

CONJUGATES TARGETING FIBROBLAST ACTIVATION PROTEIN AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/599,959, filed November 16, 2023, and U.S. Provisional Patent Application No. 63/599,970, filed November 16, 2023, each of which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

[0002] The present invention generally relates to compounds which modulate the Fibroblast Activation Protein (FAP) for the active delivery of various payloads (e.g., cytotoxic drugs, proteins, immunomodulators, radioactive components, metal chelating groups/chelating agents, fluorescent dyes or contrast agents) at the site of action. The present invention relates to the development of FAP ligands for targeting applications, in particular diagnostic methods and/or methods for therapy or surgery in relation to a disease or disorder, such as cancer, inflammation or another disease characterized by overexpression of FAP.

BACKGROUND OF THE INVENTION

[0003] Fibroblast activation protein (FAP) is highly overexpressed in stromal tissue of various cancers. Fibro-proliferative response in tumors such as breast, colon, and pancreatic cancer, the tumor stroma (TS) forms > 90% of the tumor mass. The TS consists of a large number of fibroblasts, particularly cancer-associated fibroblasts (CAFs). The CAFs contributes to tumor growth, migration, and progression, thus, its valuable for tumor diagnosis and also as a therapeutic target. The expression of fibroblast-activating protein alpha (FAP) is a unique feature of CAFs. FAP is a 97-kDa type II membrane-bound glycoprotein from the dipeptidyl peptidase IV (DPPIV4) family and shows dipeptidyl peptidase and endopeptidase activity. The endopeptidase activity of FAP distinguishes it from other members of the DPPIV family. Substrates of FAP endopeptidase activity are type I collagen, α 1-antitrypsin, and several neuropeptides. FAP is reported to play an essential role in embryonic development, and tissue remodeling. FAP expression is low in normal adult tissues but its high expression is reported in wound healing, arthritis, atherosclerotic plaques, fibrosis, and > 90% of epithelial cancers. In the CAFs of many epithelial tumors, FAP is highly

expressed and is associated with poorer cancer prognosis. Overall, FAP activity is associated with cancer development and progression.

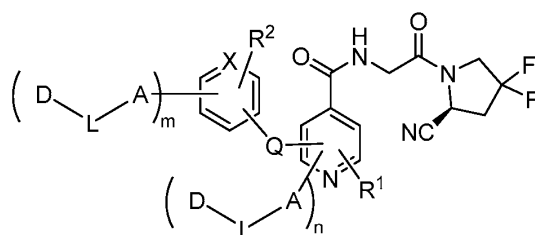
[0004] While FAP has been recognized as a potential diagnostic or therapeutic cancer target, FAP targeted delivery of a payload including one or more drugs and/or agents after systemic administration is desired. A therapeutic/diagnostic payload like proteins, immunomodulators, radioactive components, metal chelating groups or chelating agents, fluorescent dyes or contrast agents, can be conjugated to a FAP ligand. FAP-targeting antibodies have been utilized as ligands for developing antibody-drug conjugates (ADCs). OMTX705 is a novel antibody–drug conjugate (ADC) molecule targeting fibroblast-activating protein α (FAP α). It has reached clinical stages and demonstrates activities in chemotherapy and Pembrolizumab-Resistant solid tumor models (*Clin Cancer Res* (2020) 26 (13): 3420–3430).

[0005] However, small molecule ligands have several advantages compared to bigger molecules, peptides and antibodies, as it offers rapid and efficient tumor penetration, lower immunogenicity, and lower manufacturing costs. Small molecule-drug conjugates (SMDCs) have been reported as effective approaches for targeted anticancer therapy. Therefore, there remains a need for conjugates comprising improved small-molecule binders (ligands) of fibroblast activation protein (FAP).

SUMMARY OF THE INVENTION

[0006] The present invention provides a chemical conjugate comprising a fibroblast activation protein (FAP) moiety and a payload for therapeutic and/or diagnostic applications.

[0007] In one aspect, the present invention provides a compound of formula (I):



formula (I),

or a pharmaceutically acceptable salt, stereoisomer or tautomer thereof, wherein X, Q, A, L, D, m, n, R¹ and R² are as defined for formula (I).

[0008] In one aspect, the compound of formula (I) or a pharmaceutically acceptable salt, stereoisomer or tautomer thereof, is any of compounds of formula (II) to formula (VI), and their sub-formulas, a pharmaceutically acceptable salt, stereoisomer or tautomer thereof, as detailed herein.

[0009] In some another aspect, the present invention provides method of treating a disease or disorder associated with FAP (e.g., FAP-alpha), in an individual in need thereof, wherein the method comprises administering to the individual an effective amount of a compound of the present invention. In some embodiments, the disease is a cancer.

[0010] In some another aspect, the present invention provides method of imaging, diagnosis or detecting a proliferative disease associated with this FAP (e.g., FAP-alpha) in an individual in need thereof, wherein the method comprises administering to the individual an effective amount of a compound of the present invention. In some embodiments, the disease is a cancer.

[0011] In some another aspect, the present invention provides method of inhibiting FAP (e.g., FAP-alpha) in an individual in need thereof, wherein the method comprises administering to the individual an effective amount of a compound of the present invention.

[0012] In some another aspect, the present invention provides a pharmaceutical composition, for use in a method for targeted delivery of a therapeutic and/or diagnostic agent in an individual suffering from or having risk for a disease or disorder; as well as a method for targeted delivery of a therapeutically and/or diagnostically effective amount of a compound of the present invention by administering the pharmaceutically acceptable composition in an individual suffering from or having risk for a disease or disorder.

[0013] In some embodiments, the methods described herein further comprise co-administering to an individual in need thereof another therapeutic and/or diagnostic agent.

[0014] In some another aspect, the present invention provides compounds, their method of use in imaging, diagnosis or detecting and treating cancer associated with FAP, it also provides processes for preparing compounds and intermediates thereof disclosed in the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0015] FIG. 1A shows fluorescence over time for PRXS-AMC in the presence of rhFAP α .
- [0016] FIG. 1B shows fluorescence over time for Z-gly-pro-AMC in the presence of rhFAP α .
- [0017] FIG. 2A shows fluorescence over time for PRXS-AMC in the presence of rhPREP.
- [0018] FIG. 2B shows fluorescence over time for Z-gly-pro-AMC in the presence of rhPREP.
- [0019] FIG. 3A shows fluorescence over time for PRXS-AMC in the presence of rhDPPIV.
- [0020] FIG. 3B shows fluorescence over time for Z-gly-pro-AMC in the presence of rhDPPIV.
- [0021] FIG. 4A shows percentages of hFAP⁺ cells in WT HEK293 cells.
- [0022] FIG. 4B shows percentages of hFAP⁺ cells in selected HEK293-hFAP cells
- [0023] FIG. 4C shows percentages of hFAP⁺ cells in WT HT-1080 cells.
- [0024] FIG. 4D shows percentages of hFAP⁺ cells in selected HT-1080-hFAP cells.
- [0025] FIG. 5 shows the total expressions of FAP α in WT HEK293, HEK293-hFAP, WT HT-1080 and HT-1080-hFAP cells, determined by Western Blot.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

- [0026] “Alkyl” refers to and includes saturated linear and branched univalent hydrocarbon structures and combination thereof, having the number of carbon atoms designated (*i.e.*, C₁-C₁₀ means one to ten carbons). Particular alkyl groups are those having 1 to 20 carbon atoms (a “C₁-C₂₀ alkyl”). More particular alkyl groups are those having 1 to 8 carbon atoms (a “C₁-C₈ alkyl”), 3 to 8 carbon atoms (a “C₃-C₈ alkyl”), 1 to 6 carbon atoms (a “C₁-C₆ alkyl”), 1 to 5 carbon atoms (a “C₁-C₅ alkyl”), or 1 to 4 carbon atoms (a “C₁-C₄ alkyl”). Examples of alkyl include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like.
- [0027] “Alkenyl” as used herein refers to an unsaturated linear or branched univalent hydrocarbon chain or combination thereof, having at least one site of olefinic unsaturation (*i.e.*, having at least one moiety of the formula C=C) and having the number of carbon atoms designated (*i.e.*, C₂-C₁₀ means two to ten carbon atoms). The alkenyl group may be in “cis” or “trans”

configurations, or alternatively in “E” or “Z” configurations. Particular alkenyl groups are those having 2 to 20 carbon atoms (a “C₂-C₂₀ alkenyl”), having 2 to 8 carbon atoms (a “C₂-C₈ alkenyl”), having 2 to 6 carbon atoms (a “C₂-C₆ alkenyl”), or having 2 to 4 carbon atoms (a “C₂-C₄ alkenyl”). Examples of alkenyl include, but are not limited to, groups such as ethenyl (or vinyl), prop-1-enyl, prop-2-enyl (or allyl), 2-methylprop-1-enyl, but-1-enyl, but-2-enyl, but-3-enyl, buta-1,3-dienyl, 2-methylbuta-1,3-dienyl, homologs and isomers thereof, and the like.

[0028] “Alkylene” as used herein refers to the same residues as alkyl, but having bivalency. Particular alkylene groups are those having 1 to 6 carbon atoms (a “C₁-C₆ alkylene”), 1 to 5 carbon atoms (a “C₁-C₅ alkylene”), 1 to 4 carbon atoms (a “C₁-C₄ alkylene”) or 1 to 3 carbon atoms (a “C₁-C₃ alkylene”). Examples of alkylene include, but are not limited to, groups such as methylene (-CH₂-), ethylene (-CH₂CH₂-), propylene (-CH₂CH₂CH₂-), butylene (-CH₂CH₂CH₂CH₂-), and the like.

[0029] “Alkynyl” as used herein refers to an unsaturated linear or branched univalent hydrocarbon chain or combination thereof, having at least one site of acetylenic unsaturation (*i.e.*, having at least one moiety of the formula C≡C) and having the number of carbon atoms designated (*i.e.*, C₂-C₁₀ means two to ten carbon atoms). Particular alkynyl groups are those having 2 to 20 carbon atoms (a “C₂-C₂₀ alkynyl”), having 2 to 8 carbon atoms (a “C₂-C₈ alkynyl”), having 2 to 6 carbon atoms (a “C₂-C₆ alkynyl”), or having 2 to 4 carbon atoms (a “C₂-C₄ alkynyl”). Examples of alkynyl include, but are not limited to, groups such as ethynyl (or acetylenyl), prop-1-ynyl, prop-2-ynyl (or propargyl), but-1-ynyl, but-2-ynyl, but-3-ynyl, homologs and isomers thereof, and the like.

[0030] “Aryl” refers to and includes polyunsaturated aromatic hydrocarbon groups. Aryl may contain additional fused rings (*e.g.*, from 1 to 3 rings), including additionally fused aryl, heteroaryl, cycloalkyl, and/or heterocyclyl rings. In one variation, the aryl group contains from 6 to 14 annular carbon atoms. Examples of aryl groups include, but are not limited to, phenyl, naphthyl, biphenyl, and the like.

[0031] “Carbonyl” refers to the group C=O.

[0032] “Cycloalkyl” refers to and includes cyclic univalent hydrocarbon structures, which may be fully saturated, mono- or polyunsaturated, but which are non-aromatic, having the number of carbon atoms designated (*e.g.*, C₁-C₁₀ means one to ten carbons). Cycloalkyl can consist of one

ring, such as cyclohexyl, or multiple rings, such as adamantly, but excludes aryl groups. A cycloalkyl comprising more than one ring may be fused, spiro or bridged, or combinations thereof. A preferred cycloalkyl is a cyclic hydrocarbon having from 3 to 13 annular carbon atoms. A more preferred cycloalkyl is a cyclic hydrocarbon having from 3 to 8 annular carbon atoms (a "C₃-C₈ cycloalkyl"). Examples of cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, norbornyl, and the like.

[0033] "Halo" or "halogen" refers to elements of the Group 17 series having atomic number 9 to 85. Preferred halo groups include fluoro, chloro, bromo and iodo. Where a residue is substituted with more than one halogen, it may be referred to by using a prefix corresponding to the number of halogen moieties attached, e.g., dihaloaryl, dihaloalkyl, trihaloaryl etc. refer to aryl and alkyl substituted with two ("di") or three ("tri") halo groups, which may be but are not necessarily the same halo; thus 4-chloro-3-fluorophenyl is within the scope of dihaloaryl. An alkyl group in which each hydrogen is replaced with a halo group is referred to as a "perhaloalkyl." A preferred perhaloalkyl group is trifluoroalkyl (-CF₃). Similarly, "perhaloalkoxy" refers to an alkoxy group in which a halogen takes the place of each H in the hydrocarbon making up the alkyl moiety of the alkoxy group. An example of a perhaloalkoxy group is trifluoromethoxy (-OCF₃).

[0034] "Heteroaryl" refers to and includes unsaturated aromatic cyclic groups having from 1 to 10 annular carbon atoms and at least one annular heteroatom, including but not limited to heteroatoms such as nitrogen, oxygen and sulfur, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule at an annular carbon or at an annular heteroatom. Heteroaryl may contain additional fused rings (*e.g.*, from 1 to 3 rings), including additionally fused aryl, heteroaryl, cycloalkyl, and/or heterocyclyl rings. Examples of heteroaryl groups include, but are not limited to imidazolyl, pyrrolyl, pyrazolyl, 1,2,4-triazolyl, thiophenyl, furanyl, thiazolyl, isothiazolyl, thiadiazolyl, oxazolyl, isoxazolyl, oxadiazolyl, pyridyl, pyrimidyl, pyridazinyl or pyrazinyl, and the like.

[0035] "Heterocycle" or "heterocyclyl" refers to a saturated or an unsaturated non-aromatic group having from 1 to 10 annular carbon atoms and from 1 to 4 annular heteroatoms, such as nitrogen, sulfur or oxygen, and the like, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heterocyclyl group may have a

single ring or multiple condensed rings, but excludes heteroaryl groups. A heterocycle comprising more than one ring may be fused, spiro or bridged, or any combination thereof. In fused ring systems, one or more of the fused rings can be aryl or heteroaryl. Examples of heterocyclcyl groups include, but are not limited to, aziridinyl, azetidiny, oxetanyl, morpholinyl, thiomorpholinyl, azepanyl tetrahydropyranyl, dihydropyranyl, piperidinyl, piperazinyl, pyrrolidinyl, thiazolinyl, thiazolidinyl, tetrahydrofuranyl, tetrahydrothiophenyl, and the like.

[0036] “Oxo” refers to the moiety =O.

[0037] The compounds of the present disclosure may include one or more asymmetric centers depending upon the location and nature of the various desired substituents. An asymmetric carbon atom may be present in the (R) or (S) configuration, a racemic mixture is obtained with an asymmetric center, and a diastereomer mixture is obtained with a plurality of asymmetric centers. In some cases, there may also be asymmetry due to rotation around a particular bond, such as two substituted aromatic rings connecting a particular compound with the center key.

[0038] “Optionally substituted” unless otherwise specified means that a group may be unsubstituted or substituted by one or more (e.g., 1, 2, 3, 4 or 5) of the substituents listed for that group in which the substituents may be the same or different. In one embodiment, an optionally substituted group has one substituent. In another embodiment, an optionally substituted group has two substituents. In another embodiment, an optionally substituted group has three substituents. In another embodiment, an optionally substituted group has four substituents. In some embodiments, an optionally substituted group has 1 to 2, 2 to 5, 3 to 5, 2 to 3, 2 to 4, 3 to 4, 1 to 3, 1 to 4 or 1 to 5 substituents.

[0039] A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0040] As used herein, “treatment” or “treating” is an approach for obtaining beneficial or desired results including clinical results. For example, beneficial or desired results include, but are not limited to, one or more of the following: decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, delaying the progression of the disease, and/or prolonging survival of individuals. In reference to cancers or other unwanted cell proliferation, beneficial or

desired results include shrinking a tumor (reducing tumor size); decreasing the growth rate of the tumor (such as to suppress tumor growth); reducing the number of cancer cells; inhibiting, retarding or slowing to some extent and preferably stopping cancer cell infiltration into peripheral organs; inhibiting (slowing to some extent and preferably stopping) tumor metastasis; inhibiting tumor growth; preventing or delaying occurrence and/or recurrence of tumor; and/or relieving to some extent one or more of the symptoms associated with the cancer.

[0041] As used herein, “delaying development of a disease” means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0042] As used herein, an “effective dosage” or “effective amount” of compound or salt thereof or pharmaceutical composition is an amount sufficient to effect beneficial or desired results. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity of, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include ameliorating, palliating, lessening, delaying or decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. In reference to cancers or other unwanted cell proliferation, an effective amount comprises an amount sufficient to cause a tumor to shrink and/or to decrease the growth rate of the tumor (such as to suppress tumor growth) or to prevent or delay other unwanted cell proliferation. In some embodiments, an effective amount is an amount sufficient to delay development. In some embodiments, an effective amount is an amount sufficient to prevent or delay occurrence and/or recurrence. An effective amount can be administered in one or more administrations, in the case of cancer, the effective amount of the drug or composition may: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and preferably stop cancer cell infiltration into peripheral organs; (iv) inhibit (i.e., slow to

some extent and preferably stop) tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer. An effective dosage can be administered in one or more administrations. For purposes of this disclosure, an effective dosage of compound or a salt thereof, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. It is intended and understood that an effective dosage of a compound or salt thereof, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an “effective dosage” may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0043] As used herein, the term “individual” is a mammal, including humans. An individual includes, but is not limited to, human, bovine, horse, feline, canine, rodent, or primate. In some embodiments, the individual is human. The individual (such as a human) may have advanced disease or lesser extent of disease, such as low tumor burden. In some embodiments, the individual is at an early stage of a proliferative disease (such as cancer). In some embodiments, the individual is at an advanced stage of a proliferative disease (such as an advanced cancer).

[0044] Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to “about X” includes description of “X”.

[0045] “FAP” refers to fibroblast-activated protein.

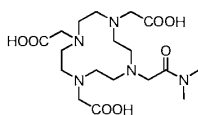
[0046] As used herein, “linker” refers to a divalent, trivalent, or multivalent moiety that covalently links one or more FAP ligands described herein to one or more payload, for instance, payload or antiviral compounds and enhancement agents. Generally, suitable linkers for the conjugates described herein are those that are sufficiently stable to exploit the circulating half-life of a conjugate and, at the same time, capable of releasing its payload after delivering the conjugate to a targeted site. Linkers can be cleavable or non-cleavable. Cleavable linkers are linkers that are cleaved by intracellular metabolism following internalization, e.g., cleavage via hydrolysis, reduction, or enzymatic reaction. Non-cleavable linkers are linkers that release an attached payload via lysosomal degradation of the antibody following internalization. Suitable linkers include, but

are not limited to, acid-labile linkers, hydrolytically-labile linkers, enzymatically cleavable linkers, reduction labile linkers, self-immolative linkers, and non-cleavable linkers. In some embodiments, linkers are or comprise peptides, glucuronides, succinimide-thioethers, polyethylene glycol (PEG) units, hydrazones, mal-caproyl units, dipeptide units, valine-citruline units, and para-aminobenzyloxycarbonyl (PABC), para-aminobenzyl (PAB) units.

[0047] A “payload” can be generally any molecule or particle. Various payloads (e.g. cytotoxic drugs, proteins, immunomodulators, radioactive components, metal chelating groups/chelating agents, fluorescent dyes or contrast agents) can be delivered at the site of disease in particular diagnostic methods and/or methods for therapy or surgery in relation to a disease or disorder, such as cancer, inflammation or another disease.

[0048] The “radionuclide” is the radioactive forms of elements which have the ability to chelate with the chelator unit of the chelating agents (eg. ^{68}Ga chelates with a chelator unit derived from DOTA). The different radionuclides are ^{18}F , ^{51}Cr , ^{67}Ga , ^{68}Ga , ^{111}In , $^{99\text{m}}\text{Tc}$, ^{186}Re , ^{188}Re , ^{139}La , ^{140}La , ^{175}Yb , ^{153}Sm , ^{166}Ho , ^{86}Y , ^{88}Y , ^{90}Y , ^{149}Pm , ^{165}Dy , ^{169}Er , ^{177}Lu , ^{47}Sc , ^{142}Pr , ^{159}Gd , ^{212}Bi , ^{213}Bi , ^{72}As , ^{72}Se , ^{97}Ru , ^{109}Pd , ^{105}Rh , $^{101\text{m}}\text{Rh}$, ^{119}Sb , ^{128}Ba , ^{123}I , ^{124}I , ^{131}I , ^{197}Hg , ^{211}At , ^{151}Eu , ^{153}Eu , ^{169}Eu , ^{201}Tl , ^{203}Pb , ^{212}Pb , ^{64}Cu , ^{67}Cu , ^{188}Re , ^{186}Re , ^{198}Au , ^{225}Ac , ^{227}Th , ^{161}Tb and ^{199}Ag . For example, the radionuclide is ^{68}Ga .

[0049] The “metal chelating groups (chelator units)” mentioned with respect to a compound of general formula (I) refers to a molecular fragment derived from a chelating agent. For example, the chelating agent unit is a molecular segment derived from 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), which may be formed amide bond through one of the carboxyl



groups of DOTA. Amide formation is introduced into the compound of general formula (I) with a help of linker (L). Other chelating agents are but not limited to ethylenediaminetetraacetic acid (EDTA), 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), triethylenetetramine (TETA), iminodiacetic acid, diethylenetriamine-N,N,N',N',N''-pentaacetic acid (DTPA), bis-(carboxymethylimidazole)glycine or 6-hydrazinopyridine-3-carboxylic acid (HYNIC).

[0050] It is understood that aspects and variations described herein also include “consisting” and/or “consisting essentially of” aspects and variations.

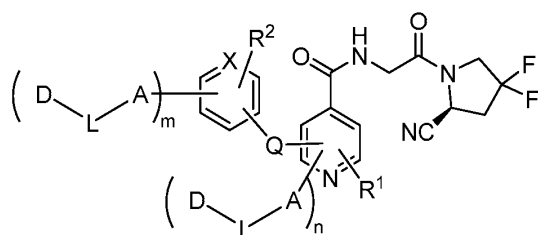
Compounds

[0051] The present invention discloses small molecule binders of fibroblast activation protein (FAP) which are suitable for targeting applications. The binders can provide high inhibition of FAP, high affinity for FAP and/or are suitable for targeted delivery of a payload, such as a therapeutic or diagnostic agent, to a site afflicted by or at risk of disease or disorder characterized by overexpression of FAP. The binders form a stable complex with FAP, display an increased affinity, increased inhibitory activity, a slower rate of dissociation from the complex, and/or prolonged residence at a disease site. The binders further can have an increased tumor-to- liver, tumor-to-kidney and/or tumor-to-intestine uptake ratio; a more potent anti-tumor effect (e.g., can be measured by mean tumor volume increase), and/or lower toxicity (e.g., can be assessed by the evaluation of changes (%) in body weight).

[0052] In some embodiments, binders can exhibit a very high, specific uptake in FAP-expressing tumors in combination with low uptake in normal organs. That is, the binders can provide advantageous therapeutic index in terms of tumor to non -tumor ratio when it is administered in vivo.

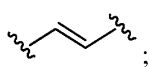
[0053] The binders further can have a high or improved affinity for human and murine fibroblast activation protein and/or cross-reactivity to the murine antigen. The binders according to the invention preferably attain FAP-specific cellular binding; FAP-selective accumulation on the cell membrane; FAP-selective accumulation inside the cytosol. In some embodiments, the binders can further rapidly and homogeneously localize at the tumor site in vivo with a high tumor-to- organs selectivity, in particular for melanoma and/or renal cell carcinoma.

[0054] The present invention provides a compound comprising a FAP binder described herein and a payload. In some embodiments, provided is a compound of formula (I):



formula (I),

or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein,

Q is a bond or ;

X is N or CR²;

A is -O-, 5- to 6-membered heterocyclyl, -(CH₂)_p-, -NR⁶-, -C(O)NR⁶-, -NR⁶C(O)-#, -C(O)O-#, -OC(O)-#, -C(O)(CH₂)_p-, -(CH₂)_pC(O)-#, -(CH₂)_pNR⁶-, -NR⁶(CH₂)_p-, -O(CH₂)_p-, or -(CH₂)_pO-#, wherein # indicates the point of attachment to L;

p is an integer from 0 to 2; and

R⁶ is hydrogen or C₁-C₆ alkyl;

or R² and R⁶ are taken together with the atom to which they are attached to form a 5- to 7-membered heterocyclyl optionally substituted by R¹¹;

D is, independently at each occurrence, a payload;

L is, independently at each occurrence, a linker;

R¹ and R² are independently hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, 3- to 6-membered heterocyclyl, -CN, halogen, C₁-C₆ alkoxy, C₁-C₆ haloalkoxy, C₁-C₆ haloalkyl, -OR³, -SR³, -S(O)₂R³, -S(O)₂NR⁴R⁵, -NR³S(O)₂R⁴, -NR⁴R⁵, -C(O)R³, -NR³C(O)R⁴, -NR³C(O)NR⁴R⁵, -C(O)OR³, -C(O)ONR⁴R⁵ or -C(O)NR⁴R⁵, wherein each of R¹ and R² is independently optionally substituted by R¹⁰;

each R³, R⁴ and R⁵ is independently hydrogen, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, 3- to 6-membered heterocyclyl, -CN, halogen, C₁-C₆ alkoxy, C₁-

C₆ haloalkoxy, C₁-C₆ haloalkyl, -OR⁷, -SR⁷, -S(O)₂R⁷, -S(O)₂NR⁸R⁹, -NR⁷S(O)₂R⁸, -NR⁸R⁹, -C(O)R⁷, -NR⁷C(O)R⁸, -NR⁷C(O)NR⁸R⁹, -C(O)OR⁷, -C(O)ONR⁸R⁹ or -C(O)NR⁸R⁹;

or R⁴ and R⁵ are taken together with the atom to which they attached to form a 3- to 6-membered heterocyclyl which is optionally substituted by R¹⁰;

each R⁷, R⁸ and R⁹ is independently hydrogen or C₁-C₆ alkyl optionally substituted by oxo, -OH or -NH₂;

or R⁸ and R⁹ are taken together with the atom to which they attached to form a 3- to 6-membered heterocyclyl which is optionally substituted by oxo, -OH or -NH₂;

R¹⁰ and R¹¹ are independently oxo, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, 3- to 6-membered heterocyclyl, -CN, halogen, C₁-C₆ alkoxy, C₁-C₆ haloalkoxy, C₁-C₆ haloalkyl, -OR¹⁴, -SR¹⁴, -S(O)₂R¹⁴, -S(O)₂NR¹⁵R¹⁶, -NR¹⁴S(O)₂R¹⁵, -NR¹⁵R¹⁶, -C(O)R¹⁴, -NR¹⁴C(O)R¹⁵, -NR¹⁴C(O)NR¹⁵R¹⁶, -C(O)OR¹⁴, -C(O)ONR¹⁵R¹⁶ or -C(O)NR¹⁵R¹⁶;

each R¹⁴, R¹⁵ and R¹⁶ is independently hydrogen or C₁-C₆ alkyl optionally substituted by oxo, -OH or -NH₂;

or R¹⁵ and R¹⁶ are taken together with the atom to which they attached to form a 3- to 6- membered heterocyclyl which is optionally substituted by oxo, -OH or -NH₂;

m is 0 or 1; and

n is 0 or 1;

provided that m + n is an integer greater than 0.

[0055] In some embodiments, D is a payload comprising one or more drugs or agents. As would be appreciated by a person skilled in the art, many known drugs/agents can be used alone or together as a payload in accordance with the present invention.

[0056] In some embodiments, D comprises or is one or more therapeutic drugs/agents, such as cytotoxic drugs. In some embodiments, D is selected from the group consisting of but not limited to vinblastine, tubulysin, paclitaxel, mitomycin C and any combination thereof (Small molecule drug conjugates (SMDCs): an emerging strategy for anticancer drug design and discovery, *New J. Chem.*, 2021, **45**, 5291-5321). In some embodiments, D is selected from the group consisting of but not limited to tubulin inhibitors (e.g., auristatins, maytansinoids, and tubulysins), DNA

damaging agents (e.g., Calicheamicins, Duocarmycins, Exatecans, and Pyrrolobenzodiazepines), immunomodulators (e.g., TLR agonists, STING agonists and inflammasome agonist), and any combination thereof (Antibody drug conjugate: the “biological missile” for targeted cancer therapy, *Sig Transduct Target Ther* 7, 93 (2022)). In some embodiments, D comprise or is anti-fibrotic agent. In some embodiments, D comprises or is Val-boroPro. In some embodiments, D comprises or is a cytolytic agent, e.g., TAM470.

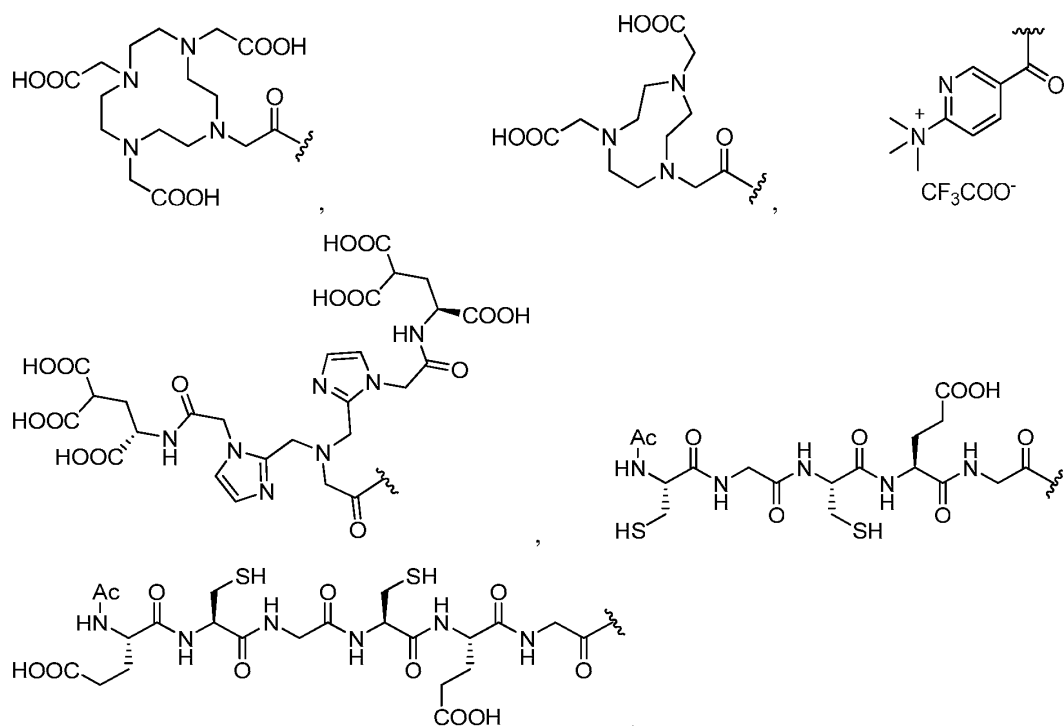
[0057] In some embodiments, D comprises or is one or more chemotherapy agents. Examples of chemotherapeutic agents used in cancer therapy include, for example, antimetabolites (e.g., folic acid, purine, and pyrimidine derivatives), alkylating agents (e.g., nitrogen mustards, nitrosoureas, platinum, alkyl sulfonates, hydrazines, triazenes, aziridines, spindle poison, cytotoxic agents, topoisomerase inhibitors and others), and hypomethylating agents (e.g., decitabine (5-azadeoxycytidine), zebularine, isothiocyanates, azacitidine (5-azacytidine), 5-fluoro-2'-deoxycytidine, 5,6-dihydro-5-azacytidine and others). Exemplary agents include Aclarubicin, Actinomycin, Alitretinoin, Altretamine, Aminopterin, Aminolevulinic acid, Amrubicin, Amsacrine, Anagrelide, Arsenic trioxide, Asparaginase, Atrasentan, Belotecan, Bexarotene, bendamustine, Bleomycin, Bortezomib, Busulfan, Camptothecin, Capecitabine, Carboplatin, Carboquone, Carmofur, Carmustine, Celecoxib, Chlorambucil, Chlormethine, Cisplatin, Cladribine, Clofarabine, Crisantaspase, Cyclophosphamide, Cytarabine, Dacarbazine, Dactinomycin, Daunorubicin, Decitabine, Demecolcine, Docetaxel, Doxorubicin, Efaproxiral, Elesclomol, Elsamitrucin, Enocitabine, Epirubicin, Estramustine, Etoglucid, Etoposide, Floxuridine, Fludarabine, Fluorouracil (5FU), Fotemustine, Gemcitabine, Gliadel implants, Hydroxycarbamide, Hydroxyurea, Idarubicin, Ifosfamide, Irinotecan, Irofulven, Ixabepilone, Larotaxel, Leucovorin, Liposomal doxorubicin, Liposomal daunorubicin, Lonidamine, Lomustine, Lucanthone, Mannosulfan, Masoprocol, Melphalan, Mercaptopurine, Mesna, Methotrexate, Methyl aminolevulinate, Mitobronitol, Mitoguazone, Mitotane, Mitomycin, Mitoxantrone, Nedaplatin, Nimustine, Oblimersen, Omacetaxine, Ortataxel, Oxaliplatin, Paclitaxel, Pegaspargase, Pemetrexed, Pentostatin, Pirarubicin, Pixantrone, Plicamycin, Porfimer sodium, Prednimustine, Procarbazine, Raltitrexed, Ranimustine, Rubitecan, Sapacitabine, Semustine, Sitimagene ceradenovec, Strataplatin, Streptozocin, Talaporfin, Tegafur-uracil, Temoporfin, Temozolomide, Teniposide, Tesetaxel, Testolactone, Tetranitrate, Thiotepa, Tiazofurine, Tioguanine, Tipifarnib, Topotecan, Trabectedin, Triaziquone, Triethylenemelamine,

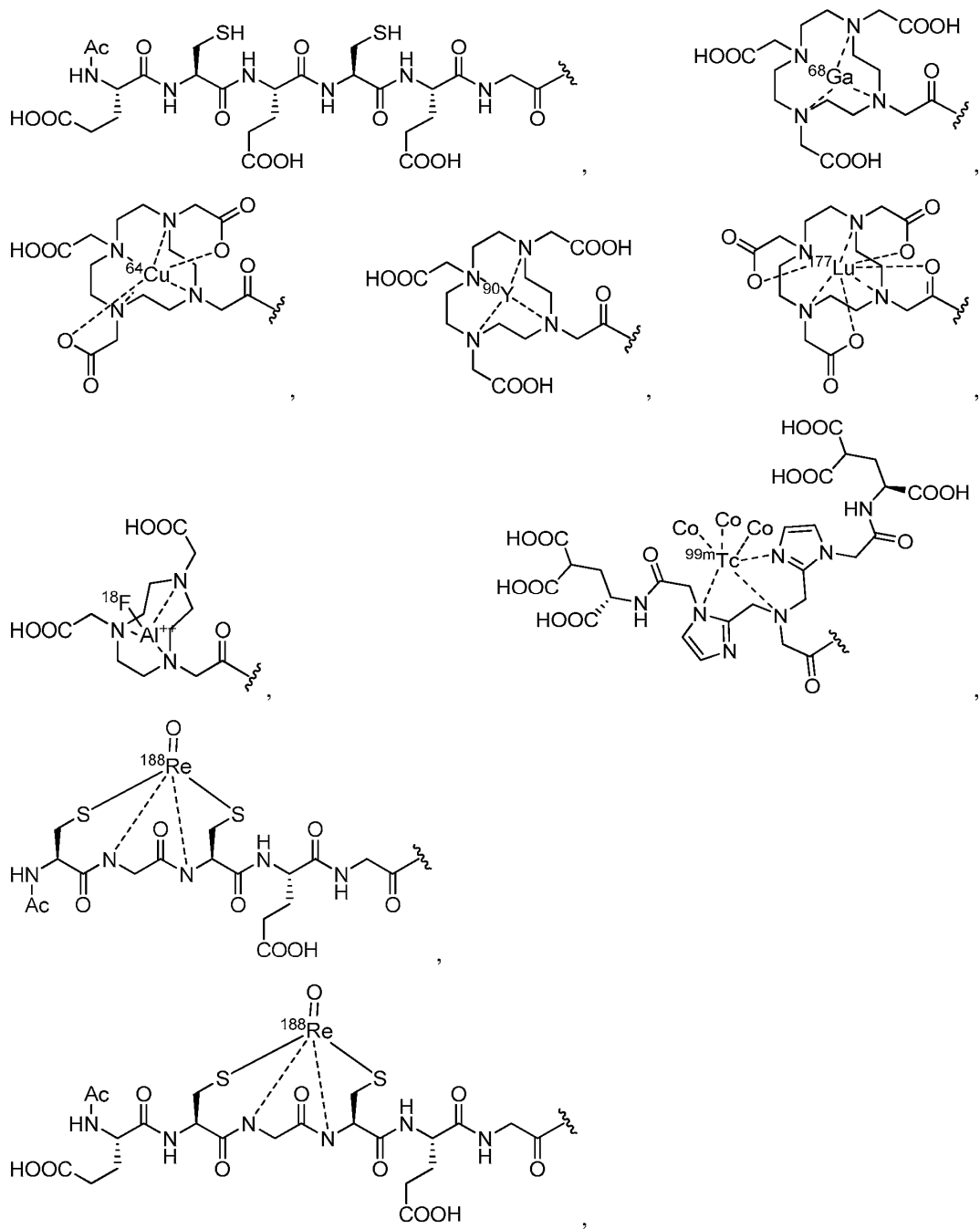
Triplatin, Tretinoin, Treosulfan, Trofosfamide, Uramustine, Valrubicin, Verteporfin, Vinblastine, Vincristine, Vindesine, Vinflunine, Vinorelbine, Vorinostat, Zorubicin, and other cytostatic or cytotoxic agents described herein.

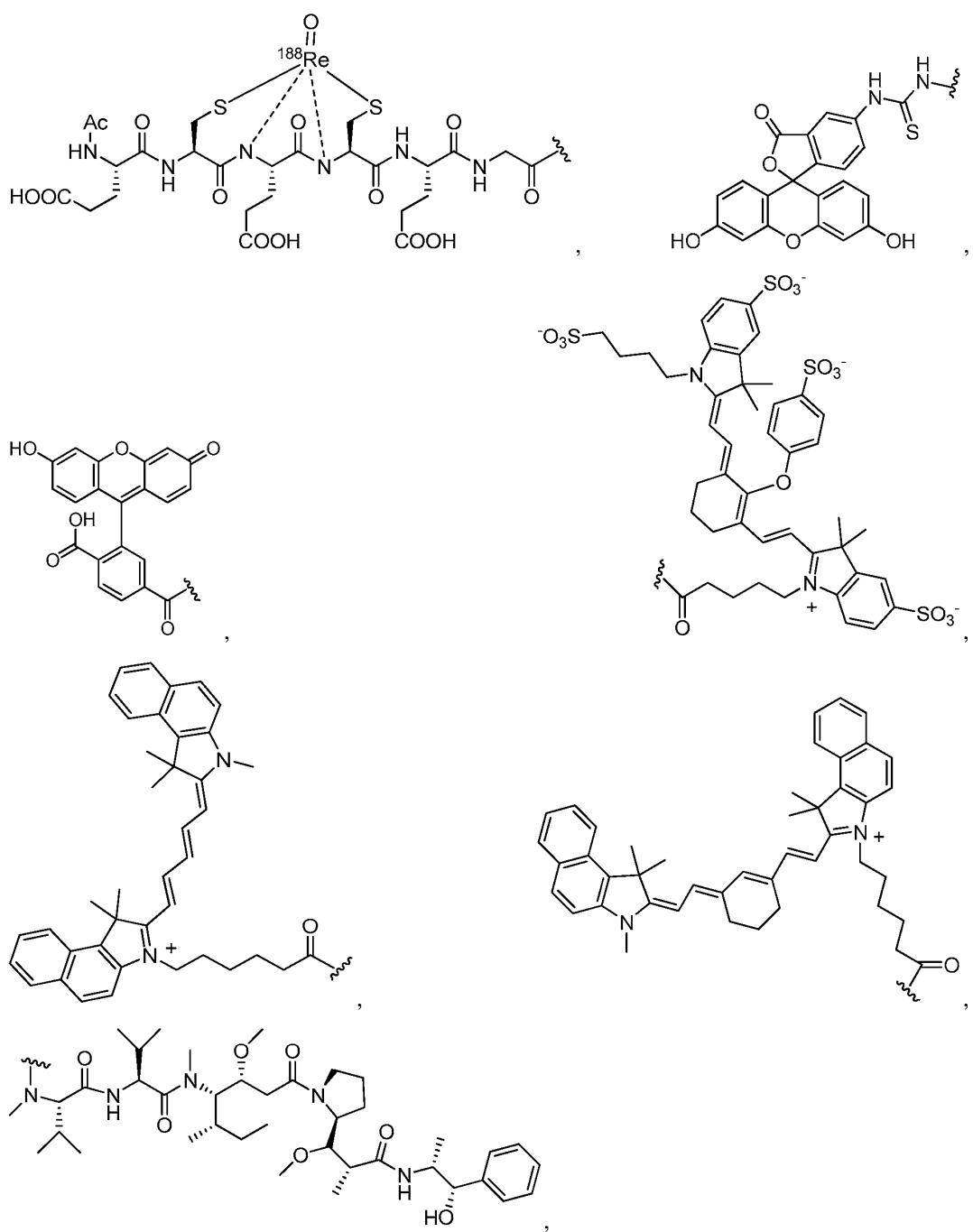
[0058] In some embodiments, the one or more drugs or agents of D are selected from the group consisting of, but not limited to, tyrosine kinase inhibitors, PI3K/mTOR inhibitors, PARP inhibitors such as Olaparib, Rucaparib, Niraparib, Talazoparib, Wee1 inhibitors, CDK4/6 inhibitors such as Palbociclib, Ribociclib, Abemaciclib, DNA-PK inhibitors, ATM inhibitors, ATR inhibitors, and any combination thereof.

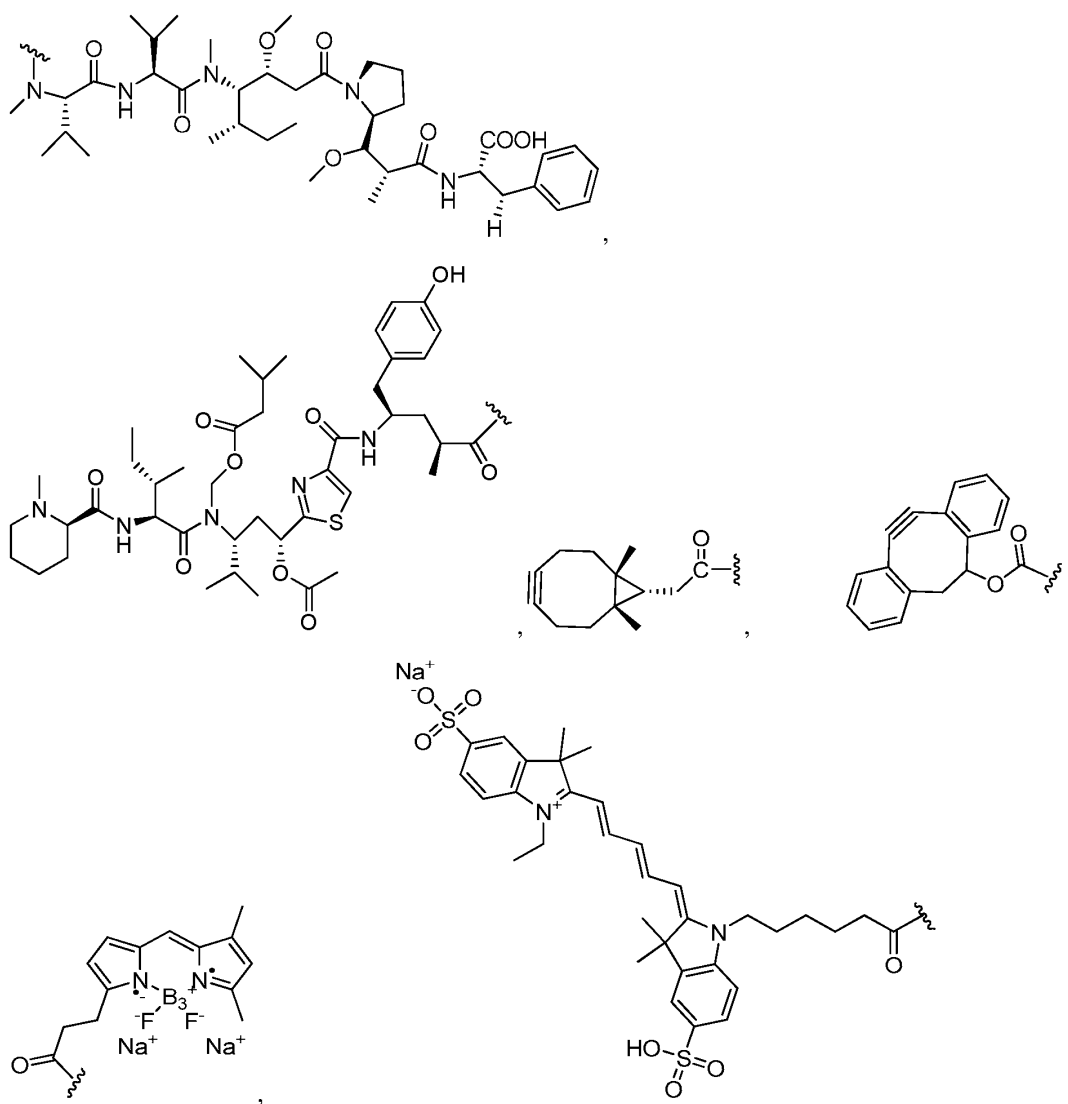
[0059] In some embodiments, D comprises or is a radioactive component, a metal chelating group, a chelating agent, a fluorescent dye, a contrast agent or any combination thereof. In some embodiment, D comprises or is a radionuclide. In some embodiments, D is a silicon-fluoride acceptor (SIFA), alone or in combination with a chelating agent. An example can be found in WO2023283627, the disclosure of which is incorporated herein by reference.

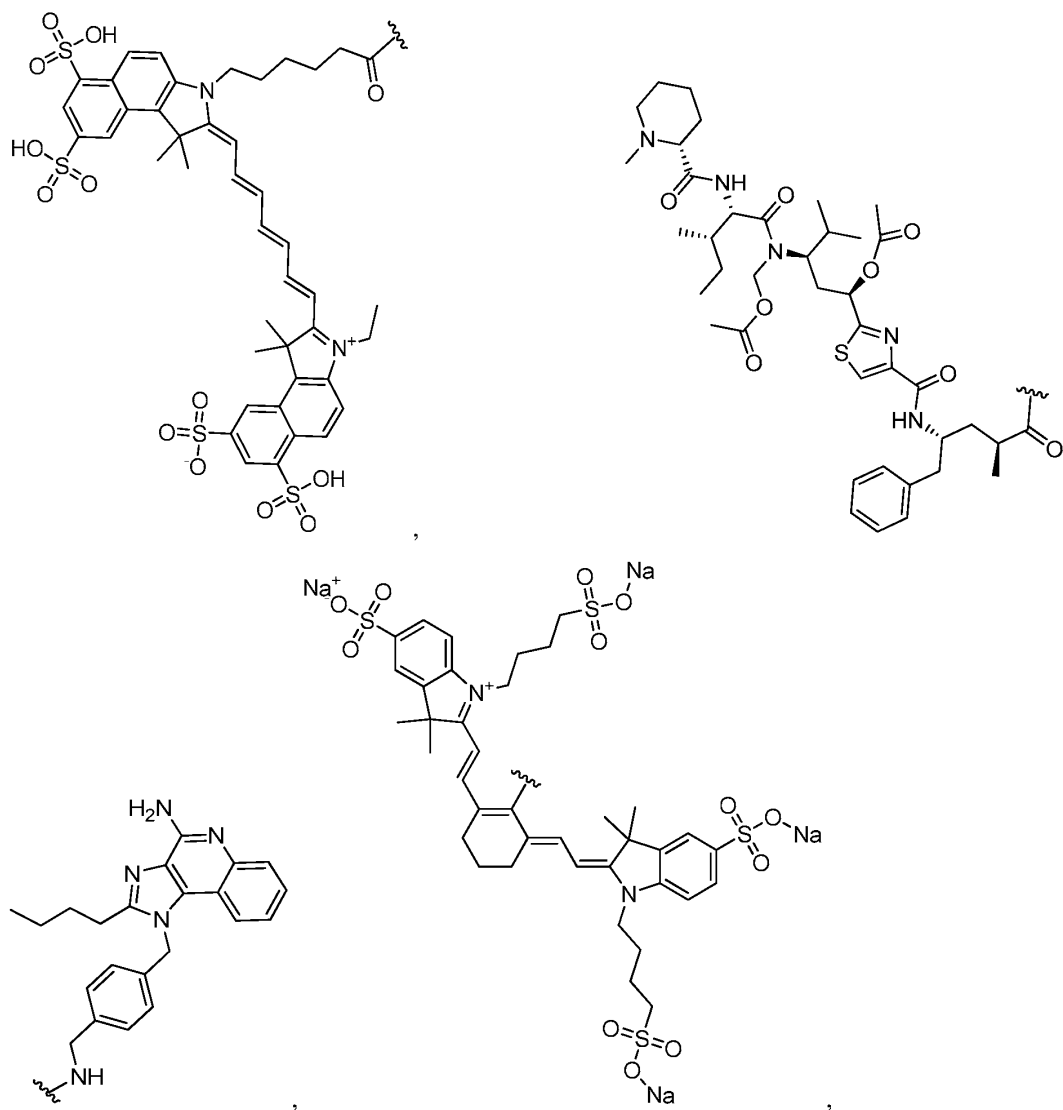
[0060] In some embodiments, D comprises or is

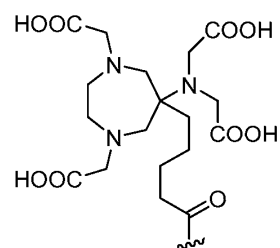
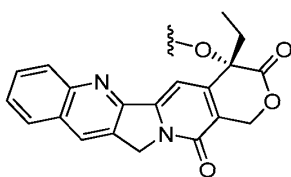
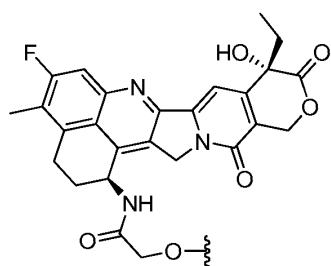
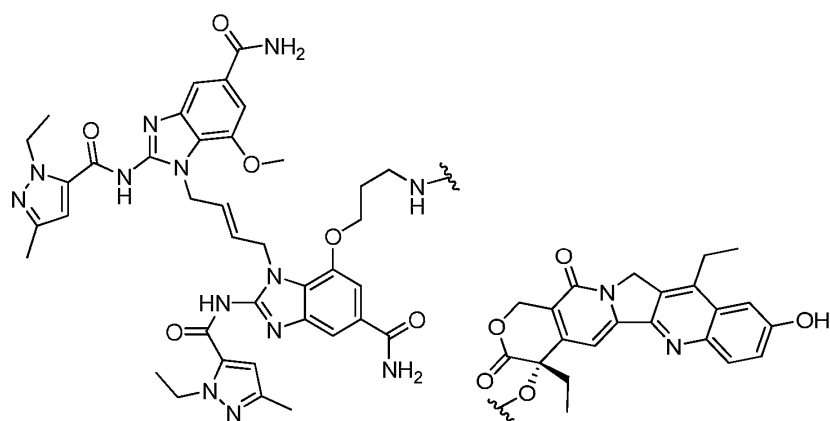
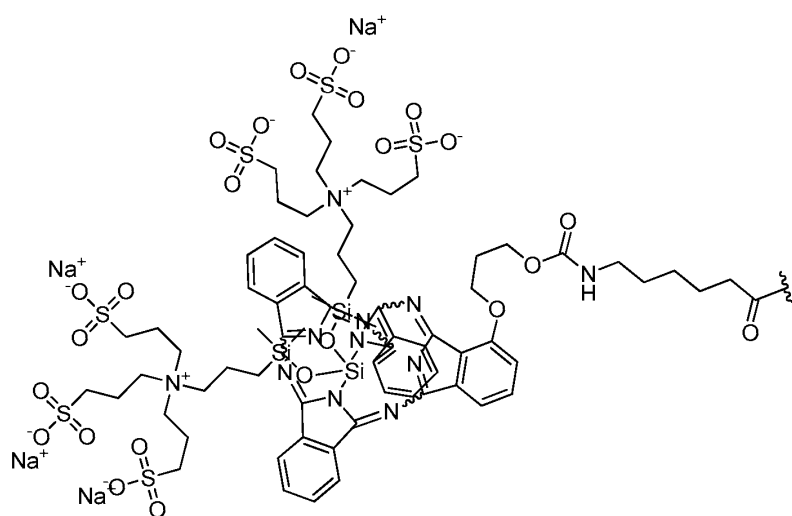


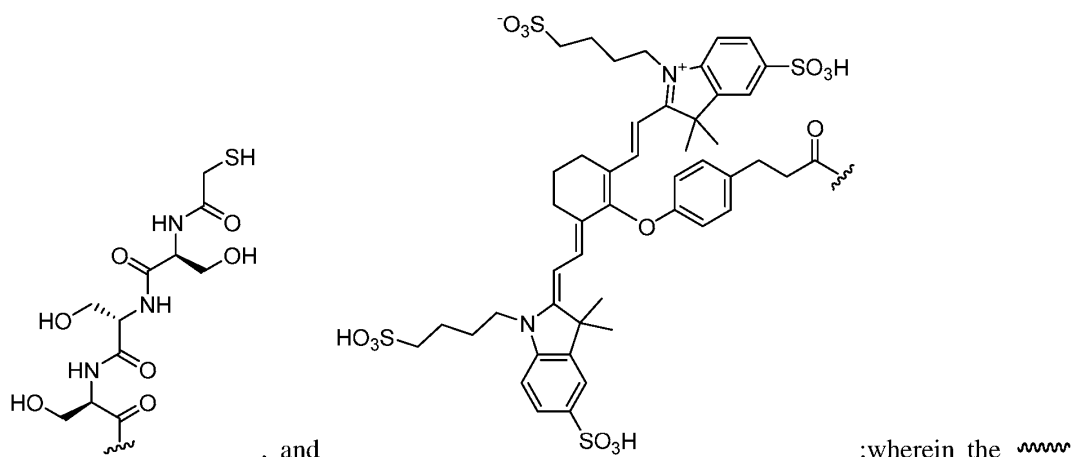




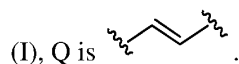








[0061] In some embodiments of a compound of formula (I), Q is a bond. In some embodiments of a compound of formula (I), Q is $-\text{CH}=\text{CH}-$. In some embodiments of a compound of formula



[0062] In some embodiments of a compound of formula (I), X is N. In some embodiments of a compound of formula (I), X is CR^2 . In some embodiments of a compound of formula (I), X is CH. In some embodiments of a compound of formula (I), X is $\text{C}-\text{OCH}_3$.

[0063] In some embodiments of a compound of formula (I), R^1 is hydrogen, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl, 3- to 6-membered heterocyclyl, $-\text{CN}$, halogen, C_1 - C_6 alkoxy, C_1 - C_6 haloalkoxy, C_1 - C_6 haloalkyl, $-\text{OR}^3$, $-\text{SR}^3$, $-\text{S}(\text{O})_2\text{R}^3$, $-\text{S}(\text{O})_2\text{NR}^4\text{R}^5$, $-\text{NR}^3\text{S}(\text{O})_2\text{R}^4$, $-\text{NR}^4\text{R}^5$, $-\text{C}(\text{O})\text{R}^3$, $-\text{NR}^3\text{C}(\text{O})\text{R}^4$, $-\text{NR}^3\text{C}(\text{O})\text{NR}^4\text{R}^5$, $-\text{C}(\text{O})\text{OR}^3$, $-\text{C}(\text{O})\text{ONR}^4\text{R}^5$ or $-\text{C}(\text{O})\text{NR}^4\text{R}^5$, wherein each of R^1 is optionally substituted by R^{10} . In some embodiments of a compound of formula (I), R^1 is hydrogen.

[0064] In some embodiments of a compound of formula (I), R^2 is hydrogen, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl, 3- to 6-membered heterocyclyl, $-\text{CN}$, halogen, C_1 - C_6 alkoxy, C_1 - C_6 haloalkoxy, C_1 - C_6 haloalkyl, $-\text{OR}^3$, $-\text{SR}^3$, $-\text{S}(\text{O})_2\text{R}^3$, $-\text{S}(\text{O})_2\text{NR}^4\text{R}^5$, $-\text{NR}^3\text{S}(\text{O})_2\text{R}^4$, $-\text{NR}^4\text{R}^5$, $-\text{C}(\text{O})\text{R}^3$, $-\text{NR}^3\text{C}(\text{O})\text{R}^4$, $-\text{NR}^3\text{C}(\text{O})\text{NR}^4\text{R}^5$, $-\text{C}(\text{O})\text{OR}^3$, $-\text{C}(\text{O})\text{ONR}^4\text{R}^5$ or $-\text{C}(\text{O})\text{NR}^4\text{R}^5$, wherein each of R^2 is optionally substituted by R^{10} . In some embodiments of a compound of formula (I), R^2 is hydrogen.

[0065] In some embodiments of a compound of formula (I), p is 0. In some embodiments of a compound of formula (I), p is 1. In some embodiments of a compound of formula (I), p is 2.

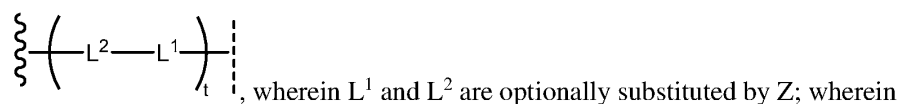
[0066] In some embodiments of a compound of formula (I), R⁶ is hydrogen. In some embodiments, R⁶ is C₁-C₆ alkyl. In some embodiments, R² and R⁶ are taken together with the atom to which they attached to form a 5- to 7- membered heterocyclyl optionally substituted by R¹¹.

[0067] In some embodiments of a compound of formula (I), m is 1 and n is 0. In some embodiments of a compound of formula (I), m is 0 and n is 1. In some embodiments of a compound of formula (I), m is 1 and n is 1.

[0068] A linker can be cleavable or non-cleavable. Without being bound to any particular theory, a linker between a payload and a FAP ligand in a conjugate can ensure the payload attached to the FAP ligand while the conjugate circulates in plasma. Many linkers that have been used in antibody-drug conjugates can be used in accordance with the present invention. Exemplary linkers can be found in An Insight into FDA Approved Antibody-Drug Conjugates for Cancer Therapy, *Molecules*. 2021 Sep 27; 26(19):5847, the disclosure of which is incorporated herein by reference. In certain embodiments, the linkers are stable in physiological conditions. In some embodiments, a linker is cleavable, for instance, able to release at least the payload portion in the presence of an enzyme or at a particular pH range or value. In some embodiments, a linker comprises an enzyme-cleavable moiety. Illustrative enzyme-cleavable moieties include, but are not limited to, peptide bonds, ester linkages, hydrazones, and disulfide linkages.

[0069] In some embodiments, a linker L of the conjugates described herein is a moiety, for instance a divalent moiety, that covalently links a FAP ligand described herein to a payload. In other embodiments, a linker L is a trivalent or multivalent moiety that covalently links one or more FAP ligands described herein to a payload.

[0070] In some embodiments of a compound of formula (I), L is, independently at each occurrence, is a linker. In some embodiments, the linker comprises or has a structure:



t is an integer from 1 to 10;

~~~~~ denotes attachment to D and ----- denotes attachment to A;

$L^1$  is, independently at each occurrence, absent or C<sub>3</sub>-C<sub>6</sub> cycloalkyl, C<sub>6</sub>-aryl, 5- to 6-membered heterocyclyl or 5- to 6-membered heteroaryl, each of which is optionally substituted by R<sup>12</sup>;

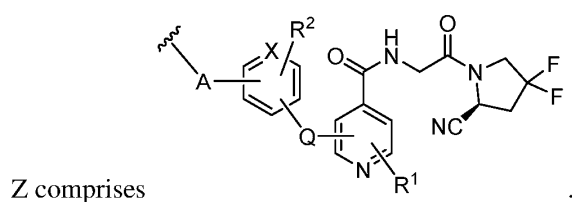
$L^2$  is, independently at each occurrence, absent or O, S, NH, N(C<sub>1</sub>-C<sub>6</sub> alkyl), C<sub>1</sub>-C<sub>10</sub> alkylene, 2- to 14-membered heteroalkylene, -C(=O)O-, -O(C=O)-, -CONH-, -CON(C<sub>1</sub>-C<sub>6</sub> alkyl)-, -NHCO-, -N(C<sub>1</sub>-C<sub>6</sub> alkyl)CO-, SO<sub>2</sub>, -SO<sub>2</sub>NH-, -SO<sub>2</sub>N(C<sub>1</sub>-C<sub>6</sub> alkyl)-, -NHSO<sub>2</sub>-, -N(C<sub>1</sub>-C<sub>6</sub> alkyl)SO<sub>2</sub>-, -S-S-, -(OCH<sub>2</sub>CH<sub>2</sub>)<sub>v</sub>-, -(CH<sub>2</sub>CH<sub>2</sub>O)<sub>v</sub>-, or -C(R<sup>m</sup>)=N-, wherein the C<sub>1</sub>-C<sub>10</sub> alkylene and 2- to 14-membered heteroalkylene of each  $L^2$ , when present, are optionally and independently substituted with R<sup>m</sup> and R<sup>n</sup>, wherein

$v$  is independently an integer from 1 to 3;

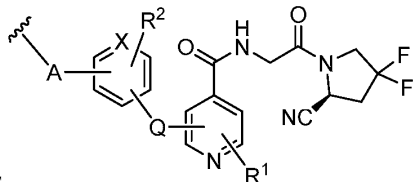
R<sup>m</sup> and R<sup>n</sup> are independently hydrogen, carboxyl, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>3</sub>-C<sub>6</sub> cycloalkyl, C<sub>6</sub>-C<sub>10</sub> aryl, 5- to 6-membered heterocyclyl or 5- to 10-membered heteroaryl, -C<sub>1</sub>-C<sub>3</sub>-alkylene(C<sub>3</sub>-C<sub>6</sub> cycloalkyl), -C<sub>1</sub>-C<sub>3</sub>-alkylene(C<sub>6</sub>-C<sub>10</sub> aryl), -C<sub>1</sub>-C<sub>3</sub>-alkylene(5- to 6-membered heterocyclyl), -C<sub>1</sub>-C<sub>3</sub>-alkylene(5- to 10-membered heteroaryl), or -C<sub>1</sub>-C<sub>3</sub>-alkylene(NHC(O)NH<sub>2</sub>), each of which is optionally substituted by R<sup>17</sup>; and

R<sup>12</sup> and R<sup>17</sup> are independently oxo, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>3</sub>-C<sub>6</sub> cycloalkyl, 3- to 6-membered heterocyclyl, -CN, halogen, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkyl, -OR<sup>14</sup>, -SR<sup>14</sup>, -S(O)<sub>2</sub>R<sup>14</sup>, -S(O)<sub>2</sub>NR<sup>15</sup>R<sup>16</sup>, -NR<sup>14</sup>S(O)<sub>2</sub>R<sup>15</sup>, -NR<sup>15</sup>R<sup>16</sup>, -C(O)R<sup>14</sup>, -NR<sup>14</sup>C(O)R<sup>15</sup>, -NR<sup>14</sup>C(O)NR<sup>15</sup>R<sup>16</sup>, -C(O)OR<sup>14</sup>, -C(O)ONR<sup>15</sup>R<sup>16</sup> or -C(O)NR<sup>15</sup>R<sup>16</sup>;

and

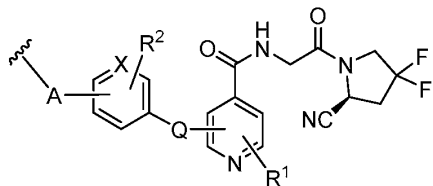


[0071] In some embodiments, at least one of  $L^1$  and  $L^2$  is substituted by Z, wherein Z comprises

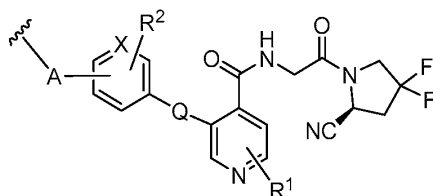


or is

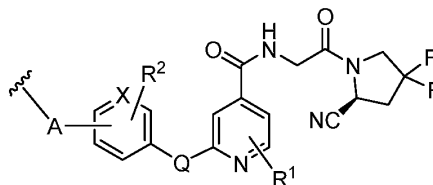
. In some embodiments, Z comprises or is



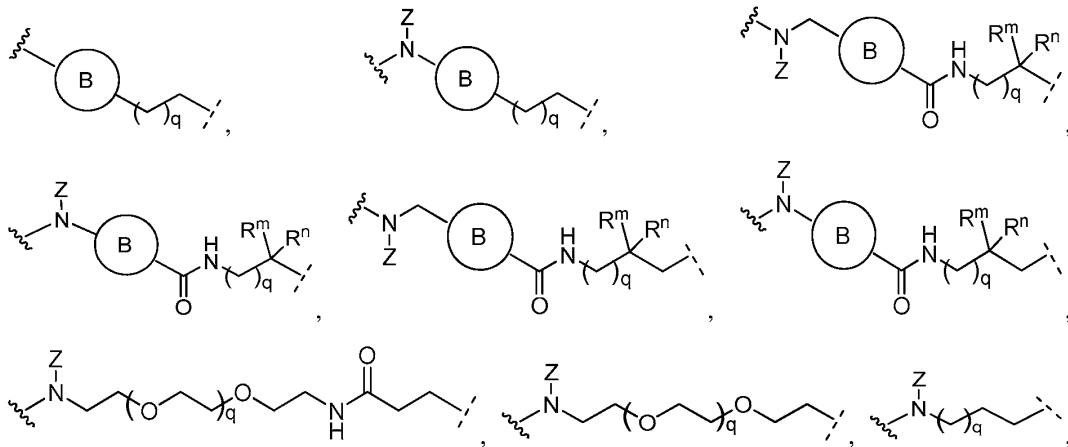
. In some embodiments, Z comprises or is

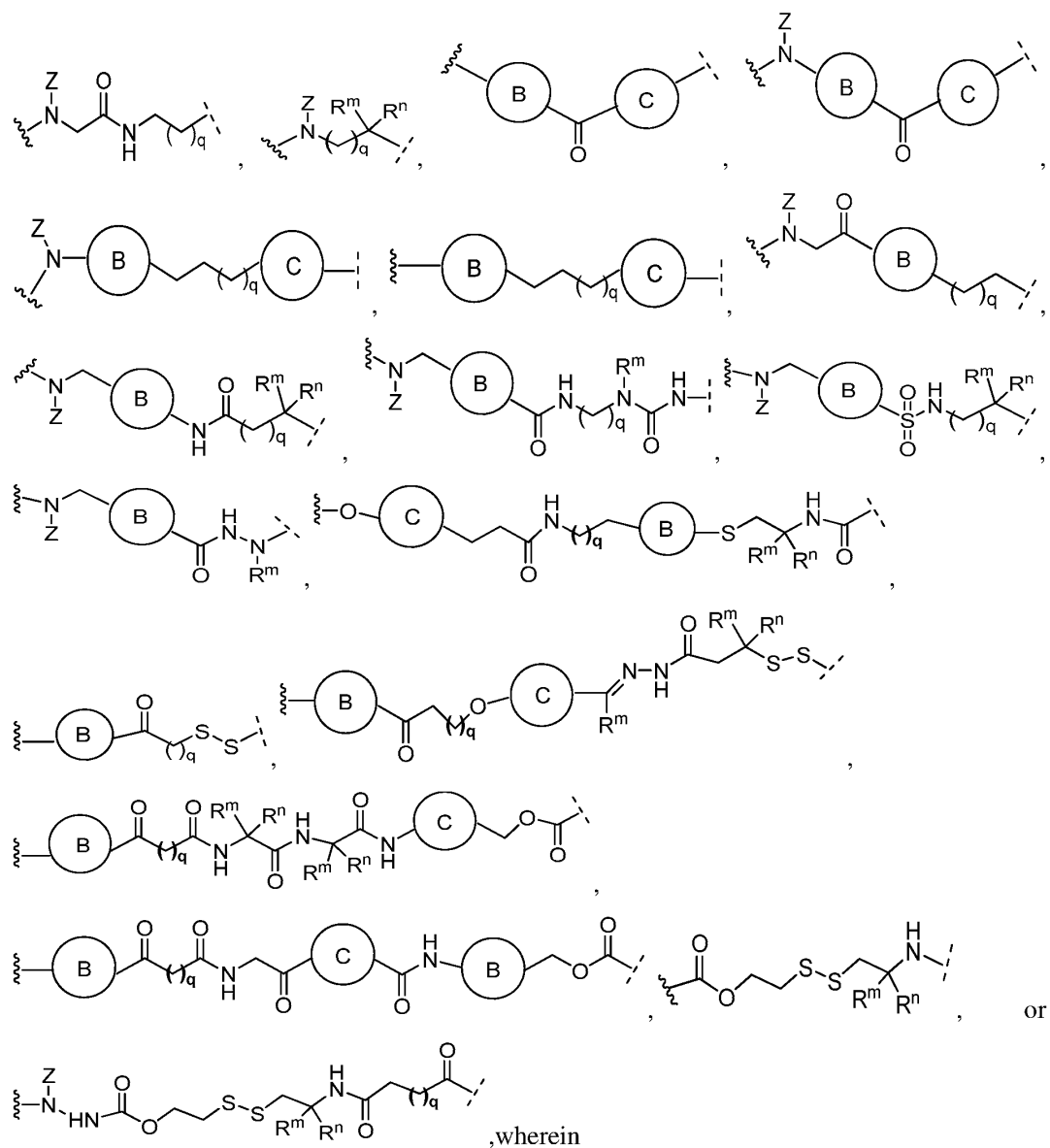


. In some embodiments, Z comprises or is



[0072] In some embodiments, L comprises or is 5- to 6-membered heterocyclyl,



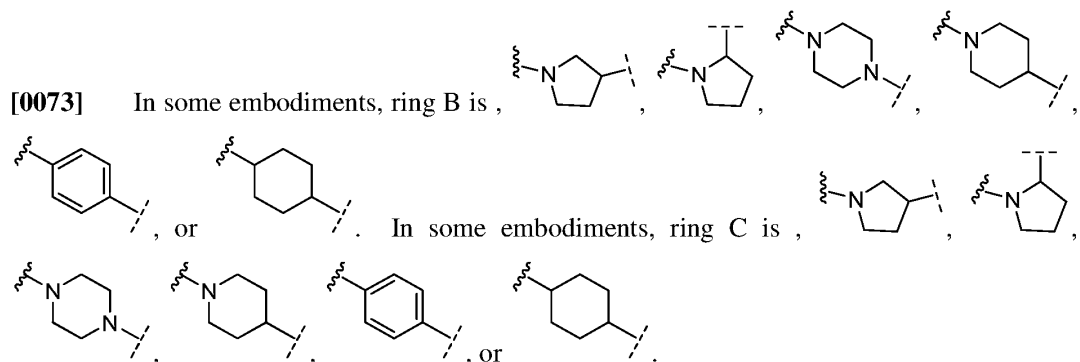


ring B is C<sub>3</sub>-C<sub>6</sub> cycloalkyl, C<sub>6</sub>-C<sub>10</sub> aryl, 5- to 6-membered heterocyclyl or 5- to 6-membered heteroaryl, each of which is optionally substituted by R<sup>b</sup>;

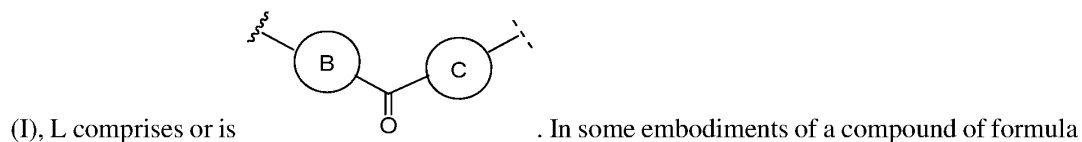
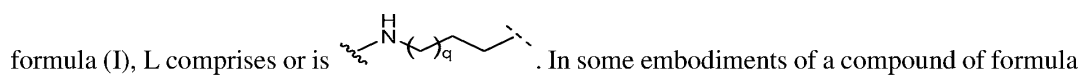
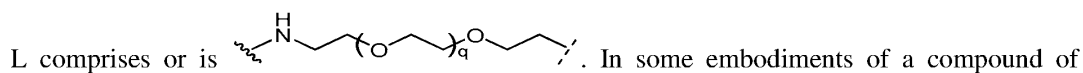
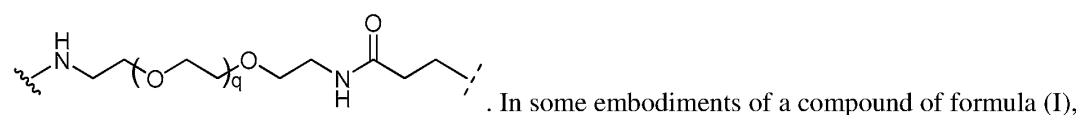
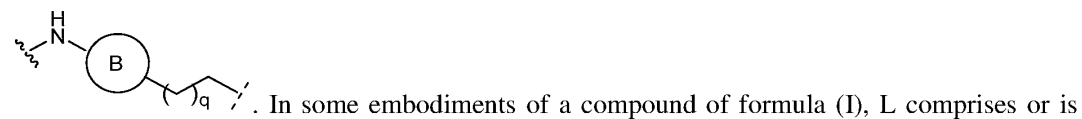
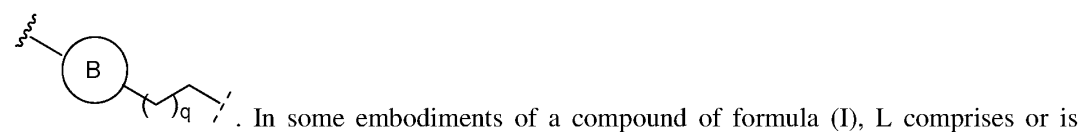
ring C is C<sub>3</sub>-C<sub>6</sub> cycloalkyl, C<sub>6</sub>-C<sub>10</sub> aryl, 5- to 6-membered heterocyclyl or 5- to 6-membered heteroaryl, each of which is optionally substituted by R<sup>c</sup>; and

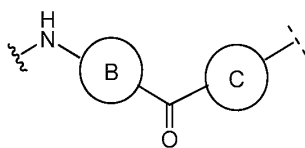
$R^b$  and  $R^c$  are independently oxo,  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_3$ - $C_6$  cycloalkyl, 3- to 6-membered heterocyclyl, -CN, halogen,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  haloalkoxy,  $C_1$ - $C_6$  haloalkyl,  $-OR^{14}$ ,  $-SR^{14}$ ,  $-S(O)_2R^{14}$ ,  $-S(O)_2NR^{15}R^{16}$ ,  $-NR^{14}S(O)_2R^{15}$ ,  $-NR^{15}R^{16}$ ,  $-C(O)R^{14}$ ,  $-NR^{14}C(O)R^{15}$ ,  $-NR^{14}C(O)NR^{15}R^{16}$ ,  $-C(O)OR^{14}$ ,  $-C(O)ONR^{15}R^{16}$  or  $-C(O)NR^{15}R^{16}$ , and

each  $q$  is independently an integer from 1 to 3.

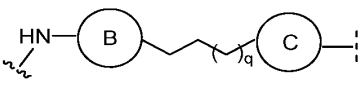


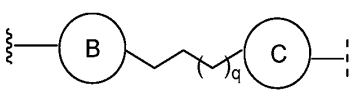
[0074] In some embodiments of a compound of formula (I), L comprises or is 5- to 6-membered heterocyclyl. In some embodiments of a compound of formula (I), L comprises or is

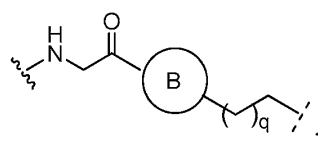


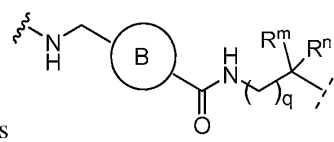


(I), L comprises or is . In some embodiments of a compound of

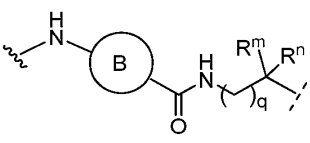
formula (I), L comprises or is  . In some embodiments of a

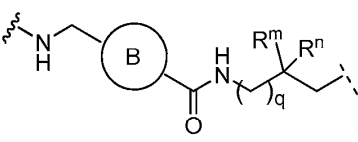
compound of formula (I), L comprises or is  . In some

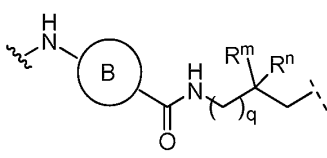
embodiments of a compound of formula (I), L comprises or is  . In

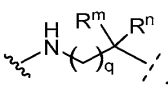
some embodiments of a compound of formula (I), L comprises or is  .

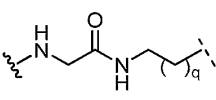
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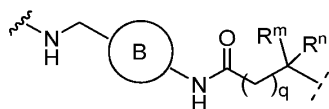
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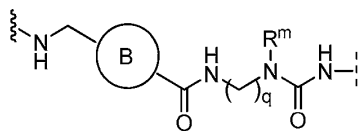
comprises or is  . In some embodiments of a compound of formula

(I), L comprises or is  . In some embodiments of a compound of formula (I), L

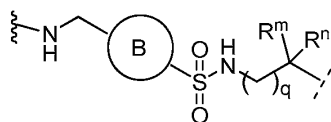
comprises or is  . In some embodiments of a compound of formula (I), L



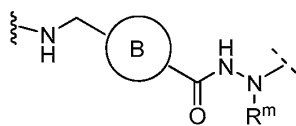
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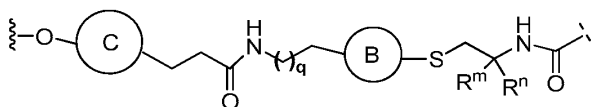
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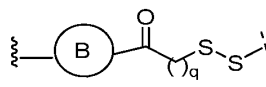
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of formula (I), L comprises or is . In some embodiments of a compound

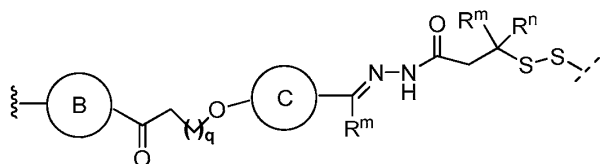


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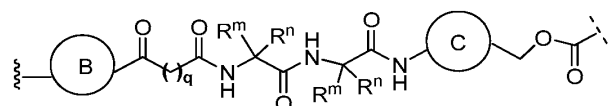


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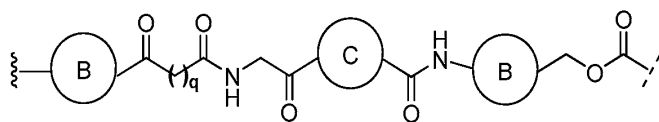


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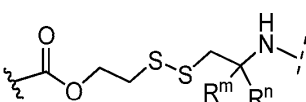
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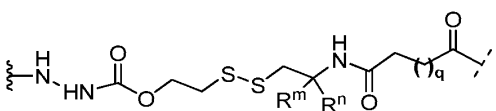
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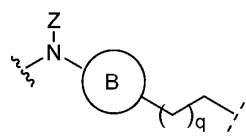
. In some embodiments of a

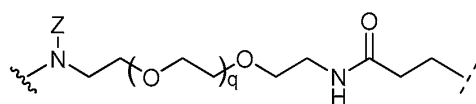


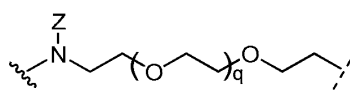
compound of formula (I), L comprises or is . In some embodiments

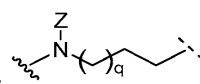
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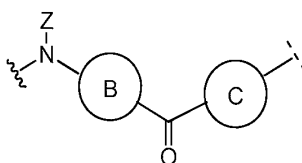
[0075] In some embodiments of a compound of formula (I), L comprises or is

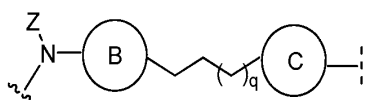
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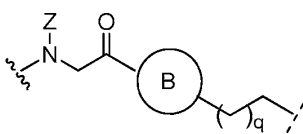
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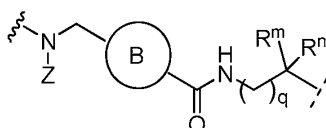
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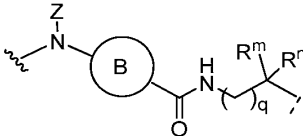
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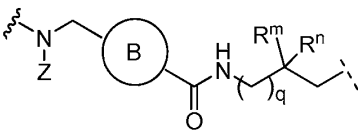
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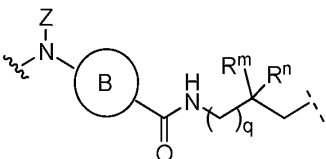
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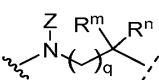
a compound of formula (I), L comprises or is . In some embodiments

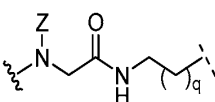
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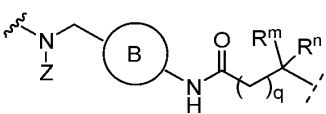
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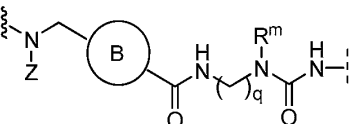
In some embodiments of a compound of formula (I), L comprises or is

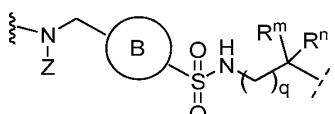
. In some embodiments of a compound of formula (I), L comprises

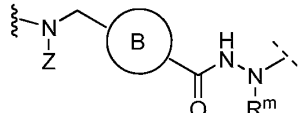
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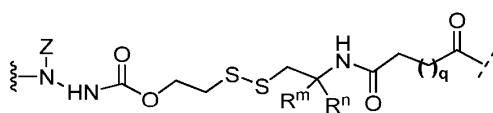
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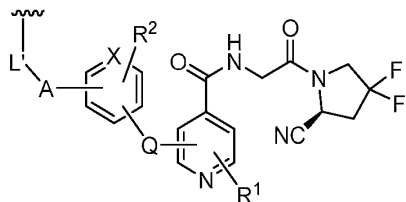
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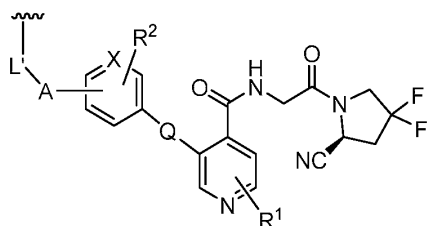
(I), L comprises or is . In some embodiments of a compound of formula

(I), L comprises or is .

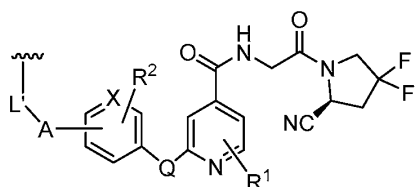
[0076] In some embodiments, Z is present and represented by



. In some embodiments, Z is present and represented by



. In some embodiments, Z is present and represented by



[0077] In some embodiments of a compound of formula (I), L' is a linker. In some embodiments, L' is different from L. In some embodiments, L' is the same as L.

[0078] In some embodiments of a compound of formula (I), B is C<sub>3</sub>-C<sub>6</sub> cycloalkyl optionally substituted by R<sup>b</sup>. In some embodiments of a compound of formula (I), B is 5- to 6-membered heterocyclyl optionally substituted by R<sup>b</sup>. In some embodiments of a compound of formula (I), B is 5- to 6-membered heteroaryl optionally substituted by R<sup>b</sup>.

[0079] In some embodiments of a compound of formula (I), B is piperazinyl optionally substituted by R<sup>b</sup>.

[0080] In some embodiments of a compound of formula (I), C is C<sub>3</sub>-C<sub>6</sub> cycloalkyl optionally substituted by R<sup>c</sup>. In some embodiments of a compound of formula (I), C is 5- to 6-membered heterocyclyl optionally substituted by R<sup>c</sup>. In some embodiments of a compound of formula (I), C is 5- to 6-membered heteroaryl optionally substituted by R<sup>c</sup>.

[0081] In some embodiments of a compound of formula (I), C is phenyl and piperazinyl, each of C is optionally substituted by R<sup>c</sup>.

**[0082]** In some embodiments of a compound of formula (I),  $q$  is 0. In some embodiments of a compound of formula (I),  $q$  is 1. In some embodiments of a compound of formula (I),  $q$  is 2. In some embodiments of a compound of formula (I),  $q$  is 3.

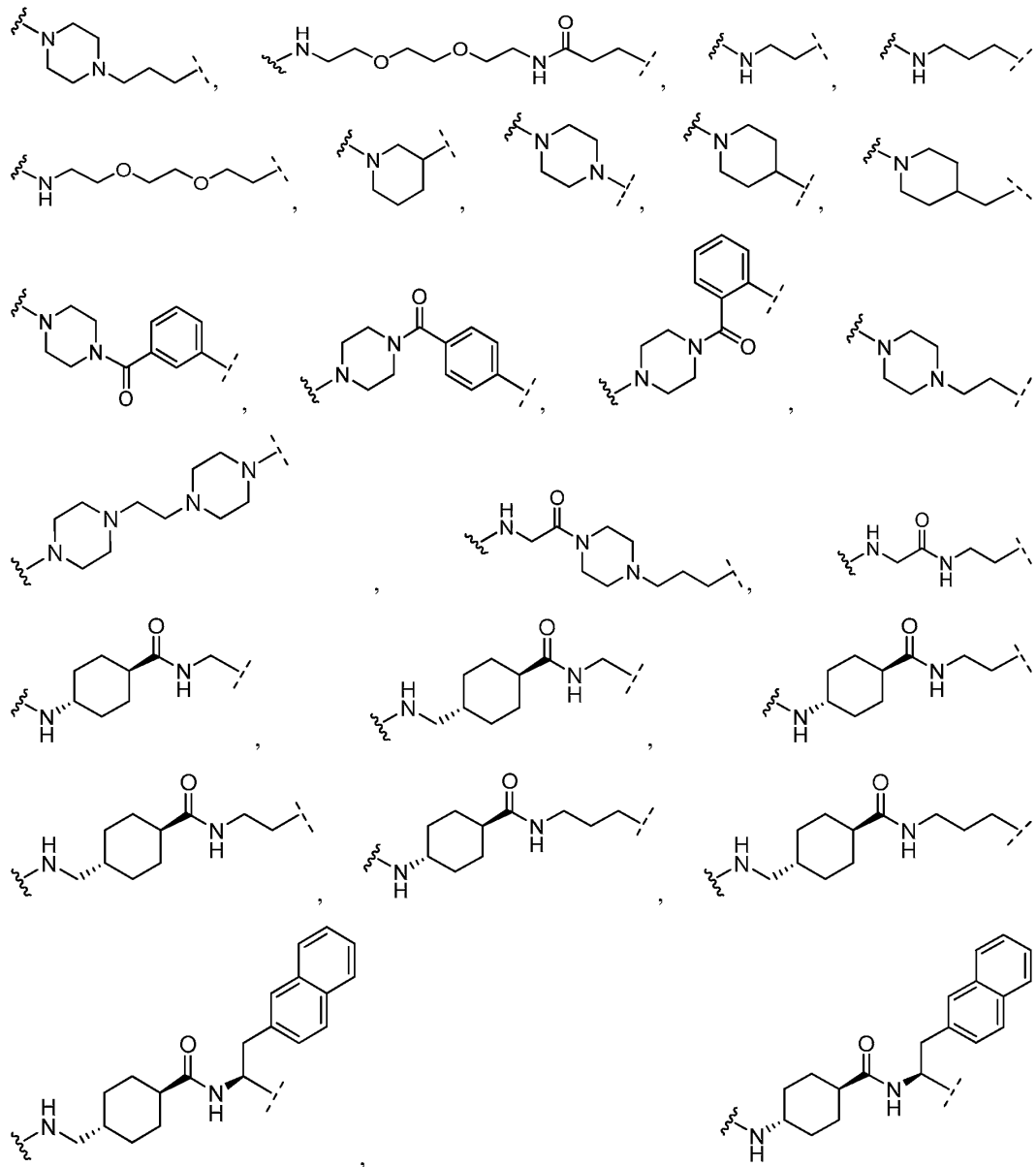
**[0083]** In some embodiments of a compound of formula (I),  $R^m$  and  $R^n$  are independently hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>3</sub>-C<sub>6</sub> cycloalkyl, C<sub>6</sub>-C<sub>10</sub> aryl, 5- to 6-membered heterocyclyl, 5- to 10-membered heteroaryl, -C<sub>1</sub>-C<sub>3</sub>-alkylene(C<sub>3</sub>-C<sub>6</sub> cycloalkyl), -C<sub>1</sub>-C<sub>3</sub>-alkylene(C<sub>6</sub>-C<sub>10</sub> aryl), -C<sub>1</sub>-C<sub>3</sub>-alkylene(5- to 6-membered heterocyclyl) or -C<sub>1</sub>-C<sub>3</sub>-alkylene(5- to 10-membered heteroaryl), each of which is optionally substituted by  $R^{17}$ ;

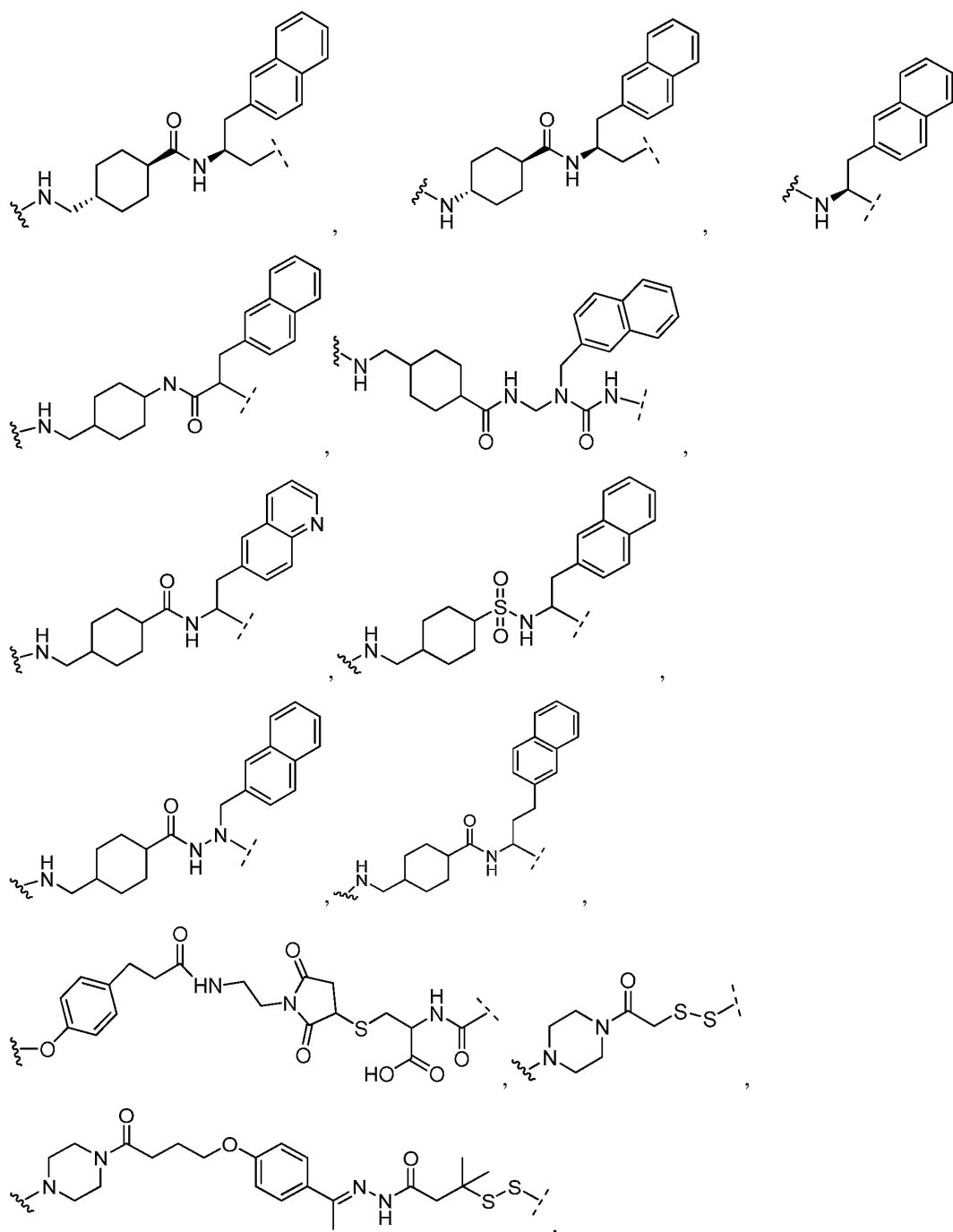
**[0084]** In some embodiments of a compound of formula (I),  $R^m$  is hydrogen. In some embodiments of a compound of formula (I),  $R^m$  is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^m$  is C<sub>3</sub>-C<sub>6</sub> cycloalkyl optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^m$  is C<sub>6</sub>-C<sub>10</sub> aryl optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^m$  is 5- to 6-membered heterocyclyl optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^m$  is 5- to 10-membered heteroaryl optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^m$  is -C<sub>1</sub>-C<sub>3</sub>-alkylene(C<sub>3</sub>-C<sub>6</sub> cycloalkyl) optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^m$  is -C<sub>1</sub>-C<sub>3</sub>-alkylene(C<sub>6</sub>-C<sub>10</sub> aryl) optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^m$  is -C<sub>1</sub>-C<sub>3</sub>-alkylene(5- to 6-membered heterocyclyl) optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^m$  is -C<sub>1</sub>-C<sub>3</sub>-alkylene(5- to 10-membered heteroaryl) optionally substituted by  $R^{17}$ .

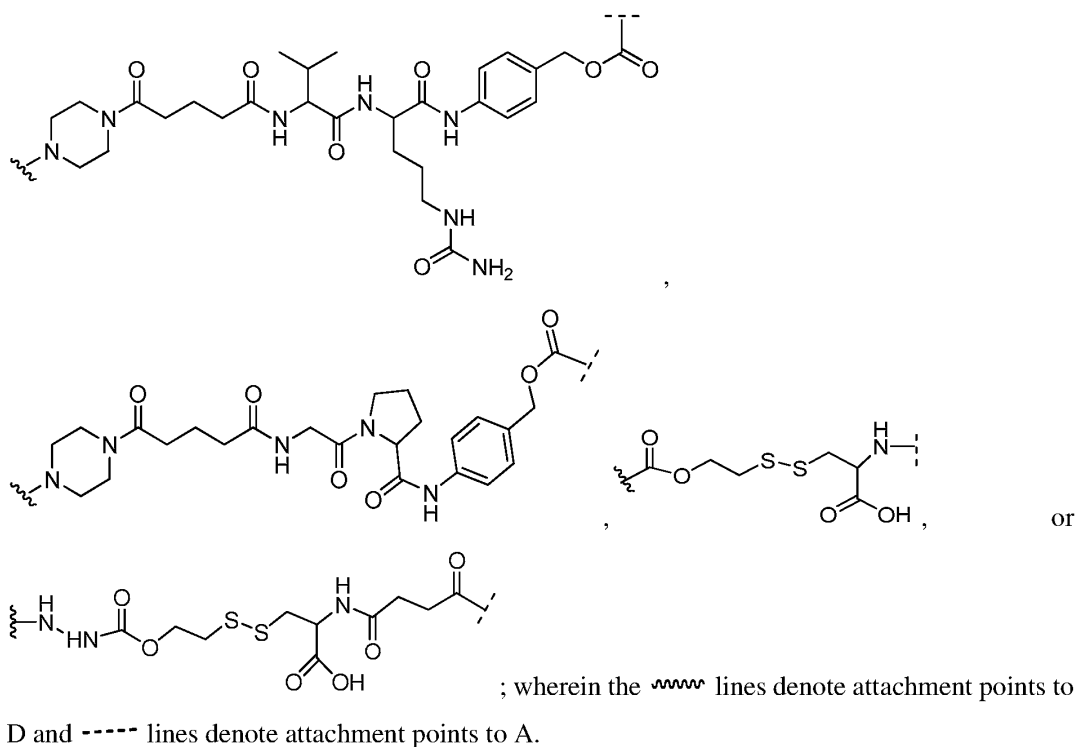
**[0085]** In some embodiments of a compound of formula (I),  $R^n$  is hydrogen. In some embodiments of a compound of formula (I),  $R^n$  is C<sub>1</sub>-C<sub>6</sub> alkyl optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^n$  is C<sub>3</sub>-C<sub>6</sub> cycloalkyl optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^n$  is C<sub>6</sub>-C<sub>10</sub> aryl optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^n$  is 5- to 6-membered heterocyclyl optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^n$  is 5- to 10-membered heteroaryl optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^n$  is -C<sub>1</sub>-C<sub>3</sub>-alkylene(C<sub>3</sub>-C<sub>6</sub> cycloalkyl) optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^n$  is -C<sub>1</sub>-C<sub>3</sub>-alkylene(C<sub>6</sub>-C<sub>10</sub> aryl) optionally substituted by  $R^{17}$ . In some embodiments of a compound of formula (I),  $R^n$  is -C<sub>1</sub>-C<sub>3</sub>-alkylene(5-

to 6-membered heterocyclyl) optionally substituted by R<sup>17</sup>. In some embodiments of a compound of formula (I), R<sup>n</sup> is -C<sub>1</sub>-C<sub>3</sub>-alkylene(5- to 10-membered heteroaryl) optionally substituted by R<sup>17</sup>.

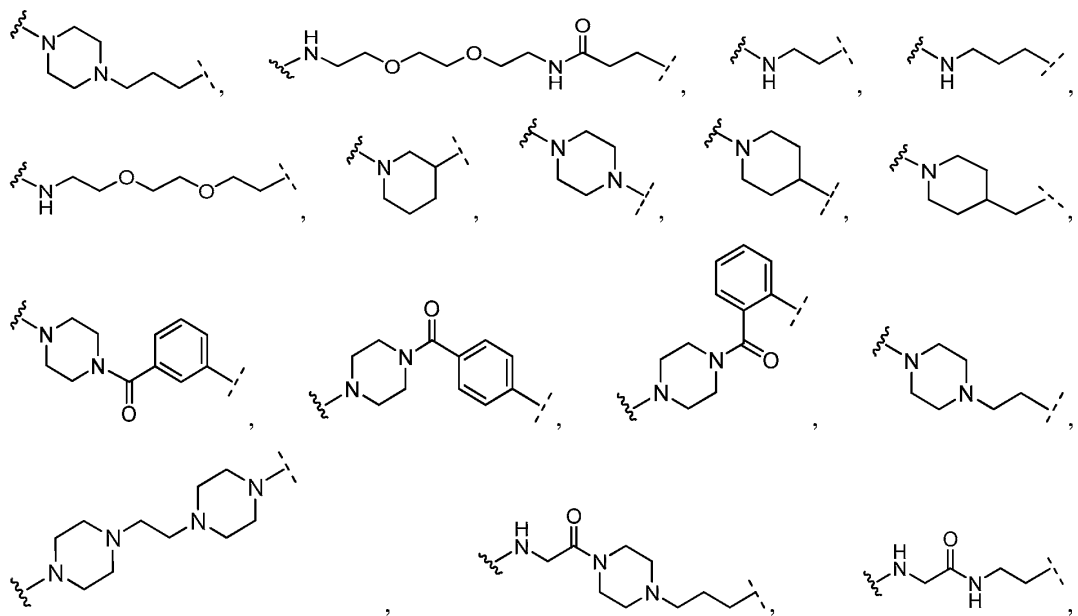
[0086] In some embodiments of a compound of formula (I), L comprises or is

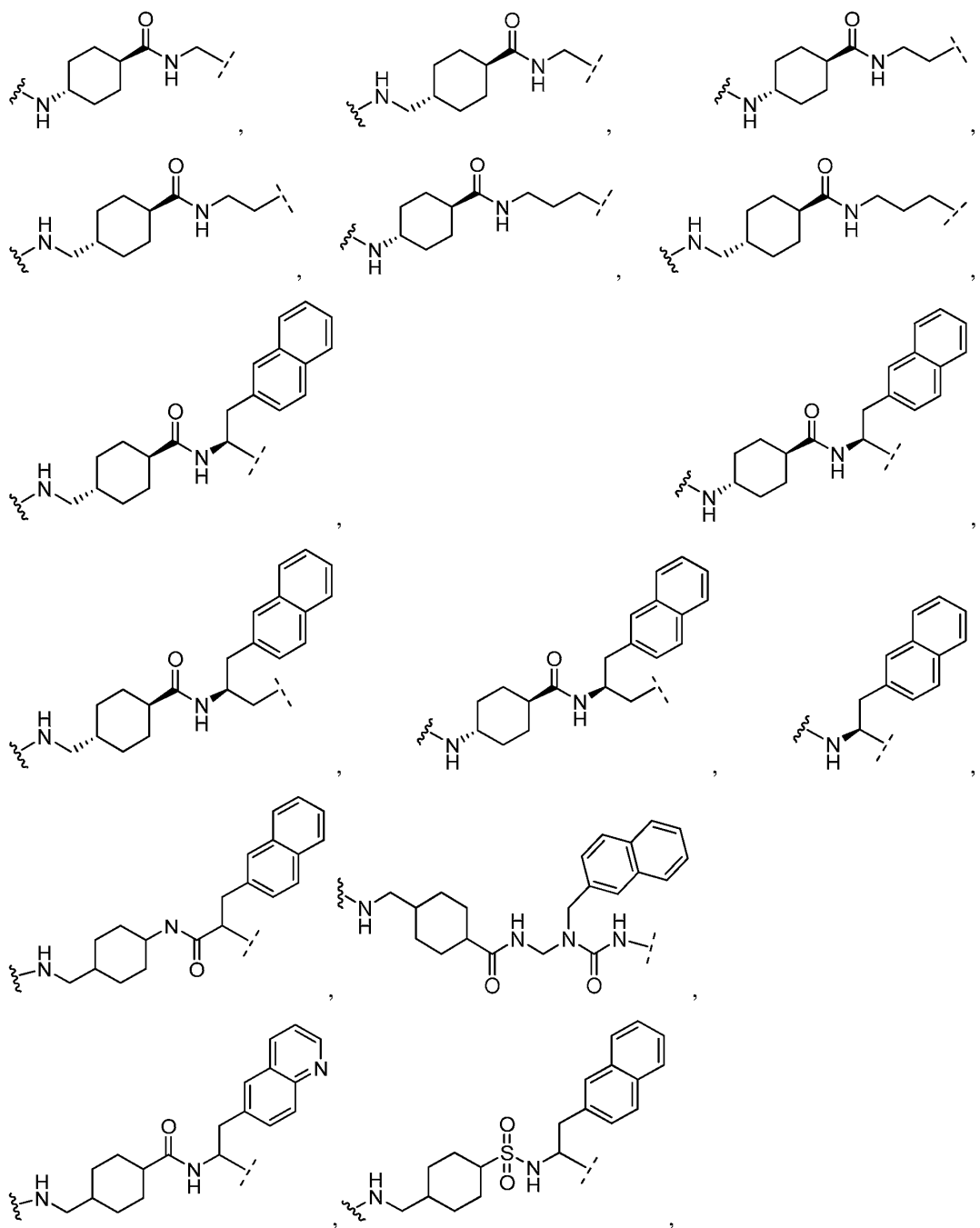




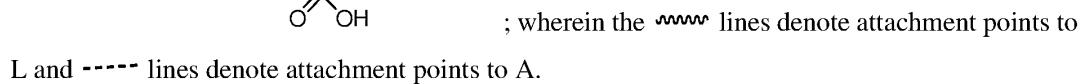


[0087] In some embodiments of a compound of formula (I), L' comprises or is







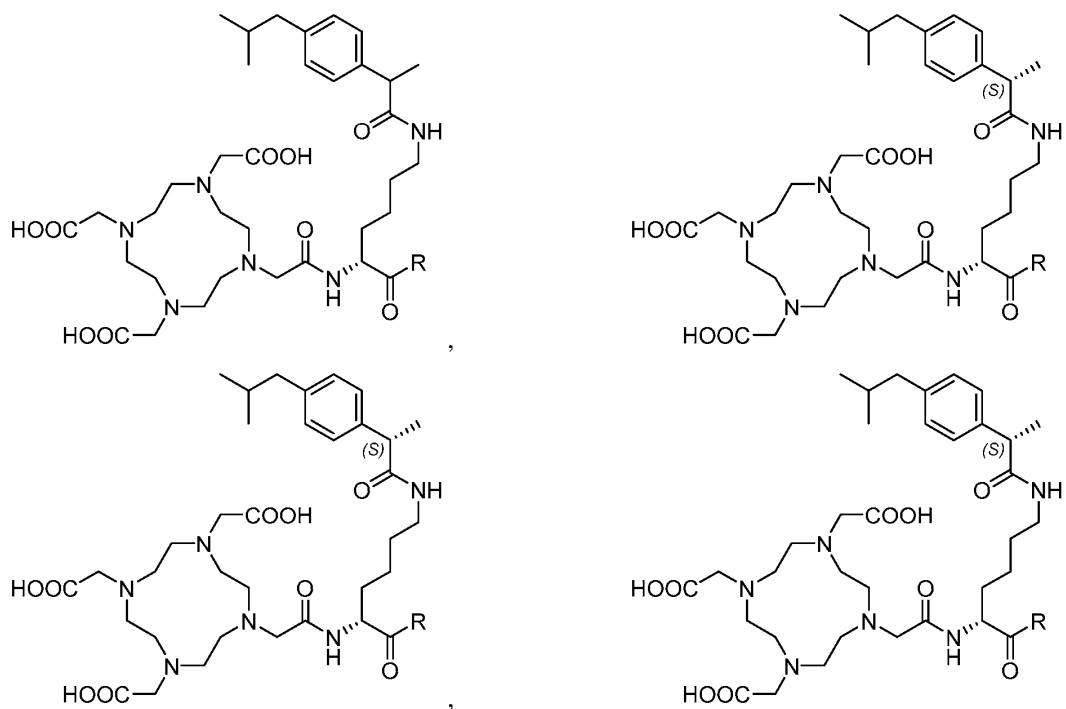


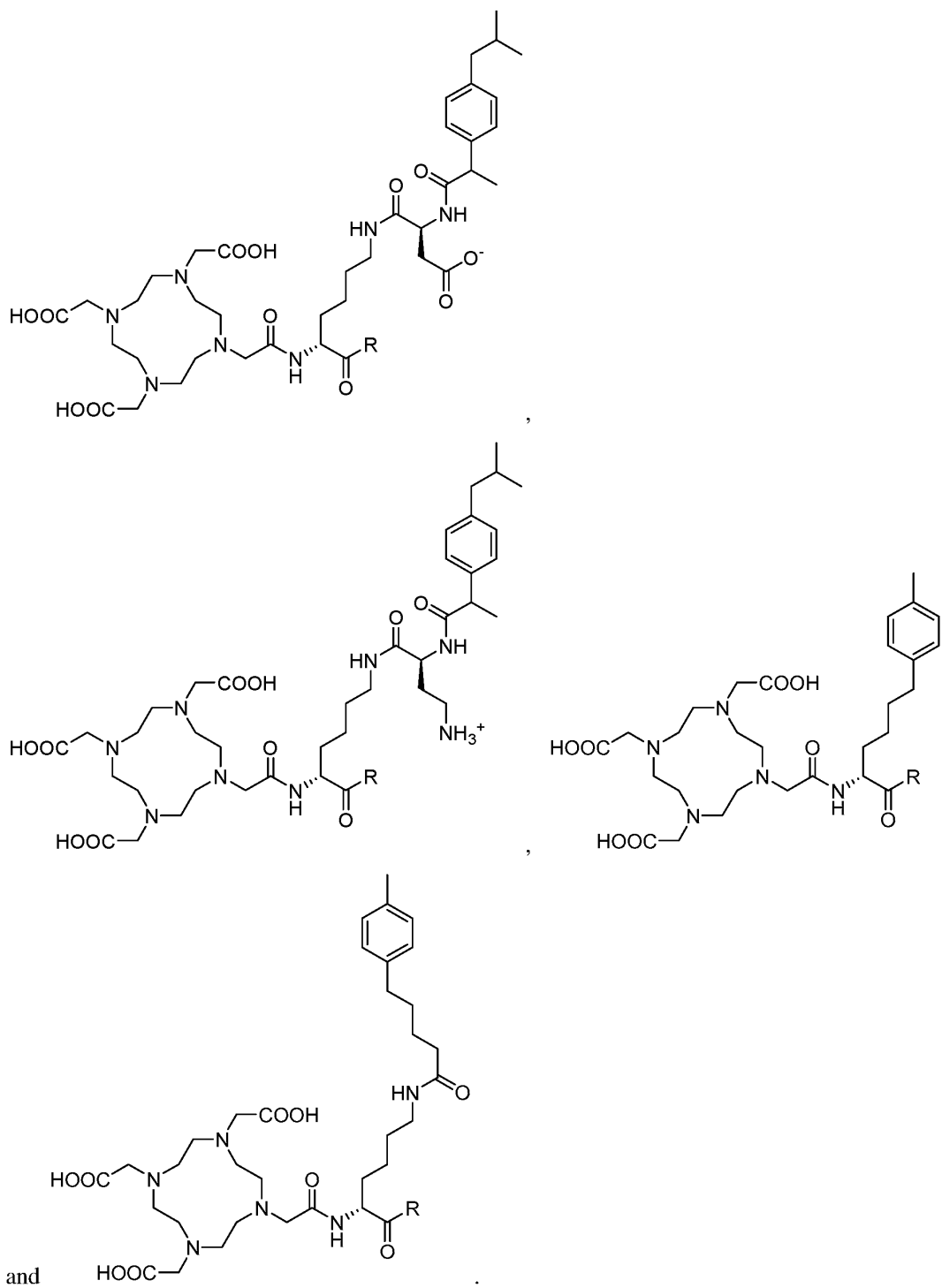
**[0088]** In some embodiments of a compound of formula (I), D is a payload. In some embodiments of a compound of formula (I), payload of D is radioactive components. In some

embodiments of a compound of formula (I), payload of D is fluorescent dyes. In some embodiments of a compound of formula (I), payload of D is contrast agents. In some embodiments of a compound of formula (I), payload of D is metal chelating groups/chelating agents.

**[0089]** In some embodiments, payload of D further comprises one or more motifs. In some embodiments, payload of D further comprises an albumin-binding motif, an amino acid motif, or any combination thereof. For example, ibuprofen can be used as an albumin-binding motif. The incorporation of albumin-binding motifs into the structure of radioligands is a validated strategy in literature to enhance blood circulation and ultimately increase tumor accumulation and activity (Preclinical Development of Novel PSMA-Targeting Radioligands: Modulation of Albumin-Binding Properties To Improve Prostate Cancer Therapy, by Christoph A. Umbricht, Martina Benešová, Roger Schibli, and Cristina Müller Molecular Pharmaceutics 2018 15 (6), 2297-2306; Development of a new class of PSMA radioligands comprising ibuprofen as an albumin-binding entity by Eberle, L.M.; Benešová, M.; Umbricht, C.A.; Borgna, F.; Büchler, M.; Zhernosekov, K.; Schibli, R.; Müller, C., Theranostics 2020, 10 (4), 1678-1693).

**[0090]** Exemplary payloads with motifs include, but are not limited to,





[0091] In some embodiments of a compound of formula (I), payload of D is a radionuclide. Representative radionuclides, which may be used in connection with the present invention are well known to the person skilled in the art and include, but are not limited, to the following ones:  $^{11}\text{C}$ ,  $^{13}\text{N}$ ,  $^{18}\text{F}$ ,  $^{24}\text{Na}$ ,  $^{28}\text{Mg}$ ,  $^{31}\text{Si}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^{38}\text{S}$ ,  $^{34\text{m}}\text{Cl}$ ,  $^{38}\text{Cl}$ ,  $^{39}\text{Cl}$ ,  $^{37}\text{Ar}$ ,  $^{41}\text{Ar}$ ,  $^{44}\text{Ar}$ ,  $^{42}\text{K}$ ,  $^{43}\text{K}$ ,  $^{44}\text{K}$ ,  $^{45}\text{K}$ ,  $^{47}\text{Ca}$ ,  $^{43}\text{Sc}$ ,  $^{44}\text{Sc}$ ,  $^{44\text{m}}\text{Sc}$ ,  $^{47}\text{Sc}$ ,  $^{48}\text{Sc}$ ,  $^{49}\text{Sc}$ ,  $^{45}\text{Ti}$ ,  $^{47}\text{V}$ ,  $^{48}\text{V}$ ,  $^{48}\text{Cr}$ ,  $^{49}\text{Cr}$ ,  $^{51}\text{Cr}$ ,  $^{51}\text{Mn}$ ,  $^{52}\text{Mn}$ ,  $^{52\text{m}}\text{Mn}$ ,  $^{56}\text{Mn}$ ,  $^{52}\text{Fe}$ ,  $^{59}\text{Fe}$ ,  $^{55}\text{Co}$ ,  $^{61}\text{Co}$ ,  $^{62\text{m}}\text{Co}$ ,  $^{56}\text{Ni}$ ,  $^{57}\text{Ni}$ ,  $^{65}\text{Ni}$ ,  $^{66}\text{Ni}$ ,  $^{60}\text{Cu}$ ,  $^{61}\text{Cu}$ ,  $^{64}\text{Cu}$ ,  $^{67}\text{Cu}$ ,  $^{62}\text{Zn}$ ,  $^{63}\text{Zn}$ ,  $^{69}\text{Zn}$ ,  $^{69\text{m}}\text{Zn}$ ,  $^{71\text{m}}\text{Zn}$ ,  $^{72}\text{Zn}$ ,  $^{65}\text{Ga}$ ,  $^{66}\text{Ga}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{70}\text{Ga}$ ,  $^{72}\text{Ga}$ ,  $^{73}\text{Ga}$ ,  $^{66}\text{Ge}$ ,  $^{67}\text{Ge}$ ,  $^{69}\text{Ge}$ ,  $^{71}\text{Ge}$ ,  $^{75}\text{Ge}$ ,  $^{77}\text{Ge}$ ,  $^{78}\text{Ge}$ ,  $^{69}\text{As}$ ,  $^{70}\text{As}$ ,  $^{71}\text{As}$ ,  $^{72}\text{As}$ ,  $^{74}\text{As}$ ,  $^{76}\text{As}$ ,  $^{77}\text{As}$ ,  $^{78}\text{As}$ ,  $^{70}\text{Se}$ ,  $^{72}\text{Se}$ ,  $^{73}\text{Se}$ ,  $^{73\text{m}}\text{Se}$ ,  $^{81}\text{Se}$ ,  $^{81\text{m}}\text{Se}$ ,  $^{83}\text{Se}$ ,  $^{74}\text{Br}$ ,  $^{74\text{m}}\text{Br}$ ,  $^{75}\text{Br}$ ,  $^{76}\text{Br}$ ,  $^{77}\text{Br}$ ,  $^{80}\text{Br}$ ,  $^{80\text{m}}\text{Br}$ ,  $^{82}\text{Br}$ ,  $^{83}\text{Br}$ ,  $^{84}\text{Br}$ ,  $^{74}\text{Kr}$ ,  $^{76}\text{Kr}$ ,  $^{77}\text{Kr}$ ,  $^{79}\text{Kr}$ ,  $^{85}\text{Kr}$ ,  $^{87}\text{Kr}$ ,  $^{88}\text{Kr}$ ,  $^{78}\text{Rb}$ ,  $^{79}\text{Rb}$ ,  $^{81}\text{Rb}$ ,  $^{82}\text{Rb}$ ,  $^{84}\text{Rb}$ ,  $^{84\text{m}}\text{Rb}$ ,  $^{86}\text{Rb}$ ,  $^{88}\text{Rb}$ ,  $^{89}\text{Rb}$ ,  $^{80}\text{Sr}$ ,  $^{81}\text{Sr}$ ,  $^{82}\text{Sr}$ ,  $^{83}\text{Sr}$ ,  $^{85\text{m}}\text{Sr}$ ,  $^{87}\text{Sr}$ ,  $^{91}\text{Sr}$ ,  $^{92}\text{Sr}$ ,  $^{84}\text{Y}$ ,  $^{85}\text{Y}$ ,  $^{85\text{m}}\text{Y}$ ,  $^{86}\text{Y}$ ,  $^{86\text{m}}\text{Y}$ ,  $^{87}\text{Y}$ ,  $^{87\text{m}}\text{Y}$ ,  $^{90}\text{Y}$ ,  $^{90\text{m}}\text{Y}$ ,  $^{91\text{m}}\text{Y}$ ,  $^9\text{Y}$ ,  $^{93}\text{Y}$ ,  $^{94}\text{Y}$ ,  $^{95}\text{Y}$ ,  $^{86}\text{Zr}$ ,  $^{87}\text{Zr}$ ,  $^{89}\text{Zr}$ ,  $^{97}\text{Zr}$ ,  $^{88}\text{Nb}$ ,  $^{89}\text{Nb}$ ,  $^{89\text{m}}\text{Nb}$ ,  $^{90}\text{Nb}$ ,  $^{92}\text{Nb}$ ,  $^{95}\text{Nb}$ ,  $^{95\text{m}}\text{Nb}$ ,  $^{96}\text{Nb}$ ,  $^{97}\text{Nb}$ ,  $^{98\text{m}}\text{Nb}$ ,  $^{101}\text{Mo}$ ,  $^{102}\text{Mo}$ ,  $^{90}\text{Mo}$ ,  $^{91}\text{Mo}$ ,  $^{93\text{m}}\text{Mo}$ ,  $^{99}\text{Mo}$ ,  $^{101}\text{Tc}$ ,  $^{104}\text{Tc}$ ,  $^{93}\text{Tc}$ ,  $^{93\text{m}}\text{Tc}$ ,  $^{94}\text{Tc}$ ,  $^{94\text{m}}\text{Tc}$ ,  $^{95}\text{Tc}$ ,  $^{96}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ ,  $^{103}\text{Ru}$ ,  $^{105}\text{Ru}$ ,  $^{94}\text{Ru}$ ,  $^{95}\text{Ru}$ ,  $^{97}\text{Ru}$ ,  $^{100}\text{Rh}$ ,  $^{101\text{m}}\text{Rh}$ ,  $^{105}\text{Rh}$ ,  $^{106\text{m}}\text{Rh}$ ,  $^{107}\text{Rh}$ ,  $^{97}\text{Rh}$ ,  $^{97\text{m}}\text{Rh}$ ,  $^9\text{Rh}$ ,  $^{99\text{m}}\text{Rh}$ ,  $^{100}\text{Pd}$ ,  $^{101}\text{Pd}$ ,  $^{103}\text{Pd}$ ,  $^{109}\text{Pd}$ ,  $^{111}\text{Pd}$ ,  $^{111\text{m}}\text{Pd}$ ,  $^{112}\text{Pd}$ ,  $^{98}\text{Pd}$ ,  $^{99}\text{Pd}$ ,  $^{101}\text{Ag}$ ,  $^{103}\text{Ag}$ ,  $^{104}\text{Ag}$ ,  $^{104\text{m}}\text{Ag}$ ,  $^{105}\text{Ag}$ ,  $^{106}\text{Ag}$ ,  $^{106\text{m}}\text{Ag}$ ,  $^{111}\text{Ag}$ ,  $^{112}\text{Ag}$ ,  $^{113}\text{Ag}$ ,  $^{115}\text{Ag}$ ,  $^{104}\text{Cd}$ ,  $^{105}\text{Cd}$ ,  $^{107}\text{Cd}$ ,  $^{111}\text{Cd}$ ,  $^{115}\text{Cd}$ ,  $^{115\text{m}}\text{Cd}$ ,  $^{117}\text{Cd}$ ,  $^{117\text{m}}\text{Cd}$ ,  $^{118}\text{Cd}$ ,  $^{107}\text{In}$ ,  $^{108\text{m}}\text{In}$ ,  $^{109}\text{In}$ ,  $^{110}\text{In}$ ,  $^{110\text{m}}\text{In}$ ,  $^{111}\text{In}$ ,  $^{112}\text{In}$ ,  $^{113}\text{In}$ ,  $^{114\text{m}}\text{In}$ ,  $^{115\text{m}}\text{In}$ ,  $^{116\text{m}}\text{In}$ ,  $^{117}\text{In}$ ,  $^{117\text{m}}\text{In}$ ,  $^{119\text{m}}\text{In}$ ,  $^{108}\text{Sn}$ ,  $^{109}\text{Sn}$ ,  $^{110}\text{Sn}$ ,  $^{111}\text{Sn}$ ,  $^{117}\text{Sn}$ ,  $^{121}\text{Sn}$ ,  $^{123\text{m}}\text{Sn}$ ,  $^{125}\text{Sn}$ ,  $^{127}\text{Sn}$ ,  $^{128}\text{Sn}$ ,  $^{115}\text{Sb}$ ,  $^{116}\text{Sb}$ ,  $^{116\text{m}}\text{Sb}$ ,  $^{117}\text{Sb}$ ,  $^{118\text{m}}\text{Sb}$ ,  $^{119}\text{Sb}$ ,  $^{120}\text{Sb}$ ,  $^{120\text{m}}\text{Sb}$ ,  $^{122}\text{Sb}$ ,  $^{126}\text{Sb}$ ,  $^{126\text{m}}\text{Sb}$ ,  $^{127}\text{Sb}$ ,  $^{128}\text{Sb}$ ,  $^{128\text{m}}\text{Sb}$ ,  $^{129}\text{Sb}$ ,  $^{129\text{m}}\text{Sb}$ ,  $^{130}\text{Sb}$ ,  $^{131}\text{Sb}$ ,  $^{114}\text{Te}$ ,  $^{116}\text{Te}$ ,  $^{117}\text{Te}$ ,  $^{118}\text{Te}$ ,  $^{119}\text{Te}$ ,  $^{119\text{m}}\text{Te}$ ,  $^{121}\text{Te}$ ,  $^{127}\text{Te}$ ,  $^{129}\text{Te}$ ,  $^{129\text{m}}\text{Te}$ ,  $^{131}\text{Te}$ ,  $^{131\text{m}}\text{Te}$ ,  $^{132}\text{Te}$ ,  $^{133}\text{Te}$ ,  $^{133\text{m}}\text{Te}$ ,  $^{134}\text{Te}$ ,  $^{118}\text{I}$ ,  $^{119}\text{I}$ ,  $^{120}\text{I}$ ,  $^{120\text{m}}\text{I}$ ,  $^{121}\text{I}$ ,  $^{123}\text{I}$ ,  $^{124}\text{I}$ ,  $^{126}\text{I}$ ,  $^{128}\text{I}$ ,  $^{130}\text{I}$ ,  $^{131}\text{I}$ ,  $^{132}\text{I}$ ,  $^{132\text{m}}\text{I}$ ,  $^{133}\text{I}$ ,  $^{134}\text{I}$ ,  $^{135}\text{I}$ ,  $^{120}\text{Xe}$ ,  $^{121}\text{Xe}$ ,  $^{122}\text{Xe}$ ,  $^{123}\text{Xe}$ ,  $^{125}\text{Xe}$ ,  $^{127}\text{Xe}$ ,  $^{133}\text{Xe}$ ,  $^{133\text{m}}\text{Xe}$ ,  $^{135}\text{Xe}$ ,  $^{135\text{m}}\text{Xe}$ ,  $^{138}\text{Xe}$ ,  $^{125}\text{Cs}$ ,  $^{127}\text{Cs}$ ,  $^{129}\text{Cs}$ ,  $^{130}\text{Cs}$ ,  $^{131}\text{Cs}$ ,  $^{132}\text{Cs}$ ,  $^{134}\text{Cs}$ ,  $^{135}\text{Cs}$ ,  $^{136}\text{Cs}$ ,  $^{138}\text{Cs}$ ,  $^{124}\text{Ba}$ ,  $^{126}\text{Ba}$ ,  $^{127}\text{Ba}$ ,  $^{128}\text{Ba}$ ,  $^{129}\text{Ba}$ ,  $^{129\text{m}}\text{Ba}$ ,  $^{131}\text{Ba}$ ,  $^{131\text{m}}\text{Ba}$ ,  $^{133}\text{Ba}$ ,  $^{135}\text{Ba}$ ,  $^{139}\text{Ba}$ ,  $^{140}\text{Ba}$ ,  $^{141}\text{Ba}$ ,  $^{142}\text{Ba}$ ,  $^{129}\text{La}$ ,  $^{131}\text{La}$ ,  $^{132}\text{La}$ ,  $^{133}\text{La}$ ,  $^{135}\text{La}$ ,  $^{140}\text{La}$ ,  $^1\text{La}$ ,  $^{41}\text{La}$ ,  $^{142}\text{La}$ ,  $^{143}\text{La}$ ,  $^{130}\text{Ce}$ ,  $^{132}\text{Ce}$ ,  $^{133}\text{Ce}$ ,  $^{133\text{m}}\text{Ce}$ ,  $^{134}\text{Ce}$ ,  $^{135}\text{Ce}$ ,  $^{137}\text{Ce}$ ,  $^{137\text{m}}\text{Ce}$ ,  $^{141}\text{Ce}$ ,  $^{143}\text{Ce}$ ,  $^{146}\text{Ce}$ ,  $^{134}\text{Pr}$ ,  $^{134\text{m}}\text{Pr}$ ,  $^{136}\text{Pr}$ ,  $^{137}\text{Pr}$ ,  $^{138\text{m}}\text{Pr}$ ,  $^{139}\text{Pr}$ ,  $^{142}\text{Pr}$ ,  $^{143}\text{Pr}$ ,  $^{144}\text{Pr}$ ,  $^{145}\text{Pr}$ ,  $^{146}\text{Pr}$ ,  $^{147}\text{Pr}$ ,  $^{135}\text{Nd}$ ,  $^{136}\text{Nd}$ ,  $^{137}\text{Nd}$ ,  $^{138}\text{Nd}$ ,  $^{139}\text{Nd}$ ,  $^{139\text{m}}\text{Nd}$ ,  $^{140}\text{Nd}$ ,  $^{141}\text{Nd}$ ,  $^{147}\text{Nd}$ ,  $^{149}\text{Nd}$ ,  $^{151}\text{Nd}$ ,  $^{152}\text{Nd}$ ,  $^{141}\text{Pm}$ ,  $^{148}\text{Pm}$ ,  $^{148\text{m}}\text{Pm}$ ,  $^{149}\text{Pm}$ ,  $^{150}\text{Pm}$ ,  $^{151}\text{Pm}$ ,  $^{140}\text{Sm}$ ,  $^{141}\text{Sm}$ ,  $^{141\text{m}}\text{Sm}$ ,  $^{142}\text{Sm}$ ,  $^{153}\text{Sm}$ ,  $^{155}\text{Sm}$ ,  $^{156}\text{Sm}$ ,  $^{145}\text{Eu}$ ,  $^{146}\text{Eu}$ ,  $^{147}\text{Eu}$ ,  $^{150}\text{Eu}$ ,  $^{152\text{m}}\text{Eu}$ ,  $^1\text{Eu}$ ,  $^{54}\text{Eu}$ ,  $^{156}\text{Eu}$ ,  $^{157}\text{Eu}$ ,  $^{158}\text{Eu}$ ,  $^{159}\text{Eu}$ ,  $^{145}\text{Gd}$ ,  $^{146}\text{Gd}$ ,  $^{147}\text{Gd}$ ,  $^{149}\text{Gd}$ ,  $^{159}\text{Gd}$ ,  $^{147}\text{Tb}$ ,  $^{148}\text{Tb}$ ,  $^{149}\text{Tb}$ ,  $^{150}\text{Tb}$ ,  $^{151}\text{Tb}$ ,  $^{152}\text{Tb}$ ,  $^{153}\text{Tb}$ ,  $^{154}\text{Tb}$ ,  $^{154\text{m}}\text{Tb}$ ,  $^{155}\text{Tb}$ ,  $^{156}\text{Tb}$ ,  $^{156\text{m}}\text{Tb}$ ,  $^{161}\text{Tb}$ ,  $^{163}\text{Tb}$ ,  $^{151}\text{Dy}$ ,  $^{152}\text{Dy}$ ,  $^{153}\text{Dy}$ ,  $^{155}\text{Dy}$ ,  $^{157}\text{Dy}$ ,  $^{165}\text{Dy}$ ,  $^{166}\text{Dy}$ ,  $^{154}\text{Ho}$ ,  $^{155}\text{Ho}$ ,  $^{156}\text{Ho}$ ,  $^{157}\text{Ho}$ ,  $^{158\text{m}}\text{Ho}$ ,  $^{159}\text{Ho}$ ,  $^{161}\text{Ho}$ ,  $^{162}\text{Ho}$ ,  $^{162\text{m}}\text{Ho}$ ,  $^{164}\text{Ho}$ ,  $^{164\text{m}}\text{Ho}$ .

Ho, <sup>166</sup>Ho, <sup>167</sup>Ho, <sup>156</sup>Er, <sup>157</sup>Er, <sup>158</sup>Er, <sup>159</sup>Er, <sup>160</sup>Er, <sup>161</sup>Er, <sup>163</sup>Er, <sup>165</sup>Er, <sup>169</sup>Er, <sup>171</sup>Er, <sup>172</sup>Er, <sup>161</sup>Tm, <sup>162</sup>Tm, <sup>163</sup>Tm, <sup>165</sup>Tm, <sup>166</sup>Tm, <sup>167</sup>Tm, <sup>172</sup>Tm, <sup>173</sup>Tm, <sup>175</sup>Tm, <sup>162</sup>Yb, <sup>163</sup>Yb, <sup>164</sup>Yb, <sup>166</sup>Yb, <sup>167</sup>Yb, <sup>169</sup>Yb, <sup>175</sup>Yb, <sup>177</sup>Yb, <sup>178</sup>Yb, <sup>167</sup>Lu, <sup>169</sup>Lu, <sup>170</sup>Lu, <sup>171</sup>Lu, <sup>172</sup>Lu, <sup>176m</sup>Lu, <sup>177</sup>Lu, <sup>178</sup>Lu, <sup>178m</sup>Lu, <sup>179</sup>Lu, <sup>168</sup>Hf, <sup>170</sup>Hf, <sup>173</sup>Hf, <sup>177m</sup>Hf, <sup>179m</sup>Hf, <sup>180m</sup>Hf, <sup>181</sup>Hf, <sup>182m</sup>Hf, <sup>183</sup>Hf, <sup>184</sup>Hf, <sup>172</sup>Ta, <sup>173</sup>Ta, <sup>174</sup>Ta, <sup>175</sup>Ta, <sup>176</sup>Ta, <sup>177</sup>Ta, <sup>178</sup>Ta, <sup>180</sup>Ta, <sup>182m</sup>Ta, <sup>183</sup>Ta, <sup>184</sup>Ta, <sup>185</sup>Ta, <sup>186</sup>Ta, <sup>174</sup>W, <sup>175</sup>W, <sup>177</sup>W, <sup>178</sup>W, <sup>179</sup>W, <sup>187</sup>W, <sup>190</sup>W, <sup>177</sup>Re, <sup>178</sup>Re, <sup>179</sup>Re, <sup>181</sup>Re, <sup>182</sup>Re, <sup>182m</sup>Re, <sup>184</sup>Re, <sup>186</sup>Re, <sup>188</sup>Re, <sup>188m</sup>Re, <sup>189</sup>Re, <sup>190m</sup>Re, <sup>180</sup>Os, <sup>181</sup>Os, <sup>182</sup>Os, <sup>183</sup>Os, <sup>183m</sup>Os, <sup>191</sup>Os, <sup>193</sup>Os, <sup>196</sup>Os, <sup>182</sup>Ir, <sup>183</sup>Ir, <sup>184</sup>Ir, <sup>185</sup>Ir, <sup>186</sup>Ir, <sup>186m</sup>Ir, <sup>187</sup>Ir, <sup>188</sup>Ir, <sup>189</sup>Ir, <sup>190</sup>Ir, <sup>194</sup>Ir, <sup>195</sup>Ir, <sup>195m</sup>Ir, <sup>196m</sup>Ir, <sup>184</sup>Pt, <sup>186</sup>Pt, <sup>187</sup>Pt, <sup>188</sup>Pt, <sup>189</sup>Pt, <sup>191</sup>Pt, <sup>195</sup>Pt, <sup>197</sup>Pt, <sup>197m</sup>Pt, <sup>199</sup>Pt, <sup>200</sup>Pt, <sup>202</sup>Pt, <sup>186</sup>Au, <sup>190</sup>Au, <sup>191</sup>Au, <sup>192</sup>Au, <sup>193</sup>Au, <sup>194</sup>Au, <sup>196</sup>Au, <sup>196m</sup>Au, <sup>198</sup>Au, <sup>198m</sup>Au, <sup>199</sup>Au, <sup>200</sup>Au, <sup>200m</sup>Au, <sup>190</sup>Hg, <sup>191</sup>Hg, <sup>192</sup>Hg, <sup>193</sup>Hg, <sup>195</sup>Hg, <sup>195m</sup>Hg, <sup>197</sup>Hg, <sup>197m</sup>Hg, <sup>199</sup>Hg, <sup>203</sup>Hg, <sup>194</sup>Tl, <sup>194m</sup>Tl, <sup>195</sup>Tl, <sup>196</sup>Tl, <sup>196m</sup>Tl, <sup>197</sup>Tl, <sup>198</sup>Tl, <sup>198m</sup>Tl, <sup>199</sup>Tl, <sup>200</sup>Tl, <sup>201</sup>Tl, <sup>202</sup>Tl, <sup>194</sup>Pb, <sup>195</sup>Pb, <sup>196</sup>Pb, <sup>197m</sup>Pb, <sup>198</sup>Pb, <sup>199</sup>Pb, <sup>199m</sup>Pb, <sup>200</sup>Pb, <sup>201</sup>Pb, <sup>202m</sup>Pb, <sup>203</sup>Pb, <sup>204</sup>Pb, <sup>209</sup>Pb, <sup>211</sup>Pb, <sup>212</sup>Pb, <sup>214</sup>Pb, <sup>200</sup>Bi, <sup>200m</sup>Bi, <sup>201</sup>Bi, <sup>202</sup>Bi, <sup>203</sup>Bi, <sup>204</sup>Bi, <sup>205</sup>Bi, <sup>206</sup>Bi, <sup>210</sup>Bi, <sup>212</sup>Bi, <sup>212m</sup>Bi, <sup>213</sup>Bi, <sup>214</sup>Bi, <sup>200</sup>Po, <sup>201</sup>Po, <sup>202</sup>Po, <sup>203</sup>Po, <sup>204</sup>Po, <sup>205</sup>Po, <sup>206</sup>Po, <sup>207</sup>Po, <sup>205</sup>At, <sup>206</sup>At, <sup>207</sup>At, <sup>208</sup>At, <sup>209</sup>At, <sup>210</sup>At, <sup>211</sup>At, <sup>208</sup>Rn, <sup>209</sup>Rn, <sup>210</sup>Rn, <sup>211</sup>Rn, <sup>212</sup>Rn, <sup>221</sup>Rn, <sup>222</sup>Rn, <sup>223</sup>Rn, <sup>212</sup>Fr, <sup>222</sup>Fr, <sup>223</sup>Fr, <sup>223</sup>Ra, <sup>224</sup>Ra, <sup>225</sup>Ra, <sup>227</sup>Ra, <sup>230</sup>Ra, <sup>224</sup>Ac, <sup>225</sup>Ac, <sup>226</sup>Ac, <sup>228</sup>Ac, <sup>229</sup>Ac, <sup>226</sup>Th, <sup>227</sup>Th, <sup>231</sup>Th, <sup>233</sup>Th, <sup>234</sup>Th, <sup>236</sup>Th, <sup>227</sup>Pa, <sup>228</sup>Pa, <sup>229</sup>Pa, <sup>230</sup>Pa, <sup>232</sup>Pa, <sup>233</sup>Pa, <sup>234</sup>Pa, <sup>235</sup>Pa, <sup>229</sup>U, <sup>230</sup>U, <sup>231</sup>U, <sup>237</sup>U, <sup>239</sup>U, <sup>240</sup>U, <sup>242</sup>U, <sup>231</sup>Np, <sup>232</sup>Np, <sup>233</sup>Np, <sup>234</sup>Np, <sup>236m</sup>Np, <sup>238</sup>Np, <sup>239</sup>Np, <sup>240</sup>Np, <sup>241</sup>Np, <sup>232</sup>Pu, <sup>235</sup>Pu, <sup>237</sup>Pu, <sup>243</sup>Pu, <sup>245</sup>Pu, <sup>246</sup>Pu, <sup>235</sup>Am, <sup>237</sup>Am, <sup>238</sup>Am, <sup>239</sup>Am, <sup>240</sup>Am, <sup>242</sup>Am, <sup>244</sup>Am, <sup>244m</sup>Am, <sup>245</sup>Am, <sup>246</sup>Am, <sup>246m</sup>Am, <sup>247</sup>Am, <sup>239</sup>Cm, <sup>240</sup>Cm, <sup>241</sup>Cm, <sup>251</sup>Cm, <sup>245</sup>Bk, <sup>246</sup>Bk, <sup>248</sup>Bk, <sup>250</sup>Bk, <sup>251</sup>Bk, <sup>244</sup>Cf, <sup>245</sup>Cf, <sup>246</sup>Cf, <sup>247</sup>Cf, <sup>253</sup>Cf, <sup>255</sup>Cf, <sup>249</sup>Es, <sup>250</sup>Es, <sup>250m</sup>Es, <sup>251</sup>Es, <sup>253</sup>Es, <sup>254m</sup>Es, <sup>255</sup>Es, <sup>256m</sup>Es, <sup>250</sup>Fm, <sup>251</sup>Fm, <sup>252</sup>Fm, <sup>254</sup>Fm, <sup>255</sup>Fm, <sup>255</sup>Md, <sup>256</sup>Md, <sup>257</sup>Md, <sup>259</sup>No.

[0092] In some embodiments, the radionuclide is selected from the group including, but not limited to, <sup>43</sup>Sc, <sup>44</sup>Sc, <sup>51</sup>Mn, <sup>52</sup>Mn, <sup>61</sup>Cu, <sup>64</sup>Cu, <sup>67</sup>Ga, <sup>68</sup>Ga, <sup>86</sup>Y, <sup>89</sup>Zr, <sup>94m</sup>Tc, <sup>99m</sup>Tc, <sup>111</sup>In, <sup>152</sup>Tb, <sup>155</sup>Tb, <sup>177</sup>Lu, <sup>201</sup>Tl, <sup>203</sup>Pb, <sup>18</sup>F, <sup>76</sup>Br, <sup>77</sup>Br, <sup>123</sup>I, <sup>124</sup>I, and <sup>125</sup>I. In some embodiments, the radionuclide is selected from <sup>18</sup>F, <sup>43</sup>Sc, <sup>44</sup>Sc, <sup>64</sup>Cu, <sup>67</sup>Ga, <sup>68</sup>Ga, <sup>86</sup>Y, <sup>89</sup>Zr, <sup>99m</sup>Tc, <sup>111</sup>In, <sup>152</sup>Tb, <sup>155</sup>Tb, and <sup>203</sup>Pb. In some embodiments, the radionuclide is selected from <sup>18</sup>F, <sup>64</sup>Cu, <sup>68</sup>Ga, and <sup>111</sup>In. In some embodiments, the radionuclide is selected from <sup>47</sup>Sc, <sup>67</sup>Cu, <sup>89</sup>Sr, <sup>90</sup>Y, <sup>111</sup>In, <sup>153</sup>Sm, <sup>149</sup>Tb, <sup>161</sup>Tb, <sup>177</sup>Lu, <sup>186</sup>Re, <sup>188</sup>Re, <sup>212</sup>Pb, <sup>213</sup>Bi, <sup>223</sup>Ra, <sup>224</sup>Ra, <sup>225</sup>Ac, <sup>226</sup>Th, <sup>227</sup>Th, <sup>131</sup>I, and <sup>211</sup>At.

[0093] In some embodiments, the radionuclide is <sup>18</sup>F, whereby <sup>18</sup>F forms a covalent bond to aluminium and aluminium forms a complex with the chelator. Methods and compositions for <sup>18</sup>F labeling of proteins, peptides and other molecules are, for example, disclosed in WO 2012/082618.

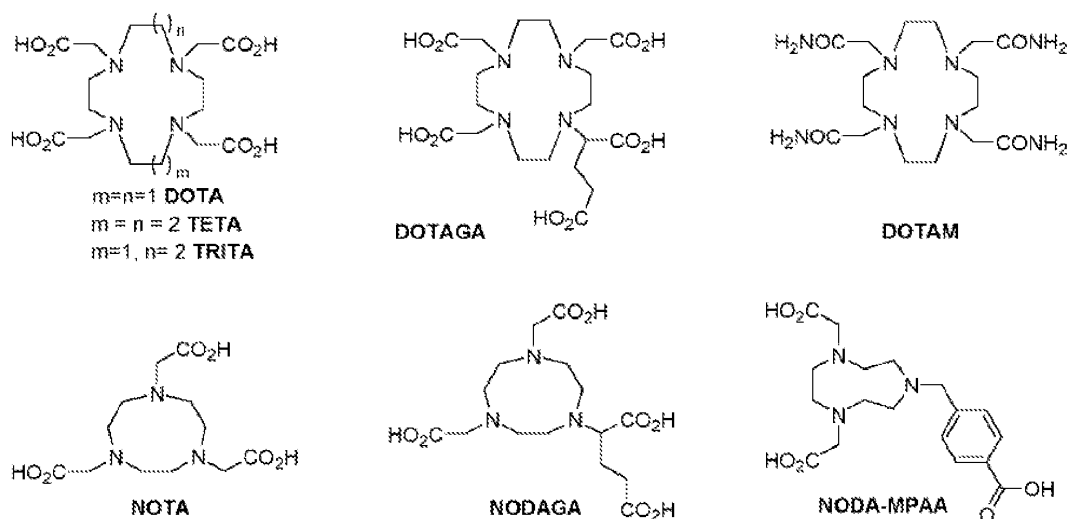
[0094] In some embodiments of a compound of formula (I), payload of D is a chelating agent. Representative chelating agents and their derivatives include, but are not limited to AAZTA, BAT, CDTA, DTA, CyEDTA, EDTMP, DTPMP, DTPA, CyDTPA, Cy2DTPA, DTPA-MA, DTPA-BA, BOPA, NT A, NOC, NOTP, CY-DTA, DTCBP, CTA, cyclam, CB-Cyclam, cyclen, TETA, sarcophagine, CPTA, TEAMA, Cyclen, D03A, D02A, TRITA, DATA, DFO, DATA(M), DATA(P), DATA(Ph), DATA(PPh), DEDPA, H<sub>10</sub>octapa, H<sub>10</sub>dedpa, H<sub>12</sub>decapa, H<sub>12</sub>azapa, H<sub>2</sub>CHX-DEDPA, DFO-Chx-MAL, DFO-p-SCN, DFO-IAC, DFO-BAC, p-SCN-Bn-DFO, DFO-pPhe-NCS, DFO-HOPO, DFC, diphosphine, DOTA, DOTAGA, DOTA-MFCO, DOTAM, DOTAM-mono-acid, DOTA-MA, DOTA-pNB, DOTA-4AMP, intro-DOTA, nitro-PA-DOTA, p-NCS-Bz-DOTA, PA-DOTA, DOTA-NCS, DOTA-NHS, CB-D02A, PCTA, p-NH<sub>2</sub>-Bn-PCTA, p-SCN-Bn-PCTA, p-SCN-Bn-DOTA, DOTMA, NB-DOTA, H<sub>4</sub>NB-DOTA, H<sub>4</sub>TCE-DOTA, HOPO, 3,4,3'-(Li-1,2-HOPO), TREN(Me-3,2-HOPO), TCE-DOTA, DOTP, DOTMP, DOTEP, DOTMPE, F-DOTPMPE, DOTPP, DOTBzP, DOTA-monoamide, DOXP, p-NCS-DOTA, p-NCS-PADOTA, p-NCS-TRITA, TRITA, TETA, 3p-C-DEPA, 3p-C-DEPA-NCS, p-NH<sub>2</sub>-BN-0X0-D03A, p-SCN-BN-TCMC, TCMC, 4-aminobutyl-DOTA, azido-mono-amide-DOTA, BCN-DOTA, butyne-DOTA, BCN-DOTA-GA, DOA3P, D02a2p, D02A(trans-H<sub>2</sub>do2a), D03A, D03A-thiol, D03AtBu-N-(2-aminoethyl)ethanamide, D03TMP-monoamide, D02AP, CB-D02A, C3B-D02A, HP-D03A, DOTA-NHS-ester, maleimide-DOTA-GA, maleimido-mono-amide-DOTA, maleimide-DOTA, NH<sub>2</sub>-DOTA-GA, NH<sub>2</sub>-PEG4-DOTA-GA, GA, p-NH<sub>2</sub>-Bn-DOTA, p-NO<sub>2</sub>-Bn-DOTA, p-SCN-Bn-DOTA, p-SCN-Bz-DOTA, TA-DOTA, TA-DOTA-GA, OTTA, DOXP, TSC, DTC, DTCBP, PTSM, ATSM, H<sub>2</sub>ATSM, H<sub>2</sub>PTSM, Dp44mT, DpC, Bp44mT, QT, hybrid thiosemicarbazone-benzothiazole, thiosemicarbazone-styrylpyridine tetradentate ligands H<sub>2</sub>L2-4, HBED, HBED-CC, dmHBED, dmEHPG, HBED-nn, SHBED, Br-Me2HBED, BPCA, HEHA, BF-HEHA, deferiprone, THP, HYNIC (2-hydrazino nicotinamide), NHS-HYNIC, HYNIC-Kp-DPPB, HYNIC-Ko-DPPB, (HYNIC)(tricine)<sub>2</sub>, (HYNIC)(EDDA)Cl, p-EDDHA, AIM, AIM A, IAM B, MAMA, MAMA-DGal, MAMA-MGal, MAMA-DA, MAMA-HAD, macropa, macropaquin, macroquin-SO<sub>3</sub>, N<sub>6</sub>S<sub>4</sub>x, N<sub>2</sub>S<sub>2</sub>, N<sub>3</sub>S, N<sub>4</sub>, MAG3B, NOTA, NODAGA, SCN-Bz-NOTA-R, NOT-P (NOTMP), NOTAM, p-NCS-NOTA, TACN, TACN-TM, NETA, NETA-monoamine, p-SCN-PhPr-NE3TA, C-NE3TA-NCS, C-NETA-NCS, 3p-C-NETA, NODASA, NOPO, NODA, NODA-MPAA, NO2A, N-benzyl-NODA, C-NOTA, BCNOT-monoamine, maleimido-mono-amide-NOTA, NO2A-azide, NO2A-butyne, NO2AP, NO3AP, N-NOTA, oxo-

DO3A, p-NH<sub>2</sub>-Bn-NOTA, p-NH<sub>2</sub>-Bn-oxo-DO3A, p-NO<sub>2</sub>-Bn-cyclen, p-SCN-Bn-NOTA, p-SCN-Bn-oxo-DO3A, TRAP, PEPA, BF-PEPA, pycup, pycup2A, pycup1A1Bn, pycup2Bn, SarAr-R, DiAmSar, AmBaSar-R, siamSar, Sar, Tachpyr, tachpyr-(6-Me), TAM A, TAM B, TAME, TAME-Hex, THP-Ph-NCS, THP-NCS, THP-TATE, NTP, H3THP, THPN, CB-TE2A, PCB-TE1A1P, TETA-NHS, CPTA, CPTA-NHS, CB-TE1K1P, CB-TE2A, TE2A, H2CB-TE2A, TE2P, CB-TE2P, MM-TE2A, DM-TE2A, 2C-TETA, 6C-TETA, BAT, BAT-6, NHS-BAT ester, SSBAT, SCN-CHX-A-DTPA-P, SCN-TETA, TMT-amine, p-BZ-HTCPP, H4pypa, H4octox, p-NO<sub>2</sub>-Bn-neunpa, p-SCN-Bn-H4neunpa, TTHA, tBu4pypa-C7-NHS, H4neunpa, H2macropa, BT-DO3A, DO3A-Nprop, DO3AP, DOTPMB, DOTAMAE, DOTAMAP, DO3AMBu, DEPA, p-NO<sub>2</sub>-Bn-PCTA, symPC2APA, symPCA2PA, asymPC2APA, asymPCA2PA, <sup>99m</sup>Tc(CO)<sub>3</sub>-Chelators, and MeO-DOTA-NCS.

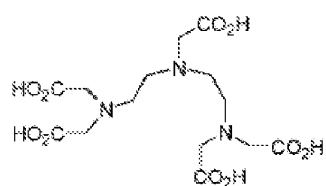
[0095] In some embodiments, a chelating agent is selected from the group consisting of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), 1,4,7,10-tetraazacyclododecane-1-(glutaric acid)-4,7,10-triacetic acid (DOTAGA), NOPO, PCTA, 1,4,7-triazacyclononanetriacetic acid (NOTA), 1,4,7-triazacyclononane-N-glutaric acid-N',N''-diacetic acid (NODAGA), 1,4,7-triazacyclononane-1,4-diacetate-methyl phenylacetic acid (NODAMPA), bis(2-hydroxybenzyl) ethylenediaminediacetic acid (HBED), 1,4,8,11-tetraazacyclododecane-1,4,8,11-tetraacetic acid (TETA), 4,11-bis-(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]-hexadecane (CB-TE2A), diethylenetriaminepentaacetic acid (DTPA), [(2-[(2-(bis-carboxymethyl-amino)-cyclohexyl)-carboxymethyl-amino]-ethyl)-carboxymethyl-amino]-acetic acid (CHX-A''-DTPA), desferal or desferrioxamine type group of chelators (DFO) (e.g., N-[5-({3-[5-(acetyl-hydroxy-amino)-pentylcarbamoyl]-propionyl]-hydroxy-amino)-pentyl]-N'-(5-amino-pentyl)-N'-hydroxy-succinamide), N,N'-bis[(6-carboxy-2-pyridyl)methyl]-4,13-diaza-18-crown (Macropa), 1,4,7,10-tetrakis[carbamoylmethyl]-1,4,7,10-tetracyclodecane (DOTAM), octadentate hydroxypyridinone-type group of chelators (HOPO), 3-({4,7-bis-[(2-carboxy-ethyl)-hydroxy-phosphinoylmethyl]-[1,4,7]triazonan-1-ylmethyl}-hydroxy-phosphinoyl)-propionic acid (TRAP), hexadentate tris(3,4-hydroxypyridinone) (THP), [4-carboxymethyl-6-(carboxymethyl-methyl-amino)-6-methyl-[1,4]diazepan-1-yl]-acetic acid (DATA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(methylene phosphonic acid) (DOTP), 3,6,10,13,16,19-hexaazabicyclo[6.6.6]icosane (sarcophagine), 3,15,27-triamino-7,19,31-trihydroxy-10,22,34-trimethyl-1,13,25-trioxa-7,19,31-triaza-cyclohexatriaconta-9,21,33-triene-

2,8,14,20,26,32-hexaone (FSC), {4-[2-(bis-carboxymethyl-amino)-ethyl]-7-carboxymethyl-[1,4,7]triazonan-1-yl}-acetic acid (NETA), {4-carboxymethyl-7-[2-(carboxymethyl-amino)-ethyl]-[1,4,7]triazonan-1-yl}-acetic acid (NE3TA), N,N'-(6-carboxy-2-pyridylmethyl)-N,N'-diacetic acid-1,2-diaminoethane (H4octapa), 1,8-(2,6-Pyridinedimethylene)-1,4,8,11-tetraazacyclo-tetradecane (Pycup),  $N_xS_{4-x}$  (also known as tetradentate chelators with N-atoms (basic amine or non-basic amide) and thiols as donors stabilizing Tc-complexes, especially Tc(V)-oxo complexes, e.g., N,N'-bis-(2-amino-ethyl)-propane-1,3-diamine (N4), N2S2, N3S), 6-hydrazino-nicotinic acid (Hynic), mTc(CO)<sub>3</sub>-Chelators (also known as bi- or tridentate chelators capable of forming stable complexes with technetium tricarbonyl fragments), AAZTA5 (6-[Bis(carboxymethyl)amino]-1,4-bis(carboxymethyl)-6-methyl-1,4-diazepane) and MATS (Mercapto-Acetyl-Tri-Serine).

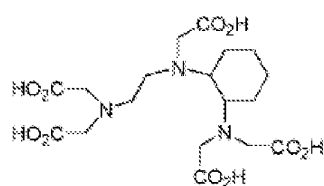
[0096] In some embodiments, a chelating agent is selected from the group consisting of



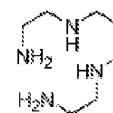




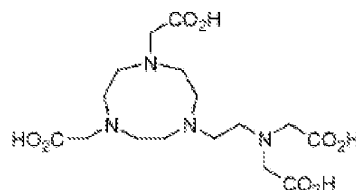
DTPA



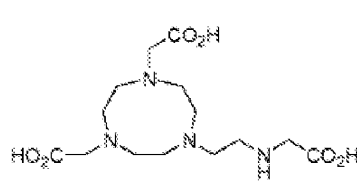
CHX-A''-DTPA



N4



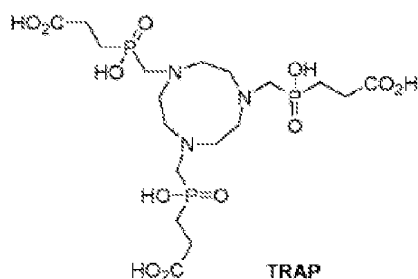
NETA



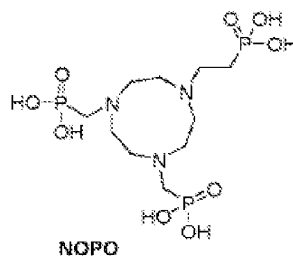
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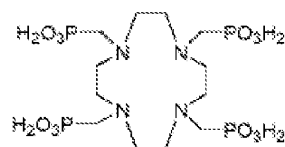
HYNIC



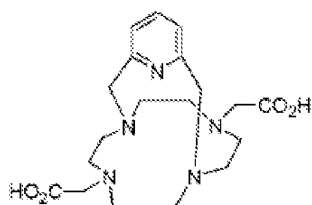
TRAP



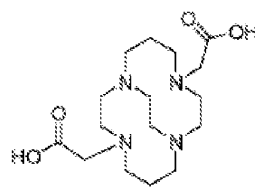
NGPO



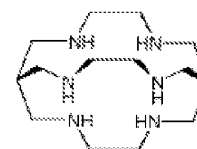
DOTP



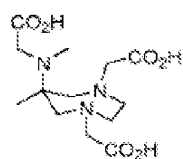
pycup



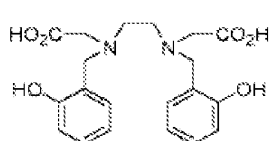
CB-TE2A



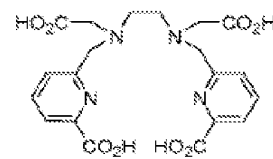
Sarcophagine



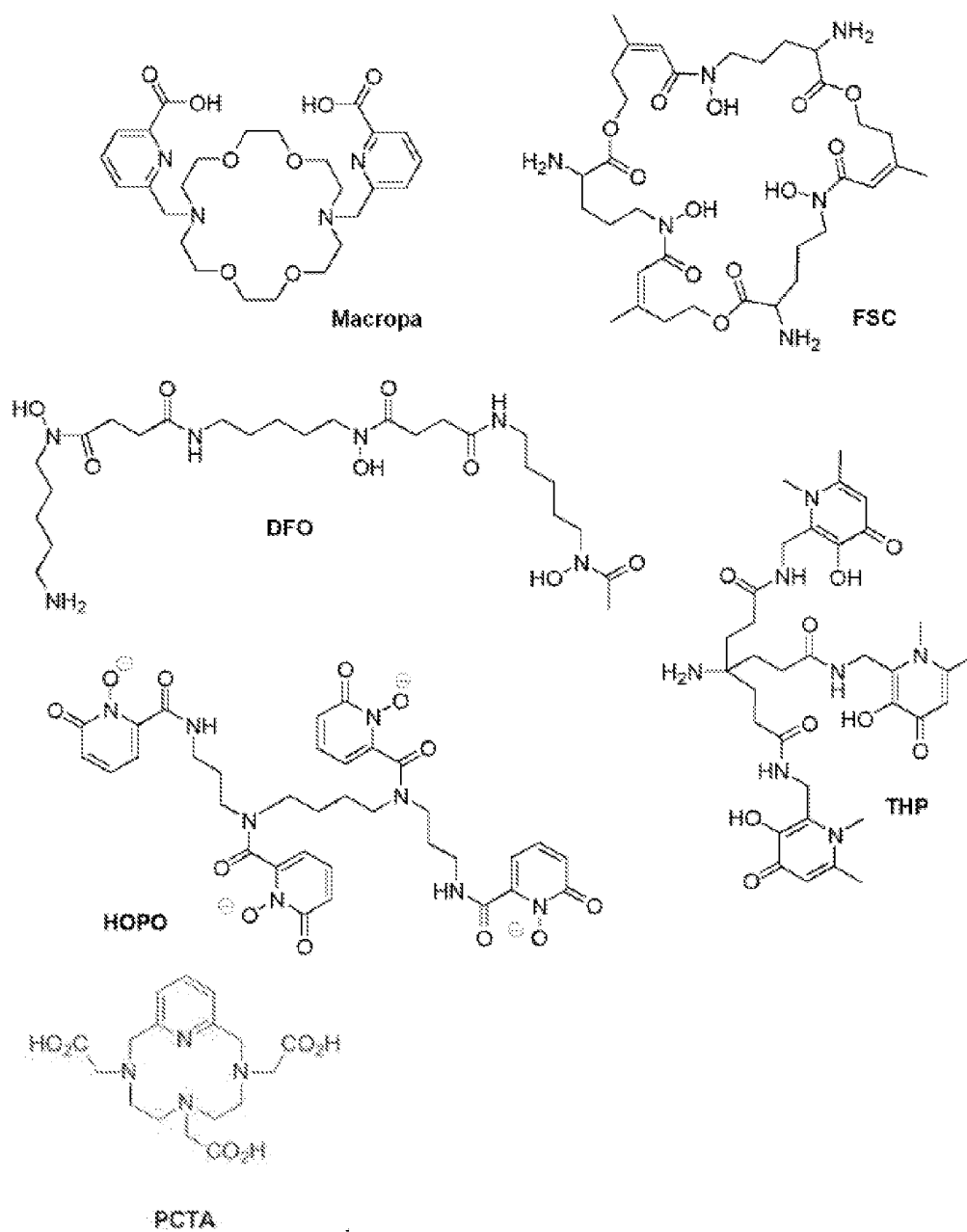
DATA



HBED



H4octapa



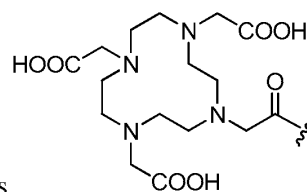
and any combination thereof.

[0097] In some embodiments, a chelating agent is selected from the group consisting of DOTA, DOTAGA, 1,4,7-triazacyclononane-1,4-bis[methylene(hydroxymethyl)phosphinic acid]-

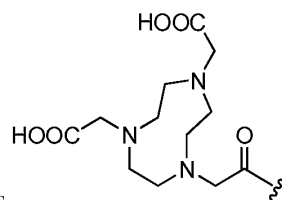
7-[methylene(2-carboxyethyl)phosphinic acid] (NOPO), 1,4,7-triazacyclononane-N,N'N''-tris(methylene phosphonic acid) (NOTP), 3,6,9,15-tetraazabicyclo[9.3.1]-pentadeca-1(15),11,13-triene-3,6,9,-triacetic acid (PCTA), DOTAM, Macropa, NOTA, NODAGA, NODA-MPAA, HBED, CB-TE2A, DFO, THP, and N4. In some embodiments, a chelating agent is selected from the group consisting of DOTA, DOTAGA, NOPO, PCTA, DOTAM, Macropa, NOTA, and NODAGA. In some embodiments, a chelating agent is selected from the group consisting of 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), ethylenediaminetetraacetic acid (EDTA), 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), triethylenetetramine (TETA), iminodiacetic acid, diethylenetriamine-N,N,N',N',N''-pentaacetic acid (DTPA), bis-(carboxymethylimidazole)glycine or 6-hydrazinopyridine-3-carboxylic acid (HYNIC).

**[0098]** In some embodiments of a compound of formula (I), payload of D is a fluorescence dye. In some embodiments, D is fluorophore derivative of coumarin, quinoline, styrene, naphthalimide, xanthone fluorescein, bodipy or rhodamine fluorescent dye (Jun JV, Chenoweth DM, Petersson EJ. Rational design of small molecule fluorescent probes for biological applications. *Org Biomol Chem.*, 2020, Aug 5;18(30):5747-5763). In some embodiments, D is near-infrared (NIR) dye. In some embodiments, D is NIR dye to guide intra-operative surgical decision-making or NIR fluorescence-guided surgery. In some embodiments, the NIR dye is S0456 NIR dye with excitation/emission bands at 789/807 nm. In some embodiments, the NIR dye is indocyanine green (ICG) NIR dye with excitation/emission bands at 780/802 nm. In some embodiments, the NIR dye is IRDye800CW NIR dye with excitation/emission bands at 774/789 nm. In some embodiments, the NIR dye is ZW800-1 NIR dye with excitation/emission bands at 768/786 nm. In some embodiments, the NIR dye is Sulfo-Cy5 NIR dye with excitation/emission bands at 646/662 nm. In some embodiments, the NIR dye is Sulfo-Cy5.5 NIR dye with excitation/emission bands at 673/691 nm. In some embodiments, the NIR dye is Sulfo-Cy7 NIR dye with excitation/emission bands at 750/773 nm. In some embodiments, the NIR dye is Sulfo-Cy7.5 NIR dye with excitation/emission bands at 778/797 nm. In some embodiments, the NIR dye is BM104 NIR dye with excitation/emission bands at 685/705 nm. In some embodiments, the NIR dye is CH1055 NIR dye with excitation/emission bands at 750/1055 nm (Debie P. and Hernot S., Emerging Fluorescent Molecular Tracers to Guide Intra-Operative Surgical Decision-Making. *Front. Pharmacol.*, 2019, 10:510).

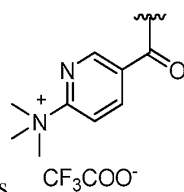
[0099] In some embodiments of a compound of formula (I), D is



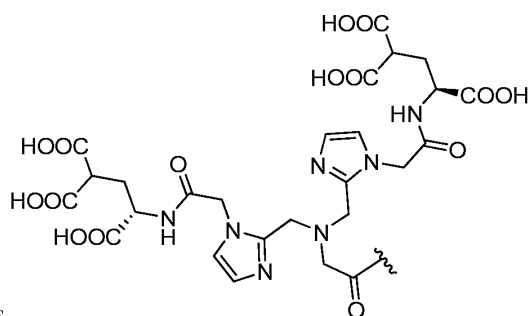
. In some embodiments of a compound of formula (I), D is



embodiments of a compound of formula (I), D is



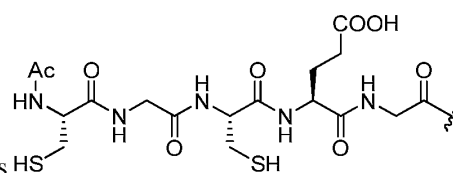
. In some embodiments of a



compound of formula (I), D is

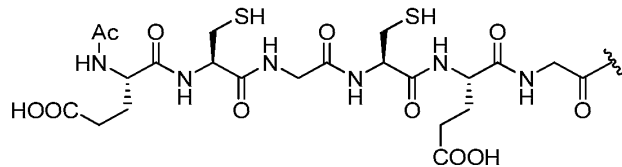
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embodiments of a compound of formula (I), D is

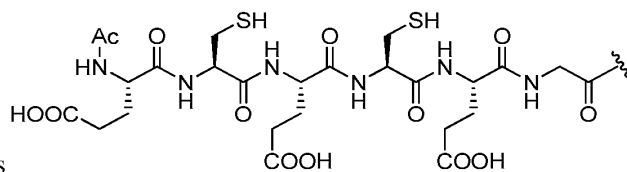


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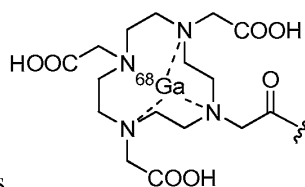
some embodiments of a compound of formula (I), D is



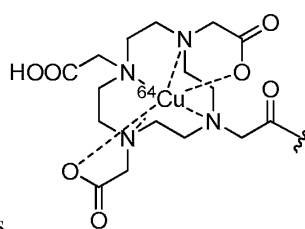
. In some embodiments of a compound



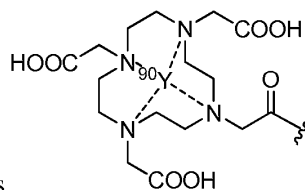
of formula (I), D is . In some



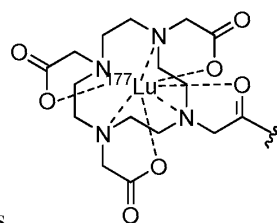
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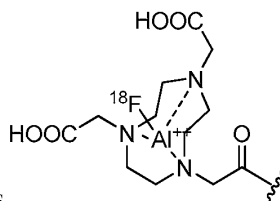
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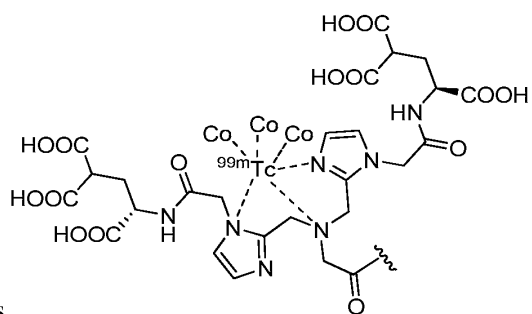
embodiments of a compound of formula (I), D is . In some



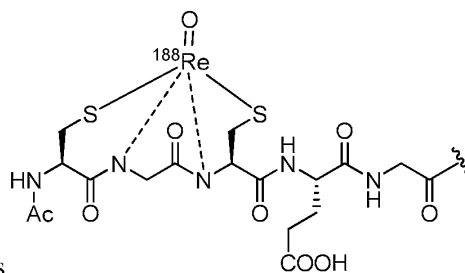
embodiments of a compound of formula (I), D is . In some embodiments



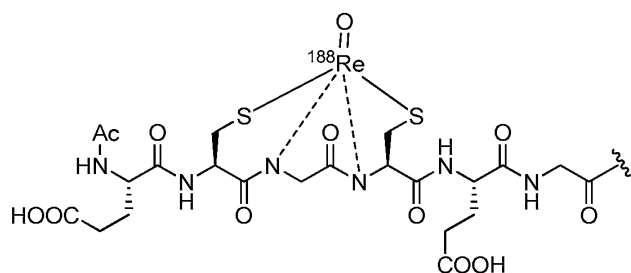
of a compound of formula (I), D is . In some embodiments of a



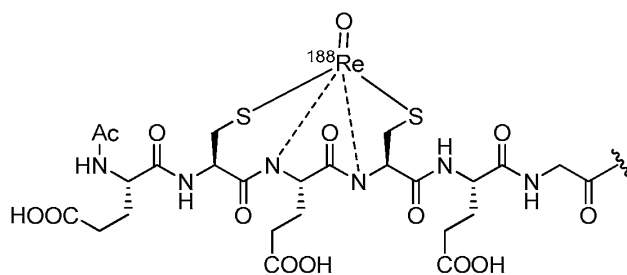
compound of formula (I), D is . In some



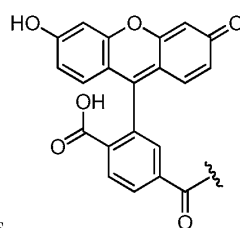
embodiments of a compound of formula (I), D is . In some embodiments of a compound of formula (I), D is



. In some embodiments of a compound

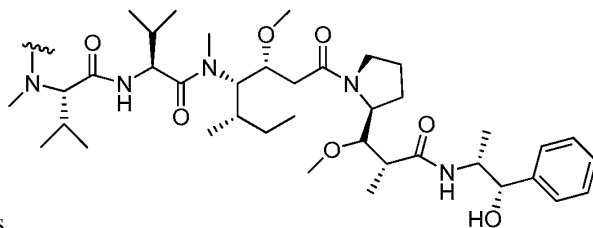


of formula (I), D is . In some

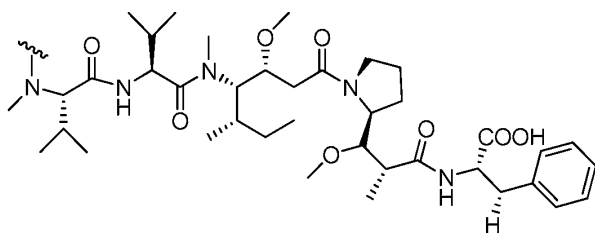


embodiments of a compound of formula (I), D is . In some embodiments of

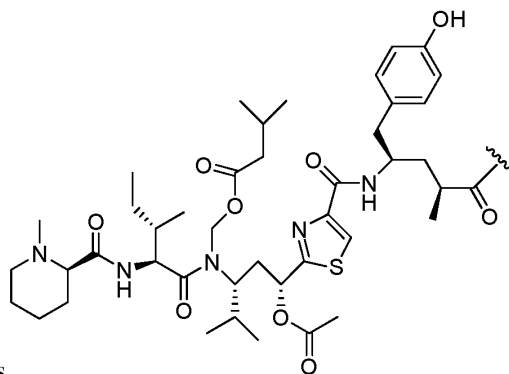




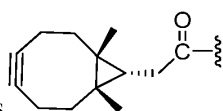
of a compound of formula (I), D is  
embodiments of a compound of formula (I), D is



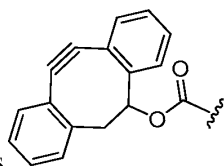
. In some embodiments of a compound of



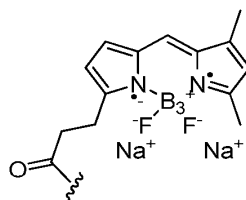
formula (I), D is . In some embodiments of a



compound of formula (I), D is . In some embodiments of a compound of

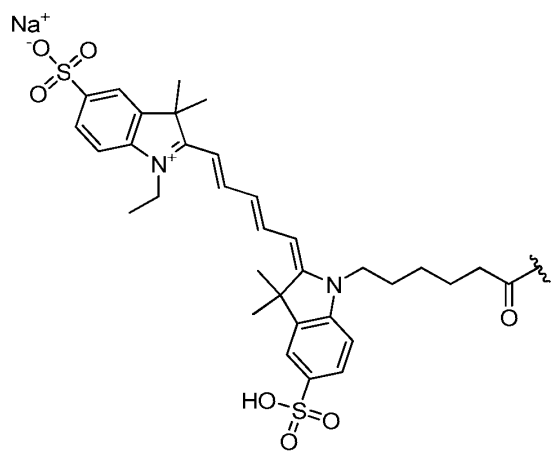


formula (I), D is . In some embodiments of a compound of formula (I), D is

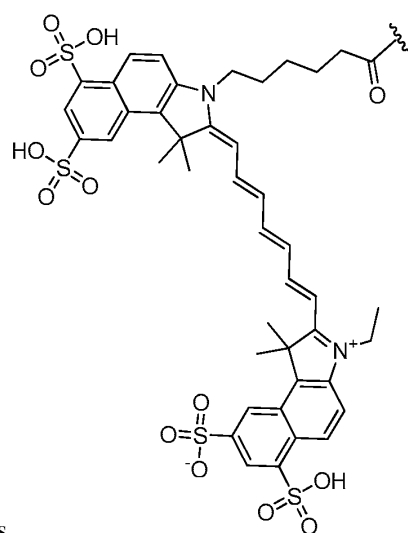


. In some embodiments of a compound of formula (I), D is



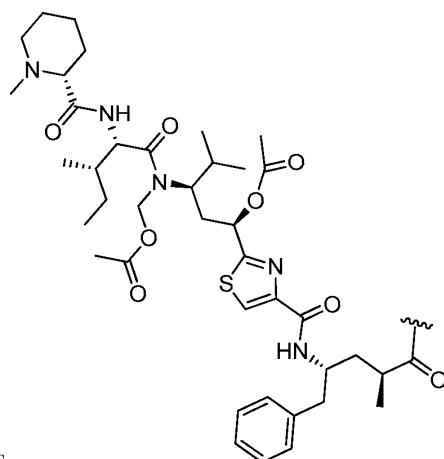


. In some embodiments of a compound of



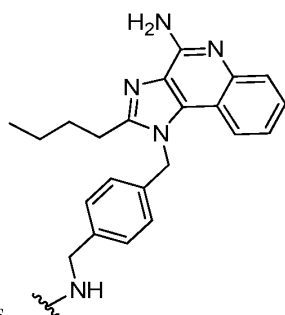
formula (I), D is

. In some embodiments of a compound of



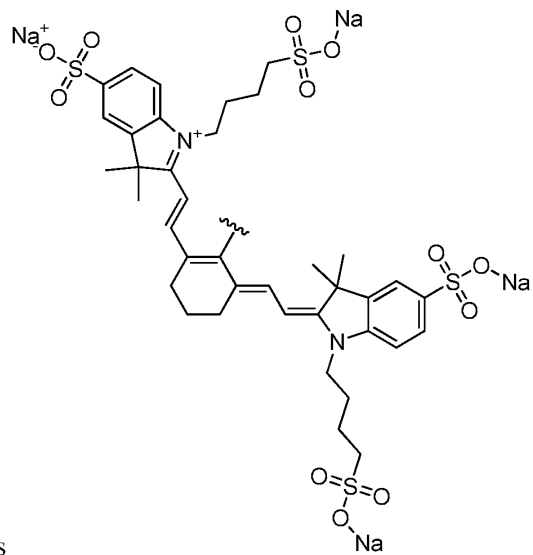
formula (I), D is

. In some embodiments of a compound



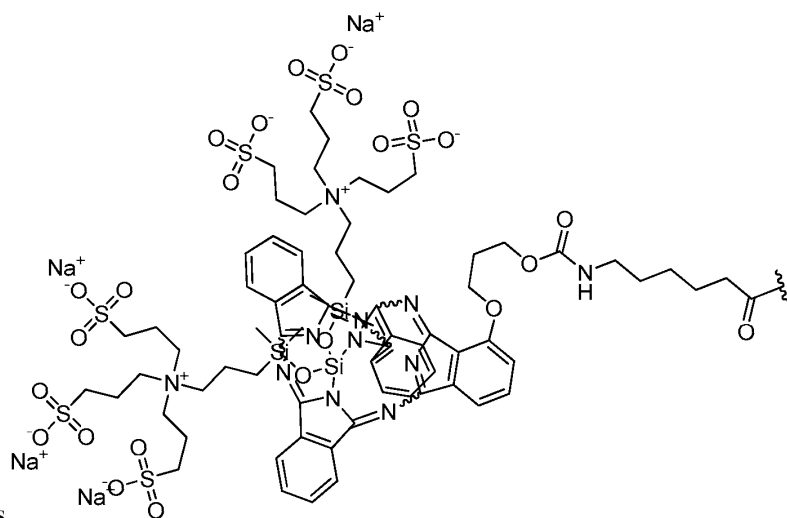
of formula (I), D is

. In some embodiments of a compound of formula



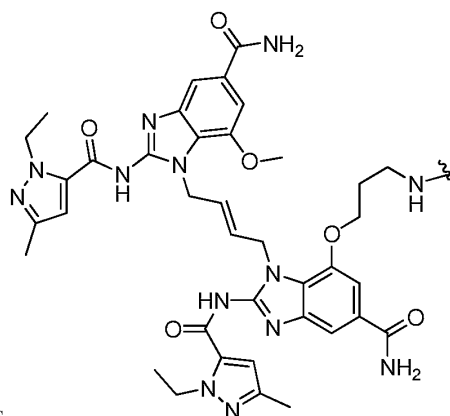
(I), D is

. In some embodiments of a compound of



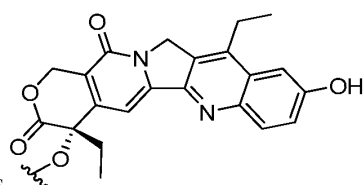
formula (I), D is

. In some



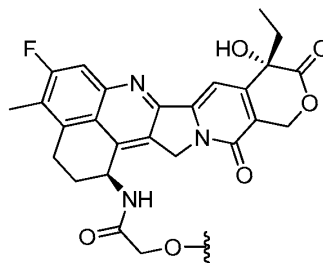
embodiments of a compound of formula (I), D is

. In



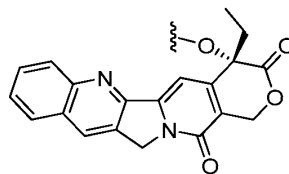
some embodiments of a compound of formula (I), D is

. In some

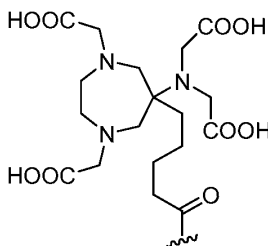


embodiments of a compound of formula (I), D is

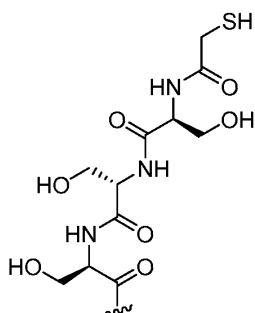
. In some



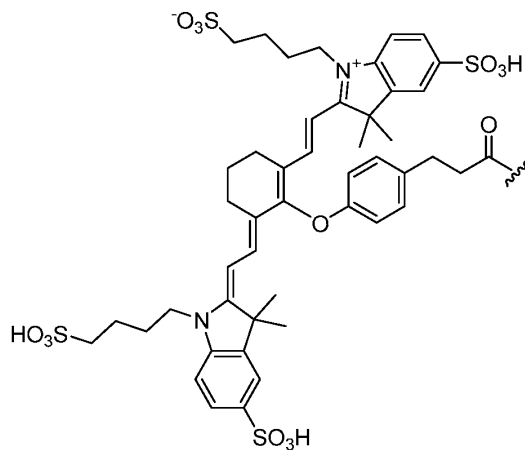
embodiments of a compound of formula (I), D is . In some



embodiments of a compound of formula (I), D is . In some embodiments



of a compound of formula (I), D is . In some embodiments of a compound

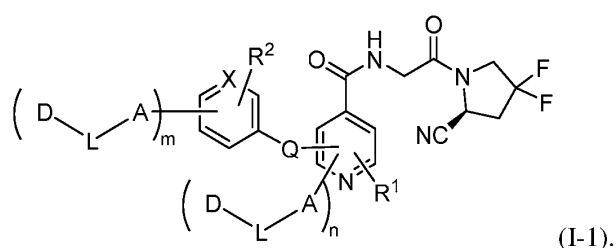


of formula (I), D is ; wherein the lines denote attachment points to L;

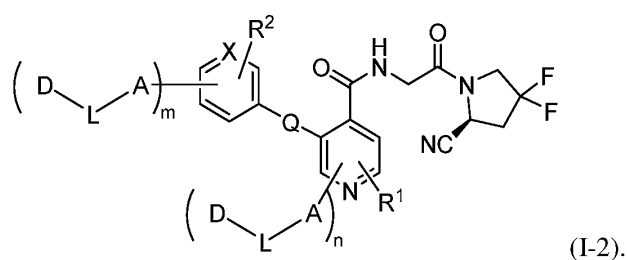
**[0100]** It is understood that each description of Q, X, A, L, D, m, n, R<sup>1</sup> and R<sup>2</sup> may be independently combined with each description of Q, X, A, L, D, m, n, R<sup>1</sup> and R<sup>2</sup> the same as if each and every combination were specifically and individually listed.

[0101] In some embodiments, A is selected from the group consisting of -O-, 5- to 6-membered heterocyclyl,  $-(CH_2)_p$ -,  $-NR^6$ -,  $-C(O)NR^6$ -,  $-NR^6C(O)$ -,  $-C(O)O$ -,  $-OC(O)$ -,  $-C(O)(CH_2)_p$ -,  $-(CH_2)_pC(O)$ -,  $-(CH_2)_p-NR^6$ -,  $-NR^6(CH_2)_p$ -,  $-O(CH_2)_p$ -, and  $-(CH_2)_p-O$ -, wherein # indicates the point of attachment to L. In some embodiments, A is -O-,  $-NR^6$ -,  $-C(O)NR^6$ -,  $-NR^6C(O)$ -,  $-C(O)O$ -, or  $-OC(O)$ -, wherein # indicates the point of attachment to L. In some embodiments, A is -O-,  $-NR^6$ -,  $-C(O)NR^6$ -, or  $-NR^6C(O)$ -, wherein # indicates the point of attachment to L. In some embodiments of a compound of formula (I), A is -O-.

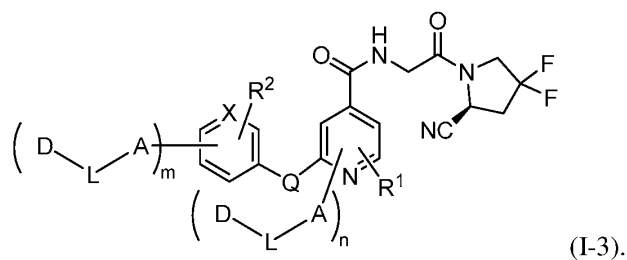
[0102] In some embodiments, a compound of formula (I) is a compound of formula (I-1):



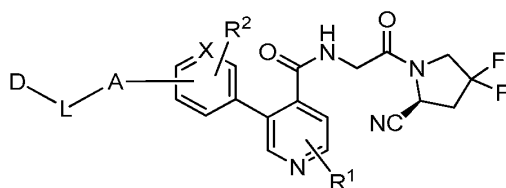
[0103] In some embodiments, a compound of formula (I) is a compound of formula (I-2):



[0104] In some embodiments, a compound of formula (I) is a compound of formula (I-3):

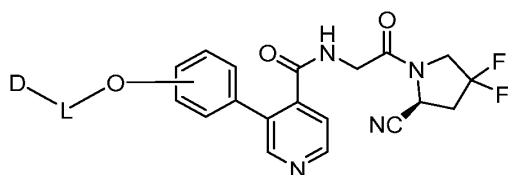


[0105] In some embodiments, a compound of formula (I) is a compound of formula (II-1),



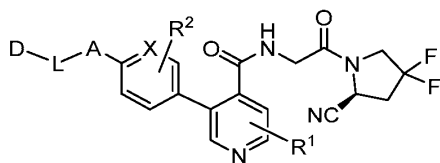
formula (II-1),

or a pharmaceutically acceptable salt, stereoisomer or tautomer thereof, wherein X, A, L, D, R<sup>1</sup> and R<sup>2</sup> are as defined for formula (I). In some embodiments, a compound of formula (II-1) is of

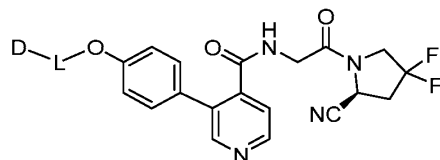


formula:

**[0106]** In some embodiments, a compound of formula (I) is a compound of formula (II-a),

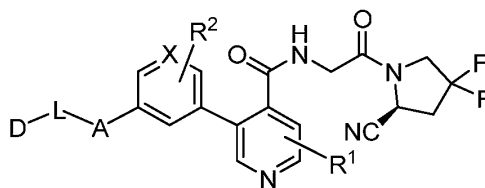


(II-a). In some embodiments, a compound of formula (II-



a) is of formula:

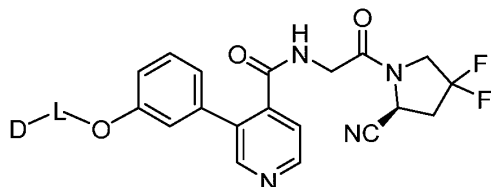
. In some embodiments, a compound of



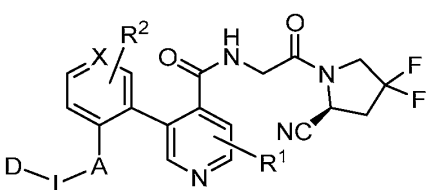
formula (I) is a compound of formula (II-b),

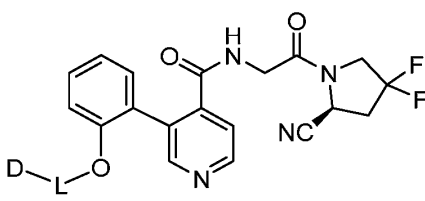
(II-b).

In some embodiments, a compound of formula (II-a) is of formula:

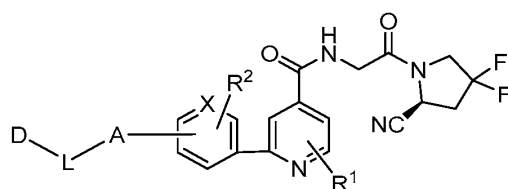


. In some embodiments, a compound of formula (I)

is a compound of formula (II-c),  (II-c). In some

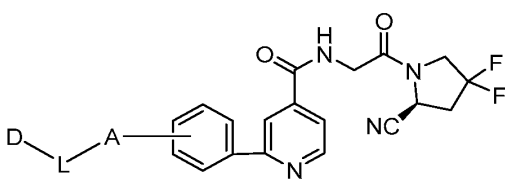
embodiments, a compound of formula (II-a) is of formula: 

[0107] In some embodiments, a compound of formula (I) is a compound of formula (II-2),

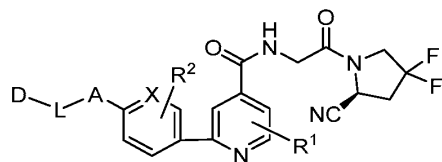


formula (II-2),

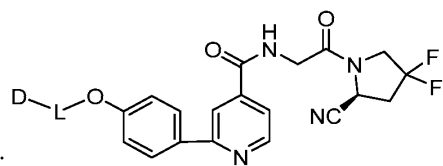
or a pharmaceutically acceptable salt, stereoisomer or tautomer thereof, wherein X, A, L, D, R<sup>1</sup> and R<sup>2</sup> are as defined for formula (I). In some embodiments, a compound of formula (II-2) is of

formula: 

[0108] In some embodiments, a compound of formula (I) is a compound of formula (II-d),



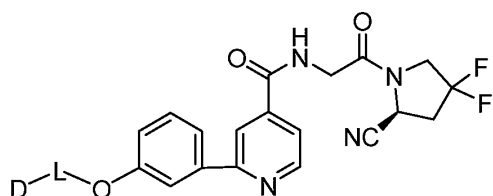
(II-d). In some embodiments, a compound of formula (II-



d) is of formula:

. In some embodiments, a compound of

formula (I) is a compound of formula (II-e), (II-e). In some embodiments, a compound of formula (II-e) is of formula:



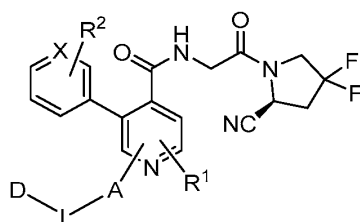
. In some embodiments, a compound of formula (I)

is a compound of formula (II-f), (II-f). In some

embodiments, a compound of formula (II-f) is of formula: .

**[0109]** In some embodiments, a compound of formula (I) is a compound of formula (III),

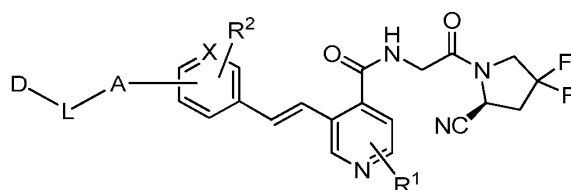




formula (III),

or a pharmaceutically acceptable salt, stereoisomer or tautomer thereof, wherein X, A, L, D, R<sup>1</sup> and R<sup>2</sup> are as defined for formula (I).

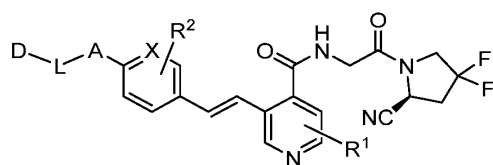
[0110] In some embodiments, a compound of formula (I) is a compound of formula (IV-1),



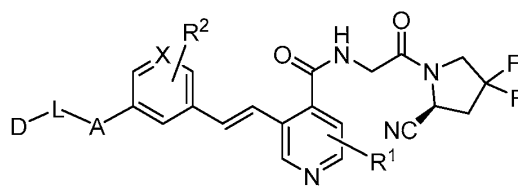
formula (IV-1),

or a pharmaceutically acceptable salt, stereoisomer or tautomer thereof, wherein X, A, L, D, R<sup>1</sup> and R<sup>2</sup> are as defined for formula (I).

