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(54) **INHIBITORS OF GROWTH FACTOR ACTIVATION ENZYMES**

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(21) Appl. No.: **17/475,290**

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A61K 49/00	(2006.01)
A61K 51/04	(2006.01)
A61K 51/08	(2006.01)
C07B 59/00	(2006.01)
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C07K 5/078	(2006.01)
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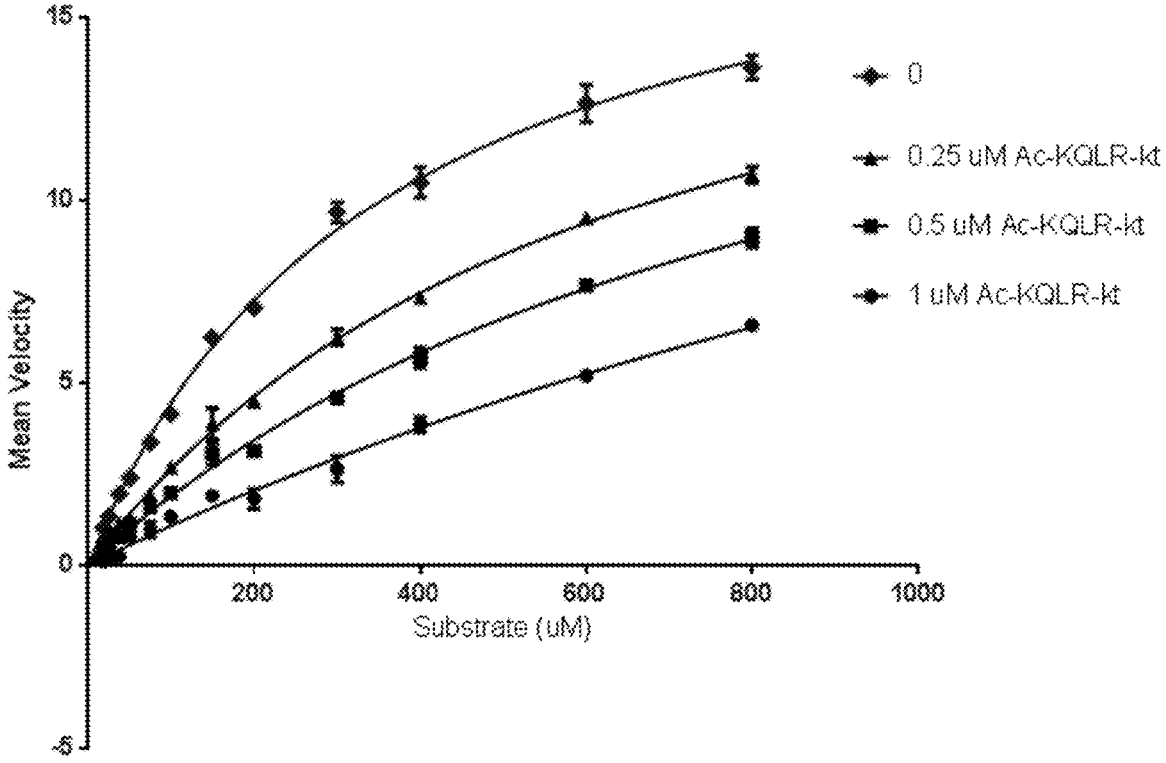
CPC ***C07K 5/0815*** (2013.01); *A61K 38/00* (2013.01); ***C07K 5/1005*** (2013.01); ***C07K 5/0817*** (2013.01); ***C07K 5/0821*** (2013.01); ***G01N 33/582*** (2013.01); ***A61K 47/64*** (2017.08); ***A61K 49/0052*** (2013.01); ***A61K 49/0056*** (2013.01); ***A61K 51/0453*** (2013.01); ***A61K 51/08*** (2013.01); ***C07B 59/002*** (2013.01); ***C07B 59/008*** (2013.01); ***C07D 277/64*** (2013.01); ***C07K 5/06095*** (2013.01); ***C07K 5/06156*** (2013.01); ***C07K 5/101*** (2013.01); ***C07K 5/1013*** (2013.01); ***C07K 5/1016*** (2013.01); ***C07K 5/126*** (2013.01); ***C07K 5/1019*** (2013.01)

(57) **ABSTRACT**

The present invention generally relates to compounds that are useful for inhibiting one or more of hepatocyte growth factor activator, matriptase, hepsin, Factor Xa, or thrombin. The present invention also relates to various methods of using the inhibitor compounds including treating a malignancy, a pre-malignant condition, or cancer by administering an effective amount of the inhibitor to a subject in need thereof.

Specification includes a Sequence Listing.

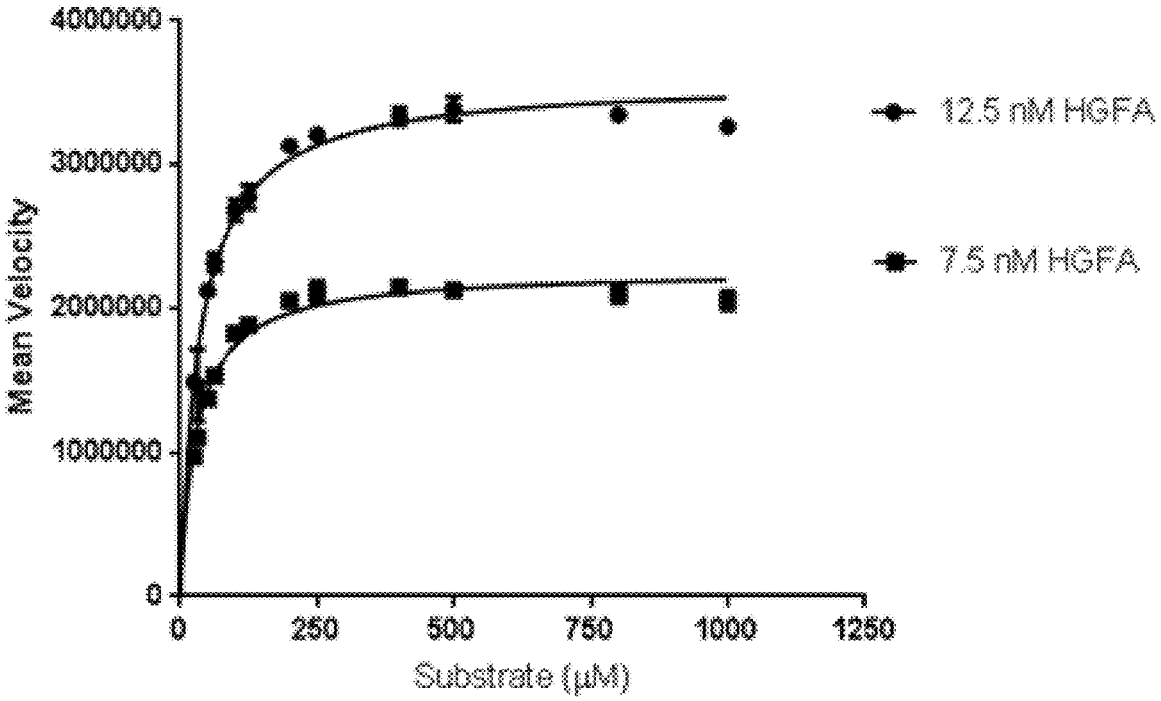
FIG. 1



	1 uM Ac-KQLR-kt	0.5 uM Ac-KQLR-kt	0.25 uM Ac-KQLR-kt	0
Vmax	23.93	19.17	19.27	19.79
Km	2137	917.7	632.9	345.7

	1 uM Ac-KQLR-kt	0.5 uM Ac-KQLR-kt	0.25 uM Ac-KQLR-kt	0
R square	0.9862	0.9944	0.9973	0.9962

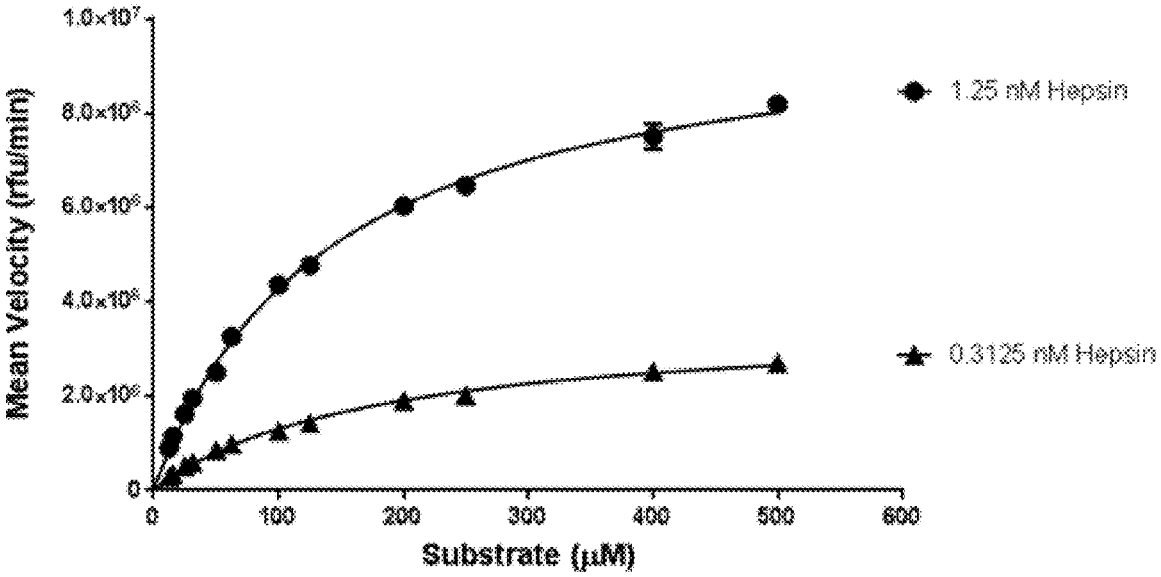
FIG. 2



	12.5 nM HGFA	7.5 nM HGFA
Km	36.06	29.88

	12.5 nM HGFA	7.5 nM HGFA
R square	0.9716	0.9610

FIG. 3

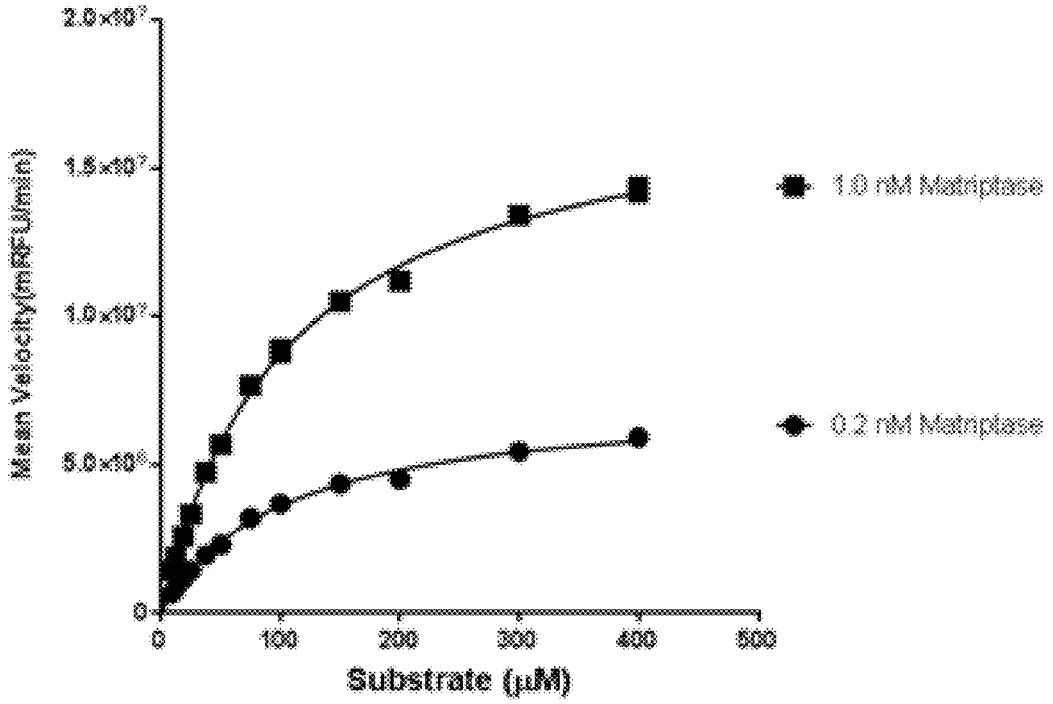


	1.25 nM Hepsin	0.3125 nM Hepsin
Km	139.7	173.0

	1.25 nM Hepsin	0.3125 nM Hepsin
Vmax	133264	61821

	1.25 nM Hepsin	0.3125 nM Hepsin
R square	0.9965	0.9952

FIG. 4



	0.2 nM Matriptase	1.0 nM Matriptase
Vmax	7.252e+006	1.793e+007
Km	101.6	106.4

	0.2 nM Matriptase	1.0 nM Matriptase
R square	0.9950	0.9969

FIG. 5

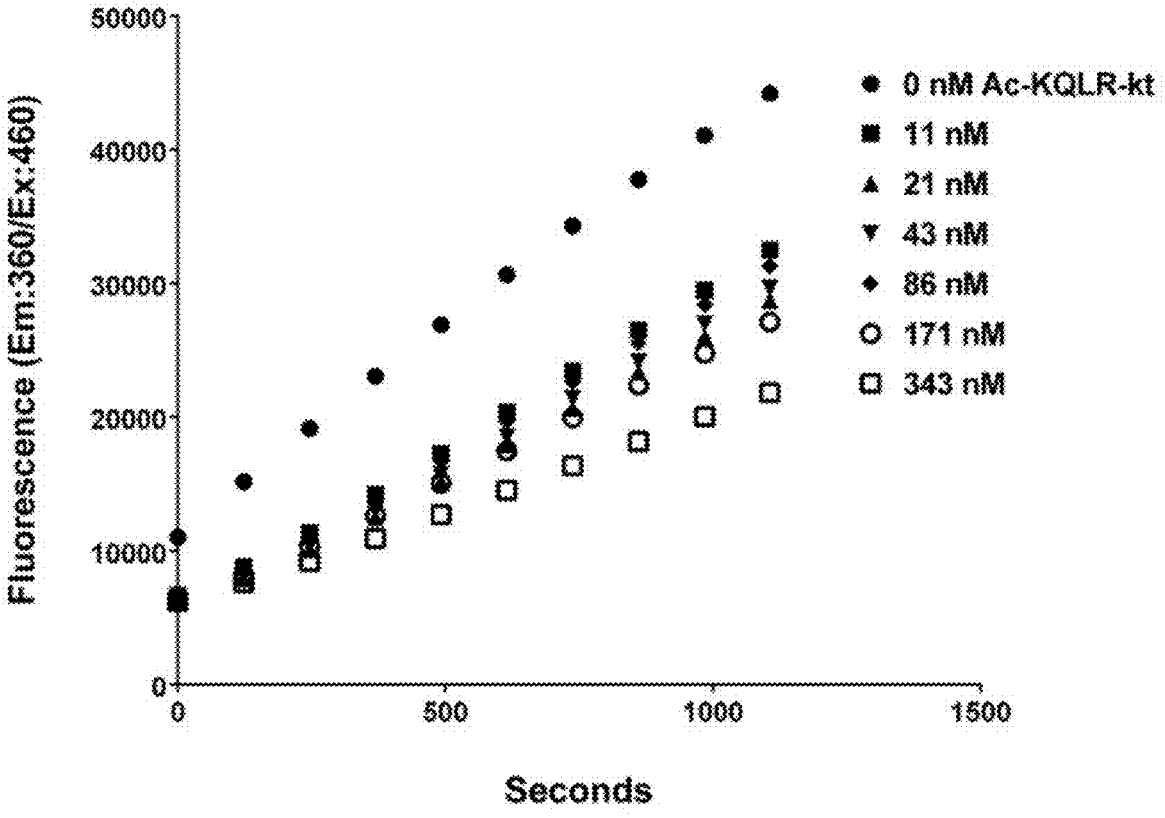

50000
40000
30000
20000
10000
0
Fluorescence (Em:360/Ex:460)
0
500
1000
1500
Seconds
0 nM Ac-KQLR-kt
11 nM
21 nM
43 nM
86 nM
171 nM
343 nM

FIG. 6

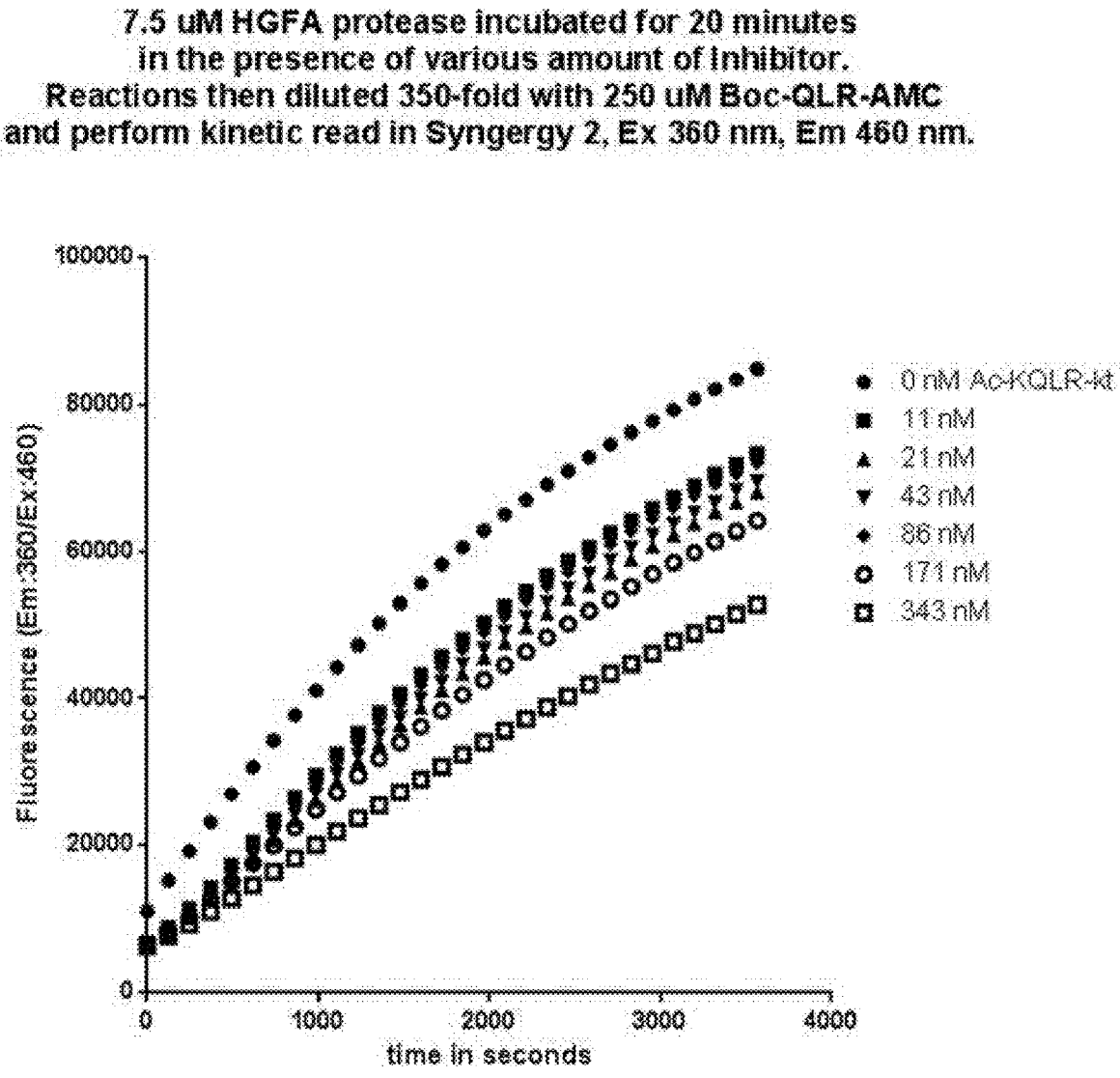

7.5 uM HGFA protease incubated for 20 minutes
in the presence of various amount of Inhibitor.
Reactions then diluted 350-fold with 250 uM Boc-QLR-AMC
and perform kinetic read in Syngergy 2, Ex 360 nm, Em 460 nm.
Fluorescence (Em:360/Ex:460)
100000
80000
60000
40000
20000
0
0
1000
2000
3000
4000
time in seconds
0 nM Ac-KQLR-kt
11 nM
21 nM
43 nM
86 nM
171 nM
343 nM

FIG. 7

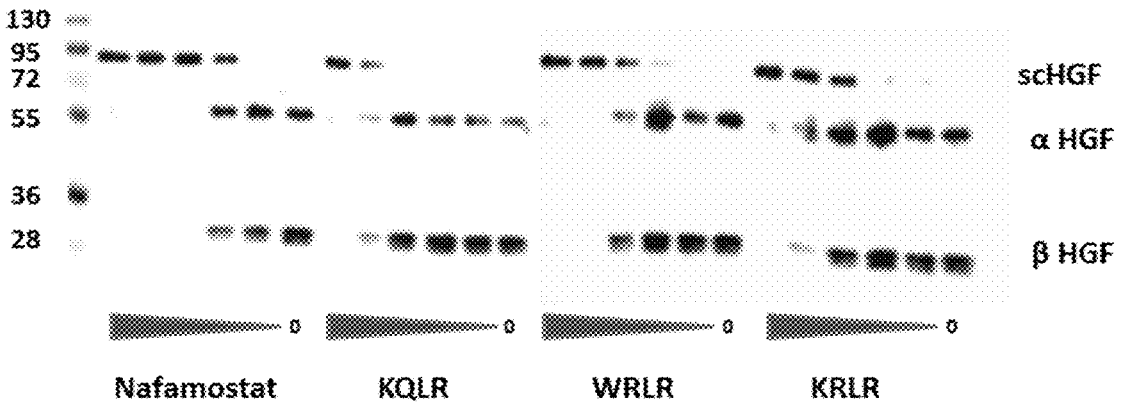

130
95
72
55
36
28
scHGF
α HGF
β HGF
0
0
0
0
Nafamostat
KQLR
WRLR
KRLR

FIG. 8

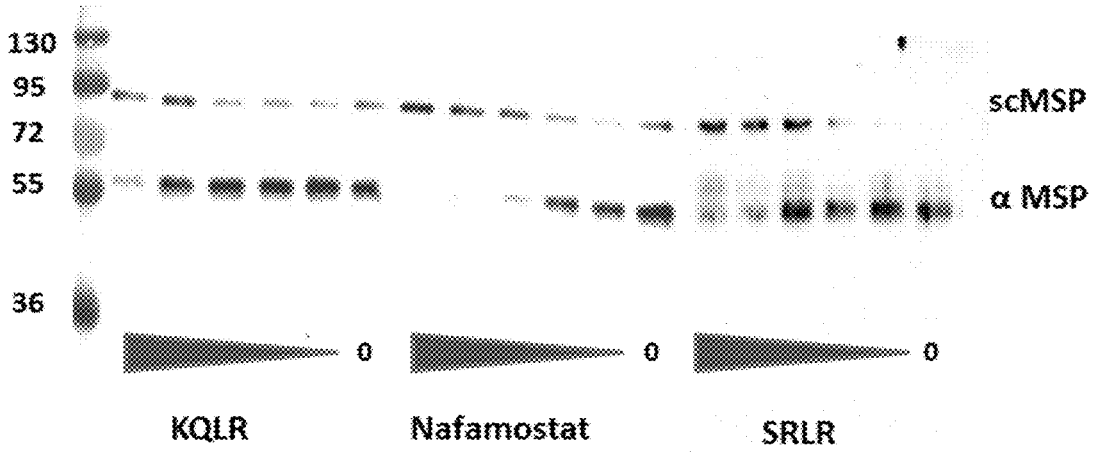

130
95
72
55
36
scMSP
α MSP
0
0
0
KQLR
Nafamostat
SRLR

FIG. 9

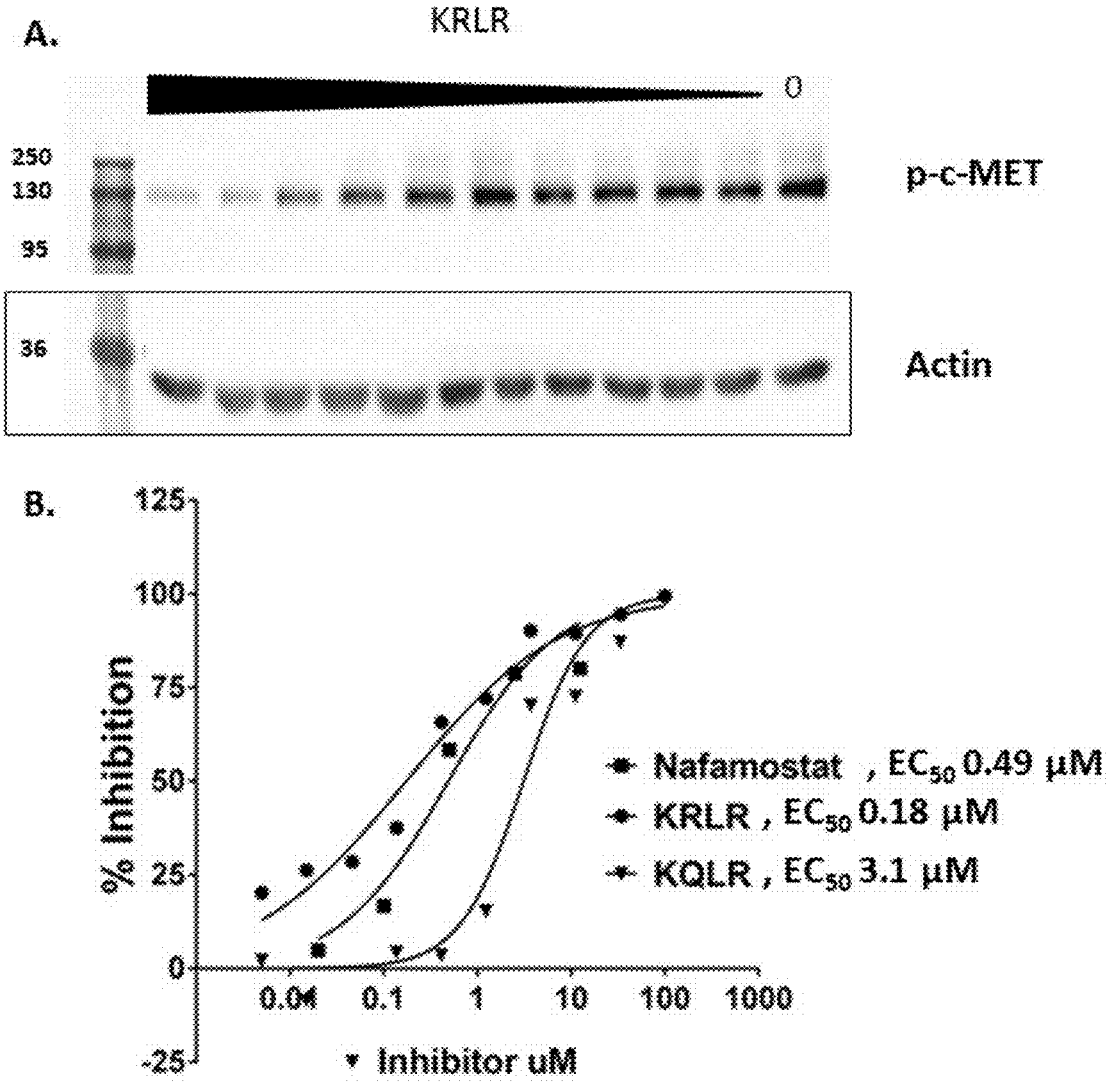
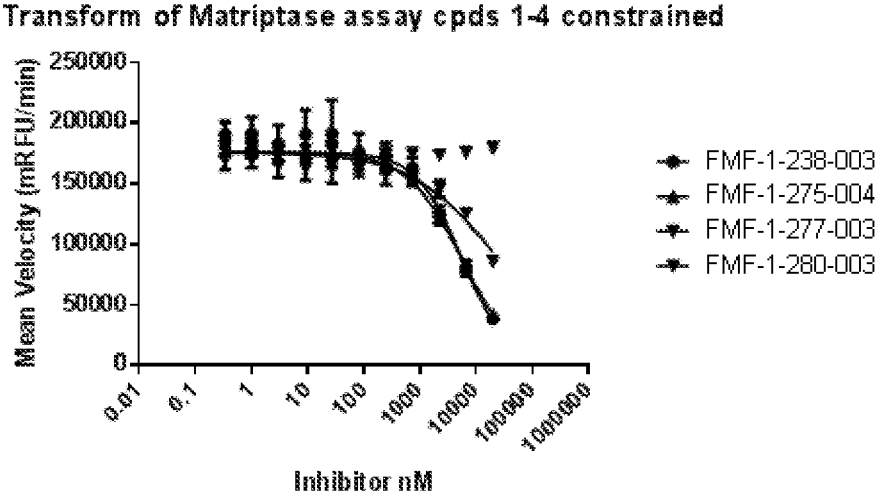

A.
KRLR
0
250
130
95
p-c-MET
36
Actin
B.
125
100
75
50
25
0
-25
% Inhibition
0.04
0.1
1
10
100
1000
Inhibitor uM
Nafamostat , EC50 0.49 µM
KRLR , EC50 0.18 µM
KQLR , EC50 3.1 µM

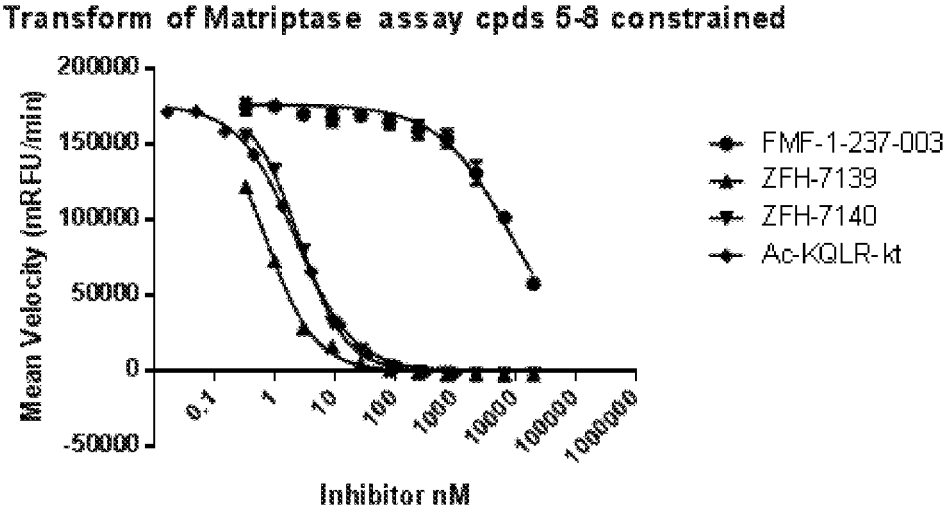
FIG. 10



	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
IC50	5645	5554		24744

	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
R square	0.9220	0.9915		0.8630

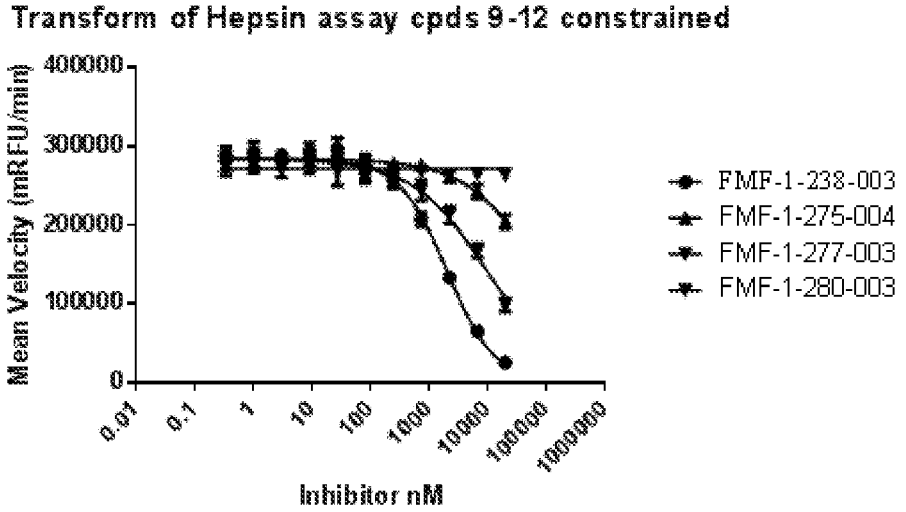
FIG. 11



	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
IC50	8884	0.7198	2.556	2.265

	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
R square	0.9745	0.9959	0.9981	0.9992

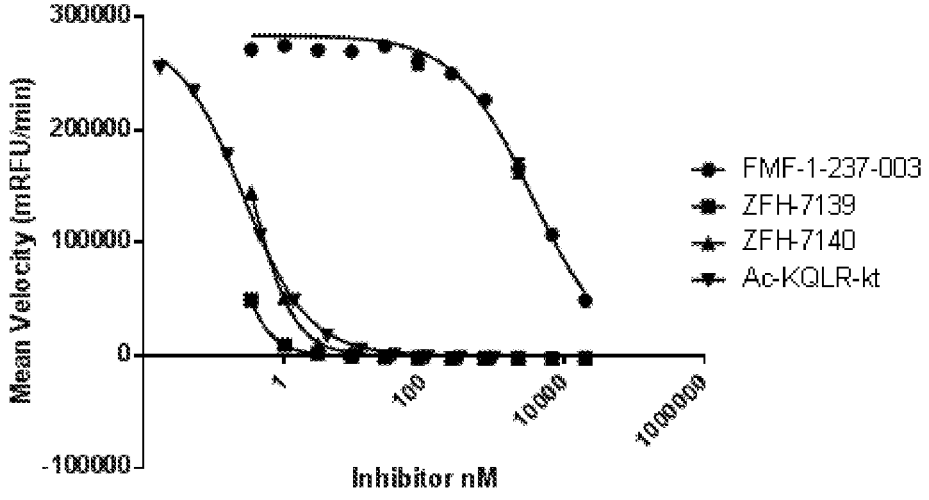
FIG. 12



	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
IC50	2026	79036	0.0	9764

	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
R square	0.9895	0.9176		0.9591

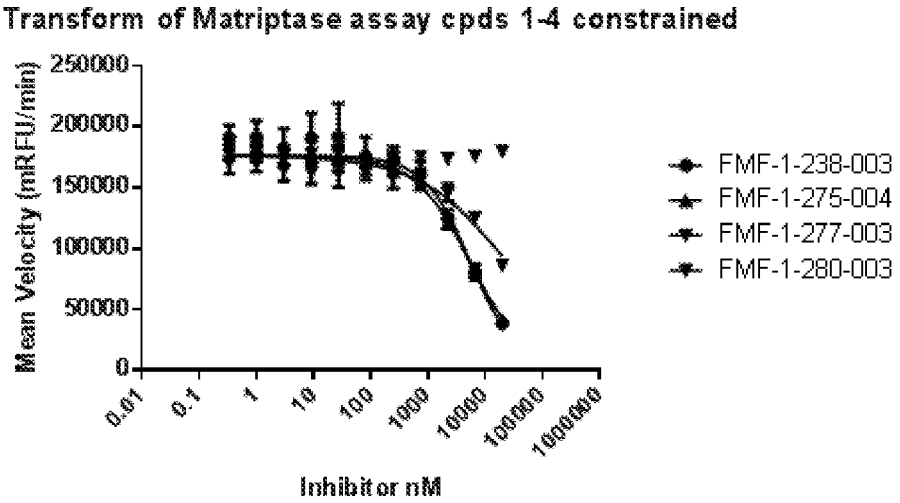
FIG. 13



	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
IC50	3396	0.1357	0.3477	0.2597

	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
R square	0.9864	0.9820	0.9975	0.9987

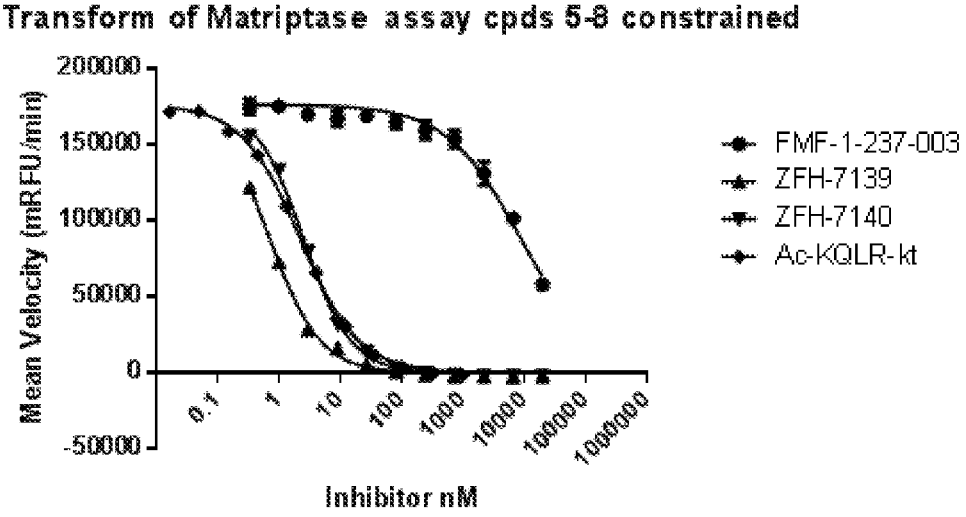
FIG. 14



	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
IC50	5645	5554		24744

	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
R square	0.9220	0.9915		0.8630

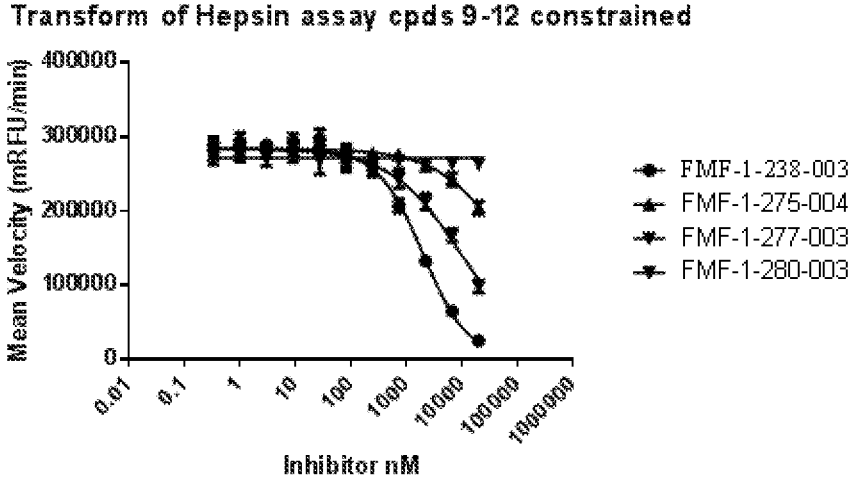
FIG. 15



	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
IC50	8884	0.7198	2.556	2.265

	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
R square	0.9745	0.9959	0.9981	0.9992

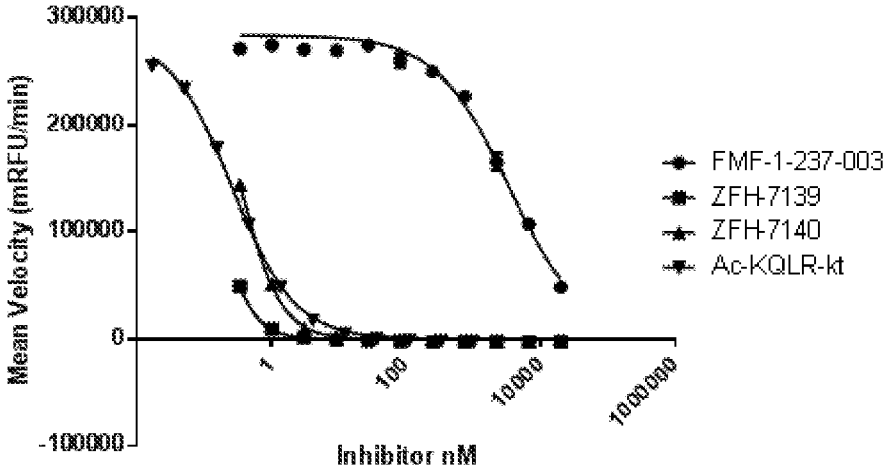
FIG. 16



	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
IC50	2026	79036	0.0	9764

	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-00
R square	0.9895	0.9176		0.9591

FIG. 17



	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
IC50	3396	0.1357	0.3477	0.2597

	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
R square	0.9864	0.9820	0.9975	0.9987

FIG. 18

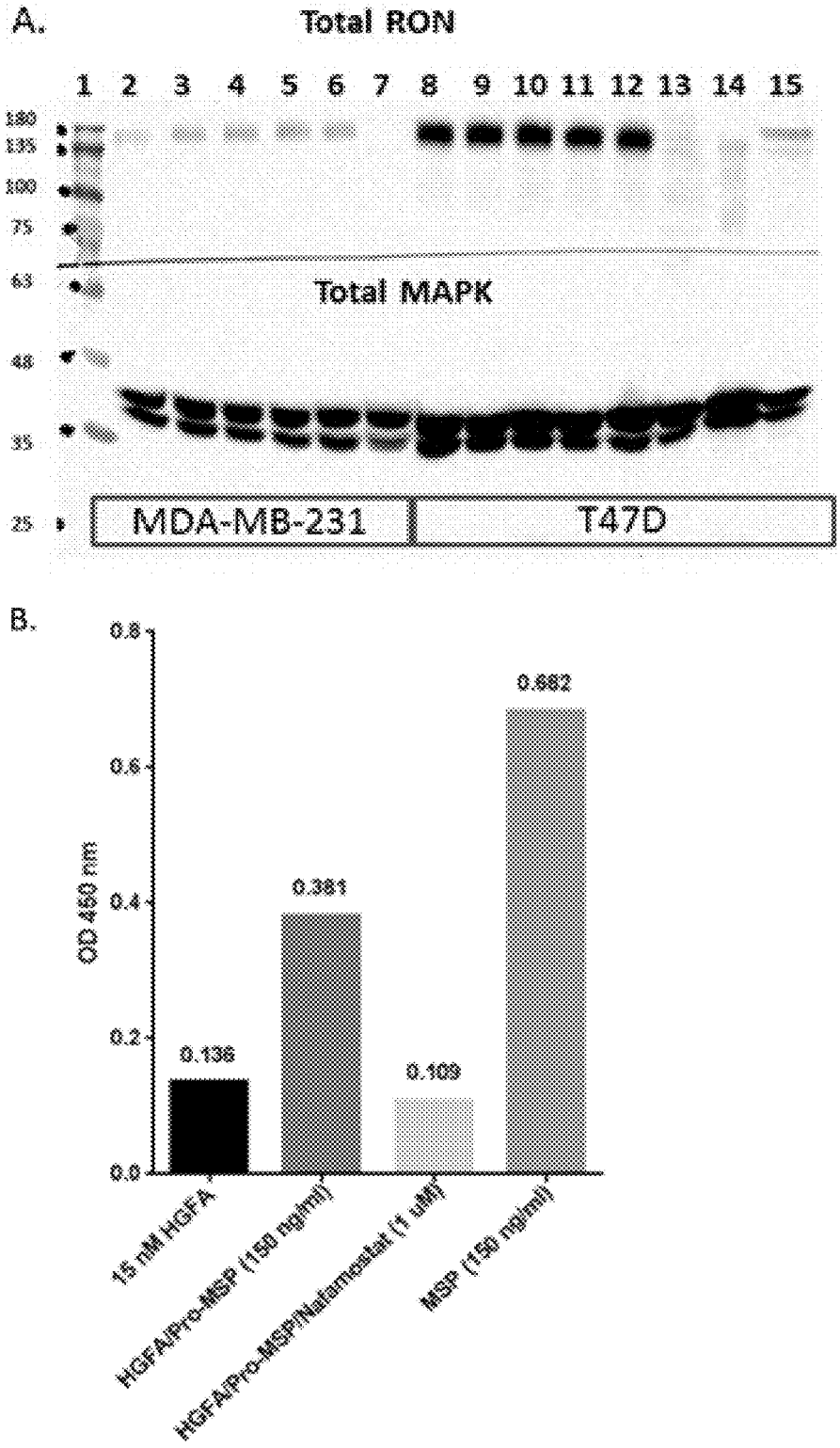

A.
Total RON
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
180
135
100
75
63
48
35
25
Total MAPK
MDA-MB-231
T47D
B.
OD 450 nm
0.8
0.6
0.4
0.2
0.0
0.136
0.381
0.109
0.682
15 nM HGFA
HGFA/Pro-MSP (150 ng/ml)
HGFA/Pro-MSP/Nafamostat (1 uM)
MSP (150 ng/ml)

FIG. 19

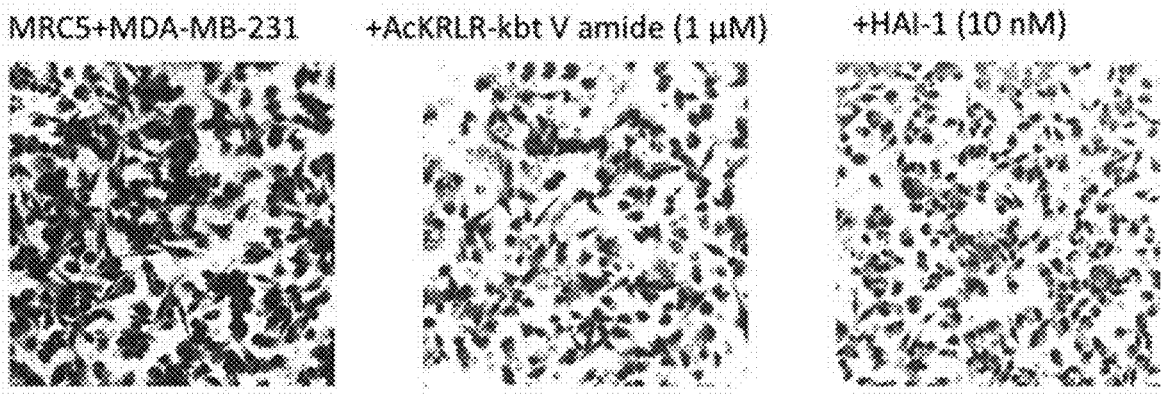

MRC5+MDA-MB-231
+AcKRLR-kbt V amide (1 μM)
+HAI-1 (10 nM)

FIG. 20

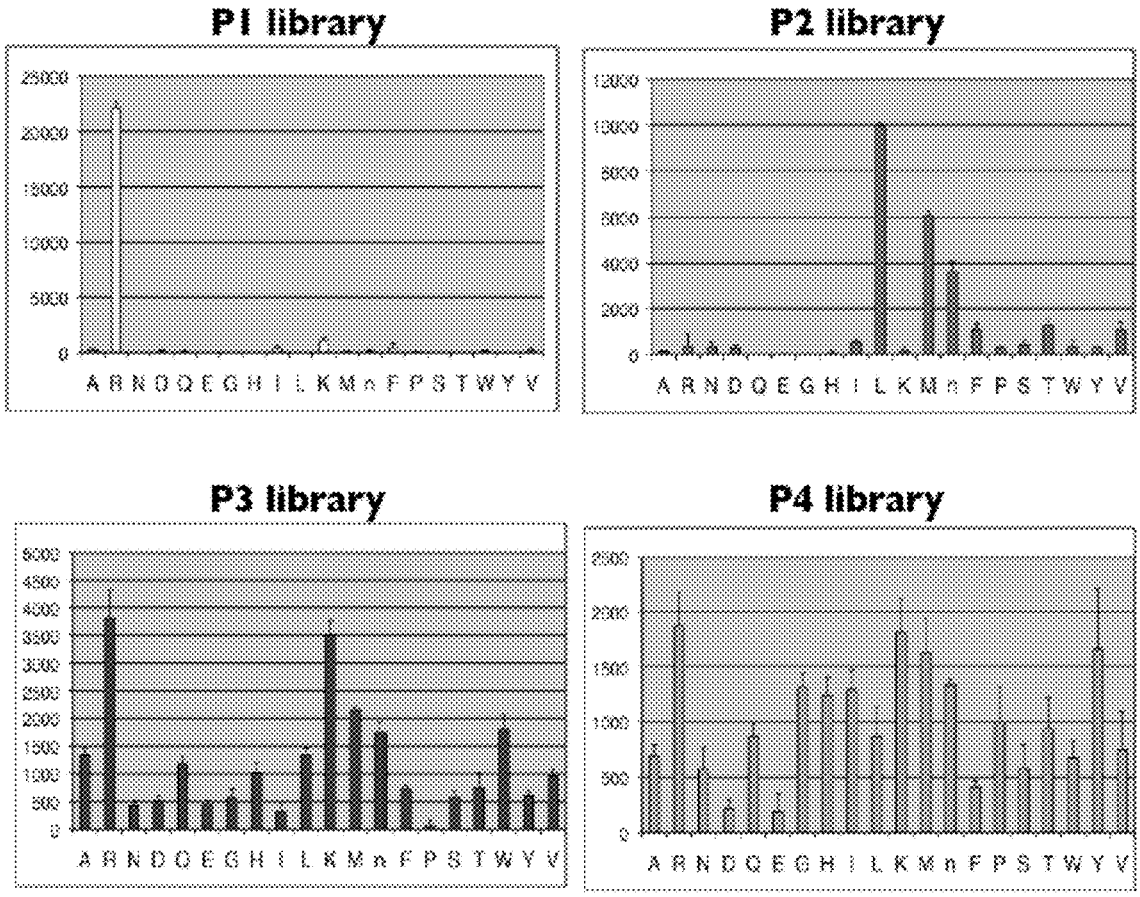
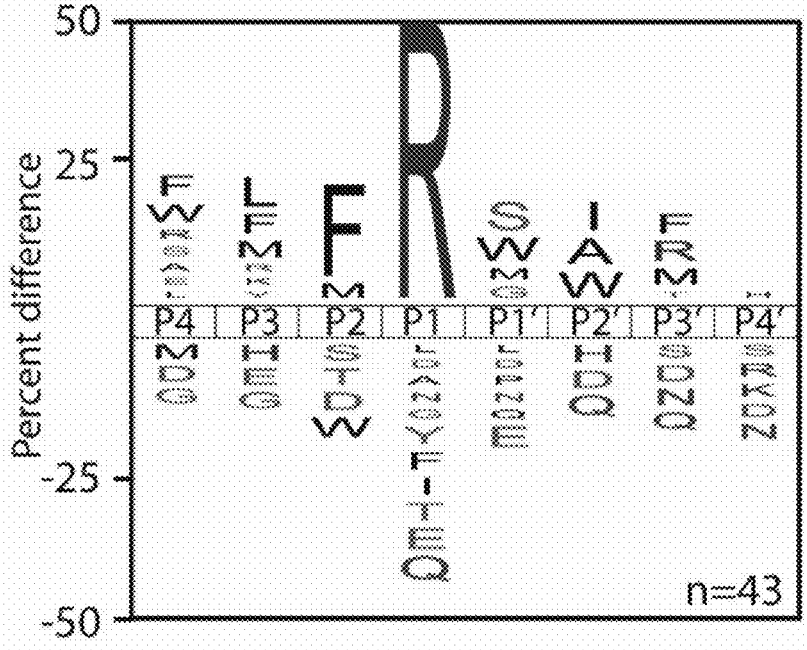
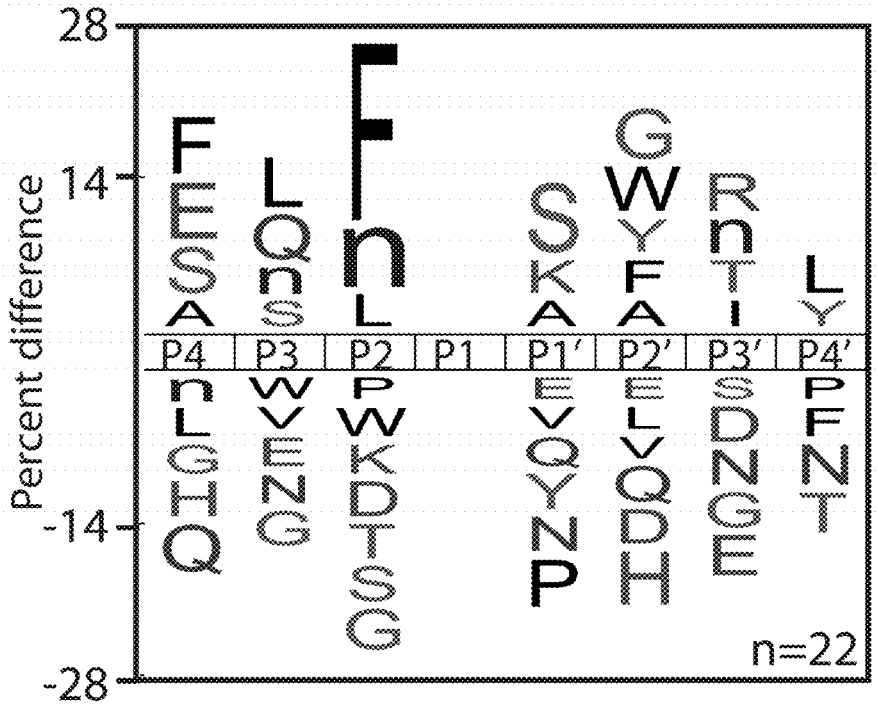

P1 library
P2 library
P3 library
P4 library
A R N D Q E G H I L K M n F P S T W Y V

FIG. 21

A.


50
25
-25
-50
Percent difference
P4
P3
P2
P1
P1′
P2′
P3′
P4′
n=43

B.


28
14
-14
-28
Percent difference
P4
P3
P2
P1
P1′
P2′
P3′
P4′
n=22

INHIBITORS OF GROWTH FACTOR ACTIVATION ENZYMES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 15/556,804, filed Sep. 8, 2017, which is the 371 National Stage of International Application No. PCT/US2016/020516, filed Mar. 2, 2016 and issued as U.S. Pat. No. 11,130,780, which claims the benefit of U.S. Provisional Patent Application No. 62/220,998, filed Sep. 19, 2015, and U.S. Provisional Patent Application No. 62/130,117, filed Mar. 9, 2015, each of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention generally relates to compounds that are useful for inhibiting one or more serine proteases including Hepatocyte Growth Factor Activator, matriptase, and hepsin. The present invention also relates to various methods of using the inhibitor compounds including treating a malignancy, a pre-malignant condition, or cancer by administering an effective amount of the inhibitor to a subject in need thereof.

BACKGROUND OF THE INVENTION

[0003] Proteases, also known as proteinases, peptidases, or proteolytic enzymes, are enzymes that degrade proteins by hydrolyzing peptide bonds between amino acid residues. It is known that proteases regulate numerous physiological processes which enable or stimulate the growth and metastasis of tumor cells. This involves the proteolytic degradation of the extracellular matrix proteins surrounding the tumor cells, which enables the invasion of tumor cells metastasizing from the tumors into the surrounding tissue and the lymph system or the blood system. Proteases are also involved in the activation of growth factors that stimulate the proliferation of cancer cells, thus enabling tumors to grow. Theses proteolytic enzymes include matrix metalloproteases, membrane-bound metalloproteases, lysosomal cysteine proteases, and a large number of serine proteases such as urokinase, plasmin, elastase, thrombin, and cathepsin G. In addition, a family of serine proteases called type II transmembrane serine proteases (TTSPs) has been found to be important in tissue homeostasis and in cancer, in particular with tumor metastasis.

[0004] One member of this enzyme class, matriptase (matriptase-1, MT-SP1, TADG-15, CAP3, epithin, and ST14), is a trypsin-like serine protease expressed by cells of epithelial origin and overexpressed in a wide variety of human cancers. Unlike most proteases, which are either secreted from or retained in the cell, matriptase, as a TTSP, is readily accessible on the cell surface and hence a good target for a variety of therapies, including vaccines, monoclonal antibodies and small molecule compounds. Inhibition of the enzyme results in concomitant inhibition of two crucial mediators of tumorigenesis, hepatocyte growth factor (HGF) and the urokinase-type plasminogen activator (uPA).

[0005] Hepsin is another member of the type II transmembrane serine protease family. Hepsin is thought to play a role in cell growth and is known to be produced at a particularly high level in the liver as well as in human hepatoma cells, some cancer cells, and nerve cells. Hepatocyte Growth Factor Activator (HGFA) is a trypsin-like protease but is a plasma protease.

[0006] Hepsin, matriptase and HGFA are differentially expressed and have upregulated function in numerous tumor types including multiple myeloma, breast, prostate, lung, liver, and pancreatic. These proteases cleave the single-chain zymogen precursors, pro-HGF (hepatocyte growth factor), and pro-MSP (macrophage stimulating protein) into active two-chain heterodimeric forms. Active HGF and MSP are activating ligands for the oncogenic receptor tyrosine kinases (RTKs), c-MET and RON, respectively.

[0007] Increased activity of hepsin, matriptase, and/or HGFA, resulting from either expression or upregulation of these proteases and/or downregulation of their endogenous serine protease inhibitors (serpins), HAI-1 (SPINT1), HAI-2 (SPINT2), and protein C inhibitor (PCI), has been demonstrated in almost every tumor type driven by c-MET and/or RON pathway signaling. This increased protease function has been clearly associated with increased metastatic cancer phenotypes and direct inhibition of this protease activity has been demonstrated to reduce this metastatic potential in multiple tumor types. The biological reason for the redundancy of activation by these three different proteases and the tight regulation by serpins in cancer is not yet understood. Furthermore, since HGF/c-MET and MSP/RON signaling are necessary for development and normal cell physiology, selective inhibitors of each protease involved in individual tumors need to be identified when developing as therapeutics in order to limit potential toxicities.

[0008] Matriptase inhibitors are of therapeutic importance, but development has been a challenging task. To date, a number of small molecule inhibitors and inhibitory antibodies have been reported. See, for example; Enyedy et al., *J. Med. Chem.* 2001, 44, 1349-1355; Steinmetzer et al., J. Med. Chem., 2006, 49: 4116-4126, and Farady et al., *J Mol Biol,* 2007, 369: 1041-1051. Also a series of peptide-based matriptase inhibitors was recently described by Marsault et al., ACS Med Chem. Lett., 2012, 3: 530-534.

