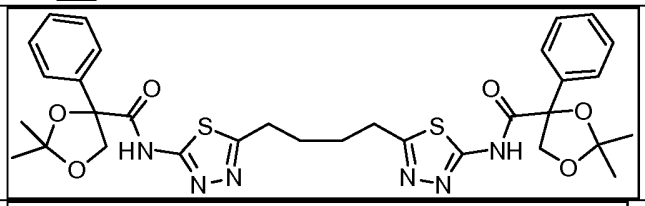
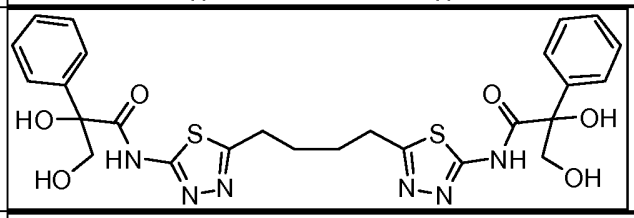
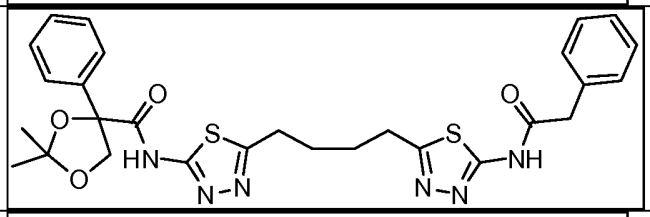
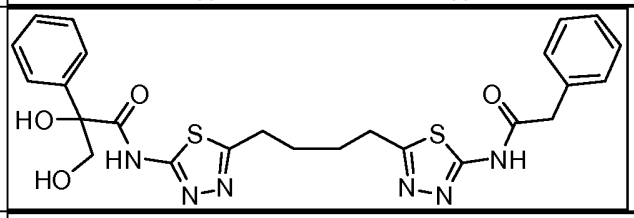
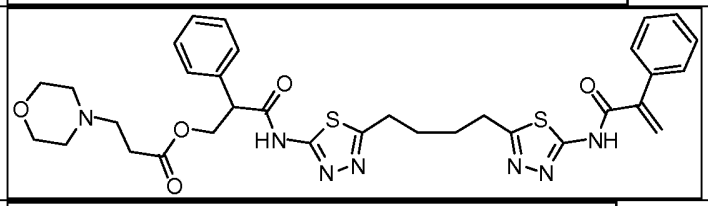
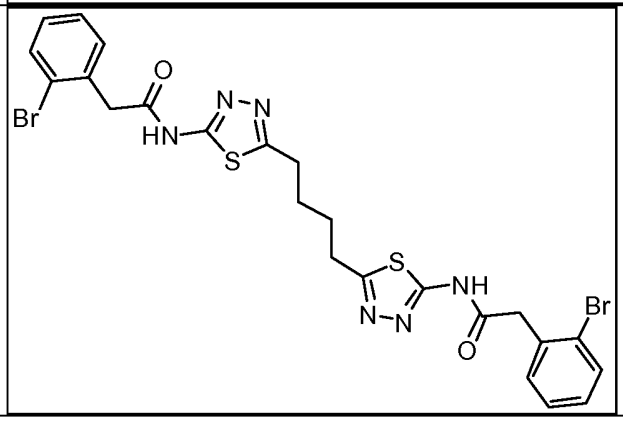
243			2.7	33
244			0.84	1.7
245			0.52	2.5
246			0.40	1.6
247			0.19	0.83
248			2.3	
249			0.12	0.16

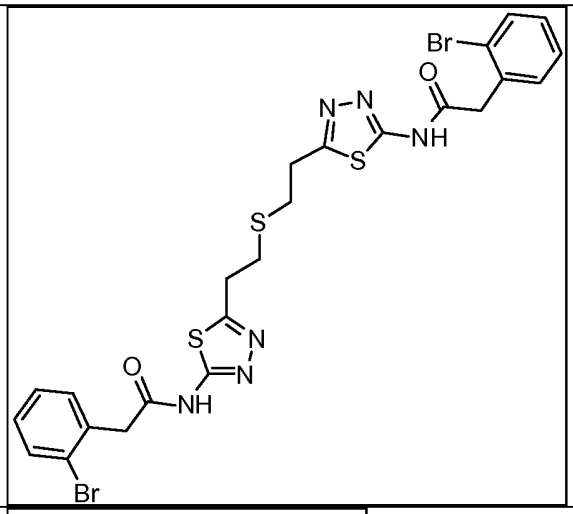
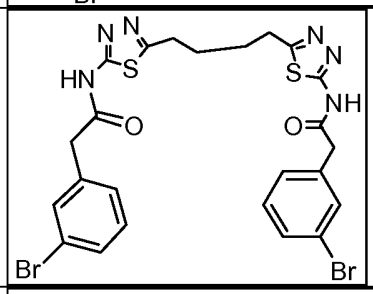
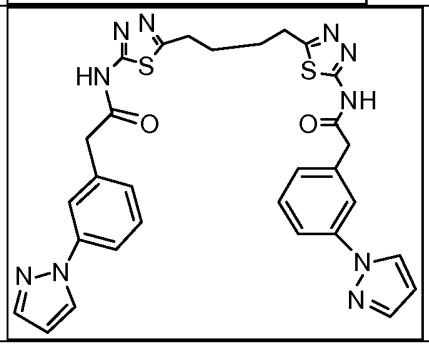
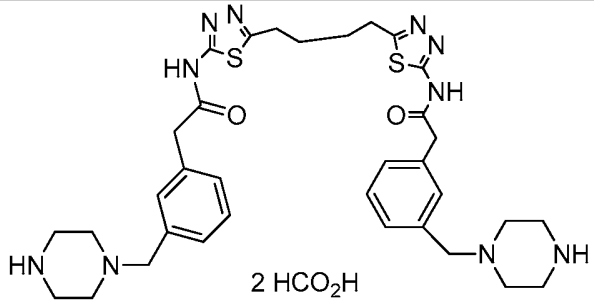
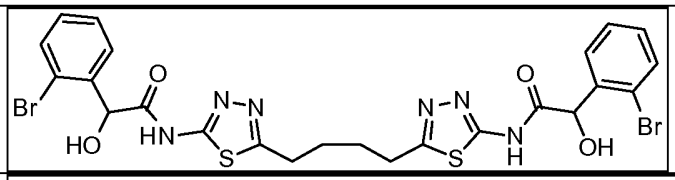
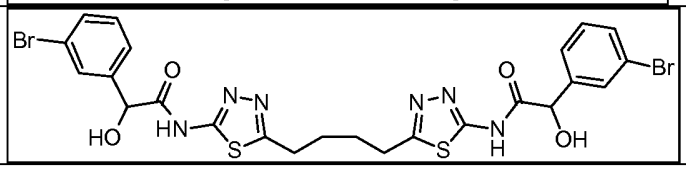
250			0.12	0.14
251			2.8	2.8
252			1.2	6.3
253			21	
254			>20	
255			0.38	
256			0.11	
257			0.12	0.073

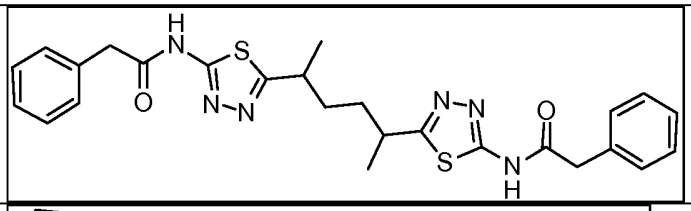
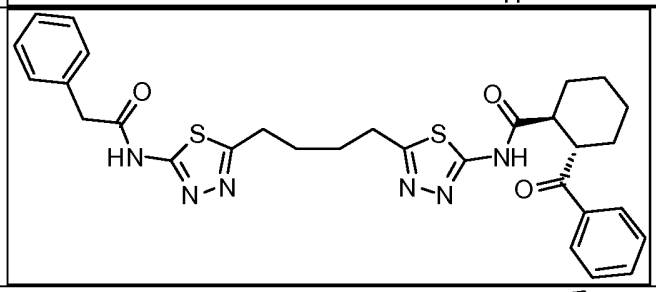
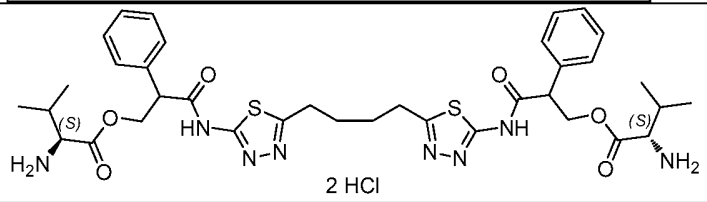
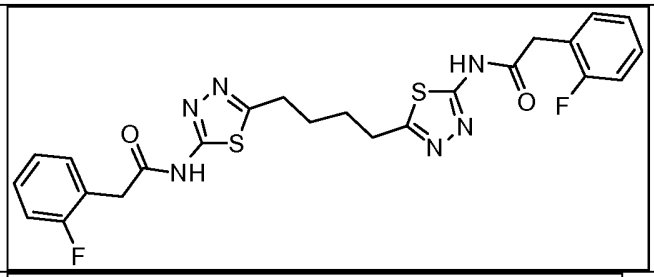
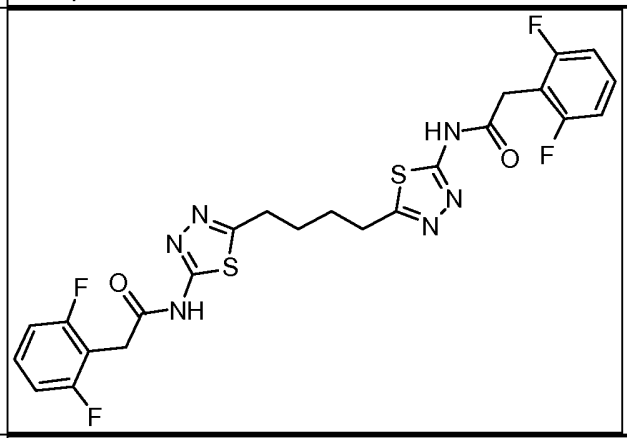
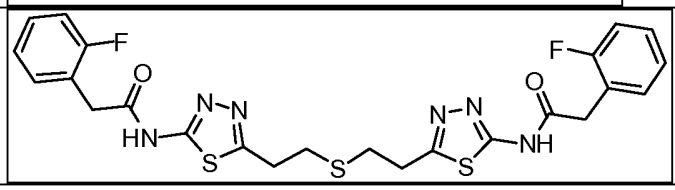
258			0.19	0.18
259			0.23	0.57
260			0.15	0.084
261			0.70	2.6
262			0.36	3.1
263			0.32	3.9

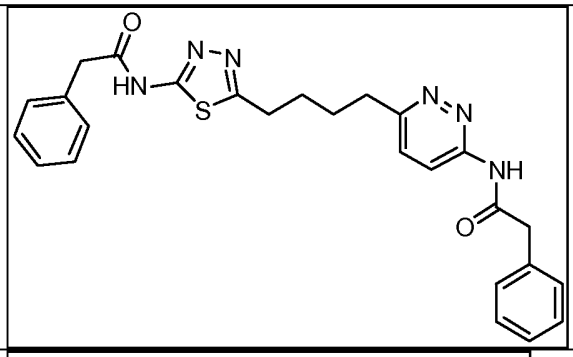
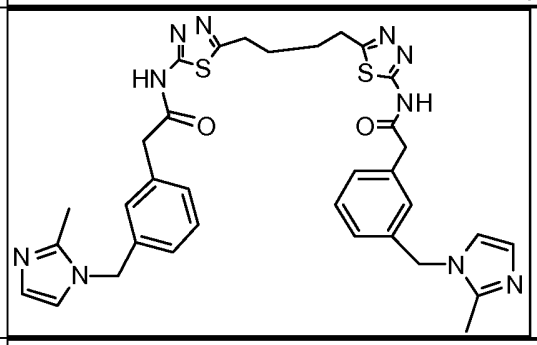
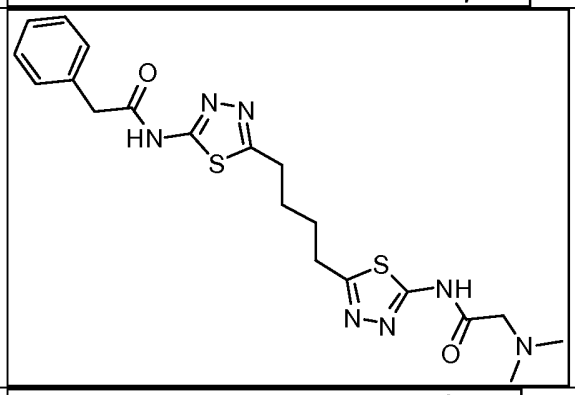
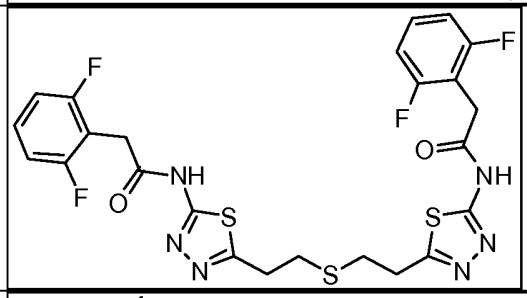
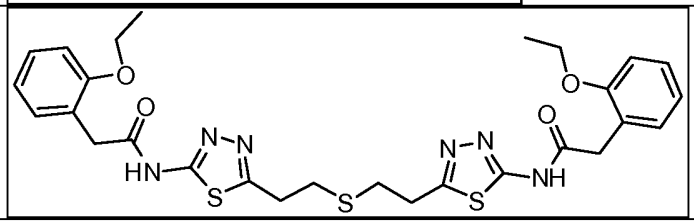
264			0.072	0.01
265			0.27	0.31
266			2.2	>50
267			0.61	0.64
268			0.60	5.4
269			0.26	0.52

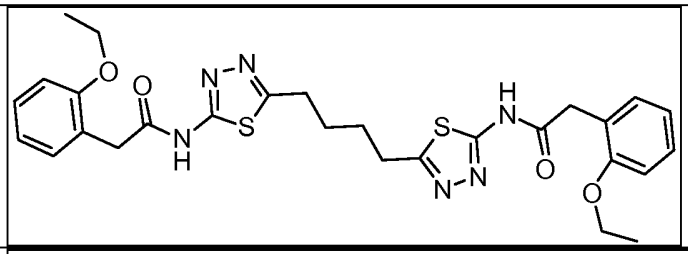
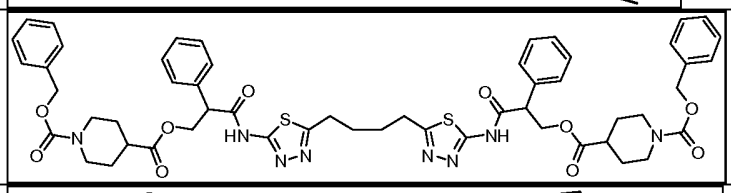
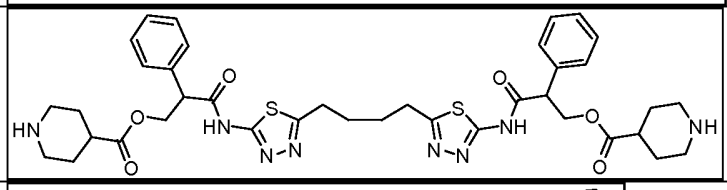
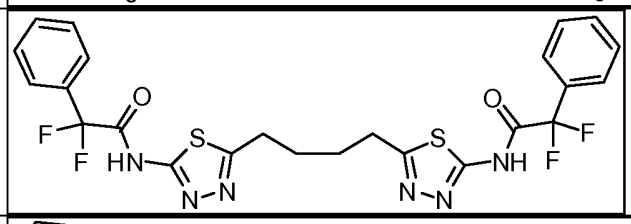
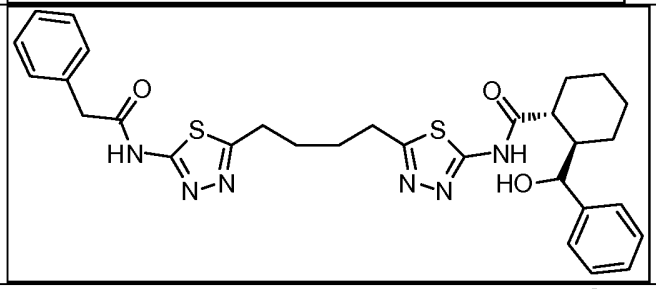
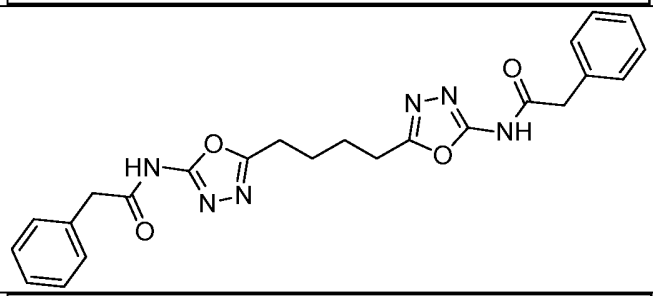
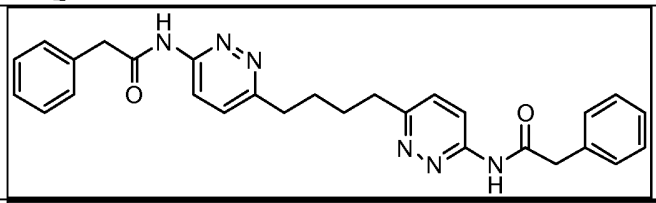
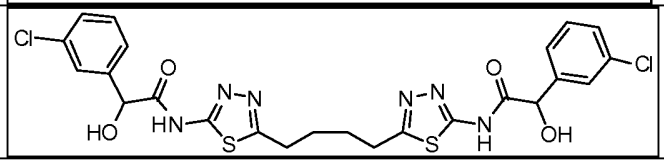
270		>5	7.4	0.85
271			0.10	0.63
272			>20	
273			0.14	0.07
274			0.75	0.68
275		0.15	2.2	0.34

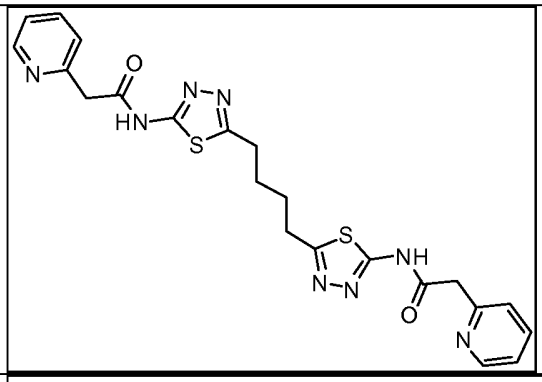
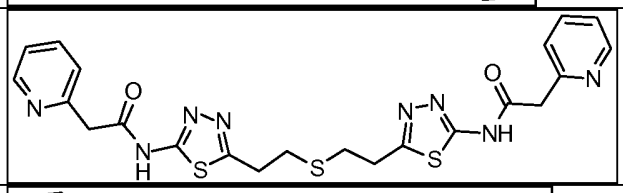
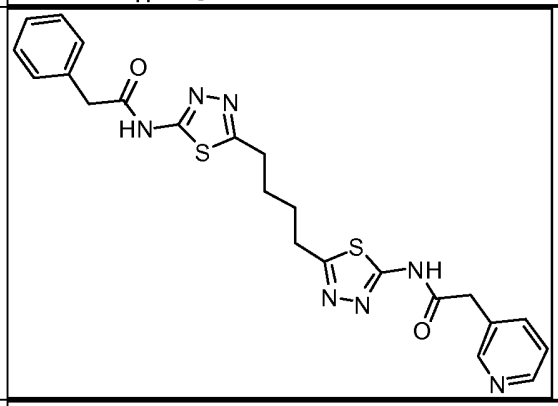
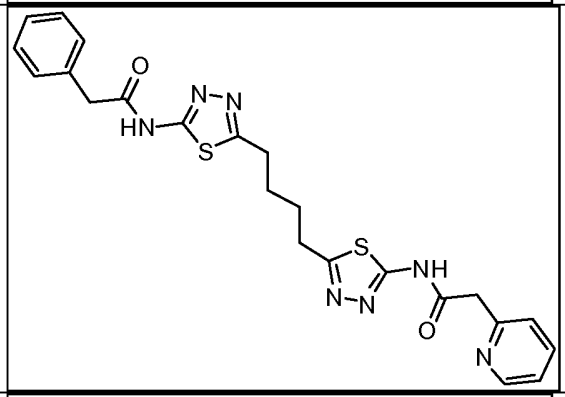
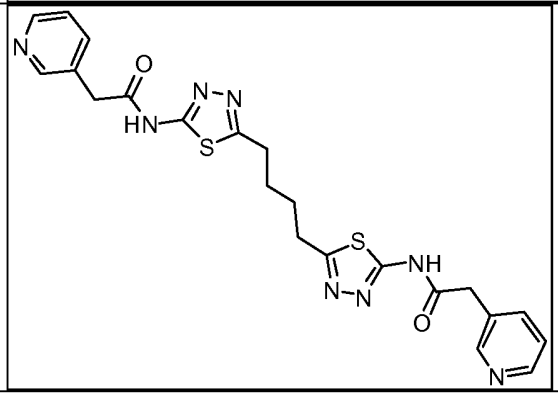
276			1.5	56
277			>20	
278			0.38	0.16
279			0.68	7.0
280			0.29	0.23
281			0.74	0.66
282			0.082	0.37

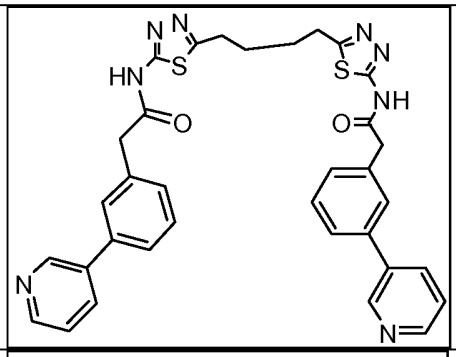
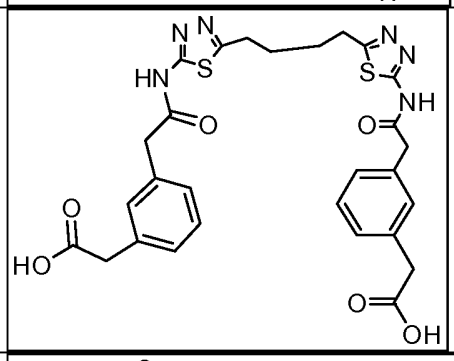
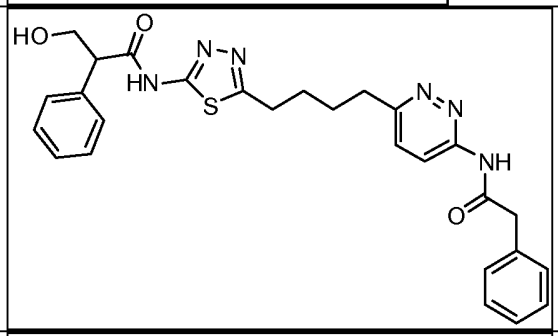
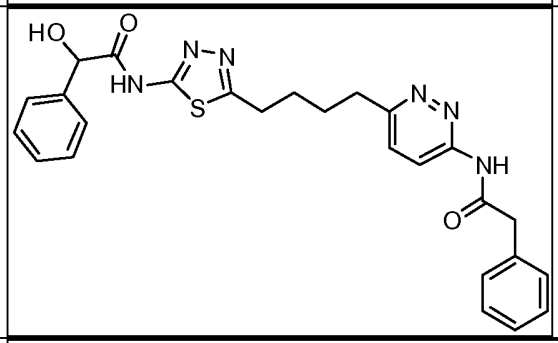
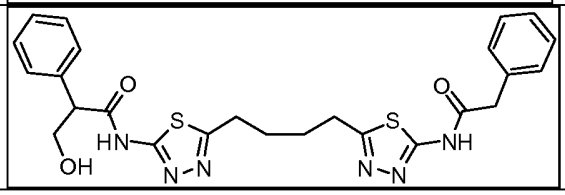
283			0.66	0.74
284		0.05	>20	
285			0.19	0.14
286			0.54	6.4
287			0.57	1.3
288		0.04	0.67	0.028

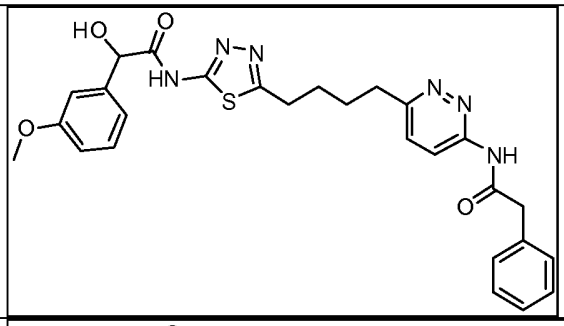
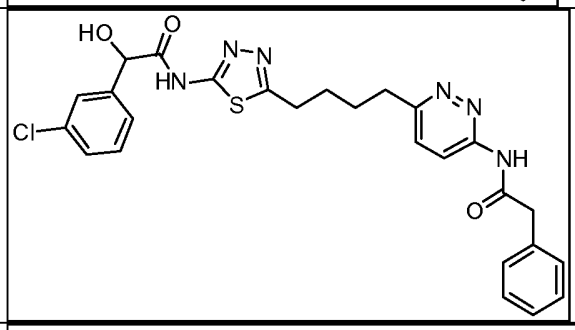
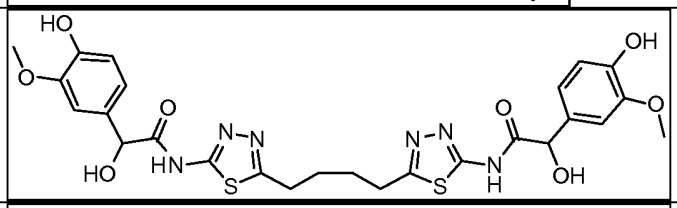
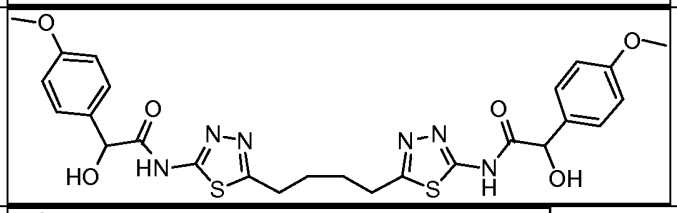
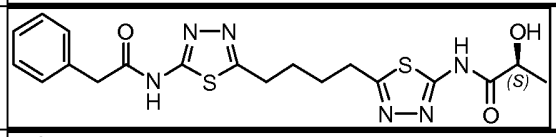
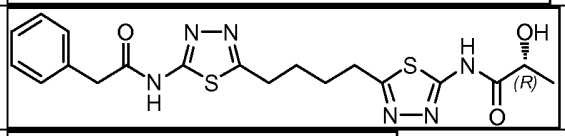
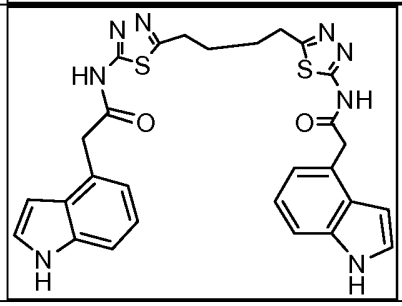
289			32	
290			0.80	0.79
291			1.5	1.8
292			0.12	0.012
293			0.24	0.04
294			0.20	1.1

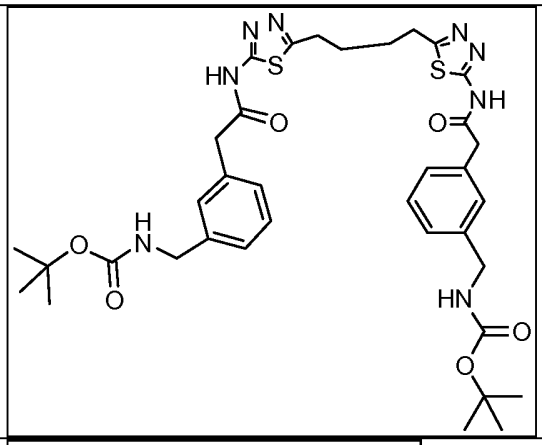
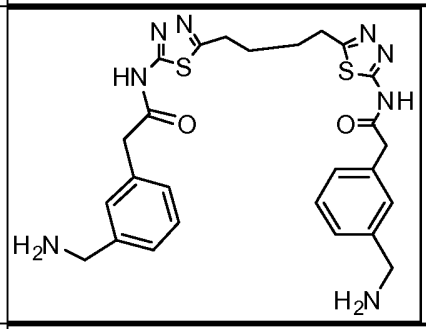
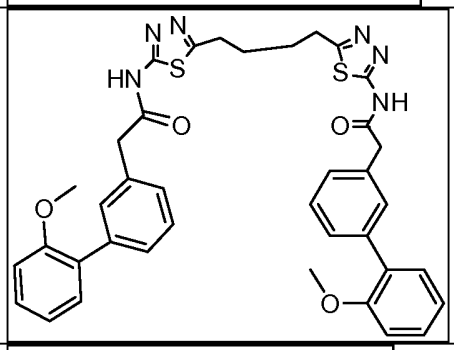
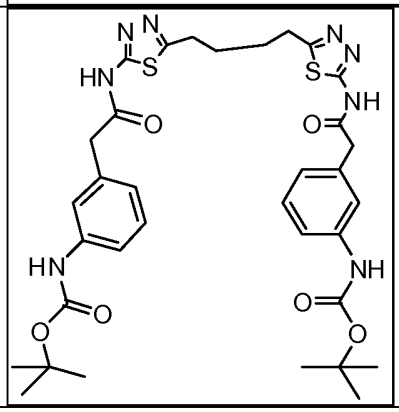
295		0.01	0.057	0.039
296			0.10	0.17
297			6.4	
298			0.73	5.1
299			0.33	

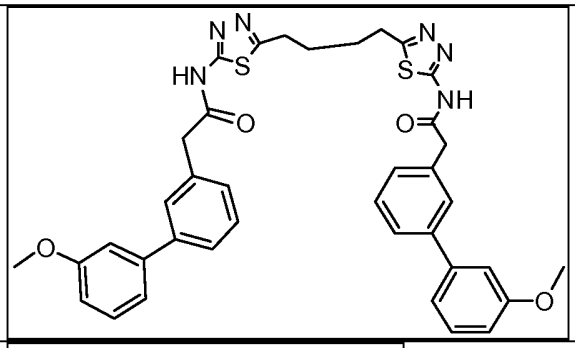
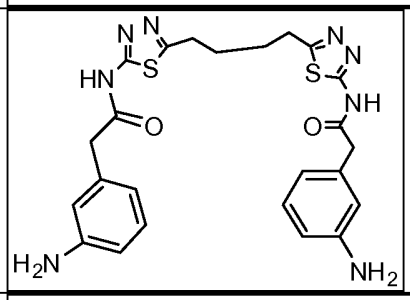
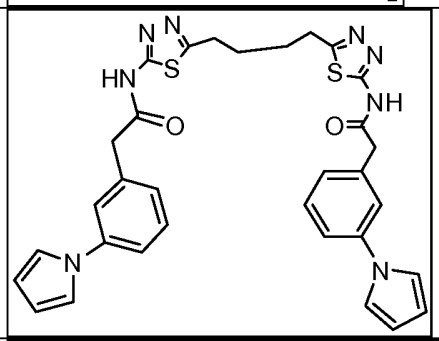
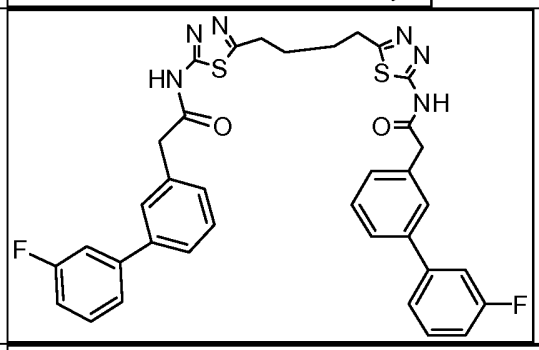
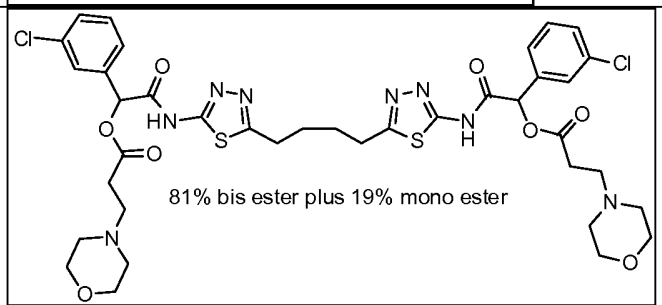
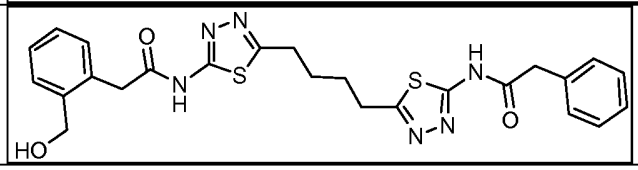
300			0.16	0.16
301			>20	0.23
302			7.0	0.87
303			>20	
304			1.2	4.9
305			>20	102
1038			0.080	1.5
306		0.031	0.52	0.066

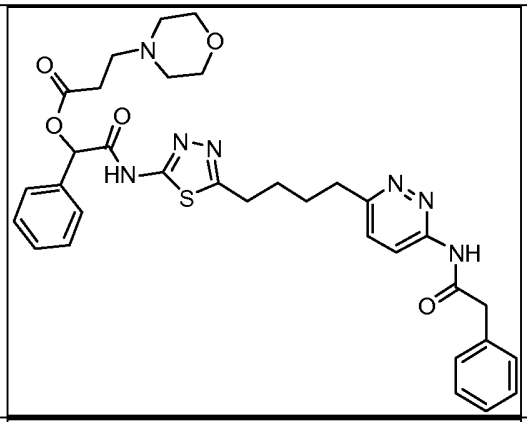
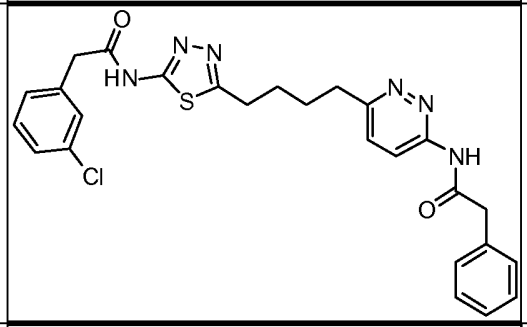
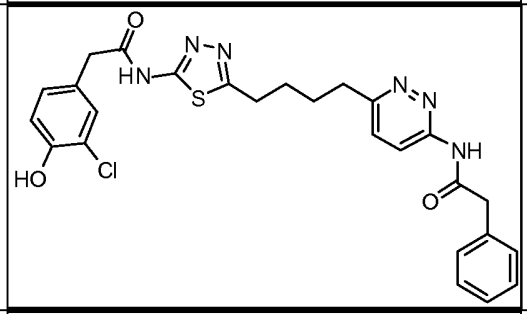
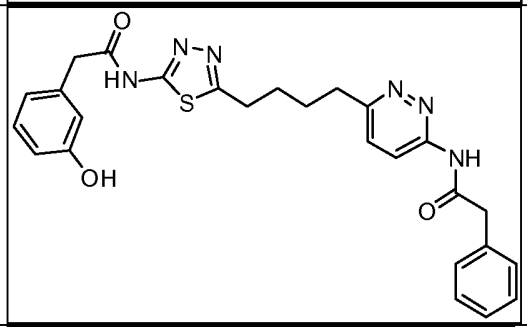
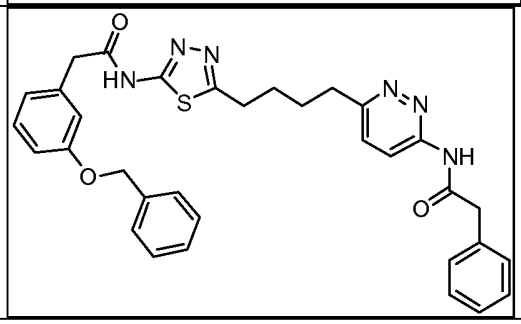
307			6.4	9.3
308			0.60	1.2
309			0.11	0.18
310			0.083	0.12
311			0.20	22.

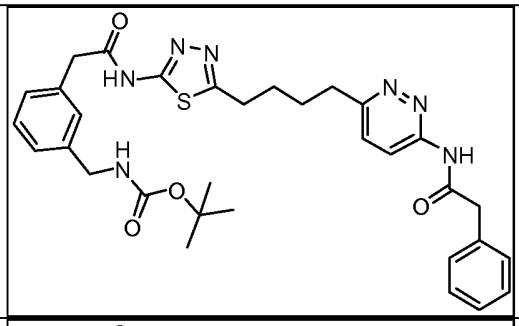
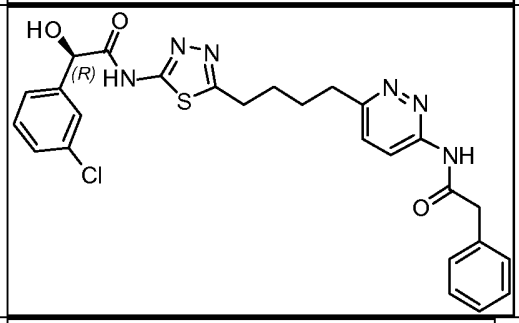
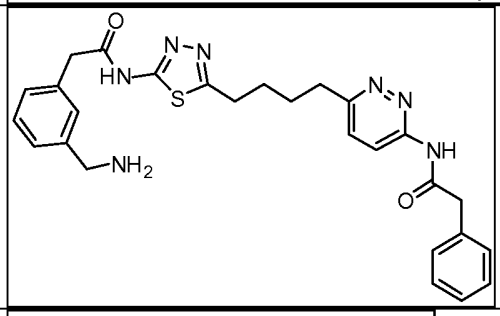
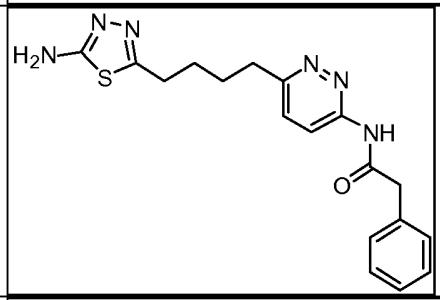
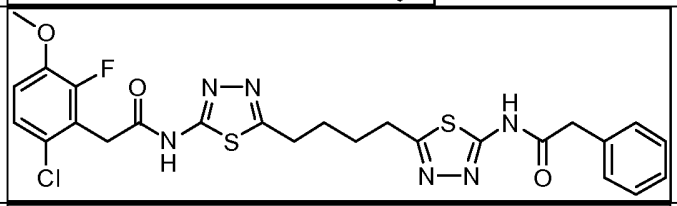
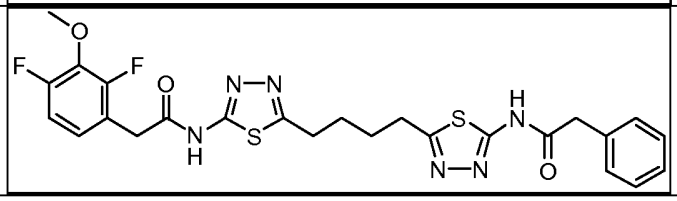
312				>20	N/D
313				0.27	94
314				0.14	0.048
315			0.017	0.12	0.035
316				0.19	0.075

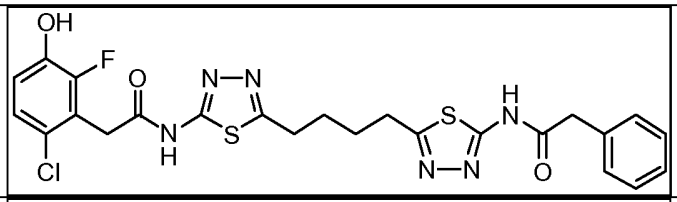
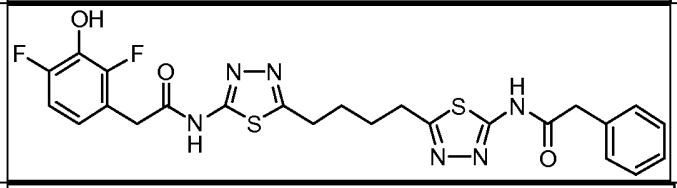
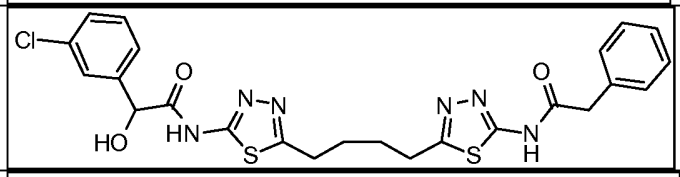
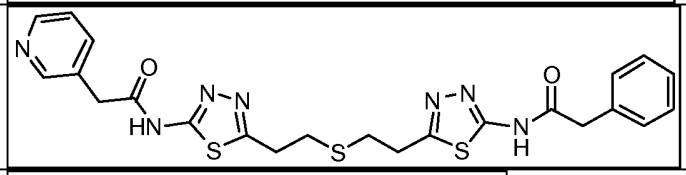
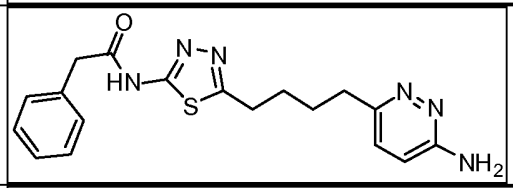
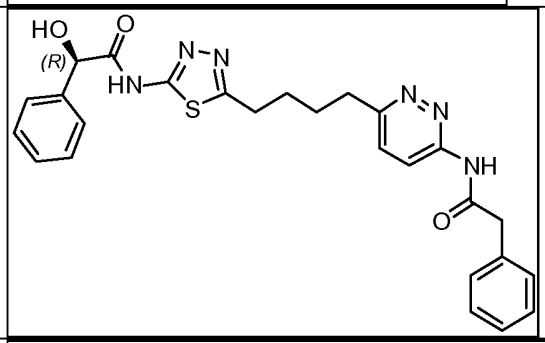
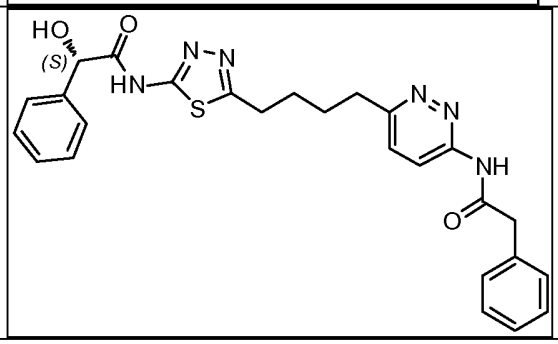
317		0.007	0.18	0.010
318		0.006	0.18	0.017
319			0.64	10
320			0.40	0.19
321			2.5	2.6
322			2.8	3.0
323			0.056	0.20

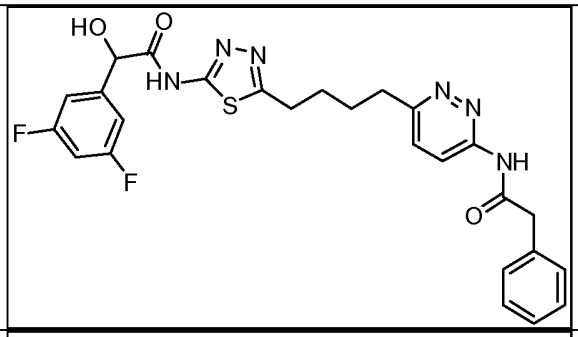
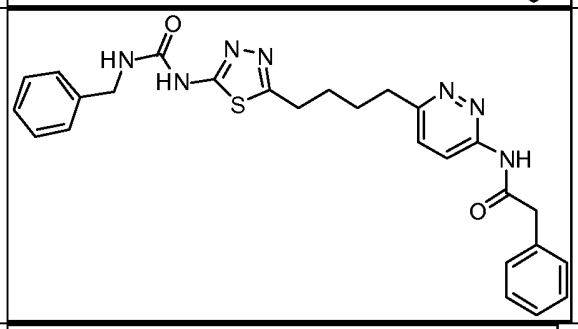
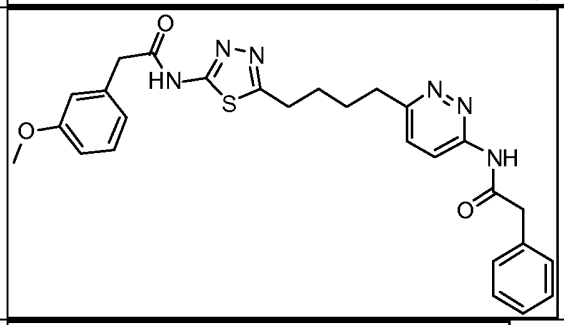
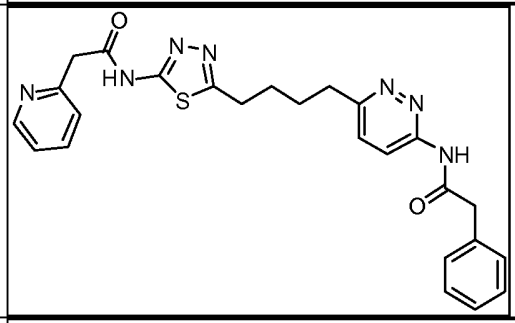
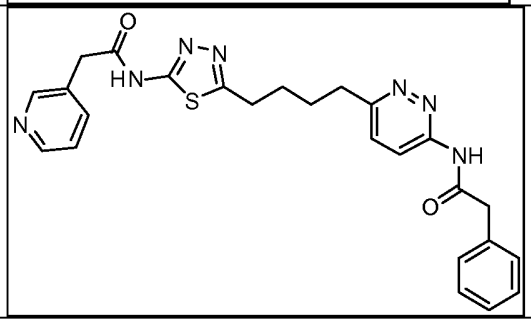
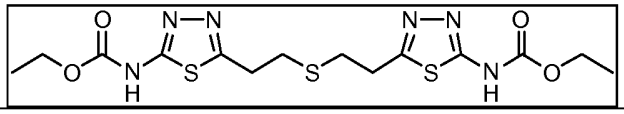
324		0.011	4.6	0.10
325		0.17	0.66	0.030
326			>20	N/D
327			>20	0.15

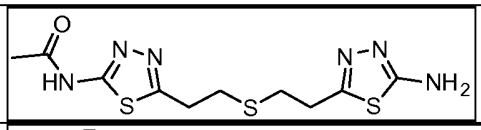
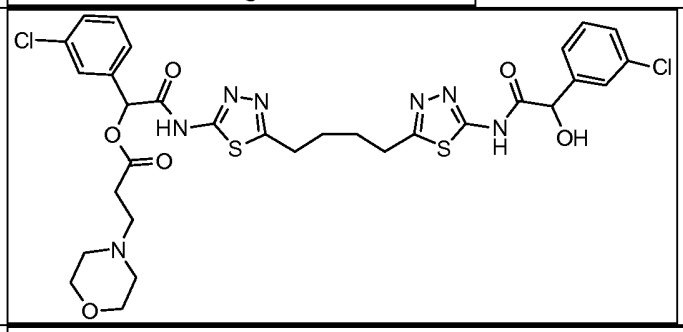
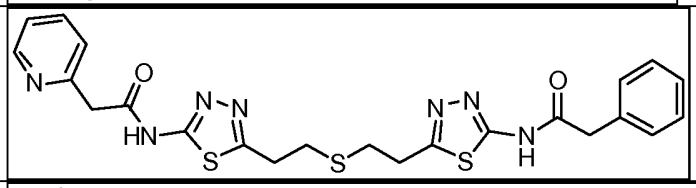
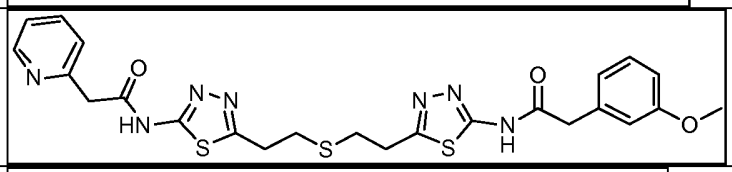
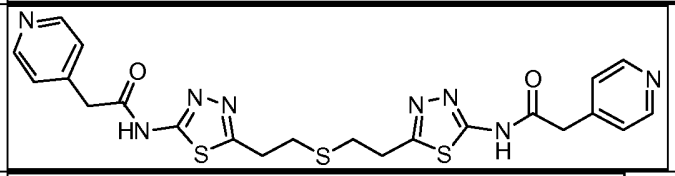
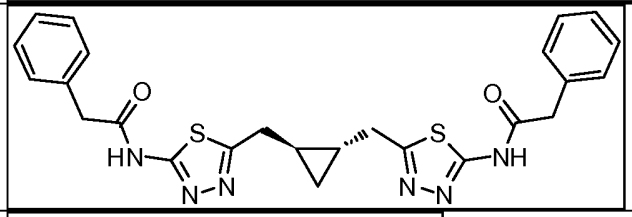
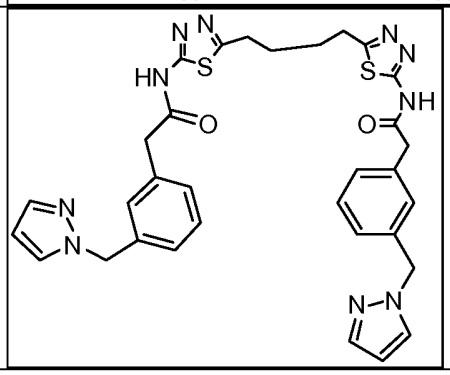
328			>20	N/D
329			0.17	0.45
330			>20	N/D
331			>20	N/D
332			3.3	0.087
333			0.10	1.6

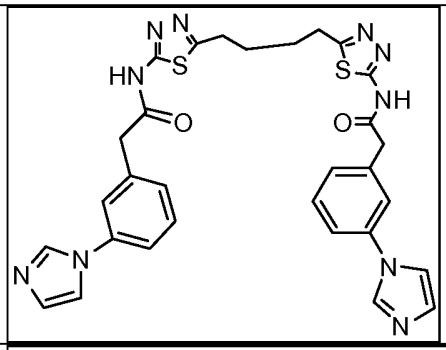
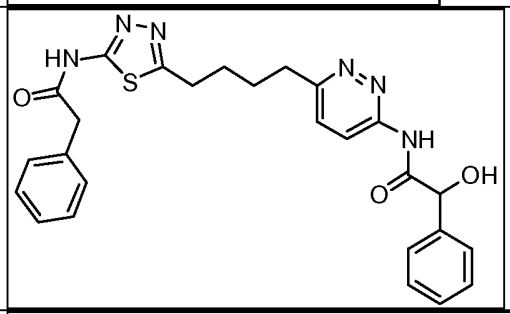
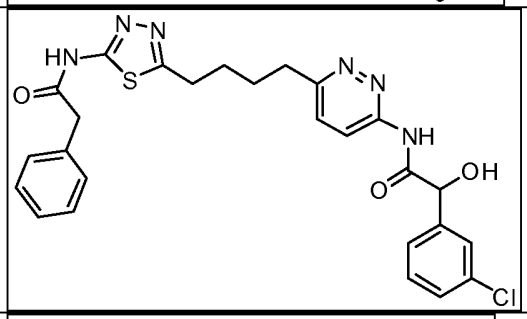
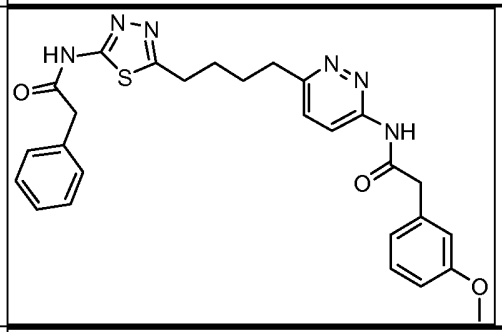
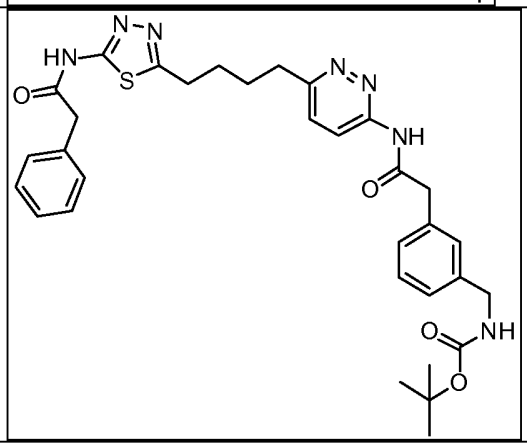
334				0.64	0.030
335				0.062	0.050
336				0.068	0.052
337				0.073	0.021
338				0.15	0.043

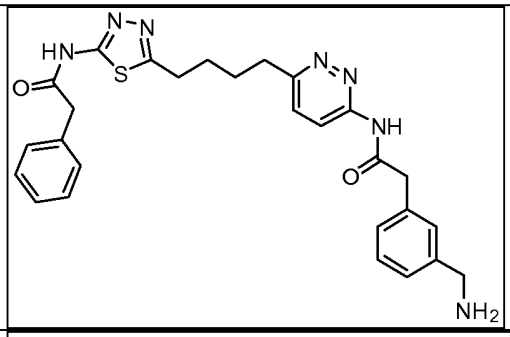
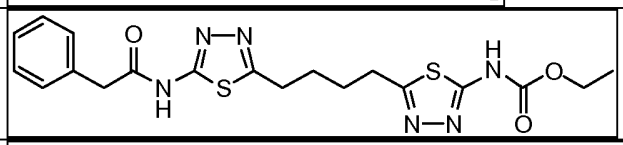
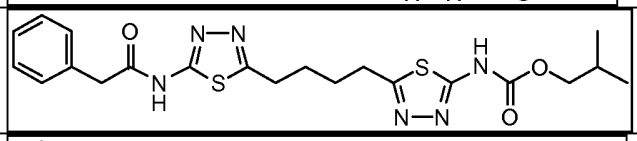
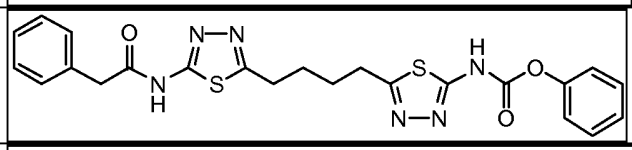
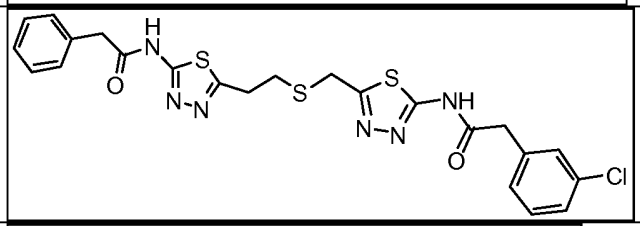
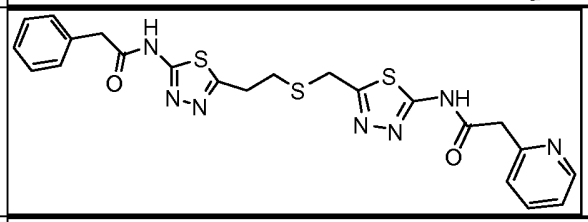
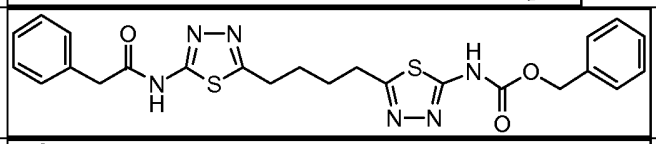
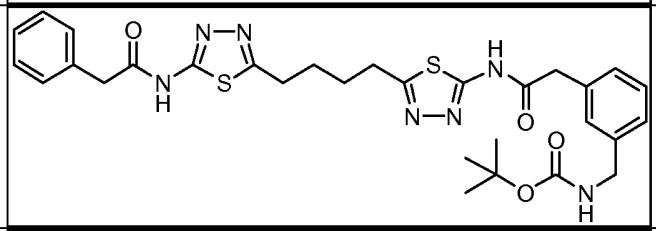
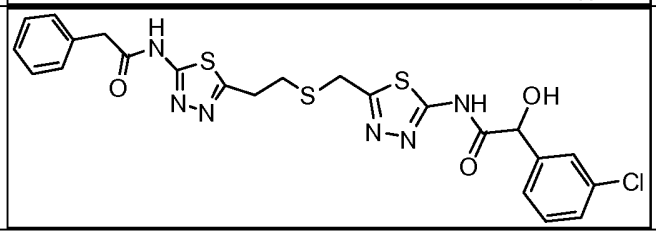
339		0.005	0.16	0.009
340			0.096	0.038
341		0.013	0.13	0.039
342			1.4	2.7
343			0.16	0.25
344				0.088

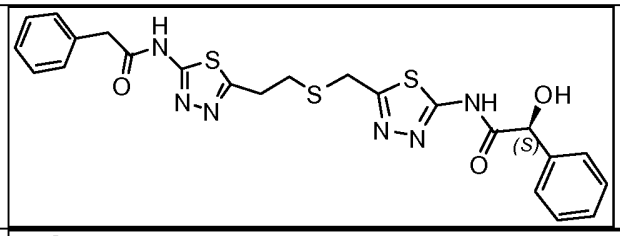
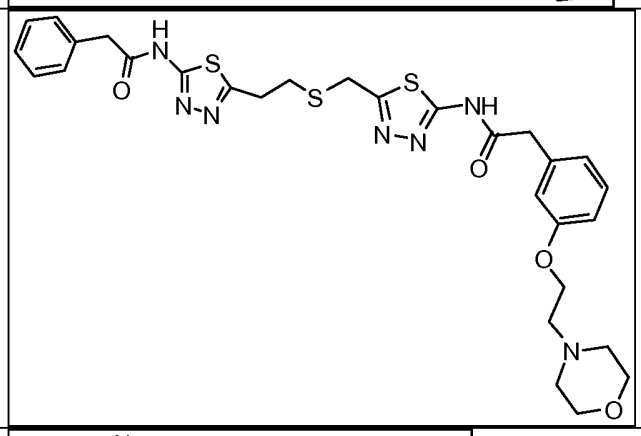
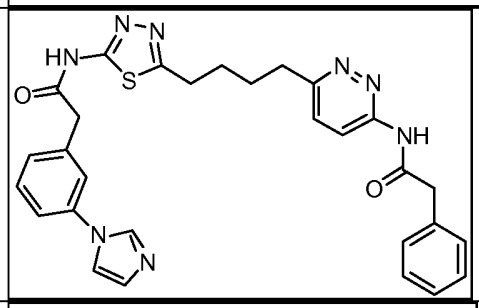
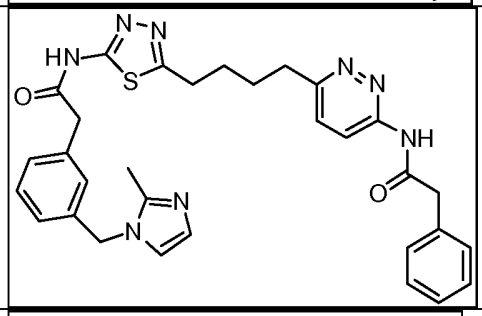
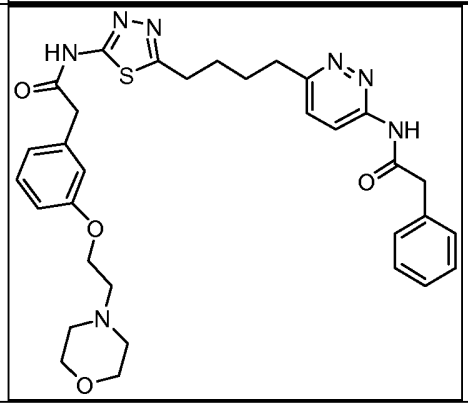
345			0.16	0.24
346			0.12	0.087
527		0.024	0.13	0.098
347			0.22	0.71
348			1.0	1.7
349			0.12	0.12
350			0.079	0.029

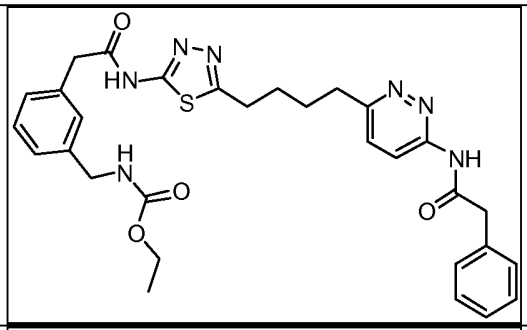
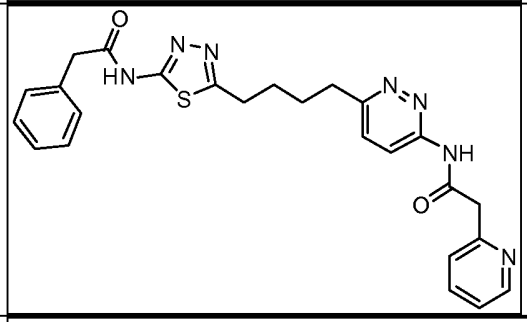
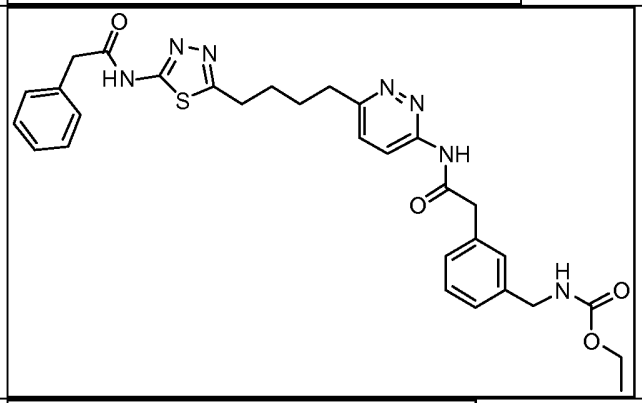
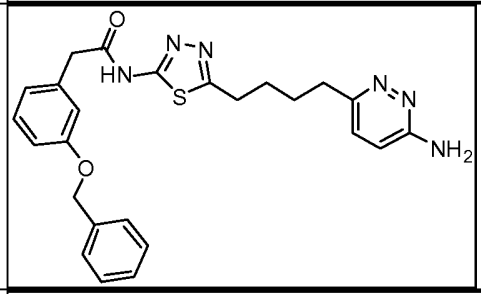
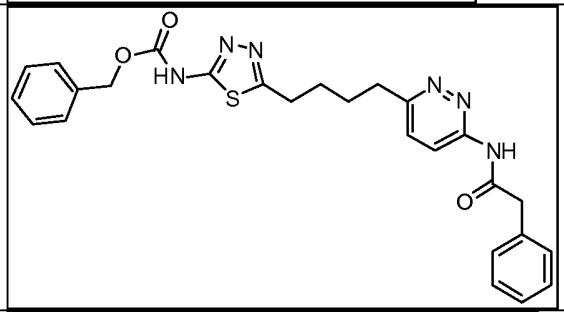
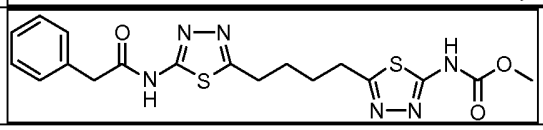
351			0.11	0.049
352			0.069	0.13
353			0.049	0.021
354			0.10	0.047
355			0.10	0.039
356			>20	N/D

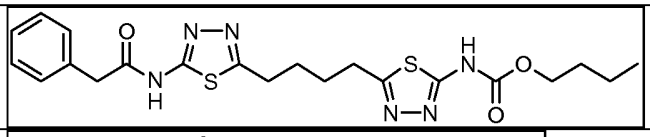
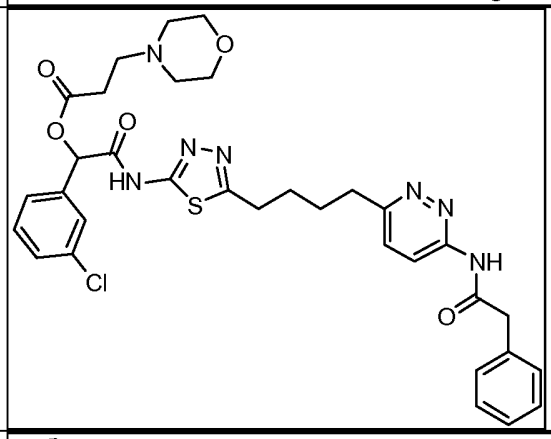
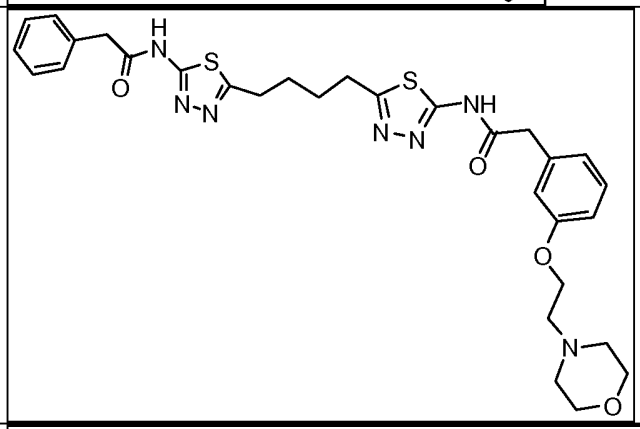
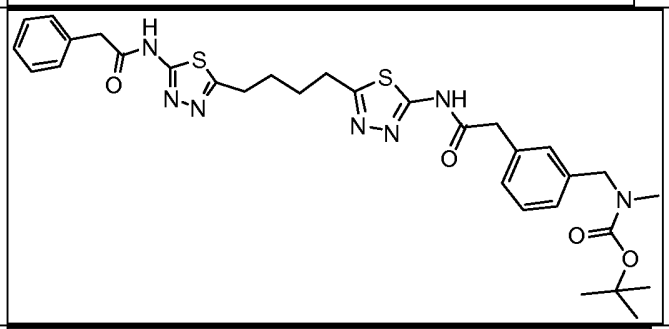
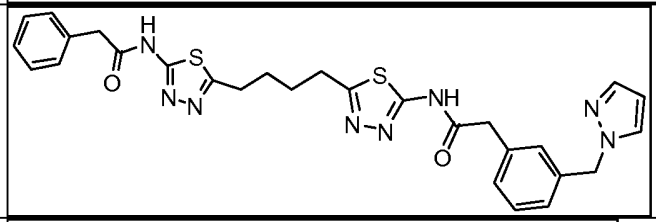
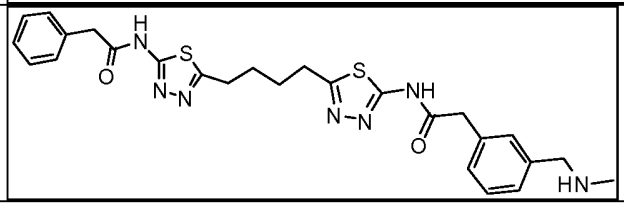
357			>20	N/D
358			1.4	0.11
359			0.38	0.91
360			0.28	0.67
361			1.8	>20
1035			>20	N/D
362			0.35	0.054

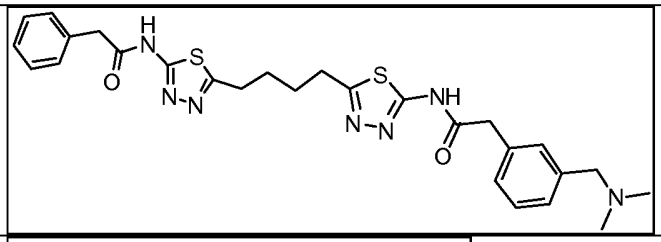
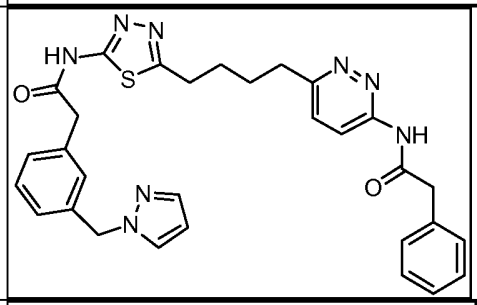
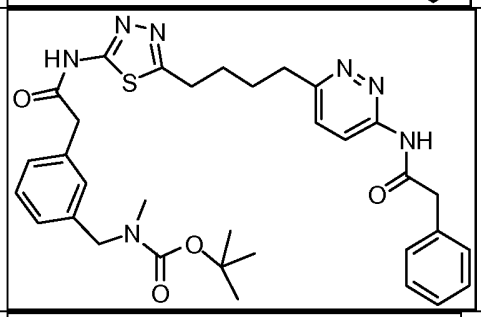
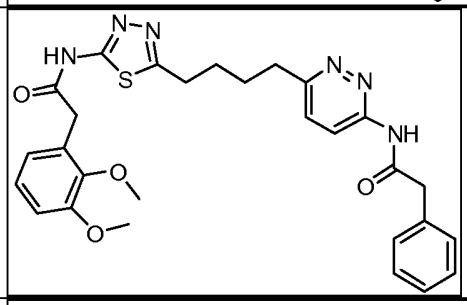
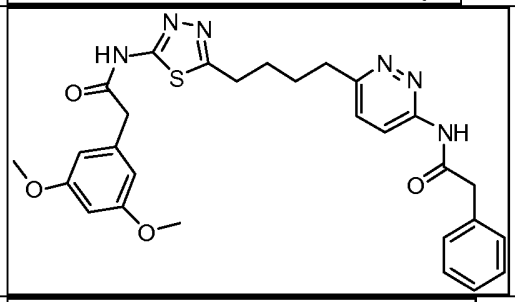
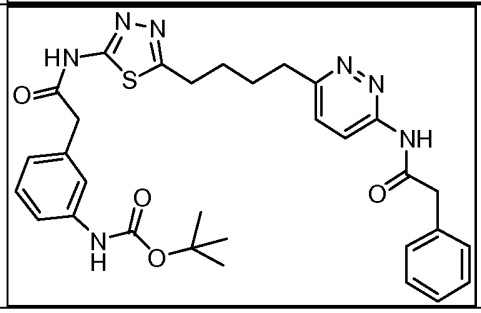
363			0.065	>20
364		0.030	0.15	0.26
365		0.009	0.092	0.089
366			0.074	0.024
367		0.002	0.12	0.006

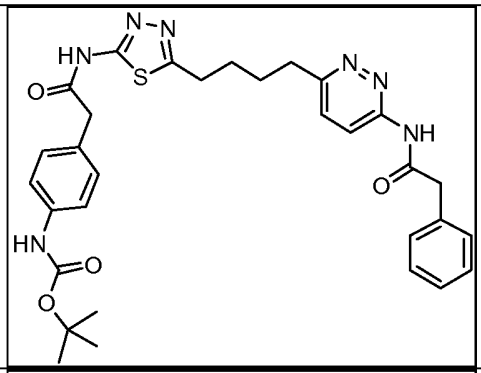
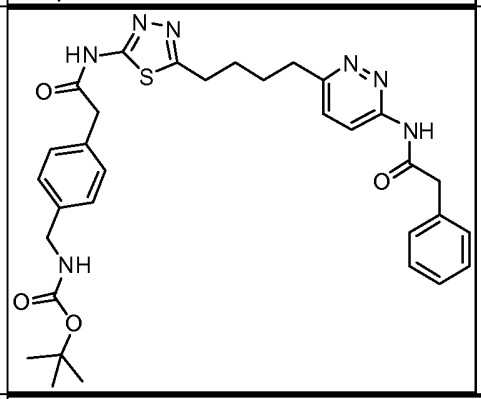
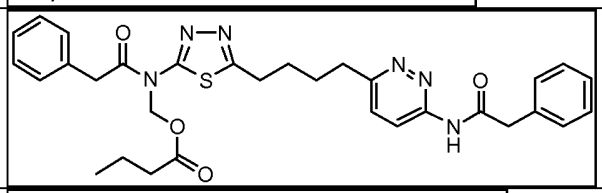
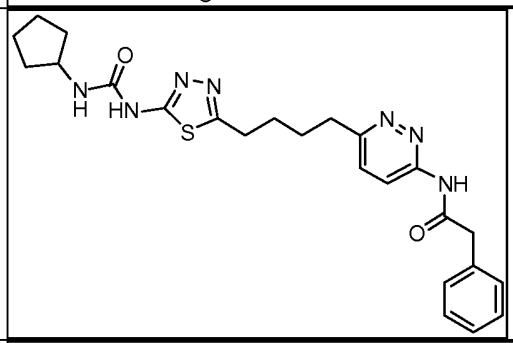
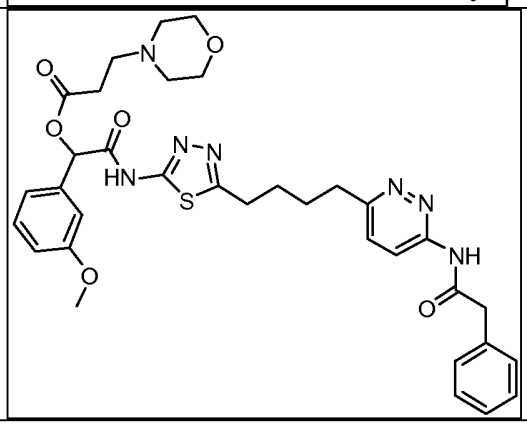
368		0.009	0.11	0.017
369			0.81	1.9
370			0.28	0.70
371			0.43	5.2
372			0.16	0.15
373			0.17	0.28
374			0.26	0.47
375		0.005	0.38	0.041
376			0.35	0.091

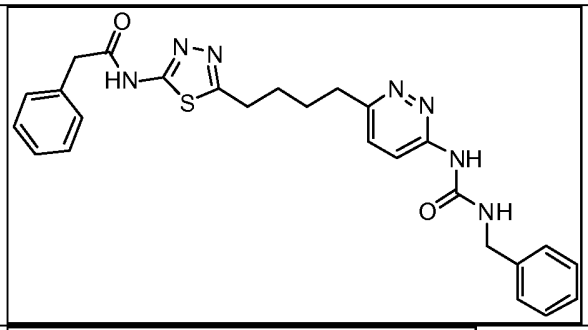
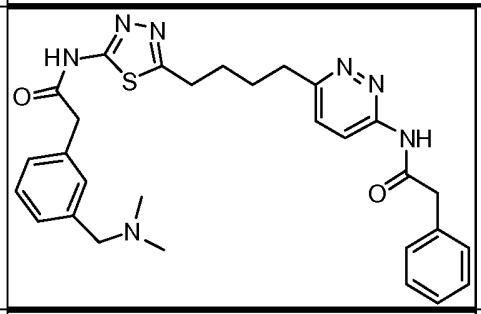
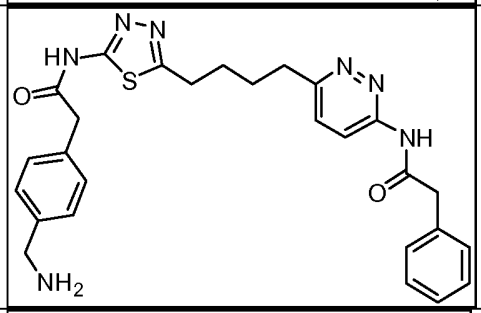
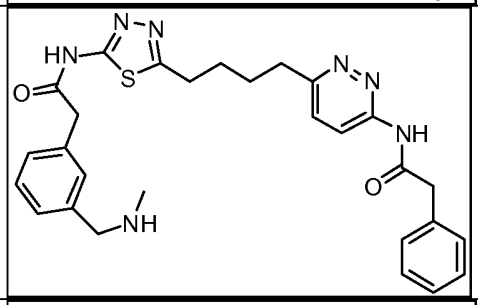
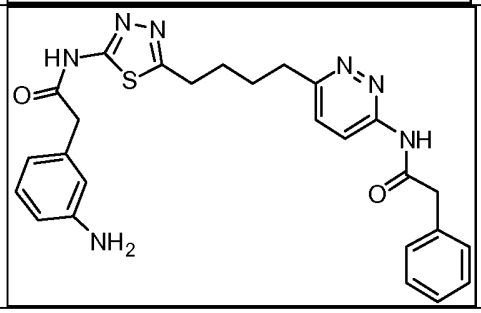
377			0.28	0.10
378			0.22	0.090
379			0.097	0.038
380			0.12	0.019
381			0.16	0.018

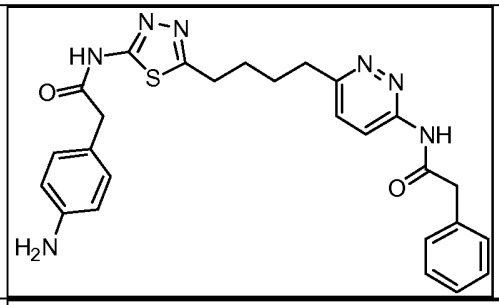
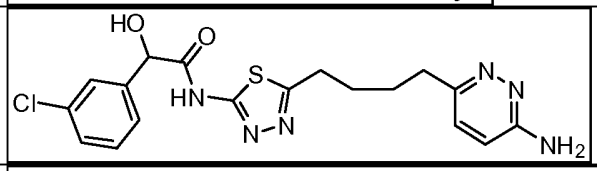
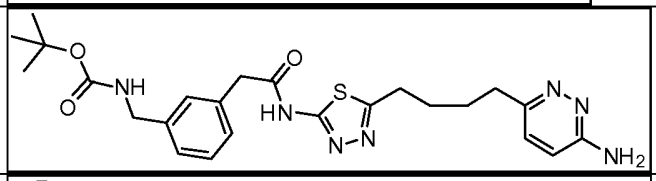
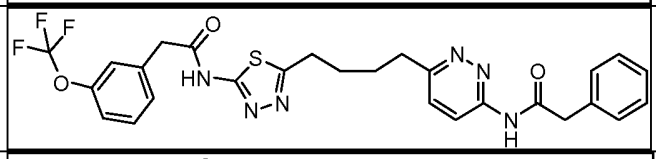
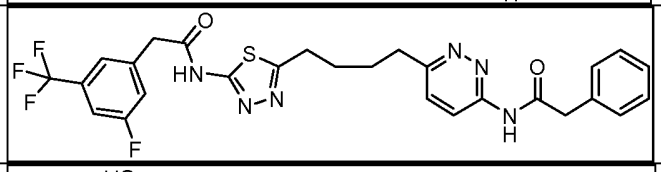
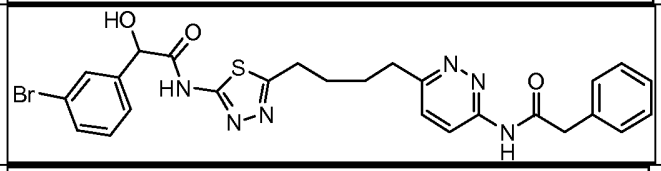
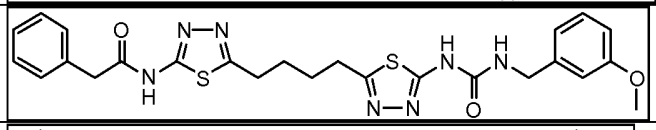
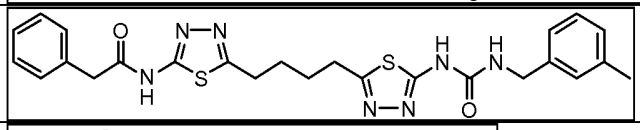
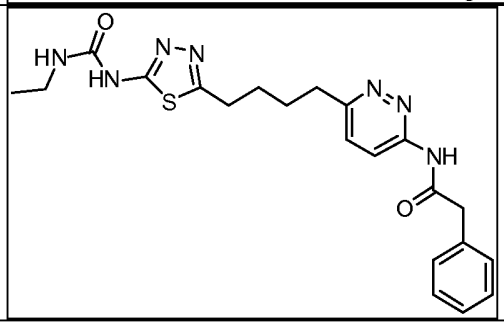
382		0.003	0.099	0.007
383			0.086	0.022
384		0.003	0.081	0.005
385			0.26	0.72
386			0.085	0.15
387			1.2	2.3

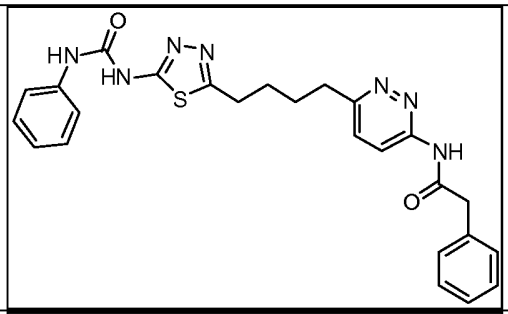
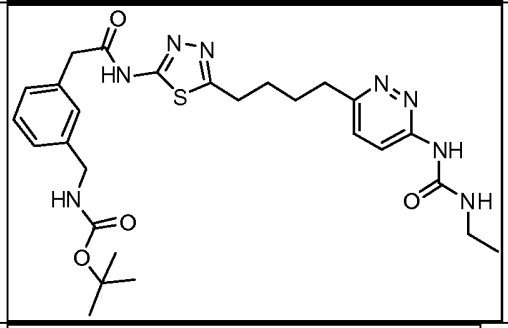
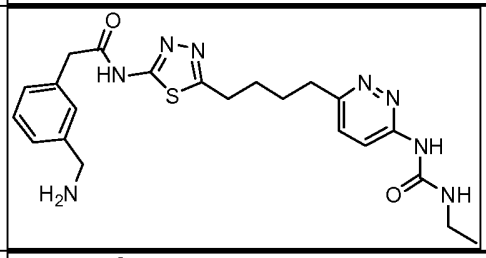
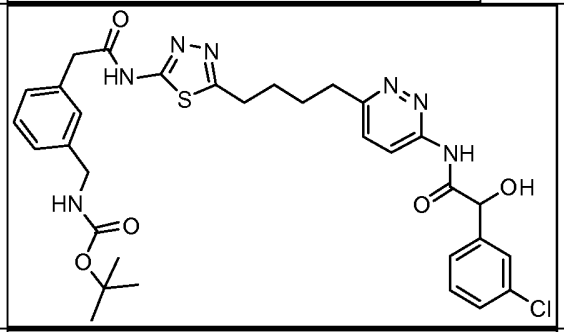
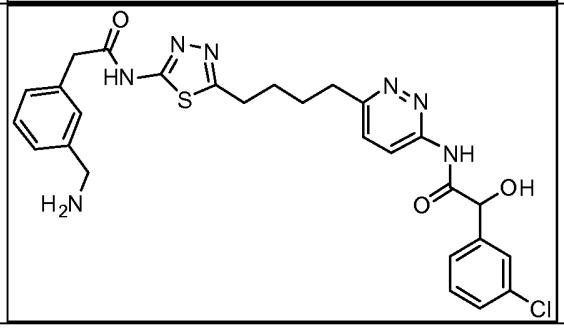
388			0.21	0.75
389		0.084		0.032
390		0.042		0.16
391		0.007		0.027
392		0.014		0.072
393		0.10		0.90

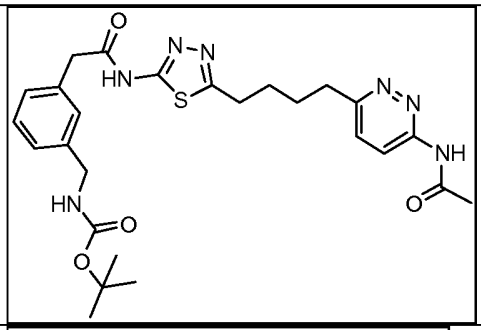
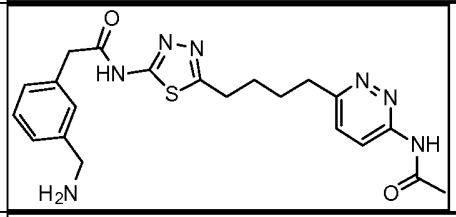
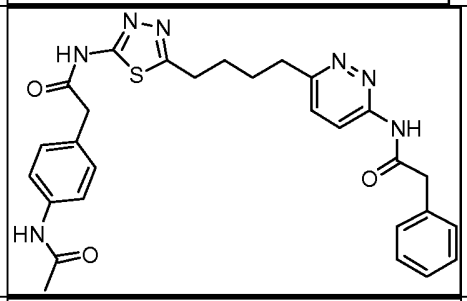
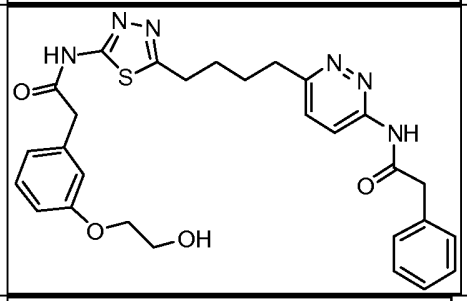
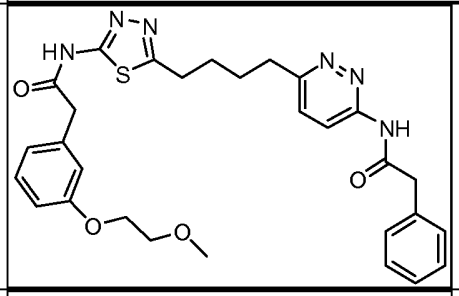
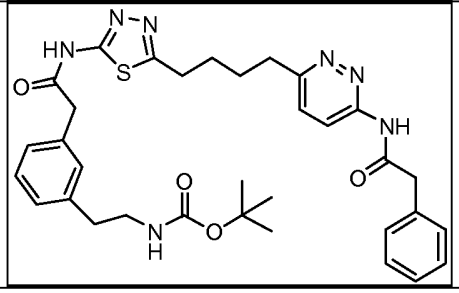
394		0.088		1.2
395		0.004		0.015
396		0.004		0.005
397		0.008		0.041
398		0.004		0.023
399		0.005		0.026

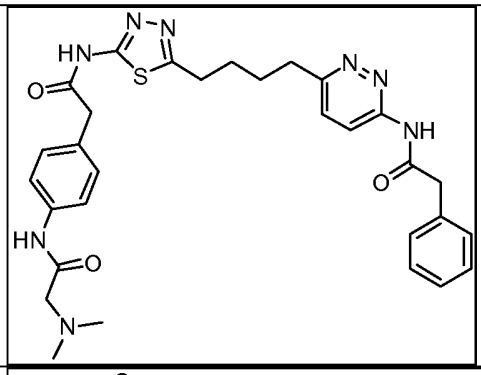
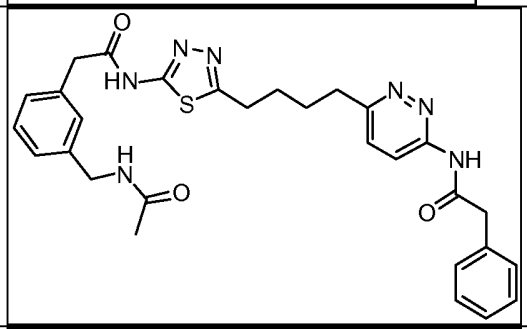
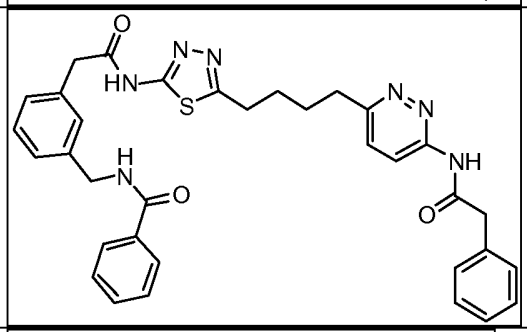
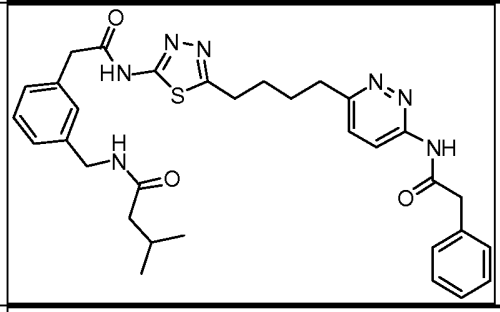
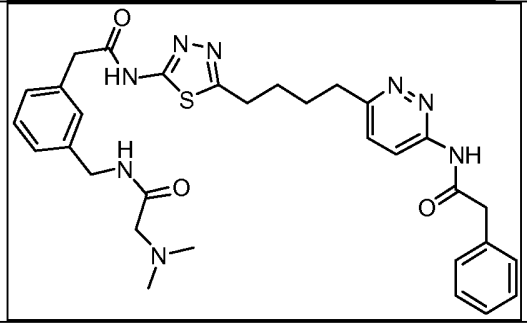
400			0.015		0.053
401			0.005		0.011
402			1.1		0.054
403			0.018		0.12
404			0.060		0.022

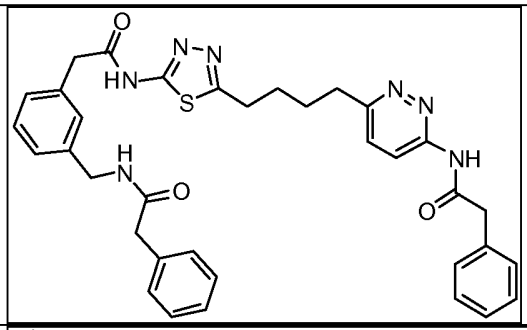
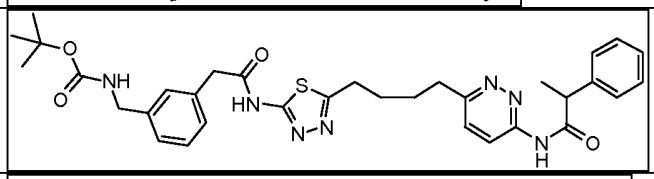
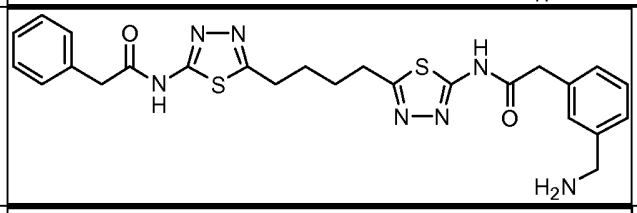
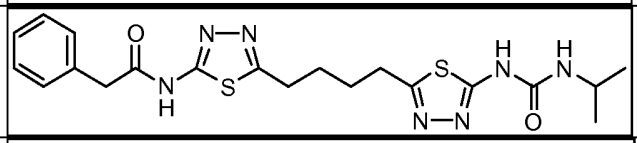
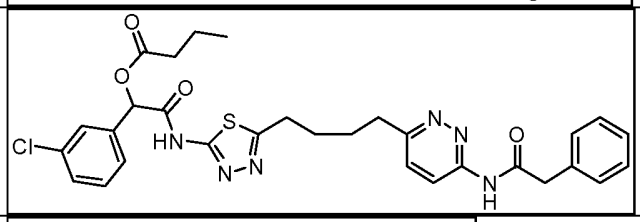
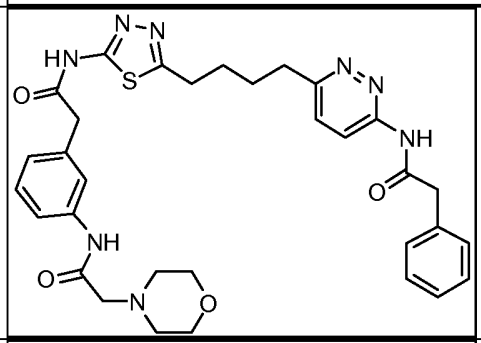
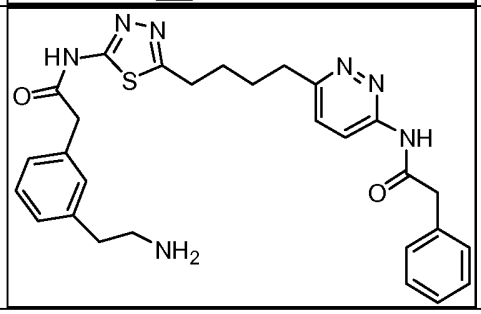
405		0.081		0.67
406		0.016		0.27
407		0.012		0.044
408		0.018		0.19
409		0.008		0.037

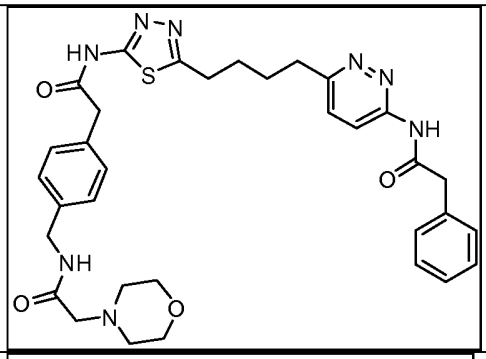
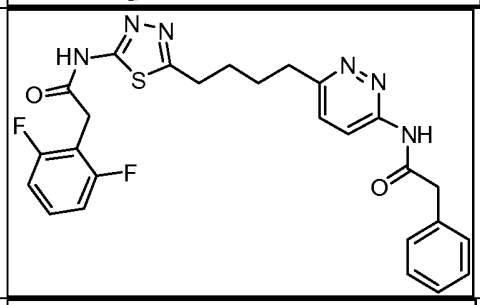
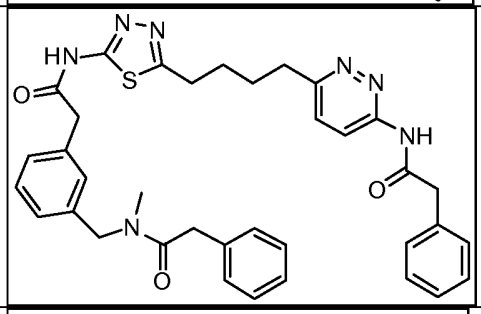
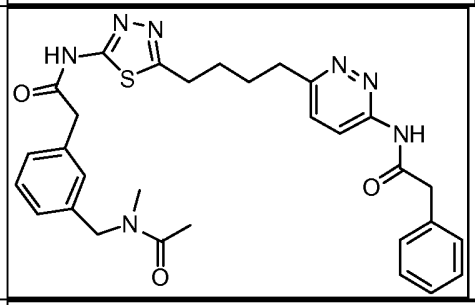
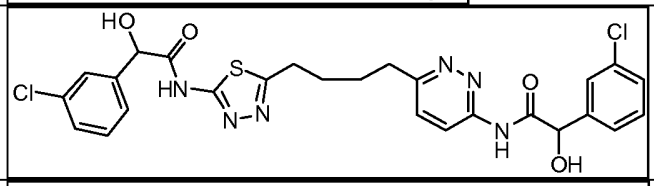
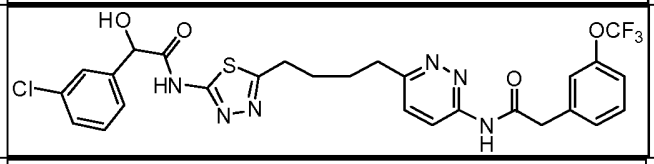
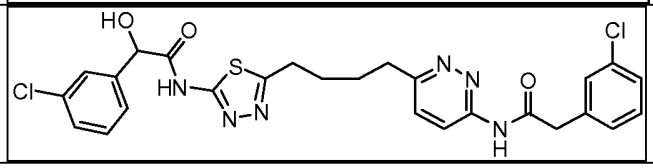
410		0.009		0.057
411		0.22		0.74
412		0.028		0.11
413		0.007		0.045
414		0.010		0.058
415		0.006		0.018
416		0.055		0.35
417		0.056		0.32
418		0.14		0.32

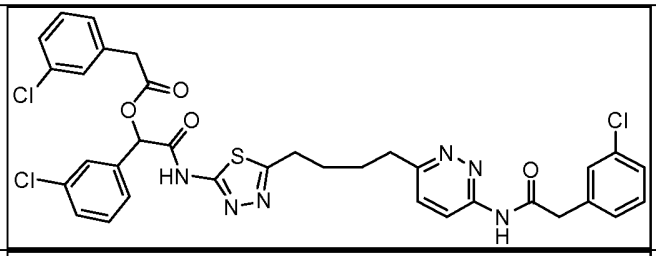
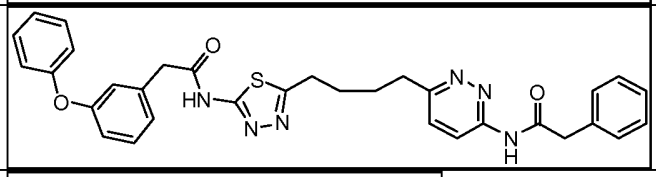
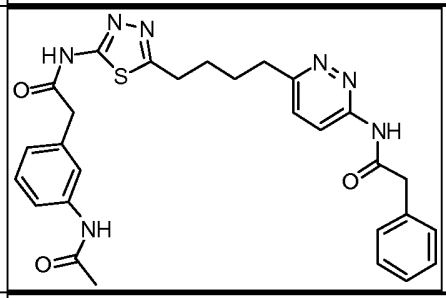
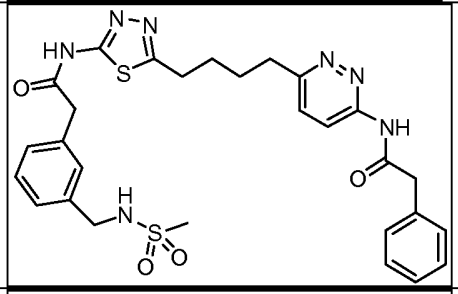
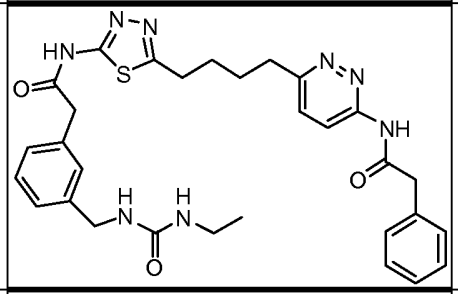
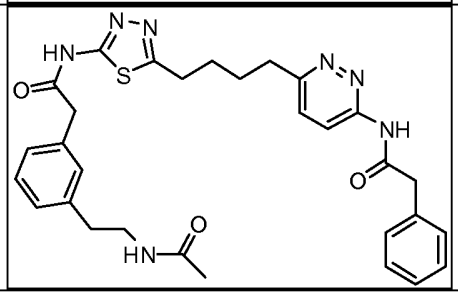
419			0.024		0.064
420			0.013		0.070
421			0.29		0.16
422			0.007		0.006
423			0.022		0.042

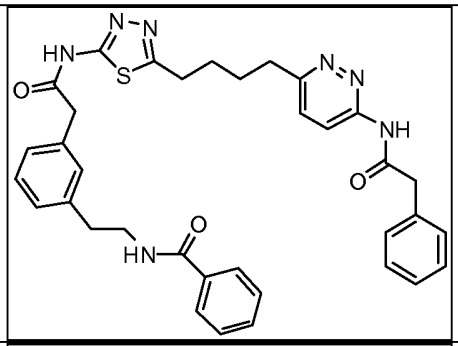
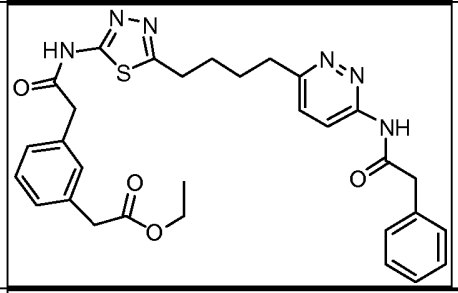
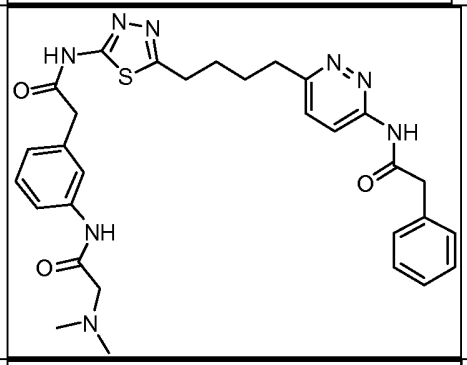
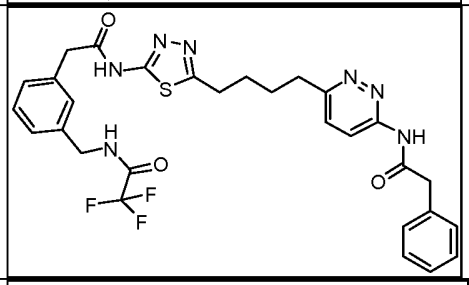
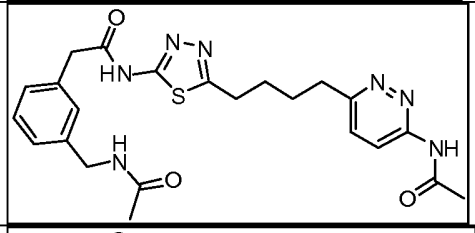
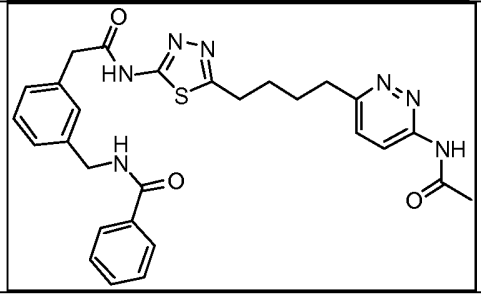
424		0.006		0.008
425		0.086		0.015
426		0.011		0.033
427		0.007		0.027
428		0.007		0.019
429		0.004		0.007

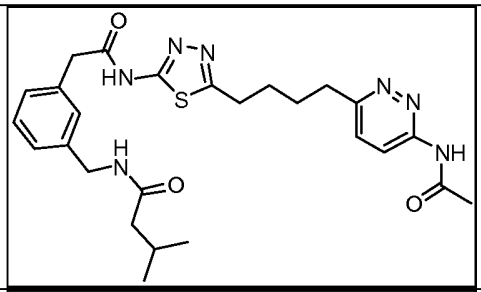
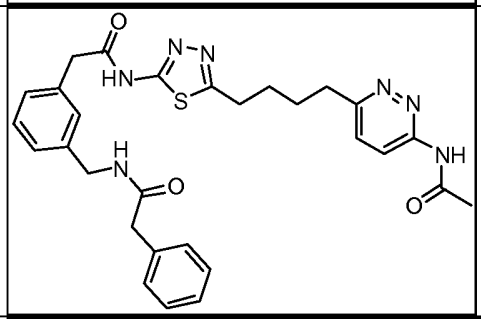
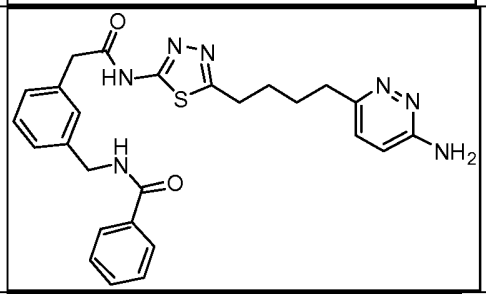
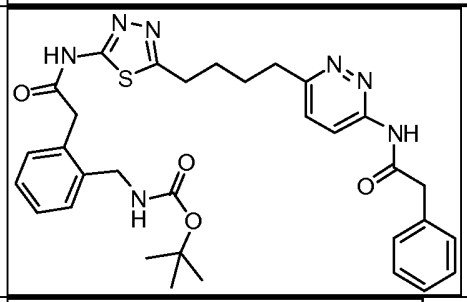
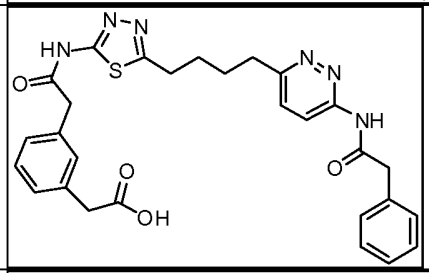
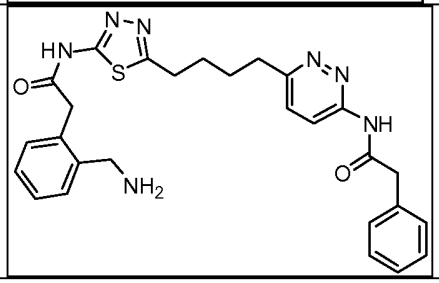
430		0.009		0.027
431		0.007		0.026
432		0.002		0.004
433		0.002		0.007
434		0.005		0.017

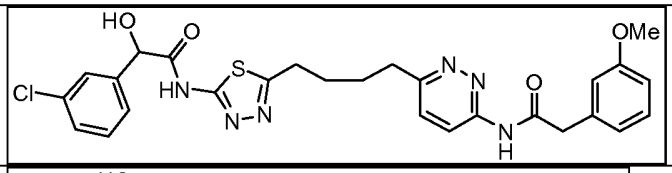
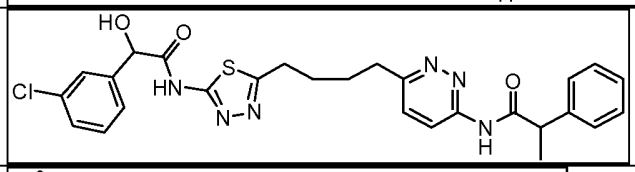
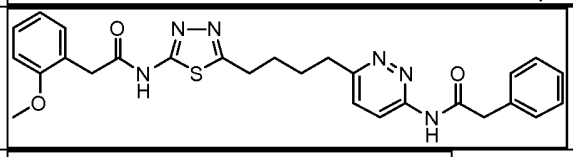
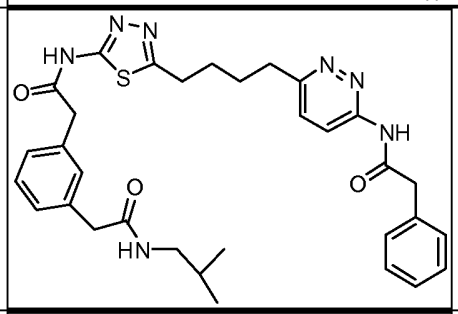
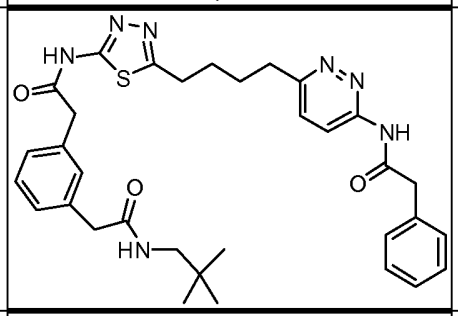
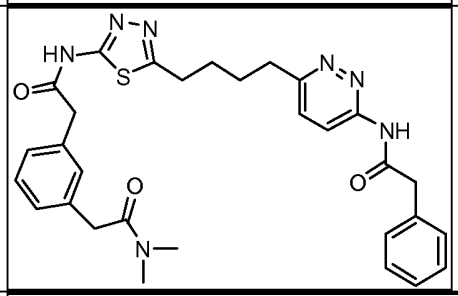
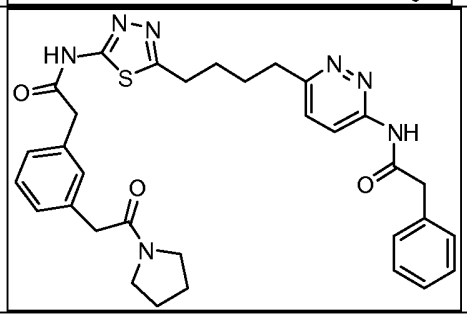
435		0.002		0.006
436		0.006		0.010
437		0.070		0.072
438		0.74		0.88
439		0.25		0.056
440		0.008		0.031
441		0.011		0.18

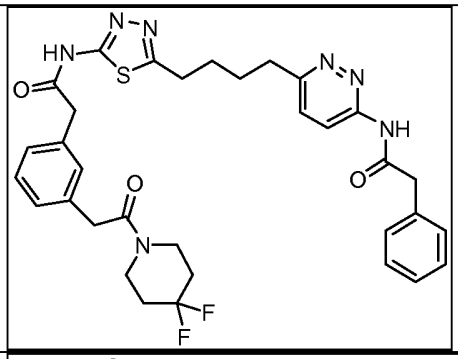
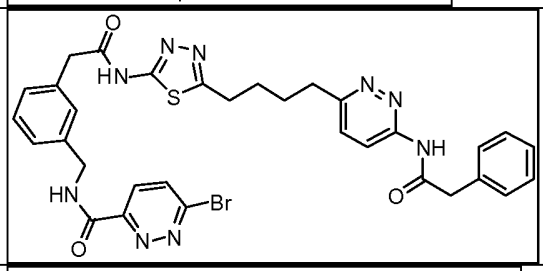
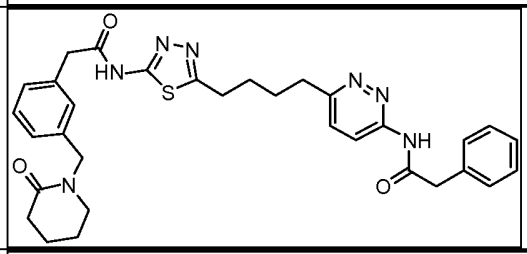
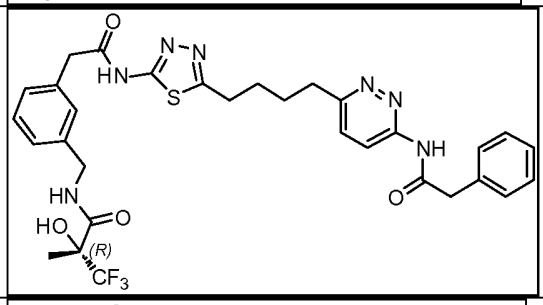
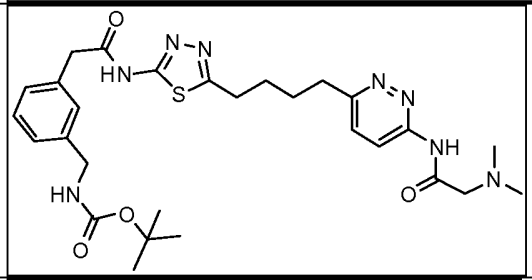
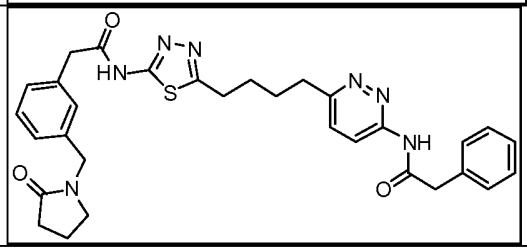
442		0.007		0.025
443		0.011		0.10
444		0.003		0.008
445		0.004		0.022
446		0.011		0.15
447		0.005		0.016
448		0.005		0.051

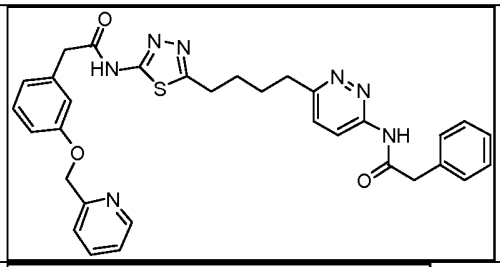
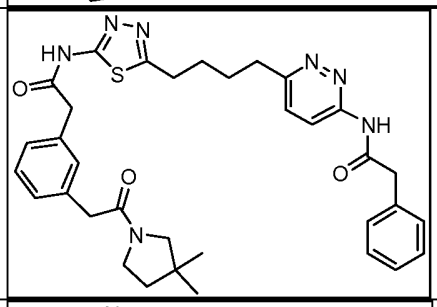
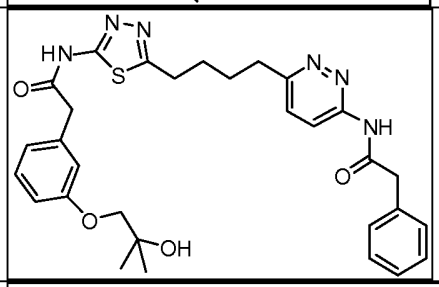
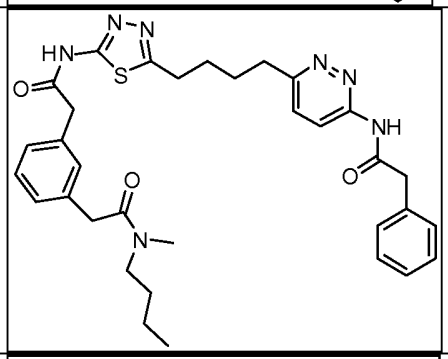
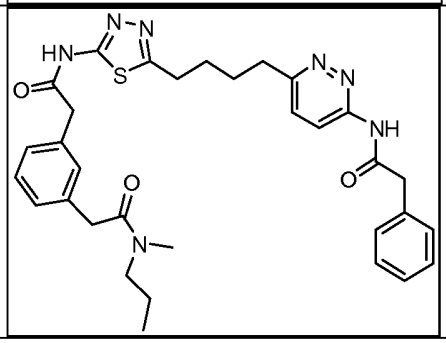
449		0.11		0.12
450		0.006		0.042
451		0.003		0.056
452		0.004		0.049
453		0.003		0.015
454		0.006		0.13

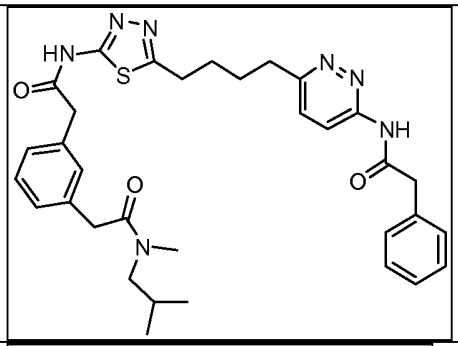
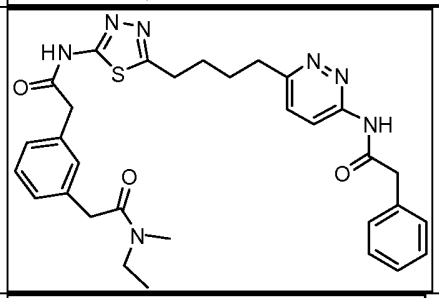
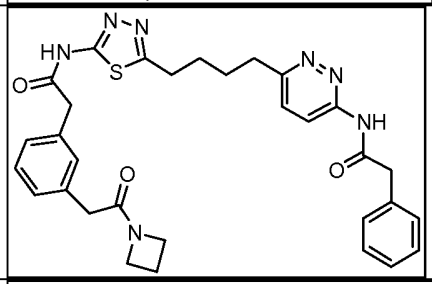
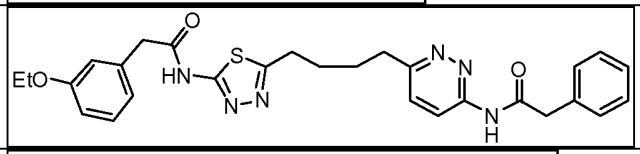
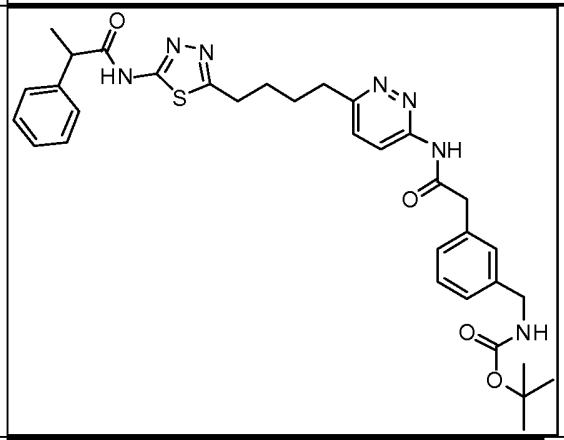
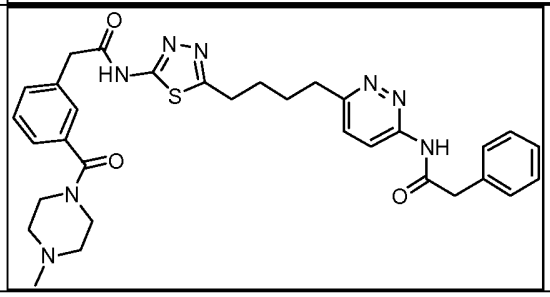
455			0.003		0.012
456			0.003		0.024
457			0.009		0.11
458			0.003		0.013
459			0.048		0.57
460			0.005		0.031

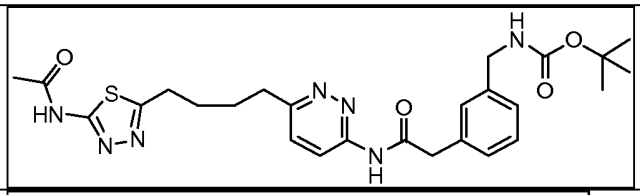
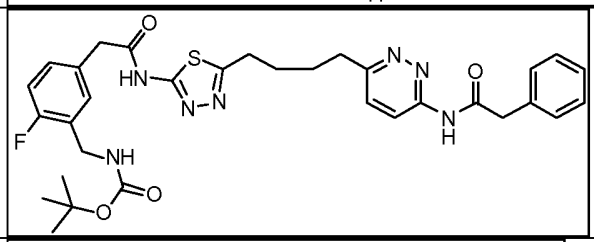
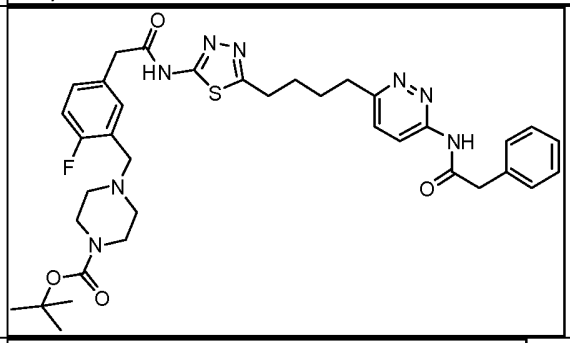
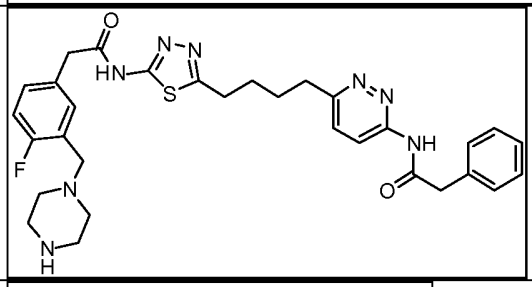
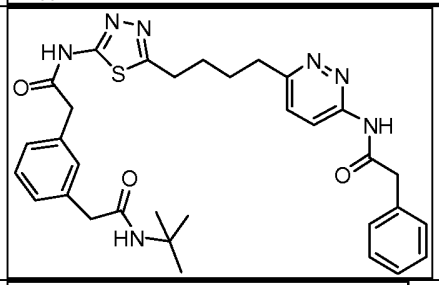
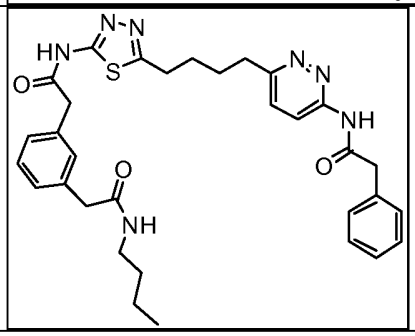
461		0.011		0.062
462		0.006		0.053
463		0.052		0.96
464		0.005		0.059
465		0.006		0.92
466		0.051		1.3

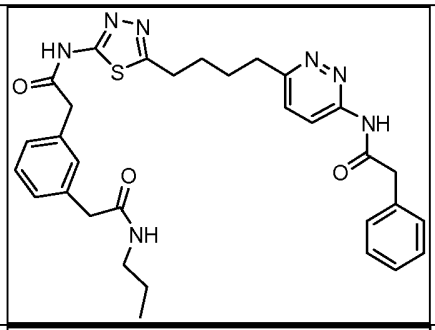
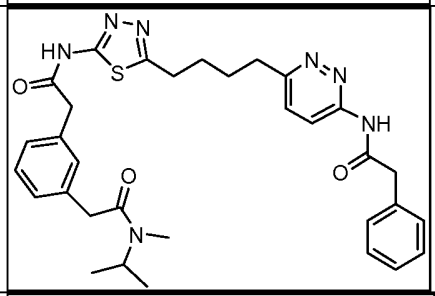
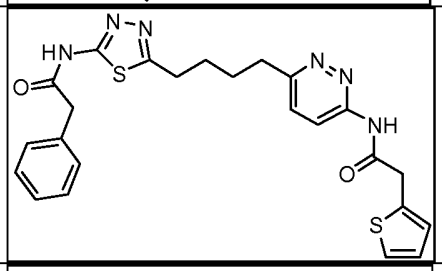
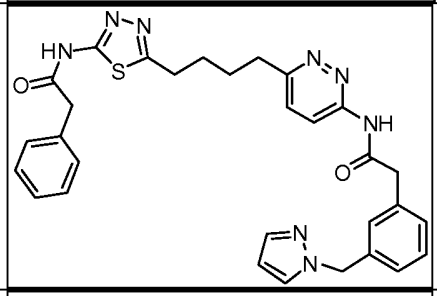
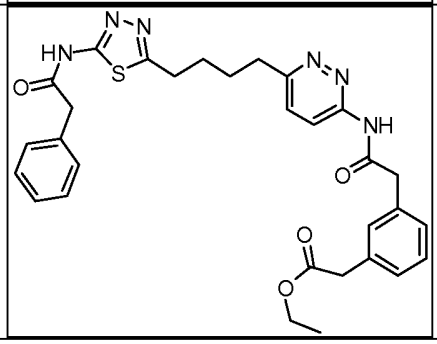
467		0.005		0.047
468		0.016		0.27
469		0.007		0.049
470		0.003		0.009
471		0.003		0.006
472		0.006		0.024
473		0.002		0.006

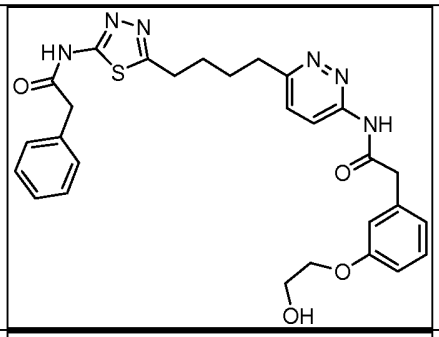
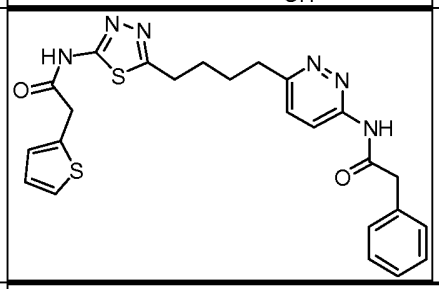
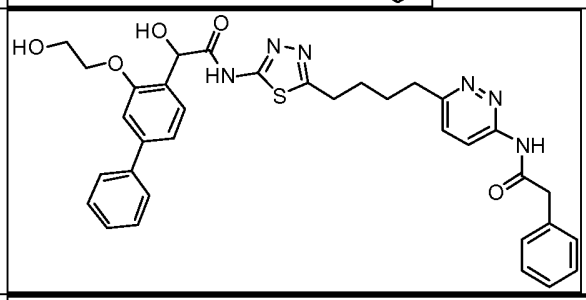
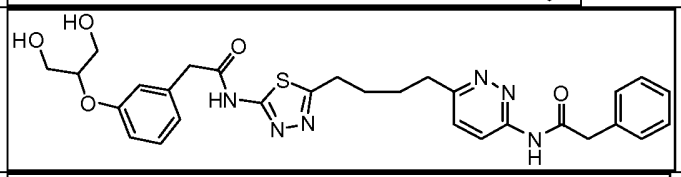
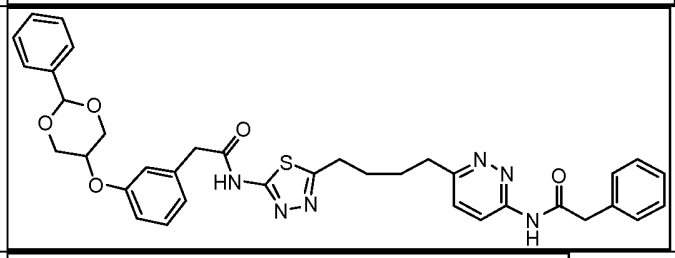
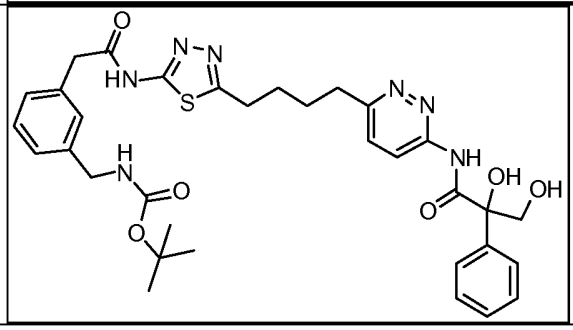
474		0.003		0.004
475		0.002		0.003
476		0.004		0.012
477		0.005		0.015
478		0.018		0.046
479		0.005		0.030

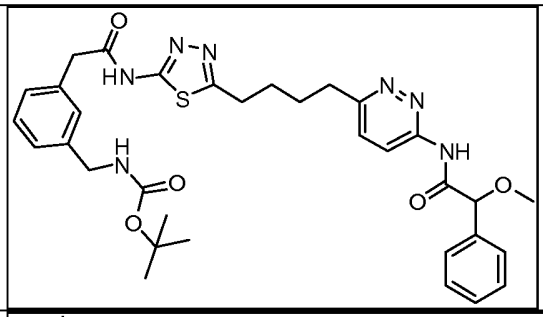
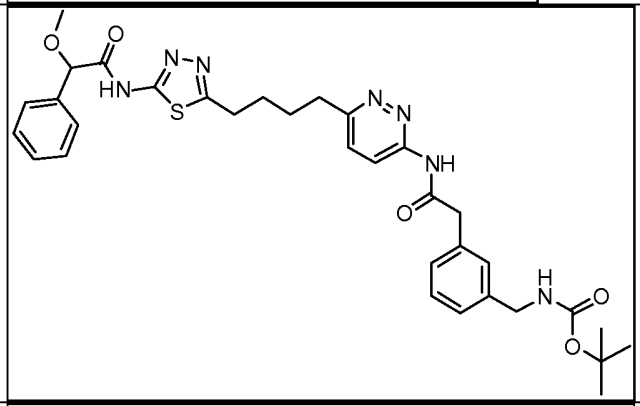
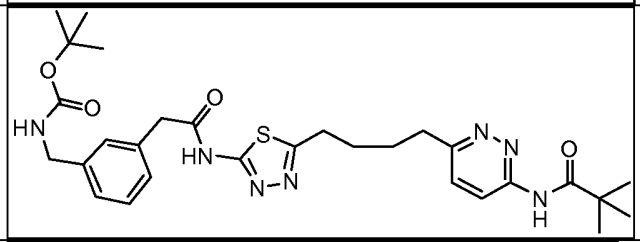
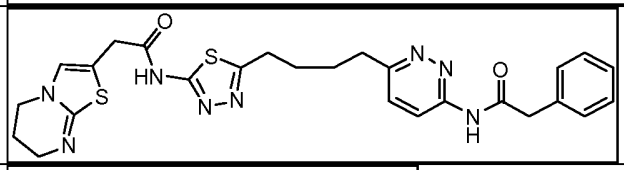
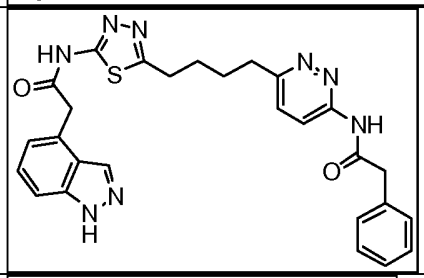
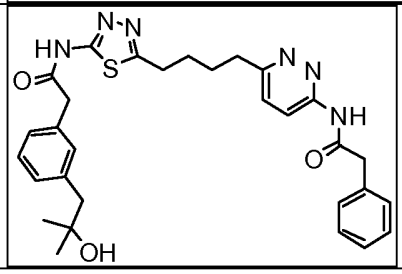
480			>20		6.3
481			0.004		0.012
482			0.007		0.038
483			0.004		0.009
484			0.003		0.011

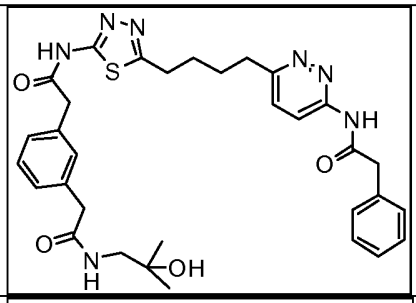
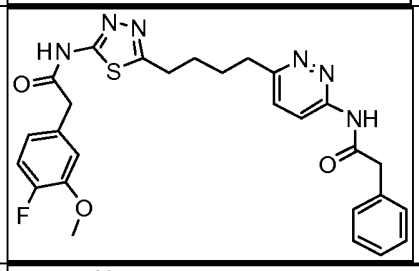
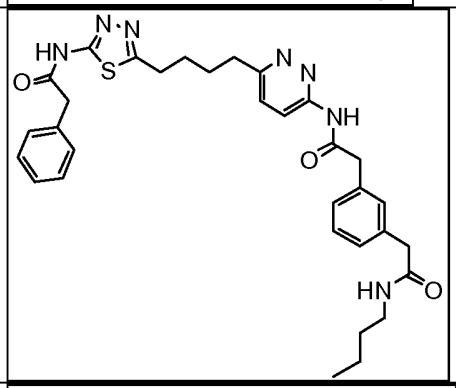
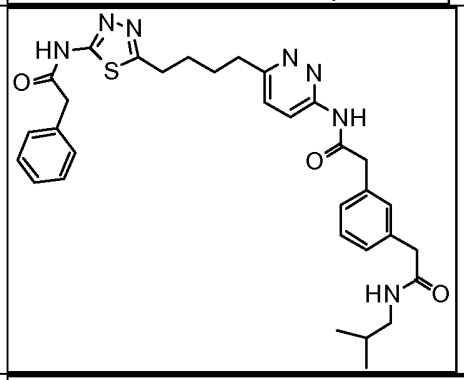
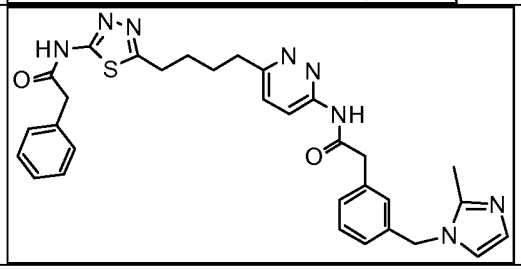
485		0.004		0.012
486		0.004		0.024
487		0.005		0.042
488		0.32		1.9
489		0.008		0.023
490		0.011		0.25

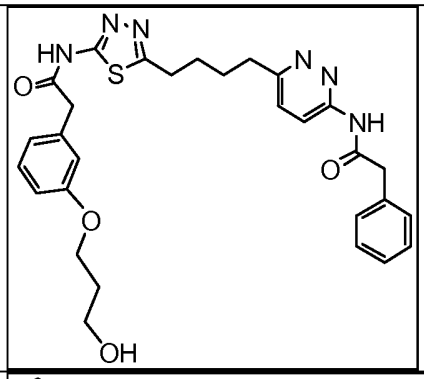
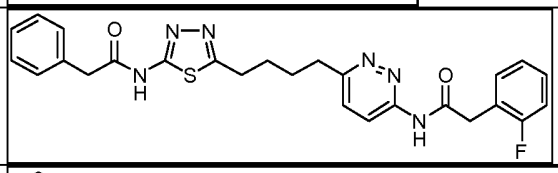
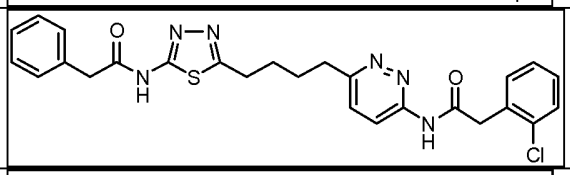
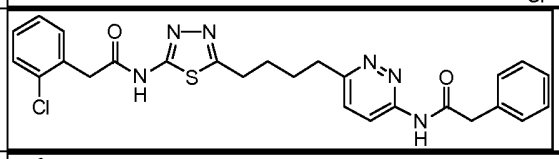
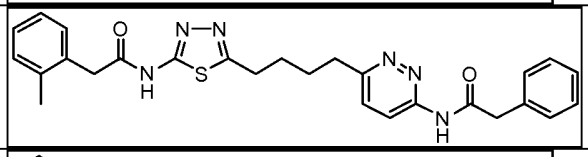
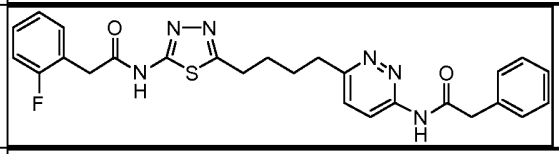
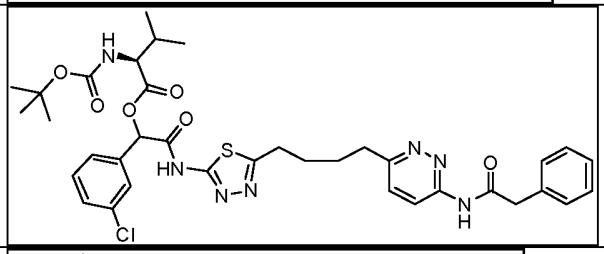
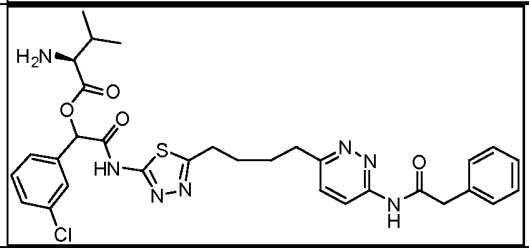
491		0.008		0.023
492		0.006		0.014
493		0.019		0.057
494		0.019		0.58
495		0.005		0.014
496		0.003		0.017

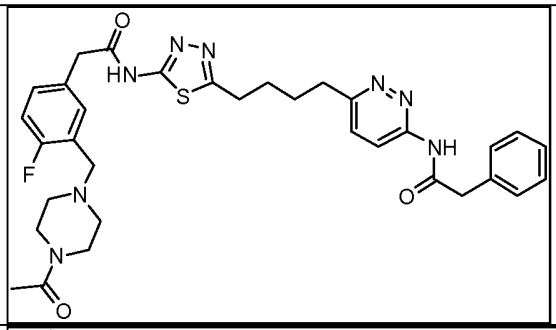
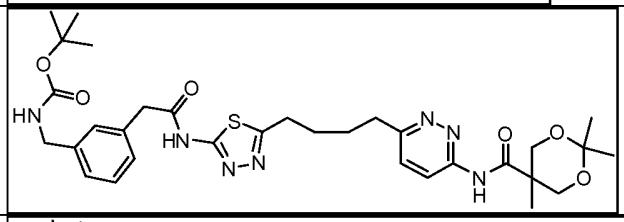
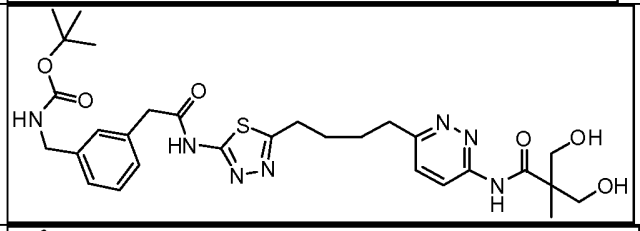
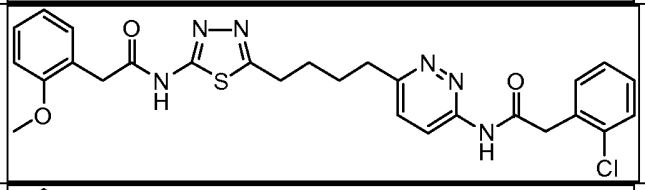
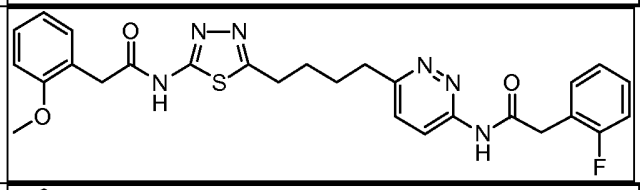
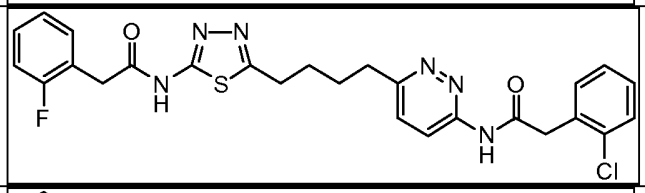
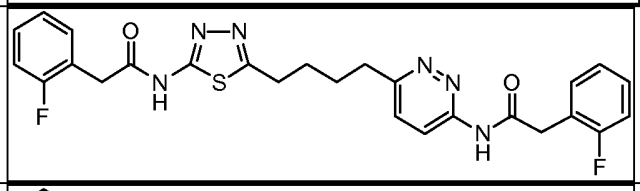
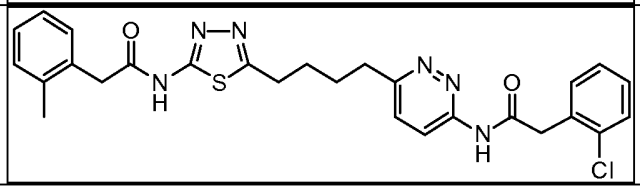
497			0.004		0.032
498			0.003		0.017
499			0.010		0.19
500			0.004		0.029
501			0.004		0.069

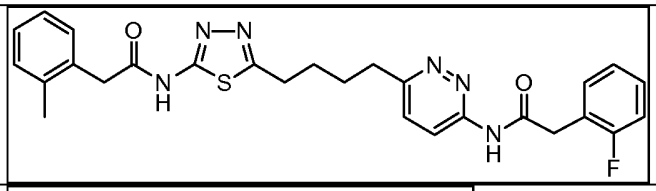
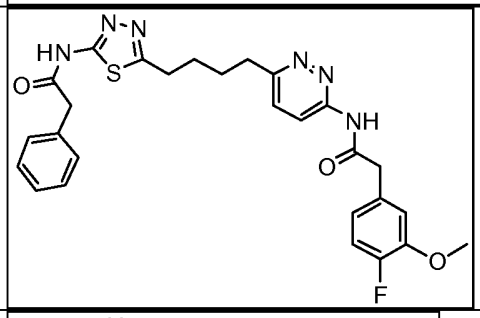
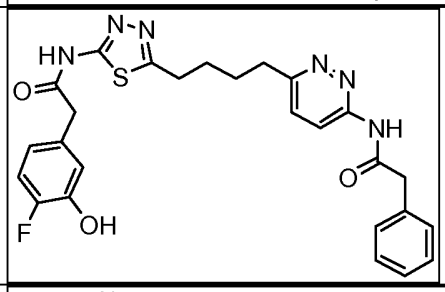
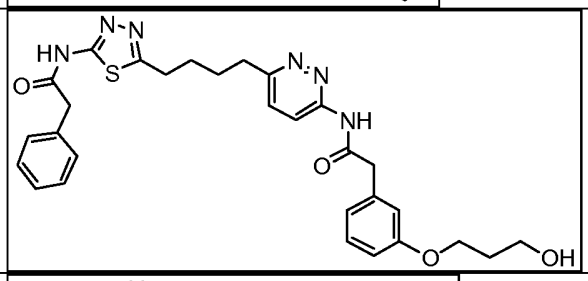
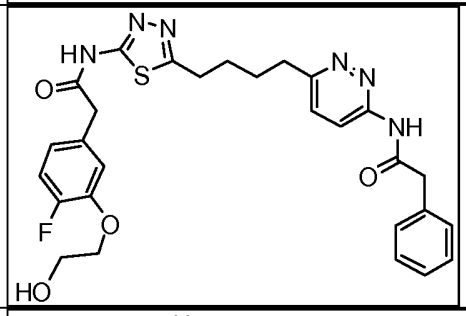
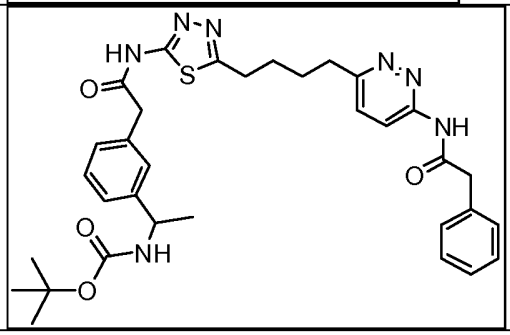
502		0.007		0.075
503		0.008		0.15
504		0.007		0.12
505		0.008		0.24
506		0.010		0.17
507		0.013		0.041

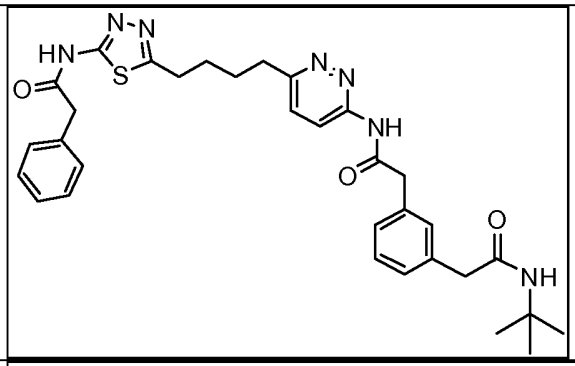
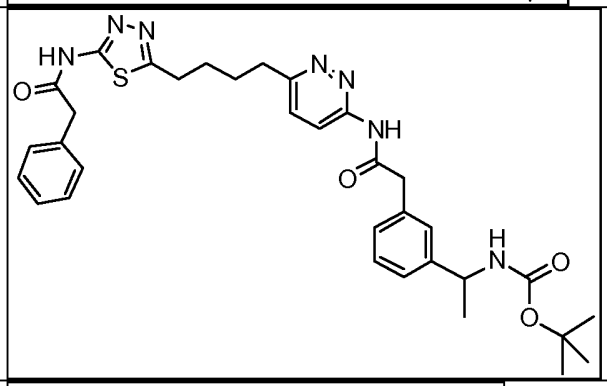
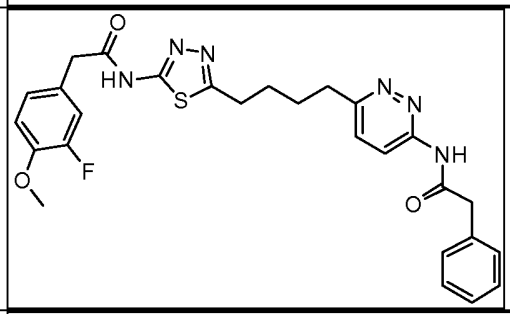
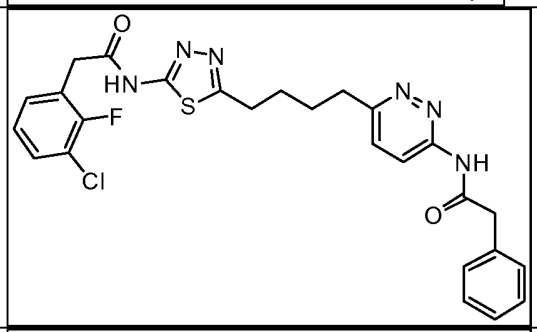
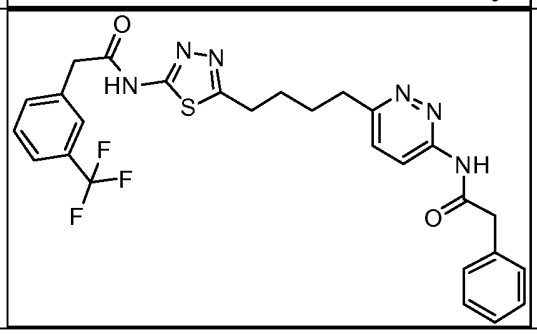
508		0.011		0.020
509		0.010		0.009
510		0.022		0.094
511		0.58		1.1
512		0.005		0.046
513		0.007		0.022

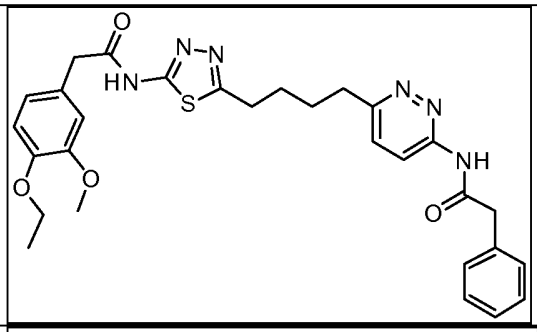
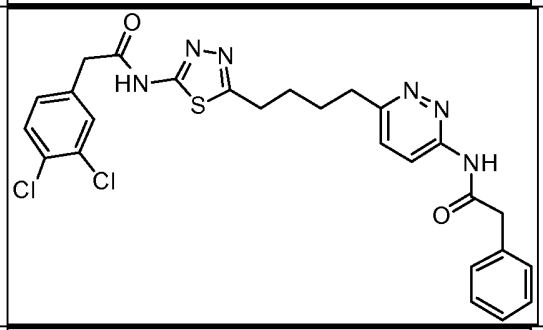
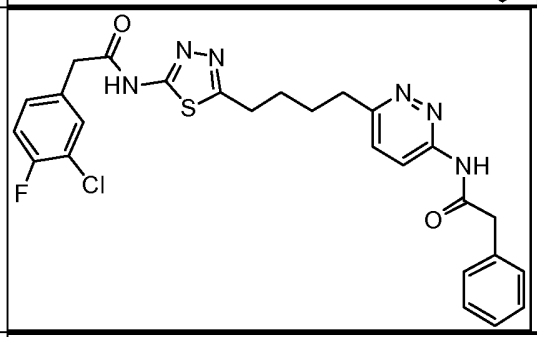
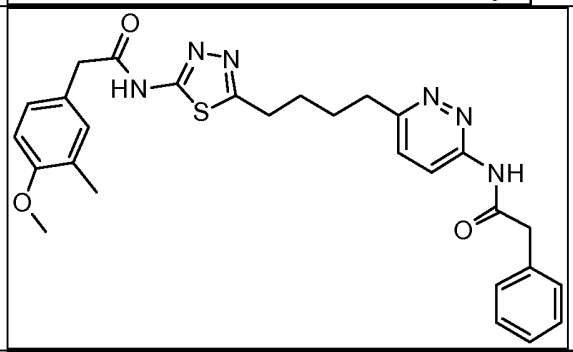
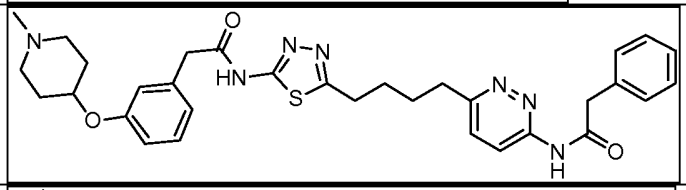
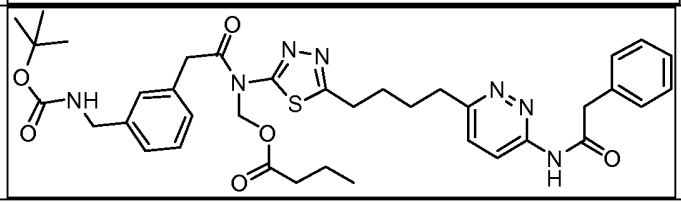
514		0.009		0.063
515		0.007		0.059
516		0.003		0.028
517		0.003		0.046
518		0.004		0.063

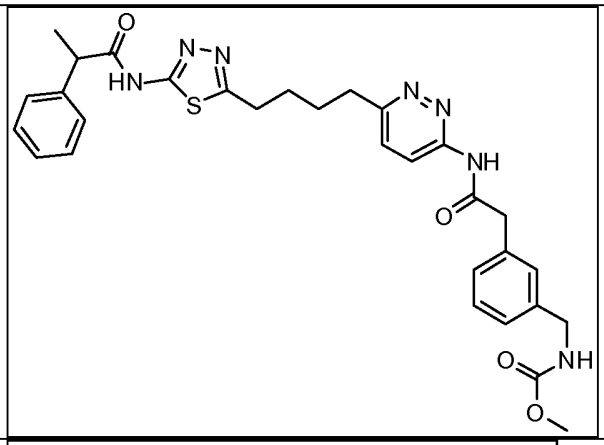
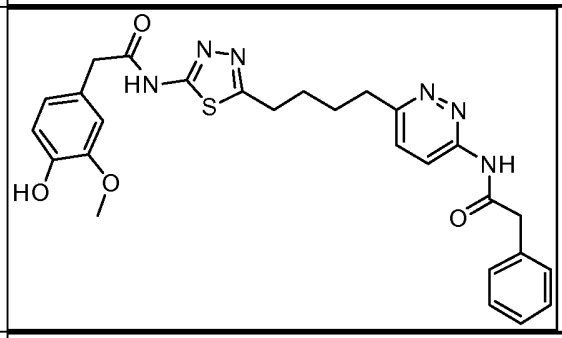
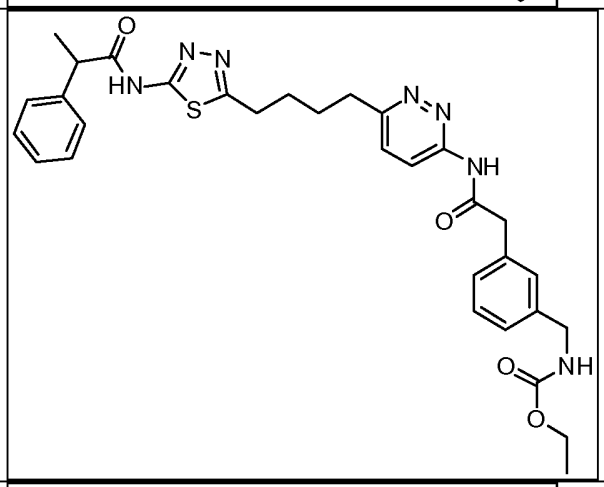
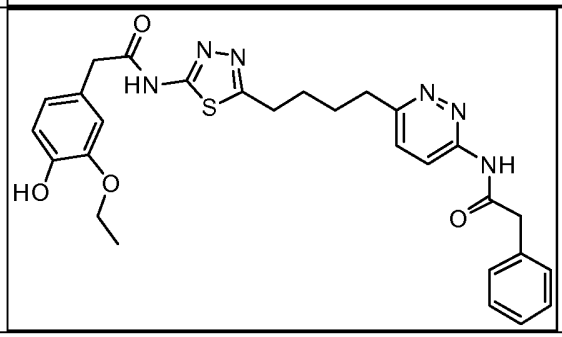
519		0.009		0.059
520		0.007		0.056
521		0.006		0.052
522		0.023		0.060
523		0.021		0.055
524				
525				
526				

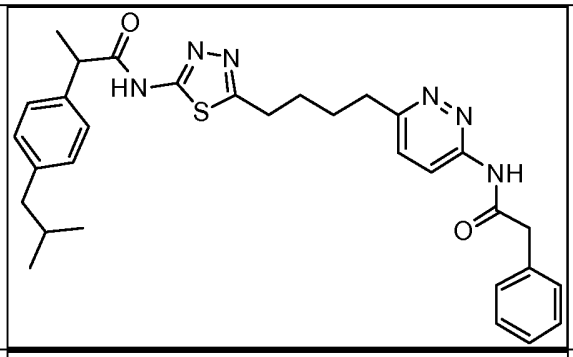
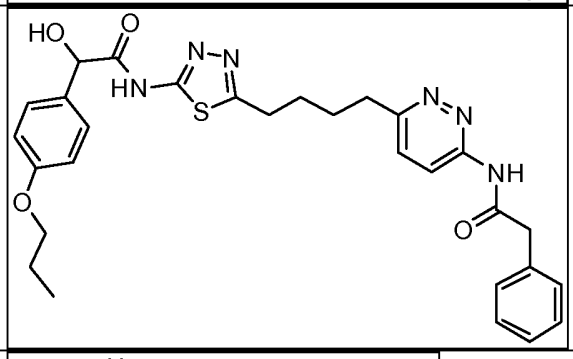
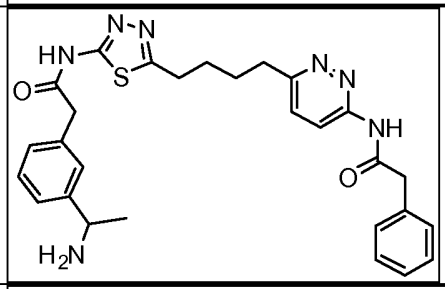
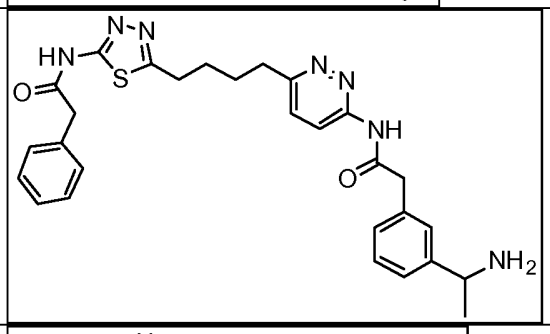
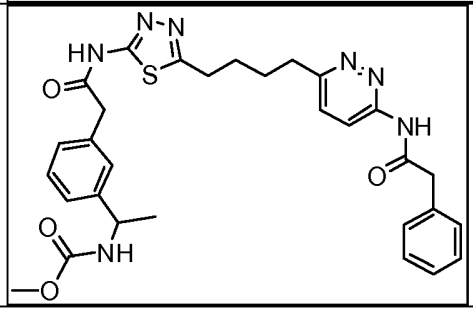
528		0.007		0.044
529		0.032		0.16
530		0.055		0.28
531		0.006		0.042
532		0.006		0.059
533		0.007		0.041
534		0.008		0.044
535		0.007		0.090

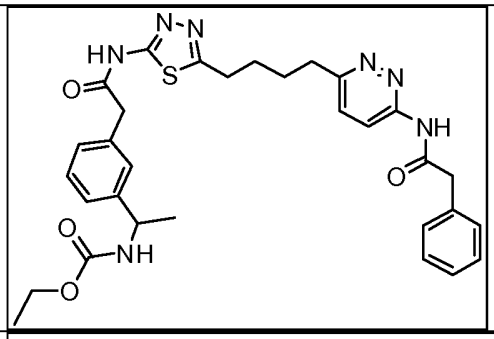
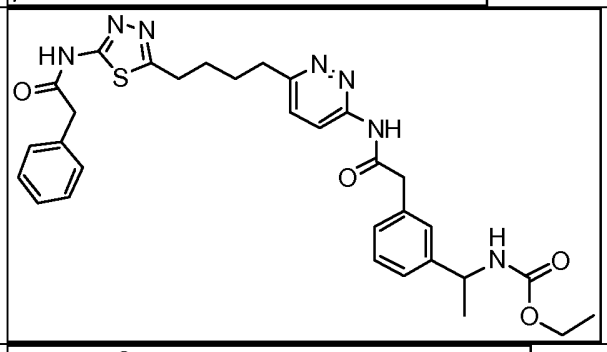
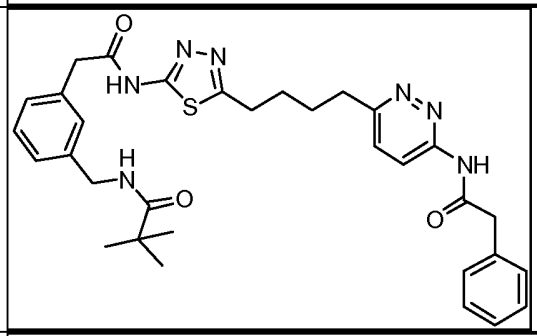
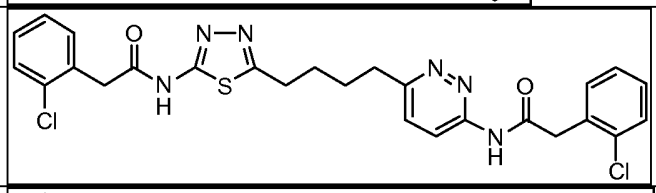
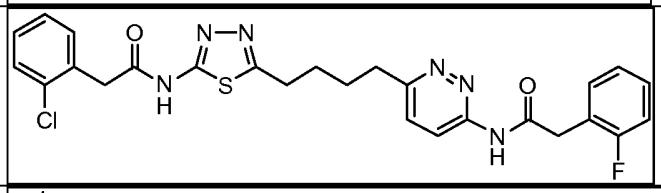
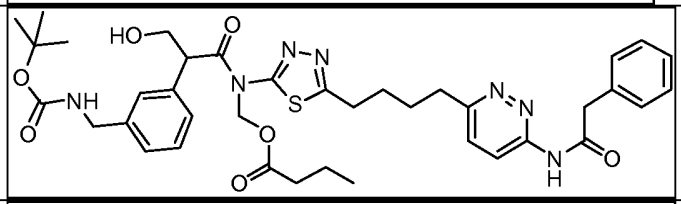
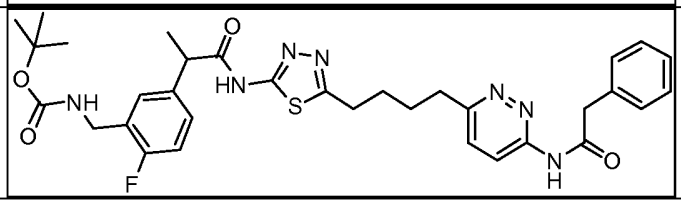
536		0.006		0.071
537		0.007		0.076
538		0.004		0.030
539		0.009		0.045
540		0.007		0.050
541		0.004		0.006

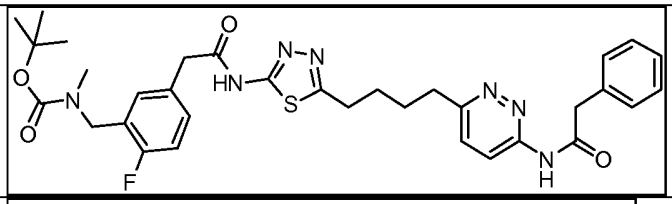
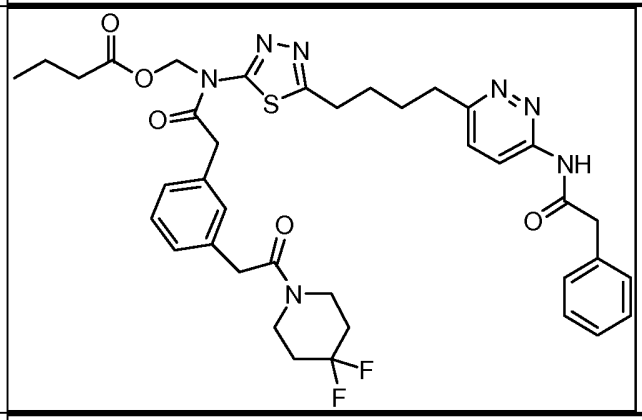
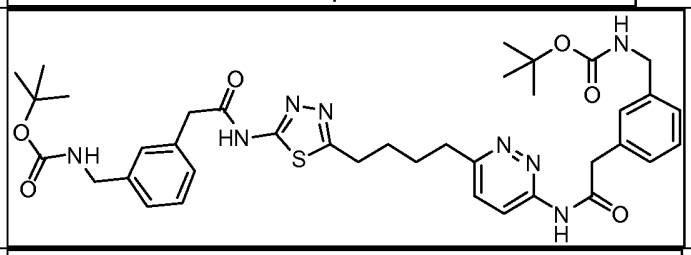
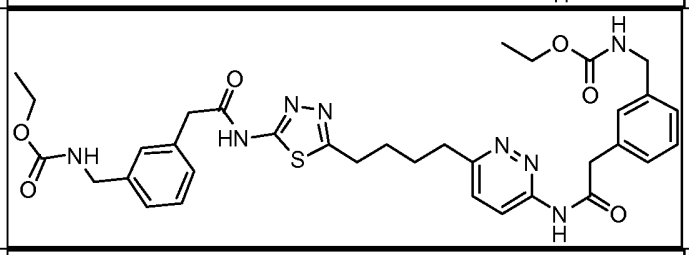
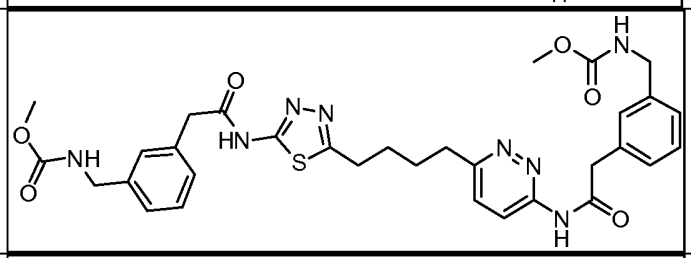
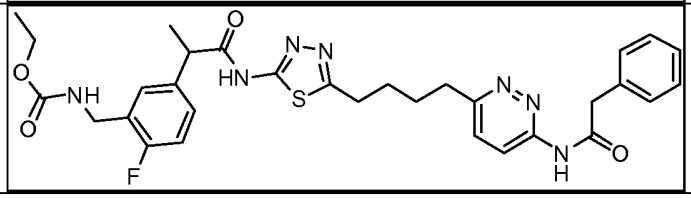
542		0.004		0.043
543		0.004		0.005
544		0.006		0.044
545		0.006		0.046
546		0.005		0.027

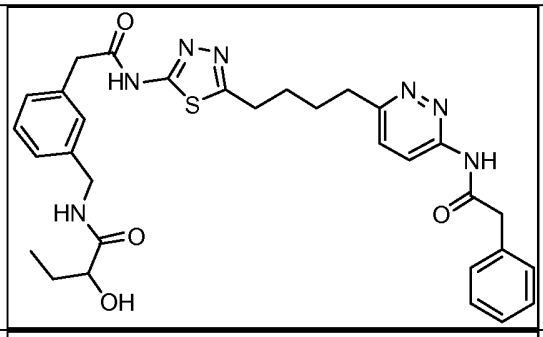
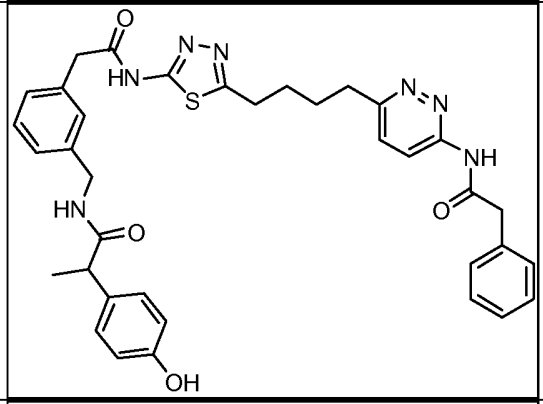
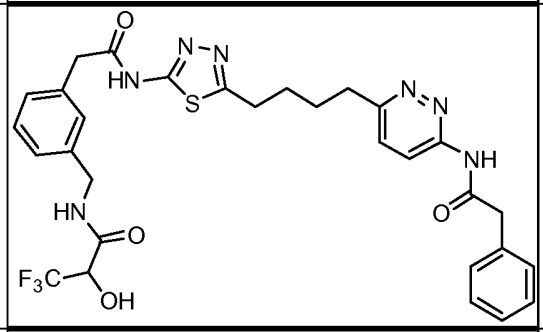
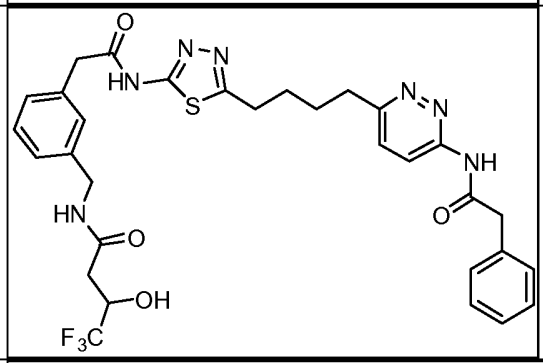
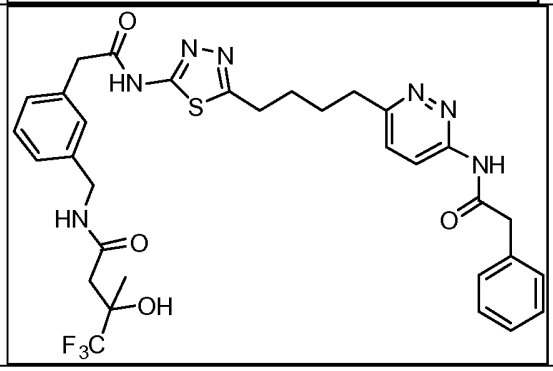
547		0.006		0.031
548		0.010		0.085
549		0.006		0.045
550		0.005		0.036
551		0.010		0.127
552		>20		0.005

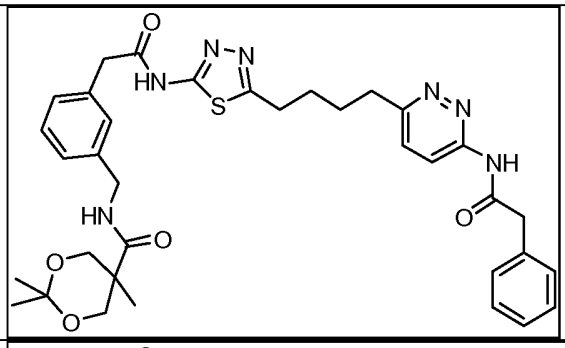
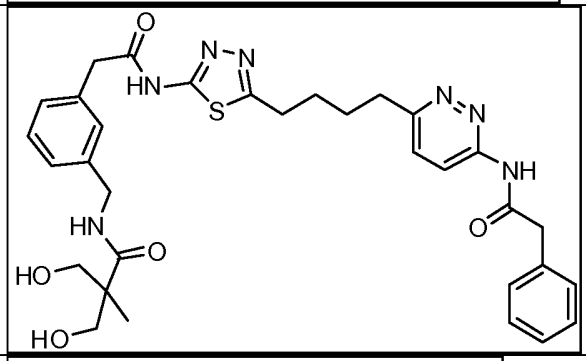
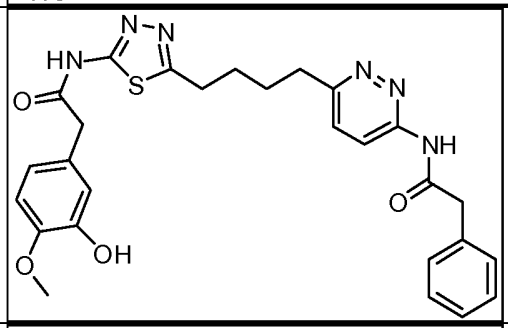
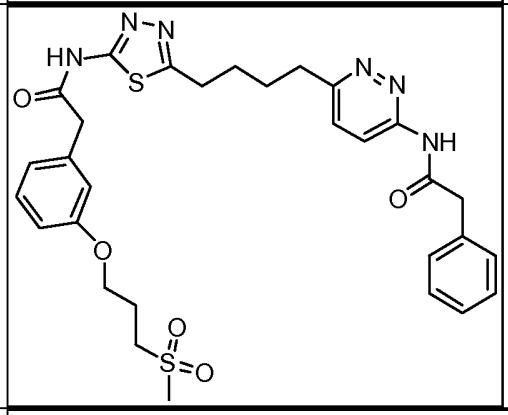
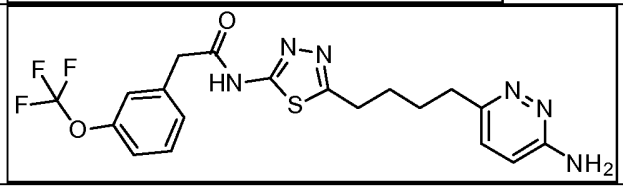
553		0.005		0.019
554		0.008		0.172
555		0.004		0.010
556		0.005		0.12

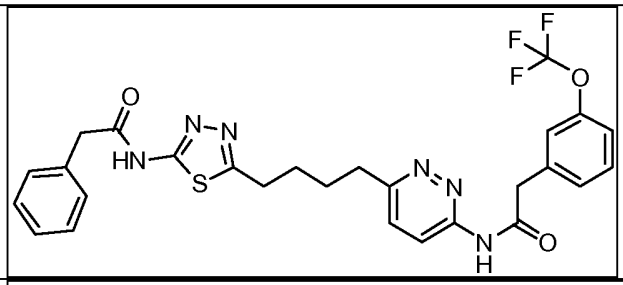
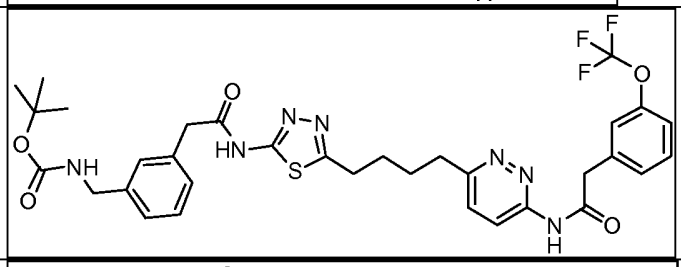
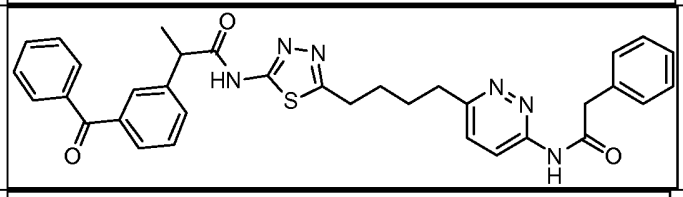
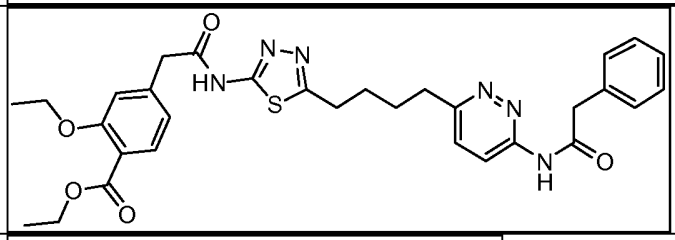
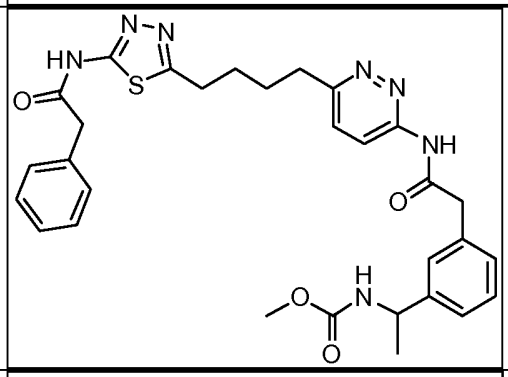
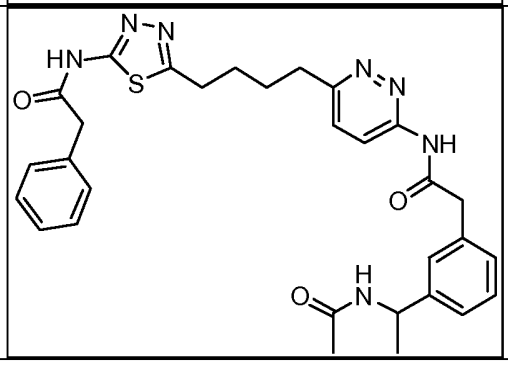
557		0.025		0.12
558		0.006		0.028
559		0.012		0.066
560		0.010		0.037
561		0.004		0.004

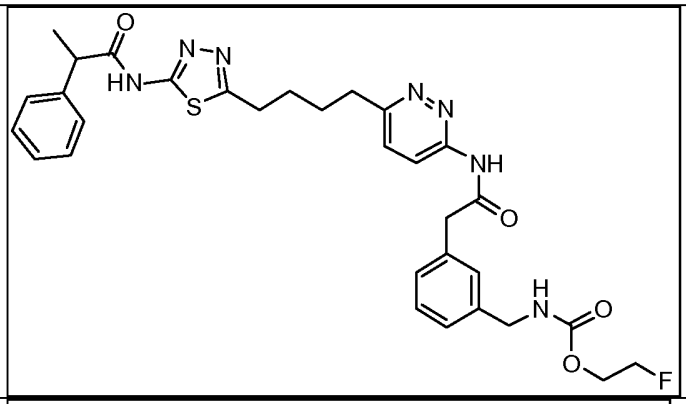
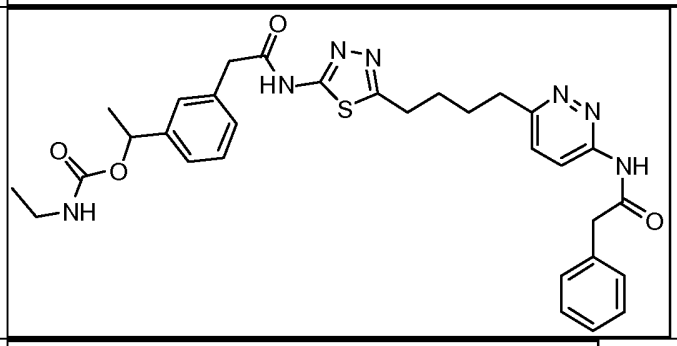
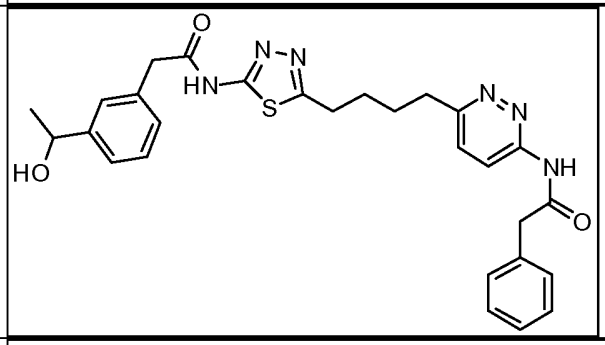
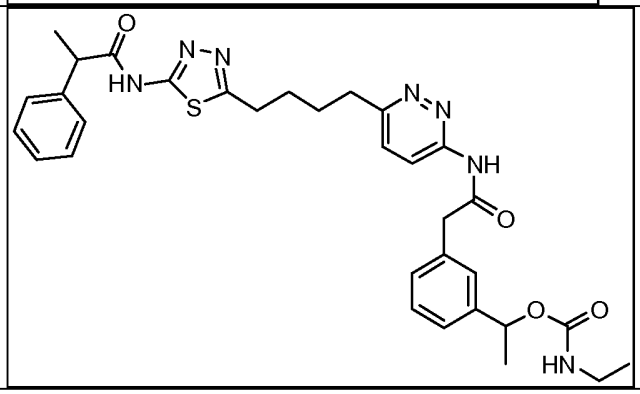
562		0.003		0.002
563		0.003		0.003
564		0.004		0.002
565		0.005		0.013
566		0.006		0.015
567		0.43		0.021
568		0.009		0.028

