(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 November 2012 (01.11.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/149102 A1

(51) International Patent Classification:
A01N 43/42 (2006.01) *C07D 513/02* (2006.01)
A61K 31/44 (2006.01)

(21) International Application Number:
PCT/US2012/035114

(22) International Filing Date:
26 April 2012 (26.04.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/480,454 29 April 2011 (29.04.2011) US

(71) Applicant *(for all designated States except US)*: **GLAXOSMITHKLINE LLC** [US/US]; One Franklin Plaza, 200 North 16th Street, Philadelphia, PA 19102 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BOBKO, Mark, A.** [US/US]; 1250 South Collegeville Road, Collegeville, PA 19426 (US). **DARCY, Michael, G.** [US/US]; 709 Swedeland Road, King Of Prussia, PA 19406 (US). **EVANS, Karen, A.** [US/US]; 1250 South Collegeville Road, Collegeville, PA 19426 (US). **FAITG, Thomas, H.** [US/US]; 1250 South Collegeville Road, Collegeville, PA 19426 (US). **KAURA, Arun, Chandar** [US/US]; 1250 South Collegeville Road, Collegeville, PA 19426 (US). **PENG, Xin** [US/US]; 1250 South Collegeville Road, Collegeville, PA 19426 (US). **SARPONG, Martha, A.** [US/US]; 1250 South Collegeville Road, Collegeville, PA 19426 (US). **SEEFELD, Mark, A.** [US/US]; 1250 South Collegeville Road, Collegeville, PA 19426 (US). **SU, Dai-shi** [US/US]; 1250 South Collegeville Road, Collegeville, PA 19426 (US).

(74) Agents: **PENG, Tony, W.** et al.; GlaxoSmithKline, Global Patents, UW2220, 709 Swedeland Road, P.O. Box 1539, King Of Prussia, PA 19406-0939 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2012/149102 A1

(54) Title: NOVEL COMPOUNDS AS WIP1 INHIBITORS

(57) Abstract: This invention relates to the use of acylated alanine derivatives for the modulation, notably the inhibition of the activity of WIP1. Suitably, the present invention relates to the use of acylated alanine derivatives in the treatment of cancer.

NOVEL COMPOUNDS AS WIP1 INHIBITORS

This application claims the benefit of US Provisional Application No. 61/480454 filed 29 April 2011, which is incorporated herein in its entirety.

FIELD OF THE INVENTION

This invention relates to the acylated alanine derivatives for the inhibition and/or induced destabilization of the Wip1 (PPM1D/PP2Cδ) phosphatase. The invention relates to the use of the acylated alanine derivatives in the treatment of cancer.

BACKGROUND OF THE INVENTION

Wild-type P53 inducible Phosphatase 1 (Wip1/PPM1D/ PP2Cδ) is a serine threonine phosphatase that plays a key role in the homeostatic down-regulation of various stress response and DNA repair signaling pathways. Wip1 is a member of the type 2C family protein phosphatase family that are dependent on Mg^{2+} or Mn^{2+} for their catalytic activity. As its name suggests, Wip1 can be induced by various DNA damaging treatments including ionizing or UV irradiation in a P53 dependent manner (Fiscella et al. *PNAS* 94, 6048-6053 (1997)). While its RNA expression is rapidly induced by various DNA damaging agents, translation of the RNA into Wip1 protein is reportedly delayed by the miR-16 micro RNA (Zhang et al., 2010). Upon its induction, Wip1 acts upon multiple effectors of the DNA damage response pathway to dephosphorylate and consequently inactivate them. Among its reported substrates are UNG2 (Lu et al., *Molecular Cell* 15, 621-634 (2004)), MDM2 (Lu et al., *Cancer Cell* 12, 342-354 (2008)), MDMX (Xinna et al., *Journal of Biological Chemistry* 281, 24847-24862 (2009)), ATM (Shreeram et al., *Journal of Experimental Medicine* 203, 2793-2799 (2006)), γH2AX (Cha et al., *Cancer Research* 70, 4112-4122 (2010)) , p38MAPK (Bulavin et al., *Nature Genetics* 36, 343-350 (2004)), Chk2 (Fujimoto et al., *Cell Death and Differentiation* 13, 1170-1180 (2006)), Chk1 and p53(Lu et al., Genes and Development 19, 1162-1174 (2005)).

Several lines of evidence have suggested an oncogenic role for Wip1. In cellular studies, overexpression of Wip1 in E1A transduced rat embryonic fibroblasts was sufficient to induce transformation foci (Nannenga et al., *Molecular Carcinogenesis* 45, 594-604 (2006)). Conversely, murine embryonic fibroblasts homozygous for Wip1 deletion showed significantly reduced tumor growth when co-transfected with the transforming

oncogenes HRAS and E1A, HRAS and ErbB2, or HRAS and Myc (Bulavin et.al., *Nature Genetics* 36, 343-350 (2004)). Wip1-/- mice also demonstrated tumor-resistance to both induced tumor models, using infection with MMTV driven ErbB2 or HRAS, as well as a decreased incidence of spontaneous leukemias and sarcomas. Similarly, Wip1-/- mice were relatively resistant to Eμ-Myc induced lymphomas (Shreeram et al., *Journal of Experimental Medicine* 203, 2793-2799 (2006)).

Overexpression of Wip1 is reported to result in delay or suppression of base-excision repair by inhibition of UNG2 and P53. Similarly, its overexpression suppresses DNA double-strand-break repair by inhibition of the ATM response, inhibiting repair by both homologous recombination and non-homologous end joining. Conversely, silencing or knockout of Wip1 has been reported to elevate the activity of multiple stress response pathways, resulting in elevated levels of phospho-p38 , phospho-p53(S15), p53 response genes p21/Waf, p16INK4A, and ARF (Bulavin et al., *Nature Genetics* 36, 343-350 (2004)), and elevated γH2AX and DNA damage-associated nuclear foci *(Moon et al., Journal of Biological Chemistry* 285, 12935-12947 (2010)).

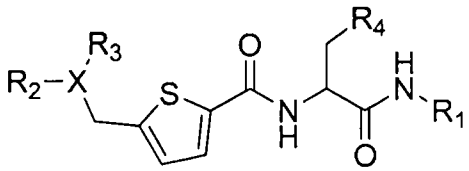
Amplified or overexpressed Wip1 has also been proposed to promote tumorigenesis in multiple cancers by suppressing the regulatory activity of a host of its tumor suppressor substrates. Amplification of the Wip1/PPM1D gene locus on 17q23 has been reported in breast cancer (Li et al., *Nature Genetics* 31, 133-134.(2002)), ovarian clear cell carcinoma (Hirasawa et al., *Clinical Cancer Research* 9, 1995-2004 (2003)), neuroblastoma (Saito-Ohara et al., *Journal of Experimental Medicine* 203, 2793-2799 (2003)), and pancreatic adenocarcinoma (Loukopoulos et al., *Cancer Science* 98, 392-400 (2007)). Moreover, elevated expression of Wip1 is reported in medulloblastomas (Castellino et al., *Journal of Neuro-Oncology* 86, 245-256 (2008)) and gastric carcinomas (Fuku et al., *Pathology International* 57, 566-571 (2007)). In several of these tumors, gene amplification has been confirmed to correlate with elevated protein expression.

Inhibition of Wip1 by small molecule inhibitors or by RNA interference has been shown to have antiproliferative effects in tumor cells. In cell culture studies, silencing of Wip1 by RNA interference results in elevated phosphorylation and activation of multiple tumor suppressors including p53, Chk2 and γH2AX, and results in tumor cell growth inhibition.(Fujimoto et al., *Cell Death and Differentiation* 13, 1170-1180 (2006); Lu et al.,

Cancer Cell 12, 342-354 (2007)). Several groups have also reportedly identified Wip1 inhibitors, describing antiproliferative activity in Wip1 amplified or overexpressing cell lines (Belova et al., *Cancer Biology and Therapy* 4, 1154-1158. (2005); Rayter et al., *Oncogene* 27, 1036-1044 (2008)). Taken together, these observations suggest that Wip1 is a compelling target for pharmacological inhibition in the treatment of cancer.

SUMMARY OF THE INVENTION

This invention relates to a compound of Formula (I);



(I)

wherein

R_1 is hydrogen, C_1-C_4alkoxy, C_1-C_4alkyl, substituted C_1-C_4alkyl, C_3-C_7cycloalkyl, or C_3-C_7heterocycloalkyl, wherein said C_3-C_7cycloalkyl may be substituted with one to three substituents selected from the group consisting of hydroxyl and -O(O)Ra, wherein Ra is C_1-C_6alkyl or substituted C_1-C_6alkyl;

R_2 is an aryl or heteroaryl ring, which may be substituted with one to four substituents selected from the group consisting of halo, C_1-C_3alkyl, substituted C_1-C_3alkyl, C_1-C_4alkoxy, hydroxyl, amino, substituted amino, C_3-C_7cycloalkyl, cyano, ester, carboxylic acid, and C_3-C_6heterocycloalkyl;

R_3 is hydrogen, C_1-C_6alkyl, substituted C_1-C_6alkyl, or R_3 is absent when X is O or S;

R_4 is cyclohexyl or cyclopentyl;

X is N, O or S;

or a pharmaceutically acceptable salt thereof.

This invention also relates to pharmaceutical compositions, which comprise compounds of Formula (I) and pharmaceutically acceptable carriers.

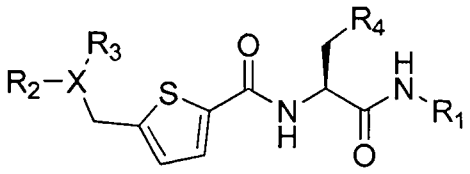
This invention also relates to methods of treating cancer which comprise administering an effective amount of a compound of Formula (I) to a human in need thereof.

This invention also relates to methods of treating cancer which comprise co-administering an compound of Formula (I) and a second compound to a human in need thereof.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to compounds of Formula (I);

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



(II);

wherein

R_1 is hydrogen, C_1-C_4alkoxy, C_1-C_4alkyl, substituted C_1-C_4alkyl, C_3-C_7cycloalkyl, or C_3-C_7heterocycloalkyl, wherein said C_3-C_7cycloalkyl may be substituted with one to three substituents selected from the group consisting of hydroxyl and -O(O)Ra, wherein Ra is C_1-C_6alkyl or substituted C_1-C_6alkyl;

R_2 is an aryl or heteroaryl ring, which may be substituted with one to four substituents selected from the group consisting of halo, C_1-C_3alkyl, substituted C_1-C_3alkyl, C_1-C_4alkoxy, hydroxyl, amino, substituted amino, C_3-C_7cycloalkyl, cyano, ester, carboxylic acid, and C_3-C_6heterocycloalkyl;

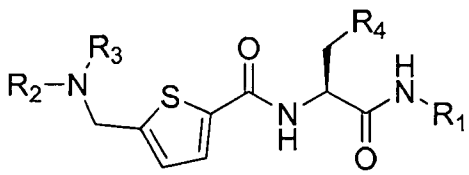
R_3 is hydrogen, C_1-C_6alkyl, substituted C_1-C_6alkyl, or R_3 is absent when X is O or S;

R_4 is cyclohexyl or cyclopentyl;

X is N, O or S;

or a pharmaceutically acceptable salt thereof.

The present invention also relates to compounds of Formula (III):



(III);

wherein

R_1 is hydrogen, C_1-C_4alkoxy, C_1-C_4alkyl, substituted C_1-C_4alkyl, C_3-C_7cycloalkyl, or C_3-C_7heterocycloalkyl, wherein said C_3-C_7cycloalkyl may be substituted with one to three substituents selected from the group consisting of hydroxyl and -O(O)Ra, wherein Ra is C_1-C_6alkyl or substituted C_1-C_6alkyl;

R_2 is an aryl or heteroaryl ring, which may be substituted with one to four substituents selected from the group consisting of halo, C_1-C_3alkyl, substituted C_1-C_3alkyl, C_1-C_4alkoxy, hydroxyl, amino, substituted amino, C_3-C_7cycloalkyl, cyano, ester, carboxylic acid, and C_3-C_6heterocycloalkyl;

R_3 is hydrogen, C_1-C_6alkyl, or substituted C_1-C_6alkyl;

R_4 is cyclohexyl or cyclopentyl;

or a pharmaceutically acceptable salt thereof.

The present invention also relates to compounds according to any one of Formulas (I)-(III), wherein R_1 is C_1-C_4alkyl or substituted C_1-C_4alkyl.

The present invention also relates to compounds according to any one of Formulas (I)-(III), wherein R_1 is C_3-C_7cycloalkyl.

The present invention also relates to compounds according to any one of Formulas (I)-(III), wherein R_1 is cyclopropyl or cyclobutyl, wherein said cyclopropyl and cyclobutyl

may be substituted with hydroxyl or –O(O)Ra, wherein Ra is C_1-C_6alkyl or substituted C_1-C_6alkyl.

The present invention also relates to compounds according to any one of Formulas (I)-(III), wherein R_2 is phenyl, which may be substituted with one to four substituents selected from the group consisting of halogen, C_1-C_3alkyl, substituted C_1-C_3alkyl, C_1-C_4alkoxy, hydroxyl, amino, substituted amino, C_3-C_7cycloalkyl, C_3-C_7heterocycloalkyl, cyano, ester, and carboxylic acid.

The present invention also relates to compounds according to any one of Formulas (I)-(III), wherein R_2 is pyridinyl, which may be substituted with one to three substituents selected from the group consisting of halo, C_1-C_3alkyl, and substituted C_1-C_3alkyl.

The present invention also relates to compounds according to any one of Formulas (I)-(III), wherein R_3 is hydrogen.

The present invention also relates to compounds according to any one of Formulas (I)-(III), wherein R_4 is cyclopentyl.

The present invention also relates to compounds selected from the group consisting of:

(S)-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(4-chloropyridin-2-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloro-pyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(4-chloro-pyridin-2-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-*N*-(1-amino-3-cyclopentyl-1-oxopropan-2-yl)-5-{[(5-chloropyridin-3-yl)amino]methyl}thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(methylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(dimethylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(methoxyamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(4-chloropyridin-2-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(4-chloropyridin-2-yl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(4-chloropyridin-2-yl)amino]methyl}-*N*-[3-cyclohexyl-1-(methylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)oxy]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloro-2-methylpyridin-3-oxy]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)oxy]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)oxy]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(3-chlorophenyl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-methoxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[*((1r,3S)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[*((1s,3R)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(cyclopropylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(1S,3r)-3-[*(S)*-2-(5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxamido)-3-cyclopentylpropanamido]cyclobutyl acetate; and

(S)-(1S,3S)-3-[*(S)*-2-(5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxamido)-3-cyclopentylpropanamido]cyclobutyl 2,6-diaminohexanoate;

This invention also relates to compounds exemplified in the Experimental section.

Typically, but not absolutely, the salts of the present invention are pharmaceutically acceptable salts. Salts encompassed within the term "pharmaceutically acceptable salts" refer to non-toxic salts of the compounds of this invention. Salts of the compounds of the present invention may comprise acid addition salts. In general, the salts are formed from pharmaceutically acceptable inorganic and organic acids. More specific examples of suitable acid salts include maleic, hydrochloric, hydrobromic, sulphuric, phosphoric, nitric, perchloric, fumic, acetic, propionic, succinic, glycolic, formic, lactic, aleic, tartaric, citric, palmoic, malonic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic,

fumaric, toluenesulfonic, methansulfonic (mesylate), naphthalene-2-sulfonic, benzenesulfonic, hydroxynaphthoic, hydroiodic, malic, teroic, tannic, and the like.

Other representative salts include acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, calcium edetate, camsylate, carbonate, clavulanate, citrate, dihydrochloride, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylsulfate, monopotassium maleate, mucate, napsylate, nitrate, oxalate, pamoate (embonate), palmitate, pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, teoclate, tosylate, triethiodide, and valerate salts.

The compound of Formula (I) or a salt thereof may exist in stereoisomeric forms (e.g., it contains one or more asymmetric carbon atoms). The individual stereoisomers (enantiomers and diastereomers) and mixtures of these are included within the scope of the present invention. The invention also covers the individual isomers of the compound or salt represented by Formula (I) as mixtures with isomers thereof in which one or more chiral centers are inverted. Likewise, it is understood that a compound or salt of Formula (I) may exist in tautomeric forms other than that shown in the formula and these are also included within the scope of the present invention. It is to be understood that the present invention includes all combinations and subsets of the particular groups defined hereinabove. The scope of the present invention includes mixtures of stereoisomers as well as purified enantiomers or enantiomerically/diastereomerically enriched mixtures. Also included within the scope of the invention are any wholly or partially deuteuriated isotopes of the compounds of Formula (I). Also included within the scope of the invention are individual isomers of the compound represented by Formula (I), as well as any wholly or partially equilibrated mixtures thereof. The present invention also includes the individual isomers of the compound or salt represented by the Formula (I) as well as mixtures with isomers thereof in which one or more chiral centers are inverted.

It will be appreciated by those skilled in the art that certain protected derivatives of compounds of formula (I), which may be made prior to a final deprotection stage, may not possess pharmacological activity as such, but may, in certain instances, be administered orally or parenterally and thereafter metabolised in the body to form compounds of the

invention which are pharmacologically active. Such derivatives may therefore be described as "prodrugs". Further, certain compounds of the invention may act as prodrugs of other compounds of the invention. All protected derivatives and prodrugs of compounds of the invention are included within the scope of the invention. It will further be appreciated by those skilled in the art, that certain moieties, known to those skilled in the art as "pro-moieties" may be placed on appropriate functionalities when such functionalities are present within compounds of the invention. Preferred prodrugs for compounds of the invention include: esters, carbonate esters, hemi-esters, phosphate esters, nitro esters, sulfate esters, sulfoxides, amides, carbamates, azo-compounds, phosphamides, glycosides, ethers, acetals and ketals. The scope of the present invention also includes prodrugs of the present compounds.

DEFINITIONS

Terms are used within their accepted meanings. The following definitions are meant to clarify, but not limit, the terms defined.

As used herein, the term "alkyl" (or "alkylene") refers to a straight or branched chain alkyl, preferably having from one to twelve carbon atoms, which may be saturated or unsaturated with multiple degrees of substitution included within the present invention. Examples of "alkyl" as used herein include methyl, ethyl, propyl, isopropyl, isobutyl, n-butyl, t-butyl, isopentyl, n-pentyl, and the like, as well as substituted versions thereof.

As used herein, the term "substituted alkyl" (or "alkylene") refers to a straight or branched chain alkyl, preferably having from one to twelve carbon atoms, which may be saturated or unsaturated with multiple degrees of substitution included within the present invention. Suitable substituents are selected from the group consisting of: halogen, amino, substituted amino, urea, cyano, hydroxyl, alkoxy, alkylthio, alkylsulfonyl, amidosulfonyl, carboxylic acid, ester, carboxamide, and aminocarbonyl.

As used herein, the term "cycloalkyl" refers to an unsubstituted or substituted mono- or polycyclic non-aromatic saturated ring, which optionally includes an alkylene linker through which the cycloalkyl may be attached. Exemplary "cycloalkyl" groups

include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and the like, as well as unsubstituted and substituted versions thereof.

As used herein, the term "alkoxy" refers to the group $-OR_a$, where R_a is C_1-C_4alkyl or C_3-$_7$cycloalkyl as defined above.

As used herein, the term "substituted amino" is meant –NR'R" wherein each R' and R" is independently selected from a group including hydrogen, unsubstituted C_1-C_6alkyl, acyl, unsubstituted C_3-C_7cycloalkyl, wherein at least one of R' and R" is not hydrogen. Examples of substituted amino includes, but are not limited to alkylamino, dialkylaminio, acylamino, and cycloalkylamino.

As used herein, the term "heterocycle" or "heterocyclyl" or "heterocycloalkyl" refers to unsubstituted and substituted mono- or polycyclic non-aromatic ring system containing one or more heteroatoms. Preferred heteroatoms include N, O, and S, including N-oxides, sulfur oxides, and dioxides. Preferably the ring is three to eight-membered and is either fully saturated or has one or more degrees of unsaturation. Multiple degrees of substitution, preferably one, two or three, are included within the present definition. Examples of "heterocyclic" groups include, but are not limited to oxetanyl, tetrahydrofuranyl, pyranyl, 1,4-dioxanyl, 1,3-dioxanyl, piperidinyl, pyrrolidinyl, morpholinyl, azetidinyl, piperazinyl, pyrrolidinonyl, piperazinonyl, pyrazolidinyl, and their various tautomers, as well as unsubstituted and substituted versions thereof.

As used herein, the term "aryl", unless otherwise defined, is meant aromatic, hydrocarbon, ring system. The ring system may be monocyclic or fused polycyclic (e.g., bicyclic, tricyclic, etc.), substituted or unsubstituted. In various embodiments, the monocyclic aryl ring is C5-C10, or C5-C7, or C5-C6, where these carbon numbers refer to the number of carbon atoms that form the ring system. A C6 ring system, i.e. a phenyl ring, is a suitable aryl group. In various embodiments, the polycyclic ring is a bicyclic aryl group, where suitable bicyclic aryl groups are C8-C12, or C9-C10. A naphthyl ring, which has 10 carbon atoms, is a suitable polycyclic aryl group. Suitable substituents for aryl are described in the definition of "optionally substituted".

As used herein, the term "heteroaryl", unless otherwise defined, is meant an aromatic ring system containing carbon(s) and at least one heteroatom. Heteroaryl may be

monocyclic or polycyclic, substituted or unsubstituted. A monocyclic heteroaryl group may have 1 to 4 heteroatoms in the ring, while a polycyclic heteroaryl may contain 1 to 10 hetero atoms. A polycyclic heteroaryl ring may contain fused, spiro or bridged ring junctions, for example, bicyclic heteroaryl is a polycyclic heteroaryl. Bicyclic heteroaryl rings may contain from 8 to 12 member atoms. Monocyclic heteroaryl rings may contain from 5 to 8 member atoms (carbons and heteroatoms). Exemplary heteroaryl groups include: benzofuran, benzothiophene, furan, imidazole, indole, isothiazole, oxazole, pyrazine, pyrazole, pyridazine, pyridine, pyrimidine, pyrrole, quinoline, quinazoline, quinoxaline, thiazole, and thiophene. Suitable substituents for heteroaryl are described in the definition of "optionally substituted".

As used herein, the term "cyano" refers to the group -CN.

As used herein, the term "acyl" refers to the group $-C(O)R_b$, where R_b is alkyl, cycloalkyl, or heterocyclyl, as each is defined herein.

As used herein, the term "optionally" means that the subsequently described event(s) may or may not occur, and includes both event(s) that occur and event(s) that do not occur.

As used herein, unless otherwise defined, the phrase "substituted" or variations thereof denote a substitution, including multiple degrees of substitution, with one or more substitutents, preferably one, two or three. The phrase should not be interpreted as duplicative of the substitutions herein described and depicted. Exemplary substituent groups include acyl, alkyl, substituted alkyl, alkylsulfonyl, alkoxy, alkoxycarbonyl, cyano, halogen, haloalkyl, hydroxyl, oxo, amide, sulfamide, urea, amino, substituted amino, acylamino, phenylcarbonyl, dialkylaminosulfonamide, morpholino, sulfonamide, thiourea, carboxylic acid, ester, and nitro.

The invention further provides a pharmaceutical composition (also referred to as pharmaceutical formulation) comprising a compound of Formula (I) or pharmaceutically acceptable salt, thereof and one or more excipients (also referred to as carriers and/or diluents in the pharmaceutical arts). The excipients are acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof (i.e., the patient).

In accordance with another aspect of the invention there is provided a process for the preparation of a pharmaceutical composition comprising mixing (or admixing) a compound of Formula (I) or salt thereof with at least one excipient.

PHARMACEUTICAL COMPOSITIONS

Pharmaceutical compositions may be in unit dose form containing a predetermined amount of active ingredient per unit dose. Such a unit may contain a therapeutically effective dose of the compound of Formula (I) or salt thereof or a fraction of a therapeutically effective dose such that multiple unit dosage forms might be administered at a given time to achieve the desired therapeutically effective dose. Preferred unit dosage formulations are those containing a daily dose or sub-dose, as herein above recited, or an appropriate fraction thereof, of an active ingredient. Furthermore, such pharmaceutical compositions may be prepared by any of the methods well-known in the pharmacy art.

Pharmaceutical compositions may be adapted for administration by any appropriate route, for example, by oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual, or transdermal), vaginal, or parenteral (including subcutaneous, intramuscular, intravenous, or intradermal) routes. Such compositions may be prepared by any method known in the art of pharmacy, for example, by bringing into association the active ingredient with the excipient(s).

When adapted for oral administration, pharmaceutical compositions may be in discrete units such as tablets or capsules; powders or granules; solutions or suspensions in aqueous or non-aqueous liquids; edible foams or whips; oil-in-water liquid emulsions or water-in-oil liquid emulsions. The compound or salt thereof of the invention or the pharmaceutical composition of the invention may also be incorporated into a candy, a wafer, and/or tongue tape formulation for administration as a "quick-dissolve" medicine.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like. Powders or granules are prepared by comminuting the compound to a suitable fine size and mixing with a similarly comminuted pharmaceutical carrier such as an edible carbohydrate, as, for example, starch or mannitol. Flavoring, preservative, dispersing, and coloring agents can also be present.

Capsules are made by preparing a powder mixture, as described above, and filling formed gelatin or non-gelatinous sheaths. Glidants and lubricants such as colloidal silica, talc, magnesium stearate, calcium stearate, solid polyethylene glycol can be added to the powder mixture before the filling operation. A disintegrating or solubilizing agent such as agar-agar, calcium carbonate, or sodium carbonate can also be added to improve the availability of the medicine when the capsule is ingested.

Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents, and coloring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars, such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth, sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, and the like. Disintegrators include, without limitation, starch, methylcellulose, agar, bentonite, xanthan gum, and the like.

Tablets are formulated, for example, by preparing a powder mixture, granulating or slugging, adding a lubricant and disintegrant, and pressing into tablets. A powder mixture is prepared by mixing the compound, suitably comminuted, with a diluent or base as described above, and optionally, with a binder such as carboxymethylcellulose, and aliginate, gelatin, or polyvinyl pyrrolidone, a solution retardant such as paraffin, a resorption accelerator such as a quaternary salt, and/or an absorption agent such as bentonite, kaolin, or dicalcium phosphate. The powder mixture can be granulated by wetting a binder such as syrup, starch paste, acadia mucilage, or solutions of cellulosic or polymeric materials and forcing through a screen. As an alternative to granulating, the powder mixture can be run through the tablet machine and the result is imperfectly formed slugs broken into granules. The granules can be lubricated to prevent sticking to the tablet forming dies by means of the addition of stearic acid, a stearate salt, talc, or mineral oil. The lubricated mixture is then compressed into tablets. The compound or salt of the present invention can also be combined with a free-flowing inert carrier and compressed into tablets directly without going through the granulating or slugging steps. A clear opaque protective coating consisting of a sealing coat of shellac, a coating of sugar, or polymeric material, and a polish coating of wax can be provided. Dyestuffs can be added to these coatings to distinguish different dosages.

