IAP BIR DOMAIN BINDING COMPOUNDS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Applications 61/303,809 filed February 12, 2010 and 61/415,638 filed November 19, 2010.

BACKGROUND OF THE INVENTION

[0002] Apoptosis, or programmed cell death, typically occurs in the normal development and maintenance of healthy tissues in multicellular organisms. It is a complex process which results in the removal of damaged, diseased or developmentally redundant cells, in the absence of signs of inflammation or necrosis.

[0003] Intrinsic apoptotic pathways are known to be dysregulated in a variety of disorders, including cancer and lymphoproliferative disorders, neurodegenerative diseases, and autoimmune and inflammatory conditions such as multiple sclerosis and rheumatoid arthritis. Cancer cells, for instance, gain the ability to overcome or circumvent apoptosis and continue with inappropriate proliferation despite strong pro-apoptotic signals such as hypoxia, endogenous cytokines, radiation treatments and chemotherapy. Abnormally apoptotic resistant cells also have been linked to autoimmune and inflammatory disease. For instance, apoptosic-resistance has been observed in fibroblast-like synoviocytes in connection with rheumatoid arthritis (RA), and in keratinocytes in connection with psoriasis. Abnormally apoptotic resistant T-cells also have been observed in several autoimmune or inflammatory diseases such as multiple sclerosis, rheumatoid arthritis, idiopathic thrombocytopenic purpura, and alopecia areata. Pathogenic effector cells also have demonstrated resistance to normal apoptotic cues. It is believed that resistance to normal apoptosis is caused, at least in part, by increased activity of anti-apoptotic pathways or expression of anti-apoptotic genes.

[0004] The caspases are an integral part of the apoptotic pathway. The caspases are a family of proteolytic enzymes from the class of cysteine proteases, which are known to initiate and execute apoptosis. In normal cells, the caspases are present as inactive zymogens, but are catalytically activated by any of several external signals. Caspase-activating signals include, for example, the release of cytokines or immunological agents following ligand-driven Death Receptor activation, or the release of mitochondrial factors,

such as cytochrome C, following genotoxic, chemotoxic, or radiation-induced cellular injury.

[0005] The Inhibitors of Apoptosis Proteins (IAPs) constitute a family of proteins that inhibit the caspases, thereby suppressing cellular apoptosis. Because of their central role in regulating caspase activity, the IAPs are capable of inhibiting programmed cell death from a wide variety of triggers. The IAPs are believed to play a role in the loss of homeostatic or endogenous cellular growth control mechanisms, as well as resistance chemotherapeutic drugs and radiation therapy.

[0006] The IAPs contain one to three homologous structural domains known as baculovirus IAP repeat (BIR) domains. They may also contain a RING zinc finger domain at the C-terminus, with a capability of inducing ubiquitinylation of IAP-binding molecules via its E3 ligase function. The human IAPs known as XIAP, HIAP1 (also referred to as cIAP2), and HIAP2 (cIAP1) each have three BIR domains, and a carboxy terminal RING zinc finger. Another IAP known as NAIP, has three BIR domains (BIR1, BIR2 and BIR3), but no RING domain. Still other IAPs known as Livin, TsIAP and MLIAP have only a single BIR domain and a single RING domain.

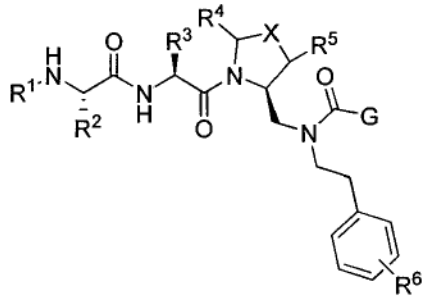
[0007] The X chromosome-linked inhibitor of apoptosis (XIAP) is an example of an IAP which can inhibit, by direct binding, the initiator caspase, known as caspase-9, and the effector caspases, known as Caspase-3 and Caspase-7. It is via the BIR3 domain that XIAP binds to and inhibits caspase-9. The linker-BIR2 domain of XIAP inhibits the activity of caspases-3 and -7. The BIR domains have also been associated with the interactions of IAPs with tumor necrosis factor-receptor associated factor (TRAFs)-1 and -2, and to TAB1, as adaptor proteins effecting survival signaling through NFkB activation. XIAP also can induce the removal of caspases by way of the E3 ligase activity of the RING zinc finger domain, which induces ubiquitylation-mediated proteasomal degradation.

[0008] The IAPs thus function as a direct brake on the apoptosis cascade by inhibiting active caspases and re-directing cellular signaling to a pro-survival mode. The sustained over-expression of one or more members of the IAP family of proteins, therefore, allows diseased cells, such as cancer cells and cells involved in autoimmune disease, to avoid apoptosis. In fact, IAP overexpression has been demonstrated to be prognostic of poor

clinical outcome in multiple cancers. Furthermore, suppressing IAP expression through RNA antisense or siRNA strategies sensitizes tumor cells to a wide variety of apoptotic insults including chemotherapy, radiotherapy, and ligand-mediated activation of the death receptors. In the case of XIAP, this has been shown in cancers as diverse as leukemia and ovarian cancer. Over expression of cIAP1 and cIAP2 also has been observed in a diverse variety of malignancies, including medulloblastomas, renal cell carcinomas, glioblastomas, and gastric carcinomas. For these reasons, the IAPs are valid therapeutic targets and compounds that inhibit their expression or function are believed to have significant utility in the treatment of proliferative diseases associated with dysregulated apoptosis, including cancer, autoimmune, and inflammatory diseases.

SUMMARY OF THE INVENTION

[0009] Provided herein is a compound of Formula 1



or a salt thereof, wherein

R^1 is H or alkyl;

R^2 is methyl or ethyl;

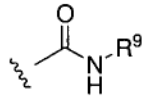
R^3 is alkyl, cycloalkyl, heterocyclyl, heteroaryl, or aryl, any of which can be optionally further substituted with an amino, alkylamino, or alkoxy;

R^4 and R^5 are each, independently, H or alkyl;

R^6 is H, halogen, or alkoxy;

X is O, S, CH_2, $-(CH_2)_2-$ or CH-R^7, wherein R^7 is NR^8, OR^8, $NC(O)OR^8$, $NHC(O)R^8$ or $NHSO_2R^8$, wherein R^8 is alkyl, cycloalkyl, heterocyclyl, aryl, arylalkyl, or heteroaryl, any of which can be optionally further substituted with an alkyl or halogen;

and G is

(1)  , wherein R^9 is substituted or unsubstituted alkyl, cycloalkyl, heterocyclyl, aryl or heteroaryl; or

(2) a substituted or unsubstituted azole or pyrrole ring, optionally fused to a substituted or unsubstituted aryl, heteroaryl, cycloalkyl or heterocyclyl. Also provided herein are methods for preparing a compound of Formula 1 or salt thereof, as well as compounds useful as intermediates in the preparation of a compound of Formula 1 or salt thereof.

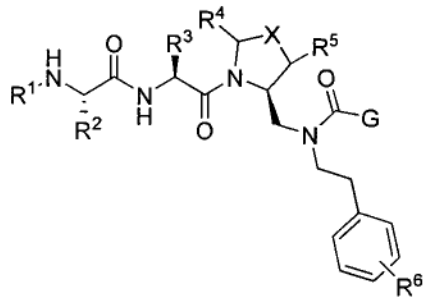
[0010] In another aspect, the invention provides a pharmaceutical composition comprising a compound of Formula 1 or salt thereof and a pharmaceutically acceptable carrier, as well as a method of preparing same comprising combining a compound of Formula 1 or salt thereof with a pharmaceutically acceptable carrier.

[0011] The invention further provides a method of enhancing apoptosis in a cell, the method comprising contacting a cell with a compound of of Formula 1 or salt thereof. A method of treating a disease or disorder characterized by insufficient apoptosis also is provided herein, the method comprising administering to a subject in need thereof a compound or pharmaceutical composition, as described above, so as to treat the disease or disorder.

[0012] Also provided herein is a probe comprising a compound of Formula 1 or salt thereof and a detectable label, as well as a method of using the probe to identify a compound that binds to an IAP BIR domain, the method comprising: (a) contacting an IAP BIR domain with the probe to form a probe:BIR domain complex, the probe being displaceable by a test compound; (b) measuring a signal from the probe so as to establish a reference level; (c) contacting the probe:BIR domain complex with a test compound; (d) measuring the signal from the probe; and (e) comparing the signal from step (d) with the reference level, wherein a modulation of the signal (e.g., an increase or decrease in the signal relative to the reference level) indicates that the test compound binds to the BIR domain.

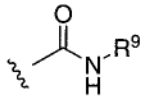
DETAILED DESCRIPTION OF THE INVENTION

[0013] Provided herein is a compound of Formula 1:



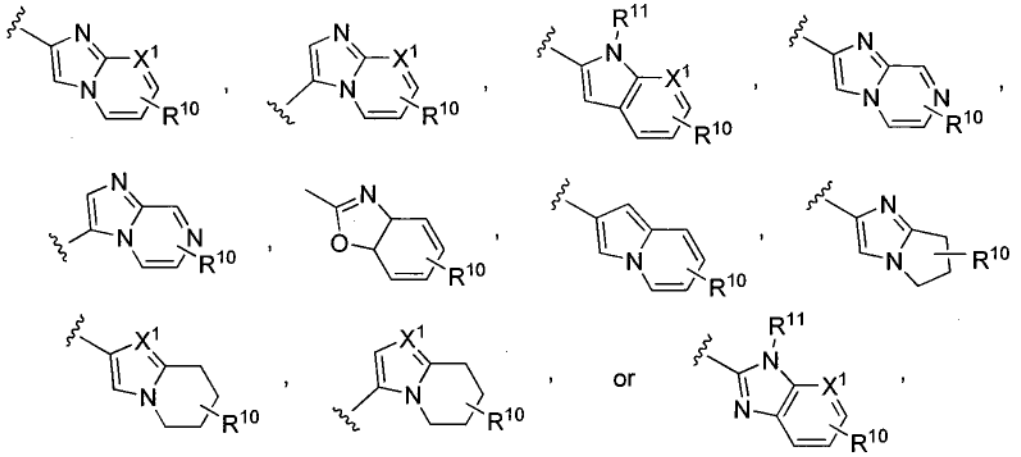
or a salt thereof. The invention encompasses all compounds described by Formula 1 and salts thereof without limitation. However, for the purposes of further illustration, preferred aspects and elements of the invention are discussed herein.

[0014] In accorandance with Formula 1, G can be a group with the structure

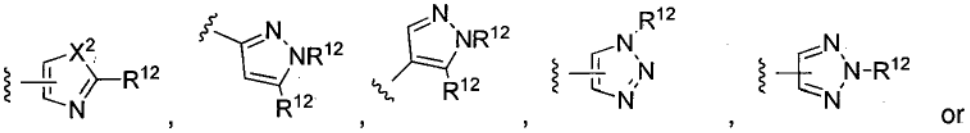


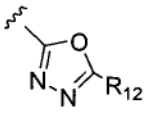
wherein R^9 is substituted or unsubstituted alkyl, cycloalkyl, heterocyclyl, aryl or heteroaryl. For example, R^9 can be a phenyl group optionally substituted with a halogen or alkoxy.

[0015] Alternatively, G can be a substituted or unsubstituted azole or pyrrole ring, optionally fused to a substituted or unsubstituted aryl, heteroaryl, cycloalkyl, or heterocyclic ring. For instance, G can be



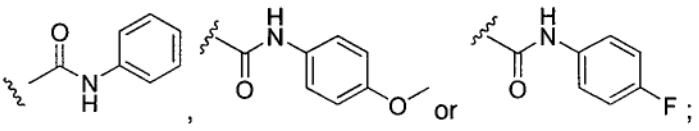
wherein X^1 is CH or N, R^{10} is H, halogen, hydroxyl, alkyl, alkoxy, aryl, amino, or NHC(O)-alkyl, and R^{11} is hydrogen, alkyl, or $NHC(O)CH_3$. G also can be



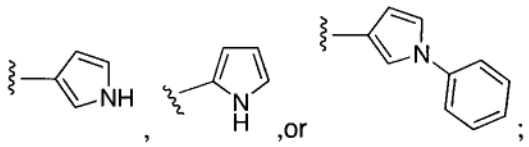


wherein X^2 is NH, NR^{12}, O, or S, and each R^{12} is independently hydrogen, alkyl, cycloalkyl, heterocyclyl, $NHC(O)CH_3$, or phenyl optionally substituted with one or more alkyl, alkoxy, or halogen groups.

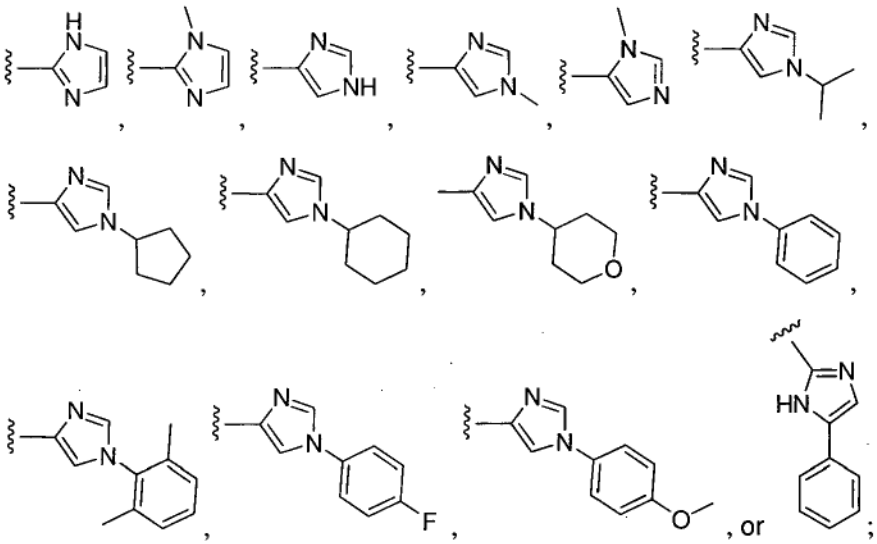
[0016] According to one embodiment G is:



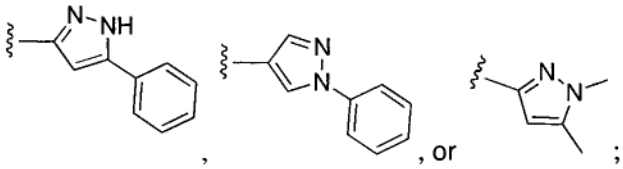
or a substituted or unsubstituted pyrrole, specific examples of which include, without limitation:



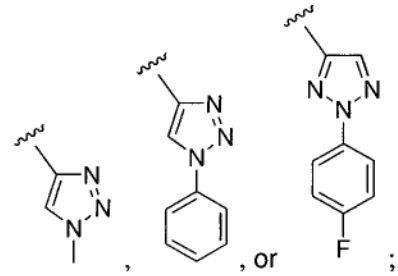
or a substituted or unsubstituted imidazole, specific examples of which include, without limitation:



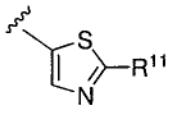
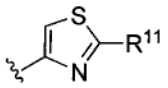
or a substituted or unsubstituted pyrazole, specific examples of which include, without limitation:



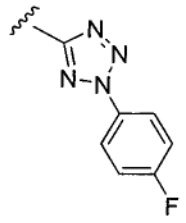
or a substituted or unsubstituted triazole, specific examples of which include, without limitation:



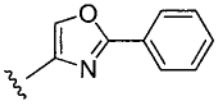
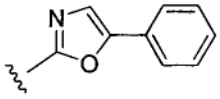
or a substituted or unsubstituted thiazole, specific examples of which include, without limitation:

 or  , wherein R^{11} is $NHC(O)CH_3$ or phenyl;

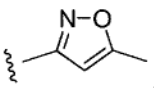
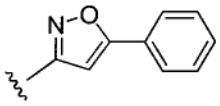
or a substituted or unsubstituted tetrazole, specific examples of which include, without limitation:

 ;

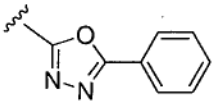
or a substituted or unsubstituted oxazole, specific examples of which include, without limitation:

 or  ;

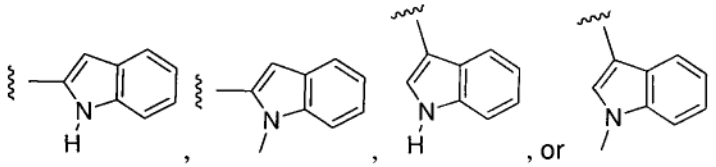
or a substituted or unsubstituted isoxazole, specific examples of which include, without limitation:

 , or  ;

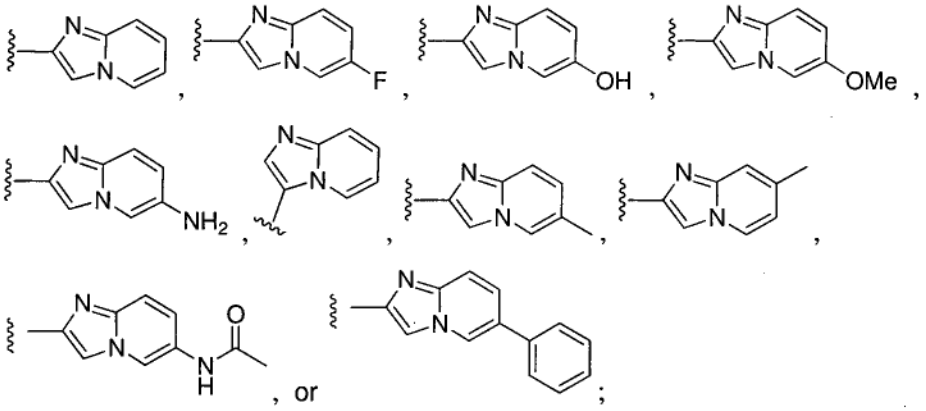
or a substituted or unsubstituted oxadiazole, specific examples of which include, without limitation:

 ;

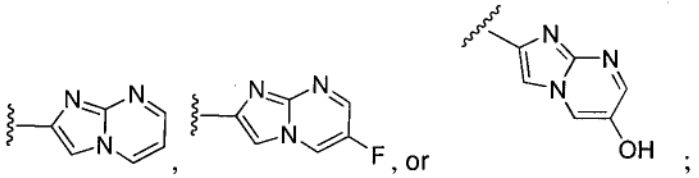
or a substituted or unsubstituted indole, specific examples of which include, without limitation:



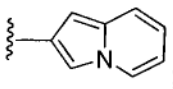
or a substituted or unsubstituted imidazo[1,2-a]pyridine, specific examples of which include, without limitation:



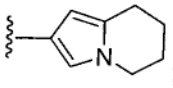
or a substituted or unsubstituted imidazo[1,2-a]pyrimidine, specific examples of which include, without limitation:



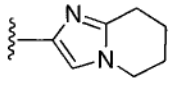
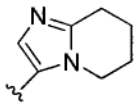
or a substituted or unsubstituted indolizine, specific examples of which include, without limitation:



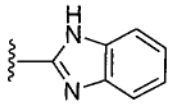
or a substituted or unsubstituted tetrahydroindolizine, specific examples of which include, without limitation:



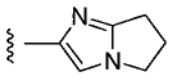
or a substituted or unsubstituted tetrahydroimidazo[1,2-a]pyridine, specific examples of which include, without limitation:

 or  ;

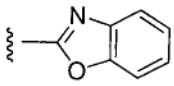
or a substituted or unsubstituted 1H-benzo[d]imidazole, specific examples of which include, without limitation:

 ;

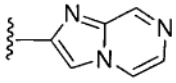
or a substituted or unsubstituted 6,7-dihydro-5H-pyrrolo[1,2-a]imidazole, specific examples of which include, without limitation:

 ;

or a substituted or unsubstituted benzo[d]oxazole, specific examples of which include, without limitation:

 ;

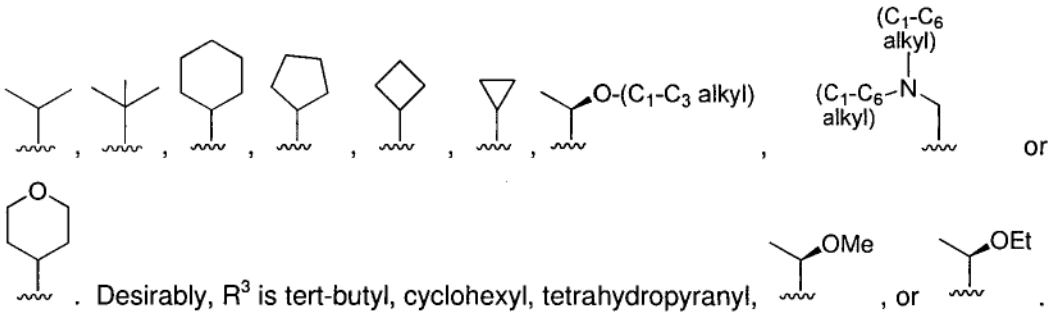
or a substituted or unsubstituted imidazo[1,2-a]pyrazine, specific examples of which include, without limitation

 .

[0017] R^1 can be any alkyl, such as a C_1-C_3 alkyl (e.g., methyl, ethyl, or propyl, including isopropyl), preferably methyl, and R^2 is methyl or ethyl.

[0018] R^3 can be alkyl, cycloalkyl, heterocyclyl, heteroaryl, or aryl, and can be optionally further substituted with an amino, alkylamino, or alkoxy. Non-limiting examples of suitable R^3 groups include C_1-C_6 or C_1-C_4 alkyl (e.g., methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, etc.), cyclohexyl, cyclopropyl, and tetrahydro-2H-pyranyl. For

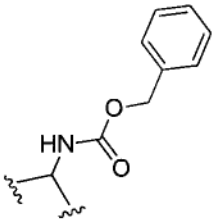
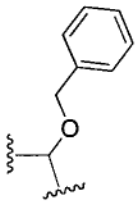
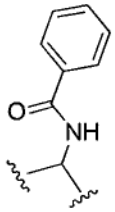
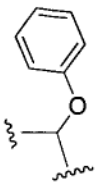
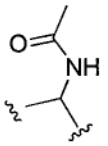
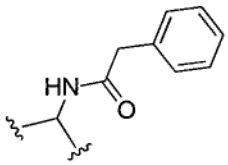
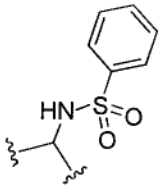
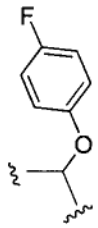
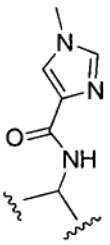
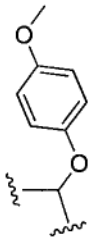
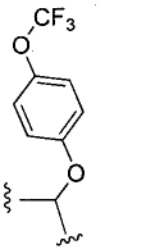
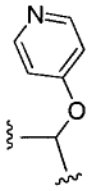
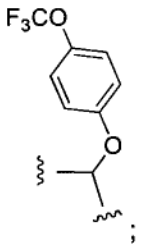
example, R^3 can be:



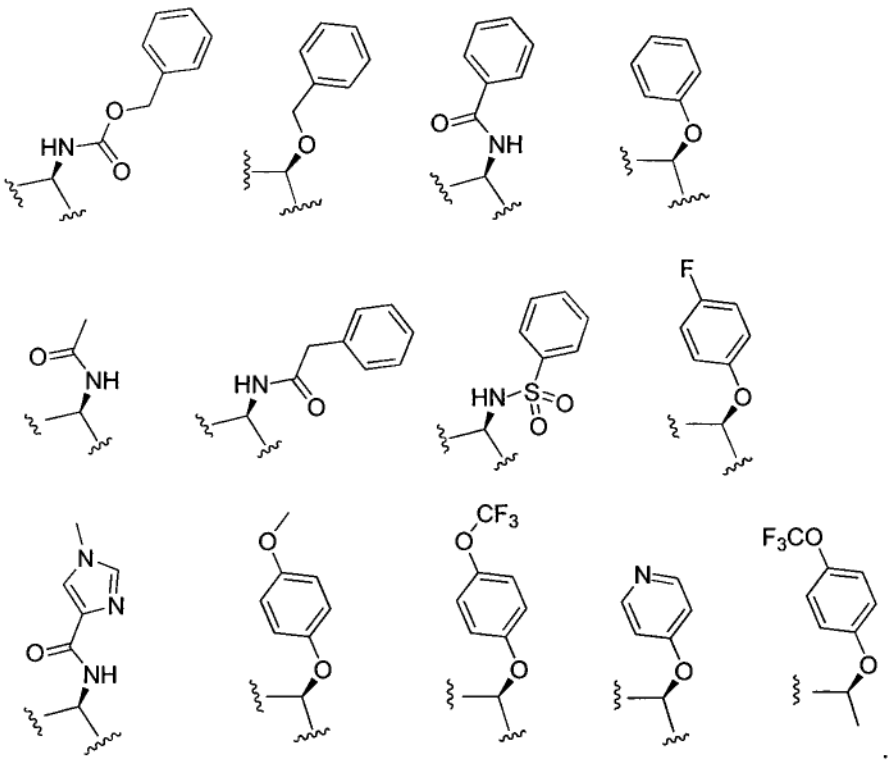
. Desirably, R^3 is tert-butyl, cyclohexyl, tetrahydropyranyl, , or .

[0019] R^4 and R^5 are, independently, hydrogen or alkyl, such as a C_1-C_6 alkyl. R^6 can be hydrogen, halogen, or an alkoxy, such as a C_1-C_6 alkoxy. Desirably, R^6 is hydrogen, fluorine, or a C_1-C_3 alkoxy, such as methoxy or ethoxy.

[0020] X can be O, S, CH_2, $-(CH_2)_2-$ or CH-R^7, wherein R^7 is NR^8, OR^8, $NHC(O)OR^8$, $NHC(O)R^8$ or $NHSO_2R^8$, and R^8 is alkyl, cycloalkyl, heterocyclyl, aryl, arylalkyl, or heteroaryl. R^8 can be further substuted with an alkyl, alkoxy, haloalkyl, or halogen. According to some embodiments, X is CH_2. In other embodiments, X is CH-$NHC(O)R^8$, and R^8 is alkyl, aryl, arylalkyl, alkoxy or heteroaryl, any of which can be optionally further substituted with an alkyl, alkoxy, haloalkyl, or halogen. In yet other embodiments, X is CH-OR^8 and R^8 is aryl or arylalkyl. which can be optionally further substituted with halogen. Specific examples of X include, without limitation:

or, more particularly:



[0021] Any of the foregoing substituent groups, in both general and preferred aspects, can be employed in any combination to provide a compound of Formula 1 or salt thereof. Specific examples of compounds of Formula 1 or salts thereof are provided in Table 1 and the Examples.

[0022] Whenever a range of the number of atoms in a structure is indicated (e.g., a C_1-C_8, C_1-C_6, C_1-C_4, or C_1-C_3 alkyl, haloalkyl, alkylamino, alkenyl, etc.), it is specifically contemplated that any sub-range or individual number of carbon atoms falling within the indicated range also can be used. Thus, for instance, the recitation of a range of 1-8 carbon atoms (e.g., C_1-C_8), 1-6 carbon atoms (e.g., C_1-C_6), 1-4 carbon atoms (e.g., C_1-C_4), 1-3 carbon atoms (e.g., C_1-C_3), or 2-8 carbon atoms (e.g., C_2-C_8) as used with respect to any chemical group (e.g., alkyl, haloalkyl, alkylamino, alkenyl, etc.) referenced herein encompasses and specifically describes 1, 2, 3, 4, 5, 6, 7, or 8 carbon atoms, as appropriate, as well as any sub-range thereof (e.g., 1-2 carbon atoms, 1-3 carbon atoms, 1-4 carbon atoms, 1-5 carbon atoms, 1-6 carbon atoms, 1-7 carbon atoms, 1-8 carbon atoms, 2-3 carbon atoms, 2-4 carbon atoms, 2-5 carbon atoms, 2-6 carbon atoms, 2-7

carbon atoms, 2-8 carbon atoms, 3-4 carbon atoms, 3-5 carbon atoms, 3-6 carbon atoms, 3-7 carbon atoms, 3-8 carbon atoms, 4-5 carbon atoms, 4-6 carbon atoms, 4-7 carbon atoms, 4-8 carbon atoms, 5-6 carbon atoms, 5-7 carbon atoms, 5-8 carbon atoms, 6-7 carbon atoms, or 6-8 carbon atoms, as appropriate).

[0023] As used herein, unless otherwise specified, the term "substituted" means a group substituted by one to four or more substituents. Examples of substitutents include, for instance, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aroyl, halo, haloalkyl (e.g., trifluoromethyl), haloalkoxy (e.g., trifluoromethoxy), hydroxy, alkoxy, alkylthioether, cycloalkyloxy, heterocylooxy, oxo, alkanoyl, aryl, arylalkyl, alkylaryl, heteroaryl, heteroarylalkyl, alkylheteroaryl, heterocyclo, aryloxy, alkanoyloxy, amino, alkylamino, arylamino, arylalkylamino, cycloalkylamino, heterocycloamino, mono- and di-substituted amino (in which the two substituents on the amino group are selected from alkyl, aryl or arylalkyl), alkanoylamino, aroylamino, aralkanoylamino, substituted alkanoylamino, substituted arylamino, substituted aralkanoylamino, thiol, alkylthio, arylthio, arylalkylthio, cycloalkylthio, heterocyclothio, alkylthiono, arylthiono, arylalkylthiono, alkylsulfonyl, arylsulfonyl, arylalkylsulfonyl, sulfonamido (e.g., SO_2NH_2), substituted sulfonamido, nitro, cyano, carboxy, carbamyl (e.g., $CONH_2$), substituted carbamyl (e.g., CONH-alkyl, CONH-aryl, CONH-arylalkyl or instances where there are two substituents on the nitrogen selected from alkyl or arylalkyl), alkoxycarbonyl, aryl, substituted aryl, guanidino, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted heteroaryl (such as, indolyl, imidazolyl, furyl, thienyl, thiazolyl, pyrrolidyl, pyridyl, pyrimidyl and the like).

[0024] As used herein, the term “azole” is intended to include a five-membered nitrogen-containing ring that contains at least one other ring-member nitrogen, sulfur, or oxygen. Non-limiting examples of azoles include pyrazole, imidazole, triazole, tetrazole, pentazole, thiazole, isothiazole, oxazole, and isooxazole.

[0025] As used herein, the term “pyrrole” is intended to include a five-membered aromatic heterocyclic ring containing one nitrogen atom. Pyrrole, as used herein, also encompass the hydrogenated derivatives 1-, 2-, and 3-pyrroline.

[0026] As used herein, the term “alkyl” is intended to include both branched and straight chain saturated aliphatic hydrocarbon groups having the specified number of

carbon atoms (e.g., C_1-C_{20} alkyl, C_1-C_8 alkyl, C_1-C_6 alkyl, etc.). For example, a C_1-C_6-alkyl includes alkyl groups with 1, 2, 3, 4, 5 or 6 carbons in a linear or branched arrangement. Similarly, a C_1-C_4 alkyl includes alkyl groups having 1, 2, 3, or 4 carbons in a linear or branched arrangement, and a C_1-C_{20}- alkyl includes alkyl groups having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 carbons in a linear or branched arrangement. Representative saturated straight chain alkyls include -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl, -n-heptyl, -n-octyl, -n-nonyl and -n-decyl; while representative saturated branched alkyls include -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, 2-methylbutyl, 3-methylbutyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2-methylhexyl, 3-methylhexyl, 4-methylhexyl, 5-methylhexyl, 2,3-dimethylbutyl, 2,3-dimethylpentyl, 2,4-dimethylpentyl, 2,3-dimethylhexyl, 2,4-dimethylhexyl, 2,5-dimethylhexyl, 2,2-dimethylpentyl, 2,2-dimethylhexyl, 3,3-dimtheylpentyl, 3,3-dimethylhexyl, 4,4-dimethylhexyl, 2-ethylpentyl, 3-ethylpentyl, 2-ethylhexyl, 3-ethylhexyl, 4-ethylhexyl, 2-methyl-2-ethylpentyl, 2-methyl-3-ethylpentyl, 2-methyl-4-ethylpentyl, 2-methyl-2-ethylhexyl, 2-methyl-3-ethylhexyl, 2-methyl-4-ethylhexyl, 2,2-diethylpentyl, 3,3-diethylhexyl, 2,2-diethylhexyl, 3,3-diethylhexyl and the like. An alkyl group can be unsubstituted or substituted. For the purposes of describing the invention, the term "alkyl" encompasses an "alkylene" where appropriate.

[0027] As used herein, the term, "alkenyl" is intended to mean an unsaturated straight or branched chain hydrocarbon group having the specified number of carbon atoms, in which at least two of the carbon atoms are bonded to each other by a double bond, and having either E or Z regeochemistry or combination thereof. For example, a C_2-C_6 alkenyl group includes hydrocarbon groups having 2, 3, 4, 5, or 6 carbons in a linear or branched arrangement, at least two of the carbon atoms being bonded together by a double bond. Examples of C_2-C_6 alkenyl include ethenyl (vinyl), 1-propenyl, 2-propenyl, 1- butenyl -2-butenyl, -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, -1-hexenyl, -2-hexenyl, -3-hexenyl, -1-heptenyl, -2-heptenyl, -3-heptenyl, -1-octenyl, -2-octenyl, -3-octenyl, -1-nonenyl, -2-nonenyl, -3-nonenyl, -1-decenyl, -2-decenyl, -3-decenyl and the like. An alkenyl group can be unsubstituted or substituted. For the purposes of describing the invention, the term "alkenyl" encompasses an "alkenylene" where appropriate.

[0028] As used herein, the term "alkynyl" is intended to mean unsaturated, straight chain hydrocarbon groups having the specified number of carbon atoms therein and in which at least two carbon atoms are bonded together by a triple bond. For example C_2-C_4 as in C_2-C_4 alkynyl is defined as including groups having 2, 3, or 4 carbon atoms in a chain, at least two of the carbon atoms being bonded together by a triple bond. Examples of such alkynyls include ethynyl, 1-propynyl, 2-propynyl, -1-butynyl, -2-butynyl, -1-pentynyl, -2-pentynyl, -3-methyl-1-butynyl, -4-pentynyl, -1-hexynyl, -2-hexynyl, -5-hexynyl, -1-heptynyl, -2-heptynyl, -6-heptynyl, -1-octynyl, -2-octynyl, -7-octynyl, -1-nonynyl, -2-nonynyl, -8-nonynyl, -1-decynyl, -2-decynyl, -9-decynyl, and the like. An alkynyl group can be unsubstituted or substituted. For the purposes of describing the invention, the term "alkynyl" encompasses an "alkynylene."

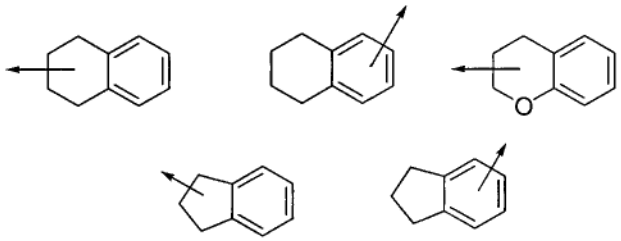
[0029] As used herein, the term "cycloalkyl" is intended to mean a monocyclic saturated aliphatic hydrocarbon group having the specified number of carbon atoms therein, for example, C_3-C_7 as in C_3-C_7 cycloalkyl is defined as including groups having 3, 4, 5, 6, or 7 carbons in a monocyclic arrangement. Examples of C_3-C_7 cycloalkyl as defined above include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl. For the purposes of describing the invention, the term "cycloalkyl" encompasses a "cycloalkylene."

[0030] As used herein, the term "cycloalkenyl" is intended to mean a monocyclic unsaturated aliphatic hydrocarbon group having the specified number of carbon atoms therein, for example, C_3-C_7 as in C_3-C_7 cycloalkenyl is defined as including groups having 3, 4, 5, 6, or 7 carbons in a monocyclic arrangement. Examples of C_3-C_7 cycloalkenyl as defined above include, but are not limited to, cyclopentenyl, and cyclohexenyl. For the purposes of describing the invention, the term "cycloalkenyl" encompasses a "cycloalkenylene."

[0031] As used herein, the term "halo" or "halogen" is intended to mean fluorine, chlorine, bromine and iodine.

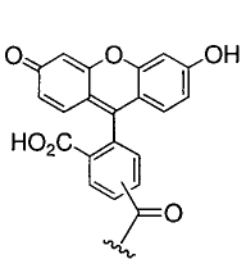
[0032] As used herein, the term "haloalkyl" is intended to mean an alkyl as defined above, in which each hydrogen atom may be successively replaced by a halogen atom. Examples of haloalkyls include, but are not limited to, CH_2F, CHF_2 and CF_3.

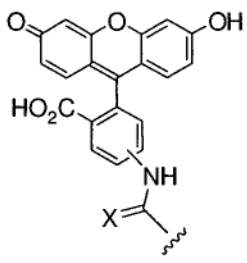
[0033] As used herein, the term "aryl", either alone or in combination with another radical, means a carbocyclic aromatic monocyclic group containing 6 carbon atoms which may be further fused to a second or a third 5- or 6-membered carbocyclic group which may be aromatic, saturated or unsaturated. Aryl includes, but is not limited to, phenyl, indanyl, 1-naphthyl, 2-naphthyl, tetrahydronaphthyl, 1-anthracenyl, 2-anthracenyl, 9-anthracenyl, 1-phenanthryl, 2-phenanthryl, 3-phenanthryl, 4-phenanthryl, and 5-phenanthryl. The aryls may be connected to another group either at a suitable position on the cycloalkyl ring or the aromatic ring. For example:



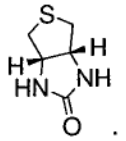
[0034] Lines drawn perpendicular to a bond between members of a ring, such as the arrowed lines above, indicate a bond that may be attached to any of the suitable ring atoms (e.g., an attachment point at any suitable member of the ring). For the purposes of describing the invention, the term "aryl" encompasses an "arylene" where appropriate.

[0035] As used herein, the term "heteroaryl" is intended to mean a monocyclic or bicyclic ring system of up to ten atoms, wherein at least one ring is aromatic, and contains from 1 to 4 hetero atoms selected from the group consisting of O, N, and S. The heteroaryl substituent may be attached either via a ring carbon atom or one of the heteroatoms. Examples of heteroaryl groups include, but are not limited to thienyl, benzimidazolyl, benzo[b]thienyl, furyl, benzofuranyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, 2H-pyrrolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, indolyl, indazolyl, purinyl, 4H-quinolizinyl, isoquinolyl, quinolyl, phthalazinyl, napthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, isothiazolyl, isochromanyl, chromanyl, isoxazolyl, furazanyl, indolinyl, isoindolinyl, thiazolo[4,5-b]-pyridine, and fluoroscein derivatives such as:



or 

. For the purposes of describing the invention, the term "heteroaryl" encompasses a "heteroarylene."

[0036] As used herein, the term "heterocyclyl" is intended to mean a 5, 6, or 7 membered non-aromatic ring system containing from 1 to 4 heteroatoms selected from the group consisting of O, N and S. Examples of heterocycles include, but are not limited to pyrrolidinyl, tetrahydrofuranyl, piperidyl, pyrrolinyl, piperazinyl, imidazolidinyl, morpholinyl, 

imidazolinyl, pyrazolidinyl, pyrazolinyl, and . For the purposes of describing the invention, the term "heterocyclyl" encompasses a "heterocyclylene."

[0037] As used herein, the term "heterobicycle" either alone or in combination with another radical, is intended to mean a heterocycle as defined above fused to another cyclic group, be it a heterocycle, an aryl or any other cyclic group defined herein. Examples of such heterobicycles include, but are not limited to, coumarin, benzo[d][1,3]dioxole, 2,3-dihydrobenzo[b][1,4]dioxine and 3,4-dihydro-2H-benzo[b][1,4]dioxepine.

[0038] As used herein, the term "heteroatom" is intended to mean O, S or N.

[0039] To the extent any indicated substituent groups may be incompatible with the synthetic methods described herein, the substituent may be protected with a suitable protecting group (PG) that is stable to the reaction conditions used in these methods. The protecting group may be removed at a suitable point in the reaction sequence of the method to provide a desired intermediate or target compound. Suitable protecting groups and the methods for protecting and de-protecting different substituents using such suitable protecting groups are well known to those skilled in the art; examples of which may be found in T. Greene and P. Wuts, Protecting Groups in Chemical Synthesis (3rd ed.), John

Wiley & Sons, NY (1999), which is incorporated herein by reference in its entirety. Examples of protecting groups used throughout include, but are not limited to Fmoc, Bn, Boc, CBz and $COCF_3$. In some instances, a substituent may be specifically selected to be reactive under the reaction conditions used in the methods of this invention. Under these circumstances, the reaction conditions convert the selected substituent into another substituent that is either useful in an intermediate compound in the methods of this invention or is a desired substituent in a target compound.

[0040] The invention encompasses any salt of a compound described herein, especially pharmaceutically acceptable salts. Pharmaceutically acceptable salts include both acid and base addition salts. Acid additional salts encompass, for instance, salts formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, or organic acids such as acetic acid, trifluoroacetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like. Base addition salts include those prepared from addition of an inorganic base or an organic base to a free acid. Salts derived from inorganic bases include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. Preferably, the salt retains the desireable biological effectiveness and properties of the free acid or base form of the compound.

[0041] The compound of Formula 1 or salt thereof preferably binds a BIR domain of an IAP. Examples of BIR binding proteins include, but are not limited to, caspases and mitochondrially derived BIR binding proteins such as Smac, Omi/WTR2A and the like.

Examples of IAPs include, but are not limited to human or mouse NAIP (Birc 1), HIAP-1 (cIAP2, Birc 3), HIAP-2 (cIAP1, Birc 2), XIAP (Birc 4), survivin (Birc 5), livin (ML-IAP, Birc 7), ILP-2 (Birc 8) and Apollon/BRUCE (Birc 6) (see for example US Patent Numbers 6,107,041; 6,133,437; 6,156,535; 6,541,457; 6,656,704; 6,689,562; Deveraux and Reed, Genes Dev. 13, 239-252, 1999; Kasof and Gomes, J. Biol. Chem., 276, 3238-3246, 2001; Vucic et al., Curr. Biol. 10, 1359-1366, 2000; Ashab et al. FEBS Lett., 495, 56-60, 2001, the contents of which are hereby incorporated by reference). The BIR domains of the IAPs are documented in the relevant literature, typically characterized by a number of invariant amino acid residue including conserved cysteines and one conserved hisitidine residue within a particular sequence. The BIR domain residues for some human IAPs are include, for instance, residues 21-93 (BIR1), 159-230 (BIR2), and 258-330 (BIR3) of XIAP (Referencing Swiss-Prot P98170), residues 41-113 (BIR1), 179-250 (BIR2), and 264-336 (BIR3) of HIAP-1 (Referencing XP-006266), and residues 24-96 (BIR1), 164-235 (BIR2), and 250-322 (BIR3) of HIAP-2 (Referencing XP-006267) (see Verhagen et al., *Genome Biology, 2(7)*: reviews 3009.1-3009.10 (2001)).

[0042] Desirably, the compound of Formula 1 or salt thereof binds to a BIR domain of XIAP, more preferably human XIAP. BIR domain binding can be detected by any suitable technique. For instance, BIR domain binding can be detected on the basis of a test compounds ability to compete with the binding of a known BIR-domain binding protein (e.g., inhibiting or preventing the binding of a known BIR-domain binding protein to a given BIR domain). Naturally occurring and synthetic BIR domain binding proteins are known in the art. In some embodiments, the compound of Formula 1 or salt thereof binds one or more IAPs (such as NAIP, HIAP-1, HIAP-2, XIAP, survivin, livin, ILP-2, or Apollon/BRUCE) with a K_i of less then or about 500 μM, 250 μM, 100 μM, 50 μM, 25 μM, 10 μM, 1 μM, 500 nM, 250 nM, 100 nM, or 50 nM (wherein a lower K_i value represents a greater binding affinity). In some embodiments, the compound of Formula 1 or salt thereof binds one or more IAPs with a K_i between about 500 μM to about 50 nM, such as about 250 μM to about 50 nM, about 100 μM to about 1 μM, or about 1 μM to about 50 nM. In some embodiments, the compound of Formula 1 or salt thereof binds both XIAP and HIAP2 with a K_i in one of the above ranges.

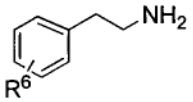
[0043] The compounds described herein may contain one or more asymmetric centers, chiral axes, and chiral planes. These compounds may, thus, give rise to enantiomers, diastereomers, and other stereoisomeric forms and may be defined in terms of absolute stereochemistry, such as (R)- or (S)- or, as (D)- or (L)- for amino acids, and/or by optical activity, such as (+) and (-). The present invention is intended to encompass any and all such possible stereoisomers, whether present in a pure or substantially form (e.g, an optically pure form) or as a mixture of isomers in any proportion, including racemic mixtures. Optically active (+) and (-), (R)- and (S)-, or (D)- and (L)-isomers may be prepared by chiral (asymmetric) synthesis using optically active reagents, substrates, catalysts, or solvents (chiral synthons or chiral reagents), or by converting one enantiomer to another by asymmetric transformation. Alternatively, isomers can be resolved from mixtures of isomeric forms (e.g., racemic mixtures) using conventional techniques, including without limitation reverse-phase HPLC, formation of diastereoisomeric salts that can be separated by crystallization, gas-liquid or liquid chromatography, selective reaction of one stereoisomer with an enantiomer-specific reagent. When the desired enantiomer is converted into another chemical entity by a separation technique, an additional step may be required to form the desired enantiomeric form.

[0044] Certain compounds of the present invention also may exist under certain conditions in anionic, cationic, or Zwitterionic forms. Compounds of Formula 1 and other formulas described herein specifically encompass such alternative forms.

[0045] According to a preferred embodiment, the compound of Formula 1 or salt thereof provides oral bioavailability when administered to a mammal, particularly a human. Desirably, the compound of Formula 1 or salt thereof exhibits an oral bioavailability of about 10% or more, about 15% or more, or about 20% or more. More preferably, the compound of Formula 1 or salt thereof, exhibits an oral bioavailability of about 25% or more, about 30% or more, about 50% or more, or even about 75% or more (e.g., about 80% or more, about 90% or more, or about 95% or more). In some embodiments, the compound of Formula 1 or salt thereof exhibits an oral bioavailability of between about 25% to about 50%, about 50% to about 75%, or about 75% to about 100%.

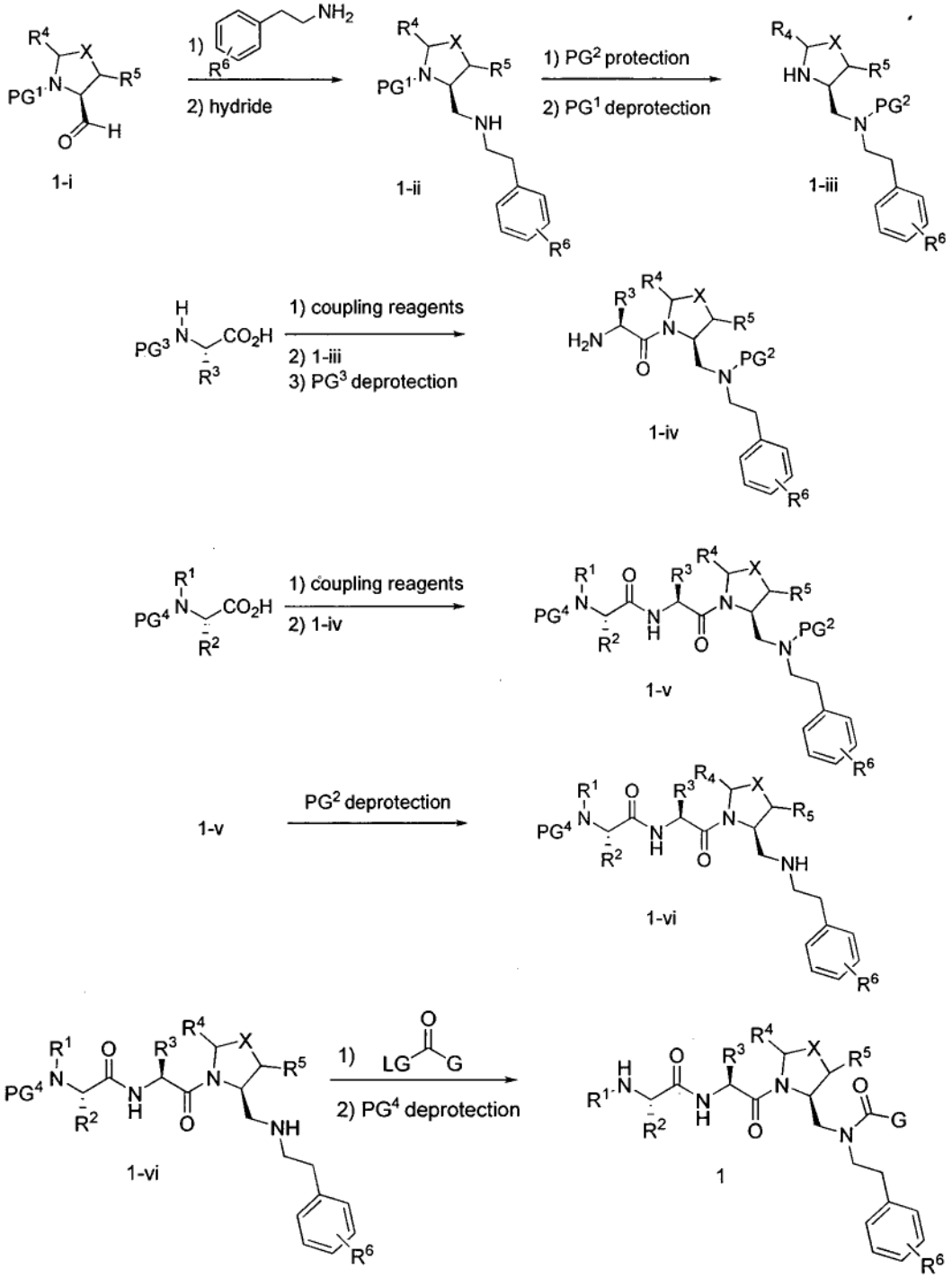
Synthesis Methods

[0046] The compounds of the invention described herein can be prepared by any of several techniques. According to one aspect of the invention, the compounds can be prepared in accordance with any of Methods A-C illustrated by Schemes 1-4.

[0047] Method A provides a method of preparing a compound of Formula 1 or salt thereof, as well as methods for preparing intermediate compounds associated therewith, comprising one or more of the following steps: (1) combining a prolinal derivative (1-i) with an amine having the formula 

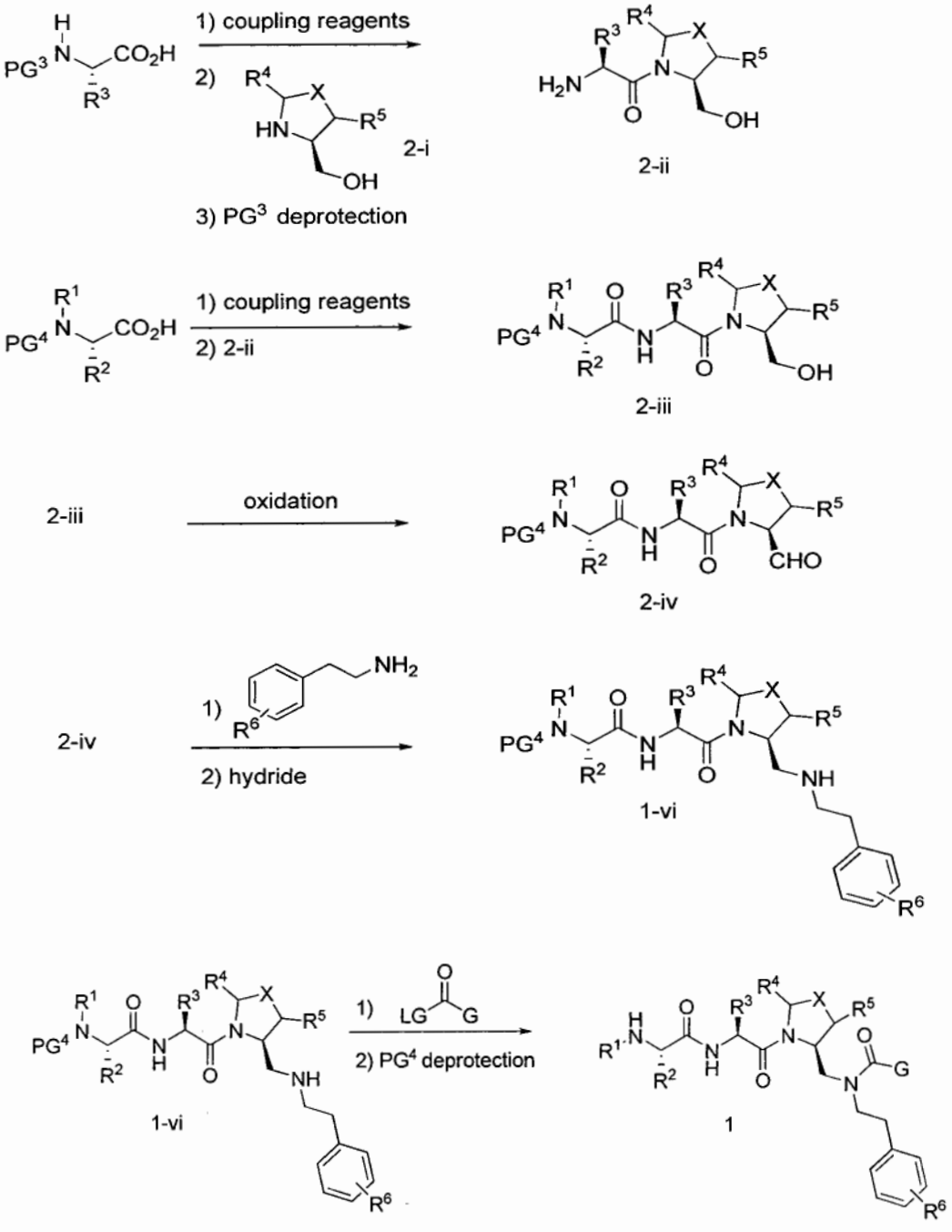
, followed by reduction with a hydride, to provide intermediate compound 1-ii, wherein PG^1 is a protecting group; (2) protecting the amine group of intermediate compound 1-ii with a protecting group (PG^2) that is different from PG^1, followed by deprotection of PG^1 to provide intermediate compound 1-iii; (3) coupling intermediate compound 1-iii with $PG^3(H)N(R^3)CHCO_2H$ using amino acid coupling agents, wherein PG^3 is a protecting group that is different from PG^2, followed by deprotection of PG^3 to provide intermediate compound 1-iv; (4) coupling intermediate compound 1-iv with $PG^4(R^1)N(R^2)CHCO_2H$ using amino acid coupling agents, wherein PG^4 is a protecting group that is different from PG^2, to provide intermediate compound 1-v; (5) deprotection of PG^2 of intermediate compound 1-v to provide intermediate compound 1-vi; and (6) acylation of intermediate compound 1-vi by combining intermediate compound 1-vi with a compound of formula LG-C(O)-G, wherein "LG" is a leaving group, followed by deprotection of PG^4 to provide a compound of Formula 1 or salt thereof. Method A is illustrated in Scheme 1, below. Each intermediate compound of Method A, as well as each individual process step for preparing the intermediate compound, is considered to be an additional aspect of the invention. Thus, provided herein is a compound of any one of Formulas 1-i through 1-v of Scheme 1, including salts thereof. Also provided herein is a method of preparing a compound of Formula 1 or salt thereof, or an intermediate compound of any of Formulas 1-i through 1-vi of Scheme 1, including salts thereof, comprising one or more of steps (1) through (6) of Method A described above.

SCHEME 1


1-i
1) 2) hydride
1-ii
1) PG2 protection
2) PG1 deprotection
1-iii
1) coupling reagents
2) 1-iii
3) PG3 deprotection
1-iv
1) coupling reagents
2) 1-iv
1-v
PG2 deprotection
1-vi
1) 2) PG4 deprotection
1

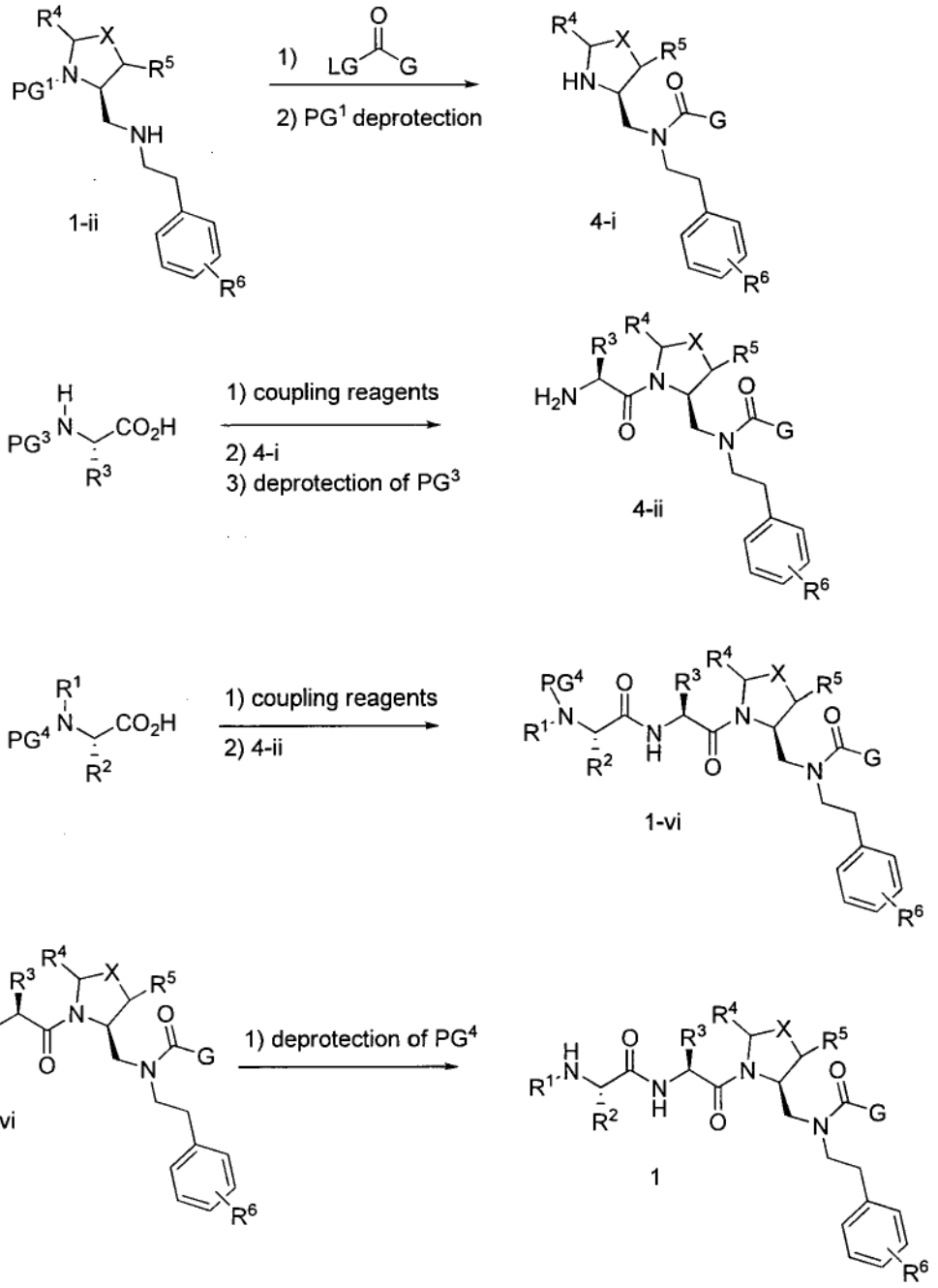
[0048] Method B provides an alternative method of preparing a compound of Formula 1 or salt thereof, as well as methods for preparing intermediate compounds associated therewith, comprising one or more of the following steps: (1) coupling a prolinol derivative (intermediate compound 2-i) with a compound of the formula $PG^3(H)N(R^3)CHCO_2H$ using amino acid coupling agents, wherein PG^3 is a protecting group, followed by deprotection of PG^3 to provide intermediate compound 2-ii; (2) coupling intermediate compound 2-ii with a compound of the formula $PG^4(R^1)N(R^2)CHCO_2H$ to provide intermediate compound 2-iii, wherein PG^4 is a protecting group; (3) oxidizing intermediate compound 2-iii to provide the corresponding aldehyde, intermediate compound 2-iv; (4) reductive amination of compound 2-iv, for example, by combining compound 2-iv with an amine followed by reduction with an appropriate hydride, to provide intermediate compound 1-vi; (5) acylation of compound 1-vi by combining compound 1-vi with a compound of formula LG-C(O)-G, wherein LG is a leaving group, followed by deprotection of PG^4, to provide a compound of Formula 1 or salt thereof. Method B is illustrated in Scheme 2, below. Each intermediate compound of Method B, as well as each individual process step for preparing the intermediate compound, is considered to be an additional aspect of the invention. Thus, provided herein is a compound of any one of Formulas 2-i through 2-iv or Formula 1-vi of Scheme 2, including salts thereof. Also provided herein is a method of preparing a compound of Formula 1 or salt thereof, or an intermediate compound of any of Formulas 2-i through 2-iv or Formula 1-vi of Scheme 2, including salts thereof, comprising one or more of steps (1) through (5) of Method B described above.

SCHEME 2


1) coupling reagents
2)
2-i
3) PG3 deprotection
2-ii
1) coupling reagents
2) 2-ii
2-iii
2-iii
oxidation
2-iv
2-iv
1)
2) hydride
1-vi
1-vi
1) LG G
2) PG4 deprotection
1

[0049] Method C provides another alternative method of preparing a compound of Formula 1 or salt thereof, as well as methods for preparing intermediate compounds associated therewith, comprising one or more of the following steps: (1) acylation of intermediate compound 1-ii (prepared as described in Method A, or by other methods) by combining intermediate compound 1-ii with a compound of formula LG-C(O)-G, wherein PG^1 is a protecting group, followed by deprotection of PG^1 to provide intermediate compound 4-i; (2) coupling compound 4-i with a compound having the formula $PG^3(H)N(R^3)CHCO_2H$ using amino acid coupling agents, wherein PG^3 is a protecting group, followed by deprotection of PG^3 to provide intermediate compound 4-ii; (3) coupling intermediate compound 4-ii with a compound having the formula $PG^4(R^1)N(R^2)CHCO_2H$ using amino acid coupling agents to provide intermediate compound 1-vi, wherein PG^4 is a protecting group, followed by deprotection of PG^4 to provide a compound of Formula 1 or salt thereof. Method C is illustrated in Scheme 3, below. Each intermediate compound of Method C, as well as each individual process step for preparing the intermediate compound, is considered to be an additional aspect of the invention. Thus, provided herein is a compound of Formula 4-i or 4-ii of Scheme 3, including salts thereof. Also provided herein is a method of preparing a compound of Formula 1 or salt thereof, or an intermediate compound of Formula 4-i or 4-ii of Scheme 3, including salts thereof, comprising one or more of steps (1) through (3) of Method C described above.

SCHEME 3


1) LG G O
2) PG1 deprotection
1-ii
4-i
1) coupling reagents
2) 4-i
3) deprotection of PG3
4-ii
1) coupling reagents
2) 4-ii
1-vi
1) deprotection of PG4
1-vi
1

Utilities

[0050] The compounds of the present invention can be used for any purpose. However, compounds of Formula 1 and salts thereof as provided herein are believed to be especially useful as IAP BIR domain binding compounds. As such the compounds of Formula 1 and salts thereof described herein can be used to enhance apoptosis in a cell or subject, particularly in cells that exhibit abnormally low levels of apoptosis or in subjects afflicted with, or having a predisposition towards, a disease or condition associated with insufficient apoptosis. Insufficient apoptosis means a level or degree of apoptosis that is abnormal under given conditions, or otherwise leads to or causes a pathological state. Thus, insufficient apoptosis encompasses, for instance, a state wherein a disease is caused or continues because cells deleterious to the subject have not apoptosed. Conditions or diseases associated with insufficient apoptosis encompass cell-proliferative diseases and disorders including, without limitation cancer, autoimmune diseases, inflammatory disorders, and cell proliferation induced by medical procedures, including, but not limited to, surgery, angioplasty, and the like.

[0051] Thus provided herein is a method of enhancing or inducing apoptosis in a cell comprising administering a compound of Formula 1 or salt thereof to a cell. Compounds of Formula 1 or salt thereof can be administered to a cell by any suitable method, for instance, contacting the cell with a compound of Formula 1 or salt thereof or a composition comprising a compound of Formula 1 or salt thereof. Target cells can include cells of any type that exhibits insufficient apoptosis, in other words, are characterized by resistance to apoptosis or which exert pathological functions that can be abrogated by apoptosis, including but not limited to cancerous and inflammatory cells. Cancerous cells can be any type of malignancy including, but not limited to, ovarian, colorectal, hematological, breast, lung or pancreatic cancer cells. Inflammatory cells can be any type including, but not limited to, B-cells, T-cells, macrophage, dendritic cells, and granulocytes. Additional examples of target cells include ectopic endometrial cells and psoriatic keratinocytes.

[0052] Apoptosis of a cell, or a population of cells, is enhanced if the level of apoptosis is increased by any degree in the presence of the compound of Formula 1 or salt thereof as compared to the level of apoptosis exhibited in the absence of the compound of

Formula 1 or salt thereof. Enhancement of apoptosis, thus, encompasses inducing apoptosis in a cell that otherwise would not apoptose, as well as increasing the rate at which a cell undergoes apoptosis, increasing the number of apoptosing cells in a cell population, or increasing the sensitivity of a cell to apoptotic stimuli. When measured in a population of cells, preferably the number of cells undergoing apoptosis is increased by at least about 25%, more preferably at least about 50%, at least about 75%, or at least about 100% (e.g., at least a 1-fold or 2-fold increase). Any technique for measuring and comparing the level of apoptosis in cells can be used to detect enhancement of apoptosis. Such techniques may be based, for instance, upon changes in cell proliferation, increases in cell membrane permeability, reduction in metabolic activity of mitochondria, fragmentation of DNA (DNA laddering) or chromatin condensation, alterations in membrane asymmetry (e.g., translocation phosphatoylserine from the cytoplasmic to the extracellular side of the membrane), activation of apoptotic caspases, release of cytochrome C or apoptosis inhibitory factor (AIF) into the cytoplasm by mitochondria, or any other basis known to be an indicator of apoptosis.

[0053] The compounds of Formula 1 and salts thereof also can be used to alter the release of inflammatory cytokines from an immune system cell, thereby reducing the inflammatory potential of the cell. Inflammatory cytokines include pro-inflammatory cytokines and anti-inflammatory cytokines. The release of cytokines is altered if the amount or rate of release of any one or more cytokines is increased or decreased by any degree in the presence of the compound of Formula 1 or salt thereof as compared to the amount or rate of release of the same one or more cytokines in the absence of the compound of Formula 1 or salt thereof. Desireably, the amount or rate of release of any one or more cytokines is altered (increased or decreased) by at least about 25%, more preferably at least about 50%, at least about 75%, or at least about 100% (e.g., at least a 1-fold or 2-fold increase). Any technique for measuring and comparing the level of cytokine release in cells can be used to detect alteration in the release of the inflammatory cytokines. Such techniques may be based, for instance, directly upon changes in the amount of cytokine in a sample or cell culture, or indirectly by detecting cellular responses to increased or decreased cytokine concentration.

[0054] Without wishing to be bound by any particular theory or mechanism, it is believed that the compounds of Formula 1 bind to or otherwise inhibit XIAP, cIAP-1, and/or cIAP-2. Thus, in a related aspect, the invention provides a method of reducing the activity or protein levels of XIAP, cIAP-1, and/or cIAP-2 in a cell comprising contacting the cell with a compound of Formula 1 or salt thereof. The activity and protein levels of XIAP, cIAP-1, and/or cIAP-2 can be measured by known assays and protein quantification techniques. All other aspects of the method are as previously described.

[0055] The compounds of the invention described herein can be administered to a cell *in vitro*. As used herein, the term "*in vitro*" means that the cell is not in a living organism. The compounds of the invention also can be administered to a cell *in vivo* or *ex vivo*. As used herein, the term "*in vivo*" means that the cell is a part of a living organism, for instance, when the cell is in a host subject. The term "*ex vivo*" as used herein refers to the administration of a compound to a cell or a population of cells *in vitro*, followed by administration of the cell or population of cells to a host subject. Often the cells are autologous to the subject.

[0056] When the compound is administered to a cell in a subject, the subject desireably is a mammal, especially a human. The methods, in accordance with this aspect of the invention, are most suitable for use in conjunction with a subject that is afflicted with a disease, or at risk for developing a disease, associated with insufficient apoptosis or an autoimmune or inflammatory disease. When the cell is in a subject, the compound of Formula 1 or salt thereof can be administered to the cell by administering the compound of Formula 1 or salt thereof, or composition comprising same (e.g., pharmaceutical composition) to the subject. Preferably, the administration of a compound of Formula 1 or salt thereof to a cell in a subject afflicted with a disease associated with insufficient apoptosis or an autoimmune or inflammatory disease is effective to treat the disease. Thus, the invention also provides a method of treating a disease associated with insufficient apoptosis or an autoimmune or inflammatory disease comprising administering to a subject in need thereof a compound of Formula 1 or salt thereof. As used herein, the term "treat" is intended to encompass alleviating to any degree, or preventing the onset of, any symptom of the disease or condition. The term "treat" also encompases inhibiting, arresting, or reversing the growth or proliferation of diseased cells or the progression or

spread (metastasis) of the disease or condition, or altering the release of inflammatory cytokines. Treatment includes preventative treatment, such as treatment of a patient after surgical removal of cancer or tumor cells to prevent regrowth of the cancer or tumor, or treatment to prevent pathogenic cell-survival, for instance, under conditions that lead to diseases such as asthma, MS, and the like.

[0057] Diseases and conditions associated with insufficient apoptosis encompass proliferative diseases characterized by inappropriately high levels of cell division, inappropriately low levels of apoptosis, or both. Such disease may include those in which there is a defect in normally programmed cell-death or the cellular apoptotic machinery (TRAIL, FAS, apoptosome).

[0058] Examples of autoimmune of inflammatory disorders where apoptotic resistance contributes to pathology or where increased apoptosis may be therapeutically beneficial include multiple sclerosis, atherosclerosis, arthritis (e.g., rheumatoid arthritis (RA)) and the like. Another aspect of the present invention provides a method of inducing apoptosis in a cell such as a rheumatoid arthritis fibroblast-like synoviocyte with a compound of Formula 1 or salt thereof alone or in combination with cytokines or death-receptor ligands such as Fas, TRAIL or TRAIL-receptor angonist antibodies.

[0059] Diseases where apoptotic resistance contributes to pathology or where increased apoptosis may be therapeutically beneficial include all types of cancer including lung, colorectal, breast and prostate cancer. Other cancers that may be treated by compounds, compositions and methods of the invention include but are not limited those listed in the following table.

Tissue	Example
Adrenal gland	neuroblastoma
Bone	osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid

Tissue	Example
	osteoma and giant cell tumors
Cardiac	sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma and teratoma
Gastrointestinal	esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma)
Genitourinary tract	kidney (adenocarcinoma, Wilm's tumor [nephroblastoma], lymphoma, leukemia), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma)
Gynecological	uterus (endometrial carcinoma), cervix (cervical carcinoma, pre-tumor cervical dysplasia), ovaries (ovarian carcinoma [serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma], granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma), fallopian tubes (carcinoma)
Hematologic	blood (myeloid leukemia [acute and chronic], acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplastic

Tissue	Example
	syndrome), Hodgkin's disease, non-Hodgkin's lymphoma [malignant lymphoma]
Liver	hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, hemangioma
Lung	bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesothelioma
Nervous system	skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma [pinealoma], glioblastoma multiform, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), spinal cord neurofibroma, meningioma, glioma, sarcoma)
Skin	malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, keloids

[0060] The compound of Formula 1 or salt thereof can be used in a pure or substantially pure form, or as part of a composition comprising the compound of Formula 1 or salt thereof and a suitable carrier. When the composition is to be administered to a subject or patient, especially a human subject or patient, the carrier should be a pharmaceutically acceptable carrier. As used herein, the terms "subject" and "patient" are intended to mean humans as well as non-human mammals such as primates, cats, dogs, swine, cattle, sheep, goats, horses, rabbits, rats, mice and the like.

[0061] The pharmaceutical compositions of the present invention can be prepared by combining a compound of the present invention with an appropriate carrier. The carrier can be any of those conventionally used and is limited only by physio-chemical considerations, such as solubility and lack of reactivity with the active compound, and by

the route of administration. It will be appreciated by one of skill in the art that, in addition to the following described pharmaceutical composition, the active compounds of the present inventive methods can be formulated as inclusion complexes, such as cyclodextrin inclusion complexes, or liposomes.

[0062] The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active agent(s) and one which has no detrimental side effects or toxicity under the conditions of use.

[0063] There are a variety of suitable formulations of the pharmaceutical composition of the present inventive methods. The formulation can be, for instance, a solid, semi-solid, or liquid, including tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols.

[0064] Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, 18th Ed., (Mack Publishing Company, Easton, Pa., 1990). The composition to be administered will, in any event, contain a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof, for treatment of a disease-state as described above.

[0065] The pharmaceutical composition can be formulated for any route of administration, including, for instance, oral, topical, transdermal, transmucosal, aerosol/inhalation, parenteral (including without limitation subcutaneous, intravenous, intramuscular, intrasernal, interperitoneal, intracerebral, intraosseous, and intradermal), rectal, sublingual, ocular, intranasal, and vaginal administration. One skilled in the art will appreciate that these routes of administering the compound of the invention are known, and, although more than one route can be used to administer a particular compound, a particular route can provide a more immediate and more effective response than another route. The following formulations are describe for the purposes of further illustration, and are in no way intended to limit the invention.

[0066] Injectable formulations are among those formulations that may be suitable in accordance with the present invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (See, e.g., Pharmaceutics and Pharmacy Practice, J.B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and ASHP Handbook on Injectable Drugs, Toissel, 4th ed., pages 622-630 (1986)). Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The compounds of the invention can be administered in a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol, isopropanol, or hexadecyl alcohol, glycols, such as propylene glycol or polyethylene glycol, dimethylsulfoxide, glycerol ketals, such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers, such as poly(ethyleneglycol) 400, an oil, a fatty acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants including pegylated or fatty acid modified glyerols and triglycerides.

[0067] Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

[0068] Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates,

alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-b-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

[0069] The parenteral formulations will typically contain from about 0.01% to about 10% by weight of the active ingredient in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include polyethylene sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[0070] Topical formulations are well-known to those of skill in the art. Such formulations are particularly suitable in the context of the present invention for application to the skin. The carrier may suitably comprise a solution, emulsion, ointment or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, bee wax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device. Topical formulations may contain a concentration of the compound of the present invention from about 0.1% to about 10% w/v (weight per unit volume).

[0071] Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the active compound dissolved in diluents, (b) capsules,

sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations may include diluents, such as water, saline, and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant. Capsule forms can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible excipients. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to the active ingredient, such excipients as are known in the art. The oral formulations will typically contain from about 0.1% to about 70% by weight of the active ingredient.

[0072] The compounds of the invention, alone or in combination with other suitable components, can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer. Such spray formulations also may be used to spray mucosa.

[0073] Additionally, the compounds of the invention, or compositions comprising such compounds, can be made into suppositories by mixing with a variety of bases, such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[0074] Alternatively, the compounds of the invention described herein can be modified into a depot form, such that the manner in which the compound of the invention is released into the body to which it is administered is controlled with respect to time and location within the body (see, e.g., U.S. Patent No. 4,450,150). Depot forms of the active compound can be, for example, an implantable composition comprising the compound and a porous material, such as a polymer, wherein the compound is encapsulated by or diffused throughout the porous material. The depot is then implanted into the desired location within the body and the compound is released from the implant at a predetermined rate by diffusing through the porous material.

[0075] In some contexts, the compounds of the invention can be advantageously administered via an implanted pump that allows intrathecal delivery. Such a delivery method is especially useful for delivery of drugs to the CNS when the drugs administered do not otherwise sufficiently penetrate the blood-brain barrier.

[0076] Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, 18th Ed., (Mack Publishing Company, Easton, Pa., 1990). The composition to be administered should, in any event, contain a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof, for treatment of a disease-state as described above.

[0077] One of ordinary skill in the art will readily appreciate that the compounds of the invention described herein can be modified in any number of ways to increase the therapeutic efficacy of the compound. For instance, the compound or inhibitor could be conjugated either directly or indirectly through a linker to a targeting moiety. The practice of conjugating compounds or inhibitors to targeting moieties is known in the art. The term "targeting moiety" as used herein, refers to any molecule or agent that specifically recognizes and binds to a cell-surface receptor, such that the targeting moiety directs the delivery of the compound or inhibitor to a population of cells on which surface the receptor is expressed. Targeting moieties include, but are not limited to, antibodies, or fragments thereof, peptides, hormones, growth factors, cytokines, and any other naturally- or non-naturally-existing ligands, which bind to cell surface receptors. The term "linker" as used herein, refers to any agent or molecule that bridges the compound or inhibitor to the

targeting moiety. One of ordinary skill in the art recognizes that sites on the compounds or inhibitors, which are not necessary for the function of the compound or inhibitor, are ideal sites for attaching a linker and/or a targeting moiety, provided that the linker and/or targeting moiety, once attached to the compound or inhibitor, do(es) not interfere with the function of the compound or inhibitor.

[0078] The amount considered to be therapeutically effective will vary depending upon a variety of factors including the the specific compound employed, the precise type and severity of the condition to be treated, and the age, body weight, general health, sex, and diet of the patient; and the mode of administration. Generally, a therapeutically effective daily dose may be from about 0.1 mg to about 40 mg/kg of body weight per day or twice per day.

Combination therapy

[0079] The compound of Formula 1 or salt thereof, or composition comprising same, can be used in accordance with the methods described herein alone or in combination with one or more additional active ingrediants. For instance, two or more different compounds of Formula 1 or salts thereof can be used together, or one or more compounds of Formula 1 or salts thereof can be used in conjuction with one or more other therapeutically effective compounds. When the compound of Formula 1 or salt thereof is used in conjunction with one or more additional active compounds (whether another compound of Formula 1 or a different compound), the one or more additional compounds can be administered simultaneously with, prior to, or after administration of the compound of Formula 1 or salt thereof. Furthermore, when administered simultaneously, the one or more additional compounds can be administered in the same composition as the compound of Formula 1 or salt thereof, or in a different composition.

[0080] The selection of additional therapeutic agents for use in combination with a compound of Formula 1 or salt thereof will depend, at least in part, on the particular disease or condition to be treated. According to one aspect of the invention, the compound of Formula 1 or salt thereof is administered in combination with an agent that directly or indirectly stimulates the death receptor apoptotic pathway. Without wishing to be bound by any particular theory, it is believed that the combined use of a compound of

Formula 1 or salt thereof and an agent that stimulates the death receptor apoptotic pathway (e.g., a death receptor agonist) produces an enhanced, and in some cases synergistic, effect. The death receptor agonist can be any agent capable of stimulating the pro apoptotic response mediated by the death-receptors. Such agents include souble TRAIL, TRAIL receptor agonists, and any agent that increases the circulating level of TRAIL in a subject, including immune system modulators such as interfereon-alpha or ionizing radiation (e.g., UVB) that can induce the release of cytokines, such as the interleukins, or TNF.

[0081] TRAIL receptor agonists include any compound that mimics TRAIL by stimulating the TRAIL death receptor. Such compounds may include, for instance, a small molecule or an antibody agonist of the TRAIL receptor. Agonist antibodies directed against the death receptors TRAIL-R1 and/or TRAIL-R2 are preferred, particularly antibodies known as HGS-ETR1 and HGS-ETR2. Exemplary agonist antibodies include those described in U.S. Pat. No. 7,244,429; in U.S. Patent Application Publication Nos. 2007/0179086, 2002/0004227, 2006/0269554 , 2005/0079172, 2007/0292411, 2006/0270837 (now U.S. Pat. No. 7,361,341), 2009/0026429, 2006/0269555, 2004/0214235, and 2007/0298039; and in International Patent Publications WO2006/017961 and WO98/51793. Each of these publications is hereby incorporated by reference in its entirety. In preferred embodiments, compounds of the invention are used in combination with one or more of these TRAIL receptor agonist antibodies for the treatment of cancer and other neoplasms.