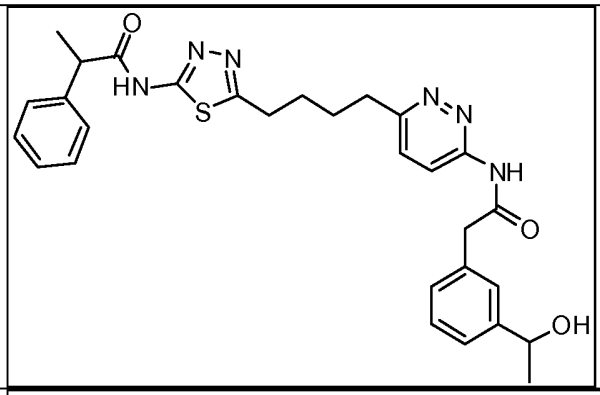
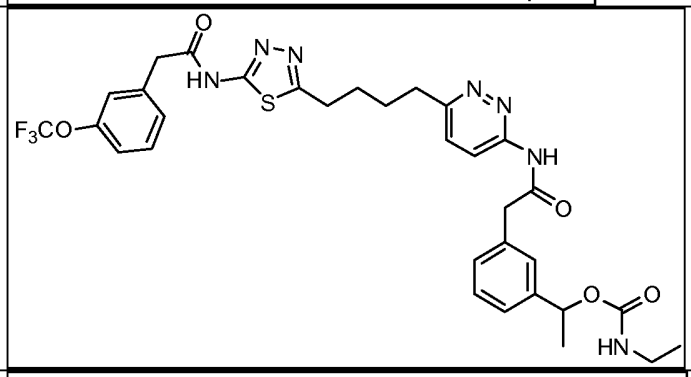
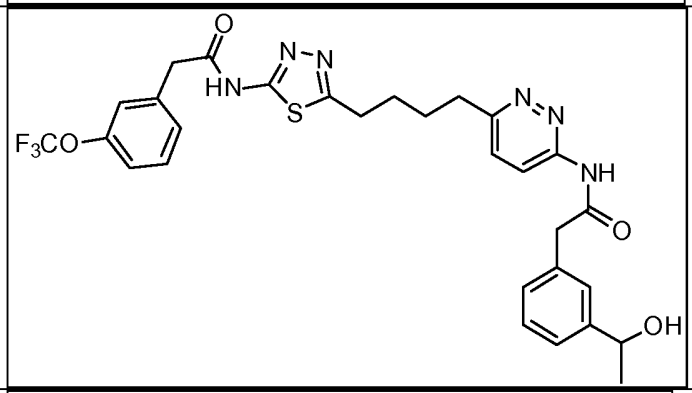
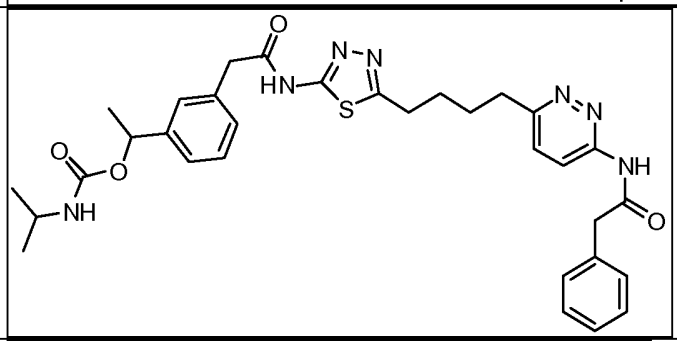
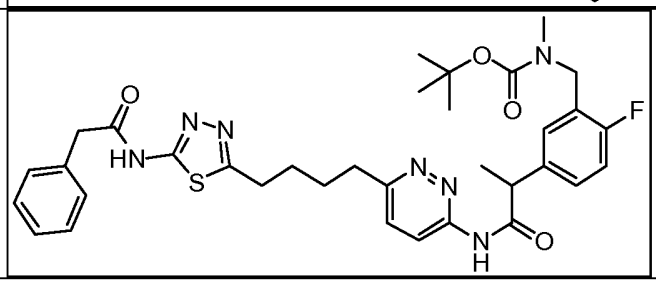
569		0.006		0.011
570		0.43		0.009
571		0.011		0.010
572		0.003		0.004
573		0.004		0.015
574		0.006		0.028

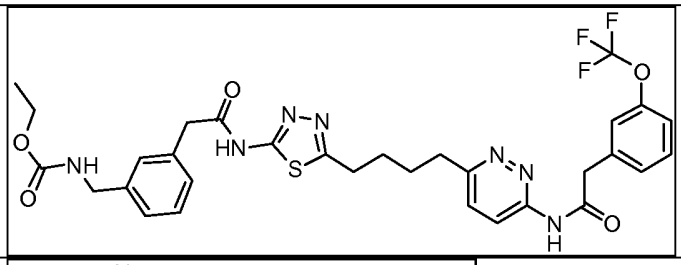
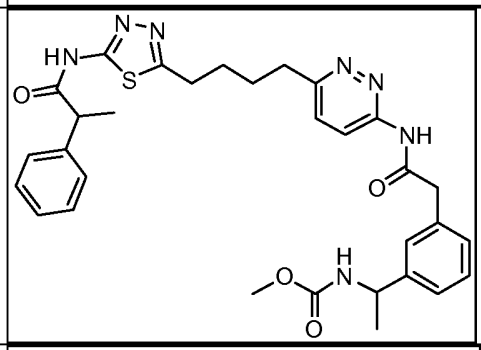
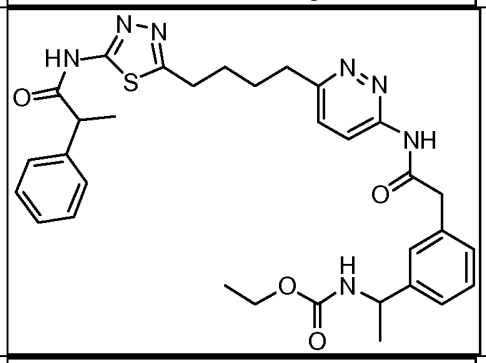
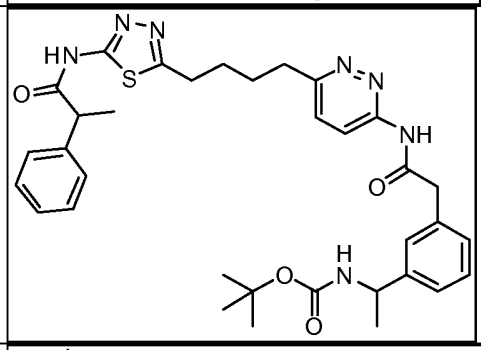
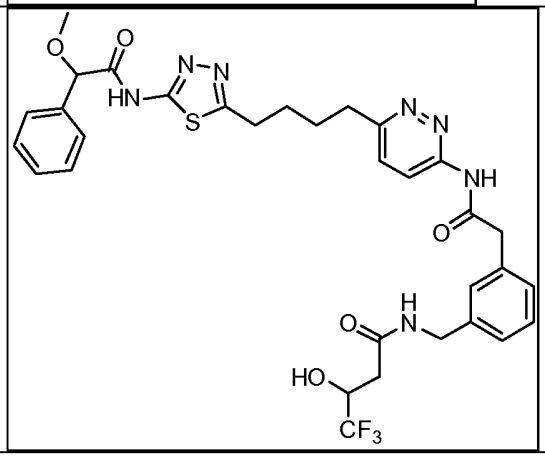
575			0.007		0.040
576			0.003		0.013
577			0.004		0.034
578			0.004		0.022
579			0.004		0.009

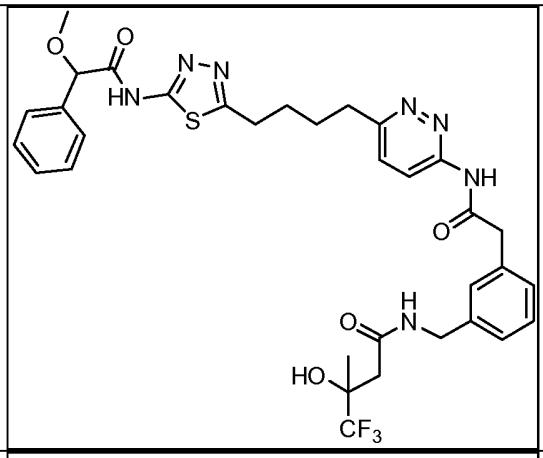
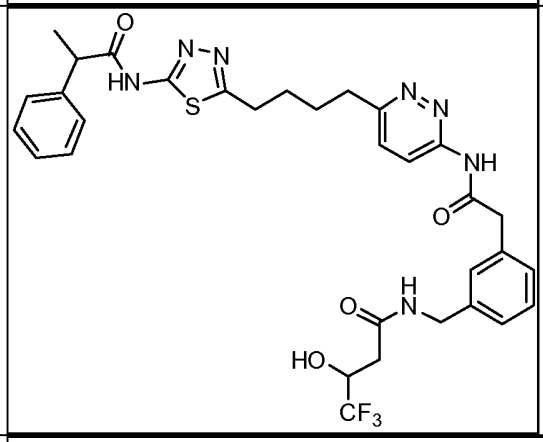
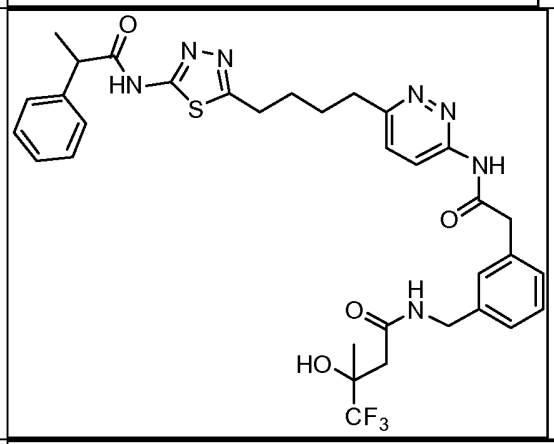
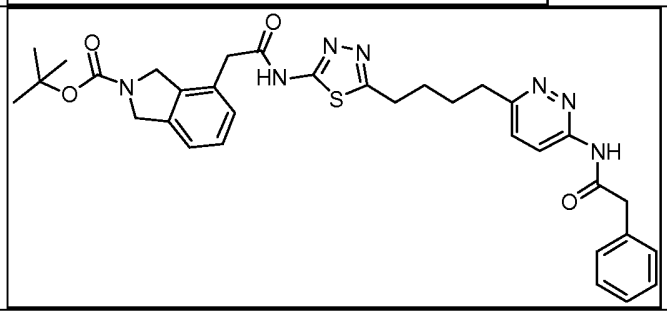
580		0.005		0.013
581		0.011		0.24
582		0.005		0.046
583		0.005		0.042
584		0.22		1.4

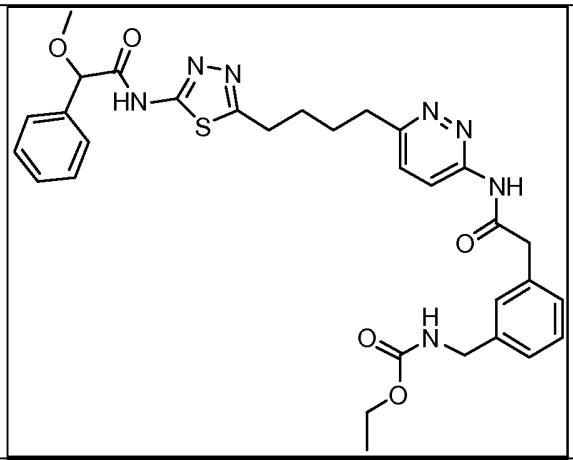
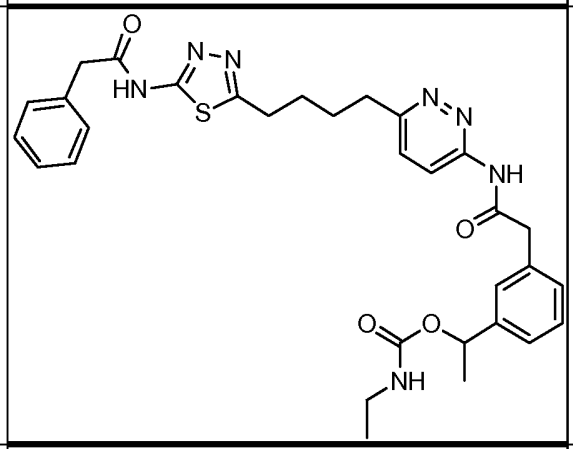
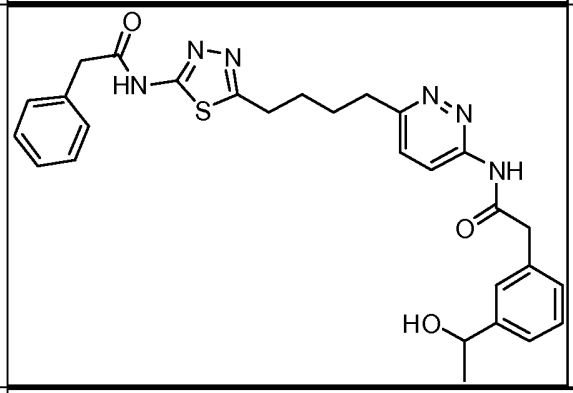
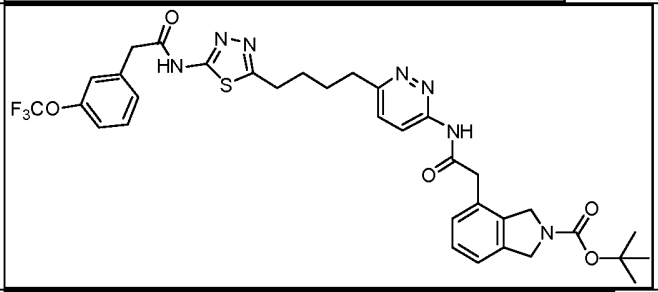
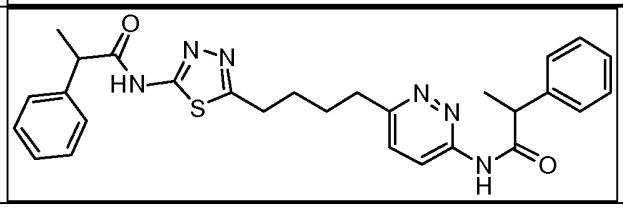
585		0.006		0.070
586		0.013		0.031
587		0.007		0.057
588		0.008		0.27
589		0.004		0.025
590		0.007		0.087

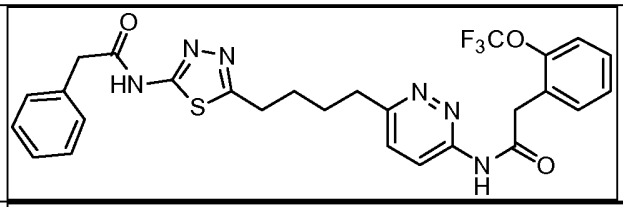
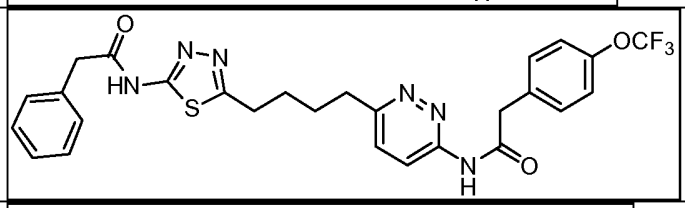
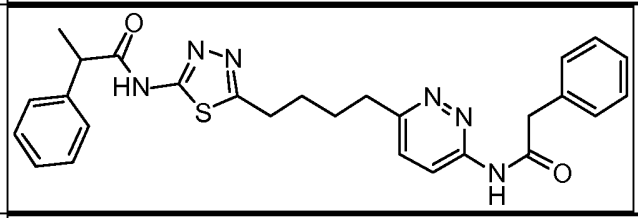
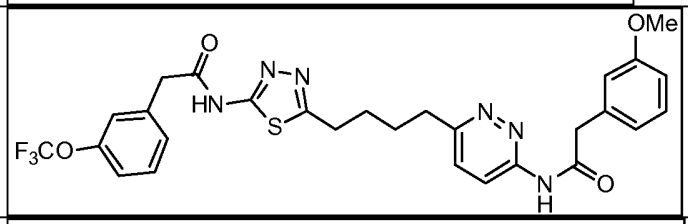
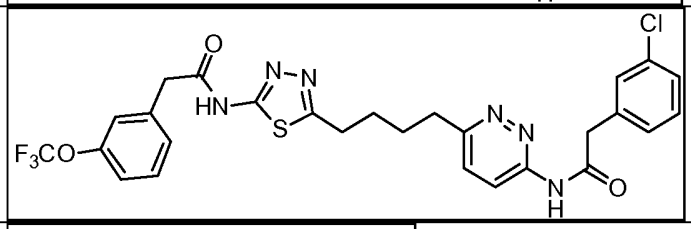
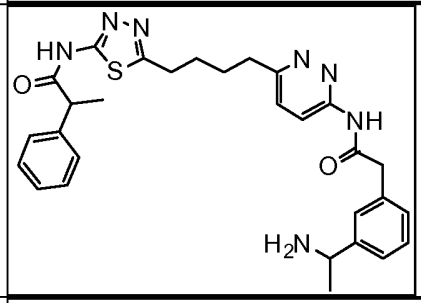
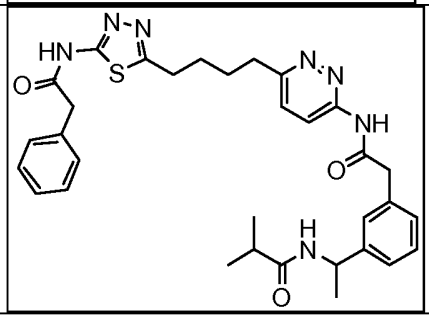
591		0.004		0.033
592		0.004		0.011
593		0.005		0.033
594		0.007		0.050

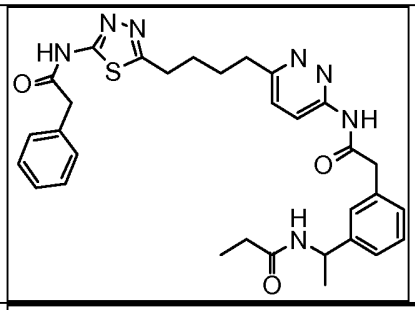
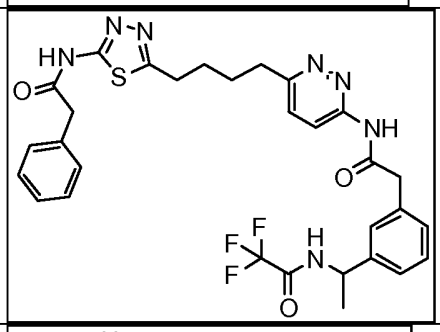
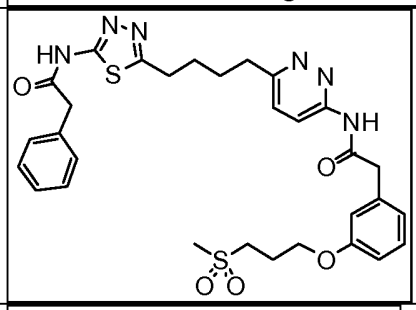
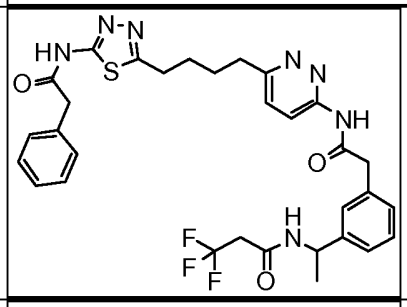
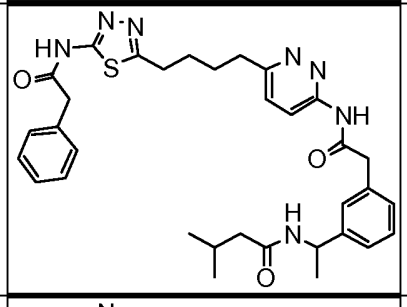
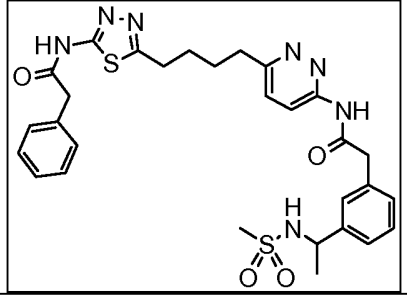
595		0.007		0.059
596		0.015		0.33
597		0.005		0.017
598		0.005		0.004
599		0.010		0.039

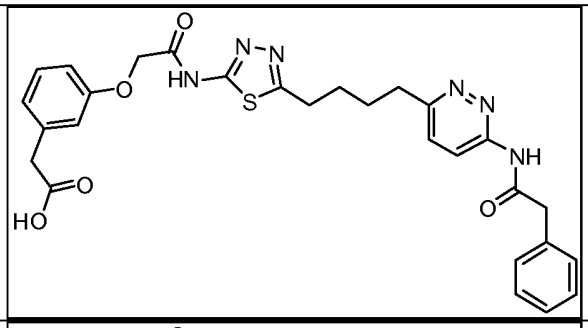
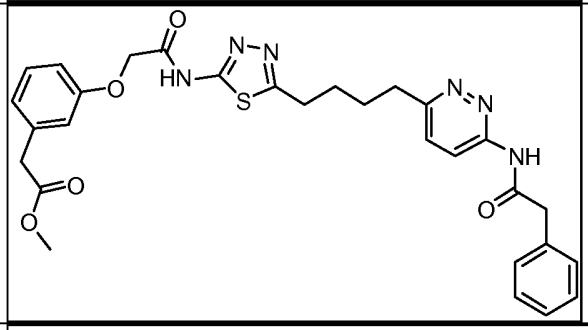
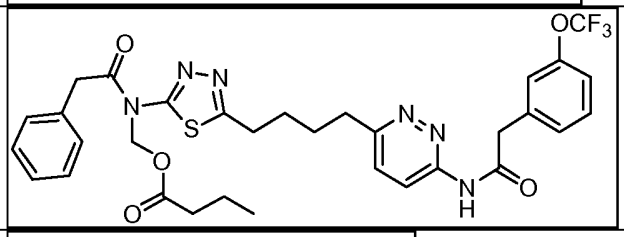
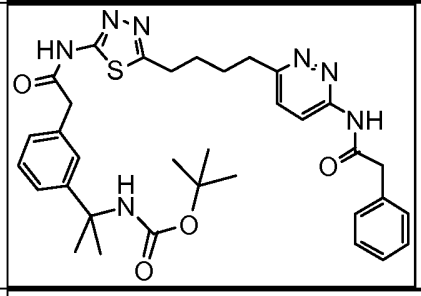
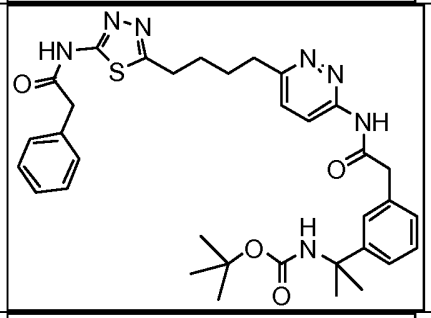
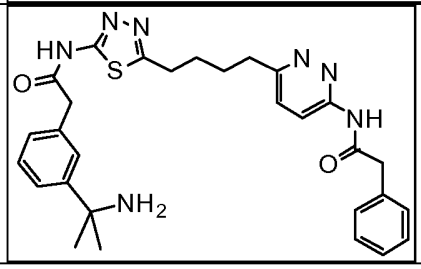
600		0.005		0.008
601		0.006		0.036
602		0.006		0.036
603		0.009		0.023
604		0.015		0.042

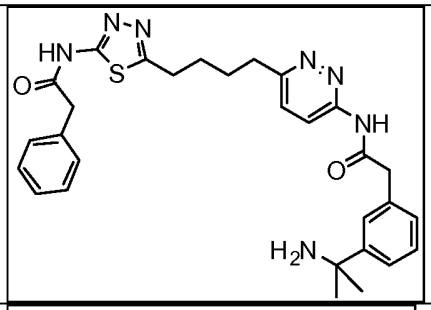
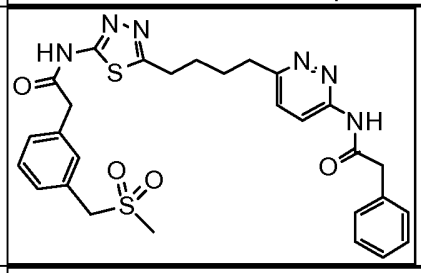
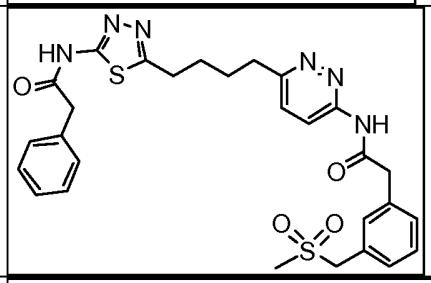
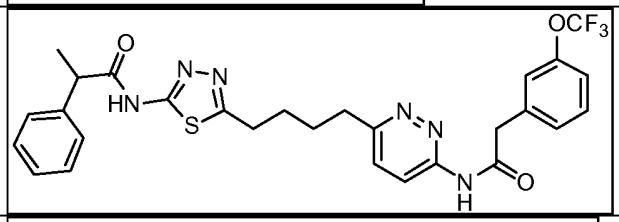
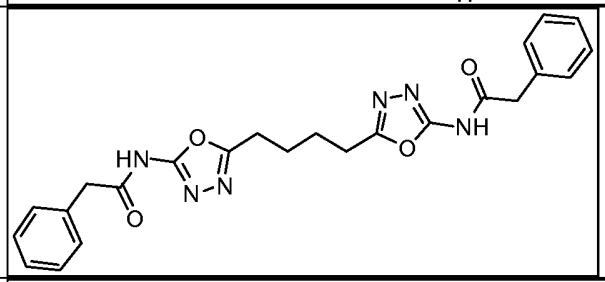
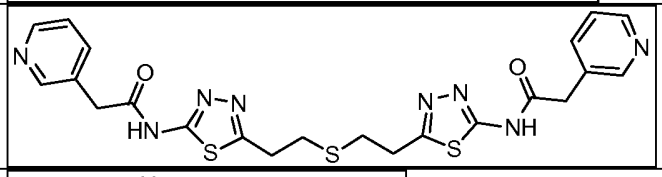
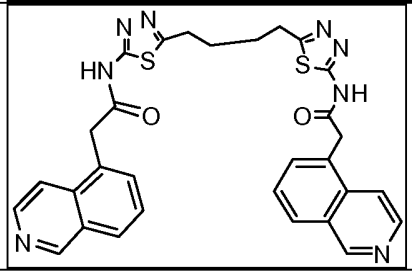
605		0.013		0.018
606		0.007		0.045
607		0.007		0.047
608		0.007		0.037

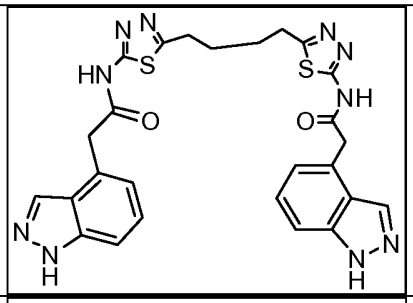
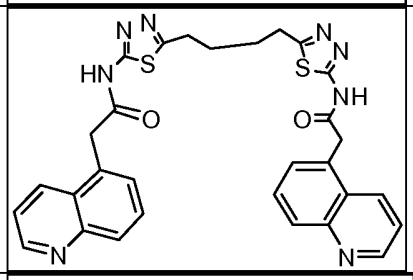
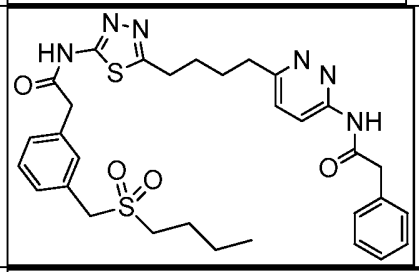
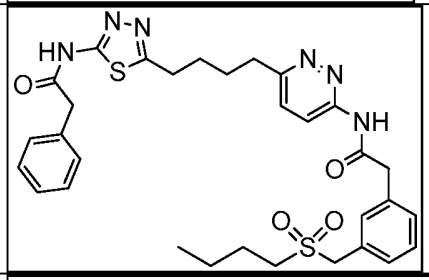
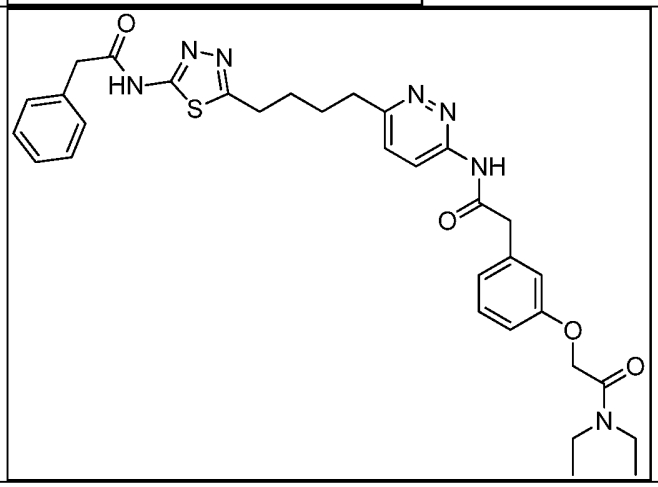
609			0.009		0.014
610			0.005		0.011
611			0.006		0.040
612			0.065		0.10
613			0.019		0.45

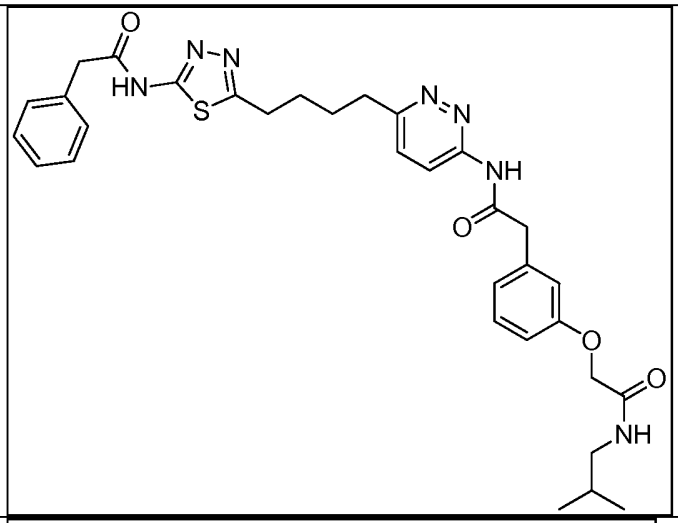
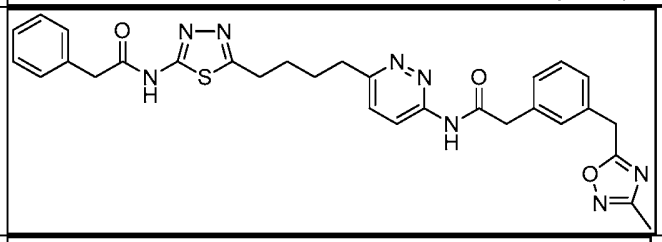
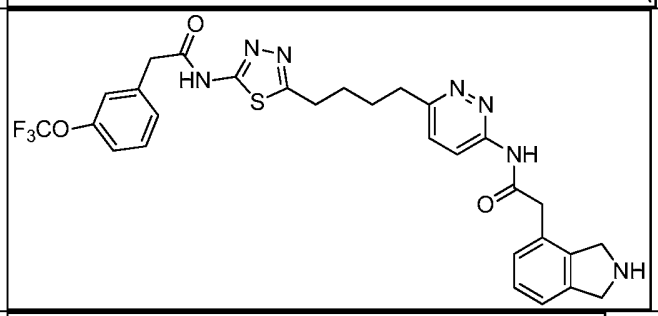
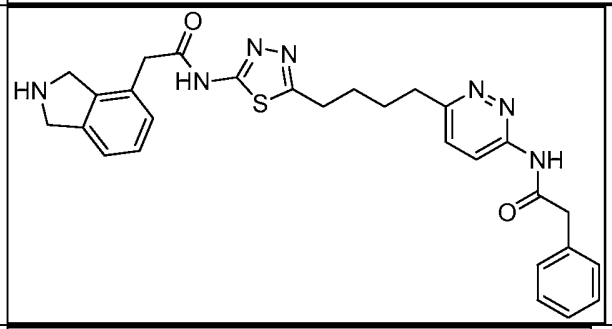
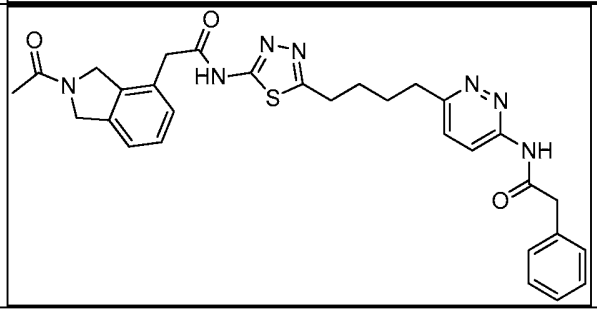
614		0.008		0.082
615		0.009		0.12
616		0.008		0.13
617		0.005		0.040
618		0.008		0.035
619		0.013		0.15
620		0.005		0.011

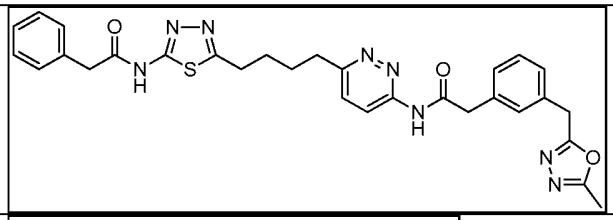
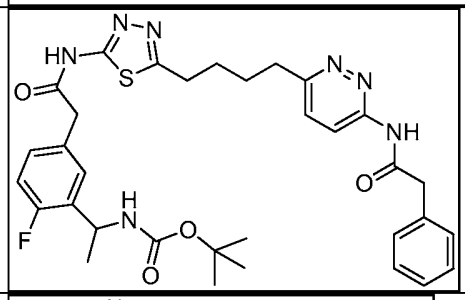
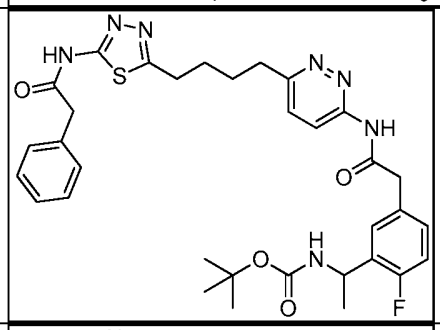
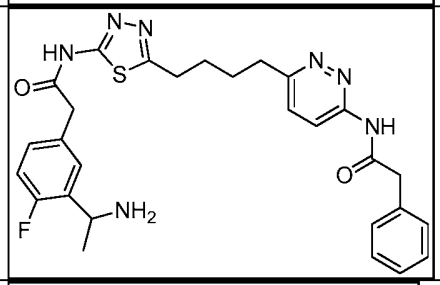
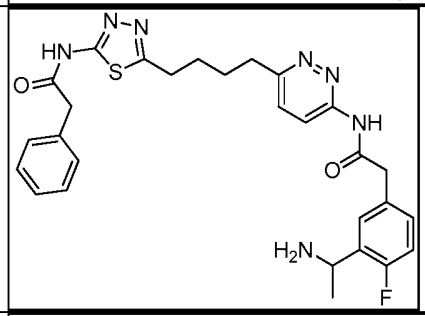
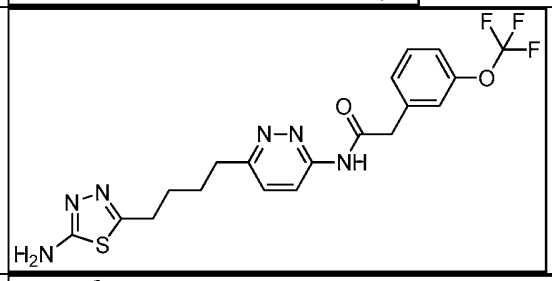
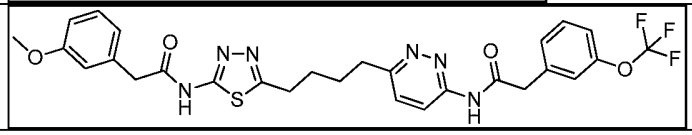
621		0.005		0.020
622		0.004		0.010
623		0.003		0.026
624		0.004		0.009
625		0.004		0.006
626		0.004		0.017

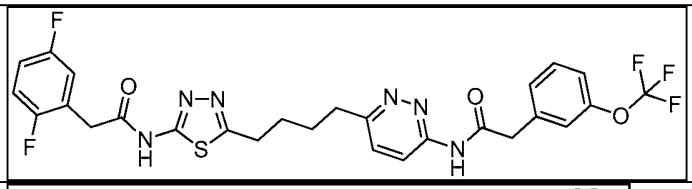
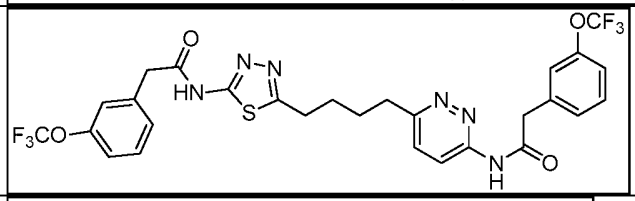
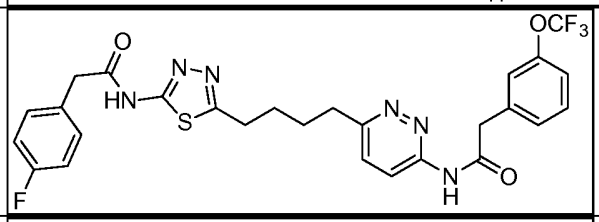
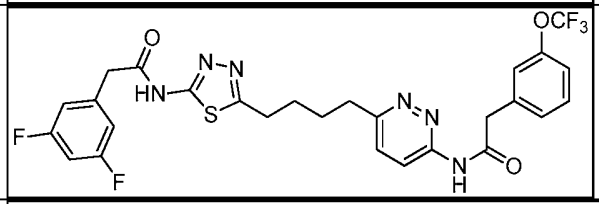
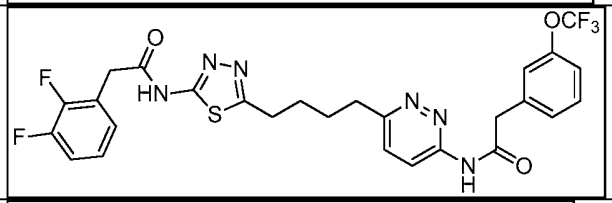
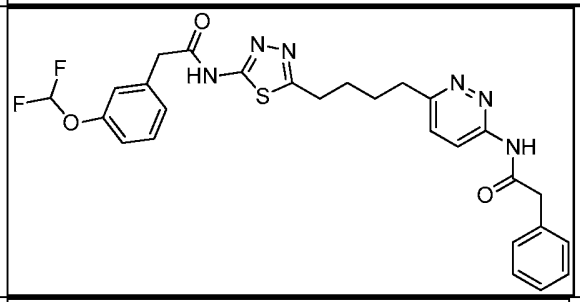
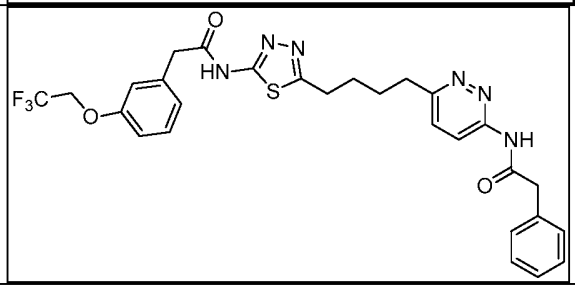
627		0.028		0.85
628		0.027		0.17
629		>20		0.065
630		0.004		0.009
631		0.005		0.006
632		0.010		0.20

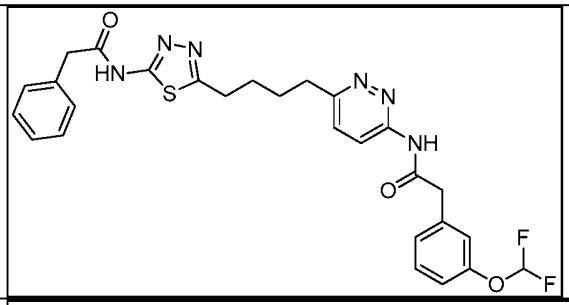
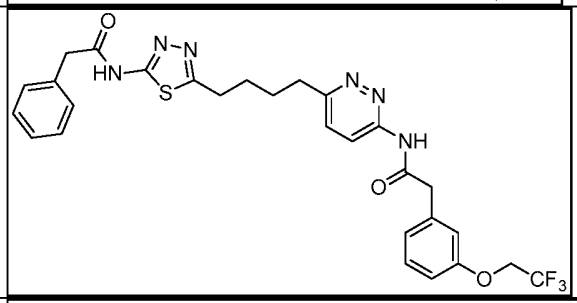
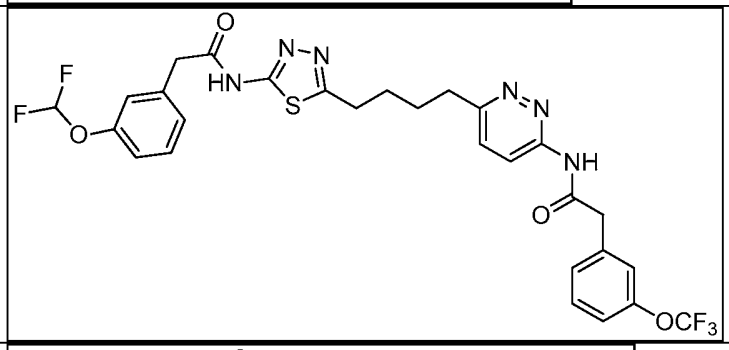
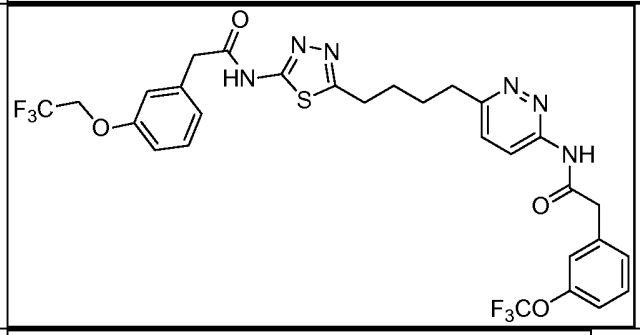
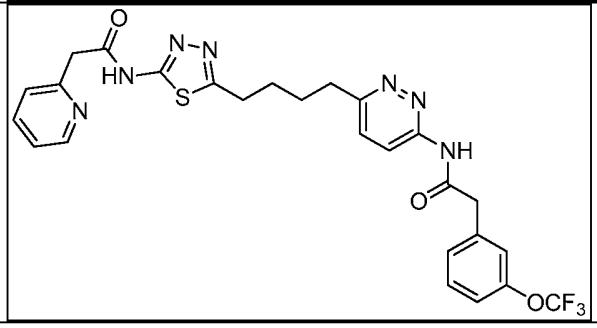
633		0.007		0.13
634		0.006		0.048
635		0.005		0.030
636		0.008		0.059
637			>20	>50
638			0.48	5.7
639			0.17	23

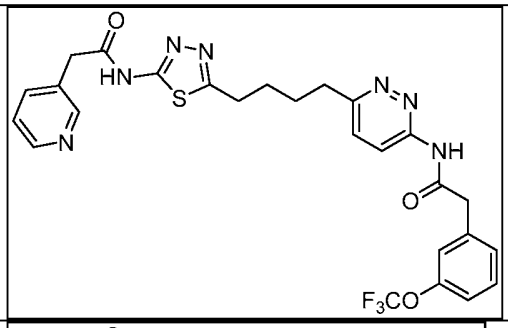
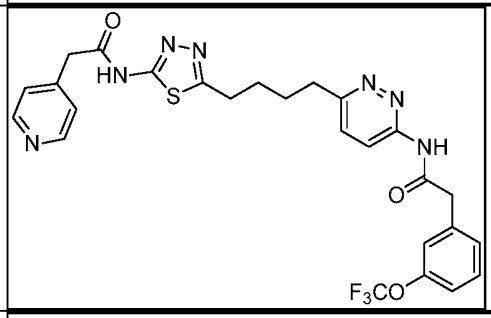
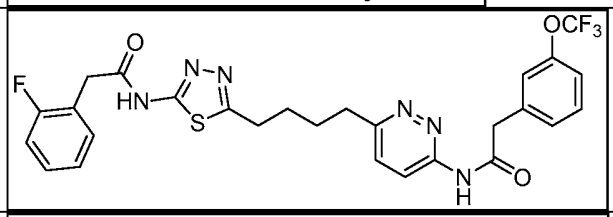
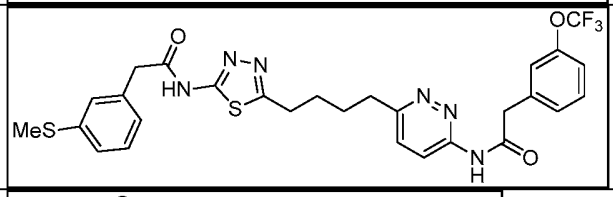
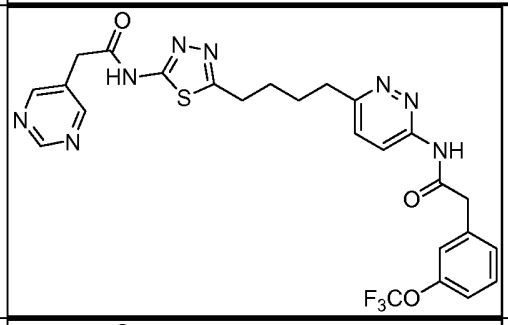
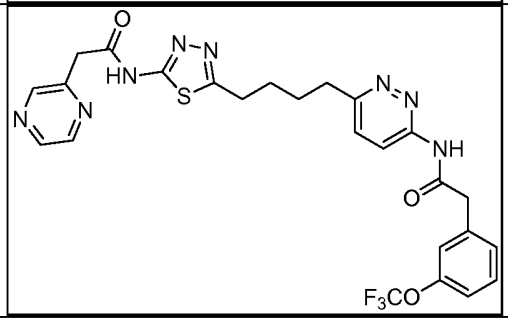
640				0.12	0.070
641				0.14	0.50
644			0.003		0.013
645			0.002		0.015
646			0.007		0.037

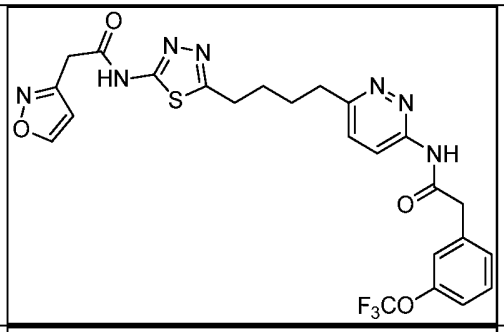
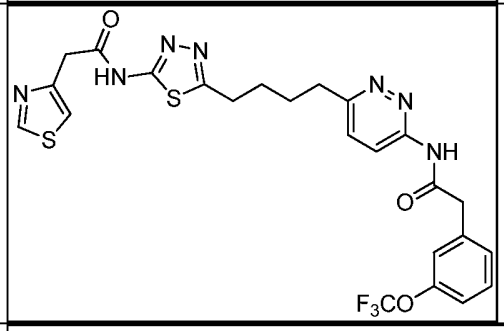
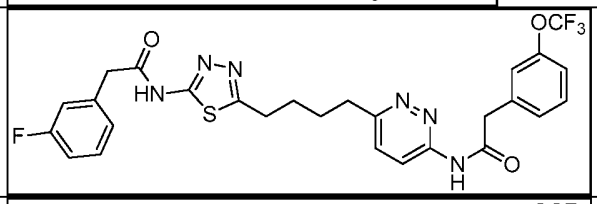
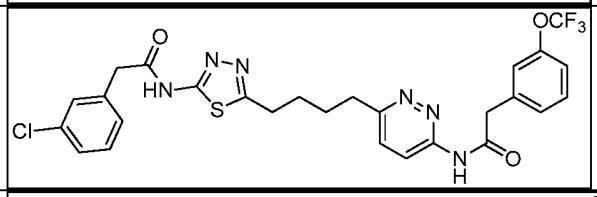
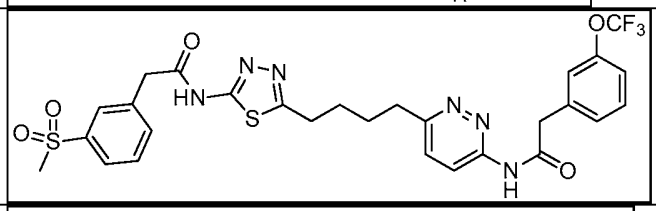
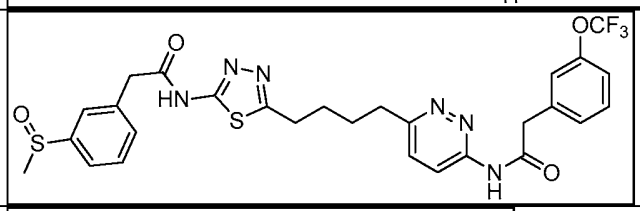
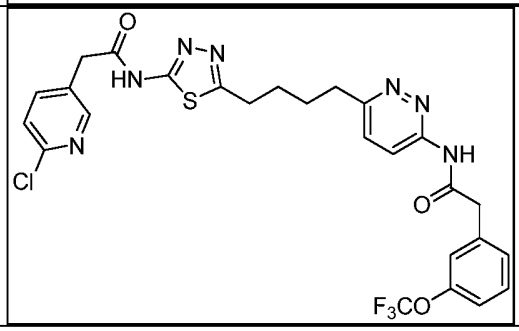
647		0.004		0.018
648		0.004		0.011
649		0.004		0.034
650		0.013		0.14
651		0.006		0.037

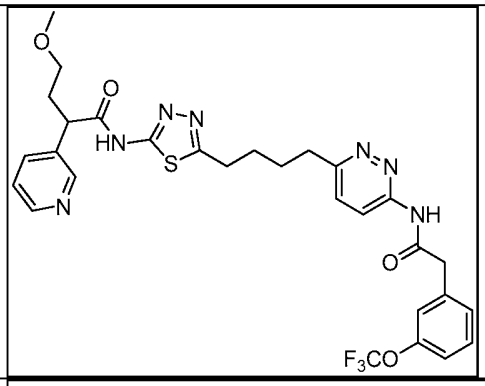
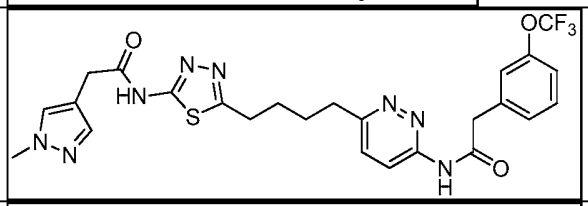
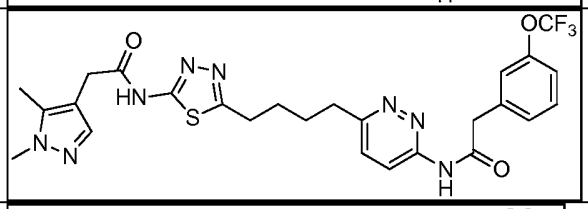
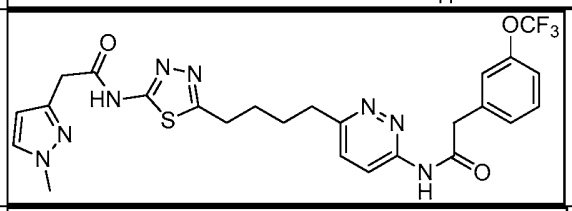
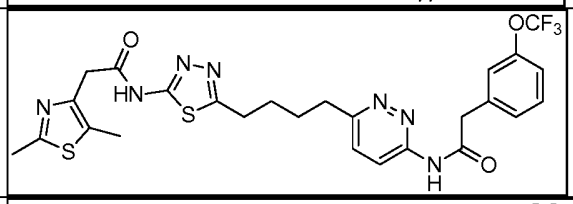
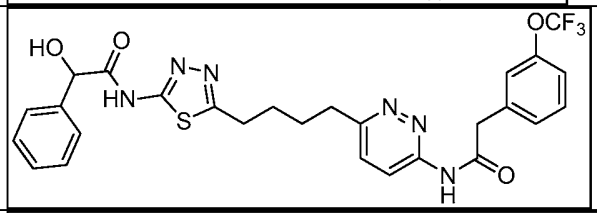
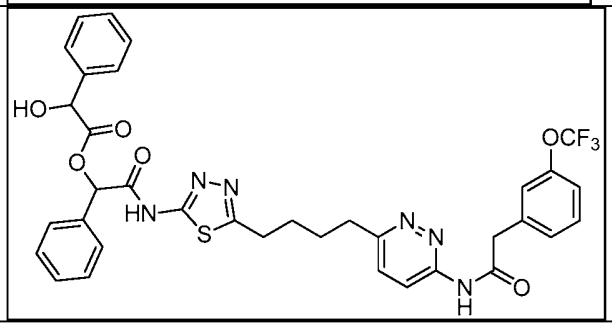
652		0.004		0.039
653		0.005		0.010
654		0.005		0.007
655		0.019		0.35
656		0.018		0.40
657		0.24		1.5
658		0.005		0.040

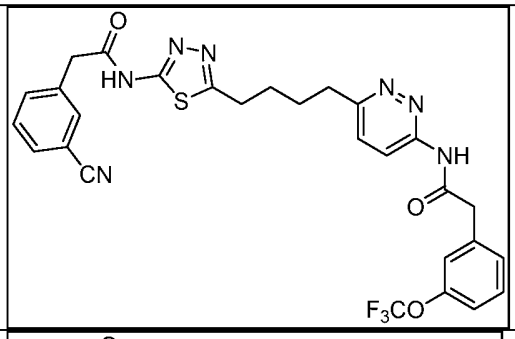
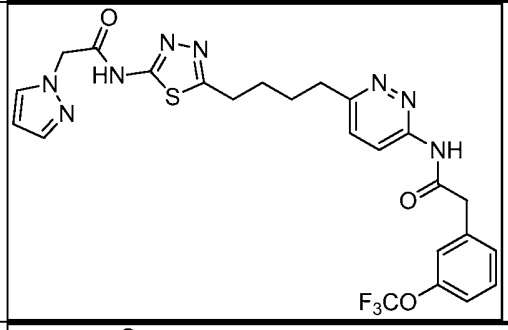
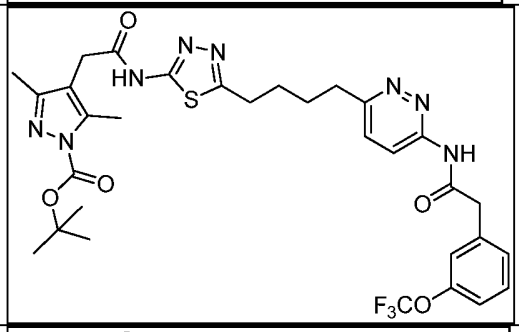
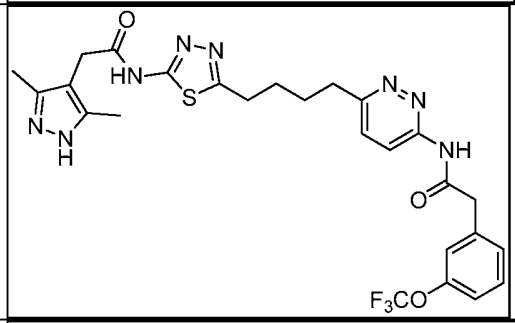
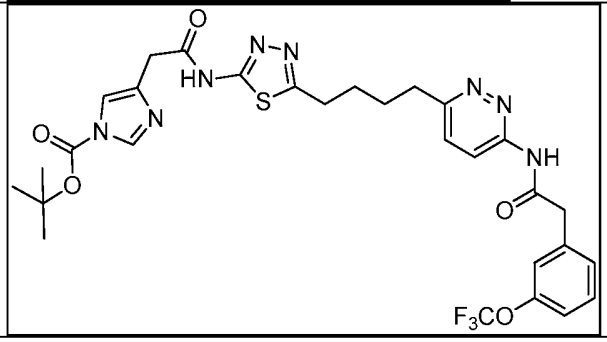
659		0.010		0.058
660		0.025		0.037
661		0.007		0.12
662		0.007		0.055
663		0.007		0.089
664		0.005		0.060
665		0.005		0.10

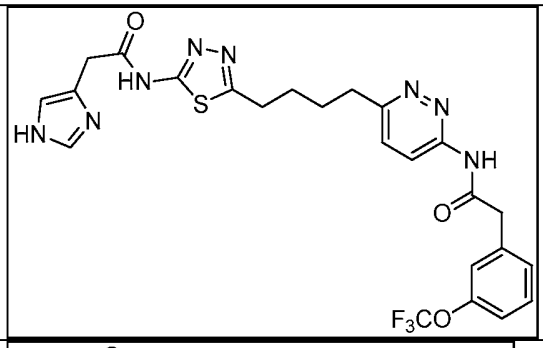
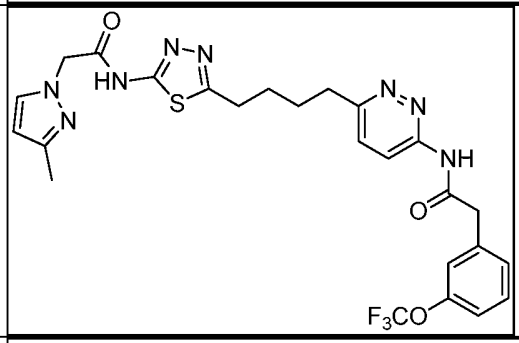
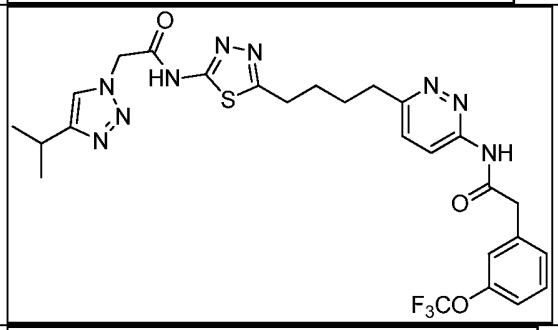
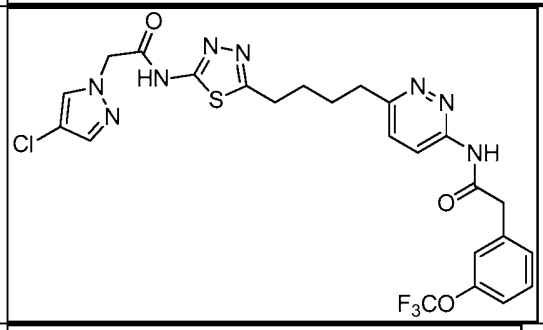
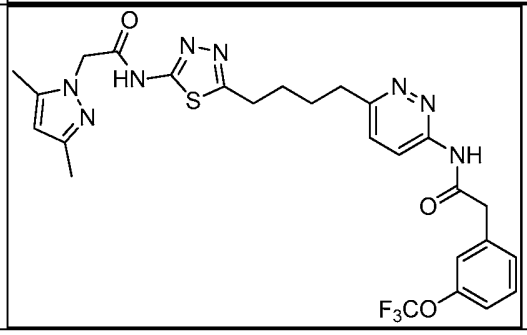
666		0.004		0.058
667		0.004		0.11
668		0.009		0.026
669		0.021		0.026
670		0.005		0.030

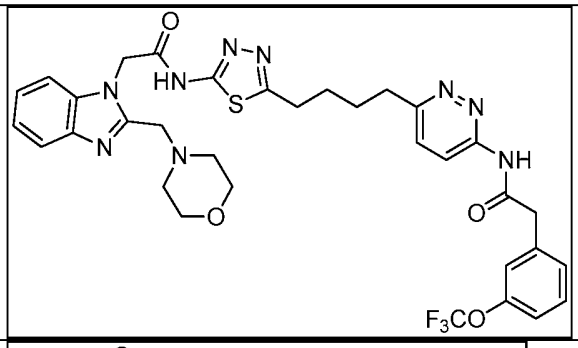
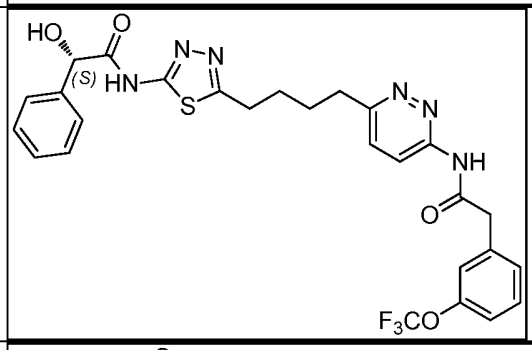
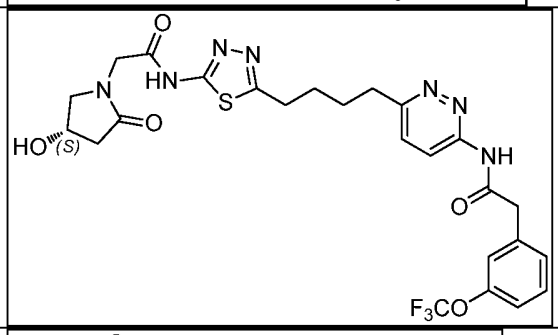
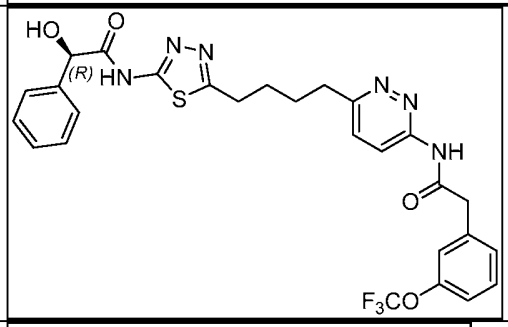
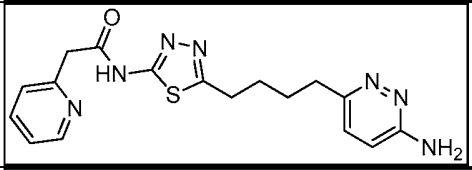
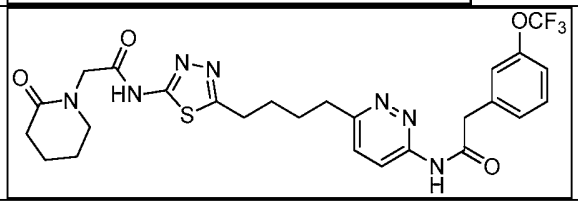
671		0.004		0.035
672		0.010		0.045
673		0.006		0.033
674		0.008		0.024
675				0.040
676				0.030

677					0.056
678					0.026
679					0.036
680					0.033
681					0.019
682					0.017
683					0.024

684				0.042
685				0.022
686				0.010
687				0.011
688				0.012
689				0.013
690				0.017

692					0.020
693					0.070
694					0.029
695					0.030
696					0.034

697					0.050
698					0.098
699					0.12
700					0.17
701					0.11

702				0.31
703				0.012
704				0.88
705				0.032
706				14
707				0.085

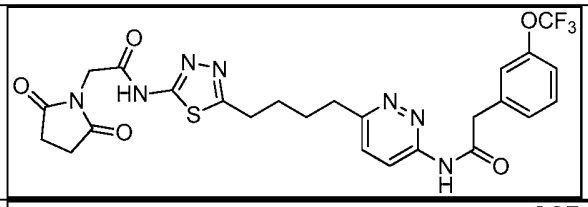
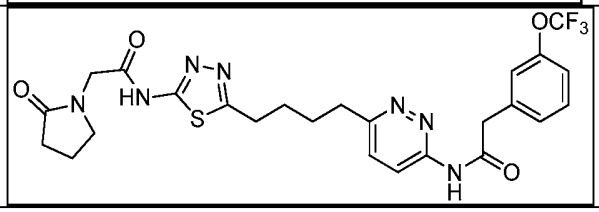
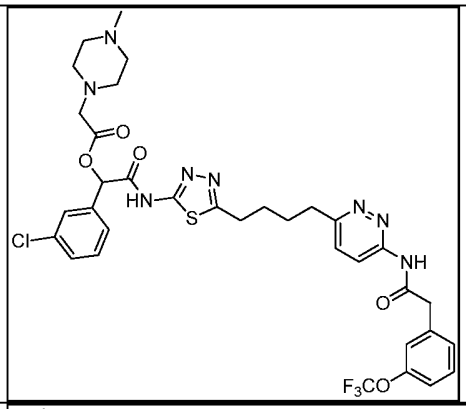
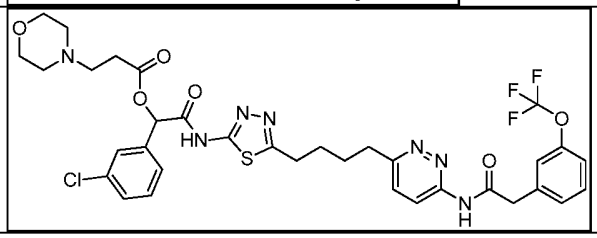
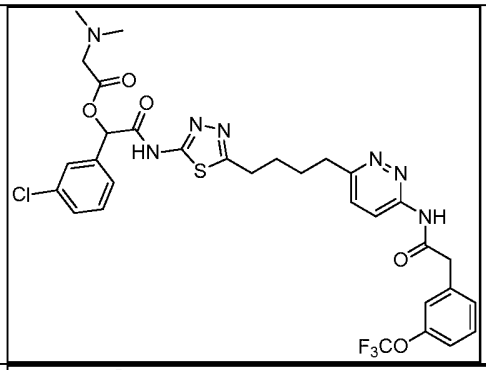
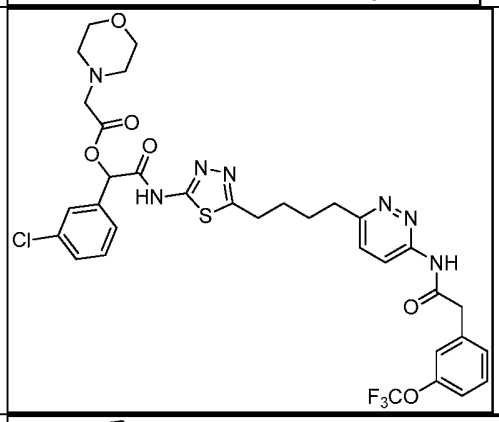
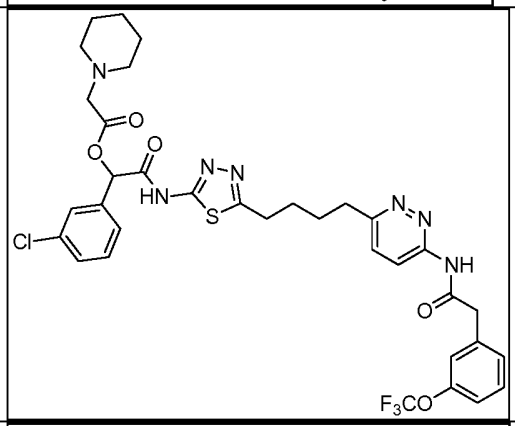
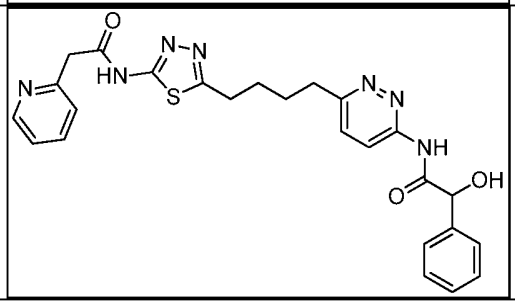
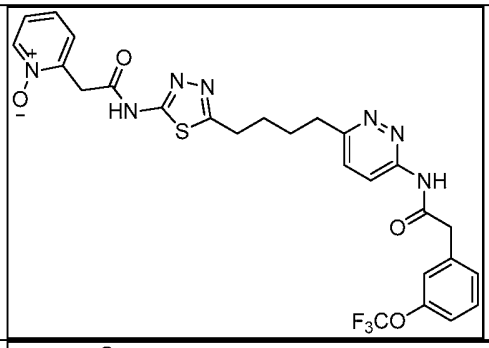
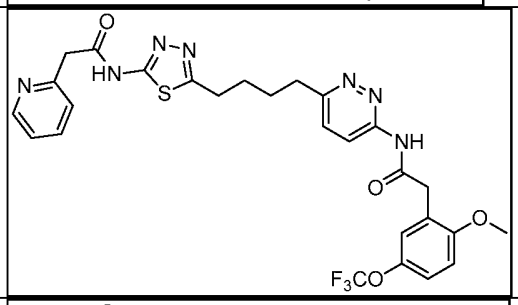
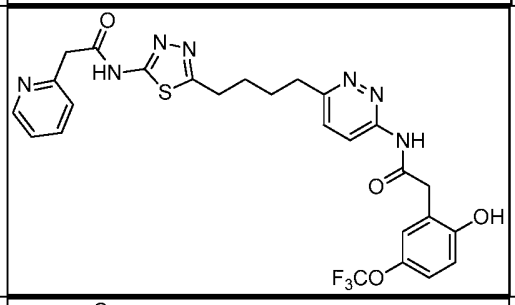
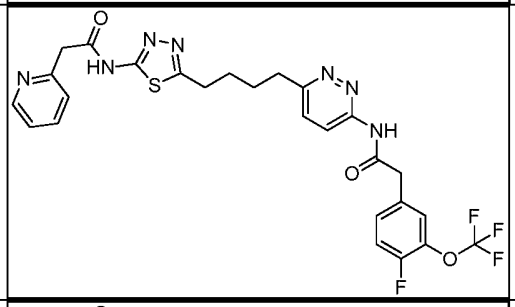
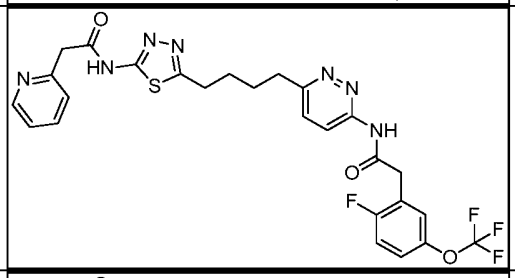
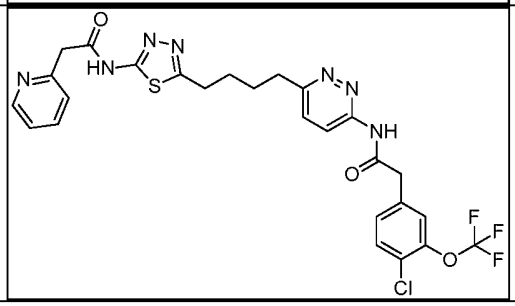
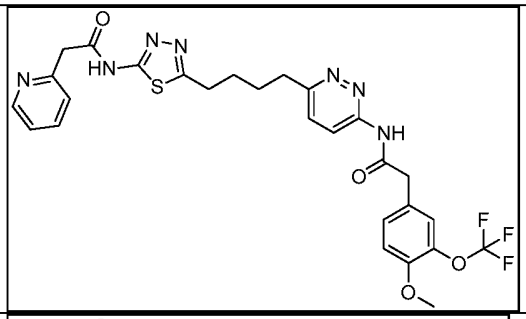
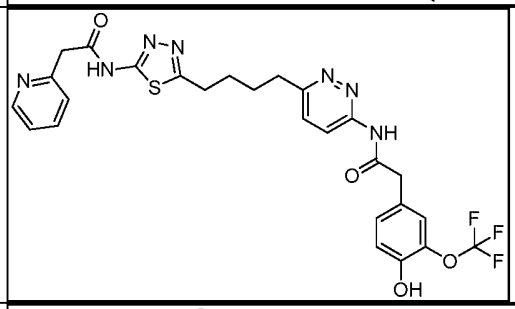
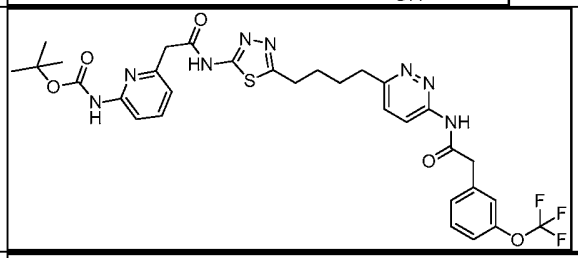
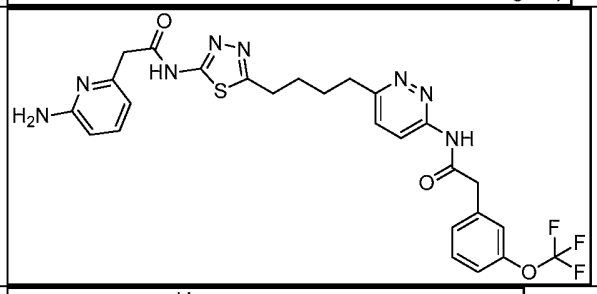
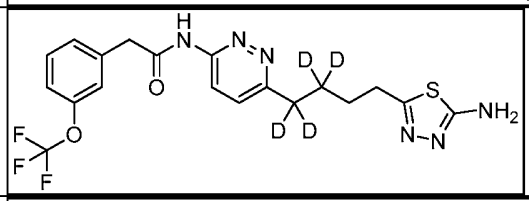
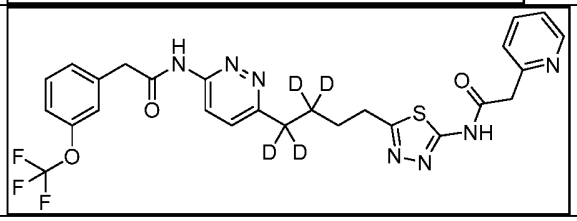
708					2.8
709					0.14

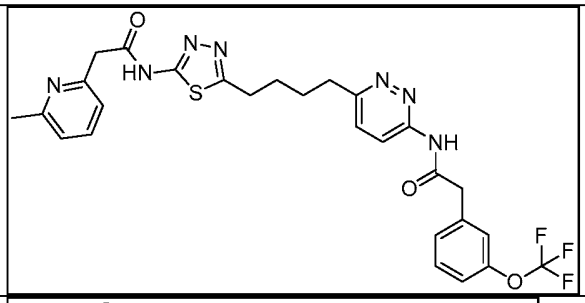
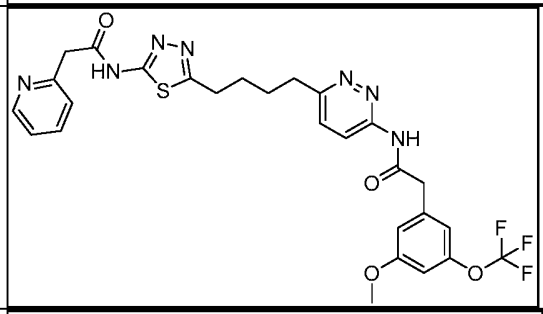
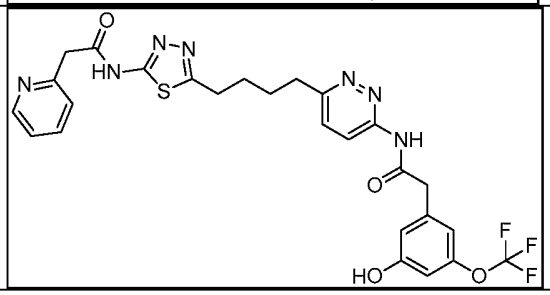
Table 3b:

Cmpd ID	Structure	Modified GAC Delta N2 IC50 60 min preinc (μM)	GAC Delta N2 IC50 60 min preinc (μM)	GAC Delta N2 IC50 no preinc (μM)	Cell prolif P493 72h IC50 (μM)
710					
711					

712						
713						
714						
715			0.19			0.39

716						0.18
717			0.034			0.019
718			0.026			0.015
719			0.033			0.01
720			0.020			0.92
721			0.016			0.022

722		0.024			0.016
723		0.042			0.02
724		0.14			0.034
725		0.050			0.15
726		0.54			0.61
727		0.023			0.012

728		0.012			0.018
729		0.016			0.026
730		0.013			0.025

Example 3: Caco-2 Permeability Assay

Caco-2 cells are commonly used in a confluent monolayer on a cell culture insert filter. When cultured in this format and under specific conditions, the cells become differentiated and polarized such that their phenotype, morphologically and functionally resembles the enterocytes lining the small intestine. The cell monolayer provides a physical and biochemical barrier to the passage of small molecules, and is widely used across the pharmaceutical industry as an in vitro model of the human small intestinal mucosa to predict the absorption of orally administered drugs (Hidalgo et al., Gastroenterology, 1989; Artursson, J. Pharm. Sci., 1990). The correlation between the in vitro apparent permeability (P¬app) across Caco-2 monolayers and the in vivo absorption is well established (Artursson et al., Biochem. Biophys. Res. Comm., 1991).

The present assay was used to determine the bidirectional permeability of the compounds of the invention through Caco-2 cell monolayers. Caco-2 cells were grown in confluent monolayers where the media of both the apical (A) and basolateral

(B) sides were at pH 7.4. Compounds were dosed at 1μM in the presence of 200μM Lucifer Yellow, on the apical side (A→B) or the basolateral side (B→A) for assessment, in duplicate. Samples from both A and B sides were taken after 120 minutes exposure, and compound concentration (reported as percent recovery) was determined using a generic LC-MS/MS method with a minimum four-point calibration curve.

The absorption potential of compounds were classified as either Low (P-app < $1X10^{-6}$ cm/s) or High (P-app > $1X10^{-6}$ cm/s). The efflux ratio was calculated as (Papp B→A)/(Papp A→B), with efflux ratios being significant when greater than or equal to 3 when the Papp (B→A) was greater than or equal to $1X10^{-6}$ cm/s. Results for certain compounds of the invention are shown in Table 4.

Table 4: Caco-2 Permeability Results

Cmpd	Direction	Recovery (%)	Papp (avg.)	Efflux Ratio	Permeability Classification	Significant Efflux
533	A→B	41	4.94	7.6	High	Yes
	B→A	52	37.5			
585	A→B	42	7.52	3.1	High	Yes
	B→A	53	23.4			
616	A→B	65	8.23	6.0	High	Yes
	B→A	76	49.5			
295	A→B	89	8.17	7.3	High	Yes
	B→A	96	59.8			
318	A→B	73	2.45	18	High	Yes
	B→A	82	44.5			
339	A→B	73	2.39	17	High	Yes
	B→A	80	41.6			
354	A→B	117	1.38	33	High	Yes
	B→A	101	45.0			
436	A→B	44	3.75	6.6	High	Yes
	B→A	57	24.7			
660	A→B	56	0.61	3.9	Low	Yes
	B→A	68	2.37			
670	A→B	70	9.64	6.2	High	Yes
	B→A	72	59.6			
679	A→B	34	7.59	2.6	High	No
	B→A	42	19.6			
447	A→B	71	7.76	3.5	High	Yes
	B→A	56	27.2			
703	A→B	51	6.26	6.6	High	Yes
	B→A	66	41.0			
705	A→B	60	8.52	6.0	High	Yes

	B→A	67	51.0			

Example 4: Solubility

Ca. 1 mg portions of test article were combined with 120 μL solvent in wells of a 96 x 2 mL polypropylene plate. The plate was vigorously vortex mixed at room temperature (*ca.* 20 C) for 18 hr and each well checked visually for undissolved solid; wells containing no visible solid were charged with additional solid test article and vortex mixed another 6 hr at room temperature after which all wells showed visible solid. The contents of all wells were then filtered through a 0.45 μm GHP filter plate to yield clear filtrates. 5 μL of each filtrate was diluted into 100 μL DMF and vortex mixed to yield HPLC samples. Duplicate quantitation standards for each test article were prepared by diluting weighed portions of solid test article in measured volumes of DMF. 2 μL of each HPLC sample and quantitation standard were analyzed by HPLC using the method outlined in Table 5. Dissolved test article concentrations were calculated by peak area ratio against the appropriate quantitation standards. Solubility results are presented in Table 6.

Table 5: Outline of HPLC Method

Instrument	Shimadzu Prominence UFLC with Diode Array UV/Vis Detector	
Column	VWR Sonoma C8(2), 3.5 μm, 2.1 x 50 mm	
Column Temp	40° C	
Mobile Phase A	0.1% (v/v) formic acid in water	
Mobile Phase B	0.1% (v/v) formic acid in acetonitrile	
Flow Rate	0.4 mL/min	
Gradient	*Time (min)*	*% Mobile Phase B*
	0	20
	8	100

	8.5	100
	8.6	20
	9.6	END

Table 6: Measured Solubilities

Solvent	**Solubility (mg/mL)**			
	1	**295**	**402**	**585**
water	< 0.002	< 0.002	< 0.004	< 0.002
0.9% NaCl	< 0.002	< 0.002	< 0.004	< 0.002
0.1 M HCl	< 0.002	0.003	< 0.004	< 0.002
50 mM Cit pH 2.3	< 0.002	< 0.002	< 0.004	< 0.002
50 mM Cit pH 3.3	< 0.002	< 0.002	< 0.004	< 0.002
50 mM Cit pH 4.4	< 0.002	< 0.002	< 0.004	< 0.002
50 mM Cit pH 5.4	< 0.002	< 0.002	< 0.004	< 0.002
PBS	< 0.002	< 0.002	< 0.004	< 0.002
0.1 M NaOH	14.420	0.268	< 0.004	0.192
10% PS80 / 50 mM cit	0.050	0.027	0.153	0.261
10% CrEL / 50 mM cit	0.076	0.055	0.157	0.228
20% SBECD / 50 mM cit	0.046	0.090	0.019	0.125
20% HPBCD / 50 mM cit	0.042	0.167	0.056	0.327
Labrasol	0.258	0.918	31.032	5.004
Capryol PGMC	0.042	1.540	11.210	1.780
Capryol 90	0.081	0.215	13.676	1.744
canola oil	< 0.002	< 0.002	0.529	0.072
PEG400	0.451	1.644	30.179	3.944
PG	0.048	0.234	1.365	1.422
EtOH	0.040	0.083	2.958	1.991
Solvent	**Solubility (mg/mL)**			
	670	**447**	**703**	
water	0.007	< 0.004	< 0.004	

0.9% NaCl	< 0.002	0.005	< 0.004	
0.1 M HCl	0.005	< 0.004	< 0.004	
50 mM Cit pH 2.3	0.066	< 0.004	< 0.004	
50 mM Cit pH 3.3	0.003	< 0.004	< 0.004	
50 mM Cit pH 4.4	< 0.002	< 0.004	< 0.004	
50 mM Cit pH 5.4	< 0.002	< 0.004	< 0.004	
PBS	< 0.002	< 0.004	< 0.004	
0.1 M NaOH	0.227	0.192	0.656	
10% PS80 / 50 mM cit	1.204	0.851	0.378	
10% CrEL / 50 mM cit	0.458	0.732	0.309	
20% SBECD / 50 mM cit	5.256	2.718	0.476	
20% HPBCD / 50 mM cit	9.685	2.177	0.651	
Labrasol	5.042	77.164	20.727	
Capryol PGMC	1.519	7.916	3.683	
Capryol 90	1.974	11.114	7.409	
canola oil	0.012	0.071	0.014	
PEG400	9.901	57.334	22.419	
PG	2.569	8.265	4.698	
EtOH	0.964	3.921	2.645	

Example 5: Anti-proliferation and glutamine dependency assay.

Breast cell lines were tested *in vitro* for their ability to grow in the absence of glutamine and for their sensitivity to compound 670 in glutamine containing media. The cells were maintained in growth media (RPMI-1640, 10%FBS, 100 units/ml penicillin and 100Ag/ml streptomycin, 0.25 µg/mL amphotericin) supplemented with 2mM glutamine at 37°C with 5% CO_2.

To determine glutamine dependence, cells were seeded in 96-well plates at a density of 3000-5000 cells/well depending on cell size and their growth characteristics. The appropriate plating density was selected to ensure that the cells did not become confluent during the 72 hour assay period. Twenty-four hours after seeding, the

plating media was removed and the cells were washed 2 times with glutamine-free growth media and then 100 uL of glutamine-free media or glutamine containing (2mM) growth media was added back to the wells. Cells were incubated for 72 hrs at 37°C with 5% CO_2 and analyzed for antiproliferative effects by Cell Titer Glo (Promega). Cell proliferation (% of DMSO control) was determined by comparing the Cell Titer Glo signal (rfu) on the day of glutamine withdrawal (t=0) measured in parallel plates with the signal observed after the 72 hour incubation period by the following formula ((rfu of cells grown in glutamine-free media for 72 hours – rfu at t=0)/(rfu of cells grown in 2mM glutamine for 72hrs – rfu at t=0)). Cell lost was determined by the following formula: (100 x rfu at 72 hours in glutamine-free media/rfu at t=0) – 100.

Sensitivity to compound 670 was determined by treating cells in 96-well plate seeded as described above. Twenty four hours after seeding, the cells were washed with growth media with 2mM glutamine and 50 uL of growth media with 2 mM glutamine was added to the well. A 10 mM DMSO stock of compound 670 was diluted into 100% DMSO at 200 uM. This was further diluted to 2 uM in growth media with 2mM glutamine. 50 ul of this mixture was added to cell plates making the final concentration of 670 uM to be 1 uM. Parallel control wells were treated with DMSO only. Cells were incubated for 72 hours at 37 °C with 5% CO2 and analyzed for antiproliferative effects by Cell Titer Glo. Cell proliferation was calculated in a manner similar to that described above with the following modifications: cell proliferation ((rfu of cells grown in 1 uM compound 670 for 72 hours – rfu at t=0)/(rfu of DMSO control at 72hrs – rfu at t=0)), cell lost (100 x rfu at 72 hours in 1 uM compound 670/rfu at t=0) – 100. The results from these assays are shown in Figure 1.

Example 6: Differential expression of glutaminase and glutamine synthetase in triple-negative breast cancer subtype.

Primary breast tumor and cell line expression datasets were downloaded [The Cancer Genome Atlas from https://genome-cancer.ucsc.edu (breast invasive carcinoma/gene expression/RNAseqV2 data) and The Cell Line Encyclopedia from http://www.broadinstitute.org/ccle/home (gene-centric RMA-normalized mRNA expression/aAffymetrix U133+2 arrays)] and the expression level within each dataset

for the following genes was evaluated: estrogen receptor (ER), progesterone receptor (PR) and Her2 (ERBB2), glutaminase (GLS) and glutamine synthetase (GLUL). The relative expression level for a given gene in each sample was calculated by comparison to the median expression of the gene in the entire dataset. Samples with the lowest relative levels of ER, PR, and Her2 ("triple-negative") were identified by analysis of individual expression distributions for the three marker genes and the relative levels of glutaminase and glutamine synthetase within this population and the non-triple-negative population was assessed. Figure 2 represents a heatmap illustrating the relatively high expression (red) of glutaminase and low expression (green) of glutamine synthetase in the triple-negative population.

Example 7: Single-agent compound 402 treatment of MDA-MB-231 orthotopic xenograft model.

Female scig/beige mice (n=20) age 6-8 weeks were implanted in the inguinal mammary fat pad with 1 x 10^7 MDA-MB-231 cells mixed 1:1 with matrigel. When tumors reached a volume of 100-150 mm^3, mice were randomized into the following two groups of n=10 mice/group: 1) Vehicle control (Gelucire) dosed PO BID for 35 days, and 2) compound 402 at 100 mg/kg (formulated at 10 mg/mL in Gelucire) dosed IP BID for 35 days. Tumors were measured with calipers twice weekly for 35 days and tumor volume calculated using the formula tumor volume (mm^3) = (a x b^2/2) where 'b' is the smallest diameter and 'a' is the largest diameter. 24 hours after the final dose, mice were sacrificed, lungs were excised, and lung metastases quantified by percent lung metastases coverage (textured lung exterior). Figure 3 shows measurement of tumor volume and metastases upon treatment with compound 402 compared to vehicle.

Example 8: Combination study with compound 389 and paclitaxel in MDA-MB-231 orthotopic xenograft model.

Female scig/beige mice (n=40) age 6-8 weeks were implanted in the inguinal mammary fat pad with 1 x 10^7 MDA-MB-231 cells mixed 1:1 with matrigel. When tumors reached a volume of 100-150 mm^3, mice were randomized into the following four groups of n=10 mice/group: 1) Vehicle control (20% HPBCD/10mM Citrate buffer pH 4.0) dosed IP BID for 35 days, 2) compound 389 at 50 mg/kg (formulated

at 5 mg/mL in 20% HPBCD/10mM Citrate buffer pH 4.0) dosed IP BID for 35 days, 3) Paclitaxel at 10 mg/kg (clinical formulation diluted to 1 mg/mL in saline) dosed IP QD x 5 days, and 4) compound 389 at 50 mg/kg IP BID x 35 days plus paclitaxel at 10 mg/kg dosed IP QD x 5 days. Tumors were measured with calipers twice weekly for 35 days and tumor volume calculated using the formula tumor volume (mm^3) = (a x b^2/2) where 'b' is the smallest diameter and 'a' is the largest diameter. Figure 4 shows measurement of tumor volume upon treatment with a combination of compound 389 and paclitaxel compared to vehicle and each agent alone.

Example 9: Determination of glutamate and glutamine in cell samples by liquid chromatography tandem mass spectrometry.

Sensitivity to compound 670 was determined as described in Example 5.

Untreated cells were examined for metabolite levels. Concentrations of glutamine and glutamate were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS). Cell pellets from in vitro cell assays were washed by PBS, mixed with methanol:water (50:50) containing internal standard (IS) for extraction of glutamine and glutamate and then stored at-70°C until analysis. The extracted cellular samples were vortexed, centrifuged and/or filtered and 10 µL of the extracts was injected for LC-MS/MS analysis. Glutamine and glutamate were quantified by comparing the peak area ratios of the analyte to IS in study samples to the standard calibration samples. The LC-MS/MS system comprised an API 4000 mass spectrometer (ABSCIEX, Foster City, CA) equipped with Shimadzu LC- 10ADvp pumps (Shimadzu, Columbia, MD) and Leap PAL HTC-xt autosampler. Chromatographic separation was achieved on an Phenomenex Luna NH2 column (2.1 × 50 mm, 3.5 µm particle size) using gradient elution. The mobile phases were (A) 10 mM ammonium acetate and 5 mM ammonium hydroxide in water and (B) 50:50 methanol:acetontrile. Mass spectrometric detection was accomplished using the Turbo ionspray interface in the negative ionization mode by MRM of the selective *m/z* transitions: 145.9→101.8 for glutamate and 144.7→108.8 for glutamine. The results from these assays are shown in Figure 9.

Example 10: Determination of glutaminase:glutamine synthetase ratios

Gene expression data were from the Barretina Cell Line dataset in Oncomine. Expression levels for each glutaminase and glutamine synthetase transcript for each primary tumor sample were quantile normalized. In any given sample, a log2 copy number of 0 indicates that the gene in question is expressed at the median expression level relative to 12,000 genes across all datasets and samples analyzed. The horizontal line indicates the ratio of the median expression of each transcript within the number of clinical samples shown. The results are represented in Figures 5, 6, 7 and 8.

Example 11: Expression and metabolite correlations extend to other tumor types

Primary tumor xenografts were provided by a commercial clinical research organization, along with microarray data for glutaminase and glutamine synthetase expression. Glutamate and glutamine cncentrations were determined as described in Example 9. Glutaminase activity was determined essentially as described in Curthoys and Bellemann (Exp Cell Res, 1979). Figure 10 shows the correlation between glutamate:glutamine ratios and glutaminase:glutamine synthetase expression ratios or glutaminase activity.

Example 12: Colon carcinoma xenograft efficacy study

Female scid/bg mice, approximately 6 weeks of age, were implanted subcutaneously on the right flank with 5 x 10^6 HCT116 cells per mouse in a volume of 100 uL of sterile PBS. When tumors reached a volume of 50 - 100mm^3, mice were randomized to groups of n=10 to receive either vehicle or test compound delivered twice daily by intraperitoneal injection. Tumors were measured three times per week using Vernier calipers and tumor volume calculated using the formula: Volume = (Length x Width^2/2), where length and width are the longest perpendicular sides of the tumor. Dosing continued twice daily until control tumors reached a size of 2000mm^3. Statistical comparisons were made using a 2-way ANOVA with Bonferroni post-test. Figure 11 shows that intraperitoneal administration of compound 188 to mice results in reduced tumor size in this HCT116 colon carcinoma xenograft model.

Example 13: Lung adenocarcinoma xenograft efficacy study

Female scid/beige mice (n=20) age 6-8 weeks were implanted subcutaneously with 1 x 10^7 H2122 lung adenocarcinoma cells per mouse suspended in PBS. Mice were randomized into the following two groups of n=10 mice/group: 1) Vehicle control (25% Hydroxypropyl-β-cyclodextrin) and 2) Compound 670 dosed orally at 200 mg/kg (formulated at 20 mg/mL in 25% HP-β-CD). For both groups, dosing was initiated 24 hours post-implant and continued orally BID for 23 days. Tumors were measured with calipers three times per week and tumor volume calculated using the formula tumor volume (mm^3) = (a x b^2/2) where 'b' is the smallest diameter and 'a' is the largest perpendicular diameter. **P-value < 0.01 (Two-sided T-test). Results are shown in Figure 12.

Example 14: mRNA expression of glutaminase and glutamine synthetase in TNBC and HR+/Her2+ breast tumor cell lines

Two publicly available databases were queried to determine the mRNA levels of glutaminase (GLS) and glutamine synthetase (GS):

- Microarray expression data for a panel of 51 breast cancer cell lines published by Neve et al., (*Cancer Cell* 10(6):515-27 (Dec 2006)) of which 20 were evaluated in the present example, and
- The Cancer Cell Line Encyclopedia (CCLE; Barretina et al., *Nature* **483**, 603–607 (29 March 2012)) which included expression data for 58 breast cancer cell lines, 25 of which were used in this example.

The Neve et al. publication included annotation of hormone and growth factor status for each cell line in the data set (25 triple negative, 26 HR+ or Her2+). For the CCLE dataset, hormone and growth factor receptor status was evaluated based on mRNA expression levels for estrogen receptor (ESR, 20/58 positive), progesterone receptor (PGR, 10/58 positive) and Her2 (ERBB2, 13/58 positive). Based on this analysis, a total of 31 cell lines were classified as TNBC and 27 as HR+ or Her2+. For the 33 cell lines represented in both datasets, there was good concordance in the hormone and growth factor receptor status assignment (32/33). The present example was carried out in a panel of cell lines that included 22 triple negative and 7 HR+ or Her2+.

The log2 transfomred mRNA expression values for the GLS splice variants KGA (probeset 203159_at) and GAC (probeset 221510_s_at) as well as GS (probeset

215001_s_at) in each cell line were median-centered based on the median expression value for all probesets across all samples in the dataset (median of 5.583 for the Neve et al. dataset and median of 4.809 for the CCLE dataset). For calculation of the GLS:GS ratio, the log2 transformed expression values for KGA, GAC, and GS were first converted back to their corresponding untransformed values. The expression levels of GLS (KGA and GAC), GS and the ratio of GLS (KGA or GAC) to GS were compared in the TNBC cell lines vs. the HR+/Her2+ cell lines. Significant differences were determined using an un-paired Student's T-test (Prism).

Differences between the TNBC cell lines and HR+/Her2+ cell lines are depicted graphically in Figure 13. For both datasets, there was significantly higher expression of the GLS splice variants KGA and GAC in the TNBC as compared to the HR+ or Her2+ cell lines. The magnitude of difference and statistical significance was greater for the GAC splice variant. For glutamine synthetase (GS), there was significantly lower expression in the TNBC cell lines relative to the HR+ or Her2+ subset for both datasets. The ratio of either KGA to GS and of GAC to GS was also significantly higher in the TNBC cell lines.

Example 15: Correlation between sensitivity to Compound 670 and expression of GLS and GS.

The cell proliferation and cell loss observed as a result of Compound 670 treatment was compared to the expression levels of glutaminase (KGA and GAC), glutamine synthetase (GS), and the ratio of glutaminase to glutamine synthetase. The antiproliferation effect of Compound 670 was determined as described in Example 5. Figure 14 displays a series of bivariate graphs plotting the Compound 670 sensitivty against each expression parameter for all tested cell lines (from either the Neve et al. or the CCLE datasets) while Table 7 summarizes the corresponding Spearman rank order correlation coefficients (and P values). For both expression datasets, significant correlations were observed between Compound 670 sensitivity and the expression of the GAC isoform of glutaminase, the expression of glutamine synthetase (GS), and the ratio of GAC:GS. The most significant correlation in each dataset was with GAC expression alone. These results support the hypothesis that cells with an elevated

GAC expression or a GAC:GS ratio are sensitive to GLS inhibition with glutaminase inhibitors. This phenotype is observed in the majority of TNBC cell lines and the minority of receptor positive breast cell lines.

Table 7. Correlation between sensitivity to Compound 670 and GLS mRNA expression, GS mRNA expression, or expression ratios[1].

			Correlation between Compound 670 sensitivity and mRNA expression parameters				
			KGA	GAC	GS	KGA: GS	GAC: GS
Dataset	Neve	Spearman correlation coefficient	-0.391	-0.7624	0.585	-0.585	-0.7353
		P-value (two-tailed)	0.0883 ns	<0.0001 ****	0.0067 **	0.0067 **	0.0002 ***
	CCLE	Spearman correlation coefficient	-0.1826	-0.5774	0.4157	-0.3339	-0.4809
		P-value (two-tailed)	0.3931 ns	0.0031 **	0.0434 *	0.1108 ns	0.0174 *

[1]Spearman rank order correlation coefficients and associated P-values for the data plotted in the bivariate graphs from Figure 14. The full panel of breast cancer cell lines (TNBC, HR+, Her2+) were combined for each correlation analysis.

Example 15: Protein expression of Gln-utilizing enzymes in breast cancer cell lines

Western analysis of extracts prepared from the panel of breast cancer cell lines was used to monitor, at the protein level, expression of GLS (GAC and KGA splice variants) and glutamine synthetase. As shown in Figure 15, consistent with the microarray mRNA expression analysis for these genes, the majority of TNBC cell lines express GAC and KGA, while GAC and KGA are expressed at lower levels (or undetectable levels) in most of the receptor-positive lines. GAC, in particular, is expressed at relatively high levels in nearly all of the TNBC cell lines tested (compared to the HR+/Her2+ cell lines). The expression of glutamine synthetase was more variable and, in contrast to the microarray data, did not display a clear distinction between TNBC and receptor-positive cells across this panel of cell lines.

Cell lysates prepared for the Western blotting were analyzed for glutaminase activity according to the method described in Example 11. Results show that the level of KGA and GAC protein corresponded with higher glutaminase activity.

Example 16: Sensitivity to glutaminase inhibitor and metabolite levels.

Glutamate and glutamine concentrations were determined as described in Example 9. Sensitivity to glutaminase inhibitor was determined as described in Example 5. Figure 16 shows the correlation between glutamate:glutamine ratios and sensitivity to compound 670.

Example 17: Multiple Myeloma xenograft study.

Female CB.17 SCID mice (n=20) age 8-12 weeks were implanted subcutaneously with 1 x 10^7 RPMI-8226 myeloma cells per mouse mixed 1:1 with Matrigel. Mice were randomized into the following two groups of n=10 mice/group: 1) Vehicle control (25% Hydroxypropyl-β-cyclodextrin) and 2) Compound 670 dosed at orally at 200 mg/kg (formulated at 20 mg/mL in 25% HP-β-CD). For both groups, dosing was initiated when tumors reached a volume of 100-150mm^3 and continued orally BID for 28 days. Tumors were measured with calipers two times per week and tumor volume calculated using the formula tumor volume (mm^3) = (a x b^2/2) where 'b' is the smallest diameter and 'a' is the largest perpendicular diameter. **P-value < 0.01 (Two-sided T-test). Results are shown in Figure 17.

Example 18: Treatment of Multiple Myeloma cells with a combination of drugs.

As shown in Figure 18, MM1S cells (panels A & B) and RPMI-8226 cells (panels C & D) were treated with a dose titration of either compound 670, pomalidomide or a mixture thereof (panels A & C) or compound 670, dexamethsone or a mixture thereof (panels B & D) for 72 hours in growth media. At the end of the incubation, cell viability was measured using Cell Titer Glo as per manufacturer's protocol (Promega, Madison, WI). Measured values for compound-treated cells were normalized to

DMSO-treated cells and data is reported as a cell survival ratio with a value of 1 (one) corresponding to maximum cell survival and a value of 0 (zero) corresponding to no cell survival. Cell survival ratios for all compound treatments are represented as bar graphs. Combination indices were calculated using the Calcusyn program (biosoft.com) and reported for individual mixtures of compound 670 and pomalidomide [POM] (panels A &C) and individual mixtures of compound 670 and dexamethasone [DEX] (panels B & D). Compound mixtures that produced a synergistic anti-tumor activity are highlighted.

Incorporation by Reference

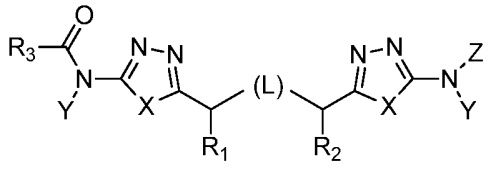
All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control. The compounds, synthetic methods, and experimental protocols and results of U.S. Application Number 13/680,582, filed November 19, 2012, are hereby incorporated by reference.

Equivalents

While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

Claims:

1. A method of treating or preventing cancer comprising administering a compound of formula I,



(I),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or , wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X, independently for each occurrence, represents S, O or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy;

R_3, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or

heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H.

2. The method of claim 1, wherein L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2.

3. The method of claim 1, wherein L represents CH_2CH_2.

4. The method of any preceding claim, wherein Y represents H.

5. The method of any preceding claim, wherein X, independently for each occurrence, represents S or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl.

6. The method of any preceding claim, wherein Z represents $R_3(CO)$.

7. The method of claim 6, wherein each occurrence of R_3 is not identical.

8. The method of any preceding claim, wherein R_1 and R_2 each represent H.

9. The method of any preceding claim, wherein R_3, independently for each occurrence, represents substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl.

10. The method of any preceding claim, wherein R_3, independently for each occurrence, represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl, heteroaryl or heteroaralkyl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl.

11. The method of claim 10, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl, or heteroaryl.

12. The method of claim 10 or 11, wherein R_{10} represents hydroxy, hydroxyalkyl, or alkoxy.

13. The method of claim 1, wherein L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and R_3, independently for each occurrence, represents substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl.

14. The method of claim 13, wherein each occurrence of R_3 is identical.

15. The method of claim 1, wherein L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and R_3, independently for each occurrence, represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl, heteroaryl or heteroaralkyl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl.

16. The method of claim 15, wherein L represents CH_2CH_2.

17. The method of claim 15 or 16, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl or heteroaryl.

18. The method of claim 17, wherein R_8 represents substituted or unsubstituted aryl.

19. The method of any of claims 15-18, wherein R_{10} represents hydroxy, hydroxyalkyl or alkoxy.

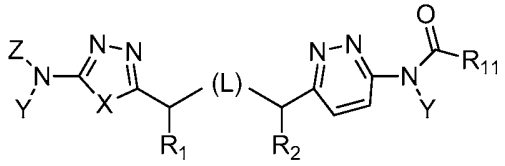
20. The method of claim 19, wherein R_{10} represents hydroxyalkyl.

21. The method of any one of claims 15-20, wherein each occurrence of R_3 is identical.

22. The method of claim 1, wherein L represents CH_2CH_2, Y represents H, X, independently for each occurrence, represents S or CH=CH, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and R_3, independently for each occurrence, represents arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl.

23. The method of claim 22, wherein each occurrence of R_3 is identical.

24. A method of treating or preventing cancer comprising administering a compound of formula Ia,



(Ia),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or , preferably CH_2CH_2, wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X represents S, O or CH=CH, preferably S or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, arylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy, preferably H;

R_3 represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H;

R_{11} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or $C(R_{12})(R_{13})(R_{14})$, $N(R_4)(R_{14})$ or OR_{14}, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_{12} and R_{13} each independently respresent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein both of R_{12} and R_{13} are not H; and

R_{14} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl.

25. The method of claim 24, wherein R_{11} represents substituted or unsubstituted arylalkyl.

26. The method of claim 25, wherein R_{11} represents substituted or unsubstituted benzyl.

27. The method of claim any of claims 24-26, wherein L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2.

28. The method of claim 27, wherein L represents CH_2CH_2.

29. The method of any of claims 24-28, wherein each Y represents H.

30. The method of any of claims 24-29, wherein X represents S or CH=CH.

31. The method of claim 30, wherein X represents S.

32. The method of any of claims 24-31, wherein Z represents $R_3(CO)$.

33. The method of claim 32, wherein R_3 and R_{11} are not identical.

34. The method of any of claims 24-33, wherein R_1 and R_2 each represent H.

35. The method of claim 32, wherein R_3 represents substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl.

36. The method of claim 35, wherein R_3 represents substituted or unsubstituted heteroarylalkyl.

37. The method of claim 32, wherein R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl, heteroaryl or heteroaralkyl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl.

38. The method of claim 37, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl, or heteroaryl.

39. The method of claim 37 or 38, wherein R_{10} represents hydroxy, hydroxyalkyl, or alkoxy.

40. The method of claim 24, wherein L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, R_3 represents substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl, and R_{11} represents substituted or unsubstituted arylalkyl.

41. The method of claim 40, wherein R_3 represents substituted or unsubstituted heteroarylalkyl.

42. The method of claim 24, wherein L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl, heteroaryl or heteroaralkyl, R_9 represents H, R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl, and R_{11} represents substituted or unsubstituted arylalkyl.

43. The method of claim 42, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl or heteroaryl.

44. The method of claim 43, wherein R_8 represents heteroaryl.

45. The method of any of claims 42-44, wherein R_{10} represents hydroxy, hydroxyalkyl or alkoxy.

46. The method of claim 24, wherein L represents CH_2CH_2, Y represents H, X represents S or CH=CH, Z represents $R_3(CO)$, R_1 and R_2 each represent H, R_3 represents substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl, and R_{11} represents substituted or unsubstituted arylalkyl.

47. The method of claim 46, wherein R_3 represents substituted or unsubstituted heteroarylalkyl.

48. The method of claim 24, wherein L represents CH_2CH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl or heteroaryl, R_9 represents H, R_{10} represents hydroxy, hydroxyalkyl or alkoxy, and R_{11} represents substituted or unsubstituted arylalkyl.

49. The method of any preceding claim, wherein the cancer is selected from breast cancer, colorectal cancer, endocrine cancer, lung cancer, melanoma, mesothelioma, renal cancer and a B cell malignancy.

50. The method of claim 49, wherein the cancer is breast cancer.

51. The method of claim 50, wherein the breast cancer comprises basal-type breast cancer cells, triple-negative breast cancer cells or claudin-low breast cancer cells.

52. The method of claim 51, wherein the breast cancer comprises basal-type breast cancer cells.

53. The method of claim 51, wherein the breast cancer comprises triple-negative breast cancer cells.

54. The method of claim 51, wherein the breast cancer comprises claudin-low breast cancer cells.

55. The method of claim 49, wherein the cancer is colorectal cancer.

56. The method of claim 49, wherein the cancer is endocrine cancer.

57. The method of claim 56, wherein the endocrine cancer is selected from adrenal cortex adenoma, adrenal cortex carcicnoma, adrenal gland pheochromocytoma and parathyroid gland adenoma.

58. The method of claim 49, wherein the cancer is melanoma.

59. The method of claim 49, wherein the cancer is renal cancer.

60. The method of claim 49, wherein the cancer is a B cell malignancy.

61. The method of claim 60, wherein the B cell malignancy is selected from multiple myeloma, leukemia and lymphoma.

62. The method of claim 61, wherein the B cell malignancy is multiple myeloma.

63. The method of claim 61, wherein the B cell malignancy is leukemia.

64. The method of claim 63, wherein the leukemia is selected from acute lymphoblastic leukemia and chronic lymphoblastic leukemia.

65. The method of claim 61, wherein the B cell malignancy is lymphoma.

66. The method of claim 65, wherein the lymphoma is selected from Burkitt's lymphoma, Diffuse large B cell lymphoma, follicular lymphoma and Hodgkin's lymphoma.

67. The method of any preceding claim, further comprising conjointly administering one or more additional chemotherapeutic agents.

68. The method of claim 67, wherein conjointly administering one or more additional chemotherapeutic agents provide improved efficacy relative to each individual administration of the compound of formula I or the one or more additional chemotherapeutic agent.

69. The method of claim 68, wherein conjointly administering one or more additional chemotherapeutic agents provide a synergistic effect.

70. The method of claim 69, wherein conjointly administering one or more additional chemotherapeutic agents provide an additive effect.

71. The method of any of claims 67-70, wherein the compound of formula I and the one or more additional chemotherapeutic agents are administered simultaneously.

72. The method of any of claims 67-70, wherein the one or more additional chemotherapeutic agents are administered within about 5 minutes to within about 168 hours prior to or after administration of the compound of formula I.

73. The method of any of claims 67-72, wherein the one or more additional chemotherapeutic agents are selected from ABT-263, aminoglutethimide, amsacrine, anastrozole, asparaginase, bcg, bicalutamide, bleomycin, bortezomib, buserelin, busulfan, campothecin, capecitabine, carboplatin, carfilzomib, carmustine, chlorambucil, chloroquine, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, demethoxyviridin, dexamethasone, dichloroacetate, dienestrol, diethylstilbestrol, docetaxel, doxorubicin, epirubicin, estradiol, estramustine, etoposide, everolimus, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil and 5-fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan,

ironotecan, lenalidomide, letrozole, leucovorin, leuprolide, levamisole, lomustine, lonidamine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, metformin, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide, oxaliplatin, paclitaxel, pamidronate, pentostatin, perifosine, PF-04691502, plicamycin, pomalidomide, porfimer, procarbazine, raltitrexed, rituximab, romidepsin, sorafenib, streptozocin, sunitinib, suramin, tamoxifen, temozolomide, temsirolimus, teniposide, testosterone, thalidomide, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, vinorelbine, and vorinostat (SAHA).

74. The method of claim 73, wherein the one or more additional chemotherapeutic agents are selected from bortezomib, capecitabine, carboplatin, carfilzomib, cyclophosphamide, daunorubicin, dexamethasone, docetaxel, doxorubicin, epirubicin, eribulin, fluorouracil, gemcitabine, ixabepilone, lenalidomide, methotrexate, mitoxantrone, mutamycin, paclitaxel, pomalidomide, rituximab, thalidomide, thiotepa, vincristine, and vinorelbine.

75. The method of claim 74, wherein the one or more additional chemotherapeutic agents are selected from bortezomib, carfilzomib, dexamethasone, doxorubicin, lenalidomide, pacilitaxel, pomalidomide, thalidomide, and rituximab.

76. A method of identifying a cancer patient that may benefit from treatment with a glutaminase inhibitor comprising determining the ratio of glutamate to glutamine in cancer cells of the cancer patient, wherein a ratio greater than or equal to 1.5 indicates the patient may benefit from treatment with a glutaminase inhibitor.

77. The method of claim 76, wherein the ratio is greater than or equal to 2.0.

78. The method of claim 76 or 77, wherein the method of determining the ratio includes measuring levels of glutamate and glutamine in the cancer cells of the cancer patient.

79. The method of any of claims 76-78, wherein the cancer is selected from a B cell malignancy, breast cancer, colorectal cancer, endocrine cancer, lung cancer, melanoma, mesothelioma and renal cancer.

80. The method of claim 79, wherein the cancer is breast cancer.

81. The method of claim 80, wherein the breast cancer comprises basal-type breast cancer cells, triple-negative breast cancer cells or claudin-low breast cancer cells.

82. The method of claim 81, wherein the breast cancer comprises basal-type breast cancer cells.

83. The method of claim 81, wherein the breast cancer comprises triple-negative breast cancer cells.

84. The method of claim 81, wherein the breast cancer comprises claudin-low breast cancer cells.

85. The method of claim 79, wherein the cancer is colorectal cancer.

86. The method of claim 79, wherein the cancer is endocrine cancer.

87. The method of claim 86, wherein the endocrine cancer is selected from adrenal cortex adenoma, adrenal cortex carcicnoma, adrenal gland pheochromocytoma and parathyroid gland adenoma.

88. The method of claim 79, wherein the cancer is melanoma.

89. The method of claim 79, wherein the cancer is renal cancer.

90. The method of claim 79, wherein the cancer is a B cell malignancy.

91. The method of claim 90, wherein the B cell malignancy is selected from multiple myeloma, leukemia and lymphoma.

92. The method of claim 91, wherein the B cell malignancy is multiple myeloma.

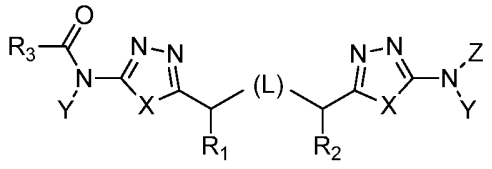
93. The method of claim 91, wherein the B cell malignancy is leukemia.

94. The method of claim 93, wherein the leukemia is selected from acute lymphoblastic leukemia and chronic lymphoblastic leukemia.

95. The method of claim 91, wherein the B cell malignancy is lymphoma.

96. The method of claim 95, wherein the lymphoma is selected from Burkitt's lymphoma, Diffuse large B cell lymphoma, follicular lymphoma and Hodgkin's lymphoma.

97. A method of treating a cancer patient comprising 1) determining the ratio of glutamate to glutamine in cancer cells of the cancer patient; and 2) if the ratio of glutamate to glutamine is greater than or equal to 1.5, administering a compound of formula I,



(I),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or , wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X, independently for each occurrence, represents S, O or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy;

R_3, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

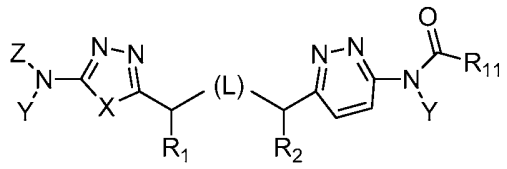
R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H.

98. The method of claim 97, wherein the compound is a compound as described in any of claims 2-23.

99. A method of treating a cancer patient comprising 1) determining the ratio of glutamate to glutamine in cancer cells of the cancer patient; and 2) if the ratio of glutamate to glutamine is greater than or equal to 1.5, administering a compound of formula Ia,



(Ia),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or , preferably CH_2CH_2, wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X represents S, O or CH=CH, preferably S or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, arylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy, preferably H;

R_3 represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or

heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H;

R_{11} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or $C(R_{12})(R_{13})(R_{14})$, $N(R_4)(R_{14})$ or OR_{14}, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_{12} and R_{13} each independently respresent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein both of R_{12} and R_{13} are not H; and

R_{14} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl.

100. The method of claim 99, wherein the compound is a compound as described in any of claims 25-48.

101. The method of any of claims 97-100, wherein the ratio is greater than or equal to 2.0.

102. The method of any of claims 97-101, wherein the method of determining the ratio includes measuring levels of glutamate and glutamine in the cancer cells of the cancer patient.

103. The method of any of claims 97-102, wherein the cancer is selected from a B cell malignancy, breast cancer, colorectal cancer, endocrine cancer, lung cancer, melanoma, mesothelioma and renal cancer.

104. The method of claim 103, wherein the cancer is breast cancer.

105. The method of claim 104, wherein the breast cancer comprises basal-type breast cancer cells, triple-negative breast cancer cells or claudin-low breast cancer cells.

106. The method of claim 105, wherein the breast cancer comprises basal-type breast cancer cells.

107. The method of claim 105, wherein the breast cancer comprises triple-negative breast cancer cells.

108. The method of claim 105, wherein the breast cancer comprises claudin-low breast cancer cells.

109. The method of claim 103, wherein the cancer is colorectal cancer.

110. The method of claim 103, wherein the cancer is endocrine cancer.

111. The method of claim 110, wherein the endocrine cancer is selected from adrenal cortex adenoma, adrenal cortex carcicnoma, adrenal gland pheochromocytoma and parathyroid gland adenoma.

112. The method of claim 103, wherein the cancer is melanoma.

113. The method of claim 103, wherein the cancer is renal cancer.

114. The method of claim 103, wherein the cancer is a B cell malignancy.

115. The method of claim 114, wherein the B cell malignancy is selected from multiple myeloma, leukemia and lymphoma.

116. The method of claim 115, wherein the B cell malignancy is multiple myeloma.

117. The method of claim 115, wherein the B cell malignancy is leukemia.

118. The method of claim 117, wherein the leukemia is selected from acute lymphoblastic leukemia and chronic lymphoblastic leukemia.

119. The method of claim 115, wherein the B cell malignancy is lymphoma.

120. The method of claim 119, wherein the lymphoma is selected from Burkitt's lymphoma, Diffuse large B cell lymphoma, follicular lymphoma and Hodgkin's lymphoma.

121. A method of identifying a cancer patient that may benefit from treatment with a glutaminase inhibitor, comprising determining the ratio of GLS:GS ratio in cancer cells of the cancer patient, wherein a ratio greater than or equal to 0.05 indicates the patient may benefit from treatment with a glutaminase inhibitor.

122. The method of claim any one of claims 121, wherein the ratio is greater than or equal to 1.

123. The method of claim 121 or 122, wherein the method of determining the ratio comprises measuring levels of GLS and GS in the cancer cells of the cancer patient.

124. The method of claim 123, wherein measuring the levels of GLS and GS comprises measuring mRNA amounts.

125. The method of claims 123, wherein measuring the levels of GLS and GS comprises measuring protein amounts.

126. The method of any one of claims 121-125, wherein GLS is GAC.

127. The method of any one of claims 121-125, wherein GLS is KGA.

128. The method of any one of claims 121-125, wherein GLS is both GAC and KGA.

129. The method of any of claims 121-128, wherein the cancer is selected from a B cell malignancy, breast cancer, colorectal cancer, endocrine cancer, lung cancer, melanoma, mesothelioma and renal cancer.

130. The method of claim 129, wherein the cancer is breast cancer.

131. The method of claim 130, wherein the breast cancer comprises basal-type breast cancer cells, triple-negative breast cancer cells or claudin-low breast cancer cells.

132. The method of claim 130, wherein the breast cancer comprises basal-type breast cancer cells.

133. The method of claim 130, wherein the breast cancer comprises triple-negative breast cancer cells.

134. The method of claim 130, wherein the breast cancer comprises claudin-low breast cancer cells.

135. The method of claim 129, wherein the cancer is colorectal cancer.

136. The method of claim 129, wherein the cancer is endocrine cancer.

137. The method of claim 136, wherein the endocrine cancer is selected from adrenal cortex adenoma, adrenal cortex carcicnoma, adrenal gland pheochromocytoma and parathyroid gland adenoma.

138. The method of claim 129, wherein the cancer is melanoma.

139. The method of claim 129, wherein the cancer is renal cancer.

140. The method of claim 129, wherein the cancer is a B cell malignancy.

141. The method of claim 140, wherein the B cell malignancy is selected from multiple myeloma, leukemia and lymphoma.

142. The method of claim 140, wherein the B cell malignancy is multiple myeloma.

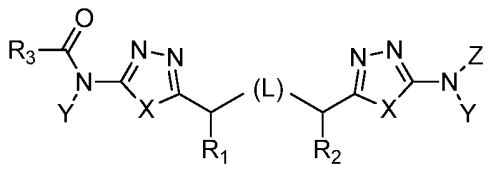
143. The method of claim 140, wherein the B cell malignancy is leukemia.

144. The method of claim 143, wherein the leukemia is selected from acute lymphoblastic leukemia and chronic lymphoblastic leukemia.

145. The method of claim 140, wherein the B cell malignancy is lymphoma.

146. The method of claim 145, wherein the lymphoma is selected from Burkitt's lymphoma, Diffuse large B cell lymphoma, follicular lymphoma and Hodgkin's lymphoma.

147. A method of treating a cancer patient comprising 1) determining the ratio of GLS to GS in cancer cells of the cancer patient; and 2) if the ratio of GSL to GS is greater than or equal to 0.05, administering a compound of formula I,



(I),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or , wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X, independently for each occurrence, represents S, O or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy;

R_3, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H.

148. The method of claim 147, wherein the compound is a compound as described in any of claims 2-23.

149. The method of claim any one of claims 147, wherein the ratio is greater than or equal to 1.

150. The method of any one of claims 147 or 149, wherein the method of determining the ratio comprises measuring the levels of GLS and GS in the cancer cells of the cancer patient.

151. The method of claim 150, wherein measuring the levels of GLS and GS comprises measuring mRNA amounts.

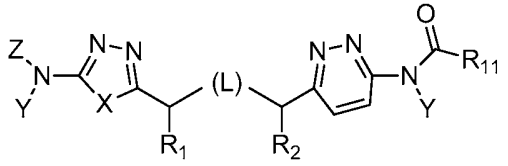
152. The method of claims 151, wherein measuring the levels of GLS and GS comprises measuring protein amounts.

153. The method of any one of claims 147-152, wherein GLS is GAC.

154. The method of any one of claims 147-152, wherein GLS is KGA.

155. The method of any one of claims 147-152, wherein GLS is both GAC and KGA.

156. A method of treating a cancer patient comprising 1) determining the ratio of GLS to GS in cancer cells of the cancer patient; and 2) if the ratio of GLS to GS is greater than or equal to 0.05, administering a compound of formula Ia,



(Ia),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or , preferably CH_2CH_2, wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X represents S, O or CH=CH, preferably S or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, arylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy, preferably H;

R_3 represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H;

R_{11} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or $C(R_{12})(R_{13})(R_{14})$, $N(R_4)(R_{14})$ or OR_{14}, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_{12} and R_{13} each independently respresent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein both of R_{12} and R_{13} are not H; and

R_{14} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl.

157. The method of claim 156, wherein the compound is a compound as described in any of claims 25-48.

158. The method of claim 156 or 157, wherein the ratio is greater than or equal to 1.

159. The method of any one of claims 156-158, wherein the method of determining the ratio comprises measuring the levels of GLS and GS in the cancer cells of the cancer patient.

160. The method of claim 159, wherein measuring the levels of GLS and GS comprises measuring mRNA amounts.

161. The method of claims 159, wherein measuring the levels of GLS and GS comprises measuring protein amounts.

162. The method of any one of claims 156-161, wherein GLS is GAC.

163. The method of any one of claims 156-161, wherein GLS is KGA.

164. The method of any one of claims 156-161, wherein GLS is both GAC and KGA.

165. The method of any of claims 156-164, wherein the cancer is selected from a B cell malignancy, breast cancer, colorectal cancer, endocrine cancer, lung cancer, melanoma, mesothelioma and renal cancer.

166. The method of claim 165, wherein the cancer is breast cancer.

167. The method of claim 166, wherein the breast cancer comprises basal-type breast cancer cells, triple-negative breast cancer cells or claudin-low breast cancer cells.

168. The method of claim 166, wherein the breast cancer comprises basal-type breast cancer cells.

169. The method of claim 166, wherein the breast cancer comprises triple-negative breast cancer cells.

170. The method of claim 166, wherein the breast cancer comprises claudin-low breast cancer cells.

171. The method of claim 165, wherein the cancer is colorectal cancer.

172. The method of claim 165, wherein the cancer is endocrine cancer.

173. The method of claim 172, wherein the endocrine cancer is selected from adrenal cortex adenoma, adrenal cortex carcicnoma, adrenal gland pheochromocytoma and parathyroid gland adenoma.

174. The method of claim 165, wherein the cancer is melanoma.

175. The method of claim 165, wherein the cancer is renal cancer.

176. The method of claim 165, wherein the cancer is a B cell malignancy.

177. The method of claim 176, wherein the B cell malignancy is selected from multiple myeloma, leukemia and lymphoma.

178. The method of claim 176, wherein the B cell malignancy is multiple myeloma.

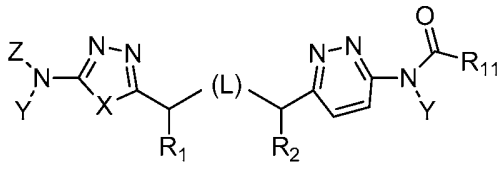
179. The method of claim 176, wherein the B cell malignancy is leukemia.

180. The method of claim 179, wherein the leukemia is selected from acute lymphoblastic leukemia and chronic lymphoblastic leukemia.

181. The method of claim 176, wherein the B cell malignancy is lymphoma.

182. The method of claim 181, wherein the lymphoma is selected from Burkitt's lymphoma, Diffuse large B cell lymphoma, follicular lymphoma and Hodgkin's lymphoma.

183. A method of treating a cancer patient comprising 1) determining glutaminase activity in cancer cells of the cancer patient; and 2) if the activity is greater than or equal to 0.005 μmol/min/mg of protein, administering a compound of formula Ia,



(Ia),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or , preferably CH_2CH_2, wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X represents S, O or CH=CH, preferably S or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, arylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy, preferably H;

R_3 represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl,

heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H;

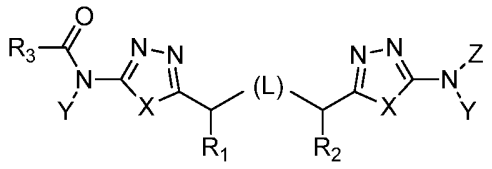
R_{11} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or $C(R_{12})(R_{13})(R_{14})$, $N(R_4)(R_{14})$ or OR_{14}, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_{12} and R_{13} each independently respresent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free

hydroxyl group may be acylated to form $C(O)R_7$, and wherein both of R_{12} and R_{13} are not H; and

R_{14} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl.

184. A method of treating a cancer patient comprising 1) 1) determining glutaminase activity in cancer cells of the cancer patient; and 2) if the activity is greater than or equal to 0.005 μmol/min/mg of protein, administering a compound of formula I,



(I),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or , wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X, independently for each occurrence, represents S, O or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy;

R_3, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy,

heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H.

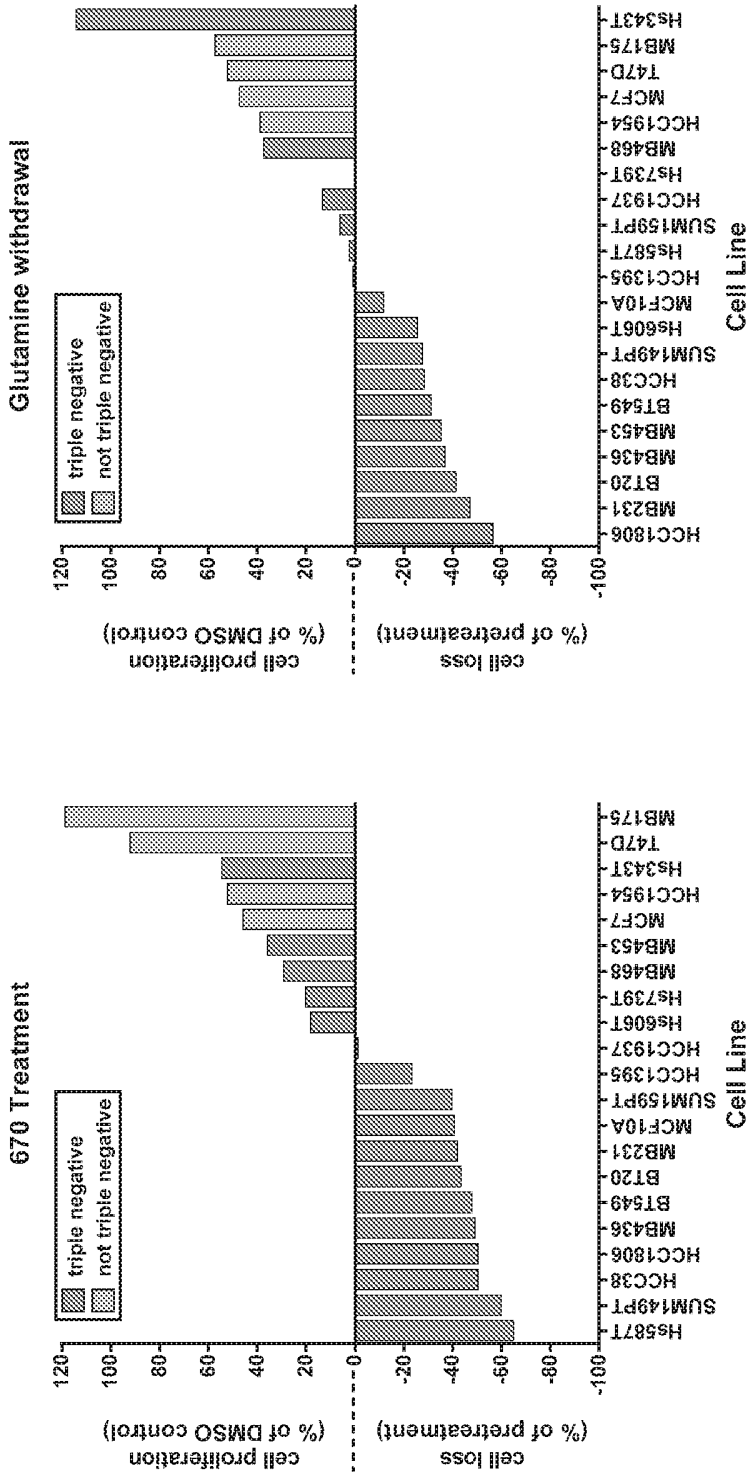

Glutamine withdrawal
triple negative
not triple negative
cell loss
(% of pretreatment)
cell proliferation
(% of DMSO control)
Cell Line
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
HCC1806
MB231
BT20
MB436
MB453
BT549
HCC38
SUM149PT
Hs606T
MCF10A
HCC1395
SUM159PT
Hs587T
HCC1937
MCF7
HCC1954
MB468
Hs739T
Hs343T
T47D
MB175
670 Treatment
triple negative
not triple negative
cell loss
(% of pretreatment)
cell proliferation
(% of DMSO control)
Cell Line
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
Hs587T
SUM149PT
HCC38
HCC1806
MB436
BT549
BT20
MB231
MCF10A
SUM159PT
HCC1395
HCC1937
Hs606T
Hs739T
MB468
MB453
MCF7
HCC1954
Hs343T
T47D
MB175

Figure 1

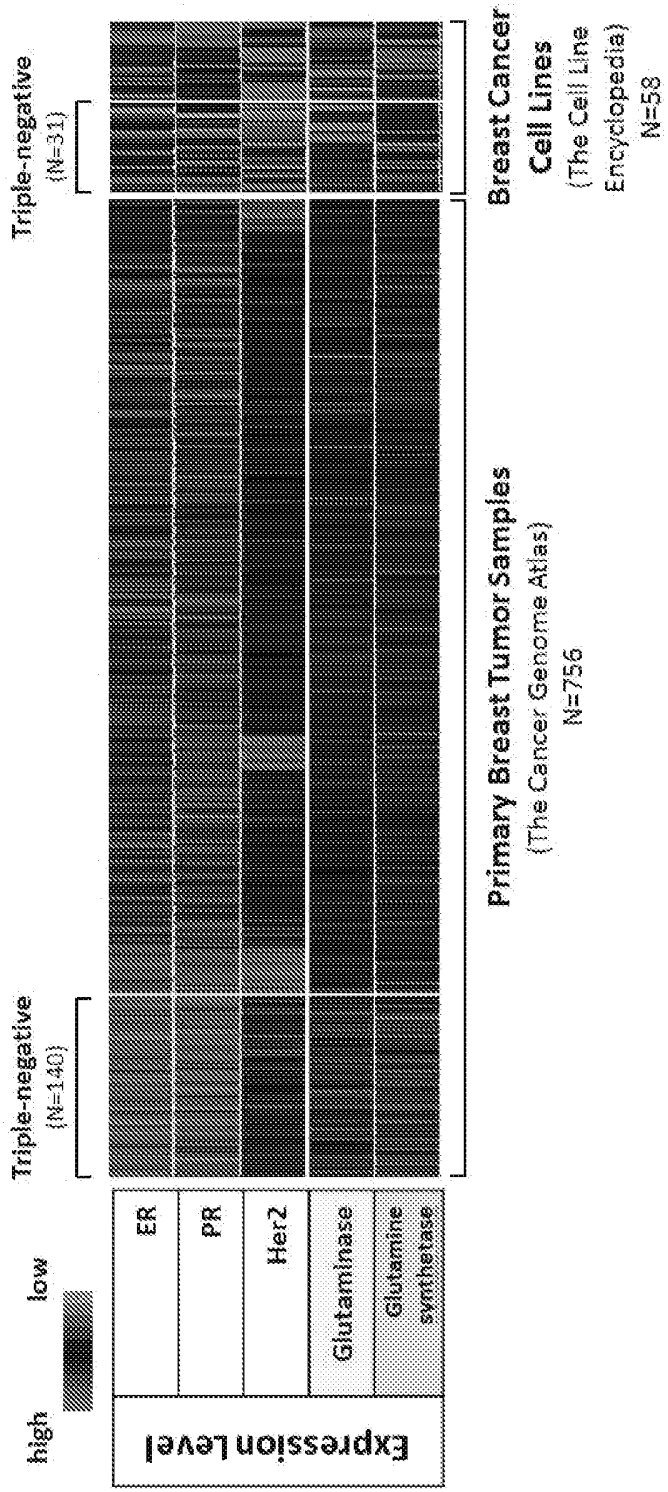

Expression Level
high
low
ER
PR
Her2
Glutaminase
Glutamine synthetase
Triple-negative (N=140)
Triple-negative (N=31)
Primary Breast Tumor Samples (The Cancer Genome Atlas) N=756
Breast Cancer Cell Lines (The Cell Line Encyclopedia) N=58

Figure 2

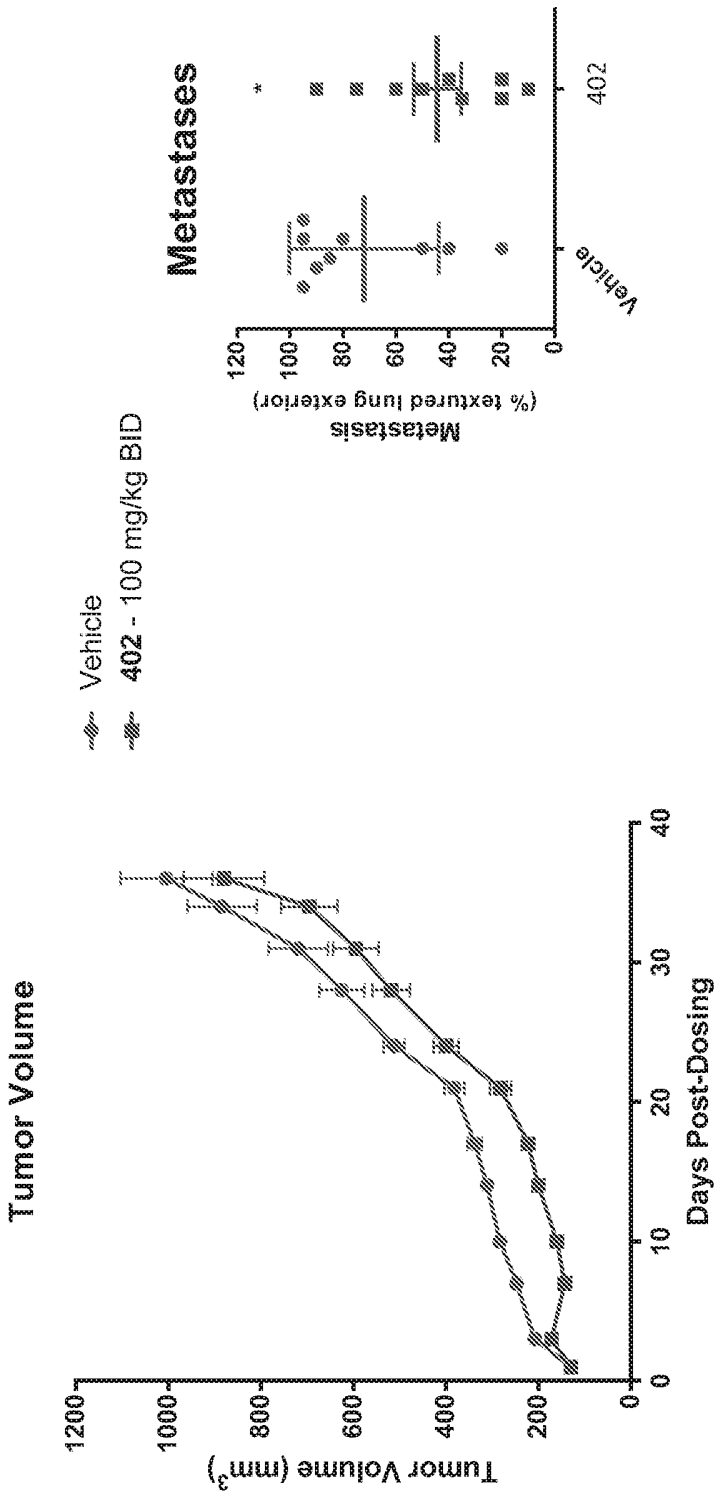

Tumor Volume
Vehicle
402 - 100 mg/kg BID
Tumor Volume (mm³)
1200
1000
800
600
400
200
0
0
10
20
30
40
Days Post-Dosing
Metastases
Metastasis
(% textured lung exterior)
120
100
80
60
40
20
0
*
Vehicle
402

Figure 3

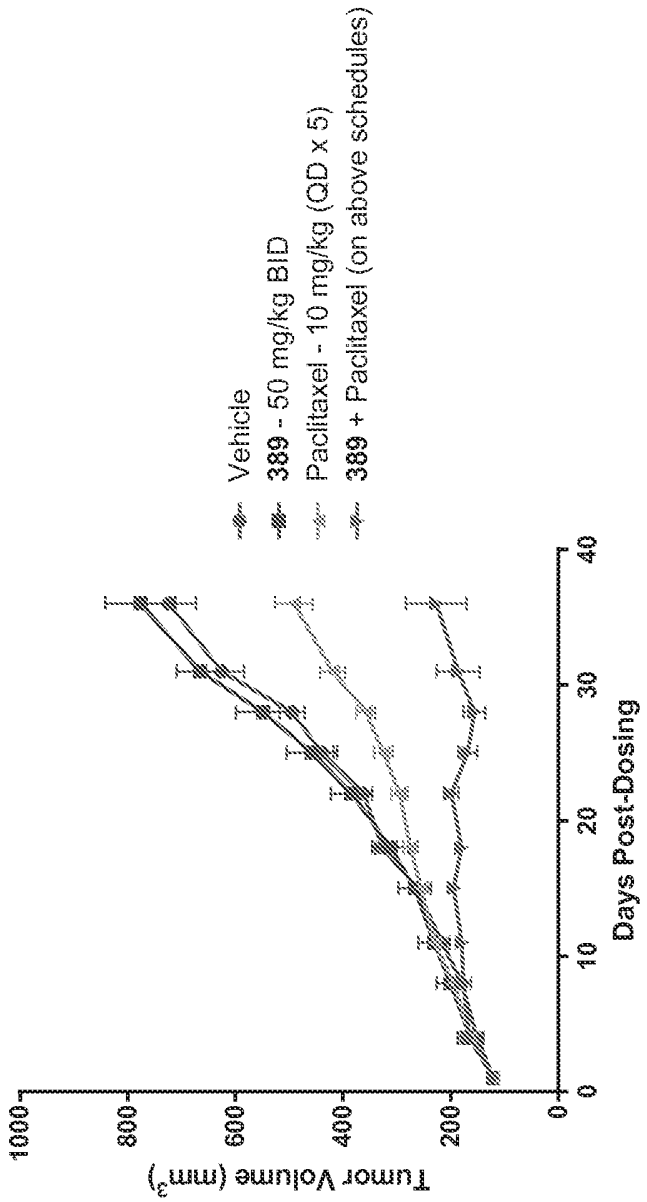

Vehicle
389 - 50 mg/kg BID
Paclitaxel - 10 mg/kg (QD x 5)
389 + Paclitaxel (on above schedules)
Tumor Volume (mm³)
1000
800
600
400
200
0
0
10
20
30
40
Days Post-Dosing

Figure 4

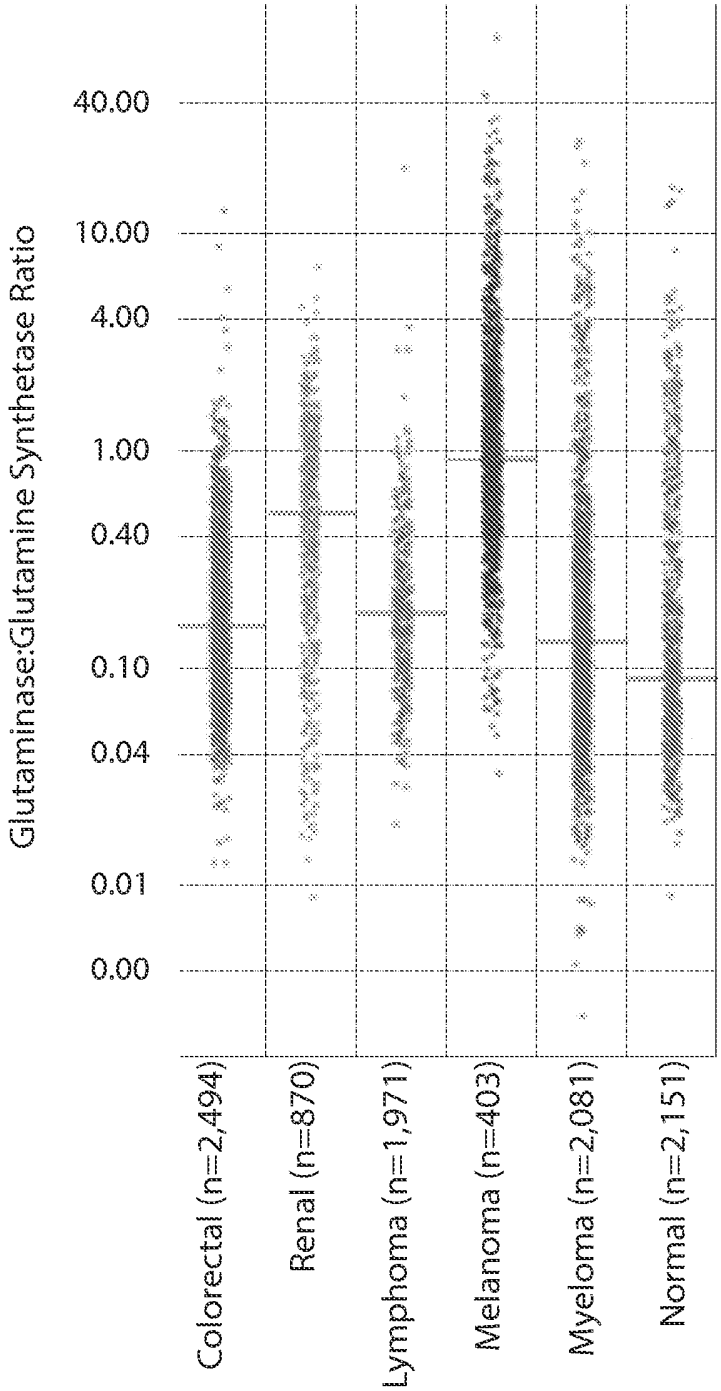

Glutaminase:Glutamine Synthetase Ratio
40.00
10.00
4.00
1.00
0.40
0.10
0.04
0.01
0.00
Colorectal (n=2,494)
Renal (n=870)
Lymphoma (n=1,971)
Melanoma (n=403)
Myeloma (n=2,081)
Normal (n=2,151)

Figure 5

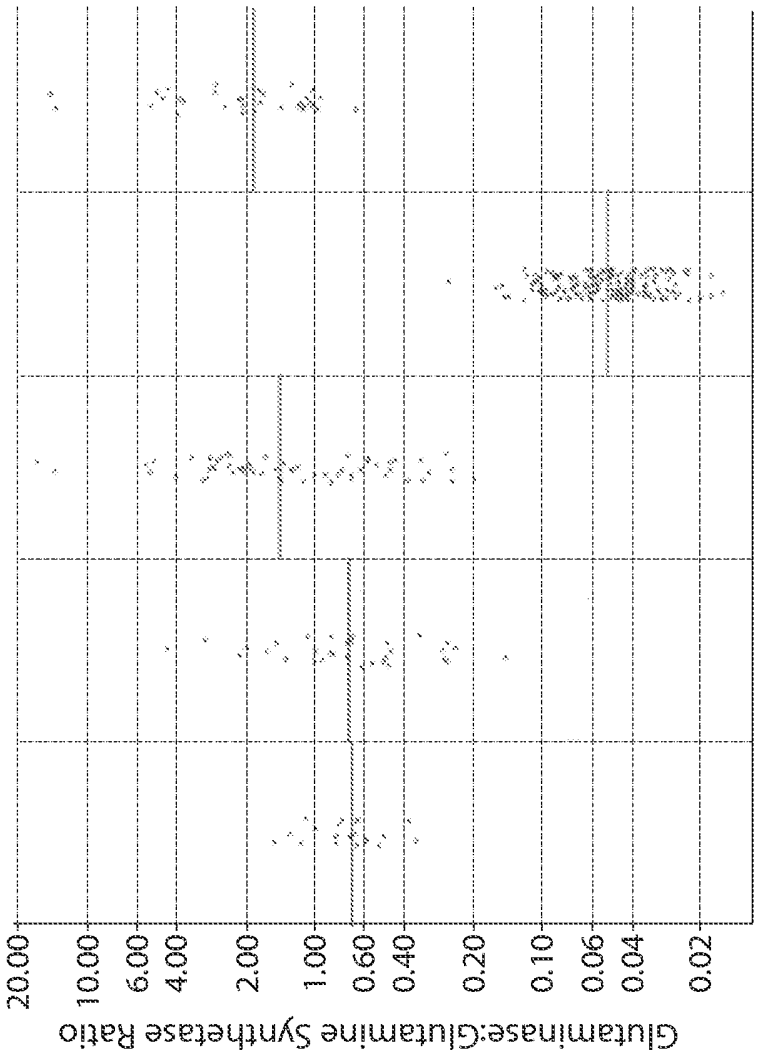

Glutaminase:Glutamine Synthetase Ratio
20.00
10.00
6.00
4.00
2.00
1.00
0.60
0.40
0.20
0.10
0.06
0.04
0.02
Adrenal cortex adenoma (n=22)
Adrenal cortex carcinoma (n=33)
Adrenal gland pheochromocytoma (n=33)
Endometrial endometrioid adenocarcinoma (n=298)
Parathyroid gland adenoma (n=19)

Figure 6

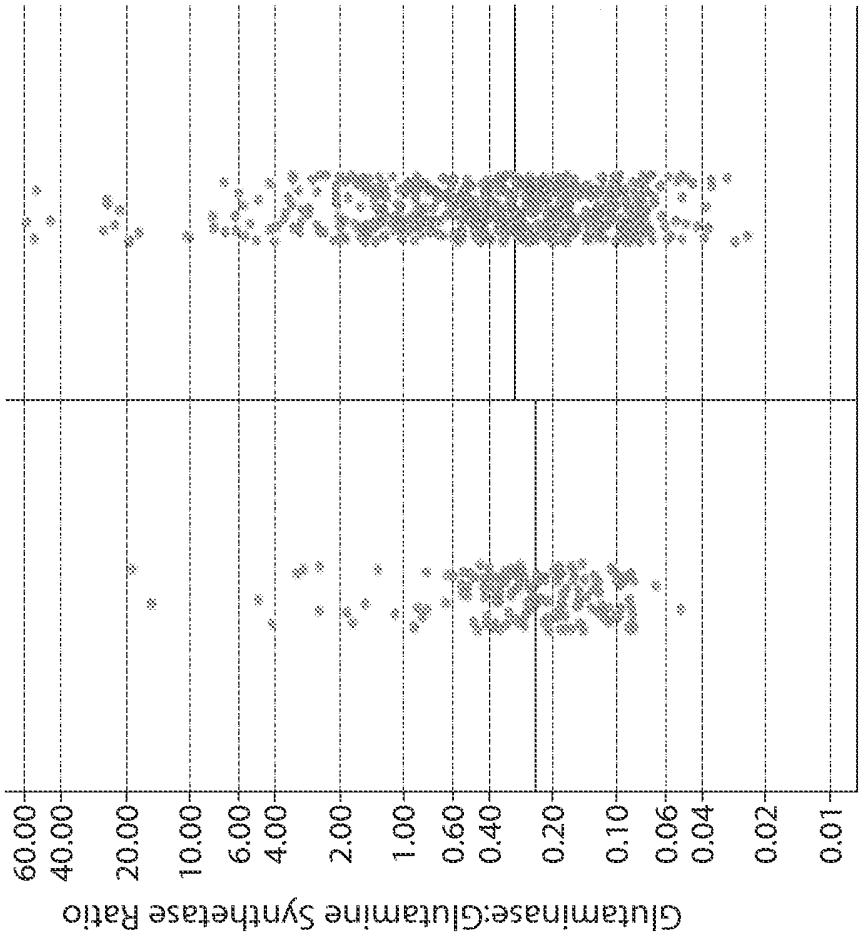

Glutaminase:Glutamine Synthetase Ratio
60.00
40.00
20.00
10.00
6.00
4.00
2.00
1.00
0.60
0.40
0.20
0.10
0.06
0.04
0.02
0.01
Acute lymphoblastic leukemia (n=161)
Chronic lymphocytic leukemia (n=588)

Figure 7

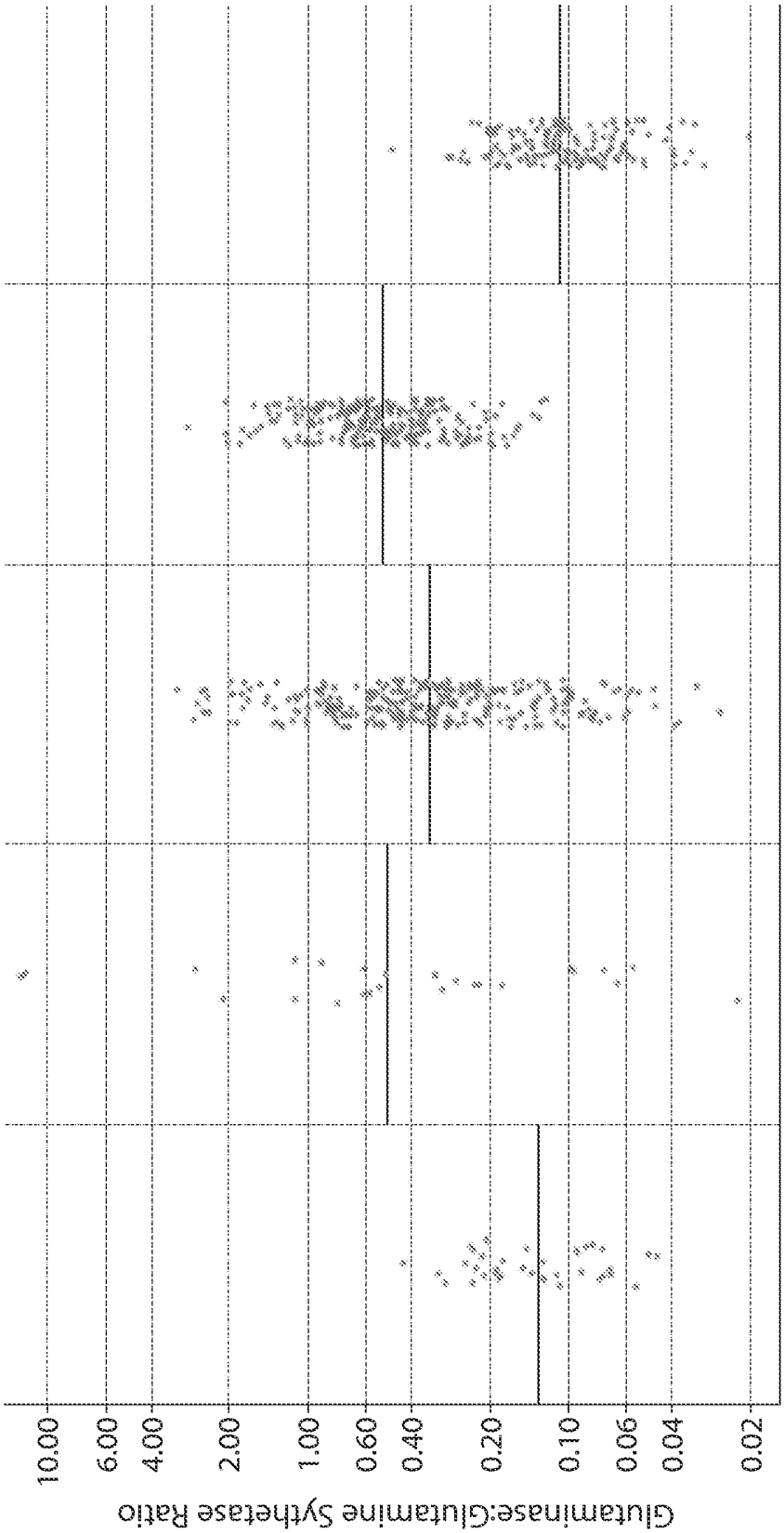

Glutaminase:Glutamine Sythetase Ratio
10.00
6.00
4.00
2.00
1.00
0.60
0.40
0.20
0.10
0.06
0.04
0.02
Anaplastic large cell lymphoma (n=38)
Burkitt's lymphoma (n=25)
Diffuse large B-cell lymphoma (n=294)
Follicular Lymphoma (n=242)
Hodgkin's lymphoma (n=146)

Figure 8

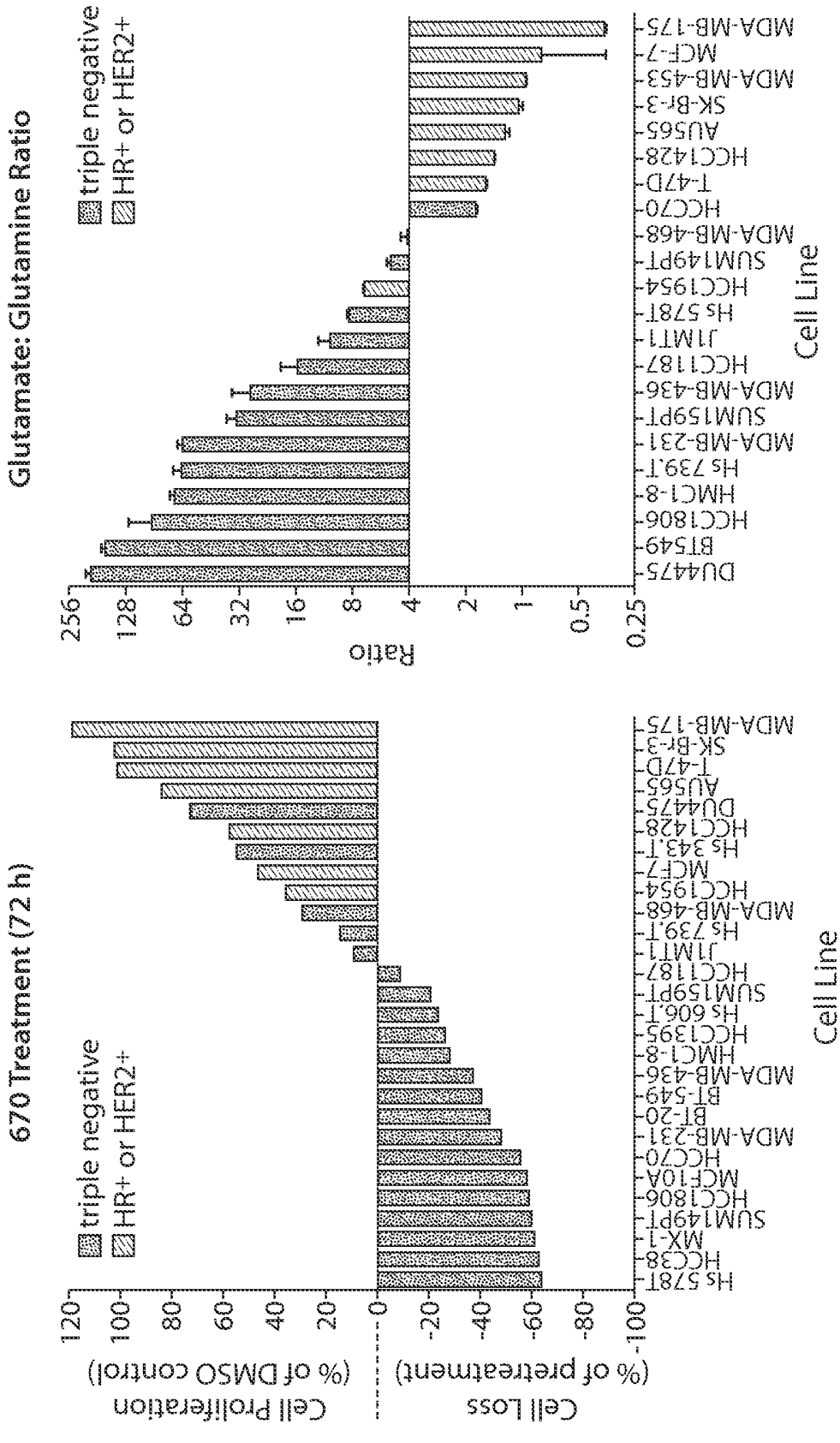

Glutamate: Glutamine Ratio
triple negative
HR+ or HER2+
Ratio
256
128
64
32
16
8
4
2
1
0.5
0.25
DU4475
BT549
HCC1806
HMC1-8
Hs 739.T
MDA-MB-231
SUM159PT
MDA-MB-436
HCC1187
J1MT1
Hs 578T
HCC1954
SUM149PT
MDA-MB-468
HCC70
T-47D
HCC1428
AU565
SK-Br-3
MDA-MB-453
MCF-7
MDA-MB-175
Cell Line
670 Treatment (72 h)
triple negative
HR+ or HER2+
Cell Proliferation
(% of DMSO control)
120
100
80
60
40
20
0
Cell Loss
(% of pretreatment)
-20
-40
-60
-80
-100
Hs 578T
HCC38
MX-1
SUM149PT
HCC1806
MCF10A
HCC70
MDA-MB-231
BT-20
BT-549
MDA-MB-436
HMC1-8
HCC1395
Hs 606.T
SUM159PT
HCC1187
J1MT1
Hs 739.T
MDA-MB-468
HCC1954
MCF7
Hs 343.T
HCC1428
DU4475
AU565
T-47D
SK-Br-3
MDA-MB-175
Cell Line

Figure 9

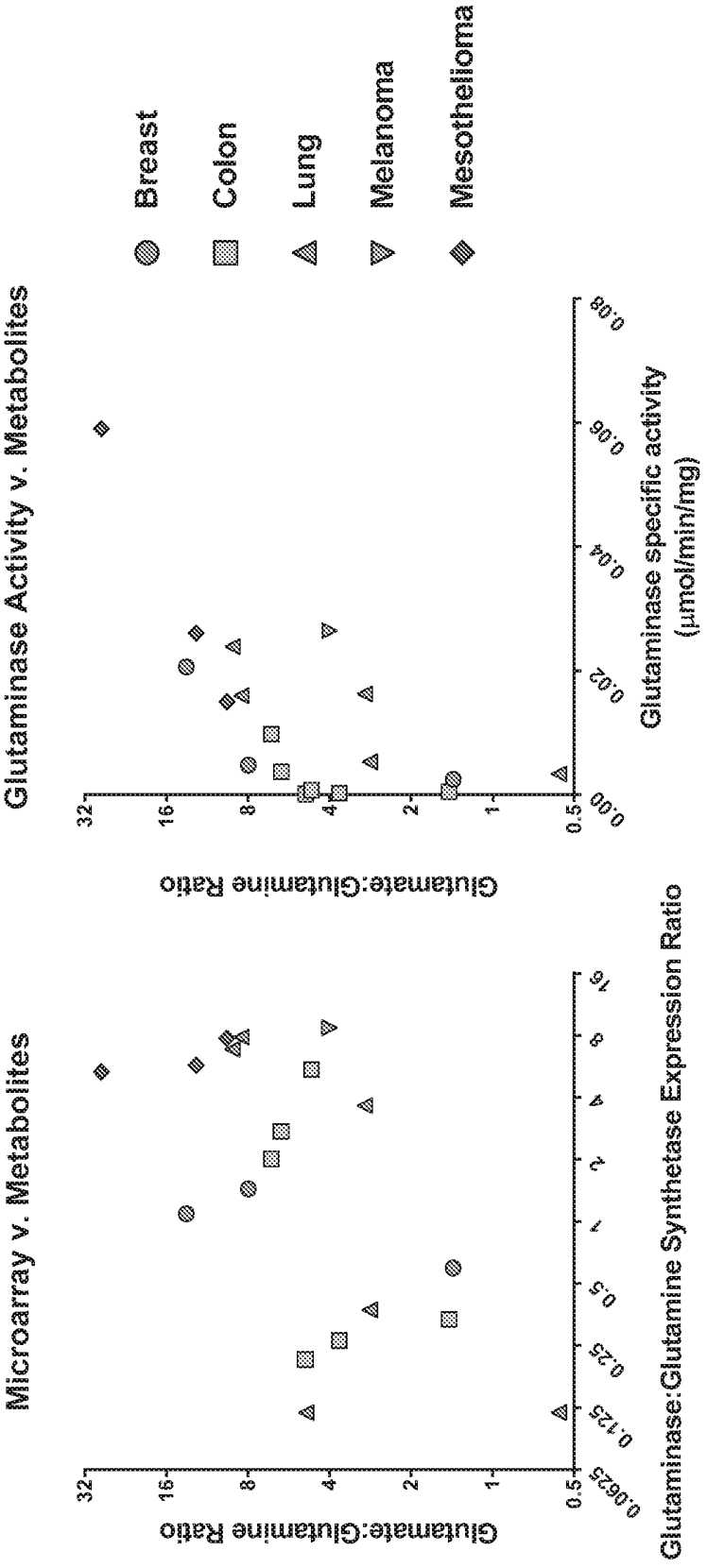

Glutaminase Activity v. Metabolites
Breast
Colon
Lung
Melanoma
Mesothelioma
Glutamate:Glutamine Ratio
32
16
8
4
2
1
0.5
0.00
0.02
0.04
0.06
0.08
Glutaminase specific activity
(µmol/min/mg)
Microarray v. Metabolites
Glutamate:Glutamine Ratio
Glutaminase:Glutamine Synthetase Expression Ratio
0.0625
0.125
0.25
0.5
1
2
4
8
16

Figure 10

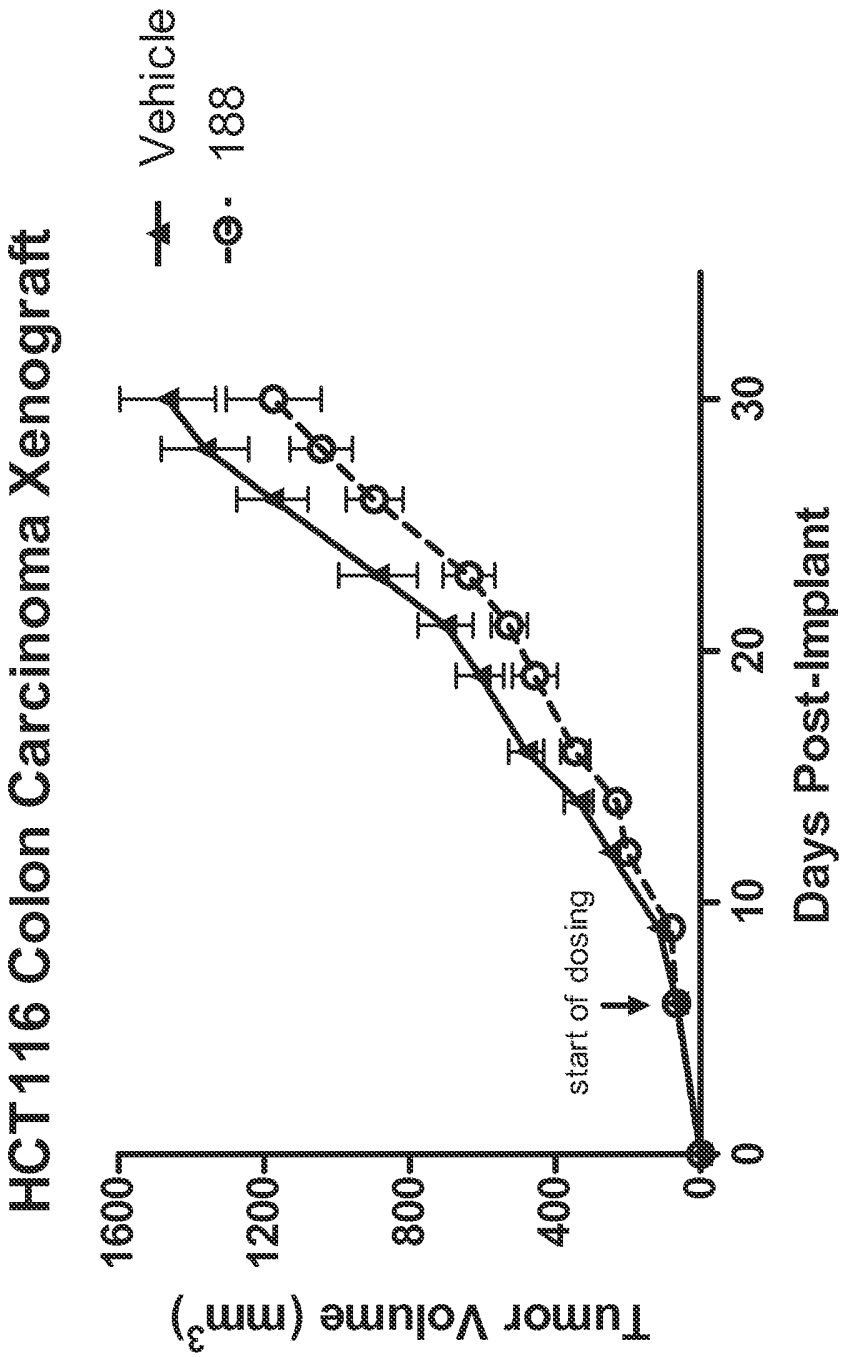

HCT116 Colon Carcinoma Xenograft
Vehicle
188
Tumor Volume (mm³)
1600
1200
800
400
0
start of dosing
0
10
20
30
Days Post-Implant

Figure 11

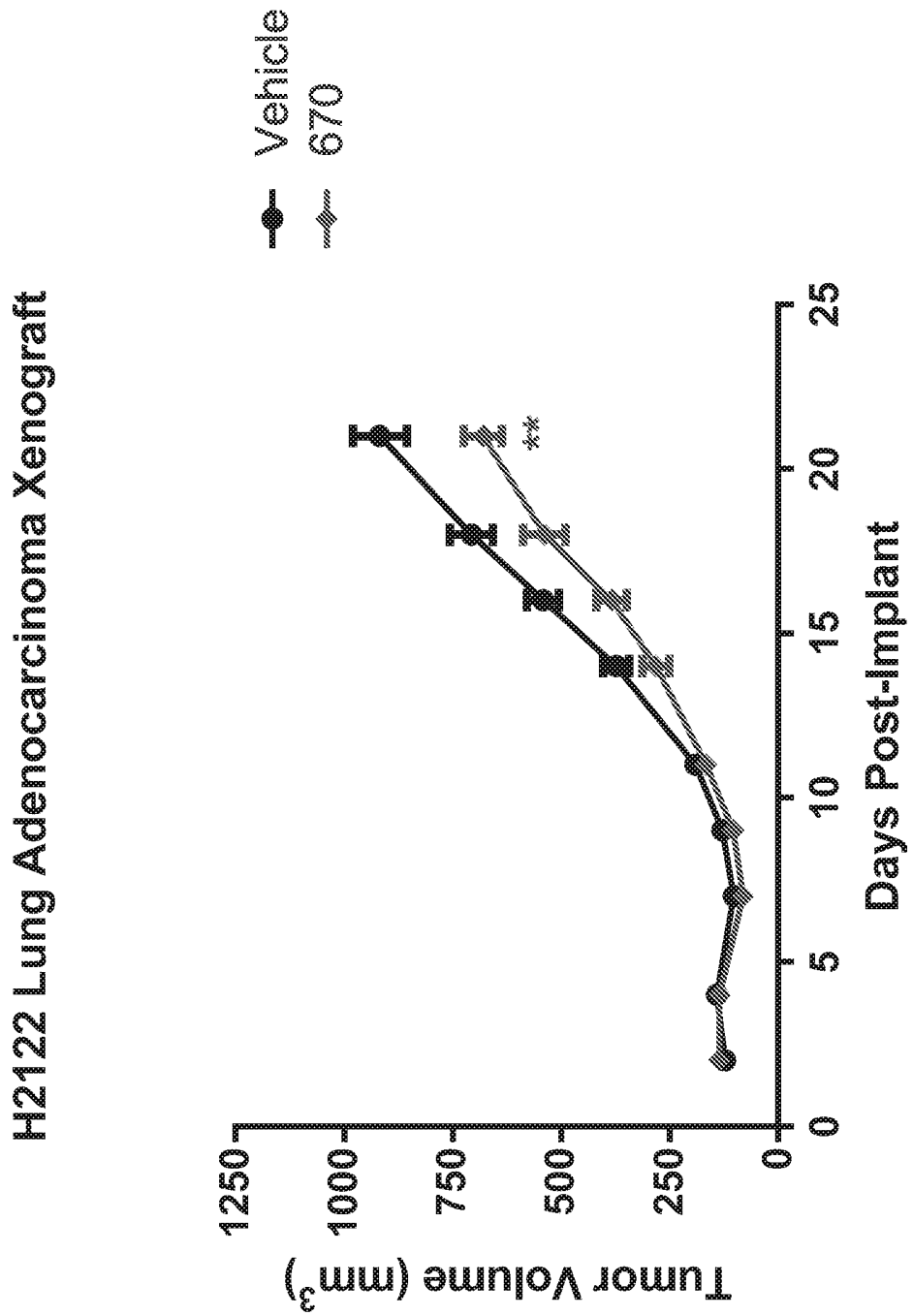

H2122 Lung Adenocarcinoma Xenograft
Vehicle
670
**
Tumor Volume (mm3)
1250
1000
750
500
250
0
0
5
10
15
20
25
Days Post-Implant

Figure 12

Figure 13

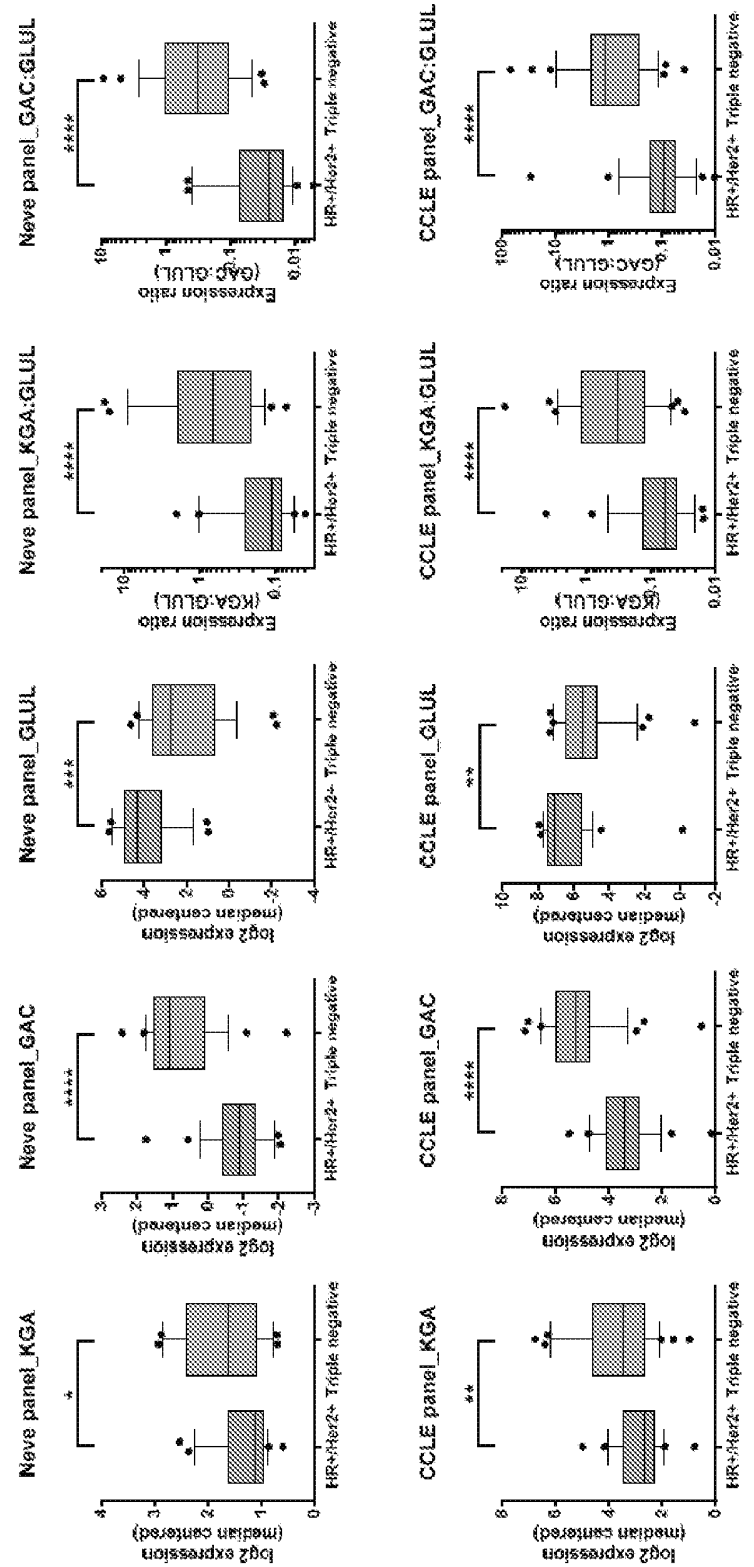
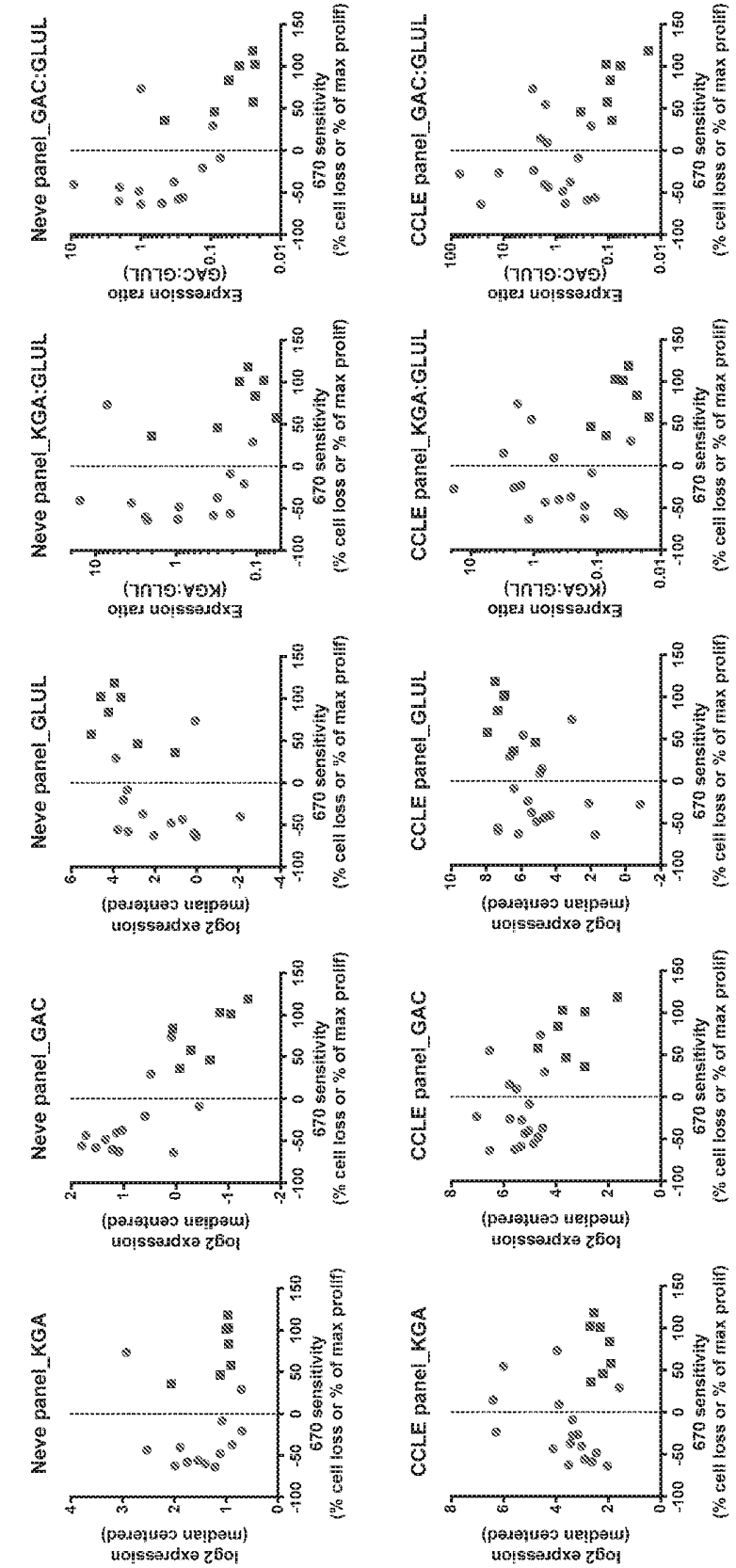

Neve panel_KGA
Neve panel_GAC
Neve panel_GLUL
Neve panel_KGA:GLUL
Neve panel_GAC:GLUL
CCLE panel_KGA
CCLE panel_GAC
CCLE panel_GLUL
CCLE panel_KGA:GLUL
CCLE panel_GAC:GLUL
log2 expression (median centered)
Expression ratio (KGA:GLUL)
Expression ratio (GAC:GLUL)
HR+/Her2+
Triple negative

Figure 14


Neve panel_KGA
Neve panel_GAC
Neve panel_GLUL
Neve panel_KGA:GLUL
Neve panel_GAC:GLUL
CCLE panel_KGA
CCLE panel_GAC
CCLE panel_GLUL
CCLE panel_KGA:GLUL
CCLE panel_GAC:GLUL
log2 expression (median centered)
Expression ratio (KGA:GLUL)
Expression ratio (GAC:GLUL)
670 sensitivity (% cell loss or % of max prolif)
triple negative
HR+ or HER2+