[0111] In some embodiments, a compound of formula (I) is a compound of formula (IV-a),

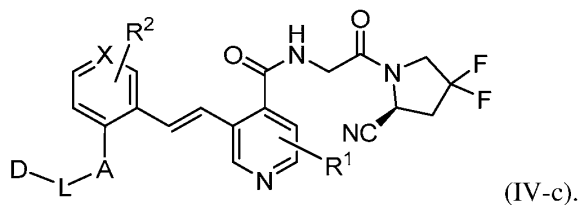


(IV-a). In some embodiments, a compound of

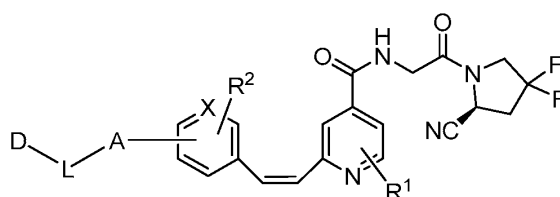


formula (I) is a compound of formula (IV-b),

(IV-b). In some embodiments, a compound of formula (I) is a compound of formula (IV-c),

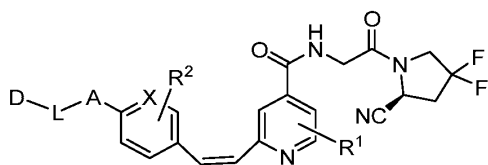


[0112] In some embodiments, a compound of formula (I) is a compound of formula (IV-2),



or a pharmaceutically acceptable salt, stereoisomer or tautomer thereof, wherein X, A, L, D, R<sup>1</sup> and R<sup>2</sup> are as defined for formula (I).

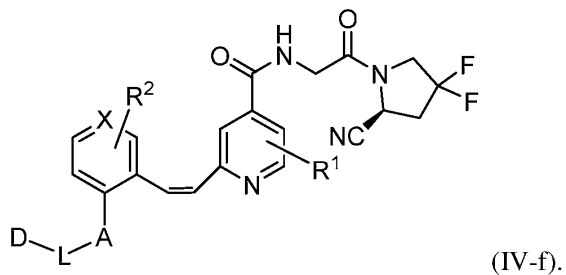
[0113] In some embodiments, a compound of formula (I) is a compound of formula (IV-d),



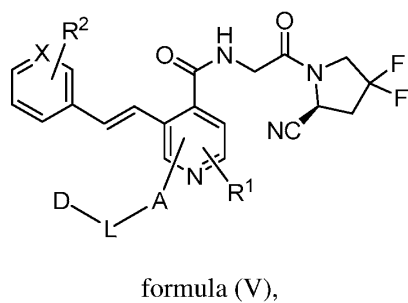
formula (I) is a compound of formula (IV-e),

(IV-

e). In some embodiments, a compound of formula (I) is a compound of formula (IV-f),

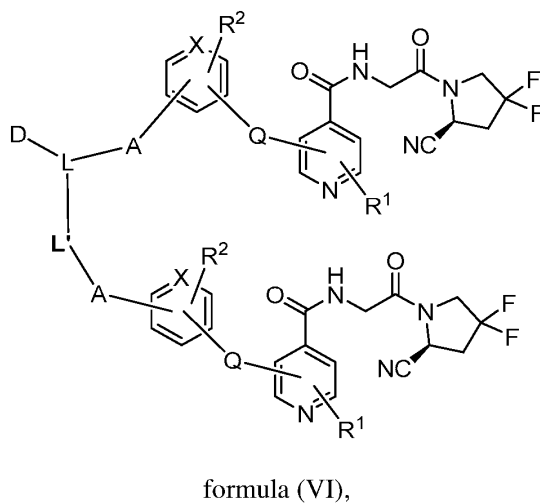


[0114] In some embodiments, a compound of formula (I) is a compound of formula (V),



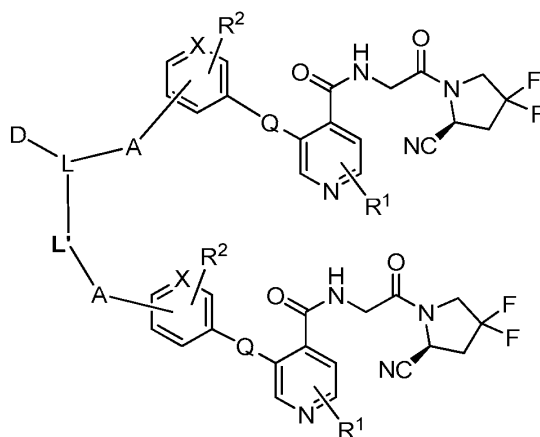
or a pharmaceutically acceptable salt, stereoisomer or tautomer thereof, wherein X, A, L, D, R<sup>1</sup> and R<sup>2</sup> are as defined for formula (I).

[0115] In some embodiments, a compound of formula (I) is a compound of formula (VI),



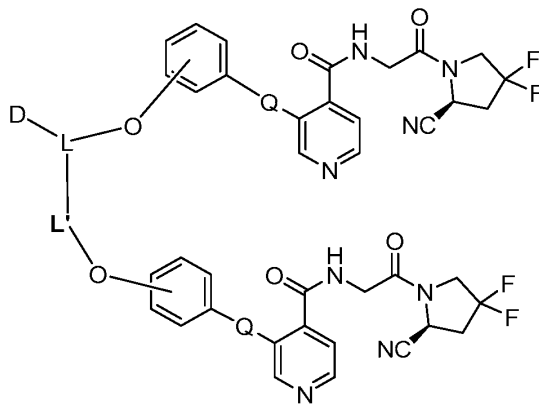
or a pharmaceutically acceptable salt, stereoisomer or tautomer thereof, wherein X, Q, A, L, L', D, R<sup>1</sup> and R<sup>2</sup> are as defined for formula (I).

[0116] In some embodiments, a compound of formula (I) is a compound of formula (VI-1),



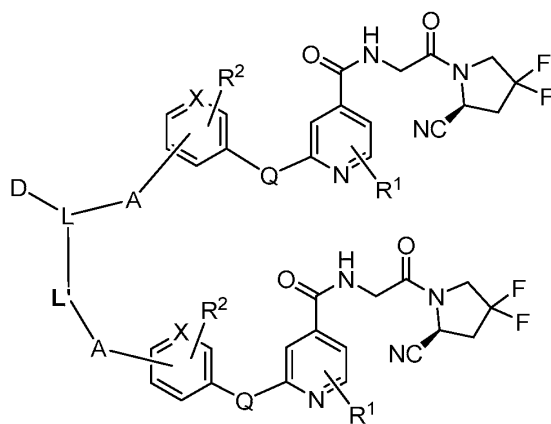
formula (VI-1),

or a pharmaceutically acceptable salt, stereoisomer or tautomer thereof, wherein X, Q, A, L, L', D, R<sup>1</sup> and R<sup>2</sup> are as defined for formula (I). In some embodiments, a compound of formula (VI-



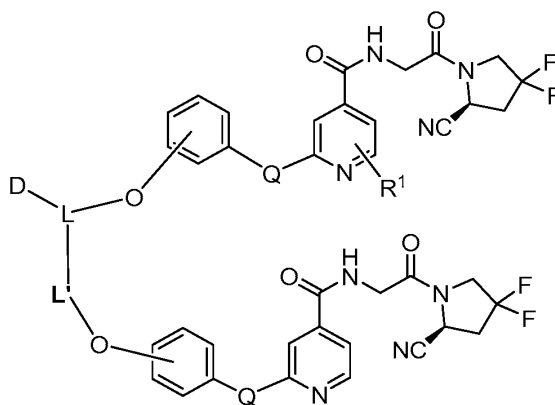
1) is a compound of formula (VI-1a):

[0117] In some embodiments, a compound of formula (I) is a compound of formula (VI-2),



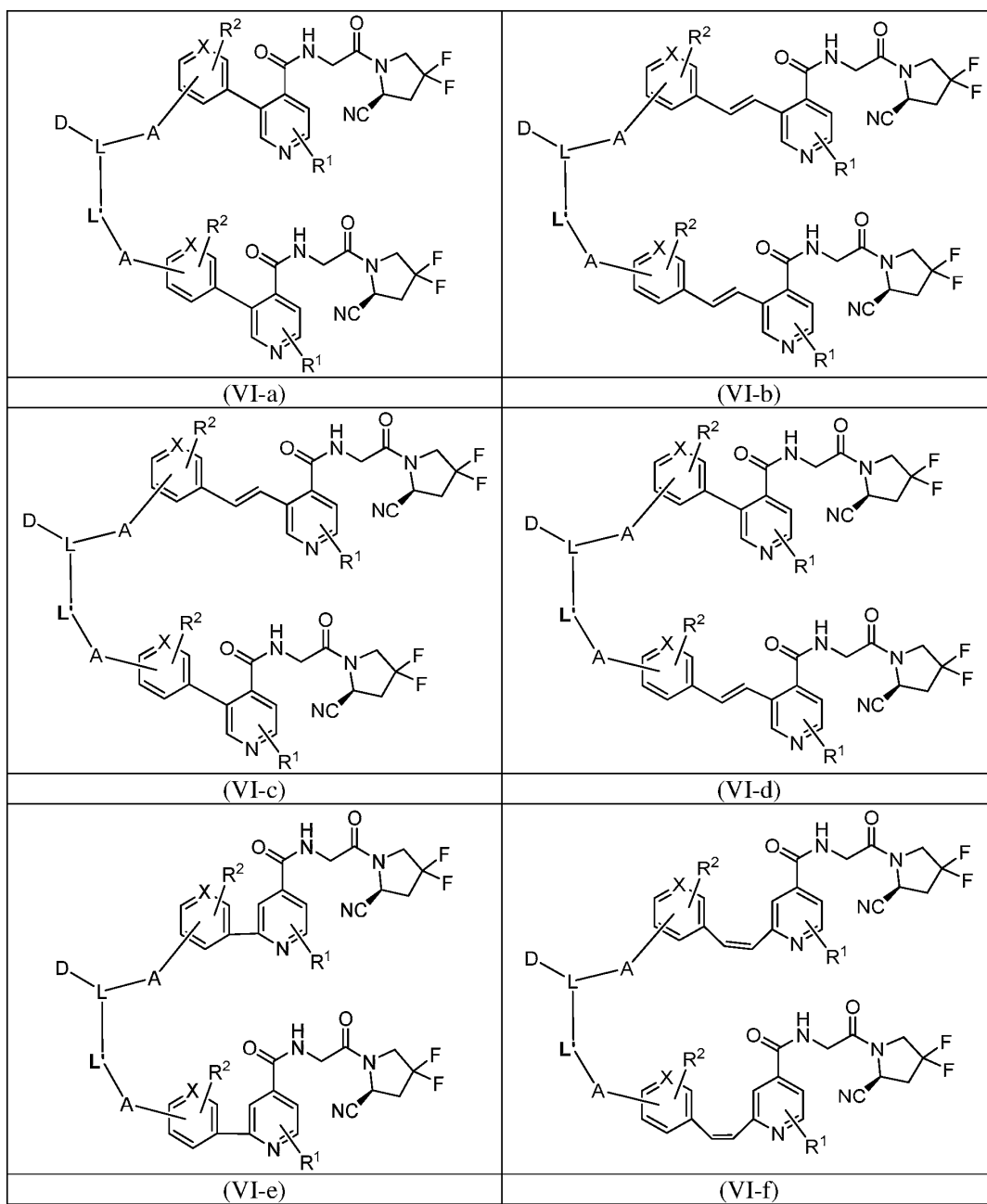
formula (VI-2),

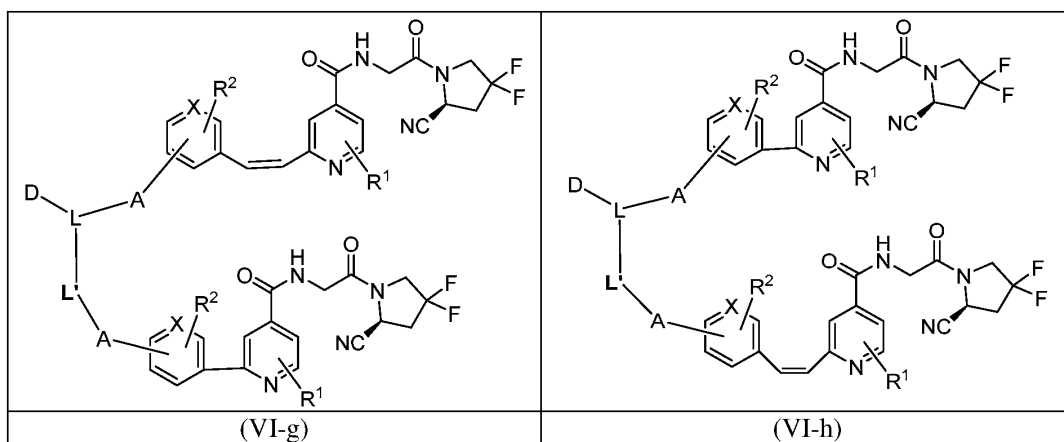
or a pharmaceutically acceptable salt, stereoisomer or tautomer thereof, wherein X, Q, A, L, L', D, R<sup>1</sup> and R<sup>2</sup> are as defined for formula (I). In some embodiments, a compound of formula (VI-



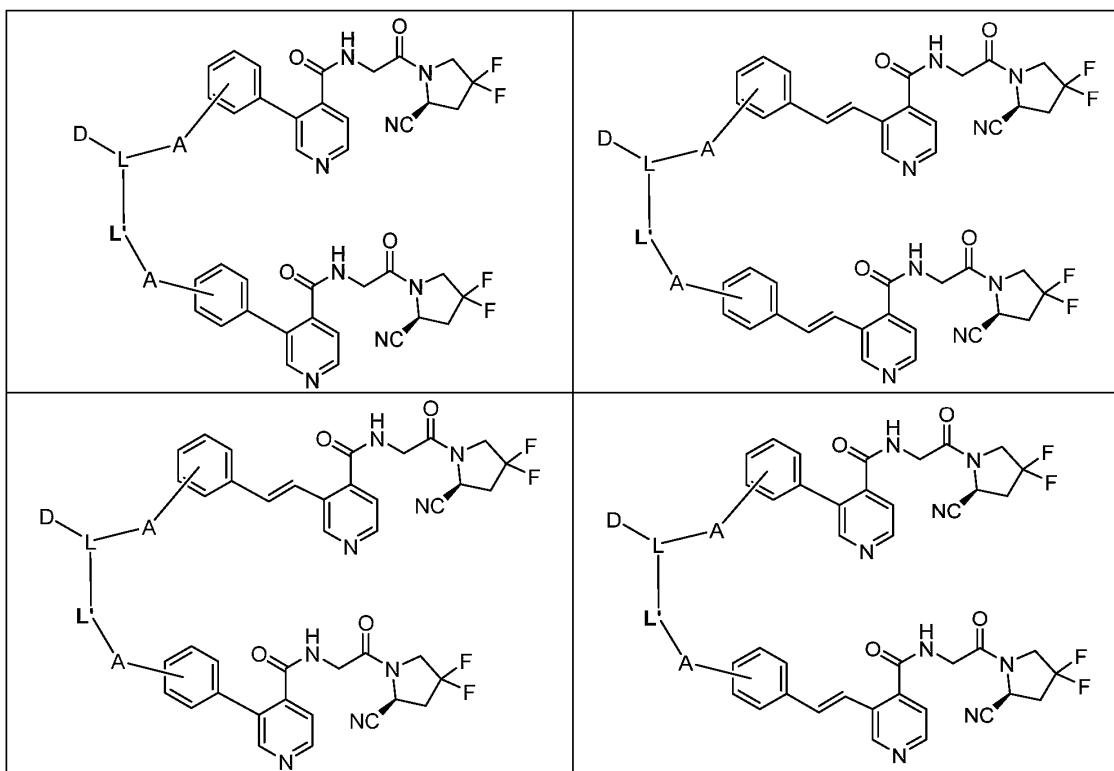
2) is a compound of formula (VI-2a):

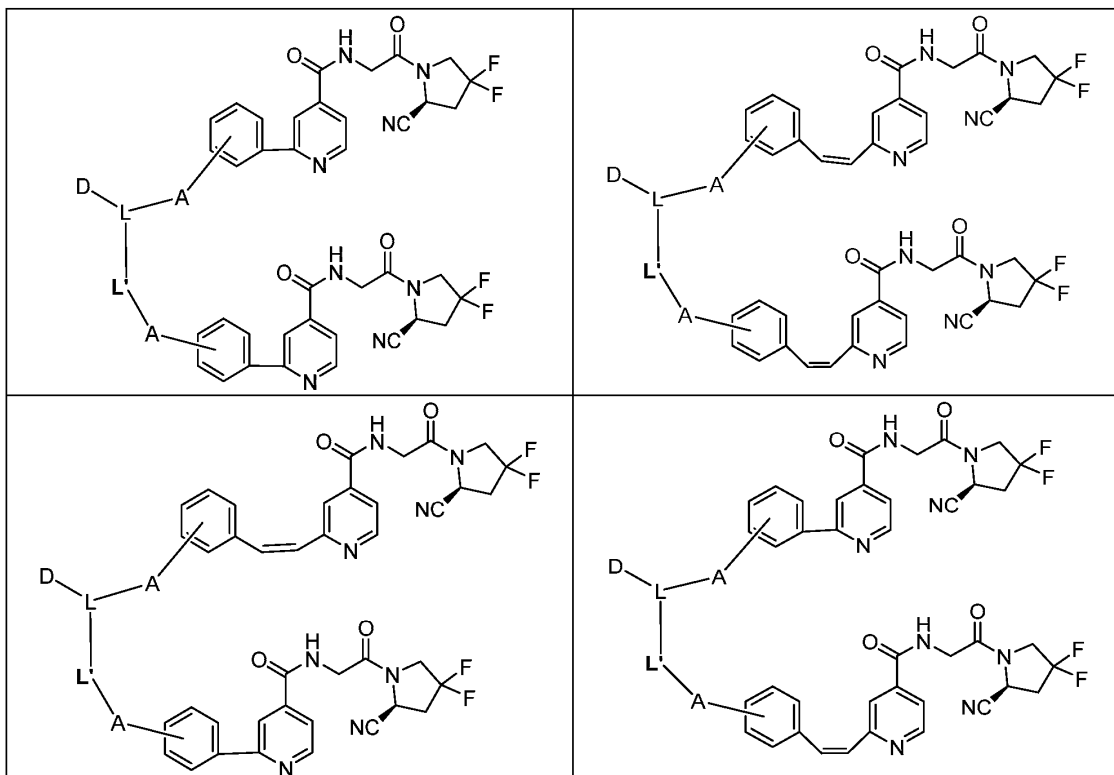
**[0118]** In some embodiments, a compound of formula (I) is a compound of any of the compounds of formula (VI-a to VI-d),



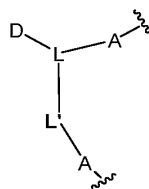


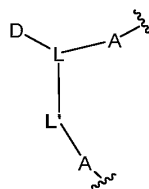
or a pharmaceutically acceptable salt, stereoisomer or tautomer thereof, wherein X, A, L, L', D, R<sup>1</sup>, and R<sup>2</sup> are as defined for formula (I). In some embodiments, a compound of formula (VI-a to VI-h) is of one of the following formulae,

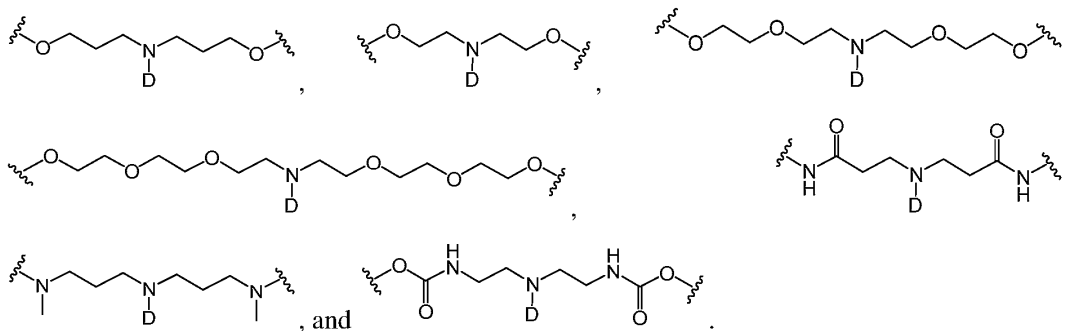




[0119] In some embodiments, a compound of formula (I) is a compound of formula (VI) or



any one of its sub-formulas, wherein the moiety of  is symmetric with respect to the payload. In some embodiments, the moiety is selected from the group consisting of



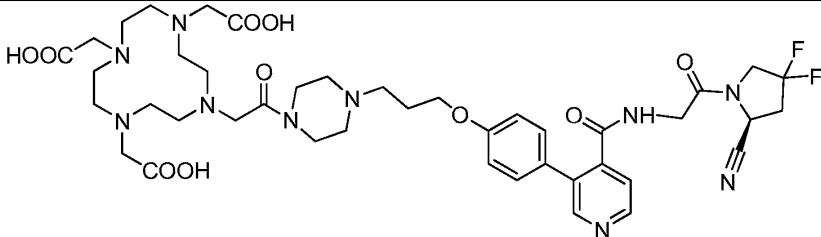


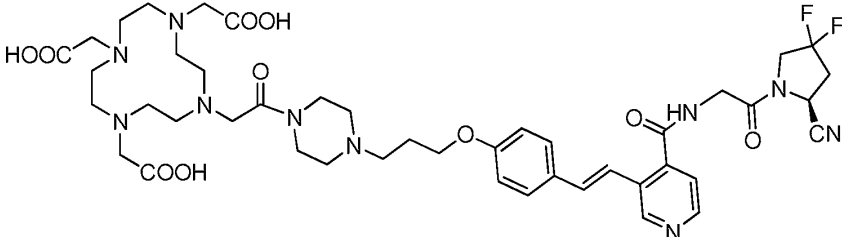
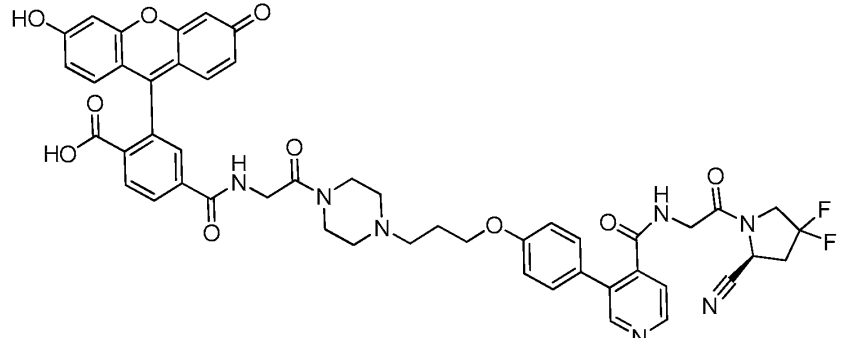
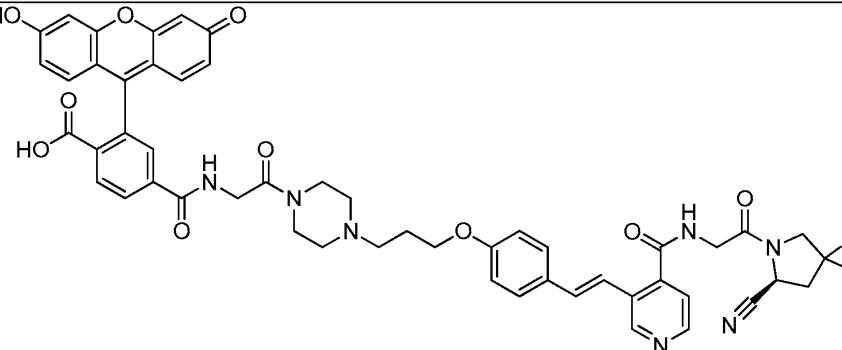
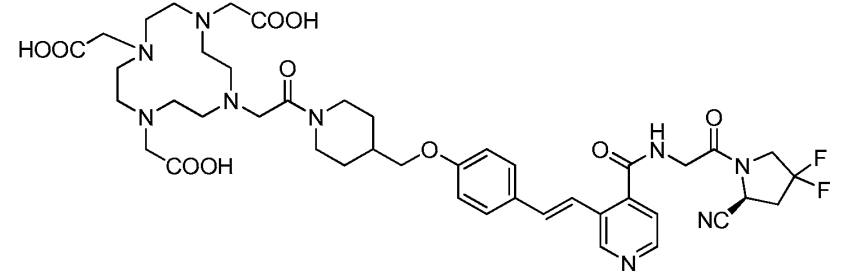
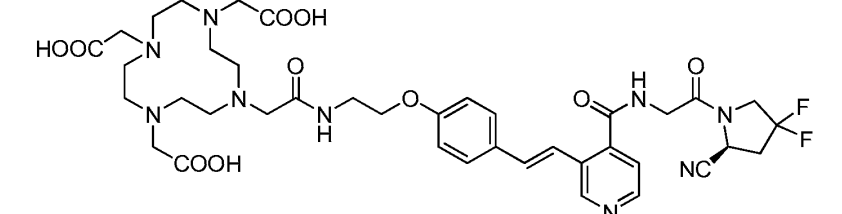
[0120] Also provided are salts of compounds referred to herein, such as pharmaceutically acceptable salts. The invention also includes any or all of the stereochemical forms, including any enantiomeric or diastereomeric forms, and any tautomers or other forms of the compounds described.

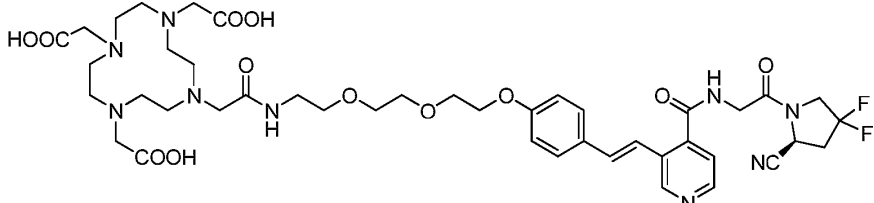
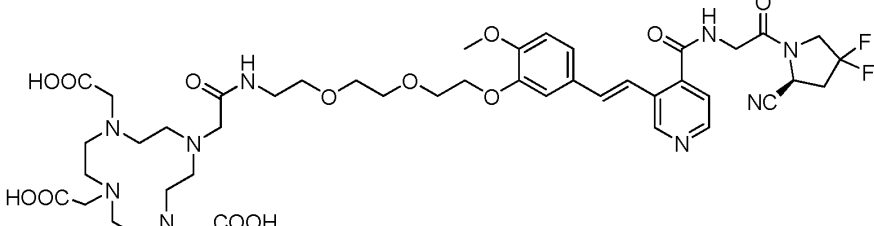
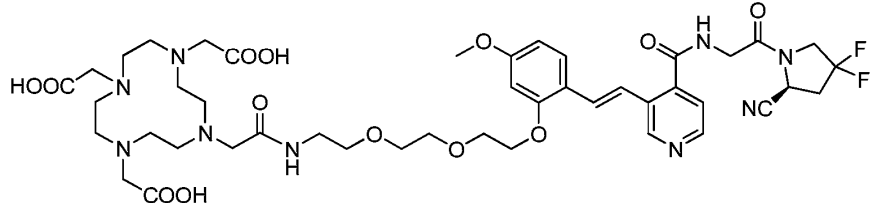
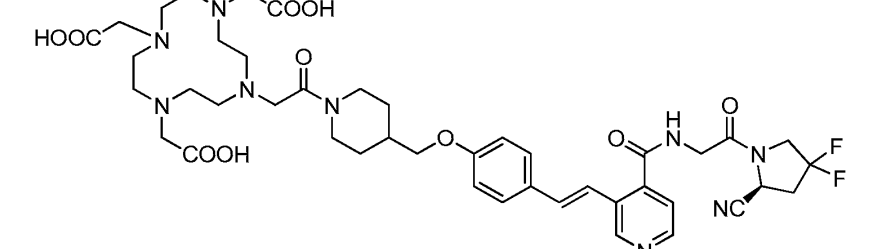
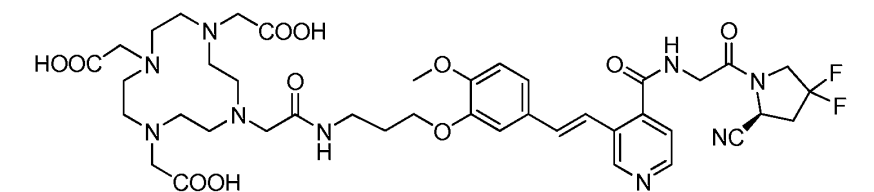
[0121] A compound as detailed herein may in one aspect be in a purified form and compositions comprising a compound in purified forms are detailed herein. Compositions comprising a compound as detailed herein or a salt thereof are provided, such as compositions of substantially pure compounds. In some embodiments, a composition containing a compound as detailed herein or a salt thereof is in substantially pure form. Unless otherwise stated, “substantially pure” intends a composition that contains no more than 35 % impurity, wherein the impurity denotes a compound other than the compound comprising the majority of the composition or a salt thereof. In some embodiments, a composition of substantially pure compound or a salt thereof is provided wherein the composition contains no more than 25 %, 20%, 15%, 10%, or 5% impurity. In some embodiments, a composition of substantially pure compound or a salt thereof is provided wherein the composition contains or no more than 3 %, 2%, 1% or 0.5% impurity.

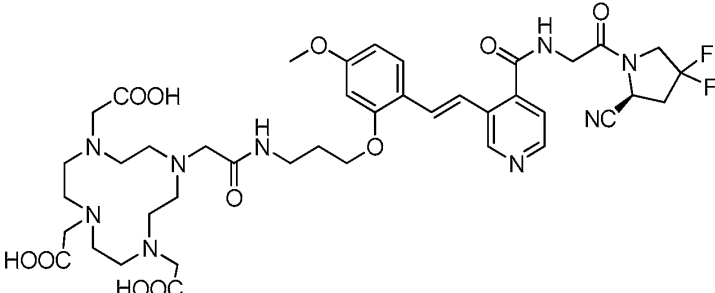
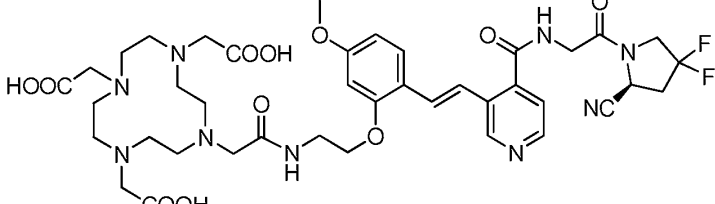
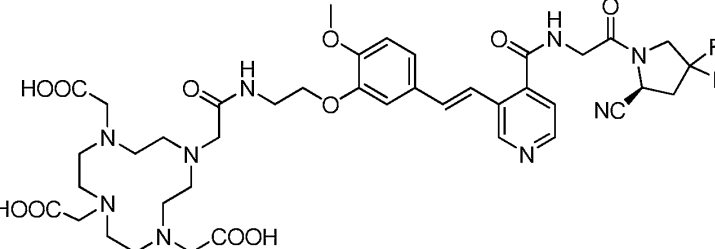
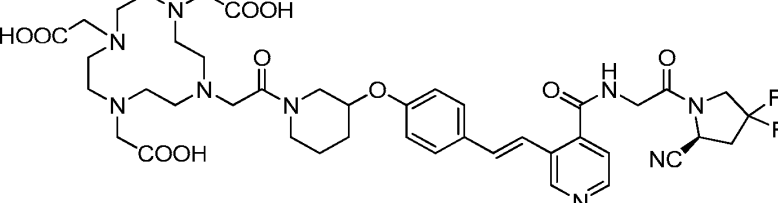
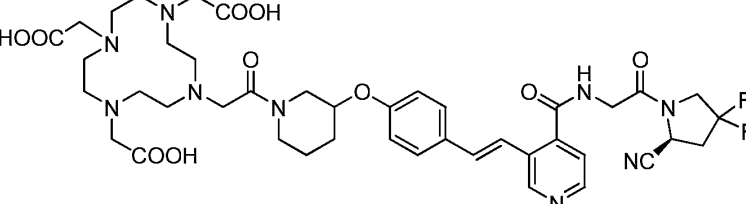
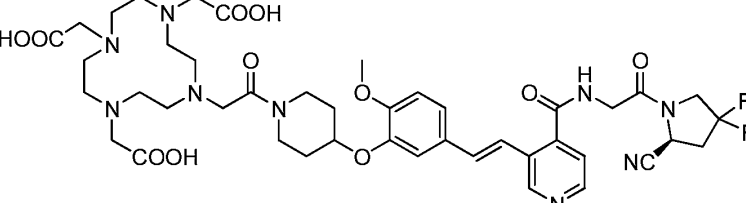
[0122] Representative compounds of the present invention are listed in table 1A and table 1B. It is understood that individual stereoisomers (e.g., enantiomers and diastereomers) and tautomers are included in the generic compound structures shown in table 1A and table 1B. Specific synthetic methods for preparing exemplary compounds are provided example herein.

**Table 1A:** Compounds

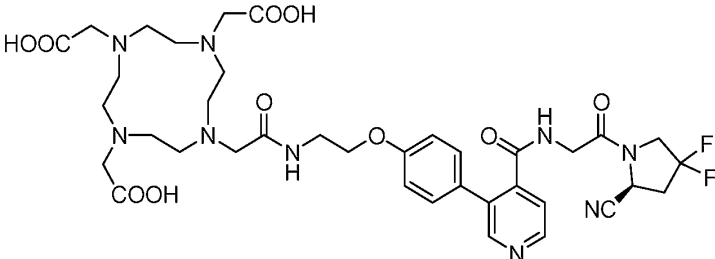
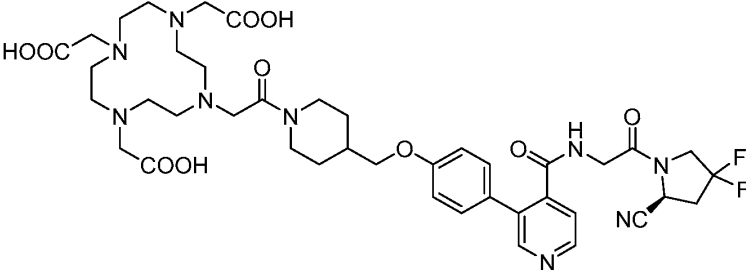
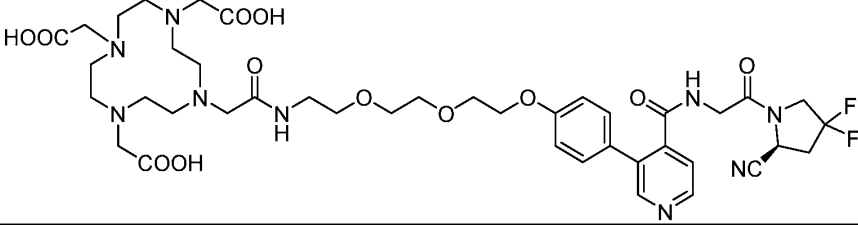
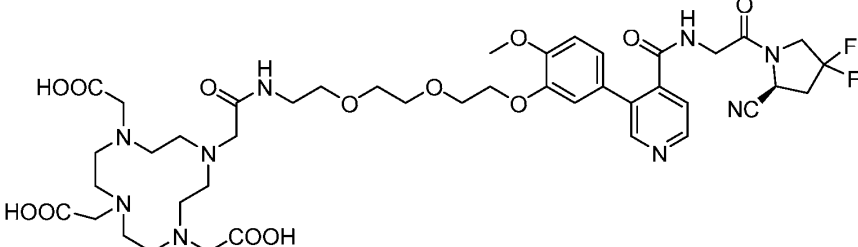
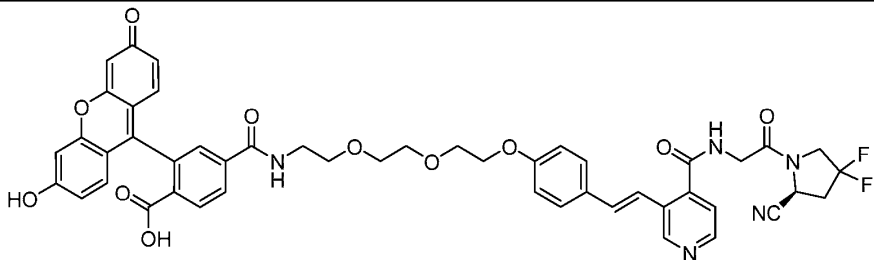
| Compound number | Compounds                                                                            |
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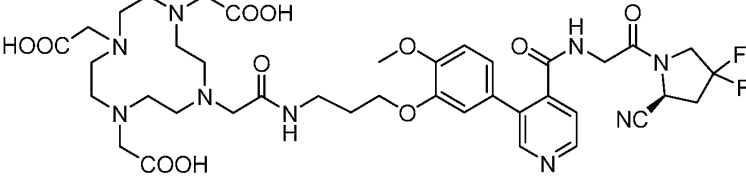
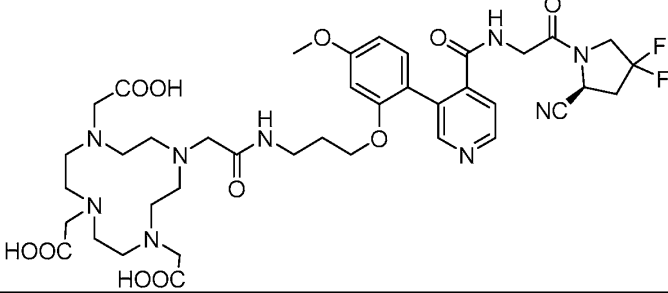
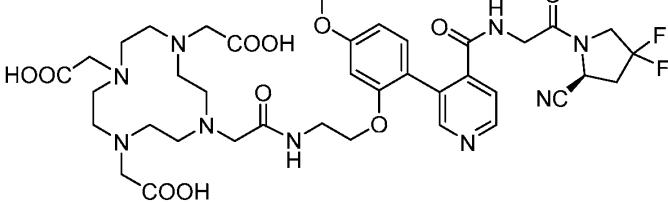
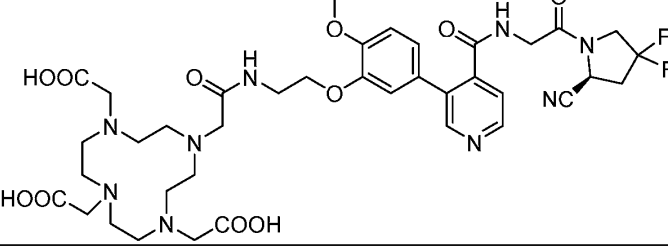
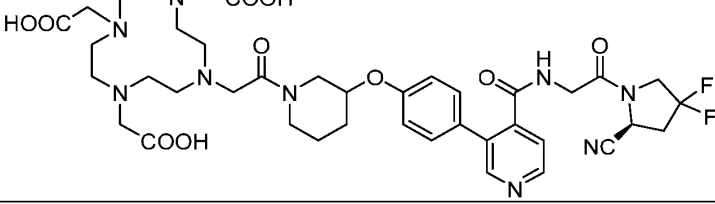
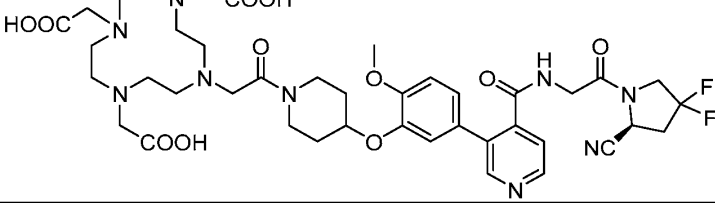
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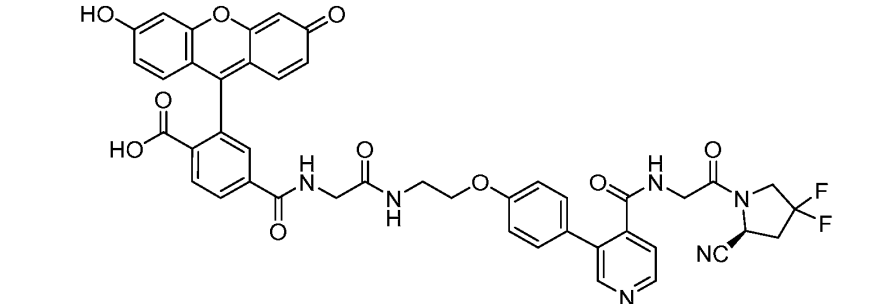
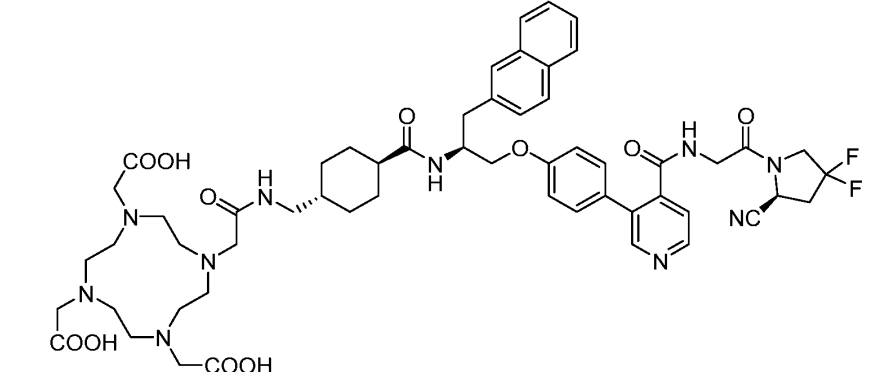
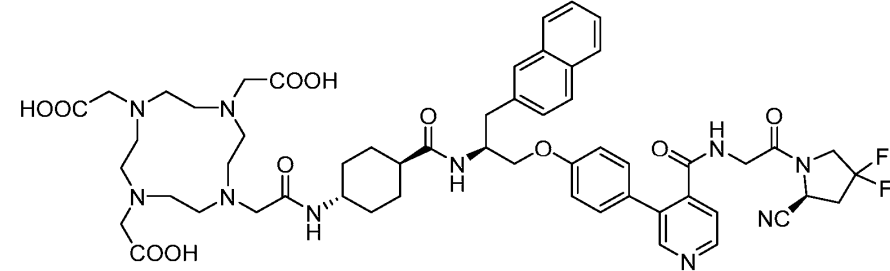
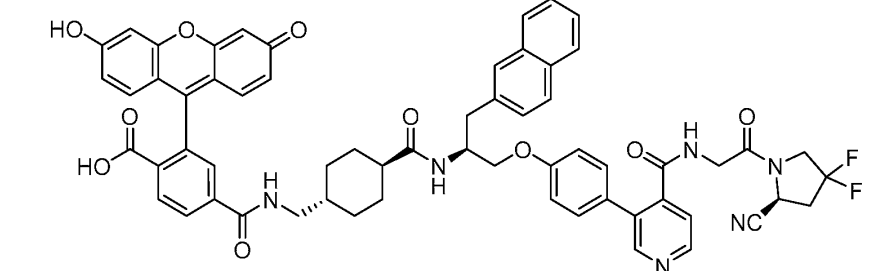
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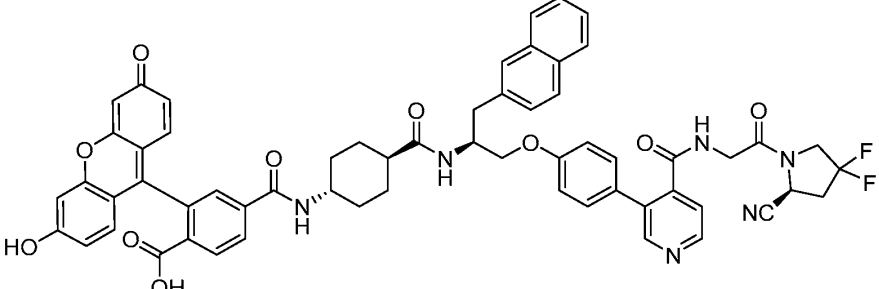
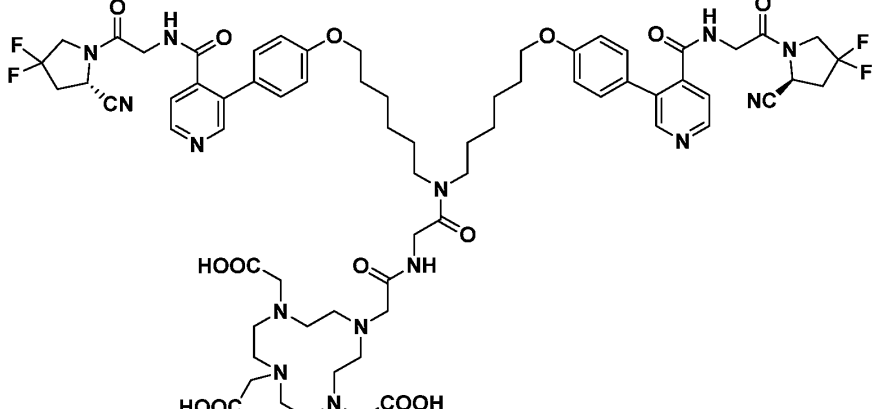
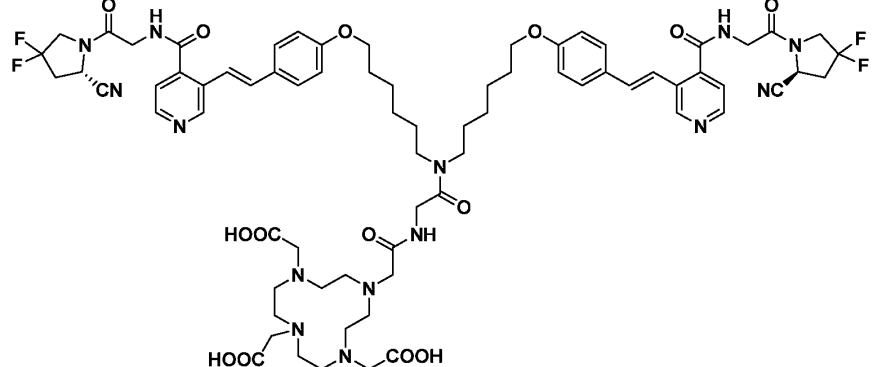
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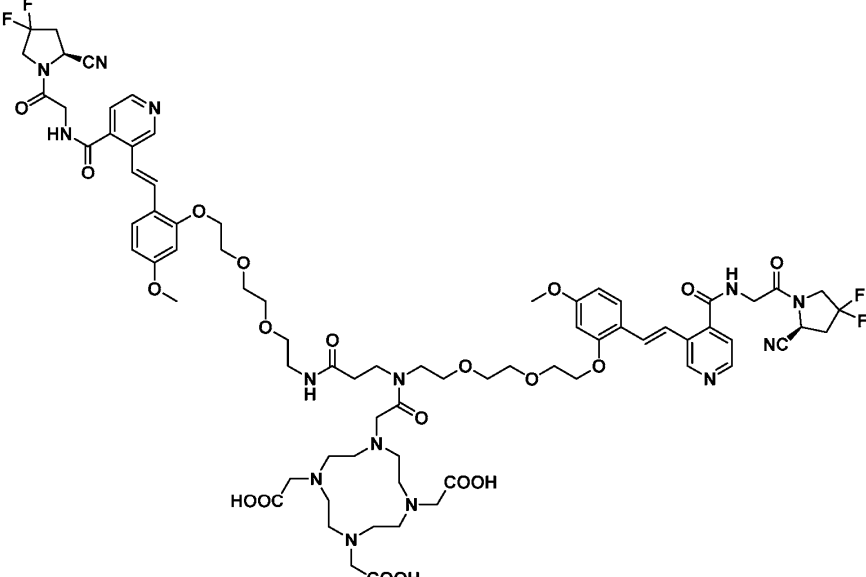
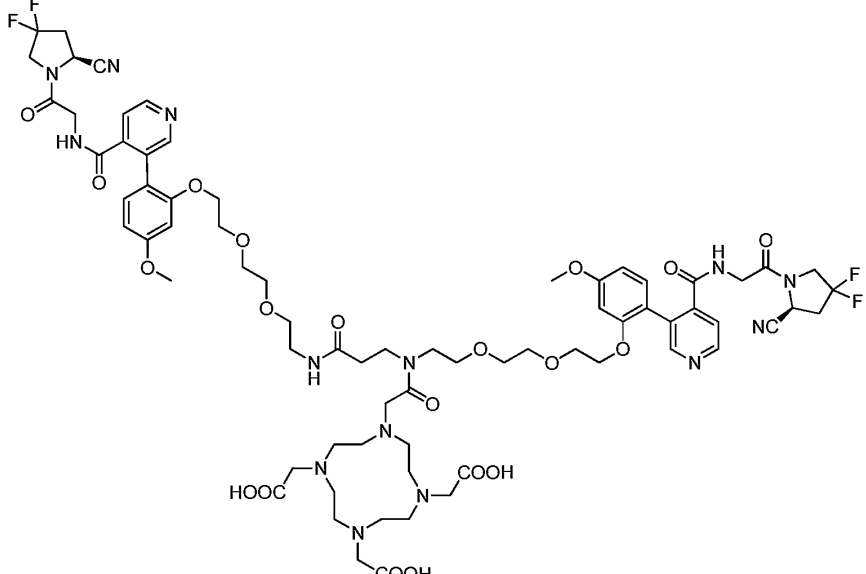
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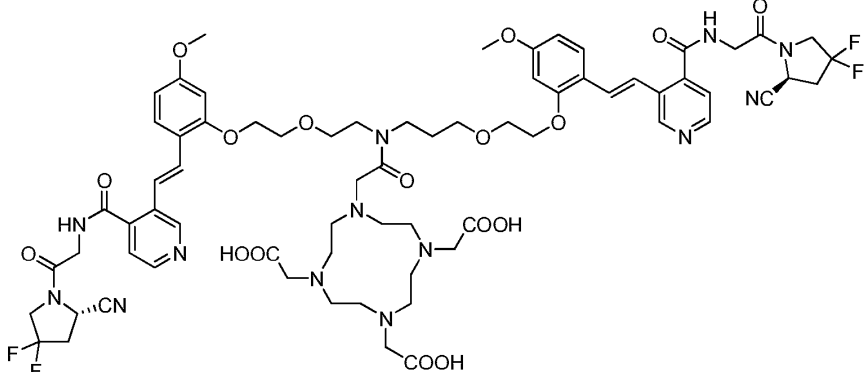
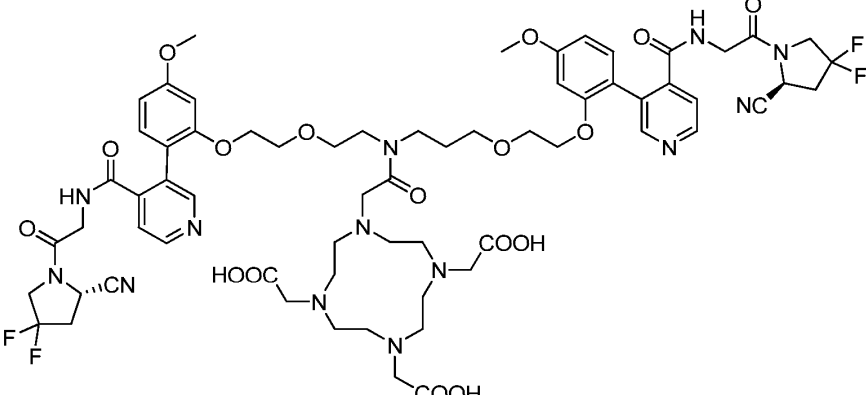
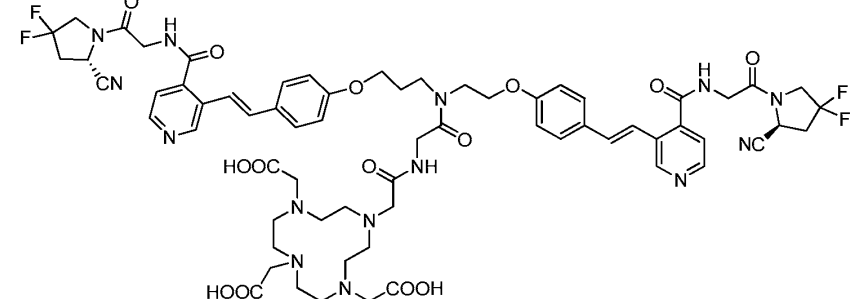
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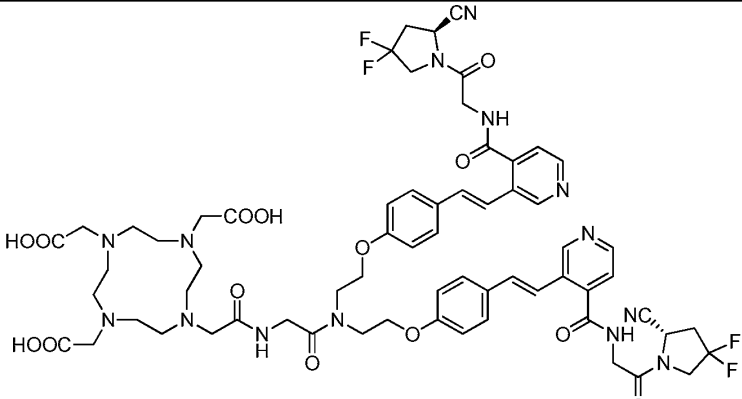
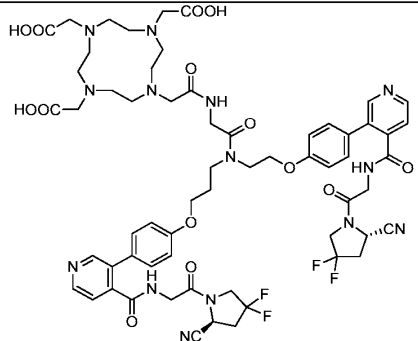
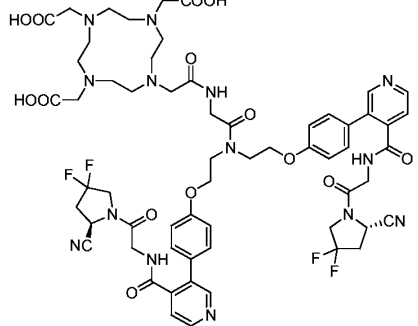
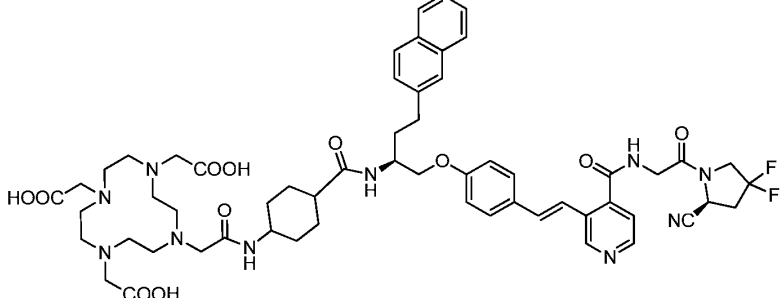
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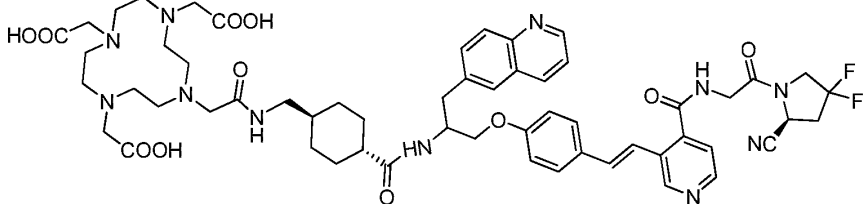
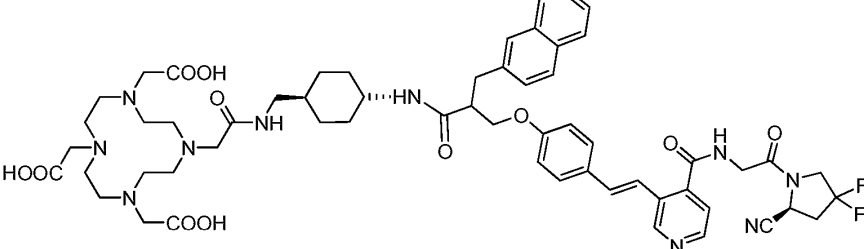
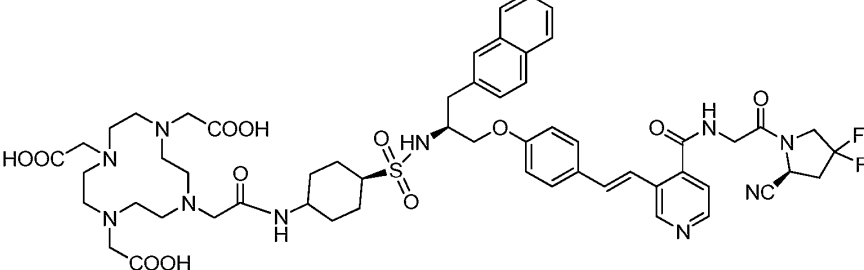
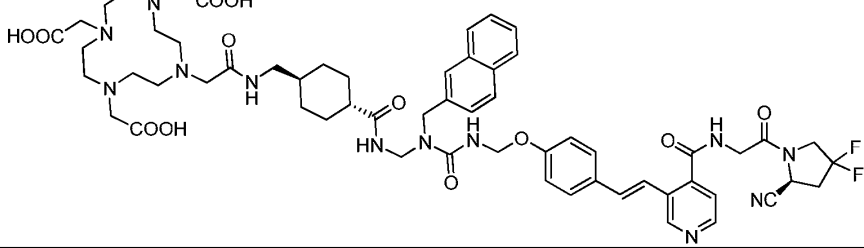
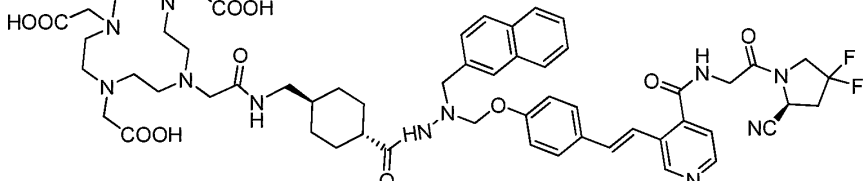


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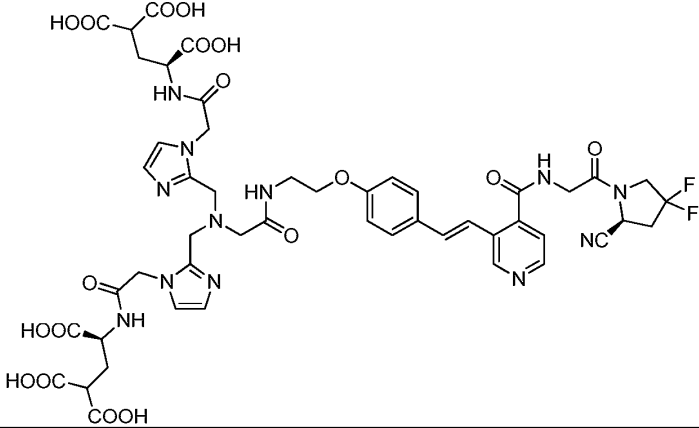
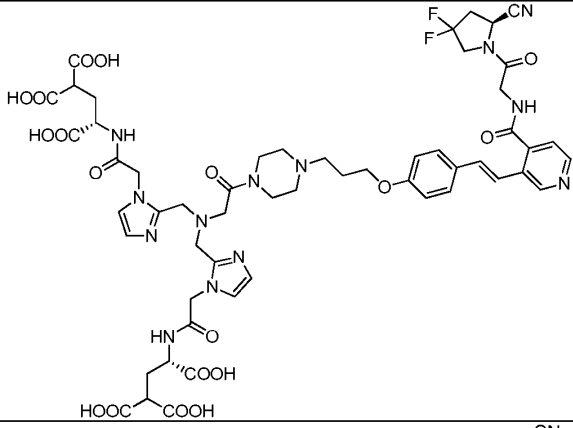
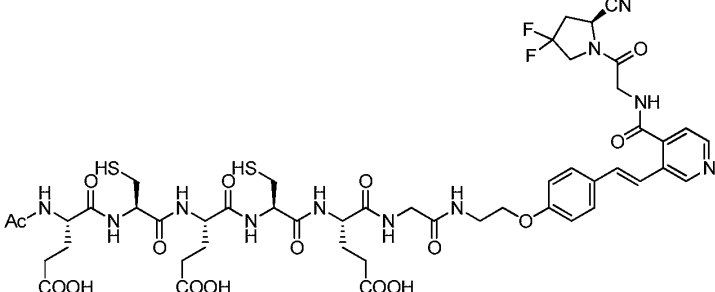
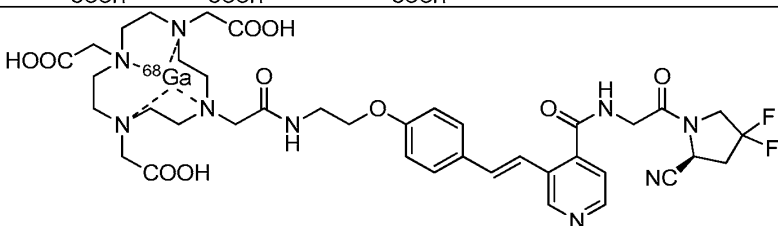
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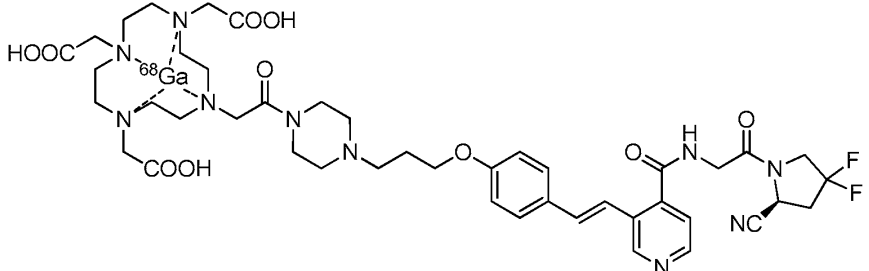
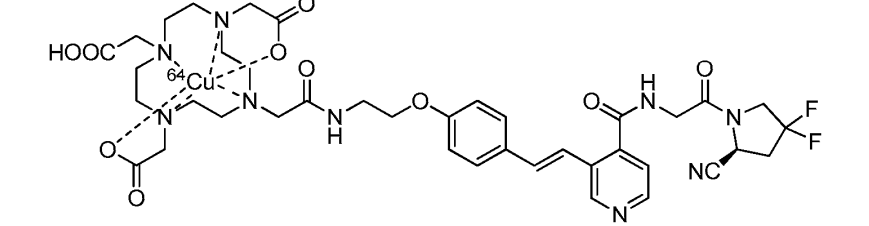
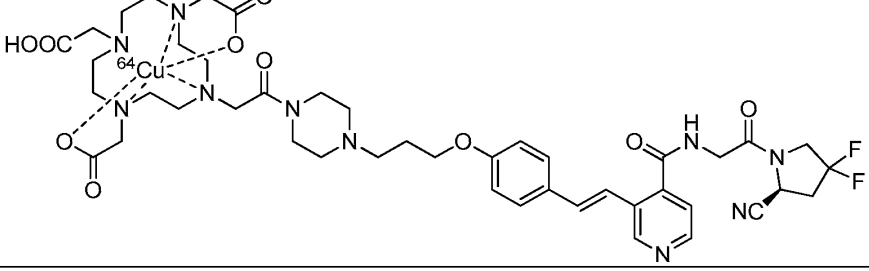
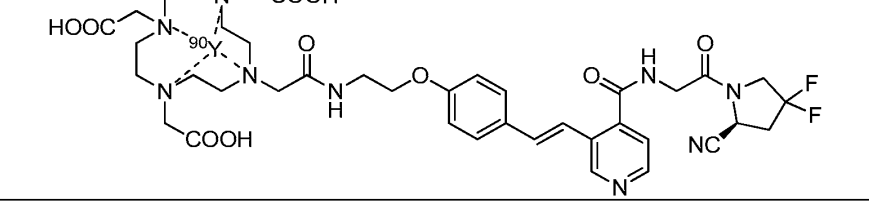
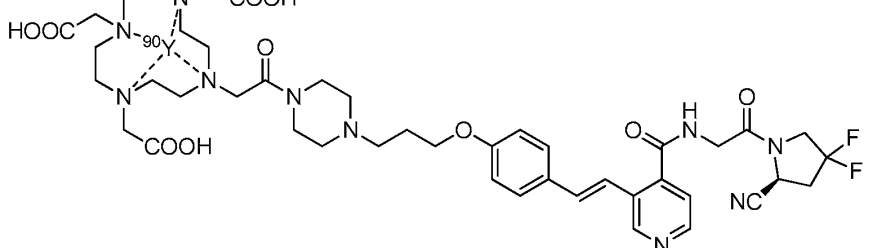
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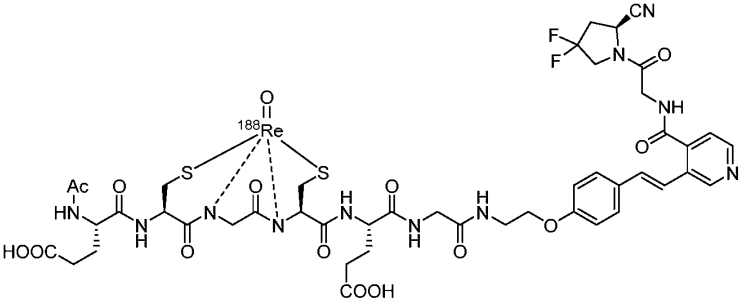
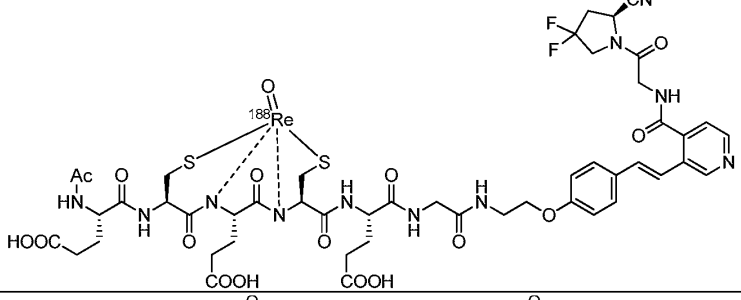
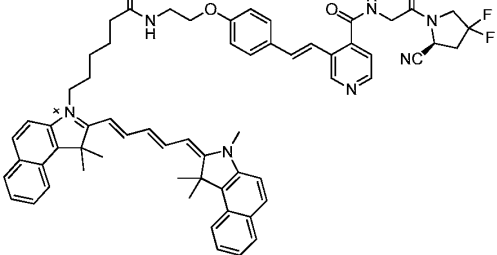
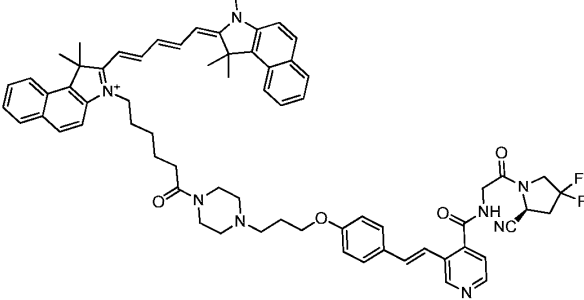
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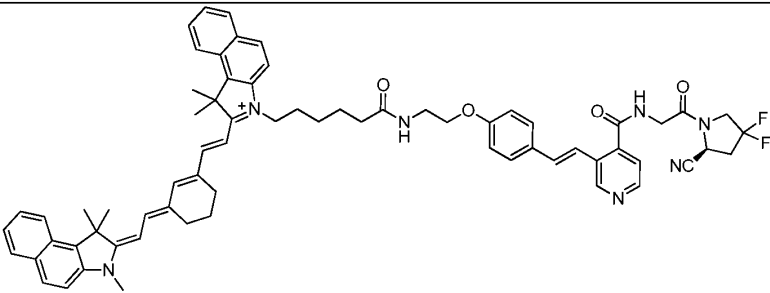
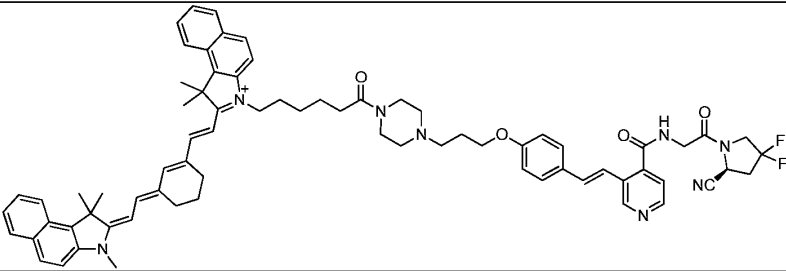
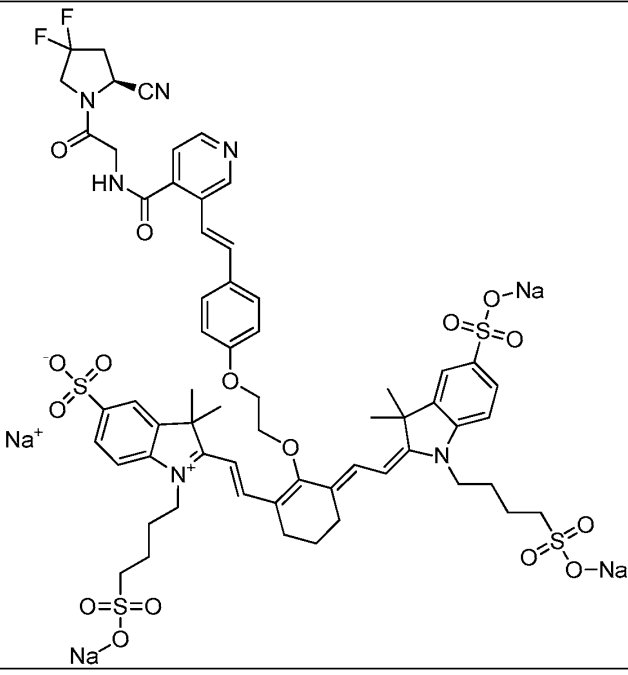
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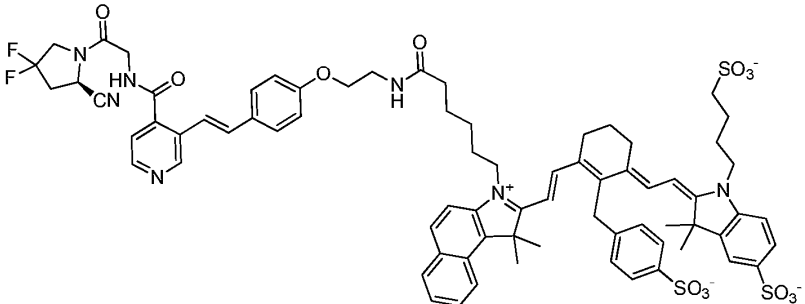
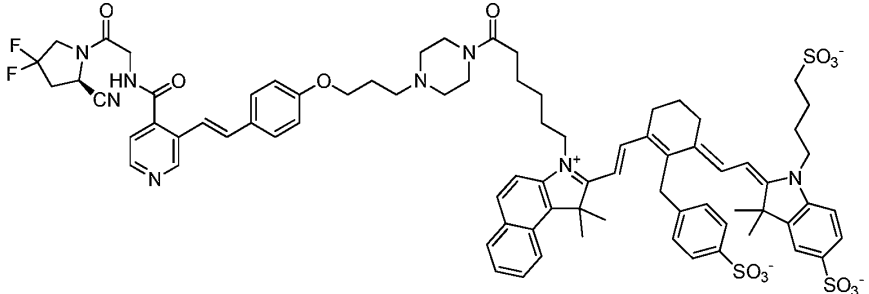
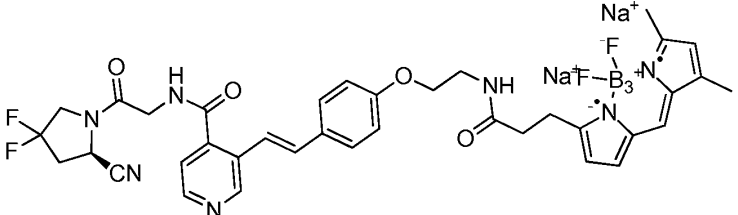
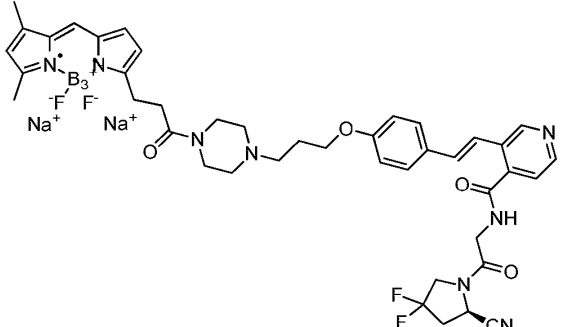


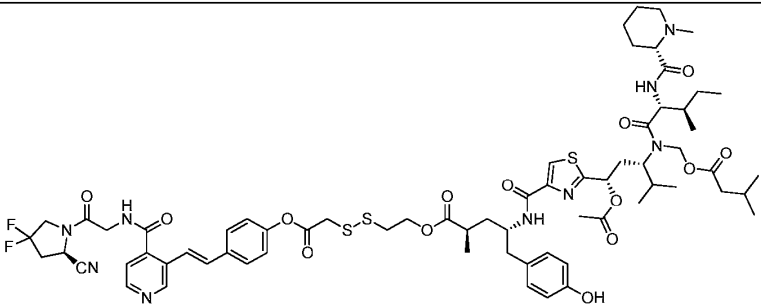
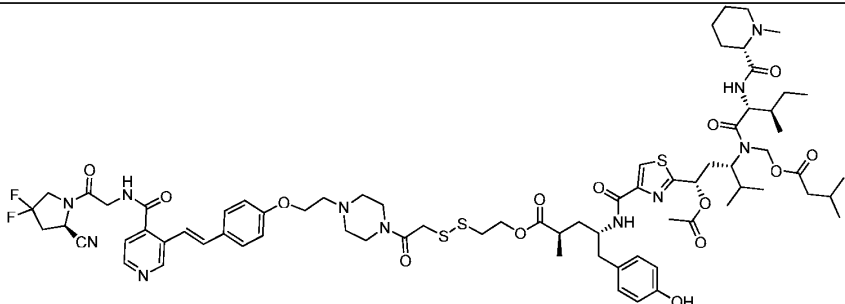
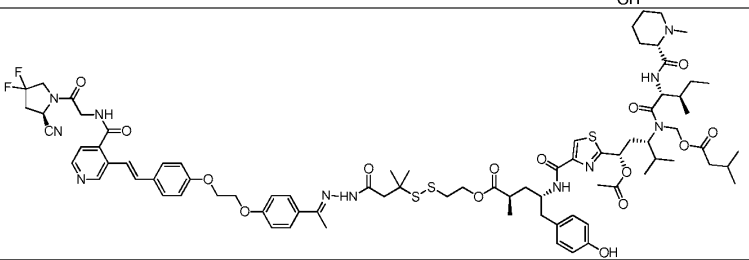
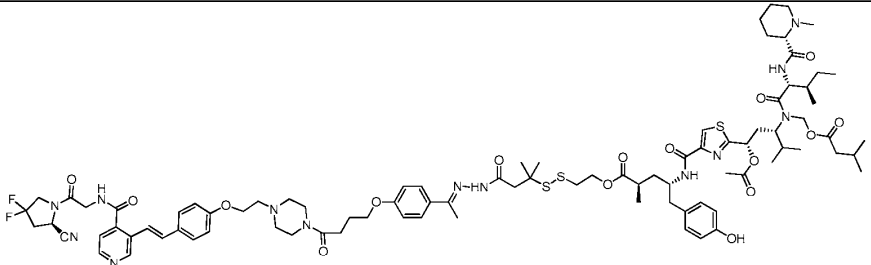
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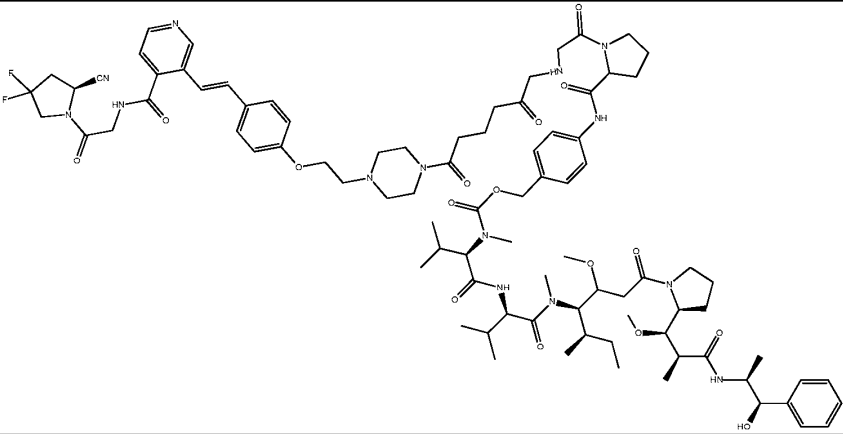
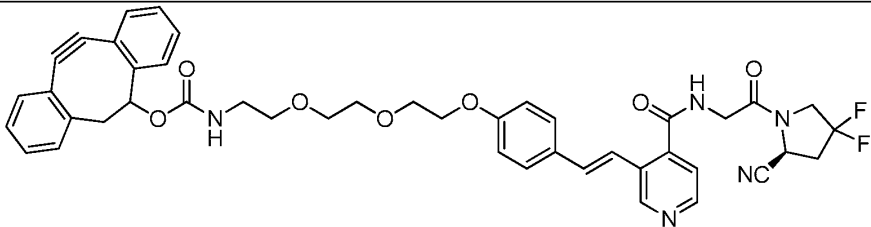
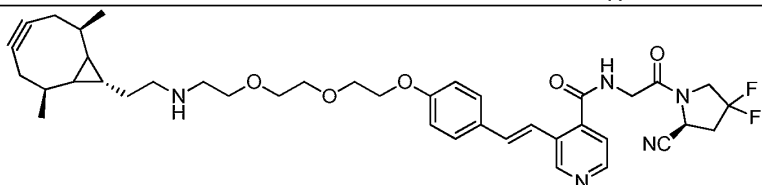
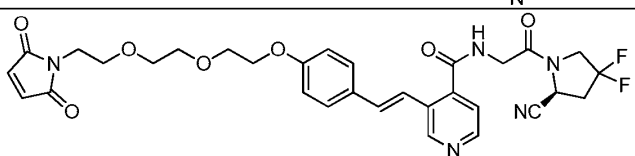
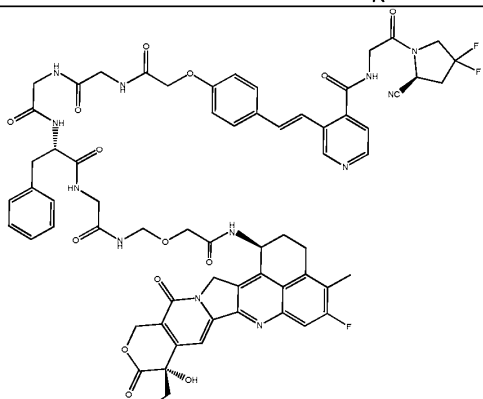
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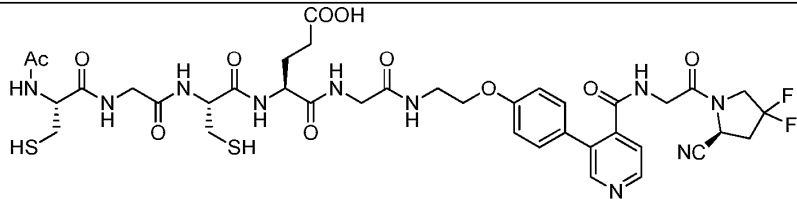
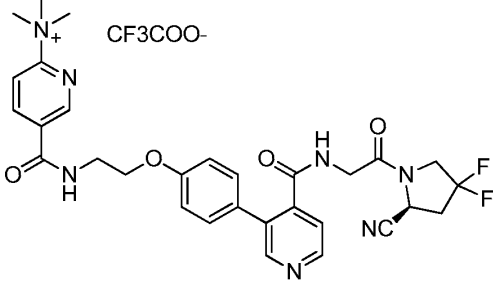
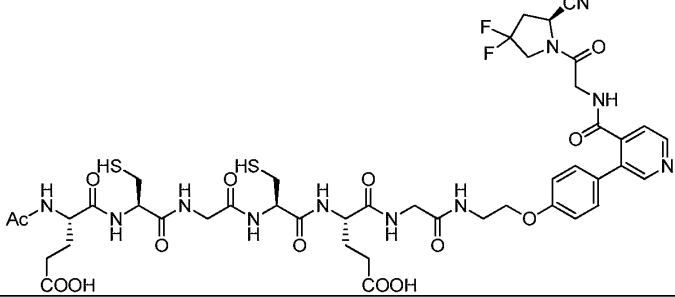
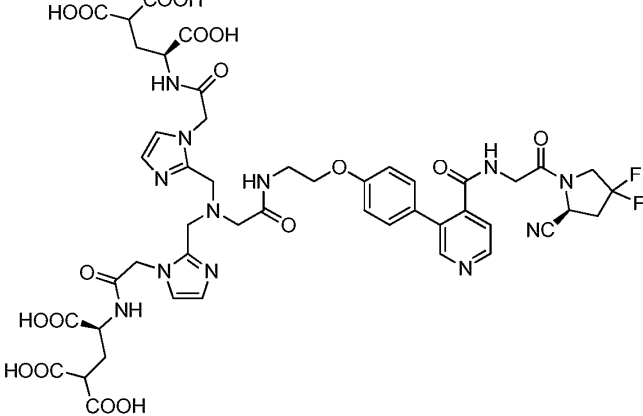
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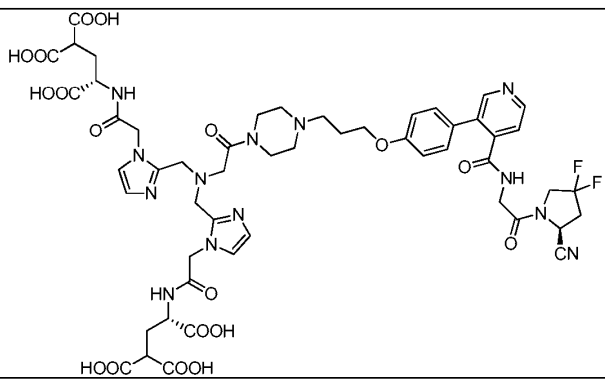
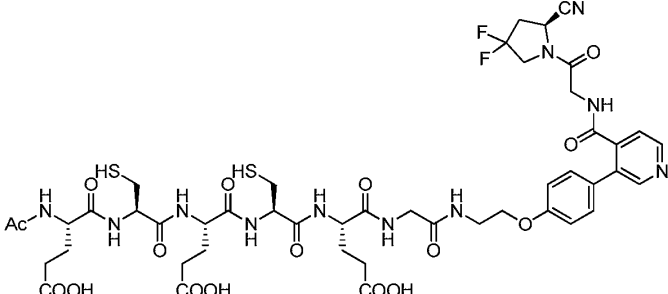
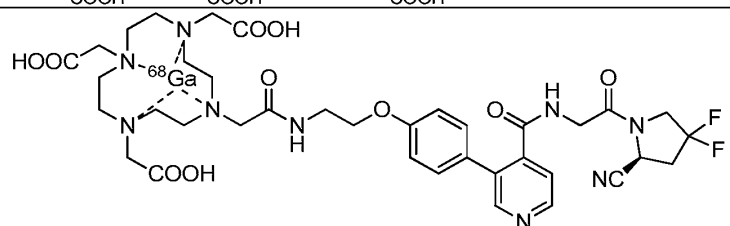
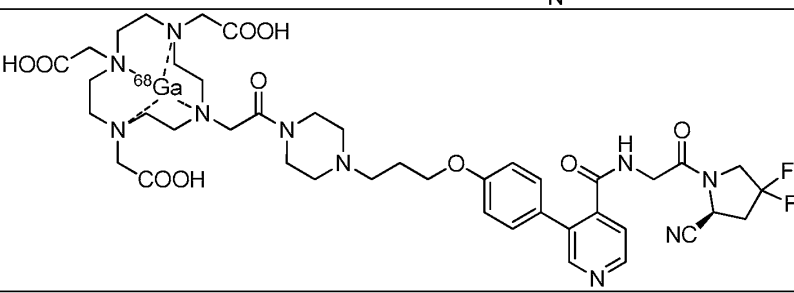
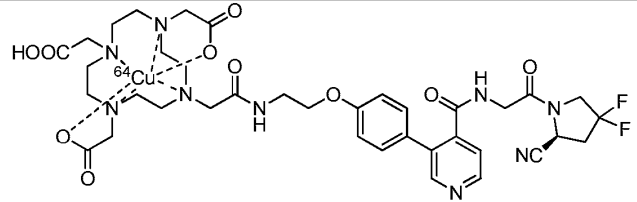
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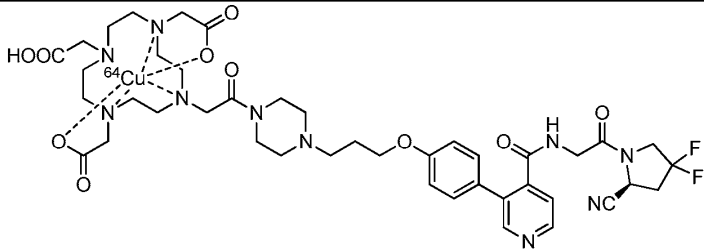
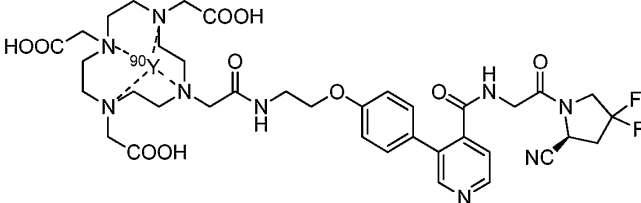
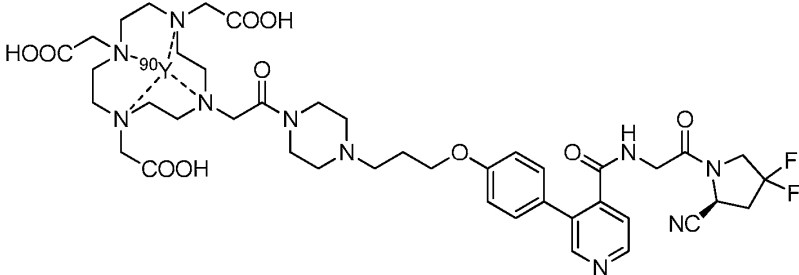
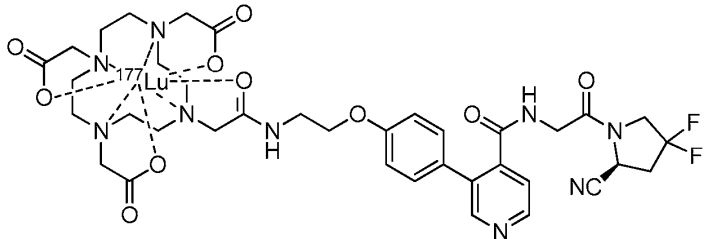
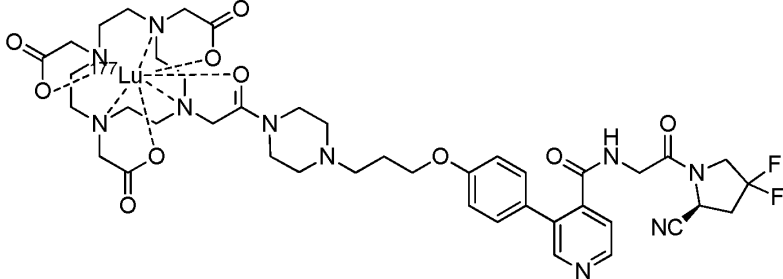


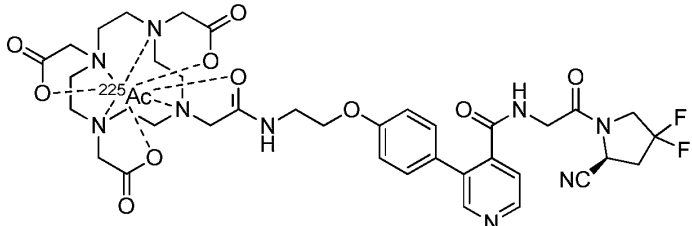
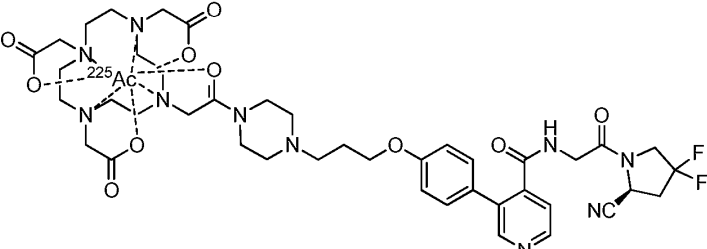
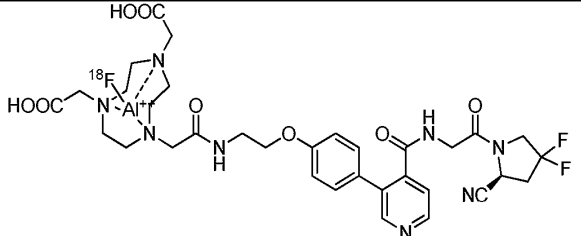
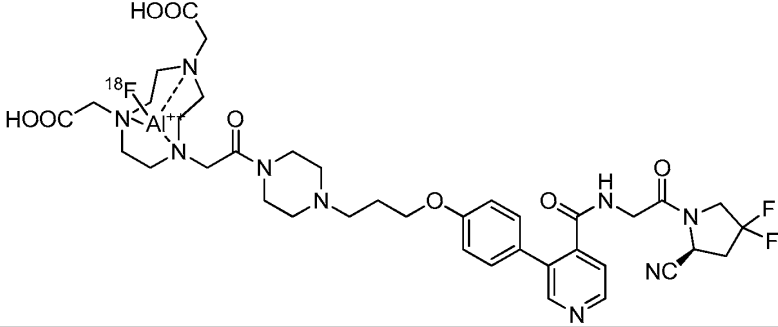
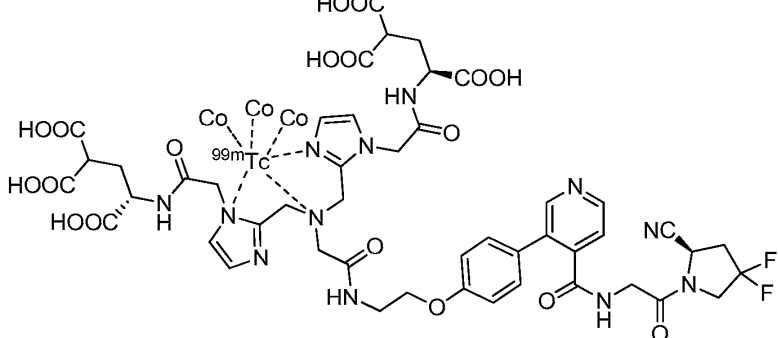
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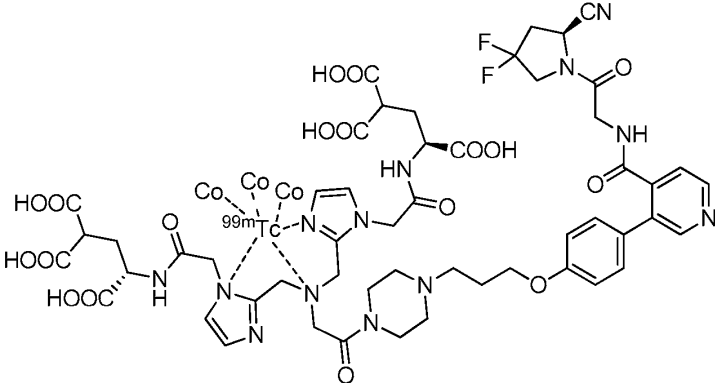
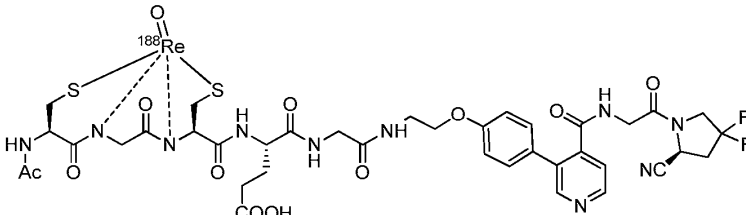
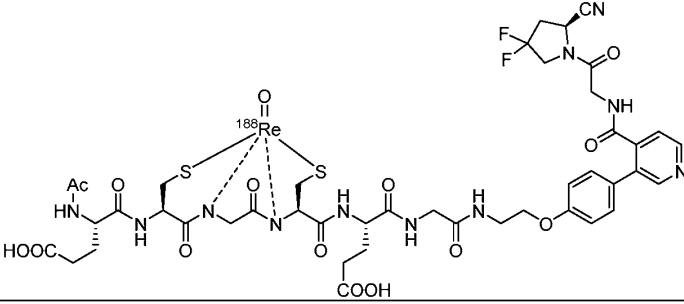
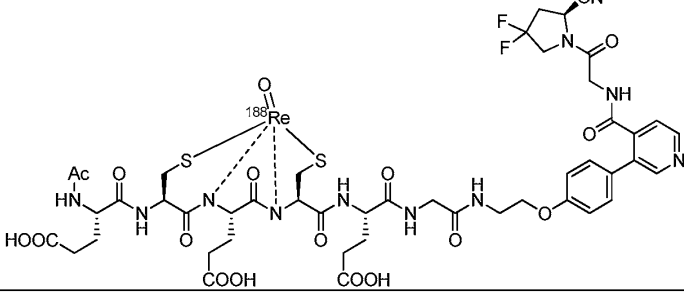
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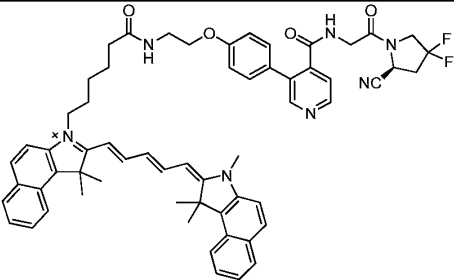
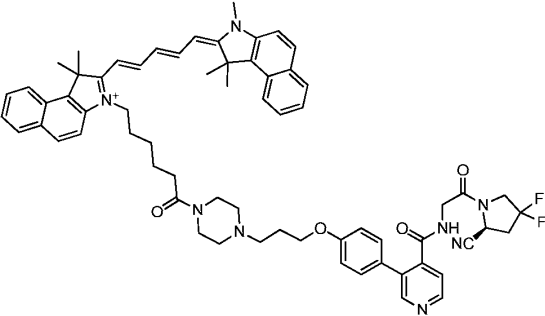
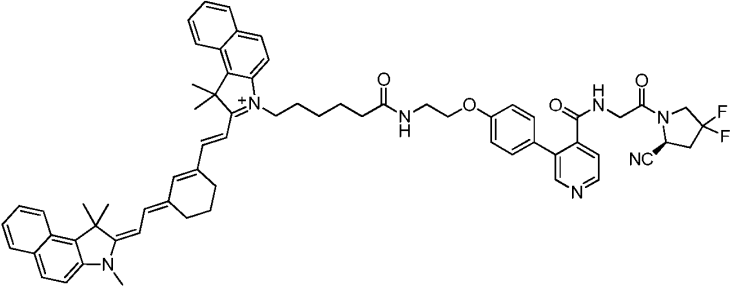
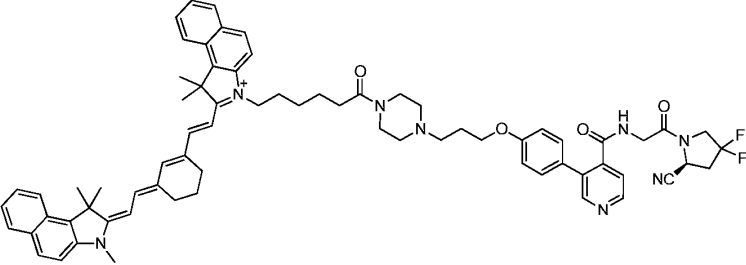
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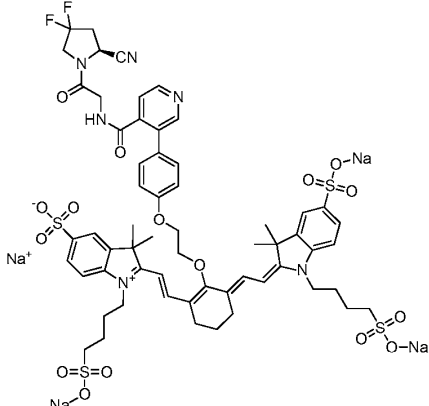
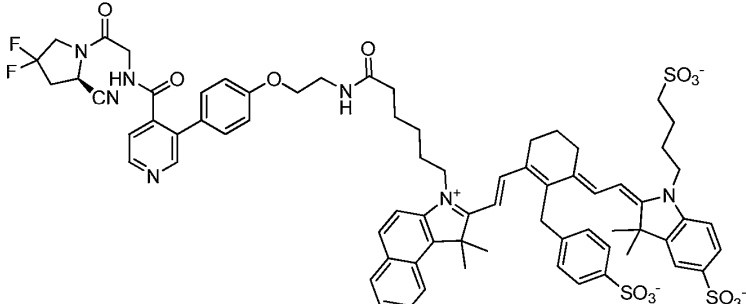
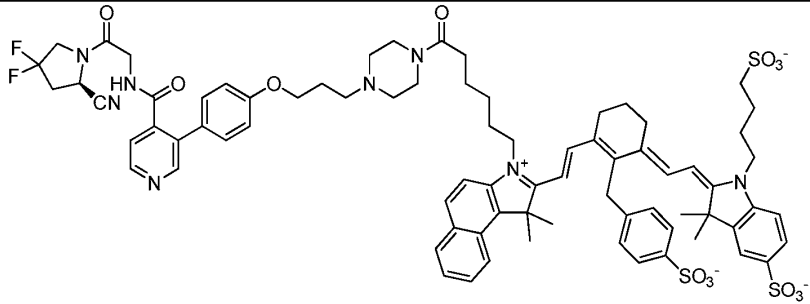
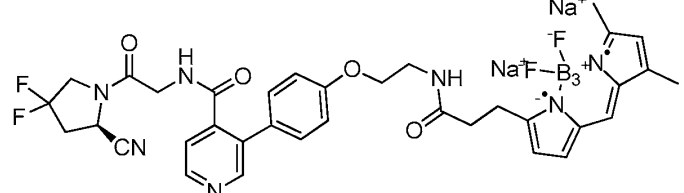
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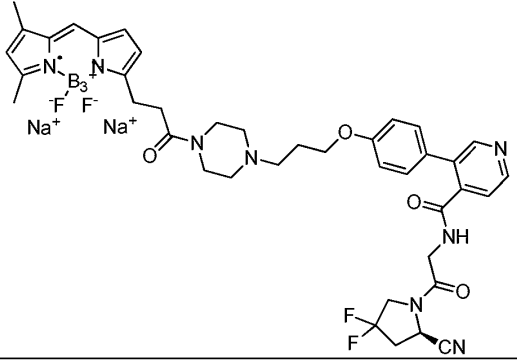
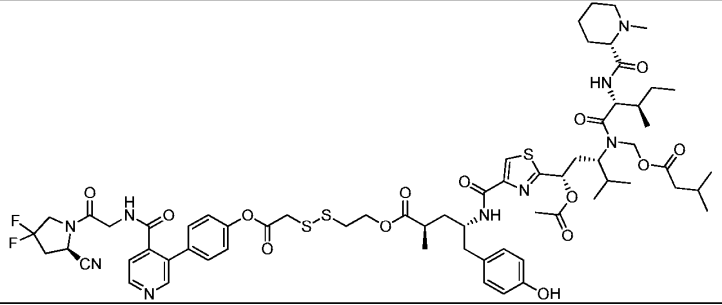
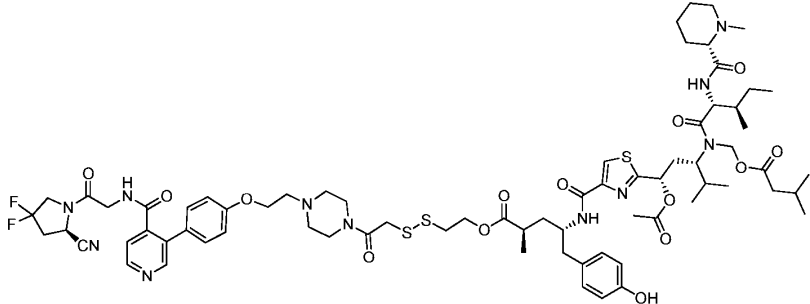
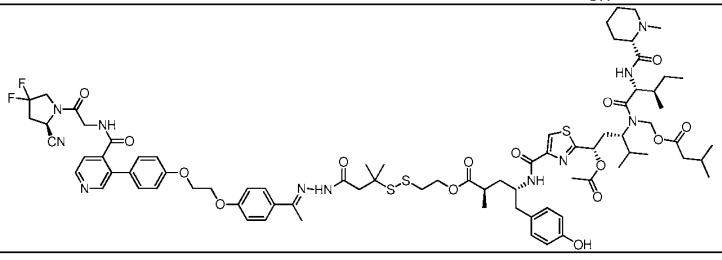
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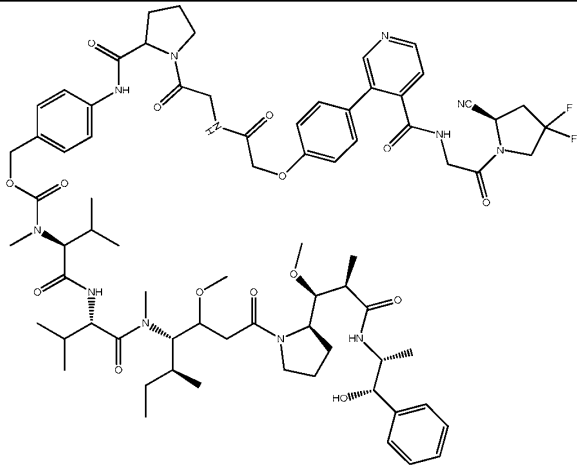
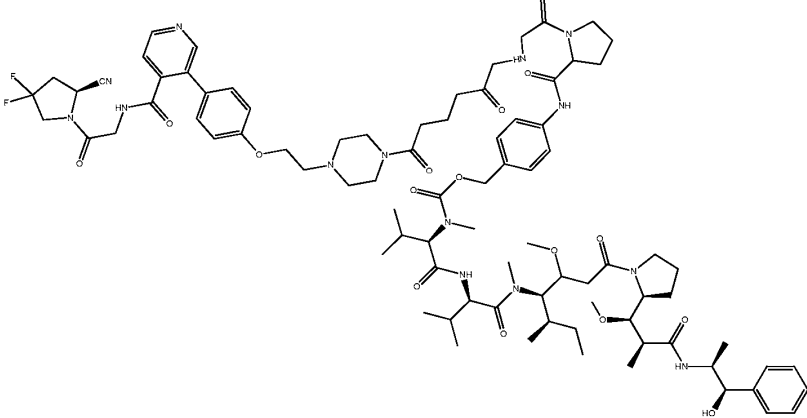
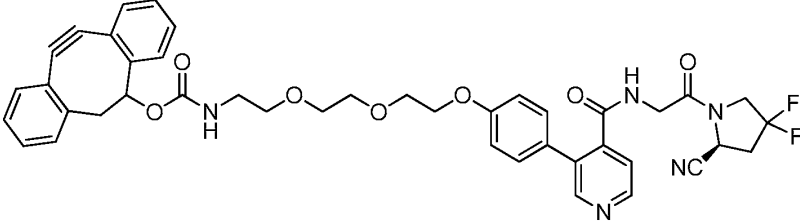
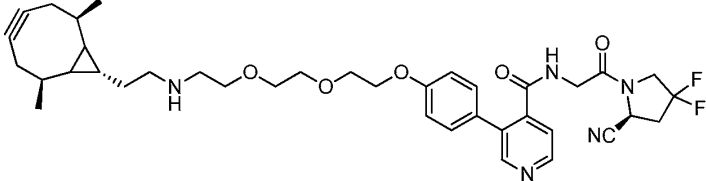
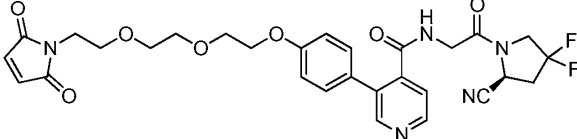
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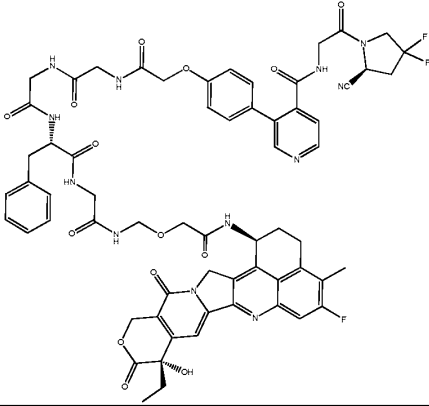
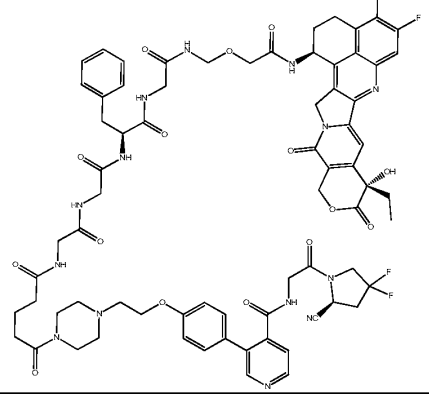
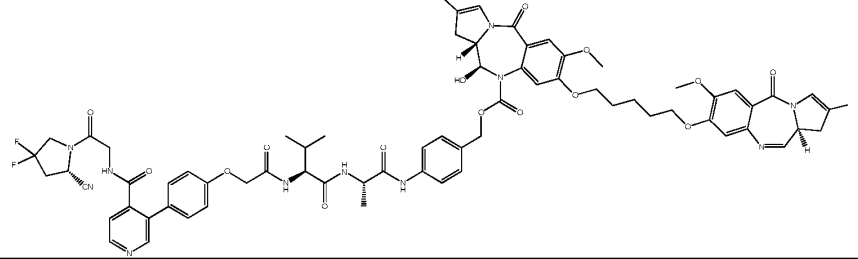
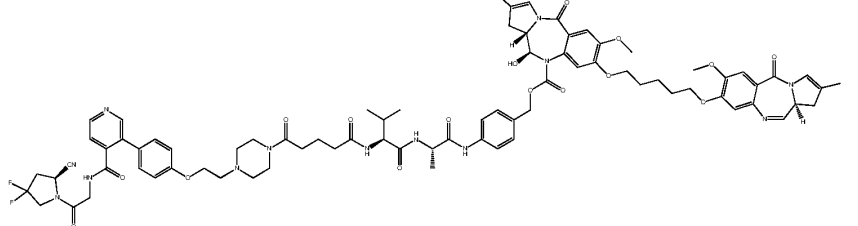


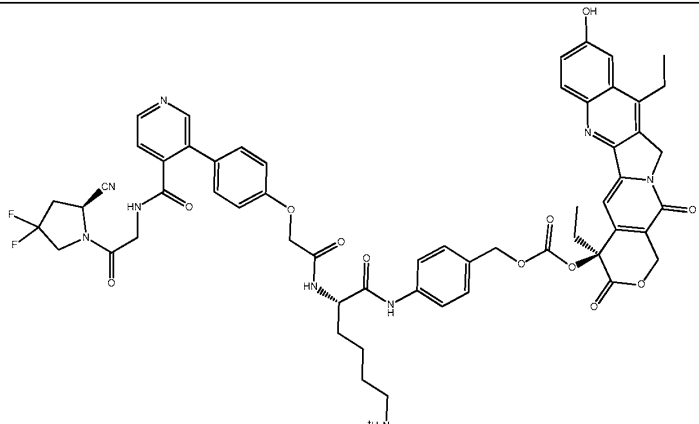
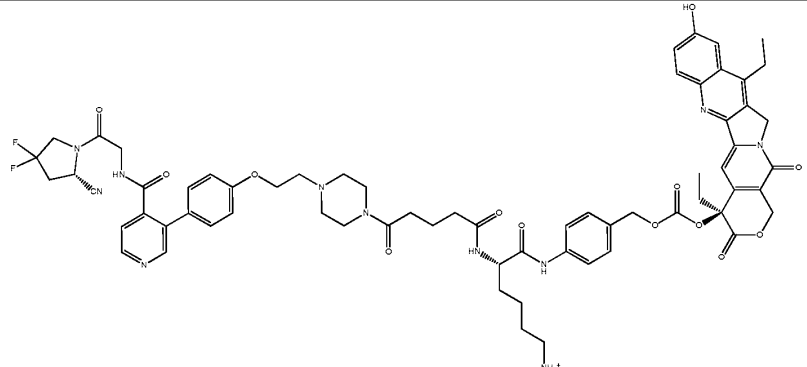
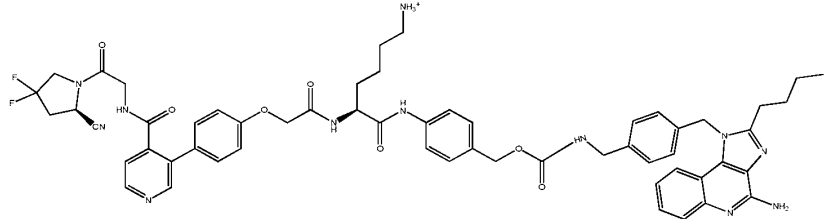
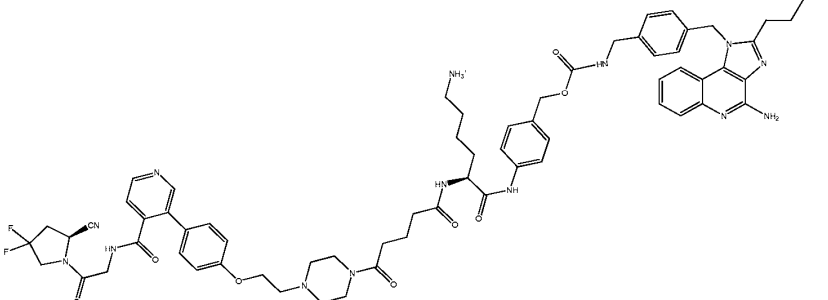
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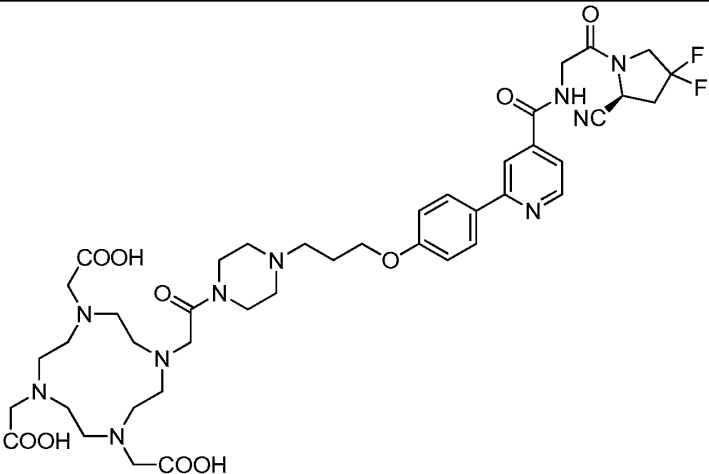
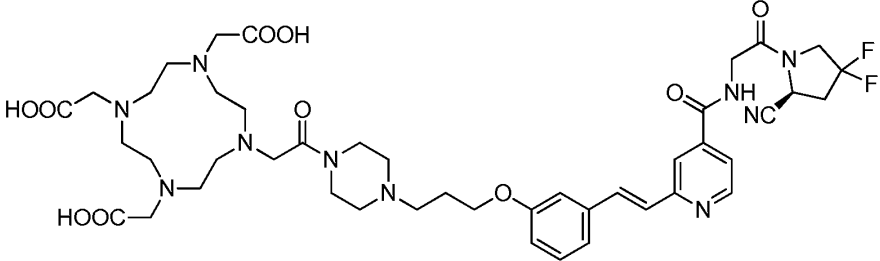
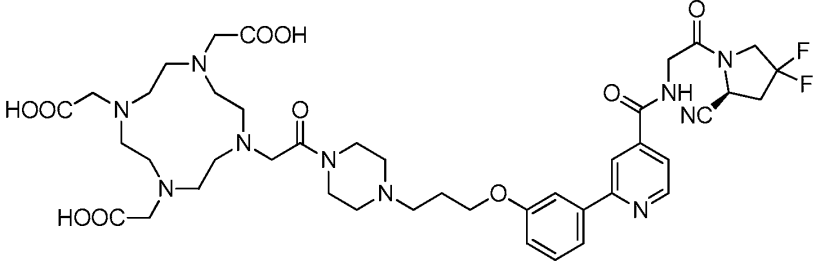


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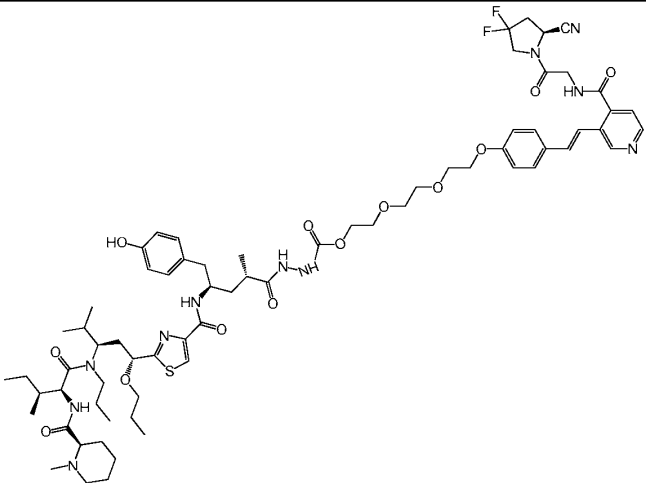
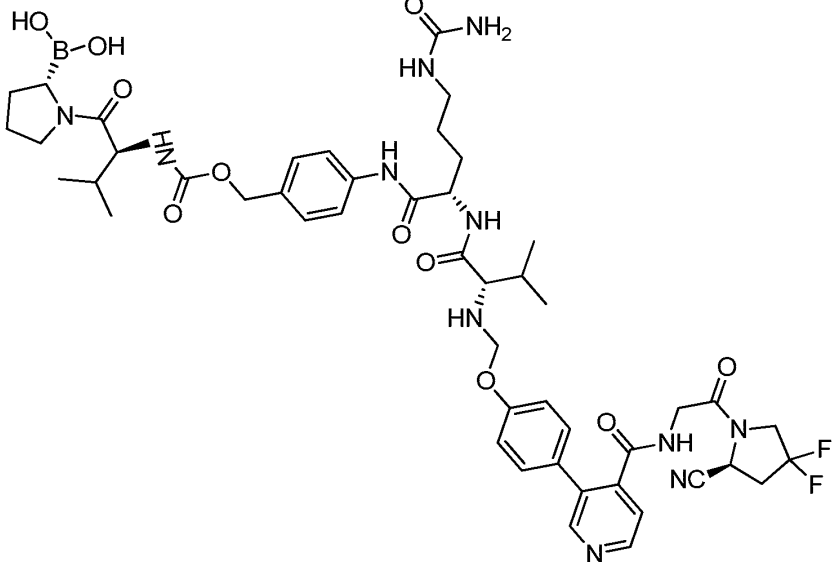
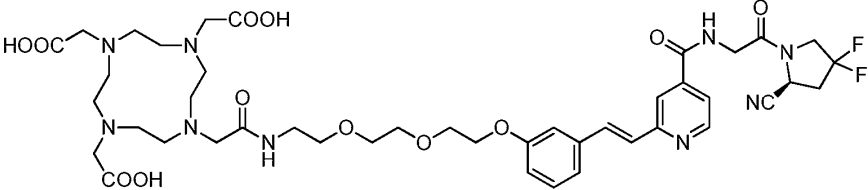
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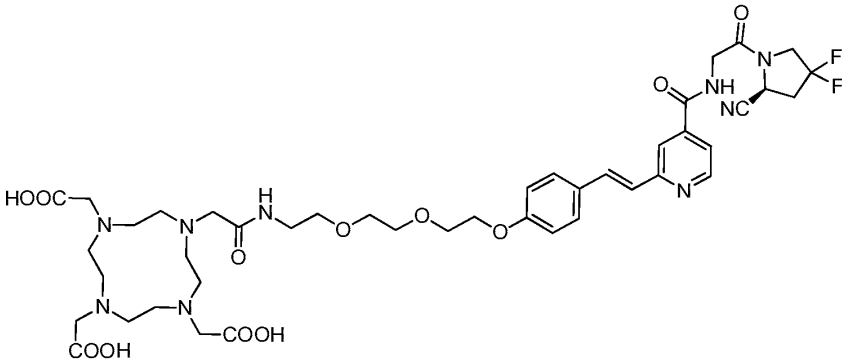
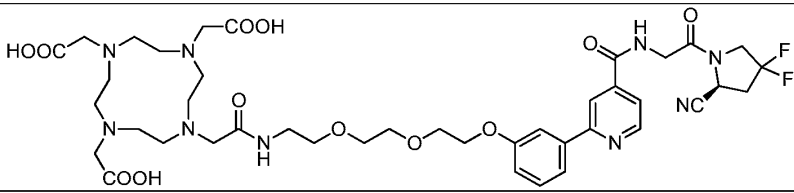
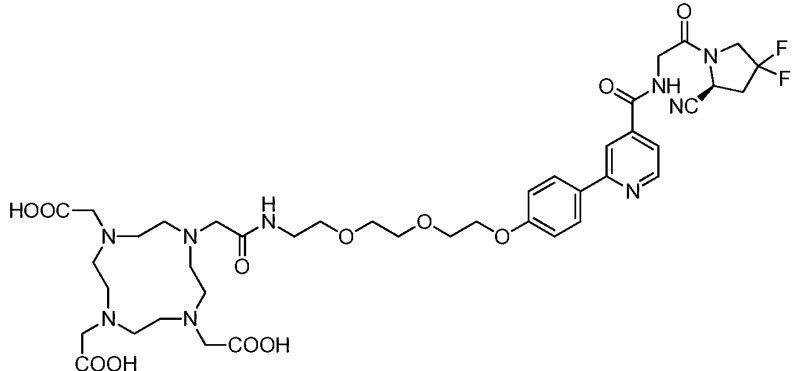
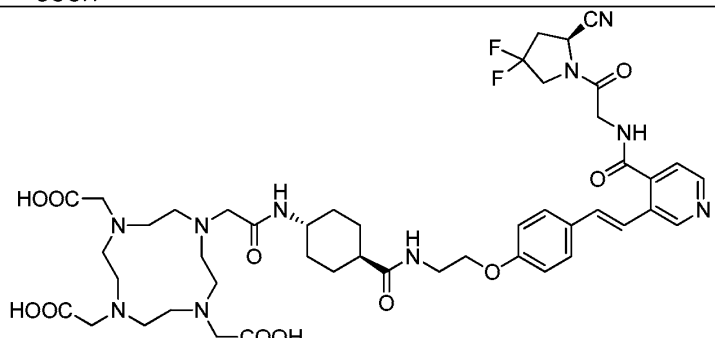
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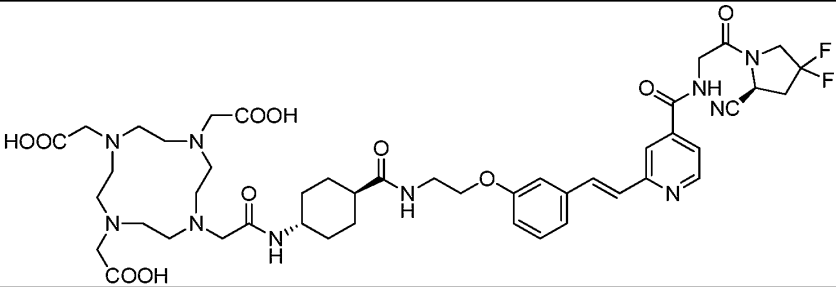
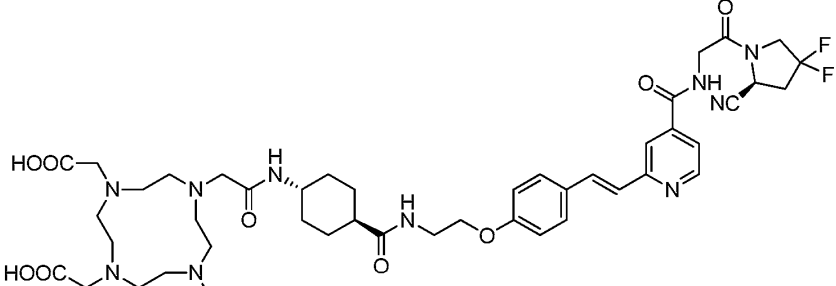
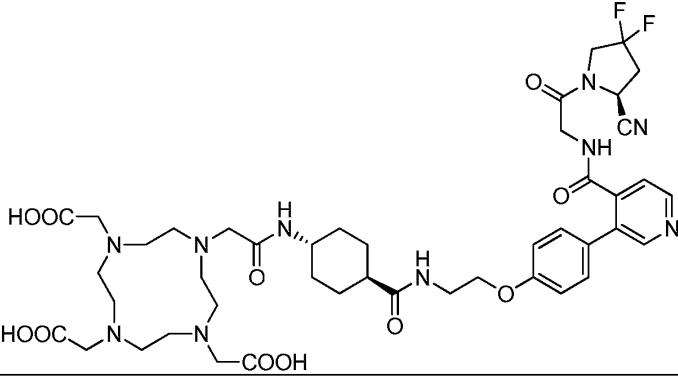
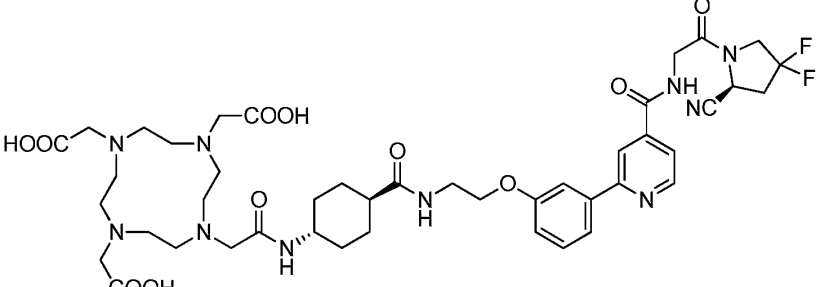
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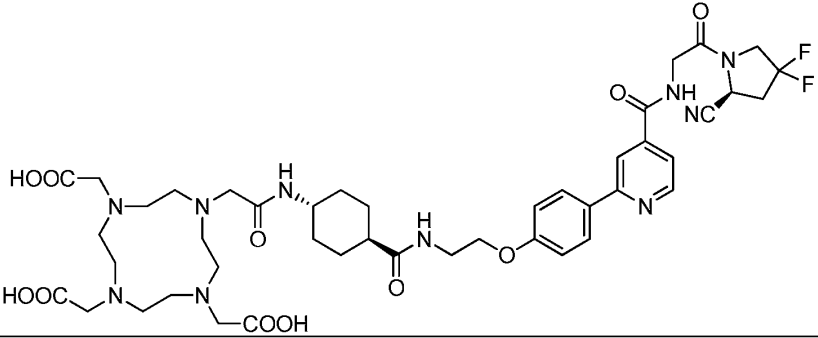
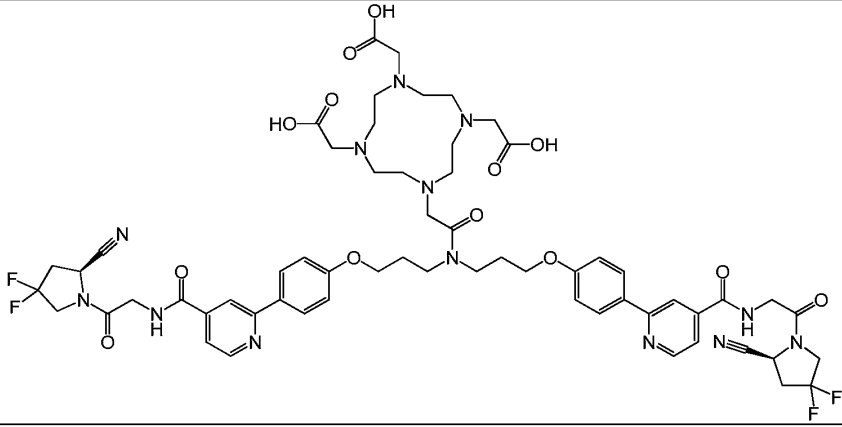
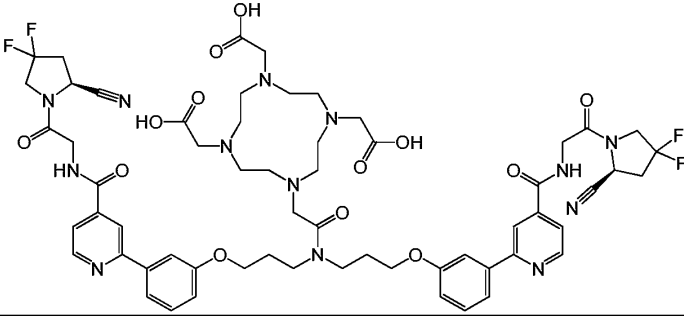
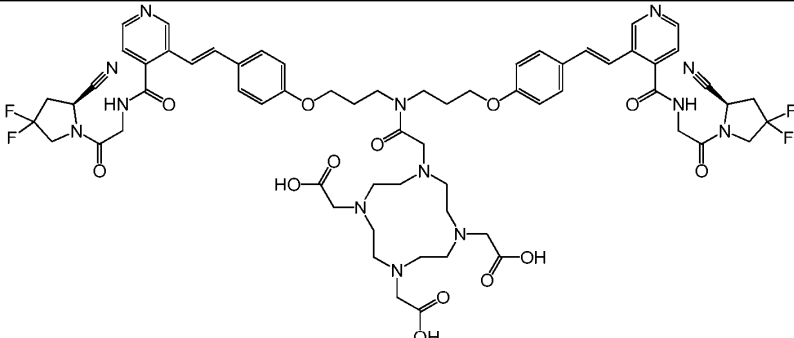
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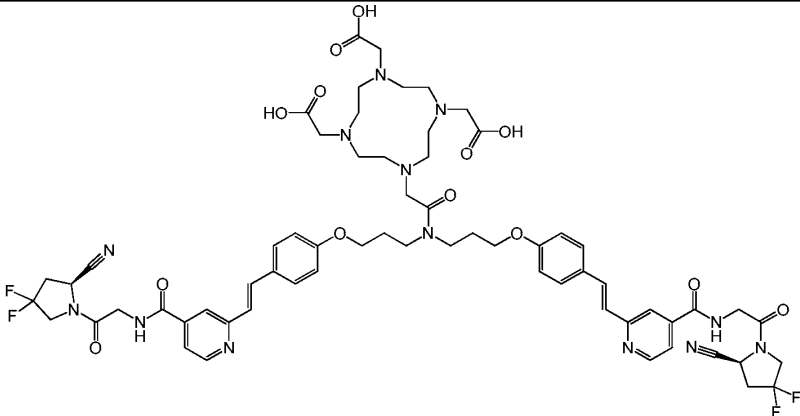
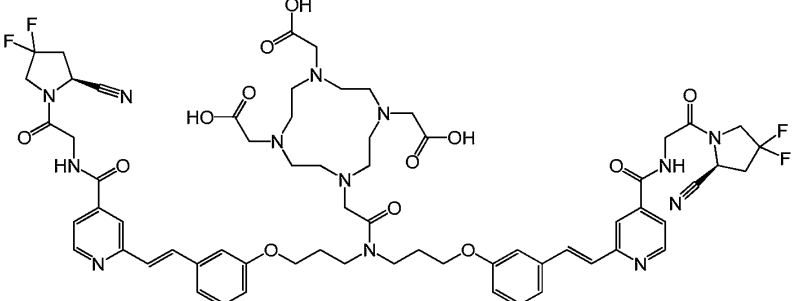
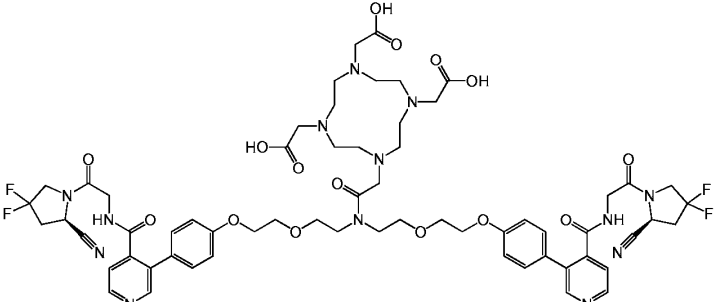


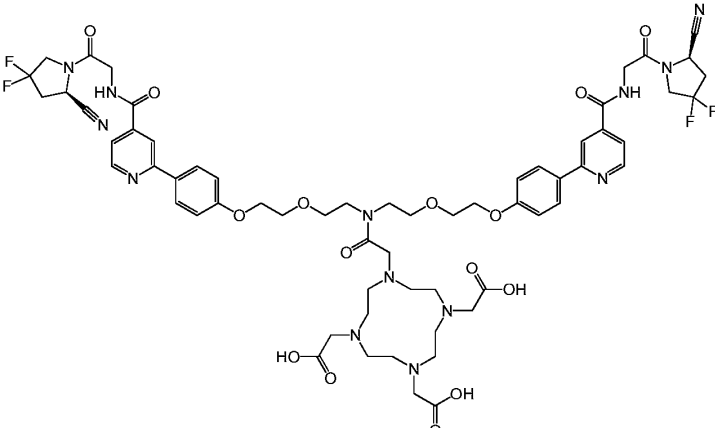
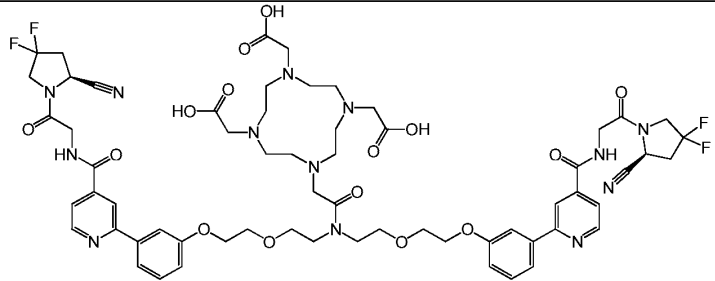
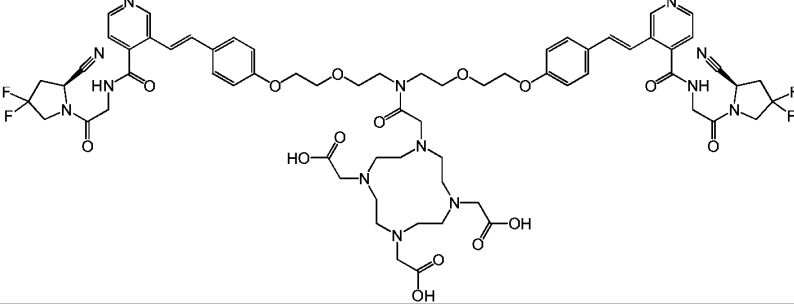
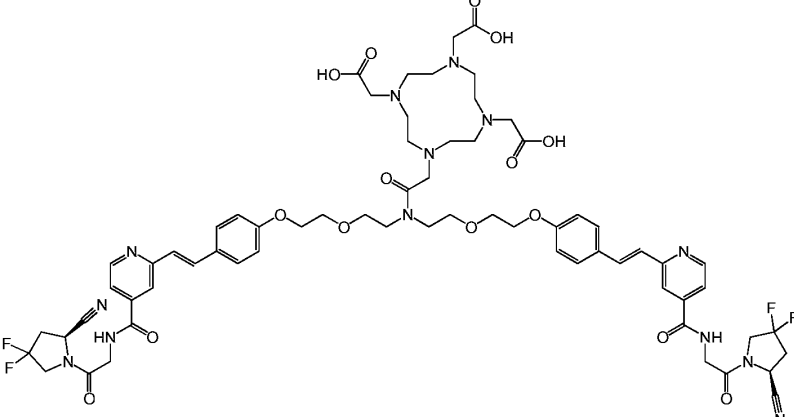
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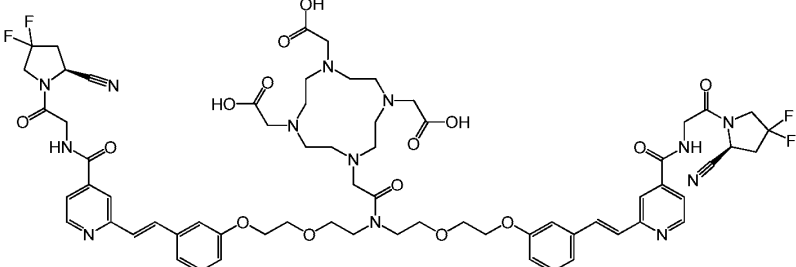
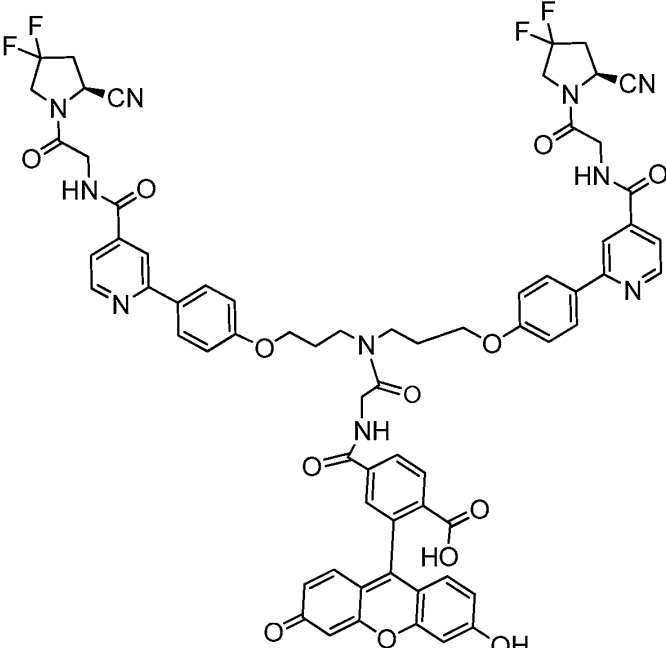
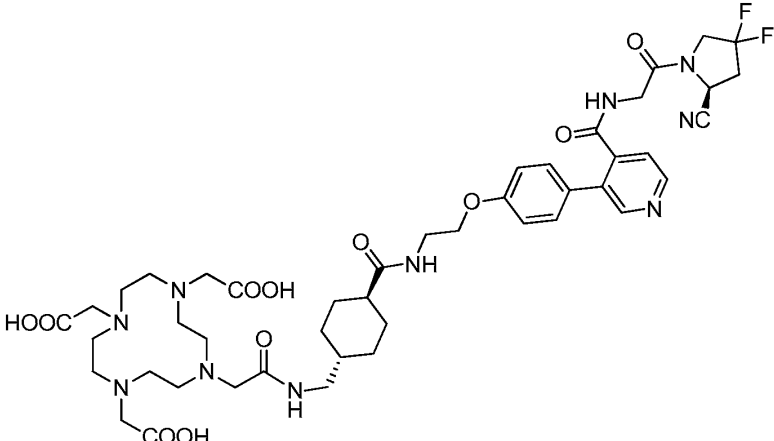
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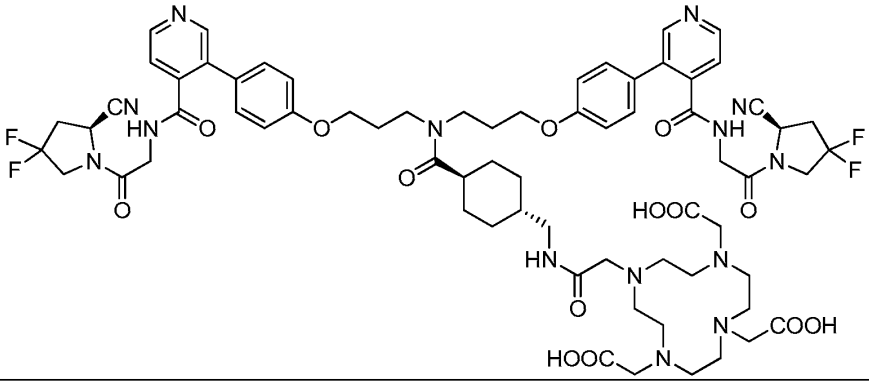
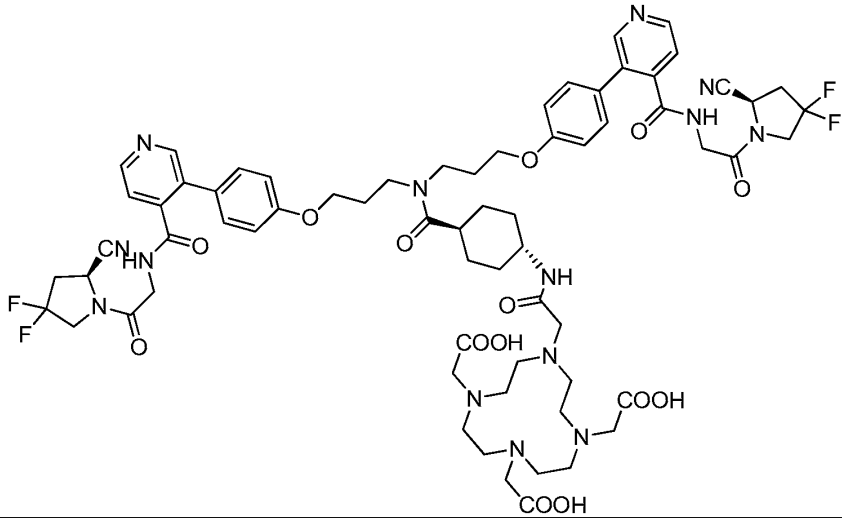
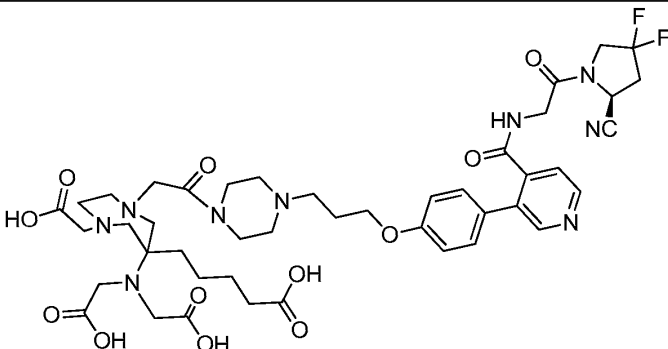
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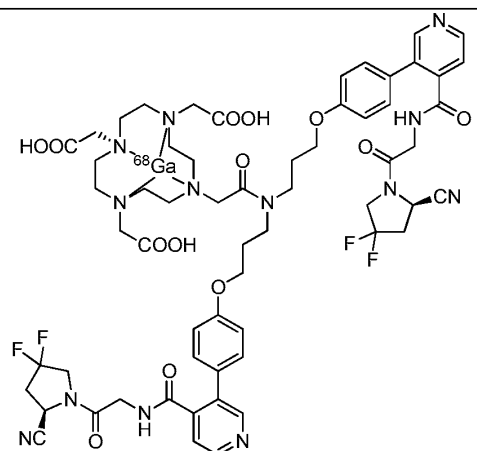
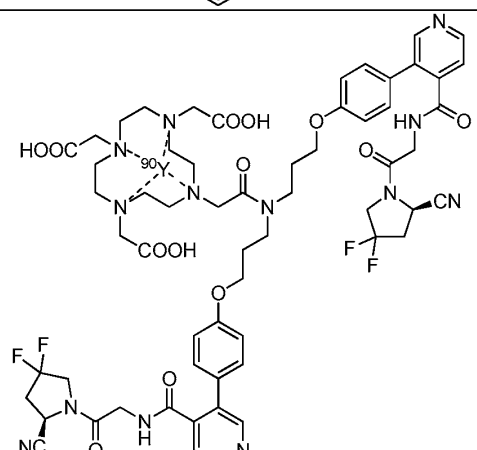
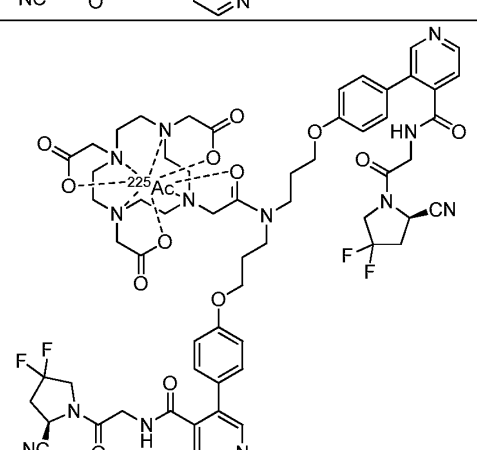
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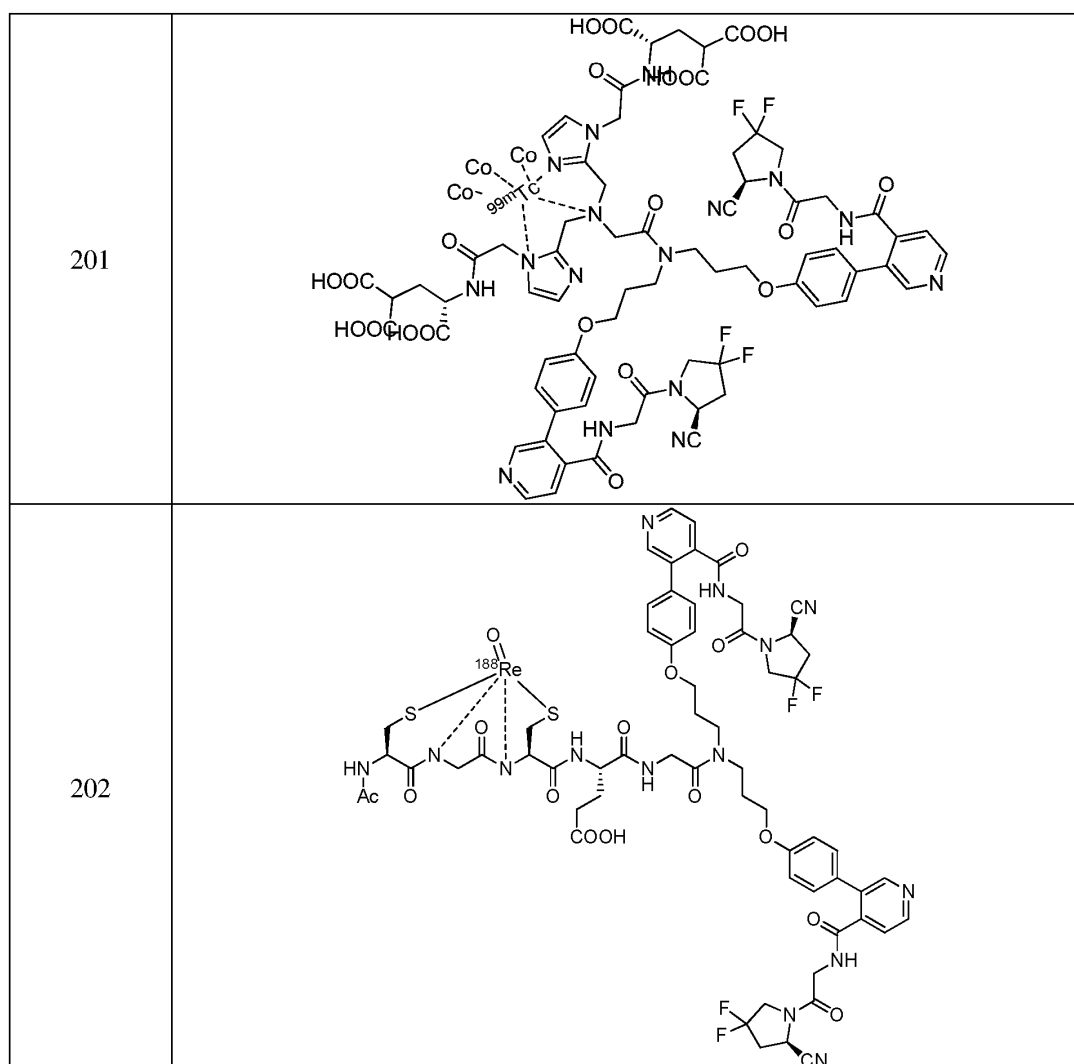
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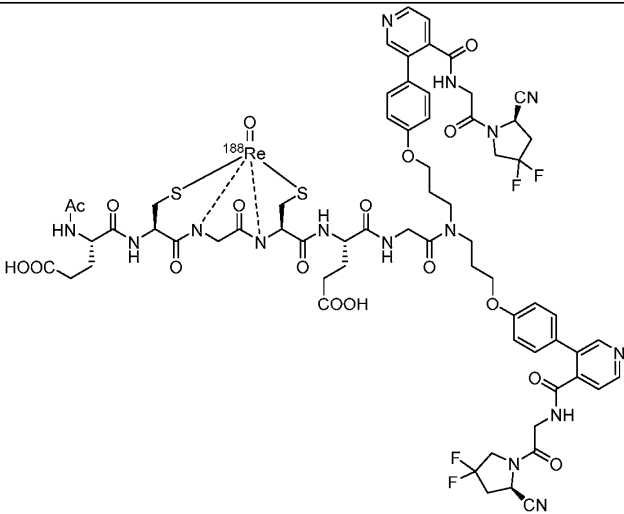
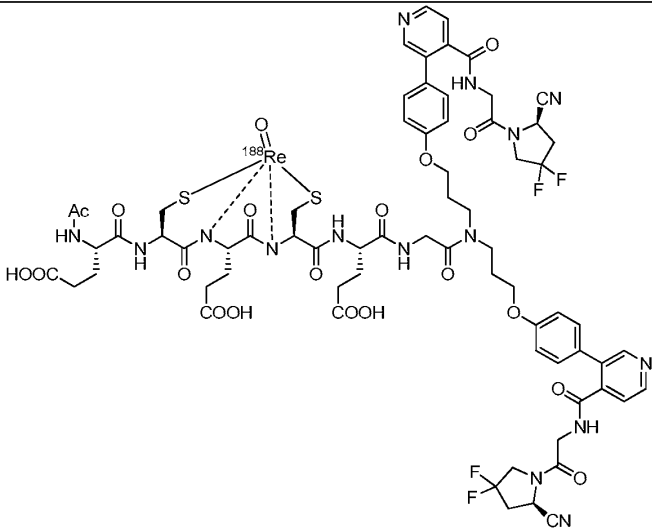
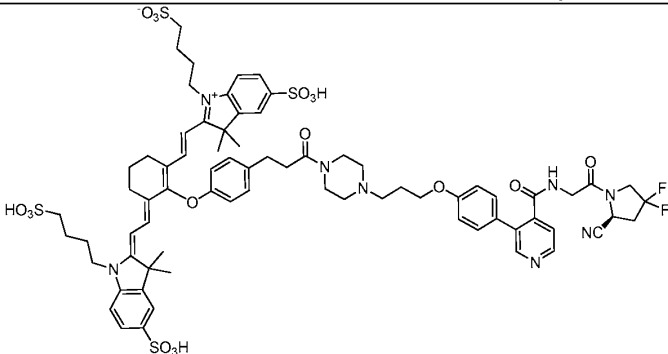
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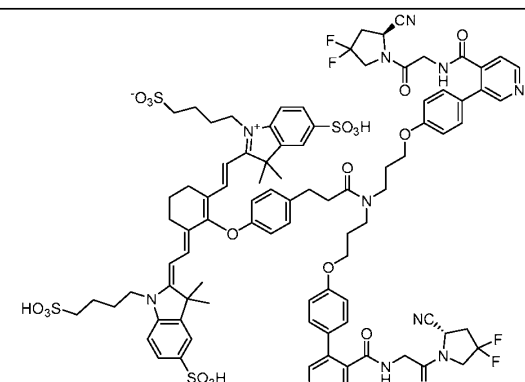
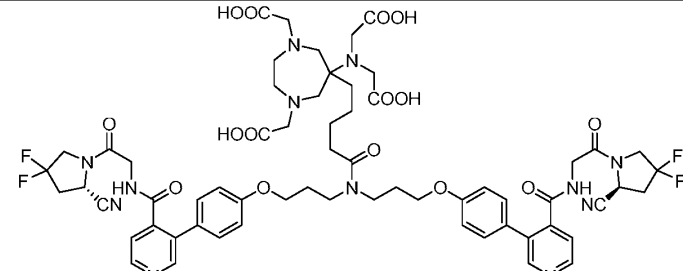
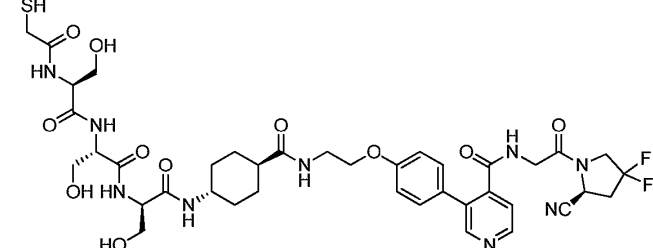
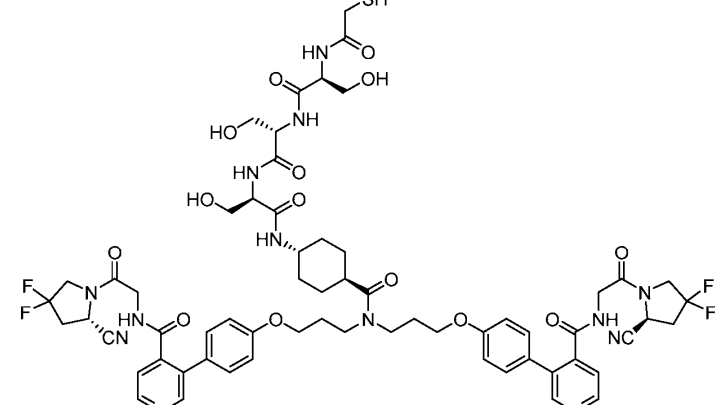


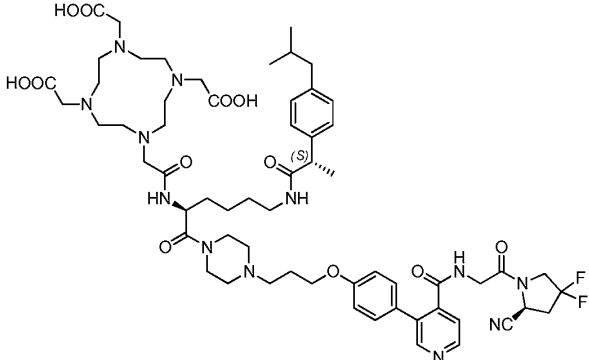
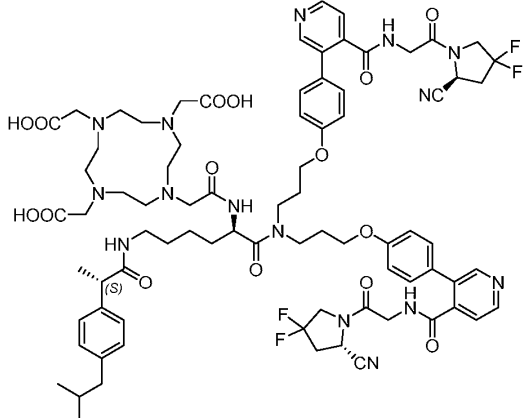
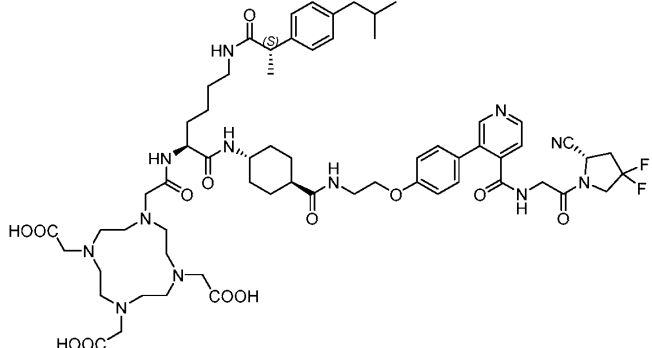
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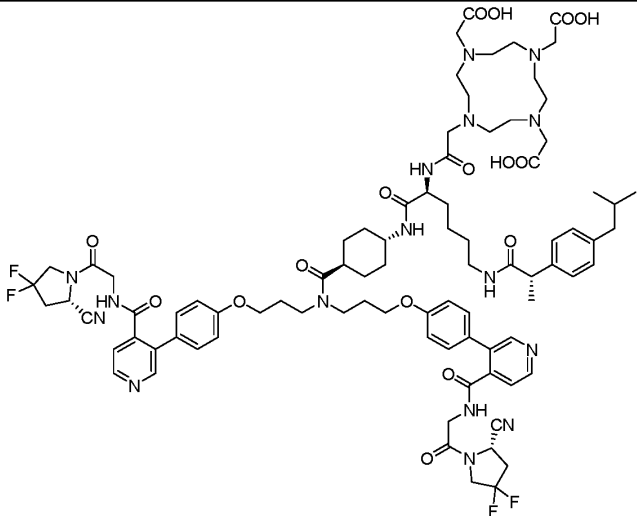
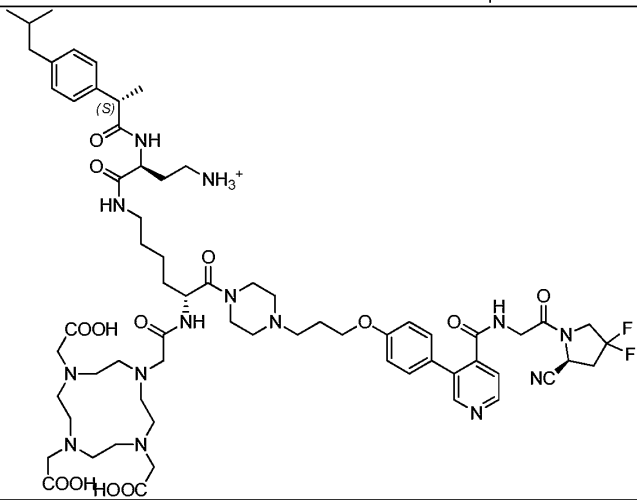
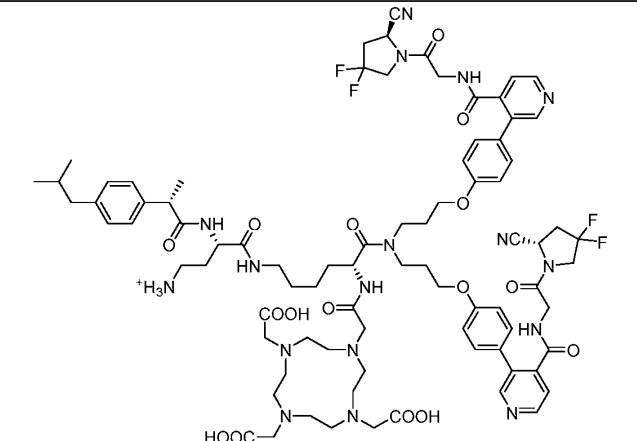
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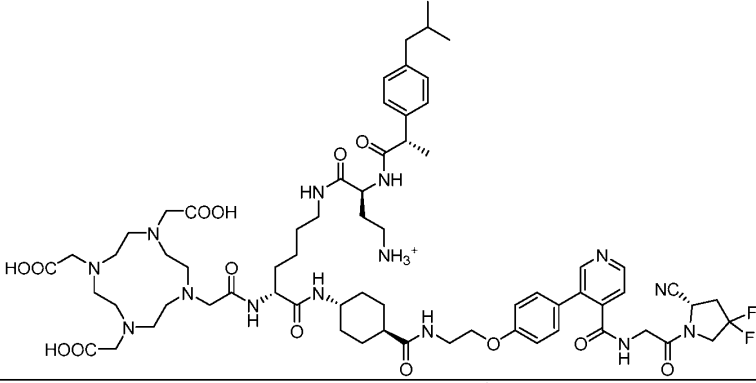
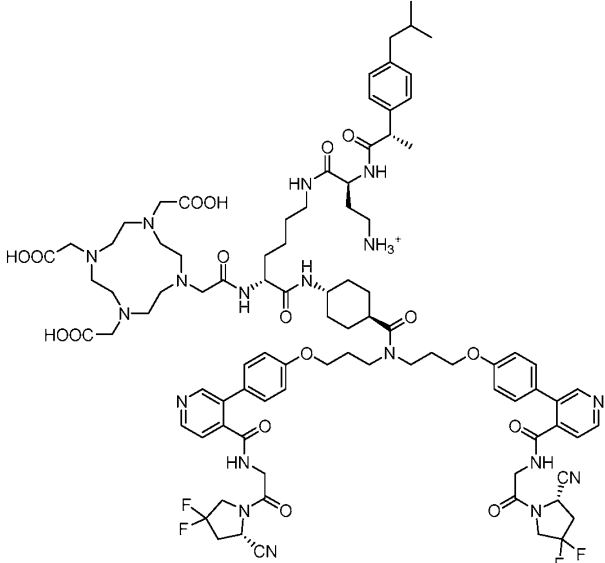
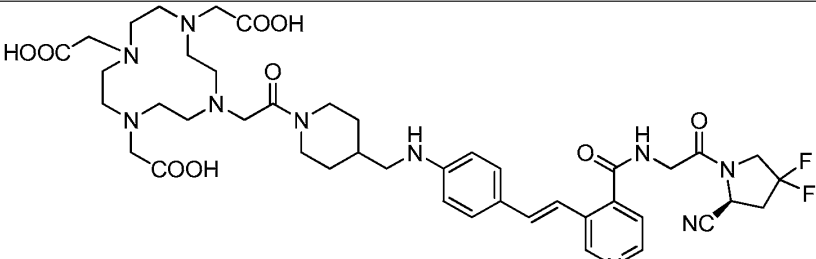
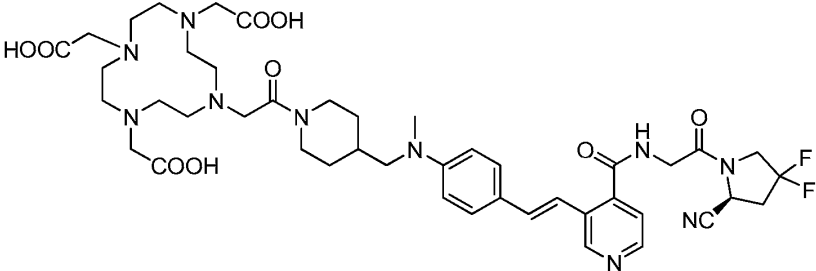
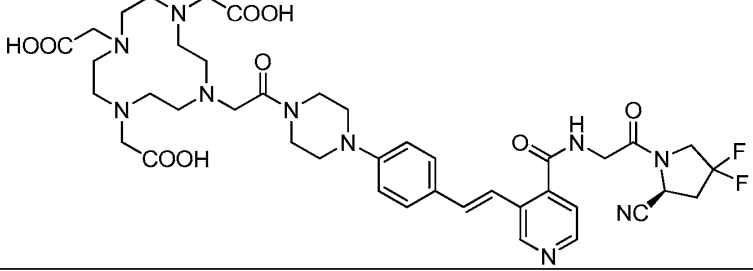
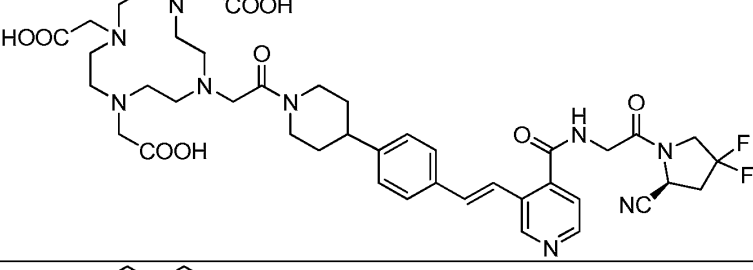
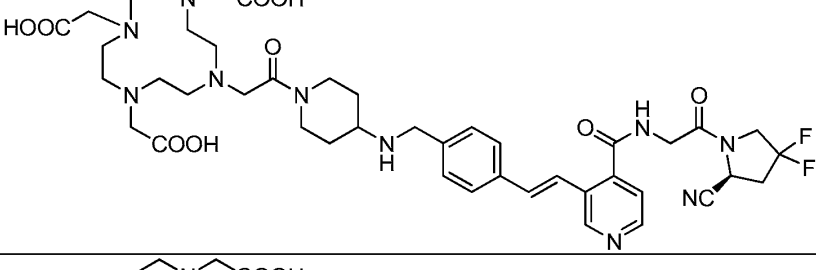
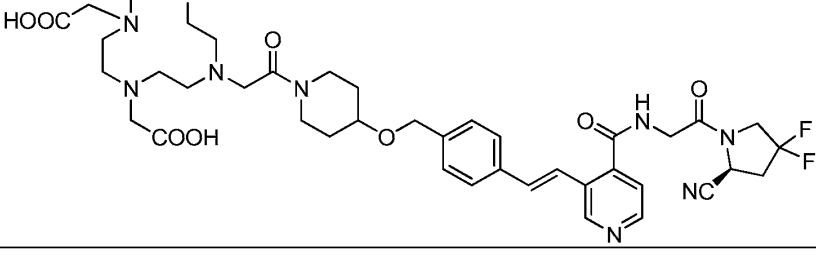
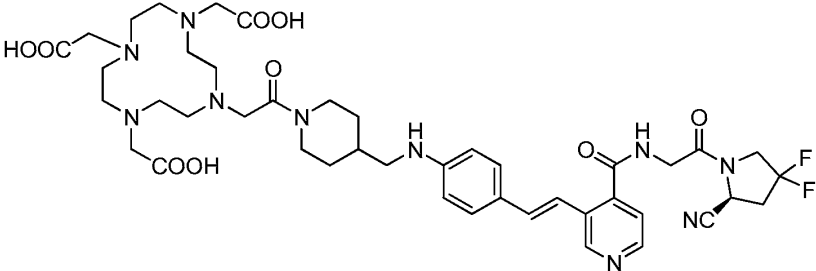
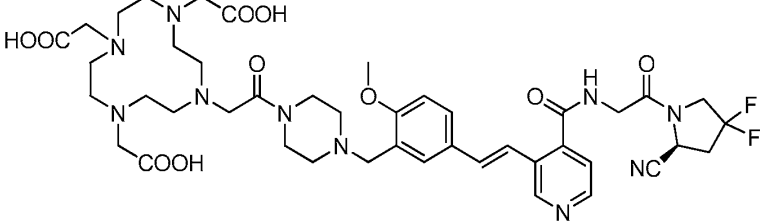
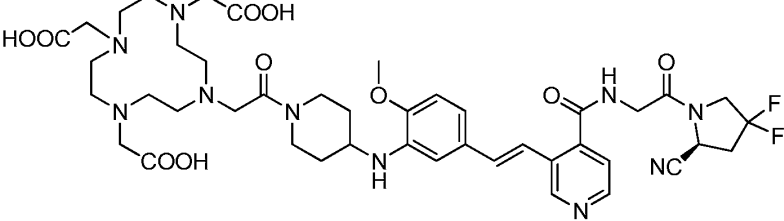
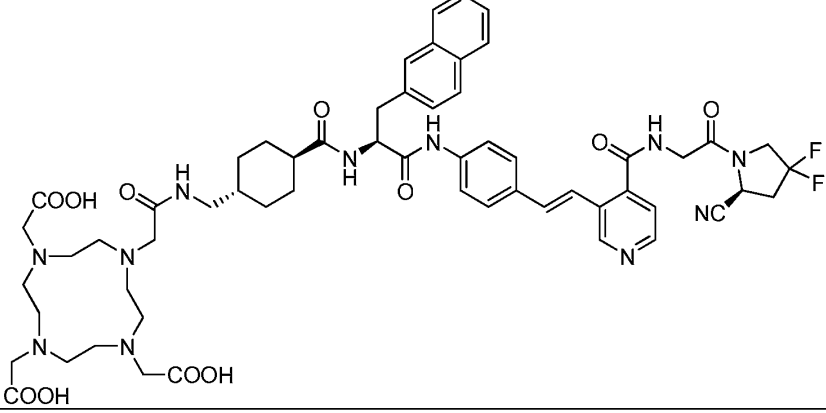
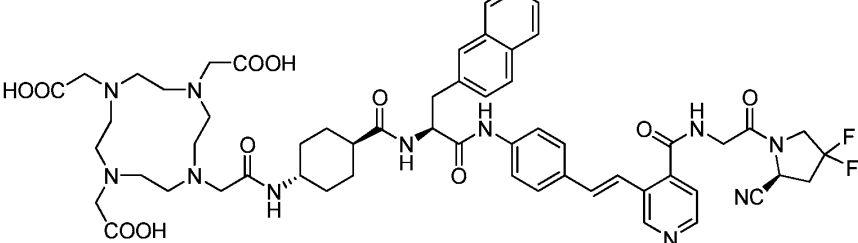
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|-----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 216 |  <p>Chemical structure of compound 216: A complex molecule featuring a central cyclohexane ring. It is substituted with a carboxylic acid group, a primary amine, and a side chain containing a pyridine ring and a fluorinated pyrrolidine ring. The structure also includes a quaternary ammonium salt group and a carboxylic acid group.</p>  |
| 217 |  <p>Chemical structure of compound 217: A complex molecule featuring a central cyclohexane ring. It is substituted with a carboxylic acid group, a primary amine, and a side chain containing a pyridine ring and a fluorinated pyrrolidine ring. The structure also includes a quaternary ammonium salt group and a carboxylic acid group.</p> |

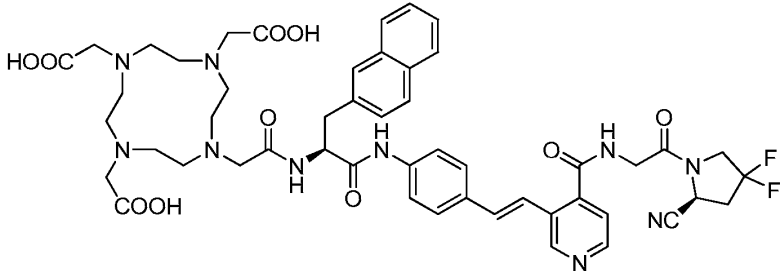
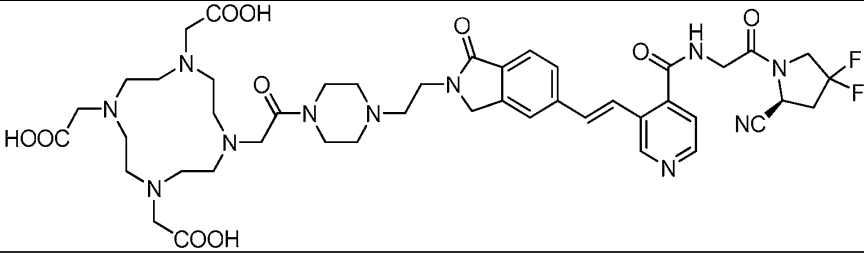
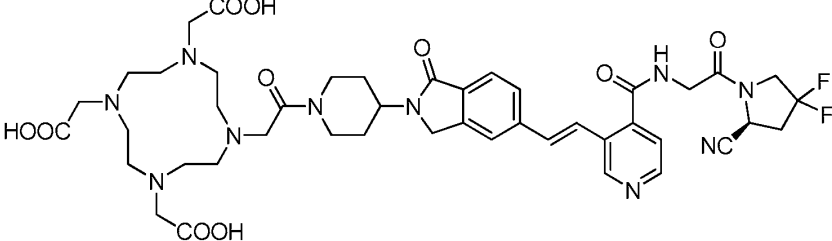
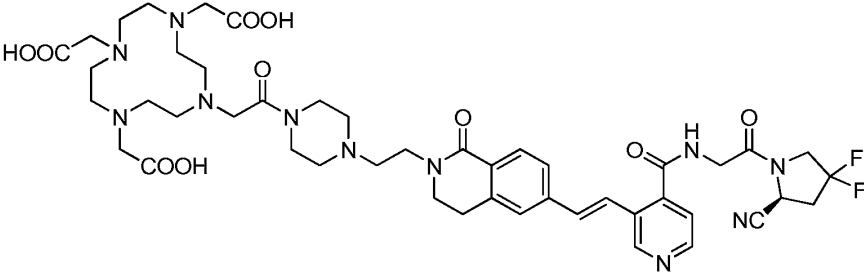
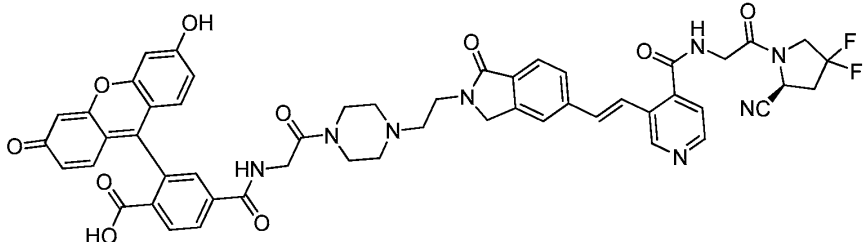
Table 1B: Compounds

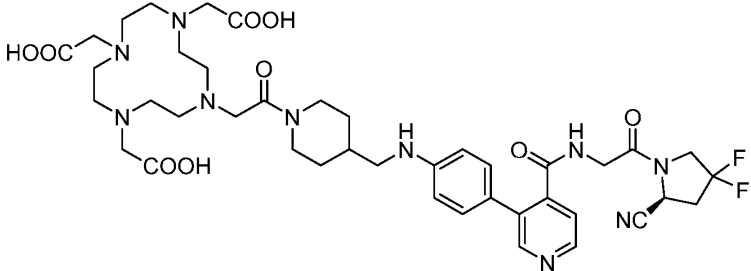
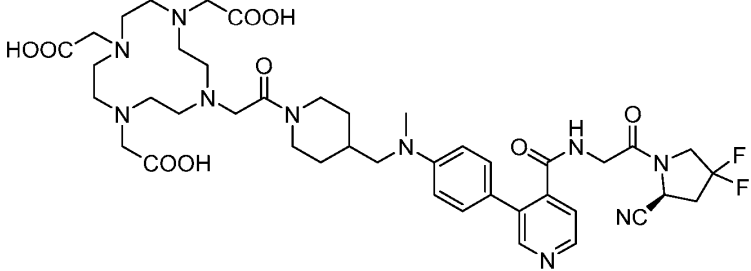
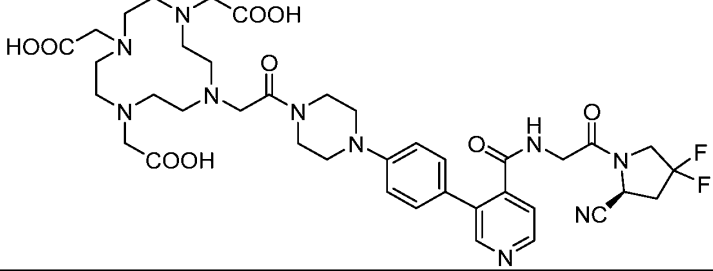
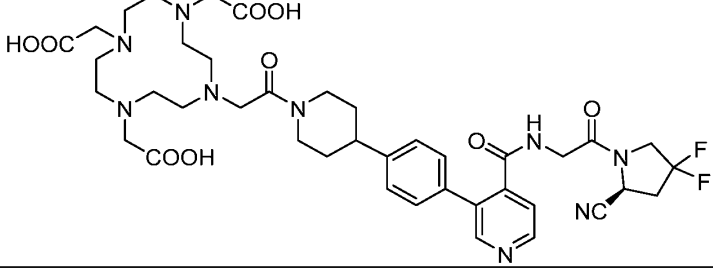
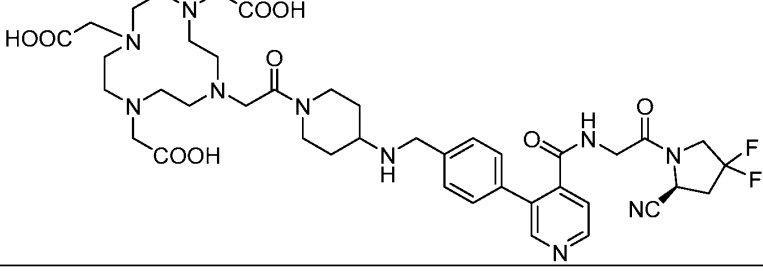
| Compound number | Compounds                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1b              |  <p>Chemical structure of compound 1b: A complex molecule featuring a central cyclohexane ring. It is substituted with a carboxylic acid group, a primary amine, and a side chain containing a pyridine ring and a fluorinated pyrrolidine ring. The structure also includes a quaternary ammonium salt group and a carboxylic acid group.</p> |

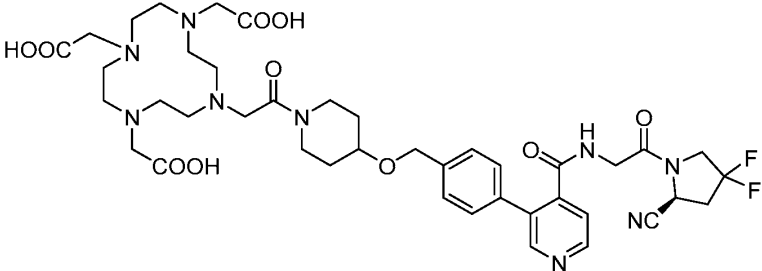
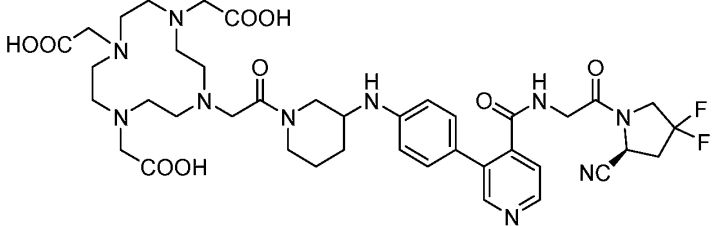
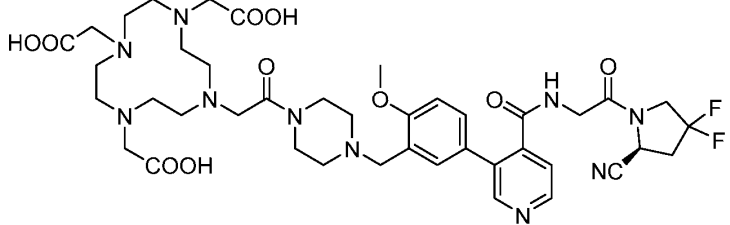
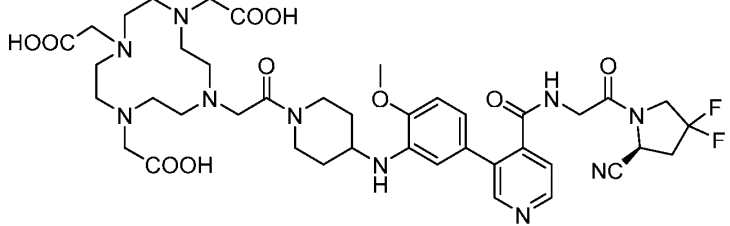
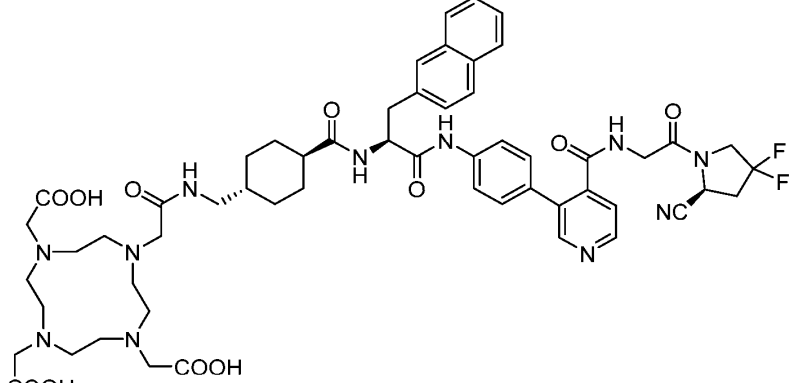


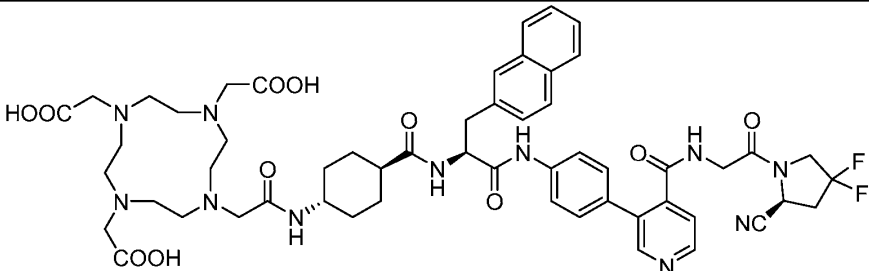
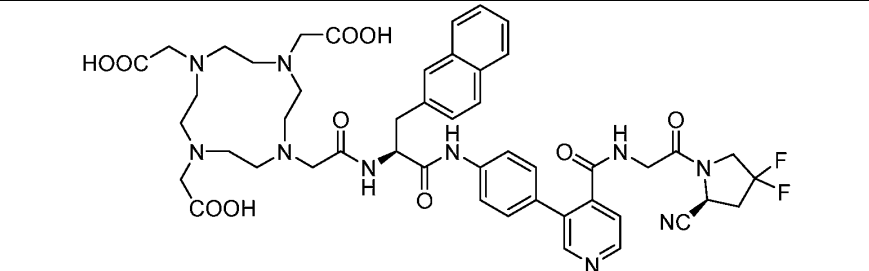
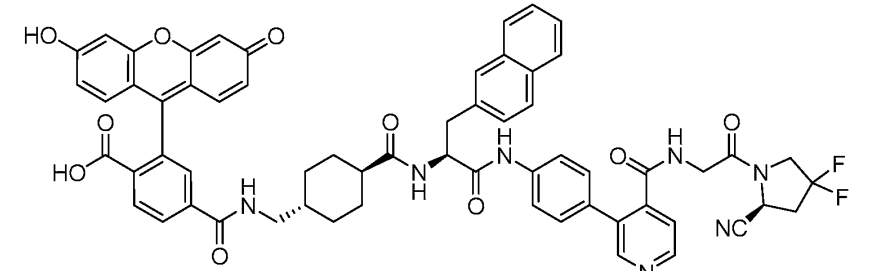
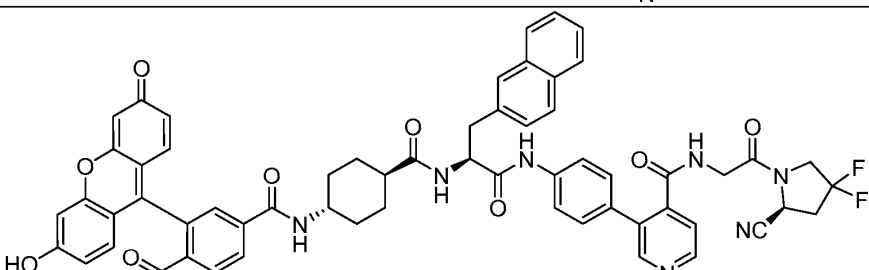
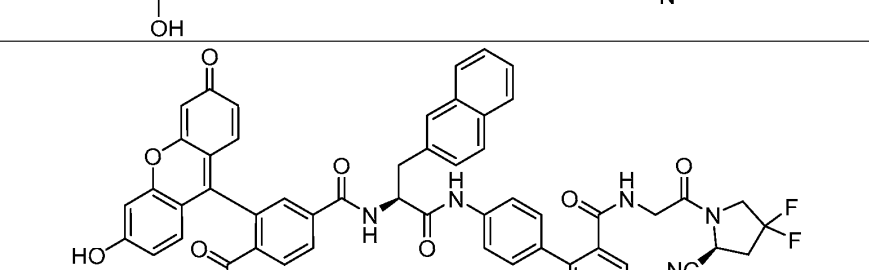
|    |                                                                                      |
|----|--------------------------------------------------------------------------------------|
| 2b |    |
| 3b |    |
| 4b |   |
| 5b |  |
| 6b |  |

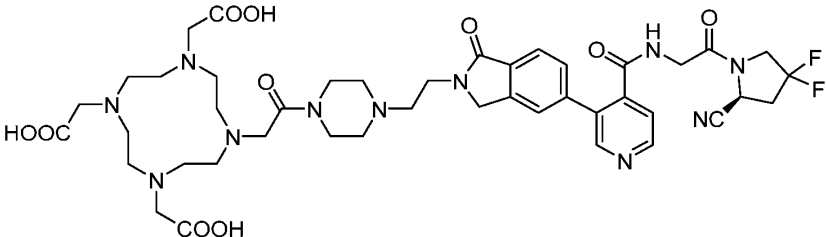
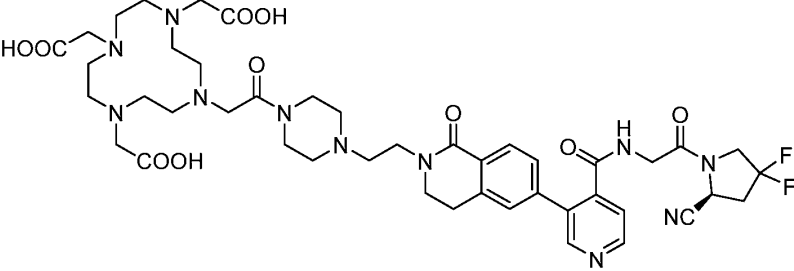
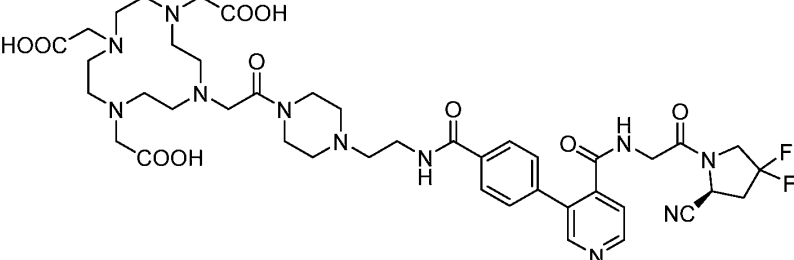
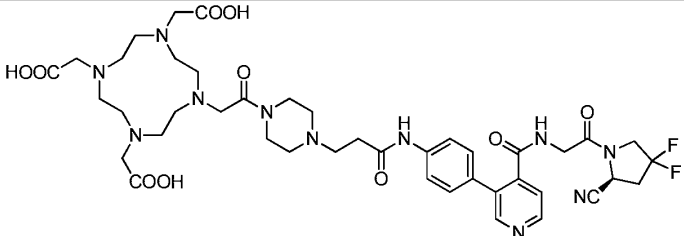
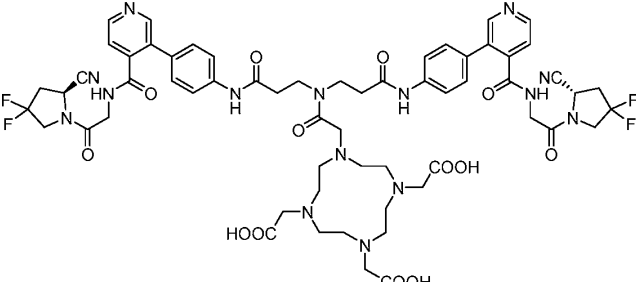
|     |                                                                                      |
|-----|--------------------------------------------------------------------------------------|
| 7b  |    |
| 8b  |    |
| 9b  |   |
| 10b |  |
| 11b |  |

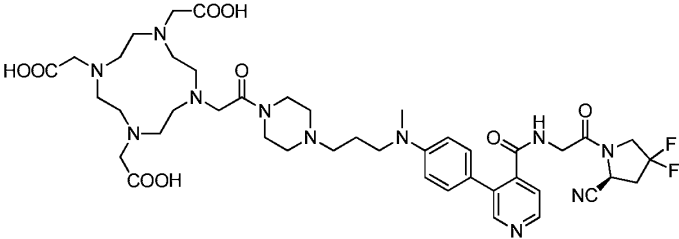
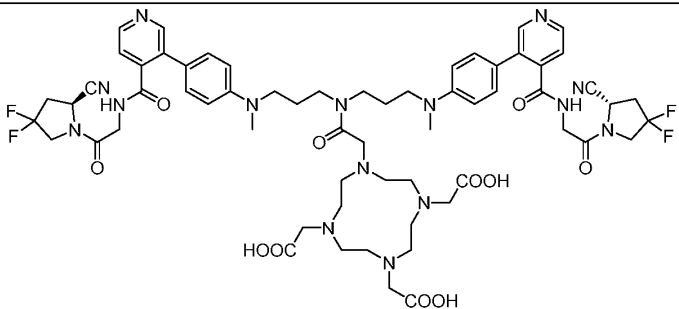
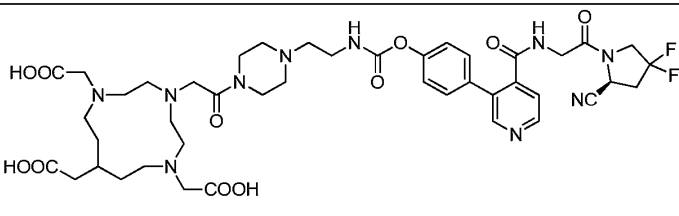
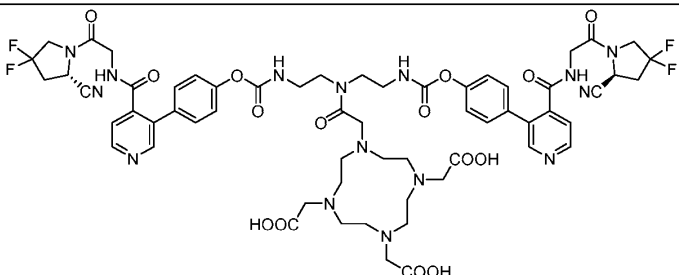
|     |                                                                                      |
|-----|--------------------------------------------------------------------------------------|
| 12b |    |
| 13b |    |
| 14b |   |
| 15b |  |
| 16b |  |

|     |                                                                                      |
|-----|--------------------------------------------------------------------------------------|
| 17b |    |
| 18b |    |
| 19b |   |
| 20b |  |
| 21b |  |

|     |                                                                                      |
|-----|--------------------------------------------------------------------------------------|
| 22b |    |
| 23b |    |
| 24b |   |
| 25b |  |
| 26b |  |

|     |                                                                                      |
|-----|--------------------------------------------------------------------------------------|
| 27b |    |
| 28b |    |
| 29b |   |
| 30b |  |
| 31b |  |

|     |                                                                                      |
|-----|--------------------------------------------------------------------------------------|
| 32b |    |
| 33b |    |
| 34b |   |
| 35b |  |
| 36b |  |

|     |                                                                                      |
|-----|--------------------------------------------------------------------------------------|
| 37b |    |
| 38b |    |
| 39b |   |
| 40b |  |

**[0123]** The compounds illustrated in table 1A and table 1B can be prepared in a manner analogous to the techniques used in connection with the preparation of the compounds given herein and in accordance, using appropriate, analogous starting materials and by utilizing the general synthetic schemes illustrated below.

**[0124]** In some embodiments, provided herein are compounds described in table 1A and table 1B, or a salt, isomer or solvate, and uses thereof.

**[0125]** The embodiments and variations described herein are suitable for compounds of any formulae detailed herein, where applicable.



[0126] Representative examples of compounds detailed herein, including intermediates and final compounds according to the present disclosure are depicted herein. It is understood that in one aspect, any of the compounds may be used in the methods detailed herein, including, where applicable, intermediate compounds that may be isolated and administered to an individual.

[0127] The compounds depicted herein may be present as salts even if salts are not depicted and it is understood that the present disclosure embraces all salts and solvates of the compounds depicted here, as well as the non-salt and non-solvate form of the compound, as is well understood by the skilled artisan. In some embodiments, the salts of the compounds provided herein are pharmaceutically acceptable salts. Where one or more tertiary amine moiety is present in the compound, the N-oxides are also provided and described.

[0128] Where tautomeric forms may be present for any of the compounds described herein, each and every tautomeric form is intended even though only one or some of the tautomeric forms may be explicitly depicted. The tautomeric forms specifically depicted may or may not be the predominant forms in solution or when used according to the methods described herein.

[0129] The present disclosure also includes any or all of the stereochemical forms, including any enantiomeric or diastereomeric forms of the compounds described. The structure or name is intended to embrace all possible stereoisomers of a compound depicted, and each unique stereoisomer has a compound number bearing a suffix "a", "b", *etc.* All forms of the compounds are also embraced by the invention, such as crystalline or non-crystalline forms of the compounds. Compositions comprising a compound of the invention are also intended, such as a composition of substantially pure compound, including a specific stereochemical form thereof, or a composition comprising mixtures of compounds of the invention in any ratio, including two or more stereochemical forms, such as in a racemic or non-racemic mixture.

[0130] The invention also intends isotopically-labeled and/or isotopically-enriched forms of compounds described herein. The compounds herein may contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. In some embodiments, the compound is isotopically-labeled, such as an isotopically-labeled compound of the formula (I) or variations thereof described herein, where a fraction of one or more atoms are replaced by an isotope of the same element. Exemplary isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, chlorine,

such as  $^2\text{H}$ ,  $^3\text{H}$ ,  $^{11}\text{C}$ ,  $^{13}\text{C}$ ,  $^{14}\text{C}$ ,  $^{13}\text{N}$ ,  $^{15}\text{O}$ ,  $^{17}\text{O}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{18}\text{F}$ ,  $^{36}\text{Cl}$ . Certain isotope labeled compounds (e.g.  $^3\text{H}$  and  $^{14}\text{C}$ ) are useful in compound or substrate tissue distribution studies. Incorporation of heavier isotopes such as deuterium ( $^2\text{H}$ ) can afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life, or reduced dosage requirements and, hence may be preferred in some instances.

[0131] Isotopically-labeled compounds of the present invention can generally be prepared by standard methods and techniques known to those skilled in the art or by procedures similar to those described in the accompanying Examples substituting appropriate isotopically-labeled reagents in place of the corresponding non-labeled reagent.

[0132] The invention also includes any or all metabolites of any of the compounds described. The metabolites may include any chemical species generated by a biotransformation of any of the compounds described, such as intermediates and products of metabolism of the compound, such as would be generated *in vivo* following administration to a human.

[0133] Articles of manufacture comprising a compound described herein, or a salt or solvate thereof, in a suitable container are provided. The container may be a vial, jar, ampoule, preloaded syringe, i.v. bag, and the like.

[0134] Preferably, the compounds detailed herein are orally bioavailable. However, the compounds may also be formulated for parenteral (e.g., intravenous) administration.

[0135] One or several compounds described herein can be used in the preparation of a medicament by combining the compound or compounds as an active ingredient with a pharmacologically acceptable carrier, which are known in the art. Depending on the therapeutic form of the medication, the carrier may be in various forms. In one variation, the manufacture of a medicament is for use in any of the methods disclosed herein, *e.g.*, for the treatment of cancer.

#### ***General synthetic schemes***

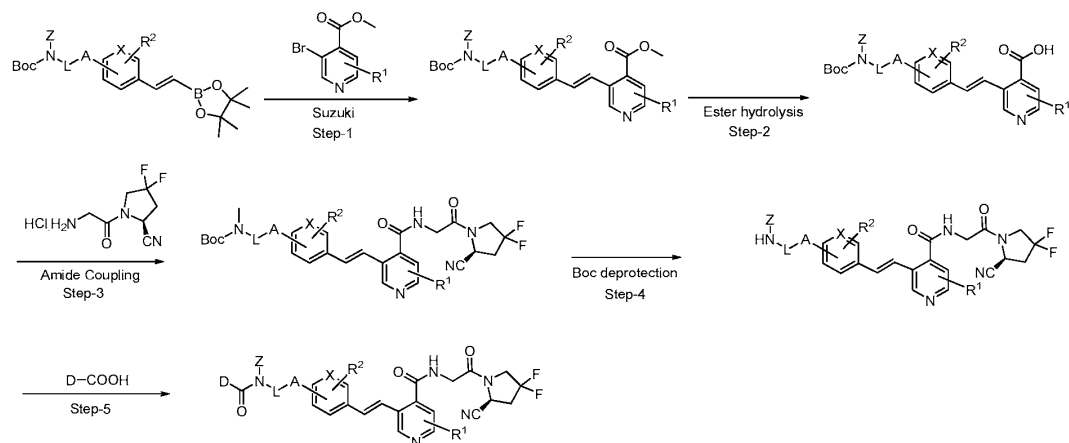
[0136] The compounds of the invention may be prepared by a number of processes as generally described below and more specifically in the Examples hereinafter (such as the schemes provided in the Examples below). In the following process descriptions, the symbols when used in the formulae depicted are to be understood to represent those groups described above in relation to the formulae herein.

[0137] Where it is desired to obtain a particular enantiomer of a compound, this may be accomplished from a corresponding mixture of enantiomers using any suitable conventional procedure for separating or resolving enantiomers. Thus, for example, diastereomeric derivatives may be produced by reaction of a mixture of enantiomers, *e.g.*, a racemate, and an appropriate chiral compound. The diastereomers may then be separated by any convenient means, for example by crystallization and the desired enantiomer recovered. In another resolution process, a racemate may be separated using chiral High Performance Liquid Chromatography. Alternatively, if desired a particular enantiomer may be obtained by using an appropriate chiral intermediate in one of the processes described.

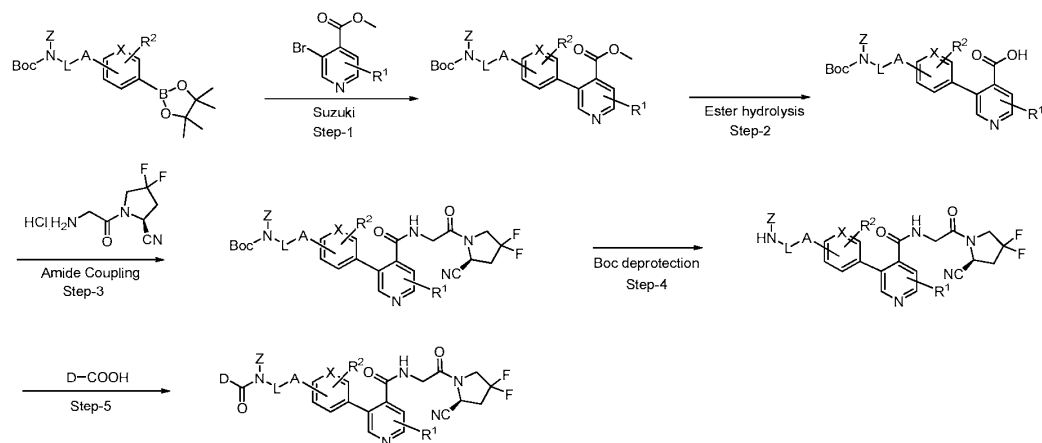
[0138] Chromatography, recrystallization and other conventional separation procedures may also be used with intermediates or final products where it is desired to obtain a particular isomer of a compound or to otherwise purify a product of a reaction.

[0139] Solvates and/or polymorphs of a compound provided herein or a pharmaceutically acceptable salt thereof are also contemplated. Solvates contain either stoichiometric or non-stoichiometric amounts of a solvent, and are often formed during the process of crystallization. Hydrates are formed when the solvent is water, or alcoholates are formed when the solvent is alcohol. Polymorphs include the different crystal packing arrangements of the same elemental composition of a compound. Polymorphs usually have different X-ray diffraction patterns, infrared spectra, melting points, density, hardness, crystal shape, optical and electrical properties, stability, and/or solubility. Various factors such as the recrystallization solvent, rate of crystallization, and storage temperature may cause a single crystal form to dominate.

[0140] In some embodiments, compounds of the present invention may be synthesized according to Scheme-1 to Scheme-2

**Scheme-1:**

wherein X, Z, A, L, D, R<sup>1</sup> and R<sup>2</sup> are as defined for formula (I).

**Scheme-2:**

wherein X, Z, A, L, D, R<sup>1</sup> and R<sup>2</sup> are as defined for formula (I).

**Pharmaceutical Compositions and Formulations**

[0141] Pharmaceutical compositions of any of the compounds detailed herein are embraced by this disclosure. Thus, the present disclosure includes pharmaceutical compositions comprising a compound as detailed herein or a salt thereof and a pharmaceutically acceptable carrier or excipient. In one aspect, the pharmaceutically acceptable salt is an acid addition salt, such as a salt formed with an inorganic or organic acid. Pharmaceutical compositions may take a form

suitable for oral, buccal, parenteral, nasal, topical or rectal administration or a form suitable for administration by inhalation.

[0142] A compound as detailed herein may in one aspect be in a purified form and compositions comprising a compound in purified forms are detailed herein. Compositions comprising a compound as detailed herein or a salt thereof are provided, such as compositions of substantially pure compounds. In some embodiments, a composition containing a compound as detailed herein or a salt thereof is in substantially pure form.

[0143] In one variation, the compounds herein are synthetic compounds prepared for administration to an individual. In another variation, compositions are provided containing a compound in substantially pure form. In another variation, the present disclosure embraces pharmaceutical compositions comprising a compound detailed herein and a pharmaceutically acceptable carrier. In another variation, methods of administering a compound are provided. The purified forms, pharmaceutical compositions and methods of administering the compounds are suitable for any compound or form thereof detailed herein.

[0144] A compound detailed herein or salt thereof may be formulated for any available delivery route, including an oral, mucosal (e.g., nasal, sublingual, vaginal, buccal or rectal), parenteral (e.g., intramuscular, subcutaneous or intravenous), topical or transdermal delivery form. A compound or salt thereof may be formulated with suitable carriers to provide delivery forms that include, but are not limited to, tablets, caplets, capsules (such as hard gelatin capsules or soft elastic gelatin capsules), cachets, troches, lozenges, gums, dispersions, suppositories, ointments, cataplasms (poultices), pastes, powders, dressings, creams, solutions, patches, aerosols (e.g., nasal spray or inhalers), gels, suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions or water-in-oil liquid emulsions), solutions and elixirs.

[0145] One or several compounds described herein or a salt thereof can be used in the preparation of a formulation, such as a pharmaceutical formulation, by combining the compound or compounds, or a salt thereof, as an active ingredient with a pharmaceutically acceptable carrier, such as those mentioned above. Depending on the therapeutic form of the system (e.g., transdermal patch vs. oral tablet), the carrier may be in various forms. In addition, pharmaceutical formulations may contain preservatives, solubilizers, stabilizers, re-wetting agents, emulgators, sweeteners, dyes, adjusters, and salts for the adjustment of osmotic pressure, buffers, coating

agents or antioxidants. Formulations comprising the compound may also contain other substances which have valuable therapeutic properties. Pharmaceutical formulations may be prepared by known pharmaceutical methods. Suitable formulations can be found, e.g., in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, PA, 20<sup>th</sup> ed. (2000), which is incorporated herein by reference.

[0146] Compounds as described herein may be administered to individuals in a form of generally accepted oral compositions, such as tablets, coated tablets, and gel capsules in a hard or in soft shell, emulsions or suspensions. Examples of carriers, which may be used for the preparation of such compositions, are lactose, corn starch or its derivatives, talc, stearate or its salts, etc. Acceptable carriers for gel capsules with soft shell are, for instance, plant oils, wax, fats, semisolid and liquid poly-ols, and so on. In addition, pharmaceutical formulations may contain preservatives, solubilizers, stabilizers, re-wetting agents, emulgators, sweeteners, dyes, adjusters, and salts for the adjustment of osmotic pressure, buffers, coating agents or antioxidants.

[0147] Any of the compounds described herein can be formulated in a tablet in any dosage form described, for example, a compound as described herein or a salt thereof can be incorporated in tablet in an amount ranging from about 1 mg to about 1000 mg.

[0148] Compositions comprising a compound provided herein are also described. In one variation, the composition comprises a compound or salt thereof and a pharmaceutically acceptable carrier or excipient. In another variation, a composition of substantially pure compound is provided.

#### ***Methods of Use***

[0149] Compounds and compositions detailed herein, such as a pharmaceutical composition containing a compound of any formula provided herein or a salt thereof and a pharmaceutically acceptable carrier or excipient, may be used in methods of administration and treatment as provided herein. In some embodiments, the compounds and compositions detailed herein is used in chemotherapy, immunotherapy, radionuclide therapy or diagnosis. The compounds and compositions may also be used in *in vitro* methods, such as *in vitro* methods of administering a compound or composition to cells for screening purposes and/or for conducting quality control assays.

[0150] Provided herein is a method of treating a disease in an individual in need thereof comprising administering an effective amount of a compound of the present invention or any embodiment, variation or aspect thereof or the present compounds or the compounds detailed or described herein) or a pharmaceutically acceptable salt thereof, to the individual (e.g., a human individual or an animal). Further provided herein is a method of treating a proliferative disease in an individual in need thereof, comprising administering an effective amount of the compound of the present invention, or a pharmaceutically acceptable salt thereof, to the individual. Also provided herein is a method of treating cancer in an individual in need thereof comprising administering an effective amount of the compound of formula (I) or a pharmaceutically acceptable salt thereof, to the individual. In some embodiments, the compound is administered to the individual according to a dosage and/or method of administration described herein.

[0151] Another aspect of the invention relates to a method of treating a disease or disorder associated with fibroblast-activated protein (FAP). The method involves administering to an individual in need thereof of a treatment for diseases or disorders associated with FAP an effective amount of the compositions and compounds of the present invention.

[0152] Another aspect of the invention is directed to a method inhibiting FAP. The method involves administering to an individual in need thereof an effective amount of the compositions or compounds of the present invention.

[0153] Provided is a method of treating cancer, comprising administering to an individual in need thereof an effective amount of the compositions and compounds of the present invention. The use of a compound of the present invention for the manufacture of a medicament for the treatment of a proliferative disease such as cancer. In some embodiments, the cancer is selected from the group consisting of carcinomas, for example carcinomas of the bladder, breast, colon, kidney, epidermis, liver, lung, oesophagus, gall bladder, ovary, pancreas, stomach, cervix, head and neck, thyroid, prostate, gastrointestinal system, or skin, hematopoietic tumors such as leukaemia, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma, or Burkett's lymphoma; hematopoietic tumors of myeloid lineage, for example acute and chronic myelogenous leukaemias, myelodysplastic syndrome, or promyelocytic leukaemia; thyroid follicular cancer; tumors of mesenchymal origin, for example fibrosarcoma or habdomyosarcoma; tumors of the central or peripheral nervous system, for example astrocytoma,

neuroblastoma, glioma or schwannoma; melanoma; seminoma; teratocarcinoma; osteosarcoma; xeroderma pigmentosum; keratoanthoma; thyroid follicular cancer; Ewing's sarcoma or Kaposi's sarcoma; Anaplastic large cell lymphoma, Tongue squamous cell carcinoma, Nasopharyngeal carcinoma, Endometrioid adenocarcinoma, Salivary gland adenoid cystic carcinoma, Testis seminoma, and Multiple myeloma. The use of a compound of the present invention for the manufacture of a medicament for the treatment of cancer, wherein the cancer is one which is characterized by a defective DNA repair mechanism or defective cell cycle.

[0154] Provided is a method of treating idiopathic pulmonary fibrosis (IPF), comprising administering to an individual in need thereof an effective amount of the compositions and compounds of the present invention. The use of a compound of the present invention for the manufacture of a medicament for the treatment of idiopathic pulmonary fibrosis (IPF).

[0155] In some embodiments, the method described herein involves FAP targeted radionuclide therapy (TRT), when the compositions and compounds of the present invention comprise chelates. (Fibroblast activation protein-targeted radionuclide therapy: background, opportunities, and challenges of first (pre)clinical studies, *European Journal of Nuclear Medicine and Molecular Imaging* (2023) 50:1906–1918). In some embodiments, the radionuclide is used for diagnosis. It will, however, also be acknowledged by a person skilled in the art that the use of said radionuclide is not limited to diagnostic purposes, but encompasses their use in therapy and theragnostics when conjugated to the compound of the invention. In some embodiments, the radionuclide is used for therapy. In some embodiments, a radionuclide is provided with a chelate. In such chelates, the chelator unit chelates directly with the radionuclide (eg.  $^{68}\text{Ga}$  chelates with a chelator unit derived from DOTA), or the radionuclide is introduced indirectly by chelation with other metals (eg.  $\text{Al}^{3+}$  is chelated with a chelator unit derived from DOTA, and the radionuclide  $^8\text{F}$  is introduced into the chelate in the form of a counter ion).

[0156] The present invention further provides a method for diagnosis of a disease or disorder, comprising administering to an individual in need thereof a diagnostically effective amount of the compositions and compounds of the present invention. In some embodiments, the method involves nuclear medicine imaging technique, such as Positron Emission Tomography (PET).

[0157] The present invention also provides a method of imaging a disease or disorder, comprising administering to an individual in need thereof a diagnostically effective amount of the



compositions and compounds of the present invention. Activated fibroblasts are found in scar formation (e.g., in ischemic tissues after myocardial infarction), chronic inflammatory and/or destructive processes (rheumatoid arthritis, Crohn disease, atherosclerotic plaque, immunoglobulin G4-related diseases), fibrosis (liver, kidney, lung), and benign tumors. (FAPI PET: Fibroblast Activation Protein Inhibitor Use in Oncologic and Nononcologic Disease, *Radiology*, 2023; 306(2):e220749). In some embodiments, the disease or disorder are the aforementioned diseases.

[0158] The disclosed compounds of the present invention can be administered in effective amounts to treat or prevent a disorder and/or prevent the development thereof in subjects. In some embodiments, the disclosed compounds have low half-maximal inhibitory concentrations, for example, IC<sub>50</sub> rhFAP (nM) and great potencies. In some embodiments, the disclosed compounds have high percentages of the compound remaining after incubation with human (Hu) or mouse (Mo) plasma, for example, Hu/Mo plasma (%) and great stability in plasma.

#### ***Combination Therapy***

[0159] The compound of the present invention may be administered either simultaneously with, or before or after, one or more other therapeutic agents. The compound of the present invention may be administered separately, by the same or different route of administration, or together in the same pharmaceutical composition as the other agents.

[0160] In some embodiments, the methods described herein comprise the additional step of co-administering to an individual in need thereof a second therapy e.g., an additional cancer therapeutic agent or an additional cancer treatment. Exemplary additional cancer therapeutic agents include for example, In one embodiment, the other active agent is selected from the group consisting of but not limited to antimetabolites, tubulin targeting agents, DNA binder and topoisomerase II inhibitors, alkylating agents, monoclonal antibodies, hormonal therapy, signal transduction inhibitors, proteasome inhibitors, DNA methyl transferases, cytokines and retinoids, hypoxia triggered DNA damaging agents, immunomodulators (e.g. CTLA-4, LAG-3, PD-1 antagonists etc.) and monoclonal antibodies.

[0161] In some embodiments, the additional cancer therapeutic agent is a chemotherapy agent. Examples of chemotherapeutic agents used in cancer therapy include, for example, antimetabolites (e.g., folic acid, purine, and pyrimidine derivatives), alkylating agents (e.g., nitrogen mustards,

nitrosoureas, platinum, alkyl sulfonates, hydrazines, triazenes, aziridines, spindle poison, cytotoxic agents, topoisomerase inhibitors and others), and hypomethylating agents (e.g., decitabine (5-azadeoxycytidine), zebularine, isothiocyanates, azacitidine (5-azacytidine), 5-fluoro-2'-deoxycytidine, 5,6-dihydro-5-azacytidine and others). Exemplary agents include Aclarubicin, Actinomycin, Alitretinoin, Altretamine, Aminopterin, Aminolevulinic acid, Amrubicin, Amsacrine, Anagrelide, Arsenic trioxide, Asparaginase, Atrasentan, Belotecan, Bexarotene, bendamustine, Bleomycin, Bortezomib, Busulfan, Camptothecin, Capecitabine, Carboplatin, Carboquone, Carmofur, Carmustine, Celecoxib, Chlorambucil, Chlormethine, Cisplatin, Cladribine, Clofarabine, Crisantaspase, Cyclophosphamide, Cytarabine, Dacarbazine, Dactinomycin, Daunorubicin, Decitabine, Demecolcine, Docetaxel, Doxorubicin, Efaproxiral, Elesclomol, Elsamitrucin, Enocitabine, Epirubicin, Estramustine, Etoglucid, Etoposide, Floxuridine, Fludarabine, Fluorouracil (5FU), Fotemustine, Gemcitabine, Gliadel implants, Hydroxycarbamide, Hydroxyurea, Idarubicin, Ifosfamide, Irinotecan, Irofulven, Ixabepilone, Larotaxel, Leucovorin, Liposomal doxorubicin, Liposomal daunorubicin, Lonidamine, Lomustine, Lucanthone, Mannosulfan, Masoprocol, Melphalan, Mercaptopurine, Mesna, Methotrexate, Methyl aminolevulinate, Mitobronitol, Mitoguazone, Mitotane, Mitomycin, Mitoxantrone, Nedaplatin, Nimustine, Oblimersen, Omacetaxine, Ortaxel, Oxaliplatin, Paclitaxel, Pegaspargase, Pemetrexed, Pentostatin, Pirarubicin, Pixantrone, Plicamycin, Porfimer sodium, Prednimustine, Procarbazine, Raltitrexed, Ranimustine, Rubitecan, Sapacitabine, Semustine, Sitimagene ceradenovec, Strataplatin, Streptozocin, Talaporfin, Tegafur-uracil, Temoporfin, Temozolomide, Teniposide, Tesetaxel, Testolactone, Tetranitrate, Thiotepa, Tiazofurine, Tioguanine, Tipifarnib, Topotecan, Trabectedin, Triaziquone, Triethylenemelamine, Triplatin, Tretinoin, Treosulfan, Trofosfamide, Uramustine, Valrubicin, Verteporfin, Vinblastine, Vincristine, Vindesine, Vinflunine, Vinorelbine, Vorinostat, Zorubicin, and other cytostatic or cytotoxic agents described herein

**[0162]** In some embodiments, the additional cancer therapeutic agents are tyrosine kinase inhibitors, PI3K/mTOR inhibitors, PARP inhibitors such as Olaparib, Rucaparib, Niraparib, Talazoparib, Wee1 inhibitors, CDK4/6 inhibitors such as Palbociclib, Ribociclib, Abemaciclib, DNA-PK inhibitors, ATM inhibitors and ATR inhibitors. Any compound of the present invention can be combined with these targeted inhibitors.

[0163] The compounds of the invention and combinations with chemotherapeutic agents or radiation therapies as described above may be administered over a prolonged term to maintain beneficial therapeutic effects or may be administered for a short period only. Alternatively, they may be administered in a pulsatile or continuous manner.

[0164] Other possible additional therapeutic modalities include gene therapy, peptide and dendritic cell vaccines, synthetic chlorotoxins, and radiolabeled drugs and antibodies.

#### ***Dosing and Method of Administration***

[0165] The dose of a compound administered to an individual (such as a human) may vary with the particular compound or salt thereof, the method of administration, and the particular disease, such as type and stage of cancer, being treated. In some embodiments, the amount of the compound or salt thereof is a therapeutically effective amount.

[0166] The effective amount of the compound may in one aspect be a dose of between about 0.01 and about 100 mg/kg. Effective amounts or doses of the compounds of the invention may be ascertained by routine methods, such as modeling, dose escalation, or clinical trials, taking into account routine factors, e.g., the mode or route of administration or drug delivery, the pharmacokinetics of the agent, the severity and course of the disease to be treated, the individual's health status, condition, and weight. An exemplary dose is in the range of about from about 0.7 mg to 7 g daily, or about 7 mg to 350 mg daily, or about 350 mg to 1.75 g daily, or about 1.75 to 7 g daily.

[0167] Any of the methods provided herein may in one aspect comprise administering to an individual a pharmaceutical composition that contains an effective amount of a compound provided herein or a salt thereof and a pharmaceutically acceptable excipient.

[0168] A compound or composition of the invention may be administered to an individual in accordance with an effective dosing regimen for a desired period of time or duration, such as at least about one month, at least about 2 months, at least about 3 months, at least about 6 months, or at least about 12 months or longer, which in some variations may be for the duration of the individual's life. In one variation, the compound is administered on a daily or intermittent schedule. The compound can be administered to an individual continuously (for example, at least once daily) over a period of time. The dosing frequency can also be less than once daily, e.g.,

about a once weekly dosing. The dosing frequency can be more than once daily, *e.g.*, twice or three times daily. The dosing frequency can also be intermittent, including a 'drug holiday' (*e.g.*, once daily dosing for 7 days followed by no doses for 7 days, repeated for any 14 day time period, such as about 2 months, about 4 months, about 6 months or more). Any of the dosing frequencies can employ any of the compounds described herein together with any of the dosages described herein.

**[0169]** The compounds provided herein or a salt thereof may be administered to an individual via various routes, including, *e.g.*, intravenous, intramuscular, subcutaneous, oral and transdermal. A compound provided herein can be administered frequently at low doses, known as 'metronomic therapy,' or as part of a maintenance therapy using compound alone or in combination with one or more additional drugs. Metronomic therapy or maintenance therapy can comprise administration of a compound provided herein in cycles. Metronomic therapy or maintenance therapy can comprise intra-tumoral administration of a compound provided herein.

**[0170]** In one aspect, the invention provides a method of treating cancer in an individual by parenterally administering to the individual (*e.g.*, a human) an effective amount of a compound or salt thereof. In some embodiments, the route of administration is intravenous, intra-arterial, intramuscular, or subcutaneous. In some embodiments, the route of administration is oral. In still other embodiments, the route of administration is transdermal.

**[0171]** The invention also provides compositions (including pharmaceutical compositions) as described herein for the use in treating, preventing, and/or delaying the onset and/or development of cancer and other methods described herein. In certain embodiments, the composition comprises a pharmaceutical formulation which is present in a unit dosage form.

**[0172]** Also provided are articles of manufacture comprising a compound of the disclosure or a salt thereof, composition, and unit dosages described herein in suitable packaging for use in the methods described herein. Suitable packaging is known in the art and includes, for example, vials, vessels, ampules, bottles, jars, flexible packaging and the like. An article of manufacture may further be sterilized and/or sealed.

***Kits***

[0173] The present disclosure further provides kits for carrying out the methods of the invention, which comprises one or more compounds described herein or a composition comprising a compound described herein. The kits may employ any of the compounds disclosed herein. In one variation, the kit employs a compound described herein or a pharmaceutically acceptable salt thereof. The kits may be used for any one or more of the uses described herein, and, accordingly, may contain instructions for the treatment of cancer.

[0174] Kits generally comprise suitable packaging. The kits may comprise one or more containers comprising any compound described herein. Each component (if there is more than one component) can be packaged in separate containers or some components can be combined in one container where cross-reactivity and shelf life permit.

[0175] The kits may be in unit dosage forms, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. For example, kits may be provided that contain sufficient dosages of a compound as disclosed herein and/or a second pharmaceutically active compound useful for a disease detailed herein to provide effective treatment of an individual for an extended period, such as any of a week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 3 months, 4 months, 5 months, 7 months, 8 months, 9 months, or more. Kits may also include multiple unit doses of the compounds and instructions for use and be packaged in quantities sufficient for storage and use in pharmacies (*e.g.*, hospital pharmacies and compounding pharmacies).

