

TRIAZACYCLODODECANSULFONAMIDE ("TCD")-BASED PROTEIN SECRETION INHIBITORS

BACKGROUND**Field of the Invention**

[0001] The present disclosure relates to triazacyclododecansulfonamide ("TCD")-based protein secretion inhibitors, including methods of making and using the same.

Incorporation by Reference of Material Submitted Electronically

[0002] This application contains, as a separate part of disclosure, a sequence listing in computer-readable form (filename 40056_Seqlisting.txt; 20,275 bytes; created February 14, 2019) which is incorporated by reference in its entirety.

Description of Related Technology

[0003] Protein translocation into the endoplasmic reticulum ("ER") constitutes the first step of protein secretion. ER protein import is essential in all eukaryotic cells and is particularly important in fast-growing tumour cells. Thus, the process of protein secretion can serve as a target both for potential cancer drugs and for bacterial virulence factors. See Kalies and Römisch, Traffic, 16(10):1027-1038 (2015).

[0004] Protein transport to the ER is initiated in the cytosol when N-terminal hydrophobic signal peptides protrude from the ribosome. Binding of signal recognition particle ("SRP") to the signal sequence allows targeting of the ribosome–nascent chain–SRP complex to the ER membrane where contact of SRP with its receptor triggers handing over of the signal peptide to Sec61. Sec61 is an ER membrane protein translocator (aka translocon) that is doughnut-shaped with 3 major subunits (heterotrimeric). It includes a "plug," which blocks transport into or out of the ER. The plug is displaced when the hydrophobic region of a nascent polypeptide interacts with the "seam" region of Sec61, allowing translocation of the polypeptide into the ER lumen. In mammals, only short proteins (<160 amino acids) can enter the ER posttranslationally, and proteins smaller than 120 amino acids are obliged to use this pathway. Some of the translocation competence is maintained by the binding of calmodulin to the signal sequence. Upon arrival at the Sec61 channel, the signal peptide or signal anchor intercalates between transmembrane domains ("TMDs") 2 and 7 of Sec61 α , which form the lateral portion of the gate, allowing the channel to open for soluble secretory proteins. As the Sec61 channel consists of 10 TMDs (Sec61 α) surrounded by a hydrophobic clamp formed by Sec61 γ , channel opening is dependent on conformational changes that involve practically all TMDs.

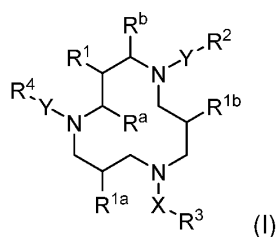
[0005] Inhibition of protein transport across the ER membrane has the potential to treat or prevent diseases, such as the growth of cancer cells and inflammation. Known secretion

inhibitors, which range from broad-spectrum to highly substrate-specific, can interfere with virtually any stage of this multistep process, and even with transport of endocytosed antigens into the cytosol for cross-presentation. These inhibitors interact with the signal peptide, chaperones, or the Sec61 channel to block substrate binding or to prevent the conformational changes needed for protein import into the ER. Examples of protein secretion inhibitors include, calmodulin inhibitors (e.g., E6 Berbamine and Ophiobolin A), Lanthanum, sterols, cyclodepsipeptides (e.g., HUN-7293, CAM741, NFI028, Cotransin, Apratoxin A, Decatransin, Valinomycin), CADA, Mycolactone, Eeyarestatin I ("ESI"), and Exotoxin A. However, the above secretion inhibitors suffer from one or more of the following: lack selectivity for the Sec61 channel, challenging manufacture due to structural complexity, and molecular weight limited administration, bio-availability and distribution.

[0006] Thus, a need exists for new small molecule inhibitors of protein secretion.

SUMMARY

[0007] In one aspect, provided herein is a compound of Formula (I), or a pharmaceutically acceptable salt thereof:



wherein: each of R^a and R^b is independently H or C_{1-3} alkyl; R^1 is H, OH, C_{1-3} alkyl, OC_{1-3} alkyl, $=CH_2$, or $=NOR^5$; or R^1 is C_{3-6} cycloalkyl or C_{3-6} heterocycloalkyl and forms a spiro group with the ring carbon to which it is attached; each of R^{1a} and R^{1b} is independently H or C_{1-3} alkyl; R^2 is C_{1-6} alkyl, $N(R^5)_2$, C_{3-8} cycloalkyl, C_{3-9} heterocycloalkyl, C_{3-9} heterocycloalkenyl, or C_{6-10} aryl; R^3 is H, C_{1-6} alkyl, C_{3-8} cycloalkyl, C_{3-8} cycloalkenyl, C_{3-7} heterocycloalkyl, C_{6-10} aryl, or C_{2-6} heteroaryl; R^4 is C_{3-8} cycloalkyl, C_{3-9} heterocycloalkyl, C_{6-10} aryl, or C_{2-9} heteroaryl; each R^5 independently is H, C_{1-3} alkyl, or C_{0-2} alkylene- C_{6-10} aryl; X is absent, C_{1-3} alkylene, C=O, or (C=O)O; Y is SO or SO_2 ; each heterocycloalkyl, heterocycloalkenyl, and heteroaryl group independently has 1, 2, or 3 ring heteroatoms selected from N, O, and S.

[0008] In some embodiments, each Y is SO. In various embodiments, each Y is SO_2 .

[0009] In various cases, R^a is H. In some cases, R^a is C_{1-3} alkyl. In some embodiments, R^a is CH_3 . In some embodiments, R^b is H. In various embodiments, R^b is C_{1-3} alkyl. In various cases, R^b is CH_3 . In various cases, each of R^a and R^b is H.

[0010] In some cases, R¹ is H, OH, or =NOR⁵. In various cases, R⁵ is each R⁵ independently is H, C₁₋₃alkyl, or C₀₋₂alkylene-C₆₋₁₀aryl. In some cases, R⁵ is H or CH₃. In some embodiments, R¹ is C₁₋₃alkyl or OC₁₋₃alkyl. In various embodiments, R¹ is CH₃ or OCH₃. In some cases, R¹ is CH₃ and exhibits *S* stereochemistry. In various embodiments, R¹ is C₃₋₆cycloalkyl or C₃₋₆heterocycloalkyl and forms a spiro group with the ring carbon to which it is attached. In some cases, R¹ together with the ring atom to which it is attached

forms , , or . In various cases, R¹ is =CH₂.

[0011] In some cases, each of R^{1a} and R^{1b} is H. In some cases, at least one of R^{1a} and R^{1b} is C₁₋₃alkyl. In some cases, each of R^{1a} and R^{1b} is CH₃.

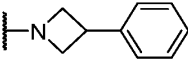
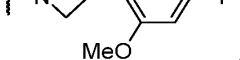
[0012] In some embodiments, R² is C₁₋₆alkyl or N(R⁵)₂. In various cases, each R⁵ independently comprises H, C₁₋₃alkyl, or benzyl. In various embodiments, R² is Et,

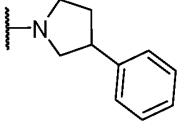
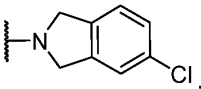
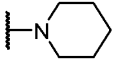
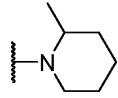
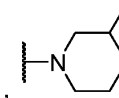
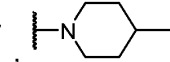
$i\text{Pr}_2\text{N}(\text{CH}_2)_2$, $\text{NH}(\text{CH}_2)_2$, $\text{NH}(\text{CH}_2)_2$, or $\text{NH}(\text{CH}_2)_2$

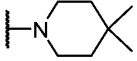
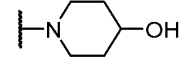
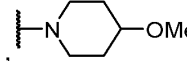
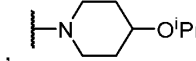
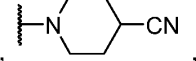
In some cases, R² comprises C₃₋₈cycloalkyl. In various cases, R² comprises cyclopentyl or cyclohexyl. In some embodiments, R² is C₃₋₉heterocycloalkyl or C₃₋₉heterocycloalkenyl. In various embodiments, R² comprises oxetanyl, azetidiny, tetrahydrofuranyl, pyrrolidinyl, tetrahydropyranyl, pyranly, piperidinyl, piperazinyl, azepanyl, morpholinyl, or tetrahydropyridinyl. In various embodiments, the C₃₋₉heterocycloalkyl or C₃₋₉heterocycloalkenyl comprises a bridge or a spiro group. In some cases, the C₃₋

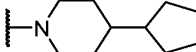
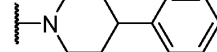
heterocycloalkyl comprising a bridge or a spiro group is selected from the group consisting of , , and , and R⁶ is C₁₋₆alkyl, C₃₋₈cycloalkyl, or C₆₋₁₀aryl. In some cases, R⁶ is phenyl optionally substituted with one to three groups independently selected from halo, C₁₋₃alkyl, C₁₋₃alkoxy, and CN. In various cases, R² is

selected from the group consisting of

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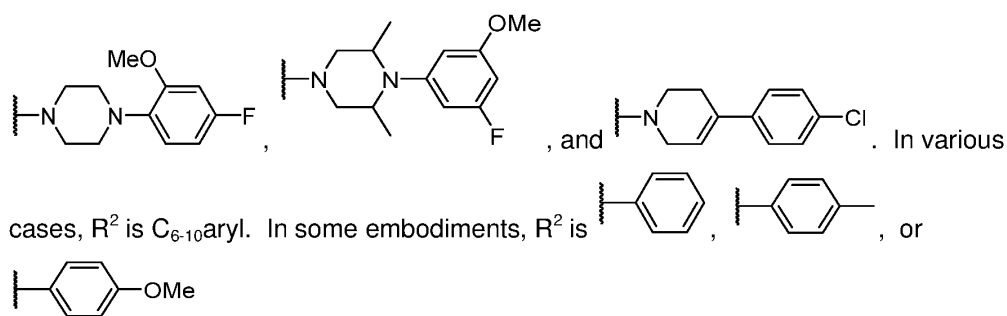
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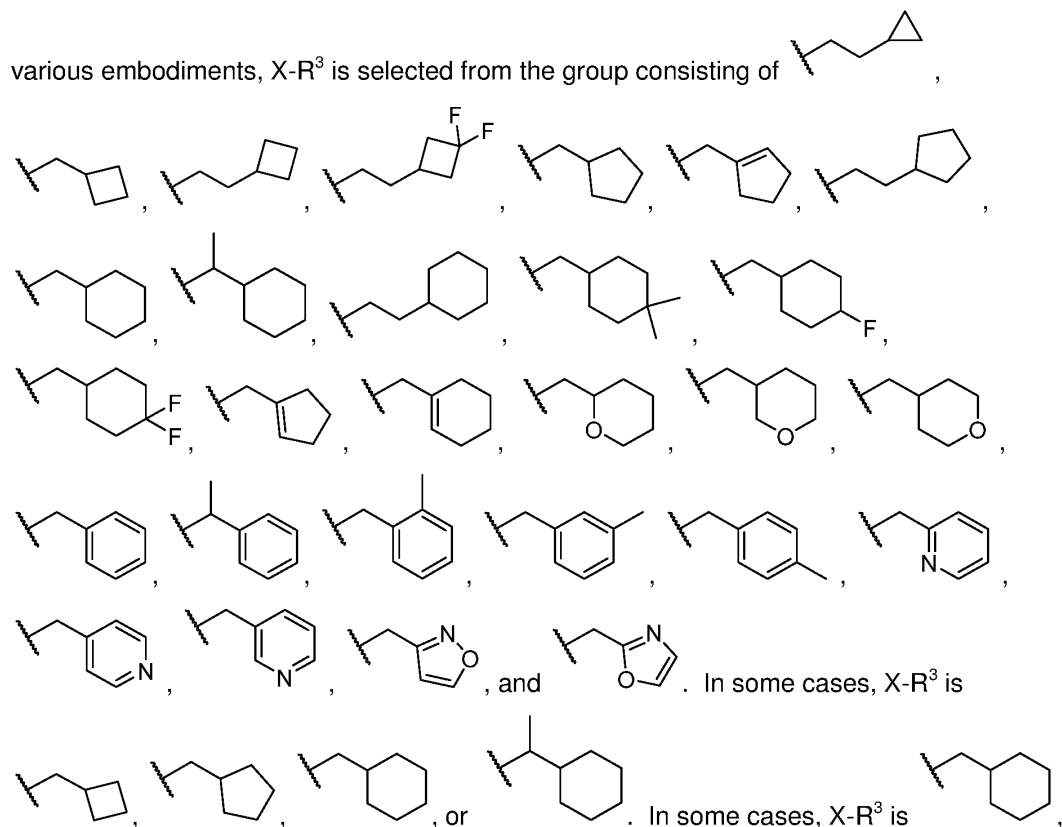


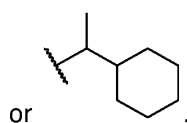




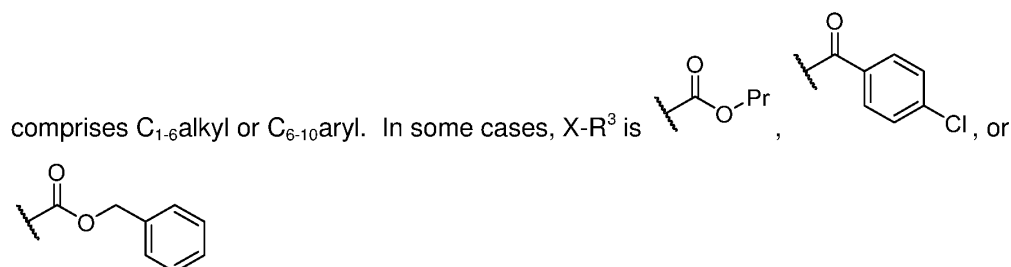
[0013] In various embodiments, X is absent. In some embodiments, R^3 comprises C_{1-6} alkyl or H. In some cases, R^3 is 2-methylbutyl, isopropyl, isopentyl, $CH_2CH_2OCH_3$, $CH_2C(CH_3)_2CN$, CH_2CF_3 , or $CH_2CH_2CF_3$. In various cases, R^3 is isobutyl.

[0014] In some embodiments, X is C_{1-3} alkylene. In some cases, X is CH_2 , CH_2CH_2 , or $CH(CH_3)$. In various cases, R^3 comprises C_{3-8} cycloalkyl, C_{3-8} cycloalkenyl, C_{3-7} heterocycloalkyl, C_{6-10} aryl, or C_{2-6} heteroaryl. In some embodiments, R^3 comprises cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclopentenyl, cyclohexenyl, tetrahydropyranyl, phenyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, furanyl, thiophenyl, thiazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiadiazolyl, pyridinyl, pyrazinyl, or pyrimidinyl. In

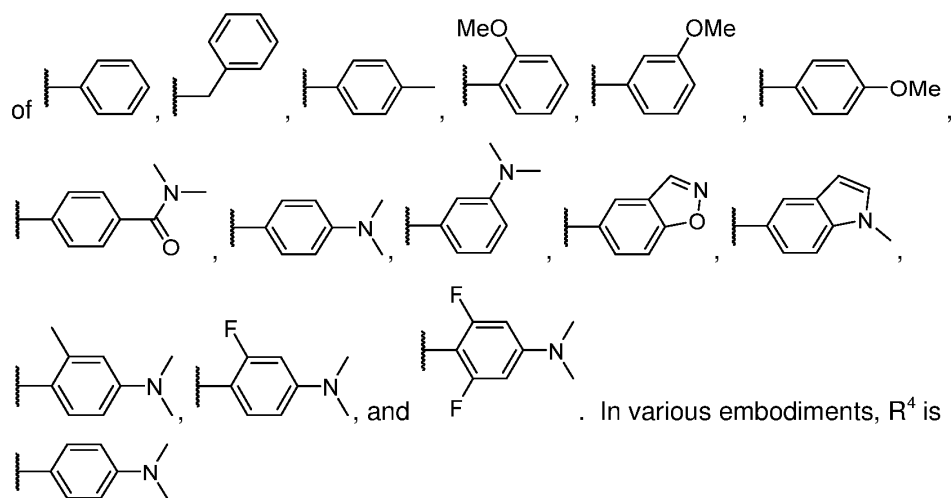




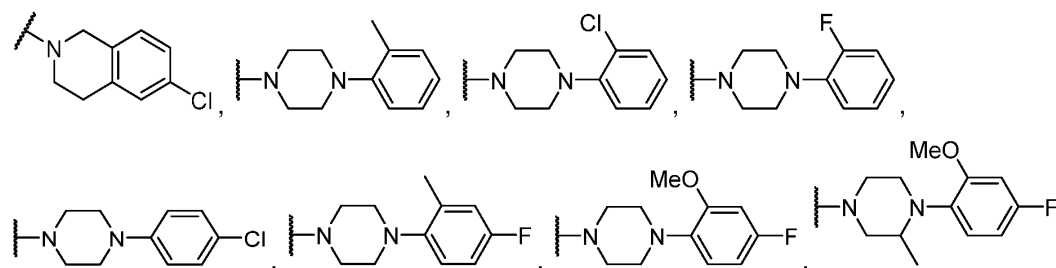
[0015] In some embodiments, X is C=O or (C=O)O. In various embodiments, R³

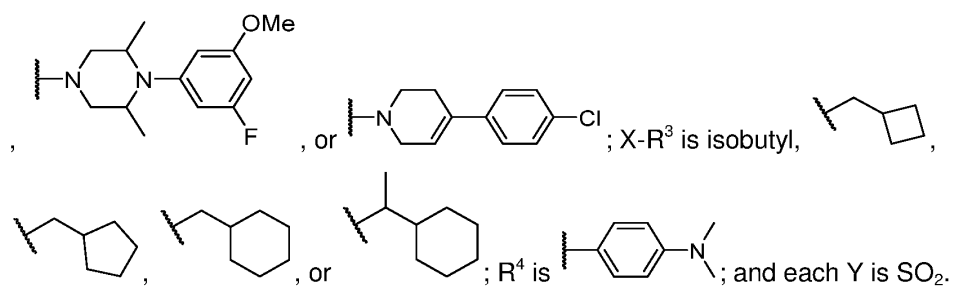


[0016] In various cases, R⁴ comprises C₃₋₈cycloalkyl or C₃₋₉heterocycloalkyl. In some embodiments, R⁴ is  or . In various embodiments, R⁴ comprises C₆₋₁₀aryl or C₂₋₉heteroaryl, and R⁴ is optionally substituted with one to three groups independently selected from halo, C₁₋₃alkyl, C₁₋₃alkoxy, C(O)N(R^N)₂, and N(R^N)₂, and each R^N is independently H or C₁₋₃alkyl. In some embodiments, R⁴ is selected from the group consisting



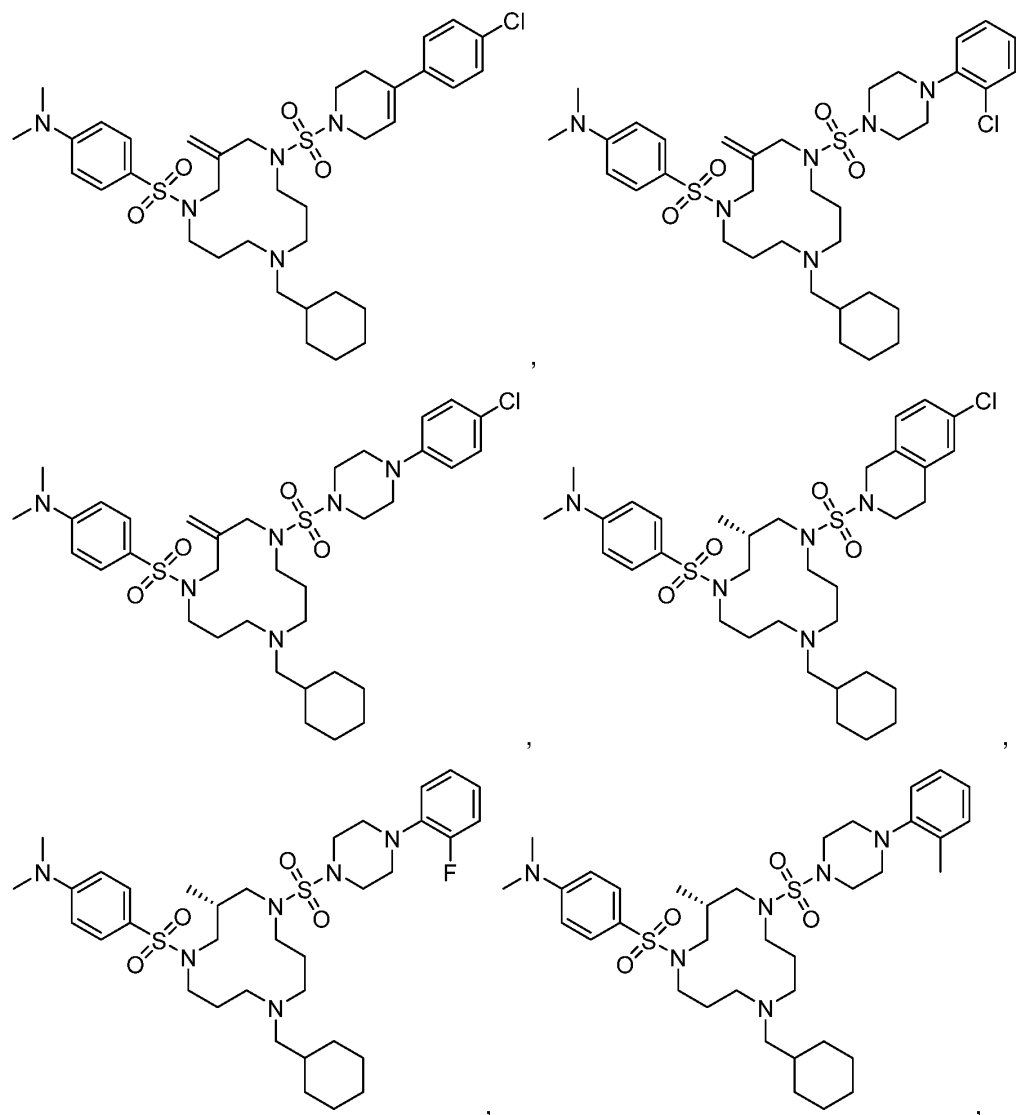
[0017] In some embodiments, R^a, R^b, R^{1a}, and R^{1b} are each H; R¹ is =CH₂ or CH₃; R² is

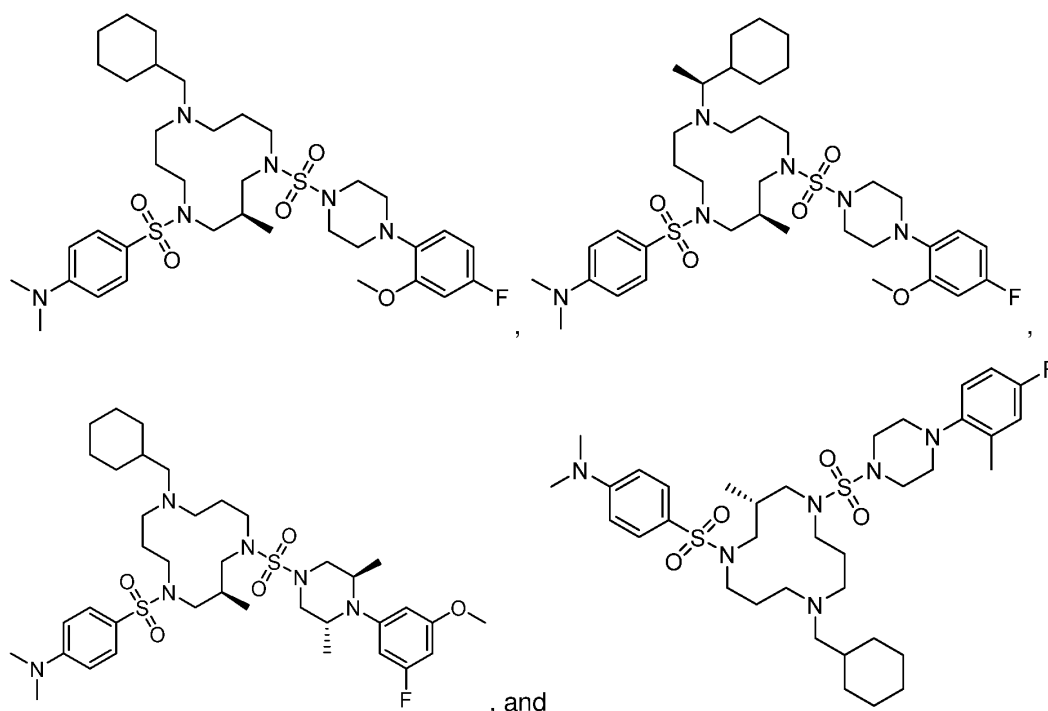




[0018] In some embodiments, R¹ is in a (*S*) configuration.

[0019] Also provided herein are compounds listed in Table A, Table B, and pharmaceutically acceptable salts thereof. In some embodiments, provided herein is a compound is selected from the group consisting of:





[0020] Further provided herein is a pharmaceutical composition comprising a compound of Formula (I), a compound listed in Table A, a compound listed in Table B, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

[0021] Also provided herein is a method of inhibiting protein secretion in a cell comprising contacting the cell with the compound or salt of Formula (I), the compound listed in Table A or a pharmaceutically acceptable salt thereof, a compound listed in Table B or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition described herein in an amount effective to inhibit secretion. In some cases, the contacting comprises administering the compound or the composition to a subject.

[0022] Further provided herein is a method for treating inflammation in a subject comprising administering to the subject a therapeutically effective amount of a compound or salt of Formula (I), a compound listed in Table A or a pharmaceutically acceptable salt thereof, a compound listed in Table B or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition described herein.

[0023] Also provided herein is a method for treating or preventing cancer or precancerous conditions in a subject comprising administering to the subject a therapeutically effective amount of the compound or salt of Formula (I), a compound listed in Table A or a pharmaceutically acceptable salt thereof, a compound listed in Table B or a

pharmaceutically acceptable salt thereof, or the pharmaceutical composition described herein.

[0024] Further aspects and advantages will be apparent to those of ordinary skill in the art from a review of the following detailed description, taken in conjunction with the drawings. The description hereafter includes specific embodiments with the understanding that the disclosure is illustrative, and is not intended to limit the invention to the specific embodiments described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

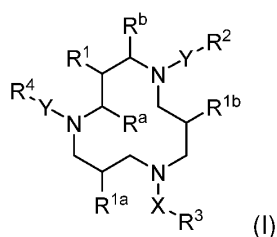
[0025] FIG. 1 depicts B16F10 efficacy data for compound A87 compared to an anti-PD-1 therapy. Compound A87 exhibited superior efficacy with once-a-week dosing over the anti-PD-1 therapy in the refractory model with respect to tumor growth inhibition and percent body weight.

[0026] Further aspects and advantages will be apparent to those of ordinary skill in the art from a review of the following detailed description. While the compounds and methods disclosed herein are susceptible of embodiments in various forms, the description hereafter includes specific embodiments with the understanding that the disclosure is illustrative, and is not intended to limit the invention to the specific embodiments described herein.

DETAILED DESCRIPTION

[0027] Provided herein are compounds that inhibit protein secretion. The compounds described herein can be used to treat or prevent diseases associated with excessive protein secretion, such as inflammation and cancer, improving the quality of life for afflicted individuals.

[0028] The compounds described herein have a structure of Formula (I)



wherein the substituents are described in detail below.

[0029] Without being bound by any particular theory, the compounds described herein inhibit protein secretion by binding to and disabling components of the translocon, including but not limited to Sec61, and in some cases, disrupting in a sequence specific fashion interactions between the nascent signaling sequence of translated proteins with components

of the translocon including but not limited to Sec61. The compounds described herein can bind specifically to the signal sequence with little to no interaction with the translocon itself.

[0030] The compounds described herein can advantageously inhibit the secretion of TNF α with an IC₅₀ of up to 5 μ M, or up to 3 μ M, or up to 1 μ M. In various cases, the compounds disclosed herein can inhibit the secretion of IL-2 with an IC₅₀ of up to 5 μ M, or up to 3 μ M, or up to 1 μ M. In some cases, the compounds disclosed herein can inhibit the secretion of PD-1 with an IC₅₀ of up to 5 μ M, or up to 3 μ M, or up to 1 μ M.

[0031] The compounds described herein are efficacious in reducing tumor growth and body weight. For example, compound A87 showed superior efficacy in reducing tumor growth with once-a-week dosing in a refractory model compared to anti-PD-1 therapy. See the Examples section and FIG. 1.

Chemical Definitions

[0032] As used herein, the term "alkyl" refers to straight chained and branched saturated hydrocarbon groups containing one to thirty carbon atoms, for example, one to twenty carbon atoms, or one to ten carbon atoms. The term C_n means the alkyl group has "n" carbon atoms. For example, C₄ alkyl refers to an alkyl group that has 4 carbon atoms. C₁₋₇alkyl refers to an alkyl group having a number of carbon atoms encompassing the entire range (i.e., 1 to 7 carbon atoms), as well as all subgroups (e.g., 1-6, 2-7, 1-5, 3-6, 1, 2, 3, 4, 5, 6, and 7 carbon atoms). Nonlimiting examples of alkyl groups include, methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *sec*-butyl (2-methylpropyl), *t*-butyl (1,1-dimethylethyl), 3,3-dimethylpentyl, and 2-ethylhexyl. Unless otherwise indicated, an alkyl group can be an unsubstituted alkyl group or a substituted alkyl group.

[0033] As used herein, the term "alkylene" refers to a bivalent saturated aliphatic radical. The term C_n means the alkylene group has "n" carbon atoms. For example, C₁₋₆alkylene refers to an alkylene group having a number of carbon atoms encompassing the entire range, as well as all subgroups, as previously described for "alkyl" groups.

[0034] As used herein, the term "alkenyl" is defined identically as "alkyl" except for containing at least one carbon-carbon double bond, and having two to thirty carbon atoms, for example, two to twenty carbon atoms, or two to ten carbon atoms. The term C_n means the alkenyl group has "n" carbon atoms. For example, C₄ alkenyl refers to an alkenyl group that has 4 carbon atoms. C₂₋₇alkenyl refers to an alkenyl group having a number of carbon atoms encompassing the entire range (i.e., 2 to 7 carbon atoms), as well as all subgroups (e.g., 2-6, 2-5, 3-6, 2, 3, 4, 5, 6, and 7 carbon atoms). Specifically contemplated alkenyl groups include ethenyl, 1-propenyl, 2-propenyl, and butenyl. Unless otherwise indicated, an alkenyl group can be an unsubstituted alkenyl group or a substituted alkenyl group.

[0035] As used herein, the term “cycloalkyl” refers to an aliphatic cyclic hydrocarbon. The term C_n means the cycloalkyl group has “n” carbon atoms. For example, C_5 cycloalkyl refers to a cycloalkyl group that has 5 carbon atoms in the ring. C_{5-8} cycloalkyl refers to cycloalkyl groups having a number of carbon atoms encompassing the entire range (i.e., 5 to 8 carbon atoms), as well as all subgroups (e.g., 5-6, 6-8, 7-8, 5-7, 5, 6, 7, and 8 carbon atoms). Nonlimiting examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Unless otherwise indicated, a cycloalkyl group can be an unsubstituted cycloalkyl group or a substituted cycloalkyl group.

[0036] As used herein, the term “cycloalkenyl” is defined similarly to “cycloalkyl” except for containing at least one carbon-carbon double bond, but is not aromatic. The term C_n means the cycloalkenyl group has “n” carbon atoms. For example, C_5 cycloalkenyl refers to a cycloalkenyl group that has 5 carbon atoms in the ring. C_{5-8} cycloalkenyl refers to cycloalkenyl groups having a number of carbon atoms encompassing the entire range (i.e., 5 to 8 carbon atoms), as well as all subgroups (e.g., 5-6, 6-8, 7-8, 5-7, 5, 6, 7, and 8 carbon atoms). Nonlimiting examples of cycloalkenyl groups include cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, and cyclooctenyl. Unless otherwise indicated, a cycloalkenyl group can be an unsubstituted cycloalkenyl group or a substituted cycloalkenyl group.

[0037] As used herein, the term “heterocycloalkyl” is defined similarly as cycloalkyl, except the ring contains one to three heteroatoms independently selected from oxygen, nitrogen, or sulfur. Nonlimiting examples of heterocycloalkyl groups include piperidine, tetrahydrofuran, tetrahydropyran, dihydrofuran, morpholine, oxazepanyl, and the like. Cycloalkyl and heterocycloalkyl groups can be saturated or partially unsaturated ring systems optionally substituted with, for example, one to three groups, independently selected alkyl, alkyleneOH, $C(O)NH_2$, NH_2 , oxo ($=O$), aryl, haloalkyl, halo, and OH. Heterocycloalkyl groups optionally can be further N-substituted as described herein.

[0038] As used herein, the term “aryl” refers to monocyclic or polycyclic (e.g., fused bicyclic and fused tricyclic) carbocyclic aromatic ring systems. Examples of aryl groups include, but are not limited to, phenyl, naphthyl, tetrahydronaphthyl, phenanthrenyl, biphenylenyl, indanyl, indenyl, anthracenyl, fluorenyl, tetralinyl. Unless otherwise indicated, an aryl group can be an unsubstituted aryl group or a substituted aryl group.

[0039] As used herein, the term “heteroaryl” refers to monocyclic or polycyclic (e.g., fused bicyclic and fused tricyclic) aromatic ring systems, wherein one to four-ring atoms are selected from oxygen, nitrogen, or sulfur, and the remaining ring atoms are carbon, said ring system being joined to the remainder of the molecule by any of the ring atoms. Nonlimiting

examples of heteroaryl groups include, but are not limited to, pyridyl, pyridazinyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, tetrazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, furanyl, thienyl, quinoliny, isoquinoliny, benzoxazolyl, benzimidazolyl, benzofuranyl, benzothiazolyl, triazinyl, triazolyl, purinyl, pyrazinyl, purinyl, indoliny, phthalziny, indazolyl, quinoliny, isoquinoliny, cinnoliny, quinazoliny, naphthyridiny, pyridopyridiny, indolyl, 3H-indolyl, pteridiny, and quinoxaliny. Unless otherwise indicated, a heteroaryl group can be an unsubstituted heteroaryl group or a substituted heteroaryl group.

[0040] As used herein, the term “hydroxy” or “hydroxyl” as used herein refers to the “—OH” group.

[0041] As used herein, the term “alkoxy” or “alkoxyl” refers to a “—O-alkyl” group.

[0042] As used herein, the term “halo” is defined as fluoro, chloro, bromo, and iodo.

[0043] As used herein, the term “carboxy” or “carboxyl” refers to a “—COOH” group.

[0044] As used herein, the term “amino” refers to a —NH₂ or —NH— group, wherein any hydrogen can be replaced with an alkyl, cycloalkyl, aryl, heteroaryl, or heterocycloalkyl group.

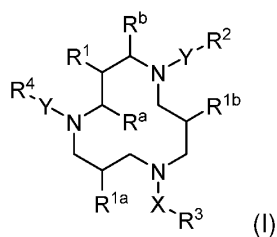


[0045] As used herein, the term “sulfonyl” refers to a group.

[0046] A “substituted” functional group (e.g., a substituted alkyl, alkylene, cycloalkyl, aryl, or heteroaryl) is a functional group having at least one hydrogen radical that is substituted with a non-hydrogen radical (i.e., a substituent). Examples of non-hydrogen radicals (or substituents) include, but are not limited to, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, ether, aryl, heteroaryl, heterocycloalkyl, hydroxyl, oxy (or oxo), alkoxyl, ester, thioester, acyl, carboxyl, cyano, nitro, amino, sulfhydryl, and halo. When a substituted alkyl group includes more than one non-hydrogen radical, the substituents can be bound to the same carbon or two or more different carbon atoms.

Protein Secretion Inhibitors

[0047] In one aspect, the compounds of the disclosure have a structure of Formula (I), or a pharmaceutically acceptable salt thereof:



wherein:

each of R^a and R^b is independently H or C_{1-3} alkyl;

R^1 is H, OH, C_{1-3} alkyl, OC_{1-3} alkyl, $=CH_2$, or $=NOR^5$; or R^1 is C_{3-6} cycloalkyl or C_{3-6} heterocycloalkyl and forms a spiro group with the ring carbon to which it is attached;

each of R^{1a} and R^{1b} is independently H or C_{1-3} alkyl;

R^2 is C_{1-6} alkyl, $N(R^5)_2$, C_{3-8} cycloalkyl, C_{3-9} heterocycloalkyl, C_{3-9} heterocycloalkenyl, or C_{6-10} aryl;

R^3 is H, C_{1-6} alkyl, C_{3-8} cycloalkyl, C_{3-8} cycloalkenyl, C_{3-7} heterocycloalkyl, C_{6-10} aryl, or C_{2-6} heteroaryl;

R^4 is C_{3-8} cycloalkyl, C_{3-9} heterocycloalkyl, C_{6-10} aryl, or C_{2-9} heteroaryl;

each R^5 independently is H, C_{1-3} alkyl, or C_{0-2} alkylene- C_{6-10} aryl;

X is absent, C_{1-3} alkylene, $C=O$, or $(C=O)O$;

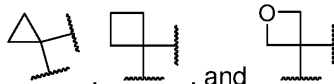
Y is SO or SO_2 ;

each heterocycloalkyl, heterocycloalkenyl, and heteroaryl group independently has 1, 2, or 3 ring heteroatoms selected from N, O, and S.

[0048] In some embodiments, each Y is SO. In various embodiments, each Y is SO_2 .

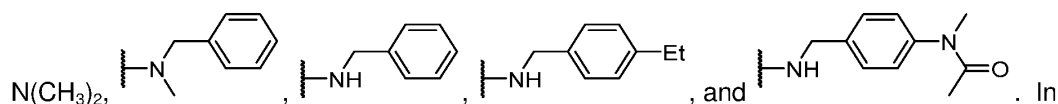
[0049] In various cases, R^a is H. In some cases, R^a is C_{1-3} alkyl. In some embodiments, R^a is CH_3 . In some embodiments, R^b is H. In various embodiments, R^b is C_{1-3} alkyl. In various cases, R^b is CH_3 . In some cases, both R^a and R^b are H.

[0050] In some cases, R^1 is H, OH, or $=NOR^5$. In various cases, R^5 is H or CH_3 . Therefore, suitable R^1 groups can include H, OH, $=NOH$, and $=NORCH_3$. In some embodiments, R^1 is C_{1-3} alkyl or OC_{1-3} alkyl. For example, R^1 can be CH_3 or OCH_3 . In some cases, R^1 is CH_3 and exhibits *S* stereochemistry. In various embodiments, R^1 is C_{3-6} cycloalkyl or C_{3-6} heterocycloalkyl and forms a spiro group with the ring carbon to which it is

attached. Suitable R^1 spiro groups can include . In various cases, R^1 is $=CH_2$. In some embodiments, each of R^{1a} and R^{1b} is H. In some embodiments,

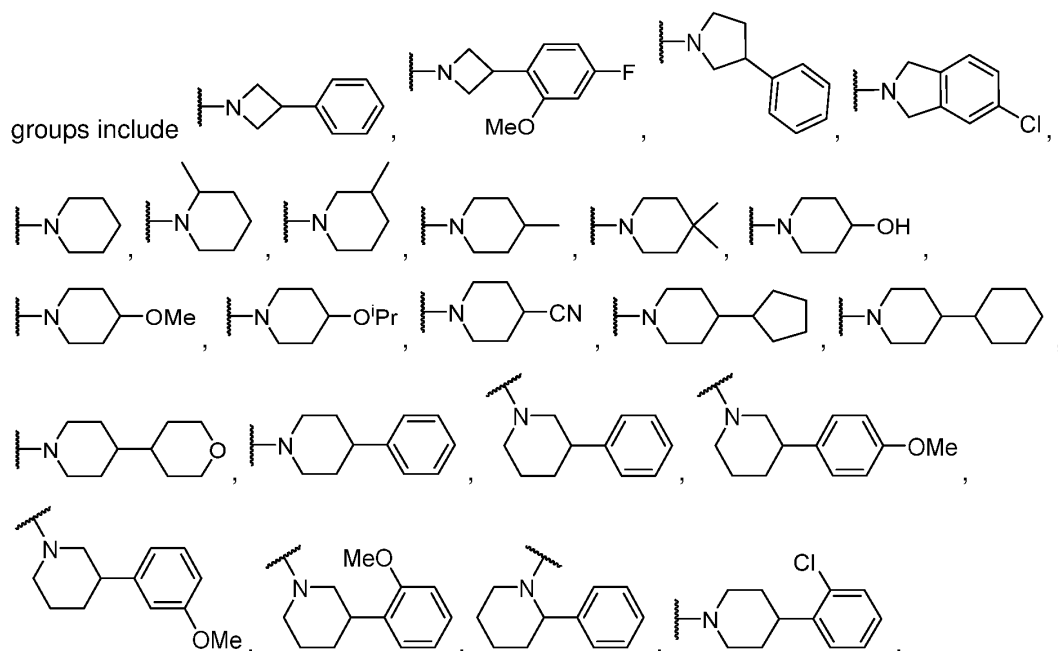
at least one of R^{1a} and R^{1b} is C_{1-3} alkyl. In some embodiments, each of R^{1a} and R^{1b} is CH_3 . In some embodiments, R^a is H, R^b is H, and R^1 is CH_3 . In various embodiments, R^a is CH_3 , R^b is H, and R^1 is H. In some cases, R^a is H, R^b is CH_3 , and R^1 is H. In various cases, R^a is CH_3 , R^b is H, and R^1 is CH_3 . In some embodiments, R^a is H, R^b is CH_3 , and R^1 is CH_3 . In various embodiments, R^a is CH_3 , R^b is CH_3 , and R^1 is H.

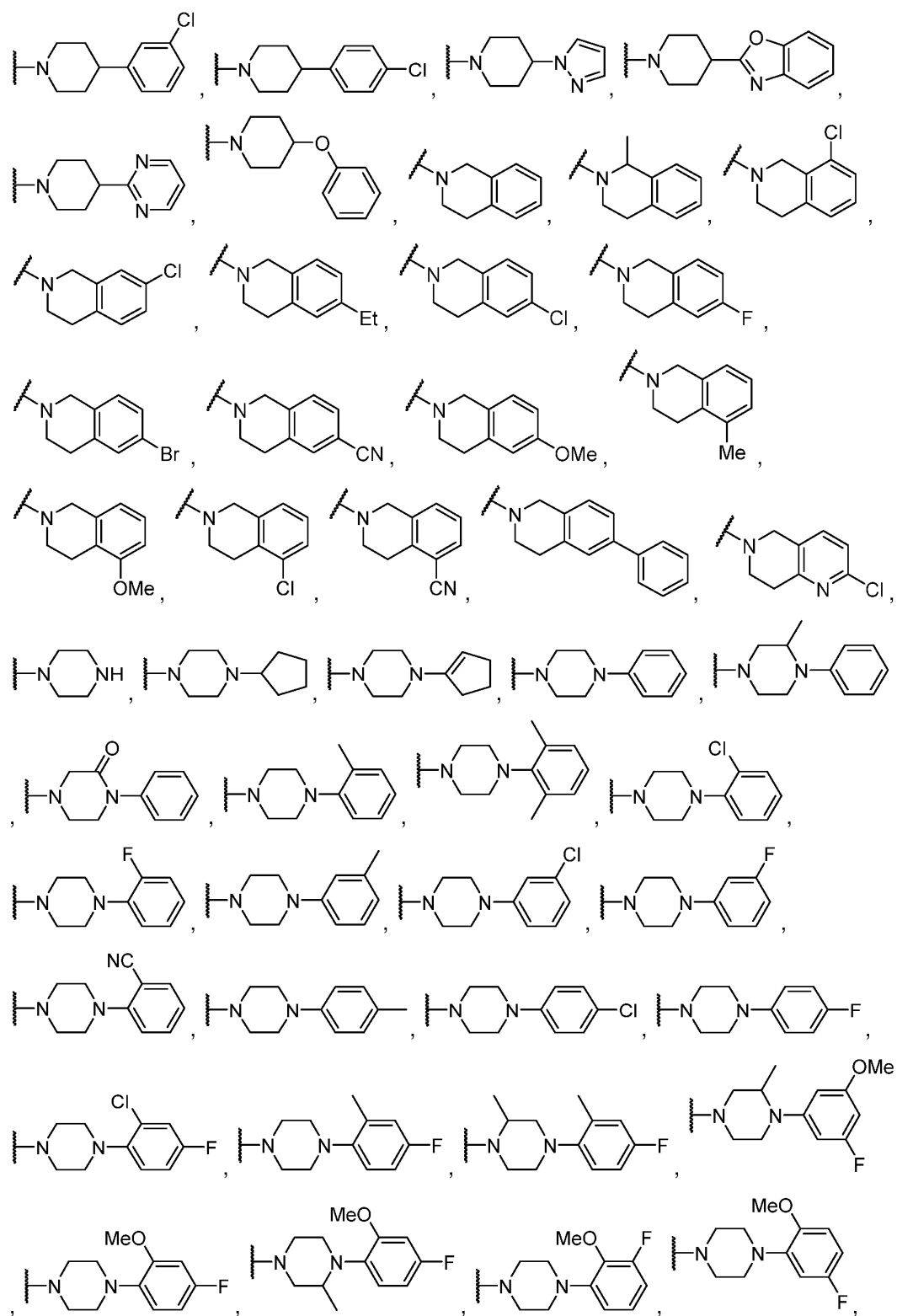
[0051] In some embodiments, R^2 is C_{1-6} alkyl or $N(R^5)_2$. In some cases, each R^5 independently comprises H, C_{1-3} alkyl, or benzyl. Suitable R^2 groups can include Et, iPr,

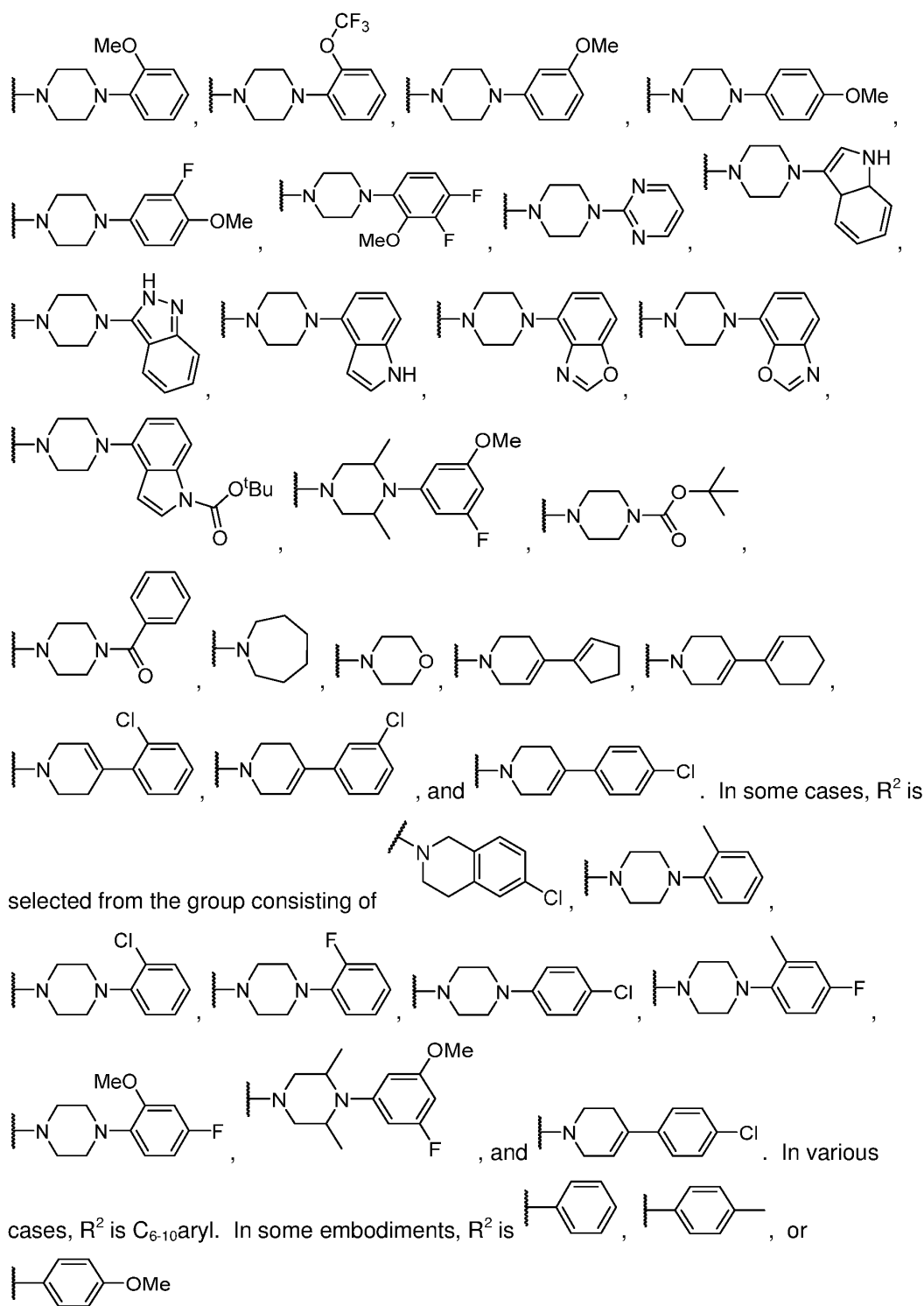


some cases, R^2 comprises C_{3-8} cycloalkyl. In various cases, R^2 comprises cyclopentyl or cyclohexyl. In some embodiments, R^2 is C_{3-9} heterocycloalkyl or C_{3-9} heterocycloalkenyl. In various embodiments, R^2 comprises oxetanyl, azetidiny, tetrahydrofuranyl, pyrrolidiny, tetrahydropyranyl, pyranly, piperidiny, piperaziny, azepanyl, morpholinyl, or tetrahydropyridiny. In various embodiments, the C_{3-9} heterocycloalkyl or C_{3-9} heterocycloalkenyl comprises a bridge or a spiro group. In some cases, the C_{3-9} heterocycloalkyl comprising a bridge or a spiro group is selected from the group consisting

of , , and , and R^6 is C_{1-6} alkyl, C_{3-8} cycloalkyl, or C_{6-10} aryl. In some cases, R^6 is phenyl optionally substituted with one to three groups independently selected from halo, C_{1-3} alkyl, C_{1-3} alkoxy, and CN. Examples of suitable R^2







[0052] In various embodiments, X is absent. In some embodiments, R^3 comprises C_{1-6} alkyl or H. In some cases, R^3 is C_{1-6} alkyl, such as methyl, ethyl, propyl, butyl, pentyl, or

hexyl. In some cases, R³ is 2-methylbutyl, isopropyl, isopentyl, CH₂CH₂OCH₃, CH₂C(CH₃)₂CN, CH₂CF₃, or CH₂CH₂CF₃. In various cases, R³ is isobutyl.