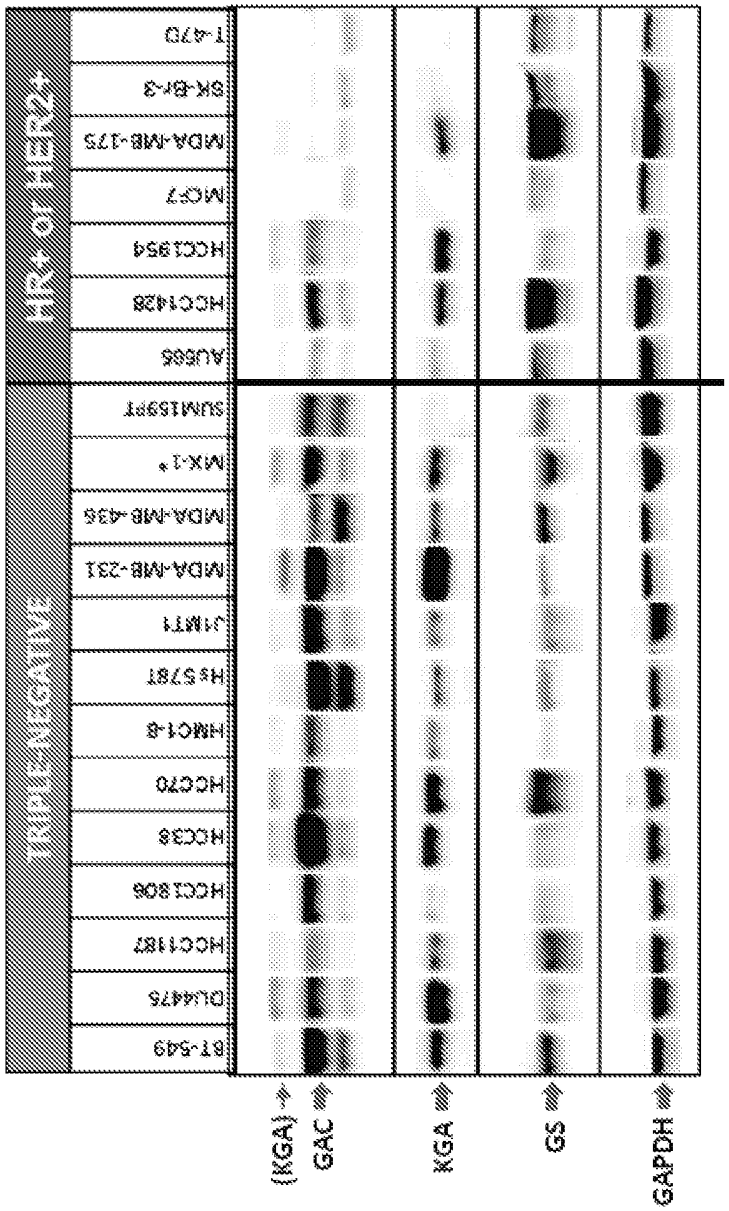

TRIPLE-NEGATIVE
HR+ or HER2+
BT-549
DU4475
HCC1187
HCC1806
HCC38
HCC70
HMC1-8
Hs 578T
J.IMT1
MDA-MB-231
MDA-MB-436
MX-1*
SUM159PT
AU565
HCC1428
HCC1954
MCF7
MDA-MB-175
SK-Br-3
T-47D
(KGA)
GAC
KGA
GS
GAPDH

Figure 15

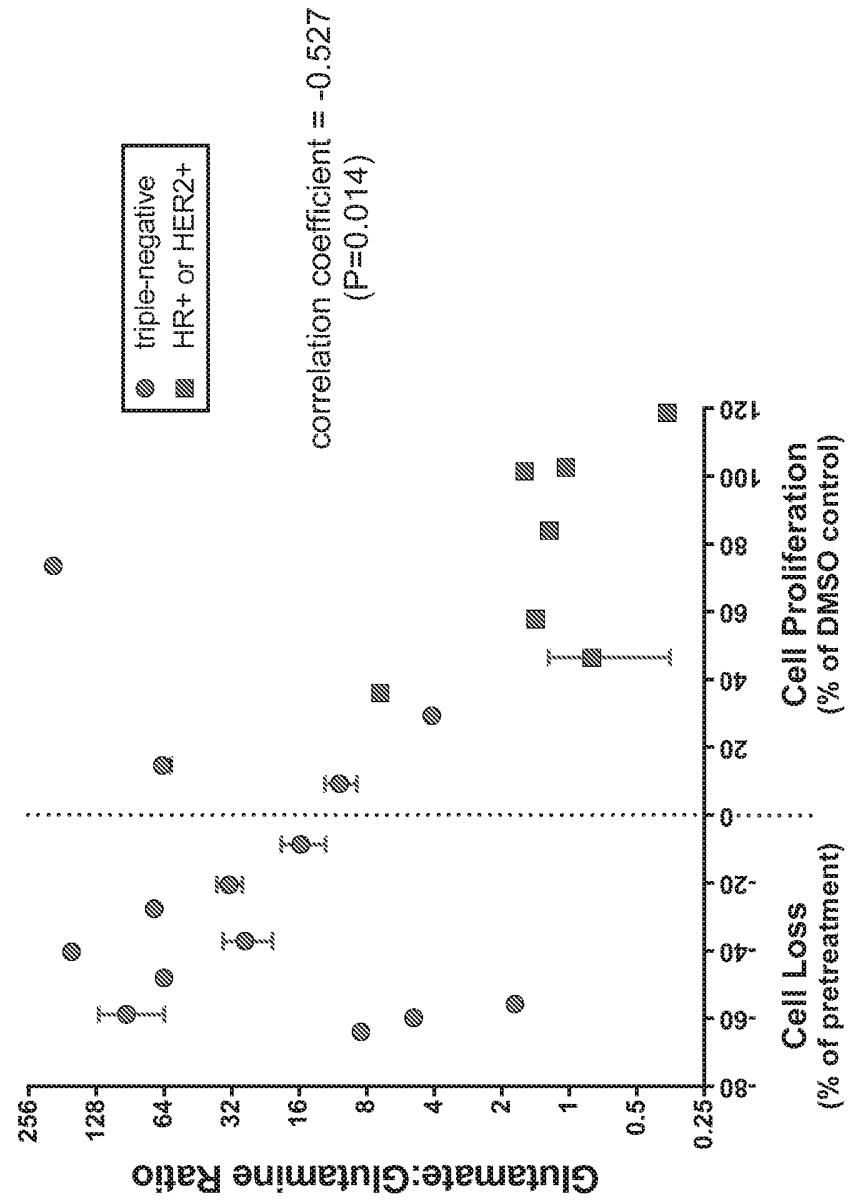

triple-negative
HR+ or HER2+
correlation coefficient = -0.527
(P=0.014)
Glutamate:Glutamine Ratio
256
128
64
32
16
8
4
2
1
0.5
0.25
Cell Loss
(% of pretreatment)
-80
-60
-40
-20
0
20
40
60
80
100
120
Cell Proliferation
(% of DMSO control)
670 Treatment (1 μM for 72 h)

Figure 16

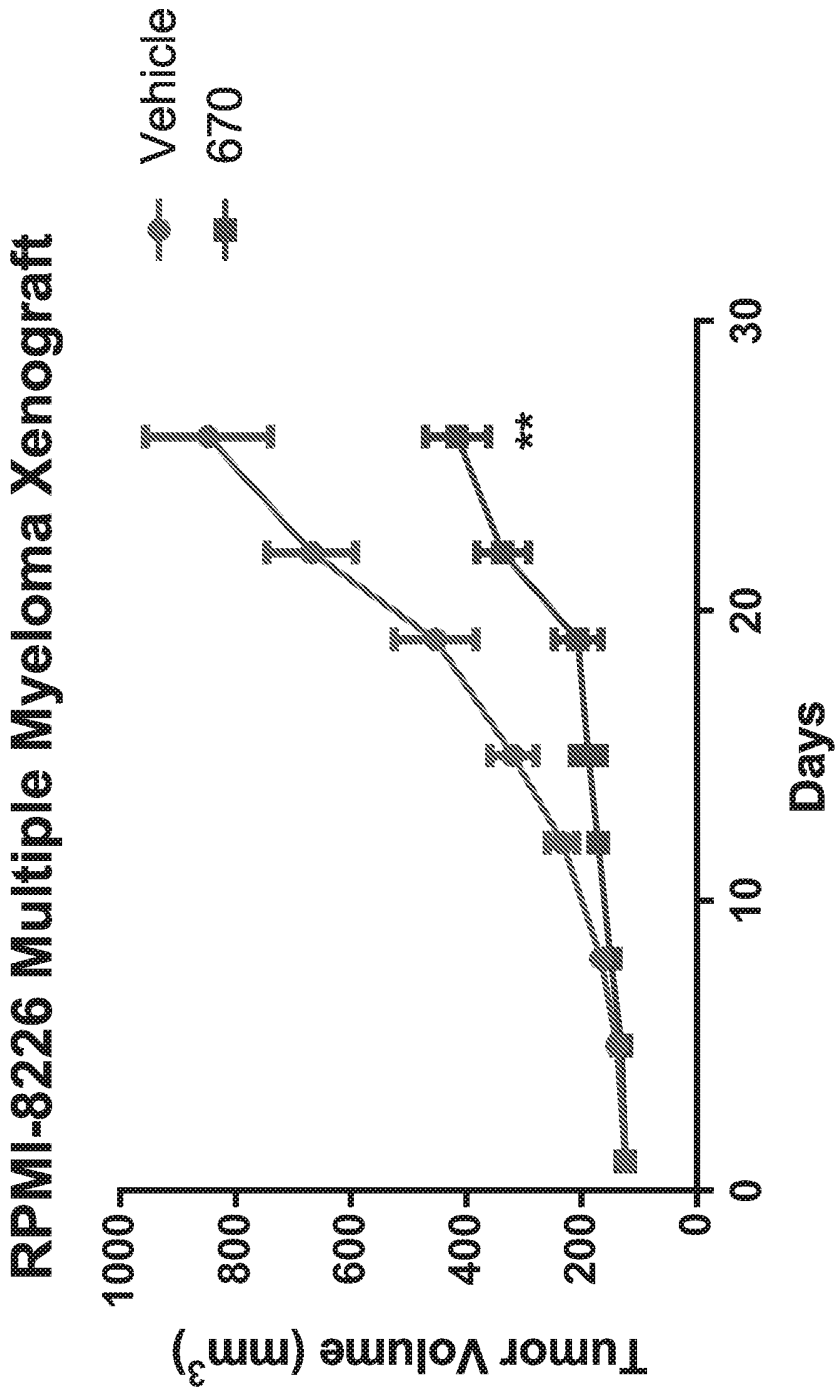

RPMI-8226 Multiple Myeloma Xenograft
Vehicle
670
**
Tumor Volume (mm3)
1000
800
600
400
200
0
0
10
20
30
Days

Figure 17

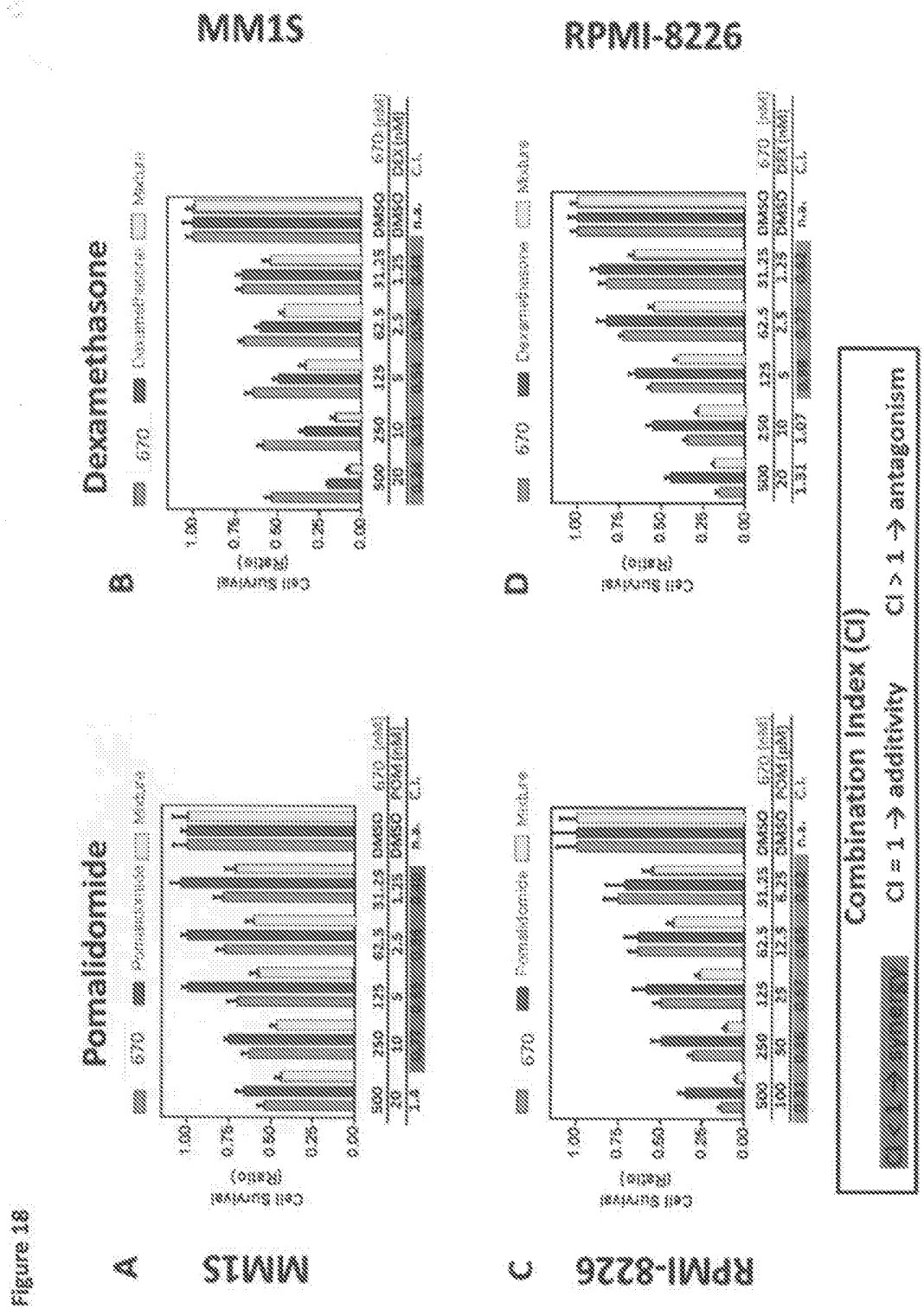

A
MM1S
Pomalidomide
B
Dexamethasone
C
RPMI-8226
D
Cell Survival (Ratio)
Combination Index (CI)
CI = 1 → additivity
CI > 1 → antagonism

Figure 18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/072830

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/433 (2006.01) ***A61K 31/4245 (2006.01)*** ***A61K 31/501 (2006.01)*** ***A61P 35/00 (2006.01)***

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA: Formulae I/Ia structure search;
WPI, EPODOC, Medline, BIOSIS: glutamine, glutamate, glutamine synthetase, glutaminase, ratio, activity, level, concentration, cancer, neoplasia, tumour, malignancy, glioma, adenoma, carcinoma, melanoma, myeloma, lymphoma, leukaemia, phaechromocytoma and related terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 March 2014	04 March 2014
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au Facsimile No.: +61 2 6283 7999	Makiko Umehara AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262833142

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	**PCT/US2013/072830**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/006506 A1 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY & THE GENERAL HOSPITAL CORPORATION D/B/A MASSACHUSETTS GENERAL HOSPITAL) 12 January 2012 See whole document - in particular, abstract, page 19 lines 1-7, page 23 lines 6-18, page 24 lines 3-8, page 25 lines 7-28 and page 29 line 4 - page 30 line 17	1-23 and 49-75
X	WO 2011/143160 A2 (THE JOHNS HOPKINS UNIVERSITY) 17 November 2011 See whole document - in particular, abstract, page 10 lines 15-25, page 11 lines 8-10, page 11 lines 12-17, page 12 lines 2-4, page 20 lines 24-33, page 21 lines 3-9, page 21 lines 14-16 and Example 13	1-23, 49, 60, 61, 63, 64 and 67-75
A	RAJAGOPALAN K.N. & DeBERARDINIS R.J.: "Role of Glutamine in Cancer: Therapeutic and Imaging Implications" *Journal of Nuclear Medicine*, 2011, vol. 52, pp. 1005-1008 See whole document - in particular, abstract, page 1005 left column paragraph 1 and page 1006 right column - page 1007 left column bridging paragraph	76-184
A	US 2005/0260697 A1 (WANG et al.) 24 November 2005 See whole document - in particular, abstract, [0122] and [0125]	76-184
P,X	WO 2013/078123 A1 (CALITHERA BIOSCIENCES INC.) 30 May 2013 See whole document, in particular abstract, page 3 line 28 - page 5 line 33, page 10 line 12 - page 13 line 13, page 18 line 15 - page 20 line 21, page 21 lines 3-13, page 25 lines 1-20, Table 3	1-75

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/072830

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT	International application No. PCT/US2013/072830

Supplemental Box

Continuation of: **Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-75 are directed to methods of treating or preventing cancer comprising administering a compound of formulae I or Ia.
- Claims 76-96 are directed to methods of identifying a cancer patient comprising determining the ratio of glutamate to glutamine in their cancer cells.
- Claims 97-120 are directed to methods of treating a cancer patient comprising determining the ratio of glutamate to glutamine in their cancer cells and administering a compound of formulae I or Ia.
- Claims 121-146 are directed to methods of identifying a cancer patient comprising determining the ratio of a glutaminase enzyme to glutamine synthetase in their cancer cells.
- Claims 147-182 are directed to methods of treating a cancer patient comprising determining the ratio of a glutaminase enzyme to glutamine synthetase in their cells and administering a compound of formulae I or Ia.
- Claims 183 and 184 are directed to methods of treating a cancer patient comprising determining glutaminase activity in their cancer cells and administering a compound of formulae I or Ia.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2013/072830**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2012/006506 A1	12 Jan 2012	WO 2012006506 A1	12 Jan 2012
WO 2011/143160 A2	17 Nov 2011	WO 2011143160 A2	17 Nov 2011
US 2005/0260697 A1	24 Nov 2005	AU 2005245785 A2	01 Dec 2005
		AU 2005245785 B2	07 Apr 2011
		CA 2578680 A1	01 Dec 2005
		EP 1747282 A2	31 Jan 2007
		EP 1747282 B1	08 Jun 2011
		JP 2007535318 A	06 Dec 2007
		JP 4885122 B2	29 Feb 2012
		US 2005260697 A1	24 Nov 2005
		US 7456027 B2	25 Nov 2008
		US 2009317805 A1	24 Dec 2009
		US 8298835 B2	30 Oct 2012
		WO 2005113798 A2	01 Dec 2005
WO 2013/078123 A1	30 May 2013	US 2013157998 A1	20 Jun 2013
		US 8604016 B2	10 Dec 2013
		US 2014050699 A1	20 Feb 2014
		WO 2013078123 A1	30 May 2013

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(43)申请公布日 2016.01.27

(21)申请号 201380072017.1

(22)申请日 2013.12.03

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61/732755 2012.12.03 US
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61/784984 2013.03.14 US
61/809795 2013.04.08 US
61/824513 2013.05.17 US

(85)PCT国际申请进入国家阶段日
2015.07.31

(86)PCT国际申请的申请数据
PCT/US2013/072830 2013.12.03

(87)PCT国际申请的公布数据
WO2014/089048 EN 2014.06.12

(71)申请人 卡利泰拉生物科技公司
地址 美国加利福尼亚州

(72)发明人 M.K. 贝内特 M.I. 格罗斯
S.D. 布罗姆利 J. 李 L. 陈
B. 戈亚尔 G. 莱迪 T.F. 斯坦顿
E.B. 舍格伦

(74)专利代理机构 中国专利代理(香港)有限公司 72001
代理人 罗文锋 徐厚才

(51)Int.Cl.
A61K 31/433(2006.01)
A61K 31/4245(2006.01)
A61K 31/501(2006.01)
A61P 35/00(2006.01)

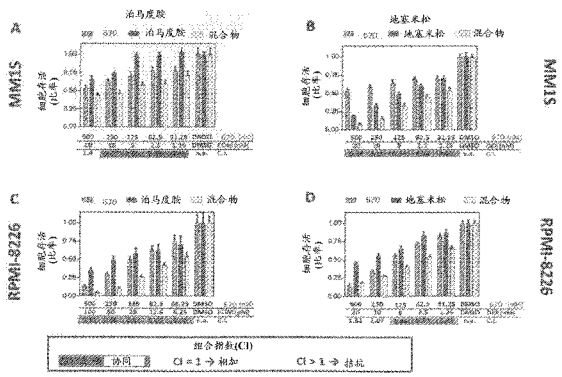
权利要求书15页 说明书265页 附图18页

(54)发明名称

用谷氨酰胺酶的杂环抑制剂治疗癌症

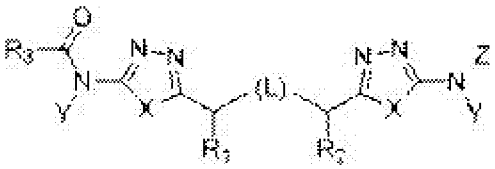
(57)摘要

本发明涉及新的杂环化合物及其药物制剂以及使用本发明的化合物治疗或预防癌症的方法。其它方面涉及鉴定可获益于用谷氨酰胺酶抑制剂治疗的癌症患者的方法，所述方法包括测定患者的癌细胞中谷氨酸与谷氨酰胺的比率、谷氨酰胺酶与谷氨酰胺合成酶比率或谷氨酰胺酶活性。



CN 105283182 A

1. 一种治疗或预防癌症的方法，所述方法包括给予式 I 的化合物或其药学上可接受的盐，



(I)，

其中：

L 表示 CH_2SCH_2、CH_2CH_2、$CH_2CH_2CH_2$、CH_2、CH_2S、SCH_2、CH_2NHCH_2、CH=CH 或 ，其中 CH 或 CH_2 单元的任何氢原子可被烷基或烷氧基置换，NH 单元的任何氢可被烷基置换，且 CH_2CH_2、$CH_2CH_2CH_2$ 或 CH_2 的 CH_2 单元的任何氢原子可被羟基置换；

X 每次出现时独立地表示 S、O 或 CH=CH，其中 CH 单元的任何氢原子可被烷基置换；

Y 每次出现时独立地表示 H 或 $CH_2O(CO)R_7$；

R_7 每次出现时独立地表示 H 或取代或未取代的烷基、烷氧基、氨基烷基、烷基氨基烷基、杂环基烷基或杂环基烷氧基；

Z 表示 H 或 $R_3(CO)$；

R_1 和 R_2 各自独立地表示 H、烷基、烷氧基或羟基；

R_3 每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基、杂芳氧基烷基或 $C(R_8)(R_9)(R_{10})$、$N(R_4)(R_5)$ 或 OR_6，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_4 和 R_5 各自独立地表示 H 或取代或未取代的烷基、羟基烷基、酰基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_6 每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；和

R_8、R_9 和 R_{10} 各自独立地表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，或 R_8 和 R_9 与它们所连接的碳一起形成碳环环系或杂环环系，其中任何游离羟基可被酰化形成 $C(O)R_7$，且其中 R_8、R_9 和 R_{10} 的至少两个不是 H。

2. 权利要求 1 的方法，其中 L 表示 CH_2SCH_2、CH_2CH_2、CH_2S 或 SCH_2。

3. 权利要求 1 的方法，其中 L 表示 CH_2CH_2。

4. 前述权利要求中任一项的方法，其中 Y 表示 H。

5. 前述权利要求中任一项的方法，其中 X 每次出现时独立地表示 S 或 CH=CH，其中 CH 单元的任何氢原子可被烷基置换。

6. 前述权利要求中任一项的方法，其中 Z 表示 $R_3(CO)$。

7. 权利要求 6 的方法，其中 R_3 的每次出现都不相同。

8. 前述权利要求中任一项的方法，其中 R_1 和 R_2 各自表示 H。

9. 前述权利要求中任一项的方法，其中 R_3 每次出现时独立地表示取代或未取代的芳基烷基、杂芳基烷基、环烷基或杂环烷基。

10. 前述权利要求中任一项的方法，其中 R_3 每次出现时独立地表示 $C(R_8)(R_9)(R_{10})$，其中 R_8 表示取代或未取代的芳基、芳基烷基、杂芳基或杂芳烷基，R_9 表示 H，R_{10} 表示羟基、羟基烷基、烷氧基或烷氧基烷基。

11. 权利要求 10 的方法，其中 R_8 表示取代或未取代的芳基、芳基烷基或杂芳基。

12. 权利要求 10 或 11 的方法，其中 R_{10} 表示羟基、羟基烷基或烷氧基。

13. 权利要求 1 的方法，其中 L 表示 CH_2SCH_2、CH_2CH_2、CH_2S 或 SCH_2，Y 表示 H，X 表示 S，Z 表示 $R_3(CO)$，R_1 和 R_2 各自表示 H，且 R_3 每次出现时独立地表示取代或未取代的芳基烷基、杂芳基烷基、环烷基或杂环烷基。

14. 权利要求 13 的方法，其中 R_3 的每次出现都是相同的。

15. 权利要求 1 的方法，其中 L 表示 CH_2SCH_2、CH_2CH_2、CH_2S 或 SCH_2，Y 表示 H，X 表示 S，Z 表示 $R_3(CO)$，R_1 和 R_2 各自表示 H，且 R_3 每次出现时独立地表示 $C(R_8)(R_9)(R_{10})$，其中 R_8 表示取代或未取代的芳基、芳基烷基、杂芳基或杂芳烷基，R_9 表示 H，且 R_{10} 表示羟基、羟基烷基、烷氧基或烷氧基烷基。

16. 权利要求 15 的方法，其中 L 表示 CH_2CH_2。

17. 权利要求 15 或 16 的方法，其中 R_8 表示取代或未取代的芳基、芳基烷基或杂芳基。

18. 权利要求 17 的方法，其中 R_8 表示取代或未取代的芳基。

19. 权利要求 15-18 中任一项的方法，其中 R_{10} 表示羟基、羟基烷基或烷氧基。

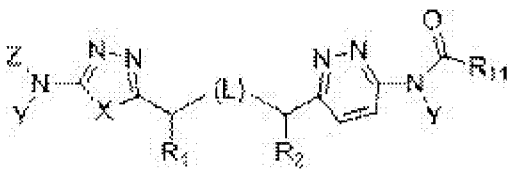
20. 权利要求 19 的方法，其中 R_{10} 表示羟基烷基。

21. 权利要求 15-20 中任一项的方法，其中 R_3 的每次出现都是相同的。

22. 权利要求 1 的方法，其中 L 表示 CH_2CH_2，Y 表示 H，X 每次出现时独立地表示 S 或 CH=CH，Z 表示 $R_3(CO)$，R_1 和 R_2 各自表示 H，且 R_3 每次出现时独立地表示芳基烷基、杂芳基烷基、环烷基或杂环烷基。

23. 权利要求 22 的方法，其中 R_3 的每次出现都是相同的。

24. 一种治疗或预防癌症的方法，所述方法包括给予式 Ia 化合物或其药学上可接受的盐，


(Ia)，

其中：

L 表示 CH_2SCH_2、CH_2CH_2、$CH_2CH_2CH_2$、CH_2、CH_2S、SCH_2、CH_2NHCH_2、CH=CH 或 ，优选 CH_2CH_2，其中 CH 或 CH_2 单元的任何氢原子可被烷基或烷氧基置换，NH 单元的任何氢可被烷基置换，且 CH_2CH_2、$CH_2CH_2CH_2$ 或 CH_2 的 CH_2 单元的任何氢原子可被羟基置换；

X 表示 S、O 或 CH=CH，优选 S 或 CH=CH，其中 CH 单元的任何氢原子可被烷基置换；

Y 每次出现时独立地表示 H 或 $CH_2O(CO)R_7$；

R_7每次出现时独立地表示H或取代或未取代的烷基、烷氧基、氨基烷基、烷基氨基烷基、杂环基烷基、芳基烷基或杂环基烷氧基；

Z 表示 H 或 $R_3(CO)$；

R_1和 R_2各自独立表示 H、烷基、烷氧基或羟基，优选 H；

R_3表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基、杂芳氧基烷基或 $C(R_8)(R_9)(R_{10})$、$N(R_4)(R_5)$ 或 OR_6，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_4和 R_5各自独立表示 H 或取代或未取代的烷基、羟基烷基、酰基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_6每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；和

R_8、R_9和 R_{10}各自独立表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，或 R_8和 R_9与它们所连接的碳一起形成碳环环系或杂环环系，其中任何游离羟基可被酰化形成 $C(O)R_7$，且其中 R_8、R_9和 R_{10}的至少两个不是 H；

R_{11}表示取代或未取代的芳基、芳基烷基、芳氧基、芳氧基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基或 $C(R_{12})(R_{13})(R_{14})$、$N(R_4)(R_{14})$ 或 OR_{14}，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_{12}和 R_{13}各自独立表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$，其中 R_{12}和 R_{13}两者都不是 H；和

R_{14}表示取代或未取代的芳基、芳基烷基、芳氧基、芳氧基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基。

25. 权利要求 24 的方法，其中 R_{11}表示取代或未取代的芳基烷基。

26. 权利要求 25 的方法，其中 R_{11}表示取代或未取代的苄基。

27. 权利要求 24-26 中任一项的方法，其中 L 表示 CH_2SCH_2、CH_2CH_2、CH_2S 或 SCH_2。

28. 权利要求 27 的方法，其中 L 表示 CH_2CH_2。

29. 权利要求 24-28 中任一项的方法，其中各个 Y 表示 H。

30. 权利要求 24-29 中任一项的方法，其中 X 表示 S 或 CH=CH。

31. 权利要求 30 的方法，其中 X 表示 S。

32. 权利要求 24-31 中任一项的方法，其中 Z 表示 $R_3(CO)$。

33. 权利要求 32 的方法，其中 R_3 和 R_{11} 不相同。

34. 权利要求 24-33 中任一项的方法，其中 R_1 和 R_2 各自表示 H。

35. 权利要求 32 的方法，其中 R_3 表示取代或未取代的芳基烷基、杂芳基烷基、环烷基或杂环烷基。

36. 权利要求 35 的方法，其中 R_3 表示取代或未取代的杂芳基烷基。

37. 权利要求 32 的方法，其中 R_3 表示 $C(R_8)(R_9)(R_{10})$，其中 R_8 表示取代或未取代的芳基、芳基烷基、杂芳基或杂芳烷基，R_9 表示 H，且 R_{10} 表示羟基、羟基烷基、烷氧基或烷氧基烷基。

38. 权利要求 37 的方法，其中 R_8 表示取代或未取代的芳基、芳基烷基或杂芳基。

39. 权利要求 37 或 38 的方法，其中 R_{10} 表示羟基、羟基烷基或烷氧基。

40. 权利要求 24 的方法，其中 L 表示 CH_2SCH_2、CH_2CH_2、CH_2S 或 SCH_2，Y 表示 H，X 表示 S，Z 表示 $R_3(CO)$，R_1 和 R_2 各自表示 H，R_3 表示取代或未取代的芳基烷基、杂芳基烷基、环烷基或杂环烷基，且 R_{11} 表示取代或未取代的芳基烷基。

41. 权利要求 40 的方法，其中 R_3 表示取代或未取代的杂芳基烷基。

42. 权利要求 24 的方法，其中 L 表示 CH_2SCH_2、CH_2CH_2、CH_2S 或 SCH_2，Y 表示 H，X 表示 S，Z 表示 $R_3(CO)$，R_1 和 R_2 各自表示 H，R_3 表示 $C(R_8)(R_9)(R_{10})$，其中 R_8 表示取代或未取代的芳基、芳基烷基、杂芳基或杂芳烷基，R_9 表示 H，R_{10} 表示羟基、羟基烷基、烷氧基或烷氧基烷基，且 R_{11} 表示取代或未取代的芳基烷基。

43. 权利要求 42 的方法，其中 R_8 表示取代或未取代的芳基、芳基烷基或杂芳基。

44. 权利要求 43 的方法，其中 R_8 表示杂芳基。

45. 权利要求 42-44 中任一项的方法，其中 R_{10} 表示羟基、羟基烷基或烷氧基。

46. 权利要求 24 的方法，其中 L 表示 CH_2CH_2，Y 表示 H，X 表示 S 或 CH=CH，Z 表示 $R_3(CO)$，R_1 和 R_2 各自表示 H，R_3 表示取代或未取代的芳基烷基、杂芳基烷基、环烷基或杂环烷基，且 R_{11} 表示取代或未取代的芳基烷基。

47. 权利要求 46 的方法，其中 R_3 表示取代或未取代的杂芳基烷基。

48. 权利要求 24 的方法，其中 L 表示 CH_2CH_2，Y 表示 H，X 表示 S，Z 表示 $R_3(CO)$，R_1 和 R_2 各自表示 H，R_3 表示 $C(R_8)(R_9)(R_{10})$，其中 R_8 表示取代或未取代的芳基、芳基烷基或杂芳基，R_9 表示 H，R_{10} 表示羟基、羟基烷基或烷氧基，且 R_{11} 表示取代或未取代的芳基烷基。

49. 前述权利要求中任一项的方法，其中所述癌症选自乳腺癌、结肠直肠癌、内分泌癌、肺癌、黑素瘤、间皮瘤、肾癌和 B 细胞恶性肿瘤。

50. 权利要求 49 的方法，其中所述癌症是乳腺癌。

51. 权利要求 50 的方法，其中所述乳腺癌包括基底型乳腺癌细胞、三阴性乳腺癌细胞或紧蛋白 - 低乳腺癌细胞。

52. 权利要求 51 的方法，其中所述乳腺癌包括基底型乳腺癌细胞。

53. 权利要求 51 的方法，其中所述乳腺癌包括三阴性乳腺癌细胞。

54. 权利要求 51 的方法，其中所述乳腺癌包括紧蛋白 - 低乳腺癌细胞。

55. 权利要求 49 的方法，其中所述癌症是结肠直肠癌。

56. 权利要求 49 的方法，其中所述癌症是内分泌癌。

57. 权利要求 56 的方法，其中所述内分泌癌选自肾上腺皮质腺瘤、肾上腺皮质癌、肾上

腺嗜铬细胞瘤和甲状旁腺腺瘤。

58. 权利要求 49 的方法，其中所述癌症是黑素瘤。

59. 权利要求 49 的方法，其中所述癌症是肾癌。

60. 权利要求 49 的方法，其中所述癌症是 B 细胞恶性肿瘤。

61. 权利要求 60 的方法，其中所述 B 细胞恶性肿瘤选自多发性骨髓瘤、白血病和淋巴瘤。

62. 权利要求 61 的方法，其中所述 B 细胞恶性肿瘤是多发性骨髓瘤。

63. 权利要求 61 的方法，其中所述 B 细胞恶性肿瘤是白血病。

64. 权利要求 63 的方法，其中所述白血病选自急性成淋巴细胞性白血病和慢性成淋巴细胞性白血病。

65. 权利要求 61 的方法，其中所述 B 细胞恶性肿瘤是淋巴瘤。

66. 权利要求 65 的方法，其中所述淋巴瘤选自伯基特淋巴瘤、弥漫性大 B 细胞淋巴瘤、滤泡性淋巴瘤和霍奇金淋巴瘤。

67. 前述权利要求中任一项的方法，所述方法还包括联合给予一种或多种其它化学治疗剂。

68. 权利要求 67 的方法，其中联合给予一种或多种其它化学治疗剂相对于各自单独给予式 I 化合物或一种或多种其它化学治疗剂提供改进功效。

69. 权利要求 68 的方法，其中联合给予一种或多种其它化学治疗剂提供协同效应。

70. 权利要求 69 的方法，其中联合给予一种或多种其它化学治疗剂提供相加效应。

71. 权利要求 67-70 中任一项的方法，其中式 I 化合物和一种或多种其它化学治疗剂同时给予。

72. 权利要求 67-70 中任一项的方法，其中一种或多种其它化学治疗剂在给予式 I 化合物之前或之后约 5 分钟 - 约 168 小时内给予。

73. 权利要求 67-72 中任一项的方法，其中一种或多种其它化学治疗剂选自 ABT-263、氨鲁米特、安吖啶、阿那曲唑、天冬酰胺酶、bcg、比卡鲁胺、博来霉素、硼替佐米、布舍瑞林、白消安、喜树碱、卡培他滨、卡铂、卡非佐米、卡莫司汀、苯丁酸氮芥、氯喹、顺铂、克拉屈滨、氯膦酸盐、秋水仙素、环磷酰胺、环丙孕酮、阿糖胞苷、达卡巴嗪、放线菌素 D、柔红霉素、脱甲绿胶霉素、地塞米松、二氯乙酸盐、己二烯雌酚、己烯雌酚、多西他赛、多柔比星、表柔比星、雌二醇、雌莫司汀、依托泊苷、依维莫司、依西美坦、非格司亭、氟达拉滨、氟氢可的松、氟尿嘧啶和 5- 氟尿嘧啶、氟甲睾酮、氟他胺、吉西他滨、染料木碱、戈舍瑞林、羟基脲、伊达比星、异环磷酰胺、伊马替尼、干扰素、伊立替康、ironotecan、来那度胺、来曲唑、亚叶酸、亮丙立德、左旋咪唑、洛莫司汀、氯尼达明、氮芥、甲羟孕酮、甲地孕酮、美法仑、巯基嘌呤、美司钠、二甲双胍、甲氨蝶呤、丝裂霉素、米托坦、米托蒽醌、尼鲁米特、诺考达唑、奥曲肽、奥沙利铂、紫杉醇、帕米膦酸盐、喷司他丁、哌立福辛、PF-04691502、普卡霉素、泊马度胺、卟吩姆钠、丙卡巴肼、雷替曲塞、利妥昔单抗、罗米地新、索拉非尼、链佐星、舒尼替尼、苏拉明、他莫昔芬、替莫唑胺、坦罗莫司、替尼泊苷、睾酮、沙利度胺、硫鸟嘌呤、塞替派、二氯二茂钛、托泊替康、曲妥珠单抗、维 A 酸、长春碱、长春新碱、长春地辛、长春瑞滨和伏林司他 (SAHA)。

74. 权利要求 73 的方法，其中一种或多种其它化学治疗剂选自硼替佐米、卡培他滨、卡铂、卡非佐米、环磷酰胺、柔红霉素、地塞米松、多西他赛、多柔比星、表柔比星、eribulin、氟

尿嘧啶、吉西他滨、伊沙匹隆、来那度胺、甲氨蝶呤、米托蒽醌、自力霉素、紫杉醇、泊马度胺、利妥昔单抗、沙利度胺、塞替派、长春新碱和长春瑞滨。

75. 权利要求 74 的方法，其中一种或多种其它化学治疗剂选自硼替佐米、卡非佐米、地塞米松、多柔比星、来那度胺、紫杉醇、泊马度胺、沙利度胺和利妥昔单抗。

76. 一种鉴定可获益于用谷氨酰胺酶抑制剂治疗的癌症患者的方法，所述方法包括测定癌症患者的癌细胞中谷氨酸与谷氨酰胺的比率，其中比率大于或等于 1.5 表明患者可获益于用谷氨酰胺酶抑制剂治疗。

77. 权利要求 76 的方法，其中所述比率大于或等于 2.0。

78. 权利要求 76 或 77 的方法，其中所述测定比率的方法包括测量癌症患者的癌细胞中谷氨酸和谷氨酰胺的水平。

79. 权利要求 76-78 中任一项的方法，所述癌症选自 B 细胞恶性肿瘤、乳腺癌、结肠直肠癌、内分泌癌、肺癌、黑素瘤、间皮瘤和肾癌。

80. 权利要求 79 的方法，其中所述癌症是乳腺癌。

81. 权利要求 80 的方法，其中所述乳腺癌包括基底型乳腺癌细胞、三阴性乳腺癌细胞或紧蛋白 - 低乳腺癌细胞。

82. 权利要求 81 的方法，其中所述乳腺癌包括基底型乳腺癌细胞。

83. 权利要求 81 的方法，其中所述乳腺癌包括三阴性乳腺癌细胞。

84. 权利要求 81 的方法，其中所述乳腺癌包括紧蛋白 - 低乳腺癌细胞。

85. 权利要求 79 的方法，其中所述癌症是结肠直肠癌。

86. 权利要求 79 的方法，其中所述癌症是内分泌癌。

87. 权利要求 86 的方法，其中所述内分泌癌选自肾上腺皮质腺瘤、肾上腺皮质癌、肾上腺嗜铬细胞瘤和甲状旁腺腺瘤。

88. 权利要求 79 的方法，其中所述癌症是黑素瘤。

89. 权利要求 79 的方法，其中所述癌症是肾癌。

90. 权利要求 79 的方法，其中所述癌症是 B 细胞恶性肿瘤。

91. 权利要求 90 的方法，其中所述 B 细胞恶性肿瘤选自多发性骨髓瘤、白血病和淋巴瘤。

92. 权利要求 91 的方法，其中所述 B 细胞恶性肿瘤是多发性骨髓瘤。

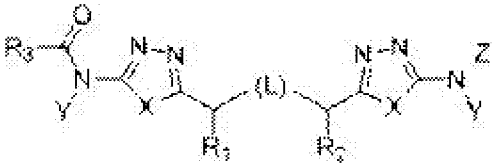
93. 权利要求 91 的方法，其中所述 B 细胞恶性肿瘤是白血病。

94. 权利要求 93 的方法，其中所述白血病选自急性成淋巴细胞性白血病和慢性成淋巴细胞性白血病。

95. 权利要求 91 的方法，其中所述 B 细胞恶性肿瘤是淋巴瘤。

96. 权利要求 95 的方法，其中所述淋巴瘤选自伯基特淋巴瘤、弥漫性大 B 细胞淋巴瘤、滤泡性淋巴瘤和霍奇金淋巴瘤。

97. 一种治疗癌症患者的方法，所述方法包括 1) 测定癌症患者的癌细胞中谷氨酸与谷氨酰胺的比率；和 2) 如果谷氨酸与谷氨酰胺的比率大于或等于 1.5，则给予式 I 的化合物或其药学上可接受的盐，



(I)，

其中：

L 表示 CH_2SCH_2、CH_2CH_2、$CH_2CH_2CH_2$、CH_2、CH_2S、SCH_2、CH_2NHCH_2、CH=CH 或 ，其中 CH 或 CH_2单元的任何氢原子可被烷基或烷氧基置换，NH 单元的任何氢可被烷基置换，且 CH_2CH_2、$CH_2CH_2CH_2$或 CH_2的 CH_2单元的任何氢原子可被羟基置换；

X 每次出现时独立地表示 S、O 或 CH=CH，其中 CH 单元的任何氢原子可被烷基置换；

Y 每次出现时独立地表示 H 或 $CH_2O(CO)R_7$；

R_7每次出现时独立地表示 H 或取代或未取代的烷基、烷氧基、氨基烷基、烷基氨基烷基、杂环基烷基或杂环基烷氧基；

Z 表示 H 或 $R_3(CO)$；

R_1和 R_2各自独立表示 H、烷基、烷氧基或羟基；

R_3每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基、杂芳氧基烷基或 $C(R_8)(R_9)(R_{10})$、$N(R_4)(R_5)$或 OR_6，其中任何游离羟基可被酰化形成 $C(O)R_7$；

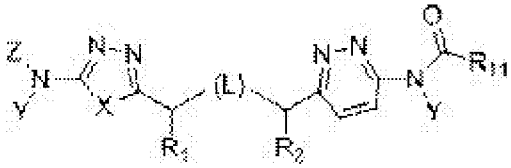
R_4和 R_5各自独立表示 H 或取代或未取代的烷基、羟基烷基、酰基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_6每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；和

R_8、R_9和 R_{10}各自独立表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，或 R_8和 R_9与它们所连接的碳一起形成碳环环系或杂环环系，其中任何游离羟基可被酰化形成 $C(O)R_7$，且其中 R_8、R_9和 R_{10}的至少两个不是 H。

98. 权利要求 97 的方法，其中所述化合物是权利要求 2-23 中任一项描述的化合物。

99. 一种治疗癌症患者的方法，所述方法包括 1) 测定癌症患者的癌细胞中谷氨酸与谷氨酰胺的比率；和 2) 如果谷氨酸与谷氨酰胺的比率大于或等于 1.5，则给予式 Ia 化合物或其药学上可接受的盐，



(Ia)，

其中：

L 表示 CH_2SCH_2、CH_2CH_2、$CH_2CH_2CH_2$、CH_2、CH_2S、SCH_2、CH_2NHCH_2、CH=CH 或 ，优选 CH_2CH_2，其中 CH 或 CH_2 单元的任何氢原子可被烷基或烷氧基置换，NH 单元的任何氢可被烷基置换，且 CH_2CH_2、$CH_2CH_2CH_2$ 或 CH_2 的 CH_2 单元的任何氢原子可被羟基置换；

X 表示 S、O 或 CH=CH，优选 S 或 CH=CH，其中 CH 单元的任何氢原子可被烷基置换；

Y 每次出现时独立地表示 H 或 $CH_2O(CO)R_7$；

R_7 每次出现时独立地表示 H 或取代或未取代的烷基、烷氧基、氨基烷基、烷基氨基烷基、杂环基烷基、芳基烷基或杂环基烷氧基；

Z 表示 H 或 $R_3(CO)$；

R_1 和 R_2 各自独立表示 H、烷基、烷氧基或羟基，优选 H；

R_3 表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基、杂芳氧基烷基或 $C(R_8)(R_9)(R_{10})$、$N(R_4)(R_5)$ 或 OR_6，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_4 和 R_5 各自独立表示 H 或取代或未取代的烷基、羟基烷基、酰基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_6 每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；和

R_8、R_9 和 R_{10} 各自独立表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，或 R_8 和 R_9 与它们所连接的碳一起形成碳环环系或杂环环系，其中任何游离羟基可被酰化形成 $C(O)R_7$，且其中 R_8、R_9 和 R_{10} 的至少两个不是 H；

R_{11} 表示取代或未取代的芳基、芳基烷基、芳氧基、芳氧基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基或 $C(R_{12})(R_{13})(R_{14})$、$N(R_4)(R_{14})$ 或 OR_{14}，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_{12} 和 R_{13} 各自独立表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$，其中 R_{12} 和 R_{13} 两者都不是 H；和

R_{14} 表示取代或未取代的芳基、芳基烷基、芳氧基、芳氧基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基。

100. 权利要求 99 的方法，其中所述化合物是权利要求 25-48 中任一项描述的化合物。

101. 权利要求 97-100 中任一项的方法，其中所述比率大于或等于 2.0。

102. 权利要求 97-101 中任一项的方法，其中所述测定比率的方法包括测量癌症患者的癌细胞中谷氨酸和谷氨酰胺的水平。

103. 权利要求 97-102 中任一项的方法，所述癌症选自 B 细胞恶性肿瘤、乳腺癌、结肠直肠癌、内分泌癌、肺癌、黑素瘤、间皮瘤和肾癌。

104. 权利要求 103 的方法，其中所述癌症是乳腺癌。

105. 权利要求 104 的方法，其中所述乳腺癌包括基底型乳腺癌细胞、三阴性乳腺癌细胞或紧蛋白 - 低乳腺癌细胞。

106. 权利要求 105 的方法，其中所述乳腺癌包括基底型乳腺癌细胞。

107. 权利要求 105 的方法，其中所述乳腺癌包括三阴性乳腺癌细胞。

108. 权利要求 105 的方法，其中所述乳腺癌包括紧蛋白 - 低乳腺癌细胞。

109. 权利要求 103 的方法，其中所述癌症是结肠直肠癌。

110. 权利要求 103 的方法，其中所述癌症是内分泌癌。

111. 权利要求 110 的方法，其中所述内分泌癌选自肾上腺皮质腺瘤、肾上腺皮质癌、肾上腺嗜铬细胞瘤和甲状旁腺腺瘤。

112. 权利要求 103 的方法，其中所述癌症是黑素瘤。

113. 权利要求 103 的方法，其中所述癌症是肾癌。

114. 权利要求 103 的方法，其中所述癌症是 B 细胞恶性肿瘤。

115. 权利要求 114 的方法，其中所述 B 细胞恶性肿瘤选自多发性骨髓瘤、白血病和淋巴瘤。

116. 权利要求 115 的方法，其中所述 B 细胞恶性肿瘤是多发性骨髓瘤。

117. 权利要求 115 的方法，其中所述 B 细胞恶性肿瘤是白血病。

118. 权利要求 117 的方法，其中所述白血病选自急性成淋巴细胞性白血病和慢性成淋巴细胞性白血病。

119. 权利要求 115 的方法，其中所述 B 细胞恶性肿瘤是淋巴瘤。

120. 权利要求 119 的方法，其中所述淋巴瘤选自伯基特淋巴瘤、弥漫性大 B 细胞淋巴瘤、滤泡性淋巴瘤和霍奇金淋巴瘤。

121. 一种鉴定可获益于用谷氨酰胺酶抑制剂治疗的癌症患者的方法，所述方法包括测定癌症患者的癌细胞中的 GLS:GS 比率，其中比率大于或等于 0.05，表明患者可获益于用谷氨酰胺酶抑制剂治疗。

122. 权利要求 121 的方法，其中所述比率大于或等于 1。

123. 权利要求 121 或 122 的方法，其中所述测定比率的方法包括测量癌症患者的癌细胞中 GLS 和 GS 的水平。

124. 权利要求 123 的方法，其中测量 GLS 和 GS 的水平包括测量 mRNA 的量。

125. 权利要求 123 的方法，其中测量 GLS 和 GS 的水平包括测量蛋白质的量。

126. 权利要求 121-125 中任一项的方法，其中 GLS 是 GAC。

127. 权利要求 121-125 中任一项的方法，其中 GLS 是 KGA。

128. 权利要求 121-125 中任一项的方法，其中 GLS 是 GAC 和 KGA 两者。

129. 权利要求 121-128 中任一项的方法，其中所述癌症选自 B 细胞恶性肿瘤、乳腺癌、结肠直肠癌、内分泌癌、肺癌、黑素瘤、间皮瘤和肾癌。

130. 权利要求 129 的方法，其中所述癌症是乳腺癌。

131. 权利要求 130 的方法，其中所述乳腺癌包括基底型乳腺癌细胞、三阴性乳腺癌细胞或紧蛋白 - 低乳腺癌细胞。

132. 权利要求 130 的方法，其中所述乳腺癌包括基底型乳腺癌细胞。

133. 权利要求 130 的方法，其中所述乳腺癌包括三阴性乳腺癌细胞。

134. 权利要求 130 的方法，其中所述乳腺癌包括紧蛋白 - 低乳腺癌细胞。

135. 权利要求 129 的方法，其中所述癌症是结肠直肠癌。

136. 权利要求 129 的方法，其中所述癌症是内分泌癌。

137. 权利要求 136 的方法，其中所述内分泌癌选自肾上腺皮质腺瘤、肾上腺皮质癌、肾上腺嗜铬细胞瘤和甲状旁腺腺瘤。

138. 权利要求 129 的方法，其中所述癌症是黑素瘤。

139. 权利要求 129 的方法，其中所述癌症是肾癌。

140. 权利要求 129 的方法，其中所述癌症是 B 细胞恶性肿瘤。

141. 权利要求 140 的方法，其中所述 B 细胞恶性肿瘤选自多发性骨髓瘤、白血病和淋巴瘤。

142. 权利要求 140 的方法，其中所述 B 细胞恶性肿瘤是多发性骨髓瘤。

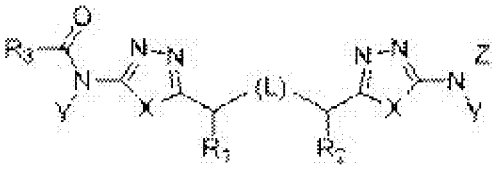
143. 权利要求 140 的方法，其中所述 B 细胞恶性肿瘤是白血病。

144. 权利要求 143 的方法，其中所述白血病选自急性成淋巴细胞性白血病和慢性成淋巴细胞性白血病。

145. 权利要求 140 的方法，其中所述 B 细胞恶性肿瘤是淋巴瘤。

146. 权利要求 145 的方法，其中所述淋巴瘤选自伯基特淋巴瘤、弥漫性大 B 细胞淋巴瘤、滤泡性淋巴瘤和霍奇金淋巴瘤。

147. 一种治疗癌症患者的方法，所述方法包括 1) 测定癌症患者的癌细胞中 GLS 与 GS 的比率；和 2) 如果 GLS 与 GS 的比率大于或等于 0.05，则给予式 I 的化合物或其药学上可接受的盐，


(I)，

其中：

L 表示 CH_2SCH_2、CH_2CH_2、$CH_2CH_2CH_2$、CH_2、CH_2S、SCH_2、CH_2NHCH_2、CH=CH 或 ，其中 CH 或 CH_2 单元的任何氢原子可被烷基或烷氧基置换，NH 单元的任何氢可被烷基置换，且 CH_2CH_2、$CH_2CH_2CH_2$ 或 CH_2 的 CH_2 单元的任何氢原子可被羟基置换；

X 每次出现时独立地表示 S、O 或 CH=CH，其中 CH 单元的任何氢原子可被烷基置换；

Y 每次出现时独立地表示 H 或 $CH_2O(CO)R_7$；

R_7 每次出现时独立地表示 H 或取代或未取代的烷基、烷氧基、氨基烷基、烷基氨基烷基、杂环基烷基或杂环基烷氧基；

Z 表示 H 或 $R_3(CO)$；

R_1 和 R_2 各自独立表示 H、烷基、烷氧基或羟基；

R_3每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基、杂芳氧基烷基或$C(R_8)(R_9)(R_{10})$、$N(R_4)(R_5)$或OR_6，其中任何游离羟基可被酰化形成$C(O)R_7$；

R_4和R_5各自独立表示H或取代或未取代的烷基、羟基烷基、酰基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成$C(O)R_7$；

R_6每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成$C(O)R_7$；和

R_8、R_9和R_{10}各自独立表示H或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，或R_8和R_9与它们所连接的碳一起形成碳环环系或杂环环系，其中任何游离羟基可被酰化形成$C(O)R_7$，且其中R_8、R_9和R_{10}的至少两个不是H。

148. 权利要求147的方法，其中所述化合物是权利要求2-23中任一项描述的化合物。

149. 权利要求147的方法，其中所述比率大于或等于1。

150. 权利要求147或149中任一项的方法，其中所述测定比率的方法包括测量癌症患者的癌细胞中GLS和GS的水平。

151. 权利要求150的方法，其中测量GLS和GS的水平包括测量mRNA的量。

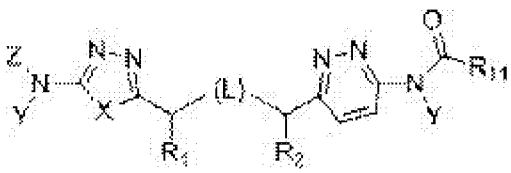
152. 权利要求151的方法，其中测量GLS和GS的水平包括测量蛋白质的量。

153. 权利要求147-152中任一项的方法，其中GLS是GAC。

154. 权利要求147-152中任一项的方法，其中GLS是KGA。

155. 权利要求147-152中任一项的方法，其中GLS是GAC和KGA两者。

156. 一种治疗癌症患者的方法，所述方法包括1)测定癌症患者的癌细胞中GLS与GS的比率；和2)如果GLS与GS的比率大于或等于0.05，则给予式Ia化合物或其药学上可接受的盐，


(Ia)，

其中：

L表示CH_2SCH_2、CH_2CH_2、$CH_2CH_2CH_2$、CH_2、CH_2S、SCH_2、CH_2NHCH_2、CH=CH或 ，优选CH_2CH_2，其中CH或CH_2单元的任何氢原子可被烷基或烷氧基置换，NH单元的任何氢可被烷基置换，且CH_2CH_2、$CH_2CH_2CH_2$或CH_2的CH_2单元的任何氢原子可被羟基置换；

X表示S、O或CH=CH，优选S或CH=CH，其中CH单元的任何氢原子可被烷基置换；

Y每次出现时独立地表示H或$CH_2O(CO)R_7$；

R_7每次出现时独立地表示H或取代或未取代的烷基、烷氧基、氨基烷基、烷基氨基烷基、杂环基烷基、芳基烷基或杂环基烷氧基；

Z 表示 H 或 $R_3(CO)$；

R_1和 R_2各自独立表示 H、烷基、烷氧基或羟基，优选 H；

R_3表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基、杂芳氧基烷基或 $C(R_8)(R_9)(R_{10})$、$N(R_4)(R_5)$ 或 OR_6，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_4和 R_5各自独立表示 H 或取代或未取代的烷基、羟基烷基、酰基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_6每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；和

R_8、R_9和 R_{10}各自独立表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，或 R_8和 R_9与它们所连接的碳一起形成碳环环系或杂环环系，其中任何游离羟基可被酰化形成 $C(O)R_7$，且其中 R_8、R_9和 R_{10}的至少两个不是 H；

R_{11}表示取代或未取代的芳基、芳基烷基、芳氧基、芳氧基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基或 $C(R_{12})(R_{13})(R_{14})$、$N(R_4)(R_{14})$ 或 OR_{14}，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_{12}和 R_{13}各自独立表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$，其中 R_{12}和 R_{13}两者都不是 H；和

R_{14}表示取代或未取代的芳基、芳基烷基、芳氧基、芳氧基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基。

157. 权利要求 156 的方法，其中所述化合物是权利要求 25-48 中任一项描述的化合物。

158. 权利要求 156 或 157 的方法，其中所述比率大于或等于 1。

159. 权利要求 156-158 中任一项的方法，其中所述测定比率的方法包括测量癌症患者的癌细胞中 GLS 和 GS 的水平。

160. 权利要求 159 的方法，其中测量 GLS 和 GS 的水平包括测量 mRNA 的量。

161. 权利要求 159 的方法，其中测量 GLS 和 GS 的水平包括测量蛋白质的量。

162. 权利要求 156-161 中任一项的方法，其中 GLS 是 GAC。

163. 权利要求 156-161 中任一项的方法，其中 GLS 是 KGA。

164. 权利要求 156-161 中任一项的方法，其中 GLS 是 GAC 和 KGA 两者。

165. 权利要求 156-164 中任一项的方法，所述癌症选自 B 细胞恶性肿瘤、乳腺癌、结肠直肠癌、内分泌癌、肺癌、黑素瘤、间皮瘤和肾癌。

166. 权利要求 165 的方法，其中所述癌症是乳腺癌。

167. 权利要求 166 的方法，其中所述乳腺癌包括基底型乳腺癌细胞、三阴性乳腺癌细胞或紧蛋白 - 低乳腺癌细胞。

168. 权利要求 166 的方法，其中所述乳腺癌包括基底型乳腺癌细胞。

169. 权利要求 166 的方法，其中所述乳腺癌包括三阴性乳腺癌细胞。

170. 权利要求 166 的方法，其中所述乳腺癌包括紧蛋白 - 低乳腺癌细胞。

171. 权利要求 165 的方法，其中所述癌症是结肠直肠癌。

172. 权利要求 165 的方法，其中所述癌症是内分泌癌。

173. 权利要求 172 的方法，其中所述内分泌癌选自肾上腺皮质腺瘤、肾上腺皮质癌、肾上腺嗜铬细胞瘤和甲状旁腺腺瘤。

174. 权利要求 165 的方法，其中所述癌症是黑素瘤。

175. 权利要求 165 的方法，其中所述癌症是肾癌。

176. 权利要求 165 的方法，其中所述癌症是 B 细胞恶性肿瘤。

177. 权利要求 176 的方法，其中所述 B 细胞恶性肿瘤选自多发性骨髓瘤、白血病和淋巴瘤。

178. 权利要求 176 的方法，其中所述 B 细胞恶性肿瘤是多发性骨髓瘤。

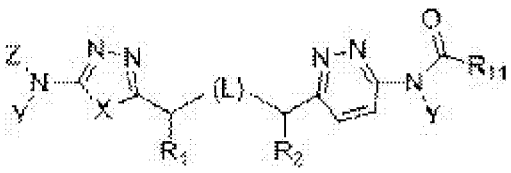
179. 权利要求 176 的方法，其中所述 B 细胞恶性肿瘤是白血病。

180. 权利要求 179 的方法，其中所述白血病选自急性成淋巴细胞性白血病和慢性成淋巴细胞性白血病。

181. 权利要求 176 的方法，其中所述 B 细胞恶性肿瘤是淋巴瘤。

182. 权利要求 181 的方法，其中所述淋巴瘤选自伯基特淋巴瘤、弥漫性大 B 细胞淋巴瘤、滤泡性淋巴瘤和霍奇金淋巴瘤。

183. 一种治疗癌症患者的方法，所述方法包括 1) 测定癌症患者的癌细胞中的谷氨酰胺酶活性；和 2) 如果活性大于或等于 0.005 μmol/ 分钟 /mg 蛋白质，则给予式 Ia 化合物或其药学上可接受的盐，


(Ia)，

其中：

L 表示 CH_2SCH_2、CH_2CH_2、$CH_2CH_2CH_2$、CH_2、CH_2S、SCH_2、CH_2NHCH_2、CH=CH 或 ，优选 CH_2CH_2，其中 CH 或 CH_2 单元的任何氢原子可被烷基或烷氧基置换，NH 单元的任何氢可被烷基置换，且 CH_2CH_2、$CH_2CH_2CH_2$ 或 CH_2 的 CH_2 单元的任何氢原子可被羟基置换；

X 表示 S、O 或 CH=CH，优选 S 或 CH=CH，其中 CH 单元的任何氢原子可被烷基置换；

Y 每次出现时独立地表示 H 或 $CH_2O(CO)R_7$；

R_7 每次出现时独立地表示 H 或取代或未取代的烷基、烷氧基、氨基烷基、烷基氨基烷基、

杂环基烷基、芳基烷基或杂环基烷氧基；

Z 表示 H 或 $R_3(CO)$；

R_1和 R_2各自独立表示 H、烷基、烷氧基或羟基，优选 H；

R_3表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基、杂芳氧基烷基或 $C(R_8)(R_9)(R_{10})$、$N(R_4)(R_5)$ 或 OR_6，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_4和 R_5各自独立表示 H 或取代或未取代的烷基、羟基烷基、酰基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_6每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；和

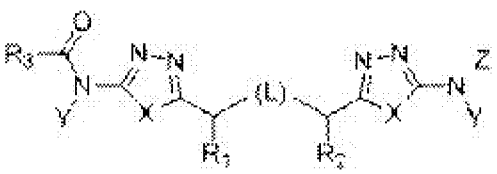
R_8、R_9和 R_{10}各自独立表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，或 R_8和 R_9与它们所连接的碳一起形成碳环环系或杂环环系，其中任何游离羟基可被酰化形成 $C(O)R_7$，且其中 R_8、R_9和 R_{10}的至少两个不是 H；

R_{11}表示取代或未取代的芳基、芳基烷基、芳氧基、芳氧基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基或 $C(R_{12})(R_{13})(R_{14})$、$N(R_4)(R_{14})$ 或 OR_{14}，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_{12}和 R_{13}各自独立表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$，其中 R_{12}和 R_{13}两者都不是 H；和

R_{14}表示取代或未取代的芳基、芳基烷基、芳氧基、芳氧基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基。

184. 一种治疗癌症患者的方法，所述方法包括 1) 测定癌症患者的癌细胞中的谷氨酰胺酶活性；和 2) 如果活性大于或等于 0.005 μmol/ 分钟 /mg 蛋白质，则给予式 I 的化合物或其药学上可接受的盐，


(I)，

其中：

L 表示 CH_2SCH_2、CH_2CH_2、$CH_2CH_2CH_2$、CH_2、CH_2S、SCH_2、CH_2NHCH_2、CH=CH 或 ，其中 CH 或

CH_2单元的任何氢原子可被烷基或烷氧基置换，NH 单元的任何氢可被烷基置换，且 CH_2CH_2、$CH_2CH_2CH_2$或 CH_2的 CH_2单元的任何氢原子可被羟基置换；

X 每次出现时独立地表示 S、O 或 CH=CH，其中 CH 单元的任何氢原子可被烷基置换；

Y 每次出现时独立地表示 H 或 $CH_2O(CO)R_7$；

R_7每次出现时独立地表示H或取代或未取代的烷基、烷氧基、氨基烷基、烷基氨基烷基、杂环基烷基或杂环基烷氧基；

Z 表示 H 或 $R_3(CO)$；

R_1和 R_2各自独立表示 H、烷基、烷氧基或羟基；

R_3每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基、杂芳氧基烷基或 $C(R_8)(R_9)(R_{10})$、$N(R_4)(R_5)$或 OR_6，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_4和 R_5各自独立表示 H 或取代或未取代的烷基、羟基烷基、酰基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；

R_6每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；和

R_8、R_9和 R_{10}各自独立表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，或 R_8和 R_9与它们所连接的碳一起形成碳环环系或杂环环系，其中任何游离羟基可被酰化形成 $C(O)R_7$，且其中 R_8、R_9和 R_{10}的至少两个不是 H。

用谷氨酰胺酶的杂环抑制剂治疗癌症

[0001] 相关申请

[0002] 本申请要求2012年12月3日提交的美国临时专利申请号61/732,755、2013年1月4日提交的美国临时专利申请号61/749,016、2013年3月14日提交的美国临时专利申请号61/784,984、2013年4月8日提交的美国临时专利申请号61/809,795和2013年5月17日提交的美国临时专利申请号61/824,513的优先权的权益，所述申请通过引用以其整体结合到本文中。

[0003] 发明背景

[0004] 谷氨酰胺通过代谢和非代谢机制支持细胞存活、生长和增殖。在活跃增殖的细胞中，谷氨酰胺代谢成为乳酸，亦称为“谷氨酰胺分解(glutaminolysis)”，是呈NADPH形式的能量的主要来源。谷氨酰胺分解中的第一步是谷氨酰胺脱氨基形成谷氨酸和氨，这被谷氨酰胺酶(GLS)催化。因此，通过谷氨酰胺酶脱氨基是谷氨酰胺代谢的控制点。

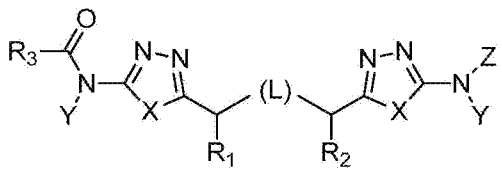
[0005] 自Warburg观察到腹水肿瘤细胞在氧气存在下显示高速率的葡萄糖消耗和乳酸分泌以来(Warburg,1956)，研究人员探索了癌细胞如何利用代谢途径以便能够继续活跃增殖。几份报告表明谷氨酰胺代谢如何支持细胞复制所必需的大分子合成(Curthoys,1995；DeBardinis,2008)。

[0006] 因此，据推理，谷氨酰胺酶为用于治疗特征在于活跃增殖细胞的疾病（例如癌症）的潜在治疗靶标。缺乏合适的谷氨酰胺酶抑制剂使得不可能验证该靶标。因此，是特异性的并且能够配制用于体内使用的谷氨酰胺酶抑制剂的产生可导致新的一类治疗剂。

[0007] 发明概述

[0008] 本发明提供治疗或预防癌症的方法，所述方法包括给予式I的化合物或其药学上可接受的盐，

[0009]



(I)，

[0010] 其中：

[0011] L表示CH_2SCH_2、CH_2CH_2、$CH_2CH_2CH_2$、CH_2、CH_2S、SCH_2、CH_2NHCH_2、CH＝CH或 ，优选CH_2CH_2，其中CH或CH_2单元的任何氢原子可被烷基或烷氧基置换，NH单元的任何氢可被烷基置换，且CH_2CH_2、$CH_2CH_2CH_2$或CH_2的CH_2单元的任何氢原子可被羟基置换；

[0012] X每次出现时独立地表示S、O或CH＝CH，优选S或CH＝CH，其中CH单元的任何氢原子可被烷基置换；

[0013] Y每次出现时独立地表示H或$CH_2O(CO)R_7$；

[0014] R_7每次出现时独立地表示H或取代或未取代的烷基、烷氧基、氨基烷基、烷基氨基烷基、杂环基烷基、芳基烷基或杂环基烷氧基；

[0015] Z 表示 H 或 $R_3(CO)$ ；

[0016] R_1和 R_2各自独立地表示 H、烷基、烷氧基或羟基 ；

[0017] R_3每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基、杂芳氧基烷基或 $C(R_8)(R_9)(R_{10})$、$N(R_4)(R_5)$ 或 OR_6，其中任何游离羟基可被酰化形成 $C(O)R_7$；

[0018] R_4和 R_5各自独立地表示 H 或取代或未取代的烷基、羟基烷基、酰基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；

[0019] R_6每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；和

[0020] R_8、R_9和 R_{10}各自独立地表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，或 R_8和 R_9与它们所连接的碳一起形成碳环环系或杂环环系，其中任何游离羟基可被酰化形成 $C(O)R_7$，且其中 R_8、R_9和 R_{10}的至少两个不是 H。

[0021] 在某些实施方案中，癌症选自乳腺癌、结肠直肠癌、内分泌癌、黑素瘤、肾癌和 B 细胞恶性肿瘤。在其中癌症是乳腺癌的某些这类的实施方案中，乳腺癌包括基底型乳腺癌细胞、三阴性乳腺癌细胞或紧蛋白-低乳腺癌 (claudin-low breast cancer) 细胞。在其中癌症是内分泌癌的某些实施方案中，内分泌癌选自肾上腺皮质腺瘤、肾上腺皮质癌、肾上腺嗜铬细胞瘤和甲状旁腺腺瘤。在其中癌症是 B 细胞恶性肿瘤的某些实施方案中，B 细胞恶性肿瘤选自多发性骨髓瘤；白血病，例如急性成淋巴细胞性白血病或慢性成淋巴细胞性白血病；和淋巴瘤，例如伯基特淋巴瘤、弥漫性大 B 细胞淋巴瘤、滤泡性淋巴瘤或霍奇金淋巴瘤。

[0022] 在某些实施方案中，本发明提供适用于在人类患者中治疗或预防癌症例如乳腺癌、结肠直肠癌、内分泌癌、黑素瘤、肾癌或 B 细胞恶性肿瘤的药物制剂，所述制剂包括有效量的本文所述任何化合物（例如本发明的化合物，例如式 I 的化合物）和一种或多种药学上可接受的赋形剂。在某些实施方案中，药物制剂可用于治疗或预防本文所述病况或疾病。在某些实施方案中，药物制剂具有足够低的致热原活性以适于人类患者静脉内使用。

[0023] 附图详述

[0024] 图 1 显示化合物 670 针对一组乳腺肿瘤细胞系的谷氨酰胺依赖性和抗增殖作用之间的相关性。

[0025] 图 2 显示三阴性乳腺癌亚型中谷氨酰胺酶和谷氨酰胺合成酶的差异性表达。

[0026] 图 3 显示 MDA-MB-231 原位异种移植物模型的单药剂化合物 402 治疗。

[0027] 图 4 显示 MDA-MB-231 原位异种移植物模型中化合物 389 和紫杉醇的联合研究。

[0028] 图 5 显示在各种癌症类型（包括结肠直肠癌、肾癌、淋巴瘤、黑素瘤和骨髓瘤）中谷氨酰胺酶：谷氨酰胺合成酶中位表达比的结果。

[0029] 图 6 显示在内分泌癌中谷氨酰胺酶：谷氨酰胺合成酶表达比随亚型变化。

[0030] 图 7 显示急性成淋巴细胞性白血病 (ALL) 和慢性淋巴细胞性白血病 (CLL) 中的谷氨酰胺酶：谷氨酰胺合成酶中位表达比。

[0031] 图 8 显示 B 细胞恶性肿瘤类别中几个淋巴瘤亚型的谷氨酰胺酶：谷氨酰胺合成酶表达比。

[0032] 图 9 显示针于一组乳腺肿瘤细胞系而言化合物 670 的抗增殖作用和谷氨酸：谷氨酰胺浓度比之间的相关性。

[0033] 图 10 显示各种原发性肿瘤异种移植物中谷氨酸：谷氨酰胺浓度比与谷氨酰胺酶：谷氨酰胺合成酶表达比和与谷氨酰胺酶比活之间的相关性。

[0034] 图 11 显示将化合物 188 腹膜内给予小鼠导致 HCT116 结肠癌异种移植物模型中肿瘤大小减小。

[0035] 图 12 显示将化合物 670 口服给予小鼠导致 H2122 肺腺癌异种移植物模型中肿瘤大小减小。

[0036] 图 13 显示在 TNBC 与 HR+ 或 Her2+ 细胞系中 GLS(KGA 或 GAC)、GS 的 mRNA 表达水平和 KGA:GS 和 GAC:GS 的比率。“箱”描述了第 2 和第 3 四分位，其中位值相当于水平线；“须”跨越第 10 和第 90 百分位数，其数据在各数据点所示的这个范围之外。

[0037] 图 14 显示对化合物 670 的敏感性和 GLS、GS 的 mRNA 表达水平或表达比之间的相关性。对于各二变量图，x 轴标绘化合物 670 敏感性，y 轴标绘表达参数，其各点代表各细胞系。

[0038] 图 15 显示乳腺癌细胞系中 KGA、GAC 和 GS 的蛋白质印迹分析。印迹用识别 KGA、GAC 和 GS 的抗体探测。CAG 抗体还识别 KGA，而且两者在印迹上通过其分子量差异是可区分的。剥离印迹，用作为上样对照的 GAPDH 再探测。

[0039] 图 16 显示谷氨酸：谷氨酰胺浓度比与对谷氨酰胺酶抑制剂化合物 670 的敏感性之间的相关性。

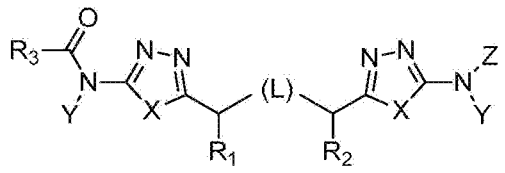
[0040] 图 17 显示在 RPMI-8226 多发性骨髓瘤异种移植物模型中将化合物 670 口服给予小鼠导致肿瘤大小减小。

[0041] 图 18 显示化合物 670 与泊马度胺 (pomalidomide) 或地塞米松协同作用在多发性骨髓瘤细胞中产生抗肿瘤作用。

[0042] 发明详述

[0043] 本发明提供治疗或预防癌症的方法，所述方法包括给予式 I 的化合物或其药学上可接受的盐，

[0044]



(I)，

[0045] 其中：

[0046] L 表示 CH_2SCH_2、CH_2CH_2、$CH_2CH_2CH_2$、CH_2、CH_2S、SCH_2、CH_2NHCH_2、CH ＝ CH 或 ，

优选 CH_2CH_2，其中 CH 或 CH_2 单元的任何氢原子可被烷基或烷氧基置换，NH 单元的任何氢可被烷基置换，且 CH_2CH_2、$CH_2CH_2CH_2$ 或 CH_2 的 CH_2 单元的任何氢原子可被羟基置换；

[0047] X 每次出现时独立地表示 S、O 或 CH = CH，优选 S 或 CH = CH，其中 CH 单元的任何氢原子可被烷基置换；

[0048] Y 每次出现时独立地表示 H 或 $CH_2O(CO)R_7$；

[0049] R_7 每次出现时独立地表示 H 或取代或未取代的烷基、烷氧基、氨基烷基、烷基氨基烷基、杂环基烷基、芳基烷基或杂环基烷氧基；

[0050] Z 表示 H 或 $R_3(CO)$；

[0051] R_1 和 R_2 各自独立地表示 H、烷基、烷氧基或羟基；

[0052] R_3 每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基、杂芳氧基烷基或 $C(R_8)(R_9)(R_{10})$、$N(R_4)(R_5)$ 或 OR_6，其中任何游离羟基可被酰化形成 $C(O)R_7$；

[0053] R_4 和 R_5 各自独立地表示 H 或取代或未取代的烷基、羟基烷基、酰基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；

[0054] R_6 每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；和

[0055] R_8、R_9 和 R_{10} 各自独立地表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，或 R_8 和 R_9 与它们所连接的碳一起形成碳环环系或杂环环系，其中任何游离羟基可被酰化形成 $C(O)R_7$，且其中 R_8、R_9 和 R_{10} 的至少两个不是 H。

[0056] 在其中烷基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基被取代的某些实施方案中，它们被选自以下的一个或多个取代基取代：取代或未取代的烷基例如全氟烷基（例如三氟甲基）、烯基、烷氧基、烷氧基烷基、芳基、芳烷基、芳基烷氧基、芳氧基、芳氧基烷基、羟基、卤素、烷氧基例如全氟烷氧基（例如三氟甲氧基）、烷氧基烷氧基、羟基烷基、羟基烷基氨基、羟基烷氧基、氨基、氨基烷基、烷基氨基、氨基烷基烷氧基、氨基烷氧基、酰氨基、酰氨基烷基例如全氟酰氨基烷基（例如三氟甲基酰氨基烷基）、酰氧基、环烷基、环烷基烷基、环烷基烷氧基、杂环基、杂环基烷基、杂环基氧基、杂环基烷氧基、杂芳基、杂芳基烷基、杂芳基烷氧基、杂芳氧基、杂芳氧基烷基、杂环基氨基烷基、杂环基氨基烷氧基、酰氨基、酰氨基烷基、脒、亚胺、氧代、羰基（例如羧基、烷氧基羰基、甲酰基或酰基包括全氟酰基（例如 $C(O)CF_3$））、羰基烷基（例如羧基烷基、烷氧基羰基烷基、甲酰基烷基或酰基烷基包括全氟酰基烷基（例如 - 烷基 $C(O)CF_3$））、氨基甲酸酯基、氨基甲酸酯基烷基、脲、脲烷基、硫酸酯基、磺酸酯基、氨基磺酰基、

砜、磺酰胺、磺酰胺烷基、氰基、硝基、叠氮基、巯基、烷硫基、硫代羰基（例如硫酯、硫代乙酸酯基或硫代甲酸酯基）、磷酰基、磷酸酯基、膦酸酯基或亚膦酸酯基。

[0057] 在某些实施方案中，L 表示 CH_2SCH_2、CH_2CH_2、$CH_2CH_2CH_2$、CH_2、CH_2S、SCH_2或 CH_2NHCH_2，其中 CH_2单元的任何氢原子可被烷基或烷氧基置换，且 CH_2CH_2、$CH_2CH_2CH_2$或 CH_2的 CH_2单元的任何氢原子可被羟基置换。在某些实施方案中，L 表示 CH_2SCH_2、CH_2CH_2、CH_2S 或 SCH_2。在某些实施方案中，L 表示 CH_2CH_2。在某些实施方案中，L 不是 CH_2SCH_2。

[0058] 在某些实施方案中，Y 表示 H。

[0059] 在某些实施方案中，X 表示 S 或 CH = CH。在某些实施方案中，一个或两个 X 表示 CH = CH。在某些实施方案中，每个 X 表示 S。在某些实施方案中，一个 X 表示 S，另一个 X 表示 CH = CH。

[0060] 在某些实施方案中，Z 表示 $R_3(CO)$。在其中 Z 为 $R_3(CO)$ 的某些实施方案中，R_3的每次出现不相同（例如式 I 的化合物不是对称的）。

[0061] 在某些实施方案中，R_1和 R_2各自表示 H。

[0062] 在某些实施方案中，R_3表示芳基烷基、杂芳基烷基、环烷基或杂环烷基。在某些实施方案中，R_3表示 $C(R_8)(R_9)(R_{10})$，其中 R_8表示芳基、芳基烷基、杂芳基或杂芳烷基，例如芳基、芳基烷基或杂芳基，R_9表示 H，R_{10}表示羟基、羟基烷基、烷氧基或烷氧基烷基，例如羟基、羟基烷基或烷氧基。

[0063] 在某些实施方案中，L 表示 CH_2SCH_2、CH_2CH_2、CH_2S 或 SCH_2，例如 CH_2CH_2、CH_2S 或 SCH_2，Y 表示 H，X 表示 S，Z 表示 $R_3(CO)$，R_1和 R_2各自表示 H，且每个 R_3表示芳基烷基、杂芳基烷基、环烷基或杂环烷基。在某些这样的实施方案中，R_3的每次出现是相同的。

[0064] 在某些实施方案中，L 表示 CH_2SCH_2、CH_2CH_2、CH_2S 或 SCH_2，Y 表示 H，X 表示 S，Z 表示 $R_3(CO)$，R_1和 R_2各自表示 H，且每个 R_3表示 $C(R_8)(R_9)(R_{10})$，其中 R_8表示芳基、芳基烷基、杂芳基或杂芳烷基，例如芳基、芳基烷基或杂芳基，R_9表示 H，且 R_{10}表示羟基、羟基烷基、烷氧基或烷氧基烷基，例如羟基、羟基烷基或烷氧基。在某些这样的实施方案中，R_3的每次出现是相同的。

[0065] 在某些实施方案中，L 表示 CH_2CH_2，Y 表示 H，X 表示 S 或 CH = CH，Z 表示 $R_3(CO)$，R_1和 R_2各自表示 H，且每个 R_3表示取代或未取代的芳基烷基、杂芳基烷基、环烷基或杂环烷基。在某些这样的实施方案中，每个 X 表示 S。在其它实施方案中，X 的一次或两次出现表示 CH = CH，例如 X 的一次出现表示 S，X 的另一次出现表示 CH = CH。在前述某些实施方案中，R_3的每次出现是相同的。在其中 X 的一次出现表示 S 和 X 的另一次出现表示 CH = CH 的前述其它实施方案中，R_3的两次出现不相同。

[0066] 在某些实施方案中，L 表示 CH_2CH_2，Y 表示 H，X 表示 S，Z 表示 $R_3(CO)$，R_1和 R_2各自表示 H，且每个 R_3表示 $C(R_8)(R_9)(R_{10})$，其中 R_8表示芳基、芳基烷基或杂芳基，R_9表示 H，且 R_{10}表示羟基、羟基烷基或烷氧基。在某些这样的实施方案中，R_8表示芳基，R_{10}表示羟基烷基。在某些这样的实施方案中，R_3的每次出现是相同的。

[0067] 在其中 L 表示 CH_2、$CH_2CH_2CH_2$或 CH_2CH_2，X 表示 O，Z 表示 $R_3(CO)$ 的某些实施方案中，两个 R_3基团均不是烷基例如甲基或 $C(R_8)(R_9)(R_{10})$，其中 R_8、R_9和 R_{10}各自独立地为氢或烷基。

[0068] 在其中 L 表示 CH_2CH_2，X 表示 S，Z 表示 $R_3(CO)$ 的某些实施方案中，两个 R_3基团均

不是苯基或杂芳基，例如 2- 呋喃基。

[0069] 在其中 L 表示 CH_2CH_2，X 表示 O，Z 表示 $R_3(CO)$ 的某些实施方案中，两个 R_3基团均不是 $N(R_4)(R_5)$，其中 R_4为芳基，例如苯基，R_5为 H。

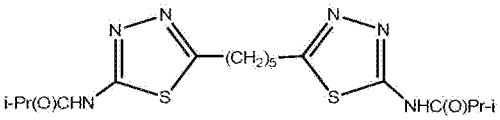
[0070] 在其中 L 表示 CH_2SCH_2，X 表示 S，Z 表示 $R_3(CO)$ 的某些实施方案中，两个 R_3基团均不是芳基，例如任选取代的苯基、芳烷基例如苄基、杂芳基例如 2- 呋喃基、2- 噻吩基或 1、2、4- 三唑基、取代或未取代的烷基例如甲基、氯甲基、二氯甲基、正丙基、正丁基、叔丁基或己基、杂环基例如嘧啶 -2,4(1H,3H)- 二酮或烷氧基例如甲氧基、戊氧基或乙氧基。

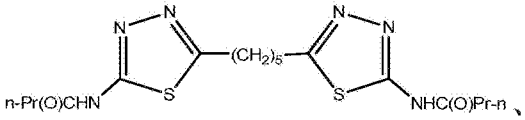
[0071] 在其中 L 表示 CH_2SCH_2，X 表示 S，Z 表示 $R_3(CO)$ 的某些实施方案中，两个 R_3基团均不是 $N(R_4)(R_5)$，其中 R_4为芳基，例如取代或未取代的苯基（例如苯基、3- 甲苯基、4- 甲苯基、4- 溴苯基或 4- 硝基苯基），R_5为 H。

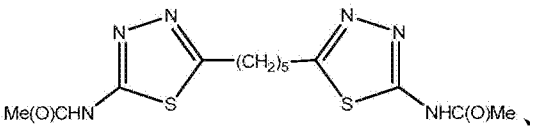
[0072] 在其中 L 表示 $CH_2CH_2CH_2$，X 表示 S，Z 表示 $R_3(CO)$ 的某些实施方案中，两个 R_3基团均不是烷基例如甲基、乙基或丙基、环烷基例如环己基或 $C(R_8)(R_9)(R_{10})$，其中 R_8、R_9和 R_{10}的任一个与它们所连接的 C 一起形成前述任一种。

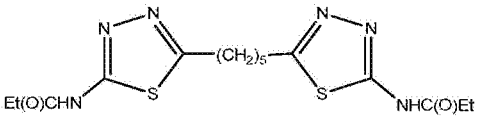
[0073] 在某些实施方案中，化合物不是以下之一：

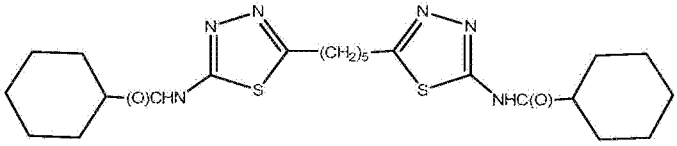
[0074]

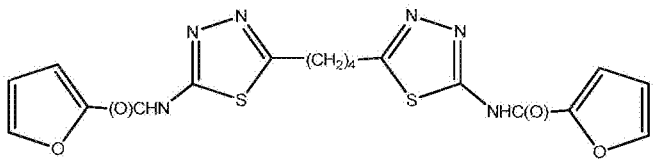

i-Pr(O)CHN
(CH2)5
NHC(O)Pr-i

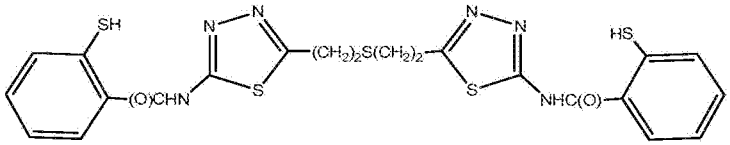

n-Pr(O)CHN
(CH2)5
NHC(O)Pr-n

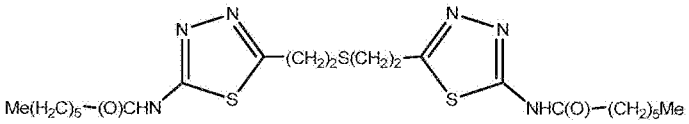

Me(O)CHN
(CH2)5
NHC(O)Me

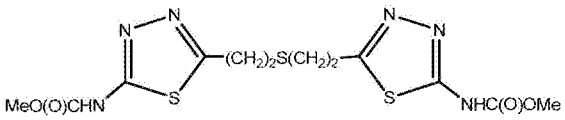

Et(O)CHN
(CH2)5
NHC(O)Et

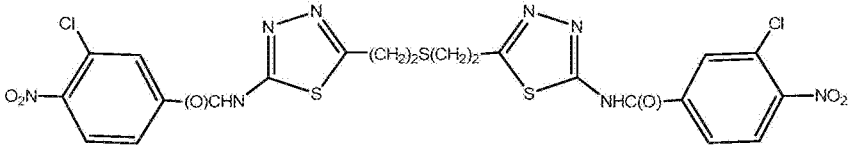

(O)CHN
(CH2)5
NHC(O)

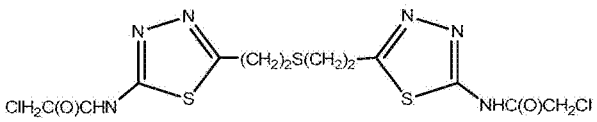

(O)CHN
(CH2)4
NHC(O)
O
O


SH
(O)CHN
(CH2)2S(CH2)2
NHC(O)
HS

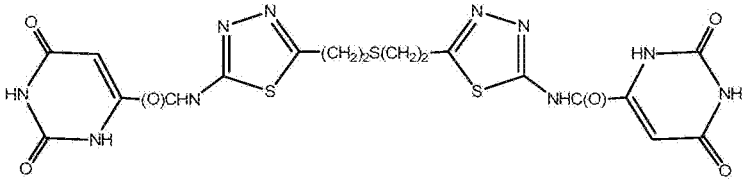

Me(H2C)5
(O)CHN
(CH2)2S(CH2)2
NHC(O)
(CH2)5Me

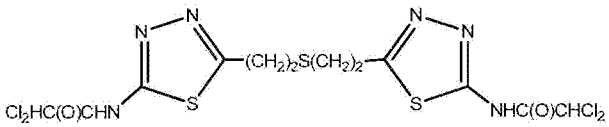

MeO(O)CHN
(CH2)2S(CH2)2
NHC(O)OMe

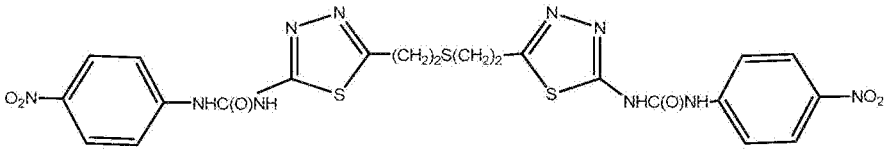

Cl
O2N
(O)CHN
(CH2)2S(CH2)2
NHC(O)
Cl
NO2

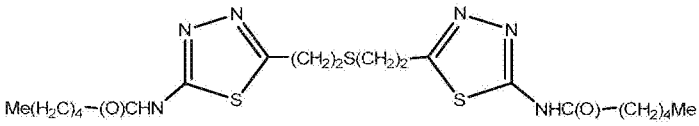

ClH2C(O)CHN
(CH2)2S(CH2)2
NHC(O)CH2Cl

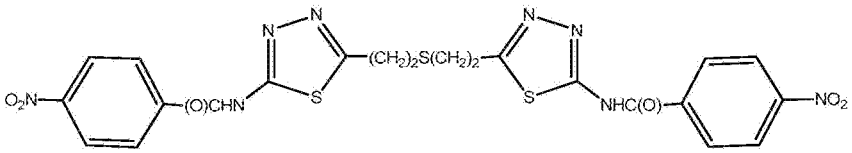
[0075]

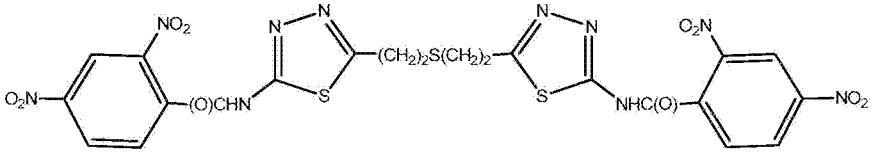


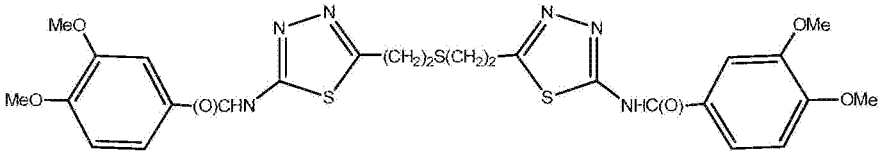


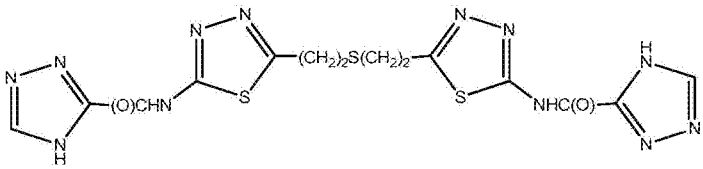


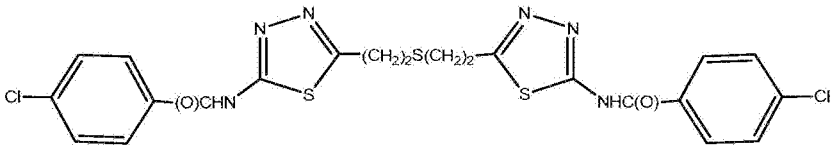


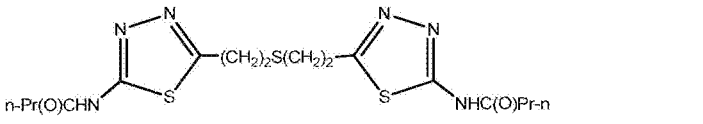




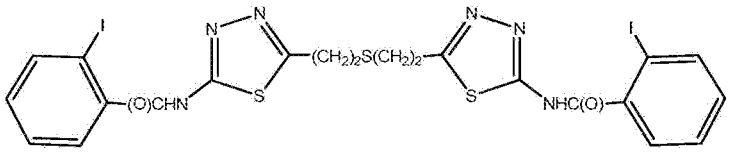








[0076]



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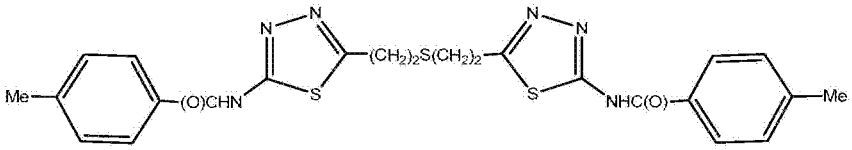

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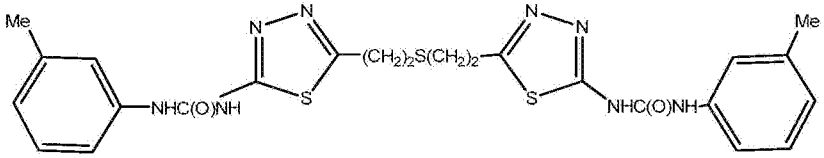

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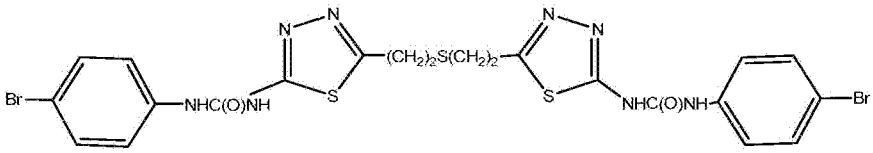

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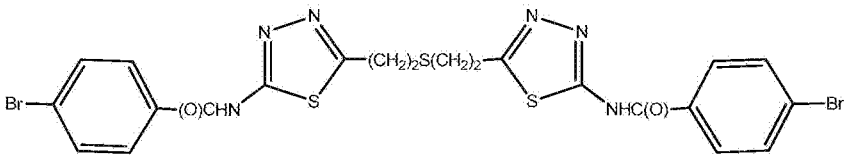

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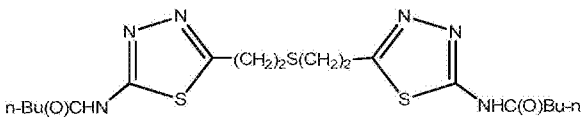

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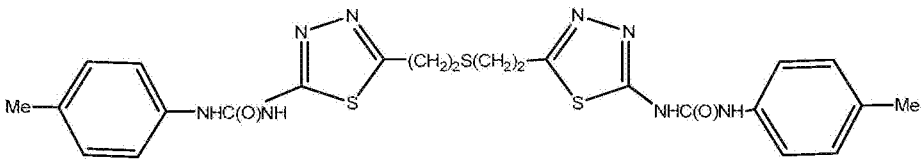

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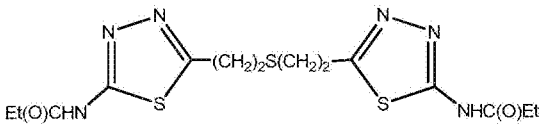

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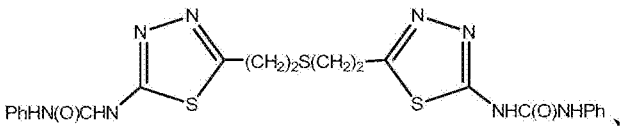

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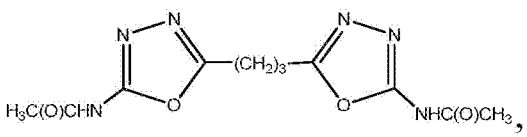

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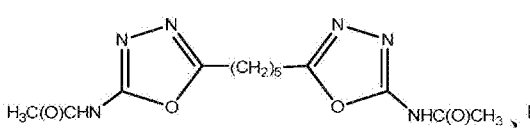

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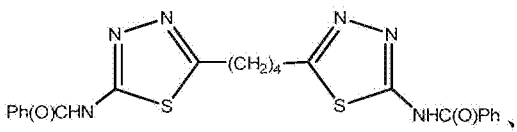

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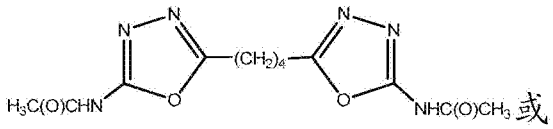
[0077]



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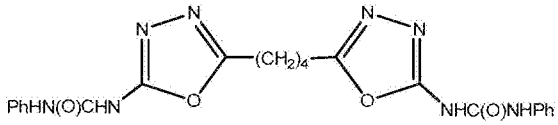

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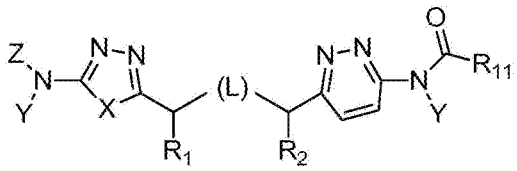
[0078] 本发明还提供治疗或预防癌症的方法，所述方法包括给予式 Ia 化合物或其药学上可接受的盐，

[0079]



(Ia)，

[0080] 其中：

[0081] L 表示 CH_2SCH_2、CH_2CH_2、$CH_2CH_2CH_2$、CH_2、CH_2S、SCH_2、CH_2NHCH_2、CH ＝ CH 或 ，优选 CH_2CH_2，其中 CH 或 CH_2 单元的任何氢原子可被烷基或烷氧基置换，NH 单元的任何氢可被烷基置换，且 CH_2CH_2、$CH_2CH_2CH_2$ 或 CH_2 的 CH_2 单元的任何氢原子可被羟基置换；

[0082] X 表示 S、O 或 CH ＝ CH，优选 S 或 CH ＝ CH，其中 CH 单元的任何氢原子可被烷基置换；

[0083] Y 每次出现时独立地表示 H 或 $CH_2O(CO)R_7$；

[0084] R_7 每次出现时独立地表示 H 或取代或未取代的烷基、烷氧基、氨基烷基、烷基氨基烷基、杂环基烷基、芳基烷基或杂环基烷氧基；

[0085] Z 表示 H 或 $R_3(CO)$；

[0086] R_1 和 R_2 各自独立地表示 H、烷基、烷氧基或羟基，优选 H；

[0087] R_3 表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基、杂芳氧基烷基或 $C(R_8)(R_9)(R_{10})$、$N(R_4)(R_5)$ 或 OR_6，其中任何游离羟基可被酰化形成 $C(O)R_7$；

[0088] R_4 和 R_5 各自独立地表示 H 或取代或未取代的烷基、羟基烷基、酰基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；

[0089] R_6 每次出现时独立地表示取代或未取代的烷基、羟基烷基、氨基烷基、酰氨基烷基、烯基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$；和

[0090] R_8、R_9 和 R_{10} 各自独立地表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、

芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，或 R_8 和 R_9 与它们所连接的碳一起形成碳环环系或杂环环系，其中任何游离羟基可被酰化形成 $C(O)R_7$，且其中 R_8、R_9 和 R_{10} 的至少两个不是 H；

[0091] R_{11} 表示取代或未取代的芳基、芳基烷基、芳氧基、芳氧基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，或 $C(R_{12})(R_{13})(R_{14})$、$N(R_4)(R_{14})$ 或 OR_{14}，其中任何游离羟基可被酰化形成 $C(O)R_7$；

[0092] R_{12} 和 R_{13} 各自独立地表示 H 或取代或未取代的烷基、羟基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烷氧基羰基、烷氧基羰基氨基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基，其中任何游离羟基可被酰化形成 $C(O)R_7$，其中 R_{12} 和 R_{13} 两者不是 H；和

[0093] R_{14} 表示取代或未取代的芳基、芳基烷基、芳氧基、芳氧基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基。

[0094] 在其中烷基、羟基烷基、氨基、酰氨基、氨基烷基、酰氨基烷基、烯基、烷氧基、烷氧基烷基、芳基、芳基烷基、芳氧基、芳氧基烷基、环烷基、环烷基烷基、杂环基、杂环基烷基、杂芳基、杂芳基烷基、杂芳氧基或杂芳氧基烷基被取代的某些实施方案中，它们被选自以下的一个或多个取代基取代：取代或未取代的烷基例如全氟烷基（例如三氟甲基）、烯基、烷氧基、烷氧基烷基、芳基、芳烷基、芳基烷氧基、芳氧基、芳氧基烷基、羟基、卤素、烷氧基例如全氟烷氧基（例如三氟甲基烷氧基）、烷氧基烷氧基、羟基烷基、羟基烷基氨基、羟基烷氧基、氨基、氨基烷基、烷基氨基、氨基烷基烷氧基、氨基烷氧基、酰氨基、酰氨基烷基例如全氟酰氨基烷基（例如三氟甲基酰氨基烷基）、酰氧基、环烷基、环烷基烷基、环烷基烷氧基、杂环基、杂环基烷基、杂环基氧基、杂环基烷氧基、杂芳基、杂芳基烷基、杂芳基烷氧基、杂芳氧基、杂芳氧基烷基、杂环基氨基烷基、杂环基氨基烷氧基、酰氨基、酰氨基烷基、脒、亚胺、氧代、羰基（例如羧基、烷氧基羰基、甲酰基、或酰基、包括全氟酰基（例如 $C(O)CF_3$））、羰基烷基（例如羧基烷基、烷氧基羰基烷基、甲酰基烷基或酰基烷基包括全氟酰基烷基（例如－烷基 $C(O)CF_3$））、氨基甲酸酯基、氨基甲酸酯基烷基、脲、脲烷基、硫酸酯基、磺酸酯基、氨基磺酰基、砜、磺酰胺、磺酰胺烷基、氰基、硝基、叠氮基、巯基、烷硫基、硫代羰基（例如硫酯、硫代乙酸酯基或硫代甲酸酯基）、磷酰基、磷酸酯基、膦酸酯基或亚膦酸酯基。

[0095] 在某些实施方案中，R_{11} 表示取代或未取代的芳基烷基，例如取代或未取代的苄基。

[0096] 在某些实施方案中，L 表示 CH_2SCH_2、CH_2CH_2、$CH_2CH_2CH_2$、CH_2、CH_2S、SCH_2 或 CH_2NHCH_2，其中 CH_2 单元的任何氢原子可被烷基或烷氧基置换，且 CH_2CH_2、$CH_2CH_2CH_2$ 或 CH_2 的 CH_2 单元的任何氢原子可被羟基置换。在某些实施方案中，L 表示 CH_2SCH_2、CH_2CH_2、CH_2S 或 SCH_2，优选 CH_2CH_2。在某些实施方案中，L 不是 CH_2SCH_2。

[0097] 在某些实施方案中，各个 Y 表示 H。在其它实施方案中，至少一个 Y 为 $CH_2O(CO)R_7$。

[0098] 在某些实施方案中，X 表示 S 或 CH ＝ CH。在某些实施方案中，X 表示 S。

[0099] 在某些实施方案中，R_1 和 R_2 各自表示 H。

[0100] 在某些实施方案中，Z 表示 $R_3(CO)$。在其中 Z 为 $R_3(CO)$ 的某些实施方案中，R_3 和 R_{11} 不相同（例如式 I 的化合物不是对称的）。

[0101] 在某些实施方案中，Z 表示 $R_3(CO)$ 和 R_3 表示芳基烷基、杂芳基烷基、环烷基或杂环

烷基。在某些实施方案中，Z 表示 $R_3(CO)$，R_3表示 $C(R_8)(R_9)(R_{10})$，其中 R_8表示芳基、芳基烷基、杂芳基或杂芳烷基例如芳基、芳基烷基或杂芳基，R_9表示 H，R_{10}表示羟基、羟基烷基、烷氧基或烷氧基烷基，例如羟基、羟基烷基或烷氧基。在某些实施方案中，Z 表示 $R_3(CO)$，R_3表示杂芳基烷基。

[0102] 在某些实施方案中，L 表示 CH_2SCH_2、CH_2CH_2、CH_2S 或 SCH_2，例如 CH_2CH_2，Y 表示 H，X 表示 S，Z 表示 $R_3(CO)$，R_1和 R_2各自表示 H，R_3表示芳基烷基、杂芳基烷基、环烷基或杂环烷基，且 R_{11}表示芳基烷基。在某些这样的实施方案中，R_3表示杂芳基烷基。

[0103] 在某些实施方案中，L 表示 CH_2SCH_2、CH_2CH_2、CH_2S 或 SCH_2，例如 CH_2CH_2，Y 表示 H，X 表示 S，Z 表示 $R_3(CO)$，R_1和 R_2各自表示 H，R_3表示 $C(R_8)(R_9)(R_{10})$，其中 R_8表示芳基、芳基烷基、杂芳基或杂芳烷基，例如芳基、芳基烷基或杂芳基，R_9表示 H，R_{10}表示羟基、羟基烷基、烷氧基或烷氧基烷基，例如羟基、羟基烷基或烷氧基，且 R_{11}表示芳基烷基。在某些这样的实施方案中，R_8表示杂芳基。

[0104] 在某些实施方案中，L 表示 CH_2CH_2，Y 表示 H，X 表示 S 或 CH ＝ CH，例如 S，Z 表示 $R_3(CO)$，R_1和 R_2各自表示 H，R_3表示取代或未取代的芳基烷基、杂芳基烷基、环烷基或杂环烷基，且 R_{11}表示芳基烷基。在某些这样的实施方案中，R_3表示杂芳基烷基。

[0105] 在某些实施方案中，L 表示 CH_2CH_2，Y 表示 H，X 表示 S，Z 表示 $R_3(CO)$，R_1和 R_2各自表示 H，R_3表示 $C(R_8)(R_9)(R_{10})$，其中 R_8表示芳基、芳基烷基或杂芳基，R_9表示 H，R_{10}表示羟基、羟基烷基或烷氧基，且 R_{11}表示芳基烷基。在某些这样的实施方案中，R_8表示芳基，且 R_{10}表示羟基烷基。在某些这样的实施方案中，R_8表示杂芳基。

[0106] 在某些实施方案中，癌症选自乳腺癌、结肠直肠癌、内分泌癌、黑素瘤、肾癌和 B 细胞恶性肿瘤。在其中癌症是乳腺癌的某些这类的实施方案中，乳腺癌包括基底型乳腺癌细胞、三阴性乳腺癌细胞或紧蛋白－低乳腺癌细胞。在其中癌症是内分泌癌的某些实施方案中，内分泌癌选自肾上腺皮质腺瘤、肾上腺皮质癌、肾上腺嗜铬细胞瘤和甲状旁腺腺瘤。在其中癌症是 B 细胞恶性肿瘤的某些实施方案中，B 细胞恶性肿瘤选自多发性骨髓瘤；白血病，例如急性成淋巴细胞性白血病或慢性成淋巴细胞性白血病；和淋巴瘤，例如伯基特淋巴瘤、弥漫性大 B 细胞淋巴瘤、滤泡性淋巴瘤或霍奇金淋巴瘤。

[0107] 在某些实施方案中，化合物选自表 3 公开的化合物的任一种。优选，化合物选自化合物 1、2、6、7、8、11、13、14、15、16、17、18、19、20、21、24、25、26、27、28、29、30、31、32、33、35、36、38、39、40、41、43、44、47、48、50、51、52、54、55、58、63、64、65、67、68、69、70、71、72、73、77、78、79、80、81、82、83、84、85、86、87、88、92、93、94、95、97、99、100、102、105、107、111、112、114、115、116、117、118、120、121、122、123、126、127、133、135、136、138、140、141、143、146、147、148、152、153、155、156、157、158、159、160、161、162、163、164、165、166、168、169、170、172、173、174、175、176、177、178、179、180、181、182、185、186、187、188、189、190、193、194、195、196、197、198、199、200、201、202、203、204、205、208、210、211、213、214、216、217、219、220、226、227、228、229、231、232、234、235、236、237、239、240、241、242、243、244、245、246、247、248、249、250、251、252、255、256、257、258、259、260、261、262、263、264、265、266、267、268、269、270、271、273、274、275、276、278、279、280、281、282、283、285、286、287、288、290、291、292、293、294、295、296、297、298、299、300、302、304、1038、306、307、308、309、310、311、313、314、315、316、317、318、319、320、321、322、323、324、325、327、329、332、333、334、

335、336、337、338、339、340、341、342、343、344、345、346、527、347、348、349、350、351、352、353、354、355、358、359、360、361、362、363、364、365、366、367、368、369、370、371、372、373、374、375、376、377、378、379、380、381、382、383、384、385、386、387、388、389、390、391、392、393、394、395、396、397、398、399、400、401、402、403、404、405、406、407、408、409、410、411、412、413、414、415、416、417、418、419、420、421、422、423、424、425、426、427、428、429、430、431、432、433、434、435、436、437、438、439、440、441、442、443、444、445、446、447、448、449、450、451、452、453、454、455、456、457、458、459、460、461、462、463、464、465、466、467、468、469、470、471、472、473、474、475、476、477、478、479、480、481、482、483、484、485、486、487、488、489、490、491、492、493、494、495、496、497、498、499、500、501、502、503、504、505、506、507、508、509、510、511、512、513、514、515、516、517、518、519、520、521、522、523、528、529、530、531、532、533、534、535、536、537、538、539、540、541、542、543、544、545、546、547、548、549、550、551、552、553、554、555、556、557、558、559、560、561、562、563、564、565、566、567、568、569、570、571、572、573、574、575、576、577、578、579、580、581、582、583、584、585、586、587、588、589、590、591、592、593、594、595、596、597、598、599、600、601、602、603、604、605、606、607、608、609、610、611、612、613、614、615、616、617、618、619、620、621、622、623、624、625、626、627、628、629、630、631、632、633、634、635、636、638、639、640、641、644、645、646、647、648、649、650、651、652、653、654、655、656、657、658、659、660、661、662、663、664、665、666、667、668、669、670、671、672、673、674、675、676、677、678、679、680、681、682、683、684、685、686、687、688、689、690、692、693、694、695、696、697、698、699、700、701、702、703、704、705、707、708、709、715、716、717、718、719、720、721、722、723、724、725、726、727、728、729 或 730。

[0108] 在某些实施方案中，本发明的化合物可以是式 I 或式 Ia 的化合物的前药，例如，其中母体化合物中的羟基作为酯或碳酸酯出现，或母体化合物中存在的羧酸作为酯出现。在某些这样的实施方案中，前药在体内代谢为活性母体化合物（例如酯水解成相应的羟基或羧酸）。

[0109] 在某些实施方案中，本发明的化合物可以是外消旋的。在某些实施方案中，本发明的化合物可富含一种对映异构体。例如，本发明的化合物可具有大于 30％对映体过量 (ee)、40％ ee、50％ ee、60％ ee、70％ ee、80％ ee、90％ ee 或甚至 95％或更大的 ee。在某些实施方案中，本发明的化合物可具有多于一个立构中心。在某些这样的实施方案中，本发明的化合物可富含一种或多种非对映体。例如，本发明的化合物可具有大于 30％非对映体过量 (de)、40％ de、50％ de、60％ de、70％ de、80％ de、90％ de 或甚至 95％或更大的 de。

[0110] 在某些实施方案中，本发明涉及用式 I 或式 Ia 化合物或其药学上可接受的盐治疗或预防癌症例如乳腺癌、结肠直肠癌、内分泌癌、黑素瘤、肾癌或 B 细胞恶性肿瘤的方法。在某些实施方案中，可富集治疗制剂以主要提供化合物（例如式 I 或式 Ia 的化合物）的一种对映异构体。对映异构体富集的混合物可包含例如一种对映异构体的至少 60mol％，或更优选至少 75、90、95 或甚至 99mol％。在某些实施方案中，富含一种对映异构体的化合物基本不含另一种对映异构体，其中基本不含意指与例如组合物或化合物混合物中的另一种对映异构体的量相比，所述物质占小于 10％，或小于 5％，或小于 4％，或小于 3％，或小于 2％，或小于 1％。例如，如果组合物或化合物混合物含有 98 克的第一对映异构体和 2 克的第二对

映异构体，则可陈述为含有 98mol％的第一对映异构体和仅 2％的第二对映异构体。

[0111] 在某些实施方案中，可富集治疗制剂以主要提供化合物（例如式 I 或式 Ia 的化合物）的一种非对映体。非对映体富集的混合物可包含例如至少 60mol％的一种非对映体，或更优选至少 75、90、95 或甚至 99mol％。

[0112] 在某些实施方案中，本发明提供适用于人类患者的药物制剂，其包含如上所示化合物的任一种（例如本发明的化合物，例如式 I 或式 Ia 化合物）和一种或多种药学上可接受的赋形剂。在某些实施方案中，药物制剂可用于治疗或预防本文所述病况或疾病。在某些实施方案中，药物制剂具有足够低的致热原活性以适用于人类患者。

[0113] 上述结构的任一个的化合物可用于制备治疗本文公开的任何疾病或病况的药物。

[0114] 酶抑制剂的用途

[0115] 作为氮、碳和能量的载体，谷氨酰胺起着重大作用。它用于肝脲合成、肾生氨作用、糖异生并作为许多细胞的呼吸燃料。细胞通过称为谷氨酰胺合成酶 (GS) 的酶内部合成谷氨酰胺或从环境中外源地得到其谷氨酰胺。

[0116] 谷氨酰胺向谷氨酸的转化由线粒体酶即谷氨酰胺酶引发。有两种主要形式的酶，K 型和 L 型，通过其对谷氨酰胺的 Km 值和对谷氨酸的反应来区分，其中 Km 值，或 Michaelis 常数，是达到最大速度一半所需要的底物浓度。L 型，亦称为“肝型”或 GLS2，对谷氨酰胺具有高 Km，并且是抗谷氨酸的。K 型，亦称为“肾型”或 GLS1 或“KGA”，对谷氨酰胺具有低 Km，并被谷氨酸抑制。最新鉴定出 GLS1 的可变剪接形式，称为谷氨酰胺酶 C 或“GAC”。

[0117] 除了作为蛋白质合成基本结构单元以外，已表明氨基酸促进对生长和分裂细胞是决定性的许多过程，而且对癌细胞尤其如此。几乎所有的癌症定义都包括提及增殖失调。对癌症中的谷氨酰胺代谢的许多研究表明许多肿瘤是急切的谷氨酰胺消耗者 (Souba, Ann. Surg., 1993 ; Collins 等, J. Cell. Physiol., 1998 ; Medina, J. Nutr., 2001 ; Shanware 等, J. Mol. Med., 2011)，这包括但不限于乳腺癌。本发明的某些实施方案涉及本文所述化合物用于治疗乳腺癌的用途。

[0118] 虽然许多癌细胞依靠外源谷氨酰胺赖以生存，但肿瘤细胞亚型中谷氨酰胺依赖程度可使细胞群对谷氨酰胺减少更敏感。例如，乳腺癌的基因表达分析已鉴定出 5 个固有亚型（腔 (luminal)A、腔 B、基底型、HER2+ 和正常样）(Sorlie 等, Proc Natl Acad Sci USA, 2001)。虽然谷氨酰胺剥夺对细胞生长和成活率有影响，但基底样细胞 (basal-like cell) 似乎对外源谷氨酰胺减少更敏感 (Kung 等, PLoS Genetics, 2011)。这支持谷氨酰胺在基底样乳腺癌细胞系中是非常重要的能量来源的观点，并且表明抑制谷氨酰胺酶可有益于治疗由基底样细胞组成的乳腺癌。图 1 进一步支持依赖于外源谷氨酰胺的细胞易受谷氨酰胺酶抑制剂的存在的影响的关联性。本发明的某些实施方案涉及治疗基底样乳腺癌细胞的方法，所述方法包括给予本申请的谷氨酰胺酶抑制剂。

[0119] 可按多种方式测定酶表达水平，根据各个测定法的具体标准，定量是相对的。结果可用来提供遗传概况，其中某些基因、mRNA 或所得表达产物的水平形成可用来表征细胞类型的特征模式 (signature pattern)。Kun 等人证实，表明谷氨酰胺依赖性的基底样乳腺癌细胞显示出这样的遗传概况，其中 GLS 表达相对高，而 GS 表达相对低。此外，GLS2 的表达水平相对低。原发性乳腺肿瘤 mRNA 表达数据集分析 (The Cancer Genome Atlas ; N = 756) 支持基底型细胞一般具有相对于 GS 表达的高的 GLS 表达。

[0120] 三阴性乳腺癌 (TNBC) 的特征在于缺乏雌激素受体 (ER)、黄体酮受体 (PR) 和人表皮生长因子受体 2(HER2) 表达。其比起其它乳腺癌亚型，具有在化学疗法后较高的复发率和较差的预后 (Dent 等，Clin Cancer Res, 2007)。引人关注的是，在 TNBC 细胞和基底样乳腺癌细胞间的代谢概况分析中似乎有显著相似性。具体地说 TNBC 细胞似乎具有高 GLS 表达和低 GS 表达的相似遗传特征 (图 2)。乳腺癌细胞系中更具体的 GLS 表达分析显示，当与激素受体 (HR) 阳性或 Her2 阳性细胞系相比时，TNBC 细胞表达较高水平的 GLS1 的两种剪接变体 KGA 和 GAC，以及显著较低水平的 GS(图 13 和 15)。本发明的方面提供用于治疗包含 TNBC 细胞的乳腺癌的方法，所述方法包括给予本申请的谷氨酰胺酶抑制剂。

[0121] 最近，鉴定出另一种乳腺癌细胞类型，称为紧蛋白 - 低 (Prat 等，Breast Cancer Res, 2010)。该细胞类型的遗传概况也显示相对高的 GLS 表达和低 GS 表达。几个紧蛋白 - 低乳腺癌细胞系的分析显示，这些细胞一般依赖于外源谷氨酰胺，并且还对谷氨酰胺酶抑制敏感。本发明的方面提供用于治疗包含紧蛋白 - 低细胞的乳腺癌的方法，所述方法包括给予本申请的谷氨酰胺酶抑制剂。

[0122] 本发明的另一个方面是本文所述化合物用于治疗包含选自基底型乳腺癌细胞、三阴性乳腺癌细胞和紧蛋白 - 低乳腺癌细胞的细胞的乳腺癌的用途。

[0123] 这就产生了这样的设想，即高 GLS 表达和低 GS 表达概况可用作遗传特征以鉴定可能特别依赖于外源谷氨酰胺并因此对谷氨酰胺酶抑制敏感的其它癌症。在分析来自商业数据库的多种原发性人类癌症时，若干癌症显示高 GLS 至低 GS 表达模式。除之前注意到的乳腺癌以外，结肠直肠癌、内分泌癌、肺癌、黑素瘤、间皮瘤、肾癌和 B 细胞恶性肿瘤具有明显高的 GLS/GS 比率 (图 5 和 10)。本发明的某些实施方案涉及本文所述化合物治疗选自结肠直肠癌、内分泌癌、肺癌、黑素瘤、间皮瘤、肾癌和 B 细胞恶性肿瘤等癌症的用途。

[0124] 与乳腺癌一样，这些癌症的一些的某些亚型似乎具有更普遍的 GLS/GS 表达比。例如，内分泌癌、肾上腺皮质腺瘤、肾上腺皮质癌、肾上腺嗜铬细胞瘤和甲状旁腺腺瘤具有的比率是子宫内膜子宫内膜样腺癌 (endometrial entometrioid adenocarcinoma) 的 3 倍 (图 6)。

[0125] 在数据集内，B 细胞恶性肿瘤包括诸如多发性骨髓瘤、白血病 (包括急性成淋巴细胞性白血病 (ALL) 和慢性成淋巴细胞性白血病 (CLL)) 和淋巴瘤 (包括伯基特淋巴瘤、弥漫性大 B 细胞淋巴瘤、滤泡性淋巴瘤和霍奇金淋巴瘤) 的癌症。所有这些癌症显示包括高 GLS/GS 表达水平比率的遗传概况，进一步说明这些癌症可能对谷氨酰胺酶抑制敏感 (图 7 和 8)。图 17 表明，在多发性骨髓瘤异种移植物模型中，给予谷氨酰胺酶抑制剂化合物减小肿瘤大小，进一步支持这个观点。本发明的某些实施方案涉及本文所述化合物用于治疗多发性骨髓瘤、白血病和淋巴瘤的用途。

[0126] 在一些实施方案中，治疗或预防例如乳腺癌、结肠直肠癌、内分泌癌、黑素瘤、肾癌或 B 细胞恶性肿瘤等癌症的方法，可包括结合一种或多种其它化学治疗剂给予本发明的化合物。可与本发明的化合物联合给予的化学治疗剂包括 :ABT-263、氨鲁米特、安吖啶、阿那曲唑、天冬酰胺酶、bcg、比卡鲁胺、博来霉素、硼替佐米、布舍瑞林、白消安、喜树碱、卡培他滨、卡铂、卡非佐米 (carfilzomib)、卡莫司汀、苯丁酸氮芥、氯喹、顺铂、克拉屈滨、氯膦酸盐、秋水仙素、环磷酰胺、环丙孕酮、阿糖胞苷、达卡巴嗪、放线菌素 D、柔红霉素、脱甲绿胶霉素 (demethoxyviridin)、地塞米松、二氯乙酸盐、己二烯雌酚、己烯雌酚、多西他赛、多柔

比星、表柔比星、表柔比星、雌莫司汀、依托泊苷、依维莫司、依西美坦、非格司亭、氟达拉滨、氟氢可的松、氟尿嘧啶和 5- 氟尿嘧啶、氟甲睾酮、氟他胺、吉西他滨、染料木碱、戈舍瑞林、羟基脲、伊达比星、异环磷酰胺、伊马替尼、干扰素、伊立替康、ironotecan、来那度胺、来曲唑、亚叶酸、亮丙立德、左旋咪唑、洛莫司汀、氯尼达明、氮芥、甲羟孕酮、甲地孕酮、美法仑、巯基嘌呤、美司钠、二甲双胍、甲氨蝶呤、丝裂霉素、米托坦、米托蒽醌、尼鲁米特、诺考达唑、奥曲肽、奥沙利铂、紫杉醇、帕米膦酸盐、喷司他丁、哌立福辛、PF-04691502、普卡霉素、泊马度胺、卟吩姆钠、丙卡巴肼、雷替曲塞、利妥昔单抗、罗米地新、索拉非尼、链佐星、舒尼替尼、苏拉明、他莫昔芬、替莫唑胺、坦罗莫司、替尼泊苷、睾酮、沙利度胺、硫鸟嘌呤、塞替派、二氯二茂钛、托泊替康、曲妥珠单抗、维 A 酸、长春碱、长春新碱、长春地辛、长春瑞滨和伏林司他 (SAHA)。例如，可与本发明的化合物联合给予的化学治疗剂包括 :氨鲁米特、安吖啶、阿那曲唑、天冬酰胺酶、bcg、比卡鲁胺、博来霉素、硼替佐米、布舍瑞林、白消安、喜树碱、卡培他滨、卡铂、卡非佐米、卡莫司汀、苯丁酸氮芥、氯喹、顺铂、克拉屈滨、氯膦酸盐、秋水仙素、环磷酰胺、环丙孕酮、阿糖胞苷、达卡巴嗪、放线菌素 D、柔红霉素、脱甲绿胶霉素、二氯乙酸盐、己二烯雌酚、己烯雌酚、多西他赛、多柔比星、表柔比星、雌二醇、雌莫司汀、依托泊苷、依维莫司、依西美坦、非格司亭、氟达拉滨、氟氢可的松、氟尿嘧啶、氟甲睾酮、氟他胺、吉西他滨、染料木碱、戈舍瑞林、羟基脲、伊达比星、异环磷酰胺、伊马替尼、干扰素、伊立替康、ironotecan、来那度胺、来曲唑、亚叶酸、亮丙立德、左旋咪唑、洛莫司汀、氯尼达明、氮芥、甲羟孕酮、甲地孕酮、美法仑、巯基嘌呤、美司钠、二甲双胍、甲氨蝶呤、丝裂霉素、米托坦、米托蒽醌、尼鲁米特、诺考达唑、奥曲肽、奥沙利铂、紫杉醇、帕米膦酸盐、喷司他丁、哌立福辛、普卡霉素、泊马度胺、卟吩姆钠、丙卡巴肼、雷替曲塞、利妥昔单抗、索拉非尼、链佐星、舒尼替尼、苏拉明、他莫昔芬、替莫唑胺、坦罗莫司、替尼泊苷、睾酮、沙利度胺、硫鸟嘌呤、塞替派、二氯二茂钛、托泊替康、曲妥珠单抗、维 A 酸、长春碱、长春新碱、长春地辛、和长春瑞滨。在其它实施方案中，可与本发明的化合物联合给予的化学治疗剂包括 :ABT-263、地塞米松、5- 氟尿嘧啶、PF-04691502、罗米地新和伏林司他 (SAHA)。在本文所述本发明的方法的某些实施方案中，与本发明的化合物联合给予的化学治疗剂是紫杉烷化学治疗剂，例如紫杉醇或多西他赛。在本文所述本发明的方法的某些实施方案中，与本发明的化合物联合给予的化学治疗剂是多柔比星。在本文所述本发明的方法的某些实施方案中，本发明的化合物与紫杉烷化学治疗剂 (例如紫杉醇) 和多柔比星联合治疗。

[0127] 开发了许多组合疗法用于治疗癌症。在某些实施方案中，本发明的化合物可与组合疗法联合给予。表 1 包括可与本发明的化合物联合给予的组合疗法的实例。

[0128] 表 1 :治疗癌症的示例性组合疗法

[0129]

名称	治疗剂
ABV	多柔比星、博来霉素、长春碱
ABVD	多柔比星、博来霉素、长春碱、达卡巴嗪
AC (乳腺)	多柔比星、环磷酰胺
AC (肉瘤)	多柔比星、顺铂
AC (成神经细胞瘤)	环磷酰胺、多柔比星
ACE	环磷酰胺、多柔比星、依托泊苷
ACe	环磷酰胺、多柔比星
AD	多柔比星、达卡巴嗪
AP	多柔比星、顺铂
ARAC-DNR	阿糖胞苷、柔红霉素
B-CAVe	博来霉素、洛莫司汀、多柔比星、长春碱
BCVPP	卡莫司汀、环磷酰胺、长春碱、丙卡巴肼、泼尼松
BEACOPP	博来霉素、依托泊苷、多柔比星、环磷酰胺、长春新碱、丙卡巴肼、泼尼松、非格司亭
BEP	博来霉素、依托泊苷、顺铂
BIP	博来霉素、顺铂、异环磷酰胺、美司钠
BOMP	博来霉素、长春新碱、顺铂、丝裂霉素
CA	阿糖胞苷、天冬酰胺酶
CABO	顺铂、甲氨蝶呤、博来霉素、长春新碱
CAF	环磷酰胺、多柔比星、氟尿嘧啶
CAL-G	环磷酰胺、柔红霉素、长春新碱、泼尼松、天冬酰胺酶

[0130]

名称	治疗剂
CAMP	环磷酰胺、多柔比星、甲氨蝶呤、丙卡巴肼
CAP	环磷酰胺、多柔比星、顺铂
CaT	卡铂、紫杉醇
CAV	环磷酰胺、多柔比星、长春新碱
CAVE ADD	CAV 和依托泊苷
CA-VP16	环磷酰胺、多柔比星、依托泊苷
CC	环磷酰胺、卡铂
CDDP/VP-16	顺铂、依托泊苷
CEF	环磷酰胺、表柔比星、氟尿嘧啶
CEPP(B)	环磷酰胺、依托泊苷、泼尼松、有或无/博来霉素
CEV	环磷酰胺、依托泊苷、长春新碱
CF	顺铂、氟尿嘧啶或卡铂氟尿嘧啶
CHAP	环磷酰胺或环磷酰胺、六甲蜜胺、多柔比星、顺铂
ChlVPP	苯丁酸氮芥、长春碱、丙卡巴肼、泼尼松
CHOP	环磷酰胺、多柔比星、长春新碱、泼尼松
CHOP-BLEO	将博来霉素加入 CHOP 中
CISCA	环磷酰胺、多柔比星、顺铂
CLD-BOMP	博来霉素、顺铂、长春新碱、丝裂霉素
CMF	甲氨蝶呤、氟尿嘧啶、环磷酰胺
CMFP	环磷酰胺、甲氨蝶呤、氟尿嘧啶、泼尼松
CMFVP	环磷酰胺、甲氨蝶呤、氟尿嘧啶、长春新碱、泼尼松
CMV	顺铂、甲氨蝶呤、长春碱
CNF	环磷酰胺、米托蒽醌、氟尿嘧啶
CNOP	环磷酰胺、米托蒽醌、长春新碱、泼尼松
COB	顺铂、长春新碱、博来霉素
CODE	顺铂、长春新碱、多柔比星、依托泊苷
COMLA	环磷酰胺、长春新碱、甲氨蝶呤、亚叶酸、阿糖胞苷
COMP	环磷酰胺、长春新碱、甲氨蝶呤、泼尼松
Cooper 方案	环磷酰胺、甲氨蝶呤、氟尿嘧啶、长春新碱、泼尼松
COP	环磷酰胺、长春新碱、泼尼松
COPE	环磷酰胺、长春新碱、顺铂、依托泊苷

[0131]

名称	治疗剂
COPP	环磷酰胺、长春新碱、丙卡巴肼、泼尼松
CP (慢性淋巴细胞性白血病)	苯丁酸氮芥、泼尼松
CP (卵巢癌)	环磷酰胺、顺铂
CT	顺铂、紫杉醇
CVD	顺铂、长春碱、达卡巴嗪
CVI	卡铂、依托泊苷、异环磷酰胺、美司钠
CVP	环磷酰胺、长春新碱、Prednisome
CVPP	洛莫司汀、丙卡巴肼、泼尼松
CYVADIC	环磷酰胺、长春新碱、多柔比星、达卡巴嗪
DA	柔红霉素、阿糖胞苷
DAT	柔红霉素、阿糖胞苷、硫鸟嘌呤
DAV	柔红霉素、阿糖胞苷、依托泊苷
DCT	柔红霉素、阿糖胞苷、硫鸟嘌呤
DHAP	顺铂、阿糖胞苷、地塞米松
DI	多柔比星、异环磷酰胺
DTIC/他莫昔芬	达卡巴嗪、他莫昔芬
DVP	柔红霉素、长春新碱、泼尼松
EAP	依托泊苷、多柔比星、顺铂
EC	依托泊苷、卡铂
EFP	依托泊苷 (Etoposie)、氟尿嘧啶、顺铂
ELF	依托泊苷、亚叶酸、氟尿嘧啶
EMA86	米托蒽醌、依托泊苷、阿糖胞苷
EP	依托泊苷、顺铂
EVA	依托泊苷、长春碱
FAC	氟尿嘧啶、多柔比星、环磷酰胺
FAM	氟尿嘧啶、多柔比星、丝裂霉素
FAMTX	甲氨蝶呤、亚叶酸、多柔比星
FAP	氟尿嘧啶、多柔比星、顺铂
F-CL	氟尿嘧啶、亚叶酸
FEC	氟尿嘧啶、环磷酰胺、表柔比星

[0132]

名称	治疗剂
FED	氟尿嘧啶、依托泊苷、顺铂
FL	氟他胺、亮丙立德
FZ	氟他胺、醋酸戈舍瑞林植入剂
HDMTX	甲氨蝶呤、亚叶酸
Hexa-CAF	六甲蜜胺、环磷酰胺、甲氨蝶呤、氟尿嘧啶
ICE-T	异环磷酰胺、卡铂、依托泊苷、紫杉醇、美司钠
IDMTX/6-MP	甲氨蝶呤、巯基嘌呤、亚叶酸
IE	异环磷酰胺、依托泊苷、美司钠
IfoVP	异环磷酰胺、依托泊苷、美司钠
IPA	异环磷酰胺、顺铂、多柔比星
M-2	长春新碱、卡莫司汀、环磷酰胺、泼尼松、美法仑
MAC-III	甲氨蝶呤、亚叶酸、放线菌素 D、环磷酰胺
MACC	甲氨蝶呤、多柔比星、环磷酰胺、洛莫司汀
MACOP-B	甲氨蝶呤、亚叶酸、多柔比星、环磷酰胺、长春新碱、博来霉素、泼尼松
MAID	美司钠、多柔比星、异环磷酰胺、达卡巴嗪
m-BACOD	博来霉素、多柔比星、环磷酰胺、长春新碱、地塞米松、甲氨蝶呤、亚叶酸
MBC	甲氨蝶呤、博来霉素、顺铂
MC	米托蒽醌、阿糖胞苷
MF	甲氨蝶呤、氟尿嘧啶、亚叶酸
MICE	异环磷酰胺、卡铂、依托泊苷、美司钠
MINE	美司钠、异环磷酰胺、米托蒽醌、依托泊苷
mini-BEAM	卡莫司汀、依托泊苷、阿糖胞苷、美法仑
MOBP	博来霉素、长春新碱、顺铂、丝裂霉素
MOP	氮芥、长春新碱、丙卡巴肼
MOPP	氮芥、长春新碱、丙卡巴肼、泼尼松
MOPP/ABV	氮芥、长春新碱、丙卡巴肼、泼尼松、多柔比星、博来霉素、长春碱
MP (多发性骨髓瘤)	美法仑、泼尼松
MP (前列腺癌)	米托蒽醌、泼尼松

[0133]

名称	治疗剂
MTX/6-MO	甲氨蝶呤、巯基嘌呤
MTX/6-MP/VP	甲氨蝶呤、巯基嘌呤、长春新碱、泼尼松
MTX-CDDPAdr	甲氨蝶呤、亚叶酸、顺铂、多柔比星
MV (乳腺癌)	丝裂霉素、长春碱
MV (急性髓细胞性白血病)	米托蒽醌、依托泊苷
M-VAC 甲氨蝶呤	长春碱、多柔比星、顺铂
MVP 丝裂霉素	长春碱、顺铂
MVPP	氮芥、长春碱、丙卡巴肼、泼尼松
NFL	米托蒽醌、氟尿嘧啶、亚叶酸
NOVP	米托蒽醌、长春碱、长春新碱
OPA	长春新碱、泼尼松、多柔比星
OPPA	将丙卡巴肼加入 OPA 中
PAC	顺铂、多柔比星
PAC-I	顺铂、多柔比星、环磷酰胺
PA-CI	顺铂、多柔比星
PC	紫杉醇、卡铂或紫杉醇、顺铂
PCV	洛莫司汀、丙卡巴肼、长春新碱
PE	紫杉醇、雌莫司汀
PFL	顺铂、氟尿嘧啶、亚叶酸
POC	泼尼松、长春新碱、洛莫司汀
ProMACE	泼尼松、甲氨蝶呤、亚叶酸、多柔比星、环磷酰胺、依托泊苷
ProMACE/cytaBOM	泼尼松、多柔比星、环磷酰胺、依托泊苷、阿糖胞苷、博来霉素、长春新碱、甲氨蝶呤、亚叶酸、复方新诺明
PRoMACE/MOPP	泼尼松、多柔比星、环磷酰胺、依托泊苷、氮芥、长春新碱、丙卡巴肼、甲氨蝶呤、亚叶酸
Pt/VM	顺铂、替尼泊苷
PVA	泼尼松、长春新碱、天冬酰胺酶
PVB	顺铂、长春碱、博来霉素
PVDA	泼尼松、长春新碱、柔红霉素、天冬酰胺酶

[0134]

名称	治疗剂
SMF	链佐星、丝裂霉素、氟尿嘧啶
TAD	氮芥、多柔比星、长春碱、长春新碱、博来霉素、依托泊苷、泼尼松
TCF	紫杉醇、顺铂、氟尿嘧啶
TIP	紫杉醇、异环磷酰胺、美司钠、顺铂
TTT	甲氨蝶呤、阿糖胞苷、氢化可的松
Topo/CTX	环磷酰胺、托泊替康、美司钠
VAB-6	环磷酰胺、放线菌素 D、长春碱、顺铂、博来霉素
VAC	长春新碱、放线菌素 D、环磷酰胺
VACAdr	长春新碱、环磷酰胺、多柔比星、放线菌素 D、长春新碱
VAD	长春新碱、多柔比星、地塞米松
VATH	长春碱、多柔比星、塞替派、氟甲睾酮(Flouxymesterone)
VBAP	长春新碱、卡莫司汀、多柔比星、泼尼松
VBCMP	长春新碱、卡莫司汀、美法仑、环磷酰胺、泼尼松
VC	长春瑞滨、顺铂
VCAP	长春新碱、环磷酰胺、多柔比星、泼尼松
VD	长春瑞滨、多柔比星
VelP	长春碱、顺铂、异环磷酰胺、美司钠
VIP	依托泊苷、顺铂、异环磷酰胺、美司钠
VM	丝裂霉素、长春碱
VMCP	长春新碱、美法仑、环磷酰胺、泼尼松
VP	依托泊苷、顺铂
V-TAD	依托泊苷、硫鸟嘌呤、柔红霉素、阿糖胞苷
5+2	阿糖胞苷、柔红霉素、米托蒽醌
7+3	阿糖胞苷以及/、柔红霉素或伊达比星或米托蒽醌
”8 合 1”	甲泼尼龙、长春新碱、洛莫司汀、丙卡巴肼、羟基脲、顺铂、阿糖胞苷、达卡巴嗪

[0135] 癌细胞的增殖需要脂质合成。通常，用于脂质合成的乙酰辅酶 A 从来源于糖酵解的丙酮酸的线粒体库形成。然而在低氧条件例如通常存在于肿瘤环境的条件下，在线粒体内丙酮酸向乙酰辅酶 A 的转化被减量调节。Metallo 等人 (2011) 和 Mullen 等人 (2011) 的最新研究显示，在所述低氧条件下，细胞反倒大多转变成利用涉及 α－酮戊二酸还原羧化以产生用于脂质合成的乙酰辅酶 A 的途径。该途径的第一步包括通过谷氨酰胺酶使谷氨酰

胺转化成谷氨酸。随后，谷氨酸转化为 α－酮戊二酸，在异柠檬酸脱氢酶介导的还原羧化步骤中所得 α－酮戊二酸转化为异柠檬酸。这种还原羧化途径的转变也发生在含有用于诱导负责使糖酵解丙酮酸转化为乙酰辅酶 A 的酶的受损线粒体或受损信号的一些肾癌细胞系中 (Mullen 等，2011)。类似的转变发生在暴露于线粒体呼吸链抑制剂（例如二甲双胍、鱼藤酮和抗霉素）的细胞中 (Mullen 等，2011)。因此，在本发明的一些实施方案中，我们提出使用线粒体呼吸链抑制剂和谷氨酰胺酶抑制剂的组合同时增加癌细胞对脂质合成的谷氨酰胺酶依赖性途径的依赖性，同时恰好抑制这些途径。

[0136] 肿瘤细胞中对糖酵解的依赖性增加是可能的，因为低氧肿瘤环境损害线粒体呼吸作用。此外，葡萄糖耗尽诱导用 MYC 癌基因转化的细胞的凋亡。这些研究结果表明，抑制糖酵解在预防癌细胞增殖中可能具有治疗价值。目前有许多已充分证明的糖酵解抑制剂 (Pelicano 等，2006)。然而，正如 Zhao 等 (2012) 指出的，“可用的糖酵解抑制剂通常不是非常有效，并且需要高剂量，这可能引起高水平的全身毒性”。因为与正常细胞相比癌细胞通常利用较高水平的葡萄糖和谷氨酰胺，所以损害这些代谢物每个的利用可能具有协同效应。因此，在本发明的一些实施方案中，我们提出使用糖酵解途径抑制剂和谷氨酰胺酶抑制剂的组合。所述糖酵解抑制剂包括 2- 脱氧葡萄糖、氯尼达明、3- 溴丙酮酸、伊马替尼、羟基硫胺素、雷帕霉素及其药理学等同物。可通过经由 DNA 损伤消耗 NAD+ 直接抑制糖酵解，所述 DNA 损伤通过 DNA 烷化剂经聚 (ADP- 核糖）聚合酶激活的途径诱导 (Zon 等，2004)。因此，在本发明的一些实施方案中，我们提出使用 DNA 烷化剂和谷氨酰胺酶抑制剂的组合。癌细胞利用戊糖磷酸途径以及糖酵解途径以产生来源于葡萄糖的代谢中间产物。因此，在本发明的一些实施方案中，我们提出使用戊糖磷酸抑制剂（例如 6- 氨基烟酰胺）以及谷氨酰胺酶抑制剂的组合。

[0137] 在某些实施方案中，本发明的化合物可与癌症治疗的非化学方法联合给予。在某些实施方案中，本发明的化合物可与放射疗法联合给予。在某些实施方案中，本发明的化合物可与手术、热切除、聚焦超声疗法、冷冻疗法或这些的任何组合联合给予。

[0138] 在某些实施方案中，本发明的不同化合物可与本发明的一种或多种其它化合物联合给予。此外，所述组合可与其它治疗剂联合给予，所述其它治疗剂例如适于治疗癌症、免疫或神经疾病的其它治疗剂，例如上文鉴定的治疗剂。在某些实施方案中，与本发明的化合物一起联合给予一种或多种其它化学治疗剂提供协同效应，例如图 18 中所示。在某些实施方案中，联合给予一种或多种其它化学治疗剂提供相加效应。

[0139] 在某些实施方案中，本发明提供试剂盒，其包含 :a) 本发明化合物的一个或多个单一剂型 ;b) 上述化学治疗剂的一个或多个单一剂型 ;和 c) 给予本发明的化合物和化学治疗剂用于治疗癌症的说明书，其中癌症选自乳腺癌、结肠直肠癌、内分泌癌、肺癌、黑素瘤、间皮瘤、肾癌和 B 细胞恶性肿瘤。

[0140] 本发明提供试剂盒，其包含 :

[0141] a) 包含本发明的化合物的药物制剂（例如一个或多个单一剂型）;和

[0142] b) 给予药物制剂例如用于治疗或预防癌症例如乳腺癌、结肠直肠癌、内分泌癌、肺癌、黑素瘤、间皮瘤、肾癌或 B 细胞恶性肿瘤的说明书。

[0143] 本发明提供试剂盒，其包含 :

[0144] a) 包含本发明的化合物的药物制剂（例如一个或多个单一剂型）;和

[0145] b) 给予药物制剂例如用于治疗或预防乳腺癌的说明书，其中乳腺癌包括基底型乳腺癌细胞、三阴性乳腺癌细胞或紧蛋白 - 低乳腺癌细胞。

[0146] 在某些实施方案中，试剂盒还包含与上述化学治疗剂联合给予包含本发明化合物的药物制剂的说明书。在某些实施方案中，试剂盒还包含含有上述化学治疗剂的第二药物制剂（例如作为一个或多个单一剂型）。

[0147] 已表明对外源谷氨酰胺的依赖性和高谷氨酰胺酶 (GLS) 和低谷氨酰胺合成酶 (GS) 水平的表达概况两者与癌细胞对谷氨酰胺酶抑制的敏感性相关。利用该信息，可以推理，癌细胞内的代谢物的量可用作预测其对谷氨酰胺酶抑制的敏感性的手段。为了彻底检验这个理论，在之前表明为谷氨酰胺依赖性并对谷氨酰胺酶抑制敏感的 TNBC 细胞中测定谷氨酸和谷氨酰胺水平（图 9）。通过液相色谱法质谱法联用 (LC-MS/MS) 测定谷氨酸和谷氨酰胺的浓度；然而，可采用测定代谢物浓度的任何方法。谷氨酸：谷氨酰胺比率大于或等于 1.5 的细胞的确显示对谷氨酰胺酶抑制敏感。当谷氨酸：谷氨酰胺比率大于或等于 2 时，相关性甚至更强。该结果提供鉴定可获益于用谷氨酰胺酶抑制剂治疗的癌症患者的方法。

[0148] 几个原发性肿瘤异种移植物的分析表明，除了这些之前论述的癌症以外，表达和代谢物相关性延伸至其它肿瘤类型，例如肺和间皮瘤（图 10）。使用 HCT116 结肠癌细胞（图 11）和 H2122 肺腺癌细胞（图 12）的异种移植物研究表明，用本文所述谷氨酰胺酶抑制剂治疗导致肿瘤大小减小。

[0149] 在某些实施方案中，本发明提供鉴定可获益于用谷氨酰胺酶抑制剂治疗的癌症患者的方法，所述方法包括测定癌症患者的癌细胞中谷氨酸与谷氨酰胺的比率，其中比率大于或等于 1.5，例如大于或等于 1.6、大于或等于 1.7、大于或等于 1.8、大于或等于 1.9 或大于或等于 2.0，表明患者可获益于用谷氨酰胺酶抑制剂治疗。在某些这样的实施方案中，测定比率的方法包括测量癌症患者的癌细胞中谷氨酸和谷氨酰胺的量。在某些实施方案中，比率大于或等于 2.0。在前述某些实施方案中，谷氨酰胺酶抑制剂是本文所述化合物（例如式 I 或式 Ia 化合物）。在某些实施方案中，癌症选自 B 细胞恶性肿瘤、乳腺癌、结肠直肠癌、内分泌癌、肺癌、黑素瘤、间皮瘤和肾癌。

[0150] 在某些实施方案中，本发明提供治疗癌症患者的方法，所述方法包括 1) 测定癌症患者的癌细胞中谷氨酸与谷氨酰胺的比率；和 2) 如果谷氨酸与谷氨酰胺的比率大于或等于 1.5，例如大于或等于 1.6、大于或等于 1.7、大于或等于 1.8、大于或等于 1.9 或大于或等于 2.0，则用式 I 或式 Ia 化合物治疗患者。在某些这样的实施方案中，测定比率的方法包括测量癌症患者的癌细胞中谷氨酸和谷氨酰胺的量。在某些实施方案中，谷氨酸与谷氨酰胺的比率大于或等于 2.0。在某些实施方案中，癌症选自 B 细胞恶性肿瘤、乳腺癌、结肠直肠癌、内分泌癌、肺癌、黑素瘤、间皮瘤和肾癌。

[0151] 如上所述，已表明高谷氨酰胺酶 (GLS) 和低谷氨酰胺合成酶 (GS) 表达水平与癌细胞对谷氨酰胺酶抑制的敏感性相关。因此可推论，癌细胞内 GLS 和 GS 的水平可用作预测其对谷氨酰胺酶抑制的敏感性的手段。为了彻底验证这个理论，在 TNBC 细胞（已知其对谷氨酰胺酶抑制更敏感）和在 HR+ 或 Her2+ 细胞（已知其对谷氨酰胺酶抑制较不敏感）中测定 GLS(KGA 和 GAC 两者）水平和 GS 水平（图 14 和表 7）。

[0152] 观察到谷氨酰胺酶抑制剂敏感性和 GLS 的 GAC 同种型的表达之间的相关性。表达

GAC的细胞显得对谷氨酰胺酶抑制更敏感。因此，具有可检测水平的GAC的细胞将对谷氨酰胺酶抑制剂（例如本文所述化合物）敏感。还对表达GAC水平等于或高于KGA的细胞观察到相关性。因此，在某些实施方案中，本发明提供鉴定可获益于用谷氨酰胺酶抑制剂治疗的癌症患者的方法，所述方法包括测定癌症患者的癌细胞中GAC和KGA表达的水平，其中GAC的表达水平大于或等于KGA的表达水平，表明患者可获益于用谷氨酰胺酶抑制剂治疗。

[0153] 在谷氨酰胺酶抑制剂敏感性和GAC:GS的比率之间观察到显著相关性。GAC:GS比率大于或等于0.05的细胞显得对谷氨酰胺酶抑制敏感。对于GAC:GS比率大于或等于1的细胞，相关性甚至更强。该结果提供鉴定可获益于用谷氨酰胺酶抑制剂治疗的癌症患者的手段。

[0154] 在某些实施方案中，本发明提供鉴定可获益于用谷氨酰胺酶抑制剂治疗的癌症患者的方法，所述方法包括测定癌症患者的癌细胞中谷氨酰胺酶与谷氨酰胺合成酶的比率，其中比率大于或等于0.05，例如大于或等于0.06、大于或等于0.07、大于或等于0.08、大于或等于0.9或大于或等于1.0，表明患者可获益于用谷氨酰胺酶抑制剂治疗。在某些这样的实施方案中，测定比率的方法包括测量癌症患者的癌细胞中谷氨酰胺酶和谷氨酰胺合成酶的水平。在某些实施方案中，比率大于或等于1。在前述某些实施方案中，谷氨酰胺酶抑制剂是本文所述化合物（例如式I或式Ia化合物）。在某些实施方案中，谷氨酰胺酶是KGA和GAC两者。在某些实施方案中，谷氨酰胺酶是KGA。在优选的实施方案中，谷氨酰胺酶是GAC。

[0155] 在某些实施方案中，本发明提供治疗癌症患者的方法，所述方法包括1)测定癌症患者的癌细胞中谷氨酰胺酶与谷氨酰胺合成酶的比率；和2)如果谷氨酰胺酶与谷氨酰胺合成酶的比率大于或等于0.05，例如大于或等于0.06、大于或等于0.07、大于或等于0.08、大于或等于0.9或大于或等于1.0，则表明患者可获益于用谷氨酰胺酶抑制剂治疗。在某些这样的实施方案中，测定比率的方法包括测量癌症患者的癌细胞中谷氨酰胺酶和谷氨酰胺合成酶的量。在某些实施方案中，比率大于或等于1。在某些实施方案中，谷氨酰胺酶是KGA和GAC两者。在某些实施方案中，谷氨酰胺酶是KGA。在优选的实施方案中，谷氨酰胺酶是GAC。

[0156] 在某些实施方案中，癌症选自B细胞恶性肿瘤、乳腺癌、结肠直肠癌、内分泌癌、肺癌、黑素瘤、间皮瘤和肾癌。

[0157] GLS(例如KGA和/或GAC)和GS的水平可采用任何合适的方法测量。一些方法包括测量蛋白质水平，其它方法包括测量mRNA的水平。

[0158] 蛋白质量可使用抗体测量。适用于本文公开的方法的抗体是市购可获得的，或可按常规制备。在目标蛋白质测定法中制备和使用抗体的方法是常规的，描述于例如Green等，Production of Polyclonal Antisera，载于Immunochemical Protocols(Manson编辑)，(Humana Press 1992)；Coligan等，载于Current Protocols in Immunology，Sec.2.4.1(1992)；Kohler和Milstein(1975)，Nature 256，495；Coligan等，第2.5.1-2.6.7节；以及Harlow等，Antibodies:A Laboratory Manual，第726页(Cold Spring Harbor Laboratory Pub.1988)。

[0159] 各种抗体的任一种都可用于本发明的方法。所述抗体包括例如多克隆、单克隆(mAb)、重组、人源化或部分人源化、单链、Fab及其片段。抗体可以是任何同种型的，例如

IgM，各种 IgG 同种型例如 IgG1、IgG2a 等，并且可来自产生抗体的任何动物物种，包括山羊、兔、小鼠、鸡等。术语对蛋白质“有特异性的抗体”意指抗体识别蛋白质中氨基酸的规定序列或表位，并且选择性地结合所述蛋白质而一般不结合非预期结合抗体的蛋白质。可采用本领域的常规方法，按常规确定实现特异性结合所需要的参数。

[0160] 在本发明的一些实施方案中，将对 KGA、GAC 和 / 或 GS 有特异性的抗体固定在表面（例如是阵列（例如微阵列）上的反应元件，或在另一种表面上，例如用于基于表面等离子体共振 (SPR) 的技术，例如 Biacore) 上，通过其特异性结合抗体的能力检测样品中的蛋白质。或者，可使样品中的蛋白质固定在表面上，通过其特异性结合抗体的能力检测。制备表面和进行分析的方法，包括对特异性结合有效的条件，是本领域常规的和众所周知的。

[0161] 合适免疫测定法的许多类型中有免疫组织化学染色法、ELISA、蛋白质印迹法（免疫印迹）、免疫沉淀法、放射免疫测定法 (RIA)、荧光激活细胞分选法 (FACS) 等。用于本发明的方法的测定法可基于比色读出、荧光读出、质谱学、目测等。

[0162] 如上所述，可通过测量 mRNA 的量，测量 GLS(KGA 和 / 或 GAC) 和 GS 的表达水平。编码 KGA、GAC 和 / 或 GS 的 mRNA 的量可采用任何合适的方法测量。所述方法的实例包括例如逆转录酶 - 聚合酶链式反应 (RT-PCR)，包括实时 PCR、微阵列分析、nanostring、RNA 印迹分析、差别杂交和核糖核酸酶保护测定。所述方法是本领域众所周知的，描述于例如 Sambrook 等，Molecular Cloning:A Laboratory Manual, 最近版本，Cold Spring Harbor Laboratory,Cold Spring Harbor,N.Y. 以及 Ausubel 等，Current Protocols in Molecular Biology,John Wiley&sons,New York,N.Y。

[0163] 在本发明的一些实施方案中，采用本领域已知的任何方法，从受试者（例如肿瘤活检）中获得组织学样品，所述样品包括但不限于组织切片、穿刺活检等。通常样品可以是“临床样品”，其是来源于患者的样品，包括针对组织学目的获取的组织切片，例如冷冻切片或石蜡切片。样品还可来源于来自细胞培养物的（细胞）上清液或细胞本身、来自组织培养和其它培养基的细胞。然后从样品中获得蛋白质或 mRNA，并用来量化 GLS(KGA 和 / 或 GAC) 和 GS 的量。

[0164] 观察谷氨酰胺酶活性和对谷氨酰胺酶抑制剂的敏感性之间的相关性的一个备选方法见图 16，其中 0.005 μmol/min/mg 蛋白质的谷氨酰胺酶活性预测了对谷氨酰胺酶抑制剂的敏感性。在某些实施方案中，本发明提供鉴定可获益于用谷氨酰胺酶抑制剂治疗的癌症患者的方法，所述方法包括测定癌症患者的癌细胞中的谷氨酰胺酶活性，其中活性大于或等于 0.005 μmol/min/mg 蛋白质，例如大于或等于 0.006 μmol/min/mg 蛋白质、大于或等于 0.007 μmol/min/mg 蛋白质、大于或等于 0.008 μmol/min/mg 蛋白质、大于或等于 0.009 μmol/min/mg 蛋白质或大于或等于 0.010 μmol/min/mg 蛋白质，表明患者可获益于用谷氨酰胺酶抑制剂治疗。在某些这样的实施方案中，测定谷氨酰胺酶活性的方法包括测量癌症患者的癌细胞中的谷氨酰胺酶活性。在某些实施方案中，谷氨酰胺酶活性大于或等于 0.010。在前述某些实施方案中，谷氨酰胺酶抑制剂是本文所述化合物（例如式 I 或式 Ia 化合物）。在某些实施方案中，癌症选自 B 细胞恶性肿瘤、乳腺癌、结肠直肠癌、内分泌癌、肺、黑素瘤、间皮瘤和肾癌。

[0165] 在某些实施方案中，本发明提供治疗癌症患者的方法，所述方法包括 1) 测定癌症患者的癌细胞中的谷氨酰胺酶活性 ；和 2) 其中活性大于或等于 0.005 μmol/min/mg 蛋白

质，例如大于或等于 0.006 μmol/min/mg 蛋白质、大于或等于 0.007 μmol/min/mg 蛋白质、大于或等于 0.008 μmol/min/mg 蛋白质、大于或等于 0.009 μmol/min/mg 蛋白质或大于或等于 0.010 μmol/min/mg 蛋白质，用式 I 或式 Ia 化合物治疗患者。在某些这样的实施方案中，测定的方法测定癌症患者的癌细胞中的谷氨酰胺酶活性。在某些实施方案中，谷氨酸与谷氨酰胺的比率大于或等于 2.0。在某些实施方案中，癌症选自 B 细胞恶性肿瘤、乳腺癌、结肠直肠癌、内分泌癌、肺癌、黑素瘤、间皮瘤和肾癌。

[0166] 本公开内容还提供检测患有癌症的受试者是否可能对谷氨酰胺酶抑制剂起反应的试剂盒。该试剂盒可包括检测本发明蛋白质的表达的量［例如蛋白质的量和 / 或编码蛋白质的核酸（例如 mRNA）的量］的一种或多种试剂。试剂盒中的试剂可包括例如对蛋白质有特异性的抗体或对 mRNA 有特异性的探针，其可用来与 RNA（或从其产生的 cDNA）杂交或进行 RT-PCR。试剂盒还可包括适于检测、测量和 / 或量化蛋白质或核酸的量的其它试剂。在其它用途中，本发明的试剂盒可用于实验应用。技术人员应认识适于实施本发明方法的试剂盒的组分。

[0167] 任选，本发明的试剂盒可包含实施方法的说明书。本发明的试剂盒的任选要素包括合适的缓冲剂、容器或包装材料。试剂盒的试剂可在其中试剂是稳定的容器中，例如呈冻干形式或稳定液体。试剂还可呈一次使用形式，例如以进行针对单个受试者的测定。

[0168] 定义

[0169] 术语“酰基”是本领域公知的，是指用通式烃基 C(O)- 表示的基团，优选烷基 C(O)-。

[0170] 术语“酰氨基”是本领域公知的，是指被酰基取代的氨基，并可用例如式烃基 C(O)NH- 表示。

[0171] 术语“酰氧基”是本领域公知的，是指用通式烃基 C(O)O- 表示的基团，优选烷基 C(O)O-。

[0172] 术语“烷氧基”是指具有与之连接的氧的烷基，优选低级烷基。代表性的烷氧基包括甲氧基、乙氧基、丙氧基、叔丁氧基等。

[0173] 术语“烷氧基烷基”是指被烷氧基取代的烷基，可用通式烷基 -O- 烷基表示。

[0174] 本文所用术语“烯基”是指含有至少一个双键的脂族基团，并意指包括“未取代的烯基”和“取代的烯基”两者，后者是指具有置换烯基的一个或多个碳上的氢的取代基的烯基部分。所述取代基可存在于包括或未包括在一个或多个双键上的一个或多个碳上。然而，所述取代基包括如下所述对烷基所预期的所有取代基，其中稳定性受抑制除外。例如，预期烯基被一个或多个烷基、碳环基、芳基、杂环基或杂芳基取代。

[0175] “烷基”或“烷烃”是完全饱和的直链或支链非芳族烃。通常，直链或支链烷基具有 1- 约 20 个碳原子，优选 1- 约 10 个，除非另有定义。直链和支链烷基的实例包括甲基、乙基、正丙基、异丙基、正丁基、仲丁基、叔丁基、戊基、己基、戊基和辛基。C_1-C_6直链或支链烷基亦称为“低级烷基”基团。

[0176] 此外，整个说明书、实施例和权利要求书中所用的术语“烷基”（或“低级烷基”）意指包括“未取代的烷基”和“取代的烷基”两者，后者是指具有置换烃骨架的一个或多个碳上的氢的取代基的烷基部分。若不是另有说明，否则所述取代基可包括例如卤素、羟基、羰基（例如羧基、烷氧基羰基、甲酰基或酰基）、硫代羰基（例如硫酯、硫代乙酸酯基或硫代甲

酸酯基）、烷氧基、磷酰基、磷酸酯基、膦酸酯基、亚膦酸酯基、氨基、酰氨基、脒、亚胺、氰基、硝基、叠氮基、巯基、烷硫基、硫酸酯基、磺酸酯基、氨基磺酰基、磺酰胺基、磺酰基、杂环基、芳烷基或芳族或杂芳族部分。本领域技术人员应了解，适当时，烃链上取代的部分本身可被取代。例如，取代烷基的取代基可包括取代的和未取代的形式的氨基、叠氮基、亚氨基、酰氨基、磷酰基（包括膦酸酯基和亚膦酸酯基）、磺酰基（包括硫酸酯基、磺酰胺基、氨基磺酰基和磺酸酯基）和甲硅烷基以及醚、烷硫基、羰基（包括酮、醛、羧酸酯和酯）、$-CF_3$、-CN 等。下面描述了示例性的取代烷基。环烷基可被烷基、烯基、烷氧基、烷硫基、氨基烷基、羰基 - 取代的烷基、$-CF_3$、-CN 等进一步取代。

[0177] 术语“C_{x-y}”当与例如酰基、酰氧基、烷基、烯基、炔基或烷氧基等化学部分联用时，意指包括链中含有 x-y 个碳的基团。例如，术语“C_{x-y}烷基”是指取代或未取代的饱和的烃基团，包括链中含有 x-y 个碳的直链烷基和支链烷基，包括卤代烷基例如三氟甲基和 2,2,2- 三氟乙基等。C_0烷基表示其中基团位于末端位置的氢，如果在内部则为键。术语“C_{2-y}烯基”和“C_{2-y}炔基”是指长度和可能取代类似于上述烷基但分别含有至少一个双键或三键的取代或未取代的不饱和脂族基团。

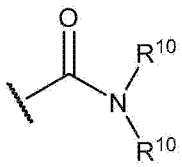
[0178] 本文所用术语“烷基氨基”是指被至少一个烷基取代的氨基。

[0179] 本文所用术语“烷硫基”是指被烷基取代的硫醇基，可用通式烷基 S- 表示。

[0180] 本文所用术语“炔基”是指含有至少一个三键的脂族基团，并意指包括“未取代的炔基”和“取代的炔基”两者，后者是指具有置换炔基的一个或多个碳上的氢的取代基的炔基部分。所述取代基可发生在包括或未包括在一个或多个三键中的一个或多个碳上。此外，所述取代基包括如上论述的烷基所预期的所有取代基，其中稳定性受抑制除外。例如，考虑炔基被一个或多个烷基、碳环基、芳基、杂环基或杂芳基取代。

[0181] 本文所用术语“酰胺”是指基团

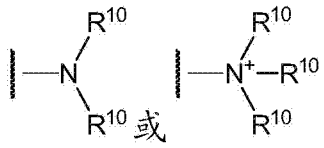
[0182]



[0183] 其中各 R^{10}独立地表示氢或烃基，或两个 R^{10}与它们所连接的 N 原子一起完成在环结构中具有 4-8 个原子的杂环。

[0184] 术语“胺”和“氨基”是本领域公知的，是指未取代的和取代的胺两者及其盐，例如可用以下表示的部分

[0185]



[0186] 其中各个 R^{10}独立地表示氢或烃基，或两个 R^{10}与它们所连接的 N 原子一起完成环结构中具有 4-8 个原子的杂环。

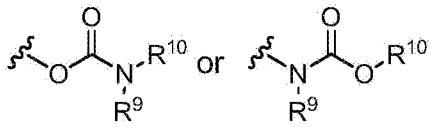
[0187] 本文所用术语“氨基烷基”是指被氨基取代的烷基。

[0188] 本文所用术语“芳烷基”是指被芳基取代的烷基。

[0189] 本文所用术语“芳基”包括其中环的每个原子是碳的取代或未取代的单环芳族基团。优选环是 5 元 -7 元环，更优选 6 元环。术语“芳基”还包括具有两个或更多个环的多环环系，其中两个或更多个碳为两个毗连环公用，其中至少一个环是芳族的，例如另一个环可以是环烷基、环烯基、环炔基、芳基、杂芳基和 / 或杂环基。芳基包括苯、萘、菲、苯酚、苯胺等。

[0190] 术语“氨基甲酸酯基”是本领域公知的，是指基团

[0191]



或

[0192] 其中 R^9和 R^{10}独立表示氢或烃基，例如烷基，或 R^9和 R^{10}与间插原子一起完成环结构中具有 4-8 个原子的杂环。

[0193] 本文所用术语“碳环”和“碳环的”是指其中环的每个原子是碳的饱和或不饱和环。术语碳环包括芳族碳环和非芳族碳环两者。非芳族碳环包括其中所有碳原子是饱和的环烷烃环和含有至少一个双键的环烯烃环两者。“碳环”包括 5-7 元单环和 8-12 元二环。二环碳环的各个环可选自饱和环、不饱和环和芳环。碳环包括其中两个环间共有 1、2 或 3 或更多个原子的二环分子。术语“稠合碳环”是指其中每个环与另一个环共有两个邻接原子的二环碳环。稠合碳环的每个环可选自饱和环、不饱和环和芳环。在一个示例性的实施方案中，芳环（例如苯基）可与饱和或不饱和环（例如环己烷、环戊烷或环己烯）稠合。如化合价允许，饱和二环、不饱和二环和芳族二环的任何组合都包括在碳环的定义中。示例性“碳环”包括环戊烷、环己烷、二环 [2.2.1] 庚烷、1,5- 环辛二烯、1,2,3,4- 四氢化萘、二环 [4.2.0] 辛 -3- 烯、萘和金刚烷。示例性稠合碳环包括萘烷、萘、1,2,3,4- 四氢化萘、二环 [4.2.0] 辛烷、4,5,6,7- 四氢 -1H- 茚和二环 [4.1.0] 庚 -3- 烯。“碳环”可在能够携带氢原子的任一个或多个位置上被取代。

[0194] “环烷基”基团是完全饱和的环烃。“环烷基”包括单环和二环。通常，单环环烷基具有 3- 约 10 个碳原子，更通常 3-8 个碳原子，除非另有定义。二环环烷基的第二个环可选自饱和环、不饱和环和芳环。环烷基包括其中两个环间共有 1、2 或 3 或更多个原子的二环分子。术语“稠合环烷基”是指其中每个环与另一个环共有两个邻接原子的二环环烷基。稠合二环环烷基的第二个环可选自饱和环、不饱和环和芳环。“环烯基”基团是含有一个或多个双键的环烃。

[0195] 本文所用术语“碳环基烷基”是指被碳环基团取代的烷基。

[0196] 术语“碳酸酯基”是本领域公知的，是指基团 $-OCO_2-R^{10}$，其中 R^{10}表示烃基。

[0197] 本文所用术语“羧基”是指用式 $-CO_2H$ 表示的基团。

[0198] 本文所用术语“酯”是指基团 $-C(O)OR^{10}$，其中 R^{10}表示烃基。

[0199] 本文所用术语“醚”是指通过氧与另一个烃基连接的烃基。因此，烃基的醚取代基可以是烃基 -O-。醚可以是对称或不对称的。醚的实例包括但不限于杂环 -O- 杂环和芳基 -O- 杂环。醚包括“烷氧基烷基”基团，其可用通式烷基 -O- 烷基表示。

[0200] 本文所用术语“卤代”和“卤素”意指卤素，包括氯、氟、溴和碘。

[0201] 本文所用术语“杂芳烷基”和“杂芳基烷基”是指被杂芳基取代的烷基。

[0202] 本文所用术语“杂烷基”是指碳原子和至少一个杂原子的饱和或不饱和链，其中无两个杂原子相邻。

[0203] 术语“杂芳基 (heteroaryl/hetaryl)”包括取代或未取代的芳族单环结构，优选 5 元 -7 元环，更优选 5-6 元环，其环结构包括至少一个杂原子，优选 1-4 个杂原子，更优选一个或两个杂原子。术语“杂芳基”还包括具有两个或更多个环的多环环系，其中两个或更多个碳为两个毗邻环公用，其中至少一个环是杂芳族的，例如另一个环可以是环烷基、环烯基、环炔基、芳基、杂芳基和 / 或杂环基。杂芳基包括例如吡咯、呋喃、噻吩、咪唑、噁唑、噻唑、吡唑、吡啶、吡嗪、哒嗪和嘧啶等。

[0204] 本文所用术语“杂原子”意指碳或氢以外的任何元素的原子。优选的杂原子为氮、氧和硫。

[0205] 术语“杂环基”、“杂环”和“杂环的”是指取代或未取代的非芳环结构，优选 3-10 元环，更优选 3-7 元环，其环结构包括至少一个杂原子，优选 1-4 个杂原子，更优选一个或两个杂原子。术语“杂环基”和“杂环的”还包括具有两个或更多个环的多环环系，其中两个或更多个碳为两个毗邻环共用，其中至少一个环是杂环，例如另一个环可以是环烷基、环烯基、环炔基、芳基、杂芳基和 / 或杂环基。杂环基包括例如哌啶、哌嗪、吡咯烷、吗啉、内酯、内酰胺等。

[0206] 本文所用术语“杂环基烷基”是指被杂环基取代的烷基。

[0207] 本文所用术语“烃基”是指没有 = O 或 = S 取代基、通过碳原子键合的基团，且通常具有至少一个碳 - 氢键和主要的碳骨架，但可任选包括杂原子。因此对于本申请的目的，像甲基、乙氧基乙基、2- 吡啶基和三氟甲基等基团被视为烃基，但取代基例如乙酰基（其在连接碳上具有 = O 取代基）和乙氧基（其通过氧而非碳连接）不是。烃基包括但不限于芳基、杂芳基、碳环、杂环基、烷基、烯基、炔基及其组合。

[0208] 本文所用术语“羟基烷基”是指被羟基取代的烷基。

[0209] 术语“低级”当与例如酰基、酰氧基、烷基、烯基、炔基或烷氧基等化学部分联用时意在包括其中取代基中有 10 个或更少个非氢原子、优选 6 个或更少个的基团。例如，“低级烷基”是指含有 10 个或更少个碳原子、优选 6 个或更少个的烷基。在某些实施方案中，本文定义的酰基、酰氧基、烷基、烯基、炔基或烷氧基取代基分别是低级酰基、低级酰氧基、低级烷基、低级烯基、低级炔基或低级烷氧基，不论它们是单独或与取代基一起出现，例如在描述羟基烷基和芳烷基（在该情况下，例如，当计算烷基取代基的碳原子时，芳基内的原子不算入）中。

[0210] 术语“多环基”、“多环”和“多环的”是指两个或更多个环（例如环烷基、环烯基、环炔基、芳基、杂芳基和 / 或杂环基），其中两个或更多个原子为两个毗邻环共用，例如环为“稠环”。多环的每个环可以是取代或未取代的。在某些实施方案中，多环的每个环在环中含有 3-10 个原子，优选 5-7 个。

[0211] 术语“甲硅烷基”是指具有 3 个烃基部分与之连接的硅部分。

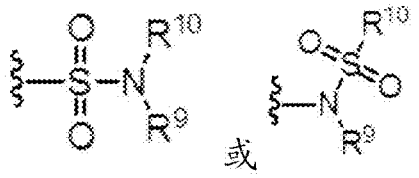
[0212] 术语“取代的”是指具有置换骨架的一个或多个碳上的氢的取代基的部分。应了解，“取代”或“被……取代”包括所述取代符合取代原子和取代基的容许的化合价且取代导致稳定化合物的隐含条件，例如所述化合物不会自发地进行例如通过重排、环化、消除等

的转化。本文所用术语“取代的”预期包括有机化合物的所有容许的取代基。从大的方面来看，可容许的取代基包括有机化合物的无环和环状、支链和无支链的、碳环和杂环、芳族和非芳族的取代基。对于适宜的有机化合物，可容许的取代基可为一个或多个、相同或不同的。对于本发明的目的，杂原子（例如氮）可具有满足杂原子化合价的本文所述有机化合物的氢取代基和/或任何可容许的取代基。取代基可包括本文所述的任何取代基，例如卤素、羟基、羰基（例如羧基、烷氧基羰基、甲酰基或酰基）、硫代羰基（例如硫酯、硫代乙酸酯基或硫代甲酸酯基）、烷氧基、磷酰基、磷酸酯基、膦酸酯基、亚膦酸酯基、氨基、酰氨基、脒、亚胺、氰基、硝基、叠氮基、巯基、烷硫基、硫酸酯基、磺酸酯基、氨基磺酰基、磺酰胺基、磺酰基、杂环基、芳烷基或芳族或杂芳族部分。本领域技术人员应了解，适当时，取代基本身可被取代。除非明确规定为“未取代的”，否则本文提及化学部分要理解为包括取代的变体。例如，提及“芳基”基团或部分无疑包括取代和未取代的变体两者。

[0213] 术语“硫酸酯基”是本领域公知的，是指基团 $-OSO_3H$ 或其药学上可接受的盐。

[0214] 术语“磺酰胺”是本领域公知的，是指用以下通式表示的基团

[0215]



[0216] 其中 R^9 和 R^{10} 独立表示氢或烃基，例如烷基，或 R^9 和 R^{10} 与间插原子一起完成环结构中具有 4-8 个原子的杂环。

[0217] 术语“亚砜”是本领域公知的，是指基团 $-S(O)-R^{10}$，其中 R^{10} 表示烃基。

[0218] 术语“磺酸酯基”是本领域公知的，是指基团 SO_3H 或其药学上可接受的盐。

[0219] 术语“砜”是本领域公知的，是指基团 $-S(O)_2-R^{10}$，其中 R^{10} 表示烃基。

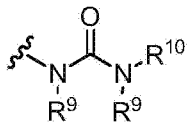
[0220] 本文所用术语“硫烷基”是指被硫醇基取代的烷基。

[0221] 本文所用术语“硫酯”是指基团 $-C(O)SR^{10}$ 或 $-SC(O)R^{10}$，其中 R^{10} 表示烃基。

[0222] 本文所用术语“硫醚”相当于醚，其中氧被硫置换。

[0223] 术语“脲”是本领域公知的，可用以下通式表示

[0224]



[0225] 其中 R^9 和 R^{10} 独立表示氢或烃基，例如烷基，或 R^9 任一个的每次出现与 R^{10} 和间插原子一起完成环结构中具有 4-8 个原子的杂环。

[0226] “保护基”是指当与分子中的反应性官能团连接时，掩蔽、降低或防止官能团的反应性的原子基团。通常，在合成过程中，可按需选择性脱去保护基。保护基的实例可参见 Greene 和 Wuts, Protective Groups in Organic Chemistry, 第 3 版，1999, John Wiley&Sons, NY 和 Harrison 等，Compendium of Synthetic Organic Methods, 第 1-8 卷，1971-1996, John Wiley&Sons, NY。代表性的氮保护基包括但不限于甲酰基、乙酰基、三氟乙酰基、苄基、苄氧基羰基（“CBZ”）、叔丁氧基羰基（“Boc”）、三甲基甲硅烷基（“TMS”）、

2- 三甲基甲硅烷基 - 乙磺酰基 (“TES”)、三苯甲基和取代的三苯甲基、烯丙氧基羰基、9- 芴基甲基氧基羰基 (“FMOC”)、硝基 - 藜芦基氧基羰基 (“NVOC”) 等。代表性的羟基保护基包括但不限于其中羟基被酰化 (酯化) 或烷基化的那些,例如苄基和三苯甲基醚,以及烷基醚、四氢吡喃基醚、三烷基甲硅烷基醚 (例如 TMS 或 TIPS 基团)、二醇醚,例如乙二醇和丙二醇衍生物和烯丙基醚。

[0227] 术语“医疗保健提供者”是指向个人、社区等提供医疗保健服务的个人或团体。“医疗保健提供者”的实例包括医生、医院、连续护理型退休社区 (continuing care retirement communities)、专业护理机构、亚急性护理机构、诊所、全科诊所、独立流动中心 (freestanding ambulatory center)、居家卫生机构 (home health agencies) 和 HMO。

[0228] 如本文所用,“预防”病症或病况的治疗剂是指在统计样品中,相对于未治疗的对照样品降低经治疗的样品的病症或病况发生率,或相对于未治疗的对照样品延迟病症或病况的一个或多个症状的发生或降低其严重性的化合物。

[0229] 术语“治疗”包括预防性和 / 或治疗性治疗。术语“预防性或治疗性”治疗是本领域公知的,包括将主题组合物的一种或多种给予宿主。如果在不需要的情况 (例如宿主动物的疾病或其它不需要的状态) 临床表现之前给予,则治疗是预防性的 (即它防止宿主出现不需要的情况),而如果在不需要的情况表现之后给予,则治疗是治疗性的 (即它旨在减轻、改善或稳定现有的不需要的情况或其副作用)。

[0230] 术语“前药”意在包括在生理条件下转化成本发明的治疗活性剂 (例如式 I 的化合物) 的化合物。制备前药的通用方法包括在生理条件下水解露出所需分子的一个或多个选择的部分。在其它实施方案中,前药被宿主动物的酶活性转化。例如,酯或碳酸酯 (例如醇或羧酸的酯或碳酸酯) 是本发明的优选前药。在某些实施方案中,上文所示制剂中的一些或所有式 I 化合物可用相应的合适前药替换,例如,其中母体化合物中的羟基作为酯或碳酸酯呈现,或者存在于母体化合物中的羧酸作为酯存在。