Oral fluids such as solutions, syrups, and elixirs can be prepared in dosage unit form so that a given quantity contains a predetermined amount of active ingredient. Syrups can be prepared by dissolving the compound or salt thereof of the invention in a suitably flavoured aqueous solution, while elixirs are prepared through the use of a non-toxic alcoholic vehicle. Suspensions can be formulated by dispersing the compound or salt of the invention in a non-toxic vehicle. Solubilizers and emulsifiers, such as ethoxylated isostearyl alcohols and polyoxyethylene sorbitol ethers, preservatives, flavor additives such as peppermint oil, natural sweeteners, saccharin, or other artificial sweeteners, and the like, can also be added.

Where appropriate, dosage unit formulations for oral administration can be microencapsulated. The formulation can also be prepared to prolong or sustain the release as, for example, by coating or embedding particulate material in polymers, wax, or the like.

In the present invention, tablets and capsules are preferred for delivery of the pharmaceutical composition.

As used herein, the term "treatment" includes prophylaxis and refers to alleviating the specified condition, eliminating or reducing one or more symptoms of the condition, slowing or eliminating the progression of the condition, and preventing or delaying the reoccurrence of the condition in a previously afflicted or diagnosed patient or subject. Prophylaxis (or prevention or delay of disease onset) is typically accomplished by administering a drug in the same or similar manner as one would to a patient with the developed disease or condition.

The present invention provides a potential treatment in a mammal, especially a human, suffering from disease conditions targeted by the present compounds. Such treatment comprises the step of administering a therapeutically effective amount of a compound of Formula (I) or salt thereof to said mammal, particularly a human. Treatment can also comprise the step of administering a therapeutically effective amount of a pharmaceutical composition containing a compound of Formula (I) or salt thereof to said mammal, particularly a human.

As used herein, the term "effective amount" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal, or human that is being sought, for instance, by a researcher or clinician.

The term "therapeutically effective amount" means any amount which, as compared to a corresponding subject who has not received such amount, results in improved treatment, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function. For use in therapy, therapeutically effective amounts of a compound of Formula (I), as well as salts thereof, may be administered as the raw chemical. Additionally, the active ingredient may be presented as a pharmaceutical composition.

While it is possible that, for use in therapy, a therapeutically effective amount of a compound of Formula (I) or salt thereof may be administered as the raw chemical, it is typically presented as the active ingredient of a pharmaceutical composition or formulation.

The precise therapeutically effective amount of a compound or salt thereof of the invention will depend on a number of factors, including, but not limited to, the age and weight of the subject (patient) being treated, the precise disorder requiring treatment and its severity, the nature of the pharmaceutical formulation/composition, and route of administration, and will ultimately be at the discretion of the attending physician or veterinarian. Typically, a compound of Formula (I) or salt thereof will be given for the treatment in the range of about 0.01 to 100 mg/kg body weight of recipient (patient, mammal) per day and more usually in the range of 0.1 to 10 mg/kg body weight per day. Acceptable daily dosages may be from about 1 to about 1000 mg/day, and preferably from about 1 to about 100 mg/day. This amount may be given in a single dose per day or in a number (such as two, three, four, five, or more) of sub-doses per day such that the total daily dose is the same. An effective amount of a salt thereof may be determined as a proportion of the effective amount of the compound of Formula (I) per se. Similar dosages should be appropriate for treatment (including prophylaxis) of the other conditions referred herein for treatment. In general, determination of appropriate dosing can be readily arrived at by one skilled in medicine or the pharmacy art.

COMBINATIONS

When a compound of Formula (I) is administered for the treatment of cancer, the term "co-administering" and derivatives thereof as used herein is meant either simultaneous administration or any manner of separate sequential administration of a LSD1 inhibiting compound, as described herein, and a further active ingredient or ingredients, known to be useful in the treatment of cancer, including chemotherapy and radiation treatment. The term further active ingredient or ingredients, as used herein, includes any compound or therapeutic agent known to or that demonstrates advantageous properties when administered to a patient in need of treatment for cancer. Preferably, if the administration is not simultaneous, the compounds are administered in a close time proximity to each other. Furthermore, it does not matter if the compounds are administered in the same dosage form, e.g. one compound may be administered topically and another compound may be administered orally.

Typically, any anti-neoplastic agent that has activity versus a susceptible tumor being treated may be co-administered in the treatment of cancer in the present invention. Examples of such agents can be found in Cancer Principles and Practice f Oncology by V.T. Devita and S. Hellman (editors), 6th edition (February 15, 2001), Lippincott Williams & Wilkins Publishers. A person of ordinary skill in the art would be able to discern which combinations of agents would be useful based on the particular characteristics of the drugs and the cancer involved. Typical anti-neoplastic agents useful in the present invention include, but are not limited to, anti-microtubule agents such as diterpenoids and vinca alkaloids; platinum coordination complexes; alkylating agents such as nitrogen mustards, oxazaphosphorines, alkylsulfonates, nitrosoureas, and triazenes; antibiotic agents such as anthracyclins, actinomycins and bleomycins; topoisomerase II inhibitors such as epipodophyllotoxins; antimetabolites such as purine and pyrimidine analogues and anti-folate compounds; topoisomerase I inhibitors such as camptothecins; hormones and hormonal analogues; signal transduction pathway inhibitors; non-receptor tyrosine kinase angiogenesis inhibitors; immunotherapeutic agents; proapoptotic agents; and cell cycle signaling inhibitors.

Examples of a further active ingredient or ingredients for use in combination or co-administered with the present LSD1 inhibiting compounds are chemotherapeutic agents.

Anti-microtubule or anti-mitotic agents are phase specific agents active against the microtubules of tumor cells during M or the mitosis phase of the cell cycle. Examples of anti-microtubule agents include, but are not limited to, diterpenoids and vinca alkaloids.

Diterpenoids, which are derived from natural sources, are phase specific anti - cancer agents that operate at the G_2/M phases of the cell cycle. It is believed that the diterpenoids stabilize the β-tubulin subunit of the microtubules, by binding with this protein. Disassembly of the protein appears then to be inhibited with mitosis being arrested and cell death following. Examples of diterpenoids include, but are not limited to, paclitaxel and its analog docetaxel.

Paclitaxel, 5β,20-epoxy-1,2α,4,7β,10β,13α-hexa-hydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine; is a natural diterpene product isolated from the Pacific yew tree *Taxus brevifolia* and is commercially available as an injectable solution TAXOL®. It is a member of the taxane family of terpenes. It was first isolated in 1971 by Wani et al. J. Am. Chem, Soc., 93:2325. 1971), who characterized its structure by chemical and X-ray crystallographic methods. One mechanism for its activity relates to paclitaxel's capacity to bind tubulin, thereby inhibiting cancer cell growth. Schiff et al., Proc. Natl, Acad, Sci. USA, 77:1561-1565 (1980); Schiff et al., Nature, 277:665-667 (1979); Kumar, J. Biol, Chem, 256: 10435-10441 (1981). For a review of synthesis and anticancer activity of some paclitaxel derivatives see: D. G. I. Kingston *et al.*, Studies in Organic Chemistry vol. 26, entitled "New trends in Natural Products Chemistry 1986", Attaur-Rahman, P.W. Le Quesne, Eds. (Elsevier, Amsterdam, 1986) pp 219-235.

Paclitaxel has been approved for clinical use in the treatment of refractory ovarian cancer in the United States (Markman et al., Yale Journal of Biology and Medicine, 64:583, 1991; McGuire et al., Ann. lntem, Med., 111:273,1989) and for the treatment of breast cancer (Holmes et al., J. Nat. Cancer Inst., 83:1797,1991.) It is a potential candidate for treatment of neoplasms in the skin (Einzig et. al., Proc. Am. Soc. Clin. Oncol., 20:46) and head and neck carcinomas (Forastire et. al., Sem. Oncol., 20:56, 1990). The compound also shows potential for the treatment of polycystic kidney disease (Woo et. al., Nature, 368:750. 1994), lung cancer and malaria. Treatment of patients with paclitaxel results in bone marrow suppression (multiple cell lineages, Ignoff, R.J. et. al, Cancer Chemotherapy

Pocket Guide, 1998) related to the duration of dosing above a threshold concentration (50nM) (Kearns, C.M. et. al., Seminars in Oncology, 3(6) p.16-23, 1995).

Docetaxel, (2R,3S)- N-carboxy-3-phenylisoserine,N-*tert*-butyl ester, 13-ester with 5β-20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate; is commercially available as an injectable solution as TAXOTERE®. Docetaxel is indicated for the treatment of breast cancer. Docetaxel is a semisynthetic derivative of paclitaxel *q.v.*, prepared using a natural precursor, 10-deacetyl-baccatin III, extracted from the needle of the European Yew tree. The dose limiting toxicity of docetaxel is neutropenia.

Vinca alkaloids are phase specific anti-neoplastic agents derived from the periwinkle plant. Vinca alkaloids act at the M phase (mitosis) of the cell cycle by binding specifically to tubulin. Consequently, the bound tubulin molecule is unable to polymerize into microtubules. Mitosis is believed to be arrested in metaphase with cell death following. Examples of vinca alkaloids include, but are not limited to, vinblastine, vincristine, and vinorelbine.

Vinblastine, vincaleukoblastine sulfate, is commercially available as VELBAN® as an injectable solution. Although, it has possible indication as a second line therapy of various solid tumors, it is primarily indicated in the treatment of testicular cancer and various lymphomas including Hodgkin's Disease; and lymphocytic and histiocytic lymphomas. Myelosuppression is the dose limiting side effect of vinblastine.

Vincristine, vincaleukoblastine, 22-oxo-, sulfate, is commercially available as ONCOVIN® as an injectable solution. Vincristine is indicated for the treatment of acute leukemias and has also found use in treatment regimens for Hodgkin's and non-Hodgkin's malignant lymphomas. Alopecia and neurologic effects are the most common side effect of vincristine and to a lesser extent myelosupression and gastrointestinal mucositis effects occur.

Vinorelbine, 3',4'-didehydro -4'-deoxy-C'-norvincaleukoblastine [R-(R*,R*)-2,3-dihydroxybutanedioate (1:2)(salt)], commercially available as an injectable solution of vinorelbine tartrate (NAVELBINE®), is a semisynthetic vinca alkaloid. Vinorelbine is indicated as a single agent or in combination with other chemotherapeutic agents, such as

cisplatin, in the treatment of various solid tumors, particularly non-small cell lung, advanced breast, and hormone refractory prostate cancers. Myelosuppression is the most common dose limiting side effect of vinorelbine.

Platinum coordination complexes are non-phase specific anti-cancer agents, which are interactive with DNA. The platinum complexes enter tumor cells, undergo, aquation and form intra- and interstrand crosslinks with DNA causing adverse biological effects to the tumor. Examples of platinum coordination complexes include, but are not limited to, cisplatin and carboplatin.

Cisplatin, cis-diamminedichloroplatinum, is commercially available as PLATINOL® as an injectable solution. Cisplatin is primarily indicated in the treatment of metastatic testicular and ovarian cancer and advanced bladder cancer. The primary dose limiting side effects of cisplatin are nephrotoxicity, which may be controlled by hydration and diuresis, and ototoxicity.

Carboplatin, platinum, diammine [1,1-cyclobutane-dicarboxylate(2-)-O,O'], is commercially available as PARAPLATIN® as an injectable solution. Carboplatin is primarily indicated in the first and second line treatment of advanced ovarian carcinoma. Bone marrow suppression is the dose limiting toxicity of carboplatin.

Alkylating agents are non-phase anti-cancer specific agents and strong electrophiles. Typically, alkylating agents form covalent linkages, by alkylation, to DNA through nucleophilic moieties of the DNA molecule such as phosphate, amino, sulfhydryl, hydroxyl, carboxyl, and imidazole groups. Such alkylation disrupts nucleic acid function leading to cell death. Examples of alkylating agents include, but are not limited to, nitrogen mustards such as cyclophosphamide, melphalan, and chlorambucil; alkyl sulfonates such as busulfan; nitrosoureas such as carmustine; and triazenes such as dacarbazine.

Cyclophosphamide, 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate, is commercially available as an injectable solution or tablets as CYTOXAN®. Cyclophosphamide is indicated as a single agent or in combination with other chemotherapeutic agents, in the treatment of malignant

lymphomas, multiple myeloma, and leukemias. Alopecia, nausea, vomiting and leukopenia are the most common dose limiting side effects of cyclophosphamide.

Melphalan, 4-[bis(2-chloroethyl)amino]-L-phenylalanine, is commercially available as an injectable solution or tablets as ALKERAN®. Melphalan is indicated for the palliative treatment of multiple myeloma and non-resectable epithelial carcinoma of the ovary. Bone marrow suppression is the most common dose limiting side effect of melphalan.

Chlorambucil, 4-[bis(2-chloroethyl)amino]benzenebutanoic acid, is commercially available as LEUKERAN® tablets. Chlorambucil is indicated for the palliative treatment of chronic lymphatic leukemia, and malignant lymphomas such as lymphosarcoma, giant follicular lymphoma, and Hodgkin's disease. Bone marrow suppression is the most common dose limiting side effect of chlorambucil.

Busulfan, 1,4-butanediol dimethanesulfonate, is commercially available as MYLERAN® TABLETS. Busulfan is indicated for the palliative treatment of chronic myelogenous leukemia. Bone marrow suppression is the most common dose limiting side effects of busulfan.

Carmustine, 1,3-[bis(2-chloroethyl)-1-nitrosourea, is commercially available as single vials of lyophilized material as BiCNU®. Carmustine is indicated for the palliative treatment as a single agent or in combination with other agents for brain tumors, multiple myeloma, Hodgkin's disease, and non-Hodgkin's lymphomas. Delayed myelosuppression is the most common dose limiting side effects of carmustine.

Dacarbazine, 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide, is commercially available as single vials of material as DTIC-Dome®. Dacarbazine is indicated for the treatment of metastatic malignant melanoma and in combination with other agents for the second line treatment of Hodgkin's Disease. Nausea, vomiting, and anorexia are the most common dose limiting side effects of dacarbazine.

Antibiotic anti-neoplastics are non-phase specific agents, which bind or intercalate with DNA. Typically, such action results in stable DNA complexes or strand breakage, which disrupts ordinary function of the nucleic acids leading to cell death. Examples of

antibiotic anti-neoplastic agents include, but are not limited to, actinomycins such as dactinomycin, anthrocyclins such as daunorubicin and doxorubicin; and bleomycins.

Dactinomycin, also know as Actinomycin D, is commercially available in injectable form as COSMEGEN®. Dactinomycin is indicated for the treatment of Wilm's tumor and rhabdomyosarcoma. Nausea, vomiting, and anorexia are the most common dose limiting side effects of dactinomycin.

Daunorubicin, (8S-cis-)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12 naphthacenedione hydrochloride, is commercially available as a liposomal injectable form as DAUNOXOME® or as an injectable as CERUBIDINE®. Daunorubicin is indicated for remission induction in the treatment of acute nonlymphocytic leukemia and advanced HIV associated Kaposi's sarcoma. Myelosuppression is the most common dose limiting side effect of daunorubicin.

Doxorubicin, (8S, 10S)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-8-glycoloyl, 7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12 naphthacenedione hydrochloride, is commercially available as an injectable form as RUBEX® or ADRIAMYCIN RDF®. Doxorubicin is primarily indicated for the treatment of acute lymphoblastic leukemia and acute myeloblastic leukemia, but is also a useful component in the treatment of some solid tumors and lymphomas. Myelosuppression is the most common dose limiting side effect of doxorubicin.

Bleomycin, a mixture of cytotoxic glycopeptide antibiotics isolated from a strain of *Streptomyces verticillus*, is commercially available as BLENOXANE®. Bleomycin is indicated as a palliative treatment, as a single agent or in combination with other agents, of squamous cell carcinoma, lymphomas, and testicular carcinomas. Pulmonary and cutaneous toxicities are the most common dose limiting side effects of bleomycin.

Topoisomerase II inhibitors include, but are not limited to, epipodophyllotoxins.

Epipodophyllotoxins are phase specific anti-neoplastic agents derived from the mandrake plant. Epipodophyllotoxins typically affect cells in the S and G_2 phases of the cell cycle by forming a ternary complex with topoisomerase II and DNA causing DNA

strand breaks. The strand breaks accumulate and cell death follows. Examples of epipodophyllotoxins include, but are not limited to, etoposide and teniposide.

Etoposide, 4'-demethyl-epipodophyllotoxin 9[4,6-0-(R)-ethylidene-β-D-glucopyranoside], is commercially available as an injectable solution or capsules as VePESID® and is commonly known as VP-16. Etoposide is indicated as a single agent or in combination with other chemotherapy agents in the treatment of testicular and non-small cell lung cancers. Myelosuppression is the most common side effect of etoposide. The incidence of leucopenia tends to be more severe than thrombocytopenia.

Teniposide, 4'-demethyl-epipodophyllotoxin 9[4,6-0-(R)-thenylidene-β-D-glucopyranoside], is commercially available as an injectable solution as VUMON® and is commonly known as VM-26. Teniposide is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia in children. Myelosuppression is the most common dose limiting side effect of teniposide. Teniposide can induce both leucopenia and thrombocytopenia.

Antimetabolite neoplastic agents are phase specific anti-neoplastic agents that act at S phase (DNA synthesis) of the cell cycle by inhibiting DNA synthesis or by inhibiting purine or pyrimidine base synthesis and thereby limiting DNA synthesis. Consequently, S phase does not proceed and cell death follows. Examples of antimetabolite anti-neoplastic agents include, but are not limited to, fluorouracil, methotrexate, cytarabine, mecaptopurine, thioguanine, and gemcitabine.

5-fluorouracil, 5-fluoro-2,4- (1H,3H) pyrimidinedione, is commercially available as fluorouracil. Administration of 5-fluorouracil leads to inhibition of thymidylate synthesis and is also incorporated into both RNA and DNA. The result typically is cell death. 5-fluorouracil is indicated as a single agent or in combination with other chemotherapy agents in the treatment of carcinomas of the breast, colon, rectum, stomach and pancreas. Myelosuppression and mucositis are dose limiting side effects of 5-fluorouracil. Other fluoropyrimidine analogs include 5-fluoro deoxyuridine (floxuridine) and 5-fluorodeoxyuridine monophosphate.

Cytarabine, 4-amino-1-β-D-arabinofuranosyl-2 (1H)-pyrimidinone, is commercially available as CYTOSAR-U® and is commonly known as Ara-C. It is

believed that cytarabine exhibits cell phase specificity at S-phase by inhibiting DNA chain elongation by terminal incorporation of cytarabine into the growing DNA chain. Cytarabine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Other cytidine analogs include 5-azacytidine and 2',2'-difluorodeoxycytidine (gemcitabine). Cytarabine induces leucopenia, thrombocytopenia, and mucositis.

Mercaptopurine, 1,7-dihydro-6H-purine-6-thione monohydrate, is commercially available as PURINETHOL®. Mercaptopurine exhibits cell phase specificity at S-phase by inhibiting DNA synthesis by an as of yet unspecified mechanism. Mercaptopurine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Myelosuppression and gastrointestinal mucositis are expected side effects of mercaptopurine at high doses. A useful mercaptopurine analog is azathioprine.

Thioguanine, 2-amino-1,7-dihydro-6H-purine-6-thione, is commercially available as TABLOID®. Thioguanine exhibits cell phase specificity at S-phase by inhibiting DNA synthesis by an as of yet unspecified mechanism. Thioguanine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Myelosuppression, including leucopenia, thrombocytopenia, and anemia, is the most common dose limiting side effect of thioguanine administration. However, gastrointestinal side effects occur and can be dose limiting. Other purine analogs include pentostatin, erythrohydroxynonyladenine, fludarabine phosphate, and cladribine.

Gemcitabine, 2'-deoxy-2', 2'-difluorocytidine monohydrochloride (β-isomer), is commercially available as GEMZAR®. Gemcitabine exhibits cell phase specificity at S-phase and by blocking progression of cells through the G1/S boundary. Gemcitabine is indicated in combination with cisplatin in the treatment of locally advanced non-small cell lung cancer and alone in the treatment of locally advanced pancreatic cancer. Myelosuppression, including leucopenia, thrombocytopenia, and anemia, is the most common dose limiting side effect of gemcitabine administration.

Methotrexate, N-[4[[(2,4-diamino-6-pteridinyl) methyl]methylamino] benzoyl]-L-glutamic acid, is commercially available as methotrexate sodium. Methotrexate exhibits cell phase effects specifically at S-phase by inhibiting DNA synthesis, repair and/or

replication through the inhibition of dyhydrofolic acid reductase which is required for synthesis of purine nucleotides and thymidylate. Methotrexate is indicated as a single agent or in combination with other chemotherapy agents in the treatment of choriocarcinoma, meningeal leukemia, non-Hodgkin's lymphoma, and carcinomas of the breast, head, neck, ovary and bladder. Myelosuppression (leucopenia, thrombocytopenia, and anemia) and mucositis are expected side effect of methotrexate administration.

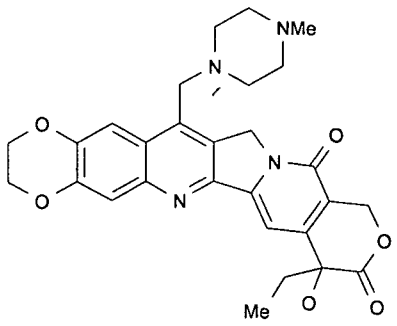
Camptothecins, including, camptothecin and camptothecin derivatives are available or under development as Topoisomerase I inhibitors. Camptothecins cytotoxic activity is believed to be related to its Topoisomerase I inhibitory activity. Examples of camptothecins include, but are not limited to irinotecan, topotecan, and the various optical forms of 7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20-camptothecin described below.

Irinotecan HCl, (4S)-4,11-diethyl-4-hydroxy-9-[(4-piperidinopiperidino) carbonyloxy]-1H-pyrano[3',4',6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione hydrochloride, is commercially available as the injectable solution CAMPTOSAR®.

Irinotecan is a derivative of camptothecin which binds, along with its active metabolite SN-38, to the topoisomerase I – DNA complex. It is believed that cytotoxicity occurs as a result of irreparable double strand breaks caused by interaction of the topoisomerase I : DNA : irintecan or SN-38 ternary complex with replication enzymes. Irinotecan is indicated for treatment of metastatic cancer of the colon or rectum. The dose limiting side effects of irinotecan HCl are myelosuppression, including neutropenia, and GI effects, including diarrhea.

Topotecan HCl, (S)-10-[(dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano[3',4',6,7]indolizino[1,2-b]quinoline-3,14-(4H,12H)-dione monohydrochloride, is commercially available as the injectable solution HYCAMTIN®. Topotecan is a derivative of camptothecin which binds to the topoisomerase I – DNA complex and prevents religation of singles strand breaks caused by Topoisomerase I in response to torsional strain of the DNA molecule. Topotecan is indicated for second line treatment of metastatic carcinoma of the ovary and small cell lung cancer. The dose limiting side effect of topotecan HCl is myelosuppression, primarily neutropenia.

Also of interest, is the camptothecin derivative of formula A following, currently under development, including the racemic mixture (R,S) form as well as the R and S enantiomers:



A

known by the chemical name "7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20(R,S)-camptothecin (racemic mixture) or "7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20(R)-camptothecin (R enantiomer) or "7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20(S)-camptothecin (S enantiomer). Such compound as well as related compounds are described, including methods of making, in U.S. Patent Nos. 6,063,923; 5,342,947; 5,559,235; 5,491,237 and pending U.S. patent Application No. 08/977,217 filed November 24, 1997.

Hormones and hormonal analogues are useful compounds for treating cancers in which there is a relationship between the hormone(s) and growth and/or lack of growth of the cancer. Examples of hormones and hormonal analogues useful in cancer treatment include, but are not limited to, adrenocorticosteroids such as prednisone and prednisolone which are useful in the treatment of malignant lymphoma and acute leukemia in children ; aminoglutethimide and other aromatase inhibitors such as anastrozole, letrazole, vorazole, and exemestane useful in the treatment of adrenocortical carcinoma and hormone dependent breast carcinoma containing estrogen receptors; progestrins such as megestrol acetate useful in the treatment of hormone dependent breast cancer and endometrial carcinoma; estrogens, androgens, and anti-androgens such as flutamide, nilutamide, bicalutamide, cyproterone acetate and 5α-reductases such as finasteride and dutasteride, useful in the treatment of prostatic carcinoma and benign prostatic hypertrophy; anti-estrogens such as tamoxifen, toremifene, raloxifene, droloxifene, iodoxyfene, as well as

selective estrogen receptor modulators (SERMS) such those described in U.S. Patent Nos. 5,681,835, 5,877,219, and 6,207,716, useful in the treatment of hormone dependent breast carcinoma and other susceptible cancers; and gonadotropin-releasing hormone (GnRH) and analogues thereof which stimulate the release of leutinizing hormone (LH) and/or follicle stimulating hormone (FSH) for the treatment prostatic carcinoma, for instance, LHRH agonists and antagagonists such as goserelin acetate and luprolide.

Signal transduction pathway inhibitors are those inhibitors, which block or inhibit a chemical process which evokes an intracellular change. As used herein this change is cell proliferation or differentiation. Signal tranduction inhibitors useful in the present invention include inhibitors of receptor tyrosine kinases, non-receptor tyrosine kinases, SH2/SH3domain blockers, serine/threonine kinases, phosphotidyl inositol-3 kinases, myo-inositol signaling, and Ras oncogenes.

Several protein tyrosine kinases catalyse the phosphorylation of specific tyrosyl residues in various proteins involved in the regulation of cell growth. Such protein tyrosine kinases can be broadly classified as receptor or non-receptor kinases.