[0176] The kits may optionally include a set of instructions, generally written instructions, although electronic storage media (*e.g.*, magnetic diskette or optical disk) containing instructions are also acceptable, relating to the use of component(s) of the methods of the present invention. The instructions included with the kit generally include information as to the components and their administration to an individual.

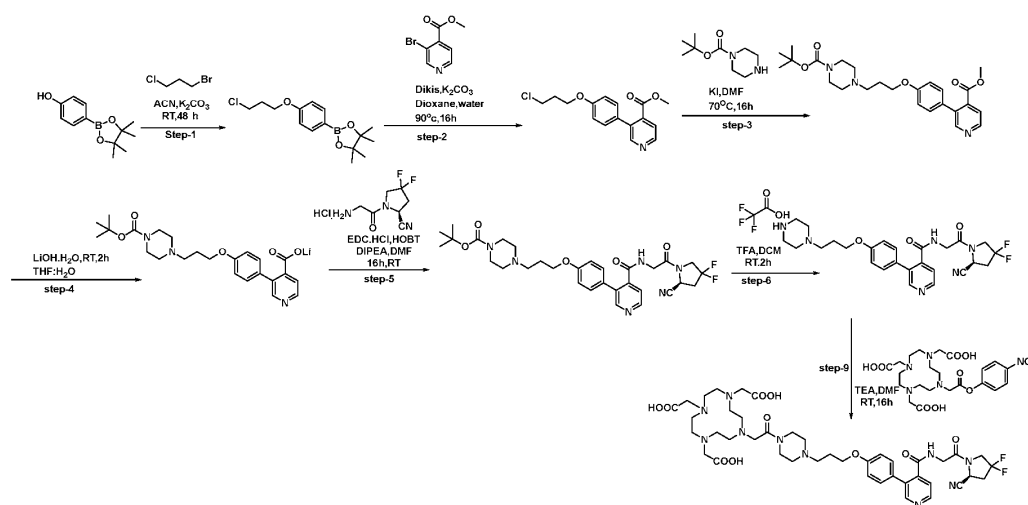
[0177] The invention can be further understood by reference to the following examples, which are provided by way of illustration and are not meant to be limiting.

[0178] All references throughout, such as publications, patents, patent applications and published patent applications, are incorporated herein by reference in their entireties.

## EXAMPLES

*Synthetic Examples*

[0179] The chemical reactions in the Synthetic Examples described can be readily adapted to prepare a number of other compounds of the invention, and alternative methods for preparing the compounds of this invention are deemed to be within the scope of this invention. For example, the synthesis of non-exemplified compounds according to the invention can be successfully performed by modifications apparent to those skilled in the art, *e.g.*, by appropriately protecting interfering groups, by utilizing other suitable reagents known in the art other than those described, or by making routine modifications of reaction conditions. Alternatively, other reactions disclosed herein or known in the art will be recognized as having applicability for preparing other compounds of the invention.

**Compound 1****Step-1: Synthesis of 2-(4-(3-chloropropoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane**

[0180] To a stirred solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (0.500 g, 2.272 mmol, 1.0 equiv) in ACN (5 mL) was added  $K_2CO_3$  (0.784 g, 5.681 mmol, 2.5 equiv), followed by the addition of 1-bromo-3-chloropropane (1.077 g, 6.818 mmol, 3.0 equiv). The Reaction mixture was allowed to stir at RT for 48 h. The progress of the reaction was monitored

by TLC and LCMS. After completion of the reaction, the reaction mixture was diluted with ethyl acetate and passed through Celite®. The filtrate was concentrated and purified by flash chromatography (0 to 20 % ethyl acetate in hexane as an eluent) to obtain 2-(4-(3-chloropropoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.455 g, 67 % Yield) as a semisolid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.61 (d, *J*=8.58 Hz, 2 H) 6.94 (d, *J*=8.58 Hz, 2 H) 4.05 - 4.17 (m, 2 H) 3.75 - 3.84 (m, 2 H) 2.11 - 2.22 (m, 2 H) 1.21 - 1.35 (m, 12 H).

### Step-2: Synthesis of methyl 3-(4-(3-chloropropoxy)phenyl)isonicotinate

[0181] To a stirred solution of methyl 3-bromoisonicotinate (0.345 g, 1.597 mmol, 1.0 equiv) in Dioxane:Water (7 : 0.7 mL) was added K<sub>2</sub>CO<sub>3</sub> (0.441 g, 3.194 mmol, 2.0 equiv), followed by the addition of 2-(4-(3-chloropropoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.709 g, 2.395 mmol, 1.5equiv). The reaction mixture was purged with N<sub>2</sub> for 5-10 minutes, followed by the addition of Bis(triphenylphosphine)palladium dichloride (0.056 g, 0.079 mmol, 0.05 equiv). The resulting reaction mixture was heated at 90 °C for 16h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the reaction mixture was diluted with ethyl acetate and passed through Celite®. The filtrate was concentrated and purified by flash chromatography (0- 30 % ethyl acetate in hexane as an eluent) to obtain methyl 3-(4-(3-chloropropoxy)phenyl)isonicotinate (0.410 g, 83 % Yield) as an off white solid. LCMS: 306 [M+1]<sup>+</sup> <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.63 - 8.72 (m, 2 H) 7.63 (d, *J*=4.77 Hz, 1 H) 7.30 (d, *J*=8.58 Hz, 2 H) 7.05 (d, *J*=8.58 Hz, 2 H) 4.14 (t, *J*=5.96 Hz, 2 H) 3.82 (t, *J*=6.44 Hz, 2 H) 3.68 (s, 3 H) 2.20 (quin, *J*=6.32 Hz, 2 H).

### Step-3: Synthesis of tert-butyl 4-(3-(4-(4-(methoxycarbonyl)pyridin-3-yl)phenoxy)propyl)piperazine-1-carboxylate

[0182] To a stirred solution of methyl 3-(4-(3-chloropropoxy)phenyl)isonicotinate (0.500 g, 1.633 mmol, 1.0 equiv) in DMF (4 mL) was added KI (0.135 g, 0.816mmol, 0.5 equiv), followed by the addition of tert-butyl piperazine-1-carboxylate (0.911 g, 4.901 mmol, 3.0equiv). The reaction mixture was stirred at 70 °C for 16 h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the reaction mixture was quenched with ice-cold water (20 mL) and extracted with ethyl acetate (20 ml × 3). Combined organic layers were separated, washed with cold water (2 X 10 ml), brine (10 ml), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography (0 to 40

% ethyl acetate in hexane as an eluent) to obtain tert-butyl 4-(3-(4-(4-(methoxycarbonyl)pyridin-3-yl)phenoxy)propyl)piperazine-1-carboxylate (0.556 g, 74 % Yield) as an off white solid.

**LCMS: 456 [M+1]<sup>+</sup>** <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*)  $\delta$  8.63 - 8.72 (m, 2 H) 7.58 (d, *J*=5.25 Hz, 1 H) 7.26 (s, 2 H) 6.96 (d, *J*=8.58 Hz, 2 H) 4.00 - 4.11 (m, 2 H) 3.73 (s, 3 H) 3.36 - 3.51 (m, 4 H) 2.56 (t, *J*=7.15 Hz, 2 H) 2.43 (br. s., 4 H) 1.94 - 2.06 (m, 2H) 1.46 (s, 9 H).

**Step-4: Synthesis of lithium 3-(4-(3-(4-(tert-butoxycarbonyl)piperazin-1-yl)propoxy)phenyl)isonicotinate**

**[0183]** To a stirred solution of tert-butyl 4-(3-(4-(4-(methoxycarbonyl)pyridin-3-yl)phenoxy)propyl)piperazine-1-carboxylate (0.350 g, 0.767 mmol, 1.0 equiv) in THF (3 mL) was added LiOH.H<sub>2</sub>O (0.065 g, 1.535 mmol, 2.0 equiv) in water (1.5 ml). The Reaction mixture was allowed to stir at RT for 2h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the organic solvent was removed under reduced pressure and freeze dried to obtain lithium 3-(4-(3-(4-(tert-butoxycarbonyl)piperazin-1-yl)propoxy)phenyl)isonicotinate (0.290 g, 84.5 % Yield) as an off white solid. **LCMS: 441 [M+1]<sup>+</sup>** <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.34 (s, 1 H) 8.28 (d, *J*=4.77 Hz, 1 H) 7.50 (d, *J*=9.06 Hz, 2 H) 7.04 (d, *J*=4.77 Hz, 1 H) 6.90 (d, *J*=8.58 Hz, 2 H) 3.95 - 4.08 (m, 2 H) 3.17 - 3.32 (m, 4 H) 2.44 (t, *J*=7.15 Hz, 2 H) 2.33 (t, *J*=4.77 Hz, 4 H) 1.82 - 1.93 (m, 2 H) 1.39 (s, 9 H) .

**Step-5: Synthesis of (S)-tert-butyl 4-(3-(4-(4-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)piperazine-1-carboxylate**

**[0184]** To a stirred solution of lithium 3-(4-(3-(4-(tert-butoxycarbonyl)piperazin-1-yl)propoxy)phenyl)isonicotinate (0.200 g, 0.452 mmol, 1.0 equiv) in DMF (2 mL) was added EDC.HCl (0.112 g, 0.587 mmol, 1.3 equiv), HOBT (0.079 g, 0.587 mmol, 1.3 equiv) and (S)-1-(2-aminoacetyl)-4,4-difluoropyrrolidine-2-carbonitrile hydrochloride (0.152 g, 0.678 mmol, 1.5 equiv) followed by the addition of DIPEA (0.25 mL, 1.356 mmol, 3.0 equiv). The resulting reaction mixture was allowed to stir at RT for 16h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the reaction mixture was quenched with water (20 mL) and extracted with ethyl acetate (10 mL  $\times$  2). The combined organic layers was washed with water (10mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure The crude product was purified by Flash chromatography (0-5 % MeOH in DCM as an eluent) to obtain (S)-tert-butyl 4-(3-(4-(4-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-



yl)phenoxy)propyl)piperazine-1-carboxylate (0.065 g 24 % Yield) as an off white solid. **LCMS: 613 [M+1]<sup>+</sup> <sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.93 (t, *J*=5.72 Hz, 1 H) 8.57 - 8.67 (m, 2 H) 7.37 - 7.51 (m, 3 H) 6.96 (d, *J*=8.58 Hz, 2 H) 5.10 (dd, *J*=9.06, 2.38 Hz, 1 H) 4.21 (t, *J*=11.44 Hz, 2 H) 3.94 - 4.11 (m, 4 H) 3.17 (d, *J*=5.25 Hz, 2 H) 2.75 - 2.97 (m, 2 H) 2.61 - 2.75 (m, 2 H) 2.44 (t, *J*=7.39 Hz, 2 H) 2.33 (br. s., 4 H) 1.81 - 1.94 (m, 2 H) 1.35 - 1.45 (m, 9H).

**Step-6: Synthesis of (S)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-3-(4-(3-(piperazin-1-yl)propoxy)phenyl)isonicotinamide 2,2,2-trifluoroacetate**

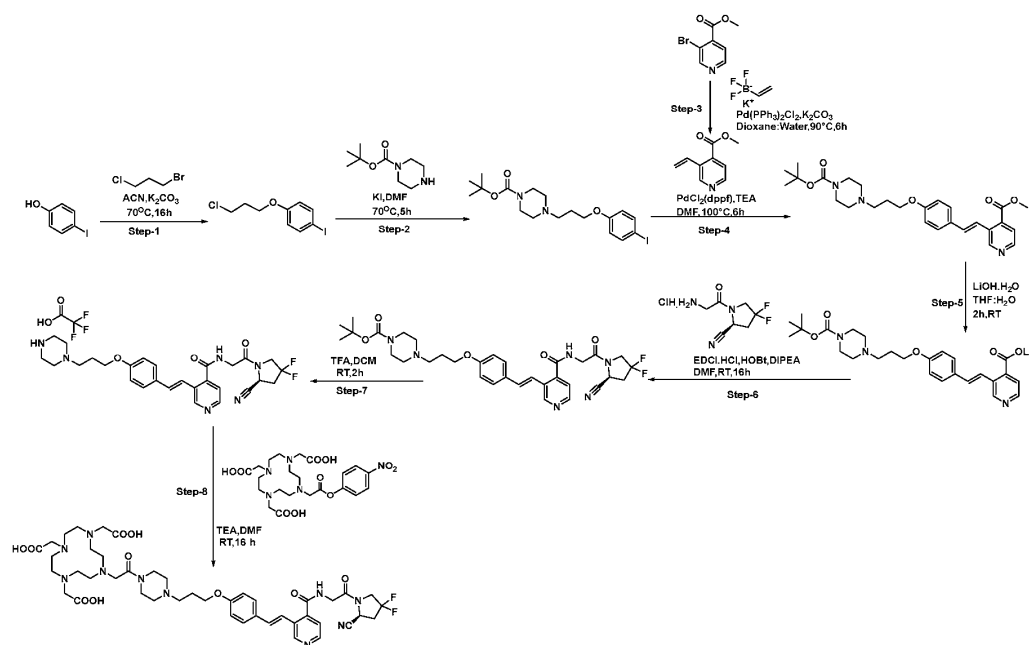
**[0185]** To a stirred solution of (S)-tert-butyl 4-(3-(4-(4-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)piperazine-1-carboxylate (0.065 g, 0.106 mmol, 1.0 equiv) in DCM (0.5 mL) was added TFA (0.2 mL) drop wise at 0°C. The Reaction mixture was allowed to stir at RT for 2h. The product formation was confirmed by TLC and LCMS. After completion of the reaction, the reaction mixture was concentrated under reduced pressure and crystallized in diethyl ether to obtain (S)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-3-(4-(3-(piperazin-1-yl)propoxy)phenyl)isonicotinamide 2,2,2-trifluoroacetate (0.060 g, 92 % Yield) as a yellow solid. **LCMS: 513 [M+1]<sup>+</sup> <sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.93 (t, *J*=5.72 Hz, 1 H) 8.79 (br. s., 1 H) 8.57 - 8.67 (m, 2 H) 7.37 - 7.51 (m, 3 H) 6.96 (d, *J*=8.58 Hz, 2 H) 5.10 (dd, *J*=9.06, 2.38 Hz, 1 H) 4.21 (t, *J*=11.44 Hz, 2 H) 3.94 - 4.11 (m, 4 H) 3.17 (d, *J*=5.25 Hz, 2 H) 2.75 - 2.97 (m, 2 H) 2.61 - 2.75 (m, 2 H) 2.44 (t, *J*=7.39 Hz, 2 H) 2.33 (br. s., 4 H) 1.81 - 1.94 (m, 2 H).

**Step-7: Synthesis of (S)-2,2',2''-(10-(2-(4-(3-(4-(4-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)piperazin-1-yl)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid**

**[0186]** To a stirred solution of (S)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-3-(4-(3-(piperazin-1-yl)propoxy)phenyl)isonicotinamide 2,2,2-trifluoroacetate (0.060 g, 0.096 mmol, 1.0 equiv) in DMF (0.3 mL) was added 2,2',2''-(10-(2-(4-(nitrophenoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.077 g, 0.147 mmol, 1.5 equiv) and TEA (0.08 mL, 0.588 mmol, 6.0 equiv). The Reaction mixture was allowed to stir at room temperature for 16 h. The product formation was confirmed by LCMS. After completion of the reaction, the mixture was concentrated under reduced pressure, crystallized in diethyl ether and further purified by reversed phase HPLC to obtain (S)-2,2',2''-(10-(2-(4-(3-(4-(4-(2-(2-cyano-4,4-

difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)piperazin-1-yl)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.050 g, 56 % Yield) as an off-white solid. **LCMS: 899 [M+1]<sup>+</sup>** <sup>1</sup>H NMR (400 MHz, DEUTERIUM OXIDE)  $\delta$  8.95 (s, 1 H) 8.86 (d,  $J=5.72$  Hz, 1 H) 8.14 (d,  $J=5.72$  Hz, 1 H) 7.53 (d,  $J=9.06$  Hz, 2 H) 7.15 (d,  $J=8.58$  Hz, 2 H) 5.11 (dd,  $J=8.11, 4.29$  Hz, 1 H) 4.61 (br. s., 2 H) 4.34 (d,  $J=6.68$  Hz, 2 H) 3.98 - 4.28 (m, 7 H) 3.85 (br. s., 5 H) 3.72 (br. s., 4 H) 3.38 - 3.62 (m, 11 H) 3.35 (br. s., 3 H) 3.16 (br. s., 8 H) 2.84 - 3.09 (m, 2 H) 2.32 (br. s., 2 H).

## Compound 2



### Step-1: Synthesis of 1-(3-chloropropoxy)-4-iodobenzene

**[0187]** To a stirred solution of 4-iodophenol (1 g, 4.545 mmol, 1.0 equiv) in ACN (10 mL) was added  $K_2CO_3$  (1.56 g, 11.36 mmol, 2.5 equiv), followed by the addition of 1-bromo-3-chloropropane (1.4 g, 9.090 mmol, 2.0 equiv). The Reaction mixture was allowed to stir at 70°C for 16 h. The progress of the reaction was monitored by TLC and LCMS. After completion of the reaction, the reaction mixture was diluted with ethyl acetate and passed through Celite®. The filtrate was concentrated and purified by flash chromatography (5 to 30 % ethyl acetate in hexane

as an eluent) to obtain 1-(3-chloropropoxy)-4-iodobenzene (1.33 g, 99 % Yield) as an off white semisolid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.48 - 7.66 (m, 2 H) 6.70 - 6.88 (m, 2 H) 4.06 (t, *J*=6.20 Hz, 2 H) 3.70 - 3.83 (m, 2 H) 2.05 - 2.22 (m, 2 H).

#### Step-2: Synthesis of tert-butyl 4-(3-(4-iodophenoxy)propyl)piperazine-1-carboxylate

[0188] To a stirred solution of 1-(3-chloropropoxy)-4-iodobenzene (0.500 g, 1.689 mmol, 1.0 equiv) in DMF (5 mL) was added KI (0.140 g, 0.844mmol, 0.5 equiv), followed by the addition of tert-butyl piperazine-1-carboxylate (1.57 g, 8.448 mmol, 5.0equiv). The reaction mixture was stirred at 70 °C for 5 h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the reaction mixture was quenched with ice-cold water (10 mL) and extracted with ethyl acetate (10 ml × 2). Combined organic layers were separated, washed with cold water (2 × 5 ml), brine (5 ml), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography (0 to 40 % ethyl acetate in hexane as an eluent) to obtain tert-butyl 4-(3-(4-iodophenoxy)propyl)piperazine-1-carboxylate (0.670 g, 88 % Yield) as an off white solid. LCMS: 447 [M+1]<sup>+</sup> <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.57 (d, *J*=8.58 Hz, 2 H) 6.78 (d, *J*=9.06 Hz, 2 H) 3.94 - 4.03 (m, 2 H) 3.24 - 3.31 (m, 4 H) 2.40 (t, *J*=6.91 Hz, 2 H) 2.24 - 2.35 (m, 4 H) 1.85 (t, *J*=6.91 Hz, 2 H) 1.39 (s, 9 H).

#### Step-3: Synthesis of methyl 3-vinylisonicotinate

[0189] To a stirred solution of methyl 6-bromoquinoline-4-carboxylate (0.600 g, 2.777 mmol, 1.0 equiv) in Dioxane:Water (6 : 0.6 mL) was added K<sub>2</sub>CO<sub>3</sub> (0.766 g, 5.554 mmol, 2.0 equiv), followed by the addition of potassium trifluoro(vinyl)borate (0.446 g, 3.333 mmol, 1.2equiv). The reaction mixture was purged with N<sub>2</sub> for 5-10 minutes, followed by the addition of Bis(triphenylphosphine)palladium dichloride (0.097 g, 0.138 mmol, 0.05 equiv). The resulting reaction mixture was heated at 90 °C for 6h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the reaction mixture was diluted with ethyl acetate and passed through Celite®. The filtrate was concentrated under reduced pressure and the crude product was purified by flash chromatography (0- 20 % ethyl acetate in hexane as an eluent) to obtain methyl 3-vinylisonicotinate (0.300 g, 66 % Yield) as a yellow oil. LCMS: 164 [M+1]<sup>+</sup> <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.95 (s, 1 H) 8.64 (d, *J*=4.77 Hz, 1 H) 7.67 (d, *J*=4.77 Hz, 1 H) 7.15 - 7.27 (m, 1 H) 5.93 (d, *J*=17.64 Hz, 1 H) 5.49 (d, *J*=11.92 Hz, 1 H) 3.83 - 3.95 (m, 3 H).

**Step-4: Synthesis of tert-butyl (E)-4-(3-(4-(2-(4-(methoxycarbonyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazine-1-carboxylate**

**[0190]** To a stirred solution of tert-butyl 4-(3-(4-iodophenoxy)propyl)piperazine-1-carboxylate (0.500 g, 1.121 mmol, 1.0 equiv) in DMF (5 mL) was added methyl 3-vinylisonicotinate (0.219 g, 1.345 mmol, 1.2 equiv), followed by the addition of TEA (0.48 mL, 3.363 mmol, 3.0 equiv). The reaction mixture was purged with N<sub>2</sub> for 5-10 minutes, followed by the addition of Pd(dppf)Cl<sub>2</sub> (0.082 g, 0.112 mmol, 0.1 equiv). The resulting reaction mixture was heated at 100 °C for 6 h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (30 mL) and passed through celite®. The filtrate was washed with water (2 × 20 mL), brine (10 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography (0-5 % MeOH in DCM as an eluent) to obtain tert-butyl (E)-4-(3-(4-(2-(4-(methoxycarbonyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazine-1-carboxylate (0.200 g, 37 % Yield) as light brown semisolid. **LCMS: 482 [M+1]<sup>+</sup>** **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>) δ 9.10 (s, 1 H) 8.58 (d, *J*=4.77 Hz, 1 H) 7.67 (d, *J*=4.77 Hz, 1 H) 7.45 - 7.62 (m, 3 H) 7.22 - 7.33 (m, 1 H) 6.98 (d, *J*=8.58 Hz, 2 H) 3.99 - 4.10 (m, 4 H) 3.91 (s, 3 H) 2.89 (s, 1 H) 2.73 (s, 1 H) 2.33 (br. s., 6 H) 1.84 - 1.92 (m, 2 H) 1.39 (s, 9 H).

**Step-5: Synthesis of lithium lithium (E)-3-(4-(3-(4-(tert-butoxycarbonyl)piperazin-1-yl)propoxy)styryl)isonicotinate**

**[0191]** To a stirred solution of tert-butyl (E)-4-(3-(4-(2-(4-(methoxycarbonyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazine-1-carboxylate (0.320 g, 0.665 mmol, 1.0 equiv) in THF (3 mL) was added LiOH.H<sub>2</sub>O (0.030 g, 0.731 mmol, 1.1 equiv) in water (0.7 mL). The reaction mixture was allowed to stir at RT for 2 h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the solvent was removed under reduced pressure and freeze dried to obtain lithium (E)-3-(4-(3-(4-(tert-butoxycarbonyl)piperazin-1-yl)propoxy)styryl)isonicotinate (0.250 g, 79 % Yield) as an off white solid. **LCMS: 468 [M+1]<sup>+</sup>** **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>) δ 9.10 (s, 1 H) 8.94 - 9.03 (m, 1 H) 8.48 (d, *J*=4.77 Hz, 1 H) 7.62 (d, *J*=8.58 Hz, 2 H) 7.48 (s, 1 H) 7.29 - 7.37 (m, 1 H) 6.91 - 7.00 (m, 2 H) 5.10 - 5.22 (m, 1 H) 4.14 - 4.22 (m, 2 H) 4.02 (t, *J*=6.20 Hz, 3 H) 2.78 - 2.99 (m, 2 H) 2.32 (br. s., 6 H) 1.83 - 1.94 (m, 2 H) 1.39 (s, 9 H).

**Step-6: Synthesis of tert-butyl (S,E)-4-(3-(4-(2-(4-((2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazine-1-carboxylate**

**[0192]** To a stirred solution of lithium (E)-3-(4-(3-(4-(tert-butoxycarbonyl)piperazin-1-yl)propoxy)styryl)isonicotinate (0.250 g, 0.535 mmol, 1.0 equiv) in DMF (2.5 mL) was added EDC.HCl (0.132 g, 0.695 mmol, 1.3 equiv), HOBT (0.093 g, 0.695 mmol, 1.3 equiv) and (S)-1-(2-aminoacetyl)-4,4-difluoropyrrolidine-2-carbonitrile hydrochloride (0.144 g, 0.642 mmol, 1.2 equiv) followed by the addition of DIPEA (0.29 mL, 1.605 mmol, 3.0 equiv). The resulting reaction mixture was allowed to stir at RT for 16h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the reaction mixture was quenched with water (10 mL) and extracted with ethyl acetate (10 mL × 3). The combined organic layers were washed with water (10 mL × 2), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by Flash chromatography (0-5 % MeOH in DCM as an eluent) to obtain tert-butyl (S,E)-4-(3-(4-(2-(4-((2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazine-1-carboxylate (0.090 g, 26 % Yield) as an off white solid. **LCMS: 639 [M+1]<sup>+</sup> <sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>) δ 9.10 (s, 1 H) 8.94 - 9.03 (m, 1 H) 8.48 (d, *J*=4.77 Hz, 1 H) 7.62 (d, *J*=8.58 Hz, 2 H) 7.48 (s, 1 H) 7.29 - 7.37 (m, 1 H) 6.91 - 7.00 (m, 2 H) 5.10 - 5.22 (m, 1 H) 4.14 - 4.22 (m, 2 H) 4.02 (t, *J*=6.20 Hz, 3 H) 3.32 (s, 5 H) 2.78 - 2.99 (m, 4 H) 2.32 (br. s., 4 H) 1.83 - 1.94 (m, 2 H) 1.39 (s, 9 H).

**Step-7: Synthesis of (S,E)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-3-(4-(3-(piperazin-1-yl)propoxy)styryl)isonicotinamide 2,2,2-trifluoroacetate**

**[0193]** To a stirred solution of tert-butyl (S,E)-4-(3-(4-(2-(4-((2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazine-1-carboxylate (0.090 g, 0.141 mmol, 1.0 equiv) in DCM (1 mL) was added TFA (0.2 mL) drop wise at 0°C. The reaction mixture was allowed to stir at RT for 2h. The product formation was confirmed by TLC and LCMS. After completion of the reaction, the reaction mixture was concentrated under reduced pressure and crystallized in diethyl ether to obtain (S,E)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-3-(4-(3-(piperazin-1-yl)propoxy)styryl)isonicotinamide 2,2,2-trifluoroacetate (0.068 g, 76 % Yield) as yellow solid. **LCMS: 539 [M+1]<sup>+</sup> <sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>) δ 9.10 (s, 1 H) 8.94 - 9.03 (m, 1 H) 8.48 (d, *J*=4.77 Hz, 1 H) 7.62 (d, *J*=8.58 Hz, 2 H) 7.48 (s, 2 H) 7.29 - 7.37 (m, 1 H) 6.91 - 7.00 (m, 2 H)

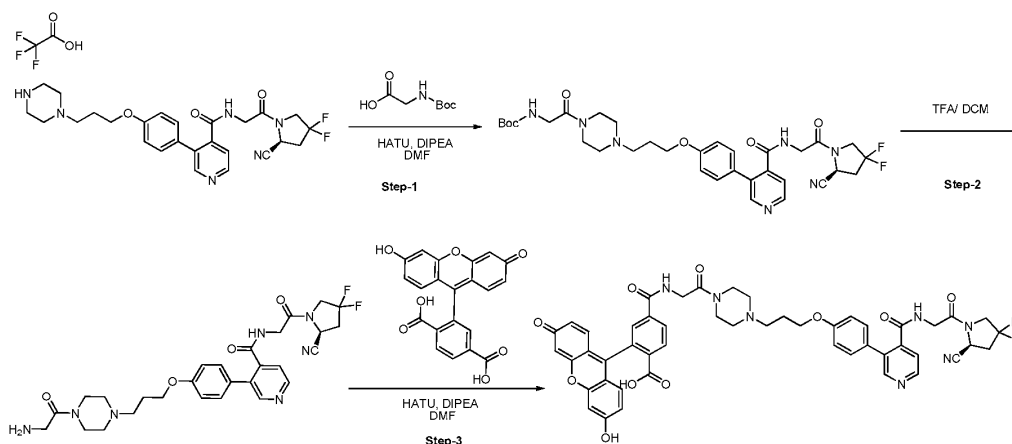
5.10 - 5.22 (m, 1 H) 4.14 - 4.22 (m, 2 H) 4.02 (t,  $J=6.20$  Hz, 3 H) 3.32 (s, 5 H) 2.78 - 2.99 (m, 4 H) 2.32 (br. s., 4 H) 1.83 - 1.94 (m, 2 H).

**Step-8: Synthesis of (S,E)-2,2',2''-(10-(2-(4-(3-(4-(2-(4-((2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazin-1-yl)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid**

**[0194]** To a stirred solution of (S,E)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-3-(4-(3-(piperazin-1-yl)propoxy)styryl)isonicotinamide 2,2,2-trifluoroacetate (0.070 g, 0.110 mmol, 1.0 equiv) in DMF (0.4 mL) was added 2,2',2''-(10-(2-(4-(3-(4-(2-(4-((2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazin-1-yl)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.086 g, 0.165 mmol, 1.5 equiv) and TEA (0.09 mL, 0.661 mmol, 6.0 equiv). The Reaction mixture was allowed to stir at room temperature for 16 h. The product formation was confirmed by LCMS. After completion of the reaction, the mixture was concentrated under reduced pressure, crystallized in diethyl ether and further purified by reversed phase HPLC to obtain (S,E)-2,2',2''-(10-(2-(4-(3-(4-(2-(4-((2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazin-1-yl)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.007 g, 7 % Yield) as an off-white solid. **LCMS: 925 [M+1]<sup>+</sup> <sup>1</sup>H NMR** (400 MHz, DEUTERIUM OXIDE)  $\delta$  9.12 - 9.25 (m, 1 H) 8.71 (d,  $J=5.72$  Hz, 1 H) 8.07 (d,  $J=6.20$  Hz, 1 H) 7.66 - 7.80 (m, 2 H) 7.32 - 7.56 (m, 2 H) 7.08 (d,  $J=9.06$  Hz, 2 H) 5.18 (dd,  $J=8.58, 4.29$  Hz, 1 H) 4.59 (d,  $J=11.44$  Hz, 2 H) 4.29 - 4.45 (m, 5 H) 4.12 - 4.29 (m, 7 H) 3.94 - 4.12 (m, 4 H) 3.85 (br. s., 9 H) 3.62 - 3.77 (m, 8 H) 3.55 (br. s., 4 H) 3.37 - 3.51 (m, 2 H) 3.35 (br. s., 2 H) 3.08 - 3.27 (m, 3 H) 2.81 - 3.08 (m, 1 H) 2.25 - 2.35 (m, 2 H).

**Compound 3**

Synthesis of (S)-4-(2-(4-(3-(4-(4-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)piperazin-1-yl)-2-oxoethylcarbamoyl)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid



**[0195]** Step1: Preparation of (S)-tert-butyl 2-(4-(3-(4-(4-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)piperazin-1-yl)-2-oxoethylcarbamate

**[0196]** To a stirred solution of 2-(tert-butoxycarbonylamino)acetic acid (0.140 g, 0.8 mmol, 1.2 eq) in DMF (8mL) was added HATU (0.38 g, 1.0 mmol, 1.5 eq) at 0 °C and the mixture was stirred at same temperature for 15 min. DIPEA (0.34 mL, 2.0 mmol, 3.0 eq) and (S)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-3-(4-(3-(piperazin-1-yl)propoxy)phenyl) isonicotinamide 2,2,2-trifluoroacetate (0.34 g, 0.66 mmol, 1.0 eq) were the added to the mixture and the mixture was stirred at RT for 1.5 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was quenched with water (10 mL) and extracted with ethyl acetate (20 mL × 3). The combined organic layers were washed with water (20 mL × 3), dried over anhydrous sodium sulfate and concentrated under reduced pressure to obtain a crude residue which was purified by CombiFlash Chromatography to afford the title compound (0.23 g , 63.30 % ).

**[0197]** LCMS: 670 [M+1]<sup>+</sup>

**[0198]** Step-2: Preparation of (S)-3-(4-(3-(4-(2-aminoacetyl)piperazin-1-yl)propoxy)phenyl)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide

**[0199]** To a stirred solution (S)-tert-butyl (2-(4-(3-(4-(4-((2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)piperazin-1-yl)-2-oxoethyl)carbamate (0.23 g, 0.333 mmol, 1.0 eq) in DCM (4 mL) was added TFA (1.1 mL) drop wise at 0 °C and the mixture was allowed to stir at RT for 1 h. The product formation was confirmed by TLC and

LCMS. After completion, the mixture was concentrated under reduced pressure and triturated in diethyl ether (10 mL x2) to obtain the title compound (0.20 g, 85.18 %).

[0200] LCMS: 570 [M+1]<sup>+</sup>

[0201] Step-3: Preparation of (S)-4-(2-(4-(3-(4-(4-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)piperazin-1-yl)-2-oxoethylcarbamoyl)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid

[0202] To a stirred solution of 3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-6-carboxylic acid (0.132 g, 0.351 mmol, 1.0 eq) in DMF (2 mL) was added HATU (0.167 g, 0.439 mmol, 1.5 eq), at 0 °C and the mixture was stirred at same temperature for 15 min, DIPEA (0.2 mL, 1.17 mmol, 4.0 eq) and (S)-3-(4-(3-(4-(2-aminoacetyl)piperazin-1-yl)propoxy)phenyl)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide 2,2,2-trifluoroacetate (0.200 g, 0.297 mmol, 1.0 eq) were added to the mixture and the mixture was stirred at RT for 16 h. The progress of the reaction was monitored by TLC and LC-MS. After completion, the mixture was concentrated under vacuum to remove solvent, residue was triturated with ether (10x3 mL), concentrated under reduced pressure to obtain a crude residue was purified by reversed phase HPLC to obtain the title compound (0.004 g, 1.48 %)

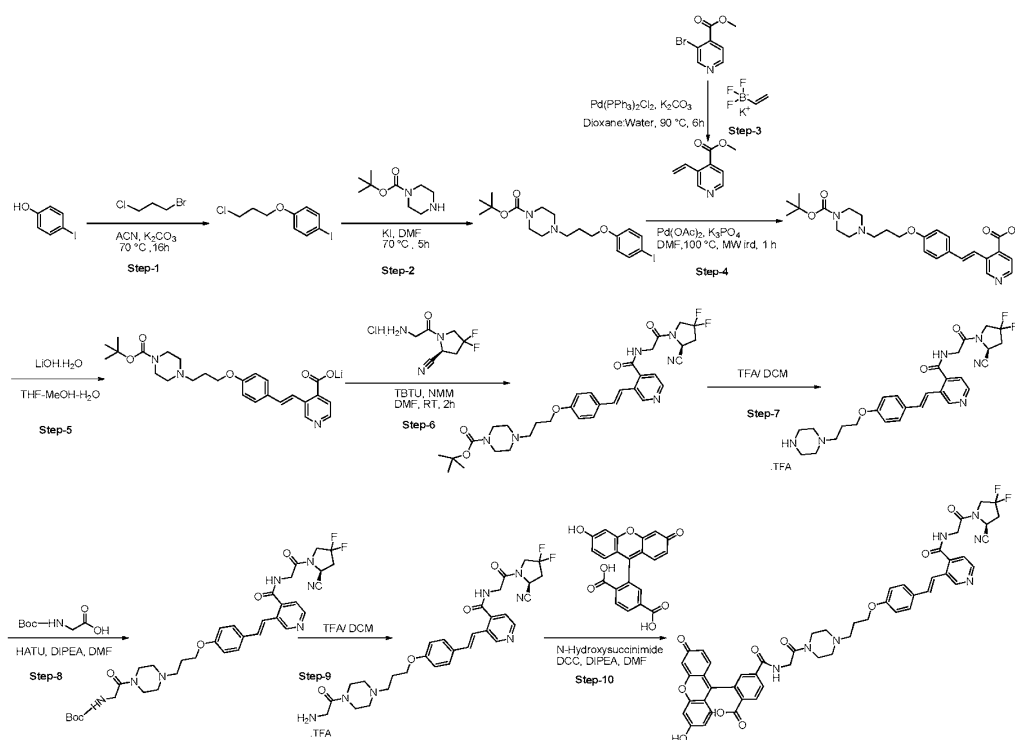
[0203] LCMS: 928 [M+1]<sup>+</sup>

[0204] <sup>1</sup>H NMR: (400 MHz, DMSO-d<sub>6</sub>) δ 10.28-10.16 (m, 1H), 8.93 (t, *J* = 6.0 Hz, 1H), 8.87-8.80 (m, 1H), 8.65-8.58 (m, 2H), 8.16 (s, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.71 (s, 1H), 7.50-7.44 (m, 2H), 7.40 (d, *J* = 5.2 Hz, 1H), 6.99-6.90 (m, *J* = 9.1 Hz, 2H), 6.69 (d, *J* = 2.4 Hz, 2H), 6.64-6.50 (m, 4H), 5.11-5.08 (m, 1H), 4.10-3.95 (m, 8H), 3.48-3.39 (m, 6H), 2.75-2.85 (m, 2H), 2.40 (d, *J* = 14.3 Hz, 2H), 2.35-2.28 (m, 2H), 1.93-1.82 (m, 2H)

#### Compound 4

Synthesis of (S,E)-4-(2-(4-(3-(4-(2-(4-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazin-1-yl)-2-oxoethylcarbamoyl)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid





[0205] Step-1: Preparation of 1-(3-chloropropoxy)-4-iodobenzene

[0206] To a stirred solution of 4-iodophenol (1 g, 4.545 mmol, 1.0 eq) in acetonitrile (10 mL) was added  $K_2CO_3$  (1.56 g, 11.36 mmol, 2.5 eq) followed by the addition of 1-bromo-3-chloropropane (1.4 g, 9.090 mmol, 2.0 eq) and the mixture was allowed to stir at 70 °C for 16 h. The progress of the reaction was monitored by TLC and  $^1H$ -NMR. After completion, the mixture was diluted with ethyl acetate and passed through Celite®. The filtrate was concentrated and purified by CombiFlash Chromatography to obtain the title compound (1.33 g, 99 %) as an off white semisolid.

[0207]  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  7.48-7.66 (m, 2H), 6.70-6.88 (m, 2H), 4.06 (t,  $J$  = 6.20 Hz, 2H), 3.70-3.83 (m, 2H), 2.05-2.22 (m, 2H)

[0208] Step-2: Preparation of tert-butyl 4-(3-(4-iodophenoxy)propyl)piperazine-1-carboxylate

[0209] To a stirred solution of 1-(3-chloropropoxy)-4-iodobenzene (0.50 g, 1.69 mmol, 1.0 eq) in DMF (5 mL) was added KI (0.14 g, 0.84 mmol, 0.5 eq) followed by the addition of tert-butyl piperazine-1-carboxylate (1.57 g, 8.44 mmol, 5.0eq) and the mixture was heated 70 °C for

5 h. Product formation was confirmed by TLC and LCMS. After completion, the mixture was quenched with ice-cold water (10 mL) and extracted with ethyl acetate (10 mL × 2). The combined organic layers were separated, washed with cold water (2 × 5 mL), brine (5 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue obtained was purified by CombiFlash Chromatography to afford the title compound (0.67 g, 88 %) as an off white solid.

[0210] LCMS: 447 [M+1]<sup>+</sup>

[0211] <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.57 (d, *J*=8.58 Hz, 2H), 6.78 (d, *J*=9.06 Hz, 2H), 3.94-4.03 (m, 2H), 3.24-3.31 (m, 4H), 2.40 (t, *J*=6.91 Hz, 2H), 2.24-2.35 (m, 4H), 1.85 (t, *J*=6.91 Hz, 2H), 1.39 (s, 9H)

[0212] Step-3: Preparation of methyl 3-vinylisonicotinate

[0213] To a stirred solution of methyl 6-bromoquinoline-4-carboxylate (2.0 g, 9.25 mmol, 1.0 eq) in 1,4-Dioxane:Water (12 mL : 2 mL) was added K<sub>2</sub>CO<sub>3</sub> (2.55 g, 18.5 mmol, 2.0 eq) followed by the addition of potassium trifluoro(vinyl)borate (1.47 g, 11.1 mmol, 1.2eq) and the mixture was degassed under N<sub>2</sub> for 5-10 min. Bis(triphenylphosphine)palladium dichloride (0.32 g, 0.46 mmol, 0.05 eq) was then added to the mixture and the mixture was further degassed under nitrogen for 5 min. The resultant reaction mixture was heated at 90 °C for 6 h. Product formation was confirmed by TLC and LCMS. After completion, the mixture was diluted with ethyl acetate and passed through Celite®. The filtrate was concentrated under reduced pressure and the crude product was purified by CombiFlash Chromatography to obtain the title compound (1.7 g, 89 %)

[0214] LCMS: 164 [M+1]<sup>+</sup>

[0215] Step-4: Preparation of (E)-tert-butyl 4-(3-(4-(2-(4-(methoxycarbonyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazine-1-carboxylate

[0216] To a stirred solution of tert-butyl 4-(3-(4-iodophenoxy)propyl)piperazine-1-carboxylate (1.3 g, 2.90 mmol, 1.0 eq) in DMF (5mL) was added methyl 3-vinylisonicotinate (0.48 g, 2.9 mmol, 1.0 eq) followed by the addition of K<sub>3</sub>PO<sub>4</sub> (1.5 g, 7.27 mmol, 2.5 eq) and the mixture was degassed under N<sub>2</sub> for 5-10 min. Pd(OAc)<sub>2</sub> (0.045 g, 0.20 mmol, 0.07 eq) was then added to the mixture and the mixture was further degassed under nitrogen for 5 min. The resultant mixture was irradiated under MW radiation at 100 °C for 1 h. Product formation was confirmed by TLC

and LC-MS. After completion, the mixture was diluted with ethyl acetate (30 mL) and passed through celite®. The filtrate was washed with water (2 × 20 mL), brine (10mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by CombiFlash Chromatography to obtain the title compound (0.620 g, 44 %) as light brown semisolid.

[0217] LCMS: 482 [M+1]<sup>+</sup>

[0218] Step-5: Preparation of lithium (E)-3-(4-(3-(4-(tert-butoxycarbonyl)piperazin-1-yl)propoxy)styryl)isonicotinate

[0219] To a stirred solution of tert-butyl (E)-4-(3-(4-(2-(4-(methoxycarbonyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazine-1-carboxylate (0.62 g, 1.288 mmol, 1.0 eq) in THF (3 mL) was added LiOH.H<sub>2</sub>O (0.162 g, 3.866 mmol, 3 eq) in water and MeOH (9.0 : 3.0 mL) and the mixture was allowed to stir at RT for 2h. Product formation was confirmed by TLC and LCMS. After completion, the mixture was concentrated under reduced pressure and dried to obtain the title compound (0.550 g, 91 %) as an off white solid.

[0220] LCMS: 474 [M+1]<sup>+</sup>

[0221] Step-6: Preparation of (S,E)-tert-butyl 4-(3-(4-(2-(4-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazine-1-carboxylate

[0222] To a stirred solution of lithium (E)-3-(4-(3-(4-(tert-butoxycarbonyl)piperazin-1-yl)propoxy)styryl)isonicotinate (0.55 g, 1.18 mmol, 1.0 eq) in DMF (12 mL) was added TBTU (0.567 g, 1.76 mmol, 1.5 eq) at 0 °C and the mixture was allowed to stir for 30 min at the same temperature. NMM (0.38 mL, 3.53 mmol, 3.0 eq) and (S)-1-(2-aminoacetyl)-4,4-difluoropyrrolidine-2-carbonitrile hydrochloride (0.318 g, 1.413 mmol, 1.2 eq) was then added to the mixture and the resultant mixture was stirred at RT for 2 h. Product formation was confirmed by TLC and LCMS. After completion, the mixture was quenched with water (10 mL) and extracted with ethyl acetate (10 mL × 3). The combined organic layers was washed with water (10 mL × 2), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by CombiFlash Chromatography to afford the title compound (0.270 g, 36 %).

[0223] LCMS: 639 [M+1]<sup>+</sup>

[0224] Step-7: Preparation of (S,E)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-3-(4-(3-(piperazin-1-yl)propoxy)styryl)isonicotinamide

[0225] To a stirred solution of tert-butyl (S,E)-4-(3-(4-(2-(4-((2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazine-1-carboxylate (0.27 g, 0.42 mmol, 1.0 eq) in DCM (10 mL) was added TFA (1.0 mL) drop wise at 0 °C and the mixture was allowed to stir at RT for 2 h. The product formation was confirmed by TLC and LCMS. After completion, the mixture was concentrated under reduced pressure and triturated in diethyl ether (10 mL × 2) to afford the title compound (0.280 g, 81 %).

[0226] LCMS: 539 [M+1]<sup>+</sup>

[0227] Step-8: Preparation of (S,E)-tert-butyl 2-(4-(3-(4-(2-(4-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazin-1-yl)-2-oxoethylcarbamate

[0228] To a stirred solution of 2-(tert-butoxycarbonylamino)acetic acid (0.09 g, 0.52 mmol, 1.2 eq) in DMF (10 mL) was added HATU (0.25 g, 0.64 mmol, 1.5 eq) at 0 °C and the mixture was allowed to stir at same temperature for 30 min. DIPEA (0.29 mL, 1.71 mmol, 4.0 eq) and (S,E)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-3-(4-(3-(piperazin-1-yl)propoxy)styryl)isonicotinamide (0.28 g, 0.43 mmol, 1.0 eq) were the added to the mixture and the resultant was stirred at RT for 1 h. Product formation was confirmed by TLC and LCMS. After completion, the mixture was quenched with water (10 mL) and extracted with ethyl acetate (10 mL × 3). The combined organic layers was washed with ice-cold water (10 mL × 2), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by CombiFlash Chromatography to afford the title compound (0.25 g, 69%).

[0229] LCMS: 696 [M+1]<sup>+</sup>

[0230] Step-9: Preparation of (S,E)-3-(4-(3-(4-(2-aminoacetyl)piperazin-1-yl)propoxy)styryl)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide

[0231] To a stirred solution of (S,E)-tert-butyl 2-(4-(3-(4-(2-(4-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazin-1-yl)-2-oxoethylcarbamate (0.250 g, 0.359 mmol, 1.0 eq) in DCM (10 mL) was added TFA (1.0 mL) drop wise at 0 °C and the mixture was allowed to stir at RT for 1h. The product formation was

confirmed by TLC and LCMS. After completion, the mixture was concentrated under reduced pressure and triturated in diethyl ether (10 mL ×2) to afford the title compound (0.20 g, 93 %).

[0232] LCMS: 596 [M+1]<sup>+</sup>

[0233] Step-10: Preparation of (S,E)-4-(2-(4-(3-(4-(2-(4-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)vinyl)phenoxy)propyl)piperazin-1-yl)-2-oxoethyl carbamoyl)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid

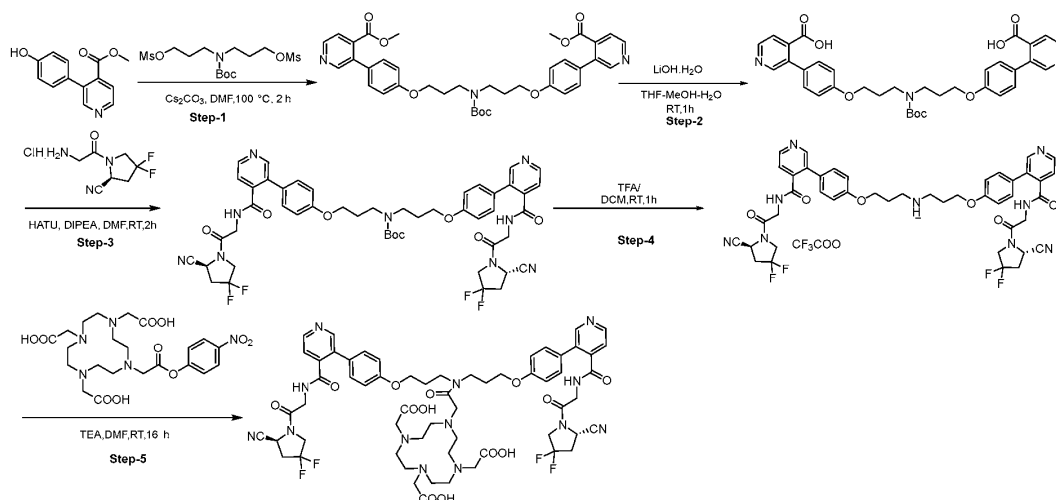
[0234] To a stirred solution of 2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)terephthalic acid (0.09 g, 0.24 mmol, 1.0 eq) in DMF (1 mL) was added NHS (0.055 g, 0.48 mmol, 2.0 eq) and DCC (0.1 g, 0.47 mmol, 2.0 eq) at 0 °C and the mixture was stirred at same temperature for 4 h. DIPEA (0.1 mL, 0.96 mmol, 4.0 eq) and (S,E)-3-(4-(3-(4-(2-aminoacetyl)piperazin-1-yl)propoxy)styryl)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide (0.17 g, 0.28 mmol, 1.2 eq) were then added to the mixture and the mixture was stirred at RT for 16 h. The progress of the reaction was monitored by LCMS. After completion, the mixture was concentrated under reduced pressure and triturated with diethyl ether to obtain a solid which was purified by Reversed Phase HPLC to afford the title compound (0.007 g, 6.4 %).

[0235] LCMS: 954 [M+1]<sup>+</sup>

[0236] <sup>1</sup>H NMR: (400 MHz, DMSO-d<sub>6</sub>) δ 10.15 (s, 1H), 9.10 (s, 1H), 8.96 (br. s., 1H), 8.84 (br. s., 1H), 8.48 (d, *J* = 5.1 Hz, 1H), 8.23-8.14 (m, 1H), 8.09 (d, *J* = 7.6 Hz, 1H), 7.70 (s, 1H), 7.62 (d, *J* = 8.9 Hz, 2H), 7.48 (s, 1H), 7.42 (s, 1H), 7.33 (d, *J* = 4.5 Hz, 1H), 6.95 (d, *J* = 8.3 Hz, 2H), 6.69 (s, 2H), 6.65-6.51 (m, 4H), 5.17 (d, *J* = 7.6 Hz, 1H), 4.29 (d, *J* = 13.4 Hz, 1H), 4.18 (d, *J* = 5.7 Hz, 3H), 4.09-3.94 (m, 5H), 3.44 (br. s., 5H), 2.93 (br. s., 1H), 2.90-2.79 (m, 2H), 2.38 (br. s., 2H), 1.94-1.81 (m, 3H)

### Compound 161

Synthesis of 2,2',2''-(10-(2-((3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid



**[0237]** Step-1: Synthesis of dimethyl 3,3'-((((tert-butoxycarbonyl)azanediyl)bis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene)diisonicotinate

**[0238]** To a stirred solution of methyl 3-(4-hydroxyphenyl)isonicotinate (0.5 g, 2.1 mmol, 1.0 eq) in DMF (10 mL) was added  $\text{Cs}_2\text{CO}_3$  (2.1 g, 6.55 mmol, 3 eq) at RT and the mixture was stirred at same temperature for 30 min. ((tert-butoxycarbonyl)azanediyl)bis(propane-3,1-diyl)dimethanesulfonate (0.638 g, 1.63 mmol, 0.75 eq) was then added to the mixture and the resultant mixture was heated at 70 °C for 2.0 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was diluted with water (20 mL) to obtain a precipitate which was filtered under vacuum to obtain a crude residue which was purified by CombiFlash Chromatography to afford dimethyl 3,3'-((((tert-butoxycarbonyl)azanediyl)bis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene)diisonicotinate (0.500 g, 35 %)

**[0239]** LCMS: 656  $[\text{M}+\text{H}]^+$

**[0240]** Step-2: Synthesis of 3,3'-((((tert-butoxycarbonyl)azanediyl)bis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene)diisonicotinic acid

**[0241]** To a stirred solution of dimethyl 3,3'-((((tert-butoxycarbonyl)azanediyl)bis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene)diisonicotinate (1.00 g, 1.52 mmol, 1.0 eq) in THF (12 mL) was added  $\text{LiOH}\cdot\text{H}_2\text{O}$  (0.639 g, 15.2 mmol, 10 eq) in water (2 mL) and methanol (6 mL) and the mixture was stirred at RT for 1 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was acidified using 2N-HCl (pH~1-2) to obtain a precipitate which

was filtered over vacuum to afford 3,3'-((((tert-butoxycarbonyl)azanediyl) bis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene))diisonicotinic acid (0.40 g, 41.79 %).

**[0242] LCMS:** 628 [M+H]<sup>+</sup>

**[0243]** Step-3: Synthesis of tert-butyl (3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)carbamate

**[0244]** To a stirred solution of 3,3'-((((tert-butoxycarbonyl)azanediyl)bis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene))diisonicotinic acid (0.600 g, 0.956 mmol, 1.0 eq) in DMF (10 mL) was added HATU (1.45 g, 3.827 mmol, 4.0 eq) at 0 °C and the mixture was stirred at same temperature for 30 min. DIPEA (1.6 mL, 9.50 mmol, 10.0 eq) and (S)-1-(2-aminoacetyl)-4,4-difluoropyrrolidine-2-carbonitrile hydrochloride (0.646 g, 2.86 mmol, 3.0 eq) were then added to the mixture and the mixture was stirred at RT for 2 h. The progress of the reaction was monitored by TLC and LCMS. After completion, ice water (30 mL) was added to the mixture to obtain the precipitate which was filtered over vacuum to obtain a crude residue which was purified by CombiFlash Chromatography to afford tert-butyl (3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)carbamate (0.800 g, 86.29 %).

**[0245] LCMS:** 970 [M+H]<sup>+</sup>

**[0246]** Step-4: Synthesis of N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-3-(4-(3-(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)amino)propoxy)phenyl)isonicotinamide Trifluoroacetate salt

**[0247]** To a stirred solution of tert-butyl (3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)carbamate (0.10 g, 0.19 mmol, 1.0 eq) in DCM (10 mL) was added TFA (0.5 mL) drop wise at 0 °C and the mixture was stirred at RT for 1 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was concentrated under reduced pressure to obtain a crude residue which was triturated with diethyl to afford N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-

3-(4-(3-((3-(4-(4-((2-((S)-2-cyano-4,4-difluoro pyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)amino)propoxy) phenyl)isonicotinamide as trifluoroacetate salt(0.098 g, 97.02 %).

**[0248] LCMS:** 870 [M+H]<sup>+</sup>

**[0249]** Step-5: Synthesis of 2,2',2''-(10-(2-((3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid

**[0250]** To a stirred solution of N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-3-(4-(3-((3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)amino)propoxy)phenyl)isonicotinamide Trifluoroacetate salt (0.150g, 0.152 mmol, 1.0 eq) in DMF (0.05 mL) were added TEA (0.06 mL, 0.762 mmol, 5 eq) followed by the addition of 2,2',2''-(10-(2-(4-nitrophenoxy)-2-oxoethyl)-1,4,7,10-tetra azacyclododecane-1,4,7-triyl)triacetic acid (0.130g, 0.182 mmol, 1.2 eq) and the mixture was stirred at RT for 16 h. The progress of the reaction was monitored by LCMS. After completion, the mixture was concentrated under reduced pressure, triturated with diethyl ether and further purified by Reversed Phase HPLC to afford 2,2',2''-(10-(2-((3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.023 g, 12.00 %)

**[0251] LCMS:** 1256 [M+H]<sup>+</sup>

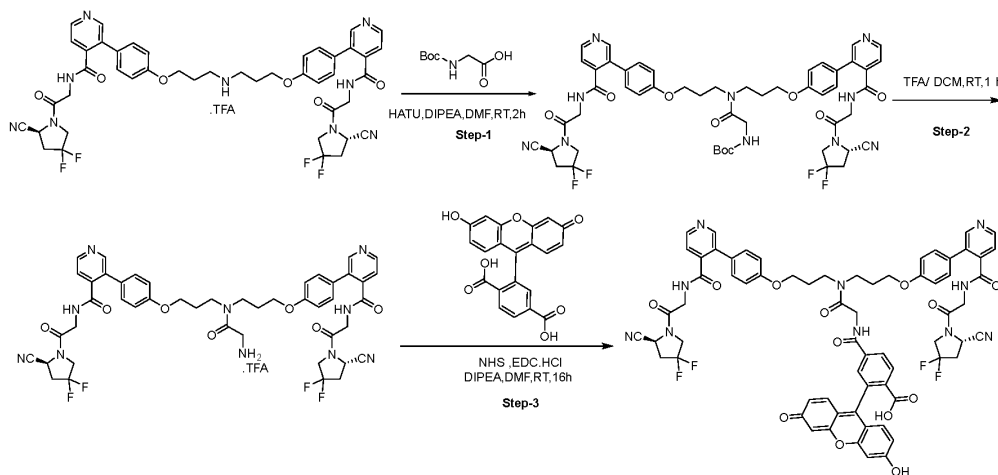
**[0252] <sup>1</sup>H NMR:** (400 MHz, DMSO-d<sub>6</sub>) δ 9.03-8.89 (m, 2H), 8.69-8.57 (m, 4H), 7.54-7.36 (m, 6H), 7.04-6.92 (m, 4H), 5.11 (dd, *J* = 3.2, 9.5 Hz, 2H), 4.29-4.14 (m, 3H), 4.13-3.94 (m, 12H), 3.94-3.82 (m, 2H), 3.59 (br. s., 1H), 3.51 (br. s., 10H), 3.00-2.85 (m, 12H), 2.85-2.76 (m, 4H), 2.12-2.03 (m, 2H), 2.00 (br. s., 2H)

#### Compound 162

Synthesis of 4-((2-((3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-((2-((S)-2-cyano-4,4-difluoro pyrrolidin-1-yl)-



2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)amino)-2-oxoethyl)carbamoyl)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid



**[0253]** Step1: Preparation of tert-butyl (2-((3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)amino)-2-oxoethyl)carbamate.

**[0254]** To a stirred solution of 2-(tert-butoxycarbonylamino)acetic acid (0.065 g, 0.366 mmol, 1.2 eq) in DMF (10 mL) was added HATU (0.174 g, 0.457 mmol, 1.5 eq) at 0 °C and the mixture was allowed to stir at same temperature for 30 min. DIPEA (0.15 mL, 0.915 mmol, 5.0 eq) and N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-3-(4-(3-((3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)amino)propoxy)phenyl)isonicotinamide trifluoroacetate salt (0.300 g, 0.305 mmol, 1.0 eq) were then successively added to the mixture and the mixture was allowed to stir at RT for 1 h. The product formation was confirmed by TLC and LCMS. After completion, the mixture was poured in ice cold water (10 mL). The resulting solid was filtered off and dried under vacuum to get the crude residue which was purified by Combi-flash chromatography to afford the tert-butyl (2-((3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)amino)-2-oxoethyl)carbamate (0.200 g, 52.49 %) as an off white solid.

[0255] LCMS: 1027 [M+1]<sup>+</sup>

[0256] Step-2: Synthesis of 3-(4-(3-(2-amino-N-(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)acetamido)propoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide Trifluoroacetate salt

[0257] To a stirred solution of tert-butyl (2-((3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)amino)-2-oxoethyl)carbamate (0.200 g, 0.215 mmol, 1.0 eq) in DCM (10 mL) was added TFA (0.5 mL) at 0 °C and allowed to stir at RT for 2 h. The product formation was confirmed by TLC and LCMS. After completion reaction mixture concentrated under reduced pressure and residue was triturated in diethyl ether to afford 3-(4-(3-(2-amino-N-(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)acetamido)propoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide as trifluoroacetate salt (0.180 g, 88.88 %).

[0258] LCMS: 927 [M+1]<sup>+</sup>

[0259] Step-3: Synthesis of 4-((2-((3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)amino)-2-oxoethyl)carbamoyl)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid

[0260] To a stirred solution of 2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)terephthalic acid (0.06 g, 0.159 mmol, 1.0 eq) in DMF (3 mL) were added NHS (0.038 g, 0.319 mmol, 2.0 eq) and EDC.HCl (0.062 g, 0.319 mmol, 2.0 eq) at 0 °C and the mixture allowed to stir at same temperature for 3 h. DIPEA (0.006 mL, 0.255 mmol, 4.0 eq) and 3-(4-(3-(2-amino-N-(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)acetamido)propoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide Trifluoroacetate salt (0.200 g, 0.191 mmol, 1.2 eq) were then successively added to the mixture and the resultant mixture was allowed to stir at RT for 16 h. The product formation was confirmed by LCMS. After completion, the mixture was acidified with 1N HCl (pH ~ 2). The resulting solid was filtered off and dried under vacuum. The crude product was purified by

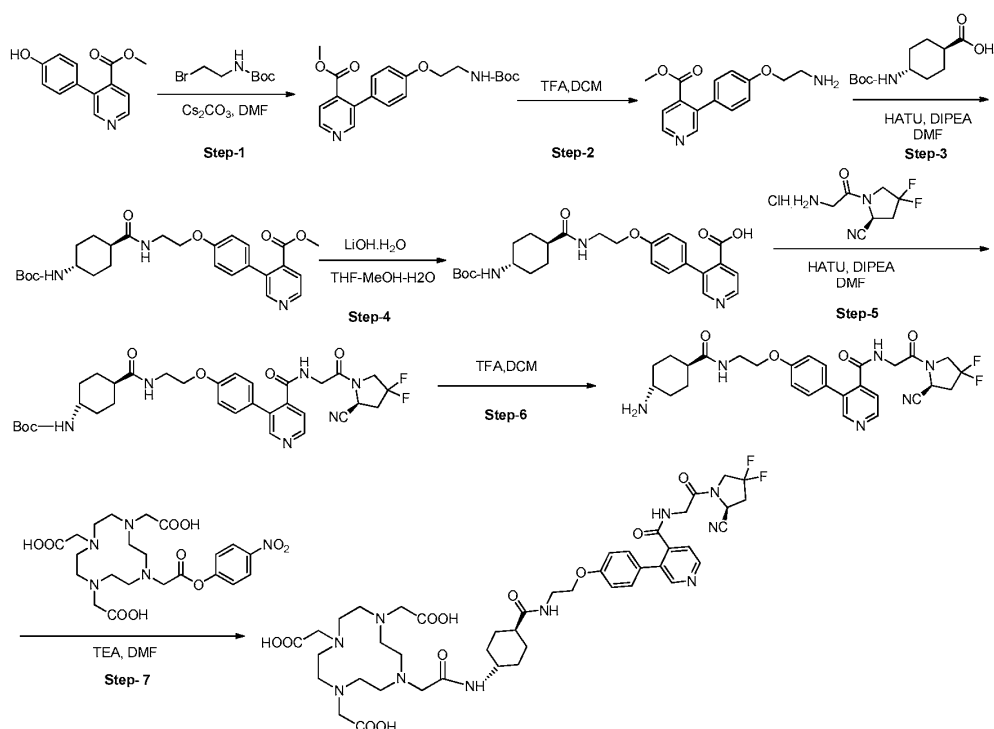
Reversed Phase HPLC to afford 4-((2-((3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)amino)-2-oxoethyl)carbamoyl)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid (0.018 g, 8.78 %).

[0261] LCMS: 1285 [M+H]<sup>+</sup>

[0262] <sup>1</sup>H NMR: (400 MHz, DMSO-d<sub>6</sub>) δ 10.18 (br. s., 2H), 8.98-8.87 (m, 3H), 8.66-8.57 (m, 4H), 8.18 (d, *J* = 7.6 Hz, 1H), 8.09 (d, *J* = 7.6 Hz, 1H), 7.73 (s, 1H), 7.53-7.34 (m, 6H), 6.94 (d, *J* = 8.9 Hz, 2H), 6.98 (d, *J* = 8.3 Hz, 2H), 6.69 (d, *J* = 2.5 Hz, 1H), 6.64-6.52 (m, 4H), 5.09 (d, *J* = 8.9 Hz, 2H), 4.24-4.16 (m, 2H), 4.16-3.88 (m, 14H), 3.50 (br. s., 2H), 2.95-2.83 (m, 2H), 2.83-2.71 (m, 2H), 2.10-1.89 (m, 4H)

### Compound 176

Synthesis of 2,2',2''-(10-(2-((1*r*,4*r*)-4-(2-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)ethylcarbamoyl)cyclohexylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid



**[0263]** Step-1: Preparation of methyl 3-(4-(2-(tert-butoxycarbonylamino)ethoxy)phenyl)isonicotinate

**[0264]** To a stirred solution of methyl 3-(4-hydroxyphenyl)isonicotinate (0.700 g, 3.05 moles, 1.0 equiv.) in DMF (10 ml),  $\text{CS}_2\text{CO}_3$  (2.90 gm, 9.15 moles, 3 eq.) was added to the RM. Then it was allowed to stir at room temperature for 30 min. tert-butyl (2-bromoethyl)carbamate (0.658 g, 3.66 moles, 1.2 eq.) was added to the reaction mixture. The Reaction mixture was allowed to stir at 80° C for 2h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the RM was washed with ice cold water and extracted with ethyl acetate. The combined organic layer was washed with brine. The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude material was purified with CombiFlash Chromatography to afford the methyl 3-(4-(2-(tert-butoxycarbonylamino)ethoxy)phenyl)isonicotinate (1.00 g, 87.95 % Yield).

**[0265]** LCMS: 373 [M+1]<sup>+</sup>

**[0266]** Step-2: Preparation of methyl 3-(4-(2-aminoethoxy)phenyl)isonicotinate

[0267] To a stirred solution of methyl 3-(4-(2-(tert-butoxycarbonylamino)ethoxy)phenyl)isonicotinate (1.00 gm, 2.68 mmol, 1.0 eq) in DCM (12 mL) was added TFA (2 mL) drop wise at 0 °C and the mixture was stirred at RT for 1 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was concentrated under reduced pressure to obtain a crude residue which was triturated with diethyl to afford the methyl 3-(4-(2-aminoethoxy)phenyl)isonicotinate (0.725 g, 69.91 %).

[0268] LCMS: 273 [M+H]<sup>+</sup>

[0269] Step-3: Preparation of methyl 3-(4-(2-((1r,4r)-4-(tert-butoxycarbonylamino)cyclohexane carboxamido)ethoxy)phenyl)isonicotinate

[0270] To a stirred solution of (1r,4r)-4-((tert-butoxycarbonyl)amino)cyclohexanecarboxylic acid (0.725 g, 2.66 mmol, 1.0 equiv.) in DMF (8 mL) HATU (1.5 gm, 3.99 moles, 1.5 eq.) was added to the mixture at 0° C and it was allowed to stir for 30 min. at 0°C, followed by the addition of DIPEA (1.9 mL, 10.64 moles, 4 eq.) and methyl 3-(4-(2-aminoethoxy)phenyl)isonicotinate (0.775 g, 3.19 moles, 1.2 eq.) was added to the RM after 5 min. The resulting reaction mixture was allowed to stir at RT for 2 h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the reaction mixture was diluted with ice cold water and extracted with ethyl acetate. The organic layer was washed with cold water. The combined organic layer was washed with sodium bicarbonate and brine and organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography to afford the methyl 3-(4-(2-((1r,4r)-4-(tert-butoxycarbonylamino)cyclohexanecarboxamido)ethoxy)phenyl) isonicotinate (0.480 g, 51.44% Yield).

[0271] LCMS: 498 [M+1]<sup>+</sup>

[0272] Step-4: Preparation of 3-(4-(2-((1r,4r)-4-(tert-butoxycarbonylamino)cyclohexane carboxamido)ethoxy)phenyl)isonicotinic acid

[0273] To a stirred solution of methyl 3-(4-(2-((1r,4r)-4-(tert-butoxycarbonylamino)cyclohexane carboxamido)ethoxy)phenyl)isonicotinate (0.480 g, 0.96 mmol, 1.0 eq) in THF (18 mL) was added LiOH.H<sub>2</sub>O (0.415 g, 9.64 mmol, 10 eq) in water (3 mL) and methanol (9 mL) and the mixture was stirred at RT for 1 h. The progress of the reaction was

monitored by TLC and LCMS. After completion, the mixture was acidified using 2N-HCl (pH~1-2) to obtain a precipitate which was filtered over vacuum to afford the 3-(4-(2-((1r,4r)-4-(tert-butoxycarbonylamino)cyclohexane carboxamido)ethoxy)phenyl)isonicotinic acid (0.300 g, 64.37 %).

[0274] LCMS: 484 [M+H]<sup>+</sup>

[0275] Step-5: Preparation of tert-butyl ((1r,4r)-4-(2-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)ethyl)carbamoyl) cyclohexylcarbamate

[0276] To a stirred solution of 3-(4-(2-((1r,4r)-4-((tert-butoxycarbonyl)amino)cyclohexane carboxamido)ethoxy)phenyl)isonicotinic acid (0.300 g, 0.62 mol, 1.0 equiv) in DMF (5 ml) HATU (0.471 g, 1.24 moles, 2 eq.) was added to the mixture at 0° C and it was allowed to stir for 30 min. at 0° C, followed by the addition of DIPEA (0.4 ml, 2.48 moles, 4 eq.) and (S)-1-(2-aminoacetyl)-4,4-difluoropyrrolidine-2-carbonitrile hydrochloride (0.210 g, 0.93 moles, 1.5 eq.) was added to the RM after 5 min. The resulting reaction mixture was allowed to stir at RT for 2 h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the reaction mixture was diluted with ice cold water and extracted with ethyl acetate. The organic layer was washed with cold water. The combined organic layer was washed with sodium bicarbonate and brine and organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography to afford the tert-butyl ((1r,4r)-4-((2-(4-(4-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)ethyl)carbamoyl) cyclohexyl)carbamate (0.230 g, 56.65% Yield).

[0277] LCMS: 655 [M+1]<sup>+</sup>

[0278] Step-6: Preparation of 3-(4-(2-((1r,4r)-4-aminocyclohexanecarboxamido)ethoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide

[0279] To a stirred solution of tert-butyl ((1r,4r)-4-((2-(4-(4-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)ethyl)carbamoyl)cyclohexyl)carbamate (0.230 g, 0.35 mmol, 1.0 eq) in DCM (7 mL) was added TFA (0.5 mL) drop wise at 0 °C and the mixture was stirred at RT for 1 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was concentrated

under reduced pressure to obtain a crude residue which was triturated with diethyl to afford the 3-(4-(2-((1*r*,4*r*)-4-aminocyclohexanecarboxamido)ethoxy)phenyl)-N-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide (0.170 g, 72.34 %).

[0280] LCMS: 555 [M+H]<sup>+</sup>