[0231] 药物组合物

[0232] 本发明的组合物和方法可用来治疗有需要的个体。在某些实施方案中,个体是哺乳动物,例如人或非人哺乳动物。当给予动物 (例如人) 时,组合物或化合物优选作为包含例如本发明的化合物和药学上可接受的载体的药物组合物给予。药学上可接受的载体是本领域众所周知的,包括例如含水溶液例如水或生理缓冲盐水或其它溶剂或溶媒,例如二醇、甘油、油 (例如橄榄油) 或注射用有机酯。在一个优选的实施方案中,当所述药物组合物是用于人类给药,特别用于侵入途径的给药 (即避开通过上皮屏障转运或扩散的途径,例如注射或植入) 时,含水溶液是无致热原或基本无致热原的。可选择赋形剂以实现例如药剂的缓释或选择性靶向一个或多个细胞、组织或器官。药物组合物可呈剂量单位形式,例如片剂、胶囊剂 (包括撒粉胶囊剂 (sprinkle capsule) 和明胶胶囊剂)、颗粒剂、复溶用冻干剂、散剂、溶液剂、糖浆剂、栓剂、注射剂等。组合物还可以透皮递送系统 (例如皮肤贴剂) 存在。组合物还可以适于局部给药的溶液剂 (例如滴眼剂) 存在。

[0233] 药学上可接受的载体可含有生理上可接受的作用剂,其起例如稳定化合物 (例如本发明的化合物)、提高化合物 (例如本发明的化合物) 溶解度或增加化合物 (例如本发明的化合物) 吸收的作用。这类生理上可接受的作用剂包括例如糖,例如葡萄糖、蔗糖或葡聚糖 ;抗氧化剂,例如抗坏血酸或谷胱甘肽 ;螯合剂 ;低分子量蛋白质或其它稳定剂或赋形

剂。所选的药学上可接受的载体，包括生理上可接受的作用剂，取决于例如组合物的给药途径。制剂或药物组合物可以是自乳化药物递送系统或自微乳化药物递送系统。药物组合物（制剂）还可以是例如本发明的化合物可掺入其中的脂质体或其它聚合物基质。例如包含磷脂或其它脂质的脂质体是相对容易制备和给予的无毒的生理上可接受的和可代谢的载体。

[0234] 本文使用短语“药学上可接受的”是指在合理医学判断范围内、适用于与人类和动物组织接触而无过度毒性、刺激性、变态反应或其它问题或并发症、与合理的利益 / 风险比相称的那些化合物、材料、组合物和 / 或剂型。

[0235] 本文所用短语“药学上可接受的载体”意指药学上可接受的材料、组合物或溶媒，例如液体或固体填充剂、稀释剂、赋形剂、溶剂或包封材料。从与制剂的其它成分相容并对患者无害的意义来看，各载体必须是“可接受的”。可用作药学上可接受的载体的材料的一些实例包括：(1) 糖，例如乳糖、葡萄糖和蔗糖；(2) 淀粉，例如玉米淀粉和马铃薯淀粉；(3) 纤维素及其衍生物，例如羧甲基纤维素钠、乙基纤维素和醋酸纤维素；(4) 西黄蓍胶粉；(5) 麦芽；(6) 明胶；(7) 滑石；(8) 赋形剂，例如可可脂和栓剂蜡；(9) 油，例如花生油、棉籽油、红花油、芝麻油、橄榄油、玉米油和大豆油；(10) 二醇，例如丙二醇；(11) 多元醇，例如甘油、山梨糖醇、甘露糖醇和聚乙二醇；(12) 酯，例如油酸乙酯和月桂酸乙酯；(13) 琼脂；(14) 缓冲剂，例如氢氧化镁和氢氧化铝；(15) 藻酸；(16) 无热原水；(17) 等渗盐水；(18) 林格氏液；(19) 乙醇；(20) 磷酸盐缓冲溶液；和 (21) 用于药物制剂的其它无毒的相容物质。

[0236] 可通过各种给药途径的任一种将药物组合物（制剂）给予受试者，包括例如口服（例如如在水性或非水溶液或混悬液的兽用顿服药、片剂、胶囊剂（包括撒粉胶囊剂和明胶胶囊剂）、大丸剂、散剂、颗粒剂、舌用糊剂）；通过口腔黏膜吸收（例如舌下）；肛门、直肠或阴道（例如作为阴道栓、乳膏剂或泡沫剂）；胃肠外（包括肌内、静脉内、皮下或鞘内、作为例如无菌溶液剂或混悬剂）；经鼻；腹膜内；皮下；透皮（例如作为施用于皮肤的贴剂）；和局部（例如作为施用于皮肤的乳膏剂、软膏剂或喷雾剂，或作为滴眼剂）。还可以配制化合物用于吸入。在某些实施方案中，可将化合物简单溶解于或悬浮于无菌水中。合适的给药途径和适于所述给药途径的组合物的详细描述可参见例如美国专利号 6,110,973、5,763,493、5,731,000、5,541,231、5,427,798、5,358,970 和 4,172,896 以及其中引用的专利。

[0237] 制剂可适宜地存在于单位剂型中，可通过制药领域众所周知的任何方法制备。可与载体材料混合以产生单一剂型的活性成分的量将随待治疗的宿主、具体的给药方式而变化。可与载体材料混合以产生单一剂型的活性成分的量一般将是产生治疗作用的化合物的量。一般而言，在 100％中计，该量的范围可为约 1％ - 约 99％的活性成分，优选约 5％ - 约 70％，最优选约 10％ - 约 30％。

[0238] 制备这些制剂或组合物的方法包括将活性化合物（例如本发明的化合物）与载体和任选一种或多种助剂成分联合的步骤。一般来说，通过将本发明的化合物与液体载体或细碎的固体载体或两者充分均匀地混合，然后如有需要，使产物成形，来制备制剂。

[0239] 适于口服给予的本发明的制剂可呈以下形式：胶囊剂（包括撒粉胶囊剂和明胶胶囊剂）、扁囊剂、丸剂、片剂、锭剂（使用调味基料、通常为蔗糖和阿拉伯树胶或西黄蓍胶）、冻干剂、散剂、颗粒剂或作为水性或非水液体中的溶液剂或混悬剂，或作为水包油或油包水

液体乳剂，或作为酏剂或糖浆剂，或作为软锭剂（使用惰性基料，例如明胶和甘油，或蔗糖和阿拉伯树胶）和/或作为漱口剂等，各自含有预定量的本发明的化合物作为活性成分。还可以大丸剂、糖药剂或糊剂给予组合物或化合物。

[0240] 为了制备口服给药的固体剂型（胶囊剂（包括撒粉胶囊剂和明胶胶囊剂）、片剂、丸剂、糖衣丸、散剂、颗粒剂等），将活性成分与一种或多种药学上可接受的载体（例如柠檬酸钠或磷酸二钙）和/或以下的任一种混合：(1) 填充剂或增量剂，例如淀粉、乳糖、蔗糖、葡萄糖、甘露糖醇，和/或硅酸；(2) 粘合剂，例如羧甲基纤维素、藻酸盐、明胶、聚乙烯吡咯烷酮、蔗糖和/或阿拉伯树胶；(3) 湿润剂，例如甘油；(4) 崩解剂，例如琼脂、碳酸钙、马铃薯或木薯淀粉、藻酸、某些硅酸盐和碳酸钠；(5) 溶液阻滞剂，例如石蜡；(6) 吸收促进剂，例如季铵化合物；(7) 润湿剂，例如鲸蜡醇和甘油单硬脂酸酯；(8) 吸收剂，例如高岭土和膨润土；(9) 润滑剂，例如滑石、硬脂酸钙、硬脂酸镁、固体聚乙二醇、十二烷基硫酸钠及其混合物；(10) 络合剂，例如改性和未改性环糊精；和 (11) 着色剂。在胶囊剂（包括撒粉胶囊剂和明胶胶囊剂），片剂和丸剂的情况下，药物组合物还可包含缓冲剂。使用诸如乳糖 (lactose/milk sugar) 等赋形剂以及高分子量聚乙二醇等，类似类型的固体组合物也可用作软充填和硬充填的明胶胶囊剂中的填充剂。

[0241] 可任选与一种或多种助剂成分一起，通过压制或模制来制备片剂。可使用粘合剂（例如明胶或羟丙基甲基纤维素）、润滑剂、惰性稀释剂、防腐剂、崩解剂（例如羟基乙酸淀粉钠或交联羧甲基纤维素钠）、表面活性剂或分散剂，来制备压制片。通过在合适的机器中模制用惰性液体稀释剂湿润的粉状化合物的混合物，来制备模制片。

[0242] 片剂和药物组合物的其它固体剂型，例如糖衣丸、胶囊剂（包括撒粉胶囊剂和明胶胶囊剂）、丸剂和颗粒剂，可任选划痕或用包衣材料和外壳材料（例如肠溶包衣和制药领域众所周知的其它包衣材料）制备。它们还可使用例如不同比例的羟丙基甲基纤维素（以提供所需释放特征）、其它聚合物基质、脂质体和/或微球体，进行配制以提供其中活性成分的慢释或控释。它们可通过例如截留细菌滤器过滤，或通过以临用前可溶于无菌水或一些其它无菌注射用介质的无菌固体组合物的形式掺入杀菌剂来灭菌。这些组合物还可任选含有遮光剂，并且可以具有只在或优先在胃肠道的某些部分，任选以延时方式释放活性成分的组成。可以使用的包埋组合物的实例包括聚合物质和蜡。活性成分还可呈微囊化形式，在适当时，与上述赋形剂的一种或多种一起。

[0243] 可用于口服给药的液体剂型包括药学上可接受的乳剂、复溶用冻干剂、微乳剂、溶液剂、混悬剂、糖浆剂和酏剂。除活性成分以外，液体剂型可含有常用于本领域的惰性稀释剂，例如水或其它溶剂、环糊精及其衍生物、增溶剂和乳化剂，例如乙醇、异丙醇、碳酸乙酯、乙酸乙酯、苄醇、苯甲酸苄酯、丙二醇、1,3-丁二醇、油（特别是棉籽油、落花生油、玉米油、胚芽油、橄榄油、蓖麻油和芝麻油）、甘油、四氢糠醇、聚乙二醇和山梨醇酐的脂肪酸酯及其混合物。

[0244] 除惰性稀释剂以外，口服组合物还可包括辅助剂，例如润湿剂、乳化剂和助悬剂、甜味剂、矫味剂、着色剂、芳香剂和防腐剂。

[0245] 除活性化合物以外，混悬剂可含有助悬剂，例如乙氧基化异硬脂醇、聚氧乙烯山梨糖醇和山梨坦酯、微晶纤维素、偏氢氧化铝 (aluminum metahydroxide)、膨润土、琼脂和西黄蓍胶及其混合物。

[0246] 用于直肠、阴道或尿道给药的药物组合物的制剂可作为栓剂呈现，其可通过将一种或多种活性化合物与一种或多种合适的无刺激的赋形剂或载体（包含例如可可脂、聚乙二醇、栓剂蜡或水杨酸酯）混合来制备，并且在室温下是固体，但在体温下是液体，因此在直肠或阴道腔中将融化并释放活性化合物。

[0247] 用于给予口腔的药物组合物的制剂可作为漱口剂，或口腔喷雾剂，或口腔软膏剂呈现。

[0248] 或者或另外地，可配制组合物用于通过导管、支架、金属丝或其它管腔内装置递送。通过这类装置的递送尤其可用于递送到膀胱、尿道、输尿管、直肠或肠。

[0249] 适于阴道给药的制剂还包括含有本领域已知是合适的这类载体的阴道栓、棉塞、乳膏剂、凝胶剂、糊剂、泡沫剂或喷雾制剂。

[0250] 用于局部或经皮给药的剂型包括散剂、喷雾剂、软膏剂、糊剂、乳膏剂、洗剂、凝胶剂、溶液、贴剂和吸入剂。可在无菌条件下将活性化合物与药学上可接受的载体和可能需要的任何防腐剂、缓冲剂或抛射剂混合。

[0251] 除活性化合物以外，软膏剂、糊剂、乳膏剂和凝胶剂可含有赋形剂，例如动物和植物脂肪、油、蜡、石蜡、淀粉、西黄蓍胶、纤维素衍生物、聚乙二醇、硅酮、膨润土、硅酸、滑石和氧化锌或其混合物。

[0252] 除活性化合物以外，散剂和喷雾剂可含有赋形剂，例如乳糖、滑石、硅酸、氢氧化铝、硅酸钙和聚酰胺粉或这些物质的混合物。喷雾剂另外可含有常规抛射剂，例如氯氟烃和挥发性未取代的烃，例如丁烷和丙烷。

[0253] 透皮贴剂具有提供将本发明的化合物受控递送到机体的附加优势。这类剂型可通过活性化合物溶解或分散在合适的介质中来制备。还可使用吸收促进剂增加化合物跨越皮肤的流动。可通过提供速率控制膜或将化合物分散在聚合物基质或凝胶中来控制这种流动的速率。

[0254] 还考虑眼用制剂、眼膏、散剂、溶液剂等在本发明的范围内。示例性眼用制剂描述于美国公布号 2005/0080056、2005/0059744、2005/0031697 和 2005/004074 和美国专利号 6,583,124，其内容通过引用结合到本文中。如有需要，液体眼用制剂具有类似于泪液、房水或玻璃体液的性质或与所述流体相当。优选的给药途径是局部给药（例如局部给药，例如滴眼剂，或通过植入剂给予）。

[0255] 本文所用短语“胃肠外给药”和“胃肠外给予”意指肠内和局部给药以外的给药方式，通常通过注射，包括而不限于静脉内、肌内、动脉内、鞘内、囊内、眶内、心内、真皮内、腹膜内、经气管、皮下、表皮下、关节内、囊下、蛛网膜下、脊柱内和胸骨内注射和输注。

[0256] 适于胃肠外给药的药物组合物包含一种或多种活性化合物以及一种或多种药学上可接受的无菌等渗的水性或非水溶液剂、分散剂、混悬剂或乳剂，或临用前可复溶为无菌注射溶液剂或分散剂的无菌粉剂，其可含有抗氧化剂、缓冲剂、抑菌剂、使制剂与预期接受者血液等渗的溶质或助悬剂或增稠剂。

[0257] 可用于本发明的药物组合物的合适的含水和非含水载体的实例包括水、乙醇、多元醇（例如甘油、丙二醇、聚乙二醇等）及其合适的混合物、植物油（例如橄榄油）和注射用有机酯（例如油酸乙酯）。例如，可通过使用包衣材料（例如卵磷脂）、在分散剂的情况下通过保持所需粒度和通过使用表面活性剂，来保持合适的流动性。

[0258] 这些组合物还可含有辅助剂例如防腐剂、润湿剂、乳化剂和分散剂。可通过包括各种抗细菌剂和抗真菌剂，例如对羟基苯甲酸酯、三氯叔丁醇、苯酚、山梨酸等，确保防止微生物的作用。可能还需要将等渗剂（例如糖、氯化钠等）包括在组合物中。另外，可通过包括延迟吸收的作用剂（例如单硬脂酸铝和明胶），引起注射药物形式的延长吸收。

[0259] 在某些情况下，为了延长药物的作用，需要减慢药物自皮下或肌内注射的吸收。这可通过使用水溶解度差的结晶或非晶形材料的液体悬浮液来实现。药物的吸收速率则取决于其溶解速率，这进而可取决于晶体大小和晶型。或者，胃肠外给予的药物形式的延长吸收通过将药物溶于或悬浮在油溶媒中来实现。

[0260] 通过在生物可降解的聚合物（例如聚丙交酯-聚乙交酯）中形成主题化合物的微囊化基质，来制备注射贮库形式。可根据药物与聚合物的比率和所用具体聚合物的性质，控制药物释放的速率。其它生物可降解的聚合物的实例包括聚原酸酯和聚酐。另通过将药物包封在与机体组织相容的脂质体或微乳剂中，来制备贮库注射制剂。

[0261] 对于用于本发明的方法，可给予活性化合物本身或作为含有例如0.1-99.5％（更优选0.5-90％）的活性成分以及药学上可接受的载体的药物组合物给予。

[0262] 还通过可再装载或生物可降解的装置提供引入方法。近年来开发了各种慢释聚合物装置并在体内测试用于药物（包括蛋白质性生物制剂）的控制递送。各种生物相容的聚合物（包括水凝胶），包括生物可降解的和不可降解的聚合物两者，都可用于形成化合物在特定靶标部位缓释的植入剂。

[0263] 药物组合物中活性成分的实际剂量水平可改变，以获得有效实现针对特定患者、组合物和给药方式所需的治疗反应而对患者无毒的一定量的活性成分。

[0264] 选择的剂量水平将取决于各种因素，包括所用的具体化合物或化合物的组合或其酯、盐或酰胺的活性、给药途径、给药时间、所用具体化合物的排泄率、治疗持续时间、与所用具体化合物联用的其它药物、化合物和/或材料、待治疗的患者的年龄、性别、体重、病况、一般健康状况和既往病史等医学领域众所周知的因素。

[0265] 具有本领域普通技术的医师或兽医可容易地确定和开给所需的治疗有效量的药物组合物。例如，医师或兽医可以低于达到所需治疗作用的水平开始药物组合物或化合物的剂量，逐渐增加剂量直到达到所需作用。所谓“治疗有效量”意指足以引发所需治疗作用的化合物的浓度。一般认为，有效量的化合物将随受试者的体重、性别、年龄和病史而变化。影响有效量的其它因素可包括但不限于患者病况的严重性、待治疗的病症、化合物的稳定性，以及如有需要，与本发明的化合物一起给予的另一种类型的治疗剂。可通过多次给予所述治疗剂，来递送较大的总剂量。确定功效和剂量的方法是本领域技术人员已知的(Isselbacher等(1996)Harrison's Principles of Internal Medicine第13版，1814-1882，通过引用结合到本文。

[0266] 一般来说，用于本发明的组合物和方法的活性化合物的合适日剂量可以是有效产生治疗作用的最低剂量的化合物的量。这类有效剂量一般将取决于上述因素。

[0267] 如有需要，可作为一整天内以合适间隔给予的1、2、3、4、5、6个或更多个亚剂量，任选以单位剂型给予有效日剂量的活性化合物。在本发明的某些实施方案中，活性化合物可每日2或3次给予。在优选的实施方案中，活性化合物可每日一次给予。

[0268] 接受该治疗的患者是有需要的任何动物，一般包括灵长类动物（特别是人）和其

它哺乳动物，例如马、牛、猪和绵羊；家禽和宠物。

[0269] 在某些实施方案中，本发明的化合物可单独使用或与另一种类型的治疗剂联合给予。本文所用短语“联合给予”是指给予两种或更多种不同的治疗化合物的任何形式使得在之前给予的治疗化合物在体内仍有效时给予第二化合物（例如两种化合物在患者中同时有效，这可包括两种化合物的协同效应）。例如，不同的治疗化合物可以相同的制剂或以单独的制剂同时或序贯给予。在某些实施方案中，不同的治疗化合物可彼此在 1 小时、12 小时、24 小时、36 小时、48 小时、72 小时或 1 周内给予。因此，接受所述治疗的个体可获益于不同治疗化合物的联合作用。

[0270] 在某些实施方案中，相对于本发明的化合物（例如式 I 或式 Ia 化合物）或一种或多种其它治疗剂的各自单独给予，本发明的化合物与一种或多种其它治疗剂（例如一种或多种其它化学治疗剂）的联合给予提供提高的功效。在某些这样的实施方案中，联合给予提供相加效应，其中相加效应是指单独给予本发明的化合物和一种或多种其它治疗剂的各自作用的总和。

[0271] 本发明包括本发明化合物的药学上可接受的盐在本发明的组合物和方法中的用途。在某些实施方案中，本发明预期的盐包括但不限于烷基、二烷基、三烷基或四烷基铵盐。在某些实施方案中，本发明预期的盐包括但不限于 L- 精氨酸、苯乙苄胺、苄星青霉素、甜菜碱、氢氧化钙、胆碱、丹醇、二乙醇胺、二乙胺、2-（二乙基氨基）乙醇、乙醇胺、乙二胺、N- 甲基葡糖胺、海巴明 (hydrabamine)、1H- 咪唑、锂、L- 赖氨酸、镁、4-(2- 羟基乙基）吗啉、哌嗪、钾、1-(2- 羟基乙基）吡咯烷、钠、三乙醇胺、氨丁三醇和锌盐。在某些实施方案中，本发明预期的盐包括但不限于 Na、Ca、K、Mg、Zn 或其它金属盐。

[0272] 药学上可接受的酸加成盐还可作为例如与水、甲醇、乙醇、二甲基甲酰胺等的各种溶剂合物存在。还可制备这类溶剂合物的混合物。这类溶剂合物的来源可来自结晶的溶剂、制备或结晶的溶剂中固有的，或对所述溶剂而言为外来的。

[0273] 润湿剂、乳化剂和润滑剂（例如十二烷基硫酸钠和硬脂酸镁）以及着色剂、脱模剂、包衣剂、甜味剂、矫味剂和芳香剂、防腐剂和抗氧化剂也可存在于组合物中。

[0274] 药学上可接受的抗氧化剂的实例包括：(1) 水溶性抗氧化剂，例如抗坏血酸、盐酸半胱氨酸、硫酸氢钠、偏亚硫酸钠、亚硫酸钠等；(2) 油溶性抗氧化剂，例如抗坏血酸棕榈酸酯、丁羟茴醚 (BHA)、丁基化羟基甲苯 (BHT)、卵磷脂、没食子酸丙酯、α - 生育酚等；和 (3) 金属 - 螯合剂，例如柠檬酸、乙二胺四乙酸 (EDTA)、山梨糖醇、酒石酸、磷酸等。

[0275] 在某些实施方案中，本发明涉及通过制备本发明化合物的制剂或本文所述试剂盒，并向医疗保健提供者营销使用制剂或试剂盒以治疗或预防本文所述任何疾病或病况的益处，来实施制药商务的方法。

[0276] 在某些实施方案中，本发明涉及通过提供用于销售本发明化合物的制剂或本文所述试剂盒的行销网，并向患者或医师提供使用制剂治疗或预防本文所述任何疾病或病况的说明资料，来实施制药商务的方法。

[0277] 在某些实施方案中，本发明包括通过确定本发明化合物治疗或预防本文所述任何疾病或病况的合适制剂和剂量，在动物中针对功效和毒性进行所鉴定的制剂的治疗概况分析，并提供销售具有可接受的治疗概况的已鉴定制剂的行销网，来实施制药商务的方法。在某些实施方案中，所述方法还包括提供向医疗保健提供者营销制剂的销售组。

[0278] 在某些实施方案中，本发明涉及通过确定本发明的化合物治疗或预防本文所述任何疾病或病况的合适的制剂和剂量，向第三方放发有权进一步开发和销售制剂的许可，来实施制药商务的方法。

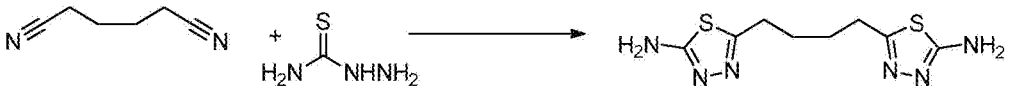
实施例

[0279] 实施例 1 :合成方案

[0280] 连接基核心的合成 :

[0281] 5,5’-(丁烷 -1,4- 二基)- 双 (1,3,4- 噻二唑 -2- 胺)(1001)

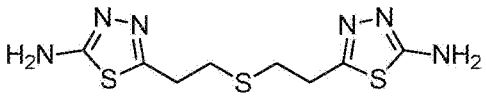
[0282]



1001

[0283] 将己二腈 (8.00g,73.98mmol) 和氨基硫脲 (13.48g,147.96mmol) 在三氟乙酸 (TFA)(75mL) 中的混合物在 80℃下加热 17 小时。使反应物冷却至室温，倒入冰和水的混合物中。将氢氧化钠小粒加入混合物中直到它为碱性 (pH 14)。白色沉淀通过吸滤收集，用水漂洗并干燥，得到 5,5’-(丁烷 -1,4- 二基)- 双 (1,3,4- 噻二唑 -2- 胺)(1001,13.07g)。^{1}H NMR(300MHz, DMSO-d_6) δ 7.00(s, 4H), 2.84(bs, 4H), 1.68(bs, 4H)。5,5’-(硫代双 (乙烷 -2,1- 二基)) 双 (1,3,4- 噻二唑 -2- 胺)(1002) 的合成

[0284]

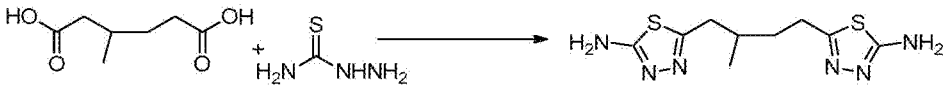


1002

[0285] 化合物 1002 按照 US/2002/0115698A1 所述制备。

[0286] 5,5’-(2- 甲基丁烷 -1,4- 二基)- 双 (1,3,4- 噻二唑 -2- 胺)(1003)

[0287]

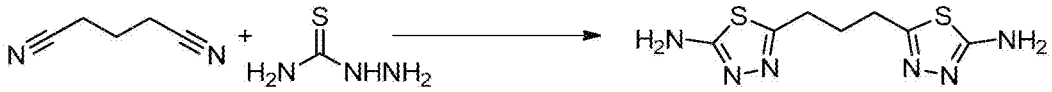


1003

[0288] 将 3- 甲基己二酸 (5.00g,31.22mmol) 和氨基硫脲 (5.69g,62.43mmol) 在 $POCl_3$(45mL) 中的混合物在 90℃下加热 4 小时。使反应物冷却至室温，倒入冰和水的混合物中。将氢氧化钠小粒加入混合物中直到它为碱性 (pH 14)。白色沉淀通过吸滤收集，用水漂洗并干燥，得到 5,5’-(2- 甲基丁烷 -1,4- 二基)- 双 (1,3,4- 噻二唑 -2- 胺)(1003,8.97g)。^{1}H NMR(300MHz, DMSO-d_6) δ 7.00(s, 4H), 2.89 - 2.81(m, 3H), 2.89 - 2.81(m, 3H), 2.69(dd, J = 7.6, 7.6Hz, 1H), 1.89 - 1.46(m, 3H), 0.94(d, J = 6.6Hz, 3H)。

[0289] 5,5’-(丙烷 -1,3- 二基)- 双 (1,3,4- 噻二唑 -2- 胺)(1004)

[0290]



1004

[0291] 将戊二腈(5.00g,53.13mmol)和氨基硫脲(9.68g,106.26mmol)在TFA(50mL)中的混合物在85℃下加热4小时。使反应物冷却至室温,倒入冰和水的混合物中。将氢氧化钠小粒加入混合物中直到它为碱性(pH 14)。白色沉淀通过吸滤收集,用水漂洗并干燥,得到5,5'-(丙烷-1,3-二基)-双(1,3,4-噻二唑-2-胺)(1004,13.72g)。^{1}H NMR(300MHz,DMSO-d_6)δ7.06-7.03(s,4H),2.87(t,J=7.5Hz,4H),2.02-1.95(m,2H)。

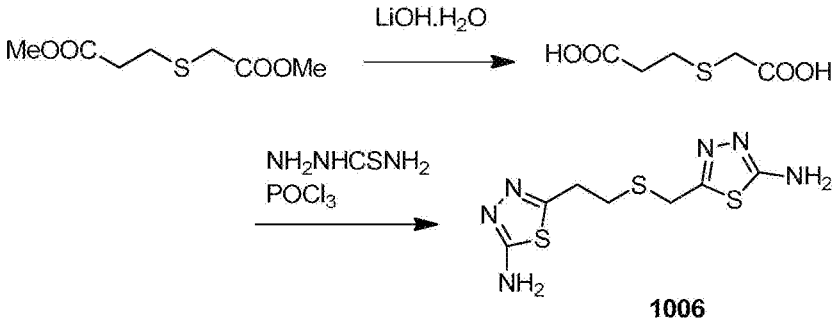
[0292] 5-(2-((2-(5-氨基-1,3,4-噻二唑-2-yl)乙基)氨基)乙基)-1,3,4-噻二唑-2-胺(1005)

[0293]

1005

[0294] 将3,3'-亚氨基二丙腈(1.50g,12.18mmol)和氨基硫脲(2.22g,24.36mmol)在TFA(10mL)中的混合物在85℃下加热4.5小时。使反应物冷却至室温,倒入冰和水的混合物中。将氢氧化钠小粒加入混合物中直到它为碱性(pH 14)。白色沉淀通过吸滤收集,用水漂洗并干燥,得到5-(2-((2-(5-氨基-1,3,4-噻二唑-2-基)乙基)氨基)乙基)-1,3,4-噻二唑-2-胺(1005,1.47g)。^{1}H NMR(300MHz,DMSO-d_6)δ6.95(s,4H),2.90(d,J=6.0Hz,4H),2.83(d,J=6.3Hz,4H)。

[0295]

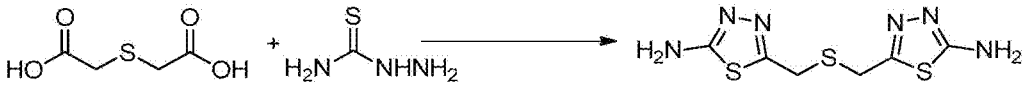


1006

[0296] 向3-((2-甲氧基-2-氧代乙基)硫代)丙酸甲酯(5.0g,26mmol)的THF/MeOH/水(60mL,4:1:1)溶液中加入一水氢氧化锂(4.375g,101mmol)。将所得混合物在室温下搅拌过夜后,将其减压浓缩。所得残余物用水(~100mL)稀释,所得溶液用6N HCl酸化。使混合物在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发,得到3-((羧基甲基)硫代)丙酸(3.64g,85%),为白色固体。^{1}H NMR(300MHz,DMSO-d6)δppm 2.55-2.57(t,2H)2.75-2.79(t,2H)3.27(s,2H)12.41(s,2H)。

[0297] 向3-((羧基甲基硫代)丙酸(3.64g,22.2mmol)和氨基硫脲(4.1g,45mmol)的混合物中慢慢加入三氯氧化磷(25mL)。将所得混合物在90℃下搅拌3小时后,慢慢倒入碎冰上。将分离的固体过滤,通过固体氢氧化钠使滤液碱化到pH~13。分离的固体经过滤,用水洗涤,在45℃下真空干燥过夜,得到1006(~3g,50%),为黄褐色固体。^{1}H NMR(300MHz,DMSO-d6)δppm 2.79-2.83(t,2H)3.06-3.10(t,2H)3.99(s,2H)7.04(s,2H)7.16(s,2H)。

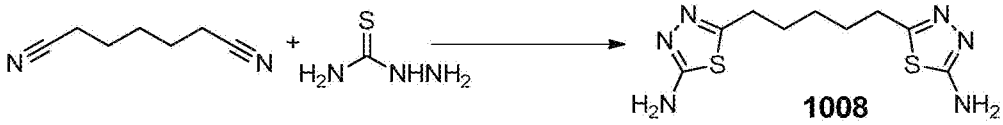
[0298]



1007

[0299] 将2,2'-硫代二乙酸(5.00g,33.3mmol)和氨基硫脲(6.07g,66.6mmol)在$POCl_3$(40mL)中的混合物在90℃下加热5小时。使反应物冷却至室温,小心地将其倒入冰和水的混合物中。将氢氧化钠小粒加入混合物中直到它为碱性(pH 14)。白色沉淀通过吸滤收集,用水漂洗并干燥,得到1007。^1H NMR(300MHz,DMSO-d_6)δ7.18(s,4H),3.96(s,4H)。

[0300]



1008

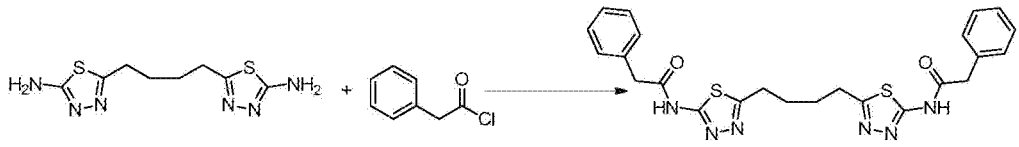
[0301] 将1,5-二氰基戊烷(1.00g,8.19mmol)和氨基硫脲(1.5g,16.40mmol)在TFA(3mL)中的混合物在85℃下加热5小时。使反应物冷却至室温,倒入冰和水的混合物中。将氢氧化钠小粒加入混合物中直到它为碱性(pH 14)。白色沉淀通过吸滤收集,用水漂洗并干燥,得到1008。^1H NMR(300MHz,DMSO-d_6)δ6.98(s,4H),2.81(t,4H),1.67(m,4H),1.20(m,2H)。

[0302] 二氨基核心的酰化:

[0303] 方法A:通过酰氯

[0304] N,N'-[5,5'-(丁烷-1,4-二基)-双(1,3,4-噻二唑-5,2-二基)]-双(2-苯基乙酰胺)(21)

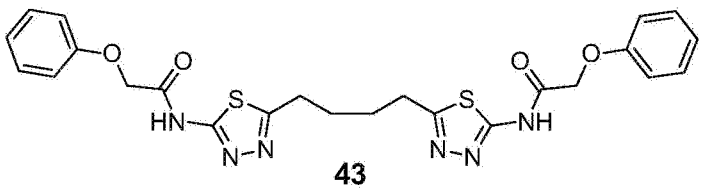
[0305]



1001 21

[0306] 在0℃下向1001(8.00g,31.21mmol)在1-甲基-2-吡咯烷酮(NMP)100mL)中的悬浮液中滴加苯乙酰氯(10.25mL,77.54mmol)。将所得混合物在0℃下搅拌1小时后,通过加入水(~200mL)将其猝灭。白色沉淀通过吸滤收集,用水漂洗并干燥,得到N,N'-[5,5'-(丁烷-1,4-二基)-双(1,3,4-噻二唑-5,2-二基)]-双(2-苯基乙酰胺)(21,14.02g)。^1H NMR(300MHz,DMSO-d_6)δ12.66(s,2H),7.34(m,10H),3.81(s,4H),3.01(bs,4H),1.76(bs,4H)。

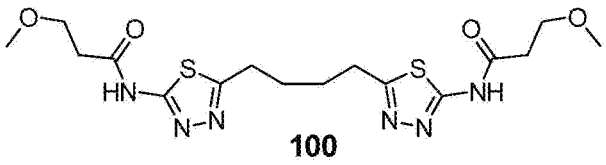
[0307]



43

[0308] 使用苯氧基乙酰氯,按照方法A制备化合物43。^1H NMR(300MHz,DMSO-d_6)δ12.68(s,2H),7.35-7.30(m,4H),6.99-6.97(m,6H),4.90(s,4H),3.05(bs,4H),1.79(bs,4H)。

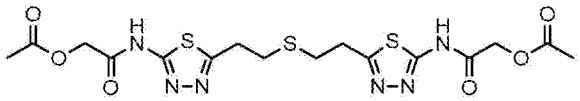
[0309]



100

[0310] 化合物 100 按照方法 A 制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.42(s,2H),3.64(t,J = 5.6Hz,4H),3.24(s,6H),3.01(bs,4H),2.72(t,J = 6.2Hz,4H),1.79(bs,4H)。

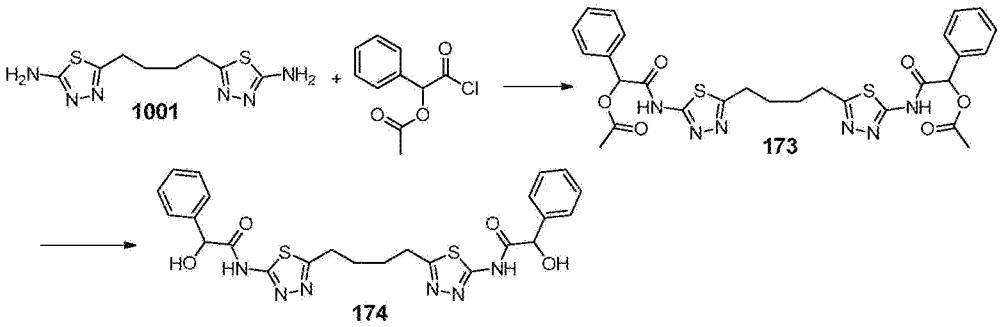
[0311]



5

[0312] 化合物 5 按照方法 A 制备 :^{1}H NMR(300MHz,DMSO-d_6) δ 12.66(s,4H),3.27(t,J = 6.99Hz,4H),2.95(t,J = 7.02Hz,4H),2.12(s,6H)。

[0313]



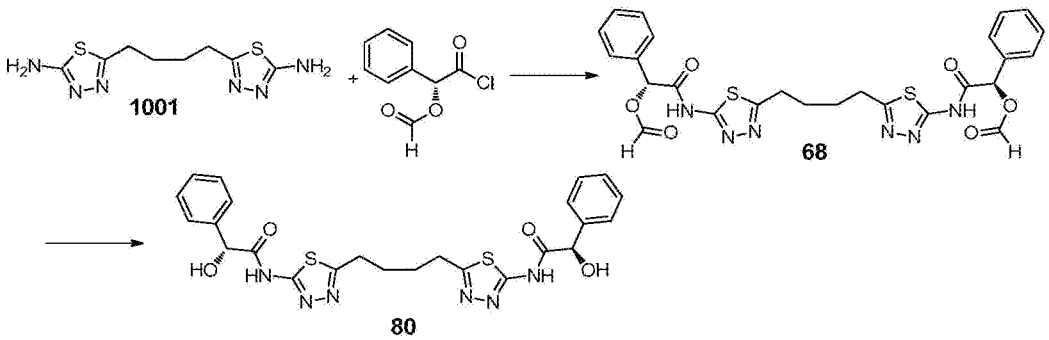
1001 173 174

[0314] 在 0℃下向 1001(200mg,0.78mmol) 在 NMP(2mL) 中的悬浮液中滴加 O- 乙酰基扁桃酸酰氯 (0.44mL,1.95mmol)。将所得混合物在 0℃下搅拌 1.5h 后,通过加入水 (～ 10mL) 将其猝灭。白色沉淀通过吸滤收集,用更多的水漂洗并干燥。粗物质用 DMSO 和 MeOH 的混合物通过重结晶纯化,得到 173。

[0315] 向烧瓶中装入 173 和 2N 氨 /MeOH(3ml),将所得混合物在室温下搅拌 6 小时。除去溶剂,将所得物质在烘箱中干燥,得到 174。^{1}H NMR(300MHz,DMSO-d_6) δ 12.42(s,2H),7.53-7.31(m,10H),6.35(s,2H),5.34(d,J = 1.14Hz,2H),3.01(bs,4H),1.76(bs,4H)。

[0316] 化合物 306 按照用于上述化合物 174 的方法制备。

[0317]



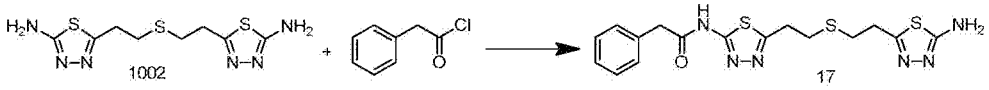
1001 68 80

[0318] 在 0 ℃ 下,向 1001(400mg,1.56mmol) 在 NMP(4mL) 中的悬浮液中滴加 (R)-(-)-O- 甲酰基扁桃酰氯 (0.61mL,3.90mmol)。将所得混合物在 0℃下搅拌 1.5h 后,通

过加入水（～ 10mL）将其猝灭。白色沉淀通过吸滤收集，用更多的水漂洗并干燥。粗物质用 DMSO 和 MeOH 的混合物通过重结晶纯化，得到 68。

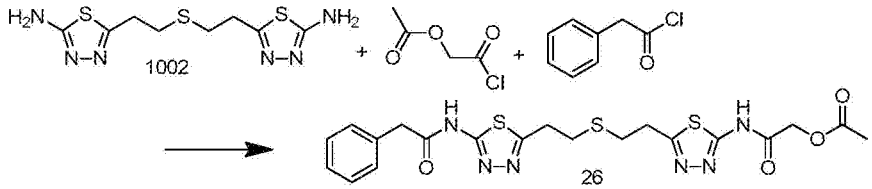
[0319] 向烧瓶中装入 68 和 2N 氨 /MeOH(5ml)，将所得混合物在室温下搅拌 2 小时。除去溶剂，将所得物质在烘箱中干燥，得到 80。^{1}H NMR(300MHz, DMSO-d_6) δ 7.53-7.31(m, 10H), 6.34(s, 2H), 5.33(s, 2H), 3.01(bs, 4H), 1.75(bs, 4H)。

[0320]



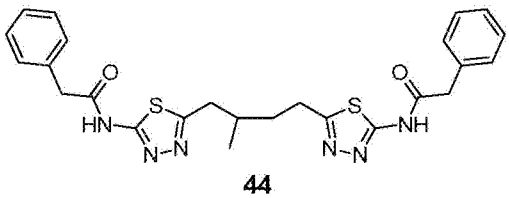
[0321] 在 -15℃下，向 1002(544mg, 1.89mmol) 在 NMP(13mL) 中的悬浮液中滴加苯乙酰氯 (0.249mL, 1.89mmol)。将所得混合物在 0℃下搅拌 1 小时，通过加入水 (54mL) 猝灭。白色沉淀通过吸滤收集，用水 (27mL) 和乙酸乙酯 (3x27mL) 漂洗。使用 2.5M NaOH 将滤液碱化至 pH11。分层，水层用二氯甲烷 (3x54mL) 萃取。合并的有机层经硫酸镁干燥并浓缩，得到 N-(5-(2-((2-(5- 氨基 -1,3,4- 噻二唑 -2- 基）乙基）硫代）乙基)-1,3,4- 噻二唑 -2- 基)-2- 苯基乙酰胺 (17,56mg)^{1}H NMR(300MHz, DMSO-d_6) δ 12.71(s, 1H), 7.32(s, 5H), 3.81(s, 2H), 3.25(t, J = 7.61Hz, 2H)3.06(t, J = 7.25Hz, 2H), 2.92(t, J = 6.90Hz, 2H), 2.85(t, J = 6.86Hz, 2H)。

[0322]



[0323] 将苯乙酰氯 (0.134mL, 1.01mmol) 和乙酰氧基乙酰氯 (0.109mL, 1.01mmol) 在 NMP(0.5mL) 中混合在一起。在室温下，将该混合物慢慢加入 1002(292mg, 1.01mmol) 在 NMP(7mL) 中的悬浮液中。将所得混合物在室温下搅拌 1 小时，通过加入水 (20mL) 猝灭。白色沉淀通过吸滤收集，用水漂洗，高真空下干燥。粗物质用制备型 HPLC 纯化。化合物 26：^{1}H NMR(300MHz, DMSO-d_6) δ 12.69(s, 2H), 7.34(3, 5H), 4.81(s, 2H), 3.82(s, 2H), 2.96(bs, 4H), 2.14(s, 3H)。

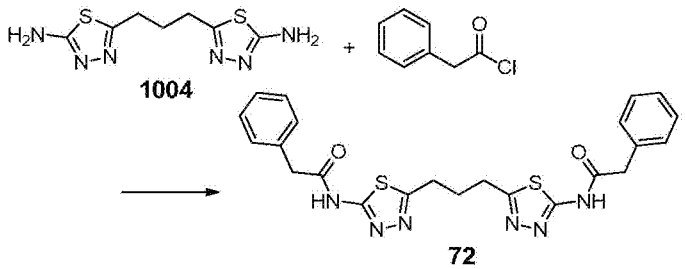
[0324]



44

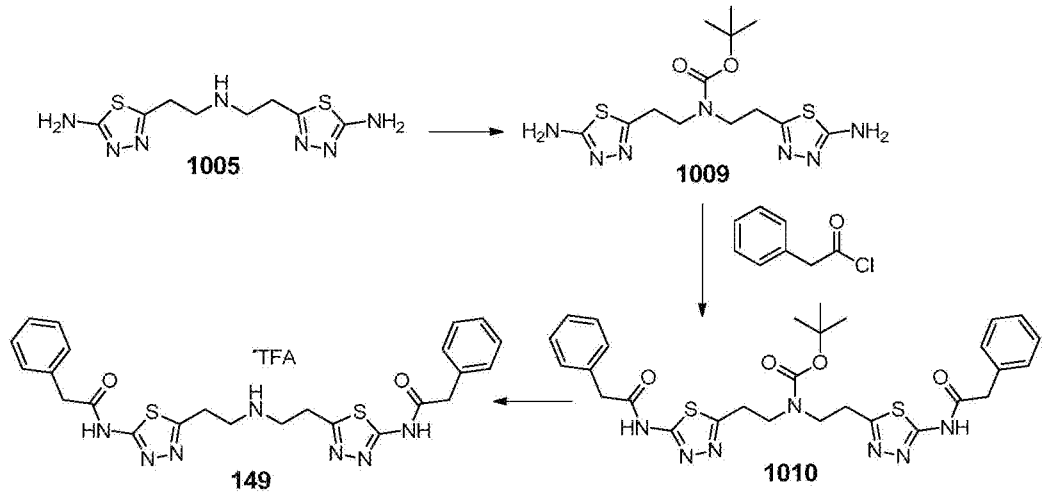
[0325] 化合物 44 按照前述化合物 21 的方法制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.66(s, 2H), 7.34 - 7.28(m, 10H), 3.81(s, 4H), 3.05 - 3.00(m, 3H), 2.87(dd, J = 7.9, 8.2Hz, 1H), 1.95 - 1.77(m, 3H), 0.94(d, J = 6.5Hz, 3H)。

[0326]



[0327] 化合物72按照前述化合物21的方法制备。在0℃下，向二胺1004(0.70g，3.07mmol)在NMP(15mL)中的悬浮液中滴加苯乙酰氯(811μL，6.13mmol)。将所得混合物在0℃下搅拌1小时后，通过加入水将其猝灭。白色沉淀通过吸滤收集，用水漂洗并干燥，得到N,N'-[5,5'-(丙烷-1,3-二基)-双(1,3,4-噻二唑-5,2-二基)]-双(2-苯基乙酰胺)(72，1.37g)。^{1}H NMR(300MHz,DMSO-d_6) δ 12.68(s,2H)，7.38-7.27(m,10H)，3.82(s,4H)，3.06(t,J = 7.2Hz,4H)，2.17-2.12(m,2H)。

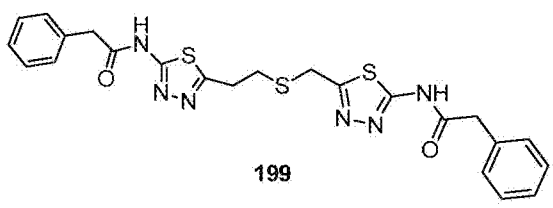
[0328]



[0329] 在室温下，向化合物1005(100mg，0.37mmol)在DMF(12mL)中的悬浮液中加入(t-Boc)$_2$O(88mg，0.41mmol)的DMF(2mL)溶液。将混合物在室温下搅拌24小时。向该反应混合物加入NMP(2mL)，接着加入苯乙酰氯(97μL，0.74mmol)。将反应物搅拌1小时后，将其倒入冰-水的混合物中。固体通过吸滤收集，用水漂洗并干燥，得到1010(180mg)。

[0330] 在室温下，将上述产物1010(160mg，0.26mmol)在TFA(1.5mL)和CH_2CH_2(10mL)的混合物中搅拌4小时后，将其浓缩。使残余物重新溶于CH_2Cl_2(3×)中并浓缩，得到N,N'-(5,5'-(氮烷二基-双(乙烷-2,1-二基))-双(1,3,4-噻二唑-5,2-二基))-双(2-苯基乙酰胺)三氟乙酸(149，122mg)。^{1}H NMR(300MHz,DMSO-d_6) δ 12.81(s,2H)，8.75(bs,2H)，7.38-7.27(m,10H)，3.84(s,4H)，3.45(d,J = 2.9Hz,4H)，3.39(d,J = 6.0Hz,4H)。

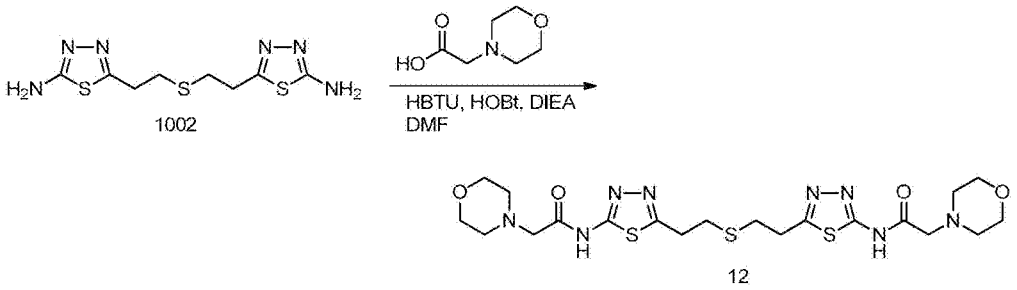
[0331]



[0332] 向 1006(0.274g,1mmol) 在 NMP(5mL) 中的悬浮液中滴加苯乙酰氯 (0.263mL,2mmol)。将混合物在室温下搅拌 1 小时,然后将其用水稀释。分离的固体经过滤,用更多的水洗涤并干燥。粗物质用制备型 HPLC 纯化,得到 199,为白色固体。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 2.87-2.91(t,2H)3.25-3.29(t,2H)3.82(s,4H)4.19(s,2H)7.26-7.33(m,10H)12.71-12.72(br s,2H)。

[0333] 方法 B :使用肽偶联试剂通过酸进行

[0334]

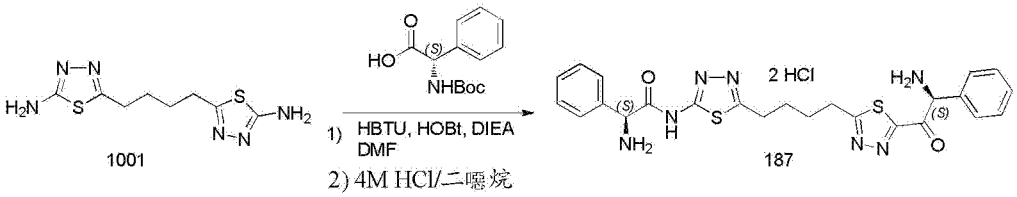


1002

12

[0335] 向含有 5,5'-(硫代双 (乙烷 -2,1- 二基)) 双 (1,3,4- 噻二唑 -2- 胺)(1002)(0.69mmol,0.20g,1.0 当量) 的烧瓶中加入 2- 吗啉代乙酸 (1.52mmol,0.22g,2.2 当量)、六氟磷酸 O-(苯并三唑 -1- 基)-N,N,N′ ,N′ - 四甲基脲鎓 (HBTU)(2.20mmol,0.83g,3.2 当量)、1- 羟基苯并三唑 (HOBT)(2.2mmol,0.29g,3.2 当量)、5mL DMF,接着加入 N,N- 二异丙基乙胺 (DIEA)(5.52mmol,0.71g,0.960mL,8.0 当量)。将混合物在室温下搅拌过夜,然后用 15mL 水稀释。混合物用 EtOAc 萃取,合并有机层,用水、盐水洗涤,经 Na_2SO_4 干燥。Na_2SO_4 经过滤除去,减压除去挥发物,得到 0.04g 的化合物 12。^{1}HNMR(300MHz,$CDCl_3$) 化合物 12 :δ 3.80(宽多重峰 ,4H),3.34(dd,4H,J = 7.2Hz),3.28(s,4H),3.00(dd,4H,J = 7.1Hz),2.63(宽多重峰 ,4H)。

[0336]



1001

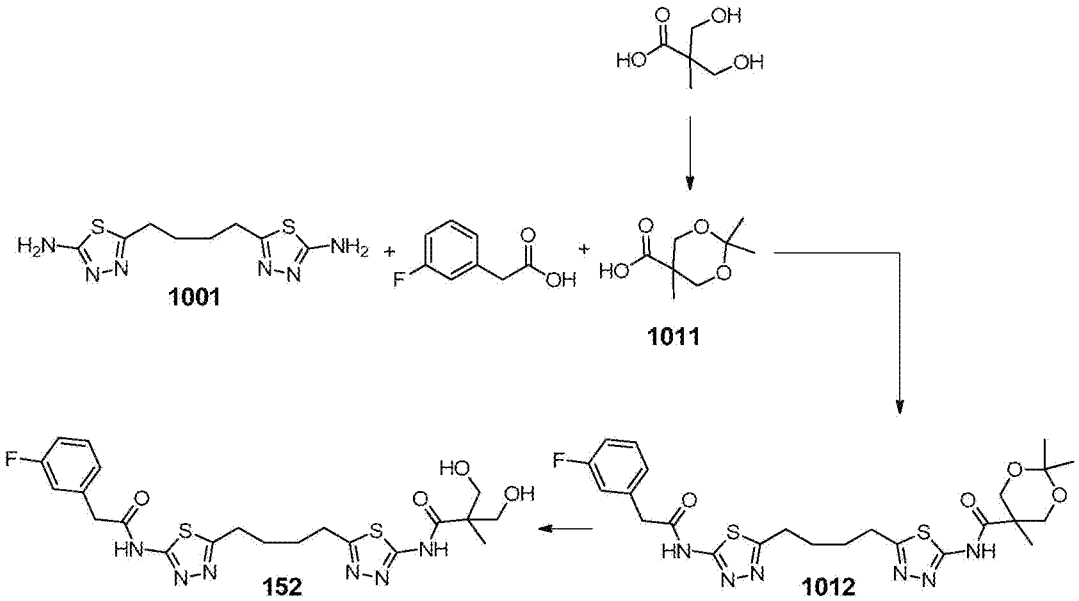
187

[0337] 向含有 5,5'-(丁烷 -1,4- 二基) 双 (1,3,4- 噻二唑 -2- 胺)(1101)(3.9mmol,1.0g,1.0 当量) 的烧瓶中加入 (S)-2-((叔丁氧基羰基) 氨基)-2- 苯乙酸 (8.58mmol,2.15g,2.2 当 量)、HBTU(12.48mmol,4.73g,3.2 当 量)、HOBt(12.48mmol,1.69g,3.2 当量)25mL 的 DMF,接着加入 DIEA(31.2mmol,4.03g,5.43mL,8.0 当量)。搅拌混合物过夜,并倒入 150mL 水中。形成的白色固体通过真空过滤收集,用水洗涤,真空干燥,得到 2.47g 的双 -Boc 保护的中间体。

[0338] 在剧烈搅拌的情况下,向双 -Boc 保护的中间体 (2.76mmol,2.0g,1.0 当量) 在 20mL 二氯甲烷 (DCM) 的浆液中加入含 4M HCl 的二噁烷 (40mmol,10mL)。混合物短时变澄清和均匀,然后形成白色沉淀。搅拌混合物过夜,用 20mL 乙醚稀释。固体通过真空过滤收集,用额外的乙醚洗涤,并真空干燥,得到 0.9g 187。^{1}HNMR(300MHz,DMSO,d_6) 化合物 187 :δ 9.13(s,4H),7.61(m,4H),7.48(m,6H),6.2(宽单峰 ,4H),5.32(s,2H),3.04(宽多重

峰，4H)，1.77(宽多重峰，4H)。

[0339]

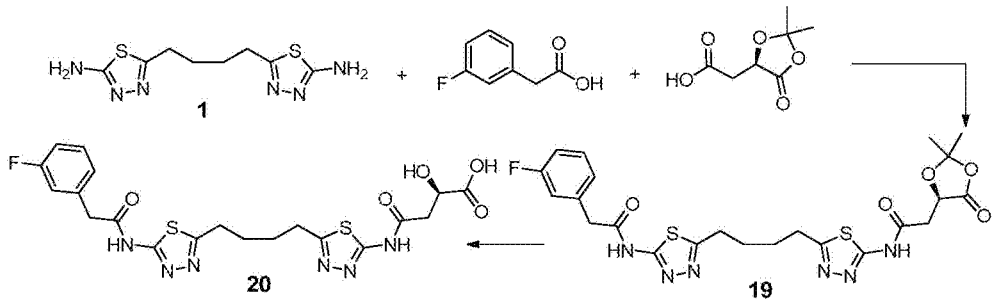


[0340] 在室温下，向2,2-双(羟基甲基)丙酸(5.00g，37.28mmol)的丙酮(80mL)溶液中加入2,2-二甲氧基丙烷(6.88mL，55.92mmol)和p-TsOH·H_2O(0.36g，1.86mmol)。将反应物搅拌2小时后，将其用Et_3N(0.30mL)猝灭。减压除去有机挥发物。使残余物在EtOAc和水之间分配。有机层用盐水洗涤，干燥($MgSO_4$)并浓缩，得到所需产物1011(5.17g)，为白色固体。

[0341] 在0℃下，向二胺1001(500mg，1.95mmol)、3-氟苯乙酸(361mg，2.34mmol)和酸1011(442mg，2.54mmol)在DMF(20mL)中的悬浮液中加入HOBt(791mg，5.85mmol)，接着加入N-(3-二甲基氨基丙基)-N′-乙基碳二亚胺盐酸盐(EDC)(1.12g，5.85mmol)。将混合物在18小时内从0℃到室温搅拌后，将其用水稀释。沉淀通过吸滤收集，用水洗涤，干燥。粗产物用硅胶色谱法纯化(用1-10％ MeOH/CH_2Cl_2洗脱)，得到N-(5-(4-(5-(2-(3-氟苯基)乙酰氨基)-1,3,4-噻二唑-2-基)丁基)-1,3,4-噻二唑-2-基)-2,2,5-三甲基-1,3-二氧杂环己烷-5-甲酰胺(1012，208mg)。

[0342] 将上述产物1012(87mg，0.16mmol)和TFA(2mL)在THF(8mL)和水(2mL)的混合物中在50℃下加热5小时后，将其减压浓缩。粗制残余物用HPLC纯化，得到N,N'-(5-(4-(5-(2-(3-氟苯基)乙酰氨基)-1,3,4-噻二唑-2-基)丁基)-1,3,4-噻二唑-2-基)-3-羟基-2-(羟基甲基)-2-甲基丙酰胺(152)。^1H NMR(300MHz，DMSO-d_6) δ 12.68(s，1H)，11.77(s，1H)，7.04-7.38(m，1H)，7.18-7.09(m，4H)，4.98(s，2H)，3.86(s，2H)，3.62(dd，J = 10.7，29.0Hz，4H)，3.03(bs，4H)，1.77(bs，4H)，1.14(s，3H)。

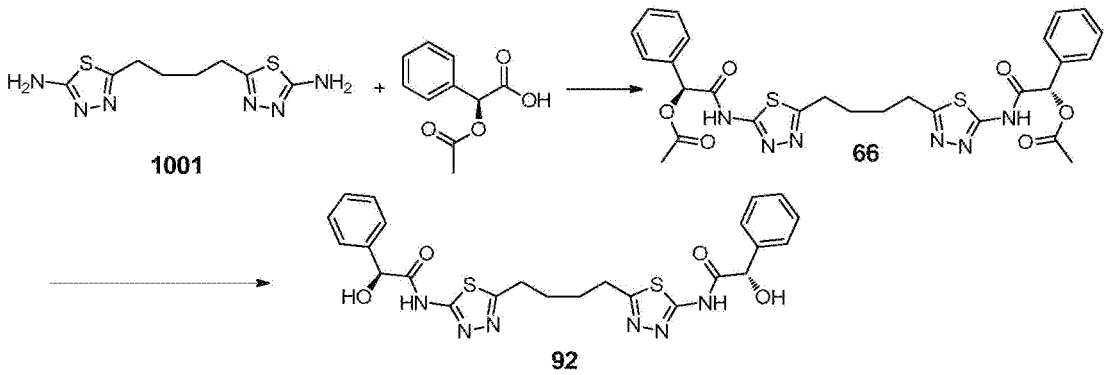
[0343]



[0344] 在 0 ℃ 下，向二胺 1001(400mg,1.56mmol)、3- 氟苯乙酸 (313mg,2.03mmol)、(R)-(-)-2,2- 二甲基 -5- 氧代 -1,3- 二氧杂环戊烷 -4- 乙酸 (353mg,2.03mmol) 和 Et_3N(200 μL) 在 DMF(20mL) 中的悬浮液中依次加入 HOBt(633mg,4.68mmol) 和 EDC(897mg,4.68mmol)。将混合物在 18 小时内从 0℃到室温搅拌后，将其用水稀释。沉淀通过吸滤收集，并用水洗涤。固体用热的 MeOH-THF 混合物进一步漂洗。合并的滤液经浓缩，并用硅胶色谱法纯化（用 1-10％ MeOH/CH_2Cl_2洗脱），得到 (R)-N-(5-(4-(5-(2-(3- 氟苯基）乙酰氨基）-1,3,4- 噻二唑 -2- 基）丁基）-1,3,4- 噻二唑 -2- 基）-3,4- 二羟基丁酰胺 (1013,93mg)。

[0345] 上述产物 1013(87mg,0.16mmol) 和 TFA(2mL) 在 THF(8mL) 和水 (2mL) 中的混合物在 50 ℃ 下加热 5 小时后，将其减压浓缩。粗制残余物用 HPLC 纯化，得到 (R)-N-(5-(4-(5-(2-(3- 氟苯基）乙酰氨基）-1,3,4- 噻二唑 -2- 基）丁基）-1,3,4- 噻二唑 -2- 基）-3,4- 二羟基丁酰胺 (153)。^1H NMR(300MHz,DMSO-d_6) δ 12.67(s,1H),12.43(s,1H),7.41 - 7.38(m,1H),7.20 - 7.12(m,4H),4.45 - 4.40(m,1H),3.86(s,2H),3.03(bs,4H),2.85 - 2.77(m,2H),1.78(bs,4H)。

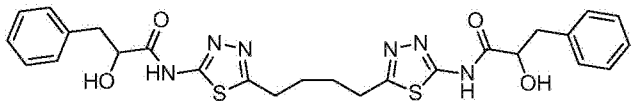
[0346]



[0347] 向 (S)-(+)-O- 乙酰基扁桃酸 (666mg,3.43mmol) 和六氟磷酸 O-(7- 氮杂苯并三唑 -1- 基）-N,N,N′,N′- 四甲基脲鎓 (HATU)(1.47g,3.86mmol) 在 DMF(4mL) 中的悬浮液中依次加入 DIEA(0.672ml,3.86mmol) 和 1001(400mg,1.56mmol)。将所得混合物在室温下搅拌过夜后，通过加入水（～ 10mL）将其猝灭。白色沉淀通过吸滤收集，用更多的水漂洗并干燥。粗物质用 DMSO 和 MeOH 的混合物通过重结晶纯化，得到 66。

[0348] 向烧瓶中装入 66 和 2N 氨 /MeOH(5ml)，将所得混合物在室温下搅拌 6 小时。除去溶剂，将所得物质在烘箱中干燥，得到 92。^1H NMR(300MHz,DMSO-d_6) δ 12.42(s,2H),7.53-7.31(m,10H),6.35(s,2H),5.33(s,2H),3.01(bs,4H),1.76(bs,4H)。

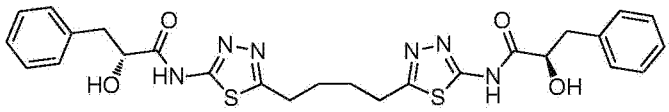
[0349]



69

[0350] 向烧瓶中装入 1001(200mg,0.78mmol)、DL-3- 苯基乳酸 (285mg,1.716mmol) 和含 HOBT(527mg,3.9mmol) 的 DMF(3ml),依次加入 EDC(897mg,4.68mmol) 和三乙胺 (0.87ml,6.24mmol)。将所得混合物在室温下搅拌过夜后,通过加入水 (～ 5mL) 将其猝灭。使混合物在水和 EtOAc 之间分配。有机萃取物用水洗涤,经硫酸钠干燥,过滤并蒸发。粗物质用硅胶色谱法纯化 (用含 0-6％ MeOH 的 CH_2Cl_2 洗脱),得到 69。^1H NMR(300MHz,DMSO-d_6) δ 12.20(s,2H),7.24(m,10H),5.75(d,J ＝ 6.87Hz,2H),4.43(m,2H),3.10(m,6H),2.89-2.81(m,2H),1.80(bs,4H)。

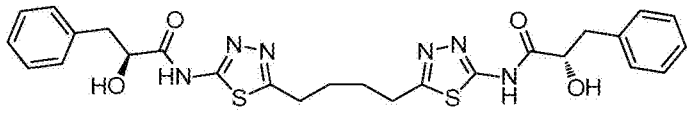
[0351]



169

[0352] 向烧瓶中装入 1001(200mg,0.78mmol)、D-(+)-3- 苯基乳酸 (285mg,1.716mmol) 和含 HOBt(464mg,3.43mmol) 的 DMF(3ml),依次加入 EDC(822mg,4.28mmol) 和三乙胺 (0.718ml,5.15mmol)。将所得混合物在室温下搅拌过夜后,通过加入水 (～ 5mL) 将其猝灭。使混合物在水和 EtOAc 之间分配。有机萃取物用水洗涤,经硫酸钠干燥,过滤并蒸发。粗物质用硅胶色谱法纯化 (用含 0-6％ MeOH 的 CH_2Cl_2 洗脱),得到 169。^1H NMR(300MHz,DMSO-d_6) δ 12.20(s,2H),7.24(m,10H),5.75(d,J ＝ 6.87Hz,2H),4.43(m,2H),3.03(m,6H),2.89-2.81(m,2H),1.80(bs,4H)。

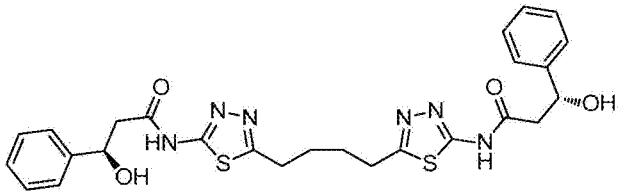
[0353]



146

[0354] 向烧瓶中装入 1001(200mg,0.78mmol)、L-(-)-3- 苯基乳酸 (285mg,1.716mmol) 和含 HOBt(464mg,3.43mmol) 的 DMF(3ml),依次加入 EDC(822mg,4.28mmol) 和三乙胺 (0.718ml,5.15mmol)。将所得混合物在室温下搅拌过夜后,通过加入水 (～ 5mL) 将其猝灭。使混合物在水和 EtOAc 之间分配。有机萃取物用更多的水洗涤,经硫酸钠干燥,过滤并蒸发。粗物质用硅胶色谱法纯化 (用含 0-6％ MeOH 的 CH_2Cl_2 洗脱),得到 146。^1H NMR(300MHz,DMSO-d_6) δ 12.27(s,2H),7.31(m,10H),5.78(m,2H),4.44(m,2H),3.05(m,6H),2.87(m,2H),1.79(bs,4H)。

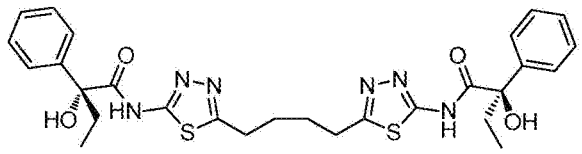
[0355]



127

[0356] 向 (R)-(+)-3- 羟基 -3- 苯基丙酸 (285mg,1.72mmol) 和 HATU(719mg,1.89mmol) 在 DMF(3mL) 中的悬浮液中依次加入 DIEA(0.329ml,1.89mmol) 和 1001(200mg,0.78mmol)。将所得混合物在室温下搅拌过夜后,通过加入水 (～ 10mL) 将其猝灭。白色沉淀通过吸滤收集,用更多的水漂洗并干燥。粗物质通过用 DMSO 和 MeOH 重结晶来纯化,得到 127。^{1}H NMR(300MHz,DMSO-d_6) δ 12.38(s,2H),7.34(m,10H),5.56(m,2H),5.10(m,2H),3.04(bs,4H),2.80(m,4H),1.80(bs,4H)。

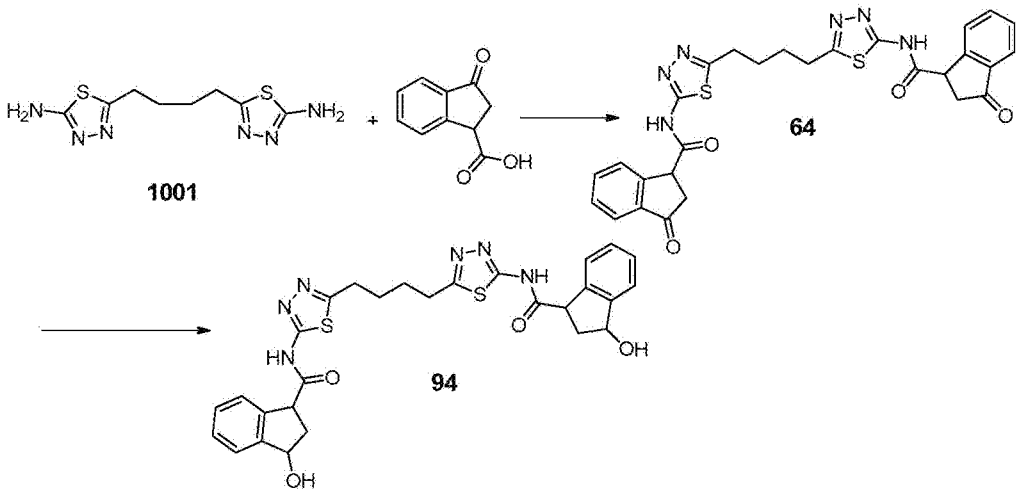
[0357]



143

[0358] 向 (R)-2- 羟基 -2- 苯基丁酸 (310mg,1.72mmol) 和 HATU(719mg,1.89mmol) 在 DMF(3mL) 中的悬浮液中依次加入 DIEA(0.329ml,1.89mmol) 和 1001(200mg,0.78mmol)。将所得混合物在室温下搅拌过夜后,通过加入水 (～ 10mL) 将其猝灭。粗物质用 HPLC 纯化,得到 143。^{1}H NMR(300MHz,DMSO-d_6) δ 7.61(d,J = 7.65Hz,4H),7.34(m,6H),2.99(bs,4H),2.26(m,2H),2.10(m,2H)1.74(bs,4H),0.80(t,6H)。

[0359]



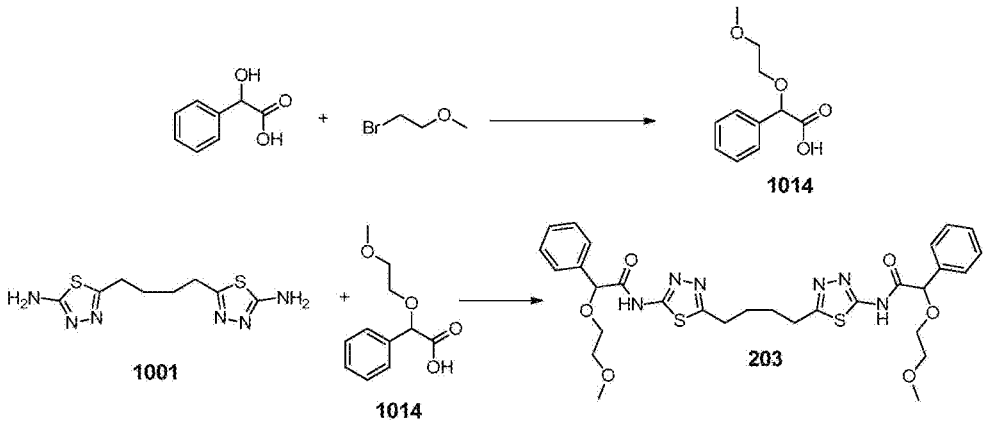
1001 64 94

[0360] 向 3- 氧代 -1- 茚满甲酸 (604mg,3.43mmol) 和 HATU(1.47g,3.86mmol) 在 DMF(5mL) 中的悬浮液中依次加入 DIEA(0.672ml,3.86mmol) 和 1001(400mg,1.56mmol)。将所得混合物在室温下搅拌过夜后,通过加入水 (～ 10mL) 将其猝灭。浅褐色沉淀通过吸滤收集,用水漂洗并干燥。粗物质用 DMSO 和 MeOH 的混合物通过重结晶纯化,得到 64。

[0361] 在 0℃下,向 64(100mg,0.175mmol) 在 EtOH(20ml) 中的悬浮液中加入 $NaBH_4$(15mg,0.384mmol),将所得混合物搅拌 1 小时后,用 1N HCl 将其猝灭。使混合物在 1N HCl 和

EtOAc 之间分配，有机萃取物经硫酸钠干燥，过滤并蒸发。粗物质用硅胶色谱法纯化（用含 0-6% MeOH 的 CH_2Cl_2 洗脱），并且用 DMSO 和 MeOH 的混合物重结晶进一步纯化，得到 94。^1H NMR(300MHz, DMSO-d_6) δ 12.81(s, 2H), 7.34(m, 8H), 5.56(m, 2H), 5.11(t, 2H), 4.15(t, 2H), 3.05(bs, 4H), 2.70(m, 2H), 2.15(m, 2H), 1.80(bs, 4H)。

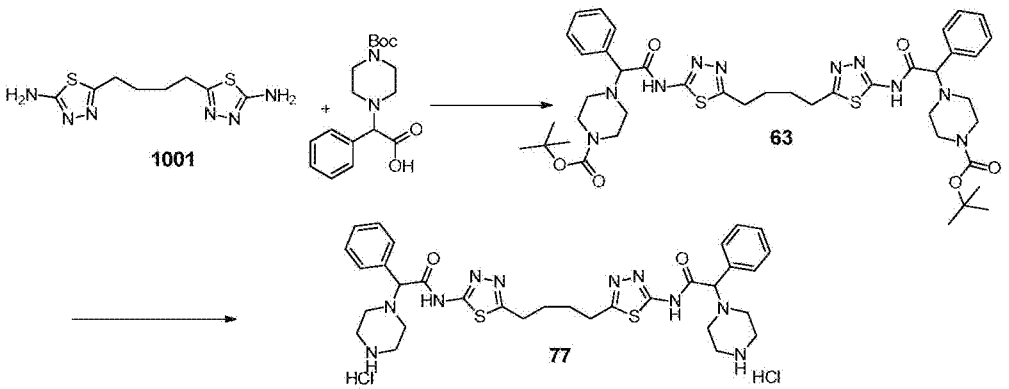
[0362]



[0363] 在 0℃下，向含 DL- 扁桃酸 (1g, 6.57mmol) 的 DMF(10ml) 溶液中加入 NaH(700mg, 19.7mmol)，将混合物搅拌 20 分钟后，滴加 2- 溴乙基甲基醚 (1.24ml, 13.1mmol)。将所得混合物在 0℃下搅拌，慢慢升温至室温过夜后，用 1N HCl 将其猝灭。使混合物在 1N HCl 和 EtOAc 之间分配，有机萃取物用水洗涤，经硫酸钠干燥，过滤并蒸发，得到 1014。

[0364] 向 1014(500mg, 2.37mmol) 和 HATU(995mg, 2.62mmol) 在 DMF(3mL) 中的悬浮液中依次加入 DIEA(0.456ml, 2.62mmol) 和 1001(277mg, 1.08mmol)。将所得混合物在室温下搅拌过夜后，通过加入水（～ 6mL）将其猝灭。使混合物在水和 EtOAc 之间分配。有机萃取物用水洗涤，经硫酸钠干燥，过滤并蒸发。粗物质用 HPLC 纯化，得到 203。^1H NMR(300MHz, DMSO-d_6) δ 12.58(s, 2H), 7.49-7.37(m, 10H), 5.22(s, 2H), 3.66-3.54(m, 8H), 3.27(s, 6H), 3.01(bs, 4H), 1.75(bs, 4H)。

[0365]

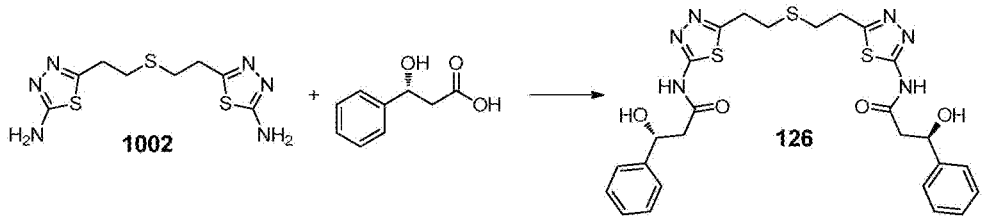


[0366] 向 2-(4-Boc- 哌嗪基)-2- 苯乙酸 (1.1g, 3.43mmol) 和 HATU(1.47g, 3.86mmol) 在 DMF(5mL) 中的悬浮液中依次加入 DIEA(0.672ml, 3.86mmol) 和 1001(400mg, 1.56mmol)。将所得混合物在室温下搅拌过夜后，通过加入水（～ 10mL）将其猝灭。白色沉淀通过吸滤收集，用水漂洗并干燥。粗物质通过用 DMSO 和 MeOH 重结晶来纯化，得到 63。

[0367] 向烧瓶中装入 63 和含 4N HCl 的 1,4- 二噁烷 (6ml)，将所得混合物在室温下搅拌 3 小时。沉淀经过滤收集，用 EtOAc/CH_2Cl_2 漂洗并干燥，得到 77。^1H NMR(300MHz, DMSO-d_6)

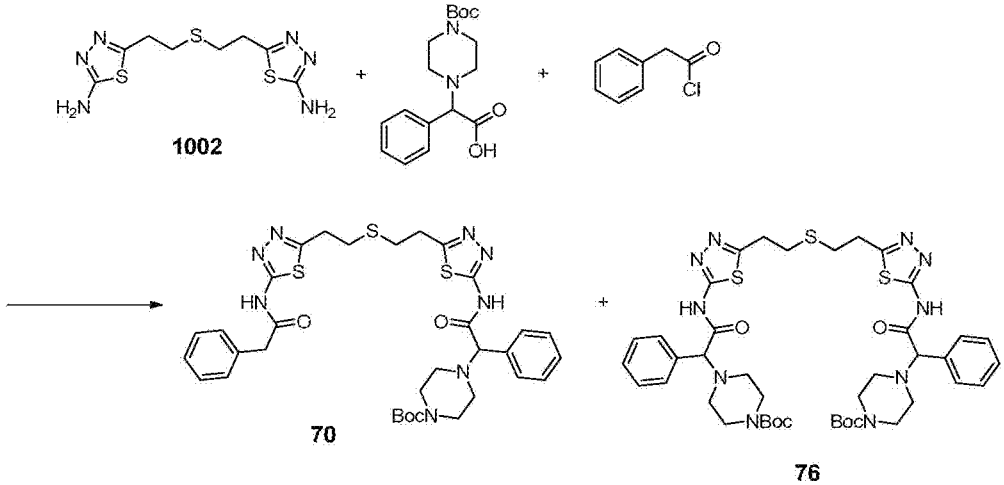
δ 9.10(bs,4H),7.51-7.41(m,10H),4.90(bs,2H),4.62(s,2H),3.15(bs,8H),3.03(bs,4H),2.73(bs,8H),1.76(bs,4H)。

[0368]



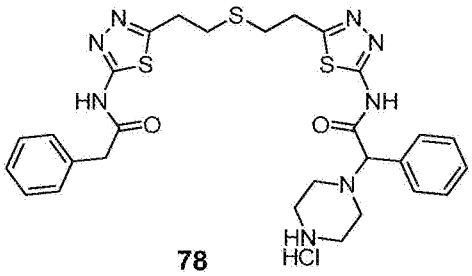
[0369] 向(R)-(+)-3-羟基-3-苯基丙酸(254mg,1.53mmol)和HATU(640mg,1.68mmol)在DMF(3mL)中的悬浮液中依次加入DIEA(0.292ml,1.68mmol)和1002(200mg,0.693mmol)。将所得混合物在室温下搅拌过夜后,通过加入水(~10mL)将其猝灭。白色沉淀通过吸滤收集,用水漂洗并干燥。粗物质用DMSO和MeOH的混合物通过重结晶纯化,得到126。^{1}H NMR(300MHz,DMSO-d_6)δ 12.40(s,2H),7.38(m,10H),5.55(m,2H),5.09(m,2H),3.27(t,4H),2.95(t,4H),2.82(m,4H)。

[0370]



[0371] 向烧瓶中装入1002(200mg,0.693mmol)、2-(4-Boc-哌嗪基)-2-苯乙酸(244mg,0.763mmol)和含HOBt(187mg,1.39mmol)的DMF(3ml),依次加入EDC(332mg,1.73mmol)和三乙胺(0.290ml,2.08mmol)。将所得混合物在室温下搅拌过夜后,在0℃下滴加苯乙酰氯(0.037ml,0.277mmol),搅拌1小时后,通过加入水(~10mL)将其猝灭。白色沉淀通过吸滤收集,用水漂洗并干燥。粗物质用HPLC纯化,得到70和76。

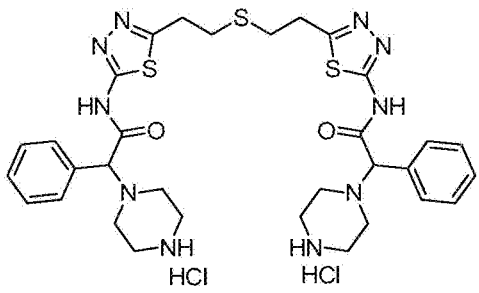
[0372]



[0373] 向烧瓶中装入70和含4N HCl的1,4-二噁烷(6ml),将所得混合物在室温下搅拌3小时。沉淀经过滤收集,用EtOAc/CH_2Cl_2漂洗并干燥,得到78。^{1}H NMR(300MHz,DMSO-d_6)

δ 12.70(s,2H),8.97(bs,2H),7.50-7.29(m,10H),4.72(bs,1H),4.59(s,1H),3.82(s,2H),3.27(t,4H),3.15(bs,4H),2.92(t,4H),2.70(bs,4H)。

[0374]

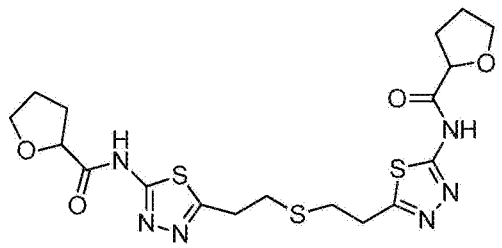


79

[0375] 向烧瓶中装入 76 和含 4N HCl 的 1,4- 二噁烷 (6ml),将所得混合物在室温下搅拌 3 小时。沉淀经过滤收集,用 EtOAc/CH_2Cl_2漂洗并干燥,得到 79。 ^{1}H NMR(300MHz,DMSO-d_6) δ 12.87(s,2H),9.03(bs,4H),7.50-7.40(m,10H),4.67(bs,2H),4.59(s,2H),3.28(t,4H),3.14(bs,8H),2.97(t,4H),2.71(bs,8H)。

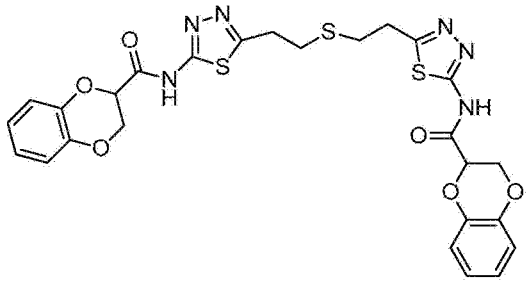
[0376] 酰胺偶联通用方法(用于下列实施例):向含羧酸 (2 当量) 的 DMF0.2 摩尔浓度悬浮液中加入 HATU(2 当量),搅拌直到反应混合物澄清,接着加入胺 (1 当量) 和 DIPEA(4 当量)。将所得混合物在室温下搅拌过夜后,通过加入水将其猝灭。分离的固体经过滤,用水洗涤,干燥。

[0377]



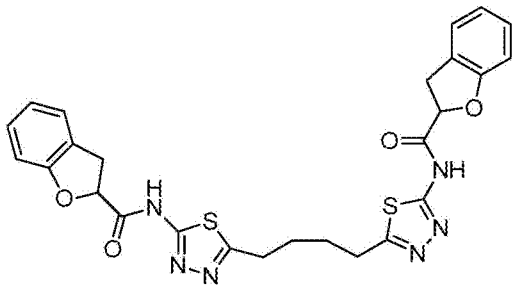
[0378] 39 :^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.89-2.01(m,6H)2.18-2.29(m,2H)2.95-3(m,4H)3.79-3.86(m,2H)3.94-4.02(m,2H)4.55-4.6(m,2H)12.29(brs,2H)。

[0379]



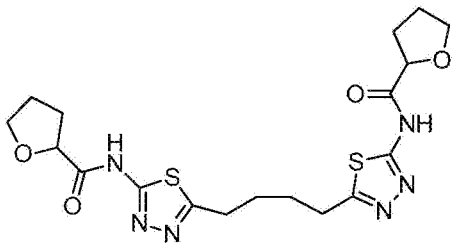
[0380] 41 :^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 2.93-2.98(m,4H)3.27-3.32(m,4H),4.46(s,4H),5.18-5.2(br s,2H)6.88-7.03(m,8H)12.87-12.92(br s,2H)。

[0381]



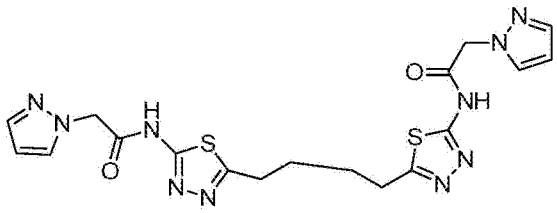
[0382] 51 :^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.78(br s,4H)3.05-3.06(br s,4H),3.38-3.40(m,2H)3.54-3.63(m,2H)5.44-5.50(m,2H)6.92-7.26(m,8H)12.78(br s,2H)。

[0383]



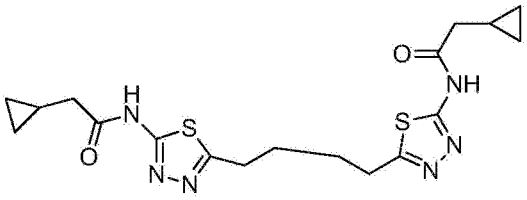
[0384] 54 :^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.92-2.03(m,10H)2.17-2.28(m,2H)3.05(br s,4H)3.79-3.85(m,2H)3.94-4.01(m,2H)4.55-4.59(m,2H)12.27(br s,2H)。

[0385]



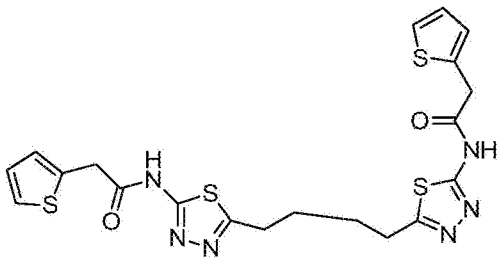
[0386] 60 :^{1}H NMR(300MHz, 二 甲 亚 砜 -d6) δ ppm 1.77(br s,4H)3.04(br s,4H)5.20(s,4H)6.31(br s,2H)7.49(br s,2H)7.79(br s,2H)12.80(br s,2H)。

[0387]



[0388] 85 :^{1}H NMR(300MHz, 二 甲 亚 砜 -d6) δ ppm 0.20-0.21(br s,4H)0.48-0.50(br s,4H)1.79(br s,4H)2.35-2.38(br s,4H)3.04(br s,4H)12.32(br s,2H)。

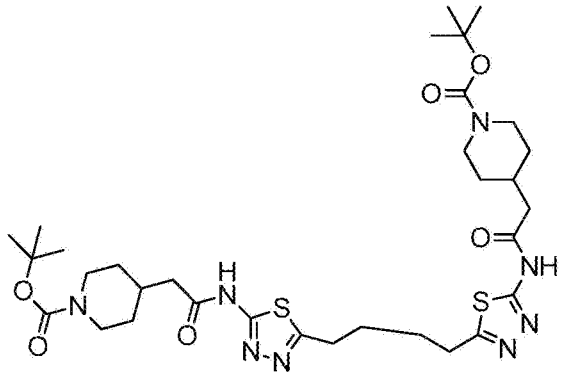
[0389]



[0390] 87 :^{1}H NMR(300MHz, 二 甲 亚 砜 -d6) δ ppm 1.78(br s,4H)3.03(br

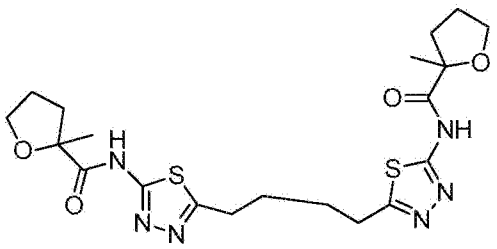
s, 4H)4.05(s, 4H)6.99(br s, 4H)7.42-7.44(m, 2H)12.68(br s, 2H)。

[0391]



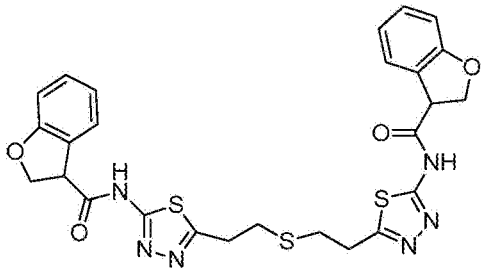
[0392] 114 :^{1}H NMR(300MHz, 二 甲 亚 砜 -d6) δ ppm 1.01-1.12(m, 4H)1, 40(s, 18H)1.61-1.65(m, 4H)1.78(br s, 4H)1.95(br s, 2H)3.84(m, 4H)2.65-2.75(m, 4H)3.03(br s, 4H)3.89-3.93(m, 4H)12.39(br s, 2H)。

[0393]



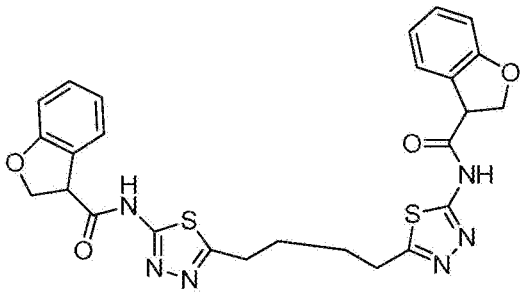
[0394] 123 :^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.43(s, 6H)1.79-1.94(m, 10H)2.22-2.31(m, 2H)3.05(br s, 4H)3.85-4.01(m, 4H)11.85(br s, 2H)。

[0395]



[0396] 133 :^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 2.92-2.97(m, 4H)3.26-3.30(m, 4H)4.61-4.87(m, 6H)6.83-6.89(m, 4H)7.16-7.21(m, 2H)7.36-7.38(m, 2H)12.95(br s, 2H)。

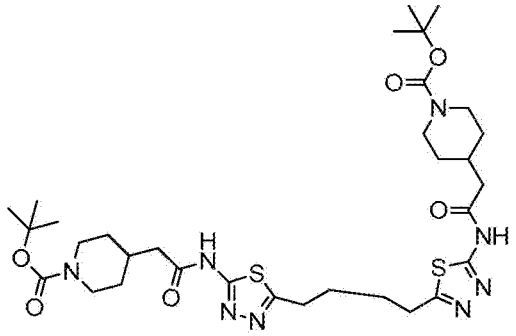
[0397]



[0398] 135 :^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.77(br s, 4H)3.03(br s, 4H)4.60-4.

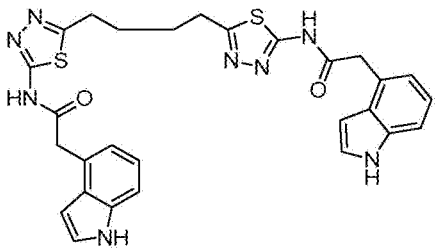
87(m,6H)6.83-6.89(m,4H)7.16-7.22(m,2H)7.36-7.38(m,2H)12.92(br s,2H)。

[0399]



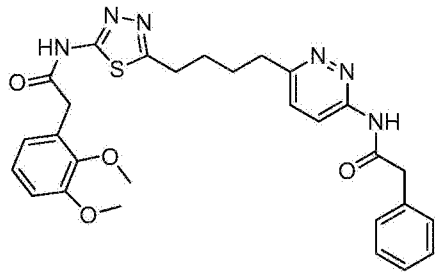
[0400] 114 :^{1}H NMR(300MHz, 二 甲 亚 砜 -d6) δ ppm 1.01-1.12(m,4H)1,40(s,18H)1.61-1.65(m,4H)1.78(br s,4H)1.95(br s,2H)3.84(m,4H)2.65-2.75(m,4H)3.03(br s,4H)3.89-3.93(m,4H)12.39(br s,2H)。

[0401]



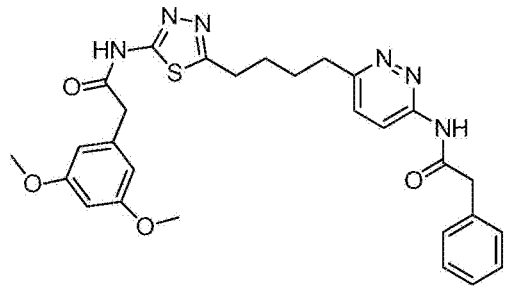
[0402] 323 :^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.76(brs,4H)3.01(brs,4H)4.02(s,4H)6.56(s,2H)6.94-7.05(m,4H)7.31-7.33(m,4H)11.12(brs,2H)12.69(s,2H)。

[0403]



[0404] 397 :^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.75(brs,4H)2.90(brs,2H)3.02(brs,2H)3.67-3.82(m,10H)6.85-7.03(m,4H)7.26-7.36(m,5H)7.55-7.58(d,1H)8.18-8.21(d,1H)11.26(s,1H)12.65(brs,1H)。

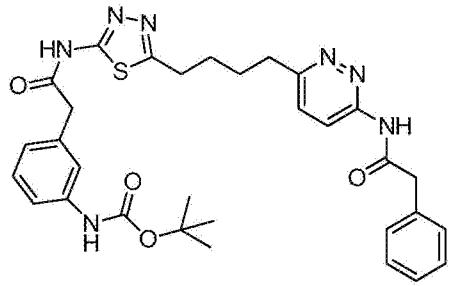
[0405]



[0406] 398 :^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm ppm 1.75(brs,4H)2.90(brs,2H)3.02(

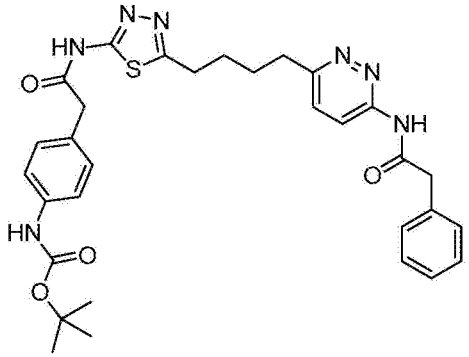
brs, 2H) 3.72-3.78 (m, 10H) 6.42-6.51 (m, 4H) 7.36 (m, 5H) 7.54-7.58 (d, 1H) 8.18-8.21 (d, 1H) 11.26 (s, 1H) 12.65 (brs, 1H)。

[0407]



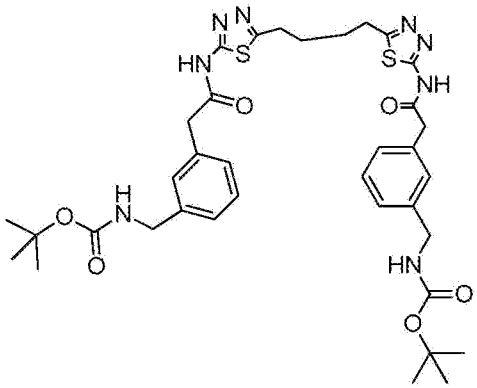
[0408] 399 : ^{1}H NMR (300MHz, 二甲亚砜-d6) δ ppm 1.48 (s, 9H) 1.75 (brs, 4H) 2.90 (brs, 2H) 3.02 (brs, 2H) 3.74-3.78 (m, 4H) 6.92-6.94 (m, 1H) 7.20-7.36 (m, 7H) 7.51-7.58 (m, 2H) 8.18-8.21 (d, 1H) 9.34 (s, 1H) 11.26 (s, 1H) 12.65 (brs, 1H)。

[0409]



[0410] 400 : ^{1}H NMR (300MHz, 二甲亚砜-d6) δ ppm 1.48 (s, 9H) 1.75 (brs, 4H) 2.90 (brs, 2H) 3.02 (brs, 2H) 3.71-3.78 (m, 4H) 7.18-7.42 (m, 9H) 7.54-7.58 (m, 2H) 8.18-8.21 (d, 1H) 9.34 (s, 1H) 11.26 (s, 1H) 12.65 (brs, 1H)。

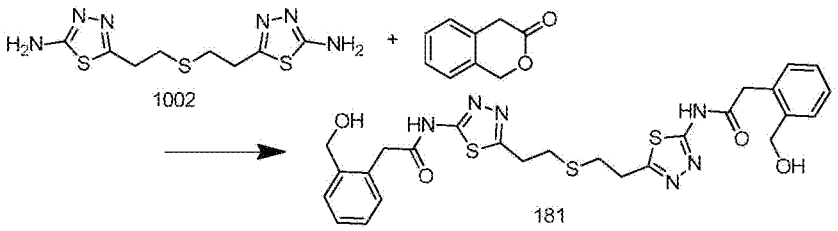
[0411]



[0412] 324 : ^{1}H NMR (300MHz, 二甲亚砜-d6) δ ppm 1.39 (s, 18H) 1.76 (brs, 4H) 3.01 (brs, 4H) 3.79 (s, 4H) 4.11-4.13 (brs, 4H) 7.13-7.38 (m, 8H) 12.65 (s, 2H)。

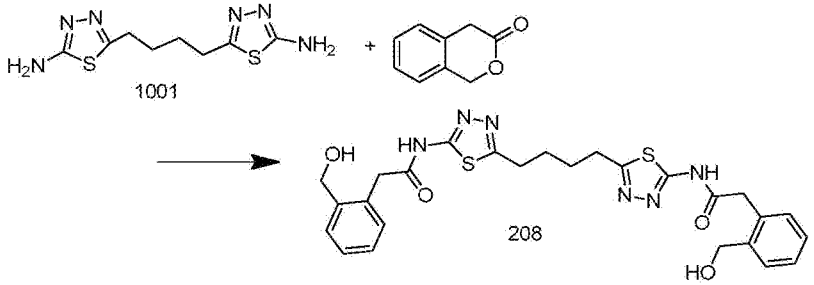
[0413] 方法 C :通过与酯 / 内酯的铝酰胺偶联

[0414]



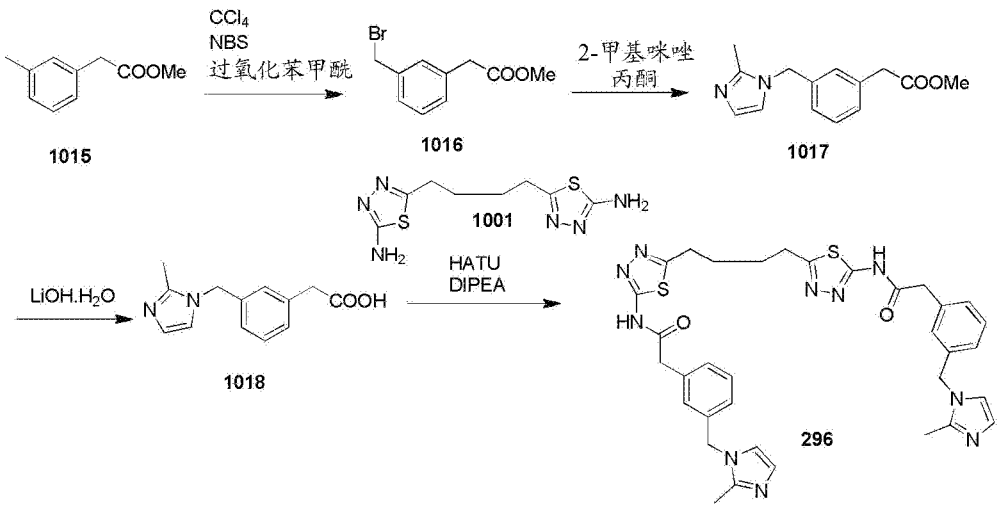
[0415] 向 1002(288mg,1.00mmol) 在甲苯 (9mL) 中的悬浮液中依次加入 3- 异色满酮 (311mg,2.10mmol) 和三甲基铝 (2M 的甲苯溶液,1.0mL,2.00mmol)。将所得混合物在 75℃下搅拌 15 小时,冷却至室温,并用乙酸乙酯 (50mL) 稀释。有机层用水 (3x20mL)、10% 氯化钠溶液 (10mL) 洗涤,干燥 (硫酸镁),并减压浓缩。粗产物用 HPLC 纯化,得到 N,N'-(5,5'-(硫代双 (乙烷 -2,1- 二基)) 双 (1,3,4- 噻二唑 -5,2- 二基)) 双 (2-(2-(羟基甲基) 苯基) 乙酰胺)(181,78mg)。^{1}H NMR(300MHz,DMSO-d_6) δ 7.42(d,J = 6.84Hz,2H),7.26(bs,6H),4.57(s,4H),3.90(s,4H),3.27(t,J = 6.62Hz,4H),2.94(t,J = 6.44Hz,4H)。

[0416]



[0417] 向 1001(256mg,1.00mmol) 在甲苯 (8mL) 中的悬浮液中依次加入 3- 异色满酮 (311mg,2.10mmol) 和三甲基铝 (2M 的甲苯溶液,1.0mL,2.00mmol)。将所得混合物在 75℃下搅拌 15 小时,冷却至室温,并用乙酸乙酯 (50mL) 稀释。有机层用水 (3x20mL)、10% 氯化钠溶液 (10mL) 洗涤,干燥 (硫酸镁),并减压浓缩。粗产物用 HPLC 纯化,得到 N,N'-(5,5'-(硫代双 (乙烷 -2,1- 二基)) 双 (1,3,4- 噻二唑 -5,2- 二基)) 双 (2-(2-(羟基甲基) 苯基) 乙酰胺)(208,62mg)。^{1}H NMR(300MHz,DMSO-d_6) δ 7.41(s,2H),7.26(s,6H),4.56(s,4H),3.01(bs,4H),1.76(bs,4H)。

[0418]



[0419] 向 1015(3.2g,19.5mmol) 的四氯化碳 (150mL) 溶液中加入 N- 溴代琥珀酰亚胺

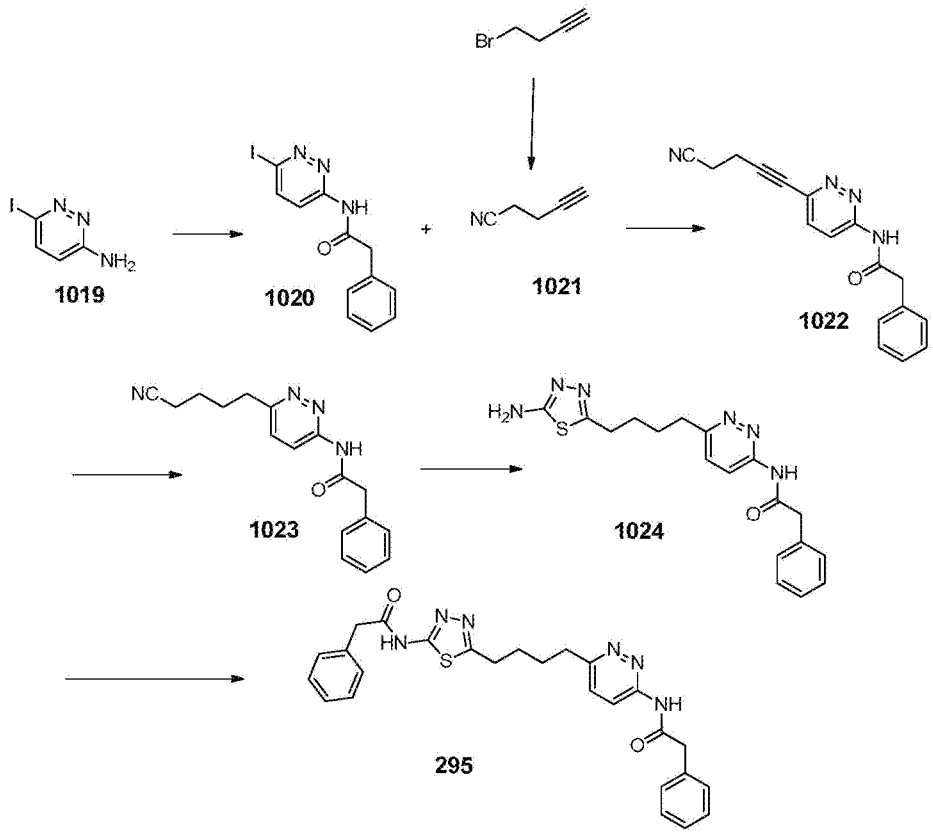
(3.47g,19.6mmol)和过氧化苯甲酰(10mg,催化性)。使所得混合物回流过夜后,将其热过滤。将滤液减压浓缩,所得残余物用硅胶色谱法纯化(用20%乙酸乙酯/己烷洗脱),得到1016(2g,42%收率),为油状物。^{1}H NMR(300MHz, 氯仿-d) δ ppm 3.66(s,2H)3.74(s,3H)4.51(s,2H)7.35(m,4H)。

[0420] 向1016(0.243g,1mmol)的丙酮(10mL)溶液中加入2-甲基咪唑(0.41g,5mmol)。使所得混合物回流过夜后,将其减压浓缩,所得残余物用水(~100mL)稀释。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发。所得残余物用硅胶色谱法纯化(用MeOH/二氯甲烷洗脱),得到1017(0.17g,69%收率),为油状物。^{1}H NMR(300MHz, 氯仿-d) δ ppm 2.37(s,3H)3.63(s,2H)3.72(s,3H)5.07(s,2H)6.87(s,1H)6.96-7.029m,2H)7.23-7.33(m,3H)。

[0421] 向1017(0.17g,0.69mmol)的THF/MeOH/水(10mL,2mL,2mL)溶液中加入一水氢氧化锂(0.06g,1.42mmol)。将所得混合物在室温下搅拌过夜后,将其减压浓缩。所得残余物用水(~20mL)稀释,所得溶液用乙酸酸化。将水层浓缩,产物用制备型HPLC分离。将所得残余物溶于水(5mL),向其中加入浓盐酸(83μL)后,将其浓缩并干燥,得到1018(0.15gm),为盐酸盐。

[0422] 向羧酸1018(105mg,0.39mmol)在DMF(3mL)中的悬浮液中加入HATU(150mg,0.39mmol),搅拌直到反应混合物澄清,接着加入胺1001(50.5mg,0.197mmol)和DIPEA(0.14mL,0.8mmol)。将所得混合物在室温下搅拌过夜后,通过加入水将其猝灭。分离的固体经过滤,用水洗涤,干燥,得到296(112mg,83%)。^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.76(brs,4H)2.38(s,6H)3.01(brs,4H)3.82(s,4H)5.25(s,4H)7.09-7.38(m,12H)12.64-12.67(brs,2H)。

[0423]



[0424] 在 0 ℃ 下，向 1019(1.5g,6.8mmol) 在 CH_2Cl_2(15mL) 中的悬浮液中依次滴加 Et_3N(1.9ml,13.6mmol) 和苯乙酰氯 (1.07ml,8.1mmol)。将所得混合物在 0℃下搅拌，然后慢慢升温至室温持续 2 天。粗物质用硅胶色谱法纯化（用含 0-25% EtOAc 的己烷洗脱），得到 1020。

[0425] 在 0℃下，向 4- 溴 -1- 丁炔 (7g,53mmol) 的 DMSO(30ml) 溶液中加入 NaI(7.94g,53mmol)。将混合物在室温下搅拌 2 小时后，使之冷却至 0℃，接着加入 NaCN(5.2g,106mmol)。所得混合物在 80℃下加热 2.5 小时，然后在室温下搅拌过夜。使混合物在水和 EtOAc 之间分配。有机萃取物用水洗涤，经硫酸钠干燥，过滤并蒸发，得到 1021。

[0426] 在氩气氛下，向 1020(400mg,1.18mmol)、$PdCl_2(PPh_3)_2$(41mg,0.059mmol) 和 CuI(11mg,0.059mmol) 在 Et_3N(3ml) 和 THF(6ml) 中的混合物中加入 1021(187mg,2.36mmol)，然后在 60℃下加热过夜。在除去溶剂后，残余物用硅胶色谱法纯化（用含 0-60% EtOAc 的己烷洗脱），得到 1022。

[0427] 向 1022(118mg,0.406mmol) 在 EtOAc(60ml) 和 EtOH(15ml) 的混合物中的溶液中加入 $Pd(OH)_2/C$(50mg,0.356mmol)。使氢气鼓泡通过所得混合物中，并搅拌 1 小时。滤出 Pd 催化剂，并将滤液浓缩，得到 1023。

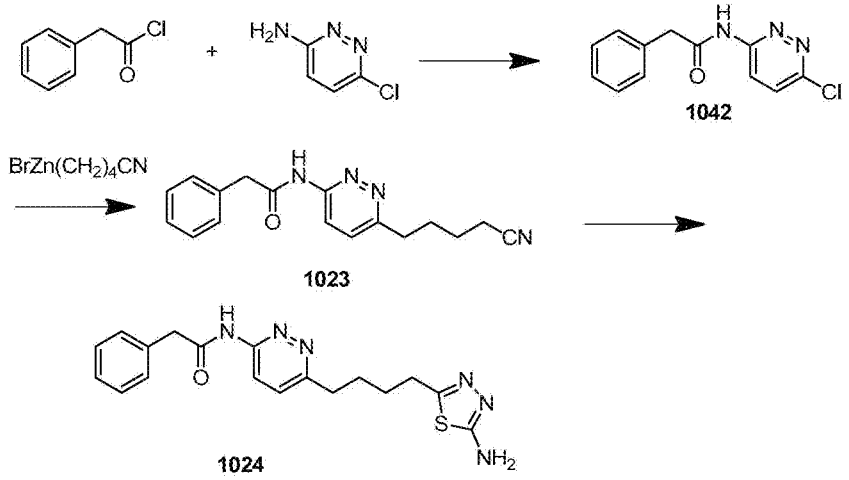
[0428] 将 1023(127mg,0.431mmol) 和氨基硫脲 (51mg,0.561mmol) 在 TFA(3mL) 中的混合物在 85℃下加热 5 小时。使反应物冷却至室温，倒入冰 - 水的混合物中。混合物用 NaOH 小粒碱化 (pH 10)。粗物质用硅胶色谱法纯化（用含 0-6% MeOH 的 CH_2Cl_2 洗脱），得到 1024。

[0429] 在 0 ℃ 下，向 1024(38.4mg,0.104mmol) 在 NMP(1mL) 中的溶液中滴加苯乙酰氯 (0.017mL,0.125mmol)。将所得混合物在 0 ℃ 下搅拌 1.5h 后，通过加入水（～10mL）将其猝灭。使混合物在水和 EtOAc 之间分配。有机萃取物用水洗涤，经硫酸

钠干燥，过滤并蒸发。粗物质用硅胶色谱法纯化（用含 0-6％ MeOH 的 CH_2Cl_2 洗脱），得 到 295。^{1}HNMR(300MHz, DMSO-d_6) δ 12.65(s, 1H), 11.26(s, 1H), 8.22-8.19(d, J = 8.82Hz, 1H), 7.58-7.54(d, J = 9.72Hz, 1H), 7.36-7.28(m, 10H), 3.81-3.78(d, J = 8.43Hz, 4H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H)。

[0430] 化合物 1024 也可按照下列方法制备：

[0431]



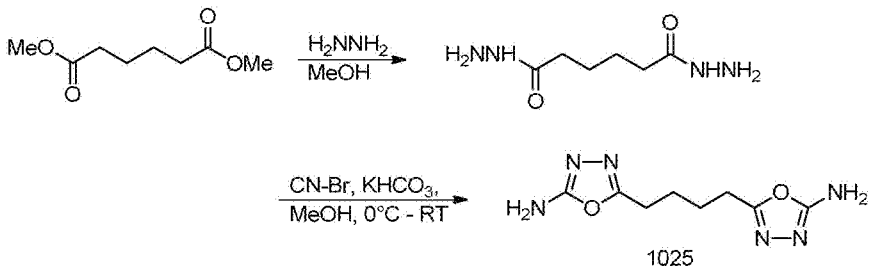
[0432] 在 19℃下，在 5 分钟内向 3-氨基-6-氯哒嗪 (11.14g, 86.0mmol) 的 NMP(279mL) 溶液中滴加苯乙酰氯 (18.2mL, 137.6mmol)，同时保持溶液的内部温度 $T_i \leq 28$℃。将所得混合物在 19℃下搅拌 90 分钟，并倒入冰水 (557mL) 中。白色沉淀通过吸滤收集，用水 (2x110mL) 和乙醚 (110mL) 漂洗。将产物高真空干燥过夜，得到 N-(6-氯哒嗪-3-基)-2-苯基乙酰胺 (xxx, 18.8g)。^{1}H NMR(300MHz, DMSO-d_6) δ 11.57(s, 1H), 8.40(d, J = 9.636Hz, 1H), 7.90(d, J = 9.516Hz, 1H), 7.36(m, 5H) 3.82(s, 2H)。

[0433] 用 $Ar_{(g)}$ 吹扫配备内部温度探针和加料漏斗的 1000mL 三颈烧瓶。在正氩气压力下，在室温下，将 4-氰基丁基溴化锌 (0.5M 的 THF 溶液，500mL，250mmol) 装入加料漏斗，然后加入反应容器中。在 $Ar_{(g)}$ 流下，在室温下将固体 N-(6-氯哒嗪-3-基)-2-苯基乙酰胺 (20.6g, 83.3mmol) 加入搅拌溶液中，接着加入 $NiCl_2$(dppp) (4.52g, 8.33mmol)。将所得混合物在 19℃下搅拌 240 分钟，然后用乙醇 (120mL) 猝灭。将水 (380mL) 加入搅拌的红色溶液中，得到浓稠的沉淀。加入乙酸乙酯 (760mL)，充分搅拌 30 分钟。通过经硅藻土垫过滤除去固体。然后将母液转移到分液漏斗中，有机层用 H_2O(380mL)、0.5％乙二胺四乙酸溶液 (380mL) 洗涤，并再次用 H_2O(380mL) 洗涤。有机层通过旋转蒸发浓缩。将所得红色油状物重新溶于 EtOAc(200mL)，将 1M HCl(380mL) 加入充分搅拌的烧瓶中。在 30 分钟后，将混合物转移到分液漏斗中，收集水层。有机层用 1M HCl(2x380mL) 萃取。然后使用 7.5％碳酸氢钠溶液调节水层的 pH 至～ 7，浅黄色沉淀通过吸滤收集，用水 (200mL) 和乙醚 (2x200mL) 漂洗。固体经高真空干燥过夜，得到 N-(6-(4-氰基丁基) 哒嗪-3-基)-2-苯基乙酰胺 (1023, 14.76g)。^{1}H NMR(300MHz, DMSO-d_6) δ 11.29(s, 1H), 8.23(d, J = 9.036Hz, 1H), 7.59(d, J = 9.246Hz, 1H), 7.32(m, 5H), 3.79(s, 2H), 2.90(t, J = 7.357Hz, 2H), 2.56(t, J = 7.038Hz, 2H), 1.79(t, J = 7.311Hz, 2H), 1.63(t, J = 7.01Hz, 2H)

[0434] 将 N-(6-(4-氰基丁基) 哒嗪-3-基)-2-苯基乙酰胺 (14.7g, 50.2mmol) 装入配备敞顶回流冷凝器的 250mL 圆底烧瓶中。向烧瓶中加入氨基硫脲 (5.03g, 55.2mmol) 和三

氟乙酸 (88mL)。使反应浆液在 65℃浴中加热 2 小时。在冷却至室温后，加入 H_2O(150mL)，搅拌 30 分钟。然后将混合物慢慢转移到在 0℃浴中冷却的搅拌的 7.5％碳酸氢钠溶液 (1400mL) 中。沉淀通过吸滤收集，用水 (2x200mL)、乙醚 (2x200mL) 漂洗，高真空下干燥过夜。将灰白色固体在 DMSO(200mL) 中调成浆液，在 80℃浴中加热直到内部温度达到 65℃。使用 DMSO(105mL) 冲洗烧瓶边侧。慢慢加入 H_2O(120mL) 直到溶液变得轻微混浊，然后从热浴中取出混合物，在搅拌同时使之冷却到环境温度。浅绿色沉淀通过吸滤收集，用水 (200mL) 和乙醚 (2x200mL) 漂洗。固体经高真空干燥过夜，得到 N-(6-(4-(5- 氨基 -1,3,4- 噻二唑 -2- 基) 丁基) 哒嗪 -3- 基)-2- 苯基乙酰胺 (1024,15.01g)。^{1}H NMR(300MHz,DMSO-d_6) δ 11.28(s,1H),8.23(d,J = 8.916Hz,1H),7.59(d,J = 8.826Hz,1H),7.36(m,5H),7.07(s,2H),3.78(s,2H),2.87(t,J = 6.799Hz,4H),1.69(bm,4H)

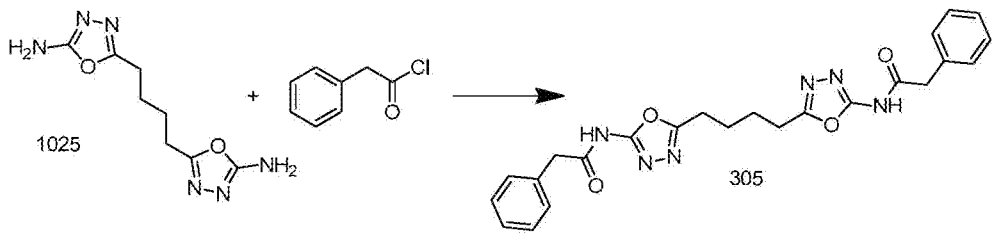
[0435]



[0436] 向己二酸二甲酯 (28.7mmol,5.0g,4.7mL,1.0 当量) 的 20mLMeOH 溶液中加入无水肼 (229.6mmol,7.36g,7.51mL,8.0 当量)，将混合物加热至 50℃，得到白色沉淀。将混合物加热 1 小时，然后使之冷却至室温。白色固体经过滤收集，用另外的 MeOH 洗涤，然后高真空干燥，得到 4.6g 脂族酰肼 (adipohydrizide)。^{1}HNMR(300MHz,DMSO-d_6) δ 8.91(s,2H),4.14(s,4H),2.00(br s,4H),1.46(br s,4H)。

[0437] 向脂族酰肼 (12.49mmol,4.0g,1.0 当量)、碳酸氢钾 (15.61mmol,1.56g,1.25 当量) 在 25mL MeOH 的 0℃冷却浆液中一次性加入固体溴化氰 (13.74mmol,1.44g,1.1 当量)。将该混合物在 0℃下搅拌，并使之在 1 小时内升温至室温，然后搅拌过夜。减压除去挥发物，将固体用水稀释。用 2.5N NaOH 调节 pH 至 12，固体经过滤收集。白色固体用水洗涤，高真空干燥，得到 1.73g 噁二唑 1025。^{1}HNMR(300MHz,DMSO-d_6) δ 6.85(s,4H),2.68(s,4H),1.68(s,4H)。

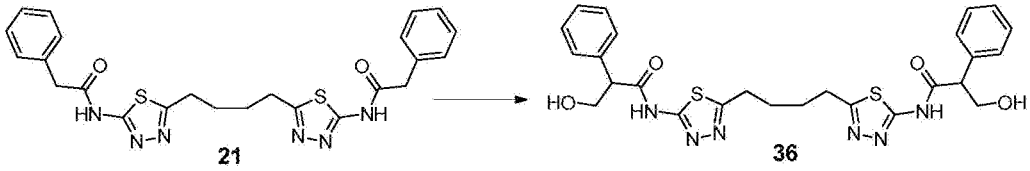
[0438]



[0439] 向噁二唑 1025(181mg,0.81mmol) 在 NMP(9mL) 中的悬浮液中加入三乙胺 (0.564mL,4.05mmol)，将混合物加热至 70℃。将混合物搅拌 30 分钟，接着加入苯乙酰氯 (0.234mL,1.77mmol)。将反应温度保持在 70℃下 15 小时，然后使冷却至室温。粗制反应混合物通过反相 HPLC 纯化，得到 305(0.015g)。^{1}HNMR(300MHz,DMSO-d_6) δ 11.74(s,2H),7.33(s,10H),3.74(s,4H),2.85(s,4H),1.76(s,4H)。

[0440] 二酰化核心的官能化：

[0441]

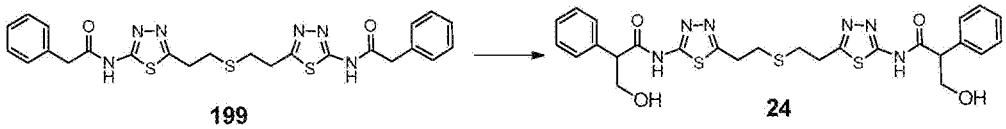


[0442] 在室温下，向 21(2.25g，4.57mmol) 在 THF(250mL) 和 H_2O(20mL) 的混合物中的悬浮液中加入 NaOH(1.83g，45.67mmol) 和甲醛溶液 (37％的水溶液，14.83mL，182.70mmol)。将所得混合物在 60℃下加热 7 小时后，使之冷却至 0℃，并用 HCl 水溶液酸化至 pH 7。白色沉淀通过吸滤收集，用水漂洗并干燥，得到 N,N'-[5,5'-(丁烷-1,4-二基)-双(1,3,4-噻二唑-5,2-二基)]-双 (3-羟基-2-苯基丙酰胺)(36，624mg)。从滤液中的第二次沉淀，得到额外的产物 (1.29g)。^1H NMR(300MHz，DMSO-d_6) δ 12.65(bs，2H)，7.35-7.30(m，10H)，5.09(bs，2H)，4.10-4.02(m，4H)，3.61(d，J = 8.1Hz，2H)，3.02(bs，4H)，1.76(bs，4H)。

[0443]

[0444] 向 199(300mg，0.572mmol) 在 THF(50mL) 和 MeOH(5ml) 的混合物的悬浮液中加入碳酸钾 (158mg，1.144mmol) 和甲醛溶液 (37％的水溶液，2mL)。将所得混合物在室温下搅拌 48 小时后，使之冷却至 0℃，并用 HCl 水溶液酸化至 pH 7。白色沉淀通过吸滤收集，用水漂洗并干燥。粗物质用 HPLC 纯化，得到 29。^1H NMR(300MHz，DMSO-d_6) δ 7.34-7.26(m，10H)，4.13-4.02(m，2H)，3.81(s，2H)，3.62(m，2H)，3.24(t，4H)，2.93(t，4H)。

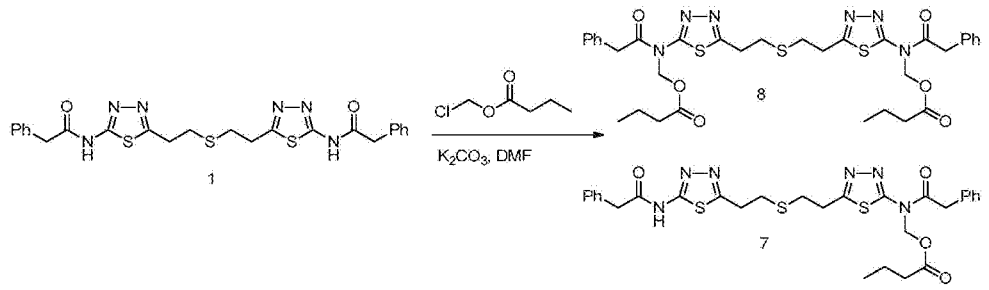
[0445]



[0446] 在室温下，向 199(2.0g，3.81mmol) 在 THF(250mL) 和 MeOH(20ml)、H_2O(20mL) 的混合物中的悬浮液中加入 1N NaOH(20ml) 和甲醛溶液 (37％的水溶液，15mL)。将所得混合物在 50℃下加热过夜后，使之冷却至 0℃，并用 HCl 水溶液酸化至 pH 7。白色沉淀通过吸滤收集，用水漂洗并干燥。粗物质用 HPLC 纯化，得到 24。^1H NMR(300MHz，DMSO-d_6) δ 12.67(bs，2H)，7.36-7.30(m，10H)，5.10(bs，2H)，4.10-4.02(m，4H)，3.61(d，2H)，3.27(t，4H)，2.95(t，4H)。

[0447] 前药：

[0448]

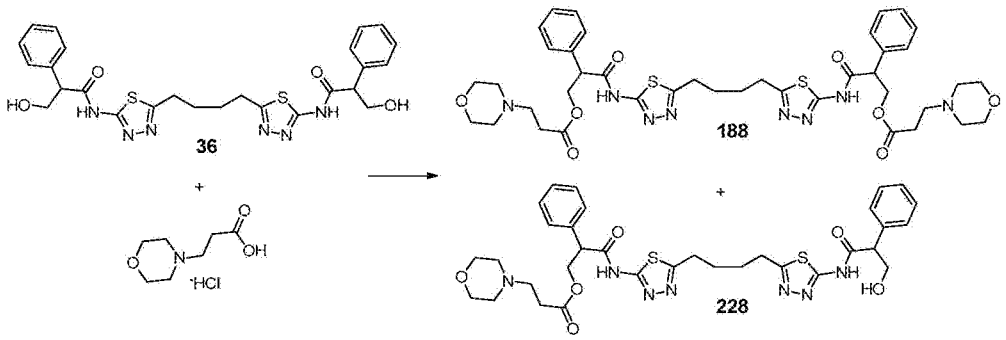


[0449] 向含有 N,N'-(5,5'-(硫代双(乙烷-2,1-二基))双(1,3,4-噻二唑-5,2-二基))双(2-苯基乙酰胺)(1)(9.4mmol,5.0g,1.0 当量)的烧瓶中加入 100mLDMF、K_2CO_3(20.98mmol,2.89g,2.2 当量)和丁酸氯甲酯(20.98mmol,2.86g,2.62mL,2.2 当量)。将混合物在室温下搅拌 15 小时,然后用 200mL 水和 200mL EtOAc 稀释。分层,水层用 EtOAc(2x 100mL)萃取,合并有机层,用水、盐水洗涤,经 Na_2SO_4干燥。Na_2SO_4经过滤除去,减压除去挥发物。化合物通过反相色谱法(MeCN,H_2O)纯化,得到 0.235g 的化合物 8 和 0.126g 的化合物 7。

[0450] ^{1}HNMR(300MHz,DMSO,d_6)化合物 8:δ 7.31(m,10H),6.18(s,4H),3.82(s,4H),3.17(dd,2H,J = 6.8Hz),2.92(dd,2H,J = 6.8Hz),2.93(m,4H),2.32(dd,2H,J = 7.2Hz),1.54(dt,2H,J = 7.2,7.4Hz),0.87(t,3H,J = 7.4Hz)。

[0451] ^{1}HNMR(300MHz,DMSO,d_6)化合物 7:δ 12.68(s,1H),7.32(m,10H),6.18(s,2H),3.82(s,4H),3.26(dd,2H,J = 7.0Hz),3.17(dd,2H,J = 6.8Hz),2.93(m,4H),2.32(dd,2H,J = 7.2Hz),1.54(dt,2H,J = 7.2,7.4Hz),0.87(t,3H,J = 7.4Hz)。

[0452]

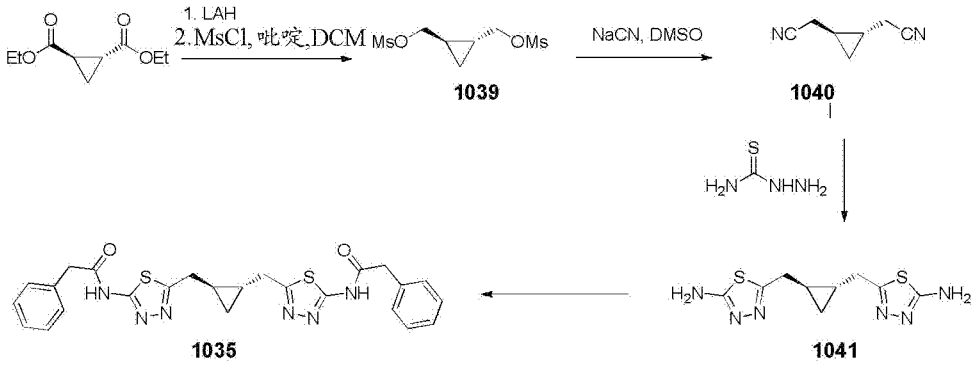


[0453] 在 0℃下,向 3-吗啉-4-基-丙酸盐酸盐(500mg,2.56mmol)在 DMF(20mL)中的悬浮液中加入 N-(3-二甲基氨基丙基)-N'-乙基碳二亚胺盐酸盐(534mg,2.79mmol)。将所得混合物在 0℃下搅拌 40 分钟,接着加入二醇 36(642mg,1.16mmol)和 4-DMAP(454mg,3.72mmol)。在 3.5 小时时间内从 0℃到室温搅拌所得混合物后,将其用 EtOAc 和冷水稀释。分离有机层,并用水(3×50mL)、盐水洗涤,干燥($MgSO_4$)并浓缩。粗产物用硅胶色谱法纯化(用含 10-25% MeOH 的 EtOAc 洗脱),得到{[5,5'-(丁烷-1,4-二基)-双(1,3,4-噻二唑-5,2-二基)]-双(氮烷二基)}-双(3-氧代-2-苯基丙烷-3,1-二基)-双(3-吗啉代丙酸酯)(188,340mg)和较少极性的产物 3-((5-{4-[5-(3-羟基-2-苯基丙酰胺基(propanamido))-1,3,4-噻二唑-2-基]丁基}-1,3,4-噻二唑-2-基)氨基)-3-氧代-2-苯基丙基 3-吗啉代丙酸酯(228,103mg)。188:^{1}H NMR(300MHz,DMSO-d_6)δ 12.80(s,2H),7.39(m,10H),4.62(t,J = 9.6Hz,2H),4.33-4.27(m,4H),3.48(bs,8H),3.02(bs,4H)

,2.45(bs,8H),2.25(bs,8H),1.76(bs,4H)。

[0454] 228:^{1}H NMR(300MHz,MeOD-d_4)δ7.43-7.37(m,10H),4.71(t,J=10.5Hz,1H),4.41(m,1H),4.30-4.24(m,2H),4.06-4.03(m,1H),3.80-3.76(m,1H),3.62(bs,4H),3.11(bs,4H),2.63-2.52(m,4H),2.40(bs,4H),1.90(bs,4H)。

[0455]



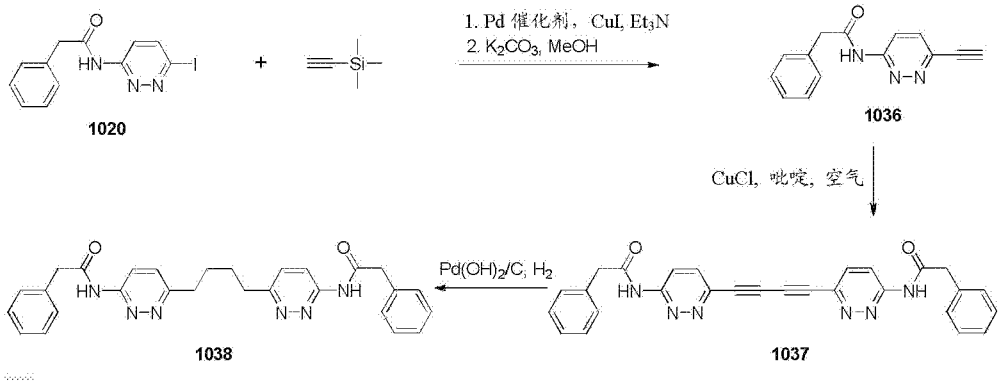
[0456] 在0℃下,向反式-1,2-环丙烷甲酸二乙酯(5.00g,26.85mmol)的THF(20mL)溶液中滴加L+AH(67.13mL,1.0M的THF溶液,67.13mmol)溶液。将所得混合物在0℃下搅拌1.5h后,将其用H_2O(20mL)、2N NaOH水溶液(20mL)和H_2O(20mL)猝灭。将混合物在室温下剧烈搅拌1小时后,使其通过硅藻土塞过滤。滤液经干燥($MgSO_4$)并浓缩,得到所需的二醇(2.73g),为无色油状物。

[0457] 在0℃下,向二醇(2.00g,19.58mmol)在CH_2Cl_2(75mL)中的混合物中依次滴加吡啶(6.34mL,78.33mmol)和MsCl(3.33mL,43.08mmol)。将所得混合物在0℃下搅拌1小时后,使之加热至室温。反应物用H_2O猝灭,用乙醚稀释。有机层用盐水洗涤,干燥($MgSO_4$)并浓缩,得到1039。将该粗产物溶于DMSO(75mL),加入NaCN(2.88g,58.75mmol)和NaI(294mg,1.96mmol)。将所得混合物在45℃下加热8小时后,使之冷却至室温,并用EtOAc和H_2O稀释。有机层经分离,用盐水洗涤,干燥($MgSO_4$)并浓缩,得到粗产物1040,无需纯化便将其用于下一步。

[0458] 将1040和氨基硫脲(3.75g,41.12mmol)在三氟乙酸(TFA)(20mL)中的混合物在80℃下加热5小时。使反应物冷却至室温,倒入冰和水的混合物中。将氢氧化钠小粒加入混合物中直到它为碱性(pH 14)。白色沉淀通过吸滤收集,用水、乙醚漂洗并干燥,得到1041(472mg)。

[0459] 在0℃下,向1041(70mg,0.26mmol)在1-甲基-2-吡咯烷酮(NMP)(5mL)中的悬浮液中滴加苯乙酰氯(72μL,0.55mmol)。将所得混合物在0℃下搅拌1小时后,通过加入水(~3mL)将其猝灭。白色沉淀通过吸滤收集,用水漂洗并干燥,得到1035(37mg)。^{1}H NMR(300MHz,DMSO-d_6)δ12.65(s,2H),7.34-7.27(m,10H),3.82(s,4H),3.04-2.75(m,4H),1.14-1.12(m,2H),0.63-0.59(m,2H)。

[0460]



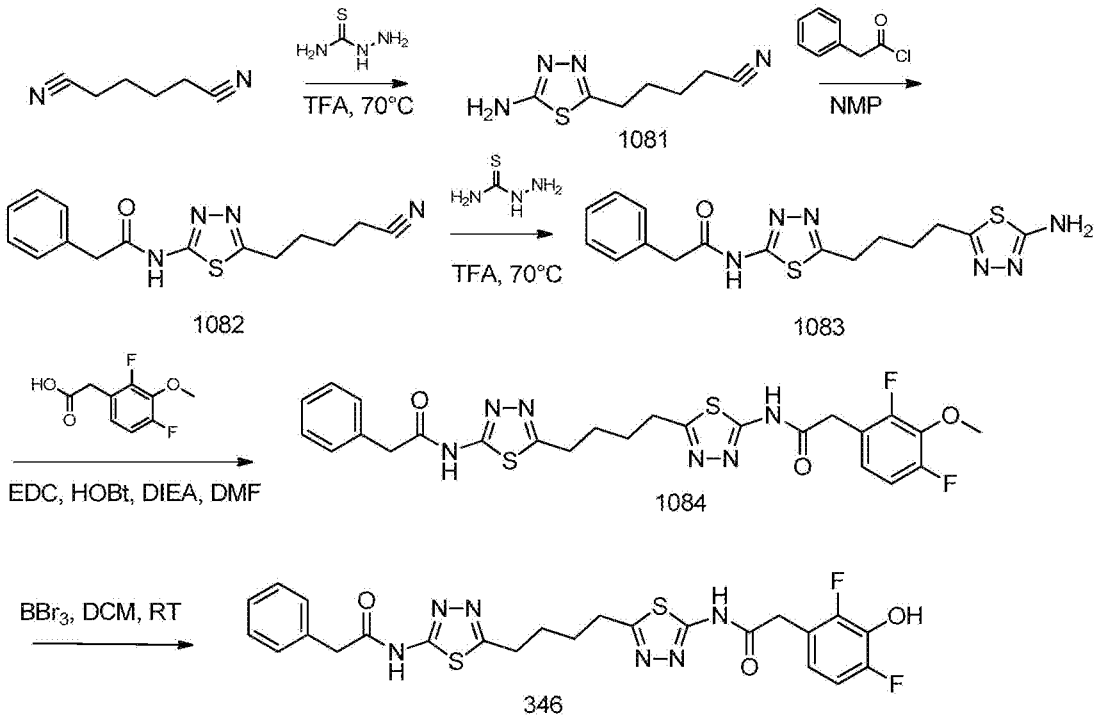
[0461] 在氩气氛、室温下，向 1020(1.50g,4.42mmol)、乙炔基三甲基甲硅烷 (813uL, 5.75mmol)、$PdCl_2(PPh_3)_2$(310mg,0.44mmol) 和 CuI(59mg,0.31mmol) 的 THF(20mL) 溶液中加入 Et_3N(6.16mL,44.23mmol)。将所得混合物在 50℃下加热 5 小时后，使之冷却至室温并经过硅藻土塞过滤。滤液经浓缩，粗制残余物用快速柱硅胶色谱法纯化（用含 10 - 50% EtOAc 的己烷洗脱），得到所需产物 (1.21g)，为固体。

[0462] 将前述中间体 (1.07g,3.48mmol) 和 K_2CO_3(0.40g,2.90mmol) 在 MeOH(100mL) 中的混合物在室温下搅拌 5 小时后，将其减压浓缩。将残余物重新溶于 EtOAc 和 H_2O 的混合物中，并用 1N HCl 水溶液中和至 pH 7。有机层经分离，用盐水洗涤，干燥 ($MgSO_4$) 并浓缩。粗制残余物用硅胶快速柱色谱法（用含 10 - 50% EtOAc 的己烷洗脱）纯化，得到所需的炔 1036(0.48g)，为白色固体。

[0463] 在室温下，向炔 1036(52mg,0.22mmol) 的吡啶 (5mL) 溶液中加入 CuCl(4.3mg, 0.04mmol)。在所有起始原料消耗时，将所得混合物在气流下搅拌 40 分钟。反应混合物用饱和 NH_4Cl 水溶液（～ 2mL）稀释。灰白色沉淀通过吸滤收集，用 H_2O 洗涤并干燥。该粗制的双 - 乙炔产物 1037(52mg) 无需进一步纯化便用于下一步骤。

[0464] 在所有起始原料消耗时，将 1037(52mg) 和 $Pd(OH)_2/C$(100mg) 在 DMF(5mL) 和 THF(10mL) 的混合物在室温下在 1 个大气压 H_2 下搅拌 3 小时。滤出钯催化剂，并将滤液浓缩。粗制残余物用硅胶柱色谱法纯化（用 1-10 % MeOH/CH_2Cl_2 洗脱），得到所需产物 1038(18mg)，为固体。^1H NMR(300MHz, DMSO-d_6) δ 11.26(s, 2H), 8.20(d, J = 8.97Hz, 2H), 7.56(d, J = 8.77Hz, 2H), 7.36 - 7.24(m, 10H), 3.78(s, 4H), 2.90(bs, 4H), 1.73(bs, 4H)。

[0465]



[0466] 向己二腈(19.02g,175.8mmol)的TFA(50mL)溶液中加入氨基硫脲(16.02g,175.8mmol),在氩气气氛下,将混合物加热至70℃达4小时。使混合物冷却至室温,并减压除去挥发物。残余物用水(200mL)稀释,并用固体NaOH调节pH至7,得到白色沉淀,经过滤收集并用水洗涤。将固体高真空干燥,得到9.22g 1081。^{1}HNMR(DMSO,d_6):δ 7.02(br s,2H)2.84(m,2H),2.55(m,2H),1.67(m,4H)。

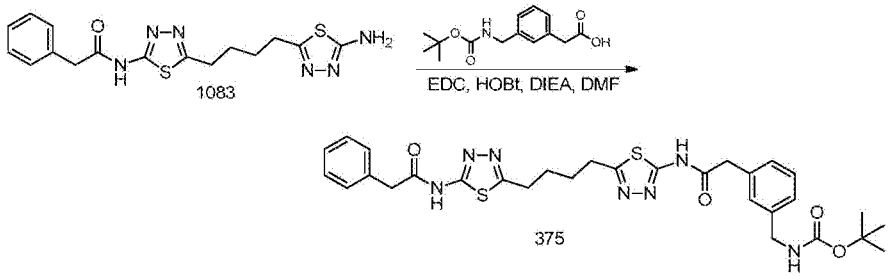
[0467] 向1081(0.625g,2.87mmol)的NMP(12.5mL)溶液中滴加苯乙酰氯(0.487g,0.42mL,3.15mmol),在氩气气氛下,将混合物在室温下搅拌1小时。将混合物倒入水(100mL)中,固体经过滤收集。固体用水洗涤,高真空干燥,得到0.805g 1082。^{1}HNMR(DMSO,d_6):δ 12.65(s,1H)7.31(m,5H),3.80(s,2H),3.00(t,2H,J = 7.3Hz),2.53(t,2H,J = 7.1Hz),1.78(dq,2H,J = 7.3,7.1Hz),1.61(dq,2H,J = 7.3,7.1Hz)。

[0468] 向1082(0.49g,1.33mmol)的TFA(10mL)溶液中加入氨基硫脲(0.23g,1.46mmol),将混合物在氩气气氛下在70℃下加热过夜。使混合物冷却至室温,并减压除去挥发物。残余物用水(50mL)稀释,并用固体NaOH调节pH至7,得到白色沉淀,其经过滤收集并用水洗涤。将固体高真空干燥,得到0.367g 1083。^{1}HNMR(DMSO,d_6):δ 12.70(s,1H)7.34(br s,5H),7.16(s,2H),3.82(s,2H),3.01(s,2H),2.84(S,2H),1.71(br s,4H)。

[0469] 向1083(0.10g,0.267mmol)、2,4-二氟-3-甲氧基苯乙酸(0.058g,0.267mmol)、EDC(0.127g,0.667mmol)、HOBt(0.090g,0.667mmol)的DMF(4mL)溶液中加入DIEA(0.171g,0.231mL,1.335mmol),将混合物在氩气气氛下搅拌过夜。将混合物倒入水(20mL)中,所形成的固体经过滤收集,用水洗涤,高真空干燥。粗产物1084无需纯化便用于下一步。向含1084(0.050g,0.091mmol)的二氯甲烷(1mL)溶液中加入BBr_3(1.0mL,1mmol,1.0M的二氢甲烷溶液),将混合物在室温、氩气气氛下拌拌4小时。减压除去挥发物,残余物用二氯甲烷(5mL)稀释。减压除去挥发物,残余物用水(15mL)稀释,并调节pH至12。水层用二氯甲烷(4x 5mL)洗涤,并调节pH至4。固体经过滤收集,用水洗涤,高真空干燥,得到0.029g 346。

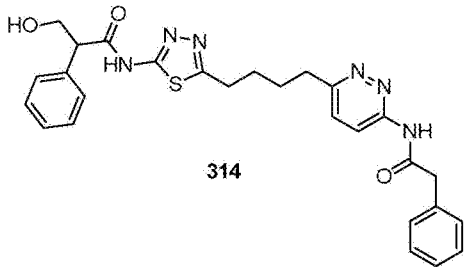
^{1}HNMR(DMSO, d_6) ：δ 12.66(s, 2H), 10.12(s, 1H), 7.33(s, 5H), 7.00(m, 1H), 6.80(m, 1H), 3.84(s, 2H), 3.81(s, 2H), 3.02(br s, 4H), 1.76(br s, 4H)。

[0470]



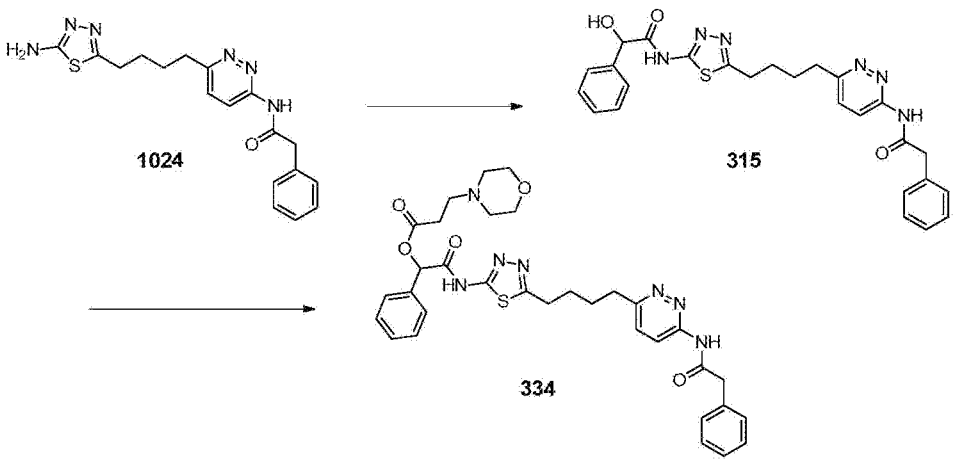
[0471] 向 1083(0.05g,0.133mmol)、Boc-3- 氨基甲基 - 苯乙酸 (0.035g,0.133mmol)、EDC(0.064g,0.332mmol)、HOBt(0.045g,0.332mmol) 的 DMF(8mL) 溶液中加入 DIEA(0.086g,0.115mL,0.665mmol)，将混合物在氩气气氛下搅拌过夜。将混合物倒入水 (20mL) 中，所形成的固体经过滤收集，用水洗涤，高真空干燥，得到 0.023g 375。^{1}HNMR(DMSO, d_6) ：δ 12.66(s, 2H), 7.27(m, 10H), 4.11(br s, 2H), 3.81(s, 2H), 3.79(s, 2H), 3.01(br s, 4H), 1.76(br s, 4H), 1.39(s, 9H)。

[0472]



[0473] 向烧瓶中装入 1024(100mg,0.27mmol)、含莨菪酸 (54mg,0.326mmol) 的 DMF(2ml)，在 0℃下依次加入 HOBT(88mg,0.652mmol) 和 EDCI(156mg,0.815mmol)。将所得混合物慢慢升温至室温，搅拌 3 小时后，通过加入水 (～ 10mL) 将其猝灭。白色沉淀通过吸滤收集，用更多的水漂洗并干燥，得到 314。^{1}H NMR(300MHz, DMSO-d_6) δ 12.65(s, 1H), 11.26(s, 1H), 8.22-8.19(d, J = 8.82Hz, 1H), 7.58-7.54(d, J = 9.72Hz, 1H), 7.36-7.28(m, 10H), 4.10-4.05(m, 2H), 3.78(s, 3H), 3.65(s, 1H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H)。

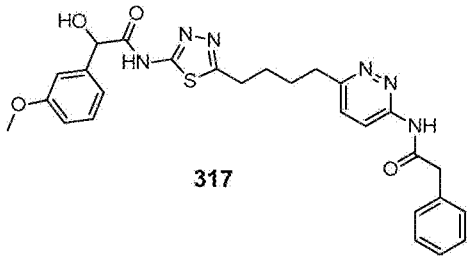
[0474]



[0475] 向烧瓶中装入1024(500mg,1.36mmol)、含DL-扁桃酸(248mg,1.63mmol)的DMF(10ml),在0℃下依次加入HOBT(441mg,3.26mmol)和EDCI(781mg,4.08mmol)。将所得混合物在0℃下搅拌10分钟,然后升温至室温,并搅拌10分钟后,在0℃下通过加入水(～50mL)将其猝灭。白色沉淀通过吸滤收集,用更多的水漂洗并干燥,得到315。^{1}H NMR(300MHz,DMSO-d_6)δ12.65(s,1H),11.26(s,1H),8.22-8.19(d,J＝8.82Hz,1H),7.58-7.50(m,3H),7.36-7.28(m,8H),6.35(s,1H),5.32(s,1H),3.78(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

[0476] 向3-吗啉-4-基-丙酸盐酸盐(209mg,1.07mmol)在DMF(10ml)中的悬浮液中加入EDCI(308mg,1.61mmol)。将所得混合物在0℃下搅拌1小时,接着加入315(447mg,0.889mmol)和4-DMAP(261mg,2.14mmol)。在6小时内将所得混合物从0℃搅拌至室温后,通过加入冰水(～50mL)将其猝灭。白色沉淀通过吸滤收集,用更多的水漂洗。粗物质用硅胶色谱法纯化(用含0-6% MeOH的EtOAc洗脱),得到334。^{1}H NMR(300MHz,DMSO-d_6)δ12.95(s,1H),11.26(s,1H),8.22-8.19(d,J＝9.45Hz,1H),7.58-7.26(m,11H),6.14(s,1H),3.78(s,2H),3.54(bs,4H),3.01(bs,2H),2.90(bs,2H),2.63(bs,4H),2.38(bs,4H),1.73(bs,4H)。

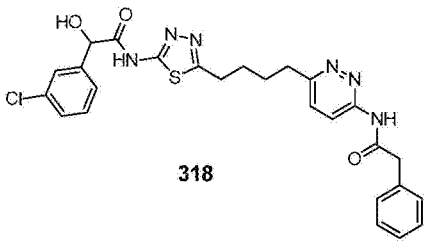
[0477]



317

[0478] 化合物317按照上述用于化合物315的方法制备。^{1}H NMR(300MHz,DMSO-d_6)δ12.40(s,1H),11.26(s,1H),8.22-8.19(d,J＝9.03Hz,1H),7.58-7.54(d,J＝9.72Hz,1H),7.36-6.87(m,9H),6.35(bs,1H),5.30(s,1H),3.78(m,5H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

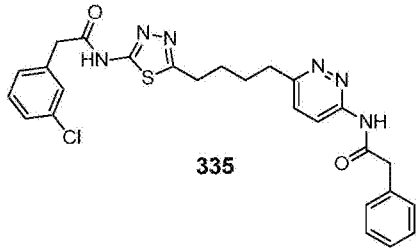
[0479]



318

[0480] 化合物318按照上述用于化合物315的方法制备。^{1}H NMR(300MHz,DMSO-d_6)δ12.50(s,1H),11.26(s,1H),8.22-8.19(d,J＝9.43Hz,1H),7.60-7.27(m,10H),6.51(bs,1H),5.35(s,1H),3.78(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

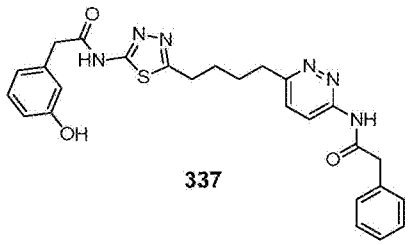
[0481]



335

[0482] 向烧瓶中装入1024(50mg,0.135mmol)、含3-氯苯乙酸(28mg,0.163mmol)的DMF(1ml),在0℃下依次加入HOBT(44mg,0.326mmol)和EDCI(78mg,0.408mmol)。使所得混合物慢慢升温至室温,搅拌1小时后,通过加入水(～5mL)将其猝灭。白色沉淀通过吸滤收集,用更多的水和乙醚漂洗,然后干燥,得到335。^{1}H NMR(300MHz,DMSO-d_6) δ 12.65(s,1H),11.26(s,1H),8.22-8.19(d,J＝8.82Hz,1H),7.58-7.54(d,J＝9.72Hz,1H),7.36-7.28(m,9H),3.84(s,2H),3.78(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

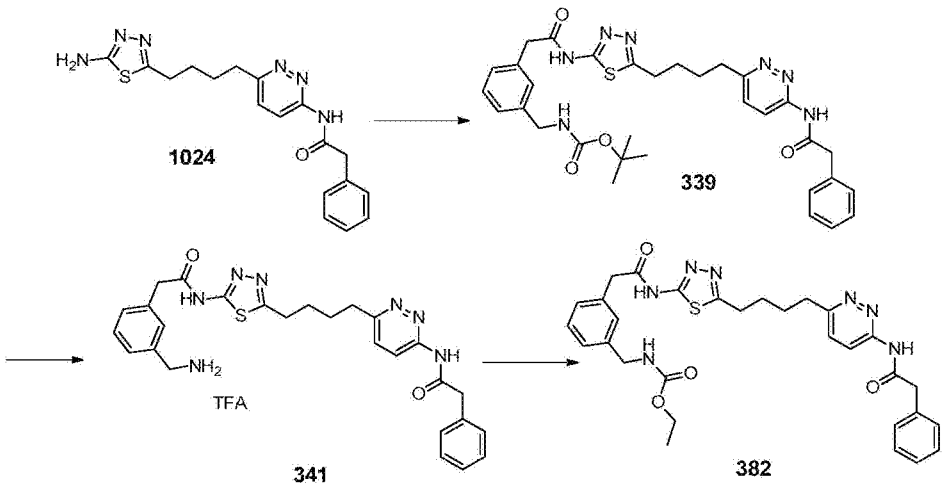
[0483]



337

[0484] 化合物337按照上述用于化合物335的方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.65(s,1H),11.26(s,1H),9.38(s,1H),8.22-8.19(d,J ＝ 8.37Hz,1H),7.58-7.54(d,J ＝ 9.63Hz,1H),7.36-7.09(m,6H),6.75-6.65(m,3H),3.78(s,2H),3.70(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

[0485]



1024 339 341 382

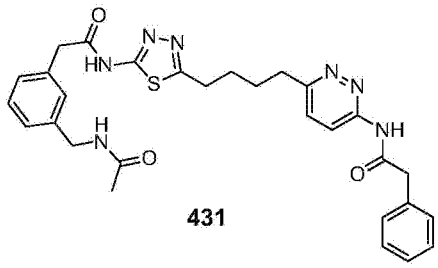
[0486] 339、341、382:向烧瓶中装入1024(100mg,0.27mmol)、含Boc-3-氨基甲基-苯乙酸(86mg,0.325mmol)的DMF(2ml),在0℃下依次加入HOBT(88mg,0.65mmol)和EDCI(156mg,0.812mmol)。将所得混合物在0℃下搅拌5分钟,然后升温至室温,并搅拌1.5小时后,在0℃下通过加入水(～10mL)将其猝灭。白色沉淀通过吸滤收集,用更多的水和乙醚漂洗,然后干燥,得到339。^{1}H NMR(300MHz,DMSO-d_6) δ 12.65(s,1H),11.26(s,1H),8.22-8.19(d,J＝8.82Hz,1H),7.58-7.54(d,J＝9.42Hz,1H),7.36-7.13(m,9H),4.13-4.11(d

, J = 10.62, 2H), 3.78(s, 4H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H), 1.38(s, 9H)。

[0487] 在 0 ℃ 下，向 339(50mg，0.081mmol) 在二氯甲烷 (2ml) 中的悬浮液中加入 TFA(2ml)。将所得混合物在室温下搅拌 20 分钟后，将其在真空下蒸发至干。加入乙醚，白色沉淀通过吸滤收集，用更多的乙醚和二氯甲烷漂洗，然后干燥，得到 341。^{1}H NMR(300MHz, DMSO-d_6) δ 12.65(s, 1H), 11.26(s, 1H), 8.22-8.19(d, J = 8.82Hz, 1H), 8.14-8.11(bs, 2H), 7.58-7.54(d, J = 9.42Hz, 1H), 7.36-7.13(m, 9H), 4.06-4.03(m, 2H), 3.84(s, 2H), 3.78(s, 2H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H)。

[0488] 在 0℃ 下，向 341(10mg，0.0159mmol) 的 DMF(1ml) 溶液中依次滴加三乙胺 (4.4ul，0.0317mmol) 和氯甲酸乙酯 (1.8ul，0.0191mmol)。将所得混合物慢慢升温至室温，搅拌 30 分钟后，在 0℃ 下通过加入水 (～ 1mL) 将其猝灭。使混合物在水和 EtOAc 之间分配。有机萃取物用水洗涤，经硫酸钠干燥，过滤并蒸发。粗物质用硅胶色谱法纯化 (用含 0-6% MeOH 的 CH_2Cl_2 洗脱)，得到 382。 ^{1}H NMR(300MHz, DMSO-d_6) δ 12.65(s, 1H), 11.26(s, 1H), 8.22-8.19(d, J = 8.82Hz, 1H), 7.67-7.58(bs, 1H), 7.58-7.54(d, J = 9.42Hz, 1H), 7.36-7.13(m, 9H), 4.18-4.16(m, 2H), 4.06-4.0(q, 2H), 3.78(s, 4H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H), 1.19-1.13(t, 3H)。

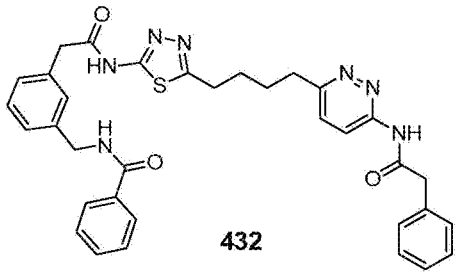
[0489]



431

[0490] 化合物 431 按照上述用于化合物 382 的方法用合适的试剂制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.65(s, 1H), 11.26(s, 1H), 8.35(s, 1H), 8.22-8.19(d, J = 8.88Hz, 1H), 7.57-7.54(d, J = 9.51Hz, 1H), 7.38-7.15(m, 9H), 4.25-4.24(d, J = 5.64Hz, 2H), 3.76(s, 4H), 3.01(bs, 2H), 2.90(bs, 2H), 1.87(s, 3H), 1.73(bs, 4H)。

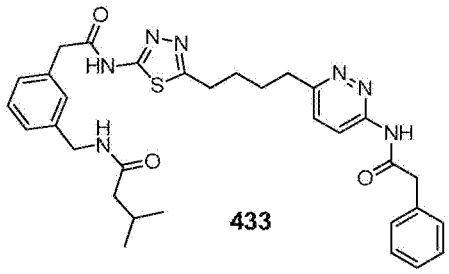
[0491]



432

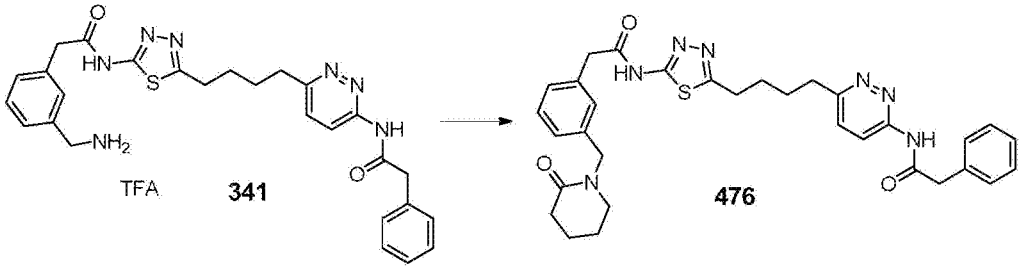
[0492] 化合物 432 按照上述用于化合物 382 的方法用合适的试剂制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.63(s, 1H), 11.26(s, 1H), 9.04-9.01(m, 1H), 8.22-8.19(d, J = 8.91Hz, 1H), 7.93-7.89(d, J = 9.51Hz, 2H), 7.58-7.25(m, 13H), 4.50-4.48(d, J = 5.91Hz, 2H), 3.78(s, 4H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H)。

[0493]



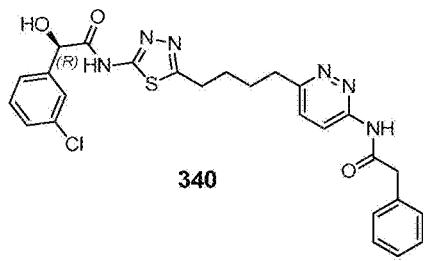
[0494] 化合物433按照上述用于化合物382的方法用合适的试剂制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.63(s,1H),11.26(s,1H),8.31-8.21(m,1H),8.20-8.19(d,J = 9.57Hz,1H),7.57-7.54(d,J = 8.73Hz,1H),7.35-7.13(m,9H),4.26-4.24(d,J = 5.52Hz,2H),3.78(s,4H),3.01(bs,2H),2.90(bs,2H),2.0(s,3H),1.73(bs,4H),0.86-0.85(d,J = 3.99Hz,6H)。

[0495]



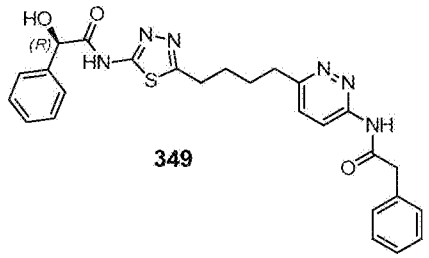
[0496] 在0℃下,向341(70mg,0.111mmol)的DMF(1ml)溶液依次滴加三乙胺(31ul,0.22mmol)和5-溴戊酰氯(12ul,0.122mmol)。将所得混合物慢慢升温至室温,搅拌1小时。然后在0℃下,将叔丁醇钾(50mg,0.445mmol)加入反应混合物中。将所得混合物慢慢升温至室温,搅拌过夜后,在0℃下通过加入水(~2mL)将其猝灭。使混合物在水和EtOAc之间分配。有机萃取物用水洗涤,经硫酸钠干燥,过滤并蒸发。粗物质用硅胶色谱法纯化(用含0-6% MeOH的CH_2Cl_2洗脱),得到476。^{1}H NMR(300MHz,DMSO-d_6) δ 12.65(s,1H),11.26(s,1H),8.22-8.19(d,J = 8.82Hz,1H),7.58-7.54(d,J = 9.42Hz,1H),7.36-7.13(m,9H),4.50(s,2H),3.78(s,4H),3.35(bs,2H),3.20(bs,2H),3.01(bs,2H),2.90(bs,2H),2.30(bs,2H),1.68-1.80(d,6H)。

[0497]



[0498] 化合物340按照上述用于化合物315的方法用合适的试剂制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.50(s,1H),11.26(s,1H),8.22-8.19(d,J = 9.24Hz,1H),7.60-7.27(m,10H),6.51(bs,1H),5.35(s,1H),3.78(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

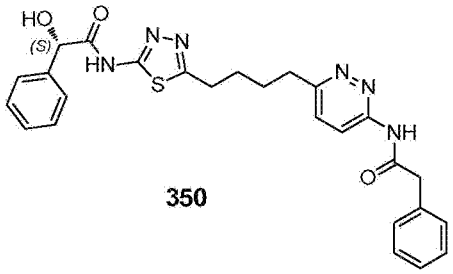
[0499]



349

[0500] 化合物349按照上述用于化合物315的方法用合适的试剂制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.41(s,1H),11.26(s,1H),8.22-8.19(d,J = 8.76Hz,1H),7.58-7.27(m,11H),6.36(s,1H),5.34(s,1H),3.78(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

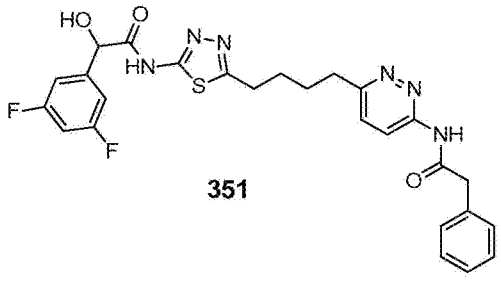
[0501]



350

[0502] 化合物350按照上述用于化合物315的方法用合适的试剂制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.41(s,1H),11.26(s,1H),8.22-8.19(d,J = 8.67Hz,1H),7.58-7.27(m,11H),6.34(s,1H),5.34(s,1H),3.78(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

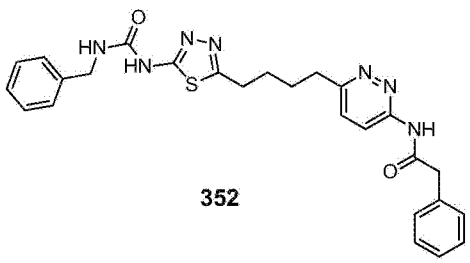
[0503]



351

[0504] 化合物351按照上述用于化合物315的方法用合适的试剂制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.50(s,1H),11.26(s,1H),8.21-8.18(d,J = 8.67Hz,1H),7.58-7.54(d,J = 9.72Hz,1H),7.36-7.23(m,8H),6.67(s,1H),5.40(s,1H),3.78(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

[0505]

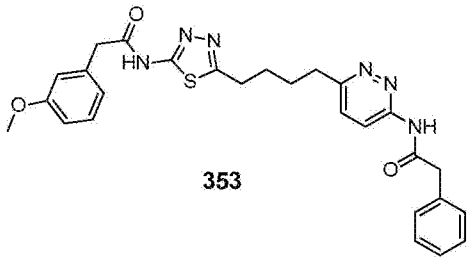


352

[0506] 在0℃下,向1024(50mg,0.136mmol)的DMF(1ml)溶液中滴加三乙胺(38ul,

0.271mmol)，接着滴加异氰酸苄酯 (20ul，0.163mmol)。将所得混合物慢慢升温至室温，搅拌 40 分钟后，在 0℃下通过加入水 (～ 5mL) 将其猝灭。白色沉淀通过吸滤收集，用更多的水漂洗。粗物质用硅胶色谱法纯化 (用含 0 - 6 % MeOH 的 CH_2Cl_2 洗脱)，得 到 352。^1H NMR(300MHz, DMSO-d_6) δ 11.26(s, 1H), 10.82(s, 1H), 8.22-8.19(d, J = 9.42Hz, 1H), 7.58-7.54(d, J = 8.79Hz, 1H), 7.36-7.31(m, 10H), 7.06(bs, 1H), 4.37-4.35(d, J = 5.22Hz, 2H), 3.78(s, 2H), 2.99-2.90(m, 4H), 1.73(bs, 4H)。

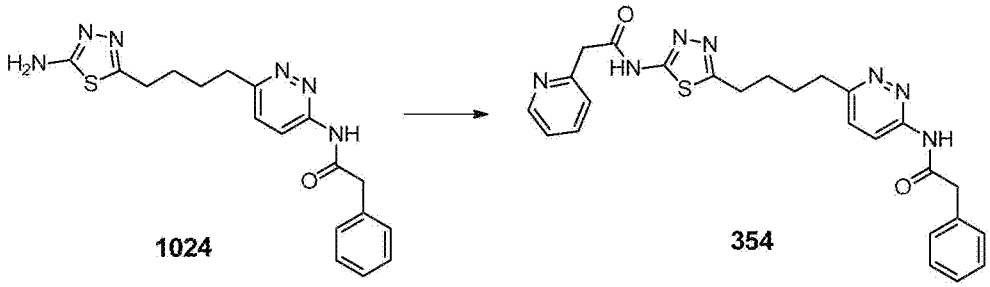
[0507]



353

[0508] 化合物 353 按照上述用于制备化合物 335 的方法制备。^1H NMR(300MHz, DMSO-d_6) δ 12.57(s, 1H), 11.26(s, 1H), 8.22-8.19(d, J = 9.45Hz, 1H), 7.57-7.54(d, J = 9.48Hz, 1H), 7.36-7.25(m, 6H), 6.91-6.84(m, 3H), 3.76(m, 7H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H)。

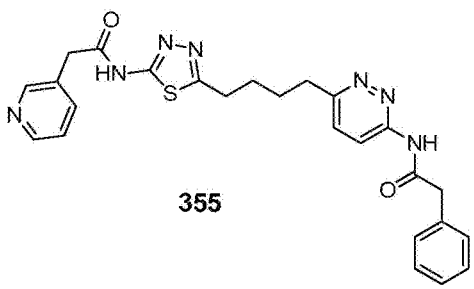
[0509]



1024 354

[0510] 向烧瓶中装入 1024(50mg，0.135mmol)、含 2- 吡啶乙酸盐酸盐 (27mg，0.156mmol) 的 DMF(1ml)，在 0℃下，依次加入丙基膦酸酐溶液 (91ul) 和三乙胺 (54ul，0.39mmol)。使所得混合物慢慢升温至室温，搅拌 1 小时后，通过加入水 (～ 5mL) 将其猝灭。白色沉淀通过吸滤收集，用更多的水和乙醚漂洗，然后干燥，得到 354。^1H NMR(300MHz, DMSO-d_6) δ 12.65(s, 1H), 11.26(s, 1H), 8.51(s, 1H), 8.22-8.19(d, J = 8.97Hz, 1H), 7.81-7.76(m, 1H), 7.58-7.54(d, J = 9.06Hz, 1H), 7.42-7.26(m, 7H), 4.02(s, 2H), 3.78(s, 2H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H)。

[0511]



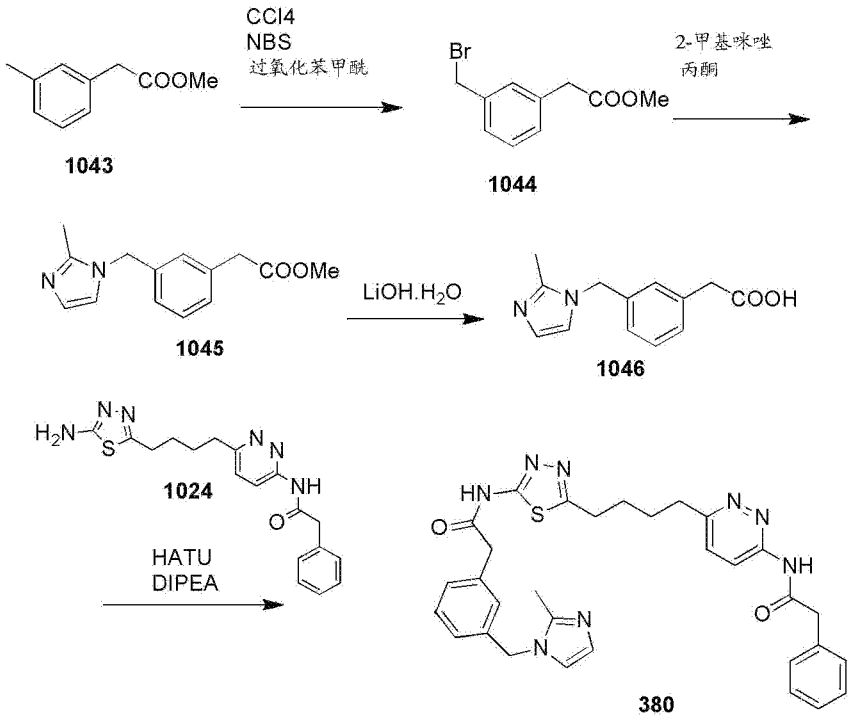
355

[0512] 化合物 355 按照上述用于制备化合物 354 的方法制备。^1H

NMR(300MHz,DMSO-d_6) δ 12.70(s,1H),11.26(s,1H),8.53-8.49(m,1H),8.22-8.19(d,J = 9.0Hz,1H),7.77-7.73(d,J = 8.46Hz,1H),7.58-7.54(d,J = 9.48Hz,1H),7.38-7.26(m,7H),3.88(s,2H),3.78(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

[0513] 化合物 309 和 310 按照上述用于制备化合物 354 的方法制备。

[0514]



[0515] 向 1043(3.2g,19.5mmol) 的四氯化碳 (150mL) 溶液中加入 N- 溴代琥珀酰亚胺 (3.47g,19.6mmol) 和过氧化苯甲酰 (10mg,催化性)。将所得混合物回流过夜后,将其热过滤。将滤液减压浓缩,所得残余物用硅胶色谱法纯化 (用 20% 乙酸乙酯 / 己烷洗脱),得到 1044(2g,42% 收率),为油状物。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 3.66(s,2H)3.74(s,3H)4.51(s,2H)7.35(m,4H)。

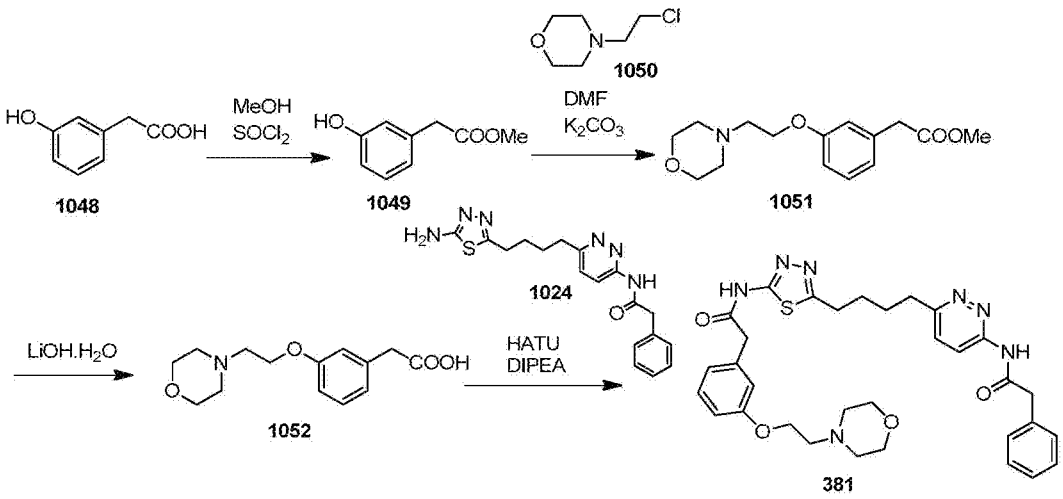
[0516] 向 1044(0.243g,1mmol) 的丙酮 (10mL) 溶液中加入 2- 甲基咪唑 (0.41g,5mmol)。将所得混合物回流过夜后,将其减压浓缩,所得残余物用水 (～ 100mL) 稀释。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发。所得残余物用硅胶色谱法纯化 (用 MeOH/ 二氯甲烷洗脱),得到 1045(0.17g,69% 收率),为油状物。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 2.37(s,3H)3.63(s,2H)3.72(s,3H)5.07(s,2H)6.87(s,1H)6.96-7.029m,2H)7.23-7.33(m,3H)。

[0517] 向 1045(0.17g,0.69mmol) 的 THF/MeOH/ 水 (10mL,2mL,2mL) 溶液中加入一水氢氧化锂 (0.06g,1.42mmol)。将所得混合物在室温下搅拌过夜后,将其减压浓缩。所得残余物用水 (～ 20mL) 稀释,所得溶液用乙酸酸化。将水层浓缩,产物用制备型 HPLC 分离。将所得残余物溶于水 (mL),向其加入浓盐酸 (mL) 后,将其浓缩并干燥,得到 1046(0.15gm),为盐酸盐。

[0518] 向羧酸 1046(41.8mg,0.157mmol) 在 DMF(3mL) 中的悬浮液中加入 HATU(61.3mg,0.161mmol),搅拌直到反应混合物澄清,接着加入胺 1024(52.5mg,0.142mmol) 和 DIPEA(50ul,0.29mmol)。将所得混合物在室温下搅拌过夜后,通过加入水将其猝灭。使

所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤，分离，经硫酸钠干燥，过滤并蒸发。所得残余物用乙醚研磨。分离的固体经过滤，用乙醚洗涤并干燥，得到380(40mg,48％)。^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.74(brs,4H)2.91-3.02(brs,4H)3.78-3.83(m,4H)5.34(s,2H)7.16-7.57(m,12H)8.19-8.22(d,1H)11.26(s,1H)12.65(brs,1H)。

[0519]



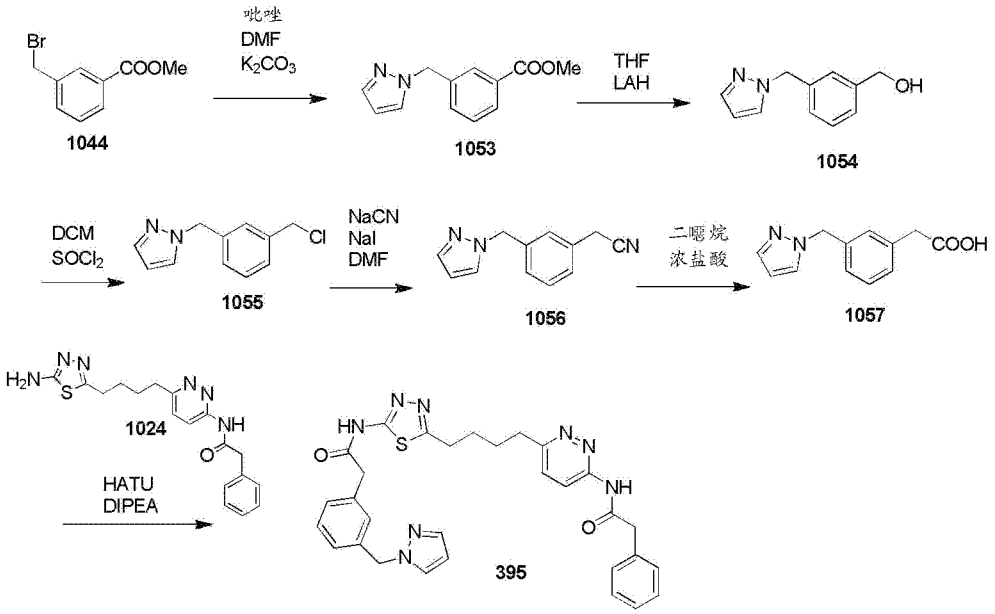
[0520] 向1048(5g,0.033mol)在甲醇(50mL)的冰冷溶液中加入亚硫酰氯(0.2mL)，将所得混合物在室温下搅拌过夜后，将其减压浓缩。使所得残余物高真空干燥过夜，得到1049(5gm)，为油状物，并照原样用于下一步骤。^{1}H NMR(300MHz, 氯仿-d) δ ppm 3.62(s,2H)3.74(s,3H)6.76-6.87(m,3H)7.18-7.21(m,1H)。

[0521] 向1049(1g,6mmol)的DMF(20mL)溶液中加入碳酸钾(2.08g,15mmol)、1050(1.225g,6.62mmol)和碘化钠(10mg)。将所得混合物在80℃下搅拌过夜后，将其用水(～100mL)稀释。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤，分离，经硫酸钠干燥，过滤并浓缩。所得残余物用硅胶色谱法纯化(用MeOH/二氯甲烷洗脱)，得到1051(1g,60％收率)，为油状物。^{1}H NMR(300MHz, 氯仿-d) δ ppm2.61(s,4H)2.83(t,2H)3.62(s,2H)3.63(s,3H)3.73-3.77(m,4H)4.14(t,2H)6.88-6.91(m,3H)7.26-7.29(m,1H)。

[0522] 向1051(1g,3.57mmol)的THF/MeOH/水(30mL,5mL,5mL)溶液中加入一水氢氧化锂(0.3g,7.14mmol)。将所得混合物在室温下搅拌过夜后，将其减压浓缩。所得残余物用水(～50mL)稀释，所得溶液用1N盐酸酸化。将水层浓缩，产物用制备型HPLC分离。将所得残余物溶于水(mL)，向其加入浓盐酸(mL)后，将其浓缩并干燥，得到1052，为盐酸盐。

[0523] 向羧酸1052(47.4mg,0.157mmol)在DMF(3mL)中的悬浮液中加入HATU(61.3mg,0.161mmol)，搅拌直到反应混合物澄清，接着加入胺1024(52.5mg,0.142mmol)和DIPEA(50ul,0.29mmol)。将所得混合物在室温下搅拌过夜后，通过加入水将其猝灭。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤，分离，经硫酸钠干燥，过滤并蒸发。所得残余物用硅胶色谱法纯化(用MeOH/二氯甲烷洗脱)，得到381(40mg,46％收率)。^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.74(brs,4H)2.72(t,2H)2.89-2.9(m,4H)3.02(brs,4H)3.336(m,2H)3.76-3.78(m,2H)4.09(m,2H)6.88-6.93(m,3H)7.24-7.36(m,6H)7.54-7.58(d,1H)8.18-8.21(d,1H)11.26(s,1H)12.65(brs,1H)。

[0524]



[0525] 向 1044(2.29g,0.01mol) 的 DMF(100mL) 溶液中加入碳酸钾 (1.38g,0.01mmol) 和吡唑 (0.68g,0.01mol)。将所得混合物在 70℃下搅拌 5 小时后,将其用水 (～ 100mL) 稀释。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发。所得残余物用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱),得到 1053(1g,50％收率)。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 3.94(s,3H)5.40(s,2H)6.33(s,1H)7.42-7.48(m,3H)7.58(s,1H)7.95(s,1H)8.00-8.02(m,1H)。

[0526] 向 1053(1g,4.62mmol) 的 THF(20mL) 冰冷溶液中滴加氢化铝锂 (2.5mL,2M/THF),将所得反应混合物在 0℃下搅拌 5 小时后,将其用饱和 Rochelle 盐溶液猝灭。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发,得到 1054(0.8g,92％收率)。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 4.71(s,2H)5.35(s,2H)6.30(s,1H)7.15-7.43(m,5H)7.58(s,1H)。

[0527] 向 1054(0.8g,4.2mmol) 的二氯甲烷 (20mL) 溶液中加入亚硫酰氯,将所得混合物在室温下搅拌 5 小时后,将其减压浓缩。使所得残余物高真空干燥过夜,得到 1055(1g,97％收率),为 HCl 盐。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 4.75(s,2H)5.38(s,2H)6.30(s,1H)7.19-7.50(m,5H)7.86(s,1H)11.49-11.60(brs,1H)。

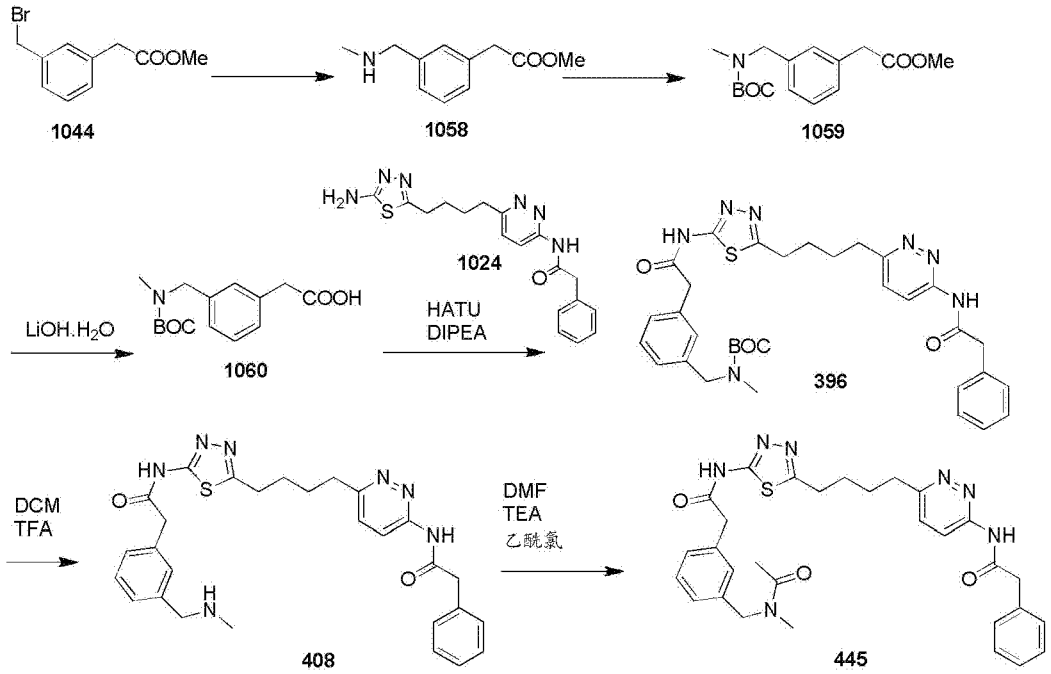
[0528] 向 1055(1g,4.1mmol) 的 DMF(20mL) 溶液中加入氰化钠 (0.625g,12.7mmol) 和碘化钠 (20mg),将所得反应混合物在 70℃下搅拌 2 小时后,将其用水稀释。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发。所得残余物用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱),得到 1056(0.664g,83％收率)。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 3.76(s,2H)5.38(s,2H)6.35(s,1H)7.19-7.46(m,5H)7.61(s,1H)。

[0529] 向 1056(0.664g,3.3mmol) 的二噁烷 (5mL) 溶液中加入浓盐酸 (5mL),将所得反应混合物在 90℃下搅拌过夜后,将其减压浓缩。所得残余物通过制备型 HPLC 纯化,并转化成 HCl 盐,得到 1057(0.5g,40％收率)。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 3.55(s,2H)5.33(s,2H)6.29(s,1H)7.14-7.20(m,4H)7.48(s,1H)7.84(s,1H)11.97-11.99(brs,1H)。

[0530] 向羧酸 1057(19.8mg,0.0785mmol) 在 DMF(2mL) 中的悬浮液中加入 HATU(30.6mg,

0.08mmol)，搅拌直到反应混合物澄清，接着加入胺 1024(26.25mg,0.07mmol) 和 DIPEA(25ul,0.15mmol)。将所得混合物在室温下搅拌过夜后，通过加入水将其猝灭。分离的固体经过滤，用水洗涤，干燥，得到 395(18mg,45％收率)。^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.74(brs,4H)2.89-3.04(m,4H)3.78(s,4H)5.33(s,2H)6.27-6.28(s,1H)7.09-7.58(m,11H)7.82(s,1H)8.19-8.21(d,1H)11.26(s,1H)12.65(brs,1H)。

[0531]



[0532] 向 1044(1g,4.1mmol) 的 THF(5mL) 溶液中加入 2M/THF 甲胺溶液 (2mL)，将所得反应混合物在室温下搅拌过夜后，将其减压浓缩。使所得残余物在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤，分离，经硫酸钠干燥，过滤并蒸发。所得残余物用硅胶色谱法纯化(用 MeOH/二氯甲烷洗脱)，得到 1058(0.26g,33％收率)。^{1}H NMR(300MHz, 氯仿-d) δ ppm 2.49(s,3H)3.66(s,2H)3.73(s,3H)3.79(s,2H)7.2-7.33(m,4H)。

[0533] 向 1058(0.26g,1.35mmol) 的二氯甲烷 (5mL) 溶液中加入 boc 酐 (0.293g,1.35mmol)，将所得反应混合物在室温下搅拌 4 小时后，并将其用硅胶色谱法纯化(用 EtOAc/己烷洗脱)，得到 1059(0.3g,77％收率)。^{1}H NMR(300MHz, 氯仿-d) δ ppm 1.5(s,9H)2.84(s,3H)3.66(s,2H)3.73(s,3H)4.44(s,2H)7.17-7.32(m,4H)。

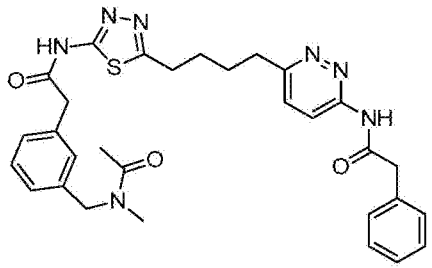
[0534] 向 1059(0.3g,1.02mmol) 在二噁烷 (3mL) 和水 (2mL) 的冰冷溶液中加入一水氢氧化锂 (0.086g,2.04mmol)，将所得反应混合物在 0℃下搅拌 3 小时后，将其用 1N HCl 酸化。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤，分离，经硫酸钠干燥，过滤并蒸发。使所得残余物高真空干燥过夜，得到 1060(0.2g,70％收率)。^{1}H NMR(300MHz, 氯仿-d) δ ppm 1.5(s,9H)2.84(s,3H)3.66(s,2H)4.43(s,2H)7.17-7.32(m,4H)。

[0535] 向羧酸 1060(51.1mg,0.183mmol) 在 DMF(3mL) 中的悬浮液中加入 HATU(69.7mg,0.183mmol)，搅拌直到反应混合物澄清，接着加入胺 1024(61.3mg,0.166mmol) 和 DIPEA(58ul,0.33mmol)。将所得混合物在室温下搅拌过夜后，通过加入水将其猝灭。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤，分离，经硫酸钠干燥，过滤并蒸发。所得残余物用硅胶色谱法纯化(用 MeOH/二氯甲烷洗脱)，得到 445(0.06g,57％

收率)。^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.37-1.38(s,9H)1.74(brs,4H)2.76(s,3H)2.89(brs,2H)3.02(brs,2H)3.78-3.80(m,4H)4.36(s,2H)7.11-7.36(m,9H)7.54-7.57(d,1H)8.18-8.21(d,1H)11.26(s,1H)12.65(brs,1H)。

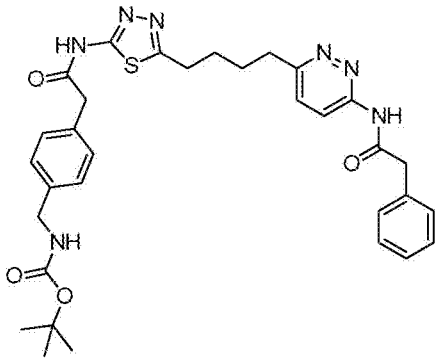
[0536] 通过396脱保护为408和再酰化制备445:

[0537]



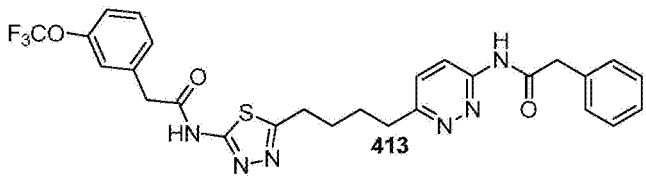
[0538] 向含408(26mg,0.04mmol)的DMF(1mL)的冰冷溶液中加入三乙胺(12.3uL,0.088mmol)和乙酰氯(3.16uL,0.044mmol)。将所得混合物在室温下搅拌2小时后,将其用水稀释。分离的固体经过滤,用水洗涤,高真空干燥过夜,得到445(10mg,48%收率)。^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.74(brs,4H)2.05(m,3H)2.91-3.02(m,7H)3.78-3.82(m,4H)4.49-4.56(m,2H)7.18-7.36(m,9H)7.55-7.58(d,1H)8.18-8.21(d,1H)8.75-8.7(brs,2H)11.26(s,1H)12.65(brs,1H)。

[0539]



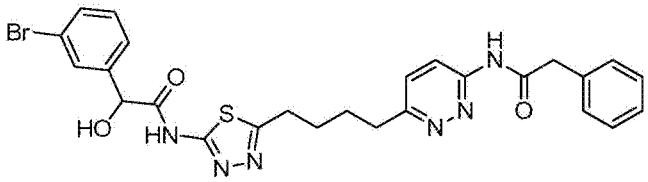
[0540] 化合物401按照上述用于制备化合物339的方法制备。^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.40(s,9H)1.75(brs,4H)2.87(brs,2H)2.89(brs,2H)3.78(s,4H)4.09-4.11(brs,2H)7.18-7.36(m,9H)7.54-7.58(d,1H)8.18-8.21(d,1H)11.26(s,1H)12.65(brs,1H)。

[0541]



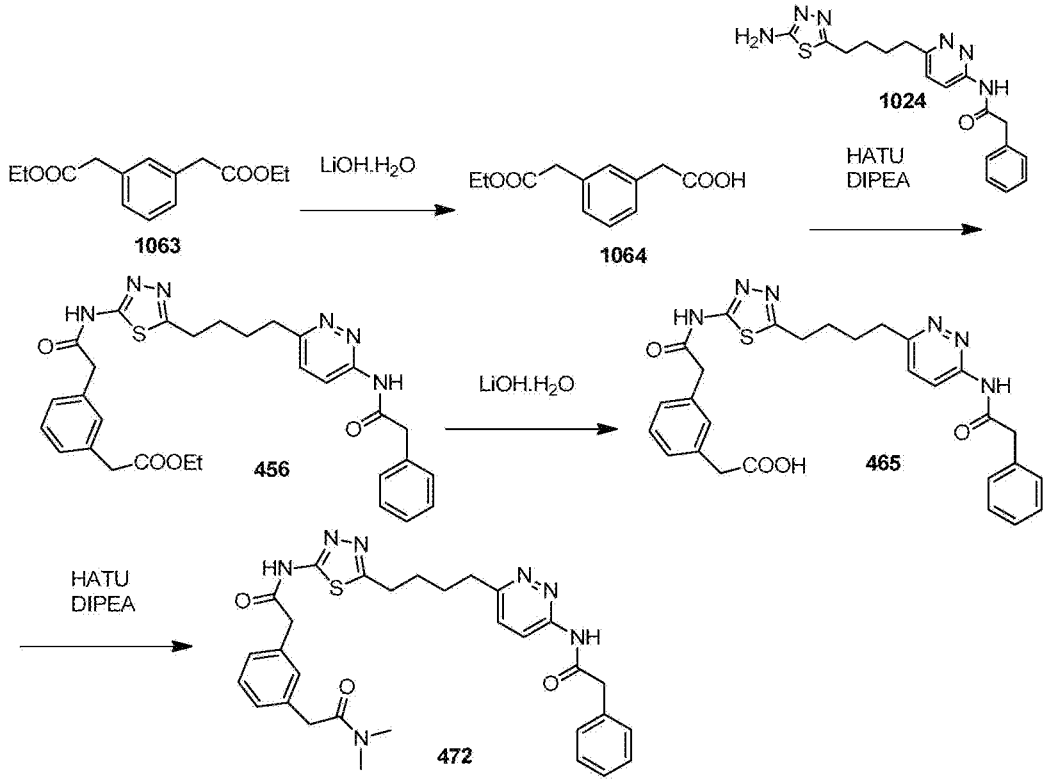
[0542] 化合物413按照上述用于制备化合物315的方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.68(bs,1H),11.26(s,1H),8.20(d,J = 9.46Hz,1H),7.58-7.26(m,10H),3.90(s,2H),3.78(s,2H),3.02(bs,2H),2.90(bs,2H),1.74(bs,4H)。

[0543]



[0544] 化合物 415 按照上述用于制备化合物 315 的方法制备：^{1}H NMR(300MHz,DMSO-d_6) δ 12.48(s,1H),11.26(s,1H),8.20(d,J = 8.95Hz,1H),7.75(s,1H),7.58 - 7.26(m,9H),6.52(m,1H),5.35(m,1H),3.78(s,2H),3.02(m,2H),2.90(m,2H),1.74(bs,4H)。

[0545]



[0546] 向 1063(6.31g,24.9mmol) 的乙醇溶液中加入一水氢氧化锂 (1.048g,24.9mmol)，将所得反应混合物在室温下搅拌 3 小时后，将其减压浓缩。所得残余物用水稀释，并用 6N HCl 酸化。溶液用乙酸乙酯萃取。有机萃取物用更多的水洗涤，分离，经硫酸钠干燥，过滤并蒸发。所得残余物用硅胶色谱法纯化（用 EtOAc/ 己烷洗脱），得到 1064(3g,53％收率）。

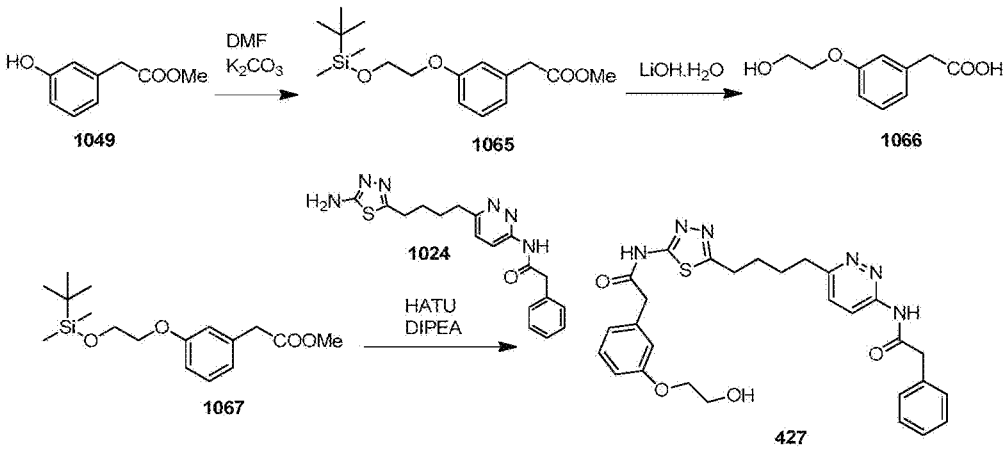
[0547] 向羧酸 1064(0.1g,0.44mmol) 在 DMF(2mL) 中的悬浮液中加入 HATU(0.17g,0.44mmol)，搅拌直到反应混合物澄清，接着加入胺 1024(0.15g,0.4mmol) 和 DIPEA(0.14mL,0.8mmol)。将所得混合物在室温下搅拌过夜后，通过加入水将其猝灭。分离的固体经过滤，用水洗涤，干燥，得到 456(0.2,86％收率）。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.18(t,3H)1.74(brs,4H)2.88-2.90(m,2H)3.01-3.04(m,2H)3.66(s,2H)3.78(s,4H)4.05-4.12(q,2H)7.19-7.36(m,9H)7.55-7.58(m,1H)8.18-8.21(d,1H)11.26(s,1H)12.65(brs,1H)。

[0548] 向 456(0.205g,0.358mmol) 的二噁烷 / 水 (20mL/6mL) 溶液中加入一水氢氧化锂 (0.06g,1.42mmol)。将所得混合物在室温下搅拌 3 小时后，将其用乙酸酸化。将溶液减压浓缩，所得残余物用水稀释。分离的固体经过滤，用水洗涤，高真空干燥过夜。所得残余物用

硅胶色谱法纯化（用 MeOH/ 二氯甲烷洗脱），得到 465(0.15g,77％收率）。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.74(brs,4H)2.90(brs,2H)3.01(brs,2H)3.5(s,2H)3.78(s,4H)7.19-7.36(m,9H)7.55-7.58(m,1H)8.18-8.21(d,1H)11.26(s,1H)12.32(brs,1H)12.65(s,1H)。

[0549] 向羧酸 465(25mg,0.046mmol) 在 DMF(1mL) 中的悬浮液中加入 HATU(19.2mg,0.05mmol)，搅拌直到反应混合物澄清，接着加入 N,N- 二甲胺 (2M/THF,30uL,0.05mmol) 和 DIPEA(16uL,0.092mmol)。将所得混合物在室温下搅拌 3 小时后，通过加入水将其猝灭。分离的固体经过滤，用水洗涤，干燥，得到 472(19mg,73％收率）。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.74(brs,4H)2.83-2.90(brs,6H)3.01(brs,4H)3.68(s,2H)3.78(s,4H)7.14-7.36(m,9H)7.55-7.58(d,1H)8.18-8.21(d,1H)11.26(s,1H)12.65(brs,1H)。

[0550]



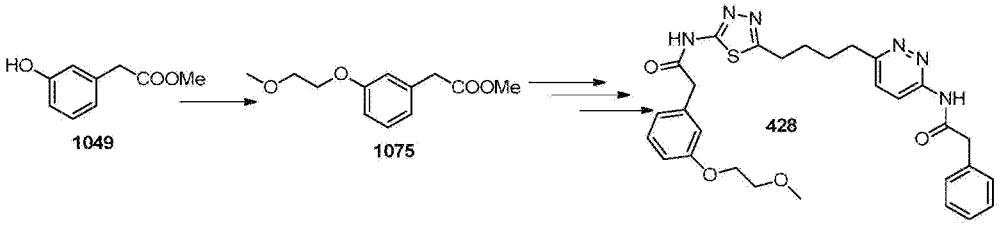
[0551] 向 1049(1g,6mmol) 的 DMF(20mL) 溶液中加入碳酸钾 (1.662g,12mmol) 和 (2.16g,9mmol)。将所得混合物在 70℃下搅拌过夜后，将其用水（～ 100mL) 稀释。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤，分离，经硫酸钠干燥，过滤并蒸发。所得残余物用硅胶色谱法纯化（用 EtOAc/ 己烷洗脱），得到 1065(1.78g,91％收率），为油状物。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 0.13(s,6H)0.95(s,9H)3.63(s,2H)3.73(s,2H)3.99-4.06(m,4H)6.87(m,3H)7.3(m,1H)。

[0552] 向 1065(1.78g,5.5mmol) 的 THF/MeOH/ 水 (30mL,3mL,3mL) 溶液中加入一水氢氧化锂 (0.46g,10.9mmol)。将所得混合物在室温下搅拌过夜后，将其减压浓缩。所得残余物用水（～ 20mL) 稀释，所得溶液用 6N 盐酸酸化。使溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤，分离，经硫酸钠干燥，过滤并蒸发。所得残余物用硅胶色谱法纯化（用 EtOAc/ 己烷洗脱），得到 1065 和 1066。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 3.54(s,2H)3.72(brs,2H)3.96-3.98(brs,2H)4.85(brs,1H)6.82-6.85(m,3H)7.0-7.22(m,1H)12.3(brs,1H)。

[0553] 向羧酸 1065(27mg,0.137mmol) 在 DMF(2mL) 中的悬浮液中加入 HATU(52.2mg,0.137mmol)，搅拌直到反应混合物澄清，接着加入胺 1024(46mg,0.125mmol) 和 DIPEA(44ul,0.25mmol)。将所得混合物在室温下搅拌过夜后，通过加入水将其猝灭。分离的固体经过滤，用水洗涤，干燥。所得固体用制备型 HPLC 纯化，得到 427(16mg,23％收率）。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.75(brs,4H)2.90(brs,2H)3.02(brs,2H)3.71-3.78(m,6H)3.98-3.99(brs,2H)4.84-4.87(brs,1H)6.83-6.92(m,3H)7.21-7.36(m,6H)7.54-7.