[0053] In some embodiments, X is C₁₋₃alkylene. In some cases, X is CH₂, CH₂CH₂, or CH(CH₃). In various cases, R³ comprises C₃₋₈cycloalkyl, C₃₋₈cycloalkenyl, C₃₋₇heterocycloalkyl, C₆₋₁₀aryl, or C₂₋₆heteroaryl. In some embodiments, R³ comprises cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclopentenyl, cyclohexenyl, tetrahydropyranyl, phenyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, furanyl, thiophenyl, thiazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiadiazolyl, pyridinyl, pyrazinyl, or pyrimidinyl.

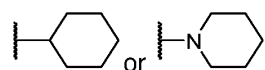
[illegible]

In some cases, X-R³ is , , , or . In some cases, X-R³ is , or .

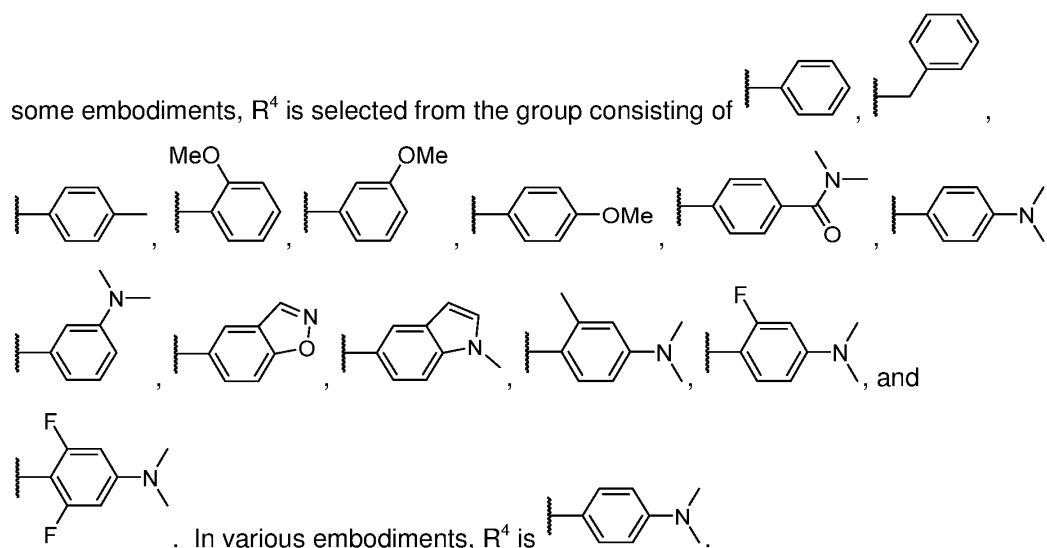
[0054] In some embodiments, X is C=O or (C=O)O. In various embodiments, R³

comprises C₁₋₆alkyl or C₆₋₁₀aryl. In some cases, X-R³ is , , , or

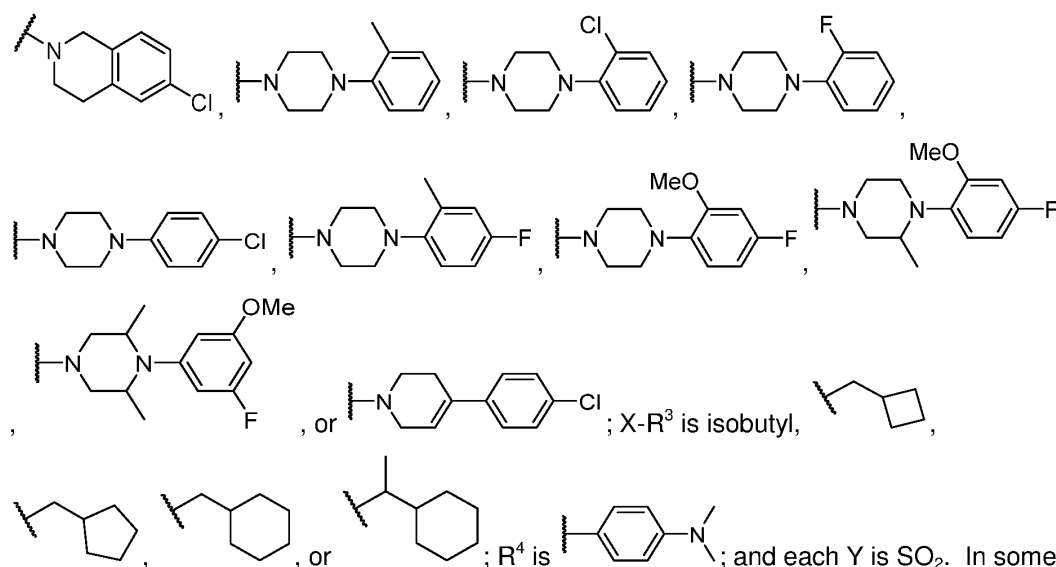
[0055] In various cases, R⁴ comprises C₃₋₈cycloalkyl or C₃₋₉heterocycloalkyl, such as



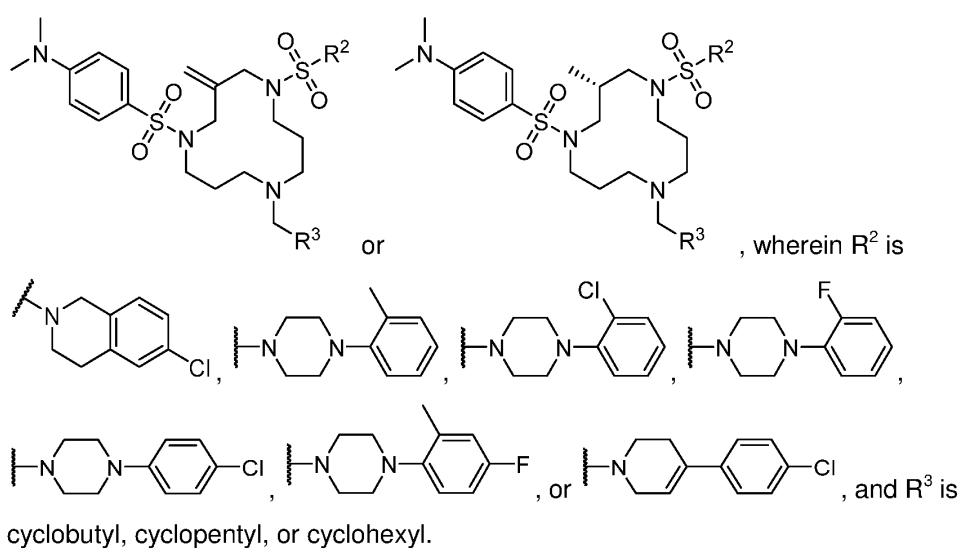
 or . In various embodiments, R⁴ comprises C₆₋₁₀aryl or C₂₋₉heteroaryl, and R⁴ is optionally substituted with one to three groups independently selected from halo, C₁₋₃alkyl, C₁₋₃alkoxy, C(O)N(R^N)₂, and N(R^N)₂, and each R^N is independently H or C₁₋₃alkyl. In



[0056] In some embodiments, R^a, R^b, R^{1a}, and R^{1b} are each H, R¹ is =CH₂ or CH₃; R² is

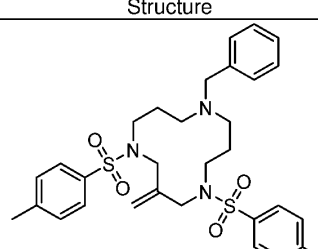
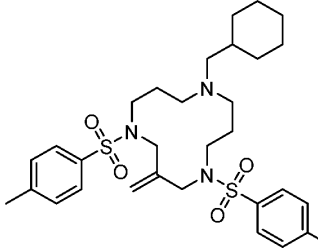
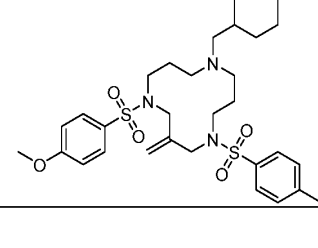
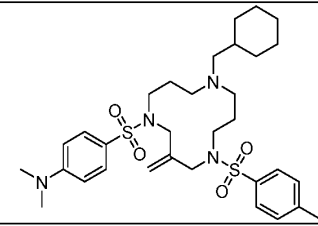
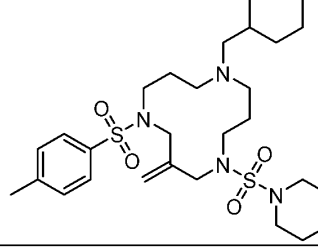
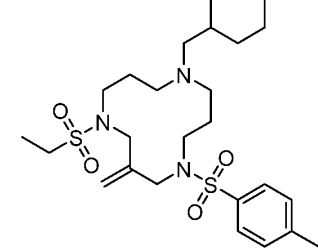


cases, R¹ is in a (S) configuration. In some cases, provided herein are compounds comprising a structure

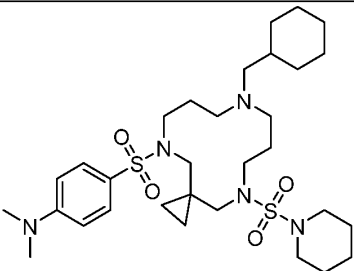
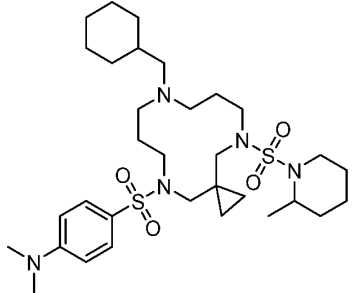
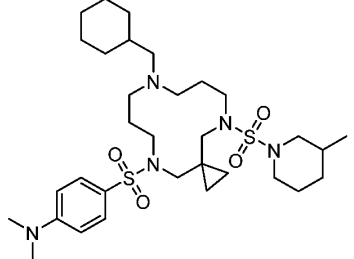
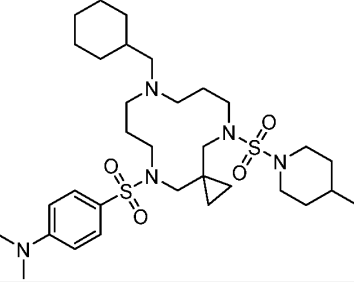
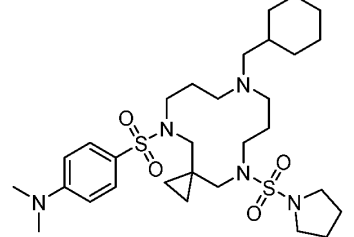
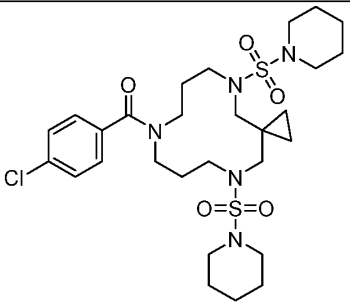
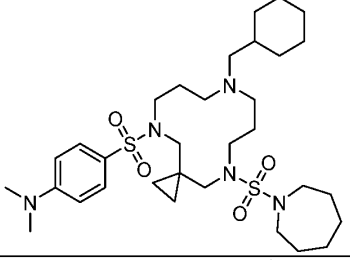
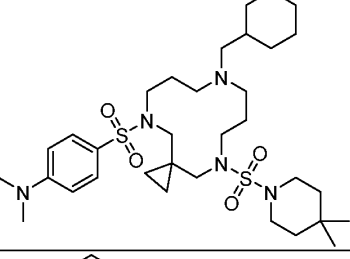
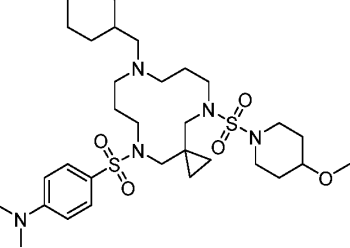
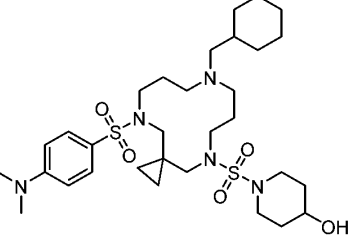
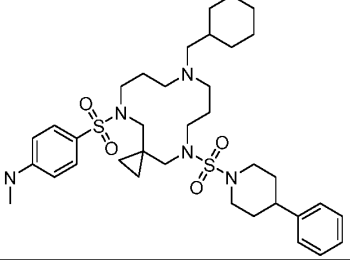


[0057] Examples of compounds of Formula (I) are shown in Table A, as compounds A1-A210, or a pharmaceutically acceptable salt thereof. In some cases, the compound is a compound of A1-A151, or pharmaceutically acceptable salt thereof.

Table A

No.	Structure
A1	
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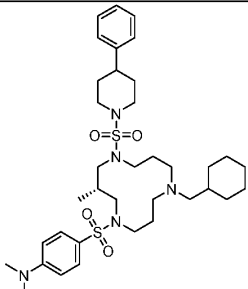
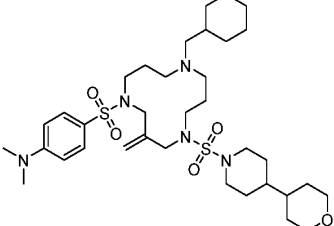
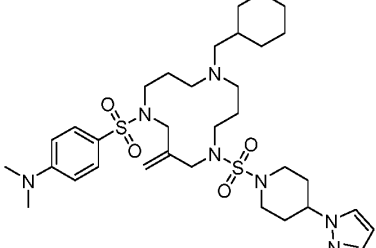
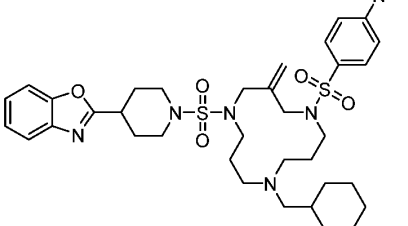
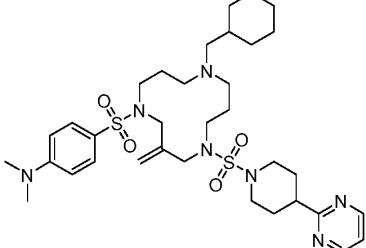
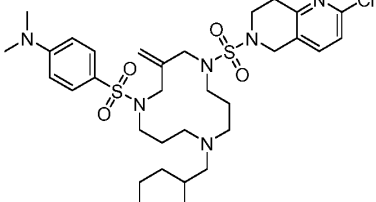
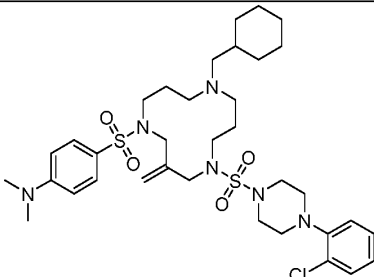
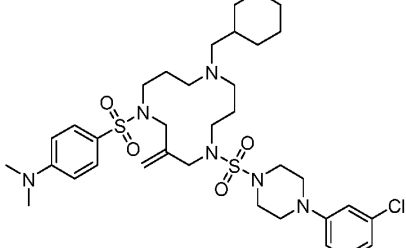
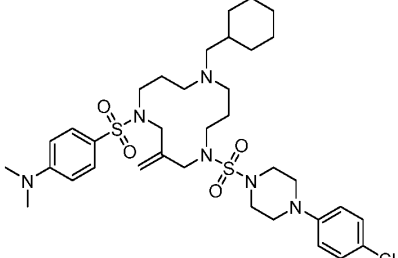
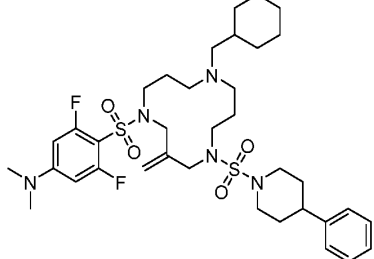
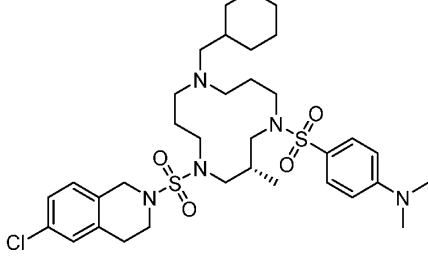
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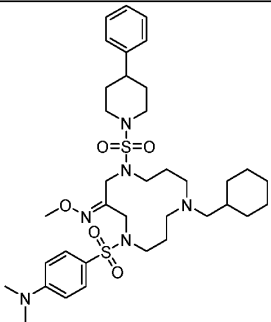
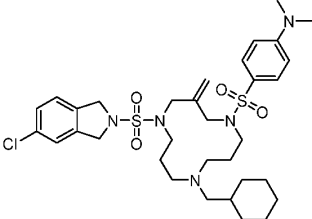
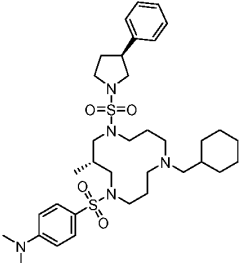
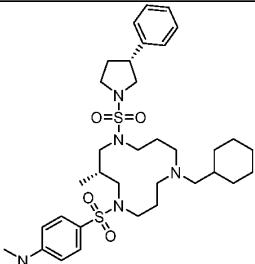
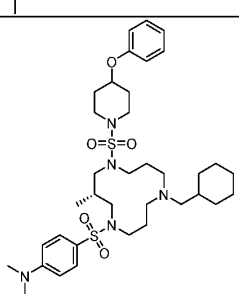
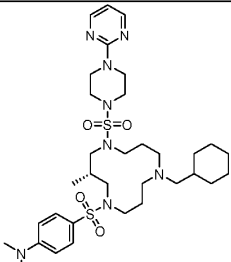
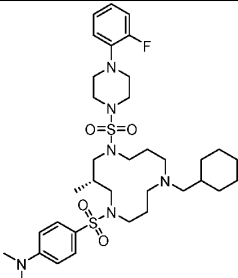
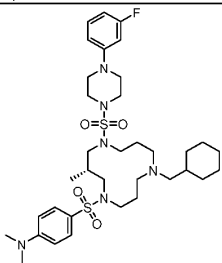
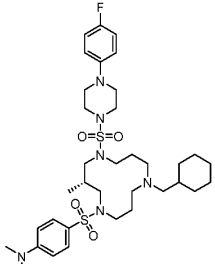
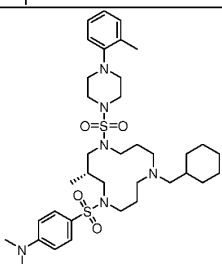
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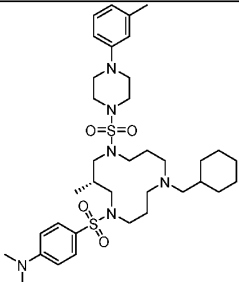
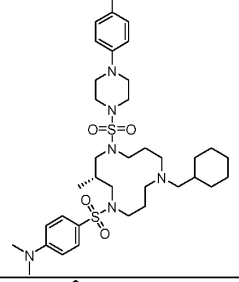
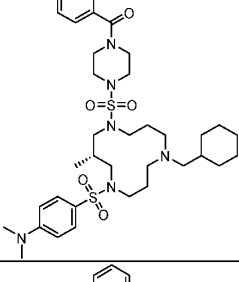
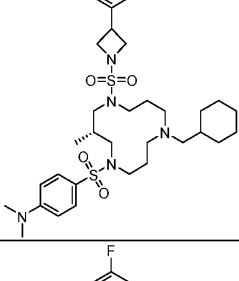
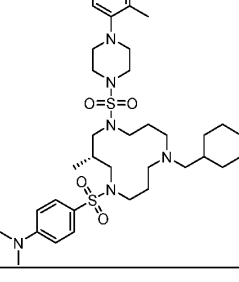
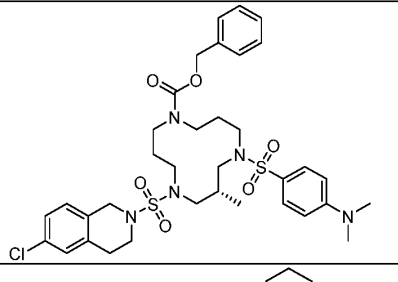
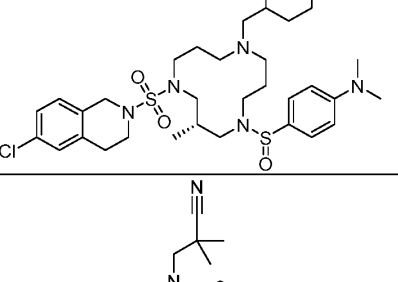
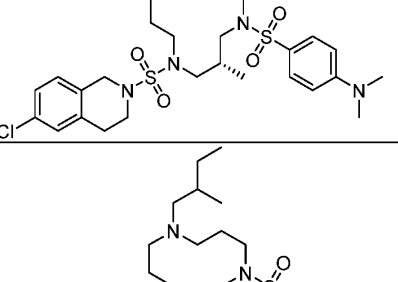
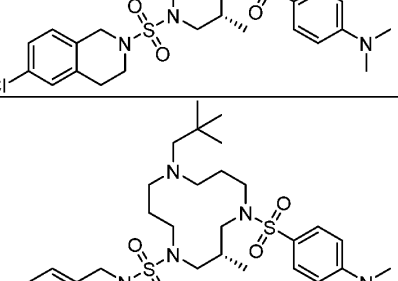
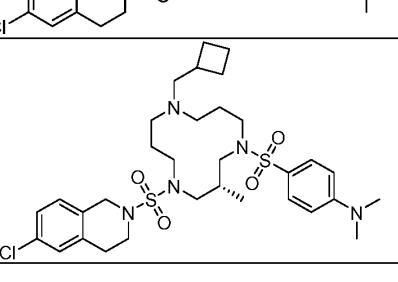
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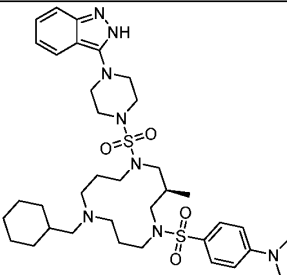
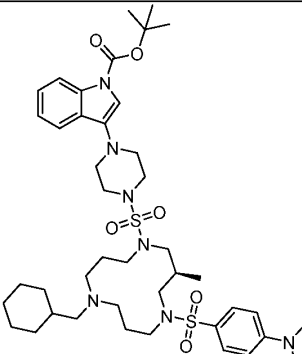
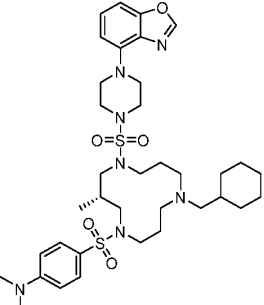
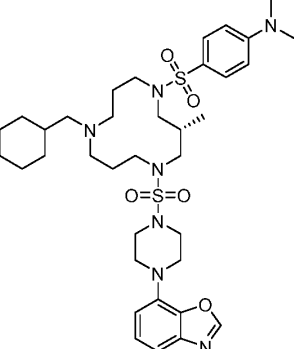
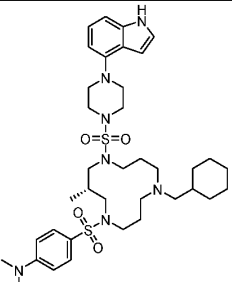
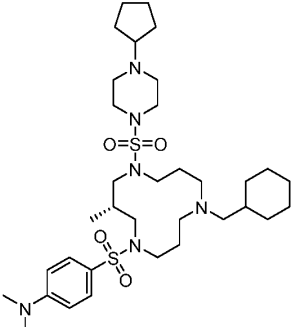
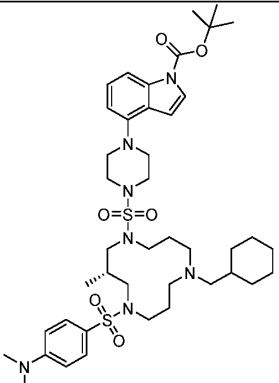
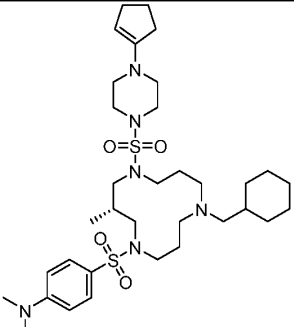
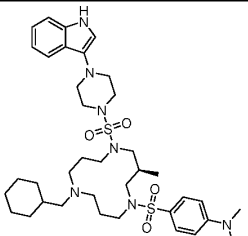
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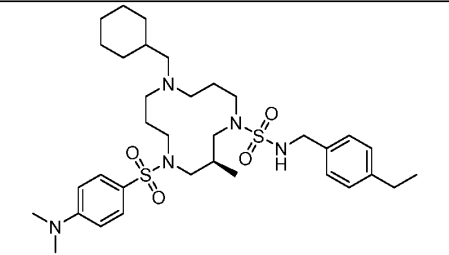
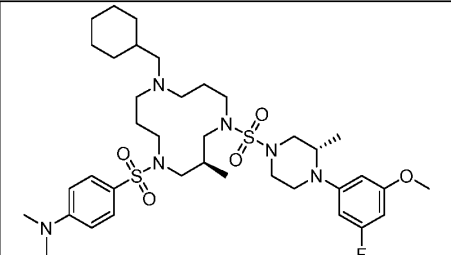
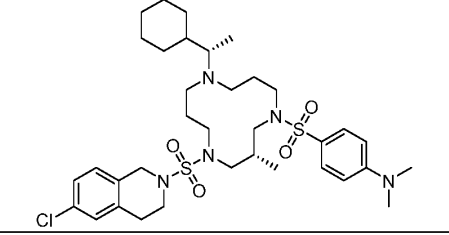
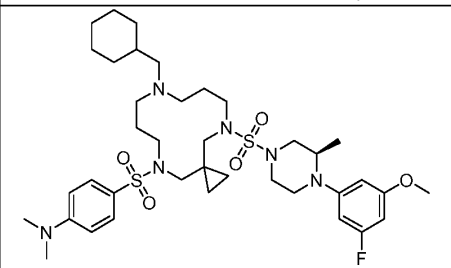
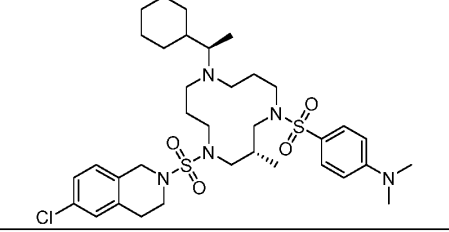
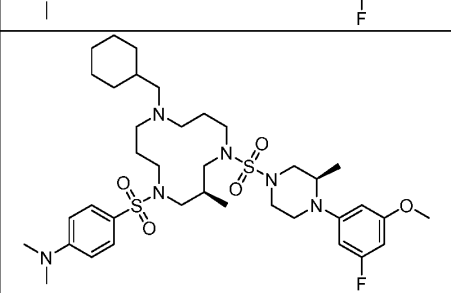
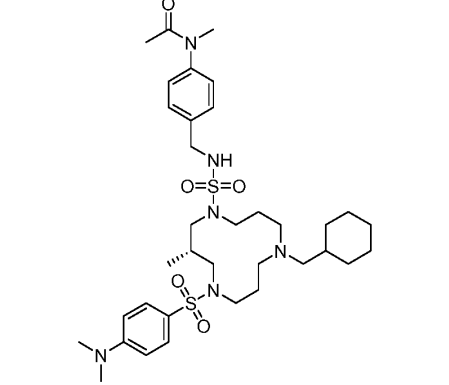
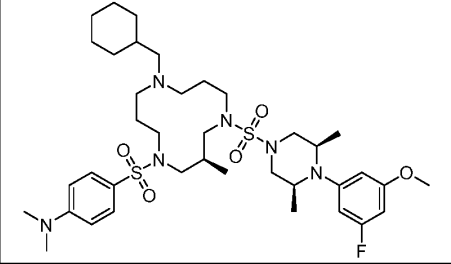
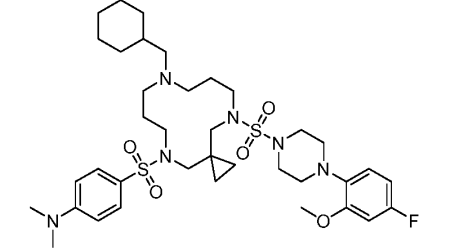
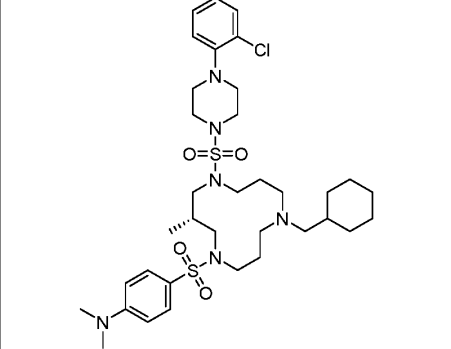
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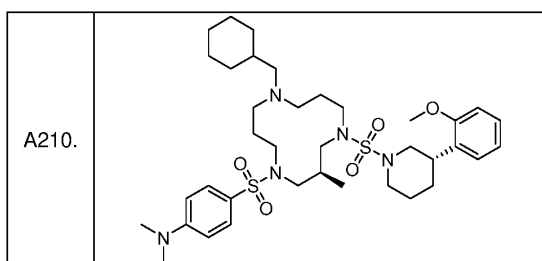
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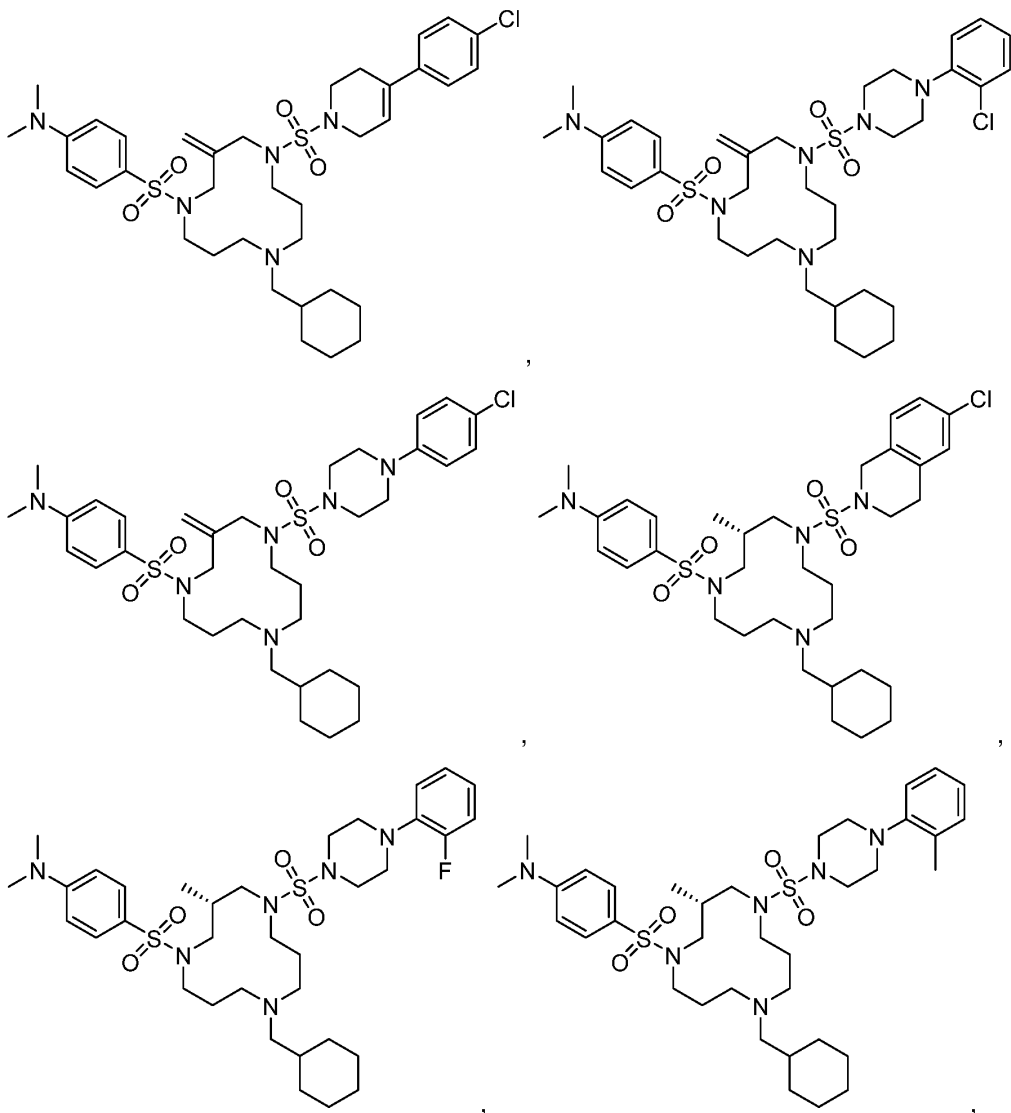
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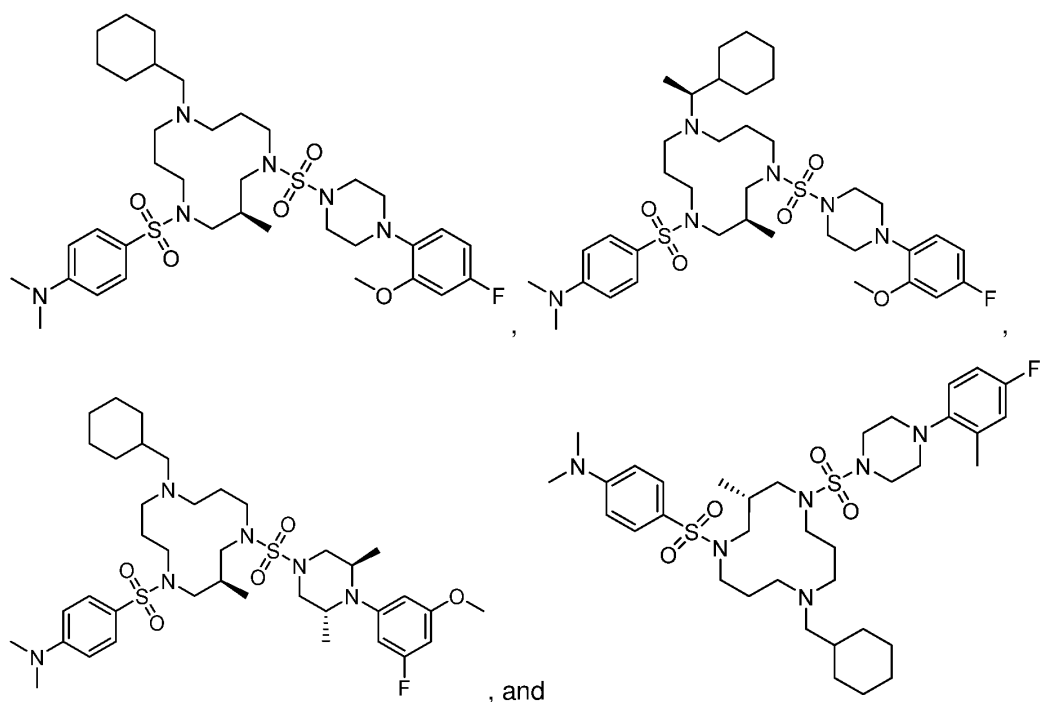
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A202	
A203	
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A209	



[0058] In some embodiments, the compounds of the disclosure include





or a pharmaceutically acceptable salt thereof.

[0059] Additional compounds of the disclosure are shown in Table B as compounds B1-B22, or a pharmaceutically acceptable salt thereof. In some cases, the compound is a compound of B1-B20, or a pharmaceutically acceptable salt thereof.

Table B

No.	Structure
B1	
B2	
B3	
B4	

No.	Structure
B5	
B6	
B7	
B8	
B9	
B10	
B11	

No.	Structure
B12	
B13	
B14	
B15	
B16	

No.	Structure
B17	
B18	
B19	

No.	Structure
B20	
B21	
B22	

[0060] The chemical structures having one or more stereocenters depicted with dashed and bold bonds (i.e., and —) are meant to indicate absolute stereochemistry of the stereocenter(s) present in the chemical structure. Bonds symbolized by a simple line do not indicate a stereo-preference. Unless otherwise indicated to the contrary, chemical structures that include one or more stereocenters which are illustrated herein without indicating absolute or relative stereochemistry, encompass all possible stereoisomeric forms of the compound (e.g., diastereomers, enantiomers) and mixtures thereof. Structures with a single bold or dashed line, and at least one additional simple line, encompass a single enantiomeric series of all possible diastereomers.

Synthesis of Protein Secretion Inhibitors

[0061] The compounds provided herein can be synthesized using conventional techniques readily available starting materials known to those skilled in the art. In general, the compounds provided herein are conveniently obtained via standard organic chemistry synthesis methods.

Synthesis of Final Compounds

[0062] In some cases, the compounds described herein can be synthesized by reacting a sulfonyl chloride having a desired R² group with propane-1,3-diamine, and reacting the resulting product with an aldehyde having a desired R³ group to form N-substituted propyl

amine compound. A propyl amino group can be introduced to the N-substituted propyl amine at the R⁴ position via reaction with 2-(3-bromopropyl)isoindoline-1,3-dione followed by hydrazine, and a desired R⁴ group can be coupled to the compound by reacting the compound with a sulfonyl chloride functionalized with the desired R⁴ group. Finally, the compound can be cyclized via a cyclization agent having a desired R¹ group, such as 3-chloro-2-chloromethyl-1-propene, 2-methylpropane-1,3-diyl diethanesulfonate, cyclopropane-1,1-diylbis(methylene) diethanesulfonate, 1,3-dichloropropane, or 1,4-dichlorobutane.

[0063] In some embodiments, compounds having a methylene group at R¹ can be synthesized by coupling a (3-aminopropyl)propane-1,3-diamine group derivatized with the desired R³ and R⁴ groups with a desired R²-sulfamoyl chloride or R²-sulfanyl chloride group, and then reacting the resulting compound with 3-chloro-2-chloromethyl-1-propene to form the desired triazacyclododecansulfonamide compound, as exemplified by **Route 1** in the Examples section.

[0064] In various embodiments, compounds having a methylene group at R¹ can be synthesized by coupling protected butane-1,3-diamine with an aldehyde having a desired R³ group to form a 3-(azaneyl)-N-butan-1-amine, reacting the amino group at the R² position with a chloro group functionalized with a desired R² group, introducing a propyl amino group to the 3-(azaneyl)-N-butan-1-amine at the R⁴ position via reaction with 2-(3-bromopropyl)isoindoline-1,3-dione followed by hydrazine, reacting the resulting amino group at the R⁴ position with a chloro group functionalized with a desired R⁴ group, and then reacting the resulting compound with 3-chloro-2-chloromethyl-1-propene to form the desired triazacyclododecansulfonamide compound, as exemplified by **Route 2** in the Examples section.

[0065] In some cases, compounds having a spiro group at R¹ can be synthesized by reacting a N-(3-(azaneyl)propyl)propane-1,3-diamino group derivatized with the desired R², R³, and R⁴ groups with 1,1-diylbis(methylene)diethanesulfonate derivatized with the desired spiro group, as exemplified by **Routes 3, 8, and 9** in the Examples section.

[0066] In various cases, compounds provided herein can be synthesized by reducing a benzyl group at R² on N-(3-(azaneyl)propyl)propane-1,3-diamino functionalized with the desired R³ and R⁴ groups, and then reacting the resulting product with a desired aldehyde derivatized with a desired R² group (e.g., dimethyl cyclohexyl), as exemplified by **Route 4** in the Examples section.

[0067] In some embodiments, compounds having a carboxylate group at R² can be prepared by reacting triazacyclododecansulfonyl group derivatized with desired R¹, R³, and R⁴

groups with a desired alkyl group and triphosgene under basic conditions, as exemplified by **Route 5** in the Examples section.

[0068] In various embodiments, compounds having piperidinyl group at R^2 can be prepared by reacting with DBU/mercaptoethanol a triazacyclododensulfonyl compound having desired R^1 , R^3 , and R^4 groups and functionalized at R^2 with a nitrophenyl group, and then coupling the resulting product with piperidinyl sulfonyl chloride, as exemplified by **Route 6** in the Examples section.

[0069] In some embodiments, compounds having a hydrogen group at R^1 can be synthesized by coupling N-(3-(azaneyl)propyl)propane-1,3-diamino functionalized with the desired R^2 , R^3 , and R^4 groups with 1,3-dichloropropane or 1,4-dichlorobutane, as exemplified by **Route 7** and **Route 17** in the Examples section.

[0070] In some cases, compounds having a chlorophenylmethanone group at R^2 can be synthesized by reacting triazacyclododensulfonyl functionalized with desired R^1 , R^3 , and R^4 groups with 4-chlorobenzoyl chloride under basic conditions, as exemplified by **Route 10** in the Examples section.

[0071] In various cases, compounds having a 4-(dimethylamino)-2-methylbenzyl group at R^4 and a spiro group at R^1 can be prepared by reacting a N-(3-(azaneyl)propyl)propane-1,3-diamino compound functionalized with the desired R^2 and R^3 groups with 4-(dimethylamino)-2-methylbenzenesulfonyl chloride, and then cyclizing the compound via coupling with 1,1-diylbis(methylene)diethanesulfonate derivatized with the desired spiro group using methods previously described herein, as exemplified by **Route 11** in the Examples section.

[0072] In some embodiments, the compounds described herein can be prepared by reacting a sulfonyl chloride having a desired R^2 group with propane-1,3-diamine, and reacting the resulting product with an aldehyde having a desired R^3 group to form a 3-(azaneyl)-N-butan-1-amine compound. A propyl amino group can be introduced to the 3-(azaneyl)-N-butan-1-amine at the R^4 position via reaction with 2-(3-bromopropyl)isoindoline-1,3-dione followed by hydrazine, and a desired R^4 group can be coupled to the compound by reacting the compound with desired sulfonyl chloride (e.g., 4-(dimethylamino)-2-methylbenzenesulfonyl chloride). Finally, the compound can be cyclized via an appropriate cyclization agent, such as 3-chloro-2-chloromethyl-1-propene, 2-methylpropane-1,3-diyl diethanesulfonate, cyclopropane-1,1-diylbis(methylene) diethanesulfonate, 1,3-dichloropropane, or 1,4-dichlorobutane, as previously described herein, as exemplified by **Route 12** in the Examples section.

[0073] In various embodiments, compounds having a 4-phenylpiperidineyl-1-yl sulfonyl group at R^2 can be synthesized by reacting a triazacyclododensulfonyl group functionalized

with desired R¹, R³, and R⁴ groups with 3-methyl-1-((4-phenylpiperidin-1-yl)sulfonyl)-1H-imidazol-3-ium trifluoromethanesulfonate, as exemplified by **Route 14** in the Examples section.

[0074] In some cases, compounds having a 4-phenylpiperidineyl-1-yl sulfonyl group at R² can be synthesized by reacting N-(3-(azaneyl)propyl)propane-1,3-diamino functionalized with the desired R³ and R⁴ groups with 3-methyl-1-((4-phenylpiperidin-1-yl)sulfonyl)-1H-imidazol-3-ium trifluoromethanesulfonate, and then cyclizing the compound with an appropriate cyclization agent, such as 2-methylpropane-1,3-diyl diethanesulfonate, 3-chloro-2-chloromethyl-1-propene, cyclopropane-1,1-diylbis(methylene) diethanesulfonate, 1,3-dichloropropane or 1,4-dichlorobutane, as previously described herein, as exemplified by **Route 15** in the Examples section. Analogous compounds having a methyl group adjacent to the R⁴ nitrogen can be similarly synthesized using 3-chloro-2-(chloromethyl)prop-1-ene, as exemplified by **Route 18** in the Examples section.

[0075] In some cases, compounds having an alcohol group for R¹ can be synthesized by cyclizing a N-(3-(azaneyl)propyl)propane-1,3-diamino compound with protected 1,3-dichloropropan-2-ol under basic conditions, as exemplified by **Route 19** in the Examples section. The alcohol group at R¹ can be methylated via reaction with MeI and NaH in toluene, as exemplified by **Route 21** in the examples section. The alcohol group at R¹ can be oxidized to a carbonyl via reaction with Dess-Martin periodinane, as exemplified by **Route 22** in the examples section. The carbonyl group at R¹ can be reacted with hydroxylamine hydrochloride to form the oxime compound, as exemplified by **Route 25** in the Examples section.

[0076] In various cases, compounds having an *S*-methyl group at R¹ can be synthesized by reacting an amino group functionalized with a desired R² group with 2,3-dimethyl-1-((2-methyl-1H-imidazol-1-yl)sulfonyl)-1H-imidazol-3-ium trifluoromethanesulfonate, followed by CF₃SO₃Me, to form 2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate functionalized with a desired R² group. The product can be reacted with (*R*)-N-(3-amino-2-methylpropyl) functionalized with a desired R⁴ group to form (*S*)-N,N'-(2-methylpropane-1,3-diamino functionalized with the desired R² and R⁴ groups. The resulting product can then be reacted with (azanediy)bis(propane-3,1-diyl) dimethanesulfonate functionalized with a desired R³ group to result in the desired compound, as exemplified by **Route 27** in the Examples section.

Synthesis of Intermediates

[0077] The intermediates used to prepare the compounds described herein also can be prepared by standard methods known to those skilled in the art, as described in the

Examples, section below (e.g., **Route 13, Route 16, Route 20, Route 24, Route 26, Route 28, Route 30, Route 31, Route 32, Route 33**).

Methods of Use

[0078] The compounds disclosed herein (e.g., the compounds of Formula (I), the compounds listed in Tables A and B, and pharmaceutically acceptable salts of the foregoing) can inhibit protein secretion of a protein of interest. The compounds disclosed herein can interfere with the Sec61 protein secretion machinery of a cell. In some cases, a compound as disclosed herein inhibits secretion of one or more of TNF α , VCAM, PRL, IL-2, INF γ , CD4, insulin, and PD-1 (mouse and/or human), or each of TNF α , VCAM, PRL, IL-2, INF γ , CD4, insulin, and PD-1 (mouse and/or human). In some cases, a compound as disclosed herein can inhibit secretion of a checkpoint protein, or inhibits secretion of a cell surface protein, endoplasmic reticulum-associated protein, or secreted protein involved in regulation of anti-tumor immune responses. In various cases, a compound disclosed herein can inhibit secretion of one or more of PD-1, PD-L1, TIM-1, LAG-3, CTLA4, BTLA, OX-40, B7H1, B7H4, CD137, CD47, CD96, CD73, CD40, VISTA, TIGIT, LAIR1, CD160, 2B4, TGFR β and combinations thereof. Protein secretion activity can be assessed in a manner as described in the Examples section below.

[0079] As used herein, the term “inhibitor” is meant to describe a compound that blocks or reduces an activity of a pharmacological target (for example, a compound that inhibits Sec61 function in the protein secretion pathway). An inhibitor can act with competitive, uncompetitive, or noncompetitive inhibition. An inhibitor can bind reversibly or irreversibly, and therefore, the term includes compounds that are suicide substrates of a protein or enzyme. An inhibitor can modify one or more sites on or near the active site of the protein, or it can cause a conformational change elsewhere on the enzyme. The term inhibitor is used more broadly herein than scientific literature so as to also encompass other classes of pharmacologically or therapeutically useful agents, such as agonists, antagonists, stimulants, co-factors, and the like.

[0080] Thus, provided herein are methods of inhibiting protein secretion in a cell. In these methods, a cell is contacted with a compound described herein (e.g., a compound of Formula (I) or a compound listed in Table A or B, and pharmaceutically acceptable salts of the foregoing), or pharmaceutical formulation thereof, in an amount effective to inhibit secretion of the protein of interest. In some embodiments, the cell is contacted *in vitro*. In various embodiments, the cell is contacted *in vivo*. In various embodiments, the contacting includes administering the compound or pharmaceutical formulation to a subject.

[0081] The biological consequences of Sec61 inhibition are numerous. For example, Sec61 inhibition has been suggested for the treatment or prevention of inflammation and/or cancer in a subject. Therefore, pharmaceutical formulations for Sec61 specific compounds, provide a means of administering a drug to a subject and treating these conditions. As used herein, the terms "treat," "treating," "treatment," and the like refer to eliminating, reducing, or ameliorating a disease or condition, and/or symptoms associated therewith. Although not precluded, treating a disease or condition does not require that the disease, condition, or symptoms associated therewith be completely eliminated. As used herein, the terms "treat," "treating," "treatment," and the like may include "prophylactic treatment," which refers to reducing the probability of redeveloping a disease or condition, or of a recurrence of a previously-controlled disease or condition, in a subject who does not have, but is at risk of or is susceptible to, redeveloping a disease or condition or a recurrence of the disease or condition. The term "treat" and synonyms contemplate administering a therapeutically effective amount of a compound of the invention to an individual in need of such treatment. Within the meaning of the invention, "treatment" also includes relapse prophylaxis or phase prophylaxis, as well as the treatment of acute or chronic signs, symptoms and/or malfunctions. The treatment can be orientated symptomatically, for example, to suppress symptoms. It can be effected over a short period, be oriented over a medium term, or can be a long-term treatment, for example within the context of a maintenance therapy. As used herein, the terms "prevent," "preventing," "prevention," are art-recognized, and when used in relation to a condition, such as a local recurrence (e.g., pain), a disease such as cancer, a syndrome complex such as heart failure or any other medical condition, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject which does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number of detectable cancerous growths in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control population, e.g., by a statistically and/or clinically significant amount. Prevention of an infection includes, for example, reducing the number of diagnoses of the infection in a treated population versus an untreated control population, and/or delaying the onset of symptoms of the infection in a treated population versus an untreated control population. Prevention of pain includes, for example, reducing the magnitude of, or alternatively delaying, pain sensations experienced by subjects in a treated population versus an untreated control population. As used herein, the terms "patient" and "subject" may be used interchangeably and mean animals, such as dogs, cats, cows, horses, and sheep (i.e., non-

human animals) and humans. Particular patients are mammals (e.g., humans). The term patient includes males and females.