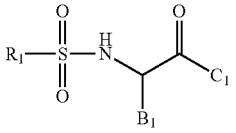
[0009] As compared to matriptase, inhibitory antibodies have also been reported but relatively few inhibitors are known for hepsin and HFGA. Small molecule hepsin inhibitors were discovered through high-throughput screening (Chevillet, J. R., et al. *Mol. Cancer Ther.* 2008, 7, 3343) but the only reported HGFA inhibitors are the non-selective serine protease inhibitors, Nafamostat and Leupeptin (Shimomura, T., et al., *Cytotechnology,* 1992, 8, 219).

[0010] Although some progress has been made toward inhibitors of matriptase, hepsin, and HGFA, there remains a need for small molecular weight inhibitors of matriptase, hepsin, and HGFA that are both potent and selective for one of more of these enzymes. Such compounds have significant therapeutic value, in particular for the treatment of cancer and other conditions involving tumor progression and migration and abnormal cell differentiation and proliferation or hematological malignancies. Compounds having improved selectivity, solubility, metabolic stability, half-life, and oral bioavailability are particularly desirable.

SUMMARY OF THE INVENTION

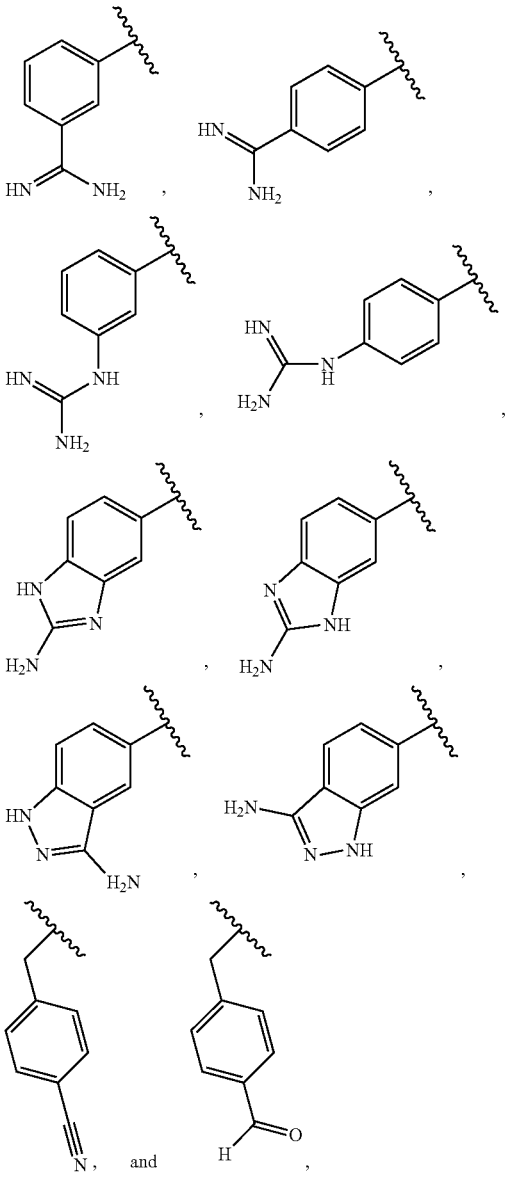
[0011] Generally, the present invention relates to compounds that are useful for inhibiting one or more serine proteases including Hepatocyte Growth Factor Activator, matriptase, hepsin, thrombin, and Factor Xa along with

various methods of use. In various aspects, the present invention is directed to compounds of Formula (I), as a single stereoisomer or as a mixture thereof:

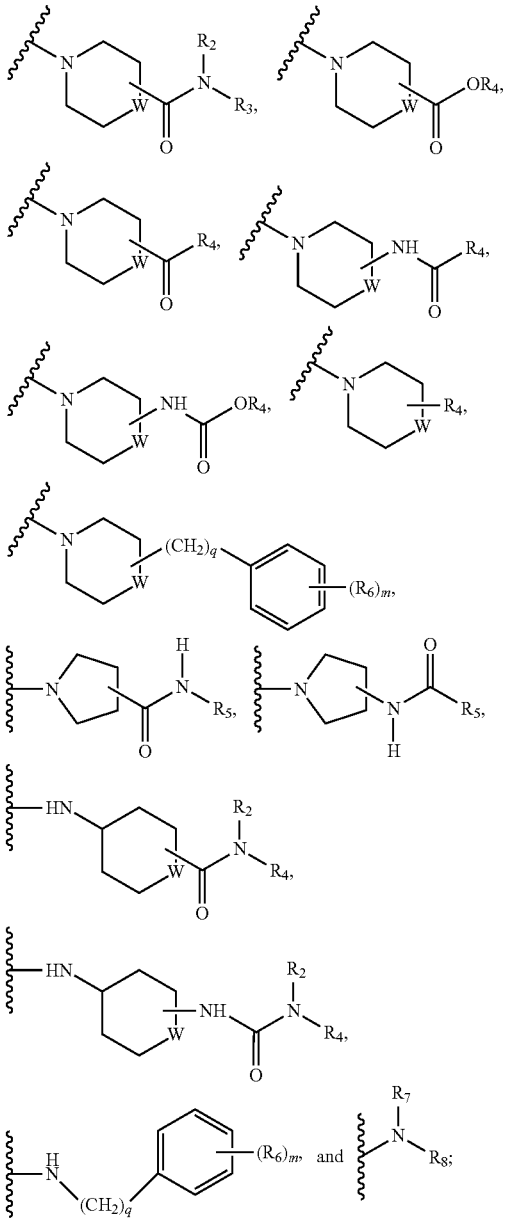


(I)

wherein R_1 is substituted or unsubstituted alkyl, substituted or unsubstituted aryl, or
substituted or unsubstituted heteroaryl;
B_1 is selected from the group consisting of:



C_1 is a group selected from the group consisting of:



W is CH, CH2, N, or NH;

[0012] R_2, R_3, R_4, R_5, R_6, R_7, and R_8 are each independently hydrogen, substituted or unsubstituted alkyl or cycloalkyl, substituted or unsubstituted alkylaryl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl or heteroaryl, with the proviso that when R_2 is methyl, then R_3 cannot also be methyl and vice versa; and
m is 0 to 5, or a pharmaceutically acceptable salt thereof.

[0013] The present invention also relates to compounds of Formula (II):

$$Y—(P_5)_b—(P_4)_n—(P_3)_m—P_2—P_1—Z \quad \text{(II)}$$

wherein

n is 0 or 1;

m is 0 or 1;

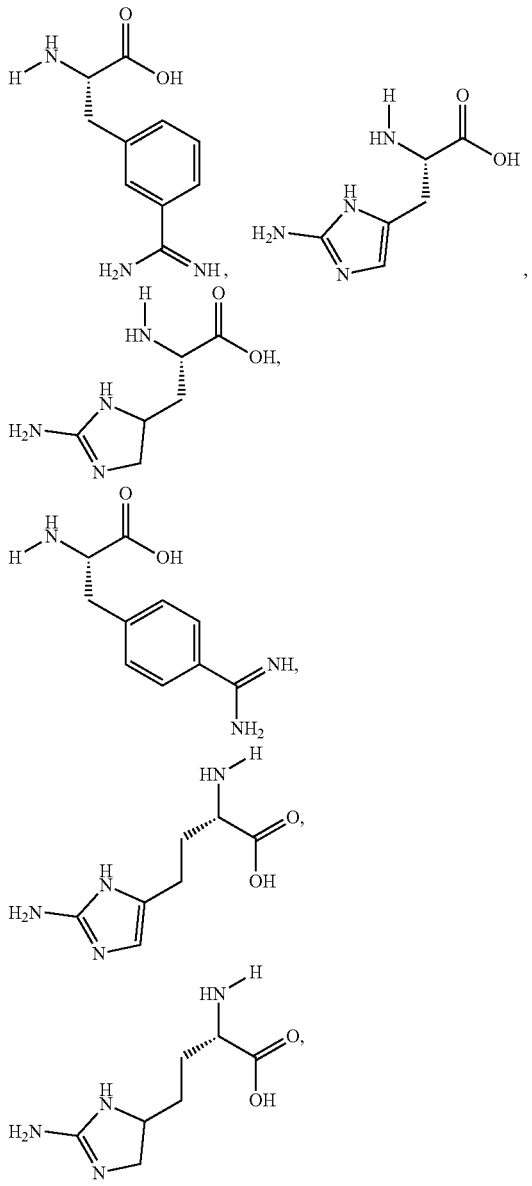
b is 0 or 1;

Y is H, acetyl, tert-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, benzyl, —$C(O)R_9$, —$SOOR_9$, —$COOR_9$, —$C(O)NHR_9$, —$(CH_2)_x$aryl-R_9, heteroaryl-R_9, -cycloalkyl-R_9, a fluorophore, biotin, or a reporter tag;

x is 0, 1, or 2;

R_9 is C_1 to C_{12} alkyl, cycloalkyl, alkylaryl, or aryl;

P_1 is a residue of an amino acid selected from the group consisting of Arg, D-Arg, Lys, substituted Lys, and an alpha-amino acid of the following:



or an unnatural amino acid residue;

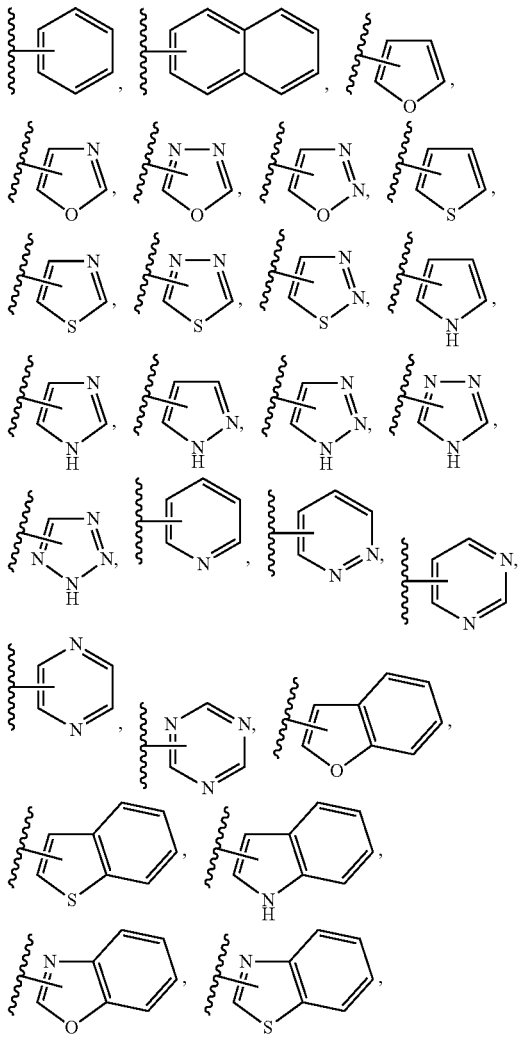
P_2 is a residue of an amino acid selected from the group consisting Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Arg, Lys, Ile, Ala, Gly, Asn, hLeu, NptGly, L-Orn, L-Cha, Nle, hTyr, Nva, Orn, Cha, and an unnatural amino acid residue;

P_3 is a residue of an amino acid selected from the group consisting Asp, Glu, Arg, Lys, Met, Trp, Leu, Gln, Phe, Tyr, His, hArg, D-Trp, L-Orn, D-Gln, L-Met(O), L-Nle(OBzl), Agp, hCha, hTyr, hPhe, D-Arg, Nle(OBzl), Orn, Met(O), and an unnatural amino acid residue;

P_4 is a residue of an amino acid selected from the group consisting Arg, Lys, Met, Try, Trp, Ser, His, Phe, Thr, Asn, Pro, Gln, Asp, Glu, Chg, Idc, dhLeu, Agp, D-Ser, Agp, His(3-Bom), Lys(2-Cl-Z), L-Orn, L-Arg(NO_2), L-Nle (OBzl), L-DAB(Z), and an unnatural amino acid residue;

P_5 is a residue of an amino acid selected from the group consisting Lys, Arg, Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Ile, Ala, Gly, Asn, and an unnatural amino acid residue; and

Z is Val, Ser, Lys, Ala, Gly, Trp, Tyr, Phe, Arg, Thr, Leu, Ile, Met, His, Nle, Phg, Pro, Gln, Asn, —CH_2Cl, or a substituted or unsubstituted ring substituent selected from the group consisting of:



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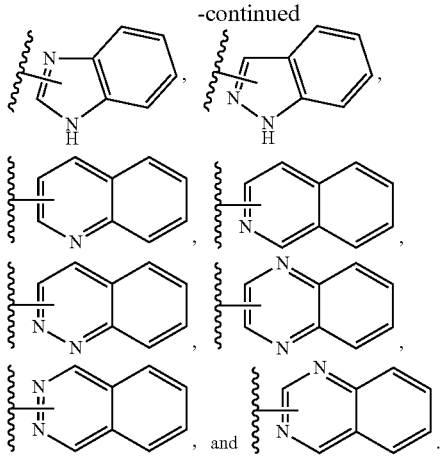


[0014] The present invention further relates to pharmaceutical compositions comprising a therapeutically effective amount of at least one compound as described herein.

[0015] The present invention also relates to various methods of use including a method of inhibiting matriptase, hepsin, or HGFA comprising administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound as described herein. Another method includes a method of inhibiting HGF/MET oncogenic signaling comprising administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound as described herein.

[0016] Other methods include a method of inhibiting carcinoma progression and a method of treating a malignancy, a pre-malignant condition, or cancer comprising administering to the subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound as described herein.

[0017] Further, the present invention relates to imaging compositions comprising a radiolabeled compound of Formula (I) or (II) as described herein and a method of detecting cancer comprising administering to a subject an imaging composition comprising the radiolabeled compound; employing a nuclear imaging technique for monitoring or visualizing a distribution of the radiolabeled compound within the body or within a portion thereof; and correlating the distribution of the radiolabeled compound to the existence of cancer.

[0018] Additionally, the present invention relates to imaging compositions comprising a fluorescent compound of Formula (I) or (II) as described herein and a method of detecting cancer comprising administering to a subject an imaging composition comprising the fluorescent compound; employing an imaging technique for monitoring or visualizing a distribution of the fluorescent compound within the body of within a portion thereof; and correlating the distribution of the fluorescent compound to the existence of cancer.

[0019] Other objects and features will be in part apparent and in part pointed out hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] FIG. **1** presents a plot of the results for the HGFA competition assay of inhibitor Ac-KQLR (SEQ ID NO: 1)-Kt.

[0021] FIG. **2** presents a plot of the results for the fluorescent inhibitor assay for inhibitor Boc-QLR-AMC and HGFA.

[0022] FIG. **3** presents a plot of the results for the fluorescent inhibitor assay for inhibitor Boc-QAR-AMC and hepsin.

[0023] FIG. **4** presents a plot of the results for the fluorescent inhibitor assay for inhibitor Boc-QAR-AMC and matriptase.

[0024] FIG. **5** presents a plot of the results of a dilution recovery experiments with Ac-KQLR (SEQ ID NO: 1)-Kt and HGFA.

[0025] FIG. **6** presents a plot of the results of a dilution recovery experiments with Ac-KQLR (SEQ ID NO: 1)-Kt and HGFA.

[0026] FIG. **7** shows the inhibition of HGFA-mediated scHGF (pro-HGF) cleavage by inhibitors. Immunoblot of scHGF cleavage reactions: Pro-HGF (30 ng) was cleaved with 1 nM HGFA in the presence of inhibitors (5-fold dilutions from 12.5 μM).

[0027] FIG. **8** shows the inhibition of HGFA-mediated scMSP (pro-MSP) cleavage by inhibitors. Immunoblot of scMSP cleavage reactions: Pro-MSP (50 ng) was cleaved with 10 nM HGFA in the presence of inhibitors (5-fold dilutions from 12.5 μM).

[0028] FIG. **9** shows MDA-MB-231 c-MET phosphorylation of cells treated with pro-HGF/HGFA reactions (3-fold dilutions of inhibitors starting at 100 μM). A. Immunoblot of pY1234/135 c-MET; B. % inhibition of c-MET phosphorylation.

[0029] FIG. **10** presents a plot of the results of a chromogenic kinetic enzyme assay for matriptase and selected inhibitor compounds.

[0030] FIG. **11** presents a plot of the results of a chromogenic kinetic enzyme assay for matriptase and selected inhibitor compounds.

[0031] FIG. **12** presents a plot of the results of a chromogenic kinetic enzyme assay for hepsin and selected inhibitor compounds.

[0032] FIG. **13** presents a plot of the results of a chromogenic kinetic enzyme assay for matriptase and selected inhibitor compounds.

[0033] FIG. **14** presents a plot of the results of a chromogenic kinetic enzyme assay for matriptase and selected inhibitor compounds.

[0034] FIG. **15** presents a plot of the results of a chromogenic kinetic enzyme assay for matriptase and selected inhibitor compounds.

[0035] FIG. **16** presents a plot of the results of a chromogenic kinetic enzyme assay for hepsin and selected inhibitor compounds.

[0036] FIG. **17** presents a plot of the results of a chromogenic kinetic enzyme assay for hepsin and selected inhibitor compounds.

[0037] FIG. **18** shows MDA-MB-231 vs T47D breast cancer cells following MSP stimulation. A. Immunoblot of total RON and MAPK of breast cancer cells; B. RON phosphorylation ELISA assay.

[0038] FIG. **19** shows the inhibition of MRC-5 mediated/pro-HGF migration and invasion of triple negative human MDA-MB-231 breast cancer cells using selected inhibitor compounds.

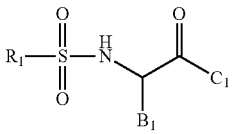
[0039] FIG. **20** shows the MSP-MS profiling for HGFA substrate cleavage and specificity.

[0040] FIG. **21** shows iceLogo figures. A. Shows frequency at which specific amino acids are present at each position. B. Comparison of P4 and P4' amino acid frequency of the 22 peptides that were cleaved versus all possible peptides that contained an arginine.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0041] In various aspects, the present invention generally relates to compounds that are useful for inhibiting one or more serine proteases, including Hepatocyte Growth Factor Activator, matriptase, hepsin, thrombin, and Factor Xa along with various methods of use. In other aspects, the present invention also relates to various methods of using the inhibitor compounds including treating a malignancy, a pre-malignant condition, or cancer by administering an effective amount of the inhibitor to a subject in need thereof.

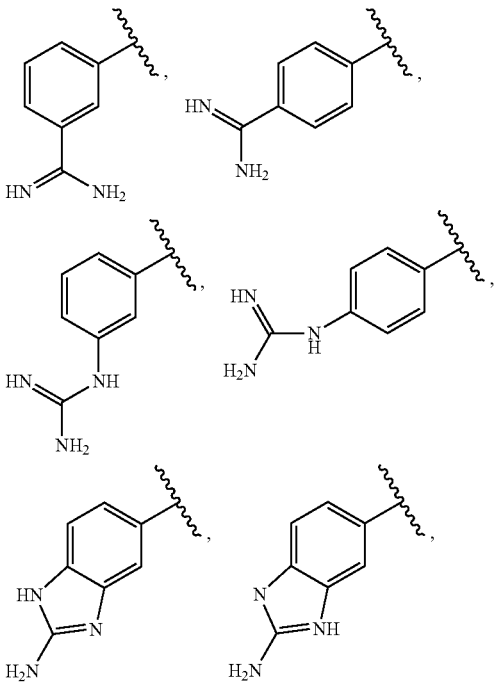
[0042] In accordance with the present invention, one class of compounds useful for inhibiting one or more of hepatocyte growth factor activator, matriptase, and hepsin includes benzamidine compounds of Formula (I), as a single stereoisomer or as a mixture thereof:



(I)

wherein R_1 is substituted or unsubstituted alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;

B_1 is selected from the group consisting of:



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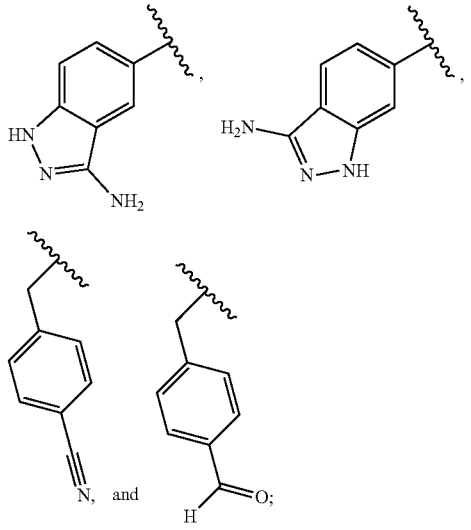
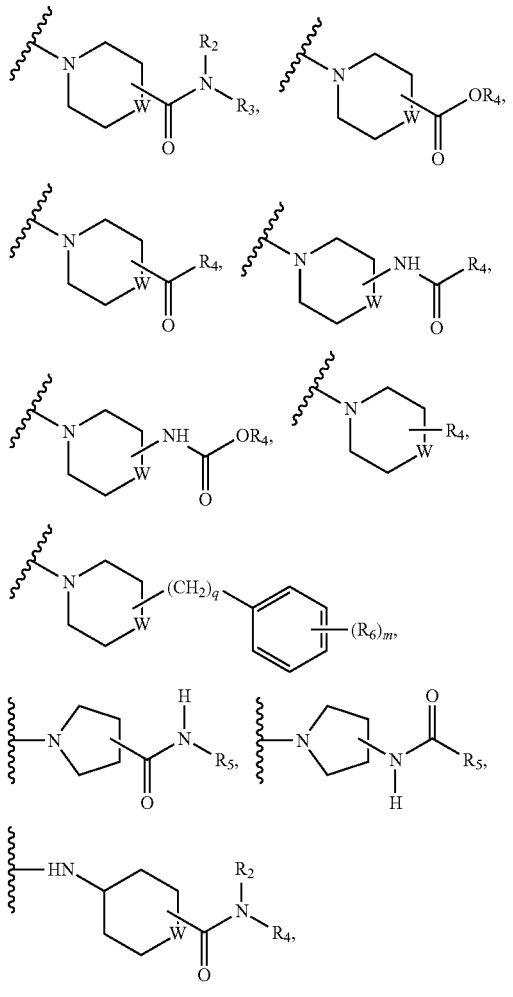


C_1 is a group selected from the group consisting of:



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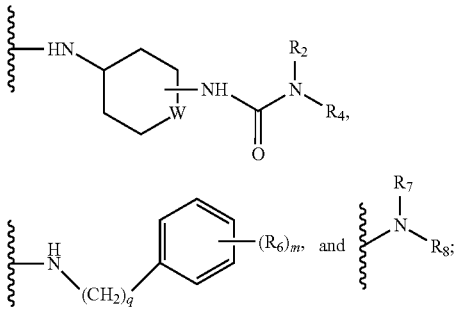
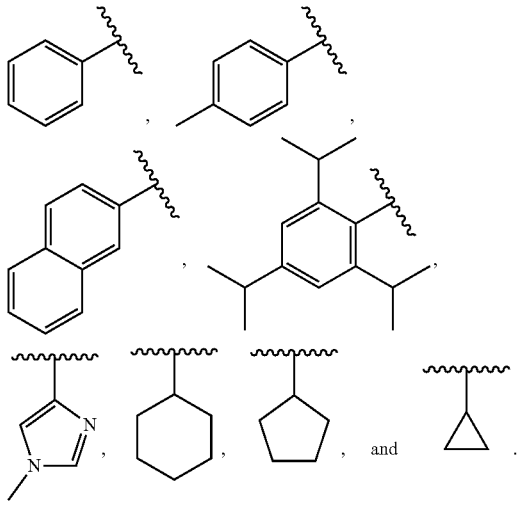


W is CH, CH_2, N, or NH;

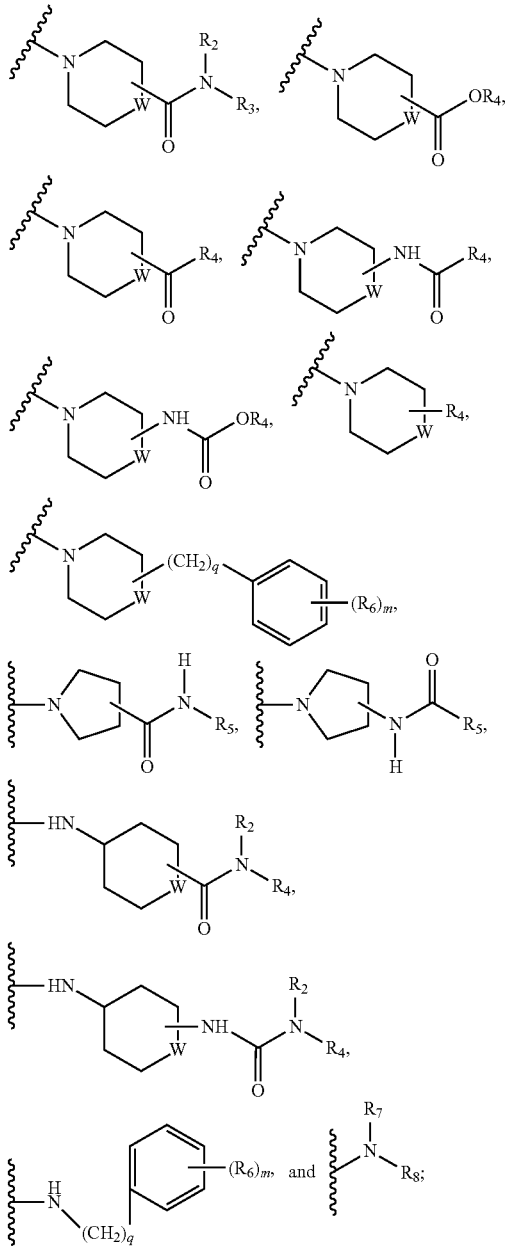
[0043] R_2, R_3, R_4, R_5, R_6, R_7, and R_8 are each independently hydrogen, substituted or unsubstituted alkyl or cycloalkyl, substituted or unsubstituted alkylaryl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl or heteroaryl, with the proviso that when R_2 is methyl, then R_3 cannot also be methyl and vice versa; and

m is 0 to 5, or a pharmaceutically acceptable salt of Formula (I).

[0044] In various embodiments, R_1 is substituted or unsubstituted C_1-C_6 alkyl, substituted or unsubstituted C_3-C_6 cycloalkyl, substituted or unsubstituted phenyl, substituted or unsubstituted naphthyl, or a substituted or unsubstituted nitrogen-containing aromatic ring. For example, the substituted C_1-C_6 alkyl, substituted C_3-C_6 cycloalkyl, substituted phenyl, substituted naphthyl, or substituted nitrogen-containing aromatic ring can comprise one or more substituents comprising halo, hydroxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, halo-substituted C_1-C_4 alkyl, or amino. In certain embodiments, R_1 is an group selected from the group consisting of:



[0045] In some embodiments, C_1 is a group selected from the group consisting of:



W is CH or N;

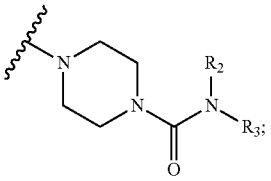
[0046] R_2, R_3, R_4, R_5, R_6, R_7, and R_8 are each independently hydrogen, substituted or unsubstituted alkyl or cycloalkyl, substituted or unsubstituted alkylaryl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl or heteroaryl, with the proviso that when R_2 is methyl, then R_3 cannot also be methyl and vice versa; and

m is 0 to 5, or a pharmaceutically acceptable salt thereof.

[0047] In some embodiments, R_2, R_3, R_4, R_5, R_6, R_7, and R_8 are each independently hydrogen, substituted or unsub-

stituted C_1-C_{10} alkyl or cycloalkyl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl. In particular embodiments, R_2 is hydrogen; R_3 is hydrogen, C_1-C_6 alkyl, benzyl, or halo-substituted benzyl; R_4 and R_5 are each independently hydrogen, C_1-C_6 alkyl, halo- or alkoxy-substituted C_1-C_6 alkyl, phenyl, phenethyl, benzyl, halo- or alkoxy-substituted benzyl; substituted or unsubstituted 3-benzothiophenyl, or substituted or unsubstituted 1-morpholinyl; R_6 is hydrogen, C_1-C_4 alkoxy; and/or R_7 and R_8 are each independently hydrogen or C_1-C_6 alkyl.

[0048] In certain embodiments, C_1 is



R_2 is hydrogen; and R_3 is hydrogen, C_1-C_6 alkyl, benzyl, or halo-substituted benzyl. In these and other embodiments, the compound of Formula (I) is a selective inhibitor of hepsin.

[0049] In accordance with the present invention, another class of compounds useful for inhibiting one or more of HGFA, matriptase, and hepsin includes polypeptide of Formula (II):

$$Y—(P_5)_b—(P_4)_n—(P_3)_m—P_2—P_1—Z \quad (II)$$

wherein

n is 0 or 1;

m is 0 or 1;

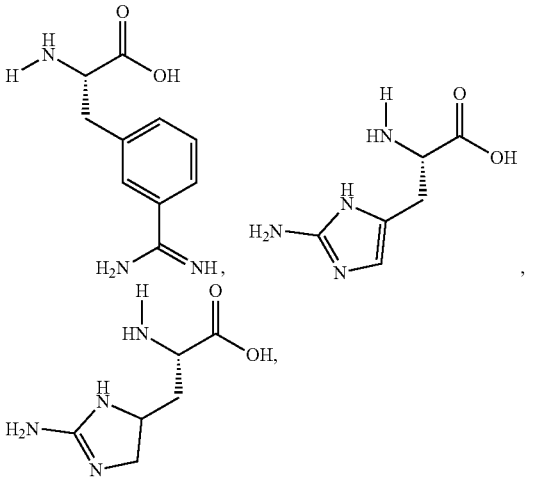
b is 0 or 1;

Y is H, acetyl, tert-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluroene, benzyl, —C(O)R_9, —SOOR_9, —COOR_9, —C(O)NHR_9, —$(CH_2)_x$aryl-R_9, heteroaryl-R_9, -cycloalkyl-R_9, a fluorophore, biotin, or a reporter tag;

x is 0, 1, or 2;

R_9 is C_1 to C_{12} alkyl, cycloalkyl, alkylaryl, or aryl;

P_1 is a residue of an amino acid selected from the group consisting of Arg, D-Arg, Lys, substituted Lys, and an alpha-amino acid of the following:



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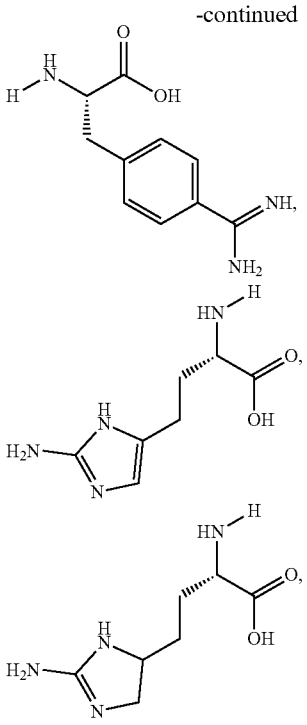


or an unnatural amino acid residue;

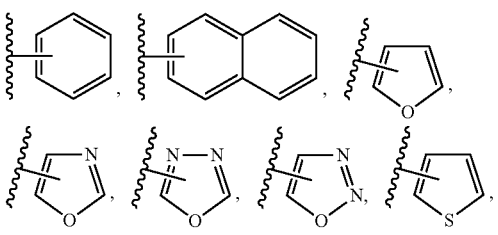
P_2 is a residue of an amino acid selected from the group consisting Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Arg, Lys, Ile, Ala, Gly, Asn, hLeu, NptGly, L-Om, L-Cha, Nle, hTyr, Nva, Orn, Cha, and an unnatural amino acid residue;

P_3 is a residue of an amino acid selected from the group consisting Asp, Glu, Arg, Lys, Met, Trp, Leu, Gln, Phe, Tyr, His, hArg, D-Trp, L-Om, D-Gln, L-Met(O), L-Nle(OBzl), Agp, hCha, hTyr, hPhe, D-Arg, Nle(OBzl), Orn, Met(O), and an unnatural amino acid residue;

P_4 is a residue of an amino acid selected from the group consisting Arg, Lys, Met, Try, Trp, Ser, His, Phe, Thr, Asn, Pro, Gln, Asp, Glu, Chg, Idc, dhLeu, Agp, D-Ser, Agp, His(3-Bom), Lys(2-Cl-Z), L-Om, L-Arg(NO_2), L-Nle (OBzl), L-DAB(Z), and an unnatural amino acid residue;

P_5 is a residue of an amino acid selected from the group consisting Lys, Arg, Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Ile, Ala, Gly, Asn, and an unnatural amino acid residue; and

Z is Val, Ser, Lys, Ala, Gly, Trp, Tyr, Phe, Arg, Thr, Leu, Ile, Met, His, Nle, Phg, Pro, Gln, Asn, —CH_2Cl, or a substituted or unsubstituted ring substituent selected from the group consisting of:



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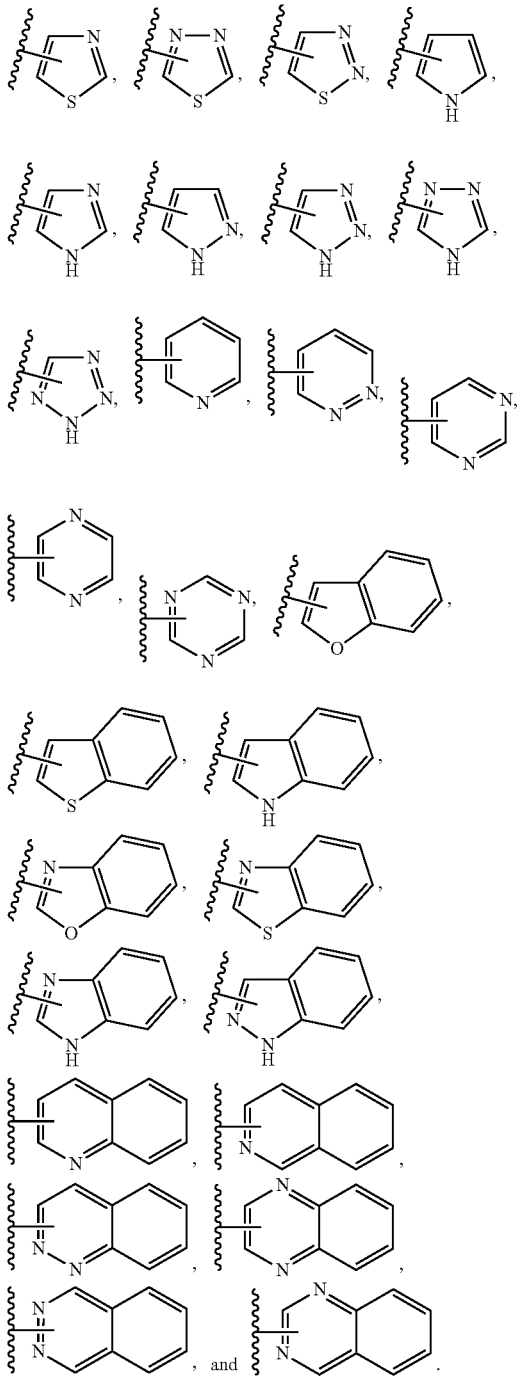


[0050] As understood herein, when two or more amino acids combine to form a peptide (e.g., of Formula (II)), the elements of water are removed, and what remains of each amino acid is called an amino-acid residue.

[0051] In various embodiments, the polypeptide of Formula (II) include one or more of the following:

Y is H, acetyl, tert-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluroene, benzyl, —C(O)R_9, —SOOR_9, —COOR_9, —C(O)NHR_9, —$(CH_2)_x$aryl-R_9, heteroaryl-R_9, -cycloalkyl-R_9, or a fluorophore;

x is 0, 1, or 2;

R_9 is C_1 to C_{12} alkyl, cycloalkyl, alkylaryl, or aryl;

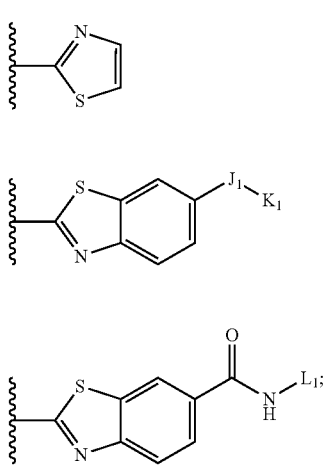
P_2 is a residue of an amino acid selected from the group consisting Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Arg, Lys, Ile, Ala, Gly, Asn, hLeu, NptGly, L-Orn, L-Cha, Nle, hTyr, Nva, Orn, Cha, and an unnatural amino acid residue;

P_3 is a residue of an amino acid selected from the group consisting Asp, Glu, Arg, Lys, Met, Trp, Leu, Gln, Phe, Tyr, His, hArg, D-Trp, L-Orn, D-Gln, L-Met(O), L-Nle(OBzl), Agp, hCha, hTyr, hPhe, D-Arg, Nle(OBzl), Orn, Met(O), and an unnatural amino acid residue;

P_4 is a residue of an amino acid selected from the group consisting Arg, Lys, Met, Try, Trp, Ser, His, Phe, Thr, Asn, Pro, Gln, Asp, Glu, Chg, Idc, dhLeu, Agp, D-Ser, Agp, His(3-Bom), Lys(2-Cl-Z), L-Orn, L-Arg(NO_2), L-Nle (OBzl), L-DAB(Z), and an unnatural amino acid residue;

P_5 is a residue of an amino acid selected from the group consisting Lys, Arg, Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Ile, Ala, Gly, Asn, and an unnatural amino acid residue; and

Z is Val, Ser, Lys, Ala, Gly, Trp, Tyr, Phe, Arg, Thr, Leu, Ile, Met, His, Nle, Phg, Pro, Gln, Asn, —CH_2Cl, a thiazole of Formula (III), or a benzothiazole of Formula (IV) or (V)



(III)

(IV)

(V)

J_1 is C(O), SO_2, CH_2 or heterocyclo;

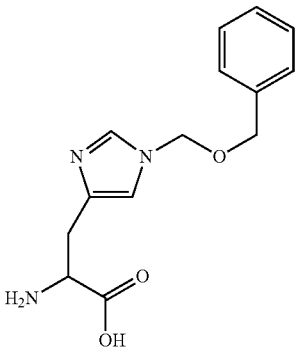
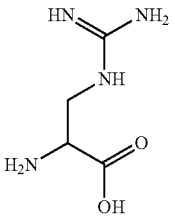
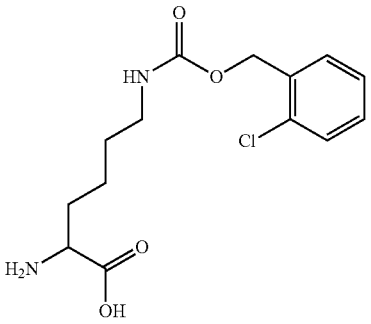
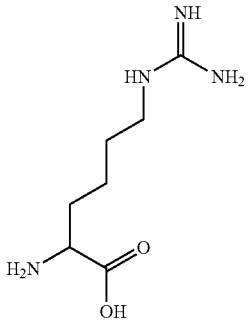
K_1 is a D- or L-amino acid, wherein the C-terminus is —COOH, —C(O)NH_2, —OH, —OR_{10}, —NH_2, —N$R_{11}R_{12}$, —H or heterocyclo;

R_{10} is C_1 to C_{12} alkyl, cycloalkyl, alkylaryl, or aryl;

R_{11} and R_{12} are each independently H, C_1 to C_{12} alkyl, cycloalkyl, alkylaryl, aryl, or heterocyclo; and R_{11} and R_{12} together can form a ring; and/or

Li is H, alkyl, cycloalkyl, alkylaryl, benzyl, substituted benzyl, 2- or 3- or 4-piperdinyl, 2- or 3- or 4-pyridinyl, alkyl, cycloalkyl, aryl, heterocyclo, or heteroaryl.

[0052] In various embodiments, P_1, P_2, P_3, P_4 and P_5 can each independently be an unnatural amino acid residue. The unnatural amino acids can be selected from the group listed in the following Table. The unnatural amino acids can be the D- and/or L-isomers.

Abbreviation	Structure
His(3-Bom)	
Agp	
Lys(2-Cl-Z)	
hArg	

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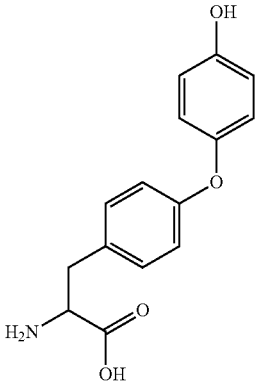
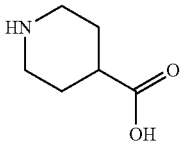
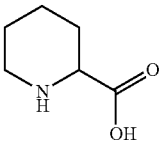
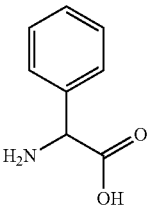
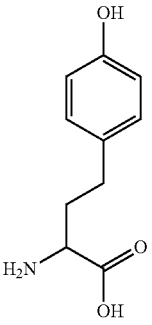
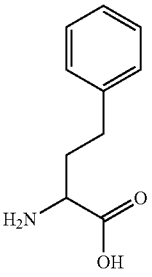
Abbreviation	Structure
Thyr	
Inp	
Pip	
Phg	
hTyr	
hPhe	

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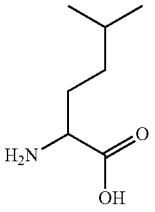
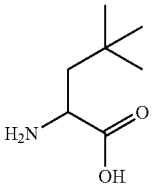
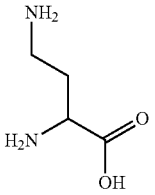
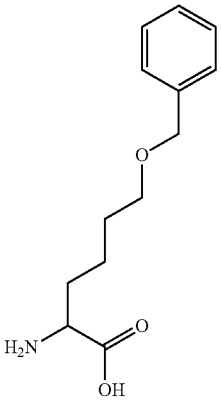
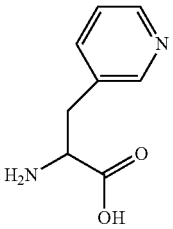
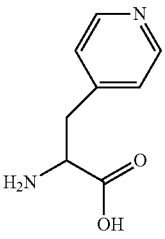
hLeu	
NptGly	
DAB(Z)	
Nle(OBzl)	
3-Pal	
4-Pal	

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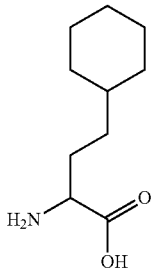
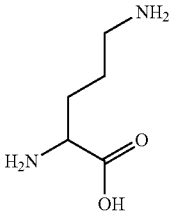
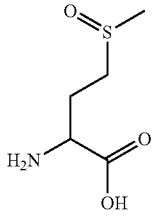
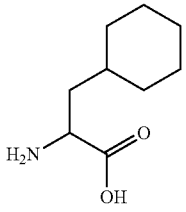
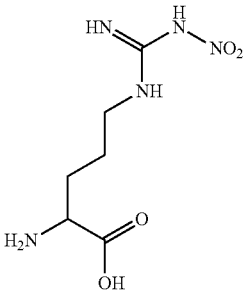
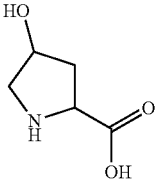
hCha	
Orn	
Met(O)	
Cha	
Arg(NO_2)	
Hyp	

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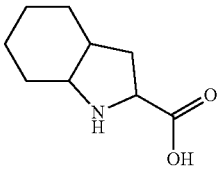
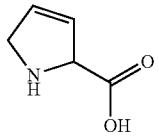
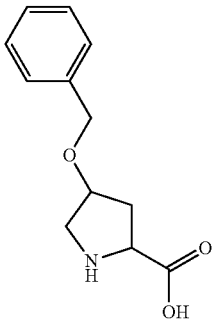
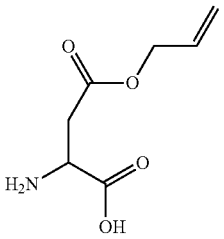
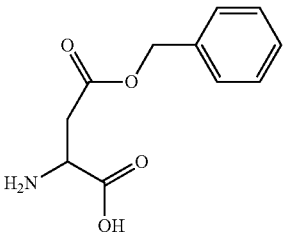
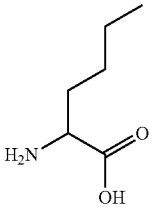
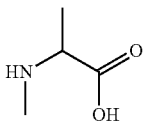
Oic	
hPro	
Hyp(Bzl)	
Asp(All)	
Asp(Bzl)	
Nle	
MeAla	

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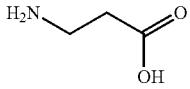
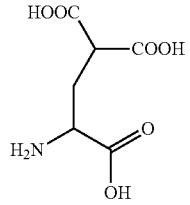
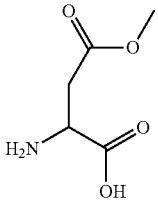
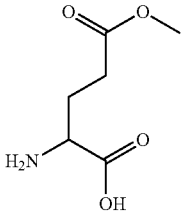
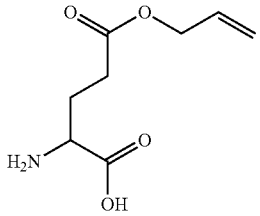
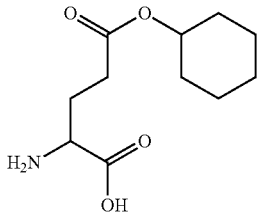
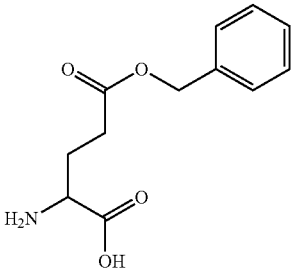
βA1a	
Gla	
Asp(Me)	
Glu(Me)	
Glu(All)	
Glu(Chx)	
Glu(Bz1)	

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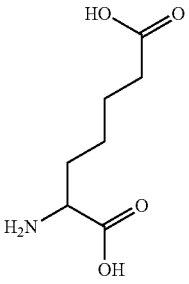
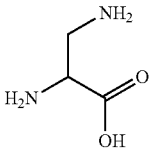
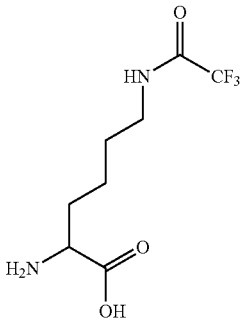
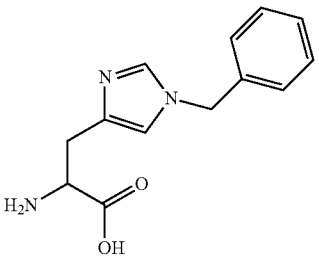
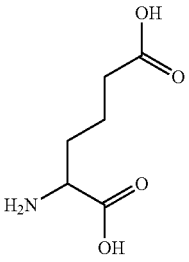
Api	
Dap	
Lys(TFA)	
His(Bzl)	
Aad	

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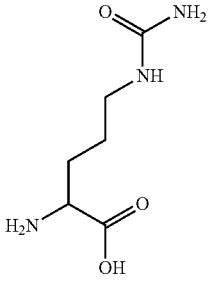
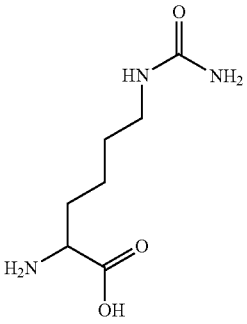
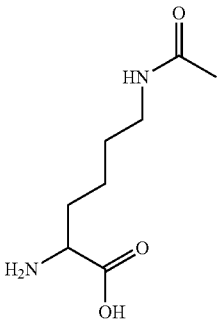
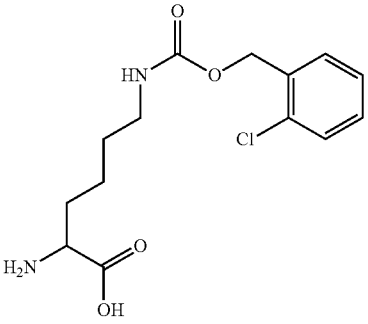
Cit	
hCit	
Lys(Ac)	
Lys(2Cl-Z)	

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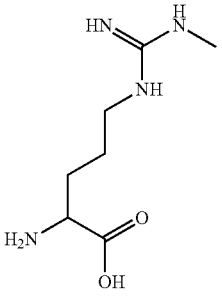
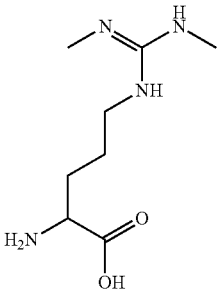
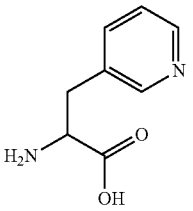
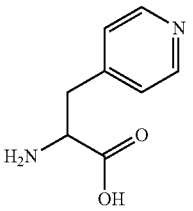
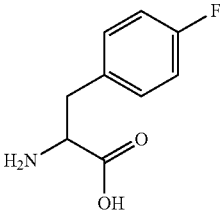
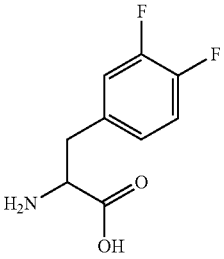
Arg(Me)	
$Arg(Me)_2$	
3-Pal	
4-Pal	
Phe(4-F)	
Phe(3,4-F)	

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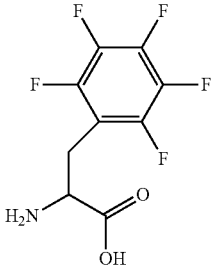
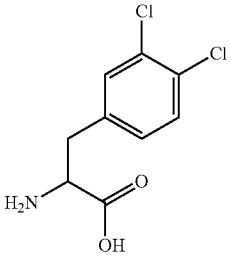
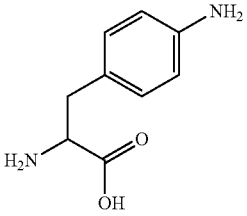
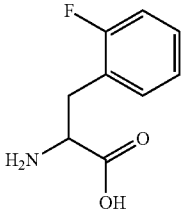
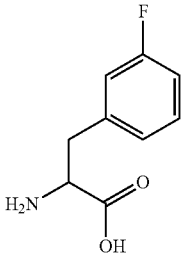
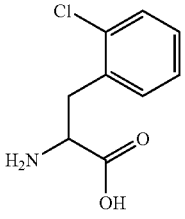
$Phe(F_5)$	
Phe(3,4-Cl)	
$Phe(4\text{-}NH_2)$	
Phe(2-F)	
Phe(3-F)	
Phe(2-Cl)	

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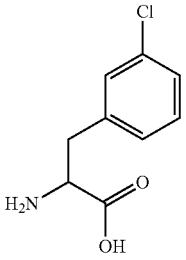
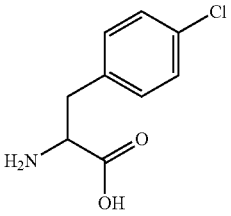
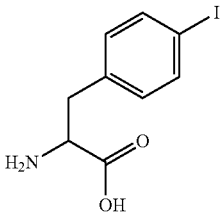
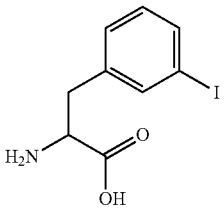
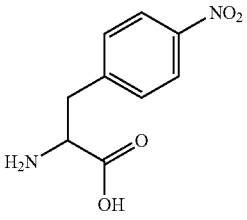
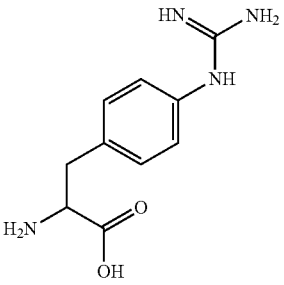
Phe(3-C1)	
Phe(4-C1)	
Phe(4-I)	
Phe(3-I)	
Phe(4-NO_2)	
Phe(4-guan)	

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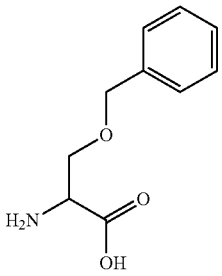
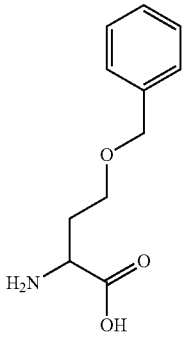
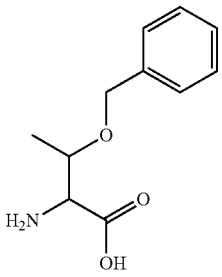
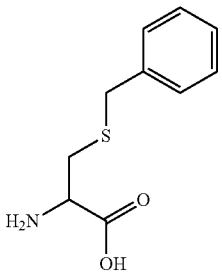
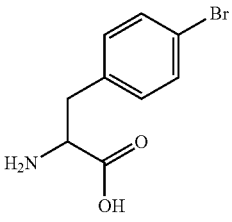
Ser(Bzl)	
hSer(Bzl)	
Thr(Bzl)	
Cys(Bzl)	
Phe(4-Br)	

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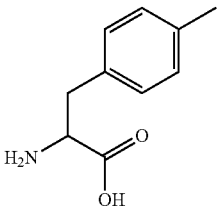
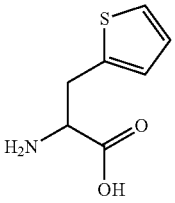
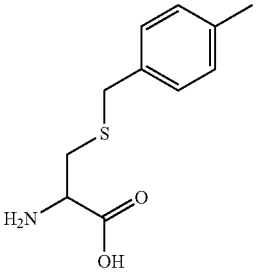
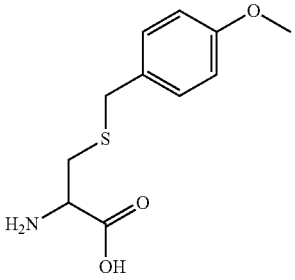
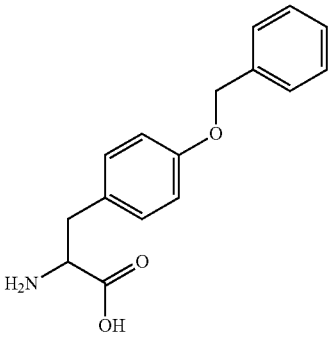
Abbreviation	Structure
Phe(4-Me)	
Ala(2-th)	
Cys(4-MeBzl)	
Cys(4-MeOBzl)	
Tyr(Bzl)	

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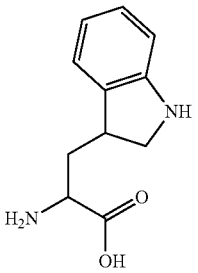
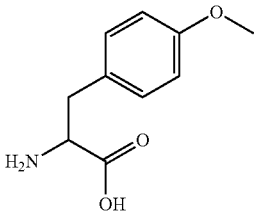
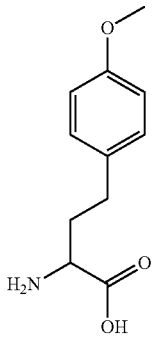
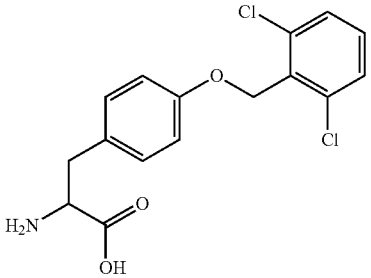
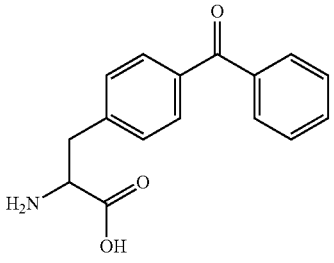
Dht	
Tyr(Me)	
hTyr(Me)	
Tyr(2,6-Cl-Bzl)	
Bpa	

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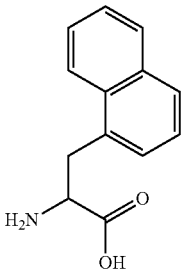
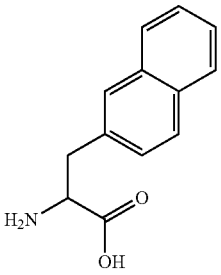
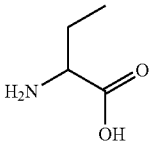
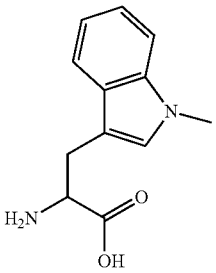
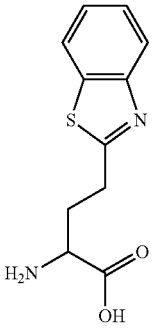
1-Nal	
2-Nal	
Abu	
Trp(Me)	
Abu(Bth)	

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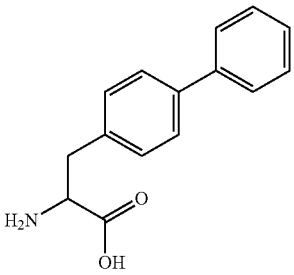
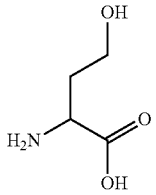
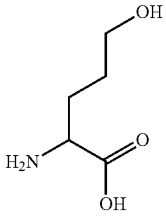
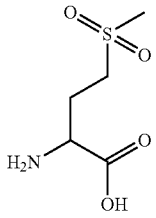
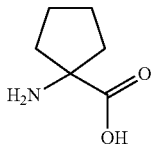
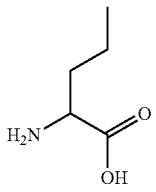
Bip	
hSer	
Hnv	
Met(O_2)	
AC5C	
Nva	

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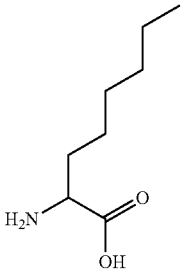
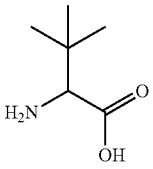
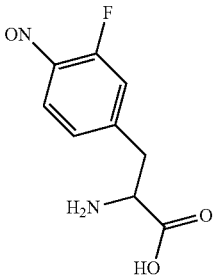
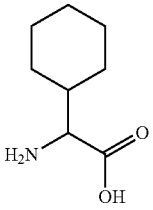
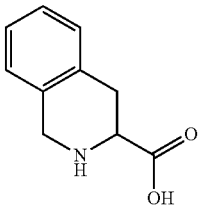
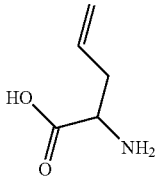
Abbreviation	Structure
2-Aoc	
Tle	
4-NO_2-3-F-Phe	
Chg	
Tic	
AllyGly	

[0053] In accordance with the present invention, another class of compounds useful for inhibiting one or more of HGFA, matriptase, and hepsin includes cyclic peptides of Formula (II):

$$Y—(P_5)_b—(P_4)_n—(P_3)_m—P_2—P_1—Z \quad (II)$$

wherein

n is 0 or 1;

m is 0 or 1;

b is 0 or 1;

Y is H, acetyl, tert-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluroene, benzyl, —C(O)R_9, —SOOR_9, —COOR_9, —C(O)NHR_9, —$(CH_2)_x$aryl-R_9, heteroaryl-R_9, -cycloalkyl-R_9, or a fluorophore;

x is 0, 1, or 2;

R_9 is C_1 to C_{12} alkyl, cycloalkyl, alkylaryl, or aryl;

P_1 is a residue of an amino acid selected from the group consisting Arg, D-Arg, Lys, substituted Lys, and an unnatural amino acid residue;

P_2 is a residue of an amino acid selected from the group consisting Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Arg, Lys, Ile, Ala, Gly, Asn, hLeu, NptGly, L-Orn, L-Cha, Nle, hTyr, Nva, Orn, Cha, and an unnatural amino acid residue;

P_3 is a residue of an amino acid selected from the group consisting Asp, Glu, Arg, Lys, Met, Trp, Leu, Gln, Phe, Tyr, His, hArg, D-Trp, L-Orn, D-Gln, L-Met(O), L-Nle(OBzl), Agp, hCha, hTyr, hPhe, D-Arg, Nle(OBzl), Orn, Met(O), and an unnatural amino acid residue;

P_4 is a residue of an amino acid selected from the group consisting Arg, Lys, Met, Try, Trp, Ser, His, Phe, Thr, Asn, Pro, Gln, Asp, Glu, Chg, Idc, dhLeu, Agp, D-Ser, Agp, His(3-Bom), Lys(2-Cl-Z), L-Orn, L-Arg(NO_2), L-Nle (OBzl), L-DAB(Z), and an unnatural amino acid residue;

P_5 is a residue of an amino acid selected from the group consisting Lys, Arg, Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Ile, Ala, Gly, Asn, and an unnatural amino acid residue;

P_3 can bond with P_5 and form a cyclic peptide; and

P_2 can bond with P_4 and form a cyclic peptide.