58(d,1H)8.2-8.23(d,1H)11.26(s,1H)12.65(brs,1H)。

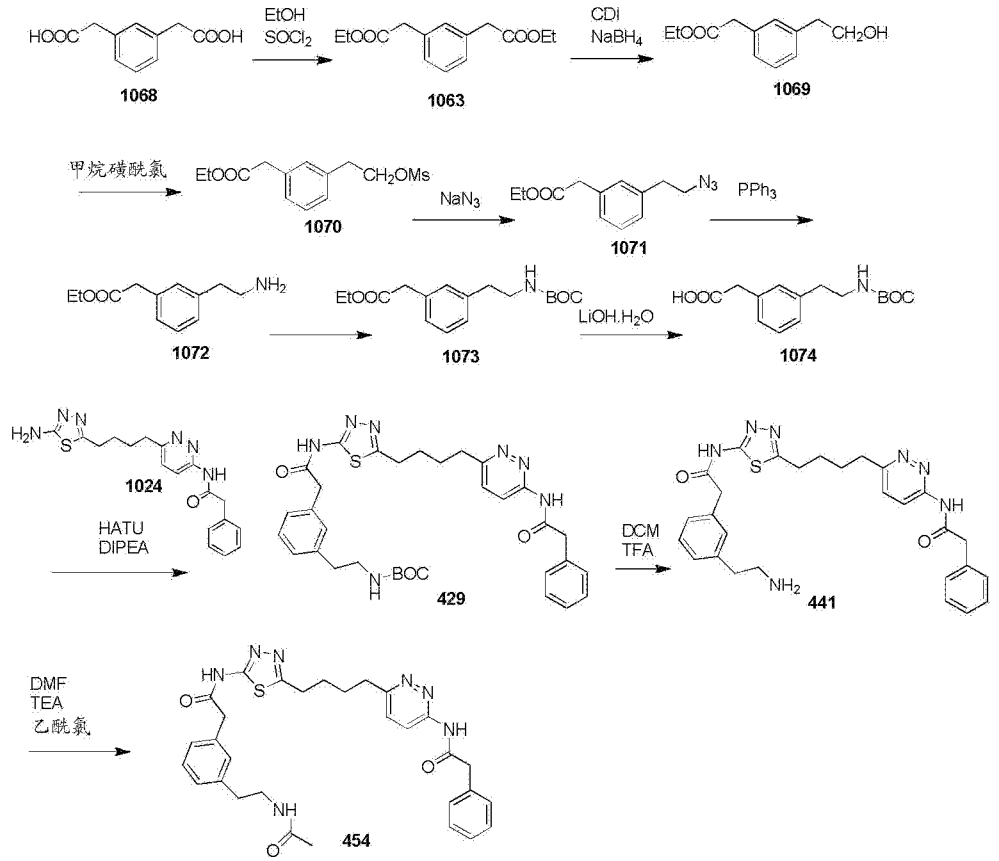
[0554]



[0555] 向1049(1g,6mmol)的丙酮(50mL)溶液中加入碳酸铯(2.545g,7.83mmol)、2-溴乙基甲基醚(0.92g,6.62mmol)和碘化钠(10mg)。将所得混合物在50℃下搅拌过夜后,将其过滤。将滤液蒸发,所得残余物用硅胶色谱法纯化(用EtOAc/己烷洗脱),得到1075(0.97g,72%收率),为油状物。^{1}H NMR(300MHz,氯仿-d)δppm 3.48(s,3H)3.63(s,2H)3.72(brs,2H)4.14-4.15(t,2H)6.86-6.9(m,3H)7.26-7.29(m,1H)。

[0556] 制备化合物428的其余部分按照上文化合物427的方法。428:^{1}H NMR(300MHz,二甲亚砜-d6)δppm 1.75(brs,4H)2.90(brs,2H)3.02(brs,2H)3.32(s,3H)3.66(brs,2H)3.78(brs,4H)4.08(brs,2H)6.88-6.92(m,3H)7.25-7.27(m,6H)7.54-7.58(d,1H)8.2-8.23(d,1H)11.26(s,1H)12.65(brs,1H)。

[0557]



[0558] 向1068(6g,30.9mmoL)的乙醇(50mL)的冰冷溶液中加入亚硫酰氯(2mL),将所得反应混合物在室温下搅拌过夜后,将其减压浓缩。使所得残余物在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发,得到1063(6gm)。

[0559] 向1063(3.35g,13.4mmol)的THF(50mL)搅拌溶液中加入CDI(2.44g,15mmol),

将所得混合物搅拌 2 小时接着加入水 (13mL)。使反应混合物冷却至 0℃，分批加入硼氢化钠 (2.87g，76mmol)。在室温下继续搅拌 3 小时后，将其用乙酸乙酯稀释，并用 6N HCl 酸化。有机层经分离，经硫酸钠干燥，过滤并蒸发。所得残余物用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱)，得到 1069(0.563g，20％收率)，为油状物。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 1.27-1.31(q, 3H)2.87-2.92(d, 2H)3.63(s, 2H)3.87-3.92(t, 2H)4.18-4.2(q, 2H)7.19-7.31(m, 4H)。

[0560] 向 1069(0.563g，2.7mmol) 的二氯甲烷 (40mL) 和三乙胺 (0.47mL，3.3mmol) 的冰冷溶液中加入甲烷磺酰氯 (0.23mL，3.3mmol)，将所得混合物在 0℃下搅拌 2 小时并在室温下搅拌 1 小时后，将其用饱和碳酸氢钠水溶液稀释。溶液用乙酸乙酯萃取。有机萃取物用更多的水洗涤，分离，经硫酸钠干燥，过滤并蒸发，得到 1070(0.78g，100％收率)。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 1.27-1.31(q, 3H)2.87(s, 3H)3.08(t, 2H)3.63(s, 2H)4.18-4.2(t, 2H)4.45(q, 2H)7.19-7.31(m, 4H)。

[0561] 向 1070(0.787g，2.7mmol) 的 DMF(6mL) 溶液中加入叠氮化钠 (0.358g，5.5mmol)，将所得反应混合物在 60℃下搅拌 3 小时后，使之在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤，分离，经硫酸钠干燥，过滤并蒸发。所得残余物用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱)，得到 1071(0.5g，78％收率)，为油状物。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 1.27-1.31(q, 3H)2.92(t, 2H)3.54(t, 2H)3.63(s, 2H)4.18-4.2(q, 2H)7.19-7.29(m, 4H)。

[0562] 向 1071(0.5g，2.1mmol) 的 THF(25mL) 溶液中加入三苯基膦 (0.787g，3mmol)，将反应混合物在室温、氩气下搅拌过夜后，将其用 1mL 水稀释。反应在 50℃下进行 1 小时后，将其减压浓缩。使残余物在饱和碳酸氢钠溶液和二氢甲烷之间分配。有机层经分离，经硫酸钠干燥，过滤并蒸发。所得残余物用硅胶色谱法纯化 (用 MeOH/ 二氯甲烷洗脱)，得到 1072(0.43g，100％收率)，为油状物。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 1.27-1.31(q, 3H)2.75-2.79(t, 2H)2.98-3.02(t, 2H)3.63(s, 2H)4.18-4.2(q, 2H)7.13-7.29(m, 4H)。

[0563] 向 1072(0.427g，2mmol) 的二氯甲烷 (30mL) 溶液中加入二碳酸二叔丁酯 (0.447g，2mmol)，将反应混合物在室温下搅拌 5 小时后，将其用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱)，得到 1073(0.577g，91％收率)，为油状物。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 1.27-1.31(q, 3H)1.59(s, 9H)2.82(t, 2H)3.4(m, 2H)3.63(s, 2H)4.18(q, 2H)7.13-7.29(m, 4H)。

[0564] 向 1073(0.577g，1.8mmol) 的二噁烷 / 水 (10mL/3mL) 溶液中加入一水氢氧化锂 (0.158g，3.6mmol)。将所得混合物在室温下搅拌过夜后，将其减压浓缩。所得残余物用水 (～ 20mL) 稀释，所得溶液用 1N 盐酸酸化。使溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤，分离，经硫酸钠干燥，过滤并蒸发，得到 1074(0.35g，67％收率)。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 2.82(m, 2H)3.4(m, 2H)3.63(s, 2H)4.6(brs, 1H)7.13-7.29(m, 4H)。

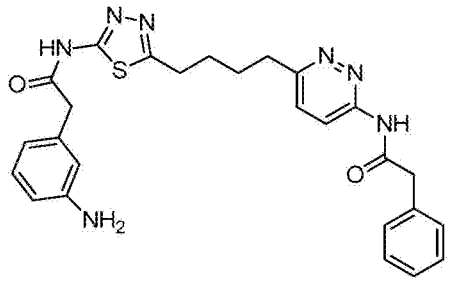
[0565] 向羧酸 1074(43.8mg，0.157mmol) 在 DMF(2mL) 中的悬浮液中加入 HATU(61.3mg，0.161mmol)，搅拌直到反应混合物澄清，接着加入胺 1024(52.5mg，0.142mmol) 和 DIPEA(50ul，0.287mmol)。将所得混合物在室温下搅拌过夜后，通过加入水将其猝灭。分离的固体经过滤，用水洗涤，干燥，得到 429(60mg，67％收率)。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.37-1.38(s, 9H)1.74(brs, 4H)2.69-2.71(m, 2H)2.87-2.88(m, 2H)2.9-3.15

(m,4H)3.78(s,4H)7.09(brs,1H)7.12-7.36(m,9H)7.54-7.57(d,1H)8.18-8.21(d,1H)11.26(s,1H)12.65(brs,1H)。

[0566] 向429(50mg,79.5mmol)在二氯甲烷(5mL)中的悬浮液中加入TFA(1mL),将反应混合物在室温下搅拌过夜后,将其减压浓缩。所得残余物用乙醚研磨。分离的固体经过滤,用乙醚洗涤,并高真空干燥过夜,得到441(45mg,88%收率),为TFA盐。^{1}H NMR(300MHz,二甲亚砜-d6)δppm 1.74(brs,4H)2.86-3.02(m,8H)3.78-3.80(s,4H)7.12-7.36(m,8H)7.58(d,1H)7.78(brs,3H)8.18-8.21(d,1H)11.26(s,1H)12.65(brs,1H)。

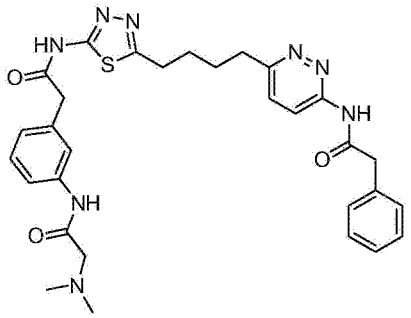
[0567] 向441(23mg,0.035mmol)的DMF(1mL)冰冷溶液中加入三乙胺(11uL,0.079mmol)和乙酰氯(2.8uL,0.038mmol)。将所得混合物在室温下搅拌2小时后,将其用水稀释。分离的固体经过滤,用水洗涤,高真空干燥过夜,得到454(10mg,50%收率)。^{1}H NMR(300MHz,二甲亚砜-d6)δppm 1.75-1.79(m,7H)2.67-2.70(m,2H)2.9(brs,2H)3.00-3.02(m,2H)3.21-3.26(m,2H)3.78(s,4H)7.12-7.36(m,9H)7.58(d,1H)7.9(brs,1H)8.18-8.21(d,1H)11.26(s,1H)12.65(brs,1H)。

[0568]



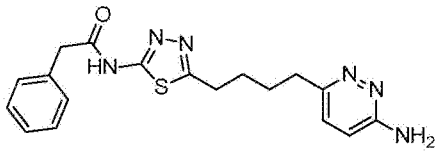
[0569] 化合物409按照上述制备化合物441的方法,通过化合物399的TFA脱保护来制备。^{1}H NMR(300MHz,二甲亚砜-d6)δppm 1.75(brs,4H)2.90(brs,2H)3.02(brs,2H)3.78(brs,4H)6.89-6.98(m,4H)7.25-7.36(m,7H)7.51-7.58(d,1H)8.2-8.23(d,1H)9.34(s,1H)11.26(s,1H)12.65(brs,1H)。

[0570]



[0571] 化合物457按照上述制备化合物39的酰胺偶联方法,通过酰化409来制备。^{1}H NMR(300MHz,二甲亚砜-d6)δppm 1.74(brs,4H)2.32(s,6H)2.89(m,2H)3.02(m,2H)3.13(s,2H)3.78(s,4H)7.01-7.04(m,1H)7.25-7.38(m,6H)7.54-7.58(m,3H)8.18-8.21(d,1H)9.77(s,1H)11.26(s,1H)12.65(brs,1H)

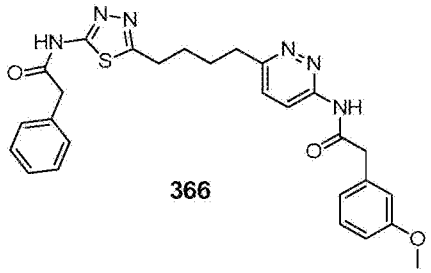
[0572]



348

[0573] 在 0 ℃ 下，向 295(30mg,0.0617mmol) 在 MeOH(2ml) 中的悬浮液中加入 2N NaOH(2ml) 溶液。将所得混合物在室温下搅拌过夜。溶剂经真空蒸发，混合物用 1N HCl 酸化至 pH 6。白色沉淀通过吸滤收集，用更多的水漂洗并干燥，得到 348。^{1}H NMR(300MHz, DMSO-d_6) δ 7.32-7.24(m, 5H), 7.15-7.12(d, J = 9.57Hz, 1H), 6.72-6.69(d, J = 9.15Hz, 1H), 6.09(s, 2H), 3.77(s, 2H), 2.99-2.96(bs, 2H), 2.76-2.70(bs, 2H), 1.70(bs, 4H)。

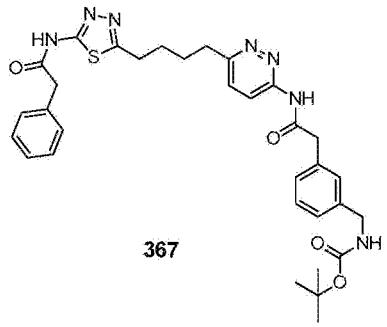
[0574]



366

[0575] 366 :^{1}H NMR(300MHz, DMSO-d_6) δ 12.65(s, 1H), 11.26(s, 1H), 8.22-8.19(d, J = 8.82Hz, 1H), 7.58-7.54(d, J = 9.32Hz, 1H), 7.33-7.25(m, 6H), 6.95-6.82(m, 3H), 3.81(s。3H), 3.75(s, 4H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H)。

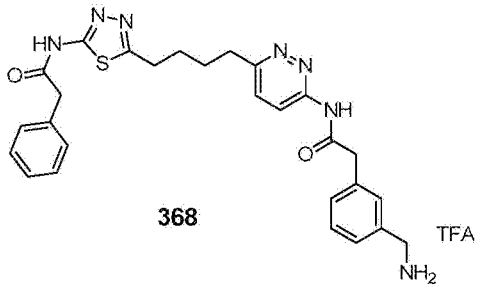
[0576]



367

[0577] 367 :向烧瓶中装入 348(100mg,0.27mmol)、含 Boc-3- 氨基甲基 - 苯乙酸 (86mg, 0.325mmol) 的 DMF(2ml)，在 0 ℃ 下依次加入 HOBT(88mg,0.65mmol) 和 EDCI(156mg, 0.812mmol)。将所得混合物在 0℃下搅拌 5 分钟，然后升温至室温过夜后，在 0℃下通过加入水 (～ 10mL) 将其猝灭。白色沉淀通过吸滤收集，用更多的水漂洗。粗物质用硅胶色谱法纯化 (用含 0 - 6% MeOH 的 CH_2Cl_2 洗脱)，得到 367。

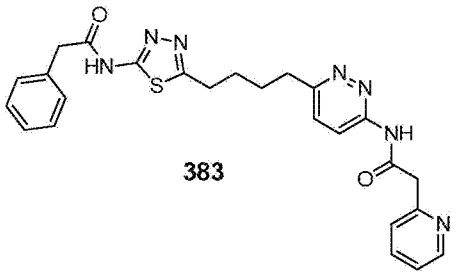
[0578]



368

[0579] 化合物368按照上述化合物341的方法，通过化合物367的脱保护来制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.65(s,1H),11.26(s,1H),8.22-8.16(m,3H),7.58-7.54(d,J = 9.27Hz,1H),7.40-7.28(m,9H),4.04(s,2H),3.81(s。4H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

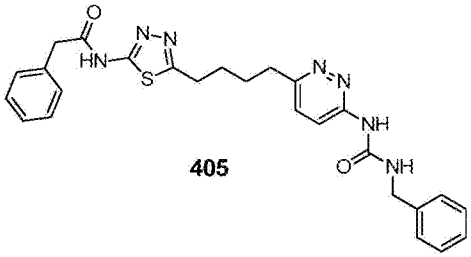
[0580]



383

[0581] 化合物383按照上述制备化合物354的方法，由化合物348制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.65(s,1H),11.26(s,1H),8.51(s,1H),8.22-8.19(d,J = 9.09Hz,1H),7.81-7.76(m,1H),7.58-7.54(d,J = 9.12Hz,1H),7.42-7.26(m,7H),4.0(s,2H),3.81(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

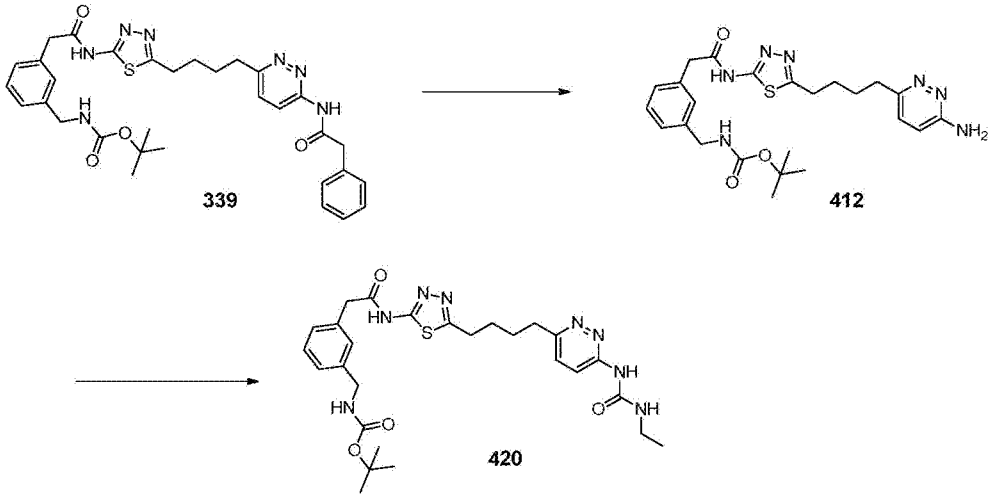
[0582]



405

[0583] 在0℃下，向348(56.5mg,0.153mmol)的DMF(1ml)溶液中依次滴加三乙胺(43ul,0.306mmol)和异氰酸苄酯(23ul,0.184mmol)。将所得混合物慢慢升温至室温，搅拌6小时后，在0℃下通过加入水(～5mL)将其猝灭。白色沉淀通过吸滤收集，用更多的水和醚和二氯甲烷漂洗，然后干燥，得到405。^{1}H NMR(300MHz,DMSO-d_6) δ 12.65(s,1H),9.57(s,1H),8.25(bs,1H),7.74-7.71(d,J = 8.61Hz,1H),7.50-7.47(d,J = 9.42Hz,1H),7.34-7.27(m,10H),4.42-4.40(d,J = 5.46Hz,2H),3.80(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

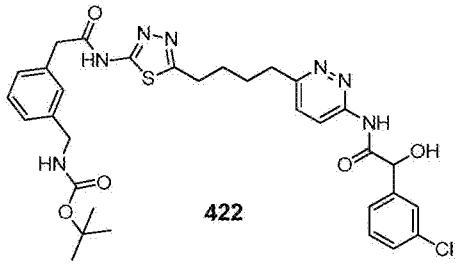
[0584]



[0585] 在0℃下，向339(1g，1.62mmol)在MeOH(10ml)中的悬浮液中加入2N NaOH(10ml)溶液。将所得混合物在室温下搅拌过夜。溶剂经真空蒸发，在0℃下，将混合物用6N HCl酸化至pH 6。混合物用EtOAc研磨，白色沉淀通过吸滤收集，用更多的EtOAc漂洗并干燥，得到412。^{1}H NMR(300MHz，DMSO-d_6) δ 12.66(s，1H)，7.29－7.22(m，2H)，7.19－7.13(m，4H)，6.72(d，J＝8.86Hz，1H)，6.12(bs，2H)，4.12(d，J＝6.09Hz，2H)，3.79(s，2H)，3.01(m，2H)，2.71(m，2H)，1.70(bs，4H)，1.39(s，9H)。

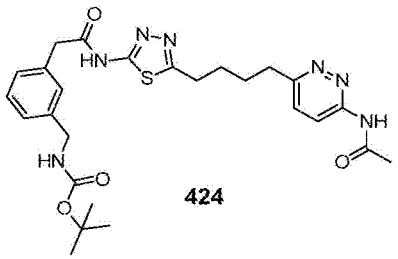
[0586] 在0℃下，向412(60mg，0.121mmol)的DMF(1ml)溶液中依次滴加三乙胺(34ul，0.242mmol)和异氰酸乙酯(11ul，0.145mmol)。将所得混合物慢慢升温至室温，搅拌6小时后，在0℃下通过加入水(～5mL)将其猝灭。白色沉淀通过吸滤收集。粗物质用硅胶色谱法纯化(用含0－6％ MeOH的CH_2Cl_2洗脱)，得到420。^{1}H NMR(300MHz，DMSO-d_6) δ 12.65(s，1H)，11.27(s，1H)，9.42(s，1H)，8.22-8.19(d，J ＝ 8.61Hz，1H)，7.77-7.13(m，5H)，6.56-6.53(bs，1H)，4.12-4.11(d，2H)，3.78(s，2H)，3.23-3.16(m，2H)，3.01(bs，2H)，2.90(bs，2H)，1.73(bs，4H)，1.38(s，9H)，1.10-1.07(t，3H)。

[0587]



[0588] 422：^{1}H NMR(300MHz，DMSO-d_6) δ 12.65(s，1H)，10.74(s，1H)，8.18-8.15(d，J ＝ 9.51Hz，1H)，7.61-7.12(m，9H)，6.62(s，1H)，5.33(s，1H)，4.13-4.11(d，J ＝ 5.58Hz，2H)，3.78(s，2H)，3.01(bs，2H)，2.90(bs，2H)，1.73(bs，4H)，1.38(s，9H)。

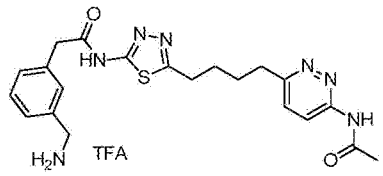
[0589]



424

[0590] 在0℃下，向412(40mg，0.0804mmol)的DMF(1ml)溶液中依次滴加三乙胺(17ul，0.121mmol)和乙酸酐(8ul，0.0844mmol)。将所得混合物慢慢升温至室温，搅拌过夜后，在0℃下通过加入水(～5mL)将其猝灭。使混合物在水和EtOAc之间分配。有机萃取物用水洗涤，经硫酸钠干燥，过滤并蒸发。粗物质用硅胶色谱法纯化(用含0-6％ MeOH的CH_2Cl_2洗脱)，得到424。^1H NMR(300MHz，DMSO-d_6) δ 12.65(s，1H)，11.01(s，1H)，8.23-8.20(d，J＝8.61Hz，1H)，7.57-7.55(d，J＝8.16Hz，1H)，7.38-7.12(m，4H)，4.13-4.11(d，J＝5.76Hz，2H)，3.78(s，2H)，3.01(bs，2H)，2.90(bs，2H)，2.14(s，3H)，1.75(bs，4H)，1.39(s，9H)。

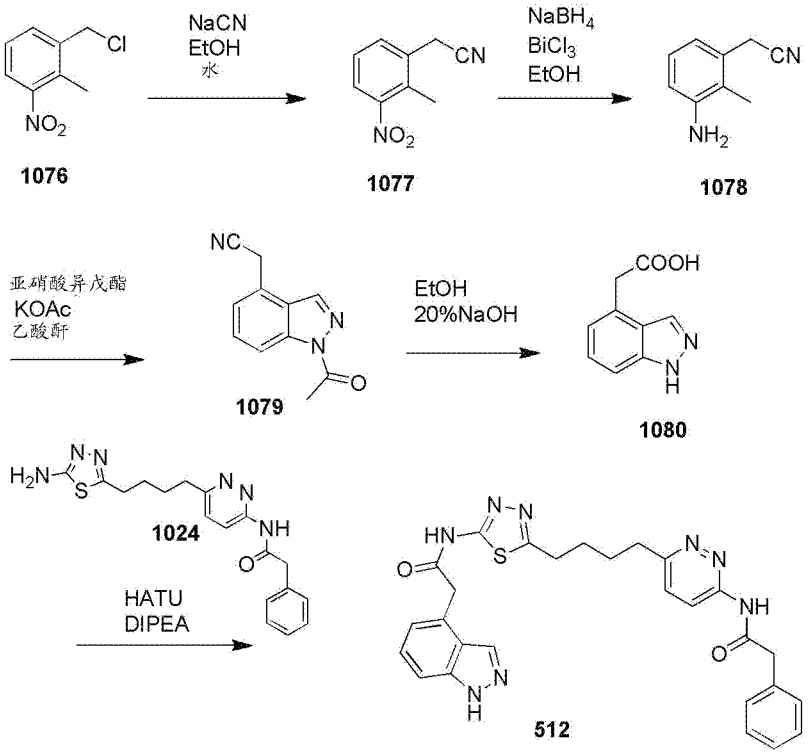
[0591]



425

[0592] 在0℃下，向424(10mg，0.018mmol)在二氯甲烷(1ml)中的悬浮液中加入TFA(1ml)。将所得混合物在室温下搅拌1小时后，将其真空蒸发至干。加入乙醚，白色沉淀通过吸滤收集，用更多的乙醚漂洗并干燥，得到425。^1H NMR(300MHz，DMSO-d_6) δ 12.70(s，1H)，11.0(s，1H)，8.22-8.19(d，J ＝ 8.82Hz，1H)，8.16-8.08(bs，2H)，7.58-7.54(d，J ＝ 9.42Hz，1H)，7.39-7.30(m，4H)，4.06-4.03(m，2H)，3.84(s，2H)，3.01(bs，2H)，2.90(bs，2H)，2.14(s，3H)，1.75(bs，4H)。

[0593]



[0594] 向 1076(1.8g,10mmmol) 的乙醇 / 水 (40mL/20mL) 溶液中加入氰化钠 (0.98g,20mmol)。将所得混合物在 90℃下搅拌 4 小时后,使之冷却至 0℃。分离的固体经过滤,用水洗涤,高真空干燥过夜,得到 1077(1.5g,85%收率)。

[0595] 向 1077(1g,5.68mmmol) 的乙醇 (50mL) 冰冷溶液中分批加入硼氢化钠 (0.86g,22.72mmol) 接着分批加入氯化铋 (2g,6.248mmol)。将所得混合物在室温下搅拌 3 小时后,使其通过硅藻土垫过滤。滤液经浓缩,使所得残余物在碳酸氢钠水溶液和乙酸乙酯之间分配。有机萃取物经分离,经硫酸钠干燥,过滤并蒸发,得到 1078(0.82g,100%收率)。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 2.17(s,3H)3.69-3.71(brs,4H)6.71-6.74(d,1H)6.80-6.83(d,1H)7.04-7.09(m,1H)。

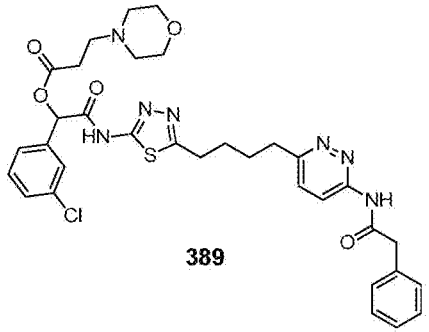
[0596] 向 1078(0.3g,2mmmol) 的甲苯 (10mL) 溶液中加入乙酸钾 (0.2g,2.04mmol) 和乙酸酐 (0.55mL,5.83mmol)。将所得混合物在 80℃下搅拌 1 小时,接着加入亚硝酸异戊酯 (0.4mL,3mmol)。继续在 80℃下搅拌过夜后,使之冷却至室温。使溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发。所得残余物用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱),得到 1079(0.22g,54%收率)。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 2.85(s,3H)4.09(s,2H)7.39-7.41(d,1H)7.58-7.63(m,1H)8.28(s,1H)8.48-8.51(d,1H)。

[0597] 向 1079(0.44g,2.21mmmol) 的乙醇 (5mL) 溶液中加入 20 % 氢氧化钠水溶液 (5mL)。将所得混合物在 90℃下搅拌过夜后,将其浓缩。所得残余物用水稀释,用乙酸酸化,并用乙酸乙酯萃取。有机萃取物经分离,经硫酸钠干燥,过滤并蒸发,得到 1080(0.1g,51%收率)。^{1}H NMR(300MHz,二甲亚砜 -d6) δ ppm 3.89(s,2H)6.98-7.0(d,1H)7.27-7.32(m,1H)7.43-7.46(d,1H)8.10(s,1H)12.3-13.2(宽双重峰 ,2H)。

[0598] 向羧酸 1080(60mg,0.34mmol) 在 DMF(2mL) 中的悬浮液中加入 HATU(130mg,0.34mmol),搅拌直到反应混合物澄清,接着加入胺 1024(114mg,0.31mmol) 和

DIPEA(108uL,0.62mmol)。将所得混合物在室温下搅拌 3 小时后,通过加入水将其猝灭。分离的固体经过滤,用水洗涤,干燥。所得残余物用硅胶色谱法纯化(用 MeOH/ 二氯甲烷洗脱),得到 512(14mg,9%收率)。^{1}H NMR(300MHz,二甲亚砜 -d6) δ ppm 1.74(brs,4H)2.89(brs,2H)2.91(brs,2H)3.78(s,2H)4.13(s,2H)7.05-7.08(m,1H)7.27-7.57(m,8H)8.19(d,2H)11.26(s,1H)12.76-12.80(brs,1H)13.11(s,1H)。

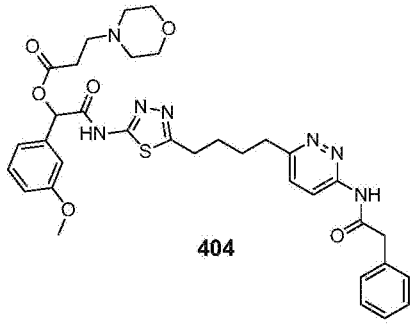
[0599]



389

[0600] 化合物 389 按照上述用于制备化合物 334 的方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.95(s,1H),11.26(s,1H),8.22-8.19(d,J = 8.91Hz,1H),7.61-7.26(m,10H),6.17(s,1H),3.78(s,2H),3.54(bs,4H),3.01(bs,2H),2.90(bs,2H),2.67-2.62(m,4H),2.38(bs,4H),1.73(bs,4H)。

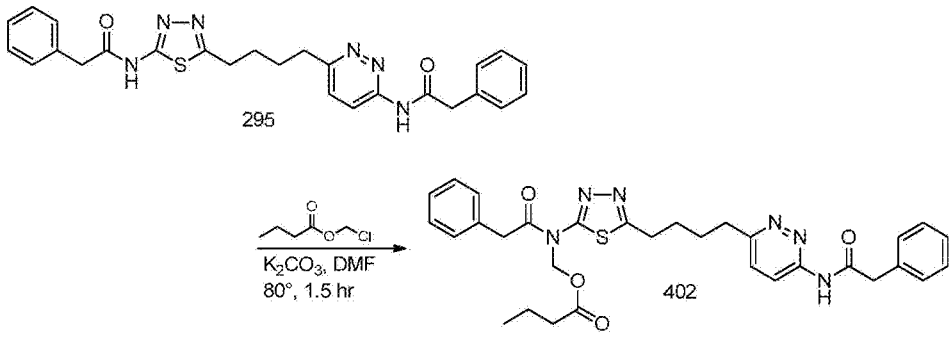
[0601]



404

[0602] 化合物 404 按照上述用于制备化合物 334 的方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.95(s,1H),11.26(s,1H),8.22-8.19(d,J = 9.60Hz,1H),7.58-7.54(d,J = 9.03Hz,1H),7.39-7.26(m,6H),7.12(s,2H),7.01-6.98(m,1H),6.10(s,1H),3.78(s,5H),3.54(bs,4H),3.01(bs,2H),2.90(bs,2H),2.64(bs,4H),2.38(bs,4H),1.74(bs,4H)。

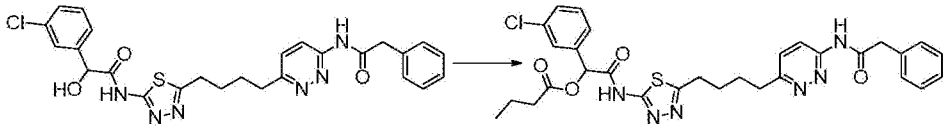
[0603]



[0604] 向烧瓶中依次加入 K_2CO_3(0.28g,2.06mmol)、化合物 295(0.5g,1.03mmol) 和 25mL DMF。将混合物搅拌 15 分钟,加入丁酸氯甲酯 (0.17g,1.23mmol),并将反应物置于氩气气

氛下。将混合物加热至 80℃ 1.5 小时，使之冷却至室温，并倒入 200ml 水中。将混合物转移到分液漏斗中，用 EtOAc(3x100mL) 萃取，分离有机层，用水 (3x50mL)、盐水 (2x50ml) 洗涤，并经 Na_2SO_4 干燥。Na_2SO_4 经过滤除去，减压除去挥发物。粗物质用反相色谱法纯化，得到 0.15g 的化合物 402。

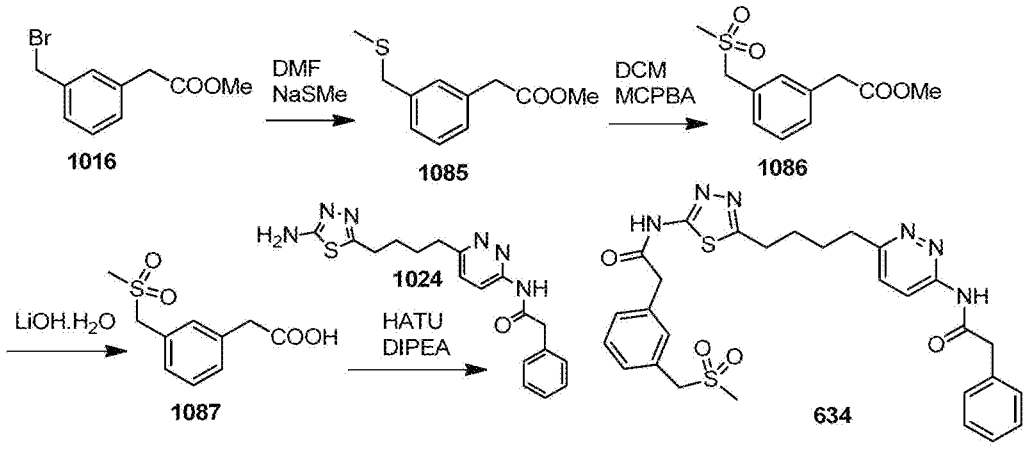
[0605]



318 439

[0606] 在 0℃下，向 318(100mg，0.19mmol) 的 CH_2Cl_2(5mL) 溶液中加入吡啶 (300μL)，接着滴加丁酰氯 (43mL，0.41mmol) 的 CH_2Cl_2(5mL) 溶液。将所得混合物在 0℃下搅拌 1 小时后，使之在 EtOAc 和 H_2O 之间分配。有机层经分离，干燥 ($MgSO_4$) 并浓缩。残余物用硅胶快速柱色谱法纯化 (用 1-10％ MeOH/CH_2Cl_2 洗脱)，得到所需产物 439(117mg)。^1H NMR(300MHz，$CDCl_3$) δ 13.01(bs，1H)，10.12(s，1H)，8.49(d，J = 9.64Hz，1H)，7.77(s，1H)，7.57(d，J = 7.11Hz，1H)，7.40 - 7.30(m，8H)，6.57(s，1H)，3.97(s，2H)，3.09(bs，2H)，3.00(bs，2H)，2.48(m，2H)，1.91(bs，4H)，1.85 - 1.62(m，2H)，0.98(t，J = 7.07Hz，3H)。

[0607]



1016 1085 1086 1024 1087 634

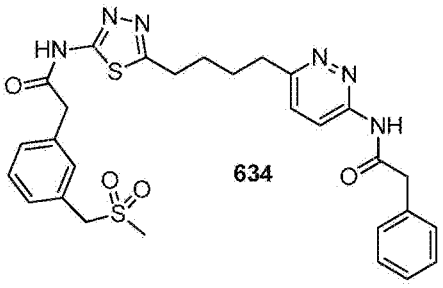
[0608] 向甲硫醇钠 (0.266g，3.8mmol) 的 DMF(10mL) 溶液中加入 1016(0.657g，2.7mmol) 的 DMF 溶液，将所得混合物在室温下搅拌过夜。使溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤，分离，经硫酸钠干燥，过滤并蒸发。所得残余物用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱)，得到 1085(0.41g，72％收率)。^1H NMR(300MHz，氯仿 -d) δ ppm 2.03-2.04(s，3H)3.66-3.73(m，7H)7.21-7.32(m，4H)。

[0609] 向 1085(0.503g，2.39mmol) 的二氯甲烷溶液中加入 MCPBA(1.338g，7.78mmol)，将所得混合物在室温下搅拌 4 小时后，将其用硫代硫酸钠水溶液稀释。有机层经分离，用饱和碳酸氢钠水溶液和水洗涤，经硫酸钠干燥，过滤并浓缩。所得残余物用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱)，得到 1086(0.5g，86％收率)。^1H NMR(300MHz，氯仿 -d) δ ppm 2.8(s，3H)3.7-3.74(m，5H)4.27(s，2H)7.30-7.4(m，4H)。

[0610] 向 1086(0.5g，2.06mmol) 的二噁烷 (10mL) 和水 (10mL) 的冰冷溶液中加入一水氢氧化锂 (0.26g，6.19mmol)，将所得反应混合物在室温下搅拌过夜后，将其浓缩。所得残余物用水稀释，并用乙酸酸化。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的

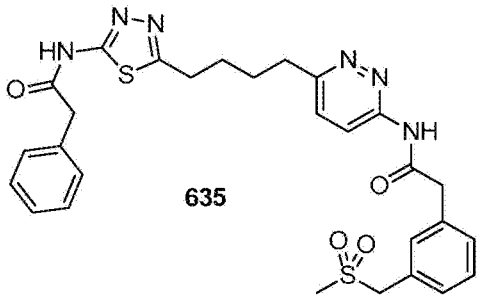
水洗涤，分离，经硫酸钠干燥，过滤并蒸发。所得残余物用乙醚研磨。分离的固体经过滤，用乙醚洗涤并高真空干燥过夜，得到 1087(0.3g，64％收率)。^{1}H NMR(300MHz，二甲亚砜-d6) δ ppm 2.92(s，3H)3.61(s，2H)4.48(s，2H)7.31-7.35(m，4H)12.37(s，1H)。

[0611]



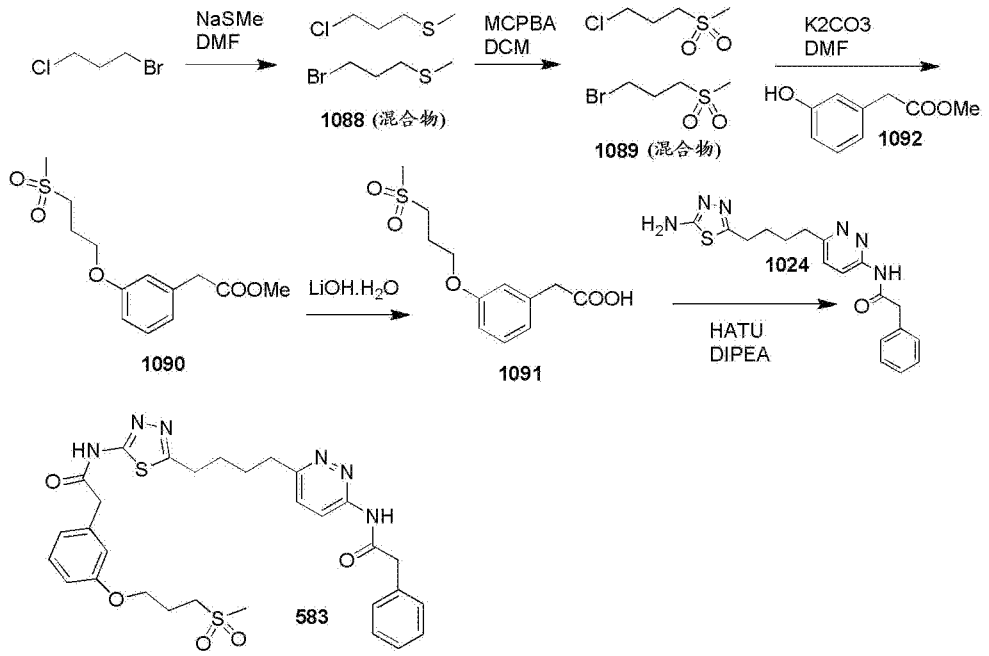
[0612] 化合物634采用类似于上述方法的方法制备。^{1}H NMR(300MHz，二甲亚砜-d6) δ ppm 1.74(brs，4H)2.91(brs，5H)3.03(brs，2H)3.78(s，2H)3.85(s，2H)4.49(s，2H)7.32-7.40(m，9H)7.55-7.58(d，1H)8.19(d，1H)11.26(s，1H)12.69(s，1H)。

[0613]



[0614] 化合物635采用类似于上述方法的方法制备。^{1}H NMR(300MHz，二甲亚砜-d6) δ ppm 1.75(brs，4H)2.91(brs，5H)3.03(brs，2H)3.82(s，4H)4.49(s，2H)7.32-7.40(m，9H)7.55-7.58(d，1H)8.19(d，1H)11.26(s，1H)12.69(s，1H)。

[0615]



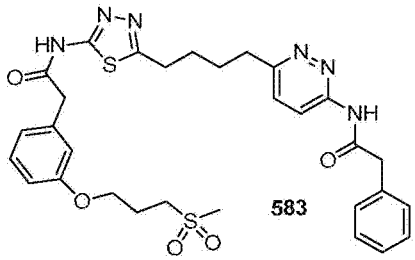
[0616] 向 1,3- 溴氯丙烷 (1.57g,10mmol) 的 DMF(10mL) 溶液中加入甲硫醇钠 (0.63g,9mmol),将所得反应混合物在室温下搅拌过夜并在 70℃下再搅拌一天。使溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发,得到 1088(1.3gm),其无需纯化便可用于下一步。

[0617] 向 1088(1.3g,7.7mmol) 的二氯甲烷 (100mL) 溶液中加入 MCPBA(5.15g,23.34mmol),将所得混合物在室温下搅拌过夜后,将其用硫代硫酸钠水溶液稀释。有机层经分离,用饱和碳酸氢钠水溶液和水洗涤,经硫酸钠干燥,过滤并浓缩。所得残余物用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱),得到 1089(0.3gm)。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 2.38-2.49(m,2H)2.99(s,3H)3.22-3.27(m,2H)3.57-3.77(m,2H)。

[0618] 向 1092(0.525g,3.16mmol) 的 DMF(15mL) 溶液中加入碳酸钾 (0.873g,6.32mmol)、1089(0.74g,4.74mmol) 和碘化钠 (10mg)。将所得混合物在 70℃下搅拌过夜后,将其用水 (～ 100mL) 稀释。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发。所得残余物用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱),得到 1090(0.53g,59％收率)。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 2.35-2.40(m,2H)2.99(s,3H)3.26-3.31(m,2H)3.63(s,2H)3.73(s,3H)4.16(t,2H)6.81-6.93(m,3H)7.25(m,1H)。

[0619] 向 1090(0.53g,1.85mmol) 的二噁烷 (8mL) 和水 (4mL) 溶液中加入一水氢氧化锂 (0.156g,3.71mmol),将所得反应混合物在室温下搅拌 5 小时后,将其用乙酸酸化。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发。所得残余物用乙醚研磨。分离的固体经过滤,用乙醚洗涤并高真空干燥过夜,得到 1091(0.2g,40％收率)。^{1}H NMR(300MHz, 氯仿 -d) δ ppm2.32-2.42(m,2H)2.99(s,3H)3.26-3.31(m,2H)3.66(s,2H)4.12-4.16(t,2H)6.83-6.94(m,3H)7.26-7.31(m,1H)。

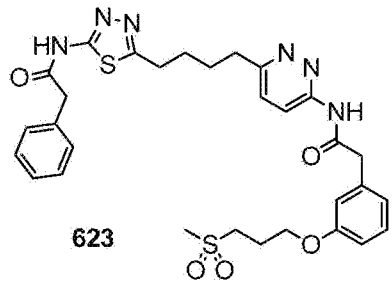
[0620]



583

[0621] 化合物 583 采用酰胺偶联通用方法所述方法通过 1091 与 1024 偶联来制备。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.74(brs,4H)2.15-2.19(m,2H)2.90-3.03(m,7H)3.27-3.39(m,2H)3.78(s,4H)4.07-4.11(t,2H)6.90-6.93(m,3H)7.24-7.37(m,6H)7.55-7.58(d,1H)8.19(d,1H)11.26(s,1H)12.69(s,1H)。

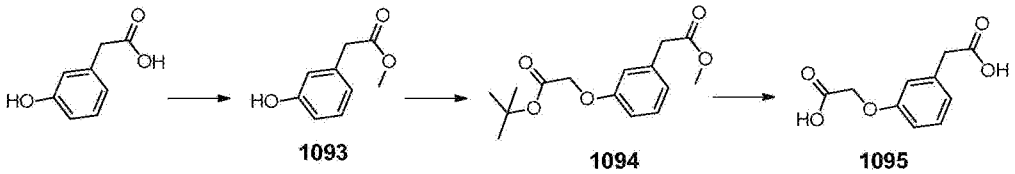
[0622]



623

[0623] 化合物623采用酰胺偶联通用方法所述方法通过11与348偶联来制备。^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.74(brs,4H)2.15-2.19(m,2H)2.90-3.03(m,7H)3.27-3.39(m,2H)3.75-3.78(m,4H)4.07-4.11(t,2H)6.90-6.97(m,3H)7.26-7.34(m,6H)7.58(d,1H)8.19(d,1H)11.26(s,1H)12.69(s,1H)。

[0624]

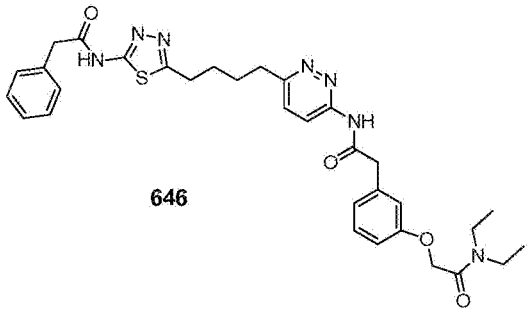


[0625] 在0℃下,向3-羟基苯乙酸(1g,0.00657mol)的MeOH(10ml)溶液中滴加(三甲基甲硅烷基)重氮甲烷溶液(2M,在己烷中,20ml)。将所得混合物在室温下搅拌30分钟后,将其蒸发至干。粗物质用硅胶色谱法纯化(用含0-25% EtOAc的己烷洗脱),得到1093。

[0626] 1094采用化合物1119所述方法制备。

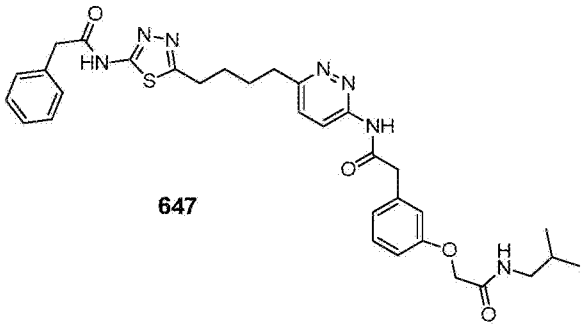
[0627] 1095采用化合物1102所述方法制备。

[0628]



[0629] 646采用化合物666所述方法制备。^{1}H NMR(300MHz,$CDCl_3$) δ 10.32(s,1H),8.50-8.47(d,J = 8.52Hz,1H),7.90-7.70(m,1H),7.40-7.36(m,6H),7.03-6.86(m,3H),4.72(s,2H),4.02(s,2H),3.90(s,2H),3.44-3.39(m,4H),3.09-2.96(d,4H),1.87(bs,4H),1.24-1.16(m,6H)。

[0630]



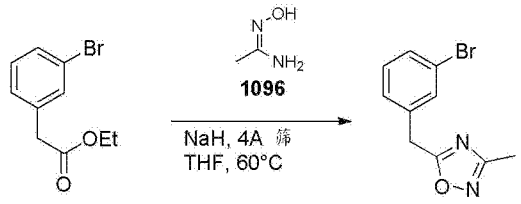
[0631] 647采用化合物666所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.61(s,1H),11.22(s,1H),8.22-8.19(d,J = 9.18Hz,1H),8.02-8.10(t,1H),7.58-7.55(d,J = 9.12Hz,1H),7.36-7.24(m,5H),6.99-6.84(m,3H),4.48(s,2H),3.82(s,2H),3.75(s,2H),3.50(s,2H),3.01-2.90(m,5H),1.73(bs,4H),0.82-0.80(d,J = 6.69Hz,6H)。

[0632]

1096

[0633] 将羟胺(50%的水溶液,7.4mL)溶液加入乙腈(60mL)中,将混合物加热至90℃16小时。使混合物冷却至室温,然后在湿冰浴中冷却,得到沉淀。固体经过滤收集,用冷的乙腈(10mL)漂洗,高真空下干燥,得到4.47g N'-羟基乙脒(hydroxyacetimidamide)1096。参见Zemolka,S.等,PCT国际申请2009118174。^{1}H NMR 300MHz $CDCl_3$:δ4.57(br s,2H),1.89(s,3H)。

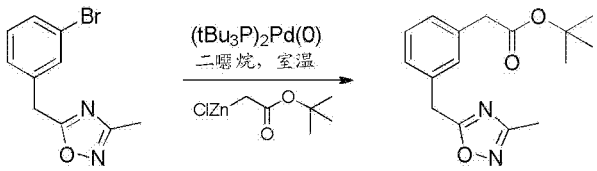
[0634]



1097 1098

[0635] 向烧瓶中装入N'-羟基乙脒1096(0.45g,6.17mmol),接着装入THF(25mL)、NaH(60%油溶液,0.246g,6.17mmol)、4A分子筛(4.5g),在氩气气氛下将混合物加热至60℃1小时。将2-(3-溴苯基)乙酸乙酯1097(1.5g,6.17mmol)的THF(12.5mL)溶液加入N'-羟基乙脒混合物中,并在60℃下加热16小时。混合物用水(100mL)稀释,用EtOAc(2x 25mL)萃取。合并有机层,用水(25mL)、盐水(2x 25mL)洗涤,经Na_2SO_4干燥。Na_2SO_4经过滤除去,减压除去挥发物。粗物质通过正相色谱法(0-30% EtOAc/己烷)纯化,得到0.56g 5-(3-溴苄基)-3-甲基-1,2,4-噁二唑1098。^{1}H NMR 300MHz $CDCl_3$:δ7.48-7.42(m,2H),7.26-7.24(m,2H),4.15(s,2H),2.38(s,3H)。

[0636]



1098 1099

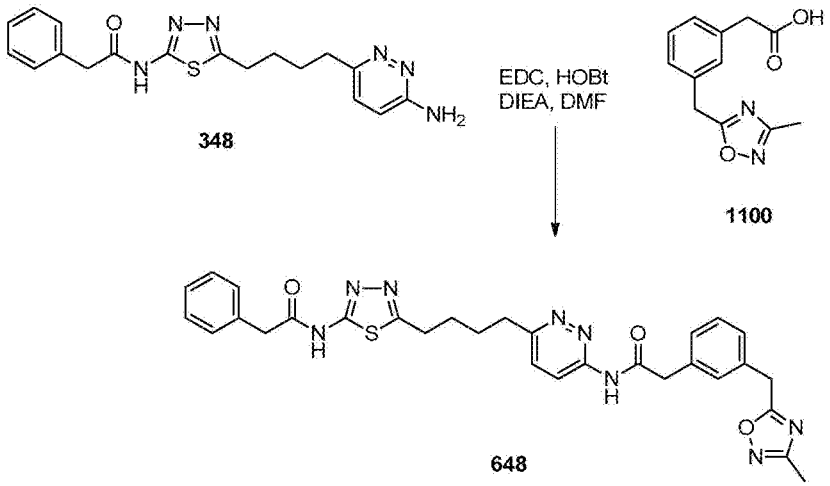
[0637] 在氩气气氛下,向5-(3-溴苄基)-3-甲基-1,2,4-噁二唑1098(0.50g,1.97mmol)的二噁烷(1mL)溶液中,加入双(三叔丁基膦)钯(0)(0.15g,0.295mmol),接着加入2-叔丁氧基-2-氧代乙基氯化锌(0.5M的乙醚溶液,4.92mmol,9.84mL)。在氩气下将混合物搅拌20小时,并减压除去挥发物。使残余物溶于EtOAc(10mL),用水(2x 5mL)、盐水(2x5mL)洗涤,并经Na_2SO_4干燥。Na_2SO_4经过滤除去,减压除去挥发物。粗产物用正相色谱法(0-50% EtOAc/己烷)纯化,得到0.300g 2-(3-((3-甲基-1,2,4-噁二唑-5-基)甲基)苯基)乙酸叔丁酯1099。^{1}H NMR 300MHz $CDCl_3$:δ7.40-7.18(m,4H),4.17(s,2H),3.51(s,2H),2.36(s,3H),1.43(s,9H)。

[0638]

1099 1100

[0639] 向2-(3-((3-甲基-1,2,4-噁二唑-5-基)甲基)苯基)乙酸叔丁酯1099(0.127g,0.44mmol)在二噁烷(3mL)中的混合物中加入含4N HCl的二噁烷(1mL),在氩气气氛下搅拌2小时。减压除去挥发物,残余物用水(5mL)稀释,并用2.5N NaOH调节pH至12。混合物用二氯甲烷(4x 2mL)洗涤,并用1N HCl调节pH至6。混合物用EtOAc(3x 2mL)萃取,合并有机层,用盐水洗涤,并经Na_2SO_4干燥。Na_2SO_4经过滤除去,减压除去挥发物,得到0.041g 2-(3-((3-甲基-1,2,4-噁二唑-5-基)甲基)苯基)乙酸1100。^1H NMR 300MHz $CDCl_3$:δ 7.40-7.18(m,4H),4.18(s,2H),3.63(s,2H),2.36(s,3H)。

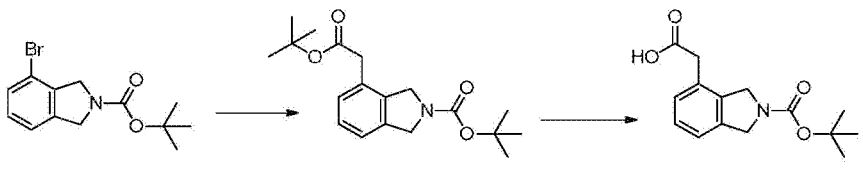
[0640]



348 1100 648

[0641] 向N-(5-(4-(6-氨基哒嗪-3-基)丁基)-1,3,4-噻二唑-2-基)-2-苯基乙酰胺348(0.061g,0.0165mmol)、2-(3-((3-甲基-1,2,4-噁二唑-5-基)甲基)苯基)乙酸1100(0.040g,0.18mmol)、1-乙基-3-(3-二甲基氨基丙基)碳二亚胺(0.078g,0.41mmol)、1-羟基苯并三唑(0.055g,0.41mmol)的DMF(3mL)溶液中加入DIEA(0.085g,0.115mL,0.66mmol),将混合物搅拌16小时。混合物用水(20mL)稀释,用EtOAc(3x 20mL)萃取。合并有机层,用水(3x 20mL)、盐水(2x 20mL)洗涤,并经Na_2SO_4干燥。Na_2SO_4经过滤除去,减压除去挥发物。粗物质用正相色谱法(0-5% MeOH/二氯甲烷)纯化,得到0.003g 2-(3-((3-甲基-1,2,4-噁二唑-5-基)甲基)苯基)-N-(6-(4-(5-(2-苯基乙酰氨基)-1,3,4-噻二唑-2-基)丁基)哒嗪-3-基)乙酰胺648。^1H NMR 300MHz $CDCl_3$:δ 12.59(s,1H),10.53(s,1H),8.45(d,1H,J = 12.2Hz),7.4-7.1(m,10H),4.15(s,2H),4.03(s,2H),3.94(s,2H),3.02(m,2H),2.94(m,2H),2.33(s,3H),1.85(m,4H)。

[0642]

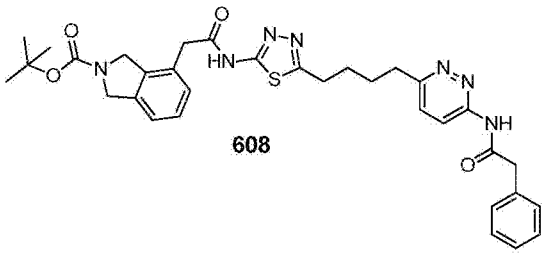


1101 1102

[0643] 1101 采用化合物 1119 所述方法制备。

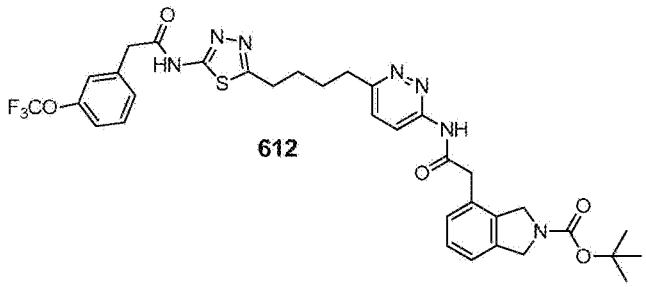
[0644] 在 0℃下，向 1101(470mg，1.41mmol) 的 MeOH(5ml) 和 H_2O(5ml) 溶液中加入一水氢氧化锂 (296mg，7.05mmol)。将所得混合物在室温下搅拌 3 天后，将其蒸发至干。混合物然后用 1N HCl 酸化 (pH 4)，并使之在水和 EtOAc 之间分配。有机萃取物用水洗涤，经硫酸钠干燥，过滤并蒸发，得到 1102。

[0645]



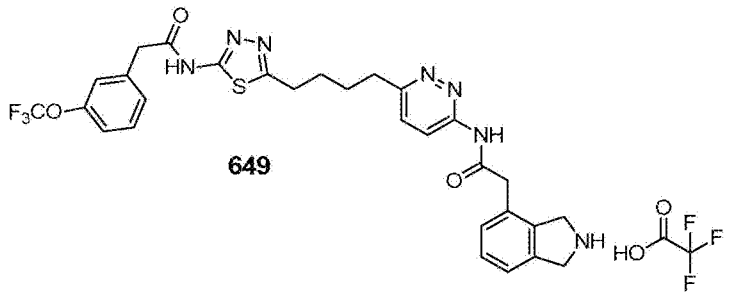
[0646] 608 采用化合物 664 所述方法制备。^{1}H NMR(300MHz，DMSO-d_6) δ 12.71(s，1H)，11.32(s，1H)，8.22-8.19(d，J = 9.15Hz，1H)，7.58-7.54(d，J = 9.27Hz，1H)，7.38-7.28(m，8H)，4.63(bs，4H)，3.82(s，2H)，3.78(s，2H)，3.01(bs，2H)，2.90(bs，2H)，1.73(bs，4H)，1.48-1.44(d，J = 5.93Hz，9H)。

[0647]



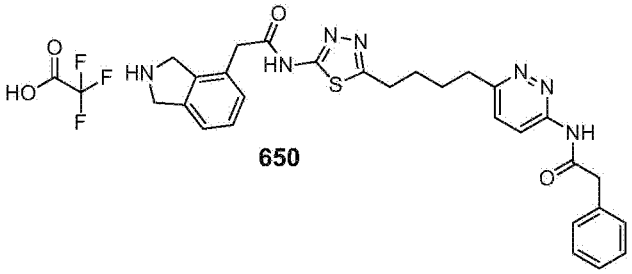
[0648] 612 采用化合物 666 所述方法制备。^{1}H NMR(300MHz，DMSO-d_6) δ 11.32(s，1H)，8.22-8.19(d，J = 9.78Hz，1H)，7.58-7.54(d，J = 9.72Hz，1H)，7.48-7.28(m，7H)，4.67-4.61(m，4H)，3.88(s，2H)，3.80(s，2H)，3.01(bs，2H)，2.90(bs，2H)，1.73(bs，4H)，1.48-1.44(d，J = 9.93Hz，9H)。

[0649]



[0650] 649 采用化合物 695 所述方法制备。^{1}H NMR(300MHz，DMSO-d_6) δ 11.36(s，1H)，8.20-8.17(d，J = 9.78Hz，1H)，7.60-7.57(d，J = 8.92Hz，1H)，7.52-7.32(m，7H)，4.61-4.56(d，J = 16.99Hz，4H)，3.91(s，2H)，3.87(s，2H)，3.01(bs，2H)，2.90(bs，2H)，1.73(bs，4H)。

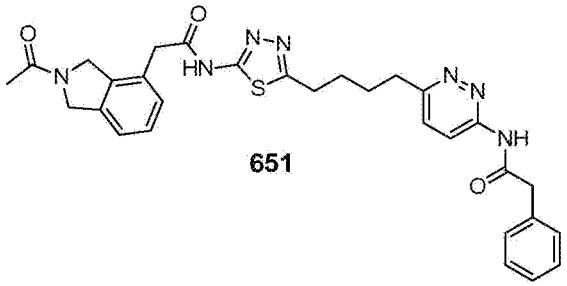
[0651]



650

[0652] 650 采用化合物 695 所述方法制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.71(s, 1H), 11.32(s, 1H), 9.40(bs, 1H), 8.22-8.19(d, J = 9.09Hz, 1H), 7.58-7.54(d, J = 9.36Hz, 1H), 7.38-7.28(m, 8H), 4.63(bs, 4H), 3.82(s, 2H), 3.78(s, 2H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H)。

[0653]



651

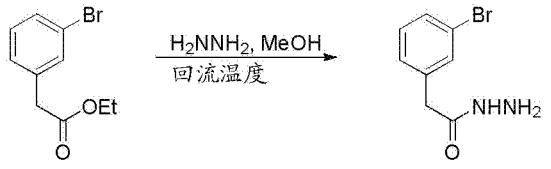
[0654] 在 0℃下，向 650(30mg, 0.0468mmol) 的 DMF(1ml) 溶液中依次滴加三乙胺 (13ul, 0.0936mmol) 和乙酸酐 (4.64ul, 0.0491mmol)。将所得混合物在 0℃下搅拌 20 分钟后，通过加入冰水 (～ 5mL) 将其猝灭。白色沉淀通过吸滤收集，用更多的水漂洗。粗物质用硅胶色谱法纯化 (用含 0-6% MeOH 的 CH_2Cl_2 洗脱)，得到 651。^{1}H NMR(300MHz, DMSO-d_6) δ 12.71(s, 1H), 11.32(s, 1H), 8.22-8.19(d, J = 9.27Hz, 1H), 7.58-7.54(d, J = 9.00Hz, 1H), 7.38-7.28(m, 8H), 4.88(bs, 2H), 4.67(bs, 2H), 3.82(s, 2H), 3.78(s, 2H), 3.01(bs, 2H), 2.90(bs, 2H), 2.11(s, 3H), 1.73(bs, 4H)。

[0655]

1103 1097

[0656] 向 2-(3- 溴苯基) 乙酸 1103(10.0g, 46.5mmol) 的 100mL EtOH 溶液中加入浓 H_2SO_4(10 滴)，将混合物加热至回流温度持续 3 小时。使混合物冷却至室温，并减压除去挥发物。使残余物溶于 EtOAc(100mL)，并用水 (2x 50mL)、饱和 $NaHCO_3$(1x 25mL)、盐水 (2x 25mL) 洗涤，经 Na_2SO_4 干燥。Na_2SO_4 经过滤除去，减压除去挥发物，得到 2-(3- 溴苯基) 乙酸乙酯 1097(11.1 克)，为液体)。^{1}H NMR 300MHz $CDCl_3$: δ 7.41(m, 2H), 7.20(m, 2H), 4.14(q, 2H, J = 9.5Hz), 3.57(s, 2H), 1.25(t, 3H, J = 9.5Hz)。

[0657]



1097 1104

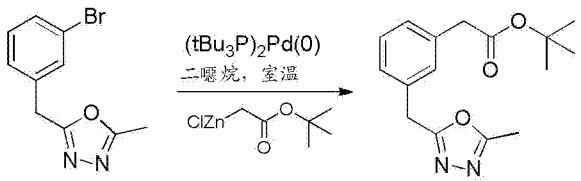
[0658] 向 2-(3- 溴 苯 基) 乙 酸 乙 酯 1097(1.5g,6.17mmol) 的 MeOH(20mL) 溶 液 中 加 入肼 (0.79g,24.7mmol),将混合物加热至回流温度持续 4 小时。使混合物冷却至室温,产生白色沉淀,其经过滤收集,并用 MeOH(10mL) 漂洗。减压干燥后,分离出 1.4 克 2-(3- 溴苯基) 乙酰肼 1104。^{1}H NMR 300MHz $CDCl_3$: δ 7.42(s,2H),7.20(s,2H),6.73(br s,1H),3.51(s,2H),1.81(br s,2H)。

[0659]

1104 1105

[0660] 向 2-(3- 溴苯基) 乙酰肼 1104(1.0g,4.37mmol) 的 AcOH(10mL) 溶液中加入原乙酸三甲酯 (2.62g,21.83mmol),将混合物加热至 115℃ 18 小时。减压除去挥发物,残余物用反相色谱法纯化,得到 0.59g 2-(3- 溴苄基)-5- 甲基 -1,3,4- 噁二唑 1105。^{1}H NMR 300MHz $CDCl_3$: δ 7.45(m,2H),7.23(m,2H),4.12(s,2H),2.49(s,3H)。

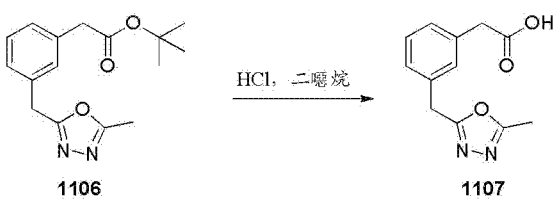
[0661]



1105 1106

[0662] 在氩气气氛下,向 2-(3- 溴苄基)-5- 甲基 -1,3,4- 噁二唑 1105(0.50g,1.97mmol) 的二噁烷 (1mL) 溶液中加入双 (三叔丁基膦) 钯 (0)(0.15g,0.295mmol),接着加入 2- 叔丁氧基 -2- 氧代乙基氯化锌 (0.5M 的乙醚溶液,4.92mmol,9.84mL)。在氩气下将混合物搅拌 20 小时,并减压除去挥发物。使残余物溶于 EtOAc(10mL),用水 (2x 5mL)、盐水 (2x 5mL) 洗涤,并经 Na_2SO_4 干燥。Na_2SO_4 经过滤除去,减压除去挥发物。粗物质用正相色谱法 (0-50% EtOAc/ 己烷) 纯化,得到 0.338g 2-(3-((5- 甲基 -1,3,4- 噁二唑 -2- 基) 甲基) 苯基) 乙酸叔丁酯 1106。^{1}H NMR 300MHz $CDCl_3$: δ 7.24(m,4H),4.12(s,2H),3.51(s,2H),2.46(s,3H),1.43(s,9H)。

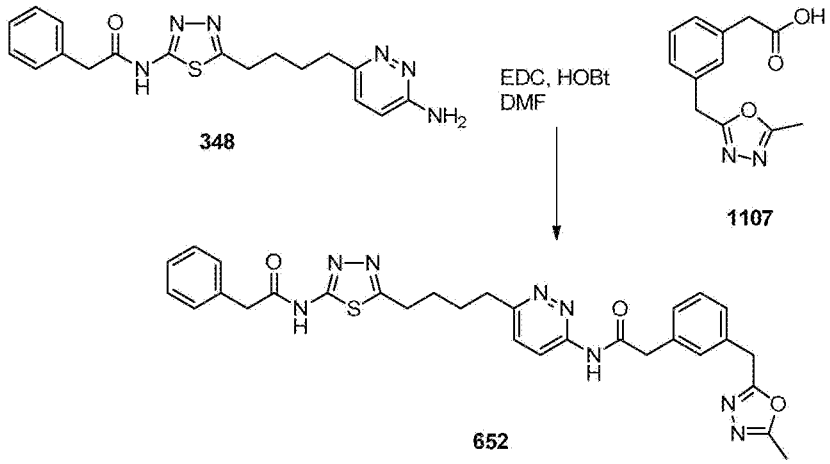
[0663]



1106 1107

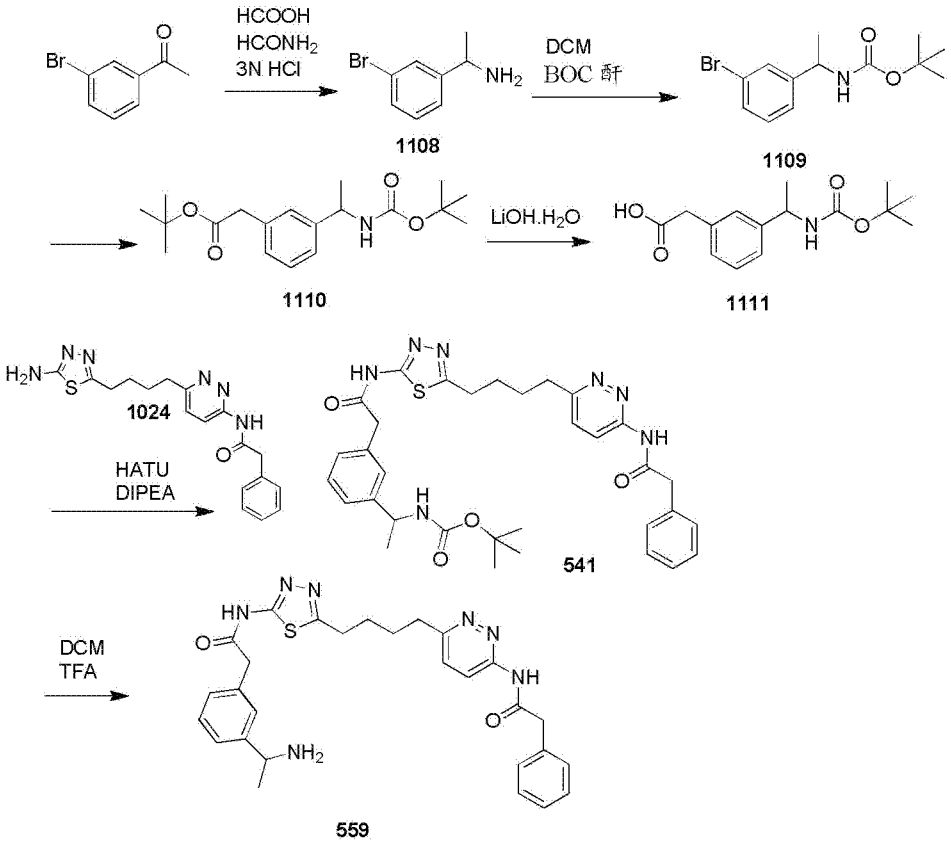
[0664] 向2-(3-((5-甲基-1,3,4-噁二唑-2-基)甲基)苯基)乙酸叔丁酯1106(0.127g,0.44mmol)在二噁烷(3mL)中的混合物中加入含4N HCl的二噁烷(1mL),在氩气气氛下搅拌2小时。减压除去挥发物,残余物用水(5mL)稀释,并用2.5N NaOH调节pH至12。混合物用二氯甲烷(4x 2mL)洗涤,并用1N HCl调节pH至6。混合物用EtOAc(3x 2mL)萃取,合并有机层,用盐水洗涤,并经Na_2SO_4干燥。Na_2SO_4经过滤除去,减压除去挥发物,得到0.023g 2-(3-((5-甲基-1,3,4-噁二唑-2-基)甲基)苯基)乙酸1107。

[0665]



[0666] 将N-(5-(4-(6-氨基哒嗪-3-基)丁基)-1,3,4-噻二唑-2-基)-2-苯基乙酰胺348(0.035g,0.094mmol)、2-(3-((5-甲基-1,3,4-噁二唑-2-基)甲基)苯基)乙酸1107(0.023g,0.094mmol)、1-乙基-3-(3-二甲基氨基丙基)碳二亚胺(0.045g,0.235mmol)、1-羟基苯并三唑(0.032g,0.235mmol)的DMF(1.75mL)溶液搅拌16小时,并用水(20mL)稀释。混合物用EtOAc(3x 20mL)萃取,合并有机层,用水(3x 20mL)、盐水(2x 20mL)洗涤,并经Na_2SO_4干燥。Na_2SO_4经过滤除去,减压除去挥发物。粗物质用反相色谱法纯化,得到0.004g 2-(3-((5-甲基-1,3,4-噁二唑-2-基)甲基)苯基)-N-(6-(4-(5-(2-苯基乙酰氨基)-1,3,4-噻二唑-2-基)丁基)哒嗪-3-基)乙酰胺652。^1H NMR 300MHz DMSO-d6:δ 12.62(s,1H),11.24(s,1H),8.16(d,1H,J = 12.2Hz),7.54(d,1H,J = 12.2Hz),7.3-7.1(m,9H),4.20(s,2H),3.78(s,2H),3.74(s,2H),2.99(m,2H),2.87(m,2H),2.41(s,3H),1.72(m,4H)。

[0667]



[0668] 将 3- 溴乙酰苯 (5g,25.1mmol) 在甲酸 (6gm) 和甲酰胺 (25mL) 的混合物加热至 170℃过夜后,将其用甲苯萃取。分离有机层,并浓缩。所得残余物用 3N HCl 稀释,将所得混合物回流过夜后,使之冷却至室温。溶液用乙醚萃取。水层经分离,用氢氧化钠水溶液碱化,并用乙醚萃取。有机层经分离,经硫酸钠干燥,过滤并浓缩,得到 1108(3g,60%收率)。^{1}H NMR(300MHz, 氯仿 -d) δ ppm 1.22-1.25(d,3H)3.97-3.99(q,1H)7.23-7.4(m,3H)7.6(s,1H)。

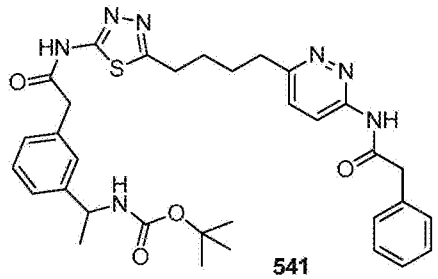
[0669] 向 1108(2.945g,14.7mmol) 的二氯甲烷 (100mL) 溶液中加入 boc 酐 (3.21g,14.7mmol),将反应混合物在室温下搅拌过夜后,将其浓缩,并通过硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱),得到 1109(3g,68 % 收率)。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.29-1.31(d,3H)1.38(s,9H)4.61-4.63(q,1H)7.3(brs,2H)7.41-7.5(m,3H)。

[0670] 在氩气下,向 1109(0.5g,1.66mmol) 和双 (三叔丁基膦) 钯 (0)(0.085g,0.166mmol) 的二噁烷 (3mL) 脱气溶液中加入 2- 叔丁氧基 -2- 氧代乙基氯化锌 (8.5mL,4.15mmol),将所得反应混合物在室温下搅拌 4 小时后,将其用饱和氯化铵水溶液猝灭。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发。所得残余物用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱),得到 1110(0.35g,62% 收率)。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.29-1.31(d,3H)1.388-1.42(brs,18H)3.53(s,2H)4.59-4.63(q,1H)7.09(brs,1H)7.12-7.20(brs,2H)7.25-7.27(m,1H)7.27-7.30(m,1H)。

[0671] 向 1110(0.44g,1.3mmol) 的甲醇 (40mL) 和水 (10mL) 溶液中加入一水氢氧化锂 (0.4gm),将所得反应混合物在室温下搅拌 2 天后,将其浓缩。所得残余物用冰冷的水稀释,并用乙酸酸化。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,

经硫酸钠干燥，过滤并蒸发。所得残余物用硅胶色谱法纯化（用 EtOAc/ 己烷洗脱），得到 1111(0.316g，86％收率）。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.22-1.39(m,12H)3.55(s,2H)4.58-4.63(q,1H)7.11-7.38(m,5H)12.29(s,1H)。

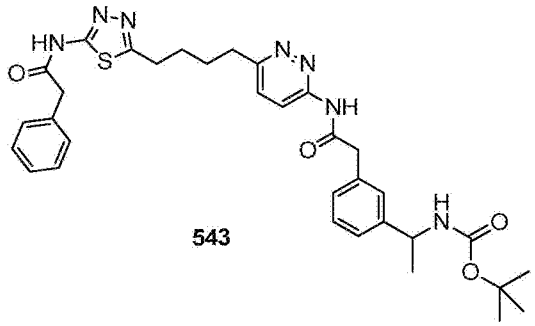
[0672]



541

[0673] ^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.43(m,12H)1.89(brs,4H)2.97-3.08(m,4H)3.95-4.03(m,4H)4.71-4.77(q,1H)7.24-7.43(m,11H)8.45-8.48(d,1H)10.99(s,1H)12.4(brs,1H)。

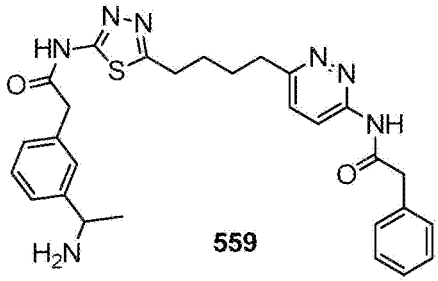
[0674]



543

[0675] ^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.43(m,12H)1.89(brs,4H)2.97-3.08(m,4H)3.95-4.03(m,4H)4.71-4.77(q,1H)7.24-7.43(m,11H)8.45-8.48(d,1H)10.22(brs,1H)12.4(brs,1H)。

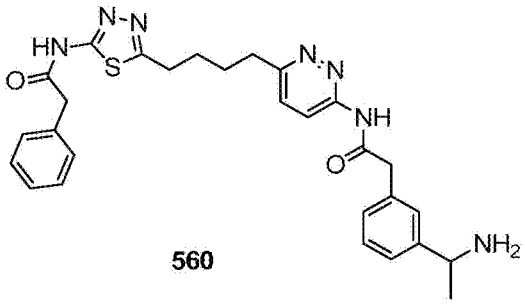
[0676]



559

[0677] ^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.5-1.52(d,3H)1.75(brs,4H)2.88-2.93(m,2H)3.03-3.05(m,2H)3.79(s,2H)3.86(s,2H)4.38-4.44(q,1H)7.27-7.59(m,10H)8.20-8.23(m,4H)11.27(s,1H)12.71(s,1H)。

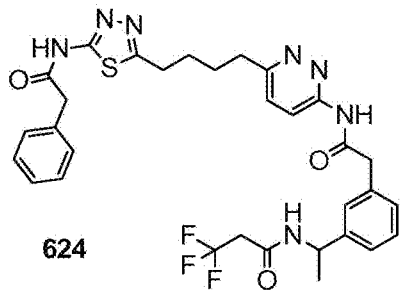
[0678]



560

[0679] ^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.5-1.52(d, 3H)1.75(brs, 4H)2.88-2.93(m, 2H)3.03-3.05(m, 2H)3.86(s, 4H)4.38-4.44(q, 1H)7.27-7.59(m, 10H)8.20-8.23(m, 4H)11.27(s, 1H)12.71(s, 1H)。

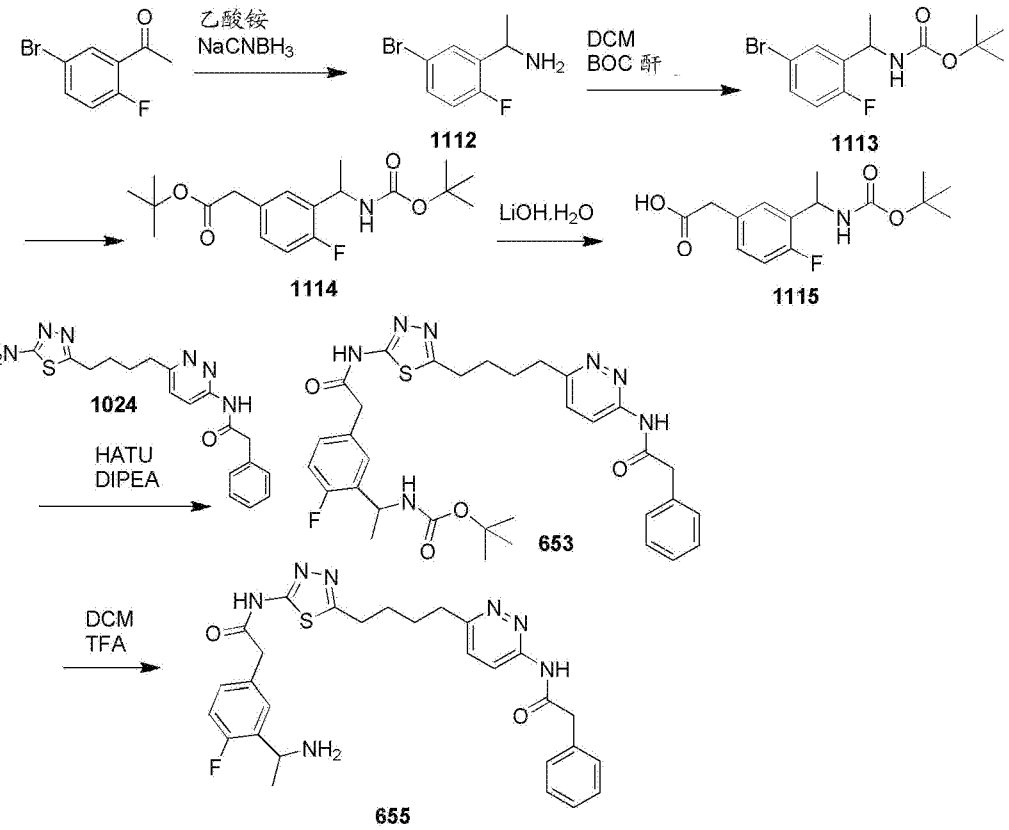
[0680]



624

[0681] H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.5-1.52(d, 3H)1.75(brs, 4H)2.88-2.93(m, 2H)3.03-3.05(m, 2H)3.78(s, 2H)3.82(s, 2H)4.91-4.96(q, 1H)7.20-7.35(m, 9H)7.55-7.58(d, 1H)8.20-8.23(d, 1H)8.68-8.71(m, 1H)11.27(s, 1H)12.71(s, 1H)。

[0682]



1112 1113 1114 1115 1024 653 655

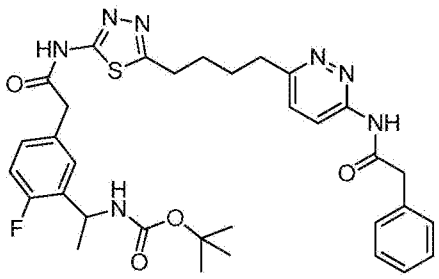
[0683] 向 1-(5- 溴 -2- 氟苯基) 乙酮 (4.5g,20.7mmol) 的甲醇 (100mL) 冰冷溶液中加入乙酸铵 (32g,414.7mmol) 和氰基硼氢化钠 (6.15g,28.98mmol)。在周末期间在室温下搅拌反应混合物后,将其浓缩。所得残余物用水稀释,用 1N NaOH 碱化至 pH ～ 13,用二氢甲烷萃取。有机萃取物经分离,经硫酸钠干燥,过滤并蒸发。所得残余物用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱),得到 1112(1.8g,40％收率)。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.24-1.26(d,3H)4.22-4.24(q,1H)7.1-7.16(t,1H)7.41-7.46(m,1H)7.76(m,1H)。

[0684] 向 1112(1.97g,9mmol) 的二氯甲烷 (100mL) 溶液中加入 boc 酐 (1.97g,9mmol),将反应混合物在室温下搅拌过夜后,将其浓缩,并通过硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱),得到 1113(2.4g,83％收率)。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.29-1.32(d,3H)1.39(s,9H)4.87(q,1H)7.14-7.21(t,1H)7.46-7.58(m,3H)。

[0685] 在氩气下,向 1113(2.4g,7.54mmol) 和双 (三叔丁基膦) 钯 (0)(0.77g,1.508mmol) 的二噁烷 (12mL) 脱气溶液中加入 2- 叔丁氧基 -2- 氧代乙基氯化锌 (38mL,18.85mmol),将所得反应混合物在室温下搅拌 4 小时后,将其用饱和氯化铵水溶液猝灭。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发。所得残余物用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱),得到 1114(2g,75％收率)。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.29-1.32(d,3H)1.38-1.41(m,18H)3.53(s,2H)4.87(q,1H)7.05-7.16(m,2H)7.26-7.29(m,1H)7.48(m,1H)。

[0686] 向 1114(2g,5.66mmol) 的甲醇 (100mL) 和水 (25mL) 溶液中加入一水氢氧化锂 (2gm),将所得反应混合物在室温下搅拌 2 天后,将其浓缩。所得残余物用冰冷的水稀释,并用乙酸酸化。使所得溶液在水和乙酸乙酯之间分配。有机萃取物用更多的水洗涤,分离,经硫酸钠干燥,过滤并蒸发。所得残余物用硅胶色谱法纯化 (用 EtOAc/ 己烷洗脱),得到 1115(1.5g,89％收率)。^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.29-1.31(d,3H)1.38(s,9H)3.53(s,2H)4.87(q,1H)7.05-7.19(m,2H)7.26-7.29(m,1H)7.45-7.48(m,1H)12.32(s,1H)。

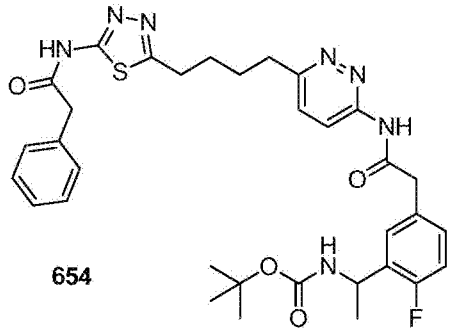
[0687]



653

[0688] ^{1}H NMR(300MHz, 二甲亚砜 -d6) δ ppm 1.30-1.33(m,12H)1.74(brs,4H)2.89(m,2H)3.02(m,2H)3.78(s,4H)4.85(q,1H)7.10-7.57(m,11H)8.19-8.22(d,1H)11.26(s,1H)12.64(s,1H)。

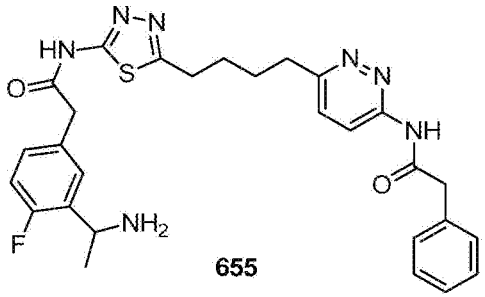
[0689]



654

[0690] ^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.28-1.32(m, 12H)1.73-1.75(brs, 4H)2.87(m, 2H)2.89(m, 2H)3.75(s, 2H)3.81(s, 2H)4.85(q, 1H)7.06-7.57(m, 11H)8.18-8.21(d, 1H)11.26(s, 1H)12.64(s, 1H)。

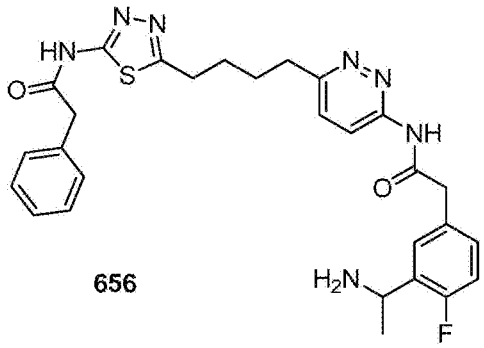
[0691]



655

[0692] ^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.51-1.53(m, 3H)1.75(brs, 4H)2.90(m, 2H)3.02(m, 2H)3.78(s, 2H)3.85(s, 2H)4.65(q, 1H)7.25-7.61(m, 10H)8.21-8.25(d, 1H)8.33-8.35(brs, 3H)11.29(s, 1H)12.68(s, 1H)。

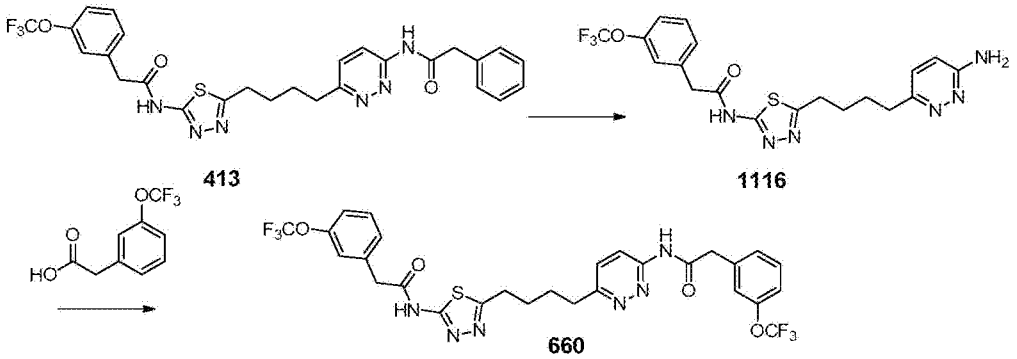
[0693]



656

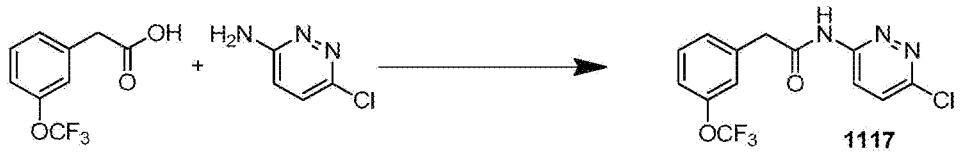
[0694] ^{1}H NMR(300MHz, 二甲亚砜-d6) δ ppm 1.54(d, 3H)1.75-1.76(brs, 4H)2.91(m, 2H)3.02(m, 2H)3.81-3.83(m, 4H)4.65(q, 1H)7.24-7.63(m, 10H)8.22-8.25(d, 1H)8.36(brs, 3H)11.35(s, 1H)12.66(s, 1H)。

[0695]



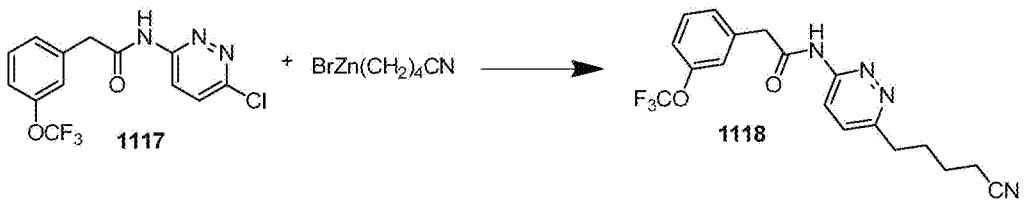
[0696] 在室温下，向413(1.62g)在MeOH(25mL)、THF(10mL)和H_2O(10mL)中的混合物中加入1N NaOH水溶液(8mL)。该将混合物搅拌24小时后，减压除去有机挥发物。残余物用1N HCl水溶液中和至pH 7，用EtOAc(2×20mL)萃取。合并的萃取物经干燥($MgSO_4$)并浓缩。粗产物用硅胶色谱法纯化(用含1-15% MeOH的二氯甲烷洗脱)，得到胺1116。按335所述将所得胺1116转化成660。^1H NMR(300MHz,DMSO-d_6) δ 12.68(bs,1H),11.31(s,1H),8.20(d,J = 9.2Hz,1H),7.57(d,J = 8.8Hz,1H),7.52-7.21(m,8H),3.90(s,2H),3.87(s,2H),3.06-2.86(m,4H),1.77-1.72(m,4H)。

[0697]



[0698] 在3000mL三颈圆底烧瓶中，将3-氨基-6-氯哒嗪(55.5g,0.428mol)和3-(三氟甲氧基)苯乙酸(1.1当量,0.471mol,104g)溶于DMF(30.0体积,1.66L)。通过加料漏斗在5分钟内加入DIEA(1.1当量,0.471mol,82mL)。将丙基膦酸酐溶液(300mL 50%的DMF溶液,1.1当量,0.471mol)装入500mL加料漏斗中，滴加到反应溶液(保持反应温度≤+30℃)中。反应通常在3小时后完成(TLC:6:4己烷-乙酸乙酯)。然后将反应混合物倒入7.5%碳酸氢钠(80.0体积,4.4L)中，其在冰浴中冷却。灰白色结晶粉经Büchner漏斗过滤，用水(20.0体积,1.1L)漂洗。在50℃下真空干燥至恒重，得到N-(6-氯哒嗪-3-基)-2-(3-(三氟甲氧基)苯基)乙酰胺1117：产量119.6g(77%)。^1H NMR(300MHz,DMSO-d_6) δ 11.63(s,1H),8.38(d,J = 9.4Hz,1H),7.88(d,J = 9.4Hz,1H),7.52-7.27(m,4H),3.90(s,2H)。

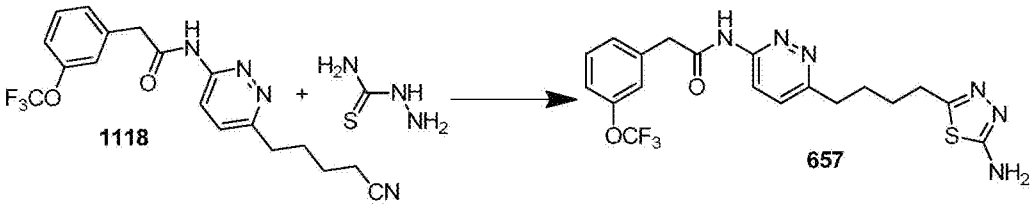
[0699]



[0700] 将4-氰基丁基溴化锌溶液(3.0当量,0.50mol,1.0L)装入经氩气吹扫的5000mL 3颈圆底烧瓶中。在氩气层下，用氩气吹扫5分钟接着加入1117(1.0当量,0.167mol,55.3g)和$NiCl_2$(dppp)(0.15当量,0.0251mol,13.6g)。反应通常在4小时后完成(TLC:1:1己烷-乙酸乙酯)。将EtOAc(15体积,832mL)加入深红色溶液中。加入水(15体积,832mL)，形成浓稠的浆液。加入1N HCl直到浆液变成浅蓝色层(～6体积,333mL)。转移到分液漏

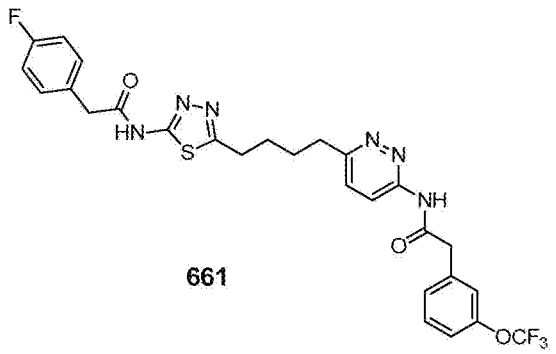
斗中，将有机层用 1N HCl(2x500mL) 洗涤，干燥 ($MgSO_4$) 并通过旋转蒸发浓缩（浴≤ 30℃）成固体淡红色油状物。将油状物溶于二氯甲烷 (15 体积，832mL)，硅胶 (100g) 被调浆成红色溶液，将其通过旋转蒸发（浴≤ 30℃）成固体淡红色粉末。加载到硅胶垫 (5cm x 11cm) 上，用含 25％己烷的乙酸乙酯 (3L) 冲洗，合并的有机物通过旋转蒸发浓缩（浴≤ 30℃）。高真空干燥至恒重，得到 N-(6-(4- 氰基丁基）哒嗪 -3- 基）-2-(3-(三氟甲氧基）苯基）乙酰胺 1118；产量 58.2g(92％）。^{1}H NMR(300MHz, DMSO-d_6) δ 11.41(s, 1H), 8.28(d, J = 9.2Hz, 1H), 7.65(d, J = 9.2Hz, 1H), 7.52 - 7.27(m, 4H), 3.89(s, 2H), 2.92(t, J = 7.5Hz, 2H), 2.56(t, J = 7.0Hz, 2H), 1.80(m, 2H), 1.61(m, 2H)。

[0701]



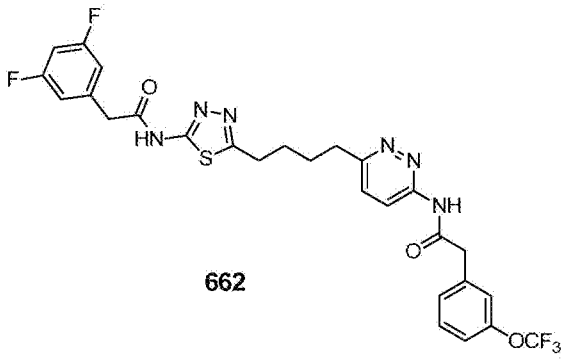
[0702] 将 1118(1.0 当量，0.154mol，58.2g) 与氨基硫脲 (1.2 当量，0.184mol，16.8g) 一起装入 500mL 圆底烧瓶中。在搅拌的同时，将 TFA(5 体积，291mL) 慢慢加入反应容器中。将反应浆液在具有敞顶回流冷凝器的 65℃浴中加热。反应通常在 5 小时后完成（通过 LC/MS 测定）。将甲苯 (10 体积，582mL) 加入深红色溶液中，通过旋转蒸发（浴≤ 30℃）共沸成红色油状物。将油状物慢慢转移到在 0℃浴中冷却的含有 7.5％碳酸氢钠溶液 (69 体积，4.0L) 的充分搅拌的 6000mL Erlenmeyer 烧瓶中。将晶体通过 Büchner 漏斗过滤，并用乙醚 (5 体积，2x250mL) 漂洗两次。高真空干燥至恒重，得到 N-(6-(4-(5- 氨基 -1,3,4- 噻二唑 -2- 基）丁基）哒嗪 -3- 基）-2-(3-(三氟甲氧基）苯基）乙酰胺 657；产量 55.7g(80％）。^{1}H NMR(300MHz, DMSO-d_6) δ 11.33(s, 1H), 8.21(d, J = 9.2Hz, 1H), 7.58(d, J = 9.2Hz, 1H), 7.51 - 7.26(m, 4H), 6.99(s, 2H), 3.88(s, 2H), 2.87(m, 4H), 1.71(m, 4H)。

[0703]



[0704] 在 0℃下，向 657(50mg，0.11mmol) 的 DMF(3mL) 溶液中加入 4- 氟苯乙酸 (22mg，0.14mmol)、HOBt(30mg，0.22mmol) 和 EDCI(42mg，0.22mmol)。将所得混合物在室温下搅拌 1.5 小时后，使之冷却至 0℃，并用 H_2O 猝灭。沉淀通过吸滤收集，用硅胶色谱法进一步纯化（用含 1 - 10％ MeOH 的二氯甲烷洗脱），得到 661。^{1}H NMR(300MHz, DMSO-d_6) δ 12.65(bs, 1H), 11.31(s, 1H), 8.20(d, J = 9.1Hz, 1H), 7.57(d, J = 9.4Hz, 1H), 7.49 - 7.14(m, 8H), 3.87(s, 2H), 3.81(s, 2H), 3.06 - 2.86(m, 4H), 1.77 - 1.72(m, 4H)。

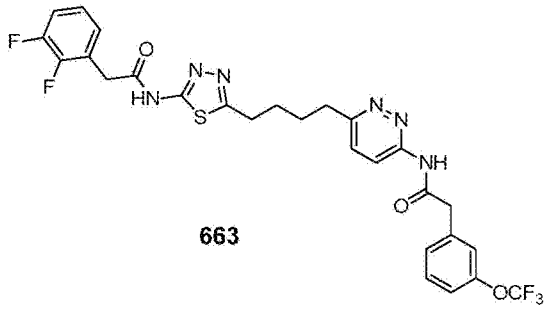
[0705]



662

[0706] 662 通过化合物 661 所述方法制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.67(bs, 1H), 11.31(s, 1H), 8.20(d, J = 9.1Hz, 1H), 7.57(d, J = 9.1Hz, 1H), 7.51 - 7.07(m, 7H), 3.89(s, 2H), 3.87(s, 2H), 3.06 - 2.86(m, 4H), 1.77 - 1.72(m, 4H)。

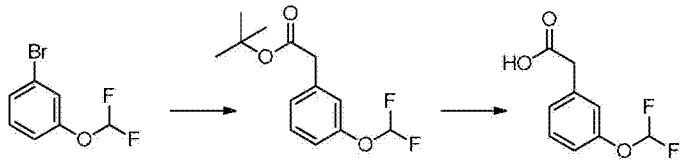
[0707]



663

[0708] 663 通过化合物 661 所述方法制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.74(bs, 1H), 11.31(s, 1H), 8.20(d, J = 9.2Hz, 1H), 7.57(d, J = 9.2Hz, 1H), 7.51 - 7.19(m, 7H), 3.97(s, 2H), 3.87(s, 2H), 3.06 - 2.86(m, 4H), 1.77 - 1.72(m, 4H)。

[0709]

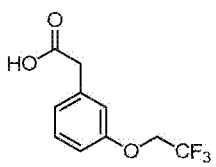


1119 1120

[0710] 在氩气氛下，向 1- 溴 -3-(二氟甲氧基) 苯 (1g, 4.5mmol)、双 (三叔丁基膦) 钯 (0)(460mg, 0.9mmol) 在 1,4- 二噁烷 (30ml) 的混合物中加入 0.5M 含 2- 叔丁氧基 -2- 氧代乙基氯化锌的乙醚 (22.5ml)。将所得混合物在室温下搅拌过夜。使混合物在饱和 NH_4Cl 和 EtOAc 之间分配。有机萃取物用盐水洗涤，经硫酸钠干燥，过滤并蒸发。粗物质用硅胶色谱法纯化 (用含 0-10% EtOAc 的己烷洗脱)，得到 1119。

[0711] 在 0℃下，向 1119(300mg, 1.16mmol) 的二氯甲烷 (5ml) 溶液中滴加 TFA(3ml)。将所得混合物在室温下搅拌过夜后，将其蒸发至干，然后将残余物用乙醚研磨，得到 1120。

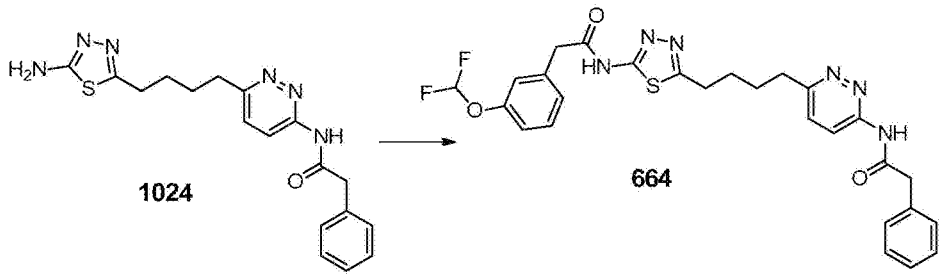
[0712]



1121

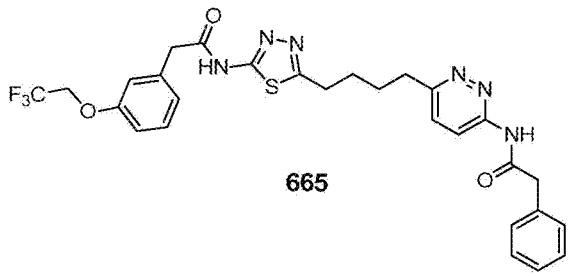
[0713] 1121 采用化合物 1120 所述方法由 1- 溴 -3-(2,2,2- 三氟乙氧基) 苯制备。

[0714]



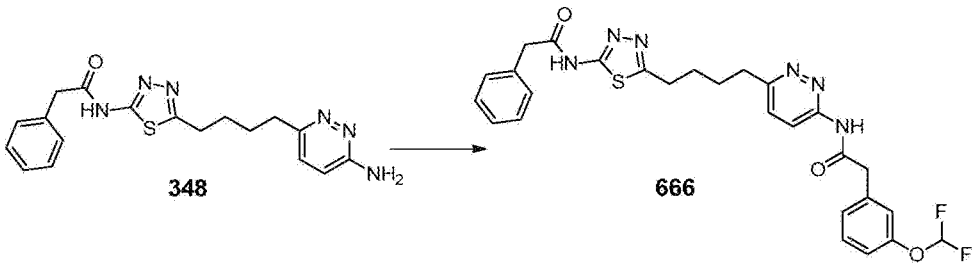
[0715] 向烧瓶中装入 1024(50mg,0.135mmol)、含 1120(28mg,0.142mmol) 的 DMF(1ml),在 0℃下,依次加入 HOBT(39mg,0.285mmol) 和 EDCI(68mg,0.356mmol)。将所得混合物慢慢升温至室温,搅拌 2 小时后,通过加入冰水 (～ 5mL) 将其猝灭。白色沉淀通过吸滤收集,用更多的水漂洗,得到 664。^{1}H NMR(300MHz,DMSO-d_6) δ 12.71(s,1H),11.32(s,1H),8.22-8.19(d,J = 9.12Hz,1H),7.58-7.54(d,J = 9.03Hz,1H),7.48-6.99(m,10H),3.85(s,2H),3.78(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

[0716]



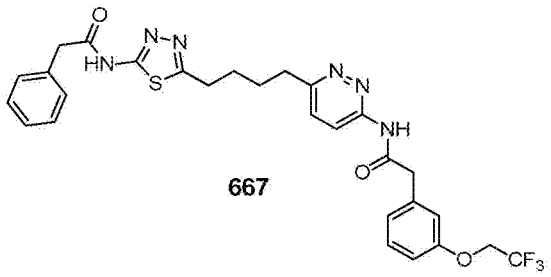
[0717] 665 采用化合物 664 所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.71(s,1H),11.32(s,1H),8.22-8.19(d,J = 9.12Hz,1H),7.58-7.54(d,J = 9.03Hz,1H),7.38-7.28(m,6H),7.03-6.97(m,3H),4.77-4.74(q,2H),3.80-3.78(d,J = 5.82Hz,4H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

[0718]



[0719] 向烧瓶中装入 348(50mg,0.135mmol)、含 1120(28mg,0.142mmol) 的 DMF(1ml),在 0℃下,依次加入 HOBT(39mg,0.285mmol) 和 EDCI(68mg,0.356mmol)。将所得混合物慢慢升温至室温,搅拌过夜后,通过加入冰水 (～ 5mL) 将其猝灭。白色沉淀通过吸滤收集,用更多的水漂洗。粗物质用硅胶色谱法纯化 (用含 0-6％ MeOH 的二氯甲烷洗脱),得 到 666。^{1}H NMR(300MHz,DMSO-d_6) δ 12.71(s,1H),11.32(s,1H),8.22-8.19(d,J = 9.12Hz,1H),7.58-7.54(d,J = 9.03Hz,1H),7.48-6.98(m,10H),3.81(bs,4H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

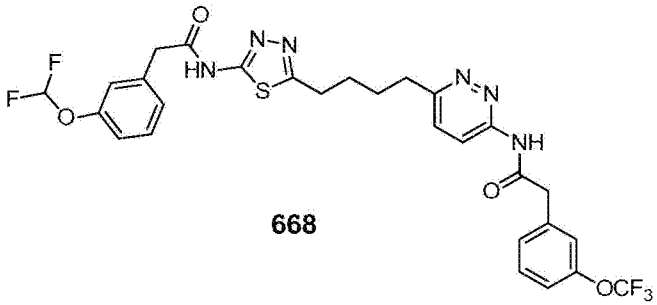
[0720]



667

[0721] 667 采用化合物 666 所述方法制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.71(s, 1H), 11.32(s, 1H), 8.22-8.19(d, J = 9.12Hz, 1H), 7.58-7.54(d, J = 8.97Hz, 1H), 7.35-7.28(m, 6H), 7.03-6.97(m, 3H), 4.77-4.74(q, 2H), 3.87(s, 2H), 3.78(s, 2H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H)。

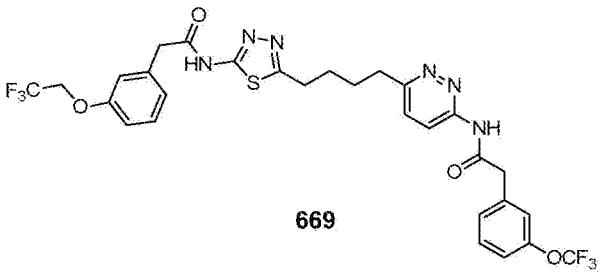
[0722]



668

[0723] 668 采用化合物 675 所述方法制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.71(s, 1H), 11.32(s, 1H), 8.22-8.19(d, J = 9.15Hz, 1H), 7.58-6.99(m, 10H), 3.87-3.84(d, 4H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H)。

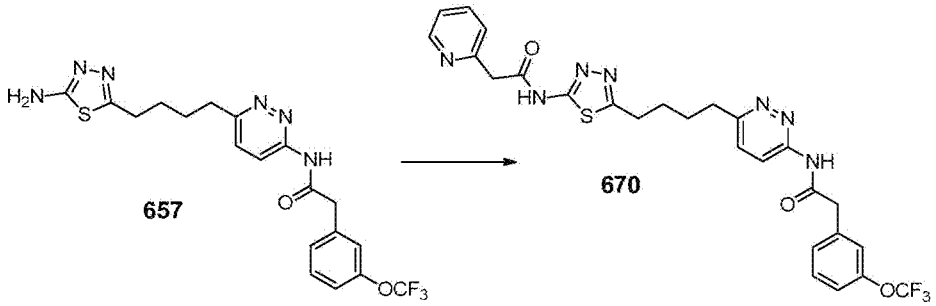
[0724]



669

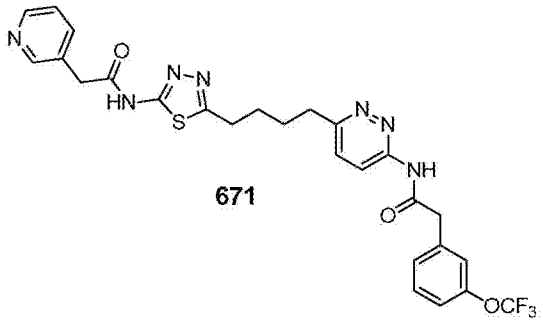
[0725] 669 采用化合物 675 所述方法制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.71(s, 1H), 11.32(s, 1H), 8.22-8.19(d, J = 9.09Hz, 1H), 7.58-7.54(d, J = 9.37Hz, 1H), 7.48-7.28(m, 6H), 7.03-6.97(m, 2H), 4.77-4.74(q, 2H), 3.87(s, 2H), 3.78(s, 2H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H)。

[0726]



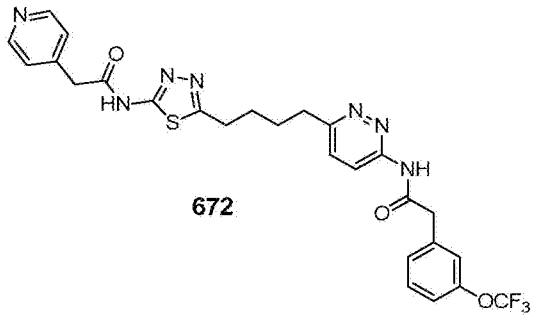
[0727] 向烧瓶中装入657(50mg,0.111mmol)、含2-吡啶乙酸盐酸盐(20mg,0.116mmol)的DMF(1ml),在0℃下,依次用丙基膦酸酐溶液(91ul)和三乙胺(40ul,0.29mmol)处理。使所得混合物慢慢升温至室温,搅拌1小时后,通过加入冰水(～5mL)将其猝灭。黄色沉淀通过吸滤收集,用更多的水漂洗。粗物质用硅胶色谱法纯化(用含0-6% MeOH的二氯甲烷洗脱),得到670。^{1}H NMR(300MHz,DMSO-d_6) δ 12.67(s,1H),11.32(s,1H),8.53-8.49(m,1H),8.22-8.19(d,J = 9.12Hz,1H),7.78-7.76(t,1H),7.58-7.26(m,7H),4.01(s,2H),3.87(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

[0728]



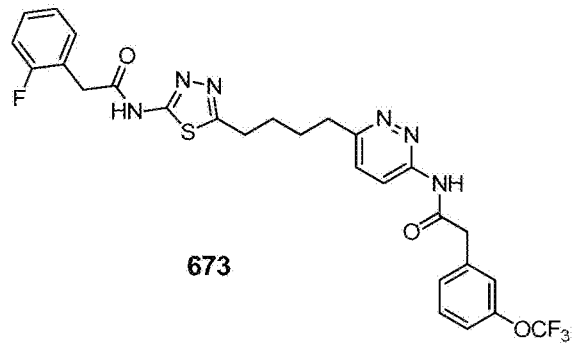
[0729] 671采用化合物670所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.70(s,1H),11.32(s,1H),8.53-8.48(m,2H),8.22-8.19(d,J = 9.12Hz,1H),7.76-7.26(m,7H),3.87(s,4H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

[0730]



[0731] 672采用化合物670所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 11.32(s,1H),8.53-8.52(bs,2H),8.22-8.19(d,J = 9.12Hz,1H),7.58-7.26(m,7H),3.87(s,4H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

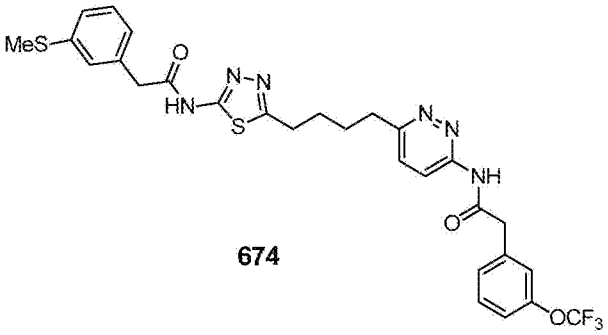
[0732]



673

[0733] 673 通过化合物 661 所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.69(bs,1H),11.31(s,1H),8.20(d,J = 9.1Hz,1H),7.57(d,J = 9.1Hz,1H),7.51 - 7.21(m,8H),3.90(s,2H),3.87(s,2H),3.06 - 2.86(m,4H),1.77 - 1.72(m,4H)。

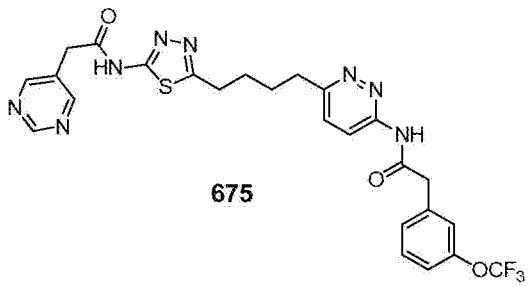
[0734]



674

[0735] 674 通过化合物 661 所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.63(bs,1H),11.32(s,1H),8.20(d,J = 9.2Hz,1H),7.57(d,J = 9.2Hz,1H),7.51 - 7.38(m,3H),7.33 - 7.09(m,5H),3.87(s,2H),3.79(s,2H),3.06 - 2.86(m,4H),2.48(s,3H),1.77 - 1.72(m,4H)。

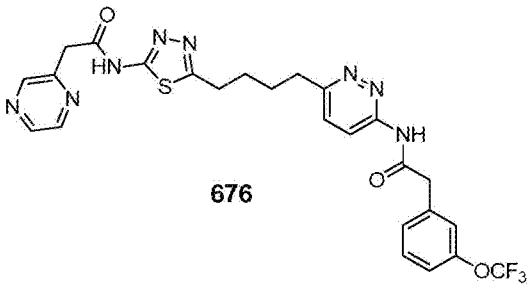
[0736]



675

[0737] 向烧瓶中装入 657(70mg,0.155mmol)、含 5- 嘧啶乙酸 (22mg,0.162mmol) 的 DMF(1ml),在 0℃下,依次加入 HOBT(44mg,0.326mmol) 和 EDCI(78mg,0.408mmol)。将所得混合物慢慢升温至室温,搅拌过夜后,通过加入冰水 (～ 5mL) 将其猝灭。白色沉淀通过吸滤收集,用更多的水漂洗。粗物质用硅胶色谱法纯化 (用含 0-6% MeOH 的二氯甲烷洗脱),得到 675。^{1}H NMR(300MHz,DMSO-d_6) δ 12.75(s,1H),11.32(s,1H),9.11(s,1H),8.76(s,1H),8.22-8.19(d,J = 9.12Hz,1H),7.59-7.26(m,6H),3.94(s,2H),3.87(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

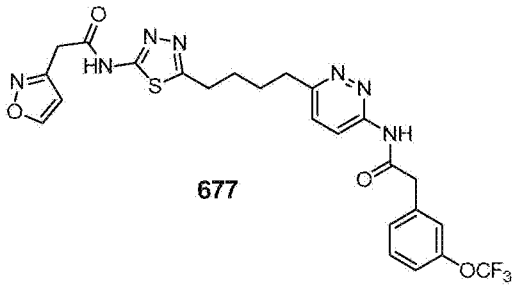
[0738]



676

[0739] 676 采用化合物 675 所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.75(s,1H),11.32(s,1H),8.70(s,1H),8.61-8.57(m,2H),8.22-8.19(d,J = 9.36Hz,1H),7.59-7.26(m,5H),4.11(s,2H),3.87(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

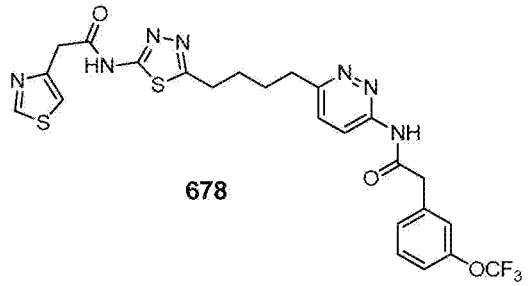
[0740]



677

[0741] 677 采用合物 675 所述方法制备化。^{1}H NMR(300MHz,DMSO-d_6) δ 12.75(s,1H),11.32(s,1H),8.89(s,1H),8.22-8.19(d,J = 9.15Hz,1H),7.59-7.26(m,5H),6.62(s,1H),3.99(s,2H),3.87(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

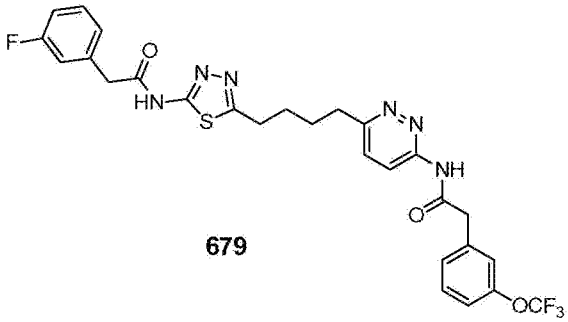
[0742]



678

[0743] 678 采用化合物 675 所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.75(s,1H),11.32(s,1H),9.06(s,1H),8.22-8.19(d,J = 9.21Hz,1H),7.59-7.26(m,6H),4.03(s,2H),3.87(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

[0744]

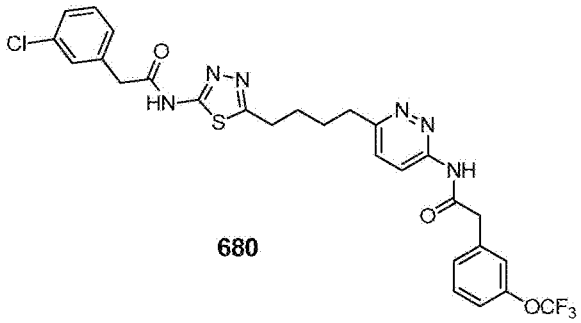


679

[0745] 679 通过化合物 661 所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.67(bs,1H),1

1.31(s,1H),8.20(d,J = 9.2Hz,1H),7.57(d,J = 9.2Hz,1H),7.51 - 7.36(m,4H),7.29 - 7.12(m,4H),3.87(s,2H),3.85(s,2H),3.06 - 2.86(m,4H),1.77 - 1.72(m,4H)。

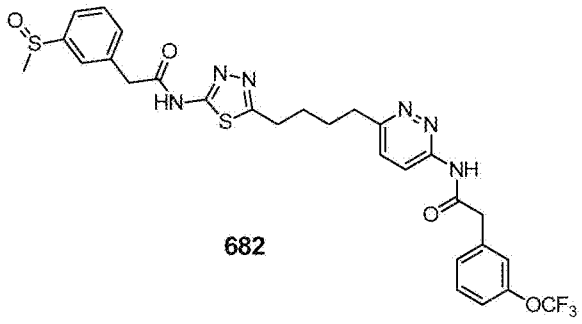
[0746]



680

[0747] 680通过化合物661所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.67(bs,1H),11.31(s,1H),8.20(d,J = 9.3Hz,1H),7.57(d,J = 9.0Hz,1H),7.51 - 7.28(m,8H),3.87(s,2H),3.84(s,2H),3.06 - 2.86(m,4H),1.77 - 1.72(m,4H)。

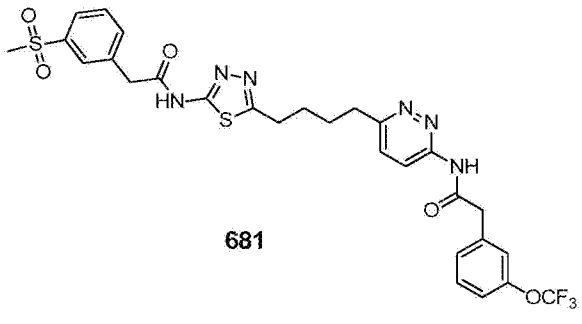
[0748]



682

[0749] 在-78℃下，分4份向674(100mg,0.16mmol)的二氯甲烷溶液中加入m-CPBA(60mg,0.24mmol)。将所得混合物在该温度下搅拌1小时后，慢慢使之升温至-10℃，并用25％ $Na_2S_2O_3$水溶液猝灭。反应物用EtOAc稀释，用饱和$NaHCO_3$水溶液(3×10mL)洗涤。合并的有机层经分离，用盐水洗涤，干燥($MgSO_4$)并浓缩。粗产物用HPLC纯化，得到682。^{1}H NMR(300MHz,DMSO-d_6) δ 12.72(bs,1H),11.31(s,1H),8.20(d,J = 9.0Hz,1H),7.68(m,1H),7.60 - 7.26(m,8H),3.91(s,2H),3.87(s,2H),3.06 - 2.86(m,4H),2.76(s,3H),1.77 - 1.72(m,4H)。

[0750]

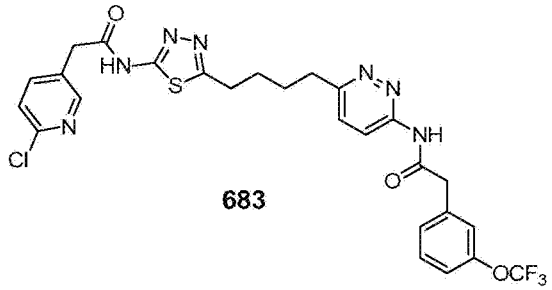


681

[0751] 681通过化合物661所述方法由657和3-甲基磺酰基苯乙酸制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.72(bs,1H),11.31(s,1H),8.20(d,J = 9.0Hz,1H),7.92 - 7.83(m,2H),7.70 - 7.26(m,7H),3.93(s,2H),3.87(s,2H),3.23(s,3H),3.06 -

2.86(m,4H),1.77－1.72(m,4H)。

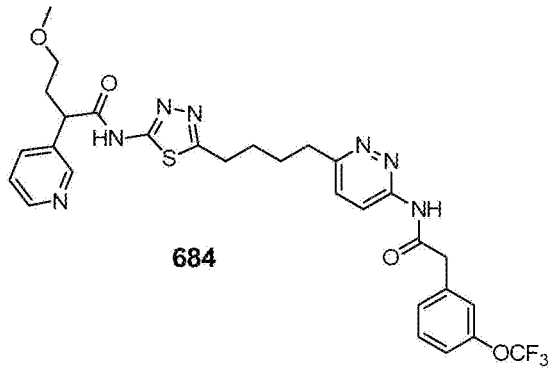
[0752]



683

[0753] 683采用化合物675所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.75(s,1H),11.32(s,1H),8.36(s,1H),8.21-8.18(d,J＝9.18Hz,1H),7.84-7.80(d,J＝9.36Hz,1H),7.59-7.26(m,6H),3.90-3.87(d,4H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

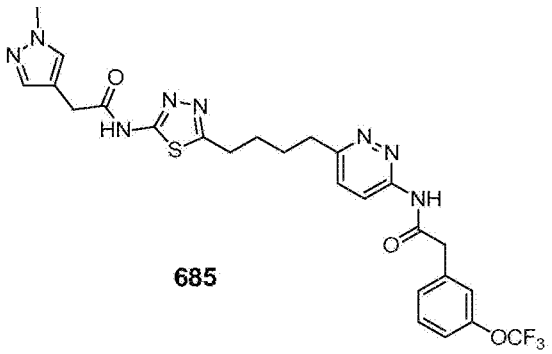
[0754]



684

[0755] 684采用化合物675所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.75(s,1H),11.32(s,1H),8.57(s,1H),8.51-8.49(d,J ＝ 9.18Hz,1H),8.21-8.18(d,J ＝ 9.06Hz,1H),7.79-7.75(d,J ＝ 9.36Hz,1H),7.59-7.26(m,6H),4.07(t,2H),3.87(s,2H),3.30-3.28(m,1H),3.19(s,3H),3.01(bs,2H),2.90(bs,2H),2.3-2.5(m,1H),1.99-1.96(m,1H),1.73(bs,4H)。

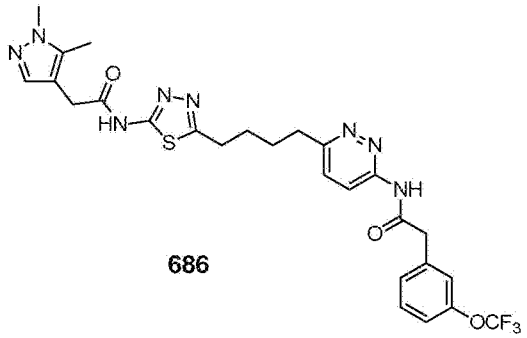
[0756]



685

[0757] 685通过化合物661所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.52(bs,1H),11.31(s,1H),8.20(d,J ＝ 9.1Hz,1H),7.61－7.25(m,7H),3.87(s,2H),3.80(s,3H),3.62(s,2H),3.06－2.86(m,4H),1.77－1.72(m,4H)。

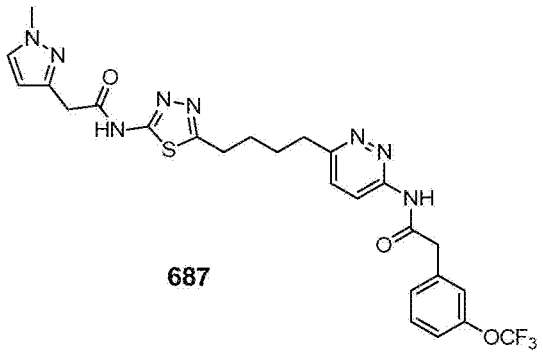
[0758]



686

[0759] 686 通过化合物 661 所述方法制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.53(bs, 1H), 11.32(s, 1H), 8.20(d, J = 9.1Hz, 1H), 7.58(d, J = 9.2Hz, 1H), 7.52 - 7.26(m, 4H), 5.96(s, 1H), 3.87(s, 2H), 3.67(s, 2H), 3.64(s, 3H), 3.06 - 2.86(m, 4H), 2.21(s, 3H), 1.77 - 1.72(m, 4H)。

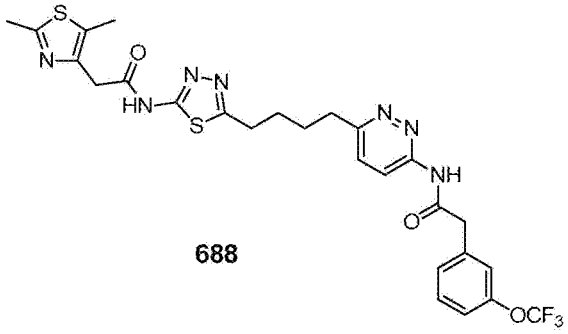
[0760]



687

[0761] 687 通过化合物 661 所述方法制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.56(bs, 1H), 11.32(s, 1H), 8.20(d, J = 9.3Hz, 1H), 7.61 - 7.38(m, 6H), 6.17(d, J = 2.2Hz, 1H), 3.87(s, 2H), 3.79(s, 3H), 3.75(s, 2H), 3.03 - 2.90(m, 4H), 1.7 - 1.72(m, 4H)。

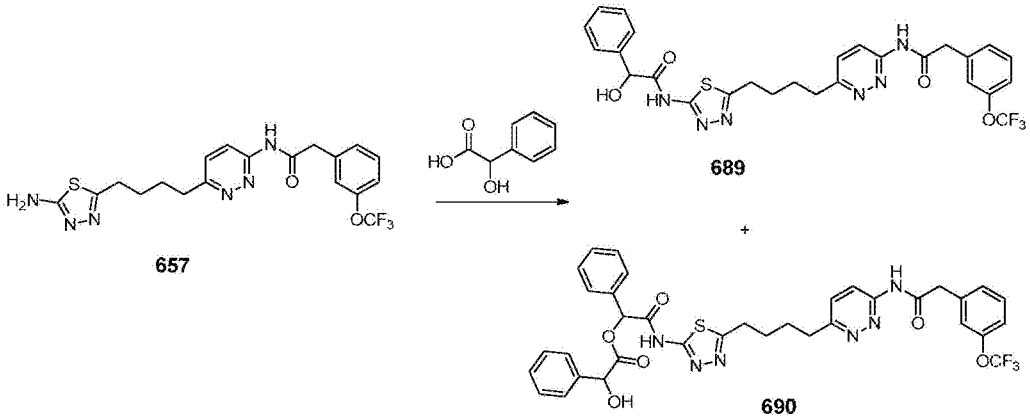
[0762]



688

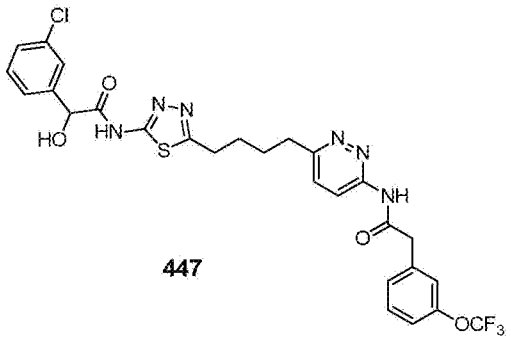
[0763] 688 通过化合物 661 所述方法制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.61(bs, 1H), 11.32(s, 1H), 8.20(d, J = 9.3Hz, 1H), 7.58(d, J = 9.3Hz, 1H), 7.51 - 7.26(m, 4H), 3.87(s, 2H), 3.84(s, 2H), 3.07 - 2.86(m, 4H), 1.77 - 1.72(m, 4H)。

[0764]



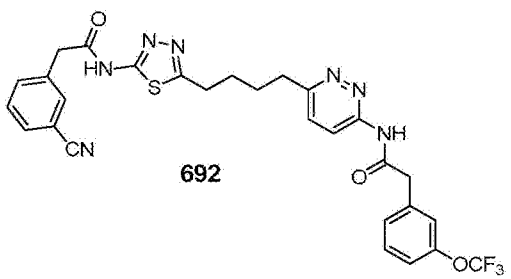
[0765] 在0℃下，向657(200mg,0.44mmol)的DMF(4mL)溶液中加入扁桃酸(124mg,0.66mmol)、HOBt(119mg,0.88mmol)和EDCI(170mg,0.88mmol)。将所得混合物在室温下搅拌1.5小时后，使之冷却至0℃，并用H_2O猝灭。沉淀通过吸滤收集，用硅胶色谱法进一步纯化(用含1-10%MeOH的二氯甲烷洗脱)，得到690和更大极性的689。689：^1H NMR(300MHz,DMSO-d_6) δ 12.42(bs,1H),11.31(s,1H),8.20(d,J = 9.2Hz,1H),7.58-7.27(m,10H),6.35(d,J = 4.4Hz,1H),5.34(d,J = 4.3Hz,1H),3.87(s,2H),3.03-2.89(m,4H),1.77-1.73(m,4H)。690：^1H NMR(300MHz,DMSO-d_6) δ 13.05(bs,1H),11.31(s,1H),8.20(d,J = 9.0Hz,1H),7.59-7.26(m,15H),6.26(d,J = 5.5Hz,1H),6.11(s,1H),5.38(d,J = 5.3Hz,1H),3.87(s,2H),3.03-2.88(m,4H),1.76-1.73(m,4H)。

[0766]



[0767] 447通过化合物689所述方法由657和3-氯扁桃酸制备。^1H NMR(300MHz,DMSO-d_6) δ 12.48(bs,1H),11.31(s,1H),8.20(d,J = 9.2Hz,1H),7.59-7.26(m,9H),6.53(m,1H),5.36(t,J = 0.7Hz,1H),3.87(s,2H),3.03-2.90(m,4H),1.75-1.71(m,4H)。

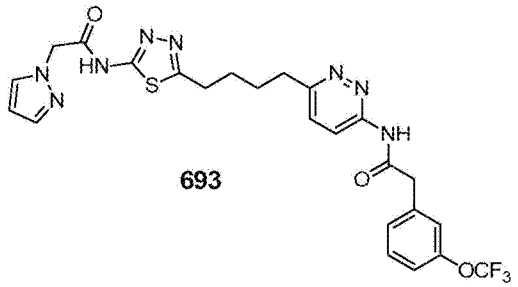
[0768]



[0769] 692采用化合物675所述方法制备。^1H NMR(300MHz,DMSO-d_6) δ 12.75(s,1H),11.