[0082] Other agents useful in combination with a compound of Formula 1 or salt thereof include, for example, estrogen receptor modulators, androgen receptor modulators, retinoid receptor modulators, cytotoxic agents, antiproliferative agents, prenyl-protein transferase inhibitors, HMG-CoA reductase inhibitors, HIV protease inhibitors, reverse transcriptase inhibitors, angiogenesis inhibitors, PPAR-γ agonists, PPAR-δ agonists, inhibitors of inherent multidrug resistance, anti-emetic agents, agents used to treat anemia or neutropenia, immunologic-enhancing drugs, proteasome inhibitors such as Velcade and MG132 (7-Leu-Leu-aldehyde) (see He at al., *Oncogene* (2004) 23, 2554-2558), HDAC inhibitors such as sodium butyrate, phenyl butyrate, hydroamic acids, cyclin tetrapeptide and the like (see Rosato et al., *Molecular Cancer Therapeutics* (2003), 1273-

1284), inhibitors of the chymotrypsin-like activity in the proteasome, and E3 ligase inhibitors. Other such agents are described in WO 03/099211 (PCT/US03/15861).

[0083] Other known chemotherapeutic agents can be used in combination with a compound of Formula 1 or salt thereof, especially for the treatment of cancers or other proliferative disease susceptible to chemotherapy. Any chemotherapeutic agent can be used in combination with the compound of Formula 1 or salt thereof. The selection of a chemotherapeutic agent may depend, in part, on the particular type of cancer or proliferative disease being treated. Exemplary chemotherapeutic agents are described in the following paragraphs. The chemotherapeutic agents described herein are merely illustrative, and are in no way limiting.

[0084] Vinca Alkaloids and Microtubule-Disrupting Compounds: Vinca alkaloids include vincristine, vinblastine, vindesine, vinflunine, vinorelbine, and anhydrovinblastine. Dolastatins are oligopeptides that primarily interfere with tubulin at the vinca alkaloid binding domain. Dolastatins include dolastatin-10 (NCS 376128), dolastatin-15, ILX651, TZT-1027, symplostatin 1, symplostatin 3, and LU103793 (cemadotin). Cryptophycins (e.g., cryptophycin 1 and cryptophycin 52 (LY355703)) bind tubulin within the vinca alkaloid-binding domain and induce G2/M arrest and apoptosis.

[0085] Other microtubule disrupting compounds are described in U.S. Pat. Nos. 6,458,765; 6,433,187; 6,323,315; 6,258,841; 6,143,721; 6,127,377; 6,103,698; 6,023,626; 5,985,837; 5,965,537; 5,955,423; 5,952,298; 5,939,527; 5,886,025; 5,831,002; 5,741,892; 5,665,860; 5,654,399; 5,635,483; 5,599,902; 5,530,097; 5,521,284; 5,504,191; 4,879,278; and 4,816,444, and U.S. patent application Publication Nos. 2003/0153505 A1; 2003/0083263 A1; and 2003/0055002 A1.

[0086] Taxanes and Other Microtubule Stabilizing Compounds: Taxanes include paclitaxel, docetaxel, RPR 109881A, SB-T-1213, SB-T-1250, SB-T-101187, BMS-275183, BRT 216, DJ-927, MAC-321, IDN5109, and IDN5390. Taxane analogs include BMS-184476, BMS-188797, and functionally related non-taxanes include epothilones (e.g., epothilone A, epothilone B (EPO906), deoxyepothilone B, and epothilone B lactam (BMS-247550)), eleutherobin, discodermolide, 2-epi-discodermolide, 2-des-

methyldiscodermolide, 5-hydroxymethyldiscoder- molide, 19-des-aminocarbonyldiscodermolide, 9(13)-cyclodiscodermolide, and laulimalide.

[0087] Other microtubule stabilizing compounds are described in U.S. Pat. Nos. 6,624,317; 6,610,736; 6,605,599; 6,589,968; 6,583,290; 6,576,658; 6,515,017; 6,531,497; 6,500,858; 6,498,257; 6,495,594; 6,489,314; 6,458,976; 6,441,186; 6,441,025; 6,414,015; 6,387,927; 6,380,395; 6,380,394; 6,362,217; 6,359,140; 6,306,893; 6,302,838; 6,300,355; 6,291,690; 6,291,684; 6,268,381; 6,262,107; 6,262,094; 6,147,234; 6,136,808; 6,127,406; 6,100,411; 6,096,909; 6,025,385; 6,011,056; 5,965,718; 5,955,489; 5,919,815; 5,912,263; 5,840,750; 5,821,263; 5,767,297; 5,728,725; 5,721,268; 5,719,177; 5,714,513; 5,587,489; 5,473,057; 5,407,674; 5,250,722; 5,010,099; and 4,939,168; and U.S. patent application Publication Nos. 2003/0186965 A1; 2003/0176710 A1; 2003/0176473 A1; 2003/0144523 A1; 2003/0134883 A1; 2003/0087888 A1; 2003/0060623 A1; 2003/0045711 A1; 2003/0023082 A1; 2002/0198256 A1; 2002/0193361 A1; 2002/0188014 A1; 2002/0165257 A1; 2002/0156110 A1; 2002/0128471 A1; 2002/0045609 A1; 2002/0022651 A1; 2002/0016356 A1; 2002/0002292 A1, each of which is hereby incorporated by reference.

[0088] Other chemotherapeutic agents that may be administered with a compound of the present invention are listed in the following table:

Alkylating agents	cyclophosphamide lomustine busulfan procarbazine ifosfamide altretamine melphalan estramustine phosphate hexamethylmelamine	mechlorethamine thiotepa streptozocin chlorambucil temozolomide dacarbazine semustine carmustine
Platinum agents	cisplatin carboplatinum oxaliplatin ZD-0473 spiroplatinum lobaplatin carboxyphthalatoplatinum satraplatin	tetraplatin BBR-3464 Ormiplatin SM-11355 iproplatin AP-5280

Antimetabolites	azacytidine tomudex gemcitabine trimetrexate capecitabine deoxycoformycin 5-fluorouracil fludarabine floxuridine pentostatin 2-chlorodeoxyadenosine raltitrexed	6-mercaptopurine hydroxyurea 6-thioguanine decitabine cytarabin clofarabine 2-fluorodeoxy cytidine irofulven methotrexate DMDC idatrexate ethynylcytidine
Topoisomerase inhibitors	amsacrine rubitecan epirubicin exatecan mesylate etoposide quinamed teniposide or mitoxantrone gimatecan irinotecan (CPT-11) diflomotecan (Beaufour-Ipsen) 7-ethyl-10-hydroxy-camptothecin	TAS-103 Topotecan elsamitrucin dexrazoxanet J-107088 pixantrone BNP-1350 rebeccamycin analogues CKD-602 (BBR-3576 KW-2170
Antitumor antibiotics	dactinomycin (actinomycin D) amonafide doxorubicin (adriamycin) azonafide deoxyrubicin anthrapyrazole valrubicin oxantrazole daunorubicin (daunomycin) losoxantrone epirubicin bleomycin sulfate (blenoxane) therarubicin	bleomycinic acid idarubicin bleomycin A rubidazone bleomycin B plicamycinp mitomycin C porfiromycin MEN-10755 cyanomorpholinodoxorubicin GPX-100 mitoxantrone (novantrone)
Antimitotic agents	paclitaxel SB 408075 docetaxel E7010 Colchicines PG-TXL vinblastine IDN 5109 Vincristine A 105972 Vinorelbine A 204197	RPR 109881A ZD 6126 TXD 258 PEG-paclitaxel epothilone B AZ10992 T 900607 IDN-5109 T 138067 AVLB

	Vindesine LU 223651 dolastatin 10 D 24851 rhizoxin ER-86526 mivobulin combretastatin A4 cemadotin isohomohalichondrin-B	cryptophycin 52 azaepothilone B vinflunine BNP-7787 auristatin PE CA-4 prodrug BMS 247550 dolastatin-10 BMS 184476 CA-4 BMS 188797 taxoprexin
Aromatase inhibitors	Aminoglutethimide Exemestane Letrozole atamestane	anastrazole YM-511 formestane
Thymidylate synthase inhibitors	pemetrexed nolatrexed	ZD-9331 CoFactor™
DNA antagonists	trabectedin mafosfamide glufosfamide apaziquone	albumin + 32P O6 benzyl guanine thymectacin edotreotide
Farnesyl-transferase inhibitors	arglabin tipifarnib lonafarnib	perillyl alcohol
Pump inhibitors	CBT-1 zosuquidar trihydrochloride	tariquidar biricodar dicitrate MS-209)
Histone acetyltransferase inhibitors	tacedinaline pivaloyloxymethyl butyrate SAHA	depsipeptide MS-275
Metallo-proteinase inhibitors	Neovastat CMT-3	marimastat BMS-275291
Ribonucleoside reductase inhibitors	gallium maltolate tezacitabine	triapine didox
TNF alpha agonists/ antagonists	virulizin revimid	CDC-394
Endothelin A	atrasentan	ZD-4054

receptor antagonist	YM-598	
Retinoic acid receptor agonists	fenretinide alitretinoin	LGD-1550
Immuno-modulators	Interferon dexosome therapy oncophage pentrix GMK ISF-154 adenocarcinoma vaccine cancer vaccine (Intercell) CTP-37	norelin IRX-2 BLP-25 PEP-005 MGV synchrovax vaccines beta.-alethine melanoma vaccine CLL therapy p21 RAS vaccine
Hormonal and antihormonal agents	estrogens Prednisone conjugated estrogens methylprednisolone ethinyl estradiol prednisolone chlortrianisen aminoglutethimide idenestrol leuprolide hydroxyprogesterone caproate goserelin medroxyprogesterone leuporelin testosterone	bicalutamide testosterone propionate; fluoxymesterone flutamide methyltestosterone octreotide diethylstilbestrol nilutamide megestrol mitotane tamoxifen P-04 (Novogen) Toremofine 2-methoxyestradiol dexamethasone arzoxifene
Photodynamic agents	talaporfin Pd-bacteriopheophorbide Theralux lutetium texaphyrin	motexafin gadolinium hypericin
Kinase Inhibitors	imatinib kahalide F leflunomide CEP-701 ZD1839 CEP-751 erlotinib MLN518 canertinib PKC412 squalamine phenoxodiol SU5416 trastuzumab	C225 ZD4190 rhu-Mab ZD6474 MDX-H210 vatalanib 2C4 PKI166 MDX-447 GW2016 ABX-EGF EKB-509 IMC-1C11

	SU6668 Sorafenib Cetuximab ZD1839 PKI 166	EKB-569 CI-1033 EKB-569 Semaxanib ZD6474 PTK-787 INC-1C11

Miscellaneous agents	
SR-27897 (CCK A inhibitor), Sanofi-Synthelabo)	gemtuzumab (CD33 antibody, Wyeth Ayerst)
BCX-1777 (PNP inhibitor, BioCryst)	CCI-779 (mTOR kinase inhibitor, Wyeth)
tocladesine (cyclic AMP agonist, Ribapharm)	PG2 (hematopoiesis enhancer, Pharmagenesis)
ranpirnase (ribonuclease stimulant, Alfacell)	exisulind (PDE V inhibitor, Cell Pathways)
alvocidib (CDK inhibitor, Aventis)	Immunol™ (triclosan oral rinse, Endo)
galarubicin (RNA synthesis inhibitor, Dong-A)	CP-461 (PDE V inhibitor, Cell Pathways)
CV-247 (COX-2 inhibitor, Ivy Medical)	triacetyluridine (uridine prodrug, Wellstat)
tirapazamine (reducing agent, SRI International)	AG-2037 (GART inhibitor, Pfizer)
P54 (COX-2 inhibitor, Phytopharm)	SN-4071 (sarcoma agent, Signature BioScience) WX-UK1 (plasminogen activator inhibitor, Wilex)
N-acetylcysteine (reducing agent, Zambon)	TransMID-107 .TM. (immunotoxin, KS Biomedix)
CapCell™ (CYP450 stimulant, Bavarian Nordic)	PBI-1402 (PMN stimulant, ProMetic LifeSciences)
R-flurbiprofen (NF-kappaB inhibitor, Encore)	PCK-3145 (apoptosis promotor, Procyon)
GCS-100 (gal3 antagonist, GlycoGenesys)	bortezomib (proteasome inhibitor, Millennium)
3CPA (NF-kappaB inhibitor, Active Biotech)	doranidazole (apoptosis promotor, Pola)
G17DT immunogen (gastrin inhibitor, Aphton)	SRL-172 (T cell stimulant, SR Pharma)
seocalcitol (vitamin D receptor agonist, Leo)	CHS-828 (cytotoxic agent, Leo)
efaproxiral (oxygenator, Allos Therapeutics)	TLK-286 (glutathione S transferase inhibitor, Telik)
131-I-TM-601 (DNA antagonist, TransMolecular)	trans-retinoic acid (differentiator, NIH)
PI-88 (heparanase inhibitor, Progen)	PT-100 (growth factor agonist, Point Therapeutics)
eflornithine (ODC inhibitor, ILEX Oncology)	MX6 (apoptosis promotor, MAXIA)
tesmilifene (histamine antagonist, YM BioSciences)	midostaurin (PKC inhibitor, Novartis)
minodronic acid (osteoclast inhibitor, Yamanouchi)	apomine (apoptosis promotor, ILEX Oncology)
histamine (histamine H2 receptor agonist, Maxim)	bryostatin-1 (PKC stimulant, GPC Biotech)
indisulam (p53 stimulant, Eisai)	urocidin (apoptosis promotor, Bioniche)
tiazofurin (IMPDH inhibitor, Ribapharm)	CDA-II (apoptosis promotor, Everlife)
aplidine (PPT inhibitor, PharmaMar)	Ro-31-7453 (apoptosis promotor, La Roche)
cilengitide (integrin antagonist, Merck KGaA)	SDX-101 (apoptosis promotor, Salmedix)
rituximab (CD20 antibody, Genentech)	brostallicin (apoptosis promotor, Pharmacia)
SR-31747 (IL-1 antagonist, Sanofi-Synthelabo)	ceflatonin (apoptosis promotor, ChemGenex)
	Herceptin

[0089] When the disease or disorder to be treated is an inflammatory or autoimmune disorder, especially rheumatoid arthritis (RA), the compound of Formula 1 or salt thereof

can be administered in combination with one or more non-steroidal anti-inflammatory drugs (NSAIDs), analgesics, corticosteroids and disease-modifying antirheumatic drugs. Other agents which may be useful in combination with Formula 1 or salt thereof for such applications include interleukin-1 (IL-1) receptor antagonist therapy such as anakinra (Kineret™), tocilizumab (Actemra™), hydroxychloroquine (Plaquenil™), sulfasalazine (Azulfidine™), leflunomide (Arava™), tumor necrosis factor inhibitors such as etanercept (Enbrel™), adalimumab (Humira™), and infliximab (Remicade™), T-cell costimulatory blocking agents such as abatacept (Orencia™), B cell depleting agents such as rituximab (Rituxan™), natalizumab (Tysabri™), intramuscular gold and other immunomodulatory and cytotoxic agents such as azathioprine (Imuran™), cyclophosphamide and cyclosporine A (Neoral™, Sandimmune™).

[0090] Still other agents which may be useful in combination with a compound of Formula 1 or salt thereof for the treatment of RA include methotrexate, alemtuzumab (Campath™), anti-RANKL MAb (denosumab), anti-Blys MAb belimumab (LymphoStat-B™), certolizumab pegol (Cimzia™), p38 inhibitors, JAK inhibitors, anti-TNF agents, anti-CD20 MAbs, anti-IL/ILR targeting agents such as those which target IL-1, IL-5, IL-6 (toclizumab), Il-4, IL-13, and IL-23.

[0091] Additional combinations may also include agents which reduce the toxicity of the aforesaid agents, such as hepatic toxicity, neuronal toxicity, nephrotoxicity and the like.

Screening assays

[0092] The compounds of the present invention may also be used in a method to screen for other compounds that bind to an IAP BIR domain. Generally speaking, to use the compounds of the invention in a method of identifying compounds that bind to an IAP BIR domain, the IAP is bound to a support, and a compound of the invention is added to the assay. Alternatively, the compound of the invention may be bound to the support and the IAP is added.

[0093] There are a number of ways in which to determine the binding of a compound of the present invention to the BIR domain. In one way, the compound of the invention, for example, may be fluorescently or radioactively labeled and binding determined directly.

For example, this may be done by attaching the IAP to a solid support, adding a detectably labeled compound of the invention, washing off excess reagent, and determining whether the amount of the detectable label is that present on the solid support. Numerous blocking and washing steps may be used, which are known to those skilled in the art.

[0094] In some cases, only one of the components is labeled. For example, specific residues in the BIR domain may be labeled. Alternatively, more than one component may be labeled with different labels; for example, using I^{125} for the BIR domain, and a fluorescent label for the probe.

[0095] The compounds of the invention may also be used as competitors to screen for additional drug candidates or test compounds. As used herein, the terms "drug candidate" or "test compounds" are used interchangeably and describe any molecule, for example, protein, oligopeptide, small organic molecule, polysaccharide, polynucleotide, and the like, to be tested for bioactivity. The compounds may be capable of directly or indirectly altering the IAP biological activity.

[0096] Drug candidates can include various chemical classes, although typically they are small organic molecules having a molecular weight of more than 100 and less than about 2,500 Daltons. Candidate agents typically include functional groups necessary for structural interaction with proteins, for example, hydrogen bonding and lipophilic binding, and typically include at least an amine, carbonyl, hydroxyl, ether, or carboxyl group. The drug candidates often include cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more functional groups.

[0097] Drug candidates can be obtained from any number of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means.

[0098] Competitive screening assays may be done by combining an IAP BIR domain and a probe to form a probe:BIR domain complex in a first sample followed by adding a test compound from a second sample. The binding of the test is determined, and a change or difference in binding between the two samples indicates the presence of a test compound capable of binding to the BIR domain and potentially modulating the IAP's activity.

[0099] Thus, provided herein as an aspect of the invention is a probe comprising a compound of the invention and a detectable label or affinity tag. Detectable labels include any chemical moeity that may be linked to a compound of the present invention such that when the compound comprising the label is associated with the BIR domain, the label allows either direct or indirect detection of the compound. Preferably, the label also allows for quantification. Affinity tags are moieties that facilitate isolation or purification of compounds to which they are attached.

[00100] As used herein, the term "probe" is intended to mean a compound of Formula 1 or salt thereof which is labeled with either a detectable label or an affinity tag, and which is capable of binding, either covalently or non-covalently, to an IAP BIR domain. When, for example, the probe is non-covalently bound, it may be displaced by a test compound. When, for example, the probe is bound covalently, it may be used to form cross-linked adducts, which may be quantified and inhibited by a test compound.

[0100] In one case, the binding of the test compound is determined through the use of competitive binding assays. In this embodiment, the probe is labeled with a fluorescent label. Under certain circumstances, there may be competitive binding between the test compound and the probe. Test compounds which display the probe, resulting in a change in fluorescence as compared to control, are considered to bind to the BIR region.

[0101] In one case, the test compound may be labeled. Either the test compound, or a compound of the present invention, or both, is added first to the IAP BIR domain for a time sufficient to allow binding to form a complex.

[0102] Formation of the probe:BIR domain complex typically require incubations of between 4 °C and 40 °C for between 10 minutes to about 1 hour to allow for high-throughput screening. Any excess of reagents are generally removed or washed away.

The test compound is then added, and the presence or absence of the labeled component is followed, to indicate binding to the BIR domain.

[0103] In one case, the probe is added first, followed by the test compound. Displacement of the probe is an indication the test compound is binding to the BIR domain and thus is capable of binding to, and potentially modulating, the activity of IAP. Either component can be labeled. For example, the presence of probe in the wash solution indicates displacement by the test compound. Alternatively, if the test compound is labeled, the presence of the probe on the support indicates displacement.

[0104] In one case, the test compound may be added first, with incubation and washing, followed by the probe. The absence of binding by the probe may indicate the test compound is bound to the BIR domain with a higher affinity. Thus, if the probe is detected on the support, coupled with a lack of test compound binding, may indicate the test compound is capable of binding to the BIR domain.

[0105] Modulation is tested by screening for a test compound's ability to modulate the activity of IAP and includes combining a test compound with an IAP BIR domain, as described above, and determining an alteration in the biological activity of the IAP. Therefore in this case, the test compound should both bind to the BIR domain (although this may not be necessary), and alter its biological activity as defined herein.

[0106] Positive controls and negative controls may be used in the assays. All control and test samples are performed multiple times to obtain statistically significant results. Following incubation, all samples are washed free of non-specifically bound material and the amount of bound probe determined. For example, where a radiolabel is employed, the samples may be counted in a scintillation counter to determine the amount of bound compound.

[0107] Typically, the signals that are detected in the assay may include fluorescence, resonance energy transfer, time resolved fluorescence, radioactivity, fluorescence polarization, plasma resonance, or chemiluminescence and the like, depending on the nature of the label. Detectable labels useful in performing screening assays in this invention include a fluorescent label such as Fluorescein, Oregon green, dansyl, rhodamine, tetramethyl rhodamine, texas red, Eu^{3+}; a chemiluminescent label

such as luciferase; colorimetric labels; enzymatic markers; or radioisotopes such as tritium, I^{125} and the like. Affinity tags, which may be useful in performing the screening assays of the present invention include be biotin, polyhistidine and the like.

EXAMPLES

[0108] The following terms and abbreviations, constructs, and general procedures are used in the Examples:

Abbreviations and Terms

Boc: *t*-butoxycarbonyl;
Boc-Chg-OH: Boc-L-2(cyclohexyl)glycine
Boc-N-MeAla-OH: N-Boc-N-methylalanine
CBz: benzyloxycarbonyl;
DIPEA: diisopropylethylamine;
DMAP: 4-(dimethylamino)pyridine;
DMF: N,N-dimethylformamide;
DTT: dithiothreitol;
EDC: 3-(dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride;
EDTA: ethylenediaminetetracetic acid;
Fmoc: N-(9-fluorenylmethoxycarbonyl);
HBTU: *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate;
HCl: hydrochloric acid;
HOAc: acetic acid;
HOBt: 1-hydroxybenzotriazole;
HPLC: high performance liquid chromatography;
LCMS: liquid chromatography-mass spectrometer;
MeOH: methanol;
$MgSO_4$: magnesium sulfate;
MS: mass spectrum;
Ms: methanesulfonyl;
$NaHCO_3$: sodium hydrogen carbonate;
Pd/C: palladium on carbon;

TEA: triethylamine;

TFA: trifluoroacetic acid;

THF: tetrahydrofuran;

TMEDA: N,N,N,N-tetramethylethylenediamine;

Ts: para-toluenesulfonyl.

IC_{50}: The amount, concentration, or dosage of a particular compound that achieves 50% of a maximum response.

EC_{50} The amount, concentration, or dosage of a particular compound that achieves 50% inhibition of cell survival.

Molecular constructs for expression

[0109] GST-XIAP linked BIR3RING: XIAP coding sequence amino acids 246-497 cloned into PGEX4T3 via BamH1 and AVA I. The plasmid was transformed into E. coli DH5α for use in protein expression and purification.

[0110] GST-HIAP2 (cIAP-1) linker BIR 3: HIAP2 coding sequence from amino acids 251-363 cloned into PGex4T3 via BamH1 and XhoI. The plasmid was transformed into E. coli DH5α for use in protein expression and purification.

[0111] GST-HIAP1(cIAP-2) linker BIR 3: HIAP1 coding sequence from amino acids 236-349, cloned into PGex4T3 via BamH1 and XhoI. The plasmid was transformed into E. coli DH5α for use in protein expression and purification.

[0112] GST- linker BIR 2 BIR3Ring: XIAP coding sequence from amino acids 93-497 cloned into PGex4T1 via BamH1 and XhoI. Amino acids 93-497 were amplified from full length XIAP in pGex4t3, using the primers: TTAATAGGATCCATCAACGGCTTTTATC and GCTGCATGTGTGTCAGAGG, using standard PCR conditions. The PCR fragment was TA cloned into pCR-2.1 (invitrogen). Linker BIR 2 BIR 3Ring was subcloned into pGex4T1 by BamHI/XhoI digestion. The plasmid was transformed into E. coli DH5α for use in protein expression and purification.

[0113] Full-length human XIAP, AEG plasmid number 23. XIAP coding sequence amino acids 1-497 cloned into GST fusion vector, PGEX4T3 via BamH1 and Xho I restriction sites. The plasmid was transformed into E. coli DH5α for use in protein purification.

[0114] GST-XIAP linker BIR 2: XIAP linker BIR 2 coding sequence from amino acids 93-497 cloned into pGex4T3 via BamHI and XhoI. The plasmid was transformed into E. coli DH5α for use in protein expression and purification.

Expression of Recombinant Proteins

[0115] Glutathione S-transferase (GST) tagged proteins were expressed in *Escherichia coli* strains DH5-alpha. For expression of full length XIAP, individual or combinations of XIAP-BIR domains, cIAP-1, cIAP-2 and Livin transformed bacteria were cultured overnight at 37 °C in Luria Broth (LB) medium supplemented with 50 ug/ml of ampicillin. The overnight culture was then diluted 25 fold into fresh LB ampicillin supplemented media and bacteria were grown up to A_{600} = 0.6 then induced with 1 mM isopropyl-D-1-thiogalactopyranoside for 3 hours. Upon induction, cells were centrifuged at 5000 RPM for 10 minutes and the media was removed. Each pellet obtained from a 1 liter culture received 10 ml of lysis buffer (50 mM Tris-HCl, 200 mM NaCl, 1 mM DTT, 1 mM PMSF, 2 mg/ml of lysosyme, 100 µg/ml)), was incubated at 4 °C with gentle shaking. After 20 minutes of incubation, the cell suspension was placed at -80 °C overnight or until needed.

Purification of recombinant proteins

[0116] For purification of recombinant proteins, the pellet was thaw on ice and resuspended with 25mL of lysis buffer (50 mM Tris-HCl pH 7.6, 0.1 mM EDTA, 100 mM NaCl, 100 µg/mL of lysozyme) /500 mL of original culture and incubated on ice for 15 min. and 5 cycles of freeze/thaw cycles were performed in liquid nitrogen and 37°C water bath. The mixture was sonicated using a probe sonicator until the suspension is no longer viscous and centrifuged at 13000g for 20 minutes to collect soluble fraction (supernatant).

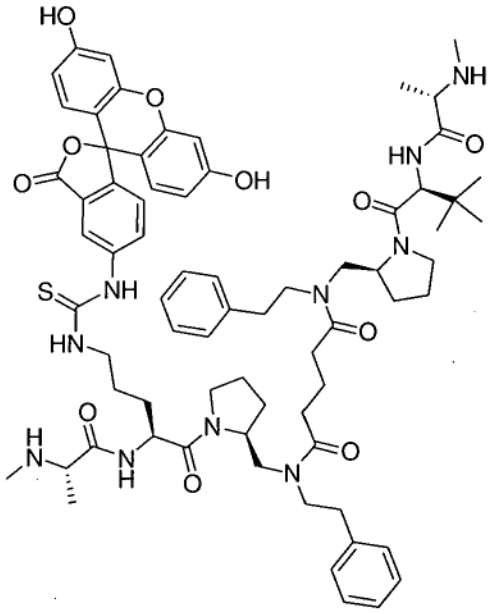
[0117] The resulting supernatant was mixed with 3mL of glutathione-Sepharose beads (Pharmacia) for 20min. at 4 °C. Afterwards, the beads were washed 3 times with 1X Tris-Buffered Saline (TBS) to remove unbound proteins. The retained proteins were eluted with 2 washes of 1mL of 50 mM TRIS pH 8.0 containing 10 mM reduced glutathione. The eluted proteins were kept separately and appropriate reagents were added to them for storage at -80 °C. As judged by SDS-PAGE the purified proteins were >90% pure. The protein concentration of purified proteins was determined from the Bradford method.

[0118] His-tag proteins were expressed in the E. Coli strain in E. coli AD494 cells using a pet28ACPP32 construct. The soluble protein fraction was prepared as described above. For protein purification, the supernatant was purified by affinity chromatography using chelating-Sepharose (Pharmacia) charged with $NiSO_4$ according to the manufacturer's instructions. Briefly, the supernatant were loaded into the $NiSO_4$ charged sepharose with 2mL of sepharose for 20min. at 4 °C . Afterwards, the beads were washed 3 times with 10 mM MOPS, pH 7.0 containing 500 mM NaCl to remove unbound proteins. The retained proteins were eluted with 2 mL of elution buffer (500 mM imidazole in Tris pH 8.0) and appropriate reagents were added to them for storage at -80 °C. Purity of the eluted protein was >90% pure as determined by SDS-PAGE. The protein concentration of purified proteins was determined from the Bradford assay.

Preparation of Probes P1 and P2

[0119] A fluorescent peptide probe P1, Fmoc-Ala-Val-Pro-Phe-Tyr(t-Bu)-Leu-Pro-Gly(t-Bu)-Gly-OH, was prepared using standard Fmoc chemistry on 2-chlorotrityl chloride resin (see Int. J. Pept. Prot. Res. 38:555-561, 1991). Cleavage from the resin was performed using 20% acetic acid in dichloromethane (dichloromethane), which left the side chain still blocked. The C-terminal protected carboxylic acid was coupled to 4'-(aminomethy)fluorescein (Molecular Probes, A-1351; Eugene, Oreg.) using excess diisopropylcarbodiimide (DIC) in dimethylformamide (DMF) at room temperature and was purified by silica gel chromatography (10% methanol in dichloromethane). The N-terminal Fmoc protecting group was removed using piperidine (20%) in DMF, and purified by silica gel chromatography (20% methanol in dichloromethane, 0.5% HOAc). Finally, the t-butyl side chain protective groups were removed using 95% trifluoroacetic acid containing 2.5% water and 2.5% triisopropyl silane, to provide probe P1 (>95% pure, HPLC).

[0120] Probe P2 was prepared using methods as described in WO 2007/131,366.

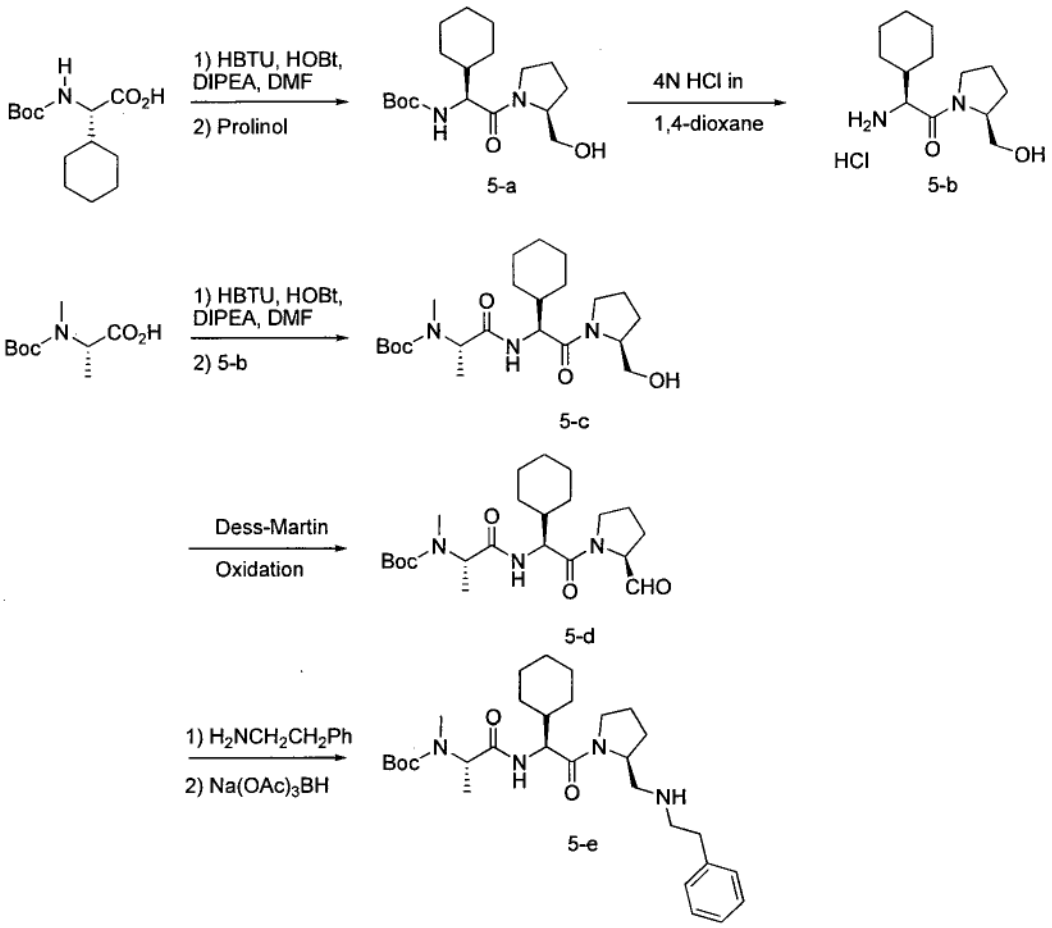


Probe P2

EXAMPLE 1

[0121] The following example illustrates the preparation of compound 5-e, which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof.

Scheme 5: Synthesis of Intermediate 5-e



[0122] *Step 1*: To a solution of Boc-Chg-OH (9.16 g, 35.6 mmol) in DMF cooled to 0 ºC were sequentially added DIPEA (10.33 mL, 59.3 mmol), HOBt (4.81 g, 35.6 mmol) and HBTU (13.50 g, 35.6 mmol). After stirring for 10 minutes (S)-prolinol (3.0 g, 29.7 mmol) was added and the reaction mixture was stirred overnight at room temperature. Water and ethyl acetate were added, the organic layer was separated, washed with 10 % citric acid, aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 5-a as colorless oil.

[0123] *Step 2*: 4N HCl in 1,4 -dioxane (30 mL) was added to intermediate 5-a (10.10 g, 29.7 mmol) and the solution was stirred for 1 hour at room temperature. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide intermediate 5-b·HCl as a white solid. MS (m/z) M+1= 240.2

[0124] *Step 3*: To a solution of Boc-N-Me-Ala-OH (6.02 g, 29.6 mmol) in DMF cooled to 0 ºC were sequentially added DIPEA (20.70 mL, 118 mmol), HOBt (6.35 g, 41.5 mmol) and HBTU (14.61 g, 38.5 mmol). After stirring for 10 minutes intermediate 5-b·HCl (8.20 g, 29.6 mmol) was added and the reaction mixture was stirred overnight at room temperature. Water and ethyl acetate were added, the organic layer was separated, washed with 10 % citric acid, aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 5-c as colorless oil.

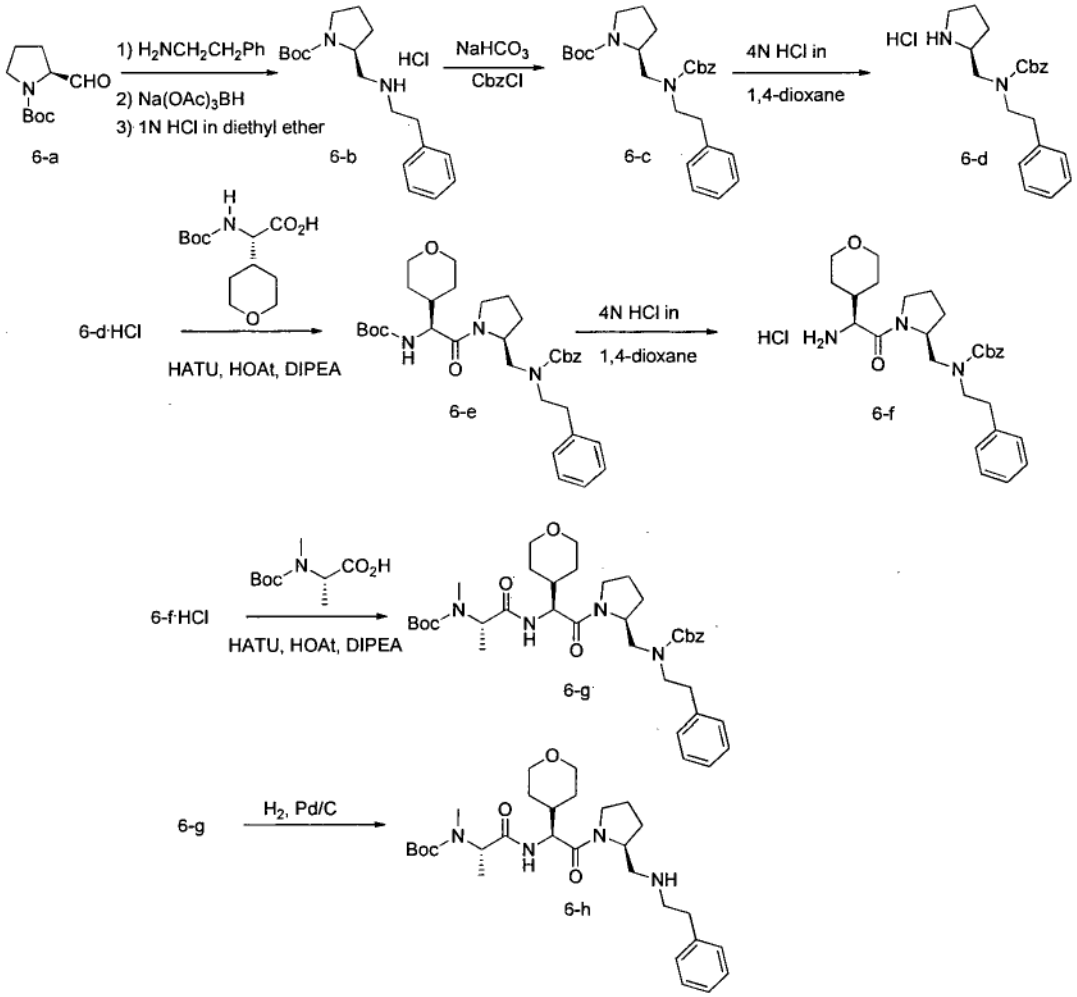
[0125] *Step 4*: To a solution of intermediate 5-c (1.20 g, 2.82 mmol) in CH_2Cl_2 cooled to 0 ºC were sequentially added sodium hydrogencarbonate (2.36 g, 28.2 mmol) and Dess-Martin periodinane (1.49 g, 3.52 mmol) and the reaction was then stirred for 2 hours at 10 ºC. Aqueous $NaHCO_3$ and ethyl acetate were added, the organic layer was separated, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 5-d as colorless oil.

[0126] *Step 5*: To a solution of intermediate 5-d (500 mg, 1.18 mmol) in CH_2Cl_2 was added phenethylamine (283 uL, 1.88 mmol). After stirring for 2 hours at room temperature sodium triacetoxyborohydride (300 mg, 1.41 mmol) and methanol were added and the reaction was stirred at room temperature overnight. Saturated aqueous $NaHCO_3$ and ethyl acetate were added, the organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 5-e as colorless oil. MS (m/z) M+1=528.4.

EXAMPLE 2

[0127] The following example illustrates the preparation of compound 6-h, which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof.

Scheme 6: Synthesis of Intermediate 6-h



[0128] *Step 1:* To a solution of N-(tert-butoxycarbonyl)-L-prolinal 6-a (10.0 g, 50.2 mmol) in dichloromethane (300 mL) was added phenethylamine (6.52 mL, 50.2 mmol). After stirring for 2 hours at room temperature, the reaction was cooled to 0 °C, sodium triacetoxyborohydride (21.0 g, 100.3 mmol) was added portionwise and the reaction mixture was then stirred at room temperature overnight. 10% aqueous Na_2CO_3 was added, the organic layer was separated, the aqueous phase was extracted with dichloromethane, the combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 6-b as a colorless oil. Intermediate 6-b was dissolved in diethyl ether (125 mL), the solution was cooled to 0 °C and 1N HCl in diethyl ether (50.0 mL, 50.0 mmol) was added. A precipitate

formed and intermediate 6-b·HCl was collected by filtration as a white solid. MS (m/z) M+1= 305.2

[0129] *Step 2:* To a solution of intermediate 6-b (6.11 g, 20.08 mmol) in 1,4-dioxane (50.0 mL) and water (50 mL) cooled to 0 °C was added $NaHCO_3$ (8.43 g, 100.0 mmol). After stirring for 15 minutes benzyl chloroformate (3.43 mL, 24.10 mmol) was added and the reaction was then stirred at room temperature for 2 hours. Water and ethyl acetate were added, the organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 6-c as a colorless oil.

[0130] *Step 3*: 4N HCl in 1,4-dioxane (67.1 mL) was added to intermediate 6-c (8.41 g, 19.18 mmol) at 0°C and the solution was stirred for 2 hours at room temperature. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide intermediate 6-d·HCl as a beige solid. MS (m/z) M+1= 339.3

[0131] *Step 4:* To a solution of intermediate 6-d (394 mg, 1.05 mmol) in DMF cooled to 0 °C were sequentially added (S)-2-Boc-2-(tetrahydro-2H-pyran-4-yl) acetic acid (300 mg, 1.15 mmol), HATU (520 mg, 1.36 mmol), HOAt (48 uL, 0.21 mmol) and DIPEA (733 uL, 4.21 mmol) and the reaction was then stirred at room temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 6-e as a yellowish oil.

[0132] *Step 5:* 4N HCl in 1,4-dioxane (3.68 mL) was added to intermediate 6-e (610 mg, 1.05 mmol) at 0 °C and the solution was stirred at 0 °C for 4 hours. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide the expected intermediate 6-f·HCl as a white solid. MS (m/z) M+1= 480.4

[0133] *Step 6:* To a solution of intermediate 6-f·HCl (271 mg, 0.52 mmol) in DMF cooled to 0 °C were sequentially added Boc-N-Me-Ala-OH (117 mg, 0.58 mmol), HATU (240 mg, 0.63 mmol), HOAt (175 uL, 0.10 mmol) and DIPEA (366 uL, 2.10 mmol) and the

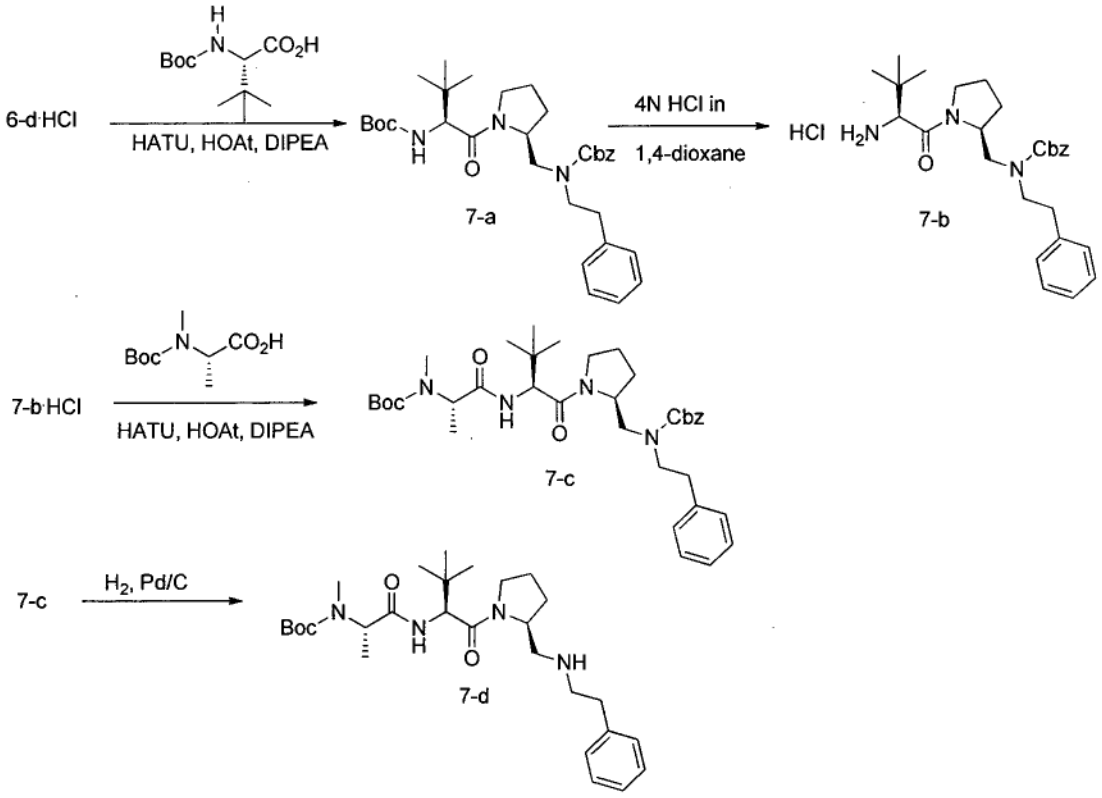
reaction was then stirred at room temperature for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 6-g as a light yellow oil.

[0134] *Step 7:* To a solution of intermediate 6-g (277 mg, 0.41 mmol) in THF and stirred under N_2 was added 10 % Pd/C (50% w/w water content) (89 mg). The reaction mixture was purged with H_2 and stirred for 3 hours. The reaction was then filtered through celite and the filtrate was concentrated in vacuo. Purification by silica gel chromatography provided intermediate 6-h as colorless oil. MS (m/z) M+1= 531.5.

EXAMPLE 3

[0135] The following example illustrates the preparation of compound 7-d, which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof.

Scheme 7: Synthesis of Intermediate 7-d



[0136] *Step 1:* To a solution of intermediate 6-d·HCl (95.90 g, 256 mmol) in DMF (1300 mL) cooled to 0 °C were sequentially added Boc-tBu-gly-OH (65.10 g, 281 mmol), HOAt (42.6 mL, 25.6 mmol), HATU (107 g, 281 mmol) and DIPEA (179 mL, 1023 mmol) and the reaction was then stirred at 0 °C for 30 min. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 7-a as a colorless oil.

[0137] *Step 2:* 4N HCl in 1,4-dioxane (480 mL) was added to intermediate 7-a (141.00 g, 256 mmol) in methanol (130 mL) at 0°C and the solution was stirred for 30 minutes at 0 °C followed by 3 hours at room temperature. Volatiles were removed under

reduced pressure and the residue was triturated with diethyl ether to provide intermediate 7-b·HCl as a white solid. MS (m/z) M+1= 452.4

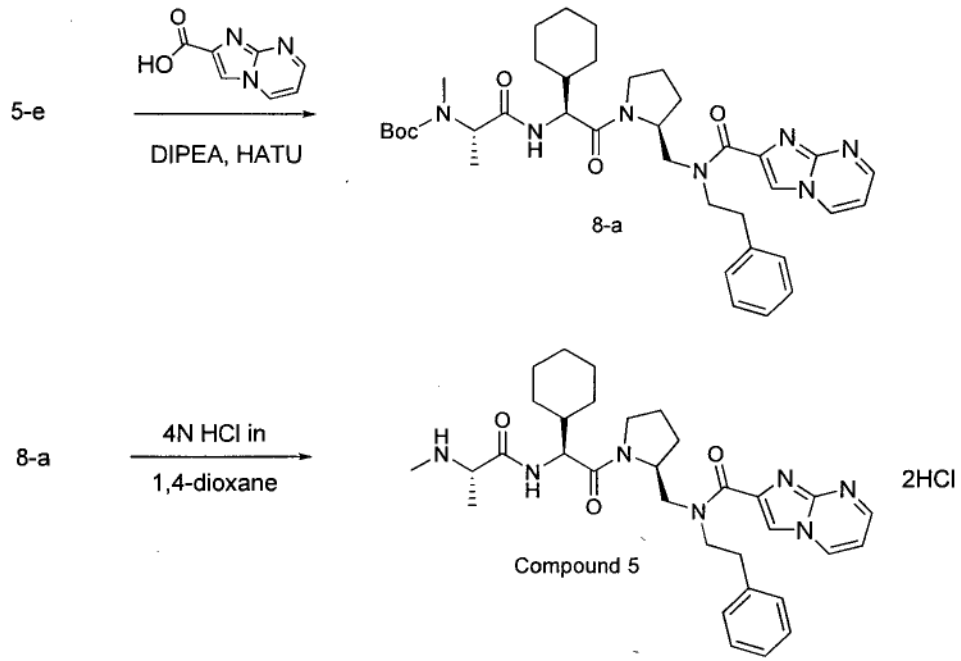
[0138] *Step 3:* To a solution of intermediate 7-b·HCl (85.00 g, 174 mmol) in DMF (870 mL) cooled to 0 °C were sequentially added Boc-N-Me-Ala-OH (38.90 g, 192 mmol), HOAt (37.70, 22.64 mmol), HATU (72.80 g, 56.3 mmol) and DIPEA (122 mL, 192 mmol) and the reaction was then stirred at 0 °C for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 7-c as white foam.

[0139] *Step 4:* To a solution of intermediate 7-c (1.56 g, 2.45 mmol) in methanol and stirred under N_2 was added 10 % Pd/C (50% w/w water content) (500 mg). The reaction mixture was purged with H_2 and stirred for 3 hours. The reaction was then filtered through celite and the filtrate was concentrated in vacuo to give intermediate 7-d as a colorless oil. MS (m/z) M+1= 503.5.

EXAMPLE 4

[0140] The following example illustrates the preparation of compound 5, which is a compound of Formula 1 or salt thereof.

Scheme 8: Synthesis of compound 5



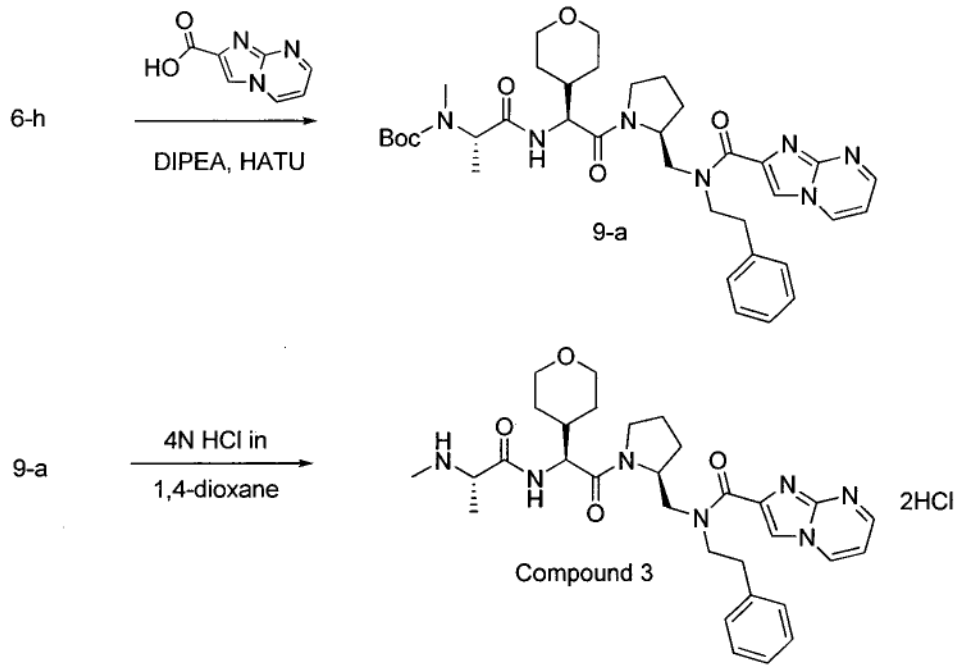
[0141] *Step 1:* To a solution of intermediate 5-e (150 mg, 0.28 mmol) in DMF, cooled to 0 ºC, were sequentially added imidazo[1,2-a]pyrimidine-2-carboxylic acid (56 mg, 0.34 mmol), HATU (162 mg, 0.42 mmol) and DIPEA (500 uL, 2.87 mmol). The reaction mixture was stirred for 2 hours at room temperature. Water and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 8-a as a white solid.

[0142] *Step 2:* 4N HCl in 1,4-dioxane (1.8 mL) was added to intermediate 8-a (99 mg, 0.14 mmol) in ethyl acetate (0.5 mL) and the solution was stirred for 1 hour at room temperature. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 5·2HCl as a white solid. MS (m/z) M+1= 574.4.

EXAMPLE 5

[0143] The following example illustrates the preparation of compound 3, which is a compound of Formula 1 or salt thereof.

Scheme 9: Synthesis of compound 3



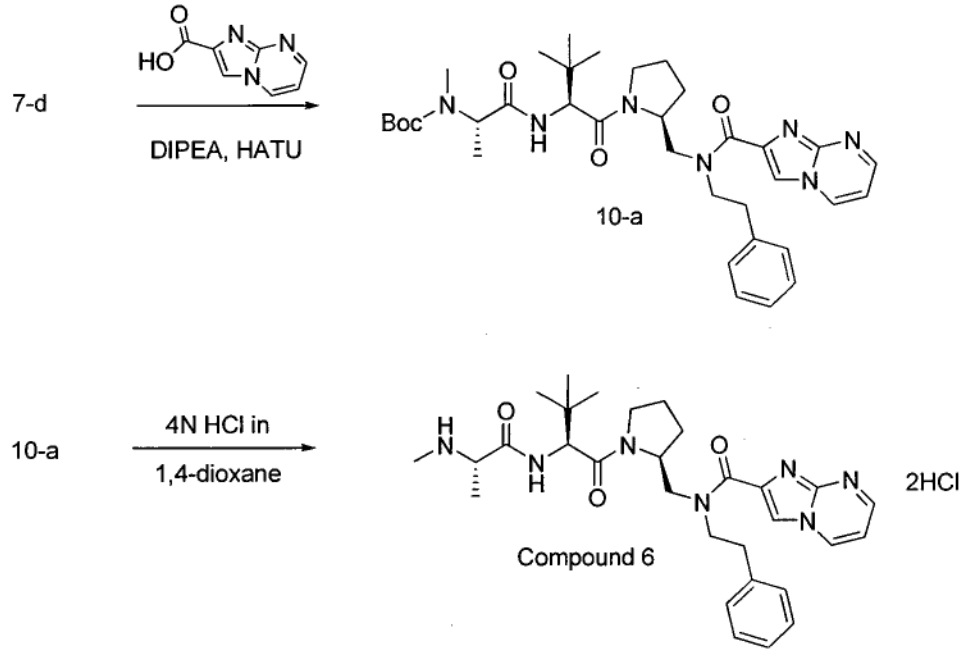
[0144] *Step 1:* To a solution of intermediate 6-h (221 mg, 0.41 mmol) in DMF cooled to 0 ºC were sequentially added imidazo[1,2-a]pyrimidine-2-carboxylic acid (74.7 mg, 0.45 mmol), HATU (206 mg, 0.54 mmol) and DIPEA (218 uL, 1.24 mmol),. The reaction mixture was stirred for 2 hours at room temperature. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 9-a as a colorless oil.

[0145] *Step 2:* 4N HCl in 1,4-dioxane (554 uL) was added to intermediate 9-a (107 mg, 0.15 mmol) in ethyl acetate (0.5 mL) at 0 ºC and the solution was stirred for 1 hour at 0ºC. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 3·2HCl as a white solid. MS (m/z) M+1= 576.4.

EXAMPLE 6

[0146] The following example illustrates the preparation of compound 6, which is a compound of Formula 1 or salt thereof.

Scheme 10: Synthesis of compound 6



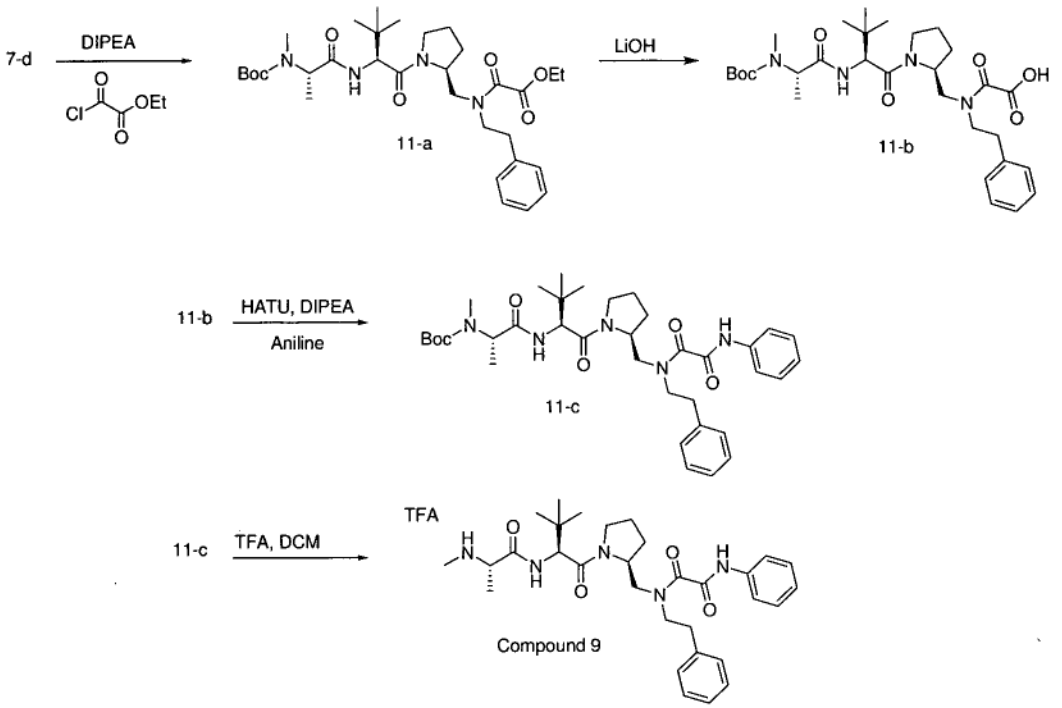
[0147] *Step 1:* To a solution of intermediate 7-d (1.97 g, 3.94 mmol) in DMF cooled to 0 ºC were sequentially added imidazo[1,2-*a*]pyrimidine-2-carboxylic acid (642 mg, 3.94 mmol), HATU (1.94 g, 5.12 mmol) and DIPEA (2.05 mL, 11.81 mmol),. The reaction mixture was stirred for 2 hours at room temperature. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 10-a as a white solid.

[0148] *Step 2:* 4N HCl in 1,4-dioxane (9.40 mL) was added to intermediate 10-a (1.74 g, 2.69 mmol) in ethyl acetate (5 mL) at 0℃ and the solution was stirred for 3 hours at 0℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 6·2HCl as a white solid. MS (m/z) M+1= 548.4

EXAMPLE 7

[0149] The following example illustrates the preparation of compound 9, which is a compound of Formula 1 or salt thereof.

Scheme 11: Synthesis of compound 9



[0150] *Step 1:* To a solution of intermediate 7-d (200 mg, 0.39 mmol) in dichloromethane cooled to 0 °C were sequentially added DIPEA (174 uL, 0.99 mmol) and ethyl 2-chloro-2-oxoacetate (109 mg, 0.79 mmol). The reaction mixture was stirred for 3 hours at room temperature. 1N HCl and ethyl acetate were added; the organic layer was separated, washed saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 11-a as a white solid.

[0151] *Step 2:* To a solution of intermediate 11-a (215 mg, 0.35 mmol) in THF cooled to 0 °C was added 2N aqueous LiOH (1.0 mL, 2.0 mmol) and the reaction was stirred for 1 hour at room temperature. 1N HCl and ethyl acetate were added; the organic

layer was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 11-b as a white solid.

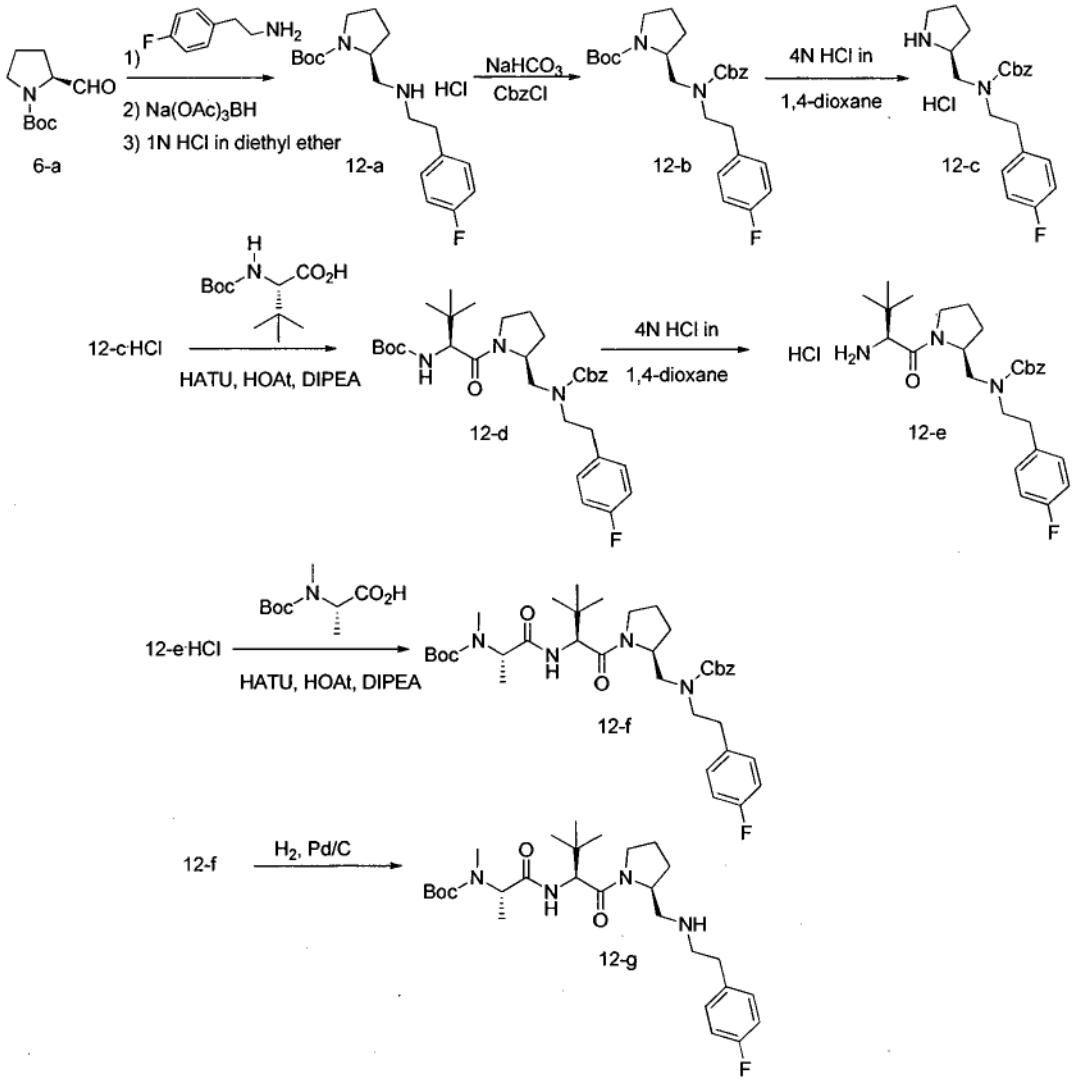
[0152] *Step 3:* To a solution of intermediate 11-b (200 mg, 0.34 mmol) in DMF cooled to 0 ºC were sequentially added aniline (41 uL, 0.45 mmol), DIPEA (61 uL, 0.34 mmol) and HATU (172 mg, 0.45 mmol) and the reaction mixture was stirred for 2 hours at 0 ºC. Water and ethyl acetate were added; the organic layer was separated, washed with 1N aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 11-c as a white solid.

[0153] *Step 4:* To a solution of intermediate 11-c (230 mg, 0.35 mmol) in dichloromethane (2 mL) cooled to 0ºC was added TFA (2 mL) and the reaction was then stirred at 0ºC for 1 hour. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 9·TFA as a white solid. MS (m/z) M+1=550.1

EXAMPLE 8

[0154] The following example illustrates the preparation of compound 12-g, which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof.

Scheme 12: Synthesis of Intermediate 12-g



[0155] *Step 1:* To a solution of N-(tert-butoxycarbonyl)-L-prolinal 6-a (30.0 g, 151.0 mmol) in dichloromethane (1000 mL) was added 2-(4-fluorophenyl)ethanamine (19.79 mL, 151.0 mmol). After stirring for 2 hours at room temperature, the reaction was cooled to 0 °C, sodium triacetoxyborohydride (38.3 g, 181.0 mmol) was added portionwise and the reaction mixture was then stirred at room temperature overnight. 10% aqueous Na_2CO_3 (800 mL) was added, the organic layer was separated, the aqueous phase was extracted with dichloromethane, the combined organic extracts were washed with brine, dried over

$MgSO_4$, filtered and concentrated in vacuo to provide intermediate 12-a as a colorless oil. Intermediate 12-a was dissolved in diethyl ether (400 mL), the solution was cooled to 0 °C and 1N HCl in diethyl ether (151.0 mL, 151.0 mmol) was added. A precipitate formed and intermediate 12-a·HCl was collected by filtration as a white solid. MS (m/z) M+1= 323.3

[0156] *Step 2:* To a solution of intermediate 12-a.HCl (40.0 g, 111.0 mmol) in 1,4-dioxane (300 mL) and water (300 mL) cooled to 0 °C was added $NaHCO_3$ (46.8 g, 557.0 mmol). After stirring for 15 minutes benzyl chloroformate (17.50 mL, 123.0 mmol) was added and the reaction was then stirred at room temperature for 1.5 hour. Water and ethyl acetate were added, the organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 12-b as a colorless oil.

[0157] *Step 3*: 4N HCl in 1,4-dioxane (139 mL) was added to intermediate 12-b (50.7 g, 111.0 mmol) at 0 °C and the solution was stirred for 2.5 hours at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether and hexanes to provide intermediate 12-c·HCl as a white foam. MS (m/z) M+1= 357.3

[0158] *Step 4:* To a solution of intermediate 12-c·HCl (38.9 g, 99.0 mmol) in DMF cooled to 0 °C were sequentially added Boc-tBu-gly-OH (15.07 g, 65.1 mmol), HATU (48.9 g, 129.0 mmol), HOAt (24.75 mL, 14.85 mmol) and DIPEA (69.0 mL, 396.0 mmol) dropwise over a period of 30 minutes and the reaction was then stirred at 0 °C for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 12-d as white foam.

[0159] *Step 5:* 4N HCl in 1,4-dioxane (108 mL) was added to a solution of intermediate 12-d (49.0 g, 86.0 mmol) in ethyl acetate (10 mL) at 0 °C and the reaction mixture was stirred for 4 hours at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide intermediate 12-e·HCl as a white foam. MS (m/z) M+1= 470.5

[0160] *Step 6:* To a solution of intermediate 12-e·HCl (10.4 g, 20.55 mmol) in DMF cooled to 0 °C were sequentially added Boc-NMe-Ala-OH (5.01 g, 24.66 mmol), HATU

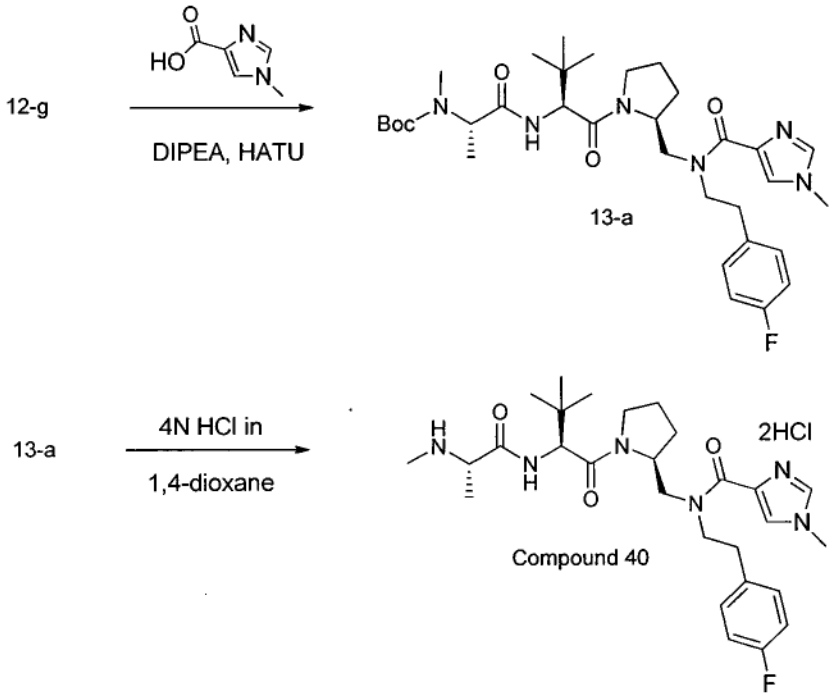
(10.94 g, 28.8 mmol), HOAt (5.14 mL, 3.08 mmol) and DIPEA (14.32 mL, 82.0 mmol) dropwise over a period of 30 minutes and the reaction was then stirred at 0 °C for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 12-f as white foam.

[0161] *Step 7:* To a solution of intermediate 12-f (8.50 g, 16.32 mmol) in MeOH (100 mL) under N_2 was added 10 % Pd/C (50% w/w water content) (3.4 g). The reaction mixture was purged with H_2 and stirred for 1 hour. The reaction was then filtered through celite and the filtrate was concentrated in vacuo. Purification by silica gel chromatography provided intermediate 12-g as a colorless oil. MS (m/z) M+1= 521.5

EXAMPLE 9

[0162] The following example illustrates the preparation of compound 40, which is a compound of Formula 1 or salt thereof.

Scheme 13: Synthesis of compound 40



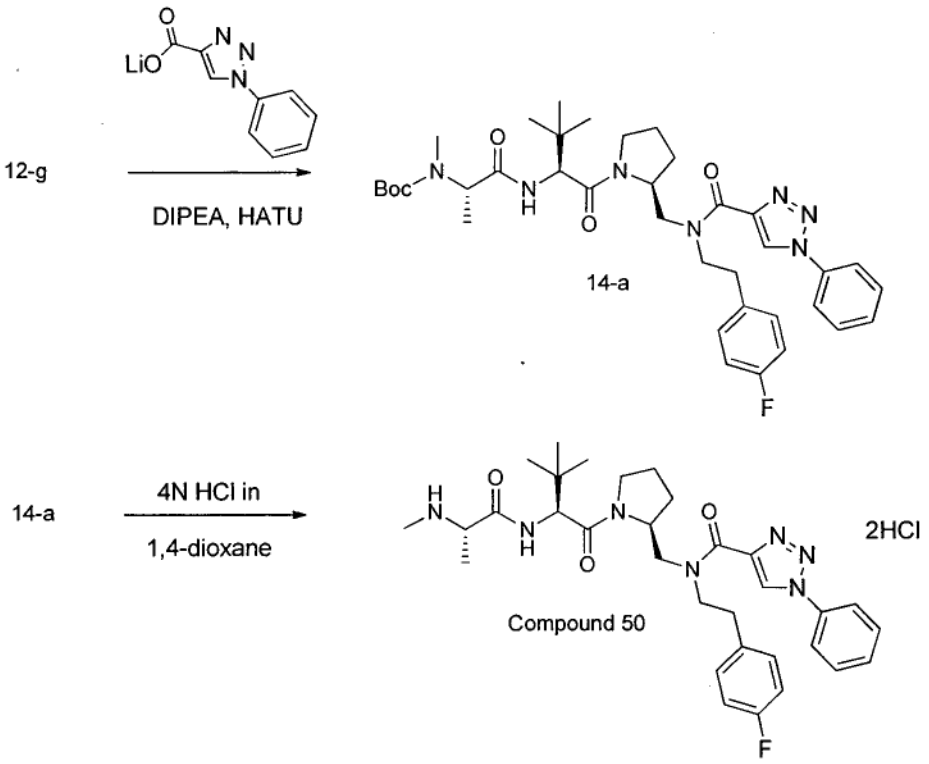
[0163] *Step 1:* To a solution of intermediate 12-g (610 mg, 1.17 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-methyl-1H-imidazole-4-carboxylic acid (177 mg, 1.40 mmol), HATU (624 mg, 1.64 mmol) and DIPEA (816 uL, 4.69 mmol) and the reaction mixture was stirred at 0 ºC for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 13-a as a white foam.

[0164] *Step 2:* 4N HCl in 1,4-dioxane (2.3 mL) was added to a solution of intermediate 13-a (590 mg, 0.93 mmol) in ethyl acetate (0.5 mL) and the mixture was stirred for 3 hours at 0 ºC. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 40·2HCl as a white solid. MS (m/z) M+1= 529.5

EXAMPLE 10

[0165] The following example illustrates the preparation of compound 50, which is a compound of Formula 1 or salt thereof.

Scheme 14: Synthesis of compound 50



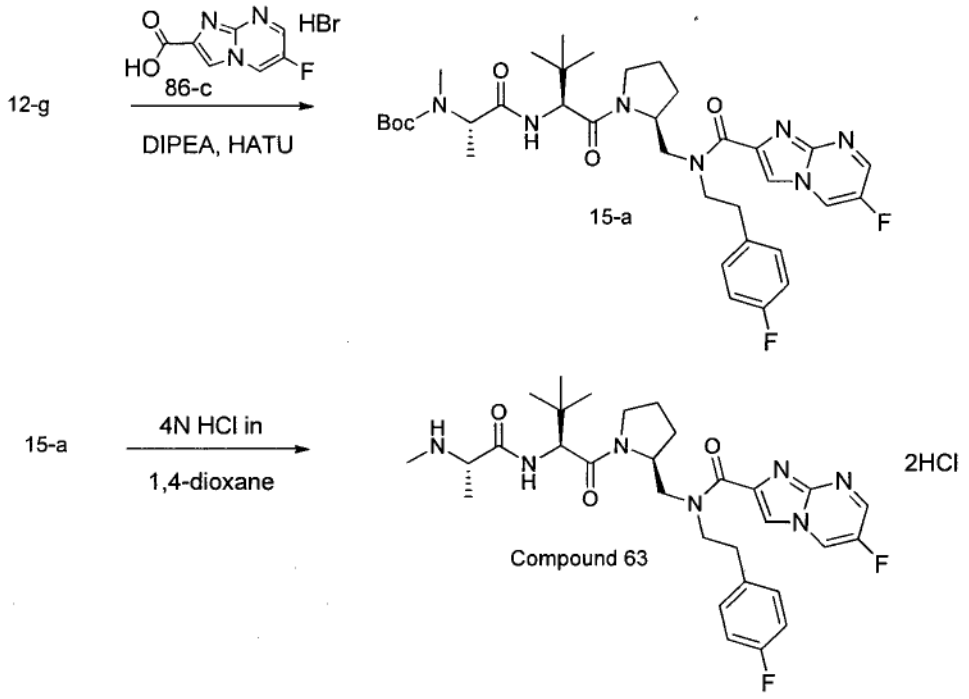
[0166] *Step 1:* To a solution of intermediate 12-g (400 mg, 0.76 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-phenyl-1H-1,2,3-triazole-4-carboxylic acid, lithium salt (226 mg, 1.15 mmol), HATU (467 mg, 1.23 mmol) and DIPEA (535 uL, 3.07 mmol) and the reaction mixture was stirred at 0℃ for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 14-a as a white foam.

[0167] *Step 2:* 4N HCl in 1,4-dioxane (1.0 mL) was added to intermediate 14-a (282 mg, 0.40 mmol) in ethyl acetate (1.0 mL) and the solution was stirred for 5 hours at 0℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 50·2HCl as a white solid. MS (m/z) M+1= 592.5

EXAMPLE 11

[0168] The following example illustrates the preparation of compound 63, which is a compound of Formula 1 or salt thereof.

Scheme 15: Synthesis of compound 63



[0169] *Step 1:* To a solution of intermediate 12-g (15.17 g, 29.1 mmol) in DMF, cooled to 0 ºC, were sequentially added 6-fluoroimidazo[1,2-a]pyrimidine-2-carboxylic acid, HBr salt (86-c) (9.16 g, 35.0 mmol), HATU (13.29 g, 35.0 mmol) and DIPEA (20.0 mL, 117 mmol) and the reaction mixture was stirred at 0°C for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 15-a as a white foam.

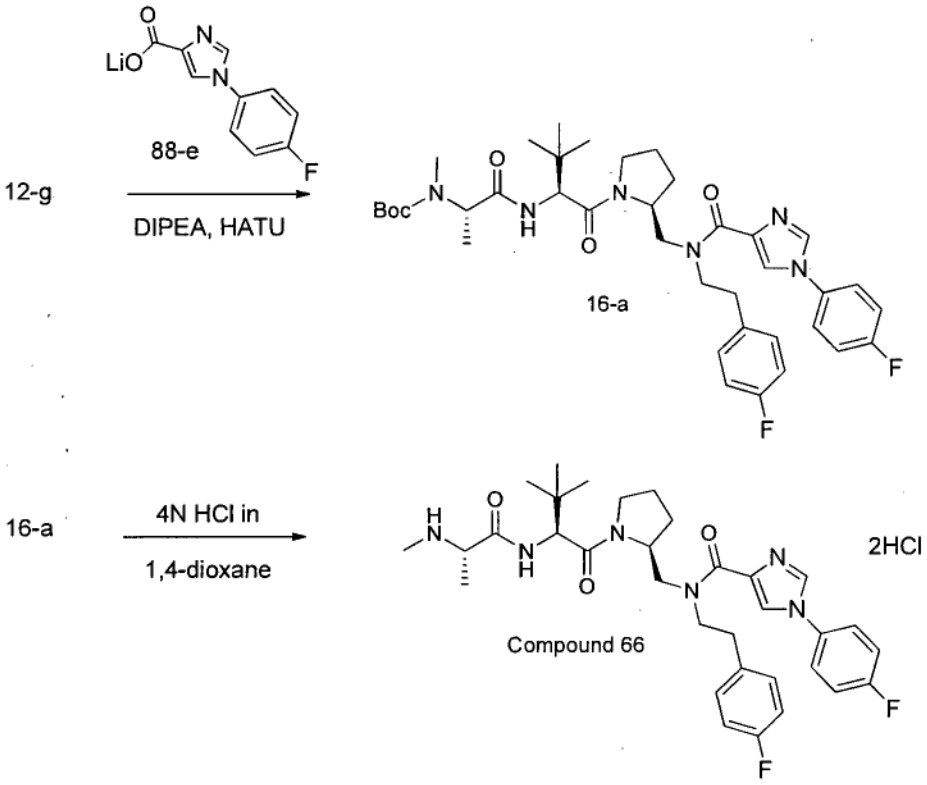
[0170] *Step 2:* 4N HCl in 1,4-dioxane (82.0 mL) was added to intermediate 15-a (14.96 g, 21.88 mmol) in ethyl acetate (11 mL) and the solution was stirred for 3 hours at

0℃. Diethyl ether was added, a precipitate formed and compound 63·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 584.5.

EXAMPLE 12

[0171] The following example illustrates the preparation of compound 66, which is a compound of Formula 1 or salt thereof.

Scheme 16: Synthesis of compound 66



[0172] *Step 1:* To a solution of intermediate 12-g (350 mg, 0.67 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-(4-fluorophenyl)-1H-imidazole-4-carboxylic acid, lithium salt (88-e) (208 mg, 1.0 mmol), HATU (435 mg, 1.14 mmol) and DIPEA (468 uL, 2.69 mmol) and the reaction mixture was stirred at 0℃ for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and

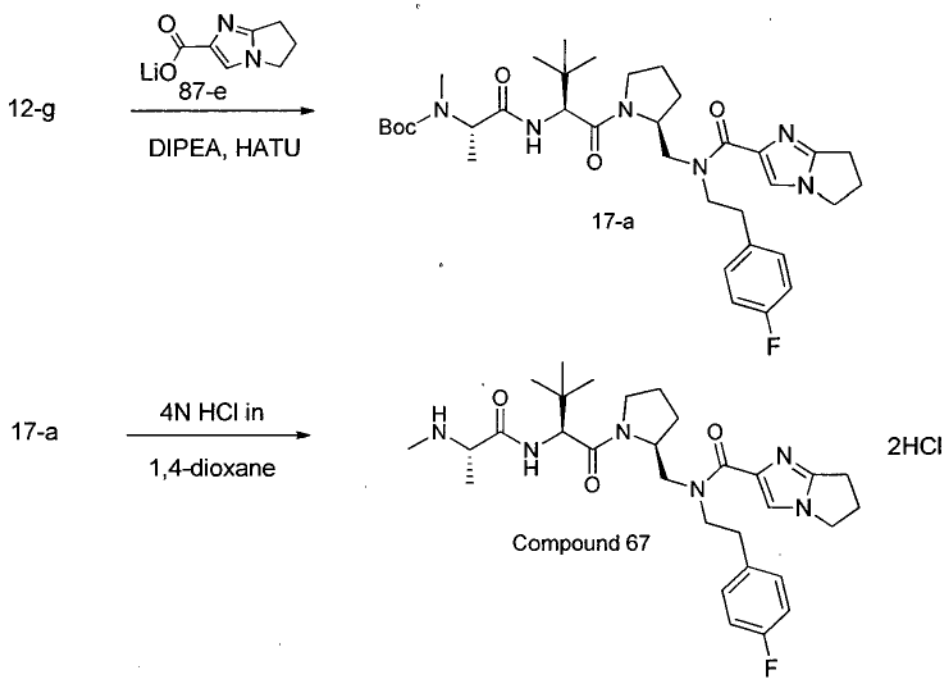
brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 16-a as a white foam.