[0281] Step-7: Preparation of 2,2',2''-(10-(2-((1*r*,4*r*)-4-(2-(4-(4-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)ethylcarbamoyl) cyclohexylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid

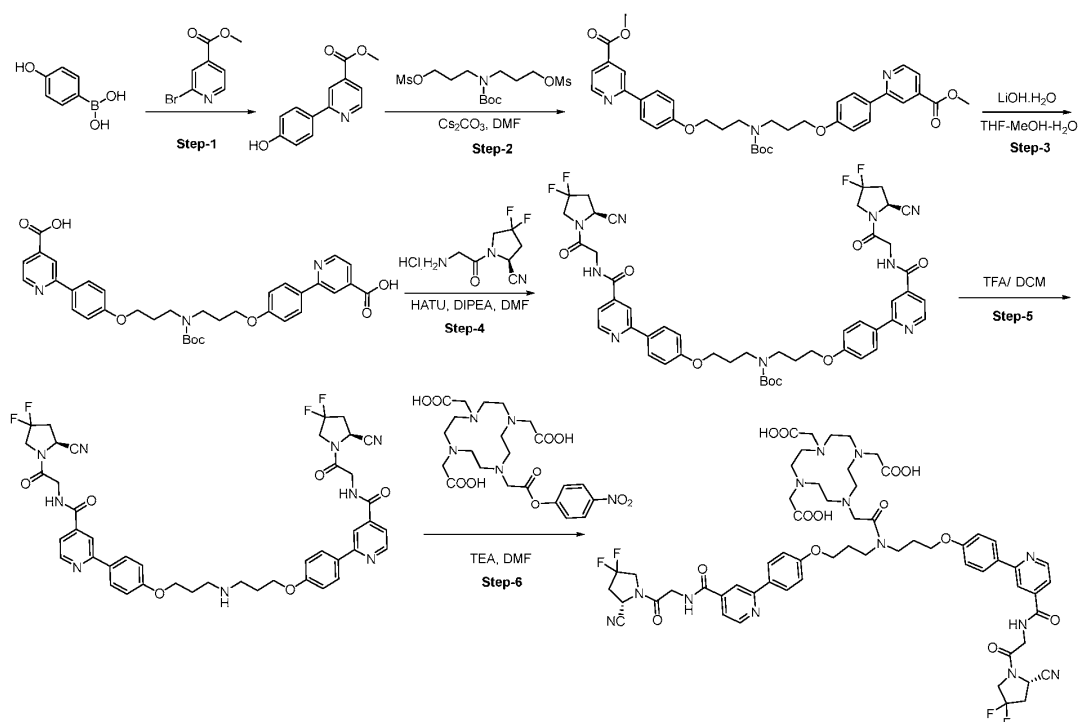
[0282] To a stirred solution of 3-(4-(2-((1*r*,4*r*)-4-aminocyclohexanecarboxamido)ethoxy)phenyl)-N-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide (0.150 g, 0.27 mol, 1.0 equiv) in DMF (0.5 mL). TEA (4 ml, 2.7 mols, 10 eq.) drop wise at RT. 2,2',2''-(10-(2-(4-nitrophenoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.168 g, 0.32 mols, 1.2 eq.) was added to the reaction mixture. The Reaction mixture was allowed to stir at RT for 16h. After completion of the reaction, the reaction mixture was concentrated under reduced pressure. The resulting residue was washed with ether for purification to afford the 2,2',2''-(10-(2-((1*r*,4*r*)-4-((2-(4-(4-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)ethyl)carbamoyl) cyclohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.012 g, 5.68 % Yield) as an off white solid.

[0283] LCMS: 941 [M+H]<sup>+</sup>

[0284] <sup>1</sup>H NMR: (400 MHz, DMSO-*d*<sub>6</sub>) 8.93 (t, *J* = 5.7 Hz, 1H), 8.67-8.53 (m, 2H), 8.10-7.96 (m, 2H), 7.53-7.36 (m, 3H), 6.97 (d, *J* = 8.9 Hz, 2H), 5.11 (dd, *J* = 3.2, 9.5 Hz, 1H), 4.27-4.12 (m, 2H), 4.11-3.92 (m, 7H), 3.50 (br. s., 6H), 3.05- 2.76 (m, 18H), 2.09 (br. s., 3H), 1.82-1.65 (m, 4H), 1.48-1.30 (m, 4H)

### Compound 179

Synthesis of 2,2',2''-(10-(2-(bis(3-(4-(4-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-2-yl)phenoxy)propyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid



[0285] Step-1: Preparation of methyl 2-(4-hydroxyphenyl)isonicotinate

[0286] To a stirred solution of methyl 2-bromoisonicotinate (0.5 g, 2.33 mmol, 1.0 eq) and (4-hydroxyphenyl)boronic acid (0.386 g, 2.8 mmol, 1.2 eq) in 1,4-Dioxane-H<sub>2</sub>O (4.5 mL : 0.5 mL) was added K<sub>2</sub>CO<sub>3</sub> (0.644 g, 4.67 mmol, 2 eq) and the mixture was degassed under nitrogen for 10 min. Pd(dppf)Cl<sub>2</sub> (0.08 g, 0.11 mmol, 0.05 eq) was then added to the mixture and the mixture was further degassed for 5 min. The resultant mixture was irradiated under MW radiation at 100 °C for 1.5 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was diluted with water (20 mL) and extracted using ethyl acetate (40 mL × 2). The combined organic layers were washed with water (20 mL), brine (20 mL), dried over anhydrous sodium sulfate and concentrated to obtain a crude residue which was purified using CombiFlash Chromatography to afford the methyl 2-(4-hydroxyphenyl)isonicotinate (0.430 g, 80 %).

[0287] LCMS: 230 [M+H]<sup>+</sup>

[0288] Step-2: Preparation of dimethyl 2,2'-((((tert-butoxycarbonyl)azanediyl)bis(propylene))bis(oxy))bis(4,1-phenylene)diisonicotinate



[0289] To a stirred solution of methyl 2-(4-hydroxyphenyl)isonicotinate (0.5 g, 2.17 mmol, 1.0 eq) in DMF (10 mL) was added CsCO<sub>3</sub> (2.1 g, 6.52 mmol, 3 eq) at RT and the mixture was stirred at same temperature for 20 min. ((tert-butoxycarbonyl)azanediy)bis(propane-3,1-diyl)dimethanesulfonate (0.63 g, 1.3 mmol, 1.63 eq) was then added to the mixture and the resultant mixture was heated at 90 °C for 2 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was diluted with water (20 mL) to obtain a precipitate which was filtered under vacuum to obtain a crude residue which was purified by CombiFlash Chromatography to afford the dimethyl 2,2'-((((tert-butoxycarbonyl)azanediy) bis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene))diisonicotinate (0.26 g, 18.18 %)

[0290] LCMS: 656 [M+H]<sup>+</sup>

[0291] Step-3: Preparation of 2,2'-((((tert-butoxycarbonyl)azanediy)bis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene))diisonicotinic acid

[0292] To a stirred solution of dimethyl 2,2'-((((tert-butoxycarbonyl)azanediy)bis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene))diisonicotinate (0.1 g, 0.15 mmol, 1.0 eq) in THF (6 mL) was added LiOH.H<sub>2</sub>O (0.03 g, 0.76 mmol, 5 eq) in water (1 mL) and methanol (3 mL) and the mixture was stirred at RT for 1 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was acidified using 2N-HCl (pH~1-2) to obtain a precipitate which was filtered over vacuum to afford the 2,2'-((((tert-butoxycarbonyl) azanediy)bis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene))diisonicotinic acid (0.09 g, 94.04 %).

[0293] LCMS: 628 [M+H]<sup>+</sup>

[0294] Step-4: Preparation of tert-butyl bis (3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)carbamate

[0295] To a stirred solution of 2,2'-((((tert-butoxycarbonyl)azanediy)bis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene))diisonicotinic acid (0.1 g, 0.159 mmol, 1.0 eq) in DMF (5 mL) was added HATU (0.09 g, 0.238 mmol, 1.5 eq) at 0 °C and the mixture was stirred at same temperature for 30 min. DIPEA (0.1 mL, 0.64 mmol, 4.0 eq) and (S)-1-(2-aminoacetyl)-4,4-difluoropyrrolidine-2-carbonitrile hydrochloride (0.04 g, 0.191 mmol, 1.2 eq) were then added to the mixture and the mixture was stirred at RT for 2 h. The progress of the reaction was monitored by TLC and LCMS. After completion, H<sub>2</sub>O (30 mL) was added to the mixture to obtain the

precipitate which was filtered over vacuum to obtain a crude residue which was purified by CombiFlash Chromatography to afford tert-butyl bis (3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)carbamate (0.09 g 58.44 %).

**[0296] LCMS:** 970 [M+H]<sup>+</sup>

**[0297] Step-5:** Preparation of 2,2'-(((azanediylbis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene))bis(N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide)

**[0298]** To a stirred solution of tert-butyl bis(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)carbamate (0.09 g, 0.092 mmol, 1.0 eq) in DCM (10 mL) was added TFA (0.8 mL) drop wise at 0 °C and the mixture was stirred at RT for 1 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was concentrated under reduced pressure to obtain a crude residue which was triturated with diethyl ether to afford the 2,2'-(((azanediylbis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene))bis(N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide) (0.075 g, 82.41 %).

**[0299] LCMS:** 870 [M+H]<sup>+</sup>

**[0300] Step-6:** Preparation of 2,2',2''-(10-(2-(bis(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid

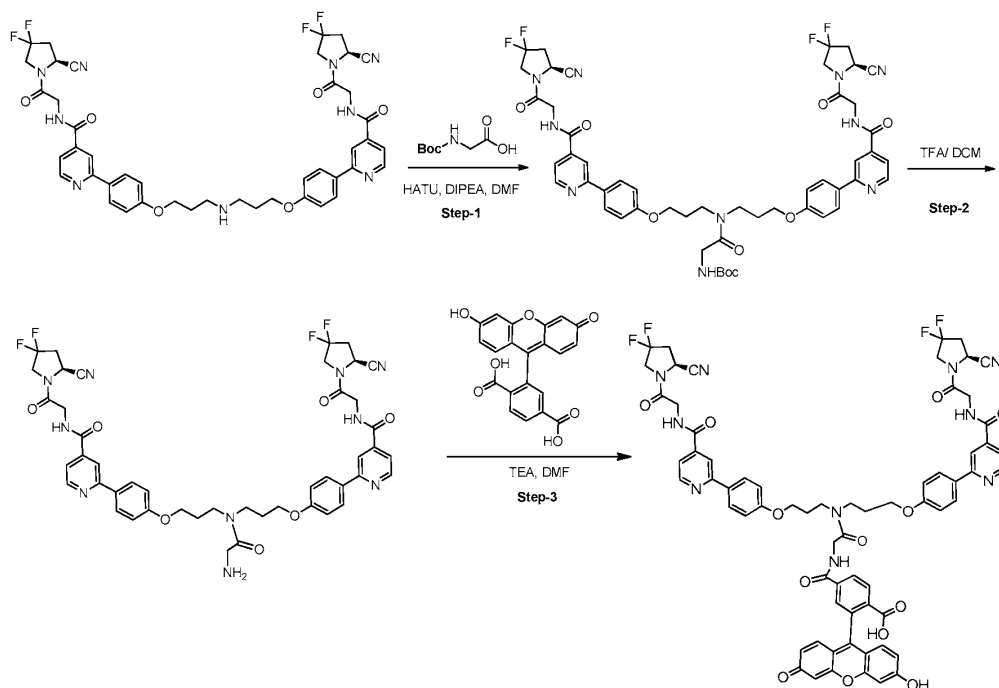
**[0301]** To a stirred solution of 2,2'-(((azanediylbis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene))bis(N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide) (0.05 g, 0.057 mmol, 1.0 eq) in DMF (3.0 mL) was added TEA (0.5 mL) and 2,2',2''-(10-(2-(4-nitrophenoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.036 g, 0.068 mmol, 1.2 eq). The mixture was stirred at RT for 16 h. The progress of the reaction was monitored by LCMS. After completion, the mixture was concentrated under reduced pressure and triturated with diethyl ether and further purified by Reversed Phase HPLC to afford the 2,2',2''-(10-(2-(bis(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl) triacetic acid (0.01 g, 15.67 %)

**[0302] LCMS:** 1256 [M+H]<sup>+</sup>

[0303]  $^1\text{H}$  NMR: (400 MHz, DMSO- $d_6$ )  $\delta$  8.69 (t,  $J$  = 4.8 Hz, 2H), 8.24-8.15 (m, 4H), 8.01-7.90 (m, 4H), 7.67 (br. s., 2H), 7.13-7.02 (m, 4H), 5.10 (d,  $J$  = 6.4 Hz, 4H), 4.28 (br. s., 2H), 4.26-4.05 (m, 8H), 3.65 (br. s., 6H), 3.62-3.52 (m, 6H), 3.40 (br. s., 4H), 3.19 (br. s., 4H), 3.05 (br. s., 6H), 2.78 (br. s., 6H), 2.20-2.07 (m, 4H)

### Compound 190

Synthesis of 4-((2-(bis(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)amino)-2-oxoethyl)carbamoyl)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid



[0304] Step-1: Preparation of tert-butyl (2-(bis(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)amino)-2-oxoethyl)carbamate To a stirred solution of 2-((tert-butoxycarbonyl)amino)acetic acid (0.05 g, 0.057 mmol, 1.0 eq) in DMF (5 mL) was added HATU (0.032 g, 0.086 mmol, 1.5 eq) at 0 °C and the mixture was stirred at same temperature for 30 min. DIPEA (0.03 mL, 0.172 mmol, 3.0 eq) and 2,2'-(((azanediylbis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene))bis(N-(2-((S)-2-cyano-

4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide) (0.012 g, 0.069 mmol, 1.2 eq) were then added to the mixture and the mixture was stirred at RT for 2 h. The progress of the reaction was monitored by TLC and LCMS. After completion, H<sub>2</sub>O (20 mL) was added to the mixture to obtain the precipitate which was filtered over vacuum to obtain a crude residue which was purified by CombiFlash Chromatography to afford the tert-butyl (2-(bis(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)amino)-2-oxoethyl)carbamate (0.058 g 19.79 %).

**[0305] LCMS:** 1027 [M+H]<sup>+</sup>

**[0306] Step-2:** Preparation of 2-(4-(3-(2-amino-N-(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)acetamido)propoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl) isonicotinamide

**[0307]** To a stirred solution of tert-butyl (2-(bis(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)amino)-2-oxoethyl)carbamate (0.06 g, 0.058 mmol, 1.0 eq) in DCM (5 mL) was added TFA (0.8 mL) drop wise at 0 °C and the mixture was stirred at RT for 1 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was concentrated under reduced pressure to obtain a crude residue which was triturated with diethyl to afford the 2-(4-(3-(2-amino-N-(3-(4-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)acetamido)propoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide (0.050 g, 82.23 %).

**[0308] LCMS:** 927 [M+H]<sup>+</sup>

**[0309] Step-3:** Preparation of 4-((2-(bis(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)amino)-2-oxoethyl)carbamoyl)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid

**[0310]** To a stirred solution of 2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)terephthalic acid (0.018 g, 0.047 mmol, 1.0 eq) in DMF (2.5 mL) was added NHS (0.011 g, 0.095 mmol, 2.0 eq) and DCC (0.019 g, 0.095 mmol, 2.0 eq) at 0 °C and the mixture was stirred at same temperature for 3 h. DIPEA (0.03 mL, 0.191 mmol, 4 eq) and 2-(4-(3-(2-amino-N-(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)

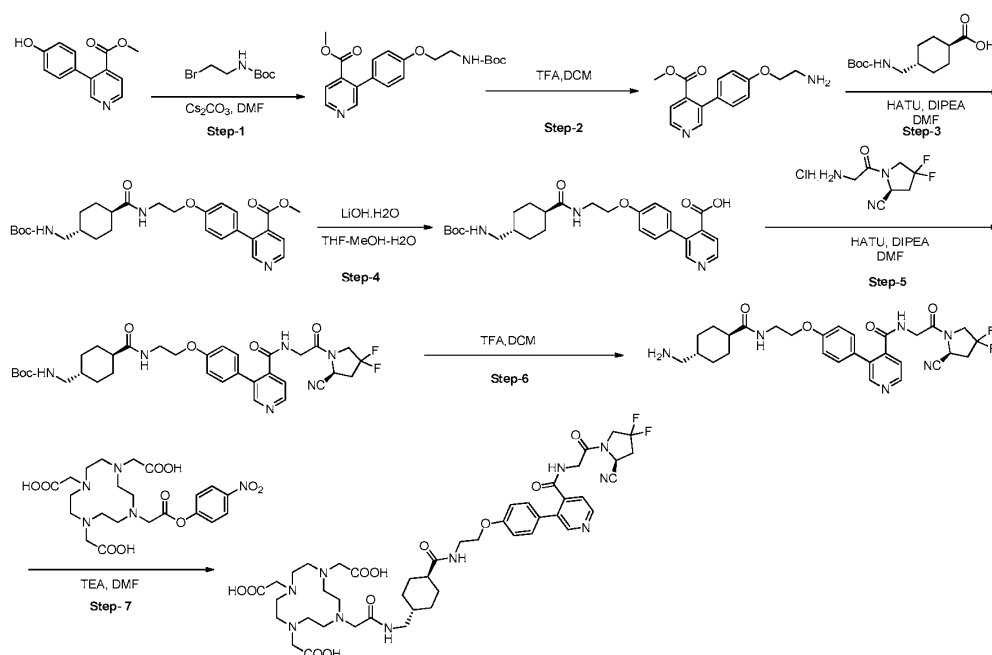
acetamido)propoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide (0.053 g, 0.057 mmol, 1.2 eq) were then added to the mixture and the mixture was stirred at RT for 16 h. The progress of the reaction was monitored by LCMS. After completion, the mixture was concentrated under reduced pressure and triturated with diethyl ether to obtain a solid which was purified by Reversed Phase HPLC to afford the 4-((2-(bis(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-2-yl)phenoxy)propyl)amino)-2-oxoethyl)carbamoyl)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid (0.004 g, 18 %).

[0311] LCMS: 1285 [M+H]<sup>+</sup>

[0312] <sup>1</sup>H NMR: (400 MHz, DMSO-d<sub>6</sub>) δ 9.21 (t, *J* = 6.0 Hz, 2H), 8.91 (t, *J* = 5.7 Hz, 1H), 8.76 (d, *J* = 5.1 Hz, 2H), 8.26 (s, 2H), 8.23-8.15 (m, 1H), 8.15-7.98 (m, 5H), 7.72 (s, 1H), 7.67-7.62 (m, 2H), 7.04 (d, *J* = 8.9 Hz, 2H), 7.08 (d, *J* = 8.3 Hz, 2H), 6.67 (s, 2H), 6.64-6.46 (m, 5H), 5.11 (dd, *J* = 3.2, 9.5 Hz, 2H), 4.32 (ddd, *J* = 4.8, 11.1, 15.9 Hz, 2H), 4.26-4.00 (m, 10H), 3.60-3.45 (m, 6H), 2.97-2.74 (m, 4H), 2.12-2.02 (m, 2H), 2.02-1.89 (m, 2H)

### Compound 191

Synthesis of 2,2',2''-(10-(2-(((1*r*,4*r*)-4-(2-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)ethylcarbamoyl)cyclohexyl)methylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid



**[0313]** Step-1: Preparation of methyl 3-(4-(2-(tert-butoxycarbonylamino)ethoxy)phenyl)isonicotinate

**[0314]** To a stirred solution of methyl 3-(4-hydroxyphenyl)isonicotinate (1.00 g, 4.36 moles, 1.0 equiv.) in DMF (15 ml),  $\text{CS}_2\text{CO}_3$  (4.20 g, 5.24 moles, 3 eq.) was added to the RM. Then it was allowed to stir at room temperature for 30 min. tert-butyl (2-bromoethyl)carbamate (0.938 g, 5.24 moles, 1.2 eq.) was added to the reaction mixture. The Reaction mixture was allowed to stir at 80° C for 2h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the RM was washed with ice cold water and extracted with ethyl acetate. The combined organic layer was washed with Brine. The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude material was purified with combi flash chromatography to afford the methyl 3-(4-(2-((tert-butoxy carbonyl)amino)ethoxy)phenyl)isonicotinate (0.850 g, 50.95 % Yield).

**[0315]** LCMS: 373 [M+1]<sup>+</sup>

**[0316]** Step-2: Preparation of methyl 3-(4-(2-aminoethoxy)phenyl)isonicotinate

**[0317]** To a stirred solution of methyl 3-(4-(2-(tert-butoxycarbonylamino)ethoxy)phenyl)isonicotinate (0.850 g, 2.28 mmol, 1.0 eq) in DCM (10 mL) was added TFA (2 mL) drop wise at

0 °C and the mixture was stirred at RT for 1 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was concentrated under reduced pressure to obtain a crude residue which was triturated with diethyl to afford the methyl 3-(4-(2-aminoethoxy)phenyl)isonicotinate (0.615 g, 69.73 %).

[0318] LCMS: 273 [M+H]<sup>+</sup>

[0319] Step-3: Preparation of methyl 3-(4-(2-((1r,4r)-4-((tert-butoxycarbonylamino)methyl)cyclohexanecarboxamido)ethoxy)phenyl)isonicotinate

[0320] To a stirred solution of (1r,4r)-4-(((tert-butoxycarbonyl)amino)methyl)cyclohexanecarboxylic acid (0.615 g, 1.23 mmol, 1.0 equiv) in DMF (8 ml ) HATU (0.701 g, 1.84 moles, 1.5 eq.) was added to the mixture at 0° C and it was allowed to stir for 30 min. at 0°C, followed by the addition of DIPEA (0.85 mL, 4.92 moles, 4 eq.) and methyl 3-(4-(2-aminoethoxy)phenyl)isonicotinate (0.332 g, 1.47 moles , 1.2 eq.) was added to the RM after 5 min. The resulting reaction mixture was allowed to stir at RT for 2 h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the reaction mixture was diluted with ice cold water and extracted with ethyl acetate. The organic layer was washed with cold water. The combined organic layer was washed with sodium bicarbonate and brine and organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography to afford the methyl 3-(4-(2-((1r, 4r)-4-((tert-butoxycarbonylamino)methyl) cyclohexanecarboxamido)ethoxy) phenyl)isonicotinate (0.815 g, 66.69% Yield).

[0321] LCMS: 512 [M+1]<sup>+</sup>

[0322] Step-4: Preparation of 3-(4-(2-((1r, 4r)-4-((tert-butoxycarbonylamino)methyl)cyclohexanecarboxamido)ethoxy)phenyl)isonicotinic acid

[0323] To a stirred solution of the methyl 3-(4-(2-((1r, 4r)-4-((tert-butoxycarbonylamino)methyl) cyclohexanecarboxamido)ethoxy)phenyl)isonicotinate (0.815 g, 2.34 mol, 1.0 equiv) in THF (18 ml) , LiOH.H<sub>2</sub>O(0.563 g, 23.45 mols,10 eq.) was dissolved in water(3 ml) and added to the RM followed by the addition of MeOH (6 ml) to the RM.It was allowed to stir for 1 hour at RT.. The resulting reaction mixture was monitored by TLC and LCMs. After completion of the reaction, the reaction mixture was quenched with ice-cold water and the

precipitation formed was filtered and dried in oven. The resulting compound was confirmed by LCMS and the desired product was 3-(4-(2-((1r, 4r)-4-(((tert-butoxycarbonyl) amino) methyl) cyclohexanecarboxamido)ethoxy)phenyl)isonicotinic acid (0.700 g, 88.38% Yield).

**[0324] LCMS:** 498 [M+1]<sup>+</sup>

**[0325] Step-5:** Preparation of tert-butyl (((1r,4r)-4-(2-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)ethyl)carbamoyl) cyclohexyl)methylcarbamate

**[0326]** To a stirred solution of 3-(4-(2-((1r, 4r)-4-(((tert-butoxycarbonyl) amino) methyl) cyclohexanecarboxamido)ethoxy)phenyl)isonicotinic acid (0.300 g, 0.603 mol, 1.0 equiv) in DMF (5 ml) HATU (0.344 g, 0.90 moles, 1.5 eq.) was added to the mixture at 0° C and it was allowed to stir for 30 min. at 0°C, followed by the addition of DIPEA (0.4 ml, 2.41 moles, 4 eq.) and (S)-1-(2-aminoacetyl)-4,4-difluoropyrrolidine-2-carbonitrile hydrochloride (0.163 g, 0.72 moles, 1.2 eq.) was added to the RM after 5 min. The resulting reaction mixture was allowed to stir at RT for 2 h. Product formation was confirmed by TLC and LCMS. After completion of the reaction, the reaction mixture was diluted with ice cold water and extracted with ethyl acetate. The organic layer was washed with cold water. The combined organic layer was washed with sodium bicarbonate and brine and organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography to afford the tert-butyl (((1r,4r)-4-(2-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)ethyl)carbamoyl)cyclohexyl)methyl)carbamate (0.250 g, 61.94% Yield).

**[0327] LCMS:** 669 [M+1]<sup>+</sup>

**[0328] Step-6:** Preparation of 3-(4-(2-((1r,4r)-4-(aminomethyl)cyclohexanecarboxamido)ethoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide

**[0329]** To a stirred solution of tert-butyl (((1r,4r)-4-(2-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)ethyl)carbamoyl) cyclohexyl)methyl)carbamate (0.250 g, 0.37 mmol, 1.0 eq) in DCM (7 mL) was added TFA (0.6 mL) drop wise at 0 °C and the mixture was stirred at RT for 1 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was concentrated under reduced



pressure to obtain a crude residue which was triturated with diethyl to afford the 3-(4-(2-((1r,4r)-4-(aminomethyl)cyclohexanecarboxamido)ethoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide (0.200 g, 78.43 %).

**[0330] LCMS:** 569 [M+H]<sup>+</sup>

**[0331] Step-7:** Preparation of 2,2',2''-(10-(2-(((1r,4r)-4-(2-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)ethylcarbamoyl)cyclohexyl)methylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid

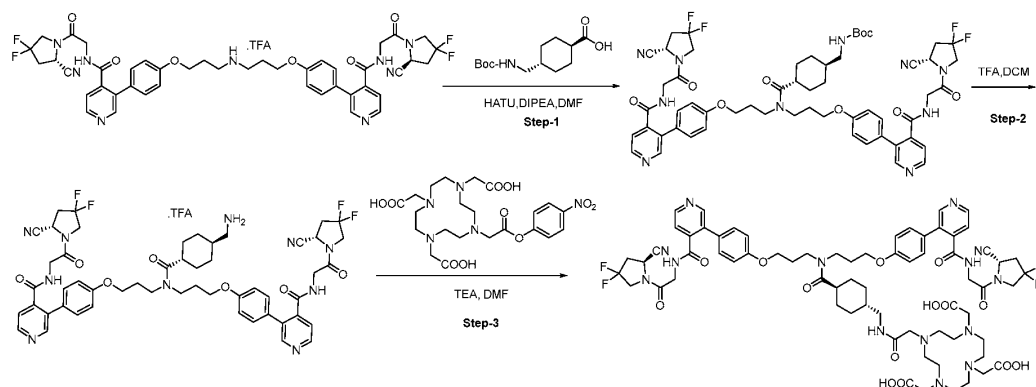
**[0332]** To a stirred solution of 3-(4-(2-((1r,4r)-4-(aminomethyl)cyclohexanecarboxamido)ethoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide (0.100 g, 0.18 mol, 1.0 equiv) in DMF (0.5 mL). TEA (0.2 ml, 1.81 mols, 10 eq.) drop wise at RT. 2,2',2''-(10-(2-(4-nitrophenoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.143 g, 0.27 mols, 1.5 eq.) was added to the reaction mixture. The Reaction mixture was allowed to stir at RT for 16h. After completion of the reaction, the reaction mixture was concentrated under reduced pressure. The resulting residue was washed with ether for purification to afford the 2,2',2''-(10-(2-(((1r,4r)-4-((2-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)ethyl)carbamoyl)cyclohexyl)methyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.004.5 g, 2.85 % Yield)

**[0333] LCMS:** 955 [M+H]<sup>+</sup>

**[0334] <sup>1</sup>H NMR:** (400 MHz, DMSO-d<sub>6</sub>) 8.98-8.88 (m, 1H), 8.69 - 8.53 (m, 2H), 8.15 (s, 1H), 7.99 (br. s., 1H), 7.52-7.36 (m, 3H), 6.97 (d, *J* = 8.3 Hz, 2H), 5.16-5.01 (m, 1H), 4.25-4.1 (m, 2H), 4.11-3.92 (m, 8H), 3.02-2.72 (m, 17H), 2.23-2.12 (m, 2H), 2.07 (d, *J* = 10.2 Hz, 4H), 2.01-1.94 (m, 1H), 1.77-1.59 (m, 6H), 1.43-1.27 (m, 6H)

## Compound 192

Synthesis of 2,2',2''-(10-(2-(((1r,4r)-4-((3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)carbamoyl)cyclohexyl)methylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid



**[0335]** Step-1: Preparation of tert-butyl ((1*r*,4*r*)-4-(bis(3-(4-(4-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)carbamoyl) cyclohexyl)methylcarbamate

**[0336]** To a stirred solution of (1*r*,4*r*)-4-(((tert-butoxycarbonyl)amino)methyl)cyclohexane carboxylic acid (0.050 g, 0.19 mmol, 1.0 eq) in DMF (3 mL) was added HATU (0.11 g, 0.29 mmol, 1.5 eq) at 0 °C and the mixture was stirred at same temperature for 30 min. DIPEA (0.10 mL, 0.58 mmol, 3.0 eq) and 3,3'-(((azanediylbis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene))bis(N-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide) (0.253g, 0.24 mmole, 1.5 eq) were then added to the mixture and the mixture was stirred at RT for 2 h. The progress of the reaction was monitored by TLC and LCMS. After completion, ice cold water (30 mL) was added to the mixture to obtain the precipitate which was filtered over vacuum to obtain a crude residue. The crude product was purified by CombiFlash Chromatography to afford tert-butyl ((1*r*,4*r*)-4-(bis(3-(4-(4-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)carbamoyl) cyclohexyl)methylcarbamate (0.210 g, 97.67 %).

**[0337]** LCMS: 1109[M+H]<sup>+</sup>

**[0338]** Step-2: Preparation of 3-(4-(3-((1*r*,4*r*)-4-(aminomethyl)-N-(3-(4-(4-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl) cyclohexanecarboxamido)propoxy)phenyl)-N-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide

**[0339]** To a stirred solution of tert-butyl ((1*r*,4*r*)-4-(bis(3-(4-(4-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)carbamoyl)

cyclohexyl)methylcarbamate (0.280 g, 0.25 mmol, 1.0 eq) in DCM (8 mL) was added TFA (1.2 mL) drop wise at 0 °C and the mixture was stirred at RT for 1 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was concentrated under reduced pressure to obtain a crude residue which was triturated with diethyl to afford 3-(4-(3-((1r,4r)-4-(aminomethyl)-N-(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl) cyclohexanecarboxamido)propoxy) phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide (0.200 g, 70.67%)

**[0340] LCMS:** 1008 [M+H]<sup>+</sup>

**[0341] Step-3:** Preparation of 2,2',2''-(10-(2-(((1r,4r)-4-((3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)carbamoyl) cyclohexyl)methylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid

**[0342]** To a stirred solution of 3-(4-(3-((1r,4r)-4-(aminomethyl)-N-(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl) cyclohexanecarboxamido)propoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide (0.150 g, 0.135 mmol, 1.0 eq) in DMF (0.2 mL) were added 2,2',2''-(10-(2-(4-nitrophenoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.119 g, 0.226 mmol, 1.5 eq) and TEA (0.07 ml, 0.675 mmol, 5.0 eq) and the mixture was stirred at RT for 16 h. The progress of the reaction was monitored by LCMS. After completion, the mixture was concentrated under reduced pressure, triturated with diethyl ether and further purified by Reversed Phase HPLC to afford 2,2',2''-(10-(2-(((1r,4r)-4-((3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl carbamoyl)pyridin-3-yl)phenoxy)propyl)carbamoyl) cyclohexyl)methylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.004 g, 2.15 %)

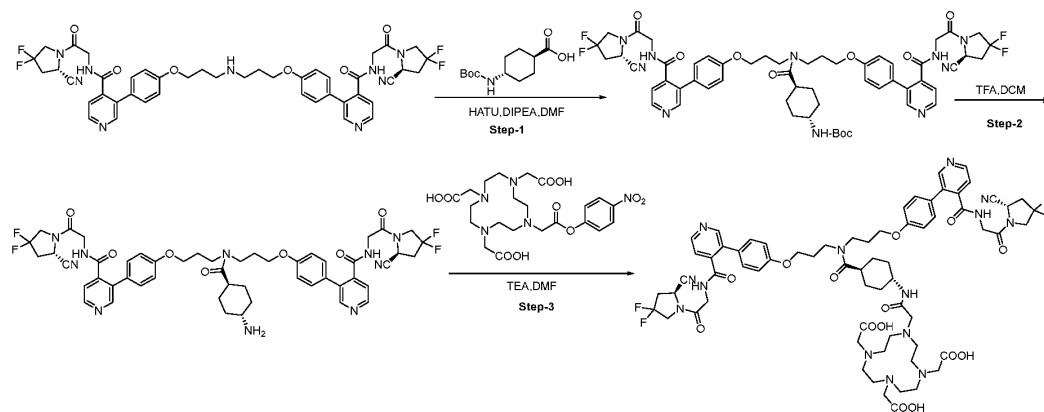
**[0343] LCMS:** 1395 [M+H]<sup>+</sup>

**[0344] <sup>1</sup>H NMR:** (400 MHz, DMSO-d<sub>6</sub>) 9.00 (br. s., 1H), 8.97-8.89 (m, 1H), 8.68-8.58 (m, 4H), 8.11 (br. s., 1H), 7.56-7.36 (m, 6H), 6.95 (d, *J* = 8.3 Hz, 2H), 6.99 (d, *J* = 8.3 Hz, 2H), 5.19-4.99 (m, 2H), 4.28-4.12 (m, 3H), 4.12-4.00 (m, 9H), 4.00-3.91 (m, 4H), 3.49 (br. s., 10H), 2.99-

2.85 (m, 10H), 2.77-2.55 (m, 4H), 1.99 (br. s., 4H), 1.92 (d,  $J = 7.6$  Hz, 4H), 1.66 (d,  $J = 11.4$  Hz, 2H), 1.58 (br. s., 2H), 1.45-1.27 (m, 6H), 1.23 (br. s., 1H), 0.83 (d,  $J = 13.4$  Hz, 2H)

### Compound 193

Synthesis of 2,2',2''-(10-(2-((1*r*,4*r*)-4-((3-(4-(4-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)carbamoyl)cyclohexylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid



**[0345]** Step-1: Preparation of tert-butyl (1*r*,4*r*)-4-(bis(3-(4-(4-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)carbamoyl)cyclohexylcarbamate

**[0346]** To a stirred solution of (1*r*,4*r*)-4-((tert-butoxycarbonyl)amino)cyclohexanecarboxylic acid (0.040 g, 0.16 mmol, 1.0 eq) in DMF (3 mL) was added HATU (0.093g, 0.246 mmol, 1.5 eq) at 0 °C and the mixture was stirred at same temperature for 30 min. DIPEA (0.08 mL, 0.49mmol, 3.0 eq) and 3,3'-(((azanediylbis(propane-3,1-diyl))bis(oxy))bis(4,1-phenylene)) bis(N-(2-((*S*)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide) (0.214 g, 0.24 mmole, 1.2 eq) were then added to the mixture and the mixture was stirred at RT for 2 h. The progress of the reaction was monitored by TLC and LCMS. After completion, ice cold water (30 mL) was added to the mixture to obtain the precipitate which was filtered over vacuum to obtain a crude residue. The crude product was purified by CombiFlash Chromatography to afford tert-butyl ((1*r*,4*r*)-4-

(bis(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)carbamoyl) cyclohexyl)carbamate (0.175 g, 97.22 %).

**[0347] LCMS:** 1095[M+H]<sup>+</sup>

**[0348]** Step-2: Preparation of 3-(4-(3-((1r,4r)-4-amino-N-(3-(4-(4-(2-((S)-2-cyano-4,4-difluoro pyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)cyclohexane carboxamido)propoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide

**[0349]** To a stirred solution of tert-butyl ((1r,4r)-4-(bis(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl) carbamoyl)cyclohexyl)carbamate (0.200g, 0.18 mmol, 1.0 eq) in DCM (5 mL) was added TFA (1.2 mL) drop wise at 0 °C and the mixture was stirred at RT for 1 h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was concentrated under reduced pressure to obtain a crude residue which was triturated with diethyl to afford 3-(4-(3-((1r,4r)-4-amino-N-(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl) cyclohexanecarboxamido) propoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide (0.150 g, 74.25 %)

**[0350] LCMS:** 995 [M+H]<sup>+</sup>

**[0351]** Step-3: Preparation of 2,2',2''-(10-(2-((1r,4r)-4-((3-(4-(4-(2-((S)-2-cyano-4,4-difluoro pyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl) carbamoyl) cyclohexylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid

**[0352]** To a stirred solution of 3-(4-(3-((1r,4r)-4-amino-N-(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl) cyclohexanecarboxamido)propoxy)phenyl)-N-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)isonicotinamide (0.150 g, 0.135 mmol, 1.0 eq) in DMF (0.2 mL) were added 2,2',2''-(10-(2-(4-nitrophenoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl) triacetic acid (0.119 g, 0.226 mmol, 1.5 eq) and TEA (0.07 ml, 0.675 mmol, 5.0 eq) and the mixture was stirred at RT for 16 h. The progress of the reaction was monitored by LCMS. After completion, the mixture was concentrated under reduced pressure, triturated with diethyl ether and further purified by Reversed

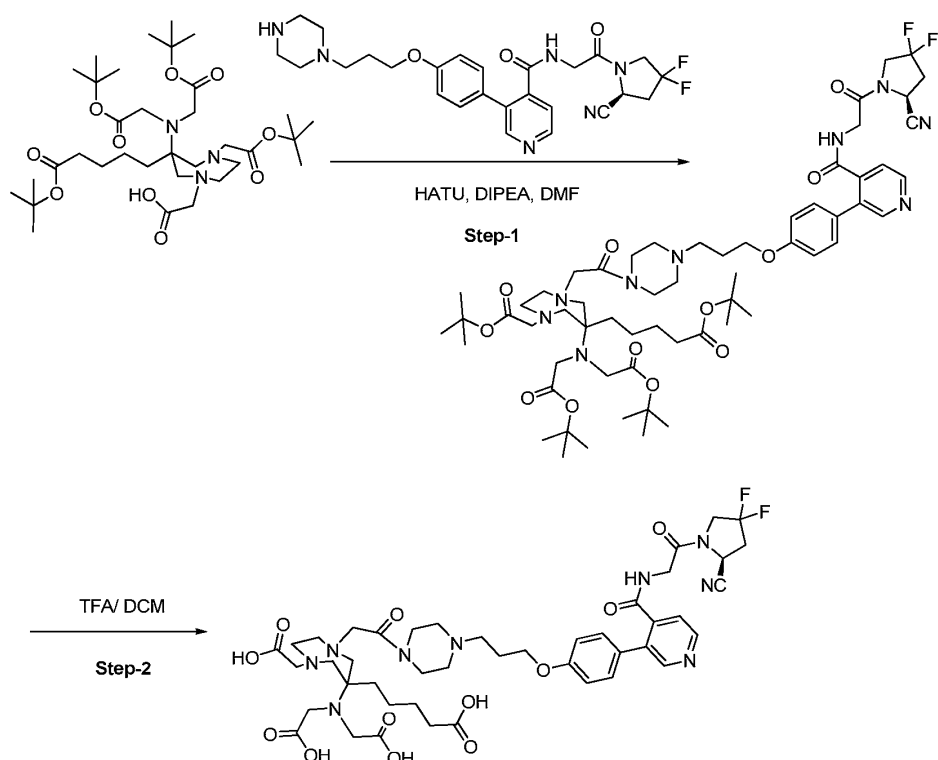
Phase HPLC to afford 2,2',2''-(10-(2-(((1r,4r)-4-((3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)(3-(4-(4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)carbamoyl)cyclohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.0018 g, 1 %)

[0353] LCMS: 1381 [M+H]<sup>+</sup>

[0354] <sup>1</sup>H NMR: (400 MHz, DMSO-d<sub>6</sub>) 8.73-8.54 (m, 4H), 8.39 (br. s., 1H), 8.16 (br. s., 2H), 7.97 (br. s., 1H), 7.57-7.36 (m, 5H), 7.07-6.91 (m, 4H), 5.18-5.01 (m, 2H), 4.31-4.15 (m, 4H), 4.15-4.01 (m, 8H), 3.98 (br. s., 8H), 3.84 (br. s., 4H), 3.10-3.05 (m, 4H), 3.05-2.88 (m, 8H), 2.78 (s, 4H), 2.82 (s, 4H), 2.66 (br. s., 4H), 2.051.96 (m, 2H), 1.93 (br. s., 4H), 1.76 (br. s., 4H)

#### Compound 194

Synthesis of 2,2'-((S)-6-(4-carboxybutyl)-1-(carboxymethyl)-4-(2-(4-(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)pyridin-3-yl)phenoxy)propyl)piperazin-1-yl)-2-oxoethyl)-1,4-diazepan-6-ylazanediyldiacetic acid



**[0355]** Step-1: Preparation of tert-butyl 2,2'-((S)-1-(2-tert-butoxy-2-oxoethyl)-6-(5-tert-butoxy-5-oxopentyl)-4-(2-(4-(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)piperazin-1-yl)-2-oxoethyl)-1,4-diazepan-6-ylazanediyldiacetate

**[0356]** To a stirred solution of (R)-2-(6-(bis(2-tert-butoxy-2-oxoethyl)amino)-4-(2-tert-butoxy-2-oxoethyl)-6-(5-tert-butoxy-5-oxopentyl)-1,4-diazepan-1-yl)acetic acid (0.180 g, 0.267 mol, 1.0 equiv) in DMF (3 ml) HATU (0.153 mg, 0.401 moles, 1.5 eq.) was added to the mixture at 0° C and it was allowed to stir for 30 min. at 0° C, followed by the addition of DIPEA (0.14 ml, 0.801 moles, 3 eq.) and (S)-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-3-(4-(3-(piperazin-1-yl)propoxy)phenyl)isonicotinamide (0.164 g, 0.320 moles, 1.2 eq.) was added to the RM after 5 min. The resulting reaction mixture was allowed to stir at RT for 2 h. The product formation was confirmed by TLC and LCMS. After completion, the mixture was diluted with ice cold water (20 mL) and extracted with ethyl acetate (30 mL). The organic layer was washed with cold water. The combined organic layer was washed with sodium bicarbonate and brine and

organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography to afford the tert-butyl 2,2'-((S)-1-(2-tert-butoxy-2-oxoethyl)-6-(5-tert-butoxy-5-oxopentyl)-4-(2-(4-(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)piperazin-1-yl)-2-oxoethyl)-1,4-diazepan-6-ylazanediyl)diacetate (0.140 g, 44.81% Yield).

[0357] **LCMS:** 1166 [M+H]<sup>+</sup>

[0358] Step-2: Preparation of 2,2'-((S)-6-(4-carboxybutyl)-1-(carboxymethyl)-4-(2-(4-(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)piperazin-1-yl)-2-oxoethyl)-1,4-diazepan-6-ylazanediyl)diacetic acid

[0359] To a stirred solution of tert-butyl 2,2'-((S)-1-(2-tert-butoxy-2-oxoethyl)-6-(5-tert-butoxy-5-oxopentyl)-4-(2-(4-(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)piperazin-1-yl)-2-oxoethyl)-1,4-diazepan-6-ylazanediyl)diacetate (0.140 g, 0.120 mmol, 1.0 eq) in DCM (5 mL) was added TFA (1.0 mL) drop wise at 0 °C and the mixture was stirred at RT for 40h. The progress of the reaction was monitored by TLC and LCMS. After completion, the mixture was concentrated under reduced pressure to obtain a crude residue which was triturated with diethyl to afford 2,2'-((S)-6-(4-carboxybutyl)-1-(carboxymethyl)-4-(2-(4-(3-(4-(4-(2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-3-yl)phenoxy)propyl)piperazin-1-yl)-2-oxoethyl)-1,4-diazepan-6-ylazanediyl)diacetic acid as trifluoroacetate salt (0.003 g, 2.6%).

[0360] **LCMS:** 942 [M+H]<sup>+</sup>

[0361] **<sup>1</sup>H NMR:** (400 MHz, DMSO-d<sub>6</sub>) 9.71 (s., 1H), 8.24 (d, 1H), 8.03 (m, 1 H), 7.98 (d, 1H), 7.68 (m, 2H), 7.05 (m, 2H), 4.46 (t, 2H), 4.06 (m, 2H), 3.85-3.70 (m, 4H), 3.44 (m, 4H), 3.32-3.3224 (m, 8H), 2.75-2.30 (m, 16H), 1.82 (m, 2H), 1.56 (m, 2H), 1.25 (m, 2H)

### ***Biological Examples***

- 1. Inhibition of FAP $\alpha$  by test compounds was assessed by in vitro enzymatic activity assays.**



FAP $\alpha$  enzymatic exopeptidase (dipeptidase) activity assay.

[0362] To assay the baseline of FAP $\alpha$  enzymatic exopeptidase activity, 20 ng of recombinant human FAP $\alpha$  (rhFAP $\alpha$ , R&S system, #3715-SE) or mouse FAP $\alpha$  (rmFAP $\alpha$ , R&S system, # 8647-SE) was incubated with 100  $\mu$ M of Z-Gly-Pro-AMC peptide (BACHEM, #L-1145) in a FAP $\alpha$  assay buffer (50 mM Tris pH 7.4, 100 mM NaCl, 0.1 mg/ml bovine serum albumin) for 1 h at 37 °C protected from light in 96-well black plates (Nunc, #237108).

[0363] To assay the FAP $\alpha$  enzymatic exopeptidase activity inhibition by the compounds of the invention, certain test compounds were pre-incubated with the enzyme for 15 min at 37 °C before starting the reaction by substrate addition. 7-Amino-4-Methylcoumarin (AMC) release was detected by measuring fluorescence at Ex/Em 380/460nm using a Multifunction Microplate Reader (Synergy 4, Biotek). All measurements were carried out as a single point in two or three independent experiments. Val-boroPro, a non-specific prolyl peptidase inhibitor, was used as a positive control.

[0364] For the calculations, the average measurements from reactions containing only vehicle and substrate, without enzyme, were used as a blank and were subtracted from the rest of the measurements. Percent inhibition was calculated using the average measurements from reactions containing vehicle, enzyme, and substrate as the maximum of enzymatic activity. The half maximal inhibitory concentration (IC<sub>50</sub>) for the rhFAP $\alpha$  or rmFAP $\alpha$  enzymatic exopeptidase activity of certain test compounds, reference compounds and Val-boroPro were determined, as shown in Table 2.

FAP $\alpha$  enzymatic endopeptidase (collagenase) activity assay.

[0365] To assay the baseline of FAP $\alpha$  enzymatic endopeptidase activity, 50 ng of recombinant human FAP $\alpha$  (rhFAP $\alpha$ ) (R&S system, #3715-SE) diluted in FAP $\alpha$  assay buffer (50 mM Tris pH 7.4, 100 mM NaCl, 0.1 mg/ml bovine serum albumin) was incubated with 5  $\mu$ g of substrate DQ-collagen (Molecular Probes #D 12060) with for 5 h at 37 °C and protected from light in 96-well black plates (Nunc, #237108).

[0366] To assay the FAP $\alpha$  enzymatic endopeptidase activity inhibition by the compounds of the invention, certain test compounds were pre-incubated with the enzyme for 30 min at 37 °C before starting the reaction by substrate addition. Collagen hydrolysis was determined by

measuring fluorescence at Ex/Em 495/515 nm using a multifunction Microplate Reader (Synergy 4, Biotek). All measurements were carried out as a single point in two or three independent experiments. Val-boroPro, a non-specific prolyl peptidase inhibitor, was used as a positive control. The half maximal inhibitory concentration ( $IC_{50}$ ) for the rhFAP $\alpha$  enzymatic endopeptidase activity (as determined by the collagenase assay) of certain test compounds, reference compounds and Val-boroPro were determined, as shown in Table 2.

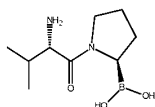
Table 2: Exopeptidase or endopeptidase inhibition of FAP $\alpha$  by test compounds.

| Compound number | rhFAP (exo $IC_{50}$ , nM) | rhFAP (endo $IC_{50}$ , nM) |
|-----------------|----------------------------|-----------------------------|
| 1               | +++                        | NT                          |
| 2               | ++                         | NT                          |
| 3               | +++                        | NT                          |
| 4               | ++                         | NT                          |
| 161             | +++                        | NT                          |
| 162             | +++                        | NT                          |
| 179             | ++                         | NT                          |
| 190             | ++                         | NT                          |
| 191             | ++                         | NT                          |
| 192             | +++                        | NT                          |
| 176             | +++                        | NT                          |
| 193             | +++                        | NT                          |
| ValboroPro      | +                          | +                           |
| Ref. Comp.1     | ++                         | ++                          |
| Ref. Comp.2     | ++                         | NT                          |
| Ref. Comp.3     | ++                         | +                           |
| Ref. Comp.4     | +                          | NT                          |
| Ref. Comp.6     | ++                         | NT                          |
| Ref. Comp.5     | ++                         | NT                          |

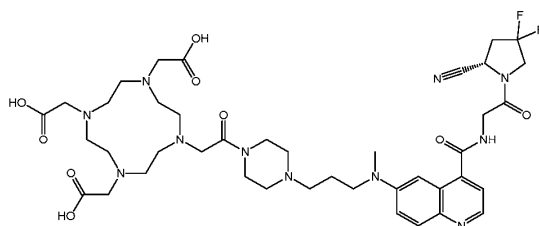
Ref. Comp. 1: FAPI-46 as described in Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-1429;  
 Ref. Comp. 2: FAPI-46-FITC adapted from Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-1429;  
 Ref. Comp. 3: FAP-2286 as described in Zboralski, D., et al., Eur J Nucl Med Mol Imaging, 2022. 49: p. 3651–3667;  
 Ref. Comp. 4: FAP-2286-FITC adapted from Zboralski, D., et al., Eur J

Nucl Med Mol Imaging, 2022. 49: p. 3651–3667; Ref. Comp. 5: 3BP-4663 as described in WO2023002045 A1; Ref. Comp. 6: 3BP-4663-FITC adapted from WO2023002045 A1; for IC<sub>50</sub>: +++ refers to IC<sub>50</sub> < 1 nM; ++ refers to 1 < IC<sub>50</sub> < 100 nM; + refers to IC<sub>50</sub> > 100 nM; rhFAPα: recombinant human fibroblast activation protein alpha; rmFAPα: recombinant mouse fibroblast activation protein alpha; endo: endopeptidase; exo: exopeptidase; NT: not tested.

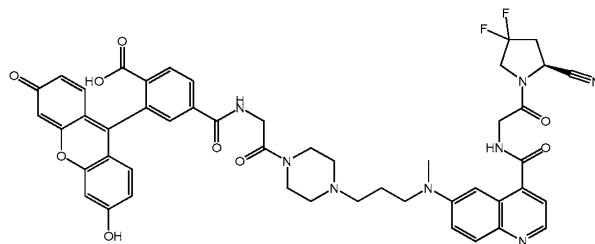
ValboroPro



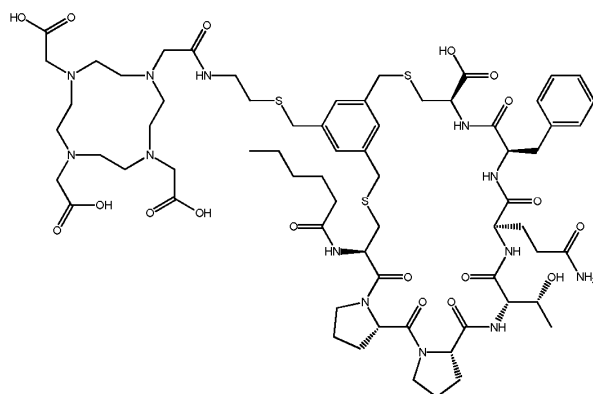
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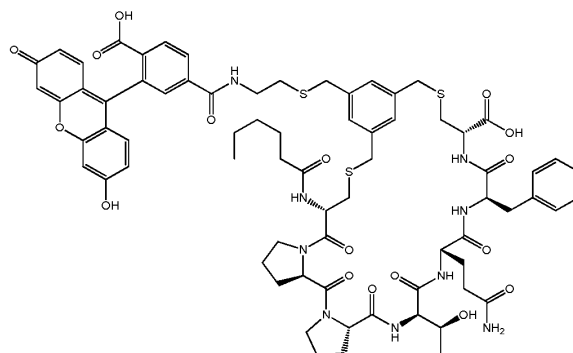
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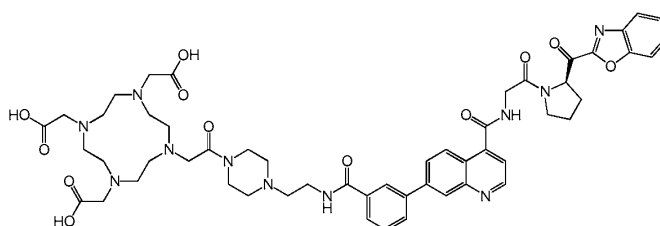
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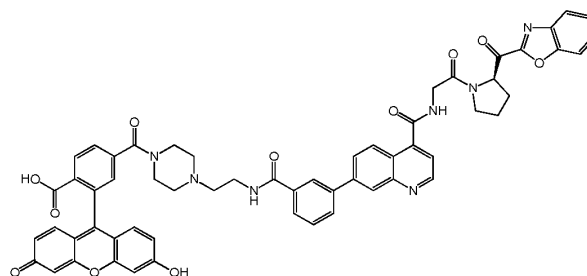
Ref. Comp.4



Ref. Comp.5



Ref. Comp.6



## 2. Selectivity of the inhibition of FAP $\alpha$ by test compounds was assessed compared to other prolyl oligopeptidase family S9 members: DPPIV, DPP8, DPP9 and PREP.

### DPPIV enzymatic activity assay

**[0367]** To assay the baseline dipeptidyl peptidase-4 (DPPIV) activity, 40 ng of recombinant human DPPIV (rhDPPIV) (R&S system, #1180-SE) was incubated with 400  $\mu$ M of H Gly-Pro-pNA substrate (BACHEM, #L-1880) in a DPPIV assay buffer (25 mM Tris, pH 8.3) for 30 min at 37 °C protected from the light in 96-well black plates (Nunc, #237108).

**[0368]** To assay the DPPIV inhibition by the compounds of the invention, certain test compounds were pre-incubated with the enzyme for 15 min at 37 °C before starting the reaction by substrate. Para-nitroaniline (pNA) release was detected by measuring absorbance at 405 nm

using a Multifunction Microplate Reader (Synergy 4, Biotek). All measurements were carried out as a single point in two or three independent experiments. Val-boroPro, a non-specific prolyl peptidase inhibitor, was used as a positive control.

#### DPP8 enzymatic activity assay

**[0369]** To assay the baseline dipeptidyl peptidase 8 (DPP8) activity, 10 ng of recombinant human DPP8 (rhDPP8) (Abcam, #ab79662) was incubated with 100  $\mu$ M of H-Gly-Pro-AMC peptide (BACHEM, #L-1215) in a DPP8 assay buffer (25 mM HEPES, pH 8.0, 0.1 mg/ml bovine serum albumin) for 30 min at 37 °C in 96-well black plates (Nunc, #237108).

**[0370]** To assay the DPP8 activity inhibition by the compounds of the invention, certain test compounds were pre-incubated with the enzyme for 15 min at 37 °C before starting the reaction by substrate addition. 7-Amino-4-Methylcoumarin (AMC) release was detected by measuring fluorescence at Ex/Em 380/460 nm using a Multifunction Microplate Reader (Synergy 4, Biotek). All measurements were carried out as a single point in two or three independent experiments. Val-boroPro, a non-specific prolyl peptidase inhibitor, was used as a positive control.

#### DPP9 enzymatic activity assay

**[0371]** To assay the baseline dipeptidyl peptidase 9 (DPP9) activity, 10 ng of recombinant human DPP9 (rhDPP9) (R&S system, #5419-SE) was incubated with 100  $\mu$ M of H-Gly-Pro-AMC peptide (BACHEM, #L-1215) in a DPP9 assay buffer (25 mM HEPES, pH 8.0, 0.1 mg/ml bovine serum albumin) for 30 min at 37 °C in 96-well black plates (Nunc, #237108).

**[0372]** To assay the DPP9 activity inhibition by the compounds of the invention, certain test compounds were pre-incubated with the enzyme for 15 min at 37 °C before starting the reaction by substrate addition. 7-Amino-4-Methylcoumarin (AMC) release was detected by measuring fluorescence at Ex/Em 380/460 nm using a Multifunction Microplate Reader (Synergy 4, Biotek). All measurements were carried out as a single point in two or three independent experiments. Val-boroPro, a non-specific prolyl peptidase inhibitor, was used as a positive control.

#### PREP enzymatic activity assay

**[0373]** To assay the baseline prolyl endopeptidase (PREP) activity, 20 ng of recombinant human PREP (rhPREP) (R&S system, #4308-SE) was incubated with 100  $\mu$ M of Z-Gly-Pro-AMC

peptide (BACHEM, #L-1145) in a PREP assay buffer (25 mM Tris, 250 mM NaCl, 10 mM DTT, pH 7.5) for 30 min at 37 °C protected from light in 96-well black plates (Nunc, #237108).

**[0374]** To assay the PREP activity inhibition by the compounds of the invention, certain test compounds were pre-incubated with the enzyme for 15 min at 37 °C before starting the reaction by substrate addition. 7 Amino-4-Methylcoumarin (AMC) release was detected by measuring fluorescence at Ex/Em 380/460nm using a Multifunction Microplate Reader (Synergy 4, Biotek). All measurements were carried out as a single point in two or three independent experiments. Val-boroPro, a non-specific prolyl peptidase inhibitor, was used as a positive control.

**[0375]** To determine if test compounds were selective or if they also inhibited other prolyl peptidases from S9 family, the half maximal inhibitory concentrations (IC<sub>50</sub>) of certain test compounds, reference compounds, and Val-boroPro were determined, as shown in Table 3.

Table 3: Inhibition of other prolyl peptidases from S9 family members by test compounds.

| Compound number | rhDPPIV (exo IC <sub>50</sub> , μM) | rhDPP8 (exo IC <sub>50</sub> , μM) | rhDPP9 (exo IC <sub>50</sub> , μM) | rhPREP (exo IC <sub>50</sub> , μM) |
|-----------------|-------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| 1               | +                                   | +                                  | +                                  | +                                  |
| 2               | +                                   | +                                  | +                                  | +                                  |
| 3               | NT                                  | NT                                 | NT                                 | NT                                 |
| 4               | NT                                  | NT                                 | NT                                 | NT                                 |
| 161             | +                                   | +++                                | +++                                | +                                  |
| 162             | NT                                  | NT                                 | NT                                 | NT                                 |
| 179             | ++                                  | +++                                | +++                                | ++                                 |
| 190             | NT                                  | NT                                 | NT                                 | NT                                 |
| 191             | +                                   | NT                                 | ++                                 | +                                  |
| 192             | +                                   | NT                                 | +++                                | +                                  |
| 176             | +                                   | NT                                 | +                                  | +                                  |
| 193             | +                                   | NT                                 | +++                                | +                                  |
| ValboroPro      | +++                                 | +++                                | +++                                | ++                                 |
| Ref. Comp.1     | +                                   | +                                  | +++                                | +                                  |
| Ref. Comp.2     | NT                                  | NT                                 | NT                                 | NT                                 |
| Ref. Comp.3     | +                                   | +                                  | ++                                 | +                                  |
| Ref. Comp.4     | NT                                  | NT                                 | NT                                 | NT                                 |

|                |    |    |    |     |
|----------------|----|----|----|-----|
| Ref.<br>Comp.6 | NT | NT | NT | NT  |
| Ref.<br>Comp.5 | +  | +  | ++ | +++ |

Ref. Comp. 1: FAPI-46 as described in Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-1429; Ref. Comp. 2: FAPI-46-FITC adapted from Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-1429; Ref. Comp. 3: FAP-2286 as described in Zboralski, D., et al., Eur J Nucl Med Mol Imaging, 2022. 49: p. 3651–3667; Ref. Comp. 4: FAP-2286-FITC adapted from Zboralski, D., et al., Eur J Nucl Med Mol Imaging, 2022. 49: p. 3651–3667; Ref. Comp. 5: 3BP-4663 as described in WO2023002045 A1; Ref. Comp. 6: 3BP-4663-FITC adapted from WO2023002045 A1; for IC<sub>50</sub>: +++ refers to IC<sub>50</sub> < 1 μM; ++ refers to 1 < IC<sub>50</sub> < 10 μM; + refers to IC<sub>50</sub> > 10 μM; rhDPPIV: recombinant human dipeptidyl peptidase-4; rhDPP8: recombinant human dipeptidyl peptidase 8; rhDPP9: recombinant human dipeptidyl peptidase 9; rhPREP: prolyl endopeptidase, NT: not tested.

### 3. Validation of selective PRXS-AMC substrate for FAP $\alpha$ activity measurements

[0376] FAP $\alpha$  activity can be measured by a general fluorescence intensity assay for dipeptidyl-peptidases using a peptide substrate attached to a chemically quenched dye, such as Ala-Pro-7-amino-4-trifluoromethyl-coumarin (AFC) or a substrate containing the consensus Gly-Pro dipeptide such as Z-Gly-Pro-AMC (Levy, M.T., et al., Hepatology, 1999, 29(6): 1768-78; Santos, A.M., et al., J Clin Invest, 2009, 119(12): 3613-25; Park, J.E., et al., J Biol Chem, 1999, 274(51): 36505-12; Niedermeyer, J., et al., Mol Cell Biol, 2000, 20(3): 1089-94; Narra, K., et al., Cancer Biol Ther, 2007, 6(11): 1691-9; Lee, K.N., et al., J Thromb Haemost, 2011, 9(5): 987-96; Li, J., et al., Bioconjug Chem, 2012, 23(8): 1704-11). These substrates are likely targeted also by other circulating proline-specific endopeptidases such as PREP that could be present in the reaction. By contrast, a proprietary substrate reagent, named PRXS-AMC, can specifically monitor FAP $\alpha$  activity.

[0377] To validate the high selectivity of this proprietary substrate, enzymatic activity assays for FAP, DPPIV, PREP and DPP9 were carried out using Z-Gly-Pro-AMC or PRXS-AMC as described in Examples 1 and 2.

[0378] To assay FAP $\alpha$ , DPPIV, DPP9 and PREP enzymatic activities, human recombinant enzymes were used at 5, 2.5, 2.5 and 5 nM final concentrations, respectively. Z-Gly-Pro-AMC or

PRXS-AMC were used at 25, 50, 100 and 200  $\mu$ M final concentrations. Reactions were carried out for 60 min at 37 °C and were protected from light. AMC release was detected by measuring fluorescence at Ex/Em 380/460 nm using a Synergy 4 multifunction microplate reader (Biotek) in kinetic mode. Measurements were performed as a single point and raw fluorescent data in the form of relative fluorescent units (RFU) were plotted versus time using the GraphPad Prism v6.02 software.

[0379] Resulting fluorescence over time for PRXS-AMC and Z-gly-pro-AMC in the presence of rhFAP $\alpha$  is shown in FIG. 1A and FIG. 1B, respectively; resulting fluorescence over time for PRXS-AMC and Z-gly-pro-AMC in the presence of rhPREP is shown in FIG. 2A and FIG. 2B, respectively; and resulting fluorescence over time for PRXS-AMC in the presence of rhDPPIV or rhDPP9 is shown in FIG. 3A and FIG. 3B, respectively.

[0380] PRXS-AMC is processed to a lesser extent than Z-Gly-Pro-AMC by the closely related prolyl oligopeptidase PREP at similar concentrations (see FIGs. 2A-2B). PRXS-AMC is not processed by DPPIV or DPP9 (FIGs. 3A-3B). In addition, PRXS-AMC showed an improved solubility in aqueous buffers.

#### 4. Enzymatic activity in mouse plasma

[0381] Whole blood from Balb/c mice was harvested into BD Microtainer® tubes (K2) EDTA (#365974, Becton Dickinson and Co.) via terminal cardiac puncture. The blood sample was immediately centrifuged at approximately 9,000 g at 4 °C for 5 minutes. Plasma was separated and stored at -80 °C in aliquots of 300  $\mu$ L.

##### FAP $\alpha$ enzymatic activity in mouse plasma

[0382] To assay the baseline enzymatic exopeptidase activity of mouse circulating FAP $\alpha$  (cFAP), 5  $\mu$ L of thawed mouse plasma was incubated with 100  $\mu$ M of PRXS-AMC substrate in a cFAP buffer (100 mM Tris-HCl, 400 mM NaCl, 50 mM salicylic acid, 1 mM EDTA, pH 7.5) for 1 h at 37 °C protected from light in 96-well black plates (Nunc, #237108).

[0383] To determine the inhibitory potency of the compounds of the invention on cFAP activity in mouse plasma, certain test compounds were pre-incubated with mouse plasma for 15 min at 37 °C before starting the reaction by substrate addition in 96-well black plates. The assay was performed for 1 h at 37 °C protected from light. 7-Amino-4-Methylcoumarin (AMC) release



was detected measuring fluorescence at an excitation wavelength of 380 nm and an emission wavelength of 460 nm using a Multifunction Microplate Reader (Synergy 4, Biotek). All measurements were carried out as a single point in two or three independent experiments. Val-boroPro, a non-specific prolyl peptidase inhibitor, was used as a positive control. The half maximal inhibitory concentration (IC<sub>50</sub>) for the cFAP enzymatic exopeptidase activity of certain test compounds, reference compounds and Val-boroPro were determined.

#### DPPIV enzymatic activity in mouse plasma

[0384] To assay the baseline enzymatic exopeptidase activity of mouse circulating dipeptidyl peptidase-4 (cDPPIV), 5 µL of thawed, mouse plasma incubated with 100 µM of dipeptide substrate H-Gly-Pro-AMC (Bachem, #L-1225) in a cFAP buffer (100 mM Tris-HCl, 400 mM NaCl, 50 mM salicylic acid, 1 mM EDTA, pH 7.5) for 30 min at 37 °C protected from light in 96-well black plates (Nunc, #237108).