32(s,1H),8.21-8.18(d,J = 9.18Hz,1H),7.80-7.26(m,9H),3.92(s,2H),3.87(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

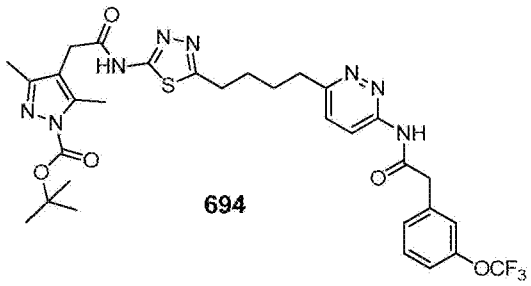
[0770]



693

[0771] 693采用化合物675所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.75(s,1H),11.32(s,1H),8.22-8.19(d,J = 9.06Hz,1H),7.79(s,1H),7.59-7.26(m,6H),6.31(s,1H),5.20(s,2H),3.87(s,2H),3.01(bs,2H),2.90(bs,2H),1.73(bs,4H)。

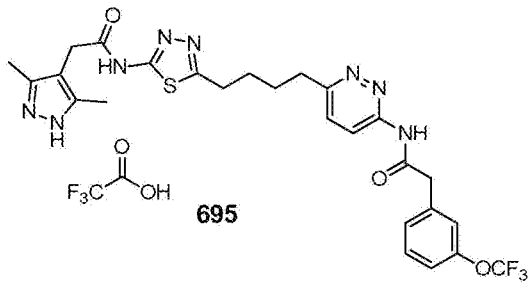
[0772]



694

[0773] 694采用化合物675所述方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.71(s,1H),11.32(s,1H),8.22-8.18(d,J = 9.15Hz,1H),7.58-7.54(d,J = 9.18Hz,1H),7.48-7.26(m,4H),3.87(s,2H),3.63(s,2H),3.01(bs,2H),2.90(bs,2H),2.39(s,3H),2.13(s,3H),1.73(bs,4H),1.57(s,9H)。

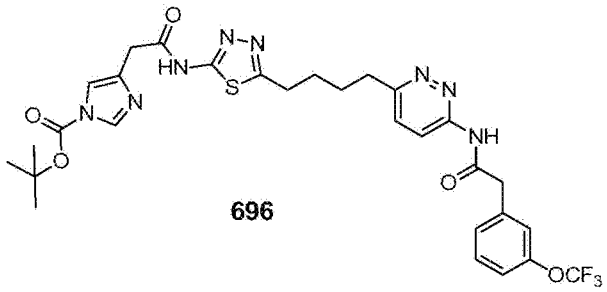
[0774]



695

[0775] 在0℃下,向694(50mg,0.081mmol)的二氯甲烷(2ml)溶液中加入TFA(2ml)。将所得混合物在室温下搅拌1小时后,将其真空蒸发至干。加入乙醚,白色沉淀通过吸滤收集,用更多的乙醚漂洗,得到695。^{1}H NMR(300MHz,DMSO-d_6) δ 12.71(s,1H),11.32(s,1H),8.22-8.19(d,J = 9.36Hz,1H),7.60-7.57(d,J = 9.27Hz,1H),7.51-7.28(m,4H),3.88(s,2H),3.57(s,2H),3.01(bs,2H),2.90(bs,2H),2.45(s,3H),2.15(s,3H),1.73(bs,4H)。

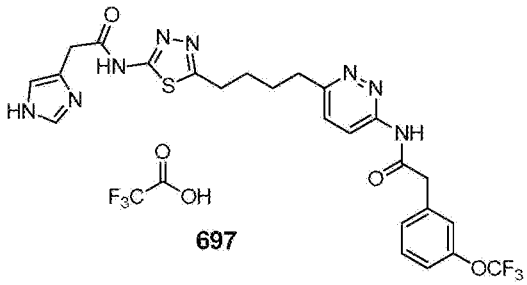
[0776]



696

[0777] 696 采用化合物 695 所述方法制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.71(s, 1H), 11.32(s, 1H), 8.22-8.19(d, J = 9.30Hz, 1H), 8.15(s, 1H), 7.58-7.54(d, J = 9.30Hz, 1H), 7.48-7.28(m, 5H), 3.87(s, 2H), 3.76(s, 2H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H), 1.59(s, 9H)。

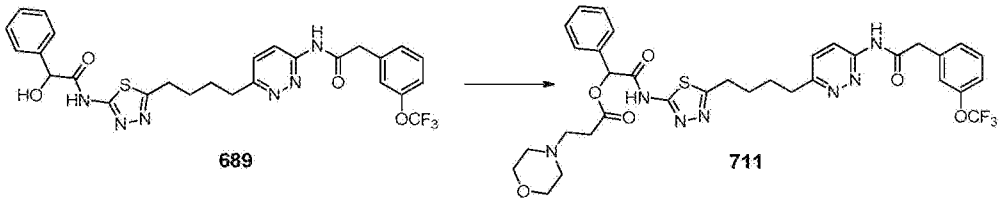
[0778]



697

[0779] 697 采用化合物 695 所述方法制备。^{1}H NMR(300MHz, DMSO-d_6) δ 14.22(s, 1H), 12.71(s, 1H), 11.32(s, 1H), 9.01(s, 1H), 8.22-8.19(d, J = 9.15Hz, 1H), 7.59-7.26(m, 6H), 4.04(s, 2H), 3.87(s, 2H), 3.01(bs, 2H), 2.90(bs, 2H), 1.73(bs, 4H)。

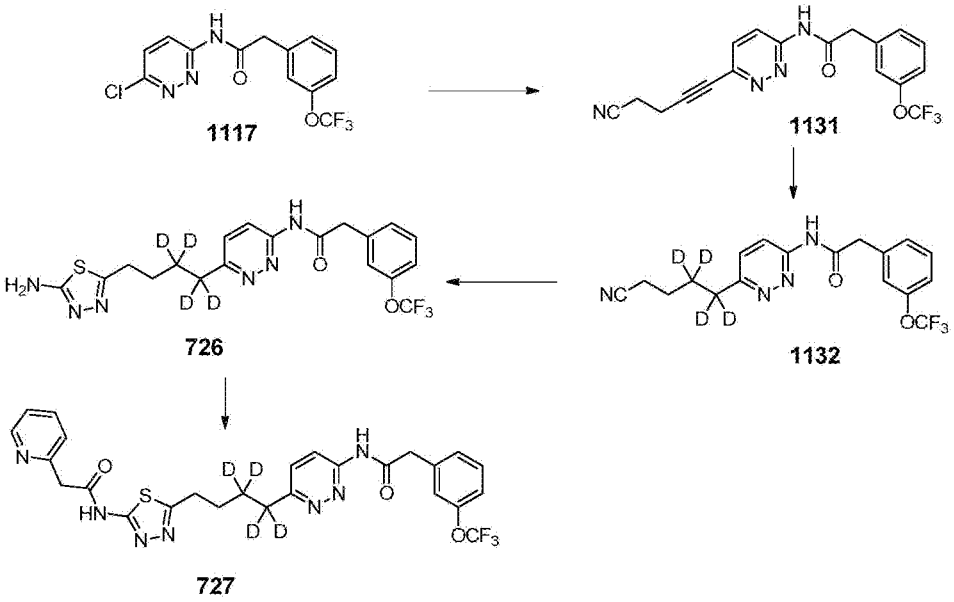
[0780]



689 711

[0781] 在 0℃下，向 3- 吗啉 -4- 基 - 丙酸盐酸盐 (113mg, 0.58mmol) 在 DMF(8mL) 中的悬浮液中加入 N-(3- 二甲基氨基丙基)-N′ - 乙基碳二亚胺盐酸盐 (130mg, 0.67mmol)。将所得混合物在 0℃下搅拌 40 分钟，接着加入 689(300mg, 0.48mmol) 和 4-DMAP(165mg, 1.35mmol)。在 3.5 小时时间内从 0℃到室温搅拌所得混合物后，将其用 EtOAc 和冷水稀释。分离有机层，并用水 (3×15mL)、盐水洗涤，干燥 ($MgSO_4$) 并浓缩。粗产物用硅胶色谱法纯化 (用含 0 - 15% MeOH 的 CH_2Cl_2 洗脱)，得到 711(297mg)，为白色固体。^{1}H NMR(300MHz, $CDCl_3$) δ 10.75(bs, 1H), 8.49(d, J = 9.0Hz, 1H), 7.64(s, 1H), 7.50 - 7.26(m, 7H), 7.16 - 7.15(m, 1H), 6.51(s, 1H), 4.04(s, 2H), 3.80 - 3.72(m, 4H), 3.88 - 2.81(m, 8H), 2.75 - 2.71(m, 5H), 1.89(m, 4H)。

[0782]



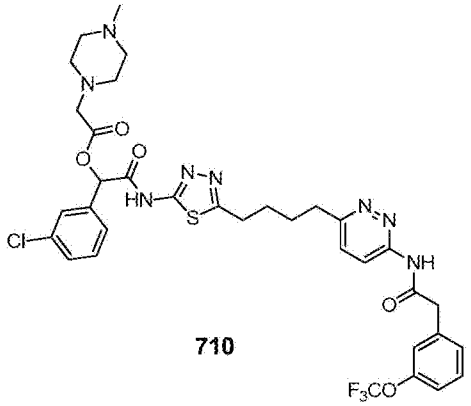
[0783] 将1117(4.00g,12.06mmol)、4-戊炔腈(2.11mL,24.12mmol)、$PdCl_2(PPh_3)_2$(847mg,1.21mmol)、CuI(184mg,0.96mmol)和Et_3N(13.44mL,96.48mmoL)在DMF(18mL)中的混合物在55℃下加热5小时。使反应物冷却至室温,倒入冰-水的混合物中。沉淀通过吸滤收集并风干。粗产物先用i-PrOH-H_2O的混合物进一步重结晶,然后再用i-PrOH进一步重结晶,得到炔1131。

[0784] 将炔1131(6.00g)和$Pd(OH)_2/C$(1.00g)在EtOAc(150mL)、THF(75mL)和MeOH(75mL)混合物中的混合物在1大气压D_2、室温下搅拌3小时后,将催化剂滤出SiO_2短塞,用EtOAc漂洗。将滤液浓缩,得到粗产物,将其用EtOAc和乙醚的混合物进一步重结晶,得到所需的烷烃1132,为灰白色固体(6.01g)

[0785] 将腈1132(5.20g,13.61mmol)和氨基硫脲(1.61g,17.69mmol)在TFA(75mL)中的混合物在80℃下加热4小时。使反应物冷却至室温,倒入冰-水的混合物中。混合物用NaOH小粒碱化(pH 14)。白色沉淀通过吸滤收集,用水漂洗并干燥,得到726(5.87g)。

[0786] 在0℃下,向726(1.40g,3.07mmol)和2-吡啶基乙酸HCl盐(1.49g,8.59mmol)的DMF(20mL)溶液中依次加入Et_3N(1.50mL,10.73mmol)和1-丙烷膦酸酐(2.73mL,50%的DMF溶液,4.29mmol)。将该混合物在室温下搅拌2.5小时后,使之冷却回到0℃,并用冰-H_2O猝灭。沉淀通过吸滤收集并风干。该粗产物用硅胶色谱法进一步纯化(用含0-15% MeOH的DCM洗脱),得到727(0.97g)。^1H NMR(300MHz,DMSO-d_6)δ12.67(s,1H),11.31(s,1H),8.52-8.50(m,1H),8.20(d,J = 9.2Hz,1H),7.78(dt,J = 1.8,7.6Hz,1H),7.58(d,J = 9.1Hz,1H),7.51-7.26(m,6H),4.02(s,2H),3.87(s,2H),3.03(t,J = 7.4Hz,2H),1.73(t,J = 7.4Hz,2H)。

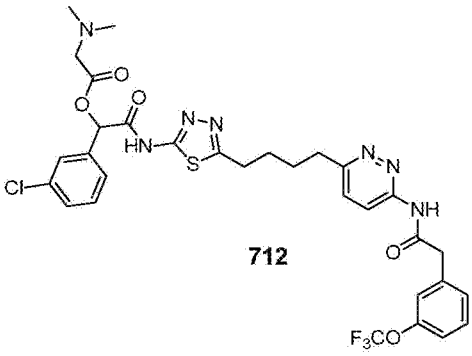
[0787]



710

[0788] 化合物710采用类似于制备化合物711所用的方法自化合物447制备。^{1}H NMR(300MHz,DMSO-d_6) δ 11.32(s,1H),8.21-8.18(d,J = 9.06Hz,1H),7.62-7.26(m,9H),6.16(s,1H),3.87(s,2H),3.52-3.50(d,2H),3.01(bs,2H),2.90(bs,2H),2.80-2.71(m,11H),1.73(bs,4H)。

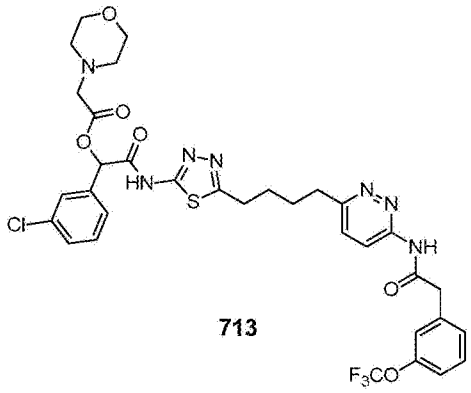
[0789]



712

[0790] 化合物712采用类似于制备化合物711所用的方法自化合物447制备。^{1}H NMR(300MHz,DMSO-d_6) δ 11.32(s,1H),8.21-8.18(d,J = 9.06Hz,1H),7.62-7.26(m,9H),6.16(s,1H),3.87(s,2H),3.38-3.36(d,2H),3.01(bs,2H),2.90(bs,2H),2.29(s,6H),1.73(bs,4H)。

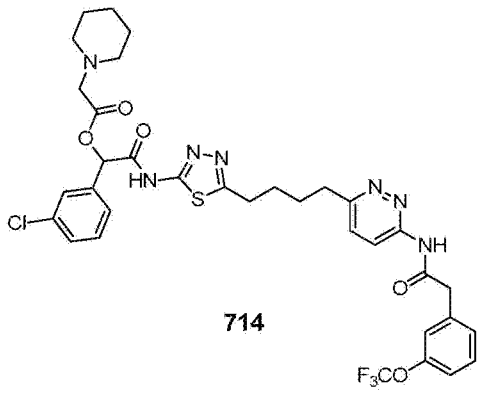
[0791]



713

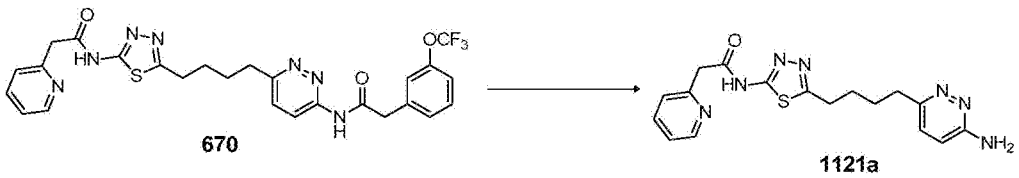
[0792] 化合物713采用类似于制备化合物711所用的方法自化合物447制备。^{1}H NMR(300MHz,DMSO-d_6) δ 13.11(bs,1H),11.32(s,1H),8.21-8.18(d,J = 9.06Hz,1H),7.62-7.26(m,9H),6.16(s,1H),3.87(s,2H),3.60-3.57(m,4H),3.44-3.42(d,2H),3.01(bs,2H),2.90(bs,2H),2.55-2.51(m,4H),1.73(bs,4H)。

[0793]



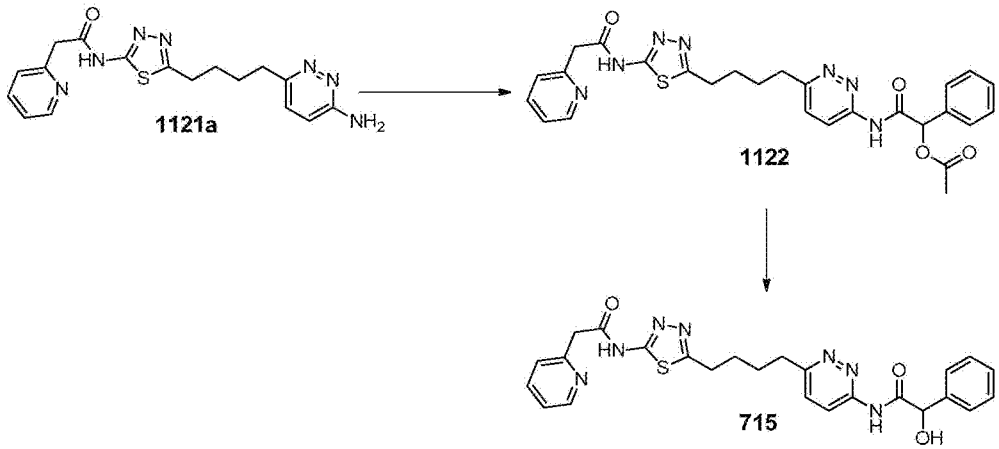
[0794] 化合物714采用类似于制备化合物711所用的方法自化合物447制备。^{1}H NMR(300MHz,DMSO-d_6) δ 11.32(s,1H),8.21-8.18(d,J = 9.06Hz,1H),7.62-7.26(m,9H),6.16(s,1H),3.87(s,2H),3.38-3.31(d,2H),3.01(bs,2H),2.90(bs,2H),2.49-2.47(m,4H),1.93(bs,4H),1.73(bs,4H),1.72(bs,2H)。

[0795]



[0796] 在0℃下,向670(3g,5.24mmol)在MeOH(50ml)中的悬浮液中加入2N NaOH(20ml)溶液。将所得混合物在室温下搅拌过夜。溶剂经真空蒸发,混合物用1N HCl酸化至pH 6。白色沉淀通过吸滤收集,用更多的水漂洗并干燥,得到1121a。^{1}H NMR(300MHz,DMSO-d_6) δ 12.66(s,1H),8.51-8.50(m,1H),7.81-7.76(m,1H),7.42-7.28(m,2H),7.16-7.13(d,1H),6.73-6.70(d,1H),6.10(s,2H),4.0(s,2H),3.01(bs,2H),2.71(bs,2H),1.70(bs,4H)。

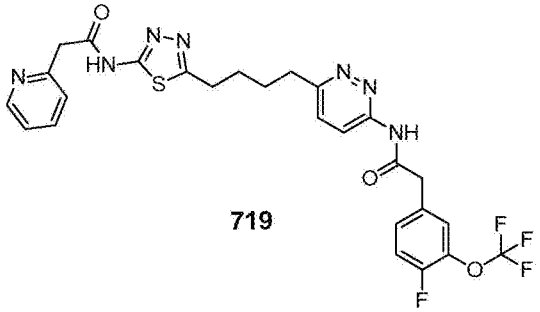
[0797]



[0798] 在0℃下,向1121a(20mg,0.054mmol)的DMF(1ml)溶液中依次滴加三乙胺(11ul,0.081mmol)和o-乙酰基扁桃酸酰氯(15ul,0.065mmol)。使所得混合物慢慢升温至室温,搅拌1小时后,在0℃下通过加入水(～3mL)将其猝灭。使混合物在水和EtOAc之间分配。有机萃取物用盐水洗涤,经硫酸钠干燥,过滤并蒸发。粗物质用硅胶色谱法纯化(用含0-5% MeOH的DCM洗脱),得到1122。

[0799] 向烧瓶中装入1122(20mg,0.037mmol)和2N氨/MeOH(5ml)。将混合物在室温下搅拌2小时。溶剂经真空蒸发,混合物用乙醚研磨。白色沉淀通过吸滤收集,用乙醚漂洗并干燥,得到715。^{1}H NMR(300MHz,DMSO-d_6)δ12.66(s,1H),10.61(s,1H),8.51-8.50(m,1H),8.21-8.18(d,J = 9.06Hz,1H),7.81-7.76(m,1H),7.61-7.53(m,3H),7.42-7.28(m,5H),6.49-6.47(d,1H),5.30-5.28(d,1H),4.0(s,2H),3.02(bs,2H),2.91(bs,2H),1.75(bs,4H)。

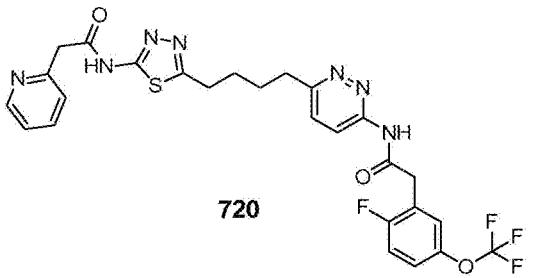
[0800]



719

[0801] 化合物719采用类似于化合物670制备所用的方法制备。^{1}H NMR(300MHz,DMSO-d_6)δ12.66(s,1H),11.32(s,1H),8.51-8.50(m,1H),8.21-8.18(d,J = 9.06Hz,1H),7.79-7.76(m,1H),7.59-7.30(m,6H),4.0(s,2H),3.87(s,2H),3.01(bs,2H),2.90(bs,2H),1.75(bs,4H)。

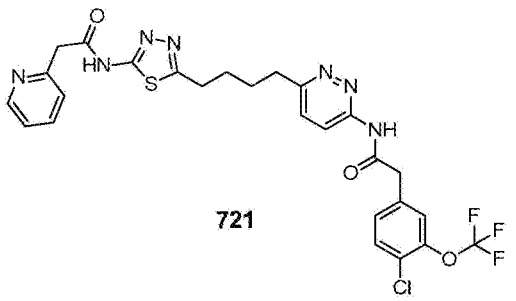
[0802]



720

[0803] 化合物720采用类似于制备化合物670所用的方法制备。^{1}H NMR(300MHz,DMSO-d_6)δ12.66(s,1H),11.32(s,1H),8.51-8.50(m,1H),8.19-8.16(d,J = 9.06Hz,1H),7.79-7.76(m,1H),7.59-7.30(m,6H),4.01(s,2H),3.95(s,2H),3.03(bs,2H),2.91(bs,2H),1.76(bs,4H)。

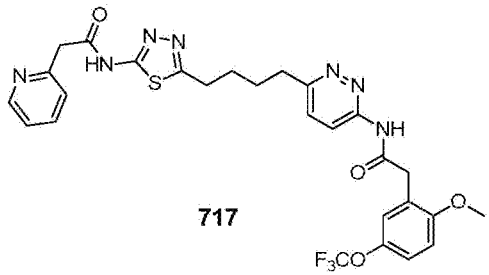
[0804]



721

[0805] 化合物721采用类似于制备化合物670所用的方法制备。^{1}H NMR(300MHz,DMSO-d_6)δ12.66(s,1H),11.32(s,1H),8.51-8.50(m,1H),8.21-8.16(d,J = 9.06Hz,1H),7.81-7.28(m,7H),4.01(s,2H),3.89(s,2H),3.03(bs,2H),2.91(bs,2H),1.76(bs,4H)。

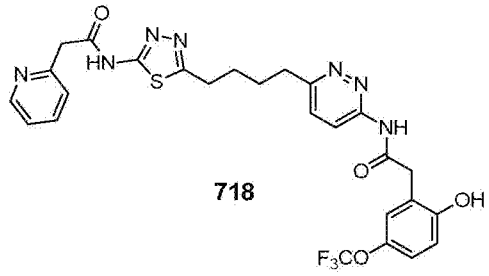
[0806]



717

[0807] 化合物717采用类似于制备化合物670所用的方法制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.66(s,1H),11.17(s,1H),8.52-8.50(m,1H),8.19-8.16(d,J = 9.06Hz,1H),7.81-7.76(m,1H),7.58-7.55(d,1H),7.42-7.09(m,4H),7.08-7.06(d,1H),4.01(s,2H),3.83(s,2H),3.79(s,3H),3.03(bs,2H),2.91(bs,2H),1.76(bs,4H)。

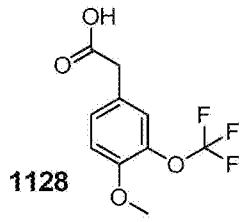
[0808]



718

[0809] 在0℃下,向717(10mg,0.017mmol)的DCM(3ml)溶液中滴加三溴化硼溶液(1N,在DCM中)(2ml)。将所得混合物慢慢升温至室温,搅拌4.5小时后,通过加入水(～3mL)将其猝灭。混合物然后用1NNaOH碱化至pH 8。使混合物在水和DCM之间分配。有机萃取物用盐水洗涤,经硫酸钠干燥,过滤并蒸发。粗物质用硅胶色谱法纯化(用含0-10% MeOH的DCM洗脱),得到718。^{1}H NMR(300MHz,DMSO-d_6) δ 11.17(s,1H),8.52-8.50(m,1H),8.21-8.18(d,J = 9.06Hz,1H),7.81-7.76(m,1H),7.58-7.55(d,1H),7.51-7.09(m,4H),6.88-6.85(d,1H),4.0(s,2H),3.79(s,2H),3.03(bs,2H),2.91(bs,2H),1.76(bs,4H)。

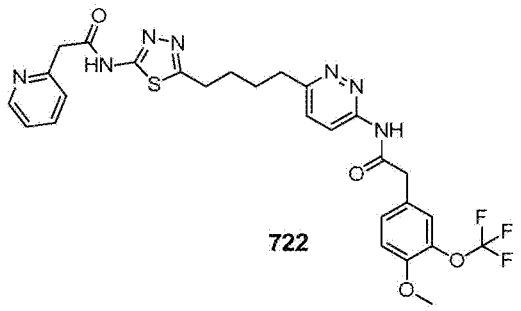
[0810]



1128

[0811] 化合物1128采用类似于下述化合物1124的方法由4-溴-2-三氟甲氧基茴香醚制备。

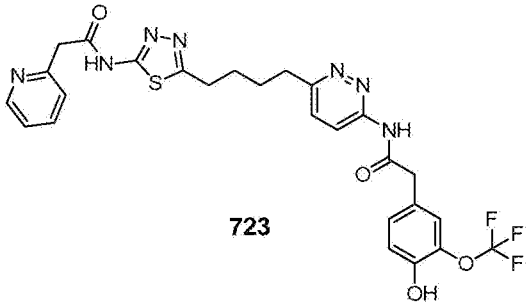
[0812]



722

[0813] 化合物722用类似于化合物670的方法使用化合物1128制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.66(s,1H),11.17(s,1H),8.52-8.50(m,1H),8.21-8.18(d,J = 9.06Hz,1H),7.81-7.76(m,1H),7.58-7.55(d,1H),7.42-7.19(m,5H),4.0(s,2H),3.85(s,3H),3.79(s,2H),3.03(bs,2H),2.91(bs,2H),1.76(bs,4H)。

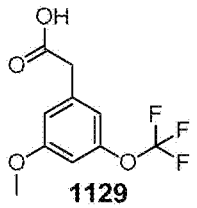
[0814]



723

[0815] 化合物723采用类似于制备上述化合物718的方法自化合物722制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.66(s,1H),11.17(s,1H),10.06(s,1H),8.52-8.50(m,1H),8.21-8.18(d,J = 9.06Hz,1H),7.81-7.76(m,1H),7.58-7.55(d,1H),7.42-7.19(m,4H),6.99-6.96(d,1H),4.0(s,2H),3.70(s,2H),3.03(bs,2H),2.91(bs,2H),1.76(bs,4H)。

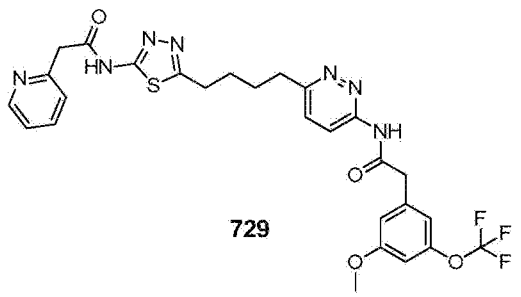
[0816]



1129

[0817] 化合物1129采用类似于下述化合物1126的方法由3-溴-5-三氟甲氧基茴香醚制备。

[0818]

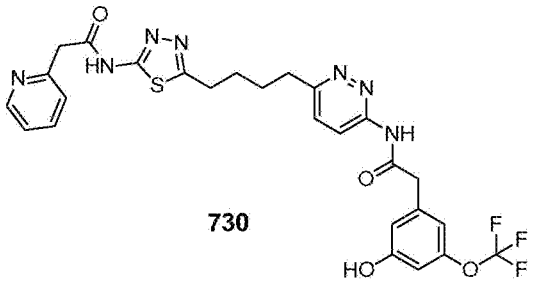


729

[0819] 化合物729采用类似于化合物670的方法使用化合物1129制备。^{1}H NMR(300MHz,DMSO-d_6) δ 12.66(s,1H),11.28(s,1H),8.52-8.50(m,1H),8.21-8.18(d,J =

9.06Hz, 1H), 7.81-7.76(m, 1H), 7.58-7.55(d, 1H), 7.42-7.29(m, 2H), 6.99-6.95(m, 2H), 6.84(s, 1H), 4.0(s, 2H), 3.80(m, 5H), 3.03(bs, 2H), 2.91(bs, 2H), 1.76(bs, 4H)。

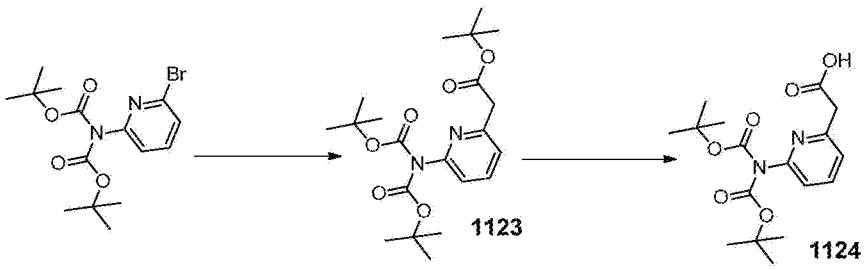
[0820]



730

[0821] 化合物730采用类似于制备上述化合物718的方法自化合物729制备。^{1}H NMR(300MHz, DMSO-d_6) δ 12.66(s, 1H), 11.28(s, 1H), 10.04(s, 1H), 8.52-8.50(m, 1H), 8.21-8.18(d, J = 9.06Hz, 1H), 7.81-7.76(m, 1H), 7.58-7.55(d, 1H), 7.42-7.29(m, 2H), 6.81-6.78(m, 2H), 6.61(s, 1H), 4.0(s, 2H), 3.74(m, 2H), 3.03(bs, 2H), 2.91(bs, 2H), 1.76(bs, 4H)。

[0822]

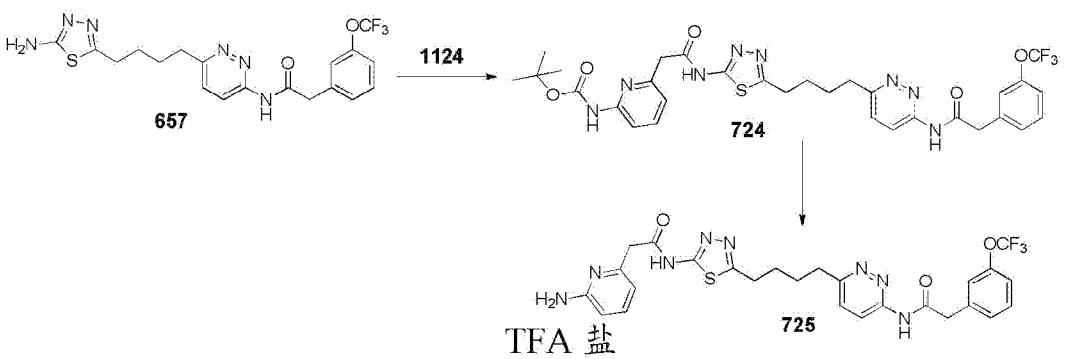


1123 1124

[0823] 在氩气氛下，向6-(二-Boc-氨基)-2-溴吡啶(1g, 2.9mmol)、双(三叔丁基膦)钯(0)(300mg, 0.59mmol)在1,4-二噁烷(30ml)中的混合物中加入含0.5M 2-叔丁氧基-2-氧代乙基氯化锌的乙醚(15ml)。将所得混合物在室温下搅拌过夜。使混合物在饱和NH_4Cl和EtOAc之间分配。有机萃取物用盐水洗涤，经硫酸钠干燥，过滤并蒸发。粗物质用硅胶色谱法纯化(用含0-20% EtOAc的己烷洗脱)，得到1123。

[0824] 在0℃下，向1123(150mg, 0.37mmol)的MeOH(6ml)和水(2ml)溶液中加入一水氢氧化锂(100mg, 2.38mmol)。将所得混合物在室温下搅拌2天后，将其蒸发至干。混合物然后用1N HCl酸化(pH 4)，并使之在水和EtOAc之间分配。有机萃取物用水洗涤，经硫酸钠干燥，过滤并蒸发，得到1124。

[0825]



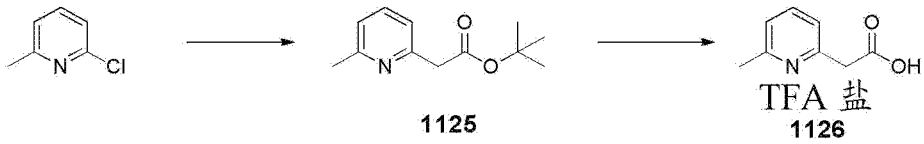
657 1124 724 725

TFA 盐

[0826] 向烧瓶中装入 657(105mg,0.232mmol)、含 1124(90mg,0.255mmol) 的 DMF(1ml),在 0℃下,依次加入丙基膦酸酐溶液 (300ul) 和三乙胺 (89ul,0.64mmol)。将所得混合物慢慢升温至室温,搅拌 3 小时后,通过加入冰水 (～ 5mL) 将其猝灭。沉淀通过吸滤收集,用更多的水漂洗。粗物质用硅胶色谱法纯化 (用含 0-6% MeOH 的 DCM 洗脱),得到 724。^{1}H NMR(300MHz,DMSO-d_6) δ 12.67(s,1H),11.32(s,1H),9.69(s,1H),8.22-8.19(d,J = 9.12Hz,1H),7.72-7.01(m,8H),3.91-3.87(d,4H),3.01(bs,2H),2.90(bs,2H),1.75(bs,4H)1.47(s,9H)。

[0827] 在 0℃下,向 724(50mg,0.07mmol) 的 DCM(3ml) 溶液中滴加 TFA(3ml)。将所得混合物在室温下搅拌 3 小时后,将其蒸发至干,然后将残余物用乙醚研磨,得到 725。^{1}H NMR(300MHz,DMSO-d_6) δ 12.67(s,1H),11.32(s,1H),8.22-8.19(d,J = 9.12Hz,1H),7.88-7.77(m,3H),7.59-7.26(m,5H),6.90-6.80(m,2H),4.05(s,2H),3.87(s,2H),3.01(bs,2H),2.90(bs,2H),1.75(bs,4H)。

[0828]

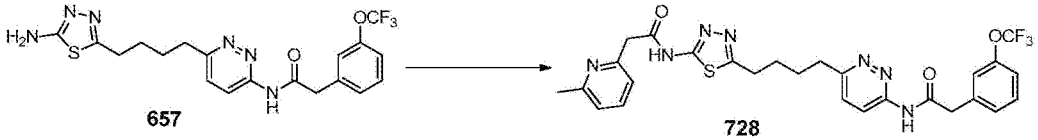


1125 1126

[0829] 在 0℃、氩气下,向乙酸叔丁酯 (789ul,5.88mmol)、2- 氯 -6- 甲基吡啶 (428ul,3.92mmol)、氯 (2- 二 - 叔丁基膦基 -2',4',6'- 三 -1- 丙基 -1,1'- 二 - 苯基)[2-(2- 氨基乙基) 苯基] 钯 (II)(27mg,0.039mmol) 在甲苯 (10ml) 的搅拌溶液加入预冷却至 0℃的 LHMDS(1M 在甲苯中)(12ml,12mmol) 溶液。将所得混合物搅拌 1 小时。使混合物在饱和 NH_4Cl 和 EtOAc 之间分配。有机萃取物用盐水洗涤,经硫酸钠干燥,过滤并蒸发。粗物质用硅胶色谱法纯化 (用含 0-15% EtOAc 的己烷洗脱),得到 1125。

[0830] 在 0℃下,向 1125(267mg,1.29mmol) 的 DCM(3ml) 溶液中滴加 TFA(1.5ml)。将所得混合物在室温下搅拌过夜后,将其蒸发至干,然后将残余物用乙醚研磨,得到 1126。

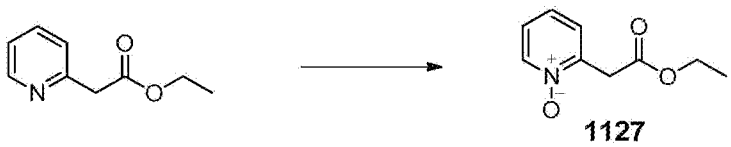
[0831]



657 728

[0832] 向烧瓶中装入 657(50mg,0.111mmol)、含 1126(35mg,0.133mmol) 的 DMF(1ml),在 0℃下,依次加入丙基膦酸酐溶液 (155ul) 和三乙胺 (57ul,0.4mmol)。将所得混合物慢慢升温至室温,搅拌 3 小时后,通过加入冰水 (～ 5mL) 将其猝灭。沉淀通过吸滤收集,用更多的水漂洗。粗物质用硅胶色谱法纯化 (用含 0-6% MeOH 的 DCM 洗脱),得到 728。^{1}H NMR(300MHz,DMSO-d_6) δ 12.67(s,1H),11.32(s,1H),8.22-8.19(d,J = 9.12Hz,1H),7.69-7.15(m,8H),3.96(s,2H),3.87(s,2H),3.01(bs,2H),2.90(bs,2H),2.52(s,3H),1.75(bs,4H)。

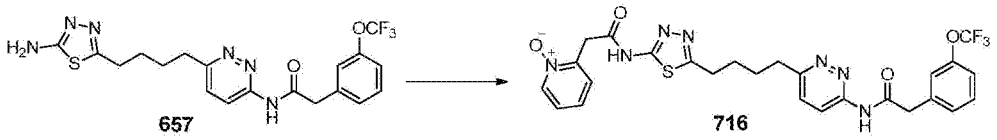
[0833]



1127

[0834] 在 0℃下，向 2- 吡啶基乙酸乙酯 (1g,6.05mmol) 的 DCM(20ml) 溶液中加入 MCPBA(77％最大)(1.77g,10.2mmol)。使所得混合物升温至室温达 3 小时后，使之在饱和碳酸氢钠和 DCM 之间分配。有机萃取物用盐水洗涤，经硫酸钠干燥，过滤并蒸发。粗物质用硅胶色谱法纯化 (用含 0-12％ MeOH 的 EtOAc 洗脱)，得到 1127。

[0835]



657 716

[0836] 向 657(331mg,0.73mmol) 的甲苯悬浮液中依次加入 1127(278mg,1.53mmol) 和三甲基铝 (2M 在甲苯中)(732ul,1.46mmol)。将所得混合物在 60℃下搅拌过夜。使反应混合物在水和 DCM 之间分配。有机萃取物用盐水洗涤，经硫酸钠干燥，过滤并蒸发。粗物质用硅胶色谱法纯化 (用含 0-5％ MeOH 的 DCM 然后含 0-15％ MeOH 的 EtOAc 洗脱)，得到 716。^{1}H NMR(300MHz,DMSO-d_6) δ 12.67(s,1H),11.32(s,1H),8.29-8.27(m,1H),8.21-8.19(d,J ＝ 9.12Hz,1H),7.61-7.26(m,8H),4.03(s,2H),3.87(s,2H),3.01(bs,2H),2.90(bs,2H),1.75(bs,4H)。

[0837] 制备型 HPLC 纯化

[0838] 所有反相制备型 HPLC 纯化在环境温度下使用带有柱的 Shimadzu Prominence 制备型液相色谱仪进行。流动相 A 和 B 分别由 0.1％甲酸 / 水和 0.1％甲酸 / 乙腈组成。将粗产物混合物以约 100mg/mL 的浓度溶于 DMF、DMSO 或其混合物中，并按表 2 所述方法进行色谱分析。然后使用 Savant Speed Vac Plus SC210A 型，将合适的色谱流分在 45℃下高真空蒸发，得到纯化的产物。

[0839] 表 2 :制备型 HPLC 方法描述

[0840]

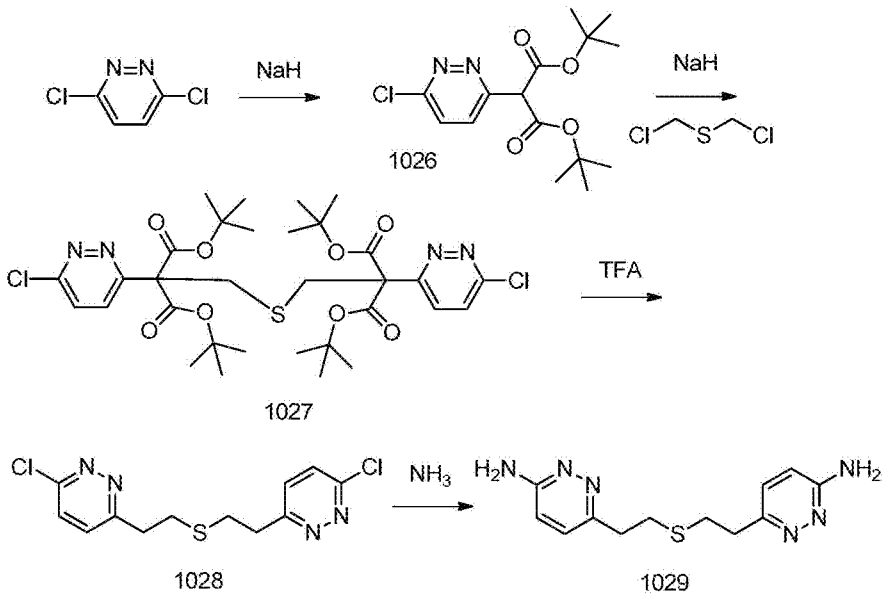
化合物 ID	柱	时间(分钟)	%MPB	流速(mL/分钟)	产物保留时间(分钟)
7	1	0	20	2	7.4
		1	20	2	
		2	20	5	
		3	70	5	
		14	100	5	
8	1	0	20	2	11.5
		1	20	2	
		2	20	5	
		3	70	5	
		14	100	5	
26	1	0	40	1	6
		1	40	2	
		3.5	40	4	
		4	40	4.73	
		10	90	4.73	
29	2	0	40	2	7.7
		1	40	3	
		2	40	18.9	
		13	50	18.9	
36	2	0	32	3	12.1
		0.5	32	5	
		1	32	18.9	
		13	35	18.9	
143	2	0	50	3	9.1
		1	50	3	
		2	50	18.9	
		5	50	18.9	
		15	80	18.9	
153	2	0	35	3	6.2
		1	35	3	
		2	35	18.9	

[0841]

		4	35	18.9	
		14	75	18.9	
199	2	0	45	3	8.3
		1	45	3	
		2	45	18.9	
		3	45	18.9	
		13	65	18.9	
203	2	0	50	3	9.6
		1	50	3	
		2	50	18.9	
		5	50	18.9	
		15	60	18.9	
208	2	0	35	3	7.6
		1	35	3	
		2	35	18.9	
		4	35	18.9	
		14	50	18.9	

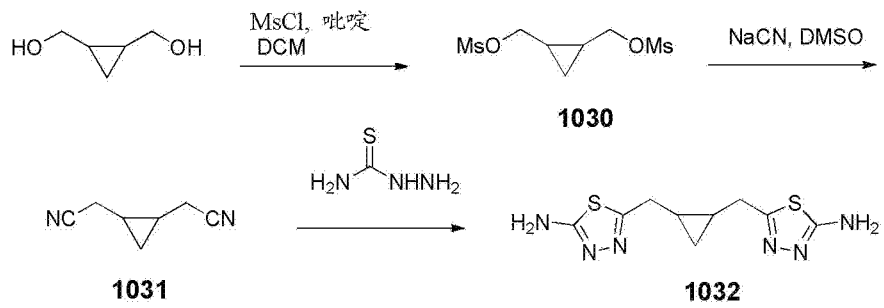
[0842] 下面的代表性合成方案也可用于产生本发明的化合物。

[0843]



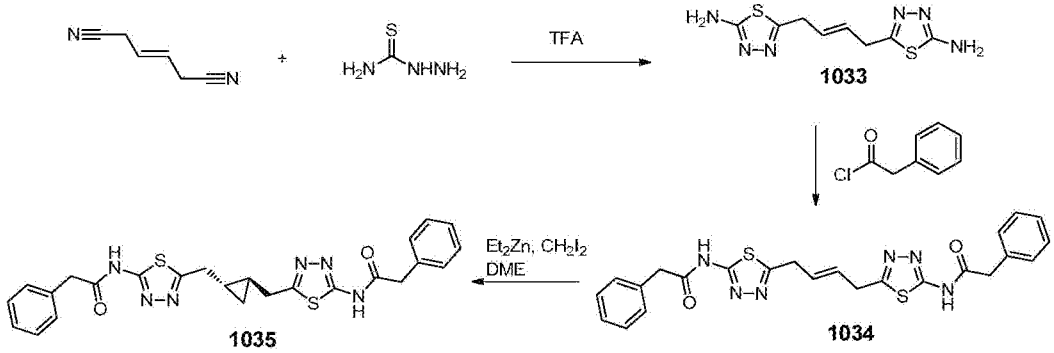
[0844] 3,6-二氯哒嗪用丙二酸二叔丁酯和含氢化钠的 THF 或 DMF 处理,得到 1026。然后将中间体 1026 用含氢化钠的 THF 或 DMF 处理,接着用双-(氯甲基)硫化物处理,得到 1027。将中间体 1027 用含 TFA 的二氯甲烷处理,得到 1028。将中间体 1028 用氨处理,得到 1029。通过依次用 2,4-二甲氧基苄胺和 TFA 处理,另使中间体 1028 转化成 1029。可采用上述用于 1001-1008 酰化的合成方案部分中描述的方法,使双-氨基中间体 1029 转化成类似于表 3 中所述的那些的酰化产物。

[0845]



[0846] 通过双-甲磺酸酯化中间体1030，使反式-和顺式-环丙烷-1,2-二基二甲醇两者转化成相应的双-腈1031。在含吡啶或三乙胺的二氢甲烷存在下，通过将二醇用甲磺酰氯处理来制备双甲磺酸酯中间体1030。在DMSO或乙醇/水中，通过将1030用氰化钠处理来制备双腈1031。采用类似于制备1001所述的方法，使双-腈1031在TFA中与氨基硫脲进行环化，得到双-氨基中间体1032。可采用上述用于1001-1008酰化的合成方案部分所述的方法，将双-氨基中间体1032转化成类似于表3中所述的那些的酰化产物。

[0847]



[0848] 烯烃类似物1033采用类似于用于制备1001所述的方法，由反式-3-己烯二腈制备。可采用上述用于1001-1008酰化的合成方案部分所述方法，将双-氨基中间体1033转化成类似于表3所述的那些的酰化产物（例如1034）。在Simmons-Smith条件下（Et_2Zn，CH_2I_2，1,2-二甲氧基乙烷），产物可进一步转化成环丙基类似物（以1035为例）。

[0849] 实施例2：化合物测定法

[0850] 在下列体外生物化学测定法和细胞增殖测定法中测定化合物。表3提供了IC50结果。

[0851] 重组酶测定法

[0852] 采用使谷氨酸（通过GAC释放）的产生与谷氨酸脱氢酶(GDH)关联并针对NAD^+还原成NADH测量吸光度的变化的生物化学测定法，评价化合物抑制重组形式的谷氨酰胺酶1(GAC)的酶活性的能力。制备底物溶液(50mM Tris-HCl pH 8.0、0.2mM EDTA、150mM K_2HPO_4、0.1mg/ml BSA、1mM DTT、20mM L-谷氨酰胺、2mM NAD^+和10ppm防沫剂)，将50μL加入96孔半面积透明板(half area clear plate)(Corning#3695)上。加入化合物(2μL)，得到在2X所需的化合物浓度下最终DMSO浓度为2%。酶促反应始于加入50μL酶溶液(50mM Tris-HCl pH 8.0、0.2mM EDTA、150mM K_2HPO_4、0.1mg/ml BSA、1mM DTT、10ppm防沫剂、4单位/ml GDH、4mM腺苷二磷酸和4nM GAC)，在Molecular Devices M5读板仪中在20℃下读数。配置读板仪以动力学模式读取吸光度（λ = 340nm)15分钟。数据以毫-吸光度单

位 / 分钟记录，与相同板上的对照化合物和仅 DMSO 对照比较斜率。斜率小于 DMSO 对照的化合物被视为抑制剂，使用对照化合物评价板变化性。

[0853] 对本发明几种化合物的该测定法的结果见表 3a 和 3b，表示为 IC50，或半最大抑制浓度，其中 IC50 是表明抑制指定生物学活性达一半需要多少化合物的定量度量。

[0854] 重组酶测定法 - 时间依赖性

[0855] 采用使谷氨酸（通过 GAC 释放）的产生与谷氨酸脱氢酶 (GDH) 关联并针对 NAD^+ 还原成 NADH 测量吸光度的变化的生物化学测定法，评价化合物抑制重组形式的谷氨酰胺酶 1(GAC) 的酶活性的能力。制备酶溶液 (50mM Tris-HCl pH 8.0、0.2mM EDTA、150mM K_2HPO_4、0.1mg/ml BSA、1mM DTT、10ppm 防沫剂、4 单位 /ml GDH、4mM 腺苷二磷酸和 4nM GAC)，将 50 μL 加入 96 孔半面积透明板 (Corning#3695) 上。加入化合物 (2 μL)，得到在 2X 所需的化合物浓度下最终 DMSO 浓度为 2%。酶 / 化合物混合物用封口箔纸 (USA Scientific) 密封，在适度搅拌下在 20℃下温育 60 分钟。酶促反应始于加入 50 μL 底物溶液 (50mM Tris-HCl pH 8.0、0.2mM EDTA、150mM K_2HPO_4、0.1mg/ml BSA、1mM DTT、20mM L- 谷氨酰胺、2mM NAD^+ 和 10ppm 防沫剂)，在 Molecular Devices M5 读板仪中在 20℃下读数。配置读板仪以动力学模式读取吸光度 (λ = 340nm) 15 分钟。数据以毫 - 吸光度单位 / 分钟记录，与相同板上的对照化合物和仅 DMSO 对照比较斜率。斜率小于 DMSO 对照的化合物被视为抑制剂，使用对照化合物评价板变化性。

[0856] 对本发明几种化合物的该测定法的结果见表 3a 和 3b，表示为 IC50，或半最大抑制浓度，其中 IC50 是表明抑制指定生物学活性达一半需要多少化合物的定量度量。

[0857] 细胞增殖测定法

[0858] 在 37℃与 5% CO_2 下，将 P493-6(myc “on”) 细胞在生长培养基 (RPMI-1640、10% FBS、2mM 谷氨酰胺、100 单位 /ml 青霉素和 100 μg/ml 链霉素）中维持。对于化合物测定，在化合物加入 50 μl 生长培养基中的当天，以 200,000 个细胞 /ml(10,000 个细胞 / 孔）的细胞密度将 P493-6 细胞铺板于 96 孔 V 底板中。按 200 倍终浓度将化合物在 100% DMSO 中系列稀释。将化合物 100 倍稀释入生长培养基中，然后将 50 μl 该混合物加入细胞板中，使得 DMSO 终浓度为 0.5%。将细胞与化合物一起在 37℃与 5% CO_2 下孵育 72 小时，使用 Guava 仪器的 Viacount(Millipore) 试剂盒，通过 Cell Titer Glo(Promega) 或 FACS 分析，分析抗增殖作用。

[0859] 对本发明几种化合物的该测定法的结果见表 3a 和 3b，表示为 IC50，或半最大抑制浓度，其中 IC50 是表明抑制指定生物学活性达一半需要多少化合物的定量度量。

[0860] 改进的重组酶测定法 - 时间依赖性

[0861] 采用使 Glu(通过谷氨酰胺酶释放) 的产生与 GDH 关联并测量由于 NADP+ 还原成 NADPH 引起的荧光增加的生物化学测定法，评价化合物抑制重组形式的谷氨酰胺酶的酶活性的能力。

[0862] 测定设置 :制备谷氨酰胺酶反应缓冲液 [50mM Tris-HCl pH 8.8、150mM K2HPO4、0.25mM EDTA、0.1mg/ml BSA(Calbiochem 号 2960)、1mM DTT、2mM NADP+(Sigma Aldrich 号 N5755) 和 0.01% TX-100]，并用来制备含 3x 酶的溶液、含 3x 底物的溶液和含 3x 抑制剂的溶液 (见下)。含抑制剂的溶液如下制备 :通过将化合物的 DMSO 原液在谷氨酰胺酶反应缓冲液中稀释以产生含有 6% DMSO 的 3x 抑制剂溶液。含 3x 酶的溶液如下制备 :通过将重组

谷氨酰胺酶和来自变形杆菌属 (Proteus) 菌种 (Sigma Aldrich 号 G4387) 的 GDH 在谷氨酰胺酶缓冲液中稀释以产生 6nM 谷氨酰胺酶加 18 单位 /mL GDH 溶液。如下制备含有 Gln、Glu 或 NADPH 的 3x 底物溶液：通过将 Gln(Sigma Aldrich 号 49419)、Glu(Sigma Aldrich 号 49449) 或 NADPH(Sigma Aldrich 号 N1630) 的原液在谷氨酰胺酶反应缓冲液中稀释以产生 3x- 底物溶液。在不需要预孵育时，通过将 5μL 含抑制剂的溶液与 5μL 含底物的溶液接着与 5μL 含酶的溶液混合，将反应物装入 384 孔小容量黑色微量滴定板 (Molecular Devices 号 0200-5202) 中。当测试化合物抑制的时间依赖性效应时，在加入含底物的溶液之前，将含酶的溶液用含抑制剂的溶液处理达规定时间。

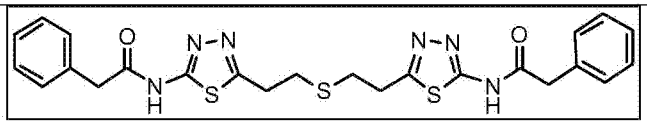
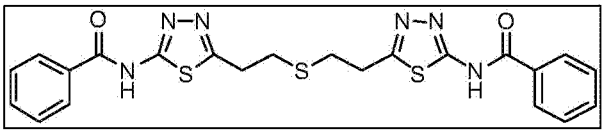
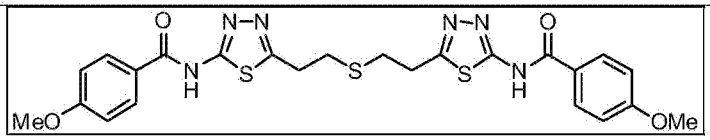
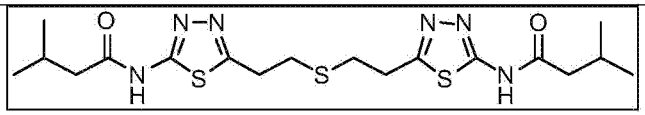
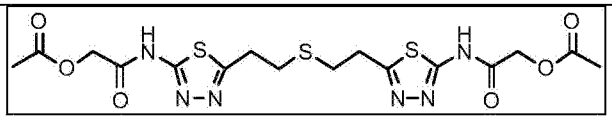
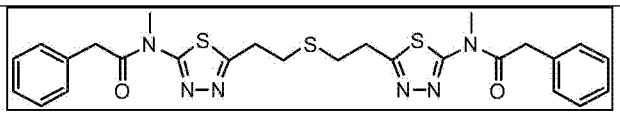
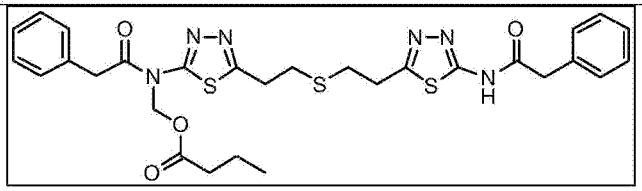
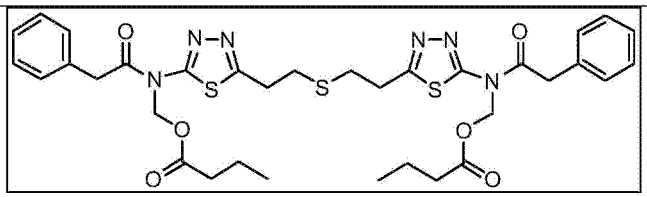
[0863] 谷氨酰胺酶活性的测量：在全部 3 种组分混合后，采用 Spectromax M5e(Molecular Devices)，记录在室温下的荧光增加 (激发 :340nM，发射 :460nm)15 分钟。

[0864] IC50 测定 :应用直线方程式 (Y = Y 截距 +(斜率)*X)，计算各进展曲线的初速度。使初速度值对化合物浓度作图，并拟合到 4 参数剂量反应方程式 (%活性 = 底部 +(顶部 - 底部)/(1+10^((LogIC50-X)*Hill 斜率))) 以计算 IC50 值。

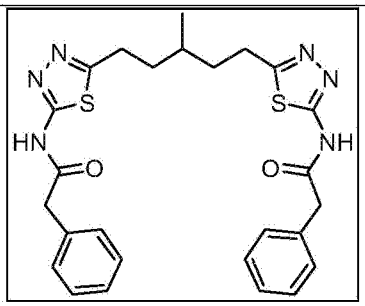
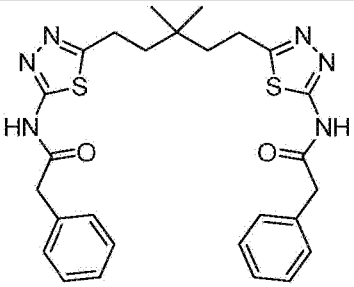
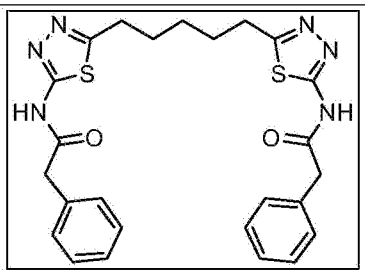
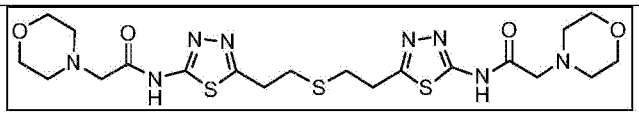
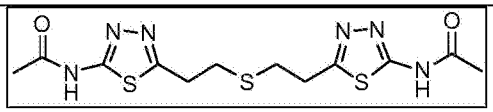
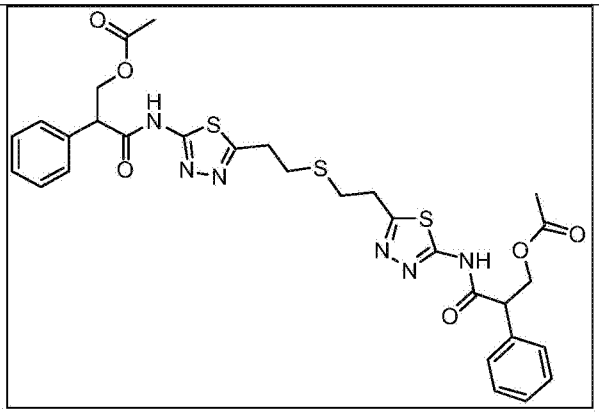
[0865] 该测定法对几种化物的结果见表 3a 和 3b，表示为 IC50，或半最大抑制浓度，其中 IC50 是表明抑制指定生物学活性达一半需要多少化合物的定量度量。

[0866] 表 3a：

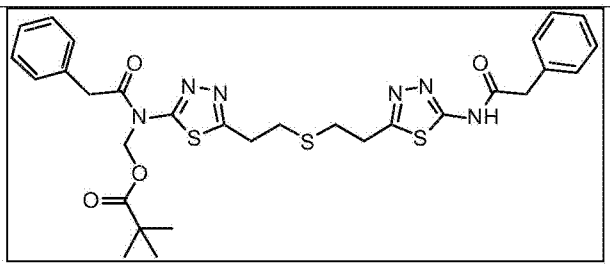
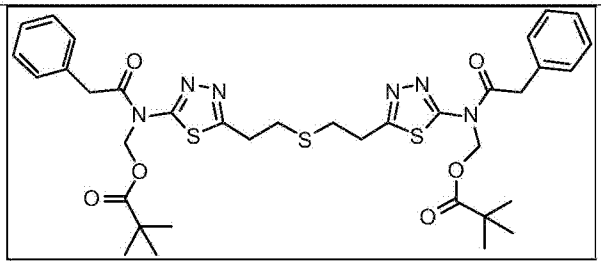
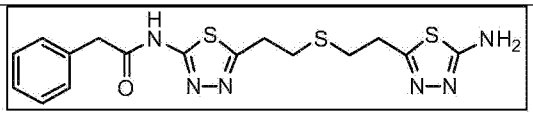
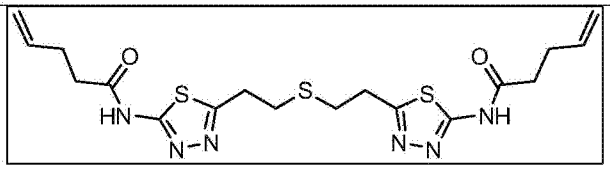
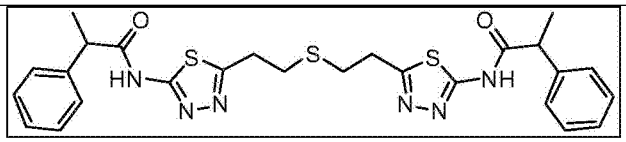
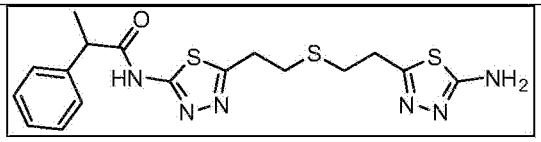
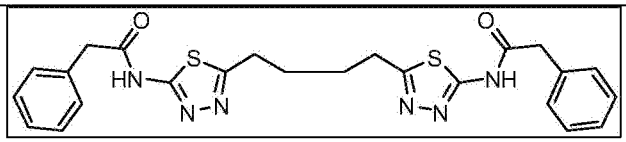
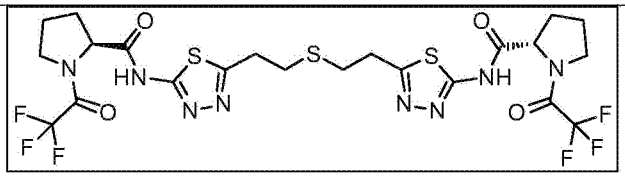
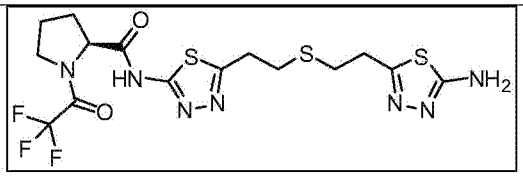
[0867]

化合物 ID	结构	GAC Δ N2 IC50 60 分钟预孵育 (μM)	GAC Δ N2 IC50 无预孵育 (μM)	细胞增殖 P493 72h IC50 (μM)
1		0.10	0.20	0.47
2			4.1	0.63
3			>50	>50
4			13	>50
5			>50	>50
6			>50	2.7
7			>50	1.0
8			>50	1.6

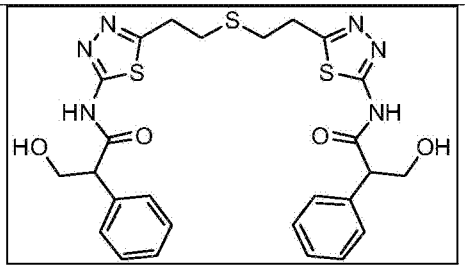
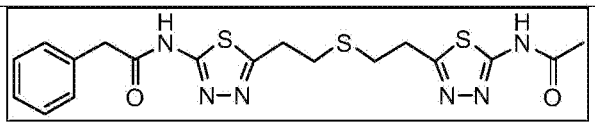
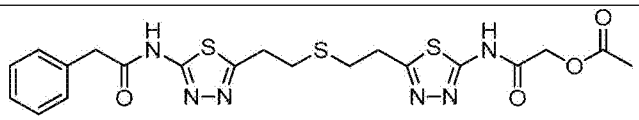
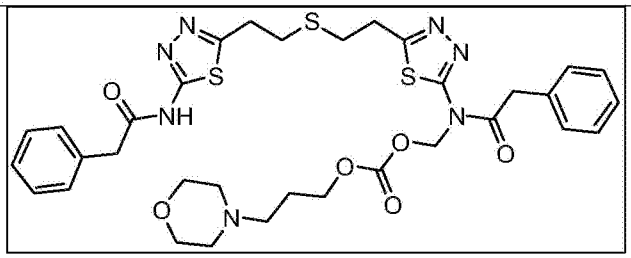
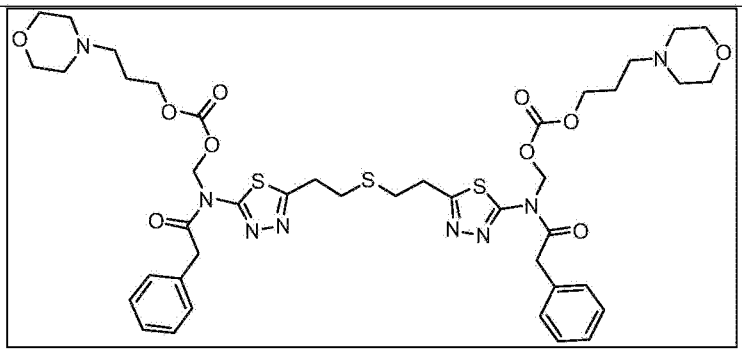
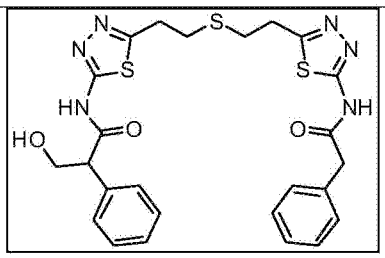
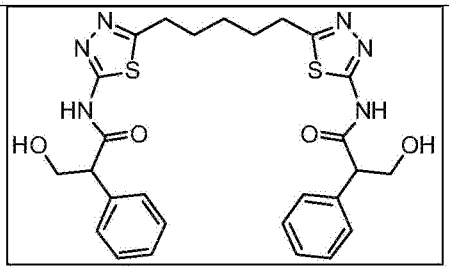
[0868]

9			>50	>50
10			>50	>50
11			1.4	0.89
12			>50	36
13			7.7	12
14			2.8	1.8

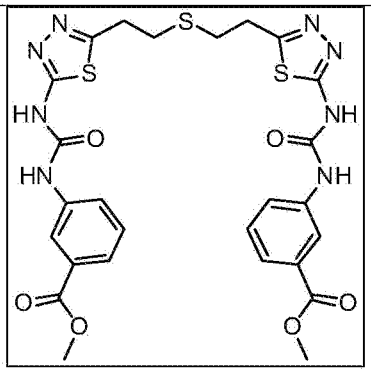
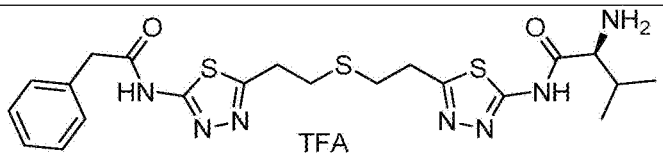
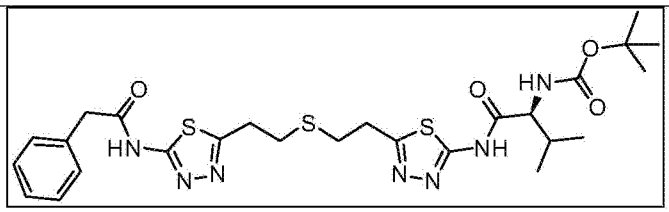
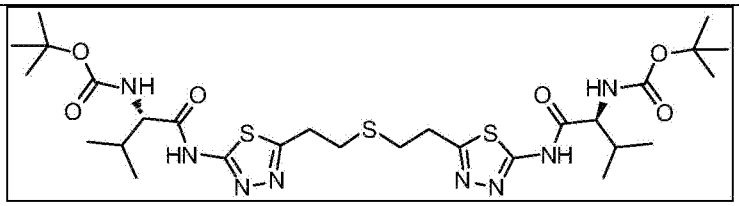
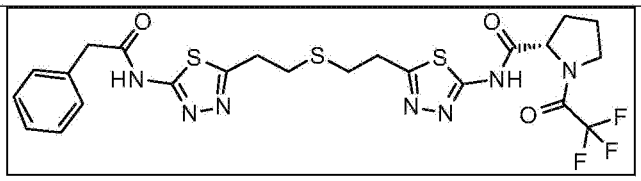
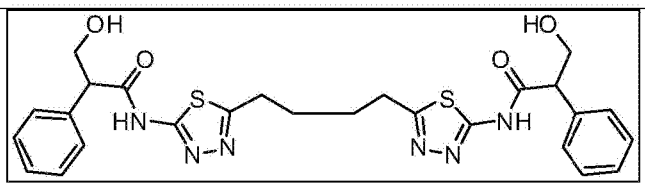
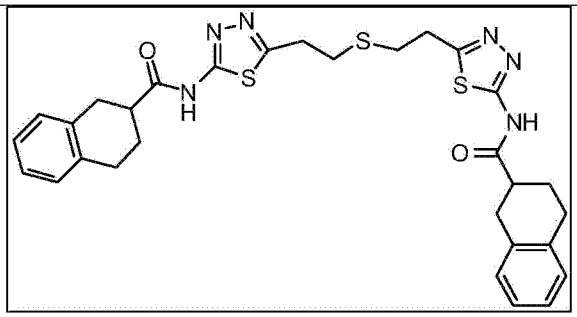
[0869]

15			>50	1.2
16			>50	0.80
17			15	4.2
18			4.5	8.2
19			11	1.7
20			6.6	2.6
21			0.16	0.02
22			>50	>50
23			>50	>50

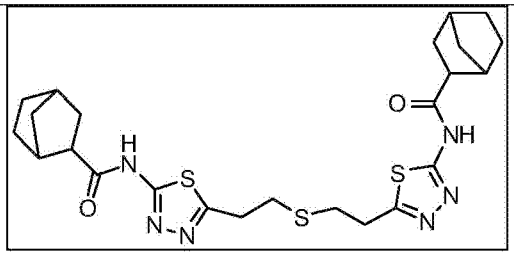
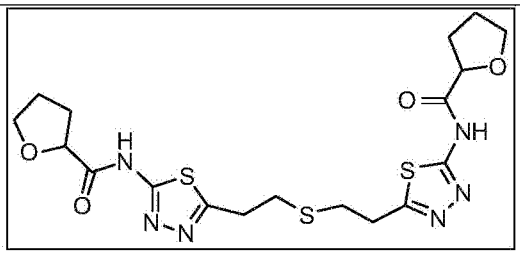
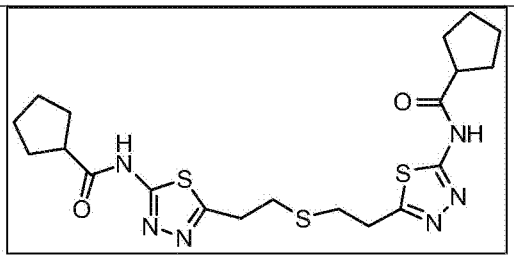
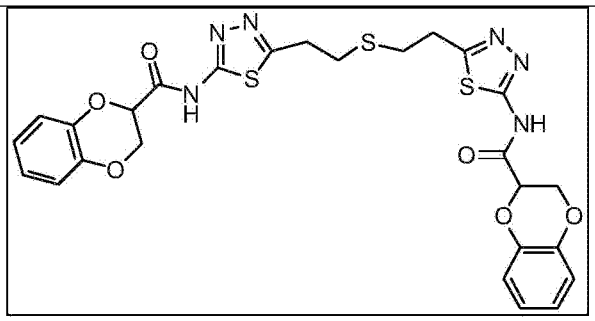
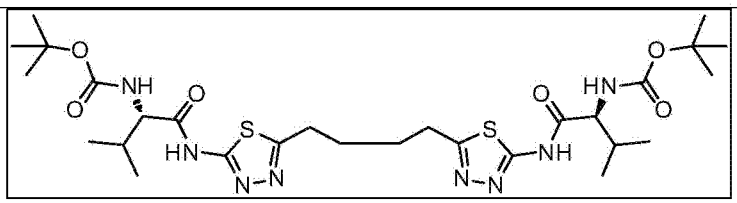
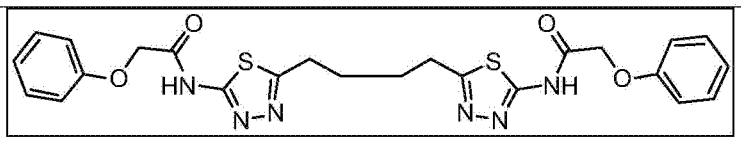
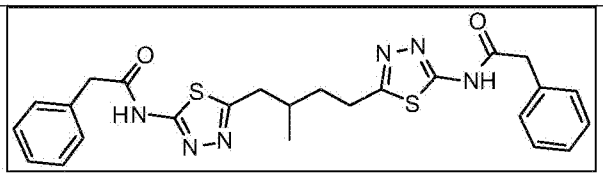
[0870]

24			0.51	2.3
25			1.2	1.5
26			5.6	0.70
27			>50	0.47
28			>50	1.0
29			0.56	4.1
30			1.2	2.5

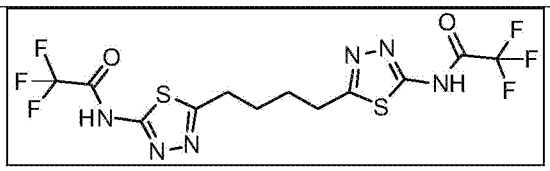
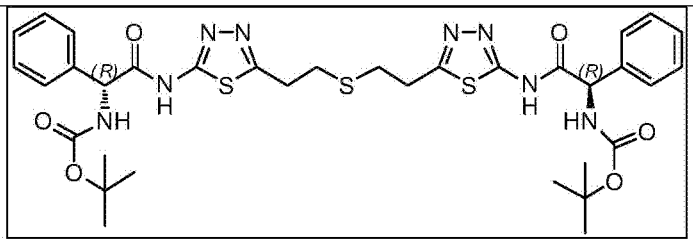
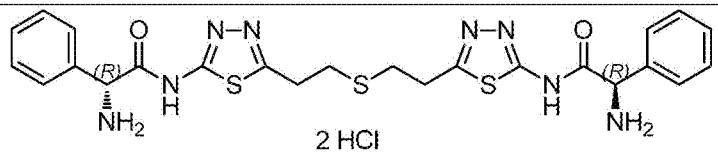
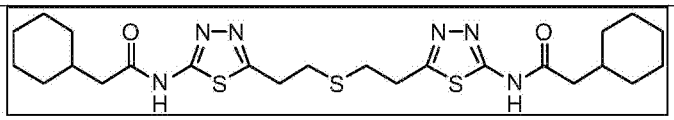
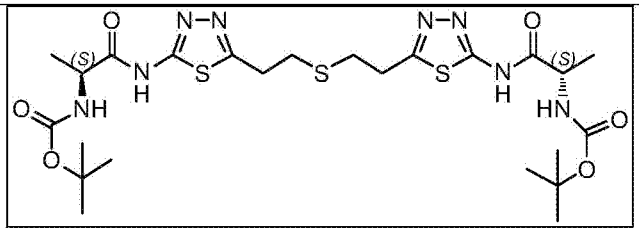
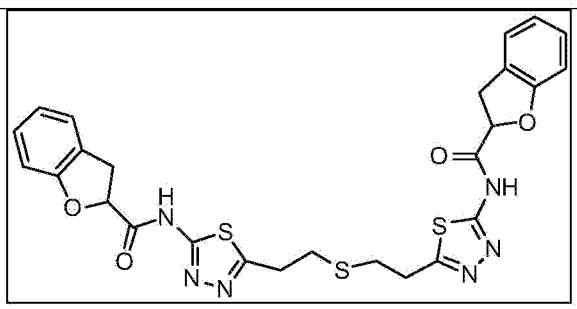
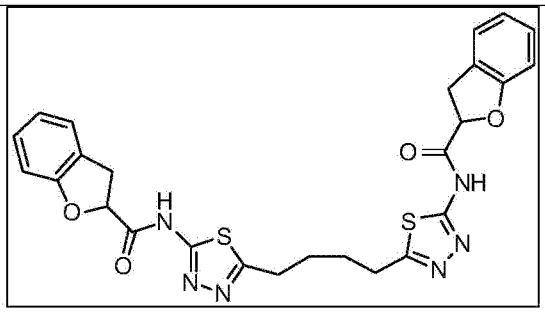
[0871]

31			>50	4.3
32			7.0	11
33			13	5.3
34			>50	>50
35			18	3.8
36		0.04	0.22	0.16
37			>50	>50

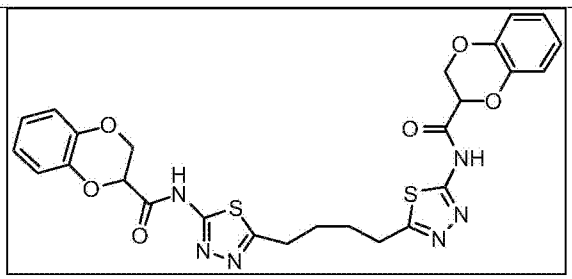
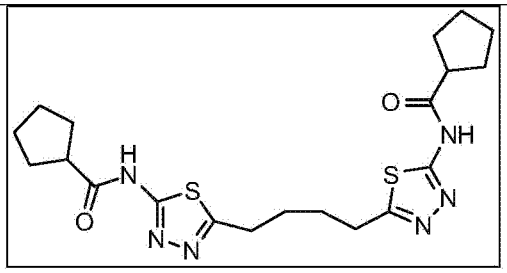
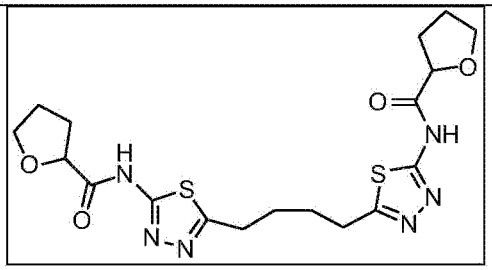
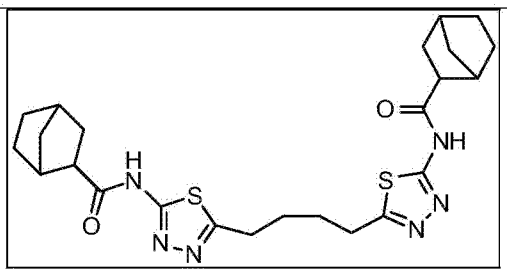
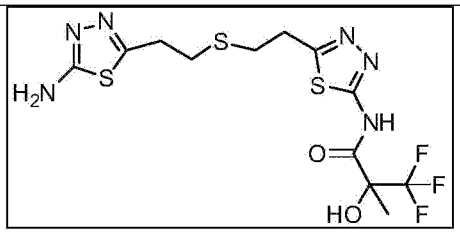
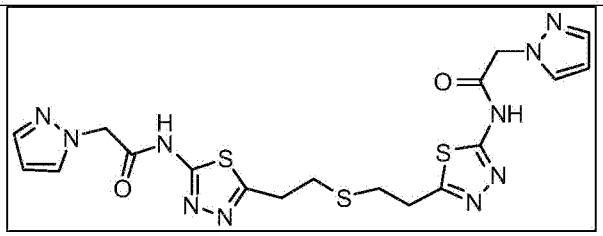
[0872]

38			>50	3.2
39			26	4.5
40			3.7	0.56
41			7.9	33
42			>50	>50
43			2.3	>50
44			4.9	2.6

[0873]

45			>50	>50
46			>50	16
47		2 HCl	8.3	35
48			>50	0.42
49			36	17
50			2.5	8.2
51			1.2	1.3

[0874]

52			8.3	30
53			>50	34
54			9.2	1.6
55			>50	3.9
56			>50	
57			40	

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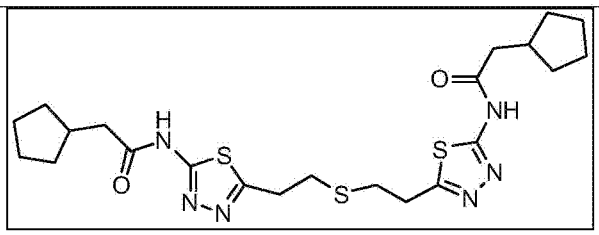
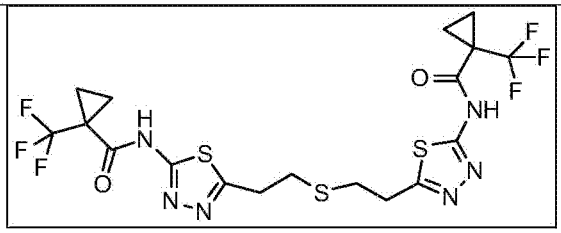
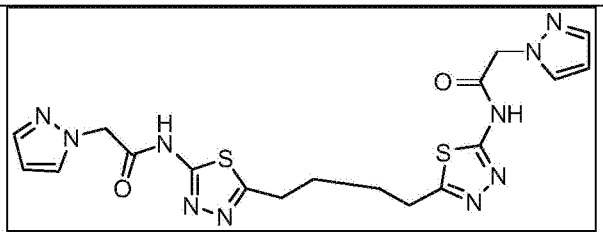
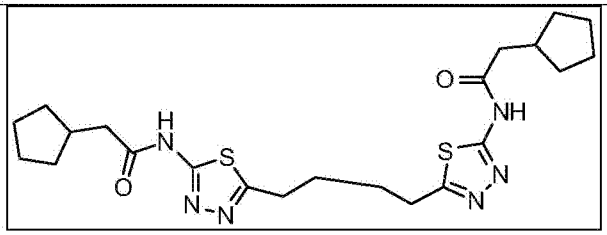
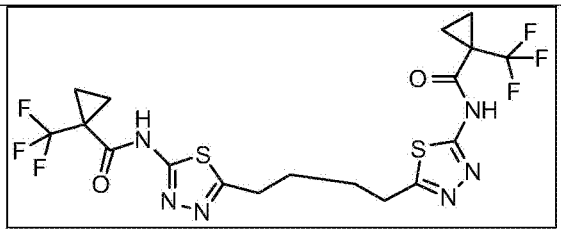
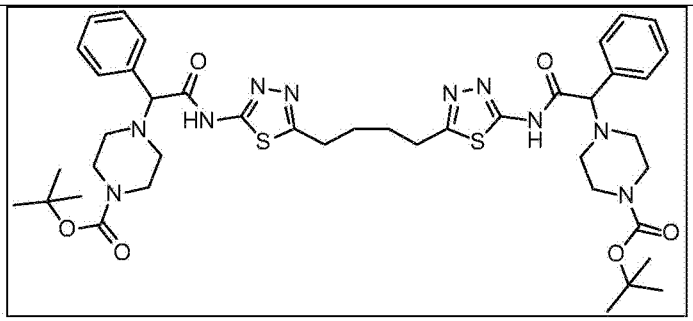
58			>50	3.7
59			>50	
60			24	14
61			>50	
62			>50	19
63			25	2.6

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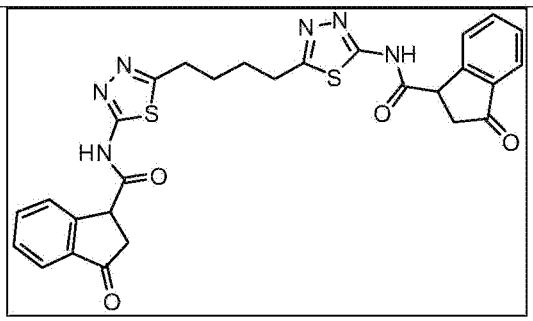
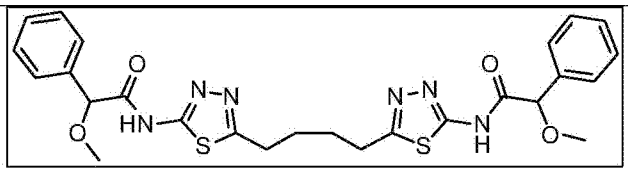
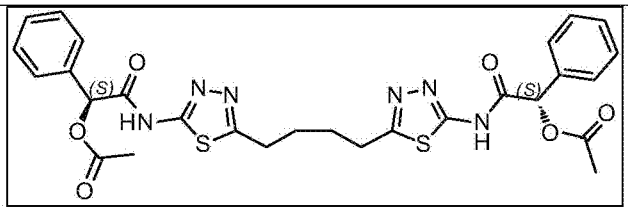
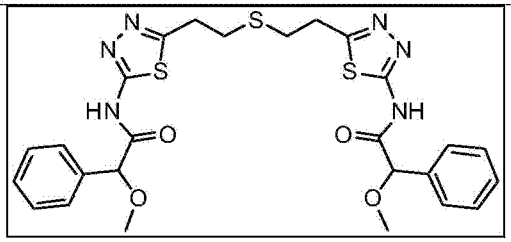
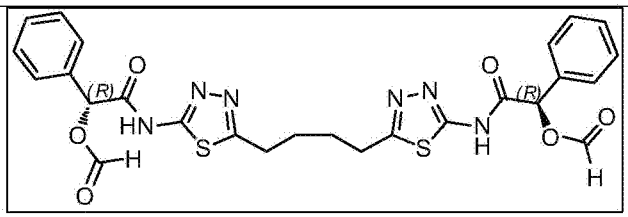
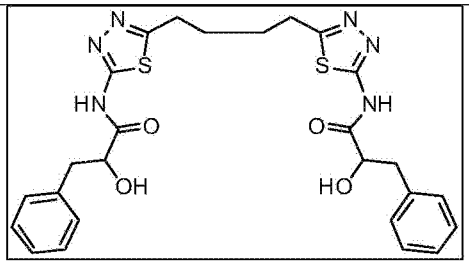
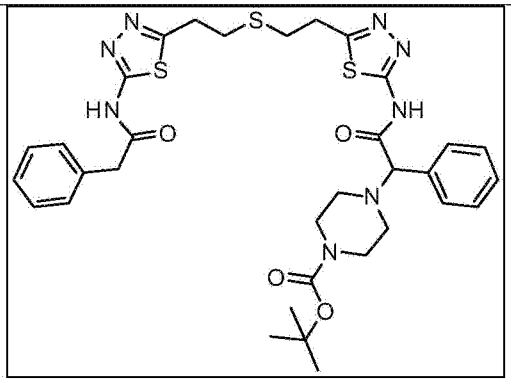
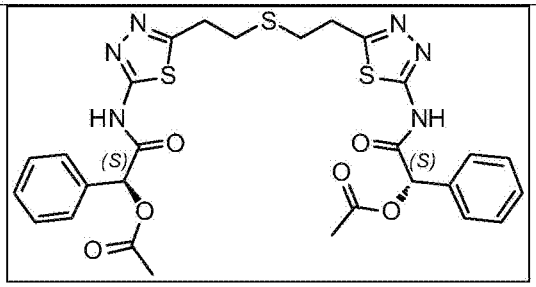
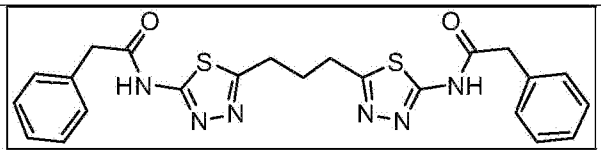
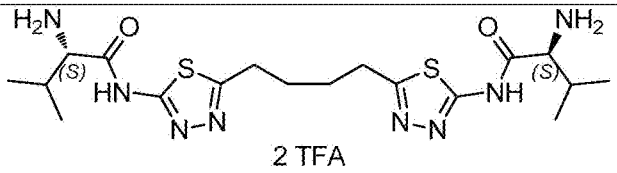
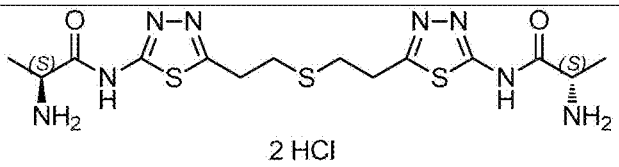
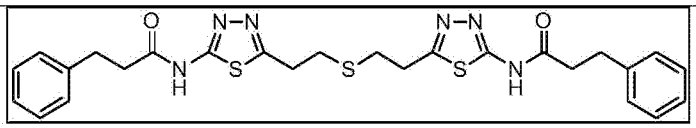
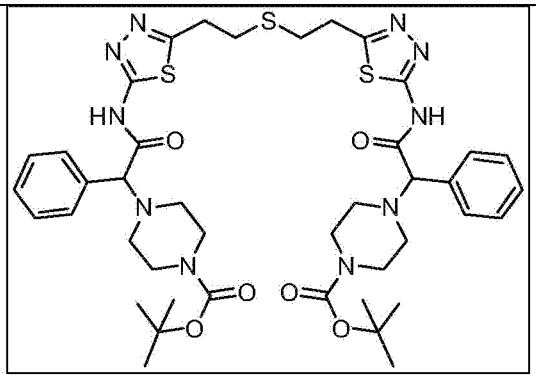
64			1.3	0.23
65			1.3	0.52
66			20	
67			3.0	1.8
68			4.9	0.34
69			0.69	0.33

[0877]

70			3.4	3.4
71			>50	6.9
72			0.59	0.47
73	 2 TFA		>50	
74	 2 HCl		>50	
75			>50	
76			>50	

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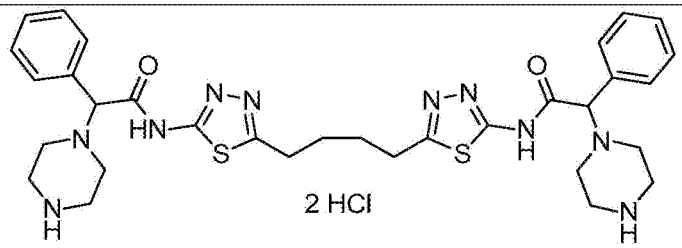
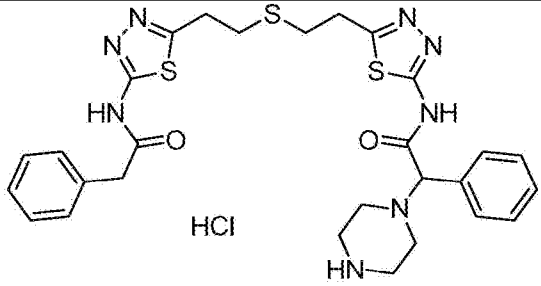
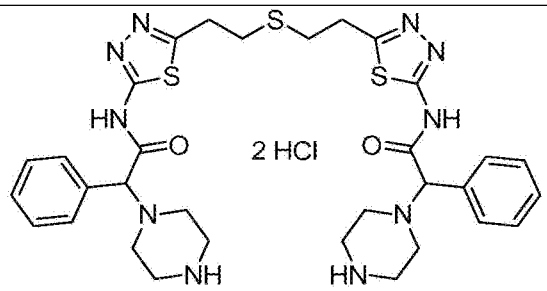
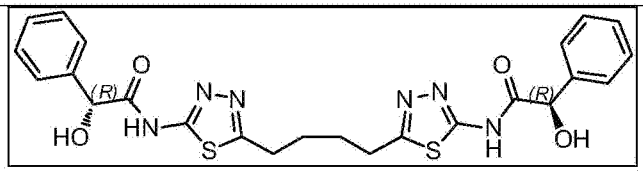
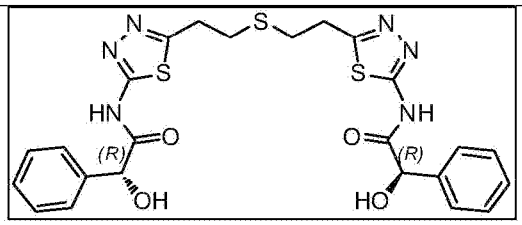
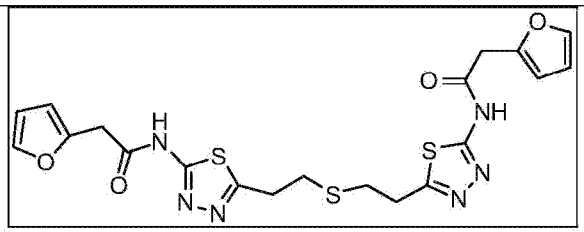
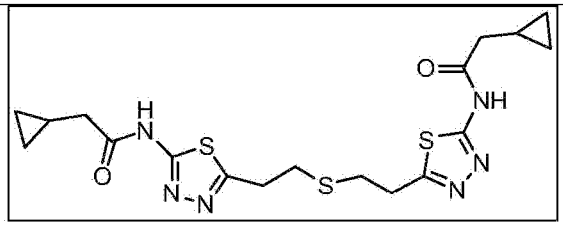
77			6.1	34
78			0.84	10
79			2.0	20
80			1.8	1.3
81			10	7.6
82			0.80	1.3
83			3.9	1.4

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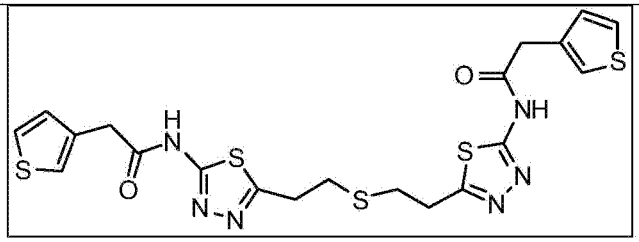
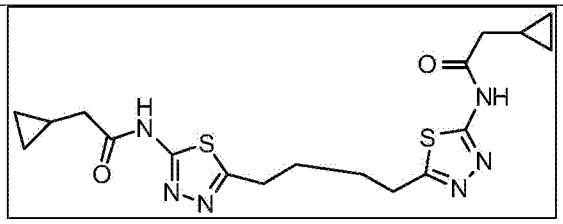
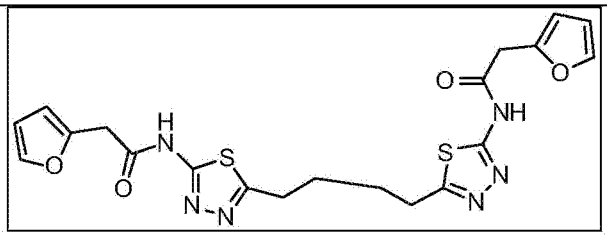
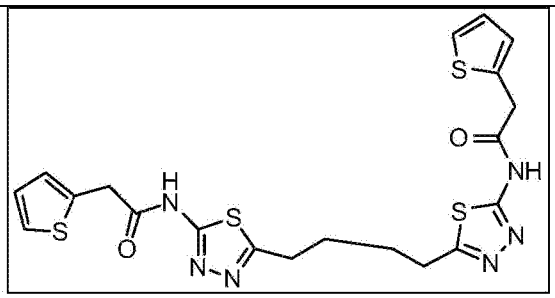
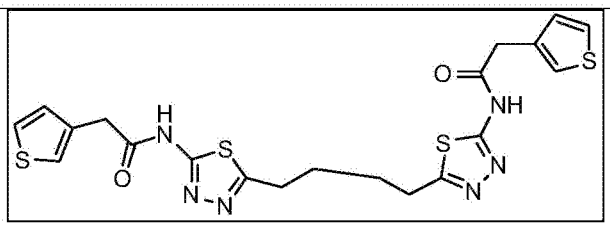
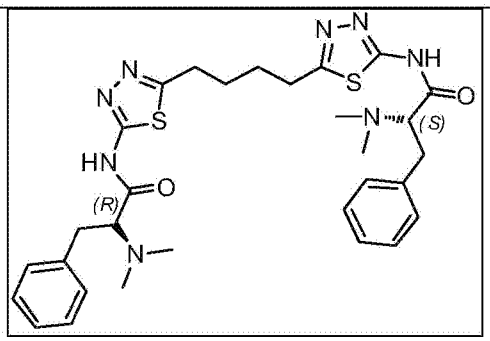
84			0.23	0.89
85			1.5	1.8
86			0.32	0.52
87			0.18	0.06
88			0.20	0.12
89			>20	

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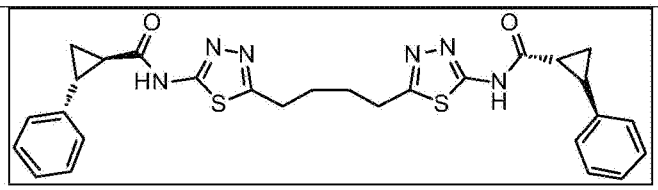
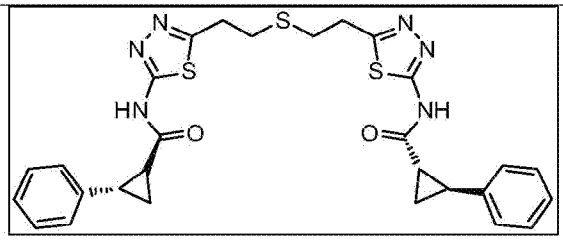
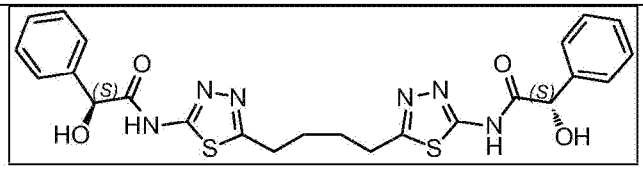
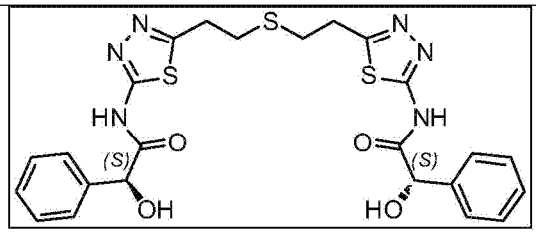
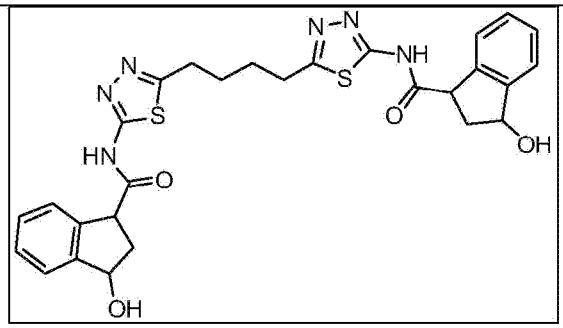
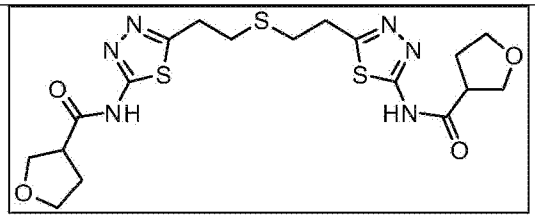
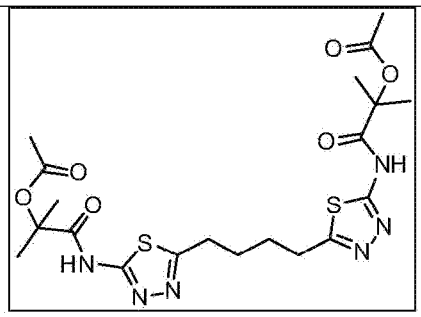
90			>20	
91			>20	
92		0.14	0.38	0.47
93			0.90	2.0
94			0.28	0.47
95			2.9	45
96			>20	

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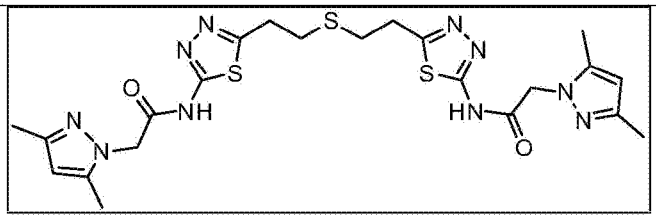
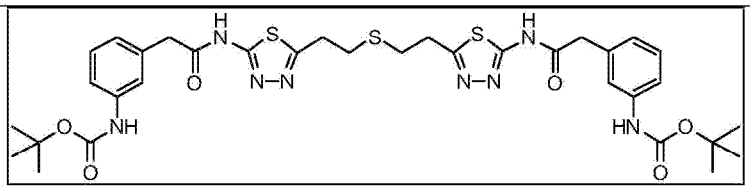
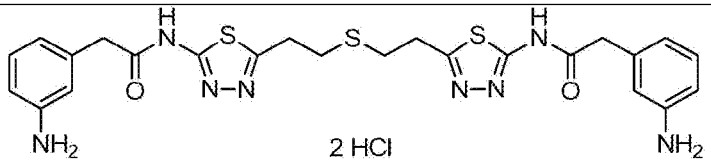
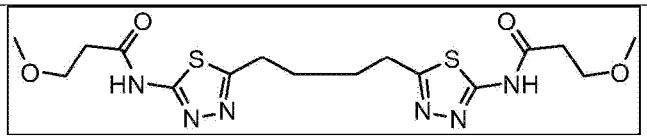
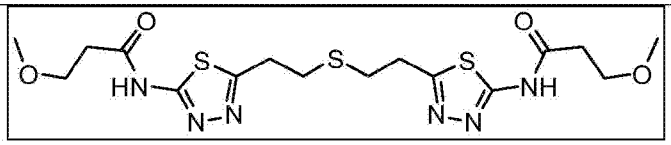
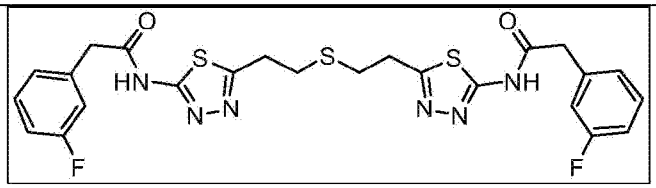
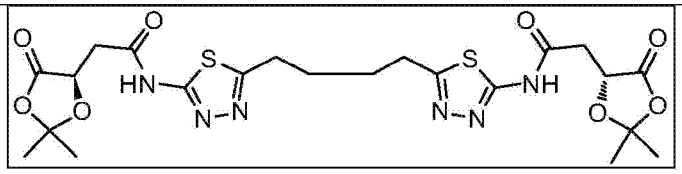
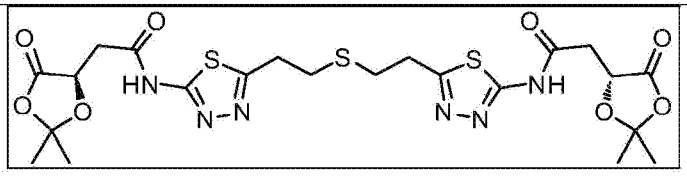
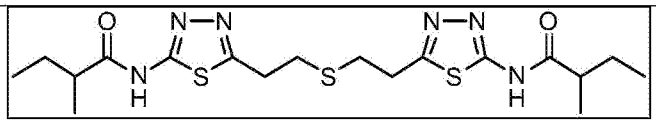
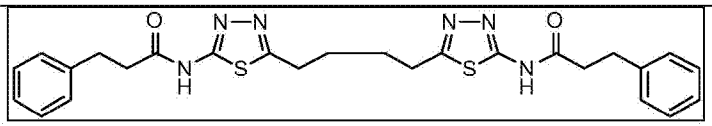
97			0.56	17
98			>20	3.9
99			2.7	1.0
100			8.1	9.0
101			24	17
102			0.24	1.4
103			19	>50
104			>20	
105			9.9	119
106			>20	

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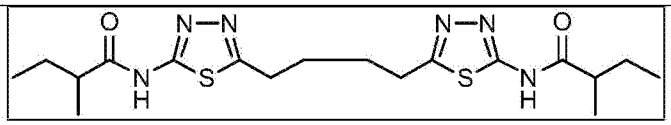
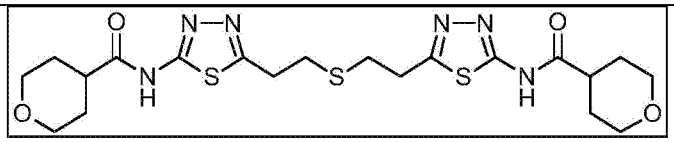
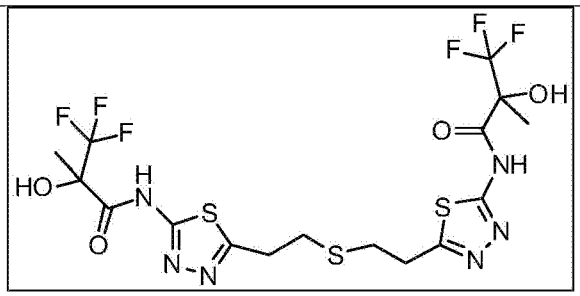
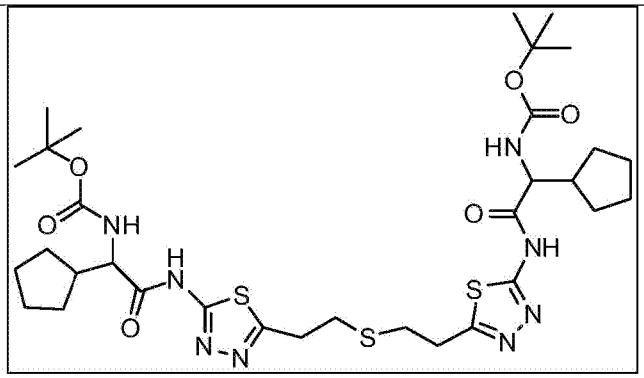
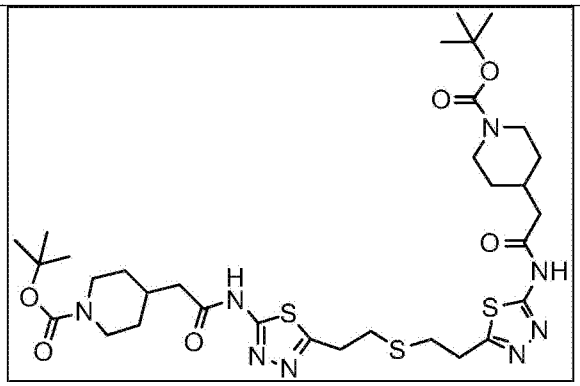
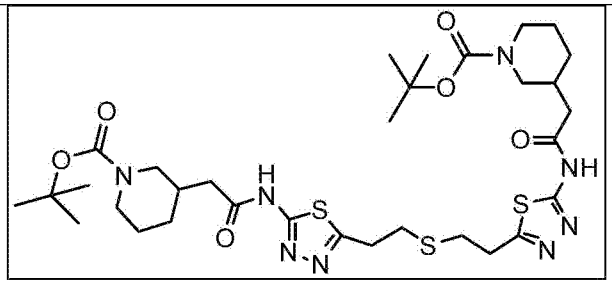
107			4.3	1.2
108			>20	
109			>20	
110			>20	
111			0.95	0.88
112			0.51	0.89

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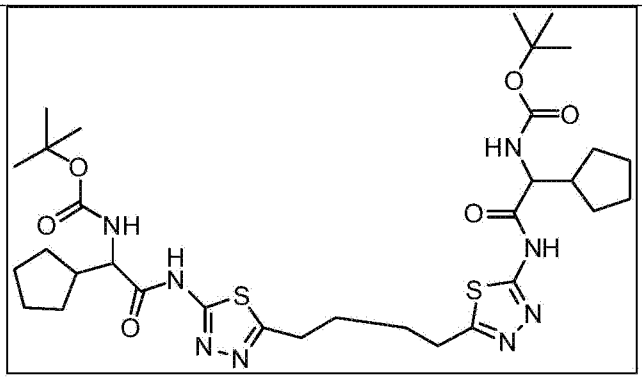
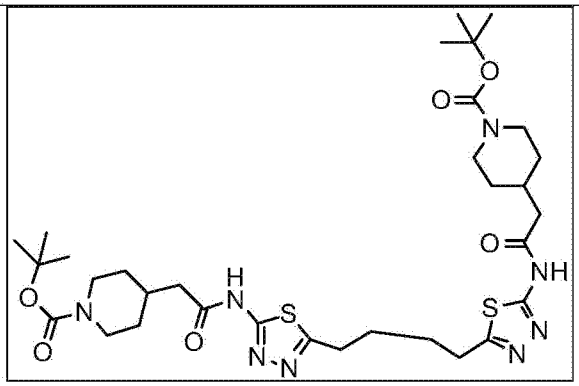
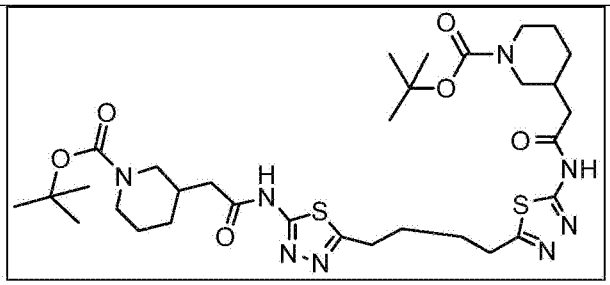
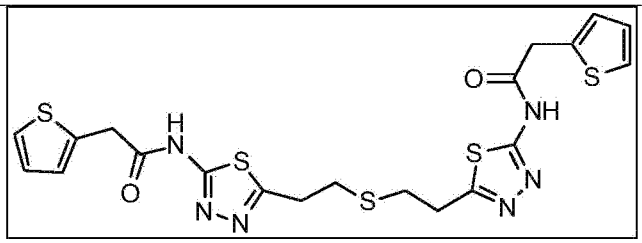
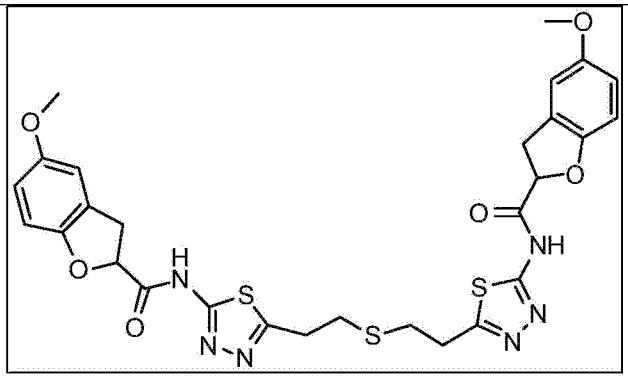
113			>20	
114			0.60	0.56
115			0.62	1.1
116			0.24	0.72
117			2.4	6.2

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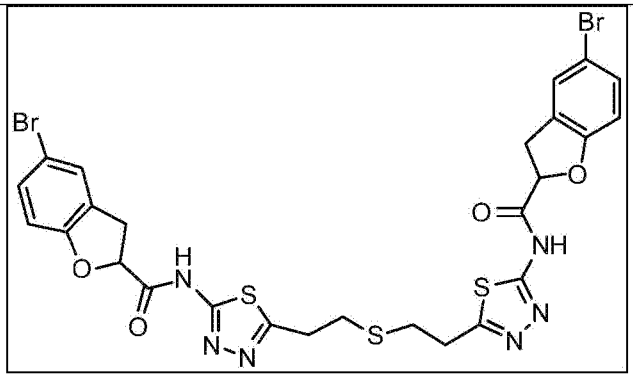
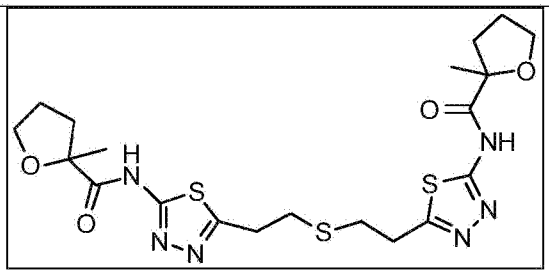
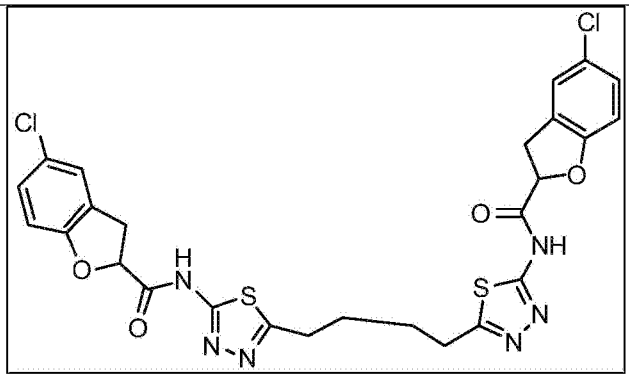
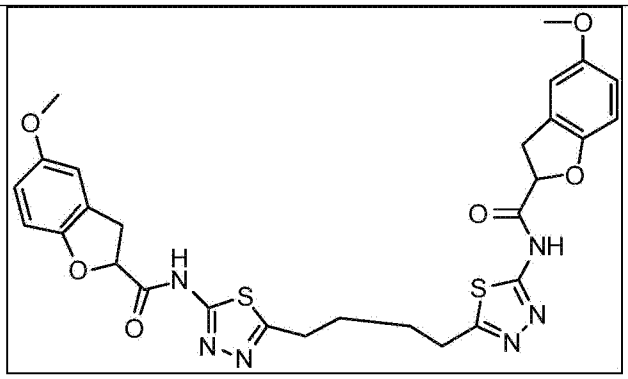
118			5.0	36
119			>20	13
120			1.8	38
121			1.7	3.5

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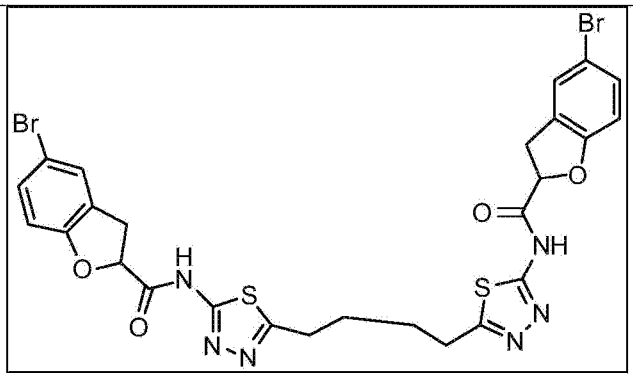
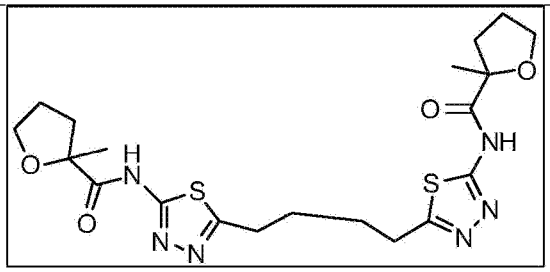
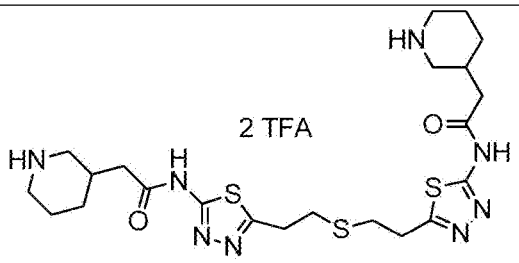
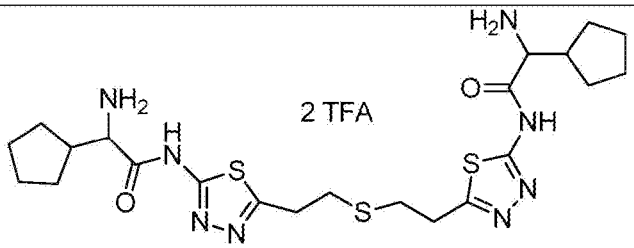
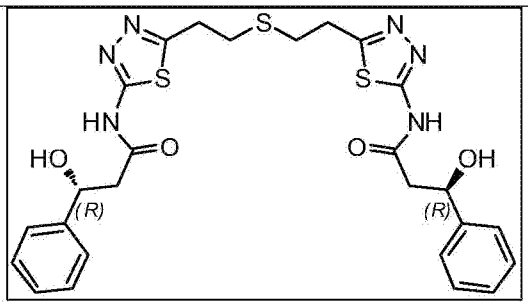
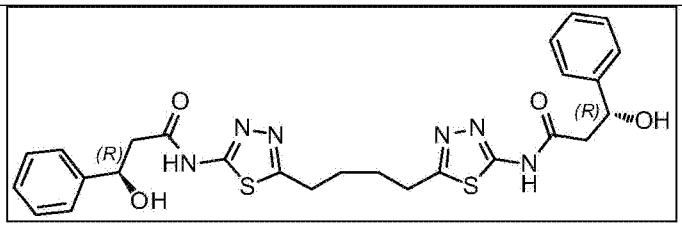
122			3.5	43
123			12	6.6
124			>20	
125			>20	
126			5.8	12
127			1.8	0.45

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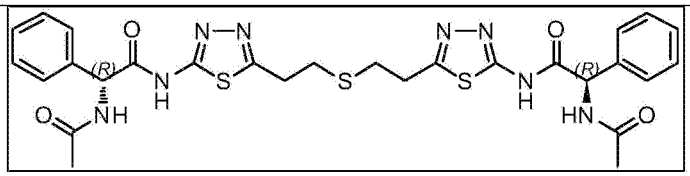
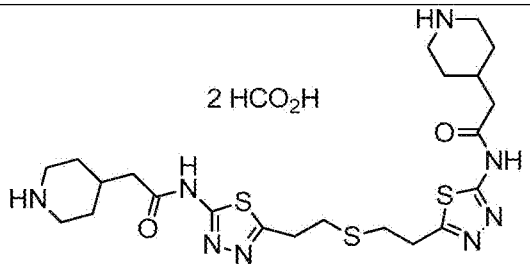
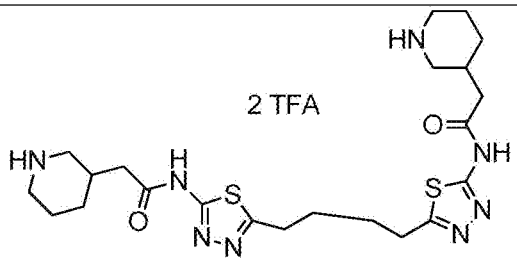
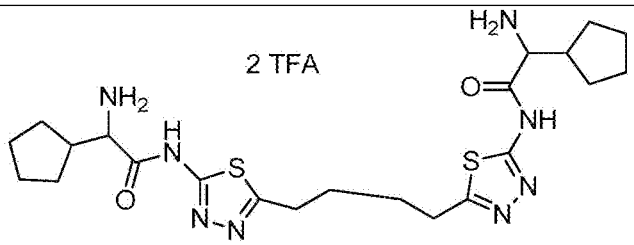
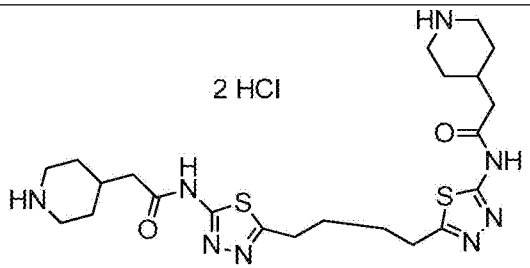
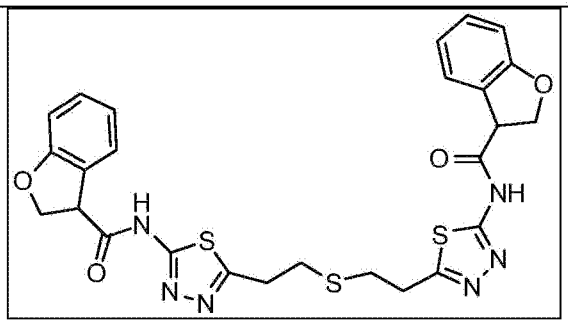
128			32	>50
129			>20	>50
130			>20	
131			19	
132			>20	
133			0.51	0.15

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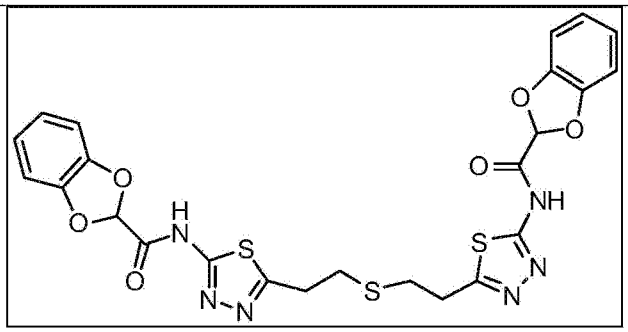
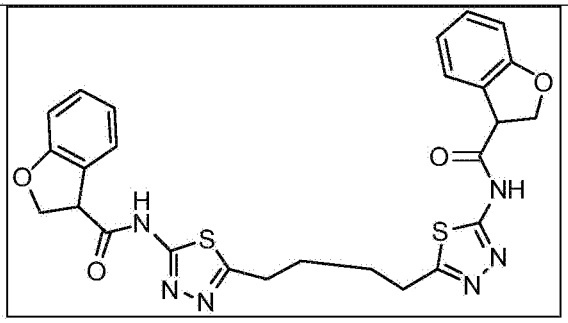
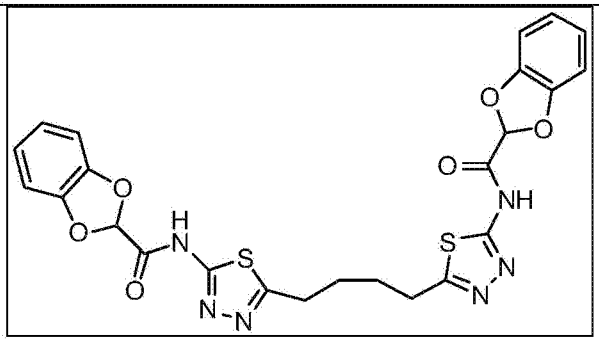
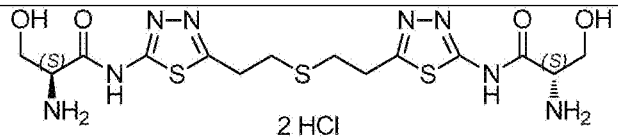
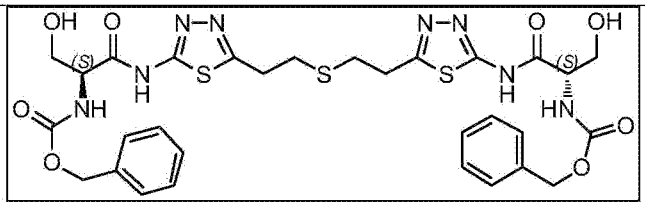
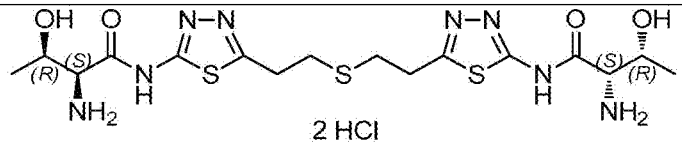
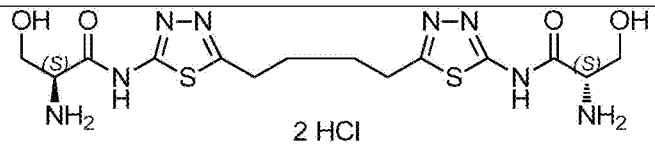
134			14	28
135			0.30	0.49
136			7.0	4.7
137			>20	
138			0.75	2.7
139			>20	
140			3.4	>50

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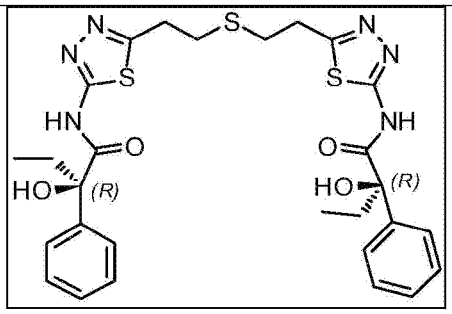
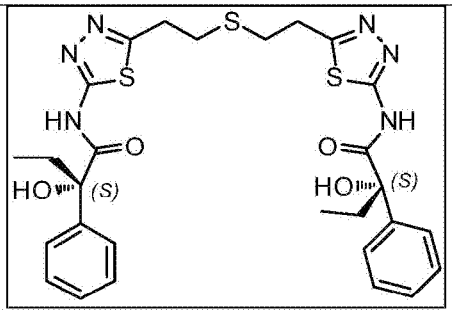
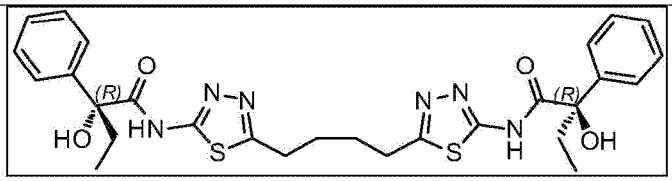
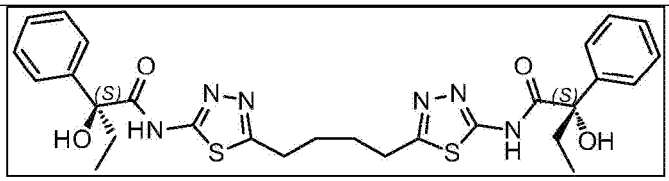
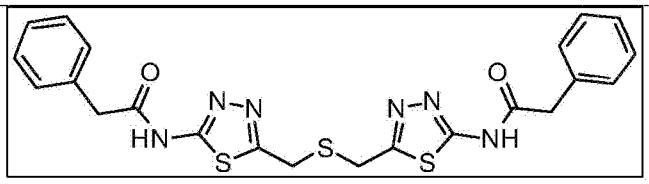
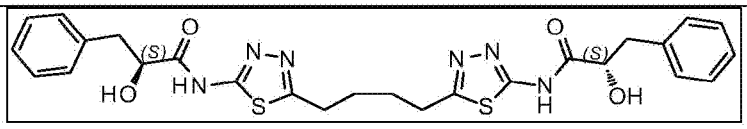
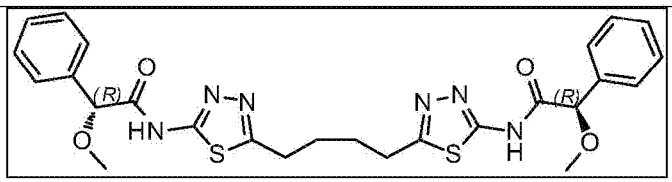
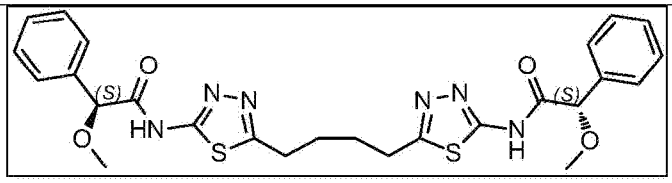
141			1.7	4.3
142			>20	
143			0.57	2.2
144			>20	
145			>20	
146			0.43	0.46
147			0.62	0.37
148			0.59	0.39

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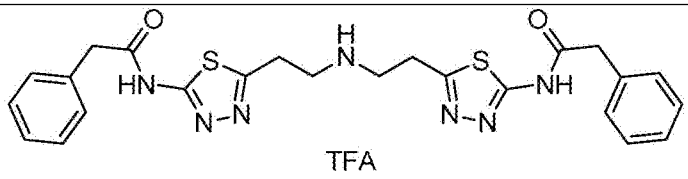
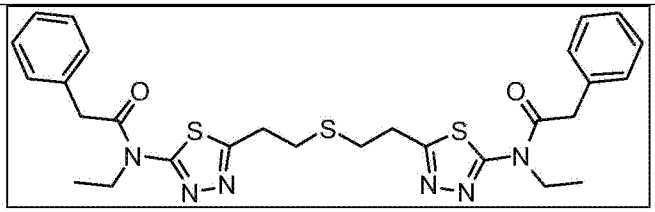
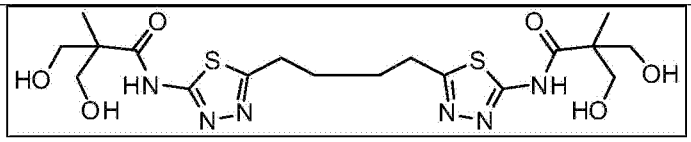
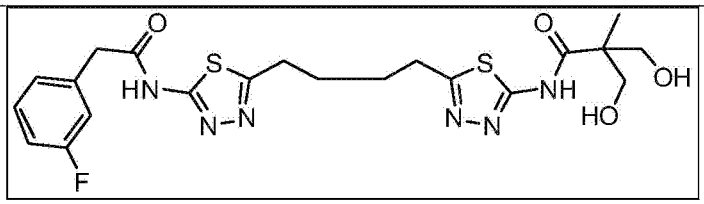
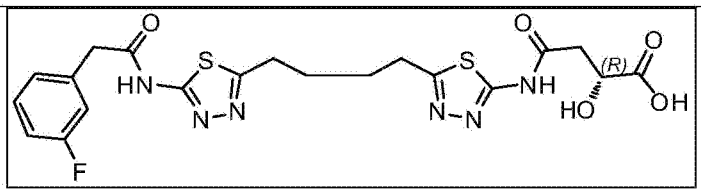
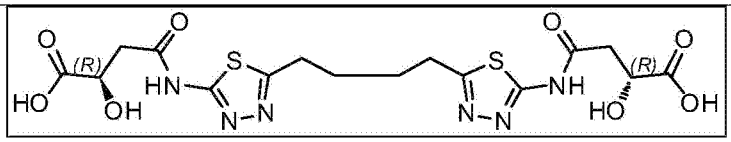
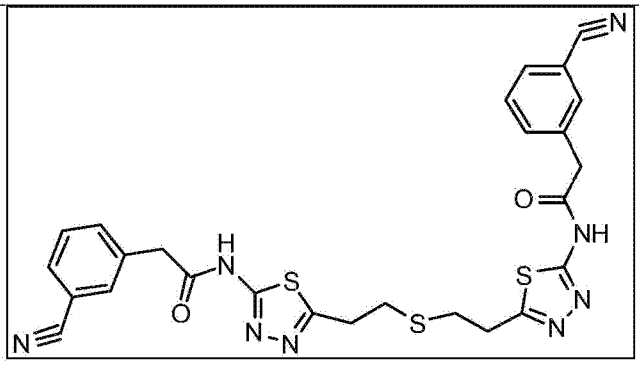
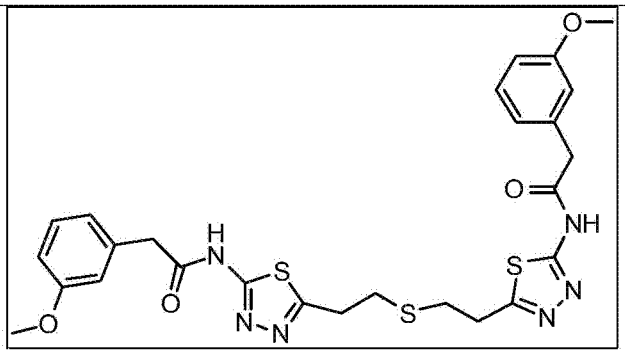
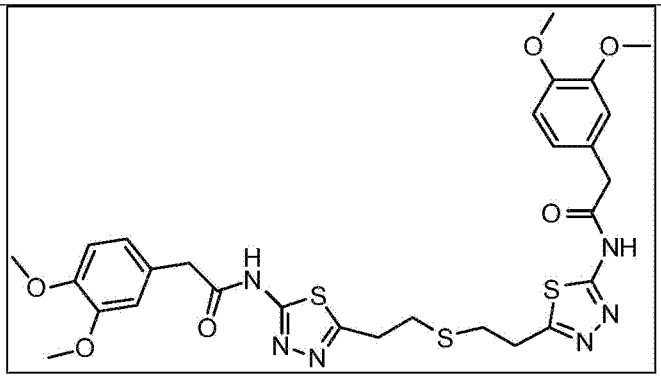
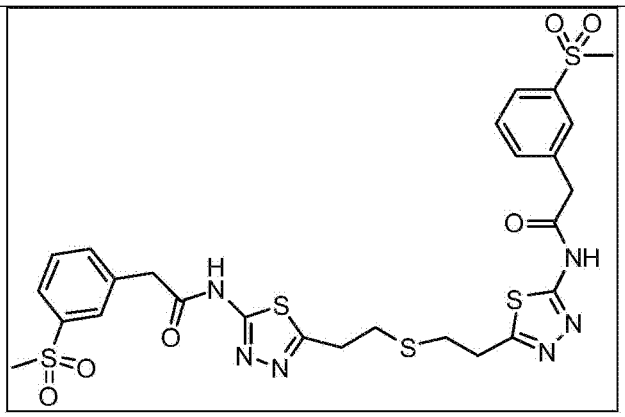
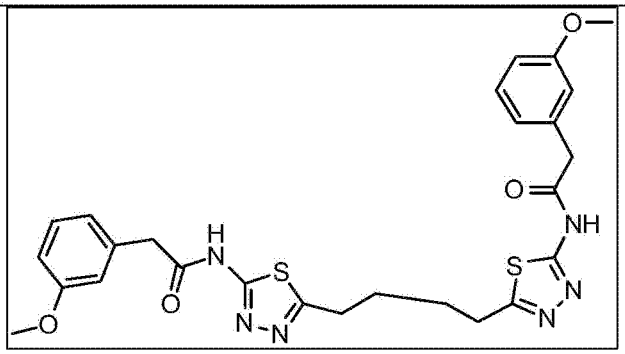
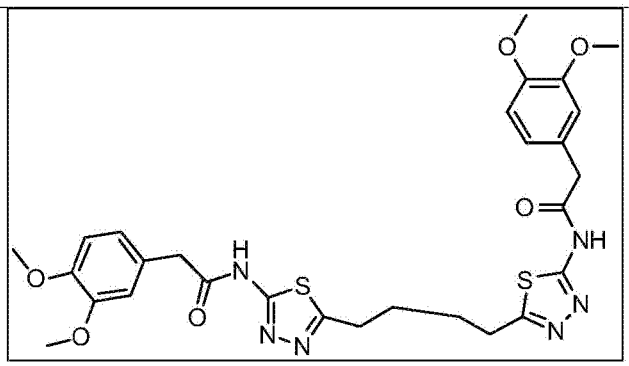
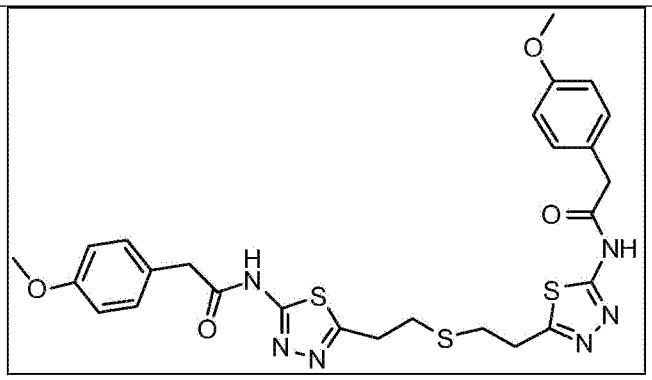
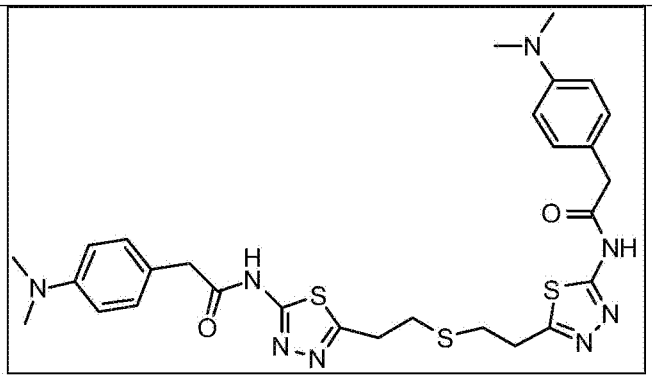
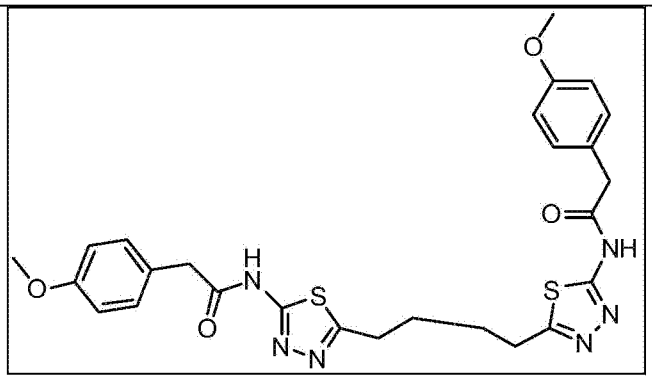
149	 TFA		15	
150			>20	
151			14	>50
152			0.73	1.1
153			1.0	>50
154			19	>50
155			0.27	1.9

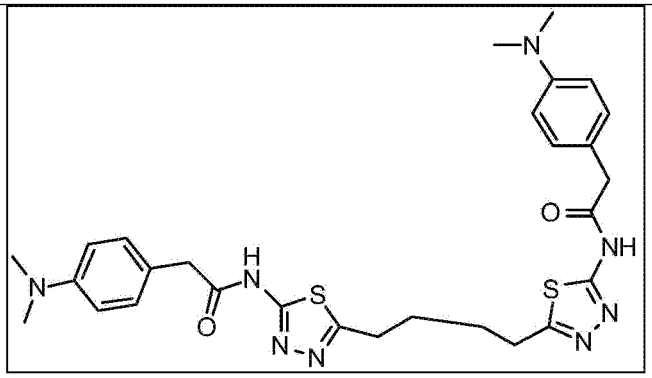
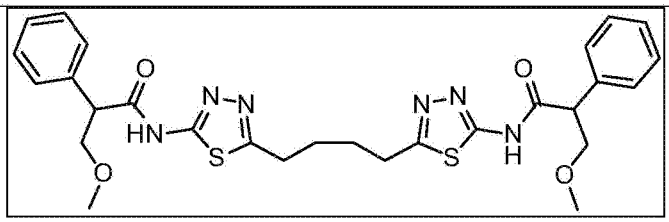
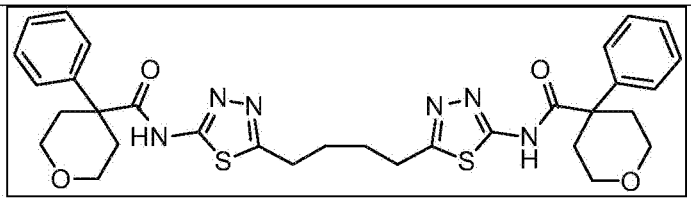
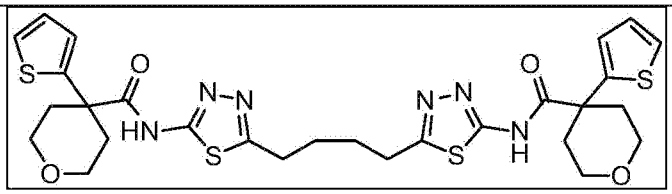
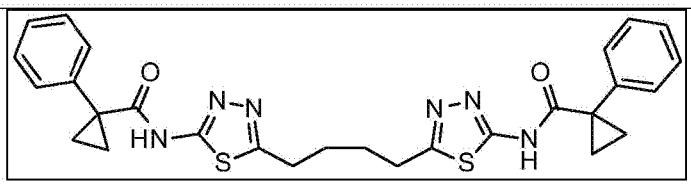
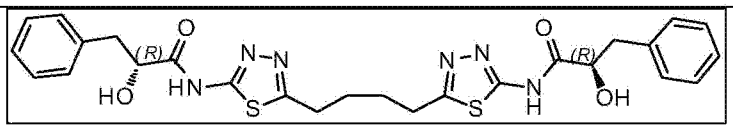
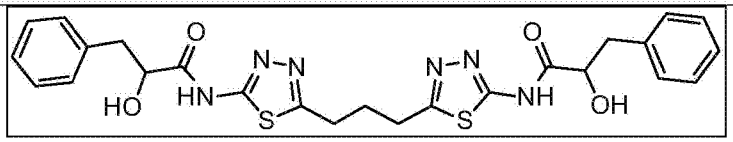
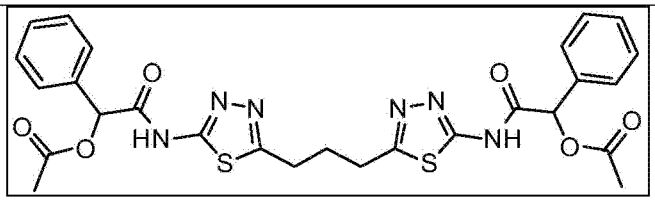
[0890]

156			0.12	0.63
157			0.34	0.18
158			0.22	8.1
159			0.11	0.05

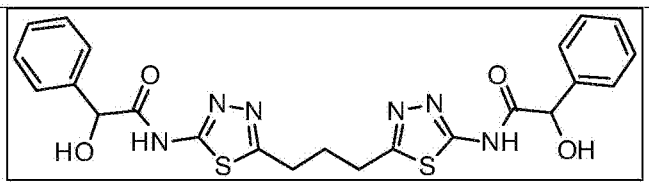
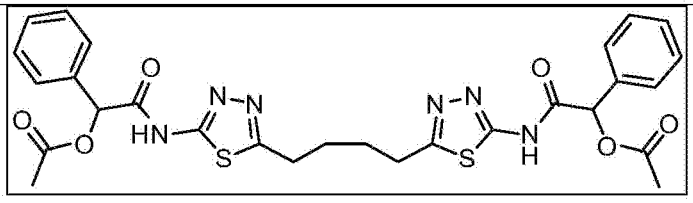
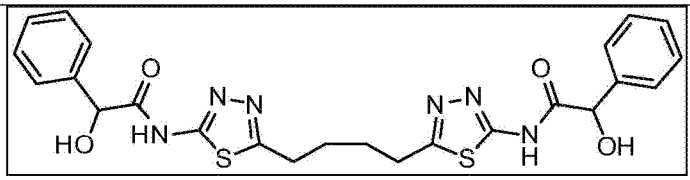
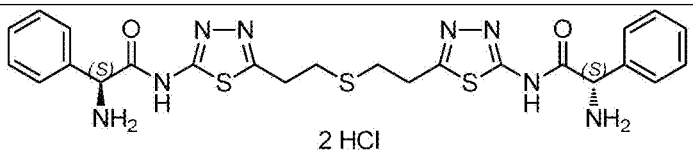
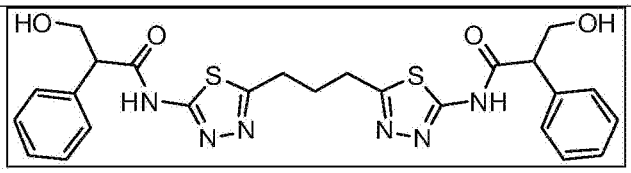
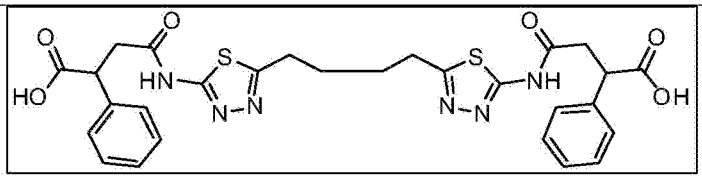
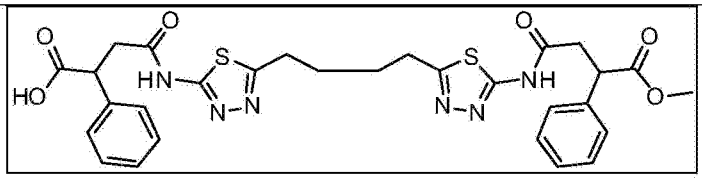
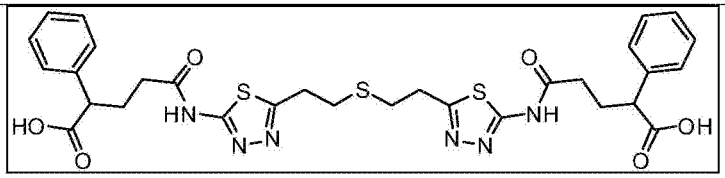
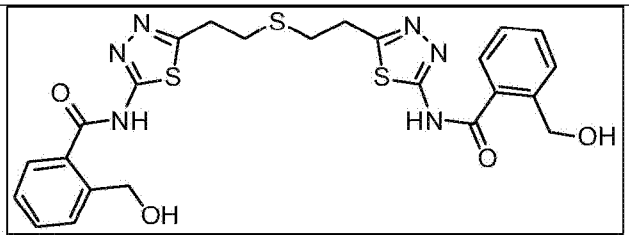
[0891]

160			0.16	>50
161			0.15	1.4
162			0.23	0.15
163			0.13	>50

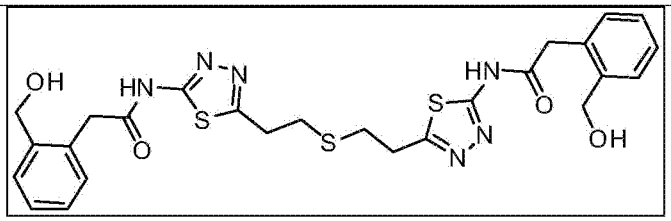
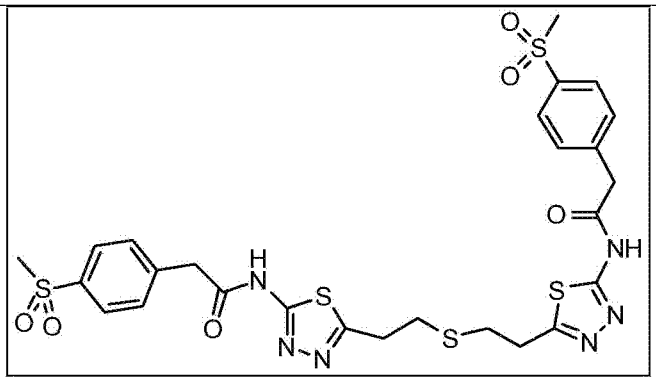
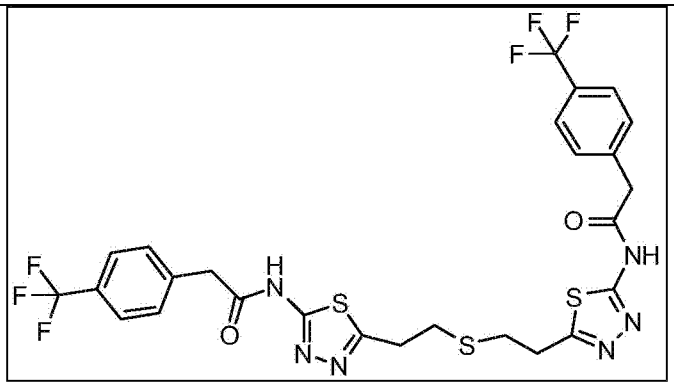
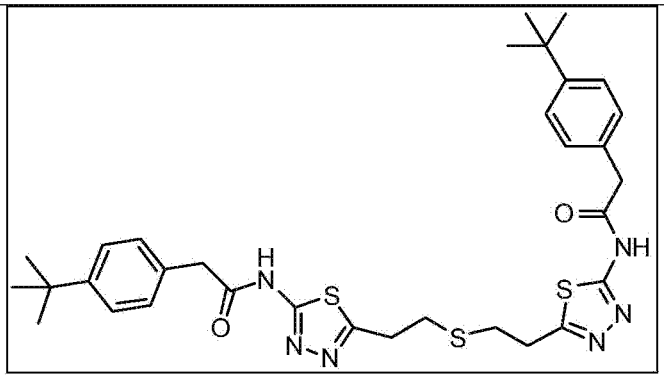
[0892]

164			0.24	0.13
165			0.51	33
166			7.4	6.8
167			11	34
168			1.3	>50
169			0.71	3.4
170			7.4	9.3
171			>20	

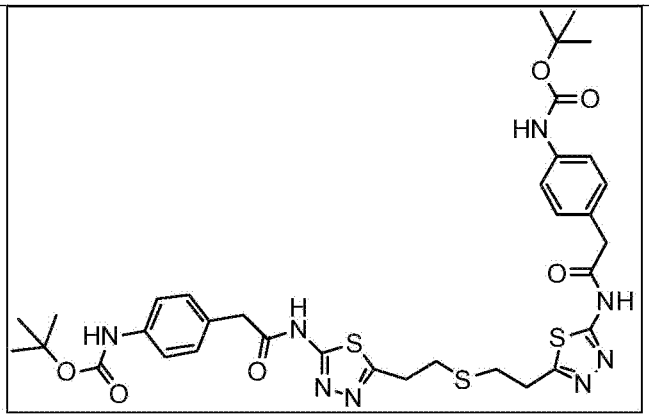
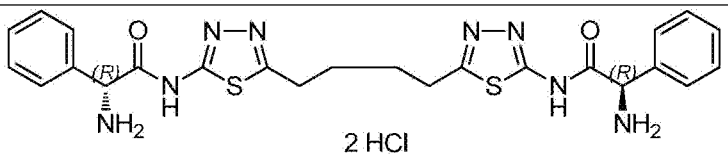
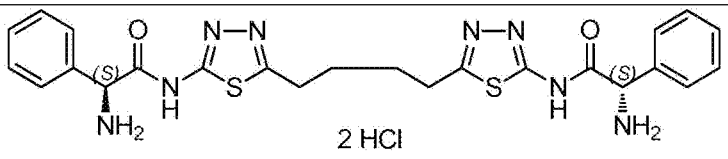
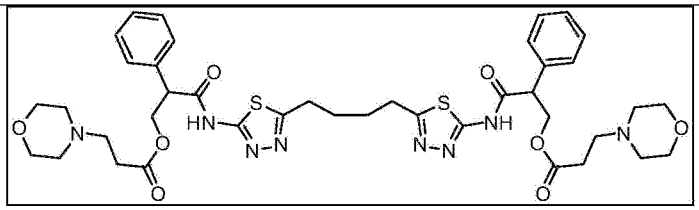
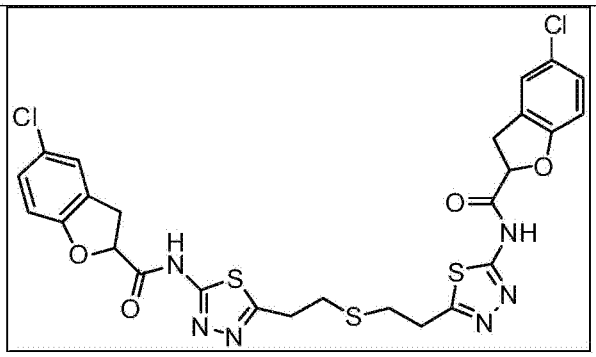
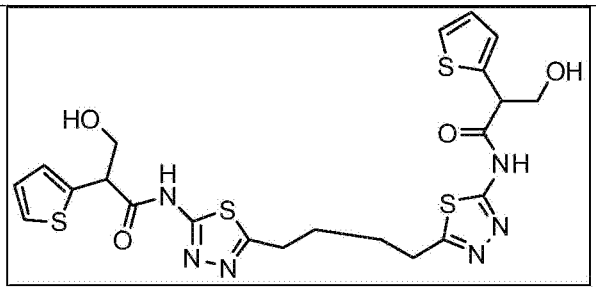
[0893]

172			1.7	3.7
173			24	0.76
174			0.29	0.44
175	 2 HCl		6.3	23
176			0.57	1.5
177			1.1	>50
178			1.5	>50
179			3.1	>50
180			8.8	>50

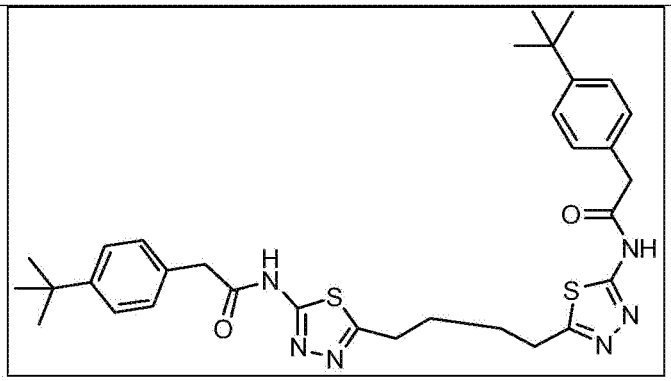
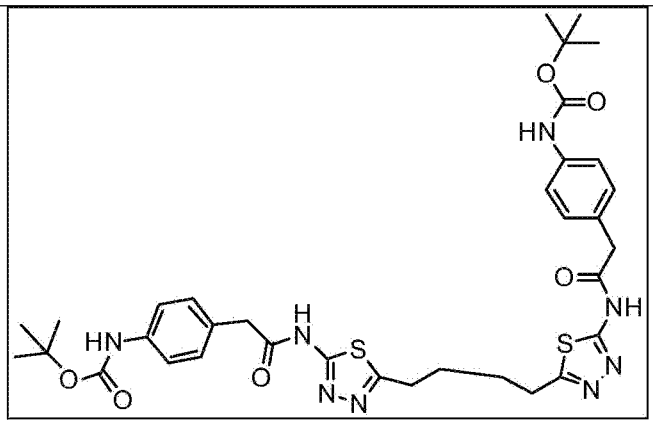
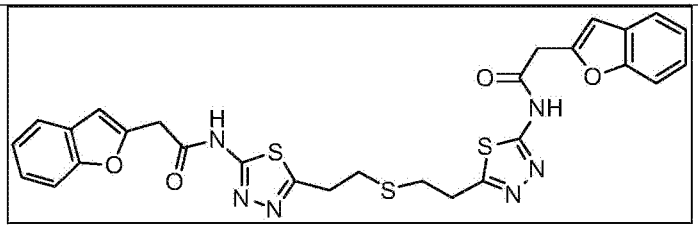
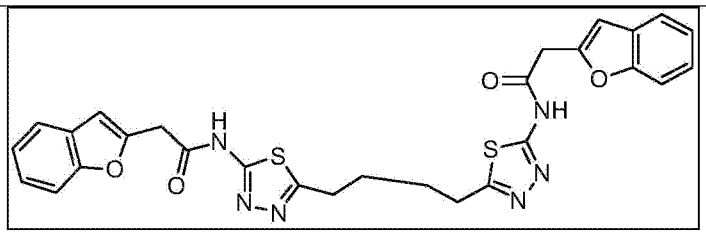
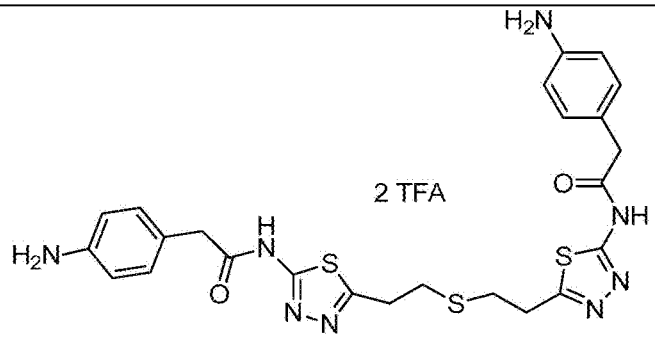
[0894]

181			0.33	30
182			0.58	>50
183			>20	
184			>20	

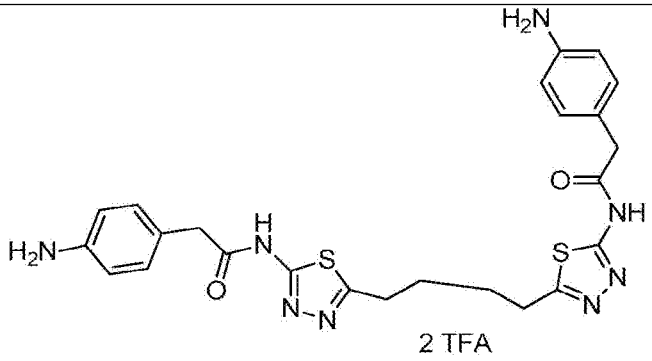
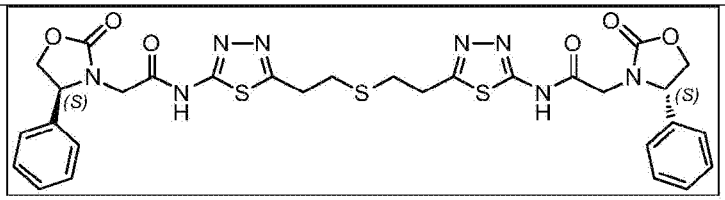
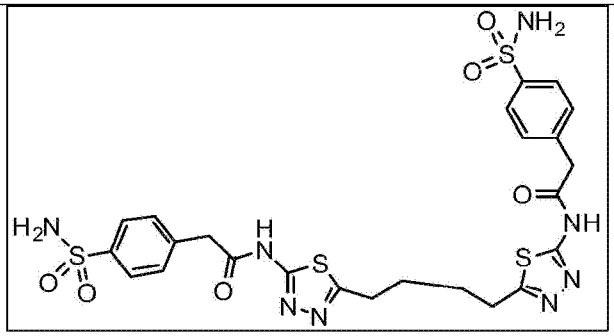
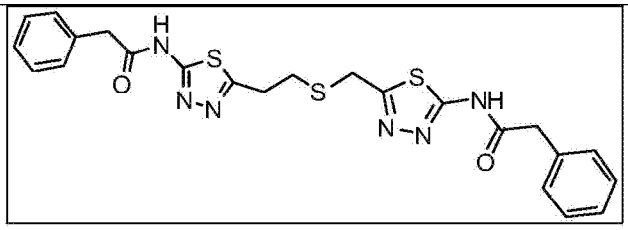
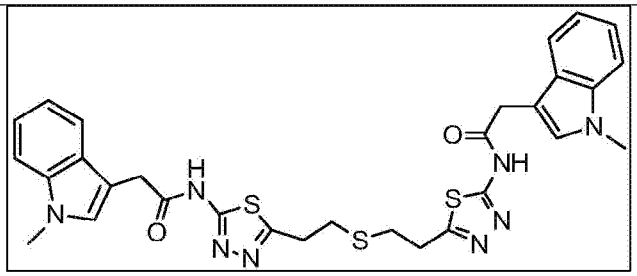
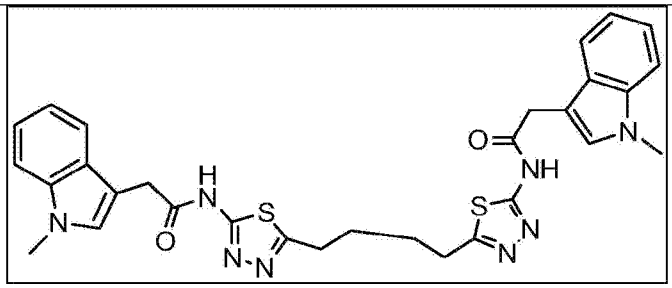
[0895]

185			>20	0.09
186	 2 HCl		3.1	13
187	 2 HCl		2.8	21
188			2.0	0.46
189			4.4	
190			0.25	0.49

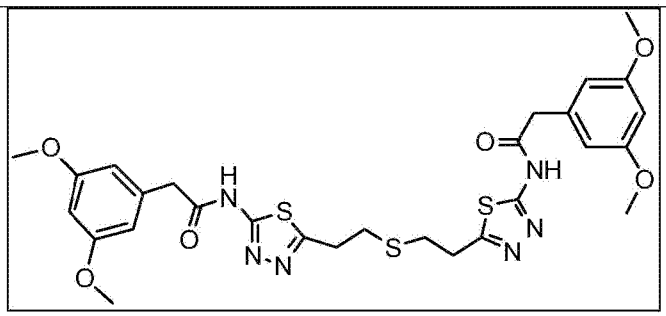
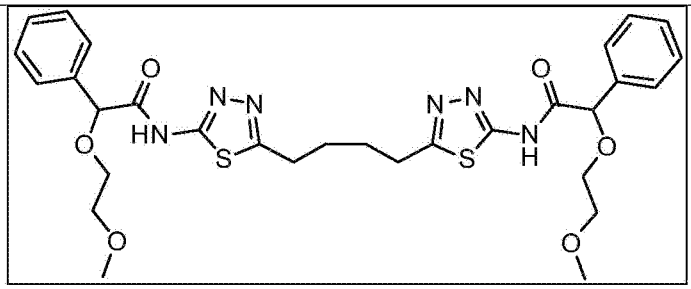
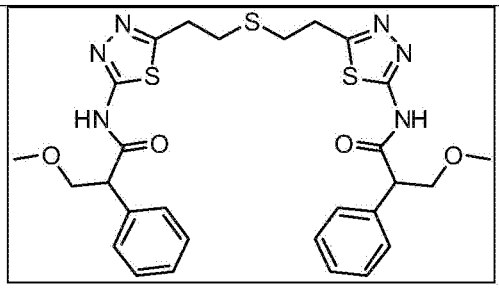
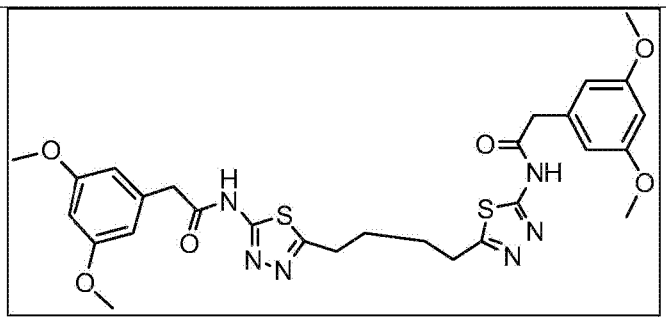
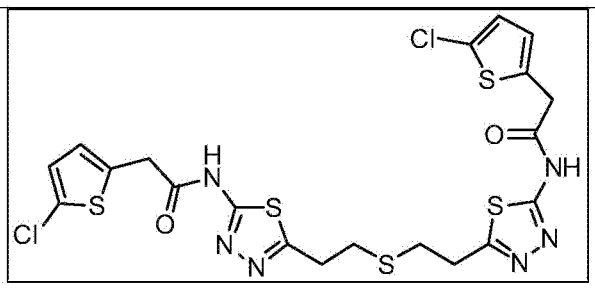
[0896]

191			>20	
192			>20	0.03
193			3.4	
194			10	
195			0.30	1.3

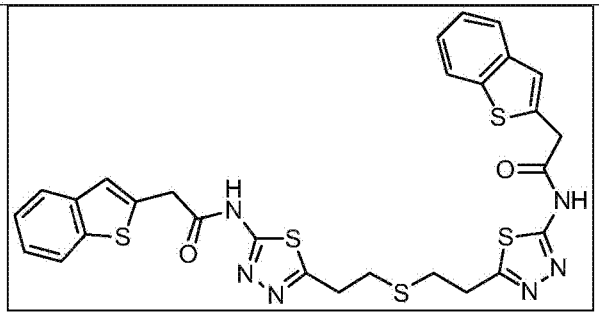
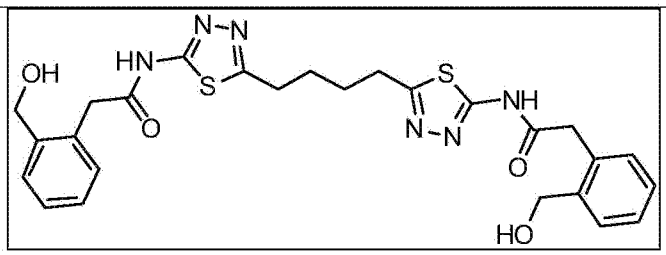
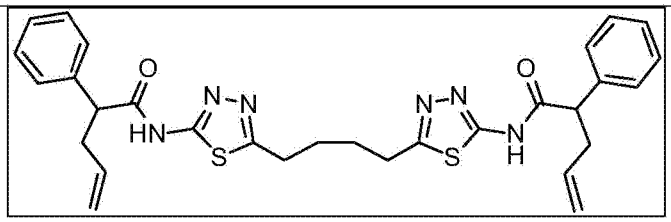
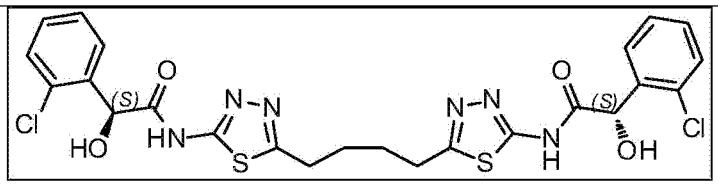
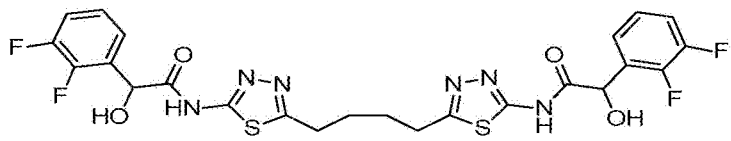
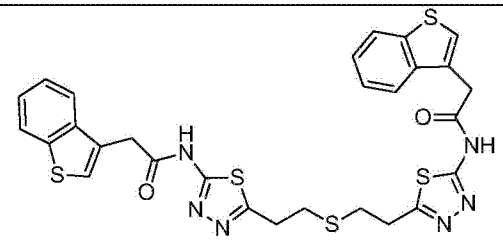
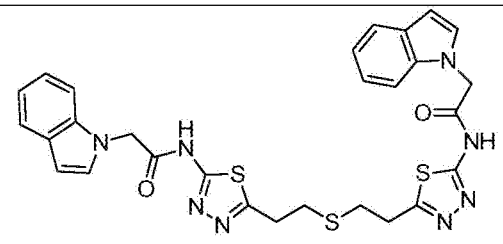
[0897]

196	 2 TFA		0.19	0.61
197			6.9	
198			0.18	>50
199			0.12	0.17
200			0.61	
201			2.7	

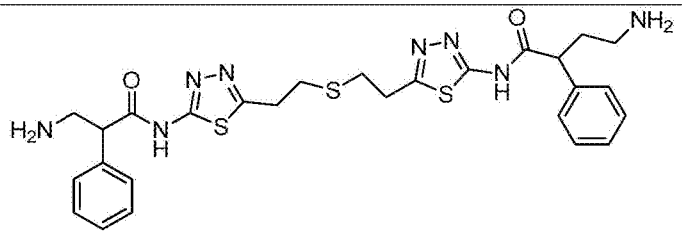
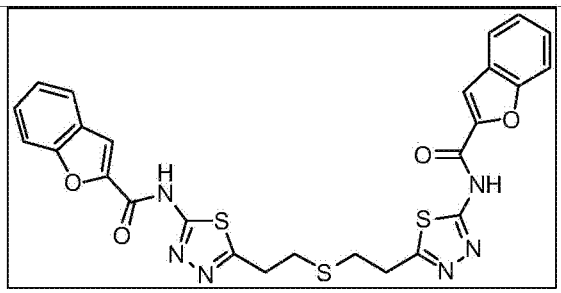
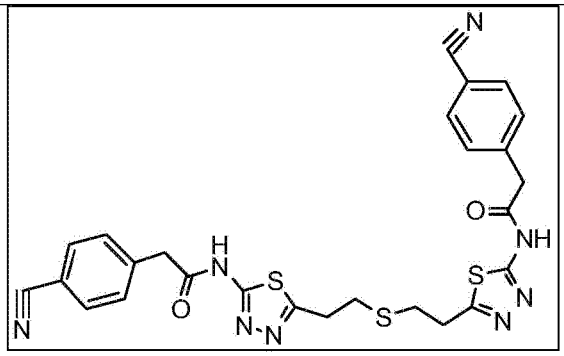
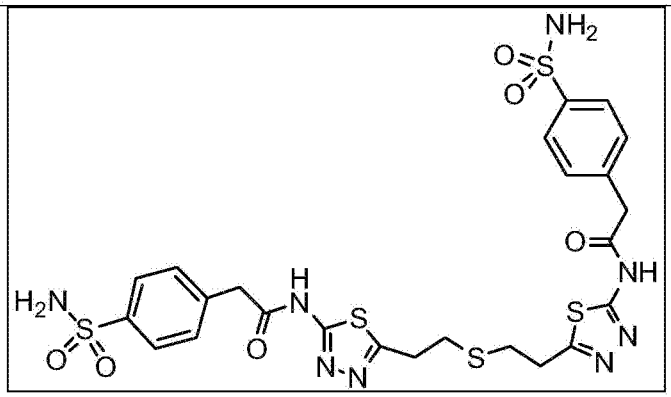
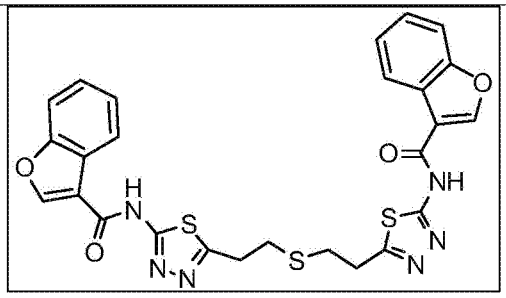
[0898]

202			0.18	0.14
203			1.7	1.7
204			0.92	2.4
205			0.38	4.1
206			>20	

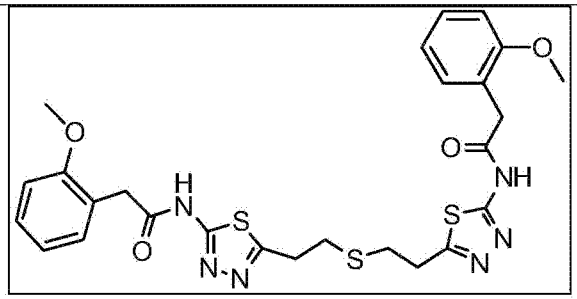
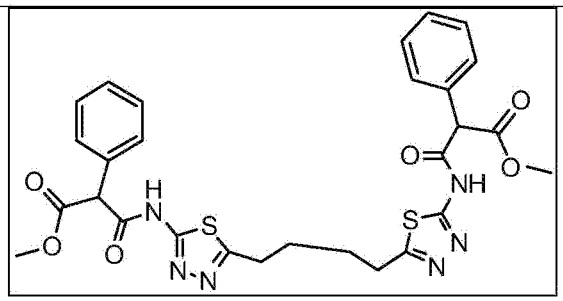
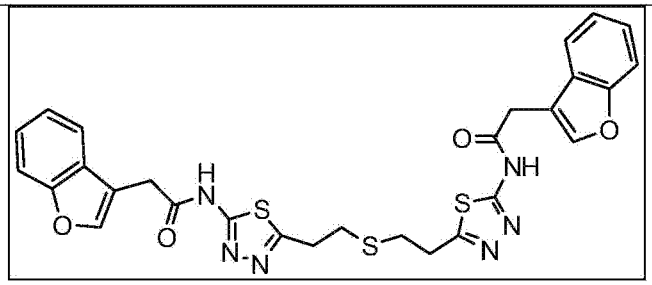
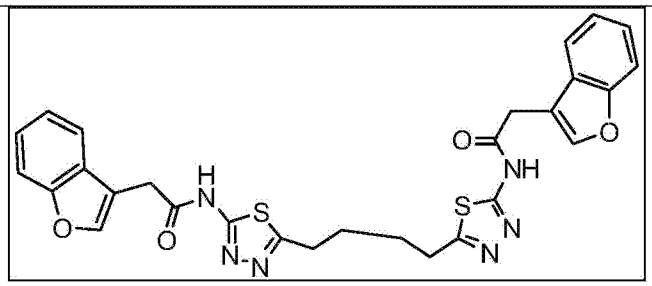
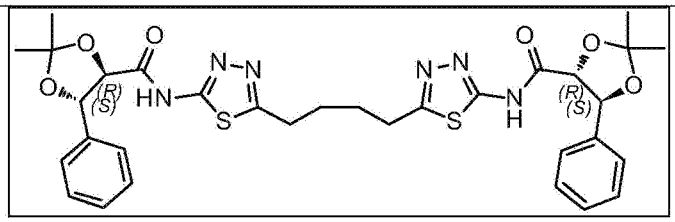
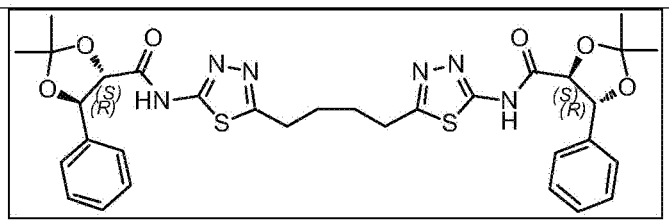
[0899]

207			13	
208			0.17	9.0
209			>20	22
210			0.38	0.42
211			1.2	1.0
212			>20	
213			2.5	4.4

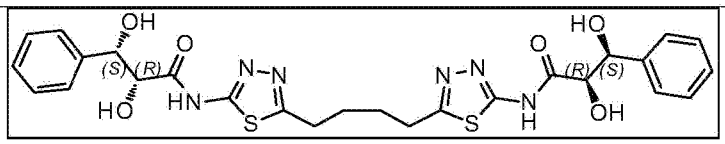
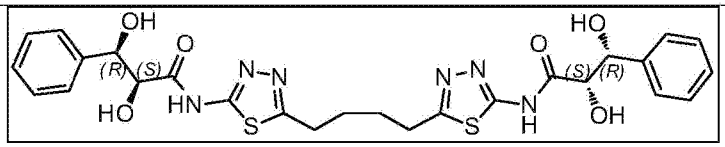
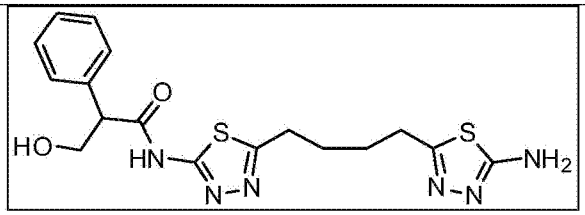
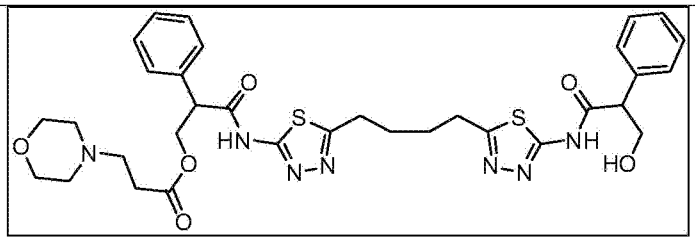
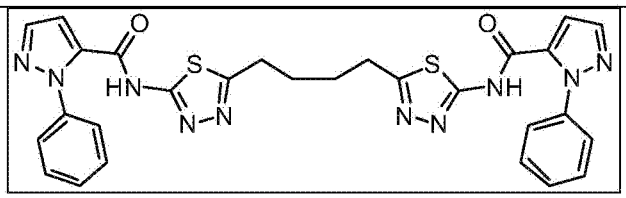
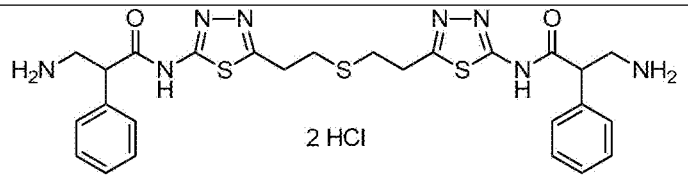
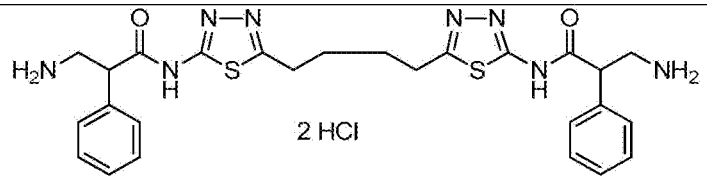
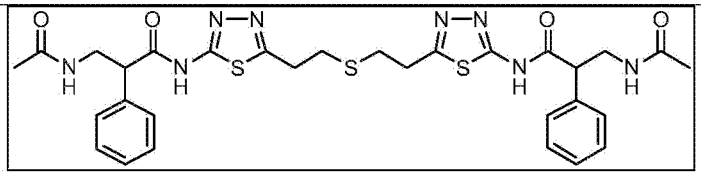
[0900]

214			0.82	1.2
215			16	
216			0.89	>50
217			0.24	>50
218			>20	

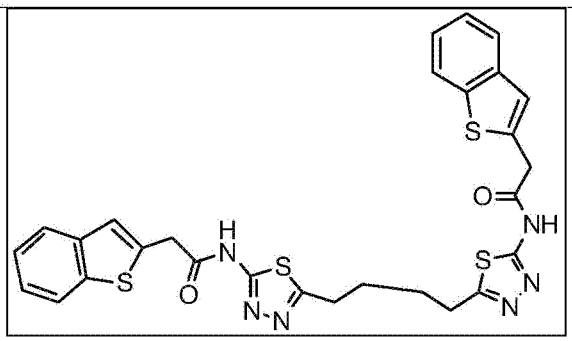
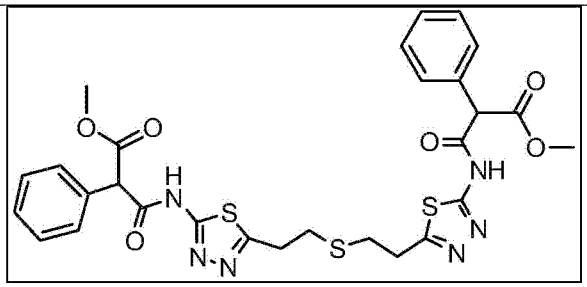
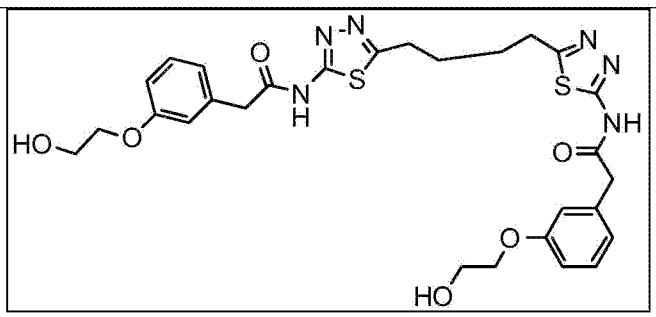
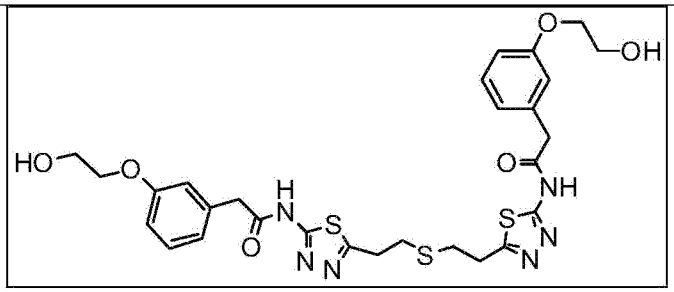
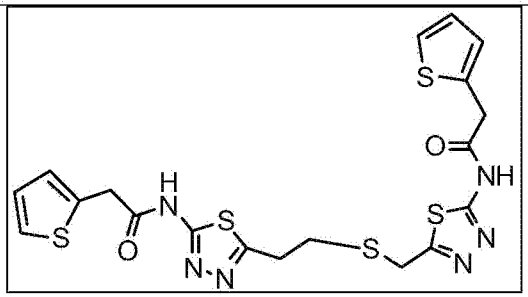
[0901]

219			0.17	0.57
220			1.6	0.31
221			>20	
222			>20	
223			>20	
224			>20	

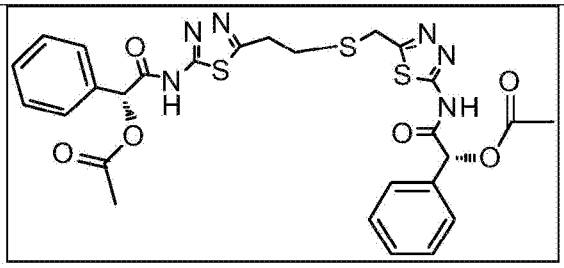
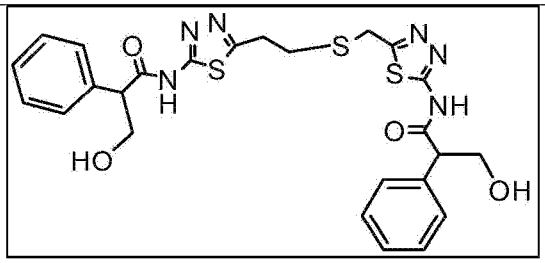
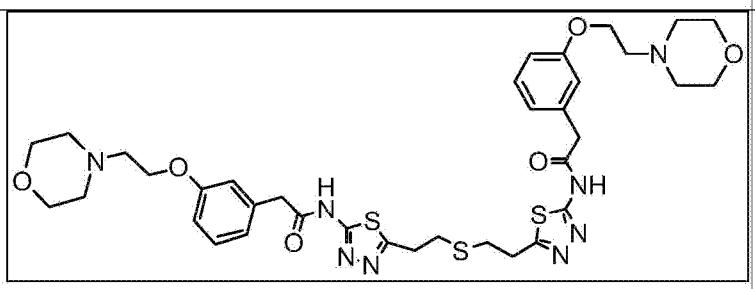
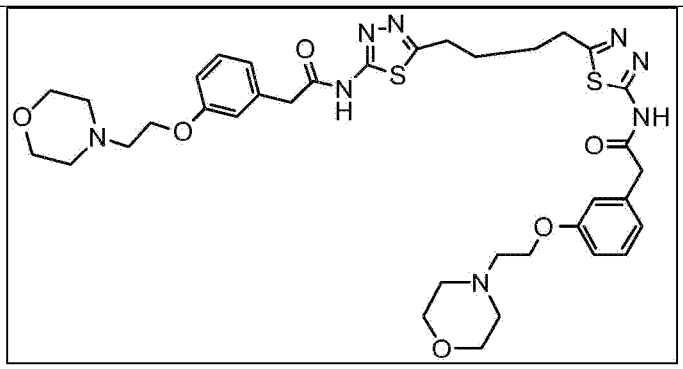
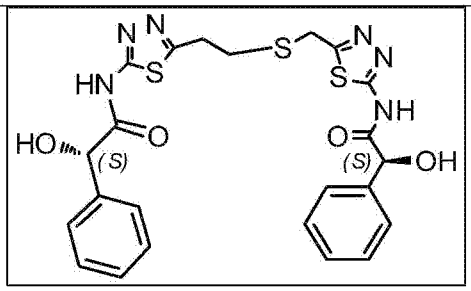
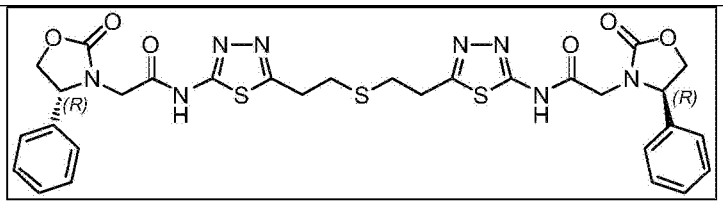
[0902]

225			>20	
226			2.3	>50
227			9.9	3.3
228			0.57	0.13
229			3.9	
230			12	
231			7.4	
232			9.8	

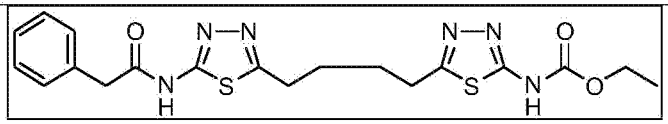
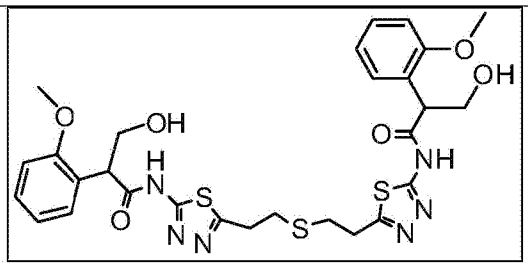
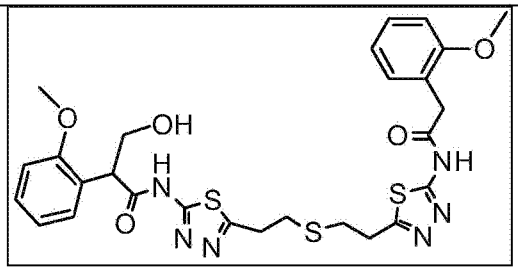
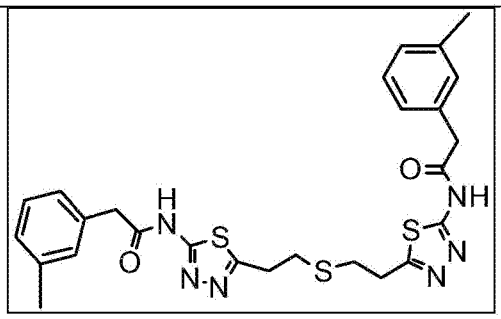
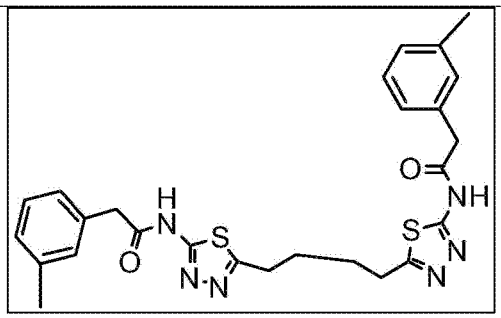
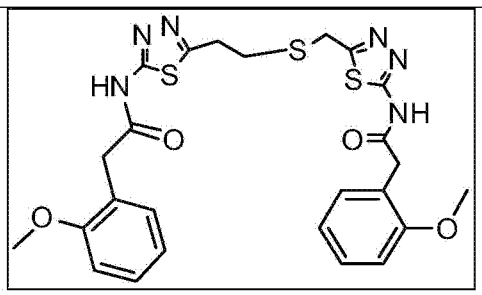
[0903]

233			15	
234			2.0	2.5
235			0.11	0.21
236			0.20	1.4
237			0.20	0.25

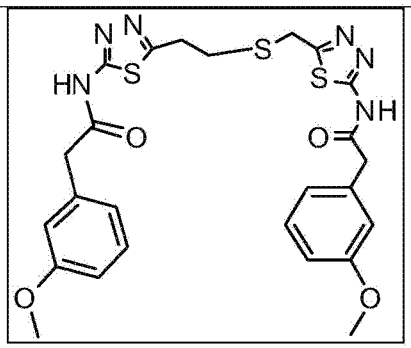
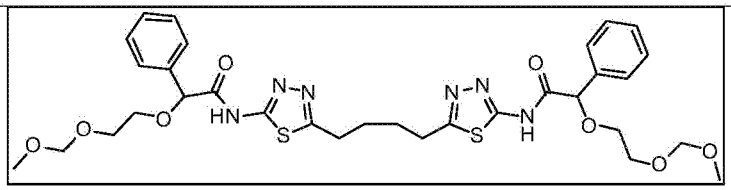
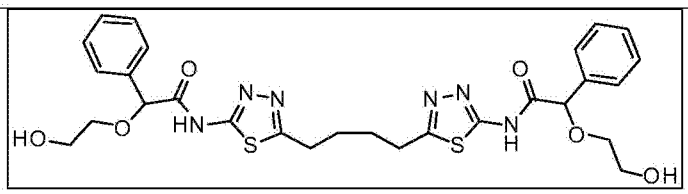
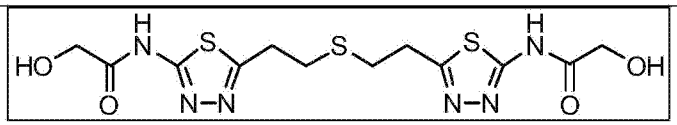
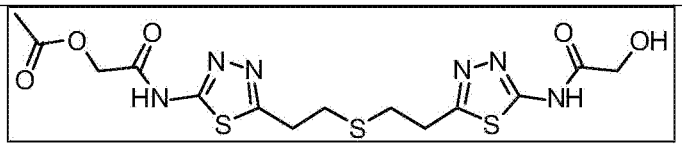
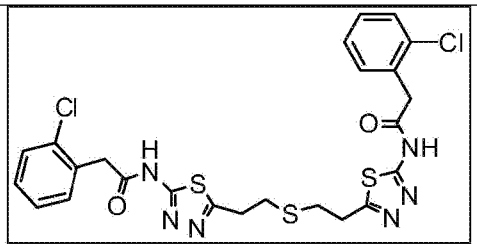
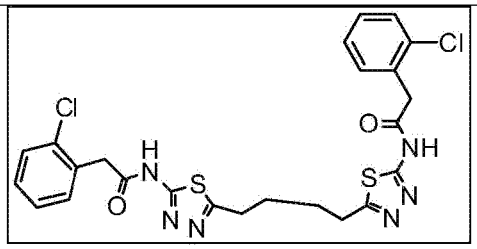
[0904]

238			13	
239			0.30	0.30
240			0.54	1.3
241			0.38	0.87
242			0.36	0.22
243			2.7	33

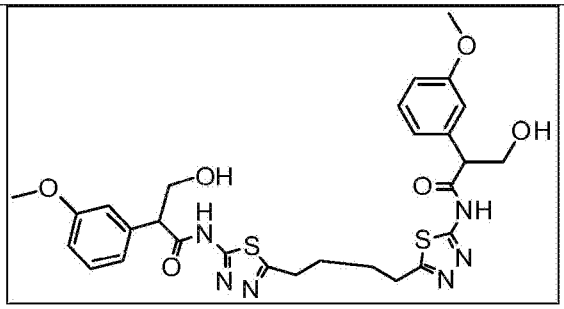
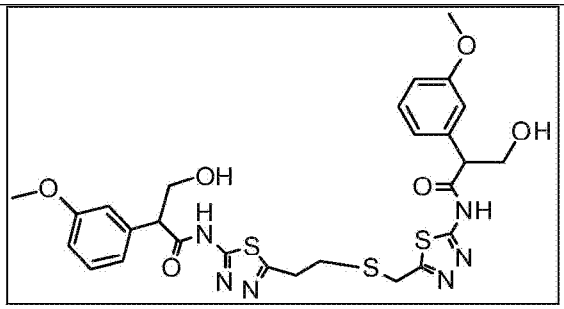
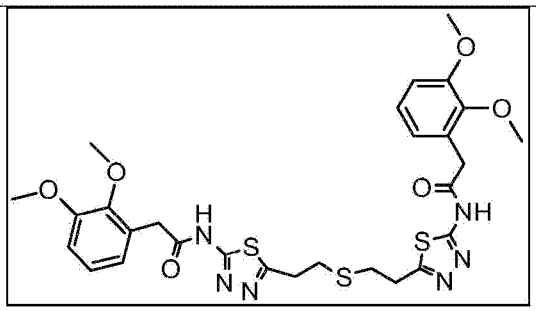
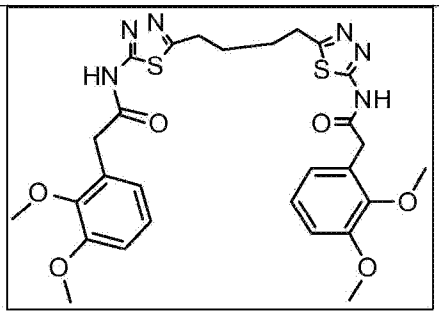
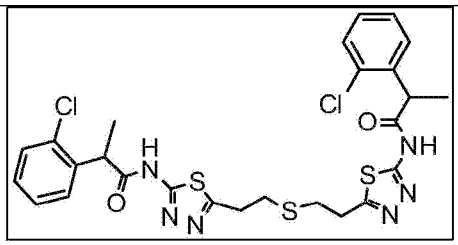
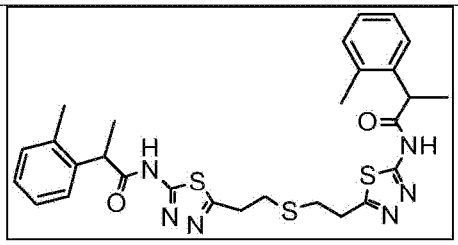
[0905]

244			0.84	1.7
245			0.52	2.5
246			0.40	1.6
247			0.19	0.83
248			2.3	
249			0.12	0.16

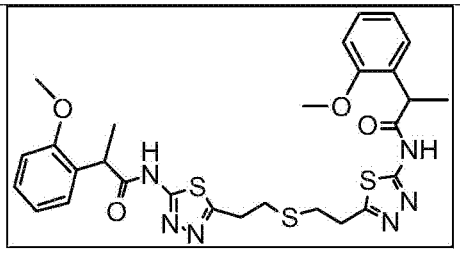
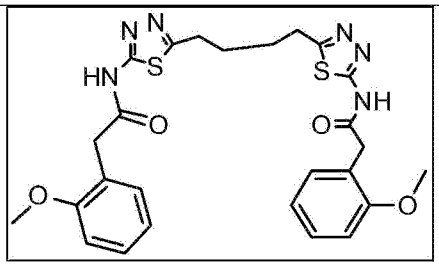
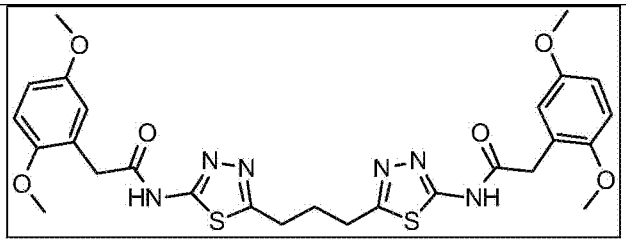
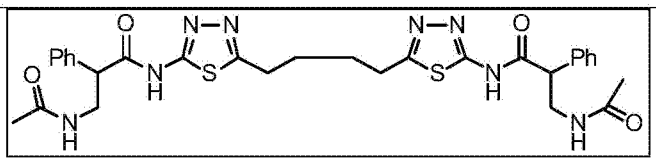
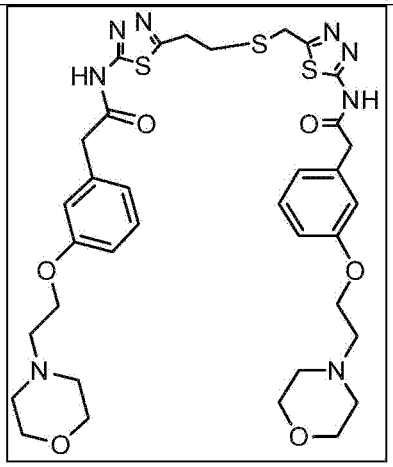
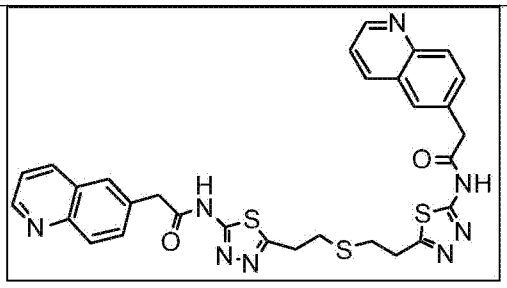
[0906]

250			0.12	0.14
251			2.8	2.8
252			1.2	6.3
253			21	
254			>20	
255			0.38	
256			0.11	