[0054] In various embodiment, a compound useful for inhibiting HGFA includes a compound of Formula (II):

$$Y—(P_5)_b—(P_4)_n—(P_3)_m—P_2—P_1—Z \quad (II)$$

wherein

n is 0 or 1;

m is 0 or 1;

b is 0 or 1;

Y is H, acetyl, tert-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluroene, benzyl, —C(O)R_9, —SOOR_9, —COOR_9, —C(O)NHR_9, —$(CH_2)_x$aryl-R_9, heteroaryl-R_9, -cycloalkyl-R_9, or a fluorophore;

x is 0, 1, or 2;

R_9 is C_1 to C_{12} alkyl, cycloalkyl, alkylaryl, or aryl;

R_1 is Arg;

[0055] R_2 is selected from the group consisting of Leu, Met, Phe, Tyr, Trp, hLeu, NptGly, Nle, hTyr, and Nva;

P_3 is selected from the group consisting of His, Gln, Arg, Lys, Leu, Phe, Trp, Tyr, hArg, D-Trp, Agp, hCha, hTyr, hPhe, and D-Arg; and

P_4 is selected from the group consisting of Thr, Asn, Ser, Arg, Lys, Phe, Trp, His(Bom), Agp, Lys(2-Cl-Z), dhLeu, Ide, and Chg.

[0056] In other embodiments, a compound useful for inhibiting matriptase includes a compound of Formula (II):

$$Y—(P_5)_b—(P_4)_n—(P_3)_m—P_2—P_1—Z \quad (II)$$

wherein

n is 0 or 1;

m is 0 or 1;

b is 0 or 1;

Y is H, acetyl, tert-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluroene, benzyl, —C(O)R_9, —SOOR_9, —COOR_9, —C(O)NHR_9, —$(CH_2)_x$aryl-R_9, heteroaryl-R_9, -cycloalkyl-R_9, or a fluorophore;

x is 0, 1, or 2;

R_9 is C_1 to C_{12} alkyl, cycloalkyl, alkylaryl, or aryl;

R_1 is selected from the group consisting of Arg, and Lys;

R_2 is selected from the group consisting of Phe, Ala, Arg, Asn, Gln, Glu, Gly, His, Leu, Lys, Met, Pro, and Ser;

R_3 is selected from the group consisting of Arg, Leu, Trp, Phe, His, Gln, Lys, D-Trp, and D-Arg; and

R_4 is selected from the group consisting of Pro, Phe, Thr, Asn, Trp, Gln, Ser, Lys, and Arg, His(Bom).

[0057] In further embodiments, a compound useful for inhibiting hepsin includes a compound of Formula (II):

$$Y—(P_5)_b—(P_4)_n—(P_3)_m—P_2—P_1—Z \quad (II)$$

wherein

n is 0 or 1;

m is 0 or 1;

b is 0 or 1;

Y is H, acetyl, tert-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluroene, benzyl, —C(O)R_9, —SOOR_9, —COOR_9, —C(O)NHR_9, —$(CH_2)_x$aryl-R_9, heteroaryl-R_9, -cycloalkyl-R_9, or a fluorophore;

x is 0, 1, or 2;

R_9 is C_1 to C_{12} alkyl, cycloalkyl, alkylaryl, or aryl;

R_1 is Arg;

[0058] R_2 is selected from the group consisting of Pro, Arg, Asn, Asp, Gln, Ile, Leu, Lys, Phe, Thr, Trp, Tyr, Orn, Cha, Nle, and Nva;

R_3 is selected from the group consisting of Leu, Trp, Phe, His, Gln, Lys, Arg, D-Gln, Agp, Nle (OBzl), Orn, Met(O), D-Trp, and D-Arg; and

R_4 is selected from the group consisting of Pro, Phe, Thr, Asn, Trp, Gln, Ser, Arg, Lys, Agp, DAB(Z), Nle (OBzl), Orn, Arg(NO_2), and His(Bom).

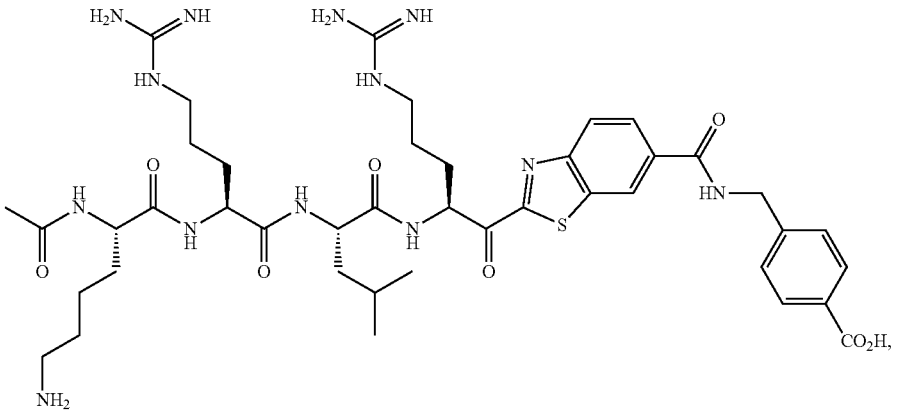
[0059] In various embodiments, P_1 can be an amino acid residue of Arg. P_2 can be an amino acid residue of Leu, Phe or Met. P_3 can be an amino acid residue of Arg, Lys, Met, or Trp. P_4 can be an amino acid residue of Arg, Lys, or Try. In these and other embodiments, the compound of Formula (II) is a selective inhibitor of HGFA.

[0060] In various embodiments, Z is a benzothiazole of Formula (IV). In these and other embodiments, J_1 is C(O) and/or K_1 is an amino acid residue of Val.

[0061] In various embodiments, when Z is a benzothiazole of Formula (V), then Li is a substituted benzyl group. Exemplary substituted benzyl groups include, but are not limited to: 2-, 3-, and 4-carboxybenzyl and the C1-C5 esters thereof.

[0062] In some embodiments, m is 1, n is 1, and P_4—P_3—P_2—P_1 of Formula (II) is a tetrapeptide selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, and mixtures thereof.

[0063] In some embodiments, the compound of Formula (II) is a tetrapeptide selected from the group consisting of:



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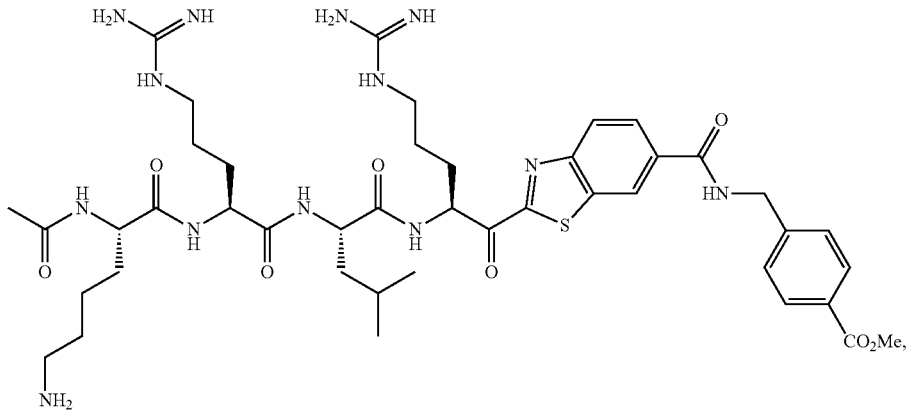

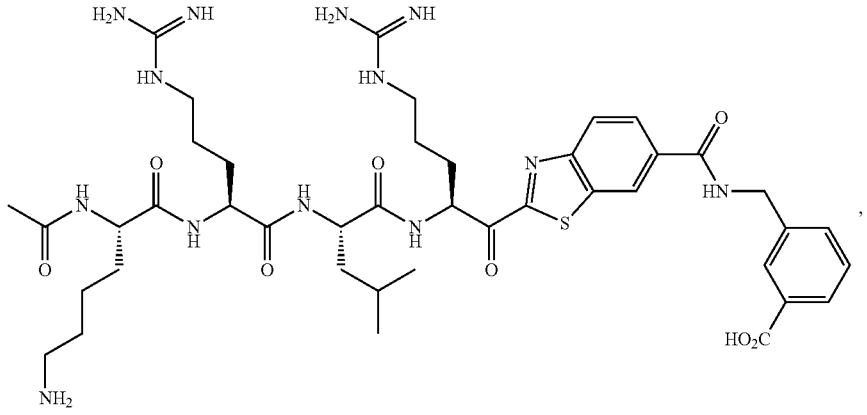

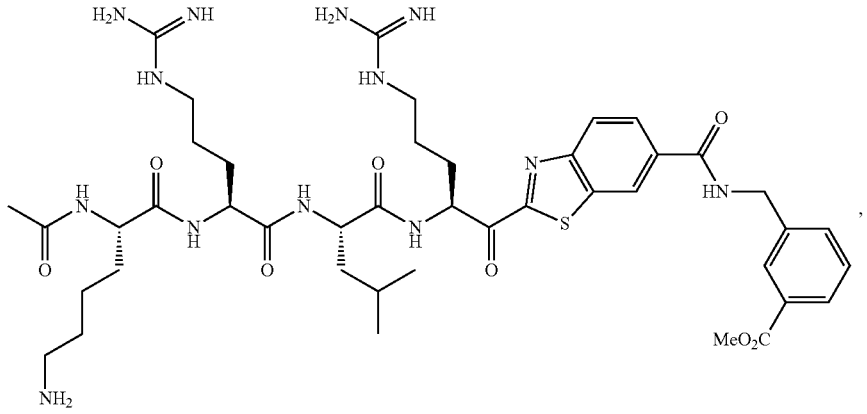

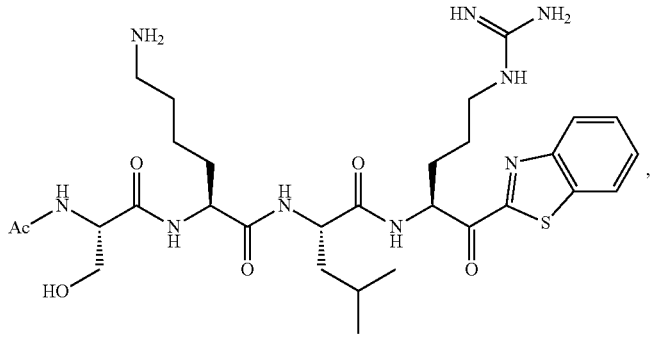

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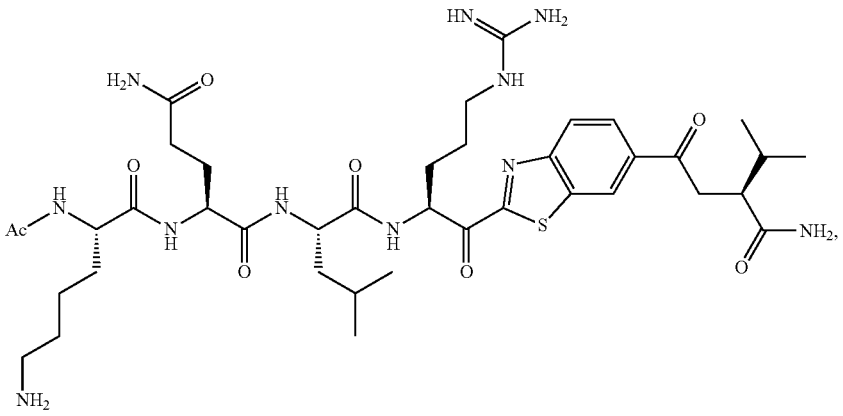

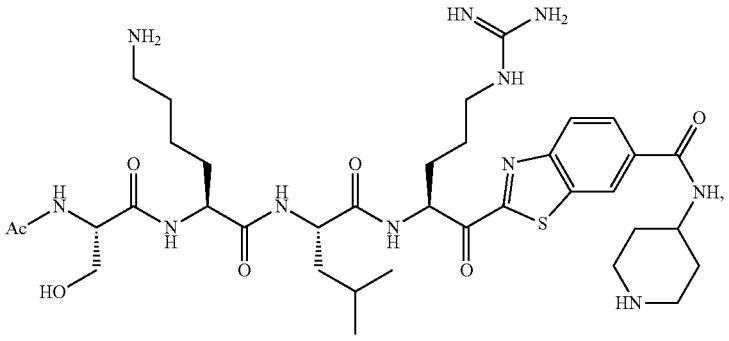

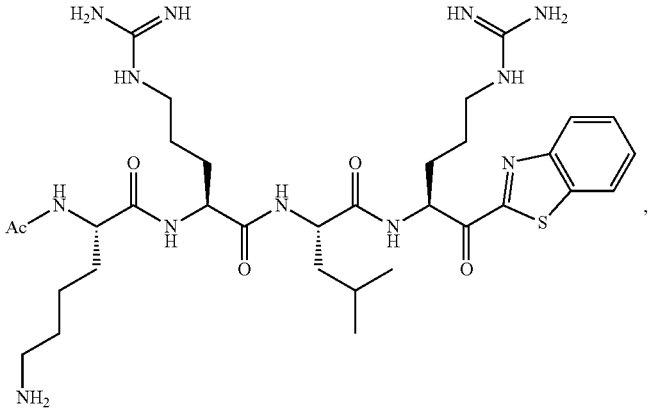

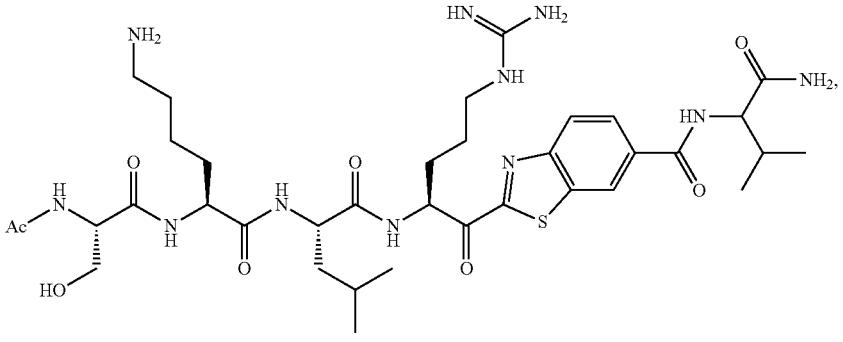

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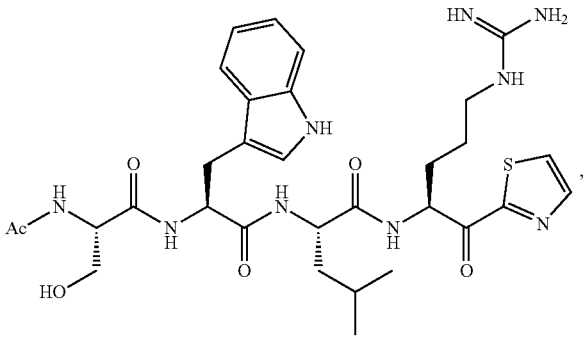

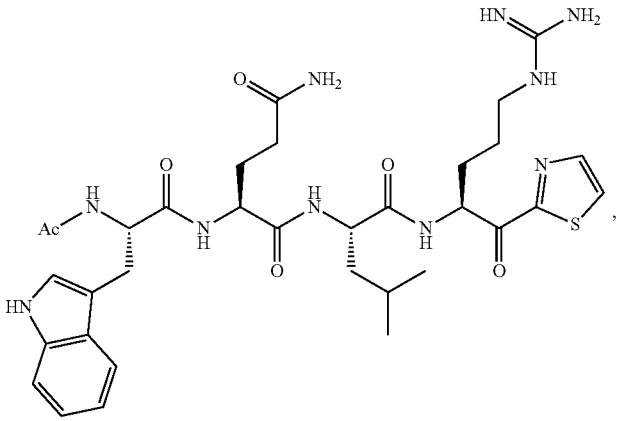

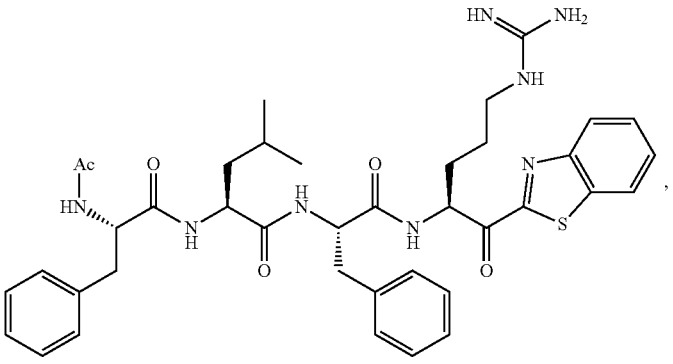

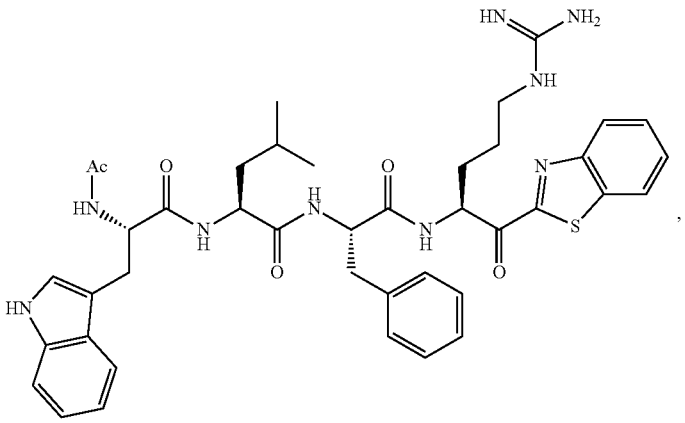

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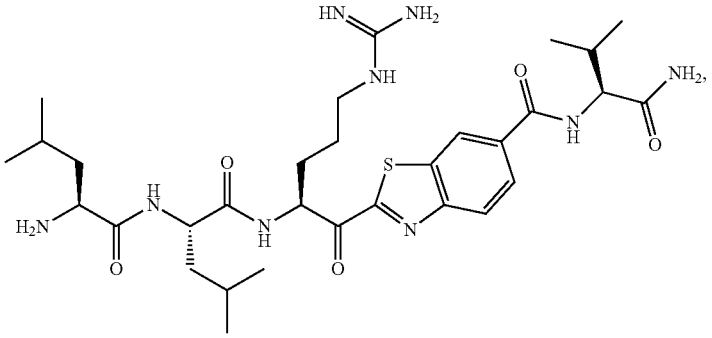

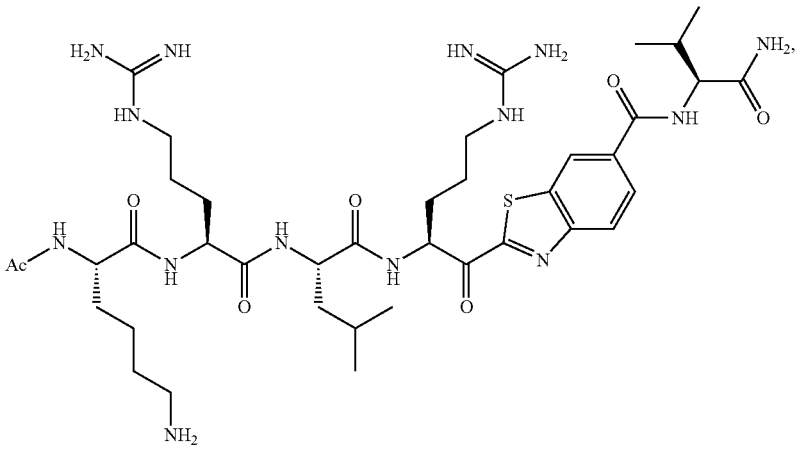

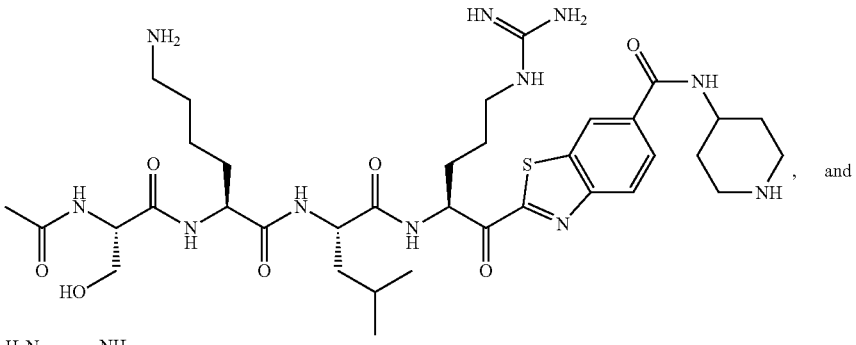

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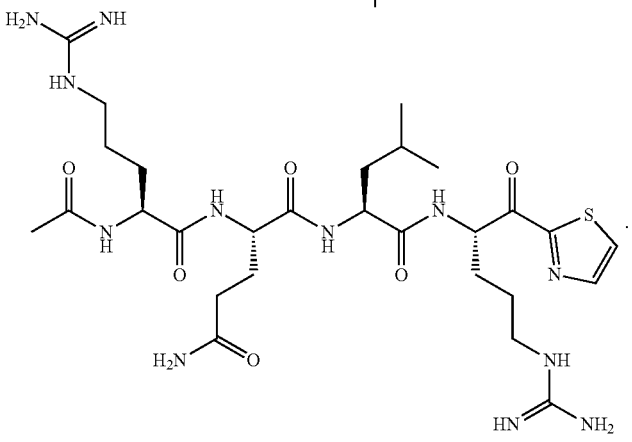
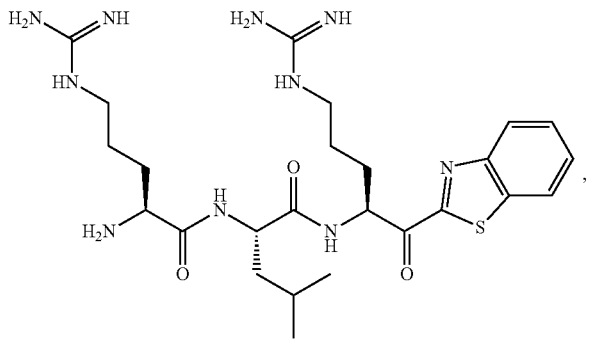
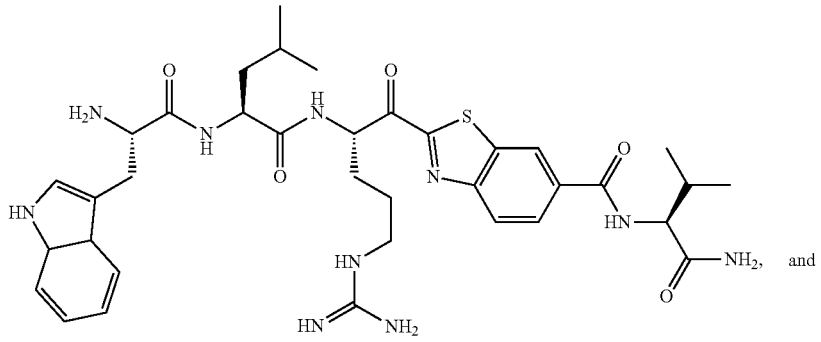


.

[0064] In some embodiments, the compound of Formula (II) is a tripeptide selected from the group consisting of.





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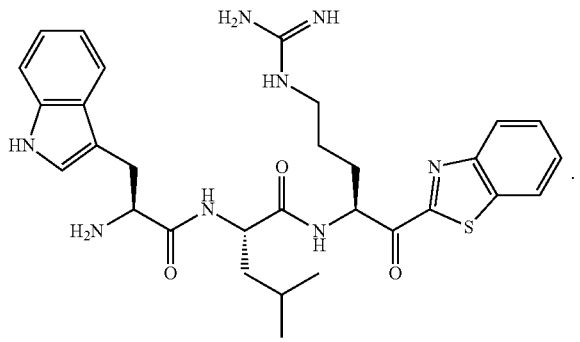
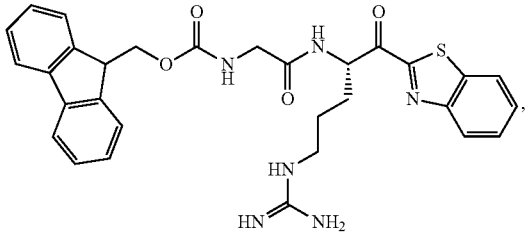


[0065] In some embodiments, the compound of Formula (II) is a dipeptide selected from the group consisting of:



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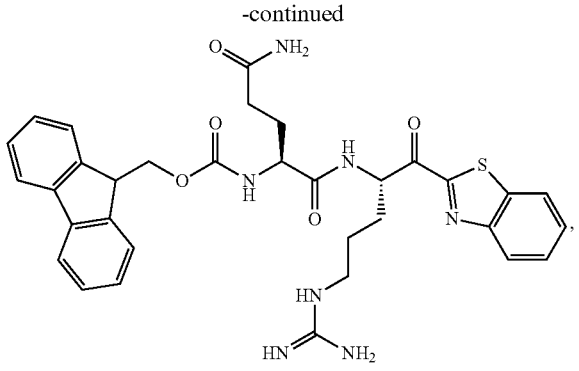
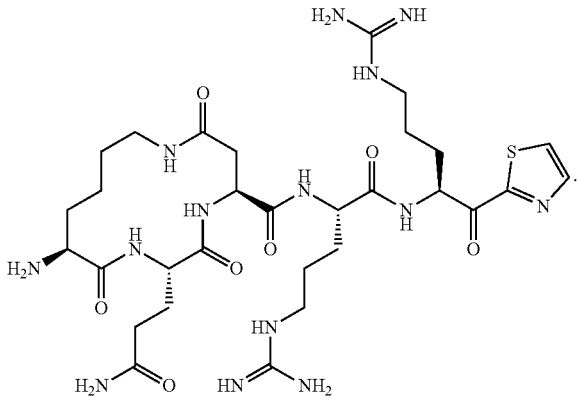


[0066] In some embodiments, the compound of Formula (II) is a cyclic peptide of the following structure:

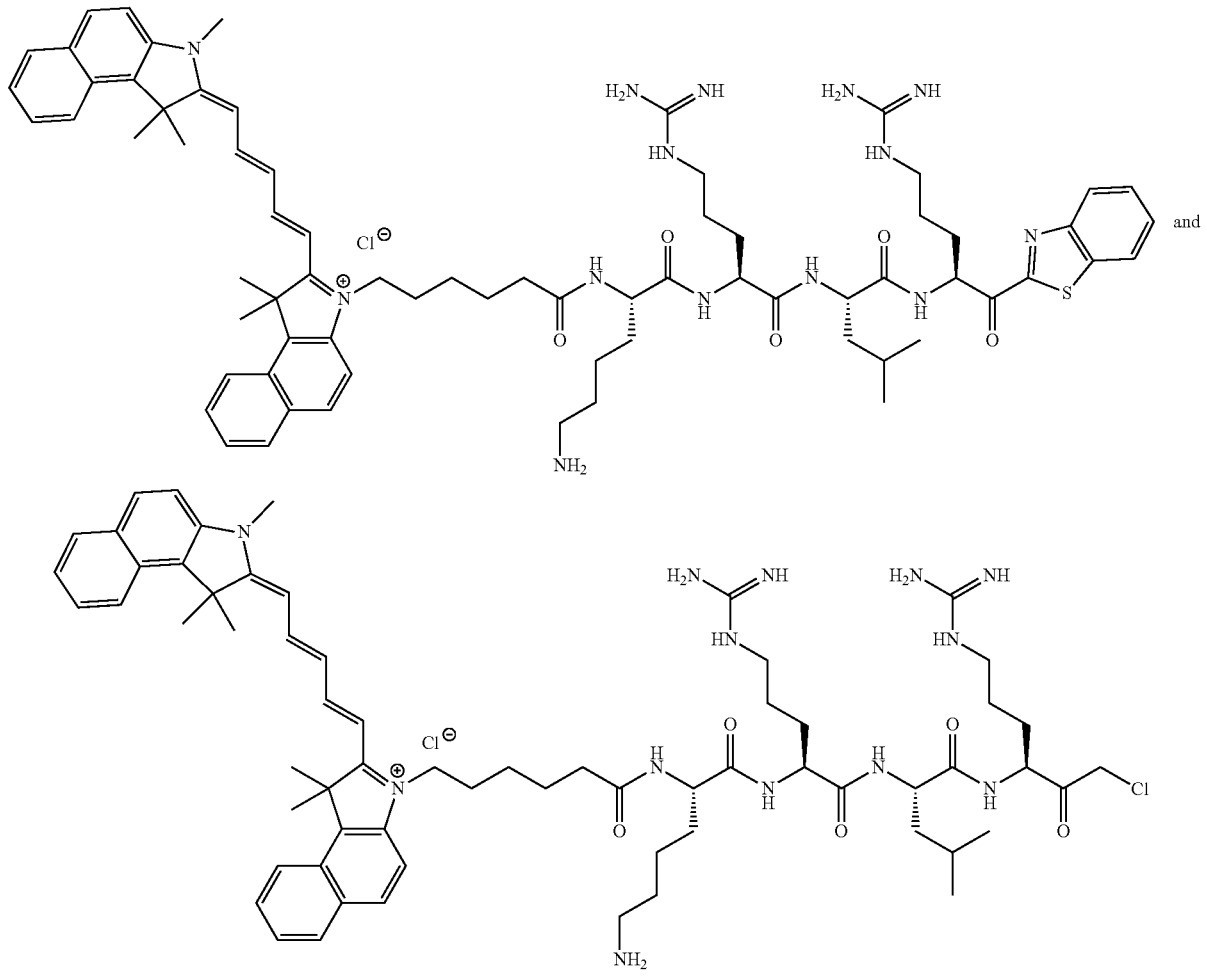


[0067] In various embodiments, Y is a fluorophore. The fluorophore can be selected from the group consisting of Cy3, Cy3.5, Cy5, Cy5.5, Cy7, and Cy7.5.

[0068] In some embodiments, the compound of Formula (II) is selected from the group consisting of.

[0069] The compounds of Formulas (I) and (II) are useful for inhibiting one or more of matriptase, hepsin, and/or HGFA. Accordingly, the present invention is also directed to a method of inhibiting matriptase, hepsin, and/or HGFA comprising administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) or (II). In certain, embodiments the compounds are highly selective for one of matriptase, hepsin, or HGFA.

[0070] As noted, matriptase, hepsin, and/or HGFA are involved in various cancerous disease conditions. Thus, the present invention is directed to various methods of using the inhibitor compounds to treat cancer in a subject (e.g., a human). One method includes inhibiting HGF/MET oncogenic signaling by administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) or (II). Another method includes inhibiting MPS/RON oncogene signaling by administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) or (II). Yet another method including reversing resistance to a kinase inhibitor by blocking HGF and/or MPS production



and/or activation by administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) or (II).

[0071] Another method includes inhibiting carcinoma progression comprising by administering to the subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) or (II).

[0072] A further method includes treating a malignancy, a pre-malignant condition, or cancer in a subject comprising administering to the subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) or (II). The cancer can be selected from the group consisting of breast, ovarian, prostate, endometrial, colon, pancreatic, head and neck, gastric, renal, brain, liver, bladder, kidney, lung, esophageal, leukemia, multiple myeloma, lymphoma, and melanoma. For example, the malignancy and the pre-malignant condition can be a condition of the breast. Also, the pre-malignant condition can be selected from the group consisting of a typical ductal hyperplasia of the breast, actinic keratosis, leukoplakia, Barrett's epithelium (columnar metaplasia) of the esophagus, ulcerative colitis, adenomatous colorectal polyps, erythroplasia of Queyrat, Bowen's disease, bowenoid papulosis, vulvar intraepthelial neoplasia, and dysplastic changes to the cervix. In various methods, the cancer can also be metastasized.

[0073] In the various methods of the present invention, the compounds of Formula (I) or (II) can also be administered in combination with an anticancer compound, radiation therapy, a compound that induces apoptosis, a surgical procedure, or any combination thereof.

[0074] In accordance with the various methods of the present invention, a pharmaceutical composition comprising an inhibitor compound of Formula (I) or (II) is administered to the subject in need thereof. The pharmaceutical composition can be administered by a routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. In various embodiments, administration is selected from the group consisting of oral, intranasal, intraperitoneal, intravenous, intramuscular, intratumoral, rectal, and transdermal.

[0075] The determination of a therapeutically effective dose for any one or more of the inhibitor compounds described herein is within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which provides the desired result. The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

[0076] Typically, the normal dosage amount of the inhibitor can vary from about 0.05 to about 100 mg per kg body weight depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. It will generally be administered so that a daily oral dose in the range, for example, from about 0.1 mg to about 75 mg, from about 0.5 mg to about 50 mg, or from about 1 mg to about 25 mg per kg body weight is given. The active ingredient can be administered in a single dose per day, or alternatively, in divided does (e.g., twice per day, three time a day, four times a day, etc.). In general, lower doses can be administered when a parenteral route is employed. Thus, for example, for intravenous administration, a dose in the range, for example, from about 0.05 mg to about 30 mg, from about 0.1 mg to about 25 mg, or from about 0.1 mg to about 20 mg per kg body weight can be used.

[0077] A pharmaceutical composition for oral administration can be formulated using pharmaceutically acceptable carriers known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the subject. In certain embodiments, the composition is formulated for parenteral administration. Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Mack Publishing Co., Easton, Pa., which is incorporated herein by reference). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

[0078] In addition to the active ingredients (e.g., the inhibitor compound), the pharmaceutical composition can contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations which can be used pharmaceutically. As used herein, the term "pharmaceutically acceptable carrier" means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material, or formulation auxiliary of any type. Some examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as lactose, glucose, and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil; and soybean oil; glycols such as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; detergents such as TWEEN 80; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; artificial cerebral spinal fluid (CSF), and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring, and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator based on the desired route of administration.

[0079] The compounds of the present invention can also be used in various nuclear imaging techniques when labeled with a suitable radionuclide. Accordingly, an imaging com-

position in accordance with the present invention comprises a radiolabeled compound of Formula (I) or (II), wherein the labeled compound comprises a radioisotope selected from the group consisting of ^{11}C, ^{13}N, ^{15}O, ^{18}F, ^{75}Br, ^{124}I, ^{125}I, and ^{131}I. Methods known in the art for radiolabeling the compounds of the present invention may be used.

[0080] Imaging methods in accordance with the present invention include a method of detecting cancer comprising:

[0081] administering to a subject a radiolabeled compound of Formula (I) or (II);

[0082] employing a nuclear imaging technique for monitoring or visualizing a distribution of the radiolabeled compound within the body or within a portion thereof; and

[0083] correlating the distribution of the radiolabeled compound to the existence of cancer.

In various embodiments, the nuclear imaging technique is positron emission tomography (PET) or photon emission computed tomography (SPECT).

[0084] Imaging methods in accordance with the present invention include a method of detecting cancer comprising:

[0085] administering to a subject a fluorescent compound of Formula (I) or (II);

[0086] employing an imaging technique for monitoring or visualizing a distribution of the fluorescent compound within the body or within a portion thereof; and

[0087] correlating the distribution of the fluorescent compound to the existence of cancer.

[0088] As used herein, the abbreviations of the naturally occurring amino acids are as follows:

Amino acid	Three letter code	One letter code
alanine	Ala	A
arginine	Arg	R
asparagine	Asn	N
aspartic acid	Asp	D
cysteine	Cys	C
glutamic acid	Glu	E
glutamine	Gln	Q
glycine	Gly	G
histidine	His	H
isoleucine	Ile	I
leucine	Leu	L
lysine	Lys	K
methionine	Met	M
phenylalanine	Phe	F
proline	Pro	P
serine	Ser	S
threonine	Thr	T
tryptophan	Trp	W
tyrosine	Tyr	Y
valine	Val	V

[0089] The naturally occurring amino acids described herein are the L-isomer unless denoted as a D-isomer.

[0090] Having described the invention in detail, it will be apparent that modifications and variations are possible without departing from the scope of the invention defined in the appended claims.

EXAMPLES

[0091] The following non-limiting examples are provided to further illustrate the present invention.

Example 1. General Synthesis, Purification, and Analytical Chemistry Procedures

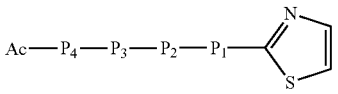
[0092] Starting materials, reagents, and solvents were purchased from commercial vendors unless otherwise noted. ^1H NMR spectra were measured on a Varian 400 MHz NMR instrument. The chemical shifts were reported ppm relative to tetramethylsilane (TMS) using residual solvent peak as the reference unless otherwise noted. The following abbreviations were used to express the multiplicities: s=singlet; d=doublet; t=triplet; q=quartet; m=multiplet; br=broad. High-performance liquid chromatography (HPLC) was carried out on GILSON GX-281 using Waters C18 5 μM, 4.6*50 mm and Waters Prep C18 5 μM, 19*150 mm reverse phase columns, eluted with a gradient system of 5:95 to 95:5 acetonitrile:water with a buffer consisting of 0.05% TFA. Purity assessment and mass spectra (MS) data were obtained using a Hewlett-Packard HPLC/MSD using electrospray ionization (ESI) for detection. All reactions were monitored by thin layer chromatography (TLC) carried out on Merck silica gel plates (0.25 mm thick, 60F254), visualized by using UV absorbance (254 nm) or dyes such as ninhydrin, $KMnO_4$, p-anisaldehyde or ceric ammonium molybdate (CAM). Silica gel chromatography was carried out on a Teledyne ISCO CombiFlash purification system using prepacked silica gel columns (12 g-330 g sizes). All compounds used for biological assays are greater than 95% purity based on NMR and HPLC by UV absorbance at 210 nm and 254 nm wavelengths.

Example 2. Synthesis of Tetrapeptide Ketothiazole Inhibitor Compounds

[0093] The tetrapeptide ketothiazoles (kt) listed in Table 2.1 were synthesized in accordance with the general schemes 2A and 2B shown below and the procedures described below.

TABLE 2.1

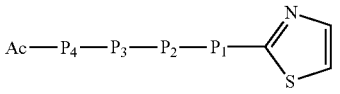
Tetrapeptide ketothiazoles.



Compound No.	Ac—P_4—P_3—P_2—P_1—kt
5	Ac-KQLR(SEQ ID NO: 1)-kt
5-1	Ac-KQLdR-kt
5a	Ac-KHLR(SEQ ID NO: 2)-kt
5a-1	Ac-KHLdR-kt
5b	Ac-WQLR(SEQ ID NO: 3)-kt
5b-1	Ac-WQLdR-kt
5c	Ac-KQFR(SEQ ID NO: 4)-kt
5c-1	Ac-KQFdR-kt
5d	Ac-KFLR(SEQ ID NO: 5)-kt
5e	Ac-RQLR(SEQ ID NO: 6)-kt
5e-1	Ac-RQLdR-kt

TABLE 2.1-continued

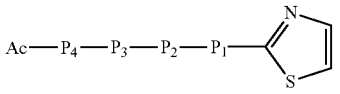
Tetrapeptide ketothiazoles.



Compound No.	Ac—P_4—P_3—P_2—P_1—kt
5f	Ac-SQLR(SEQ ID NO: 7)-kt
5g	Ac-KRLR(SEQ ID NO: 8)-kt
5h	Ac-WRLR(SEQ ID NO: 9)-kt
6	Ac-SKLR(SEQ ID NO: 10)-kt
6-1	Ac-SKLdR-kt
6a	Ac-SHLR(SEQ ID NO: 11)-kt
6a-1	Ac-SHLdR-kt
6b	Ac-WKLR(SEQ ID NO: 12)-kt
6b-1	Ac-WKLdR-kt
6c	Ac-SKFR(SEQ ID NO: 13)-kt

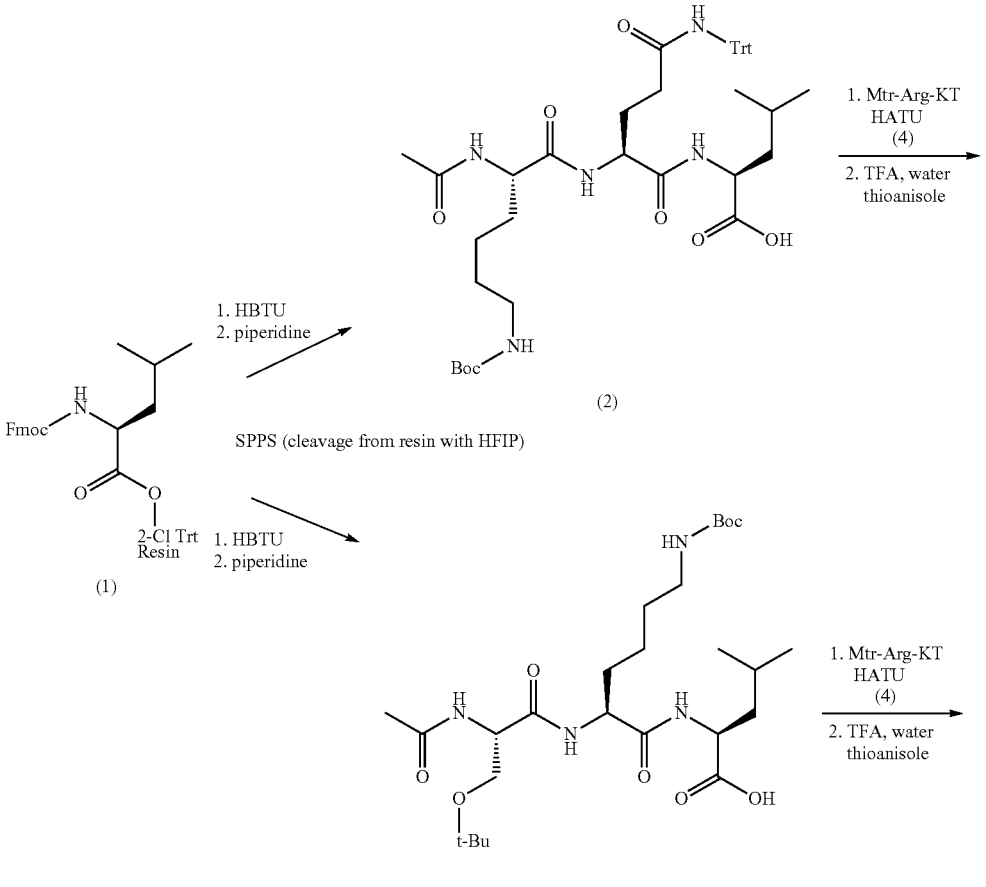
TABLE 2.1-continued

Tetrapeptide ketothiazoles.



Compound No.	Ac—P_4—P_3—P_2—P_1—kt
6c-1	Ac-SKFdR-kt
6d	Ac-NKLR(SEQ ID NO: 14)-kt
6e	Ac-SRLR(SEQ ID NO: 15)-kt
6e-1	Ac-SdRLR-kt
6e-2	Ac-SRLdR-kt
6e-3	Ac-SdRLdR-kt
6f	Ac-TKLR(SEQ ID NO: 16)-kt
6g	Ac-SWLR(SEQ ID NO: 17)-kt
6h	Ac-RKLR(SEQ ID NO: 18)-kt

Scheme 2A: Synthesis of pro-HGF and pro-MSP P4-P1 tetrapeptide ketothiazoles



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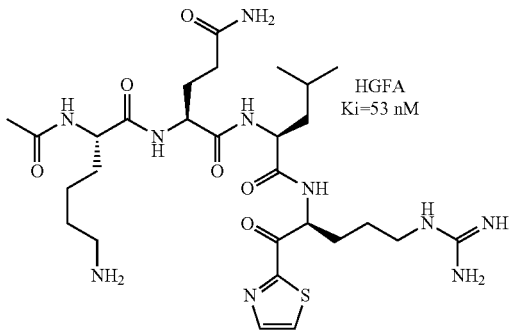
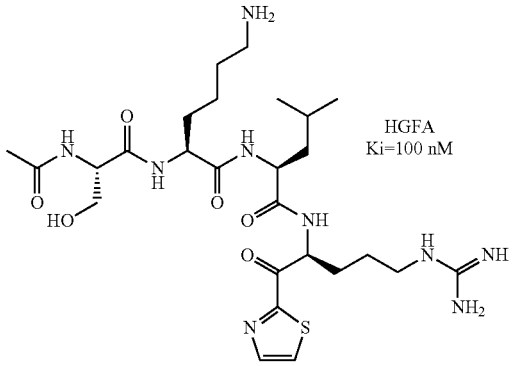
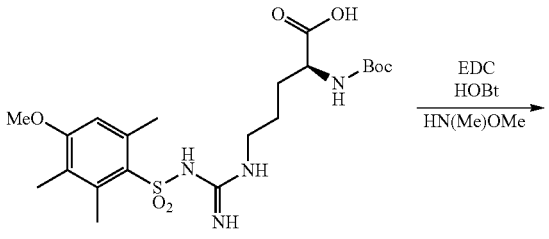


Ac-Lys-Gln-Leu-Arg-KT
pro-HGF tetrapeptide (5)



Ac-Ser-Lys-Leu-Arg-KT
pro-MSP tetrapeptide (6)

Scheme 2B. Synthesis of protected arginine ketothiazole



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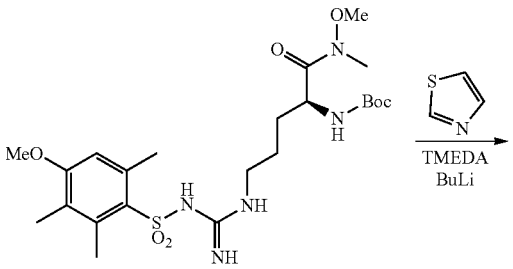

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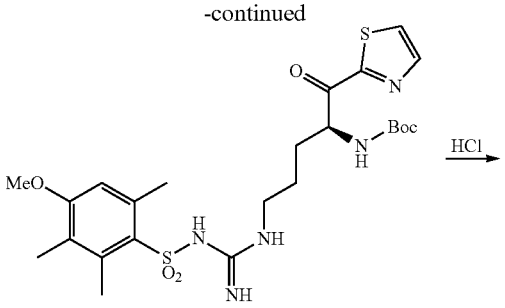
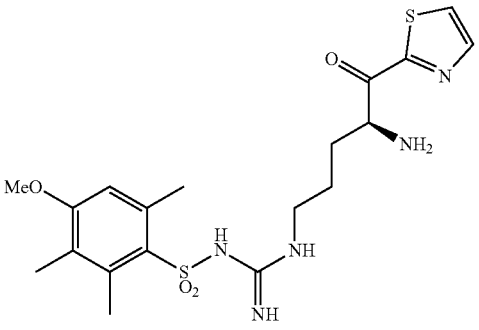


(4c)



Mtr-Arg-kt
(4)

[0094] Tripeptide synthesis: Tripeptide intermediates were synthesized on a 0.1 mmol scale using a CEM Liberty Microwave Peptide Synthesizer and Fmoc-amino acid-preloaded 2-Cl-trityl resins. Standard peptide coupling conditions were employed utilizing 5 equiv. of Fmoc-amino acid/4.5 equiv. HBTU/10 equiv. N,N-Diisopropylethylamine ($^{i}Pr_2NEt$) in DMF was added and the mixture was heated at 75° C. for 5 minutes by microwave. Piperidine/DMF (20% v/v) was employed for the deprotection of the Fmoc protecting group using the microwave for 5 minutes.

[0095] Acetyl (Ac) capping of the tripeptides: The tripeptide resin was suspended in 15 mL of 0.5 M Ac_2O/DMF and 1 M $^{i}Pr_2NEt$/DMF. The mixture was shaken at room temperature for 1 hour. The resin was filtered and washed with DMF (10 mL×4) followed by CH_2Cl_2 (10 mL×4).

[0096] Cleavage of tripeptide resin: Ac-capped tripeptide resin was suspended and shaken in 15 mL of 25% v/v HFIP/CH_2Cl_2 for 1 hour. The mixture was filtered. The filtrate was concentrated then dried in vacuo, giving rise to crude Ac-capped tripeptide product.

[0097] Ac-KQFR (SEQ ID NO: 4) ketothiazole (5c). Under nitrogen atmosphere, at 0° C. anhydrous DMF (5 mL) was added into the round bottom flask containing Ac-capped KQF tripeptide (0.081 g, 0.1 mmol) and HATU (0.042 g, 0.11 mmol). After stirring for 10 minutes, arginine ketothiazole (0.042 g, 0.1 mmol), then N,N-diisopropylethylamine (0.065 g, 0.5 mmol) were added. The mixture was stirred overnight while being warmed to room temperature naturally. The majority of DMF was removed and to the resulting residue was added 20 mL of water. The precipitate that formed was filtered and dried. To this precipitate was added 5 mL of TFA/thioanisole/water (95/2.5/2.5 (v/v/v)). The mixture was stirred at room temperature for 4 hours and then was added to 40 mL of cold ether. The precipitated crude product was collected by centrifugation, followed by carefully decanting out the ether solvent. The crude product was purified by HPLC (C18, 15*150 mm column; eluent: acetonitrile/water (0.1% TFA)) to give Ac-KQFR (SEQ ID NO: 4) ketothiazole (0.032 g) in 46% yield. At the same time the isomer KQFR (SEQ ID NO: 4)* ketothiazole (0.020 g) was also collected. ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) 6 ppm 1.35 (m, 2H) 1.45-1.77 (m, 7H) 1.78-2.04 (m, 6H) 2.06-2.29 (m, 2H) 2.92 (t, J=7.63 Hz, 2H) 3.00 (m, 2H) 3.13 (m, 2H) 4.12-4.20 (m, 1H) 4.21-4.29 (m, 1H) 4.59 (t, J=7.63 Hz, 1H) 5.30-5.45 (m, 1H) 7.17 (m, 5H) 8.04 (br. s., 1H) 8.07 (br. s., 1H). MS (ESI): found: $[M+H]^+$, 687.5.

[0098] Ac-SQLR (SEQ ID NO: 7) ketothiazole (5f) (yield: 42%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.85 (d, J=5.87 Hz, 3H) 0.90 (d, J=5.87 Hz, 3H) 1.48-1.76 (m, 5H) 1.77-1.88 (m, 1H) 1.89-2.02 (m, 1H) 2.02-2.20 (m, 5H) 2.26-2.47 (m, 2H) 3.11-3.32 (m, 2H) 3.84 (tt, J=11.44, 5.77 Hz, 2H) 4.25-4.45 (m, 3H) 5.47 (dd, J=9.39, 4.30 Hz, 1H) 8.06 (d, J=3.13 Hz, 1H) 8.11 (d, J=3.13 Hz, 1H). MS (ESI): found: $[M+H]^+$, 612.5.

[0099] Ac-SRLR (SEQ ID NO: 15) ketothiazole (6e) (yield: 26%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.85 (d, J=5.87 Hz, 3H) 0.91 (d, J=5.87 Hz, 3H) 1.52-1.77 (m, 8H) 1.79-1.89 (m, 2H) 1.99-2.18 (m, 4H) 3.09-3.34 (m, 4H) 3.83 (tt, J=11.35, 5.67 Hz, 2H) 4.29-4.45 (m, 3H) 5.46 (dd, J=9.20, 4.11 Hz, 1H) 8.06 (d, J=3.13 Hz, 1H) 8.12 (d, J=3.13 Hz, 1H). MS (ESI): found: $[M+H]^+$, 640.5.

[0100] Ac-TKLR (SEQ ID NO: 16) ketothiazole (6f) (yield: 59%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.85 (d, J=5.87 Hz, 3H) 0.91 (d, J=5.48 Hz, 3H) 1.12-1.24 (m, 3H) 1.32-1.90 (m, 12H) 1.99-2.18 (m, 4H) 2.98 (t, J=7.43 Hz, 2H) 3.15-3.30 (m, 2H) 4.07-4.19 (m, 1H) 4.24 (d, J=5.48 Hz, 1H) 4.34 (td, J=8.90, 5.67 Hz, 2H) 5.45 (dd, J=9.19, 4.50 Hz, 1H) 8.07 (d, J=3.13 Hz, 1H) 8.12 (d, J=3.13 Hz, 1H). MS (ESI): found: $[M+H]^+$, 626.5.

[0101] Ac-KFLR (SEQ ID NO: 5) ketothiazole (5d) (yield: 43%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.85 (d, J=6.26 Hz, 3H) 0.90 (d, J=6.26 Hz, 3H) 1.13-1.38 (m, 2H) 1.40-1.77 (m, 9H) 1.78-1.91 (m, 1H) 1.99 (s, 3H) 2.04-2.17 (m, 1H) 2.82-3.05 (m, 3H) 3.09-3.32 (m, 3H) 4.08-4.21 (m, 1H) 4.37 (dd, J=8.80, 6.06 Hz, 1H) 4.65 (dd, J=8.61, 6.26 Hz, 1H) 5.44 (dd, J=9.19, 4.50 Hz, 1H) 7.15-7.43 (m, 5H) 8.08 (d, J=3.13 Hz, 1H) 8.13 (d, J=3.13 Hz, 1H). MS (ESI): found: $[M+H]^+$, 672.5.

[0102] Ac-NKLR (SEQ ID NO: 14) ketothiazole (6d) (yield: 39%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.85 (d, J=5.48 Hz, 3H) 0.91 (d, J=5.87 Hz, 3H) 1.32-1.48 (m, 2H) 1.48-1.91 (m, 10H) 1.93-2.17 (m, 4H) 2.62-2.84 (m, 2H) 2.98 (t, J=7.63 Hz, 2H) 3.14-3.30 (m, 2H) 4.20-4.42 (m, 2H) 4.63 (t, J=7.04 Hz, 1H) 5.45 (dd, J=9.39, 4.30 Hz, 1H) 8.06 (d, J=3.13 Hz, 1H) 8.11 (d, J=3.13 Hz, 1H). MS (ESI): found: $[M+H]^+$, 639.5.

[0103] Ac-RQLR (SEQ ID NO: 6) ketothiazole (5e) (yield: 13%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.85 (d, J=5.87 Hz, 3H) 0.91 (d, J=5.87 Hz, 3H) 1.45-1.89 (m, 10H) 1.90-2.19 (m, 6H) 2.35 (td, J=7.43, 3.91 Hz, 2H) 3.14-3.28 (m, 4H) 4.25 (dd, J=8.02, 6.06 Hz, 1H) 4.30-4.43 (m, 2H) 5.47 (dd, J=9.39, 4.30 Hz, 1H) 8.07 (d, J=3.13 Hz, 1H) 8.12 (d, J=3.13 Hz, 1H). MS (ESI): found: $[M+H]^+$, 681.5.

[0104] Ac-WOLR(SEQ ID NO: 3) ketothiazole (5b) (yield: 13%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.81 (d, J=5.87 Hz, 3H) 0.88 (d, J=5.48 Hz, 3H) 1.37-1.69 (m, 6H) 1.70-1.91 (m, 3H) 1.91-2.10 (m, 5H) 2.98-3.35 (m, 4H) 3.92-4.06 (m, 1H) 4.11-4.23 (m, 1H) 4.48 (t, J=6.65 Hz, 1H) 5.39 (m, 1H) 7.09 (t, J=7.24 Hz, 1H) 7.14-7.26 (m, 2H) 7.43 (d, J=8.22 Hz, 1H) 7.52 (d, J=7.43 Hz, 1H) 8.00 (d, J=2.74 Hz, 1H) 8.05 (d, J=2.74 Hz, 1H). MS (ESI): found: $[M+H]^+$, 711.5.

[0105] Ac-KHLR (SEQ ID NO: 2) ketothiazole (5a) (yield: 52%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.82 (d, J=4.30 Hz, 3H) 0.87 (d, J=4.70 Hz, 3H) 1.22-1.44 (m, 2H) 1.44-1.73 (m, 9H) 1.74-1.87 (m, 1H) 1.96 (s, 3H) 2.06 (m, 1H) 2.83-2.99 (m, 2H) 3.02-3.36 (m, 4H) 4.06-4.22 (m, 1H) 4.34 (m, 1H) 4.58-4.72 (m, 1H) 5.43 (dd, J=8.61, 4.30 Hz, 1H) 7.23 (s, 1H) 8.03 (d, J=2.35 Hz, 1H) 8.08 (d, J=2.35 Hz, 1H) 8.58 (s, 1H). MS (ESI): found: $[M+H]^+$, 662.5.

[0106] Ac-SKLR (SEQ ID NO: 10) ketothiazole (6) (yield: 57%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.81 (d, J=4.70 Hz, 3H) 0.87 (d, J=4.30 Hz, 3H) 1.26-1.91 (m, 12H) 1.93-2.16 (m, 4H) 2.94 (t, J=7.43 Hz, 2H) 3.18 (m, 2H) 3.79 (t, J=6.46 Hz, 2H) 4.18-4.46 (m, 3H) 5.43 (dd, J=9.00, 3.91 Hz, 1H) 8.03 (d, J=2.74 Hz, 1H) 8.08 (d, J=2.74 Hz, 1H). MS (ESI): found: $[M+H]^+$, 612.5.