[0082] Inhibition of Sec61-mediated secretion of inflammatory proteins (e.g., TNF α) can disrupt inflammation signaling. Thus, provided herein is a method of treating inflammation in a subject by administering to the subject a therapeutically effective amount of a compound described herein, (i.e., a compound of Formula (I) or a compound listed in Table A or B, or a pharmaceutically acceptable salt of the foregoing).

[0083] Further, the viability of cancer cells relies upon increased protein secretion into the ER for survival. Therefore, non-selective or partially selective inhibition of Sec61 mediated protein secretion may inhibit tumor growth. Alternatively, in the immune-oncology setting, selective secretion inhibitors of known secreted or transmembrane immune checkpoint proteins (e.g., PD-1, TIM-3, LAG3, etc.) can result in activation of the immune system to against various cancers.

[0084] Accordingly, also provided herein is a method of treating cancer in a subject by administering to the subject a therapeutically effective amount of a compound described herein, (e.g., a compound of Formula (I), a compound listed in Table A or B, or a pharmaceutically acceptable salt of the foregoing). Specifically contemplated cancers that can be treated using the compounds and compositions described herein include, but are not limited to melanoma, multiple myeloma, prostate, lung, non small cell lung carcinoma, squamous cell carcinoma, leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, lymphoma, NPM/ALK-transformed anaplastic large cell lymphoma, diffuse large B cell lymphoma, neuroendocrine tumors, breast, mantle cell lymphoma, renal cell carcinoma, rhabdomyosarcoma, ovarian cancer, endometrial cancer, small cell carcinoma, adenocarcinoma, gastric carcinoma, hepatocellular carcinoma, pancreatic cancer, thyroid carcinoma, anaplastic large cell lymphoma, hemangioma, head and neck cancer, bladder, and colorectal cancers. In some cases, the cancer is a solid tumor. In various cases, the cancer is head and neck cancer, squamous cell carcinoma, gastric carcinoma, or pancreatic cancer.

[0085] The compounds described herein are also contemplated to be used in the prevention and/or treatment of a multitude of diseases including, but not limited to, proliferative diseases, neurotoxic/degenerative diseases, ischemic conditions, autoimmune and autoinflammatory disorders, inflammation, immune-related diseases, HIV, cancers, organ graft rejection, septic shock, viral and parasitic infections, conditions associated with acidosis, macular degeneration, pulmonary conditions, muscle wasting diseases, fibrotic diseases, bone and hair growth diseases.

[0086] Examples of proliferative diseases or conditions include diabetic retinopathy, macular degeneration, diabetic nephropathy, glomerulosclerosis, IgA nephropathy, cirrhosis, biliary atresia, congestive heart failure, scleroderma, radiation-induced fibrosis, and lung fibrosis (idiopathic pulmonary fibrosis, collagen vascular disease, sarcoidosis, interstitial lung diseases and extrinsic lung disorders).

[0087] Inflammatory diseases include acute (e.g., bronchitis, conjunctivitis, myocarditis, pancreatitis) and chronic conditions (e.g., chronic cholecystitis, bronchiectasis, aortic valve stenosis, restenosis, psoriasis and arthritis), along with conditions associated with inflammation such as fibrosis, infection and ischemia.

[0088] Immunodeficiency disorders occur when a part of the immune system is not working properly or is not present. They can affect B lymphocytes, T lymphocytes, or phagocytes and be either inherited (e.g., IgA deficiency, severe combined immunodeficiency (SCID), thymic dysplasia and chronic granulomatous) or acquired (e.g., acquired immunodeficiency syndrome (AIDS), human immunodeficiency virus (HIV) and drug-induced immunodeficiencies). Immune-related conditions include allergic disorders such as allergies, asthma and atopic dermatitis like eczema. Other examples of such immune-related conditions include lupus, rheumatoid arthritis, scleroderma, ankylosing spondylitis, dermatomyositis, psoriasis, multiple sclerosis and inflammatory bowel disease (such as ulcerative colitis and Crohn's disease).

[0089] Tissue/organ graft rejection occurs when the immune system mistakenly attacks the cells being introduced to the host's body. Graft versus host disease (GVHD), resulting from allogeneic transplantation, arises when the T cells from the donor tissue go on the offensive and attack the host's tissues. In all three circumstances, autoimmune disease, transplant rejection and GVHD, modulating the immune system by treating the subject with a compound or composition of the disclosure could be beneficial.

[0090] Also provided herein is a method of treating an autoimmune disease in a patient comprising administering a therapeutically effective amount of the compound described herein. An "autoimmune disease" as used herein is a disease or disorder arising from and directed against an individual's own tissues. Examples of autoimmune diseases include, but are not limited to, inflammatory responses such as inflammatory skin diseases including psoriasis and dermatitis (e.g., atopic dermatitis); systemic scleroderma and sclerosis; responses associated with inflammatory bowel disease (such as Crohn's disease and ulcerative colitis); respiratory distress syndrome (including adult respiratory distress syndrome (ARDS)); dermatitis; meningitis; encephalitis; uveitis; colitis; glomerulonephritis; allergic conditions such as eczema and asthma and other conditions involving infiltration of T

cells and chronic inflammatory responses; atherosclerosis; leukocyte adhesion deficiency; rheumatoid arthritis; systemic lupus erythematosus (SLE); diabetes mellitus (e.g., Type I diabetes mellitus or insulin dependent diabetes mellitus); multiple sclerosis; Reynaud's syndrome; autoimmune thyroiditis; allergic encephalomyelitis; Sjorgen's syndrome; juvenile onset diabetes; and immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes typically found in tuberculosis, sarcoidosis, polymyositis, granulomatosis and vasculitis; pernicious anemia (Addison's disease); diseases involving leukocyte diapedesis; central nervous system (CNS) inflammatory disorder; multiple organ injury syndrome; hemolytic anemia (including, but not limited to cryoglobulinemia or Coombs positive anemia); myasthenia gravis; antigen-antibody complex mediated diseases; anti-glomerular basement membrane disease; antiphospholipid syndrome; allergic neuritis; Graves' disease; Lambert-Eaton myasthenic syndrome; pemphigoid bullous; pemphigus; autoimmune polyendocrinopathies; Reiter's disease; stiff-man syndrome; Behcet disease; giant cell arteritis; immune complex nephritis; IgA nephropathy; IgM polyneuropathies; immune thrombocytopenic purpura (ITP) or autoimmune thrombocytopenia. Compounds provided herein may be useful for the treatment of conditions associated with inflammation, including, but not limited to COPD, psoriasis, asthma, bronchitis, emphysema, and cystic fibrosis.

[0091] Also provided herein is the use of a compound as disclosed herein for the treatment of neurodegenerative diseases. Neurodegenerative diseases and conditions includes, but not limited to, stroke, ischemic damage to the nervous system, neural trauma (e.g., percussive brain damage, spinal cord injury, and traumatic damage to the nervous system), multiple sclerosis and other immune-mediated neuropathies (e.g., Guillain-Barre syndrome and its variants, acute motor axonal neuropathy, acute inflammatory demyelinating polyneuropathy, and Fisher Syndrome), HIV/AIDS dementia complex, axonopathy, diabetic neuropathy, Parkinson's disease, Huntington's disease, multiple sclerosis, bacterial, parasitic, fungal, and viral meningitis, encephalitis, vascular dementia, multi-infarct dementia, Lewy body dementia, frontal lobe dementia such as Pick's disease, subcortical dementias (such as Huntington or progressive supranuclear palsy), focal cortical atrophy syndromes (such as primary aphasia), metabolic-toxic dementias (such as chronic hypothyroidism or B12 deficiency), and dementias caused by infections (such as syphilis or chronic meningitis).

[0092] Further guidance for using compounds and compositions described herein (e.g., a compound of Formula (I), a compound listed in Table A or B, or a pharmaceutically acceptable salt of the foregoing) for inhibiting protein secretion can be found in the Examples section, below.

Pharmaceutical Formulations and Administration

[0093] The methods provided herein include the manufacture and use of pharmaceutical compositions, which include one or more of the compounds provided herein. Also included are the pharmaceutical compositions themselves. Pharmaceutical compositions typically include a pharmaceutically acceptable carrier. Thus, provided herein are pharmaceutical formulations that include a compound described herein (e.g., a compound of Formula (I), a compound listed in Table A or B, or a pharmaceutically acceptable salt of the foregoing), as previously described herein, and one or more pharmaceutically acceptable carriers.

[0094] The phrase “pharmaceutically acceptable” is employed herein to refer to those ligands, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0095] The phrase “pharmaceutically acceptable carrier” as used herein means a pharmaceutically acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material. As used herein the language “pharmaceutically acceptable carrier” includes buffer, sterile water for injection, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose, and sucrose; (2) starches, such as corn starch, potato starch, and substituted or unsubstituted β -cyclodextrin; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil, and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol, and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations. In certain embodiments, pharmaceutical compositions provided herein are non-pyrogenic, i.e., do not induce significant temperature elevations when administered to a patient.

[0096] The term “pharmaceutically acceptable salt” refers to the relatively non-toxic,

inorganic and organic acid addition salts of a compound provided herein. These salts can be prepared *in situ* during the final isolation and purification of a compound provided herein, or by separately reacting the compound in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, laurylsulphonate salts, and amino acid salts, and the like. (See, for example, Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66: 1-19.)

[0097] In some embodiments, a compound provided herein may contain one or more acidic functional groups and, thus, is capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances refers to the relatively non-toxic inorganic and organic base addition salts of a compound provided herein. These salts can likewise be prepared *in situ* during the final isolation and purification of the compound, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate, or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary, or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts, and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, and the like (see, for example, Berge et al., *supra*).

[0098] Wetting agents, emulsifiers, and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring, and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[0099] Compositions prepared as described herein can be administered in various forms, depending on the disorder to be treated and the age, condition, and body weight of the patient, as is well known in the art. For example, where the compositions are to be administered orally, they may be formulated as tablets, capsules, granules, powders, or syrups; or for parenteral administration, they may be formulated as injections (intravenous, intramuscular, or subcutaneous), drop infusion preparations, or suppositories. For application by the ophthalmic mucous membrane route, they may be formulated as eye drops or eye ointments. These formulations can be prepared by conventional means in conjunction with the methods described herein, and, if desired, the active ingredient may be mixed with any conventional additive or excipient, such as a binder, a disintegrating agent, a

lubricant, a corrigent, a solubilizing agent, a suspension aid, an emulsifying agent, or a coating agent.

[00100] Actual dosage levels of the active ingredients in the pharmaceutical compositions provided herein may be varied so as to obtain “therapeutically effective amount,” which is an amount of the active ingredient effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

[00101] The concentration of a compound provided herein in a pharmaceutically acceptable mixture will vary depending on several factors, including the dosage of the compound to be administered, the pharmacokinetic characteristics of the compound(s) employed, and the route of administration. In some embodiments, the compositions provided herein can be provided in an aqueous solution containing about 0.1-10% w/v of a compound disclosed herein, among other substances, for parenteral administration. Typical dose ranges can include from about 0.01 to about 50 mg/kg of body weight per day, given in 1-4 divided doses. Each divided dose may contain the same or different compounds. The dosage will be a therapeutically effective amount depending on several factors including the overall health of a patient, and the formulation and route of administration of the selected compound(s).

[00102] Dosage forms or compositions containing a compound as described herein in the range of 0.005% to 100% with the balance made up from non-toxic carrier may be prepared. Methods for preparation of these compositions are known to those skilled in the art. The contemplated compositions may contain 0.001%-100% active ingredient, in one embodiment 0.1-95%, in another embodiment 75-85%. Although the dosage will vary depending on the symptoms, age and body weight of the patient, the nature and severity of the disorder to be treated or prevented, the route of administration and the form of the drug, in general, a daily dosage of from 0.01 to 2000 mg of the compound is recommended for an adult human patient, and this may be administered in a single dose or in divided doses. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect.

[00103] In jurisdictions that forbid the patenting of methods that are practiced on the human body, the meaning of “administering” of a composition to a human subject shall be restricted to prescribing a controlled substance that a human subject will self-administer by any technique (e.g., orally, inhalation, topical application, injection, insertion, etc.). The broadest reasonable interpretation that is consistent with laws or regulations defining patentable subject matter is intended. In jurisdictions that do not forbid the patenting of methods that are practiced on the human body, the “administering” of compositions includes

both methods practiced on the human body and also the foregoing activities.

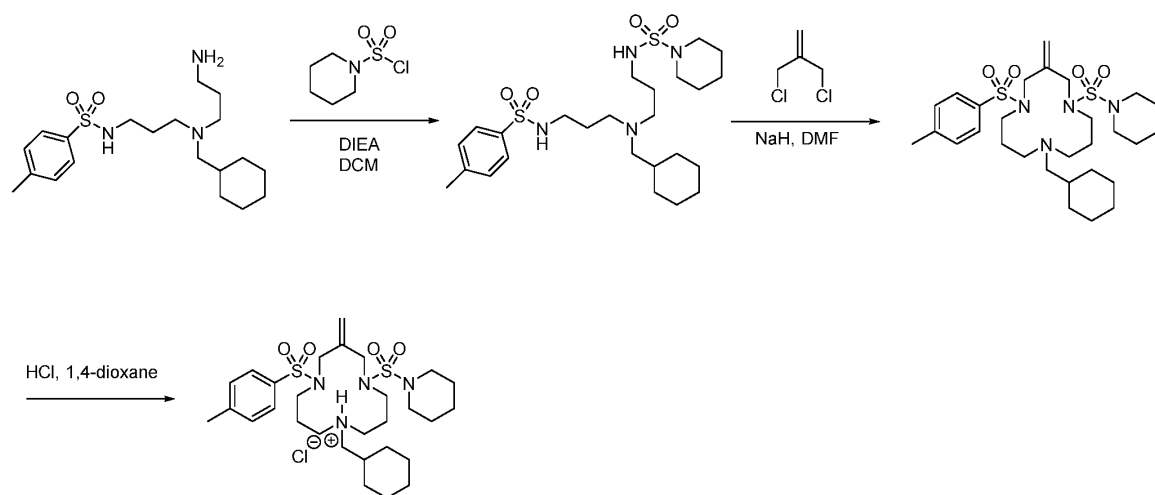
Other Embodiments

[00104] It is to be understood that while the disclosure is read in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the disclosure, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

EXAMPLES

[00105] The following examples are provided for illustration and are not intended to limit the scope of the invention.

Route 1



[00106] The starting material was synthesized using the method described in *J. Med. Chem.* **2016**, 59, 2633-2647. To N-(3-((3-aminopropyl)(cyclohexylmethyl)amino)propyl)-4-methylbenzenesulfonamide (0.200 g, 0.523 mmol) in DCM (2.0 mL) was added DIEA (2 eq, 1.05 mmol, 179 μ L) followed by piperidine-1-sulfonyl chloride (0.096 g, 0.523 mmol). After 16 h the reaction was quenched with sodium bicarbonate (sat.), extracted with DCM, dried with sodium sulfate, filtered, and concentrated.

[00107] To the crude N-(3-((cyclohexylmethyl)(3-((4-methylphenyl)sulfonamido)propyl)amino)propyl)piperidine-1-sulfonamide in DMF (10 mL) was added NaH (2.5 eq, 1.31 mmol, 0.052 g of a 60% dispersion in mineral oil). The mixture was stirred for 1 h then was heated to 85 $^{\circ}$ C and 3-chloro-2-chloromethyl-1-propene (0.9 eq, 0.471 mmol, 55 μ L dissolved in 0.5 mL DMF) was added over 4 h. After heating for 16 h the mixture was cooled to ambient temp, diluted with brine and water, extracted with ethyl acetate (3X), washed with brine, dried with sodium sulfate, filtered, and concentrated. Flash

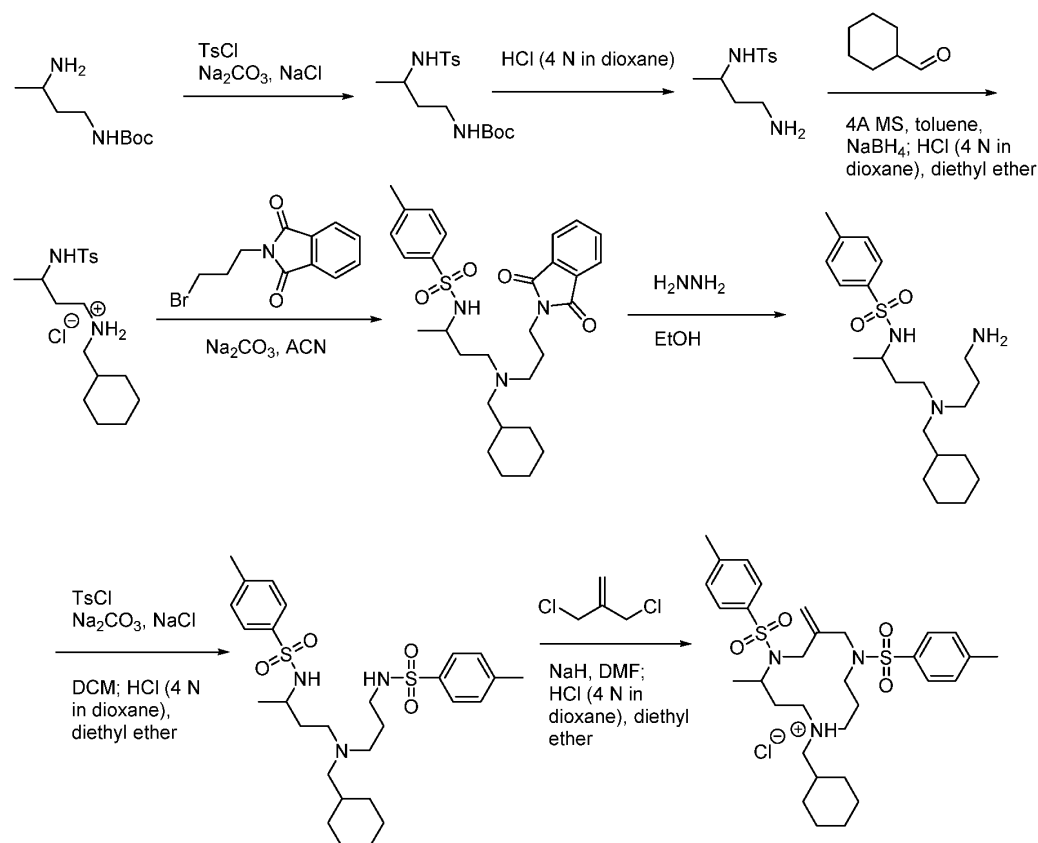
column chromatography (0-60% hexanes/ethyl acetate + 1% DEA) provided the freebase product.

[00108] To the freebase was added diethyl ether (1.0 mL) to dissolve and HCl (4 N in dioxane, 1.046 mmol, 262 μ L). The HCl salt was filtered and dried under high vacuum to provide compound **A5**. MS(EI) for $C_{29}H_{48}N_4O_4S_2$, found 581 $[M+H]^+$.

[00109] The following compounds were synthesized in a similar manner:

No.	IUPAC	$[M+H]^+$
A6	9-(cyclohexylmethyl)-1-(ethanesulfonyl)-5-(4-methylbenzenesulfonyl)-3-methylidene-1,5,9-triazacyclododecane	526
A7	9-(cyclohexylmethyl)-1-(4-methylbenzenesulfonyl)-3-methylidene-5-(propane-2-sulfonyl)-1,5,9-triazacyclododecane	540
A8	1-(cyclohexanesulfonyl)-9-(cyclohexylmethyl)-5-(4-methylbenzenesulfonyl)-3-methylidene-1,5,9-triazacyclododecane	580

Route 2



[00110] To tert-butyl (3-aminobutyl)carbamate (2.5 g, 13.3 mmol) in DCM (12.5 mL), sat.

Na₂CO₃ (12.5 mL), and sat. NaCl (12.5 mL) was added TsCl (13.3 mmol, 2.54 g). After 1 h the organic layer was washed with brine, dried with sodium sulfate, filtered, and concentrated to provide tert-butyl (3-((4-methylphenyl)sulfonamido)butyl)carbamate which was carried forward without further purification.

[00111] To the tosylate (13.3 mmol) was added TFA (66.5 mmol, 5.09 mL) and DCM (5.09 mL). After 2 h the reaction was concentrated, diluted with NaOH (1N, 30 mL), extracted with DCM (3X30 mL), dried with sodium sulfate, filtered, and concentrated to provide the amine.

[00112] To the amine (10.94 mmol) was added toluene (49 mL) and cyclohexanecarboxaldehyde (1.34 g, 12.0 mmol). The mixture was refluxed with a dean stark apparatus overnight (external temperature of 185 °C) then concentrated, diluted with ethanol (absolute, 19 mL) and NaBH₄ (0.827 g, 21.8 mmol) was added portion-wise while the internal temperature was maintained at <20 °C. After stirring for 2 h the mixture was diluted with water (10 mL), stirred for 20 min, extracted with DCM (3X), washed with brine, dried with sodium sulfate, filtered, and concentrated to provide the amine. The amine was diluted with diethyl ether (20 mL) and HCl (conc., ~0.3 mL) was added dropwise. The HCl salt crashed out within 5 min and was filtered using diethyl ether to wash. The product was dried under vacuum overnight to provide N-(4-((cyclohexylmethyl)amino)butan-2-yl)-4-methylbenzenesulfonamide hydrochloride.

[00113] To the HCl salt (3.5 mmol) was added acetonitrile (9 mL), sodium carbonate (0.99 g, 9.2 mmol), Lil (0.114 g), and 3-bromopropylphthalimide (2.30 g). The mixture was heated to 70 °C for 5 h at which time it was filtered and concentrated. The crude phthalimide was carried forward without further purification.

[00114] To the phthalimide (3.5 mmol) was added hydrazine monohydrate (4.2 mL) and ethanol (15 mL). After heating to 80 °C for 30 min the reaction was complete and the precipitate was filtered washing with ethanol. The filtrate was concentrated and diluted with HCl (2 N, 30 mL) then allowed to stand for 1 h. To the aqueous mixture was added NaOH (1 N) to basify to pH 10. The mixture was then extracted with DCM (3X), dried with sodium sulfate, filtered, and concentrated to provide crude N-(4-((3-aminopropyl)(cyclohexylmethyl)amino)butan-2-yl)-4-methylbenzenesulfonamide.

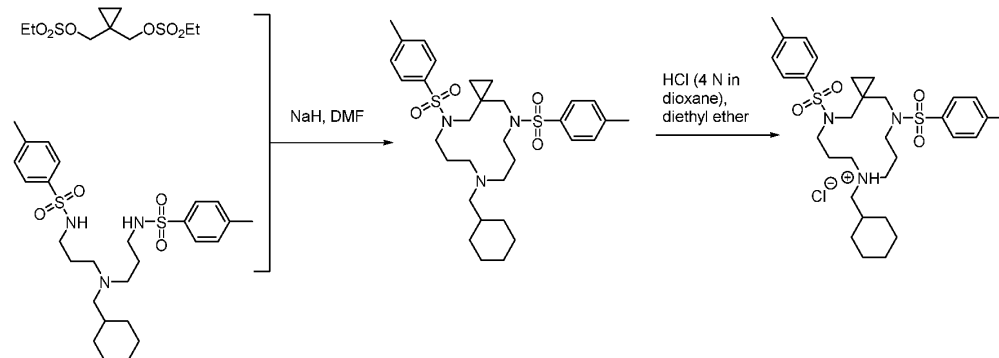
[00115] To the amine (695 mg, 1.76 mmol) was added DCM (9 mL), sat. Na₂CO₃ (9 mL), sat. NaCl (9 mL), TsCl (1.76 mmol, 336 mg). After stirring for 1 h the mixture was extracted with DCM (3X), dried with sodium sulfate, filtered, and concentrated to provide the freebase. The HCl salt was made by diluting the freebase in diethyl ether (50 mL) and adding HCl (conc., 300 uL). After placing under high vacuum for 2 h the HCl salt (1.02 g) was collected.

[00116] To the HCl salt (0.400 g, 0.683 mmol) in DMF (14 mL) was added NaH (3.5 eq, 2.39 mmol, 60% dispersion in mineral oil, 80.3 mg). After stirring for 1 h, 3-chloro-2-chloromethyl-1-propene (0.9 eq, 0.615 mmol, 71 μ L in 7 mL DMF) was added dropwise over 3 h. After heating an additional 2 h the mixture was cooled to ambient temperature, diluted with brine, water, and ethyl acetate. The mixture was extracted with ethyl acetate (2X), washed with brine, dried with sodium sulfate, filtered, and concentrated. Flash column chromatography (0-90% hexanes/ethyl acetate + 1% DEA) provided the product. The HCl salt was formed by diluting the freebase in diethyl ether (20 vol.) and adding HCl (4N in dioxane, 4 eq) to provide 5-(cyclohexylmethyl)-2-methyl-11-methylene-1,9-ditosyl-1,5,9-triazacyclododecane hydrochloride, compound **B1**. MS(EI) for $C_{32}H_{47}N_3O_4S_2$, found 602 $[M+H]^+$.

[00117] The following compounds were synthesized in a similar manner:

No.	IUPAC	$[M+H]^+$
B2	1-(cyclohexylmethyl)-3-methyl-5,9-bis(4-methylbenzenesulfonyl)-7-methylidene-1,5,9-triazacyclododecane	602
A9	N,N-dimethyl-4-[[5-(4-methylbenzenesulfonyl)-3-methylidene-9-[(oxan-4-yl)methyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]aniline	619

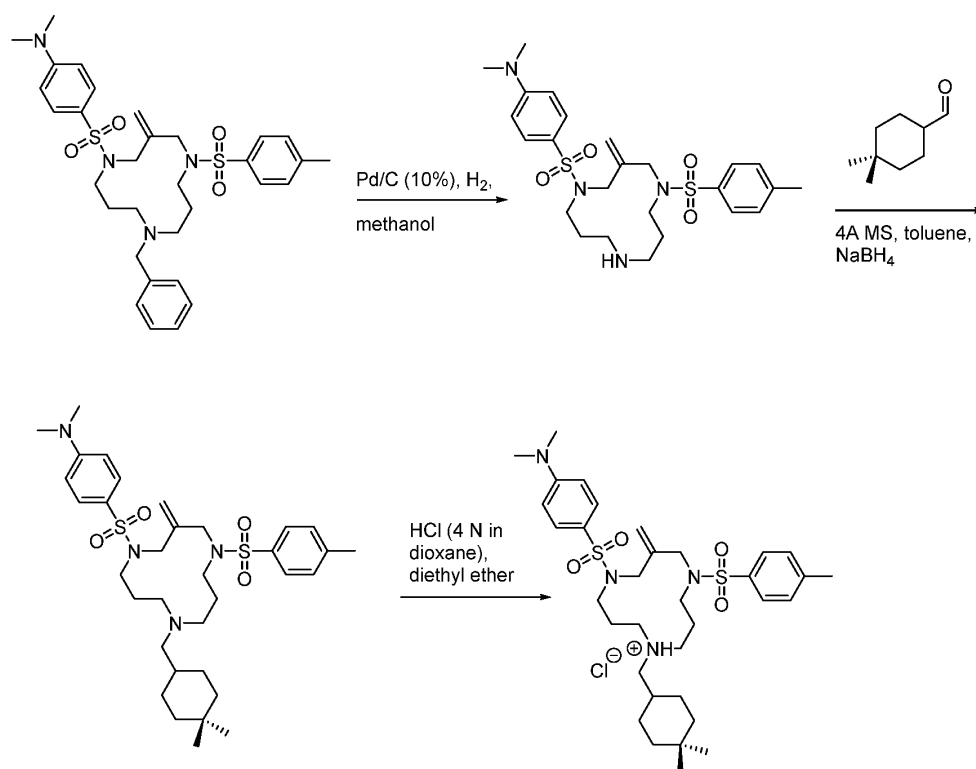
Route 3



[00118] To N-(3-((cyclohexylmethyl)(3-((4-methylphenyl)sulfonamido)butyl)amino)propyl)-4-methylbenzenesulfonamide (0.099 mmol) in DMF (10.0 mL) was added sodium hydride (0.350 mmol of a 60% dispersion in mineral oil). The mixture was stirred at ambient temperature for 30 min then heated to 80 °C before cyclopropane-1,1-diylbis(methylene) diethanesulfonate (0.099 mmol dissolved in 1.0 mL DMF) over the course of 2 h. After stirring an additional 4 h at 80 °C the mixture was cooled to ambient temperature, quenched with brine, extracted with ethyl acetate (3X). The combined organics were washed with brine (2X), dried with sodium sulfate, filtered, and concentrated. Flash column chromatography (0-

60% hexanes (1% DEA)/ethyl acetate) provided the freebase product which was dissolved in diethyl ether (2 mL) and HCl (0.1 mL of a 4 N solution in 1,4-dioxane) was added. The mixture was concentrated to provide 4-[[9-(cyclohexylmethyl)-13-(4-methylbenzenesulfonyl)-5,9,13-triazaspiro[2.11]tetradecan-5-yl]sulfonyl]-N,N-dimethylaniline hydrochloride, compound **A11**. MS(EI) for $C_{33}H_{50}N_4O_4S_2$, found 631 $[M+H]^+$.

Route 4

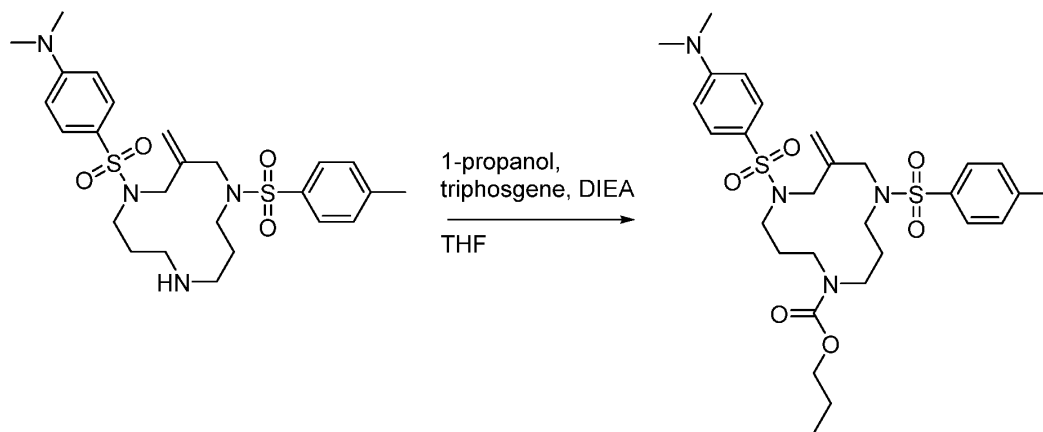


[00119] The starting material was synthesized using the method described in *J. Med. Chem.* **2016**, 59, 2633-2647. To the starting material (0.327 mmol) in methanol (2.0 mL) under argon was added Pd/C (20 mg). A hydrogen atmosphere was established and the mixture was stirred at ambient temp. After 3 h the reaction was filtered and purified by flash column chromatography (0-80% hexanes (1% DEA)/ethyl acetate) to provide the product .

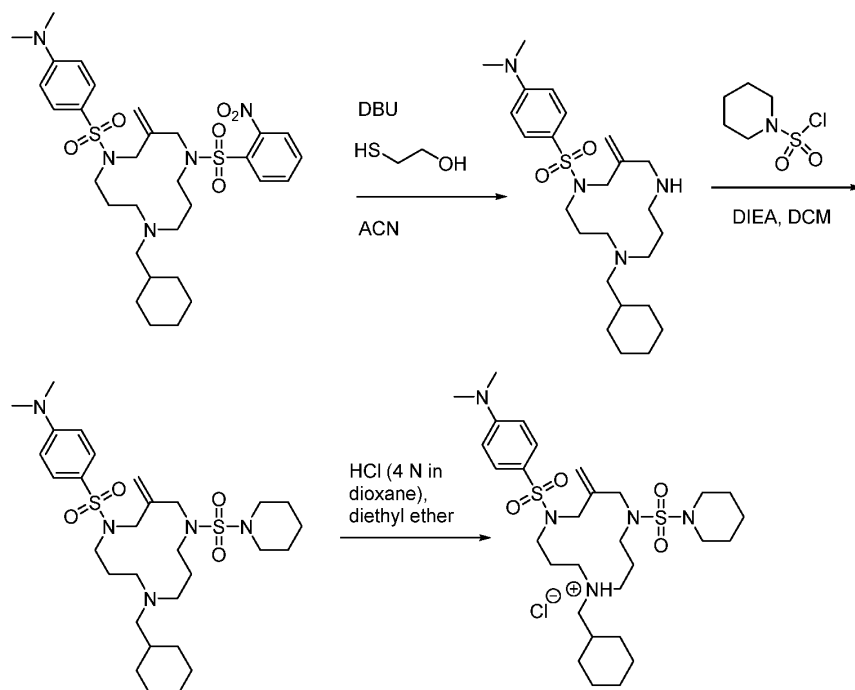
[00120] To the starting material (0.028 mmol) was added toluene (5 mL) followed by the aldehyde (0.028 mmol). The mixture was stirred for 3 h at reflux then concentrated. To the imine was added ethanol followed by sodium borohydride. The mixture was stirred at ambient temp for 30 min then diluted with water (1 mL), extracted, with DCM (3X1 mL), dried with sodium sulfate, filtered, and concentrated. The product was purified by flash column chromatography (0-60% hexanes (1% DEA)/ethyl acetate). The freebase was diluted with ethyl ether (1 mL) and HCl (7 μ L, 4 N in dioxane) was added. The mixture was filtered to

provide 4-((9-((4,4-dimethylcyclohexyl)methyl)-3-methylene-5-tosyl-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline hydrochloride (compound **A10**).
MS(EI) for $C_{34}H_{52}N_4O_4S_2$, found 645 $[M+H]^+$.

Route 5



[00121] To triphosgene (0.0316 mmol, 9.4 mg) in DCM (100 μ L) was added 1-propanol (0.190 mmol 14 μ L). After stirring for 45 min this solution was added to the amine (0.0158 mmol, 8.2 mg), DIEA (0.126 mmol, 22 μ L), and DCM (100 μ L). The mixture was stirred at ambient temperature for 1 h then purified directly by flash column chromatography (0-70% hexanes/ethyl acetate) to provide propyl 5-((4-(dimethylamino)phenyl)sulfonyl)-7-methylene-9-tosyl-1,5,9-triazacyclododecane-1-carboxylate (compound **A12**). MS(EI) for $C_{29}H_{42}N_4O_6S_2$, found 607 $[M+H]^+$.

Route 6

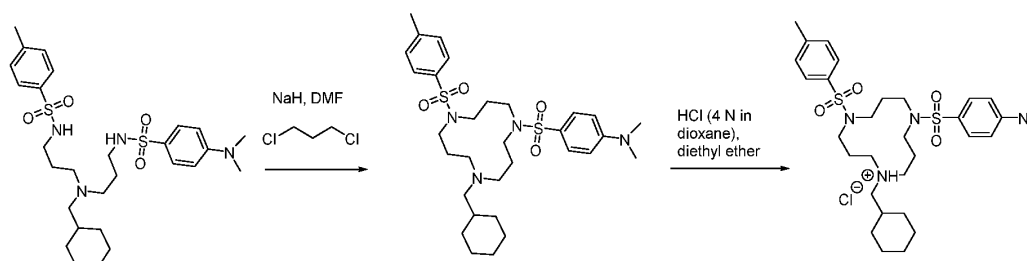
[00122] The starting material, 4-((9-(cyclohexylmethyl)-3-methylene-5-((2-nitrophenyl)sulfonyl)-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline, was synthesized using a similar method described in **Route 2** substituting with the appropriate sulfonyl chlorides.

[00123] To the starting material (1.09 g, 1.68 mmol) in ACN (12.3 mL) was added DBU (0.780 mL, 5.22 mmol) followed by 2-mercaptoethanol (0.135 mL, 1.92 mmol). The mixture was stirred at ambient temperature for 2 h then an additional aliquot of 2-mercaptoethanol (1.14 eq) was added. After stirring an additional 2 h the mixture was concentrated and purified directly by FCC (0-100% hexanes (1% DEA)/ethyl acetate). 4-((9-(Cyclohexylmethyl)-3-methylene-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline.

[00124] To the amine (70 mg, 0.152 mmol) in DCM (2 mL) was added DIEA (0.303 mmol, 52 μ L) followed by piperidine-1-sulfonyl chloride (0.152 mmol, 28 mg). After allowing to stand overnight the reaction was purified directly by flash column chromatography (hexanes (1% DEA)/ethyl acetate 0-60%). The HCl salt of the product was made by dissolving the freebase in diethyl ether (1 mL) and adding HCl (4 N in dioxane, 38 μ L). The filtrate was collected to provide 4-((9-(cyclohexylmethyl)-3-methylene-5-(piperidin-1-ylsulfonyl)-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline hydrochloride (compound **A15**). MS(EI) for $C_{30}H_{51}N_5O_4S_2$, found 610 $[M+H]^+$.

[00125] The following compounds were synthesized in a similar manner:

No.	IUPAC	[M+H] ⁺
A16	9-(cyclohexylmethyl)-5-[4-(dimethylamino)benzenesulfonyl]-N,N-dimethyl-3-methylidene-1,5,9-triazacyclododecane-1-sulfonamide	570
A13	4-[[9-(cyclohexylmethyl)-3-methylidene-5-(piperidine-1-sulfonyl)-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	610

Route 7

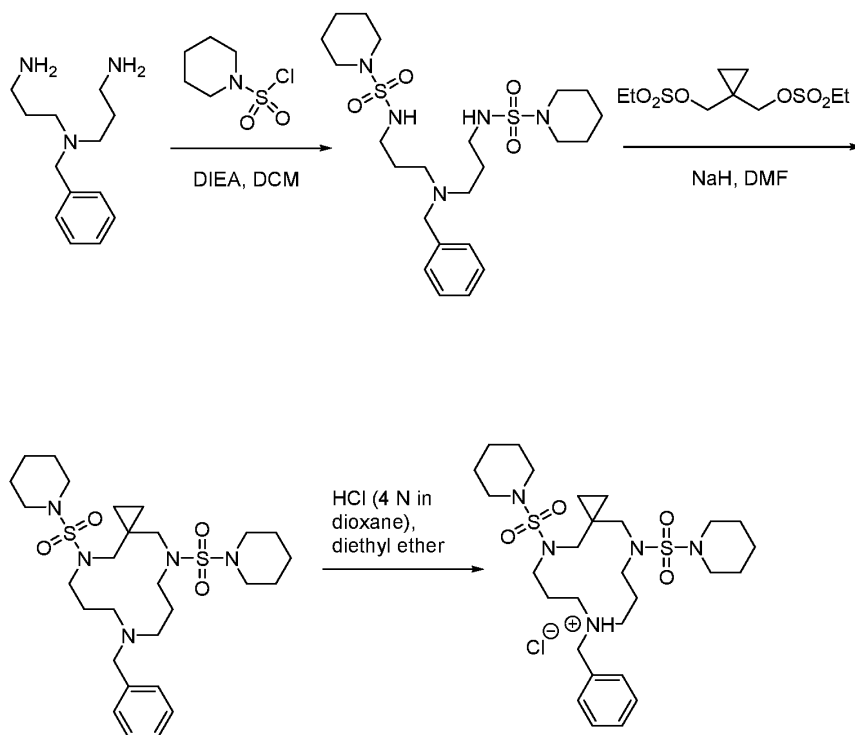
[00126] The starting material, N-(3-((cyclohexylmethyl)(3-((4-(dimethylamino)phenyl)sulfonamido)propyl)amino)propyl)-4-methylbenzenesulfonamide, was synthesized using the same method described in **Route 1** substituting with the appropriate sulfonyl chloride.

[00127] To the diamine (49 mg, 0.087 mmol) in DMF (2.5 mL) was added NaH (0.22 mmol, 9 mg). After stirring for 30 min at ambient temperature, 1,3-dichloropropane (0.087 mmol, 8.3 uL in 0.5 mL DMF) was slowly added over 1 h while heating at 80 °C. After 3 h the mixture was cooled to ambient temp, diluted with brine (10 mL), water (5 mL), and ethyl acetate (10 mL). The aqueous layer was extracted with ethyl acetate (3X5 mL), the organics were combined and washed with brine (1X5 mL), dried with sodium sulfate, and concentrated. Flash column chromatography (0-50% hexanes (1% DEA/ethyl acetate) provided the freebase product that was converted to the HCl salt with the addition of ether (1 mL) then HCl (20 uL, 4 N in dioxane). The filtrate was collected and concentrated to provide 4-((5-(cyclohexylmethyl)-9-tosyl-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline (compound **A14**). MS(EI) for C₃₁H₄₈N₄O₄S₂, found 605 [M+H]⁺.

[00128] The following compound was synthesized in a similar manner:

No.	IUPAC	[M+H] ⁺
A17	4-[[10-(cyclohexylmethyl)-14-(piperidine-1-sulfonyl)-2-oxa-6,10,14-triazaspiro[3.1]pentadecan-6-yl]sulfonyl]-N,N-dimethylaniline	640

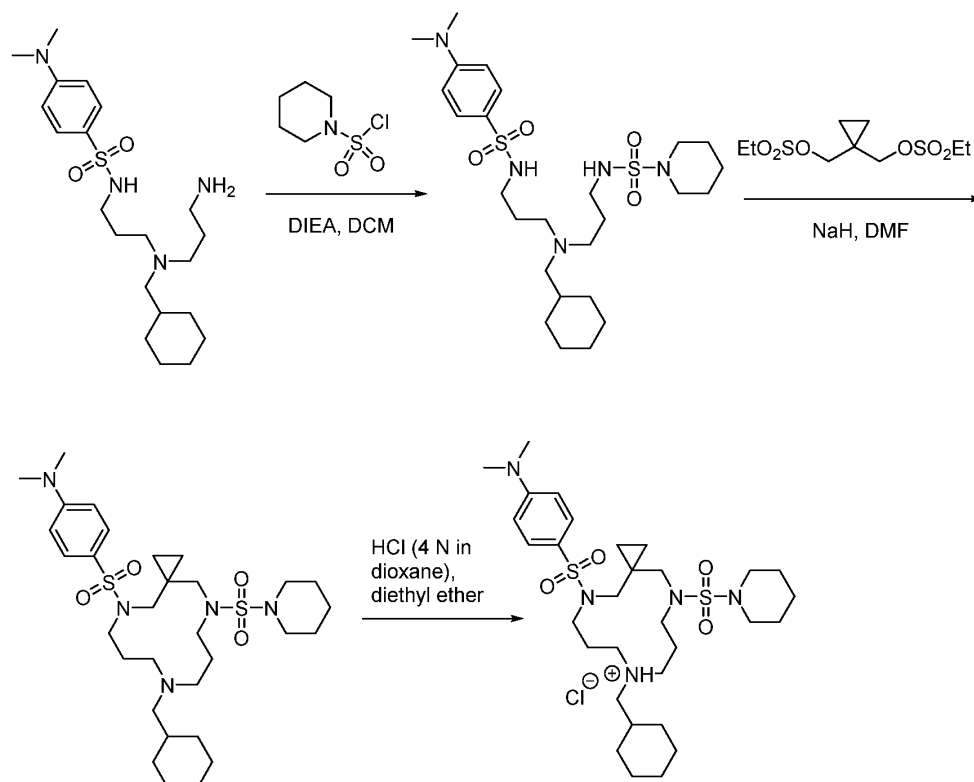
Route 8



[00129] The starting material was synthesized using the method described in *J. Med. Chem.* **2006**, 49, 1291-1312. To N1-(3-aminopropyl)-N1-benzylpropane-1,3-diamine (363 mg, 1.64 mmol) in DCM (2 mL) was added DIEA (1.14 mL, 6.56 mL) then the sulfonyl chloride (345 μ L, 2.46 mmol). The solution was allowed to stand overnight at ambient temp. Concentration and purification by flash column chromatography (0-80% hexanes (1% DEA)/ethyl acetate) provided the product.

[00130] To the starting material (260 mg, 504 μ L) in DMF (20 mL) was added NaH (50 mg of a 60% dispersion in mineral oil, 1.26 mmol). The mixture was stirred at 80 °C for 20 min then the bis-sulfonate (144 mg, 504 μ L) was added over the course of 1 h. After heating for 4 h the reaction was complete and was diluted with ethyl acetate (40 mL) and brine (40 mL), extracted with ethyl acetate (2X20 mL). The combined organics were washed with brine (20 mL), dried with sodium sulfate, filtered, and concentrated. Flash column chromatography (hexanes (1% DEA)/ethyl acetate 0-50%) provided the product which was diluted with ethyl ether (5 mL) and HCl (4 N in dioxane, 100 μ L) was added. The mixture was concentrated, washed with diethyl ether, and dried to provide 9-benzyl-5,13-bis(piperidin-1-ylsulfonyl)-5,9,13-triazaspiro[2.11]tetradecane hydrochloride (compound **A18**). MS(EI) for $C_{28}H_{47}N_5O_4S_2$, found 581 $[M+H]^+$.

Route 9



[00131] The starting material, N-(3-((3-aminopropyl)(cyclohexylmethyl)amino)propyl)-4-(dimethylamino)benzenesulfonamide, was synthesized using a similar method as described in **Route 2**.

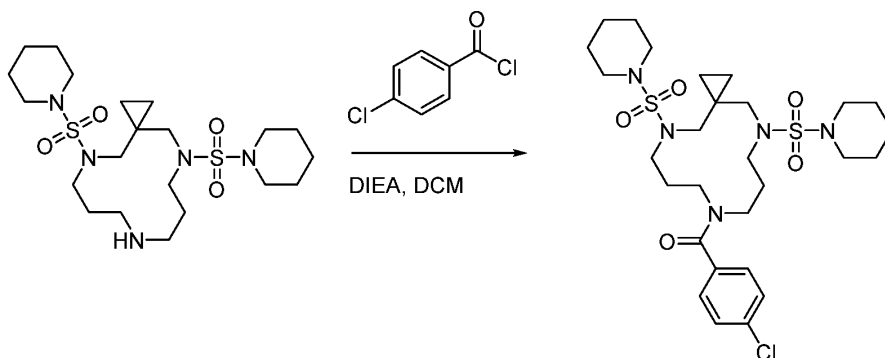
[00132] To N-(3-((3-aminopropyl)(cyclohexylmethyl)amino)propyl)-4-(dimethylamino)benzenesulfonamide (559 mg, 1.36 mmol) in DCM (2 mL) was added DIEA (0.71 mL, 4.08 mmol) then the sulfonyl chloride (250 mg, 1.36 mmol). The solution was allowed to stand overnight at ambient temp. Concentration and purification by flash column chromatography (0-80% hexanes (1% DEA)/ethyl acetate) provided the product.

[00133] The cyclization of N-(3-((cyclohexylmethyl)(3-((4-(dimethylamino)phenyl)sulfonamido)propyl)amino)propyl)piperidine-1-sulfonamide and salt formation was carried out in a similar manner to **Route 8** to provide 4-((9-(cyclohexylmethyl)-13-(piperidin-1-ylsulfonyl)-5,9,13-triazaspiro[2.11]tetradecan-5-yl)sulfonyl)-N,N-dimethylaniline hydrochloride (compound **A19**). MS(EI) for $C_{31}H_{53}N_5O_4S_2$, found 624 $[M+H]^+$.

[00134] The following compounds were synthesized in a similar manner. The cyclization of compounds **A25**, **A26**, **A27**, **A28**, and **A29** was carried out using NMP as the solvent.

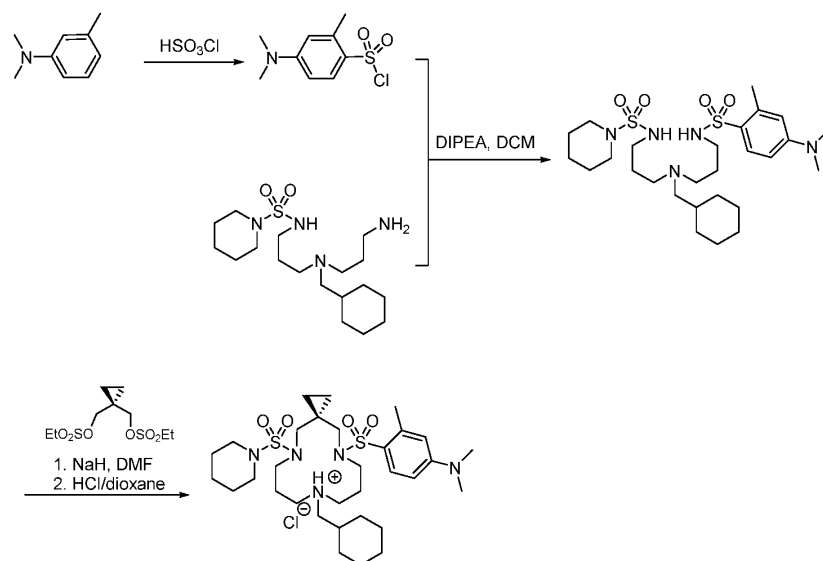
No.	IUPAC	[M+H] ⁺
A20	4-{{9-(cyclohexylmethyl)-13-[(2-methylpiperidin-1-yl)sulfonyl]-5,9,13-triazaspiro[2.1]tetradecan-5-yl}sulfonyl}-N,N-dimethylaniline	638
A21	4-{{9-(cyclohexylmethyl)-13-[(3-methylpiperidin-1-yl)sulfonyl]-5,9,13-triazaspiro[2.1]tetradecan-5-yl}sulfonyl}-N,N-dimethylaniline	638
A22	4-{{9-(cyclohexylmethyl)-13-[(4-methylpiperidin-1-yl)sulfonyl]-5,9,13-triazaspiro[2.1]tetradecan-5-yl}sulfonyl}-N,N-dimethylaniline	638
A23	4-{{9-(cyclohexylmethyl)-13-(pyrrolidine-1-sulfonyl)-5,9,13-triazaspiro[2.1]tetradecan-5-yl}sulfonyl}-N,N-dimethylaniline	610
B3	4-{{8-(cyclohexylmethyl)-4-(piperidine-1-sulfonyl)-1,4,8-triazacycloundecan-1-yl}sulfonyl}-N,N-dimethylaniline	584
B4	4-{{7-(cyclohexylmethyl)-3-(piperidine-1-sulfonyl)-1,3,7-triazecan-1-yl}sulfonyl}-N,N-dimethylaniline	570
A25	4-{{13-(azepane-1-sulfonyl)-9-(cyclohexylmethyl)-5,9,13-triazaspiro[2.1]tetradecan-5-yl}sulfonyl}-N,N-dimethylaniline	638
A26	4-{{9-(cyclohexylmethyl)-13-[(4,4-dimethylpiperidin-1-yl)sulfonyl]-5,9,13-triazaspiro[2.1]tetradecan-5-yl}sulfonyl}-N,N-dimethylaniline	652
A27	4-{{9-(cyclohexylmethyl)-13-[(4-methoxypiperidin-1-yl)sulfonyl]-5,9,13-triazaspiro[2.1]tetradecan-5-yl}sulfonyl}-N,N-dimethylaniline	654
A28	1-{{9-(cyclohexylmethyl)-13-[4-(dimethylamino)benzenesulfonyl]-5,9,13-triazaspiro[2.1]tetradecan-5-yl}sulfonyl}piperidin-4-ol	640
A29	4-{{9-(cyclohexylmethyl)-13-[(4-phenylpiperidin-1-yl)sulfonyl]-5,9,13-triazaspiro[2.1]tetradecan-5-yl}sulfonyl}-N,N-dimethylaniline	700

Route 10



[00135] To the amine (67 mg, 0.14 mmol) in DCM (12 mL) was added DIEA (35 mg, 0.27 mmol) followed by 4-chlorobenzoyl chloride (24 mg, 0.14 mmol). After standing at ambient temperature for 30 min the reaction was quenched with sodium bicarbonate (sat.), extracted with DCM (3X), dried with sodium sulfate, and filtered. Flash column chromatography (0-60% hexanes (1% DEA)/ethyl acetate) provided (5,13-bis(piperidin-1-ylsulfonyl)-5,9,13-triazaspiro[2.11]tetradecan-9-yl)(4-chlorophenyl)methanone (compound **A24**). MS(EI) for $C_{28}H_{44}ClN_5O_5S_2$, found 630 $[M+H]^+$.