Receptor tyrosine kinases are transmembrane proteins having an extracellular ligand binding domain, a transmembrane domain, and a tyrosine kinase domain. Receptor tyrosine kinases are involved in the regulation of cell growth and are generally termed growth factor receptors. Inappropriate or uncontrolled activation of many of these kinases, i.e. aberrant kinase growth factor receptor activity, for example by over-expression or mutation, has been shown to result in uncontrolled cell growth. Accordingly, the aberrant activity of such kinases has been linked to malignant tissue growth. Consequently, inhibitors of such kinases could provide cancer treatment methods. Growth factor receptors include, for example, epidermal growth factor receptor (EGFr), platelet derived growth factor receptor (PDGFr), erbB2, erbB4, vascular endothelial growth factor receptor (VEGFr), tyrosine kinase with immunoglobulin-like and epidermal growth factor homology domains (TIE-2), insulin growth factor –I (IGFI) receptor, macrophage colony stimulating factor (cfms), BTK, ckit, cmet, fibroblast growth factor (FGF) receptors, Trk receptors (TrkA, TrkB, and TrkC), ephrin (eph) receptors, and the RET protooncogene. Several inhibitors of growth receptors are under development and include ligand antagonists, antibodies, tyrosine kinase inhibitors and anti-sense

oligonucleotides. Growth factor receptors and agents that inhibit growth factor receptor function are described, for instance, in Kath, John C., Exp. Opin. Ther. Patents (2000) 10(6):803-818; Shawver et al DDT Vol 2, No. 2 February 1997; and Lofts, F. J. et al, "Growth factor receptors as targets", New Molecular Targets for Cancer Chemotherapy, ed. Workman, Paul and Kerr, David, CRC press 1994, London.

Tyrosine kinases, which are not growth factor receptor kinases are termed non-receptor tyrosine kinases. Non-receptor tyrosine kinases useful in the present invention, which are targets or potential targets of anti-cancer drugs, include cSrc, Lck, Fyn, Yes, Jak, cAbl, FAK (Focal adhesion kinase), Brutons tyrosine kinase, and Bcr-Abl. Such non-receptor kinases and agents which inhibit non-receptor tyrosine kinase function are described in Sinh, S. and Corey, S.J., (1999) Journal of Hematotherapy and Stem Cell Research 8 (5): 465 – 80; and Bolen, J.B., Brugge, J.S., (1997) Annual review of Immunology. 15: 371-404.

SH2/SH3 domain blockers are agents that disrupt SH2 or SH3 domain binding in a variety of enzymes or adaptor proteins including, PI3-K p85 subunit, Src family kinases, adaptor molecules (Shc, Crk, Nck, Grb2) and Ras-GAP. SH2/SH3 domains as targets for anti-cancer drugs are discussed in Smithgall, T.E. (1995), Journal of Pharmacological and Toxicological Methods. 34(3) 125-32.

Inhibitors of Serine/Threonine Kinases including MAP kinase cascade blockers which include blockers of Raf kinases (rafk), Mitogen or Extracellular Regulated Kinase (MEKs), and Extracellular Regulated Kinases (ERKs); and Protein kinase C family member blockers including blockers of PKCs (alpha, beta, gamma, epsilon, mu, lambda, iota, zeta). IkB kinase family (IKKa, IKKb), PKB family kinases, AKT kinase family members, and TGF beta receptor kinases. Such Serine/Threonine kinases and inhibitors thereof are described in Yamamoto, T., Taya, S., Kaibuchi, K., (1999), Journal of Biochemistry. 126 (5) 799-803; Brodt, P, Samani, A., and Navab, R. (2000), Biochemical Pharmacology, 60. 1101-1107; Massague, J., Weis-Garcia, F. (1996) Cancer Surveys. 27:41-64; Philip, P.A., and Harris, A.L. (1995), Cancer Treatment and Research. 78: 3-27, Lackey, K. et al Bioorganic and Medicinal Chemistry Letters, (10), 2000, 223-226; U.S. Patent No. 6,268,391; and Martinez-Iacaci, L., et al, Int. J. Cancer (2000), 88(1), 44-52.

Inhibitors of Phosphotidyl inositol-3 Kinase family members including blockers of PI3-kinase, ATM, DNA-PK, and Ku are also useful in the present invention. Such kinases are discussed in Abraham, R.T. (1996), Current Opinion in Immunology. 8 (3) 412-8; Canman, C.E., Lim, D.S. (1998), Oncogene 17 (25) 3301-3308; Jackson, S.P. (1997), International Journal of Biochemistry and Cell Biology. 29 (7):935-8; and Zhong, H. et al, Cancer res, (2000) 60(6), 1541-1545.

Also useful in the present invention are Myo-inositol signaling inhibitors such as phospholipase C blockers and Myoinositol analogues. Such signal inhibitors are described in Powis, G., and Kozikowski A., (1994) New Molecular Targets for Cancer Chemotherapy ed., Paul Workman and David Kerr, CRC press 1994, London.

Another group of signal transduction pathway inhibitors are inhibitors of Ras Oncogene. Such inhibitors include inhibitors of farnesyltransferase, geranyl-geranyl transferase, and CAAX proteases as well as anti-sense oligonucleotides, ribozymes and immunotherapy. Such inhibitors have been shown to block ras activation in cells containing wild type mutant ras , thereby acting as antiproliferation agents. Ras oncogene inhibition is discussed in Scharovsky, O.G., Rozados, V.R., Gervasoni, S.I. Matar, P. (2000), Journal of Biomedical Science. 7(4) 292-8; Ashby, M.N. (1998), Current Opinion in Lipidology. 9 (2) 99 – 102; and BioChim. Biophys. Acta, (19899) 1423(3):19-30.

As mentioned above, antibody antagonists to receptor kinase ligand binding may also serve as signal transduction inhibitors. This group of signal transduction pathway inhibitors includes the use of humanized antibodies to the extracellular ligand binding domain of receptor tyrosine kinases. For example Imclone C225 EGFR specific antibody (see Green, M.C. et al, Monoclonal Antibody Therapy for Solid Tumors, Cancer Treat. Rev., (2000), 26(4), 269-286); Herceptin ® erbB2 antibody (see Tyrosine Kinase Signalling in Breast cancer:erbB Family Receptor Tyrosine Kniases, Breast cancer Res., 2000, 2(3), 176-183); and 2CB VEGFR2 specific antibody (see Brekken, R.A. et al, Selective Inhibition of VEGFR2 Activity by a monoclonal Anti-VEGF antibody blocks tumor growth in mice, Cancer Res. (2000) 60, 5117-5124).

Non-receptor kinase angiogenesis inhibitors may also find use in the present invention. Inhibitors of angiogenesis related VEGFR and TIE2 are discussed above in regard to signal transduction inhibitors (both receptors are receptor tyrosine kinases).

Angiogenesis in general is linked to erbB2/EGFR signaling since inhibitors of erbB2 and EGFR have been shown to inhibit angiogenesis, primarily VEGF expression. Thus, the combination of an erbB2/EGFR inhibitor with an inhibitor of angiogenesis makes sense. Accordingly, non-receptor tyrosine kinase inhibitors may be used in combination with the EGFR/erbB2 inhibitors of the present invention. For example, anti-VEGF antibodies, which do not recognize VEGFR (the receptor tyrosine kinase), but bind to the ligand; small molecule inhibitors of integrin ($alpha_v$ $beta_3$) that will inhibit angiogenesis; endostatin and angiostatin (non-RTK) may also prove useful in combination with the disclosed erb family inhibitors. (See Bruns CJ et al (2000), Cancer Res., 60: 2926-2935; Schreiber AB, Winkler ME, and Derynck R. (1986), Science, 232: 1250-1253; Yen L et al. (2000), Oncogene 19: 3460-3469).

Agents used in immunotherapeutic regimens may also be useful in combination with the compounds of formula (I). There are a number of immunologic strategies to generate an immune response against erbB2 or EGFR. These strategies are generally in the realm of tumor vaccinations. The efficacy of immunologic approaches may be greatly enhanced through combined inhibition of erbB2/EGFR signaling pathways using a small molecule inhibitor. Discussion of the immunologic/tumor vaccine approach against erbB2/EGFR are found in Reilly RT et al. (2000), Cancer Res. 60: 3569-3576; and Chen Y, Hu D, Eling DJ, Robbins J, and Kipps TJ. (1998), Cancer Res. 58: 1965-1971.

Agents used in proapoptotic regimens (e.g., bcl-2 antisense oligonucleotides) may also be used in the combination of the present invention. Members of the Bcl-2 family of proteins block apoptosis. Upregulation of bcl-2 has therefore been linked to chemoresistance. Studies have shown that the epidermal growth factor (EGF) stimulates anti-apoptotic members of the bcl-2 family (i.e., mcl-1). Therefore, strategies designed to downregulate the expression of bcl-2 in tumors have demonstrated clinical benefit and are now in Phase II/III trials, namely Genta's G3139 bcl-2 antisense oligonucleotide. Such proapoptotic strategies using the antisense oligonucleotide strategy for bcl-2 are discussed in Water JS et al. (2000), J. Clin. Oncol. 18: 1812-1823; and Kitada S et al. (1994), Antisense Res. Dev. 4: 71-79.

Cell cycle signalling inhibitors inhibit molecules involved in the control of the cell cycle. A family of protein kinases called cyclin dependent kinases (CDKs) and their

interaction with a family of proteins termed cyclins controls progression through the eukaryotic cell cycle. The coordinate activation and inactivation of different cyclin/CDK complexes is necessary for normal progression through the cell cycle. Several inhibitors of cell cycle signalling are under development. For instance, examples of cyclin dependent kinases, including CDK2, CDK4, and CDK6 and inhibitors for the same are described in, for instance, Rosania et al, Exp. Opin. Ther. Patents (2000) 10(2):215-230. Further, p21WAF1/CIP1 has been described as a potent and universal inhibitor of cyclin-dependent kinases (Cdks) (Ball et al., *Progress in Cell Cycle Res., 3:* 125 (1997)). Compounds that are known to induce expression of p21WAF1/CIP1 have been implicated in the suppression of cell proliferation and as having tumor suppressing activity (Richon et al., *Proc. Nat Acad. Sci. U.S.A. 97(18):* 10014-10019 (2000)), and are included as cell cycle signaling inhibitors.

Modulators of the Retinoid Acid Receptor have been used to treat leukemias. The pathology of the leukemia is associated with the abnormal accumulation of immature progenitor cells that are sensitive to retinoc acid therapy. The majority of cases of acute promyelocytic leukemia (APL), also called acute myeloid leukemia subtype M3, involve a chromosomal translocation of chromosomes 15 and 17 that causes genetic fusion of the retinoic acid receptor (*RAR*) gene to the promyelocytic leukemia (*PML*) gene. This fusion PML-RAR protein is responsible for preventing immature myeloid cells from differentiating into more mature cells. This block in differentiation is and subsequent accumulation of less differentiated cells is thought to cause leukemia. ATRA, Tretinoin, acts on PML-RAR to lift this block, causing the immature promyelocytes to differentiate to normal mature blood cells thus decreasing promyelocytes and promoting a population of terminally differentiated cells with a restricted lifespan. Talazorole is an experimental drug in the same class as Tretinoin.

Epigenetic alterations have been implicated in virtually all types of human cancers. Cancer specific changes are often associated with silencing of tumor suppressor genes via histone modifications and modifications to DNA including DNA hypermethylation. Epigenetic pharmaceuticals control regulatory regions associated with tumor suppressor genes by causing conformational changes in histones and removing repressive modifications to DNA. These changes directly affect the formation and progression of

cancer. Examples of epigenetic agents include histone deacetylase inhibitors and DNA methylation inhibitors.

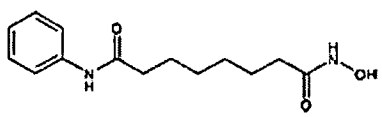
Histone deacetylase inhibitors (HDAC inhibitors, HDI) are a class of compounds that interfere with the function of histone deacetylases. Inhibitors of histone deacetylases have been shown to be useful in the treatment of cutaneous T-cell lymphoma. They are being investigated in the clinic for multiple other tumor types. Examples of HDAC inhibitors approved for use are Vorinostat and Romidepsin. These compounds are thought to inhibit the activity of HDACs and result in the accumulation of acetylation to histones promoting gene expression.

Azacitidine (INN) or 5-azacytidine, sold under the trade name Vidaza, is a chemical analogue of cytidine, a nucleoside present in DNA and RNA. Azacitidine and its deoxy derivative, decitabine (also known as 5-aza-2'deoxycytidine), are used in the treatment of myelodysplastic syndrome and are currently under study for other tumor indications. Azacitidine acts as a false substrate and potent inhibitor of DNA methyltransferases leading to reduction of DNA methylation. DNA methyltransferases incorporate azacitidine into DNA during replication and into RNA during transcription in the cell. Inhibition of DNA methylation occurs through the formation of stable complexes between the molecule and DNA methyltransferases, thereby saturating cell methylation machinery.This results in a loss of DNA methylation and can affect the way cell regulation proteins, such as transcriptional machinery, are able to associate with the DNA.

Examples of such HDAC inhibitors include:

1. Vorinostat, including pharmaceutically acceptable salts thereof. Marks et al., *Nature Biotechnology 25,* 84 to 90 (2007); Stenger, *Community Oncology 4,* 384-386 (2007).

Vorinostat has the following chemical structure and name:

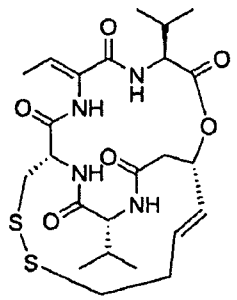


N-hydroxy-*N'*-phenyl-octanediamide

2. Romidepsin, including pharmaceutically acceptable salts thereof.

Vinodhkumar et al., *Biomedicine & Pharmacotherapy 62* (2008) 85-93.

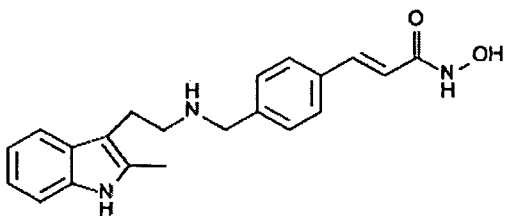
Romidepsin, has the following chemical structure and name:



(1S,4S,7Z,10S,16E,21R)-7-ethylidene-4,21-di(propan-2-yl)-2-oxa-12,13-dithia-5,8,20,23-tetrazabicyclo[8.7.6]tricos-16-ene-3,6,9,19,22-pentone

3. Panobinostat, including pharmaceutically acceptable salts thereof. *Drugs of the Future 32(4): 315-322 (2007).*

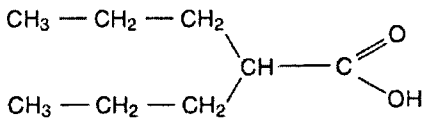
Panobinostat, has the following chemical structure and name:



(2*E*)-*N*-hydroxy-3-[4-({[2-(2-methyl-1*H*-indol-3-yl)ethyl]amino}methyl)phenyl]acrylamide

4. Valproic acid, including pharmaceutically acceptable salts thereof. Gottlicher, et al., EMBO J. 20(24): 6969-6978 (2001).

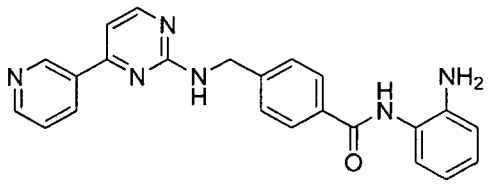
Valproic acid, has the following chemical structure and name:



2-propylpentanoic acid

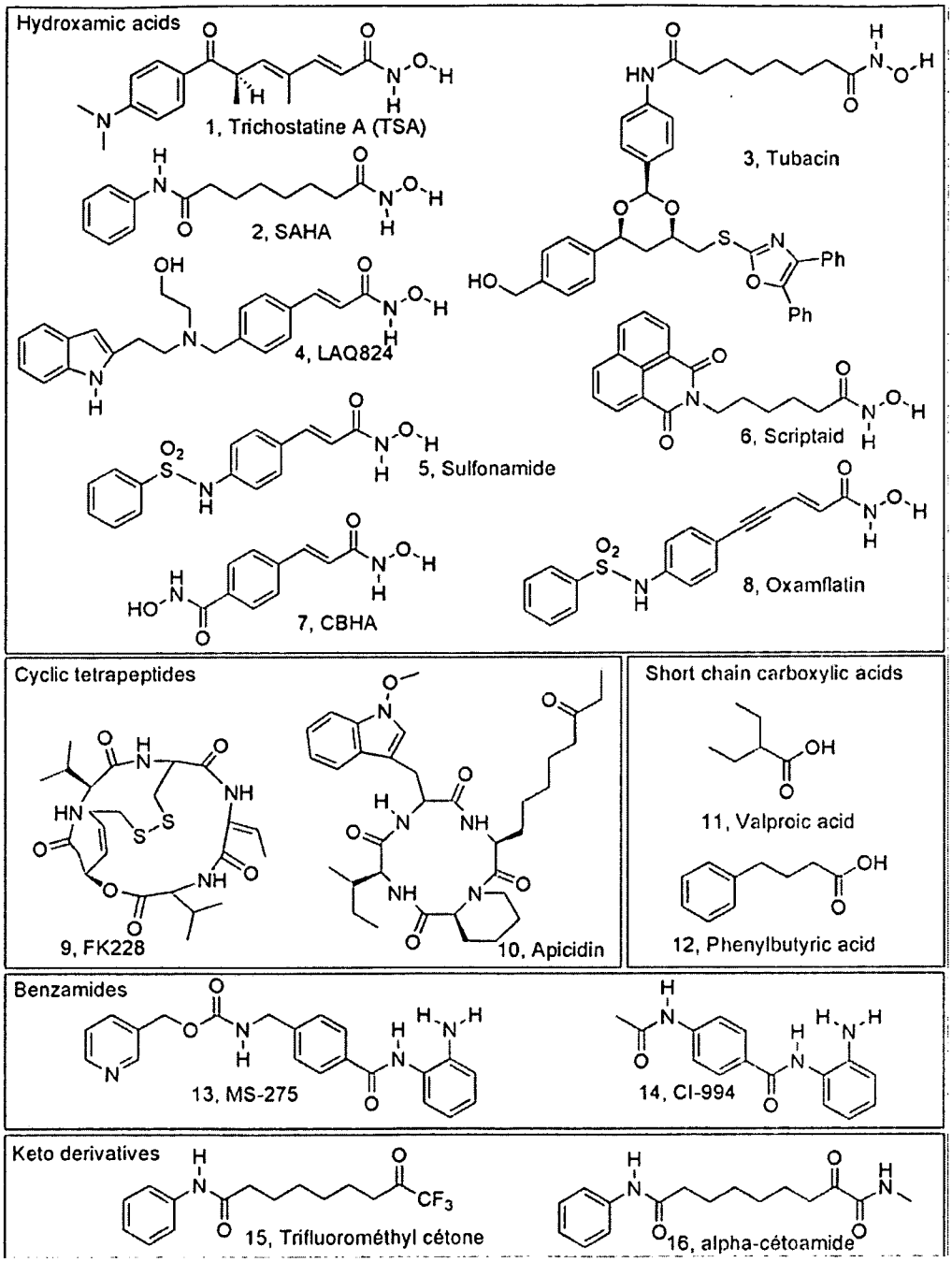
5. Mocetinostat (MGCD0103), including pharmaceutically acceptable salts thereof. Balasubramanian et al., Cancer Letters 280: 211-221 (2009).

Mocetinostat, has the following chemical structure and name:



N-(2-Aminophenyl)-4-[[(4-pyridin-3-ylpyrimidin-2-yl)amino]methyl] benzamide

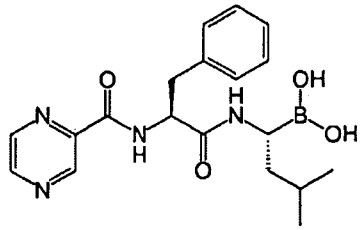
Further examples of such HDAC inhibitors are included in Bertrand European Journal of Medicinal Chemistry 45, (2010) 2095-2116, particularly the compounds of table 3 therein as indicated below.



Proteasome inhibitors are drugs that block the action of proteasomes, cellular complexes that break down proteins, like the p53 protein. Several proteasome inhibitors are marketed or are being studied in the treatment of cancer. Suitable proteasome inhibitors for use in combination herein include:

1. Bortezomib (Velcade®), including pharmaceutically acceptable salts thereof. Adams J, Kauffman M (2004), *Cancer Invest* **22** (2): 304–11.

Bortezomib has the following chemical structure and name.

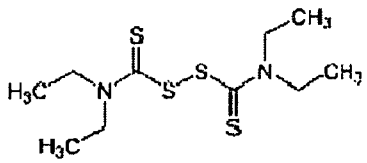


[(1*R*)-3-methyl-1-({(2*S*)-3-phenyl-2-[(pyrazin-2-ylcarbonyl)amino]propanoyl}amino)butyl]boronic acid

2. Disulfiram, including pharmaceutically acceptable salts thereof.

Bouma et al. (1998). *J. Antimicrob. Chemother.* **42** (6): 817–20.

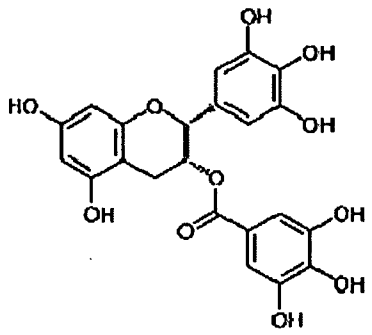
Disulfiram has the following chemical structure and name.



1,1',1",1'"-[disulfanediylbis(carbonothioylnitrilo)]tetraethane

3. Epigallocatechin gallate (EGCG), including pharmaceutically acceptable salts thereof. Williamson et al., (December 2006), *The Journal of Allergy and Clinical Immunology* **118** (6): 1369–74.

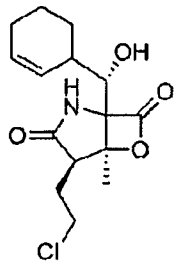
Epigallocatechin gallate has the following chemical structure and name.



[(2*R*,3*R*)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl]3,4,5-trihydroxybenzoate

4. Salinosporamide A, including pharmaceutically acceptable salts thereof. Feling et at., (2003), *Angew. Chem. Int. Ed. Engl.* **42** (3): 355–7.

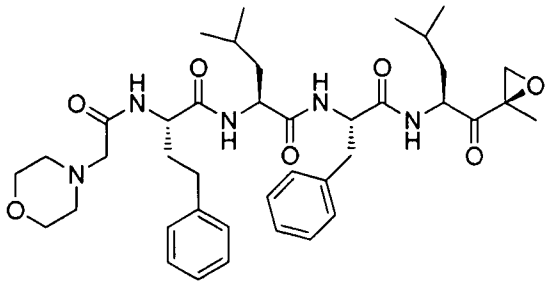
Salinosporamide A has the following chemical structure and name.



(4R,5S)-4-(2-chloroethyl)-1-((1S)-cyclohex-2-enyl(hydroxy)methyl) -5-methyl-6-oxa-2-azabicyclo3.2.0heptane-3,7-dione

5. Carfilzomib, including pharmaceutically acceptable salts thereof. Kuhn DJ, et al, Blood, 2007, 110:3281-3290.

Carfilzomib has the following chemical structure and name.

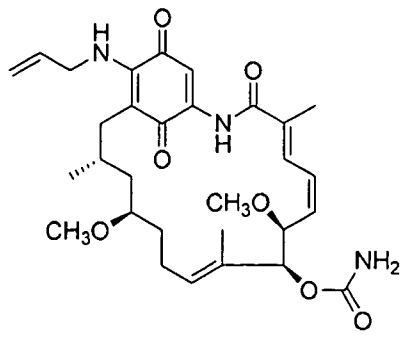


(S)-4-methyl-N-((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)pentanamide

The 70 kilodalton heat shock proteins (Hsp70s) and 90 kilodalton heat shock proteins (Hsp90s) are a families of ubiquitously expressed heat shock proteins. Hsp70s and Hsp90s are over expressed certain cancer types. Several Hsp70s and Hsp90s inhibitors are being studied in the treatment of cancer. Suitable Hsp70s and Hsp90s inhibitors for use in combination herein include:

1. 17-AAG(Geldanamycin), including pharmaceutically acceptable salts thereof. Jia W et al. Blood. 2003 Sep 1;102(5):1824-32.

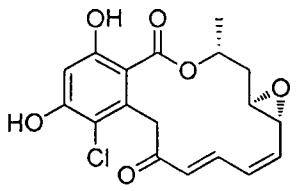
17-AAG(Geldanamycin) has the following chemical structure and name.



17-(Allylamino)-17-demethoxygeldanamycin

2. Radicicol, including pharmaceutically acceptable salts thereof. (Lee et al., Mol Cell Endocrinol. 2002, 188,47-54)

Radicicol has the following chemical structure and name.



(1aR,2Z,4E,14R,15aR)-8-chloro-9,11-dihydroxy-14-methyl-15,15a-dihydro-1aH-benzo[c]oxireno[2,3-k][1]oxacyclotetradecine-6,12(7H,14H)-dione

Inhibitors of cancer metabolism - Many tumor cells show a markedly different metabolism from that of normal tissues. For example, the rate of glycolysis, the metabolic process that converts glucose to pyruvate, is increased, and the pyruvate generated is reduced to lactate, rather than being further oxidized in the mitochondria via the tricarboxylic acid (TCA) cycle. This effect is often seen even under aerobic conditions and is known as the Warburg Effect.

Lactate dehydrogenase A (LDH-A), an isoform of lactate dehydrogenase expressed in muscle cells, plays a pivotal role in tumor cell metabolism by performing the reduction of pyruvate to lactate, which can then be exported out of the cell. The enzyme has been shown to be upregulated in many tumor types. The alteration of glucose metabolism described in the Warburg effect is critical for growth and proliferation of cancer cells and knocking down LDH-A using RNA-i has been shown to lead to a reduction in cell proliferation and tumor growth in xenograft models.

D. A. Tennant et. al., Nature Reviews, 2010, 267.

P. Leder, et. al., Cancer Cell, 2006, 9, 425.

High levels of fatty acid synthase (FAS) have been found in cancer precursor lesions. Pharmacological inhibition of FAS affects the expression of key oncogenes involved in both cancer development and maintenance.