[0107] Ac-SHLR (SEQ ID NO: 11) ketothiazole (6a) (yield: 32%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.81 (d, J=5.09 Hz, 3H) 0.86 (d, J=5.09 Hz, 3H) 1.41-1.60 (m, 3H) 1.60-1.72 (m, 2H) 1.73-1.88 (m, 1H) 1.90-2.15 (m, 4H) 2.99-3.37 (m, 4H) 3.63-3.83 (m, 2H) 4.20-4.41 (m, 2H) 4.68 (dd, J=8.61, 5.48 Hz, 1H) 5.44 (dd,

J=8.61, 3.13 Hz, 1H) 7.24 (s, 1H) 8.03 (d, J=2.35 Hz, 1H) 8.08 (d, J=2.35 Hz, 1H) 8.57 (s, 1H). MS (ESI): found: $[M+H]^+$, 621.5.

[0108] Ac-SKFR (SEQ ID NO: 13) ketothiazole (6c) (yield: 46%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 1.25 (m, 2H) 1.42-1.81 (m, 7H) 1.86-2.11 (m, 4H) 2.80-3.06 (m, 4H) 3.13 (m, 2H) 3.64-3.86 (m, 2H) 4.17-4.28 (m, 1H) 4.32 (t, J=5.67 Hz, 1H) 4.56 (t, J=7.83 Hz, 1H) 5.38 (dd, J=8.61, 4.30 Hz, 1H) 7.00-7.36 (m, 5H) 8.04 (d, J=2.74 Hz, 1H) 8.07 (d, J=2.74 Hz, 1H). MS (ESI): found: $[M+H]^+$, 646.5.

[0109] Ac-WKLR (SEQ ID NO: 12) ketothiazole (6b) (yield: 38%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.86 (d, J=5.48 Hz, 3H) 0.93 (d, J=5.48 Hz, 3H) 1.09-1.20 (m, 2H) 1.39-1.71 (m, 9H) 1.73-1.88 (m, 1H) 1.98 (s, 3H) 2.01-2.14 (m, 1H) 2.80-2.94 (m, 2H) 3.10-3.27 (m, 4H) 4.04-4.13 (m, 1H) 4.15-4.24 (m, 1H) 4.45-4.59 (m, 1H) 5.42 (dd, J=9.00, 3.91 Hz, 1H) 7.08-7.17 (m, 1H) 7.18-7.28 (m, 2H) 7.47 (d, J=7.83 Hz, 1H) 7.57 (d, J=7.83 Hz, 1H) 7.93 (dd, J=13.50, 6.85 Hz, 1H) 8.05 (d, J=2.74 Hz, 1H) 8.09 (d, J=2.74 Hz, 1H). MS (ESI): found: $[M+H]^+$, 711.8.

[0110] Ac-KQLR (SEQ ID NO: 1) ketothiazole (5) (yield: 32%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.80 (d, J=5.09 Hz, 3H) 0.86 (d, J=5.09 Hz, 3H) 1.26-1.84 (m, 12H) 1.84-2.13 (m, 6H) 2.29 (m, 2H) 2.93 (t, J=7.43 Hz, 2H) 3.17 (m, 2H) 4.18 (t, J=7.04 Hz, 1H) 4.23-4.37 (m, 2H) 5.35-5.51 (m, 1H) 8.01 (d, J=2.74 Hz, 1H) 8.07 (d, J=2.74 Hz, 1H). MS (ESI): found: $[M+H]^+$, 653.5.

[0111] Ac-KRLR (SEQ ID NO: 8)-ketothiazole (5g) (yield: 44%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.77-0.91 (m, 6H) 1.26-1.45 (m, 3H) 1.46-1.58 (m, 5H) 1.58-1.70 (m, 7H) 1.70-1.85 (m, 4H) 1.97 (s, 3H) 1.99-2.11 (m, 2H) 2.93 (t, J=7.63 Hz, 2H) 3.15 (dt, J=13.79, 6.99 Hz, 4H) 4.17 (dd, J=8.22, 5.87 Hz, 1H) 4.24-4.30 (m, 1H) 4.30-4.37 (m, 1H) 5.41 (dd, J=9.20, 4.11 Hz, 1H) 8.02 (d, J=2.74 Hz, 1H) 8.07 (d, J=3.13 Hz, 1H) MS (ESI): found: $[M+H]^+$, 681.7.

[0112] Ac-WRLR (SEQ ID NO: 9)-ketothiazole (5h) (yield: 24%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.82 (d, J=5.87 Hz, 3H) 0.89 (d, J=5.87 Hz, 3H) 1.10-1.32 (m, 3H) 1.34-1.45 (m, 2H) 1.48 (d, J=6.65 Hz, 3H) 1.53-1.68 (m, 3H) 1.68-1.83 (m, 1H) 1.95 (s, 3H) 2.02 (d, J=10.56 Hz, 1H) 2.97 (t, J=5.87 Hz, 2H) 3.12 (t, J=6.85 Hz, 2H) 3.15-3.22 (m, 2H) 4.02 (dd, J=8.02, 5.67 Hz, 1H) 4.15 (t, J=7.63 Hz, 1H) 4.47 (t, J=7.04 Hz, 1H) 5.37 (dd, J=9.00, 4.30 Hz, 1H) 7.02-7.12 (m, 1H) 7.17 (t, J=7.63 Hz, 1H) 7.21 (s, 1H) 7.42 (d, J=8.22 Hz, 1H) 7.51 (d, J=7.83 Hz, 1H) 8.00 (d, J=3.13 Hz, 1H) 8.05 (d, J=3.13 Hz, 1H) MS (ESI): found: $[M+H]^+$, 739.7.

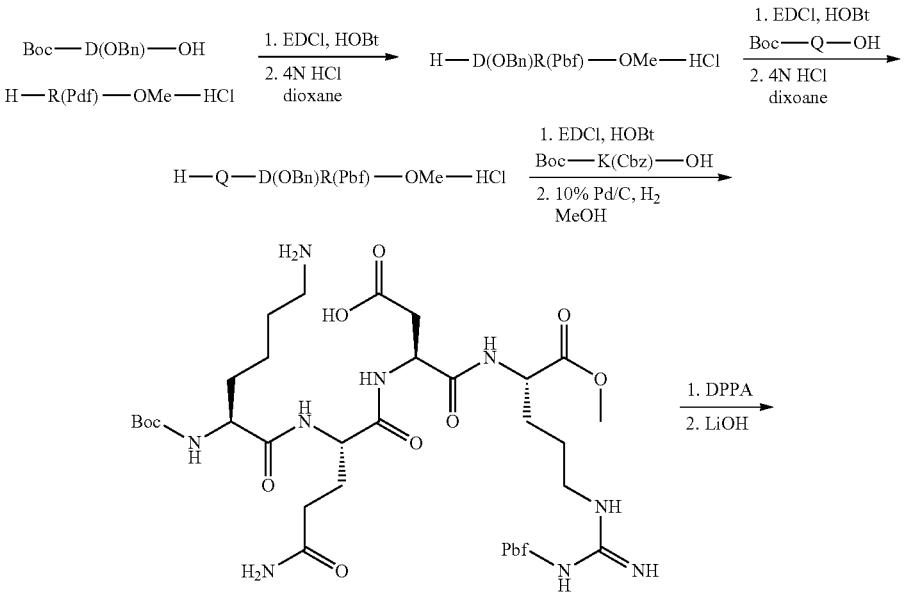
[0113] Ac-SWLR (SEQ ID NO: 17)-ketothiazole (62) (yield: 20%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.74 (d, J=6.65 Hz, 3H) 0.71 (d, J=6.26 Hz, 3H) 0.99-1.15 (m, 2H) 1.35 (t, J=7.04 Hz, 2H) 1.51-1.64 (m, 3H) 1.72 (s, 3H) 1.76 (br. s., 1H) 2.00 (dd, J=13.69, 4.70 Hz, 2H) 3.00-3.18 (m, 3H) 3.25 (d, J=5.48 Hz, 2H) 3.60-3.81 (m, 3H) 4.11-4.26 (m, 2H) 4.62 (t, J=5.87 Hz, 1H) 5.33 (dd, J=9.59, 4.11 Hz, 1H) 7.08-7.15 (m, 1H) 7.15 (s, 1H) 7.17-7.23 (m, 1H) 7.43 (d, J=8.22 Hz, 1H) 7.57 (d, J=8.22 Hz, 1H) 7.63 (br. s., 1H) 7.77 (br. s., 1H) 8.00 (d, J=3.13 Hz, 1H) 8.06 (d, J=2.74 Hz, 1H) MS (ESI): found: $[M+H]^+$, 670.5.

[0114] Ac-RKLR (SEQ ID NO: 18)-ketothiazole (6h) (yield: 31%) ^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.76-0.91 (m, 6H) 1.24-1.47 (m, 3H) 1.47-1.56 (m, 3H) 1.56-1.70 (m, 7H) 1.70-1.86 (m, 4H) 1.97 (s, 3H) 2.00-2.12 (m, 1H) 2.93 (t, J=7.63 Hz, 2H) 3.06-3.24 (m, 4H) 4.19 (t, J=7.04 Hz, 1H) 4.27 (dd, J=8.80, 6.06 Hz, 1H) 4.33 (d, J=9.00 Hz, 1H) 5.41 (dd, J=9.00, 4.70 Hz, 1H) 8.03 (d, J=3.13 Hz, 1H) 8.08 (d, J=3.13 Hz, 1H) MS (ESI): found: $[M+H]^+$, 681.7.

Example 3. Synthesis of Cyclic Peptides

[0115] The cyclic peptide is listed in Table 3.1 were synthesized in accordance with the general schemes 3A and 3B and cyclization schemes 3C, 3D, 3E, and 3F shown below and the procedures described below.

Scheme 3A



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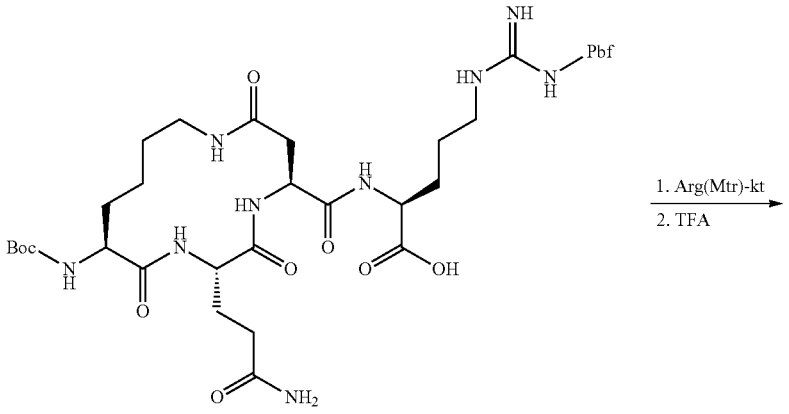
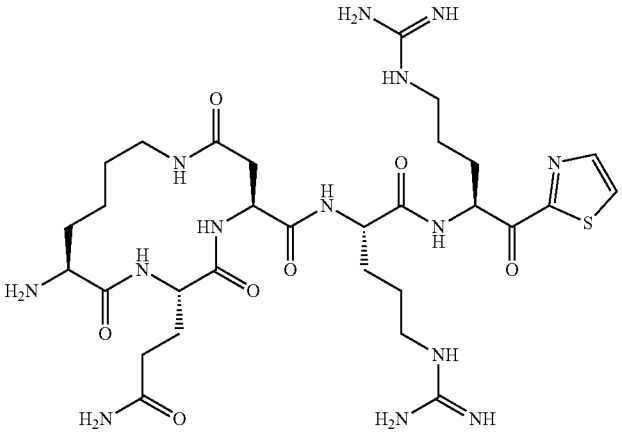
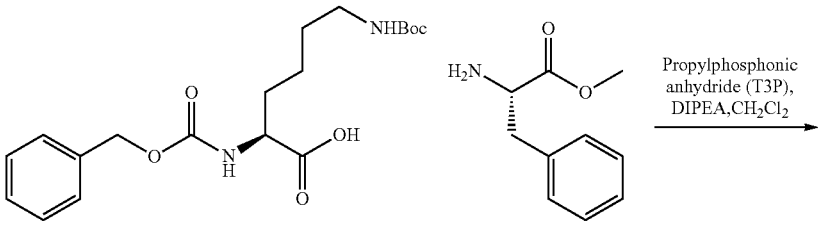




Scheme 3B



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-continued

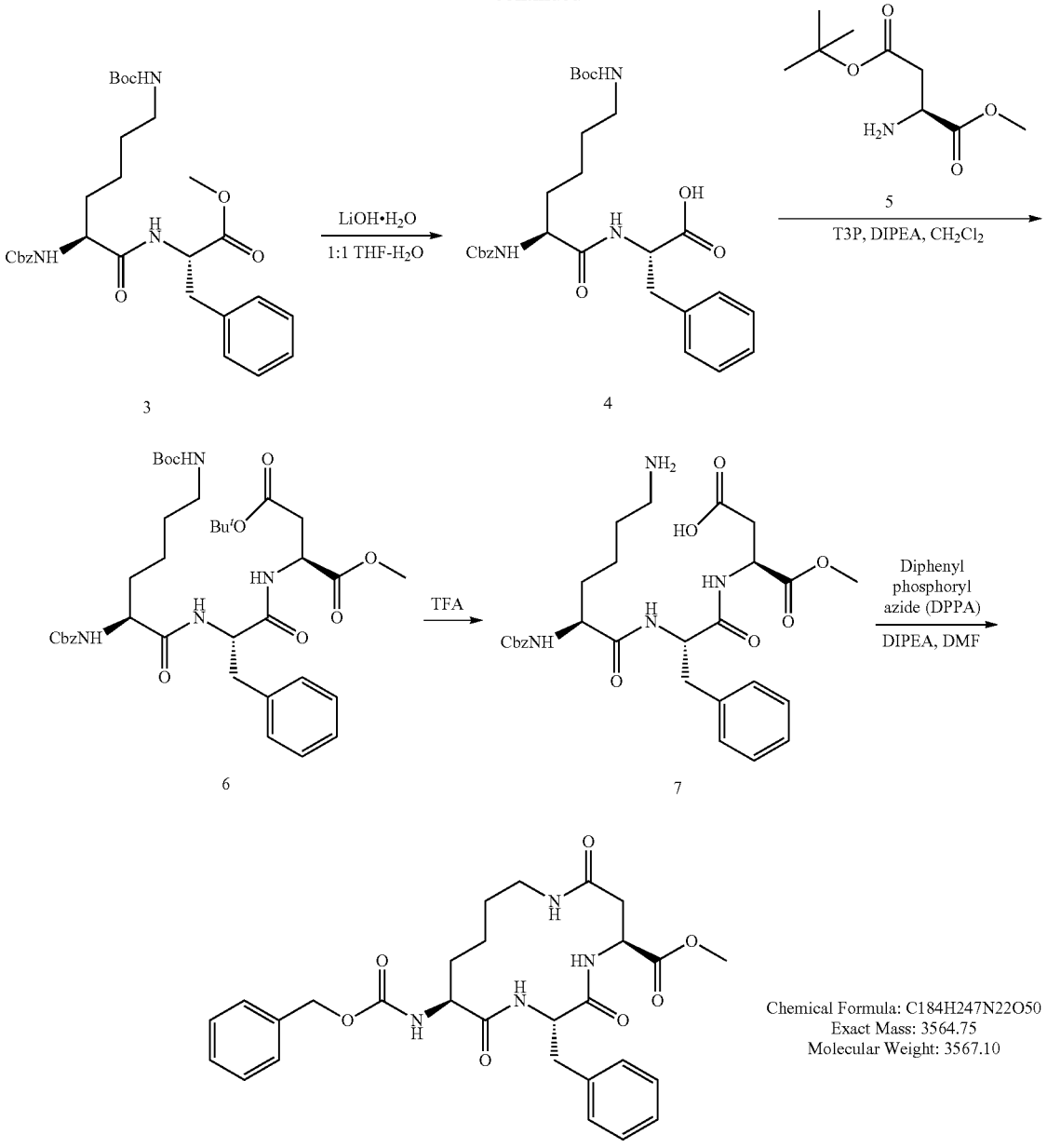


TABLE 3.1

Y—P_5—P_4—P_3—P_2—P_1—Z		
Compound No.	Y-cyclo(P_5-P_4-P_3)-P_2-P_1-Z	Cyclization
7074	H-cyclo(KQD)RR-kt	P_3-P_5

[0116] Cbz-K(Boc)F-OMe (3): Compound 1 (0.1 mmol) and compound 2 (0.1 mmol) was taken in dry CH_2Cl_2 (5 mL) and cooled to 0° C. and diisopropyletheylamine (DIPEA) (0.3 mmol) and propylphosphonic anhydride (T3P) (0.1 mmol, 50% solution in ethyl acetate) were added dropwise to the reaction mixture respectively. The reaction mixture was stirred for 1 hr at room temperature under nitrogen atmosphere. The completion of the reaction was confirmed by LCMS monitoring. On completion, the reaction mixture was diluted with 5 mL and washed with 10% citric acid, saturated Na_2CO_3 solution and brine respectively. The organic layer was dried over sodium sulfate and concentrated under reduced pressure to obtain the compound 3 as white solid (yield: 80%, Chemical Formula: $C_{29}H_{39}N_3O_7$, Exact Mass: 541.28, ESIMS: 564.6 [M+Na^+]).

[0117] Cbz-K(Boc)F-OH (4): Compound 3 (0.1 mmol) was taken in 4 mL THF-H_2O (1:1) mixture and LiOH.H_2O

(0.3 mmol) was added to the solution. The reaction mixture was stirred for 15 minutes at room temperature and the completion of the reaction was confirmed by LCMS monitoring. On completion, the THF was evaporated under reduced pressure and the water layer was cooled to 0° C. The water layer was brought to pH 6.5 by dropwise addition of cold 0.5 M HCl solution and kept on ice for 15 minutes to complete the precipitation of the product as white solid. The precipitate was isolated by filtration and dried under reduced pressure to obtain the compound 4 as white solid (Yield: 83%, Chemical Formula: $C_{28}H_{37}N_3O_7$, Exact Mass: 527.26, ESIMS: 528.3 [M+H+]).

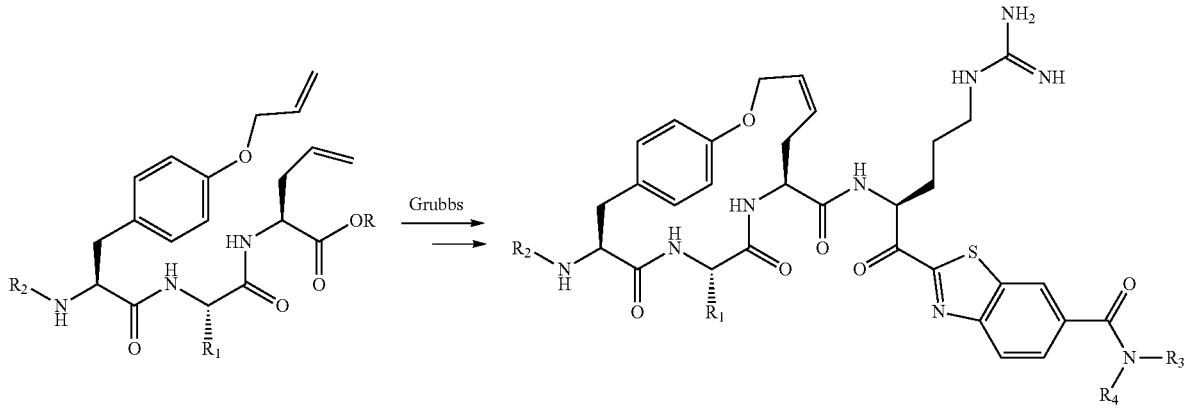
[0118] Cbz-K(Boc)FD(t-Bu)-OMe (6): Compound 4 (0.1 mmol) and compound 5 (0.1 mmol) was taken in dry CH_2Cl_2 (5 mL) and cooled to 0° C. and diisopropyletheylamine (DIPEA) (0.3 mmol) and propylphosphonic anhydride (T3P) (0.1 mmol, 50% solution in ethyl acetate) were added dropwise to the reaction mixture respectively. The reaction mixture was stirred for 1 hr at room temperature under nitrogen atmosphere. The completion of the reaction was confirmed by LCMS monitoring. On completion, the reaction mixture was diluted with 5 mL and washed with 10% citric acid, saturated Na_2CO_3 solution and brine respectively. The organic layer was dried over sodium sulfate and concentrated under reduced pressure to obtain the compound 3 as white solid (yield: 80%, Chemical Formula: $C_{37}H_{52}N_4O_{10}$ Exact Mass: 712.37, ESIMS: 713.4 [M+H+]).

[0119] Cbz-KFD-OMe (7): Trifluoroacetic acid (TFA) (3 mL) was added to the compound 6 and the reaction mixture was stirred for 1 hr. The completion of the reaction was confirmed by LCMS monitoring. On completion, the TFA was evaporated under reduced pressure by azeotroping with toluene and the dry mass was triturated with diethyl ether to obtain the pure compound 7 as white solid (Yield: 92%, Chemical Formula: $C_{28}H_{36}N_4O_8$, Exact Mass: 556.25, ESIMS: 557.3 [M+H+]).

[0120] Cbz-cyclo(KFD)-OH (PK-1-71): Compound 7 (0.09 mmol) was taken in 25 mL DMF and DIPEA (0.27 mmol) and diphenyl phosphoryl azide (0.1 mmol) was added to the solution. The reaction mixture was stirred for 12 hr at room temperature under nitrogen atmosphere and the completion of the reaction was confirmed by LCMS monitoring. The DMF was evaporated under reduced pressure and the crude product was purified by normal phase column chromatography using silica gel column and 5% MeOH in dichloromethane as eluent to obtain the product PK-1-71 as white solid (yield: 68%, Chemical Formula: $C_{28}H_{34}N_4O_7$, Exact Mass: 538.24, ESIMS: 539.4 [M+H+],) ^{1}H NMR (400 MHz, METHANOL-d4) d 8.03 (s, 1H), 7.97 (s, 1H), 7.42-7.49 (m, 2H), 7.29-7.35 (m, 6H), 7.17-7.23 (m, J=7.80 Hz, 5H), 7.10-7.16 (m, 2H), 3.69 (td, J=6.65, 13.30 Hz, 1H), 3.19 (q, J=7.43 Hz, 1H), 2.98 (s, 1H), 2.85 (s, 1H), 2.74 (s, 1H), 1.36 (s, 3H), 1.34 (t, J=3.33 Hz, 8H).

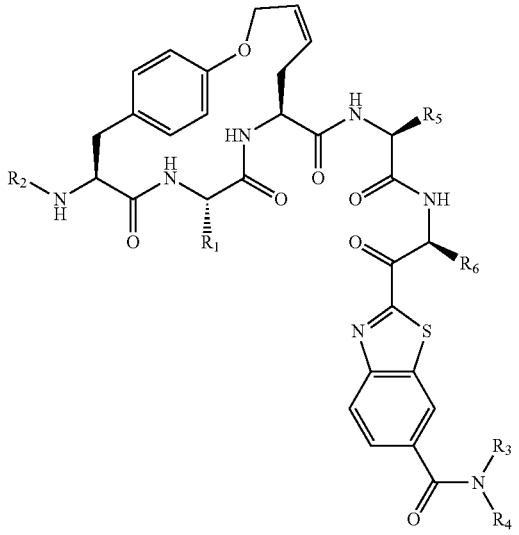
[0121] ((7S,10S,13S,Z)-13-acetamido-10-(3-amino-3-oxopropyl)-9,12-dioxo-2-oxa-8,11-diaza-1(1,4)-benzenacyclotetradecaphan-4-ene-7-carbonyl)-L-arginine (CJ-1-55). methyl N2-((S)-2-((S)-2-((S)-2-acetamido-3-(4-(allyloxy)phenyl)propanamido)-5-amino-5-oxopentanamido)pent-4-enoyl)-Nw-((2,2,4,5,7-pentamethyl-2,3-dihydrobenzofuran-6-yl)sulfonyl)-L-argininate [calculated for Chemical Formula: $C_{44}H_{62}N_8O_{11}S$, Exact Mass: 910.43, MS(ESI): found: $[M+H]^+$, 911.4, (0.18 g, 0.2 mmol)] was dissolved in DCM (125 mL). Grubbs second generation catalyst (50 mg, 0.06 mmol) was added and then the stirred reaction mixture was heated to reflux. The reaction as filtered and purified by silica gel chromatography (17 mg, 0.019 mmol). The product was dissolved in water (1 mL) and THF (1 mL), then LiOH (2 mg, 0.084 mmol) was added, and the reaction stirred for 1 hour. The THF was removed and 2 drops of 3N HCl were added to precipitate the Pbf-protected acid. The solid was dissolved in 3 mL of TFA/water/thioanisole (95:2.5:2.5) and stirred for 3 hours. The solvent was removed under reduced pressure and the residue was purified by C18 reverse phase HPLC (5-95% Acetonitrile/water/0.05% TFA). After lyopholization of pure fractions the title compound (4.4 mg) was obtained as the TFA salt. Calculated for Chemical Formula: $C_{28}H_{40}N_8O_8$, Exact Mass: 616.30, MS(ESI): found: $[M+H]^+$ 617.3.

Scheme 3C

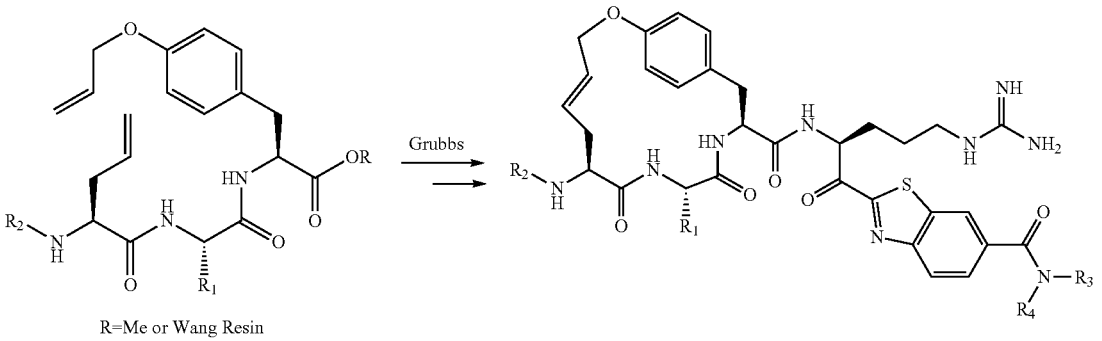


cyclo P2-P4

-continued



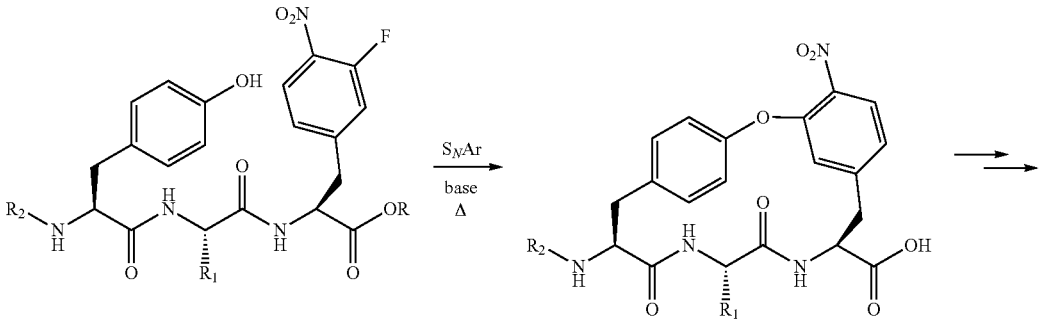
cyclo P3-P5



R=Me or Wang Resin

cyclo P2-P4

Scheme 3D



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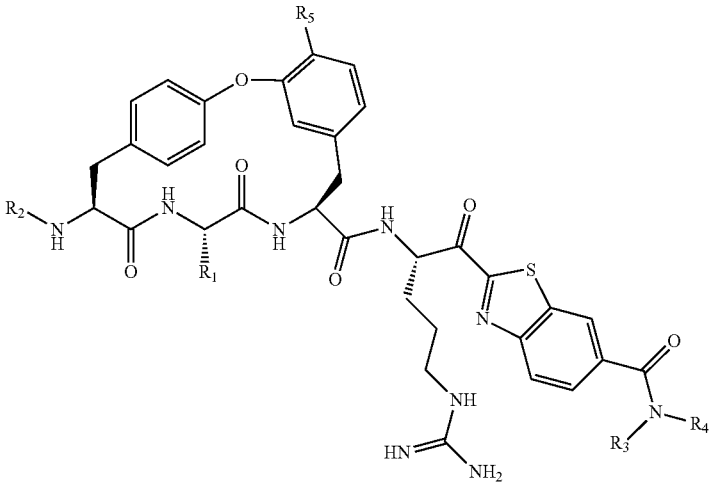
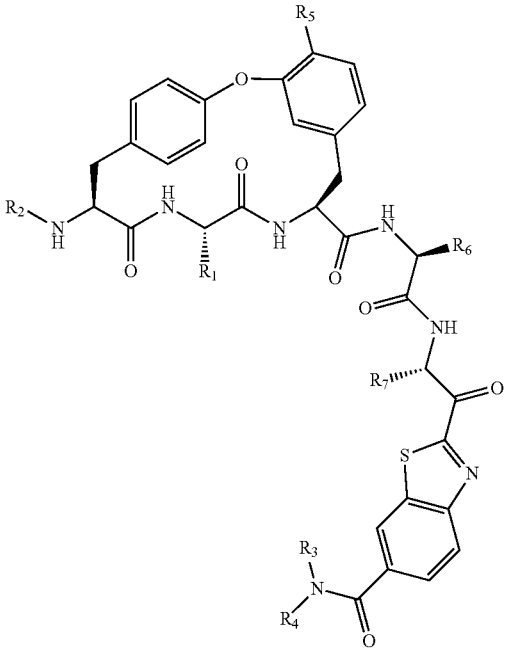
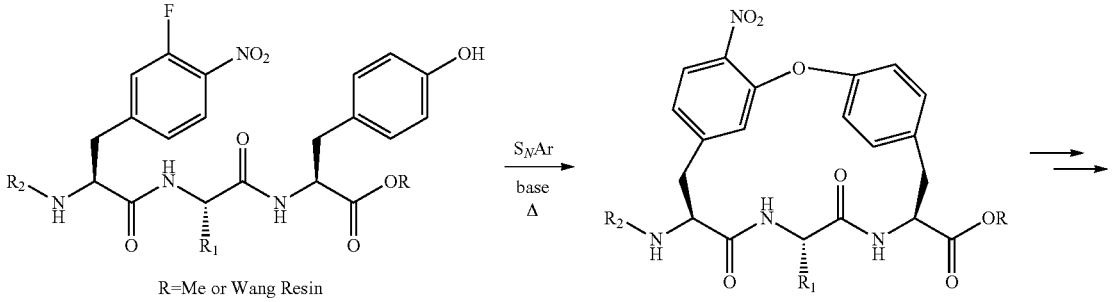


cyclo P2-P4

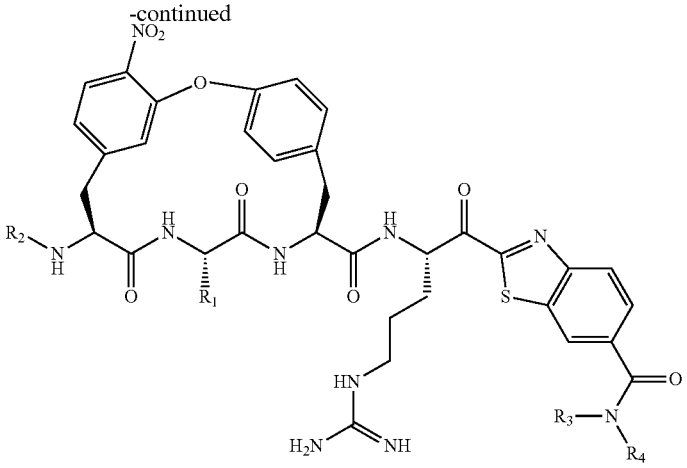


cyclo P3-P5



R=Me or Wang Resin

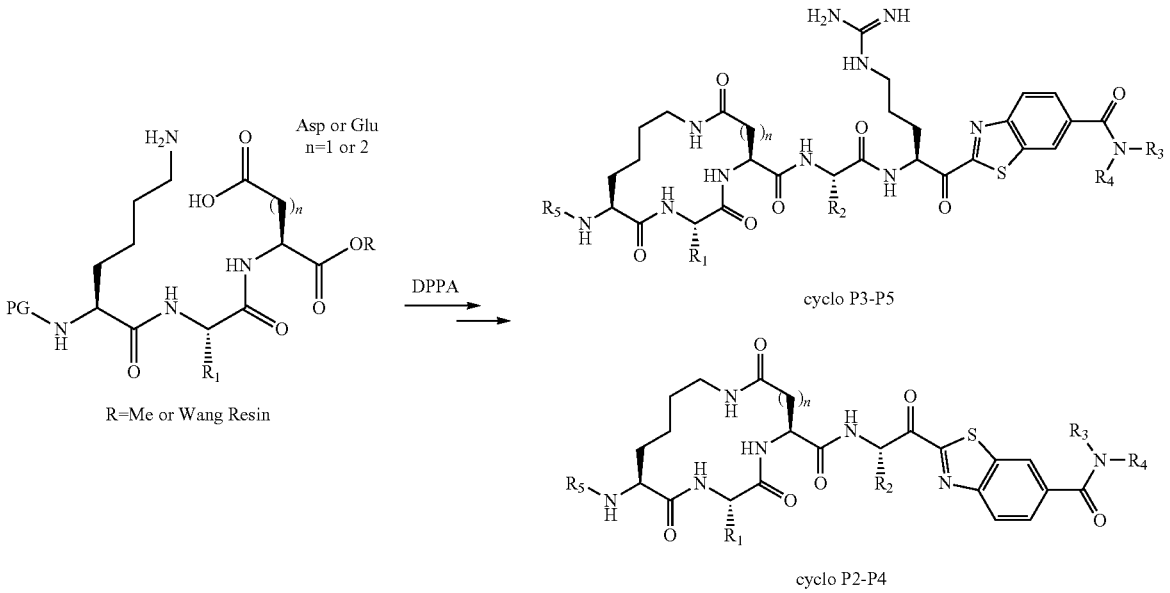
-continued



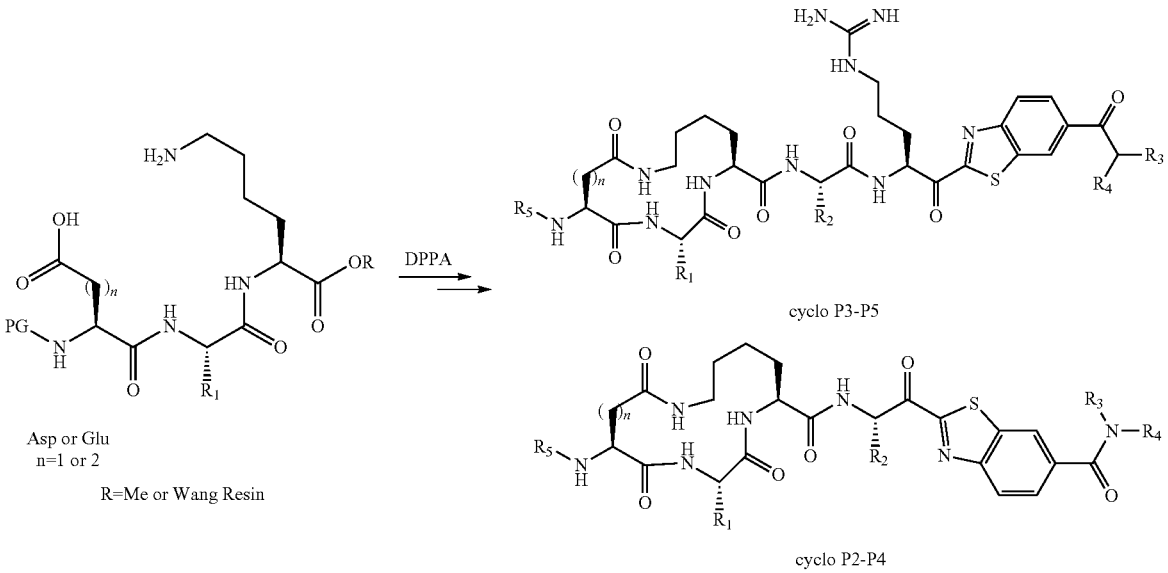
cyclo P2-P4

[0122] (2S,5S,13S)-13-amino-2-(3-amino-3-oxopropyl)-N—((S)-5-guanidino-1-(((S)-5-guanidino-1-oxo-1-(thiazol-2-yl)pentan-2-yl)amino)-1-oxopentan-2-yl)-3,7,14-trioxo-1,4,8-triazacyclotetradecane-5-carboxamide (7074). A solution of (6S,9S,12S)-9-(3-amino-3-oxopropyl)-6-(4-aminobutyl)-12-(((S)-1-methoxy-1-oxo-5-(3-((2,2,4,5,7-pentamethyl-2,3-dihydrobenzofuran-6-yl)sulfonyl)guanidino)pentan-2-yl)carbamoyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatetradecan-14-oic acid [calculated for Chemical Formula: $C_{40}H_{65}N_9O_{13}S$, Exact Mass: 911.44, MS(ESI): found: $[M+H]^+$, 912.4; (0.3 g, 0.33 mmol)] in DMF (10 mL) was slowly added to a stirred solution of DPPA (0.66 mmol, 0.15 mL) and DIPEA (0.66 mmol, 0.12 mL) in DMF (140 mL). The solvent was concentrated under reduced pressure and the residue purified by silica gel chromatography to give 100 mg of the fully protected cycloamide. This product (56 mg, 0.063 mmol) was dissolved in water (2 mL) and THF (2 mL) and then 2 mL of MeOH. LiOH (5 mg, 0.21 mmol) was added and the reaction stirred for 1 hour. 2 drops of 3N HCl was added and the reaction was then concentrated in vacuo to give 52 mg of Boc/Pbf-protected acid. This product (52 mg, 0.059 mmol) was dissolved in 5 mL of DMF, placed under a nitrogen atmosphere and cooled to with an ice-bath. To this stirred solution was added HATU (27 mg, 0.071 mmol), DIPEA (0.36 mmol, 0.063 mL), and then a solution of H-Arg(Mtr)-kt-HCl salt (29 mg, 0.06 mmol) in DMF. The reaction was allowed to come to room temperature and then stirred overnight. The reaction was added to water and the precipitate filtered to give the product as a mixture of two peaks by LCMS (51 mg crude). The solid was dissolved in 5 mL of TFA/water/thioanisole (95:2.5:2.5) and stirred for 6 hours. The solvent was removed under reduced pressure and the residue was purified by C18 reverse phase HPLC (5-95% Acetonitrile/water/0.05% TFA). After lyopholization of fractions, the title compound (12 mg) was obtained as a 3:1 ratio of D and L diastereomers (mixture of R and S isomers of the alpha keto carbon). Calculated for Chemical Formula: $C_{30}H_{50}N_{14}O_7S$, Exact Mass: 750.37, MS(ESI): found: $[M+H]^+$, 751.4.

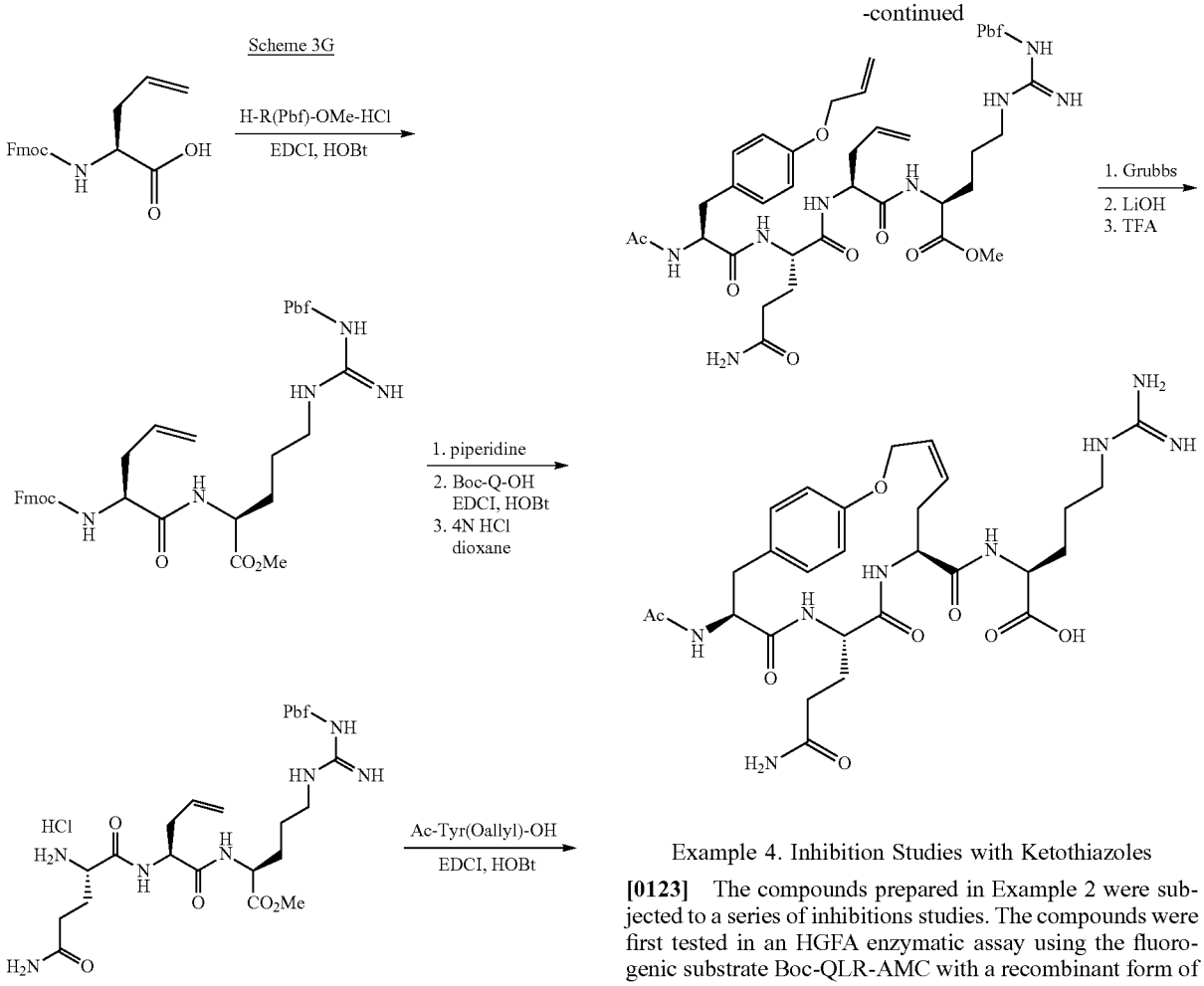
Scheme 3E



Scheme 3F



Scheme 3G



Example 4. Inhibition Studies with Ketothiazoles

[0123] The compounds prepared in Example 2 were subjected to a series of inhibitions studies. The compounds were first tested in an HGFA enzymatic assay using the fluorogenic substrate Boc-QLR-AMC with a recombinant form of the HGFA serine protease domain.

Synthesis of Boc-QLR-AMC Fluorogenic Substrate

[0124] Boc-R(NO_2)-AMC. Under nitrogen atmosphere, pyridine (60 mL) was added into the round bottom flask containing Boc-R(NO_2)—OH (4.653 g, 14.6 mmol) and 7-amino-4-methylcoumarin (3.829 g, 21.9 mmol). Diisopropylcarbodiimide (2.023 g, 16.0 mmol) was added and the mixture was stirred overnight. The mixture was filtered. The filtrate was concentrated then dried in vacuo. The resultant residue was purified by silica gel chromatography with dichloromethane/methanol combinations as eluent giving rise to Boc-R(NO_2)-AMC (2.964 g) in 43% yield. MS (ESI): found $[M+H]^+$, 477.4.

[0125] HCl.H_2N—R(NO_2)-AMC. 4 N HCl in dioxane (25 mL) was added into the round bottom flask containing Boc-R(NO_2)-AMC (2.964 g, 6.2 mmol) and the mixture stirred for 2 hours. The dioxane was removed in vacuo and to the resultant residue methanol was added then concentrated in vacuo three times, giving rise to the title compound in quantitative yield. MS (ESI): found $[M+H]^+$, 377.3.

[0126] Boc-QL-OH. Under nitrogen atmosphere, anhydrous DMF (10 mL) was added into the round bottom flask containing Boc-Q-OH (0.500 g, 2.0 mmol), H-L-OMe-HCl (0.406 g, 2.2 mmol), EDCI.HCl (0.467 g, 2.4 mmol), and HOBt (0.466 g, 3.1 mmol). N,N-diisopropylethylamine (0.787 g, 6.1 mmol) was added and the mixture was stirred overnight.

[0127] The majority of DMF was removed in vacuo and to the resulting residue was added 20 mL of water. The precipitate was isolated by filtration then purified by silica gel chromatography with dichloromethane/methanol combinations as eluent to give Boc-QL-OMe (0.711 g) in 95% yield. MS (ESI): found $[M+Na]^+$, 396.4. Methanol/water (1:1 v/v, 10 mL) was added into the round bottom flask containing the Boc-QL-OMe (0.711 g, mmol) and LiOH (0.068 g, 2.8 mmol). The reaction was stirred overnight. The mixture was concentrated in vacuo and to the resulting residue was added 30 mL of water. 0.5 M HCl was added dropwise until pH=4.5 was reached, then the mixture was extracted three times with ethyl acetate. The ethyl acetate layers were collected, dried with Na_2SO_4, then concentrated in vacuo to give rise to Boc-QL-OH (0.603 g) in 49% yield. MS (ESI): found $[M+Na]^+$, 373.4.

[0128] Boc-QLR(NO_2)-AMC. Under nitrogen atmosphere, anhydrous DMF (10 mL) was added into the round bottom flask containing Boc-QL-OH (0.603 g, 1.7 mmol), HCl.H_2N—R(NO_2)-AMC (0.406 g, 2.2 mmol), EDCI.HCl (0.322 g, 1.7 mmol), and HOBt (0.257 g, 1.7 mmol). N,N-diisopropylethylamine (0.904 g, 7.0 mmol) was added and the mixture was stirred overnight. The majority of DMF was removed in vacuo and to the resulting residue was added 20 mL of water. The mixture was extracted three times with ethyl acetate. The ethyl acetate layers were collected, dried with Na_2SO_4, then concentrated in vacuo. The resultant residue was purified by silica gel chromatography with dichloromethane/methanol combinations as eluent giving rise to Boc-QLR(NO_2)-AMC (0.250 g) in 20% yield. MS (ESI): found $[M+H]^+$, 718.5.

[0129] Boc-QLR-AMC. Into the solution of Boc-QLR (NO_2)-AMC (0.250 g, 0.35 mmol) in MeOH (15 mL) was added Pd/C (10%) (0.111 g) followed by several drops of acetic acid. The mixture was stirred under hydrogen atmosphere for 21 hours. Additional Pd/C (10%) (0.184 g) was added with a few drops of acetic acid. The mixture was stirred for 24 hours, then filtered. The filtrate was concentrated. ⅕ of the resulting residue was purified by HPLC (C18, 15*150 mm column; eluent: acetonitrile/water (0.05% TFA)) to give the title compound (0.037 g) in 78% yield. ^1H NMR (400 MHz, METHANOL-d_4) δ ppm 0.92 (d, J=6.30 Hz, 3H) 0.98 (d, J=6.26 Hz, 3H) 1.45 (s, 9H) 1.54-2.14 (m, 9H) 2.25-2.41 (m, 2H) 2.49 (s, 3H) 3.17-3.29 (m, 2H) 4.00-4.14 (m, 1H) 4.34-4.47 (m, 1H) 4.49-4.61 (m, 1H) 6.28 (s, 1H) 7.46-7.60 (m, 1H) 7.71-7.80 (m, 1H) 7.80-7.89 (m, 1H). MS (ESI): found: $[M+H]^+$, 673.6.

[0130] Expression and purification of N-terminal His-tag HGFA serine protease domain: Using primers identified as SEQ ID NO 1, SEQ ID NO 2, and standard PCR protocols, the nucleotide sequence encoding amino acids 373-655 of HGFA was synthesized. This PCR product was cloned into the SfoI-HindIII sites of a modified pFastBac HT baculovirus expression vector (Addgene, Cambridge, Mass.). This vector contains a six amino His tag followed by a seven amino spacer and a seven amino acid TEV cleavage site placed immediately downstream of the Honey Bee melittin signal peptide. Using a modified Bac to Bac Expression System (Life Technologies, Carlsbad, Calif.), recombinant HGFA bacmids were obtained by transforming DH10Bac *Escherichia coli* cells. To obtain HGFA containing baculovirus, purified bacmids were transfected into Sf9 insect cells. After 5 days in culture at 27° C., media was harvested from transfected Sf9 cells. This media was used to prepare baculovirus infected insect cells (BIICs). These BIIICs were used to infect High 5 insect cells. Four days post infection, media was harvested and recombinant protein prepared as follows. Media was chilled to 4° C. and spun at 4000×g for 20 minutes (all subsequent steps were performed at 4° C. unless noted). Clarified media was passed first through a Whatman GF/B 1 um (#1821-047, GE Healthcare Life Sciences, Piscataway, N.J.) and then a 0.22 um PES membrane (#99955, TPP Techno Plastic Products AG, Trasadingen, Switzerland) and then concentrated using a Pall Centramate tangential flow system and Centramate T-series Cassette (#OS010T12, Pall Corporation, Port Washington, N.Y.). Concentrated media was then buffer exchanged in two steps, five volumes of 50 mM Na-phosphate, 500 mM NaCl, pH 6.2, followed by five volumes of 50 mM Na-phosphate, 500 mM NaCl, pH 7.5. The concentrated and buffered exchanged insect cell media was again filtered as above and made 25 mM imidazole (#I202, Sigma-Aldrich, St. Louis, Mo.) and was mixed with nickel agarose beads (#H-321-25, Gold Biotechnology, Inc., St. Louis Mo.). After mixing this slurry for 12 hours, nickel agarose beads were allowed to settle by gravity and then loaded into a column. Beads were washed with buffer (25 mM Na-phosphate, 500 mM NaCl, 25 mM imidazole, pH 8) and the bound protein eluted using (25 mM Na-phosphate, 500 mM NaCl, 250 mM imidazole, pH 8). Using a Amicon Ultra-4 Centrifugal filters (#UFC801008, Merck Millipore, Ltd., Tullagreen, Ireland), peak protein fractions were concentrated and run over a Superdex-200 10/300 GL column (GE Healthcare Life Sciences, Piscataway, N.J.) in 10 mM Tris, 200 mM NaCl, 0.2 mM EDTA, pH 8. HGFA containing fractions were pooled, concentrated, made 50% glycerol, and stored at minus 20° C. Protein was quantitated using a modified Lowry protein assay (#500-0006, Bio-Rad Laboratories, Hercules, Calif.).

[0131] Chromogenic Kinetic Enzyme InhibitorAssays of HGFA: The inhibitors (0-50 μM final concentration in reaction) were diluted in DMSO (2% DMSO final concentration in reaction) and then mixed with recombinant HGFA

(12.5 nM final concentration in reaction) in TNC buffer (25 mM Tris, 150 mM NaCl, 5 mM $CaCl_2$), 0.01% TRITON X-100, pH 8). After incubating for thirty minutes at 25° C., chromogenic substrate, Pefachrome FVIIa, (#093-01, Enzyme Research Laboratories, South Bend, Ind.)) was added to a final concentration of 250 μM in a final reaction volume of 40 microliters. Changes in absorbance at 405 nm were measured over time in a Biotek Synergy 2 plate reader (Winnoski, Vt.). Using Gen 5 2.00 software program (Biotek, Winnoski, Vt.), a four parameter curve fit was used to determine the inhibitor IC_{50}s from a plot of the mean reaction velocity versus the inhibitor concentration. The IC_{50} values represent the average of three separate experimental determinations.

[0132] Chromogenic Kinetic Enzyme Assays of Thrombin and Factor Xa: Inhibitors (11-pt serial dilutions, 0-20 μM final concentration) were serially diluted in DMSO (2% DMSO final concentration) and then mixed with recombinant thrombin (0.15 nM final concentration) or Factor Xa (0.35 nM final concentration) in TNC buffer (25 mM Tris, 150 mM NaCl, 5 mM $CaCl_2$), 0.01% TRITON X-100, pH 8) using clear 384 well plates. After incubating for 30 minutes at 25° C., the chromogenic substrate (S2238; D-Phe-Pip-Arg-pNA) for thrombin (K_m=14.5 μM) or (S2222; Bz-Ile-Glu-Gly-Arg-pNA) for Factor Xa (K_m=200 μM) was added to a final concentration of K_m (4×K_m (50 μM) for thrombin) in a final reaction volume of 40 microliters. Changes in absorbance at 405 nm were measured over time in a Biotek Synergy 2 plate (Winnoski, Vt.). Using GraphPad Prism version 6.04 software program, (GraphPad Software, San Diego, Calif., www.graphpad.com), a four parameter curve fit was used to determine the inhibitor IC_{50}s from a plot of the mean reaction velocity versus the inhibitor concentration. K_i values were calculated from the IC_{50} values using the Cheng and Prusoff equation (K_i=IC_{50}/(1+[S]/K_m).

TABLE 4.1

Structure	HGFA IC_{50} (μM)	Matriptase IC_{50} (μM)	Hepsin IC_{50} (μM)	Thrombin Ki (nM)	Factor Xa Ki (nM)
Nafamostat	0.80	0.001	0.002		
Leupeptin (Ac-LLR-H	0.535	1.150	0.256		
Ac-KQLR(SEQ ID NO: 1)-kt (5)	0.445	0.005	0.002	>20000	170
Ac-KQLdR-kt (5-1)	5.526	—	—	—	—
Ac-KHLR(SEQ ID NO: 2)-kt (5a)	0.650	—	—	—	—
Ac-KHLdR-kt (5a-1)	10.931	—	—	—	—
Ac-WQLR(SEQ ID NO: 3)-kt (5b)	0.471	0.049	0.001	—	—
Ac-WQLdR-kt (5b-1)	7.123	—	—	—	—
Ac-KQFR(SEQ ID NO: 4)-kt (5c)	0.470	—	—	>20000	78.8
Ac-KQFdR-kt (5c-1)	7.824	—	—	—	—
Ac-KFLR(SEQ ID NO: 5)-kt (5d)	0.330	0.035	0.022	—	—
Ac-RQLR(SEQ ID NO: 6)-kt (5e)	0.270	0.001	0.001	>20000	8.10
Ac-RQLdR-kt (5e-1)	3.118	—	—	—	—
Ac-SQLR(SEQ ID NO: 7)-kt (5f)	0.180	—	—	—	—
Ac-KRLR(SEQ ID NO: 8)-kt (5g)	0.225	0.014	0.005	—	—
Ac-WRLR(SEQ ID NO: 9)-kt (5h)	0.275	0.031	0.0003	—	—
Ac-SKLR(SEQ ID NO: 10)-kt (6)	0.56	0.048	0.00	>20000	3062
Ac-SKLdR-kt (6-1)	6.311	—	—	—	—
Ac-SHLR(SEQ ID NO: 11)-kt (6a)	1.800	0.560	0.005	—	—

TABLE 4.1-continued

Structure	HGFA IC_{50} (μM)	Matriptase IC_{50} (μM)	Hepsin IC_{50} (μM)	Thrombin Ki (nM)	Factor Xa Ki (nM)
Ac-SHLdR-kt (6a-1)	21.218	—	—	—	—
Ac-WKLR(SEQ ID NO: 12)-kt (6b)	0.320	0.097	0.004	—	—
Ac-WKLdR-kt (6b-1)	6.500	—	—	—	—
Ac-SKFR(SEQ ID NO: 13)-kt (6c)	0.520	0.034	0.051	>2000	530.00
Ac-SKFdR-kt (6c-1)	10.795	—	—	—	—
Ac-NKLR(SEQ ID NO: 14)-kt (6d)	0.397	—	—	—	—
Ac-SRLR(SEQ ID NO: 15)-kt (6e)	0.303	0.024	0.005	—	—
Ac-SdRLR-kt (6e-1)	1.420	—	—	—	—
Ac-SRLdR-kt (6e-2)	2.241	—	—	—	—
Ac-SdRLdR-kt (6e-3)	11.920	—	—	—	—
Ac-TKLR(SEQ ID NO: 16)-kt (6f)	0.567	—	—	—	—
Ac-SWLR(SEQ ID NO: 17)-kt (6g)	0.435	0.175	0.009	3698	759
Ac-RKLR(SEQ ID NO: 18)-kt (6h)	0.370	—	—	—	—
Ac-NKLR(SEQ ID NO: 14)-kt	0.4	—	—	—	—
H-KQLdR-kt	11.31	—	—	—	—
H-KQLR(SEQ ID NO: 1)-Kt	0.44	—	—	—	—
Ac-LLR-kt	1.48				

[0133] HGFA Competition Assay of inhibitor Ac-KQLR (SEQ ID NO: 1)-Kt (5) and substrate: To determine whether the ketothiazoles are behaving as competitive inhibitors, different amounts of Ac-KQLR (SEQ ID NO: 1)-Kt (5) (0, 0.25, 0.5, and 1.0 μM) were mixed with Pefachrome serially diluted in TNC buffer. HGFA was added to 12.5 nM and changes in absorbance at 405 nm were measured over time in a Biotek Synergy 2 plate reader. From the plots of the mean reaction velocity versus substrate concentration and the Michaelis-Menten enzyme kinetics equation within GraphPad Prism version 6.04 for Windows, the reaction V_{max} and subsequent K_m for each concentration of inhibitor was determined as shown in the FIG. **1**.