[0173] *Step 2:* 4N HCl in 1,4-dioxane (1.0 mL) was added to intermediate 16-a (515 mg, 0.78 mmol) in MeOH (0.5 mL) and the solution was stirred for 2 hours at 0°C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 66·2HCl as a white solid. MS (m/z) M+1= 609.5

EXAMPLE 13

[0174] The following example illustrates the preparation of compound 67, which is a compound of Formula 1 or salt thereof.

Scheme 17: Synthesis of compound 67



[0175] *Step 1:* To a solution of intermediate 12-g (856 mg, 1.64 mmol) in DMF, cooled to 0 ºC, were sequentially added 6,7-dihydro-5H-pyrrolo[1,2-a]imidazole-2-carboxylic acid, lithium salt (87-e) (340 mg, 2.13 mmol), HATU (875 mg, 2.30 mmol) and DIPEA (859 uL, 4.93 mmol) and the reaction mixture was stirred at 0°C for 30 minutes. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer

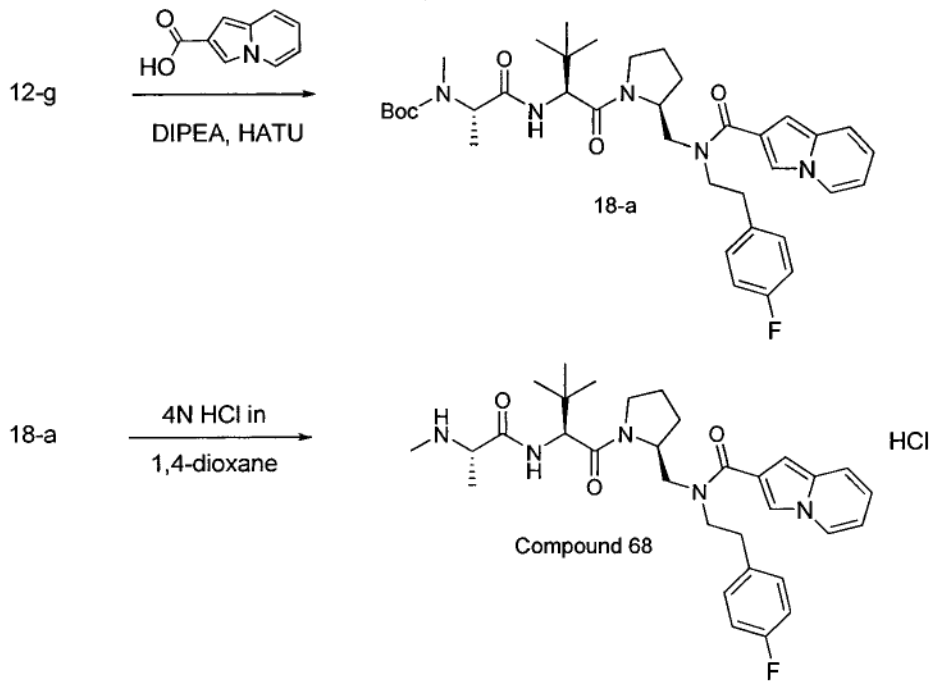
was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 17-a as a white foam.

[0176] *Step 2:* 4N HCl in 1,4-dioxane (1.0 mL) was added to intermediate 17-a (515 mg, 0.78 mmol) in ethyl acetate (0.5 mL) and the solution was stirred for 2 hours at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 67·2HCl as a white solid. MS (m/z) M+1= 555.6

EXAMPLE 14

[0177] The following example illustrates the preparation of compound 68, which is a compound of Formula 1 or salt thereof.

Scheme 18: Synthesis of compound 68



[0178] *Step 1:* To a solution of intermediate 12-g (300 mg, 0.57 mmol) in DMF, cooled to 0 ºC, were sequentially added indolizine-2-carboxylic acid (107 mg, 0.66 mmol), HATU (285 mg, 0.75 mmol) and DIPEA (301 uL, 1.72 mmol) and the reaction mixture was

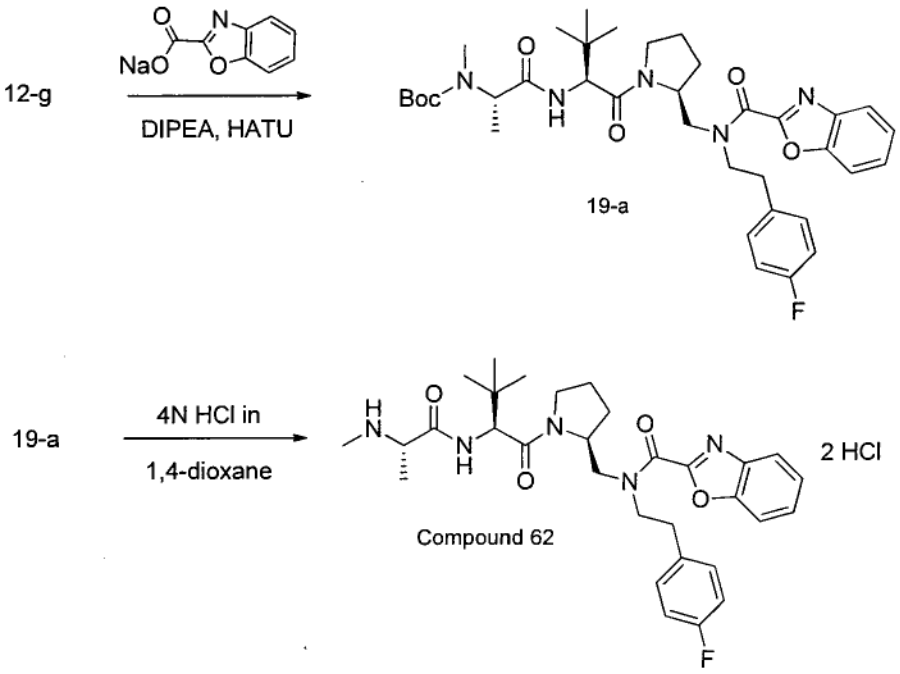
stirred at 0℃ for 30 minutes. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 18-a as a white foam.

[0179] *Step 2:* 4N HCl in 1,4-dioxane (1.0 mL) was added to intermediate 18-a (360 mg, 0.54 mmol) in ethyl acetate (0.5 mL) and the solution was stirred for 2 hours at 0℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 68·HCl as a white solid. MS (m/z) M+1= 564.5

EXAMPLE 15

[0180] The following example illustrates the preparation of compound 62, which is a compound of Formula 1 or salt thereof.

Scheme 19: Synthesis of compound 62



[0181] *Step 1:* To a solution of intermediate 12-g (300 mg, 0.57 mmol) in DMF, cooled to 0 ºC, were sequentially added sodium benzo[d]oxazole-2-carboxylate (139 mg,

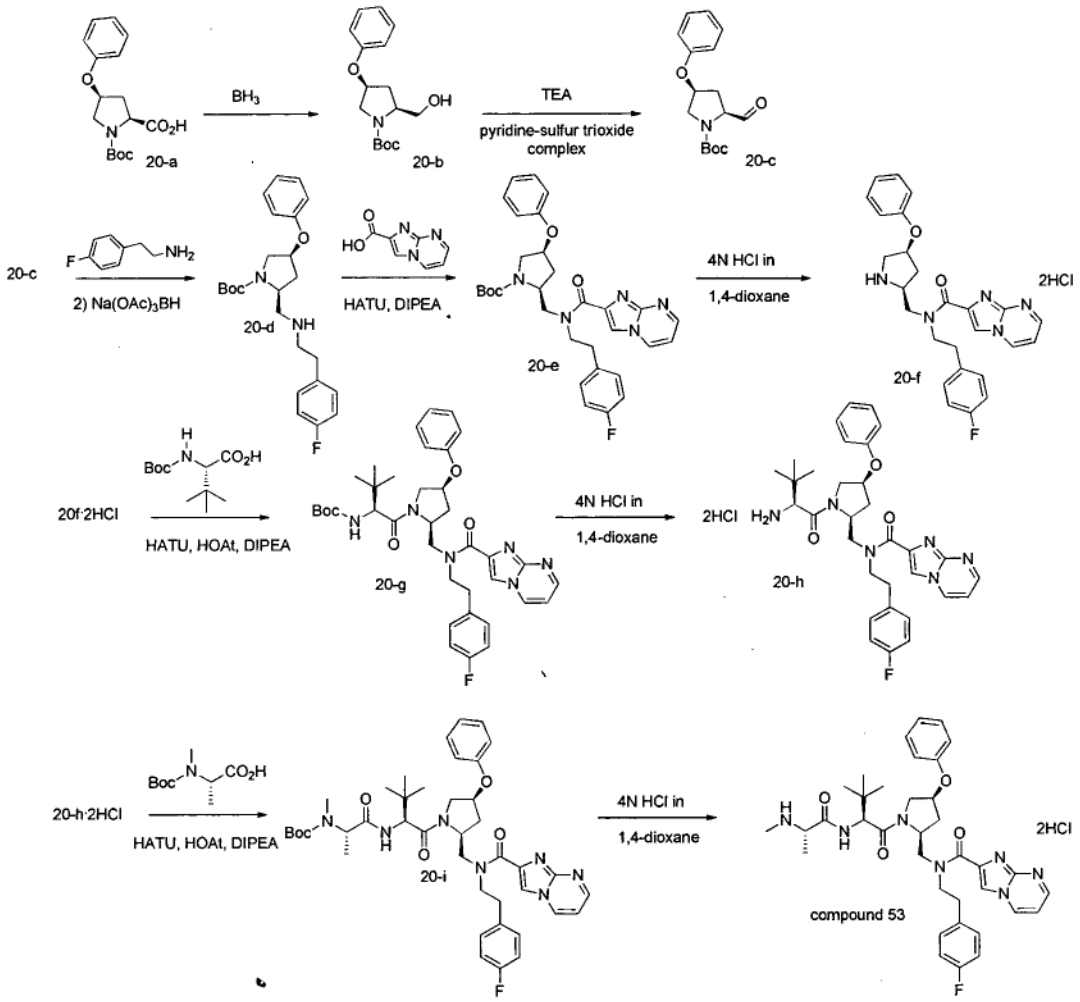
0.74 mmol), HATU (263 mg, 0.69 mmol) and DIPEA (401 uL, 2.30 mmol) and the reaction mixture was stirred at 0℃ for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 19-a as a white foam.

[0182] *Step 2:* 4N HCl in 1,4-dioxane (1.20 mL) was added to intermediate 19-a (294 mg, 0.44 mmol) and the solution was stirred for 2 hours at 0℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 62·HCl as a white solid. MS (m/z) M+1= 566.5

EXAMPLE 16

[0183] The following example illustrates the preparation of compound 53, which is a compound of Formula 1 or salt thereof.

Scheme 20: Synthesis of compound 53



[0184] *Step 1:* To a solution of (2S,4S)-1-(tert-butoxycarbonyl)-4-phenoxypyrrolidine-2-carboxylic acid 20-a (1.40 g, 4.56 mmol) in THF cooled to 0 °C was added borane tetrahydrofuran complex (18.22 ml, 18.2 mmol), the reaction was stirred at 0 °C for 15 minutes and room temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 20-b as a colorless oil.

[0185] *Step 2:* To a solution of intermediate 20-b (1.33 g, 4.53 mmol) in DMSO (5.24 mL, 73.9 mmol) and dichloromethane (10 mL) was added TEA (2.53 mL, 18.13 mmol) and pyridine- sulfur trioxide complex (1.44 g, 9.07 mmol), the reaction was then stirred at 0℃ for 30 minutes and room temperature for 30 minutes. Water and ethyl acetate were added; the organic layer was separated, washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 20-c as colorless oil.

[0186] *Step 3:* To a solution of intermediate 20-c (1.32 g, 4.53 mmol) in dichloromethane was added 2-(4-fluorophenyl)ethanamine (595 µL, 4.53 mmol). After stirring for 30 minutes sodium triacetoxyborohydride (1.21 g, 5.44 mmol) was added at 0 °C and the reaction mixture was then stirred at room temperature overnight. Water and ethyl acetate were added; the organic layer was separated, washed with 1N aqueous NaOH, water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 20-d as colorless oil.

[0187] *Step 4:* To a solution of intermediate 20-d (1.87 g, 4.51 mmol) in DMF, cooled to 0 ºC, were sequentially added imidazo[1,2-a]pyrimidine-2-carboxylic acid (1.21 g, 4.96 mmol), HATU (2.05 g, 5.41 mmol) and DIPEA (2.36 mL, 13.53 mmol) and the reaction mixture was then stirred at room temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 20-e as a white foam.

[0188] *Step 5:* 4N HCl in 1,4-dioxane (1.0 mL) was added to intermediate 20-e (800 mg, 1.43 mmol) and the solution was stirred at 0℃ for 1 hour. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide intermediate 20-f·2HCl as a white solid. MS (m/z) M+1=460.4

[0189] *Step 6:* To a solution of intermediate 20-f·2HCl (709 mg, 1.43 mmol) in DMF cooled to 0 ℃ were sequentially added Boc-tBu-gly-OH (397 mg, 1.71 mmol), HOAt (357 uL, 0.21 mmol), HATU (707 mg, 1.85 mmol) and DIPEA (1.0 mL, 5.72 mmol) and the reaction was then stirred at room temperature for 1 hour. Saturated aqueous ammonium

chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 20-g as white foam.

[0190] *Step 7:* 4N HCl in 1,4-dioxane (1.0 mL) was added to intermediate 20-g (651 mg, 0.96 mmol) and the solution was stirred for 1 hour at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide intermediate 20-h·2HCl as a white solid. MS (m/z) M+1=573.5

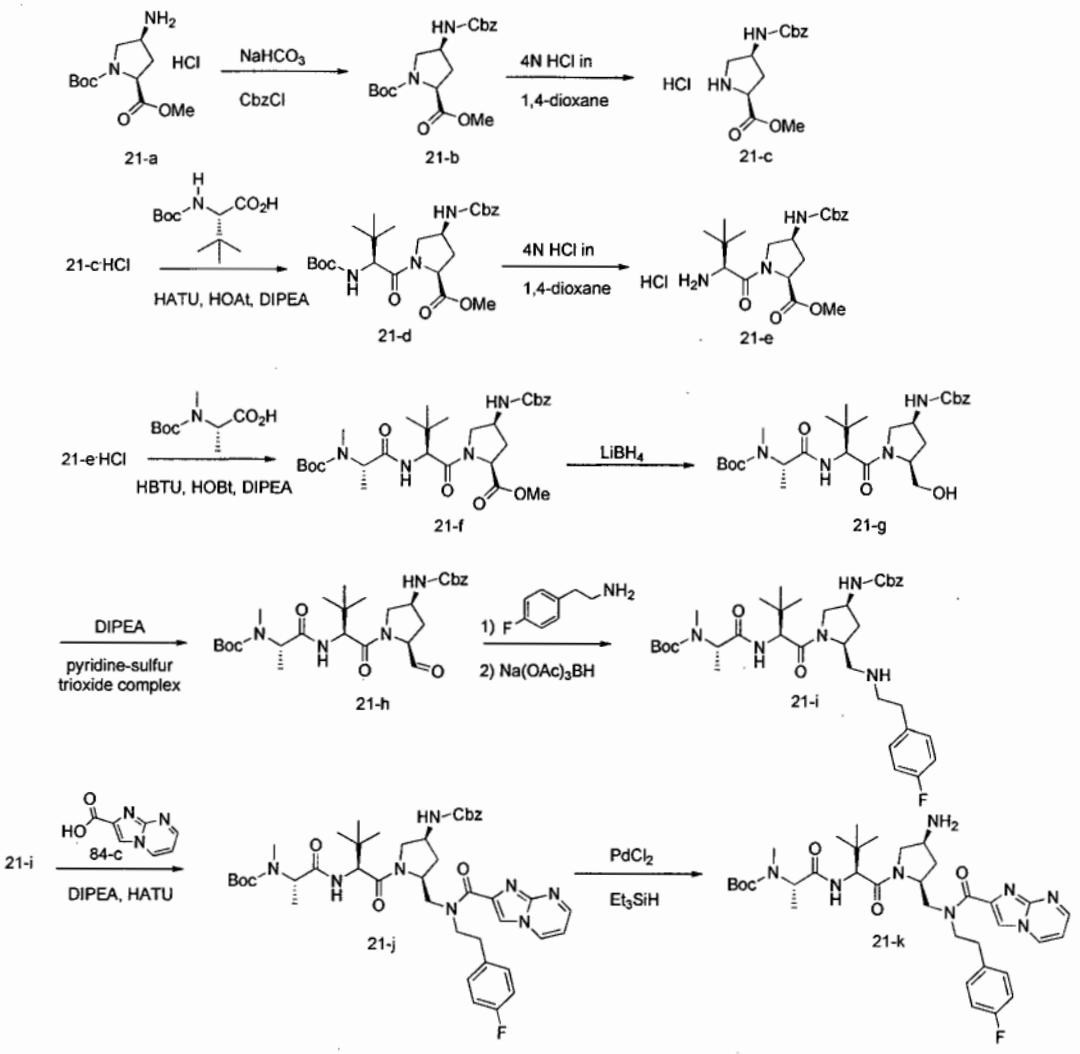
[0191] *Step 8:* To a solution of intermediate 20-h·2HCl (300 mg, 0.49 mmol) in DMF cooled to 0 °C were sequentially added Boc-NMe-Ala-OH (140 mg, 0.69 mmol), HOAt (123 uL, 0.07 mmol), HATU (281 mg, 0.73 mmol), and DIPEA (344 uL, 1.97 mmol) and the reaction was then stirred at room temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 20-i as a white foam.

[0192] *Step 9:* 4N HCl in 1,4-dioxane (1.0 mL) was added to intermediate 20-i (348 mg, 0.45 mmol) and the solution was stirred for 1 hour at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 53·2HCl as a white solid. MS (m/z) M+1= 658.5

EXAMPLE 17

[0193] The following example illustrates the preparation of compound 21-k, which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof.

Scheme 21: Synthesis of Intermediate 21-k



[0194] *Step 1:* To a solution of intermediate 21-a·HCl (10.0 g, 35.6 mmol) in 1,4-dioxane (89 mL) and water (89 mL) cooled to 0 °C were sequentially added sodium bicarbonate (8.98 g, 107.0 mmol) and benzyl chloroformate (6.72 g, 37.4 mmol) and the reaction was then stirred at room temperature for 3 hours. Diethyl ether (200 mL) was added; the organic layer was separated, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 21-b as colorless oil.

[0195] *Step 2:* 4N HCl in 1,4-dioxane (30.0 mL) was added to intermediate 21-b (13.0 g, 34.4 mmol) and the solution was stirred for 1 hour at 0 °C. Volatiles were removed

under reduced pressure and the residue was triturated with diethyl ether to provide intermediate 21-c·HCl as a white solid. MS (m/z) M+1= 279.3

[0196] *Step 3:* To a solution of intermediate 21-c·HCl (10.52 g, 33.4 mmol) in DMF cooled to 0 °C were sequentially added Boc-tBu-gly-OH (8.50 g, 36.8 mmol), HOAt (5.57 mL, 3.34 mmol), HATU (13.98 g, 36.8 mmol), and DIPEA (23.35 mL, 134.0 mmol) and the reaction was then stirred at room temperature for 3 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 21-d as colorless oil.

[0197] *Step 4:* 4N HCl in 1,4-dioxane (30.0 mL) was added to intermediate 21-d (15.8 g, 32.1 mmol) and the solution was stirred for 1 hour at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide intermediate 21-e·HCl as a white solid. MS (m/z) M+1= 392.5

[0198] *Step 5:* To a solution of intermediate 21-e·HCl (15.2 g, 35.5 mmol) in DMF cooled to 0 °C were sequentially added Boc-NMe-Ala-OH (7.58 g, 37.3 mmol), HOAt (5.92 mL, 3.55 mmol), HATU (14.86 g, 39.1 mmol), and DIPEA (24.8 mL, 142.0 mmol) and the reaction was then stirred at room temperature for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 21-f as white foam.

[0199] *Step 6:* To a solution of intermediate 21-f (20.2 g, 35.0 mmol) in THF cooled to 0 °C was added lithium borohydride (1.60 g, 73.6 mmol) and the reaction was stirred at room temperature for 1 hour. Water and ethyl acetate were added; the organic layer was separated, washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 21-g as a white foam.

[0200] *Step 7:* To a solution of intermediate 21-g (10.1 g, 18.41 mmol) in DMSO (5.23 mL, 73.6 mmol) and dichloromethane (184 mL) cooled to 0 °C was added DIPEA

(11.22 mL, 64.4 mmol) and pyridine sulfur trioxide complex (8.79 g, 55.2 mmol), the reaction was then stirred at 0°C for 2 hours. Water and ethyl acetate were added; the organic layer was separated, washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 21-h as white foam.

[0201] *Step 8:* To a solution of intermediate 21-h (10.60 g, 19.39 mmol) in dichloromethane was added 2-(4-fluorophenyl)ethanamine (2.70 g, 19.39 mmol). After stirring at room temperature overnight sodium triacetoxyborohydride (4.93 g, 23.27 mmol) was added portion wise at 0 °C and the reaction mixture was then stirred at room temperature for 2 hours. Saturated aqueous $NaHCO_3$ was added; the organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 21-i as white foam. MS (m/z) M+1= 670.6

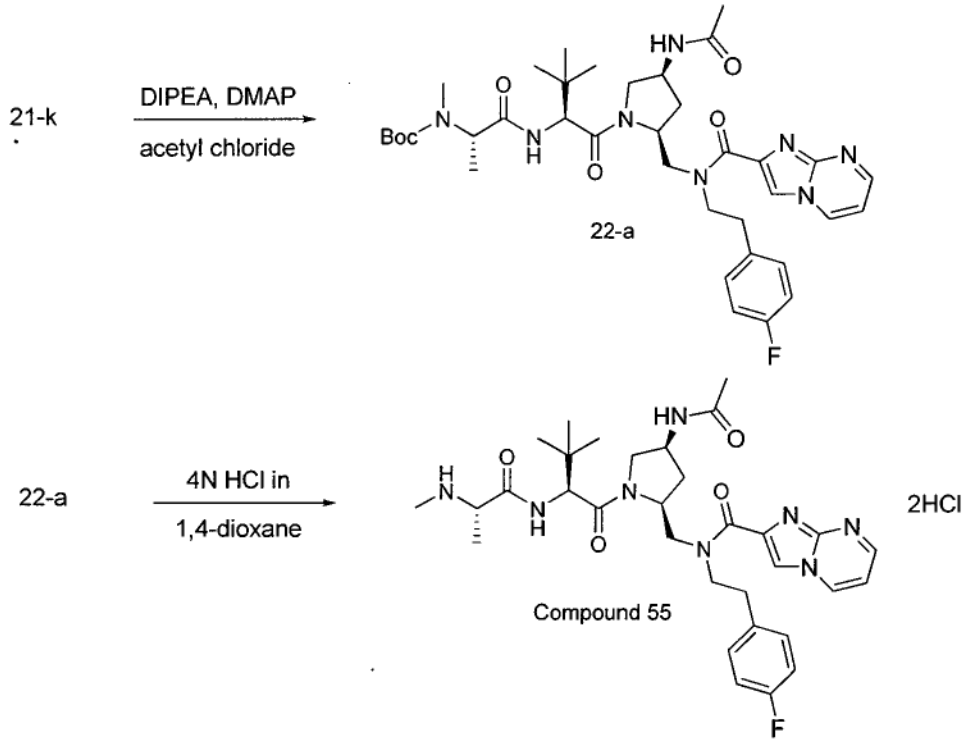
[0202] *Step 9:* To a solution of intermediate 21-i (10.0 g, 14.93 mmol) and imidazo[1,2-a]pyrimidine-2-carboxylic acid, HBr salt (84-c) (3.64 g, 14.93 mmol) in DMF, cooled to 0 ºC, were sequentially added HATU (6.24 g, 16.42 mmol) and DIPEA (10.43 mL, 59.70 mmol) and the reaction mixture was stirred at room temperature for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 21-j as a beige foam.

[0203] *Step 10:* To a solution of intermediate 21-j (2.75 g, 3.37 mmol) in TEA (4.0 mL, 28.7 mmol) were sequentially added palladium (II) chloride (60 mg, 0.34 mmol) and triethylsilane (1.34 mL, 8.45 mmol). The reaction mixture was purged with H_2 and stirred at room temperature for 2 hours. The reaction was then filtered through celite and the filtrate was concentrated in vacuo to give intermediate 21-k as a beige solid. MS (m/z) M+1= 681.7

EXAMPLE 18

[0204] The following example illustrates the preparation of compound 55, which is a compound of Formula 1 or salt thereof.

Scheme 22: Synthesis of compound 55



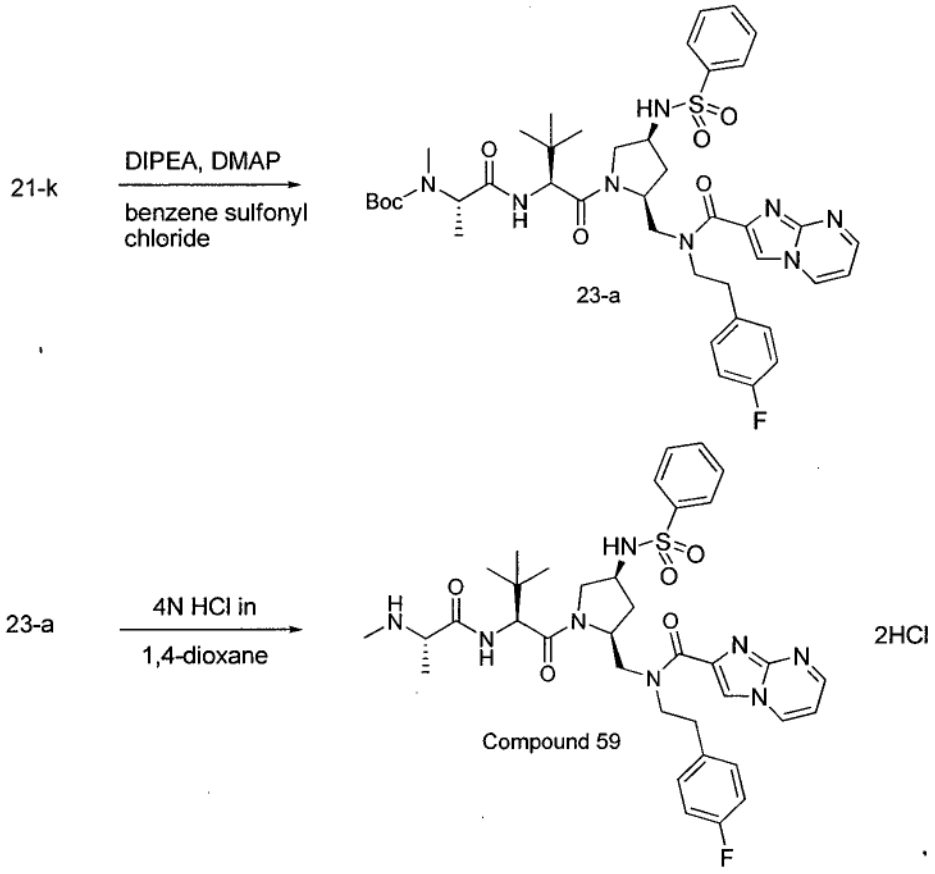
[0205] *Step 1:* To a solution of intermediate 21-k (420 mg, 0.61 mmol) in dichloromethane (2.0 mL) cooled to 0°C were sequentially added DIPEA (385 uL, 2.20 mmol), DMAP (4.50 mg, 0.03 mmol) and acetyl chloride (63 uL, 0.88 mmol) and the reaction was stirred at room temperature for 18 hours. Water and ethyl acetate were added; the organic layer was separated, washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 22-a as white solid

[0206] *Step 2:* 4N HCl in 1,4-dioxane (2.0 mL) was added to intermediate 22-a (230 mg, 0.32 mmol) and the solution was stirred for 1 hour at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 55·2HCl as a white solid. MS (m/z) M+1= 623.5

EXAMPLE 19

[0207] The following example illustrates the preparation of compound 59, which is a compound of Formula 1 or salt thereof.

Scheme 23: Synthesis of compound 59



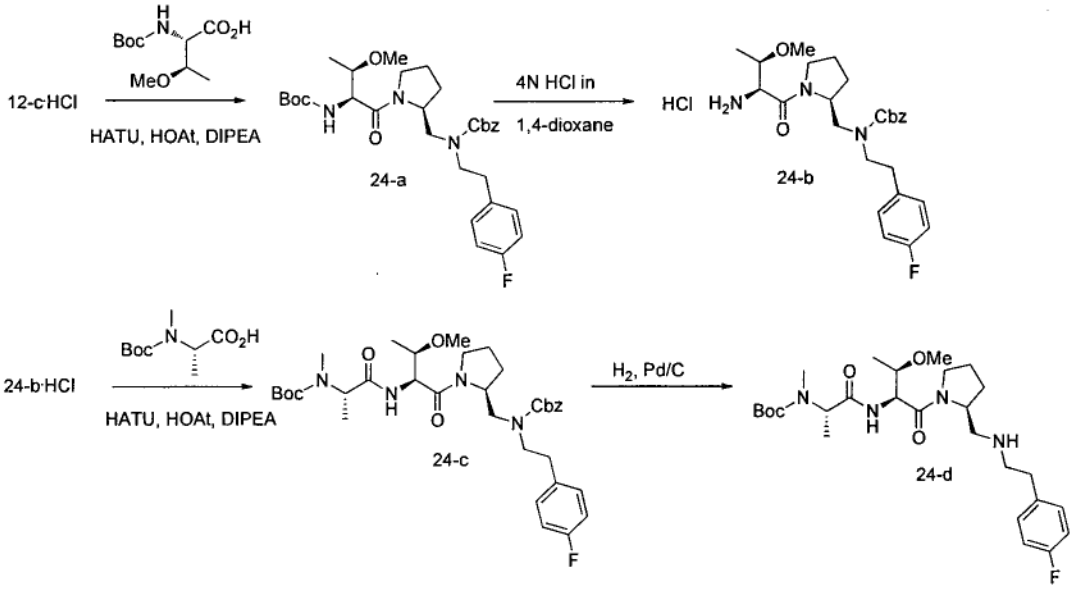
[0208] *Step 1:* To a solution of intermediate 21-k (420 mg, 0.61 mmol) in pyridine (4.0 mL) cooled to 0℃ were sequentially added DIPEA (323 uL, 1.85 mmol), DMAP (3.8 mg, 0.03 mmol) and benzenesulfonyl chloride (79 uL, 0.61 mmol) and the reaction was stirred at room temperature overnight. Water and ethyl acetate were added; the organic layer was separated, washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 23-a as a white solid.

[0209] *Step 2:* 4N HCl in 1,4-dioxane (2.0 mL) was added to intermediate 23-a (100 mg, 0.12 mmol) and the solution was stirred for 1 hour at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 59·2HCl as a white solid. MS (m/z) M+1= 721.5

EXAMPLE 20

[0210] The following example illustrates the preparation of compound 24-d, which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof.

Scheme 24: Synthesis of Intermediate 24-d



[0211] *Step 1:* To a solution of intermediate 12-c·HCl (13.7 g, 34.9 mmol) in DMF cooled to - 10 °C were sequentially added Boc-Thr(Me)-OH (8.1 g, 34.9 mmol), HATU (14.6 g, 38.4 mmol), HOAt (63.9 mL, 38.4 mmol) and DIPEA (24.4 mL, 139.0 mmol) and the reaction was then stirred at 0 °C for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 24-a as white foam.

[0212] *Step 2:* 4N HCl in 1,4-dioxane (94.0 mL) was added to intermediate 24-a (10.8 g, 18.9 mmol) in ethyl acetate (10 mL) at 0 °C and the solution was stirred for 3 hours at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide intermediate 24-b·HCl as a white foam. MS (m/z) M+1= 472.5

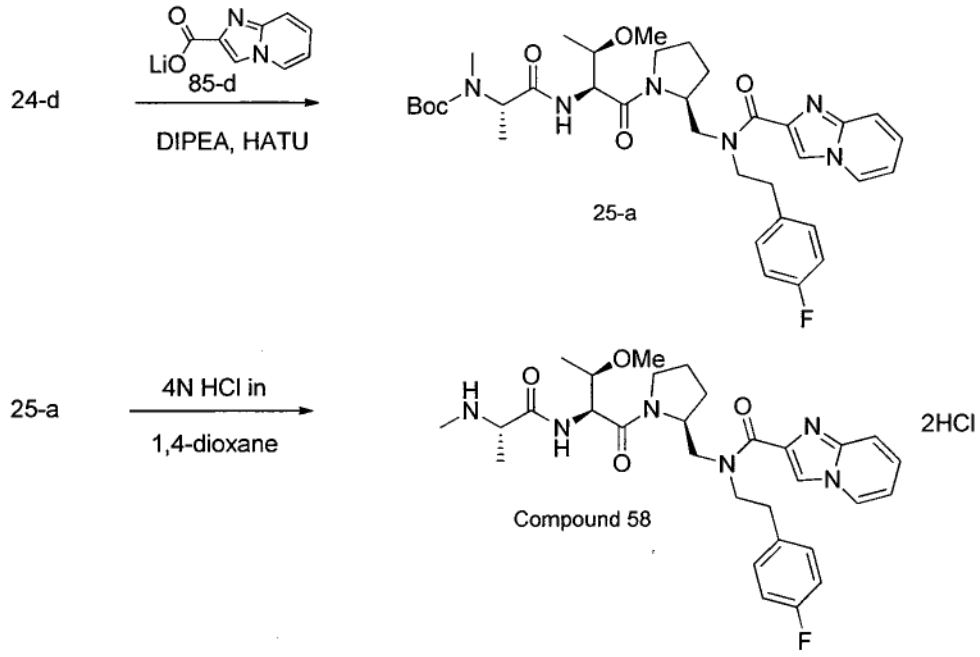
[0213] *Step 3:* To a solution of intermediate 24-b·HCl (25.0 g, 49.2 mmol) in DMF cooled to 0 °C were sequentially added Boc-NMe-Ala-OH (12.0 g, 59.0 mmol), HATU (26.2 g, 68.9 mmol), HOAt (12.3 mL, 7.38 mmol) and DIPEA (34.3 mL, 197 mmol) and the reaction was then stirred at 0 °C for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 24-c as white foam.

[0214] *Step 4:* To a solution of intermediate 24-c (15.88 g, 23.67 mmol) in MeOH (118 mL) under N_2 was added 10 % Pd/C (50% w/w water content) (3.53 g). The reaction mixture was purged with H_2 and stirred for 5 hours. The reaction was then filtered through celite and the filtrate was concentrated in vacuo to give intermediate 24-d as colorless oil. MS (m/z) M+1= 537.5

EXAMPLE 21

[0215] The following example illustrates the preparation of compound 58, which is a compound of Formula 1 or salt thereof.

Scheme 25: Synthesis of compound 58



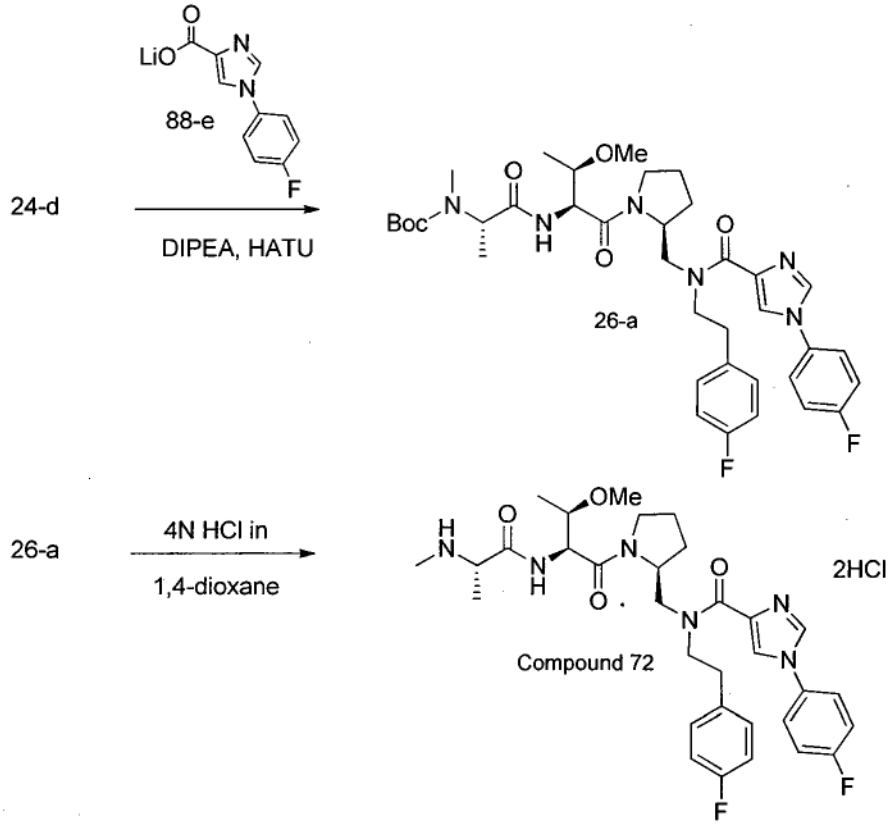
[0216] *Step 1:* To a solution of intermediate 24-d (15.0 g, 28.7 mmol) in DMF, cooled to 0 ºC, were sequentially added imidazo [1,2-a]pyridine-2-carboxylic acid, lithium salt (85-d) (5.79 g, 34.40 mmol), HATU (13.10 g, 34.40 mmol) and DIPEA (20.0 mL, 115.0 mmol) and the reaction mixture was stirred at 0 ℃ for 3 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 25-a as a white foam.

[0217] *Step 2:* 4N HCl in 1,4-dioxane (69.20 mL) was added to intermediate 25-a (12.30 g, 18.45 mmol) in ethyl acetate (9.20 mL) and the solution was stirred for 3 hours at 0 ℃. Diethyl ether was added, a precipitate formed and compound 58·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 567.5

EXAMPLE 22

[0218] The following example illustrates the preparation of compound 72, which is a compound of Formula 1 or salt thereof.

Scheme 26: Synthesis of compound 72



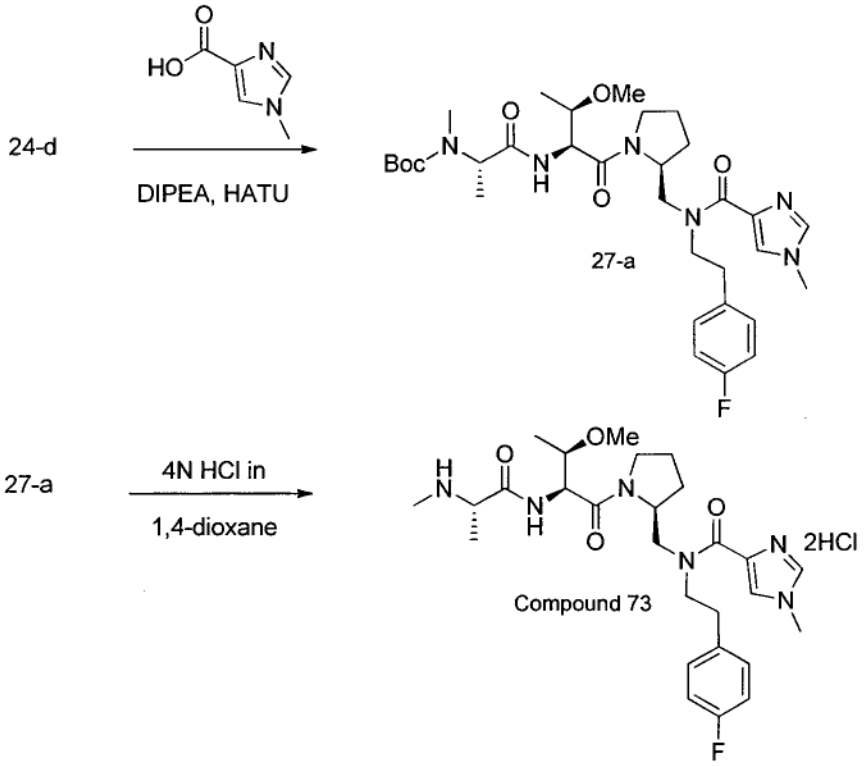
[0219] *Step 1:* To a solution of intermediate 24-d (400 mg, 0.76 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-(4-fluorophenyl)-1H-imidazole-4-carboxylic acid, lithium salt (88-e) (212 mg, 1.0 mmol), HATU (437 mg, 1.14 mmol) and DIPEA (400 uL, 2.29 mmol) and the reaction mixture was stirred at room temperature for 30 minutes. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 26-a as a white foam.

[0220] *Step 2:* 4N HCl in 1,4-dioxane (1.30 mL) was added to intermediate 26-a (385 mg, 0.54 mmol) in ethyl acetate (0.5 mL) and the solution was stirred for 2 hours at 0 ºC. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 72·2HCl as a white solid. MS (m/z) M+1= 611.5

EXAMPLE 23

[0221] The following example illustrates the preparation of compound 73, which is a compound of Formula 1 or salt thereof.

Scheme 27: Synthesis of compound 73



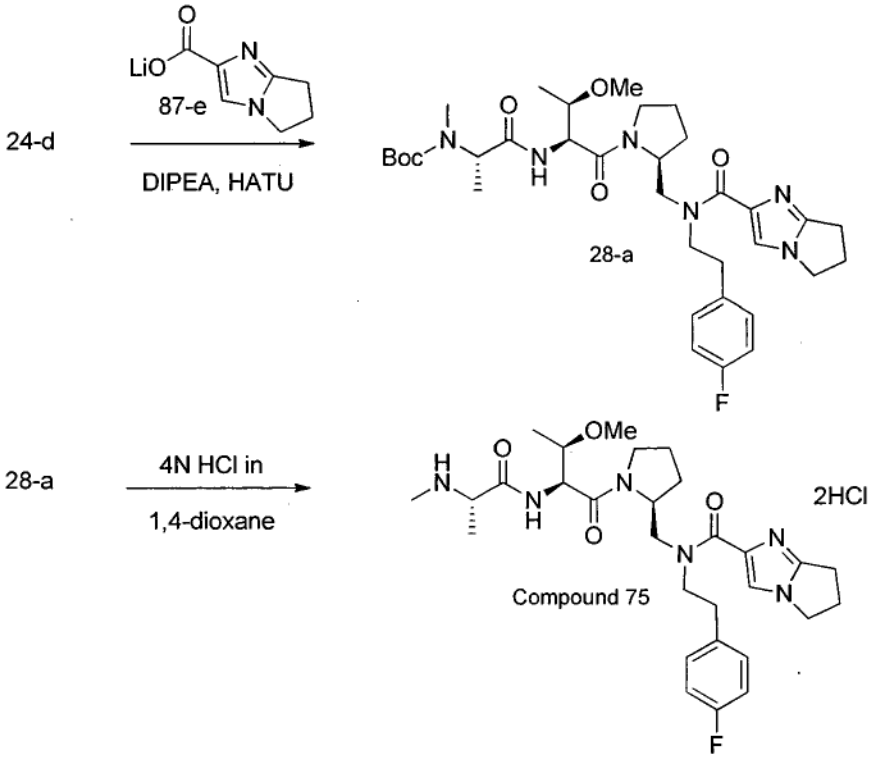
[0222] *Step 1:* To a solution of intermediate 24-d (580 mg, 1.11 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-methyl-1H-imidazole-4-carboxylic acid (168 mg, 1.33 mmol), HATU (591 mg, 1.55 mmol) and DIPEA (581 uL, 3.33 mmol) and the reaction mixture was stirred at 0℃ for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 27-a as a white foam.

[0223] *Step 2:* 4N HCl in 1,4-dioxane (2.0 mL) was added to intermediate 27-a (510 mg, 0.81 mmol) in ethyl acetate (0.5 mL) and the solution was stirred for 3 hours at 0 ℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 73·2HCl as a white solid. MS (m/z) M+1= 531.5

EXAMPLE 24

[0224] The following example illustrates the preparation of compound 75, which is a compound of Formula 1 or salt thereof.

Scheme 28: Synthesis of compound 75



[0225] *Step 1:* To a solution of intermediate 24-d (1.8 g, 3.44 mmol) in DMF, cooled to 0 ºC, were sequentially added 6,7-dihydro-5-H-pyrrolo[1,2-a]imidazole-2-carboxylic acid, lithium salt (87-e) (743 mg, 4.48 mmol), HATU (2.0 g, 5.51 mmol) and DIPEA (1.80 mL, 10.33 mmol) and the reaction mixture was stirred at 0 ℃ for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer

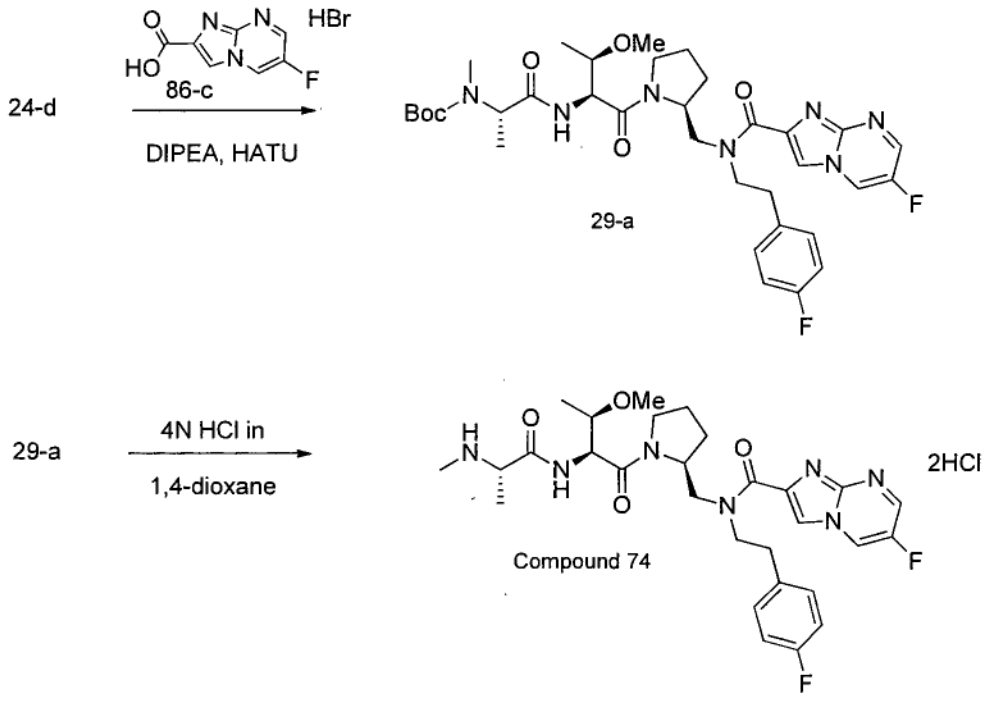
was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 28-a as a white foam.

[0226] *Step 2:* 4N HCl in 1,4-dioxane (6.17 mL) was added to intermediate 28-a (1.62 g, 2.46 mmol) in ethyl acetate (1.0 mL) and the solution was stirred for 2 hours at 0℃. Diethyl ether was added, a precipitate formed and compound 75·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 557.4

EXAMPLE 25

[0227] The following example illustrates the preparation of compound 74, which is a compound of Formula 1 or salt thereof.

Scheme 29: Synthesis of compound 74



[0228] *Step 1:* To a solution of intermediate 24-d (956 mg, 1.82 mmol) in DMF, cooled to 0 ºC, were sequentially added 6-fluoroimidazo[1,2-a]pyrimidine-2-carboxylic acid, HBr salt (86-c) (575 mg, 2.19 mmol), HATU (1.04 g, 2.74 mmol) and DIPEA (958 uL,

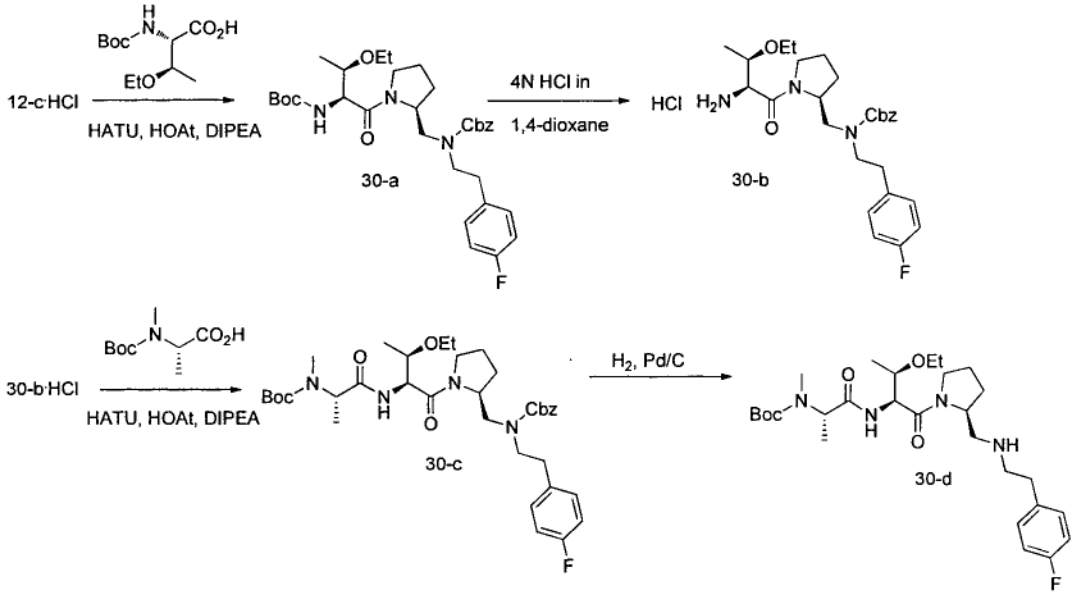
5.49 mmol) and the reaction mixture was stirred at 0 °C for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 29-a as a white foam.

[0229] *Step 2:* 4N HCl in 1,4-dioxane (3.65 mL) was added to intermediate 29-a (1.0 g, 1.45 mmol) in ethyl acetate (1.0 mL) and the solution was stirred for 2 hours at 0 °C. Diethyl ether was added, a precipitate formed and compound 74·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 586.4

EXAMPLE 26

[0230] The following example illustrates the preparation of compound 30-d, which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof.

Scheme 30: Synthesis of intermediate 30-d



[0231] *Step 1:* To a solution of intermediate 12-c·HCl (13.7 g, 34.9 mmol) in DMF cooled to - 10 °C were sequentially added Boc-Thr(Et)-OH (8.1 g, 34.9 mmol), HATU

(14.6 g, 38.4 mmol), HOAt (63.9 mL, 38.4 mmol) and DIPEA (24.4 mL, 139.0 mmol) and the reaction was then stirred at 0 °C for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 30-a as white foam.

[0232] *Step 2:* 4N HCl in 1,4-dioxane (94.0 mL) was added to intermediate 30-a (10.8 g, 18.9 mmol) in ethyl acetate (10 mL) at 0°C and the solution was stirred for 3 hours at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide intermediate 30-b·HCl as a white foam. MS (m/z) M+1= 486.5

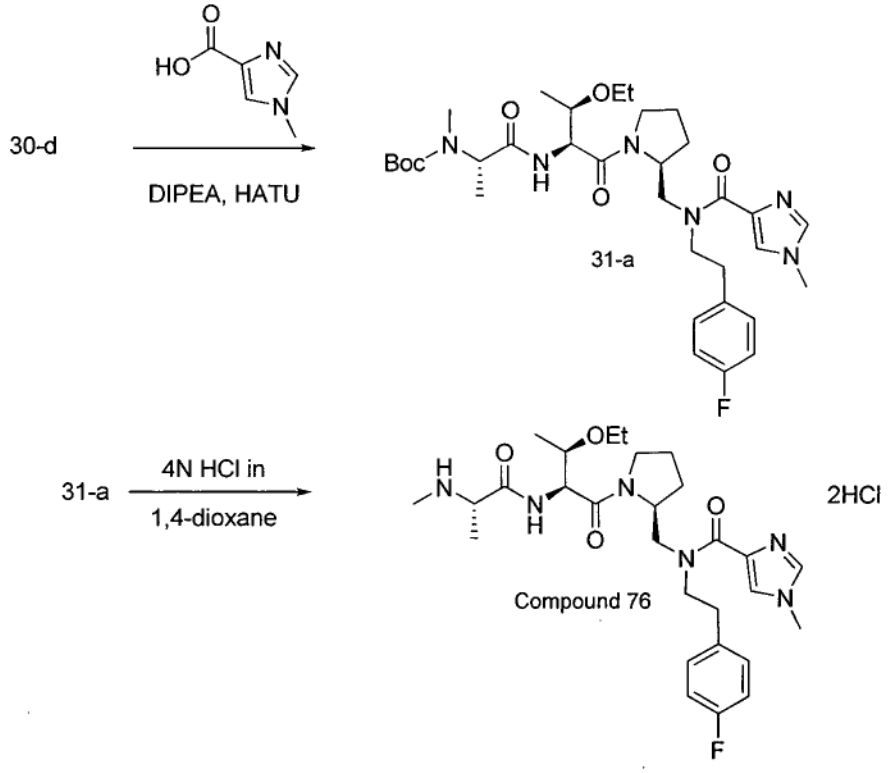
[0233] *Step 3:* To a solution of intermediate 30-b·HCl (25.0 g, 49.2 mmol) in DMF cooled to 0 °C were sequentially added Boc-NMe-Ala-OH (12.0 g, 59.0 mmol), HATU (26.2 g, 68.9 mmol), HOAt (12.3 mL, 7.38 mmol) and DIPEA (34.3 mL, 197 mmol) and the reaction was then stirred at 0 °C for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 30-c as a white foam.

[0234] *Step 4:* To a solution of intermediate 30-c (15.88 g, 23.67 mmol) in MeOH (118 mL) under N_2 was added 10 % Pd/C (50% w/w water content) (3.53 g). The reaction mixture was purged with H_2 and stirred for 1 hour. The reaction was then filtered through celite and the filtrate was concentrated in vacuo to provide intermediate 30-d as a colorless oil. MS (m/z) M+1= 537.5

EXAMPLE 27

[0235] The following example illustrates the preparation of compound 76, which is a compound of Formula 1 or salt thereof.

Scheme 31: Synthesis of compound 76



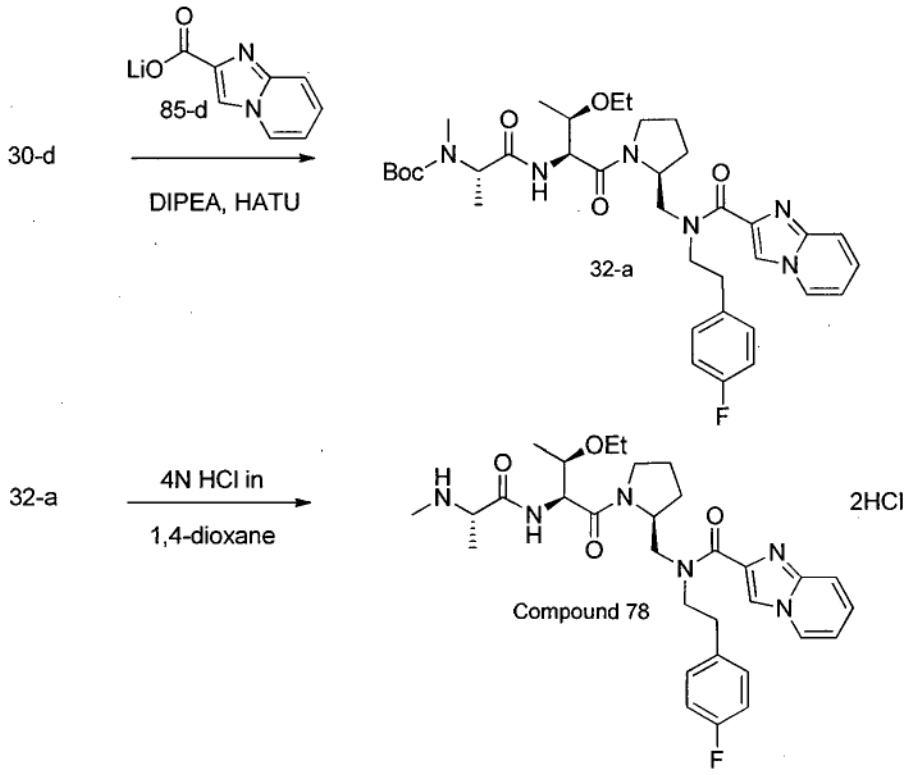
[0236] *Step 1:* To a solution of intermediate 30-d (2.0 g, 3.73 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-methyl-1H-imidazole-4-carboxylic acid (564 mg, 4.47 mmol), HATU (1.70 g, 4.47 mmol) and DIPEA (2.60 mL, 14.91 mmol) and the reaction mixture was stirred at 0 ºC for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 31-a as a white foam.

[0237] *Step 2:* 4N HCl in 1,4-dioxane (11.05 mL) was added to intermediate 31-a (1.90 g, 2.95 mmol) and the solution was stirred for 3 hours at 0 ºC. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 76·2HCl as a white solid. MS (m/z) M+1= 545.5

EXAMPLE 28

[0238] The following example illustrates the preparation of compound 78, which is a compound of Formula 1 or salt thereof.

Scheme 32: Synthesis of compound 78



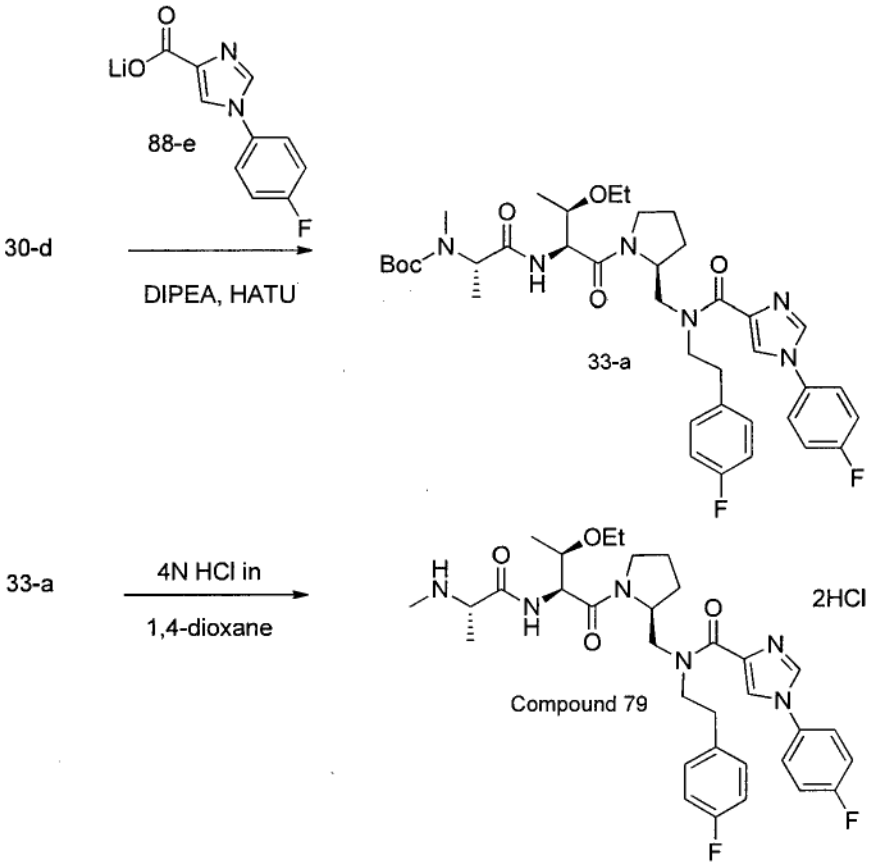
[0239] *Step 1:* To a solution of intermediate 30-d (2.0 g, 3.73 mmol) in DMF, cooled to 0 ºC, were sequentially added imidazo [1,2-a]pyridine-2-carboxylic acid, lithium salt (85-d) (752 mg, 4.47 mmol), HATU (1.70 g, 4.47 mmol) and DIPEA (2.60 mL, 14.91 mmol) and the reaction mixture was stirred at 0 ℃ for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 32-a as a white foam.

[0240] *Step 2:* 4N HCl in 1,4-dioxane (8.81 mL) was added to intermediate 32-a (1.60 g, 2.35 mmol) in ethyl acetate (0.783 mL) and the solution was stirred for 3 hours at 0 ℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 78·2HCl as a white solid. MS (m/z) M+1= 581.4

EXAMPLE 29

[0241] The following example illustrates the preparation of compound 79, which is a compound of Formula 1 or salt thereof.

Scheme 33: Synthesis of compound 79



[0242] *Step 1:* To a solution of intermediate 30-d (1.6 g, 2.98 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-(4-fluorophenyl)-1H-imidazole-4-carboxylic acid, lithium salt (88-e) (632 mg, 2.98 mmol), HATU (1.36 g, 3.58 mmol) and DIPEA (2.10L, 11.93 mmol) and the reaction mixture was stirred at 0 ℃ for 2 hours. Saturated

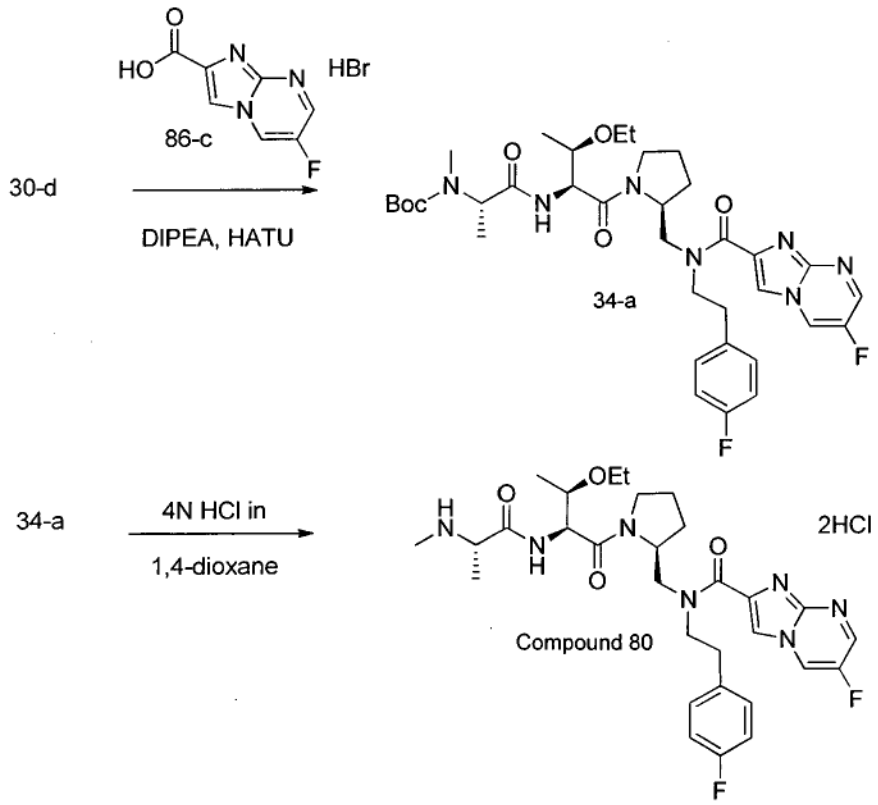
aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 33-a as a white foam.

[0243] *Step 2:* 4N HCl in 1,4-dioxane (6.62 mL) was added to intermediate 33-a (1.28 g, 1.76 mmol) and the solution was stirred for 3 hours at 0°C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 79·2HCl as a white solid. MS (m/z) M+1= 625.5

EXAMPLE 30

[0244] The following example illustrates the preparation of compound 80, which is a compound of Formula 1 or salt thereof.

Scheme 34: Synthesis of compound 80



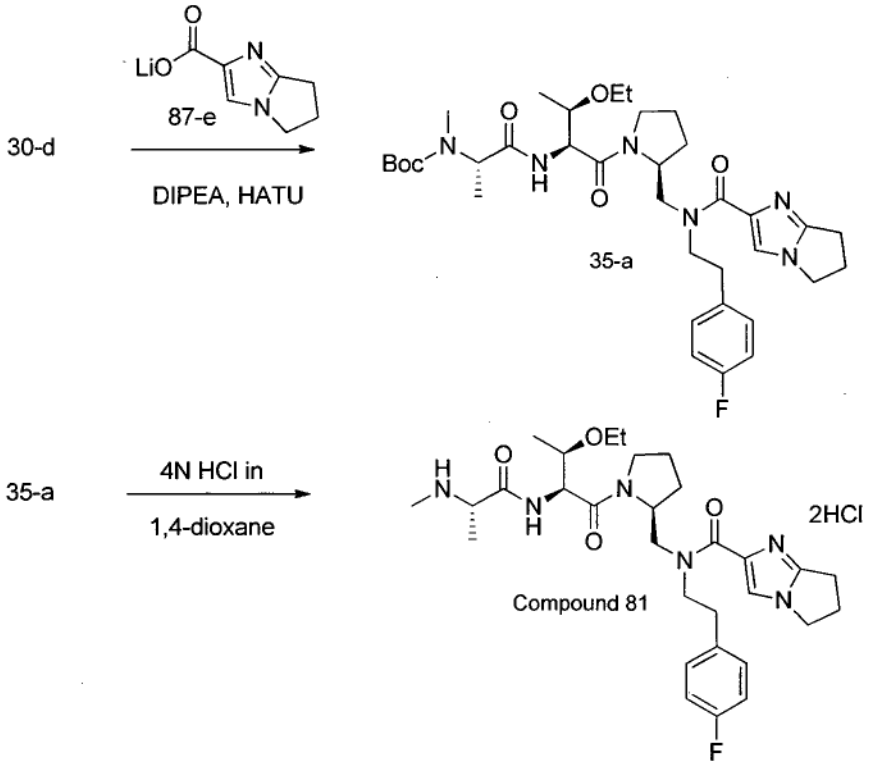
[0245] *Step 1:* To a solution of intermediate 30-d (1.60 g, 2.98 mmol) in DMF, cooled to 0 ºC, were sequentially added 6-fluoroimidazo[1,2-a]pyrimidine-2-carboxylic acid, HBr salt (86-c) (937 mg, 3.58 mmol), HATU (1.36 g, 3.58 mmol) and DIPEA (2.07 mL, 11.93 mmol) and the reaction mixture was stirred at 0 ºC for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 34-a as a white foam.

[0246] *Step 2:* 4N HCl in 1,4-dioxane (7.93 mL) was added to intermediate 34-a (1.48 g, 2.11 mmol) and the solution was stirred for 3 hours at 0 ºC. Diethyl ether was added, a precipitate formed and compound 80·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 600.5

EXAMPLE 31

[0247] The following example illustrates the preparation of compound 81, which is a compound of Formula 1 or salt thereof.

Scheme 35: Synthesis of compound 81



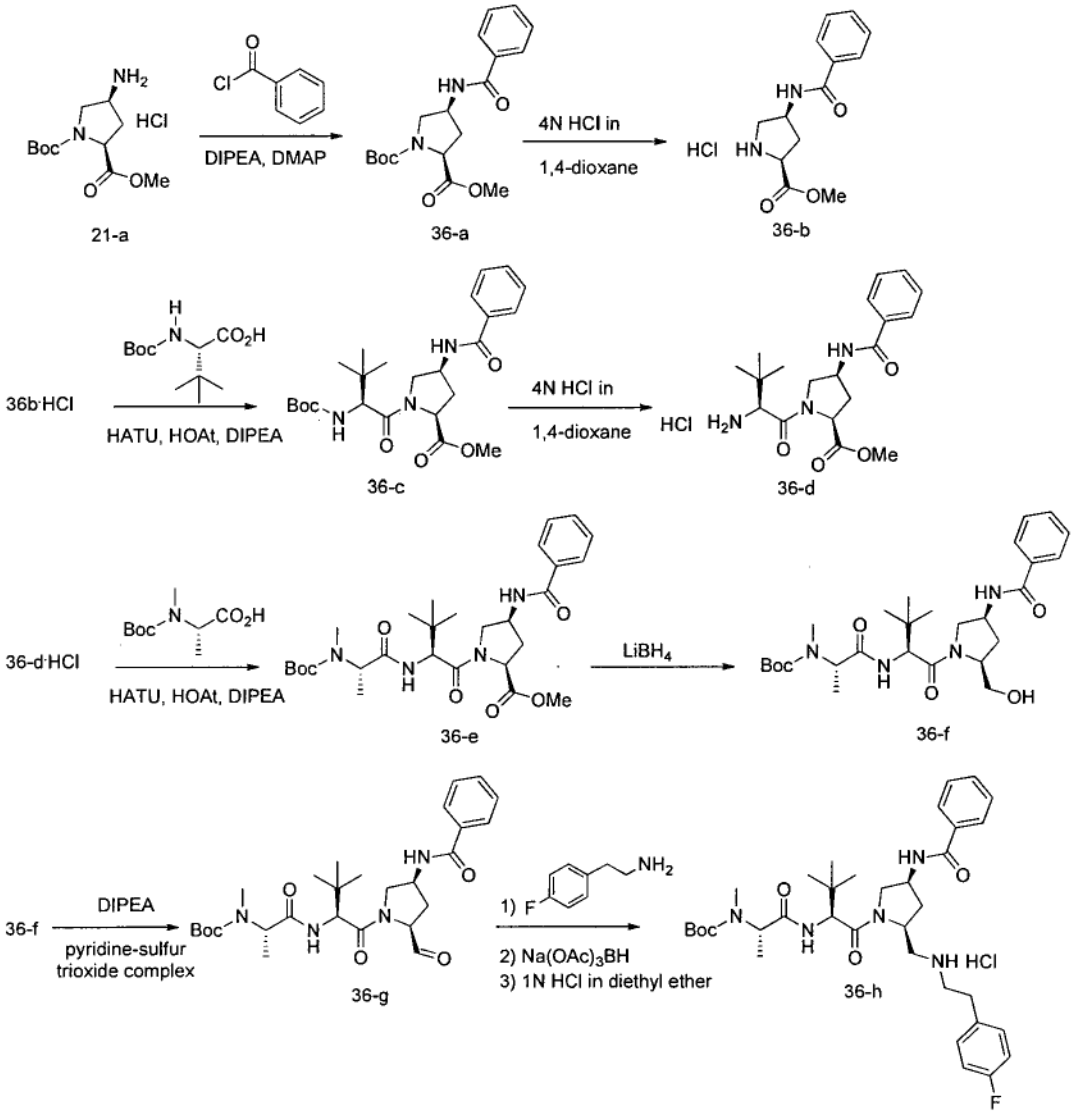
[0248] *Step 1:* To a solution of intermediate 30-d (1.6 g, 2.98 mmol) in DMF, cooled to 0 ºC, were sequentially added 6,7-dihydro-5-H-pyrrolo[1,2-a]imidazole-2-carboxylic acid, lithium salt (87-e) (566 mg, 3.58 mmol), HATU (1.36 g, 3.58 mmol) and DIPEA (2.0 mL, 11.93 mmol) and the reaction mixture was stirred at 0 ºC for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 35-a as a white foam.

[0249] *Step 2:* 4N HCl in 1,4-dioxane (6.48 mL) was added to intermediate 35-a (1.45 g, 2.16 mmol) and the solution was stirred for 3 hours at 0 ºC. Diethyl ether was added, a precipitate formed and compound 81·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 571.5

EXAMPLE 32

[0250] The following example illustrates the preparation of compound 36-h, which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof.

Scheme 36: Synthesis of Intermediate 36-h



21-a 36-a 36-b 36-c 36-d 36-e 36-f 36-g 36-h

[0251] *Step 1:* To a solution of intermediate 21-a·HCl (15.0 g, 53.4 mmol) in dichloromethane cooled to 0 °C were sequentially added DIPEA (37.3 mL, 214.0 mmol), DMAP (326 mg, 2.67 mmol) and benzoyl chloride (6.82 mL, 58.8 mmol) and the reaction was then stirred at room temperature overnight. Water and ethyl acetate were added, the organic layer was separated, washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 36-a as white solid.

[0252] *Step 2:* 4N HCl in 1,4-dioxane (141.0 mL) was added to intermediate 36-a (19.6 g, 56.3 mmol) and the solution was stirred for 1 hour at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide intermediate 36-b·HCl as a white solid. MS (m/z) M+1=249.2

[0253] *Step 3:* To a solution of intermediate 36-b·HCl (14.7 g, 51.6 mmol) in DMF cooled to 0 °C were sequentially added Boc-tBu-Gly-OH (13.13 g, 56.8 mmol), HOAt (8.60 mL, 5.16 mmol), HATU (21.59 g, 56.8 mmol), and DIPEA (36.1 mL, 207.0 mmol) and the reaction was then stirred at room temperature for 3 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 36-c as colorless oil.

[0254] *Step 4:* 4N HCl in 1,4-dioxane (130.0 mL) was added to intermediate 36-c (24.0 g, 52.0 mmol) and the solution was stirred for 1 hour at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide intermediate 36-d·HCl as a white solid. MS (m/z) M+1= 362.2

[0255] *Step 5:* To a solution of intermediate 36-d·HCl (10.73 g, 52.8 mmol) in DMF cooled to 0 °C were sequentially added Boc-NMe-Ala-OH (10.73 g, 52.8 mmol), HOAt (8.80 mL, 5.28 mmol), HATU (22.07 g, 58.1 mmol), and DIPEA (36.9 mL, 211.0 mmol) and the reaction was then stirred at room temperature for 3 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and

brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 36-e as white foam.

[0256] *Step 6:* To a solution of intermediate 36-e (29.0 g, 53.0 mmol) in THF cooled to 0 °C was added lithium borohydride (2.42 g, 111.0 mmol) and the reaction was stirred at room temperature for 1 hour. Water and ethyl acetate were added; the organic layer was separated, washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 36-f as a white foam.

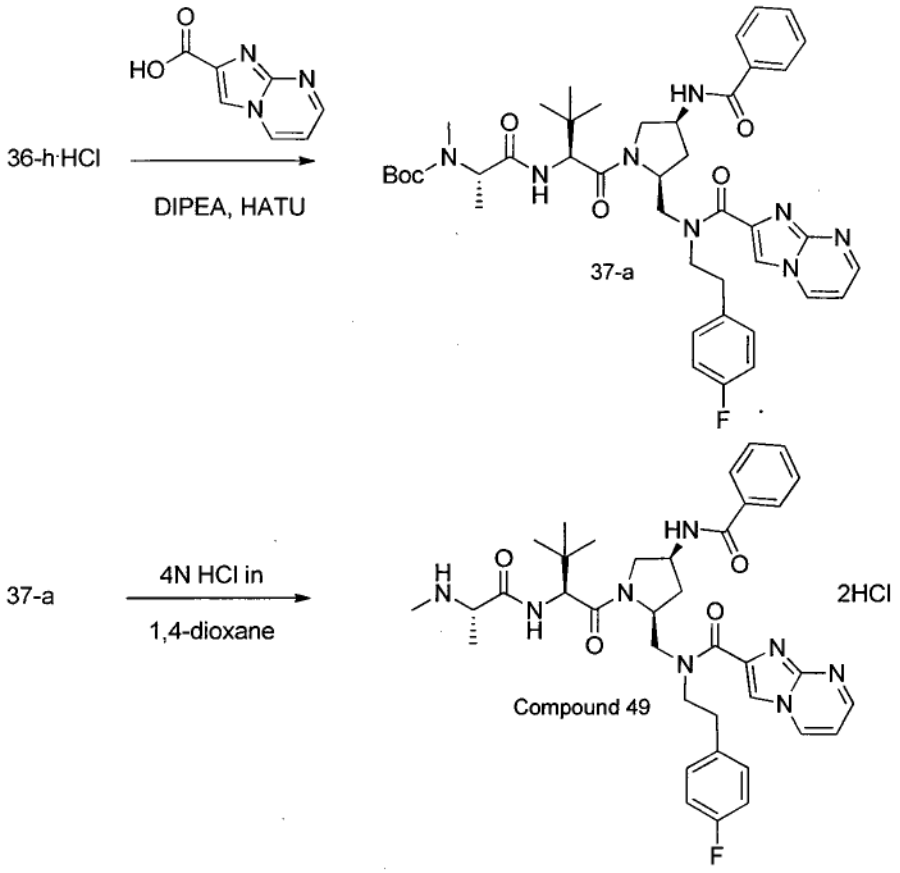
[0257] *Step 7:* To a solution of intermediate 36-f (25.8 g, 49.7 mmol) in DMSO (14.13 mL, 199.0 mmol) and dichloromethane (200 mL) cooled to 0°C was added DIPEA (30.3 mL, 174.0 mmol) and pyridine sulfur trioxide complex (23.75 g, 149.0 mmol), the reaction was then stirred at 0°C for 1 hour. Water and ethyl acetate were added; the organic layer was separated, washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 36-g as white foam.

[0258] *Step 8:* To a solution of intermediate 36-g (25.7 g, 49.7 mmol) in dichloromethane was added 2-(4-fluorophenyl)ethanamine (6.21 mL, 47.4 mmol). After stirring at room temperature overnight, sodium triacetoxyborohydride (21.14 g, 95.0 mmol) was added portion wise at 0 °C and the reaction mixture was then stirred at room temperature for 2 hours. Saturated aqueous $NaHCO_3$ was added; the organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 36-h as white foam. To a solution of intermediate 36-h (27.4 g, 42.8 mmol) in diethyl ether (500 mL) was added 1N HCl in diethyl ether (52.1 mL, 52.1 mmol), a precipitate formed and intermediate 36-h·HCl was collected by filtration as a white solid. MS (m/z) M+1= 640.6

EXAMPLE 33

[0259] The following example illustrates the preparation of compound 49, which is a compound of Formula 1 or salt thereof.

Scheme 37: Synthesis of compound 49



[0260] *Step 1:* To a solution of intermediate 36-h·HCl (2.0 g, 2.96 mmol) in DMF, cooled to 0 ºC, were sequentially added imidazo[1,2-a]pyrimidine-2-carboxylic acid (507 mg, 3.11 mmol), HATU (1.23 g, 3.25 mmol) and DIPEA (2.06 mL, 11.83 mmol) and the reaction mixture was stirred at room temperature for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 37-a as a white foam.