Alli *et al. Oncogene* (2005) 24, 39–46. doi:10.1038

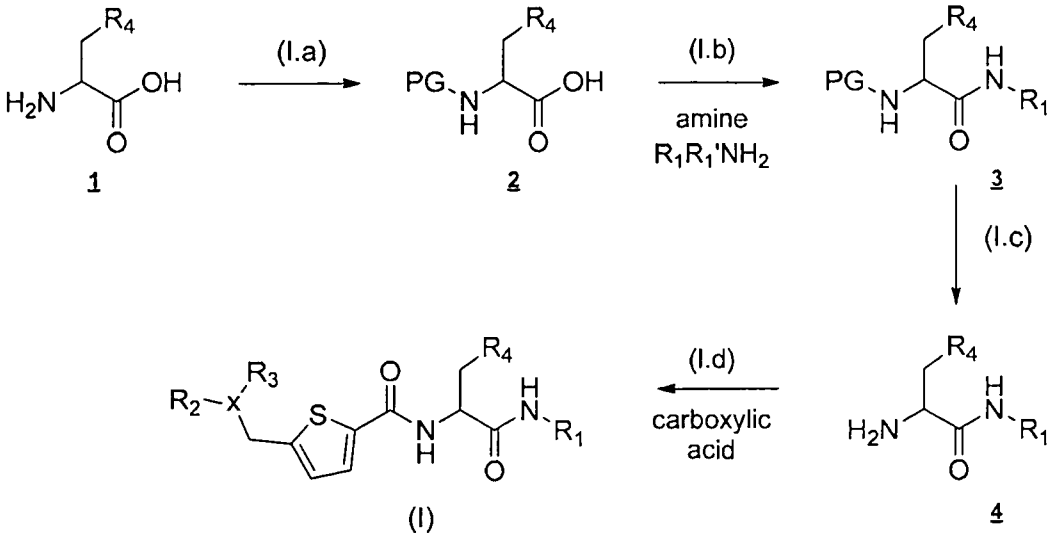
Inhibitors of cancer metabolism, including inhibitors of LDH-A and inhibitors of fatty acid biosynthesis (or FAS inhibitors), are suitable for use in combination with the compounds of this invention.

In one embodiment, the cancer treatment method of the claimed invention includes the co-administration a compound of Formula (I) and/or a pharmaceutically acceptable salt, hydrate, solvate or pro-drug thereof and at least one anti-neoplastic agent, such as one selected from the group consisting of anti-microtubule agents, platinum coordination complexes, alkylating agents, antibiotic agents, topoisomerase II inhibitors, antimetabolites, topoisomerase I inhibitors, hormones and hormonal analogues, signal transduction pathway inhibitors, non-receptor tyrosine kinase angiogenesis inhibitors,

immunotherapeutic agents, proapoptotic agents, FAS inhibitors, HDAC inhibitors, LDH-A inhibitors and cell cycle signaling inhibitors.

GENERAL SYNTHETIC SCHEMES

Scheme I

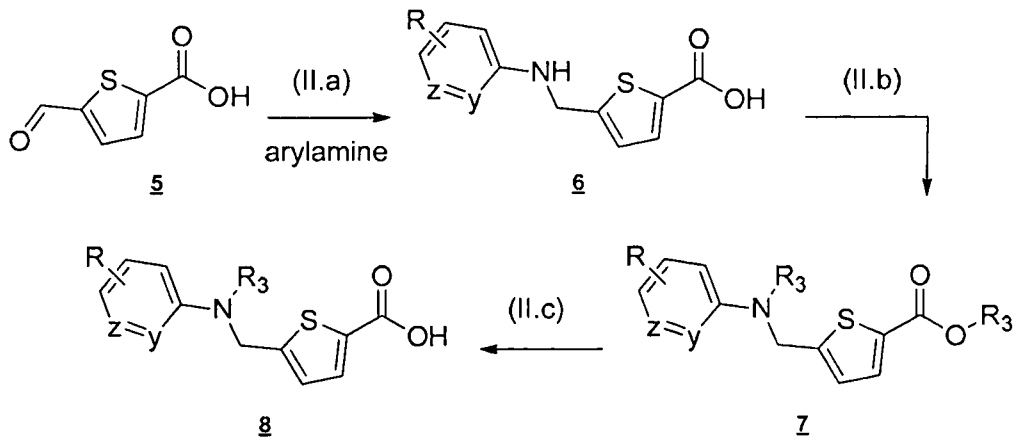


I.a: Protection step, for example: CbzCl, NaOH, water ; I.b, I.d: Amidification step, for example: EDC, HOAt, DIEA, DMF ; I.c: Deprotection step, for example: H_2, Pd/C, EtOAc, EtOH.

Many different protecting groups are available to one skilled in the art and can be used here as long as they do not interfere with the processes listed herein. Methods for the protection of amines are described in standard reference volumes, such as Greene "Protective Groups in Organic Synthesis" (published by Wiley-Interscience). For example, the amino group of Aminoacid 1 can be protected by, but not exclusively, *tert*-butyl carbamate (Boc), (9H-fluoren-9-yl)methyl (Fmoc) or benzyl carbamate (Cbz). Typical conditions include $(Boc)_2O$, FmocCl or CbzCl, with or without a base like NaOH, $NaHCO_3$ or triethylamine in an organic solvent like THF, ACN, DMF, EtOAc or even in water. Protected amino-acid 2 can be transformed into the amide 3 by typical amide coupling conditions. A variety of amide coupling reagents such as EDC, HOBt, HOAt, HATU, PyBrop, etc. are commercially available and can be used here. Amide coupling

reactions are generally run in solvents such as DCM or DMF, utilizing an organic base like Et_3N or $(i\text{-}Pr)_2NEt$. The amino group of 3 was deprotected to afford 4. Many deprotection of the amino group are available to one skilled in the art and can be used here as long as they do not interfere with the processes listed herein. Methods for the deprotection of amines are described in standard reference volumes, such as Greene "Protective Groups in Organic Synthesis" (published by Wiley-Interscience). They can include HCl in dioxane, TFA in DCM, piperidine in DMF, thermal or hydrogenation conditions. Compound 4 could then be coupled to any substituted thiophene-2-carboxylic acid like any intermediate from Scheme II and result in a final product of formula (I). A variety of amide coupling reagents such as EDC, HOBt, HOAt, HATU, PyBrop, etc. are commercially available and can be used here. Amide coupling reactions are generally run in solvents such as DCM or DMF, utilizing an organic base like Et_3N or $(i\text{-}Pr)_2NEt$.

Scheme II (x=N)

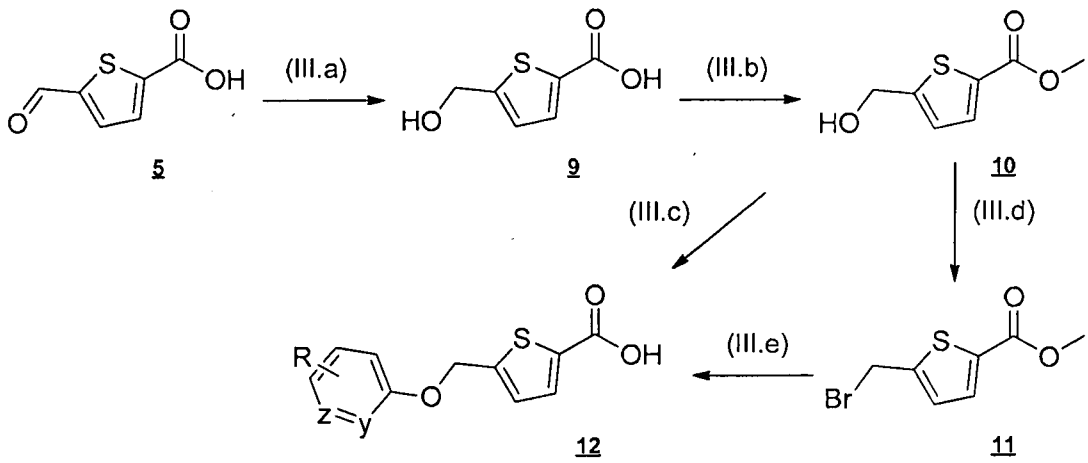


II.a: Reductive amination step, for example: 1) MeOH, 2) $NaCNBH_3$; II.b : Alkylation step for example: EtI, NaH, DMF ; II.c: Hydrolysis step, for example: 1N LiOH, THF.

Reductive amination of 5 with an arylamine can afford the carboxylic acid 6, which can eventually be alkylated to give 7. Subsequent saponification can give the carboxylic acid 8. There are a lot of conditions available to one skilled in the art to perform the reductive amination of the formyl group of 5. They can be in one-pot or sequential, using acid (for example AcOH) as a catalyst and/or a dehydrating agent (for example molecular sieves),

for the imine formation. The reactions are often conducted in methanol or a chlorinated solvent like DCM or 1,2-dichloroethane, using a reduction agent like $NaBH_4$, $NaCNBH_3$, $NaBH(OAc)_3$ or even LAH. The alkylation reaction can be typically conducted with an alkylated agent such as an alkyl halide (EtI for example), a strong base like NaH in a polar solvent like DMF. The hydrolysis/saponification step can be conducted with a base like LiOH or NaOH in water, or in acidic conditions like (1N HCl for example).

Scheme III (x=O)

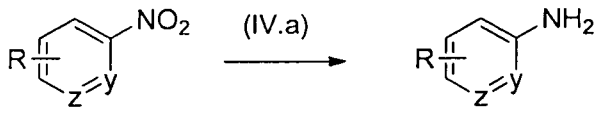


III.a: Reduction step, for example: $NaBH_4$, MeOH ; III.b : Esterification step for example: MeOH, H_2SO_4 ; III.c: Mitsunobu reaction step, for example : ArOH, DEAD, Ph_3P, THF ; III.d: Bromination step, for example: CBr_4, DCM ; III.e: Alkylation step, for example: ArOH, Cs_2CO_3, ACN, DMF.

Reduction of the formyl group of compound **5** using for example $NaBH_4$ can afford compound **9**. Such reduction could also be conducted with other reducing agents like lithium aluminium hydride, diisobutylaluminium hydride, sodium borohydride, L-selectride, diborane, diazene or aluminum hydride. Sodium cyanoborohydride, 9-BBN-pyridine and tributyltin hydride are also known to be selective for aldehydes. Hydrogenation using platinum or ruthenium as catalysts could also be an option. The esterification of **9** into **10** could typically be conducted in methanol as solvent in the presence of catalytic sulfuric acid. Alcohol **10** may be converted to bromo derivative **11**

using CBr_4, hydrobromic acid or phosphorus tribromide (PBr_3). This transformation can also take place using radical conditions in water instead of an organic solvent and the bromine is obtained by oxidation of hydrobromic acid with hydrogen peroxide. An incandescent light bulb is then sufficient for bromine radical generation. Finally compound **12** can be obtained from a phenol or hydroxy-pyridine by Mitsunobu reaction with **10** or alkylation with **11**. Mitsunobu reactions are well known to those skilled in the art of organic synthesis. Methods and reaction conditions for such transformations are discussed in Synthesis 1981, 1-28. Alkylation conditions include but are not limited to using an inorganic base (like Cs_2CO_3) and in a polar solvent like ACN or DMF.

Scheme IV



y,z = N or CH or C-alkyl

IV.a: Reduction step, for example: $SnCl_2$, HCl, EtOH.

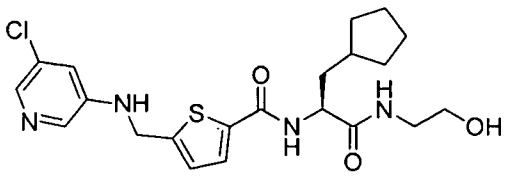
Reduction of the nitro group can be achieved by a wide variety of methods known to the one skilled in the art, including using SnCl2 with HCl in EtOH, or metal Fe in AcOH, or hydrazine or even hydrogenation using a catalyst such as palladium on carbon.

EXPERIMENTAL

The following examples are intended for illustration only and are not intended to limit the scope of the invention in any way, the invention being defined by the claims. Unless otherwise noted, reagents are commercially available or are prepared according to procedures in the literature. The symbols and conventions used in the descriptions of processes, schemes, and examples are consistent with those used in the contemporary scientific literature, for example, the Journal of the American Chemical Society or the Journal of Biological Chemistry.

Example 1

Preparation of *(S)*-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide



(*S*)-2-Amino-3-cyclopentyl-*N*-(2-hydroxyethyl)propanamide.

A solution of *(S)*-2-[(tert-butoxycarbonyl)amino]-3-cyclopentylpropanoic acid (5 g, 19.43mmol), ethanolamine (1.175mL, 19.43mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (7.45g, 38.9mmol), 1-hydroxy-7-azabenzotriazole (5.29g, 38.9mmol), and *N*-methylmorpholine (8.54mL, 78mmol) was stirred in DCM (50mL) for 3h at room temperature. LCMS showed desired intermediate, LC-MS(ES) m/z = 301.3 [M+H]+. The reaction was poured into water and washed three times with water. Organic layer was separated, dried over sodium sulfate, filtered, and concentrated.

The product was then redissolved in DCM (50mL) and treated with 4M HCl/dioxane (50.0mL). The reaction stirred overnight at room temperature. LCMS showed desired product, LC-MS(ES) m/z = 201.3 [M+H]+. The reaction was then concentrated and dried under vacuum overnight to afford a white solid (3.5g).

5-Formyl-thiophene-2-carboxylic acid.

as described in Renouard, Thierry; Graetzel, Michael, *Tetrahedron*, 2001, 57(38), 8145 - 8150

5-{[(5-Chloropyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid.

A mixture of 5-formyl-2-thiophenecarboxylic acid (6.88g, 44.1mmol) and 5-chloro-3-pyridinamine (5.667g, 44.1mmol) was dissolved in MeOH (80mL) and heated at 50°C for 2 hours. An aliquot was removed and pumped under vacuum to dryness. NMR indicated formation of the intermediate imine. A tan precipitate was observed. The reaction was cooled to 0°C under nitrogen and solid sodium cyanoborohydride (3.32g, 52.9mmol) was added portion wise over 10 minutes. The ice bath was removed and the heterogeneous reaction was allowed to stir at room temperature for 3h. The reaction was quenched by adding 1N HCl until the pH = 4. The reaction was concentrated to remove the MeOH and the residue was diluted with water (not soluble) and a few drops of 1N HCl to bring the pH to 3-4. The tan suspension was filtered and dried over a weekend in a Buchner funnel to provide the desired product as a tan solid (10.5g). LC/MS indicated 97% purity, LC-MS(ES) m/z = 269.2 [M+H]+.

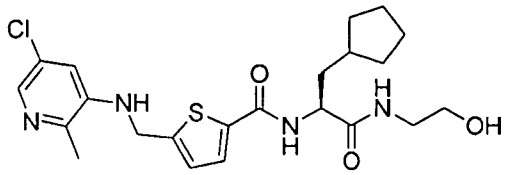
(*S*)-5-{[(5-Chloropyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.

A mixture of 5-{[(5-chloro-3-pyridinyl)amino]methyl}-2-thiophenecarboxylic acid (1.249g, 4.65mmol), *(S)*-2-amino-3-cyclopentyl-*N*-(2-hydroxyethyl)propanamide hydrochloride (1.10g, 4.65mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.891g, 4.65mmol), 1-hydroxy-7-azabenzotriazole (0.712g, 4.65mmol), and diispropylethylamine (2.435mL, 13.94mmol) in DCM (31mL) was stirred overnight at room temperature. LC/MS indicated desired product. Added an additional 0.2 eq of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (179mg) and 0.2 eq of 1-hydroxybenzotriazole (143mg) and stirred at room temperature for 5 hours. The reaction mixture was diluted with DCM and transferred to a separatory funnel. The separated organic layer was washed with saturated aqueous solution of NaHCO3. The addition of bicarbonate caused much of the product to gum up and stick to the walls of the funnel. IPA was added (25mL) to the 225mL of DCM (to give a 10% IPA:DCM mixture). This resulted in two homogeneous layers. The layers were separated and the aqueous layer was extracted with 10%IPA:DCM. The organic layers were combined and washed with brine, dried over Na2SO4, filtered, and concentrated to give a tan solid (2.09g). Crude was purified via chromatography on silica gel (2.5-7.5% MeOH:DCM over 40 min then 7.5-

8.5% MeOH:DCM for 20 min) and dried for 3 days at 55°C in a vacuum oven to give a pale yellow solid (1.8g). Data indicated desired product : 100% purity, LC-MS(ES) m/z = 451.3, 453.3 (MH+1).

Example 2

Preparation of *(S)*-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide:



5-Chloro-2-methyl-3-nitropyridine.

To a suspension of sodium hydride (8.76 g, 60% dispersion in mineral oil) in THF (340mL) under nitrogen atmosphere was added 2,5-dichloro-3-nitropyridine (40g, 207mmol followed by diethylmalonate (63.2mL, 412mmol dissolved in 63mL of THF) drop wise at room temperature for 20min. A vigorous evolution of gas was observed. After 2 h, additional sodium hydride (1.19 g, 60% dispersion in mineral oil) was added and the reaction mixture was then stirred at room temperature for 1.5h. The reaction mixture was concentrated under reduced pressure and diluted with 6N HCl (400mL) and refluxed for 12.5 h. The resulting mixture was concentrated under reduced pressure and diluted with saturated sodium carbonate solution (400mL) until pH=9, then diluted with dichloromethane (400 mL), stirred for 10 min, and filtered to remove an insoluble green solid; from the filtrate, the organic layer was separated and washed with brine, dried over anhydrous Na2SO4 and concentrated to afford 5-chloro-2-methyl-3-nitropyridine (26 g crude) as a brown liquid which was directly used in the next step without further purification.

5-Chloro-2-methylpyridine-3-amine.

To a solution of 5-chloro-2-methyl-3-nitropyridine (26g, 151mmol) in ethyl acetate (850mL) under nitrogen atmosphere was added SnCl2.2H2O (136g, 603mmol) at room temperature. The reaction mixture was heated to 85°C for 3h. Concentration of the reaction mixture under reduced pressure afforded a pale yellow slurry which was basified with 1N NaOH solution (520mL). The resulting mixture was diluted with dichloromethane (750mL) and stirred for 10 minutes. The mixture was then filtered through a celite pad to remove undissolved solids. The filtrate's organic layer was separated, dried over anhydrous Na2SO4 and concentrated under reduced pressure. The crude compound was purified by column chromatography on silica gel (100-200 mesh) using 0-35% ethyl acetate in petroleum ether to afford desired 5-chloro-2-methylpyridine-3-amine (18 g) as a brown solid.

5-{[(5-Chloro-2-methyl-3-pyridinyl)amino]methyl}-2-thiophenecarboxylic acid.

To a solution of 5-formyl-thiophene-2-carboxylic acid (19.7g, 126mmol) in methanol (200mL) under nitrogen atmosphere was added 5-chloro-2-methylpyridine-3-amine (17.99g, 126mmol). The mixture was heated to 50°C for 2h; the reaction mixture was concentrated under reduced pressure to obtain a solid. The solid material was dissolved in methanol (200mL) and cooled to 0°C then sodium cyanoborohydride (9.51 g, 151mmol) was added over 10 minutes and the resulting mixture was stirred at room temperature for 14 h. The reaction mixture was quenched by adding 1N HCl (100mL) to bring pH=4, concentrated under vacuum. The residue was diluted with water (100mL). The resulting tan suspension was filtered and dried to get crude solid (28 g, crude). The solid was triturated with ethyl acetate (50mL) to get 5-{[(5-chloro-2-methyl-3-pyridinyl)amino]methyl}-2-thiophenecarboxylic acid (27g) as a pale yellow solid.

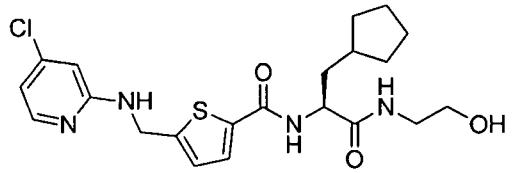
***(S)*-5-{[(5-Chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.**

A mixture of 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (1.314g, 4.65mmol), *(S)*-2-amino-3-cyclopentyl-*N*-(2-hydroxyethyl)propanamide

hydrochloride (1.10g, 4.65mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.336g, 6.97mmol), 1-hydroxybenzotriazole (1.067g, 6.97mmol), and diispropylethylamine (2.435mL, 13.94mmol) in DCM (31 mL) was stirred overnight at room temperature. LC/MS indicated mostly desired product. The reaction was diluted with DCM and extracted sequentially with saturated aqueuous solution of NaHCO3 and brine. The DCM was dried over Na2SO4, filtered, and concentrated to give a tan solid (2.63 g). Crude was purified via chromatography on silica gel (3.5%-8.5% MeOH:DCM over 40 min). Desired fractions were isolated and concentrated to give a pale yellow solid (0.926g) with 100% purity by HPLC, LC-MS(ES) m/z = 465.3, 467.3 (MH+1).

Example 3

Preparation of *(S)*-5-{[(4-chloropyridin-2-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide:



5-[(4-Chloropyridin-2-ylamino)methyl]thiophene-2-carboxylic acid.

To a suspension of 5-formylthiophene-2-carboxylic acid (22g, 141mmol) in toluene (220mL) was added 4-chloropyridin-2-amine (18.05g, 141mmol) and MeOH (20mL) under nitrogen atmosphere at room temperature and the resulting reaction mixture was heated to reflux using Dien-Stark condenser for 1 h. The reaction mixture was concentrated under reduced pressure. To the resulting solid residue MeOH (220mL) was added, the mixture was cooled to 0°C and then treated with NaBH3CN (13.29g, 210mmol) which was added portion wise over a period of 10 min. The resulting mixture was stirred at room temperature for 15h. The reaction was quenched by addition of 1N HCl until pH = 5-6 and concentrated under reduced pressure. The gummy residue was diluted with water (100mL, note: gummy residue was insoluble) to which additional few drops of 1N HCl to bring pH to 5 were added, before collection of the solid by filtration. Resulting

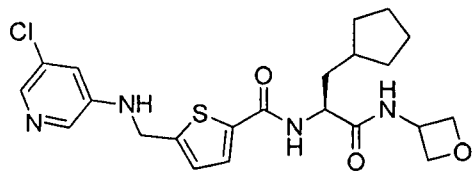
solid was dried to afford the crude compound (30 g). The final product was obtained by recrystallizing twice from ethanol (10 volumes). 5-[(4-chloropyridin-2-ylamino)methyl]thiophene-2-carboxylic acid (12.5 g) was obtained as an off-white solid.

(*S*)-5-{[(4-Chloropyridin-2-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.

A mixture of 5-{[(4-chloropyridin-2-yl)amino]methyl}thiophene-2-carboxylic acid (1.249g, 4.65mmol), *(S)*-2-amino-3-cyclopentyl-*N*-(2-hydroxyethyl)propanamide hydrochloride (1.10g, 4.65mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.336g, 6.97mmol), 1-hydroxybenzotriazole (1.067g, 6.97mmol), and diispropylethylamine (2.435mL, 13.94mmol) in DCM (31mL) was stirred overnight at room temperature. LC/MS indicated mostly desired product. The reaction was diluted with DCM and washed sequentially with saturated aqueous solution of NaHCO3 and brine, dried over Na2SO4, filtered, and concentrated to give a tan solid (2.365g). LC/MS indicated 85% desired product. Crude product was purified via chromatography on silica gel (2.5-8.5% MeOH:DCM over 40 min) and placed in a vacuum oven overnight at 55°C to give a white solid (1.50g). Data indicated desired product: 100% purity, LC-MS(ES) m/z = 451.3, 453.3 (MH+1).

Example 4

Preparation of *(S)*-5-{[(5-chloro-pyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide:



(*S*)-(9H-Fluoren-9-yl)methyl [3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]carbamate.

A solution of *(S)*-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)-3-cyclopentylpropanoic acid (1 g, 2.64 mmol), oxetan-3-amine (193mg, 2.64mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (606mg, 3.16mmol), 1-hydroxy-7-azabenzotriazole (430 mg, 3.16 mmol), and *N*-methylmorpholine (1.159mL, 10.54mmol), was stirred in DCM (20mL) for 18h at room temperature.

The reaction mixture was washed with water three times. The combined aqueous layers were extracted with DCM (2x25mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, concentrated, and vacuum pumped to a white solid (1.14 g, >90% pure by LC-MS) that was used directly in the next reaction without further purification.

_(S)_-2-Amino-3-cyclopentyl-_N_-(oxetan-3-yl)propanamide.

A solution of *(S)*-(9H-fluoren-9-yl)methyl [3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]carbamate (1.14g, 2.62mmol) and piperidine (520µL, 5.25mmol) in THF (10mL) was stirred at room temperature for 18h. The reaction was checked by TLC using KMnO4 stain. Desired compound was purified on silica column using 50%EtOAc/Hexanes followed by 10%MeOH/EtOAc. The desired fractions were combined, concentrated, and vacuum pumped to afford *(S)*-2-amino-3-cyclopentyl-*N*-(oxetan-3-yl)propanamide as a yellow oil (80%pure) used as is in subsequent reaction.

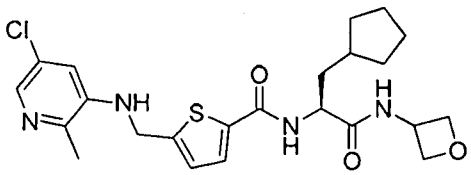
_(S)_-5-{[(5-Chloro-pyridin-3-yl)amino]methyl}-_N_-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide.

(S)-2-amino-3-cyclopentyl-*N*-(oxetan-3-yl)propanamide (200mg, 0.942mmol) and 5-{[(5-chloropyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (253mg, 0.942mmol) were dissolved in 10mL of DMF. Triethylamine (0.394mL, 2.83mmol), 1-hydroxybenzotriazole (144mg, 0.942mmol) and finally 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (181mg, 0.942mmol) were added and the contents allowed to stir at room temperature for 3 days. The reaction was then poured into 250mL of water and extracted (3 x 150mL) with ethyl acetate. The organic fractions were pooled, washed (2 x 200mL) with water and (2 x 200mL) with brine, dried over sodium sulfate,

filtered and evaporated to dryness. The crude material was purified by automated flash chromatography and eluted using a 0 - 4% MeOH / DCM gradient over 40min. The desired fractions were then pooled and evaporated to dryness to give 206mg of a slightly off-white solid, LC-MS(ES) m/z = 463.2, 465.2 [M+H]+.

Example 5

Preparation of *(S)*-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide:

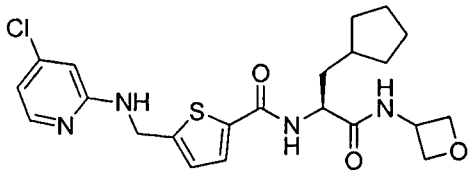


***(S)*-5-{[(5-Chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide.**

A solution of *(S)*-2-amino-3-cyclopentyl-*N*-(oxetan-3-yl)propanamide (113mg, 0.531mmol), 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (150mg, 0.531mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (203mg, 1.061mmol), 1-hydroxy-7-azabenzotriazole (144mg, 1.061mmol), and *N*-methylmorpholine (0.233mL, 2.122mmol), was stirred in DCM (3mL) with a couple drops of DMF for 2h at room temperature. The reaction mixture was purified directly on silica using 50-80% AcOEt/Hexanes. Desired fractions were combined and concentrated in-vacuum, transferred into submission vial using DCM. Concentrated and vacuum pumped to afford a white solid (190 mg). LCMS-showed 99% pure desired product, with a desired mass peak of LC-MS(ES) m/z = 477.2, 479.2 [M+H]+.