[0134] Fluorescent Kinetic Enzyme Inhibitor Assays of HGFA, matriptase hepsin, and thrombin: Inhibitors (11-pt serial dilutions, 0-20 μM final concentration in reaction) were serially diluted in DMSO (2% DMSO final concentration) and then mixed with either recombinant serine protease domain of HGFA [Z. Han, P. K. Harris, D. E. Jones, R. Chugani, T. Kim, M. Agarwal, W. Shen, S. A. Wildman, J. W. Janetka, *Acs Med Chem Lett* 2014, 5, 1219-1224], matriptase (Charles Craik, UCSF) or hepsin* (#4776-SE-010, R&D Systems, Minneapolis, Minn.) in black 384 well plates (Corning #3575. Corning, N.Y.). The final assay concentration for HGFA, matriptase and hepsin 7.5 nM, 0.2 nM, and 0.3 nM, respectively in TNC buffer (25 mM Tris, 150 mM NaCl, 5 mM $CaCl_2$), 0.01% TRITON X-100, pH 8). After thirty minutes incubation at room temperature, Boc-QLR-AMC substrate (K_m=37 μM) was added to the HGFA assays and Boc-QAR-AMC substrate was added to the matriptase (K_m=93 μM) and hepsin (K_m=156 μM) assays. The final substrate concentrations for all assays were at the K_m for the respective enzymes. Changes in fluorescence (excitation at 380 nm and emission at 460 nm) were measured at room temperature over time in a Biotek Synergy 2 plate reader (Winnoski, Vt.). Using GraphPad Prism version 6.04 software program, (GraphPad Software, San Diego, Calif., www.graphpad.com), a four parameter curve fit was used to determine the inhibitor IC_{50}s from a plot of the mean reaction velocity versus the inhibitor concentration. The IC_{50} values represent the average of three separate experimental determinations. K_i values were calculated using the Cheng and Prusoff equation (K_i=IC_{50}/(1+[S]/K_m).

[0135] *Hepsin Activation: Recombinant Hepsin (10 μg, 0.44 mg/mL as received from R&D Systems, Catalogue #4776-SE) was diluted to 2.4 μM in TNC buffer (25 mM Tris, 150 mM NaCl, 5 mM $CaCl_2$), 0.01% TRITON X-100, pH 8) and incubated at 37° C. After twenty-four hours, the

hepsin was diluted in glycerol to 50%. This stock hepsin (1.2 μM) was stored in a −20° C. freezer and diluted in TNC buffer for use in assays.

[0136] As shown in the following table, the pro-HGF and pro-MSP tetrapeptides 5 and 6 showed K_is of 53 nM and 81 nM, respectively. These results demonstrate that inhibitor 5 was competitive with substrate to the active-site. The majority of compounds evaluated are more selective for matriptase and hepsin; most pronounced with hepsin where some inhibitors having K_is <1 nM. There are several compounds which are equipotent for HGFA compared to matriptase but leupeptin is 3-fold selective for HGFA over matriptase and only 3-fold favoring hepsin. The pro-HGF peptide (5) is 200-fold more potent for hepsin and 50-fold for matriptase while the pro-MSP peptide (6) is only slightly more potent for matriptase and 70-fold potent for hepsin versus HGFA. Ac-SWLR (SEQ ID NO: 17)-Kt (6g) is equipotent for both HGFA and matriptase with a K_i=66 nM but K_i=1.2 nM for hepsin. 6g, Ac-SKFR (SEQ ID NO: 13)-Kt (6c), Ac-KFLR (SEQ ID NO: 5)-Kt (5d) and Ac-SRLR(SEQ ID NO: 15)-Kt (6e) show HGFA selectivity over both hepsin and matriptase.

TABLE 4.2

Structure	HGFA Ki (nM)	Matriptase Ki (nM)	Hepsin Ki (nM)
Nafamostat	25	0.02	0.53
Leupeptin (Ac-LLR-H)	188	696	61
Ac-KQLR(SEQ ID NO: 1)-kt (5)	70	1.60	0.19
Ac-KQLdR-kt (5-1)	—	—	—
Ac-KHLR(SEQ ID NO: 2)-kt (5a)	96	22.3	0.41
Ac-KHLdR-kt (5a-1)	—	—	—
Ac-WQLR(SEQ ID NO: 3)-kt (5b)	65	32.4	0.21
Ac-WQLdR-kt (5b-1)	—	—	—
Ac-KQFR(SEQ ID NO: 4)-kt (Sc)	58	0.69	0.58
Ac-KQFdR-kt (5c-1)	—	—	—
Ac-KFLR(SEQ ID NO: 5)-kt (5d)	80	15	2.1
Ac-RQLR(SEQ ID NO: 6)-kt (5e)	60	0.32	0.28
Ac-RQLdR-kt (5e-1)	—	—	—
Ac-SQLR(SEQ ID NO: 7)-kt (5f)	182	9.2	0.34
Ac-KRLR(SEQ ID NO: 8)-kt (5g)	12	1.1	0.57
Ac-WRLR(SEQ ID NO: 9)-kt (5h)	21	5.5	0.21
Ac-SKLR(SEQ ID NO: 10)(SEQ ID NO: 10)-kt (6)	81	58	1.2
Ac-SKLdR-kt (6-1)	—	—	—
Ac-SHLR(SEQ ID NO: 11)-kt (6a)	332	104.2	0.60
Ac-SHLdR-kt (6a-1)	—	—	—
Ac-WKLR(SEQ ID NO: 12)-kt (6b)	56	8.6	0.55
Ac-WKLdR-kt (6b-1)	—	—	—
Ac-SKFR(SEQ ID NO: 13)-kt (6c)	57	3.03	8.5
Ac-SKFdR-kt (6c-1)	—	—	—
Ac-NKLR(SEQ ID NO: 14)-kt (6d)	79	12	1.4
Ac-SRLR(SEQ ID NO: 15)-kt (6e)	24	5.8	0.68
Ac-SdRLR-kt (6e-2)	—	—	—
Ac-SRLdR-kt (6e-1)	—	—	—
Ac-SdRLdR-kt (6e-3)	—	—	—

TABLE 4.2-continued

Structure	HGFA Ki (nM)	Matriptase Ki (nM)	Hepsin Ki (nM)
Ac-TKLR(SEQ ID NO: 16)-kt (6f)	103	8.7	0.61
Ac-SWLR(SEQ ID NO: 17)-kt (6g)	63	69	1.2
Ac-RKLR(SEQ ID NO: 18)-kt (6h)	17	0.83	0.47
Ac-NKLR(SEQ ID NO: 14)-kt	79	12.4	1.41
Ac-LLR-kt	253.00	27.950	2.288

Not measured. K_m Determinations:

[0137] In black 384 well plates (Corning #3575), 12.5 nM HGFA was mixed with various amounts of Boc-QLR-AMC and 1 nM matriptase and 0.3 nM hepsin were mixed with various amounts of Boc-QAR-AMC. Changes in fluorescence (excitation at 380 nm and emission at 460 nn) were measured at room temperature over time in a Biotek Synergy 2 plate reader (Winnoski, Vt.). Using plots of the mean reaction velocity versus substrate concentration and the Michaelis-Menten enzyme kinetics equation within GraphPad Prism version 6.04 for Windows (GraphPad Software, San Diego, Calif., www.graphpad.com), the reaction V_{max} and subsequent K_m for each of the substrates were determined. The plots are presented in FIGS. **2-4**.

[0138] Dilution recovery experiments with Ac-KQLR (SEQ ID NO: 1)-Kt (5) and HGFA: To demonstrate reversibility of HGFA ketothiazole inhibitors and to examine the dissociation of the enzyme-inhibitor complex, dilution recovery experiments were performed. High concentration HGFA (7.5 μM) were mixed with different concentrations of Ac-KQLR (SEQ ID NO: 1)-Kt (5), between 0-120 μM. After incubating for 20 minutes at room temperature, these reactions were diluted rapidly (1:350) in TNC buffer containing 250 μM Boc-QLR-AMC substrate (~7 times K_m). Activity was monitored by recording the change in fluorescence (excitation at 380 nm and emission at 460 nn) over time in a Biotek Synergy 2 plate reader. GraphPad Prism was used to plot the change in fluorescence over time. The results are provided in FIGS. **5** and **6**. These results show that Ac-KQLR (SEQ ID NO: 1)-Kt (5) is a reversible inhibitor of HGFA as the enzyme activity is recovered slowly over time even at an inhibitor concentrations $7\times K_i$.

[0139] Biochemical Assay for Proteolysis of pro-HGF and pro-MSP by HGFA: A biochemical assay was employed in order to demonstrate that inhibitors can block the proteolytic activation of the endogenous growth factors, pro-HGF and pro-MSP by HGFA in a dose-dependent manner. In order to determine efficiency of our recombinant HGFA we performed a concentration response of HGFA using a fixed concentration of pro-HGF and pro-MSP. Since both the single chain inactive pro-HGF or pro-MSP and the active heterodimers have the same molecular weight, SDS gels were developed under reducing conditions.

[0140] For pro-HGF proteolysis, inhibitors (0-12.5 μM final concentration in reaction) were diluted in DMSO (2% DMSO final concentration in reaction) and then mixed with recombinant HGFA (1.0 nM final concentration) in TNC buffer. After 30 minutes incubation at room temperature (25° C.), 25 ng of pro-HGF (R&D Systems, NKG011306A) was added. After 1 hour incubation at 37° C., reactions were stopped by adding SDS gel loading buffer containing DTT (reducing) and then run on 12% PAGE. Proteins were transferred to Millipore Immobilon-P membranes (Billerica, Mass.) and then immunoblotted at 4° C. with anti-HGF antibody (R&D Systems, AF-294-NA) and HRP conjugated donkey anti-goat IgG (Santa Cruz, SC-2020) diluted 1:500 and 1:5000 in 5% milk/TBST (5% Carnation nonfat dried milk/10 mM Tris, 150 mM NaCl, 0.05% TWEEN 20, pH 8.0), respectively. Membranes were washed in TBST, immersed in Millipore Luminata Crescendo Western-HRP Substrate for 5 minutes, and then exposures made on a BioRad ChemiDoc MP Imaging System (Hercules, Calif.). For pro-MSP proteolysis assays, the procedures were similar, except that the HGFA concentration was 75 nM, pro-MSP (R&D Systems ZN081306A) concentration was 50 ng per reaction, and 1:500 dilution of anti-MSP antibody (R&D Systems, AF352) was used for MSP detection during immunoblotting.

[0141] The results are shown in FIGS. **7** and **8** below. Lanes with pro-HGF (FIG. **7**) and pro-MSP (FIG. **8**) contain one band at 90 and 75 KDa, respectively, whereas activated HGF and MSP appear as two bands as the 60 KDa α-chain and 30 KDa β-chain in HGF and 50 KDa and 25 KDa for MSP (note: MSP Ab only recognizes the α-chain). Shown in FIG. **4**, we found that nafamostat and the three inhibitors 5, 5h, 5g all showed a dose-dependent inhibition of pro-HGF activation with similar EC50 values in direct correlation with those found from the HGFA enzyme assay. These inhibitors in addition to 6e also all show dose-dependent inhibition of pro-MSP proteolysis by HGFA as shown in FIG. **7**. While it is difficult to quantitate the level of MSP activation since the pro-MSP has some active two-chain MSP present, the EC50 values correlate with the level of potency seen in the enzyme assay. These results show the inhibitors inhibit the processing of both known protein substrates of HGFA.

[0142] c-MET Phosphorylation (Y1234/1235) in cells treated with HGFA processed pro-HGF and MSP: To show the inhibition provided by the tetrapeptide compounds would have effects on cell signaling through c-MET kinase, a phosphorylation assay using the invasive breast cancer cell line, MDA-MB-231 was developed. This cell line has high expression of c-MET and pro-HGFA but not pro-HGF.

[0143] MDA-MB-231 cells were maintained in RPMI medium (Sigma-Aldrich R8758, St. Louis, Mo.) containing 10% fetal bovine serum (Sigma F2442) and 1× Penicillin/Streptomycin (Pen/Strep) antibiotics (Thermo Fisher SV30010). For cMet phosphorylation measurements, MDA-MB-231 cells were switched to starve medium (RPMI medium containing 1 mM Sodium Pyruvate (Corning 25-000-ci), 10 mM HEPES (Corning 25-060-ci), 0.225% Glucose (Corning 25-037-ci), 1×PenStrep). After 18 hours, cells were switched to fresh starve media and then treated

with HGFA Proteolysis reactions containing 1 nM HGFA, 50 ng pro-HGF and various amounts of inhibitors. After 15 minutes incubation, media was removed by aspiration and the remaining cells were washed twice with cold Dulbeccos Phosphate Buffered Saline (Life Technologies #14190-136). Next cells were scraped into in Lysis Buffer (50 mM Tris, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.25% Sodium Deoxcholate, pH 7.5) containing 1 mM sodium fluoride, 1 mM sodium orthovanandate, 1× Sigma (P8340) Inhibitor Cocktail, and one Roche Complete Mini, EDTA-free Protease Inhibitor Cocktail tablet per 10 ml buffer and stored frozen. Lysate aliquots were mixed with reducing SDS gel loading buffer and run on 10% PAGE. Immunoblots were performed as described previously, except primary antibodies, anti-phospho cMet (Cell Signaling, CS3077) and anti-total cMet (Cell Signaling, CS3127) were used and diluted in 5% BSA/TBST. Secondary antibodies, HRP-anti rabbit antibody (CS7074) and HRP-anti mouse (CS7076) were used, respectively. All antibodies were used at a 1:1000 dilution. Images were obtained and quantitated using Bio-Rad ChemiDoc MP Imaging System. cMet phosphorylation signals were normalized using the total cMet signals and % inhibition calculated using the ratio of inhibited versus uninhibited HGFA Proteolytic reactions. Results were plotted in GraphPad Prism and a four parameter curve fit was used to determine the inhibitor EC_{50}s.

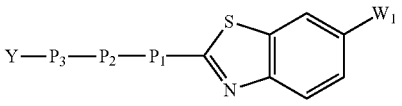
[0144] As shown in FIG. **9**, several inhibitors were effective at decreasing c-MET phosphorylation in a dose-dependent manner. Thus, these results show that these inhibitors of HGFA can function as non-kinase inhibitors of HGF-mediated c-MET kinase signaling in cancer. The most potent compound 5g had an EC50 of 180 nM.

Example 5. Synthesis of Ketobenzothiazole Inhibitor Compounds

[0145] The polypeptide ketobenzothiazoles (kbt) listed in Table 5.1 were synthesized in accordance with the general scheme 5A shown below and the procedures described below.

TABLE 5.1

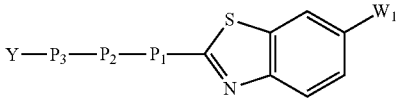
Polypeptide Compounds



Compound No.	Ac—P_3—P_2—P_1—Kt	W_1
1-13A1	H-WFR-kbt	H
1-18A1	H-dWFR-kbt	H
1-15A1	H-dWLR-kbt	H
1-56A1	H-WLR-kbt-COOH	COOH
1-56A1	H-dWLR-kbt-COOH	COOH
1-54A1	H-His(Bom)WLR-kbt	H
7182	H-LLR-kbt V amide	Va1-NH_2
7185-1	Fmoc-AR-kbt	H
7185-2	Fmoc-RR-kbt	H
7185-3	Fmoc-NR-kbt	H
7185-4	Fmoc-DR-kbt	H
7185-6	Fmoc-QR-kbt	H
7185-7	Fmoc-ER-kbt	H
7185-8	Fmoc-GR-kbt	H
7185-9	Fmoc-HR-kbt	H
7185-10	Fmoc-IR-kbt	H
7185-11	Fmoc-LR-kbt	H

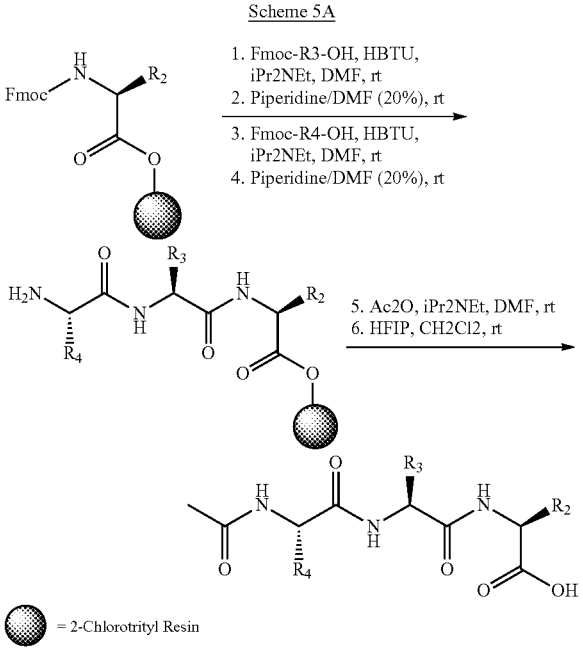
TABLE 5.1-continued

Polypeptide Compounds



Compound No.	Ac—P_3—P_2—P_1—Kt	W_1
7185-12	Fmoc-KR-kbt	H
7187-13	Fmoc-MR-kbt	H
7187-14	Fmoc-FR-kbt	H
7187-15	Fmoc-PR-kbt	H
7187-16	Fmoc-SR-kbt	H
7188-17	Fmoc-TR-kbt	H
7188-18	Fmoc-WR-kbt	H
7188-19	Fmoc-YR-kbt	H
7188-20	Fmoc-VR-kbt	H

Scheme 5A



[0146] Tripeptides were synthesized in 0.5 mmol scale through consecutive coupling of amino acid-preloaded 2-Cl-trityl resin with Fmoc protected amino acids followed by the deprotection of Fmoc group. In the coupling reaction, 5 equiv. Fmoc-amino acid/5 equiv. HBTU/10 equiv. $^{i}Pr_2NEt$ was used and the mixture was shaken at room temperature overnight. In the deprotection of Fmoc group, piperidine/DMF (20% v/v) was employed and the mixture was shaken from 1 to 4 hours at room temperature to ensure complete reaction.

[0147] Peptide coupling and deprotection steps of the Fmoc group: Into the reaction vial (with a fritted glass resin support) containing H-Leu-2-Cl trityl, H-Phe-2-Cl trityl resin or H-Xxx-2-Cl trityl resin (0.714 g, 0.5 mmol), DMF/CH_2Cl_2 (15/15 mL) was added. The mixture was shaken at room temperature for 30 minutes, then filtered. The resulting resin was washed with DMF (10 mL) 2 times. Into another vial containing Fmoc-AA-OH (2.5 mmol) in DMF (20 mL),

HBTU (0.853 g, 2.25 mmol) and $^{i}Pr_2NEt$ was added (0.87 mL, 5 mmol). The mixture was stirred at room temperature for 10 minutes, then added into the reaction vial containing the resin. The mixture was shaken at room temperature overnight, then filtered. The resin was washed with DMF (20 mL×4). To the resulting resin piperidine/DMF (20% v/v, 30 mL) was added. The mixture was shaken for 1-4 hours at room temperature, then filtered. The resin was washed with DMF (10 mL×4).

[0148] Acetyl capping of the tripeptides: The tripeptide resin was suspended in 30 mL of 0.5 M Ac_2O/DMF and 1 M $^{i}Pr_2NEt$/DMF. The mixture was shaken at room temperature for 1 hour. The resin was filtered and washed with DMF (10 mL×4) followed by CH_2Cl_2 (10 mL×4).

[0149] Cleavage of tripeptide resin: Ac-capped tripeptide resin was suspended and shaken in 30 ml of 25% v/v HFIP/CH_2Cl_2 for 1 hour. The mixture was filtered. The filtrate was concentrated then dried in vacuo, giving rise to crude product of Acetyl-capped tripeptides.

[0150] Boc-WF-OMe: Boc-Trp-OH (1 g, 3.28 mmol) and HCl.Phe-OMe (0.708g, 3.28 mmol) was taken in dry dichloromethane (10 mL) under nitrogen atmosphere and the reaction mixture was cooled to 0° C. and N,N-diisopropylethylamine (1.7 mL, 9.84 mmol) and propylphosphonic anhydride (1.9 mL, 3.28 mmol, 50% solution in EtOAc) were added to the solution drop wise respectively. The reaction mixture was then stirred at 25° C. under nitrogen atmosphere for 1 hour and the completion of the reaction was confirmed by LC-MS monitoring. On completion the reaction mixture was diluted with 10 mL dichloromethane and washed with 10% citric acid solution, saturated sodium bicarbonate solution and brine respectively. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was triturated with hexane to obtain the title product in pure form as a white solid. Yield: 1.3 g (92.8%). Chemical formula: $C_{23}H_{33}N_3O_5$, Exact Mass: 465.55, MS(ESI): found: $[M+Na]^+$, 488.58.

[0151] Boc-WF-OH: Boc-WF-OMe (0.130 g, 0.279 mmol) was taken in a 1:1 mixture of THF and water and $LiOH.H_2O$ (0.035g, 0.873 mmol) was added to it. The reaction mixture was stirred for 30 minutes at 25° C. and the completion of the reaction was confirmed by LCMS monitoring. On completion, the THF was evaporated under reduced pressure and the remaining water layer was cooled to 0° C. The water layer was then brought to pH 6.5 by slow addition of 0.5 M HCl solution in water. The crude product precipitates out on addition of HCl and it was isolated by filtration. The crude product was dried under reduced pressure and triturated with diethyl ether to obtain the pure title product in pure form as white solid. Yield: 0.102g (80%). Exact Mass: 451.21, MS(ESI): found: $[M+H]^+$, 452.26.

[0152] Boc-WFR(Mtr)-kbt: Boc-WF-OH (35 mg, 0.077 mmol) and HATU (43.9 mg, 0.115 mmol) was taken in dry DMF under nitrogen atmosphere and the reaction mixture was cooled to 0° C. N,N-diisopropylethylamine (0.04 mL, 0.231 mmol) was then added drop wise to the reaction mixture and the reaction mixture was allowed to stir for 15 minutes followed by addition of HCl.Arg(Mtr)-kbt (41.75 mg, 0.077 mmol). The reaction mixture was stirred for 12 hours at 25° C. under nitrogen atmosphere and the completion of the reaction was confirmed by LC-MS monitoring. On completion, the reaction mixture was diluted with EtOAc and washed with 10% citric acid solution, saturated sodium bicarbonate solution and brine respectively. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was directly taken to the next step without further purification. Chemical formula: $C_{48}H_{56}N_8O_8S_2$, Exact Mass: 936.37, MS(ESI): found: $[M+H]^+$, 937.17.

[0153] H-WFR-kbt (1-13A1): Boc-WFR(Mtr)-kbt (85 mg, crude product from previous step) was taken in 5 mL TFA:thioanisole:H_2O (95:2.5:2.5) and the reaction mixture was stirred for 6 hours at 25° C. The completion of the reaction was confirmed by LC-MS monitoring. On completion, the reaction mixture was concentrated under reduced pressure and triturated with diethyl ether to obtain the crude product as brown solid. The crude product was then subjected to reverse phase semi-preparative HPLC (Stationary phase: C18 column, mobile phase: H_2O-Acetonitrile with 0.1% TFA in each, 15-65% Acetonitrile in H_2O gradient for 20 minutes) to obtain the pure title product as yellow solid. Yield: 20 mg (41% over two steps). ^1H NMR (400 MHz, CHLOROFORM-d) δ ppm 8.08 (br. s., 1H), 7.67 (d, J=8.22 Hz, 1H), 7.37 (d, J=8.22 Hz, 1H), 7.10-7.25 (m, 5H), 6.94-7.05 (m, 1H), 6.82 (d, J=7.04 Hz, 2H), 6.14-6.29 (m, 1H), 5.12 (d, J=2.74 Hz, 1H), 4.69-4.82 (m, 1H), 4.45 (br. s., 1H), 3.62 (s, 3H), 3.09-3.37 (m, 1H), 2.95 (d, J=5.48 Hz, 2H), 1.62 (s, 1H), 1.43 (br. s., 9H). Chemical formula: $C_{33}H_{36}N_8O_3S$, Exact Mass: 624.26, MS (ESI): found: $[M+H]^+$, 625.5.

[0154] Boc-dWF-OMe: The title compound was synthesized using the same procedure as Boc-WF-OMe starting with Boc-dTrp-OH. Yield: 90%. Chemical formula: $C_{23}H_{33}N_3O_5$, Exact Mass: 465.55, MS(ESI): found: $[M+Na]^+$, 488.58.

[0155] Boc-dWF-OH: The title compound was synthesized using the same procedure as Boc-WF-OH starting with Boc-dWF-OMe. Yield: 80%. Exact Mass: 451.21, MS(ESI): found: $[M+H]^+$, 452.26.

[0156] Boc-dWFR(Mtr)-kbt: The title compound was synthesized using the same procedure as Boc-WFR(Mtr)-kbt starting with Boc-dWF-OH. The crude product was directly taken to the next step. Chemical formula: $C_{48}H_{56}N_8O_8S_2$, Exact Mass: 936.37, MS(ESI): found: $[M+H]^+$, 937.17.

[0157] H-dWFR-kbt (1-18A1): The title compound was synthesized using the same procedure as H-WFR(Mtr)-kbt starting with Boc-dWF-OH. Yield: 25 mg (42% over two steps). Chemical formula: $C_{33}H_{36}N_8O_3S$, ^1H NMR (400 MHz, METHANOL-d4) δ ppm 8.21 (d, J=6.65 Hz, 1H), 8.11 (d, J=7.43 Hz, 1H), 7.57-7.67 (m, 4H), 7.12-7.36 (m, 10H), 7.01-7.08 (m, 2H), 6.84 (s, 1H), 3.48 (d, J=1.96 Hz, 1H), 3.13 (d, J=6.65 Hz, 4H), 3.01-3.07 (m, 1H), 2.75-2.84 (m, 1H), 2.65 (s, 2H), 2.03 (s, 5H), 1.41 (d, J=8.61 Hz, 1H) Exact Mass: 624.26, MS (ESI): found: $[M+H]^+$, 625.5.

[0158] Boc-dWL-OMe: The title compound was synthesized using the same procedure as Boc-WF-OMe starting with Boc-dTrp-OH and HCl.H-Leu-OMe. Yield: 92%. Chemical formula: $C_{23}H_{33}N_3O_5$, Exact Mass: 431.24, MS(ESI): found: $[M+Na]^+$, 454.3.

[0159] Boc-dWL-OH: The title compound was synthesized using the same procedure as Boc-WF-OH starting with Boc-dWF-OMe. Yield: 83%. Exact Mass: 417.23, MS(ESI): found: $[M+H]^+$, 418.26.

[0160] Boc-dWLR(Mtr)kbt: The title compound was synthesized using the same procedure as Boc-WFR(Mtr)-kbt starting with Boc-dWL-OH. The crude product was directly taken to the next step. Chemical formula: $C_{45}H_{58}N_8O_8S_2$, Exact Mass: 902.38, MS(ESI): found: $[M+H]^+$, 903.5.

[0161] H-dWLR-kbt (1-15A1): The title compound was synthesized using the same procedure as H-WFR(Mtr)-kbt starting with Boc-dWLR(Mtr)kbt. Yield: 22 mg (41% over two steps). Chemical formula: C $C_{30}H_{38}N_8O_3S$, ^{1}H NMR (400 MHz, METHANOL-d4) δ ppm 10.58 (br. s., OH), 8.82 (s, 1H), 8.22-8.32 (m, 2H), 7.70 (dd, J=8.02, 16.24 Hz, 0H), 7.57-7.64 (m, 1H), 7.37 (d, J=6.26 Hz, 1H), 7.22 (d, J=13.30 Hz, 0H), 7.11-7.18 (m, 2H), 7.02-7.10 (m, 1H), 5.55-5.67 (m, 1H), 4.47-4.58 (m, OH), 4.35 (dd, J=5.09, 10.17 Hz, 0H), 4.05-4.31 (m, 2H), 3.37-3.55 (m, 1H), 3.07-3.27 (m, 3H), 2.65 (s, 1H), 2.18 (dd, J=6.06, 13.11 Hz, 1H), 1.57-1.95 (m, 3H), 1.16-1.52 (m, 2H), 1.06 (td, J=7.14, 13.89 Hz, 1H), 0.91-1.00 (m, 2H), 0.65-0.81 (m, 6H) Exact Mass: 624.26, MS (ESI): found: $[M+H]^+$, 625.5.

[0162] H-dWFRkbt-COOH (1-45A1): The title compound was synthesized using the same procedure as WFR(Mtr)kbt starting with Boc-dWFR(Mtr)kbt-COOH. Yield: 24 mg (43% over two steps). Chemical formula: $C_{34}H_{36}N_8O_5S$, ^{1}H NMR (400 MHz, METHANOL-d4) δ ppm 10.58 (br. s., OH), 8.82 (s, 1H), 8.22-8.32 (m, 2H), 7.70 (dd, J=8.02, 16.24 Hz, 0H), 7.57-7.64 (m, 1H), 7.37 (d, J=6.26 Hz, 1H), 7.22 (d, J=13.30 Hz, 0H), 7.11-7.18 (m, 2H), 7.02-7.10 (m, 1H), 5.55-5.67 (m, 1H), 4.47-4.58 (m, OH), 4.35 (dd, J=5.09, 10.17 Hz, 0H), 4.05-4.31 (m, 2H), 3.37-3.55 (m, 1H), 3.07-3.27 (m, 3H), 2.65 (s, 1H), 2.18 (dd, J=6.06, 13.11 Hz, 1H), 1.57-1.95 (m, 3H), 1.16-1.52 (m, 2H), 1.06 (td, J=7.14, 13.89 Hz, 1H), 0.91-1.00 (m, 2H), 0.65-0.81 (m, 6H) Exact Mass: 668.253, MS (ESI): found: $[M+H]^+$, 669.5.

[0163] H-WLRkbt-COOH (1-56A1): The title compound was synthesized using the same procedure as WLR(Mtr)kbt starting with Boc-WLR(Mtr)kbt-COOH. Yield: 26 mg (44% over two steps). Chemical formula: $C_{31}H_{38}N_8O_5S$, ^{1}H NMR (400 MHz, METHANOL-d4) δ ppm 8.77-8.84 (m, 1H), 8.22-8.32 (m, 3H), 7.57-7.71 (m, 1H), 7.31-7.42 (m, 2H), 7.04-7.23 (m, 3H), 4.09-4.13 (m, 1H), 3.48 (s, 1H), 3.22-3.27 (m, 2H), 3.13 (s, 1H), 2.65 (s, 2H), 1.62 (d, J=7.04 Hz, 1H), 1.17 (s, 1H), 0.90-1.00 (m, 6H), 0.65-0.80 (m, 7H) Exact Mass: 634.269, MS (ESI): found: $[M+H]^+$, 635.8.

[0164] H-dWLRkbt-COOH (1-56A1): The title compound was synthesized using the same procedure as dWLR(Mtr) kbt starting with Boc-dWLR(Mtr)kbt-COOH. Yield: 25 mg (42% over two steps). Chemical formula: $C_{31}H_{38}N_8O_5S$, ^{1}H NMR (400 MHz, METHANOL-d4) δ ppm 10.58 (br. s., OH), 8.82 (s, 1H), 8.22-8.32 (m, 2H), 7.70 (dd, J=8.02, 16.24 Hz, 0H), 7.57-7.64 (m, 1H), 7.37 (d, J=6.26 Hz, 1H), 7.22 (d, J=13.30 Hz, 0H), 7.11-7.18 (m, 2H), 7.02-7.10 (m, 1H), 5.55-5.67 (m, 1H), 4.47-4.58 (m, OH), 4.35 (dd, J=5.09, 10.17 Hz, 0H), 4.05-4.31 (m, 2H), 3.37-3.55 (m, 1H), 3.07-3.27 (m, 3H), 2.65 (s, 1H), 2.18 (dd, J=6.06, 13.11 Hz, 1H), 1.57-1.95 (m, 3H), 1.16-1.52 (m, 2H), 1.06 (td, J=7.14, 13.89 Hz, 1H), 0.91-1.00 (m, 2H), 0.65-0.81 (m, 6H) Exact Mass: 634.269, MS (ESI): found: $[M+H]^+$, 635.8.

[0165] Fmoc-H(Bom)WL-OMe: The title compound was synthesized using same procedure described as Boc-WF-OMe starting from Fmoc-His(Bom)-OH and HCl.H-WL-OMe. The crude product was directly taken to the next step. Chemical formula: $C_{47}H_{50}N_6O_7$, Exact Mass: 810.5, MS(ESI): found: $[M+H]^+$, 811.78.

[0166] Fmoc-H(Bom)WL-OH: The title compound was synthesized using same procedure described as Boc-WF-OH starting from Fmoc-His(Bom)WL-OMe. Yield: 76%. Chemical formula: $C_{46}H_{48}N_6O_7$, Exact Mass: 796.35, MS(ESI): found: $[M+H]^+$, 797.78.

[0167] Fmoc-H(Bom)WLR(Mtr)-Kbt: The title compound was synthesized using same procedure described as Boc-WF-RKbt starting from Fmoc-His(Bom)WL-OH and HCl.H-Arg(Mtr)-Kbt. Yield: 65%. Exact Mass: 1281.514, MS(ESI): found: $[M+H]^+$, 1262.7.

[0168] H(Bom)WLR-Kbt (1-54A1): The title compound was synthesized using same procedure described as H-WF-RKbt starting from Fmoc-His(Bom)WLR(Mtr)Kbt, followed by a treatment with 4M HCl solution. Yield: 20% over three steps. Exact Chemical formula: $C_{44}H_{53}N_{11}O_5S$, ^{1}H NMR (400 MHz, METHANOL-d4) δ ppm 7.89 (d, J=7.83 Hz, 4H), 7.71 (d, J=7.43 Hz, 4H), 7.46-7.52 (m, 4H), 7.39-7.45 (m, 4H), 4.49 (t, J=5.28 Hz, 2H), 3.83 (d, J=5.48 Hz, 5H), 3.42 (d, J=12.13 Hz, 4H), 2.99 (t, J=11.54 Hz, 4H), 1.88 (br. s., 6H), 1.75 (br. s., 8H), 1.44-1.60 (m, 2H) Mass: 847.3, MS(ESI): found: $[M+H]^+$, 848.7.

[0169] H(Bom)dWLR-Kbt (1-58A1): The title compound was synthesized using same procedure described as H-WF-RKbt starting from Fmoc-dHis(Bom)WLR(Mtr)Kbt, followed by a treatment with 4M HCl solution. Yield: 36% over three steps. Exact Chemical formula: $C_{44}H_{53}N_{11}O_5S$, ^{1}H NMR (400 MHz, METHANOL-d4) δ ppm 7.89 (d, J=7.83 Hz, 4H), 7.71 (d, J=7.43 Hz, 4H), 7.46-7.52 (m, 4H), 7.39-7.45 (m, 4H), 4.49 (t, J=5.28 Hz, 2H), 3.83 (d, J=5.48 Hz, 5H), 3.42 (d, J=12.13 Hz, 4H), 2.99 (t, J=11.54 Hz, 4H), 1.88 (br. s., 6H), 1.75 (br. s., 8H), 1.44-1.60 (m, 2H) Mass: 847.3, MS(ESI): found: $[M+H]^+$, 848.7.

[0170] Boc-LL-OMe: The title compound was synthesized using the same procedure as Boc-dWL-OMe starting with Boc-Leu-OH and HCl.H-Leu-OMe.

[0171] Boc-LL-OH: The title compound was synthesized using the same procedure as Boc-dWL-OH starting with Boc-LL-OMe. Exact Mass: 417.5 MS(ESI): found: $[M+H]^+$ 418.6

[0172] H-LLR-kbt V amide (7182). Synthesized in a similar manner to H-WFRkbt from Boc-LLR(Mtr)-Kbt V amide. Yield 4.6 mg. Chemical Formula: $C_{31}H_{49}N_9O_5S$, Exact Mass: 659.36, MS(ESI): found: $[M+H]^+$, 660.6.

[0173] Boc-WL-OH: The title compound was synthesized using the same procedure as Boc-dWL-OH starting with Boc-WL-OMe. Exact Mass: 417.2, MS(ESI): found: $[M+H]^+$, 418.3.

[0174] H-WLR-kbt V amide (7181). Synthesized in a similar manner to H-dWLR-kbt from Boc-WLR(Mtr)-kbt V amide. Yield 4.5 mg. Chemical Formula: $C_{36}H_{48}N_{10}O_5S$, Exact Mass: 732.35, MS(ESI): found: $[M+H]^+$, 733.6.

[0175] Boc-R(Mtr)L-OH: The title compound was synthesized using the same procedure as Boc-dWL-OH starting with Boc-R(Mtr)L-OMe. Exact Mass: MS(ESI): 599.7 found: $[M+H]^+$, 600.8.

[0176] H-RLR-kbt V amide (7180). Yield 5 mg. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 8.90 (d, J=7.04 Hz, 0H), 8.65 (s, 1H), 8.35 (d, J=8.61 Hz, 1H), 8.28 (d, J=8.61 Hz, 1H), 8.10 (d, J=9.00 Hz, 1H), 5.53-5.61 (m, 1H), 4.42-4.55 (m, 2H), 3.92 (t, J=5.87 Hz, 1H), 3.18 (t, J=6.85 Hz, 2H), 2.20 (qd, J=6.81, 13.60 Hz, 2H), 1.86-1.98 (m, 3H), 1.70-1.82 (m, 3H), 1.56-1.68 (m, 3H), 1.07 (dd, J=4.30, 6.65 Hz, 6H), 0.96 (d, J=6.26 Hz, 16H). Chemical Formula: $C_{31}H_{50}N_2O_5S$, Exact Mass: 702.37, MS(ESI): found: $[M+H]^+$, 703.6.

[0177] Fmoc-A-R-kbt (7185-1). Under nitrogen atmosphere, Fmoc-A-OH (0.02 mmol) was dissolved in DMF (10 mL) and then HATU (0.02 mmol) was added. After stirring for 10 minutes, H-R(Mtr)-kbt-HCl (0.0185 mmol) and N,N-

diisopropylethylamine (0.1 mmol) were added. The mixture was stirred at room temperature for 4 hours. The solvent was removed and then 1.5 mL of cleavage cocktail (38:1:1 TFA/water/thioanisole) was added and then the reaction was stirred for 4 hours. After concentration in vacuo, the residue was purified by C18 reverse phase HPLC to give the title compound as a lyophilized white powder.

[0178] Fmoc-RR-kbt (7185-2). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{34}H_{39}N_9O_4S$, Exact Mass 669.28, MS(ESI): found $[M+H]^+$ 670.4.

[0179] Fmoc-NR-kbt (7185-3). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{32}H_{33}N_7O_5S$, Exact Mass 627.23, MS(ESI): found $[M+H]^+$ 628.3.

[0180] Fmoc-DR-kbt (7185-4). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{32}H_{32}N_6O_6S$, Exact Mass, 628.21, MS(ESI): found $[M+H]^+$ 629.3.

[0181] Fmoc-QR-kbt (7185-6). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{33}H_{35}N_7O_5S$, Exact Mass 641.24, MS(ESI): found $[M+H]^+$ 642.4.

[0182] Fmoc-ER-kbt (7185-7). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{33}H_{34}N_6O_6S$, Exact Mass 642.23, MS(ESI): found $[M+H]^+$ 643.3.

[0183] Fmoc-GR-kbt (7185-8). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{30}H_{30}N_6O_4S$, Exact Mass 570.2, MS(ESI): found $[M+H]^+$ 571.3.

[0184] Fmoc-HR-kbt (7185-9). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{34}H_{34}N_8O_4S$, Exact Mass 650.24, MS(ESI): found $[M+H]^+$ 651.4.

[0185] Fmoc-IR-kbt (7185-10). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{34}H_{38}N_6O_4S$, Exact Mass 626.27, MS(ESI): found $[M+H]^+$ 627.4.

[0186] Fmoc-LR-kbt (7185-11). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{34}H_{38}N_6O_4S$, Exact Mass 626.27, MS(ESI): found $[M+H]^+$ 627.4.

[0187] Fmoc-KR-kbt (7185-12). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{34}H_{39}N_7O_4S$, Exact Mass 641.28, MS(ESI): found $[M+H]^+$ 642.5.

[0188] Fmoc-MR-kbt (7187-13). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{32}H_{34}N_6O_4S_2$, Exact Mass 630.21, MS(ESI): found $[M+H]^+$ 631.3.

[0189] Fmoc-FR-kbt (7187-14). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{37}H_{36}N_6O_4S$, Exact Mass 660.25, MS(ESI): found $[M+H]^+$ 661.4.

[0190] Fmoc-PR-kbt (7187-15). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{33}H_{34}N_6O_4S$, Exact Mass 610.24, MS(ESI): found $[M+H]^+$ 611.5.

[0191] Fmoc-SR-kbt (7187-16). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{31}H_{32}N_6O_5S$, Exact Mass 600.22, MS(ESI): found $[M+H]^+$ 601.3.

[0192] Fmoc-TR-kbt (7188-17). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{32}H_{34}N_6O_5S$, Exact Mass 614.23, MS(ESI): found $[M+H]^+$ 615.4.

[0193] Fmoc-WR-kbt (7188-18). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{39}H_{37}N_7O_4S$, Exact Mass 699.26, MS(ESI): found $[M+H]^+$ 700.4.

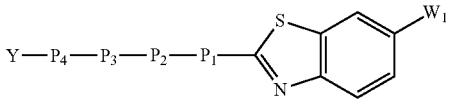
[0194] Fmoc-YR-kbt (7188-19). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{37}H_{36}N_6O_5S$, Exact Mass 676.25, MS(ESI): found $[M+H]^+$ 677.4.

[0195] Fmoc-VR-kbt (7188-20). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{33}H_{36}N_6O_4S$, Exact Mass 612.25, MS(ESI): found $[M+H]^+$ 613.5.

[0196] The tetrapeptide ketothiazoles listed in Table 5.2 were synthesized in accordance with the procedures and schemes described below.

TABLE 5.2

Tetrapeptide ketobenzothiazoles.



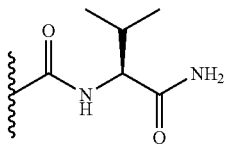
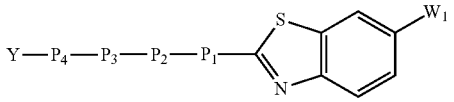
Compound No.	Y—P_4—P_3—P_2—P_1—Z	W_1
7115	Ac-KRLR(SEQ ID NO: 8)-Kbt-Va1-NH_2	
7054	Ac-KRLR(SEQ ID NO: 8)-Kbt	H
7117	H-WRLR(SEQ ID NO: 9)-Kbt	H
1-45A1	H-dWFR-kbt-COOH	COOH

TABLE 5.2-continued

Tetrapeptide ketobenzothiazoles.



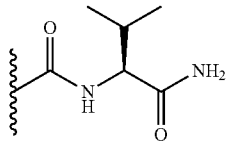
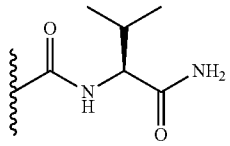
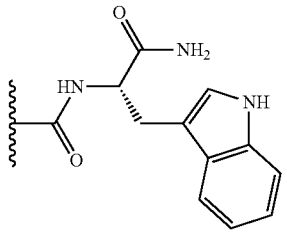
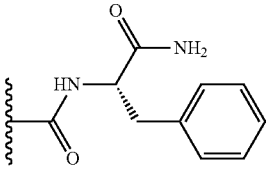
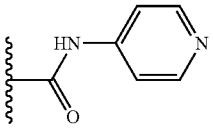
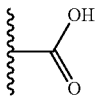
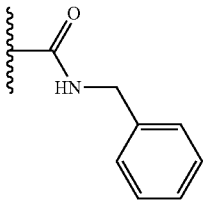
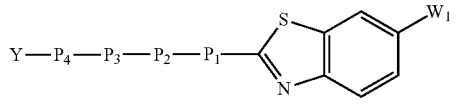
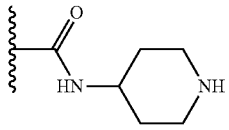
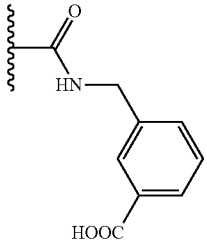
Compound No.	Y—P_4—P_3—P_2—P_1—Z	W_1
7055	Ac-KQLR(SEQ ID NO: 1)-Kbt-Val-NH_2	
7116	Ac-SKLR(SEQ ID NO: 10)-Kbt-Val-NH_2	
7124	Ac-SKLR(SEQ ID NO: 10)-Kbt-Trp-NH_2	
7125	Ac-SKLR(SEQ ID NO: 10)-Kbt-Phe-NH_s	
7006	Ac-KQLR(SEQ ID NO: 1)-Kbt	H
7126	Ac-SKLR(SEQ ID NO: 10)-Kbt-4-pyridinylamide	
7053	Ac-SKLR(SEQ ID NO: 10)-Kbt	H
7063	Ac-FLFR(SEQ ID NO: 19)-Kbt	H
7118	Ac-SKLR(SEQ ID NO: 10)-Kbt-COOH	
7064	Ac-WLFR(SEQ ID NO: 20)-Kbt	H
7139	Ac-SKLR(SEQ ID NO: 10)-Kbt-benzylamide	

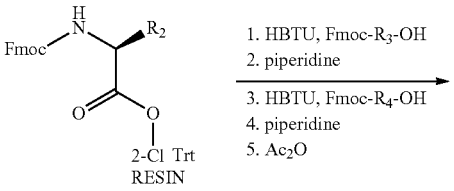
TABLE 5.2-continued

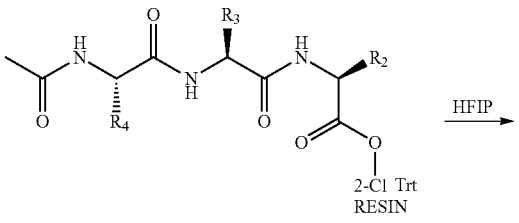
Tetrapeptide ketobenzothiazoles.



Compound No.	Y—P_4—P_3—P_2—P_1—Z	W_1
7140	Ac-SKLR(SEQ ID NO: 10)-Kbt-4-piperidinylamide	
7165	Ac-KRLR(SEQ ID NO: 8)-Kbt-5-COOH	

[0197] Procedure for the Preparation of Tetrapeptide Ketobenzothiazole: Tripeptides were synthesized in 0.5 mmol scale through consecutive coupling of amino acid-preloaded 2-Cl-trityl resin with Fmoc protected amino acids followed by the deprotection of Fmoc group. In the coupling reaction, 5 equiv. Fmoc-amino acid/4.5 equiv. HBTU/10 equiv. $^{i}Pr_2NEt$ was used and the mixture was shaken at room temperature overnight. In the deprotection of Fmoc group, piperidine/DMF (20% v/v) was employed and the mixture was shaken for 4 hours at room temperature.





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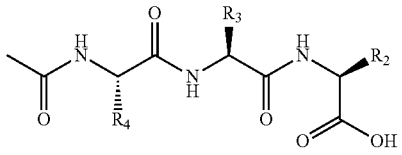
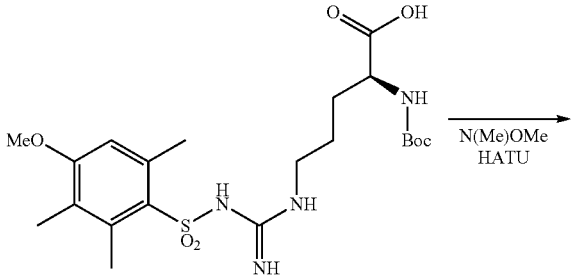


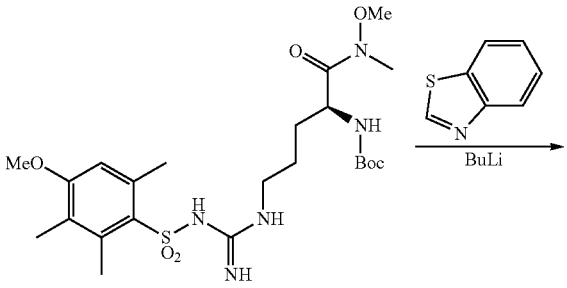
[0198] Peptide coupling and deprotection of Fmoc: Into the reaction vial (with a fritted glass resin support) containing H-Leu-2-Cl-trityl resin (0.714 g, 0.5 mmol), DMF/CH_2Cl_2 (15/15 mL) was added. The mixture was shaken at room temperature for 30 minutes, then filtered. The resulting resin was washed with DMF (10 mL×2). Into another vial containing Fmoc-AA-OH (2.5 mmol) in DMF (20 mL), HBTU (0.853 g, 2.25 mmol) and $^{i}Pr_2NEt$ was added (0.87 mL, 5 mmol). The mixture was stirred at room temperature for 10 minutes, then added into the reaction vial containing the resin. The mixture was shaken at room temperature overnight, then filtered. The resin was washed with DMF (20 mL×4). To the resulting resin piperidine/DMF (20% v/v, 30 mL) was added. The mixture was shaken for 4 hours at room temperature, then filtered. The resin was washed with DMF (10 mL×4).

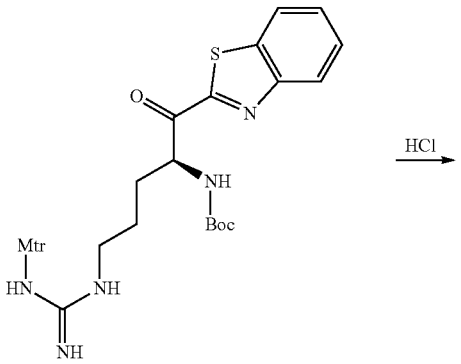
[0199] Acetyl capping of the tripeptides: The tripeptide resin was suspended in 30 mL of 0.5 M Ac_2O/DMF and 1 M $^{i}Pr_2NEt$/DMF. The mixture was shaken at room temperature for 1 hour. The resin was filtered and washed with DMF (10 mL×4) followed by CH_2Cl_2 (10 mL×4).

[0200] Cleavage of tripeptide resin: Ac-capped tripeptide resin was suspended and shaked in 30 ml of 25% v/v HFIP/CH_2Cl_2 for 1 hour. The mixture was filtered. The

filtrate was concentrated then dried in vacuo, giving rise to crude product of Ac-capped tripeptide.







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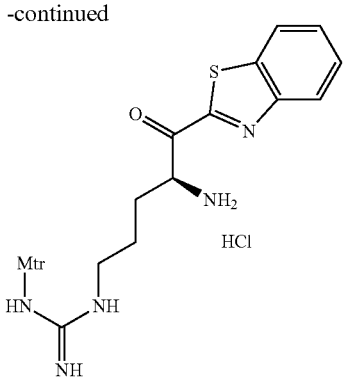
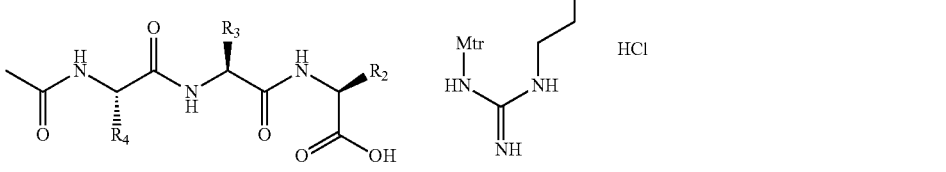


[0201] BocHN-Arg(Mtr) ketobenzothiazole. At −78° C., n-BuLi/Hex (2.5M) (14 mL, 35 mmol) was added dropwise into the solution of benzothiazole (4.87 g, 36 mmol) in THF (50 mL) over 15 minutes. After the mixture was stirred for additional half an hour, the solution of Boc-HN-Arg(Mtr) Weinreb amide (1.06 g, 2 mmol) in THF (15 mL) was added slowly over 50 min. For synthesis of Weinreb amide, see Han et al., "Inhibitors of HGFA, Matriptase, and Hepsin Serine Proteases: A Nonkinase Strategy to Block Cell Signaling in Cancer, *Acs Med Chem Lett* (2014) 5, 1219-1224. The mixture was stirred at −78° C. for additional 2 hours. The reaction was quenched with aqueous NH_4Cl and the aqueous layer was extracted with AcOEt. The organic phase was collected, dried with Na_2SO_4, then concentrated. The resulting residue was purified by silica gel chromatography with $CHCl_3$/MeOH combination as eluent to give the title compound (1.14 g) in 94% yield. 1H NMR (400 MHz, METHANOL-d_4) δ ppm 1.43 (s, 9H) 1.53-1.78 (m, 4H) 2.05 (s, 3H) 2.55 (s, 3H) 2.61 (s, 3H) 3.17-3.29 (m, 2H) 3.82 (s, 3H) 5.19-5.43 (m, 1H) 6.57 (s, 1H) 7.53-7.71 (m, 2H) 8.06-8.28 (m, 2H). MS(ESI): found: $[M+H]^+$, 604.4.

[0202] HCl.H_2N-Arg(Mtr) ketobenzothiazole. The mixture of BocHN-Arg(Mtr) ketobenzothiazole (1.0 g, 1.66 mmol) in 30 mL of HCl/dioxane (1.5 M) was stirred at room temperature. The reaction was monitored by LCMS until completion. The solvent was removed then the resulting residue was dried in vacuo to the tile product in quantitative yield. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 1.60-1.86 (m, 2H) 2.00-2.20 (m, 4H) 2.24-2.36 (m, 1H) 2.53 (s, 3H) 2.61 (s, 3H) 3.84 (s, 3H) 5.14-5.40 (m, 1H) 6.65 (s, 1H) 7.59-7.78 (m, 2H)_8.10-8.34 (m, 2H). MS(ESI): found: $[M+H]^+$504.4.



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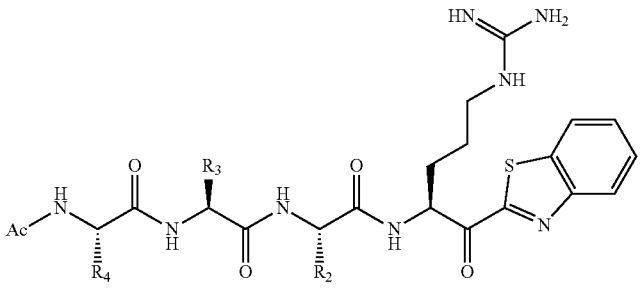


[0203] Ac-KQLR (SEQ ID NO: 1) ketobenzothiazole (7006). Under nitrogen atmosphere, at 0° C. anhydrous DMF (5 mL) was added into the round bottom flask containing AcHN-K(Boc)-Q(Trt)-L-OH tripeptide (0.064 g, 0.083 mmol) and HATU (0.035 g, 0.091 mmol). After stirring for 10 minutes, Mtr-protected arginine ketobenzothiazole (0.068 g, 0.013 mmol), then N,N-diisopropylethylamine (0.053 g, 0.41 mmol) were added. The mixture was stirred at room temperature for 4 hours while being warmed to room temperature naturally. DMF was removed and to the resulting residue water (20 mL) was added. The precipitate formed was filtered and dried. To this precipitate 5 mL of TFA/thioanisole/water (95/2.5/2.5 (v/v/v)) was added. The mixture was stirred at room temperature for 4 hours. Then cold ether (40 mL) was added. The resulting precipitate, which is the crude product, was collected by centrifugation, then by decanting out carefully ether solvent. The crude product was purified by HPLC (C18, 15*150 mm column; eluent: acetonitrile/water (0.05% TFA)) to give Ac-KQLR (SEQ ID NO: 1) ketobenzothiazole (0.030 g) in 43% yield. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.88 (d, J=6.26 Hz, 3H) 0.92 (d, J=6.26 Hz, 3H) 1.36-1.90 (m, 12H) 1.90-2.26 (m, 3H) 2.02 (s, 3H) 2.26-2.44 (m, 2H) 2.87-2.97 (m, 2H) 3.20-3.40 (m, 2H) 4.21-4.29 (m, 1H) 4.31-4.38 (m, 1H) 4.39-4.48 (m, 1H) 5.63-5.78 (m, 1H) 7.54-7.74 (m, 2H) 8.08-8.16 (m, 1H) 8.19-8.29 (m, 1H). MS(ESI): found: $[M+H]^+$, 703.6.

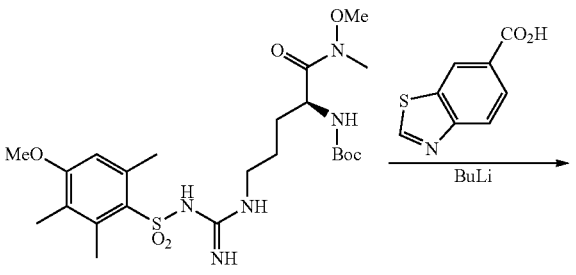
[0204] Ac-SKLR (SEQ ID NO: 10) ketobenzothiazole (7053). (yield: 23%). Synthesized using the same procedure as for Ac-KQLR (SEQ ID NO: 1) ketobenzothiazole. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.87 (d, J=6.65 Hz, 3H) 0.91 (d, J=6.65 Hz, 3H) 1.36-2.08 (m, 12H) 2.01 (s, 3H) 2.10-2.29 (m, 1H) 2.82-3.04 (m, 2H) 3.22-3.38 (m, 2H) 3.65-3.93 (m, 2H) 4.24-4.53 (m, 3H) 5.58-5.74 (m, 1H) 7.56-7.71 (m, 2H) 8.08-8.16 (m, 1H) 8.18-8.25 (m, 1H). MS(ESI): found: $[M+H]^+$, 662.5.

[0205] Ac-KRLR (SEQ ID NO: 8) ketobenzothiazole (7054). (yield: 38%). Synthesized using the same procedure as for Ac-KQLR (SEQ ID NO: 1) ketobenzothiazole. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.90 (d, J=6.80 Hz, 3H) 0.94 (d, J=6.80 Hz, 3H) 1.36-1.96 (m, 16H) 1.99 (s, 3H) 2.09-2.34 (m, 1H) 2.87-2.98 (m, 2H) 3.14-3.23 (m, 2H) 3.25-3.33 (m, 2H) 4.20-4.30 (m, 1H) 4.33-4.52 (m, 2H) 5.54-5.76 (m, 1H) 7.59-7.70 (m, 2H) 8.10-8.16 (m, 1H) 8.18-8.23 (m, 1H). MS(ESI): found: $[M+H]^+$, 731.7.

[0206] Ac-FLFR (SEQ ID NO: 19) ketobenzothiazole (7063). (yield: 47%). Synthesized using the same procedure as for Ac-KQLR (SEQ ID NO: 1) ketobenzothiazole. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.85 (d, J=6.26 Hz, 3H) 0.90 (d, J=6.26 Hz, 3H) 1.36-1.63 (m, 3H) 1.66-1.99 (m, 3H) 1.91 (s, 3H) 2.05-2.25 (m, 1H) 2.80-2.91 (m, 1H) 2.92-3.02 (m, 1H) 3.02-3.18 (m, 2H) 3.20-3.33 (m, 2H) 4.19-4.39 (m, 1H) 4.47-4.70 (m, 2H) 5.64-5.75 (m, 1H) 7.01-7.33 (m, 10H) 7.57-7.71 (m, 2H) 8.10-8.17 (m, 1H) 8.19-8.25 (m, 1H). MS(ESI): found: $[M+H]^+$, 741.6.