[0385] To determine the inhibitory potency of the compounds of the invention on cDPPIV activity in mouse plasma, certain test compounds were pre-incubated with mouse plasma for 15 min at 37 °C before starting the reaction by substrate addition in 96-well black plates. The assay was performed for 30 min at 37 °C protected from light. 7-Amino-4-Methylcoumarin (AMC) release was detected measuring fluorescence at an excitation wavelength of 380 nm and an emission wavelength of 460 nm using a Multifunction Microplate Reader (Synergy 4, Biotek). All measurements were carried out as a single point in two or three independent experiments. Val-boroPro, a non-specific prolyl peptidase inhibitor, was used as a positive control. The half maximal inhibitory concentration (IC<sub>50</sub>) for the cDPPIV enzymatic exopeptidase activity of certain test compounds, reference compounds and Val-boroPro were determined.

### **5. Surface plasmon resonance assay**

[0386] The binding kinetics of certain test compounds of the invention to an immobilized human FAPα (hFAP) were measured by Surface Plasmon Resonance (SPR) and calculated using single cycle kinetic measurements.

[0387] SPR studies were performed using a Biacore™ 8K (Cytiva) and Series S CM5 sensor chips. The hFAP protein (AcroBiosystems, #FAP-H5244) was diluted in 10 mM sodium acetate buffer, pH4.5 and directly immobilized onto the flow cells at 25°C using the Amine Coupling Kit

(Cytiva, BR100050) according to the manufacturer's recommendations. A preinstalled program for immobilization was used with an immobilization level of 7000 - 8000 RU.

**[0388]** To measure their affinity for the hFAP protein, a series of concentrations of test compounds ranging from 0.00153 - 100 nM were prepared in running buffer (10 mM Na<sub>2</sub>HPO<sub>4</sub> x 12 H<sub>2</sub>O, 2 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl, 2.7 mM KCl, pH7.4 with 0.05% Tween-20) and injected over the sensor surface for 120 seconds at a flow rate of 30 µl/min in single-cycle kinetics at 25 °C. The dissociation was monitored for 1800 seconds. The SPR raw data in the form of resonance units (RU) were plotted as sensorgrams using the Biacore T200 control software. The association rate ( $k_{on}$ ), dissociation rate ( $k_{off}$ ), dissociation constant ( $K_D$ ) were calculated from blank-normalized SPR data using the kinetics 1:1 binding model from the Biacore T200 evaluation software (GE Healthcare Life Sciences). Half-time values ( $t_{1/2}$ ) numbers were calculated from the respective  $k_{off}$  values ( $t_{1/2} = \ln(2)/k_{off}$ ).

## **6. Generation of stable cell lines overexpressing human FAP**

**[0389]** The ORF of human FAP (hFAP) was subcloned from the pCMV6-XL5 vector into the pLenti-CMV-IRES-Puro vector using the restriction and ligation cloning method. To introduce a DYKDDDDK sequence tag at the cytoplasmic N-terminal and restriction enzyme sites, the insert was amplified by PCR using EcoRI-FAP-FW and PmeI-FAP-RV primers (Macrogen Inc.) plus the Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific, F530L) according to the manufacturer's instructions. The final pLenti-CMV-Flag-FAP-IRES-Puro construct was assembled from the insert and the linearized vector using the T4 DNA Ligase (New England Biolabs, M0202L) as per instructions then transformed in *E. coli* Stbl3 (Invitrogen, C737303). Resulting clones were verified by sequencing later.

**[0390]** To produce lentiviral particles, wild-type (WT) HEK293T cells were seeded in 10-cm tissue culture dishes using DMEM-F12 culture medium supplemented with fetal bovine serum and penicillin/streptomycin cocktail and cultured overnight (80% confluence) at 37 °C and 5% CO<sub>2</sub> in a humidified incubator. At the next day, HEK293T cells were transfected with the pLenti-CMV-Flag-FAP-IRES-Puro vector using the Lenti-X™ Packaging Single Shots system (Takara Bio Inc.) following the manufacturer's instructions. Lentivirus-containing supernatants were harvested 48 h after transfection and centrifuged to eliminate the cell debris. Lentiviral particles were concentrated using the Lenti-X Concentrator (Clontech Laboratories Inc.) according the

manufacturer's instructions. Lentiviral particles were resuspended in DMEM-F12 medium and stored at -80°C until use.

**[0391]** For transduction with lentiviral particles, the WT HEK293 or HT-1080 target cells (ATCC) were seeded in 24-well plates in DMEM-F12 culture medium supplemented with fetal bovine serum and penicillin/streptomycin cocktail and cultured overnight at 37 °C and 5% CO<sub>2</sub> in a humidified incubator. WT target cells were cultured overnight and then transduced with different lentiviral titers diluted in DMEM-F12 medium supplemented with tetracycline-free fetal bovine serum and 5 µg/mL polybrene. Plates were centrifuged at 1,200 g for 60 min at room temperature and then incubated for 6 h at 37°C and 5% CO<sub>2</sub> in a humidified incubator before replacing the transduction medium with fresh complete medium. Cells incubated only with supplemented DMEM-F12 medium plus polybrene were used as negative control. 48 h post-transduction, HEK293-hFAP and HT-1080-hFAP were selected by incubation in complete medium supplemented with 1 µg/mL Puromycin for 7 days.

**[0392]** For the analysis by flow cytometry of surface hFAP expression in the selected cells, WT HEK293, WT HT-1080, HEK293-hFAP and HT-1080-hFAP cells were cultured as a sub-confluent monolayer in T75 flasks for 48-72h and then detached using StemPro™ Accutase™ (Thermo Fisher Scientific, #A1110501) and transferred to microfuge tubes. The cells were washed once with PBS and then stained with Live/dead™ Fixable Aqua Dead Cell dye (Thermo Fisher Scientific, #L34966) according to the manufacturer's instructions. Then, cells were washed once with PBS and incubated with Human FAP Alexa Fluor® 647-conjugated Antibody (R&D Systems, #FAB3715R) in PBE buffer (PBS 1x supplemented with 0.5% bovine serum albumin (Rockland Immunochemicals inc., #BSA-50) plus 2mM EDTA) for 20 min at 4°C in the dark. Cells were washed with PBE once and resuspended in the same buffer. Stained cells were analyzed by flow cytometry using a MACSQuant Analyzer 10 (Miltenyi Biotec).

**[0393]** Acquired raw data in the form of hFAP positive (hFAP<sup>+</sup>) cells and mean fluorescence intensity of anti-hFAP Alexa Fluor 647 (anti-FAP-AF647 MFI) were plotted as dot-plots and histograms respectively.

**[0394]** Resulting percentages of hFAP<sup>+</sup> cells in WT HEK293 and selected HEK293-hFAP cells are shown in the Figure 4A-B. Resulting percentages of hFAP<sup>+</sup> cells in WT HT-1080 and selected HT-1080-hFAP cells are shown in the Figure 4C-D.

[0395] WT HEK293 and WT HT-1080 cells do not express detectable levels of hFAP on surface (see FIG. 4A and 4C respectively). Selected HEK293-hFAP cells corresponds to a polyclonal population with high and homogeneous expression of hFAP on surface (see FIG. 4B). Selected HT-1080-hFAP cells corresponds to a polyclonal population with lower and heterogeneous expression of hFAP on surface (see FIG.4D).

[0396] For the analysis of total hFAP expression by western blot, HEK293-hFAP or HT-1080-hFAP cells were cultured as a sub-confluent monolayer in T75 flasks for 48 or 72h at 37°C and 5% CO<sub>2</sub> in a humidified incubator, respectively. The culture medium was removed and cells were washed with ice-cold PBS 1x and homogenized immediately by scrapping in presence of Cell Lysis Buffer 1x (Cell Signaling Technology, #9803) supplemented with cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail (Roche, #4693159001). Homogenates were clarified by centrifugation and protein samples were quantified using the BCA Protein Assay Kit (Thermo Fisher Scientific, #23225). Protein samples were boiled in Laemmli protein sample buffer (Bio-Rad, #161-0747) supplemented with  $\beta$ -mercaptoethanol at 96°C during 5 min.

[0397] For immunoblot analysis, aliquots of each protein sample containing 30ug total proteins were loaded in 10% Acrilamide/bis-acrilamide gels and separated by SDS-PAGE. Gels were transferred to PVDF membranes (Bio-Rad, #1620177).

[0398] For immunodetection, membranes were blocked in TBS 1x buffer with 3% BSA and 0.1% Tween for 1 h at room temperature. The sheep polyclonal anti- human FAP $\alpha$  antibody (R&D systems, #AF3715), the rabbit monoclonal anti- human FAP $\alpha$  antibody (Abcam, #ab207178) or the mouse anti- $\beta$ -actin (Sigma-Aldrich, #A5441) were used as primary antibodies for or overnight at 4 °C or 1h at room temperature. The donkey polyclonal anti-sheep IgG HRP-conjugated antibody (R&D systems, #HAF016), the goat polyclonal anti-rabbit IgG HRP-conjugated antibody (Rockland Immunochemicals, #611-1322-0100) or the goat anti-mouse IgG HRP-conjugated antibody (Rockland Immunochemicals, #610-1302) were used as secondary antibodies for 1h at room temperature. Proteins were detected using the ECL Western Blotting Substrate (Thermo Fisher Scientific, #32106) and visualized using a ChemiDoc™ Imaging System (Bio-Rad) and Image lab software v5.2.1 build 11.

[0399] The cell surface expressions of FAP $\alpha$  in WT HEK293 and WT HT-1080 cells determined by flow cytometry are shown in FIG. 4A and 4C, respectively. The cell surface

expressions of human FAP $\alpha$  in transduced HEK293-hFAP and HT-1080-hFAP cells determined by flow cytometry are shown in FIG. 4B and 4D, respectively. The total expressions of FAP $\alpha$  in above wild type and transduced cell lines determined by Western Blot are shown in FIG. 5.

[0400] The FAP $\alpha$  protein is not expressed WT HEK293 and WT HT-1080 cells (see FIG. 4A and 4C). After transduction, the human FAP $\alpha$  is expressed significantly in higher extent in HEK293-hFAP cells than HT-1080-hFAP cells (see FIG. 4B, 4D and 5).

## 7. Cell FAP binding assays

[0401] To investigate the binding kinetics of compounds of the invention to the cell surface FAP $\alpha$ , the dissociation constant ( $K_D$ ) of certain test compounds was determined by flow cytometry in the HEK-hFAP or HT-1080-hFAP cell lines (generated above) using direct or competition methods as described S.A. Hunter & J.R. Cochran (*Methods in Enzymology*, Vol. 580, 2016, pp.21-44).

[0402] For this purpose, cell lines were cultured as a sub-confluent monolayer in T75 flasks for 48-72h as described above. At the day of the experiment, cells were detached using StemPro Accutase (Thermo Fisher Scientific, #A1110501), washed and counted using a LUNA-II Automated Cell Counter (Logos Biosystems). Cells were stained with LiveDead Aqua solution (Molecular Probes, #L34957) following the manufacturer's indications. After staining, cells were diluted in ice-cold PBE buffer to a final concentration of  $1.6 \times 10^6$  cells/mL and 100 $\mu$ L of the cell suspension were transferred to a V-shaped bottom, polypropylene 96-well plate (Thermo Scientific, #249935).

[0403] A direct method was used to assess the  $K_D$  of fluorescent compounds. For this, cells were incubated for 1h at 4°C in the dark with different concentrations of the examined compound from 0 to 1 $\mu$ M diluted in ice-cold PBE buffer. After incubation, cells were washed once with ice-cold PBE buffer and then centrifuged to remove the supernatant containing the non-bound compound. Cells were resuspended in ice-cold PBE and analyzed by flow cytometry in a MACSQuant Analyzer 10 (Miltenyi Biotec). Cells were gated to include only single, viable cells and 10,000 events were analyzed per condition. The percentage of fluorescently labeled cells and mean fluorescence intensity of each sample were calculated and corrected for autofluorescence (expression only) using the FlowJo software v10.4.

[0404] The  $K_D$  values of fluorescent test compounds were determined plotting the mean fluorescence intensity against the compound concentration (log scale) of each sample and fitting a sigmoidal curve using non-linear regression analysis (dose response-inhibition,  $\log_{inh}$  vs normalized response, variable slope) using GraphPad Prism v6.02 software.

[0405] In order to assess the  $K_D$  of non-fluorescent compounds of the invention, a competition method was established. For this purpose, cells were incubated for 1h at 4°C in the dark with different concentrations of the non-fluorescent test compound (from 0 to 1μM diluted in ice-cold PBE buffer) plus a constant concentration of the corresponding fluorescent competitor. The competitor was used at a value lower than its  $K_D$ , such that it can still be detected but is able to be competed off. After incubation, cells were washed once with ice-cold PBE buffer and then centrifuged to remove the supernatant containing the non-bound compounds. Cells were resuspended in ice-cold PBE and analyzed by flow cytometry in a MACSQuant Analyzer 10 (Miltenyi Biotec) as described above. The percentage of fluorescently labeled cells and mean fluorescence intensity (MFI) of each sample were calculated and corrected for autofluorescence using the FlowJo software v10.4.

[0406] The  $IC_{50}$  values of non-fluorescent test compounds were determined plotting the MFI against the compound concentrations (log scale) of each sample and fitting a sigmoidal curve using non-linear regression analysis (dose response-inhibition,  $\log_{inh}$  vs normalized response, variable slope) using GraphPad Prism v6.02 software. Then, the  $K_D$  were calculated using the Cheng–Prusoff equation (*Biochem Pharmacol*, Vol. 22, 1973, pp.3099-3108):

$$K_D = \frac{IC_{50}}{1 + \frac{[competitor]}{K_D competitor}}$$

[0407] The binding of compounds to the cell surface FAPα was calculated as the dissociation constant ( $K_D$ ) using direct or competition methods, as shown in Table 4.

Table 4 – Binding of test compounds to the cell surface FAPα.

| Compound number | Method | $K_D$ HEK293-hFAP @ 4°C (nM) |
|-----------------|--------|------------------------------|
| 1               | C      | ++                           |
| 2               | NT     | NT                           |
| 3               | D      | +++                          |

|             |    |     |
|-------------|----|-----|
| 4           | D  | +++ |
| 161         | NT | NT  |
| 162         | D  | +++ |
| 179         | NT | NT  |
| 190         | D  | +++ |
| 191         | NT | NT  |
| 192         | NT | NT  |
| 176         | NT | NT  |
| 193         | NT | NT  |
| ValboroPro  | NT | NT  |
| Ref. Comp.1 | C  | +++ |
| Ref. Comp.2 | D  | ++  |
| Ref. Comp.3 | NT | NT  |
| Ref. Comp.4 | D  | +   |
| Ref. Comp.6 | D  | ++  |
| Ref. Comp.5 | NT | NT  |

Ref. Comp. 1: FAPI-46 as described in Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-1429; Ref. Comp. 2: FAPI-46-FITC adapted from Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-1429; Ref. Comp. 3: FAP-2286 as described in Zboralski, D., et al., Eur J Nucl Med Mol Imaging, 2022. 49: p. 3651–3667; Ref. Comp. 4: FAP-2286-FITC adapted from Zboralski, D., et al., Eur J Nucl Med Mol Imaging, 2022. 49: p. 3651–3667; Ref. Comp. 5: 3BP-4663 as described in WO2023002045 A1; Ref. Comp. 6: 3BP-4663-FITC adapted from WO2023002045 A1; for  $K_D$ : +++ refers to  $K_D < 25$  nM; ++ refers to  $25 < K_D < 50$  nM; + refers to  $K_D > 50$  nM;  $K_D$ : dissociation constant; D: direct method; C: competition method; NT: not tested.

## 8. Cellular uptake & retention assay

[0408] In order to investigate the internalization of compounds of the invention into FAP $\alpha$ -expressing cells, the cellular uptake and retention of certain test compounds were determined by flow cytometry in the HEK-hFAP or HT-1080-hFAP cell lines generated above.

[0409] For this purpose, cell lines were seeded in 12-well plates and incubated for 48-72h at 37°C, 5% CO<sub>2</sub> in a humidified incubator. At the day of the experiment, the culture medium was

replaced with assay medium (DMEM-F12 supplemented with 1% FBS) plus a constant concentration of the fluorescent test compound. Cells were allowed to interact with the test compounds for 1h at 37°C and then the medium containing the unbound fraction was removed and replaced with fresh assay medium. The fluorescence of the cell-bound fraction was determined immediately (time point = 0h) or after 5, 16 and 24h by flow cytometry to determine the uptake and retention of test compounds.

**[0410]** To perform the flow cytometry, cells were detached using StemPro Accutase (Thermo Fisher Scientific, #A1110501), diluted in culture medium for inactivation and then collected by centrifugation. Cells were washed once with PBS1x and then stained with LiveDead Aqua solution (Molecular Probes, #L34957) following the manufacturer's indications. After staining, cells were resuspended in PBE at room temperature and analyzed by flow cytometry in a MACSQuant Analyzer 10 (Miltenyi Biotec). Cells were gated to include only single, viable cells and 10,000 events were analyzed per condition. The percentage of fluorescently labeled cells and mean fluorescence intensity of each sample were calculated and corrected for autofluorescence (expression only) using the FlowJo software v10.4.

**[0411]** For uptake and retention analysis, the integrated mean fluorescence intensity (iMFI) was introduced as a metric to represent the total fluorescence of the remaining bound fraction at different time points after removal of the unbound fraction. The iMFI was computed by multiplying the relative frequency (% positive) of cells labeled with the fluorescent test compound with the mean fluorescence intensity (MFI) of that population as described Parisa Shooshtari et al. (*Cytometry, Vol. 77A, Issue 9, 2010, pp.873-880*). The uptake and retention of fluorescent test compounds were determined calculating the area under curve (AUC) of the iMFI across time using GraphPad Prism v6.02 software.

**[0412]** The uptake and retention of certain test compounds in HEK-hFAP or HT-1080-hFAP cell lines was determined as shown in Table 5.

Table 5 – Uptake and retention of test compounds in FAP $\alpha$ - expressing cells.

| Compound number | AUC HEK293-hFAP @ 100nM (RUA) |
|-----------------|-------------------------------|
| 1               | NT                            |
| 2               | NT                            |
| 3               | +++                           |



|             |     |
|-------------|-----|
| 4           | ++  |
| 161         | NT  |
| 162         | +++ |
| 179         | NT  |
| 190         | ++  |
| 191         | NT  |
| 192         | NT  |
| 176         | NT  |
| 193         | NT  |
| ValboroPro  | NT  |
| Ref. Comp.1 | NT  |
| Ref. Comp.2 | +   |
| Ref. Comp.3 | NT  |
| Ref. Comp.4 | NT  |
| Ref. Comp.6 | +++ |
| Ref. Comp.5 | NT  |

Ref. Comp. 1: FAPI-46 as described in Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-1429; Ref. Comp. 2: FAPI-46-FITC adapted from Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-1429; Ref. Comp. 3: FAP-2286 as described in Zboralski, D., et al., Eur J Nucl Med Mol Imaging, 2022. 49: p. 3651–3667; Ref. Comp. 4: FAP-2286-FITC adapted from Zboralski, D., et al., Eur J Nucl Med Mol Imaging, 2022. 49: p. 3651–3667; Ref. Comp. 5: 3BP-4663 as described in WO2023002045 A1; Ref. Comp. 6: 3BP-4663-FITC adapted from WO2023002045 A1; for AUC: +++ refers to AUC > 20,000 RUA; ++ refers to 5,000 < AUC < 20,000 RUA; + refers to AUC < 5,000 RUA; AUC: area under curve; RUA: relative units of area; NT: not tested.

#### 9. Microsomal stability assay in species.

[0413] To investigate the liver metabolic stability of certain test compounds of the invention, compounds were incubated at a final concentration of 1 $\mu$ M with human (hLM) (Thermo Scientific, #HMMCPL), mouse (mLM) (Thermo Scientific, #MSMCPL) or rat (rLM) (Thermo Scientific, #RTMCPL) liver microsomes (0.5 mg/mL final concentration) and 1mM NADPH (Sigma-Aldrich, #N1630) in 100 mM phosphate buffer, pH 7.4 at 37 °C. The reactions were terminated at

0, 5, 15 and 30 min by the addition of ice-cold acetonitrile containing 50ng/mL propranolol as an internal standard. The reaction mixtures were partitioned by centrifugation at 15,000 rpm for 15 min. The resulting supernatants were analyzed for remaining percentage (%), half-life ( $t_{1/2}$ , min) and intrinsic clearance ( $CL_{int}$ ) by LC-MS/MS (Shimadzu Nexera UPLC with an AB Sciex 4500 detector). Verapamil hydrochloride (Sigma-Aldrich, #V4369) was used as a positive control in all studies.

**[0414]** Remaining fraction after 30 min of reaction and in vitro PK parameters were calculated for certain compounds, as shown in Table 6.

Table 6 – Stability of test compounds in liver microsomes of different species.

| Molecule name | HLM<br>(% Rem.<br>x 30 min) | MLM<br>(% Rem.<br>x 30 min) | RLM<br>(% Rem. x<br>30 min) | $t_{1/2}$<br>(HLM) (min) | $t_{1/2}$<br>(MLM) (min) | $t_{1/2}$<br>(RLM) (min) |
|---------------|-----------------------------|-----------------------------|-----------------------------|--------------------------|--------------------------|--------------------------|
| 1             | +                           | +                           | NT                          | +                        | +                        | NT                       |
| 2             | +                           | +                           | NT                          | +                        | +                        | NT                       |
| 3             | NT                          | NT                          | NT                          | NT                       | NT                       | NT                       |
| 4             | NT                          | NT                          | NT                          | NT                       | NT                       | NT                       |
| 161           | +                           | ++                          | NT                          | ++                       | +++                      | NT                       |
| 162           | NT                          | NT                          | NT                          | NT                       | NT                       | NT                       |
| 179           | +                           | +                           | NT                          | +                        | +                        | NT                       |
| 190           | NT                          | NT                          | NT                          | NT                       | NT                       | NT                       |
| 191           | +                           | +                           | NT                          | ++                       | ++                       | NT                       |
| 192           | +                           | +                           | NT                          | +                        | ++                       | NT                       |
| 176           | ++                          | ++                          | NT                          | ++                       | ++                       | NT                       |
| 193           | +                           | +                           | NT                          | +                        | +                        | NT                       |
| ValboroPro    | ++                          | +                           | ++                          | ++                       | ++                       | +++                      |
| Ref. Comp.1   | ++                          | +                           | ++                          | +++                      | ++                       | +++                      |
| Ref. Comp.2   | ++                          | +                           | ++                          | ++                       | ++                       | +++                      |
| Ref. Comp.3   | +                           | ++                          | NT                          | +                        | ++                       | NT                       |
| Ref. Comp.4   | NT                          | NT                          | NT                          | NT                       | NT                       | NT                       |
| Ref. Comp.6   | NT                          | NT                          | NT                          | NT                       | NT                       | NT                       |
| Ref. Comp.5   | +                           | +                           | NT                          | ++                       | +                        | NT                       |

Ref. Comp. 1: FAPI-46 as described in Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-1429;

Ref. Comp. 2: FAPI-46-FITC adapted from Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-

1429; Ref. Comp. 3: FAP-2286 as described in Zboralski, D., et al., Eur J Nucl Med Mol Imaging,

2022. 49: p. 3651–3667; Ref. Comp. 4: FAP-2286-FITC adapted from Zboralski, D., et al., Eur J

Nucl Med Mol Imaging, 2022. 49: p. 3651–3667; Ref. Comp. 5: 3BP-4663 as described in WO2023002045 A1; Ref. Comp. 6: 3BP-4663-FITC adapted from WO2023002045 A1; for %: +++ refers to % > 95%; ++ refers to 85 < % < 95 %; + refers to % < 85 %; for  $t_{1/2}$ : +++ refers to  $t_{1/2}$  > 180 min; ++ refers to 90 <  $t_{1/2}$  < 180 min; + refers to  $t_{1/2}$  < 90 min; %: remaining percentage;  $t_{1/2}$ : half-life; NT: not tested.

#### 10. Plasma stability assay in species.

[0415] To investigate the plasma stability of certain test compounds of the invention, compounds were incubated at 1  $\mu$ M final concentration in 50  $\mu$ L of plasma (having K<sub>2</sub>EDTA as anticoagulant) human, rat and mouse neat plasma at 37 °C. After 0.5, 1, 2, 4, and 5 h of incubation, respective time point samples were quenched for precipitation of plasma proteins with 250  $\mu$ L of ice-cold acetonitrile:methanol (50:50, v/v%) mixture containing 50 ng/mL propranolol as an internal standard. Then all the samples were centrifuged at 10,000 rpm and 100  $\mu$ L of supernatants were transferred to 96-deep well plate for analysis. Non-incubated samples (zero time points) were also prepared in which plasma was quenched with same chilled solvent containing internal standard. Procaine hydrochloride (#46608 Aldrich) was used as control for human plasma, Enalapril (# E6888 Aldrich) for rat plasma and Propentherline (#P8891 Aldrich) for mouse plasma.

[0416] The resulting samples were analyzed for remaining percentage (%) using Nexera X2 UHPLC system coupled to Sciex 4500 QTrap/Triple Quad mass spectrometer. Chromatographic separation was carried out on a suitable column using a linear gradient. Mass spectrometry detection was performed in positive ion mode. For the quantitative data analysis, the Sciex Analyst 1.6.1 software was used.

[0417] The stability of compounds was determined by comparing the area ratio of the respective time point (0.5, 1, 2, 4 and 5 h) sample with non-incubated (zero time point) samples. The remaining fraction of compounds after 5h post- incubation with cross-species plasmas was calculated as remaining percentage (%), as shown in Table 7.

Table 7 - Stability of test compounds in plasma of different species.

| Compound number | Rem. Fraction in human plasma (% , 5h) | Rem. Fraction in mouse plasma (% , 5h) | Rem. Fraction in rat plasma (% , 5h) |
|-----------------|----------------------------------------|----------------------------------------|--------------------------------------|
| 1               | ++                                     | ++                                     | NT                                   |
| 2               | ++                                     | ++                                     | NT                                   |

|             |     |     |     |
|-------------|-----|-----|-----|
| 3           | NT  | NT  | NT  |
| 4           | NT  | NT  | NT  |
| 161         | ++  | ++  | NT  |
| 162         | NT  | NT  | NT  |
| 179         | +   | +   | NT  |
| 190         | NT  | NT  | NT  |
| 191         | +++ | +++ | NT  |
| 192         | +++ | +   | NT  |
| 176         | ++  | ++  | NT  |
| 193         | ++  | ++  | NT  |
| ValboroPro  | +++ | +++ | NT  |
| Ref. Comp.1 | +++ | +++ | +++ |
| Ref. Comp.2 | +++ | +++ | +++ |
| Ref. Comp.3 | +   | +++ | NT  |
| Ref. Comp.4 | NT  | NT  | NT  |
| Ref. Comp.6 | NT  | NT  | NT  |
| Ref. Comp.5 | ++  | ++  | NT  |

Ref. Comp. 1: FAPI-46 as described in Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-1429; Ref. Comp. 2: FAPI-46-FITC adapted from Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-1429; Ref. Comp. 3: FAP-2286 as described in Zboralski, D., et al., Eur J Nucl Med Mol Imaging, 2022. 49: p. 3651–3667; Ref. Comp. 4: FAP-2286-FITC adapted from Zboralski, D., et al., Eur J Nucl Med Mol Imaging, 2022. 49: p. 3651–3667; Ref. Comp. 5: 3BP-4663 as described in WO2023002045 A1; Ref. Comp. 6: 3BP-4663-FITC adapted from WO2023002045 A1; for Rem. Fraction: +++ refers to Rem. Fraction > 75 %; ++ refers to 50 < Rem. Fraction < 75 %; + refers to Rem. Fraction < 50 %; %: remaining fraction; NT: not tested.

### 11. Plasma protein binding assay in species.

**[0418]** The plasma protein binding (PPB) of certain test compounds of the invention was investigated in mouse and human plasma samples and determined using equilibrium dialysis. The method comprised of a dialyzer insert (Thermo Scientific, # 89809) containing two chambers of 250  $\mu$ L volume each, separated by a semi permeable membrane. Plasma samples containing 2  $\mu$ M of test compound was aliquoted in one of the chambers and the other chamber was filled with phosphate buffer saline (PBS), pH 7.4. The assembly was incubated for 4 h at 37 °C in a water bath. At the end of incubation, an aliquot of plasma and PBS were precipitated by using ice-cold acetonitrile. The samples were vortexed for 30 s, centrifuged at 3,500 rpm for 20 min at 4 °C.

[0419] The resulting supernatant was collected and analyzed for remaining free drug by LC-MS/MS using a Shimadzu Nexera UPLC with an AB Sciex 4500 detector. Miconazole (#M3512 Aldrich) was used as positive control.

[0420] The plasma protein binding (PPB) of compounds was determined using the following equation:

$$\% \text{ bound drug} = \% \text{ total drug} - \% \text{ free drug}$$

[0421] The PPB of compounds to cross-species plasma samples was calculated as bound fraction (PPB %), as shown in Table 8.

Table 8 – Binding of test compounds to plasma proteins of different species.

| Compound number | Bound Fraction in human plasma (% , 4h) | Bound Fraction in mouse plasma (% , 4h) | Bound Fraction in rat plasma (% , 4h) |
|-----------------|-----------------------------------------|-----------------------------------------|---------------------------------------|
| 1               | +                                       | +                                       | NT                                    |
| 2               | ++                                      | ++                                      | NT                                    |
| 3               | NT                                      | NT                                      | NT                                    |
| 4               | NT                                      | NT                                      | NT                                    |
| 161             | ++                                      | ++                                      | NT                                    |
| 162             | NT                                      | NT                                      | NT                                    |
| 179             | +++                                     | +++                                     | NT                                    |
| 190             | NT                                      | NT                                      | NT                                    |
| 191             | ++                                      | +                                       | NT                                    |
| 192             | ++                                      | ++                                      | NT                                    |
| 176             | +                                       | ++                                      | NT                                    |
| 193             | ++                                      | +++                                     | NT                                    |
| ValboroPro      | NT                                      | NT                                      | NT                                    |
| Ref. Comp.1     | ++                                      | ++                                      | ++                                    |
| Ref. Comp.2     | NT                                      | NT                                      | NT                                    |
| Ref. Comp.3     | +                                       | ++                                      | NT                                    |
| Ref. Comp.4     | NT                                      | NT                                      | NT                                    |
| Ref. Comp.6     | NT                                      | NT                                      | NT                                    |
| Ref. Comp.5     | NT                                      | NT                                      | NT                                    |

Ref. Comp. 1: FAPI-46 as described in Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-1429; Ref. Comp. 2: FAPI-46-FITC adapted from Loktev, A., et al., J Nucl Med, 2019. 60(10): p. 1421-1429; Ref. Comp. 3: FAP-2286 as described in Zboralski, D., et al., Eur J Nucl Med Mol Imaging, 2022. 49: p. 3651–3667; Ref. Comp. 4: FAP-2286-FITC adapted from Zboralski, D., et al., Eur J Nucl Med Mol Imaging, 2022. 49: p. 3651–3667; Ref. Comp. 5: 3BP-4663 as described in WO2023002045 A1; Ref. Comp. 6: 3BP-4663-FITC adapted from WO2023002045 A1; for PPB: +++ refers to PPB > 75 %; ++ refers to 50 < PPB < 75 %; + refers to PPB < 50 %; PPB: plasma protein binding; NT: not tested.

## 12. Radio-labeling of selected compounds.

[0422] In order to serve as a diagnostically, therapeutically, or theragnostically active agent, a compound needs to be labeled with a radioactive isotope. The labeling procedure needs to be appropriate to ensure a high radiochemical yield and purity of the radiolabeled compound of the invention. This example shows that the exemplary compounds of the invention are appropriate for radiolabeling and can be labeled in high radiochemical yield and purity.

[0423] As a general procedure for radiolabeling of certain compounds of this invention,  $^{177}\text{Lu}$  and  $^{68}\text{Ga}$  are chelated after pH adjustment with sodium acetate. The reaction mixtures are heated to 95°C for 10 min, and the completeness of the reaction is checked by radio-high-performance liquid chromatography. The  $^{177}\text{Lu}$ -labeled exemplary compounds can be used directly for in vitro studies or diluted with 0.9% saline and directly applied for organ distribution studies. The  $^{68}\text{Ga}$ -labeled exemplary compounds are processed by solid phase extraction before PET imaging.

[0424] As a general procedure for the in vitro stability of radio- labeled compounds in serum, a  $^{177}\text{Lu}$ - or  $^{68}\text{Ga}$ - labeled exemplary compound is incubated with human or mouse serum for 1, 2, 4 and 24 h at 37 °C. At the end of each incubation, resulting samples are precipitated and supernatants are subjected to radio chromatographic analysis.

## 13. Radioligand binding studies.

[0425] The FAP-binding affinities of certain radiolabeled compounds of the invention are investigated using direct or competition scintigraphy methods in the HEK-hFAP or HT-1080-hFAP cell lines generated above.

[0426] For this purpose, cell lines are seeded and incubated for 48-72h (final confluence of approximately 80 – 90 %) at 37°C, 5% CO<sub>2</sub> in a humidified incubator. At the day of the experiment, the culture medium is replaced with assay medium (DMEM-F12 supplemented with 1% FBS) plus the radio-labelled exemplary compound. The radio-labelled compound is allowed to interact with cells for different intervals ranging from 10 min to 24 h at 37°C.

[0427] Competition experiments are performed by simultaneous exposure to unlabeled precursor ( $10^{-5}$  to  $10^{-10}$  M) and the radiolabeled compound for 60 min.

[0428] At the end of each incubation, the cells are washed twice with PBS and subsequently lysed with lysis buffer (0.3 M NaOH, 0.2% sodium dodecyl sulfate). Radioactivity is determined in a  $\gamma$ -counter, normalized to 1 million cells, and calculated as percentage injected dose (%ID/ $10^6$  cells).

#### 14. Biodistribution and imaging studies.

[0429] Radioactively labeled compounds can be detected in vivo by imaging methods such as SPECT and PET. Furthermore, the data acquired by such techniques can be confirmed by direct measurement of radioactivity contained in the individual organs prepared from an animal injected with a radioactively labeled compound of the invention. Thus, the biodistribution (the measurement of radioactivity in individual organs) of a radioactively labeled compound can be determined and analyzed. This example shows that the compounds of the present invention show a biodistribution appropriate for diagnostic imaging and therapeutic treatment of tumors.

[0430] In a general procedure for biodistribution experiments, immunocompromised mice are subcutaneously engrafted with the either antigen-positive (e.g. HEK-hFAP, HT-1080-hFAP, U87MG), HT-29 cells, patient-derived explants and/or antigen-negative tumor cells (e.g. WT HEK293 or WT HT-1080 cells). Antigen-negative tumors are engrafted to perform the specificity studies with selected radio- labeled compounds. When tumors reach an appropriate size, mice are assigned to the experimental groups to receive an intravenous injection of the <sup>177</sup>Lu- labeled exemplary compound via tail vein. For organ distribution, the animals are sacrificed after the indicated time points (from 30 min to 24 h). The distributed radioactivity is measured in all dissected organs and in blood using a  $\gamma$ -counter. The values are expressed as percentage injected dose per gram of tissue (%ID/g).

[0431] For PET imaging studies, tumor-bearing mice are injected with  $^{68}\text{Ga}$ -labeled compounds and images are acquired using a small-animal PET scanner. Within the first 60 min, a dynamic scan is performed, followed by a static scan from 120 to 140 min after injection. Images are reconstructed iteratively using the 3-dimensional maximum a priori ordered-subset expectation maximization and are converted to standardized uptake value (SUV) images. Quantification is done using a region-of-interest technique and expressed as SUV.

[0432] A blocking experiment can be also performed by adding 30-100 nmol of the unlabeled precursor or reference compound to the solution of  $^{68}\text{Ga}$ -labeled exemplary compound before injection.

#### **15. Efficacy and survival study with radiolabeled compounds.**

[0433] In order to investigate the therapeutic efficacy of a radiolabeled compound of this invention, the evaluation of tumor growth delay and median survival after the injection of  $^{177}\text{Lu}$ -labeled compound is assessed in tumor-bearing mice.

[0434] For this purpose, immunocompromised mice are subcutaneously engrafted with antigen-positive cells (e.g. HEK-hFAP, HT-1080-hFAP, U87MG), HT-29 cells or patient-derived explants. When tumors reach an appropriate size, mice are assigned to the experimental groups to receive a single intravenous injection via tail vein of the vehicle, the unlabeled precursor or a specific dose of the  $^{177}\text{Lu}$ -labeled exemplary compound.

[0435] Mouse health checks are performed throughout the study on a weekly basis including body weight measurements. Tumor growth is monitored daily and measured thrice a week using a digital caliper. The measurement of the tumor is performed in two dimensions and the volume is expressed in  $\text{mm}^3$  using the formula:  $V = 0.5 \times a \times b^2$ , where  $a$  and  $b$  are the long and short diameters of the tumor, respectively. In addition, mice are followed for survival. Study endpoints include tumor size  $> 2\text{cm}$  in any dimension, tumor ulceration, mouse is moribund, and  $> 20\%$  body weight lost from the last measurement.

#### **16. Efficacy and survival study with conjugated compounds.**

[0436] In order to investigate the therapeutic efficacy of a cytotoxic drug- conjugated compound of this invention, the evaluation of tumor growth delay and median survival is assessed in tumor-bearing mice.



[0437] For this purpose, immunocompromised mice are subcutaneously engrafted with the either antigen-positive cells (e.g. HEK-hFAP, HT-1080-hFAP, U87MG), HT-29 cells or patient-derived explants. When tumors reach an appropriate size, mice are assigned to the experimental groups to receive a specific regimen of intravenous injections of vehicle, unconjugated precursor or cytotoxic drug- conjugated exemplary compound.

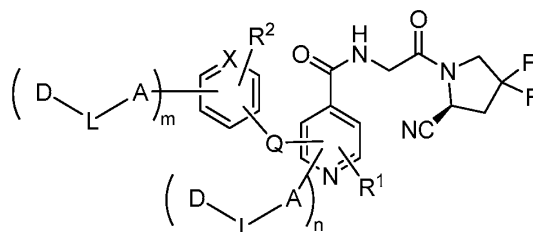
[0438] Mouse health checks are performed throughout the study on a weekly basis including body weight measurements. Tumor growth, survival and study endpoints are monitored as expressed above.

#### **17. Clinical PET/CT imaging study.**

[0439] A human patient diagnosed with metastatic cancer is selected for the clinical imaging study. The patient receives an intravenous injection of a medical recommended dose of a radio-labeled exemplary compound of the invention. The imaging procedure takes place at 10 min, 1 h, and 3 h after tracer administration. The PET/CT scans are obtained with a PET/CT scanner. Immediately after CT scanning, a whole-body PET scan is acquired in 3 dimensions. SUVs are quantitatively assessed using a region-of-interest technique. The data are analyzed retrospectively with approval of the local ethics committee.

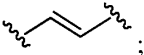
## CLAIMS

1. A compound of formula (I):



formula (I),

or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein,

Q is a bond or ;

X is N or CR<sup>2</sup>;

A is -O-, 5- to 6-membered heterocyclyl, -(CH<sub>2</sub>)<sub>p</sub>-, -NR<sup>6</sup>-, -C(O)NR<sup>6</sup>-, -NR<sup>6</sup>C(O)-#, -C(O)O-#, -OC(O)-#, -C(O)(CH<sub>2</sub>)<sub>p</sub>-, -(CH<sub>2</sub>)<sub>p</sub>C(O)-#, -(CH<sub>2</sub>)<sub>p</sub>-NR<sup>6</sup>-, -NR<sup>6</sup>(CH<sub>2</sub>)<sub>p</sub>-, -O(CH<sub>2</sub>)<sub>p</sub>-, or - (CH<sub>2</sub>)<sub>p</sub>-O-#, wherein # indicates the point of attachment to L;

p is an integer from 0 to 2; and

R<sup>6</sup> is hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

or R<sup>2</sup> and R<sup>6</sup> are taken together with the atom to which they attached to form a 5- to 7- membered heterocyclyl optionally substituted by R<sup>11</sup>;

D is, independently at each occurrence, a payload;

L is, independently at each occurrence, a linker;

R<sup>1</sup> and R<sup>2</sup> are independently hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>3</sub>-C<sub>6</sub> cycloalkyl, 3- to 6-membered heterocyclyl, -CN, halogen, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkyl, -OR<sup>3</sup>, -SR<sup>3</sup>, -S(O)<sub>2</sub>R<sup>3</sup>, -S(O)<sub>2</sub>NR<sup>4</sup>R<sup>5</sup>, -NR<sup>3</sup>S(O)<sub>2</sub>R<sup>4</sup>, -NR<sup>4</sup>R<sup>5</sup>, -C(O)R<sup>3</sup>, -NR<sup>3</sup>C(O)R<sup>4</sup>, -NR<sup>3</sup>C(O)NR<sup>4</sup>R<sup>5</sup>, -C(O)OR<sup>3</sup>, -C(O)ONR<sup>4</sup>R<sup>5</sup> or -C(O)NR<sup>4</sup>R<sup>5</sup>, wherein each of R<sup>1</sup> and R<sup>2</sup> is independently optionally substituted by R<sup>10</sup>;

each  $R^3$ ,  $R^4$  and  $R^5$  is independently hydrogen,  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_3$ - $C_6$  cycloalkyl, 3- to 6-membered heterocyclyl, -CN, halogen,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  haloalkoxy,  $C_1$ - $C_6$  haloalkyl,  $-OR^7$ ,  $-SR^7$ ,  $-S(O)_2R^7$ ,  $-S(O)_2NR^8R^9$ ,  $-NR^7S(O)_2R^8$ ,  $-NR^8R^9$ ,  $-C(O)R^7$ ,  $-NR^7C(O)R^8$ ,  $-NR^7C(O)NR^8R^9$ ,  $-C(O)OR^7$ ,  $-C(O)ONR^8R^9$  or  $-C(O)NR^8R^9$ ;

or  $R^4$  and  $R^5$  are taken together with the atom to which they attached to form a 3- to 6-membered heterocyclyl which is optionally substituted by  $R^{10}$ ;

each  $R^7$ ,  $R^8$  and  $R^9$  is independently hydrogen or  $C_1$ - $C_6$  alkyl optionally substituted by oxo, -OH or  $-NH_2$ ;

or  $R^8$  and  $R^9$  are taken together with the atom to which they attached to form a 3- to 6-membered heterocyclyl which is optionally substituted by oxo, -OH or  $-NH_2$ ;

$R^{10}$  and  $R^{11}$  are independently oxo,  $C_1$ - $C_6$  alkyl,  $C_2$ - $C_6$  alkenyl,  $C_2$ - $C_6$  alkynyl,  $C_3$ - $C_6$  cycloalkyl, 3- to 6-membered heterocyclyl, -CN, halogen,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  haloalkoxy,  $C_1$ - $C_6$  haloalkyl,  $-OR^{14}$ ,  $-SR^{14}$ ,  $-S(O)_2R^{14}$ ,  $-S(O)_2NR^{15}R^{16}$ ,  $-NR^{14}S(O)_2R^{15}$ ,  $-NR^{15}R^{16}$ ,  $-C(O)R^{14}$ ,  $-NR^{14}C(O)R^{15}$ ,  $-NR^{14}C(O)NR^{15}R^{16}$ ,  $-C(O)OR^{14}$ ,  $-C(O)ONR^{15}R^{16}$  or  $-C(O)NR^{15}R^{16}$ ;

each  $R^{14}$ ,  $R^{15}$  and  $R^{16}$  is independently hydrogen or  $C_1$ - $C_6$  alkyl optionally substituted by oxo, -OH or  $-NH_2$ ;

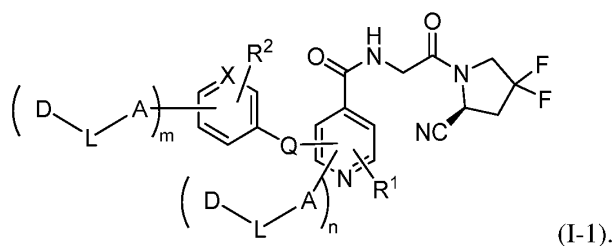
or  $R^{15}$  and  $R^{16}$  are taken together with the atom to which they attached to form a 3- to 6- membered heterocyclyl which is optionally substituted by oxo, -OH or  $-NH_2$ ;

m is 0 or 1; and

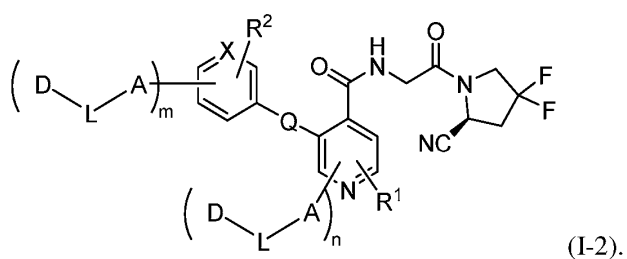
n is 0 or 1;

provided that m + n is greater than 0.

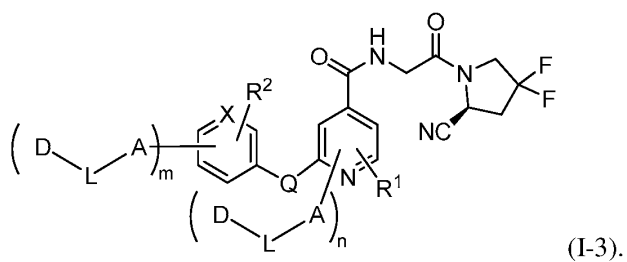
2. The compound of claim 1, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is of formula (I-1):



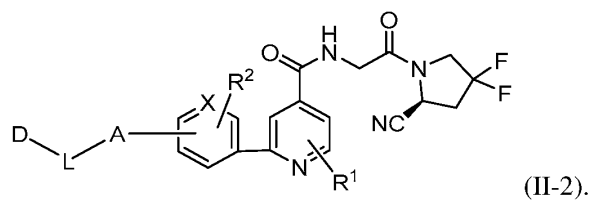
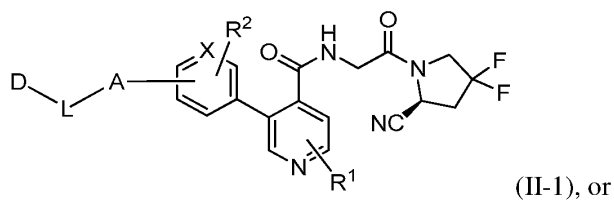
3. The compound of claim 1 or 2, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is of formula (I-2):



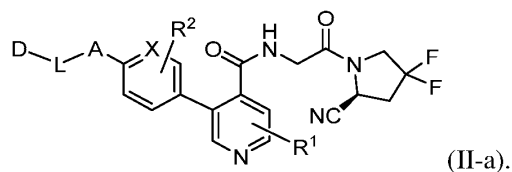
4. The compound of claim 1 or 2, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is of formula (I-3):



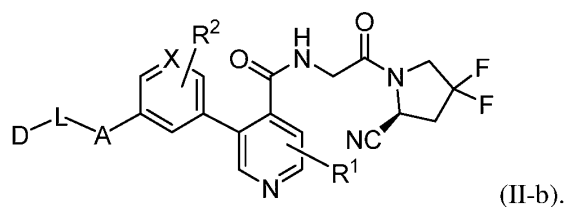
5. The compound of any one of claims 1-4, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein Q is a bond.
6. The compound of any one of claims 1-5, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is of formula (II-1) or formula (II-2):



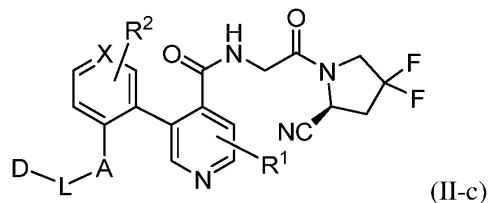
7. The compound of claim 6, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is of formula (II-a):



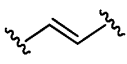
8. The compound of claim 6, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is of formula (II-b):



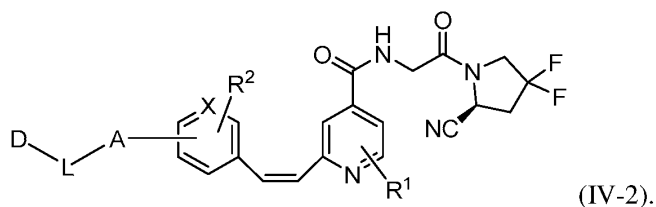
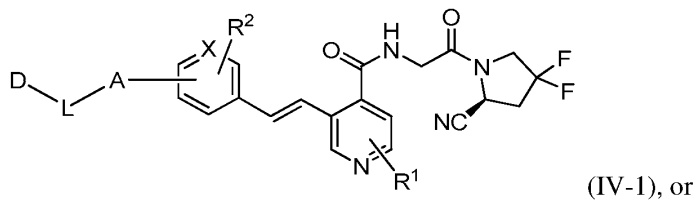
9. The compound of claim 6, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is of formula (II-c):



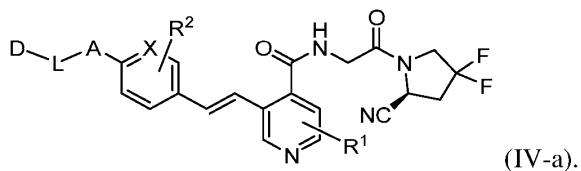
10. The compound of any one of claims 1-4, or a pharmaceutically acceptable salt,

stereoisomer, or tautomer thereof, wherein Q is .

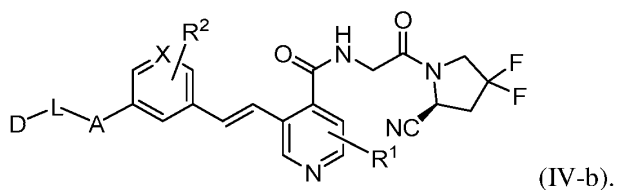
11. The compound of any one of claims 1-4 and 10, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is of formula (IV-1) or formula (IV-2):



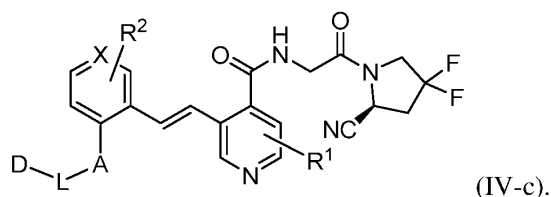
12. The compound of claim 11, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is of formula (IV-a):



13. The compound of claim 11, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is of formula (IV-b):



14. The compound of claim 11, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is of formula (IV-c):



15. The compound of any one of claims 1-14, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein X is CH.

16. The compound of any one of claims 1-14, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein X is N.

17. The compound of any one of claims 1-16, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein R<sup>1</sup> is hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>3</sub>-C<sub>6</sub> cycloalkyl, 3- to 6-membered heterocyclyl, -CN, halogen, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, or C<sub>1</sub>-C<sub>6</sub> haloalkyl.

18. The compound of any one of claims 1-17, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein R<sup>2</sup> is hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>3</sub>-C<sub>6</sub> cycloalkyl, 3- to 6-membered heterocyclyl, -CN, halogen, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> haloalkoxy, or C<sub>1</sub>-C<sub>6</sub> haloalkyl.

19. The compound of any one of claims 1-5, 10 and 15-18, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein m is 1 and n is 0.

20. The compound of any one of claims 1-5, 10 and 15-18, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein m is 0 and n is 1.

21. The compound of any one of claims 1-20, or a pharmaceutically acceptable salt,

stereoisomer, or tautomer thereof, wherein L comprises or has the structure:

$$\begin{array}{c} \text{---} \\ \text{---} \\ \text{---} \end{array} \left( \text{---} \text{L}^2 \text{---} \text{L}^1 \text{---} \right)_t \begin{array}{c} \text{---} \\ \text{---} \\ \text{---} \end{array}$$

wherein L<sup>1</sup> and L<sup>2</sup> are optionally substituted by Z; wherein

t is an integer from 1 to 10;