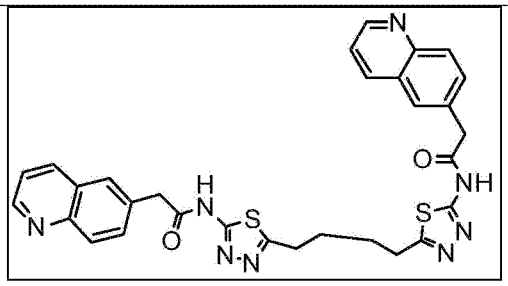
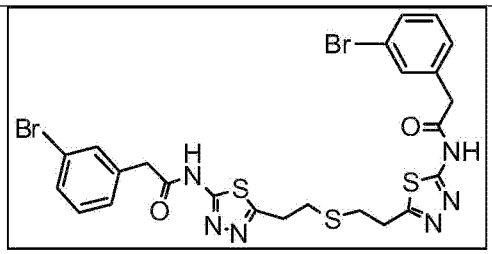
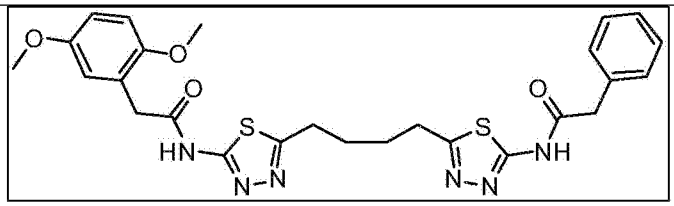
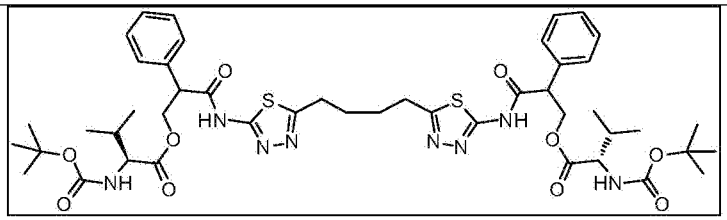
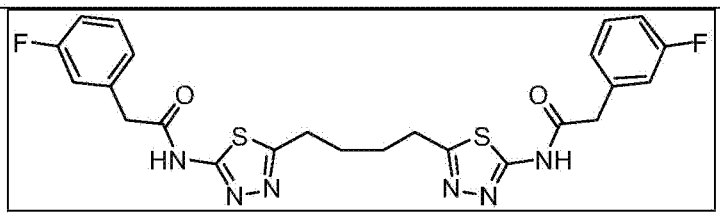
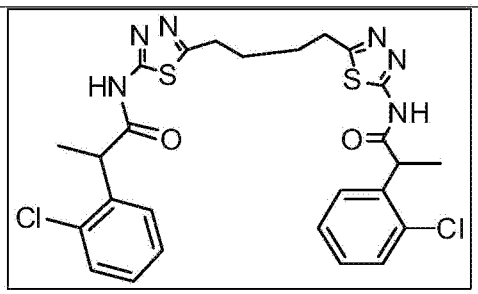
[0907]

257			0.12	0.073
258			0.19	0.18
259			0.23	0.57
260			0.15	0.084
261			0.70	2.6
262			0.36	3.1

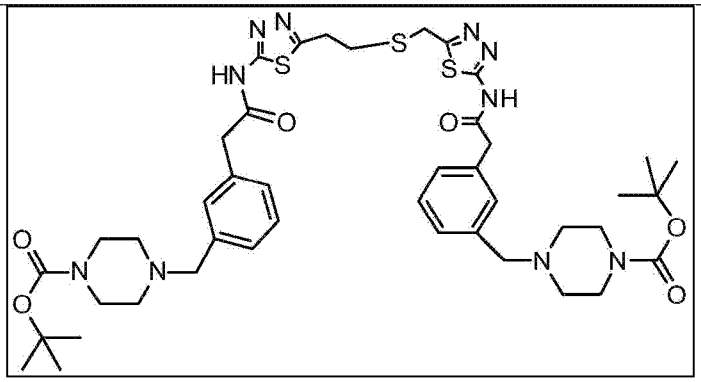
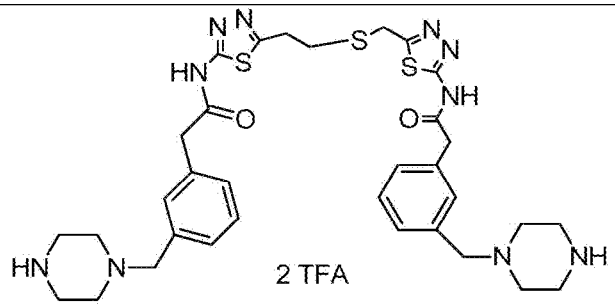
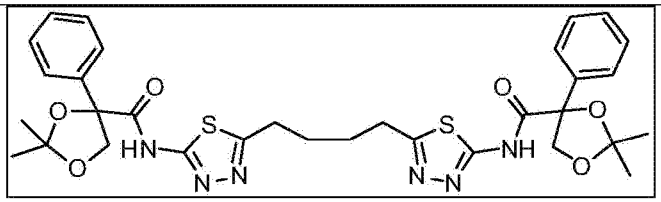
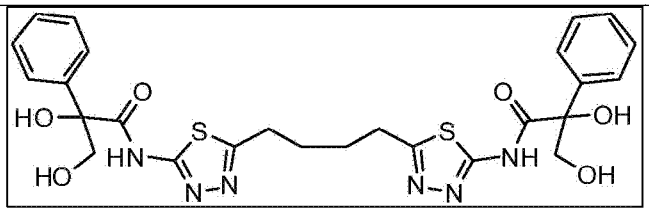
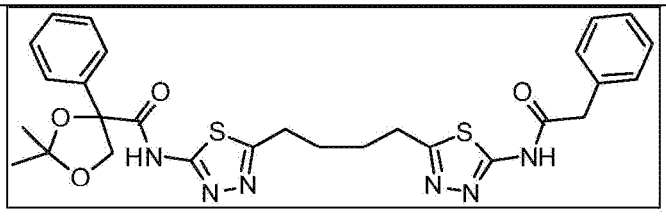
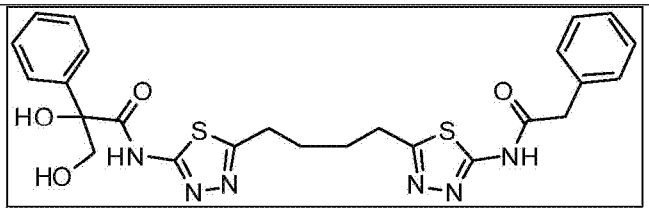
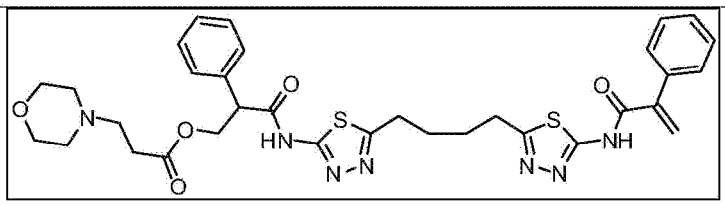
[0908]

263			0.32	3.9
264			0.072	0.01
265			0.27	0.31
266			2.2	>50
267			0.61	0.64
268			0.60	5.4

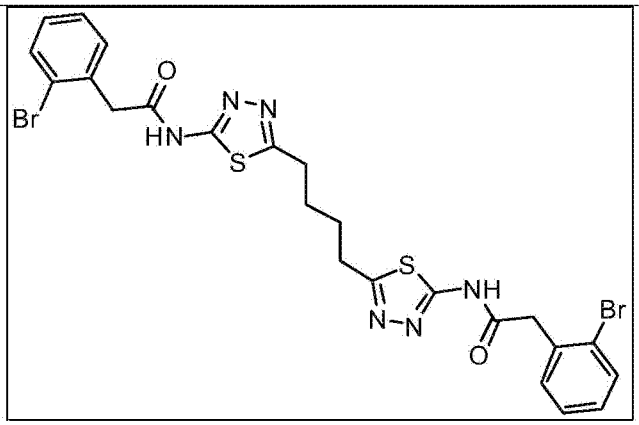
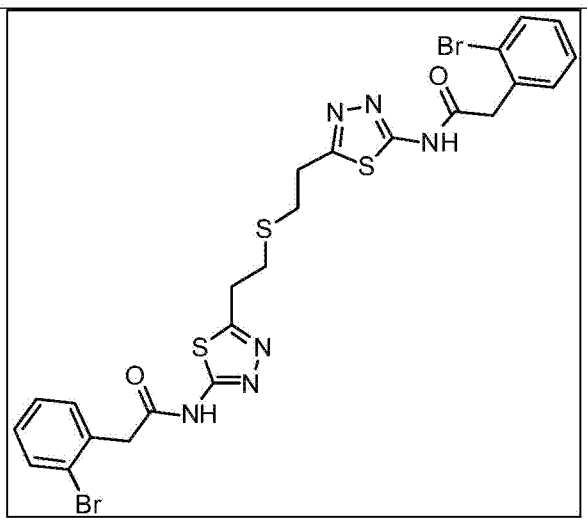
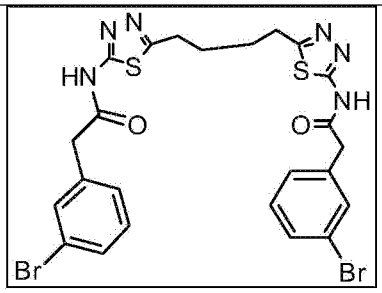
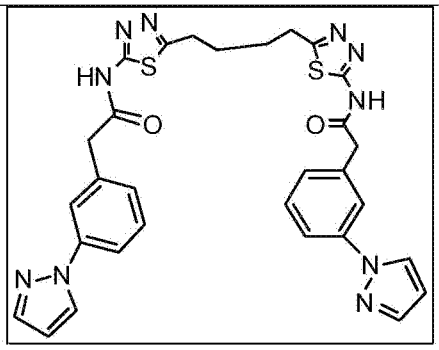
[0909]

269			0.26	0.52
270		>5	7.4	0.85
271			0.10	0.63
272			>20	
273			0.14	0.07
274			0.75	0.68

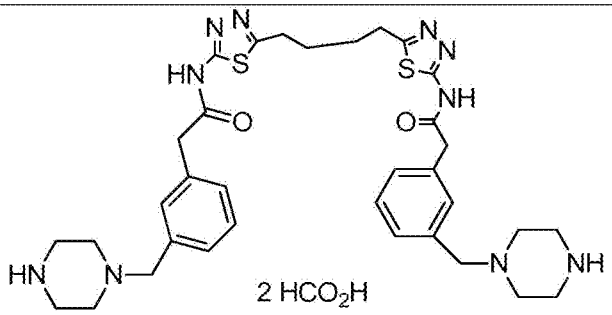
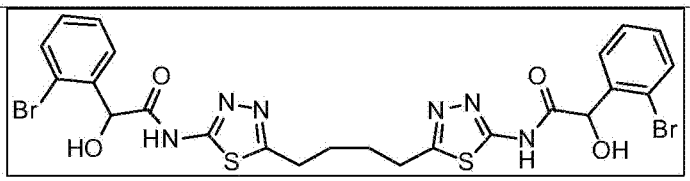
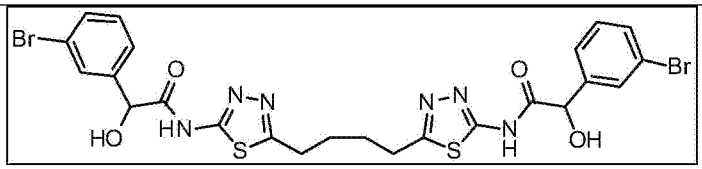
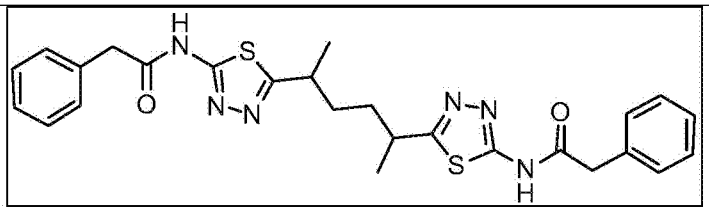
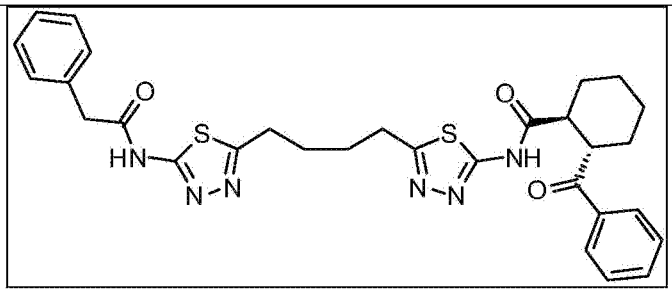
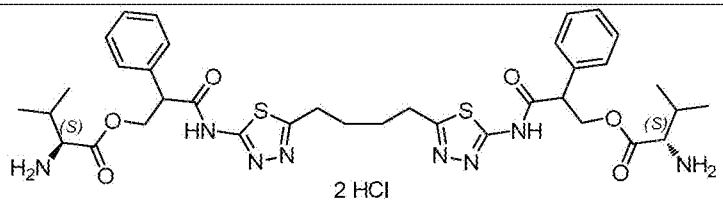
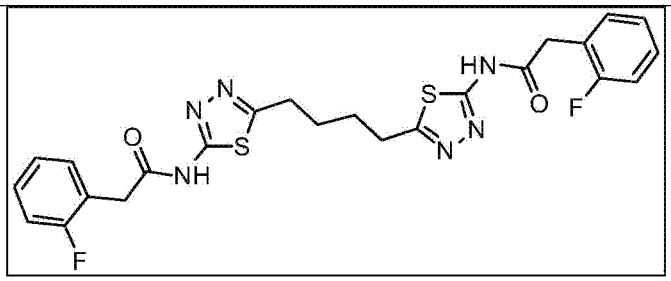
[0910]

275		0.15	2.2	0.34
276			1.5	56
277			>20	
278			0.38	0.16
279			0.68	7.0
280			0.29	0.23
281			0.74	0.66

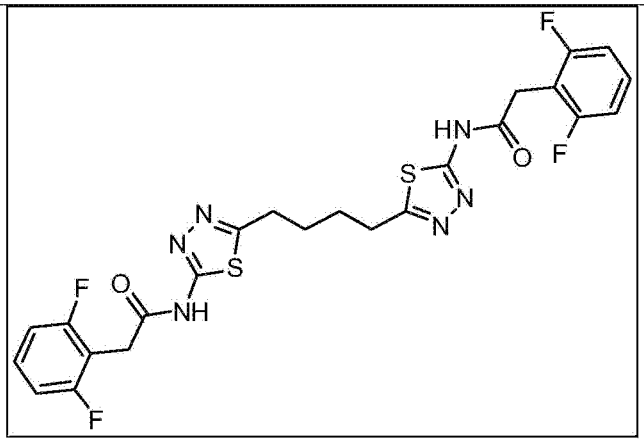
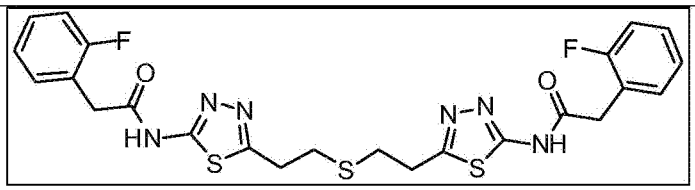
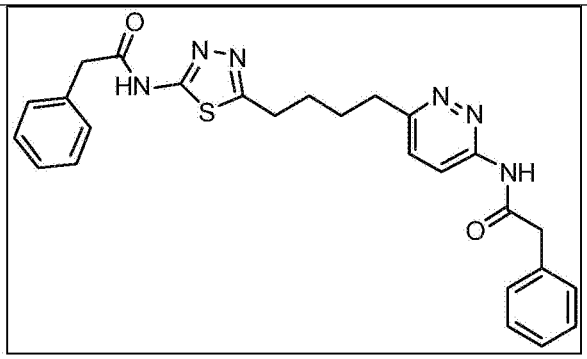
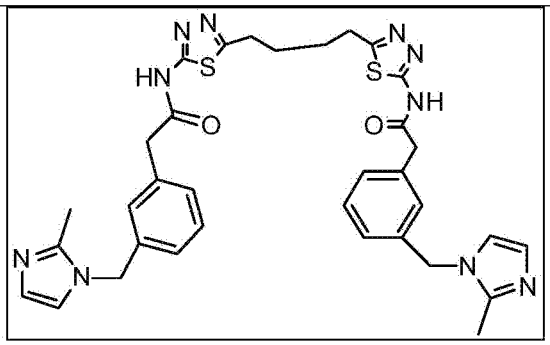
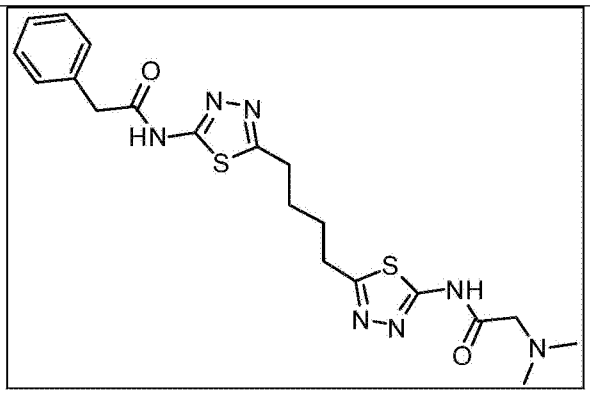
[0911]

282			0.082	0.37
283			0.66	0.74
284		0.05	>20	
285			0.19	0.14

[0912]

286			0.54	6.4
287			0.57	1.3
288		0.04	0.67	0.028
289			32	
290			0.80	0.79
291			1.5	1.8
292			0.12	0.012

[0913]

293			0.24	0.04
294			0.20	1.1
295		0.01	0.057	0.039
296			0.10	0.17
297			6.4	

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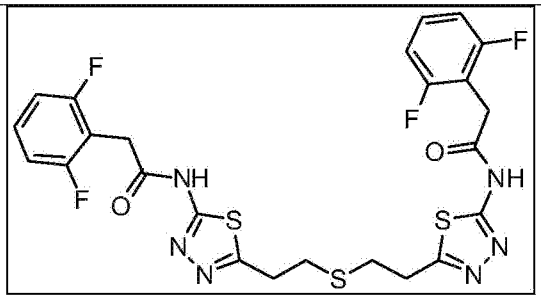
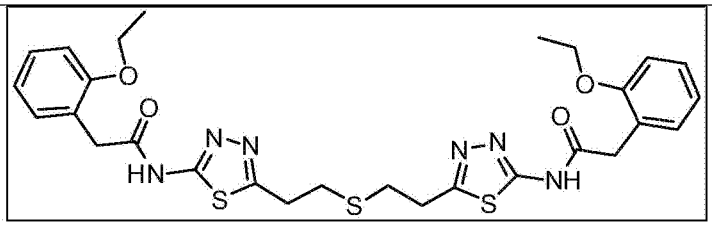
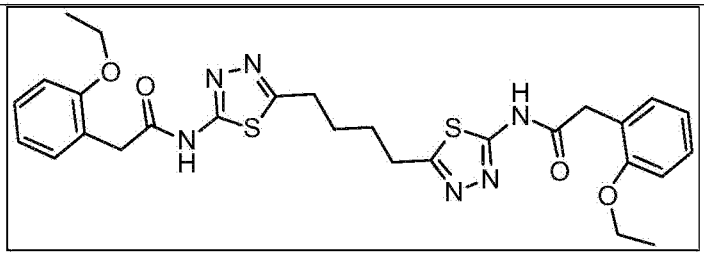
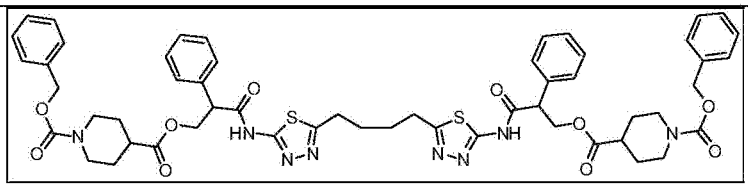
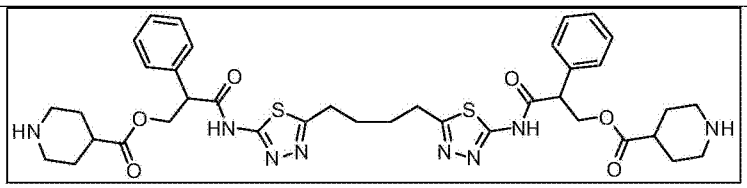
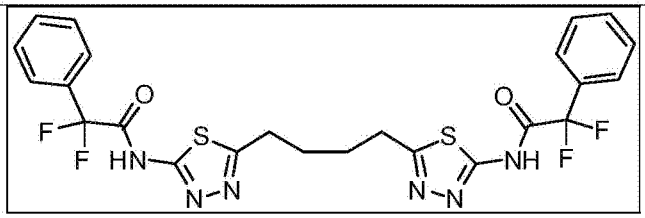
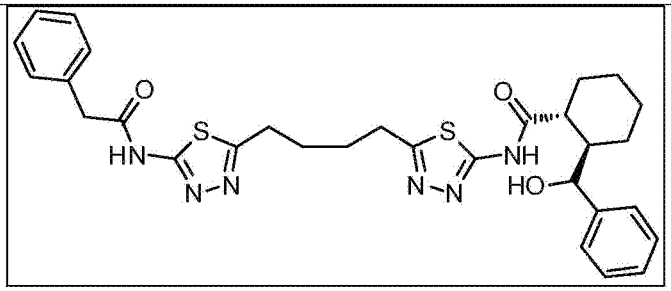
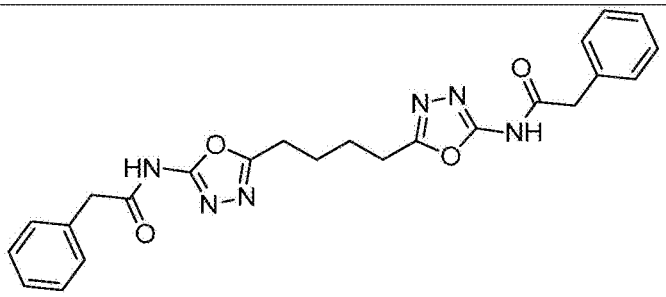
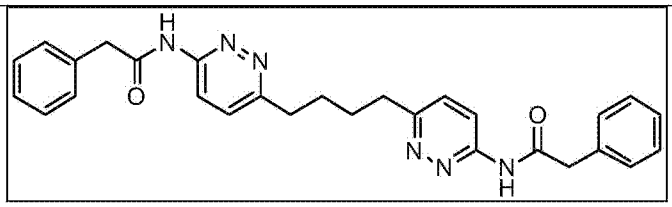
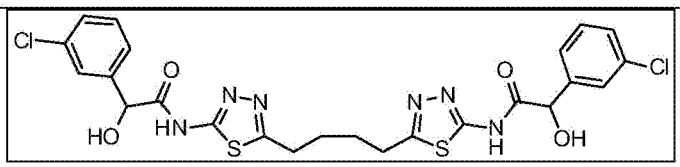
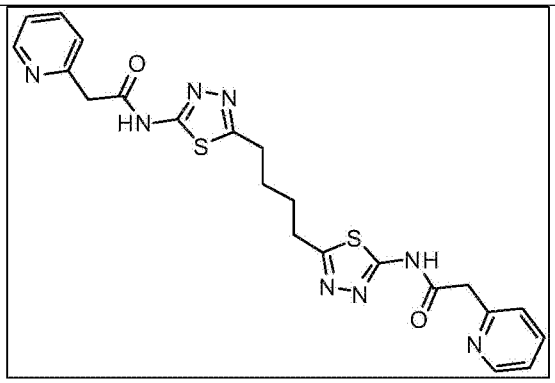
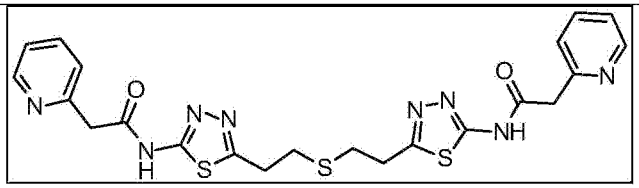
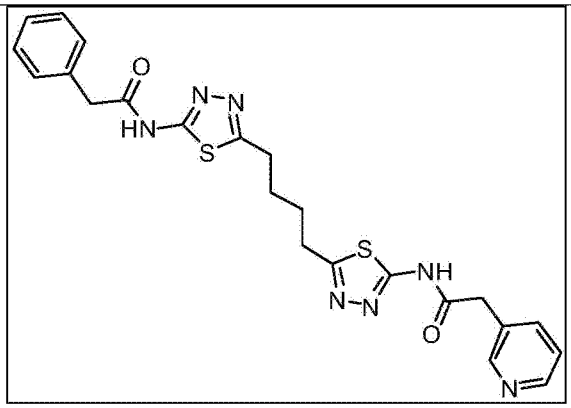
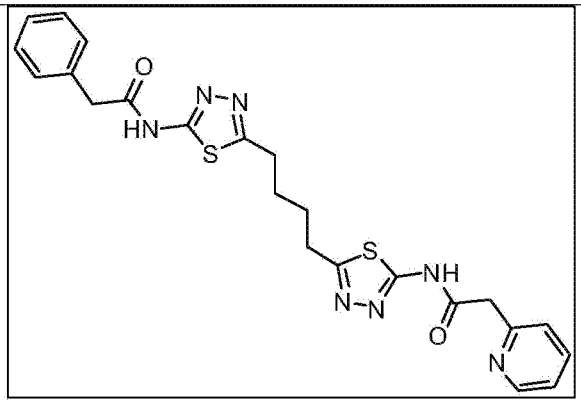
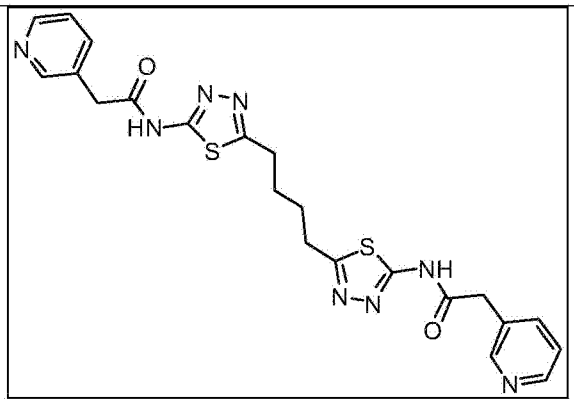
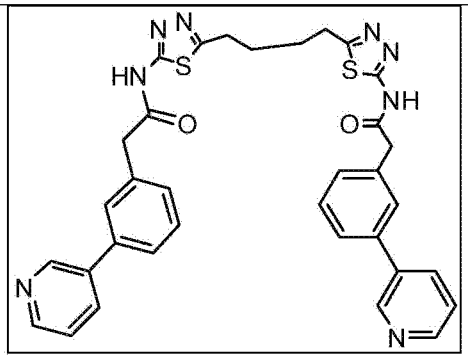
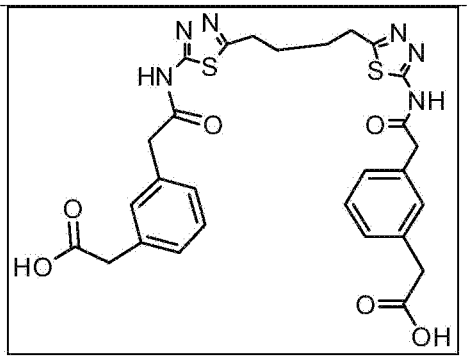
298			0.73	5.1
299			0.33	
300			0.16	0.16
301			>20	0.23
302			7.0	0.87
303			>20	
304			1.2	4.9

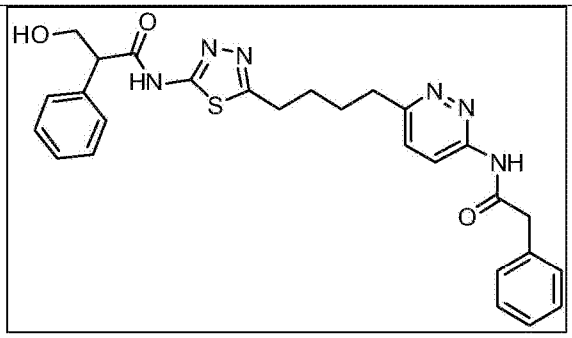
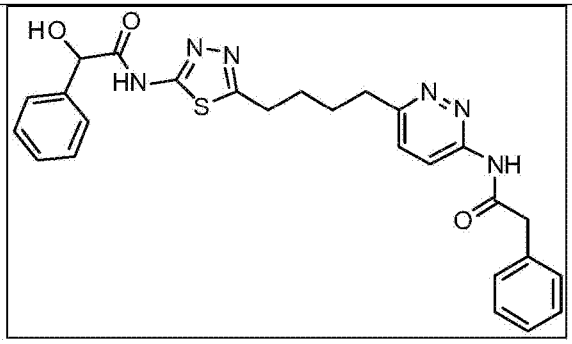
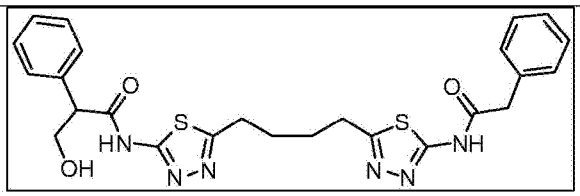
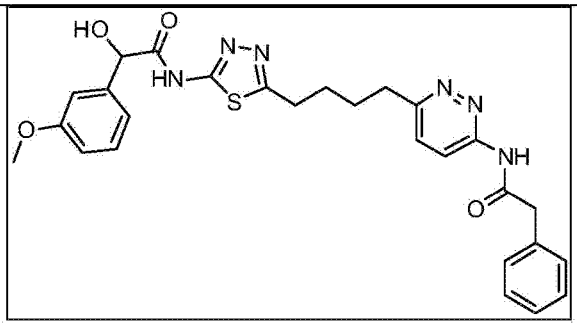
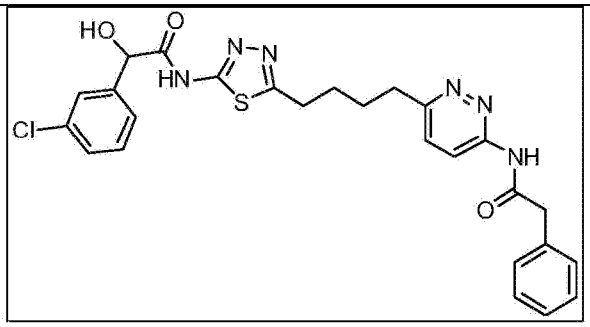
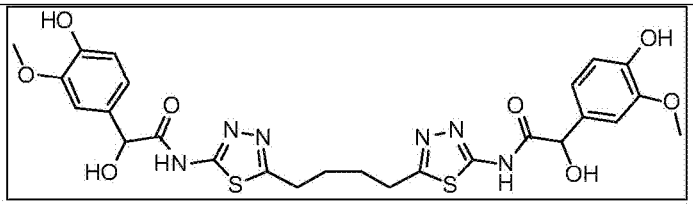
[0915]

305			>20	102
1038			0.080	1.5
306		0.031	0.52	0.066
307			6.4	9.3
308			0.60	1.2
309			0.11	0.18

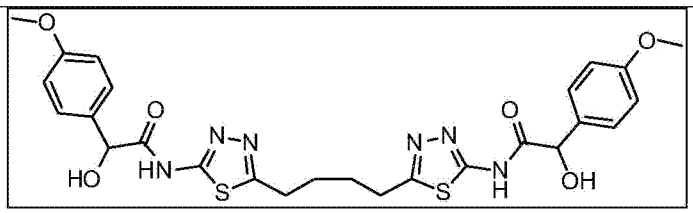
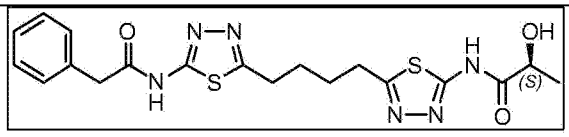
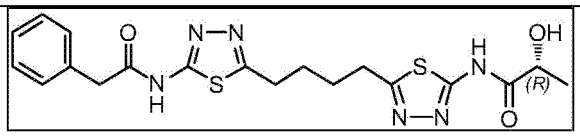
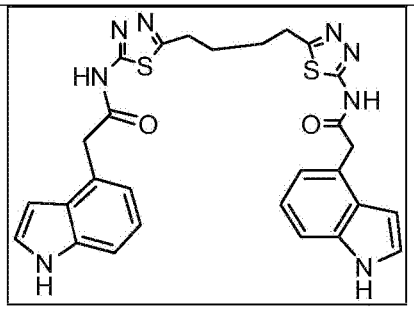
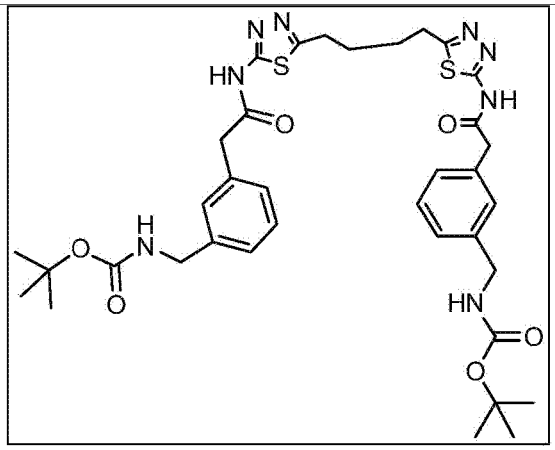
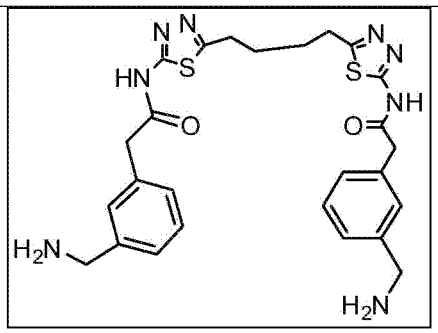
[0916]

310			0.083	0.12
311			0.20	22.
312			>20	N/D
313			0.27	94

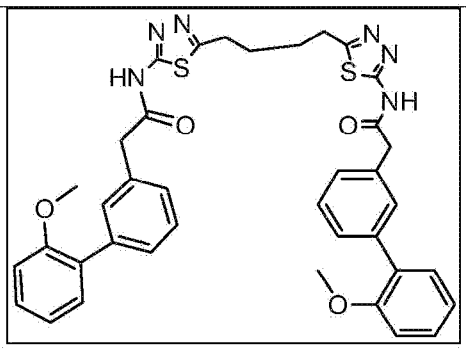
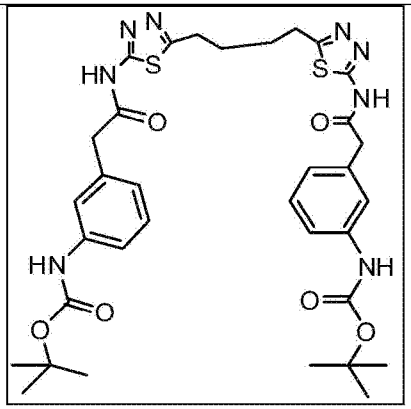
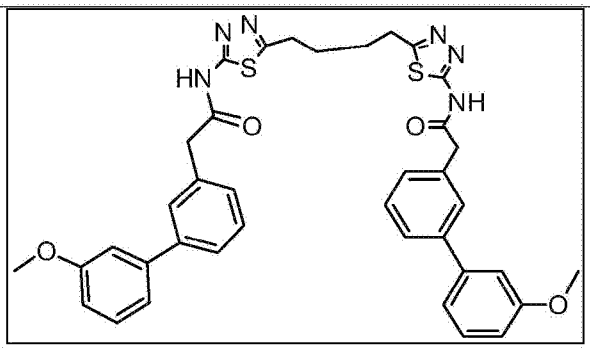
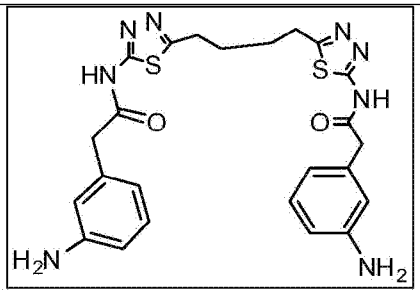
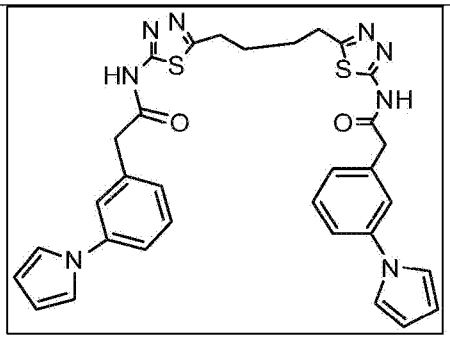
[0917]

314			0.14	0.048
315		0.017	0.12	0.035
316			0.19	0.075
317		0.007	0.18	0.010
318		0.006	0.18	0.017
319			0.64	10

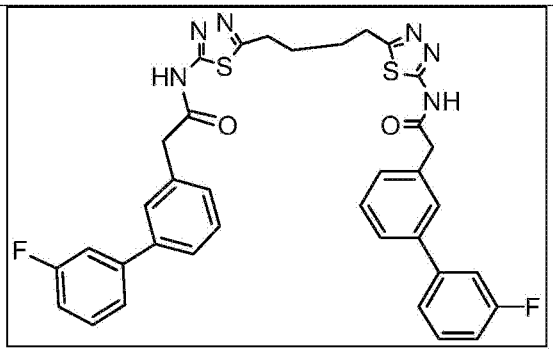
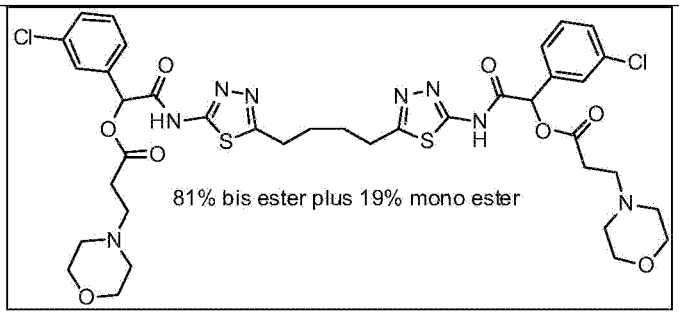
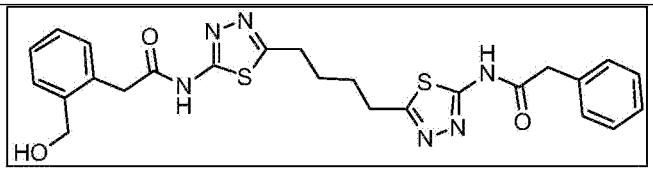
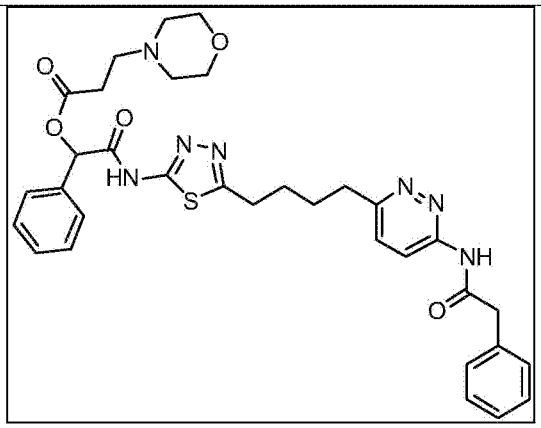
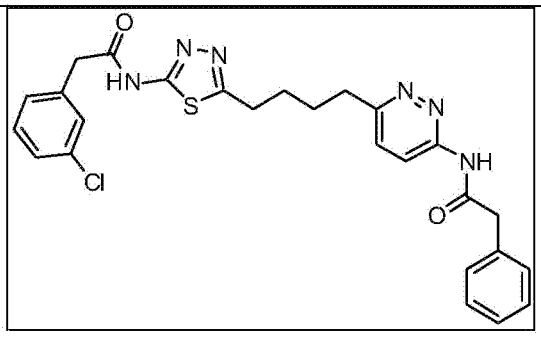
[0918]

320			0.40	0.19
321			2.5	2.6
322			2.8	3.0
323			0.056	0.20
324		0.011	4.6	0.10
325		0.17	0.66	0.030

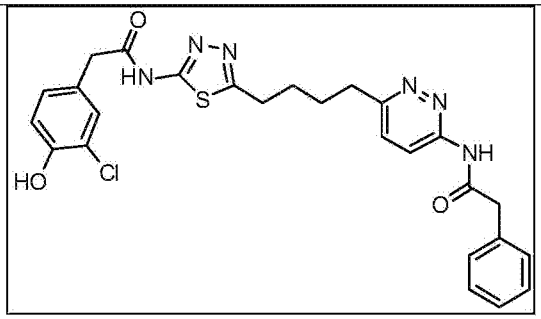
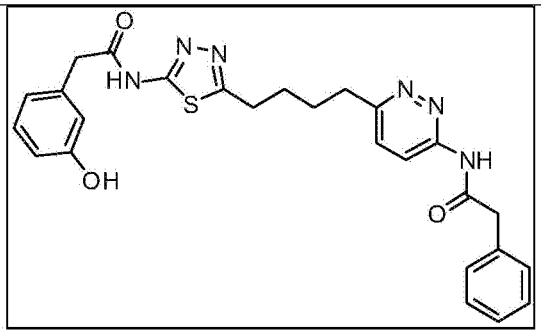
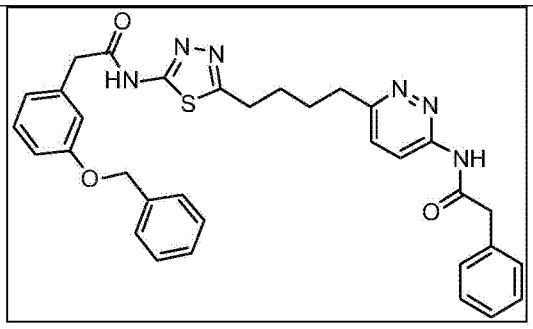
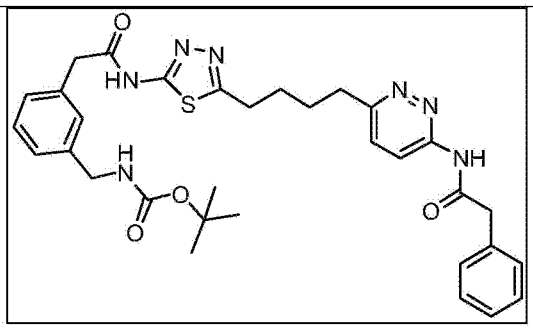
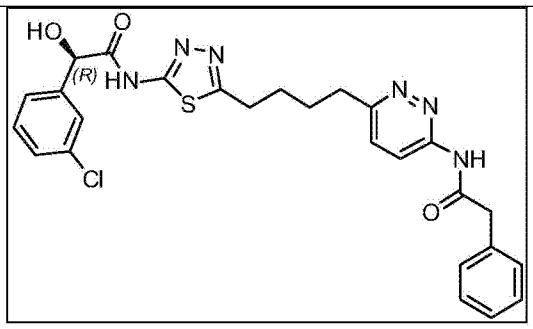
[0919]

326			>20	N/D
327			>20	0.15
328			>20	N/D
329			0.17	0.45
330			>20	N/D

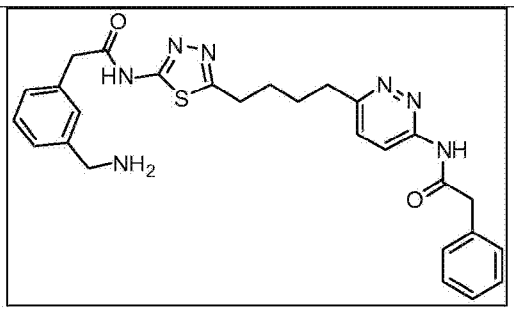
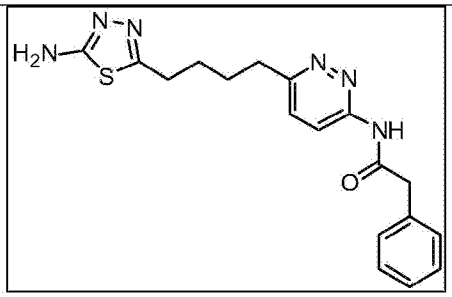
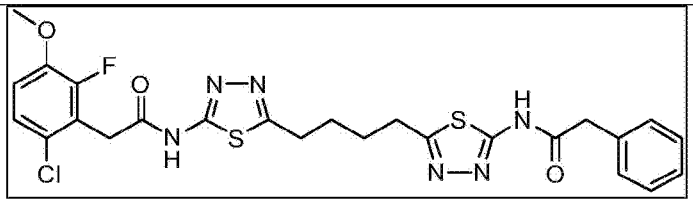
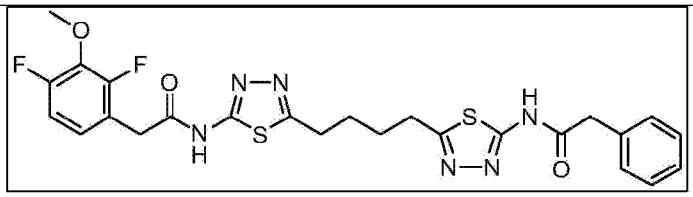
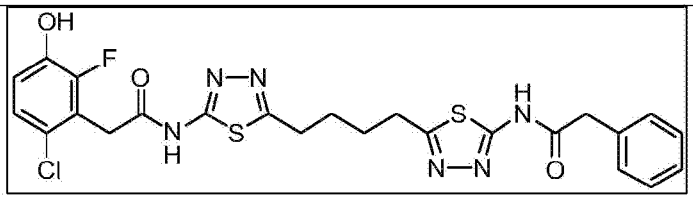
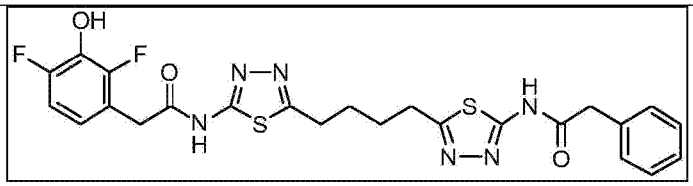
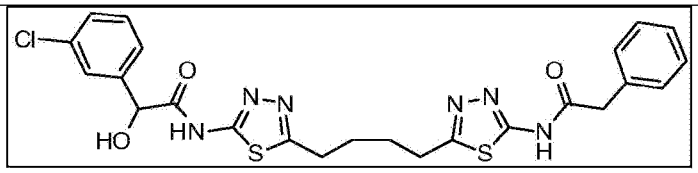
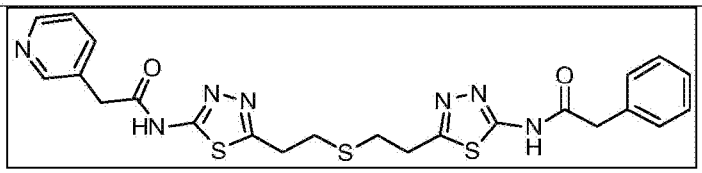
[0920]

331			>20	N/D
332			3.3	0.087
333			0.10	1.6
334			0.64	0.030
335			0.062	0.050

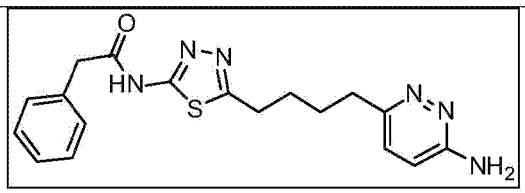
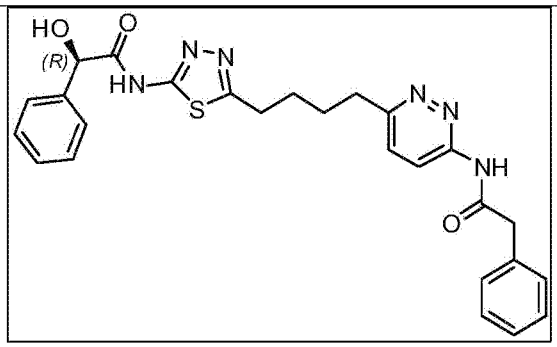
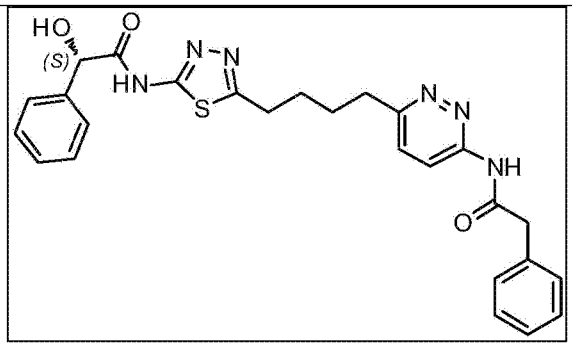
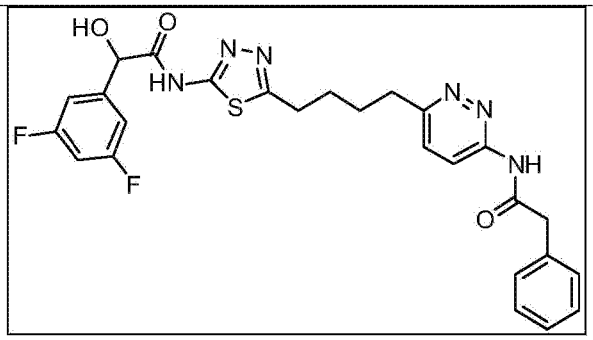
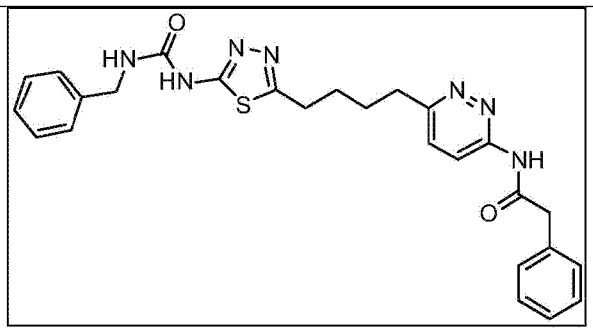
[0921]

336			0.068	0.052
337			0.073	0.021
338			0.15	0.043
339		0.005	0.16	0.009
340			0.096	0.038

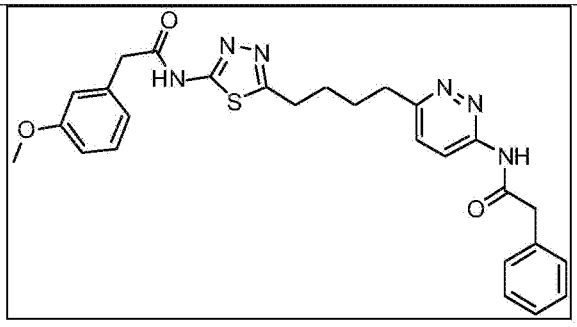
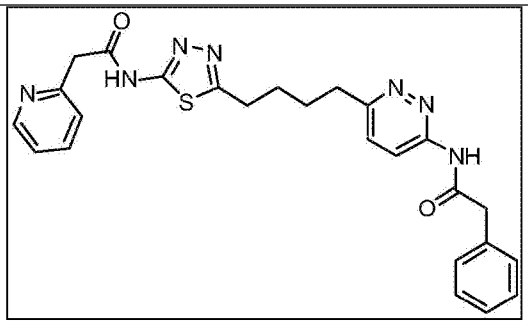
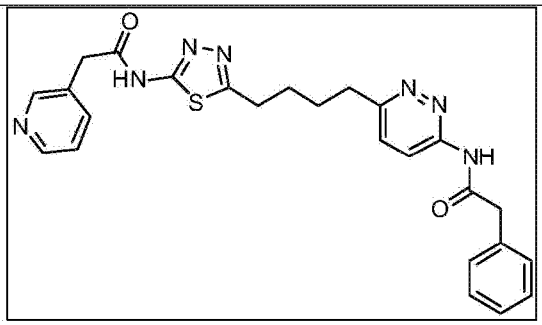
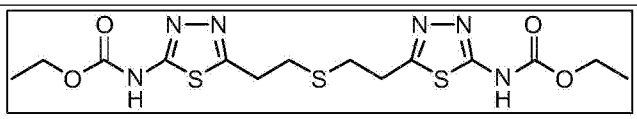
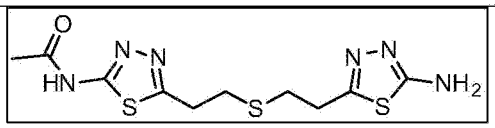
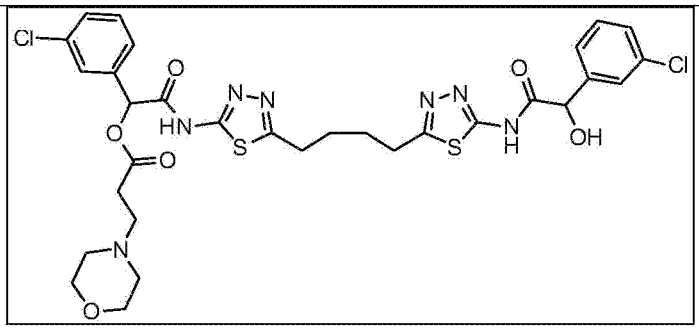
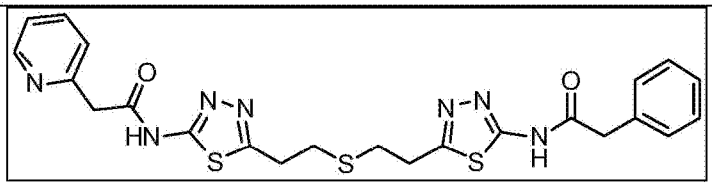
[0922]

341		0.013	0.13	0.039
342			1.4	2.7
343			0.16	0.25
344				0.088
345			0.16	0.24
346			0.12	0.087
527		0.024	0.13	0.098
347			0.22	0.71

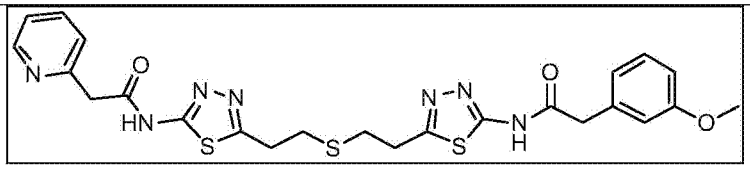
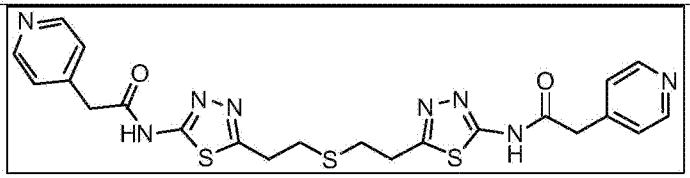
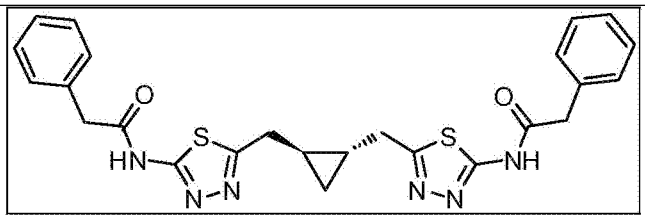
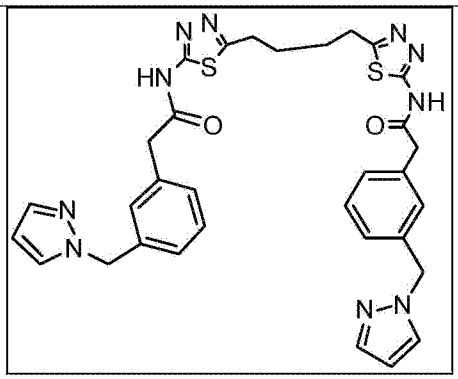
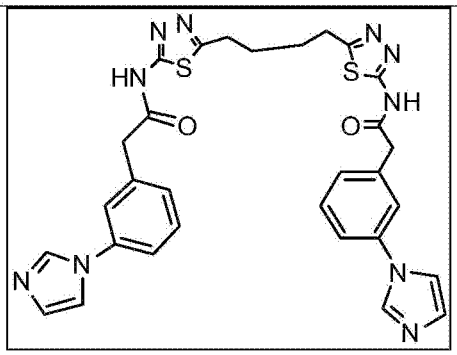
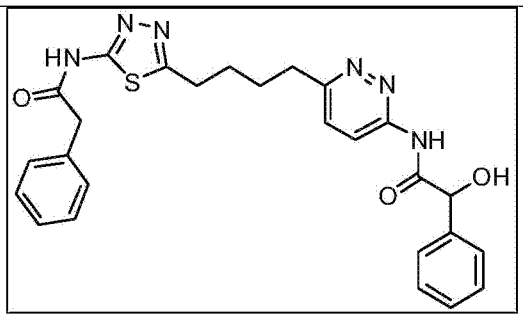
[0923]

348			1.0	1.7
349			0.12	0.12
350			0.079	0.029
351			0.11	0.049
352			0.069	0.13

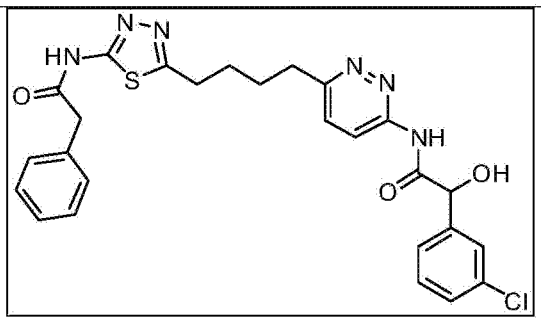
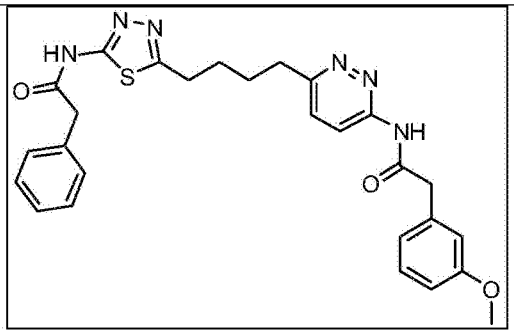
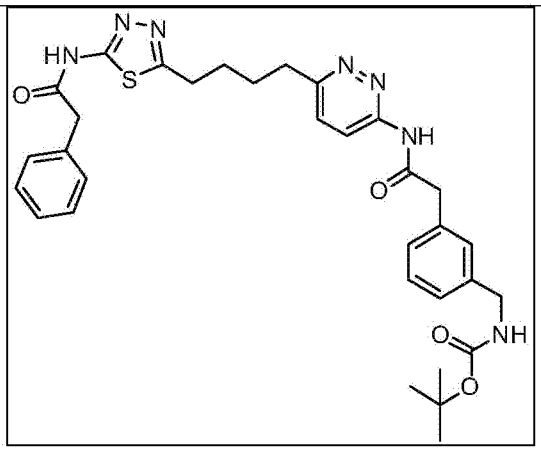
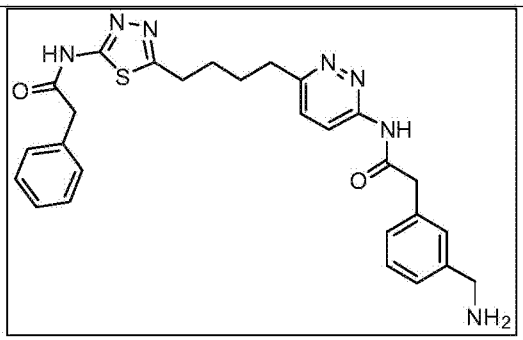
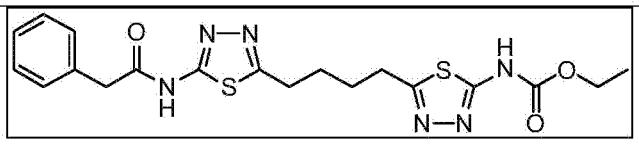
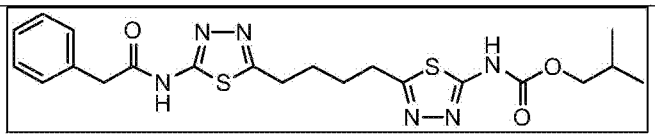
[0924]

353			0.049	0.021
354			0.10	0.047
355			0.10	0.039
356			>20	N/D
357			>20	N/D
358			1.4	0.11
359			0.38	0.91

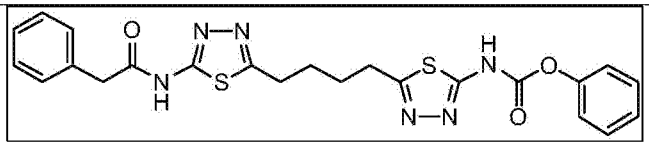
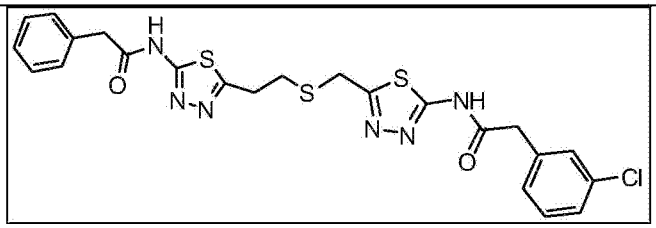
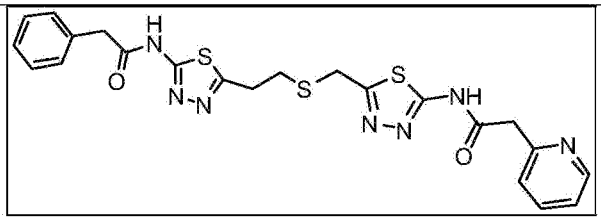
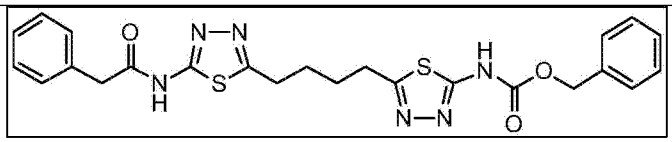
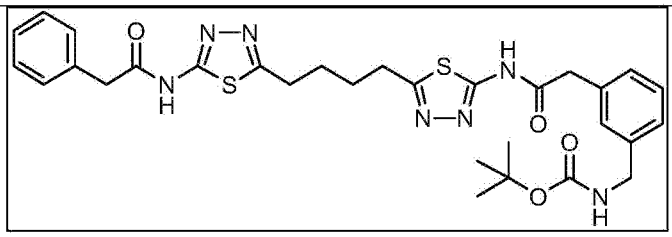
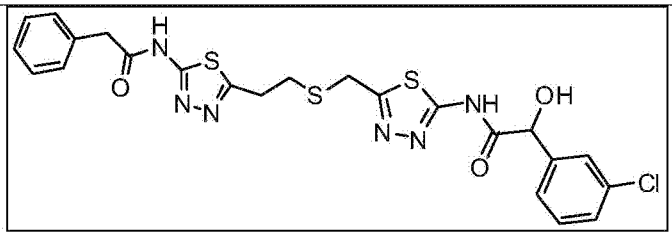
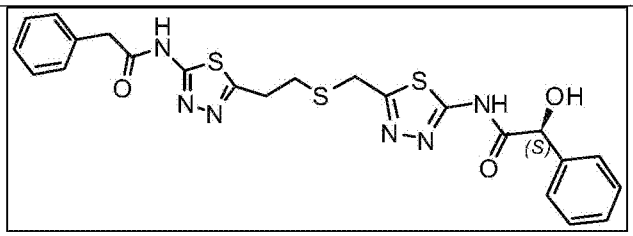
[0925]

360			0.28	0.67
361			1.8	>20
1035			>20	N/D
362			0.35	0.054
363			0.065	>20
364		0.030	0.15	0.26

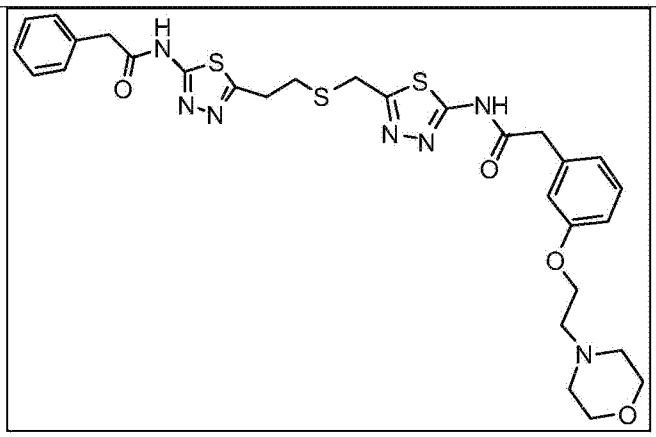
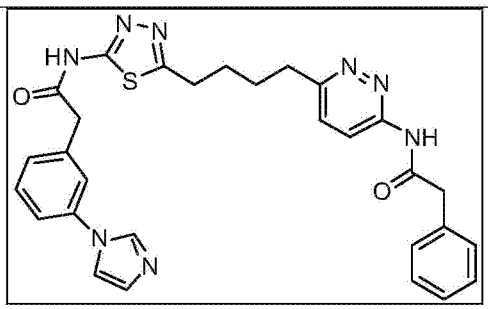
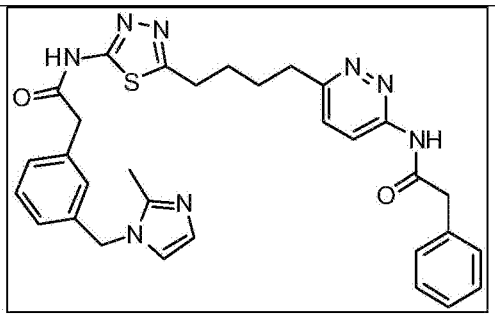
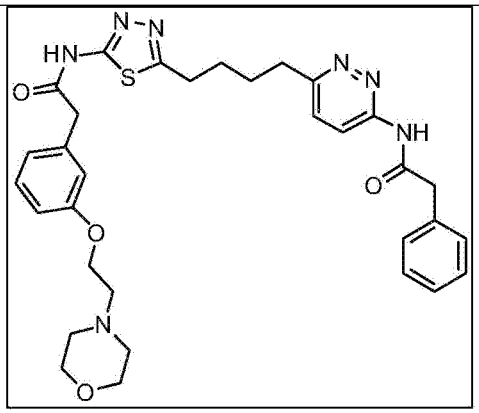
[0926]

365		0.009	0.092	0.089
366			0.074	0.024
367		0.002	0.12	0.006
368		0.009	0.11	0.017
369			0.81	1.9
370			0.28	0.70

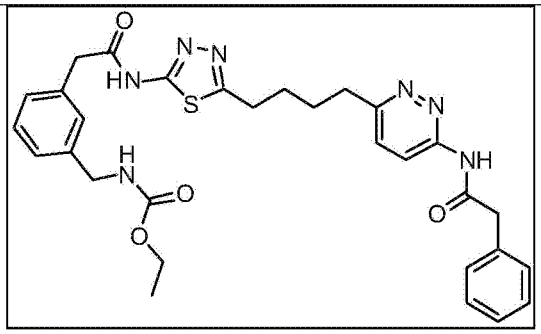
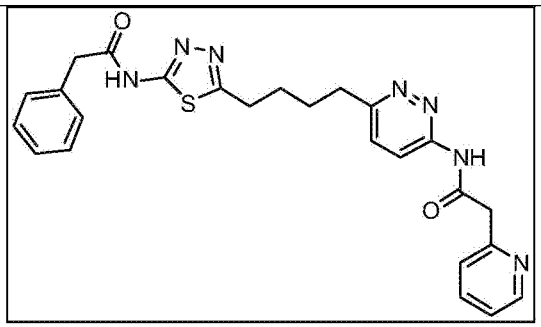
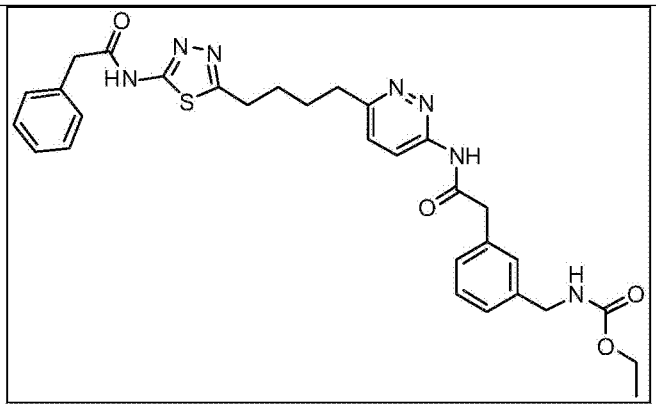
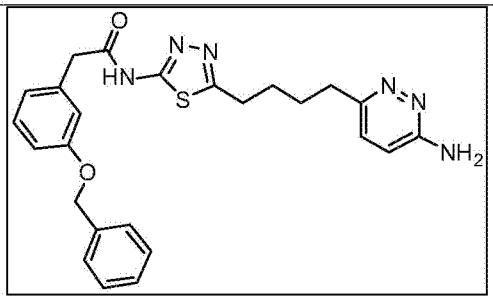
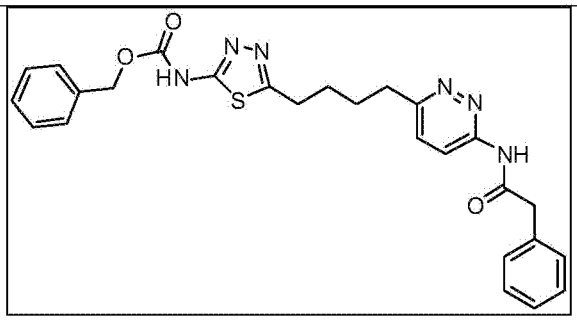
[0927]

371			0.43	5.2
372			0.16	0.15
373			0.17	0.28
374			0.26	0.47
375		0.005	0.38	0.041
376			0.35	0.091
377			0.28	0.10

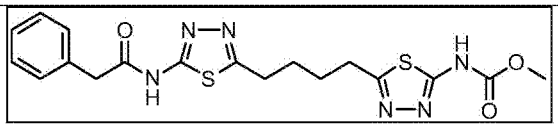
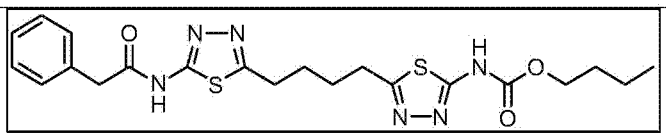
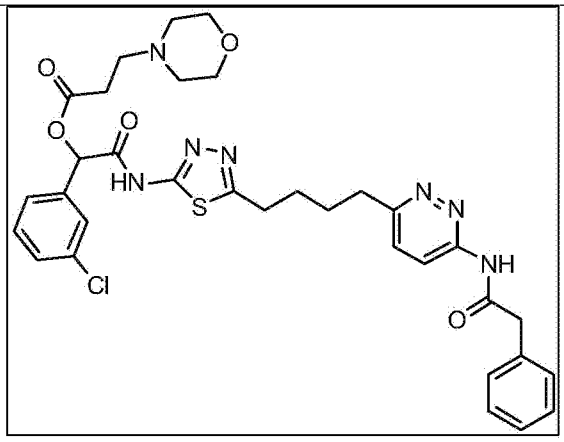
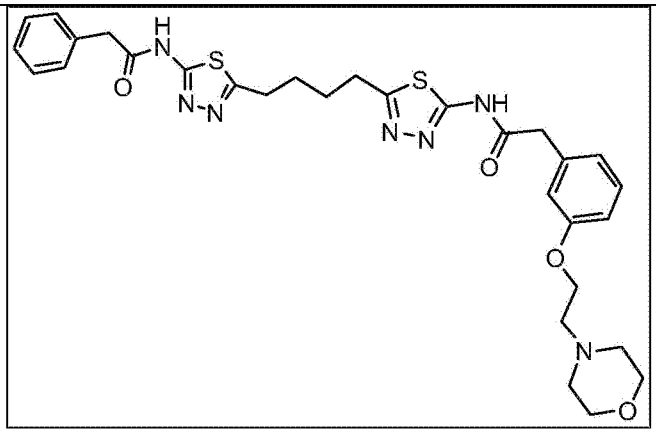
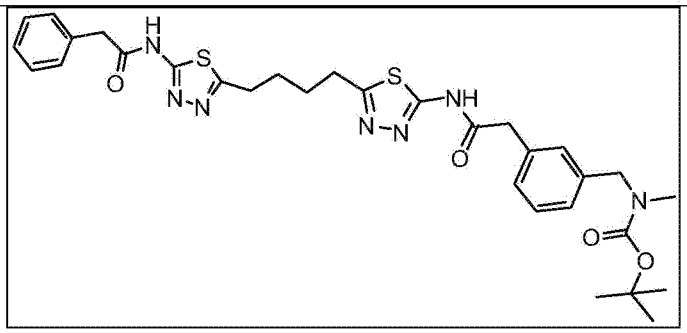
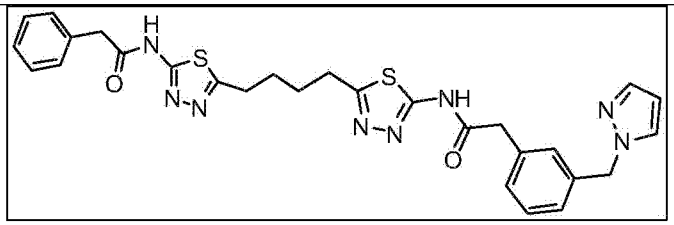
[0928]

378			0.22	0.090
379			0.097	0.038
380			0.12	0.019
381			0.16	0.018

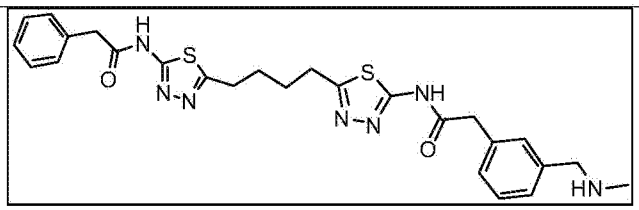
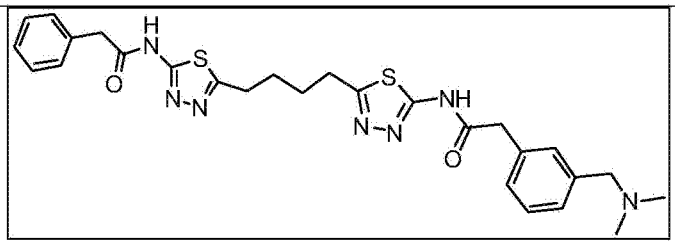
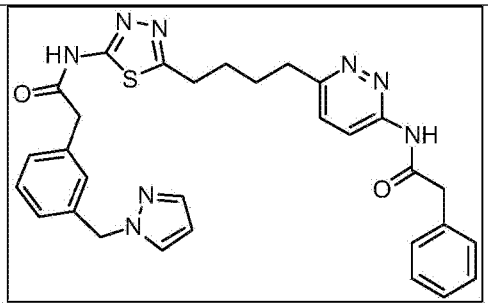
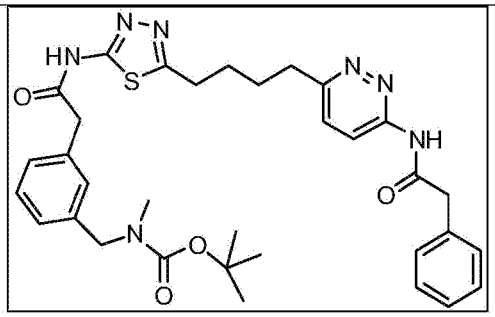
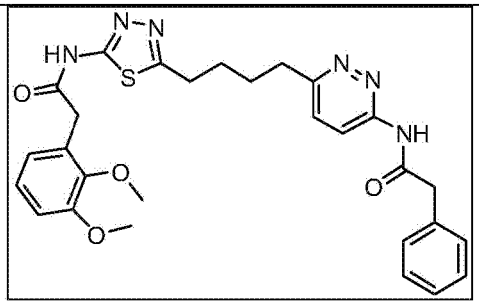
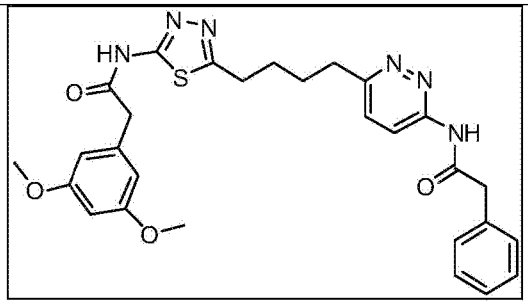
[0929]

382		0.003	0.099	0.007
383			0.086	0.022
384		0.003	0.081	0.005
385			0.26	0.72
386			0.085	0.15

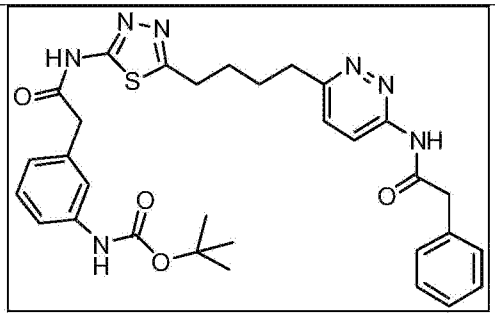
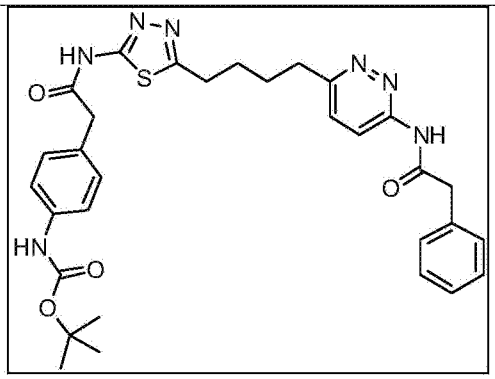
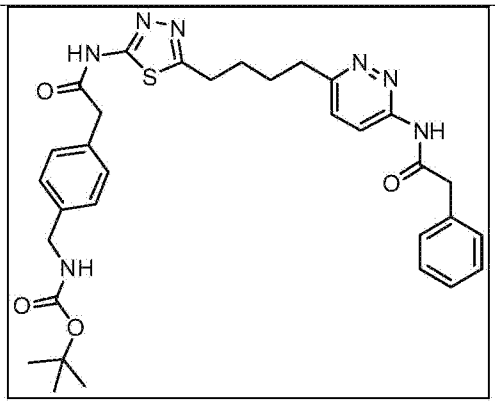
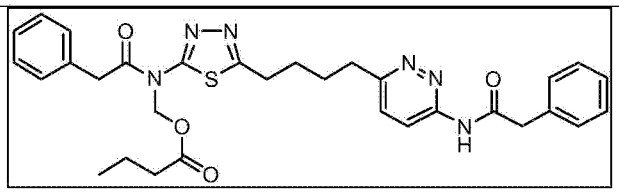
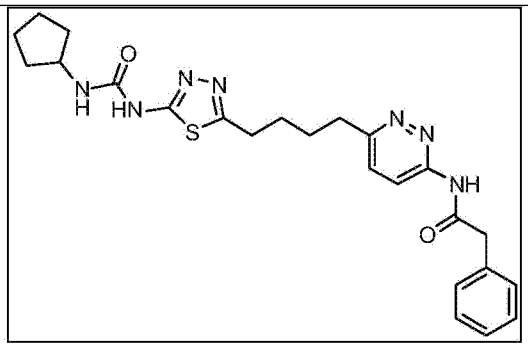
[0930]

387			1.2	2.3
388			0.21	0.75
389		0.084		0.032
390		0.042		0.16
391		0.007		0.027
392		0.014		0.072

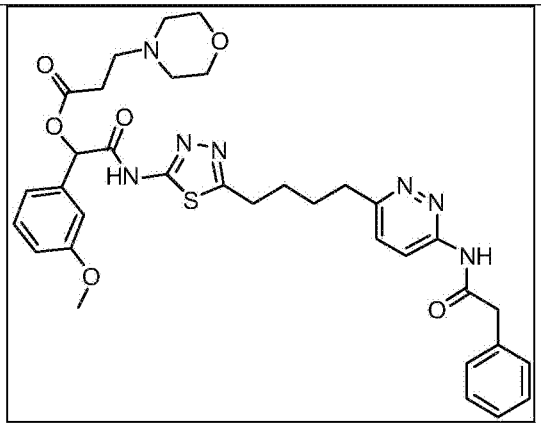
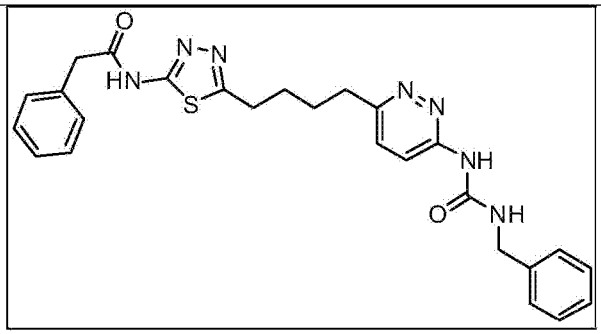
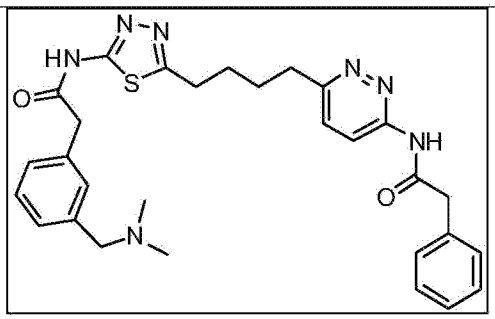
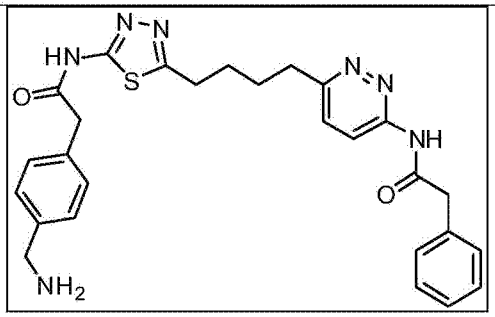
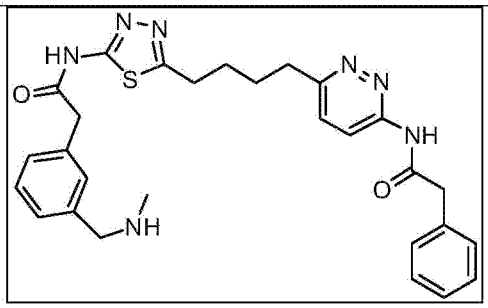
[0931]

393		0.10		0.90
394		0.088		1.2
395		0.004		0.015
396		0.004		0.005
397		0.008		0.041
398		0.004		0.023

[0932]

399		0.005		0.026
400		0.015		0.053
401		0.005		0.011
402		1.1		0.054
403		0.018		0.12

[0933]

404		0.060		0.022
405		0.081		0.67
406		0.016		0.27
407		0.012		0.044
408		0.018		0.19

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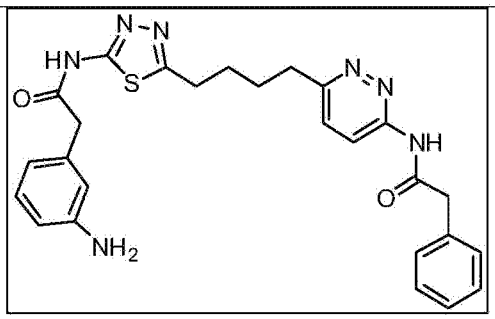
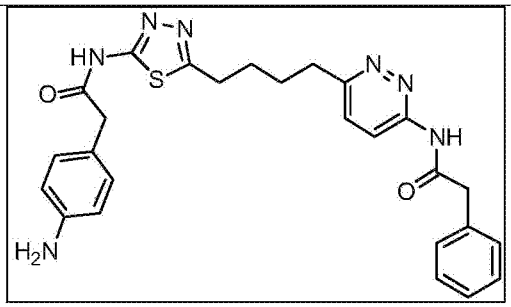
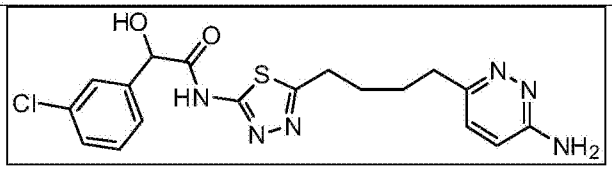
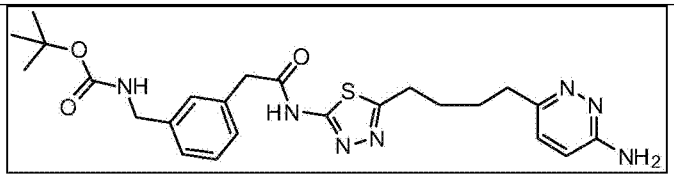
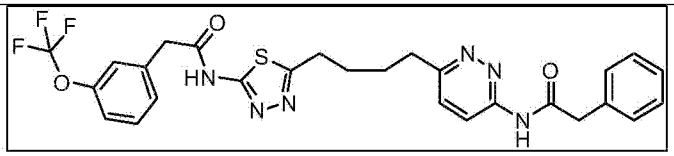
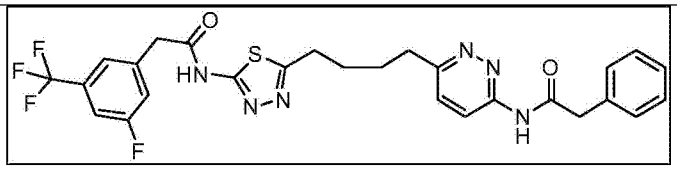
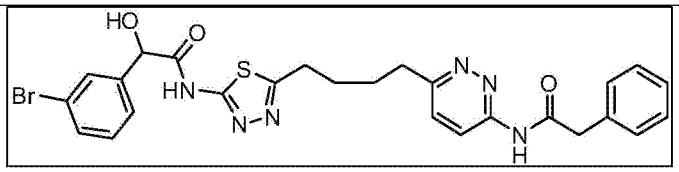
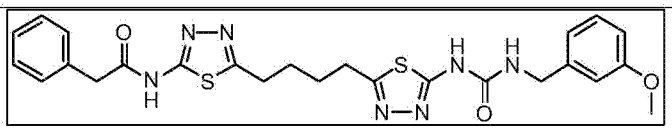
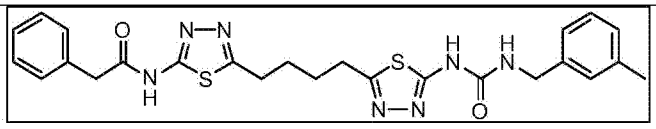
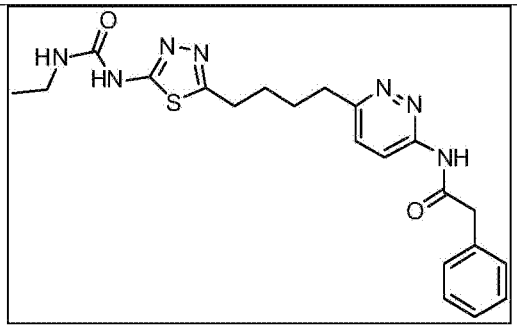
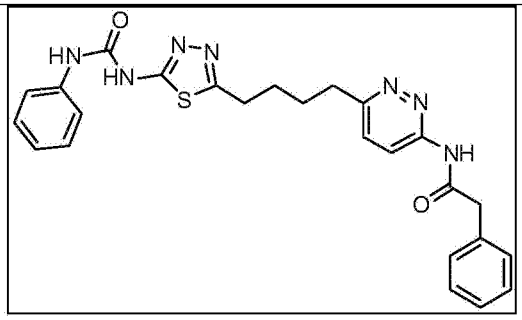
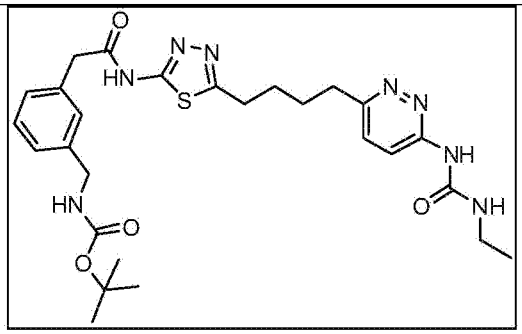
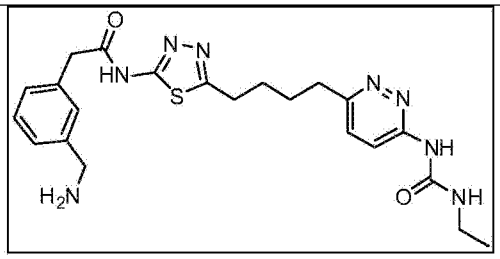
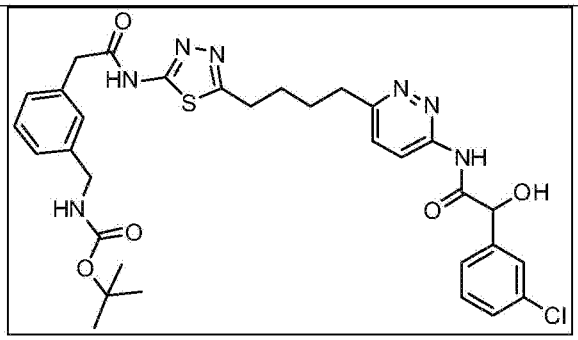
409		0.008		0.037
410		0.009		0.057
411		0.22		0.74
412		0.028		0.11
413		0.007		0.045
414		0.010		0.058
415		0.006		0.018
416		0.055		0.35
417		0.056		0.32

[0935]

418		0.14		0.32
419		0.024		0.064
420		0.013		0.070
421		0.29		0.16
422		0.007		0.006

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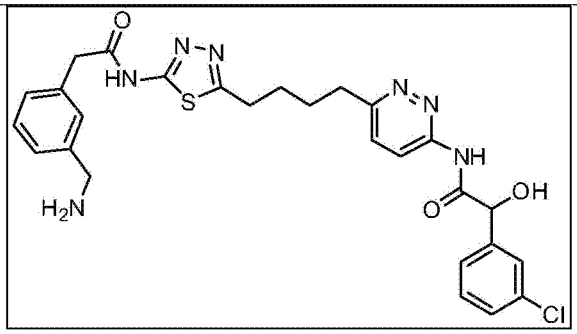
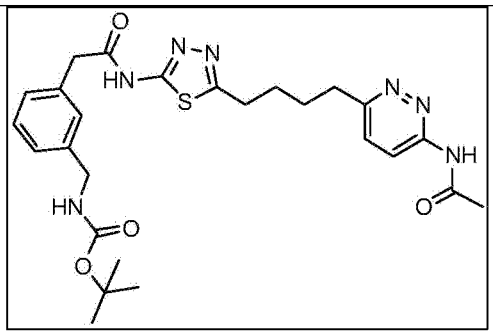
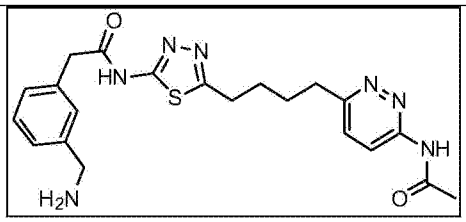
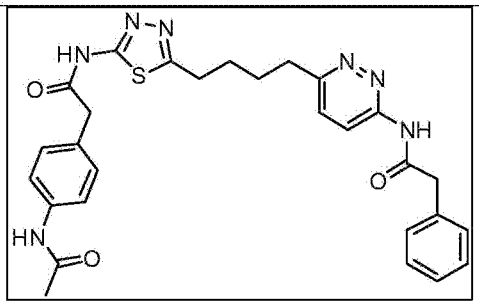
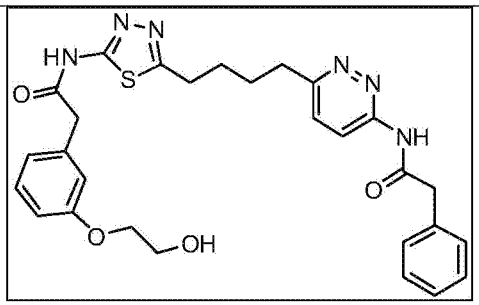
423		0.022		0.042
424		0.006		0.008
425		0.086		0.015
426		0.011		0.033
427		0.007		0.027

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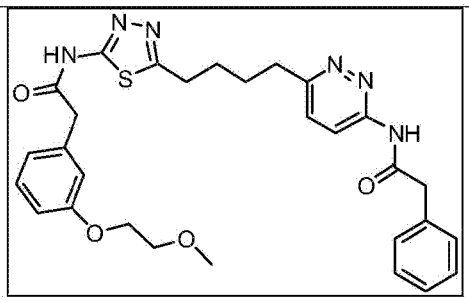
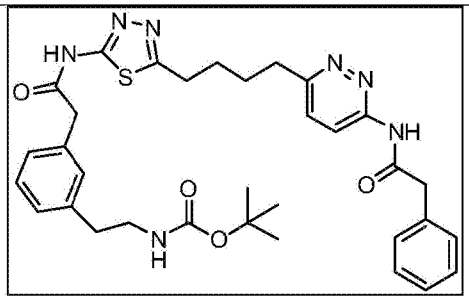
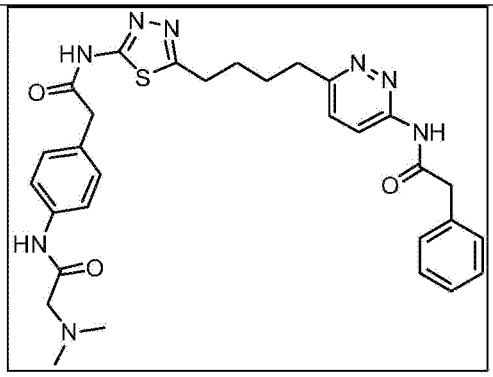
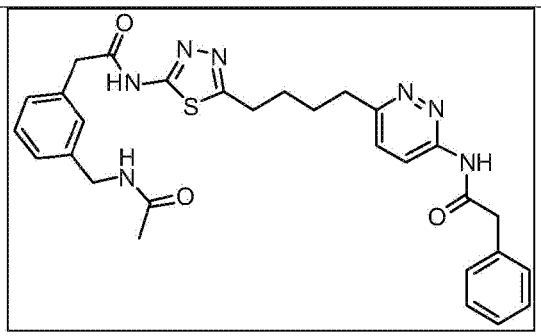
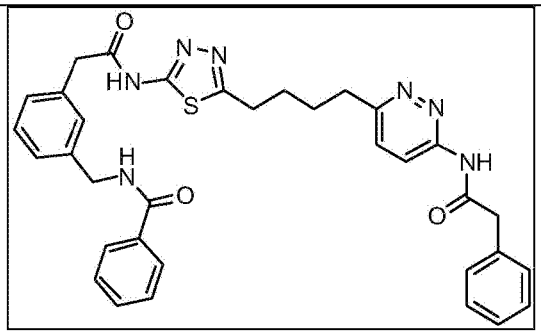
428		0.007		0.019
429		0.004		0.007
430		0.009		0.027
431		0.007		0.026
432		0.002		0.004

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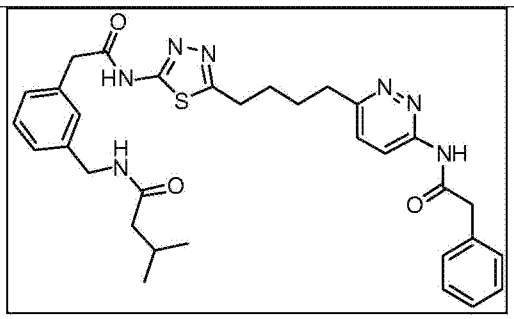
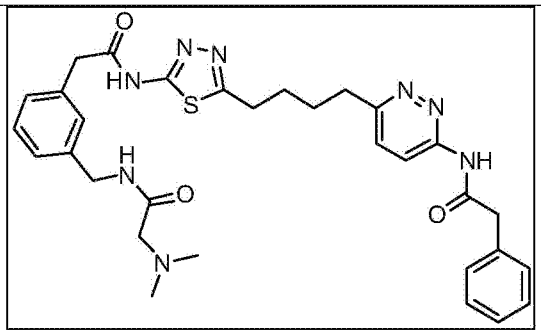
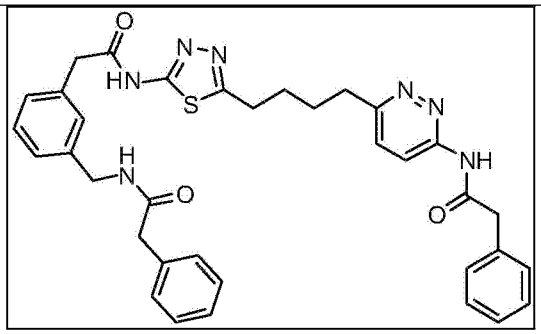
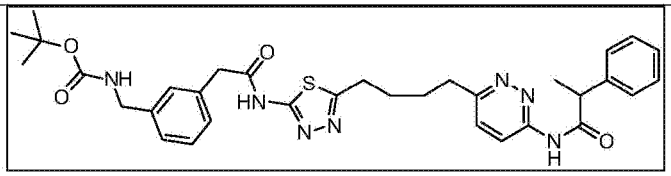
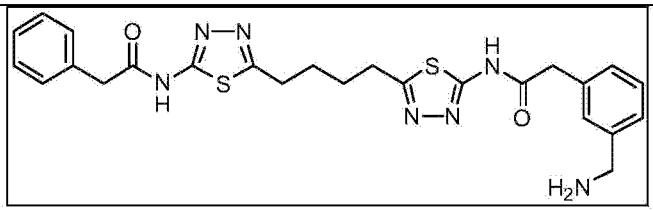
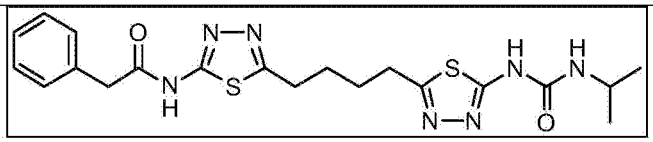
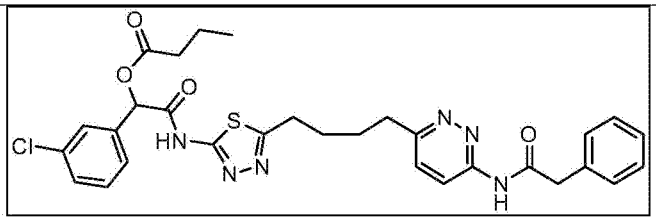
433		0.002		0.007
434		0.005		0.017
435		0.002		0.006
436		0.006		0.010
437		0.070		0.072
438		0.74		0.88
439		0.25		0.056

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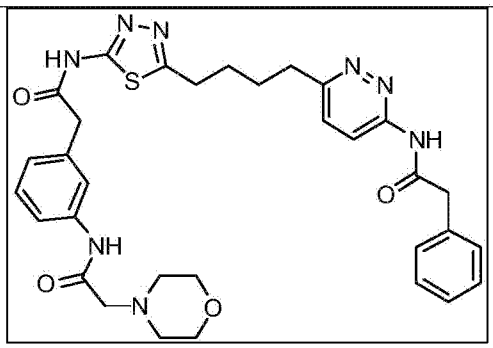
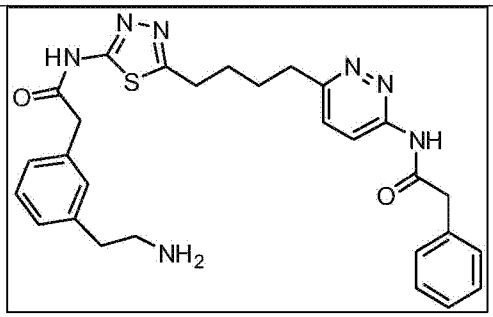
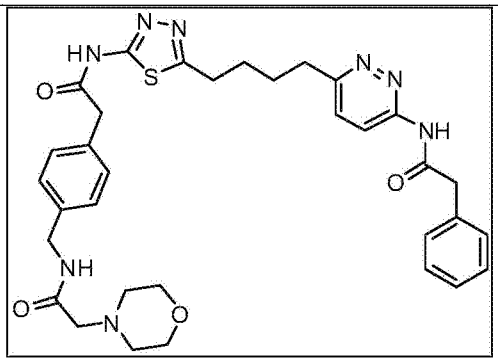
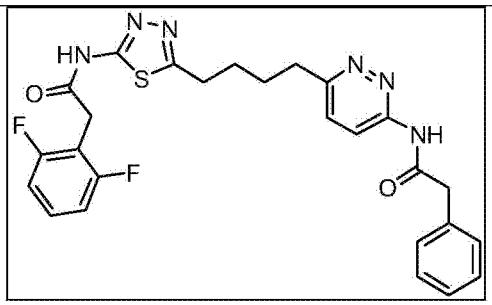
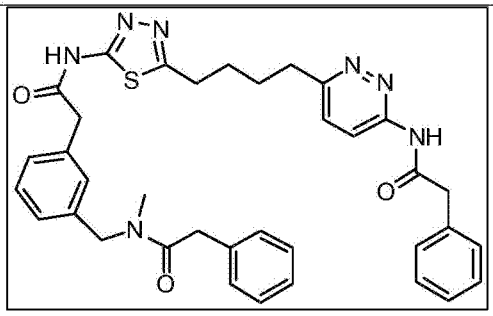
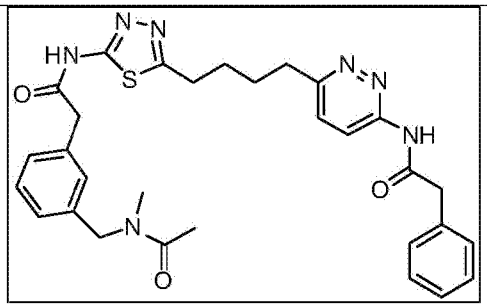
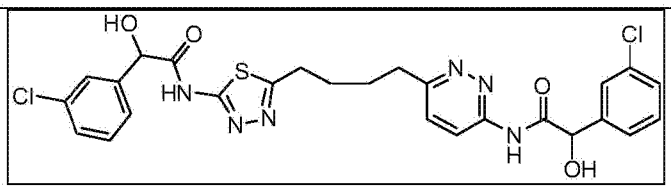
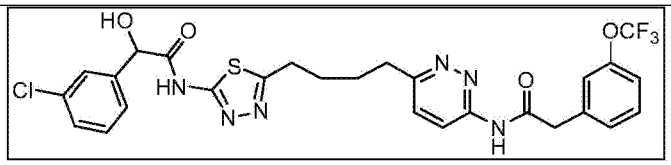
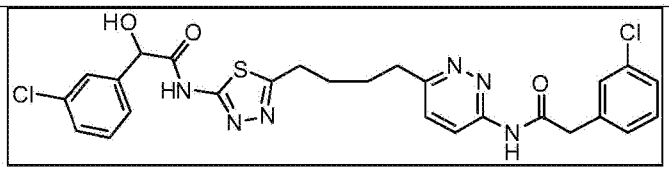
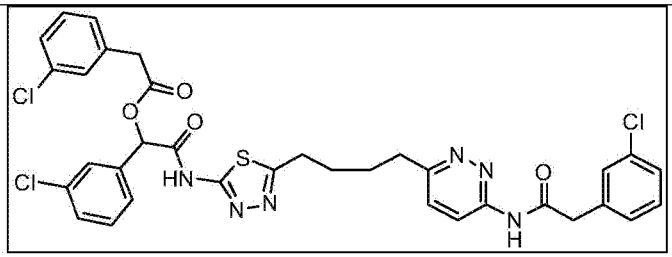
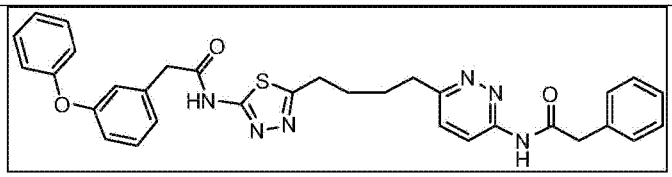
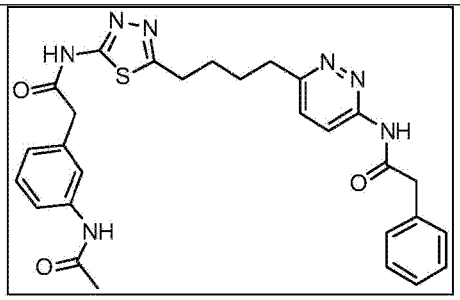
440		0.008		0.031
441		0.011		0.18
442		0.007		0.025
443		0.011		0.10
444		0.003		0.008

[0940]

445		0.004		0.022
446		0.011		0.15
447		0.005		0.016
448		0.005		0.051
449		0.11		0.12
450		0.006		0.042
451		0.003		0.056

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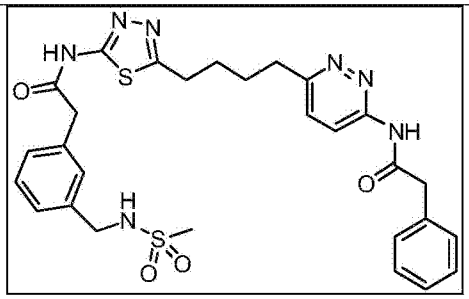
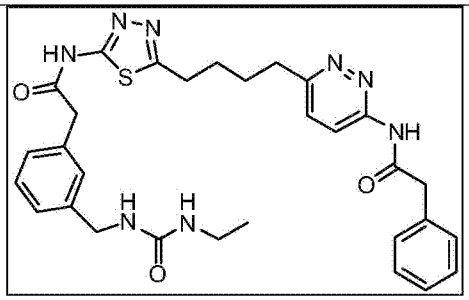
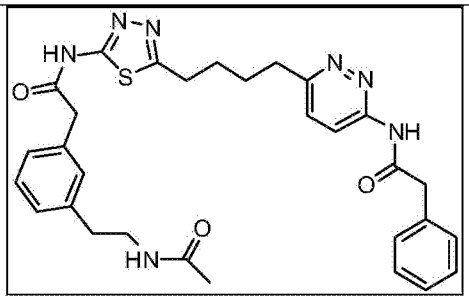
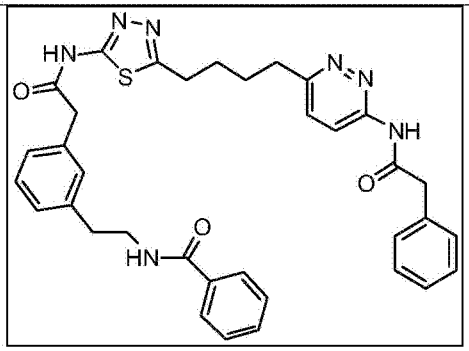
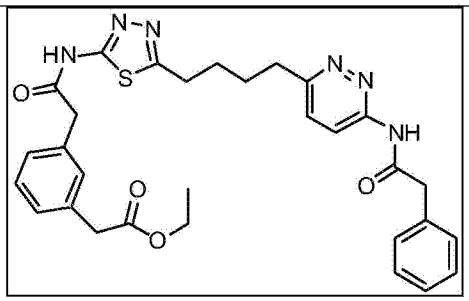
452		0.004		0.049
453		0.003		0.015
454		0.006		0.13
455		0.003		0.012
456		0.003		0.024

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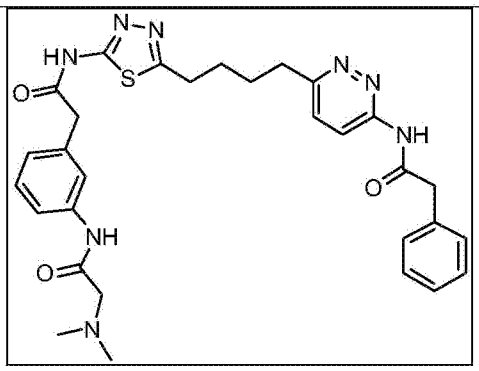
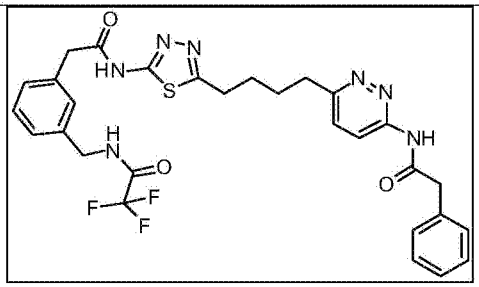
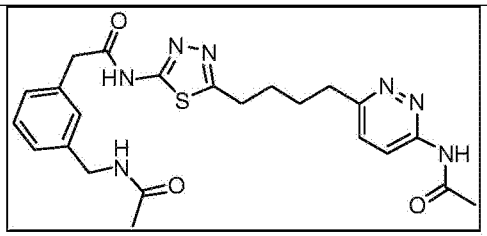
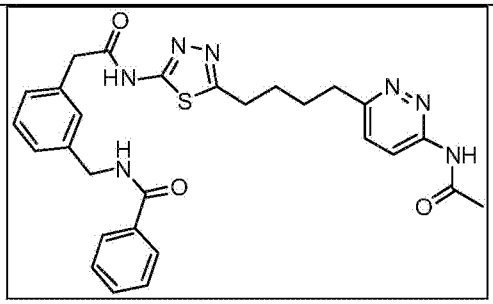
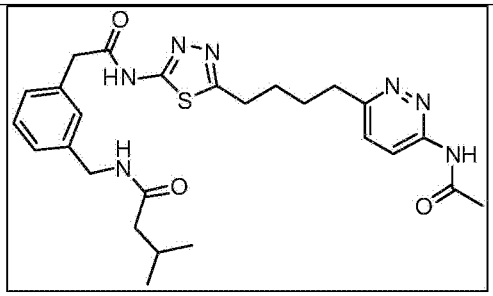
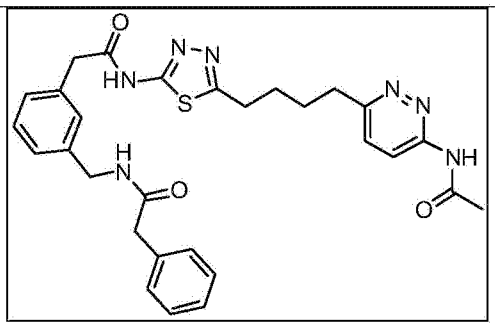
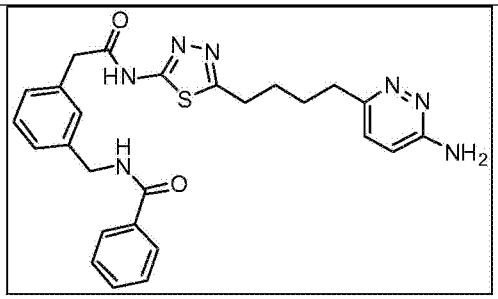
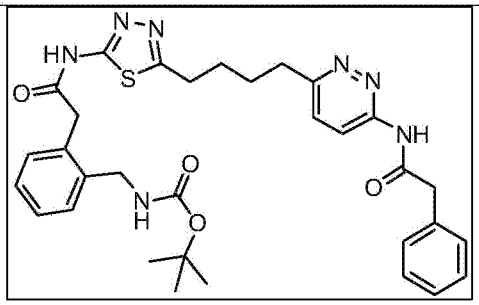
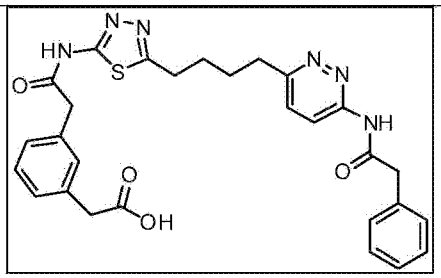
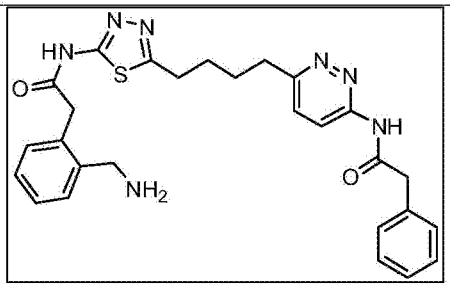
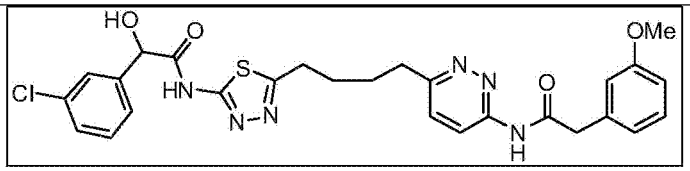
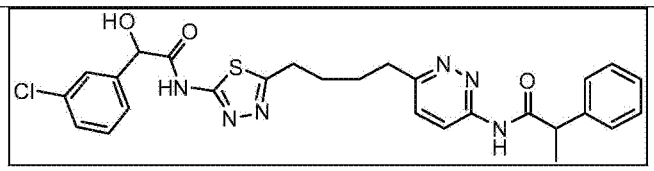
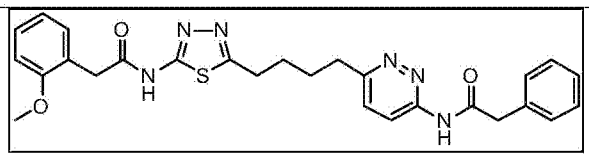
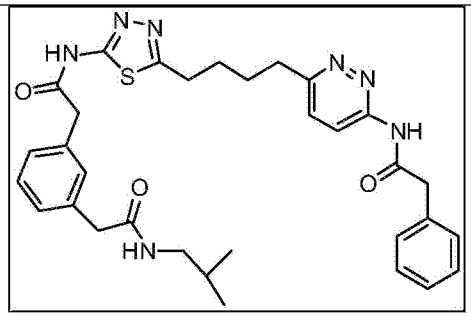
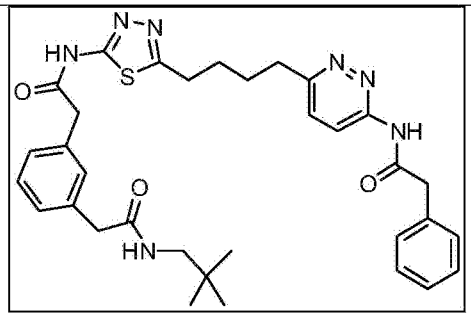
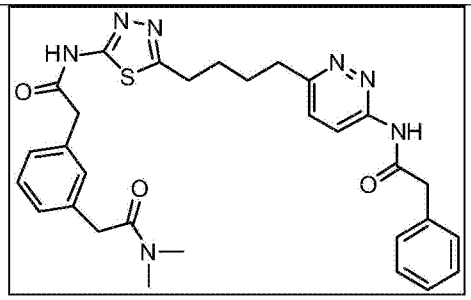
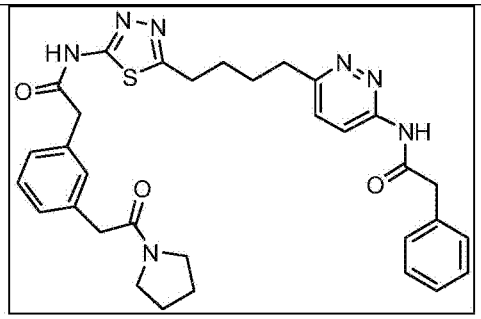
457		0.009		0.11
458		0.003		0.013
459		0.048		0.57
460		0.005		0.031
461		0.011		0.062

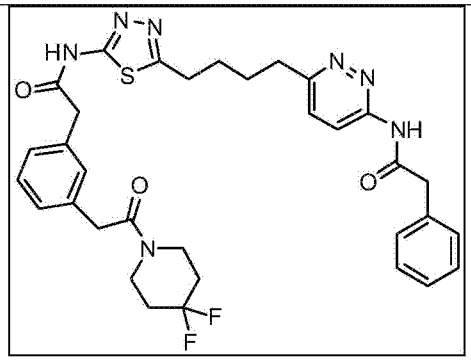
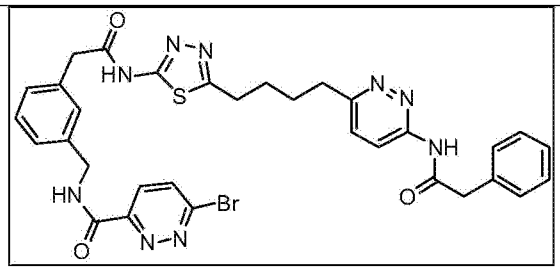
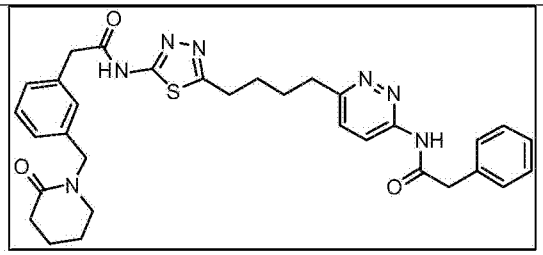
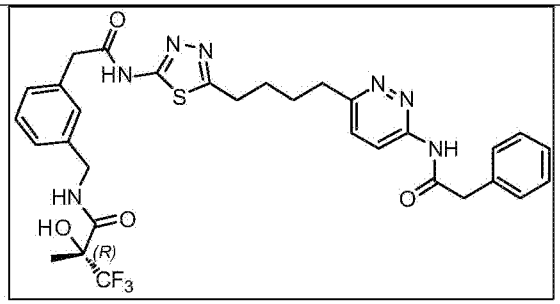
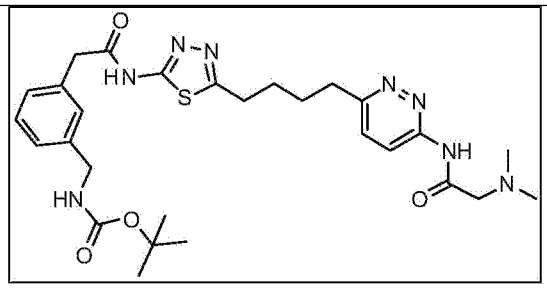
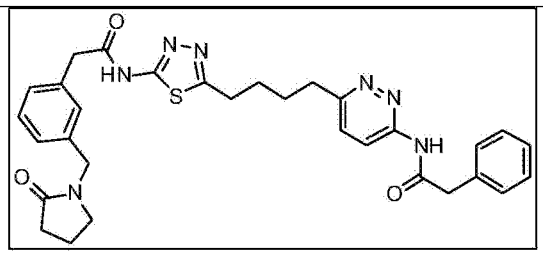
[0943]

462		0.006		0.053
463		0.052		0.96
464		0.005		0.059
465		0.006		0.92
466		0.051		1.3
467		0.005		0.047

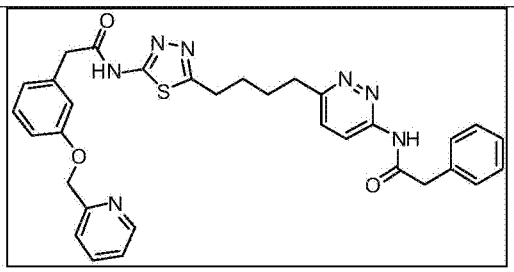
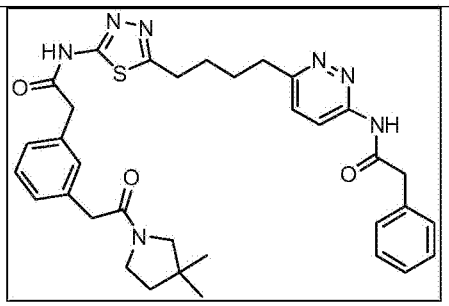
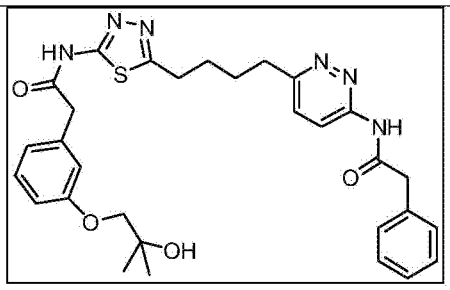
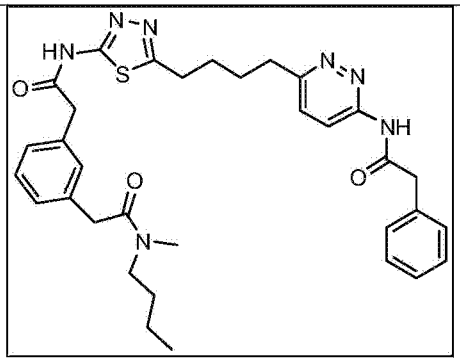
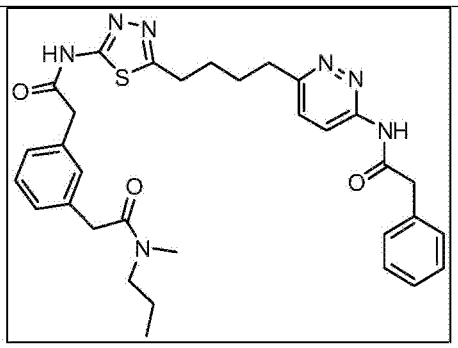
[0944]

468		0.016		0.27
469		0.007		0.049
470		0.003		0.009
471		0.003		0.006
472		0.006		0.024
473		0.002		0.006

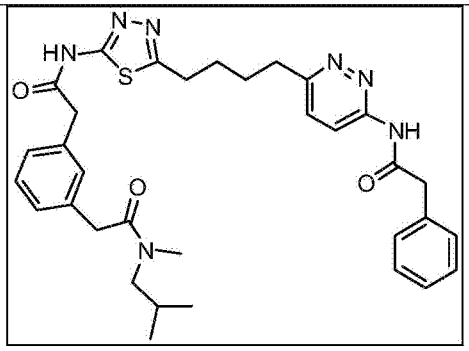
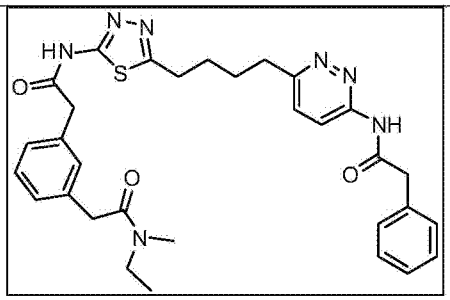
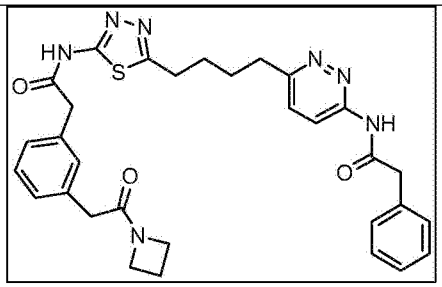
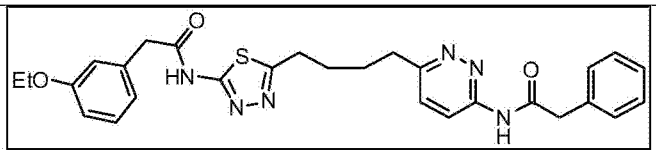
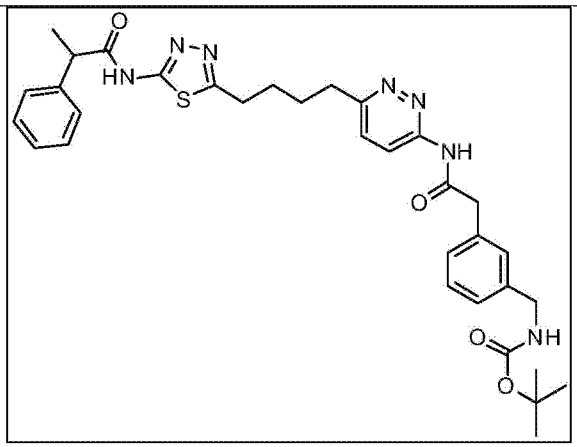
[0945]

474		0.003		0.004
475		0.002		0.003
476		0.004		0.012
477		0.005		0.015
478		0.018		0.046
479		0.005		0.030

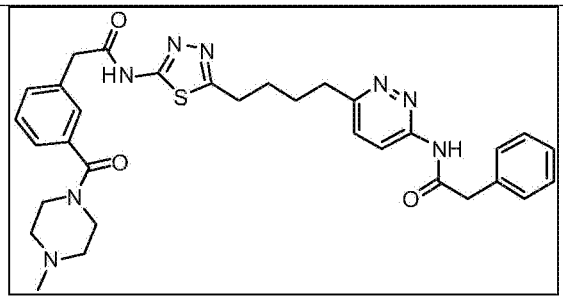
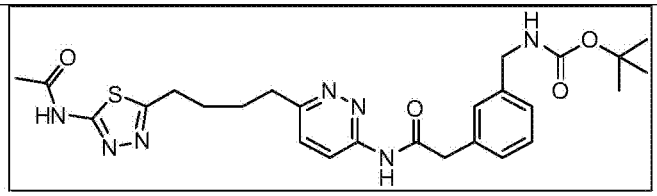
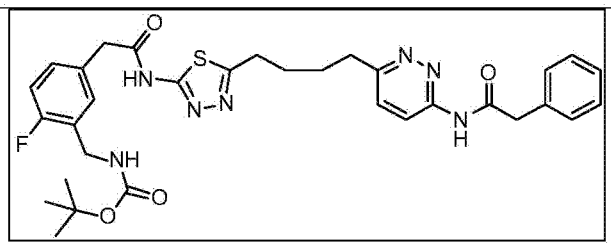
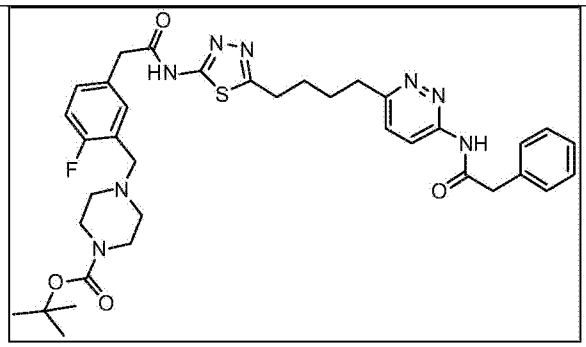
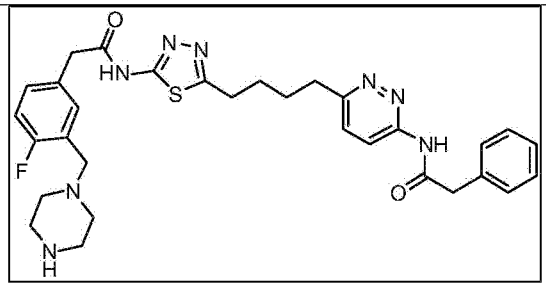
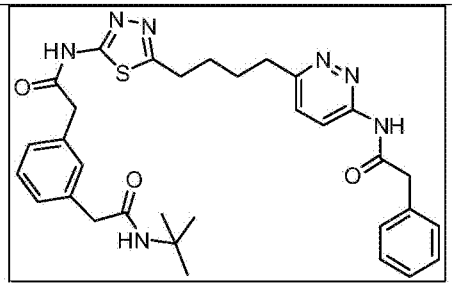
[0946]

480		>20		6.3
481		0.004		0.012
482		0.007		0.038
483		0.004		0.009
484		0.003		0.011

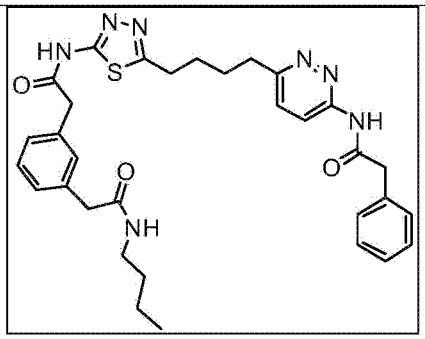
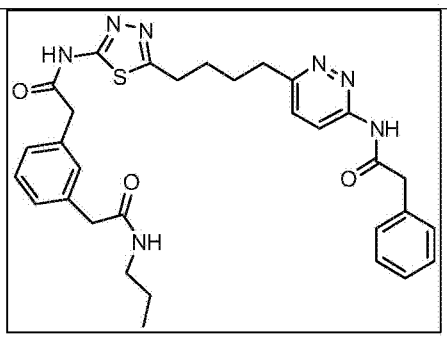
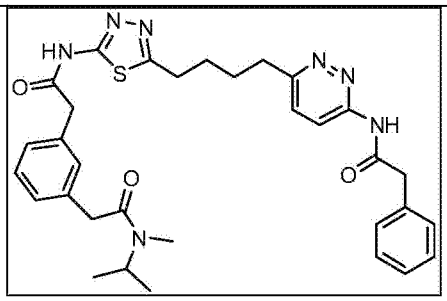
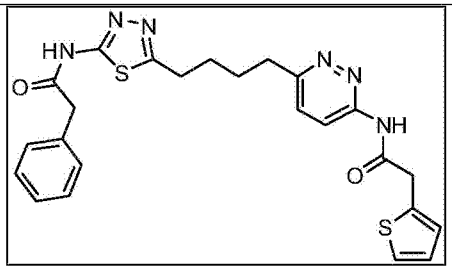
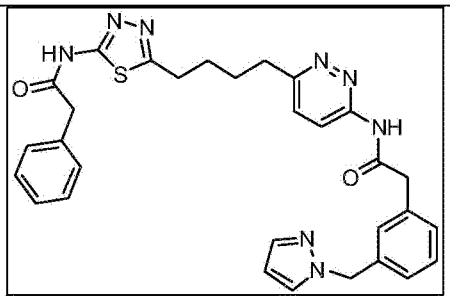
[0947]

485		0.004		0.012
486		0.004		0.024
487		0.005		0.042
488		0.32		1.9
489		0.008		0.023

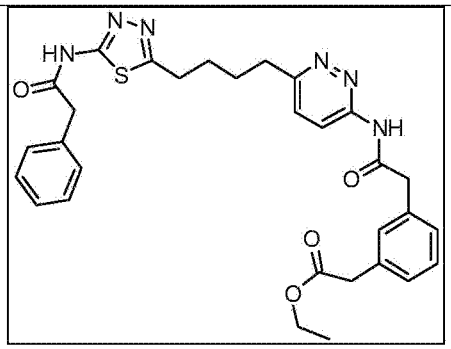
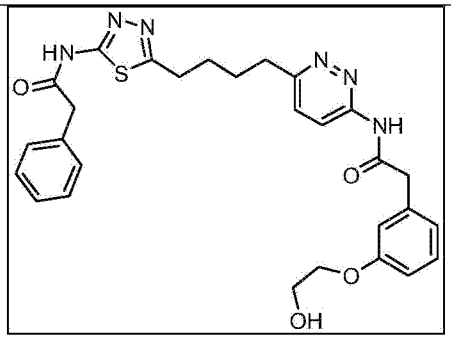
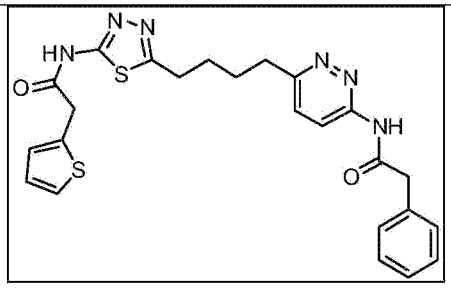
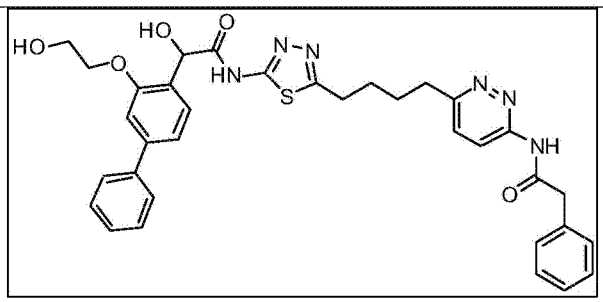
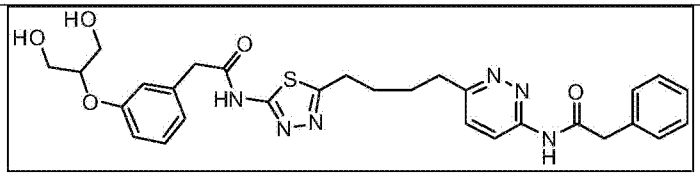
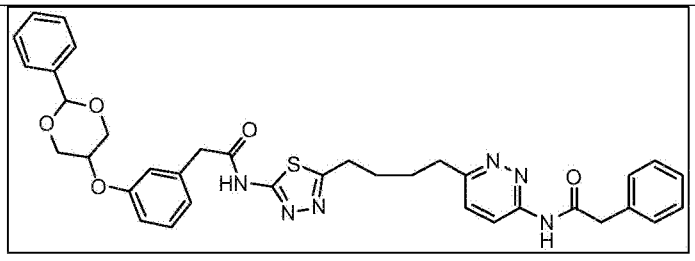
[0948]

490		0.011		0.25
491		0.008		0.023
492		0.006		0.014
493		0.019		0.057
494		0.019		0.58
495		0.005		0.014

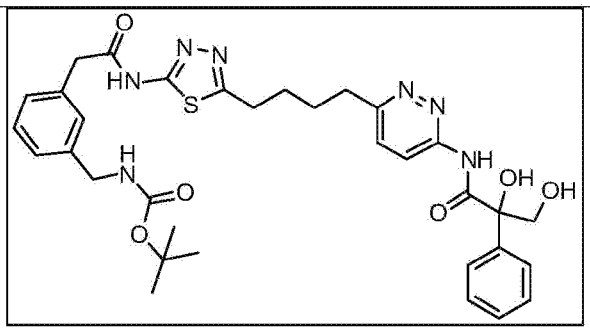
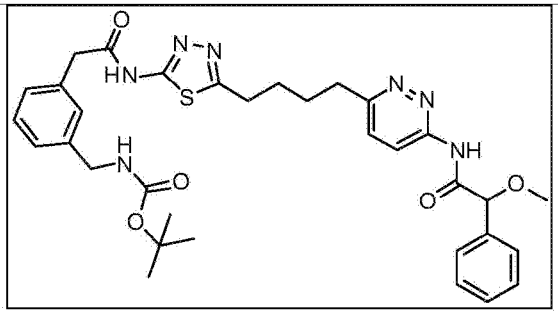
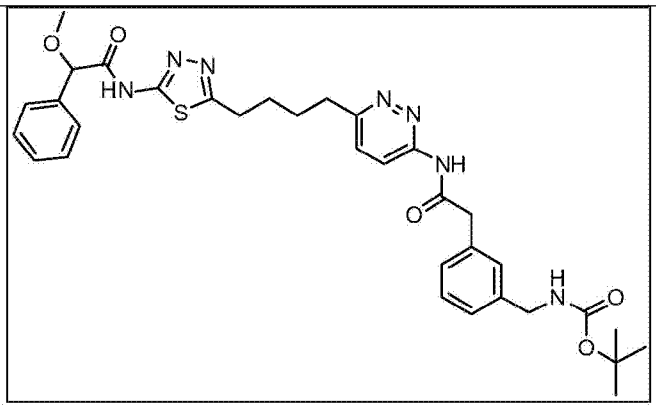
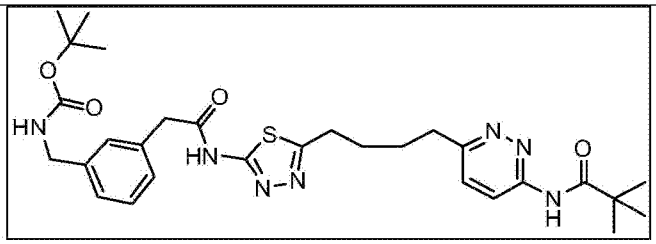
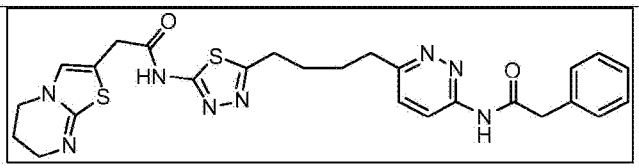
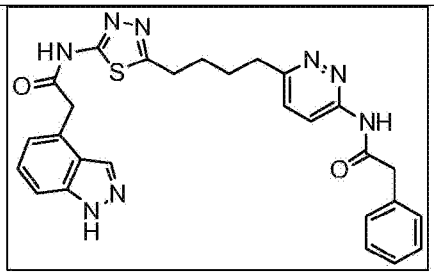
[0949]

496		0.003		0.017
497		0.004		0.032
498		0.003		0.017
499		0.010		0.19
500		0.004		0.029

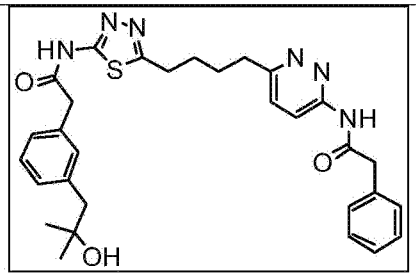
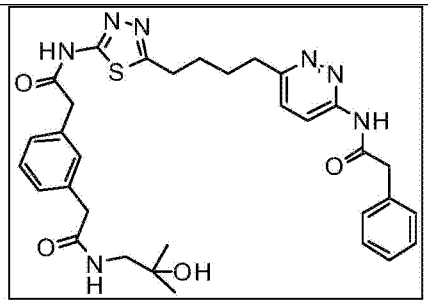
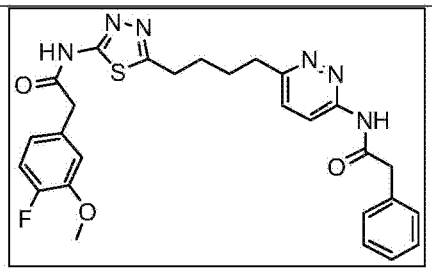
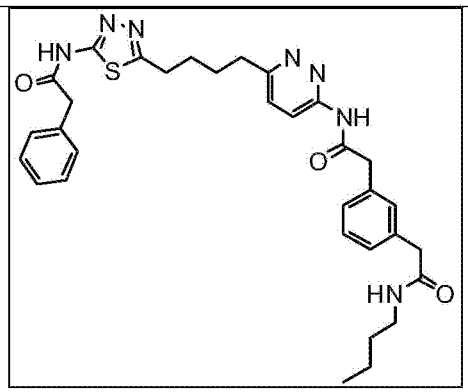
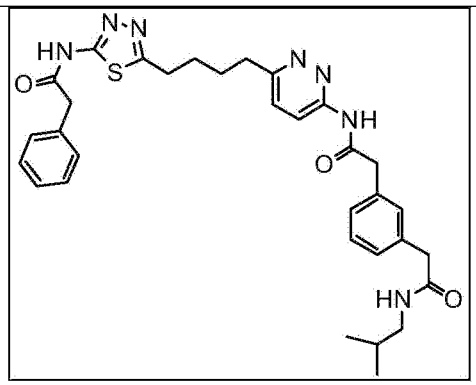
[0950]

501		0.004		0.069
502		0.007		0.075
503		0.008		0.15
504		0.007		0.12
505		0.008		0.24
506		0.010		0.17

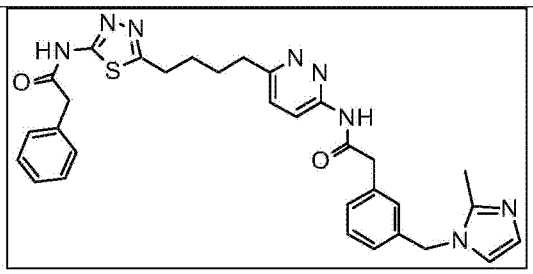
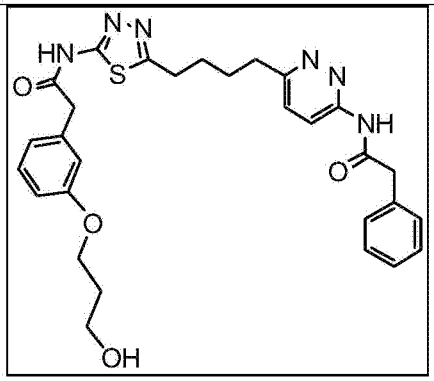
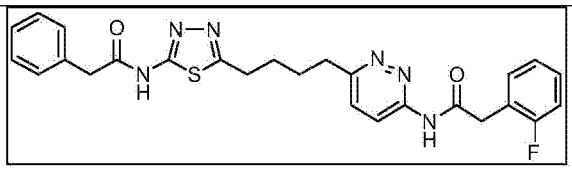
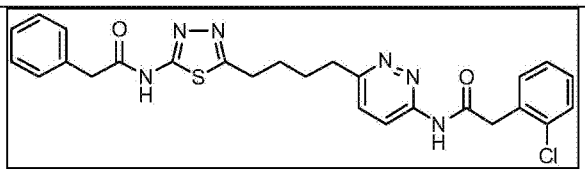
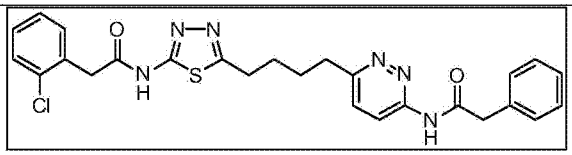
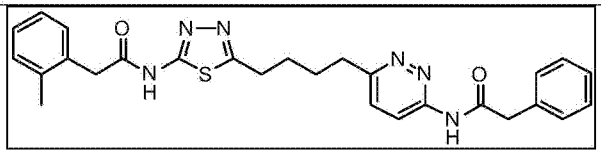
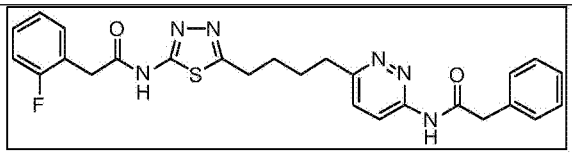
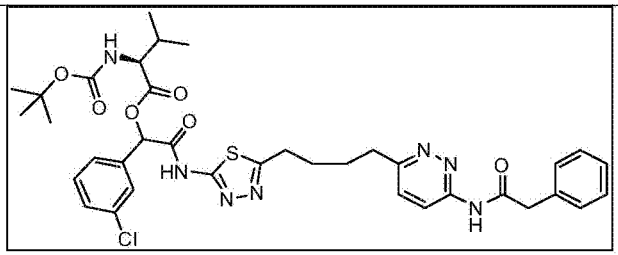
[0951]

507		0.013		0.041
508		0.011		0.020
509		0.010		0.009
510		0.022		0.094
511		0.58		1.1
512		0.005		0.046

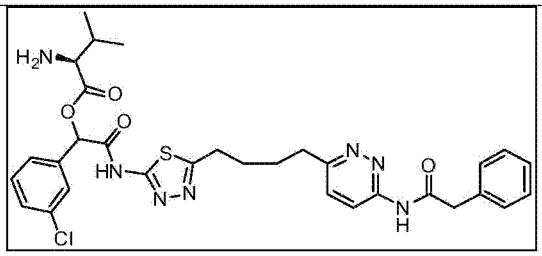
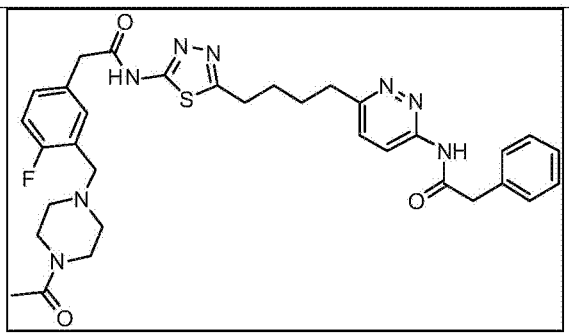
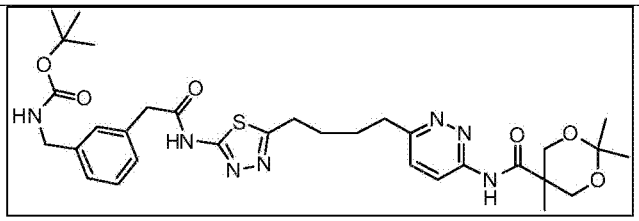
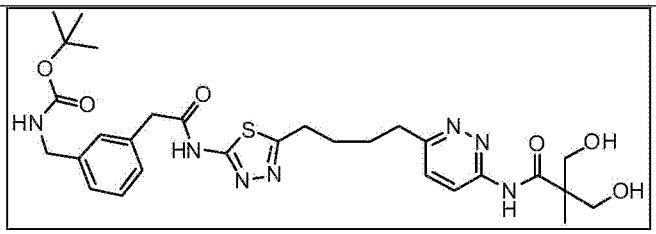
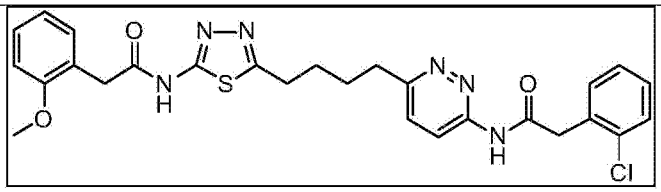
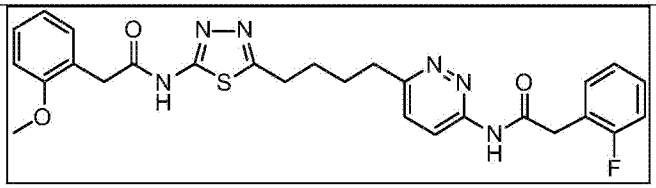
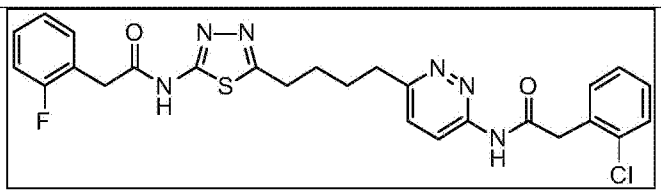
[0952]

513		0.007		0.022
514		0.009		0.063
515		0.007		0.059
516		0.003		0.028
517		0.003		0.046

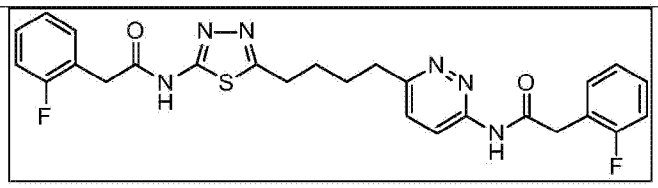
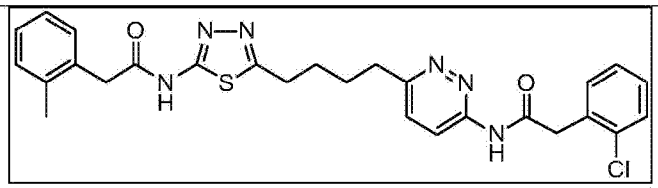
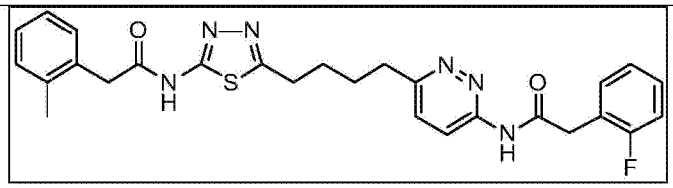
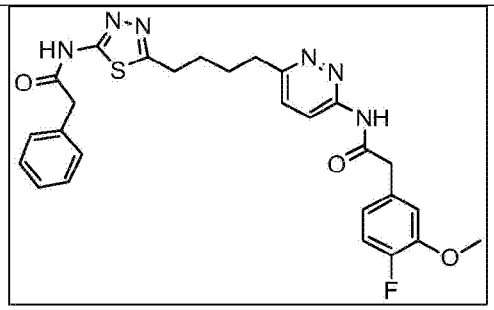
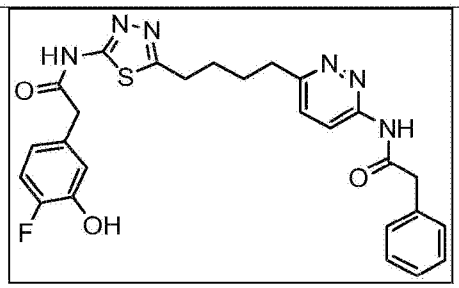
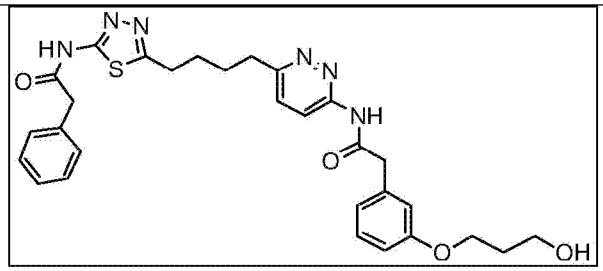
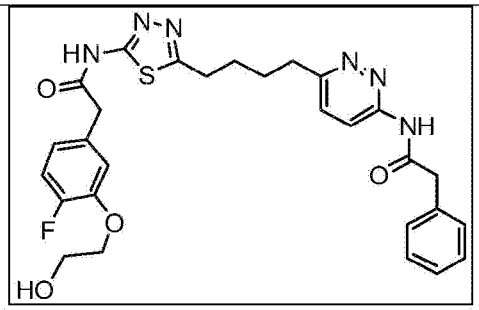
[0953]

518		0.004		0.063
519		0.009		0.059
520		0.007		0.056
521		0.006		0.052
522		0.023		0.060
523		0.021		0.055
524				
525				

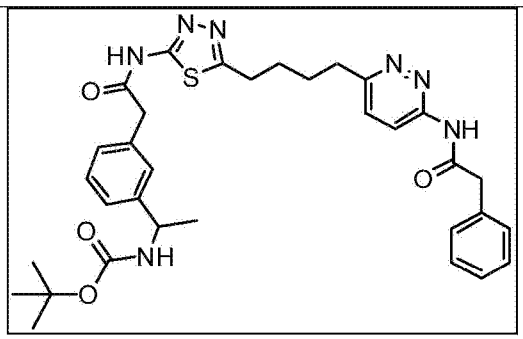
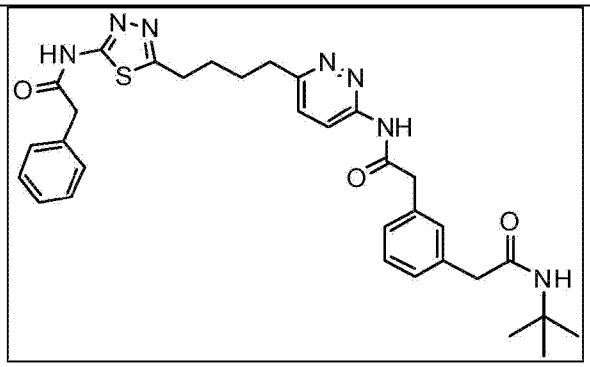
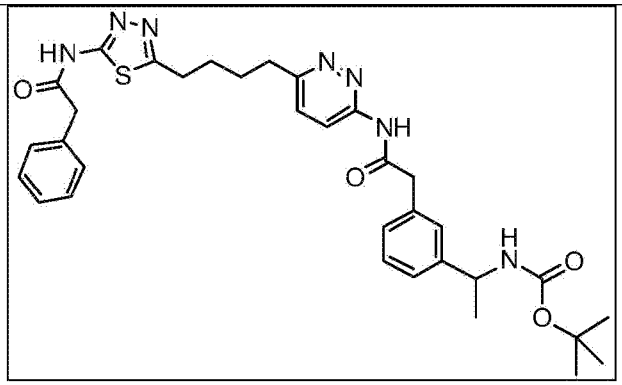
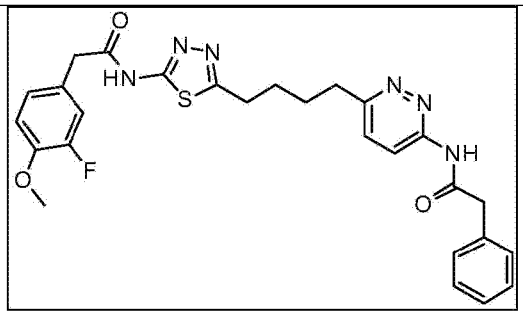
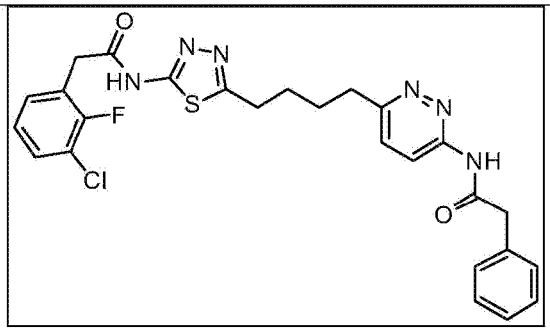
[0954]

526				
528		0.007		0.044
529		0.032		0.16
530		0.055		0.28
531		0.006		0.042
532		0.006		0.059
533		0.007		0.041

[0955]

534		0.008		0.044
535		0.007		0.090
536		0.006		0.071
537		0.007		0.076
538		0.004		0.030
539		0.009		0.045
540		0.007		0.050

[0956]

541		0.004		0.006
542		0.004		0.043
543		0.004		0.005
544		0.006		0.044
545		0.006		0.046

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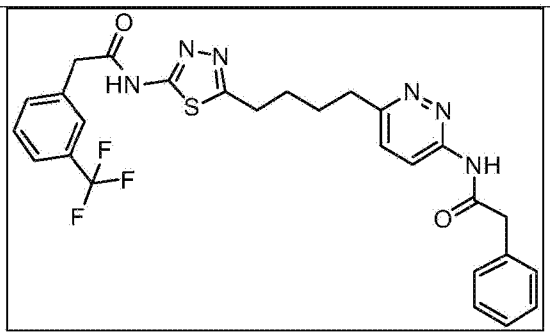
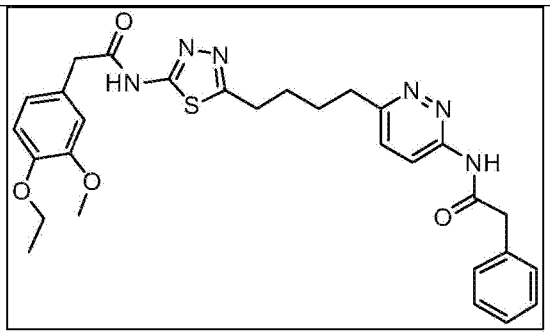
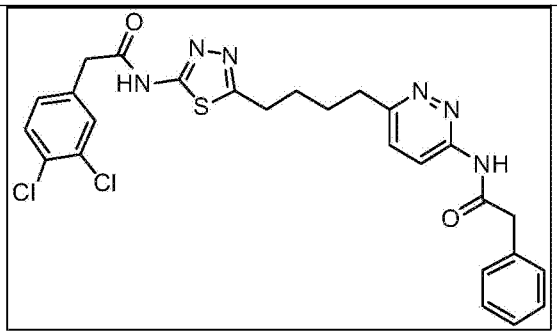
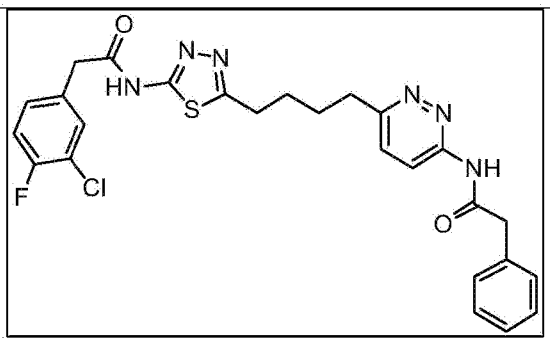
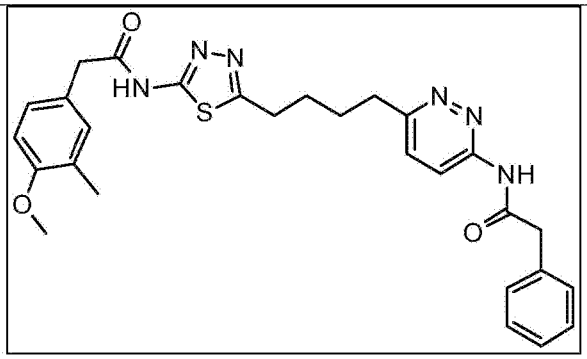
546		0.005		0.027
547		0.006		0.031
548		0.010		0.085
549		0.006		0.045
550		0.005		0.036

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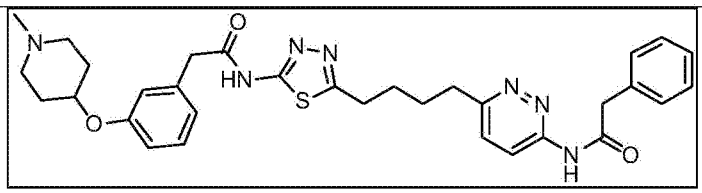
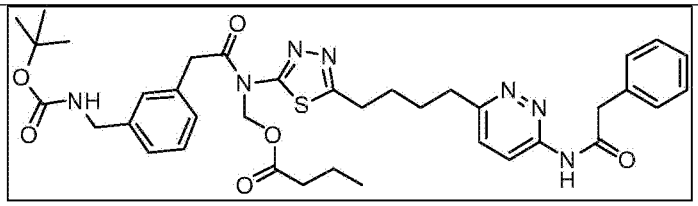
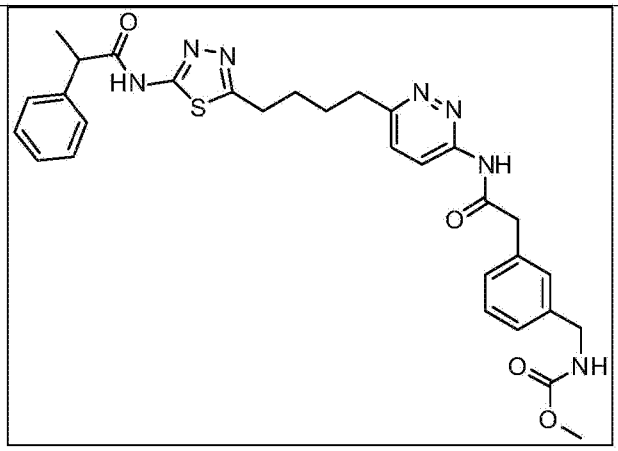
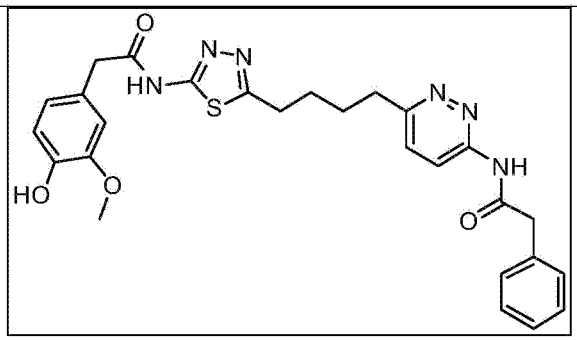
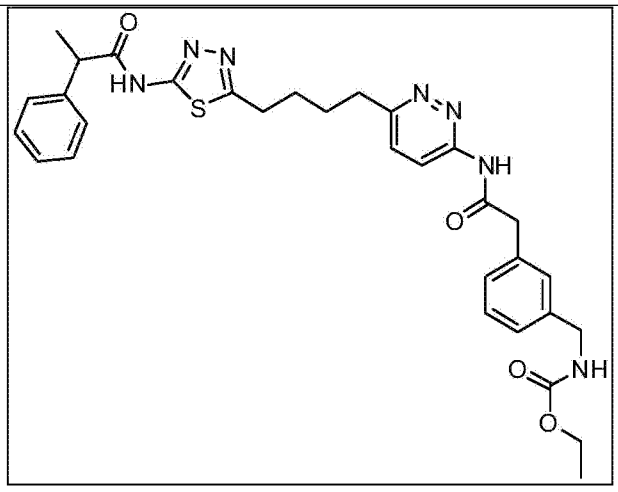
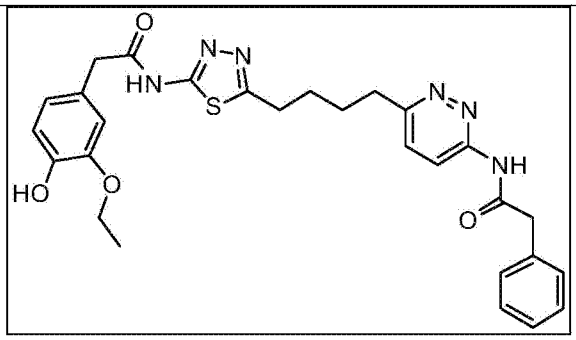
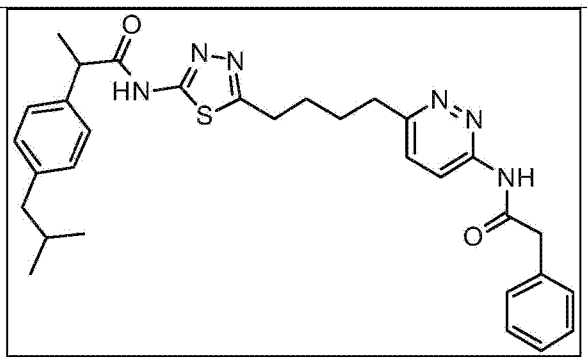
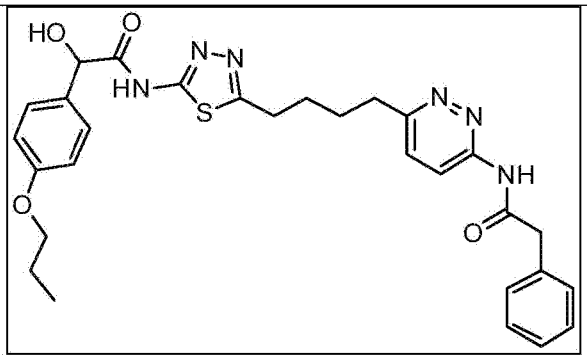
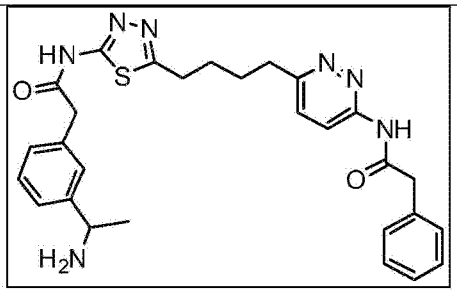
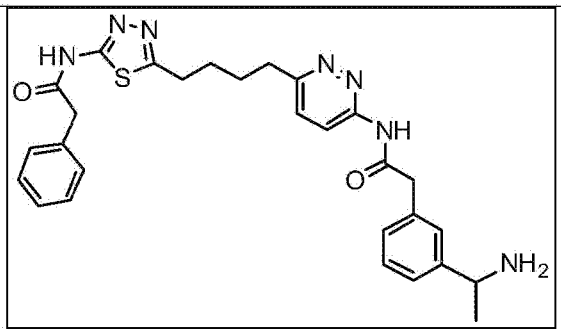
551		0.010		0.127
552		>20		0.005
553		0.005		0.019
554		0.008		0.172
555		0.004		0.010

[0959]

556		0.005		0.12
557		0.025		0.12
558		0.006		0.028
559		0.012		0.066
560		0.010		0.037

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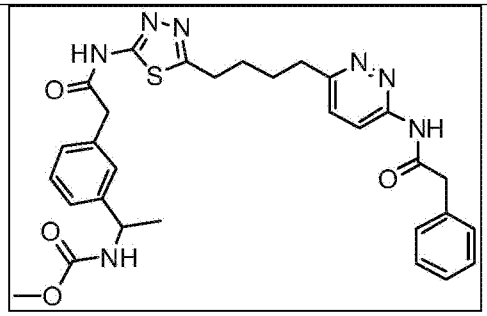
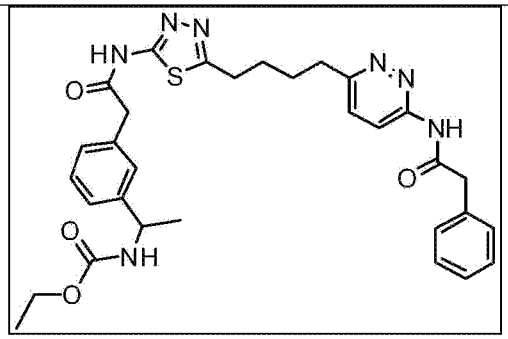
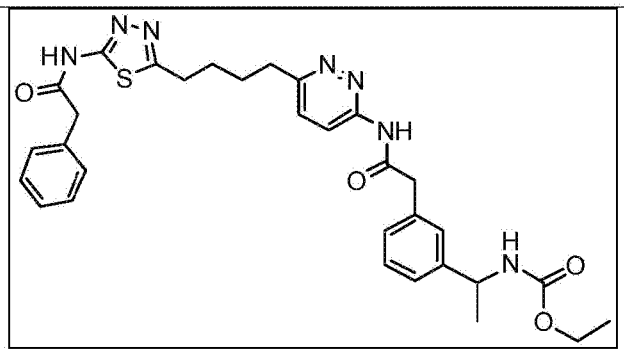
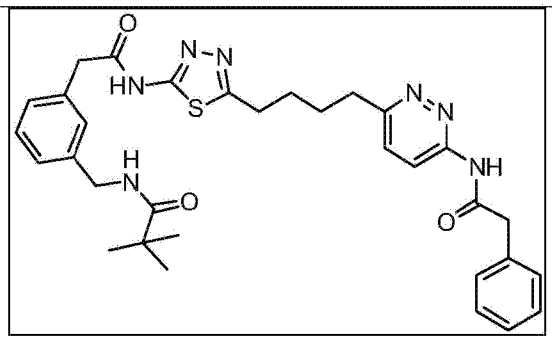
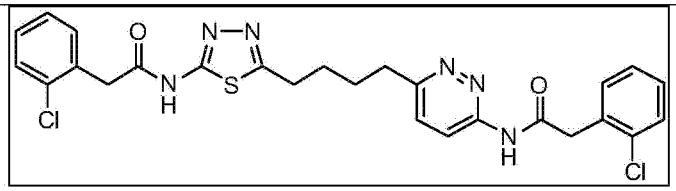
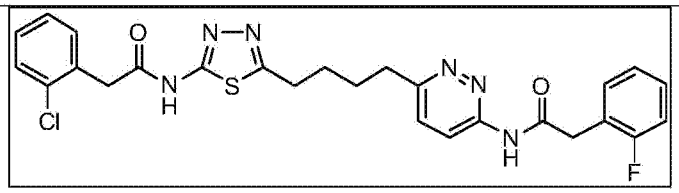
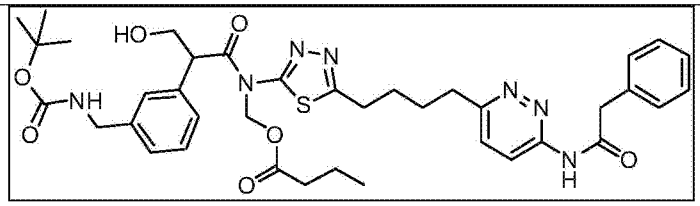
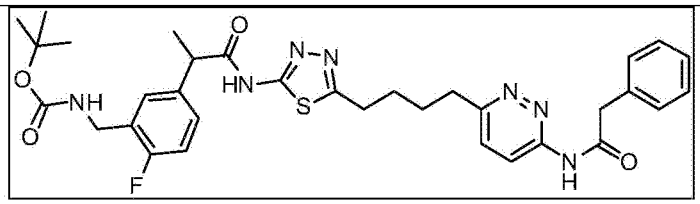
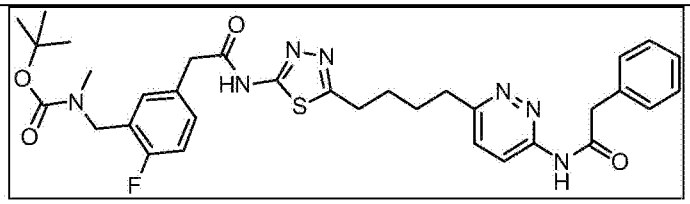
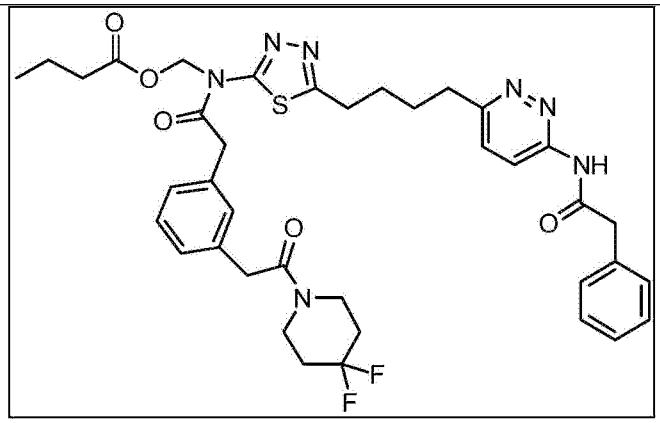
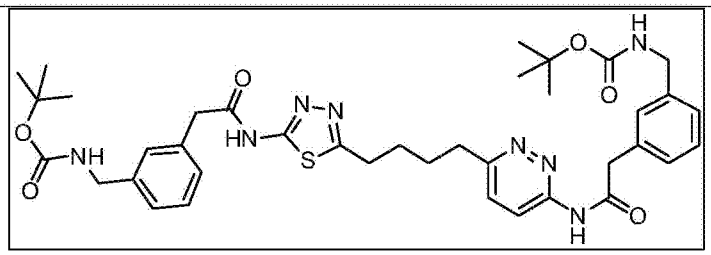
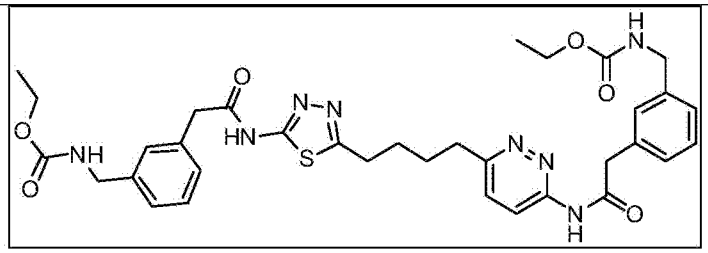
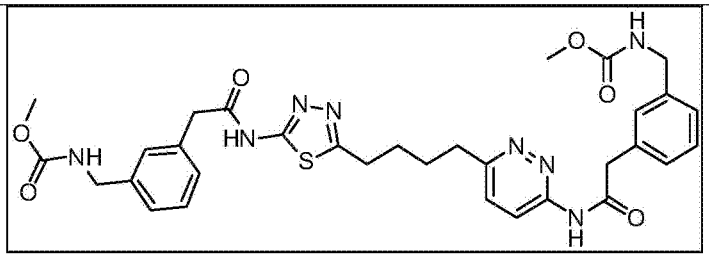
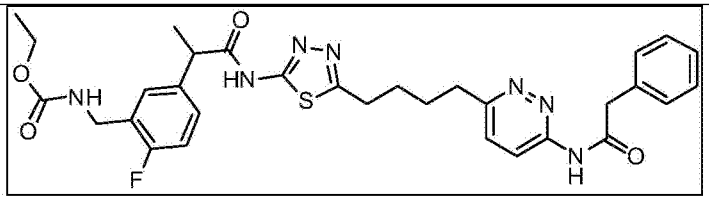
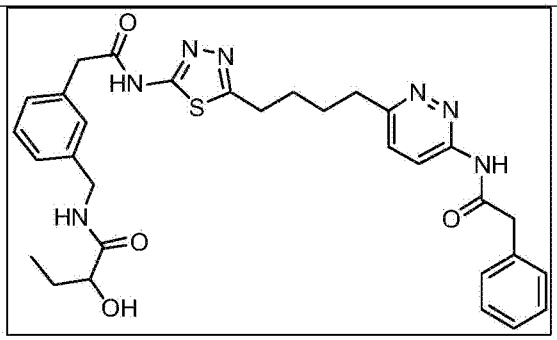
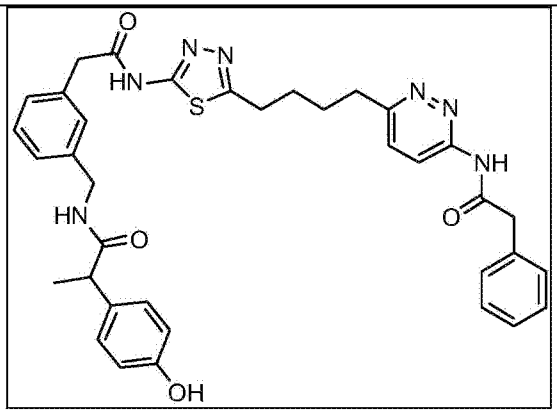
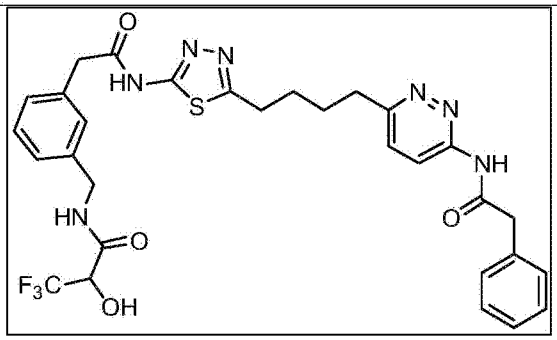
561		0.004		0.004
562		0.003		0.002
563		0.003		0.003
564		0.004		0.002
565		0.005		0.013
566		0.006		0.015

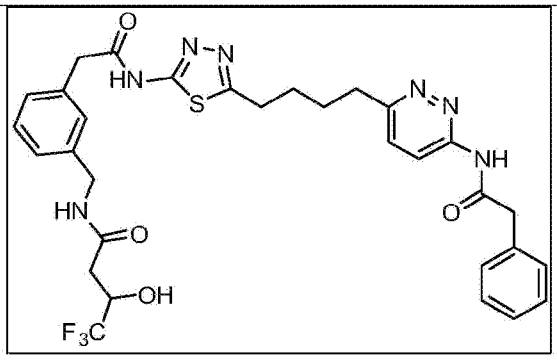
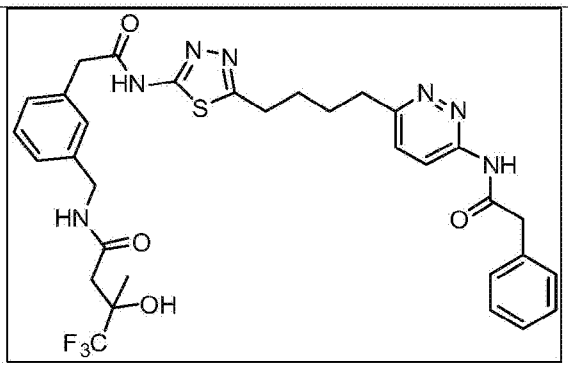
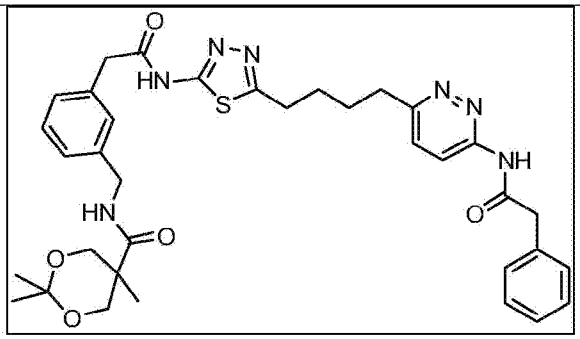
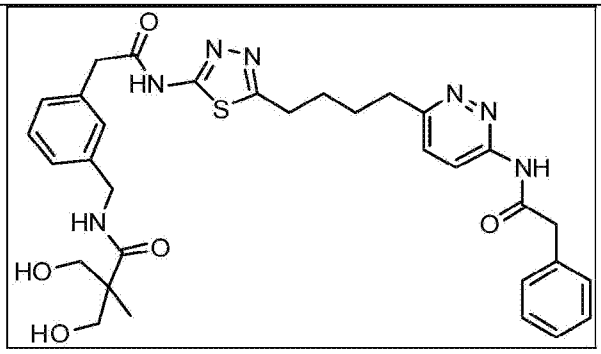
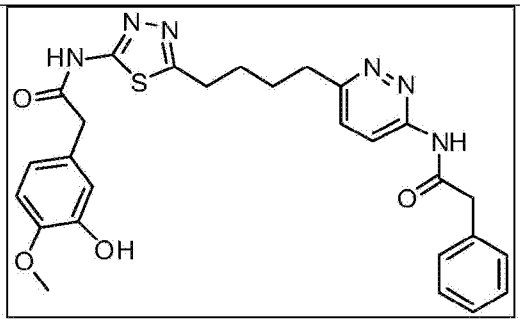
[0961]

567		0.43		0.021
568		0.009		0.028
569		0.006		0.011
570		0.43		0.009
571		0.011		0.010
572		0.003		0.004