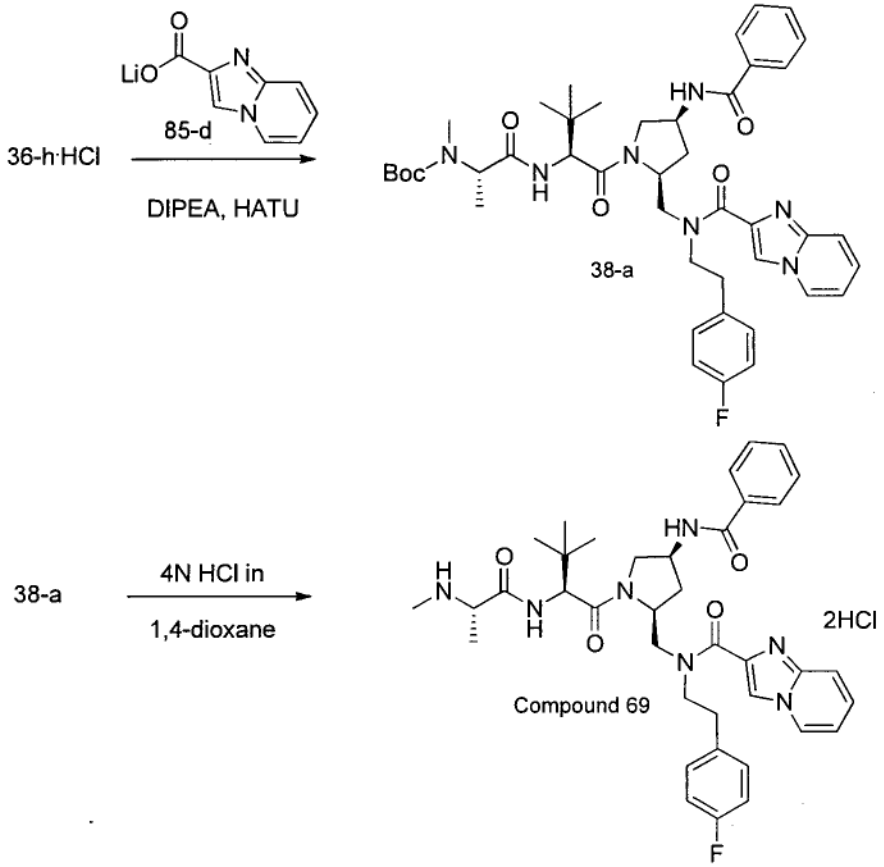
[0261] *Step 2:* 4N HCl in 1,4-dioxane (3.19 mL) was added to intermediate 37-a (1.0 g, 1.27 mmol) and the solution was stirred for 1 hour at 0 °C. Volatiles were removed

under reduced pressure and the residue was triturated with diethyl ether to provide compound 49·2HCl as a white solid. MS (m/z) M+1=685.5

EXAMPLE 34

[0262] The following example illustrates the preparation of compound 69, which is a compound of Formula 1 or salt thereof.

Scheme 38: Synthesis of compound 69



[0263] *Step 1:* To a solution of intermediate 36-h·HCl (2.0 g, 2.96 mmol) in DMF, cooled to 0 ºC, were sequentially added imidazo [1,2-a]pyridine-2-carboxylic acid, lithium salt (85-d) (525 mg, 3.11 mmol), HATU (1.23 g, 3.25 mmol) and DIPEA (2.06 mL, 11.83 mmol) and the reaction mixture was stirred at room temperature for 3 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous

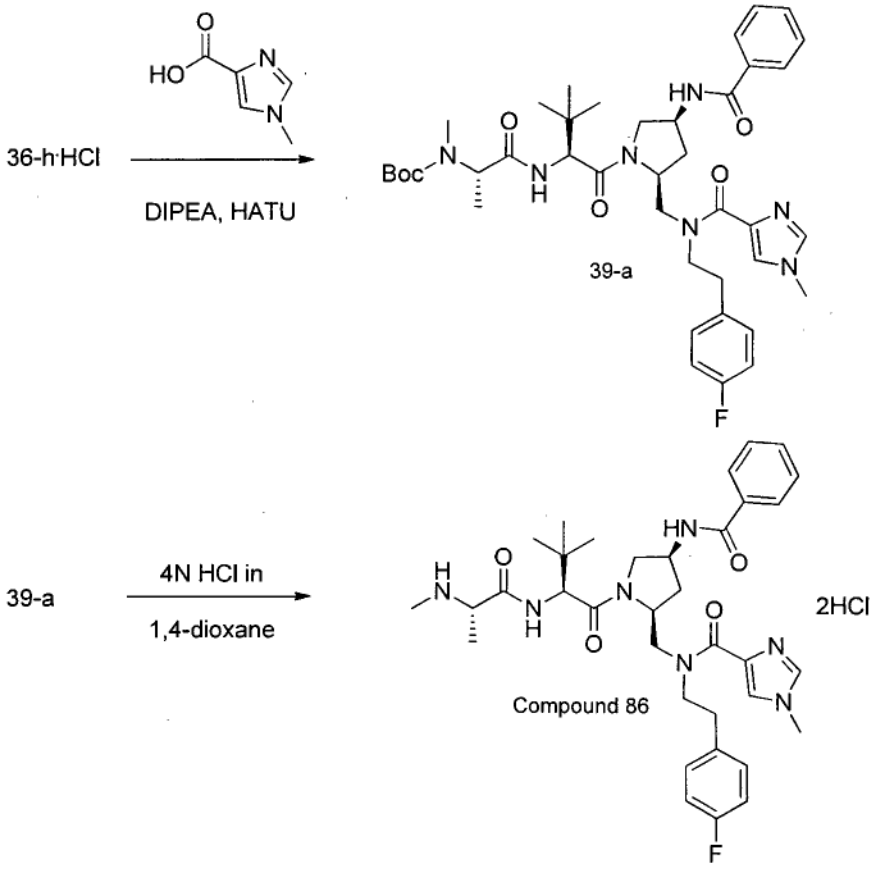
$NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 38-a as a white foam.

[0264] *Step 2:* 4N HCl in 1,4-dioxane (3.19 mL) was added to intermediate 38-a (1.24 g, 1.58 mmol) and the solution was stirred for 1 hour at 0℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 69·2HCl as a white solid. MS (m/z) M+1=684.4

EXAMPLE 35

[0265] The following example illustrates the preparation of compound 86, which is a compound of Formula 1 or salt thereof.

Scheme 39: Synthesis of compound 86



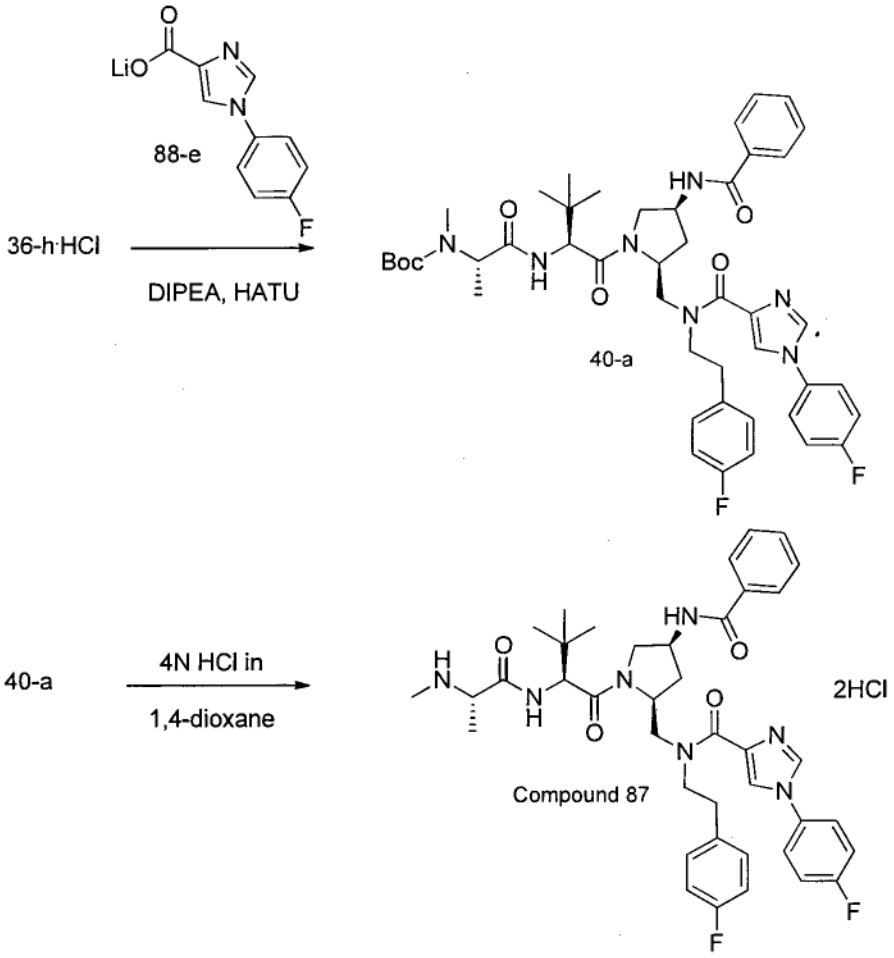
[0266] *Step 1:* To a solution of intermediate 36-h·HCl (2.0 g, 2.96 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-methyl-1H-imidazole-4-carboxylic acid (448 mg, 3.55 mmol), HATU (1.23 g, 3.25 mmol) and DIPEA (2.06 mL, 11.83 mmol) and the reaction mixture was stirred at room temperature for 3 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 39-a as a white foam.

[0267] *Step 2:* 4N HCl in 1,4-dioxane (4.68 mL) was added to intermediate 39-a (1.40 g, 1.87 mmol) and the solution was stirred for 1 hour at 0 ºC. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 86·2HCl as a white solid. MS (m/z) M+1=648.5

EXAMPLE 36

[0268] The following example illustrates the preparation of compound 87, which is a compound of Formula 1 or salt thereof.

Scheme 40: Synthesis of compound 87



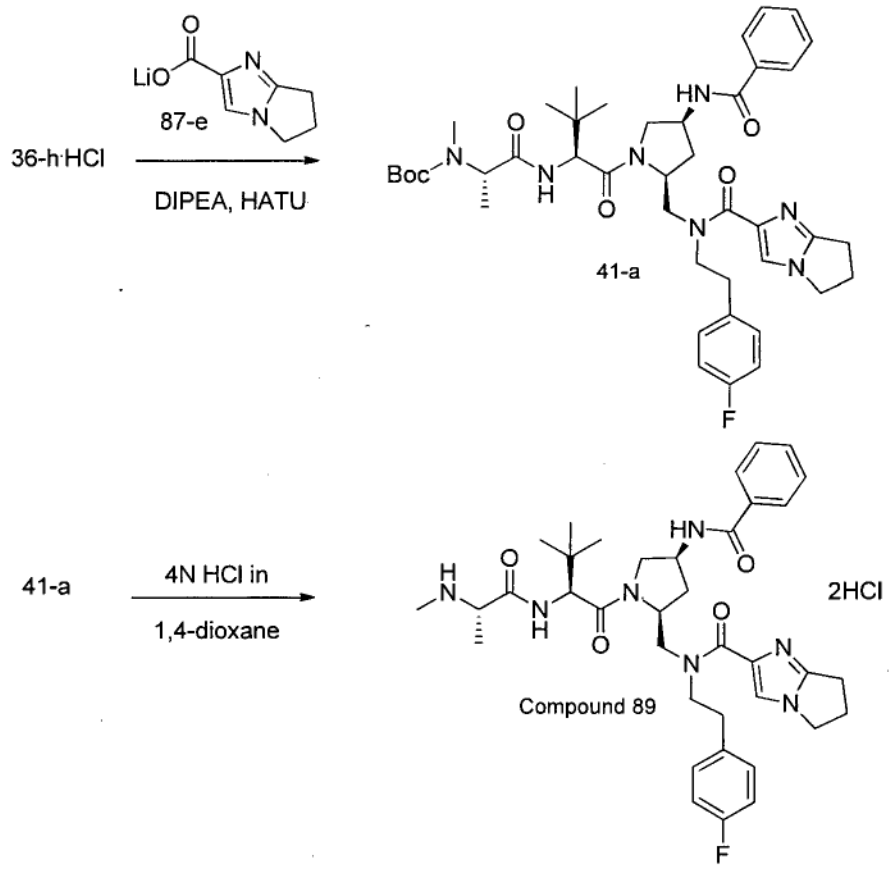
[0269] *Step 1:* To a solution of intermediate 36-h·HCl (2.0 g, 2.96 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-(4-fluorophenyl)-1H-imidazole-4-carboxylic acid, lithium salt (88-e) (662 mg, 3.11 mmol), HATU (1.23 g, 3.25 mmol) and DIPEA (2.06 mL, 11.83 mmol) and the reaction mixture was stirred at room temperature for 3 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 40-a as a white foam.

[0270] *Step 2:* 4N HCl in 1,4-dioxane (5.92 mL) was added to intermediate 40-a (1.40 g, 1.87 mmol) and the solution was stirred for 1 hour at 0°C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 87·2HCl as a white solid. MS (m/z) M+1=728.5

EXAMPLE 37

[0271] The following example illustrates the preparation of compound 89, which is a compound of Formula 1 or salt thereof.

Scheme 41: Synthesis of compound 89



[0272] *Step 1:* To a solution of intermediate 36-h·HCl (2.0 g, 2.96 mmol) in DMF, cooled to 0 ºC, were sequentially added 6,7-dihydro-5-H-pyrrolo[1,2-a]imidazole-2-carboxylic acid, lithium salt (87-e) (589 mg, 3.55 mmol), HATU (1.23 g, 3.25 mmol) and

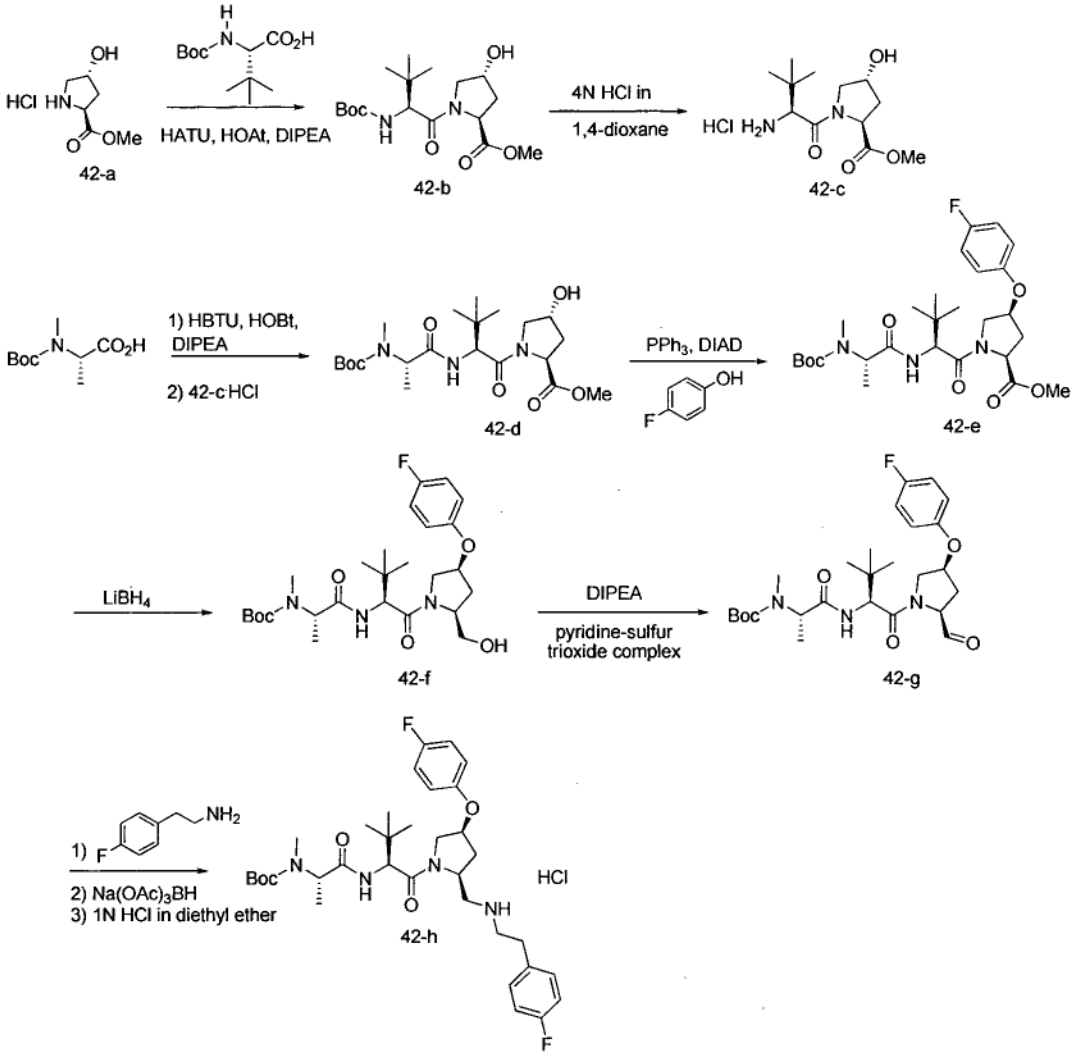
DIPEA (2.06 mL, 11.83 mmol) and the reaction mixture was stirred at room temperature for 3 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 41-a as a white foam.

[0273] *Step 2:* 4N HCl in 1,4-dioxane (3.29 mL) was added to intermediate 41-a (1.02 g, 1.32 mmol) and the solution was stirred for 1 hour at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 89·2HCl as a white solid. MS (m/z) M+1=674.5

EXAMPLE 38

[0274] The following example illustrates the preparation of compound 42-h , which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof.

Scheme 42: Synthesis of Intermediate 42-h



[0275] *Step 1:* To a solution of (2S, 4R)-methyl 4-hydroxypyrrolidine-2-carboxylate, HCl salt 42-a (4.0 g, 22.02 mmol) in DMF cooled to 0 °C were sequentially added Boc-tBu-Gly-OH (6.11 g, 26.4 mmol), HOAt (5.51 mL, 3.30 mmol), HATU (10.89 g, 28.6 mmol), and DIPEA (15.39 mL, 88.0 mmol) and the reaction was then stirred at room temperature for 3 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and

concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 42-b as a beige solid.

[0276] *Step 2:* 4N HCl in 1,4-dioxane (80 mL) was added to intermediate 42-b (7.89 g, 22.0 mmol) and the solution was stirred for 3 hours at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide intermediate 42-c·HCl as a white foam. MS (m/z) M+1= 259.1

[0277] *Step 3:* To a solution of Boc-NMe-Ala-OH (7.16 g, 35.2 mmol) in DMF cooled to 0 °C were sequentially added HOBt (5.73 g, 37.4 mmol), HBTU (14.19 g, 37.4 mmol), and DIPEA (19.23 mL, 40.0 mmol). After stirring for 10 minutes, intermediate 42-c (6.49 g, 25.1 mmol) was added and the reaction was then stirred at room temperature overnight. Water and ethyl acetate were added; the organic layer was separated, washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 42-d as a white solid.

[0278] Step 4: To a solution of intermediate 42-d (2.32 g, 5.23 mmol), 4-fluorophenol (704 mg, 6.28 mmol) and triphenylphosphine (1.92 g, 7.32 mmol) in THF was added DIAD (1.42 mL, 7.32 mmol) dropwise and the reaction was then stirred at room temperature for 2 days. Diethyl ether and hexane were added, a precipitate formed and triphenyl phosphine oxide was removed by filtration. Volatiles were removed under reduced pressure and the residue was purified by silica gel chromatography to provide the expected intermediate 42-e as a colorless oil.

[0279] *Step 5:* To a solution of intermediate 42-e (4.6 g, 8.56 mmol) in THF cooled to 0°C was added lithium borohydride (559 mg, 25.7 mmol) and the reaction was stirred at room temperature for 3 hours. Water and ethyl acetate were added; the organic layer was separated, washed with 10% citric acid, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 42-f as colorless oil.

[0280] *Step 6:* To a solution of intermediate 42-f (4.2 g, 8.24 mmol) in DMSO (2.33 mL, 33.0 mmol) and dichloromethane (80 mL) cooled to 0°C was added DIPEA (5.04 mL, 28.8 mmol) and pyridine sulfur trioxide complex (3.94 g, 24.72 mmol), the

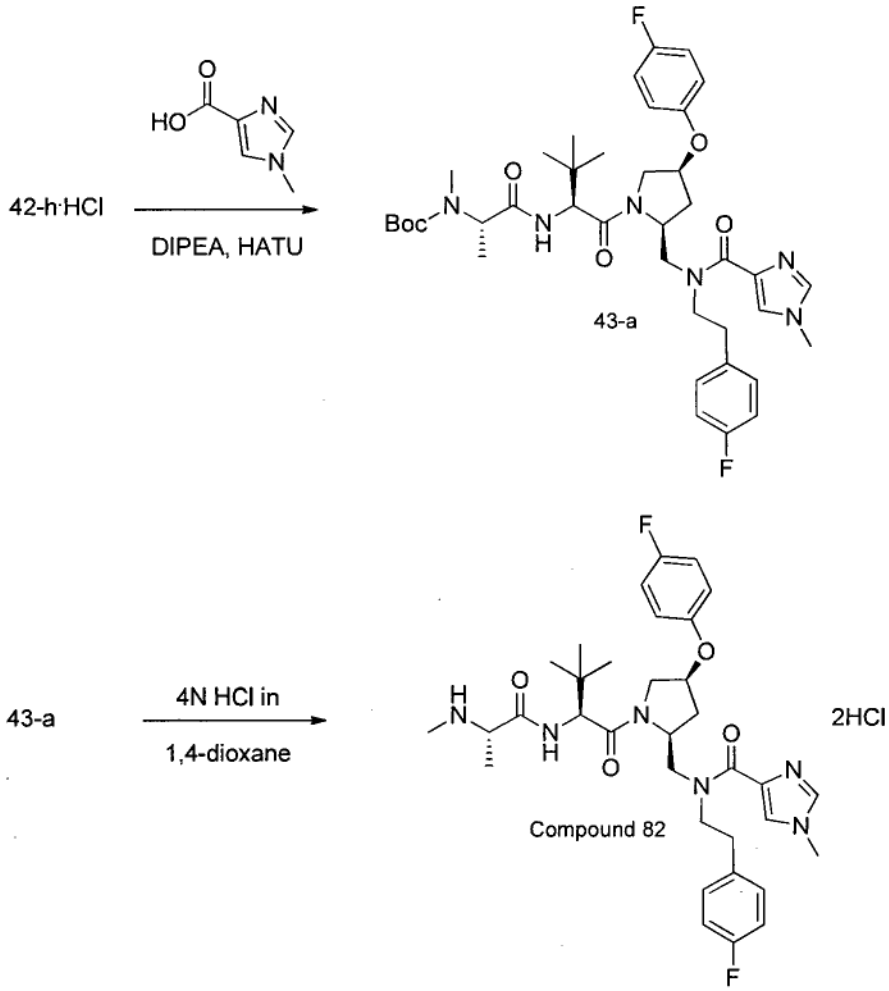
reaction was then stirred at 0 °C for 1 hour. Water and ethyl acetate were added; the organic layer was separated, washed with 10% citric acid, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 42-g as beige solid.

[0281] *Step 7:* To a solution of intermediate 42-g (3.8 g, 7.49 mmol) in dichloromethane was added 2-(4-fluorophenyl)ethanamine (935 uL, 7.13 mmol). After stirring at room temperature overnight, sodium triacetoxyborohydride (3.18 g, 14.26 mmol) was added portion wise at 0 °C and the reaction mixture was then stirred at room temperature for 2 hours. Saturated aqueous $NaHCO_3$ was added; the organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 42-g as yellow oil. To a solution of intermediate 42-g in diethyl ether (100 mL) was added 1N HCl in diethyl ether (7.84 mL, 7.84 mmol), a precipitate formed and intermediate 42-h·HCl was collected by filtration as beige solid. MS (m/z) M+1= 631.5

EXAMPLE 39

[0282] The following example illustrates the preparation of compound 82, which is a compound of Formula 1 or salt thereof.

Scheme 43: Synthesis of compound 82



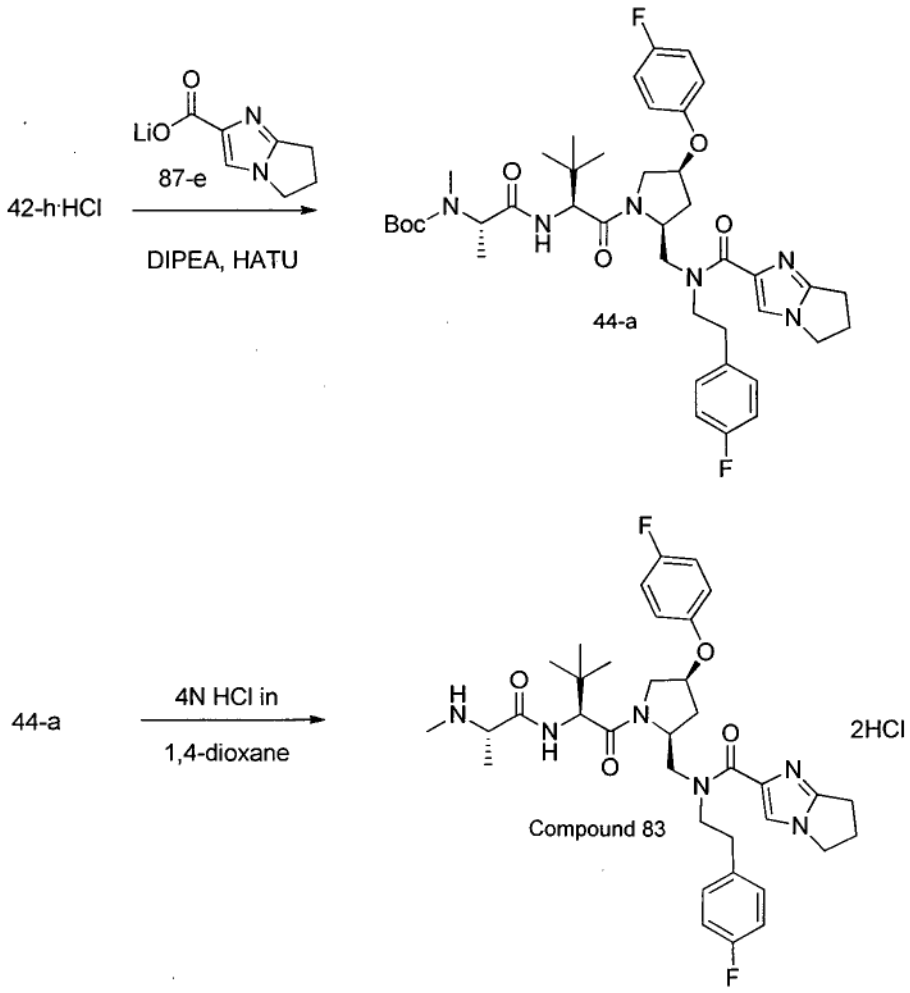
[0283] *Step 1:* To a solution of intermediate 42-h·HCl (1.50 g, 2.24 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-methyl-1H-imidazole-4-carboxylic acid (397 mg, 3.15 mmol), HATU (1.19 g, 3.15 mmol) and DIPEA (1.57 mL, 8.99 mmol) and the reaction mixture was stirred at room temperature for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 43-a as a white foam.

[0284] *Step 2:* 4N HCl in 1,4-dioxane (4.50 mL) was added to intermediate 43-a (1.10 g, 1.48 mmol) and the solution was stirred for 2 hour at 0 ℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 82·2HCl as a white solid. MS (m/z) M+1=639.5

EXAMPLE 40

[0285] The following example illustrates the preparation of compound 83, which is a compound of Formula 1 or salt thereof.

Scheme 44: Synthesis of compound 83



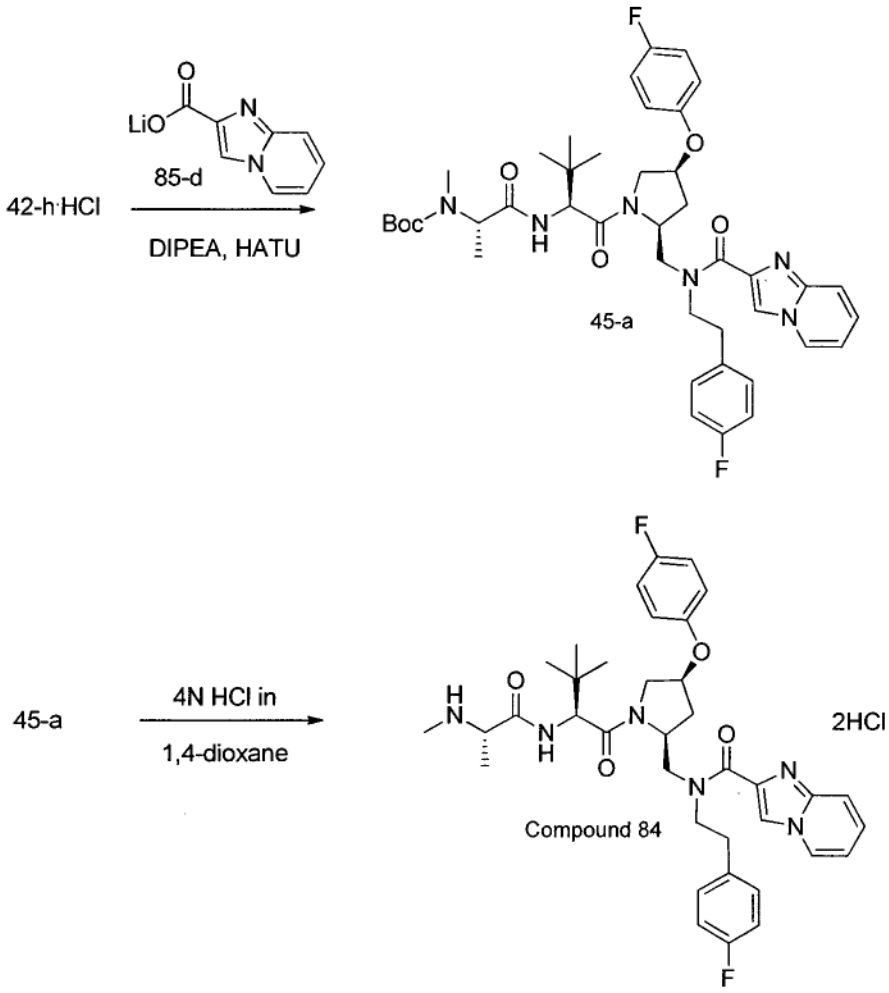
[0286] *Step 1:* To a solution of intermediate 42-h·HCl (1.40 g, 2.01 mmol) in DMF, cooled to 0 ºC, were sequentially added 6,7-dihydro-5-H-pyrrolo[1,2-a]imidazole-2-carboxylic acid, lithium salt (87-e) (447 mg, 2.94 mmol), HATU (1.12 g, 2.94 mmol) and DIPEA (1.46 mL, 8.39 mmol) and the reaction mixture was stirred at room temperature for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 44-a as a white foam.

[0287] *Step 2:* 4N HCl in 1,4-dioxane (6.69 mL) was added to intermediate 44-a (1.02 g, 1.33 mmol) and the solution was stirred for 2 hours at 0 ºC. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ethe r to provide compound 83·2HCl as a white solid. MS (m/z) M+1=665.5

EXAMPLE 41

[0288] The following example illustrates the preparation of compound 84, which is a compound of Formula 1 or salt thereof.

Scheme 45: Synthesis of compound 84



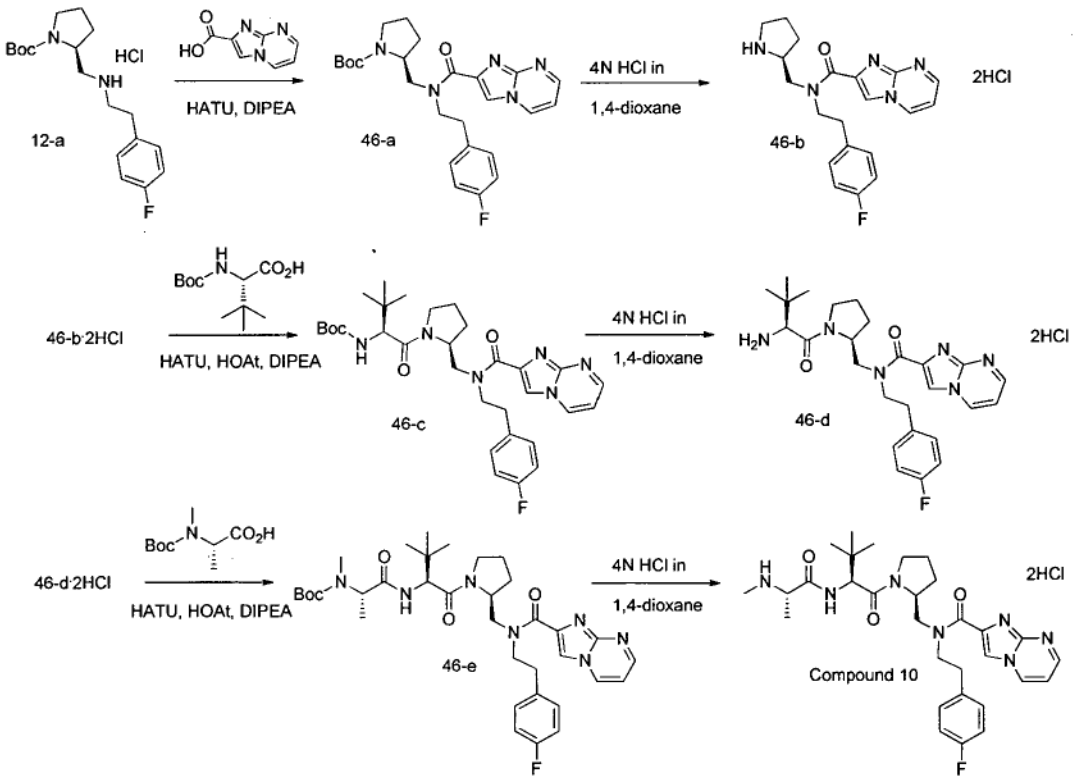
[0289] *Step 1:* To a solution of intermediate 42-h·HCl (2.0 g, 2.96 mmol) in DMF, cooled to 0 ºC, were sequentially added imidazo [1,2-a]pyridine-2-carboxylic acid, lithium salt (85-d) (494 mg, 2.94 mmol), HATU (1.11 g, 2.94 mmol) and DIPEA (1.46 mL, 8.39 mmol) and the reaction mixture was stirred at room temperature for 3 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 45-a as a white foam.

[0290] *Step 2:* 4N HCl in 1,4-dioxane (5.97 mL) was added to intermediate 45-a (926 mg, 1.19 mmol) and the solution was stirred for 2 hours at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 84·2HCl as a white solid. MS (m/z) M+1=675.5

EXAMPLE 42

[0291] The following example illustrates the preparation of compound 10, which is a compound of Formula 1 or salt thereof.

Scheme 46: Synthesis of compound 10



[0292] *Step 1:* To a solution of intermediate 12-a·HCl (25.0 g, 69.7 mmol) in DMF cooled to 0 °C were sequentially added imidazo [1,2-a]pyrimidine-2-carboxylic acid (13.64 g, 84.0 mmol), HATU (34.4 g, 91.0 mmol) and DIPEA (48.5 mL, 279.0 mmol) dropwise over a period of 45 minutes and the reaction was then stirred at 0 °C for 30 minutes.

Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 46-a as a beige foam.

[0293] *Step 2:* 4N HCl in 1,4-dioxane (171 mL) was added to intermediate 46-a (32.0 g, 68.4 mmol) and the solution was stirred for 1 hour at 0 °C. Diethyl ether was added and intermediate 46-b·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 368.3

[0294] *Step 3:* To a solution of intermediate 46-b·2HCl (25.0 g, 56.8 mmol) in DMF cooled to 0 °C were sequentially added Boc-tBu-gly-OH (14.44 g, 62.5 mmol), HATU (28.1 g, 73.8 mmol), HOAt (14.19 mL, 8.52 mmol) and DIPEA (39.6 mL, 227.0 mmol) dropwise over a period of 30 minutes and the reaction was then stirred at 0 °C for 45 minutes. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 46-c as white foam.

[0295] *Step 4:* 4N HCl in 1,4-dioxane (62.4 mL) was added to intermediate 46-c (14.5 g, 24.97 mmol) and the solution was stirred for 2 hours at 0 °C. Diethyl ether was added and intermediate 46-d·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 481.5

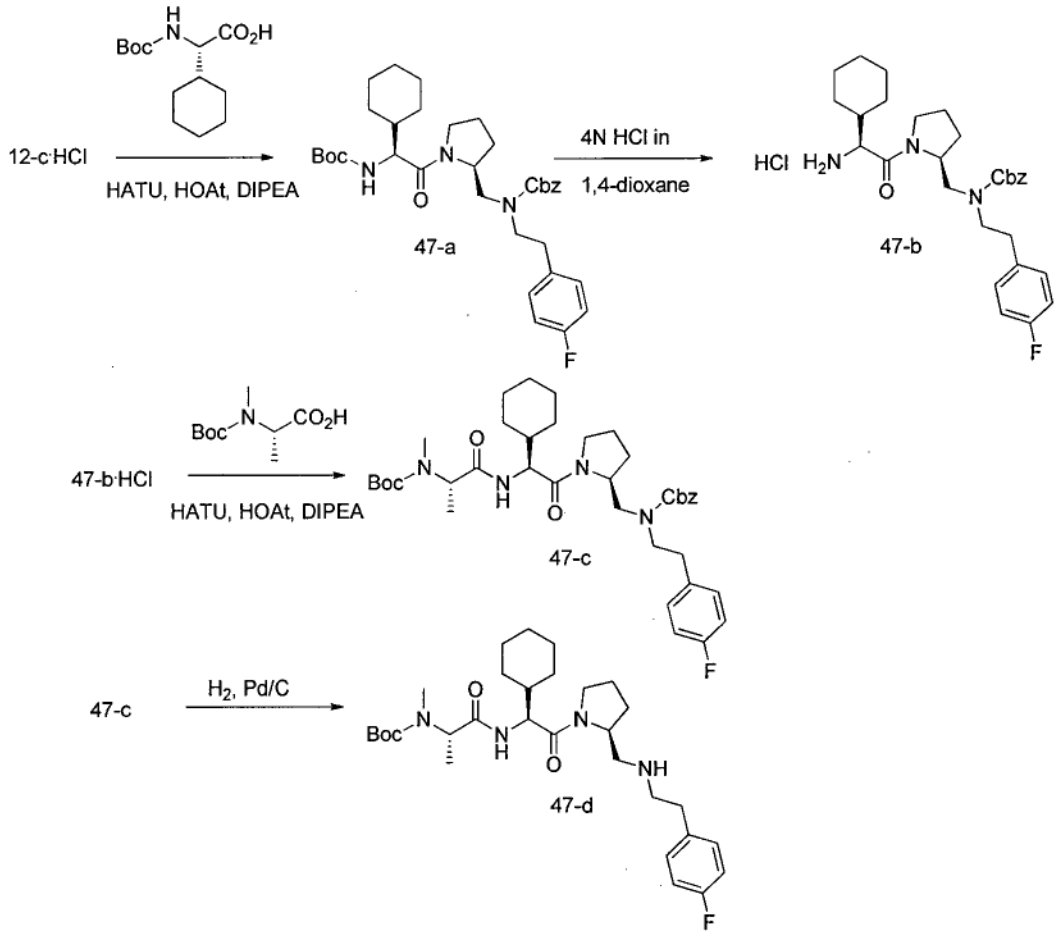
[0296] *Step 5:* To a solution of intermediate 46-d·2HCl (13.8 g, 26.7 mmol) in DMF cooled to 0 °C were sequentially added Boc-NMe-Ala-OH (5.97 g, 29.4 mmol), HATU (13.19 g, 34.7 mmol), HOAt (6.67 mL, 4.0 mmol) and DIPEA (18.6 mL, 107.0 mmol) dropwise over a period of 30 minutes and the reaction was then stirred at 0 °C for 30 minutes. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 46-e as white foam.

[0297] *Step 6:* 4N HCl in 1,4-dioxane (58.2 mL) was added to intermediate 46-e (15.5 g, 23.28 mmol) in ethyl acetate (5 mL) and the solution was stirred for 1.5 hours at 0℃. Diethyl ether was added and compound 10·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 566.5

EXAMPLE 43

[0298] The following example illustrates the preparation of compound 47-d, which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof.

Scheme 47: Synthesis of intermediate 47-d



[0299] *Step 1:* To a solution of intermediate 12-c·HCl (29.57 g, 75.0 mmol) in DMF cooled to 0 °C were sequentially added Boc-Chg-OH (22.27 g, 87.0 mmol), HATU (42.9 g, 113.0 mmol), HOAt (18.82 mL, 11.29 mmol) and DIPEA (41.4 mL, 226.0 mmol) over a period of 30 minutes and the reaction was then stirred at room temperature for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 47-a as a white foam.

[0300] *Step 2:* 4N HCl in 1,4-dioxane (188 mL) was added to intermediate 47-a (44.7 g, 75.0 mmol) in ethyl acetate (10 mL) at 0 °C and the solution was stirred for 4 hours at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide intermediate 47-b·HCl as a white foam. MS (m/z) M+1= 596.4

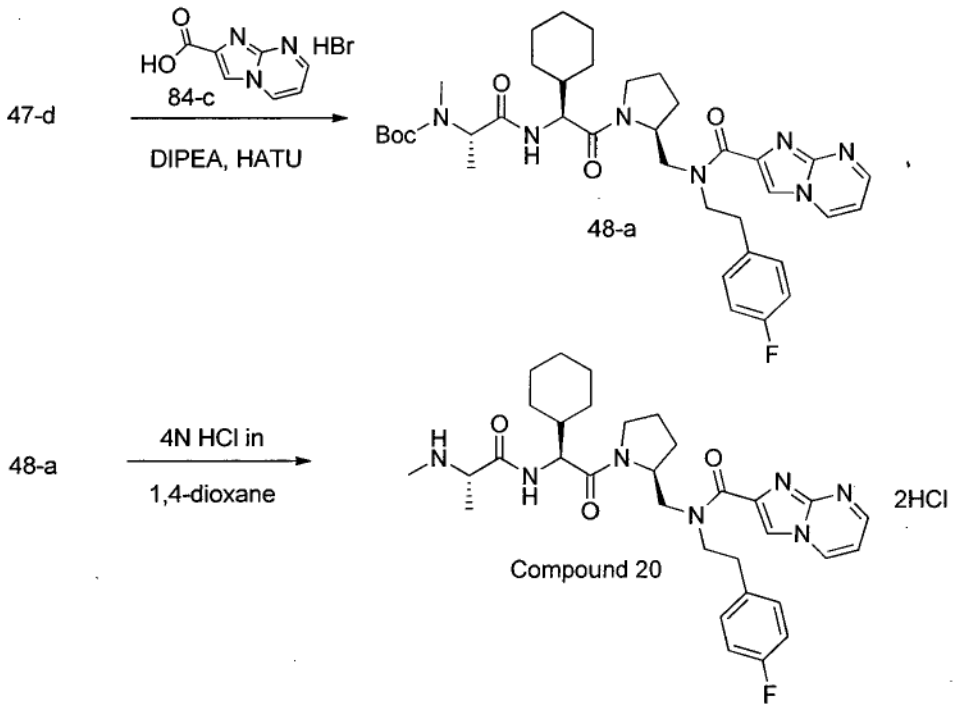
[0301] *Step 3:* To a solution of intermediate 47-b·HCl (25.8 g, 48.50 mmol) in DMF cooled to 0 °C were sequentially added Boc-NMe-Ala-OH (11.33 g, 55.8 mmol), HATU (25.8 g, 67.9 mmol), HOAt (8.08 mL, 4.85 mmol) and DIPEA (33.8 mL, 194.0 mmol) dropwise over a period of 30 minutes and the reaction was then stirred at 0 °C for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 47-c as a white foam.

[0302] *Step 4:* To a solution of intermediate 47-c (23.7 g, 34.8 mmol) in MeOH (100 mL) under N_2 was added 10 % Pd/C (50% w/w water content) (7.4 g). The reaction mixture was purged with H_2 and stirred for 2 hours. The reaction was then filtered through celite and the filtrate was concentrated in vacuo to provide intermediate 47-d as a white foam. MS (m/z) M+1= 547.4

EXAMPLE 44

[0303] The following example illustrates the preparation of compound 20, which is a compound of Formula 1 or salt thereof.

Scheme 48: Synthesis of compound 20



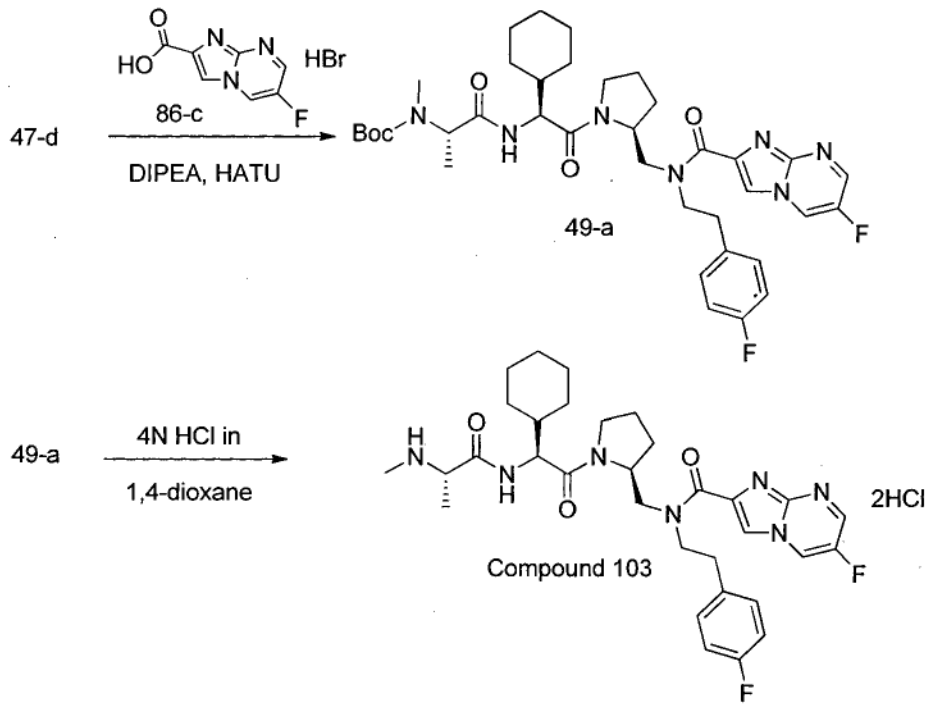
[0304] *Step 1:* To a solution of intermediate 47-d (17.9 g, 32.7 mmol) in DMF cooled to 0 ºC were sequentially added imidazo[1,2-a]pyrimidine-2-carboxylic acid, HBr salt (84-c) (9.59 g, 39.3 mmol), HATU (18.67 g, 49.1 mmol) and DIPEA (17.11 mL, 98.0 mmol) over a period of 30 minutes and the reaction mixture was stirred at 0 ℃ for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 48-a as white foam.

[0305] *Step 2:* 4N HCl in 1,4-dioxane (44.5 mL) was added to intermediate 48-a (12.32 g, 17.81 mmol) in ethyl acetate (10 mL) at 0 ℃ and the solution was stirred for 4 hours at 0 ℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 20·2HCl as a white solid. MS (m/z) M+1= 592.4

EXAMPLE 45

[0306] The following example illustrates the preparation of compound 103, which is a compound of Formula 1 or salt thereof.

Scheme 49: Synthesis of compound 103



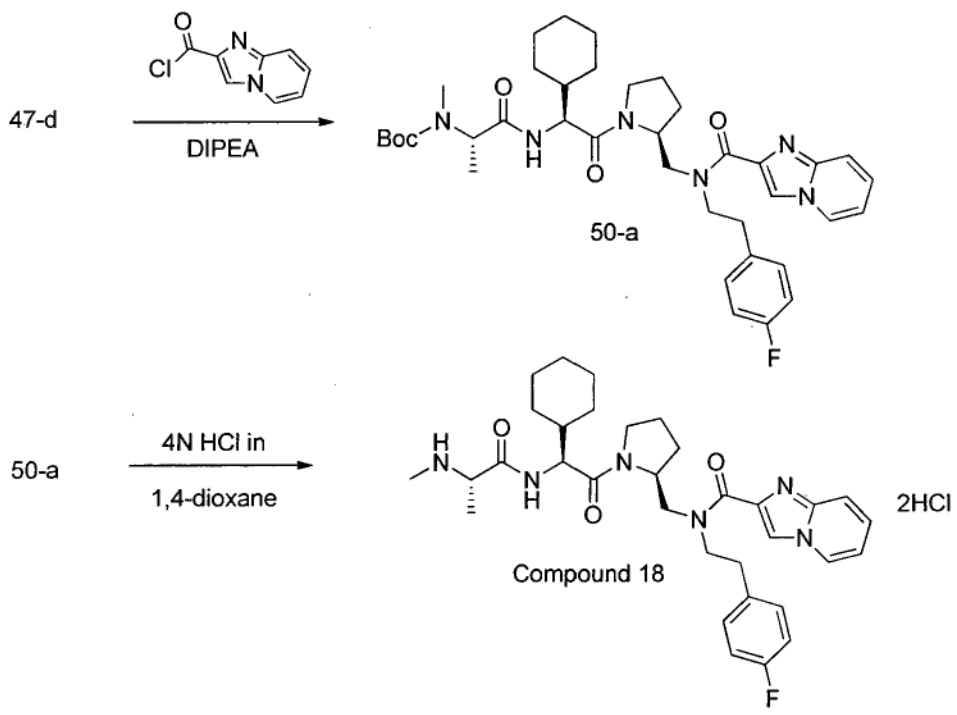
[0307] *Step 1:* To a solution of intermediate 47-d (500 mg, 0.91 mmol) in DMF, cooled to 0 ºC, were sequentially added 6-fluoroimidazo[1,2-a]pyrimidine-2-carboxylic acid, HBr salt (86-c) (276 mg, 1.05 mmol), HATU (522 mg, 1.37 mmol) and DIPEA (478 uL, 2.74 mmol) and the reaction mixture was stirred at 0 ° for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 49-a as a white foam.

[0308] *Step 2:* 4N HCl in 1,4-dioxane (882 uL) was added to intermediate 49-a (250 mg, 0.35 mmol) in ethyl acetate (500 uL) and the solution was stirred for 2 hours at 0 ℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 103·2HCl as a white solid. MS (m/z) M+1= 610.3

EXAMPLE 46

[0309] The following example illustrates the preparation of compound 18, which is a compound of Formula 1 or salt thereof.

Scheme 50: Synthesis of compound 18



[0310] *Step 1:* To a solution of intermediate 47-d (303 mg, 0.55 mmol) in DMF, cooled to 0 ºC, were sequentially added DIPEA (483 uL, 2.77 mmol) and imidazo[1,2-a]pyrimidine-2-carbonyl chloride (250 mg, 1.38 mmol) and the reaction mixture was stirred at room temperature for 30 minutes. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous

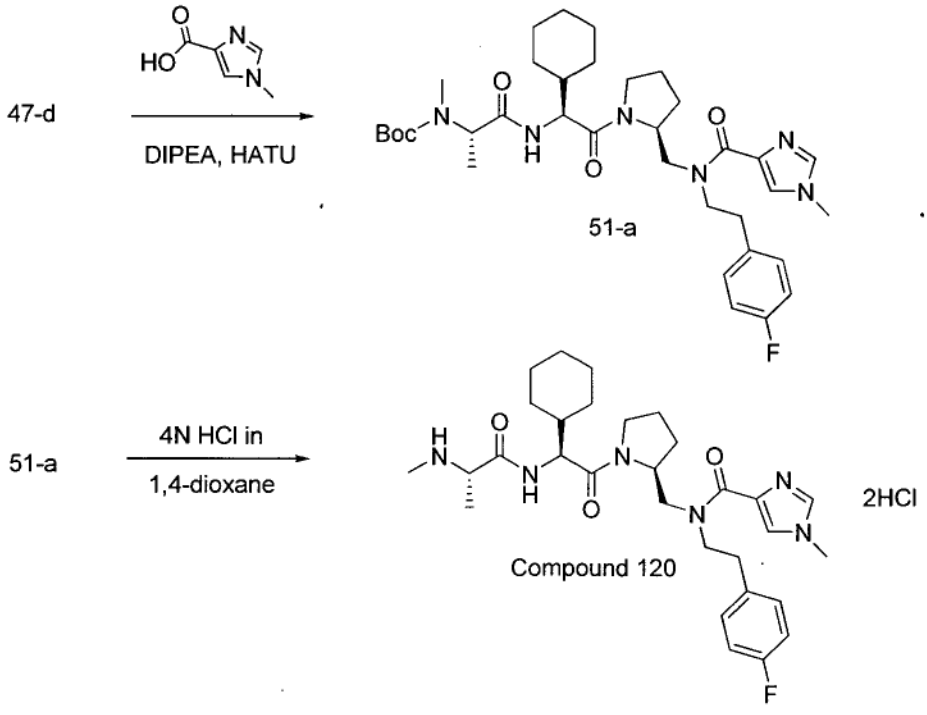
ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 50-a as a white foam.

[0311] *Step 2:* 4N HCl in 1,4-dioxane (1.85 mL) was added to intermediate 50-a (366 mg, 0.53 mmol) in methanol (200 uL) and the solution was stirred for 2 hours at 0 ºC. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 18·2HCl as white solid. MS (m/z) M+1= 591.4

EXAMPLE 47

[0312] The following example illustrates the preparation of compound 120, which is a compound of Formula 1

Scheme 51: Synthesis of compound 120



[0313] *Step 1:* To a solution of intermediate 47-d (500 mg, 0.91 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-methyl-1H-imidazole-4-carboxylic acid (138 mg,

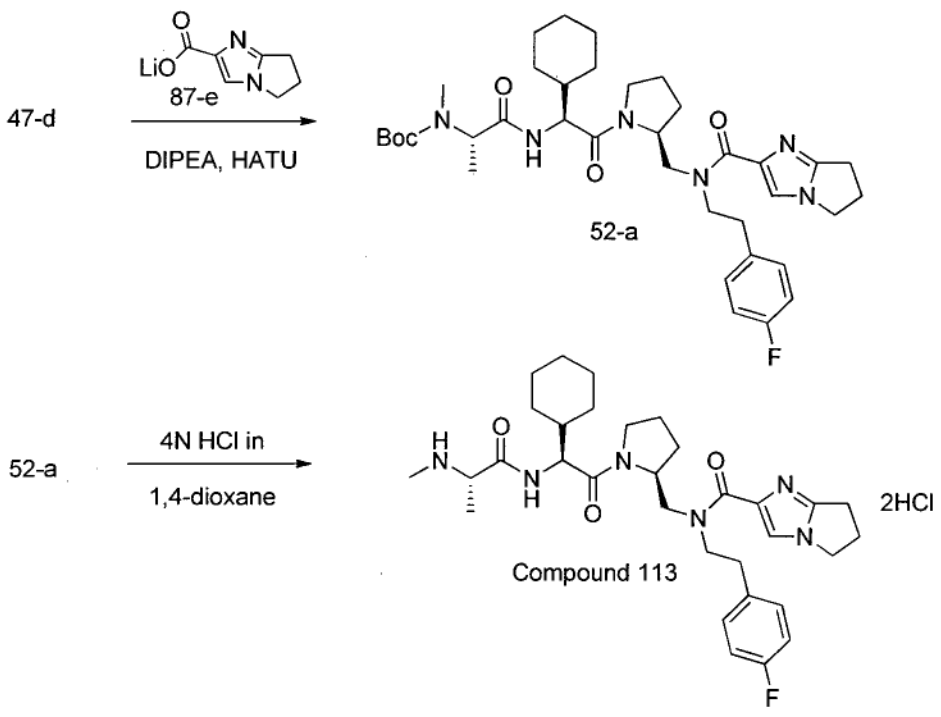
1.09 mmol), HATU (522 mg, 1.37 mmol) and DIPEA (478 uL, 2.74 mmol) and the reaction mixture was stirred at 0 °C for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 51-a as a white foam.

[0314] *Step 2:* 4N HCl in 1,4-dioxane (918 uL) was added to intermediate 51-a (240 mg, 0.37 mmol) in ethyl acetate (500 uL) and the solution was stirred for 2 hours at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 120·2HCl as a white solid. MS (m/z) M+1= 555.3

EXAMPLE 48

[0315] The following example illustrates the preparation of compound 113, which is a compound of Formula 1

Scheme 52: Synthesis of compound 113



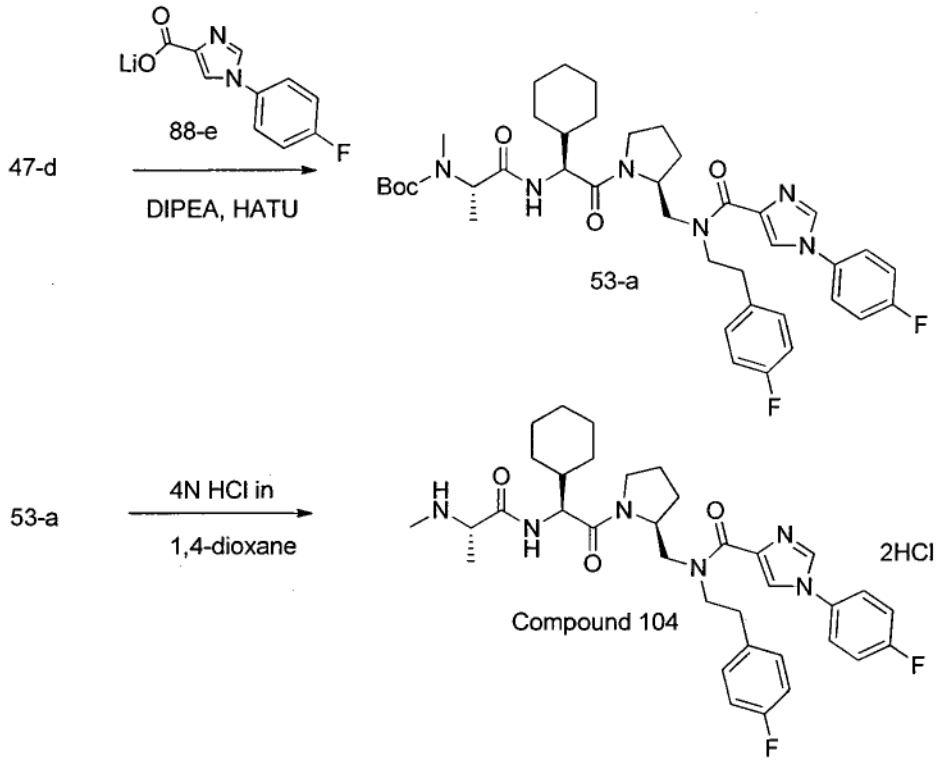
[0316] *Step 1:* To a solution of intermediate 47-d (500 mg, 0.91 mmol) in DMF, cooled to 0 ºC, were sequentially added 6,7-dihydro-5-H-pyrrolo[1,2-a]imidazole-2-carboxylic acid, lithium salt (87-e) (152 mg, 915 mmol), HATU (522 g, 1.37 mmol) and DIPEA (478 uL, 2.74 mmol) and the reaction mixture was stirred at 0 ºC for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 52-a as a white foam.

[0317] *Step 2:* 4N HCl in 1,4-dioxane (1.37 mL) was added to intermediate 52-a (373 mg, 0.54 mmol) in ethyl acetate (500 uL) and the solution was stirred for 2 hours at 0 ºC. Diethyl ether was added, a precipitate formed and compound 113·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 581.3

EXAMPLE 49

[0318] The following example illustrates the preparation of compound 104, which is a compound of Formula 1

Scheme 53: Synthesis of compound 104



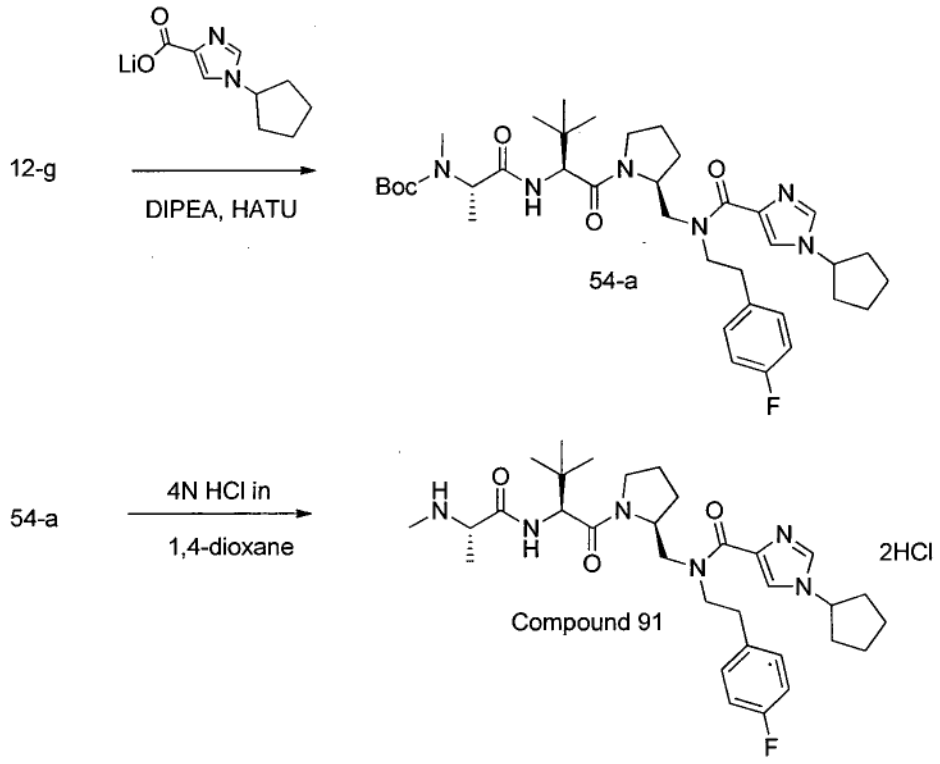
[0319] *Step 1:* To a solution of intermediate 47-d (500 mg, 0.91 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-(4-fluorophenyl)-1H-imidazole-4-carboxylic acid, lithium salt (88-e) (224 mg, 1.05 mmol), HATU (522 mg, 1.37 mmol) and DIPEA (478 uL, 2.74 mmol) and the reaction mixture was stirred at 0 ºC for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 53-a as a white foam.

[0320] *Step 2:* 4N HCl in 1,4-dioxane (886 uL) was added to intermediate 53-a (260 mg, 0.35 mmol) in ethyl acetate (0.5 mL) and the solution was stirred for 2 hours at 0 ºC. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 104·2HCl as a white solid. MS (m/z) M+1= 635.3

EXAMPLE 50

[0321] The following example illustrates the preparation of compound 91, which is a compound of Formula 1

Scheme 54: Synthesis of compound 91



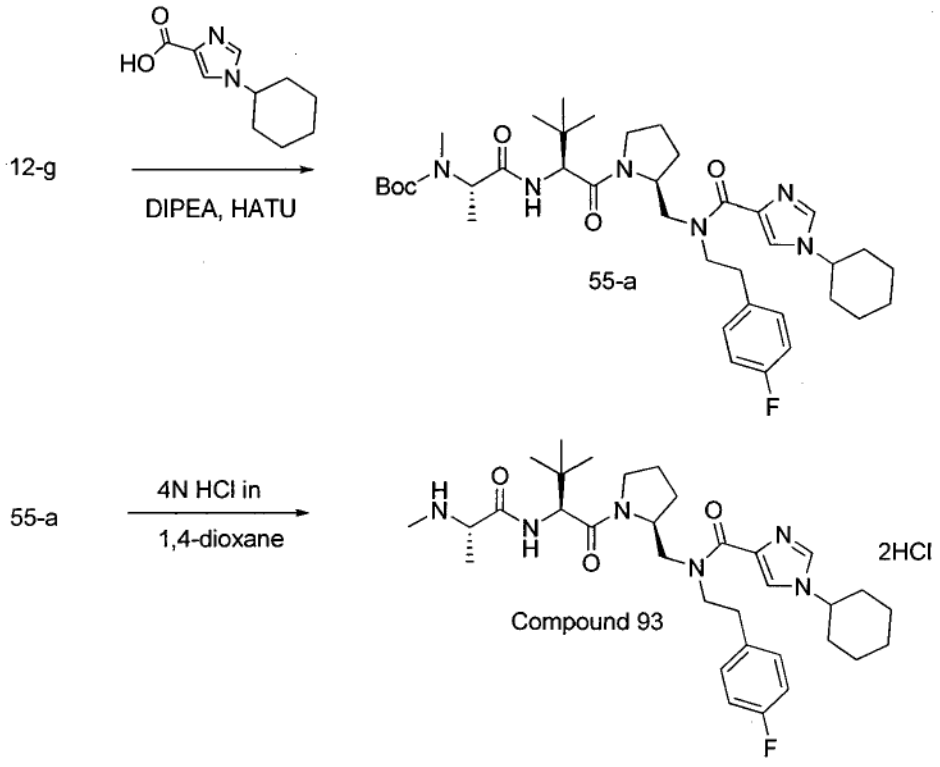
[0322] *Step 1:* To a solution of intermediate 12-g (1.50 g, 2.88 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-cyclopentyl-1H-imidazole-4-carboxylic acid, lithium salt (831 mg, 4.47 mmol), HATU (1.31 g, 3.46 mmol) and DIPEA (2.0 mL, 11.52 mmol) and the reaction mixture was stirred at 0 ºC for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 54-a as a white foam.

[0323] *Step 2:* 4N HCl in 1,4-dioxane (8.79 mL) was added to intermediate 54-a (1.20 g, 1.75 mmol) in ethyl acetate (500 uL) and the solution was stirred for 1.5 hour at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 91·2HCl as a white solid. MS (m/z) M+1= 583.5

EXAMPLE 51

[0324] The following example illustrates the preparation of compound 93, which is a compound of Formula 1

Scheme 55: Synthesis of compound 93



[0325] *Step 1:* To a solution of intermediate 12-g (1.40 g, 2.69 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-cyclohexyl-1H-imidazole-4-carboxylic acid (783 mg, 4.03 mmol), HATU (1.23 g, 3.23 mmol) and DIPEA (1.87 mL, 10.76 mmol) and the

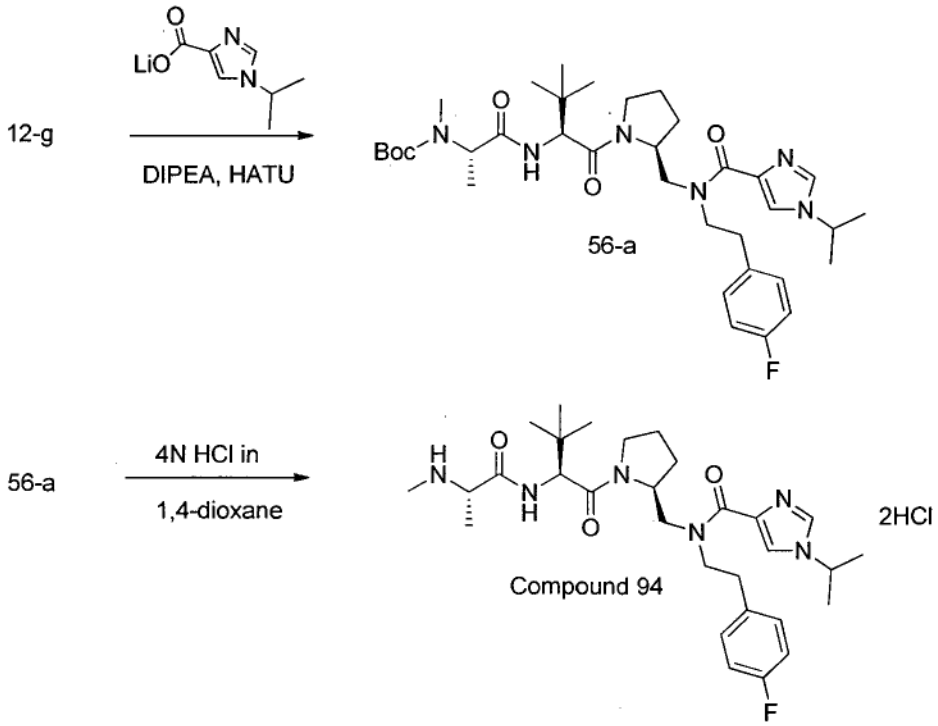
reaction mixture was stirred at 0℃ for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 55-a as a white foam.

[0326] *Step 2:* 4N HCl in 1,4-dioxane (5.17 mL) was added to intermediate 55-a (960 mg, 1.37 mmol) in ethyl acetate (1.0 mL) and the solution was stirred for 1.5 hours at 0 ℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 93·2HCl as a white solid. MS (m/z) M+1= 597.4

EXAMPLE 52

[0327] The following example illustrates the preparation of compound 94, which is a compound of Formula 1

Scheme 56: Synthesis of compound 94



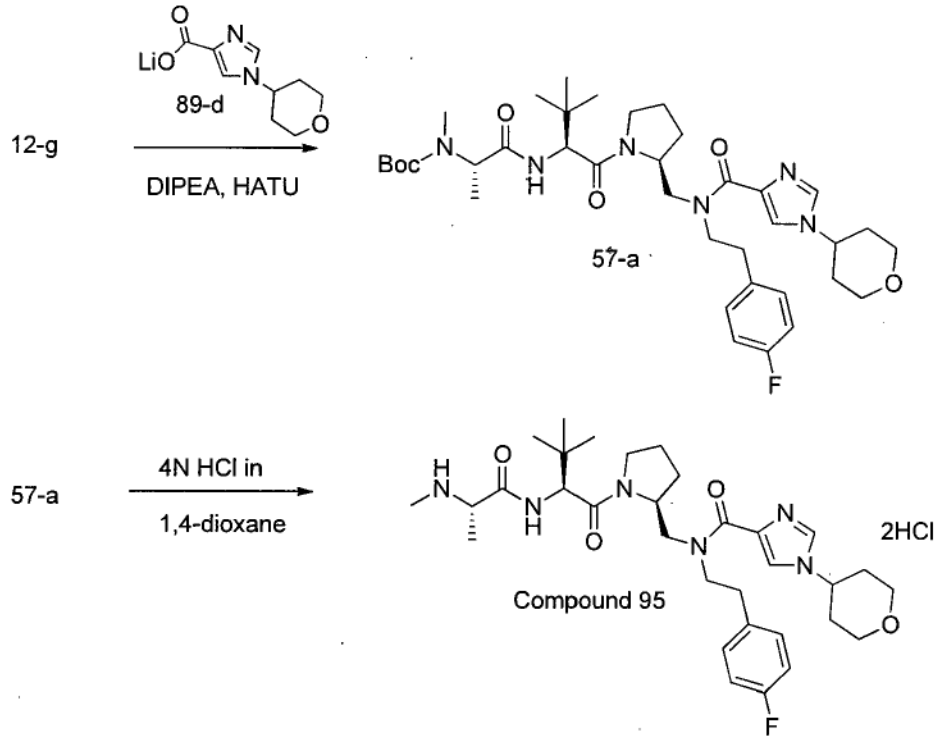
[0328] *Step 1:* To a solution of intermediate 12-g (1.60 g, 3.07 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-isopropyl-1H-imidazole-4-carboxylic acid, lithium salt (734 mg, 4.76 mmol), HATU (1.40 g, 3.69 mmol) and DIPEA (2.14 mL, 12.29 mmol) and the reaction mixture was stirred at 0 ℃ for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 56-a as a white foam.

[0329] *Step 2:* 4N HCl in 1,4-dioxane (4.14 mL) was added to intermediate 56-a (850 mg, 1.10 mmol) in ethyl acetate (1.0 mL) and the solution was stirred for 2 hours at 0 ℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 94·2HCl as a white solid. MS (m/z) M+1= 557.4

EXAMPLE 53

[0330] The following example illustrates the preparation of compound 95, which is a compound of Formula 1

Scheme 57: Synthesis of compound 95



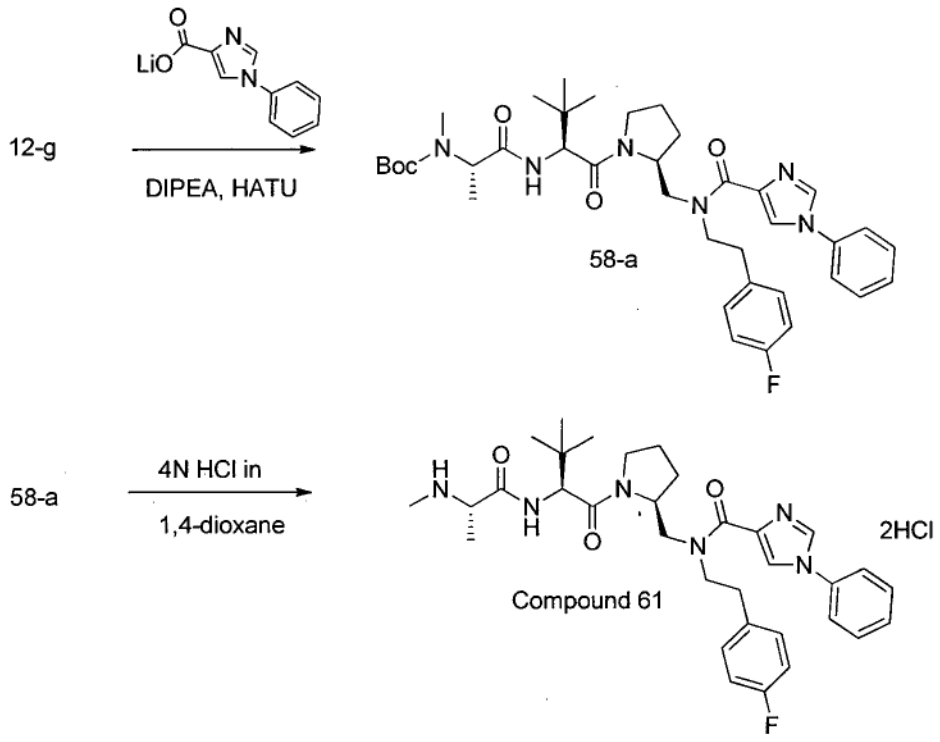
[0331] *Step 1:* To a solution of intermediate 12-g (1.30 g, 2.49 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-(tetrahydro-2H-pyran-4-yl)-1H-imidazole-4carboxylic acid, lithium salt (89-d) (656 mg, 3.25 mmol), HATU (1.23 g, 3.25 mmol) and DIPEA (1.74 mL, 10.0 mmol) and the reaction mixture was stirred at 0 ºC for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 57-a as a white foam.

[0332] *Step 2:* 4N HCl in 1,4-dioxane (4.43 mL) was added to intermediate 57-a (960 mg, 1.18 mmol) in ethyl acetate (1.0 mL) and the solution was stirred for 2 hours at 0 ºC. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 95·2HCl as a white solid. MS (m/z) M+1= 599.4

EXAMPLE 54

[0333] The following example illustrates the preparation of compound 61, which is a compound of Formula 1

Scheme 58: Synthesis of compound 61



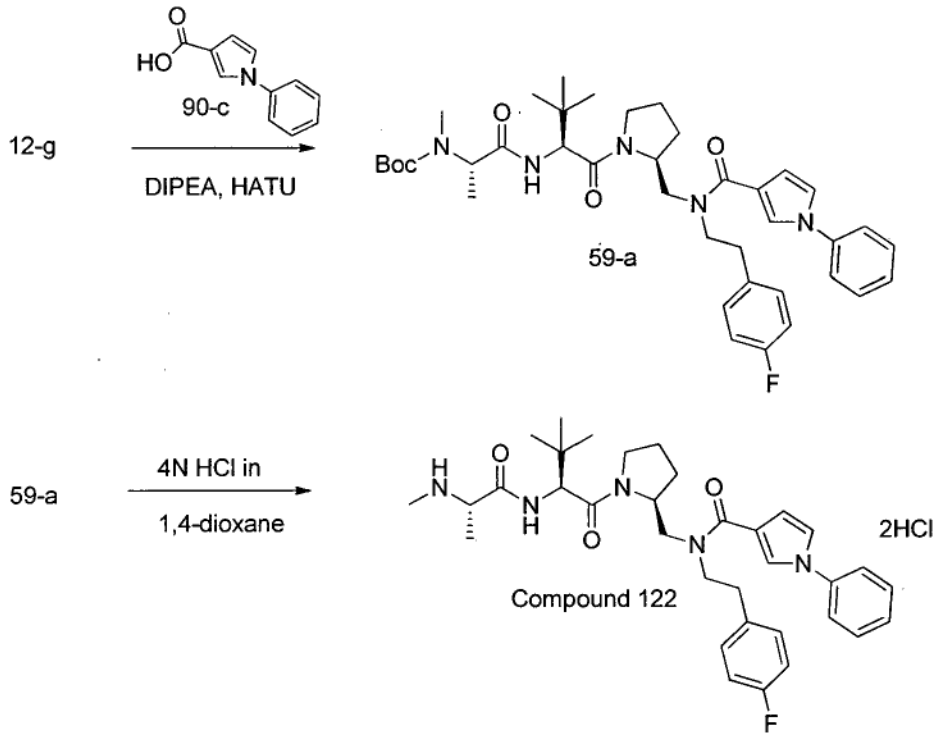
[0334] *Step 1:* To a solution of intermediate 12-g (350 mg, 0.67 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-phenyl-1H-imidazole-4-carboxylic acid, lithium salt (190 mg, 1.0 mmol), HATU (435 mg, 1.14 mmol) and DIPEA (468 uL, 2.69 mmol) and the reaction mixture was stirred at 0 ℃ for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 58-a as a white foam.

[0335] *Step 2:* 4N HCl in 1,4-dioxane (1.25 mL) was added to intermediate 58-a (345 mg, 0.50 mmol) in methanol (500 uL) and the solution was stirred for 2 hours at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 61·2HCl as a white solid. MS (m/z) M+1= 591.5

EXAMPLE 55

[0336] The following example illustrates the preparation of compound 122, which is a compound of Formula 1

Scheme 59: Synthesis of compound 122



[0337] *Step 1:* To a solution of intermediate 12-g (213 mg, 0.41 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-phenyl-1H-pyrrole-3-carboxylic acid (90-c) (100 mg, 0.53 mmol), HATU (203 mg, 0.53 mmol) and DIPEA (286 uL, 1.64 mmol) and the reaction mixture was stirred at 0 °C for 1 hour. Saturated aqueous ammonium chloride

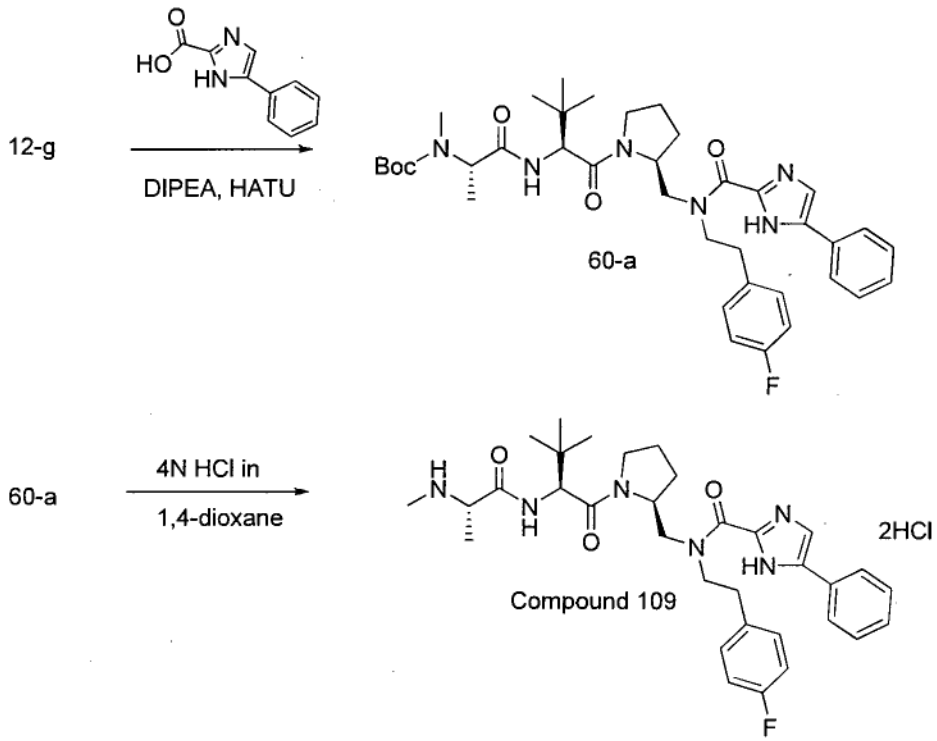
and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 59-a as a white foam.