Route 11



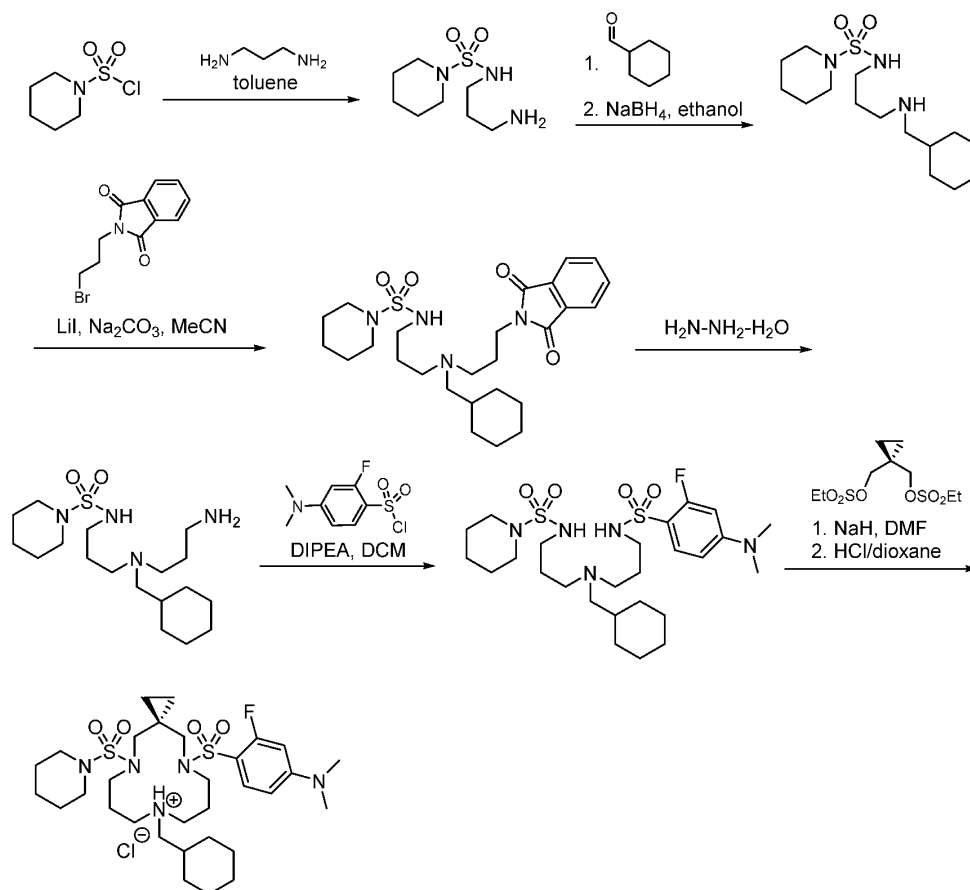
[00136] To a solution of *N,N*-3-trimethylaniline (1.35 g, 10.0 mmol) in $CHCl_3$ (4 mL) was added chlorosulfonic acid (4 mL). The reaction mixture was heated at 80 °C for 18 h. The mixture was cooled to ambient temperature and diluted with DCM (20 mL). The resulting mixture was poured into ice-water (30 mL) and then adjusted to pH=7~8 with saturated aqueous Na_2CO_3 . The organic layer was separated, washed with saturated aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4 and concentrated. The residue was recrystallized from petroleum ether/EtOAc (10:1, 10 mL) to provide 4-(dimethylamino)-2-methylbenzenesulfonyl chloride. 1H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, J = 9.6 Hz, 1H), 6.53 (m, 2H), 3.09 (s, 6H), 2.70 (s, 3H).

[00137] 4-[[9-(cyclohexylmethyl)-13-(piperidine-1-sulfonyl)-5,9,13-triazaspiro[2.11]tetradecan-5-yl]sulfonyl]-*N,N*,3-trimethylaniline was prepared following the procedure described in **Route 12** to provide compound **A30**. 1H NMR (400 MHz, $DMSO-d_6$): δ 9.57 (br s, 1H), 7.50 (d, J = 8.8 Hz, 2H), 6.85 (m, 2H), 3.95 (br s, 1H), 2.89~3.55 (m, 24H), 1.45~1.90 (m, 15H), 1.28 (m, 4H), 0.95 (m, 2H), 0.66 (br s, 4H). MS(EI) for $C_{32}H_{55}N_5O_4S_2$, found 638 $[M+H]^+$.

[00138] The following compound was synthesized in a similar manner:

No.	IUPAC	[M+H] ⁺	¹ H NMR
A41	5-(cyclohexanesulfonyl)-9-(cyclohexylmethyl)-13-(piperidine-1-sulfonyl)-5,9,13-triazaspiro[2.11]tetradecane	587	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆): δ 9.46 (br s, 1H), 3.37-2.95 (m, 15H), 2.01-1.01 (m, 33H), 0.95 (m, 2H), 0.73 (m, 4H).

Route 12



[00139] To a solution of piperidine-1-sulfonyl chloride (68.3 g, 0.37 mol) in toluene (350 mL) was added dropwise a solution of propane-1,3-diamine (83.4 g, 1.12 mol) in toluene (150 mL). The reaction mixture was stirred overnight at ambient temperature. The resulting slurry was filtered and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (DCM/MeOH = 50:1 to 20:1) to afford *N*-(3-aminopropyl)piperidine-1-sulfonamide. ¹H NMR (400 MHz, CDCl₃): δ 3.17 (m, 6H), 2.88 (t, *J* = 6.0 Hz, 2H), 1.65 (m, 6H), 1.54 (m, 2H).

[00140] A mixture of *N*-(3-aminopropyl)piperidine-1-sulfonamide (22.62 mmol) and cyclohexanecarbaldehyde (2.8 g, 24.89 mmol) in toluene (90.0 mL) was heated under reflux

overnight with removal of water by means of a Dean-Stark apparatus. The mixture was cooled to ambient temperature and concentrated under vacuum. The resulting product was dissolved in ethanol (35 mL) and NaBH₄ (1.66 g, 43.7 mmol) was added. The reaction mixture was stirred at ambient temperature overnight. The reaction was quenched with 2*N* aqueous HCl (30 mL) and then adjusted to pH=10 with aqueous NaOH. The resulting mixture was extracted with ethyl acetate (50 mL) and the organic layer was concentrated. The residue was purified by column chromatography on silica gel (DCM/MeOH= 50:1 to 40:1) to provide *N*-(3-(cyclohexylmethylamino)propyl)piperidine-1-sulfonamide. ¹H NMR (400 MHz, CDCl₃): δ 3.17 (m, 6H), 2.78 (t, *J* = 6.0 Hz, 2H), 2.45 (d, *J* = 6.4 Hz, 2H), 1.75 (m, 12H), 1.44 (m, 1H), 1.23 (m, 3H), 0.96 (m, 2H). MS(EI) for C₁₅H₃₁N₃O₂S, found 318 [M+H]⁺.

[00141] To a solution of *N*-(3-(cyclohexylmethylamino)propyl)piperidine-1-sulfonamide (2.8 g, 8.83 mmol) in CH₃CN (50 mL) were added Na₂CO₃ (0.97 g, 9.19 mmol), LiI (0.28 g, 2.12 mmol) and 3-bromopropylphthalimide (5.66 g, 21.19 mmol). The reaction mixture was heated under reflux overnight. The mixture was cooled to ambient temperature and filtered. The filtrate was concentrated and the residue was purified by column chromatography on silica gel (EtOAc/Petroleum ether = 1:3) to provide *N*-(3-((cyclohexylmethyl)(3-(1,3-dioxoisindolin-2-yl)propyl)amino)propyl)piperidine-1-sulfonamide. ¹H NMR (400 MHz, CDCl₃): δ 7.85 (m, 2H), 7.73 (m, 2H), 5.82 (br s, 1 H), 3.68 (t, *J* = 7.2 Hz, 2H), 3.17 (m, 6H), 2.46 (m, 4H), 2.14 (m, 2H), 1.82 (m, 2H), 1.67 (m 12H), 1.45 (m, 3H), 1.18 (m, 2H), 0.87 (m, 2H).

[00142] To a solution of *N*-(3-((cyclohexylmethyl)(3-(1,3-dioxoisindolin-2-yl)propyl)amino)propyl)piperidine-1-sulfonamide (500 mg, 0.10 mmol) in EtOH (5 mL) was added NH₂NH₂ (1.5 mL, 2.55 mmol). The reaction mixture was stirred at ambient temperature overnight. The solvent was removed under reduced pressure and the residue was diluted with ethyl acetate (20 mL). The resulting mixture was washed with water (2 × 10 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated to provide *N*-(3-((3-aminopropyl)(cyclohexylmethyl)amino)propyl)piperidine-1-sulfonamide (380 mg, quantitative). ¹H NMR (400 MHz, CDCl₃): δ 3.17 (m, 6H), 2.78 (t, *J* = 6.8 Hz, 2H), 2.45 (m, 4H), 2.14 (d, *J* = 7.2 Hz, 2H), 1.82 (m, 2H), 1.67 (m 12H), 1.45 (m, 3H), 1.18 (m, 2H), 0.87 (m, 2H). MS(EI) for C₁₈H₃₈N₄O₂S, found 375 [M+H]⁺.

[00143] To a solution of *N*-(3-((3-aminopropyl)(cyclohexylmethyl)amino)propyl)piperidine-1-sulfonamide (2.42 g, 6.49 mmol) and DIEA (1.31 g, 10.3 mmol) in DCM (50 mL) was added the sulfonyl chloride (1.69 g, 7.13 mmol). The reaction mixture was stirred at ambient temperature for 2 h. The mixture was washed with water (50 mL). The organic phase was dried over anhydrous Na₂SO₄ and concentrated. The residue was purified by column chromatography on silica gel (MeOH/DCM = 1:80 to 1:30) to provide *N*-(3-

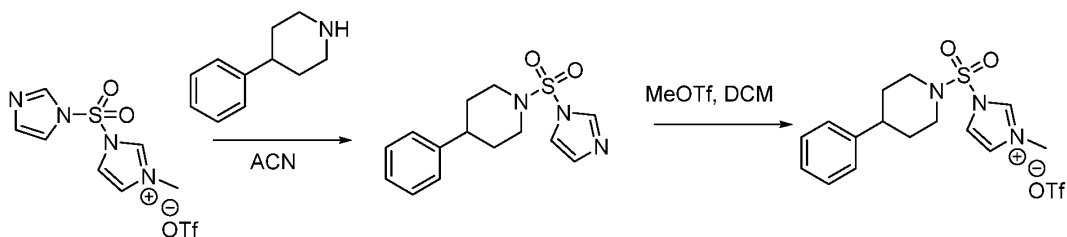
((Cyclohexylmethyl)(3-(4-(dimethylamino)-2-fluorophenylsulfonamido)propyl)amino)propyl)piperidine-1-sulfonamide. ^1H NMR (400 MHz, CDCl_3): δ 7.66 (m, 1H), 6.43 (m, 2H), 5.58 (br s, 2H), 3.15 (m, 6H), 3.03 (s, 6H), 2.99 (br s, 2H), 2.46 (br s, 4H), 2.12 (br s, 2H), 1.30~1.70 (m, 18H), 1.21 (m, 4H), 0.82 (m, 2H). MS(EI) for $\text{C}_{26}\text{H}_{46}\text{FN}_5\text{O}_4\text{S}_2$, found 575 $[\text{M}+\text{H}]^+$.

[00144] To a solution of *N*-(3-((Cyclohexylmethyl)(3-(4-(dimethylamino)-2-fluorophenylsulfonamido)propyl)amino)propyl)piperidine-1-sulfonamide (2.28 g, 3.97 mmol) in DMF (20 mL) was added sodium hydride (400 mg, 3.97 mmol). The mixture was stirred for 30 min at ambient temperature. Cyclopropane-1,1-diylbis(methylene) diethanesulfonate (1.3 g, 3.97 mmol) was added and the reaction mixture was stirred at 80 °C for 2 h. The mixture was cooled to ambient temperature and poured into water (150 mL). The resulting mixture was extracted with ethyl acetate (100 mL). The organic layer was washed with water (50 mL $\times$ 2), dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/Petroleum ether = 1:15 to 1:8) to provide the freebase which was dissolved in ether (5 mL). Hydrochloric acid (1.5 mL or a 3 N solution in 1,4-dioxane) was added and the mixture was stirred for 0.5 h. The solvent was removed under vacuum and the residue was triturated with ether three times to provide compound **A40**. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.89 (br s, 1H), 7.54 (m, 1H), 6.63 (m, 2H), 3.67 (br s, 1H), 3.55-2.89 (m, 23H), 1.90-1.45 (m, 15H), 1.28 (m, 4H), 0.95 (m, 2H), 0.69 (m, 4H). MS(EI) for $\text{C}_{31}\text{H}_{52}\text{FN}_5\text{O}_4\text{S}_2$, found 643 $[\text{M}+\text{H}]^+$.

[00145] The following compounds were synthesized in a similar manner:

No.	IUPAC	$[\text{M}+\text{H}]^+$	^1H NMR
A31	9-(cyclohexylmethyl)-5-(4-methoxybenzenesulfonyl)-13-(piperidine-1-sulfonyl)-5,9,13-triazaspiro[2.11]tetradecane	611	^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.75 (br s, 1H), 7.73 (d, $J = 8.8$ Hz, 2H), 7.16 (d, $J = 8.8$ Hz, 2H), 3.85 (s, 3H), 3.55-2.89 (m, 18H), 1.90-1.45 (m, 15H), 1.28 (m, 4H), 0.95 (m, 2H), 0.66 (m, 4H).
A32	9-(cyclohexylmethyl)-5-(3-methoxybenzenesulfonyl)-13-(piperidine-1-sulfonyl)-5,9,13-triazaspiro[2.11]tetradecane	611	^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.89 (br s, 1H), 7.57 (m, 1H), 7.32 (m, 2H), 7.23 (s, 1H), 3.85 (s, 3H), 3.55-2.89 (m, 18H), 2.04-1.45 (m, 15H), 1.28 (m, 4H), 0.95 (m, 2H), 0.67 (m, 4H).
A33	9-(cyclohexylmethyl)-5-(2-methoxybenzenesulfonyl)-13-(piperidine-1-sulfonyl)-5,9,13-triazaspiro[2.11]tetradecane	611	^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.35 (br s, 1H), 7.80 (m, 1H), 7.68 (m, 1H), 7.28 (m, 1H), 7.13 (m, 1H), 3.89 (s, 3H), 3.55-2.89 (m, 18H), 1.90-1.45 (m, 15H), 1.28 (m, 4H), 0.95 (m, 2H), 0.66 (br s, 2H), 0.51 (br s, 2H).
A34	5-(benzenesulfonyl)-9-(cyclohexylmethyl)-13-(piperidine-1-sulfonyl)-5,9,13-triazaspiro[2.11]tetradecane	581	^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.26 (br s, 1H), 7.76 (m, 4H), 3.41-2.89 (m, 18H), 1.90-1.45 (m, 15H), 1.28 (m, 4H), 0.95 (m, 2H), 0.73 (br s, 4H).

Route 13



[00146] To 1-((1H-imidazol-1-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (1.69 g, 4.65 mmol, reference: *J. Org. Chem.* **2002**, 68, 115-119.) and 4-phenylpiperidine (750 mg, 4.65 mmol) was added ACN (16 mL) and the solution was allowed to stand for 2 h then concentrated and purified directly by flash column chromatography (0-80% hexanes/ethyl acetate) to provide the product.

[00147] To 1-((1H-imidazol-1-yl)sulfonyl)-4-phenylpiperidine (600 mg, 2.06 mmol) in DCM at 0 °C was added methyl triflate (0.23 mL, 2.06 mmol) over 5 min. After allowing to stand for 30 min the mixture was concentrated to provide 3-methyl-1-((4-phenylpiperidin-1-yl)sulfonyl)-1H-imidazol-3-ium trifluoromethanesulfonate which was carried forward without further purification. MS(EI) for $C_{15}H_{20}N_3O_2S$, found 307 $[M]^+$.

[00148] The following compounds were synthesized in a similar manner:

1-((8-chloro-3,4-dihydroisoquinolin-2(1H)-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A51**).

1-((7-chloro-3,4-dihydroisoquinolin-2(1H)-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A52**).

1-((6-chloro-3,4-dihydroisoquinolin-2(1H)-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A53**).

1-((5-chloro-3,4-dihydroisoquinolin-2(1H)-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A54**).

1-((6-fluoro-3,4-dihydroisoquinolin-2(1H)-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A59**).

1-((6-bromo-3,4-dihydroisoquinolin-2(1H)-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A60**).

1-((4-isopropoxy-piperidin-1-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate hydrochloride (Intermediate for compound **A66**).

1-((4-(cyclopent-1-en-1-yl)-3,6-dihydropyridin-1(2H)-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A67**).

1-((4-(cyclohex-1-en-1-yl)-3,6-dihydropyridin-1(2H)-yl)sulfonyl)-3-methyl-1H-imidazol-

3-ium trifluoromethanesulfonate (Intermediate for compound **A68**).

1-((4-(3-chlorophenyl)-3,6-dihydropyridin-1(2H)-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A69**).

1-((4-(2-chlorophenyl)-3,6-dihydropyridin-1(2H)-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A73**).

1-((4-(4-chlorophenyl)-3,6-dihydropyridin-1(2H)-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A74**).

1-((4-(1H-pyrazol-1-yl)piperidin-1-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A79**).

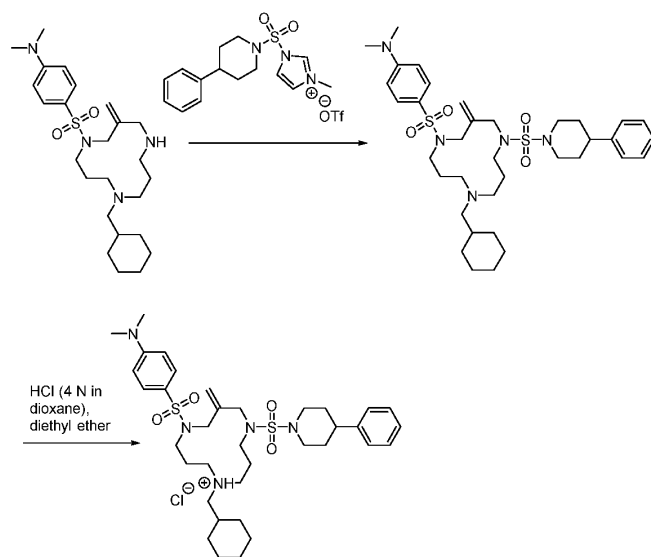
1-((4-(benzo[d]oxazol-2-yl)piperidin-1-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A80**).

3-methyl-1-((4-(pyrimidin-2-yl)piperidin-1-yl)sulfonyl)-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A81**).

1-((2-chloro-7,8-dihydro-1,6-naphthyridin-6(5H)-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A82**).

1-((5-chloroisindolin-2-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A89**).

Route 14



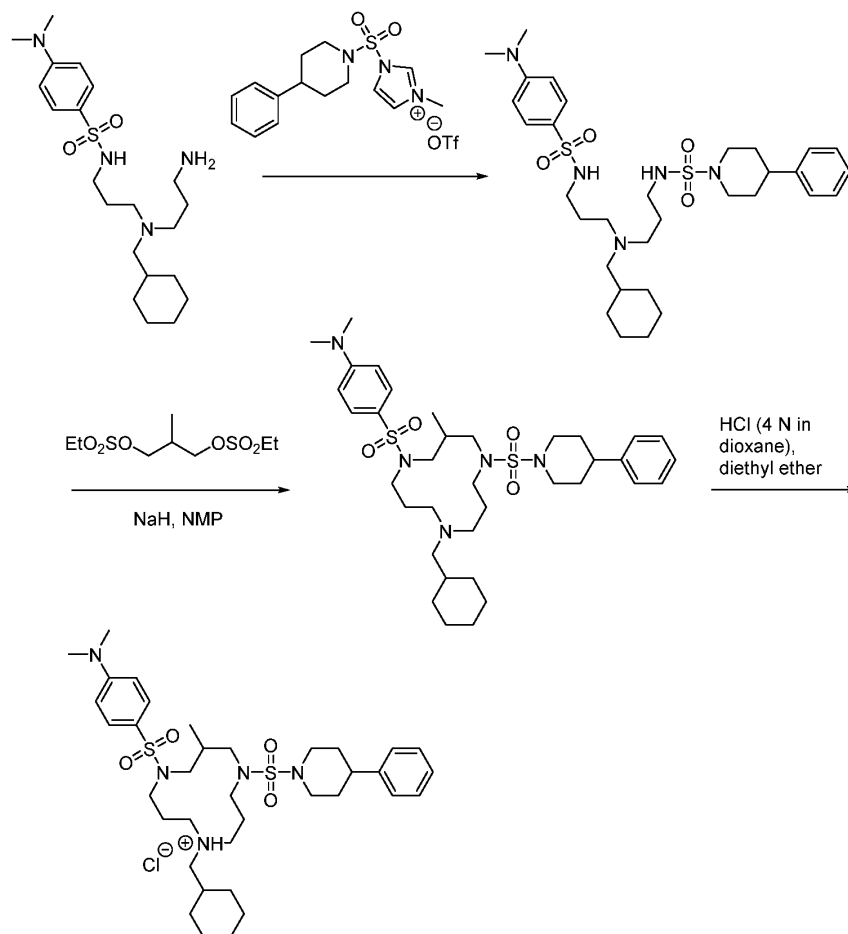
[00149] To 4-((9-(cyclohexylmethyl)-3-methylene-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline (97 mg, 0.21 mmol) in ACN (0.8 mL) was added 3-methyl-1-((4-phenylpiperidin-1-yl)sulfonyl)-1H-imidazol-3-ium trifluoromethanesulfonate (95 mg, 0.21 mmol) and the mixture was heated to 80 °C for 8 h then concentrated and purified directly by flash column chromatography (0-70% hexanes (1% DEA)/ethyl acetate) to provide the

freebase. To this was added diethyl ether (5 mL) and HCl (52 μ L of a 4 N HCl in 1,4-dioxane) and the mixture was allowed to stand for 10 min then concentrated to provide 4-[[9-(cyclohexylmethyl)-3-methylidene-5-[(4-phenylpiperidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline hydrochloride (compound **A35**). MS(EI) for $C_{36}H_{55}N_5O_4S_2$, found 686 $[M+H]^+$.

[00150] The following compounds were synthesized in a similar manner:

No.	IUPAC	$[M+H]^+$
A36	4-[[9-(cyclohexylmethyl)-3-methylidene-5-[(4-phenylpiperazin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	687
A37	4-[[9-(cyclohexylmethyl)-3-methylidene-5-[(4-phenoxy-piperidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	702
A38	4-[[9-(cyclohexylmethyl)-3-methylidene-5-[(3-phenylpiperidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	686
A39	1-[[9-(cyclohexylmethyl)-5-[4-(dimethylamino)benzenesulfonyl]-3-methylidene-1,5,9-triazacyclododecan-1-yl]sulfonyl]piperidine-4-carbonitrile	635
A42	4-[[5-(cyclohexylmethyl)-9-[(4-phenylpiperidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	674
A43	4-[[9-(cyclohexylmethyl)-3-methylidene-5-[(3S)-3-phenylpiperidin-1-yl]sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	686
A46	4-[[9-(cyclohexylmethyl)-5-[(3R)-3-methyl-4-phenylpiperazin-1-yl]sulfonyl]-3-methylidene-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	701
A47	4-[[9-(cyclohexylmethyl)-5-[(3S)-3-methyl-4-phenylpiperazin-1-yl]sulfonyl]-3-methylidene-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	701
A48	4-[[9-(cyclohexylmethyl)-5-[4-(dimethylamino)benzenesulfonyl]-3-methylidene-1,5,9-triazacyclododecan-1-yl]sulfonyl]-1-phenylpiperazin-2-one	701
A49	4-[[9-(cyclohexylmethyl)-3-methylidene-5-[(3R)-3-phenylpiperidin-1-yl]sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	686
A50	4-[[9-(cyclohexylmethyl)-3-methylidene-5-(1,2,3,4-tetrahydroisoquinoline-2-sulfonyl)-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	658

Route 15



[00151] To N-(3-((3-aminopropyl)(cyclohexylmethyl)amino)propyl)-4-(dimethylamino)benzenesulfonamide (838 mg, 1.84 mmol) in ACN (5 mL) was added 3-methyl-1-((4-phenylpiperidin-1-yl)sulfonyl)-1H-imidazol-3-ium trifluoromethanesulfonate (838 mg, 1.84 mmol) and the mixture was heated to 80 °C for 8 h the concentrated and purified directly by flash column chromatography (0-70% hexanes (1% DEA)/ethyl acetate) to provide the product. ¹H NMR (400 MHz, CDCl₃): δ 7.68 (d, *J* = 8.8 Hz, 2H), 7.33 (m, 5H), 6.68 (d, *J* = 8.8 Hz, 2H), 5.75 (br s, 2H), 3.84 (m, 2H), 3.18 (m, 2H), 3.05 (s, 6H), 2.91 (m, 4H), 2.65 (m, 1H), 2.43 (m, 4H), 2.08 (m, 2H), 1.73 (m, 12H), 1.40 (m, 1H), 1.15 (m, 4H), 0.85 (m, 2H).

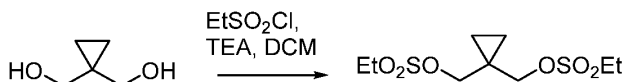
[00152] To 4-((9-(cyclohexylmethyl)-3-methyl-5-((4-phenylpiperidin-1-yl)sulfonyl)-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline (123 mg, 0.194 mmol) in NMP (10 mL) was added sodium hydride (19 mg of a 60% dispersion in mineral oil, 0.485 mmol) over 20 min. The mixture was stirred at ambient temperature for 30 min then 80 °C for an additional 20 min. The bis-sulfonate was then added over the course of 1 h. After 8 h at 80 °C the

mixture was cooled to ambient temp., diluted with brine and ethyl acetate, extracted with ethyl acetate (3X), the combined organics were washed with brine (3X), dried with sodium sulfate, filtered, and concentrated. Flash column chromatography (0-50% hexanes (1% DEA)/ethyl acetate) provided the freebase. The freebase was converted to the HCl salt with the addition of ethyl ether (5 mL) and HCl (4N in dioxane, 50 μ L). Concentration after 10 min provided 4-[[9-(cyclohexylmethyl)-3-methyl-5-[(4-phenylpiperidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline hydrochloride (compound **A44**). MS(EI) for $C_{36}H_{57}N_5O_4S_2$, found 688 $[M+H]^+$.

[00153] The following compound was synthesized in a similar manner:

No.	IUPAC	$[M+H]^+$
B7	N-{3-[(cyclohexylmethyl)(3-[[[(4-phenylpiperidin-1-yl)sulfonyl]amino]propyl]amino]propyl)-4-(dimethylamino)benzene-1-sulfonamide	634

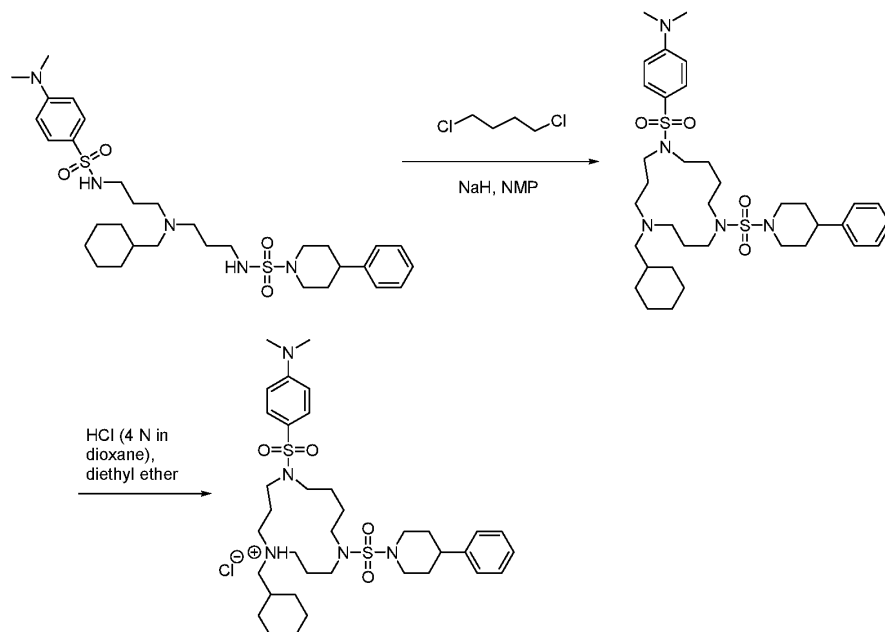
Route 16



[00154] To 1,1-bis(hydroxymethyl)cyclopropane (5.00, 49.0 mmol) in acetone (20 mL) was added TEA (2.2 eq, 108 mmol) and the mixture was cooled to 0-5 °C. Ethanesulfonyl chloride was added at a rate to maintain the internal temperature below 10 °C (~1 h). After stirring an additional 2 h at ambient temperature the mixture was diluted with 150 mL of water, extracted with ethyl acetate (2X50 mL), washed with brine, dried with sodium sulfate, filtered, and concentrated to provide the product. ¹H NMR (400 MHz, CDCl₃): δ 4.16 (s, 4H), 3.20 (m, 4H), 1.46 (t, J = 7.2 Hz, 6H), 0.83 (s, 4H). MS(EI) for $C_9H_{18}O_6S_2$, found 287 $[M+H]^+$.

[00155] The following compound was synthesized in a similar manner 2-methylpropane-1,3-diyl diethanesulfonate (Intermediate for compound **A44**).

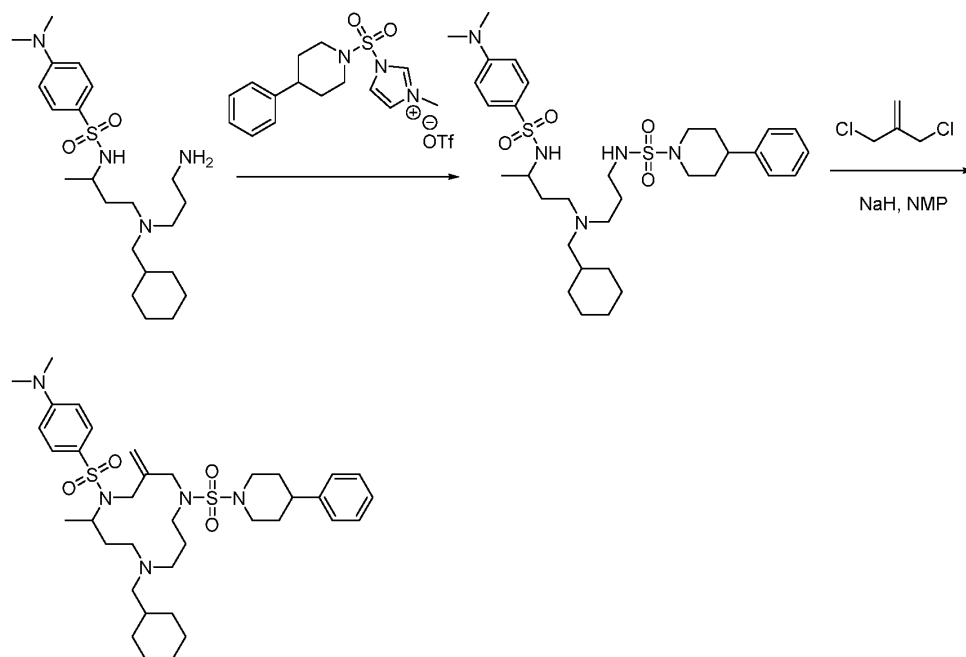
Route 17



[00156] To N-(3-((cyclohexylmethyl)(3-((4-(dimethylamino)phenyl)sulfonamido)propyl)amino)propyl)-4-phenylpiperidine-1-sulfonamide (89 mg, 0.14 mmol) in NMP (7 mL) was added NaH (14 mg of a 60% dispersion in mineral oil, 0.35 mmol) portion-wise over 20 min. The mixture was stirred at ambient temperature for 30 min then 80 °C for 20 min. The dichloride was then added and the mixture was heated at 80 °C. After 8 h at 80 °C the mixture was cooled to ambient temp, diluted with brine and ethyl acetate, extracted with ethyl acetate (3X), the combined organics were washed with brine (3X), dried with sodium sulfate, filtered, and concentrated. Flash column chromatography (0-50% hexanes (1% DEA)/ethyl acetate) provided the product. The product was converted to the HCl salt with the addition of ethyl ether (5 mL) and HCl (4N in dioxane, 35 uL). The mixture was concentrated to provide 4-[[5-(cyclohexylmethyl)-9-[(4-phenylpiperidin-1-yl)sulfonyl]-1,5,9-triazacyclotridecan-1-yl]sulfonyl]-N,N-dimethylaniline hydrochloride (compound **B5**). MS(EI) for $C_{36}H_{57}N_5O_4S_2$, found 688 $[M+H]^+$.

[00157] The following compounds were synthesized in a similar manner:

No.	IUPAC	$[M+H]^+$
B8	4-[[[(11Z)-5-(cyclohexylmethyl)-9-[(4-phenylpiperidin-1-yl)sulfonyl]-1,5,9-triazacyclotridec-11-en-1-yl]sulfonyl]-N,N-dimethylaniline	686
B9	4-[[[(11E)-5-(cyclohexylmethyl)-9-[(4-phenylpiperidin-1-yl)sulfonyl]-1,5,9-triazacyclotridec-11-en-1-yl]sulfonyl]-N,N-dimethylaniline	686

Route 18

[00158] N-(4-((3-aminopropyl)(cyclohexylmethyl)amino)butan-2-yl)-4-(dimethylamino)benzenesulfonamide was synthesized following **Route 2** with substitution of the appropriate sulfonyl chloride.

[00159] 4-((5-(cyclohexylmethyl)-2-methyl-11-methylene-9-((4-phenylpiperidin-1-yl)sulfonyl)-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline was synthesized following the procedure described in **Route 15** substituting with 3-chloro-2-(chloromethyl)prop-1-ene to provide 4-((5-(cyclohexylmethyl)-2-methyl-11-methylene-9-((4-phenylpiperidin-1-yl)sulfonyl)-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline (compound **B6**). MS(EI) for $C_{37}H_{57}N_5O_4S_2$, found 700 $[M+H]^+$.

[00160] The following compounds were synthesized in a similar manner:

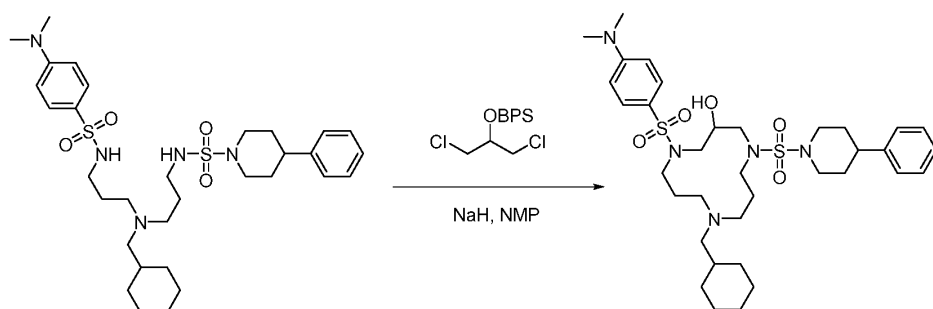
No.	IUPAC	$[M+H]^+$	1H NMR
B19	4-{{[5-(cyclohexylmethyl)-2-methyl-11-methylen-9-[(4-phenylpiperidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl}-N,N-dimethylaniline	700	
A51	4-{{[5-[(8-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methylen-1,5,9-triazacyclododecan-1-yl]sulfonyl}-N,N-dimethylaniline	692	

A52	4-({5-[(7-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl}sulfonyl)-N,N-dimethylaniline	692	
A53	4-({5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl}sulfonyl)-N,N-dimethylaniline	692	¹ H NMR (400 MHz, CDCl ₃): δ 7.59 (d, <i>J</i> = 8.8 Hz, 2H), 7.15 (m, 2H), 7.04 (m, 1H), 6.70 (d, <i>J</i> = 8.8 Hz, 2H), 5.25 (s, 1H), 5.13 (s, 1H), 4.34 (s, 2H), 4.12 (s, 2H), 4.10 (s, 2H), 3.51 (m, 6H), 3.05 (s, 6H), 2.88 (m, 4H), 2.41 (m, 2H), 2.25 (m, 2H), 1.95 (m, 2H), 1.85 (m, 2H), 1.67 (m, 6H), 1.23 (m, 4H), 0.85 (m, 1H).
A54	4-({5-[(5-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl}sulfonyl)-N,N-dimethylaniline	692	
A56	4-({5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl}sulfonyl)-3-fluoro-N,N-dimethylaniline	710	
A57	4-({5-[(4-(2-chlorophenyl)piperidin-1-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl}sulfonyl)-N,N-dimethylaniline	720	
A58	4-({5-[(4-(3-chlorophenyl)piperidin-1-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl}sulfonyl)-N,N-dimethylaniline	720	
A59	4-({9-(cyclohexylmethyl)-5-[(6-fluoro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methylidene-1,5,9-triazacyclododecan-1-yl}sulfonyl)-N,N-dimethylaniline	676	
A60	4-({5-[(6-bromo-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-	736	

	(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline		
A64	4-[[9-(cyclohexylmethyl)-5-[(4-cyclopentyl)piperidin-1-yl)sulfonyl]-3-methylidene-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	678	
A65	4-[[9-(cyclohexylmethyl)-5-[(4-cyclohexyl)piperidin-1-yl)sulfonyl]-3-methylidene-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	692	
A66	4-[[9-(cyclohexylmethyl)-3-methylidene-5-[[4-(propan-2-yloxy)piperidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	668	
A67	4-[[9-(cyclohexylmethyl)-5-[[4-(cyclopent-1-en-1-yl)-1,2,3,6-tetrahydropyridin-1-yl)sulfonyl]-3-methylidene-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	674	
A68	4-[(5-[[4-(cyclohex-1-en-1-yl)-1,2,3,6-tetrahydropyridin-1-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	688	
A69	4-[(5-[[4-(3-chlorophenyl)-1,2,3,6-tetrahydropyridin-1-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	718	
A70	2-[[9-(cyclohexylmethyl)-5-[4-(dimethylamino)benzenesulfonyl]-3-methylidene-1,5,9-triazacyclododecan-1-yl)sulfonyl]-1,2,3,4-tetrahydroisoquinoline-6-carbonitrile	683	
A71	4-[[9-(cyclohexylmethyl)-5-[(6-methoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methylidene-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	688	

A72	4-[(5-{[4-(4-chlorophenyl)piperidin-1-yl]sulfonyl}-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	720	
A73	4-[(5-{[4-(2-chlorophenyl)-1,2,3,6-tetrahydropyridin-1-yl]sulfonyl}-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	718	
A74	4-[(5-{[4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridin-1-yl]sulfonyl}-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	718	
A78	4-{[9-(cyclohexylmethyl)-3-methylidene-5-{[4-(oxan-4-yl)piperidin-1-yl]sulfonyl}-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	694	
A79	4-{[9-(cyclohexylmethyl)-3-methylidene-5-{[4-(1H-pyrazol-1-yl)piperidin-1-yl]sulfonyl}-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	676	
A80	4-[(5-{[4-(1,3-benzoxazol-2-yl)piperidin-1-yl]sulfonyl}-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	727	
A81	4-{[9-(cyclohexylmethyl)-3-methylidene-5-{[4-(pyrimidin-2-yl)piperidin-1-yl]sulfonyl}-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	688	
A82	4-[(5-{[2-chloro-5,6,7,8-tetrahydro-1,6-naphthyridin-6-yl]sulfonyl}-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	693	
A88	(3Z)-9-(cyclohexylmethyl)-1-[4-(dimethylamino)benzenesulfonyl]-N-methoxy-5-[(4-phenylpiperidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-3-	717	

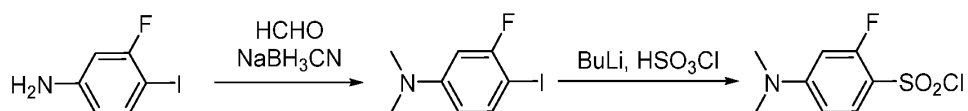
	imine		
A83	4-[(5-[[4-(2-chlorophenyl)piperazin-1-yl]sulfonyl]-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	721	¹ H NMR (400 MHz, CDCl ₃): δ 7.61 (d, <i>J</i> = 8.8 Hz, 2H), 7.39 (m, 2H), 7.05 (m, 2H), 6.82 (d, <i>J</i> = 8.8 Hz, 2H), 5.35 (m, 2H), 4.10 (m, 2H), 3.41 (m, 6H), 3.10 (m, 4H), 3.05 (s, 6H), 2.88 (m, 2H), 2.41 (m, 3H), 2.21 (m, 4H), 2.01 (m, 3H), 1.85 (m, 6H), 1.55 (m, 11H), 1.15 (m, 9H), 0.82 (m, 3H).
A84	4-[(5-[[4-(3-chlorophenyl)piperazin-1-yl]sulfonyl]-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	721	¹ H NMR (400 MHz, CDCl ₃): δ 7.59 (d, <i>J</i> = 8.8 Hz, 2H), 7.18 (m, 1H), 6.85 (m, 3H), 6.78 (d, <i>J</i> = 8.8 Hz, 2H), 5.25 (m, 2H), 4.10 (m, 2H), 3.51 (m, 2H), 3.20 (m, 10H), 3.05 (s, 6H), 2.88 (m, 2H), 2.41 (m, 2H), 2.01 (m, 2H), 1.85 (m, 4H), 1.65 (m, 6H), 1.25 (m, 5H), 0.82 (m, 1H).
A85	4-[(5-[[4-(4-chlorophenyl)piperazin-1-yl]sulfonyl]-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	721	¹ H NMR (400 MHz, CDCl ₃): δ 7.59 (d, <i>J</i> = 8.8 Hz, 2H), 7.26 (d, <i>J</i> = 8.0 Hz, 2H), 6.87 (d, <i>J</i> = 8.0 Hz, 2H), 6.70 (d, <i>J</i> = 8.8 Hz, 2H), 5.25 (m, 2H), 4.10 (m, 2H), 3.51 (m, 2H), 3.20 (m, 10H), 3.05 (s, 6H), 2.88 (m, 2H), 2.41 (m, 2H), 2.01 (m, 2H), 1.85 (m, 4H), 1.65 (m, 6H), 1.27 (m, 5H), 0.85 (m, 1H).
A89	4-[(5-[[5-(5-chloro-2,3-dihydro-1H-isoindol-2-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methylidene-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	678	¹ H NMR (400 MHz, CDCl ₃): δ 7.59 (d, <i>J</i> = 8.8 Hz, 2H), 7.25 (m, 4H), 6.68 (d, <i>J</i> = 8.8 Hz, 2H), 5.28 (s, 1H), 5.12 (s, 1H), 4.64 (m, 4H), 4.12 (s, 2H), 3.45 (m, 4H), 3.05 (s, 6H), 2.88 (m, 2H), 2.25 (m, 2H), 1.95 (m, 2H), 1.65 (m, 8H), 1.23 (m, 8H), 0.85 (m, 1H).

Route 19

[00161] To N-(3-((cyclohexylmethyl)(3-((4-(dimethylamino)phenyl)sulfonamido)propyl)amino)propyl)-4-phenylpiperidine-1-sulfonamide (33 mg, 0.052 mmol) in NMP (3 mL) was added sodium hydride (4.6 mg of a 60% dispersion in mineral oil, 0.11 mmol). The mixture was stirred at ambient temperature for 30 min then 80 °C for an additional 20 min. The dichloride (19 mg, 0.052 mmol) was then added. After 2 h at 80 °C the mixture was cooled to ambient temp, diluted with brine and ethyl acetate,

extracted with ethyl acetate (3X), the combined organics were washed with brine (3X), dried with sodium sulfate, filtered, and concentrated. Flash column chromatography (0-50% hexanes (1% DEA)/ethyl acetate) provided 9-(cyclohexylmethyl)-1-[4-(dimethylamino)benzenesulfonyl]-5-[(4-phenylpiperidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-3-ol (compound **A55**). MS(EI) for $C_{35}H_{55}N_5O_5S_2$, found 690 $[M+H]^+$.

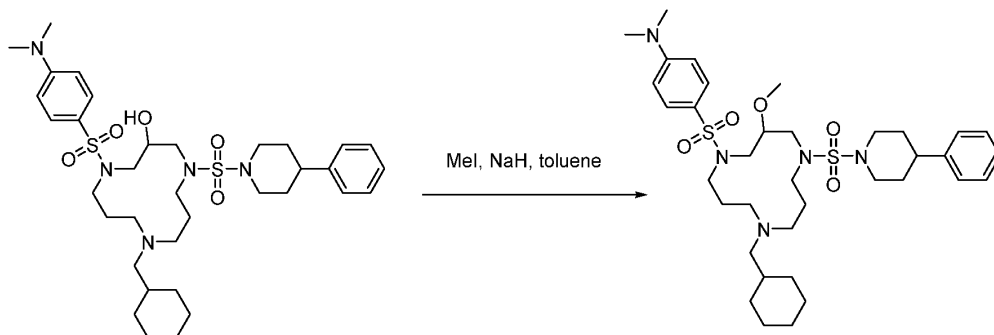
Route 20



[00162] To a solution of 3-fluoro-4-iodoaniline (1.11 g, 4.68 mmol) in acetonitrile (15.00 mL) were added 37% aqueous formaldehyde (8.0 mL, 99.9 mmol) and sodium cyanoborohydride (1.88 g, 29.97 mmol). Then acetic acid (1 mL) was added dropwise over 10 min and the reaction mixture was stirred at ambient temperature overnight. Aqueous NaOH (1N, 30 mL) was added and the resulting mixture was extracted with ethyl acetate (50 mL). The organics were washed with brine, dried over anhydrous sodium sulfate and concentrated to provide 3-fluoro-4-iodo-*N,N*-dimethylaniline. 1H NMR (400 MHz, $CDCl_3$): δ 7.47 (m, 1H), 6.43 (m, 1H), 6.28 (m, 1H), 2.94 (s, 6H).

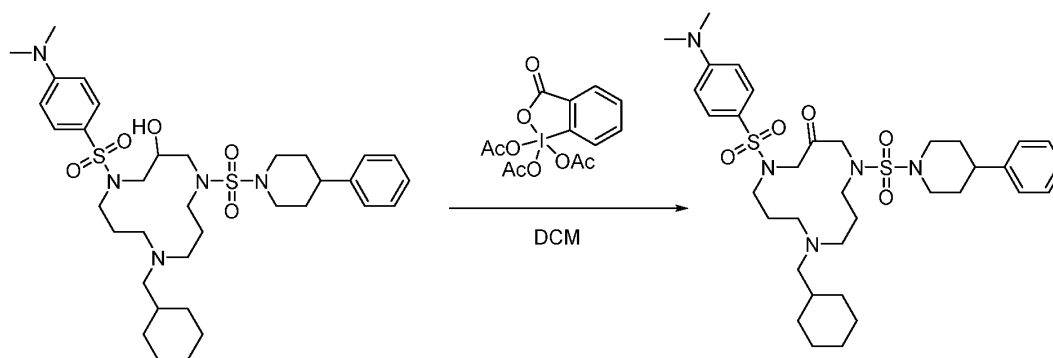
[00163] To a solution of 3-fluoro-4-iodo-*N,N*-dimethylaniline (500 mg, 1.89 mmol) in ether (5 mL) was added *n*-BuLi (0.8 mL, 1.89 mmol) at $-78^\circ C$. The mixture was stirred for 0.5 h at this temperature. Sulfuryl dichloride (405 mg, 3 mmol) was added and the reaction mixture was stirred for 1 h. The reaction was quenched with water (10 mL) and then adjusted to pH=8 with saturated aqueous $NaHCO_3$. The resulting mixture was extracted with ethyl acetate (10 mL). The organic phase was separated and concentrated. The residue was purified by column chromatography on silica gel to provide 4-(dimethylamino)-2-fluorobenzoyl chloride. 1H NMR (400 MHz, $CDCl_3$): δ 7.73 (m, 1H), 6.43 (m, 2H), 3.15 (s, 3H).