Example 6

Preparation of *(S)*-5-{[(4-chloro-pyridin-2-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide:

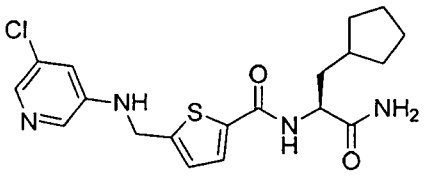


(S)-5-{[(4-Chloro-pyridin-2-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide.

To a solution of 5-{[(4-chloropyridin-2-yl)amino]methyl}thiophene-2-carboxylic acid (190mg, 0.707mmol) in DCM (100mL) were added *(S)*-2-amino-3-cyclopentyl-*N*-(oxetan-3-yl)propanamide (150mg, 0.707mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (163mg, 0.848mmol), 1-hydroxy-7-azabenzotriazole (115mg, 0.848mmol) and *N*-methylmorpholine (0.233mL, 2.121mmol). The reaction mixture was stirred at 25°C for 10h. The mixture was then concentrated and purified by reverse phase (eluting with 30 - 50% CH_3CN/water containing 0.1% HCOOH) to afford an off-white solid as the desired product (257.3mg), LC-MS(ES) m/z = 463.2, 465.2 [M+H]+.

Example 7

Preparation of *(S)*-*N*-(1-amino-3-cyclopentyl-1-oxopropan-2-yl)-5-{[(5-chloropyridin-3-yl)amino]methyl}thiophene-2-carboxamide:



(S)-tert-Butyl (1-amino-3-cyclopentyl-1-oxopropan-2-yl)carbamate.

A mixture of (S)-2-[(tert-butoxycarbonyl)amino]-3-cyclopentylpropanoic acid (1.388g, 5.39mmol), ammonium chloride (0.577g, 10.79mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.551g, 8.09mmol), 1-hydroxybenzotriazole (1.239g, 8.09mmol), and diisopropylethylamine (3.77mL, 21.58mmol) was stirred overnight at room temperature. The reaction was diluted with DCM and washed sequentially with 1N HCl, water, saturated aqueous solution of NaHCO3, water, and brine. The DCM solution

was dried over Na2SO4, filtered, and concentrated to give a white solid(1.0g). 1H-NMR indicated desired product (>95% purity). No further purification at this stage.

(S)-2-Amino-3-cyclopentylpropanamide hydrochloride.

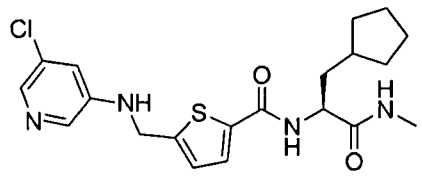
4M HCl (9.75mL, 39.0mmol) in 1,4-dioxane was added to solid (S)-tert-butyl (1-amino-3-cyclopentyl-1-oxopropan-2-yl)carbamate (1.00g, 3.90mmol) at room temperature under nitrogen and the resulting slurry was stirred for 1 hour. The white slurry was concentrated in vacuum and the residue was pumped under high vacuum overnight to give a white solid (750.0 mgs). 1H-NMR indicated removal of the protecting group. No further purification at this stage.

(S)-N-(1-Amino-3-cyclopentyl-1-oxopropan-2-yl)-5-{[(5-chloropyridin-3-yl)amino]methyl}thiophene-2-carboxamide.

A mixture of (S)-2-amino-3-cyclopentylpropanamide hydrochloride (250.5mg, 1.300mmol) , 5-{[(5-chloropyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (349mg, 1.300mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (374mg, 1.950mmol), 1-hydroxybenzotriazole (299mg, 1.950mmol), and diisopropylethylamine (0.681mL, 3.90mmol) in DCM (10mL) was stirred overnight at room temperature. The reaction was diluted with DCM and washed sequentially with an saturated aqueous solution of NaHCO3, water, and brine. The DCM was dried over Na_2SO_4, filtered, and concentrated to give a pale yellow solid (346.6mg), which was purified via chromatography on silica gel (2.5-9.0% MeOH:CHCl3 over 40min) and placed in a vacuum oven overnight at 55°C to give a white solid (109.4mg). LC-MS(ES) m/z = 407.3, 409.3 [M+H]+

Example 8

Preparation of (S)-5-{[(5-chloropyridin-3-yl)amino]methyl}-N-[3-cyclopentyl-1-(methylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide:



(S)-2-Amino-3-cyclopentyl-*N*-methylpropanamide hydrochloride.

To a suspension of *(S)*-2-[(tert-butoxycarbonyl)amino]-3-cyclopentylpropanoic acid (1.2g, 4.68mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.898g, 4.68mmol) in EtOAc (15mL) was added 1-hydroxybenzotriazole (0.72g, 4.68mmol) and methylamine (9.37mL, 2M in THF). The reaction mixture was stirred at room temperature for 20h. Additional reagents (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, 1-hydroxybenzotriazole, and methylamine) were added with DMF (2mL) for solubility. The reaction mixture stirred at room temperature for 9 hours and was then diluted with water and EtOAc until two clear layers were observed. The aqueous layer was extracted with EtOAc (2x). The combined organic layers were washed with saturated aqueous sodium bicarbonate solution (1x), brine (1x), dried over MgSO4, filtered, and concentrated in vacuum to afford an off-white solid (716.1 mg). The solid was readily dissolved in DCM and subjected to silica gel purification (0-60% ethyl acetate/hexanes). The desired fractions were combined, and concentrated in vacuum to afford (S)-tert-butyl (3-cyclopentyl-1-(methylamino)-1-oxopropan-2-yl)carbamate as a white solid (452.4 mg), LC-MS(ES) m/z = 171.1 [M+H]+

To (S)-tert-butyl (3-cyclopentyl-1-(methylamino)-1-oxopropan-2-yl)carbamate (452.4 mg, 1.67 mmol) was added 4N HCl in dioxane (6 mL, 24 mmol). The clear colorless solution was stirred at RT for 1h. The solution was concentrated in vacuo to afford *(S)*-2-amino-3-cyclopentyl-*N*-methylpropanamide hydrochloride as a white solid (356 mg). ^{1}H NMR (DMSO-d_6) δ 0.98 - 1.16 (m, 2 H) 1.39 - 1.64 (m, 4 H) 1.64 - 1.87 (m, 5 H) 2.65 (d, *J*=4.55 Hz, 3 H) 3.63 (t, *J*=6.95 Hz, 1 H) 8.21 (br. s., 3 H) 8.53 (d, *J*=4.55 Hz, 1 H).

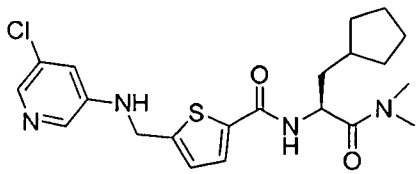
(S)-5-{[(5-Chloropyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(methylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide.

To a suspension of 5-{[(5-chloropyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (130mg, 0.48mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (93 mg, 0.48 mmol), diisopropylethylamine (169 uL, 0.97 mmol) and 1-hydroxybenzotriazole (74 mg, 0.48mmol) in EtOAc (5mL) was added *(S)*-2-amino-3-cyclopentyl-*N*-methylpropanamide hydrochloride (100mg, 0.48mmol) and DMF (2mL). The solution was stirred at room temperature for 20h. The reaction was diluted with water and EtOAc. The organic layer was washed with brine, dried over MgSO4, filtered, and concentrated in vacuum to afford a yellow oil (226.6 mg). This oil was readily dissolved in DMSO and subjected to reversed-phase HPLC purification (15-35% ACN/water). The desired product fractions were poured into ethyl acetate and saturated aqueous NaHCO3 solution. The combined organics were washed with brine (1x), dried over MgSO4, filtered, and concentrated in vacuum to afford a white solid (123.2 mg),

LC-MS(ES) m/z = 421.3, 423.3 [M+H]+.

Example 9

Preparation of *(S)*-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(dimethylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide:



(S)-(9H-Fluoren-9-yl)methyl(3-cyclopentyl-1-(dimethylamino)-1-oxopropan-2-yl)carbamate.

To a solution of *(S)*-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)-3-cyclopentylpropanoic acid (1.0 g, 2.63 mmol) in DCM (10ml) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.605g, 3.15mmol), 1-hydroxy-7-azabenzotriazole (0.429g, 3.15mmol), *N*-methylmorpholine (0.867mL, 7.89mmol) and dimethylamine hydrochloride (0.214g, 2.63mmol). The mixture was stirred at 25°C for 4h. The mixture

was concentrated and purified with silica gel column eluting with 10 - 50% EtOAc / hexane to afford the desired product (2.497g). LC-MS(ES) m/z = 407.3 [M+H]+.

(S)-2-Amino-3-cyclopentyl-*N,N*-dimethylpropanamide.

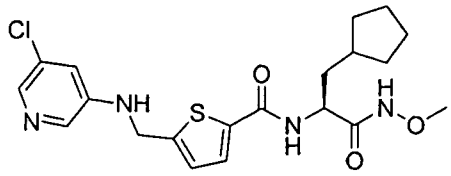
To a solution of *(S)*-(9H-fluoren-9-yl)methyl(3-cyclopentyl-1-(dimethylamino)-1-oxopropan-2-yl)carbamate (0.887g, 2.182mmol) in THF (10mL) was treated with piperidine (1.080mL, 10.91mmol). The mixture was stirred at 25°C for 18h. The mixture was concentrated and purified with silica gel column eluting with 10 - 100% EtOAc/hexanes followed by 0 - 5% $CHCl_3$ / MeOH containing 0.1% NH_4OH. Desired fractions were collected and concentrated to afford the desired product (0.351g) as a colorless oil. LC-MS(ES) m/z = 185.4 [M+H]+.

(S)-5-{[(5-Chloropyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(dimethylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide.

To a solution of 5-{[(5-chloropyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (117mg, 0.434mmol) in DCM (3mL) were added (S)-2-amino-3-cyclopentyl-*N,N*-dimethylpropanamide (80mg, 0.434mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (100mg, 0.521mmol), 1-hydroxy-7-azabenzotriazole (70.8mg, 0.521mmol) and *N*-methylmorpholine (0.143mL, 1.302mmol). The mixture was stirred at 25°C for 1h. The mixture was concentrated and purified by reverse phase HPLC eluting with 35 - 55% acetonitrile in water containing 0.1% HCOOH. The desired fractions were concentrated to afford 128 mg of the desired product as an off-white solid. LC-MS(ES) m/z = 435.2, 437.2 [M+H]+.

Example 10

Preparation of *(S)*-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(methoxyamino)-1-oxopropan-2-yl]thiophene-2-carboxamide:



(*S*)-tert-Butyl [3-cyclopentyl-1-(methoxyamino)-1-oxopropan-2-yl]carbamate.

To a clear solution of *(S)*-2-[(tert-butoxycarbonyl)amino]-3-cyclopentylpropanoic acid (1.0g, 3.89mmol), O-methylhydroxylamine hydrochloride (1.558g, 4.66mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.894g, 4.66mmol) in DMF (6.1mL) was added in one portion 1-hydroxy-7-azabenzotriazole (0.635g, 4.66mmol). The reaction mixture was stirred at room temperature for 12h, then was diluted with DCM and washed with water (20 mL) and brine (20mL) before being dried over Na2SO4 and filtered. Most of solvent was removed and the residual crude material was absorbed onto a dry-loading cartridge. Purification was performed by elution with EtOAc in Hexane (10 to 70%). Desired fractions were collected and concentrated to afford the desired product (920 mg). LC-MS(ES) m/z = 287.3 [M+H]+.

(*S*)-2-amino-3-cyclopentyl-*N*-methoxypropanamide hydrochloride.

To a solution of *(S)*-tert-butyl (3-cyclopentyl-1-(methoxyamino)-1-oxopropan-2-yl)carbamate (900mg, 3.14mmol) in DCM (20.75mL) was added 4N HCl (3929 µl, 15.71 mmol) in dioxane. Reaction mixture stirred at room temperature overnight, then concentrated down to afford the desired product (840 mg) as HCl salt. No purification at this stage. LC-MS(ES) m/z = 187.1 [M+H]+.

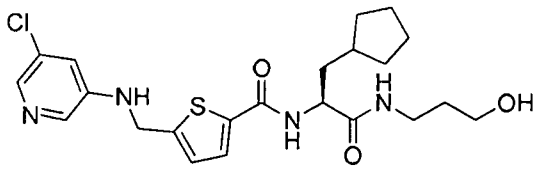
(*S*)-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(methoxyamino)-1-oxopropan-2-yl]thiophene-2-carboxamide.

To a clear solution of *(S)*-2-amino-3-cyclopentyl-*N*-methoxypropanamide hydrochloride (70mg, 0.314mmol) and, 5-{[(5-chloropyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (84mg, 0.314mmol) and diisopropylethylamine (54.9µL, 0.314mmol) in DCM

(3.1mL) was added as one portion of solid 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (120mg, 0.314mmol). The reaction mixture was stirred at room temperature for 12h. The solvent was then evaporated to dryness. The residure was dissolved in DMSO (2.0mL) then subjected to reverse phase HPLC purification eluting with 10-80% CH3CN in water with 0.1% of Formic acid. The desired fractions were collected and the solvent was removed by vacuum in order to afford the final product (24mg) as a white solid. LC-MS(ES) m/z = 437.3, 439.3 [M+H]+.

Example 11

Preparation of *(S)*-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide:



***(S)*-tert-Butyl {3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}carbamate.**

In a 250mL round bottomed flask with stirbar were placed *(S)*-2-[(tert-butoxycarbonyl)amino]-3-cyclopentylpropanoic acid (5g, 19.43mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (3.72g, 19.43mmol), and EtOAc (100mL). To the resulting mixture were added diisopropylethylamine (6.79mL, 38.9mmol) and 1-hydroxybenzotriazole (2.98g, 19.43mmol). This mixture was stirred at room temperature for 5min and then to it was added 3-amino-1-propanol (1.477mL, 19.43mmol). The reaction mixture stirred at room temperature overnight. LCMS after 16 hr showed full conversion to desired mass, although the product is not UV active. The reaction mixture was quenched by addition of water (about 50mL) and extracted with 3 x 75mL EtOAc. The combined organics were washed with 1N HCl, saturated aqueous solution of NaHCO3, and brine; dried with MgSO4; filtered; and concentrated by rotary evaporation to give the desired product (5.96g) as a colorless oil. LC-MS(ES) m/z = 315.3 [M+H]+.

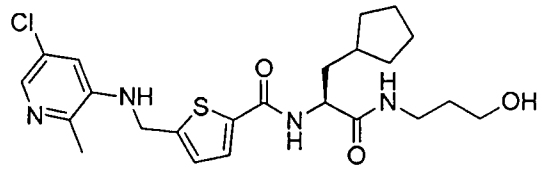
(S)-5-{[(5-Chloropyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.

In a 20mL reaction vial with stirbar were placed (S)-tert-butyl {3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}carbamate (150mg, 0.324mmol) and HCl, 4M in dioxane (3mL, 12.00mmol). The vial was capped and the reaction mixture stirred at room temperature. LCMS after 45 min showed full deprotection. The reaction mixture was then concentrated under vacuum. The residue was taken up in DMF (0.5 mL).

In a separate 20mL reaction vial with stirbar were combined 5-{[(5-chloropyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (87mg, 0.324mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (62.2mg, 0.324mmol), 1-hydroxybenzotriazole (49.7mg, 0.324mmol), EtOAc (1.5mL), and diisopropylethylamine (0.170 mL, 0.973 mmol). The reaction mixture stirred at room temperature for about 5 minutes and then to it was added the DMF solution of the deprotected amine from above. The reaction mixture stirred at room temperature overnight. LCMS after 19h showed full consumption of amine intermediate. The reaction mixture was quenched by addition of water (10mL) and extracted with 3 x 20mL of EtOAc. The combined organics were washed with brine, dried with MgSO4, filtered, and concentrated by rotary evaporation to give 160mg of crude material. The crude residue was taken up in DMSO, filtered, and subjected to reverse phase HPLC purification using 0.1%TFA/acetonitrile, 0.1%TFA/water conditions with a 2min hold and a 16-36%, 7.5min gradient followed with a 3min 100% organic hold time and a flow rate of 47mL/min. The desired fractions were combined and converted to the free base form by addition of saturated aqueous solution of NaHCO3 and extraction with EtOAc. The combined organics were combined, washed with brine, dried with Na2SO4, concentrated, and lyophilized to give the desired product as a monohydrate (84.2 mg) as a white solid. LC-MS(ES) m/z = 465.3, 467.3 [M+H]+.

Example 12

Preparation of *(S)*-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide:

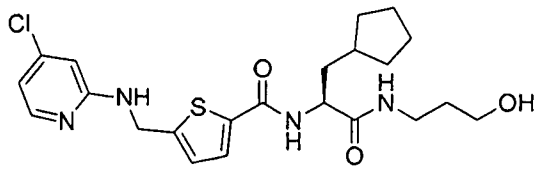


(*S*)-5-{[(5-Chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.

In a 20 mL reaction vial with stirbar were placed *(S)*-tert-butyl {3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}carbamate (150mg, 0.324mmol) and HCl, 4M in dioxane (3mL, 12.00mmol). The vial was capped and the reaction mixture stirred at room temperature. LCMS after 45 min showed full deprotection. The reaction mixture was concentrated under vacuum. The residue was taken up in EtOAc (1.5mL) and DMF (0.5mL). To the resulting solution was added diisopropylethylamine (0.170mL, 0.973mmol), 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (92mg, 0.324mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (62.2mg, 0.324mmol), and 1-hydroxybenzotriazole (49.7mg, 0.324mmol). The reaction mixture stirred at room temperature overnight. The reaction mixture was then quenched by addition of water (about 10mL) and extracted with 3 x 20mL EtOAc. The combined organics were washed with brine, dried with MgSO4, filtered, and concentrated by rotary evaporation to give 170mg of crude material. The crude residue was taken up in DMSO, filtered, and subjected to reverse phase HPLC purification, eluted using 0.1%TFA/acetonitrile, 0.1%TFA/water conditions with a 2min hold and a 13-33%, 7.5min gradient followed with a 3min 100% organic hold time and a flow rate of 47mL/min. The desired fractions were combined and converted to the free base form by addition of saturated aqueous solution of NaHCO3 and extraction with EtOAc. The combined organics were combined, washed with brine, dried with Na2SO4, concentrated, and lyophilized to give the desired product (79.5 mg). LC-MS(ES) m/z = 479.4, 481.4 [M+H]+.

Example 13

Preparation of *(S)*-5-{[(4-chloropyridin-2-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide:

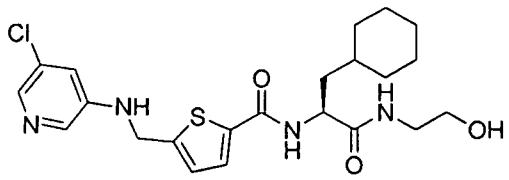


(*S*)-5-{[(4-Chloropyridin-2-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.

In a 20mL vial with stirbar were placed *(S)*-tert-butyl {3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}carbamate (150mg, 0.324mmol) and HCl, 4M in dioxane (2mL, 8.00mmol). The reaction mixture stirred at room temperature. LCMS after 1 hr showed reaction completion. The reaction mixture was then concentrated and the residue was taken up in EtOAc (2mL) and DMF (0.5mL). To the resulting mixture was added 5-{[(4-chloropyridin-2-yl)amino]methyl}thiophene-2-carboxylic acid (87mg, 0.324mmol), 1-hydroxybenzotriazole (49.7mg, 0.324mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (62.2mg, 0.324mmol), and diisopropylethylamine (0.170mL, 0.973mmol). The reaction mixture stirred at room temperature overnight. LCMS after 16.5 hr showed full consumption of limiting reagent. To the reaction mixture was added water (5mL) and the resulting mixture was extracted with 3 x 10mL EtOAc. The combined organics were washed with brine, dried with Na2SO4, filtered, and concentrated by rotary evaporation. The crude residue was taken up in DMSO, filtered, and subjected to reverse phase HPLC purification using acidic: 0.1%TFA/acetonitrile, 0.1%TFA/water conditions with a 2min hold and a 12-32%, 7.5mingradient followed with a 3min 100% organic hold time and a flow rate of 47mL/min. The desired fractions were combined and converted to the free base form by addition of saturated aqueous solution of NaHCO3 followed by extraction with EtOAc. The combined organics were combined, washed with brine, dried with Na2SO4, concentrated, and lyophilized to give the desired product (68.6 mg) as a white solid.). LC-MS(ES) m/z = 465.3, 467.3 [M+H]+.LCMS shows 100% desired material by UV (0.64 min, M+H=465.3).

Example 14

Preparation of *(S)*-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide:



(*S*)-tert-Butyl {3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}carbamate.

A solution of *(S)*-2-((tert-butoxycarbonyl)amino)-3-cyclohexylpropanoic acid (20g, 73.7mmol,), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (16.96g, 88mmol), 1-hydroxybenzotriazole (12.04g, 88mmol), ethanolamine (6.72mL, 111mmol) and *N*-methylmorpholine (32.4mL, 295mmol), stirred in DCM (300 mL) for 2h at room temperature. Reaction continued to stir overnight in order to push to completion, which was confirmed by LCMS, LC-MS(ES) m/z = 315.4 [M+H]+. Reaction mixture was transferred to a separatory funnel, then washed with water, saturated aqueous NaHCO3 solution, brine, dried over sodium sulfate, filtered, concentrated and vacuum pumped overnight to afford a yellow oil. The product was redissolved in DCM and purified thru a silica plug using 100% EtOAc to remove baseline impurities. The filtrate was concentrated and vacuum pumped to afford a fluffy white solid (23.17g) which was used directly in the next reaction.

(*S*)-2-Amino-3-cyclohexyl-*N*-(2-hydroxyethyl)propanamide hydrochloride.

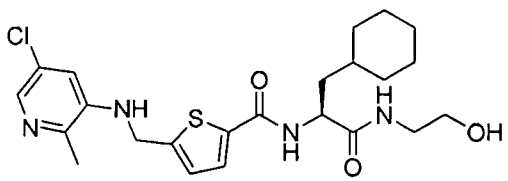
To a solution of *(S)*-tert-butyl {3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}carbamate (18g, 57.2mmol), in DCM (300mL) was added aqueous 4M HCl solution(71.6mL, 286mmol). The reaction stirred for 2h at room temperature. The reaction was concentrated and vacuum pumped overnight to afford a white solid (14g), LC-MS(ES) m/z = 215.3 [M+H]+.

(S)-5-{[(5-Chloropyridin-3-yl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.

A mixture of 5-{[(5-chloro-3-pyridinyl)amino]methyl}-2-thiophenecarboxylic acid (1.620g, 6.03mmol), *(S)*-2-amino-3-cyclohexyl-*N*-(2-hydroxyethyl)propanamide (1.512g, 6.03mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.156g, 6.03mmol), 1-hydroxybenzotriazole (0.923g, 6.03mmol), and diisopropylethylamine (3.16mL, 18.09mmol) in DCM (40mL) was stirred at room temperature for 16 hours. The reaction was diluted with DCM and a saturated aqueous NaHCO3 solution then transferred to a separatory funnel. It was observed that much of the desired product had gummed up and was stuck to the walls of the separatory funnel. The upper aqueous layer was neutralized by addition of 2N HCl. 35mL of IPA were then added to the 350mL of DCM already in the funnel. This made the brown gum dissolve and gave two homogeneous layers. The layers were separated and the aqueous layer was extracted with 50mL more of 10% IPA:DCM. The combined IPA/DCM layers were dried over Na2SO4, filtered, and concentrated to give a tan foam (2.44g). Crude product was purified via chromatography on silica gel (2.5-7.5% MeOH:DCM). Desired Fractions were concentrated and pumped under high vacuum to give a white solid, (0.881 g). Data indicated desired product (100% purity by analytical HPLC), LC-MS(ES) m/z = 465.3, 467.4 [M+H]+.

Example 15

Preparation of *(S)*-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide:

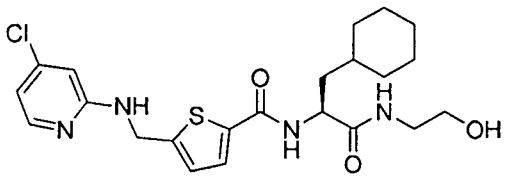


(S)-5-{[(5-Chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.

A solution of 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (2.5g, 8.84 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (2.034g, 10.61mmol), 1-hydroxy-7-azabenzotriazole (1.444g, 10.61mmol), *(S)*-2-amino-3-cyclohexyl-*N*-(2-hydroxyethyl)propanamide hydrochloride (2.217g, 8.84mmol) and *N*-methylmorpholine (3.89mL, 35.4mmol) was stirred in DCM (80mL) for 4h at room temperature. The reaction was poured into water and extracted with DCM (3x50mL). The aqueous layer formed a gummy residue which stuck to sides of the separatory funnel. The aqueous layer was then extracted again with EtOAc (3x50mL) which dissolved all the gummy residue. The combined organic layers were washed with brine, dried over Na2SO4, filtered and concentrated to an off-white solid. The product was triturated with DCM, filtered, and dried overnight to afford 2.6 g of a white solid (100% pure by HPLC), LC-MS(ES) m/z = 479.3, 481.3 [M+H]+.