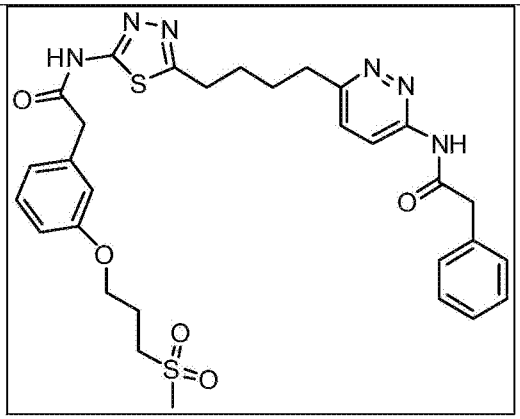
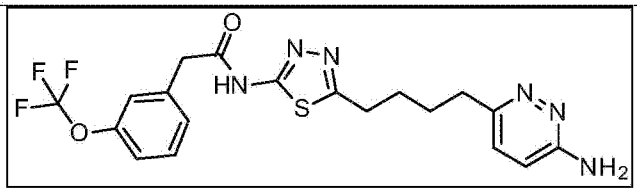
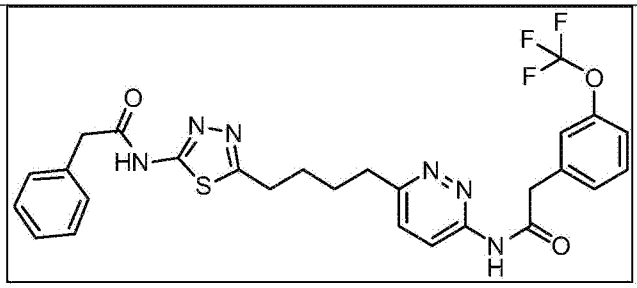
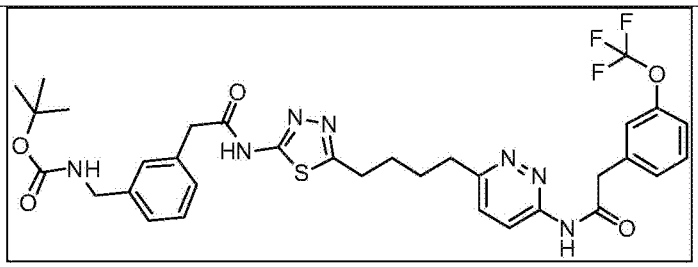
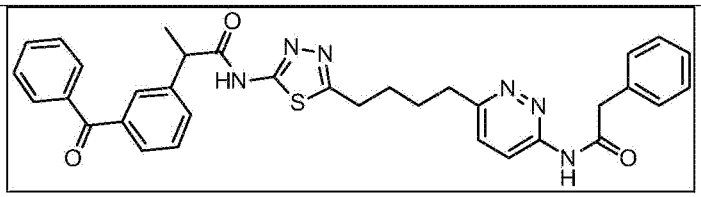
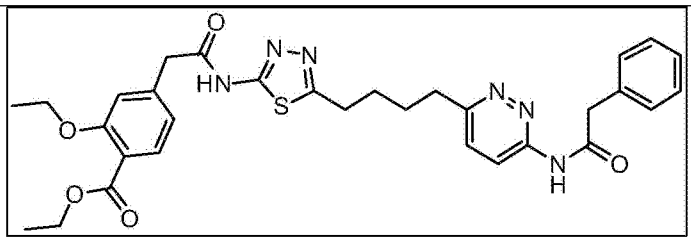
[0962]

573		0.004		0.015
574		0.006		0.028
575		0.007		0.040
576		0.003		0.013
577		0.004		0.034

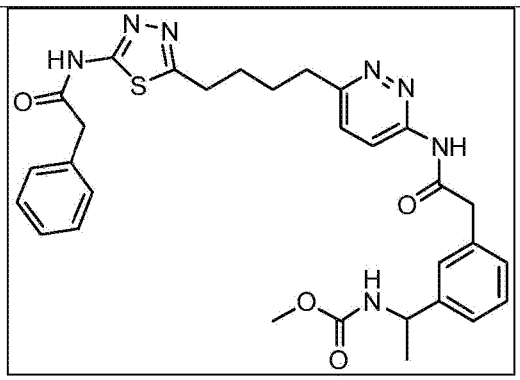
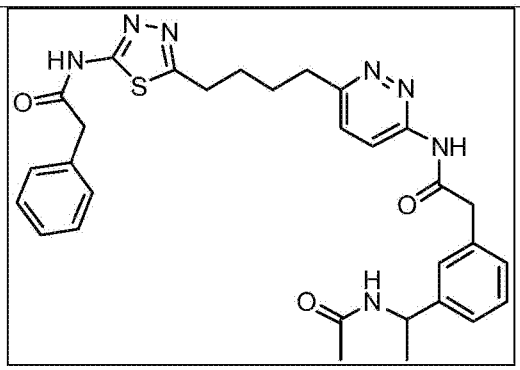
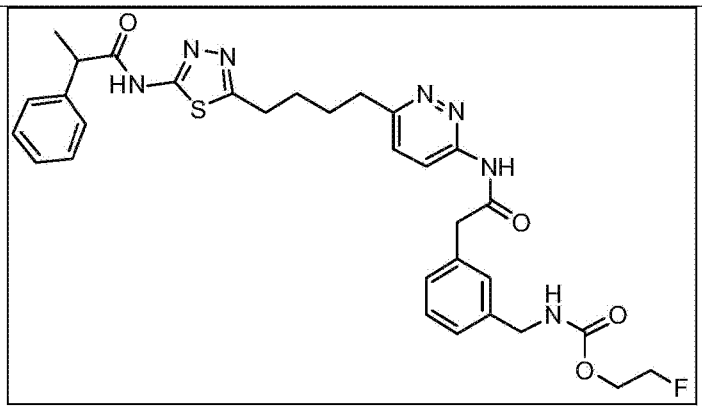
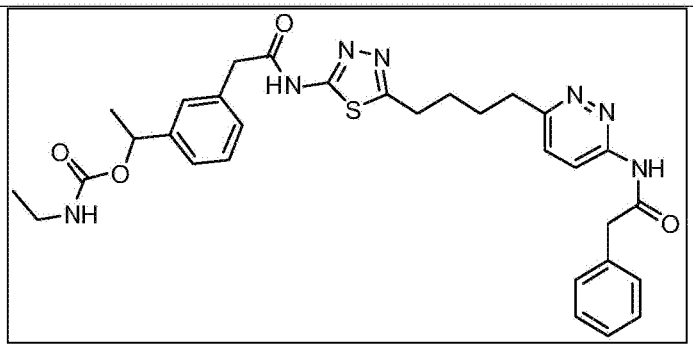
[0963]

578		0.004		0.022
579		0.004		0.009
580		0.005		0.013
581		0.011		0.24
582		0.005		0.046

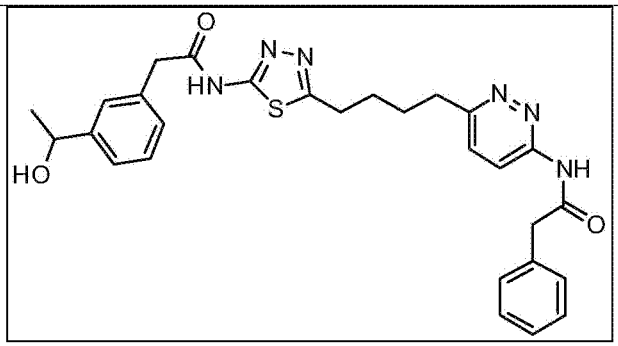
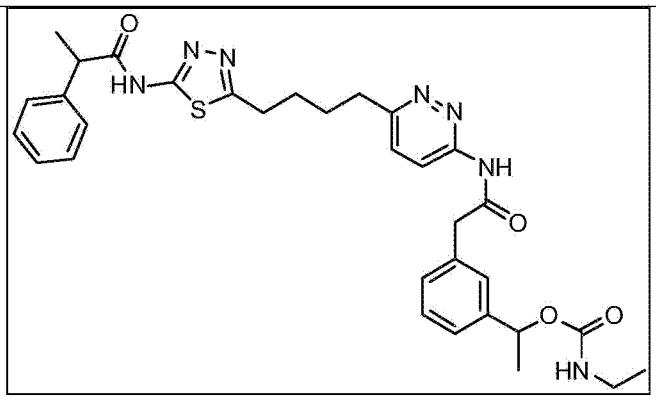
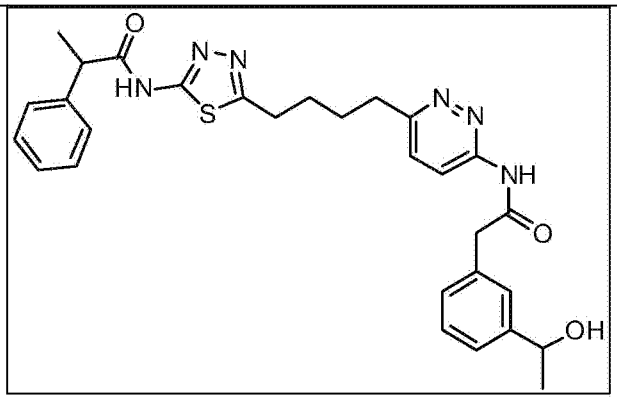
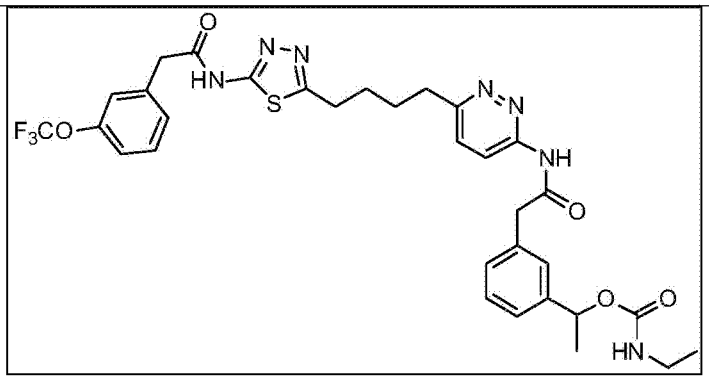
[0964]

583		0.005		0.042
584		0.22		1.4
585		0.006		0.070
586		0.013		0.031
587		0.007		0.057
588		0.008		0.27

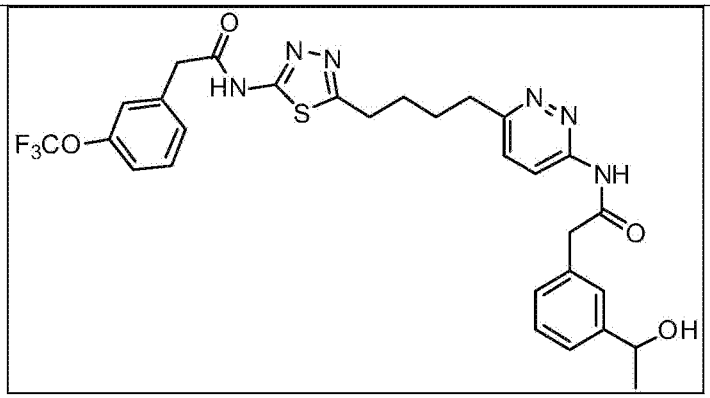
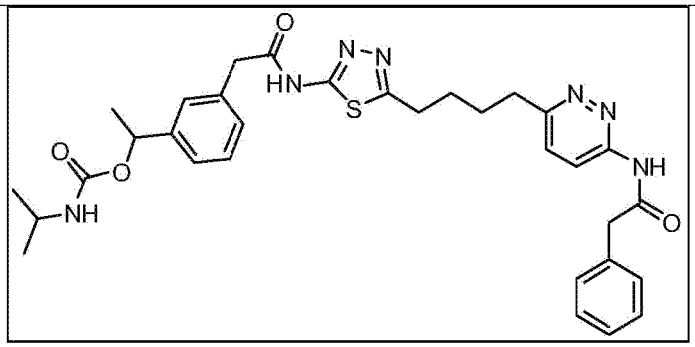
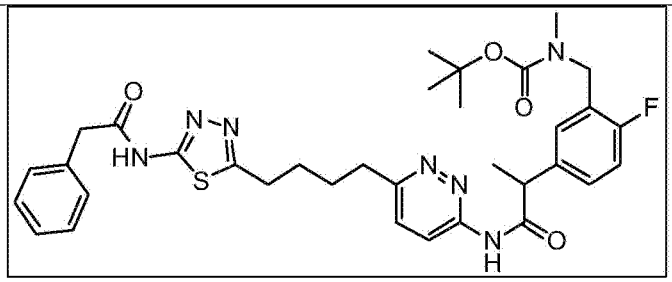
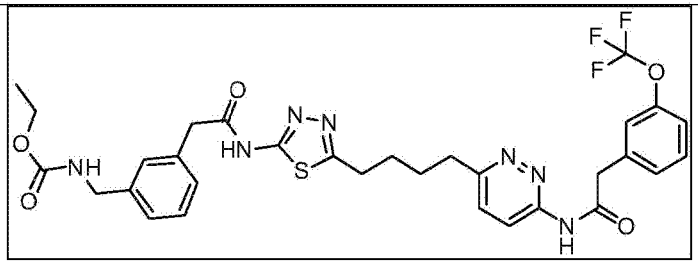
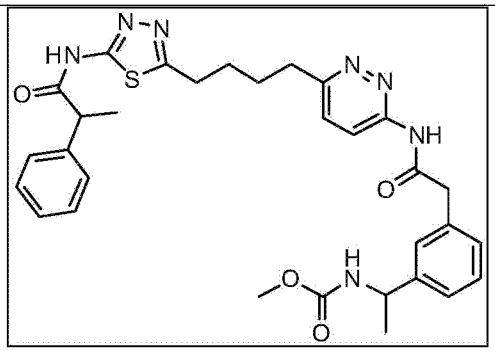
[0965]

589		0.004		0.025
590		0.007		0.087
591		0.004		0.033
592		0.004		0.011

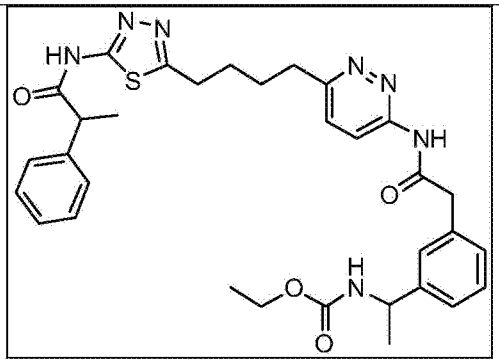
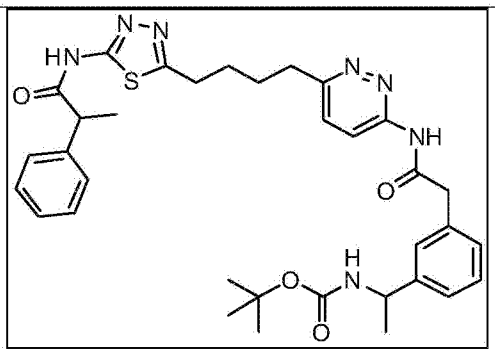
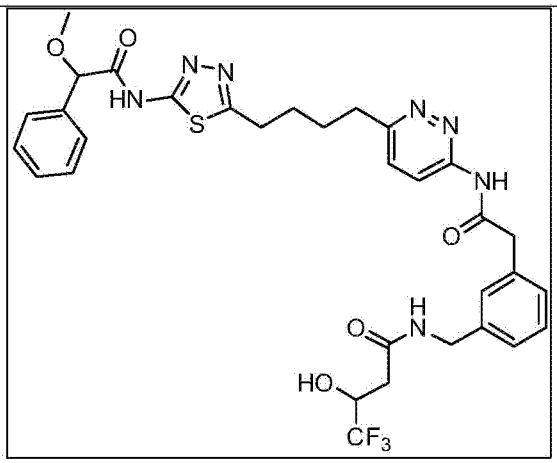
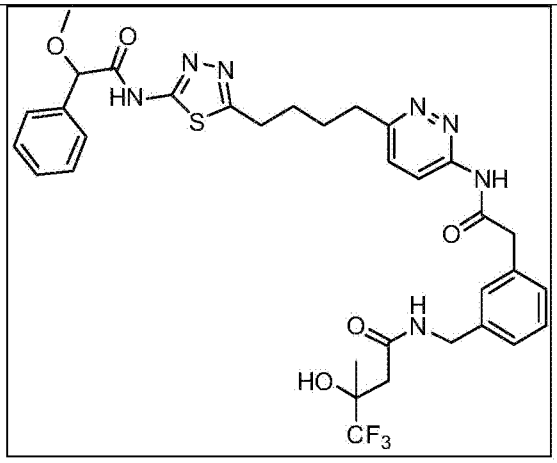
[0966]

593		0.005		0.033
594		0.007		0.050
595		0.007		0.059
596		0.015		0.33

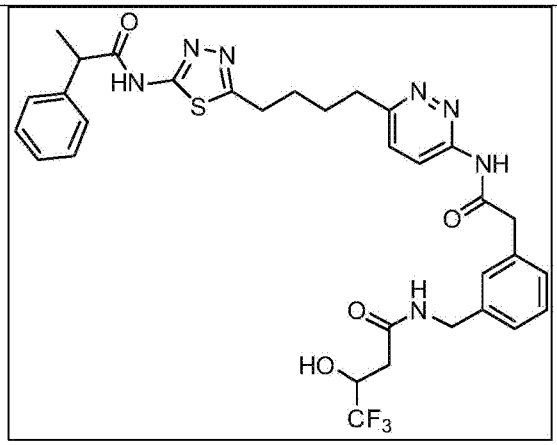
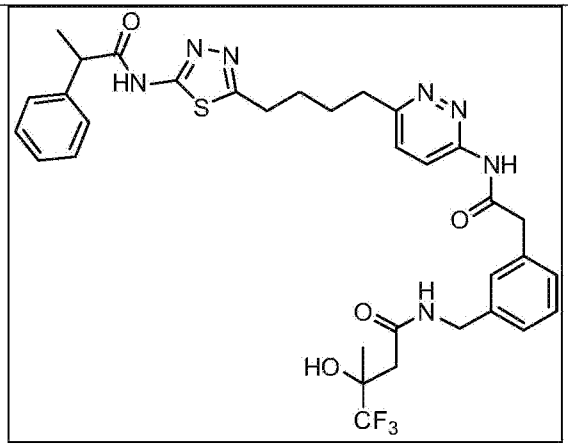
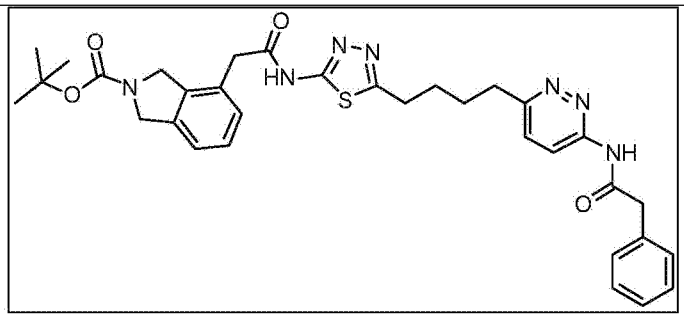
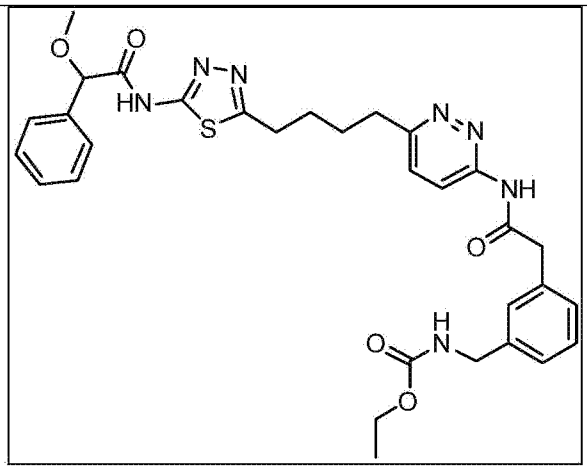
[0967]

597		0.005		0.017
598		0.005		0.004
599		0.010		0.039
600		0.005		0.008
601		0.006		0.036

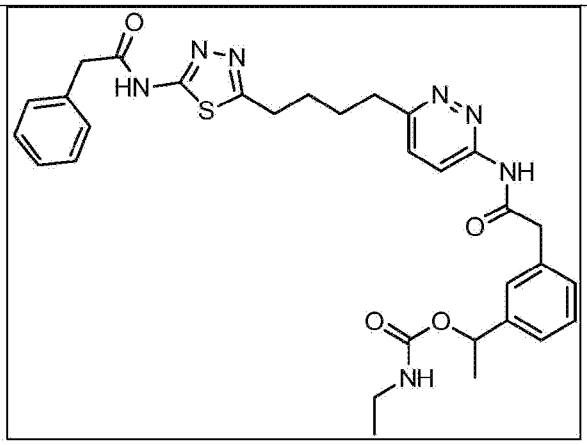
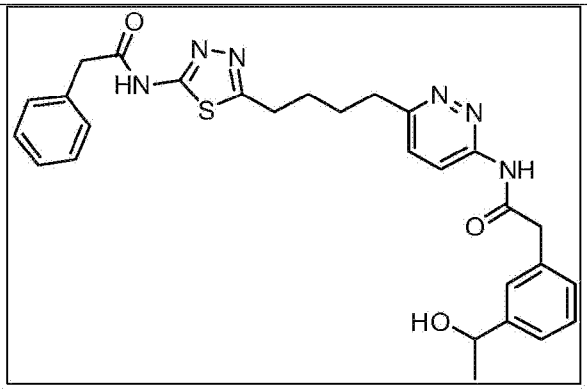
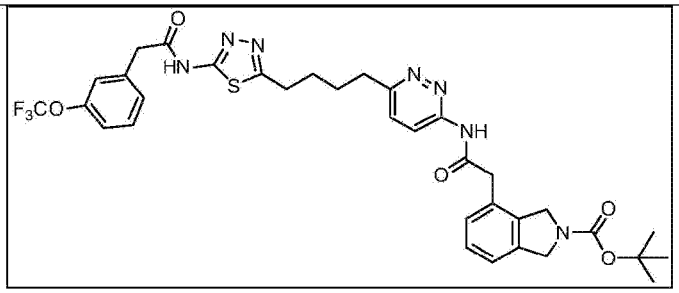
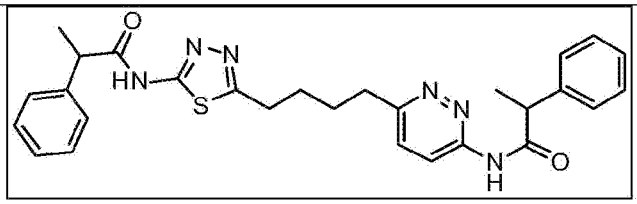
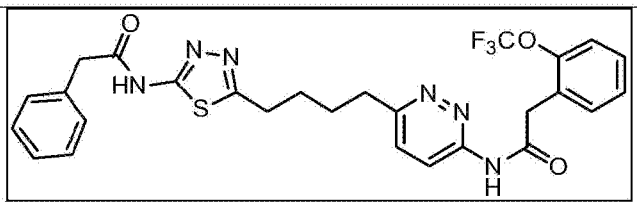
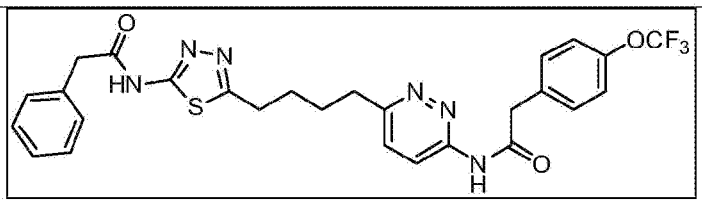
[0968]

602		0.006		0.036
603		0.009		0.023
604		0.015		0.042
605		0.013		0.018

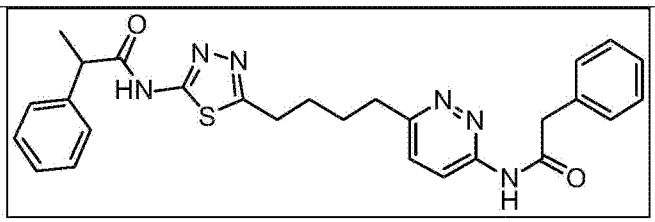
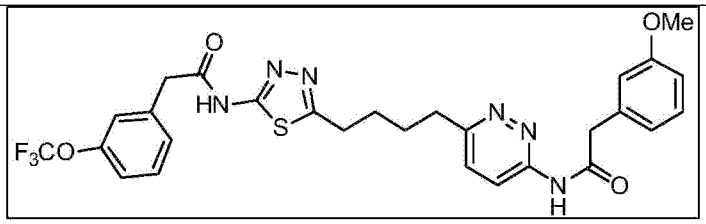
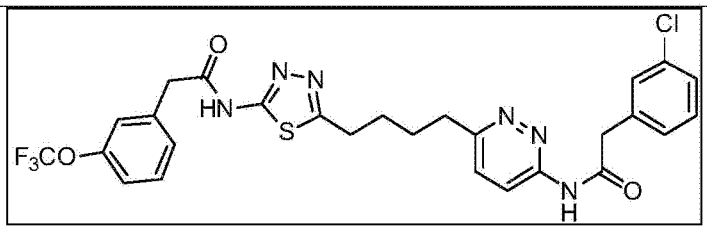
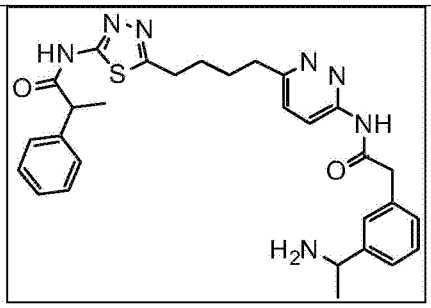
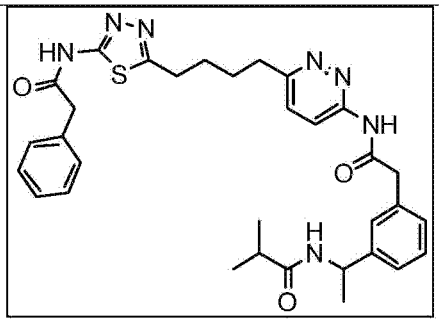
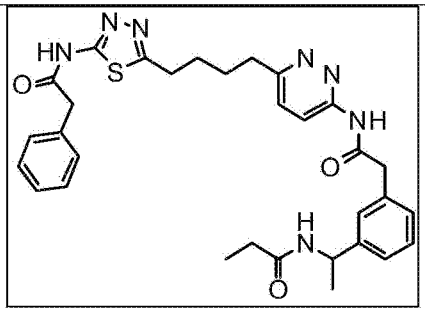
[0969]

606		0.007		0.045
607		0.007		0.047
608		0.007		0.037
609		0.009		0.014

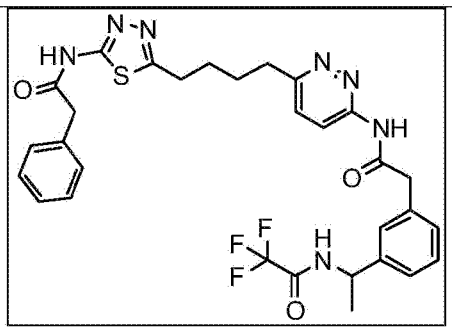
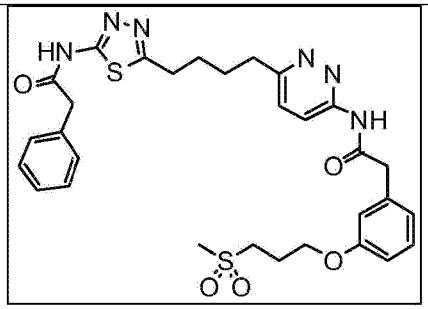
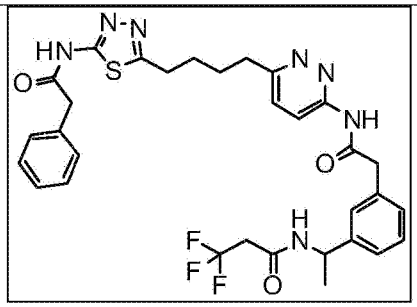
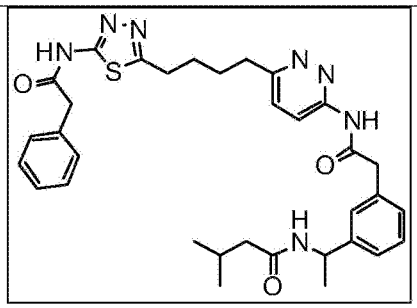
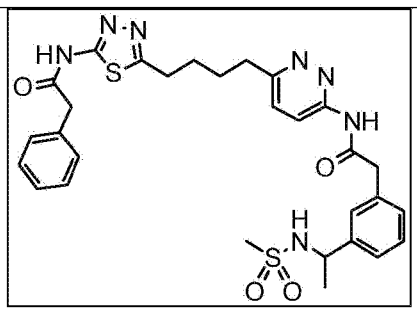
[0970]

610		0.005		0.011
611		0.006		0.040
612		0.065		0.10
613		0.019		0.45
614		0.008		0.082
615		0.009		0.12

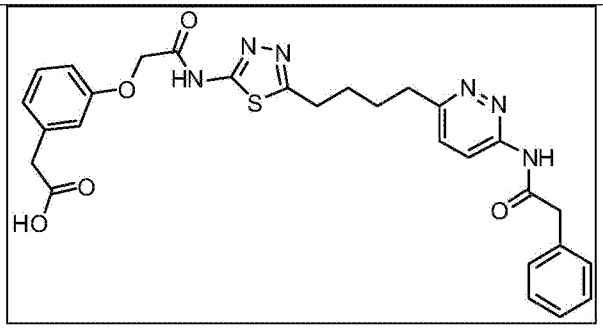
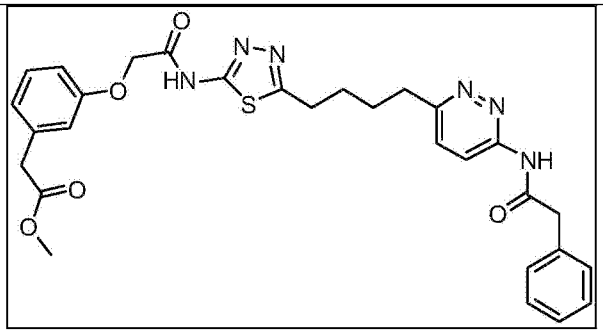
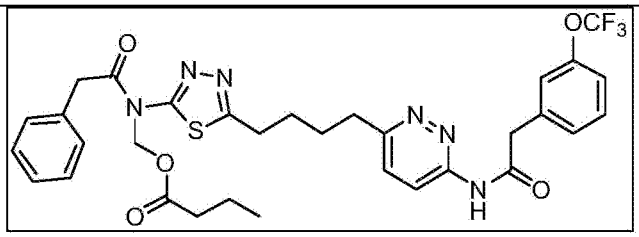
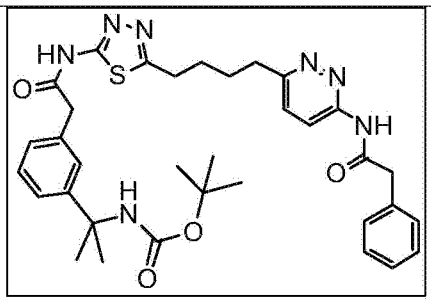
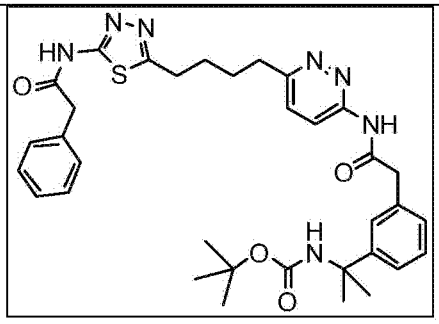
[0971]

616		0.008		0.13
617		0.005		0.040
618		0.008		0.035
619		0.013		0.15
620		0.005		0.011
621		0.005		0.020

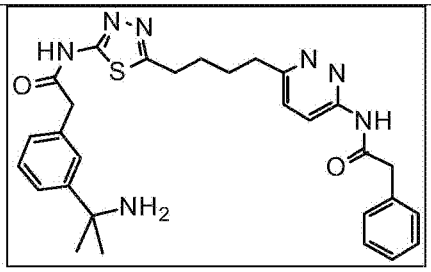
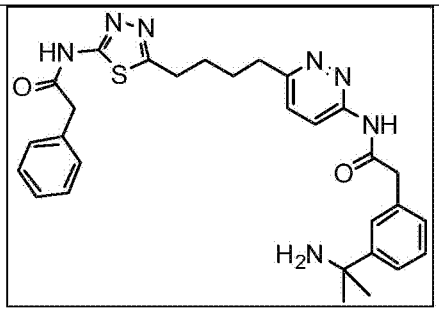
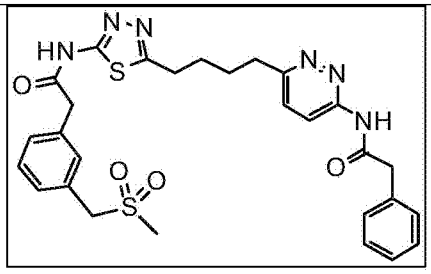
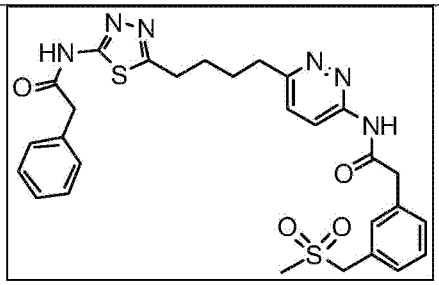
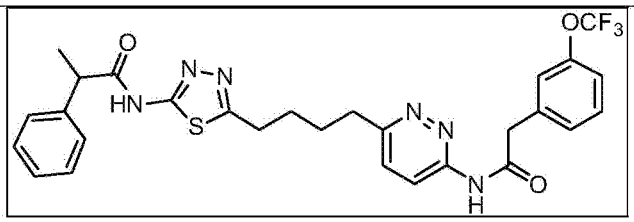
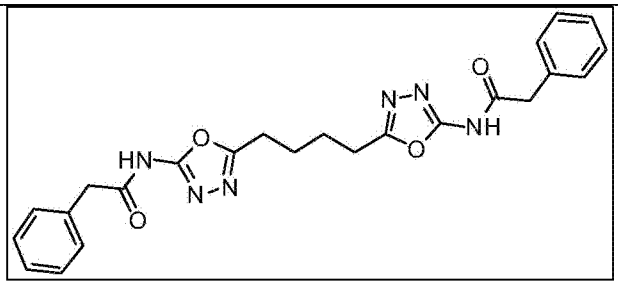
[0972]

622		0.004		0.010
623		0.003		0.026
624		0.004		0.009
625		0.004		0.006
626		0.004		0.017

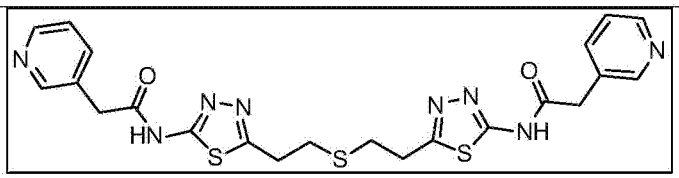
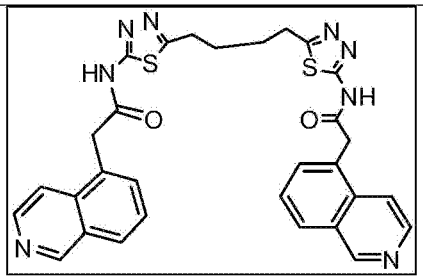
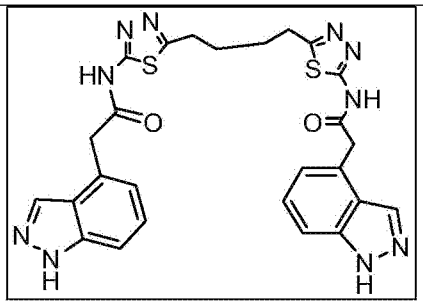
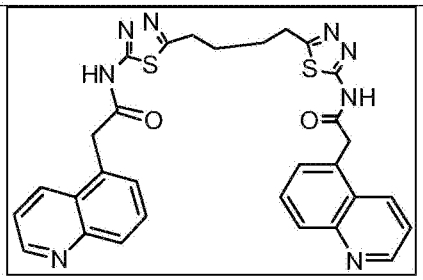
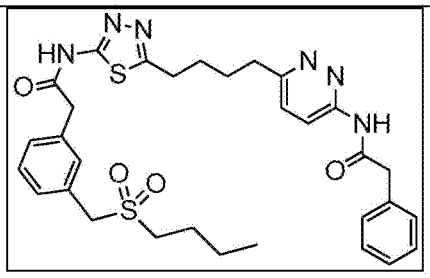
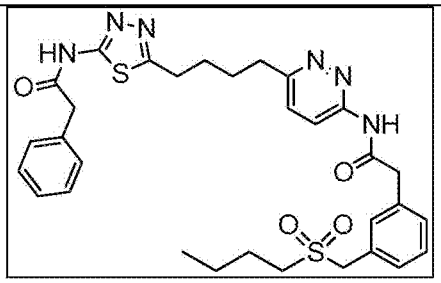
[0973]

627		0.028		0.85
628		0.027		0.17
629		>20		0.065
630		0.004		0.009
631		0.005		0.006

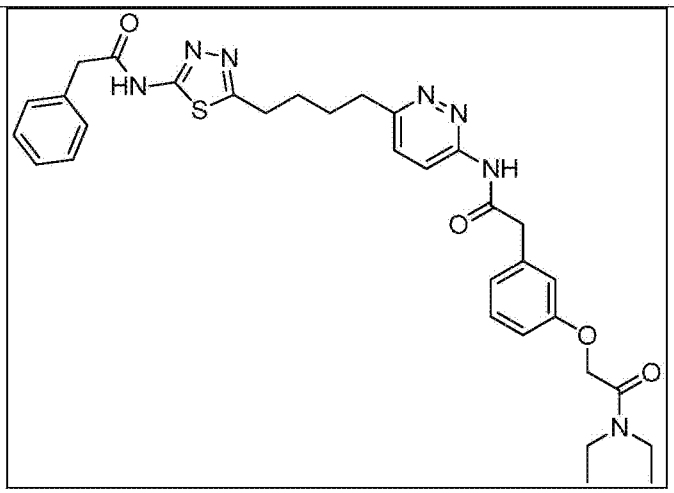
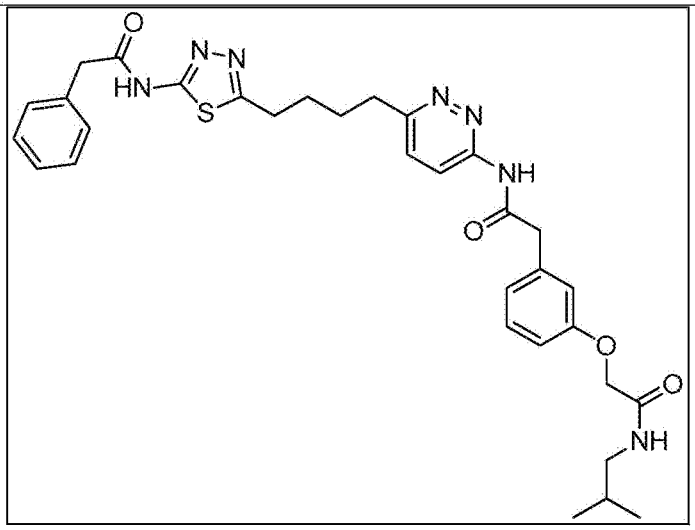
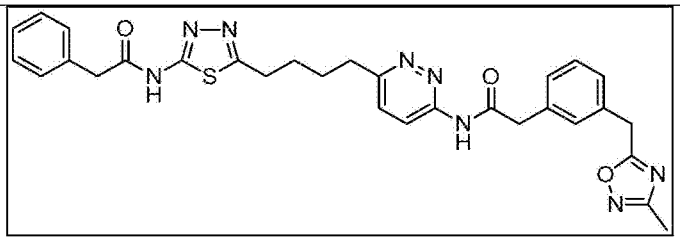
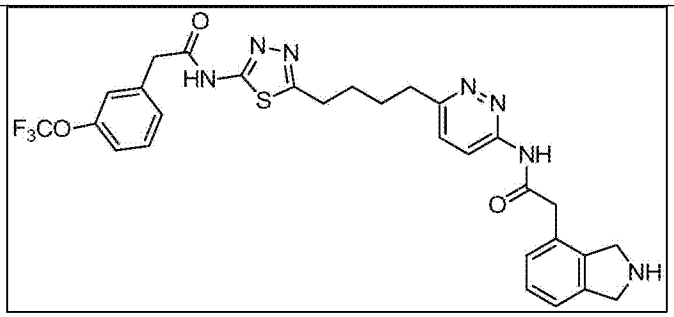
[0974]

632		0.010		0.20
633		0.007		0.13
634		0.006		0.048
635		0.005		0.030
636		0.008		0.059
637			>20	>50

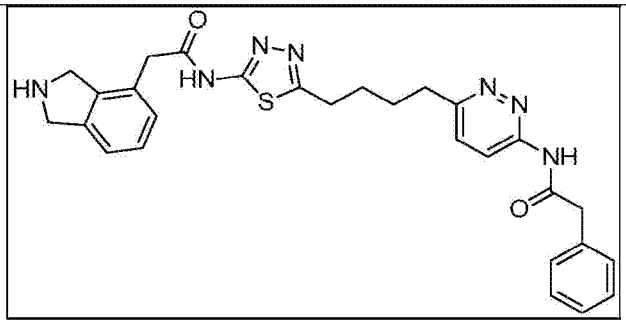
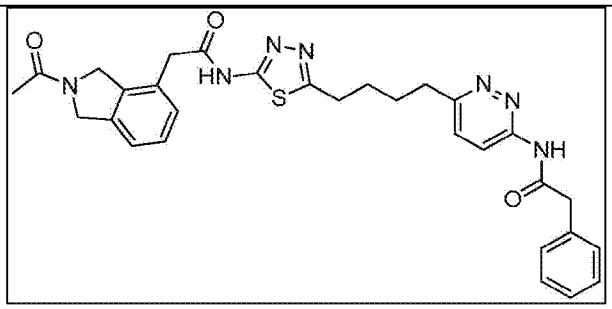
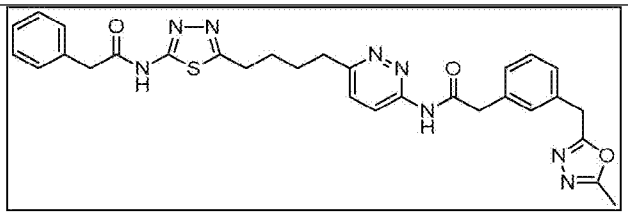
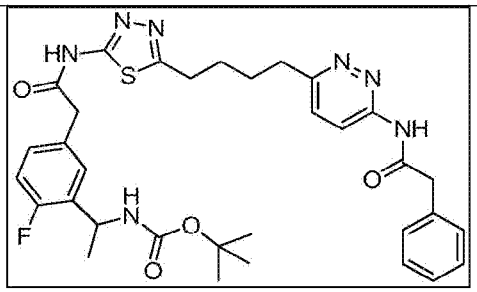
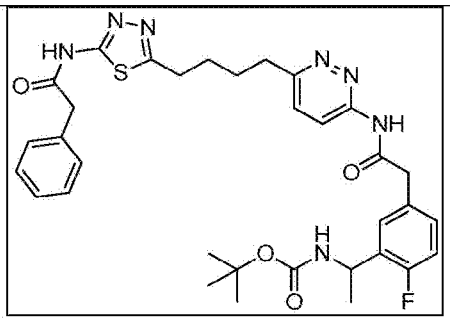
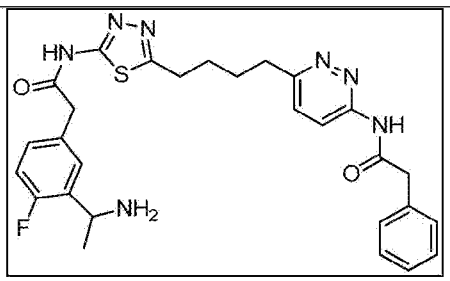
[0975]

638			0.48	5.7
639			0.17	23
640			0.12	0.070
641			0.14	0.50
644		0.003		0.013
645		0.002		0.015

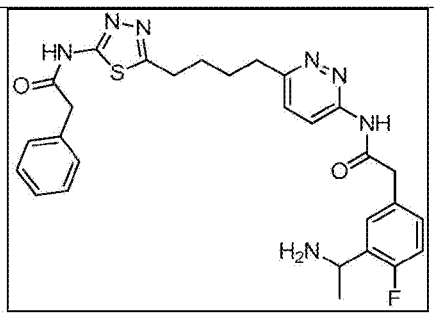
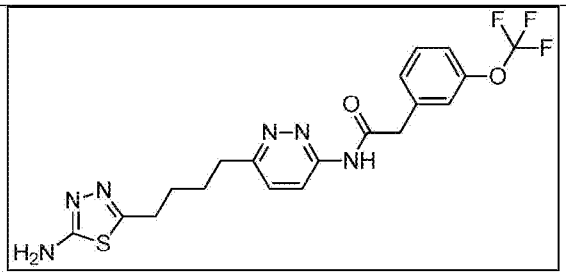
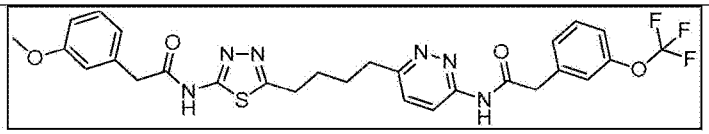
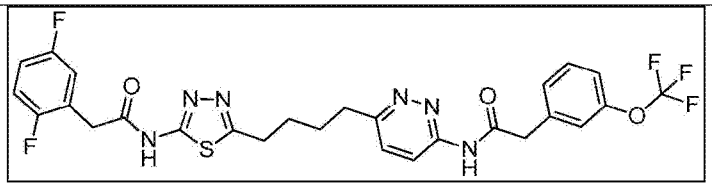
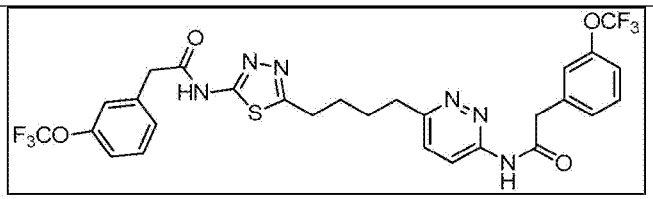
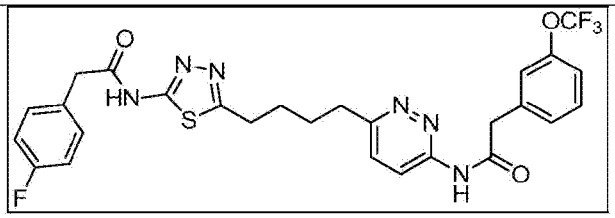
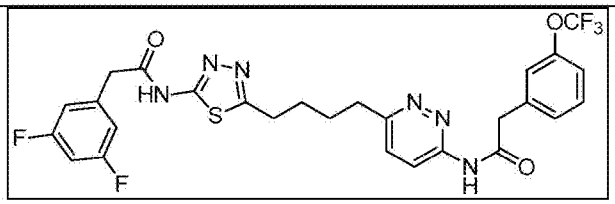
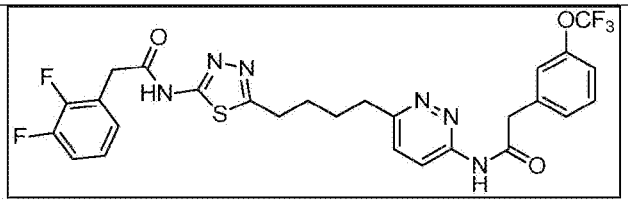
[0976]

646		0.007		0.037
647		0.004		0.018
648		0.004		0.011
649		0.004		0.034

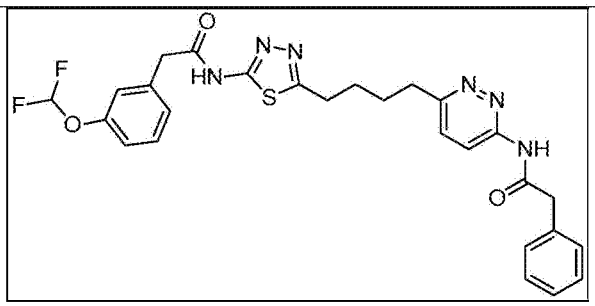
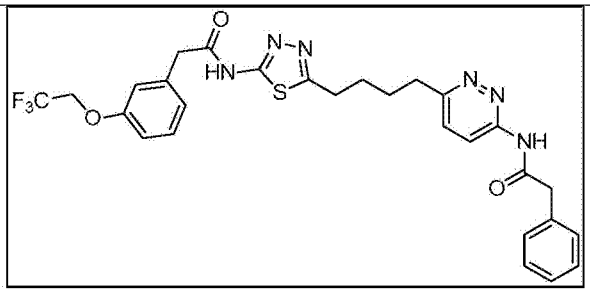
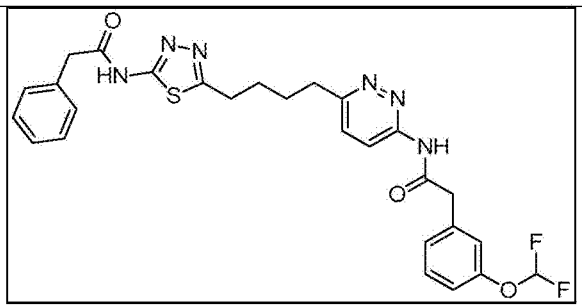
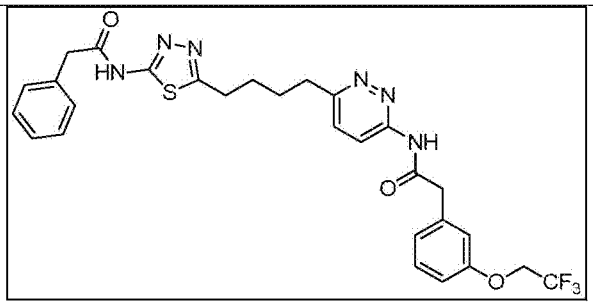
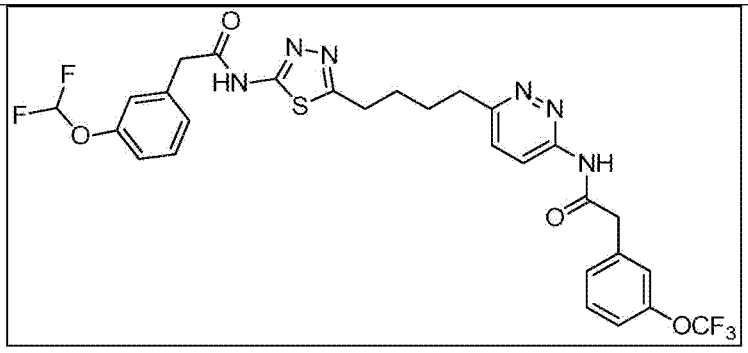
[0977]

650		0.013		0.14
651		0.006		0.037
652		0.004		0.039
653		0.005		0.010
654		0.005		0.007
655		0.019		0.35

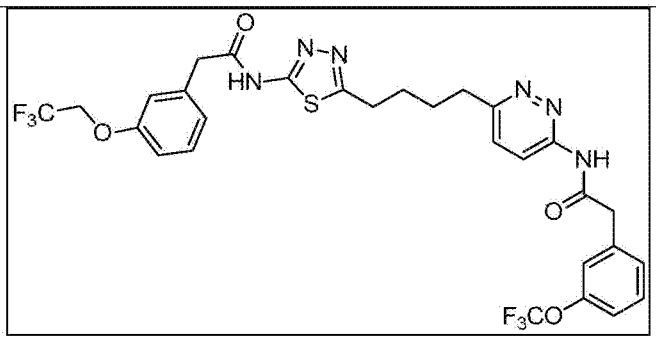
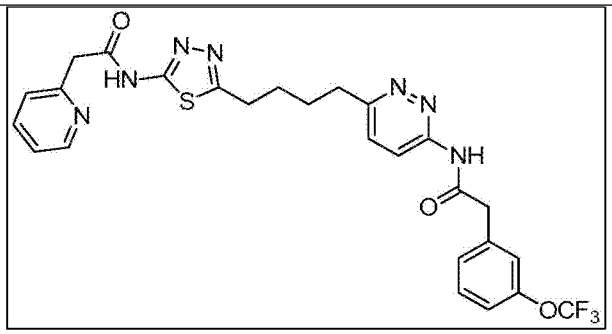
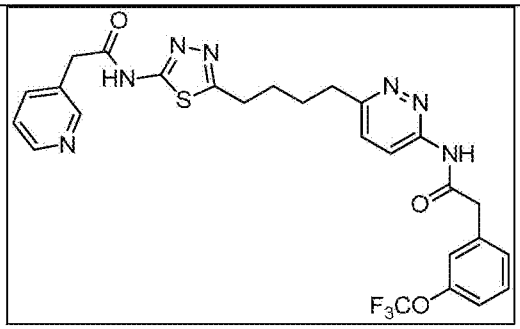
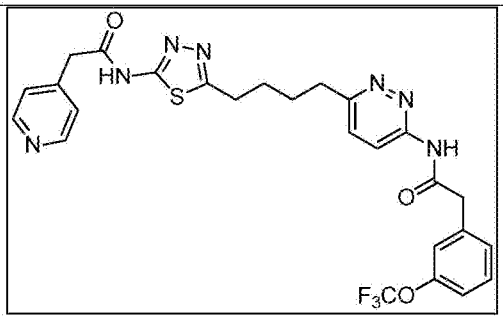
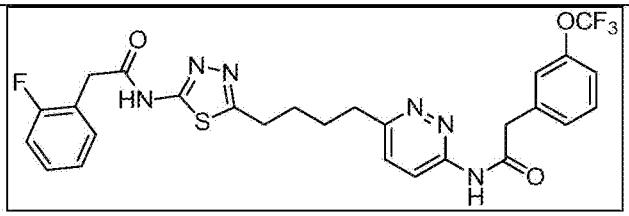
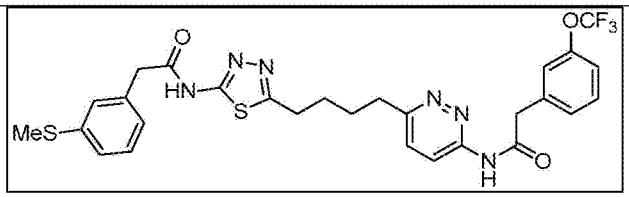
[0978]

656		0.018		0.40
657		0.24		1.5
658		0.005		0.040
659		0.010		0.058
660		0.025		0.037
661		0.007		0.12
662		0.007		0.055
663		0.007		0.089

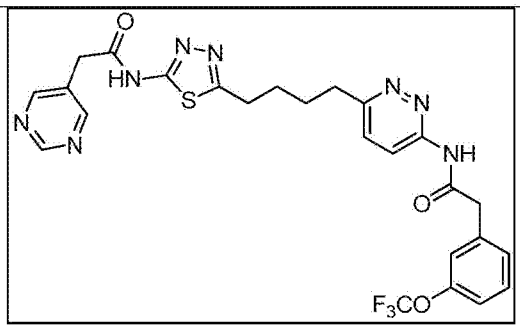
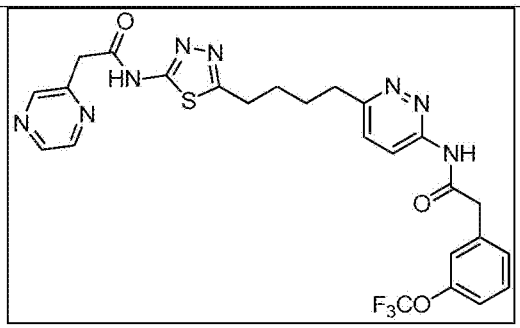
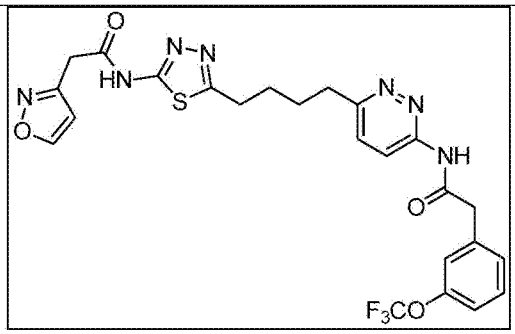
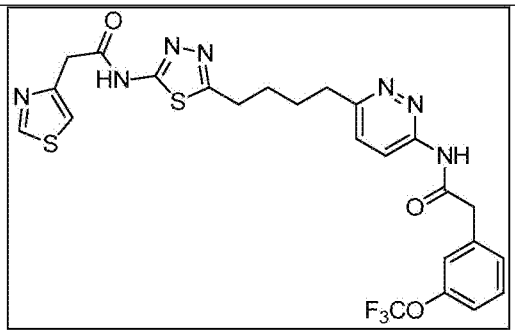
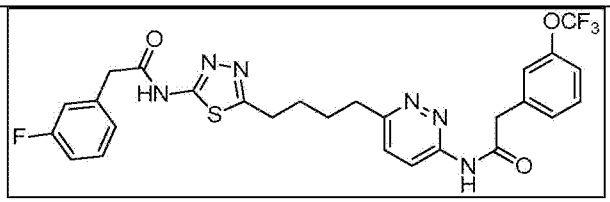
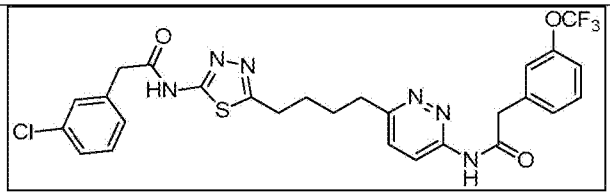
[0979]

664		0.005		0.060
665		0.005		0.10
666		0.004		0.058
667		0.004		0.11
668		0.009		0.026

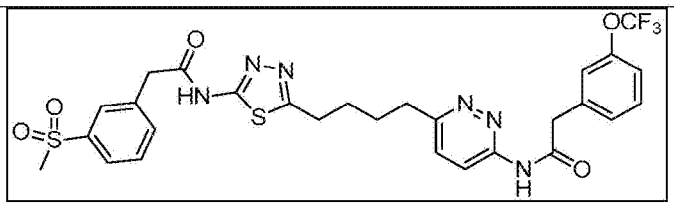
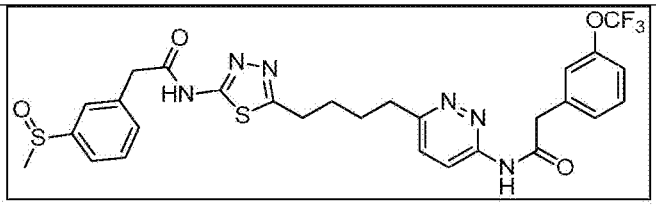
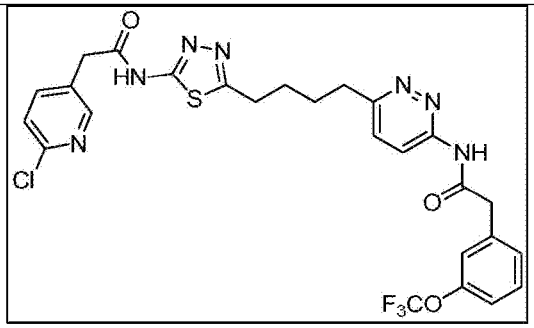
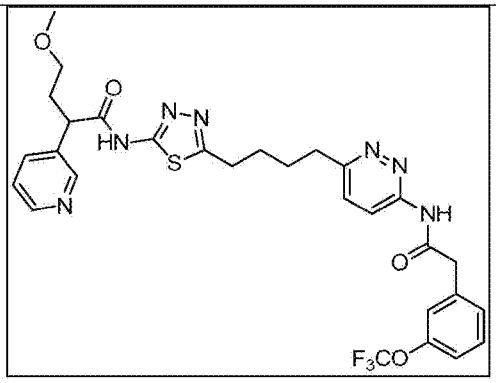
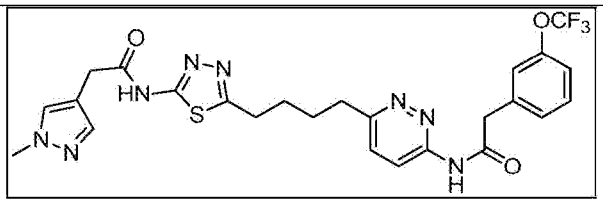
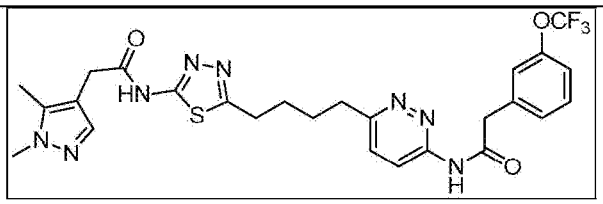
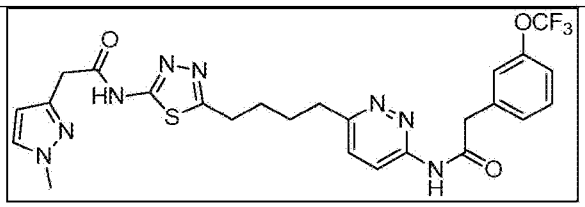
[0980]

669		0.021		0.026
670		0.005		0.030
671		0.004		0.035
672		0.010		0.045
673		0.006		0.033
674		0.008		0.024

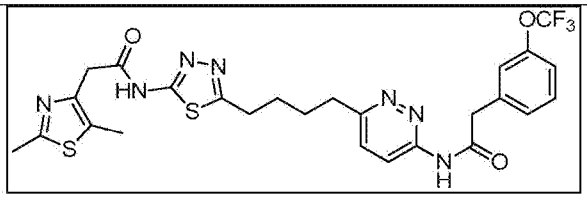
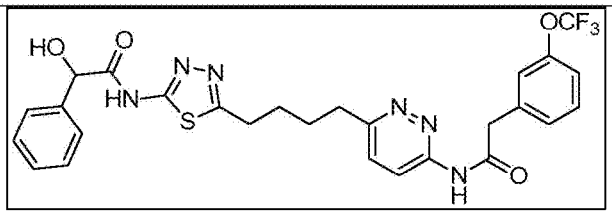
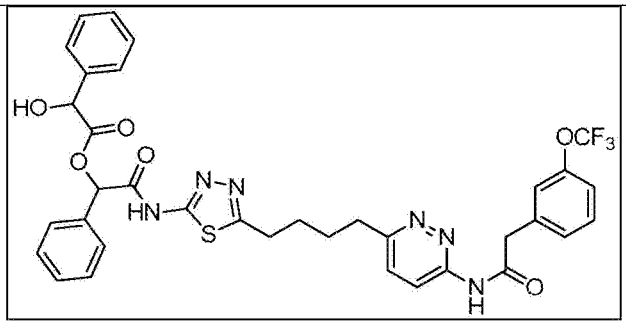
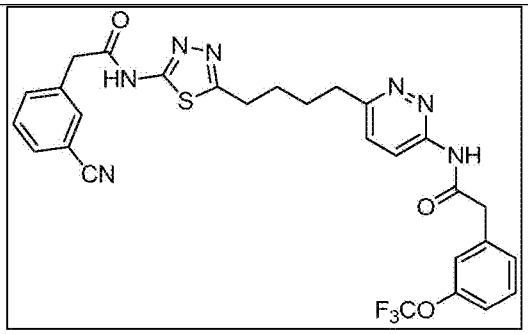
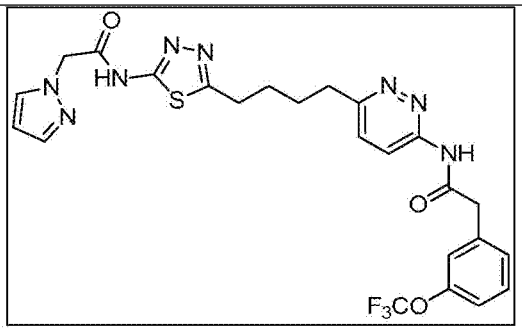
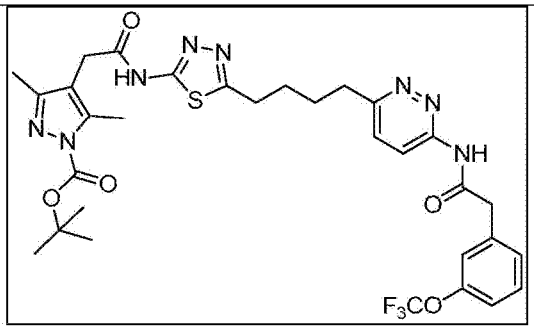
[0981]

675				0.040
676				0.030
677				0.056
678				0.026
679				0.036
680				0.033

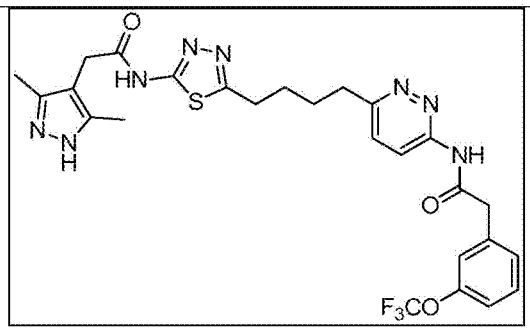
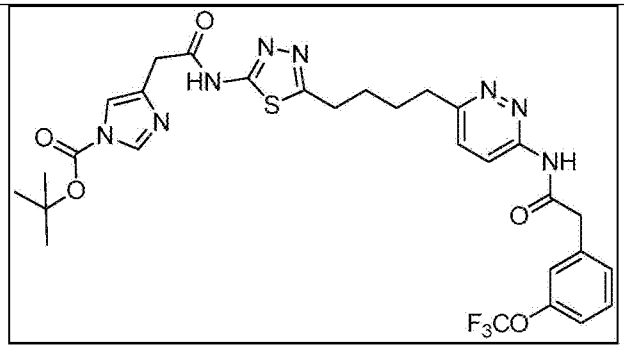
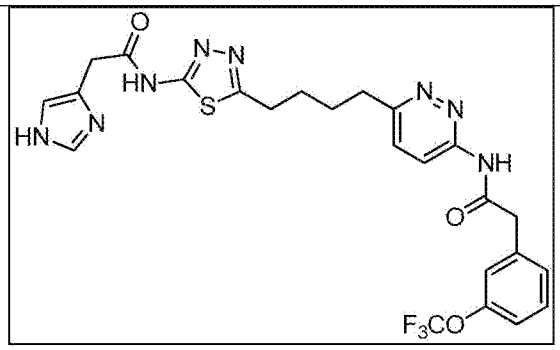
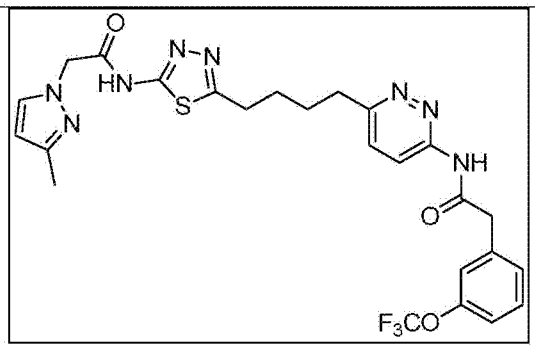
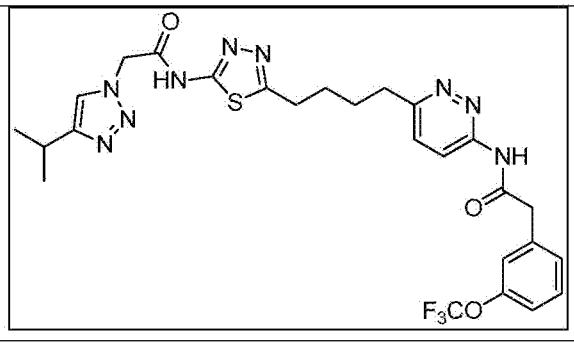
[0982]

681				0.019
682				0.017
683				0.024
684				0.042
685				0.022
686				0.010
687				0.011

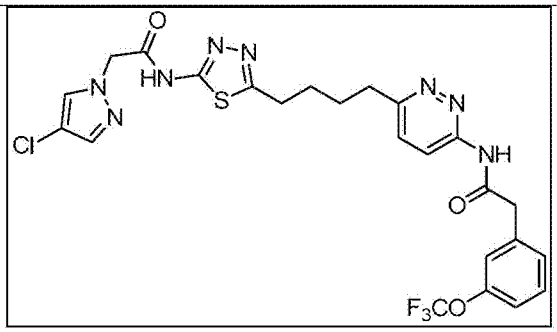
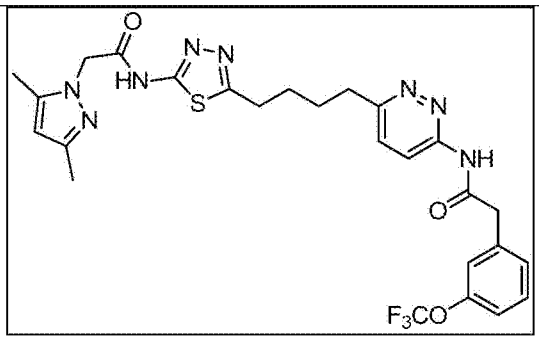
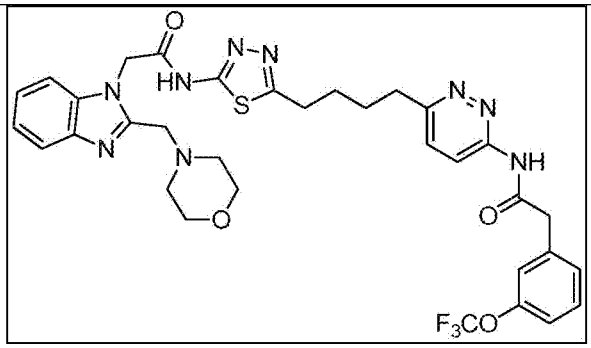
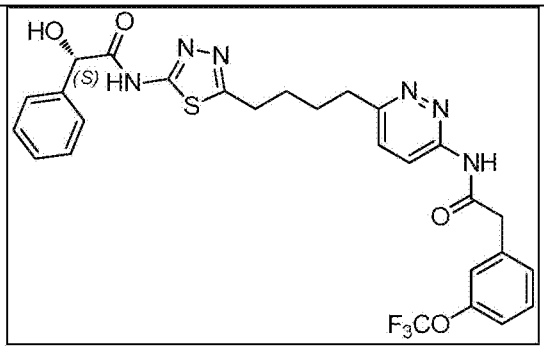
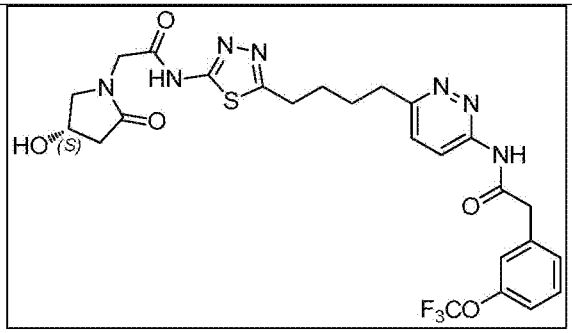
[0983]

688				0.012
689				0.013
690				0.017
692				0.020
693				0.070
694				0.029

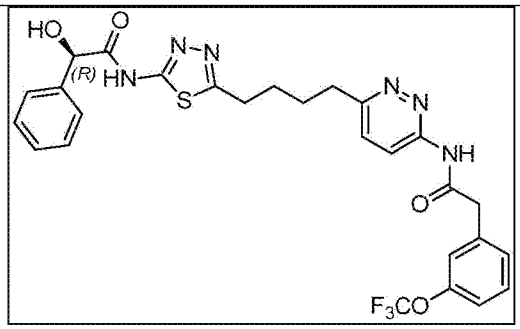
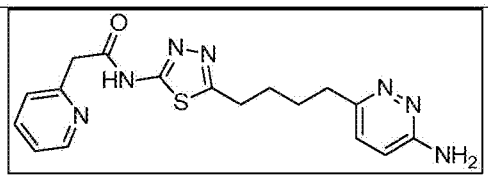
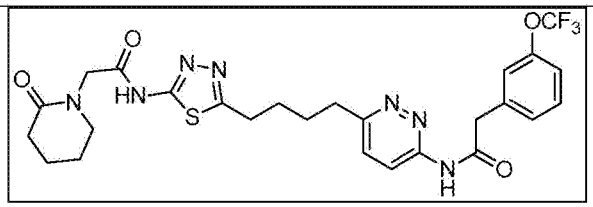
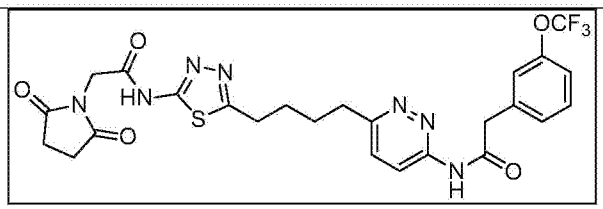
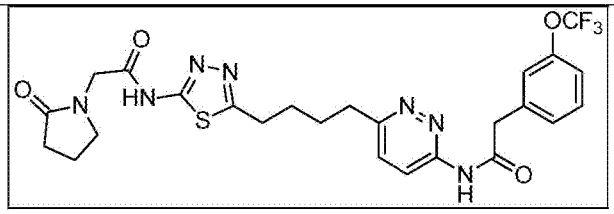
[0984]

695				0.030
696				0.034
697				0.050
698				0.098
699				0.12

[0985]

700				0.17
701				0.11
702				0.31
703				0.012
704				0.88

[0986]

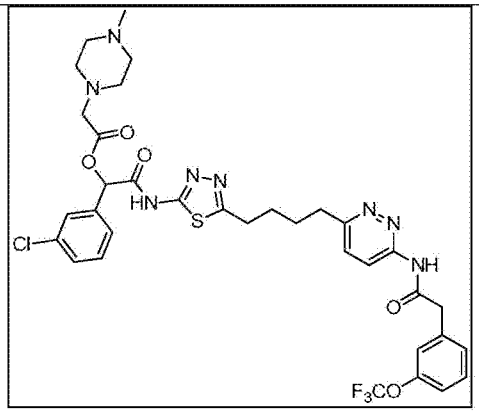
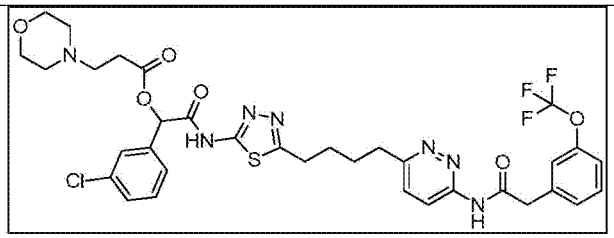
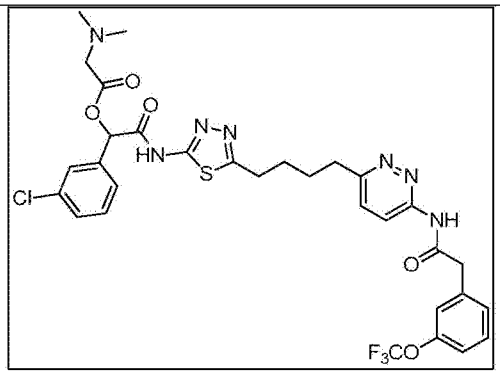
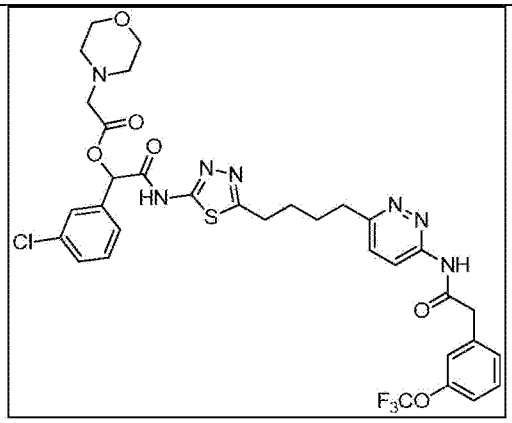
705				0.032
706				14
707				0.085
708				2.8
709				0.14

[0987] 表 3b：

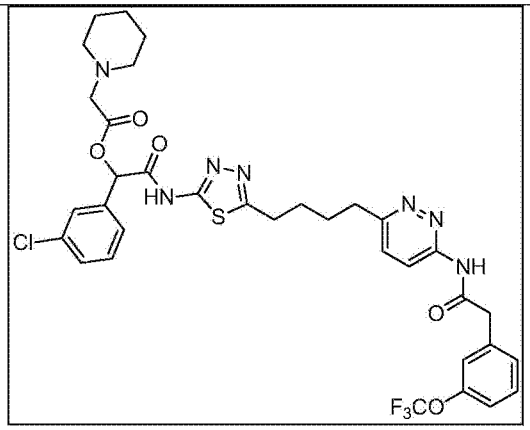
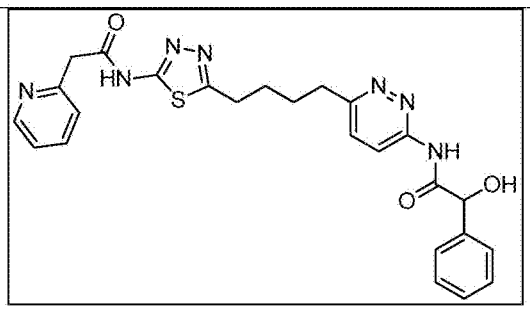
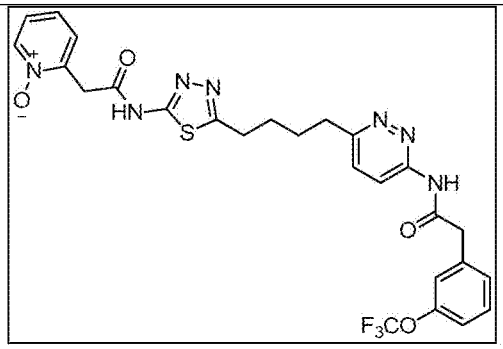
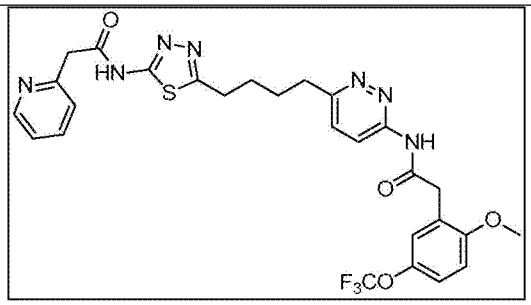
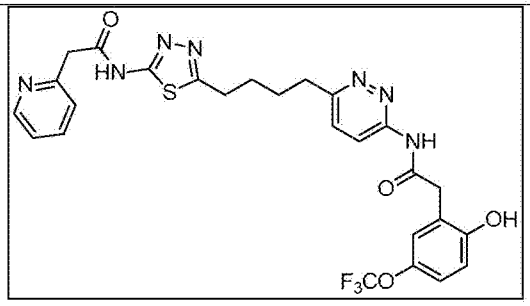
[0988]

化合物 ID	结构	改良的 GAC Δ N2 IC50 60 分钟预孵育 (μM)	GAC Δ N2 IC50 60 分钟预孵育 (μM)	GAC Δ N2 IC50 无预孵育 (μM)	细胞增殖 P493 72h IC50 (μM)

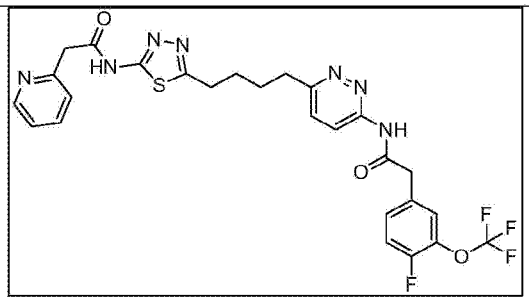
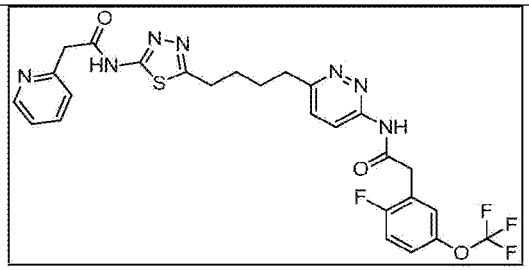
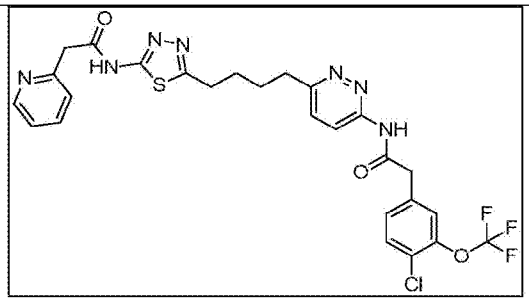
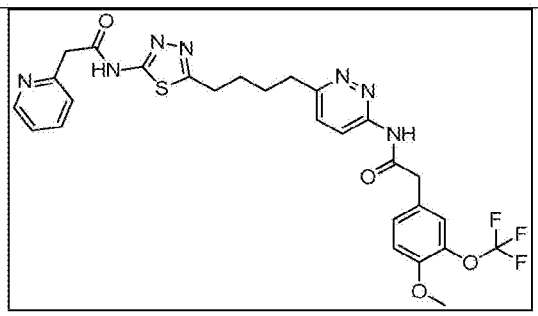
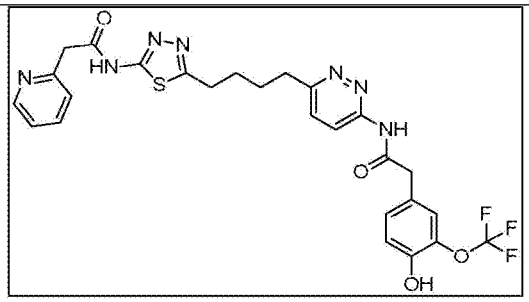
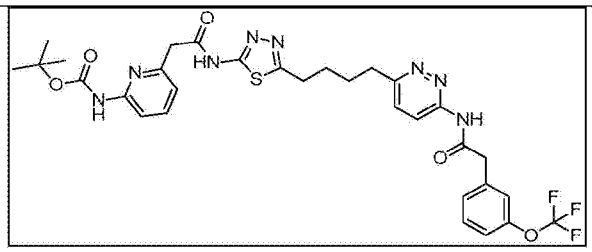
[0989]

710					
711					
712					
713					

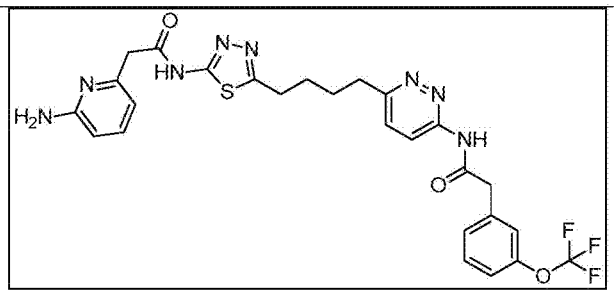
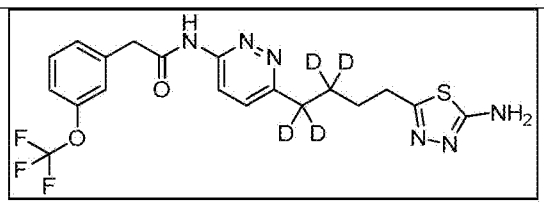
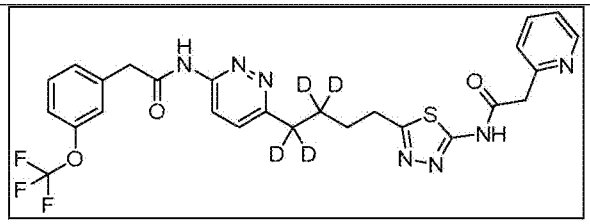
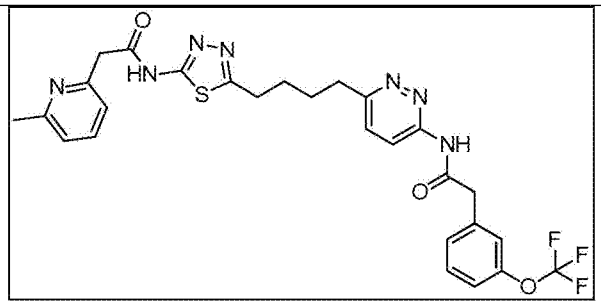
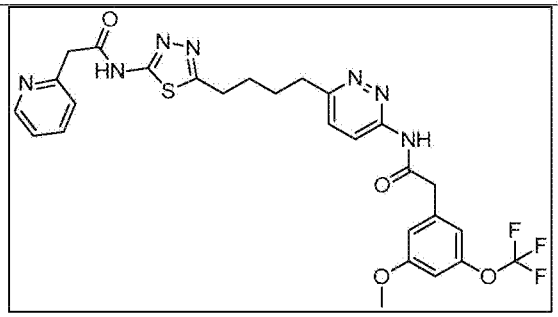
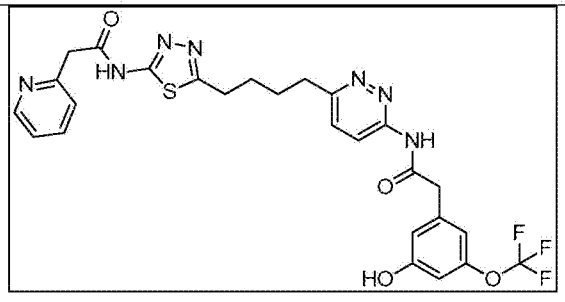
[0990]

714					
715		0.19			0.39
716					0.18
717		0.034			0.019
718		0.026			0.015

[0991]

719		0.033			0.01
720		0.020			0.92
721		0.016			0.022
722		0.024			0.016
723		0.042			0.02
724		0.14			0.034

[0992]

725		0.050			0.15
726		0.54			0.61
727		0.023			0.012
728		0.012			0.018
729		0.016			0.026
730		0.013			0.025

[0993] 实施例 3 :Caco-2 通透性测定法

[0994] Caco-2 细胞通常用于细胞培养插入滤片上的汇合单层。当以这种方式并在特定条件下培养时,细胞发生分化和极化,使得其表型在形态和功能上类似于内衬小肠的肠细胞。细胞单层对小分子的通过提供物理和生物化学屏障,并广泛用于制药业作为人小肠

粘膜的体外模型以预测口服给予的药物的吸收 (Hidalgo 等 , Gastroenterology, 1989 ; Artursson, J. Pharm. Sci., 1990)。跨越 Caco-2 单层的体外表观通透性 (P-app) 和体内吸收间的相关性已被充分证实 (Artursson 等 , Biochem. Biophys. Res. Comm., 1991)。

[0995] 采用本测定法以测定本发明的化合物通过 Caco-2 细胞单层的双向通透性。使 Caco-2 细胞在汇合单层中生长,在单层中顶面 (A) 和底侧面 (basolateral side)(B) 两者的培养基为 pH 7.4。在 200μM 萤光黄存在下,一式两份将 1μM 化合物给予顶面 (A → B) 或底侧面 (B → A) 上用于评价。在 120 分钟暴露后,取 A 面和 B 面两者的样品,采用通用 LC-MS/MS 方法,以最小 4 点校正曲线,测定化合物浓度 (以%回收率报告)。

[0996] 化合物的吸收潜力归类为低 (P-app<$1X10^{-6}$cm/s) 或高 (P-app>$1X10^{-6}$cm/s)。流出比率计算为 (Papp B → A)/(Papp A → B),当 Papp(B → A) 大于或等于 $1X10^{-6}$cm/s 时,流出比率如大于或等于 3 则显著。本发明某些化合物的结果见表 4。

[0997] 表 4 :Caco-2 通透性结果

[0998]

化合物	方向	回收率 (%)	Papp (平均)	流出比率	通透性分类	显著流出
533	A→B	41	4.94	7.6	高	是
	B→A	52	37.5			
585	A→B	42	7.52	3.1	高	是
	B→A	53	23.4			
616	A→B	65	8.23	6.0	高	是
	B→A	76	49.5			
295	A→B	89	8.17	7.3	高	是
	B→A	96	59.8			
318	A→B	73	2.45	18	高	是

[0999]

	B→A	82	44.5			
339	A→B	73	2.39	17	高	是
	B→A	80	41.6			
354	A→B	117	1.38	33	高	是
	B→A	101	45.0			
436	A→B	44	3.75	6.6	高	是
	B→A	57	24.7			
660	A→B	56	0.61	3.9	低	是
	B→A	68	2.37			
670	A→B	70	9.64	6.2	高	是
	B→A	72	59.6			
679	A→B	34	7.59	2.6	高	否
	B→A	42	19.6			
447	A→B	71	7.76	3.5	高	是
	B→A	56	27.2			
703	A→B	51	6.26	6.6	高	是
	B→A	66	41.0			
705	A→B	60	8.52	6.0	高	是
	B→A	67	51.0			

[1000] 实施例 4 :溶解度

[1001] 将试验品的约 1mg 部分与 120 μL 溶剂在 96x 2mL 聚丙烯板的孔中混合。将板在室温（约 20℃）下剧烈涡旋混合 18 小时，目视检查各孔未溶解的固体；不含可见固体的孔加入额外的固体试验品，在室温下再涡旋 6 小时，之后所有孔显示可见固体。然后将所有孔的内含物通过 0.45 μm GHP 滤板过滤得到透明滤液。将 5 μL 各滤液在 100 μL DMF 中稀释，涡旋混合得到 HPLC 样品。通过在测量体积的 DMF 中稀释称重部分的固体试验品，来制备各试验品的一式两份定量标准品。采用表 5 中概述的方法，通过 HPLC 分析 2 μL 各 HPLC 样品和定量标准品。通过针对合适的定量标准品的峰面积比，计算溶解的试验品浓度。溶解度结果见表 6。

[1002] 表 5 :HPLC 方法概述

[1003]

仪器	带二极管阵列 UV/Vis 检测器的 Shimadzu

[1004]

	Prominence UFLC	
柱	VWR Sonoma C8(2)，3.5 μm，2.1 x 50 mm	
柱温	40℃	
流动相 A	0.1% (v/v)甲酸/水	
流动相 B	0.1% (v/v)甲酸/乙腈	
流速	0.4 mL/分钟	
梯度	*时间(分钟)*	*%流动相 B*
	0	20
	8	100
	8.5	100
	8.6	20
	9.6	结束

[1005] 表 6 :所测溶解度

[1006]

溶剂	**溶解度(mg/mL)**			
	1	**295**	**402**	**585**
水	< 0.002	< 0.002	< 0.004	< 0.002
0.9% NaCl	< 0.002	< 0.002	< 0.004	< 0.002
0.1 M HCl	< 0.002	0.003	< 0.004	< 0.002
50 mM Cit pH 2.3	< 0.002	< 0.002	< 0.004	< 0.002
50 mM Cit pH 3.3	< 0.002	< 0.002	< 0.004	< 0.002
50 mM Cit pH 4.4	< 0.002	< 0.002	< 0.004	< 0.002
50 mM Cit pH 5.4	< 0.002	< 0.002	< 0.004	< 0.002
PBS	< 0.002	< 0.002	< 0.004	< 0.002
0.1 M NaOH	14.420	0.268	< 0.004	0.192
10% PS80/50 mM cit	0.050	0.027	0.153	0.261
10% CrEL/50 mM cit	0.076	0.055	0.157	0.228

[1007]

20% SBECD/50 mM cit	0.046	0.090	0.019	0.125
20% HPBCD/50 mM cit	0.042	0.167	0.056	0.327
Labrasol	0.258	0.918	31.032	5.004
Capryol PGMC	0.042	1.540	11.210	1.780
Capryol 90	0.081	0.215	13.676	1.744
低芥酸菜籽油	< 0.002	< 0.002	0.529	0.072
PEG400	0.451	1.644	30.179	3.944
PG	0.048	0.234	1.365	1.422
EtOH	0.040	0.083	2.958	1.991
溶剂	**溶解度(mg/mL)**			
	670	**447**	**703**	
水	0.007	< 0.004	< 0.004	
0.9% NaCl	< 0.002	0.005	< 0.004	
0.1 M HCl	0.005	< 0.004	< 0.004	
50 mM Cit pH 2.3	0.066	< 0.004	< 0.004	
50 mM Cit pH 3.3	0.003	< 0.004	< 0.004	
50 mM Cit pH 4.4	< 0.002	< 0.004	< 0.004	
50 mM Cit pH 5.4	< 0.002	< 0.004	< 0.004	
PBS	< 0.002	< 0.004	< 0.004	
0.1 M NaOH	0.227	0.192	0.656	
10% PS80/50 mM cit	1.204	0.851	0.378	
10%	0.458	0.732	0.309	

[1008]

CrEL/50 mM cit				
20% SBECD/50 mM cit	5.256	2.718	0.476	
20% HPBCD/50 mM cit	9.685	2.177	0.651	
Labrasol	5.042	77.164	20.727	
Capryol PGMC	1.519	7.916	3.683	
Capryol 90	1.974	11.114	7.409	
低芥酸菜籽油	0.012	0.071	0.014	
PEG400	9.901	57.334	22.419	
PG	2.569	8.265	4.698	
EtOH	0.964	3.921	2.645	

[1009] 实施例 5 :抗增殖和谷氨酰胺依赖性测定法

[1010] 体外测试乳腺细胞系在缺乏谷氨酰胺下生长的能力和在含有谷氨酰胺的培养基中其对化合物 670 的敏感性。在 37℃、5% CO_2下将细胞保持在补充 2mM 谷氨酰胺的生长培养基 (RPMI-1640、10% FBS、100 单位 /ml 青霉素和 100Ag/ml 链霉素、0.25 μg/mL 两性霉素) 中。

[1011] 为了测定谷氨酰胺依赖性,根据细胞大小及其生长特性,将细胞以 3000-5000 个细胞 / 孔的密度接种在 96 孔板中。选择合适的接板密度以确保细胞在 72 小时测定期限内不发生汇合。接种后 24 小时,除去接板培养基,将细胞用不含谷氨酰胺的生长培养基洗涤 2 次,然后将 100uL 不含谷氨酰胺的培养基或含有的谷氨酰胺 (2mM) 的生长培养基加回到孔中。将细胞在 37℃与 5% CO_2下孵育 72 小时,并通过 Cell Titer Glo(Promega) 分析抗增殖作用。通过经由下式 ((在不含谷氨酰胺的培养基中生长 72 小时的细胞的 rfu - 在 t = 0 时的 rfu)/(在 2mM 谷氨酰胺中生长 72 小时的细胞的 rfu - 在 t = 0 时的 rfu)) 比较谷氨酰胺撤出当天 (t = 0) 平行板中测量的 Cell Titer Glo 信号 (rfu) 与在 72 小时孵育期后观察到的信号,计算细胞增殖 (DMSO 对照的%)。通过下式测定细胞损失 :(100x 不含谷氨酰胺的培养基中 72 小时时的 rfu/ 在 t = 0 时的 rfu) - 100。

[1012] 通过处理如上所述接种在 96 孔板中的细胞,来测定对化合物 670 的敏感性。在接种后 24 小时,将细胞用含 2mM 谷氨酰胺的生长培养基洗涤,将 50uL 含 2mM 谷氨酰胺的生长培养基加入孔中。将 10mM 化合物 670 的 DMSO 原液以 200uM 稀释入 100% DMSO 中。将其在含 2mM 谷氨酰胺的生长培养基中进一步稀释成 2uM。将 50ul 的该混合物加入细胞板,使得 670uM 的终浓度为 1uM。平行对照孔只用 DMSO 处理。将细胞在 37℃与 5% CO2 下孵育 72 小时,并通过 Cell Titer Glo 分析抗增殖作用。以带有下列修改的类似于上述的方式,

计算细胞增殖：细胞增殖（（在 1uM 化合物 670 中生长 72 小时的细胞的 rfu - 在 t = 0 时的 rfu)/（在 72 小时时 DMSO 对照的 rfu - 在 t = 0 时的 rfu)），细胞损失（100x 1uM 化合物 670 中在 72 小时时的 rfu/ 在 t = 0 时的 rfu) - 100。这些测定的结果见图 1。

[1013] 实施例 6：三阴性乳腺癌亚型中谷氨酰胺酶和谷氨酰胺合成酶的差异性表达。

[1014] 下载原发性乳腺肿瘤和细胞系表达数据集 [The Cancer Genome Atlas，来自 https://genome-cancer.ucsc.edu（乳腺侵润性癌 / 基因表达 /RNAseqV2 数据）和 The Cell Line Encyclopedia，来自 http://www.broadinstitute.org/ccle/home（基因中心 RMA 归一化 mRNA 表达 /aAffymetrix U133+2 阵列）]，并评价下列基因各数据集内的表达水平：雌激素受体 (ER)、黄体酮受体 (PR) 和 Her2(ERBB2)、谷氨酰胺酶 (GLS) 和谷氨酰胺合成酶 (GLUL)。通过与整个数据集中基因的表达中位值进行比较，来计算各样品中指定基因的相对表达水平。通过分析该群中 3 个标志物基因各自的表达分布和谷氨酰胺酶和谷氨酰胺合成酶的相对水平，来鉴定具有最低相对水平的 ER、PR 和 Her2（“三阴性”）的样品，并且评价非三阴性群。图 2 表示表明三阴性群中相对高表达（红色）的谷氨酰胺酶和低表达（绿色）的谷氨酰胺合成酶的热图。

[1015] 实施例 7：MDA-MB-231 原位异种移植物模型的单药剂化合物 402治疗。

[1016] 将与基质胶 1:1 混合的 1x 10^7个 MDA-MB-231 细胞植入 6-8 周龄雌性 scig/beige 小鼠 (n = 20) 的腹股沟乳房脂垫。当肿瘤达到 100-150mm^3的体积时，将小鼠随机分成以下两组，n = 10 只小鼠 / 组：1) 溶媒对照 (Gelucire)PO BID 给予 35 天，和 2)100mg/kg 的化合物 402（以 10mg/mL 在 Gelucire 中配制）IP BID 给予 35 天。用测径器每周两次测量肿瘤 35 天，应用下式计算肿瘤体积：肿瘤体积 (mm^3) = (a x b^2/2)，其中‘b’是最小直径，‘a’是最大直径。最终剂量后 24 小时，处死小鼠，切下肺，肺转移用百分比肺转移覆盖度（肺外部质地粗糙）量化。图 3 显示与溶媒相比用化合物 402 治疗时肿瘤体积和转移的测量结果。

[1017] 实施例 8：MDA-MB-231 原位异种移植物模型中用化合物 389 和紫杉醇的联合研究。

[1018] 用与基质胶 1:1 混合的 1x 10^7个 MDA-MB-231 细胞植入 6-8 周龄的雌性 scig/beige 小鼠 (n = 40) 的腹股沟乳房脂垫。当肿瘤达到 100-150mm^3的体积时，将小鼠随机分成以下 4 组，n = 10 只小鼠 / 组：1) 溶媒对照 (20％ HPBCD/10mM 柠檬酸盐缓冲液 pH 4.0) IP BID 给予 35 天，2)50mg/kg 的化合物 389(5mg/mL 在 20％ HPBCD/10mM 柠檬酸盐缓冲液 pH 4.0 中配制）IP BID 给予 35 天，3)10mg/kg 的紫杉醇（在盐水稀释至 1mg/mL 的临床制剂）IP QD 给予 5 天，和 4)50mg/kg 的化合物 389IP BID x 35 天加 10mg/kg 的紫杉醇 IP QD 给予 x 5 天。用测径器每周两次测量肿瘤 35 天，应用下式计算肿瘤体积：肿瘤体积 (mm^3) = (a x b^2/2)，其中‘b’是最小直径，‘a’是最大直径。图 4 显示与溶媒和仅各药物相比，用化合物 389 和紫杉醇的组合治疗时肿瘤体积的测量结果。

[1019] 实施例 9：通过液相色谱法串联质谱法测定细胞样品中的谷氨酸和谷氨酰胺。

[1020] 如实施例 5 所述测定对化合物 670 的敏感性。

[1021] 针对代谢物水平检查未处理的细胞。通过液相色谱法串联质谱法 (LC-MS/MS) 测定谷氨酰胺和谷氨酸的浓度。来自体外细胞测定法的细胞沉淀用 PBS 洗涤，与含有内标 (IS) 的甲醇：水 (50:50) 混合以提取谷氨酰胺和谷氨酸，然后在 -70℃下保存直到分析。提取的细胞样品经涡旋，离心和 / 或过滤，注入 10 μL 提取物用于 LC-MS/MS 分析。通过比较研究样品中与标准校准样品中分析物与 IS 的峰面积比，量化谷氨酰胺和谷氨酸。LC-MS/MS

系统包括配备 Shimadzu LC-10ADvp 泵 (Shimadzu, Columbia, MD) 和 Leap PAL HTC-xt 自动进样器的 API 4000 质谱仪 (ABSCIEX, Foster City, CA)。使用梯度洗脱,在 Phenomenex Luna NH2 柱 (2.1×50mm,3.5μm 粒度) 上实现色谱分离。流动相为 (A)10mM 乙酸铵和 5mM 氢氧化铵水溶液,和 (B)50:50 甲醇:乙腈。通过选择性 m/z 转换的 MRM:对于谷氨酸为 145.9 → 101.8 和对于谷氨酰胺为 144.7 → 108.8,使用负电离模式的 Turbo 离子喷雾界面实现质谱检测。这些测定的结果见图 9。

[1022] 实施例 10:谷氨酰胺酶:谷氨酰胺合成酶比率的测定

[1023] 基因表达数据来自 Oncomine 的 Barretina Cell Line 数据集。使各个原发性肿瘤样品的各个谷氨酰胺酶和谷氨酰胺合成酶转录物的表达水平分位数归一化。在任何指定样品中,log2 拷贝数为 0 表明所述基因以相对于跨越所有数据集和所分析的样品的 12,000 个基因的表达中位值水平表达。水平线表明所示临床样品数内各个转录物的表达中位值的比率。结果见图 5、6、7 和 8。

[1024] 实施例 11:表达和代谢物相关性延伸到其它肿瘤类型

[1025] 原发性肿瘤异种移植物连同谷氨酰胺酶和谷氨酰胺合成酶表达的微阵列数据由商业临床研究组织提供。如实施例 9 中所述测定谷氨酸和谷氨酰胺。谷氨酰胺酶活性基本上按 Curthoys 和 Bellemann(Exp Cell Res,1979) 的描述测定。图 10 显示谷氨酸:谷氨酰胺比率与谷氨酰胺酶:谷氨酰胺合成酶表达比或谷氨酰胺酶活性之间的相关性。

[1026] 实施例 12:结肠癌异种移植物功效研究

[1027] 以 100uL 无菌 PBS 的体积用 5x 10^6 个 HCT116 细胞 / 小鼠皮下植入约 6 周龄的雌性 scid/bg 小鼠右胁。当肿瘤达到 50-100mm^3 的体积时,小鼠随机分成 n = 10 的组以接受通过腹膜内注射每日递送 2 次的溶媒或试验化合物。使用 Vernier 测径器,每周 3 次测量肿瘤,应用下式计算肿瘤体积:体积 = (长度 x 宽度 ^{2}/2),其中长度和宽度是肿瘤的最长垂直边。继续给药每天两次直到对照肿瘤达到 2000mm^3 的大小。应用 2 方向 ANOVA 与 Bonferroni 事后检验进行统计比较。图 11 显示在该 HCT116 结肠癌异种移植物模型中将化合物 188 腹膜内给予小鼠导致肿瘤大小减小。

[1028] 实施例 13:肺腺癌异种移植物功效研究

[1029] 用悬浮于 PBS 中的 1x 10^7 个 H2122 肺腺癌细胞 / 小鼠皮下植入 6-8 周龄的雌性 scid/beige 小鼠 (n = 20) 中。将小鼠随机分成以下两组,n = 10 只小鼠 / 组:1) 溶媒对照 (25% 羟基丙基 -β- 环糊精),和 2) 以 200mg/kg 口服给予的化合物 670(以 20mg/mL 在 25% HP-β-CD 中配制)。对于两个组,在植入后 24 小时开始给药,并继续口服 BID 23 天。用测径器每周 3 次测量肿瘤,应用下式计算肿瘤体积:肿瘤体积 (mm^3) = (a x b^2/2),其中 'b' 是最小直径,'a' 是最大垂直直径。**P 值 <0.01(双侧 T 检验)。结果见图 12。

[1030] 实施例 14:TNBC 和 HR+/Her2+ 乳腺肿瘤细胞系中谷氨酰胺酶和谷氨酰胺合成酶的 mRNA 表达

[1031] 查询 2 个可公开获取的数据库以测定谷氨酰胺酶 (GLS) 和谷氨酰胺合成酶 (GS) 的 mRNA 水平:

[1032] -Neve 等,(Cancer Cell 10(6):515-27(2006 年 12 月)) 公开的一组 51 个乳腺癌细胞系的微阵列表达数据,在本实施例中评价了其中的 20 个,和

[1033] -The Cell Line Encyclopedia(CCLE;Barretina 等,Nature 483,603-607(2012

年 3 月 29 日），其包括 58 个乳腺癌细胞系的表达数据，本实施例中使用其中的 25 个。

[1034] Neve 等人的出版物包括数据集中各个细胞系的激素和生长因子状态的注释（25 个三阴性，26 个 HR+ 或 Her2+）。对于 CCLE 数据集，根据雌激素受体（ESR，20/58 阳性）、黄体酮受体（PGR，10/58 阳性）和 Her2（ERBB2，13/58 阳性）的 mRNA 表达水平，评价激素和生长因子受体状态。根据该分析，总共 31 个细胞系归类于 TNBC，27 个归类为 HR+ 或 Her2+。对于两个数据集提供的 33 个细胞系，在激素和生长因子受体状态分配中有良好的一致性（32/33）。本实施例在包括 22 个三阴性和 7 个 HR+ 或 Her2+ 的一组细胞系中进行。

[1035] 根据跨越数据集中所有样品的所有探针集（probeset）的表达中位值，使各个细胞系中的 GLS 剪接变体 KGA（探针集 203159_at）和 GAC（探针集 221510_s_at）以及 GS（探针集 215001_s_at）的 log2 换算的 mRNA 表达值进行中位值居中（Neve 等数据集的中位值为 5.583，CCLE 数据集的中位值为 4.809）。对于 GLS:GS 比率计算，首先将 KGA、GAC 和 GS 的 log2 换算表达值转换回相应的未换算值。在 TNBC 细胞系与 HR+/Her2+ 细胞系中比较 GLS（KGA 和 GAC）、GS 的表达水平和 GLS（KGA 或 GAC）与 GS 的比率。应用不配对斯氏 T 检验（Prism），测定显著差异。

[1036] TNBC 细胞系和 HR+/Her2+ 细胞系间的差异通过图表描述于图 13 中。对于两个数据集，与 HR+ 或 Her2+ 细胞系相比，在 TNBC 中有显著较高的 GLS 剪接变体 KGA 和 GAC 表达。GAC 剪接变体的差异数量和统计显著性较大。对于谷氨酰胺合成酶（GS），相对于两个数据集的 HR+ 或 Her2+ 亚集，在 TNBC 细胞系中有显著较低的表达。KGA 与 GS 比率和 GAC 与 GS 的比率在 TNBC 细胞系中也显著较高。

[1037] 实施例 15：对化合物 670 的敏感性和 GLS 和 GS 的表达之间的相关性。

[1038] 将所观察到的作为化合物 670 治疗结果的细胞增殖和细胞损失与谷氨酰胺酶（KGA 和 GAC）、谷氨酰胺合成酶（GS）的表达水平和谷氨酰胺酶与谷氨酰胺合成酶的比率进行比较。按实施例 5 中所述测定化合物 670 的抗增殖作用。图 14 显示绘出所有测试的细胞系的化合物 670 敏感性对各表达参数（来自 Neve 等或 CCLE 数据集）的曲线的一系列二变量图，而表 7 概括相应的 Spearman 秩序相关系数（和 P 值）。对于两个表达数据集，在化合物 670 敏感性和谷氨酰胺酶的 GAC 同种型的表达、谷氨酰胺合成酶（GS）的表达和 GAC:GS 的比率之间观察到显著相关性。各个数据集中的最大显著相关性为仅用 GAC 表达进行的。这些结果支持这样的设想，即 GAC 表达或 GAC:GS 比率升高的细胞对用谷氨酰胺酶抑制剂的 GLS 抑制敏感。在多数 TNBC 细胞系和少数受体阳性乳腺细胞系中观察到这种表型。

[1039] 表 7. 化合物 670 的敏感性与 GLS mRNA 表达、GS mRNA 表达或表达比之间的相关性[1]。

[1040]

			化合物670敏感性和mRNA表达参数之间的相关性				
			KGA	GAC	GS	KGA:GS	GAC:GS
数据集	Neve	Spearman相关系数	-0.391	-0.7624	0.585	-0.585	-0.7353
		P值(双尾)	0.0883 ns	<0.0001 ****	0.0067 **	0.0067 **	0.0002 ***
	CCLE	Spearman相关系数	-0.1826	-0.5774	0.4157	-0.3339	-0.4809
		P值(双尾)	0.3931 ns	0.0031 **	0.0434 *	0.1108 ns	0.0174 *

[1041] [1]对于图 14 中二变量图绘出的数据的 Spearman 秩序相关系数和相关 P 值。对于各相关性分析，汇总整组的乳腺癌细胞系 (TNBC、HR+、Her2+)。

[1042] 实施例 15 :乳腺癌细胞系中利用 Gln 的酶的蛋白质表达

[1043] 使用从乳腺癌细胞系组制备的提取物的蛋白质印迹分析监测蛋白质水平上的 GLS(GAC 和 KGA 剪接变体）和谷氨酰胺合成酶的表达。如图 15 所示，与这些基因的微阵列 mRNA 表达分析一致，多数 TNBC 细胞系表达 GAC 和 KGA，而在大多数受体阳性系中 GAC 和 KGA 以较低水平（或未检出水平）表达。具体地说，GAC 在所测的几乎所有 TNBC 细胞系以相对高的水平表达（与 HR+/Her2+ 细胞系相比）。谷氨酰胺合成酶的表达更易变，并且与微阵列数据不同，在整个该细胞系组中在 TNBC 和受体阳性细胞间不显示明显区别。

[1044] 按照实施例 11 所述方法，针对谷氨酰胺酶活性对用于蛋白质印迹法所制备的细胞裂解物进行分析。结果表明，KGA 和 GAC 蛋白的水平与较高谷氨酰胺酶活性对应。

[1045] 实施例 16 :对谷氨酰胺酶抑制剂的敏感性和代谢物水平。

[1046] 谷氨酸和谷氨酰胺浓度按实施例 9 所述测定。对谷氨酰胺酶抑制剂的敏感性按实施例 5 所述测定。图 16 显示谷氨酸：谷氨酰胺比率和对化合物 670 的敏感性之间的相关性。

[1047] 实施例 17 :多发性骨髓瘤异种移植物研究。

[1048] 给 8-12 周龄的雌性 CB.17SCID 小鼠 (n = 20) 皮下植入与基质胶 1:1 混合的 $1x10^7$个 RPMI-8226 骨髓瘤细胞 / 小鼠。将小鼠随机分成以下两组，n = 10 个小鼠 / 组 :1) 溶媒对照 (25％羟基丙基 -β- 环糊精），和 2) 以 200mg/kg 口服给予的化合物 670（以 20mg/mL 在 25％ HP-β-CD 中配制）。对于两个组，当肿瘤达到 100-150mm^3的体积时开始给药，继续口服 BID 28 天。用测径器每周两次测量肿瘤，应用下式计算肿瘤体积 :肿瘤体积 (mm^3) = ($a \times b^2/2$)，其中 'b' 是最小直径，'a' 最大垂直直径。**P 值 <0.01（双侧 T 检验）。结果见图 17。

[1049] 实施例 18 :用药物组合处理多发性骨髓瘤细胞。

[1050] 如图 18 所示，将 MM1S 细胞（图 A 和 B）和 RPMI-8226 细胞（图 C 和 D）在生长培

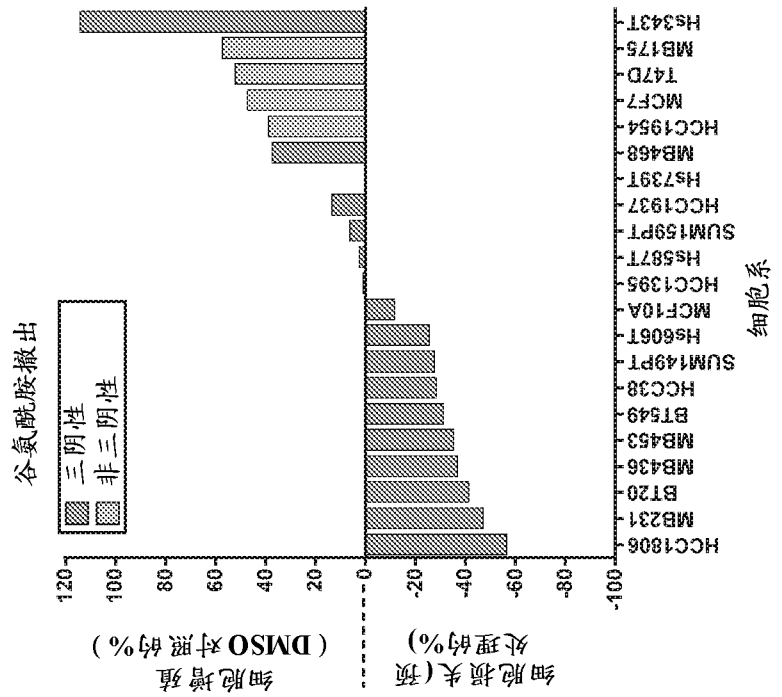
养基中用剂量滴定的化合物670、泊马度胺或其混合物（图A和C）或化合物670、地塞米松或其混合物（图B和D）处理72小时。按照生产商的方案，采用Cell Titer Glo(Promega，Madison，WI)，在孵育结束时测量细胞成活率。使化合物处理细胞的测量值归一化至DMSO处理细胞，数据以细胞存活比率报告，其1(一）的值对应于最大细胞存活，0(零）的值对应于无细胞存活。所有化合物处理的细胞存活比率用柱状图表示。组合指数应用Calcusyn程序(biosoft.com)计算，针对化合物670和泊马度胺[POM]的各混合物（图A和C）和化合物670和地塞米松[DEX]的各混合物（图B和D）报告。突出显示产生协同抗肿瘤活性的化合物混合物。

[1051] 通过引用结合

[1052] 本文提及的所有出版物和专利通过引用以其整体结合到本文中，就像各出版物或专利具体而单独指明通过引用予以结合一样。在有冲突的情况下，以本申请（包括本文的任何定义）为准。2012年11月19日提交的美国申请号13/680,582的化合物、合成方法和实验方案和结果通过引用结合到本文中。

[1053] 等同内容

[1054] 虽然论述了主题发明的具体实施方案，但上述说明书是说明性的和非限制性的。在阅读本说明书和随附权利要求书时，本发明的许多变化对本领域技术人员而言将变得显而易见。通过参考权利要求书及其等同内容的整个范围和说明书及所述变化，确定本发明的整个范围。


谷氨酰胺撤出
三阴性
非三阴性
细胞增殖
（DMSO 对照的%）
细胞损失（预处理的%）
细胞系
HCC1806
MB231
BT20
MB436
MB453
BT549
HCC38
SUM149PT
Hs606T
MCF10A
HCC1395
Hs587T
SUM159PT
HCC1937
Hs739T
MB468
HCC1954
MCF7
T47D
MB175
Hs343T

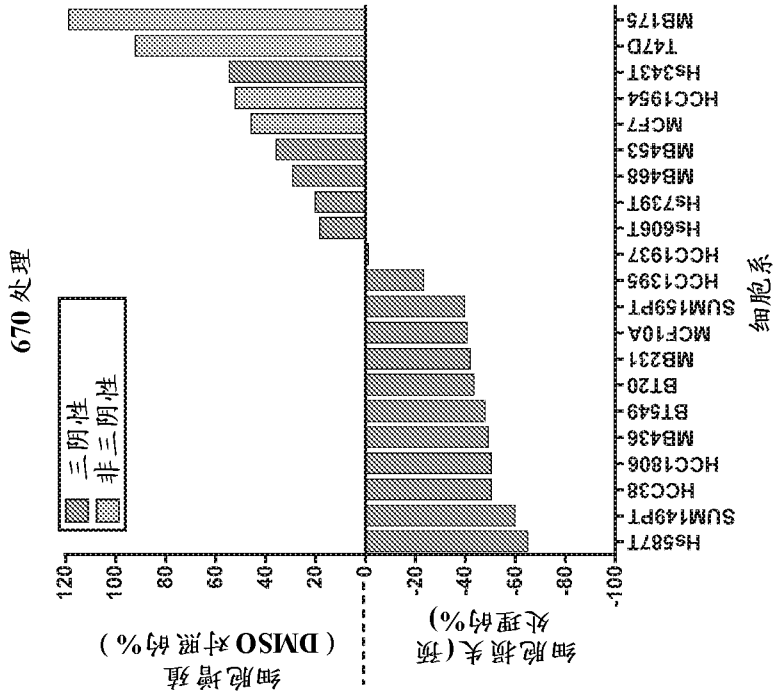

670 处理
三阴性
非三阴性
细胞增殖
（DMSO 对照的%）
细胞损失（预处理的%）
细胞系
Hs587T
SUM149PT
HCC38
HCC1806
MB436
BT549
BT20
MB231
MCF10A
SUM159PT
HCC1395
HCC1937
Hs606T
Hs739T
MB468
MB453
MCF7
HCC1954
Hs343T
T47D
MB175

图 1

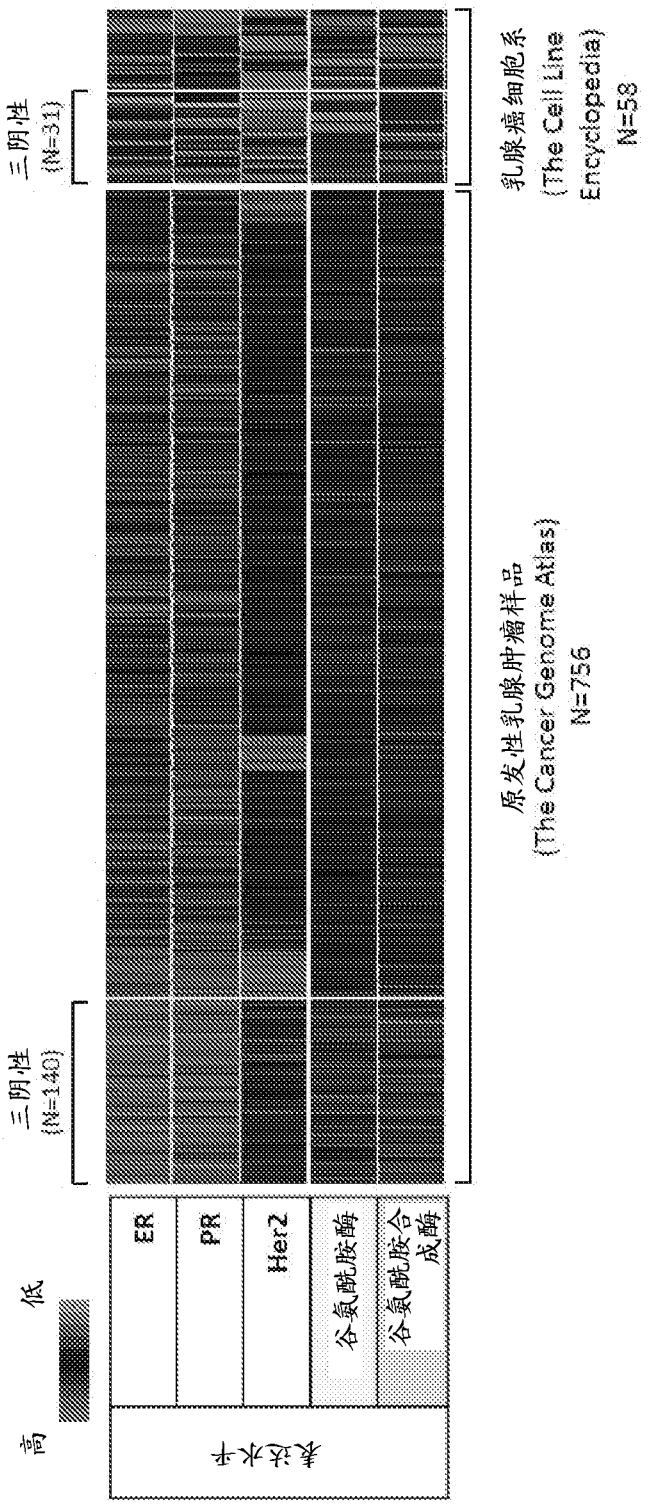

表达水平
高
低
ER
PR
Her2
谷氨酰胺酶
谷氨酰胺合成酶
三阴性
(N=140)
三阴性
(N=31)
原发性乳腺肿瘤样品
(The Cancer Genome Atlas)
N=756
乳腺癌细胞系
(The Cell Line Encyclopedia)
N=58

图 2

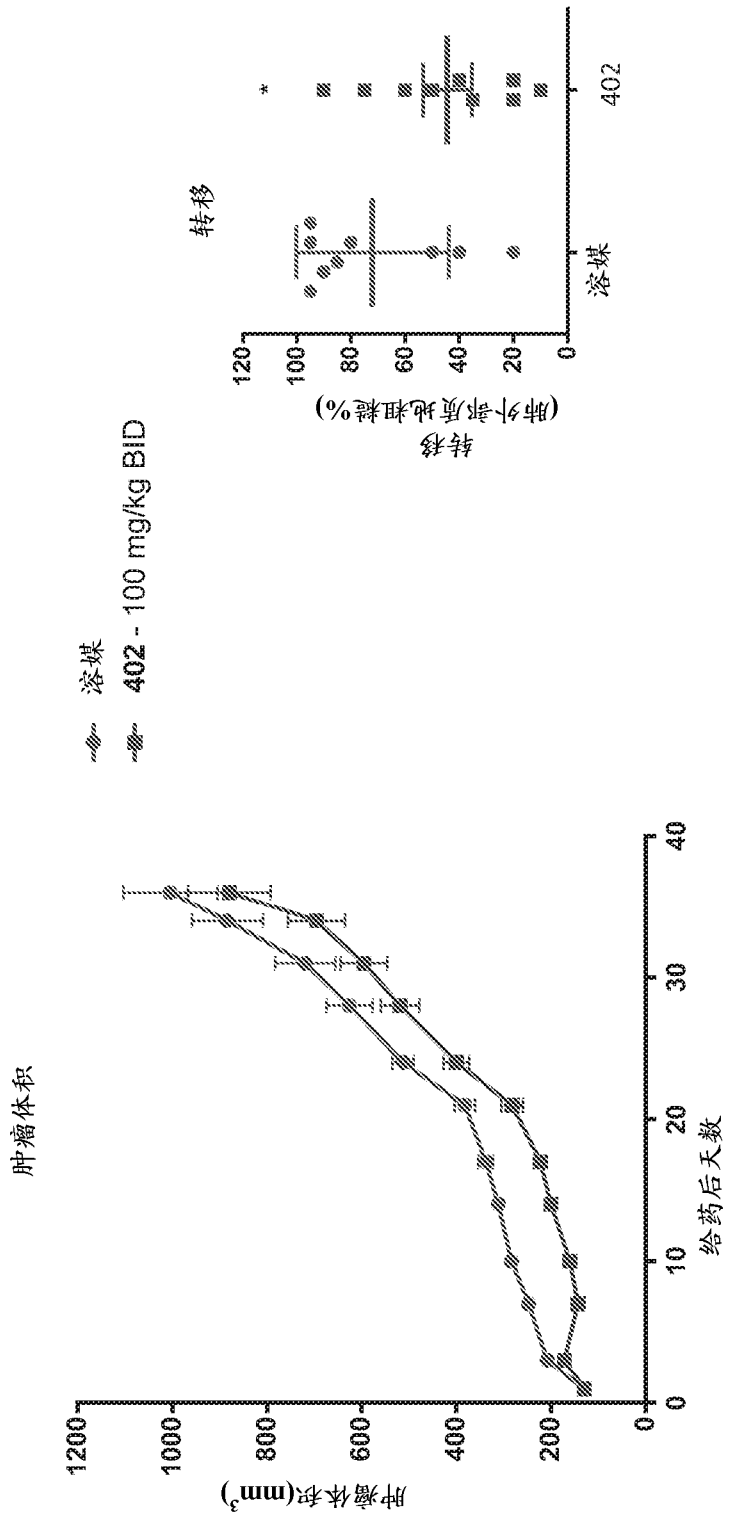

肿瘤体积
肿瘤体积(mm³)
1200
1000
800
600
400
200
0
0
10
20
30
40
给药后天数
溶媒
402 - 100 mg/kg BID
转移
转移
(肺外非原发组织%)
120
100
80
60
40
20
0
溶媒
402
*

图 3

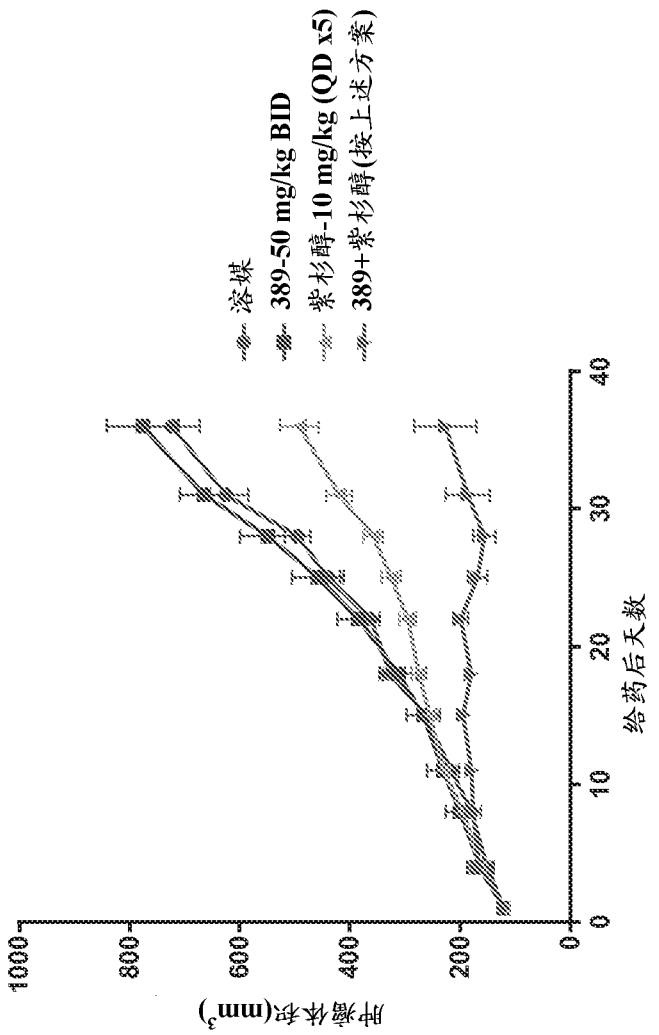

溶媒
389-50 mg/kg BID
紫杉醇-10 mg/kg (QD x5)
389+紫杉醇(按上述方案)
肿瘤体积(mm³)
1000
800
600
400
200
0
0
10
20
30
40
给药后天数

图 4

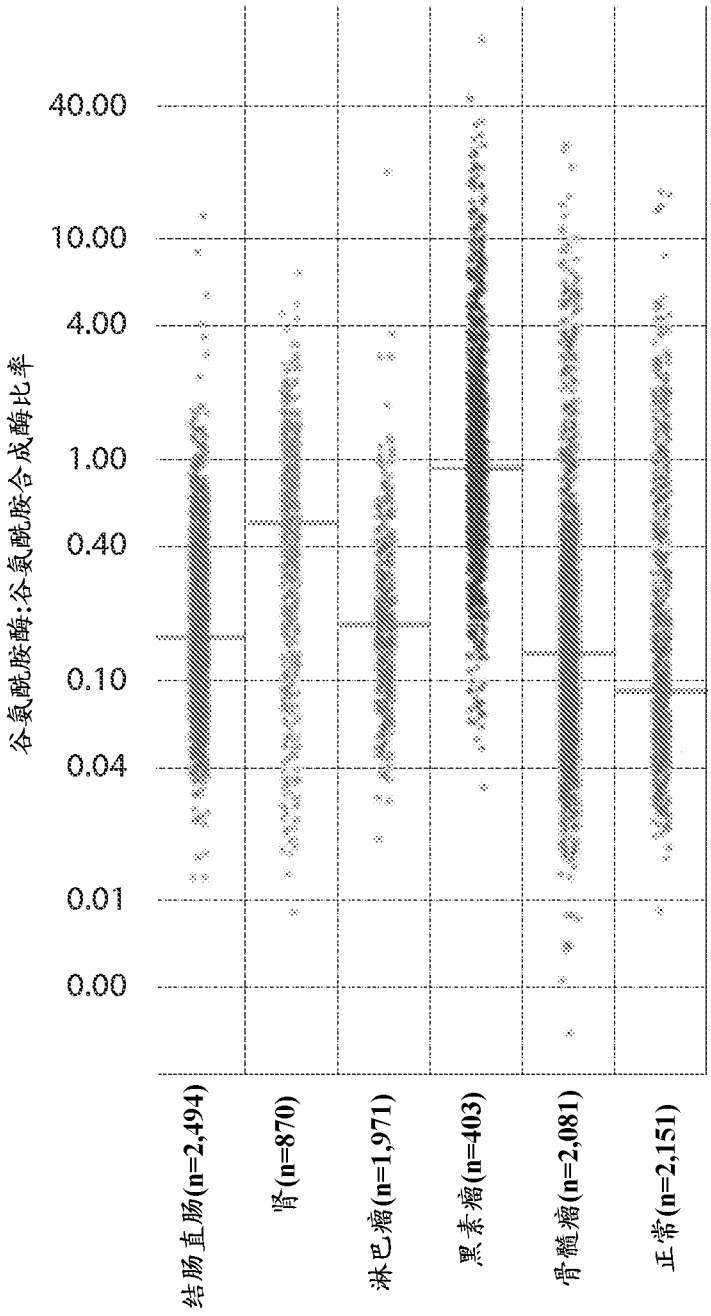

谷氨酰胺酶:谷氨酰胺合成酶比率
40.00
10.00
4.00
1.00
0.40
0.10
0.04
0.01
0.00
结肠直肠(n=2,494)
肾(n=870)
淋巴瘤(n=1,971)
黑素瘤(n=403)
骨髓瘤(n=2,081)
正常(n=2,151)

图 5

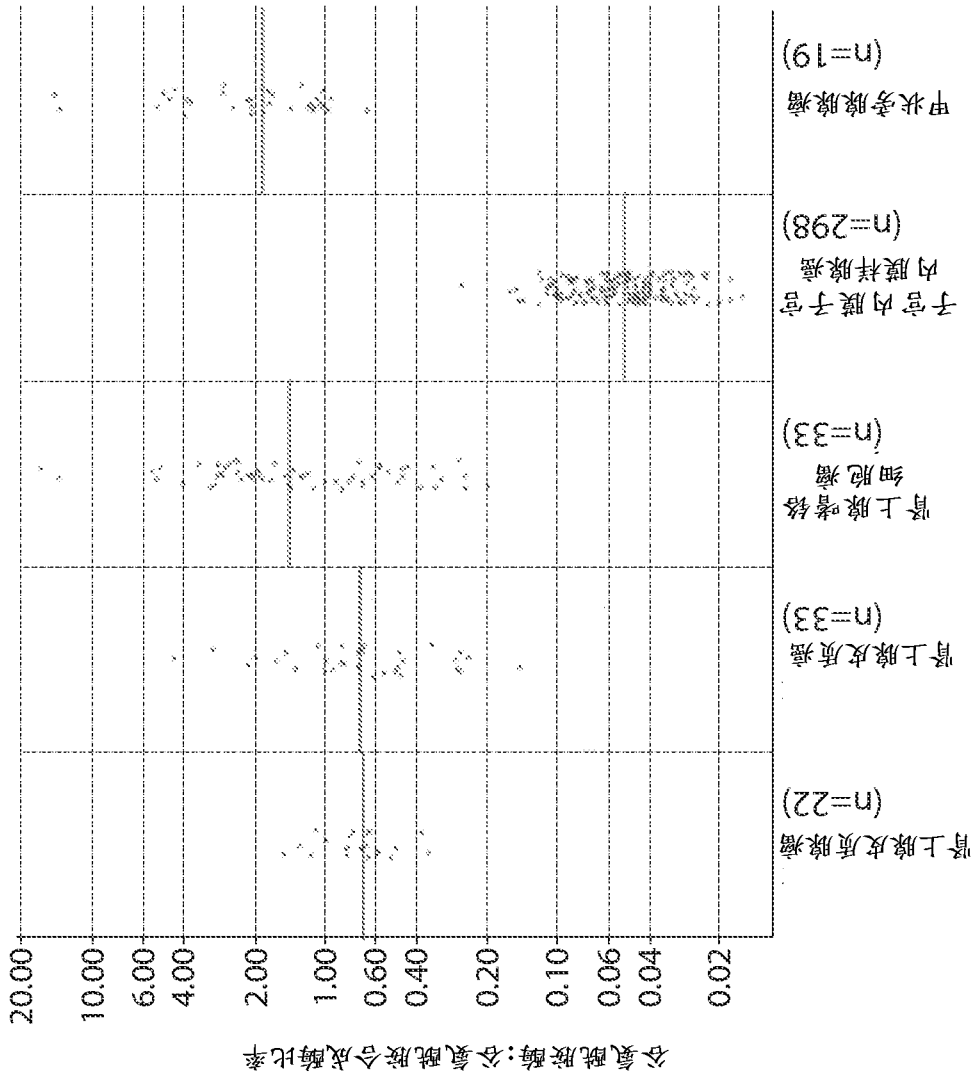

谷氨酰胺酶:谷氨酰胺合成酶比率
20.00
10.00
6.00
4.00
2.00
1.00
0.60
0.40
0.20
0.10
0.06
0.04
0.02
肾上腺皮质腺癌
(n=22)
肾上腺皮质癌
(n=33)
肾上腺嗜铬
细胞瘤
(n=33)
子宫内膜子宫
内膜样腺癌
(n=298)
甲状旁腺腺瘤
(n=19)

图 6

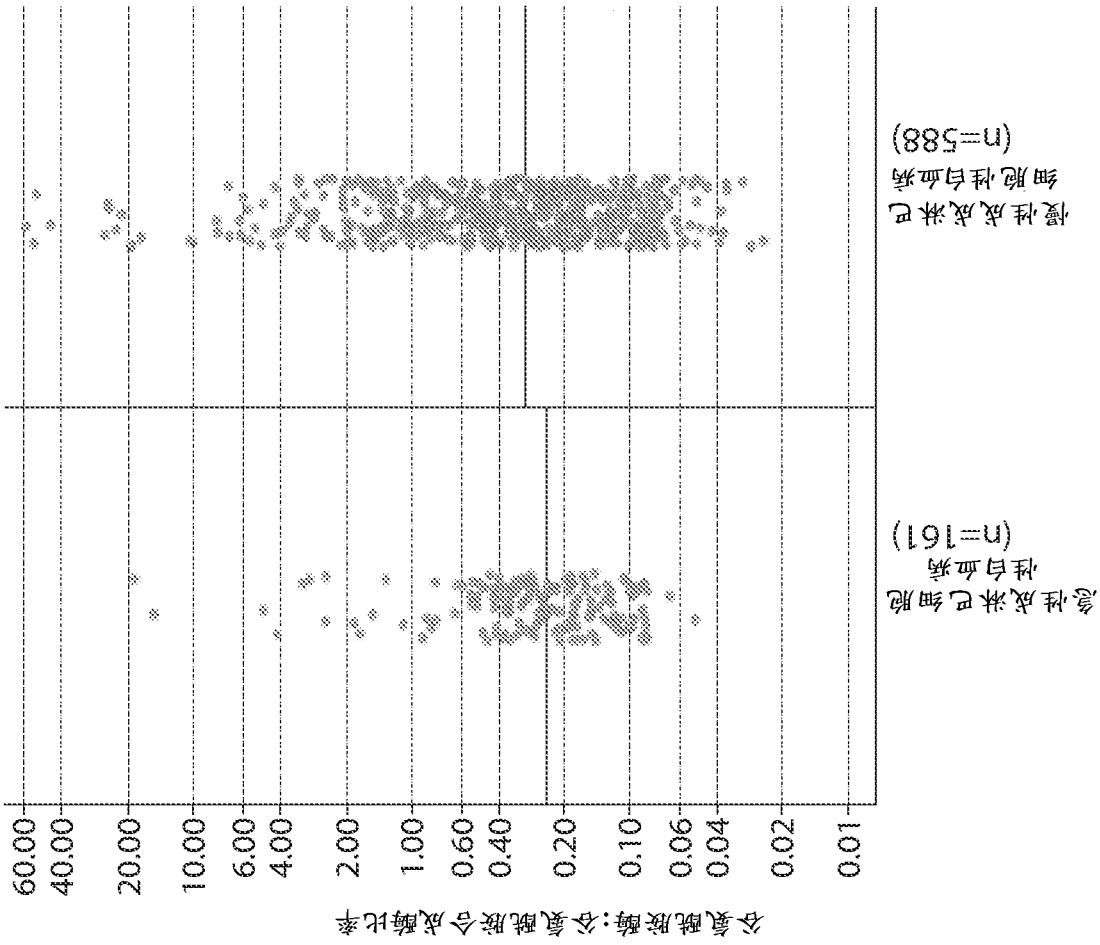

谷氨酰胺酶:谷氨酰胺合成酶比率
60.00
40.00
20.00
10.00
6.00
4.00
2.00
1.00
0.60
0.40
0.20
0.10
0.06
0.04
0.02
0.01
急性成淋巴细胞
性白血病
(n=161)
慢性成成淋巴
细胞性白血病
(n=588)

图 7

谷氨酰胺酶:谷氨酰胺合成酶比率
10.00
6.00
4.00
2.00
1.00
0.60
0.40
0.20
0.10
0.06
0.04
0.02
间变性大细胞淋巴瘤 (n=38)
伯基特淋巴瘤 (n=25)
弥漫性大 B 细胞淋巴瘤 (n=294)
滤泡性淋巴瘤 (n=242)
霍奇金淋巴瘤 (n=146)

图 8

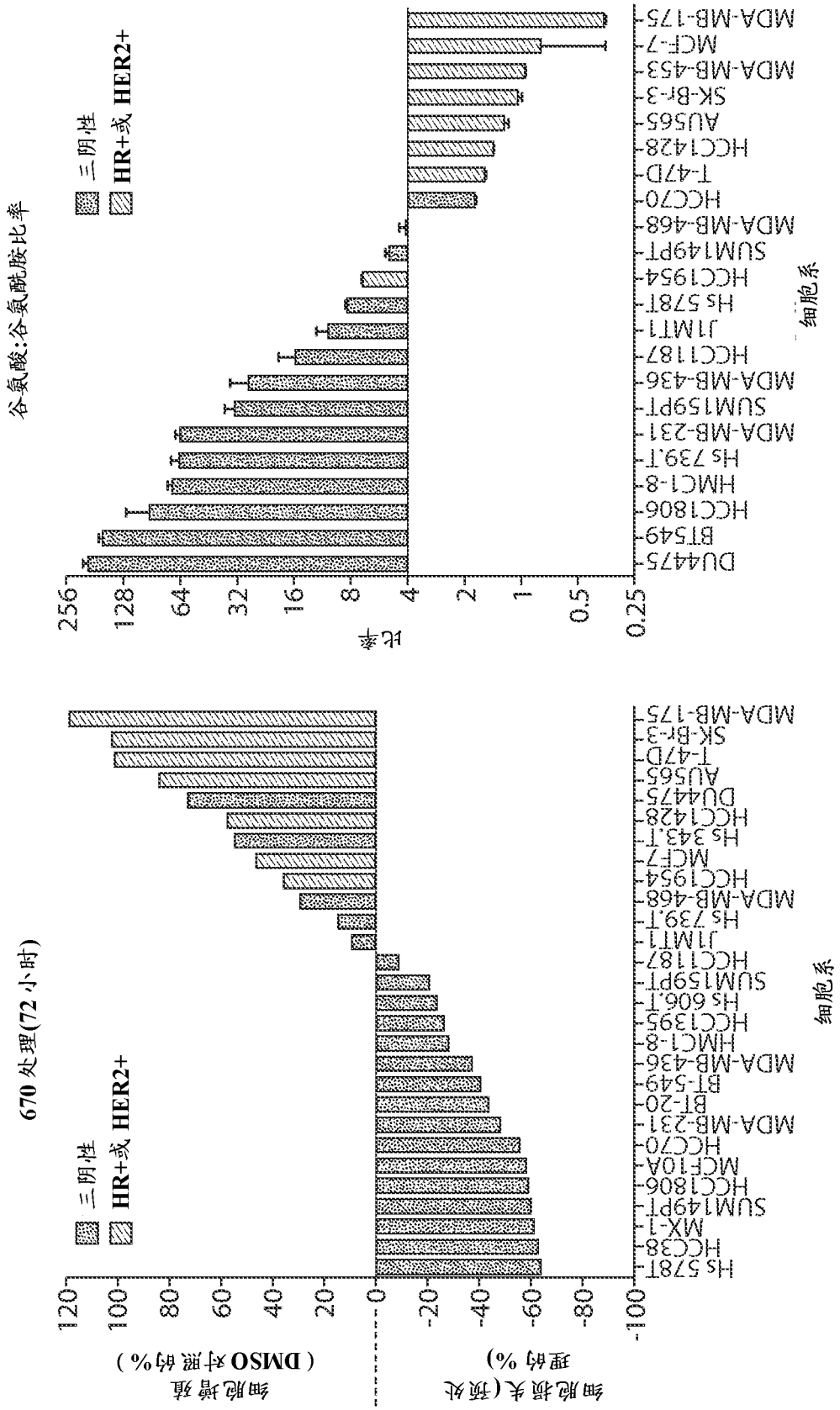

谷氨酸:谷氨酰胺比率
比率
256
128
64
32
16
8
4
2
1
0.5
0.25
三阴性
HR+或 HER2+
DU4475
BT549
HCC1806
HMC1-8
Hs 739.T
MDA-MB-231
SUM159PT
MDA-MB-436
HCC1187
JIMT1
Hs 578T
HCC1954
SUM149PT
MDA-MB-468
HCC70
T-47D
HCC1428
AU565
SK-Br-3
MDA-MB-453
MCF-7
MDA-MB-175
细胞系
670 处理(72 小时)
细胞损失(预处理的 %)
细胞增殖(DMSO 对照的 %)
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
Hs 578T
HCC38
MX-1
SUM149PT
HCC1806
MCF10A
HCC70
MDA-MB-231
BT-20
BT-549
MDA-MB-436
HMC1-8
HCC1395
Hs 606.T
SUM159PT
HCC1187
JIMT1
Hs 739.T
MDA-MB-468
HCC1954
MCF7
Hs 343.T
HCC1428
DU4475
AU565
T-47D
SK-Br-3
MDA-MB-175
细胞系

图 9

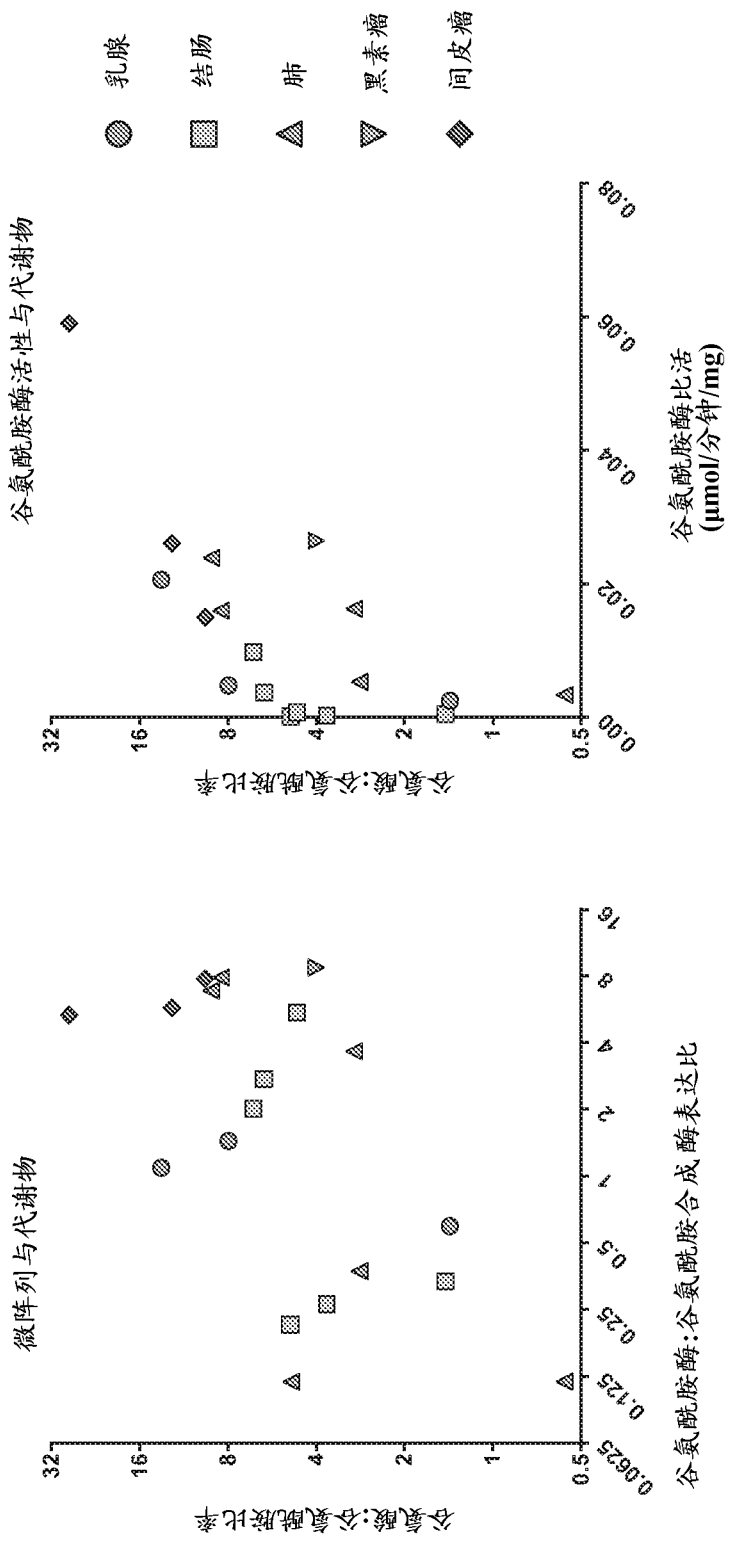

乳腺
结肠
肺
黑素瘤
间皮瘤
谷氨酰胺酶活性与代谢物
谷氨酸:谷氨酰胺比率
32
16
8
4
2
1
0.5
0.00
0.02
0.04
0.06
0.08
谷氨酰胺酶比活
(μmol/分钟/mg)
微阵列与代谢物
谷氨酸:谷氨酰胺比率
32
16
8
4
2
1
0.5
0.0625
0.125
0.25
0.5
1
2
4
8
16
谷氨酰胺酶:谷氨酰胺合成酶表达比

图 10

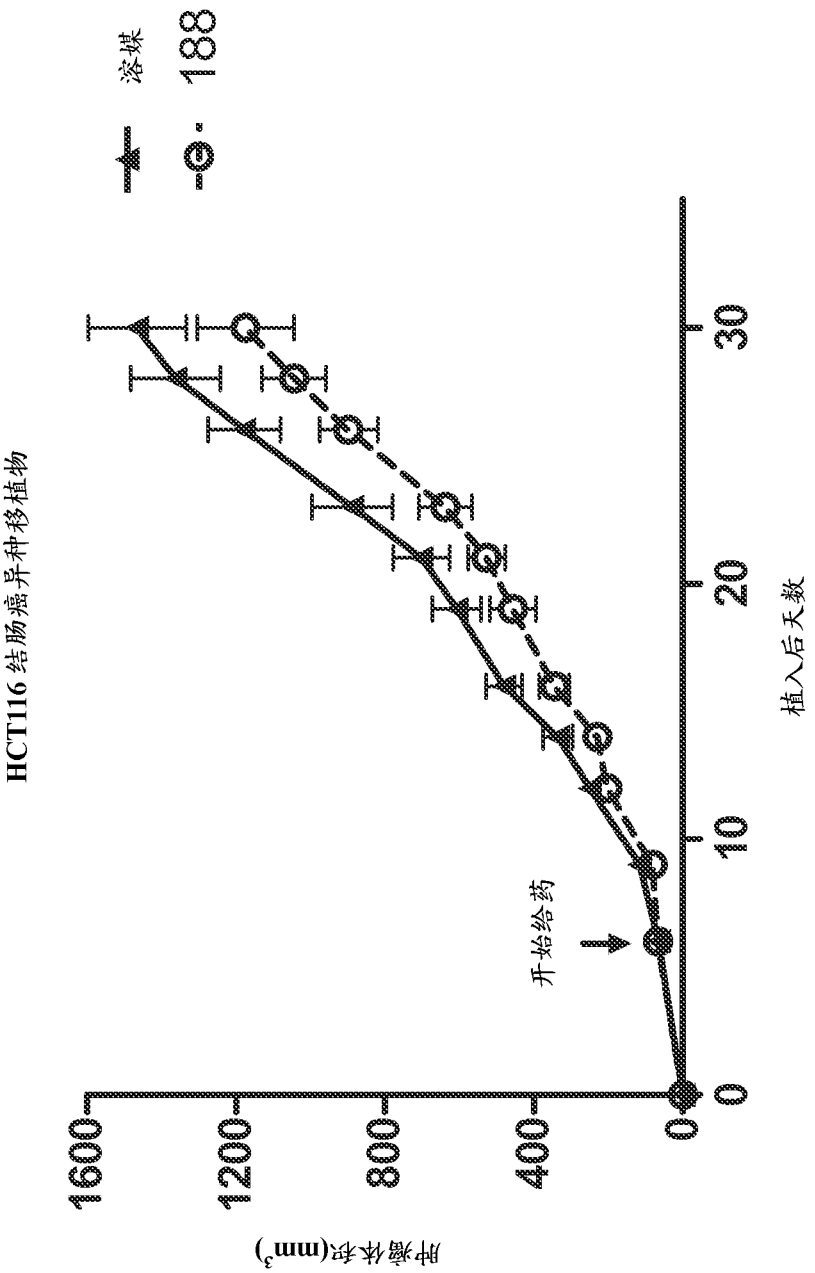

HCT116 结肠癌异种移植物
溶媒
188
肿瘤体积(mm³)
1600
1200
800
400
0
开始给药
0
10
20
30
植入后天数

图 11

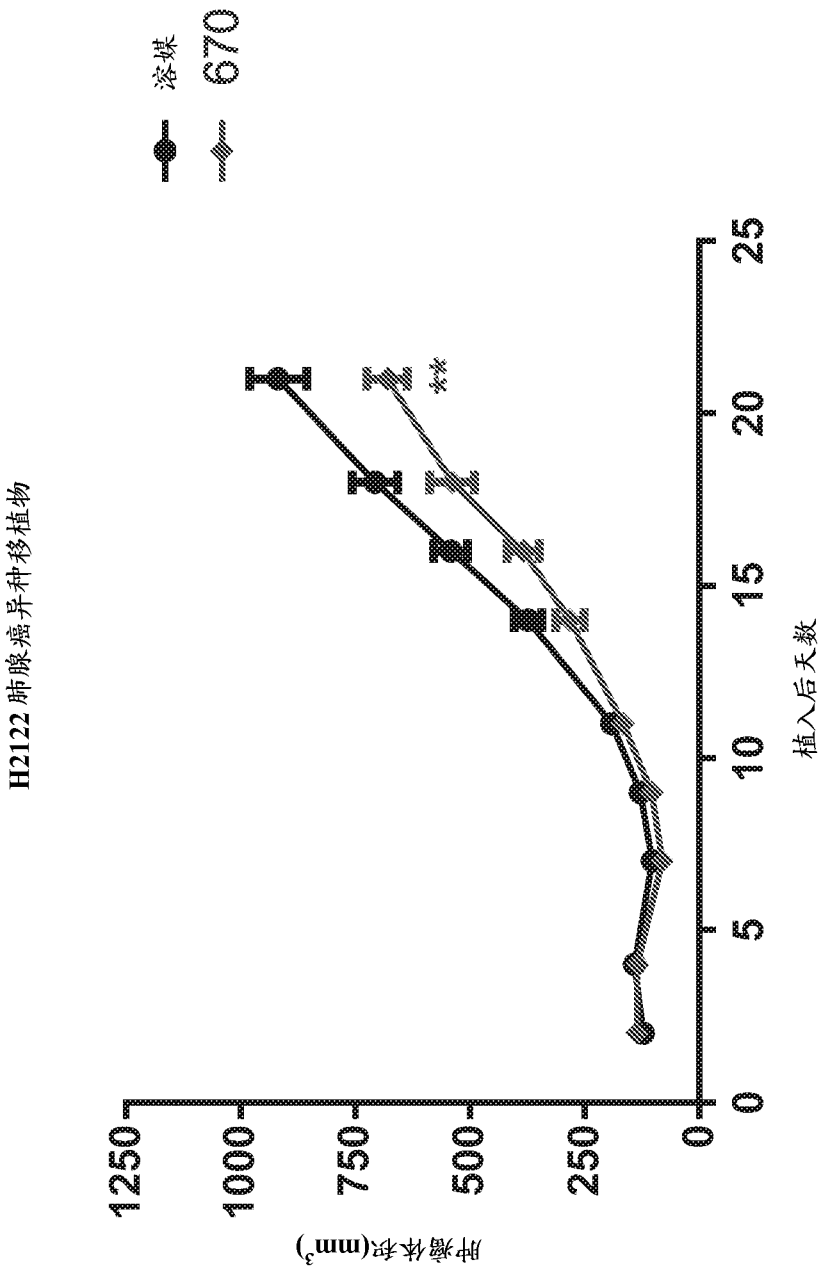

H2122 肺腺癌异种移植物
溶媒
670
**
肿瘤体积(mm³)
1250
1000
750
500
250
0
0
5
10
15
20
25
植入后天数

图 12

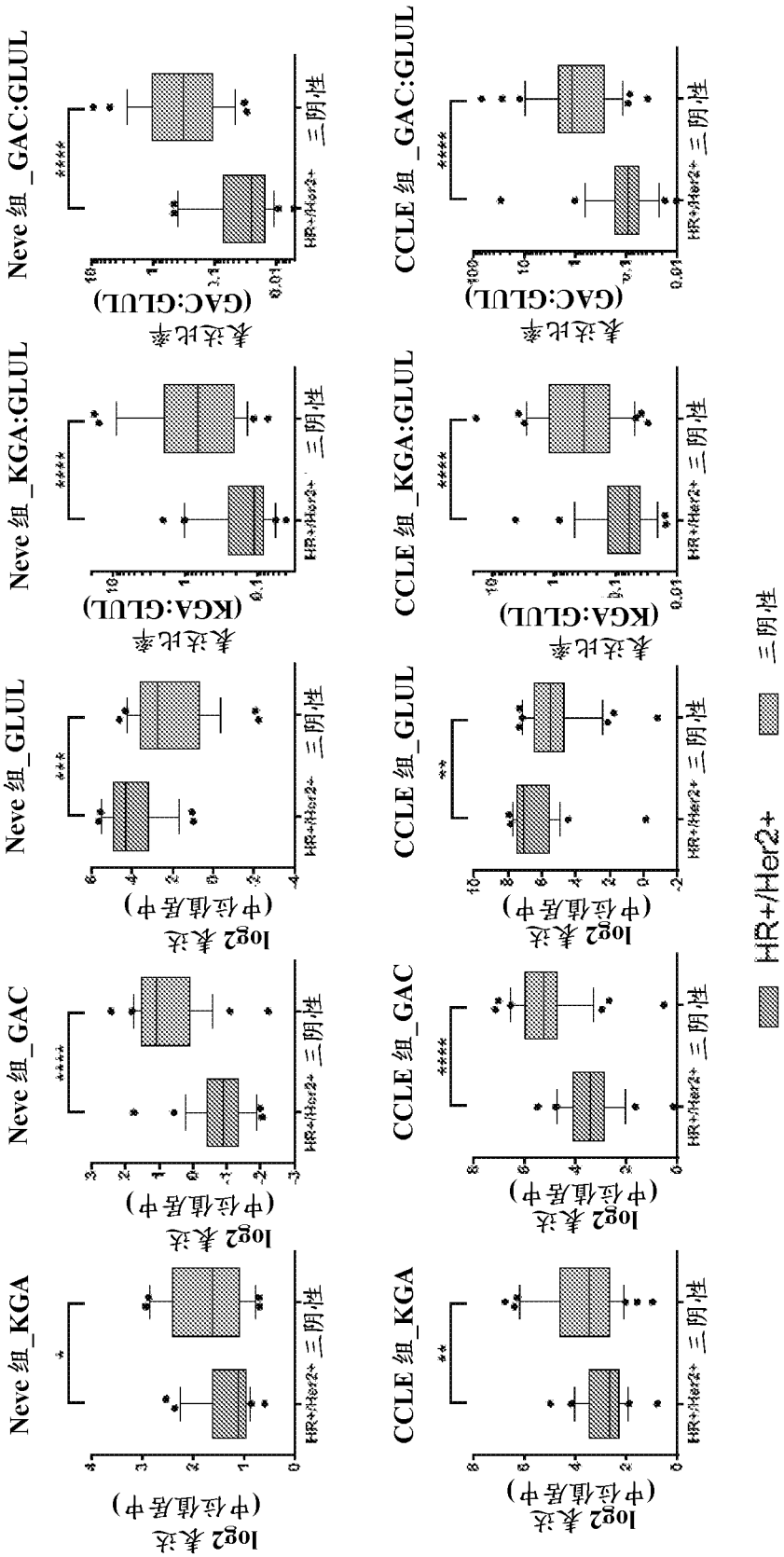

Neve 组_KGA
Neve 组_GAC
Neve 组_GLUL
Neve 组_KGA:GLUL
Neve 组_GAC:GLUL
CCLE 组_KGA
CCLE 组_GAC
CCLE 组_GLUL
CCLE 组_KGA:GLUL
CCLE 组_GAC:GLUL
log2 表达
(中位值居中)
表达比率
(KGA:GLUL)
(GAC:GLUL)
HR+/Her2+
三阴性

图 13

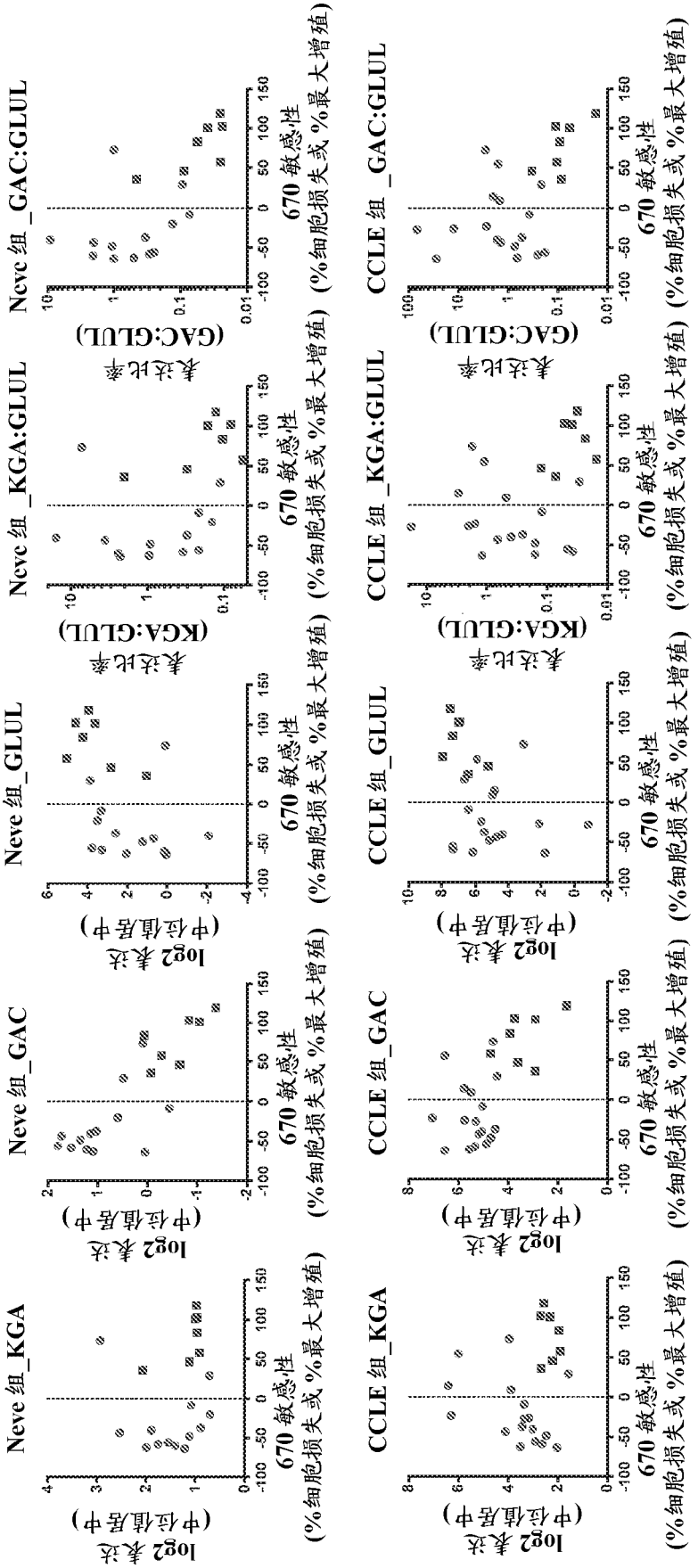

Neve 组_KGA
Neve 组_GAC
Neve 组_GLUL
Neve 组 _KGA:GLUL
Neve 组 _GAC:GLUL
CCLE 组_KGA
CCLE 组_GAC
CCLE组_GLUL
CCLE 组 _KGA:GLUL
CCLE 组 _GAC:GLUL
log2 表达 (中位值居中)
表达比率 (KGA:GLUL)
表达比率 (GAC:GLUL)
670 敏感性
(%细胞损失或%最大增殖)
三阴性
HR+或 HER2+

图 14

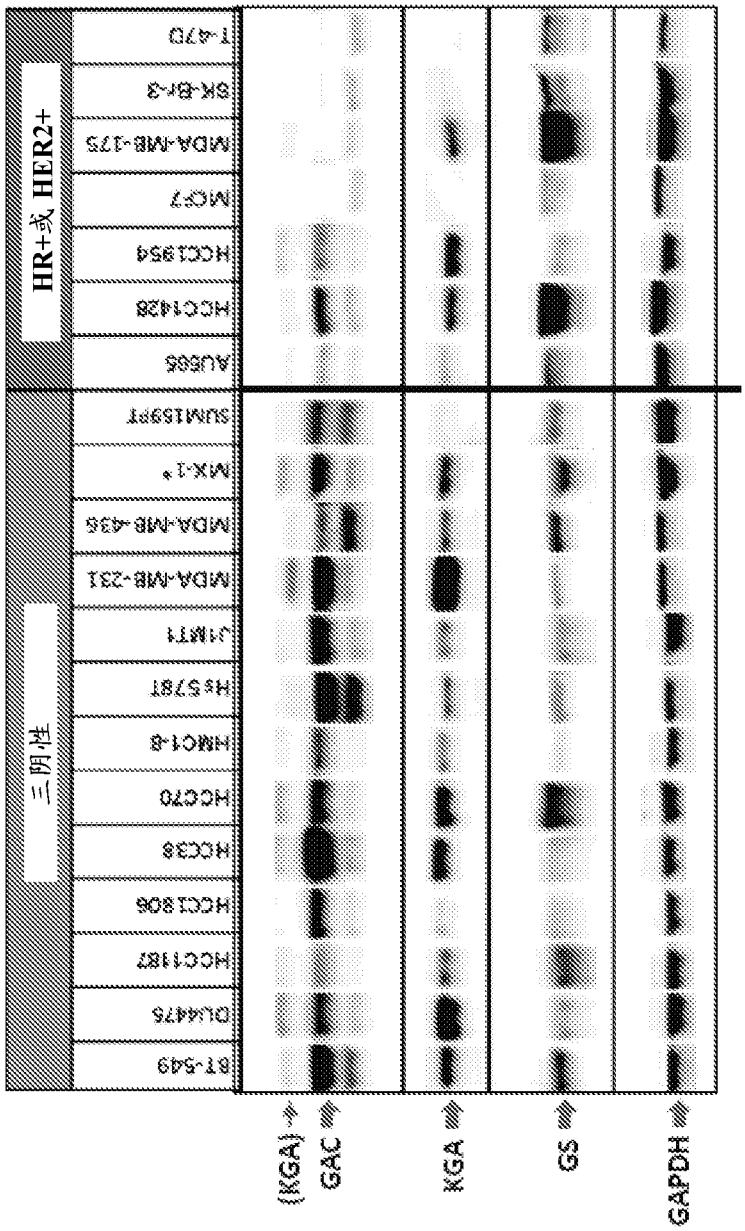

HR+或 HER2+
三阴性
T-47D
SK-BR-3
MDA-MB-175
MCF7
HCC1954
HCC1428
AU565
BT-549
DU4475
HCC1187
HCC1806
HCC38
HCC70
HMC1-8
Hs 578T
LY MT1
MDA-MB-231
MDA-MB-436
MX-1*
SUM159PT
(KGA)
GAC
KGA
GS
GAPDH

图 15

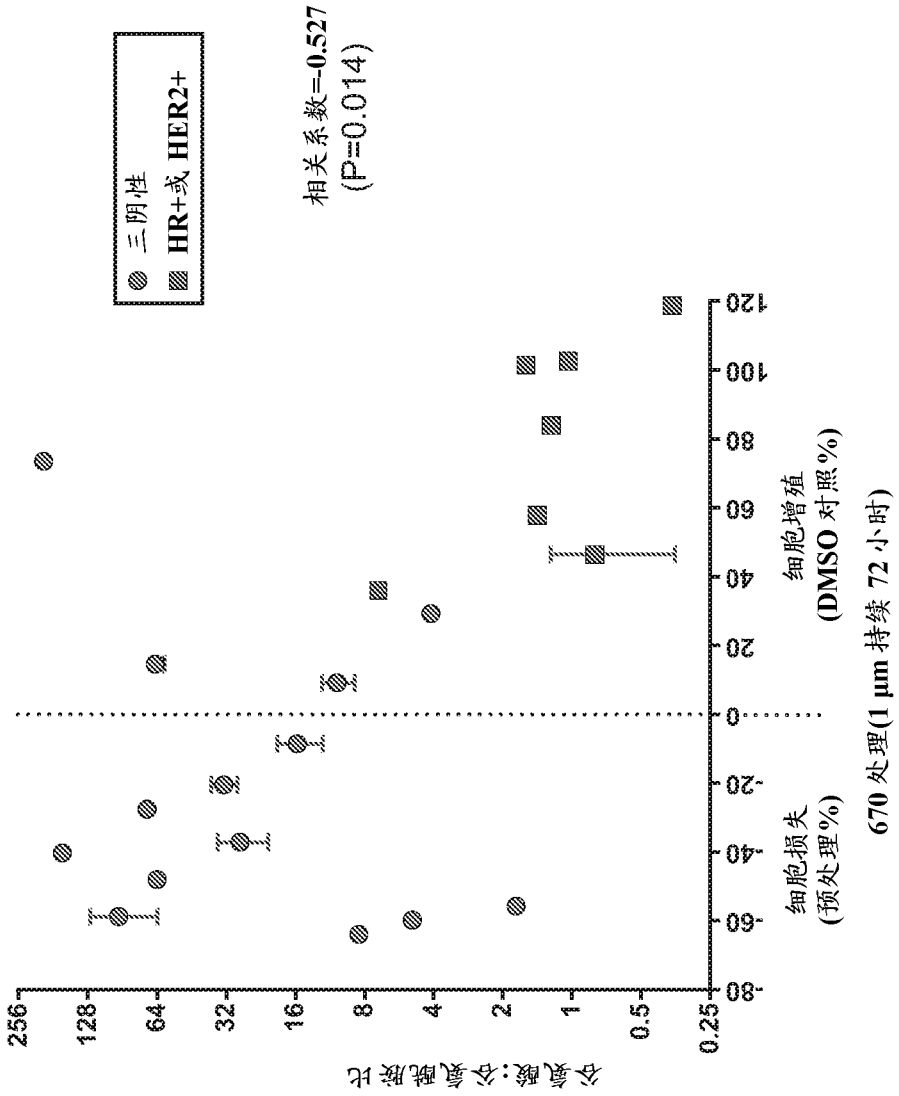

三阴性
HR+或 HER2+
相关系数=-0.527
(P=0.014)
谷氨酸:谷氨酰胺比
256
128
64
32
16
8
4
2
1
0.5
0.25
-80
-60
-40
-20
0
20
40
60
80
100
120
细胞损失
(预处理%)
细胞增殖
(DMSO 对照%)
670 处理(1 μm 持续 72 小时)

图 16

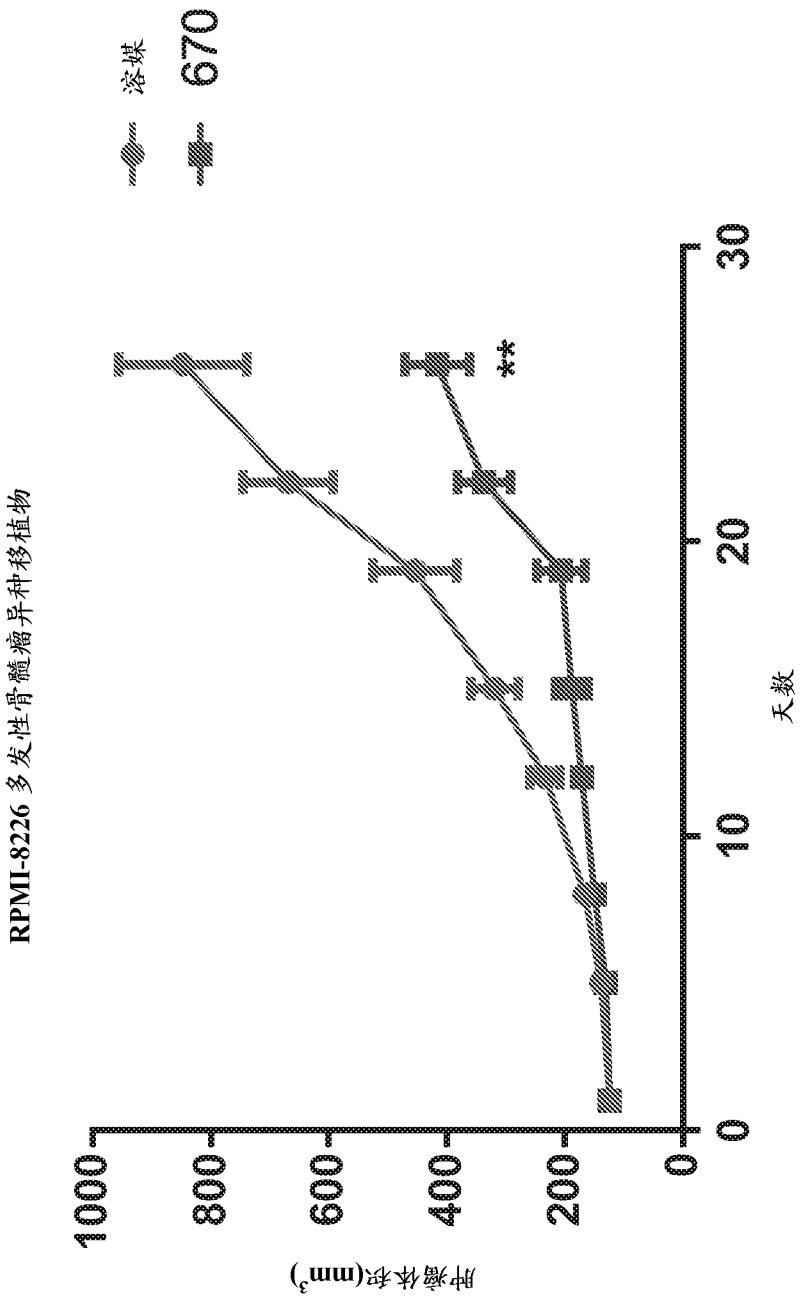

RPMI-8226 多发性骨髓瘤异种移植物
溶媒
670
**
肿瘤体积(mm³)
1000
800
600
400
200
0
0
10
20
30
天数

图 17

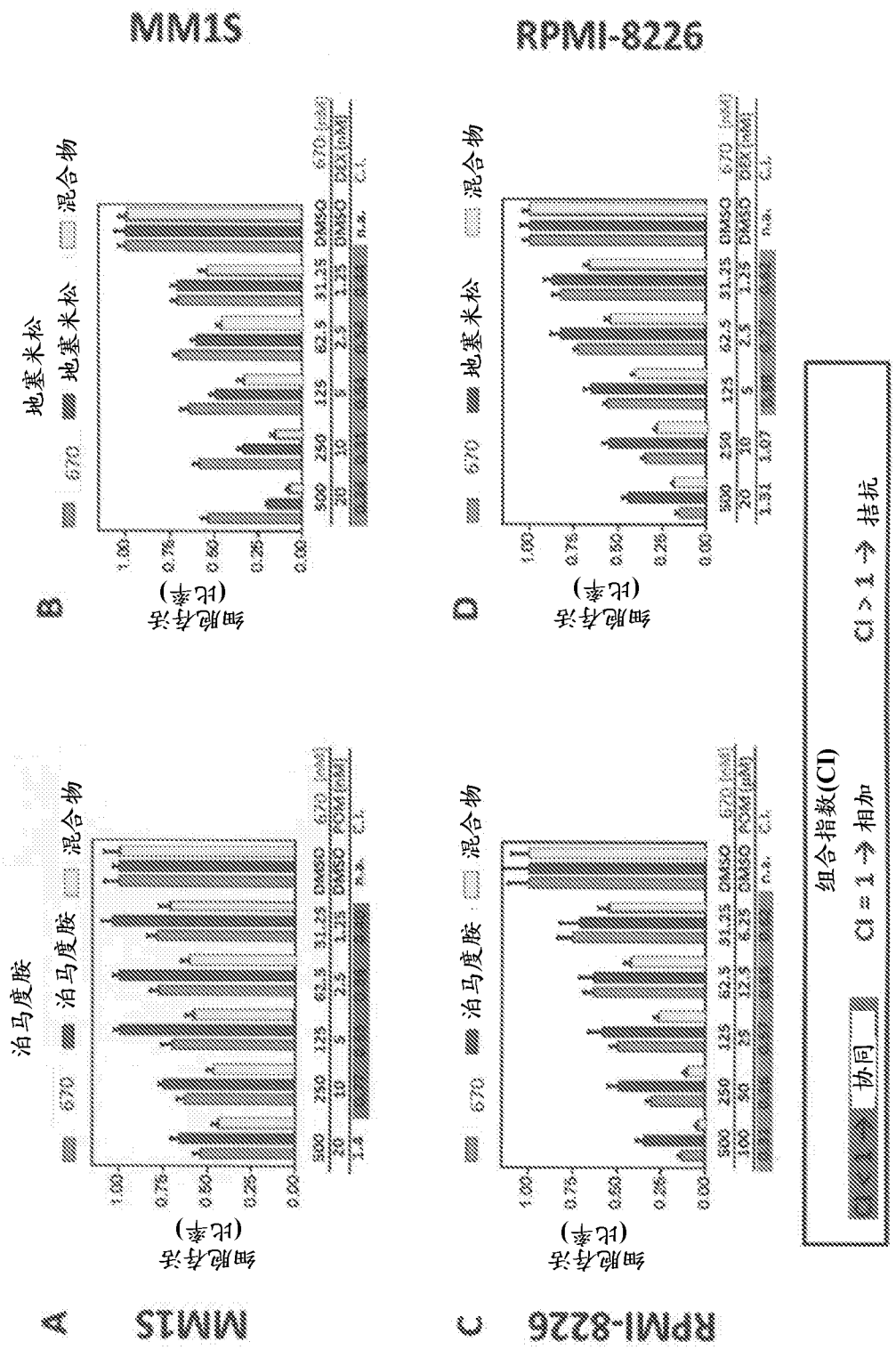

MM1S
RPMI-8226
A
B
C
D
泊马度胺
地塞米松
混合物
细胞存活
(比率)
组合指数(CI)
协同
CI = 1 → 相加
CI > 1 → 拮抗

图 18