Route 21



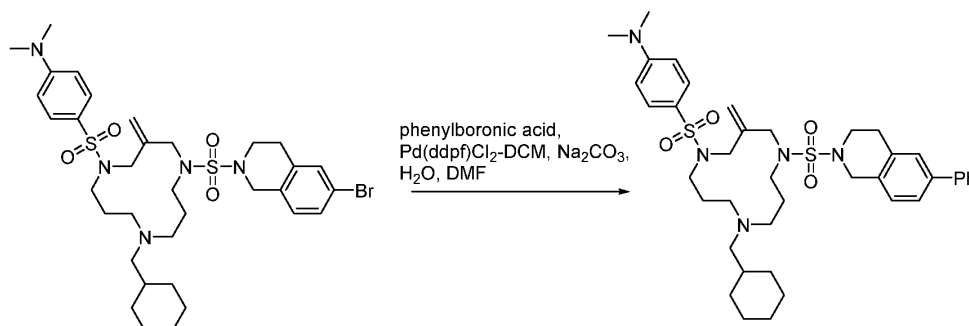
[00164] To 9-(cyclohexylmethyl)-1-((4-(dimethylamino)phenyl)sulfonyl)-5-((4-phenylpiperidin-1-yl)sulfonyl)-1,5,9-triazacyclododecan-3-ol (30 mg, 0.043 mmol) in toluene (0.5 mL) was added NaH (1.6 mg of a 60% dispersion in mineral oil, 0.065 mmol) followed by iodomethane (14 μ L, 0.22 mmol). After stirring for 1 h the mixture was quenched with water, extracted with DCM (2X), the organics were combined and dried with sodium sulfate, filtered, and concentrated. Flash column chromatography (0-50% hexanes/ethyl acetate +1% DEA) provided 4-([9-(cyclohexylmethyl)-3-methoxy-5-[(4-phenylpiperidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl)-N,N-dimethylaniline (compound **A61**). MS(EI) for $C_{36}H_{57}N_5O_5S_2$, found 704 $[M+H]^+$.

Route 22



[00165] To 9-(cyclohexylmethyl)-1-((4-(dimethylamino)phenyl)sulfonyl)-5-((4-phenylpiperidin-1-yl)sulfonyl)-1,5,9-triazacyclododecan-3-ol (171 mg, 0.248 mmol) was added DCM (10 mL) followed by Dess-Martin periodinane (126 mg, 0.297 mmol). The mixture was allowed to stand for 2 h at rt then it was concentrated and purified directly by FCC (0-60% hexanes (1% DEA)/ethyl acetate) to provide 9-(cyclohexylmethyl)-1-((4-(dimethylamino)phenyl)sulfonyl)-5-((4-phenylpiperidin-1-yl)sulfonyl)-1,5,9-triazacyclododecan-3-one (compound **B20**). MS(EI) for $C_{35}H_{53}N_5O_5S_2$, found 688 $[M+H]^+$.

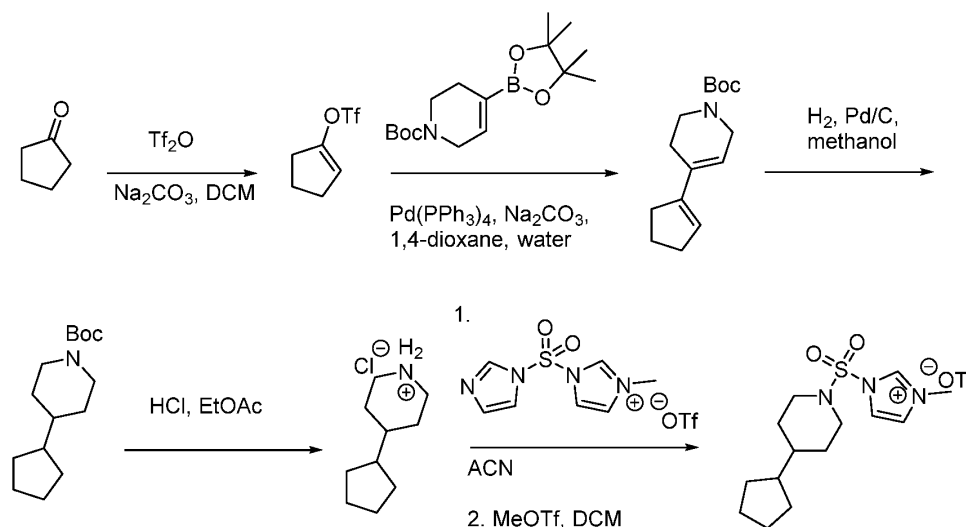
Route 23



[00166] To 4-((5-((6-bromo-3,4-dihydroisoquinolin-2(1H)-yl)sulfonyl)-9-(cyclohexylmethyl)-

3-methylene-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline (70 mg, 0.095 mmol) in DMF (0.7 mL) was added water (0.1 mL), phenylboronic acid (13 mg, 0.10 mmol), Pd(dppf)Cl₂-DCM (7.8 mg, 0.001 mmol), and sodium carbonate (30 mg, 0.29 mmol). The mixture was heated to 80 °C for 1 h then diluted with brine, extracted with ethyl acetate (2X), dried with sodium sulfate, filtered, and concentrated. Flash column chromatography (0-50% hexanes (1% DEA)/ethyl acetate) provided 4-((9-(cyclohexylmethyl)-3-methylene-5-((6-phenyl-3,4-dihydroisoquinolin-2(1H)-yl)sulfonyl)-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline (compound **A63**). MS(EI) for C₄₀H₅₃N₅O₄S₂, found 734 [M+H]⁺.

Route 24



[00167] To a mixture of cyclopentanone (5.50 g, 65.5 mmol) and Na₂CO₃ (10.4 g, 98.2 mmol) in DCM (130 mL) was added Tf₂O (18.6 g, 72.0 mmol) at -20 °C. The reaction mixture was allowed to warm to ambient temperature and stirred overnight. The solid was filtered off and the filtrate was concentrated to provide cyclopentenyl trifluoromethanesulfonate.

[00168] A solution of cyclopentenyl trifluoromethanesulfonate (7.7 g, 35.6 mmol) in 1,4-dioxane (105 mL) and water (50 mL) was degassed and then filled with argon. *tert*-Butyl 4-(4,4,5,5-tetramethyl-1,3-dioxolan-2-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate (6.0 g, 19.4 mmol), Pd(PPh₃)₄ (1.02 g, 0.88 mmol) and sodium carbonate (10.3 g, 97.1 mmol) were added. The reaction mixture was stirred overnight at 90 °C and then cooled to ambient temperature. The mixture was poured into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. Purification by flash column chromatography on silica gel (ethyl acetate/hexanes = 1:99 to 5:95) provided *tert*-Butyl 4-cyclopentenyl-5,6-dihydropyridine-1(2*H*)-carboxylate. ¹H NMR (400 MHz, CDCl₃): δ 5.73 (br s, 1H), 5.56 (br s, 1H), 4.00 (br s, 2H), 3.45 (br s, 2H), 2.47 (m, 4H), 2.33 (br s, 2H), 1.94 (m, 2H), 1.48 (s, 9H).

[00169] To a solution of *tert*-Butyl 4-cyclopentenyl-5,6-dihydropyridine-1(2*H*)-carboxylate (3.3 g, 15.3 mmol) in methanol (30 mL) was added Pd/C (0.6 g). The mixture was hydrogenated under 15 psi pressure for 5 h. The mixture was filtered and the filtrate was concentrated under reduced pressure to provide *tert*-Butyl 4-cyclopentylpiperidine-1-carboxylate.

[00170] To a solution of *tert*-Butyl 4-cyclopentylpiperidine-1-carboxylate (2.9 g, 11.5 mmol) in ethyl acetate (15 mL) was added EtOAc/HCl (4*N*, 20 mL). The mixture was stirred for 2 h and the solvent was removed. The resulting product was washed with ether to provide 4-cyclopentylpiperidine hydrochloride. ¹H NMR (400 MHz, D₂O): δ 3.25 (m, 2H), 2.75 (m, 2H), 1.84 (m, 2H), 1.61 (m, 2H), 1.45 (m, 8H), 0.96 (m, 2H).

[00171] 4-Cyclopentylpiperidine hydrochloride (1.4 g, 9.5 mmol) and 1-(1*H*-imidazol-1-ylsulfonyl)-3-methyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (3.45 g, 9.5 mmol) were mixed in CH₃CN (20 mL). The mixture was stirred at ambient temperature overnight. The solvent was removed. The residue was purified by column chromatography on silica gel (EtOAc/petroleum ether = 1:9 to 1:3) to provide 1-(1*H*-imidazol-1-ylsulfonyl)-4-cyclopentylpiperidine. ¹H NMR (400 MHz, CDCl₃): δ 7.91 (s, 1H), 7.24 (s, 1H), 7.14 (s, 1H), 3.87 (m, 2H), 2.51 (m, 2H), 1.83 (m, 4H), 1.54 (m, 5H), 1.33 (m, 2H), 1.08 (m, 3H).

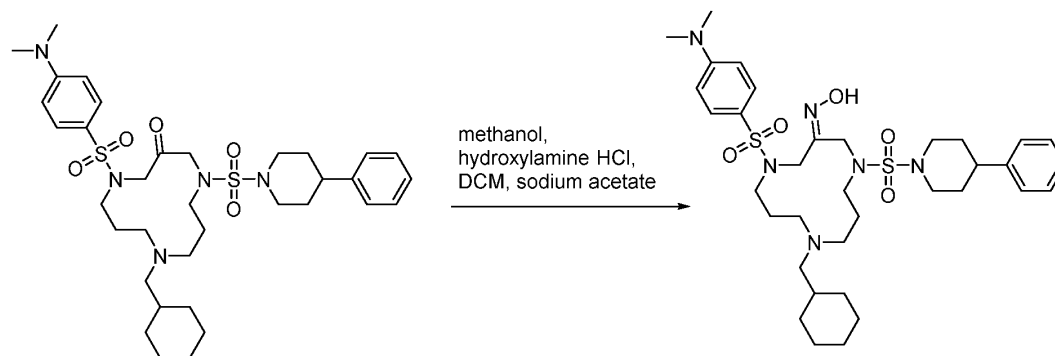
[00172] To a solution of 1-(1*H*-imidazol-1-ylsulfonyl)-4-cyclopentylpiperidine (500 mg, 1.8 mmol) in DCM (5 mL) was added MeOTf (0.3 g, 1.8 mmol). The reaction mixture was stirred at ambient temperature overnight. The solvent was removed and the resulting product was washed with ether to provide 1-((4-cyclopentylpiperidin-1-yl)sulfonyl)-3-methyl-1*H*-imidazol-3-ium trifluoromethanesulfonate. ¹H NMR (400 MHz, CD₃OD): δ 9.59 (s, 1H), 8.02 (s, 1H), 7.78 (s, 1H), 4.00 (s, 3H), 3.95 (m, 2H), 2.87 (m, 2H), 1.81 (m, 4H), 1.54 (m, 6H), 1.23 (m, 4H).

[00173] The following compounds were synthesized in a similar manner:

1-((4-cyclohexylpiperidin-1-yl)sulfonyl)-3-methyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A65**).

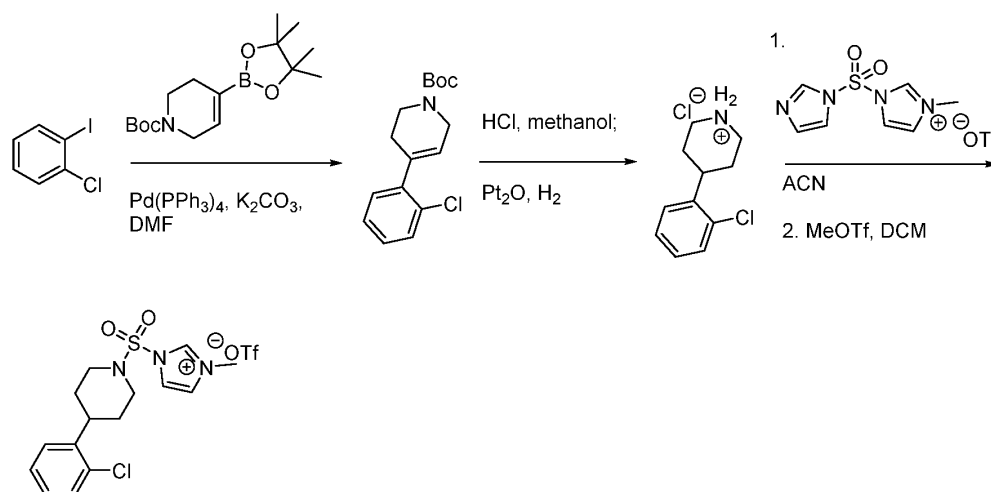
3-methyl-1-((4-(tetrahydro-2*H*-pyran-4-yl)piperidin-1-yl)sulfonyl)-1*H*-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A78**).

Route 25



[00174] To 9-(cyclohexylmethyl)-1-((4-(dimethylamino)phenyl)sulfonyl)-5-((4-phenylpiperidin-1-yl)sulfonyl)-1,5,9-triazacyclododecan-3-one (19 mg, 0.028 mmol) in DCM (0.5 mL) and methanol (0.05 mL) was added hydroxylamine hydrochloride (5.8 mg, 0.083 mmol) and sodium acetate (6.8 mg, 0.083 mmol). The mixture was stirred for 6 h at ambient temperature then concentrated and purified by flash column chromatography (0-60% hexanes (1% DEA)/ethyl acetate) to provide 9-(cyclohexylmethyl)-1-((4-(dimethylamino)phenyl)sulfonyl)-5-((4-phenylpiperidin-1-yl)sulfonyl)-1,5,9-triazacyclododecan-3-one oxime (compound **A75**). MS(EI) for $C_{35}H_{54}N_6O_5S_2$, found 703 $[M+H]^+$.

Route 26



[00175] A solution of 1-chloro-2-iodobenzene (5.0 g, 21.1 mmol) in DMF (150 mL) was degassed and then filled with argon. *tert*-Butyl 4-(4,4,5,5-tetramethyl-1,3-dioxolan-2-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate (7.13 g, 23.0 mmol), Pd(dppf)Cl₂ (1.7 g, 2.1 mmol) and potassium carbonate (8.69 g, 63.0 mmol) were added. The reaction mixture was stirred

overnight at 110°C and then cooled to ambient temperature. The mixture was poured into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Purification by flash column chromatography on silica gel (ethyl acetate/hexanes = 1:9 to 1:4) provided *tert*-Butyl 4-(2-chlorophenyl)-5,6-dihydropyridine-1(2*H*)-carboxylate. ¹H NMR (400 MHz, CDCl₃): δ 7.37 (m, 1H), 7.18 (m, 3H), 5.67 (m, 1H), 4.06 (m, 2H), 3.64 (m, 2H), 2.46 (br s, 2H), 1.51 (s, 9H).

[00176] To a solution of *tert*-Butyl 4-(2-chlorophenyl)-5,6-dihydropyridine-1(2*H*)-carboxylate (2.0 g, 6.8 mmol) in MeOH (20 mL) was added HCl/MeOH (3*M*, 20 mL). The mixture was stirred at ambient temperature for 2 h. The resulting solution of 4-(2-chlorophenyl)-1,2,3,6-tetrahydropyridine hydrochloride was used directly in the next step.

[00177] To the solution of 4-(2-chlorophenyl)piperidine hydrochloride in MeOH/HCl was added PtO₂ (0.1 g). The mixture was hydrogenated at ambient temperature under 15psi for 3 h. The catalyst was filtered off and the filtrate was concentrated to provide the product. ¹H NMR (400 MHz, DMSO-*d*₆): δ 9.19 (br s, 2H), 7.37 (m, 1H), 7.36 (m, 4H), 3.44 (m, 1H), 3.33 (m, 2H), 3.08 (m, 2H), 1.95 (m, 4H).

[00178] The free base (0.95 g, 4.8 mmol) and 1-(1*H*-imidazol-1-ylsulfonyl)-3-methyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (1.76 g, 4.8 mmol) were mixed in CH₃CN (10 mL). The mixture was stirred at ambient temperature overnight. The solvent was removed, and the residue was purified by column chromatography on silica gel to provide 1-(1*H*-imidazol-1-ylsulfonyl)-4-(2-chlorophenyl)piperidine. ¹H NMR (400 MHz, CDCl₃): δ 8.01 (s, 1H), 7.36 (m, 2H), 7.18 (m, 4H), 4.07 (m, 2H), 3.06 (m, 1H), 2.74 (m, 2H), 1.99 (m, 2H), 1.81 (m, 2H).

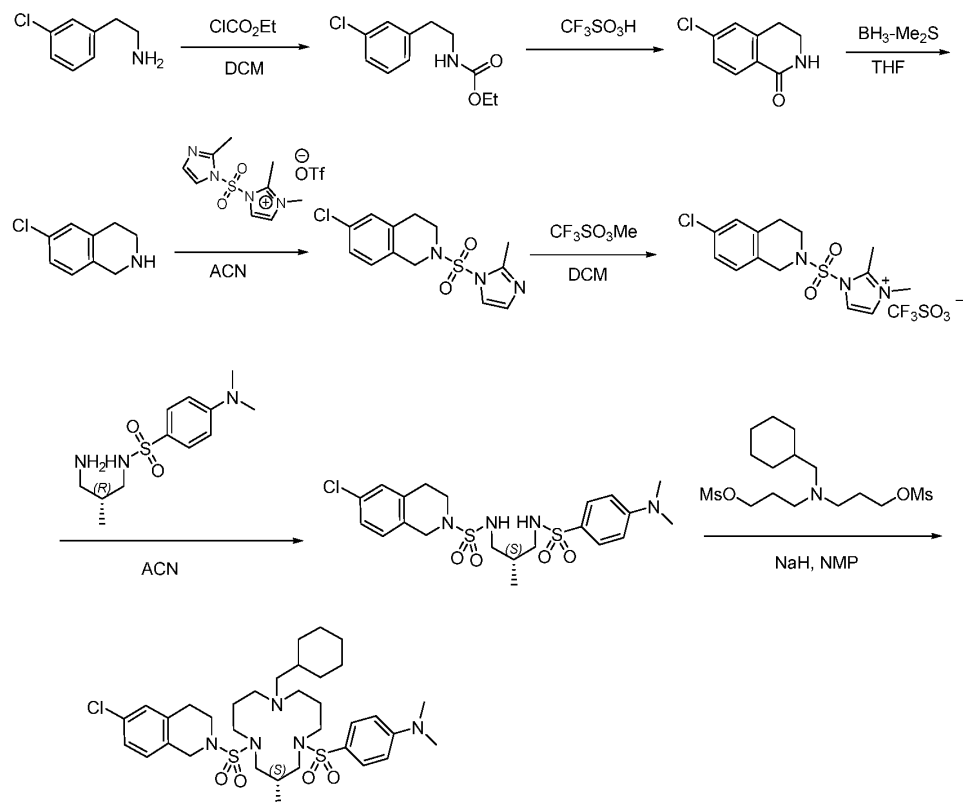
[00179] To a solution of 1-(1*H*-imidazol-1-ylsulfonyl)-4-(2-chlorophenyl)piperidine (501 mg, 1.5 mmol) in DCM (5 mL) was added MeOTf (0.27 g, 1.63 mmol). The mixture was stirred at ambient temperature overnight. The solvent was removed. The resulting product was washed with ether to provide 1-((4-(2-chlorophenyl)piperidin-1-yl)sulfonyl)-3-methyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A57**). ¹H NMR (400 MHz, CD₃OD): δ 9.65 (s, 1H), 8.08 (s, 1H), 7.82 (s, 1H), 7.39 (m, 4H), 4.16 (m, 2H), 4.02 (s, 3H), 3.31 (m, 3H), 2.03 (m, 2H), 1.85 (m, 2H).

[00180] The following compounds were synthesized in a similar manner:

1-((4-(3-chlorophenyl)piperidin-1-yl)sulfonyl)-3-methyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A58**).

1-((4-(4-chlorophenyl)piperidin-1-yl)sulfonyl)-3-methyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (Intermediate for compound **A72**).

Route 27



[00181] To a solution of 2-(3-chlorophenyl)ethanamine (149 g, 0.95 mol) and TEA (144 g, 1.42 mol) in DCM (1.5 L) was added dropwise ethyl chloroformate (107 g, 1.14 mol) at 0°C. The reaction mixture was stirred for 3 h and then washed with 1N aqueous HCl (1 L) and saturated aqueous NaHCO₃ (600 mL). The DCM layer was dried over anhydrous sodium sulfate and concentrated to provide methyl 3-chlorophenethylcarbamate. ¹H NMR (400 MHz, CDCl₃): δ 7.27 (m, 3H), 7.08 (m, 1H), 4.77 (m, 1H), 3.78 (s, 3H), 3.44 (m, 2H), 2.80 (m, 2H).

[00182] Methyl 3-chlorophenethylcarbamate (193 g, 0.9 mol) was dissolved in CF₃SO₃H (1.36 kg). The mixture was heated at 120 °C overnight. The mixture was cooled to ambient temperature and then poured into ice-water (4 L). The resulting product was collected by filtration and washed with ether to provide 6-chloro-3,4-dihydroisoquinolin-1(2H)-one. ¹H NMR (400 MHz, CDCl₃): δ 8.01 (m, 1H), 7.35 (m, 1H), 7.25 (m, 2H), 6.21 (br s, 1H), 3.60 (m, 2H), 3.01 (m, 2H).

[00183] To a solution of 6-chloro-3,4-dihydroisoquinolin-1(2H)-one (33 g, 182 mmol) in THF (330 mL) was added dropwise BH₃-Me₂S (73 mL, 729 mmol). The reaction mixture was refluxed overnight. The mixture was cooled to ambient temperature and then quenched with

6*N* aqueous HCl (300 mL). THF was removed under reduced pressure and the remaining solution was refluxed overnight. The mixture was concentrated to a certain volume and then basified with 2*N* aqueous NaOH. The resulting mixture was extracted with DCM. The DCM layer was dried over anhydrous sodium sulfate and concentrated to provide 6-chloro-1,2,3,4-tetrahydroisoquinoline.

[00184] To a solution of 6-chloro-1,2,3,4-tetrahydroisoquinoline (18.5 g, 0.11 mol) in CH₃CN (180 mL) was added compound 2,3-dimethyl-1-((2-methyl-1*H*-imidazol-1-yl)sulfonyl)-1*H*-imidazol-3-ium trifluoromethanesulfonate (43.5 g, 0.11 mol, Reference: *J. Org. Chem.* **2002**, *68*, 115-119.). The reaction mixture was stirred overnight at 30 °C. The solvent was removed and the residue was purified by column chromatography on silica gel (EtOAc/Petroleum ether = 1:1) to provide 6-chloro-2-(2-methyl-1*H*-imidazol-1-ylsulfonyl)-1,2,3,4-tetrahydroisoquinoline. ¹H NMR (400 MHz, CDCl₃): δ 7.21 (m, 1H), 7.18 (s, 1H), 7.02 (m, 1H), 6.94 (m, 1H), 4.45 (s, 2H), 3.62 (m, 2H), 2.92 (m, 2H), 2.67 (s, 3H).

[00185] To a solution of 6-chloro-2-(2-methyl-1*H*-imidazol-1-ylsulfonyl)-1,2,3,4-tetrahydroisoquinoline (25.5 g, 82 mmol) in DCM (260 mL) was added CF₃SO₃Me (13.45 g, 82 mmol). The reaction mixture was stirred at ambient temperature overnight. The solvent was removed and the residue was washed with ether to provide 1-(6-chloro-3,4-dihydroisoquinolin-2(1*H*)-ylsulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate.

[00186] To a solution of 1-(6-Chloro-3,4-dihydroisoquinolin-2(1*H*)-ylsulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (8.7 g, 18.4 mmol) in CH₃CN (100 mL) was added (R)-*N*-(3-amino-2-methylpropyl)-4-(dimethylamino)benzenesulfonamide (5.0 g, 18.4 mmol). The reaction mixture was stirred overnight at 30 °C. The solvent was removed and the residue was purified by column chromatography on silica gel (EtOAc/Petroleum ether = 1:3 to 1:1) to provide (S)-6-chloro-*N*-(3-((4-(dimethylamino)phenyl)sulfonamido)-2-methylpropyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (compound **B10**). ¹H NMR (400 MHz, CDCl₃): δ 7.85 (m, 2H), 7.43 (m, 2H), 7.18 (m, 2H), 7.03 (m, 1H), 4.35 (s, 2H), 3.48 (m, 2H), 3.18 (s, 6H), 3.15 (m, 1H), 2.95 (m, 6H), 2.85 (m, 1H), 1.89 (m, 1H), 0.89 (m, 3H).

[00187] (S)-6-Chloro-*N*-(3-((4-(dimethylamino)phenyl)sulfonamido)-2-methylpropyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (300 mg, 0.6 mmol) was dissolved in NMP (3 mL) and NaH (60%, 96 mg, 2.4 mmol) was added. The mixture was stirred for 30 min at ambient temperature and then heated at 80 °C for 20 min. The mixture was cooled to ambient temperature. A solution of ((cyclohexylmethyl)azanediyl)bis(propane-3,1-diyl)dimethanesulfonate (231 mg, 0.6 mmol) in NMP (1 mL) was added. The reaction mixture was heated at 80 °C for 1 h then cooled to ambient temperature and quenched with water

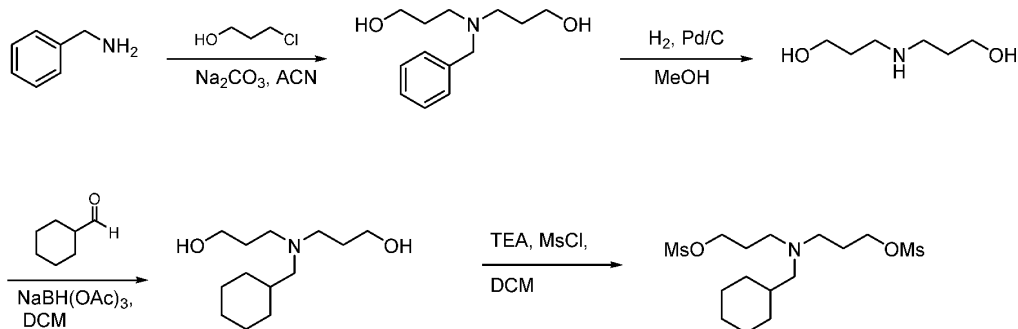
(10 mL). The resulting mixture was extracted with ethyl acetate (15 mL). The extract was washed with brine and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/petroleum ether = 1:8 to 1:3) to provide compound **A87**. MS(EI) for $C_{34}H_{52}ClN_5O_4S_2$, found 695 $[M+H]^+$. 1H NMR (400 MHz, $CDCl_3$): δ 7.59 (d, J = 8.8 Hz, 2H), 7.15 (m, 2H), 7.00 (m, 1H), 6.68 (d, J = 8.8 Hz, 2H), 4.34 (m, 2H), 3.71 (m, 2H), 3.45 (m, 2H), 3.17 (m, 3H), 3.05 (s, 6H), 2.88 (m, 4H), 2.25 (m, 2H), 2.15 (m, 6H), 1.95 (m, 2H), 1.65 (m, 8H), 1.23 (m, 6H), 0.95 (m, 5H).

[00188] The following compounds were synthesized in a similar manner:

No.	IUPAC	$[M+H]^+$	1H NMR
B11	N-[(2R)-3-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]amino}-2-methylpropyl]-4-(dimethylamino)benzene-1-sulfonamide	501	
A90	4-[[[(3S)-9-(cyclohexylmethyl)-3-methyl-5-[[[(3S)-3-phenylpyrrolidin-1-yl]sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	674	
A91	4-[[[(3S)-9-(cyclohexylmethyl)-3-methyl-5-[[[(3R)-3-phenylpyrrolidin-1-yl]sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	674	
A92	4-[[[(3S)-9-(cyclohexylmethyl)-3-methyl-5-[(4-phenoxy piperidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	704	
A93	4-[[[(3S)-9-(cyclohexylmethyl)-3-methyl-5-[[4-(pyrimidin-2-yl)piperazin-1-yl]sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	691	
A94	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[4-(2-fluorophenyl)piperazin-1-yl]sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	707	
A95	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[4-(3-fluorophenyl)piperazin-1-yl]sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	707	
A96	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[4-(4-fluorophenyl)piperazin-1-yl]sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	707	

A97	4-[[[(3S)-9-(cyclohexylmethyl)-3-methyl-5-[[4-(2-methylphenyl)piperazin-1-yl]sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	703	
A98	4-[[[(3S)-9-(cyclohexylmethyl)-3-methyl-5-[[4-(3-methylphenyl)piperazin-1-yl]sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	703	
A99	4-[[[(3S)-9-(cyclohexylmethyl)-3-methyl-5-[[4-(4-methylphenyl)piperazin-1-yl]sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	703	
A100	4-[[[(3S)-5-[(4-benzoylpiperazin-1-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	717	
A101	4-[[[(3S)-9-(cyclohexylmethyl)-3-methyl-5-[(3-phenylazetidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	660	
A102	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[4-(4-fluoro-2-methylphenyl)piperazin-1-yl]sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	721	
A76	4-[[[(3R)-9-(cyclohexylmethyl)-3-methyl-5-[(4-phenylpiperidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	688	¹ H NMR (400 MHz, CDCl ₃): δ 7.60 (d, <i>J</i> = 8.8 Hz, 2H), 7.33 (m, 5H), 6.82 (d, <i>J</i> = 8.8 Hz, 2H), 3.77 (m, 4H), 3.28 (m, 1H), 3.18 (m, 3H), 3.05 (s, 6H), 2.88 (m, 4H), 2.61 (m, 1H), 2.41 (m, 1H), 2.21 (m, 4H), 2.01 (m, 3H), 1.85 (m, 8H), 1.25 (m, 6H), 0.93 (m, 3H), 0.82 (m, 4H).
A77	4-[[[(3S)-9-(cyclohexylmethyl)-3-methyl-5-[(4-phenylpiperidin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	688	¹ H NMR (400 MHz, CDCl ₃): δ 7.60 (d, <i>J</i> = 8.8 Hz, 2H), 7.33 (m, 5H), 6.82 (d, <i>J</i> = 8.8 Hz, 2H), 3.77 (m, 4H), 3.28 (m, 1H), 3.18 (m, 3H), 3.05 (s, 6H), 2.88 (m, 4H), 2.61 (m, 1H), 2.41 (m, 1H), 2.21 (m, 4H), 2.01 (m, 3H), 1.85 (m, 8H), 1.25 (m,

			6H), 0.93 (m, 3H), 0.82 (m, 4H).
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Route 28

[00189] To a solution of benzylamine (37.7 g, 0.350 mol) in CH_3CN (1 L) were added 3-chloropropan-1-ol (100 g, 1.06 mol) and Na_2CO_3 (131 g, 1.24 mol). The reaction mixture was refluxed for 50 h and then cooled to ambient temperature. The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (MeOH/DCM = 1:99 to 3:97) to provide 3,3'-(benzylazanediyldipropyl-1-ol). ^1H NMR (400 MHz, CDCl_3): δ 7.34 (m, 5H), 3.70 (m, 4H), 3.61 (s, 2H), 3.48 (s, 2H), 2.68 (m, 4H), 1.78 (m, 4H).

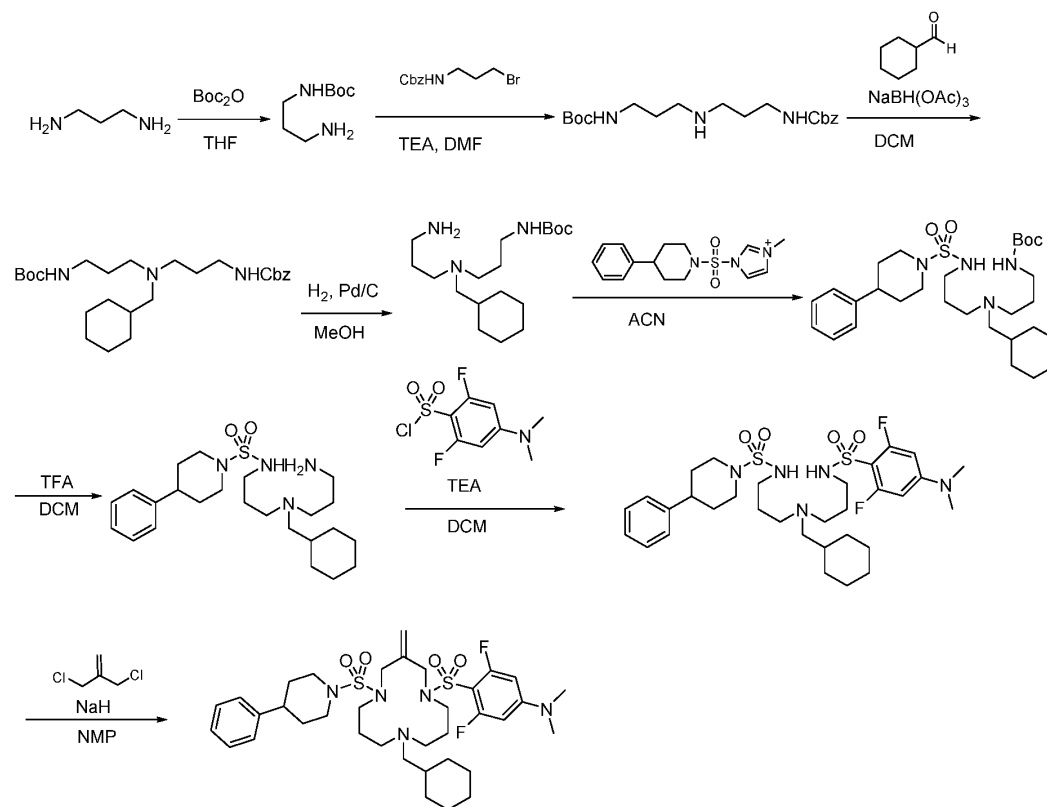
[00190] To a solution of 3,3'-(benzylazanediyldipropyl-1-ol (55 g) in MeOH (500 mL) was added Pd/C (10 g). The mixture was hydrogenated at ambient temperature under 15 kg pressure for 6 h. The mixture was filtered and the filtrate was concentrated to provide 3,3'-azanediyldipropyl-1-ol. ^1H NMR (400 MHz, CDCl_3): δ 3.78 (m, 4H), 3.46 (s, 2H), 2.84 (m, 4H), 1.75 (m, 4H).

[00191] To a solution of 3,3'-azanediyldipropyl-1-ol (15 g, 113 mmol) in DCM (300 mL) were added cyclohexanecarbaldehyde (18.8 g, 141 mmol) and $\text{NaBH}(\text{OAc})_3$ (72.5 g, 0.34 mol). The mixture was stirred for 0.5 h and HOAc (20.4 g, 0.34 mol) was added. The reaction mixture was stirred at ambient temperature overnight. The mixture was quenched with water (150 mL) and then adjusted to pH=12 with NaOH. The organic layer was separated, dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography on silica gel (MeOH/DCM = 3:97 to 1:9) to provide 3,3'-((cyclohexylmethyl)azanediyldipropyl-1-ol). ^1H NMR (400 MHz, CDCl_3): δ 3.75 (m, 4H), 2.64 (m, 4H), 2.18 (m, 2H), 1.75 (m, 9H), 1.55 (m, 1H), 1.21 (m, 3H), 0.89 (m, 1H).

[00192] To a solution of compound 3,3'-((cyclohexylmethyl)azanediyldipropyl-1-ol) (1.2 g, 5.2 mmol) and TEA (1.0 g, 10.4 mmol) in DCM (15 mL) was added MsCl (1.18 g, 10.4 mmol) at 0 °C. The reaction mixture was stirred for 4 h and then quenched with water (15

mL). The DCM layer was separated, dried over anhydrous sodium sulfate and concentrated to provide compound ((cyclohexylmethyl)azanediyl)bis(propane-3,1-diyl) dimethanesulfonate. The dimesylate was used immediately without further purification.

Route 29



[00193] To a solution of propane-1,3-diamine (100 g, 1.35 mol) in THF (800 mL) was added a solution of Boc_2O (73.6 g, 0.34 mol) in THF (200 mL) at 0~10 °C. The reaction mixture was stirred for 3 h and water (1 L) was added. The resulting mixture was extracted with ethyl acetate (500 mL x 2). The organic layers were combined and concentrated to a volume of 400 mL. Hexane (300 mL) was added and to the resulting solution was added 15% aqueous oxalic acid (1 L). The mixture was stirred for 0.5 h. The organic layer was decanted and the aqueous layer was adjusted to pH=10 with 3N aqueous NaOH. The resulting mixture was extracted with DCM (600 mL x 2). The combined extracts were dried over anhydrous sodium sulfate and concentrated to provide *tert*-Butyl 3-aminopropylcarbamate. ^1H NMR (400 MHz, CDCl_3): δ 5.03 (br s, 1H), 3.23 (br s, 2H), 2.69 (m, 2H), 1.71 (m, 2H), 1.44 (s, 9H).

[00194] Triethylamine (4.70 g, 46.5 mmol) was added dropwise to a stirred mixture of benzyl 3-bromopropylcarbamate (4.2 g, 15.5 mmol) to provide *tert*-Butyl 3-

aminopropylcarbamate (2.7 g, 15.5 mmol) in DMF (50 mL) at ambient temperature. The reaction mixture was heated at 70 °C for 1 h. The majority of DMF was removed under vacuum and the remaining mixture was diluted with water (120 mL) and washed with ether (150 mL×4) to remove most of the dialkylated by-product. The aqueous layer was adjusted to pH=11 with 1 *N* aqueous NaOH and extracted with ether (200 mL). The extract was washed with water (150 mL×3) to remove unreacted *tert*-Butyl 3-aminopropylcarbamate, dried with anhydrous sodium sulfate and concentrated to provide *tert*-butyl 3-((3-(((benzyloxy)carbonyl)amino)propyl)amino)propyl)carbamate. ¹H NMR (400 MHz, CDCl₃): δ 7.36 (m, 5H), 5.60 (br s, 1H), 5.11 (m, 3H), 3.30 (m, 2H), 3.20 (m, 2H), 2.68 (m, 4H), 1.70 (m, 5H), 1.44 (s, 9H).

[00195] To a solution of *tert*-butyl 3-((3-(((benzyloxy)carbonyl)amino)propyl)amino)propyl)carbamate (100 mg, 0.27 mmol) in DCM (5 mL) was added cyclohexanecarbaldehyde (31 mg, 0.27 mmol) and HOAc (30 mg, 1.1 mmol). The mixture was stirred for 0.5 h and NaBH(OAc)₃ (235 g, 1.1 mmol) was added in one portion. The reaction mixture was stirred for 4 h. TLC analysis showed 50% conversion and another portion of cyclohexane carbaldehyde (15 mg, 0.13 mmol) was added. The reaction mixture was stirred overnight at ambient temperature. Water (10 mL) was added to quench the reaction and the mixture was adjusted to pH=2 by addition of HOAc. The mixture was stirred for another 0.5 h and then adjusted to pH=12 with aqueous NaOH (1 *M*). The organic layer was separated and concentrated to provide *tert*-butyl 3-((3-(((benzyloxy)carbonyl)amino)propyl)(cyclohexylmethyl)amino)propyl)carbamate. ¹H NMR (400 MHz, CDCl₃): δ 7.27 (m, 5H), 6.12 (br s, 1H), 5.55 (br s, 1H), 5.01 (s, 3H), 3.34 (m, 2H), 3.18 (m, 2H), 3.08 (m, 2H), 2.80 (m, 4H), 1.65 (m, 9H), 1.44 (s, 9H), 1.12 (m, 6H).

[00196] To a solution of *tert*-butyl 3-((3-(((benzyloxy)carbonyl)amino)propyl)(cyclohexylmethyl)amino)propyl)carbamate (0.50 g, 1.1 mmol) in MeOH (5 mL) was added Pd/C (0.1 g). The mixture was hydrogenated under 15 psi pressure for 3 h. The mixture was filtered and the filtrate was concentrated to provide *tert*-butyl 3-((3-aminopropyl)(cyclohexylmethyl)amino)propyl)carbamate. ¹H NMR (400 MHz, CDCl₃): δ 5.78 (br s, 1H), 3.19 (br s, 2H), 2.78 (m, 2H), 2.40 (m, 4H), 2.13 (m, 2H), 1.98 (br s, 2H), 1.67 (m, 9H), 1.44 (s, 9 H), 1.12 (m, 4H), 0.89 (m, 2H).

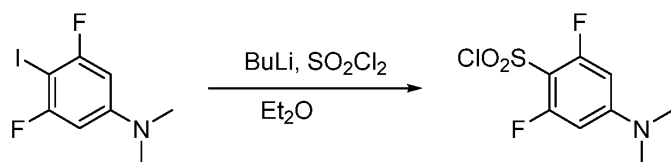
[00197] To a solution of *tert*-butyl 3-((3-aminopropyl)(cyclohexylmethyl)amino)propyl)carbamate (1.2 g, 3.7 mmol) in CH₃CN (10 mL) was added 3-methyl-1-(4-phenylpiperidin-1-ylsulfonyl)-1 *H*-imidazol-3-ium (1.47 g, 3.7 mmol). The reaction mixture was stirred at ambient temperature overnight. The solvent was removed and the residue was purified by column chromatography on silica gel to provide *tert*-butyl 3-((cyclohexylmethyl)(3-(4-phenylpiperidine-1-sulfonamido)propyl) amino)propyl)carbamate.

[00198] To a solution of *tert*-butyl 3-((cyclohexylmethyl)(3-(4-phenylpiperidine-1-sulfonamido)propyl) amino)propylcarbamate (0.85 g, 1.5 mmol) in DCM (15 mL) was added TFA (10 mL). The reaction mixture was stirred at ambient temperature for 3 h. The solvent was removed and the residue was dissolved in DCM (50 mL). The solution was basified with 1 *N* aqueous NaOH. The DCM layer was dried over anhydrous Na₂SO₄ and concentrated to provide *N*-(3-((3-aminopropyl)(cyclohexylmethyl)amino)propyl)-4-phenylpiperidine-1-sulfonamide.

[00199] To a solution of *N*-(3-((3-aminopropyl)(cyclohexylmethyl)amino)propyl)-4-phenylpiperidine-1-sulfonamide (90 mg, 0.20 mmol) in DCM (5 mL) was added TEA (40 mg, 0.4 mmol). The mixture was cooled 0 °C and a solution of 4-(dimethylamino)-2,6-difluorobenzene-1-sulfonyl chloride (60 mg, 0.24 mmol) in DCM (1 mL) was added. The reaction mixture was stirred at ambient temperature for 2 h. The mixture was diluted with DCM (30 mL) and then washed with water (20 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated. The residue was purified by column chromatography on silica gel to provide *N*-(3-((cyclohexylmethyl)(3-(4-(dimethylamino)phenylsulfonamido)propyl) amino)propyl)-4-phenylpiperidine-1-sulfonamide.

[00200] To a solution of *N*-(3-((cyclohexylmethyl)(3-(4-(dimethylamino)phenylsulfonamido)propyl) amino)propyl)-4-phenylpiperidine-1-sulfonamide (80 mg, 0.123 mmol) in NMP (2 mL) was added NaH (12 mg, 0.29 mmol). The mixture was heated at 80 °C for 0.5 h. The mixture was cooled to ambient temperature and a solution of 3-chloro-2-(chloromethyl)prop-1-ene (15 mg, 0.12 mmol) in NMP (0.1 mL) was added. The reaction mixture was heated at 80 °C for 2 h. The mixture was cooled to ambient temperature and water (20 mL) was added. The resulting mixture was extracted with ethyl acetate (15 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane = 10:30 to 20:10) to provide compound **A86**. MS(EI) for C₃₆H₅₃F₂N₅O₄S₂, found 733 [M+H]⁺. ¹H NMR (400 MHz, CDCl₃): δ 7.31 (m, 5H), 6.18 (m, 2H), 5.25 (m, 2H), 4.05 (s, 2H), 3.87 (m, 4H), 3.20 (m, 4H), 3.05 (s, 6H), 2.88 (m, 2H), 2.41 (m, 4H), 2.05 (m, 5H), 1.75 (m, 9H), 1.27 (m, 6H), 0.85 (m, 2H).

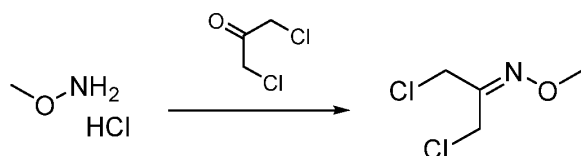
Route 30



[00201] To a solution of 3,5-difluoro-4-iodo-*N,N*-dimethylaniline (500 mg, 1.77 mmol) in

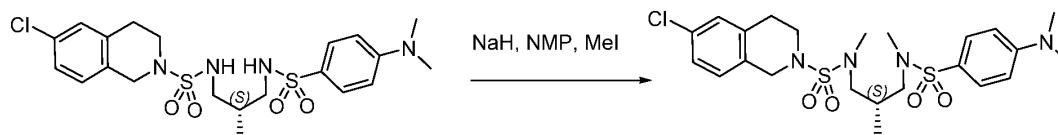
ether (5 mL) was added dropwise *n*-BuLi (0.71 mL, 1.77 mmol) at -78 °C. The mixture was stirred for 0.5 h at -78 °C. Sulfuryl dichloride (358 mg, 1.5 mmol) was added. The reaction mixture was stirred for another 0.5 h. The mixture was quenched with water (10 mL) and then extracted with ethyl acetate (15 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated. The residue was purified by column chromatography on silica gel to provide the crude product, which was further recrystallized from hexane/EtOAc (10:1) to provide 4-(dimethylamino)-2,6-difluorobenzene-1-sulfonyl chloride. ¹H NMR (400 MHz, CDCl₃): δ 6.21 (s, 1H), 6.18 (s, 1H), 3.09 (s, 6H).

Route 31



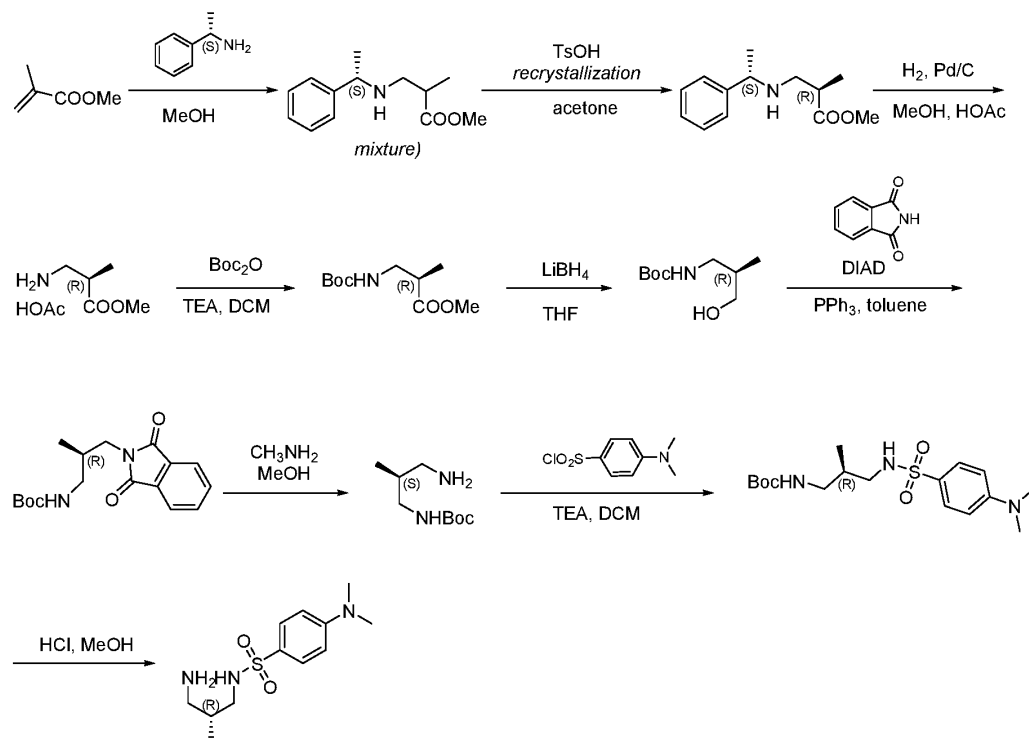
[00202] To a suspension of 1, 3-dichloropropan-2-one (5.0 g, 39.5 mmol) in H₂O (50 mL) was added *O*-methylhydroxylamine hydrochloride (3.5 g, 41.5 mmol) and the reaction mixture was stirred at ambient temperature for 1 h. The organic layer was separated, diluted with ether and concentrated under vacuum (<30 °C) to provide 1,3-dichloropropan-2-one *O*-methyl oxime. ¹H NMR (400 MHz, CDCl₃): δ 4.33 (s, 2H), 4.27 (s, 2H), 3.94 (s, 3H).

Route 32



[00203] To (S)-6-chloro-N-(3-((4-(dimethylamino)phenyl)sulfonamido)-2-methylpropyl)-3,4-dihydroisoquinoline-2(1H)-sulfonamide (100.0 mg, 0.200 mmol) in dry NMP (3 mL) at 0 °C was added NaH (24.0 mg, 0.600 mmol) and the mixture was stirred at ambient temp for 20 min and turned from a cloudy mixture to nearly transparent during this time. Iodomethane (27 μL, 0.440 mmol) was added and the mixture was allowed to stir at ambient temp for 1 h and did not progress past ~50% completion, so the same amount of NaH and methyl iodide was added again and the reaction was complete after an additional hour at ambient temp. Brine (10 mL) and EA (10 mL) was added. The aqueous layer was extracted with EA (1X5 mL) and the organics were combined, washed with brine (3X10 mL), dried with sodium sulfate, filtered and concentrated. FCC (hexanes/EA 0-70%) provided compound **B12**. MS(EI) for C₂₃H₃₃ClN₄O₄S₂, found 529 [M+H]⁺.

Route 33



[00204] To a solution of methyl methacrylate (300 g, 3.0 mol) in MeOH (1.5 L) was added (S)-1-phenylethanamine (363 g, 3 mol). The reaction mixture was refluxed for 5 days. The mixture was concentrated and the residue was purified by column chromatography on silica gel (EtOAc/Petroleum ether = 1:9 to 1:1) to provide methyl 2-methyl-3-((S)-1-phenylethylamino)propanoate. 1H NMR (400 MHz, $CDCl_3$): δ 7.25 (m, 5H), 3.75 (m, 1H), 3.69 (s, 3H), 2.75 (m, 3H), 1.39 (m, 1H), 1.28 (m, 3H), 1.09 (m, 3H).