Example 16

Preparation of *(S)*-5-{[(4-chloropyridin-2-yl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide:



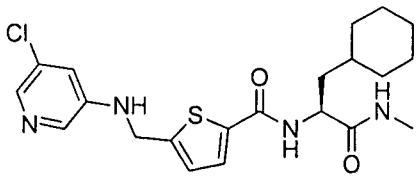
***(S)*-5-{[(4-Chloropyridin-2-yl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.**

To a clear solution of 5-{[(4-chloropyridin-2-yl)amino]methyl}thiophene-2-carboxylic acid (268mg, 0.997mmol) and *(S)*-2-amino-3-cyclohexyl-*N*-(2-hydroxyethyl)propanamide hydrochloride (250mg,0.997 mmol) and diisopropylethylamine (696µL, 3.99mmol) in DCM (9273 µL) was added solid 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (417mg, 1.097mmol) in one portion. The reaction was stirred overnight at room temperature, before being diluted with DCM and washed with water and brine. The organic phase was dried with Na2SO4. The solvent

was removed by vacuum and crude material was purified on silica gel (0-80 % MeOH in DCM). However purification was only partial. Pure fractions were combined to afford a first crop of the desired product as an off-white solid (112 mg). The impure fractions were collected and evaporated to dryness, before being dissolved in CH3CN (2.0mL) and purified by reverse phase HPLC (CH3CN in water 10-60% with 0.1% of formic acid). The new resulting pure fractions were combined and concentrated under vacuum to give a second crop of desired compound (124 mg) as a white solid. Both batches had HPLC purities>99%, LC-MS(ES) m/z = 465.2, 467.3 [M+H]+.

Example 17

Preparation of *(S)*-5-{[(4-chloropyridin-2-yl)amino]methyl}-*N*-[3-cyclohexyl-1-(methylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide:



***(S)*-tert-Butyl [3-cyclohexyl-1-(methylamino)-1-oxopropan-2-yl]carbamate.**

To a stirred solution of *(S)*-2-[(tert-butoxycarbonyl)amino]-3-cyclohexylpropanoic acid (10g, 36.9mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (7.06g, 36.9mmol), 1-hydroxybenzotriazole (5.64g, 36.9mmol) in DCM (10mL) at 0°C was added Methylamine (2 M, THF) (73.7mL, 147mmol) via syringe. The reaction was allowed to warm to room temperature after 1 hour, and stirred at room temperature overnight. The reaction mixture was diluted with DCM (200mL) and poured into water (200mL). The organic layer was separated and washed with additional water (200mL) then dried over sodium sulfate, filtered and concentrated. The residual oil was purified by flash chromatography (0-50% EtOAc in hexanes) to afford the desired product (8.4g) as a white solid, LC-MS(ES) m/z = 285 [M+H]+.

***(S)*-2-Amino-3-cyclohexyl-*N*-methylpropanamide, hydrochloride.**

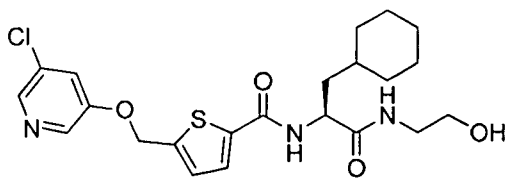
(S)-tert-Butyl [3-cyclohexyl-1-(methylamino)-1-oxopropan-2-yl]carbamate (8.4g, 29.5 mmol) and HCl (4M, dioxane) (100mL, 400mmol) were stirred overnight at room temperature. The reaction mixture was concentrated to afford the desired product (7.48g) as a white solid, LS-MS(ES) m/z = 185 [M+H]+.

(S)-5-{[(4-Chloropyridin-2-yl)amino]methyl}-*N*-[3-cyclohexyl-1-(methylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide.

A mixture of 5-{[(5-chloropyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (0.339g, 1.262mmol), *(S)*-2-amino-3-cyclohexyl-*N*-methylpropanamide, hydrochloride (0.334g, 1.514mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.242g, 1.262mmol), 1-hydroxybenzotriazole (0.193 g, 1.262 mmol), and diisopropylethylamine (0.661mL, 3.78mmol) in DCM (10mL) was stirred at room temperature overnight. The reaction was diluted with DCM, washed with a saturated aqueous NaHCO3 solution and brine, dried over Na2SO4, filtered, and concentrated to give a tan solid. Crude product was purified via chromatography on silica gel (2.5-7.5% MeOH:DCM) and placed in a vacuum oven at 50°C for 3 days to give the desired product as a white solid(231.4mg), LC-MS(ES) m/z = 435.2, 437.3 [M+H]+.

Example 18

Preparation of *(S)*-5-{[(5-chloropyridin-3-yl)oxy]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide:



Methyl 5-{[(5-chloropyridin-3-yl)oxy]methyl}thiophene-2-carboxylate.

To a solution of 5-chloropyridin-3-ol (3.64g, 28.1mmol) in ACN (77mL) and DMF (7.73mL)was added Cs2CO3 (12.47g, 38.3mmol). The reaction mixture was stirred for 10min and cooled down in an ice bath. A solution of methyl 5-(bromomethyl)thiophene-2-carboxylate (6.0g, 25.5mmol) in ACN (20mL) was then added dropwise using an addition funnel. The reaction mixture was stirred for 2h and was then concentrated down, before being taken back in water and EtOAc. The organic phase was washed by water and brine, then dried over Na2SO4 and filtered. Crude product was filtrated through a silica gel column with 30% EtOAc in hexanes, to afford the desired product (5.3g) as a light brown solid, LC-MS (ES) m/z = 284.0, 286.0 [M+H]+.

5-{[(5-Chloropyridin-3-yl)oxy]methyl}thiophene-2-carboxylic acid.

To a slurry of methyl 5-{[(5-chloropyridin-3-yl)oxy]methyl}thiophene-2-carboxylate (1.6 g, 5.24mmol) in water (4.72mL) and THF (11.01mL) was added 6N NaOH aqueous solution (10.49mL, 26.2mmol). The reaction mixture was stirred overnight. Most volatiles were then removed under vacuum, and the resulting solution was acidified with 6N HCl aqueous solution and was then extracted with EtOAc(2x). The combined organic extracts were washed with water then brine, dried with Na2SO4 and filtered. Concentration to dryness, followed by trituration with DCM afforded the desired product (1.3g) as off white solid, LC-MS (ES) m/z = 270.0, 272.0 [M+H]+.

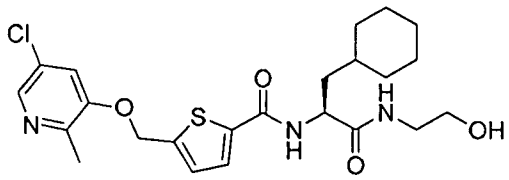
(*S*)-5-{[(5-Chloropyridin-3-yl)oxy]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.

A solution of *(S)*-2-amino-3-cyclohexyl-*N*-(2-hydroxyethyl)propanamide, hydrochloride (1.5g, 5.98mmol), 5-{[(5-chloropyridin-3-yl)oxy]methyl}thiophene-2-carboxylic acid (1.613g, 5.98mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.376g, 7.18mmol), 1-hydroxy-7-azabenzotriazole (0.977g, 7.18mmol) and *N*-methylmorpholine (2.63mL, 23.93mmol) in DCM (30mL) was stirred at room temperature for 2h. The reaction mixture was diluted with DCM and water. The separated organic layer was washed with water(2x), then brine, and dried over Na2SO4. After filtration, the solution was concentrated down with silica and purified by silica column (using 0-50% of mixture

(9% MeOH+1%NH4Cl+90%CHCl3) in CHCl3 as eluant. Desired fractions were collected and concentrated under reduced pressure to afford the desired product (2.1g) as white solid, LC-MS(ES) m/z = 466.2, 468.3 [M+H]+.

Example 19

Preparation of *(S)*-5-{[(5-chloro-2-methylpyridin-3-oxy]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide:



5-Chloro-2-methylpyridin-3-ol.

To a solution of sulfuric acid (270mL) and water (900mL) was added 5-chloro-2-methylpyridine-3-amine (18g, 126mmol) at room temperature and cooled down to 0°C. A solution of NaNO2 (9.58g, 139mmol, dissolved in 90mL of water) was then added drop wise over 20min. The reaction mixture was then stirred at 0°C for additional 30 min before being diluted with sulfuric acid (180mL) and water (720mL). The resulting mixture was then heated at 100°C for 1h; then cooled down to room temperature and adjusted to pH=6 with NaOH solution. The resulting mixture was then extracted with EtOAc (2 x 500mL). The organic phases were combined, dried over Na2SO4 and concentrated under reduced pressure to afford the desired product (17g) as a pale yellow solid, LC-MS (ES) m/z = 143.0, 145.0 [M+H]+

Methyl 5-{[(5-chloro-2-methylpyridin-3-yl)oxy]methyl}thiophene-2-carboxylate.

To a solution of methyl-5-(bromomethyl)thiophene-2-carboxylate (27.8g, 118mmol) in DMF (170mL) under nitrogen atmosphere was added at room temperature 5-chloro-2-methylpyridin-3-ol (17g, 118mmol) followed by K2CO3 (16.36g, 118mmol). The

reaction mixture was stirred for 5 h, before being diluted with water (340mL) and extracted with EtOAc (600mL). The separated organic phase was dried over Na2SO4, filtered and concentrated under reduced pressure to afford the desired product (27g) as an off white solid, LC-MS (ES) m/z = 297.0, 299.0 [M+H]+

5-{[(5-Chloro-2-methylpyridin-3-yl)oxy]methyl}thiophene-2-carboxylic acid.

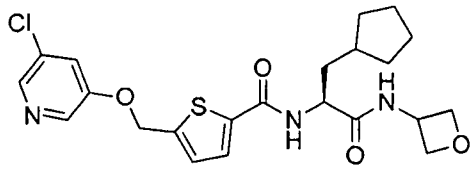
To a solution of methyl-5-{[(5-chloro-2-methylpyridin-3-yl)oxy]methyl}thiophene-2-carboxylate (27g, 91mmol) in THF (540mL) and water (270mL) was added at room temperature lithium hydroxide mono hydride (19.04g, 453mmol). The reaction mixture was heated at 45°C for 16 h, then concentrated under reduced pressure. The obtained solid residue was diluted with water (200mL) then pH adjusted to 6 with 1N HCl aqueous solution (100mL). The resulting solution was extracted with EtOAc (600 mL) and the separated organic phase was dried over Na2SO4, filtered and concentrated under reduced pressure to afford the desired product (13.75 g) as a pale yellow solid, LC-MS (ES) m/z = 283.0, 285.0 [M+H]+

***(S)*-5-{[(5-Chloro-2-methylpyridin-3-oxy]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.**

The reaction mixture of *(S)*-2-amino-3-cyclohexyl-*N*-(2-hydroxyethyl)propanamide, hydrochloride (66mg, 0.263mmol) and 5-{[(5-chloro-2-methylpyridin-3-yl)oxy]methyl}thiophene-2-carboxylic acid (74.7mg, 0.263mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (60.5mg, 0.316mmol), 1-hydroxy-7-azabenzotriazole (43.0mg, 0.316mmol) and *N*-methylmorpholine (0.116mL, 1.053mmol) in DMF (3mL) was stirred overnight. LCMS showed completion. The reaction mixture was purified by reverse phase HPLC (0.1% formic acid in mobile phase, water/ACN) using 20-70% gradient, but the resulting product was not pure enough. Fractions were combined and concentrated then an additional purification was conducted by flash chromatography using a 0-100% gradient of the mixture CHCl3/(9% MeOH+1%NH4Cl+90%CHCl3). Desired fractions were combined and concentrated to afford the desired product (69 mg) as beige solid. LC-MS (ES) m/z = 480.3, 482.3 [M+H]+

Example 20

Preparation of *(S)*-5-{[(5-chloropyridin-3-yl)oxy]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide:

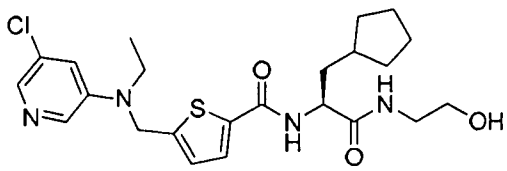


(*S*)-5-{[(5-Chloropyridin-3-yl)oxy]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide.

To a solution of 5-{[(5-chloropyridin-3-yl)oxy]methyl}thiophene-2-carboxylic acid (191mg, 0.707mmol) in DCM (100mL) were added *(S)*-2-amino-3-cyclopentyl-*N*-(oxetan-3-yl)propanamide (150mg, 0.707mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (163mg, 0.848mmol), 1-hydroxy-7-azabenzotriazole (115mg, 0.848mmol) and *N*-methylmorpholine (0.233mL, 2.121mmol). The mixture was stirred at 25 °C for 10h. The mixture was then concentrated and purified by silica gel chromatography (0 - 3% MeOH/CHCl3) to afford the desired product as an off-white solid (164 mg). LC-MS (ES) m/z = 464.2, 466.2 [M+H]+

Example 21

Preparation of *(S)*-5-{[(5-chloropyridin-3-yl)oxy]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide:



Ethyl 5-{[(5-chloropyridin-3-yl)(ethyl)amino]methyl}thiophene-2-carboxylate.

A 400 mL round-bottomed flask was charged with 5-{[(5-chloropyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (6g, 22.33mmol) in DMF (60mL) to give a yellow solution at 0°C under nitrogen. Sodium hydride 60% in mineral oil (2.77g, 69.2mmol) was added to the reaction mixture. Ethyl iodide (3.97mL, 49.1mmol) was added to the reaction mixture. The reaction was stirred to room temperature. After overnight stirring, the reaction mixture was checked by LCMS and showed some product mixed with various other alkylated products. The reaction mixture was diluted with water (200mL) and extracted with EtOAc (200mL x3). The EtOAc layers were dried over Na2SO4, filtered, and concentrated. The residue was chromatographed on silicagel column and eluted with EtOAc and hexanes (5% to 25%) and the clean fractions were concentrated to dryness to obtain the desired product (2.5 g). LC-MS (ES) m/z = 325.2, 327.2 [M+H]+.

Lithium 5-{[(5-chloropyridin-3-yl)(ethyl)amino]methyl}thiophene-2-carboxylate.

A 50 mL round-bottomed flask was charged with ethyl 5-{[(5-chloropyridin-3-yl)(ethyl)amino]methyl}thiophene-2-carboxylate (2.5g, 7.70mmol) and 1N LiOH (7.70mL, 7.70mmol) in THF (20mL) to give a brown suspension at 25°C under nitrogen. The reaction was stirred and warmed up to 50°C overnight. The reaction was then concentrated to the desired product (2.4g). LC-MS (ES) m/z = 297.1, 299.1 [M+H]+.

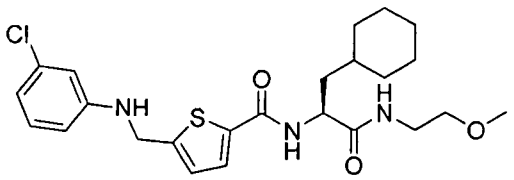
(*S*)-5-{[(5-Chloropyridin-3-yl)oxy]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide.

A solution of *(S)*-2-amino-3-cyclopentyl-*N*-(2-hydroxyethyl)propanamide, hydrochloride (122mg, 0.514mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (126 mg, 0.655 mmol), lithium 5-{[(5-chloropyridin-3-yl)(ethyl)amino]methyl}thiophene-2-carboxylate (150mg, 0.468mmol), 1-hydroxy-7-azabenzotriazole (89mg, 0.655mmol) and DMF (5mL) was stirred at room temperature over the weekend. The reaction mixture was then diluted with water (20mL) and extracted with DCM (20mL). The organic layer was washed with water then dried over Na2SO4, filtered, and concentrated. The residue was purified via chromatography on silica gel column using DCM and DCM with MeOH (5% to 40%) and

the clean fraction were concentrated to dryness to obtain the desired product (80 mg) as a yellow solid. LC-MS (ES) m/z = 479.0, 481.0 [M+H]+

Example 22

Preparation of *(S)*-5-{[(3-chlorophenyl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-methoxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide:



***(S)*-tert-Butyl [3-cyclohexyl-1-(methoxyethylamino)-1-oxopropan-2-yl]carbamate.**

A solution of 3-cyclohexyl-*N*-{[(1,1-dimethylethyl)oxy]carbonyl}-L-alanine (10 g, 36.9 mmol), 2-methoxyethylamine (3.17 mL, 36.9 mmol), HBTU (14.01 g, 36.9 mmol), and N-methylmorpholine (6.08 mL, 55.3 mmol) stirred in N,N-Dimethylformamide (DMF) (200 mL) at RT for 2h. The reaction was monitored by LCMS to show 79% (BPC) desired product, MW = 329.3 (M+H). TLC with KMnO4 stain shows product Rf = 0.5 and baseline spot with 1:1 (EtOAc:Hexanes). The reaction was poured into water and extracted with EtOAc (4 x 100 mL). The combined orgnanic layers were combined, washed with brine, dried over sodium sulfate, filtered, concentrated to an orange oil. Seemed wet. Redisolved in EtOAC and washed with brine, dried over sodium sulfate, and concentrated to a thick orange oil. Redissolved in DCM, loaded onto 80g pre-packed silica gel column. Purified using 20-40% EtOAc/Hexanes over 30 minutes. Collecting waste in tubes (since product not UV active).

LCMS-shows 100% desired product, MW = 329.3 (M+H)

1H NMR (400 MHz, DMSO-d6) d 7.78 (br. s., 1H), 6.79 (d, J = 8.34 Hz, 1H), 3.71 - 4.22 (m, 1H), 3.07 - 3.42 (m, 7H), 2.46 (br. s., 2H), 1.63 (d, J = 9.85 Hz, 4H), 1.25 - 1.46 (m, 11H), 0.95 - 1.28 (m, 3H), 0.67 - 0.98 (m, 2H)

Used directly in next reaction.

(S)-2-Amino-3-cyclohexyl-*N*-(2-methoxyethyl)propanamide hydrochloride.

4M HCl/Dioxane (15.22mL, 60.9mmol) was added to a solution of *(S)*-tert-butyl [3-cyclohexyl-1-(methoxyethylamino)-1-oxopropan-2-yl]carbamate (4g, 12.18mmol) in DCM (50mL). The reaction stirred 2h at room temperature under nitrogen. The reaction was concentrated and vacuum pumped to afford a pale yellow foamy solid. LC-MS (ES) m/z = 229.2 [M+H]+

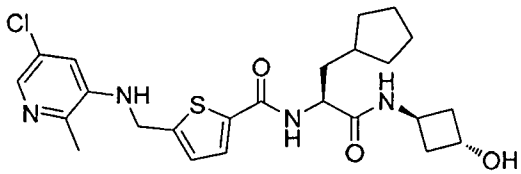
(S)-5-{[(3-Chlorophenyl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-methoxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.

A solution of 5-{[(3-chlorophenyl)amino]methyl}-2-thiophenecarboxylic acid (761mg, 2.84mmol), *(S)*-2-amino-3-cyclohexyl-*N*-(2-methoxyethyl)propanamide hydrochloride (800mg, 2.369mmol), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (1078mg, 2.84mmol), and *N*-methylmorpholine (0.781mL, 7.11mmol) stirred in DMF (20mL) at room temperature for 2h. The reaction was poured into water and extracted with EtOAc (3 x 20mL). The organic layers were combined, washed with brine, dried over sodium sulfate & decolorizing carbon, filtered thru a pad of celite, concentrated and vacuum pumped to afford a yellow oil. This oil was dissolved in DCM and purified on silica gel using 20%-40% EtOAc/Hexanes gradient. Desired fractions were combined, concentrated, and the resulting solid was dried in vacuum-oven to afford a pale yellow gummy solid (405mg). Solid was then dissolved in EtOAc (10mL) and Hexanes (10mL) was added. Product formed on sides of round-bottomed flask. Decanted off solution and then redissolved remaining solids in ether, concentrated, and vacuum pumped to an off-white fluffy solid. HPLC-still shows 99.1%. Dissolved the solid in DMSO and purified by reverse phase HPLC using 0.1% TFA. Combined desired fractions, removed organic solvent by rotary evaporation in-vacuum. Aqueous solution

was poured into a saturated aqueous solution of NaHCO3, then extracted with EtOAc (3 x 20mL). The resulting combined orgnanic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. Residue was redissolved in ether, concentrated, and vacuum pumped to a fluffy white solid. LC-MS (ES) m/z = 478.3, 480.3 [M+H]+

Example 23

Preparation of 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1r,3S)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide:



Mixture of *tert*-butyl {*(S)*-3-cyclopentyl-1-[(*(1r,3S)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}carbamate and *tert*-butyl {*(S)*-3-cyclopentyl-1-[(*(1s,3R)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}carbamate.

To a clear solution of *(S)*-2-[(tert-butoxycarbonyl)amino]-3-cyclopentylpropanoic acid (3.0g, 11.66mmol), a (1:1) mixture of cis/trans 3-aminocyclobutanol (1.117g, 12.82mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (2.68g, 13.99mmol) in Dichloromethane (DCM) (41.2mL) was added 1-hydroxy-7-azabenzotriazole (1.904g, 13.99mmol) as one portion. The reaction mixture was stirred at room temperature for 12 hours, then diluted with DCM and washed with water, an aqueous saturated solution of NaHCO3 (20 mL x2) ; an aqueous saturated solution of NH4Cl (20 mL x2) and brine (20mL x3). The decanted organic layer was separated and dried over Na2SO4. The organic solvent was evaporated under reduced pressure to afford a wax-like solid which was then triturated with water. The resulted solid was collected by filtration to afford the titled mixture (3.7g, 10.77mmol, 92% yield). LC-MS (ES) m/z = 327.3 [M+H]+

Mixture of *(S)*-2-amino-3-cyclopentyl-*N*-((*1r,3S)*-3-hydroxycyclobutyl)propanamide and *(S)*-2-amino-3-cyclopentyl-*N*-((*1s,3R)*-3-hydroxycyclobutyl)propanamide, bis hydrochloride.

The mixture of *tert*-butyl {*(S)*-3-cyclopentyl-1-[(*(1r,3S)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}carbamate and *tert*-butyl {*(S)*-3-cyclopentyl-1-[(*(1s,3R)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}carbamate (3.7g, 10.77mmol) was then dissolved in Dichloromethane (DCM) (41.2mL), treated with 4M HCl in dioxane (14.57mL, 58.3mmol) and stirred at room temperature overnight. The solvent was then evaporated and the residue was triturated with Et2O which converted it into a white solid. The ether layer was decanted and the trituration repeated with fresh Et2O. The suspension was allowed to stand for 10 minutes before careful removal of supernatant ether. The resulting solid was dried under high vacuum to afford the titled mixture (2.6g, 8.25mmol, 70.8% yield). LC-MS (ES) m/z = 227.2 [M+H]+

Mixture of 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1r,3S)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide and 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1s,3R)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.

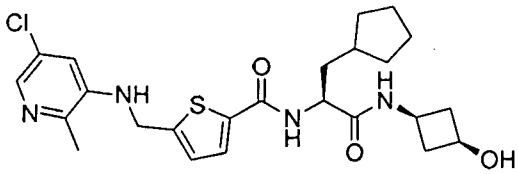
To a clear solution of the mixture of *(S)*-2-amino-3-cyclopentyl-*N*-(*(1r,3S)*-3-hydroxycyclobutyl)propanamide and *(S)*-2-amino-3-cyclopentyl-*N*-(*(1s,3R)*-3-hydroxycyclobutyl)propanamide, bis hydrochloride (600mg, 2.005mmol) and 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (567mg, 2.005mmol) and diisopropylethylamine (1.4mL, 8.02mmol) in Dichloromethane (DCM) (18.7mL) was added as one portion of solid *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (915mg, 2.406mmol). The reaction mixture was stirred at room temperature for 12 hours then diluted with DCM and washed with water (20mL x3) and brine (20mL). The decanted organic phase was isolated and concentrated by evaporation under low pressure. LC-MS (ES) m/z = 491.4, 493.4 [M+H]+

5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1r,3S)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.

Chiral separation of the mixture of the two diastereoisomers 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1r,3S)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide and 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1s,3R)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide was performed transferring the analytical conditions (on AD-H column with 30:70 EtOH:Heptane and 0.1% isopropylamine added as modifier) to a semi-prepaprative column AD-H column using a 40:60 EtOH:Heptane with 0.1%isopropylamine. Injection samples were dissolved using EtOH and methanol MeOH (up to a 3:1 ratio in volume). (a) 1st eluting diastereomer (165mg) is titled desired compound. (b) 2nd eluting diastereomer (240mg) is 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1s,3R)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide. LC-MS (ES) m/z = 491.4, 493.4 [M+H]+

Example 24

Preparation of 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1s,3R)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide:

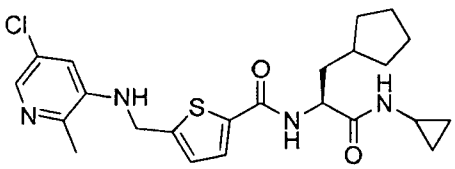


5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1s,3R)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide.

Chiral separation of the mixture of the two diastereoisomers 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1r,3S)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide and 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1s,3R)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide was performed transferring the analytical conditions (on AD-H column with 30:70 EtOH:Heptane and 0.1% isopropylamine added as modifier) to a semi-prepaprative column AD-H column using a 40:60 EtOH:Heptane with 0.1%isopropylamine. Injection samples were dissolved using EtOH and methanol MeOH (up to a 3:1 ratio in volume). (a) 1st eluting diastereomer (165mg) is 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1r,3S)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide. (b) 2nd eluting diastereomer (240mg) is the titled desired compound. LC-MS (ES) m/z = 491.4, 493.4 [M+H]+

Example 25

Preparation of *(S)*-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(cyclopropylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide:



***(S)*-tert-Butyl [3-cyclopentyl-1-(cyclopropylamino)-1-oxopropan-2-yl]carbamate.**

To a solution of *(S)*-2-[(tert-butoxycarbonyl)amino]-3-cyclopentylpropanoic acid (10g, 38.9mmol) in Dichloromethane (DCM) (175mL) were added cyclopropylamine (4.44g, 78mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (8.94g, 46.6mmol), and 1-hydroxy-7-azabenzotriazole (6.34g, 46.6mmol), The mixture was stirred at room temperature overnight then diluted with DCM and washed with saturated aqueous

NaHCO3, water and brine. The organic phase was decanted, and concentrated under reducing pressure to give an oil. This oil was dissolved in DMF(10 mL). To this DMF solution, water was added slowly while stirring. A white solid formed and was collected then washed with water several times. The solid was dried in a 65°C oven for 18 hours to afford a white solid which was directly used in the Boc deprotection step described below.

(S)-Amino-3-cyclopentyl-*N*-(2-cyclopropyl)propanamide bis-hydrochloride.

The (S)-tert-Butyl [3-cyclopentyl-1-(cyclopropylamino)-1-oxopropan-2-yl]carbamate obtained from the preceding step was dissolved in DCM (20mL) and 4M hydrochloric acid in dioxane (19.43mL, 78mmol) was added. The mixture was stirred at room temperature overnight. The solvent was then removed by distillation under reduced pressure to afford the titled compound as a white solid (7.56g, 26.7mmol, 68.6 % yield).