[0207] Ac-WLFR (SEQ ID NO: 20) ketobenzothiazole (7064). (yield: 19%). Synthesized using the same procedure as for Ac-KQLR (SEQ ID NO: 1) ketobenzothiazole. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.79 (d, J=6.26 Hz, 3H) 0.84 (d, J=6.26 Hz, 3H) 1.32-1.50 (m, 3H) 1.62-1.91 (m, 3H) 1.95 (s, 3H) 2.06-2.21 (m, 1H) 2.88-3.00 (m, 1H) 3.05-3.29 (m, 5H) 4.14-4.33 (m, 1H) 4.50-4.72 (m, 2H) 5.61-5.77 (m, 1H) 6.96-7.03 (m, 1H) 7.04-7.20 (m, 7H) 7.29-7.37 (m, 1H) 7.54-7.71 (m, 3H) 7.85-7.97 (m, 1H) 8.10-8.16 (m, 1H) 8.18-8.26 (m, 1H). MS(ESI): found: $[M+H]^+$, 780.6.

[0208] WRLR (SEQ ID NO: 9) ketobenzothiazole (7117). (yield: 39%). Synthesized using the same procedure as for Ac-KQLR (SEQ ID NO: 1) ketobenzothiazole, starting with the coupling of BocHN-W-R(Mtr)-L-OH tripeptide and Mtr-protected arginine ketobenzothiazole. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.94 (d, J=6.65 Hz, 3H) 0.95 (d, J=6.65 Hz, 3H) 1.48-1.99 (m, 10H) 2.12-2.30 (m, 1H) 3.14-3.23 (m, 3H) 3.24-3.29 (m, 2H) 3.39-3.52 (m, 1H) 4.18-4.29 (m, 1H) 4.37-4.53 (m, 2H) 5.61-5.68 (m, 1H) 7.01-7.09 (m, 1H) 7.10-7.17 (m, 1H) 7.22 (s, 1H) 7.34-7.41 (m, 1H) 7.58-7.71 (m, 3H) 8.10-8.16 (m, 1H) 8.17-8.24 (m, 1H) 8.25-8.33 (m, 1H). MS(ESI): found: $[M+H]^+$, 747.7.



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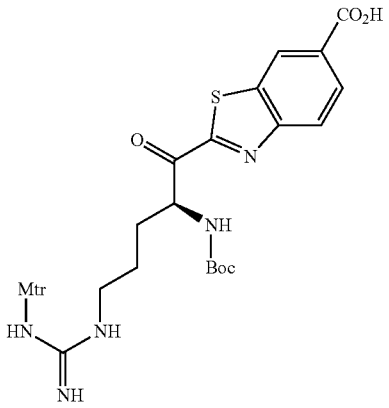
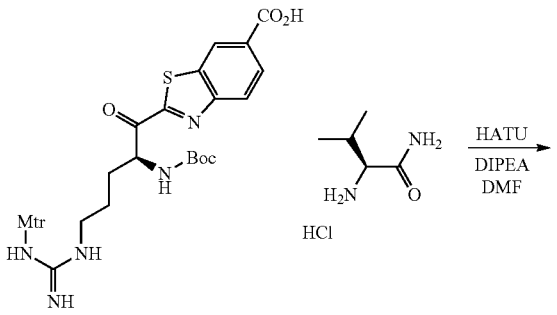


[0209] BocHN-Arg(Mtr) ketobenzothiazole-6-COOH. At −78° C., ″BuLi/Hex (2.5M) (28.5 mL, 71.4 mmol) was added dropwise into the solution of 6-carboxybenzothiazole (6.57 g, 37 mmol) in THF (450 mL) over 25 minutes. After the mixture was stirred for additional half an hour, the solution of Boc-HN-Arg(Mtr) Weinreb amide (1.63 g, 3.08 mmol) in THF (60 mL) was added slowly over 20 minutes at −78° C. After the addition then the mixture was stirred at −24° C. to −20° C. for 1.5 hours. The reaction was quenched with saturated aqueous NH_4Cl (270 mL). The layers were separated and the aqueous layer was extracted with AcOEt. The organic phase was collected and washed with water, 5% citric acid, then dried with Na_2SO_4 and concentrated. The resulting residue was purified by silica gel chromatography with CH_2Cl_2/MeOH combination as eluent to give the title compound (0.91 g) in 46% yield. ^1H NMR (400 MHz, METHANOL-d_4) δ ppm 1.43 (s, 9H) 1.66 (m, 4H) 2.03 (s, 3H) 2.54 (s, 3H) 2.60 (s, 3H) 3.22-3.29 (m, 2H) 3.80 (s, 3H) 5.18-5.41 (m, 1H) 6.55 (s, 1H) 8.20 (m, 2H) 8.79 (s, 1H). MS(ESI): found: $[M+H]^+$, 648.4.



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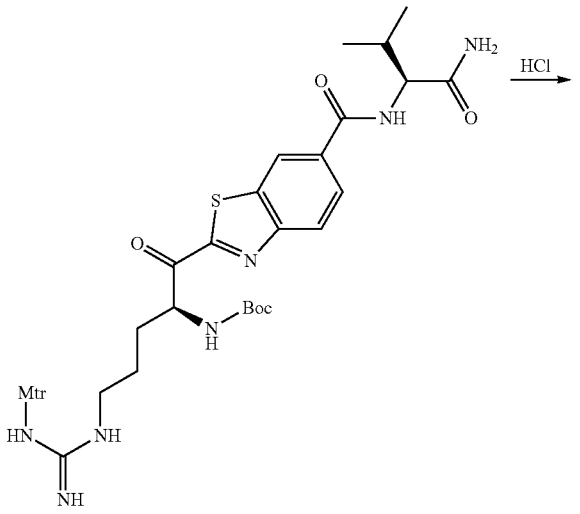
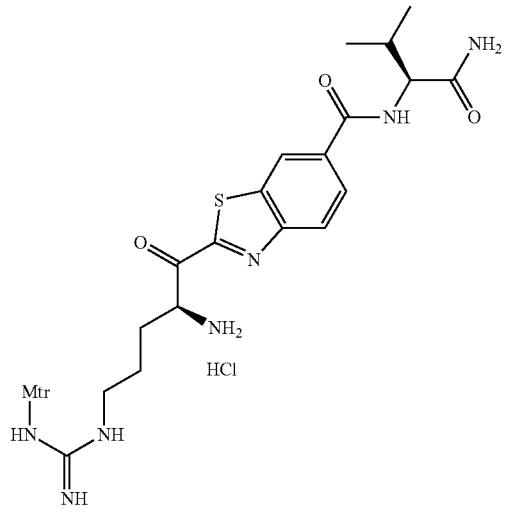


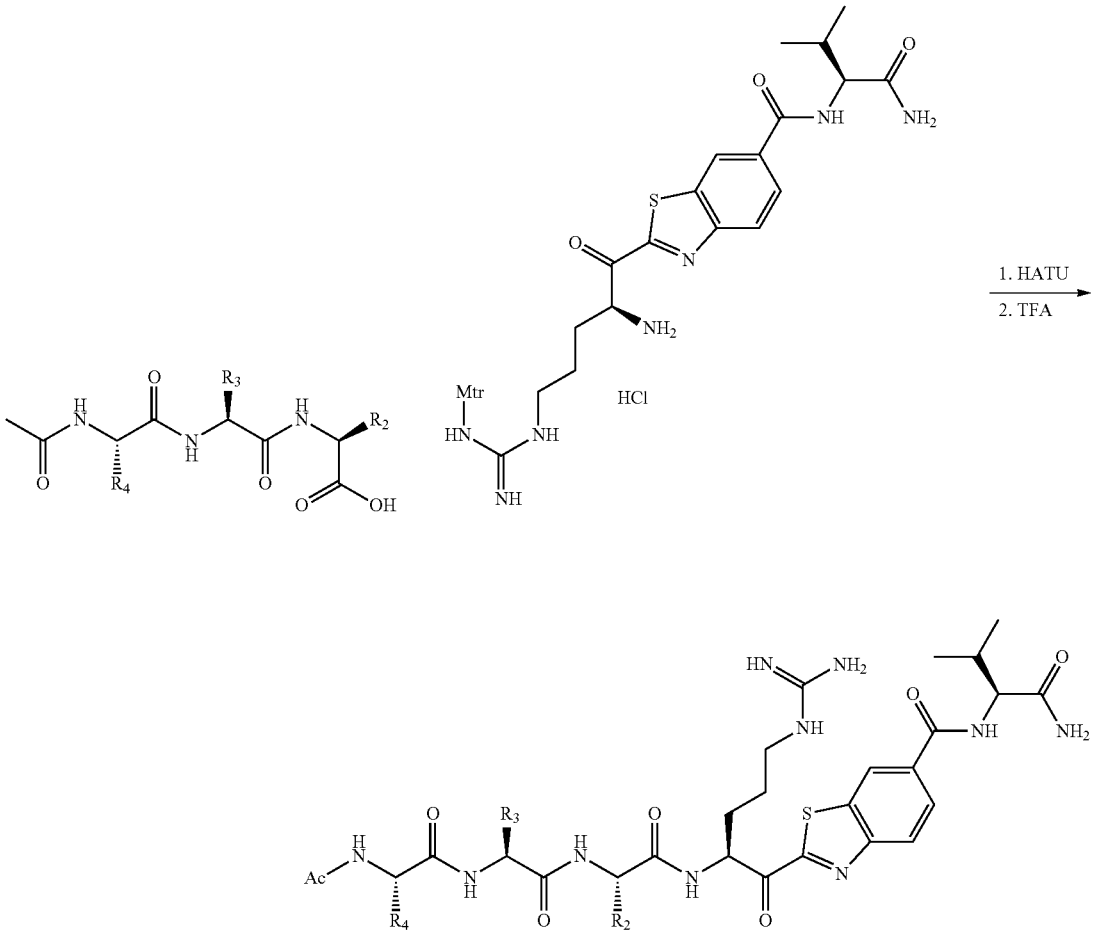


[0210] BocHN-Arg(Mtr) ketobenzothiazole-6-CONH-Val-amide. Under nitrogen atmosphere, at 0° C. anhydrous DMF (5 mL) was added into the round bottom flask containing BocHN-Arg(Mtr) ketobenzothiazole-COOH (0.060 g, 0.093 mmol) and HATU (0.042 g, 0.11 mmol). After stirring for 10 minutes, L-valine amide hydrochloride (0.017 g, 0.11 mmol), then N,N-diisopropylethylamine (0.062 g, 0.55 mmol) were added. The mixture was stirred overnight while being warmed to room temperature naturally. DMF was removed and to the resulting residue 20 mL water was added. The precipitate formed was filtered and dried. The product was purified by silica gel chromatography with $CHCl_3$/MeOH combination as eluent to give the title compound (0.049 g) in 70% yield ^1H NMR (400 MHz, METHANOL-d_4) δ ppm 1.06 (d, J=1.57 Hz, 3H) 1.08 (d, J=1.96 Hz, 3H) 1.42 (s, 9H) 1.56-1.79 (m, 4H) 2.04 (s, 3H) 2.15-2.30 (m, 1H) 2.55 (s, 3H) 2.60 (s, 3H) 3.20-3.28 (m, 2H) 3.80 (s,

3H) 4.45 (d, J=7.43 Hz, 1H) 5.19-5.41 (m, 1H) 6.57 (s, 1H) 8.00-8.12 (m, 1H) 8.13-8.29 (m, 1H) 8.62 (s, 1H). MS(ESI): found: $[M+H]^+$, 746.5.

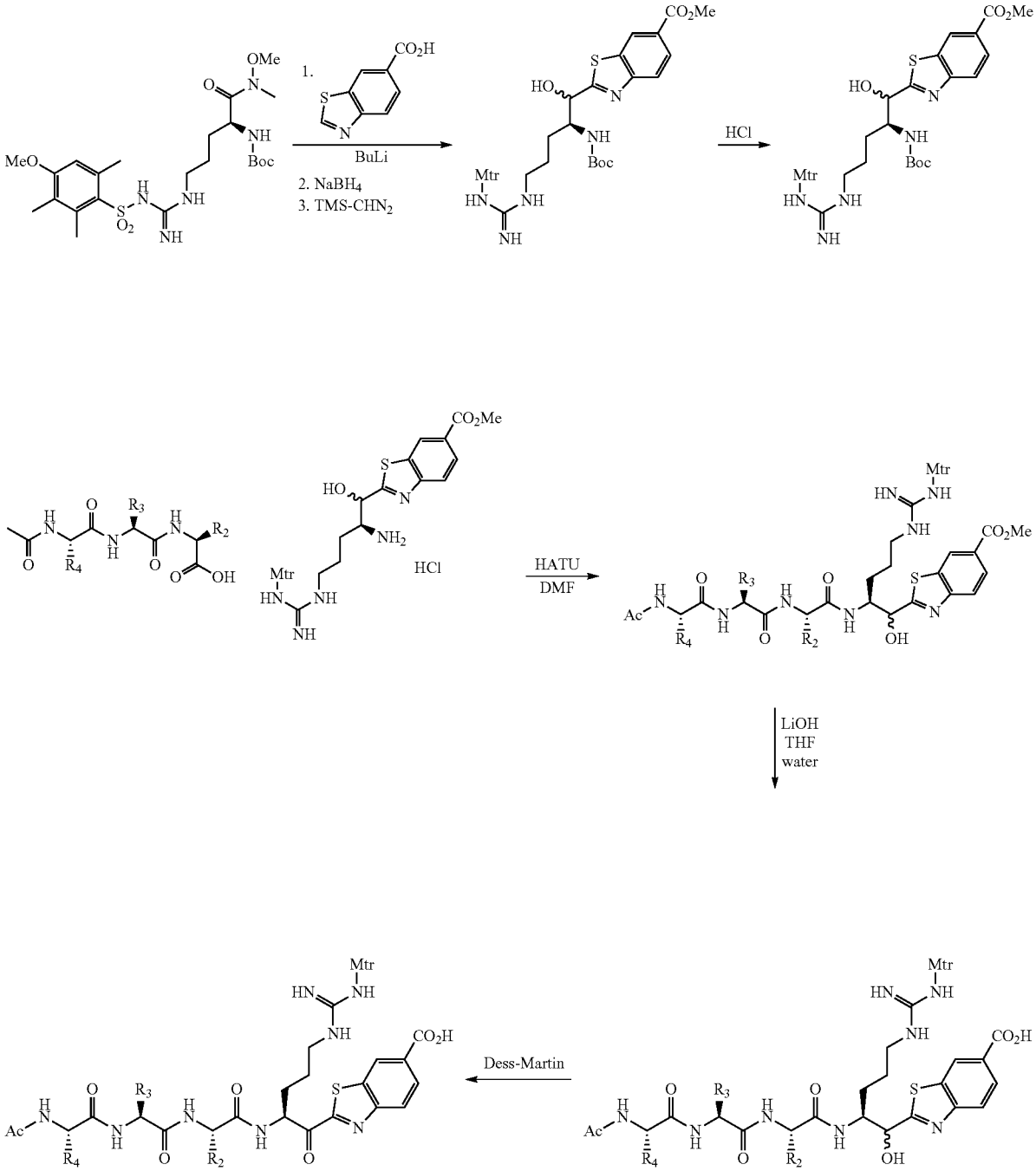
[0211] HCl.H_2N-Arg(Mtr) ketobenzothiazole-6-CONH-Val-amide. The mixture of BocHN-Arg(Mtr) ketobenzothiazole-(C═O)-V-amide (0.049 g, 0.066 mmol) in 10 mL of HCl/dioxane (2.5 M) was stirred at room temperature. The reaction was monitored by LCMS until completion. The solvent was removed then the resulting residue was dried in vacuo to the tile product in quantitative yield. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 1.06 (d, J=3.13 Hz, 3H) 1.08 (d, J=3.13 Hz, 3H) 1.58-1.91 (m, 2H) 1.95-2.41 (m, 6H) 2.52 (s, 3H) 2.61 (s, 3H) 3.85 (s, 3H) 4.46 (d, J=7.40 Hz, 1H) 5.16-5.38 (m, 1H) 6.66 (bs, 1H) 7.99-8.18 (m, 1H) 8.22-8.39 (m, 1H) 8.55-8.78 (m, 1H). MS(ESI): found: $[M+H]^+$, 646.5.

[0213] Ac-SKLR (SEQ ID NO: 10) ketobenzothiazole-6-CONH-Val-amide (7116). The title compound was synthesized using the same procedure as for Ac-KQLR (SEQ ID NO: 1) ketobenzothiazole-(C═O)-V-amide. Yield: 24%. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.87 (d, J=6.46 Hz, 3H) 0.91 (d, J=6.46 Hz, 3H) 1.06 (d, J=2.54 Hz, 3H) 1.07 (d, J=2.54 Hz, 3H) 1.37-1.97 (m, 12H) 2.01 (s, 3H)

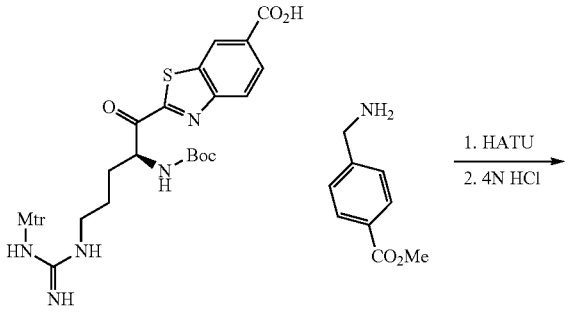


[0212] Ac-KQLR (SEQ ID NO: 1) ketobenzothiazole-6-CONH-Val-amide (7055). The title compound was synthesized using the same procedure as for the tetrapeptide ketothiazoles, starting with the coupling of protected Ac-K(Boc)-Q(Trt)-L-OH tripeptide and HCl.H_2N-Arg(Mtr) ketobenzothiazole-(C═O)-V-amide. Yield: 11%. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.87 (d, J=6.00 Hz, 3H) 0.92 (d, J=6.00 Hz, 3H) 1.06 (d, J=2.74 Hz, 3H) 1.08 (d, J=2.74 Hz, 3H) 1.37-2.14 (m, 14H) 2.02 (s, 3H) 2.14-2.43 (m, 4H) 2.87-3.00 (m, 2H) 3.22-3.38 (m, 2H) 4.20-4.29 (m, 1H) 4.29-4.38 (m, 1H) 4.39-4.50 (m, 2H) 5.58-5.75 (m, 1H) 8.05-8.14 (m, 1H) 8.24-8.33 (m, 1H) 8.62-8.67 (m, 1H). MS(ESI): found: $[M+H]^+$, 845.7.

[0214] Ac-KRLR (SEQ ID NO: 8) ketobenzothiazole-6-CONH-Val-amide (7115). The title compound was synthesized using the same procedure as for Ac-KQLR (SEQ ID NO: 1) ketobenzothiazole-(C═O)-V-amide. Yield: 28%. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.92 (dd, J=11.15, 6.46 Hz, 6H) 1.07 (dd, J=6.65, 2.74 Hz, 6H) 1.35-1.95 (m, 16H) 1.99 (s, 3H) 2.14-2.27 (m, 2H) 2.87-2.98 (m, 2H) 3.14-3.21 (m, 2H) 3.25-3.33 (m, 2H) 4.21-4.28 (m, 1H) 4.33-4.39 (m, 1H) 4.40-4.49 (m, 2H) 5.46-5.74 (m, 1H) 8.07-8.12 (m, 1H) 8.24-8.33 (m, 1H) 8.61-8.68 (m, 1H). MS(ESI): found: $[M+H]^+$, 873.7.



[0215] H-R (Mtr) ketobenzothiazole-(C═O)-NHBn(4-COOMe)-HCl. The title compound was synthesized from using the same procedure as HCl.H-Arg(Mtr) ketobenzothiazole-6-CONH-Val-amide. Yield, 85 mg, MS(ESI): found: $[M+H]^+$, 695.4.



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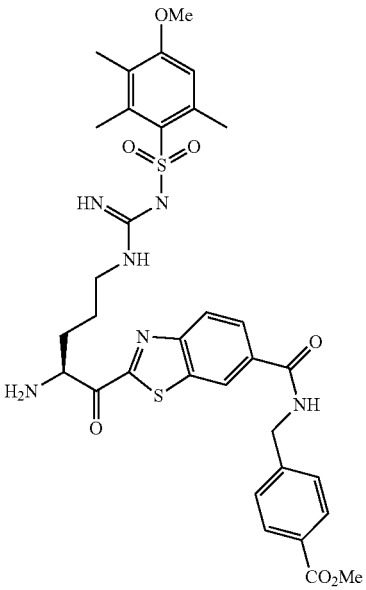


[0216] Ac-KRLR (SEQ ID NO: 8) ketobenzothiazole-(C═O)-NHBn(4-COOMe) (23a). The title compound was synthesized from H-R ketobenzothiazole-(C═O)-NHBn(4-COOMe)-HCl using the same procedure as Ac-KQLR (SEQ ID NO: 1) ketobenzothiazole-6-CONH-Val-amide and was purified by HPLC. Yield: 23% over two steps. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.90 (d, J=6.26 Hz, 3H) 0.93 (d, J=6.65 Hz, 3H) 1.37-1.95 (m, 16H) 1.99 (s, 3H) 2.13-2.28 (m, 1H) 2.87-2.97 (m, 2H) 3.13-3.23 (m, 2H) 3.25-3.32 (m, 2H) 3.89 (s, 3H) 4.21-4.28 (m, 1H) 4.32-4.41 (m, 1H) 4.41-4.50 (m, 1H) 4.69 (s, 2H) 5.56-5.66 (m, 1H) 7.50 (d, J=7.83 Hz, 2H) 8.00 (d, J=8.22 Hz, 2H) 8.09-8.13 (m, 1H) 8.27-8.32 (m, 1H) 8.62-8.67 (m, 1H). MS(ESI): found: $[M+H]^+$, 922.8.

[0217] H-R (Mtr) ketobenzothiazole-(C═O)-NHBn(3-COOMe)-HCl. The title compound was synthesized from using the same procedure as HCl.H-Arg(Mtr) ketobenzothiazole-6-CONH-Val-amide. Yield, 80 mg, MS(ESI): found: $[M+H]^+$, 695.4.

[0218] Ac-KRLR (SEQ ID NO: 8) ketobenzothiazole-(C═O)-NHBn(3-COOMe) (23b). The title compound was synthesized from H-R ketobenzothiazole-(C═O)-NHBn(3-COOMe)-HCl using the same procedure as Ac-KQLR (SEQ ID NO: 1) ketobenzothiazole-6-CONH-Val-amide and was purified by HPLC. Yield: 19% over two steps. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.90 (d, J=6.65 Hz, 3H) 0.93 (d, J=6.26 Hz, 3H) 1.40-1.95 (m, 16H) 1.99 (s, 3H) 2.13-2.27 (m, 1H) 2.88-2.97 (m, 2H) 3.13-3.23 (m, 2H) 3.25-3.31 (m, 2H) 3.89 (s, 3H) 4.21-4.28 (m, 1H) 4.33-4.41 (m, 1H) 4.41-4.50 (m, 1H) 4.68 (s, 2H) 5.57-5.66 (m, 1H) 7.43-7.52 (m, 1H) 7.61-7.69 (m, 1H) 7.90-7.97 (m, 1H) 8.03-8.08 (m, 1H) 8.09-8.13 (m, 1H) 8.26-8.31 (m, 1H) 8.58-8.68 (m, 1H). MS(ESI): found: $[M+H]^+$, 922.8.

[0219] Ac-KRLR (SEQ ID NO: 8) ketobenzothiazole-(C═O)-NHBn(3-COOH) (23c). Compound 23b (0.01 g, 0.010 mmol) was taken in 0.5 mL 0.05M LiOH solution and the reaction mixture was stirred for 1.5 hours at room temperature. On completion, the pH of the reaction mixture was brought to 2 by dropwise addition of 1N HCl solution. The crude product was purified by HPLC. Yield: 44%. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.90 (d, J=6.65 Hz, 3H) 0.93 (d, J=6.65 Hz, 3H) 1.37-1.93 (m, 16H) 1.99 (s, 3H) 2.13-2.27 (m, 1H) 2.88-2.96 (m, 2H) 3.14-3.21 (m, 2H) 3.25-3.30 (m, 2H) 4.20-4.28 (m, 1H) 4.32-4.41 (m, 1H) 4.41-4.50 (m, 1H) 4.64-4.73 (m, 2H) 5.56-5.65 (m, 1H) 7.43-7.50 (m, 1H) 7.61-7.67 (m, 1H) 7.91-7.97 (m, 1H) 8.04-8.09 (m, 1H) 8.09-8.15 (m, 1H) 8.26-8.31 (m, 1H) 8.62-8.67 (m, 1H). MS(ESI): found: $[M+H]^+$, 908.8.

[0220] Ac-KRLR (SEQ ID NO: 8) ketobenzothiazole-(C═O)-NHBn(4-COOH) (23d). The title compound was synthesized using the same procedure as 23c starting with 23a. Yield: 44%. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.91 (d, J=6.65 Hz, 3H) 0.93 (d, J=6.26 Hz, 3H) 1.33-1.95 (m, 16H) 1.99 (s, 3H) 2.14-2.27 (m, 1H) 2.88-2.96 (m, 2H) 3.14-3.21 (m, 2H) 3.25-3.30 (m, 2H) 4.19-4.28 (m, 1H) 4.33-4.41 (m, 1H) 4.41-4.50 (m, 1H) 4.61-4.76 (m, 2H) 5.54-5.69 (m, 1H) 7.46-7.52 (m, 2H) 7.98-8.04 (m, 2H) 8.09-8.15 (m, 1H) 8.27-8.32 (m, 1H) 8.62-8.68 (m, 1H). MS(ESI): found: $[M+H]^+$, 908.8.

[0221] Ac-S(^{t}Bu)-K(Boc)-L-R(Mtr)-ketobenzothiazole-6-COOH. Under nitrogen atmosphere at −78° C., ^{n}BuLi/Hex (2.5M) (28.5 mL, 71.4 mmol) was added dropwise into the solution of 6-carboxybenzothiazole (6.566 g, 37 mmol) in THF (450 mL) over 25 minutes. After the mixture was stirred for additional half an hour, the solution of Boc-HN-Arg(Mtr) Weinreb amide (1.633 g, 3.08 mmol) in THF (60 mL) was added slowly over 20 min at −78° C. After the addition then the mixture was stirred at −24° C. to −20° C. for 1.5 hours. The reaction was quenched with saturated aqueous NH_4Cl (270 mL). The layers were separated and the aqueous layer was extracted with AcOEt. The organic phase was collected and washed with water and brine, dried with Na_2SO_4, then concentrated in vacuo. To the resulting residue MeOH (50 mL) was added. The mixture was cooled at −25° C. and sodium borohydride (0.706 g, 18.7 mmol) was added. The mixture was stirred at −25~−20° C. for 1 hour. Acetone (10 mL) was added to quench the reaction and the mixture was stirred for 15 minutes then concentrated in vacuo. The residue was suspended in water, acidified to pH 3-4, and extracted with AcOEt. The organic phase was washed with brine, dried with Na_2SO_4, then concentrated in vacuo. The resulting residue was dissolved in CH_2Cl_2/MeOH (17/3 v/v, 40 mL), cooled to 0° C. Into it, (trimethylsilyl)diazomethane

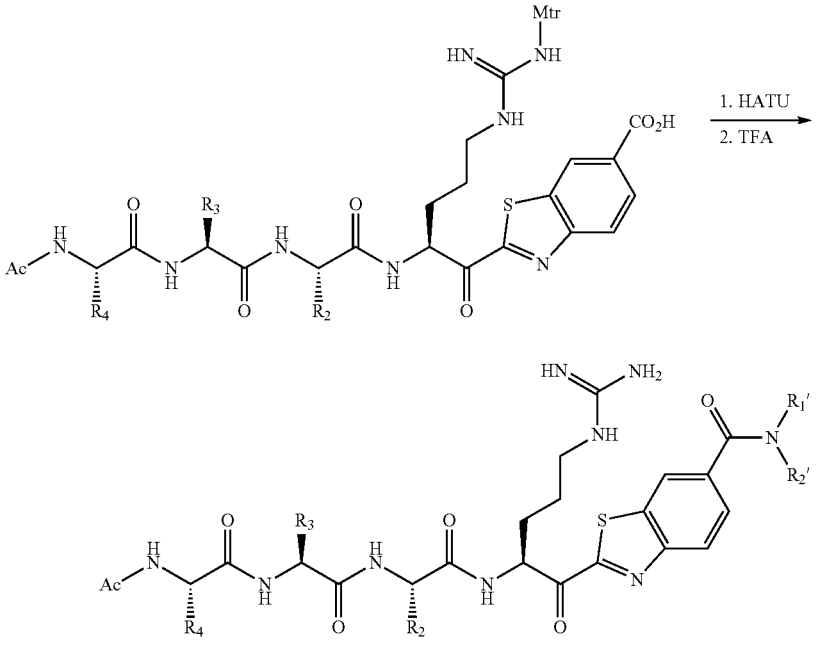
(2 M in hexane, 9.2 mL, 18.4 mmol) was added dropwise over 25 minutes. The mixture was stirred at 0° C. for 1 hour. MeOH (5 mL) was added and the mixture was concentrated in vacuo. The residue was purified by silica gel chromatography with $CHCl_3$/MeOH combination as eluent to give BocHN-Arg(Mtr)-CH(OH)benzothiazole-6-COOMe a (0.445 g, mixture of diastereomers) in 22% yield. MS(ESI): found: $[M+H]^+$, 664.5.

[0222] The mixture of a (0.44 g, 0.66 mmol) in TFA/CH_2Cl_2 (1/4 v/v, 18 mL) was stirred at room temperature for 2 hours. The solvents were removed and the residue was dried in vacuo to give TFA-H_2N-Arg(Mtr)-CH(OH)benzothiazole-6-COOMe b (0.45 g, mixture of diastereomers) in quantitative yield. MS(ESI): found: $[M+H]^+$, 564.5.

[0223] Under nitrogen atmosphere, at 0° C. anhydrous DMF (13 mL) was added into the round bottom flask containing Ac-S(^{t}Bu)-K(Boc)-L-OH (0.3650 g, 0.67 mmol) and HATU (0.255 g, 0.67 mmol). The mixture was stirred for 10 minutes, then b (0.40 g, 0.56 mmol) and N,N-diisopropylethylamine (0.49 mL, 2.8 mmol) were added sequentially. The mixture was stirred overnight while being warmed to room temperature naturally. DMF was removed and to the resulting residue, water (20 mL) was added. The precipitate formed was collected and purified by silica gel chromatography with $CHCl_3$/MeOH combination as eluent. The fractions with Ac-S(^{t}Bu)-K(Boc)-L-Arg(Mtr)-CH(OH)benzothiazole-6-COOMe c (mixture of diastereomers) confirmed by LCMS [MS(ESI): found: $[M+H]^+$, 1090.8], were collected, and concentrated in vacuo. The residue c was dissolved in THF/H_2O (15 mL/10 mL) and LiOH (0.036 g, 1.5 mmol) was added. The mixture was stirred overnight, then concentrated. The concentrated mixture was acidified to pH 4.0 with 0.25 N HCl aqueous solution and extracted with AcOEt. The organic phase was collected, dried with Na_2SO_4 and concentrated in vacuo to give Ac-S(^{t}Bu)-K(Boc)-L-Arg(Mtr)-CH(OH)benzothiazole-6-COOH d (mixture of diastereomers) (0.52 g) in 86% yield. MS(ESI): found: $[M+H]^+$, 1076.8. d was used in the next step without further purification.

[0224] Into the solution of d (0.30 g, 0.28 mmol) in CH_2Cl_2 (100 mL), Dess-Martin periodinane (DMP) (0.198 g, 0.47 mmol) was added. The mixture was stirred at room temperature for 3 hours, then additional DMP (0.12 g, 0.28 mmol) was added. The mixture was stirred overnight, then quenched with 1 M $Na_2S_2O_3$ aqueous solution. The organic phase was separated and the aqueous was extracted with CH_2Cl_2 2 times. The organic phases were combined, dried with Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel chromatography with CH_2Cl_2/MeOH combination as eluent to give the title compound Ac-S(^{t}Bu)-K(Boc)-L-R(Mtr)-ketobenzothiazole-COOH (0.186 g) in 62% yield. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.88 (d, J=5.87 Hz, 3H) 0.92 (d, J=6.26 Hz, 3H) 1.18 (s, 9H) 1.27-1.90 (m, 21H) 1.92-2.19 (m, 7H) 2.54 (s, 3H) 2.60 (s, 3H) 2.95-3.08 (m, 2H) 3.23-3.33 (m, 2H) 3.53-3.70 (m, 2H) 3.79 (s, 3H) 4.18-4.54 (m, 3H) 5.37-5.69 (m, 1H) 6.55 (s, 1H) 8.14-8.25 (m, 2H) 8.79 (s, 1H). MS(ESI): found: $[M+H]^+$, 1074.9.

[0225] Ac-SKLR (SEQ ID NO: 10)-ketobenzothiazole-6-COOH (7118). To the crude Ac-S(^{t}Bu)-K(Boc)-L-R(Mtr)-ketobenzothiazole-COOH received from the DMP oxidation of d (0.053 g, 0.049 mmol), 5 mL of TFA/thioanisole/water (95/2.5/2.5 (v/v/v)) was added. The mixture was stirred at room temperature for 4 hours. Then cold ether (40 mL) was added. The resulting precipitate, which is the crude product, was collected by centrifugation, then by decanting out carefully ether solvent. The crude product was purified by HPLC (C18, 15*150 mm column; eluent: acetonitrile/water (0.05% TFA)) to give the title compound (0.01 g) in 29% yield. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.75-1.00 (m, 6H) 1.40-2.05 (m, 15H) 2.13-2.27 (m, 1H) 2.87-2.98 (m, 2H) 3.29-3.33 (m, 2H) 3.73-3.91 (m, 2H) 4.25-4.46 (m, 3H) 5.58-5.67 (m, 1H) 8.25-8.29 (m, 2H) 8.83 (s, 1H). MS(ESI): found: $[M+H]^+$, 706.5.

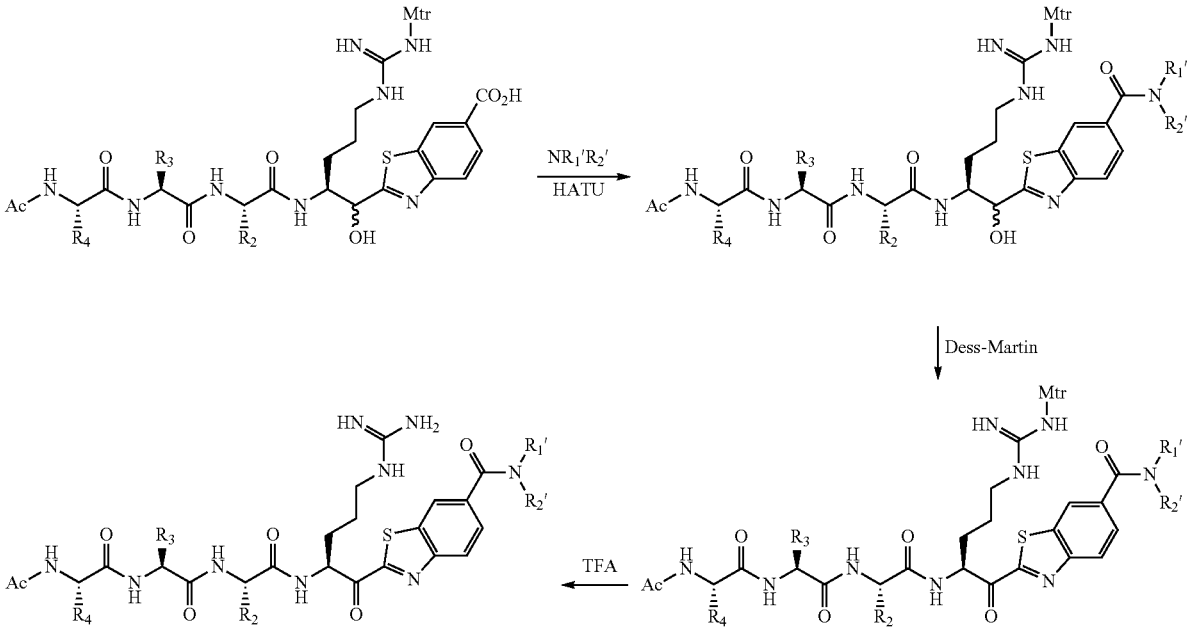


[0226] Ac-SKLR (SEQ ID NO: 10)-ketobenzothiazole-6-CONH-Phe-amide (7125). The title compound was synthesized using the same procedure as for BocHN-Arg(Mtr) ketobenzothiazole-6-CONH-Val-amide, starting with the coupling of Ac-S(^{t}Bu)-K(Boc)-L-R(Mtr)-ketobenzothiazole-COOH and L-phenylalanine amide hydrochloride. Yield: 25%. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.83-0.93 (m, 6H) 1.35-2.05 (m, 15H) 2.12-2.25 (m, 1H) 2.86-2.97 (m, 2H) 3.02-3.12 (m, 1H) 3.21-3.38 (m, 3H) 3.72-3.90 (m, 2H) 4.25-4.45 (m, 3H) 4.85-4.94 (m, 1H) 5.56-5.64 (m, 1H) 7.17-7.22 (m, 1H) 7.24-7.35 (m, 4H) 7.93-7.99 (m, 1H) 8.20-8.26 (m, 1H) 8.47-8.51 (m, 1H). MS(ESI): found: $[M+H]^+$, 852.7.

[0227] Ac-SKLR (SEQ ID NO: 10)-ketobenzothiazole-6-CONH-Trp-amide (7124). The title compound was synthesized using the same procedure as for Ac-SKLR(SEQ ID NO: 10)-ketobenzothiazole-6-CONH-Phe-amide. Yield: 24%. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.84-0.93 (m, 6H) 1.33-2.05 (m, 15H) 2.11-2.25 (m, 1H) 2.83-2.97 (m, 2H) 3.20-3.36 (m, 3H) 3.40-3.50 (m, 1H) 3.71-3.90 (m, 2H) 4.24-4.46 (m, 3H) 4.91-5.00 (m, 1H) 5.54-5.65 (m, 1H) 6.97-7.03 (m, 1H) 7.06-7.11 (m, 1H) 7.18 (s, 1H) 7.30-7.35 (m, 1H) 7.65-7.71 (m, 1H) 7.91-7.97 (m, 1H) 8.00-8.06 (m, 1H) 8.18-8.23 (m, 1H) 8.36-8.39 (m, 1H). MS(ESI): found: $[M+H]^+$, 891.7.

[0228] Ac-SKLR (SEQ ID NO: 10)-ketobenzothiazole-6-CONH-4-pyridine (7126). The title compound was synthesized using the same procedure as for Ac-SKLR(SEQ ID NO: 10)-ketobenzothiazole-6-CONH-Phe-amide. Yield: 18%. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.84-0.93 (m, 6H) 1.35-2.05 (m, 15H) 2.15-2.29 (m, 1H) 2.86-2.98 (m, 2H) 3.24-3.37 (m, 2H) 3.73-3.98 (m, 2H) 4.23-4.47 (m, 3H) 5.57-5.69 (m, 1H) 8.23-8.29 (m, 1H) 8.35-8.44 (m, 3H) 8.67-8.72 (m, 2H) 8.85 (s, 1H). MS(ESI): found: $[M+H]^+$, 782.7.

[0230] Into the solution of e (0.083 g, 0.066 mmol) in CH_2Cl_2 (15 mL), Dess-Martin periodinane (DMP) (0.042 g, 0.099 mmol) was added. The mixture was stirred at room temperature for 3 hours, then quenched with 1 M $Na_2S_2O_3$ aqueous solution. The organic phase was separated and the aqueous was extracted with CH_2Cl_2 2 times. The organic phases were combined, dried with Na_2SO_4 and concentrated in vacuo. Into the resulting residue 3 mL of TFA/thioanisole/water (95/2.5/2.5 (v/v/v)) was added. The mixture was stirred at room temperature for 4 hours. Then cold ether (40 mL) was added. The resulting precipitate, which is the crude product, was collected by centrifugation, then by decanting out carefully ether solvent. The crude product was purified by HPLC (C18, 15*150 mm column; eluent: acetonitrile/water (0.05% TFA)) to give the title compound Ac-SKLR (SEQ ID NO: 10)-ketobenzothiazole-6-CONH-4-piperidine (0.022 g) in 42% yield. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.87 (d, J=6.26 Hz, 3H) 0.91 (d, J=6.26 Hz, 3H) 1.33-2.05 (m, 17H) 2.15-2.30 (m, 3H) 2.85-2.98 (m, 2H)

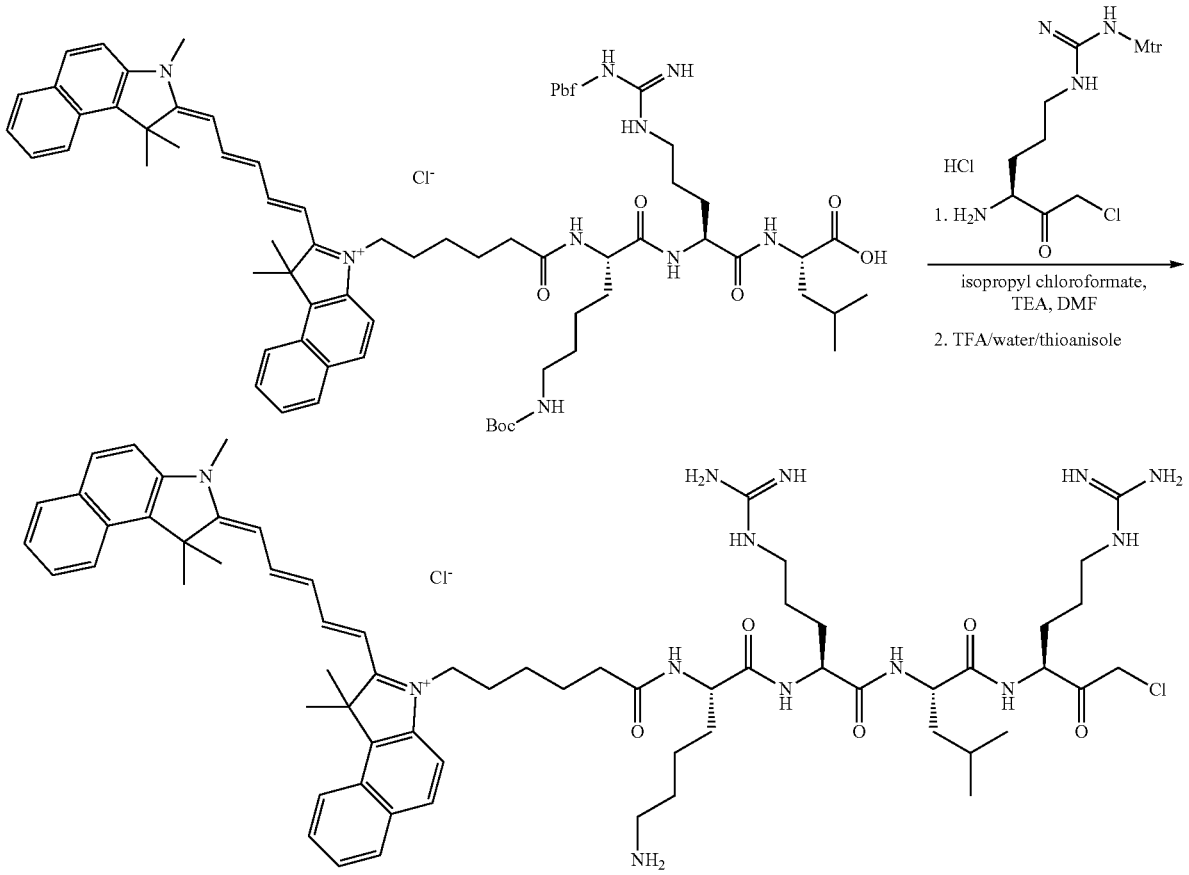


[0229] Ac-SKLR (SEQ ID NO: 10)-ketobenzothiazole-6-CONH-4-piperidine (7140). Under nitrogen atmosphere, at 0° C. anhydrous DMF (5 mL) was added into the RB flask containing Ac-S(^{t}Bu)-K(Boc)-L-Arg(Mtr)-CH(OH)benzothiazole-6-COOH d (0.088 g, 0.081 mmol) and HATU (0.037 g, 0.097 mmol). The mixture was stirred for 10 minutes, then 4-amino-1-Boc-piperidine (0.019 g, 0.097 mmol) and N,N-diisopropylethylamine (0.031 g, 0.24 mmol) were added sequentially. The mixture was stirred while being warmed to room temperature naturally. The reaction was monitored by LCMS until completion. DMF was removed and to the resulting residue, water (20 mL) was added. The precipitate formed was collected and purified by silica gel chromatography with CH_2Cl_2/MeOH combination as eluent to give Ac-S(^{t}Bu)-K(Boc)-L-Arg(Mtr)-CH(OH) benzothiazole-6-CONH-4-1-Boc-piperidine e (0.088 g, mixture of diastereomers) in 86% yield. MS(ESI): found: $[M+H]^+$, 1259.0.

3.10-3.23 (m, 2H) 3.23-3.38 (m, 2H) 3.43-3.55 (m, 2H) 3.72-3.90 (m, 2H) 4.15-4.46 (m, 4H) 5.55-5.71 (m, 1H)

8.06-8.11 (m, 1H) 8.25-8.30 (m, 1H) 8.60-8.63 (m, 1H) MS(ESI): found: $[M+H]^+$, 788.7.

[0231] Ac-SKLR (SEQ ID NO: 10)-ketobenzothiazole-6-CONH-Bn (7139). The title compound was synthesized



using the same procedure as for Ac-SKLR (SEQ ID NO: 10)-ketobenzothiazole-6-CONH-4-piperidine. Yield: 33%. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 0.81-0.95 (m, 6H) 1.31-2.09 (m, 15H) 2.12-2.26 (m, 1H) 2.84-2.98 (m, 2H) 3.20-3.40 (m, 2H) 3.65-3.91 (m, 2H) 4.20-4.46 (m, 3H) 4.59-4.66 (m, 2H) 5.57-5.67 (m, 1H) 7.22-7.29 (m, 1H) 7.30-7.41 (m, 4H) 8.08-8.13 (m, 1H) 8.21-8.34 (m, 1H) 8.57-8.67 (m, 1H). MS(ESI): found: $[M+H]^+$, 795.6.

[0232] Cy5.5-KRLR (SEQ ID NO: 8)-OH (RSC-1177). To H-K(Boc)R(Pbf)L-2-Cl Trt resin [(190 mg, 0.067 mmol), synthesized in a similar fashion to 7-AMINE)] was added 5 mL of DMF and 5 mL of DCM. After 30 min, the solvent was filtered and a solution of Cy5.5-NHS ester (71 mg, 0.10 mmol, Lumiprobe) in DMF (5 mL) and Hunig's Base (0.025 mL, 0.13 mmol) were added, then stirred overnight at room temperature. The solvent was filtered off and the resin washed thoroughly with DMF and DCM, then dried under vacuum. To the resin was added a 1:3 mixture of HFIP and DCM (15 mL) and after 45 minutes of gently shaking, the solvent was removed and then replenished. After 30 minutes of additional shaking, the solvent was removed. The combined filtrates were concentrated in vacuo to give 107 mg of crude material. Purification was achieved with 5-50% MeOH/DCM on silica gel. Yield 65 mg, 73%. Chemical Formula: $C_{76}H_{102}N_9O_{10}S^+Cl^-$, Exact Mass: 1332.75, MS(ESI): found: $[M]^+$, 1332.9.

[0233] Cy5.5-KRLR (SEQ ID NO: 8)-cmk (RSC-1179). To a cooled (ice bath) solution of Cy5.5-KRLR (SEQ ID NO: 8)-OH (15 mg, 0.011 mmol) in THF (1 mL) was added N-methylmorpholine (0.011 mmol) and isopropyl chloroformate (0.011 mL, 1M solution in toluene. The reaction mixture was stirred for 10-15 minutes and then trimethylamine (0.011 mmol) and (S)—N—(N-(4-amino-6-chloro-5-oxohexl)carbamimidoyl)-4-methoxy-2,3,6-trimethylbenzenesulfonamide hydrochloride [aE. Gherardi, W. Birchmeier, C. Birchmeier, G. Vande Woude, *Nature reviews. Cancer* 2012, 12, 89-103; bE. C. Smyth, F. Sclafani, D. Cunningham, *Oncotargets Ther* 2014, 7, 1001-1014.] (5 mg, 0.011 mmol) in DMF (0.2 mL) were added followed by stirring for an additional 2 hours. The solvent was removed and then 1.5 mL of cleavage cocktail (38:1:1 TFA/water/thioanisole) was added to the resin. After gentle shaking overnight, the solvent was removed in vacuo, triturated in toluene, and then concentrated in vacuo. The residue was purified by C_{18} reverse phase HPLC to give 10 mg of crude product which was re-purified to give 4 mg of pure title compound as the TFA salt. Chemical Formula: $C_{65}H_{91}ClN_{13}O_5$+Cl−, Exact Mass: 1168.69, MS(ESI): found: $[M]^+$, 1168.8.

[0234] Cy5.5-KRLR (SEQ ID NO: 8)-kbt (RSC-1178). Synthesized in a similar manner to Ac-KQLR (SEQ ID NO:

1) kbt (3) from Cy5.5-KRLR (SEQ ID NO: 8)-OH. Chemical Formula: $C_{71}H_{93}N_{14}O_5S+Cl-$, Exact Mass: 1253.72, MS(ESI): found: $[M]^+$, 1253.7.

Example 6. Inhibition Studies with Polypeptide Ketobenzothiazoles

[0235] Fluorescent inhibitor and chromogenic proteolytic assays were performed in general accordance with the procedures described in Example 4 for compounds prepared in Examples 5 and 6.

[0236] The results of the assays are presented in Table 6.1. The results show increased potency of 2-fold of the ketobenzothiazoles over thiazole 5. More importantly when we substitute a Val on the benzothiazole ring 36c as in 8, we increase the HGFA potency another two-fold (Ki 13 nM) but decreased matriptase activity >10-fold (Ki 7 nM) and >3-fold for hepsin (Ki 0.3 nM). Ac-SKLR (SEQ ID NO: 10)-kbt V amide (9) showed a 4-fold enhancement in HGFA potency with no effect on hepsin or matriptase. We discovered Ac-KQLR (SEQ ID NO: 1)-kbt (7) improves potency 2-fold and further substitution of the benzothiazole ring with Valine, Ac-KQLR (SEQ ID NO: 1)-kbt V amide (8) increases potency another 2-fold for HGFA (Ki 13 nM) but decreased matriptase activity >10-fold (Ki 7 nM) and >3-fold for hepsin (Ki 0.3 nM). This result demonstrates that these inhibitor compounds have improved selectivity for HGFA.

TABLE 6.1

		Fluorogenic Assay		
Compound No.	$Y-P_4-P_3-P_2-P_1-Z$	HGFA Ki (nM) (nM)	Matriptase Ki (nM)	Hepsin Ki
7185-1	Fmoc-AR-kbt	7983.5	0.5	272.37
7185-2	Fmoc-RR-kbt	8633.5	2.0	99.92
7185-3	Fmoc-NR-kbt	>20000	84.1	1.92
7185-4	Fmoc-DR-kbt	>20000	397.9	232.92
7185-6	Fmoc-QR-kbt	6827	1.6	170.07
7185-7	Fmoc-ER-kbt	>20000	8.4	337.63
7185-8	Fmoc-GR-kbt	9375	0.1	831.00
7185-9	Fmoc-HR-kbt	6356.19	66.7	268.07
7185-10	Fmoc-IR-kbt	6466.05	657.1	297.32
7185-11	Fmoc-LR-kbt	449.776	425.3	114.90
7185-12	Fmoc-KR-kbt	>20000	0.2	159.25
7187-13	Fmoc-MR-kbt	1208.00	62.1	489.97
7187-14	Fmoc-FR-kbt	963.50	1281.5	777.88
7187-15	Fmoc-PR-kbt	>20000	40.4	776.25
7187-16	Fmoc-SR-kbt	>20000	90.00	885.50
7188-17	Fmoc-TR-kbt	>20000	1217.00	436.75
7188-18	Fmoc-WR-kbt	3197.00	823.5	410.62
7188-19	Fmoc-YR-kbt	2961.00	331.6	217.38
7188-20	Fmoc-VR-kbt	4633.83	1488.0	313.60
1-13A1	H-WFR-kbt	5681.25	1871.5	240.15
1-18A1	H-dWFR-kbt	2250	135.3	369.80
1-15A1	H-dWLR-kbt	127.98	34.6	3.58
1-45A1	H-WFR-kbt-COOH	981	232	1.41
7171	H-RLR-kbt	83.07	3.3	0.50
1-56A1	H-WLR-kbt-COOH	1379	1206.5	446.30
1-57A1	H-dWLR-kbt-COOH	345.9	363.6	136.50

TABLE 6.1-continued

Compound No.	Y-P_4-P_3-P_2-P_1-Z	Fluorogenic Assay HGFA Ki (nM) (nM)	Matriptase Ki (nM)	Hepsin Ki
1-54A1	H-His(Bom)-WLR-kbt	3711.83	1549.8	2.08
1-58A1	H-His(Bom)-dWLR-kbt	2282.17	1504.7	1.13
7182	H-LLR-kbt-Val-NH_2	82.09	23.6	0.88
7181	H-WLR-kbt-Val-NH_2	73.89	49.2	1.31
7180	H-RLR-kbt-Val-NH_2	12.50	4.2	0.44
7170	H-WLR-kbt	103.98	33.5	1.52
7115	Ac-KRLR(SEQ ID NO: 8)-kbt-Val-NH_2	5.83	7.415	0.2265
7054	Ac-KRLR(SEQ ID NO: 8)-kbt	6.00	0.540	0.112
7117	H-WRLR(SEQ ID NO: 9)-kbt	8.75	3.79	0.12
7055	Ac-KQLR(SEQ ID NO: 1)kbt-Val-NH_2	12.00	6.98	0.29
7116	Ac-SKLR(SEQ ID NO: 10)-kbt-Val-NH_2	12.98	10.3	0.19
7124	Ac-SKLR(SEQ ID NO: 10)-kbt-Trp-NH_2	15.75	3.74	0.098
7125	Ac-SKLR(SEQ ID NO: 10)-kbt-Phe-NH_S	17.28	6.26	0.14
7006	Ac-KQLR(SEQ ID NO: 1)-kbt	30.00	0.550	0.09
7126	Ac-SKLR(SEQ ID NO: 10)-kbt-4-pyridinylamide	31.76	3.95	0.14
7053	Ac-SKLR(SEQ ID NO: 10)-kbt	33.00	3.05	0.16
7063	Ac-FLFR(SEQ ID NO: 19)-kbt	114.00	3.60	1.44
7118	Ac-SKLR(SEQ ID NO: 10)-kbt-COOH	131.97	54.8	0.173
7064	Ac-WLFR(SEQ ID NO: 20)-kbt	133.00	5.960	0.347
7139	Ac-SKLR(SEQ ID NO: 10)-kbt-benzylamide	9.10	0.360	0.80
7140	Ac-SKLR(SEQ ID NO: 10)-kbt-4-piperidinylamide	7.70	1.28	0.20
7165	Ac-KRLR(SEQ ID NO: 8)-kbt-Phe-COOH	6.07	5.14	0.37
7164	Ac-KRLR(SEQ ID NO: 8)-kbt-Benzyl-3-COOH	3.33	3.60	0.30
7159	Ac-KRLR(SEQ ID NO: 8)-kbt-Benzyl-3-COOMe	7.76	1.80	0.36
7158	Ac-KRLR(SEQ ID NO: 8)-kbt-Benzyl-4-COOMe	5.85	0.98	0.26
1179	Cy5.5-KRLR(SEQ ID NO: 8)-cmk	34.14	172.2	323.53
1178-2	Cy5.5-KRLR(SEQ ID NO: 8)-kbt	608	87.5	3.89

[0237] The results of selectivity assays are presented in Table 6.2.

TABLE 6.2

Compound No.	Y-P_4-P_3-P_2-P_1-Z	Fluorogenic Assay Thrombin Ki (nM)	Factor Xa Ki (nM)	Trypsin Ki (nM)
7118	Ac-SKLR(SEQ ID NO: 10)-kbt-COOH	>2000	1247	0.03
7006	Ac-KQLR(SEQ ID NO: 1)-kbt	>20000	129	0.24
7055	Ac-KQLR(SEQ ID NO: 1)-kbt-Val-NH_2	3212	66	0.07
7053	Ac-SKLR(SEQ ID NO: 10)-kbt	>20000	1901	0.16
7116	Ac-SKLR(SEQ ID NO: 10)-kbt-Val-NH_2	4505	385	0.34
7117	H-WRLR(SEQ ID NO: 9)-kbt	4890	2.4	0.96
7063	Ac-FLFR(SEQ ID NO: 19)-kbt	>20000	13.1	0.27
7054	Ac-KRLR(SEQ ID NO: 8)-kbt	>20000	91.9	0.82
7064	Ac-WLFR(SEQ ID NO: 20)-kbt	>20000	1.0	
7115	Ac-KRLR(SEQ ID NO: 8)-kbt-Val-NH_2	1560	17.0	
7124	Ac-SKLR(SEQ ID NO: 10)-kbt-Trp-NH_2	1231	163	0.02
7125	Ac-SKLR(SEQ ID NO: 10)-kbt-Phe--NH_2	3640	544	0.27
7126	Ac-SKLR(SEQ ID NO: 10)-kbt-pyridine-NH_2	3431	221	1.06
7139	Ac-SKLR(SEQ ID NO: 10)-kbt-benzyl	2467	190	0.05
7140	Ac-SKLR(SEQ ID NO: 10)-kbt-piperidine	2948	447	0.41
7158	Ac-KRLR(SEQ ID NO: 8)-kbt-benzyl-4-COOMe	20.92	9.0	0.05
7159	Ac-KRLR(SEQ ID NO: 8)-kbt-Benzyl-3-COOMe	85.38	14	0.04
7164	Ac-KRLR(SEQ ID NO: 8)-kbt-benzyl-4-COOH	1618		
7170	H-WLR-kbt	457.00	532.00	0.050
7171	H-RLR-kbt	8198	56	0.13
7182	H-LLR-Val-NH_2	101.4	159	0.61
7181	H-WLR-kbt-Val-NH_2	41.04	159	0.01
7180	H-RLR-kbt-Val-NH_2	548	18	0.31
7165	Ac-KRLR(SEQ ID NO: 8)-kbt-benzyl-3-COOH	1944	20.2	1.06
7187-14	Fmoc-FR-kbt	>2000	28.4	14.350
7185-11	Fmoc-LR-kbt	995	410	19.78

Example 7. Inhibition Studies with Cyclic Peptides

[0238] Fluorescent inhibitor and chromogenic proteolytic assays were performed in general accordance with the procedures described in Example 4 for compounds prepared in Example 3. Table 7.1 presents the results for a cyclic peptide compound 7074.