~~~~~ denotes attachment to D and ----- denotes attachment to A;

L^1 is, independently at each occurrence, absent or C_3 - C_6 cycloalkyl, C_6 -aryl, 5- to 6-membered heterocyclyl or 5- to 6-membered heteroaryl, each of which is optionally substituted by R^{12} ;

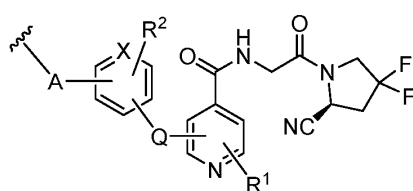
L^2 is, independently at each occurrence, absent or O, S, NH, $N(C_1$ - C_6 alkyl), C_1 - C_{10} alkylene, 2- to 14-membered heteroalkylene, $-C(=O)O-$, $-O(C=O)-$, $-CONH-$, $-CON(C_1$ - C_6 alkyl)-, $-NHCO-$, $-N(C_1$ - C_6 alkyl)CO-, SO_2 , $-SO_2NH-$, $-SO_2N(C_1$ - C_6 alkyl)-, $-NHSO_2-$, $-N(C_1$ - C_6 alkyl) SO_2- , $-S-S-$, $-(OCH_2CH_2)_v-$, $-(CH_2CH_2O)_v-$, or $-C(R^m)=N-$, wherein the C_1 - C_{10} alkylene and 2- to 14-membered heteroalkylene of each L^2 , when present, are optionally and independently substituted with R^m and R^n , wherein