[00205] To a solution of 2-methyl-3-((S)-1-phenylethylamino)propanoate (440 g, 1.99 mol) in acetone (6 L) was added TsOH- H_2O (378 g, 1.99 mol). The mixture was stirred at ambient temperature for 1 h. The precipitated solid was collected by filtration and dried to provide a salt (300 g) which was suspended in acetone (2 L) and refluxed for 1 h. The mixture was cooled to ambient temperature and the resulting precipitate was collected by filtration to provide a salt which was suspended in DCM (2 L) and saturated aqueous K_2CO_3 (1.5 L). The mixture was stirred for 0.5 h and the solid was dissolved. The DCM layer was separated, dried over anhydrous Na_2SO_4 and concentrated to provide (R)-methyl 2-methyl-3-((S)-1-phenylethylamino)propanoate.

[00206] To a solution of (R)-methyl 2-methyl-3-((S)-1-phenylethylamino)propanoate (140 g, 0.630 mol) in MeOH (1.4 L) were added HOAc (30 mL) and Pd/C (15 g). The mixture was hydrogenated under 20 kg pressure at 60 °C for 6 h. The catalyst was removed by filtration

and the filtrate was concentrated to provide (*R*)-methyl 3-amino-2-methylpropanoate. ¹H NMR (400 MHz, CDCl₃): δ 5.09 (br, 3H), 3.72 (s, 3H), 3.05 (m, 1H), 2.93 (m, 1H), 2.75 (m, 1H), 1.23 (d, *J* = 7.2 Hz, 3H).

[00207] To a solution of (*R*)-methyl 3-amino-2-methylpropanoate (112 g, 0.63 mol) in dichloromethane (1 L) were added TEA (160 g, 1.58 mol) and Boc₂O (152 g, 0.69 mol) at 0~5 °C. The reaction mixture was allowed to warm to ambient temperature and stirred overnight. The mixture was washed with water (1 L), 2*N* aqueous HCl (1 L) and saturated aqueous NaHCO₃ (1 L), respectively. The organic layer was dried over anhydrous Na₂SO₄ and concentrated. The residue was purified by column chromatography on silica gel to provide (*R*)-methyl 3-(*tert*-butoxycarbonylamino)-2-methylpropanoate. ¹H NMR (400 MHz, CDCl₃): δ 4.94 (br s, 1H), 3.70 (s, 3H), 3.31 (m, 2H), 2.70 (m, 1H), 1.52 (s, 9H), 1.18 (d, *J* = 7.2 Hz, 3H).

[00208] To a solution of (*R*)-methyl 3-(*tert*-butoxycarbonylamino)-2-methylpropanoate (6 g, 51.3 mmol) in THF (60 mL) was added LiBH₄ (1.12 g, 102 mmol). The reaction mixture was stirred overnight at ambient temperature. The mixture was quenched with water (30 mL) followed by addition of 1*N* aqueous HCl to adjust pH=2~3. The resulting mixture was extracted with ethyl acetate and the extract was washed with saturated aqueous NaHCO₃, dried over anhydrous Na₂SO₄ and concentrated to provide (*R*)-*tert*-Butyl 3-hydroxy-2-methylpropylcarbamate. ¹H NMR (400 MHz, CDCl₃): δ 3.57 (m, 1H), 3.37 (m, 2H), 3.05 (m, 1H), 1.77 (m, 1H), 1.48 (s, 9H), 0.88 (d, *J* = 7.2 Hz, 3H).

[00209] To a solution of (*R*)-*tert*-Butyl 3-hydroxy-2-methylpropylcarbamate (2.5 g, 13.2 mmol) in toluene (25 mL) were added PPh₃ (4.15 g, 15.8 mmol) and isoindoline-1,3-dione (2.72 g, 18.5 mmol). The mixture was cooled to 0~5°C and DIAD (3.2 g, 15.8 mmol) was added dropwise. The reaction mixture was stirred at ambient temperature for 3 h. The precipitated solid was filtered off and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (EtOAc/Petroleum ether = 1:9 to 1:1) to provide (*R*)-*tert*-Butyl 3-(1,3-dioxoisindolin-2-yl)-2-methylpropylcarbamate. ¹H NMR (400 MHz, CDCl₃): δ 7.85 (m, 2H), 7.47 (m, 2H), 5.23 (br s, 1H), 3.64 (m, 2H), 3.03 (br s, 2H), 2.13 (m, 1H), 1.43 (s, 9H), 0.96 (d, *J* = 6.4 Hz, 3H).

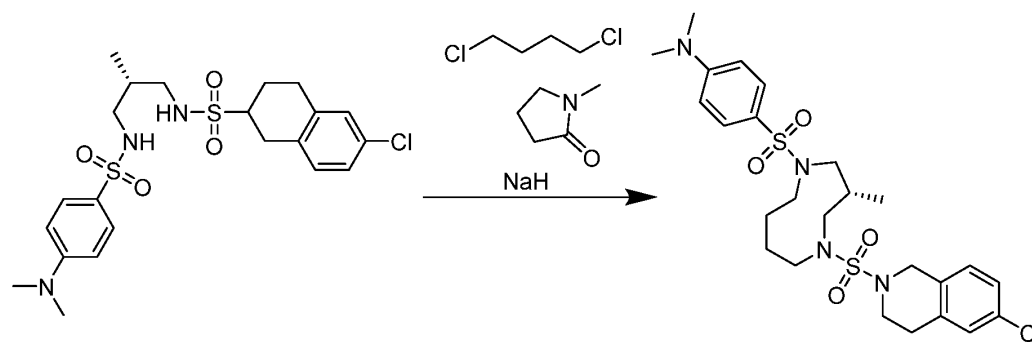
[00210] To a solution of (*R*)-*tert*-Butyl 3-(1,3-dioxoisindolin-2-yl)-2-methylpropylcarbamate (40 g, 8.83 mmol) in MeOH (200 mL) was added methylamine solution (7.8 g, 30% in MeOH). The reaction mixture was heated at 60 °C for 6 h. The solvent was removed under reduced pressure. The residue was suspended in ether (300 mL) and then stirred for 0.5 h. The solid was filtered off and the filtrate was concentrated under reduced pressure to provide (*S*)-*tert*-Butyl 3-amino-2-methylpropylcarbamate.

[00211] To a solution of (*S*)-*tert*-Butyl 3-amino-2-methylpropylcarbamate (12.5 g, 66.5 mmol) in DCM (250 mL) was added TEA (10.3 g, 101.7 mmol). The mixture was cooled to 0~5 °C and 4-(dimethylamino) benzene-1-sulfonyl chloride (16 g, 72 mmol) was added. The reaction mixture was stirred at ambient temperature for 3 h. The mixture was washed with water and the organic phase was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc/Petroleum ether = 1:9 to 3:7) to provide (*R*)-*tert*-Butyl 3-(4-(dimethylamino)phenylsulfonamido)-2-methylpropylcarbamate. ¹H NMR (400 MHz, CDCl₃): δ 7.70 (d, *J* = 8.8 Hz, 2H), 6.71 (d, *J* = 8.8 Hz, 2H), 5.45 (m, 1H), 4.72 (m, 1H), 3.18 (m, 1H), 3.04 (s, 6H), 2.98 (m, 2H), 2.75 (m, 1H), 1.78 (m, 1H), 1.41 (s, 9H), 0.87 (m, *J* = 6.8 Hz, 3H).

[00212] To a solution of (*R*)-*tert*-Butyl 3-(4-(dimethylamino)phenylsulfonamido)-2-methylpropylcarbamate (21 g, 59.3 mmol) in MeOH (150 mL) was added 4*N* HCl/MeOH (150 mL). The mixture was stirred for 3 h at ambient temperature. The solvent was removed under reduced pressure. The resulting solid was suspended in DCM (200 mL) and saturated aqueous K₂CO₃ (150 mL) was added. The mixture was stirred for 5 min and the organic layer was separated, dried over anhydrous Na₂SO₄, and concentrated to provide compound (*R*)-*N*-(3-amino-2-methylpropyl)-4-(dimethylamino)benzenesulfonamide. ¹H NMR (400 MHz, CDCl₃): δ 7.70 (d, *J* = 8.8 Hz, 2H), 6.68 (d, *J* = 8.8 Hz, 2H), 3.04 (s, 6H), 2.98 (m, 1H), 2.75 (m, 2H), 2.58 (m, 1H), 1.78 (m, 2H), 0.87 (m, *J* = 7.2 Hz, 3H).

[00213] Using a similar method (*S*)-*N*-(3-amino-2-methylpropyl)-4-(dimethylamino)benzenesulfonamide was synthesized (Intermediate for compound **A76**).

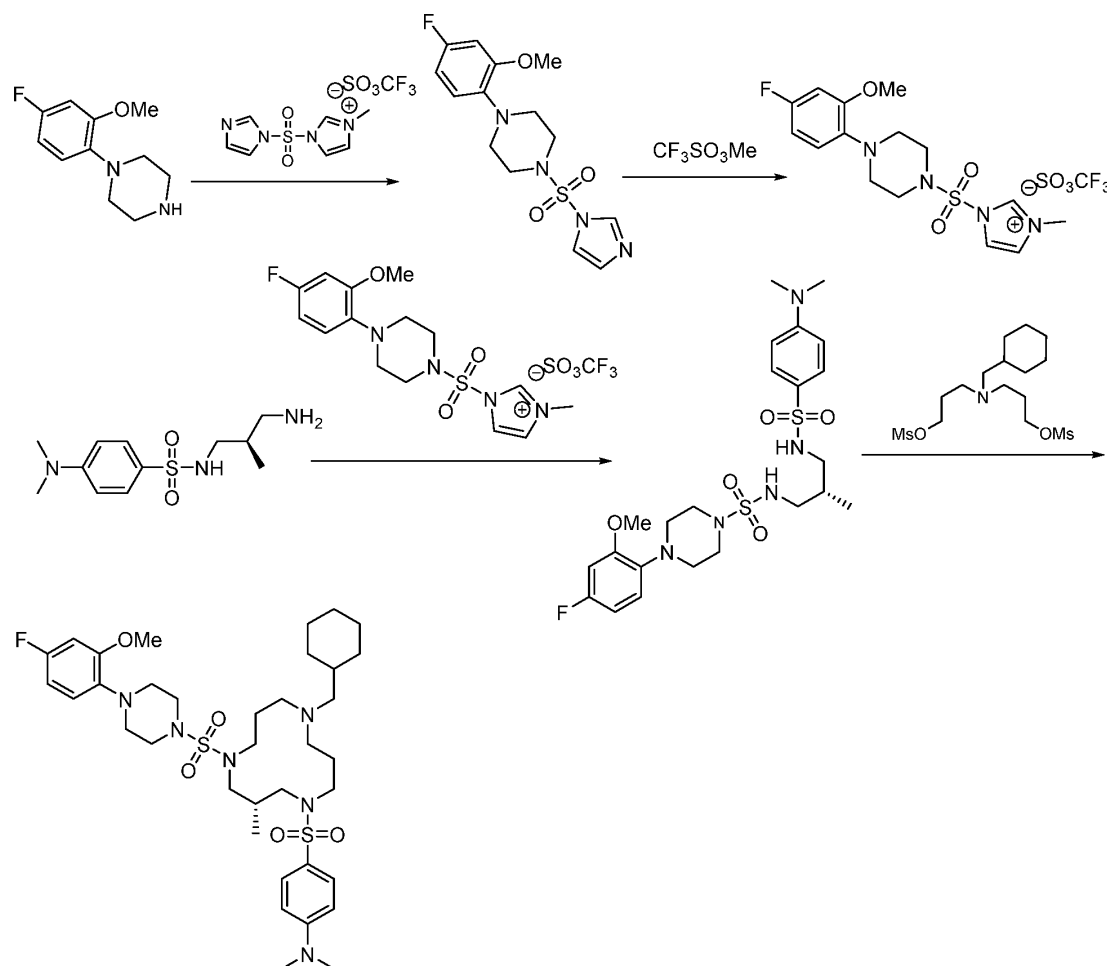
Route 34



[00214] To 6-chloro-N-((*R*)-3-((4-(dimethylamino)phenyl)sulfonamido)-2-methylpropyl)-1,2,3,4-tetrahydronaphthalene-2-sulfonamide (100 mg, 200 μM) in dry NMP (4 mL) at 0 °C was added NaH (24 mg, 600 μmol) and the mixture was stirred at ambient temp for 20 min over which time it turned from a cloudy mixture to nearly transparent. The dichloride (21.3 μL, 200 μmol) was added and the mixture was heated to 80 °C. After 40 min the reaction

was quenched with brine and the aqueous layer was extracted with ethyl acetate (2X), the combined organics were washed with brine (3X), dried with sodium sulfate, filtered, and concentrated. Column chromatography (0-50% hexanes + 1% diethylamine)/ethyl acetate provided the product (compound **B21**). MS(EI) for $C_{25}H_{35}ClN_4O_4S_2$, found 555 $[M+H]^+$.

Route 35



[00215] To a solution of 1-(4-fluoro-2-methoxyphenyl)piperazine (1.4 g, 6.8 mmol) in CH_3CN (15 mL) was added 1-(1*H*-imidazol-1-ylsulfonyl)-3-methyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (2.7 g, 6.8 mmol). The reaction mixture was stirred at ambient temperature overnight. The mixture was then concentrated and the residue was purified by column chromatography to provide 1-((1*H*-imidazol-1-yl)sulfonyl)-4-(4-fluoro-2-methoxyphenyl)piperazine.

[00216] To a solution of 1-((1*H*-imidazol-1-yl)sulfonyl)-4-(4-fluoro-2-methoxyphenyl)piperazine (0.92 g, 2.7 mmol) in DCM (10 mL) was added CF_3SO_3Me (0.45 g,

2.7 mmol) at 0 °C. The reaction mixture was stirred at ambient temperature for 5 h then concentrated and the resulting product was washed with ether to provide 1-((4-(4-fluoro-2-methoxyphenyl)piperazin-1-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate.

[00217] To a solution of (R)-N-(3-amino-2-methylpropyl)-4-(dimethylamino)benzenesulfonamide (0.6 g, 2.2 mmol) in CH₃CN (6 mL) was added 1-((4-(4-fluoro-2-methoxyphenyl)piperazin-1-yl)sulfonyl)-3-methyl-1H-imidazol-3-ium trifluoromethanesulfonate (1.1 g, 2.2 mmol). The reaction mixture was stirred at ambient temperature overnight. The mixture was concentrated and the residue was purified by column chromatography to provide (S)-N-(3-((4-(dimethylamino)phenyl)sulfonamido)-2-methylpropyl)-4-(4-fluoro-2-methoxyphenyl)piperazine-1-sulfonamide.

[00218] To a solution of (S)-N-(3-((4-(dimethylamino)phenyl)sulfonamido)-2-methylpropyl)-4-(4-fluoro-2-methoxyphenyl)piperazine-1-sulfonamide (771 mg, 1.42 mmol) in NMP (7 mL) was added NaH (140 mg, 3.48 mmol, 60%). The mixture was stirred for 0.5 h at 80 °C. The mixture was cooled to 70 °C and ((cyclohexylmethyl)azanediyl)bis(propane-3,1-diyl) dimethanesulfonate (820 mg, 2.1 mmol) was added. The reaction mixture was stirred for 2 h at 70 °C then cooled and poured into water. The resulting mixture was extracted with ethyl acetate. The extract was concentrated and the residue was purified by column chromatography (petroleum ether/EtOAc = 10:1) to provide compound **A137**. MS (EI) for C₃₆H₅₇FN₆O₅S₂, found 737 [M+H]⁺. ¹H NMR (400 MHz, CDCl₃): δ 7.62 (d, *J* = 8.8 Hz, 2H), 7.16 (m, 2H), 7.05 (m, 1H), 6.86 (d, *J* = 8.8 Hz, 2H), 4.35 (m, 2H), 3.62 (m, 4H), 3.19 (m, 2H), 3.04 (s, 6H), 2.87 (m 4H), 2.46 (m, 5H), 1.92 (m, 1H), 1.79 (m, 2H), 1.66 (m, 4H).

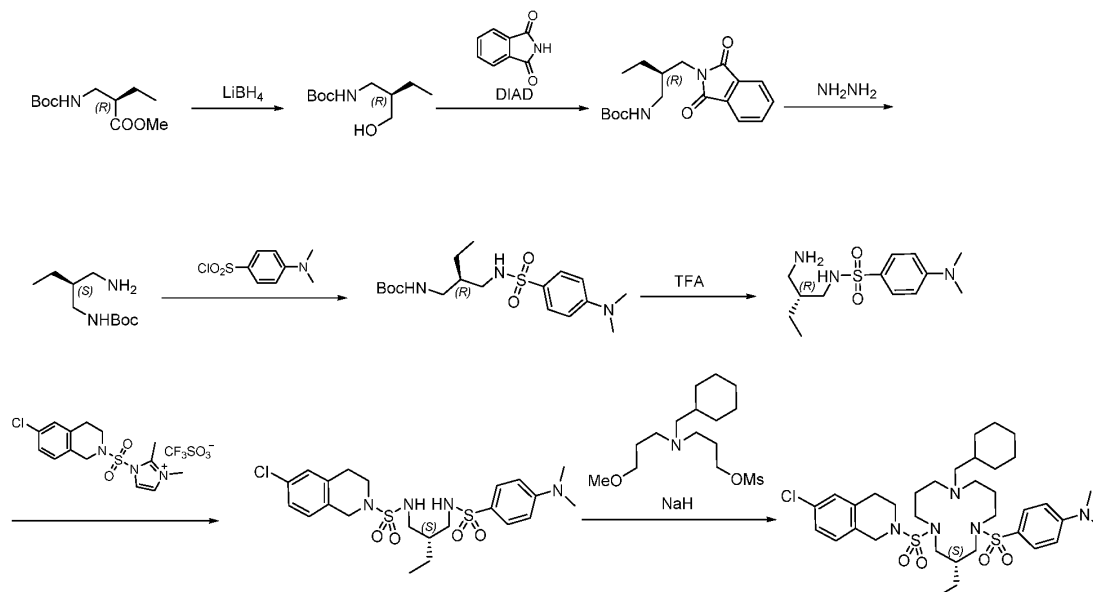
[00219] The following compounds were synthesized in a similar manner:

No.	IUPAC	[M+H] ⁺
A153	tert-butyl 4-(((3S)-9-(cyclohexylmethyl)-5-[4-(dimethylamino)benzenesulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl)piperazine-1-carboxylate	714
A118	2-(4-(((3S)-9-(cyclohexylmethyl)-5-[4-(dimethylamino)benzenesulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl)piperazin-1-yl)benzonitrile	715
A154	4-(((3S)-9-(cyclohexylmethyl)-5-[4-(2-methoxyphenyl)piperazin-1-yl)sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline	720
A155	4-(((3S)-9-(cyclohexylmethyl)-3-methyl-5-(piperazine-1-sulfonyl)-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline	613
A119	4-(((3S)-9-(cyclohexylmethyl)-5-[4-(2,6-dimethylphenyl)piperazin-1-yl)sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl)-N,N-dimethylaniline	717

A136	4-[[[(3S)-9-(cyclohexylmethyl)-5-[(6-ethyl-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	688
A161	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[6-(4-fluoro-2-methylphenyl)-2,6-diazaspiro[3.3]heptan-2-yl)sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	733
A162	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[[(2S)-4-(4-fluoro-2-methylphenyl)-2-methylpiperazin-1-yl)sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	735
A163	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[[(2R)-4-(4-fluoro-2-methylphenyl)-2-methylpiperazin-1-yl)sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	735
A166	4-[[[(3S)-9-(cyclohexylmethyl)-3-methyl-5-[(5-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	674
A138	4-[[[(3S)-9-(cyclohexylmethyl)-5-[(5-methoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	690
A151	2-[[[(3S)-9-(cyclohexylmethyl)-5-[4-(dimethylamino)benzenesulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl]-1,2,3,4-tetrahydroisoquinoline-5-carbonitrile	685
A170	4-[[[(3S)-9-(cyclohexylmethyl)-3-methyl-5-[(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	674
A172	4-[[[(3S)-9-(cyclohexylmethyl)-3-methyl-5-[(3-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	674
A173	4-[[[(3S)-9-(cyclohexylmethyl)-3-methyl-5-[(4-[2-(trifluoromethoxy)phenyl]piperazin-1-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	773
A174	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[4-(3,4-difluoro-2-methoxyphenyl)piperazin-1-yl)sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	755
A175	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[4-(3-fluoro-2-methoxyphenyl)piperazin-1-yl)sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	737
A176	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[4-(5-fluoro-2-methoxyphenyl)piperazin-1-yl)sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	737
A177	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[[(3R)-4-(4-fluoro-2-methoxyphenyl)-3-methylpiperazin-1-yl)sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	751
A178	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[3-(4-fluoro-2-methoxyphenyl)azetidin-1-yl)sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	708

A181	4-[[[(3S)-5-[(5-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	694
A187	(3S)-N-benzyl-9-(cyclohexylmethyl)-5-[4-(dimethylamino)benzenesulfonyl]-3-methyl-1,5,9-triazacyclododecane-1-sulfonamide	634
A198	4-[[[(3S)-5-[[4-(2-chlorophenyl)piperazin-1-yl]sulfonyl]-9-(cyclohexylmethyl)-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	723
A199	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[4-(4-methoxyphenyl)piperazin-1-yl]sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	719
A201	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[4-(3-methoxyphenyl)piperazin-1-yl]sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	719
A202	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[4-(3-fluoro-4-methoxyphenyl)piperazin-1-yl]sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	737
A206	4-[[[(3S)-5-[[4-(2-chloro-4-fluorophenyl)piperazin-1-yl]sulfonyl]-9-(cyclohexylmethyl)-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	741
A147	4-[[[(4S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-4-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	694
A148	4-[[[(4R)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-4-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	694
A150	4-[[[(2S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-2-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	694
A149	4-[[[(2R)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-2-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	694
A190	4-[[[(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-[(1S)-1-cyclohexylethyl]-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	708
A191	4-[[[(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-[(1R)-1-cyclohexylethyl]-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	708
A207	4-[[[(3S)-9-[(1S)-1-cyclohexylethyl]-5-[[4-(4-fluoro-2-methoxyphenyl)-3-methylpiperazin-1-yl]sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	765
A205	N,N-dimethyl-4-[[[(3S)-3-methyl-9-[(1S)-1-phenylethyl]-5-(1,2,3,4-tetrahydroisoquinoline-2-sulfonyl)-1,5,9-triazacyclododecan-1-yl]sulfonyl]aniline	668

Route 36



[00220] To a solution of (*R*)-methyl 2-((tert-butoxycarbonylamino)methyl)butanoate (4.8 g, 20.8 mmol) in THF (50 mL) was added LiBH_4 (0.7 g, 33 mmol). The reaction mixture was stirred overnight at ambient temperature. The mixture was quenched with water (30 mL) followed by addition of 1*N* aqueous HCl dropwise to adjust to pH=2~3. The resulting mixture was extracted with ethyl acetate and the extract was washed with saturated aqueous NaHCO_3 , dried over anhydrous sodium sulfate, and concentrated to provide (*R*)-*tert*-butyl 2-(hydroxymethyl)butylcarbamate.

[00221] To a solution of (*R*)-*tert*-butyl 2-(hydroxymethyl)butylcarbamate (3.4 g, 16.7 mmol) in toluene (40 mL) was added PPh_3 (6.2 g, 17.4 mmol) and isoindoline-1,3-dione (3.7 g, 25 mmol). The mixture was cooled to 0~5 °C and DIAD (4.3 g, 22 mmol) was added dropwise. The reaction mixture was stirred at ambient temperature for 3 h. The resulting precipitate was filtered and the filtrate was concentrated. The residue was purified by column chromatography (petroleum ether/EtOAc = 9:1 to 1:1) to provide 1-(4-fluoro-2-methoxyphenyl)-4-(2-methyl-1*H*-imidazol-1-ylsulfonyl)piperazine.

[00222] To a solution of 1-(4-fluoro-2-methoxyphenyl)-4-(2-methyl-1*H*-imidazol-1-ylsulfonyl)piperazine (3.8 g, 11.4 mmol) in EtOH (70 mL) was added hydrazine hydrate (1.1 g, 80%). The reaction mixture was refluxed for 6 h. The resulting product was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in 1*N* aqueous NaOH (20 mL) then extracted with DCM (50 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated to provide (*S*)-*tert*-butyl 2-

(aminomethyl)butylcarbamate.

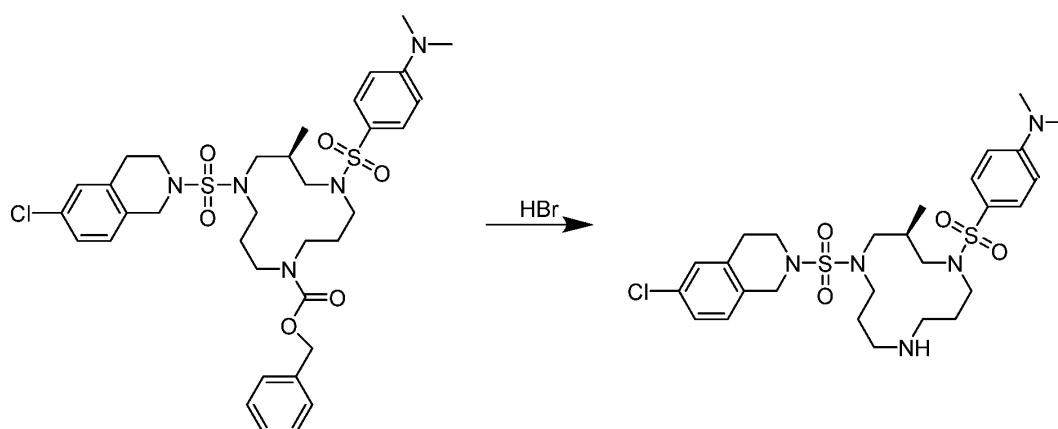
[00223] To a solution of (*S*)-*tert*-butyl 2-(aminomethyl)butylcarbamate (1.87 g, 9.2 mmol) in DCM (20 mL) was added TEA (1.01 g, 10.1 mol). The mixture was cooled to 0~5 °C and 4-(dimethylamino)benzene-1-sulfonyl chloride (2.0 g, 9.2 mmol) was added. The reaction mixture was stirred at ambient temperature for 3 h. The mixture was washed with water then concentrated. The residue was purified by column chromatography (petroleum ether/EtOAc = 9:1 to 7:3) to provide (*R*)-*tert*-butyl 2-((4-(dimethylamino)phenylsulfonamido)methyl)butylcarbamate.

[00224] To a solution of (*R*)-*tert*-butyl 2-((4-(dimethylamino)phenylsulfonamido)methyl)butylcarbamate (2.9 g, 7.5 mmol) in MeOH (10 mL) was added 4*N* HCl/MeOH (15 mL). The mixture was stirred for 3 h at ambient temperature. The solvent was removed under reduced pressure. The resulting product was suspended in DCM (100 mL) and saturated aqueous K₂CO₃ (450 mL) was added. The mixture was stirred for 5 min. The DCM layer was separated, dried over anhydrous sodium sulfate, and concentrated to provide (*R*)-*N*-(2-(aminomethyl)butyl)-4-(dimethylamino)benzenesulfonamide.

[00225] To a solution of (*R*)-*N*-(2-(aminomethyl)butyl)-4-(dimethylamino)benzenesulfonamide (0.9 g, 3.2 mmol) in CH₃CN (9 mL) was added 1-((6-chloro-3,4-dihydroisoquinolin-2(1*H*)-yl)sulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (0.59 g, 3.2 mmol). The reaction mixture was stirred overnight at ambient temperature. The mixture was concentrated and the residue was purified by column chromatography (petroleum ether/EtOAc = 8:1 to 2:1) to provide (*S*)-6-chloro-*N*-(2-((4-(dimethylamino)phenylsulfonamido)methyl)butyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide.

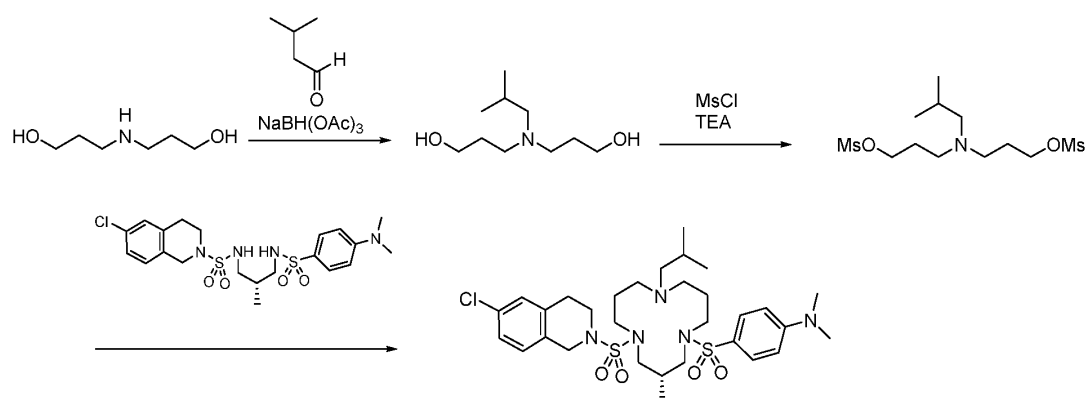
[00226] To a solution of (*S*)-6-chloro-*N*-(2-((4-(dimethylamino)phenylsulfonamido)methyl)butyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (0.8 g, 1.5 mmol) in NMP (3mL) was added NaH (159 mg, 3.9 mmol, 60%). The mixture was stirred for 0.5 h at 80 °C. The mixture was cooled to 70 °C and cyclopropane-1,1-diylbis(methylene) dimethanesulfonate (510 mg, 2.0 mmol) was added. The reaction mixture was stirred for 2 h at 70 °C. The solvent was removed and the residue was purified by column chromatography (petroleum ether/EtOAc = 15:1) to provide compound **A182**. LC-MS: *m/z* 708.2 [M+H]⁺. ¹H NMR (400 MHz, CDCl₃): δ 7.59 (m, 2H), 7.15 (m, 2H), 7.05 (m, 1H), 6.68 (m, 2H), 4.34 (m, 2H), 3.55 (m, 4H), 3.19 (m, 6H), 3.04 (s, 6H), 2.87 (m, 4H), 2.15 (m, 4H), 1.89 (m, 10H), 1.32 (m, 8H), 0.88 (m, 3H).

Route 37



[00227] To benzyl (S)-5-((6-chloro-3,4-dihydroisoquinolin-2(1H)-yl)sulfonyl)-9-((4-(dimethylamino)phenyl)sulfonyl)-7-methyl-1,5,9-triazacyclododecane-1-carboxylate (25 mg) was added HBr (33% in acetic acid, 0.5 mL). After 30 min at ambient temperature the mixture was diluted with diethyl ether and water (1:1 mix, 10 mL) and the organic layer was removed. The aqueous layer was washed with diethyl ether (1X5 mL), basified with NaOH (4 N) to pH ~12, then extracted with DCM (3X2 mL). The organics were dried with sodium sulfate, filtered, and concentrated. Addition of hexanes (~2 mL) followed by concentration was used to facilitate precipitation of the product. Drying under vacuum provided compound **A183**. MS(EI) for $C_{27}H_{40}ClN_5O_4S_2$, found 598 $[M+H]^+$.

Route 38



[00228] To a solution of 3,3'-azanediylidipropen-1-ol (1.0 g, 7.5 mmol) in DCM (20 mL) was added isobutyraldehyde (1.22 g, 11.3 mmol). The mixture was stirred for 30 min at ambient temperature. Acetic acid (1.8 g, 30.1 mmol) was added and the mixture was stirred for another 30 min. Sodium triacetoxymethylborohydride (6.40 g, 30.1 mmol) was then added and the reaction mixture was stirred overnight at ambient temperature. The mixture was adjusted

to pH=1-2 with 3*N* aqueous HCl and then stirred for 1 h. To the mixture was added 20% aqueous NaOH (to adjust pH to 10-11) then it was extracted with DCM (50 mL). The extract was washed with brine, dried over anhydrous sodium sulfate, and concentrated to provide 3,3'-(isobutylazanediyl)bis(propan-1-ol).

[00229] To a solution of 3,3'-(isobutylazanediyl)bis(propan-1-ol) (780 mg, 4.1 mmol) in DCM (10 mL) was added NEt₃ (820 mg, 8.2 mmol). MsCl (935 mg, 8.2 mmol) was then added slowly at 0 °C. The reaction mixture was stirred for 3 h at ambient temperature. The mixture was diluted with DCM (20 mL) and then washed with water (25 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated to provide 3,3'-(Isobutylazanediyl)bis(propane-3,1-diyl) dimethanesulfonate.

[00230] To a solution of (S)-6-chloro-N-(3-((4-(dimethylamino)phenyl)sulfonamido)-2-methylpropyl)-3,4-dihydroisoquinoline-2(1H)-sulfonamide (0.515 g, 1.03 mmol) in dry *N*-methyl pyrrolidone was added NaH (0.25 g, 2.26 mmol) at ambient temperature. The mixture was heated at 80 °C with stirring for 30 min and then cooled to ambient temperature. 3,3'-(Isobutylazanediyl)bis(propane-3,1-diyl) dimethanesulfonate (0.5 g, 1.5 mmol) was added and the reaction mixture was heated at 80 °C for 2 h. The reaction was quenched with water then extracted with ethyl acetate. The extract was dried over anhydrous sodium sulfate and concentrated. The residue was purified by flash column chromatography to afford compound **A152**. MS (EI) for C₃₁H₄₈ClN₅O₄S₂, found 654.4 [M+H]⁺. ¹H NMR (400 MHz, CDCl₃): δ 7.59 (d, 2H), 7.16 (m, 2H), 7.04 (m, 1H), 6.69 (d, 2H), 4.32 (m, 2H), 3.60 (m, 4H), 3.31 (m, 3H), 3.04 (s, 6H), 2.90 (m, 4H), 2.27 (m, 4H), 1.93 (m, 2H), 1.79 (m, 1H), 0.87 (m, 9H).

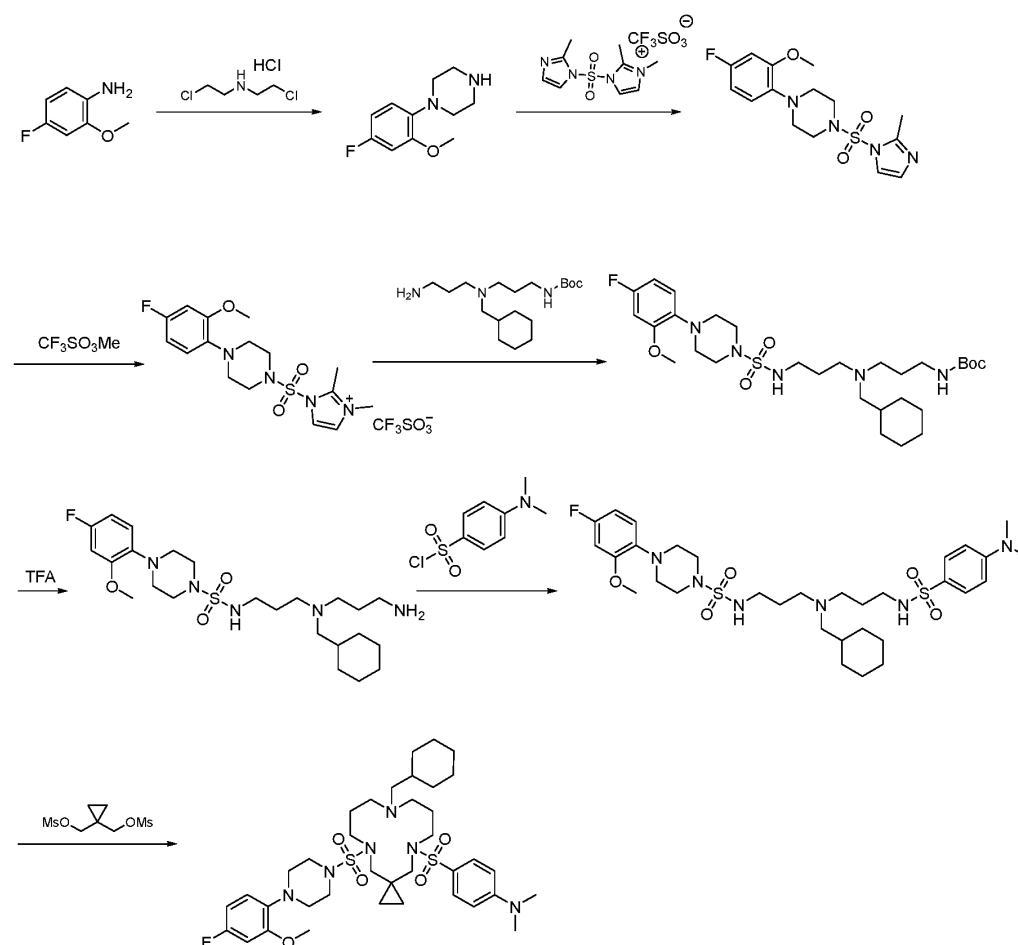
[00231] The following compounds were synthesized in a similar manner:

No.	IUPAC	[M+H] ⁺
A123	4-[[[(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methyl-9-(3-methylbutyl)-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	668
A156	4-[[[(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methyl-9-[(3-methylphenyl)methyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	702
A131	4-[[[(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methyl-9-[(4-methylphenyl)methyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	702
A157	4-[[[(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclopentylmethyl)-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	680
A106	4-[[[(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methyl-9-(2-methylbutyl)-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	668

A108	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclobutylmethyl)-3-methyl-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	666
A134	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(2-cyclohexylethyl)-3-methyl-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	708
A122	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(2-cyclopropylethyl)-3-methyl-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	666
A120	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(2-cyclopentylethyl)-3-methyl-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	694
A165	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methyl-9-(3,3,3-trifluoropropyl)-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	694
A141	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methyl-9-(propan-2-yl)-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	640
A176	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-[(cyclopent-1-en-1-yl)methyl]-3-methyl-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	678
A135	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-[(cyclohex-1-en-1-yl)methyl]-3-methyl-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	692
A167	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methyl-9-(2,2,2-trifluoroethyl)-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	680
A169	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-[(3,3-difluorocyclobutyl)methyl]-3-methyl-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	702
A133	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methyl-9-[(2-methylphenyl)methyl]-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	702
A140	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(2-methoxyethyl)-3-methyl-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	656
A125	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methyl-9-[(1,3-oxazol-2-yl)methyl]-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	679
A124	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methyl-9-[(1,2-oxazol-3-yl)methyl]-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	679
A183	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl}sulfonyl}-N,N-dimethylaniline	598
A185	4-{{{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-[(4,4-difluorocyclohexyl)methyl]-3-methyl-1,5,9-	730

	triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	
A107	4-[[[(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(2,2-dimethylpropyl)-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	668
A204	4-[[[(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-[(4-fluorocyclohexyl)methyl]-3-methyl-1,5,9-triazacyclododecan-1-yl)sulfonyl]-N,N-dimethylaniline	712

Route 39



[00232] To a mixture of 4-fluoro-2-methoxyaniline (25.0 g, 177 mmol), K_2CO_3 (36.7 g, 266 mmol) and NaI (10.6 g, 0.07 mmol) in *i*-PrOH (250 mL) was added bis(2-chloroethyl)amine hydrochloride (47 g, 177 mmol). The reaction mixture was refluxed overnight. The solvent was removed and the residue was purified by column chromatography (petroleum ether/EtOAc = 10:1) to provide 1-(4-fluoro-2-methoxyphenyl)piperazine. MS: m/z 212.36 $[M+H]^+$.

[00233] A solution of 1-(4-fluoro-2-methoxyphenyl)piperazine (4.0 g, 19 mmol) in MeCN (40 mL) was added 2,3-dimethyl-1-(2-methyl-1*H*-imidazol-1-ylsulfonyl)-1*H*-imidazol-3-ium (7.5 g, 19 mmol). The reaction mixture was stirred overnight at ambient temperature. The solvent was removed and the residue was purified by column chromatography (petroleum ether/EtOAc = 20:1) to provide 1-(4-fluoro-2-methoxyphenyl)-4-(2-methyl-1*H*-imidazol-1-ylsulfonyl)piperazine. MS: *m/z* 355.36 [M+H]⁺.

[00234] To a solution of 1-(4-fluoro-2-methoxyphenyl)-4-(2-methyl-1*H*-imidazol-1-ylsulfonyl)piperazine (6.0 g, 17 mmol) in DCM (60 mL) was added CF₃SO₃Me (2.78 g, 17 mmol). The reaction mixture was stirred for 2 h at ambient temperature. The solvent was removed and the residue was washed with ether to provide 1-(4-(4-fluoro-2-methoxyphenyl)piperazin-1-ylsulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate.

[00235] To a solution of 1-(4-(4-fluoro-2-methoxyphenyl)piperazin-1-ylsulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (740 mg, 2.3 mmol) in CH₃CN (8 mL) was added *tert*-butyl 3-((3-aminopropyl)(cyclohexylmethyl)amino)propylcarbamate (1.17 g, 2.3 mmol). The reaction mixture was stirred overnight at ambient temperature. The mixture was concentrated and the residue was purified by column chromatography (petroleum ether/EtOAc = 8:1 to 2:1) to provide *tert*-butyl 3-((cyclohexylmethyl)(3-(4-(4-fluoro-2-methoxyphenyl)piperazine-1-sulfonamido)propyl)amino)propylcarbamate.

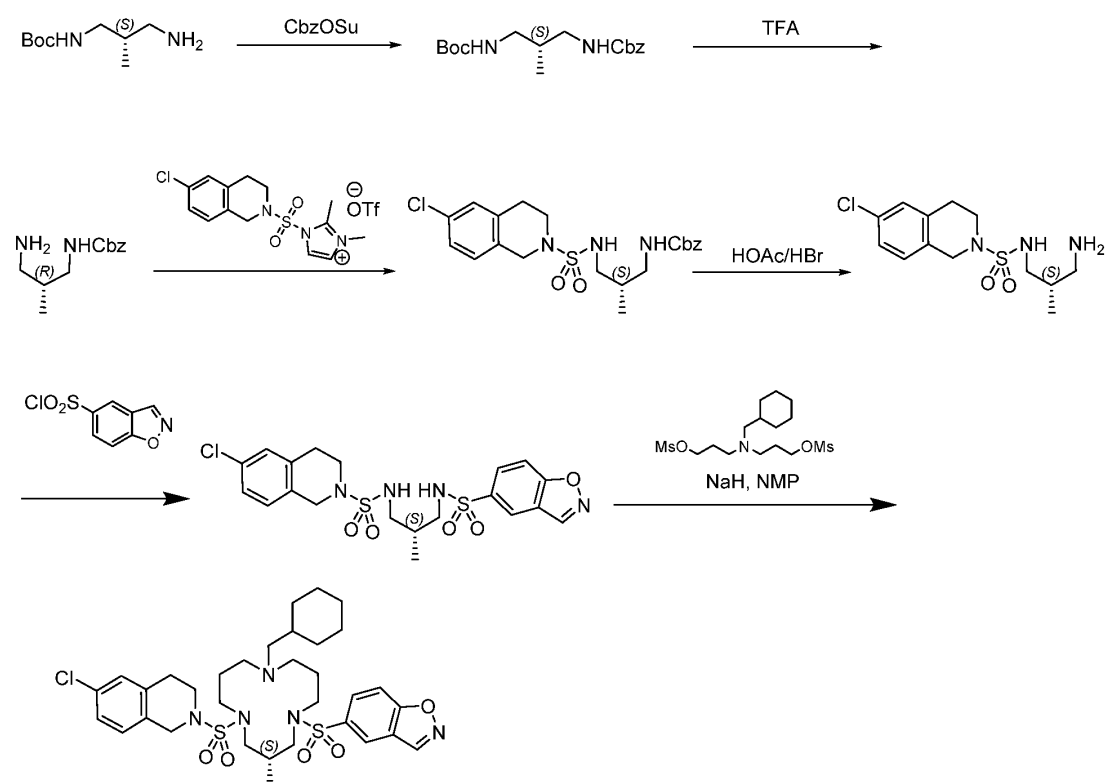
[00236] To a solution of *tert*-butyl 3-((cyclohexylmethyl)(3-(4-(4-fluoro-2-methoxyphenyl)piperazine-1-sulfonamido)propyl)amino)propylcarbamate (600 mg, 1 mmol) in DCM (30 mL) was added TFA (30 mL). The mixture was stirred for 2 h at ambient temperature. The solvent was removed and the residue was dissolved in ethyl acetate then washed with saturated aqueous NaHCO₃. The organic phase was dried over anhydrous sodium sulfate and concentrated to provide *N*-(3-((3-aminopropyl)(cyclohexylmethyl)amino)propyl)-4-(4-fluoro-2-methoxy phenyl)piperazine-1-sulfonamide.

[00237] To a solution of *N*-(3-((3-aminopropyl)(cyclohexylmethyl)amino)propyl)-4-(4-fluoro-2-methoxy phenyl)piperazine-1-sulfonamide (416 mg, 0.83 mmol) in DCM (5 mL) was added Et₃N (170 mg, 1.66 mmol) and 4-(dimethylamino)benzenesulfonyl chloride (365 mg, 1.66 mmol). The reaction mixture was stirred overnight at ambient temperature. The mixture was concentrated and purified by column chromatography (petroleum ether/EtOAc = 8:1 to 2:1) to provide *N*-(3-((cyclohexylmethyl)(3-(4-(dimethylamino)phenylsulfonamido)propyl)amino)propyl)-4-(4-fluoro-2-methoxyphenyl)piperazine-1-sulfonamide.

[00238] To a solution of *N*-(3-((cyclohexylmethyl)(3-(4-

(dimethylamino)phenylsulfonamido)propyl) amino)propyl)-4-(4-fluoro-2-methoxyphenyl)piperazine-1-sulfonamide (320 mg, 0.45 mmol) in NMP (3 mL) was added NaH (53 mg, 1.32 mmol, 60% dispersion). The mixture was stirred for 0.5 h at 80 °C then cooled to 70 °C and cyclopropane-1,1-diylbis(methylene) dimethanesulfonate (170 mg, 0.66 mmol) was added. The reaction mixture was stirred for 2 h at 70 °C. The solvent was removed and the residue was purified by column chromatography (petroleum ether/EtOAc = 15:1) to provide compound **A193**. MS (EI) for $C_{37}H_{57}FN_6O_5S_2$, found 745.0 $[M+H]^+$. 1H NMR (400 MHz, $CDCl_3$): δ 7.57 (t, 2H), 6.64 (t, 1H), 6.63 (br s, 4H), 3.86 (s, 3H), 3.19 (m, 4H), 3.04 (m, 8H), 2.87 (m, 12H), 2.36 (m, 2H), 1.56 (m, 15H), 0.43 (m, 4H).

Route 40



[00239] To a solution of (*S*)-*tert*-butyl 3-amino-2-methylpropylcarbamate (4.0 g, 21.3 mmol) in THF (20 mL) and water (20 mL) was added CbzOSu (5.83 g, 23.5 mmol). Aqueous NaOH (4*M*) was added to adjust to pH=11. The reaction mixture was stirred at ambient temperature for 2 h. The mixture was diluted with water (15 mL) and then extracted with ethyl acetate (50 mL). The organic phase was washed with 1*N* aqueous HCl, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column chromatography to provide benzyl *tert*-butyl (2-methylpropane-1,3-diyl)(*S*)-dicarbamate.

[00240] To a solution of benzyl tert-butyl (2-methylpropane-1,3-diyl)(S)-dicarbamate (4.5 g, 14.0 mmol) in DCM (25 mL) was added TFA (20 mL). The reaction mixture was stirred at ambient temperature for 3 h. The mixture was concentrated and the residue was dissolved in DCM (50 mL). The resulting solution was washed with saturated aqueous K₂CO₃ and the DCM layer was dried over anhydrous sodium sulfate and concentrated to provide (*R*)-benzyl 3-amino-2-methylpropylcarbamate.

[00241] To a solution of (*R*)-benzyl 3-amino-2-methylpropylcarbamate (2.7 g, 12.2 mmol) in CH₃CN (25 mL) was added 1-(6-chloro-3,4-dihydroisoquinolin-2(1*H*)-ylsulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium (5.7 g, 12.2 mmol). The reaction mixture was stirred at ambient temperature overnight. The mixture was concentrated and the residue was purified by column chromatography to provide (*S*)-benzyl 3-(6-chloro-1,2,3,4-tetrahydroisoquinoline-2-sulfonamido)-2-methyl propylcarbamate.

[00242] (*S*)-Benzyl 3-(6-chloro-1,2,3,4-tetrahydroisoquinoline-2-sulfonamido)-2-methyl propylcarbamate (2.7 g, 6.0 mmol) was dissolved in HBr (48%)/HOAc (20 mL). The mixture was stirred at ambient temperature for 1 h. The mixture was diluted with water (100 mL) and then washed with ether (50 mL). The aqueous layer was basified with NaOH to pH=10. The resulting mixture was extracted with DCM (100 mL). The DCM layer was dried over anhydrous sodium sulfate and concentrated to provide (*S*)-*N*-(3-amino-2-methylpropyl)-6-chloro-3,4-dihydroisoquinoline-2(1*H*)- sulfonamide.

[00243] To a solution of (*S*)-*N*-(3-amino-2-methylpropyl)-6-chloro-3,4-dihydroisoquinoline-2(1*H*)- sulfonamide (0.6 g, 1.9 mmol) and TEA (270 mg, 2.7 mmol) in DCM (6 mL) was added benzo[d]isoxazole-5-sulfonyl chloride (420 mg, 1.9 mmol) at 0 °C. The reaction mixture was stirred at ambient temperature for 2 h. The mixture was washed with water and then concentrated. The residue was purified by column chromatography to provide (*S*)-*N*-(3-(6-chloro-1,2,3,4-tetrahydroisoquinoline-2-sulfonamido)-2- methylpropyl)benzo[d]isoxazole-5-sulfonamide.