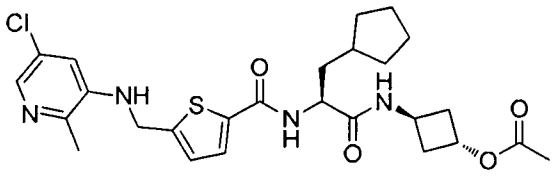
(S)-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(cyclopropylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide.

A round bottom flask was charged with 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxylic acid (4.86g, 17.19mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (4.28g, 22.34mmol) and 1-hydroxy-7-azabenzotriazole (3.04 g, 22.34 mmol) in *N*,*N*-Dimethylformamide (DMF) (54.5mL). To this mixture *N*-methylmorpholine (5.67mL, 51.6mmol) was added drop wise. The reaction mixture was continuously stirred at room temperature for 0.5 h. Another round bottom flask was charged with (S)-Amino-3-cyclopentyl-*N*-(2-cyclopropyl)propanamide, bis-hydrochloride (4.0g, 17.19mmol) in *N*,*N*-Dimethylformamide (DMF) (54.5mL) and *N*-methylmorpholine (5.67mL, 51.6 mmol). After complete dissolution, the contents of the second flask were slowly added to the first flask. The reaction mixture was stirred at room temperature overnight. The DMF solution was carefully poured into icy water (250 mL) while stirring and a white solid precipitated. The solid was collected by filtration and washed with water (50mL x3) then Hexanes (50mL x2). The off-white solid was heated in an oven 60°C under high vacuum to obtain a solid (7.13g, 14.69mmol), which was redissolved in EtOAc (20mL) and stirred at reflux-using an oil bath and a condenser for 10

min. The hot EtOAc solution was allowed to cool down slowly to room temperature. The recrystallized material was collected by filtration to afford the titled compound (6.13g, 13.03mmol, 76 % yield) as a white solid. LC-MS (ES) m/z = 461.5, 463.4 [M+H]+

Example 26

Preparation of *(1S,3r)*-3-[*(S)*-2-(5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxamido)-3-cyclopentylpropanamido]cyclobutyl acetate:



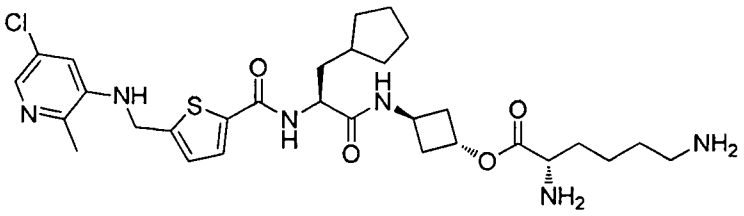
(1S,3r)-3-[(S)-2-(5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxamido)-3-cyclopentylpropanamido]cyclobutyl acetate.

A round bottom was charged with 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1r,3S)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide (600mg, 1.222mmol), triethylamine (341µL, 2.444mmol) and 4-(dimethylamino)pyridine (74.6mg, 0.611mmol) in Tetrahydrofuran (THF) (11.9mL). The reaction mixture was cooled down to 0°C using an icy water bath. To this mixture, acetic anhydride (187mg, 1.833mmol) was added via syringe. The reaction mixture was then allowed to warm up to room temperature. Disappearance of alcohol was followed by LCMS and more acetic acid had to be added for a total of 1.8eq. After 6h of additional stirring, the reaction was worked up by addition of water followed by extraction using EtOAc (20mL x3). The decanted organic phase was washed by a saturated aqueous solution of NaHCO3 then brine. The EtOAc solution was dried over Na_2SO4, then filtered and concentrated under low pressure.

The resulting crude solid was recrystallized from hot EtOAc to afford the final desired product (440mg, 0.809mmol, 66.2 % yield). LC-MS (ES) m/z = 533.3, 535.3 [M+H]+

Example 27

Preparation of *(S)-(1S,3S)*-3-[*(S)*-2-(5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxamido)-3-cyclopentylpropanamido]cyclobutyl 2,6-diaminohexanoate:

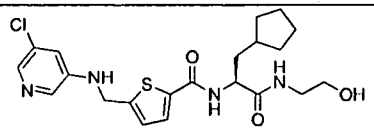
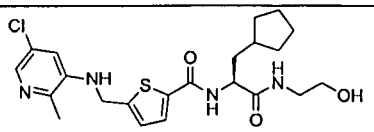


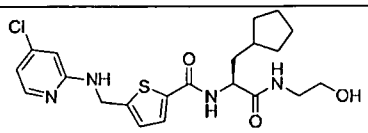
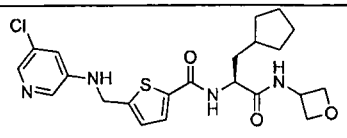
***(S)-(1S,3S)*-3-[*(S)*-2-(5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxamido)-3-cyclopentylpropanamido]cyclobutyl bis[(tert-butoxycarbonyl)amino]hexanoate.**

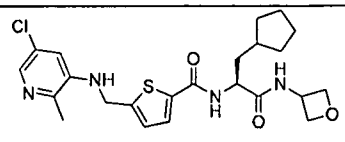
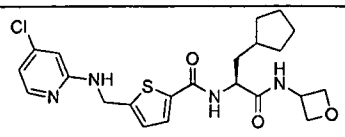
A solution of *(S)*-2,6-bis[(tert-butoxycarbonyl)amino]hexanoic acid (300mg, 0.866mmol), 4-(dimethylamino)pyridine (159mg, 1.299mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 332mg, 1.732mmol) in *N,N*-dimethylformamide (DMF, 8.7mL) was stirred at room temperature for 4h. To this reaction mixture 5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1r,3S)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide (425mg, 0.866mmol) in DMF (2.0mL) was added. After 30 h of stirring at room temperature 2.0 additional equivalents of EDC reagent were added. The reaction mixture was continuously stirred at room temperature for another 10 hours, then poured into water and the formed solid was collected by filtration. The solid was then dried by air and redissolved in EtOAc. The solution was washed by water and brine, decanted then dried over Na2SO4 and finally filtered and concentrated. The crude was finally purified by flash chromatography on silica gel column by elution with a mixture of 2 to 5% MeOH in DCM. The desired fractions were collected and concentrated to afford the titled compound (267mg, 0.310mmol, 35.7% yield). LC-MS (ES) m/z = 819.4, 821.4, 820.4 [M+H]+

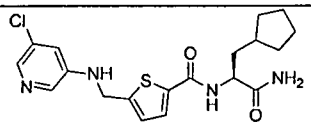
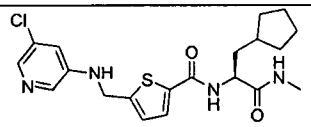
(S)-(1S,3S)-3-[(S)-2-(5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxamido)-3-cyclopentylpropanamido]cyclobutyl 2,6-diaminohexanoate, trihydrocholride.

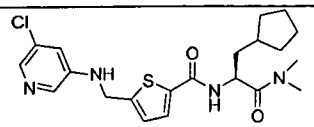
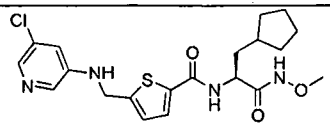
To a solution of *(S)-(1S,3S)*-3-[*(S)*-2-(5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxamido)-3-cyclopentylpropanamido]cyclobutyl 2,6-bis[(tert-butoxycarbonyl)amino]hexanoate (640mg, 0.781mmol) in Dichloromethane (10.0mL) was added 4M HCl in dioxane (3.91mL, 15.62mmol). The reaction mixture was stirred at room temperature for 5 hours. A white solid precipitated. The reaction solvent was removed by distillation under vacuum and residual HCl was driven off by co-evaporating twice with DCM (20mL). The solid was suspended a third time in DCM (10mL) and collected by filtration to afford the titled compound (450mg, 0.611mmol, 78% yield). LC-MS (ES) m/z = 619.3, 622.2 [M+H]+

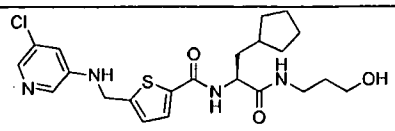
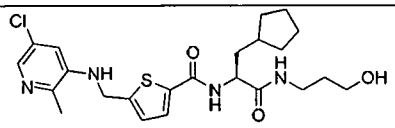
Example 1		1H NMR (400 MHz, DMSO-d6) δ ppm 8.40 (d, J=8.3 Hz, 1 H), 7.96 (s, 2 H), 7.77 (d, J=2.0 Hz, 2 H), 7.08 (d, J=3.8 Hz, 1 H), 7.03 (s, 1 H), 6.98 (s, 1 H), 4.63 (s, 1 H), 4.53 (d, J=6.1 Hz, 2 H), 4.37 (d, J=4.0 Hz, 1 H), 3.38 (d, J=5.8 Hz, 2 H), 3.12 (s, 2 H), 1.60 - 1.82 (m, 5 H), 1.55 (br. s., 2 H), 1.44 (d, J=3.8 Hz, 2 H), 1.10 (dd, 2 H)
Example 2		1H NMR (400 MHz, DMSO-d6) δ ppm 8.38 (d, J=8.3 Hz, 1 H), 7.94 (s, 1 H), 7.74 (d, J=3.8 Hz, 1 H), 7.67 (d, J=2.0 Hz, 1 H),

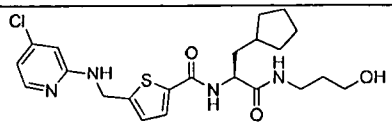
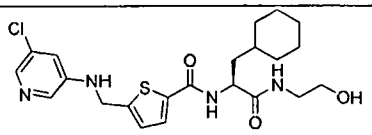
		7.08 (d, J=3.8 Hz, 1 H), 6.85 (d, J=2.0 Hz, 1 H), 6.43 (s, 1 H), 4.63 (t, J=5.4 Hz, 1 H), 4.57 (d, J=6.1 Hz, 2 H), 4.36 (s, 1 H), 3.37 (d, J=5.6 Hz, 2 H), 3.11 (d, J=6.3 Hz, 2 H), 2.33 (s, 3 H), 1.60 - 1.84 (m, 5 H), 1.49 - 1.59 (m, 2 H), 1.37 - 1.48 (m, 2 H), 1.11 (br. s., 2 H)
Example 3		1H NMR (400 MHz, DMSO-d6) δ ppm 8.37 (d, J=8.3 Hz, 1 H), 7.98 (d, J=5.6 Hz, 2 H), 7.72 (d, J=3.8 Hz, 1 H), 7.49 (s, 1 H), 7.00 (d, J=3.8 Hz, 1 H), 6.53 - 6.67 (m, 2 H), 4.65 (s, 3 H), 4.29 - 4.44 (m, 1 H), 3.38 (d, 2 H), 3.12 (s, 2 H), 1.59 - 1.88 (m, 5 H), 1.54 (dd, J=6.7, 3.7 Hz, 2 H), 1.36 - 1.50 (m, 2 H), 1.11 (br. s., 2 H)
Example 4		1H NMR (400MHz ,DMSO-d6) δ ppm 8.77 (d, J = 6.6 Hz, 1 H), 8.43 (d, J = 8.3 Hz, 1 H), 7.96 (d, J = 2.5 Hz, 1 H), 7.76 (dd, J =3.0, 4.5 Hz, 2 H), 7.08 (d, J = 3.8 Hz, 1 H), 7.03 (t, J = 2.3 Hz, 1 H), 6.98 (t, J = 6.1 Hz, 1 H),

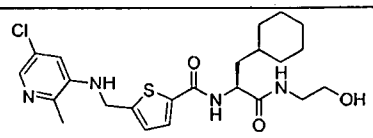
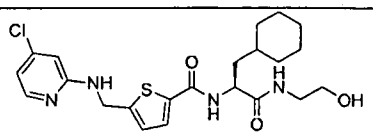
		4.81 - 4.72 (m, J = 6.6, 6.6, 13.5 Hz, 1 H), 4.71 - 4.65 (m, 2 H), 4.53 (d, J = 6.1 Hz, 2 H), 4.47 - 4.39 (m, 2 H), 4.39 - 4.32 (m, 1 H), 1.88 - 1.61 (m, 5 H), 1.56 (dt, J = 3.6, 6.9 Hz, 2 H), 1.45 (dd, J = 3.9, 7.2 Hz, 2 H), 1.22 - 1.03 (m, 2 H)
Example 5		1H NMR (400MHz ,DMSO-d6) δ ppm 8.77 (d, J = 5.8 Hz, 1 H), 8.42 (d, J = 7.8 Hz, 1 H), 7.75 (d, J = 2.8 Hz, 1 H), 7.67 (s, 1 H), 7.08 (br. s., 1 H), 6.85 (s, 1 H), 6.43 (t, J = 4.9 Hz, 1 H), 4.82 - 4.72 (m, 1 H), 4.72 - 4.64 (m, 2 H), 4.57 (d, J = 5.6 Hz, 2 H), 4.42 (q, J = 6.5 Hz, 2 H), 4.38 - 4.26 (m, 1 H), 2.33 (s, 3 H), 1.87 - 1.61 (m, 5 H), 1.56 (br. s., 2 H), 1.45 (br. s., 2 H), 1.12 (br. s., 2 H)
Example 6		1H NMR (400 MHz, DMSO-d6) δ ppm 8.76 (d, J = 6.3 Hz, 1H), 8.38 (d, J = 8.1 Hz, 1H), 7.97 (d, J = 5.6 Hz, 1H), 7.72 (d, J = 3.8 Hz, 1H), 7.48 (t, J = 6.2 Hz, 1H), 7.00 (d, J = 3.8 Hz, 1H), 6.61

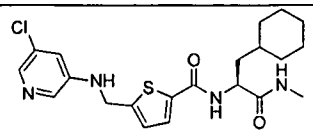
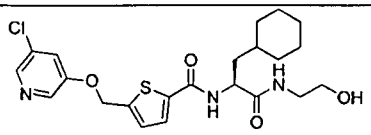
		(dd, J = 1.9, 5.4 Hz, 1H), 6.58 (d, J = 1.5 Hz, 1H), 4.71 - 4.79 (m, 1H), 4.66 - 4.71 (m, 2H), 4.64 (d, J = 6.1 Hz, 2H), 4.42 (dt, J = 6.2., 7.8 Hz, 2H), 4.37 (td, J = 3.1, 4.8. Hz, 1H), 1.73 (dd, J = 3.8, 9.4 Hz, 3H), 1.65 (dd, J = 5.1, 8.3 Hz, 2H), 1.50 - 1.60 (m, 2H), 1.39 - 1.49 (m, 2H), 1.14 (s, 2H)
Example 7		1H NMR (400 MHz, DMSO-d6) δ ppm 8.33 (d, J=8.3 Hz, 1 H), 7.96 (d, J=2.3 Hz, 1 H), 7.76 (dd, J=9.2, 2.9 Hz, 2 H), 7.39 (s, 1 H), 7.07 (d, J=3.8 Hz, 1 H), 7.03 (t, J=2.3 Hz, 1 H), 6.94 - 7.00 (m, 2 H), 4.53 (d, J=6.3 Hz, 2 H), 4.32 (d, J=3.8 Hz, 1 H), 1.41 - 1.84 (m, 9 H), 1.02 - 1.18 (m, 2 H)
Example 8		1H NMR (400 MHz, DMSO-d6) δ ppm 8.42 (d, J=8.34 Hz, 1 H) 7.96 (d, J=2.27 Hz, 1 H) 7.90 (q, J=4.63 Hz, 1 H) 7.77 (d, J=2.02 Hz, 1 H) 7.75 (d, J=3.79 Hz, 1 H) 7.08 (d, J=3.54 Hz, 1 H)

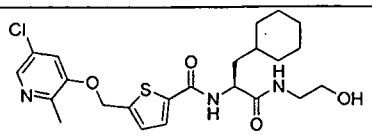
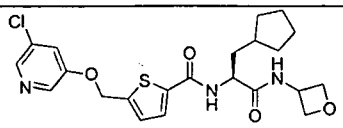
		7.03 (t, J=2.27 Hz, 1 H) 6.98 (t, J=6.19 Hz, 1 H) 4.53 (d, J=5.81 Hz, 2 H) 4.33 (td, J=8.84, 5.05 Hz, 1 H) 2.56 (d, J=4.80 Hz, 3 H) 1.61 - 1.85 (m, 5 H) 1.55 (dt, J=6.82, 3.41 Hz, 2 H) 1.37 - 1.50 (m, 2 H) 1.03 - 1.15 (m, 2 H)
Example 9		1H NMR (400 MHz, DMSO-d6) δ ppm 8.54 (d, J = 8.3 Hz, 1H), 7.96 (d, J = 2.5 Hz, 1H), 7.75 - 7.80 (m, 2H), 7.07 (d, J = 3.5 Hz, 1H), 7.03 (t, J = 2.3 Hz, 1H), 6.97 (t, J = 6.2 Hz, 1H), 4.78 - 4.87 (m, 1H), 4.53 (d, J = 6.1 Hz, 2H), 3.05 (s, 3H), 2.83 (s, 3H), 1.66 - 1.86 (m, 4H), 1.52 - 1.65 (m, 3H), 1.46 (td, J = 3.7, 7.52 Hz, 2H), 1.06 - 1.18 (m, 2H), 1.04 (d, J = 6.1 Hz, 1H)
Example 10		1H NMR (400 MHz, DMSO-d6) δ ppm 7.91 (d, J=2.3 Hz, 1 H), 7.78 (d, J=2.0 Hz, 1 H), 7.68 (d, J=3.8 Hz, 1 H), 7.00 - 7.15 (m, 2 H), 4.60 (s, 2 H), 4.34 - 4.49 (m, 1 H), 3.70 (s, 3 H), 1.73 - 1.98 (m, 5 H),

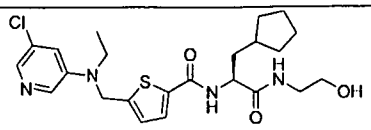
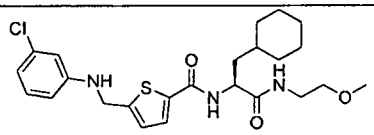
		1.62 - 1.73 (m, 2 H), 1.57 (dt, J=7.71, 4.0 Hz, 2 H), 1.07 - 1.28 (m, 2 H)
Example 11		1H NMR (400 MHz, DMSO-d6) δ ppm 8.39 (d, J=8.3 Hz, 1 H), 7.91 - 8.01 (m, 2 H), 7.76 (dd, J=4.4, 2.9 Hz, 2 H), 7.08 (d, J=3.8 Hz, 1 H), 7.03 (t, J=2.4 Hz, 1 H), 6.98 (t, J=6.1 Hz, 1 H), 4.53 (d, J=6.1 Hz, 2 H), 4.40 (t, J=5.3 Hz, 1 H), 4.33 (dq, J=8.2, 4.9 Hz, 1 H), 3.38 (q, J=6.3 Hz, 2 H), 3.03 - 3.15 (m, 2 H), 1.60 - 1.84 (m, 5 H), 1.49 - 1.59 (m, 4 H), 1.44 (tt, J=7.4, 3.4 Hz, 2 H), 1.04 - 1.17 (m, 2 H)
Example 12		1H NMR (400 MHz, DMSO-d6) δ ppm 8.37 (d, J=8.3 Hz, 1 H), 7.95 (t, J=5.8 Hz, 1 H), 7.75 (d, J=3.8 Hz, 1 H), 7.67 (d, J=2.0 Hz, 1 H), 7.07 (d, J=3.8 Hz, 1 H), 6.85 (d, J=2.0 Hz, 1 H), 6.43 (t, J=6.2 Hz, 1 H), 4.57 (d, J=6.1 Hz, 2 H), 4.40 (t, J=5.2 Hz, 1 H), 4.33 (dq, J=8.3, 5.0 Hz, 1 H), 3.38 (q, J=6.3 Hz, 2 H), 3.03 - 3.14

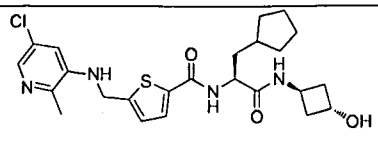
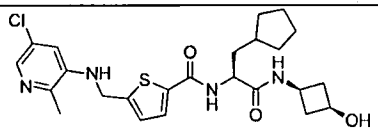
		(m, 2 H), 2.33 (s, 3 H), 1.60 - 1.84 (m, 5 H), 1.49 - 1.58 (m, 4 H), 1.44 (tt, J=7.4, 3.6 Hz, 2 H),1.04 - 1.17 (m, 2 H)
Example 13		1H NMR (400 MHz, DMSO-d6) δ ppm 8.35 (d, J=8.3 Hz, 1 H), 7.91 - 8.01 (m, 2 H), 7.72 (d, J=3.8 Hz, 1 H), 7.48 (t, J=6.1 Hz, 1 H), 7.00 (d, J=3.8 Hz, 1 H), 6.61 (dd, J=5.4, 1.9 Hz, 1 H), 6.59 (d, J=1.8 Hz, 1 H), 4.64 (d, J=6.1 Hz, 2 H), 4.40 (t, J=5.3 Hz, 1 H), 4.34 (dq, J=8.3, 5.0 Hz, 1 H), 3.38 (q, J=6.3 Hz, 2 H), 3.02 - 3.16 (m, 2 H), 1.61 - 1.84 (m, 5 H), 1.49 - 1.61 (m, 4 H), 1.37 - 1.48 (m, 2 H), 1.03 - 1.17 (m, 2 H)
Example 14		1H NMR (400 MHz, DMSO-d6) δ ppm 8.39 (d, J=8.6 Hz, 1 H) 7.96 (d, J=2.5 Hz, 1 H) 7.92 (t, J=5.7 Hz, 1 H) 7.76 (dd, J=7.2, 2.9 Hz, 2 H) 7.08 (d, J=3.8 Hz, 1 H) 7.03 (t, J=2.3 Hz, 1 H) 6.98 (t, J=6.2 Hz, 1 H) 4.63 (t, J=5.4 Hz, 1 H) 4.53 (d, J=6.1 Hz, 2 H) 4.36

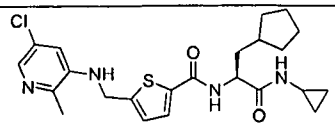
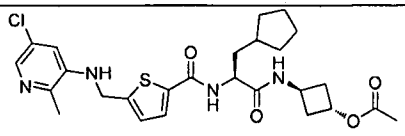
		- 4.48 (m, 1 H) 3.37 (q, J=6.1 Hz, 2 H) 3.11 (m, J=5.8 Hz, 2 H) 1.47 - 1.76 (m, 7 H) 1.29 (d, J=8.3 Hz, 1 H) 1.02 - 1.23 (m, 3 H) 0.77 - 0.98 (m, 2 H)
Example 15		1H NMR (400MHz ,DMSO-d6) δ ppm 8.37 (d, J = 8.3 Hz, 1 H), 7.91 (t, J = 5.6 Hz, 1 H), 7.74 (d, J = 3.8 Hz, 1 H), 7.67 (d, J = 2.0 Hz, 1 H), 7.08 (d, J = 3.8 Hz, 1 H), 6.86 (d, J = 2.0 Hz, 1 H), 6.43 (t, J = 6.1 Hz, 1 H), 4.62 (t, J = 5.4 Hz, 1 H), 4.57 (d, J = 6.1 Hz, 2 H), 4.47 - 4.30 (m, 1 H), 3.37 (q, J = 6.1 Hz, 2 H), 3.33 (s, 1 H), 3.19 - 3.02 (m, 2 H), 2.33 (s, 3 H), 1.75 - 1.47 (m, 6 H), 1.29 (br. s., 1 H), 1.22 - 1.01 (m, 3 H), 0.99 - 0.75 (m, 2 H)
Example 16		1H NMR (400 MHz, DMSO-d6) δ ppm 8.35 (d, J=8.34 Hz, 1 H) 7.98 (d, J=5.56 Hz, 1 H) 7.90 (t, J=5.68 Hz, 1 H) 7.72 (d, J=3.79 Hz, 1 H) 7.48 (t, J=6.19 Hz, 1 H) 7.00 (d, J=3.54 Hz, 1 H) 6.53 - 6.66

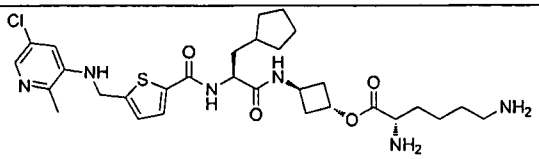
		(m, 2 H) 4.57 - 4.68 (m, 2 H) 4.30 - 4.50 (m, 1 H) 3.37 (q, J=6.15 Hz, 2 H) 3.01 - 3.17 (m, 2 H) 1.47 - 1.75 (m, 6 H) 1.27 (br. s., 1 H) 1.18 (br. s., 1 H) 1.10 (d, J=9.09 Hz, 2 H) 0.90 (d, J=12.13 Hz, 2 H)
Example 17		1H NMR (400MHz ,DMSO-d6) δ ppm 8.41 (d, J=8.3 Hz, 1 H), 7.96 (d, J=2.3 Hz, 1 H), 7.87 (d, J=4.8 Hz, 1 H), 7.76 (dd, J=6.2, 2.9 Hz, 2 H), 7.08 (d, J=3.8 Hz, 1 H), 7.03 (s, 1 H), 6.98 (s, 1 H), 4.53 (d, J=6.1 Hz, 2 H), 4.39 (td, J=5.0, 3.4 Hz, 1 H), 2.56 (d, J=4.5 Hz, 3 H), 1.51 - 1.73 (m, 7 H), 1.23 - 1.32 (m, 1 H), 1.11 (d, J=8.3 Hz, 3 H), 0.92 (br. s., 2 H)
Example 18		1H NMR (400MHz ,DMSO-d6) δ ppm 8.51 (d, J = 8.34 Hz, 1H), 8.29 - 8.36 (m, 1H), 8.25 (d, J = 1.77 Hz, 1H), 7.95 (t, J = 5.56 Hz, 1H), 7.82 (d, J = 3.79 Hz, 1H), 7.70 - 7.76 (m, 1H), 7.25 (d, J = 3.79 Hz, 1H), 5.45 (s, 2H), 4.64 (t, J = 5.43 Hz, 1H), 4.41 -