TABLE 7.1

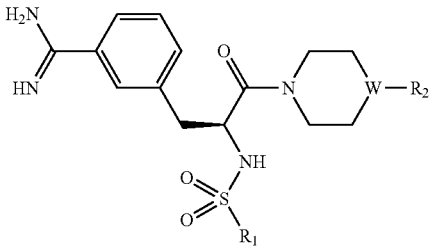
		Fluorogenic Assay		
Compound	Y-P_5-P_4-P_3-P_2-P_1-Z	HGFA Ki (nM)	Matriptase Ki (nM)	HGFA Ki (nM)
7074	H-cyclo(KQD)RR-kt	5941.67	0.71	1.55

Example 8. Synthesis of Benzamidine Inhibitor Compounds

[0239] The benzamidine inhibitors listed in Table 8.1 were synthesized in accordance with the general scheme 8A shown below and the procedures described below. Phenylalanine naphthyl sulfonamides were synthesized in accordance with general scheme B and the procedures described below.

TABLE 8.1

Benzamidine inhibitors.



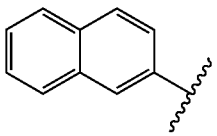
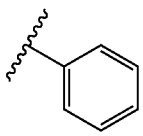
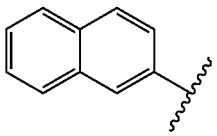
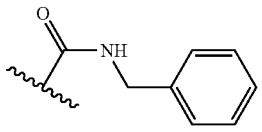
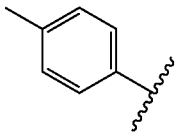
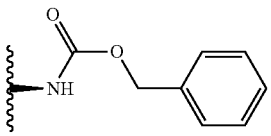
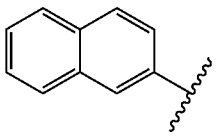
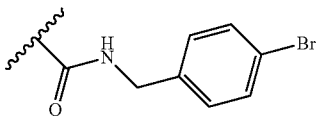
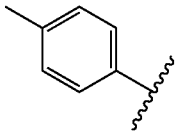
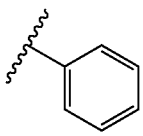
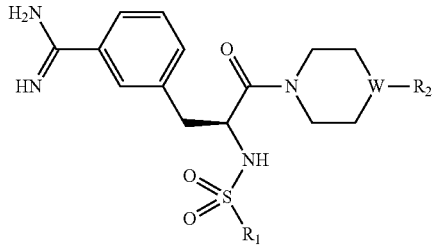
Compound	R_1	R_2	W
16			C
17a			N
18*			C
25a			N
25b			C

TABLE 8.1-continued

Benzamidine inhibitors.



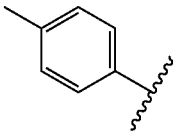
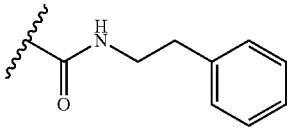
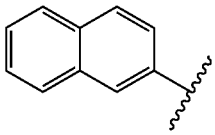
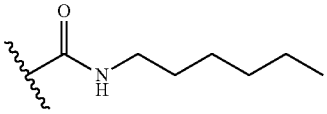
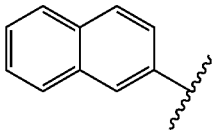
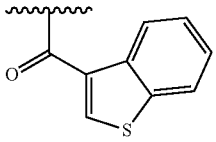
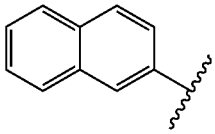
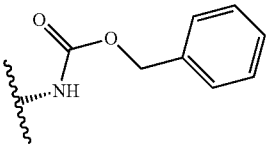
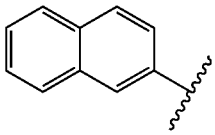
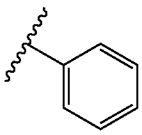
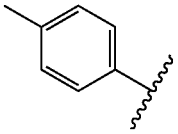
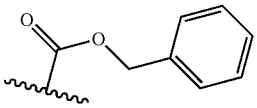
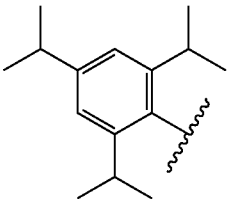
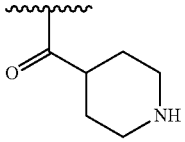
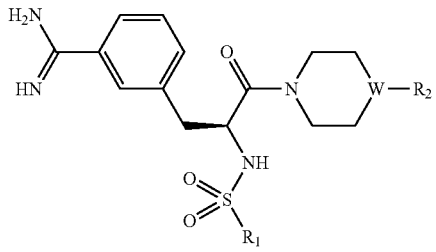
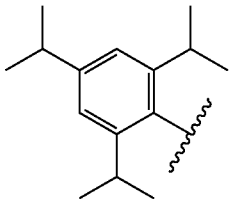
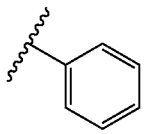
Compound	R_1	R_2	W
25c*			C
25d			N
25e			N
25f*			C
25g			N
25h			N
26			N

TABLE 8.1-continued

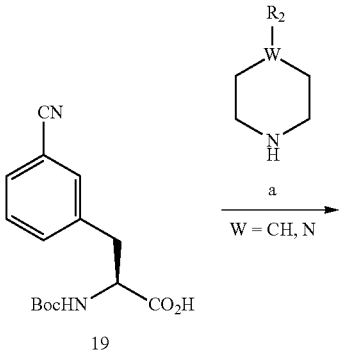
Benzamidine inhibitors.



Compound	R_1	R_2	W
27			N

*R2 is substituted on the 3-position of the piperidine ring.

Scheme 8A. Synthesis of 3-amidinophenylalanine sulfonamide analogs



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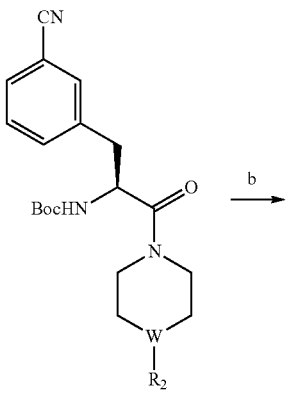

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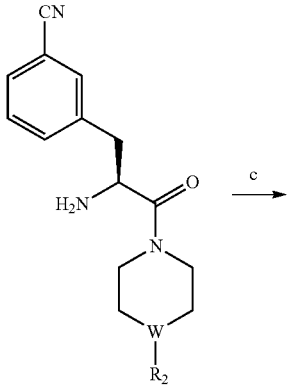

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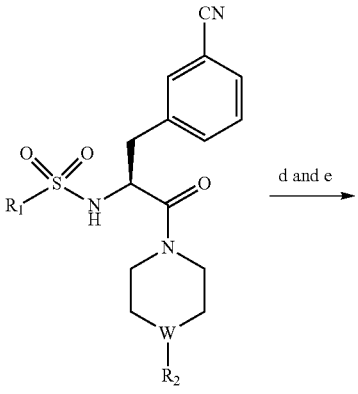

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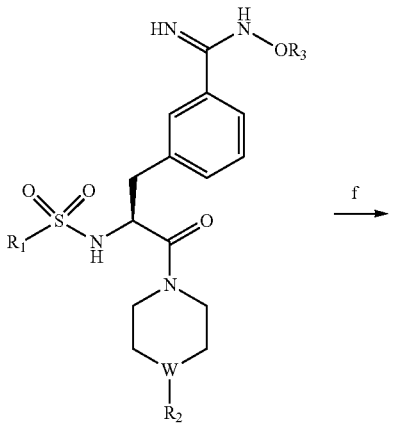
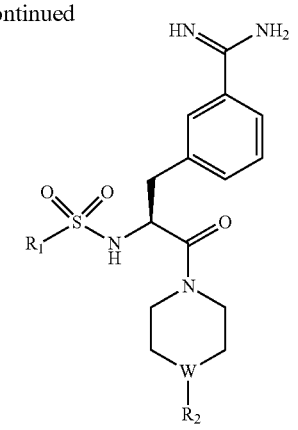


23 R3 = H
24 R3 = Ac

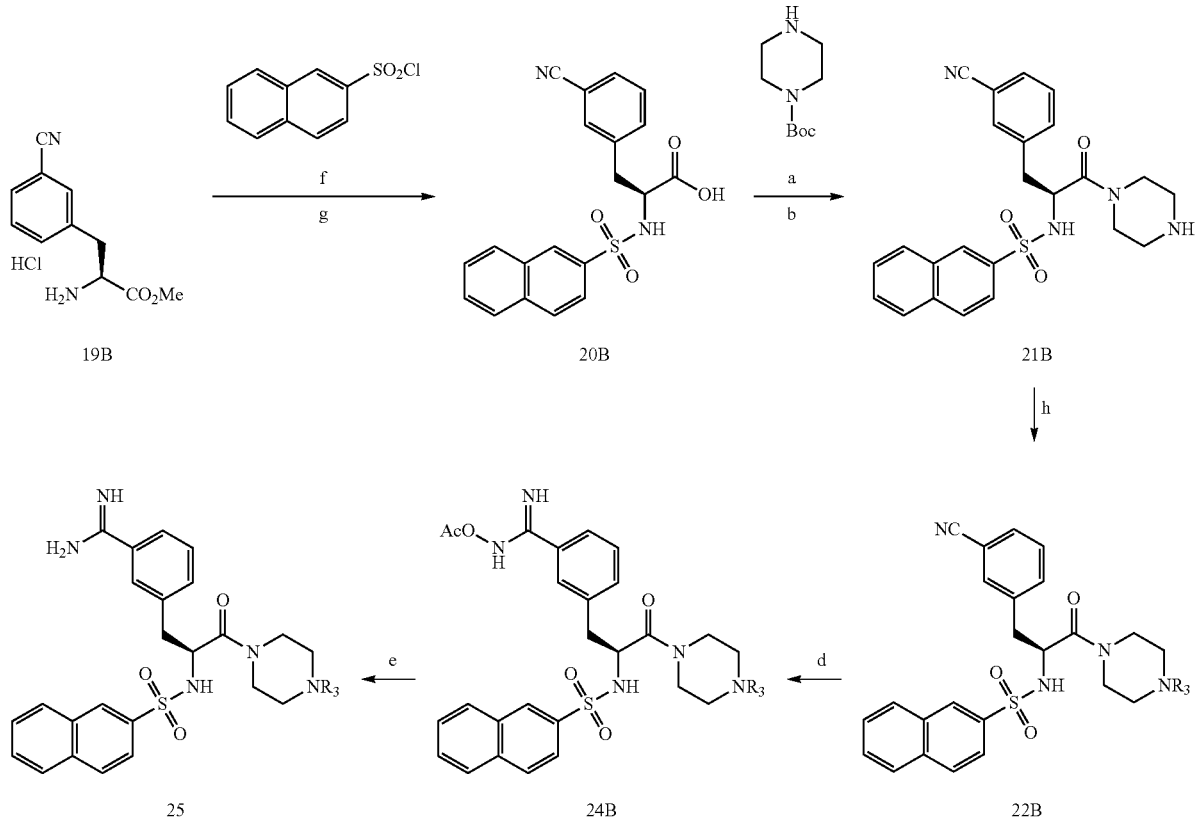
-continued



25 R1 = 4-tosyl or
2-naphthyl
26 and 27 R1 = 2,4,6-triisopropylphenyl

[0240] Reagents and conditions in Scheme A are as follows a) HATU, THF (or DMF), DIPEA; b) 4N HCl in 1,4-doxane; c) R_1SO_2Cl, DMAP, pyridine, THF; d) i. NH_2OH—HCl, $K2CO_3$ (or DIPEA), EtOH; e) Ac_2O, AcOH; f) Zn, AcOH.

Scheme 8B. Synthesis of 3-amidinophenylalanine sulfonamide analogs



[0241] Reagents and conditions in Scheme B are as follows a) HATU, THF (or DMF), DIPEA; b) 4N HCl in 1,4-doxane; d) i. NH_2OH—HCl, K_2CO_3 (or DIPEA), EtOH; ii. Ac_2O, AcOH; e) Zn, AcOH; f) DMAP, pyridine, THF; g) LiOH, THF; h) HATU, RCO_2H (R_3═COR) or R—N═C═O (R_3═CONHR).

[0242] General Procedure A: General procedure for the synthesis of amides (20): A solution of N-Boc-3-cyanophenylalanine (19) in dry DMF (0.5 M) was added Hunig's Base (4.0 equiv) followed by HATU (1.3 equiv). The yellow solution was allowed to stir for 10 minutes and was then added amine (1.3 equiv). The resulting mixture was stirred at room temperature. After two hours, the reaction was checked by LCMS and was complete. The reaction mixture was diluted with ethyl acetate, washed with water (1×), brine (2×), dried ($MgSO_4$), filtered and concentrated to oil. The crude product was purified by MPLC (0-75% hexanes/ethyl acetate) to afford pure product.

[0243] General Procedure B: General procedure for BOC deprotection (21): A solution of N-Boc protected amide in dioxane (0.4 M) was added 4.0 M HCl in dioxane (10 equiv). The resulting mixture was allowed to stir at room temperature. After 4 hours, the reaction was checked by LCMS and was complete. The solvent was removed under reduced pressure to afford pure product.

[0244] General Procedure C: General procedure for sulfonamideformation (22): A mixture of amide in THF (0.2 M) was added Hunig's Base (10 equiv) and the mixture was stirred until all solids dissolved. The solution was then added DMAP (10 mol %) followed by arylsulfonyl chloride (1.1 equiv) and stirred at room temperature. After 2 hours the reaction was checked by LCMS and was complete. The reaction was diluted with ethyl acetate, washed with saturated $NaHCO_3$ (2×), brine (1×), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude material was purified via MPLC (0-75% ethyl acetate/hexanes) to afford pure product as a solid.

[0245] General Procedure D: General procedure for formation of the amidoxime (23): A suspension of sulfonamide in dry ethanol (0.2M) was added hydroxylamine hydrochloride (2.2 equiv) followed by Hunig's base (2.3 equiv). The resulting mixture was heated to 75° C. for approximately 3 hours which resulted in a homogeneous solution. The reaction mixture was allowed to cool to room temperature, checked by LCMS and was complete. The solvent was removed under reduced pressure to afford the crude amidoxime intermediate as a white solid.

[0246] General Procedure E: General procedure for acetylation of amidoxime (24). A solution of crude amidoxime in acetic acid (0.2 M) was added acetic anhydride at room temperature. The resulting mixture was allowed to stir at room temperature. After approximately 2 hours the reaction was checked by LCMS and was complete. The solvent was removed under reduced pressure to afford the crude product.

[0247] General Procedure F: General procedure for preparation of amidines (25) and (26): A solution of O-acetylamidoxime in acetic acid (0.2 M) was added zinc dust (15 equiv) at room temperature. The resulting suspension was stirred for 3 hours at room temperature. The reaction was checked by LCMS and was complete. The mixture was filtered and concentrated under reduced pressure to give the crude product as an oil. The crude product was purified via reverse phase HPLC (5-65% acetonitrile/water/0.05% trifluoroacetic acid) to afford pure product as a white solid after lyophilization.

[0248] (S)-3-(2-(Naphthalene-2-sulfonamido)-3-oxo-3-(4-phenylpiperidin-1-yl)propyl)benzimidamide (16). Prepared from N-acetoxy-3-[(2S)-2-[(2-naphthylsulfonyl) amino]-3-oxo-3-(4-phenylpiperidin-1-yl)propyl] benzenecarboximidamide using General Procedure F. Purified by reversed-phase HPLC (5-75% acetonitrile/water/0.05% TFA), Yield (0.092 g, 52.5%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.26 (s, 2H), 8.23-8.50 (m, 2H), 7.94-8.10 (m, 2H), 7.56-7.78 (m, 4H), 7.35-7.56 (m, 2H), 7.07-7.33 (m, 3H), 6.96 (d, J=7.42 Hz, 1H), 6.78 (d, J=7.14 Hz, 1H), 4.49-4.79 (m, 1H), 3.82-4.21 (m, 2H), 3.61-3.79 (m, 1H), 3.41-3.56 (m, 2H), 2.89-3.06 (m, 1H), 2.72-2.89 (m, 1H), 2.59 (t, J=12.22 Hz, 1H), 1.31-1.64 (m, 2H), 0.75-1.08 (m, 1H), 0.38-0.67 (m, 1H). LRMS (ESI) $C_{31}H_{32}N_4O_3S$ found [M+H]: 541.2.

[0249] N-Acetoxy-3-[(2S)-2-[(2-naphthylsulfonyl) amino]-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzenecarboximidamide. Prepared from N-hydroxy-3-[(2S)-2-[(2-naphthylsulfonyl)amino]-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzenecarboximidamide using General Procedure E. Purification by MPLC (0-90% EtOAc/hexanes). Yield (0.17 g, 47.7%). LRMS (ESI) $C_{33}H_{34}N_4O_5S$ found [M+H]: 599.2.

[0250] N-Hydroxy-3-[(2S)-2-[(2-naphthylsulfonyl) amino]-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzenecarboximidamide. Prepared from tert-butyl (S)-(3-(3-cyanophenyl)-1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl) carbamate (0.312 g, 0.589 mmol) using General Procedure D. LRMS (ESI) $C_{31}H_{32}N_4O_4S$ found [M+H]: 557.2.

[0251] N-[(2S)-3-(3-Cyanophenyl)-1-oxo-1-(4-phenylpiperidin-1-yl)propan-2-yl]naphthalene-2-sulfonamide. Prepared from 3-[(2S)-2-amino-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzonitrile (4.1 g, 20.1 mmol) using General Procedure C. Yield (0.312 g, 79%). LRMS (ESI) $C_{31}H_{29}N_3O_3S$ found [M+H]: 524.2.

[0252] 3-[(2S)-2-Amino-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzonitrile. Prepared from tert-butyl [(2S)-3-(3-cyanophenyl)-1-oxo-1-(4-phenylpiperidin-1-yl)propan-2-yl]carbamate (0.67 g, 1.54 mmol) using General Procedure B. LRMS (ESI) $C_{21}H_{23}N_3O$ found [M+H]: 334.2.

[0253] tert-butyl [(2S)-3-(3-Cyanophenyl)-1-oxo-1-(4-phenylpiperidin-1-yl)propan-2-yl]carbamate. Prepared from N-Boc-3-cyanophenylalanine (19) (0.56 g, 1.81 mmol) and N-phenylpiperidine (0.32 g, 2.01 mmol) using General Procedure A. Purified by MPLC (0-75% EtOAc/Hexanes). Yield (0.67 g, 85%). LRMS (ESI) $C_{26}H_{31}N_3O_3$ found [M+H]: 434.2.

[0254] (S)-3-(2-(Naphthalene-2-sulfonamido)-3-oxo-3-(4-phenylpiperazin-1-yl)propyl)benzimidamide (25g). Prepared from (S)—N-acetoxy-3-(2-(naphthalene-2-sulfonamido)-3-oxo-3-(4-phenylpiperazin-1-yl)propyl) benzimidamide (0.123 g, 0.205 mmol) using General Procedure F. Purified by reversed-phase HPLC (5-60% acetonitrile/water/0.05% TFA), Yield (0.069 g, 54.7%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.42 (s, 2H), 9.29 (s, 2H), 8.22-8.51 (m, 2H), 7.85-8.12 (m, 3H), 7.36-7.74 (m, 7H), 7.08-7.28 (m, 2H), 6.61-6.90 (m, 3H), 4.49-4.75 (m, 1H), 3.37-3.61 (m, 1H), 3.19-3.37 (m, 1H), 3.03-3.18 (m, 1H), 2.96 (dd, J=7.00, 13.32 Hz, 2H), 2.52-2.89 (m, 4H), 2.25-2.45 (m, 1H). LRMS (ESI) $C_{30}H_{31}N_5O_3S$ found [M+H]: 542.2.

[0255] (S)—N-Acetoxy-3-(2-(naphthalene-2-sulfonamido)-3-oxo-3-(4-phenylpiperazin-1-yl)propyl)benzimidamide. Prepared from N-hydroxy-3-[(2S)-2-[(2-naphthylsulfonyl)amino]-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzenecarboximidamide (0.26 mmol) using General Procedure E. Purification by MPLC (0-40% EtOAc/hexanes). Yield (0.123 g, 79%). LRMS (ESI) $C_{32}H_{33}N_5O_5S$ found [M+H]: 600.2.

[0256] (S)—N-Hydroxy-3-(2-(naphthalene-2-sulfonamido)-3-oxo-3-(4-phenylpiperazin-1 yl)propyl)benzimidamide. Prepared from (S)—N-(3-(3-cyanophenyl)-1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl)naphthalene-2-sulfonamide (0.312 g, 0.589 mmol) using General Procedure D. LRMS (ESI) $C_{30}H_{31}N_5O_4S$ found [M+H]: 558.2.

[0257] (S)—N-(3-(3-Cyanophenyl)-1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl)naphthalene-2-sulfonamide. Prepared from (S)-3-(2-amino-3-oxo-3-(4-phenylpiperazin-1-yl)propyl)benzonitrile (0.121 g, 0.327 mmol) using General Procedure C. Purified by MPLC (0-75% EtOAc/Hexanes). Yield (0.133 g, 79.5%). LRMS (ESI) $C_{30}H_{28}N_4O_3S$ found [M+H]: 525.2.

[0258] (S)-3-(2-Amino-3-oxo-3-(4-phenylpiperazin-1-yl)propyl)benzonitrile. Prepared from tert-butyl [(2S)-3-(3-cyanophenyl)-1-oxo-1-(4-phenylpiperidin-1-yl)propan-2-yl]carbamate (0.347 g, 0.80 mmol) using General Procedure B. Purified product obtained by trituration in ether followed by filtration and drying. Yield (0.29 g, 97%). LRMS (ESI) $C_{20}H_{22}N_4O$ found [M+H]: 335.2.

[0259] tert-butyl (S)-(3-(3-Cyanophenyl)-1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl)carbamate. Prepared from N-Boc-3-cyanophenylalanine (19) (0.24 g, 0.83 mmol) and N-phenylpiperazine (0.92 mmol) using General Procedure A. Purified by MPLC (0-75% EtOAc/Hexanes). Yield (0.35 g, 96%). LRMS (ESI) $C_{25}H_{30}N_4O_3$ found [M+H]: 435.2.

[0260] (S)-Benzyl 4-(3-(3-carbamimidoylphenyl)-2-(4-methylphenylsulfonamido)propanoyl)piperazine-1-carboxylate (25h). Prepared from benzyl (S)-4-(3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-((4-methylphenyl) sulfonamido)propanoyl)piperazine-1-carboxylate (0.141 g, 0.228 mmol) using General Procedure F. Purified by reversed-phase HPLC (5-75% acetonitrile/water/0.05% TFA), Yield (0.058 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.39 (br. s., 2H), 9.29 (br. s., 2H), 8.15 (d, J=9.34 Hz, 1H), 7.63-7.82 (m, 2H), 7.22-7.62 (m, 10H), 5.07 (s, 2H), 4.37-4.63 (m, 1H), 3.44-3.71 (m, 2H), 3.10-3.29 (m, 3H), 2.84-3.10 (m, 3H), 2.60-2.84 (m, 2H), 2.32 (s, 3H). LRMS (ESI) $C_{29}H_{33}N_5O_5S$ found [M+H]: 564.2.

[0261] Benzyl (S)-4-(3-(3-(N-acetoxycarbamimidoyl) phenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperazine-1-carboxylate. Prepared from benzyl (S)-4-(3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-((4-methylphenyl) sulfonamido)propanoyl)piperazine-1-carboxylate (0.132 g, 0.228 mmol) using General Procedure E. LRMS (ESI) $C_{31}H_{35}N_5O_7S$ found [M+H]: 622.2.

[0262] Benzyl (S)-4-(3-(3-(N-hydroxycarbamimidoyl) phenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperazine-1-carboxylate. Prepared from benzyl (S)-4-(3-(3-cyanophenyl)-2-((4-methylphenyl)sulfonamido)propanoyl) piperazine-1-carboxylate (0.24 g, 0.228 mmol) using General Procedure D. LRMS (ESI) $C_{29}H_{33}N_5O_6S$ found [M+H]: 580.2.

[0263] Benzyl (S)-4-(3-(3-cyanophenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperazine-1-carboxylate. Prepared from benzyl (S)-4-(2-amino-3-(3-cyanophenyl) propanoyl)piperazine-1-carboxylate (0.243 g, 0.522 mmol) using General Procedure C. Purified by MPLC (0-75% EtOAc/Hexanes). Yield (0.124 g, 45.6%). LRMS (ESI) $C_{29}H_{30}N_4O_5S$ found [M+H]: 547.2.

[0264] Benzyl (S)-4-(2-amino-3-(3-cyanophenyl)propanoyl)piperazine-1-carboxylate. Prepared from benzyl (S)-4-(2-((tert-butoxycarbonyl)amino)-3-(3-cyanophenyl)propanoyl)piperazine-1-carboxylate (0.257 g, 0.522 mmol) using General Procedure B. LRMS (ESI) $C_{22}H_{24}N_4O_3$ found [M+H]: 393.2.

[0265] Benzyl (S)-4-(2-((tert-butoxycarbonyl)amino)-3-(3-cyanophenyl)propanoyl)piperazine-1-carboxylate. Prepared from N-Boc-3-cyanophenylalanine (19) (1.02 g, 3.51 mmol) and N-carboxybenzyl piperazine (3.89 mmol) using General Procedure A. Purified by MPLC (0-80% EtOAc/Hexanes). Yield (0.257 g). LRMS (ESI) $C_{27}H_{32}N_4O_5$ found [M+H]: 493.2.

[0266] (S)-3-(3-Cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoic acid (20B). Prepared from methyl (S)-2-amino-3-(3-cyanophenyl)propanoate (19B) (4.1 g, 20.1 mmol) using General Procedure C to give methyl (S)-3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoate. Yield (8.19 g). LRMS (ESI) $C_{21}H_{18}N_2O_4S$ found [M+H]: 395.1. A solution of the crude ester in 1M HCl/AcOH (2:1) was refluxed for ~8 hours until no starting material was remaining by LCMS. After cooling to room temperature, the precipitate was filtered to give the title compound, Yield (8.0 g). LRMS (ESI) $C_{20}H_{16}N_2O_4S$ found [M+H]: 381.1.

[0267] (S)—N-(3-(3-Cyanophenyl)-1-oxo-1-(piperazin-1-yl)propan-2-yl)naphthalene-2-sulfonamide (21B). Using General Procedure A, the crude 20B (2.0 g, 5.26 mmol) and N-tert-butoxycarbonyl-piperazine (0.98 g, 5.26 mmol) were combined to give tert-butyl (S)-4-(3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxylate after MPLC (0-75% EtOAc/hexanes), Yield (2.15 g). LRMS (ESI) $C_{29}H_{32}N_4O_5S$ found [M+H]: 549.2. To a solution of the product in 1,4-dioxane was added HCl and stirred at room temperature overnight. After concentrating in vacuo, the residue was dissolved in MeOH and then concentrated to dryness. Purification by MPLC (0-10% MeOH/DCM gave the title product, Yield (0.8 g). ^{1}H NMR (300 MHz, dmso-d_6) δ 2.30-2.47 (m, 2H) 2.71 (dd, J=13.46, 9.07 Hz, 2H) 2.87 (dd, J=13.46, 5.77 Hz, 2H) 3.09-3.21 (m, 1H) 3.31-3.43 (m, 3H) 4.51 (dd, J=8.65, 6.46 Hz, 1H) 7.33 (t, J=7.83 Hz, 1H) 7.49 (d, J=7.69 Hz, 2H) 7.57-7.74 (m, 5H) 7.93-8.10 (m, 3H) 8.24 (s, 1H) ppm. LRMS (ESI) $C_{24}H_{24}N_4O_3S$ found [M+H]: 449.2.

[0268] (S)—N-Benzyl-4-(3-(3-carbamimidoylphenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide (17a). Prepared from (S)-4-(3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido) propanoyl)-N-benzylpiperazine-1-carboxamide using General Procedure F. Purification by reverse phase HPLC (10-60% acetonitrile/water/0.05% TFA). Yield: 24.2 mg (44%). ^{1}H NMR (300 MHz, dmso-d_6) 6 ppm 2.56-2.83 (m, 3H) 2.86-3.10 (m, 3H) 3.18 (br. s., 2H) 3.79-4.02 (m, 1H) 4.17 (m, 2H) 4.56 (m, 2H) 6.96 (s, 1H) 7.02-7.08 (m, 2H) 7.13-7.22-7.31 (m, 3H) 7.43 (m, 2H) 7.51-7.77 (m, 4H) 7.97 (br. s., 1H) 8.00-8.21 (m, 2H) 8.22-8.49 (m, 2H) 8.92 (m, 2H) 8.63 (br. S. 1H) 9.29 (br s, 1H). LRMS (ESI) $C_{32}H_{34}N_6O_4S$ found [M+H]: 599.2.

[0269] (S)-4-(3-(3-(N-Acetoxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-benzylpiperazine-1-carboxamide. Prepared from (S)—N-benzyl-4-(3-(3-

(N-hydroxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide using General Procedure E. LRMS (ESI) $C_{34}H_{36}N_6O_6S$ found [M+H]: 657.2.

[0270] (S)—N-Benzyl-4-(3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl) piperazine-1-carboxamide. Prepared from (S)—N-benzyl-4-(3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido) propanoyl)piperazine-1-carboxamide (54.4 mg, 0.094 mmol) using General Procedure D. LRMS (ESI) $C_{34}H_{36}N_6O_6S$ found [M+H]: 615.2.

[0271] (S)—N-Benzyl-4-(3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide. To a solution of 21B (50 mg, 0.119 mmol) in THF (1 mL) under a nitrogen atmosphere was added benzyl isocyanate (41 uL, 0.335 mmol). The reaction mixture was stirred overnight at room temperature. The reaction was concentrated in vacuo and the residue purified by MPLC (10-75% EtOAc/hexanes) to give the title compound. Yield (54.4 mg). LRMS (ESI) $C_{32}H_{31}N_5O_4S$ found [M+H]: 582.2.

[0272] (S)—N-(4-Bromobenzyl)-4-(3-(3-carbamimidoylphenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide (25a). Prepared from (S)-4-(3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-(4-bromobenzyl)piperazine-1-carboxamide using General Procedure F. Purification by reverse phase HPLC (25-75% acetonitrile/water/0.05% TFA). Yield: 9.8 mg (12%). ^{1}H NMR (300 MHz, dmso-d_6) δ ppm 2.56-2.83 (m, 3H) 2.83-3.00 (m, 2H) 3.04 (d, J=9.39 Hz, 1H) 3.11-3.28 (m, 2H) 3.39-3.51 (m, 1H) 3.57-3.76 (m, 1H) 4.11 (d, J=5.48 Hz, 1H) 4.42-4.61 (m, 1H) 6.99-7.27 (m, 3H) 7.27-7.52 (m, 4H) 7.52-7.74 (m, 4H) 7.82 (s, 1H) 7.89-8.17 (m, 3H) 8.17-8.43 (m, 2H) 9.09 (s, 2H) 9.32 (s, 2H). LRMS (ESI) $C_{32}H_{33}BrN_6O_4S$ found [M+H]: 677.1/679.1.

[0273] (S)-4-(3-(3-(N-Acetoxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanol)-N-(4-bromobenzyl) piperazine-1-carboxamide. Prepared from (S)—N-(4-bromobenzyl)-4-(3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide using General Procedure E. LRMS (ESI) $C_{34}H_{35}BrN_6O_6S$ found [M+H]: 635.1/637.1.

[0274] (S)—N-(4-Bromobenzyl)-4-(3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide. Prepared from (S)—N-(4-bromobenzyl)-4-(3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide (78 mg, 0.112 mmol) using General Procedure D. LRMS (ESI) $C_{32}H_{33}BrN_6O_5S$ found [M+H]: 693.1/695.1.

[0275] (S)—N-(4-Bromobenzyl)-4-(3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide. To a solution of 21B (50 mg, 0.115 mmol) in THF (1 mL) under a nitrogen atmosphere was added 4-bromobenzyl isocyanate (32 μL, 0.23-0 mmol). The reaction mixture was stirred overnight at room temperature. The reaction was concentrated in vacuo and the triturated with THF, filtered, and dried to give the title compound. Yield (78 mg). LRMS (ESI) $C_{32}H_{30}BrN_5O_4S$ found [M+H]: 660.1/662.1.

[0276] (S)-4-(3-(3-Carbamimidoylphenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-hexylpiperazine-1-carboxamide (25d). Prepared from (S)-4-(3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido) propanoyl)-N-hexylpiperazine-1-carboxamide using General Procedure F. The crude product was purified using MPLC (0-10% MeOH/DCM) to the give the title compound. Yield: 20 mg (50%). ^{1}H NMR (300 MHz, dmso-d_6) 6 ppm 0.75-0.93 (m, 3H) 1.12-1.31 (m, 5H) 1.72 (s, 3H) 2.54 (s, 4H) 2.62-2.74 (m, 2H) 2.86 (d, J=7.43 Hz, 1H) 2.93 (d, J=7.04 Hz, 2H) 3.00 (s, 1H) 3.50 (s, 1H) 4.28 (br. s., 1H) 4.45 (br. s., 1H) 4.56 (d, J=7.43 Hz, 1H) 6.41 (br. s., 2H) 6.54-6.79 (m, 2H) 6.95 (d, J=8.61 Hz, 1H) 7.14 (d, J=7.04 Hz, 1H) 7.23 (br. s., 1H) 7.34 (br. s., 1H) 7.54 (br. s., 1H) 7.57-7.75 (m, 2H) 7.89-8.13 (m, 2H) 8.22-8.38 (m, 1H) 8.49 (s, 1H). LRMS (ESI) $C_{31}H_{40}N_6O_4S$ found [M+H]: 593.3.

[0277] (S)-4-(3-(3-(N-Acetoxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-hexylpiperazine-1-carboxamide. Prepared from (S)—N-hexyl-4-(3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide using General Procedure E. LRMS (ESI) $C_{33}H_{42}N_6O_6S$ found [M+H]: 651.3.

[0278] (S)—N-Hexyl-4-(3-(3-(N-hydroxycarbamimidoyl) phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide. Prepared from (S)-4-(3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-hexylpiperazine-1-carboxamide (40 mg, 0.069 mmol) using General Procedure D. LRMS (ESI) $C_{31}H_{40}N_6O_5S$ found [M+H]: 609.3.

[0279] (S)-4-(3-(3-Cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-hexylpiperazine-1-carboxamide. To a solution of 21B (50 mg, 0.115 mmol) in THF (1 mL) under a nitrogen atmosphere was added hexyl isocyanate (49 uL, 0.335 mmol). The reaction mixture was stirred overnight at room temperature. The reaction was concentrated in vacuo and the residue purified by MPLC (10-70% EtOAc/hexanes) to give the title compound. Yield (40 mg). LRMS (ESI) $C_{31}H_{37}N_5O_4S$ found [M+H]: 576.3.

[0280] (S)-3-(3-(4-(Benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-2-(naphthalene-2-sulfonamido)-3-oxopropyl) benzimidamide (25e). Prepared from (S)—N-acetoxy-3-(3-(4-(benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-2-(naphthalene-2-sulfonamido)-3-oxopropyl)benzimidamide using General Procedure F. Purification by reverse phase HPLC (5-60% acetonitrile/water/0.05% TFA). Yield: 11 mg (21%). ^{1}H NMR (300 MHz, dmso-d_6) δ ppm 2.71 (br. s., 1H) 2.74-2.86 (m, 1H) 2.87-2.96 (m, 1H) 3.00 (br. s., 1H) 3.15 (d, J=19.17 Hz, 2H) 3.92 (s, 2H) 4.54 (s, 2H) 6.57 (s, 2H) 7.04-7.20 (m, 1H) 7.20-7.34 (m, 1H) 7.34-7.54 (m, 2H) 7.59 (br. s., 1H) 7.63-7.77 (m, 2H) 7.87 (s, 2H) 7.97-8.22 (m, 3H) 8.33 (br. s., 1H) 8.39 (m, 1H) 8.60 (m, 1H) 8.90 (m, 2H) 9.25 (m, 2H). LRMS (ESI) $C_{33}H_{31}N_5O_4S_2$ found [M+H]: 626.2.

[0281] (S)—N-Acetoxy-3-(3-(4-(benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-2-(naphthalene-2-sulfonamido)-3-oxopropyl)benzimidamide. Prepared from (S)-1-(3-(4-(benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-2-(naphthalene-2-sulfonamido)-3-oxopropyl)-N-hydroxy-1l4-pyran-3-carboximidamide using General Procedure E. LRMS (ESI) $C_{35}H_{33}N_5O_6S_2$, found [M+H]: 684.2.

[0282] (S)-1-(3-(4-(Benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-2-(naphthalene-2-sulfonamido)-3-oxopropyl)-N-hydroxy-1l4-pyran-3-carboximidamide. Prepared from (S)—N-(1-(4-(benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-3-(3-cyanophenyl)-1-oxopropan-2-yl)naphthalene-2-sulfonamide (51 mg, 0.0139 mmol) using General Procedure D. LRMS (ESI) $C_{33}H_{31}N_5O_5S_2$, found [M+H]: 642.2.

[0283] (S)—N-(1-(4-(Benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-3-(3-cyanophenyl)-1-oxopropan-2-yl)naphtha-

lene-2-sulfonamide. To a solution of 3-carboxy benzothiophene (18 mg, 0.101 mmol), HBTU (42 mg, 0.115 mmol), and DIPEA (88 uL, 0.506 mmol) in THF (1.5 mL) under a nitrogen atmosphere, was added 21B (50 mg, 0.115 mmol). The reaction mixture was stirred overnight at room temperature. The reaction was concentrated in vacuo and the residue purified by MPLC (10-70% EtOAc/hexanes) to give the title compound. Yield (51 mg). LRMS (ESI) $C_{33}H_{28}N_4O_4S_2$ found [M+H]: 609.2.

[0284] (S)-3-(3-Oxo-3-(4-phenylpiperazin-1-yl)-2-((2,4,6-triisopropylphenyl)sulfonamido)propyl)benzimidamide (27). Prepared from (S)—N-acetoxy-3-(3-oxo-3-(4-phenylpiperazin-1-yl)-2-((2,4,6-triisopropylphenyl)sulfonamido)propyl)benzimidamide using General Procedure F. Purified by reversed-phase HPLC (5-80% acetonitrile/water/0.05% TFA), Yield (0.166g, 42.8%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.08-9.47 (m, 4H), 8.15-7.91 (d, 1H), 7.57-7.74 (m, 2H), 7.39-7.56 (m, 2H), 7.07-7.29 (m, 4H), 6.71-6.91 (m, 3H), 4.41-4.64 (m, 1H), 3.93-4.20 (m, 2H), 3.27-3.52 (m, 2H), 3.15-3.27 (m, 2H), 2.99-3.15 (m, 2H), 2.78-2.99 (m, 4H), 2.59-2.78 (m, 2H), 0.98-1.31 (m, 18H). LRMS (ESI), $C_{35}H_{47}N_5O_3S$, [M+H]: 618.3.

[0285] (S)—N-Acetoxy-3-(3-oxo-3-(4-phenylpiperazin-1-yl)-2-((2,4,6-triisopropylphenyl)sulfonamido)propyl)benzimidamide. Prepared from (S)—N-hydroxy-3-(3-oxo-3-(4-phenylpiperazin-1-yl)-2-((2,4,6-triisopropylphenyl)sulfonamido)propyl)benzimidamide using General Procedure E. LRMS (ESI), $C_{37}H_{49}N_5O_5S$, [M+H]: 676.3.

[0286] (S)—N-Hydroxy-3-(3-oxo-3-(4-phenylpiperazin-1-yl)-2-((2,4,6-triisopropylphenyl)sulfonamido)propyl)benzimidamide. Prepared from (S)—N-(3-(3-cyanophenyl)-1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl)-2,4,6-triisopropylbenzenesulfonamide using General Procedure D. LRMS (ESI), $C_{35}H_{47}N_5O_4S$, [M+H]: 634.3

[0287] (S)—N-(3-(3-Cyanophenyl)-1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl)-2,4,6-triisopropylbenzenesulfonamide. Prepared from (S)-3-(3-cyanophenyl)-2-((2,4,6-triisopropylphenyl)sulfonamido)propanoic acid (0.36 g, 0.96 mmol)[35] and 4-phenyl piperazine (1.19 mmol) using General Procedure A. Purification by MPLC (0-50% EtOAc/hexanes), Yield (0.34 g, 72%). LRMS (ESI), $C_{35}H_{44}N_4O_3S$, [M+H]: 601.3

[0288] (S)-3-(2-(4-Methylphenylsulfonamido)-3-oxo-3-(4-phenylpiperidin-1-yl)propyl)benzimidamide (25b). Prepared from using General Procedure F. Yield (0.13 g, 55%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.32 (s, 2H), 9.37 (s, 2H), 8.00-8.36 (m, 1H), 7.63-8.00 (m, 2H), 7.40-7.63 (m, 4H), 7.06-7.40 (m, 5H), 7.00 (d, J=7.42 Hz, 1H), 4.41-4.73 (m, 1H), 4.21-4.39 (m, 1H), 3.92 (t, J=9.75 Hz, 1H), 3.63-3.81 (m, 1H), 2.90-3.02 (m, 1H), 2.49-2.88 (m, 4H), 2.36 (s, 2H), 1.95-2.33 (m, 1H), 1.65 (d, J=11.81 Hz, 1H), 1.39-1.60 (m, 1H), 1.08-1.39 (m, 1H), 0.78-1.08 (m, 1H). LRMS (ESI), $C_{28}H_{32}N_4O_3S$, [M+H]: 505.2.

[0289] (S)—N-Acetoxy-3-(2-((4-methylphenyl)sulfonamido)-3-oxo-3-(4-phenylpiperidin-1-yl)propyl)benzimidamide. Prepared from (S)—N-hydroxy-3-(2-((4-methylphenyl)sulfonamido)-3-oxo-3-(4-phenylpiperidin-1-yl)propyl)benzimidamide using General Procedure E. Purification by MPLC (0-90% EtOAc/hexanes). Yield (0.24 g, 89%). LRMS (ESI), $C_{30}H_{34}N_4O_5S$, [M+H]: 563.2.

[0290] (S)—N-Hydroxy-3-(2-((4-methylphenyl)sulfonamido)-3-oxo-3-(4-phenylpiperidin-1-yl)propyl)benzimidamide. Prepared from (S)—N-(3-(3-cyanophenyl)-1-oxo-1-(4-phenylpiperidin-1-yl)propan-2-yl)-4-methylbenzenesulfonamide using General Procedure D. LRMS (ESI), $C_{28}H_{32}N_4O_4S$, [M+H]: 521.2.

[0291] (S)—N-(3-(3-Cyanophenyl)-1-oxo-1-(4-phenylpiperidin-1-yl)propan-2-yl)-4-methylbenzenesulfonamide. Prepared from 3-[(2S)-2-amino-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzonitrile (0.256 g, 0.69 mmol) using General Procedure C. Yield (0.23 g, 68%). LRMS (ESI), $C_{28}H_{29}N_3O_3S$, [M+H]: 488.2.

[0292] Benzyl (S)-1-((S)-3-(3-carbamimidoylphenyl)-2-(4-methylphenylsulfonamido)propanoyl)piperidin-3-ylcarbamate (18). Prepared from benzyl ((S)-1-((S)-3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperidin-3-yl)carbamate using General Procedure F. Yield (0.14 g, 44%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.13-9.47 (m, 4H), 7.60-7.77 (m, 2H), 7.40-7.59 (m, 4H), 7.12-7.40 (m, 7H), 5.00 (s, 1H), 4.29-4.60 (m, 1H), 3.95-4.15 (m, 1H), 3.62-3.85 (m, 2H), 3.56 (d, J=1.10 Hz, 1H), 3.29-3.46 (m, 2H), 2.64-2.99 (m, 3H), 2.53-2.63 (m, 1H), 2.34 (s, 3H), 1.69-1.98 (m, 1H), 1.56-1.69 (m, 1H), 1.26-1.49 (m, 1H), 1.08-1.24 (m, 1H). LRMS (ESI), $C_{30}H_{35}N_5O_5S$, [M+H]: 578.2.

[0293] Benzyl ((S)-1-((S)-3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((S)-1-((S)-3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperidin-3-yl)carbamate using General Procedure E. LRMS (ESI), $C_{32}H_{37}N_5O_7S$, [M+H]: 636.2.

[0294] Benzyl ((S)-1-((S)-3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((S)-1-((S)-3-(3-cyanophenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperidin-3-yl)carbamate using General Procedure D. LRMS (ESI), $C_{30}H_{35}N_5O_6S$, [M+H]: 594.2.

[0295] Benzyl ((S)-1-((S)-3-(3-cyanophenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((S)-1-((S)-2-amino-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate using General Procedure C. Purification by MPLC (0-75% EtOAc/hexanes). Yield (0.27 g, 69%). LRMS (ESI), $C_{30}H_{32}N_4O_5S$, [M+H]: 561.2.

[0296] Benzyl ((S)-1-((S)-2-amino-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate using General Procedure B. LRMS (ESI), $C_{23}H_{26}N_4O_3$, [M+H]: 407.2.

[0297] Benzyl ((S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate. Prepared from 19 (0.46 g, 1.59 mmol) and benzyl (S)-piperidin-3-ylcarbamate (0.48 g, 2.04 mmol) using General Procedure A. LRMS (ESI), $C_{28}H_{34}N_4O_5$, [M+H]: 507.2.

[0298] Benzyl (R)-1-((S)-3-(3-carbamimidoylphenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-ylcarbamate (25f). Prepared from benzyl ((R)-1-((S)-3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-yl)carbamate using General Procedure F. Yield (0.136 g, 53%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.20-9.48 (m, 3H), 9.15 (br. s., 1H), 8.15-8.43 (m, 2H), 7.85-8.15 (m, 3H), 7.77 (br. s., 1H), 7.55-7.69 (m, 3H), 7.29-7.53 (m, 8H), 4.86-5.23 (m, 3H), 4.35-4.68 (m, 1H), 3.87-4.17 (m, 1H), 3.57-3.87 (m, 1H), 2.81-3.09 (m, 2H), 2.52-2.81 (m, 3H), 1.74-1.94 (m, 1H), 1.58-1.74 (m, 1H), 1.34-1.58 (m, 1H), 0.90-1.29 (m, 2H). LRMS (ESI), $C_{33}H_{35}N_5O_5S$, [M+H]: 614.2.

[0299] Benzyl ((R)-1-((S)-3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((R)-1-((S)-3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-yl)carbamate using General Procedure E. LRMS (ESI), $C_{35}H_{37}N_5O_7S$, [M+H]: 672.2.

[0300] Benzyl ((R)-1-((S)-3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((R)-1-((S)-3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-yl)carbamate using General Procedure D. LRMS (ESI), $C_{33}H_{35}N_5O_6S$, [M+H]: 630.2.

[0301] Benzyl ((R)-1-((S)-3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((R)-1-((S)-2-amino-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate using General Procedure C. Purification by MPLC (0-85% EtOAc/hexanes). Yield (0.22 g, 57%). LRMS (ESI), $C_{33}H_{32}N_4O_5S$, [M+H]: 597.2.

[0302] Benzyl ((R)-1-((S)-2-amino-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((R)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate using General Procedure B. Yield (0.29 g). LRMS (ESI), $C_{23}H_{26}N_4O_3$, [M+H]: 407.2.

[0303] Benzyl ((R)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate. Prepared from 19 (0.42 g, 1.46 mmol) and benzyl (R)-piperidin-3-ylcarbamate (0.48 g, 2.04 mmol) using General Procedure A. Purification by MPLC (5-80% EtOAc/hexanes), Yield (0.346 g, 47%). LRMS (ESI), $C_{28}H_{34}N_4O_5$, [M+H]: 507.2.

[0304] 1-((S)-3-(3-Carbamimidoylphenyl)-2-(4-methylphenylsulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide (25c). Prepared from 1-((S)-3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide using General Procedure F. Yield (0.28 g, 39%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.52-9.78 (m, 2H), 9.30-9.52 (m, 2H), 8.03-8.40 (m, 2H), 7.76-7.98 (m, 2H), 7.49-7.76 (m, 4H), 7.24-7.49 (m, 7H), 4.51-4.78 (m, 1H), 3.90-4.36 (m, 1H), 3.78-3.90 (m, 1H), 3.59-3.68 (m, 1H), 3.29-3.46 (m, 2H), 3.00-3.19 (m, 1H), 2.69-3.00 (m, 4H), 2.56-2.69 (m, 1H), 2.01-2.25 (m, 1H), 1.80-2.01 (m, 1H), 1.23-1.80 (m, 3H), 0.30-1.21 (m, 1H). LRMS (ESI), $C_{31}H_{37}N_5O_4S$, [M+H]: 576.3.

[0305] 1-((S)-3-(3-(N-Acetoxycarbamimidoyl)phenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide. Prepared from 1-((S)-3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide using General Procedure E. LRMS (ESI), $C_{33}H_{39}N_5O_6S$, [M+H]: 634.3.

[0306] 1-((S)-3-(3-(N-Hydroxycarbamimidoyl)phenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide. Prepared from 1-((S)-3-(3-cyanophenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide using General Procedure D. LRMS (ESI), $C_{31}H_{37}N_5O_5S$, [M+H]: 592.3.

[0307] 1-((S)-3-(3-Cyanophenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide. Prepared from (S)-3-(3-cyanophenyl)-2-((4-methylphenyl)sulfonamido)propanoic acid (0.67 g, 1.95 mmol) and N-phenethylpiperidine-3-carboxamide (0.68 g, 2.53 mmol) using General Procedure A. Yield (0.96 g, 88%). LRMS (ESI), $C_{31}H_{34}N_4O_4S$, [M+H]: 559.2.

[0308] (S)-3-(3-Cyanophenyl)-2-((4-methylphenyl)sulfonamido)propanoic acid. To a solution of methyl (S)-3-(3-cyanophenyl)-2-((4-methylphenyl)sulfonamido)propanoate (1.85g, 5.17 mmol) in THF (30 mL) was added LiOH (0.80 g) in water (10 mL). The reaction was stirred at room temperature for 2 h. The organic layer was extracted with water and the combined aqueous fractions were adjusted to pH 3 with conc. HCl and then extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and the filtrate concentrated in vacuo to give the title compound. Yield (1.68 g, 95%). LRMS (ESI), $C_{17}H_{16}N_2O_4S$, [M+H]: 345.1.

[0309] Methyl (S)-3-(3-cyanophenyl)-2-((4-methylphenyl)sulfonamido)propanoate. Prepared from 19B (1.53 g, 6.36 mmol) using General Procedure C. Yield (1.85 g, 81%). LRMS (ESI), $C_{18}H_{18}N_2O_4S$, [M+H]: 359.1.

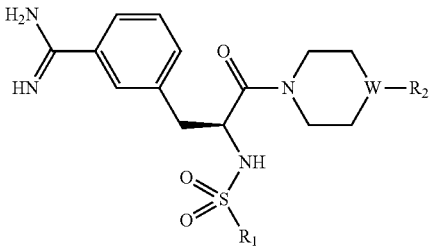
Example 9 Inhibition Studies with Benzamidine Compounds

[0310] Fluorescent inhibitor and chromogenic proteolytic assays were performed in general accordance with the procedures described in Example 4 for compounds prepared in Example 8.

[0311] The results for these studies are presented in Tables 9.1, 9.2, and 9.3 and FIGS. **10-17**.

[0312] The results for selective studies are presented in Table 9.4.

TABLE 9.1



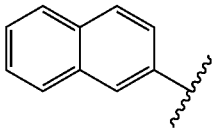
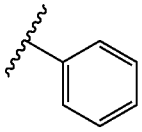
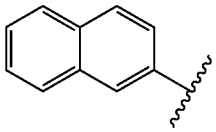
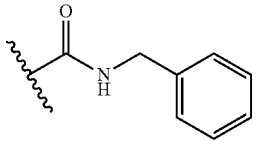
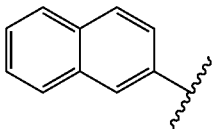
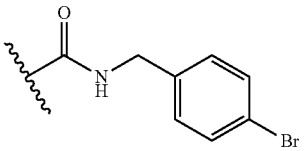
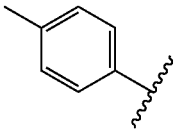
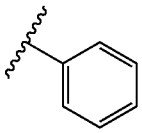
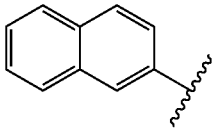
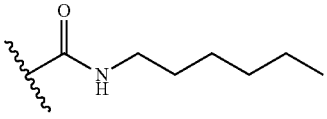
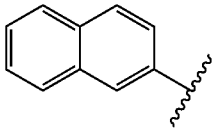
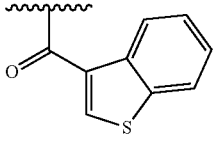
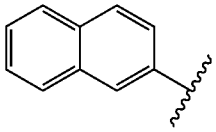
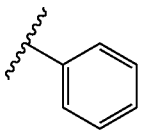
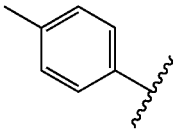
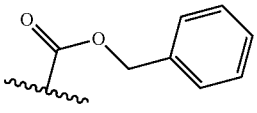
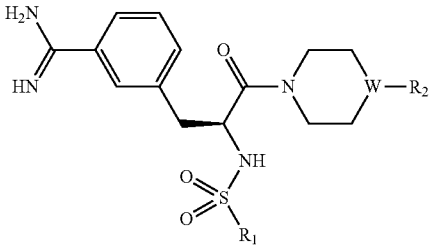
Compound No.	R_1	R_2	W	Fluorogenic Assay HGFA K_i (μM)	Matriptase K_i (μM)	Hepsin K_i (μM)
16 (DEJ-1-69-002)			C	10.1	0.3	3.28
17a (FMF-1-279-003)			N	14.0	2.06	1.0
25a (FMF-1-236-003)			N	16.2	4.2	0.048
25b (DEJ-1-65-001)			C	19.85	0.57	4.8
25d (FMF-1-278-003)			N	>50	14.9	1.3
25e (FMF-1-281-003)			N	>50	1.2	3.75
25g (DEJ-1-63-001)			N	18.3	1.8	7.0
25h (DEJ-1-112-004)			N	>50	0.43	2.4

TABLE 9.1-continued



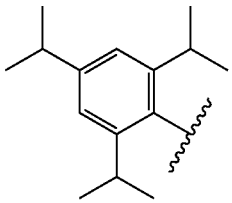
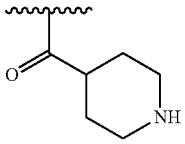
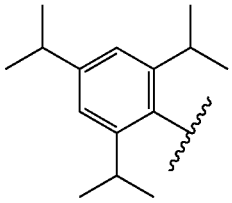
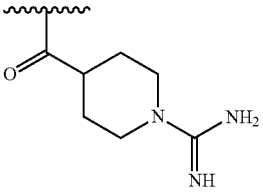
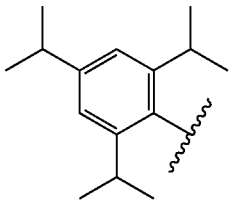
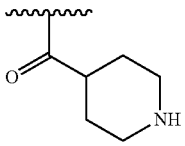
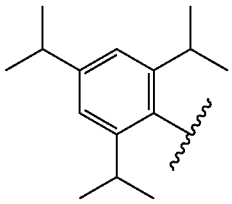
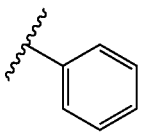
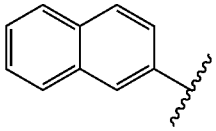
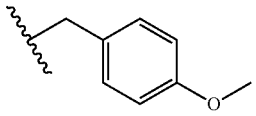
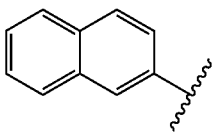
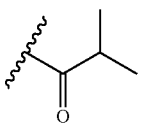
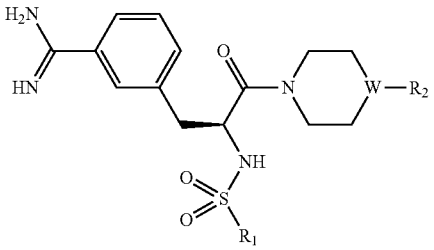
Compound No.	R_1	R_2	W	Fluorogenic Assay HGFA K_i (μM)	Matriptase K_i (μM)	Hepsin K_i (μM)
26 (JJ-I-134-2)			N	5.5	0.12	8.14
JWJ-I-135			N	17.84	0.0043	1.58
JJ-I-134-2			N	5.45	0.12	8.13
27 (DEJ-1-173-004)			N	22.2	0.76	7.4
28 (FMF-1-238-003)			N		2.286-002	
29 (FMF-1-276-003)			N		>20	>20

TABLE 9.1-continued



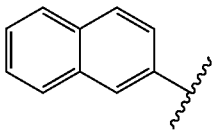
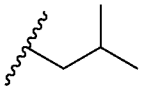
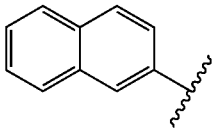
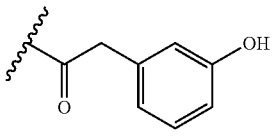
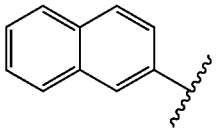
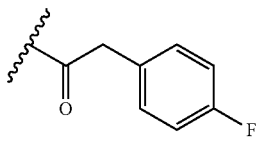
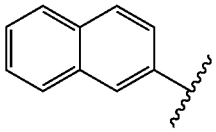
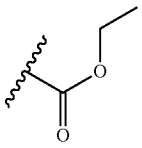
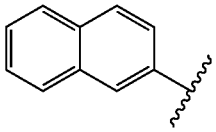
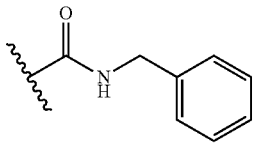
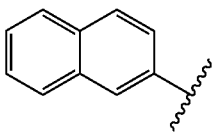
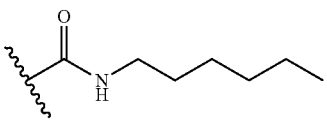
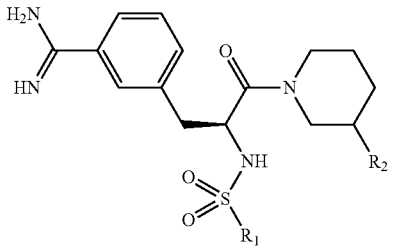
Compound No.	R_1	R_2	W	Fluorogenic Assay HGFA K_i (μM)	Matriptase K_i (μM)	Hepsin K_i (μM)
30 (FMF-1-277-004)			N		12.4	4.9
31 (FMF-1-280-003)			N		4.4	1.7
32 (FMF-1-237-003)			N		2.8	1.0
FMF-1-275-004			N		2.78	39.5
FMF-1-279-003			N	14.03	2.56	1.04
FMF-1-278-003			N	>50	14.87	1.28

TABLE 9.2



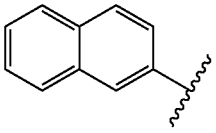
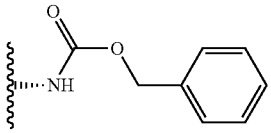
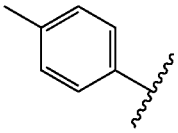
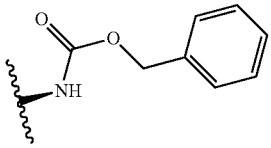
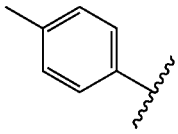
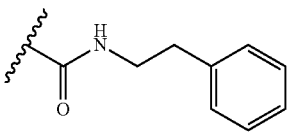
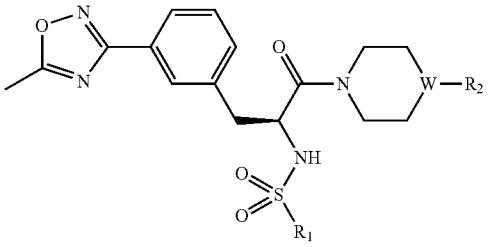
Compound No.	R_1	R_2	Fluorogenic Assay HGFA K_i (μM)	Matriptase K_i (μM)	Hepsin K_i (μM)
25f* (DEJ-1-86-002)			>50	4.9	2.8
18* (DEJ-1-85-002)			>50	1	2.3
25c* (DEJ-1-194-002)			>50	1.3	0.67

TABLE 9.3



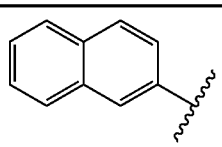
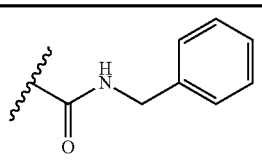
Compound No.	R_1	R_2	W	Fluorogenic Assay HGFA K_i (μM)	Matriptase K_i (μM)	Hepsin K_i (μM)
(FMF-1-254-004)			N	>50	>10	>10

TABLE 9.4

Compound No.	Thrombin K_i (nM)	Factor Xa K_i (nM)	Trypsin K_i (nM)
FMF-1-236-003	3106.0	4564.0	2.20
JWJ-1-135	>2000	—	1.93

Example 10. Tumor Microenvironment and Drug Sensitivity

[0313] The microenvironment of solid tumors is characterized by the presence of activated stromal cells, which produce an abundance of inflammatory mediators and growth factors that have been shown to play a critical role in tumor progression. Macrophages and myofibroblasts are two major components of the tumor microenvironment. HGF and other factors produced by macrophages and myofibroblasts that modulate oncogenic signaling pathways in tumor cells, include MET were investigated. An objective of this example is to understand the nature of tumor-derived factors that are responsible for activation of stromal cells and determine how the presence of genetic alterations in tumor cells alters communication of tumor cells with the stroma. In addition to their effect on tumor growth, HGF or MSP from the tumor microenvironment are also important regulators of responsiveness of cancer cells to antineoplastic therapies.