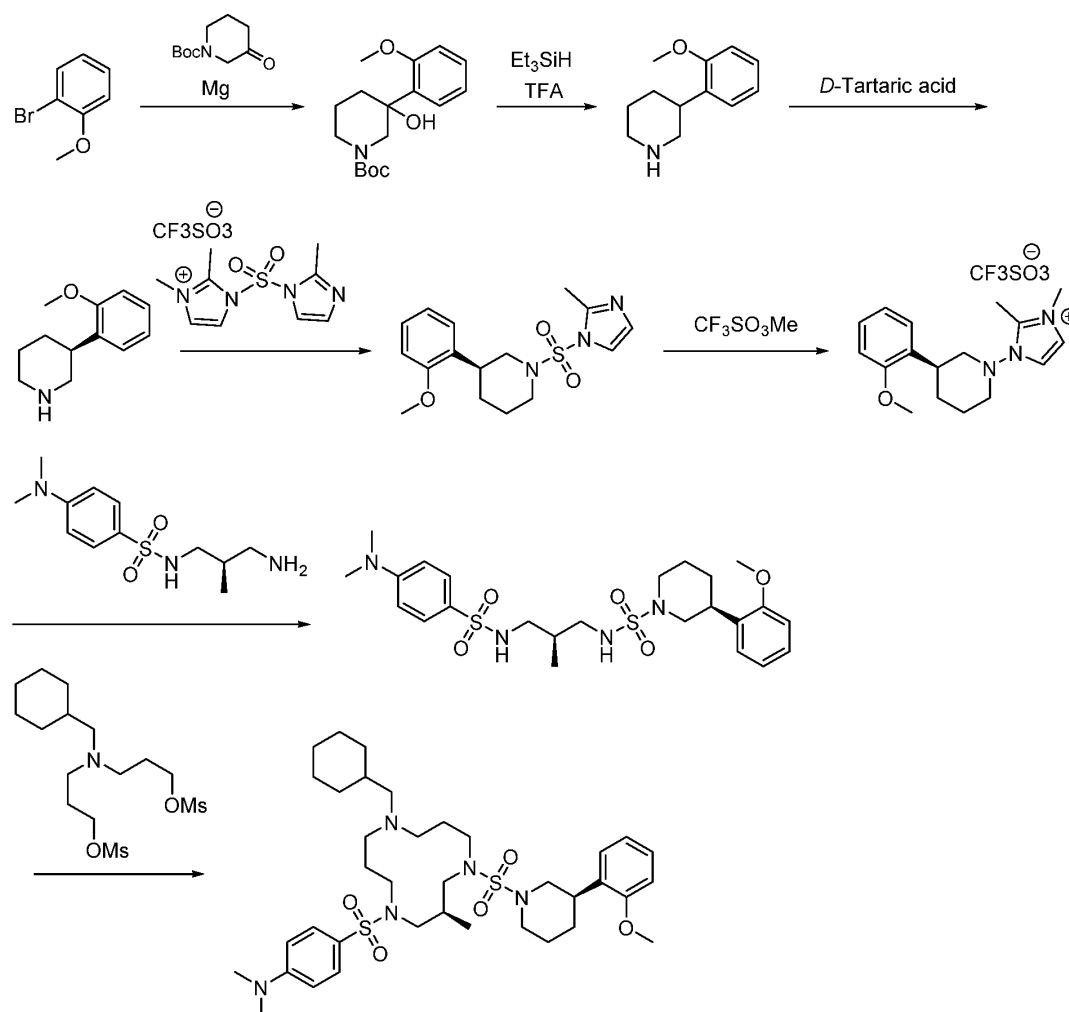
[00244] To a solution of (*S*)-*N*-(3-(6-chloro-1,2,3,4-tetrahydroisoquinoline-2-sulfonamido)-2- methylpropyl)benzo[d]isoxazole-5-sulfonamide (0.61 g, 1.22 mmol) in NMP (6 mL) was added NaH (122 mg, 3.05 mmol, 60%). The mixture was stirred for 0.5 h at 80 °C. The mixture was cooled to 70 °C and 3,3'-(cyclohexylmethylazanediyl)bis(propene-3,1-diyl) dimethanesulfonate (0.74 g, 1.83 mmol) was added. The reaction mixture was stirred for 2 h at 70 °C. The mixture was cooled and poured into water. The resulting mixture was extracted with ethyl acetate. The organic phase was concentrated and the residue was purified by column chromatography (petroleum ether/EtOAc = 12:1) to provide compound **A180**. LC-MS (ESI): *m/z* 692.2 [M+H]⁺. ¹H NMR (400 MHz, CDCl₃): δ 7.84 (s, 1H), 7.61 (m, 1H), 7.13 (m,

3H), 6.69 (m, 2H), 4.32 (m, 2H), 3.66 (m, 5H), 3.21 (m, 5H), 2.91 (m, 4H), 2.77 (m, 4H), 1.91 (m, 4H), 1.57 (m, 6H), 1.13 (m, 6H), 0.89 (m, 3H).

[00245] The following compounds were synthesized in a similar manner:

No.	IUPAC	[M+H] ⁺
A168	3-[[{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	694
A171	6-chloro-2-[[{(3S)-9-(cyclohexylmethyl)-3-methyl-5-phenylmethanesulfonyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-1,2,3,4-tetrahydroisoquinoline	665
A179	4-[[{(3S)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylbenzamide	722
A184	(3S)-N-benzyl-9-(cyclohexylmethyl)-5-[4-(dimethylamino)benzenesulfonyl]-N,3-dimethyl-1,5,9-triazacyclododecane-1-sulfonamide	648
A188	6-chloro-2-[[{(3S)-9-(cyclohexylmethyl)-3-methyl-5-[(1-methyl-1H-indol-5-yl)sulfonyl]-1,5,9-triazacyclododecan-1-yl]sulfonyl]-1,2,3,4-tetrahydroisoquinoline	704
A189	(3S)-9-(cyclohexylmethyl)-5-[4-(dimethylamino)benzenesulfonyl]-N-[(4-ethylphenyl)methyl]-3-methyl-1,5,9-triazacyclododecane-1-sulfonamide	662
A192	N-{4-[[[(3S)-9-(cyclohexylmethyl)-5-[4-(dimethylamino)benzenesulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]amino)methyl]phenyl}-N-methylacetamide	705

Route 41



[00246] To a suspension of Mg (4.4 g, 181 mmol) in THF (200 mL) was added 1-bromo-2-methoxybenzene (40 g, 214 mmol). The mixture was stirred at 80 °C for 0.5 h. The mixture was cooled to 0 °C and a solution of *tert*-butyl 3-oxopiperidine-1-carboxylate (35.5 g, 181 mmol) in THF was added. The reaction mixture was stirred at ambient temperature for 2 h. The residue was purified by column chromatography to provide *tert*-butyl 3-hydroxy-3-(2-methoxyphenyl)piperidine-1-carboxylate. ¹H NMR (400 MHz, CDCl₃): δ 7.48 (m, 1H), 7.32 (m, 1H), 6.98 (m, 2H), 4.08 (m, 1H), 3.96 (s, 3H), 3.82 (m, 1H), 3.31 (s, 1H), 2.98 (m, 1H), 2.25 (m, 1H), 1.99 (m, 2H), 1.62 (m, 2H), 1.63 (m, 9H).

[00247] A solution of *tert*-butyl 3-hydroxy-3-(2-methoxyphenyl)piperidine-1-carboxylate (23.0 g, 75 mmol) and triethylsilane (44 mL) in DCM (230 mL) was cooled to -30 °C and TFA (27 mL) was added. The reaction mixture was stirred at -30 °C for 2.5 h and then allowed to

warm to ambient temperature and stirred for another 3.5 h. The mixture was poured into ice-water and the solution was adjusted to pH=9 with saturated aqueous sodium hydroxide. The resulting mixture was extracted three times with DCM. The combined organic phases were dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography to provide 3-(2-methoxyphenyl)piperidine.

[00248] A solution of 3-(2-methoxyphenyl)piperidine (6.5 g, 34 mmol) and D-tartaric acid (5.1 g, 34 mmol) in MeOH (25 mL) was refluxed for 2 h. The mixture was cooled to ambient temperature and filtered to provide the tartrate salt. This tartrate salt was recrystallized twice from MeOH and then treated with aqueous NaOH. The resulting mixture was extracted with DCM. The DCM layer was dried over anhydrous sodium sulfate and concentrated to provide (S)-3-(2-methoxyphenyl)piperidine.

[00249] A solution of (S)-3-(2-methoxyphenyl)piperidine (500 mg, 2.6 mmol) and 2,3-dimethyl-1-((2-methyl-1H-imidazol-1-yl)sulfonyl)-1H-imidazol-3-ium trifluoromethanesulfonate (1.1 g, 2.7 mmol) in MeCN (15 ml) was stirred overnight at ambient temperature. The solvent was removed. The residue was purified by column chromatography (petroleum ether/EtOAc = 20:1) to provide (S)-3-(2-methoxyphenyl)-1-((2-methyl-1H-imidazol-1-yl)sulfonyl)piperidine. LC-MS (ESI): m/z 336.06 $[M+H]^+$.

[00250] To a solution of (S)-3-(2-methoxyphenyl)-1-((2-methyl-1H-imidazol-1-yl)sulfonyl)piperidine (800 g, 2.4 mmol) in DCM (10 ml) was added CF₃SO₃Me (398 mg, 2.4 mmol). The reaction mixture was stirred for 2 h at ambient temperature. The solvent was removed and the residue was washed with ether to provide (S)-1-(3-(2-methoxyphenyl)piperidin-1-yl)-2,3-dimethyl-1H-imidazol-3-ium trifluoromethanesulfonate.

[00251] To a solution of (S)-1-(3-(2-methoxyphenyl)piperidin-1-yl)-2,3-dimethyl-1H-imidazol-3-ium trifluoromethanesulfonate (1 g, 2 mmol) in CH₃CN (24 ml) was added (R)-N-(3-amino-2-methylpropyl)-4-(dimethylamino)benzenesulfonamide (0.55 g, 2 mmol). The reaction mixture was stirred overnight at ambient temperature. The mixture was concentrated. The residue was purified by column chromatography (petroleum ether/EtOAc = 8:1 to 2:1) to provide (S)-N-((S)-3-((4-(dimethylamino)phenyl)sulfonamido)-2-methylpropyl)-3-(2-methoxyphenyl)piperidine-1-sulfonamide.

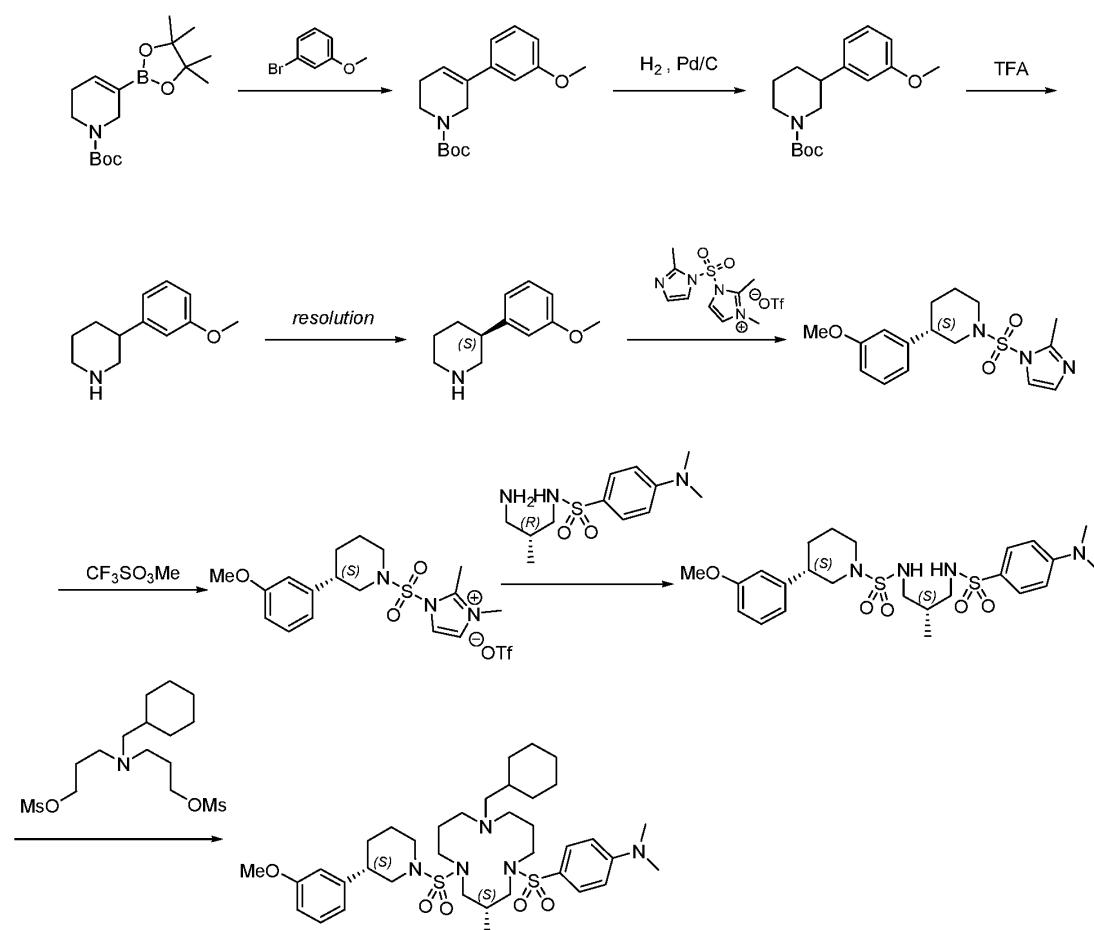
[00252] To a solution of (S)-N-((S)-3-((4-(dimethylamino)phenyl)sulfonamido)-2-methylpropyl)-3-(2-methoxyphenyl)piperidine-1-sulfonamide (454 mg, 0.86 mmol) in NMP (14 mL) was added NaH (104 mg, 2.6 mmol, 60%). The mixture was stirred for 0.5 h at 80 °C. The mixture was cooled to 70 °C and ((cyclohexylmethyl)azanediyl)bis(propane-3,1-diyl)dimethanesulfonate (500 mg, 1.3 mmol) was added. The reaction mixture was stirred for 2 h at 70 °C. The mixture was cooled to 0-5 °C and poured into water. The resulting mixture was

extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by prep-HPLC to provide compound **A210**. LC-MS (ESI): m/z 718.17 $[M+H]^+$. 1H NMR (400 MHz, $CDCl_3$): δ 7.60 (m, 2H), 7.23 (m, 2H), 6.95(m, 2H), 6.68 (m, 2H), 3.82 (s, 3H), 3.73 (m, 5H), 3.26 (m, 4H), 3.12 (s, 6H), 2.76 (m, 5H), 2.36 (m, 4H), 1.76 (m, 9H), 1.33 (m, 10H), 0.86 (m, 6H).

[00253] The following compound was synthesized in a similar manner:

No.	IUPAC	$[M+H]^+$
A208	4-[[[(3S)-9-(cyclohexylmethyl)-5-[[[(3S)-3-(4-methoxyphenyl)piperidin-1-yl]sulfonyl]-3-methyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	718

Route 42



[00254] To a solution of *tert*-butyl 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate (10.92 g, 35 mmol), 1-bromo-3-methoxybenzene (6.6 g,

35 mmol) and Cs_2CO_3 (3.44 g, 106 mmol) in 1,4-dioxane (50 mL) and H_2O (10 mL) was added $\text{Pd}(\text{dppf})\text{Cl}_2$ (1.32 g, 1.75 mmol). The reaction mixture was stirred overnight at 80 °C. The solvent was removed. The residue was purified by column chromatography (petroleum ether/EtOAc = 10:1) to provide *tert*-butyl 3-(3-methoxyphenyl)-5,6-dihydropyridine-1(2*H*)-carboxylate. LC-MS (ESI): m/z 290.12 $[\text{M}+\text{H}]^+$. ^1H NMR (400 MHz, CDCl_3): δ 7.26 (m, 1H), 6.96 (m, 1H), 6.89 (s, 1H), 6.81 (m, 1H), 6.19 (s, 1H), 4.25 (s, 2H), 3.81 (s, 3H), 3.54 (m, 2H), 2.30 (s, 2H), 1.49 (s, 9H).

[00255] To a solution of *tert*-butyl 3-(3-methoxyphenyl)-5,6-dihydropyridine-1(2*H*)-carboxylate (9.46 g, 32 mmol) in MeOH (75 mL) and EtOAc (25 mL) was added Pd/C (2 g). The mixture was stirred at ambient temperature for 4 h under H_2 atmosphere. The mixture was filtered and the filtrate was concentrated to provide *tert*-butyl 3-(3-methoxyphenyl)piperidine-1-carboxylate. ^1H NMR (400 MHz, CDCl_3): δ 7.25 (m, 1H), 6.81 (m, 3H), 3.99 (m, 2H), 3.73 (s, 3H), 2.65 (m, 1H), 2.55 (m, 1H), 2.50 (m, 2H), 1.98 (m, 1H), 1.65 (m, 2H), 1.44 (s, 9H).

[00256] A solution of *tert*-butyl 3-(3-methoxyphenyl)piperidine-1-carboxylate (7.85 g, 27 mmol) in HCl/MeOH (80 mL) was stirred at ambient temperature for 4 h. The mixture was treated with water and then adjusted to pH=12 with 3*N* aqueous NaOH. The resulting mixture was extracted with EtOAc (100 mL $\times$ 2). The combined extracts were dried over anhydrous sodium sulfate and concentrated to provide 3-(3-methoxyphenyl). LC-MS (ESI): m/z 192.09 $[\text{M}+\text{H}]^+$.

[00257] To a solution of *D*-(-)-tartaric acid (2.5 g, 16.7 mmol) in MeOH (12.5 mL) was added 3-(3-methoxyphenyl)piperidine (3.2 g, 16.7 mmol). The mixture was stirred at 80 °C for 24 h then cooled to ambient temperature. The resulting precipitate was collected by filtration and then recrystallized three times from MeOH to provide (*S*)-3-(3-methoxyphenyl)piperidine.

[00258] To a solution of (*S*)-3-(3-methoxyphenyl)piperidine (1.28 g, 6.7 mmol) in CH_3CN (10 mL) was added 1-(1*H*-imidazol-1-ylsulfonyl)-3-methyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (2.87 g, 7.3 mmol). The reaction mixture was stirred at ambient temperature overnight. The solvent was removed. The residue was purified by column chromatography (petroleum ether/EtOAc = 9:1 to 8:2) to provide (*S*)-3-(3-methoxyphenyl)-1-(2-methyl-1*H*-imidazol-1-ylsulfonyl)piperidine. ^1H NMR (400 MHz, CDCl_3): δ 7.27 (m, 1H), 7.21 (s, 1H), 6.93 (s, 1H), 6.79 (m, 2H), 6.72 (s, 1H), 3.93 (m, 2H), 3.82 (s, 3H), 2.68 (m, 1H), 2.65 (m, 2H), 2.62 (s, 3H), 2.06 (m, 1H), 1.95 (m, 1H), 1.82 (m, 1H), 1.62 (m, 1H).

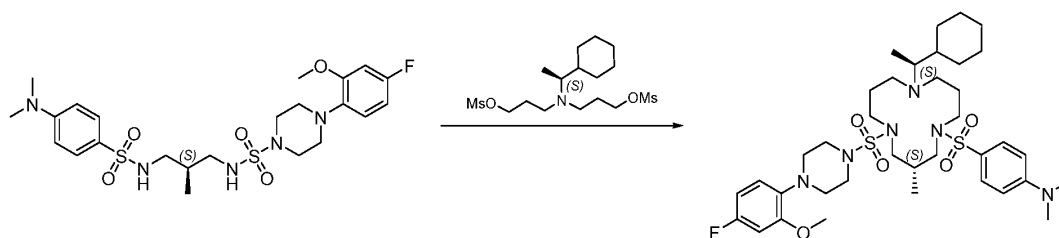
[00259] To a solution of (*S*)-3-(3-methoxyphenyl)-1-(2-methyl-1*H*-imidazol-1-ylsulfonyl)piperidine (1.6 mg, 4.7 mmol) in DCM (20 mL) was added $\text{CF}_3\text{CO}_2\text{Me}$ (782 mg, 4.7

mmol). The reaction mixture was stirred at ambient temperature for 2 h. The solvent was removed. The resulting product was washed with ether to provide (S)-1-(3-(3-methoxyphenyl)piperidin-1-ylsulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate.

[00260] To a solution of (S)-1-(3-(3-methoxyphenyl)piperidin-1-ylsulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (1.1 g, 2.2 mmol) in CH₃CN (11 mL) was added (R)-N-(3-amino-2-methylpropyl)-4-(dimethylamino)benzenesulfonamide (896 mg, 33 mmol). The mixture was stirred at ambient temperature overnight. The solvent was removed. The residue was purified by column chromatography to provide (S)-N-((S)-3-(4-(dimethylamino)phenylsulfonamido)-2-methylpropyl)-3-(3-methoxyphenyl)piperidine-1-sulfonamide. ¹H NMR (400 MHz, CDCl₃): δ 7.68 (m, 2H), 7.28 (m, 1H), 6.85 (m, 1H), 6.80 (m, 2H), 6.68 (m, 2H), 4.81 (m, 2H), 3.82 (s, 3H), 3.76 (m, 2H), 3.12 (m, 1H), 3.07 (s, 6H), 2.82 (m, 4H), 2.11 (m, 1H), 1.93 (m, 2H), 1.77 (m, 1H), 1.29 (m, 3H), 0.91 (m, 3H).

[00261] To a solution of (S)-N-((S)-3-(4-(dimethylamino)phenylsulfonamido)-2-methylpropyl)-3-(3-methoxyphenyl)piperidine-1-sulfonamide (550 mg, 1.05 mmol) in NMP (6 mL) was added sodium hydride (75.6 mg, 3.15 mmol). The mixture was stirred for 30 min at 80 °C. The mixture was cooled to 70 °C and a solution of ((cyclohexylmethyl)azanediyl)bis(propane-3,1-diyl) dimethanesulfonate (606 mg, 1.57 mmol) in NMP (3 mL) was added. The reaction mixture was stirred at 70 °C for 1 h. The mixture was cooled to ambient temperature and poured into water (10 mL). The resulting mixture was extracted with ethyl acetate (20 mL). The organic layer was washed with water (15 mL × 2), dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography (EtOAc/Petroleum ether = 1:15 to 1:5) to provide compound **A209**. LC-MS (ESI): *m/z* 719.2 [M+H]⁺. ¹H NMR (400 MHz, CDCl₃): δ 7.61 (m, 2H), 7.28 (m, 2H), 6.84 (m, 3H), 6.70 (m, 2H), 3.83 (s, 3H), 3.70 (m, 2H), 3.55 (m, 2H), 3.30 (m, 1H), 3.11 (s, 9H), 2.79 (m, 5H), 2.41 (m, 1H), 2.33 (m, 3H), 2.05 (m, 8H), 1.67 (m, 4H), 1.27 (m, 7H), 0.94 (m, 3H), 0.85 (m, 2H).

Route 43



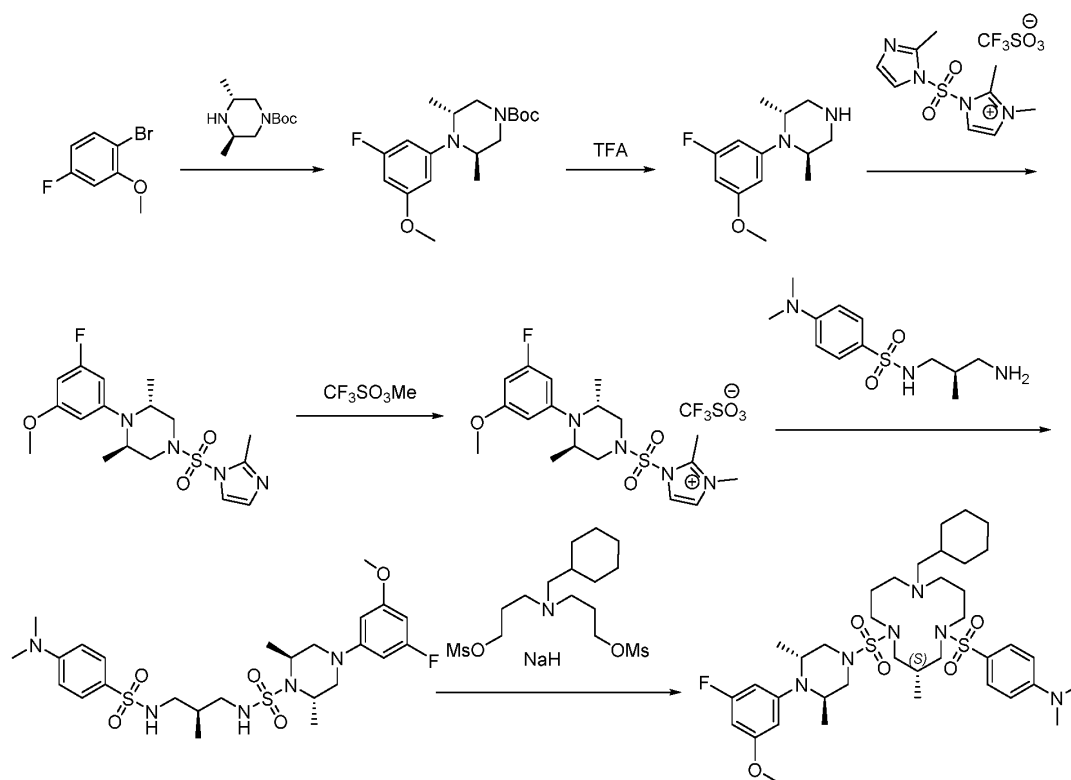
[00262] To a solution of (S)-N-(3-((4-(dimethylamino)phenyl)sulfonamido)-2-

methylpropyl)-4-(4-fluoro-2-methoxyphenyl)piperazine-1-sulfonamide (900 mg, 1.65 mmol) in NMP (5 mL) was added NaH (198 mg, 4.92 mmol, 60%). The mixture was stirred for 0.5 h at 80 °C. The mixture was cooled to 70 °C and (*S*)-3,3'-(1-cyclohexylethylazanediyl)bis(propane-3,1-diyl) dimethanesulfonate (844 mg, 2.49 mmol) was added. The reaction mixture was stirred for 2 h at 70 °C then cooled to 0-5 °C and poured into water. The resulting mixture was extracted with ethyl acetate. The extract was concentrated. The residue was purified by column chromatography (petroleum ether/EtOAc = 15:1) to provide compound **A203**. LC-MS (ESI): *m/z* 751.33 [M+H]⁺. ¹H NMR (400 MHz, CDCl₃): δ 7.68 (m, 2H), 6.92 (m, 1H), 6.78 (m, 4H), 3.92 (s, 4H), 3.86 (m, 1H), 3.62 (m, 1H), 3.40 (m, 2H), 3.19 (m, 3H), 3.04 (s, 9H), 2.87 (m 2H), 2.26 (m, 3H), 1.92 (m, 3H), 1.79 (m, 4H), 1.66 (m, 2H), 1.54 (m, 2H), 1.38 (m, 4H), 1.26 (m, 5H), 0.98 (m, 3H), 0.83 (m, 4H).

[00263] The following compounds were synthesized in a similar manner:

No.	IUPAC	[M+H] ⁺
B22	4-[[[(1 <i>S</i> ,12 <i>R</i>)-10-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-6-(cyclohexylmethyl)-2,6,10-triazabicyclo[10.2.0]tetradecan-2-yl]sulfonyl]-N,N-dimethylaniline	706
A160	4-[[[(3 <i>S</i> ,7 <i>S</i> ,11 <i>S</i>)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-3,7,11-trimethyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	722
B16	4-[[[(3 <i>S</i> ,7 <i>R</i> ,11 <i>S</i>)-5-[(6-chloro-1,2,3,4-tetrahydroisoquinolin-2-yl)sulfonyl]-9-(cyclohexylmethyl)-3,7,11-trimethyl-1,5,9-triazacyclododecan-1-yl]sulfonyl]-N,N-dimethylaniline	722

Route 44



[00264] To a solution of 1-bromo-4-fluoro-2-methoxybenzene (910 mg, 4.4 mmol) and 1M KHMDS (18 mL, 18 mmol) in 1,4-dioxane (9 mL) was added *tert*-butyl (3*R*,5*R*)-3,5-dimethylpiperazine-1-carboxylate (950 mg, 4.4 mmol). The reaction mixture was stirred at 100 °C for 3 h. The solvent was removed and the residue was purified by column chromatography (petroleum ether/EtOAc = 20:1) to provide *tert*-butyl (3*R*,5*R*)-4-(3-fluoro-5-methoxyphenyl)-3,5-dimethylpiperazine-1-carboxylate. LC-MS (ESI): *m/z* 339.0 [M+H]⁺.

[00265] To a solution of *tert*-butyl (3*R*,5*R*)-4-(3-fluoro-5-methoxyphenyl)-3,5-dimethylpiperazine-1-carboxylate (800 mg, 2.4 mmol) in DCM (4 mL) was added TFA (4 mL). The mixture was stirred at ambient temperature for 1 h. The solvent was removed. The residue was dissolved in DCM (50 mL) and then washed with saturated aqueous K₂CO₃. The organic layer was dried over anhydrous sodium sulfate and concentrated to provide (2*R*,6*R*)-1-(3-methoxy-5-methylphenyl)-2,6-dimethylpiperazine.

[00266] To a solution of (2*R*,6*R*)-1-(3-methoxy-5-methylphenyl)-2,6-dimethylpiperazine (500 mg, 2.1 mmol) in MeCN (5 mL) was added 2,3-dimethyl-1-((2-methyl-1H-imidazol-1-yl)sulfonyl)-1H-imidazol-3-ium trifluoromethanesulfonate (825 mg, 2.1 mmol). The reaction mixture was stirred overnight at ambient temperature. The solvent was removed. The

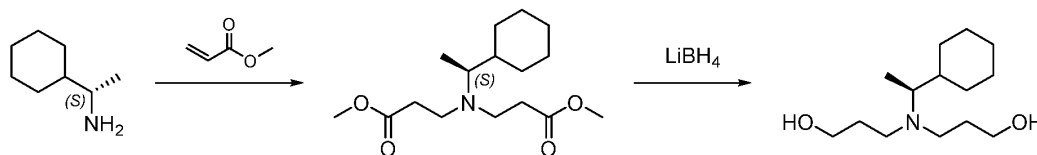
residue was purified by column chromatography (petroleum ether/EtOAc = 20:1) to provide (2*R*,6*R*)-1-(3-fluoro-5-methoxyphenyl)-2,6-dimethyl-4-((2-methyl-1*H*-imidazol-1-yl)sulfonyl)piperazine. LC-MS (ESI): m/z 383.53 [M+H]⁺.

[00267] To a solution of (2*R*,6*R*)-1-(3-methoxy-5-methylphenyl)-2,6-dimethylpiperazine (450 mg, 1.2 mmol) in DCM (5 mL) was added CF₃SO₃Me (193 mg, 1.2 mmol). The reaction mixture was stirred for 2 h at ambient temperature. The solvent was removed and the residue was washed with ether to provide 1-(((3*R*,5*R*)-4-(3-fluoro-5-methoxyphenyl)-3,5-dimethylpiperazin-1-yl)sulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate.

[00268] To a solution of 1-(((3*R*,5*R*)-4-(3-fluoro-5-methoxyphenyl)-3,5-dimethylpiperazin-1-yl)sulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (500 mg, 0.91 mmol) in CH₃CN (5 mL) was added (R)-*N*-(3-amino-2-methylpropyl)-4-(dimethylamino)benzenesulfonamide (248 mg, 0.91 mmol). The reaction mixture was stirred overnight at ambient temperature. The mixture was concentrated. The residue was purified by column chromatography (petroleum ether/EtOAc = 8:1 to 2:1) to provide (2*S*,6*S*)-*N*-((*S*)-3-((4-(dimethylamino)phenyl)sulfonamido)-2-methylpropyl)-4-(3-fluoro-5-methoxyphenyl)-2,6-dimethylpiperazine-1-sulfonamide. LC-MS (ESI): m/z 572.4 [M+H]⁺.

[00269] To a solution of (2*S*,6*S*)-*N*-((*S*)-3-((4-(dimethylamino)phenyl)sulfonamido)-2-methylpropyl)-4-(3-fluoro-5-methoxyphenyl)-2,6-dimethylpiperazine-1-sulfonamide (430 mg, 0.75 mmol) in NMP (4 mL) was added NaH (91 mg, 2.3 mmol, 60%). The mixture was stirred for 0.5 h at 80 °C. The mixture was cooled to 70 °C and ((cyclohexylmethyl)azanediyl)bis(propane-3,1-diyl) dimethanesulfonate (435 mg, 1.1 mmol) was added. The reaction mixture was stirred for 2 h at 70 °C. The solvent was removed and the residue was purified by column chromatography (petroleum ether/EtOAc = 15:1) to provide compound **A200**. LC-MS (ESI): m/z 764.3 [M+H]⁺. ¹H NMR (400 MHz, CDCl₃): δ 7.60 (d, 2H), 6.69 (d, 2H), 6.32 (d, 3H), 3.78 (s, 3H), 3.06 (q, 2H), 3.04 (s, 8H), 3.00 (s, 2H), 2.87 (d, 2H), 2.76 (s, 2H), 2.34 (br s, 4H), 2.09 (br s, 2H), 1.33 (s, 17H), 1.04 (d, 7H), 0.93 (d, 3H).

(*S*)-3,3'-(1-Cyclohexylethylazanediyl)dipropen-1-ol (Intermediate for compounds A203, A190, A207)



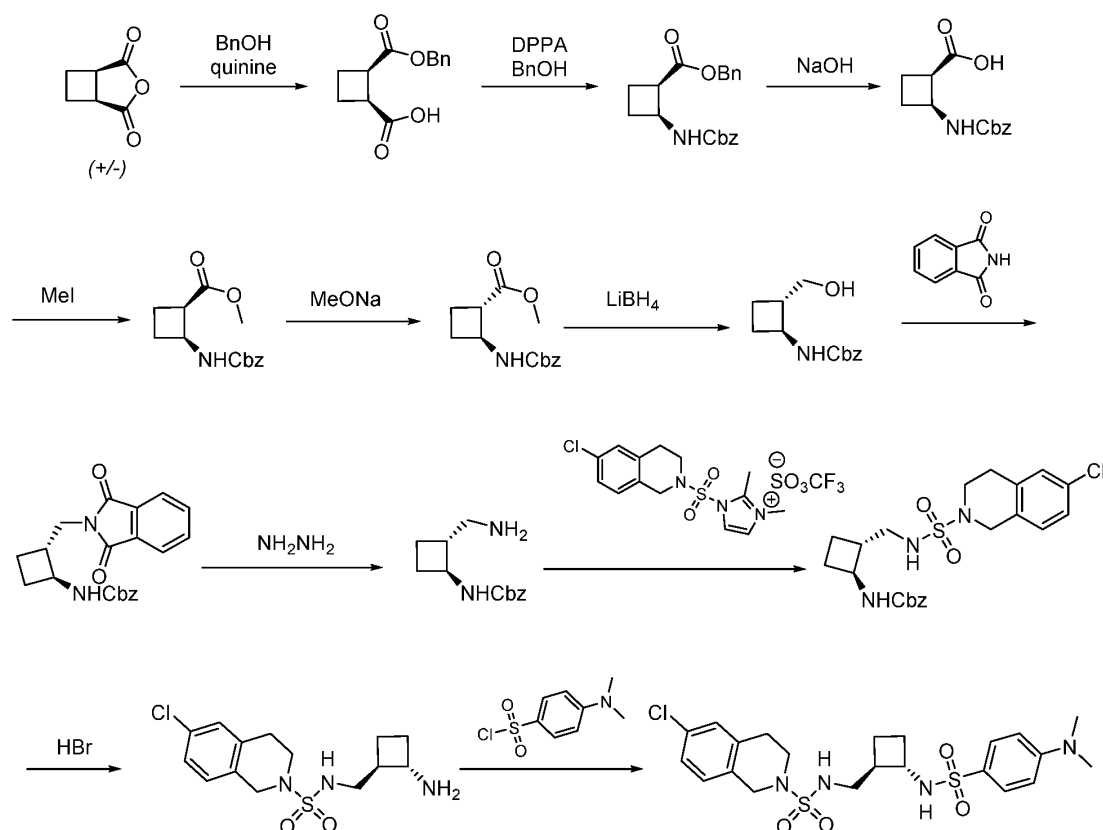
[00270] To a solution of (*S*)-1-cyclohexylethanamine (4.0 g, 31.46 mmol) in methanol (50 mL) was added methyl acrylate (8.1 g, 94.38 mmol). The reaction mixture was stirred for 72

h at 45 °C. The mixture was concentrated and the residue was purified by column chromatography (EtOAc/petroleum ether = 30:1) to provide (S)-dimethyl 3,3'-(1-cyclohexylethylazanediyl)dipropionate.

[00271] To a solution of (S)-dimethyl 3,3'-(1-cyclohexylethylazanediyl)dipropionate (4.0 g, 13.37 mmol) in THF (50 mL) was added LiBH₄ (1.45 g, 66.84 mmol). The reaction mixture was stirred at 60 °C for 2 h. The mixture was cooled to 0-5 °C and quenched with water (30 mL). The mixture was adjusted to pH=3 with 1 N aqueous hydrochloric acid and stirred for 30 minutes. The resulting mixture was washed with dichloromethane. The aqueous layer was adjusted to pH = 9-10 with saturated aqueous sodium carbonate and then extracted with DCM (3X). The combined organic layers were dried over anhydrous sodium sulfate and concentrated to provide (S)-3,3'-(1-cyclohexylethylazanediyl)diprop-1-ol. ¹H NMR (400 MHz, CDCl₃): δ 3.66 (s, 1H), 2.82 (m, 2H), 2.42 (m, 2H), 2.21 (m, 1H), 1.96 (m, 2H), 1.67 (m, 4H), 1.22 (m, 4H), 0.91 (m, 3H), 0.81 (m, 1H), 0.72 (m, 1H).

[00272] The following compounds were synthesized in a similar manner: (*R*)-3,3'-(1-Cyclohexylethylazanediyl)diprop-1-ol (Intermediate for compound **A191**) and (S)-3,3'-((1-phenylethyl)azanediyl)bis(prop-1-ol) (Intermediate for compound **A205**)

6-Chloro-N(((1*R*,2*S*)-2-(4-(dimethylamino)phenylsulfonamido)cyclobutyl) methyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (Intermediate for compound **B22**)



[00273] To a suspension of *cis*-3-oxabicyclo[3.2.0]heptane-2,4-dione (32.8 g, 200 mmol) and quinine (71.4 g, 220 mmol) in toluene (1 L) was added benzyl alcohol (64.9 g, 600 mmol) dropwise over a period of 0.5 h at -55°C . The reaction mixture was stirred at -55°C for 96 h. The resulting clear solution was concentrated to dryness and the residue was dissolved in diethyl ether (1.2 L). The solution was washed with 2*N* aqueous HCl (1 L), the organic layer was extracted with saturated aqueous sodium bicarbonate (500×5 mL), and the resulting combined aqueous phases were washed with diethyl ether (500 mL) to remove the traces of benzyl alcohol. The aqueous phase was acidified with 8*N* aqueous HCl then extracted with DCM (1.0 L×2). The combined organic layers were dried over anhydrous sodium sulfate and concentrated to provide (1*S*,2*R*)-2-(benzyloxycarbonyl)cyclobutanecarboxylic acid.

[00274] To a solution of (1*S*,2*R*)-2-(benzyloxycarbonyl)cyclobutanecarboxylic acid (40.1 g, 171.4 mmol) and triethylamine (26.0 g, 257 mmol) in dichloroethane (400 mL) was added dropwise DPPA (51.8 g, 188.5 mmol) at 0°C . The mixture was stirred for 2 h at ambient temperature then washed with water and dried over anhydrous NaSO_4 . To the organic solution was added BnOH (18.5 g, 171 mmol) and triethylamine (34.6 g, 342 mmol). The

reaction mixture was refluxed overnight and then diluted with DCM (500 mL). The mixture was washed with 1 *N* aqueous HCl (1 L) and saturated aqueous NaHCO₃. The organic phase was dried over anhydrous sodium sulfate, concentrated, and purified by column chromatography to provide (1*R*,2*S*)-benzyl 2-(benzyloxycarbonylamino)cyclobutanecarboxylate.

[00275] To a solution of (1*R*,2*S*)-benzyl 2-(benzyloxycarbonylamino)cyclobutanecarboxylate (20 g, 59 mmol) in THF (100 mL) was added 4 *N* aqueous NaOH (50 mL) at 0 °C. The reaction mixture was stirred overnight at ambient temperature. The mixture was diluted with water (30 mL) then washed with ether (50 mL×2). The aqueous layer was acidified with 3 *N* aqueous HCl and extracted with ethyl acetate. The extracts were dried over anhydrous sodium sulfate and concentrated to provide (1*R*,2*S*)-2-(benzyloxycarbonylamino)cyclobutanecarboxylic acid. ¹H NMR (400 MHz, CDCl₃): δ 7.34 (m, 5H), 5.12 (m, 2H), 4.62 (m, 1H), 3.58 (m, 1H), 2.28 (m, 2H), 1.89 (m, 2H).

[00276] To a solution of (1*R*,2*S*)-2-(benzyloxycarbonylamino)cyclobutanecarboxylic acid (4.0 g, 16.1 mmol) and K₂CO₃ (4.4 g, 32 mmol) in DMF (40 mL) was added MeI (2.96 g, 20.8 mmol). The reaction mixture was stirred at ambient temperature overnight. The mixture was poured into water (150 mL) then extracted with ether (200 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography to provide (1*R*,2*S*)-methyl 2-(benzyloxycarbonylamino)cyclobutanecarboxylate. ¹H NMR (400 MHz, CDCl₃): δ 7.34 (m, 5H), 5.69 (m, 1H), 5.08 (s, 2H), 4.62 (m, 1H), 3.66 (s, 3H), 3.38 (m, 1H), 2.32 (m, 2H), 1.99 (m, 2H).

[00277] To a solution of (1*R*,2*S*)-methyl 2-(benzyloxycarbonylamino)cyclobutanecarboxylate (8.9 g, 33.8 mmol) in MeOH (250 mL) was added NaOMe (9.1 g, 169 mmol). The mixture was heated at 45 °C for 4 h then cooled to 0 °C and HOAc (10.2 g, 169 mmol) was added dropwise. The mixture was then poured into water (500 mL) and extracted with ethyl acetate (300 mL). The extract was washed with saturated aqueous NaHCO₃ and dried over anhydrous sodium sulfate. The organic solution was concentrated and the residue was purified by column chromatography to provide (1*S*,2*S*)-methyl 2-(benzyloxycarbonylamino)cyclobutanecarboxylate. ¹H NMR (400 MHz, CDCl₃): δ 7.34 (m, 5H), 5.08 (m, 3H), 4.38 (m, 1H), 3.72 (s, 3H), 3.38 (m, 1H), 2.32 (m, 2H), 1.89 (m, 2H).

[00278] To a solution of (1*S*,2*S*)-methyl 2-(benzyloxycarbonylamino)cyclobutanecarboxylate (1.3 g, 4.9 mmol) in THF (10 mL) was added LiBH₄ (0.38 g, 9.8 mmol). The reaction mixture was stirred for 3 h at ambient temperature then poured into water (15 mL) and extracted with ethyl acetate. The organic phase was dried over

anhydrous sodium sulfate and concentrated to provide benzyl (1*S*,2*S*)-2-(hydroxymethyl)cyclobutylcarbamate. ¹H NMR (400 MHz, CDCl₃): δ 7.34 (m, 5H), 5.08 (m, 3H), 3.72 (m, 1H), 3.57 (m, 2H), 2.32 (m, 2H), 1.89 (m, 2H), 1.45 (m, 1H).

[00279] To a solution of benzyl (1*S*,2*S*)-2-(hydroxymethyl)cyclobutylcarbamate (1.1 g, 4.94 mmol), PPh₃ (1.5 g, 5.9 mmol) and isoindoline-1,3-dione (1.0 g, 6.9 mmol) in toluene (10 mL) was added DIAD (1.2 g, 5.9 mmol) at 0 °C. The reaction mixture was stirred for 3 h. The precipitate was filtered off and the filtrate was concentrated and purified by column chromatography to provide benzyl (1*S*,2*R*)-2-((1,3-dioxoisindolin-2-yl)methyl)cyclobutylcarbamate.

[00280] To a solution of benzyl (1*S*,2*R*)-2-((1,3-dioxoisindolin-2-yl)methyl)cyclobutylcarbamate (2.0 g, 5.5 mmol) in EtOH (40 mL) was added hydrazine hydrate (0.64 g). The reaction mixture was refluxed for 3 h. The mixture was cooled, filtered, and the filtrate was concentrated. The residue was dissolved in 1*N* aqueous HCl (30 mL) and then washed with ether (20 mL). The aqueous layer was adjusted to pH=10. The resulting mixture was extracted with DCM (50 mL×2). The combined organics were dried over anhydrous sodium sulfate and concentrated to provide benzyl (1*S*,2*R*)-2-(aminomethyl)cyclobutylcarbamate.

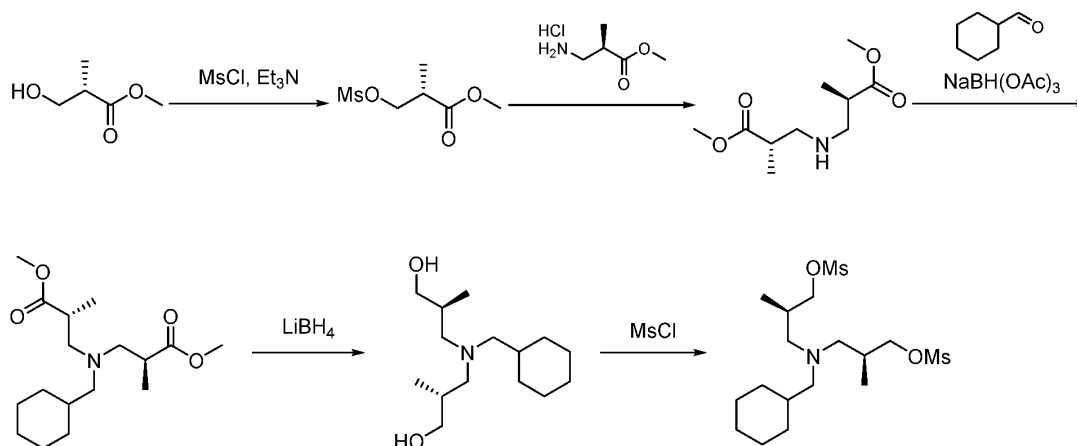
[00281] To a solution of benzyl (1*S*,2*R*)-2-(aminomethyl)cyclobutylcarbamate (0.8 g, 3.4 mmol) in CH₃CN (10 mL) was added 1-((6-chloro-3,4-dihydroisoquinolin-2(1*H*)-yl)sulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (2.4 g, 5.12 mmol). The reaction mixture was stirred at ambient temperature overnight. The mixture was concentrated and the residue was purified by column chromatography to provide benzyl (1*S*,2*R*)-2-((6-chloro-1,2,3,4-tetrahydroisoquinoline-2-sulfonamido)methyl) cyclobutylcarbamate.

[00282] To 40% HBr solution in HOAc (10 mL) was added benzyl (1*S*,2*R*)-2-((6-chloro-1,2,3,4-tetrahydroisoquinoline-2-sulfonamido)methyl) cyclobutylcarbamate (1.2 g, 2.6 mmol). The mixture was stirred for 3 h then poured into water (50 mL) and washed with ether. The aqueous layer was adjusted to pH=10 with 8*N* aqueous NaOH. The mixture was extracted with DCM (50 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated to provide *N*-(((1*R*,2*S*)-2-aminocyclobutyl)methyl)-6-chloro-3,4-dihydroisoquinoline-2(1*H*)- sulfonamide.

[00283] To a solution of *N*-(((1*R*,2*S*)-2-aminocyclobutyl)methyl)-6-chloro-3,4-dihydroisoquinoline-2(1*H*)- sulfonamide (0.66 g, 2.0 mmol) and NEt₃ (350 mg, 3.5 mmol) in DCM (5 mL) was added 4-(dimethylamino)benzenesulfonyl chloride (426 mg, 2.0 mmol) at 0 °C. The reaction mixture was stirred at ambient temperature for 2 h then concentrated. The residue was dissolved in DCM and washed with water. The organic phase was dried over

anhydrous sodium sulfate and concentrated to provide 6-chloro-*N*-(((1*R*,2*S*)-2-(4-(dimethylamino)phenylsulfonamido)cyclobutyl) methyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide. ¹H NMR (400 MHz, CDCl₃): δ 7.77 (m, 2H), 7.18 (m, 2H), 7.05 (m, 1H), 6.75 (m, 2H), 5.28 (m, 1H), 5.05 (m, 1H), 4.36 (s, 2H), 3.50 (m, 2H), 3.35 (m, 1H), 3.15 (m, 1H), 3.09 (s, 6H), 2.92 (m, 3H), 2.42 (m, 1H), 2.03 (m, 1H), 1.65 (m, 2H), 1.25 (m, 1H).

(*S*)-3-((Cyclohexylmethyl)((*R*)-2-methyl-3-(methylsulfonyloxy)propyl)amino)-2-methylpropyl methanesulfonate (Intermediate for compound **A178**)



[00284] To a solution of (*S*)-methyl 3-hydroxy-2-methylpropanoate (4.0 g, 25.42 mmol) and triethylamine (5.3 g, 50.84 mmol) in DCM (60 mL) was added slowly methanesulfonyl chloride (5.6 g, 10.20 mmol) at 0–5 °C. The reaction mixture was stirred for 1 h at ambient temperature. The mixture was then diluted with DCM (100 mL) and washed with water (50 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated to provide (*S*)-methyl 2-methyl-3-(methylsulfonyloxy)propanoate.

[00285] To a solution of (*R*)-methyl 2-aminopropanoate hydrochloride (1.60 g, 10.20 mmol) in acetonitrile (200 mL) was added (*S*)-methyl 2-methyl-3-(methylsulfonyloxy)propanoate (4.2 g, 21.43 mmol) and potassium carbonate (4.20 g, 30.60 mmol). The reaction mixture was refluxed for 16 h then filtered and the filtrate was concentrated to provide (*2R,2'S*)-dimethyl 2,2'-azanediylbis(methylene)dipropionate.