[0338] *Step 2:* 4N HCl in 1,4-dioxane (1.22 mL) was added to intermediate 59-a (244 mg, 0.32 mmol) in ethyl acetate (325 uL) and the solution was stirred for 2 hours at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 122·2HCl as a white solid. MS (m/z) M+1= 590.2

EXAMPLE 56

[0339] The following example illustrates the preparation of compound 109, which is a compound of Formula 1

Scheme 60: Synthesis of compound 109



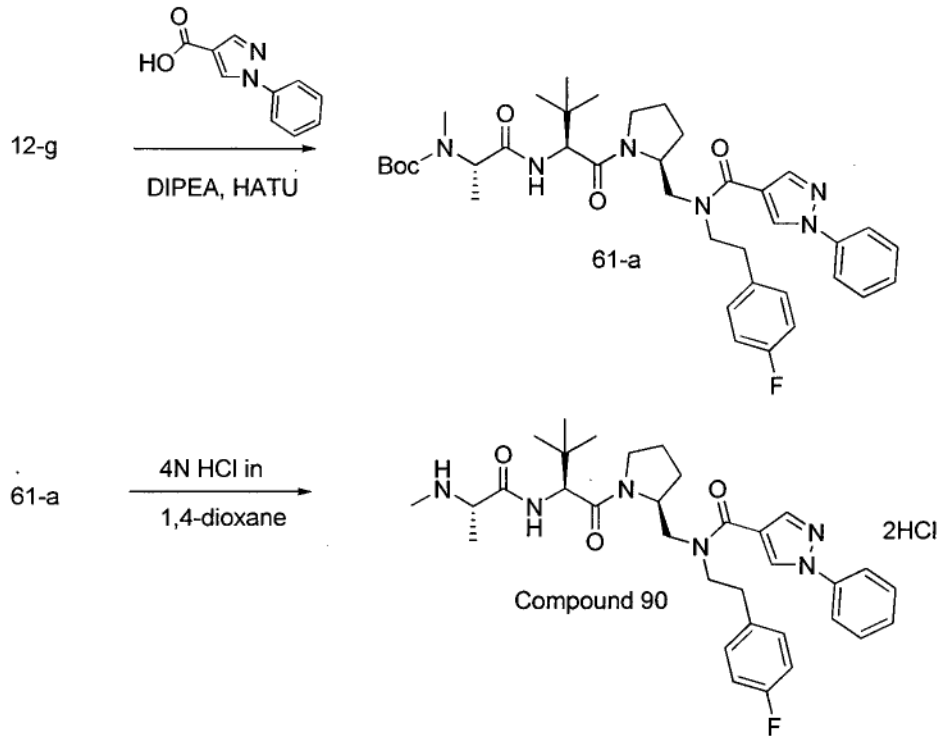
[0340] *Step 1:* To a solution of intermediate 12-g (229 mg, 0.44 mmol) in DMF, cooled to 0 ºC, were sequentially added 5-phenyl-1H-imidazole-2-carboxylic acid (195 mg, 0.87 mmol), HATU (220 mg, 0.57 mmol) and DIPEA (310 uL, 1.78 mmol) and the reaction mixture was stirred at 0 ºC for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 60-a as a white foam.

[0341] *Step 2:* 4N HCl in 1,4-dioxane (2.22 mL) was added to intermediate 60-a (307 mg, 0.44 mmol) in ethyl acetate (445 uL) and the solution was stirred for 2 hours at 0 ºC. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 109·2HCl as a white solid. MS (m/z) M+1= 591.2

EXAMPLE 57

[0342] The following example illustrates the preparation of compound 90, which is a compound of Formula 1

Scheme 61: Synthesis of compound 90



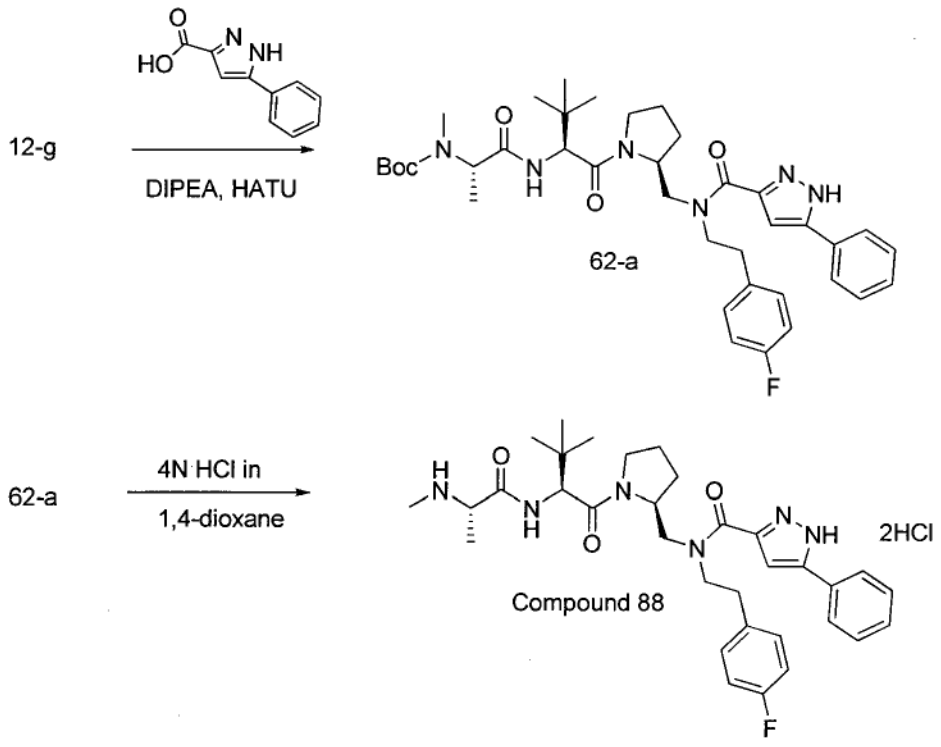
[0343] *Step 1:* To a solution of intermediate 12-g (598 mg, 0.44 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-phenyl-1H-pyrazole-4-carboxylic acid (217 mg, 1.15 mmol), HATU (526 mg, 1.38 mmol) and DIPEA (803 uL, 4.61 mmol) and the reaction mixture was stirred at 0℃ for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 61-a as a white foam.

[0344] *Step 2:* 4N HCl in 1,4-dioxane (3.13 mL) was added to intermediate 61-a (577 mg, 0.83 mmol) in ethyl acetate (500 uL) and the solution was stirred for 2 hours at 0 ℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 90·2HCl as a white solid. MS (m/z) M+1= 591.4

EXAMPLE 58

[0345] The following example illustrates the preparation of compound 88, which is a compound of Formula 1

Scheme 62: Synthesis of compound 88



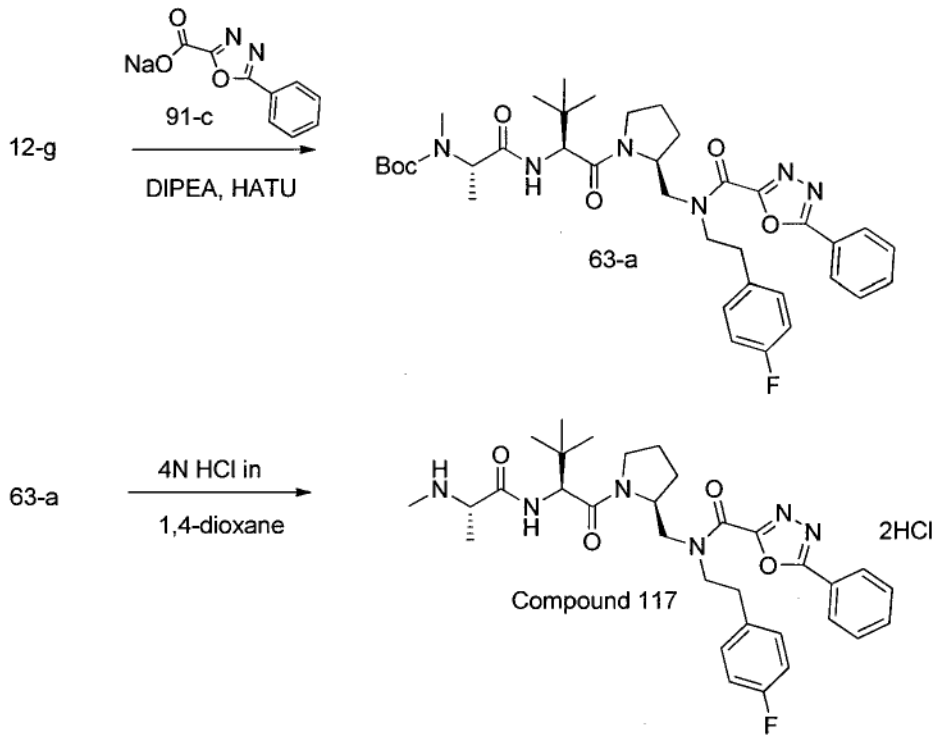
[0346] *Step 1:* To a solution of intermediate 12-g (1.2 g, 2.30 mmol) in DMF, cooled to 0 ºC, were sequentially added 5-phenyl-1H-pyrazole-3-carboxylic acid (455 mg, 2.42 mmol), HATU (1.0 g, 2.77 mmol) and DIPEA (1.60 mL, 9.22 mmol) and the reaction mixture was stirred at 0 ℃ for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 62-a as a white solid.

[0347] *Step 2:* 4N HCl in 1,4-dioxane (6.15 mL) was added to intermediate 62-a (850 mg, 1.23 mmol) in ethyl acetate (500 uL) and the solution was stirred for 1.5 hour at 0 ℃. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 88·2HCl as a white solid. MS (m/z) M+1= 591.4

EXAMPLE 59

[0348] The following example illustrates the preparation of compound 117, which is a compound of Formula 1

Scheme 63: Synthesis of compound 117



[0349] *Step 1:* To a solution of intermediate 12-g (1.0 g, 1.92 mmol) in DMF, cooled to 0 ºC, were sequentially added 5-phenyl-1,3,4-oxadiazole-2-carboxylic acid sodium salt (91-c) (1.36 g, 6.41 mmol), HATU (2.19 g, 5.76 mmol) and DIPEA (1.34 mL, 7.68 mmol) and the reaction mixture was stirred at room temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was

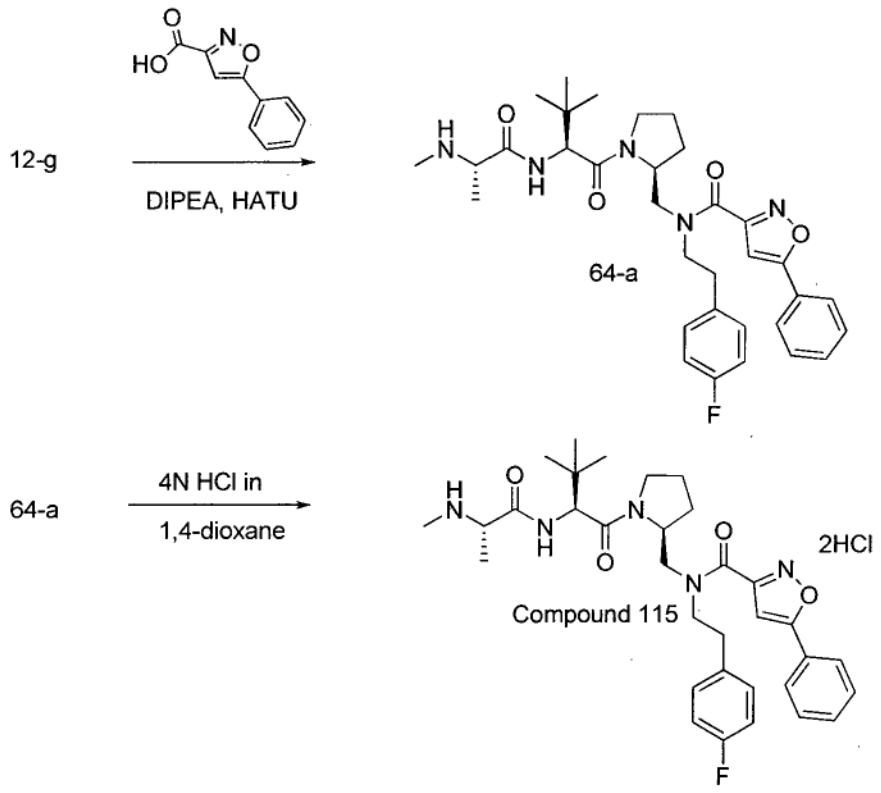
separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 63-a as a white solid.

[0350] *Step 2:* 4N HCl in 1,4-dioxane (10.0 mL) was added to intermediate 63-a (453 mg, 0.65 mmol) and the solution was stirred for 3 hours at 0 °C. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 117·2HCl as a white solid. MS (m/z) M+1= 593.2

EXAMPLE 60

[0351] The following example illustrates the preparation of compound 115, which is a compound of Formula 1

Scheme 64: Synthesis of compound 115



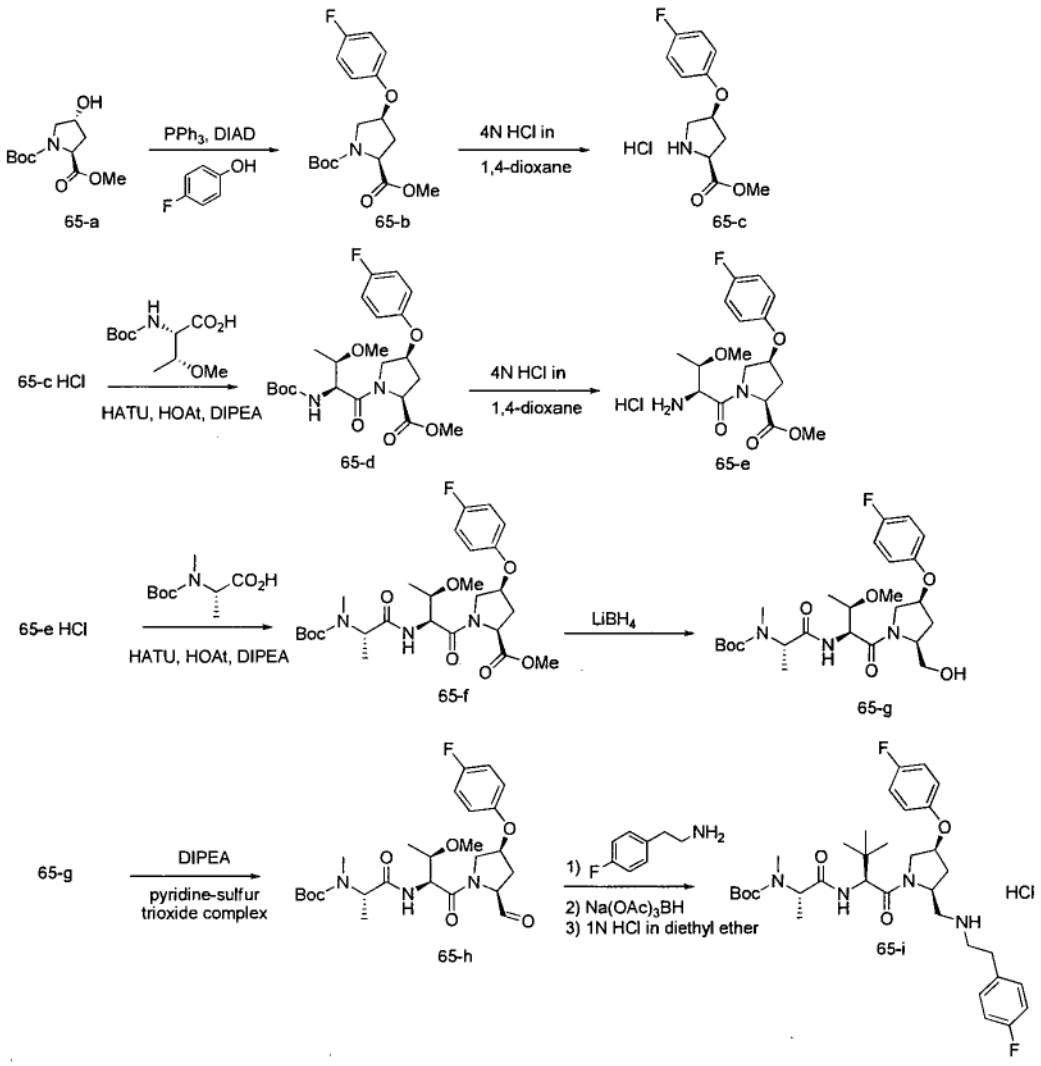
[0352] *Step 1:* To a solution of intermediate 12-g (847 g, 1.62 mmol) in DMF, cooled to 0 ºC, were sequentially added 5-phenylisoxazole-3-carboxylic acid (431 mg, 2.27 mmol), HATU (1.05 g, 2.77 mmol) and DIPEA (1.13 mL, 6.51 mmol) and the reaction mixture was stirred at room temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 64-a as a white foam.

[0353] *Step 2:* 4N HCl in 1,4-dioxane (5.0 mL) was added to intermediate 64-a (356 mg, 0.51 mmol) and the solution was stirred for 2 hours at 0 ºC. Diethyl ether was added, a precipitate formed and compound 115·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 592.1

EXAMPLE 61

[0354] The following example illustrates the preparation of compound 65-i, which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof

Scheme 65: Synthesis of intermediate 65-i



[0355] Step 1: To a solution of (2S,4R)-1-Boc-2-methyl-4-hydroxypyrrolidine-2-carboxylate (18.0 g, 73.4 mmol), 4-fluorophenol (9.05 g, 81.0 mmol) and triphenylphosphine (21.17 g, 81.0 mmol) in THF was added DIAD (17.07 g, 84.0 mmol) in THF (20 mL) dropwise and the reaction was then stirred at room temperature for 2 days. Diethyl ether and hexane were added, a precipitate formed and triphenyl phosphine oxide was removed by filtration. Volatiles were removed under reduced pressure and the

residue was purified by silica gel chromatography to provide the expected intermediate 65-b as yellow solid.

[0356] *Step 2:* 4N HCl in 1,4-dioxane (78 mL, 312 mmol) was added to intermediate 65-b (21.2 g, 62.5 mmol) and the solution was stirred for 1 hour at 0 °C and then 30 minutes at room temperature. Diethyl ether was added, a precipitate formed and intermediate 65-c·HCl was collected by filtration as a white solid. MS (m/z) M+1= 240.0

[0357] *Step 3:* To a solution of intermediate 65-c·HCl (14.2 g, 51.5 mmol) in DMF cooled to 0 °C were sequentially added Boc-Thr(Me)-OH (13.22 g, 56.7 mmol), HATU (21.54 g, 56.7 mmol), HOAt (8.58 mL, 5.15 mmol) and DIPEA (36.0 mL, 206 mmol) and the reaction was then stirred at room temperature for 3 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 65-d as white foam.

[0358] *Step 4:* 4N HCl in 1,4-dioxane (63.3 mL) was added to intermediate 65-d (23.0 g, 50.6 mmol) and the solution was stirred for 1 hour at 0 °C and then 30 minutes at room temperature. Diethyl ether was added, a precipitate formed and intermediate 65-e·HCl was collected by filtration as a white solid. MS (m/z) M+1= 355.2

[0359] *Step 5:* To a solution of intermediate 65-e·HCl (16.2 g, 41.4 mmol) in DMF cooled to 0 °C were sequentially added Boc-NMe-Ala-OH (9.27 g, 45.6 mmol), HATU (1734 g, 45.6 mmol), HOAt (6.91 mL, 4.14 mmol) and DIPEA (29.0 mL, 166.0 mmol) and the reaction was then stirred at room temperature for 3 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided the expected intermediate 65-f as a white foam.

[0360] *Step 6:* To a solution of intermediate 65-f (23.0 g, 42.6 mmol) in THF cooled to 0 °C was added lithium borohydride (1.95 g, 90.0 mmol) and the reaction was stirred at room temperature overnight. Water and ethyl acetate were added; the organic layer was separated, washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine,

dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 65-g as white foam.

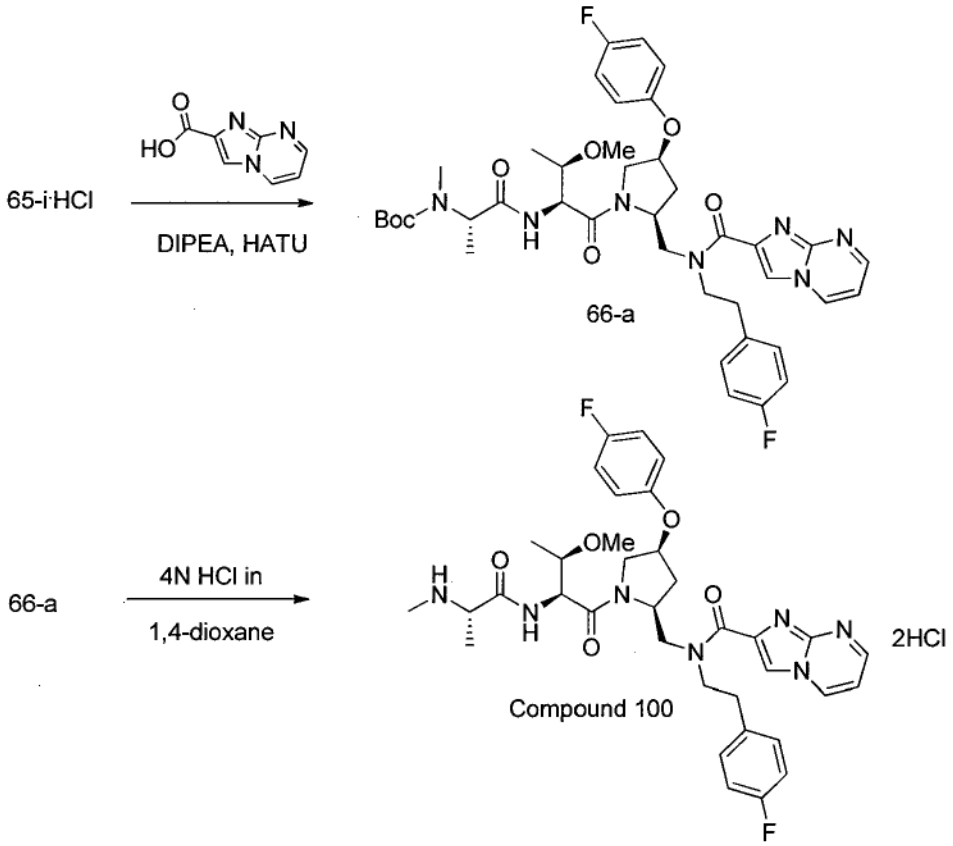
[0361] *Step 7:* To a solution of intermediate 65-g (8.7 g, 17.01 mmol) in DMSO (4.83 mL, 68.0 mmol) and dichloromethane (150 mL) cooled to 0°C was added DIPEA (10.40 mL, 59.5 mmol) and pyridine sulfur trioxide complex (8.12 g, 51.0 mmol), the reaction was then stirred at room temperature overnight. Water and ethyl acetate were added; the organic layer was separated, washed with 10% citric acid, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 65-h as white foam.

[0362] *Step 8:* To a solution of intermediate 65-h (8.0 g, 15.70 mmol) in dichloromethane was added 2-(4-fluorophenyl)ethanamine (1.87 mL, 14.3 mmol). After stirring at room temperature for 2 hours, sodium triacetoxyborohydride (6.37 g, 28.5 mmol) was added portion wise and the reaction mixture was then stirred at room temperature for 2 hours. Saturated aqueous $NaHCO_3$ was added; the organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 65-i as a yellow oil. To a solution of intermediate 65-i in diethyl ether (100 mL) was added 1N HCl in diethyl ether (15.7 mL), a precipitate formed and intermediate 65-i·HCl was collected by filtration as beige solid. MS (m/z) M+1= 633.4

EXAMPLE 62

[0363] The following example illustrates the preparation of compound 100, which is a compound of Formula 1

Scheme 66: Synthesis of compound 100



[0364] *Step 1:* To a solution of intermediate 65-i·HCl (1.5 g, 2.24 mmol) in DMF cooled to 0 ºC were sequentially added imidazo[1,2-a]pyrimidine-2-carboxylic acid (464 mg, 2.84 mmol), HATU (992 mg, 2.61 mmol) and DIPEA (1.65 mL, 9.48 mmol) and the reaction mixture was stirred at room temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 66-a as white foam.

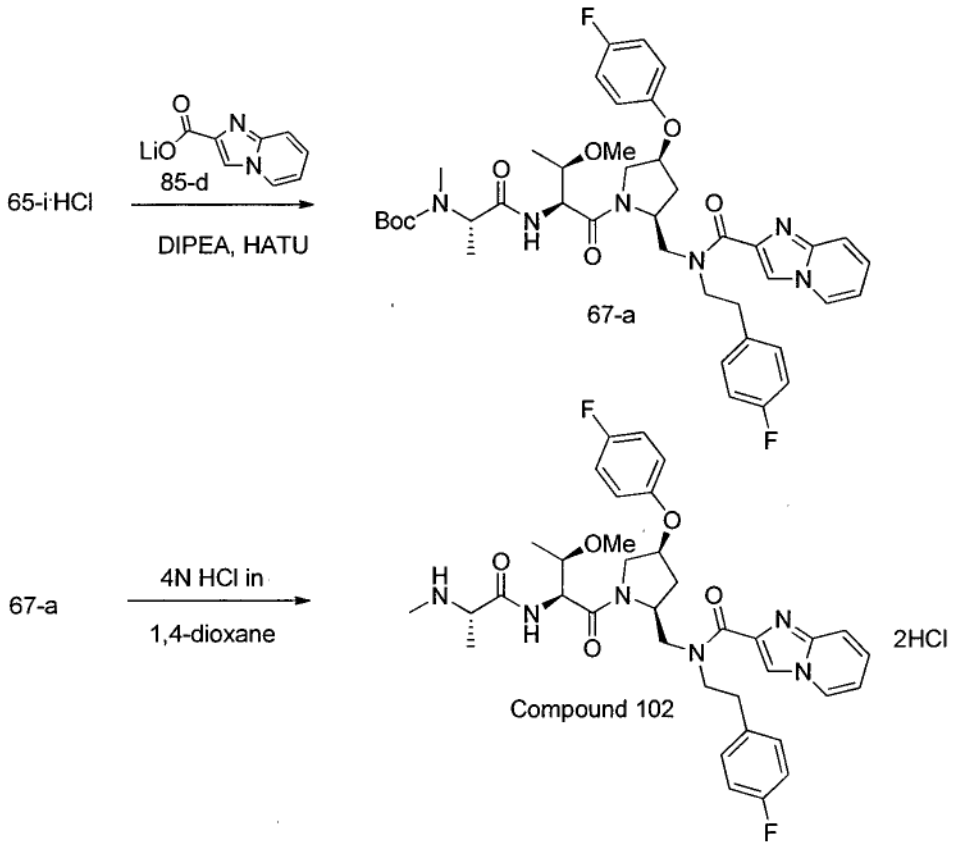
[0365] *Step 2:* 4N HCl in 1,4-dioxane (2.0 mL) was added to intermediate 66-a (760 mg, 0.97 mmol) at 0 ºC and the solution was stirred for 1 hour at 0 ºC and then 30

minutes at room temperature. Ethyl acetate was added, a precipitate formed and compound100·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 678.5

EXAMPLE 63

[0366] The following example illustrates the preparation of compound 102, which is a compound of Formula 1

Scheme 67: Synthesis of compound 102



[0367] *Step 1:* To a solution of intermediate 65-i·HCl (2.0 g, 2.98 mmol) in DMF cooled to 0 ºC were sequentially added imidazo[1,2-a]pyridine-2-carboxylic acid lithium salt (85-d) (641 mg, 3.79 mmol), HATU (1.32 g, 3.48 mmol) and DIPEA (2.20 mL, 12.64 mmol) and the reaction mixture was stirred at room temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was

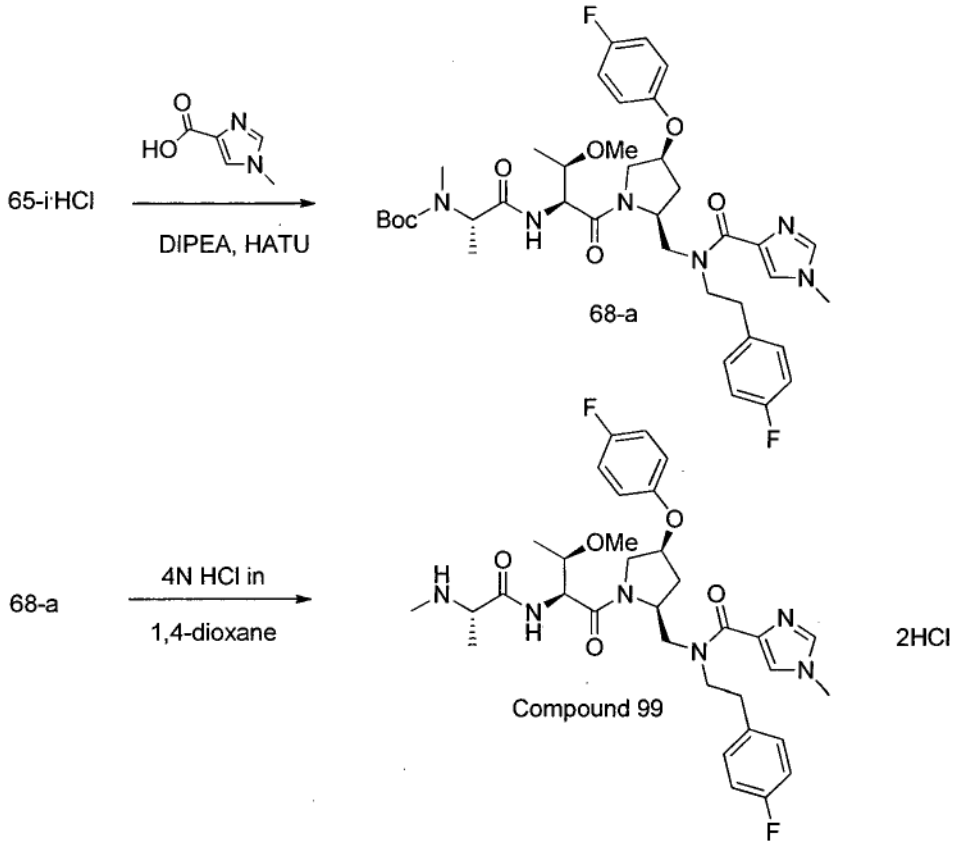
separated, washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 67-a as white foam.

[0368] *Step 2:* 4N HCl in 1,4-dioxane (3.0 mL) was added to intermediate 67-a (900 mg, 1.16 mmol) at 0 °C and the solution was stirred for 1 hour at 0 °C and then 30 minutes at room temperature. Ethyl acetate was added, a precipitate formed and compound 102·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 677.5

EXAMPLE 64

[0369] The following example illustrates the preparation of compound 99, which is a compound of Formula 1

Scheme 68: Synthesis of compound 99



[0370] *Step 1:* To a solution of intermediate 65-i·HCl (2.0 g, 2.98 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-methyl-1H-imidazole-4-carboxylic acid (478 mg, 3.79 mmol), HATU (1.32 g, 3.48 mmol) and DIPEA (2.20 mL, 12.64 mmol) and the reaction mixture was stirred at 0 ºC for 1 hour and room temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 68-a as a white foam.

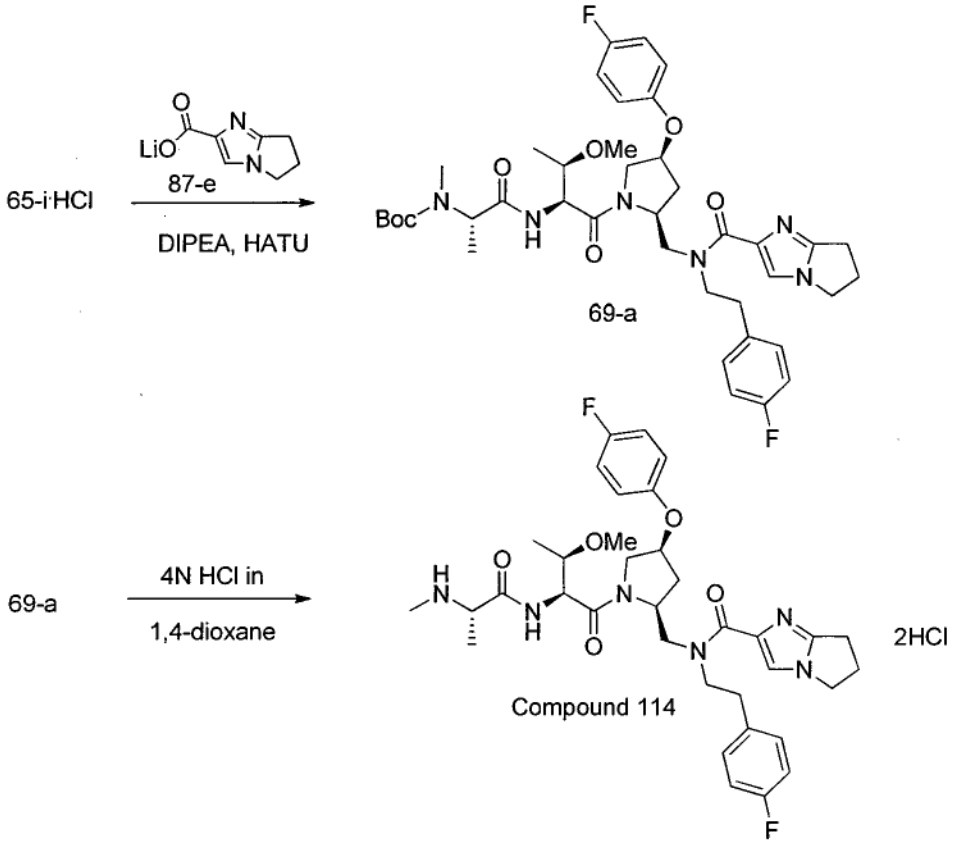
[0371] *Step 2:* 4N HCl in 1,4-dioxane (3.0 mL) was added to intermediate 68-a (900 mg, 1.16 mmol) at 0 ºC and the solution was stirred for 1 hour at 0 ºC and then 30

minutes at room temperature. Ethyl acetate was added, a precipitate formed and compound 99·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 641.4

EXAMPLE 65

[0372] The following example illustrates the preparation of compound 114, which is a compound of Formula 1

Scheme 69: Synthesis of compound 114



[0373] *Step 1:* To a solution of intermediate 65-i·HCl (600 mg, 0.90 mmol) in DMF, cooled to 0 ºC, were sequentially added 6,7-dihydro-5-H-pyrrolo[1,2-a]imidazole-2-carboxylic acid, lithium salt (87-e) (236 mg, 1.42 mmol), HATU (397 mg, 1.04 mmol) and

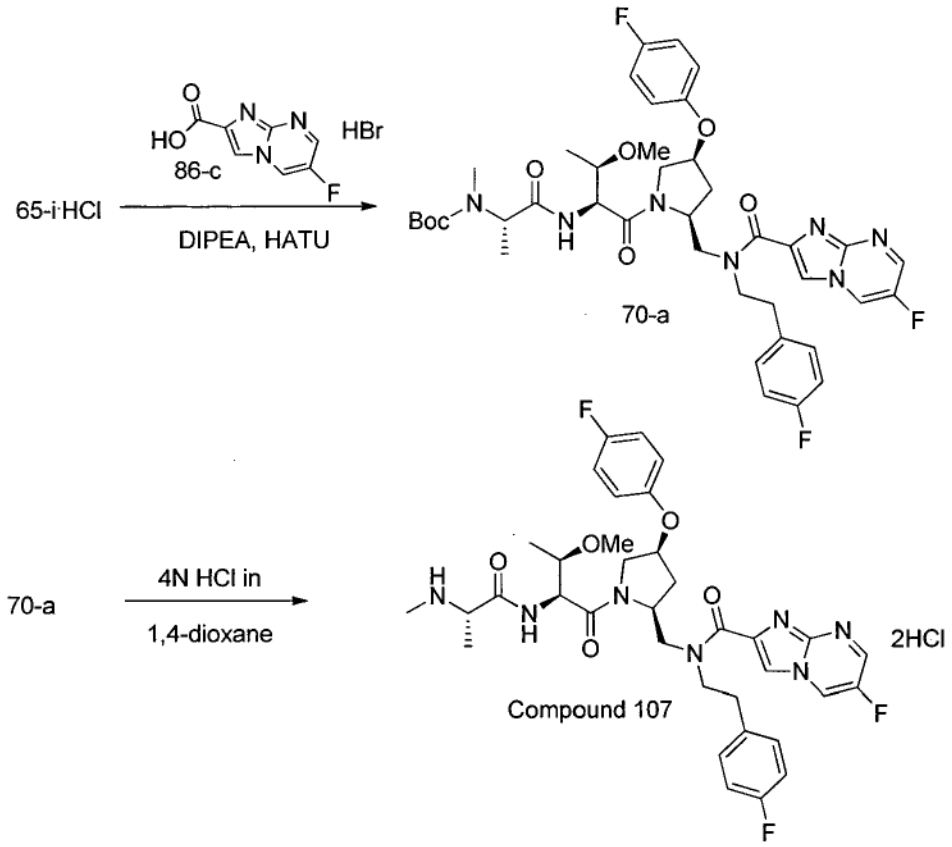
DIPEA (662 uL, 3.79 mmol) and the reaction mixture was stirred at 0 °C for 1 hour and room temperature for 2 hours. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 69-a as a white foam.

[0374] *Step 2:* 4N HCl in 1,4-dioxane (750 uL) was added to intermediate 69-a (230 mg, 0.30 mmol) at 0 °C and the solution was stirred for 1 hour at 0 °C and then 30 minutes at room temperature. Ethyl acetate was added, a precipitate formed and compound 114·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 667.1

EXAMPLE 66

[0375] The following example illustrates the preparation of compound 107, which is a compound of Formula 1

Scheme 70: Synthesis of compound 107



[0376] *Step 1:* To a solution of intermediate 65-i·HCl (600 mg, 0.87 mmol) in DMF, cooled to 0 ºC, were sequentially added 6-fluoroimidazo[1,2-a]pyrimidine-2-carboxylic acid, HBr salt (86-c) (298 mg, 1.13 mmol), HATU (397 mg, 1.04 mmol) and DIPEA (662 uL, 3.79 mmol) and the reaction mixture was stirred at 0 ºC for 1 hour and room temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 70-a as a white foam.

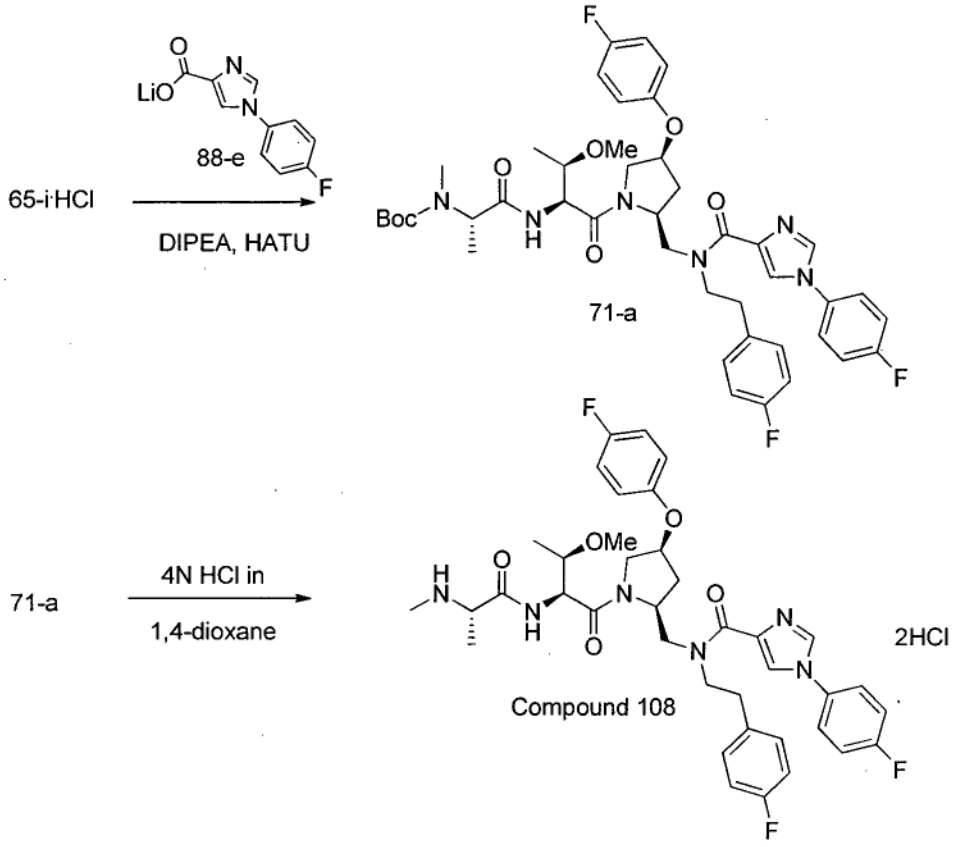
[0377] *Step 2:* 4N HCl in 1,4-dioxane (1.41 mL) was added to intermediate 70-a (450 mg, 0.56 mmol) at 0 ºC and the solution was stirred for 1 hour at 0 ºC. Ethyl acetate

was added, a precipitate formed and compound 107·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 696.3

EXAMPLE 67

[0378] The following example illustrates the preparation of compound 108, which is a compound of Formula 1

Scheme 71: Synthesis of compound 108



[0379] *Step 1:* To a solution of intermediate 65-i·HCl (600 mg, 0.87 mmol) in DMF, cooled to 0 ºC, were sequentially added 1-(4-fluorophenyl)-1H-imidazole-4-carboxylic acid, lithium salt (88-e) (303 mg, 1.42 mmol), HATU (397 mg, 1.04 mmol) and DIPEA (662 uL, 3.79 mmol) and the reaction mixture was stirred at 0 ºC for 1 hour and room

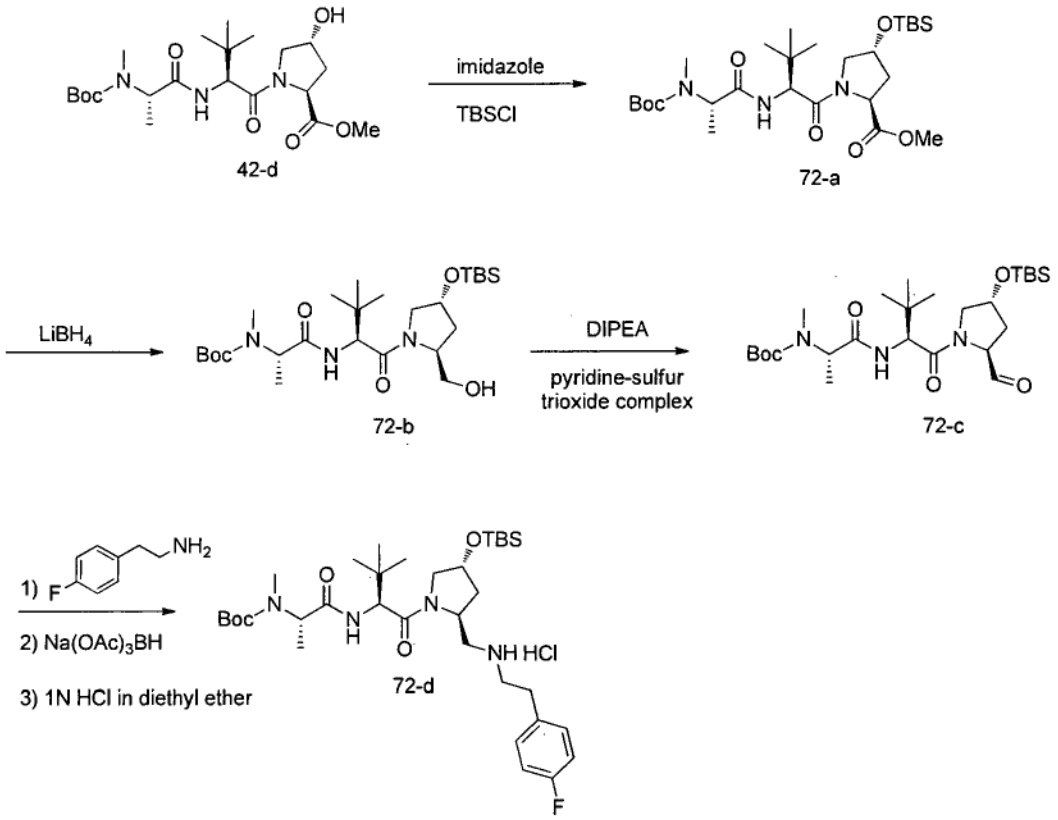
temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 71-a as a white foam.

[0380] *Step 2:* 4N HCl in 1,4-dioxane (670 uL) was added to intermediate 71-a (220 mg, 0.26 mmol) at 0 °C and the solution was stirred for 1 hour at 0 °C. Ethyl acetate was added, a precipitate formed and compound 108·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 721.1

EXAMPLE 68

[0381] The following example illustrates the preparation of compound 72-d, which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof

Scheme 72: Synthesis of intermediate 72-d



[0382] *Step 1:* To a solution of intermediate 42-d (18.0 g, 40.6 mmol) in DMF cooled to 0 ºC were sequentially added imidazole (3.32 g, 48.7 mmol), DMAP (496 mg, 4.06 mmol) and *tert*-butylchlorodimethylsilane (6.73 mL, 44.6 mmol) and the reaction mixture was stirred at room temperature overnight. Water and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 72-a as a colorless oil.

[0383] *Step 2:* To a solution of intermediate 72-a (6.0 g, 10.76 mmol) in THF cooled to 0 ºC was added lithium borohydride (1.17 g, 53.8 mmol) and the reaction was stirred at room temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with 10% aqueous citric

acid, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 72-b as a white foam.

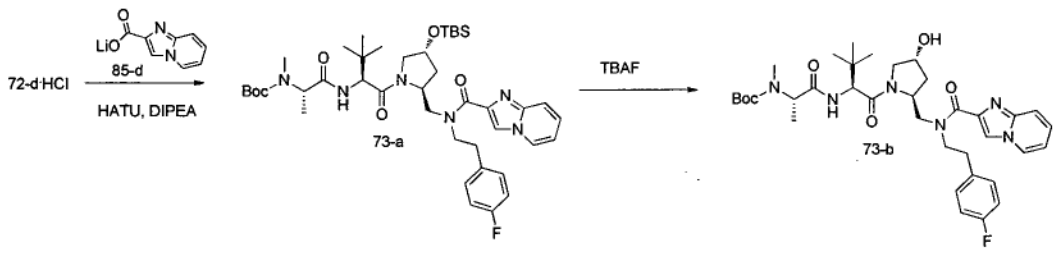
[0384] *Step 3:* To a solution of intermediate 72-b (5.6 g, 10.57 mmol) in DMSO (3.0 mL, 42.3 mmol) and dichloromethane (80 mL) cooled to 0 °C was added TEA (5.89 mL, 42.3 mmol) and pyridine sulfur trioxide complex (5.05 g, 31.7 mmol), the reaction was then stirred at 0 °C for 30 minutes and room temperature for 1 hour. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 72-c as white foam.

[0385] *Step 4:* To a solution of intermediate 72-c (3.0 g, 5.68 mmol) in dichloromethane was added 2-(4-fluorophenyl)ethanamine (746 uL, 5.68 mmol). After stirring at room temperature overnight, the reaction was cooled to -5 °C, sodium triacetoxyborohydride (2.54 g, 11.37 mmol) was added portion wise and the reaction mixture was then stirred at room temperature for 2 hours. Saturated aqueous $NaHCO_3$ was added; the organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 72-d as yellow foam. To a solution of intermediate 72-d in diethyl ether (100 mL) was added 1N HCl in diethyl ether (5.68 mL), a precipitate formed and intermediate 72-d·HCl was collected by filtration as beige solid. MS (m/z) M+1= 651.6

EXAMPLE 69

[0386] The following example illustrates the preparation of compound 73-b, which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof

Scheme 73: Synthesis of intermediate 73-b



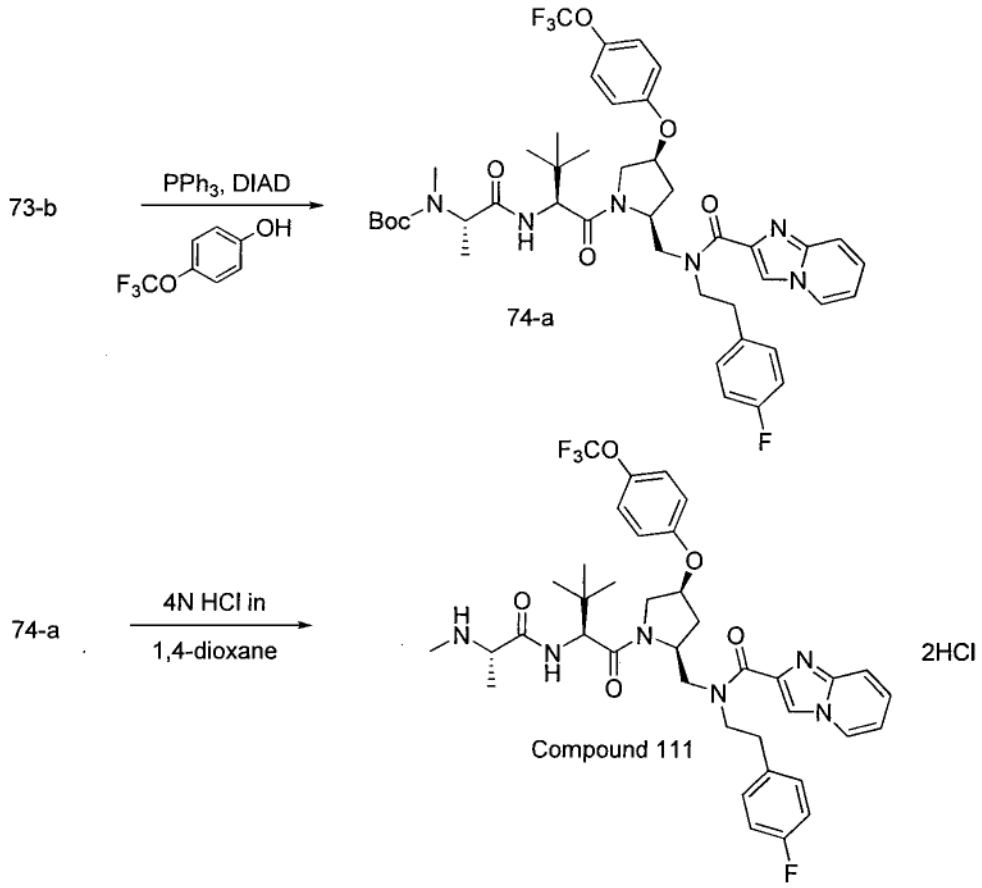
[0387] *Step 1:* To a solution of intermediate 72-d·HCl (7.02 g, 10.78 mmol) in DMF cooled to 0 ºC were sequentially added imidazo[1,2-a]pyridine-2-carboxylic acid, lithium salt (85-d) (3.08 g, 18.33 mmol), HATU (6.97 g, 18.33 mmol) and DIPEA (7.53 mL, 43.1 mmol) and the reaction mixture was stirred at room temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 73-a as white foam.

[0388] *Step 2:* To a solution of intermediate 73-a (6.14 g, 7.72 mmol) in THF cooled to 0 ºC was added 1.0 M solution of TBAF in THF (10.04 mL, 10.04 mmol) and the reaction mixture was stirred at room temperature for 2 hours. Water and ethyl acetate were added; the organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 73-b as a white foam.

EXAMPLE 70

[0389] The following example illustrates the preparation of compound 111, which is a compound of Formula 1

Scheme 74: Synthesis of compound 111



[0390] *Step 1:* To a solution of intermediate 73-b (1.95 g, 2.86 mmol), 4-(trifluoromethoxy)phenol (561 mg, 3.15 mmol) and triphenylphosphine (901 mg, 3.44 mmol) in THF was added DIAD (724 uL, 3.72 mmol) dropwise and the reaction was then stirred at room temperature for 2 days. Diethyl ether and hexane were added, a precipitate formed and triphenyl phosphine oxide was removed by filtration. Volatiles were removed under reduced pressure and the residue was purified by silica gel chromatography to provide the expected intermediate 74-a as colorless oil.

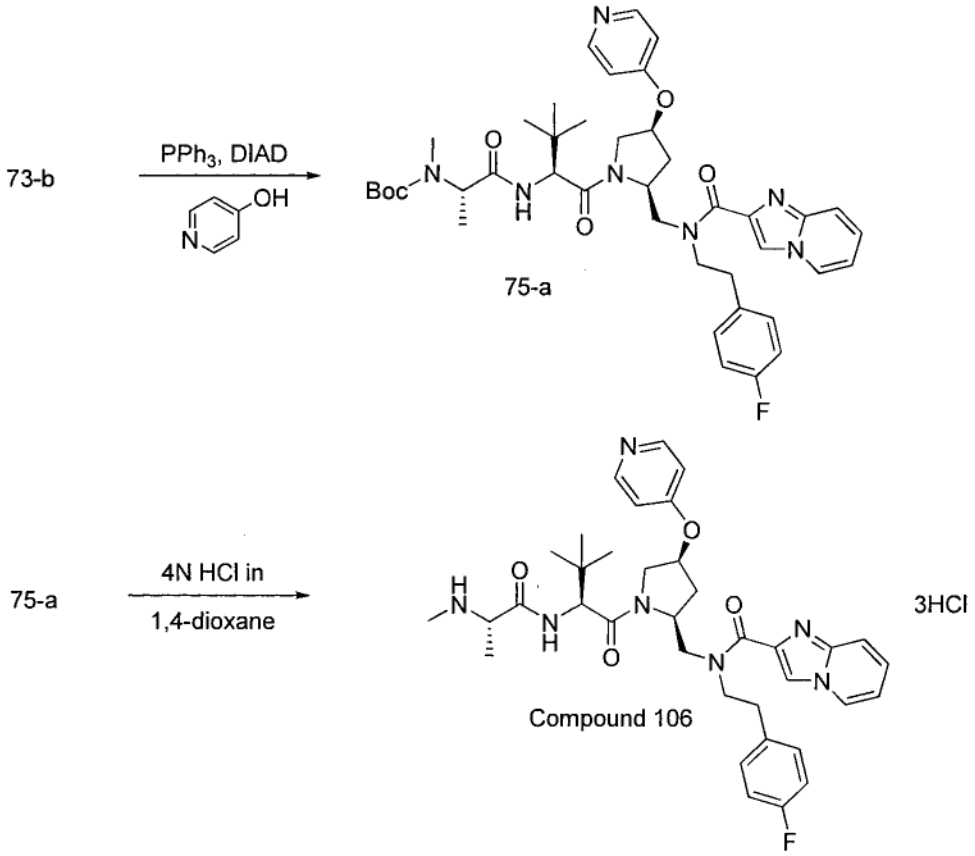
[0391] *Step 2:* 4N HCl in 1,4-dioxane (6.85 mL) was added to intermediate 74-a (921 mg, 1.09 mmol) in ethyl acetate (500 uL) and the solution was stirred for 2 hours at 0

°C. Diethyl ether was added, a precipitate formed and compound 111·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 741.3

EXAMPLE 71

[0392] The following example illustrates the preparation of compound 106, which is a compound of Formula 1

Scheme 75: Synthesis of compound 106



[0393] *Step 1:* To a solution of intermediate 73-b (2.30 g, 3.38 mmol), pyridine-4-ol (353 mg, 3.72 mmol) and triphenylphosphine (1.06 g, 4.05 mmol) in THF was added DIAD (854 uL, 4.39 mmol) dropwise and the reaction was then stirred at room temperature for 2

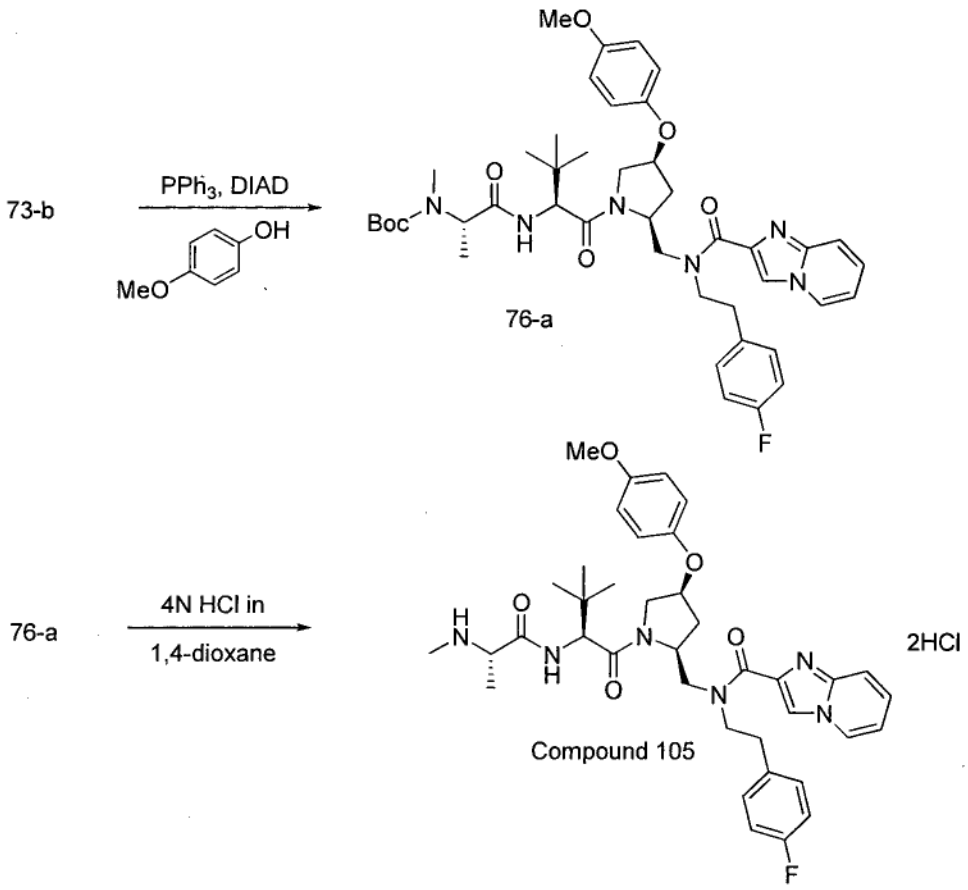
days. Diethyl ether and hexane were added, a precipitate formed and triphenyl phosphine oxide was removed by filtration. Volatiles were removed under reduced pressure and the residue was purified by silica gel chromatography to provide the expected intermediate 75-a as colorless oil.

[0394] *Step 2:* 4N HCl in 1,4-dioxane (9.24 mL) was added to intermediate 75-a (1.12 g, 1.47 mmol) in ethyl acetate (500 uL) and the solution was stirred for 2 hours at 0 °C. Diethyl ether was added, a precipitate formed and compound 106·3HCl was collected by filtration as a white solid. MS (m/z) M+1= 658.1

EXAMPLE 72

[0395] The following example illustrates the preparation of compound 105, which is a compound of Formula 1

Scheme 76: Synthesis of compound 105



[0396] *Step 1:* To a solution of intermediate 73-b (1.79 g, 2.63 mmol), 4-methoxyphenol (359 mg, 2.89 mmol) and triphenylphosphine (828 mg, 3.16 mmol) in THF was added DIAD (665 uL, 3.42 mmol) dropwise and the reaction was then stirred at room temperature for 2 days. Diethyl ether and hexane were added, a precipitate formed and triphenyl phosphine oxide was removed by filtration. Volatiles were removed under reduced pressure and the residue was purified by silica gel chromatography to provide the expected intermediate 76-a as a colorless oil.

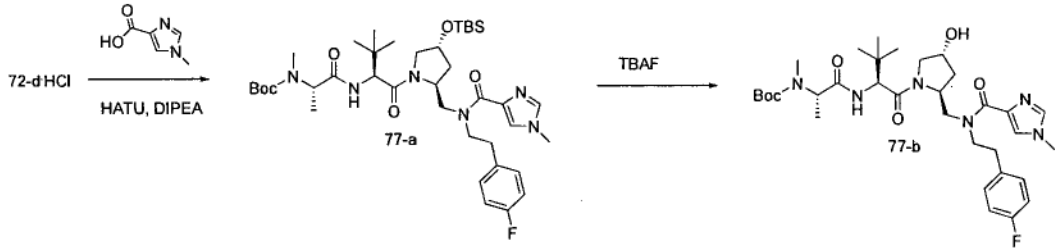
[0397] *Step 2:* 4N HCl in 1,4-dioxane (4.05 mL) was added to intermediate 76-a (510 mg, 0.64 mmol) in ethyl acetate (500 uL) and the solution was stirred for 2 hours at 0

°C. Diethyl ether was added, a precipitate formed and compound 105·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 687.1

EXAMPLE 73

[0398] The following example illustrates the preparation of compound 77-b, which can be used as an intermediate in the preparation of a compound of Formula 1 or salt thereof

Scheme 77: Synthesis of intermediate 77-b



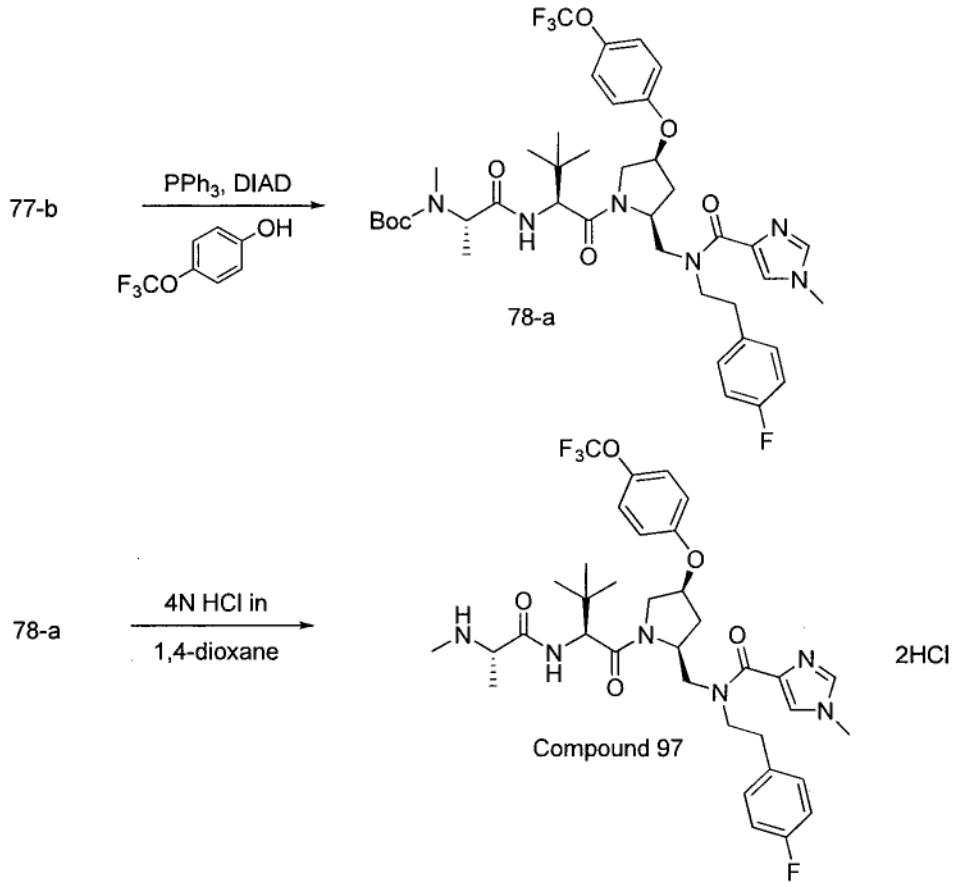
[0399] *Step 1:* To a solution of intermediate 72-d·HCl (3.0 g, 4.61 mmol) in DMF cooled to 0 ºC were sequentially added 1-N-methylimidazole carboxylic acid (988 mg, 7.83 mmol), HATU (2.98 g, 7.83 mmol) and DIPEA (3.22 mL, 18.43 mmol) and the reaction mixture was stirred at room temperature overnight. Saturated aqueous ammonium chloride and ethyl acetate were added; the organic layer was separated, washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 77-a as white foam.

[0400] *Step 2:* To a solution of intermediate 77-a (570 mg, 0.75 mmol) in THF cooled to 0 ºC was added 1.0 M solution of TBAF in THF (970 uL, 0.97 mmol) and the reaction mixture was stirred at room temperature for 2 hours. Water and ethyl acetate were added; the organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 77-b as white foam.

EXAMPLE 74

[0401] The following example illustrates the preparation of compound 97, which is a compound of Formula 1

Scheme 78: Synthesis of compound 97



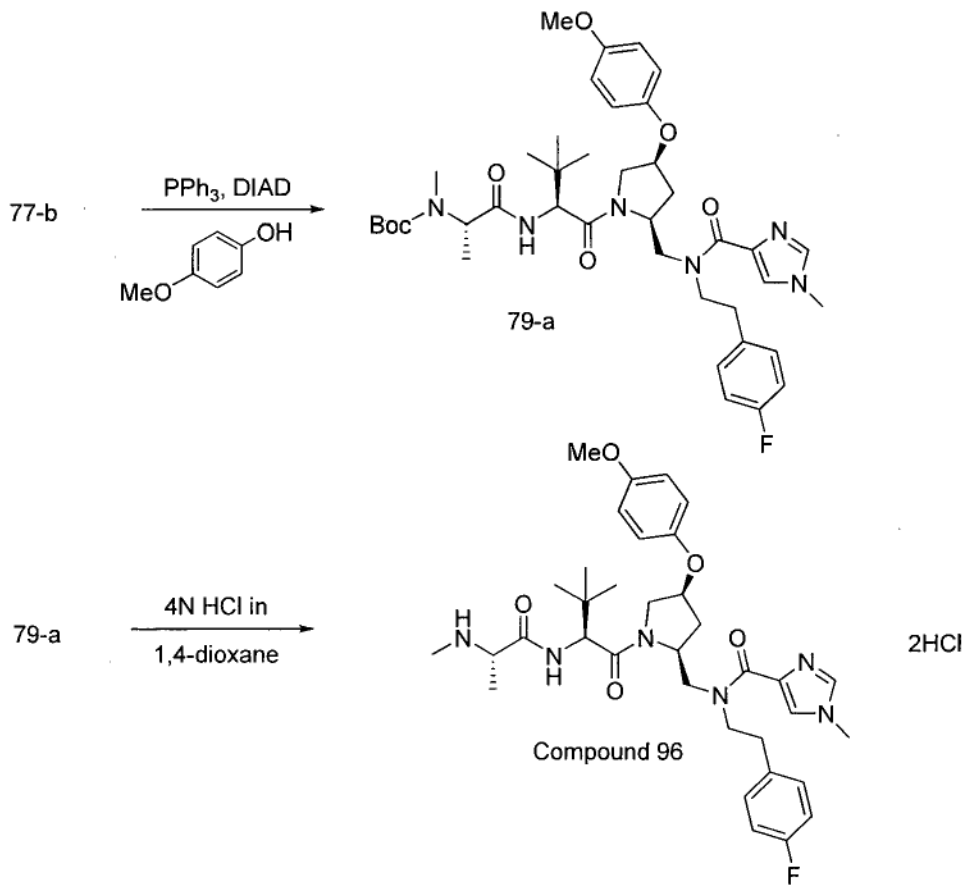
[0402] Step 1: To a solution of intermediate 77-b (700 mg, 1.08 mmol), 4-(trifluoromethoxy)phenol (445 mg, 2.49 mmol) and triphenylphosphine (712 mg, 2.71 mmol) in THF was added DIAD (549 uL, 2.82 mmol) and the reaction was then stirred at room temperature for 2 days. Diethyl ether and hexane were added, a precipitate formed and triphenyl phosphine oxide was removed by filtration. Volatiles were removed under reduced pressure and the residue was purified by silica gel chromatography to provide the expected intermediate 78-a as colorless oil.

[0403] *Step 2:* 4N HCl in 1,4-dioxane (6.0 mL) was added to intermediate 78-a (450 mg, 0.56 mmol) in ethyl acetate (500 uL) and the solution was stirred for 2 hours at 0 °C. Diethyl ether was added, a precipitate formed and compound 97·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 705.4

EXAMPLE 75

[0404] The following example illustrates the preparation of compound 96, which is a compound of Formula 1

Scheme 79: Synthesis of compound 96



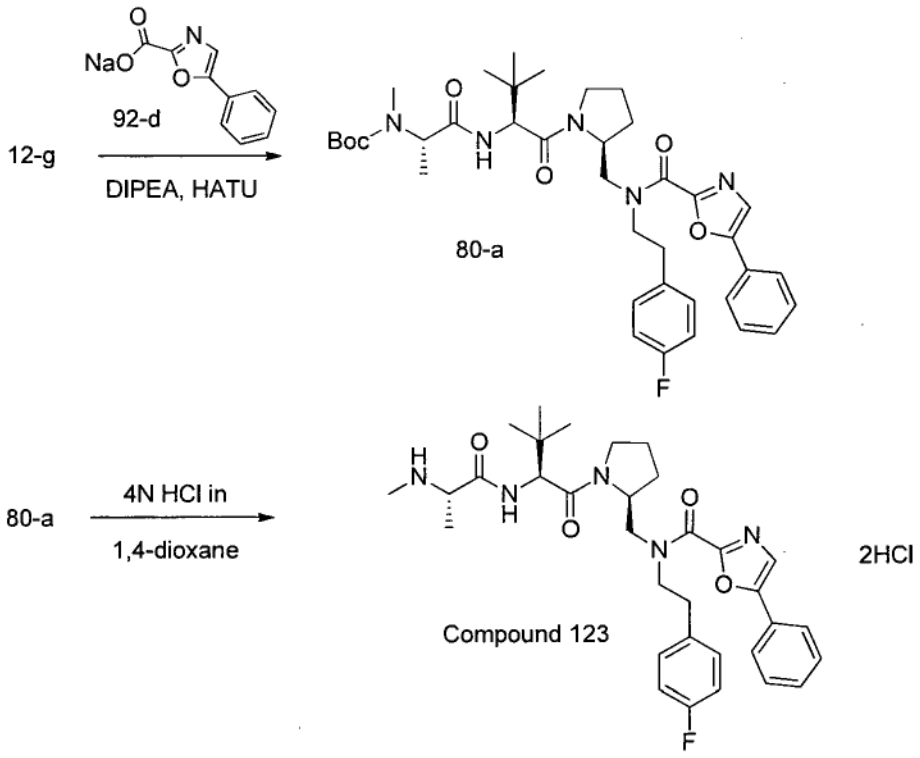
[0405] Step 1: To a solution of intermediate 77-b (484 mg, 0.75 mmol), 4-methoxyphenol (214 mg, 1.72 mmol) and triphenylphosphine (492 mg, 1.87 mmol) in THF was added DIAD (379 uL, 195 mmol) and the reaction was then stirred at room temperature for 2 days. Diethyl ether and hexane were added, a precipitate formed and triphenyl phosphine oxide was removed by filtration. Volatiles were removed under reduced pressure and the residue was purified by silica gel chromatography to provide the expected intermediate 79-a as beige solid.

[0406] *Step 2:* 4N HCl in 1,4-dioxane (2.80 mL) was added to intermediate 79-a (420 mg, 0.56 mmol) in ethyl acetate (500 uL) and the solution was stirred for 2 hours at 0 °C. Diethyl ether was added, a precipitate formed and compound 96·2HCl was collected by filtration as a white solid. MS (m/z) M+1= 651.4

EXAMPLE 76

[0407] The following example illustrates the preparation of compound 123, which is a compound of Formula 1

Scheme 80: Synthesis of compound 123



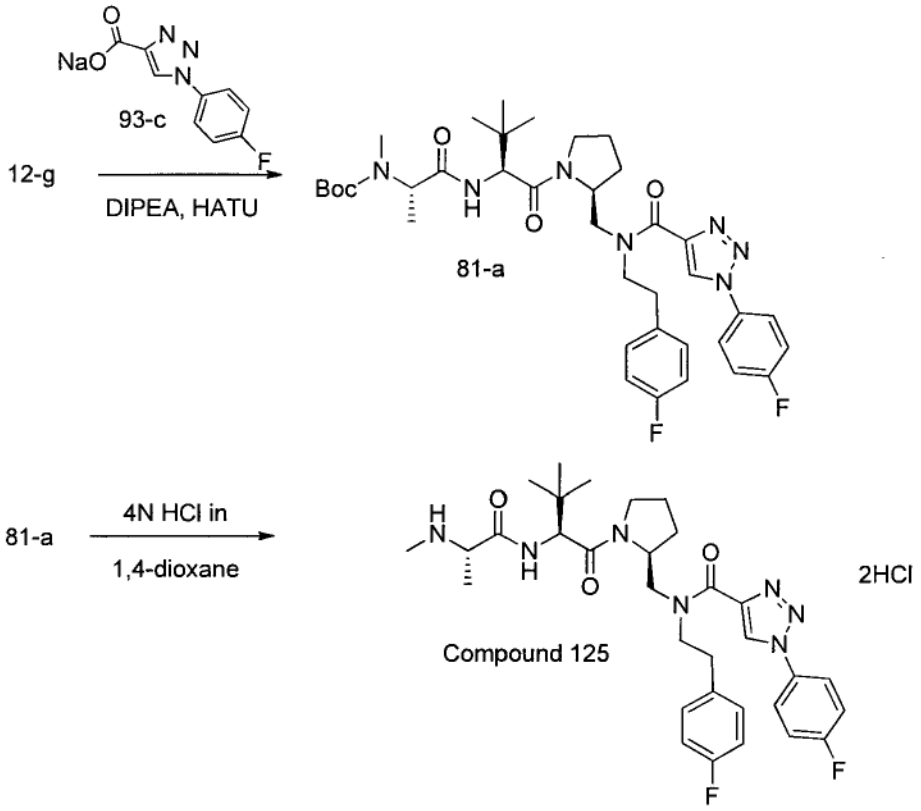
[0408] *Step 1*: To a solution of intermediate 12-g (0.478 g, 0.919 mmol) in DMF at 0 ºC were sequentially added 5-phenyloxazole-2-carboxylic acid, sodium salt (92-d) (0.194 g, 0.919 mmol), HATU (1.05 g, 2.76 mmol) and DIPEA (0.642 mL, 3.68 mmol) and the reaction mixture was stirred at room temperature overnight. Ethyl acetate and saturated aqueous ammonium chloride were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 80-a as a white solid.

[0409] *Step 2*: 4N HCl in 1,4-dioxane (5 mL, 20.0 mmol) was added to intermediate 80-a (390 mg, 0.564 mmol) at 0 ºC and the solution was stirred for 3 hours at 0 ºC. Diethyl ether was added, a precipitate was formed and compound 123·2HCl was collected by filtration as a white solid. MS *m/z* M+1= 592.2

EXAMPLE 77

[0410] The following example illustrates the preparation of compound 125, which is a compound of Formula 1

Scheme 81: Synthesis of compound 125



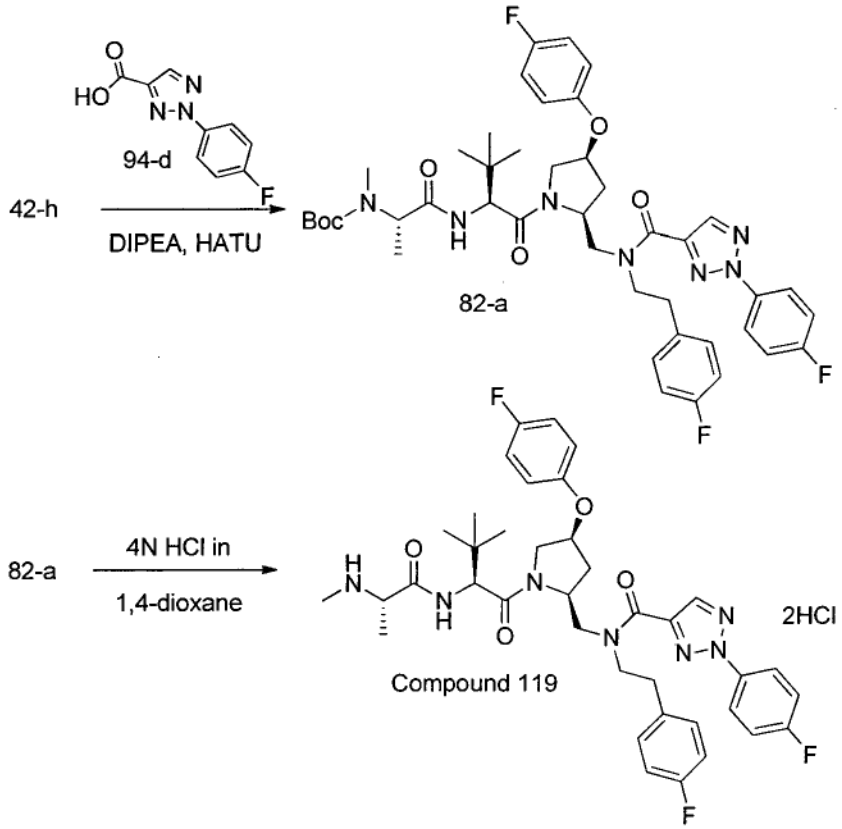
[0411] *Step 1*: To a solution of intermediate 12-g (316 mg, 0.608 mmol) in DMF at 0 ºC were sequentially added 1-(4-fluorophenyl)-1*H*-1,2,3-triazole-4-carboxylic acid, sodium salt (93-c) (0.2 g, 0.790 mmol), HATU (0.347 g, 0.912 mmol) and DIPEA (0.423 mL, 2.431 mmol) and the reaction mixture was stirred at room temperature overnight. Ethyl acetate and saturated aqueous ammonium chloride were added; the organic layer was separated, washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 81-a as a white solid.

[0412] *Step 2*: 4N HCl in 1,4-dioxane (1.5 mL, 6.06 mmol) was added to a solution of intermediate 81-a (0.43 g, 0.606 mmol) in ethyl acetate (1.2 mL) and the solution was stirred for 2 hours at 0 ºC. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 125·2HCl as an off-white solid. MS *m/z* M+1= 610.1

EXAMPLE 78

[0413] The following example illustrates the preparation of compound 119, which is a compound of Formula 1

Scheme 82: Synthesis of compound 119



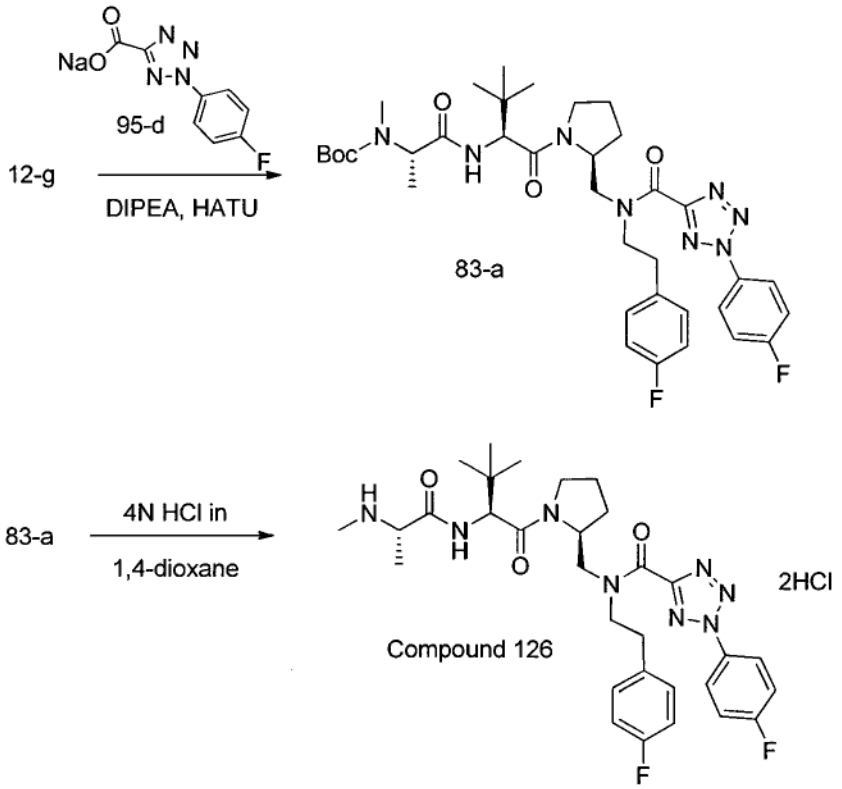
[0414] *Step 1*: To a solution of intermediate 42-h (250 mg, 0.396 mmol) in DMF at 0 ºC were sequentially added 2-(4-fluorophenyl)-2*H*-1,2,3-triazole-4-carboxylic acid (94-d) (130 mg, 0.630 mmol), HATU (271 mg, 0.713 mmol) and DIPEA (207 uL, 1.189 mmol). The reaction mixture was stirred for 3 hours at 0 °C and at room temperature overnight. Ethyl acetate and saturated aqueous ammonium chloride were added; the organic layers were separated. The aqueous phase was extracted with ethyl acetate; the combined organic extracts were washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 82-a as a white solid.