v is independently an integer from 1 to 3;

R^m and R^n are independently hydrogen, carboxyl, C_1 - C_6 alkyl, C_3 - C_6 cycloalkyl, C_6 - C_{10} aryl, 5- to 6-membered heterocyclyl or 5- to 10-membered heteroaryl, $-C_1$ - C_3 -alkylene(C_3 - C_6 cycloalkyl), $-C_1$ - C_3 -alkylene(C_6 - C_{10} aryl), $-C_1$ - C_3 -alkylene(5- to 6-membered heterocyclyl), $-C_1$ - C_3 -alkylene(5- to 10-membered heteroaryl), or $-C_1$ - C_3 -alkylene($NHC(O)NH_2$), each of which is optionally substituted by R^{17} ; and

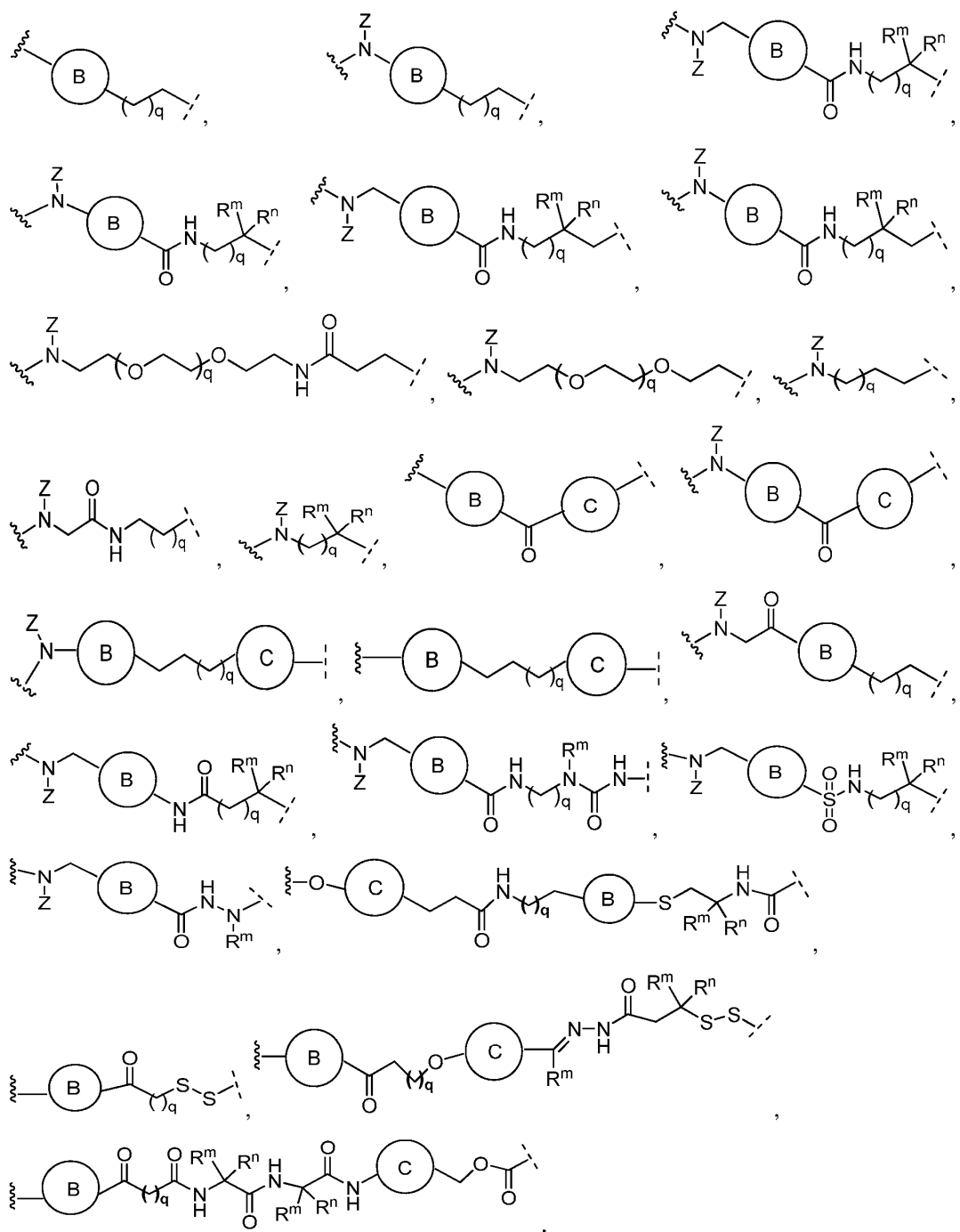
R^{12} and R^{17} are independently oxo, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl, 3- to 6-membered heterocyclyl, $-CN$, halogen, C_1 - C_6 alkoxy, C_1 - C_6 haloalkoxy, C_1 - C_6 haloalkyl, $-OR^{14}$, $-SR^{14}$, $-S(O)_2R^{14}$, $-S(O)_2NR^{15}R^{16}$, $-NR^{14}S(O)_2R^{15}$, $-NR^{15}R^{16}$, $-C(O)R^{14}$, $-NR^{14}C(O)R^{15}$, $-NR^{14}C(O)NR^{15}R^{16}$, $-C(O)OR^{14}$, $-C(O)ONR^{15}R^{16}$ or $-C(O)NR^{15}R^{16}$;

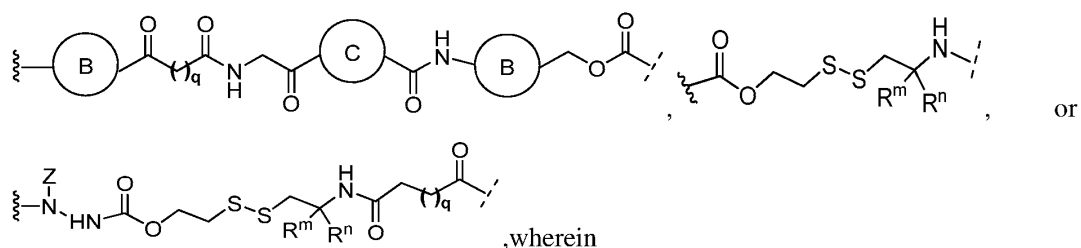
and



Z comprises

22. The compound of any one of claims 1-21, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein L comprises or is 5- to 6-membered heterocyclyl,





the lines denote attachment points to D;

lines denote attachment points to A;

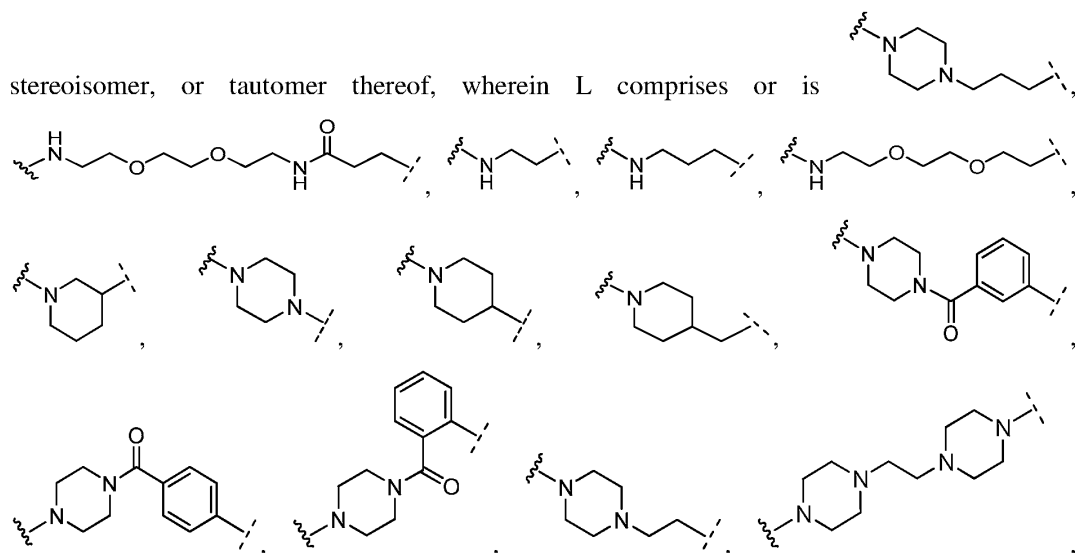
ring B is C₃-C₆ cycloalkyl, C₆-C₁₀ aryl, 5- to 6-membered heterocyclyl or 5- to 6-membered heteroaryl, each of which is optionally substituted by R^b;

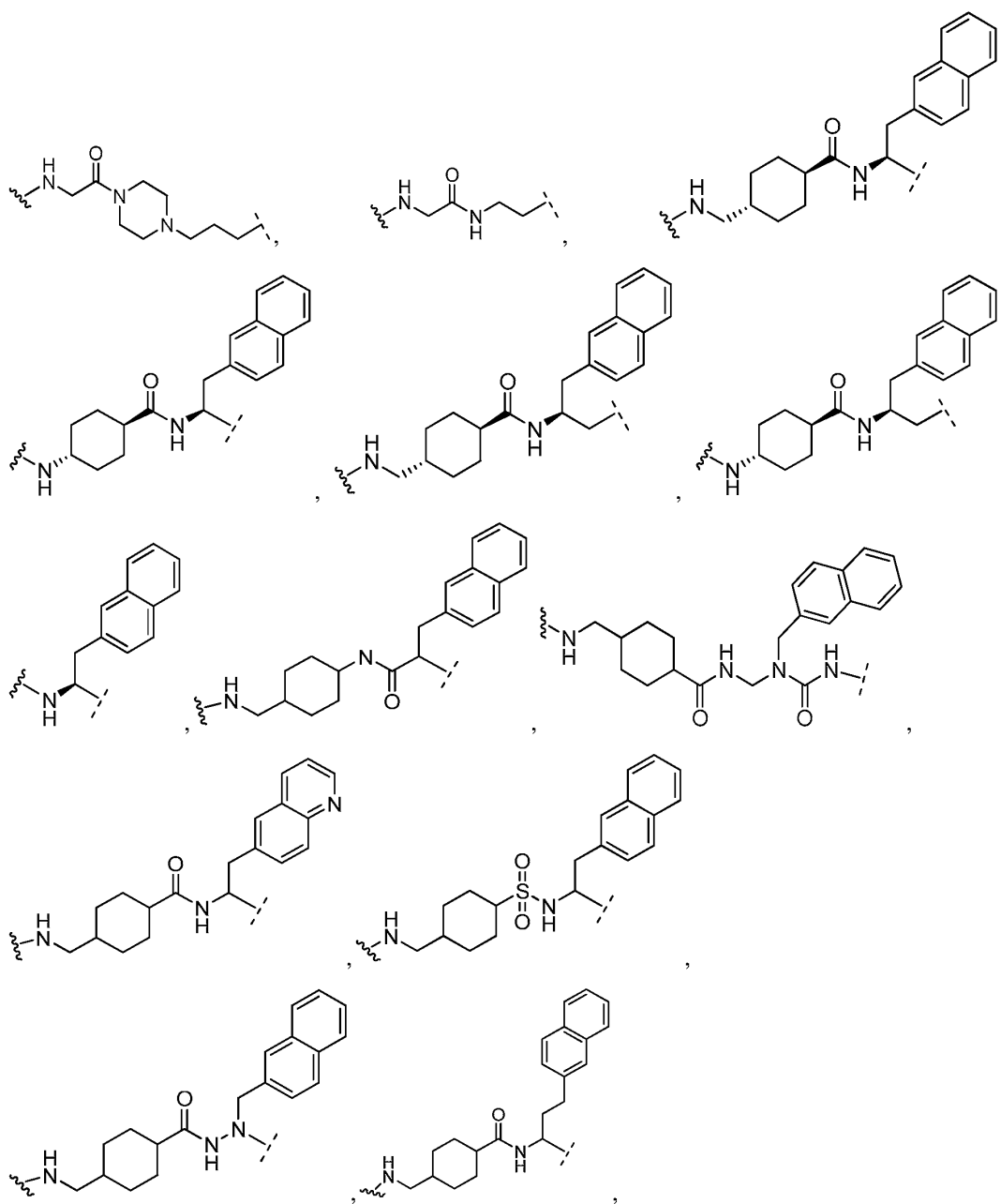
ring C is C₃-C₆ cycloalkyl, C₆-C₁₀ aryl, 5- to 6-membered heterocyclyl or 5- to 6-membered heteroaryl, each of which is optionally substituted by R^c; and

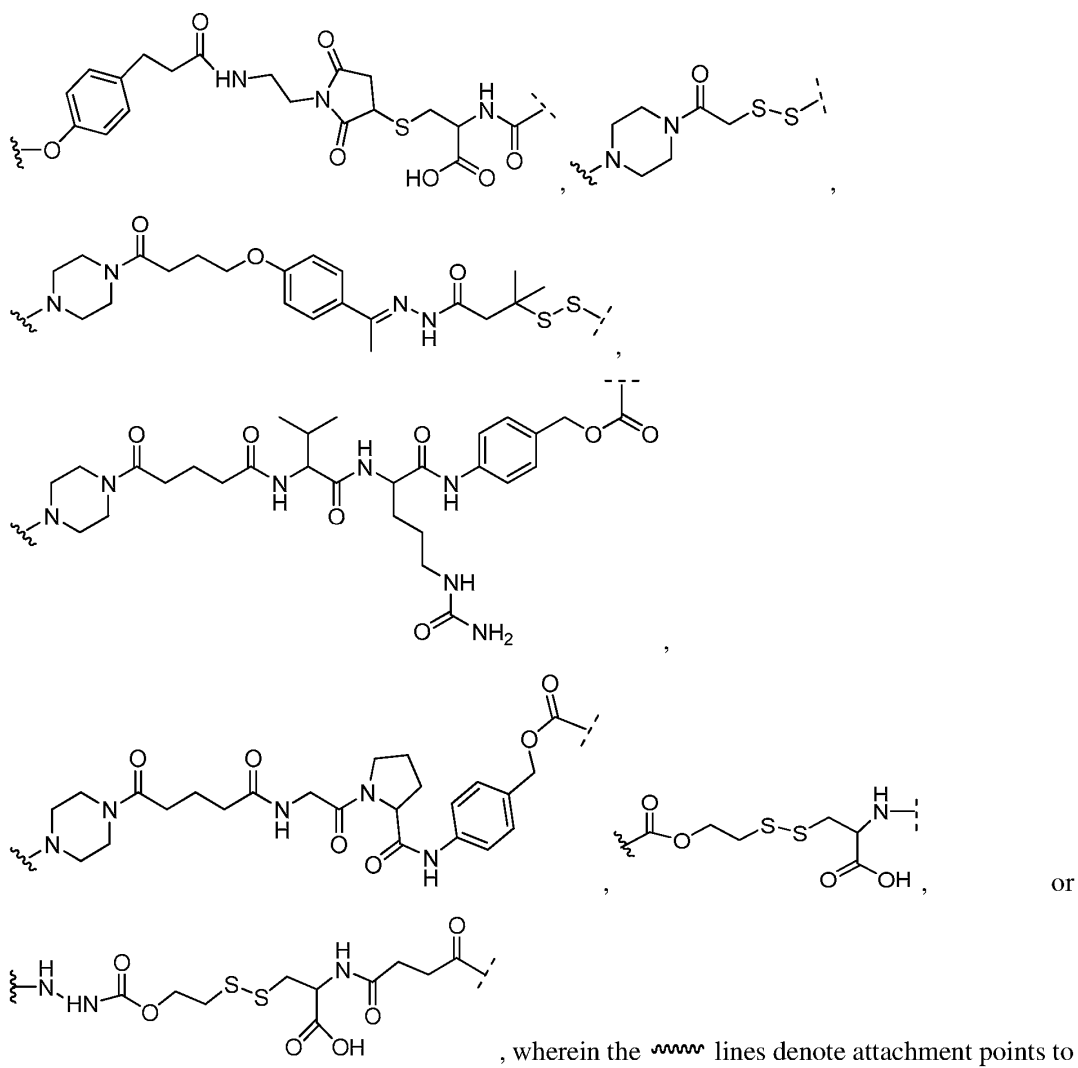
R^b and R^c are independently oxo, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, 3- to 6-membered heterocyclyl, -CN, halogen, C₁-C₆ alkoxy, C₁-C₆ haloalkoxy, C₁-C₆ haloalkyl, -OR¹⁴, -SR¹⁴, -S(O)₂R¹⁴, -S(O)₂NR¹⁵R¹⁶, -NR¹⁴S(O)₂R¹⁵, -NR¹⁵R¹⁶, -C(O)R¹⁴, -NR¹⁴C(O)R¹⁵, -NR¹⁴C(O)NR¹⁵R¹⁶, -C(O)OR¹⁴, -C(O)ONR¹⁵R¹⁶ or -C(O)NR¹⁵R¹⁶, and

each q is independently an integer from 1 to 3.

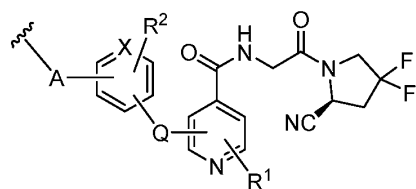
23. The compound of any one of claims 1-22, or a pharmaceutically acceptable salt,



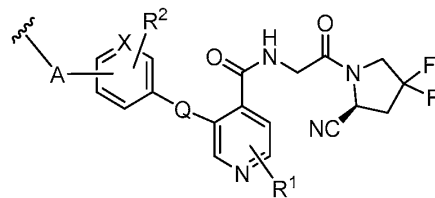




24. The compound of any one of claims 21-23, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein Z is present and comprises

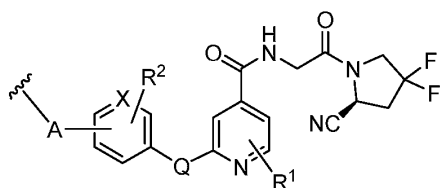


25. The compound of claim 24, or a pharmaceutically acceptable salt, stereoisomer, or

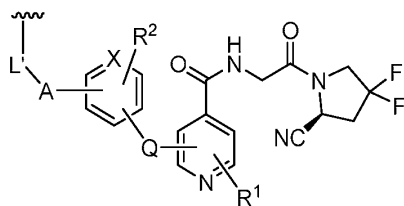


tautomer thereof, wherein Z is present and comprises

or

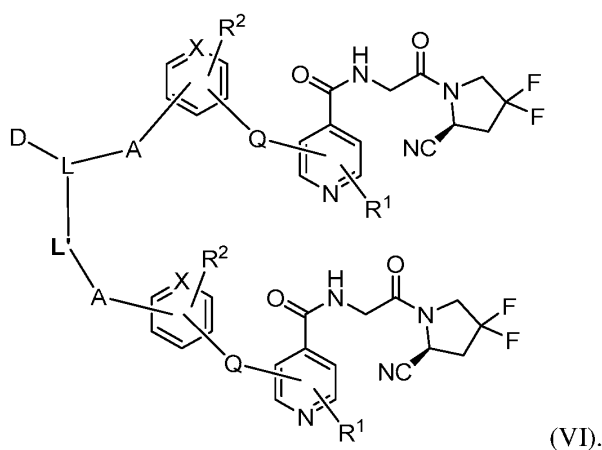


26. The compound of any one of claims 21-25, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein Z is present and represented by

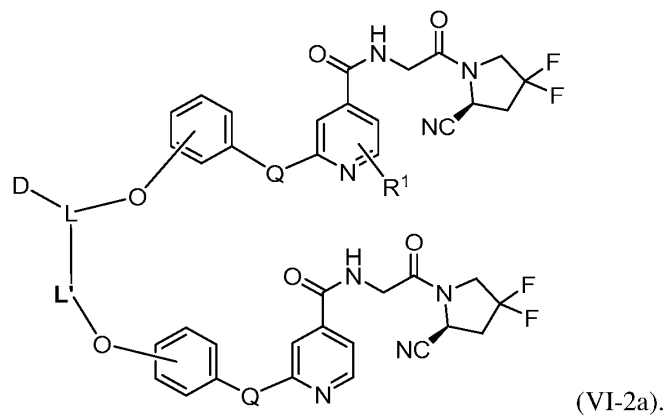
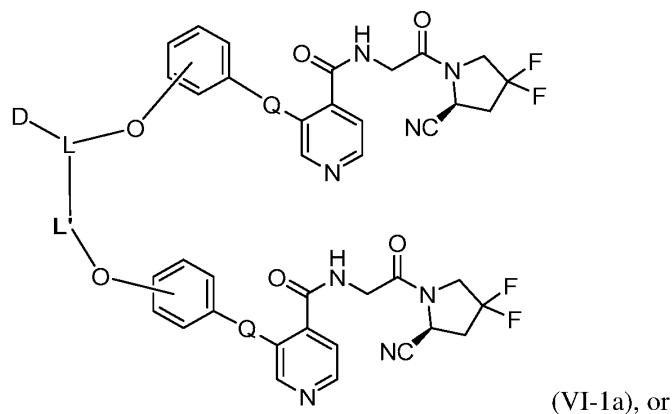


, and L' is a linker.

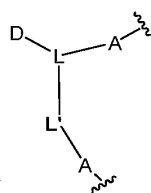
27. The compound of claim 26, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is of formula (VI):



28. The compound of claim 27, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is of formula (VI-1a) or formula (VI-2a):

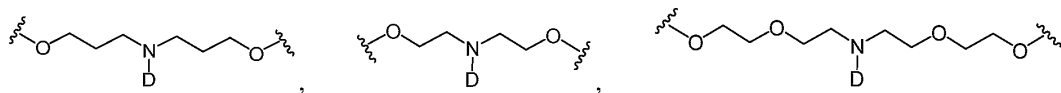


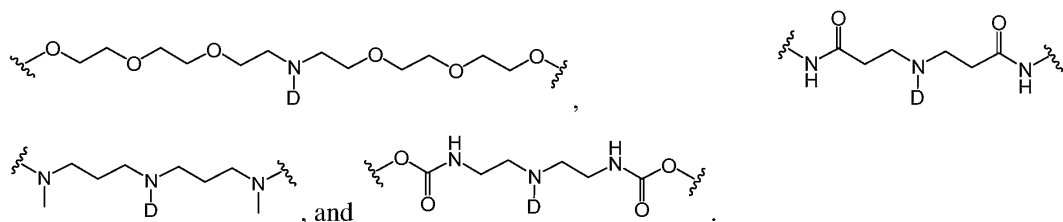
29. The compound of claim 27, or a pharmaceutically acceptable salt, stereoisomer, or



tautomer thereof, wherein the moiety of  is symmetric with respect to the payload.

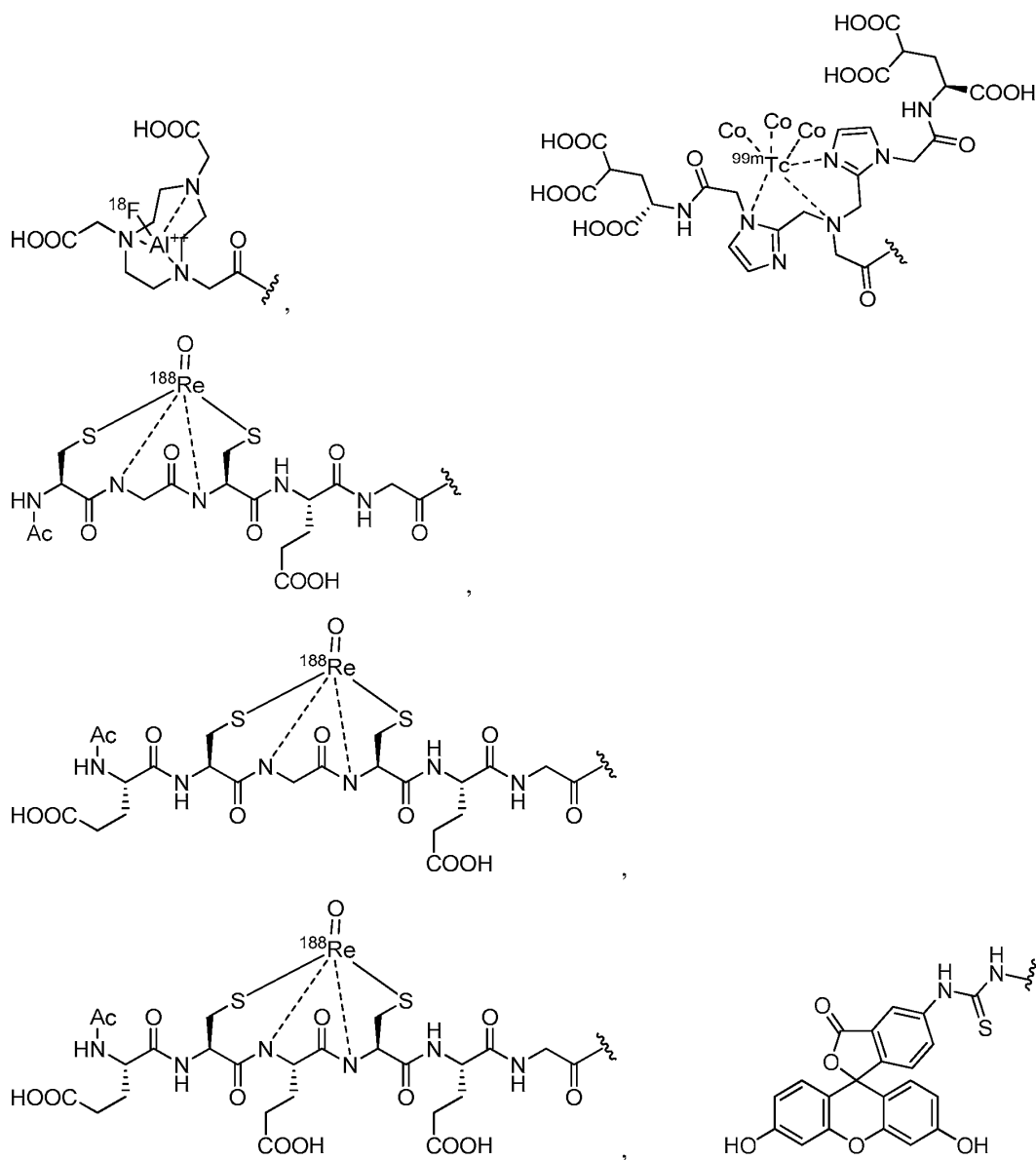
30. The compound of claim 29, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the moiety is selected from the group consisting of

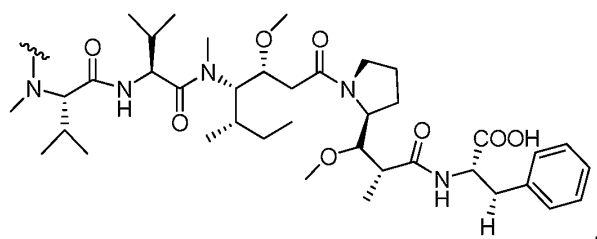
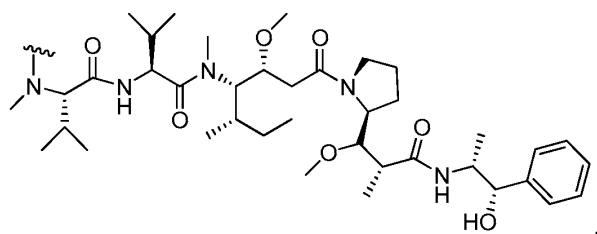
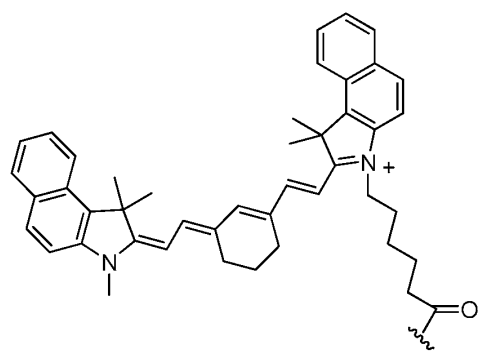
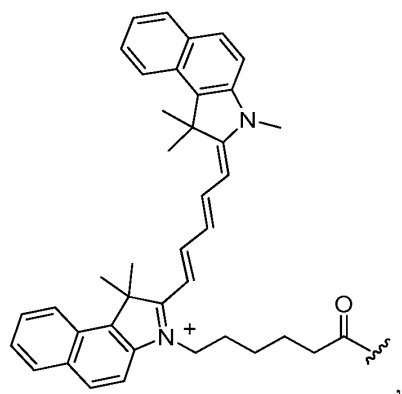
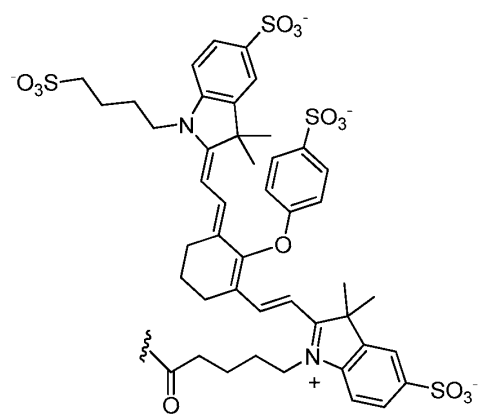
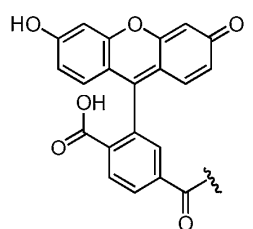


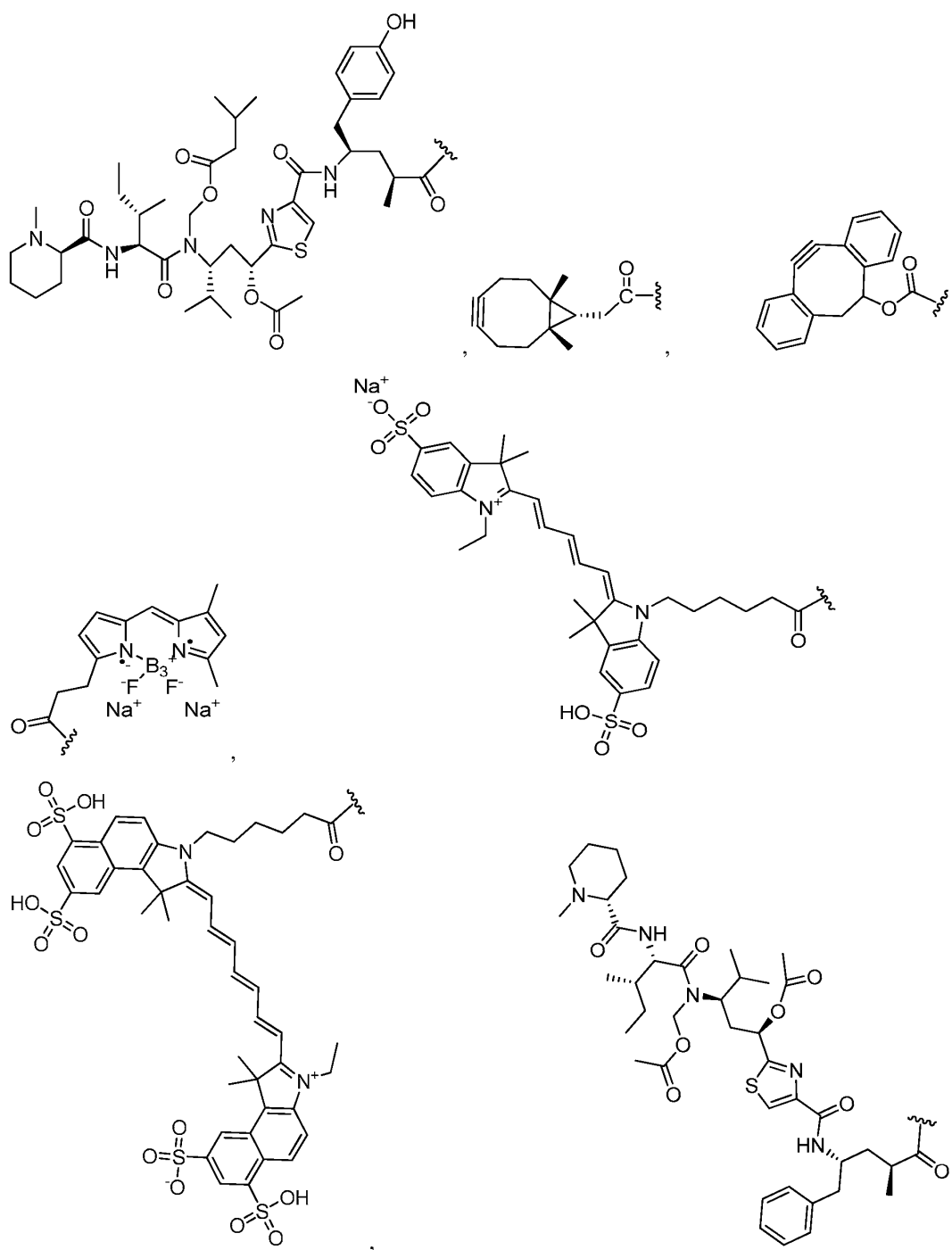


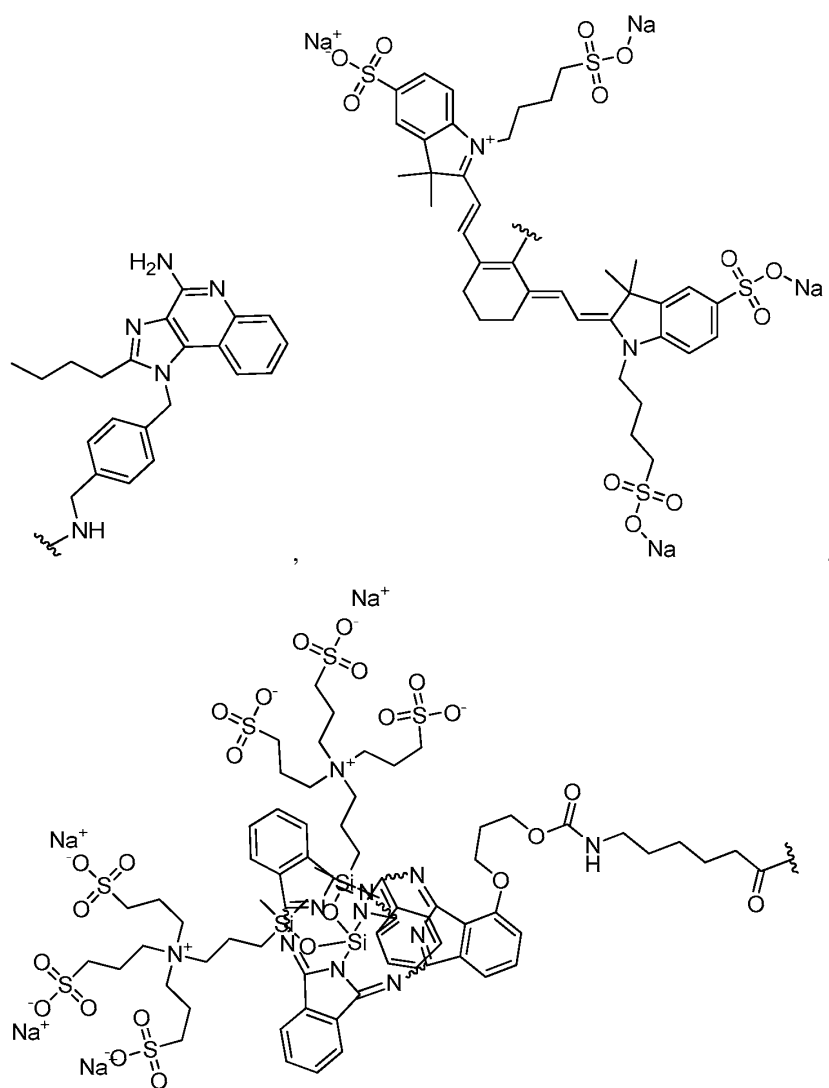
31. The compound of any one of claims 1-30, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein A is -O-, -NR⁶-, -C(O)NR⁶-, -NR⁶C(O)-#, -C(O)O-#, or -OC(O)-#, wherein # indicates the point of attachment to L.
32. The compound of claim 31, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein A is -O-.
33. The compound of any one of claims 1-32, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the payload comprises or is, independently at each occurrence, one or more drugs and agents.
34. The compound of any one of claims 1-33, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the payload comprises or is, independently at each occurrence, a cytotoxic drug.
35. The compound of any one of claims 1-33, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the payload comprises or is, independently at each occurrence, a radioactive component, a metal chelating group, a chelating agent, a fluorescent dye, a contrast agent, or any combination thereof.
36. The compound of any one of claims 1-33, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the payload comprises or is, independently at each occurrence, a radionuclide.
37. The compound of any one of claims 1-33, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the payload comprises or is, independently at each

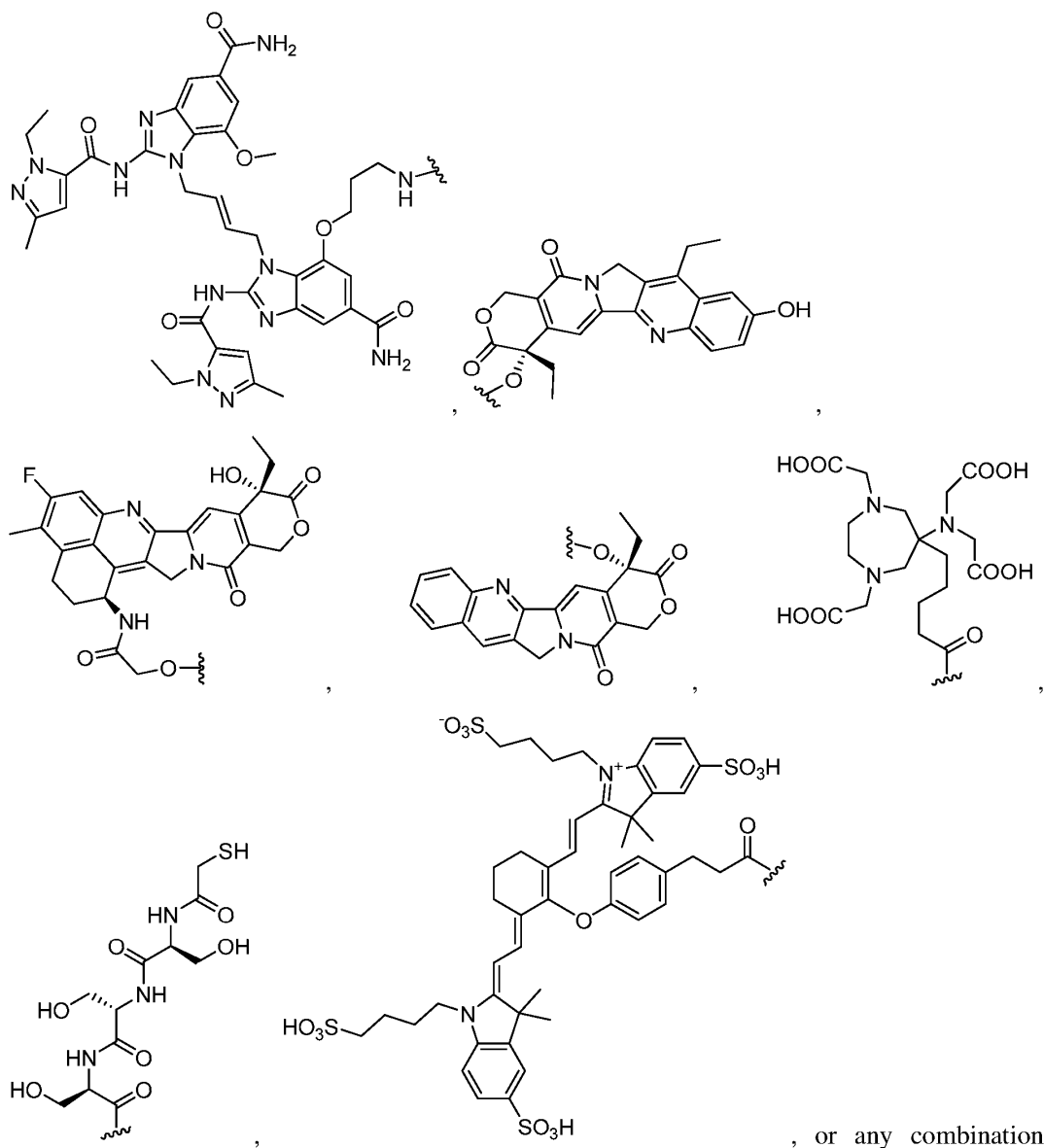
[illegible]









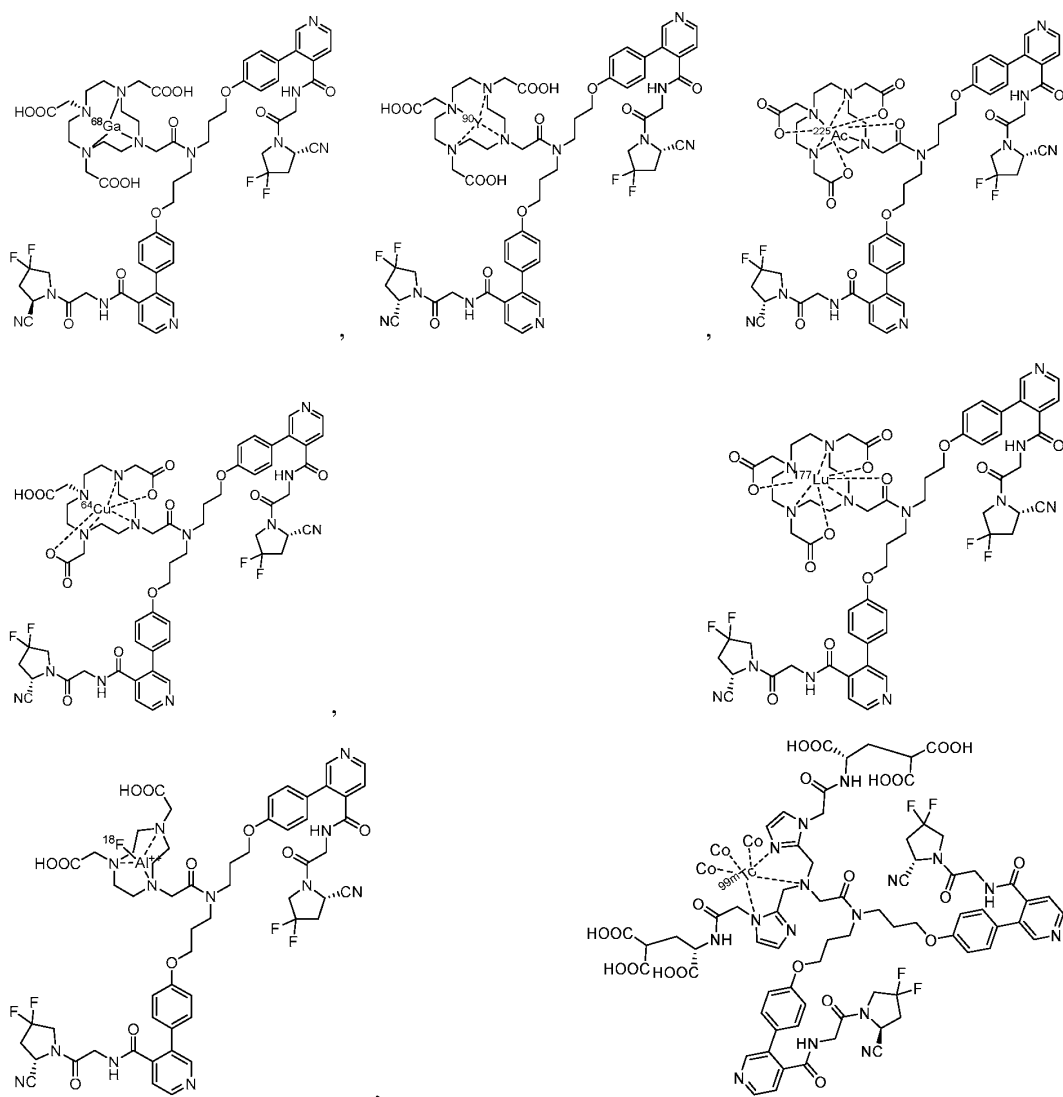


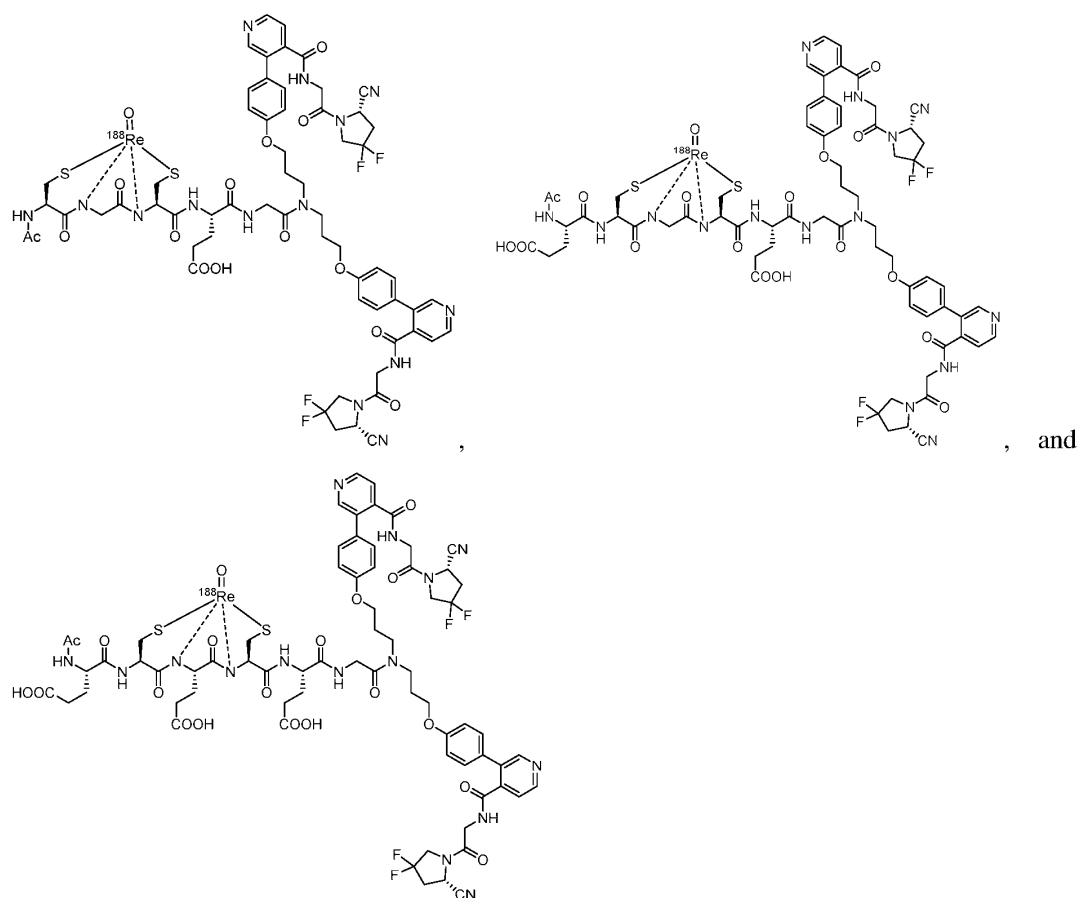
thereof, wherein the lines denote attachment points to L.

38. The compound of any one of claims 1-37, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the payload further comprises one or more motifs.

39. The compound of claim 38, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the motif is an albumin-binding motif.

40. The compound of claim 39, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the motif is an ibuprofen motif.
41. The compound of claim 1, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is a compound of Table 1A and Table 1B.
42. The compound of claim 1 or 41, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, wherein the compound is selected from the group consisting of





43. A pharmaceutical composition comprising a compound of any one of claims 1-42, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof, and a pharmaceutically acceptable carrier.

44. A method of treating a disease or disorder mediated by fibroblast activation protein (FAP) in an individual in need thereof comprising administering to the individual a compound of any one of claims 1-42, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof.

45. The method of claim 44, wherein the disease or disorder is breast cancer, colorectal cancer, ovarian cancer, prostate cancer, pancreatic cancer, kidney cancer, lung cancer, bladder cancer, liver cancer, head and neck cancer, esophagus cancer, thyroid cancer, brain cancer, neuroblastoma, glioma, melanoma, lymphoma, fibrosarcoma, osteosarcoma, bone sarcoma,

connective tissue sarcoma, renal cell carcinoma, giant cell carcinoma, squamous cell carcinoma, leukemia, skin cancer, soft tissue cancer, gastrointestinal carcinoma, or adenocarcinoma.

46. A method of inhibiting FAP in an individual comprising administering to the individual a compound of any one of claims 1-42, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof.

47. A method of imaging, diagnosing, or detecting a disease or disorder mediated by FAP in an individual in need thereof comprising administering to the individual a compound of any one of claims 1-42, or a pharmaceutically acceptable salt, stereoisomer, or tautomer thereof.

48. The method of claim 47, wherein the disease or disorder is a chronic inflammatory and/or destructive process, fibrosis, or a benign tumor.