[0314] RON phosphorylation and migration and invasion assays of breast cancer cells: RON receptor tyrosine kinase activation confers resistance to tamoxifen in the MCF7 and T47D breast cancer cell lines. MDA-MB-231 express RON but we have not been able to detect phosphorylated RON (pRON) following MSP stimulation.

[0315] Detection and quantification of pRON in T47D cells was performed using a sandwich ELISA assy. RON capture antibody (R&D Systems, AF691) was immobilized on high protein binding 96-well plates. Next, cell lysates are added to wells, followed by incubation with an anti-phosphotyrosine-HRP antibody and then addition of a TMB substrate. Optical density of wells was determined by reading plates at 450 nm in a plate reader.

[0316] It can been seen in FIG. **18** that RON phosphorylation in T47D cells following active MSP stimulation, or HGFA-mediated pro-MSP stimulation, results in similar levels of RON phosphorylation. Inhibition with the triplex HGFA, matriptase, hepsin inhibitor Nafamostat, reduces pRON to background levels.

[0317] Triple-negative MDA-MB-231 breast cancer cells co-express MET, hepsin and HGFA but lower levels of RON and matriptase while T47D cells have good expression of RON.

[0318] MRC5 fibroblasts were plated in the bottom well of a Boyden chamber, and incubated overnight to condition the attractant media. The following day, MDA-MB-231 or T47D cells +/− inhibitors were plated above on Matrigel coated inserts (8.0 μm membrane inserts, 12-well; Corning 3422) and incubated in FBS media overnight. Inhibitors were added to fresh media and then cells were plated above on the inserts. The cells were fixed and stained at the 24 hour time point. The inserts were then removed and mounted on glass plates where the number of migrated cells were imaged and counted at 4 fields per membrane using a light microscope at 20×.

[0319] It can be seen in FIG. **19** that the two triplex inhibitors of HGFA, matriptase, and hepsin, 7115 and 7054 (1 μM) abrogate the MRC5-mediated migration and invasion of MDA-MB-231 cells through Matrigel by blocking active HGF production. The inhibitors tested are as effective as HAI-1, an endogenous inhibitor of matriptase, hepsin, and HGFA.

Example 11. PS-SCL and MSP-MS Substrate Screening of Natural and Unnatural Amino Acids

[0320] Characterization of hepsin and HGFA P4-P2 substrate specificity using HyCoSuL.: Each of the P_4, P_3, and P_2 sublibraries were screened at the 100 μM concentration with hepsin either HGFA in a 100 μL final volume per well (99 μL of protease in buffer+1 μL of 10 mM substrates mixture). The total time of the assay was 30 minutes, however only linear portions of the progress curve (5-15 minutes) were used for velocity (RFU/s) calculations. Each sublibrary screening was repeated at least 3-times and the average value calculated from each measurement was used to create the substrate specificity matrix—the best recognized amino acid in each position was set to 100%, and other amino acids were adjusted accordingly.

[0321] Recombinant human hepsin (10 μg in 20 uL, 4776-SE-010, R&D Systems) was added to the 100 μL of activation buffer (0.1 M Tris, 0.15 M NaCl, 0.01 M $CaCl_2$), 0.05% TRITON-X, pH=8.0) and incubated at 37° C. for 24 hours (hepsin concentration in activation buffer 2 μM). Once activated hepsin was diluted in assay buffer (0.05 M Tris, pH=9.0) to the final concentration of 4 nM and incubated at 37° C. for 15 minutes before added to a substrate.

[0322] HFGA enzyme was diluted in the assay buffer (0.15 M NaCl, 0.025 M Tris, 0.005 M $CaCl_2$), pH=8.0) to the final concentration of 10-20 nM (depending of sublibrary) and incubated at 25° C. for 30 minutes before added to a substrate.

[0323] Characterization of hepsin and HGFA P1 substrate specificity using Ac-Ala-Arg-Leu-P1-ACC individual substrate library: The 142-membered Ac-Ala-Arg-Leu-P1-ACC fluorogenic substrate library containing 19 natural (except cysteine) and 123 unnatural amino acids was used to determine hepsin and HGFA preferences in P_1 position. The protocol for both enzymes activation and incubation are as the same as in HyCoSuL screening. The P_1 individual substrate library was used at the final concentration of 5 μM. The total time of the assay was 30 min, however only linear portions of the progress curve (5-15 minutes) were used for velocity (RFU/s) calculations. Each screening for hepsin either HGFA was repeated 3-times and the average value for each measurement was used to create the substrate specificity matrix.

[0324] The detailed protocol for the use of ACC-labeled combinatorial peptide libraries in protease substrate specificity screening can be found in: Poreba M, Szalek A, Kasperkiewicz P, Drag M., *Methods Mol Biol.* 2014, 1133, 41-59, which is incorporated herein by reference.

[0325] Results for MSP-MS profiling for HGFA substrate cleavage and specificity can be seen in FIG. **20** and Tables 11.1-11.4. IceLogo graphs can be seen in FIGS. **21**A and **21**B.

TABLE 11.1

P4-P4'-All	P4-P4'-Cleaved by HGFA (4 hr)
ADARKYWN	AMFRKYPI
AGKRRDWX	ASMRIYIE
AMFRKYPI	EIFRKWHX
AMWRLDII	ESARDXXX
ASHRMGKN	EYFRMIRW
ASMRIYIE	FLVRTWKM
DAQRWKNI	FRIRSGTX
DEPRGHMY	FTQRAGIL
DQVRRMNX	IDLRWMAY
DSIRHQGP	KLFRFNWX
EIFRKWHX	NFLRGPXX
EKQRFHPX	NQMRGFXX
ELGRSANA	PQRRGMXX
ESARDXXX	RMIRWAVL
EVARPLGX	SLYRMIRQ
EYFRMIRW	SYMRWPXX
EYPRPQEW	TYFRAWXX
FDNRVGKW	VQHRLFTY
FHWRIMQG	WAFRSRYH
FLVRTWKM	WPQRRGMX
FPVRPTEX	XKARSAFA
FRIRSGTX	YAFRSTMV

TABLE 11.2

	P1 position % activity	
Amino Acid	Hepsin	HGFA
L-Ala (A)	0.49	0.00
L-Arg (R)	100.00	100.00
L-Asn (N)	0.00	0.00
L-Asp (D)	0.00	0.00
L-Gln (Q)	0.00	0.00
L-Glu (E)	0.13	0.00
Gly (G)	0.07	0.00
L-His (H)	0.17	0.00
L-Ile (I)	0.08	0.00
L-Leu (L)	0.03	0.00
L-Lys (K)	2.34	0.99
L-Nle	0.07	0.00
L-Phe (F)	0.12	0.00
L-Pro (P)	0.04	0.00
L-Ser (S)	0.20	0.00
L-Thr (T)	0.45	0.00
L-Trp (W)	0.70	0.73
L-Tyr (Y)	0.30	0.00
L-Val (V)	0.00	0.00
L-MeAla	0.09	0.00

TABLE 11.2-continued

	P1 position % activity	
Amino Acid	Hepsin	HGFA
B-Ala	0.00	0.00
dhPro 2/2	0.00	0.00
L-Oic	0.00	2.10
L-Hyp	0.00	0.00
L-Hyp(Bzl)	0.00	0.30
L-Gla	0.00	0.00
L-Asp(Me)	2.77	3.11
L-Asp(Oall)	2.34	0.00
L-Asp(Bzl)	0.93	12.28
L-Glu(Me)	0.41	0.00
L-Glu(All)	1.26	0.00
L-Glu(Ochx)	0.17	0.00
L-Glu(Bzl)	0.09	0.00
L-Aad	0.00	0.00
L-Api	0.00	0.00
L-Dap	0.03	0.00
L-Orn	0.00	0.00
L-Cit	0.42	0.00
L-hCit	0.13	0.00
L-Lys(Ac)	0.00	0.00
L-Lys(tfa)	0.00	0.00
L-Lys(2-Cl-Z)	0.09	0.00
His(Bzl)	0.12	0.00
Arg(Me)	0.00	0.00
Arg(Me)2 1/2 sym	0.00	0.00
L-hArg	0.24	0.00
L-3-Pal	0.00	0.00
L-4-Pal	0.00	0.00
L-Phe(4-NH2)	0.08	0.00
L-Phe(2-F)	0.04	0.00
L-Phe(3-F)	0.01	0.00
L-Phe(4-F)	0.08	0.00
L-Phe(3,4-F2)	0.25	0.00
L-Phe(F5)	0.00	0.00
L-Phe(2-Cl)	0.04	0.00
L-Phe(3-Cl)	0.21	0.00
L-Phe(4-Cl)	0.07	0.00
L-Phe(3,4-Cl2)	0.02	0.00
L-Phe(3-I)	0.00	0.00
L-Phe(4-I)	0.34	0.00
L-Phe(4-Br)	0.10	0.00
L-Phe(NO2)	0.39	1.37
L-Phe(guan)	9.45	0.00
L-Phe(4-Me)	0.00	0.00
L-hPhe	0.16	0.00
L-Ala(2th)	0.28	0.42
L-Ser(Bzl)	0.30	0.00
L-Hse(Bzl)	0.06	0.00
L-Thr(Bzl)	0.04	0.00
L-Cys(Bzl)	0.00	0.00
L-Cys(Me-Bzl)	0.13	0.00
L-Cys(4-MeOBzl)	0.25	0.00
L-Tyr(Bzl)	0.45	0.00
L-Dht	0.00	0.00
L-Trp(Me)	0.13	0.48
L-Tyr(Me)	0.14	0.00
L-hTyr(Me)	0.00	0.56
L-Tyr(2,6-Cl-Bzl)	0.41	0.00
L-Abu(Bth)	0.00	0.00
L-Bip	0.13	0.00
L-Bpa	0.56	1.57
Nle(Obzl)	0.15	0.00
L-1-Nal	0.20	0.00
L-2-Na1	0.61	0.00
L-Hse	0.00	0.00
L-Hnv	1.55	0.00
L-Met (M)	0.00	0.00
L-Met(O)	0.00	0.00
L-Met(O_2)	0.00	0.00
L-2-Abu	0.00	0.00
L-Nva	0.14	0.00
L-Tle	0.42	0.00

TABLE 11.2-continued

Amino Acid	P1 position % activity Hepsin	HGFA
L-hLeu	0.00	0.00
L-2-Aoc	2.82	0.00
AC5C	0.15	6.43
L-Chg	0.00	0.00
L-Cha	0.00	0.00
L-hCha	0.21	0.00
L-Thyr	0.28	0.00
L-Inp	0.07	0.00
D-Ala	0.00	0.00
D-Asn	0.00	0.00
D-Asp	0.05	0.00
D-Gln	0.00	0.00
D-Glu	0.00	0.00
D-Leu	0.00	0.00
D-Lys	0.23	0.00
D-Phe	0.38	0.00
D-Pro	1.08	0.00
D-Phe	0.09	0.00
D-Ser	0.02	0.00
D-Thr	0.24	0.00
D-Trp	0.50	0.00
D-Tyr	0.23	0.00
D-Pip	0.42	5.57
D-Tic	0.14	1.12
D-Gla	0.00	0.00
D-Chg	0.60	0.65
D-Cha	0.13	0.00
D-Phg	0.09	0.00
D-3-Pal	0.00	0.00
D-4-Pal	0.00	0.00
D-Phe(4-Me)	0.48	0.00
D-Phe(2-F)	0.00	0.00
D-Phe(3-F)	0.44	0.00
D-Phe(4-F)	0.00	0.00
D-Phe(3,4-F2)	0.31	0.00
D-Phe(F5)	0.34	0.00
D-Phe(2-Cl)	0.66	0.00
D-Phe(3-Cl)	0.12	0.00
D-Phe(4-Cl)	0.00	0.00
D-Phe(3,4-Cl2)	0.07	0.00
D-Phe(4-I)	0.32	0.00
D-Phe(4-Br)	0.11	4.00
D-Phe(4-NO2)	1.45	0.74
D-Ser(Bzl)	0.00	0.00
D-Thr(Bzl)	0.00	0.00
D-hPhe	0.04	0.00
D-Bip	0.30	0.00
D-Bpa	0.34	0.00
D-1-Nal	0.23	0.49
D-2-Nal	0.11	0.00

TABLE 11.3

Amino Acid	P4 Position % Activity Hepsin	HGFA	P3 Position % Activity Hepsin	HGFA	P2 Position % Activity Hepsin	HGFA
L-Ala (A)	41.30	6.68	28.07	8.97	17.85	2.37
L-Arg (R)	80.32	23.62	70.75	43.98	57.23	2.08
L-Asn (N)	25.25	8.20	24.47	6.12	63.72	4.79
L-Asp (D)	0.22	1.90	3.89	2.81	0.33	1.21
L-Gln (Q)	29.82	9.01	58.69	15.98	12.19	3.35
L-Glu (E)	0.76	1.96	16.72	3.47	0.00	0.89
Gly (G)	29.19	10.84	26.14	10.42	0.00	1.14
L-His (H)	25.35	8.90	43.05	11.93	31.24	4.00
L-Ile (I)	47.02	14.09	11.37	9.06	45.68	4.94
L-Leu (L)	33.10	10.18	19.51	13.71	100.00	100.00
L-Lys (K)	75.43	18.29	80.71	44.63	46.70	1.04

TABLE 11.3-continued

Amino Acid	P4 Position % Activity Hepsin	HGFA	P3 Position % Activity Hepsin	HGFA	P2 Position % Activity Hepsin	HGFA
L-Nle	40.93	11.76	24.09	19.15	62.04	45.52
L-Phe (F)	30.80	9.42	2.91	13.51	25.22	16.60
L-Pro (P)	56.00	10.30	2.09	2.04	13.10	2.21
L-Ser (S)	31.63	7.04	38.70	14.57	18.40	3.24
L-Thr (T)	29.29	7.67	35.34	14.51	70.75	8.87
L-Trp (W)	6.82	8.78	0.00	17.50	6.00	4.04
L-Tyr (Y)	22.39	16.47	7.75	11.59	22.79	3.17
L-Val (V)	43.08	11.66	16.77	9.51	51.85	8.33
D-Ala	18.44	7.11	48.09	9.84	0.00	0.00
D-Arg	44.37	23.20	66.08	29.10	0.00	0.00
D-Asn	18.48	7.38	38.75	15.16	0.00	0.00
D-Asp	1.98	2.25	17.18	5.37	0.00	0.00
D-Gln	20.13	8.03	97.80	13.84	0.00	0.00
D-Glu	2.98	2.62	34.55	3.61	0.00	0.00
D-His	19.33	7.79	40.09	11.09	0.00	0.00
D-Leu	24.76	10.56	20.14	7.08	0.00	0.00
D-Lys	49.84	17.66	64.56	21.11	0.00	0.00
D-Phe	13.15	8.13	24.92	15.22	0.00	0.00
D-Pro	20.45	10.30	38.88	4.96	0.00	0.00
D-Ser	21.84	7.94	54.84	10.86	11.55	0.00
D-Phg	22.48	9.06	57.07	40.01	13.53	4.40
D-Thr	25.74	8.61	36.69	6.45	0.00	0.00
D-Trp	1.48	7.65	24.90	87.59	0.00	0.00
D-Tyr	12.66	10.09	29.94	17.32	0.00	0.00
D-Val	22.58	8.25	27.04	5.76	0.00	0.00
β-Ala	30.74	10.07	22.78	15.95	0.00	0.24
L-Hyp	37.81	10.66	4.70	2.18	38.67	3.95
L-Hyp(Bzl)	33.33	8.91	1.29	4.34	32.68	4.39
L-Thz	45.46	12.65	9.93	7.76	6.16	2.80
L-Oic	53.72	16.76	2.92	2.78	0.67	20.71
L-Idc	0.00	32.64	0.00	6.43	0.00	0.96
L-Pip	34.68	9.25	1.64	0.83	10.66	1.75
L-Tic	13.31	10.84	8.10	25.13	2.45	3.58
dhAbu	44.51	24.45	7.29	9.44	7.72	14.12
dhLeu	52.44	42.50	5.85	11.02	0.00	0.48
L-Dap	35.65	13.31	48.71	24.27	10.01	1.49
L-Dab	47.53	16.76	48.55	29.40	13.49	0.50
L-Dab(Z)	96.34	16.55	31.58	21.38	20.86	4.84
L-Cit	36.68	6.44	29.53	13.41	32.27	6.32
L-hCit	40.51	10.53	27.31	19.71	36.25	7.59
L-Orn	84.52	20.23	79.70	54.04	78.48	0.00
L-Lys(TFA)	50.71	11.66	35.84	21.11	38.29	5.16
L-Lys(Ac)	35.02	8.71	29.15	16.02	23.04	7.36
L-Lys(2-ClZ)	41.91	47.56	2.55	14.21	21.20	29.53
L-Agp	98.08	80.01	97.35	74.18	35.36	11.83
L-Agb	37.96	11.59	26.80	19.62	3.53	1.05
L-Arg(NO2)	82.11	8.33	41.82	16.01	17.44	2.76
L-Arg(Z)2	61.73	16.44	50.93	33.31	52.91	4.93
L-hArg	56.24	22.07	47.00	100.00	57.96	5.83
L-His(3-Bom)	38.40	100.00	15.18	13.20	0.00	0.43
L-Phe(NH2)	24.64	9.11	11.65	17.32	30.51	2.45
L-Phe(guan)	35.43	20.91	9.10	42.11	34.36	11.74
L-Trp(Me)	0.00	2.49	0.00	18.14	7.81	1.12
L-Dht	1.56	10.19	0.00	58.65	13.91	20.10
L-Asp(Me)	18.55	9.31	13.81	10.52	0.00	0.75
L-Asp(Chx)	27.32	10.34	19.99	22.46	0.79	2.16
L-Asp(Bzl)	19.34	9.60	7.29	6.92	0.00	1.35
L-Glu(Me)	36.79	9.13	64.70	32.95	13.49	6.65
L-Glu(Chx)	45.93	11.19	43.91	30.08	14.72	6.42
L-Glu(Bzl)	60.31	13.89	50.15	33.35	36.33	6.88
L-Phe(2-F)	24.33	10.49	7.80	13.39	1.78	5.98
L-Phe(3-F)	24.03	10.21	10.43	15.12	30.55	11.72
L-Phe(4-F)	27.78	11.22	5.16	15.01	32.86	4.84
L-Phe(3,4-F2)	19.65	8.79	0.71	15.06	38.16	3.73
L-Phe(F5)	26.60	12.25	5.13	22.36	0.36	1.55
L-Phe(2-Cl)	14.92	10.91	0.98	10.78	2.71	11.87
L-Phe(3-Cl)	9.26	10.25	0.35	21.45	33.86	12.49
L-Phe(4-Cl)	26.01	12.60	0.31	13.32	2.85	1.74
L-Phe(3,4-Cl2)	2.40	9.92	0.00	16.15	0.00	2.22
L-Phe(4-Br)	5.08	13.65	0.00	13.96	2.42	2.41
L-Phe(4-Me)	15.51	11.98	2.39	16.82	6.44	0.97
L-3-Pal	31.27	7.69	28.37	11.00	17.32	4.29

TABLE 11.3-continued

	P4 Position % Activity		P3 Position % Activity		P2 Position % Activity	
Amino Acid	Hepsin	HGFA	Hepsin	HGFA	Hepsin	HGFA
L-4-Pal	33.68	9.27	21.91	14.57	0.11	4.34
L-Ala(2th)	35.30	11.16	11.54	20.51	10.54	11.48
L-Ala(Bth)	14.64	10.26	20.97	23.90	6.52	2.16
L-Bta	13.63	9.79	0.13	24.11	0.00	3.71
L-Abu	38.68	9.87	28.98	15.31	0.00	1.81
L-Abu(Bth)	38.68	11.84	4.21	21.63	0.64	14.29
L-Ser(Ac)	37.96	7.56	42.09	21.69	6.73	1.67
L-Ser(Bzl)	21.57	14.49	11.56	19.45	20.43	5.27
L-hSer	26.55	7.34	33.48	16.59	31.76	2.12
L-hSer(Bzl)	40.00	12.03	21.24	20.03	46.09	17.39
L-Thr(Bzl)	40.13	13.14	16.36	21.72	36.14	7.19
L-Cys(Bzl)	23.44	16.89	3.06	25.14	1.50	7.46
L-Cys(MeBzl)	15.53	13.63	0.87	17.21	3.49	6.24
L-Cys(4-MeOBzl)	12.84	13.88	0.10	16.76	7.07	9.44
L-Nle(O-Bzl)	91.83	16.90	82.17	30.67	0.00	0.00
L-Phg	34.01	11.24	28.27	23.25	48.33	14.18
L-hPhe	44.79	11.14	10.63	61.77	4.71	32.14
L-Chg	46.64	25.12	6.60	18.69	10.88	27.27
L-Cha	25.44	13.42	3.64	22.61	74.49	6.03
L-hCha	5.44	12.92	0.13	73.74	0.00	2.90
L-Igl	25.47	10.62	0.00	52.15	2.20	19.79
L-1-Nal	5.18	6.36	0.00	23.07	3.00	4.19
L-2-Nal	1.34	11.72	0.00	12.75	0.00	5.10
L-Bpa	0.86	12.77	0.00	8.16	0.00	1.57
L-2-Aoc	19.98	12.15	12.74	33.06	8.90	14.37
L-hLeu	41.61	14.86	13.84	23.44	14.44	72.69
L-NptGly	27.66	8.34	9.92	12.95	37.46	53.86
L-Hnv	33.75	7.92	33.40	15.07	34.62	5.49
L-Tle	38.05	9.71	7.48	6.70	0.00	0.00
L-Tyr(Me)	31.38	9.54	4.55	17.41	10.89	1.79
L-Tyr(2,6Cl2-Z)	0.08	9.94	0.09	4.47	0.00	2.83
L-Tyr(Bzl)	3.10	9.39	0.00	14.03	0.28	3.71
L-Tyr(2-Br-Z)	22.51	12.67	8.05	17.09	7.72	2.39
L-hTyr	31.56	13.69	7.46	73.59	0.00	42.92

TABLE 11.3-continued

	P4 Position % Activity		P3 Position % Activity		P2 Position % Activity	
Amino Acid	Hepsin	HGFA	Hepsin	HGFA	Hepsin	HGFA
L-hTyr(Me)	36.30	10.62	10.00	25.48	0.00	23.13
L-Nva	41.99	11.78	25.85	17.60	58.40	39.80
L-Met(O)	22.86	6.64	68.16	16.63	11.40	1.68

TABLE 11.4

	Ac-Ala-Arg-Leu-Arg-ACC	NH_2-Leu-DTrp-Nle-Arg-ACC	Ac-His(3Bom)-Agp-hLeu-Arg-ACC	Ac-Agp-hArg-Leu-Arg-ACC
K_M	107.2	73.4	52.1	37.8
k_{cat}	0.337	1.30	1.47	1.56
k_{cat}/K_M	3,144	17,662	28,140	41,254

[0326] When introducing elements of the present invention or the preferred embodiments(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

[0327] In view of the above, it will be seen that the several objects of the invention are achieved and other advantageous results attained.

[0328] As various changes could be made in the above compositions and methods without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying figures shall be interpreted as illustrative and not in a limiting sense.

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Gln Ala Gln Ala Leu Thr Glu Asp Gly Arg Pro Cys Arg Phe Pro Phe
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Trp Gly Tyr Cys Val Glu Ala Thr Pro Pro Pro Gly Gly Pro Ala Ala
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Leu Asp Pro Cys Ala Ser Gly Pro Cys Leu Asn Gly Gly Ser Cys Ser
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Asn Thr Gln Asp Pro Gln Ser Tyr His Cys Ser Cys Pro Arg Ala Phe
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Thr Gly Lys Asp Cys Gly Thr Glu Lys Cys Phe Asp Glu Thr Arg Tyr
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Glu Tyr Leu Glu Gly Gly Asp Arg Trp Ala Arg Val Arg Gln Gly His
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His Leu Ile Val Ala Thr Gly Thr Thr Val Cys Ala Cys Pro Pro Gly
            260                 265                 270

Phe Ala Gly Arg Leu Cys Asn Ile Glu Pro Asp Glu Arg Cys Phe Leu
        275                 280                 285

Gly Asn Gly Thr Gly Tyr Arg Gly Val Ala Ser Thr Ser Ala Ser Gly
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Leu Ser Cys Leu Ala Trp Asn Ser Asp Leu Leu Tyr Gln Glu Leu His
305                 310                 315                 320

Val Asp Ser Val Gly Ala Ala Ala Leu Leu Gly Leu Gly Pro His Ala
                325                 330                 335

Tyr Cys Arg Asn Pro Asp Asn Asp Glu Arg Pro Trp Cys Tyr Val Val
            340                 345                 350

Lys Asp Ser Ala Leu Ser Trp Glu Tyr Cys Arg Leu Glu Ala Cys Glu
        355                 360                 365

Ser Leu Thr Arg Val Gln Leu Ser Pro Asp Leu Leu Ala Thr Leu Pro
    370                 375                 380

Glu Pro Ala Ser Pro Gly Arg Gln Ala Cys Gly Arg Arg His Lys Lys
385                 390                 395                 400

Arg Thr Phe Leu Arg Pro Arg Ile Ile Gly Gly Ser Ser Ser Leu Pro
                405                 410                 415

Gly Ser His Pro Trp Leu Ala Ala Ile Tyr Ile Gly Asp Ser Phe Cys
            420                 425                 430

Ala Gly Ser Leu Val His Thr Cys Trp Val Val Ser Ala Ala His Cys
        435                 440                 445

Phe Ser His Ser Pro Pro Arg Asp Ser Val Ser Val Val Leu Gly Gln
    450                 455                 460

His Phe Phe Asn Arg Thr Thr Asp Val Thr Gln Thr Phe Gly Ile Glu
465                 470                 475                 480

Lys Tyr Ile Pro Tyr Thr Leu Tyr Ser Val Phe Asn Pro Ser Asp His
                485                 490                 495

Asp Leu Val Leu Ile Arg Leu Lys Lys Lys Gly Asp Arg Cys Ala Thr
            500                 505                 510

Arg Ser Gln Phe Val Gln Pro Ile Cys Leu Pro Glu Pro Gly Ser Thr
        515                 520                 525

Phe Pro Ala Gly His Lys Cys Gln Ile Ala Gly Trp Gly His Leu Asp
    530                 535                 540

Glu Asn Val Ser Gly Tyr Ser Ser Ser Leu Arg Glu Ala Leu Val Pro
545                 550                 555                 560

Leu Val Ala Asp His Lys Cys Ser Ser Pro Glu Val Tyr Gly Ala Asp
                565                 570                 575

Ile Ser Pro Asn Met Leu Cys Ala Gly Tyr Phe Asp Cys Lys Ser Asp
            580                 585                 590

Ala Cys Gln Gly Asp Ser Gly Gly Pro Leu Ala Cys Glu Lys Asn Gly
        595                 600                 605

Val Ala Tyr Leu Tyr Gly Ile Ile Ser Trp Gly Asp Gly Cys Gly Arg
    610                 615                 620
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Leu His Lys Pro Gly Val Tyr Thr Arg Val Ala Asn Tyr Val Asp Trp
625                 630                 635                 640

Ile Asn Asp Arg Ile Arg Pro Pro Arg Arg Leu Val Ala Pro Ser
                645                 650                 655


<210> SEQ ID NO 24
<211> LENGTH: 855
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

Met Gly Ser Asp Arg Ala Arg Lys Gly Gly Gly Gly Pro Lys Asp Phe
1               5                   10                  15

Gly Ala Gly Leu Lys Tyr Asn Ser Arg His Glu Lys Val Asn Gly Leu
            20                  25                  30

Glu Glu Gly Val Glu Phe Leu Pro Val Asn Asn Val Lys Lys Val Glu
        35                  40                  45

Lys His Gly Pro Gly Arg Trp Val Val Leu Ala Ala Val Leu Ile Gly
    50                  55                  60

Leu Leu Leu Val Leu Leu Gly Ile Gly Phe Leu Val Trp His Leu Gln
65                  70                  75                  80

Tyr Arg Asp Val Arg Val Gln Lys Val Phe Asn Gly Tyr Met Arg Ile
                85                  90                  95

Thr Asn Glu Asn Phe Val Asp Ala Tyr Glu Asn Ser Asn Ser Thr Glu
            100                 105                 110

Phe Val Ser Leu Ala Ser Lys Val Lys Asp Ala Leu Lys Leu Leu Tyr
        115                 120                 125

Ser Gly Val Pro Phe Leu Gly Pro Tyr His Lys Glu Ser Ala Val Thr
    130                 135                 140

Ala Phe Ser Glu Gly Ser Val Ile Ala Tyr Tyr Trp Ser Glu Phe Ser
145                 150                 155                 160

Ile Pro Gln His Leu Val Glu Glu Ala Glu Arg Val Met Ala Glu Glu
                165                 170                 175

Arg Val Val Met Leu Pro Pro Arg Ala Arg Ser Leu Lys Ser Phe Val
            180                 185                 190

Val Thr Ser Val Val Ala Phe Pro Thr Asp Ser Lys Thr Val Gln Arg
        195                 200                 205

Thr Gln Asp Asn Ser Cys Ser Phe Gly Leu His Ala Arg Gly Val Glu
    210                 215                 220

Leu Met Arg Phe Thr Thr Pro Gly Phe Pro Asp Ser Pro Tyr Pro Ala
225                 230                 235                 240

His Ala Arg Cys Gln Trp Ala Leu Arg Gly Asp Ala Asp Ser Val Leu
                245                 250                 255

Ser Leu Thr Phe Arg Ser Phe Asp Leu Ala Ser Cys Asp Glu Arg Gly
            260                 265                 270

Ser Asp Leu Val Thr Val Tyr Asn Thr Leu Ser Pro Met Glu Pro His
        275                 280                 285

Ala Leu Val Gln Leu Cys Gly Thr Tyr Pro Pro Ser Tyr Asn Leu Thr
    290                 295                 300

Phe His Ser Ser Gln Asn Val Leu Leu Ile Thr Leu Ile Thr Asn Thr
305                 310                 315                 320

Glu Arg Arg His Pro Gly Phe Glu Ala Thr Phe Phe Gln Leu Pro Arg
                325                 330                 335
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Met Ser Ser Cys Gly Gly Arg Leu Arg Lys Ala Gln Gly Thr Phe Asn
            340                 345                 350

Ser Pro Tyr Tyr Pro Gly His Tyr Pro Pro Asn Ile Asp Cys Thr Trp
        355                 360                 365

Asn Ile Glu Val Pro Asn Asn Gln His Val Lys Val Arg Phe Lys Phe
    370                 375                 380

Phe Tyr Leu Leu Glu Pro Gly Val Pro Ala Gly Thr Cys Pro Lys Asp
385                 390                 395                 400

Tyr Val Glu Ile Asn Gly Glu Lys Tyr Cys Gly Glu Arg Ser Gln Phe
                405                 410                 415

Val Val Thr Ser Asn Ser Asn Lys Ile Thr Val Arg Phe His Ser Asp
            420                 425                 430

Gln Ser Tyr Thr Asp Thr Gly Phe Leu Ala Glu Tyr Leu Ser Tyr Asp
        435                 440                 445

Ser Ser Asp Pro Cys Pro Gly Gln Phe Thr Cys Arg Thr Gly Arg Cys
    450                 455                 460

Ile Arg Lys Glu Leu Arg Cys Asp Gly Trp Ala Asp Cys Thr Asp His
465                 470                 475                 480

Ser Asp Glu Leu Asn Cys Ser Cys Asp Ala Gly His Gln Phe Thr Cys
                485                 490                 495

Lys Asn Lys Phe Cys Lys Pro Leu Phe Trp Val Cys Asp Ser Val Asn
            500                 505                 510

Asp Cys Gly Asp Asn Ser Asp Glu Gln Gly Cys Ser Cys Pro Ala Gln
        515                 520                 525

Thr Phe Arg Cys Ser Asn Gly Lys Cys Leu Ser Lys Ser Gln Gln Cys
    530                 535                 540

Asn Gly Lys Asp Asp Cys Gly Asp Gly Ser Asp Glu Ala Ser Cys Pro
545                 550                 555                 560

Lys Val Asn Val Val Thr Cys Thr Lys His Thr Tyr Arg Cys Leu Asn
                565                 570                 575

Gly Leu Cys Leu Ser Lys Gly Asn Pro Glu Cys Asp Gly Lys Glu Asp
            580                 585                 590

Cys Ser Asp Gly Ser Asp Glu Lys Asp Cys Asp Cys Gly Leu Arg Ser
        595                 600                 605

Phe Thr Arg Gln Ala Arg Val Val Gly Gly Thr Asp Ala Asp Glu Gly
    610                 615                 620

Glu Trp Pro Trp Gln Val Ser Leu His Ala Leu Gly Gln Gly His Ile
625                 630                 635                 640

Cys Gly Ala Ser Leu Ile Ser Pro Asn Trp Leu Val Ser Ala Ala His
                645                 650                 655

Cys Tyr Ile Asp Asp Arg Gly Phe Arg Tyr Ser Asp Pro Thr Gln Trp
            660                 665                 670

Thr Ala Phe Leu Gly Leu His Asp Gln Ser Gln Arg Ser Ala Pro Gly
        675                 680                 685

Val Gln Glu Arg Arg Leu Lys Arg Ile Ile Ser His Pro Phe Phe Asn
    690                 695                 700

Asp Phe Thr Phe Asp Tyr Asp Ile Ala Leu Leu Glu Leu Glu Lys Pro
705                 710                 715                 720

Ala Glu Tyr Ser Ser Met Val Arg Pro Ile Cys Leu Pro Asp Ala Ser
                725                 730                 735
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His Val Phe Pro Ala Gly Lys Ala Ile Trp Val Thr Gly Trp Gly His
            740                 745                 750

Thr Gln Tyr Gly Gly Thr Gly Ala Leu Ile Leu Gln Lys Gly Glu Ile
        755                 760                 765

Arg Val Ile Asn Gln Thr Thr Cys Glu Asn Leu Leu Pro Gln Gln Ile
    770                 775                 780

Thr Pro Arg Met Met Cys Val Gly Phe Leu Ser Gly Gly Val Asp Ser
785                 790                 795                 800

Cys Gln Gly Asp Ser Gly Gly Pro Leu Ser Ser Val Glu Ala Asp Gly
                805                 810                 815

Arg Ile Phe Gln Ala Gly Val Val Ser Trp Gly Asp Gly Cys Ala Gln
            820                 825                 830

Arg Asn Lys Pro Gly Val Tyr Thr Arg Leu Pro Leu Phe Arg Asp Trp
        835                 840                 845

Ile Lys Glu Asn Thr Gly Val
    850                 855


<210> SEQ ID NO 25
<211> LENGTH: 417
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

Met Ala Gln Lys Glu Gly Gly Arg Thr Val Pro Cys Cys Ser Arg Pro
1               5                   10                  15

Lys Val Ala Ala Leu Thr Ala Gly Thr Leu Leu Leu Leu Thr Ala Ile
            20                  25                  30

Gly Ala Ala Ser Trp Ala Ile Val Ala Val Leu Leu Arg Ser Asp Gln
        35                  40                  45

Glu Pro Leu Tyr Pro Val Gln Val Ser Ser Ala Asp Ala Arg Leu Met
    50                  55                  60

Val Phe Asp Lys Thr Glu Gly Thr Trp Arg Leu Leu Cys Ser Ser Arg
65                  70                  75                  80

Ser Asn Ala Arg Val Ala Gly Leu Ser Cys Glu Glu Met Gly Phe Leu
                85                  90                  95

Arg Ala Leu Thr His Ser Glu Leu Asp Val Arg Thr Ala Gly Ala Asn
            100                 105                 110

Gly Thr Ser Gly Phe Phe Cys Val Asp Glu Gly Arg Leu Pro His Thr
        115                 120                 125

Gln Arg Leu Leu Glu Val Ile Ser Val Cys Asp Cys Pro Arg Gly Arg
    130                 135                 140

Phe Leu Ala Ala Ile Cys Gln Asp Cys Gly Arg Arg Lys Leu Pro Val
145                 150                 155                 160

Asp Arg Ile Val Gly Gly Arg Asp Thr Ser Leu Gly Arg Trp Pro Trp
                165                 170                 175

Gln Val Ser Leu Arg Tyr Asp Gly Ala His Leu Cys Gly Gly Ser Leu
            180                 185                 190

Leu Ser Gly Asp Trp Val Leu Thr Ala Ala His Cys Phe Pro Glu Arg
        195                 200                 205

Asn Arg Val Leu Ser Arg Trp Arg Val Phe Ala Gly Ala Val Ala Gln
    210                 215                 220

Ala Ser Pro His Gly Leu Gln Leu Gly Val Gln Ala Val Val Tyr His
225                 230                 235                 240
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Gly Gly Tyr Leu Pro Phe Arg Asp Pro Asn Ser Glu Glu Asn Ser Asn
                245                 250                 255

Asp Ile Ala Leu Val His Leu Ser Ser Pro Leu Pro Leu Thr Glu Tyr
            260                 265                 270

Ile Gln Pro Val Cys Leu Pro Ala Ala Gly Gln Ala Leu Val Asp Gly
        275                 280                 285

Lys Ile Cys Thr Val Thr Gly Trp Gly Asn Thr Gln Tyr Tyr Gly Gln
    290                 295                 300

Gln Ala Gly Val Leu Gln Glu Ala Arg Val Pro Ile Ile Ser Asn Asp
305                 310                 315                 320

Val Cys Asn Gly Ala Asp Phe Tyr Gly Asn Gln Ile Lys Pro Lys Met
                325                 330                 335

Phe Cys Ala Gly Tyr Pro Glu Gly Gly Ile Asp Ala Cys Gln Gly Asp
            340                 345                 350

Ser Gly Gly Pro Phe Val Cys Glu Asp Ser Ile Ser Arg Thr Pro Arg
        355                 360                 365

Trp Arg Leu Cys Gly Ile Val Ser Trp Gly Thr Gly Cys Ala Leu Ala
    370                 375                 380

Gln Lys Pro Gly Val Tyr Thr Lys Val Ser Asp Phe Arg Glu Trp Ile
385                 390                 395                 400

Phe Gln Ala Ile Lys Thr His Ser Glu Ala Ser Gly Met Val Thr Gln
                405                 410                 415

Leu
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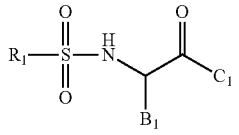
1.-**26**. (canceled)

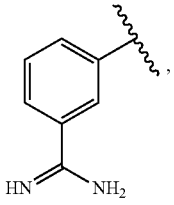
27. A compound of Formula (I), as a single stereoisomer or as a mixture thereof:

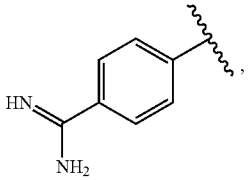


(I)

wherein R_1 is substituted or unsubstituted alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;

B_1 is selected from the group consisting of:





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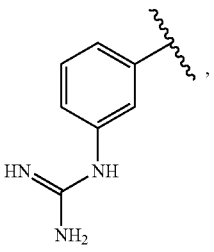

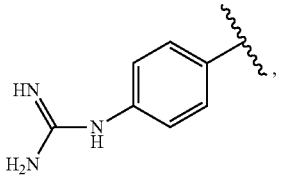

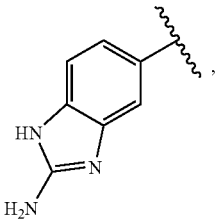

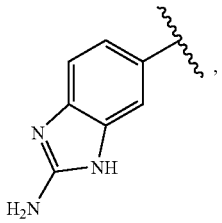

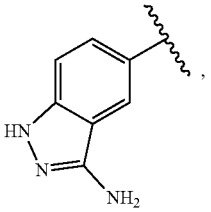

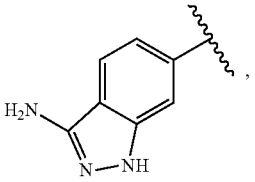

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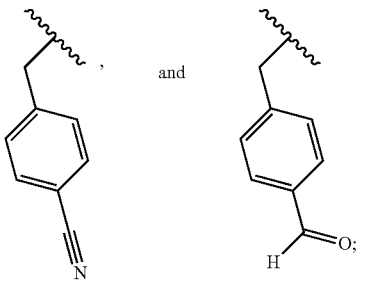
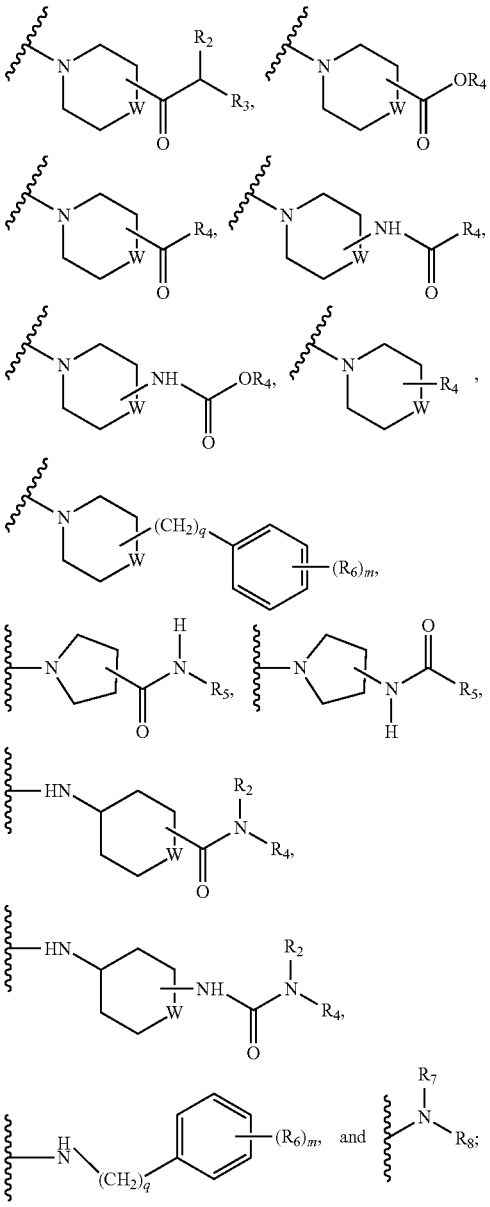


C_1 is a group selected from the group consisting of:



W is CH, CH_2, N, or NH;

R_2, R_3, R_4, R_5, R_6, R_7, and R_8 are each independently hydrogen, substituted or unsubstituted alkyl or cycloalkyl, substituted or unsubstituted alkylaryl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl or heteroaryl, with the proviso that when R_2 is methyl, then R_3 cannot also be methyl and vice versa; and

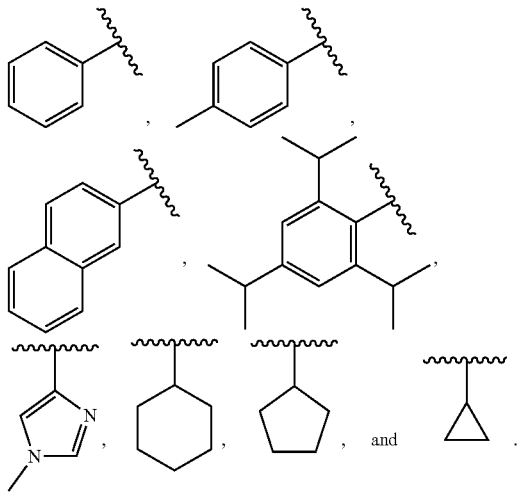
m is 0 to 5, or a pharmaceutically acceptable salt thereof.

28. The compound of claim **27** wherein R_1 is substituted or unsubstituted C_1-C_6 alkyl, substituted or unsubstituted C_3-C_6 cycloalkyl, substituted or unsubstituted phenyl, substituted or unsubstituted naphthyl, or a substituted or unsubstituted nitrogen-containing aromatic ring.

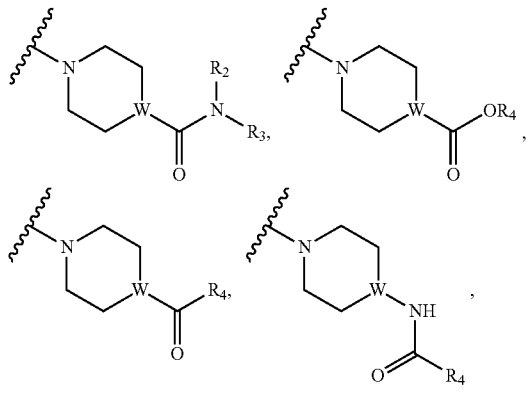
29. The compound of claim **28** wherein R_1 is substituted or unsubstituted phenyl or substituted or unsubstituted naphthyl.

30. The compound of claim **27** wherein the substituted C_1-C_6 alkyl, substituted C_3-C_6 cycloalkyl, substituted phenyl, substituted naphthyl, or substituted nitrogen-containing aromatic ring comprise one or more substituents comprising halo, hydroxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, halo-substituted C_1-C_4 alkyl, or amino.

31. The compound of claim **27** wherein R_1 is a group selected from the group consisting of:



32. The compound of claim **27** wherein C_1 is a group selected from the group consisting of:



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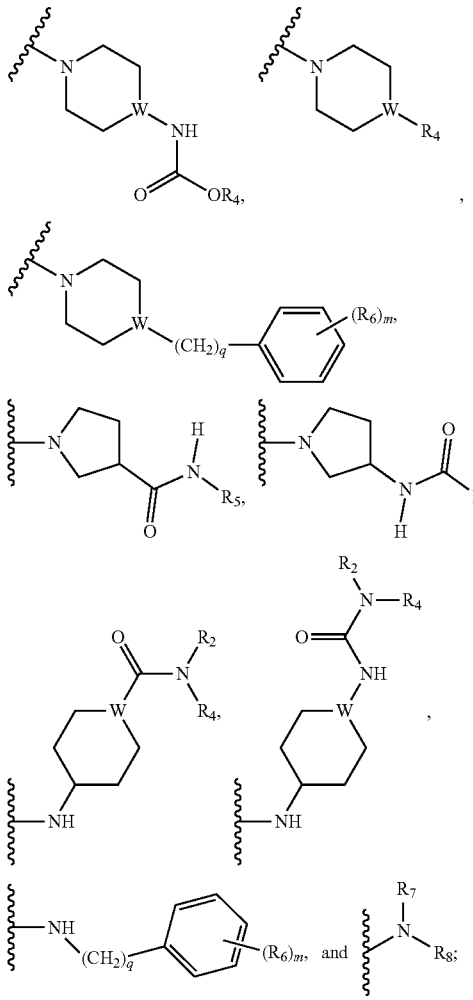

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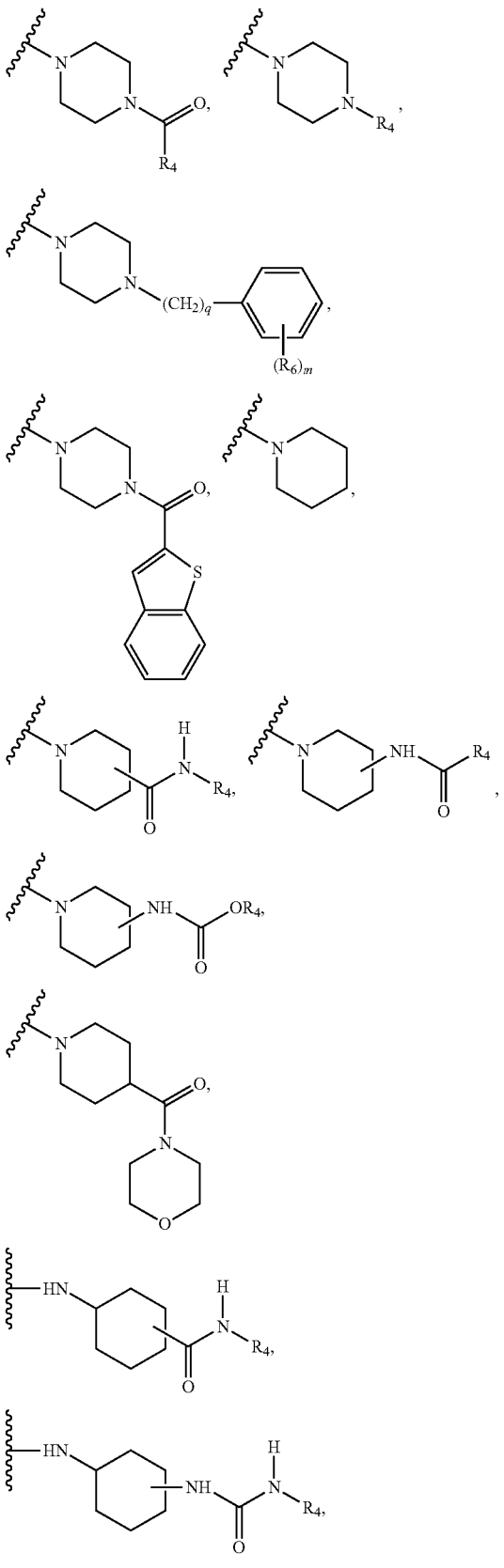
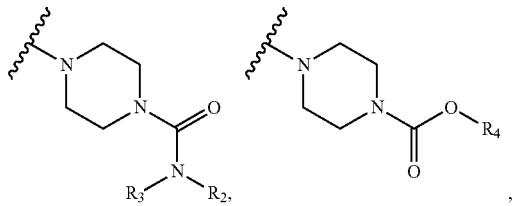


W is CH or N;

R_2, R_3, R_4, R_5, R_6, R_7, and R_8 are each independently hydrogen, substituted or unsubstituted alkyl or cycloalkyl, substituted or unsubstituted alkylaryl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl or heteroaryl, with the proviso that when R_2 is methyl, then R_3 cannot also be methyl and vice versa; and

m is 0 to 5, or a pharmaceutically acceptable salt thereof.

33. The compound of claim **27** wherein C_1 is a group selected from the group consisting of:



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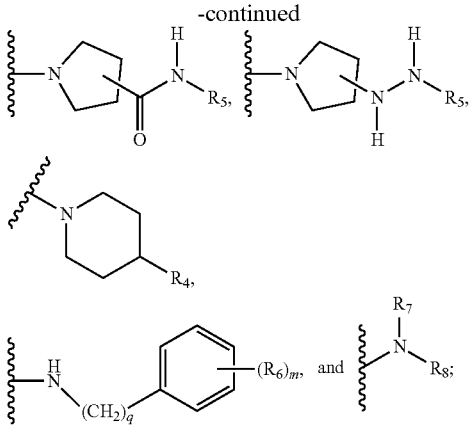


R_2, R_3, R_4, R_5, R_6, R_7, and R_8 are each independently hydrogen, substituted or unsubstituted alkyl or cycloalkyl, substituted or unsubstituted alkylaryl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl or heteroaryl, with the proviso that when R_2 is methyl, then R_3 cannot also be methyl and vice versa; and

m is 0 to 5, or a pharmaceutically acceptable salt thereof.

34. The compound of claim **27** wherein R_2, R_3, R_4, R_5, R_6, R_7, and R_8 are each independently hydrogen, substituted or unsubstituted C_1-C_{10} alkyl or cycloalkyl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

35. The compound of claim **27** wherein:

R_2 is hydrogen;

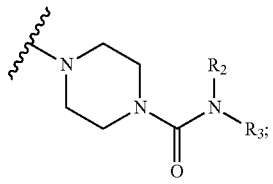
R_3 is hydrogen, C_1-C_6 alkyl, benzyl, or halo-substituted benzyl;

R_4 and R_5 are each independently hydrogen, C_1-C_6 alkyl, halo- or alkoxy-substituted C_1-C_6 alkyl, phenyl, phenethyl, benzyl, halo- or alkoxy-substituted benzyl; substituted or unsubstituted 3-benzothiophenyl, or substituted or unsubstituted 1-morpholinyl;

R_6 is hydrogen, C_1-C_4 alkoxy; and/or

R_7 and R_8 are each independently hydrogen or C_1-C_6 alkyl.

36. The compound of claim **27** wherein C_1 is



R_2 is hydrogen; and

R_3 is hydrogen, C_1-C_6 alkyl, benzyl, halo-substituted benzyl, aryl, cycloalkyl, alkylaryl, or hetercyclo.

37.-**41**. (canceled)

42. A method of inhibiting tumor progression comprising administering to the subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of claim **27**.

43. A method of treating a malignancy, a pre-malignant condition, or cancer in a subject comprising administering to the subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of claim **27**.

44. The method of claim **43**, wherein the cancer is selected from the group consisting of breast, ovarian, prostate, endometrial, colon, pancreatic, head and neck, gastric, renal, brain, liver, bladder, kidney, lung, esophageal, leukemias, multiple myeloma, lymphoma, and melanoma.

45.-**52**. (canceled)

53. A pharmaceutical composition comprising a therapeutically effective amount of at least one compound of claim **27**.

54. An imaging composition comprising a fluorescent compound of Formula (I) or (II) of claim **27**, wherein the labeled compound comprises a fluorophore selected from the group consisting of Cy3, Cy3.5, Cy5, Cy5.5, Cy7, and Cy7.5.

55. A method of detecting cancer comprising:

(i) administering to a subject an imaging composition of claim **54**;

(ii) employing a fluorescence imaging technique for monitoring or visualizing a distribution of the fluorescent compound within the body or within a portion thereof; and

(iii) correlating the distribution of the fluorescent compound to the existence of cancer.

56. An imaging composition comprising a radiolabeled compound of Formula (I) or (II) of claim **27**, wherein the labeled compound comprises a radioisotope selected from the group consisting of ^{11}C, ^{13}N, ^{15}O, ^{18}F, ^{75}Br, ^{124}I, ^{125}I, and ^{131}I.

57. A method of detecting cancer comprising:

(iv) administering to a subject an imaging composition of claim **56**;

(v) employing a nuclear imaging technique for monitoring or visualizing a distribution of the radiolabeled compound within the body or within a portion thereof; and

(vi) correlating the distribution of the radiolabeled compound to the existence of cancer.

58. The method of claim **57**, wherein the nuclear imaging technique is positron emission tomography (PET) or photon emission computed tomography (SPECT).

* * * * *