[0415] *Step 2*: 4N HCl in 1,4-dioxane (924 uL, 3.70 mmol) was added to intermediate 82-a (303 mg, 0.370 mmol) and the solution was stirred for 1 hour at 0 ºC. Volatiles were removed under reduced pressure and the residue was triturated with diethyl ether to provide compound 119·2HCl as a white solid. MS *m/z* M+1= 720.1

EXAMPLE 79

[0416] The following example illustrates the preparation of compound 126, which is a compound of Formula 1

Scheme 83: Synthesis of compound 126



[0417] *Step 1*: To a solution of intermediate 12-g (300 mg, 0.576 mmol) in DMF at 0 ºC were sequentially added 2-(4-fluorophenyl)-2*H*-tetrazole-5-carboxylic acid, sodium salt (95-d) (190 mg, 0.749 mmol), HATU (329 mg, 0.864 mmol) and DIPEA (400 uL, 2.305 mmol). The reaction mixture was stirred at 0 °C for 1 hour. Ethyl acetate and saturated aqueous ammonium chloride were added; the organic layers were separated. The aqueous phase was extracted with ethyl acetate; the combined organic extracts were washed with saturated aqueous ammonium chloride, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to give intermediate 83-a.

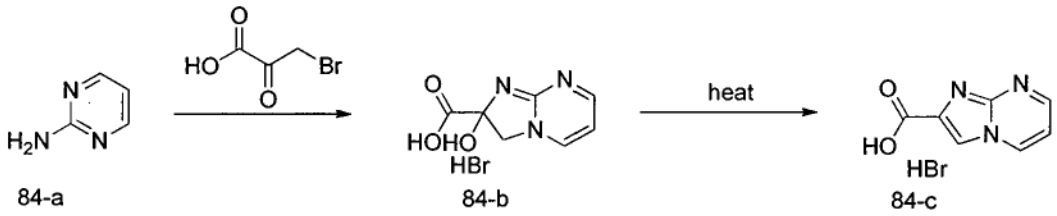
[0418] *Step 2*: 4N HCl in 1,4-dioxane (1.76 mL, 7.03 mmol) was added to a solution of intermediate 83-a (303 mg, 0.370 mmol) and the solution was stirred at 0 ºC for 2

hours. Volatiles were removed under reduced pressure. Purification by reverse phase chromatography provided compound 126·2HCl as a white solid. MS *m/z* M+1= 611.1

EXAMPLE 80

[0419] The following example illustrates the preparation of imidazo[1,2-*a*]pyrimidine-2-carboxylic acid, HBr salt (84-c)

Scheme 84: Synthesis of 84-c



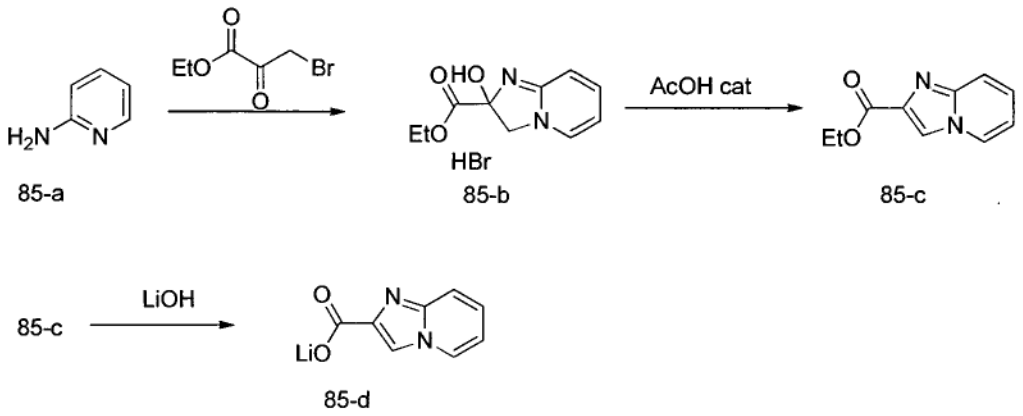
[0420] *Step 1*: To a solution of 2-aminopyrimidine 84-a (50.00 g, 526 mmol) in DMF (375 mL) cooled to 0 °C was added 3-bromo-2-oxopropanoic acid (88.00 g, 526 mmol). The reaction was stirred at 0 °C for 2 hours and then warmed to room temperature over 2 hours and stirred for 18 hours. Acetonitrile (650 mL) was added; precipitate formed and was collected by filtration. The precipitate was rinsed with acetonitrile and diethyl ether and dried in vacuo to obtain intermediate 84-b·HBr as a white solid. MS *m/z* M+1=182.0

[0421] *Step 2*: To a suspension of intermediate 84-b·HBr (98.80 g, 377 mmol) in THF (600 mL) was added N,N-dimethylacetamide (48 mL). The reaction was stirred at 80 °C for 48 hours, cooled to room temperature and poured into acetonitrile (700 mL). A precipitate was formed and collected by filtration, rinsed with acetonitrile and diethyl ether and dried in vacuo to give imidazo[1,2-*a*]pyrimidine-2-carboxylic acid, HBr salt (84-c) as a beige solid. MS *m/z* M+1= 164.2

EXAMPLE 81

[0422] The following example illustrates the preparation of imidazo[1,2-*a*]pyridine-2-carboxylic acid, lithium salt (85-d)

Scheme 85: Synthesis of 85-d



[0423] *Step 1*: To a solution of 2-aminopyridine 85-a (20.00 g, 213 mmol) in THF (417 mL) was added ethyl bromopyruvate (30.0 mL, 215 mmol). The reaction mixture was stirred at room temperature for 18 hours. A precipitate formed and was collected by filtration and rinsed with THF to provide intermediate 85-b·HBr as a yellow solid.

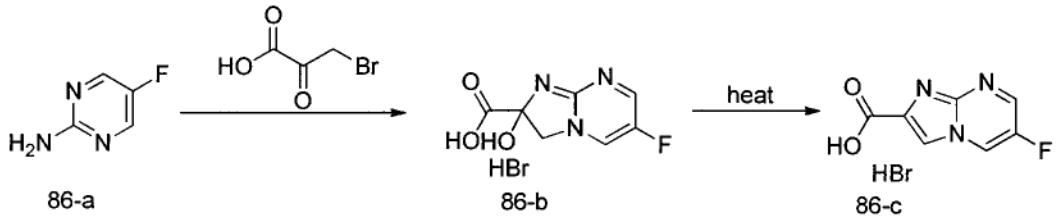
[0424] *Step 2*: To a suspension of intermediate 85-b·HBr in ethanol (550 mL) was added AcOH (5 mL) and the mixture was heated at reflux for 3 hours to give a clear solution. The solution was concentrated in vacuo, precipitate was formed and triturated with diethyl ether; intermediate 85-c·HBr was collected by filtration as a beige solid, dissolved in water (0.5 L) and basified to pH 8 by using NaOH pellets. This solution was extracted with ethyl acetate four times; the combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to provide intermediate 85-c as an off-white solid.

[0425] *Step 3*: To a solution of intermediate 85-c (26.40 g, 0.139 mmol) in ethanol (140 mL) was added 2M aqueous LiOH (70 mL, 140 mmol) and the reaction was stirred at room temperature for 5 hours. The reaction mixture was cooled to 0 °C, precipitate was formed, collected by filtration and rinsed with diethyl ether to obtain imidazo[1,2-*a*]pyridine-2-carboxylic acid, lithium salt (85-d) as a white solid. ^{1}H NMR (200 MHz, CD_3OD), δ ppm 8.41 (d, *J*= 6.6 Hz, 1H), 8.11 (s, 1H), 7.53 (d, *J*= 9.2 Hz, 1H), 7.29 (dd, *J*= 8.0 Hz, *J*= 7.3 Hz, 1H), 6.90 (dd, *J*= 6.7 Hz, *J*= 6.7 1H)

EXAMPLE 82

[0426] The following example illustrates the preparation of 6-fluoroimidazo[1,2-*a*]pyrimidine-2-carboxylic acid, HBr salt (86-c)

Scheme 86: Synthesis of 86-c



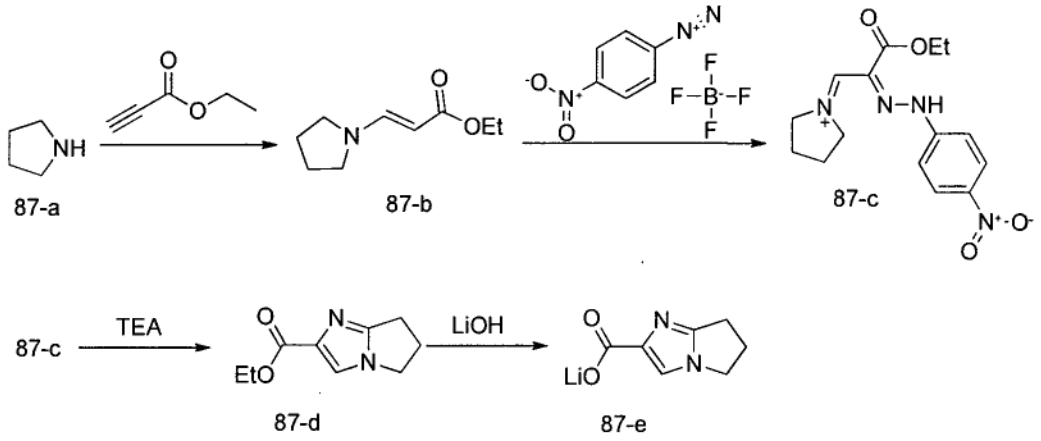
[0427] *Step 1*: To a solution of 5-fluoropyrimidin-2-amine 86-a (3.17 g, 28.0 mmol) in DMF (14 mL) at 0 °C was added bromopyruvic acid (7.73 g, 53.4 mmol). The reaction mixture was stirred for 2 hours at 0 °C and then for 2 days at room temperature. The reaction was diluted with acetone; precipitate formed and intermediate 86-b·HBr was collected by filtration as a beige solid.

[0428] *Step 2*: A suspension of intermediate 86-b·HBr (8.40 g, 30.0 mmol) in THF (100 mL) and AcOH (10 mL) was stirred at 70 °C for 72 hours. THF was added and the precipitate was collected by filtration, rinsed with THF and diethyl ether and dried in vacuo to provide 6-fluoroimidazo[1,2-*a*]pyrimidine-2-carboxylic acid, HBr salt (86-c) as a pale beige solid. MS *m/z* M+1= 182.0

EXAMPLE 83

[0429] The following example illustrates the preparation of 6,7-dihydro-5*H*-pyrrolo[1,2-*a*]imidazole-2-carboxylic acid, lithium salt (87-e)

Scheme 87: Synthesis of 87-e



[0430] *Step 1*: To a solution of intermediate 87-a (5.00 g, 70.3 mmol) in THF was added ethyl propiolate (6.90 g, 70.3 mmol) at 10 °C. The reaction mixture was stirred for 1 hour at room temperature and concentrated in vacuo to provide intermediate 87-b as a yellow oil.

[0431] *Step 2*: To a solution of intermediate 87-b (11.90 g, 70.3 mmol) in acetonitrile (1400 mL) was added 4-nitrobenzenediazonium tetrafluoroborate (16.66 g, 70.3 mmol) and the reaction was stirred for 1 hour at room temperature to provide a solution of intermediate 87-c.

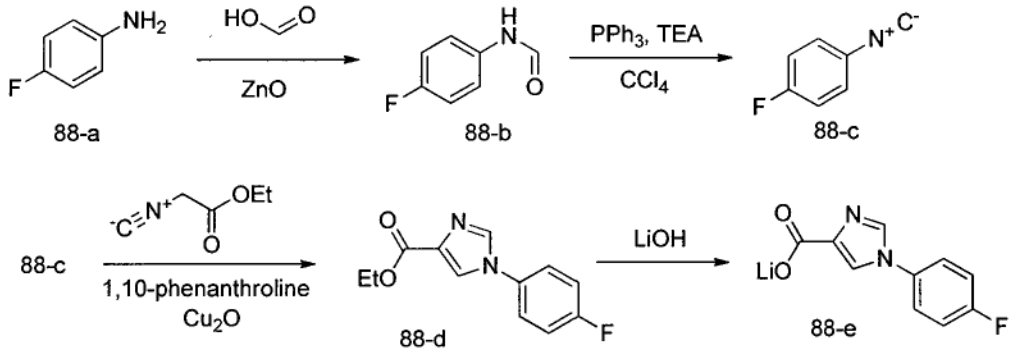
[0432] *Step 3*: To a solution of intermediate 87-c was added TEA (19.0 mL, 141 mmol) and the reaction mixture was heated at reflux for 2 hours, cooled to room temperature and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 87-d as a brown solid. MS *m/z* M+Na= 202.9

[0433] *Step 4*: To a solution of intermediate 87-d (5.36 g, 29.7 mmol) in THF was added 2N aqueous LiOH (32.7 mL, 65.4 mmol). The reaction mixture was stirred at room temperature for 48 hours and concentrated in vacuo to obtain 6,7-dihydro-5*H*-pyrrolo[1,2-*a*]imidazole-2-carboxylic acid, lithium salt (87-e) as a pale beige solid. MS *m/z* M+1= 153.0

EXAMPLE 84

[0434] The following example illustrates the preparation of 1-(4-fluorophenyl)-1*H*-imidazole-4-carboxylic acid, lithium salt (88-e)

Scheme 88: Synthesis of 88-e



[0435] *Step 1*: To a solution of zinc oxide (7.32 g, 90 mmol) in formic acid (20.7 mL, 540 mmol) at 0 °C was added 4-fluoroaniline 88-a (20.00 g, 180 mmol). The reaction mixture was stirred at 70 °C for 1 hour, cooled to room temperature, diluted with ethyl acetate and filtered over a pad of Celite. The filtrate was washed with water, 10% aqueous Na_2CO_3 and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to give intermediate 88-b as a beige solid. MS *m/z* M+1= 140.0

[0436] *Step 2*: To a solution of intermediate 88-b (8.35 g, 60.0 mmol) in dichloromethane (111 mL) were sequentially added triphenylphospine (17.79 g, 67.8 mmol), TEA (8.32 mL, 60.0 mmol) and carbon tetrachloride (6.21 mL, 64.2 mmol). After stirring for 72 hours at room temperature, the reaction mixture was cooled to 0 °C. A precipitate was formed, collected by filtration and discarded. The filtrate was concentrated and suspended in cold diethyl ether. An insoluble residue was collected by filtration and discarded. The filtrate was concentrated in vacuo to provide intermediate 88-c as a brown oil.

[0437] *Step 3*: To a suspension of copper oxide (1.20 g, 8.40 mmol) and 1,10-phenanthroline (3.03 g, 16.80 mmol) in THF (400 mL) purged with nitrogen were

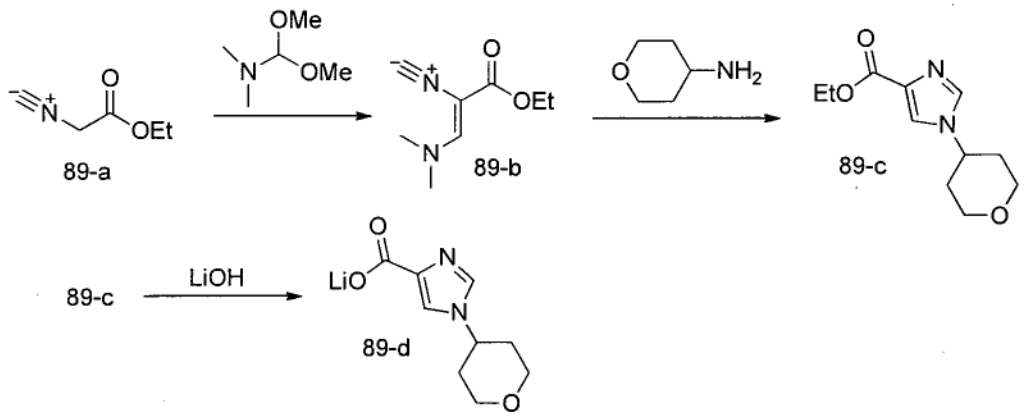
sequentially added intermediate 88-c (7.27 g, 60.0 mmol) and ethyl isocyanoacetate (9.2 mL, 84 mmol). After stirring at 70 °C for 18 hours the reaction mixture was cooled to room temperature and filtered over a pad of Celite. The filtrate was concentrated in vacuo. Purification by silica gel chromatography provided intermediate 88-d as a light yellow solid. MS *m/z* M+1= 235.0

[0438] *Step 4*: To a solution of intermediate 88-d (10.47 g, 44.7 mmol) in THF was added 2N aqueous LiOH (24.6 mL, 49.2 mmol). After stirring at room temperature overnight, the reaction mixture was concentrated in vacuo. The resulting residue was diluted with water and extracted with dichloromethane. The organic fractions were discarded and the aqueous layer was concentrated in vacuo to provide 1-(4-fluorophenyl)-1*H*-imidazole-4-carboxylic acid, lithium salt (88-e) as a light yellow solid. MS *m/z* M+1= 207

EXAMPLE 85

[0439] The following example illustrates the preparation of 1-(tetrahydro-2*H*-pyran-4-yl)-1*H*-imidazole-4-carboxylic acid, lithium salt (89-d)

Scheme 89: Synthesis of 89-d



[0440] *Step 1*: To a solution of ethyl 2-isocyanoacetate 89-a (5 g, 44.2 mmol) in anhydrous ethanol (50 mL) at 0 °C was added 1,1-dimethoxy-N,N-dimethylmethanamine (10.53 g, 88 mmol). After stirring at room temperature overnight, the reaction mixture was

concentrated in vacuo. Purification by chromatography on neutral aluminum provided intermediate 89-b as a yellow oil.

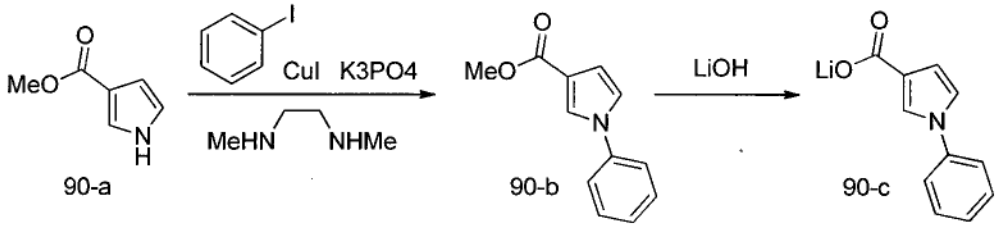
[0441] *Step 2*: To a seal-tube were added intermediate 89-b (1.00 g, 5.95 mmol) and tetrahydro-*2H*-pyran-4-amine (1.80 g, 17.84 mmol). The tube was filled with nitrogen, sealed and heated at 70 °C for 2 hours. After cooling to room temperature, the reaction mixture was purified by silica gel chromatography to provide intermediate 89-c as a beige solid.

[0442] *Step 3*: To a solution of intermediate 89-c (0.72 g, 3.22 mmol) in a 3:1 methanol/water mixture was added LiOH (0.116 g, 4.83 mmol). After stirring at room temperature for 18 hours, the reaction mixture was concentrated in vacuo to give 1-(tetrahydro-2*H*-pyran-4-yl)-1*H*-imidazole-4-carboxylic acid, lithium salt (89-d) as a white foam. MS *m/z* M+1= 197.0

EXAMPLE 86

[0443] The following example illustrates the preparation of 1-phenyl-1*H*-pyrrole-3-carboxylic acid, lithium salt (90-c)

Scheme 90: Synthesis of 90-c



[0444] *Step 1*: To a dried seal-tube were added methyl 1*H*-pyrrole-3-carboxylate 90-a (150 mg, 1.199 mmol), copper(I) iodide (11.4 mg, 0.060 mmol), K_3PO_4 (534 mg, 2.52 mmol), iodobenzene (160 uL, 1.439 mmol) and toluene (1.2 mL). Nitrogen was bubbled through the mixture for 5 min followed by addition of N^1,N^2-dimethylethane-1,2-diamine (21 mg, 0.240 mmol). The tube was sealed and the reaction was stirred overnight at 100 ºC. It was then diluted with ethyl acetate and filtered over a pad of Celite. The filtrate was

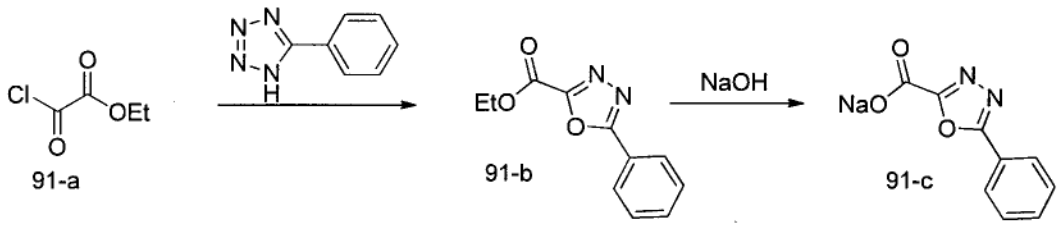
washed with saturated aqueous ammonium chloride and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to give intermediate 90-b. MS *m/z* M+1= 202.2

[0445] *Step 2*: To a solution of intermediate 90-b (241 mg, 1.198 mmol) in a 2:1 THF/ /water mixture was added LiOH (92 mg, 3.832 mmol). The reaction mixture was heated at 50 ºC overnight and concentrated in vacuo. The residue was dissolved in water, extracted with dichloromethane and diethyl ether and the organic extracts were discarded. The aqueous phase was acidified to pH 2 using 37% aqueous HCl and extracted twice with dichloromethane. The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo to give 1-phenyl-1*H*-pyrrole-3-carboxylic acid, lithium salt (90-c) as a white solid. MS *m/z* M+Na=210.2

EXAMPLE 87

[0446] The following example illustrates the preparation of 5-phenyl-1,3,4-oxadiazole-2- carboxylic acid, sodium salt (91-c)

Scheme 91: synthesis of 91-c



[0447] *Step 1*: To a solution of ethyl 2-chloro-2-oxoacetate 91-a (5.61 g, 41.1 mmol) in toluene was added 5-phenyl-1H-tetrazole (6.00 g, 41.1 mmol). After stirring at reflux for 2 hours, the reaction mixture was diluted with ethyl acetate and water. The organic layer was washed twice with 1N aqueous HCl, water and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 91-b as a yellow solid. MS *m/z* M+1= 219.1

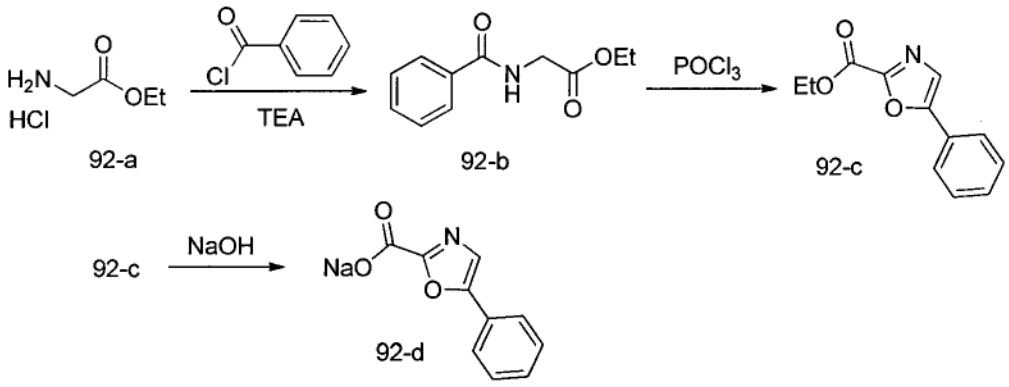
[0448] *Step 2*: To a solution of intermediate 91-b (1.40 g, 6.42 mmol) in THF was added 1N aqueous NaOH (6.7 mL, 6.74 mmol) at 0 ºC. The reaction mixture was stirred at

room temperature for 2 hours and concentrated in vacuo to provide 5-phenyl-1,3,4-oxadiazole-2- carboxylic acid, sodium salt (91-c) as a white solid. MS *m/z* M+1= 191.1

EXAMPLE 88

[0449] The following example illustrates the preparation of 5-phenyloxazole-2-carboxylic acid, sodium salt (92-d)

Scheme 92: Synthesis of 92-d



[0450] *Step 1*: To a solution of glycine ethyl ester hydrochloride 92-a (4.64 g, 33.2 mmol) in dichloromethane at 0 ºC were sequentially added benzoyl chloride (3.22 mL, 27.7 mmol) and TEA (11.49 mL, 83 mmol). The reaction mixture was stirred at 0 ºC for 2 hours and at room temperature overnight. Ethyl acetate and water were added; the organic layer was separated, washed with 1N aqueous HCl twice, followed by water and brine wash, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to give intermediate 92-b as an oil.

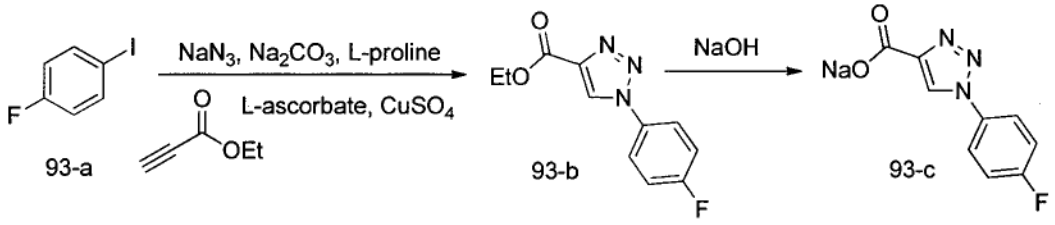
[0451] *Step 2*: To a solution of intermediate 92-b (1.10 g, 4.68 mmol) in toluene (5.0 mL) was added phosphorus oxychloride (2.18 mL, 23.38 mmol). The reaction mixture was heated at reflux overnight and cooled to room temperature. Ethyl acetate and saturated aqueous $NaHCO_3$ were added; the organic layer was separated, washed with saturated aqueous $NaHCO_3$, water and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 92-c as a white solid. MS *m/z* M+1= 218.1

[0452] *Step 2*: To a solution of intermediate 92-c (200 mg, 0.921 mmol) in THF was added 1 N aqueous NaOH (1.1 mL, 1.105 mmol) at 0 ºC. After stirring at room temperature for 3 hours, the reaction mixture was concentrated in vacuo to give 5-phenyloxazole-2-carboxylic acid, sodium salt (92-d) as a white solid. MS *m/z* M+1= 190.2

EXAMPLE 89

[0453] The following example illustrates the preparation of 1-(4-fluorophenyl)-1*H*-1,2,3-triazole-4-carboxylic acid, sodium salt (93-c)

Scheme 93: Synthesis of 93-c



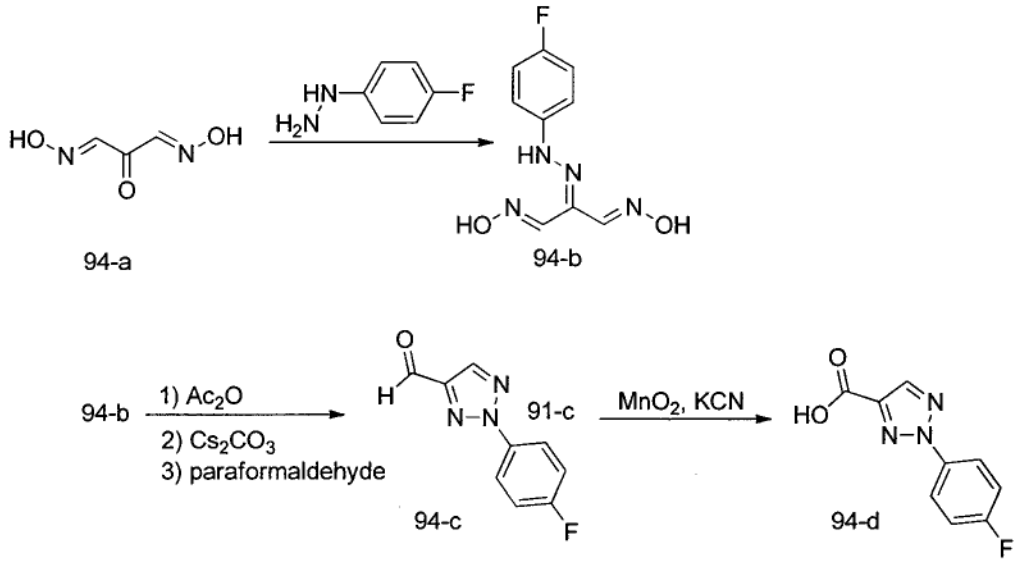
[0454] *Step 1*: To a solution of 1-fluoro-4-iodobenzene 93-a (0.926 g, 4.17 mmol) in DMSO (4.0 mL) and water (0.44 mL) were sequentially added sodium azide (0.325 g, 5.01 mmol), sodium carbonate (0.088 g, 0.833 mmol), L-proline (0.096 g, 0.833 mmol), ethyl propiolate (0.409 g, 4.17 mmol), sodium L-ascorbate (0.207 g, 1.043 mmol) and copper(II) sulfate (0.052 g, 0.208 mmol). The reaction mixture was stirred at 65 ºC for 18 hours and cooled to room temperature. Aqueous ammonia (1 mL), ethyl acetate (25 mL) and water (20 mL) were added. The phases were separated and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by reverse phase chromatography provided intermediate 93-b. MS *m/z* M+1= 236.1

[0455] *Step 2*: To a solution of intermediate 93-b (200 mg, 0.850 mmol) in THF was added 2N aqueous NaOH (0.425 mL, 0.850 mmol). After stirring at room temperature for 18 hours, the reaction mixture was concentrated in vacuo to provide 1-(4-fluorophenyl)-1*H*-1,2,3-triazole-4-carboxylic acid, sodium salt (93-c) as a yellow solid. MS *m/z* M+Na=230.0

EXAMPLE 90

[0456] The following example illustrates the preparation of 2-(4-fluorophenyl)-2*H*-1,2,3-triazole-4-carboxylic acid, sodium salt (94-d)

Scheme 94: Synthesis of 94-d



[0457] *Step 1*: To a solution of 94-a (1.75 g, 15.08 mmol) in ethanol (16 mL) was added 4-fluorophenylhydrazine (1.90 g, 15.08 mmol) and the reaction was stirred at 70 °C for 30 minutes. Water (16 mL) was added, the reaction mixture was heated to 85 °C, concentrated by one third and cooled in an ice bath. A yellow precipitate formed and was collected by filtration. The precipitate was dissolved in a 25% ethanol/toluene mixture (40 mL). The flask was equipped with a condenser and a Dean-Stark trap and the reaction mixture was heated at reflux until all ethanol was collected in the Dean-Stark trap. The mixture was cooled to room temperature; precipitate formed and intermediate 94-b was collected by filtration as a brown solid.

[0458] *Step 2*: A solution of intermediate 94-b (1.64 g, 7.32 mmol) in acetic anhydride (13.8 mL, 146 mmol) was stirred at room temperature for 30 minutes. The reaction was diluted with water (60 mL), stirred for 30 min and the resulting precipitate

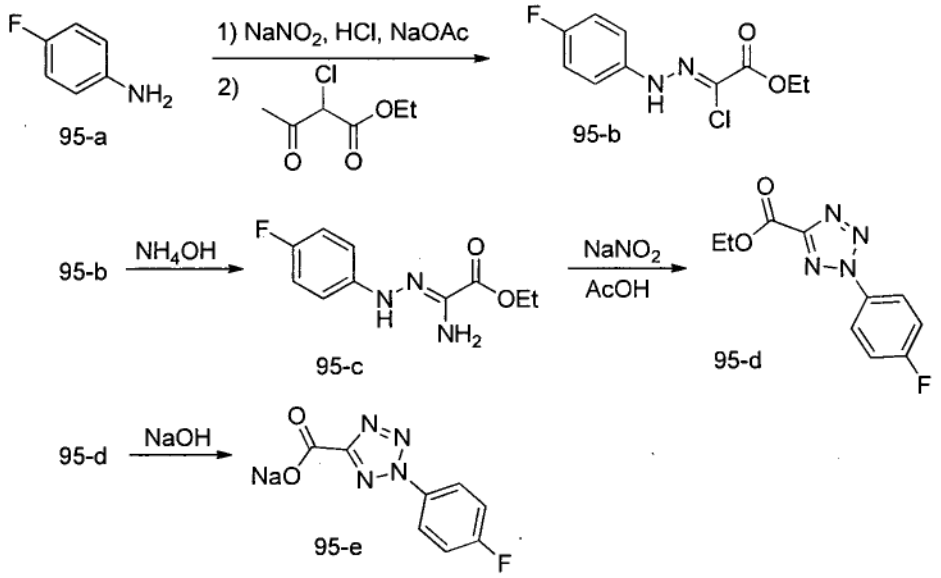
was collected by filtration. The solid was partitioned between ethyl acetate and water. The layers were separated; the organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The residue was dissolved in THF and cesium carbonate (2.62 g, 8.05 mmol) was added. The mixture was stirred at room temperature for 30 minutes and filtered. The filtrate was concentrated in vacuo; the residue was dissolved in diethyl ether and washed with water, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resulting solid was dissolved in 2M aqueous HCl (50 mL), paraformaldehyde (0.433 g, 14.41 mmol) was added and the reaction mixture was heated at reflux for 2 hours. Upon cooling to room temperature, the mixture was extracted with diethyl ether. The organic layer was separated and washed with water, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel chromatography provided intermediate 94-c as a pale yellow solid.

[0459] *Step 3*: To a solution of intermediate 94-c (300 mg, 1.569 mmol) in methanol (15 mL) at 0 °C were added potassium cyanide (171 mg, 2.62 mmol) and manganese (IV) oxide (1078 mg, 12.40 mmol). The reaction mixture was stirred at room temperature for 18 hours, filtered over a pad of Celite and the cake was rinsed with methanol. The filtrate was concentrated in vacuo. Purification by silica gel chromatography provided 2-(4-fluorophenyl)-2*H*-1,2,3-triazole-4-carboxylic acid, sodium salt (94-d) as a pale yellow solid. MS *m/z* M+1= 244.2

EXAMPLE 91

[0460] The following example illustrates the preparation of 2-(4-fluorophenyl)-2*H*-tetrazole-5-carboxylic acid, sodium salt (95-e)

Scheme 95: synthesis of 95-e



[0461] *Step 1*: To a solution of 4-fluoroaniline 95-a (2.00 g, 18.00 mmol) in ethanol (5.7 mL) and water (2.0 mL) was added 37% aqueous HCl (3.7 mL, 45.0 mmol) and the reaction mixture was cooled to -5 °C. To the mixture was added 5N aqueous sodium nitrite (3.96 mL, 19.80 mmol) drop-wise followed by an addition of 6N aqueous sodium acetate (9.0 mL, 54.0 mmol). The reaction mixture was stirred for 10 minutes at 0 °C then ethyl 2-chloro-3-oxobutanoate (2.96 g, 18.00 mmol) was added and stirring continued overnight at 27 ºC. THF (20 mL) was added and the reaction mixture was warmed up to 40 ºC and transferred to a separation funnel. The aqueous layer was separated and discarded. The organic layer contained intermediate 95-b as a THF solution. MS *m/z* M+Na= 267.1

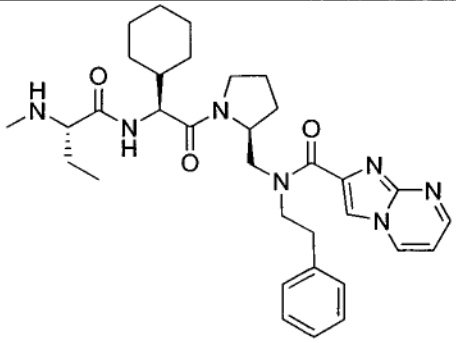
[0462] *Step 2*: To a solution of intermediate 95-b (4.40 g, 18 mmol) in THF was added 25% aqueous NH_4OH (16 mL, 108 mmol) at 0 ºC. The reaction mixture was stirred at room temperature for 3 hours and transferred to a separation funnel. The aqueous layer was separated and discarded. The organic layer was concentrated and the residue was triturated with diethyl ether/hexanes mixture; precipitate was formed and intermediate 95-c was collected as a red-brick solid.

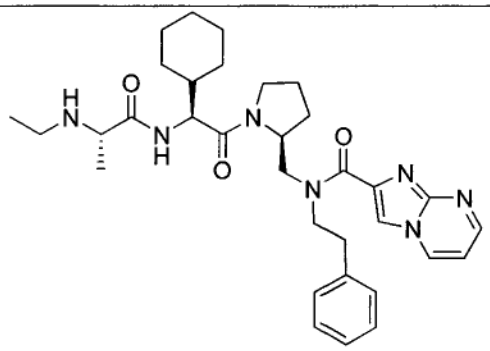
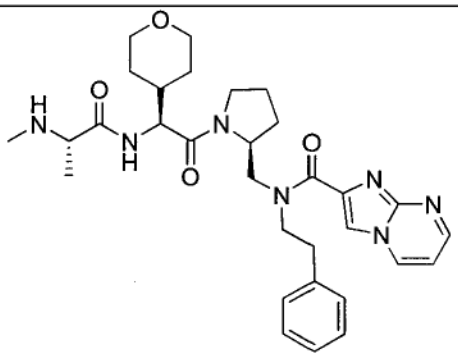
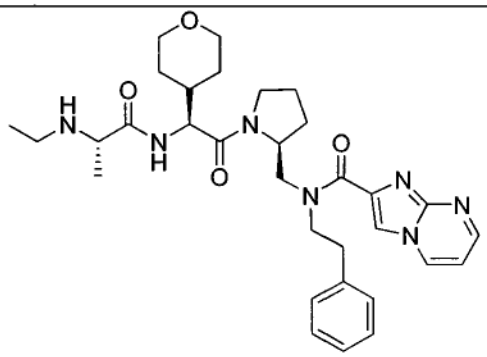
[0463] *Step 3*: To a solution of intermediate 95-c (1.36 g, 6.04 mmol) in THF (27 mL) was added acetic acid (1.1 mL, 18.12 mmol) and the reaction mixture was warmed up to 85 °C. To the reaction was added 2.6N aqueous sodium nitrite (2.8 mL, 7.25 mmol), the mixture was stirred at 85 °C for 4 hours and concentrated in vacuo. The residue was dissolved in ethyl acetate and washed with saturated aqueous $NaHCO_3$, the layers were separated and the aqueous phase was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by silica gel flash chromatography provided intermediate 95-d as a yellow solid. MS *m/z* M+Na= 258.9

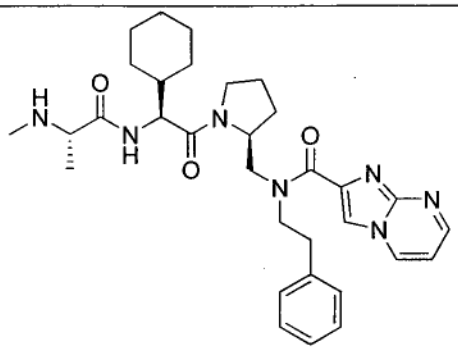
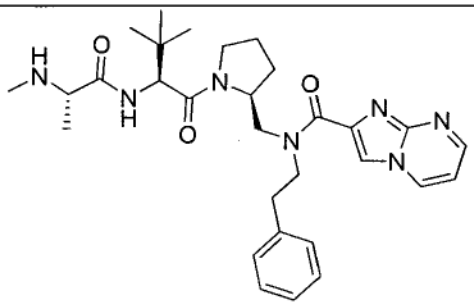
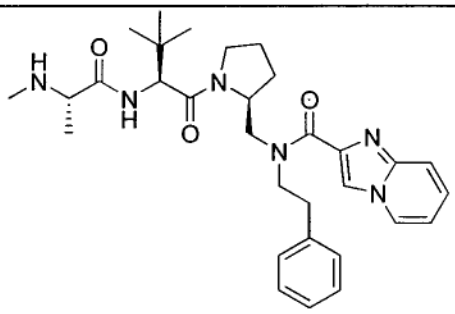
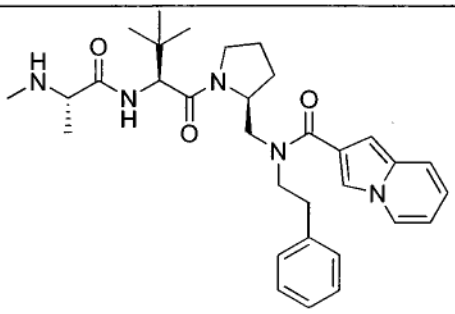
[0464] *Step 4*: To a solution of intermediate 95-d (840 mg, 3.56 mmol) in THF was added 2N aqueous NaOH (1.78 mL, 3.56 mmol). The reaction mixture was stirred at 50 °C for 18 hours and concentrated in vacuo to provide 2-(4-fluorophenyl)-2*H*-tetrazole-5-carboxylic acid, sodium salt (95-e) as a yellow solid. MS *m/z* M+1= 209.1

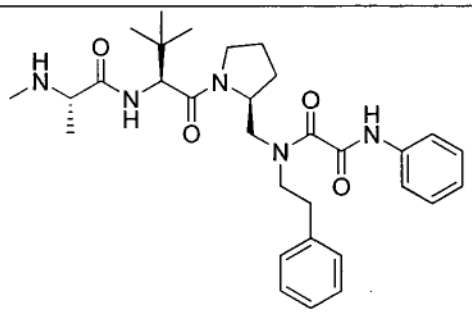
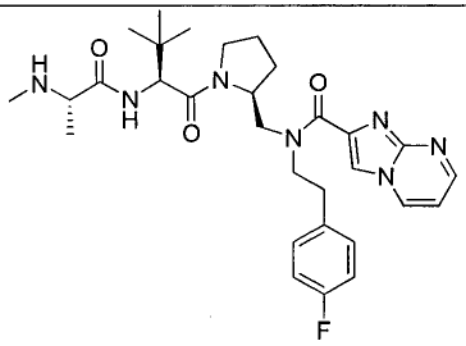
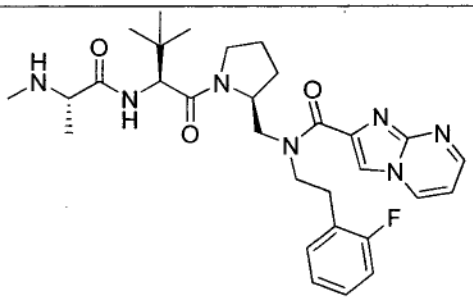
[0465] Compounds prepared according to the above procedures, or minor modifications thereof, are illustrated in table 1. Minor modifications to the procedures above and use of the appropriate starting materials as needed to arrive at the compounds of table 1 will be apparent to one skilled to the art.

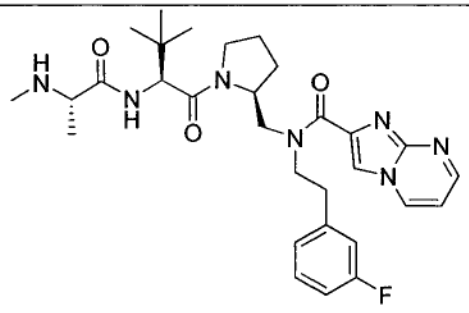
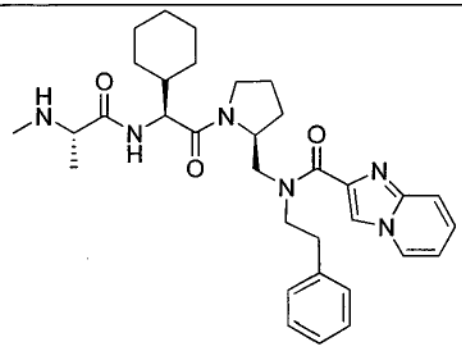
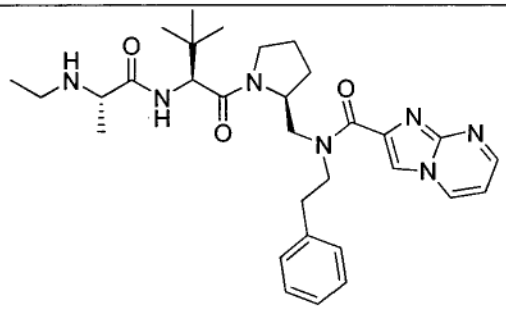
TABLE 1

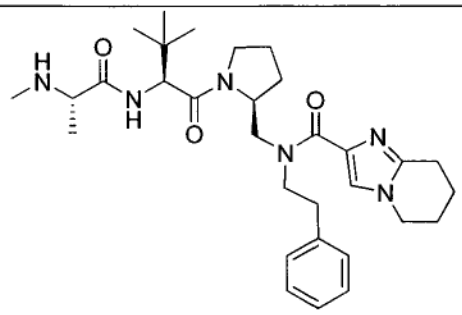
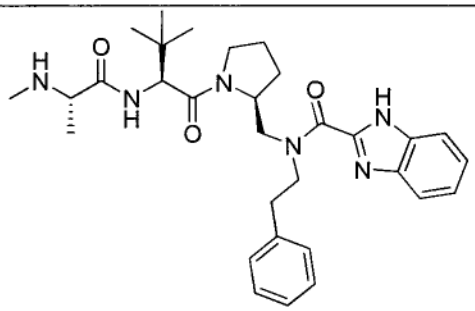
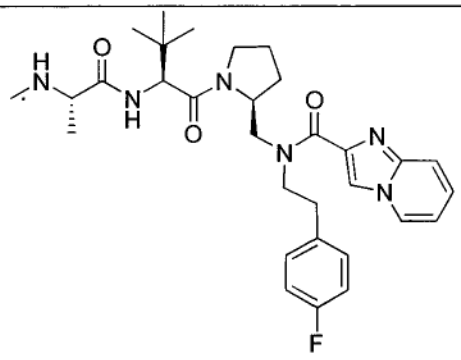
Cmpd #	Structure	MS
1		M+1=588.5

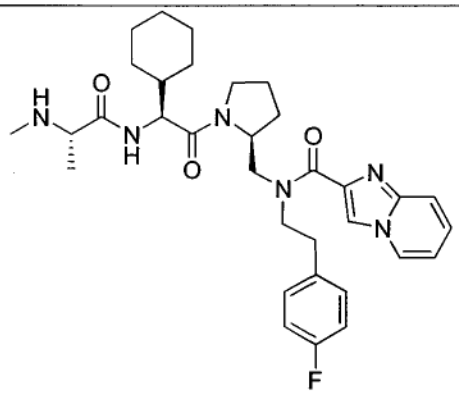
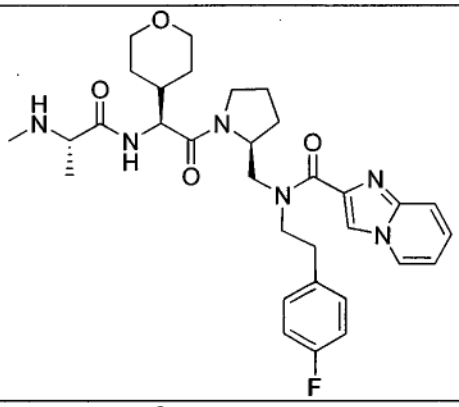
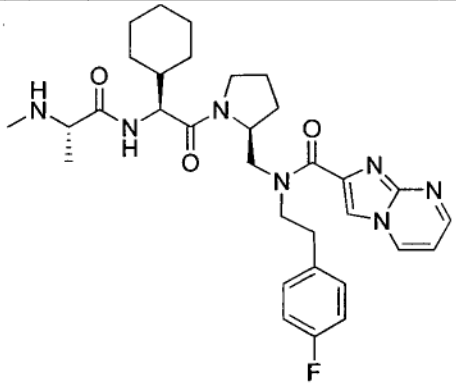
Cmpd #	Structure	MS
2		M+1=588.4
3		M+1=576.4
4		M+1=590.4

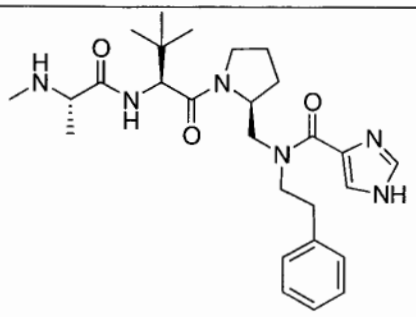
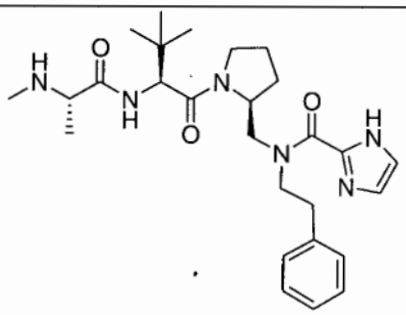
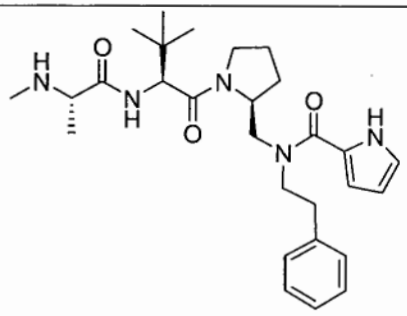
Cmpd #	Structure	MS
5		M+1=574.4
6		M+1=548.4
7		M+1=547.5
8		M+1=546.5

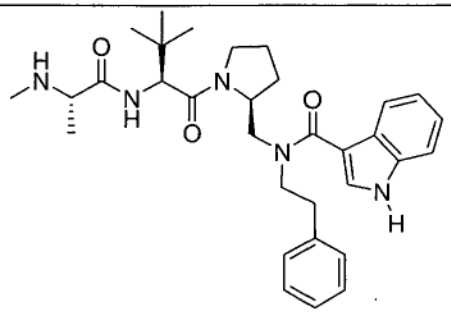
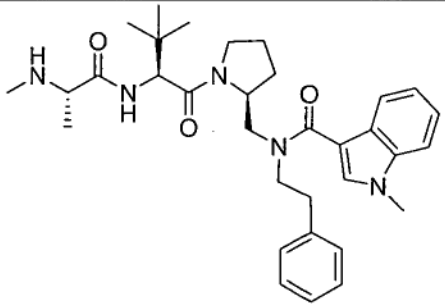
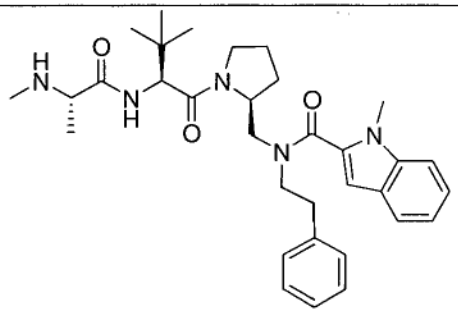
Cmpd #	Structure	MS
9		M+1=550.1
10		M+1=566.4
11		M+1=566.4

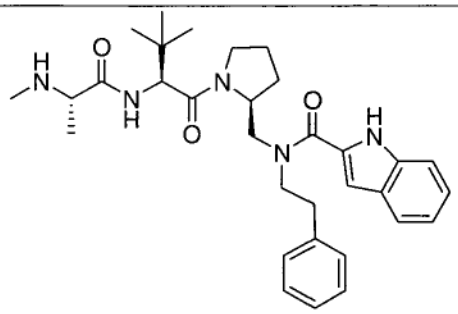
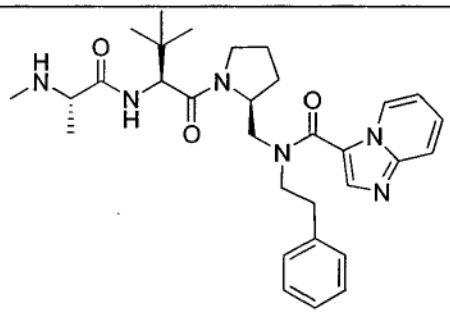
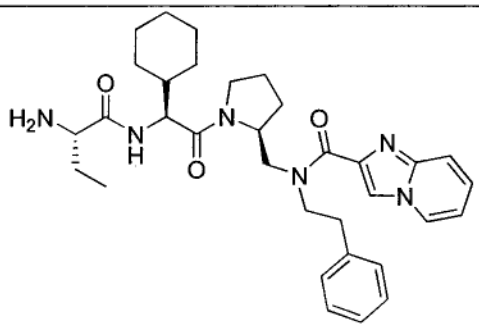
Cmpd #	Structure	MS
12		M+1=566.4
13		M+1=573.5
14		M+1=562.4

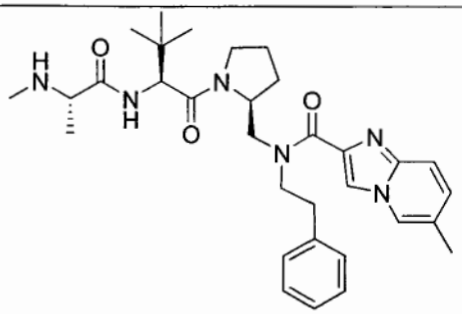
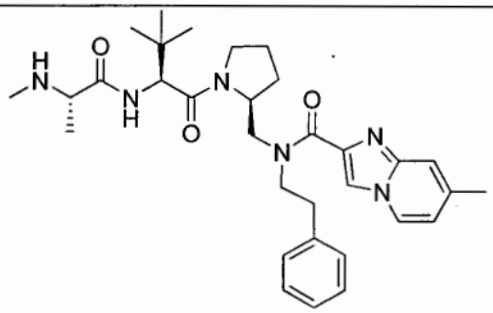
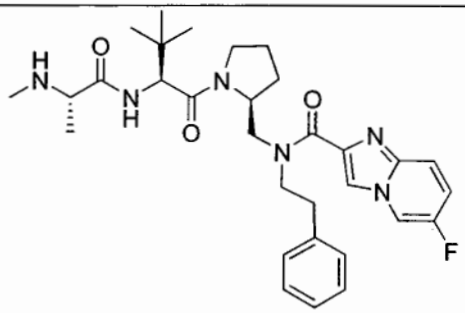
Cmpd #	Structure	MS
15		M+1=551.5
16		M+1=547.4
17		M+1=565.4

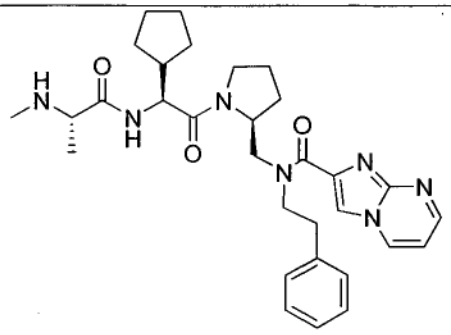
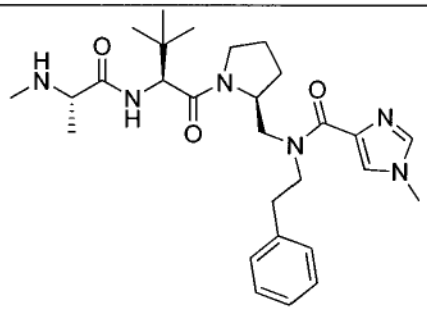
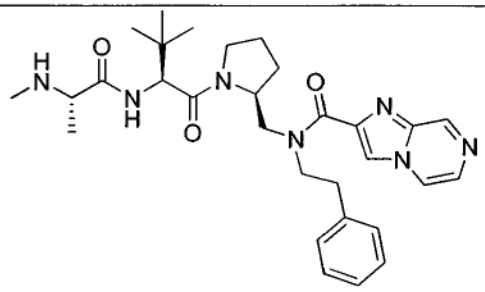
Cmpd #	Structure	MS
18		M+1=591.4
19		M+1=593.4
20		M+1=592.4

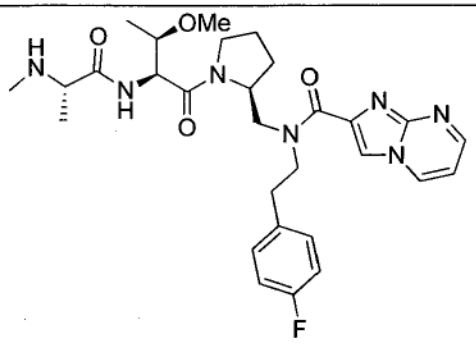
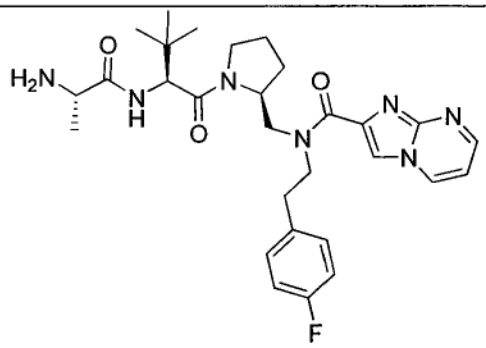
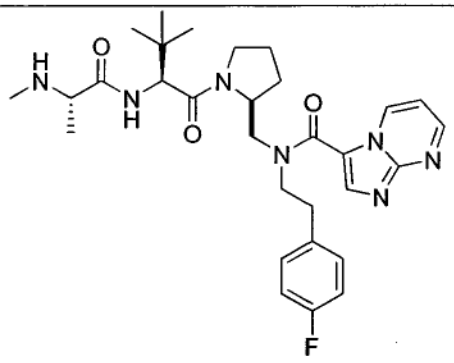
Cmpd #	Structure	MS
21		M+1=497.2
22		M+1=497.2
23		M+1=496.4

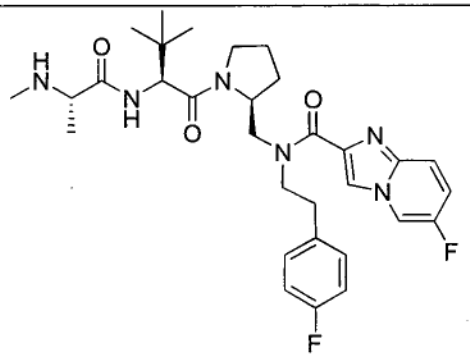
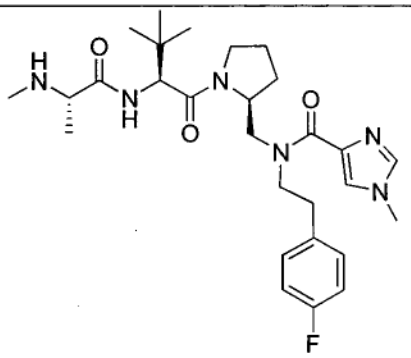
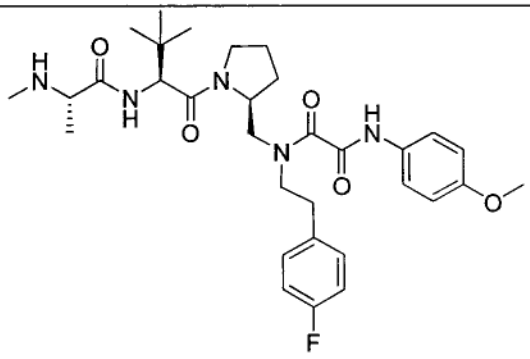
Cmpd #	Structure	MS
24		M+1=546.2
25		M+1=560.4
26		M+1=560.4

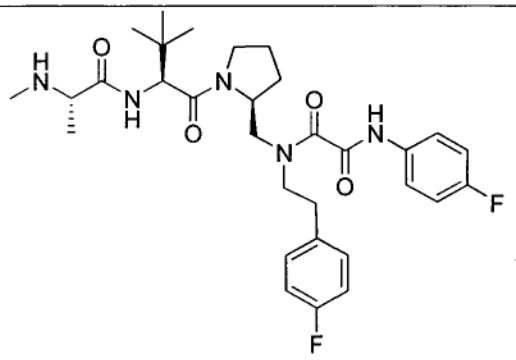
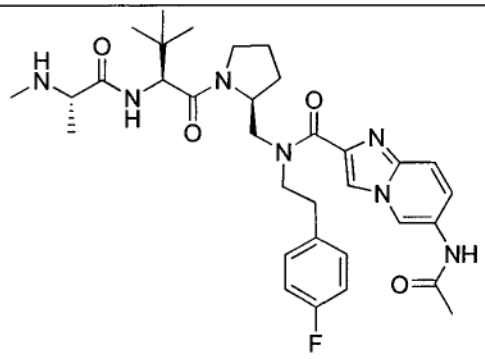
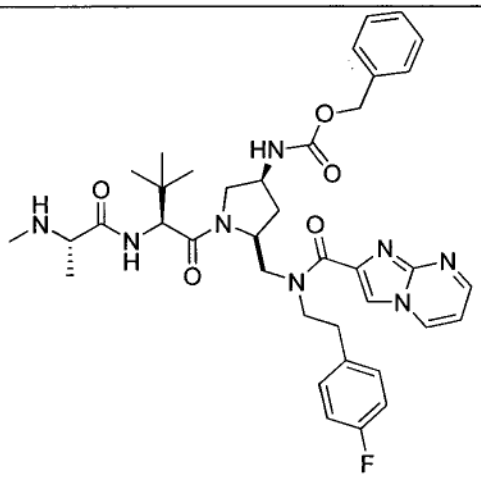
Cmpd #	Structure	MS
27		M+1=546.2
28		M+1=548.5
29		M+1=573.4

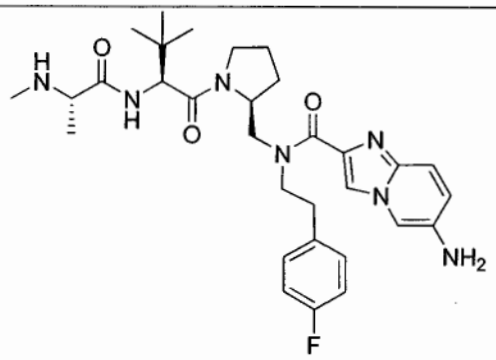
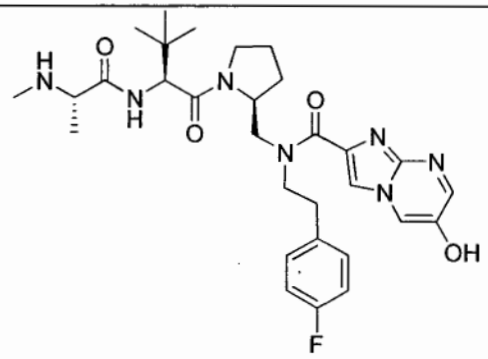
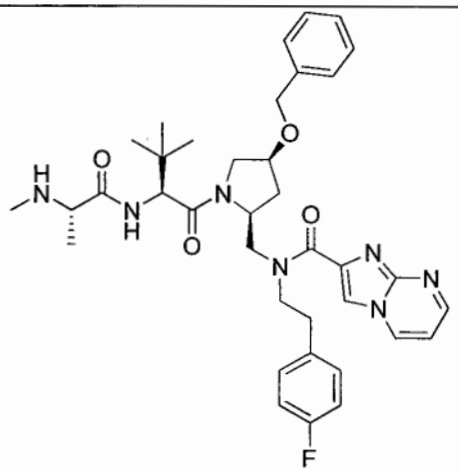
Cmpd #	Structure	MS
30		M+1=561.4
31		M+1=561.4
32		M+1=565.4

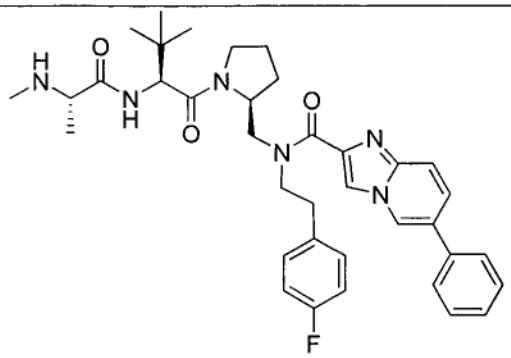
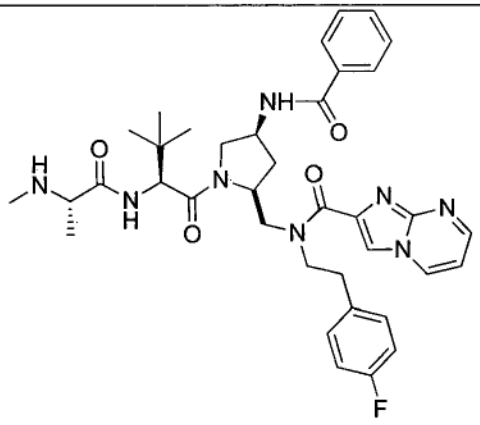
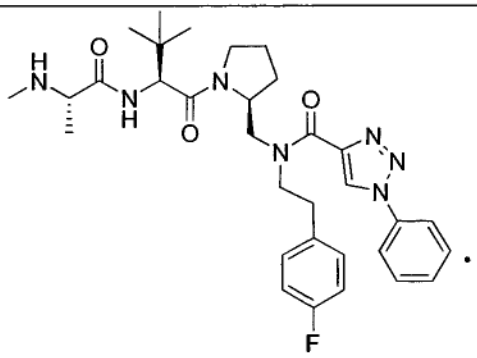
Cmpd #	Structure	MS
33		M+1=560.4
34		M+1=511.4
35		M+1=548.5

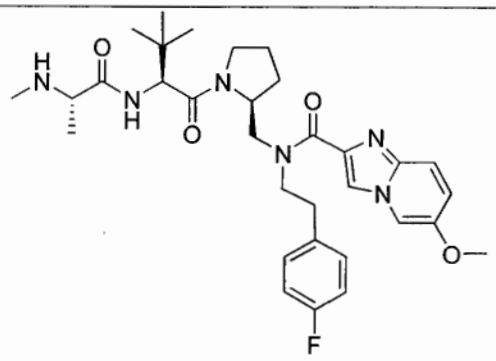
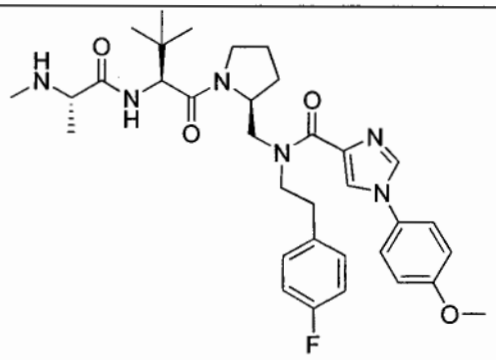
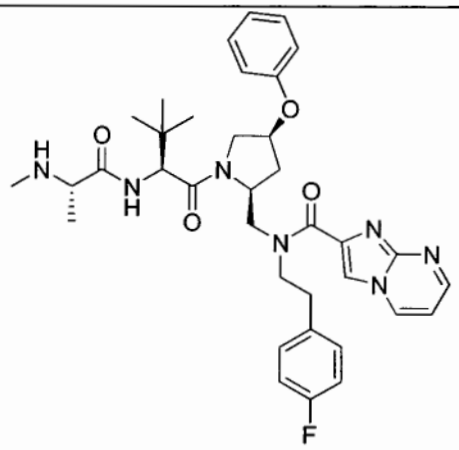
Cmpd #	Structure	MS
36		M+1=568.5
37		M+1=552.5
38		M+1=566.5

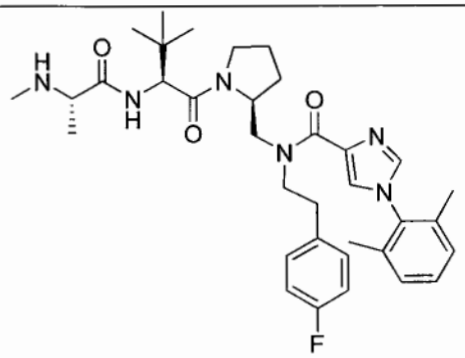
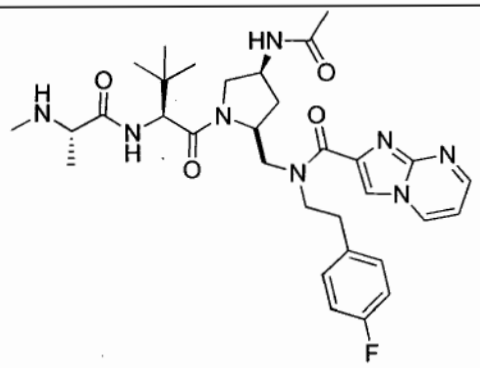
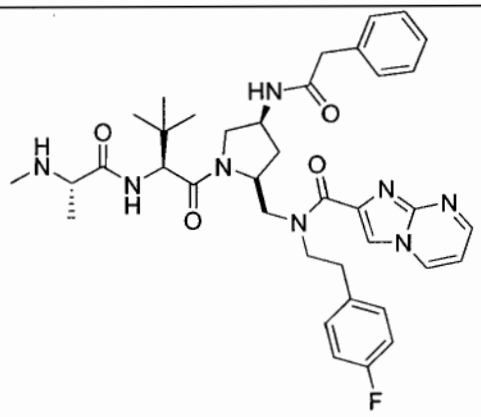
Cmpd #	Structure	MS
39		M+1=583.4
40		M+1=529.5
41		M+1=598.5

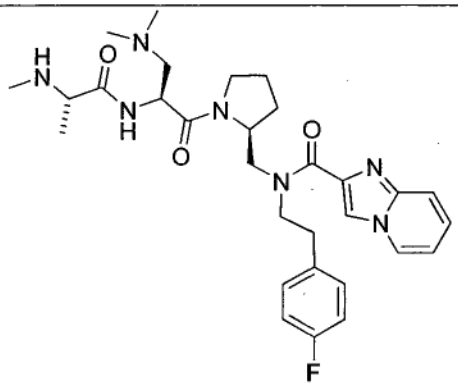
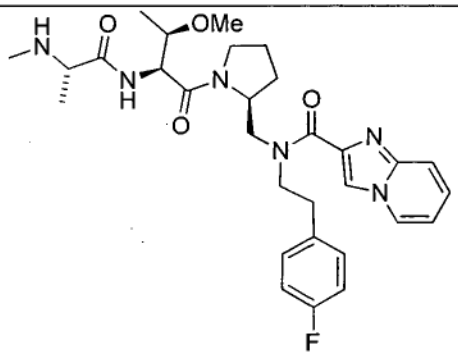
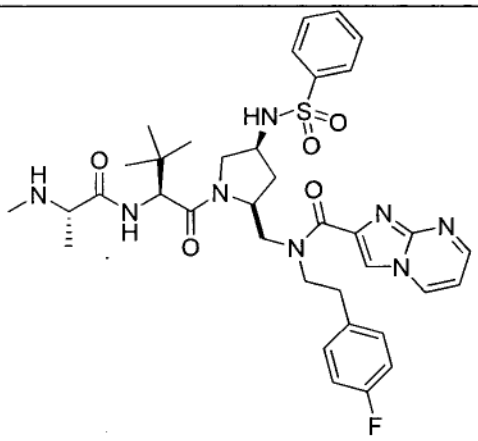
Cmpd #	Structure	MS
42		M+1=586.5
43		M+1=622.6
44		M+1=715.5

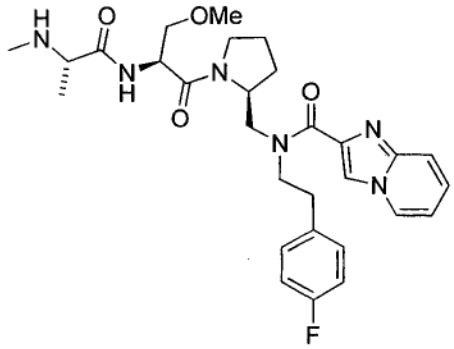
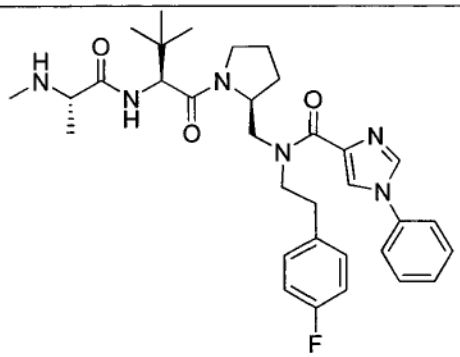
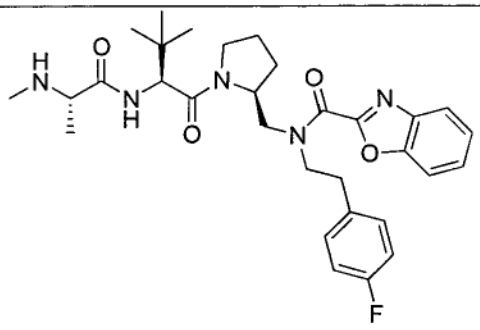
Cmpd #	Structure	MS
45		M+1=580.5
46		M+1=582.5
47		M+1=672.5

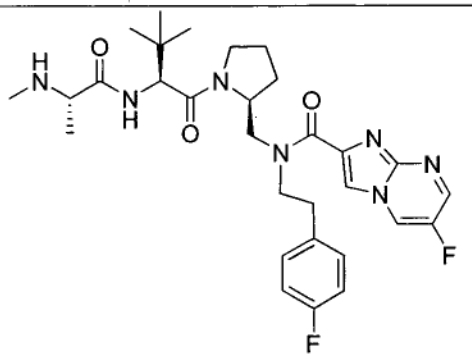
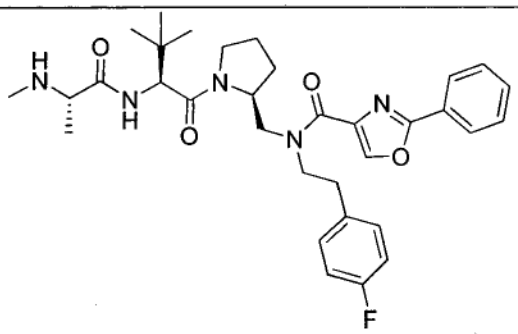
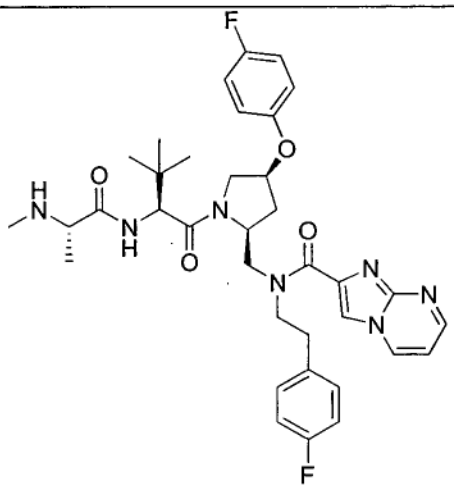
Cmpd #	Structure	MS
48		M+1=641.5
49		M+1=685.5
50		M+1=592.5

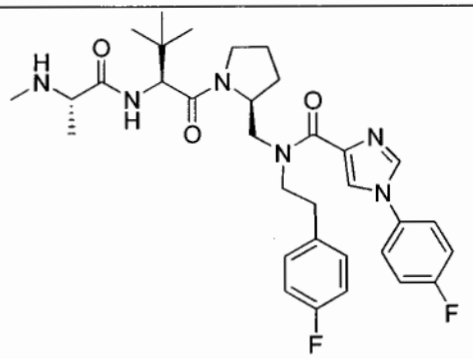
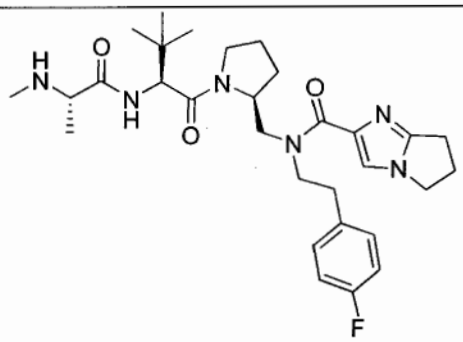
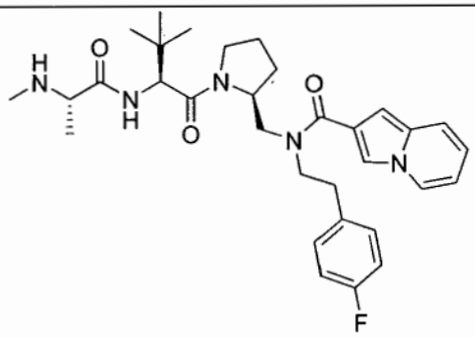
Cmpd #	Structure	MS
51		M+1=595.5
52		M+1=621.5
53		M+1=658.5

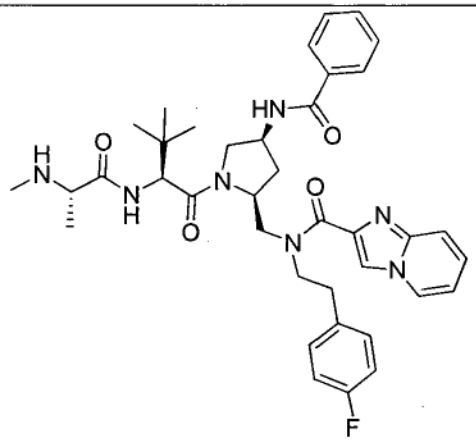
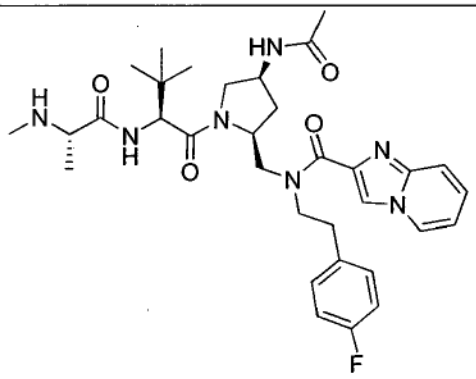
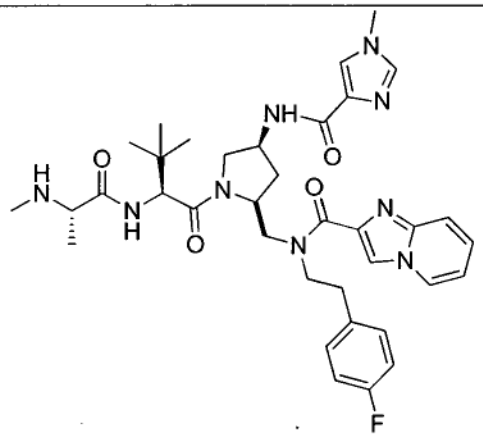
Cmpd #	Structure	MS
54		M+1=619.6
55		M+1=623.5
56		M+1=699.5

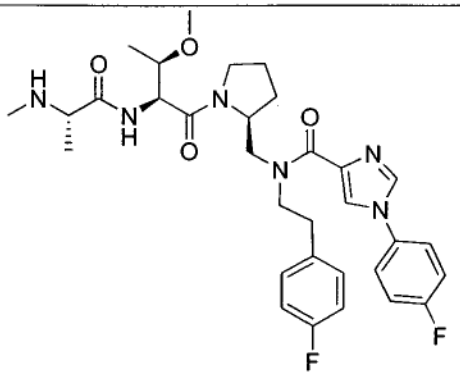
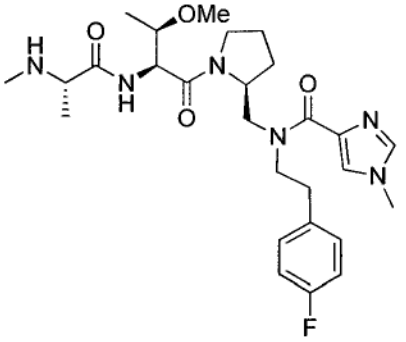
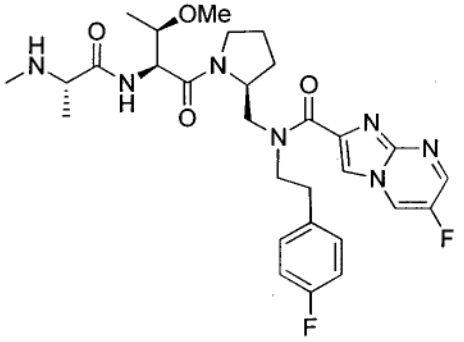
Cmpd #	Structure	MS
57		M+1=566.6
58		M+1=567.5
59		M+1=721.5