[00286] To a solution of (*2R,2'S*)-dimethyl 2,2'-azanediylbis(methylene)dipropionate (650 mg, 3.0 mmol) in DCM (10 mL) was added cyclohexanecarbaldehyde (336 mg, 3.0 mmol) and sodium triacetoxyborohydride (954 mg, 4.50 mmol). The reaction mixture was stirred overnight at ambient temperature. The mixture was quenched with water and adjusted to pH=10. The resulting mixture was extracted with DCM. The extract was concentrated and the residue was purified by column chromatography to provide (*2R,2'S*)-dimethyl 2,2'-

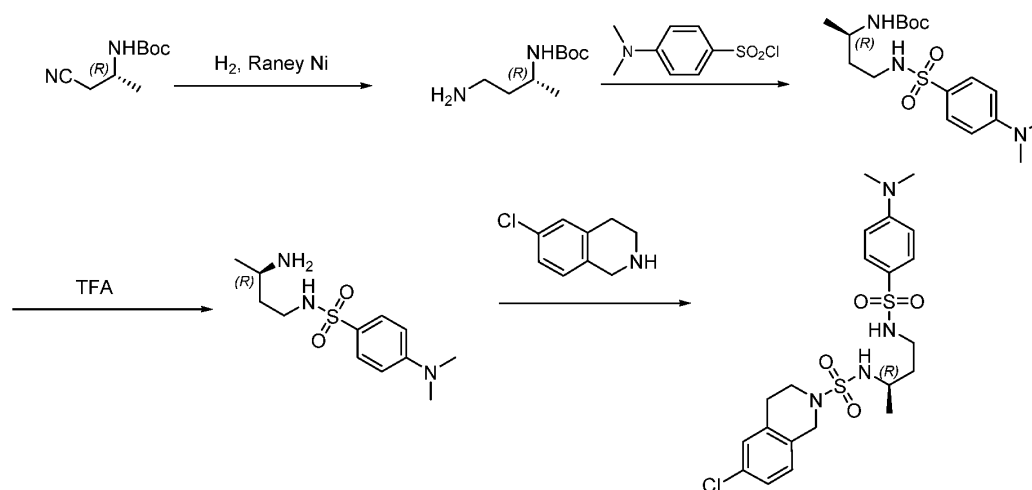
(cyclohexylmethylazanediy)bis(methylene)dipropionate.

[00287] To a solution of (2*R*,2'*S*)-dimethyl 2,2'-(cyclohexylmethylazanediy)bis(methylene)dipropionate (350 mg, 1.28 mmol) in THF (10 mL) was added LiBH₄ (486 mg, 12.80 mmol). The reaction mixture was stirred at 80 °C overnight. The mixture was cooled and quenched with water. The resulting mixture was adjusted to pH=5 with 1*N* aqueous hydrochloric acid and stirred for 0.5 h then adjusted to pH=10 and extracted with ethyl acetate three times. The combined organics were dried over anhydrous sodium sulfate and concentrated to provide (2*R*,2'*S*)-2,2'-(cyclohexylmethylazanediy)bis(methylene)diprop-1-ol.

[00288] To a solution of (2*R*,2'*S*)-2,2'-(cyclohexylmethylazanediy)bis(methylene)diprop-1-ol (153 mg, 0.59 mmol) and triethylamine (0.12 g, 1.2 mmol) in DCM (5 mL) was added MsCl (135 mg, 1.18 mmol) at 0 °C. The reaction mixture was stirred for 3 h at ambient temperature. The mixture was diluted with DCM (15 mL) and then washed with water (20 mL×2). The organics were dried over anhydrous sodium sulfate and concentrated to provide (*S*)-3-((cyclohexylmethyl)((*R*)-2-methyl-3-(methylsulfonyloxy)propyl)amino)-2-methylpropyl methanesulfonate. MS (EI) C₁₇H₃₅NO₆S₂, found 414 [M+H]⁺.

[00289] The following compound was synthesized in a similar manner: (2*S*,2'*S*)-((cyclohexylmethyl)azanediy)bis(2-methylpropane-3,1-diyl) dimethanesulfonate (Intermediate for compound **A160**)

(*R*)-6-Chloro-*N*-(4-(4-(dimethylamino)phenylsulfonamido)butan-2-yl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (Intermediate for compound **A148**)



[00290] A solution of (*R*)-*tert*-butyl 1-cyanopropan-2-ylcarbamate (13.0 g, 71 mmol) in ethanol (250 mL) saturated with anhydrous ammonia was treated with Raney-Ni (24 g) under 55 psi of H₂ overnight. The mixture was filtered through a pad of celite and the filtrate

was concentrated. The residue was purified by column chromatography to provide (*R*)-*tert*-Butyl 4-aminobutan-2-ylcarbamate.

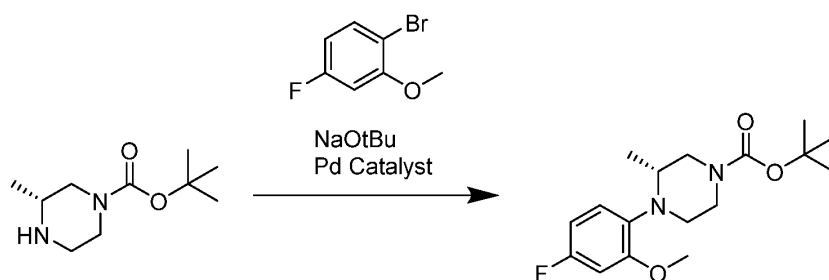
[00291] To a solution of (*R*)-*tert*-butyl 4-aminobutan-2-ylcarbamate (2.5 g, 13.3 mmol) and triethylamine (1.33 g, 13.3 mmol) in DCM (25 mL) was added slowly a solution of 4-(dimethylamino)benzene-1-sulfonyl chloride (2.93 g, 13.3 mmol) in DCM (15 mL) at 0-5 °C. The reaction mixture was stirred for 1 h at ambient temperature. The mixture was diluted with DCM (50 mL) and washed with water (30 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography to provide (*R*)-*tert*-butyl 4-(4-(dimethylamino)phenylsulfonamido)butan-2-ylcarbamate.

[00292] To a solution of (*R*)-*tert*-butyl 4-(4-(dimethylamino)phenylsulfonamido)butan-2-ylcarbamate (1.2 g, 3.2 mmol) in DCM (15 mL) was added TFA (7 mL). The mixture was stirred for 2 h and the solvent was removed. The residue was dissolved in DCM (30 mL) and then washed with saturated aqueous NaHCO₃. The organic phase was dried over anhydrous sodium sulfate and concentrated to provide (*R*)-*N*-(3-aminobutyl)-4-(dimethylamino)benzenesulfonamide (0.88 g, quantitative).

[00293] To a solution of (*R*)-*N*-(3-aminobutyl)-4-(dimethylamino)benzenesulfonamide (0.88 g, 4.0 mmol) in CH₃CN (10 mL) was added 6-chloro-1,2,3,4-tetrahydroisoquinoline (1.98 g, 4.0 mmol). The reaction mixture was stirred at ambient temperature overnight. The mixture was concentrated and the residue was purified by column chromatography to provide (*R*)-6-chloro-*N*-(4-(4-(dimethylamino)phenylsulfonamido)butan-2-yl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide.

[00294] The following compounds were synthesized in a similar manner: (S)-6-chloro-*N*-(4-((4-(dimethylamino)phenyl)sulfonamido)butan-2-yl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (Intermediate for compound **A147**); (S)-6-chloro-*N*-(3-((4-(dimethylamino)phenyl)sulfonamido)butyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (Intermediate for compound **A150**); and (R)-6-chloro-*N*-(3-((4-(dimethylamino)phenyl)sulfonamido)butyl)-3,4-dihydroisoquinoline-2(1*H*)-sulfonamide (Intermediate for compound **A149**)

(R)-1-(4-fluoro-2-methoxyphenyl)-2-methylpiperazine (Intermediate for compound **A177**)



[00295] To tert-butyl (R)-3-methylpiperazine-1-carboxylate (5.37 g, 26.8 mmol) and 1-bromo-4-fluoro-2-methoxybenzene (5.00 g, 24.4 mmol) in toluene (50 mL) was added sodium tert-butoxide (4.69 g, 48.8 mmol) followed by BrettPhos palladacycle G3 (66.4 mg, 0.0732 mmol). The mixture was sealed under nitrogen and stirred at 100 °C and after 1 h the mixture was cooled to ambient temp, diluted with ethyl acetate and water. The organic layer was dried with sodium sulfate, filtered, and concentrated. Crude product was purified by column chromatography (0-40%, ethyl acetate/hexanes) to provide the product. MS(EI) for $C_{17}H_{25}FN_2O_3$, found 325 $[M+H]^+$.

[00296] To tert-butyl (R)-4-(4-fluoro-2-methoxyphenyl)-3-methylpiperazine-1-carboxylate (0.51 g, 1.6 mmol) was added HCl (1.6 mL, 4 N in dioxane) and the mixture was allowed to stand at ambient temp for 16 h then diluted with water (5 mL) and ethyl acetate (5 mL). The organic layer was removed and washed with water (1X5 mL). The combined aqueous layers were washed with ethyl acetate (1X5 mL), basified with NaOH (5 N to pH ~12), extracted with DCM (3X5 mL), dried with sodium sulfate, filtered, and concentrated to provide (R)-1-(4-fluoro-2-methoxyphenyl)-2-methylpiperazine which was carried forward without further purification. MS(EI) for $C_{12}H_{17}FN_2O$, found 225 $[M+H]^+$.

[00297] The following compounds were synthesized in a similar manner:

- 1-(4-fluoro-2-methoxyphenyl)piperazine (Intermediate for **A137**)
- 1-(2-methoxyphenyl)piperazine (Intermediate **A154**)
- 2-(piperazin-1-yl)benzonitrile (Intermediate for **A118**)
- 1-(2,6-dimethylphenyl)piperazine (Intermediate for **A119**)
- 2-(4-fluoro-2-methyl)-2,6-diazaspiro[3.3]heptane (Intermediate for **A161**)
- (S)-1-(4-fluoro-2-methylphenyl)-3-methylpiperazine (Intermediate for **A162**)
- (R)-1-(4-fluoro-2-methylphenyl)-3-methylpiperazine (Intermediate for **A163**)
- 1-(2-(trifluoromethoxy)phenyl)piperazine (Intermediate for **A173**)
- 1-(3,4-difluoro-2-methoxyphenyl)piperazine (Intermediate for **A174**)
- 1-(3-fluoro-2-methoxyphenyl)piperazine (Intermediate for **A175**)
- 1-(5-fluoro-2-methoxyphenyl)piperazine (Intermediate for **A176**)
- 1-(2-chlorophenyl)piperazine (Intermediate for **A198**)

1-(4-methoxyphenyl)piperazine (Intermediate for **A199**)
1-(3-methoxyphenyl)piperazine (Intermediate for **A201**)
1-(3-fluoro-4-methoxyphenyl)piperazine (Intermediate for **A202**)
1-(2-chloro-4-fluorophenyl)piperazine (Intermediate for **A206**)
(S)-1-(3-fluoro-5-methoxyphenyl)-2-methylpiperazine (Intermediate for **A194**)
(R)-1-(3-fluoro-5-methoxyphenyl)-2-methylpiperazine (Intermediate for **A195**)
(2S,6R)-1-(3-fluoro-5-methoxyphenyl)-2,6-dimethylpiperazine (Intermediate for **A197**)
(2R,6R)-1-(3-fluoro-5-methoxyphenyl)-2,6-dimethylpiperazine (Intermediate for **A200**)

Assays

Dox Induced PD1-ss-Gluc Assay

[00298] Flp-In 293 T-RExTM cells were transfected with pcDNATM5/FRT/TO plasmid inserted with cDNA encoding Gaussia Luciferase fused to the 3' end of cDNA encoding PD1 signal sequence plus 10 amino acids (N-MQIPQAPWPVWAVLQLGWRPGWFLDSPDR-C) (SEQ ID NO: 1). Transfected cells were selected for resistance to the selectable markers Hygromycin and Blasticidin to create a stable cell line that contained the PD1-ss+10aa/Gaussia Luciferase cDNA insert whose expression was regulated under the T-RExTM system. The day before assay, cells were trypsinized and plated in 384-well tissue culture plates. The next day, compound dilutions in DMSO/media containing doxycycline were added to the wells and incubated at 37°C, 5% CO₂. 24 hours later, coelenterazine substrate was added to each well and luciferase signal was quantified using Tecan Infinite M1000 Pro for potency determination.

[00299] Results for select compounds provided herein are shown in Table 1, below. For chemical structures that include one or more stereoisomers, but are illustrated without indicating stereochemistry, the assay data refers to a mixture of stereoisomers.

Dox Induced PD1-FL-Gluc Assay

[00300] Flp-In 293 T-RExTM cells were transfected with pcDNATM5/FRT/TO plasmid inserted with cDNA encoding Firefly Luciferase fused to the 3' end of cDNA encoding full length PD1 (amino acids 1-288). Transfected cells were selected for resistance to the selectable markers Hygromycin and Blasticidin to create a stable cell line that contained the PD1-FL/Firefly Luciferase cDNA insert whose expression was regulated under the T-RExTM system. The day before assay, cells were trypsinized and plated in 384-well tissue culture plates. The next day, compound dilutions in DMSO/media containing doxycycline were added to the wells and incubated at 37°C, 5% CO₂. 24 hours later, coelenterazine substrate was added to each well and luciferase signal was quantified using Tecan Infinite M1000 Pro for potency determination.

[00301] Results for select compounds provided herein are shown in Table 1, below. For chemical structures that include one or more stereoisomers, but are illustrated without indicating stereochemistry, the assay data refers to a mixture of stereoisomers.

Dox Induced TNF α -FL-Gluc Assay

[00302] Flp-In 293 T-RExTM cells were transfected with pcDNATM5/FRT/TO plasmid inserted with cDNA encoding Gaussia Luciferase fused to the 3' end of cDNA encoding full length TNF α (amino acids 1-233). Transfected cells were selected for resistance to the selectable markers Hygromycin and Blasticidin to create a stable cell line that contained the TNF α -FL/Gaussia Luciferase cDNA insert whose expression was regulated under the T-RExTM system. The day before assay, cells were trypsinized and plated in 384-well tissue culture plates. The next day, compound dilutions in DMSO/media containing doxycycline were added to the wells and incubated at 37°C, 5% CO₂. 24 hours later, coelenterazine substrate was added to each well and luciferase signal was quantified using Tecan Infinite M1000 Pro for potency determination.

[00303] Results for select compounds provided herein are shown in Table 1, below. For chemical structures that include one or more stereoisomers, but are illustrated without indicating stereochemistry, the assay data refers to a mixture of stereoisomers.

Dox Induced IL2-FL-Gluc Assay

[00304] Flp-In 293 T-RExTM cells were transfected with pcDNATM5/FRT/TO plasmid inserted with cDNA encoding Gaussia Luciferase fused to the 3' end of cDNA encoding full length IL-2 (amino acids 1-153). Transfected cells were selected for resistance to the selectable markers Hygromycin and Blasticidin to create a stable cell line that contained the IL-2-FL/Gaussia Luciferase cDNA insert whose expression was regulated under the T-RExTM system. The day before assay, cells were trypsinized and plated in 384-well tissue culture plates. The next day, compound dilutions in DMSO/media containing doxycycline were added to the wells and incubated at 37°C, 5% CO₂. 24 hours later, coelenterazine substrate was added to each well and luciferase signal was quantified using Tecan Infinite M1000 Pro for potency determination.

[00305] Results for select compounds provided herein are shown in Table 1, below. For chemical structures that include one or more stereoisomers, but are illustrated without indicating stereochemistry, the assay data refers to a mixture of stereoisomers.

Dox Induced HER3-ss-Gluc Assay

[00306] Flp-In 293 T-RExTM cells were transfected with pcDNATM5/FRT/TO plasmid inserted with cDNA encoding Gaussia Luciferase fused to the 3' end of cDNA encoding

HER3 signal sequence plus 4 amino acids (N- MRANDALQVLGLLFLSLARGSEVG-C) (SEQ ID NO: 2). Transfected cells were selected for resistance to the selectable markers Hygromycin and Blasticidin to create a stable cell line that contained the HER3-ss+4aa/Gaussia Luciferase cDNA insert whose expression was regulated under the T-REx™ system. The day before assay, cells were trypsinized and plated in 384-well tissue culture plates. The next day, compound dilutions in DMSO/media containing doxycycline were added to the wells and incubated at 37°C, 5% CO₂. 24 hours later, coelenterazine substrate was added to each well and luciferase signal was quantified using Tecan Infinite M1000 Pro for potency determination.

[00307] Results for select compounds provided herein are shown in the below table. For chemical structures that include one or more stereoisomers, but are illustrated without indicating stereochemistry, the assay data refers to a mixture of stereoisomers.

Table for HER3 activity

No.	24hr Dox Inducible Her3(ss+4) Gluc 293FRT/TO: Mean IC50 (nM)	No.	24hr Dox Inducible Her3(ss+4) Gluc 293FRT/TO: Mean IC50 (nM)	No.	24hr Dox Inducible Her3(ss+4) Gluc 293FRT/TO: Mean IC50 (nM)
A122	110.63	A189	959.52	A203	34.13
A120	31.42	A190	35.6	A204	134.41
A137	10.85	A191	116.79	A205	230.38
A151	276.03	A192	1709.12	A206	38.19
A177	10.86	A193	50.85	A207	17.98
A179	399.69	A194	303.25	A208	427.53
A124	912.67	A195	381.8	A209	497.91
A182	203.11	A196	78.19	A210	342.58
A183	2145.26	A197	77.48	B22	57.77
A184	155.56	A198	44.08		
A185	85.5	A199	131.52		
A107	44.21	A200	29.08		
A187	802.1	A201	211.04		
A188	88.04	A202	129.54		

H929 Cell Viability Assay

[00308] The human multiple myeloma cell line NCI-H929 was cultured in Advanced RPMI 1640 media (Gibco®) supplemented with 6% fetal bovine serum, 2mM Glutamine, and 1x Penicillin/Streptomycin. On the day of assay, cells were resuspended in RPMI 1640 media supplemented with 10% fetal bovine serum, 2mM Glutamine, and 1x Penicillin/Streptomycin and plated in 384-well tissue culture plates and treated with compound dilutions in DMSO/media. Plates were incubated at 37°C, 5% CO₂ for 48 hours. After 48 hours, Celltiter-Glo® (Promega) was added to each well and luciferase signal was quantified using Tecan Infinite M1000 Pro for cell viability determination.

[00309] Results for select compounds provided herein are shown in Table 1, below. For chemical structures that include one or more stereoisomers, but are illustrated without indicating stereochemistry, the assay data refers to a mixture of stereoisomers.

U266 Cell Viability Assay

[00310] The human multiple myeloma cell line U266B1 was cultured in RPMI 1640 media supplemented with 10% fetal bovine serum, 2mM Glutamine, and 1x Penicillin/Streptomycin. Cells were plated in 384-well tissue culture plates and treated with compound dilutions in DMSO/media. Plates were incubated at 37°C, 5% CO₂ for 48 hours. After 48 hours, Celltiter-Glo® (Promega) was added to each well and luciferase signal was quantified using Tecan Infinite M1000 Pro for cell viability determination.

[00311] Results for select compounds provided herein are shown in Table 1, below. For chemical structures that include one or more stereoisomers, but are illustrated without indicating stereochemistry, the assay data refers to a mixture of stereoisomers

In Vitro Microsome Stability Assays

[00312] Potassium phosphate (423 µL of a 0.1 M solution) was added to 8-strip deep well tubes followed by human, mouse, rat, or monkey microsomes (25 µL of a 20 mg/mL solution). The tubes were then placed on ice and the test article (2 µL of a 0.25 mM solution in DMSO) was added. The mixture was preincubated at 37 °C for 3 to 5 minutes (shaking at 150 rpm) then the reaction was initiated by adding 50 µL NADPH. An aliquot of samples (100 µL) were collected at 0 and 30 min and 200 µL of an acetonitrile mixture containing IS (compound 914) was added to quench the reaction. After centrifuging for 10 min at 4000 rpm, a mixed solution of the 20 µL of the supernatant (20 µL) plus 100 µL ACN/H₂O (1:1) were injected for LC-MS/MS analysis. Liver microsome stability is assessed by the %remaining of compound at 30 min which is calculated using the following equation:
$$[(AUC_{\text{AnalyteT} = 30} / AUC_{\text{IS}}) / (AUC_{\text{AnalyteT} = 0} / AUC_{\text{IS}})] * 100$$

[00313] Results for select compounds provided herein are shown in Table 1, below. For chemical structures that include one or more stereoisomers, but are illustrated without indicating stereochemistry, the assay data refers to a mixture of stereoisomers.

Transiently Expressed Dox Induced Signal Sequence-Gluc Assay

[00314] Flp-In 293 T-REx™ cells were transfected with 500ng pcDNA™5/FRT/TO plasmids inserted with cDNA encoding Gaussia Luciferase fused to the 3' end of cDNA encoding target signal sequences plus 10 amino acids. Transfected cells were incubated overnight then cells were trypsinized and plated in 384-well tissue culture plates. The next day, compound dilutions in DMSO/media containing doxycycline were added to the wells and incubated at 37°C, 5% CO₂. 24 hours later, coelenterazine substrate was added to each well and luciferase signal was quantified using Tecan Infinite M1000 Pro for potency determination.

Target	Signal Peptide Sequence	+10 Amino Acids	A87	A137	A151
TNFR1	MGLSTVPDLLPLVLLELLVGIYPS GVIG (SEQ ID NO: 3)	LVPHLGDREK (SEQ ID NO: 4)	307	152	1625
TNFR2	MAPVAVWAALAVGLELWAAAH A (SEQ ID NO: 5)	LPAQVAFTPY (SEQ ID NO: 6)	564	186	2114
IL-17a	MTPGKTSLSLLLLSLEAIVKA (SEQ ID NO: 7)	GITIPRNPGC (SEQ ID NO: 8)	447	216	1816
IL-17Ra	MGAARSPPSAVPGPLGLLLLLG VLAPGGAS (SEQ ID NO: 9)	LRLDHRALV (SEQ ID NO: 10)	424	176	1862
IL-23a	MLGSRAVMLLLLPWTAQG (SEQ ID NO: 11)	RAVPGGSSPA (SEQ ID NO: 12)	567	223	1924
IL-12b (p40)	MCHQQLVISWFSLVFLASPLVA (SEQ ID NO: 13)	IWELKKDVYV (SEQ ID NO: 14)	155	74	913
IL-23R	MNQVTIQWDAVIALYILFSWCH G (SEQ ID NO: 15)	GITNINCSGH (SEQ ID NO: 16)	111	47	666
IL-6	MNSFSTSAFGPVAFSLGLLLVLPA AFPAP (SEQ ID NO: 17)	VPPGEDSKDV (SEQ ID NO: 18)	66	38	437
IL-6R	MLAVGCALLAALLAAPGAA (SEQ ID NO: 19)	LAPRRCPAQE (SEQ ID NO: 20)	78	48	445
GP130	MLTLQTWLVQALFIFLTTESTG (SEQ ID NO: 21)	ELLDPCGYIS (SEQ ID NO: 22)	201	161	969
IL-1R1	MKVLLRLICFIALLISS (SEQ ID NO: 23)	LEADKCKERE (SEQ ID NO: 24)	374	282	1474

IL-1R2	MLRLYLVMGVSA (SEQ ID NO: 25)	FTLQPAHTG (SEQ ID NO: 26)	23	17	168
IL-1Ra (isoform 1)	MEICRGLRSHLITLLFLFHSETIC (SEQ ID NO: 27)	RPSGRKSSKM (SEQ ID NO: 28)	111	47	666
IL-2Ra (CD25)	MDSYLLMWGLLTFIMVPGCQA (SEQ ID NO: 29)	ELCDDDPPEI (SEQ ID NO: 30)	159	55	876
IFN-a1	MASPFALLMVLVLSCKSSCSLG (SEQ ID NO: 31)	CDLPETHSLD (SEQ ID NO: 32)	128	57	820
IFN-a2	MALTFALLVALLVLSCKSSCSVG (SEQ ID NO: 33)	CDLPQTHSLG (SEQ ID NO: 34)	51	44	404
IFN-a4	MALSFLLMAVLVLSYKSICSLG (SEQ ID NO: 35)	CDLPQTHSLG (SEQ ID NO: 36)	221	89	1129
IFN-b	MTNKCLLQIALLCFSTTALS (SEQ ID NO: 37)	MSYNLLGFLQ (SEQ ID NO: 38)	818	288	4305
IFNAR1	MMVVLLGATTLLVAVAPWVLS AAAGG (SEQ ID NO: 39)	KNLKSPQKVE (SEQ ID NO: 40)	14	6	145
IFNAR2	MLLSQNAFIFRSLNLVLMVYISLV FG (SEQ ID NO: 41)	ISYDSPDYTD (SEQ ID NO: 42)	133	42	778
CTLA-4	MACLGFRHKAQLNLATRTWPC TLLFFLLFIPVFC (SEQ ID NO: 43)	KAMHVAQPAV (SEQ ID NO: 44)	259	112	1145
PD-L1	MRIFAVFIFMTYWHLLNA (SEQ ID NO: 45)	FTVTVPKDLV (SEQ ID NO: 46)	56	19	366
LAG3	MWEAQFLGLLFLQPLWVAPVKP LQPGAE (SEQ ID NO: 47)	VPVWVAQEGA (SEQ ID NO: 48)	75	48	464
TIM3	MFSHLPFDCVLLLLLLLTRS (SEQ ID NO: 49)	SEVEYRAEVG (SEQ ID NO: 50)	282	132	1354
TIGIT	MRWCLLLIWAQGLRQAPLASG (SEQ ID NO: 51)	MMTGTIETT (SEQ ID NO: 52)	162	79	737
CD96	MEKKWKYCAVYIIQIHFKG (SEQ ID NO: 53)	VWEKTVNTEE (SEQ ID NO: 54)	12	7	107
VISTA	MGVPTALEAGSWRWGSLFALF LAASLGPVAA (SEQ ID NO: 55)	FKVATPYSLY (SEQ ID NO: 56)	211	150	911
B7H3	MLRRRGSPGMGVHVGAALGAL WFCLTGA (SEQ ID NO: 57)	LEVQVPEDPV (SEQ ID NO: 58)	27	9	201
CD73	MCPRAARAPATLLALGAVLWP AAGA (SEQ ID NO: 59)	WELTILHTND (SEQ ID NO: 60)	58	44	375
PDGFRa	MGTSHPAFLVLGCLLTGLSLILC (SEQ ID NO: 61)	QLSLPSILPN (SEQ ID NO: 62)	90	37	565

VGFR2	MQSKVLLAVALWLCVETRA (SEQ ID NO: 63)	ASVGLPSVSL (SEQ ID NO: 64)	684	213	2591
IL-7R	MTILGTTFGMVFSLLQVVSG (SEQ ID NO: 65)	ESGYAQNGDL (SEQ ID NO: 66)	58	16	392
EGFR	MRPSGTAGAALLALLAALCPASR A (SEQ ID NO: 67)	LEEKVCQGT (SEQ ID NO: 68)	60	47	412
HER3	MRANDALQVLGLLFLARG (SEQ ID NO: 69)	SEVGNSQAVC (SEQ ID NO: 70)	89	57	522
VEGF	MNFLLSWVHWSLALLYLHHAK WSQA (SEQ ID NO: 71)	APMAEGGGQN (SEQ ID NO: 72)	111	51	645
TACE (ADAM17)	MRQSLLFTSVVPFVLA (SEQ ID NO: 73)	PRPPDDPGFG (SEQ ID NO: 74)	66	40	391
ADAM10	MVLLRVLILLWSAAGMGG (SEQ ID NO: 75)	QYGNPLNKYI (SEQ ID NO: 76)	231	127	849

Human Peripheral Blood Mononuclear Cell (PBMC) Cytokine Assay

[00315] Human PBMC were freshly isolated from whole blood collections of 3 normal donors utilizing density gradient centrifugation followed by red blood cell lysis. PBMC from each donor were assayed separately. Cells were suspended in RPMI 1640 media supplemented with 5% fetal bovine serum, 2 mM L-glutamine, 10 mM HEPES, and 1X penicillin/streptomycin. PBMC were seeded at 200,000 cells/well in 96-well round-bottom plates either without stimulation or with stimulation by lipopolysaccharide (LPS, 1 µg/mL) or antibodies against human CD3 (plate-bound, 2 µg/mL) and human CD28 (soluble, 2 µg/mL). Serial dilutions of compound in DMSO/media were simultaneously added and PBMC were incubated at 37°C, 5% CO₂ for 24 hours. After 24 hours, plates were centrifuged and half of the supernatant volume removed for cytokine analysis by electrochemiluminescent immunoassay (U-PLEX® Biomarker Multiplex, Meso Scale Discovery). For viability analysis, CellTiter-Glo® (Promega) was added to the remaining material in each well and quantified via luminescent plate reader.

Mean IC50 (nM) [Stimulation Condition]	A87	A137	A133	A177
GM-CSF [Anti-CD3/CD28]	165	25	404	19
IFN γ [Anti-CD3/CD28]	152	21	234	19
IL-1 β [LPS]	>25000	>25000	>25000	>25000
IL-2 [Anti-CD3/CD28]	31	8	197	10

Mean IC50 (nM) [Stimulation Condition]	A87	A137	A133	A177
IL-6 [LPS]	61	12	193	24
IL-23 [LPS]	200	79	1054	54
TNFα [Anti-CD3/CD28]	82	17	216	13
CellTiter-Glo Viability [No Stimulation]	>25000	>25000	>25000	>25000

Efficacy Study: Effect of Compound A87 on tumor growth and body weight in a B16F10 model compared to anti-PD-1 therapy.

[00316] Female C57BL/6 mice (7-8 week old) were injected in the flank subcutaneously with 5×10^6 B16F10 cells in 0.1 ml on Day 0. On Day 3, compound **A87** formulated in 10% ETOH/10% Kolliphor EL was administered IV 30 mg/kg QW, IV 10 mg/kg D1D2, or IP 15 mg/kg QOD. Anti-PD1 antibody treatment (RMP1-14 from Bioxcell, 200 µg) was administered i.p. on a QODx3 dose schedule. Tumor size was monitored with a digital caliper (Fowler) every 2–3 d until reaching a size of 1.5-2.0 cm and expressed as volume (length × width × height). B16F10, a melanoma line, was obtained from ATCC. C57/BL6 mice were purchased from Charles rivers laboratories.

Table 1. Activities of Compounds

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A1	1026.3	1396.1	I.A.	1170.58	I.A.	I.A.	0.21 0.12 0.18 0.18
A2	344.75	807.39	I.A.		10141	I.A.	0.09 0.59 0.09 0.04
A3	241.49	637.58	11412		5080.51	I.A.	0.09 0.37 0.83 0.25
A4	93.87	310.79	3763.69		1220.45	I.A.	0.56 1.17 1.17 0.25

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A5	651.38		I.A.		8429.37	I.A.	0.27 0.24 0.31 0.24
A6	4747.33		I.A.		24073.2	I.A.	0.12 0.17 0.44 0.53
A7	2314.7		I.A.		22428.6	I.A.	0.17 0.23 0.99 0.29
A8	2681.93		I.A.		I.A.	I.A.	0.17 0.61 0.19 0.09
A9	1106.83		I.A.		I.A.	I.A.	0.34 0.14 0.66 0.09
A10	123.42		4687.09		2863.07	I.A.	0.2 8.85 0.14 0.77
A11	365.31	865.78	I.A.	623.7	I.A.	I.A.	0.18 0.17 0.08 0.21
A12	857.59		I.A.		I.A.	I.A.	0.1 0.07 0.22 0.11
A13	25.01		1471.73		987.14	I.A.	0.04 0.1 0.1 0.65
A14	640.11	1234.03	I.A.	1163.27	I.A.	I.A.	5.39 1.13 1.4 0.08
A15	95.1		3063.1		2955.87	14604.7	0.29 0.061 0.13 0.07
A16	108.07		6341.65		4977.83	I.A.	2.09 2.62 41.1 0.11
A17	942.97		I.A.		I.A.	I.A.	0.41 1.06 0.36 0.49
A18	I.A.		I.A.		I.A.	15473.2	0.05 0.11 0.05 0.04

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A19	325.83		I.A.		I.A.	21895.5	0.24 0.1 0.08 0.05
A20	206.77		I.A.		12798.2	I.A.	0.07 0.19 0.06 0.05
A21	184.02		10002.6		8288.81	I.A.	1.77 0.62 0.11 0.14
A22	155.66	404.42	6377.93	370.18	6673.8	I.A.	0.12 0.4 0.11 0.16
A23	758.88		I.A.		I.A.	I.A.	0.17 0.13 0.03 0.04
A24	I.A.		I.A.		I.A.	I.A.	0.13 0.04 0.03 0.03
A25	435.17		I.A.		I.A.	I.A.	0.32 4.16 0.2 0.12
A26	224.49		I.A.		16068.5	I.A.	3.4 1.84 0.13 6.65
A27	492.55		I.A.		I.A.	I.A.	2.15 0.98 2.07 2.52
A28	5251.11		I.A.		10426	I.A.	0.06 0.14 0.06 --
A29	241.1	591.29	I.A.	446.59	I.A.	I.A.	32.4 43.3 19.5 39.6
A30	792.84		I.A.		I.A.	I.A.	0.35 0.28 0.03 0.12
A31	619.25		I.A.		I.A.	I.A.	0.25 0.09 0.03 0.05
A32	I.A.		I.A.		I.A.	I.A.	0.51 0.27 0.09 0.06

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A33	I.A.		I.A.		I.A.	I.A.	0.08 0.13 0.07 0.05
A34	I.A.		I.A.		I.A.	I.A.	0.24 0.12 0.09 0.03
A35	83.03	243.99	> 11897.98		I.A.	I.A.	20.9 30 13.6 24.8
A36	118.49	319.35	> 21809.41	300.59	> 15385.93	I.A.	1.85 0.94 1.54 6.69
A37	202.16		I.A.		I.A.	I.A.	6.03 13.5 5.69 8.26
A38	75.12		5490.99		> 11844.59	I.A.	12.6 24.2 9.04 11.4
A39	761.18		I.A.		I.A.	10421.2	0.26 6 0.11 0.05
A40	308.26		> 15070.18		I.A.	I.A.	0.19 0.12 0.04 0.04
A41	17532.8		I.A.		I.A.	I.A.	0.2 0.14 0.1 0.08
A42	696.43		I.A.		I.A.	I.A.	0.79 7.69 0.81 2.65
A43	46.87	160.55	1208.05	132.63	2974.53	I.A.	20.1 22.3 12 15.4
A44	132.1		12652.5		I.A.	I.A.	43.8 54 38 47.6
A46	50.13		1758.33		3336.62	I.A.	3.63 4.46 1.44 6.12
A47	258.84		I.A.		I.A.	I.A.	5.68 10.7 1.68 12.6

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A48	991.35		I.A.		I.A.	I.A.	0.07 0.4 0.1 0.19
A49	407.05		I.A.		I.A.	I.A.	11.8 16.4 9.36 3.5
A50	22.21	74.12	660.73	56.97	1007.74	6536.29	70.1 3.6 0.79 1.32
A51	61.36		3068.97		6153.41	I.A.	28 34.7 23.6 26.7
A52	45.56		1035.16		2105.88	I.A.	29.3 40.1 38.3 31
A53	35.33		1014.07		1717.71	> 10260.36	39.2 50.5 39 38
A54	26.51		952.37		1907.23	I.A.	34.6 42.4 30.5 33.8
A55	1540.39		I.A.		I.A.	I.A.	1.18 0.97 0.34 4.87
A56	32.76		1255.87		1634.05	I.A.	53.2 57.3 42.6 44.5
A57	73.41		7807.65		I.A.	I.A.	60.2 84 28.5 31.9
A58	72.14		8500.05		I.A.	I.A.	72.8 66.7 53.1 31.6
A59	24.23		615.54		1442.63	I.A.	3.73 3.9 1.94 1.89
A60	18.54		886.17		1483.8	I.A.	60.8 50.3 38.7 46.7
A61	955.94		I.A.		I.A.	I.A.	17.9 19.6 8.96 13.3

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A63	43.65		1873.85		7433.97	I.A.	85.2 88.2 84.4 76.4
A64	74.98		3293.48		6402.59	I.A.	53.1 63.1 48 53.3
A65	153.77		8715.63		I.A.	I.A.	73.4 78 57.8 53.1
A66	187.59		3495.74		9211	I.A.	0.02 0.39 0.35 0.13
A67	40.77		937.29		2398.75	I.A.	28 45.4 27.6 33.2
A68	156.12		6440.61		I.A.	I.A.	55.1 57.1 57.1 50.5
A69	136.39		I.A.		I.A.	I.A.	64.6 74.3 58.2 63
A70	46.08		1301.94		3023.7	I.A.	3.14 6.75 3.26 1.36
A71	63.32		2014.74		7724.78	I.A.	11.5 17.2 6.75 5.55
A72	96.18		6972.14		10286.3	I.A.	43.3 54.6 53.2 62.5
A73	37.27		1861.53		3471.61	I.A.	16.3 71.5 25.4 18.2
A74	77.38	257.97	I.A.	248.26	I.A.	I.A.	53.7 55.7 60.6 56.3
A75	104.41	255.94	3782.3	233.05	3029.96	I.A.	2.75 7.54 0.28 3.08
A76	I.A.	I.A.	I.A.	I.A.	I.A.	I.A.	28.6 33.9 35.7 53.1

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A77	95.05	338	5544.21	359.68	4012.61	I.A.	37.3 32.5 20.8 30.1
A78	359.83		I.A.		I.A.	I.A.	0.15 0.25 0.11 0.2
A79	408.23		I.A.		I.A.	I.A.	10.6 0.1 0.06 0.16
A80	272.95		I.A.		I.A.	I.A.	13.4 14.1 2.56 10.8
A81	476.93		I.A.		I.A.	I.A.	0.12 0.12 0.03 0.2
A82	30.52		1547.55		2298.14	I.A.	0.08 0.16 0.23 0.3
A83	28.17		961.05		959.61	I.A.	23.4 47.6 34.9 39.3
A84	175.94		I.A.		I.A.	I.A.	68.3 67.3 65.8 60.6
A85	87.46		I.A.		I.A.	I.A.	52.2 64.9 42.9 25.6
A86		428.72	I.A.	394.88	5625.91	I.A.	34.1 39.9 29.2 41.3
A87	19.15		492.19	50.23	223.57	I.A.	49.6 43.6 32.7 39.7
A88	4330.54		I.A.	I.A.	I.A.	I.A.	15.9 15.2 1.33 11.7
A89	82.05		1208.13	186.53	1751.25	I.A.	17.1 22.2 13.5 18.5
A90	207.02		2413.57	493.18	3786.86	I.A.	28.2 20.2 14.1 20.1

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A91	193.42		2459.27	376.81	3011.92	14653.1	25.4 21.6 21.8 23.7
A92	304.02		I.A.	518.65	I.A.	I.A.	40.8 51.8 40.4 44.3
A93	670.27		I.A.	911.37	10427.9	I.A.	0.4 0.51 0.18 0.84
A94	36.37		550.17	78.29	800.16	I.A.	45.7 45.5 30.3 42.1
A95	198.02		4897.04	390.92	6711.7	I.A.	54.2 43.1 46.1 43.9
A96	121.85		1847.65	231.78	5104.13	I.A.	37.6 41.9 32.8 40.5
A97	41.78		565.68	87.04	725.76	I.A.	77.7 71.4 66.1 71.1
A98	201.79		7335.28	351.2	I.A.	I.A.	55.2 32.8 26.9 57.5
A99	115.58		3535.19	349.8	I.A.	I.A.	60.8 50.8 37 41.9
A100	1150.93		I.A.	1560.04	I.A.	I.A.	0.1 2.18 0.04 0.22
A101	220.23		3425.89	564.3	I.A.	I.A.	20.8 25.4 18.8 30.2
A102	33.39		477.31	90.53	558.29	I.A.	86.6 101.9 70 62.3
A103	445.68		I.A.	698.23	I.A.	I.A.	42.2 36.5 38.5 31.6

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A106	52.35		1118.61	126.18	902.63	I.A.	8.81 18.9 30.7 7.18
A107	26.11		609.25	72.97	1176.64	I.A.	16.1 19.3 25.0 83.9
A108	29.58		546.31	68.29	420.52	I.A.	0.62 2.05 0.88 1.06
A118	67.79		2093.86	172.7	5345.11	I.A.	22.1 18.3 14.7 14.2
A119	52.34		1077.79	170.3	7675.81	I.A.	50.8 66.1 53.9 71.7
A120	15.58		238.67	27.34	672.16	I.A.	19.2 28.5 16.3 4.78
A122	51.93		885.26	134.57	2420.21	I.A.	36.4 0.17 0.15 0.38
A123	13.98		424.92	45.93	834.51	I.A.	6.13 7.41 3.47 5.02
A124	606.76		I.A.	802.05	I.A.	I.A.	1.01 1.25 1.91 18.9
A125	829.42		11524.98	1090.55	I.A.	I.A.	0.14 0.37 8.67 0.2
A131	145.44		2403.07	426.19	4744.68	I.A.	101 73.9 70.2 84.5
A133	36.84		748.55	134.51	5121.95	I.A.	82.2 87.3 67.3 71.3
A134	11.04		309.22	27.28	540.66	I.A.	27.9 41.3 9.76 31.9
A135	21.58		625.08	48.58	1239.26	I.A.	26.1 56.3 39.2 9.36

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A136	26.46		543.65	101.39	1726.93	I.A.	14.3 26.6 23.2 65.3
A137	8.30		191.24	21.45	281.93	I.A.	28 31.6 14.2 48.8
A138	73.91		947.28	156.27	2396.92	I.A.	13.2 17.1 12.5 8.84
A140	491.56		3009.42	808.08	I.A.	I.A.	3.68 0.56 1.56 0.41
A141	431.02		4235.2	862.9	9106.16	I.A.	0.48 1.14 0.66 0.26
A142	38.25		741.05	67.02	1446.85	I.A.	16 22.6 3.89 11.8
A147	4122.89		I.A.	I.A.	I.A.	I.A.	0.74 1.16 0.99 2.76
A148	925.61		I.A.	1117.32	I.A.	I.A.	1.59 1.29 0.67 0.52
A149	I.A.		I.A.	I.A.	I.A.	I.A.	0.83 0.47 0.96 0.44
A150	30.29		467.79	41.34	2000.66	I.A.	1.38 2.02 2.2 4.6
A151	152.57		1216.54	305.64	2711.98	I.A.	4.55 4.2 3.98 4.28
A152	81.5		1028.9	173.82	2171.97	I.A.	5.06 3.6 3.22 3.69
A153	1293.98		I.A.	1255.35	I.A.	I.A.	0.87 6.23 1.26 2.57
A154	14.94		504.45	58.66	749.43	I.A.	17.5 12.3 10.2 7.33

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A155	1350.05			3484.03	7795.99	I.A.	16.8 38.8 4.86 17.8
A156	92.46		1825.18	206.72	3707.48	I.A.	75.6 82.1 80.6 81.3
A157	23.33		584.47	77.41	858.16	I.A.	23.3 32.2 27.3 21.1
A160	152.83		10687.02	452.72	I.A.	I.A.	61.3 51.1 46.1 71.8
A161	577.46		I.A.	725.52	I.A.	I.A.	26.5 27.3 16.7 20.2
A162	101.25		6263.53	200.98	I.A.	I.A.	62.5 69.6 60.3 70.5
A163	55.56		1928.36	81.21	13622.1	I.A.	72.8 54.9 57.5 64.2
A164	454.69		I.A.	974.47	I.A.	I.A.	69.0 57.9 40.9 64.8
A165	131.14		1857.05	293.51	4086.76	I.A.	14.9 11.9 12.8 2.23
A166	16.68		278.28	30.25	547.83	I.A.	26.4 31.3 18.7 14.0
A167	200.61		2656.44	412.45	6495.51	I.A.	18.3 9.19 73 5.68
A168	1491.98		I.A.	1144.17	I.A.	I.A.	42 42.1 73 25.7
A169	81.52		1048.04	192.27	3272.71	I.A.	22.5 13.1 4.23 34.7
A170	56.13		797.63	133.66	1775.44	I.A.	3.65 10.8 1.37 7.2

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A171	I.A.		I.A.	I.A.	I.A.	I.A.	0.91 0.90 0.26 0.60
A172	29.18		436.2	66.4	1750.08	I.A.	3.65 2.23 2.63 3.46
A173	78.83		5638.24	248.3	I.A.	I.A.	49.2 53.8 55.9 56.1
A174	15.79		565.56	53.31	1861.24	I.A.	36.9 44.4 28.4 41.7
A175	9.35		393.21	40.35	1436.98	I.A.	6.73 6.28 2.27 4.26
A176	17.11		631.2	62.1	3109.99	I.A.	32.4 23.0 15.2 31.9
A177	4.95		154.54	12.57	179.88	I.A.	11.6 14.5 2.8 13.7
A178	134.76		4820.51	255.26	I.A.	I.A.	22.8 29.2 25.8 24.0
A179	269.79		3249.91	72.57	11347.74	I.A.	0.5 0.18 0.26 0.04
A180	2742.03		I.A.	13843.24	20179.62	I.A.	0.44 1.97 0.08 0.80
A181	38.69		983.85	122.41	1479.93	I.A.	17.1 22.0 27.9 23.3
A182	78.84		1957.98	255.55	I.A.	I.A.	61.7 41.9 41.5 53.7
A183	1443.55			2524.48	6116.1	I.A.	7.39 3.17 0.75 10.1
A184	100.13		1079.53	232.68	6916.08	I.A.	5.64 7.47 1.23 2.56

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A185	43.18		573.1	85.38	1468.19	I.A.	30.1 37.8 10.0 21.8
A187	427.09		6171.01	1087.5	3866.88	I.A.	0.03 0.07 0.15 0.05
A188	38.94		714.46	99.28	1555.52	I.A.	4.48 27.8 12.4 27
A189	512.4		8138.23	1358.47	10609.19	I.A.	0.14 0.07 0.01 0.06
A190	16.23		294.54	45.7	867.11	I.A.	69.3 85.3 71.6 74.1
A191	57.62		1095.62	144.64	6734.93	I.A.	55.3 65.0 68.3 48.6
A192	1153.54		I.A.	2332.92	9362.89	I.A.	0.18 0.08 0.10 0.02
A193	23.8		748.16	81.77	6806.9	I.A.	10.1 11.3 7.72 34.2
A194	261.32		I.A.	401.51	I.A.	I.A.	68.5 59.2 65.0 63.6
A195	210.96		11201.43	668.05	I.A.	I.A.	58.5 63.3 32.8 53.6
A196	30.96		1474.7	104.78	I.A.	I.A.	
A197	34.25		666.07	95.53	4125.97	I.A.	65.4 76.6 92.8 70.7
A198	18.54		372.95	53.28	1170.31	I.A.	68.2 61.4 65.1 39.2
A199	80.8		1420.53	162.14	I.A.	I.A.	23.7 28.3 25.5 8.0

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
A200	11.23		284.85	27.87	435.72	I.A.	71.9 83.1 73.9 78.1
A201	117.7		7086.36	282.88	I.A.	I.A.	39.1 35.4 26.5 32.8
A202	56.59		2052.14	175.52	2914.63	I.A.	55.0 50.8 38.3 13.3
A203	14.76		434.38	30.44	396.26	I.A.	80.3 78.7 77.9 83.4
A204	58.42		981.88	157.89	1487.11	I.A.	62.6 58.3 49.7 48.4
A205	128.76		1806.9	305.32	2362.23	I.A.	38.9 38.7 31.7 37.2
A206	17.37		395.41	38.95	657.05	I.A.	84.3 80.5 65 54.7
A207	7.93		221.47	10.6	105.74	I.A.	84.3 98.8 63.0 62.7
A208	343		I.A.	615.95	I.A.	I.A.	51.8 68.9 37.5 23.3
A209	326.73		I.A.	672.98	I.A.	I.A.	31.8 44.7 45.7 22.6
A210	202.76		I.A.	446.86	I.A.	I.A.	76.0 54.6 55.4 47.4
B1	423.7		I.A.		I.A.	I.A.	0.02 0.01 0.02 0.04
B2	1721.99		I.A.		I.A.	I.A.	0.55 1.46 0.23 0.35
B3	7382.27		I.A.		9441.64	12597.4	0.15 0.1 0.08 0.26

No.	24hr Dox Inducible PD1ssGluc 293FRT/TO : Mean IC50 (nM)	24hr Dox Inducible PD1FLFluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible TNFaFLGluc 293FRT/TO: Mean IC50 (nM)	24hr Dox Inducible IL2FLGluc 293FRT/TO: Mean IC50 (nM)	48hr H929 Viability Celltiter-Glo: Mean EC50 (nM)	48hr U266 Viability Celltiter-Glo: Mean EC50 (nM)	Liver Microsome Stability- % Left after 30min w/ NADPH (%) Mouse Rat Monkey Human
B4	I.A.		I.A.		I.A.	16627.8	2.21 2.79 1.7 12.1
B5	1497.99		I.A.		I.A.	I.A.	0.24 7.08 0.21 0.54
B6	416.05		I.A.		I.A.	I.A.	81.8 13.2 4.57 7.66
B7	6900.19		24323		6858.59	7481.69	1.47 0.58 0.04 0.03
B8	I.A.		I.A.		I.A.	I.A.	17.1 23.8 15.5 22.1
B9	3642.93		I.A.		I.A.	I.A.	0.36 3.74 0.26 0.49
B10	16476.4		I.A.	6923.68	I.A.	I.A.	
B11	I.A.		I.A.	I.A.	I.A.	I.A.	
B12	5655.74		I.A.	I.A.	I.A.	I.A.	
B16	449.25		13885	825.91	I.A.	I.A.	58.4 72.3 44.7 55.2
B19	220.99		15364.2		I.A.	I.A.	13.5 15.7 0.98 9.46
B20	794.07		I.A.		I.A.	I.A.	3.41 11.1 1.73 5.43
B21	3951.77		I.A.	I.A.	I.A.	I.A.	0.16 0.06 0.03 0.17
B22	31.31		519.36	80.48	1648.2	I.A.	8.31 10.1 1.77 1.77

*I.A. means and IC50 or EC50 of greater than 25μM