		4.51 (m, 1H), 3.36 - 3.42 (m, 2H), 3.05 - 3.20 (m, 2H), 1.52 - 1.75 (m, 7H), 1.23 - 1.36 (m, 1H), 1.04 - 1.22 (m, 3H), 0.79 - 0.98 (m, 2H)
Example 19		1H NMR (400MHz ,DMSO-d6) δ ppm 8.52 (d, J = 8.3 Hz, 1H), 8.09 (d, J = 2.0 Hz, 1H), 7.96 (t, J = 5.6 Hz, 1H), 7.82 (d, J = 3.8 Hz, 1H), 7.67 (d, J = 1.8 Hz, 1H), 7.23 (d, J = 3.8 Hz, 1H), 5.43 (s, 2H), 4.64 (br. s., 1H), 4.41 - 4.51 (m, 1H), 3.36 - 3.43 (m, 2H), 3.06 - 3.19 (m, 2H), 2.35 (s, 3H), 1.52 - 1.75 (m, 7H), 1.31 (d, J = 3.5 Hz, 1H), 1.04 - 1.22 (m, 3H), 0.79 - 0.99 (m, 2H)
Example 20		1H NMR (400MHz ,DMSO-d6) δ ppm 8.80 (d, J = 6.6 Hz, 1H), 8.55 (d, J = 8.1 Hz, 1H), 8.34 (d, J = 2.5 Hz, 1H), 8.25 (d, J = 1.8 Hz, 1H), 7.82 (d, J = 3.8 Hz, 1H), 7.72 - 7.77 (m, 1H), 7.25 (d, J = 3.8 Hz, 1H), 5.45 (s, 2H), 4.74 - 4.83 (m, 1H), 4.64 - 4.73 (m, 2H), 4.36 -

		4.46 (m, 3H), 1.64 - 1.83 (m, 5H), 1.53 - 1.61 (m, 2H), 1.41 - 1.49 (m, 2H), 1.15 (d, J = 11.87 Hz, 2H)
Example 21		1H NMR (400MHz ,DMSO-d6) δ ppm 8.41 (d, J=8.3 Hz, 1 H), 8.07 (d, J=2.5 Hz, 1 H), 7.94 (t, J=5.6 Hz, 1 H), 7.84 (d, J=1.8 Hz, 1 H), 7.76 (d, J=3.8 Hz, 1 H), 7.19 (t, J=2.3 Hz, 1 H), 7.05 (d, J=3.8 Hz, 1 H), 4.78 (s, 2 H), 4.72 - 4.53 (m, 1 H), 4.43 - 4.27 (m, 1 H), 3.51 (q, J=7.0 Hz, 2 H), 3.42 - 3.28 (m, 2 H), 3.21 - 3.01 (m, 2 H), 1.81 – 1.39 (m, 9 H), 1.21 - 0.97 (m, 5 H).
Example 22		1H NMR (400MHz ,DMSO-d6) δ ppm 8.37 (d, J = 8.3 Hz, 1H), 8.01 (t, J = 5.6 Hz, 1H), 7.74 (d, J = 3.8 Hz, 1H), 7.06 - 7.11 (m, 1H), 7.01 - 7.06 (m, 1H), 6.70 (t, J = 6.1 Hz, 1H), 6.62 (t, J = 2.2 Hz, 1H), 6.57 (t, J = 1.8 Hz, 1H), 6.55 (d, J = 1.8 Hz, 1H), 4.47 (d, J = 6.1 Hz, 2H), 4.38 - 4.45 (m, 1H), 3.33 (s, 3H), 3.29 - 3.33 (m,

		2H), 3.16 - 3.22 (m, 2H), 1.42 - 1.77 (m, 7H), 1.29 (br. s., 1H), 1.03 - 1.17 (m, 3H), 0.74 - 0.96 (m, 2H)
Example 23		1H NMR (400MHz ,DMSO-d6) δ ppm 8.34 (d, J = 8.3 Hz, 1H), 8.24 (d, J = 6.8 Hz, 1H), 7.75 (d, J = 3.8 Hz, 1H), 7.67 (d, J = 2.0 Hz, 1H), 7.07 (d, J = 3.8 Hz, 1H), 6.85 (d, J = 2.3 Hz, 1H), 6.42 (t, J = 6.1 Hz, 1H), 4.99 (d, J=5.6 Hz, 1H), 4.57 (d, J = 5.8 Hz, 2H), 4.29 - 4.41 (m, 1H), 4.28 - 4.20 (m, 1H), 4.16 (d, J=7.1 Hz, 1 H), 2.33 (s, 3H), 2.20 -1.98 (m, 4H), 1.81-1.43 (m, 9 H), 1.22 - 1.01 (m, 2H)
Example 24		1H NMR (400MHz ,DMSO-d6) δ ppm 8.33 (d, J = 8.3 Hz, 1H), 8.17 (d, J = 7.6 Hz, 1H), 7.74 (d, J = 3.8 Hz, 1H), 7.67 (d, J = 2.0 Hz, 1H), 7.07 (d, J = 3.8 Hz, 1H), 6.85 (d, J = 2.0 Hz, 1H), 6.42 (t, J = 6.1 Hz, 1H), 5.04 (d, J = 5.8 Hz, 1H), 4.57 (d, J = 5.8 Hz, 2H), 4.33 (td, J =

		9.1, 5.1 Hz, 1H), 3.72 - 3.85 (m, 1H), 3.52 - 3.71 (m, 1H), 2.40 - 2.49 (m, 2H), 2.33 (s, 3H), 1.64 - 1.79 (m, 6H), 1.52 - 1.63 (m, 3H), 1.36 - 1.50 (m, 2H), 1.01 - 1.20 (m, 2H)
Example 25		1H NMR (400MHz ,DMSO-d6) δ ppm 8.35 (d, J = 8.3 Hz, 1H), 8.06 (d, J = 4.3 Hz, 1H), 7.74 (d, J = 3.8 Hz, 1H), 7.67 (d, J = 2.0 Hz, 1H), 7.07 (d, J = 3.5 Hz, 1H), 6.85 (d, J = 2.0 Hz, 1H), 6.42 (t, J = 5.9 Hz, 1H), 4.57 (d, J = 5.8 Hz, 2H), 4.29 (td, J = 8.9, 5.4 Hz, 1H), 2.61 (td, J = 7.5, 3.8 Hz, 1H), 2.33 (s, 3H), 1.66 - 1.78 (m, 3H), 1.52 - 1.64 (m, 3H), 1.33 - 1.49 (m, 2H), 0.98 - 1.20 (m, 2H), 0.50 - 0.68 (m, 2H), 0.30 - 0.48 (m, 2H)
Example 26		1H NMR (400MHz ,DMSO-d6) δ ppm 8.40 (d, J = 7.1 Hz, 1H), 8.37 (d, J = 8.3 Hz, 1H), 7.75 (d, J = 3.8 Hz, 1H), 7.67 (d, J = 2.0 Hz, 1H), 7.07 (d, J = 3.8 Hz, 1H), 6.85 (d, J = 2.0 Hz, 1H),

		6.42 (t, J = 6.1 Hz, 1H), 5.02 (t, J = 5.9 Hz, 1H), 4.57 (d, J = 6.1 Hz, 2H), 4.17 - 4.42 (m, 2H), 2.19 - 2.37 (m, 6H), 2.00 (s, 3H), 1.51 - 1.81 (m, 6H), 1.44 (tt, J = 7.3, 3.7 Hz, 2H), 0.92 - 1.20 (m, 2H)
Example 27		1H NMR (400MHz ,DMSO-d6) δ ppm 7.83 (s, 1H), 7.66 - 7.77 (m, 1H), 7.05 - 7.16 (m, 2H), 5.64 (d, J = 1.5 Hz, 1H), 5.10 (br. s., 1H), 4.61 (s, 2H), 4.29 (br. s., 2H), 3.95 (s, 1H), 2.75 (t, J = 7.5 Hz, 2H), 2.38 (s, 7H), 1.72 (s, 3H), 1.75 (s, 2H), 1.55 (d, J=7.6 Hz, 6H), 1.40 (br. s., 4H), 1.08 (br. s., 2H)

BIOCHEMICAL ASSAY FOR LSD-1 ACTIVITY

Wip1 Dephosphorylation Assay with Fluorescein Diphosphate (FDP) Substrate

Recombinant Wip1 protein (Wip1(A2-K420)) was expressed in Sf9 cells, lysed in 0.5% CHAPS, purified over 1.) Nickel Column, 2.) Superdex 200 pool3, then 3.) MonoQ p2 , and finally stored at -20°C in storage buffer (50mM Tris-HCl (pH 8.0), 20% Glycerol, 0.2M NaCl,1mM TCEP, 1mM CHAPS, 0.25mM Imidazole). Assay reaction was carried out in reaction buffer containing 50mM Tris pH7.5, 30mM $MgCl_2$, 0.05mg/mL BSA, 0.05% CHAPS ,and 1mM DTT. Fluorescein diphosphate (FDP, tetraammonium salt;

Invitrogen) solution was prepared as 10mM stock made in 50mM Tris (pH7.5), and stored at -20°C.

For the assay, compounds were serially-diluted from a maximum final concentration of 5μM, 3-fold drops for 11points to a minimum final concentration of 0.085nM, and 0.1μL of compounds in DMSO were added to Non-binding 384 well plate (Corning). Subsequently, buffer and Wip1 enzyme (1nM final) were added for a 15min preincubation. Reaction was then started with the addition of FDP substrate (50μM final) in buffer. The reaction was allowed to proceed for2 hours at room temperature. Fluorescence was read using a Viewlux Microplate Imager (Perkin Elmer) Fluorescein 480/540 protocol. Readout in Relative Fluorescent units (RFUs) were compared with vehicle (1% DMSO) treated control wells. Curves were analyzed using ActivityBase and XLfit, and XC50 results expressed as pIC50 values.

Wip1 Dephosphorylation Assay with phospho-P38 Substrate

Recombinant full-length Wip1 protein (FL-Wip1) was expressed in Sf9 cells, lysed in 0.5% CHAPS, purified over 1.) NiNTA column, then 2.)Superdex 200 pool4, and finally stored at -20°C in storage buffer (50mM Tris-HCl (pH 8.0), 20% Glycerol, 0.15M NaCl, 0.1M EGTA, 1mM TCEP). Recombinant GST-tagged full-length p38 was expressed in Sf9 cells, lysed, purified over 1.) NiNTA column, 2.)Superdex 200 pool 3. Recovered protein was then treated with recombinant MKK6 (U. Dundee; DU1671) at 1/50 molar ratio to achieve phosphorylation of p38 (T180/Y182). Following reaction with MKK6, p38 underwent a buffer exchange and repurification over a Nickel column, and was then stored at -80°C in storage buffer (50mM Tris-HCl (pH 8.0), 0.15M NaCl, 0.1mM EGTA, 1mM TCEP).

For the assay, compounds were serially-diluted from a maximum final concentration of 50μM, 3-fold drops for 11points to a minimum final concentration of 0.85nM, and 0.1μL of compounds in DMSO (or neat DMSO for vehicle control) were added to Non-binding 384 well plate (Corning). A final concentration of 15nM FL-Wip1 was added to the assay plate in assay buffer (50mM Tri (pH 7.5), 30mM $MgCl_2$, 0.1mM EGTA, 0.1mg/mL BSA, 1mM CHAPS, 1mM DTT) for a 15min preincubation. Reaction was then started with the addition of 8nM phosphorylated GST-p38 (T180/Y182). The reaction was allowed to proceed for 90 minutes at room temperature. A mixture of detection reagents were added containing final concentrations of 50 mM EDTA, 1:300 phospho-p38 MAPK (Thr180/

Tyr182) rabbit mAB (D3F9), 5 ug/mL anti-GST-IgG conjugated to SureLight Allophycocyanin, and 1nM LANCE E-W1024 labelled anti rabbit IgG. The reaction was covered and incubated for 60 minutes at room temperature. Finally, fluorescence was read using a Viewlux Microplate Imager (Perkin Elmer) or alternatively an Envision MultiLabel Reader (Perkin Elmer). Readout in Relative Fluorescent units (RFUs) were compared with vehicle (1% DMSO) treated control wells. Curves were analyzed using ActivityBase and XLfit, and XC50 results expressed as pIC50 values.

Cellular Assay for p53-Transcriptional Activation

A p53-Luciferase reporter BacMam construct was made that contains a p53 response element ((CCTGGACTTGCCTGGCCTG)15) upstream of a minimal promoter derived from HSV TK and the firefly Luciferase open reading frame. The construct was cloned into a BacMam expression vector, BacMam virus was cultured from DH10bac cells, and titer was determined. MX-1 breast carcinoma cell line was obtained from the NCI and cultured as recommended in RPMI+10% fetal bovine serum (FBS).

For the assay, MX-1 cells were trypsinized and viable cells were counted and diluted in OptiMEM (Invitrogen)+ 5% FBS to achieve 10,000 cells per 48uL (2.1×10^5 cells/mL). For transduction of the p53-luciferase reporter, BacMam was added to diluted cells such that the Multiplicity of Tranduction (MOT) is 5 pfu/cell. Transduced MX-1 cells were then seeded 48uL/well into 384 well black polystyrene cell culture microplates (Greiner) and placed in tissue culture incubator at 37°C and 5% CO_2 for 24 hours. The following day, compounds were serially-diluted by 3-fold drops for 10 point curves. Initial compound serial dilutions in DMSO were then diluted 20-fold further in cell media, and 2.0µL of compound dilutions were added to cells (0.2% DMSO final). The dilution curve spanned from a maximum final concentration of 20µM to a minimum final concentration of 1.0nM. Microplates were then returned to tissue culture incubator at 37°C and 5%CO_2. After an additional 24 hours incubation, plates were removed from the incubator and allowed to sit on bench-top for 15 minutes at room temperature. At this point, 25µls of resuspended Bright Glo (Promega) solution was added to all wells, and plates were shaken for 2 minutes at room temperature, and luminescence was read on the EnVision 2100 plate reader.). Readout in Relative luminescence units (RLUs) were compared with vehicle

(0.2% DMSO) treated control wells. Curves were analyzed using XLfit. Results were expressed as several parameters including: 1.) maximum-fold induction, 2.) concentration of 2-fold induction, 3.) concentration of 3-fold induction, and 4.) XC50.

Wip1 Cellular Outgrowth Assay

Cell lines used for the cellular outgrowth assay included MX-1 breast carcinoma line (NCI), A549 lung carcinoma (ATCC), Molt3 T-Cell leukemia (ATCC), and HN5 head and neck carcinoma cell line (ICR-UK). All cell lines were cultured according to recommended conditions. Cell lines were split in duplicate T-75 flasks two to three days prior to assay set-up in ratios which yielded70-80% confluence at time of harvest for plate seeding. Cells were trypsinized, viable cell concentrations were determined using a using ViCell XR (Beckman Coulter), and cells were resuspended to yield 2,000 cells per ml. Into 96 well black polystyrene cell culture microplates (Nalgene) 96 µls cell suspension was added and seeded cells were allowed to adhere overnight in 37°C + 5% CO2 incubator.

Compounds were diluted in U-bottom 384-well plate over 10 points in DMSO. Compound dilutions in DMSO or neat DMSO (for vehicle control) were then diluted 20-fold with media (RPMI-1640 + 10% FBS) in an intermediate compound plate and mixed thoroughly. Titrations were 3-fold for a 10 point series with a maximum final concentration of 30µM and a minimum final concentration of 1.5nM. From the intermediate plate, 4 µls were transferred to each well of the 48 µl/well of cells (total volume on cells, 50 µls; 0.2% DMSO). Microplates were then returned to tissue culture incubator at 37°C and 5%CO_2 for an additional 7 day incubation. For reference, on the day of compound addition (Time-0), one additional plate for each cell type is developed with the addition of 50µls CellTiter-Glo (Promega), plates are shaken gently for 2 minutes, allowed to react for 20 minutes, and luminescence is read on a Tecan Safire (Tecan). This T=0 value indicates the signal of the starting number of cells. At day 7, plates are harvested by the addition of 50µls Cell Titer-Glo (Promega) to all wells as described above. Readout in Relative luminescence units (RLUs) were compared with vehicle (0.2% DMSO) treated control wells. Curves were analysed using XLfit and reported as IC50s.

Biological Data

Exemplified compounds of the present invention were tested according to the above assays and were found to be inhibitors of WIP1. The pIC_{50} values ranged from about 5.3-8.5. The IC_{50} values of the present compounds range from about 3 to 5000 nM. The IC_{50} values of the more active compounds range from about 3 to 100 nM. The most active compounds are under 10 nM.

The pIC50 of compounds of Examples 1-3 and 6-9 ranges from 7.5 to 8.0.

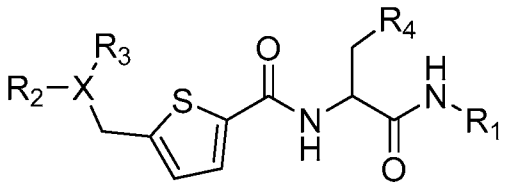
The pIC50 of compounds of Examples 14, 17, 4 ranges from 8.0-8.5.

Exemplified enzymatic activity (Wip1 Dephosphorylation assay with Fluorescein Diphosphate (FDP) Substrate)

Example No.	pIC50
Example 5	8.2
Example 10	7.7
Example 15	8.4
Example 21	8.1
Example 22	8.0
Example 25	8.2

What is claimed is:

1. A compound of Formula (I);



(I)

wherein

R_1 is hydrogen, C_1-C_4alkoxy, C_1-C_4alkyl, substituted C_1-C_4alkyl, C_3-C_7cycloalkyl, or C_3-C_7heterocycloalkyl, wherein said C_3-C_7cycloalkyl may be substituted with one to three substituents selected from the group consisting of hydroxyl and -O(O)Ra, wherein Ra is C_1-C_6alkyl or substituted C_1-C_6alkyl;

R_2 is an aryl or heteroaryl ring, which may be substituted with one to four substituents selected from the group consisting of halo, C_1-C_3alkyl, substituted C_1-C_3alkyl, C_1-C_4alkoxy, hydroxyl, amino, substituted amino, C_3-C_7cycloalkyl, cyano, ester, carboxylic acid, and C_3-C_6heterocycloalkyl;

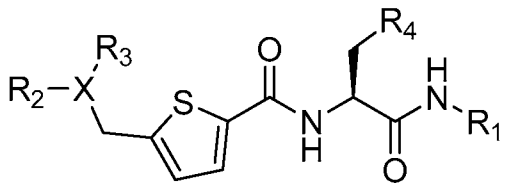
R_3 is hydrogen, C_1-C_6alkyl, substituted C_1-C_6alkyl, or R_3 is absent when X is O or S;

R_4 is cyclohexyl or cyclopentyl;

X is N, O or S;

or a pharmaceutically acceptable salt or prodrug thereof.

2. A compound of claim 1, which is represented by Formula (II):



(II);

wherein

R_1 is hydrogen, C_1-C_4alkoxy, C_1-C_4alkyl, substituted C_1-C_4alkyl, C_3-C_7cycloalkyl, or C_3-C_7heterocycloalkyl, wherein said C_3-C_7cycloalkyl may be substituted with one to three substituents selected from the group consisting of hydroxyl and -O(O)Ra, wherein Ra is C_1-C_6alkyl or substituted C_1-C_6alkyl;

R_2 is an aryl or heteroaryl ring, which may be substituted with one to four substituents selected from the group consisting of halo, C_1-C_3alkyl, substituted C_1-C_3alkyl, C_1-C_4alkoxy, hydroxyl, amino, substituted amino, C_3-C_7cycloalkyl, cyano, ester, carboxylic acid, and C_3-C_6heterocycloalkyl;

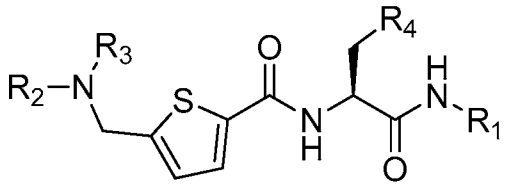
R_3 is hydrogen, C_1-C_6alkyl, substituted C_1-C_6alkyl, or R_3 is absent when X is O or S;

R_4 is cyclohexyl or cyclopentyl;

X is N, O or S;

or a pharmaceutically acceptable salt thereof.

3. A compound of claim 1, which is represented by Formula (III):



(III);

wherein

R_1 is hydrogen, C_1-C_4alkoxy, C_1-C_4alkyl, substituted C_1-C_4alkyl, C_3-C_7cycloalkyl, or C_3-C_7heterocycloalkyl, wherein said C_3-C_7cycloalkyl may be substituted with one to three substituents selected from the group consisting of hydroxyl and -O(O)Ra, wherein Ra is C_1-C_6alkyl or substituted C_1-C_6alkyl;

R_2 is an aryl or heteroaryl ring, which may be substituted with one to four substituents selected from the group consisting of halo, C_1-C_3alkyl, substituted C_1-C_3alkyl, C_1-

C_4alkoxy, hydroxyl, amino, substituted amino, C_3-C_7cycloalkyl, cyano, ester, carboxylic acid, and C_3-C_6heterocycloalkyl;

R_3 is hydrogen, C_1-C_6alkyl or substituted C_1-C_6alky;

R_4 is cyclohexyl or cyclopentyl;

or a pharmaceutically acceptable salt thereof.

4. A compound according to any one of claims 1-3, wherein R_1 is C_1-C_4alkyl or substituted C_1-C_4alkyl.

5. A compound according to any one of claims 1-3, wherein R_1 is C_3-C_7cycloalkyl.

6. A compound according to any one of claims 1-3, wherein R_1 is cyclopropyl or cyclobutyl, wherein said cyclopropyl and cyclobutyl may be substituted with hydroxyl or –O(O)Ra, wherein Ra is C_1-C_6alkyl or substituted C_1-C_6alkyl.

7. A compound according to any one of claims 1-6, wherein R_2 is phenyl, which may be substituted with one to four substituents selected from the group consisting of halogen, C_1-C_3alkyl, substituted C_1-C_3alkyl, C_1-C_4alkoxy, hydroxyl, amino, substituted amino, C_3-C_7cycloalkyl, C_3-C_7heterocycloalkyl, cyano, ester, and carboxylic acid.

8. A compound according to any one of claims 1-6, wherein R_2 is pyridinyl, which may be substituted with one to three substituents selected from the group consisting of halo, C_1-C_3alkyl, and substituted C_1-C_3alkyl.

9. A compound according to any one of claims 1-8, wherein R_3 is hydrogen.

10. A compound according to any one of the above claims, wherein R_4 is cyclopentyl.

11. A compound according to claim 1, which is selected from the group consisting of:

(S)-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(4-chloropyridin-2-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloro-pyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(4-chloro-pyridin-2-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-*N*-(1-amino-3-cyclopentyl-1-oxopropan-2-yl)-5-{[(5-chloropyridin-3-yl)amino]methyl}thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(methylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(dimethylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(methoxyamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(4-chloropyridin-2-yl)amino]methyl}-*N*-{3-cyclopentyl-1-[(3-hydroxypropyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(4-chloropyridin-2-yl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(4-chloropyridin-2-yl)amino]methyl}-*N*-[3-cyclohexyl-1-(methylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)oxy]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloro-2-methylpyridin-3-oxy]methyl}-*N*-{3-cyclohexyl-1-[(2-hydroxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)oxy]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(5-chloropyridin-3-yl)oxy]methyl}-*N*-[3-cyclopentyl-1-(oxetan-3-ylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(S)-5-{[(3-chlorophenyl)amino]methyl}-*N*-{3-cyclohexyl-1-[(2-methoxyethyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1r,3S)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-{*(S)*-3-cyclopentyl-1-[(*(1s,3R)*-3-hydroxycyclobutyl)amino]-1-oxopropan-2-yl}thiophene-2-carboxamide;

(S)-5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}-*N*-[3-cyclopentyl-1-(cyclopropylamino)-1-oxopropan-2-yl]thiophene-2-carboxamide;

(1S,3r)-3-[*(S)*-2-(5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxamido)-3-cyclopentylpropanamido]cyclobutyl acetate; and

(S)-(1S,3S)-3-[*(S)*-2-(5-{[(5-chloro-2-methylpyridin-3-yl)amino]methyl}thiophene-2-carboxamido)-3-cyclopentylpropanamido]cyclobutyl 2,6-diaminohexanoate;

or a pharmaceutically acceptable salt thereof.

12. A pharmaceutical composition comprising a compound according to any one of the above claims or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

13. A method of treating cancer which comprises administering to a human in need thereof an effective amount of a compound as described in any one of claims 1-11.

14. A method of claim 13 wherein the cancer is selected from the group consisting of brain, glioblastomas, leukemias, lymphomas, Bannayan-Zonana syndrome, Cowden disease, Lhermitte-Duclos disease, breast, inflammatory breast cancer, Wilm's tumor, Ewing's sarcoma, Rhabdomyosarcoma, ependymoma, medulloblastoma, neuroblastoma, small cell lung, endometrium, cervix, esophagus, colon, gastric, bladder, head and neck, kidney, lung, liver, melanoma, ovarian, pancreatic, prostate, mesothelioma, sarcoma, osteosarcoma, giant cell tumor of bone and thyroid.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/35114

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 43/42; A61K 31/44; C07D 513/02 (2012.01)
USPC - 514/301, 546/114
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A01N 43/42; A61K 31/44; C07D 513/02 (2012.01)
USPC: 514/301, 546/114

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8): A01N 43/42; A61K 31/44; C07D 513/02 (2012.01)
USPC: 514/301, 546/114

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST; PGPB, USPT, EPAB, JPAB, SureChem, PubChem, Dialog
Wild-type P53 inducible Phosphatase 1 (Wip1),
(S)-5-{[(5-chloropyridin-3-yl)amino] methyl}-N-{3-cyclopentyl-1- (1-oxetan-3ylamino)]-1-oxopropan-2-yl] thiophene-2-carboxamide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2005/0277628 A1 (PFAU et al.) 15 December 2005 (15.12.2005) para [0001]-[0003], [0018]-[0022]	1-6 and 11
Y	US 2008/0167346 A1 (WAGNER et al.) 10 July 2008 (10.07.2008) para [0005], [0007], [0009], [0011], [0016], [0020], [0023], [0025], [0077]-[0078], [0082], [0088]	1-6 and 11
A	US 2004/0254221 A1 (YAMAZAKI et al.) 16 December 2004 (16.12.2004) para[0009]-para[0022], para[0033]-para[0038], para[0040], para[0057], para[0063] page 78, compound #102, page 86, compound # 132	1-6 and 11
A	US 2004/0167189 A1 (BULAVIN et al.) 26 August 2004 (26.08.2004) Abstract	1-6 and 11
A	US 2010/0256098 A1 (APPELLA et al.) 7 October 2010 (07.10.2010) Abstract	1-6 and 11

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 July 2012 (09.07.2012)	23 JUL 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/35114

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-10 and 12-14
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.