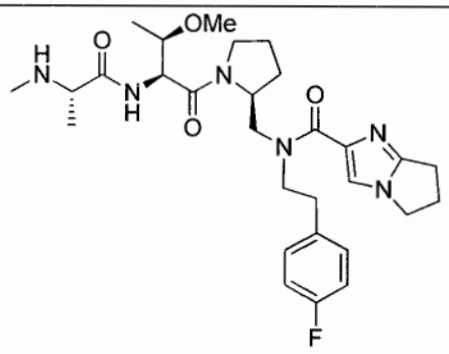
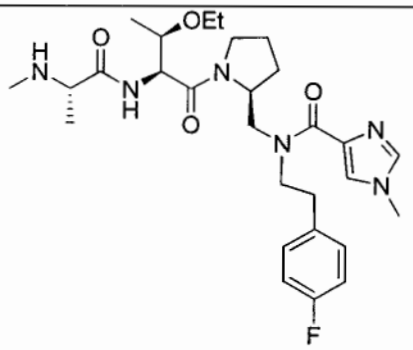
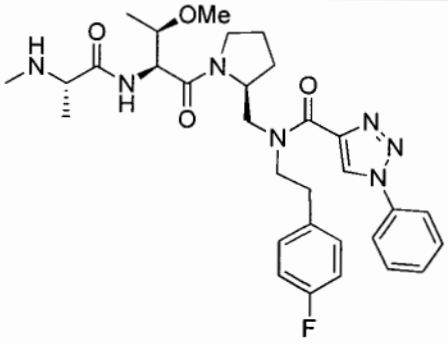
Cmpd #	Structure	MS
60		M+1=553.5
61		M+1=591.5
62		M+1=566.5

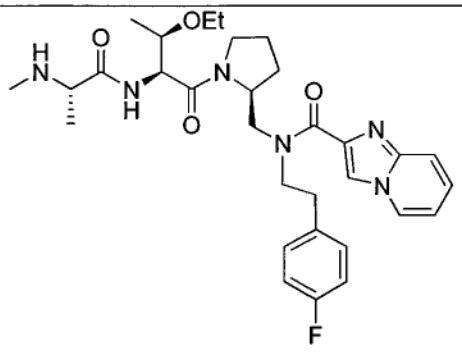
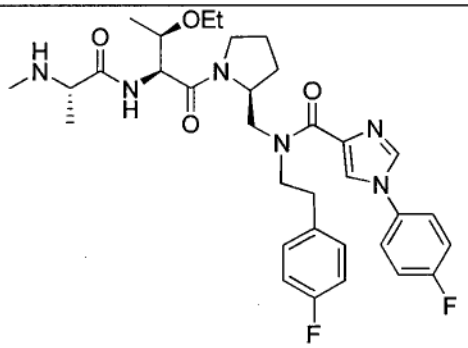
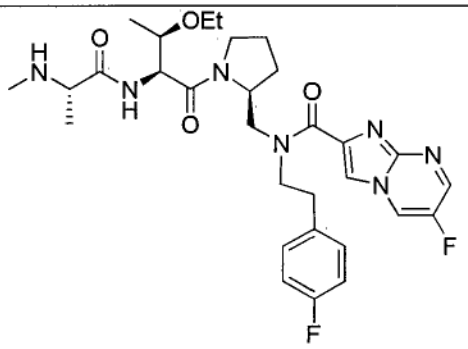
Cmpd #	Structure	MS
63		M+1=584.5
64		M+1=592.5
65		M+1=676.6

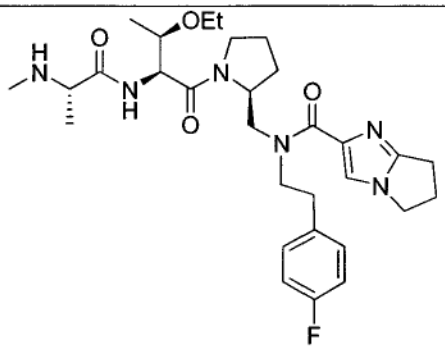
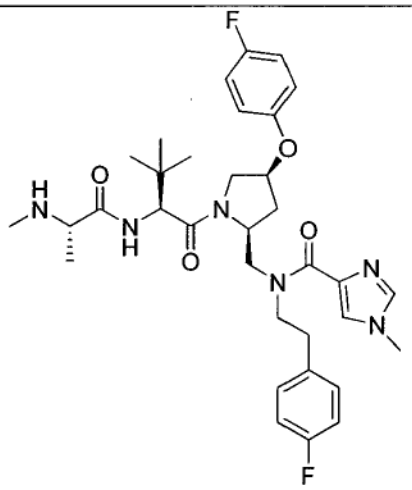
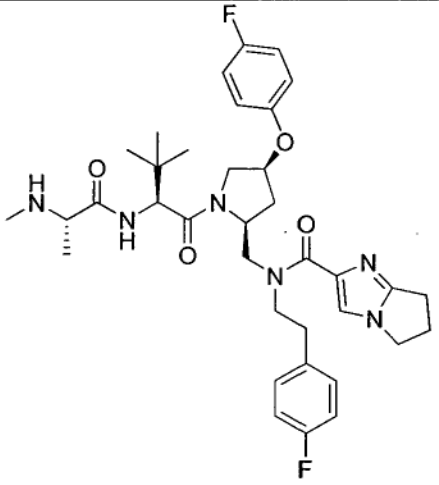
Cmpd #	Structure	MS
66		M+1=609.5
67		M+1=555.6
68		M+1=564.5

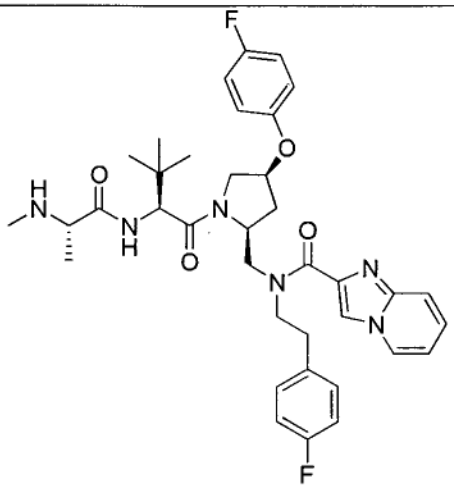
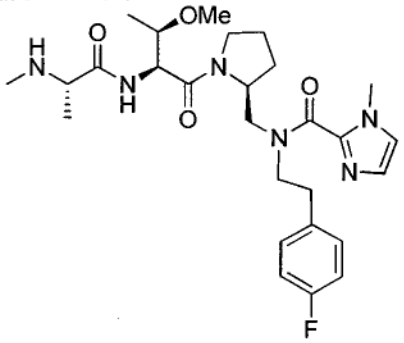
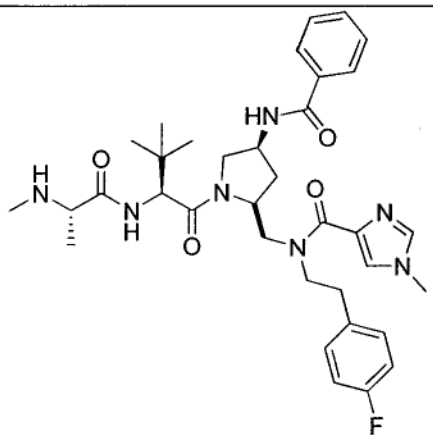
Cmpd #	Structure	MS
69		M+1=684.5
70		M+1=622.5
71		M+1=668.5

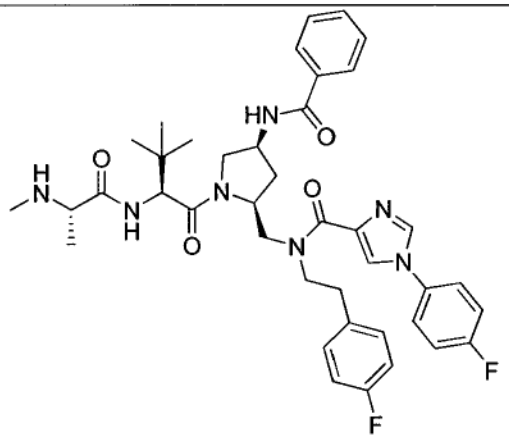
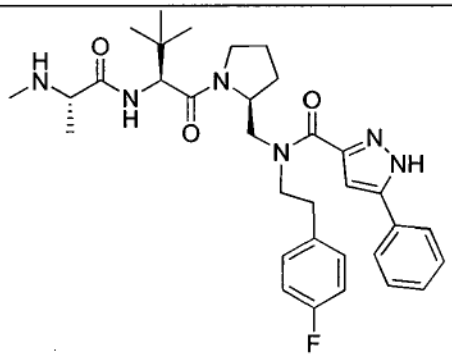
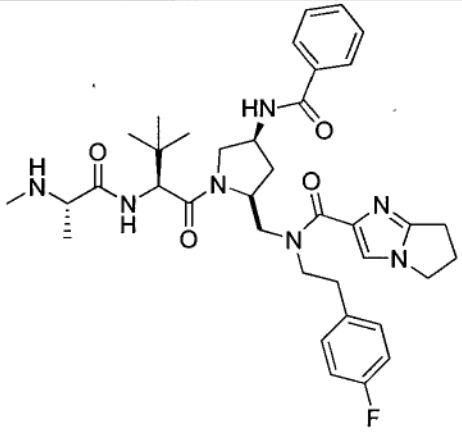
Cmpd #	Structure	MS
72		M+1=611.5
73		M+1=531.5
74		M+1=586.4

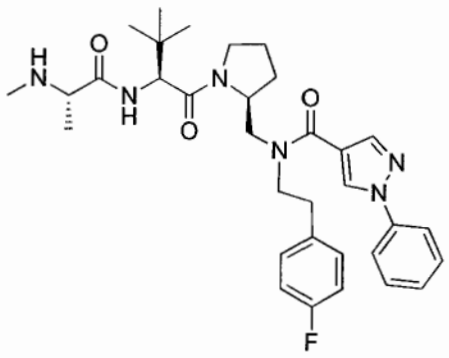
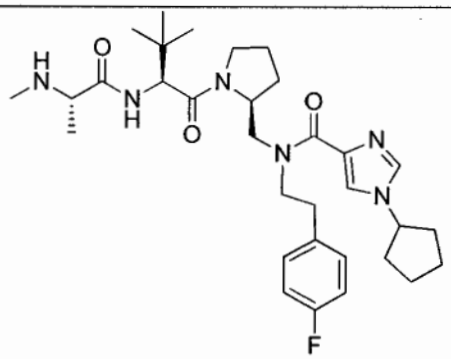
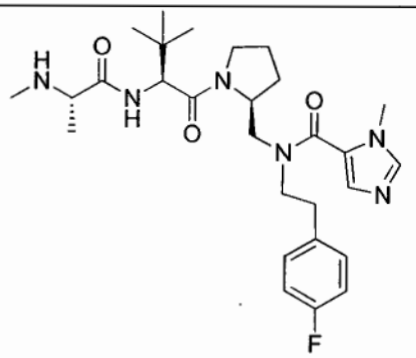
Cmpd #	Structure	MS
75		M+1=557.5
76		M+1=545.5
77		M+1=594.4

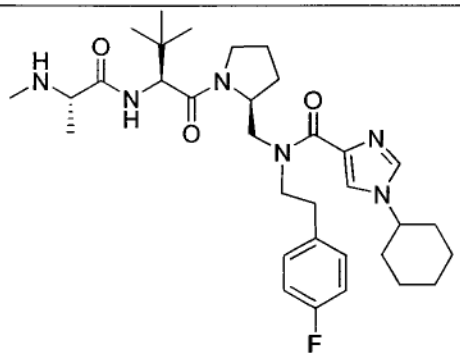
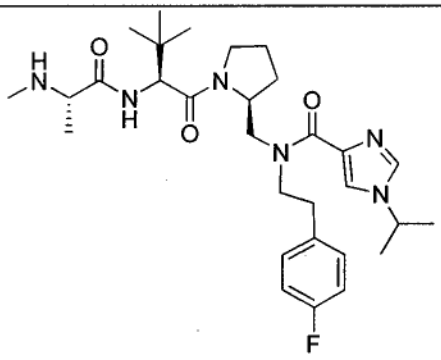
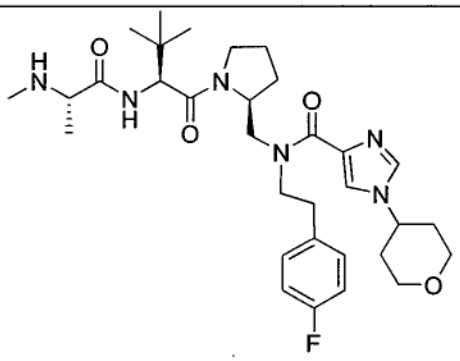
Cmpd #	Structure	MS
78		M+1=581.4
79		M+1=625.5
80		M+1=600.5

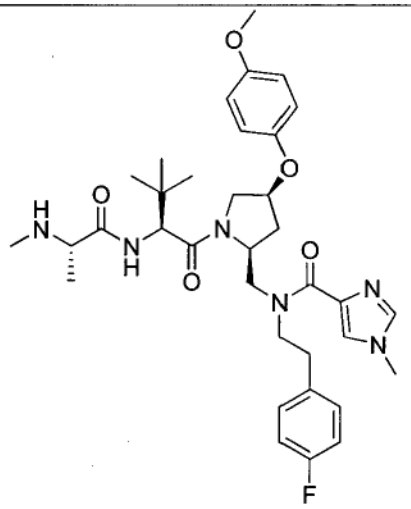
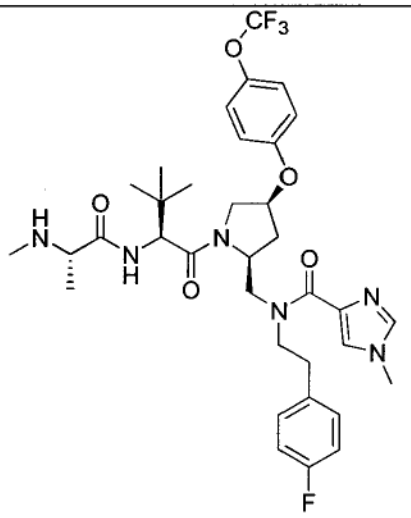
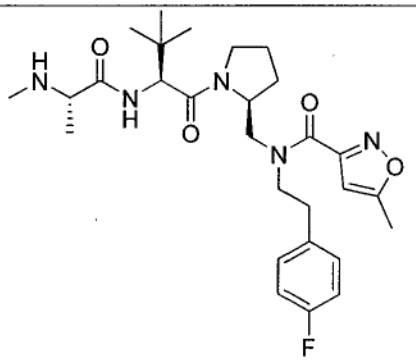
Cmpd #	Structure	MS
81		M+1=571.5
82		M+1=639.5
83		M+1=665.5

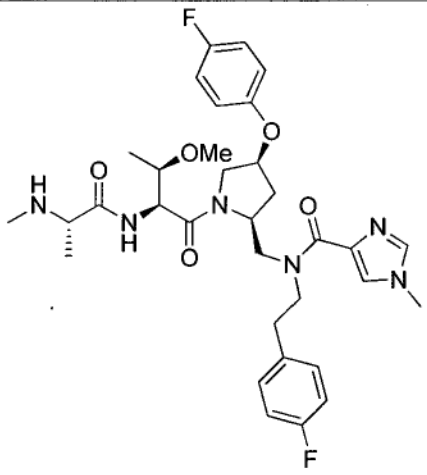
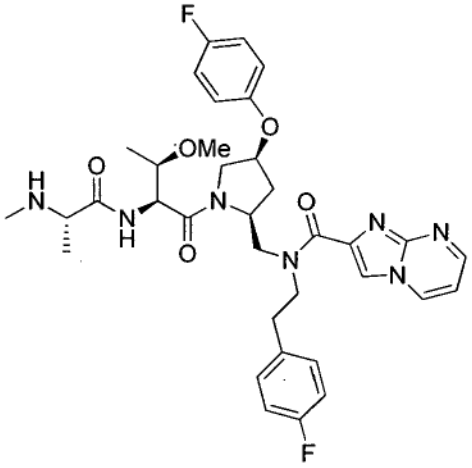
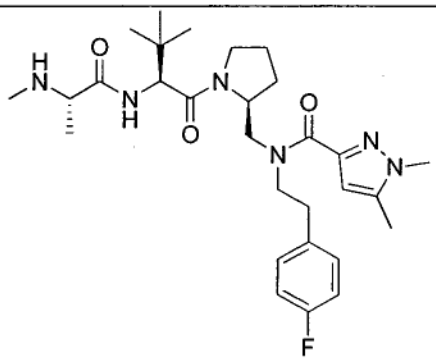
Cmpd #	Structure	MS
84		M+1=675.5
85		M+1=531.5
86		M+1=648.5

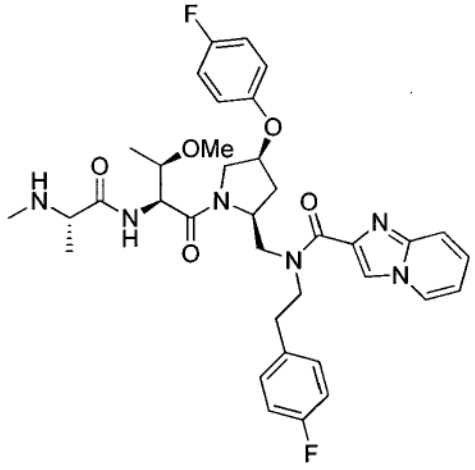
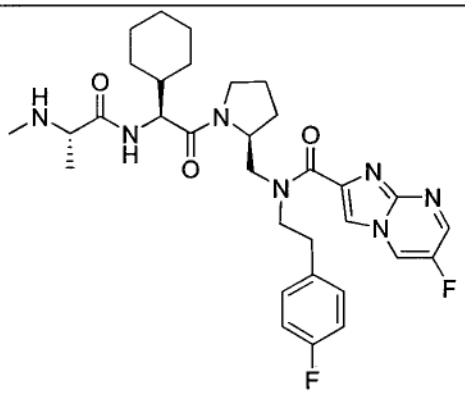
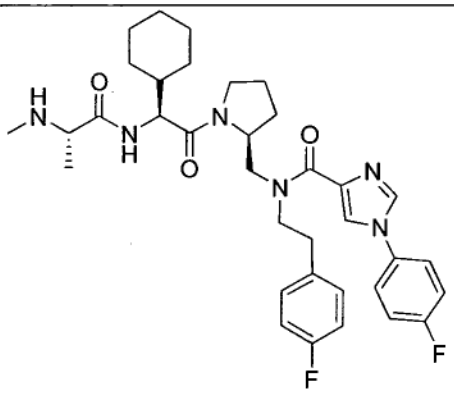
Cmpd #	Structure	MS
87		M+1=728.5
88		M+1=591.4
89		M+1=674.5

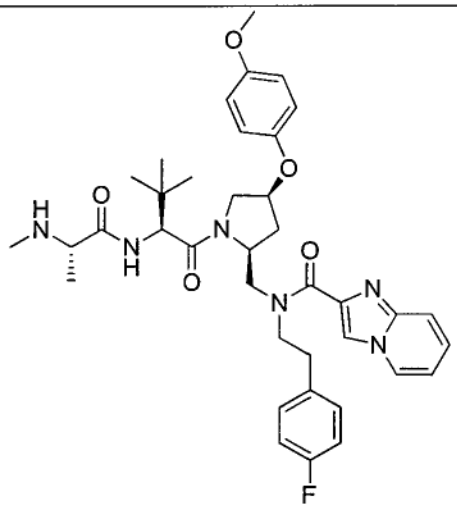
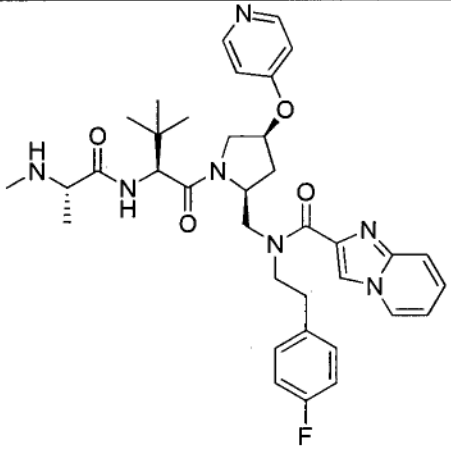
Cmpd #	Structure	MS
90		M+1=591.4
91		M+1=583.5
92		M+1=529.4

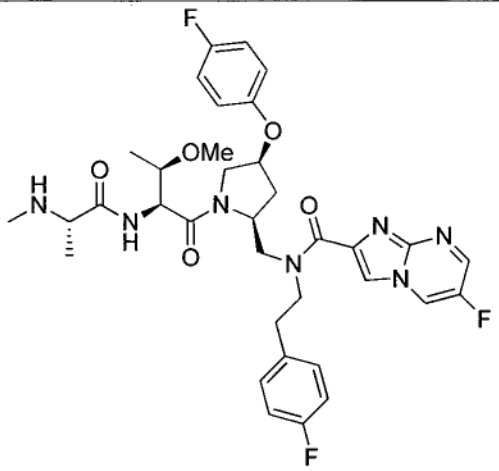
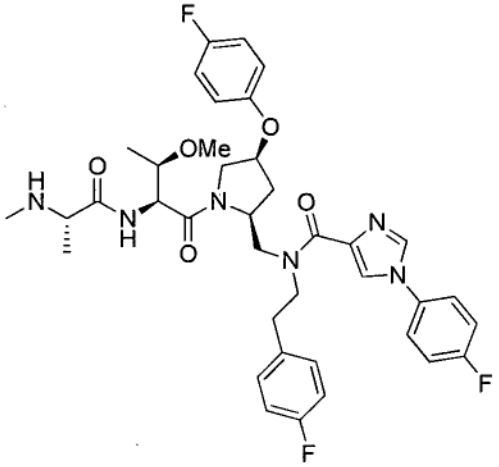
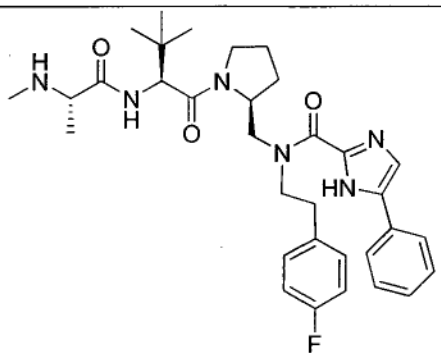
Cmpd #	Structure	MS
93		M+1=597.4
94		M+1=557.4
95		M+1=599.4

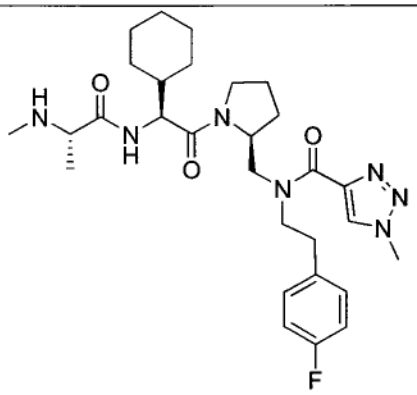
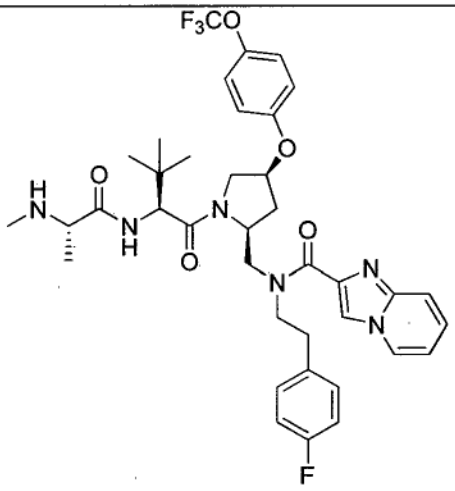
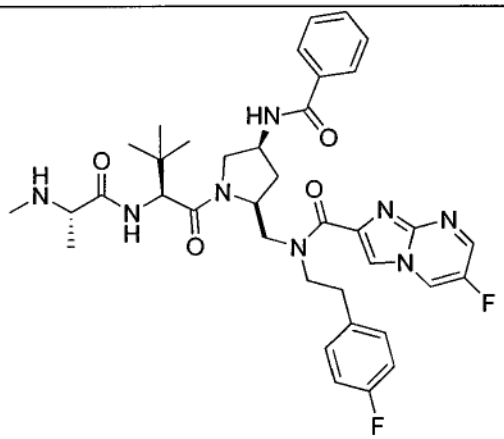
Cmpd #	Structure	MS
96		M+1=651.4
97		M+1=705.4
98		M+1=530.3

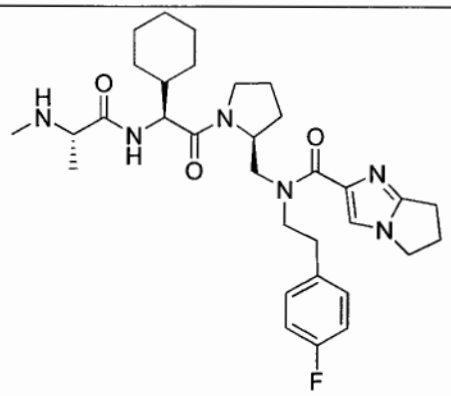
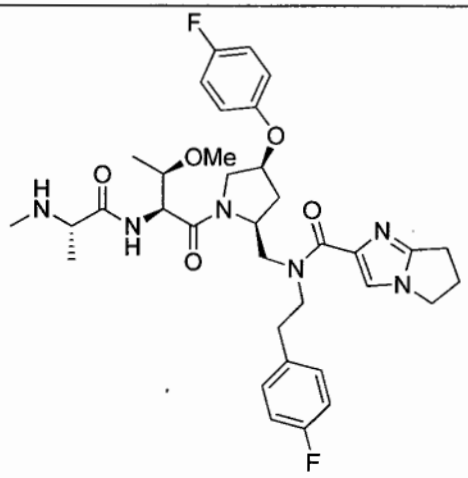
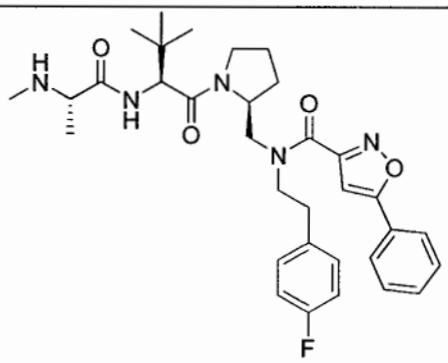
Cmpd #	Structure	MS
99		M+1=641.4
100		M+1=678.5
101		M+1=543.4

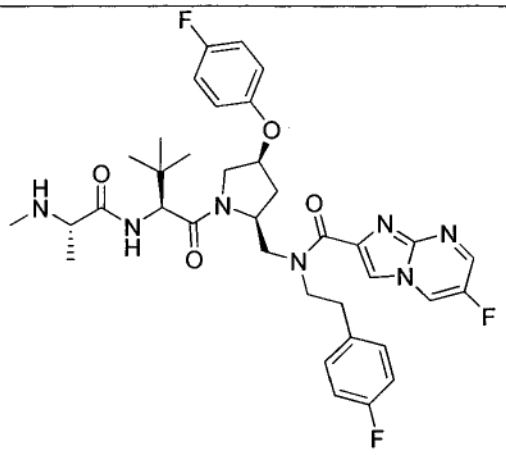
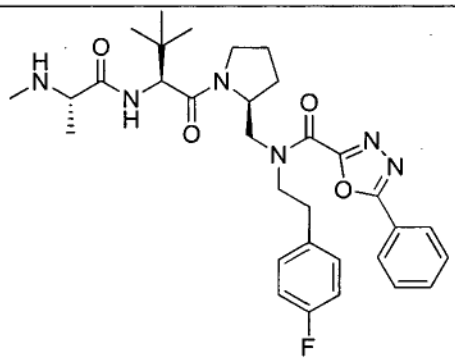
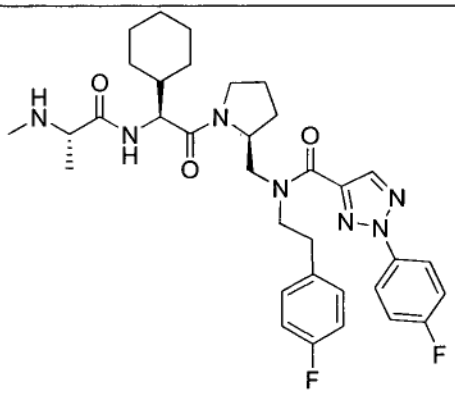
Cmpd #	Structure	MS
102		M+1=677.5
103		M+1=610.3
104		M+1=635.3

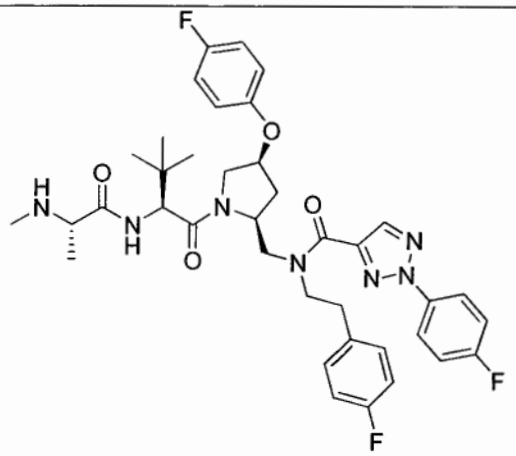
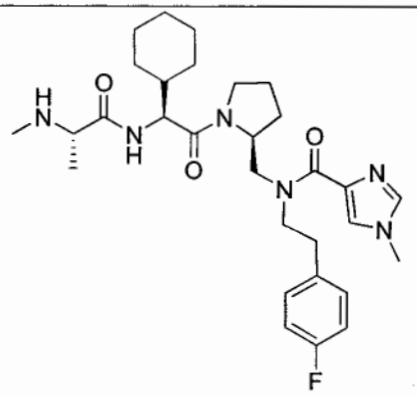
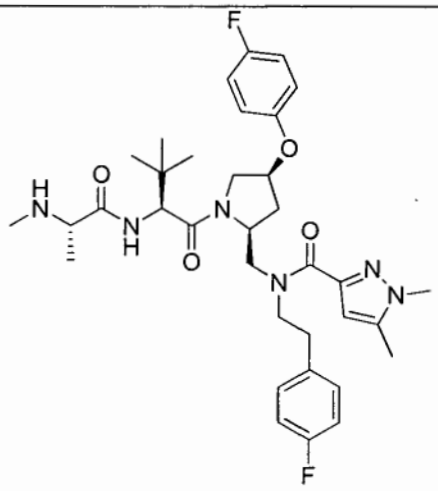
Cmpd #	Structure	MS
105		M+1=687.1
106		M+1=658.1

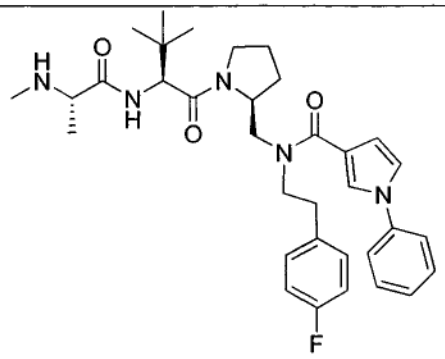
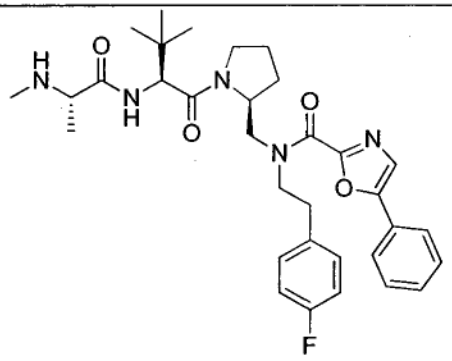
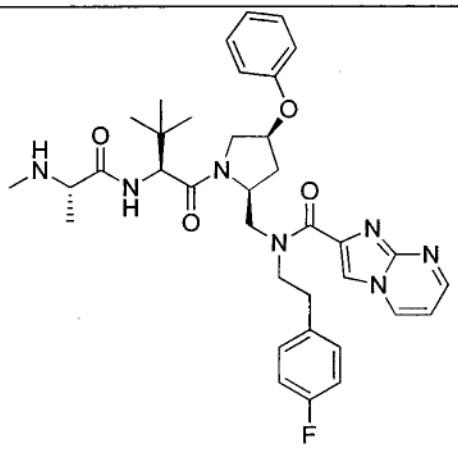
Cmpd #	Structure	MS
107		M+1=696.3
108		M+1=721.1
109		M+1=591.2

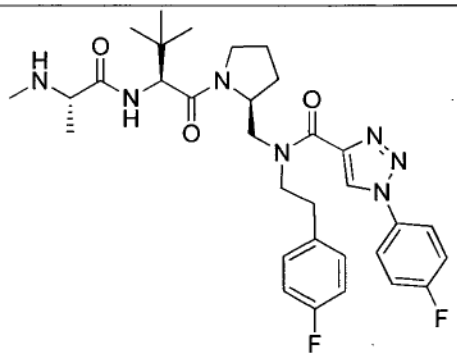
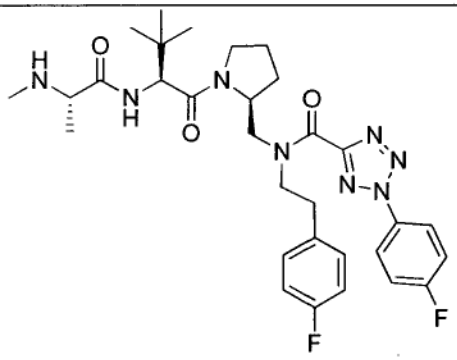
Cmpd #	Structure	MS
110		M+1=556.3
111		M+1=741.3
112		M+1=703.1

Cmpd #	Structure	MS
113		M+1=581.3
114		M+1=667.1
115		M+1=592.1

Cmpd #	Structure	MS
116		M+1=694.0
117		M+1=593.2
118		M+1=636.2

Cmpd #	Structure	MS
119		M+1=720.1
120		M+1=555.3
121		M+1=653.1

Cmpd #	Structure	MS
122		M+1=590.2
123		M+1=592.2
124		M+1=658.5

Cmpd #	Structure	MS
125		M+1=610.1
126		M+1=611.1

EXAMPLE 9

[0466] The following example illustrates the use of a compound of Formula 1 or salt thereof.

Fluorescence Polarization-Based Competition Assay

[0467] Binding of compounds of Formula 1 to target BIR domains was measured using a fluorescence-polarization (FP) assay. Fluorescence-polarization was evaluated using a GENios microplate reader (TECAN) Pro instrument with the excitation filter set at 485 nm and the emission filter set at 535 nm. The final amount of protein used in the assay corresponds to the amount of protein necessary to obtain 80% of the maximum FP value in a P1 or P2 fluorescent (FITC) probe saturation experiment. The compounds potency (IC_{50}) and selectivity, was assessed in the presence of the amount of target

protein established, fluorescent probe, and a 10 point serial dilution in assay buffer (50mM Hepes, pH 7.5, 250 mcg/mL γ-globulin, 2mM DTT, 1% DMSO) of the selected compounds.

[0468] Assays were carried out in duplicate using untreated black 96-well plates (Corning #3915) and a total volume of 100 microliters, containing 25 microliters of fluorescent probe at a final concentration of 2 nM in assay buffer, 25 microliters of diluted compound, 25 microliters of BIR protein into assay buffer and 25 microliters of assay buffer. Buffer only (blank) or probe only in buffer (G-factor) were used as controls for calibration. The plate was incubated at room temperature in the dark for one hour and FP values in millipolarization units (mP) were recorded using Genios Pro FP reader.

[0469] The measured FP values (mP) were then plotted against compound concentration, and IC_{50} values were calculated based on a sigmoidal dose-response (variable-slope) curve fit using GraphPad Prism version 4.02 for Windows, GraphPad Software, San Diego California USA and/or CambridgeSoft BioAssay Enterprise Version 10.1. The IC_{50} value is the concentration of test compound at which 50% of the tracer was displaced. k_i values were derived from the calculated IC_{50} values as described above and according to the equation described in Nikolovska-Coleska, Z. (2004) *Anal Biochem 332*, 261-273. The results are presented in Table 2, wherein A = less than 25 nM; B = less than 250 nM; C = less than 1000 nM; and D = greater than 1000 nM. Compounds aree identified in Table 3 according to the compound numbers presented in Table 1. As the results show, compounds of Formula 1 demonstrating good binding affinity.

Table 2

Cpd No.	XIAP k_i (nM)	c-IAP1 k_i (nM)	cIAP2 k_i (nM)	Cpd No.	XIAP k_i (nM)	c-IAP1 k_i (nM)	cIAP2 k_i (nM)
1	C	A	-	44	B	A	A
2	D	B	B	45	A	A	-
3	B	A	-	46	B	A	-
4	C	B	B	47	B	A	A
5	B	A	-	48	B	A	-
6	B	A	A	49	C	A	A
7	B	A	A	50	B	A	A
8	A	A	-	51	B	A	-
9	B	A	-	52	B	A	-
10	B	A	A	53	B	A	A
11	B	A	-	54	B	A	-
12	C	A	B	55	B	A	A
13	B	A	-	56	C	A	A
14	C	A	B	57	C	A	-
15	B	A	-	58	B	A	A
16	B	A	-	59	B	A	A
17	B	A	A	60	C	A	-
18	B	A	A	61	B	A	A
19	B	A	-	62	B	A	-
20	B	A	A	63	B	A	A
21	B	A	-	64	B	A	-
22	B	A	-	65	A	A	A
23	A	A	-	66	B	A	A
24	B	A	-	67	B	A	A
25	B	A	-	68	B	A	A
26	B	A	-	69	B	A	A
27	B	A	-	70	C	A	B
28	B	A	-	71	C	A	A
29	C	A	-	72	B	A	A
30	B	A	-	73	B	A	A
31	B	A	-	74	B	A	A
32	B	A	A	75	B	A	A
33	B	A	-	76	B	A	A
34	B	A	-	77	B	A	A
35	A	A	-	78	B	A	A
36	B	A	A	79	B	A	A
37	B	A	A	80	B	A	A
38	-	-	-	81	B	A	A
39	B	A	-	82	A	A	A
40	B	A	A	83	A	A	A
41	B	A	-	84	A	A	A
42	B	A	-	85	C	A	A
43	B	A	-	86	B	A	A

Cpd No.	XIAP k_i (nM)	c-IAP1 k_i (nM)	cIAP2 k_i (nM)	Cpd No.	XIAP k_i (nM)	c-IAP1 k_i (nM)	cIAP2 k_i (nM)
87	B	A	A	107	B	A	A
88	B	A	A	108	B	A	A
89	B	A	A	109	B	A	A
90	B	A	A	110	B	A	A
91	B	A	A	111	A	A	A
92	C	A	B	112	B	A	A
93	B	A	A	113	B	A	A
94	B	A	A	114	A	A	A
95	B	A	A	115	B	A	A
96	B	A	A	116	A	A	A
97	B	A	A	117	C	A	A
98	B	A	A	118	-	-	-
99	B	A	A	119	-	-	-
100	B	A	A	120	B	A	A
101	-	-	-	121	-	-	-
102	B	A	A	122	B	A	A
103	B	A	A	123	B	A	A
104	B	A	A	124	B	A	A
105	A	A	A	125	B	A	A
106	B	A	A	126	B	A	A

Cell Culture and Cell Death Assays

[0470] Colorectal carcinoma HCT116 cells were cultured as monolayers in 96 well plates at a density of 2000 cells per well in McCoy's 5a medium (HyClone) supplemented with 2.2 g/L sodium bicarbonate , 10% FBS ($HyClo_{ne}$) and 1% penicillin/streptomycin (HyClone). 24 hours later, triplicate wells were treated with HGS ETR1 (30 ng/ml) in combination wit_h compound. Cells were incubated in the presence of compound and HGS agonistic Trail receptor antibod*y, ETR1 (Mapatum*amab, 30ng/ml) for 72 hours. Metabolic viability of remaining cells was assessed by MTT (thiazolyl blue tetrazolium bromide, Sigma) assay.

[0471] EC_{50} values (50% cell survival in the presence of compound as compared to untreated controls) were calculated from survival curves using BioAssay software (CambridgeSoft) and GraphPad Prism (Graph Pad Software Inc.). The results are provided in Table 3, below, wherein A = less than 50 nM; B = less than 250 nM; C= less than 1000 nM; and D = greater than 1000 nM. Compounds aree identified in Table 3

according to the compound numbers presented in Table 1. As the results show, tested compounds of Formula 1 demonstrated strong potency.

Table 3

Compound Number	HCT116+ mapatubimap EC_{50} (nM)	Compound Number	HCT116+ mapatubimap EC_{50} (nM)
1	B	41	C
2	C	42	C
3	C	43	B
4	D	44	B
5	A	45	A
6	B	46	C
7	B	47	B
8	B	48	A
9	B	49	B
10	A	50	B
11	B	51	B
12	B	52	B
13	B	53	A
14	B	54	A
15	B	55	D
16	C	56	C
17	A	57	C
18	A	58	A
19	A	59	C
20	A	60	C
21	C	61	B
22	B	62	C
23	B	63	A
24	B	64	B
26	C	65	A
27	C	66	A
28	C	67	A
29	D	68	B
30	B	69	B
31	B	70	C
32	B	71	C
33	B	72	A
34	B	73	B
35	B	74	A
36	B	75	A
37	D	76	A
38	B	77	A
39	B	78	A
40	A	79	A

Compound Number	HCT116+ mapatubimap EC_{50} (nM)	Compound Number	HCT116+ mapatubimap EC_{50} (nM)
80	A	103	A
81	A	104	A
82	A	105	A
83	A	106	A
84	A	107	A
85	A	108	B
86	A	109	A
87	B	110	A
88	B	111	A
89	A	112	B
90	B	113	A
91	A	114	A
92	B	115	B
93	A	116	A
94	A	117	B
95	A	118	B
96	A	119	B
97	A	120	A
98	B	121	A
99	A	122	B
100	B	123	A
101	A	124	A
102	A	--	--

Adjuvant Induced Arthritis (AIA) Model

[0472] Male Lewis rats (Charles River, 125-150 g) were habituated to the animal facility for one week prior to the day of challenge with synthetic adjuvant lipoidal amine (LA). On experimental day (d)=0, when the mean body weight (BW) of the cohort was 165-200 g, rats were anesthetized with isoflurane, the lower back was shaved and wiped with alcohol and then 0.1 mL of LA dissolved in Complete Freunds Adjuvant (CFA) (50 mg/mL LA in CFA) was injected subcutaneously at the base of the tail. On experimental d=7, BW was recorded for each animal and the ankle width measured using electronic calipers (QuantuMike micrometer; Mitutoyo). Balanced groups were formed based on ankle width and treatment was initiated. Animals were treated orally (PO) once a day (QD) or twice a day (BID) with test compounds 5, 6, 7, or 10 (see Table 1) at 10, 30 & 60 mg/kg, PO, dexamethasone (0.15 mg/kg, PO), or vehicle alone (5 mL/kg, PO) from d=7 until d=14, with BW and ankle width recorded daily.

[0473] The tested compounds at the highest doses demonstrated greater than 50% reduction in paw swelling as compared to controls. Some compounds showed similar efficacy at the lower tested dosages. These results indicate the effectiveness of the tested compounds for this indication.

Collagen-Induced Arhritis (CIA): Semi-Therapeutic Dosing

[0474] Male, B10 RIII mice (7-8 weeks old, Jackson Labs) were habituated to the animal facility for 1 wk. They were then challenged with collagen, which was emulsified in CFA supplemented with mycobacterium tuberculosis, on experimental day 0 and 15. From experimental day 12 onwards animals were dosed with test compound 6, 7, or 10 (see Table 1) at 3, 10, 30 mg/kg (PO BID), or dexamethasone (0.2 mg/kg, positive control). Clinical arthritis severity was assessed using an established scoring system for the duration of the study.

[0475] The tested compounds demonstrated greater than 50% reduction in Arthritc score at the highest doses. Some compounds showed similar efficacy at the lower tested dosages. These results indicate the effectiveness of the tested compounds for this indication.

CIA: Therapeutic Dosing

[0476] Male, B10 RIII mice (7-8 weeks old, Jackson Labs) were habituated to the animal facility for 1 wk. They were then challenged with collagen, which was emulsified in CFA supplemented with mycobacterium tuberculosis, on experimental day 0 and 15. From experimental day 15 onwards animals were dosed with test compound 10, 40, 45, or 66 (see Table 1) at 3, 10, 30 mg/kg (PO BID), or dexamethasone (0.2 mg/kg, positive control). Clinical arthritis severity was assessed using an established scoring system for the duration of the study.

[0477] The tested compounds reduced the mean arthritic score in the treated animals as compared to vehicle (water) control at the highest doses.

MDA-MB-231 Compound + Mapatubamab Xenograft Study

[0478] Female, nude mice received 5 X 10^6 MDA-MB-231 (CD) cells subcutaneously in the right flank (0.1 mL volume) on day 0 of the experiment. When the average tumor size reached ~100 mm^3, groups were formed using a balanced design based on tumor

size, and dosing was initiated. Test compound 10 or 7 (see Table 1) (or corresponding vehicle alone) was given daily, and mapatubamab (or corresponding vehicle alone) was given twice weekly. Tumor size and body weight were measured twice weekly throughout the study.

[0479] Compounds 10 and 7 when dosed at (30 and 60 mg/kg, PO, QD) in combination with mapatubamab (10 mg/kg, IV) reduced tumor volume by 50% as compared to control, demonstrating combinational efficacy between the tested compounds and mapatubamab.

MDA-MB-231 Compound plus Taxol Xenograft Study

[0480] Female, CD-1, nude mice received 5 X 10^6 MDA-MB-231(CD) cells subcutaneously in the right flank (0.1 mL volume, cells suspended in serum-free media) on day 1 of the experiment. When the average tumor size reached ~100 mm^3, groups were formed using a balanced design based on tumor size. Compounds 10, 7, 20, 40, 45, 58, 63, and 66 were then administered twice weekly at 30 and 100 mg/kg, PO, for two weeks. Taxol (20 mg/kg, IP) was co-administered with the initial treatment with test compound, and then once weekly for the duration of the study. Tumor size and body weight were measured twice weekly.

[0481] The tested compounds achieved stasis or tumor regression in the xenograft study.

Maximum tolerated Dose (MTD) Study:

[0482] Male, CD-1 mice (20-25 g on arrival, Charles River) were habituated to the animal facility for four days and then randomly assigned to treatment groups. Animals were dosed by oral gavage twice weekly, for a total of six treatments, with compounds 63, 58, 45, 10, 20, 40, and 66 at 15, 70, or 350 mg/kg in water, PO, or water alone (5 mL/kg, PO). Body weight and general health were monitored throughout the experiment. Twenty four hours after the last dose (day 19), animals were anesthetized with isoflurane, and blood was collected for serum biochemistry. Serum biochemical parameters were restricted to random glucose, urea, creatinine, total protein, albumin, globulin, A:G ratio, total bilirubin, AST, ALT, alkaline phosphatase, GGT and amylase. Once exsanguinated the animals underwent full necropsy and organ weights were recorded for brain, heart,

liver, spleen, kidneys, stomach (empty) and intestine (empty, from stomach to cecum). Because some treatments were suppressing weight gain (see below), organ weights were normalized to brain weight, and then expressed as percent change from the vehicle treated control group.

[0483] Mice showed positive growth curves and minimal or no clinical signs as a result of treatment, with slight weight loss observed for compounds 63 and 40 at the 350 mg/kg dose.

Pharmacokinetics (PK) of Compound in Plasma

[0484] CD-1 mice were dosed with compounds 5, 6, 7, 10, 17, 34, 58, 63, 66, 77, 78, 80, 81, 86, 87, 99, and 114 by IV or oral administration. Post-administration, the concentration of drugin the plasma of the treated mice was determined by HPLC-mass spectrometry, and drug concentrations were estimated relative to standard curves of the test compounds. The area under the time-concentration curve (AUC) was determined from the plasma concentrations, and the oral bioavailablity (%F) was calculated.

[0485] Tested compounds of Formula 1 demonstrated good oral bioavailability (19% to 100% F) in CD-1 mice. Additionally, compounds selected from the tested group of compounds demonstrated positive allometric scaling to human equivalent doses.

[0486] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

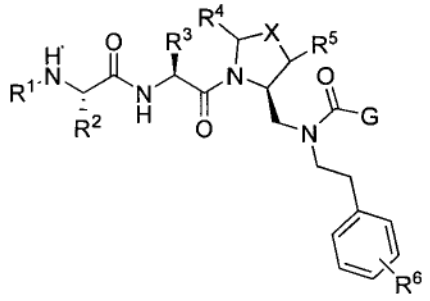
[0487] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is

incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0488] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIMS

1. A compound of Formula 1:



or a salt thereof, wherein

R^1 is H or alkyl;

R^2 is methyl or ethyl;

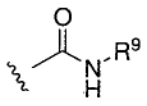
R^3 is alkyl, cycloalkyl, heterocyclyl, heteroaryl, or aryl, any of which can be optionally further substituted with an amino, alkylamino, or alkoxy;

R^4 and R^5 are each, independently, H or alkyl;

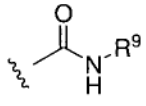
R^6 is H, halogen, or alkoxy;

X is O, S, CH_2, $-(CH_2)_2-$ or CH-R^7, wherein R^7 is NR^8, OR^8, $NC(O)OR^8$, $NHC(O)R^8$ or $NHSO_2R^8$, wherein R^8 is alkyl, cycloalkyl, heterocyclyl, aryl, arylalkyl, or heteroaryl, any of which can be optionally further substituted with an alkyl or halogen;

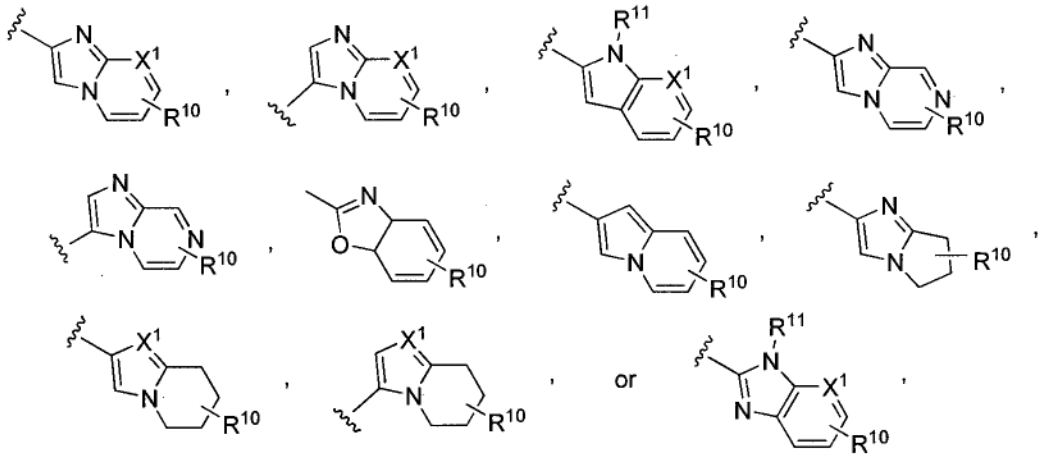
and G is

(1)  , wherein R^9 is substituted or unsubstituted alkyl, cycloalkyl, heterocyclyl, aryl or heteroaryl; or

(2) a substituted or unsubstituted azole or pyrrole ring, optionally fused to a substituted or unsubstituted aryl, heteroaryl, cycloalkyl or heterocyclyl.

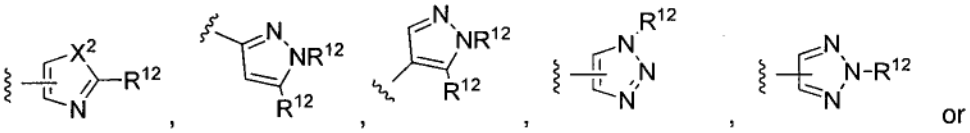
2. The compound of claim 1, wherein G is  , and R^9 is substituted or unsubstituted alkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl.

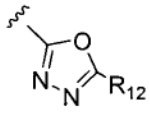
3. The compound of claim 1, wherein G is



and X^1 is CH or N, R^{10} is H, halogen, hydroxyl, alkyl, alkoxy, aryl, amino, or NHC(O)-alkyl, and R^{11} is hydrogen, alkyl, or $NHC(O)CH_3$;

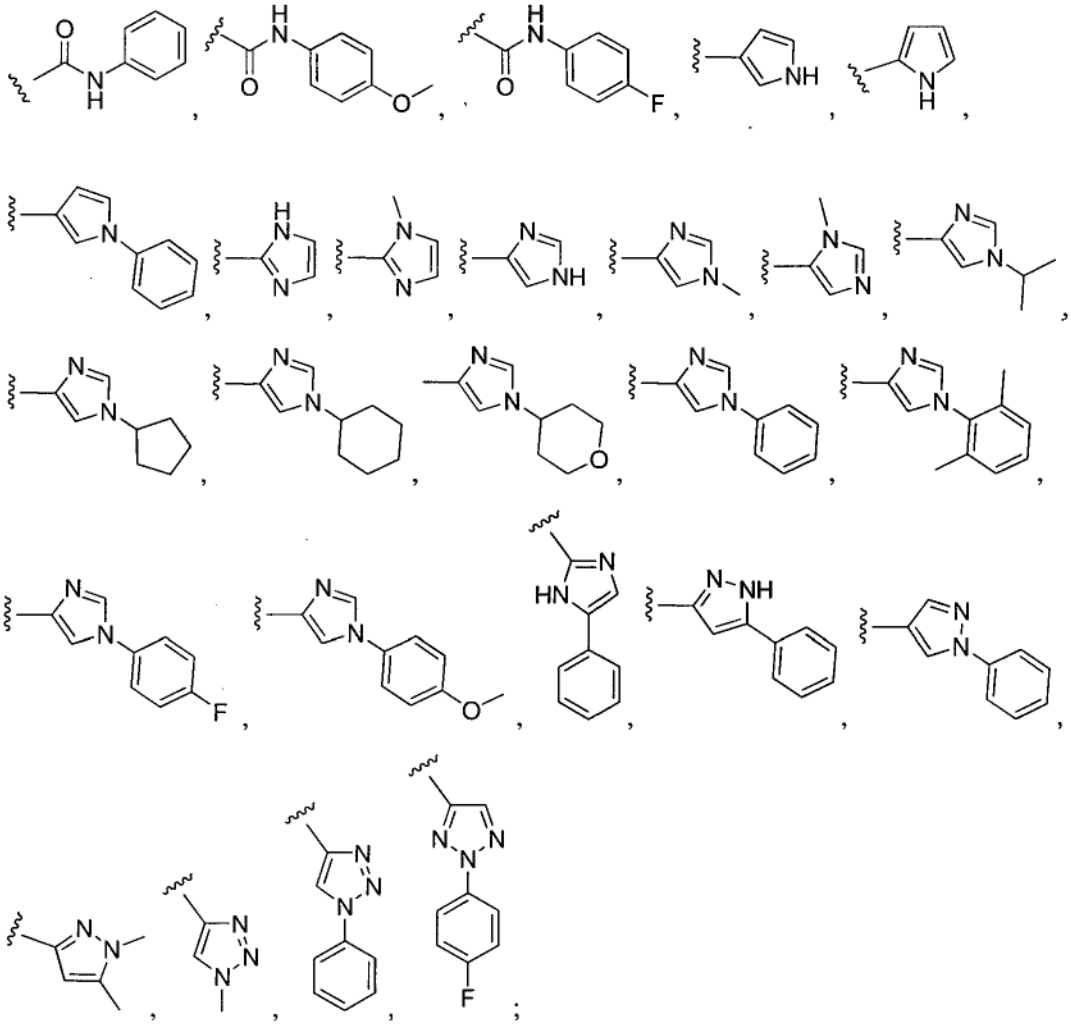
or G is



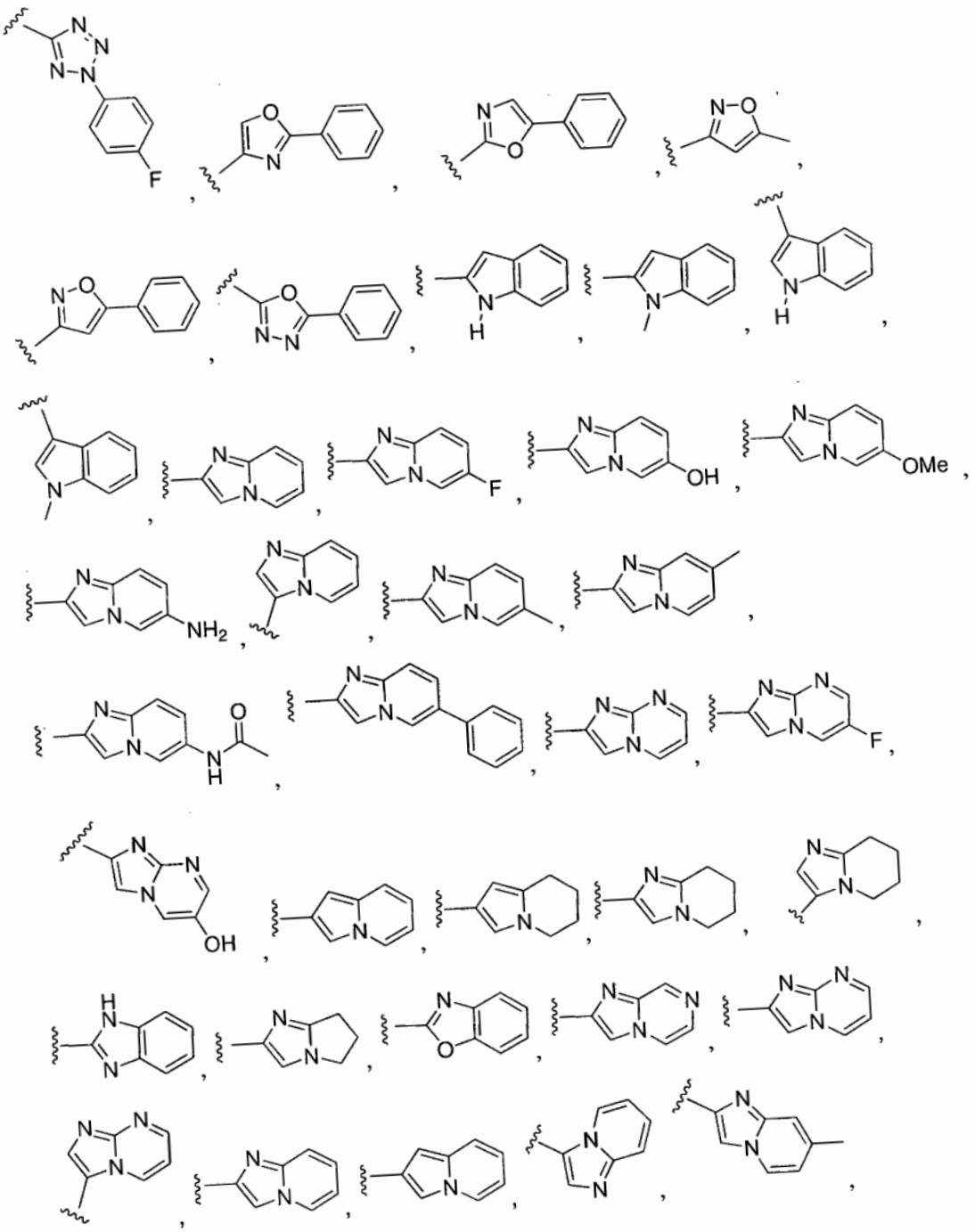


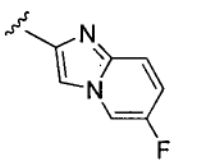
wherein X^2 is NH, NR^{12}, O, or S, and each R^{12} is independently hydrogen, alkyl, cycloalkyl, heterocyclyl, $NHC(O)CH_3$, or phenyl optionally substituted with one or more alkyl, alkoxy, or halogen groups.

4. The compound of claim 1, wherein G is



, wherein R^{11} is $NHC(O)CH_3$ or phenyl;

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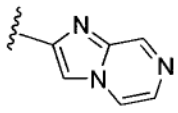
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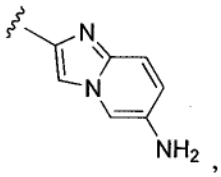
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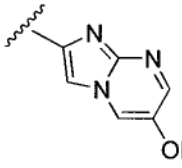
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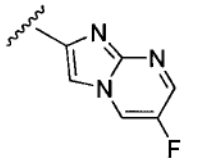
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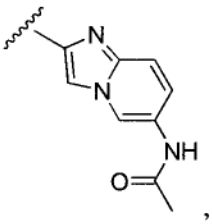
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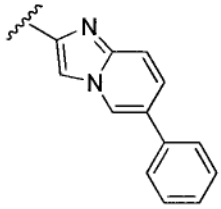
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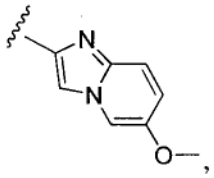
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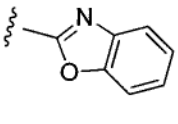
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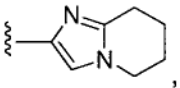
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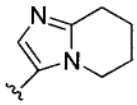
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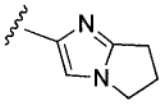
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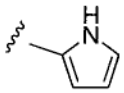
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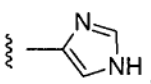
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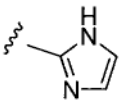
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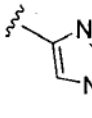
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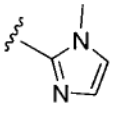
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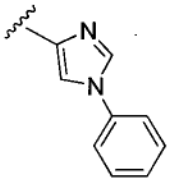
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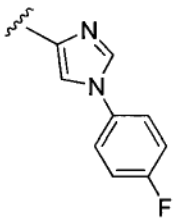
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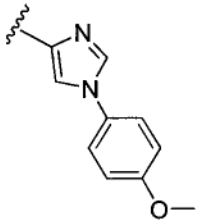
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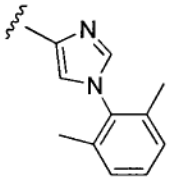
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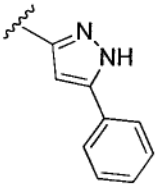
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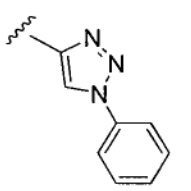
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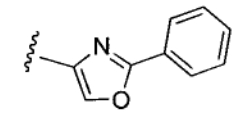
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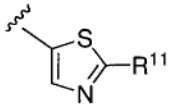
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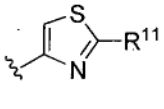
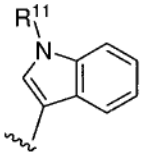
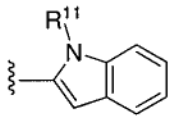
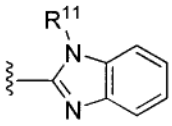

, wherein R^{11} is $NHC(O)CH_3$ or phenyl; or

 ,  , or  , wherein R^{11} is H or alkyl.

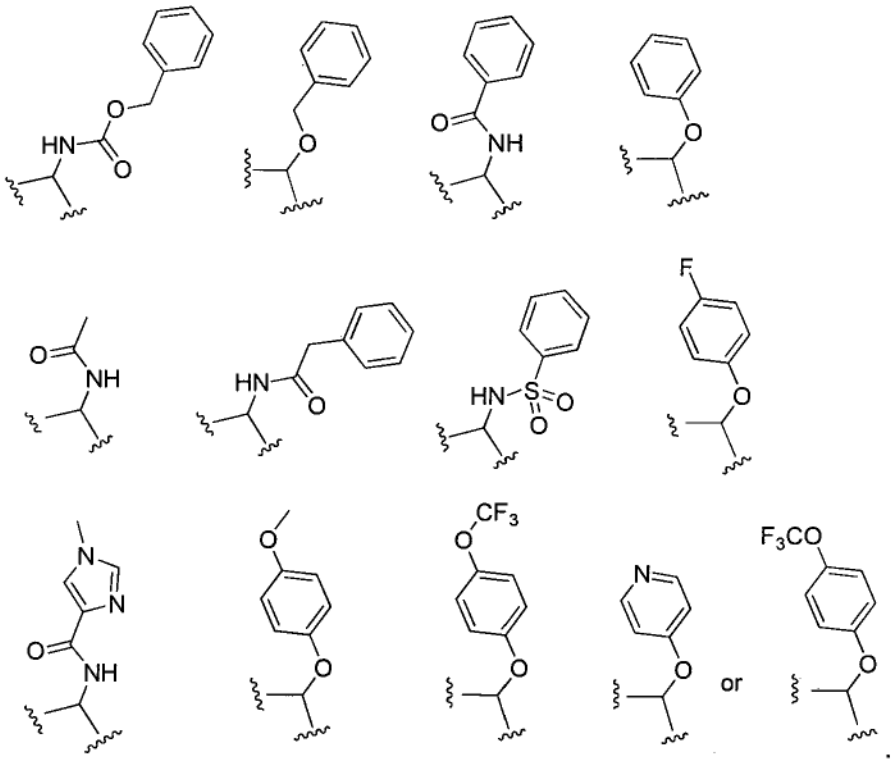
5. The compound of any of claims 1-4, wherein R^6 is H, F, or methoxy.

6. The compound of any of claims 1-5, wherein X is $-CH_2-$.

7. The compound of any of claims 1-5, wherein X is $CH\text{-}NHC(O)R^8$ and R^8 is alkyl, aryl, arylalkyl, alkoxy or heteroaryl, any of which can be optionally further substituted with an alkyl or halogen.

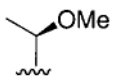
8. The compound of any of claims 1-5, wherein X is $CH\text{-}OR^8$ and R^8 is aryl or arylalkyl, which can be optionally further substituted with halogen.

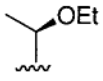
9. The compound of any of claims 1-5, wherein X is:



10. The compound of any of claims 1-9, wherein R^4 and R^5 both are hydrogen.

11. The compound of any of claims 1-10, wherein R^1 and R^2 both are methyl.

12. The compound of any of claims 1-11, wherein R^3 is tert-butyl, cyclohexyl, tetrahydropyranyl, 

, or 

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13. The compound of claim 1, wherein the compound is:

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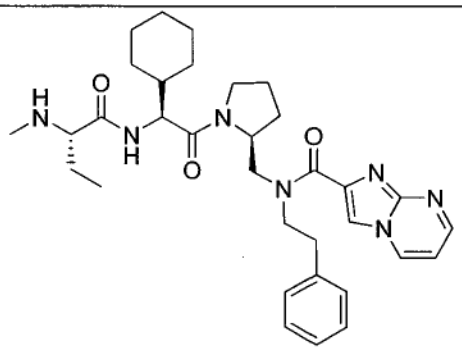
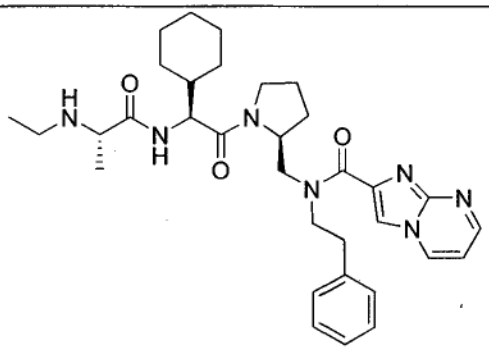
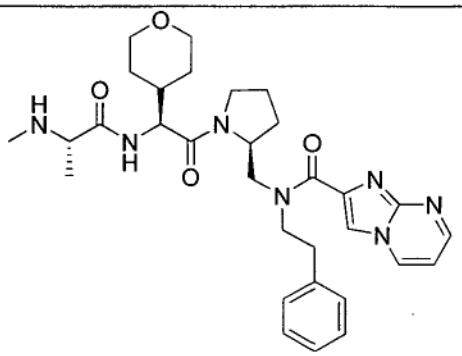
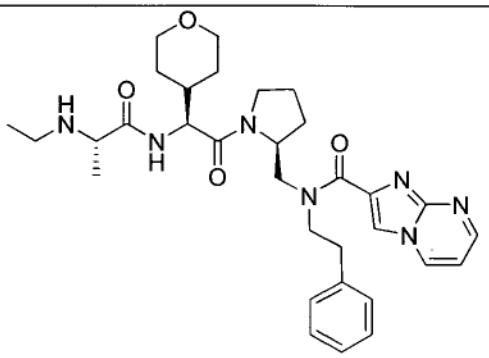
1	
2	
3	
4	

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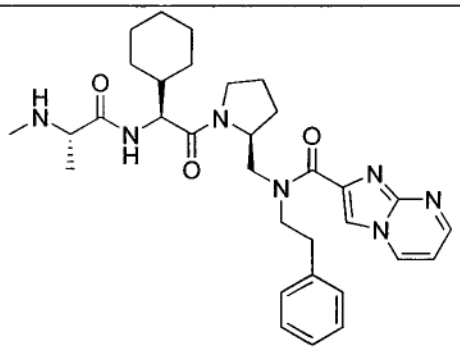
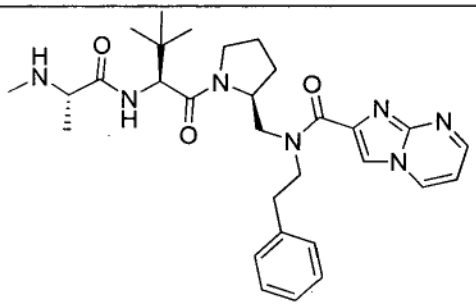
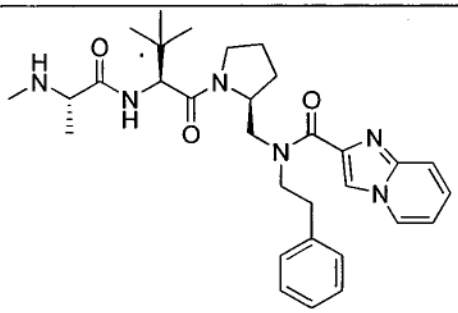
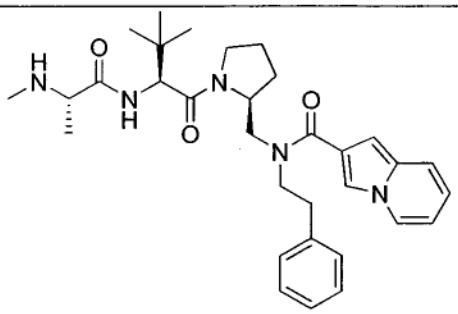
5	
6	
7	
8	

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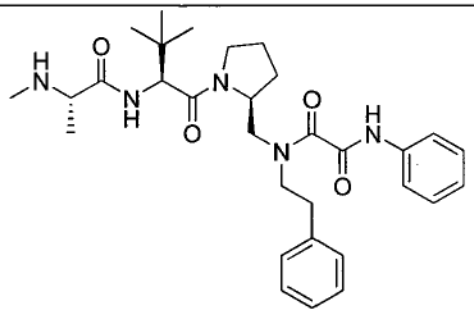
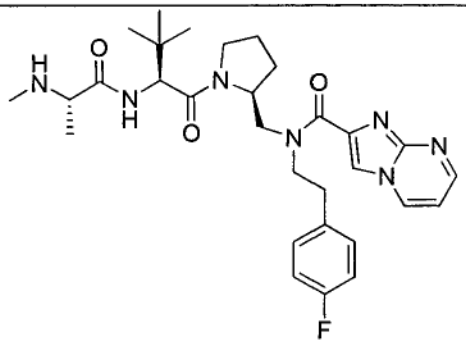
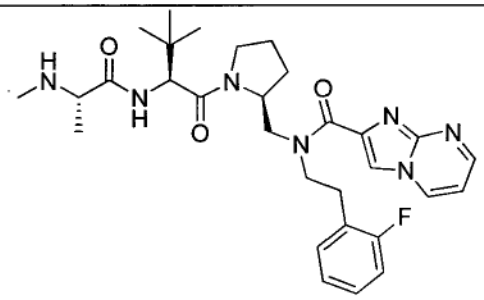
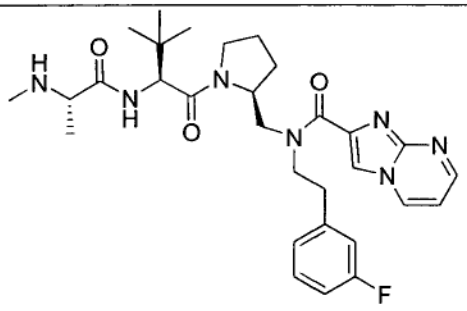
9	
10	
11	
12	

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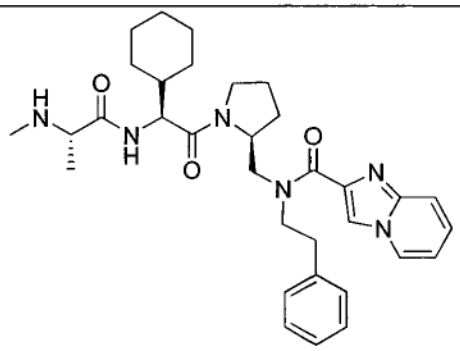
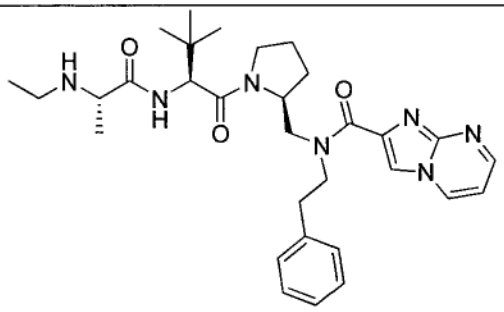
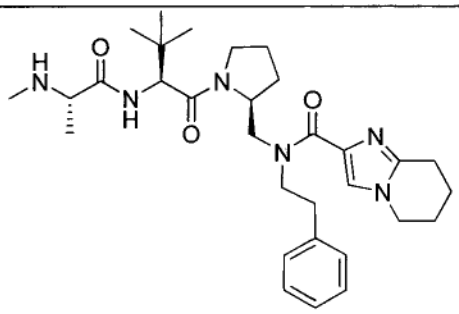
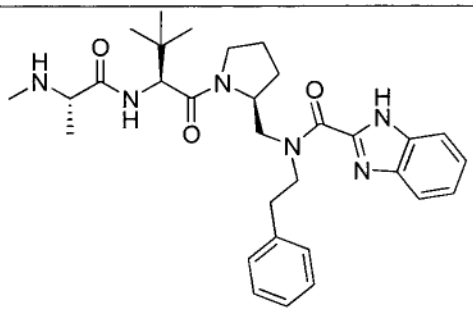
13	
14	
15	
16	

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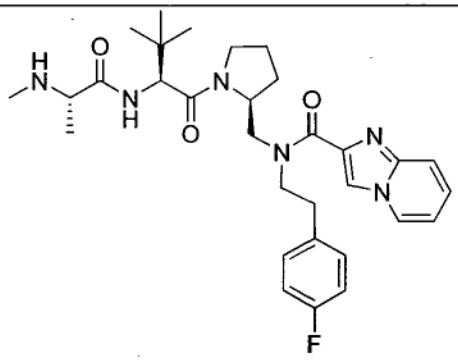
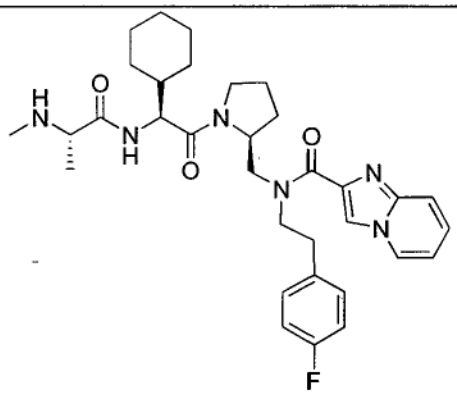
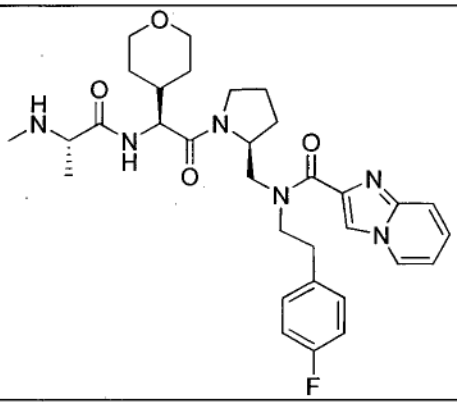
17	
18	
19	

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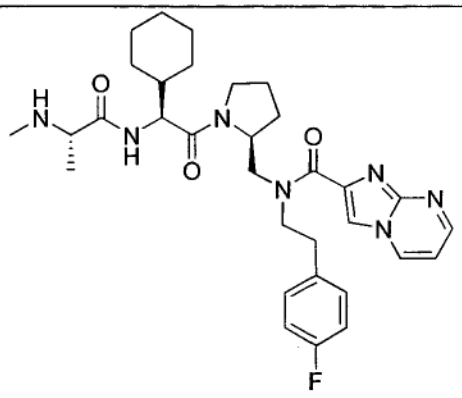
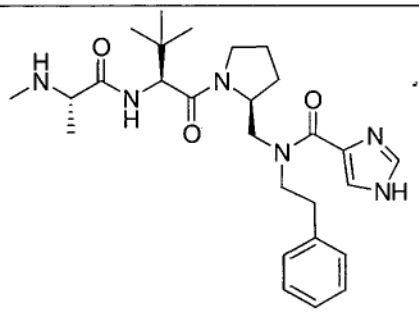
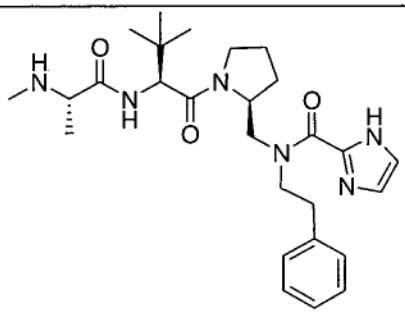
20	
21	
22	

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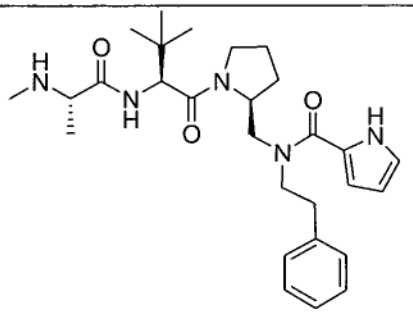
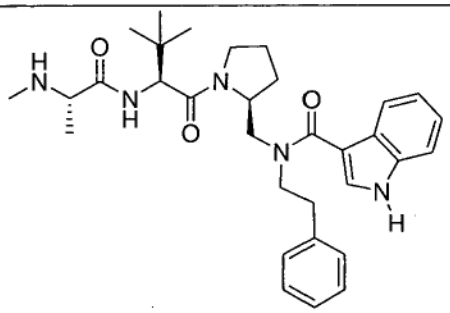
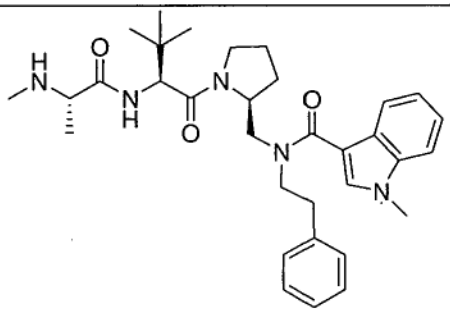
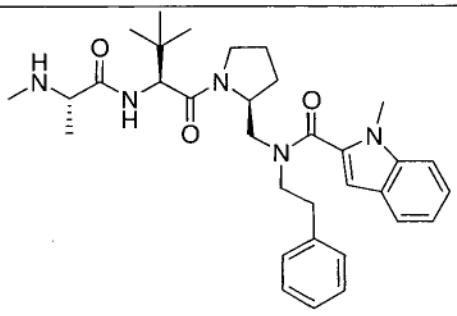
23	
24	
25	
26	

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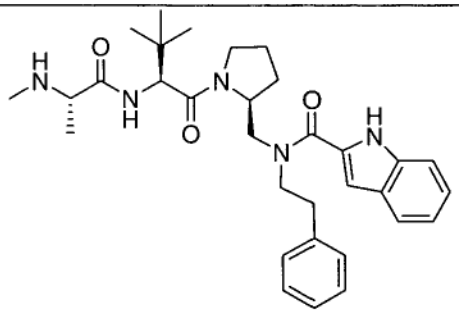
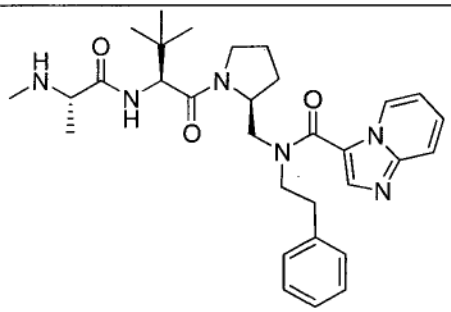
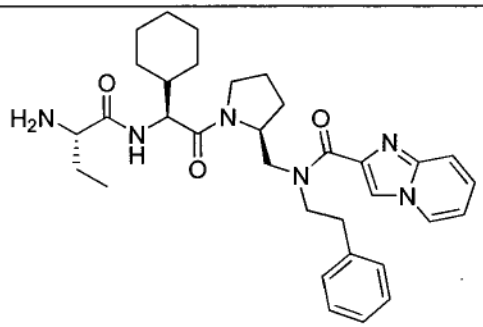
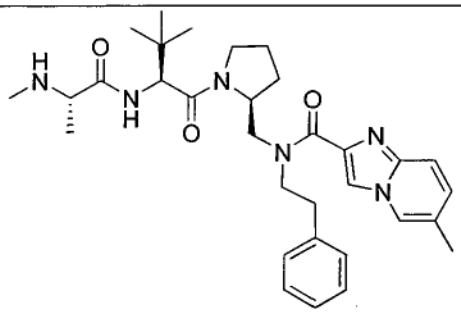
27	
28	
29	
30	

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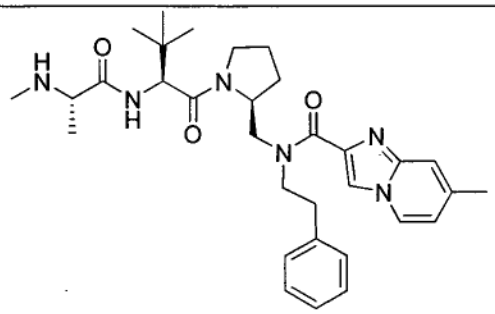
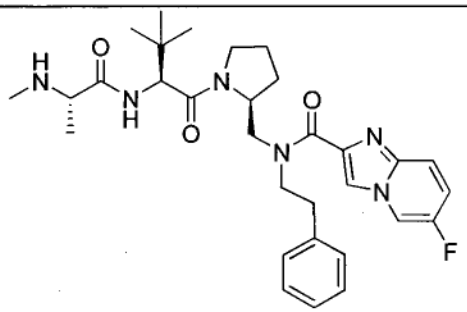
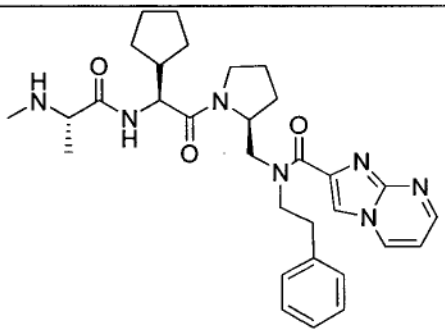
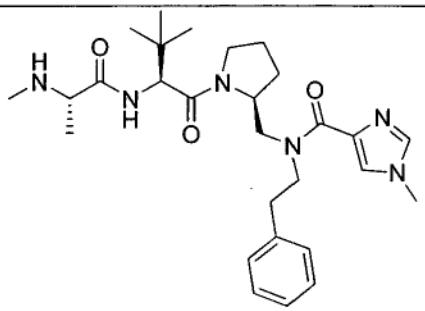
31	
32	
33	
34	

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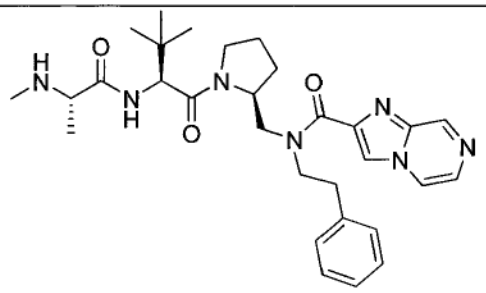
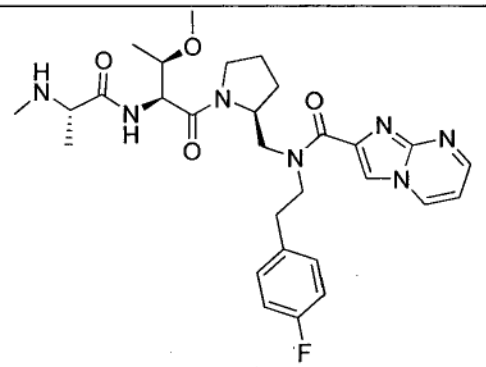
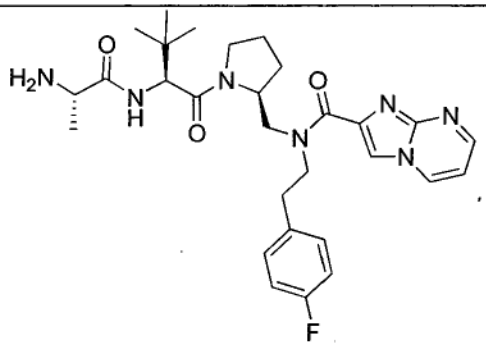
35	
36	
37	

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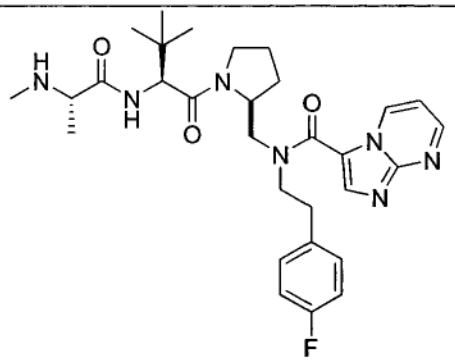
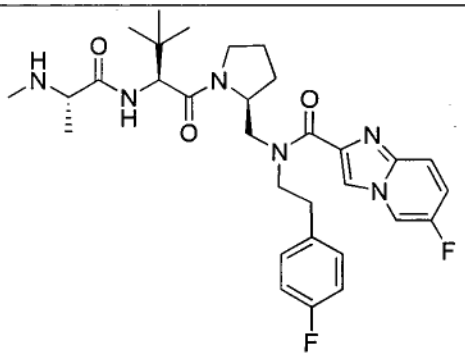
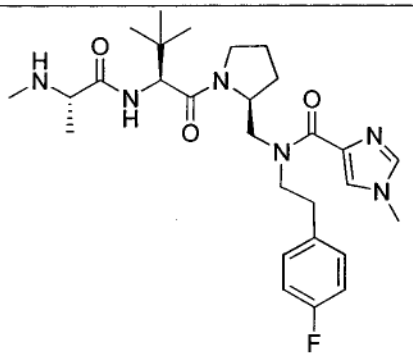
38	
39	
40	

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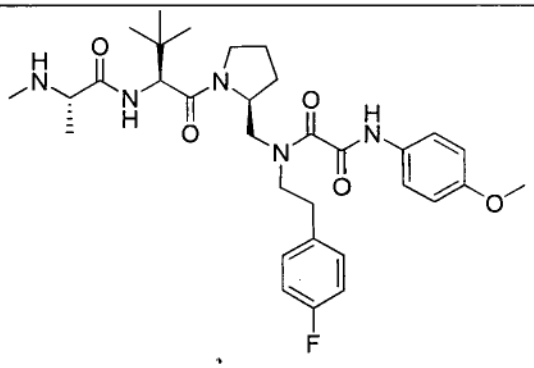
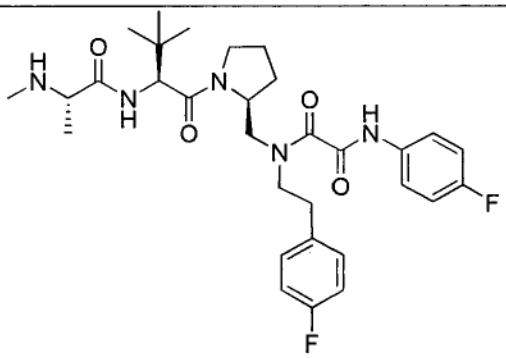
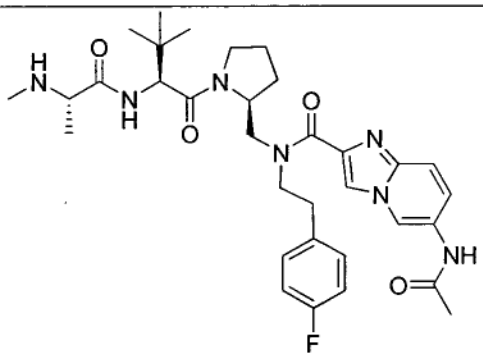
41	
42	
43	

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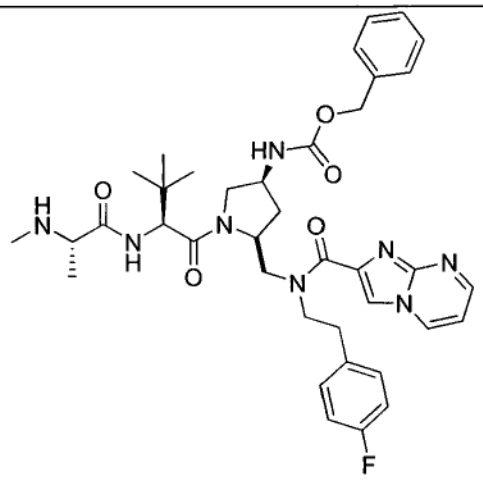
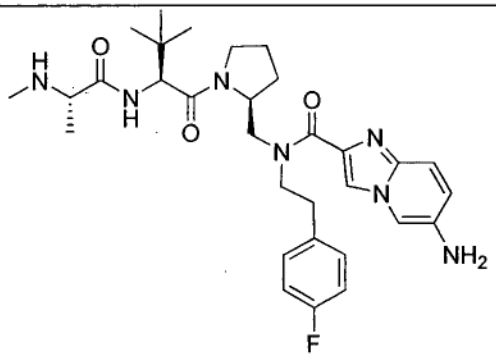
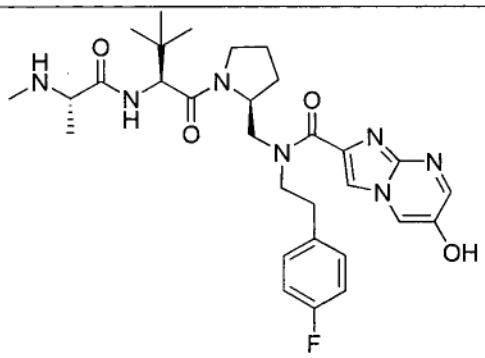
44	
45	
46	

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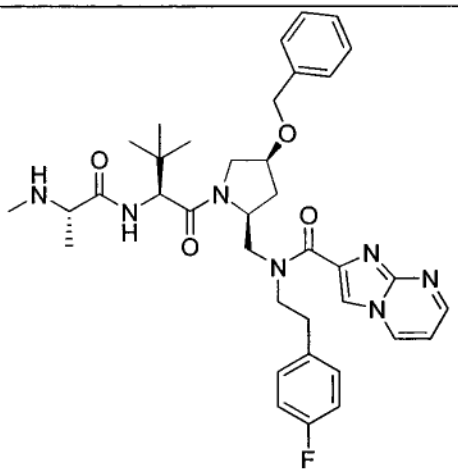
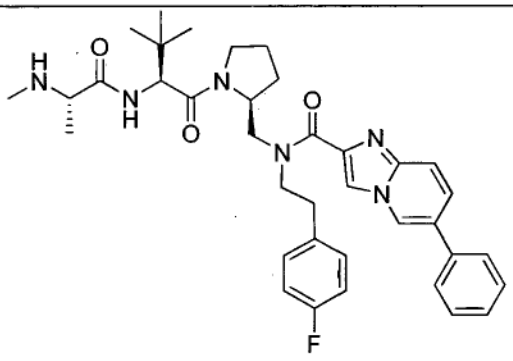
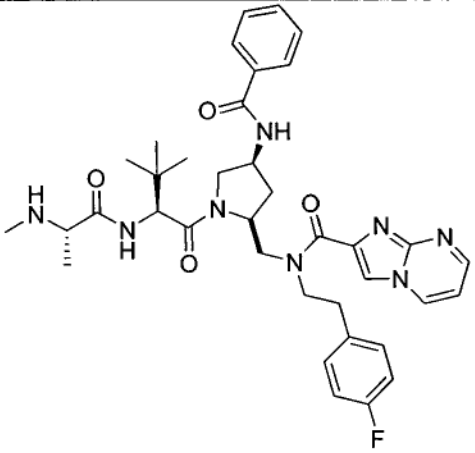
47	
48	
49	

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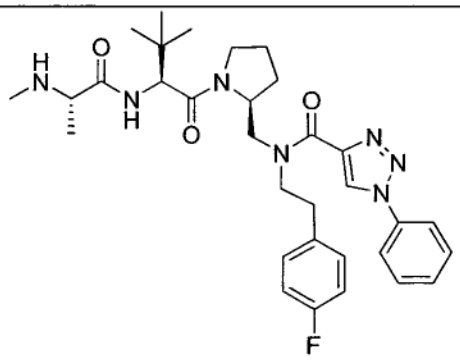
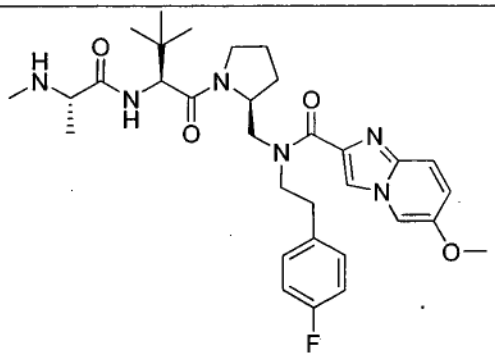
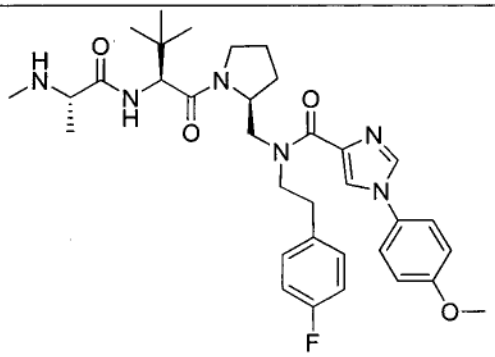
50	
51	
52	

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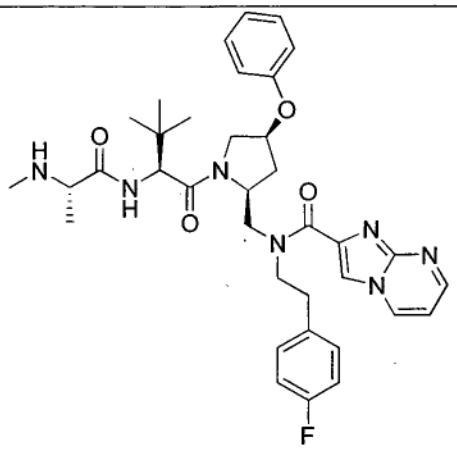
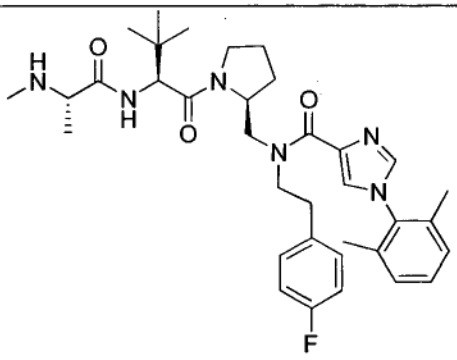
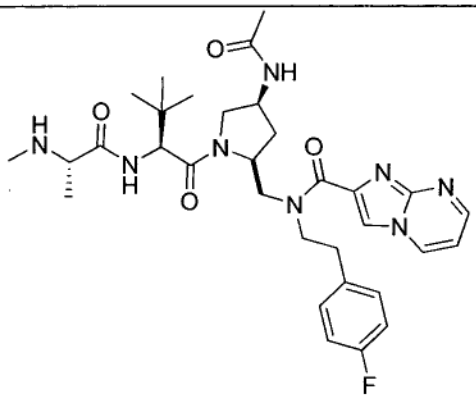
53	
54	
55	

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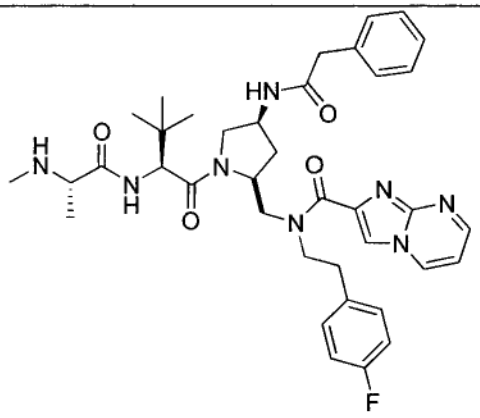
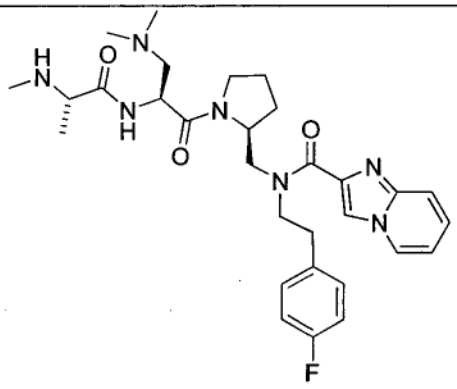
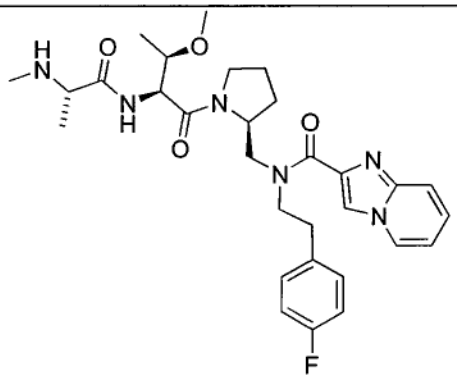
56	
57	
58	

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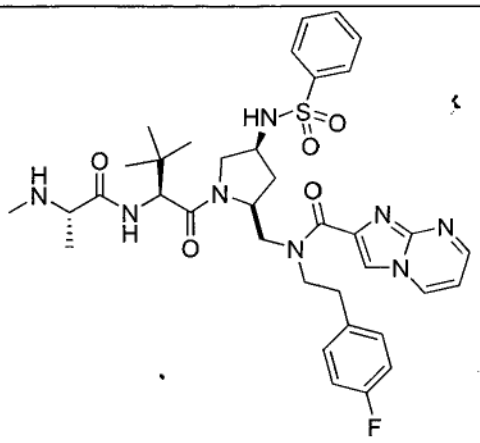
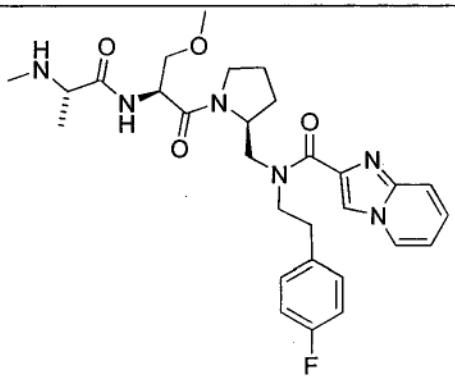
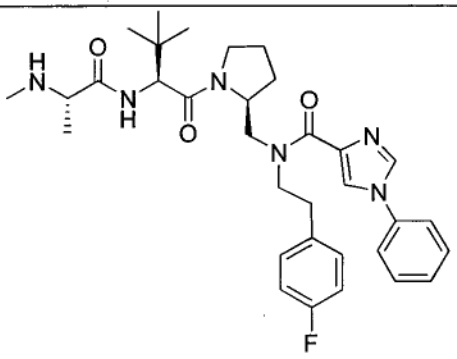
59	
60	
61	

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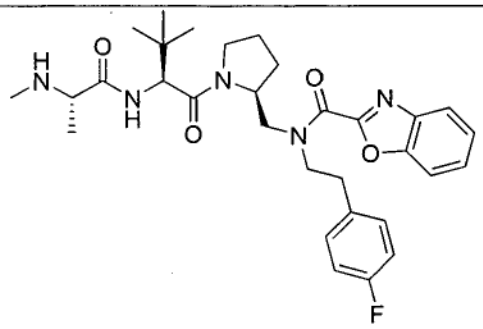
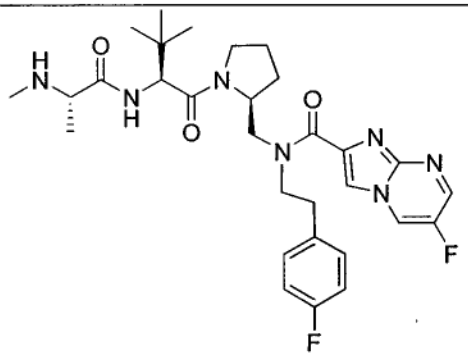
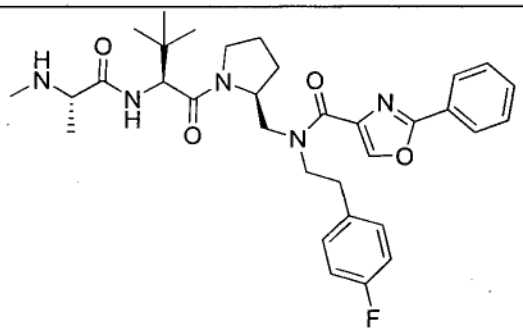
62	
63	
64	

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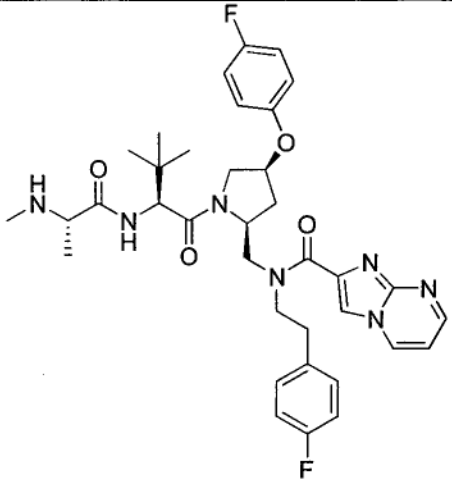
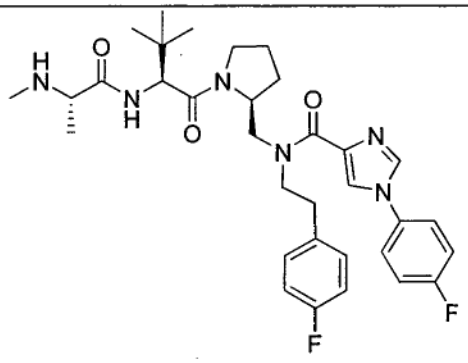
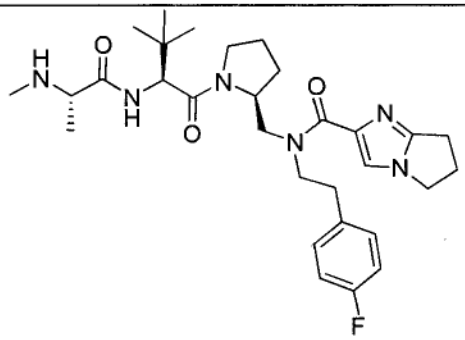
65	
66	
67	

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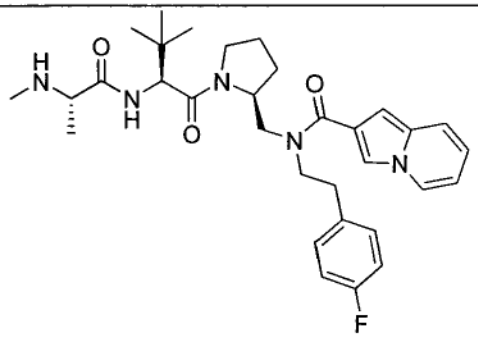
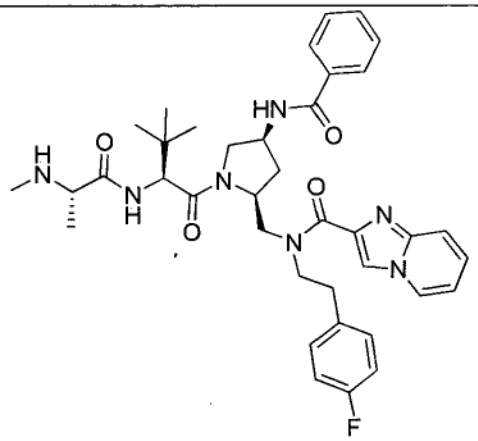
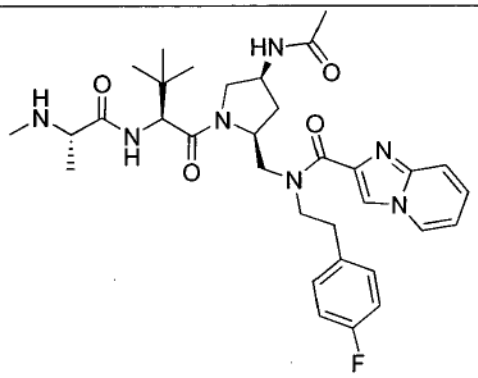
68	
69	
70	

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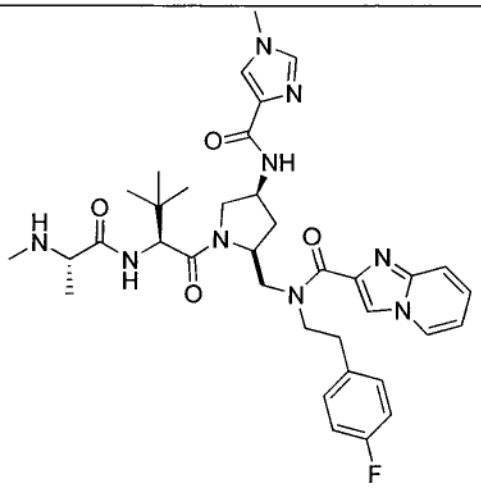
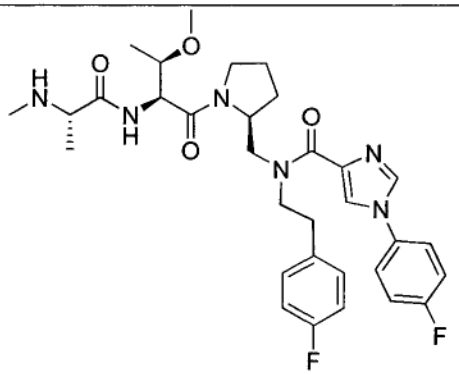
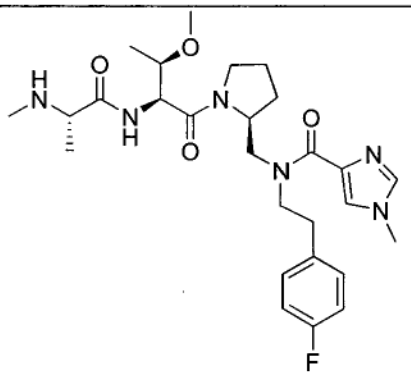
71	
72	
73	

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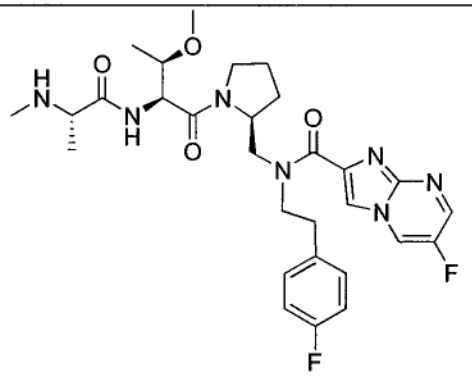
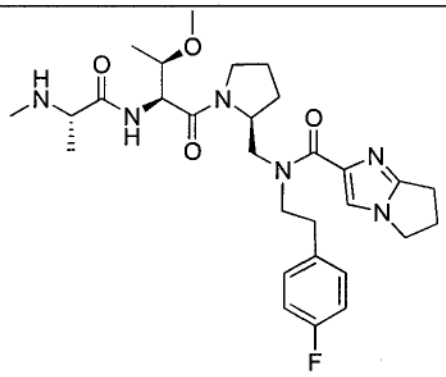
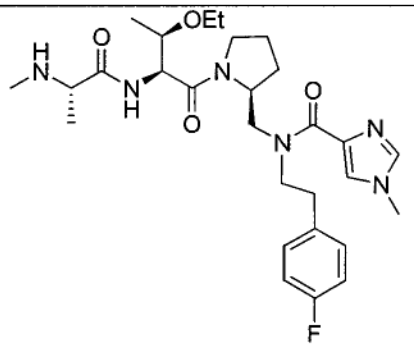
74	
75	
76	

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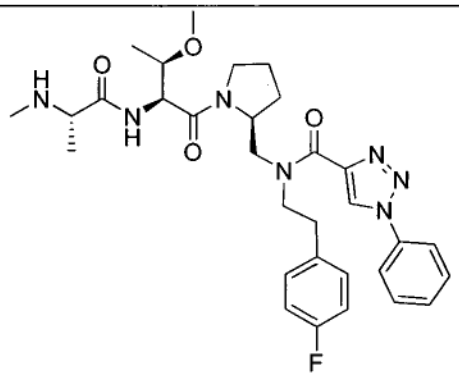
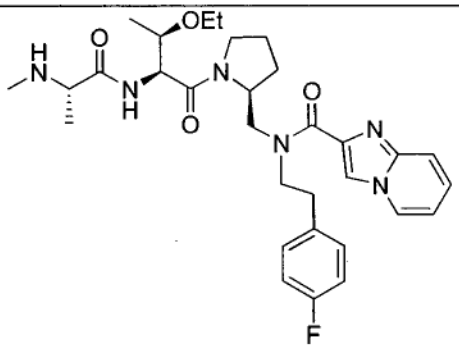
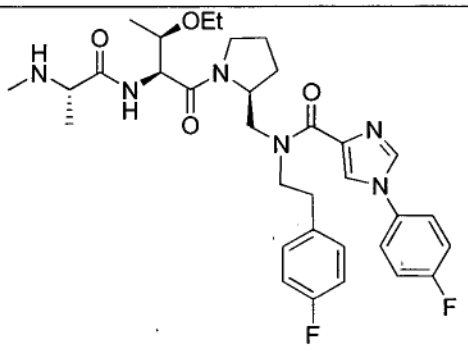
77	
78	
79	

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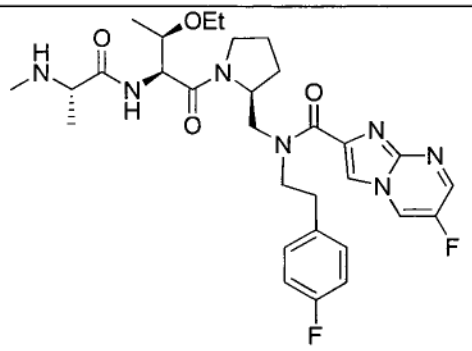
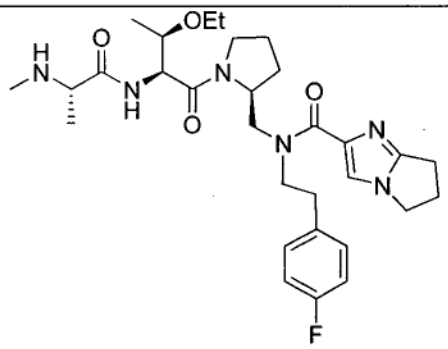
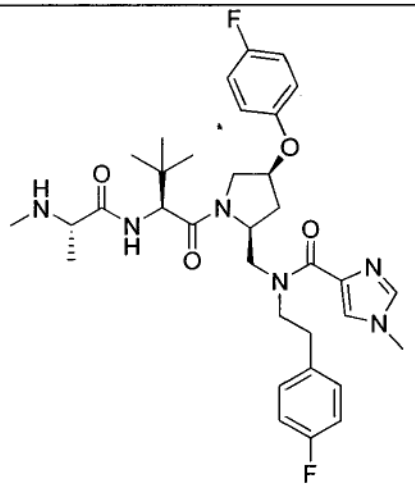
80	
81	
82	

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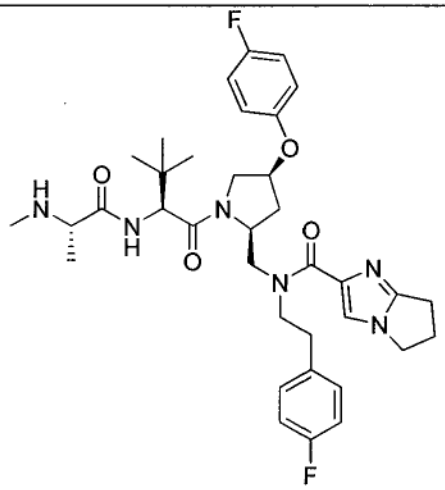
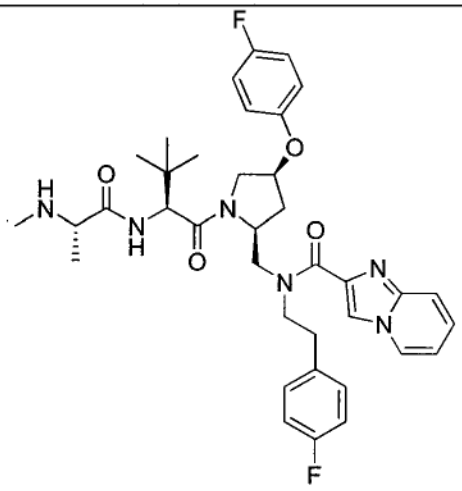
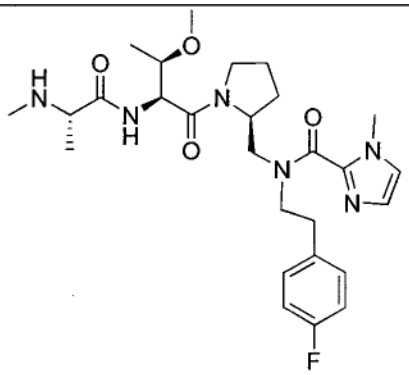
83	
84	
85	

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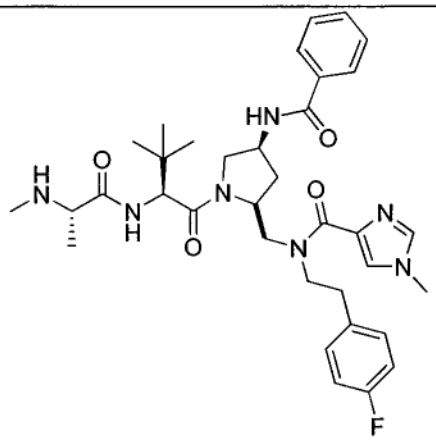
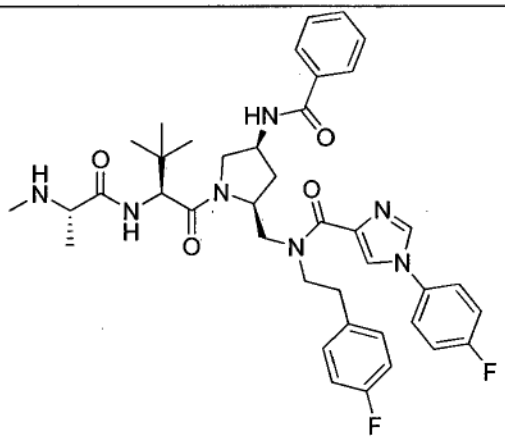
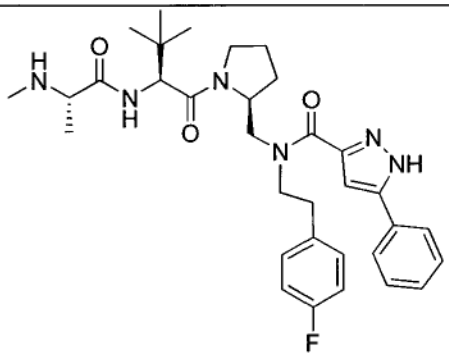
86	
87	
88	

or

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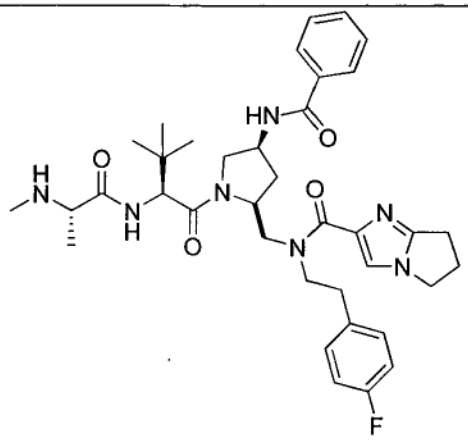
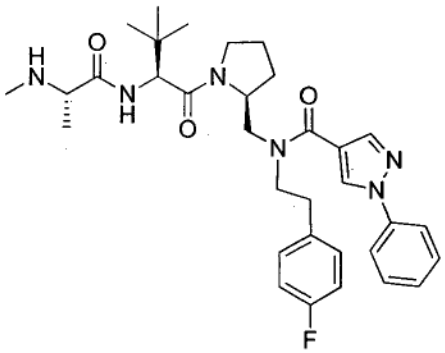
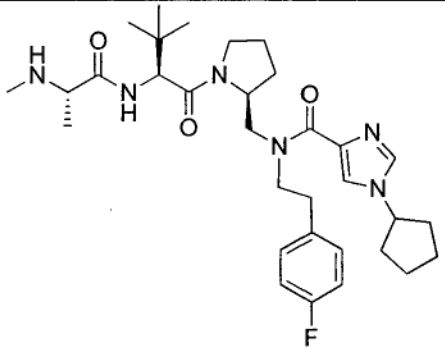
89	
90	
91	

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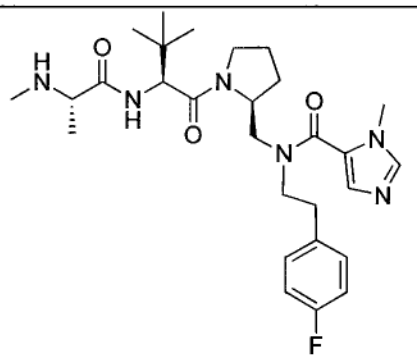
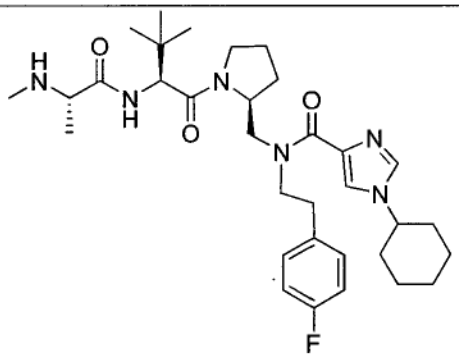
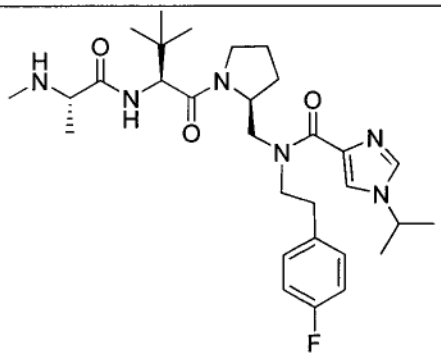
92	
93	
94	

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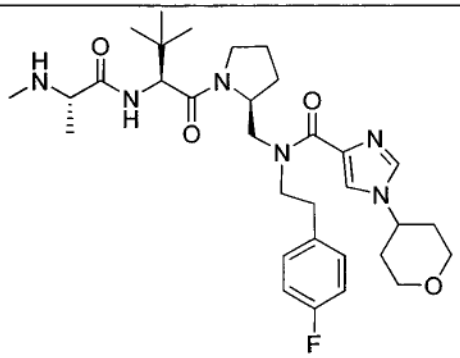
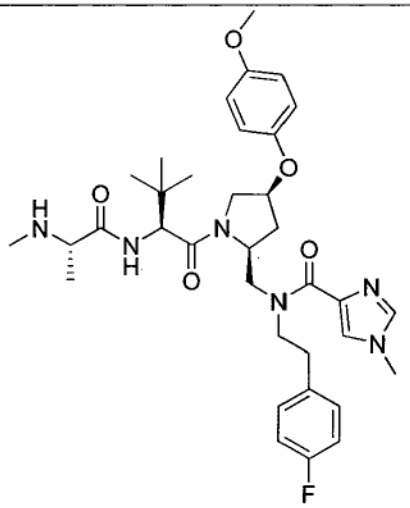
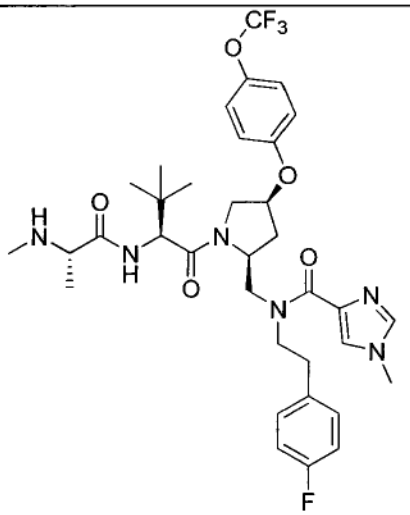
95	
96	
97	

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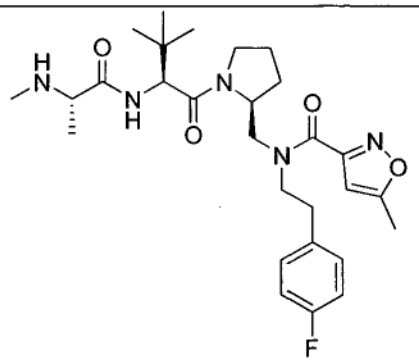
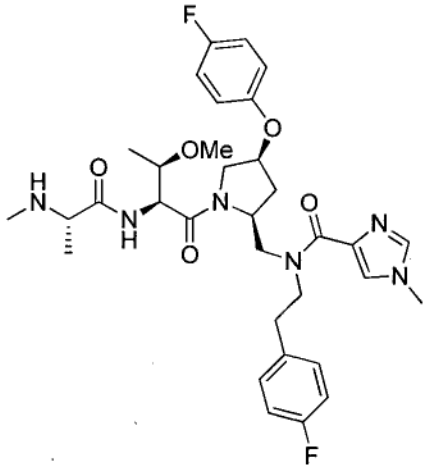
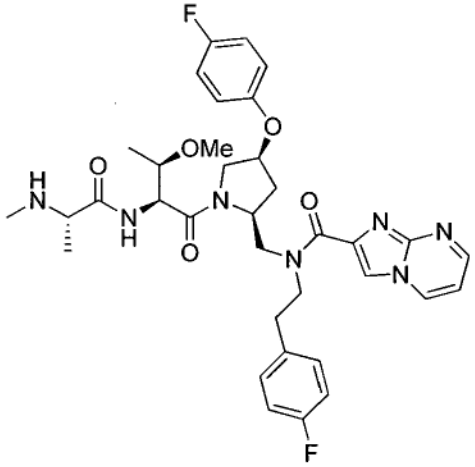
98	
99	
100	

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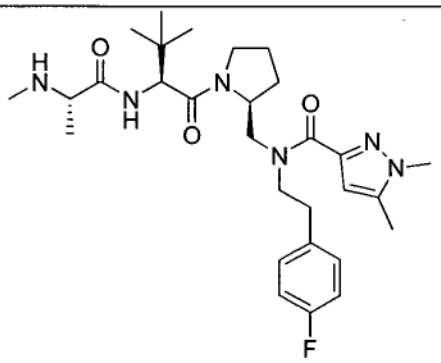
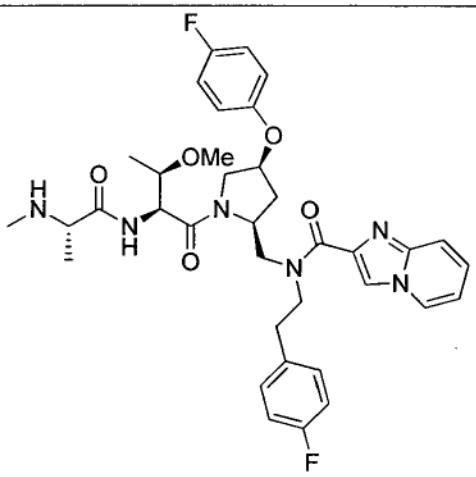
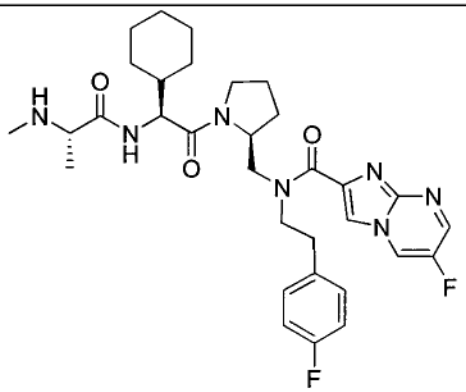
101	
102	
103	

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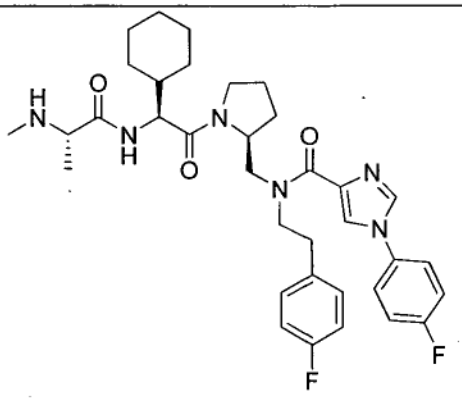
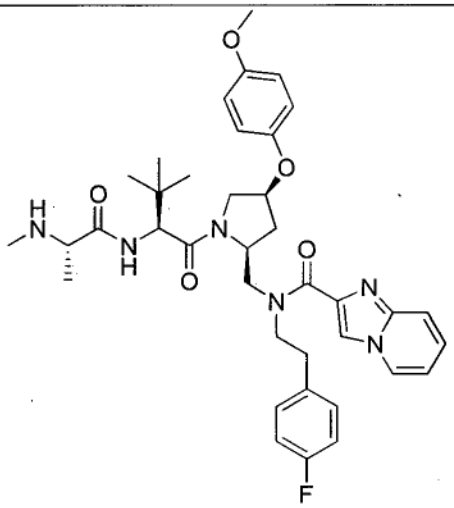
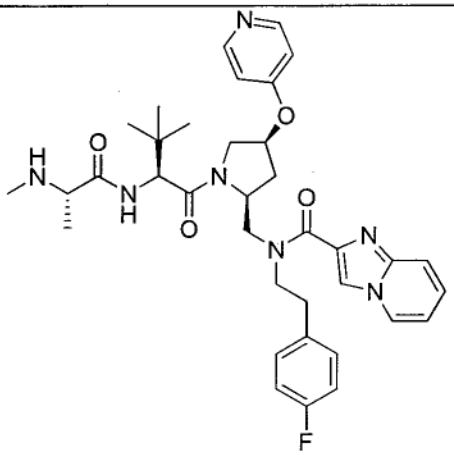
104	
105	
106	

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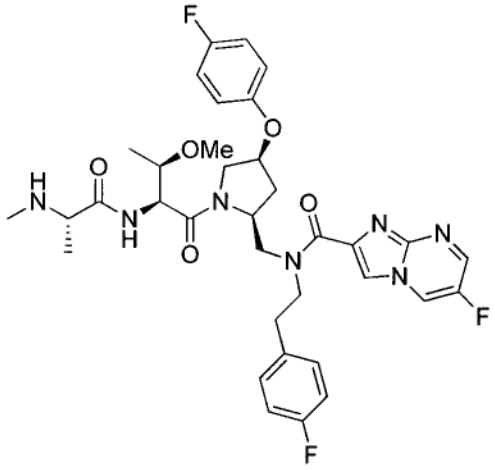
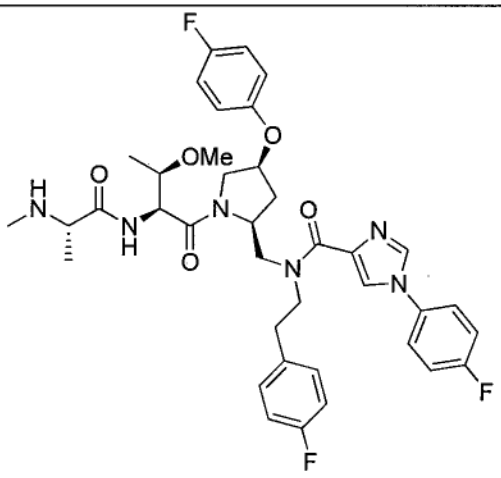
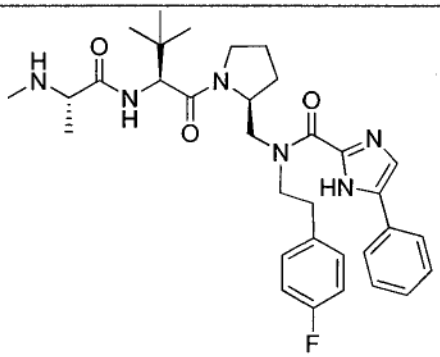
107	
108	
109	

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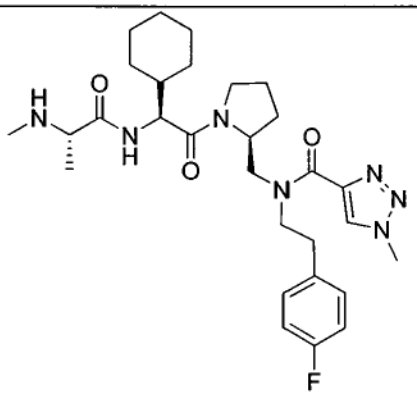
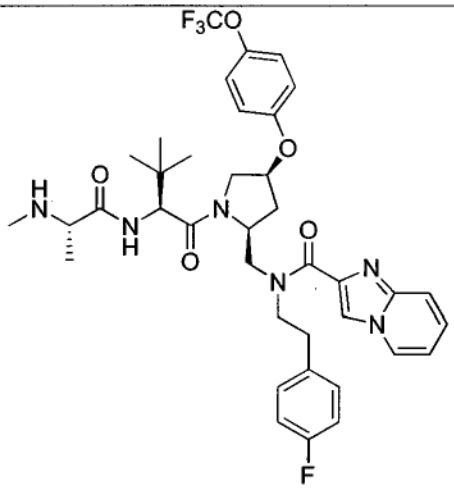
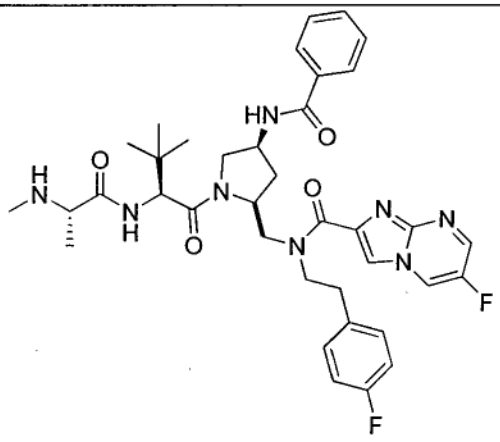
110	
111	
112	

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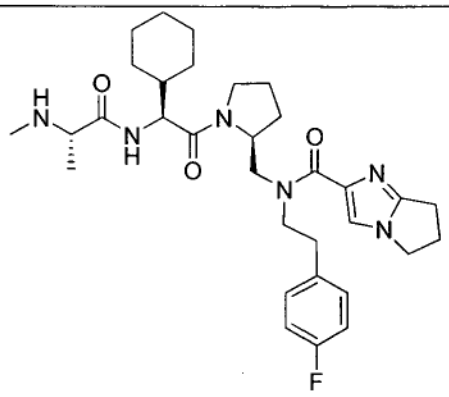
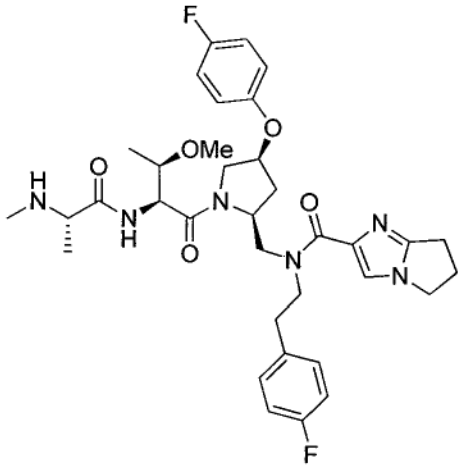
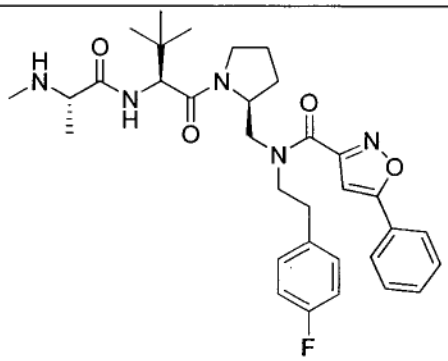
113	
114	
115	

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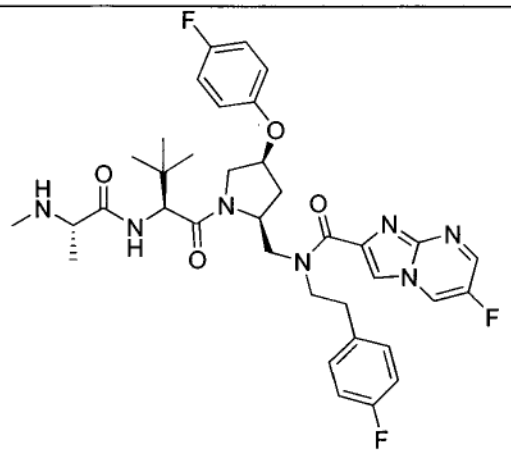
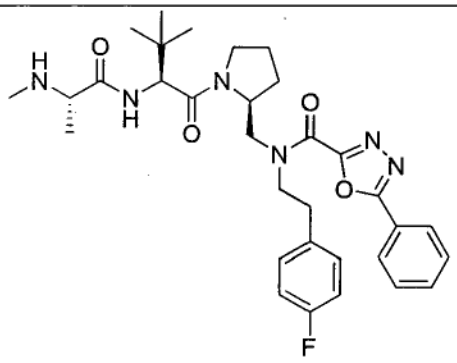
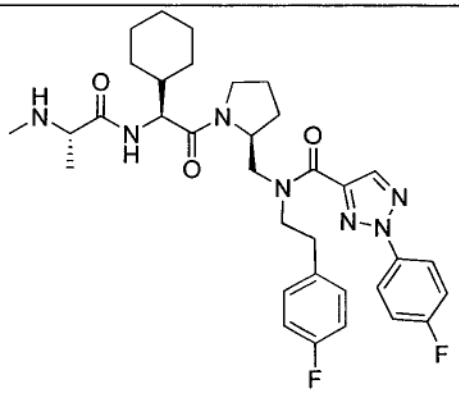
116	
117	
118	

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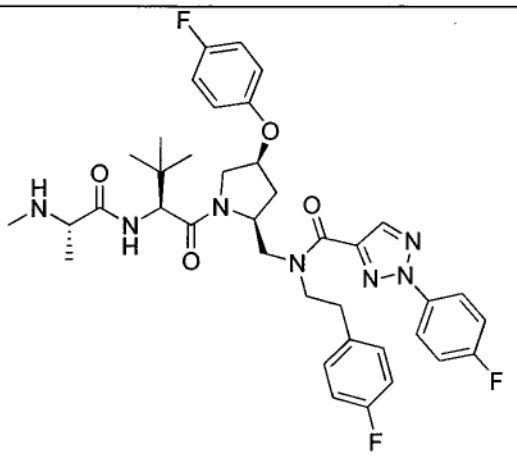
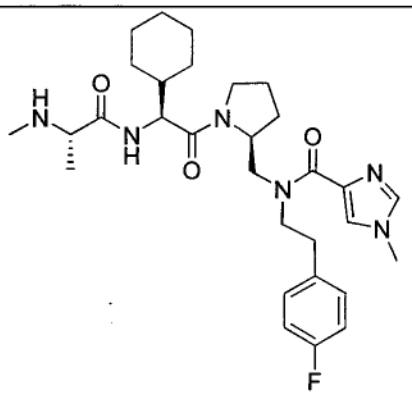
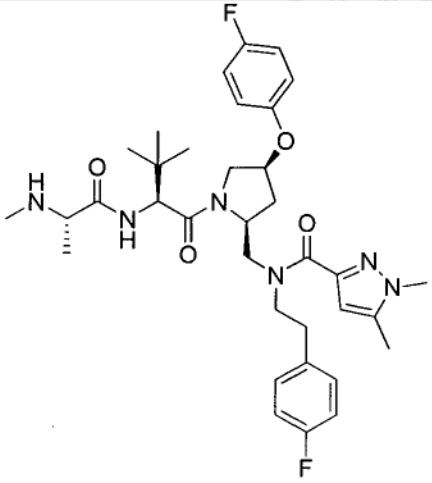
119	
120	
121	

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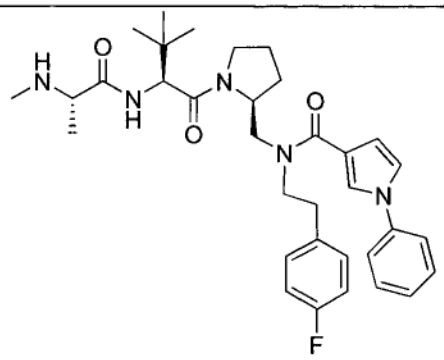
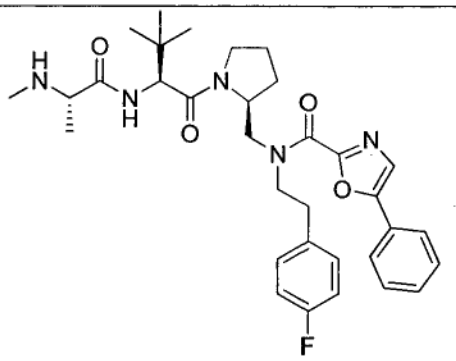
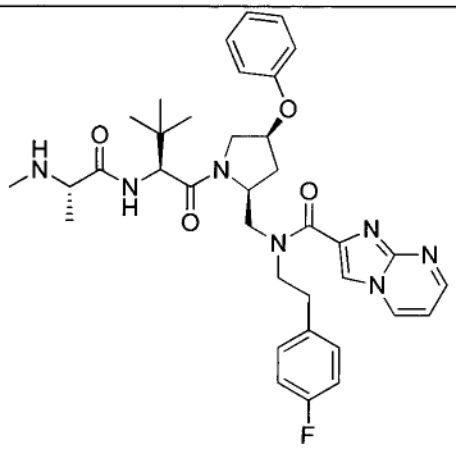
122	
123	
124	

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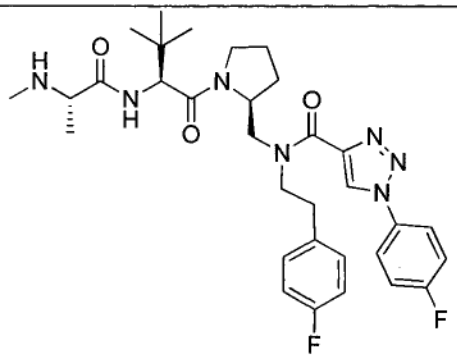
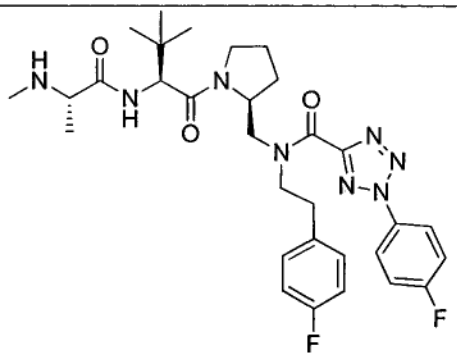
125	
126	

or pharmaceutically acceptable salt thereof.

14. A method of enhancing apoptosis in a cell comprising contacting a cell with a compound of any of claims 1-13.

15. The method of claim 14, wherein the cell is a cancer cell.

16. A method of altering the release of inflammatory cytokines from a cell comprising contacting a cell with a compounds of any of claims 1-13.

17. The method of claim 16, wherein the cell is a lymphocyte, granulocyte or an antigen presenting cell.

18. The method of claim 14, wherein the cell is in a subject, and the cell is contacted with the compound of any of claims 1-13 by administering the compound to the subject.

19. The method of any of claims 14-18, further comprising administering to the subject a chemotherapeutic agent prior to, simultaneously with, or after administration of the compound of any of claims 1-13.

20. The method of any of claims 14-19, further comprising administering to the subject a death receptor agonist prior to, simultaneously with, or after administration of the compound of any of claims 1-13.

21. The method of claim 20, wherein the death receptor agonist is TRAIL.

22. The method of claim 21, wherein the death receptor agonist is a TRAIL receptor antibody.

23. The method of any of claims 20-22, in which the death receptor agonist is administered in an amount that produces a synergistic effect.

24. The method of any of claims 14-23, in which the subject is a human.

25. The method of claim 24, wherein the subject is afflicted with a proliferative disease.

26. The method of claim 25, wherein the proliferative disease is cancer.

27. The method of claim 25, wherein the proliferative disease is an autoimmune disease or inflammatory disorder.

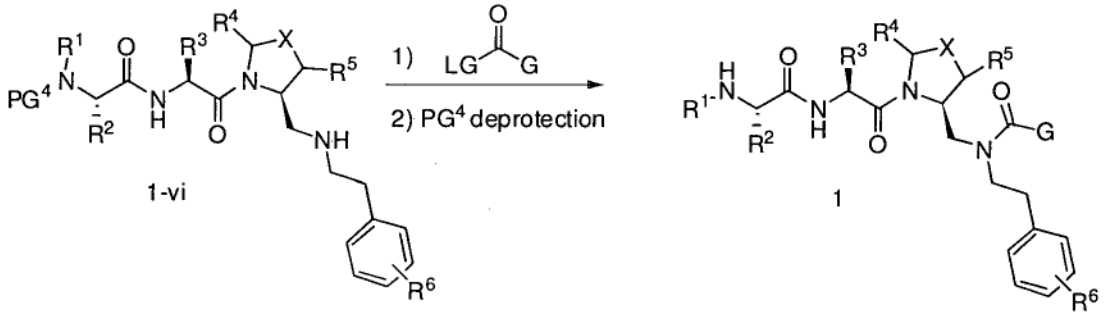
28. A compound of any of claims 1-13 for enhancing apoptosis in a cell in accordance with the method of any of claims 14-27.

29. A compound of any of claims 1-13 for use as a medicament for the treatment of a proliferative disease.

30. Use of a compound of any of claims 1-13 to prepare a medicament for the treatment of a proliferative disease.

31. An compound of any of Formulas 1-i through 1-vi, 2-i through 2-iv, 4-i, or 4-ii.

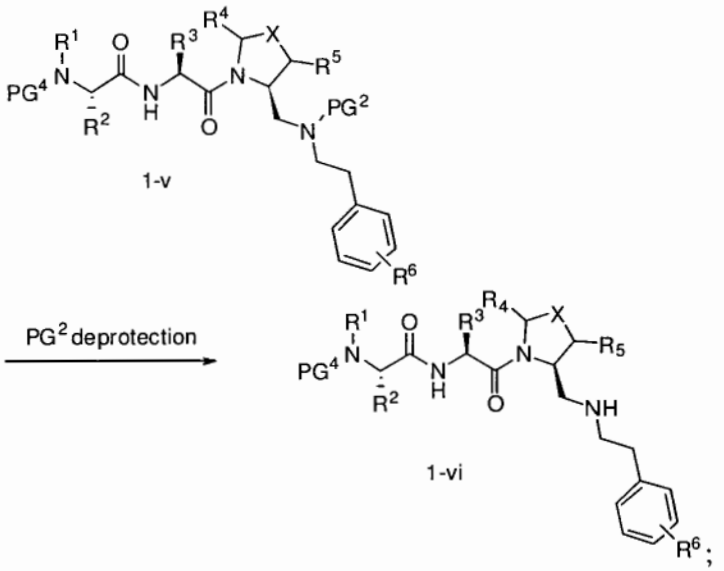
32. A method of preparing a compound of claim 1 comprising combining compound 1-vi with a compound of formula LG-C(O)-G, wherein LG is a leaving group and PG^4 is a protecting group, followed by deprotection of PG^4 to provide a compound of Formula 1:



;

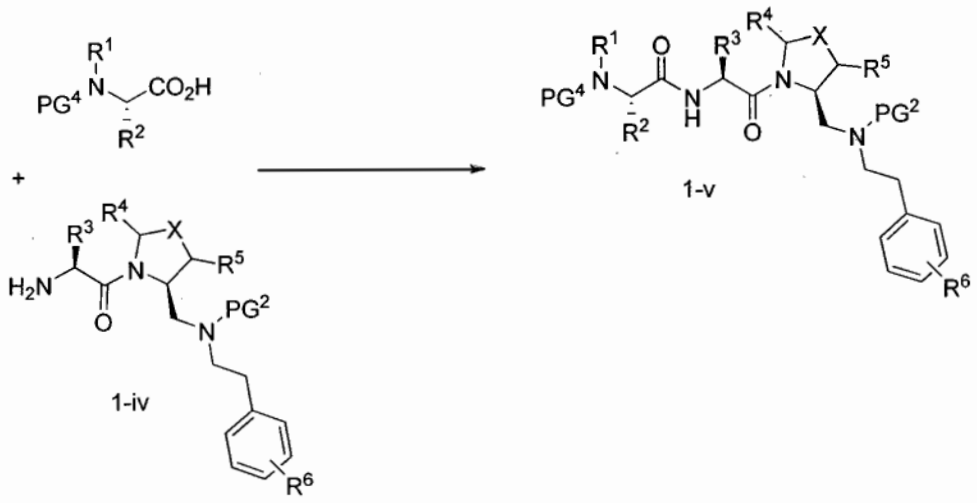
wherein the method optionally further comprises

(a) deprotection of PG^2 of compound 1-v to provide compound 1-vi, wherein PG^2 is a protecting group;



wherein the method optionally further comprises

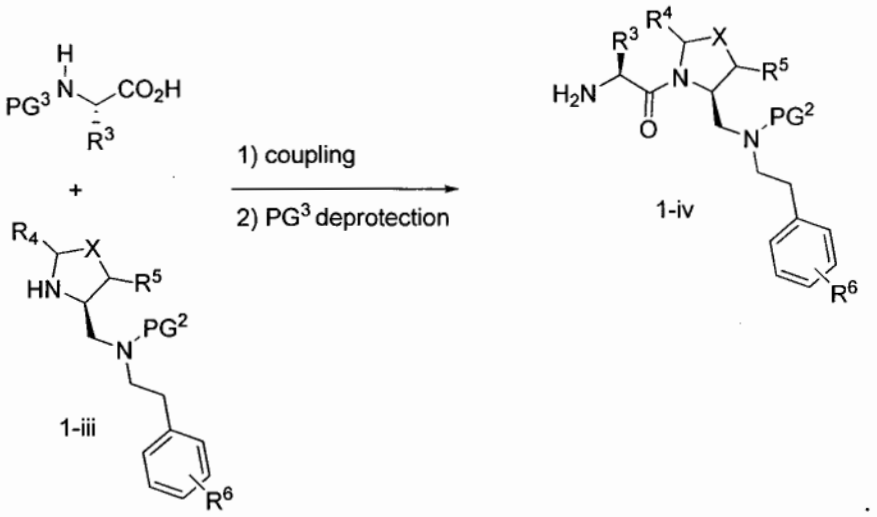
(b) coupling compound 1-iv with $PG^4(R^1)N(R^2)CHCO_2H$, wherein PG^4 is a protecting group that is different from PG^2, to provide compound 1-v;



;

wherein the method optionally further comprises

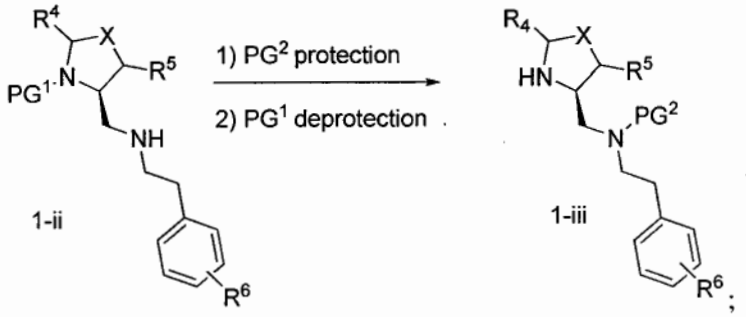
(c) coupling compound 1-iii with $PG^3(H)N(R^3)CHCO_2H$, wherein PG^3 is a protecting group that is different from PG^2, followed by deprotection of PG^3 to provide compound 1-iv;



;

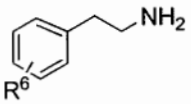
wherein the method optionally further comprises

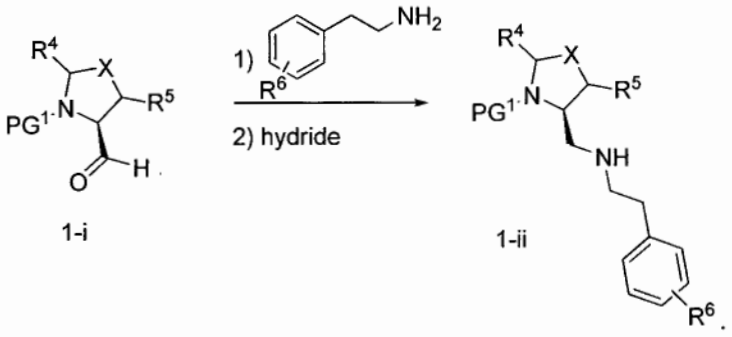
(d) protecting the amine group of compound 1-ii with a protecting group (PG^2) that is different from PG^1, followed by deprotection of PG^1 to provide compound 1-iii;



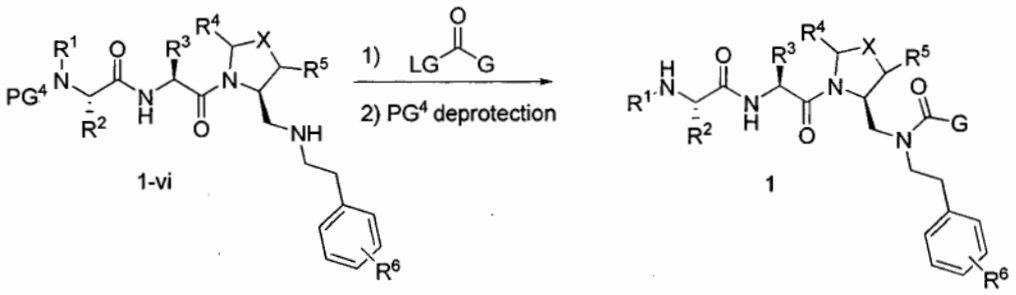
;

wherein the method optionally further comprises

(e) combining compound 1-i with an amine having the formula  , followed by reduction with a hydride, to provide compound 1-ii, wherein PG^1 is a protecting group:

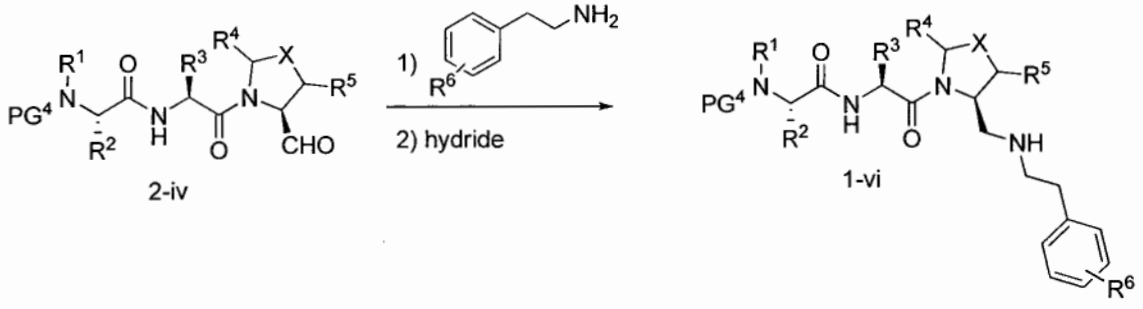


33. A method of preparing a compound of claim 1 comprising combining compound 1-vi with a compound of formula LG-C(O)-G, wherein LG is a leaving group, followed by deprotection of PG^4, to provide a compound of Formula 1:



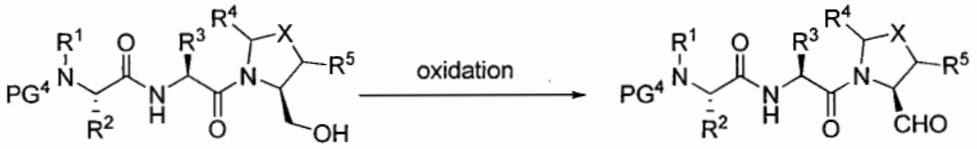
wherein the method optionally further comprises

(a) reductive amination of compound 2-iv to provide compound 1-vi:



wherein the method optionally further comprises

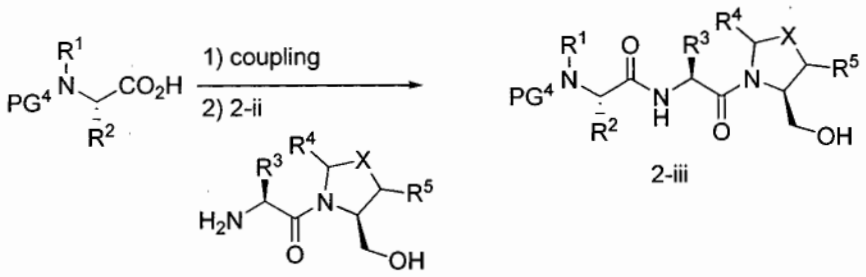
(b) oxidizing compound 2-iii to provide compound 2-iv:



2-iii 2-iv

wherein the method optionally further comprises

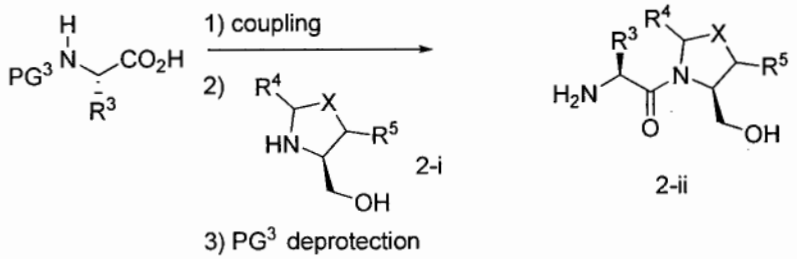
(c) coupling compound 2-ii with a compound of the formula $PG^4(R^1)N(R^2)CHCO_2H$ to provide compound 2-iii, wherein PG^4 is a protecting group:



2-iii

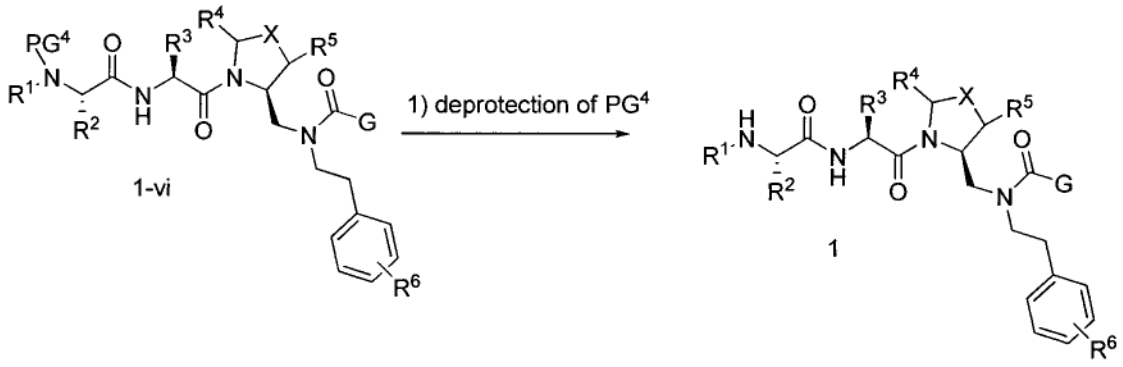
wherein the method optionally further comprises

(d) coupling compound 2-i with a compound of the formula $PG^3(H)N(R^3)CHCO_2H$, wherein PG^3 is a protecting group, followed by deprotection of PG^3 to provide intermediate compound 2-ii:



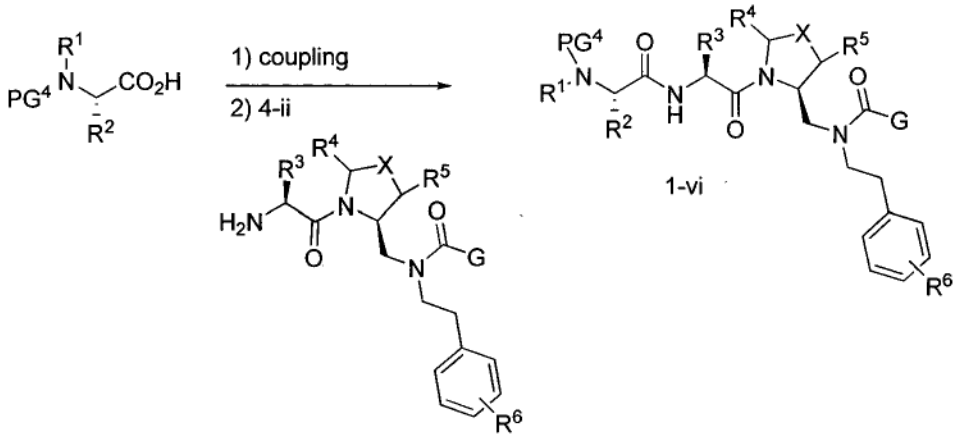
2-ii

34. A method of preparing a compound of claim 1 comprising deprotecting compound 1-vi to provide a compound of Formula 1:



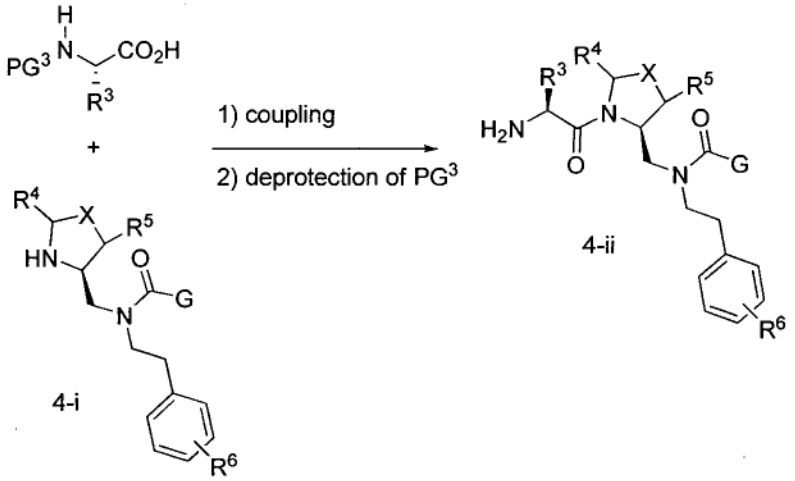
wherein the method optionally further comprises

(a) coupling compound 4-ii with a compound having the formula $PG^4(R^1)N(R^2)CHCO_2H$ to provide compound 1-vi, wherein PG^4 is a protecting group:



wherein the method optionally further comprises

(b) coupling compound 4-i with a compound having the formula $PG^3(H)N(R^3)CHCO_2H$, wherein PG^3 is a protecting group, followed by deprotection of PG^3 to provide compound 4-ii:



wherein the method optionally further comprises

(c) combining compound 1-ii with a compound of formula LG-C(O)-G, wherein PG^1 is a protecting group, followed by deprotection of PG^1 to provide intermediate compound 4-i:

