



(51) International Patent Classification:

C07D 401/12 (2006.01) C07D 403/14 (2006.01)
C07D 403/12 (2006.01) A61P 31/12 (2006.01)
C07D 401/14 (2006.01) A61K 31/498 (2006.01)

(21) International Application Number:

PCT/IB2024/000314

(22) International Filing Date:

14 June 2024 (14.06.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/508,204 14 June 2023 (14.06.2023) US

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Street, New York, NY 10016 (US).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM,

DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG,
KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY,
MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA,
NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO,
RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH,
TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS,
ZA, ZM, ZW.

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

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

(54) Title: SMALL MOLECULE LIGANDS AND APTAMERS

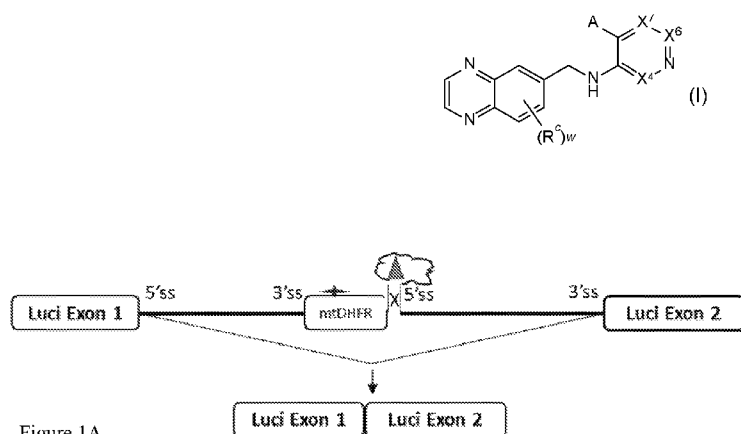


Figure 1A

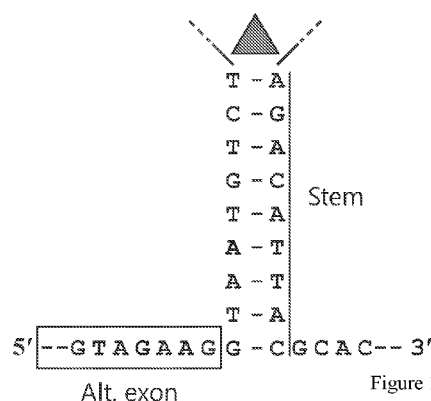


Figure 1B

(57) Abstract: The present disclosure provides small molecules of Formula (I) that bind to aptamers. Also contemplated are riboswitches and polynucleotide cassettes for regulating the expression of a target gene in response to the small molecule, wherein the polynucleotide cassettes comprise the aptamers disclosed herein. The small molecules disclosed herein that bind to the aptamers disclosed herein are modulators of target gene expression where the target gene contains a riboswitch comprising an aptamer described herein.

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

SMALL MOLECULE LIGANDS AND APTAMERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application No. 63/508,204, filed June 14, 2023, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] This application includes a Sequence Listing submitted electronically as an xml file named SeqList-162027-54076.xml, created on June 13, 2023, with a size of 9,477 bytes. The Sequence Listing is incorporated herein by reference.

FIELD

[0003] The present disclosure relates to small molecules that are modulators of target gene expression where the target gene contains a riboswitch comprising an aptamer as described herein. Also disclosed are riboswitches and polynucleotide cassettes for regulating the expression of a target gene, wherein the polynucleotide cassettes comprise the aptamer as disclosed herein.

BACKGROUND

[0004] Aptamers are oligonucleotides that bind to a target ligand with high affinity and specificity. These nucleic acid sequences have proven to be of high therapeutic and diagnostic value with recent FDA approval of the first aptamer drug and additional ones in the clinical pipelines. Their high degree of specificity and versatility have established RNA aptamers as one of the pivotal tools of the emerging RNA nanotechnology field in the fight against human diseases including cancer, viral infections and other diseases.

[0005] In addition, aptamers may be utilized as part of a riboswitch that has certain effects in the presence or absence of an aptamer ligand. For example, riboswitches may be used to regulate gene expression in response to the presence or absence of the aptamer ligand.

[0006] However, aptamers/ligands derived from prokaryotic sources or generated using in vitro selection methods often fail to demonstrate the functionality required for the expression of therapeutic targets genes in eukaryotic systems. For example, the ligand for the aptamer

may be a cellular molecule that would not be appropriate for use in systems for regulating a therapeutic gene product, for example, because presence of the ligand would interfere in the regulation of target gene expression, or because the ligand is not otherwise appropriate for administration to cell or tissue. As such, new aptamer sequences, small molecule ligands, and aptamer/ligand combinations able to regulate gene expression in response to the presence or absence of the small molecule ligand are needed.

SUMMARY

[0007] Provided herein are small molecules of Formula I, including those listed in Table A, that bind to aptamer sequences. The small molecules are modulators of target gene expression where the target gene contains a riboswitch comprising an aptamer, also described herein. Also disclosed are riboswitches and polynucleotide cassettes for regulating the expression of a target gene, wherein the polynucleotide cassettes comprise the aptamers disclosed herein, where the aptamer binds one or more of the small molecules disclosed herein. Further provided are methods of using the small molecules with the aptamers, riboswitches, and/or polynucleotide cassettes for the regulation of target genes, including therapeutic genes.

[0008] In embodiments, the aptamer that binds to one or more small molecules disclosed herein (i.e., one or more of the compounds according to Formulas I-XIV) is encoded by an aptamer encoding sequence disclosed in PCT/IB2022/000762 (WO2023/111686), incorporated herein by reference in its entirety. In embodiments, the aptamer encoding sequence comprises a sequence that is at least 95% identical, or at least 99% identical, to an aptamer encoding sequence disclosed in PCT/IB2022/000762. In embodiments, the aptamer encoding sequence (i) comprises a sequence that is at least 95% identical, or at least 99% identical, to SEQ ID NO: 1; or (ii) comprises SEQ ID NO: 1 (which is referred to herein as the 12C6-1 aptamer).

SEQ ID NO:1 (12C6-1 aptamer):

CTGGGGAGTCCTTCATGCGGGGCTGAGAGGATGGAA
GCAATCGACCATCGACCCATTGCACCTGATCCGGATC
ATGCCGGCGCAGGGAG

[0009] In embodiments, the aptamer sequence disclosed herein, further comprises additional sequence at the 5' and 3' ends that is complementary and capable of forming part of the aptamer P1 stem. In embodiments, this P1 stem of the aptamer is, comprises, or overlaps with the effector region of the riboswitches disclosed herein. In embodiments, the

aptamer P1 stem comprises a 5' splice site sequence of a 3' intron and sequence complementary thereto. For example, the P1 stem may comprise all of, or the intronic portion of: A G || G G T G A G T; A A A || G T A A G C; G C A || G T A A G T; G A G || G T G T G G; A/C A G || G T A/G A G T; N A G || G T A/G A G T; N A G || G T A A G T; A/C A/T G || G T A N G T; or N A G/A || G T A A G T (where N can be A, G, C, or T, and where || represents the exon-intron boundary).

[0010] In embodiments, the aptamers disclosed (including referenced) herein bind to one or more of the small molecules of Formula I to XIV, including those listed in Table A.

[0011] In one aspect, the disclosure provides the RNA aptamer encoded by the aptamer encoding sequences disclosed herein.

[0012] In one aspect, the disclosure provides nucleic acid sequence encoding a recombinant riboswitch for the regulation of target gene expression in response to a small molecule, wherein the riboswitch comprises an aptamer disclosed herein.

[0013] In another aspect, the disclosure provides a polynucleotide cassette for regulating the expression of a target gene, wherein the polynucleotide cassette comprises a sequence encoding an aptamer that binds to a small molecule, wherein the aptamer encoding sequence comprises an aptamer encoding sequence disclosed herein.

[0014] In embodiments, the polynucleotide cassette comprises sequence encoding:

- (a) a riboswitch; and
- (b) an alternatively-spliced exon, flanked by a 5' intron and a 3' intron,

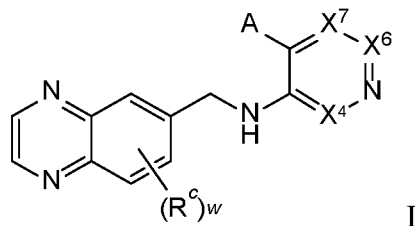
wherein the riboswitch comprises (i) an effector region comprising a stem forming sequence that includes the 5' splice site sequence of the 3' intron (and sequence complementary to the 5' splice site sequence of the 3' intron), and (ii) the aptamer comprises an aptamer sequence disclosed herein; and

wherein the alternatively-spliced exon comprises a stop codon that is in-frame with the target gene when the alternatively-spliced exon is spliced into the target gene mRNA.

[0015] In embodiments, the effector stem is, or comprises, a P1 stem of the aptamers disclosed herein. In other words, the effector stem comprises a first sequence that is linked to the 5' end of the aptamers disclosed herein and a second sequence that is linked to the 3' end of the aptamers disclosed herein, wherein the first and second sequence comprise sequence that is complementary and capable of forming a stem.

[0016] In embodiments, the polynucleotide cassette is located in the protein coding sequence of the target gene. In embodiments, the polynucleotide cassette is located in an untranslated region of the target gene or in an intron of the target gene.

[0017] In embodiments, the small molecule has the structure according to Formula I:



I

or a pharmaceutically acceptable salt thereof, wherein

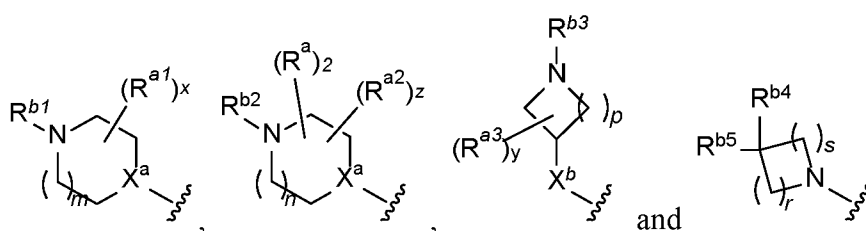
X^4 is selected from CH, CR^d and N;

X^6 is selected from CH, CR^d and N;

X^7 is selected from CH, CR^d and N;

wherein 0 or 1 of X^4 , X^6 or X^7 is N;

A is selected from the group consisting of:



X^a is selected from N and CH;

X^b is selected from O, NH, and NCH_3 ;

each R^{a1} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a1} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

each R^a is independently selected from C_1 to C_3 alkyl both of which C_1 to C_3 alkyl groups are bonded to the same ring carbon atom, or alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring;

each R^{a2} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, and halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

each R^{a3} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b1} is C_1 to C_3 alkyl or C_3 to C_6 cycloalkyl, each optionally substituted with halo, OH, or $O-C_{1-3}$ alkyl;

R^{b2} is selected from H, C_1 to C_3 alkyl optionally substituted with halo, OH, or $O-C_{1-3}$ alkyl;

R^{b3} is C_1 to C_3 alkyl optionally substituted with halo, OH, or $O-C_{1-3}$ alkyl;

R^{b4} is selected from OH, $-NH_2$, $-NH(C_1 \text{ to } C_3 \text{ alkyl})$, and $-N(C_1 \text{ to } C_3 \text{ alkyl})_2$;

R^{b5} is selected from H and C_1 to C_3 alkyl;

m is 1 or 2; n is 1 or 2; p is 1 or 2; r is 1 or 2; s is 1 or 2;

x is 0, 1, 2 or 3;

y is 0, 1, 2 or 3;

z is 0, 1, or 2;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH; and

w is 0, 1 or 2.

[0018] In embodiments, the small molecule has a structure according to Formula II-XIV, including, e.g., a structure provided in Table A. The small molecule ligands of the Formula I-XIV and Table A may provide one or more beneficial properties, including improved penetration of the blood-brain barrier, improved partitioning into the tissue of the eye, low toxicity; and good bioavailability.

[0019] In one aspect the disclosure provides a vector comprising a polynucleotide cassette, an aptamer encoding sequence/aptamer sequence, and/or riboswitch disclosed herein. In embodiments, the vector is a viral vector or a non-viral vector. In embodiments, the viral vector is an adenoviral vector, an adeno-associated virus vector, and a lentiviral vector.

[0020] In one aspect, the disclosure provides a cell comprising a vector, a polynucleotide cassette, an aptamer encoding sequence/aptamer sequence, or riboswitch disclosed herein.

[0021] The disclosure also provides methods for modulating the expression of a target gene using a polynucleotide cassette, an aptamer encoding sequence/aptamer sequence, or riboswitch disclosed herein, by provided to a cell or tissue a small molecule of Formula I-XIV, including, e.g., a small molecule provided in Table A.

BRIEF DESCRIPTION OF THE FIGURES

[0022] **Figures 1A and 1B.** **Fig. 1A:** schematic of an embodiment of a synthetic riboswitch cassette containing a riboswitch in the context of an intron-alternative exon-aptamer-intron. **Fig. 1B:** In the presence of aptamer ligand, aptamer ligand binding facilitates the formation of the effector stem that sequesters the accessibility of the splice site sequence on the 3' end of the alternative exon (e.g., the 5' splice site sequence of the 3' intron), resulting in the exclusion of the stop codon containing alternative exon from the target gene mRNA and target gene expression.

[0023] **Figures 2A and 2B** show the exposure levels in the plasma (P), brain (B) and eye (E) for mice dosed with selected compounds at 100 mg/kg PO. Fold induction of luciferase expression, in response to the compounds, in HEK293 cells from a luciferase gene containing the 12C6-1 gene regulation cassette (SEQ ID NO: 4) is also provided.

DETAILED DESCRIPTION

[0024] Provided herein are aptamer sequences that bind to, or otherwise respond to the presence of, small molecules of Formula I-XIV. In some embodiments, the aptamer sequences provided herein are useful for the regulation of the expression of a target gene in response to the presence or absence of the small molecule ligand. Also contemplated are recombinant riboswitches comprising the aptamer sequences disclosed herein, as well as recombinant polynucleotide cassettes for regulating the expression of a target gene, wherein the polynucleotide cassettes comprise sequences encoding the riboswitches disclosed herein. Also provided herein are methods of using the aptamers, riboswitches, and/or polynucleotide cassettes for the regulation of target genes, including therapeutic genes, and for the treatment of subjects in need thereof.

[0025] Aptamers

[0026] Aptamers are single-stranded nucleic acid molecules that non-covalently bind to specific ligands with high affinity and specificity by folding into three-dimensional

structures. Aptamer ligands include ions, small molecules, proteins, viruses, and cells.

[0027] Aptamer ligands can be, for example, an organic compound, amino acid, steroid, carbohydrate, or nucleotide. Non-limiting examples of small molecule aptamer ligands include antibiotics, therapeutics, dyes, cofactors, metabolites, molecular markers, neurotransmitters, pollutants, toxins, food adulterants, carcinogens, drugs of abuse. As such, aptamers are useful for the detection of small molecules. Application of small-molecule detection by aptamers include environmental monitoring, food safety, medicine (including diagnostics), microbiology, analytical chemistry, forensic science, agriculture, and basic biology research.

[0028] The term "aptamer" as used herein refers to an RNA polynucleotide (or DNA sequence encoding the RNA polynucleotide) that specifically binds to a ligand or class of ligands. The term "ligand" refers to a molecule that is specifically bound by an aptamer. Aptamers have binding regions that are capable of forming complexes with an intended target molecule (i.e., the ligand). An aptamer will typically be between about 15 and about 200 nucleotides in length. More commonly, an aptamer will be between about 30 and about 100 nucleotides in length, for example, 70 to 90 nucleotides in length. Aptamers typically comprise multiple paired (P) regions in which the aptamer forms a stem and unpaired regions where the aptamer forms a joining (J) region or a loop (L) region. The paired regions can be numbered sequentially starting at the 5' end (P1) and numbering each stem sequentially (P2, P3, etc.). The loops (L1, L2, etc.) are numbered based on the adjacent paired region and the joining regions are numbered according to the paired regions that they link.

[0029] In embodiments, the aptamer that binds to one or more small molecules disclosed herein (i.e., one or more of the compounds according to Formulas I-XIV) is encoded by an aptamer encoding sequence disclosed in PCT/IB2022/000762 (WO2023/111686), incorporated herein by reference in its entirety. In embodiments, the aptamer encoding sequence comprises a sequence that is at least 95% identical, or at least 99% identical, to an aptamer encoding sequence disclosed in PCT/IB2022/000762. In embodiments, the aptamer encoding sequence (i) comprises a sequence that is at least 95% identical, or at least 99% identical to SEQ ID NO: 1; or (ii) comprises SEQ ID NO: 1 (which is referred to herein as the 12C6-1 aptamer).

[0030] In embodiments, the first and the last nucleotide of the aptamer encoding sequence disclosed herein can be any nucleotide or no nucleotide. In embodiments, the first two and the last two nucleotides of the aptamer encoding sequence disclosed herein can be any nucleotide or no nucleotide. In these embodiments, additional sequence that is 5' and 3' of

the aptamer encoding sequence may be present and form part of the stem forming sequence of the riboswitch.

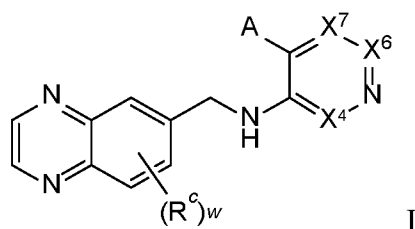
[0031] In one aspect, the disclosure provides the aptamer encoded by the aptamer encoding sequences disclosed herein.

[0032] The ordinarily skilled artisan would understand that the aptamers described herein may be ribonucleic acid (RNA) molecules. In embodiments, the aptamers described herein are part of a longer RNA polynucleotide, including, for example, hnRNA, mRNA, siRNA, or miRNA.

[0033] Aptamer Ligands

[0034] In embodiments, an aptamer disclosed herein binds to, or otherwise responds to the presence or addition of, a small molecule (ligand) disclosed herein, including small molecules having the structure according to Formula I to XIV, including the small molecules in Table A.

[0035] In embodiments, the small molecule has a structure according to Formula I:



or a pharmaceutically acceptable salt thereof, wherein

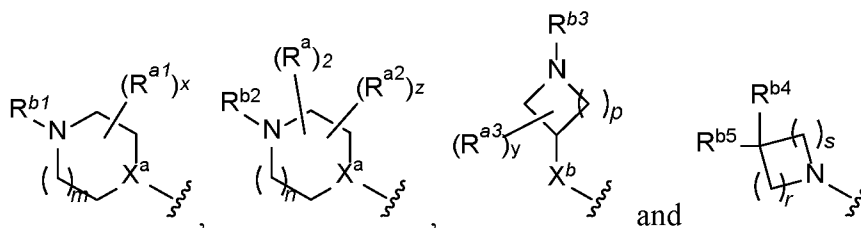
X^4 is selected from CH, CR^d and N;

X^6 is selected from CH, CR^d and N;

X^7 is selected from CH, CR^d and N;

wherein 0 or 1 of X^4 , X^6 or X^7 is N;

A is selected from the group consisting of:



X^a is selected from N and CH;

X^b is selected from O, NH, and NCH_3 ;

each R^{a1} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$,

halo, hydroxyl and amino; or additionally or alternatively, two R^{a1} attached to the

same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

each R^a is independently selected from C₁ to C₃ alkyl both of which C₁ to C₃ alkyl groups are bonded to the same ring carbon atom, or alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring;

each R^{a2} is independently selected from C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, and halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

each R^{a3} is independently selected from C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b1} is C₁ to C₃ alkyl or C₃ to C₆ cycloalkyl, each optionally substituted with halo, OH, or O-C₁₋₃ alkyl;

R^{b2} is selected from H, C₁ to C₃ alkyl optionally substituted with halo, OH, or O-C₁₋₃ alkyl;

R^{b3} is C₁ to C₃ alkyl optionally substituted with halo, OH, or O-C₁₋₃ alkyl;

R^{b4} is selected from OH, -NH₂, -NH(C₁ to C₃ alkyl), and -N(C₁ to C₃ alkyl)₂;

R^{b5} is selected from H and C₁ to C₃ alkyl;

m is 1 or 2; *n* is 1 or 2; *p* is 1 or 2; *r* is 1 or 2; *s* is 1 or 2;

x is 0, 1, 2 or 3;

y is 0, 1, 2 or 3;

z is 0, 1, or 2;

each R^c is independently selected from halo, C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, -CN, hydroxyl and amino;

each R^d is independently selected from halo, C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, -CN, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH; and

w is 0, 1 or 2.

[0036] For the compounds according to Formula I, x may be selected to be 1, 2 or 3; x may be selected to be 1 or 2; or x may be selected to be 1. R^{a1} may be selected to be methyl, fluoro or chloro; or R^{a1} may be selected to be methyl. Alternatively, x may be 0.

[0037] For the compounds according to Formula I, y may be selected to be 0 or 1. R^{a3} may be selected from halo or methyl; or R^{a3} may be selected to be methyl. Alternatively, y may be 0.

[0038] For the compounds according to Formula I, z may be selected to be 1, 2 or 3; z may be selected to be 1 or 2; or z may be selected to be 1. R^{a2} may be selected to be methyl, fluoro or chloro; or R^{a2} may be selected to be methyl. Alternatively, z may be 0.

[0039] For the compounds according to Formula I, w may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl. Alternatively, w may be 0.

[0040] For the compounds according to Formula I, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 . Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

[0041] For the compounds according to Formula I, m may be 1.

[0042] For the compounds according to Formula I, n may be 1.

[0043] For the compounds according to Formula I, p may be 1. Alternatively, p may be 2.

[0044] For the compounds according to Formula I, r and s may be independently selected from 1 or 2. In embodiments, one of r and s are selected to be 1 and the other is 2. In embodiments, both r and s are 2. In other embodiments, both r and s are 1.

[0045] For the compounds according to Formula I, X^a may be N.

[0046] For the compounds according to Formula I, X^b may be O. Alternatively, X^b may be NH. Alternatively, X^b may be NCH_3 .

[0047] For the compounds according to Formula I, R^{b1} may be C_1 to C_3 alkyl, which may be unsubstituted or substituted with halo, OH, or O- C_{1-3} alkyl. Alternatively, R^{b1} may be C_3 to C_6 cycloalkyl, which may be unsubstituted or substituted with halo, OH, or O- C_{1-3} alkyl. For example, R^{b1} may be $-CH_3$, $-CH_2CH_3$, cyclopropyl, $-CH_2CH_2OH$, $-CH_2CH_2F$ and the like. For compounds of the Formula I, R^{b1} may be methyl.

[0048] For the compounds according to Formula I, R^{b2} may be C_1 to C_3 alkyl, which may be unsubstituted or substituted with halo, OH, or O- C_{1-3} alkyl. Alternatively, R^{b2} may be C_3

to C₆ cycloalkyl, which may be unsubstituted or substituted with halo, OH, or O-C₁₋₃ alkyl. For example, R^{b2} may be -CH₃, -CH₂CH₃, cyclopropyl, -CH₂CH₂OH, -CH₂CH₂F and the like. For compounds of the Formula I, R^{b2} may be H or methyl. Alternatively, R^{b2} may be H.

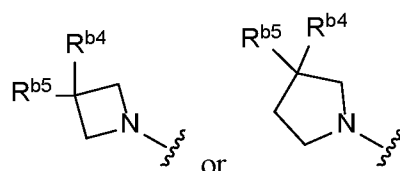
[0049] For the compounds according to Formula I, R^{b3} may be C₁ to C₃ alkyl, which may be unsubstituted or substituted with halo, OH, or O-C₁₋₃ alkyl. For example, R^{b3} may be -CH₃, -CH₂CH₃, -CH₂CH₂OH, -CH₂CH₂F and the like. For compounds of the Formula I, R^{b3} may be methyl.

[0050] For compounds of Formula I, each R^a is independently selected from C₁ to C₃ alkyl both of which C₁ to C₃ alkyl groups are bonded to the same ring carbon atom; or each R^a is methyl. Alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring; or two R^a taken together with the carbon to which they are attached may form a spiro-cyclopropyl group.

[0051] For compounds of Formula I, R^{b4} is selected from OH, -NH₂, -NH(C₁ to C₃ alkyl), and -N(C₁ to C₃ alkyl)₂. In embodiments, R^{b4} may be -NH₂, -NH(CH₃), -NH(CH₂CH₃), -N(CH₃)₂, or N(CH₂CH₃)₂.

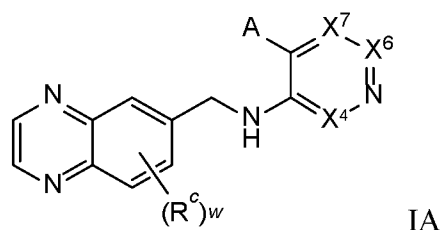
[0052] For compounds of Formula I, R^{b5} may be C₁ to C₃ alkyl, such as CH₃, CH₂CH₃, CH₂CH₂CH₃, CH(CH₃)₂. Alternatively, R^{b5} may be H.

[0053] For compounds of the Formula I, the substructure from the following substructures:



wherein R^{b4} and R^{b5} are as described above.

[0054] In embodiments, the small molecule has a structure according to Formula IA



IA

or a pharmaceutically acceptable salt thereof, wherein

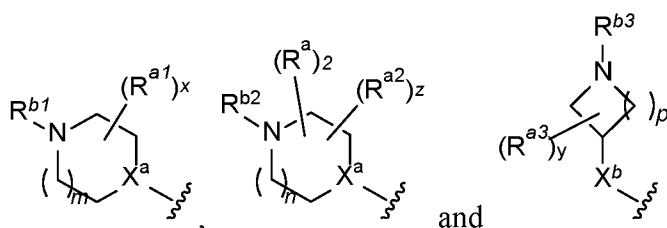
X^4 is selected from CH, CR^d and N;

X^6 is selected from CH, CR^d and N;

X^7 is selected from CH, CR^d and N;

wherein 0 or 1 of X^4 , X^6 or X^7 is N;

A is selected from the group consisting of:



X^a is selected from N and CH;

X^b is selected from O, NH, and NCH_3 ;

each R^{a1} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a1} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

each R^a is independently selected from C_1 to C_3 alkyl both of which C_1 to C_3 alkyl groups are bonded to the same ring carbon atom, or alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring;

each R^{a2} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, and halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

each R^{a3} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b1} is C_1 to C_3 alkyl;

R^{b2} is selected from H and C_1 to C_3 alkyl;

R^{b3} is C_1 to C_3 alkyl;

m is 1 or 2; n is 1 or 2; p is 1 or 2;

x is 0, 1, 2 or 3;

y is 0, 1, 2 or 3;

z is 0, 1, or 2;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH; and

w is 0, 1 or 2.

[0055] For the compounds according to Formula IA, X^4 may be CH, X^6 may be CH, and X^7 may be CH.

[0056] For the compounds according to Formula IA, x may be selected to be 1, 2 or 3; x may be selected to be 1 or 2; or x may be selected to be 1. R^{a1} may be selected to be methyl, fluoro or chloro; or R^{a1} may be selected to be methyl. Alternatively, x may be 0.

[0057] For the compounds according to Formula IA, y may be selected to be 0 or 1. R^{a3} may be selected from halo or methyl; or R^{a3} may be selected to be methyl. Alternatively, y may be 0.

[0058] For the compounds according to Formula IA, z may be selected to be 1, 2 or 3; z may be selected to be 1 or 2; or z may be selected to be 1. R^{a2} may be selected to be methyl, fluoro or chloro; or R^{a2} may be selected to be methyl. Alternatively, z may be 0.

[0059] For the compounds according to Formula IA, w may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl. Alternatively, w may be 0.

[0060] For the compounds according to Formula IA, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 . Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

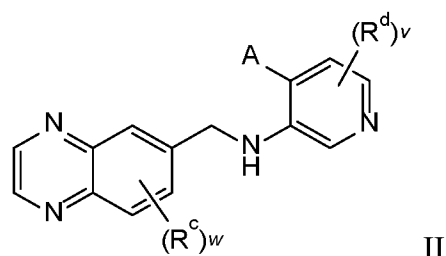
[0061] For the compounds according to Formula IA, m may be 1.

[0062] For the compounds according to Formula IA, n may be 1.

[0063] For the compounds according to Formula IA, p may be 1. Alternatively, p may be 2.

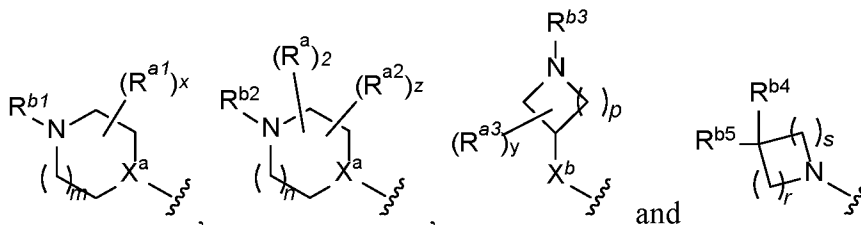
- [0064] For the compounds according to Formula IA, X^a may be N.
- [0065] For the compounds according to Formula IA, X^b may be O.
- [0066] For compounds of the Formula IA, R^{b1} may be methyl.
- [0067] For compounds of the Formula IA, R^{b3} may be methyl.
- [0068] For compounds of the Formula IA, R^{b2} may be H or methyl.
- [0069] For compounds of Formula IA, each R^a is independently selected from C_1 to C_3 alkyl both of which C_1 to C_3 alkyl groups are bonded to the same ring carbon atom; or each R^a is methyl. Alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring; or two R^a taken together with the carbon to which they are attached may form a spiro-cyclopropyl group.

[0070] In other embodiments, the small molecule has a structure according to Formula II:



or a pharmaceutically acceptable salt thereof, wherein

A is selected from the group consisting of:



X^a is selected from N and CH;

X^b is selected from O, NH, and NCH₃;

each R^{a1} is independently selected from C_1 to C_3 alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, halo, hydroxyl and amino; or additionally or alternatively, two R^{a1} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

each R^a is independently selected from C_1 to C_3 alkyl both of which C_1 to C_3 alkyl groups are bonded to the same ring carbon atom, or alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring;

each R^{a2} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, and halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

each R^{a3} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b1} is C_1 to C_3 alkyl or C_3 to C_6 cycloalkyl, each optionally substituted with halo, OH, or O- C_{1-3} alkyl;

R^{b2} is selected from H, C_1 to C_3 alkyl optionally substituted with halo, OH, or O- C_{1-3} alkyl;

R^{b3} is C_1 to C_3 alkyl optionally substituted with halo, OH, or O- C_{1-3} alkyl;

R^{b4} is selected from OH, $-NH_2$, $-NH(C_1 \text{ to } C_3 \text{ alkyl})$, and $-N(C_1 \text{ to } C_3 \text{ alkyl})_2$;

R^{b5} is selected from H and C_1 to C_3 alkyl;

m is 1 or 2; n is 1 or 2; p is 1 or 2; r is 1 or 2; s is 1 or 2;

x is 0, 1, 2 or 3;

y is 0, 1, 2 or 3;

z is 0, 1, or 2;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

[0071] For the compounds according to Formula II, x may be selected to be 1, 2 or 3; x may be selected to be 1 or 2; or x may be selected to be 1. R^{a1} may be selected to be methyl, fluoro or chloro; or R^{a1} may be selected to be methyl. Alternatively, x may be 0.

[0072] For the compounds according to Formula II, y may be selected to be 0 or 1. R^{a3} may be selected from halo or methyl; or R^{a3} may be selected to be methyl. Alternatively, y may be 0.

[0073] For the compounds according to Formula II, z may be selected to be 1, 2 or 3; z may be selected to be 1 or 2; or z may be selected to be 1. R^{a2} may be selected to be methyl, fluoro or chloro; or R^{a2} may be selected to be methyl. Alternatively, z may be 0.

[0074] For the compounds according to Formula II, w may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl. Alternatively, w may be 0.

[0075] For the compounds according to Formula II, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 . Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

[0076] For the compounds according to Formula II, m may be 1.

[0077] For the compounds according to Formula II, n may be 1.

[0078] For the compounds according to Formula II, p may be 1. Alternatively, p may be 2.

[0079] For the compounds according to Formula II, r and s may be independently selected from 1 or 2. In embodiments, one of r and s are selected to be 1 and the other is 2. In embodiments, both r and s are 2. In other embodiments, both r and s are 1.

[0080] For the compounds according to Formula II, X^a may be N.

[0081] For the compounds according to Formula II, X^b may be O. Alternatively, X^b may be NH.

[0082] For the compounds according to Formula II, R^{b1} may be C_1 to C_3 alkyl, which may be unsubstituted or substituted with halo, OH, or O- C_{1-3} alkyl. Alternatively, R^{b1} may be C_3 to C_6 cycloalkyl, which may be unsubstituted or substituted with halo, OH, or O- C_{1-3} alkyl. For example, R^{b1} may be $-CH_3$, $-CH_2CH_3$, cyclopropyl, $-CH_2CH_2OH$, $-CH_2CH_2F$ and the like. For compounds of the Formula II, R^{b1} may be methyl.

[0083] For the compounds according to Formula II, R^{b2} may be C_1 to C_3 alkyl, which may be unsubstituted or substituted with halo, OH, or O- C_{1-3} alkyl. Alternatively, R^{b2} may be C_3

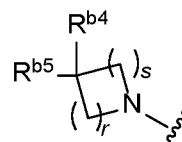
to C₆ cycloalkyl, which may be unsubstituted or substituted with halo, OH, or O-C₁₋₃ alkyl. For example, R^{b2} may be -CH₃, -CH₂CH₃, cyclopropyl, -CH₂CH₂OH, -CH₂CH₂F and the like. For compounds of the Formula II, R^{b2} may be H or methyl. Alternatively, R^{b2} may be H.

[0084] For the compounds according to Formula II, R^{b3} may be C₁ to C₃ alkyl, which may be unsubstituted or substituted with halo, OH, or O-C₁₋₃ alkyl. For example, R^{b3} may be -CH₃, -CH₂CH₃, -CH₂CH₂OH, -CH₂CH₂F and the like. For compounds of the Formula II, R^{b3} may be methyl.

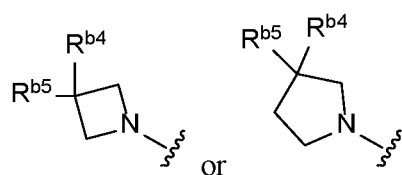
[0085] For compounds of Formula II, each R^a is independently selected from C₁ to C₃ alkyl both of which C₁ to C₃ alkyl groups are bonded to the same ring carbon atom; or each R^a is methyl. Alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring; or two R^a taken together with the carbon to which they are attached may form a spiro-cyclopropyl group.

[0086] For compounds of Formula II, R^{b4} is selected from OH, -NH₂, -NH(C₁ to C₃ alkyl), and -N(C₁ to C₃ alkyl)₂. In embodiments, R^{b4} may be -NH₂, -NH(CH₃), -NH(CH₂CH₃), -N(CH₃)₂, or N(CH₂CH₃)₂.

[0087] For compounds of Formula II, R^{b5} may be C₁ to C₃ alkyl, such as CH₃, CH₂CH₃, CH₂CH₂CH₃, CH(CH₃)₂. Alternatively, R^{b5} may be H.

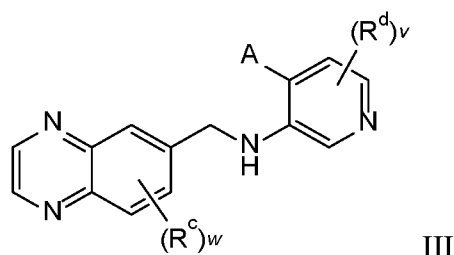


[0088] For compounds of the Formula II, the substructure may be selected to from the following substructures:



wherein R^{b4} and R^{b5} are as described above.

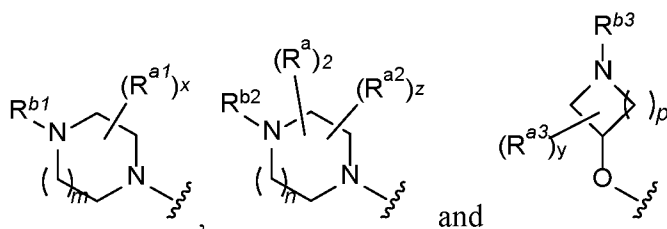
[0089] In other embodiments, the small molecule has a structure according to Formula III:



III

or a pharmaceutically acceptable salt thereof, wherein

A is selected from the group consisting of:



each R^{a1} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a1} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

each R^a is independently selected from C_1 to C_3 alkyl both of which C_1 to C_3 alkyl groups are bonded to the same ring carbon atom, or alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring;

each R^{a2} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, and halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

each R^{a3} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b1} is C_1 to C_3 alkyl or C_3 to C_6 cycloalkyl, each optionally substituted with halo, OH, or O- C_{1-3} alkyl;

R^{b2} is selected from H, C_1 to C_3 alkyl optionally substituted with halo, OH, or O- C_{1-3} alkyl;

R^{b3} is C_1 to C_3 alkyl optionally substituted with halo, OH, or O- C_{1-3} alkyl;

m is 1 or 2; n is 1 or 2; p is 1 or 2;

x is 0, 1, 2 or 3;

y is 0, 1, 2 or 3;

z is 0, 1, or 2;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, -CN, hydroxyl and amino;

each R^d is independently selected from halo, C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, -CN, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

[0090] For the compounds according to Formula III, *x* may be selected to be 1, 2 or 3; *x* may be selected to be 1 or 2; or *x* may be selected to be 1. R^{a1} may be selected to be methyl, fluoro or chloro; or R^{a1} may be selected to be methyl. Alternatively, *x* may be 0.

[0091] For the compounds according to Formula III, *y* may be selected to be 0 or 1. R^{a3} may be selected from halo or methyl; or R^{a3} may be selected to be methyl. Alternatively, *y* may be 0.

[0092] For the compounds according to Formula III, *z* may be selected to be 1, 2 or 3; *z* may be selected to be 1 or 2; or *z* may be selected to be 1. R^{a2} may be selected to be methyl, fluoro or chloro; or R^{a2} may be selected to be methyl. Alternatively, *z* may be 0.

[0093] For the compounds according to Formula III, *w* may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl. Alternatively, *w* may be 0.

[0094] For the compounds according to Formula III, each R^d may be selected from halo, C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, and -CHF₂; or R^d may be selected from CH₃, CH₂F, CHF₂, CF₃, F, Cl, Br, and OCH₃. Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

[0095] For the compounds according to Formula III, *m* may be 1.

[0096] For the compounds according to Formula III, *n* may be 1.

[0097] For the compounds according to Formula III, *p* may be 1. Alternatively, *p* may be 2.

[0098] For the compounds according to Formula III, X^a may be N.

[0099] For the compounds according to Formula III, X^b may be O. Alternatively, X^b may be NH.

[0100] For the compounds according to Formula III, R^{b1} may be C₁ to C₃ alkyl, which may be unsubstituted or substituted with halo, OH, or O-C₁₋₃ alkyl. Alternatively, R^{b1} may be C₃ to C₆ cycloalkyl, which may be unsubstituted or substituted with halo, OH, or O-C₁₋₃

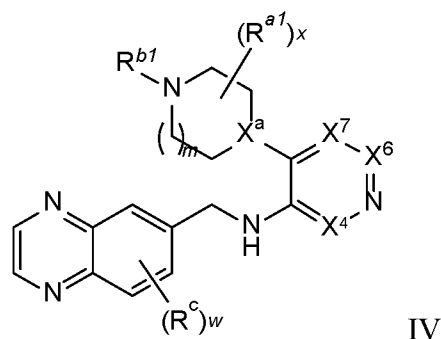
alkyl. For example, R^{b1} may be $-\text{CH}_3$, $-\text{CH}_2\text{CH}_3$, cyclopropyl, $-\text{CH}_2\text{CH}_2\text{OH}$, $-\text{CH}_2\text{CH}_2\text{F}$ and the like. For compounds of the Formula III, R^{b1} may be methyl.

[0101] For the compounds according to Formula III, R^{b2} may be C_1 to C_3 alkyl, which may be unsubstituted or substituted with halo, OH, or $\text{O}-\text{C}_{1-3}$ alkyl. Alternatively, R^{b2} may be C_3 to C_6 cycloalkyl, which may be unsubstituted or substituted with halo, OH, or $\text{O}-\text{C}_{1-3}$ alkyl. For example, R^{b2} may be $-\text{CH}_3$, $-\text{CH}_2\text{CH}_3$, cyclopropyl, $-\text{CH}_2\text{CH}_2\text{OH}$, $-\text{CH}_2\text{CH}_2\text{F}$ and the like. For compounds of the Formula III, R^{b2} may be H or methyl. Alternatively, R^{b2} may be H.

[0102] For the compounds according to Formula III, R^{b3} may be C_1 to C_3 alkyl, which may be unsubstituted or substituted with halo, OH, or $\text{O}-\text{C}_{1-3}$ alkyl. For example, R^{b3} may be $-\text{CH}_3$, $-\text{CH}_2\text{CH}_3$, $-\text{CH}_2\text{CH}_2\text{OH}$, $-\text{CH}_2\text{CH}_2\text{F}$ and the like. For compounds of the Formula III, R^{b3} may be methyl.

[0103] For compounds of Formula III, each R^a is independently selected from C_1 to C_3 alkyl both of which C_1 to C_3 alkyl groups are bonded to the same ring carbon atom; or each R^a is methyl. Alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring; or two R^a taken together with the carbon to which they are attached may form a spiro-cyclopropyl group.

[0104] In other embodiments, the small molecule has a structure according to Formula IV:



or a pharmaceutically acceptable salt thereof, wherein

X^4 is selected from CH, CR^d and N;

X^6 is selected from CH, CR^d and N;

X^7 is selected from CH, CR^d and N;

wherein 0 or 1 of X^4 , X^6 or X^7 is N;

X^a is selected from N and CH;

each R^{a1} is independently selected from C_1 to C_3 alkyl, $-\text{OCH}_3$, $-\text{CF}_3$, $-\text{CH}_2\text{F}$, $-\text{CHF}_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a1} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to

different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b1} is C₁ to C₃ alkyl or C₃ to C₆ cycloalkyl, each optionally substituted with halo, OH, or O-C₁₋₃ alkyl;

m is 1 or 2;

x is 0, 1, 2 or 3;

each R^c is independently selected from halo, C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, -CN, hydroxyl and amino;

each R^d is independently selected from halo, C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, -CN, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH; and

w is 0, 1 or 2.

[0105] For the compounds according to Formula IV, *x* may be selected to be 1, 2 or 3; *x* may be selected to be 1 or 2; or *x* may be selected to be 1. R^{a1} may be selected to be methyl, fluoro or chloro; or R^{a1} may be selected to be methyl. Alternatively, *x* may be 0.

[0106] For the compounds according to Formula IV, *w* may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl. Alternatively, *w* may be 0.

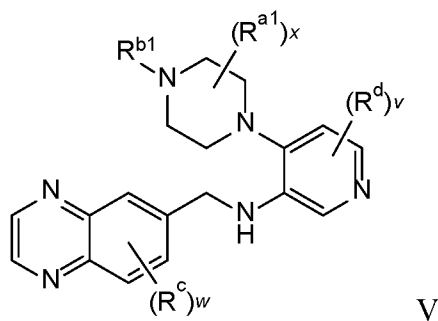
[0107] For the compounds according to Formula IV, each R^d may be selected from halo, C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, and -CHF₂; or R^d may be selected from CH₃, CH₂F, CHF₂, CF₃, F, Cl, Br, and OCH₃. Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

[0108] For the compounds according to Formula IV, *m* may be 1.

[0109] For the compounds according to Formula IV, X^a may be N.

[0110] For compounds of the Formula IV, R^{b1} may be C₁ to C₃ alkyl, which may be unsubstituted or substituted with halo, OH, or O-C₁₋₃ alkyl. Alternatively, R^{b1} may be C₃ to C₆ cycloalkyl, which may be unsubstituted or substituted with halo, OH, or O-C₁₋₃ alkyl. For example, R^{b1} may be -CH₃, -CH₂CH₃, cyclopropyl, -CH₂CH₂OH, -CH₂CH₂F and the like. For compounds of the Formula IV, R^{b1} may be methyl.

[0111] In other embodiments, the small molecule has a structure according to Formula V:



or a pharmaceutically acceptable salt thereof, wherein

each R^{a1} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a1} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b1} is C_1 to C_3 alkyl or C_3 to C_6 cycloalkyl, each optionally substituted with halo, OH, or O- C_{1-3} alkyl;

x is 0, 1, 2 or 3;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

[0112] For the compounds according to Formula V, x may be selected to be 1, 2 or 3; x may be selected to be 1 or 2; or x may be selected to be 1. R^{a1} may be selected to be methyl, fluoro or chloro; or R^{a1} may be selected to be methyl. Alternatively, x may be 0.

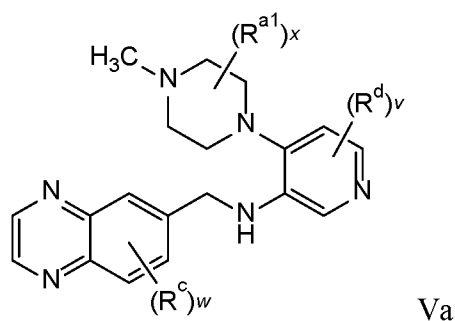
[0113] For compounds of the Formula V, R^{b1} may be C_1 to C_3 alkyl, which may be unsubstituted or substituted with halo, OH, or O- C_{1-3} alkyl. Alternatively, R^{b1} may be C_3 to C_6 cycloalkyl, which may be unsubstituted or substituted with halo, OH, or O- C_{1-3} alkyl. For example, R^{b1} may be $-CH_3$, $-CH_2CH_3$, cyclopropyl, $-CH_2CH_2OH$, $-CH_2CH_2F$ and the like. For compounds of the Formula V, R^{b1} may be methyl.

[0114] For the compounds according to Formula V, w may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl.

Alternatively, w may be 0.

[0115] For the compounds according to Formula V, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 . Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

[0116] In other embodiments, the small molecule has a structure according to Formula Va:



or a pharmaceutically acceptable salt thereof, wherein

each R^{a1} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a1} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

x is 0, 1, 2 or 3;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, -CN, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, -CN, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH; and

w is 0, 1 or 2;

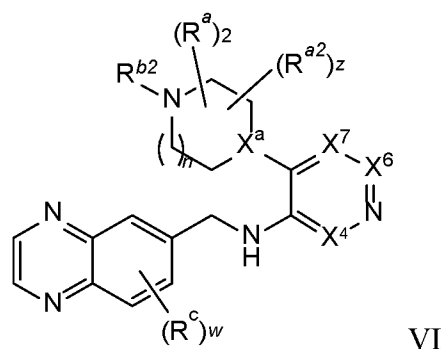
v is 0, 1 or 2.

[0117] For the compounds according to Formula Va, x may be selected to be 1, 2 or 3; x may be selected to be 1 or 2; or x may be selected to be 1. R^{a1} may be selected to be methyl, fluoro or chloro; or R^{a1} may be selected to be methyl. Alternatively, x may be 0.

[0118] For the compounds according to Formula Va, w may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl. Alternatively, w may be 0.

[0119] For the compounds according to Formula Va, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 . Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

[0120] In other embodiments, the small molecule has a structure according to Formula VI:



VI

or a pharmaceutically acceptable salt thereof, wherein

X^4 is selected from CH, CR^d and N;

X^6 is selected from CH, CR^d and N;

X^7 is selected from CH, CR^d and N;

wherein 0 or 1 of X^4 , X^6 or X^7 is N;

X^a is selected from N and CH;

each R^a is independently selected from C_1 to C_3 alkyl both of which C_1 to C_3 alkyl groups are bonded to the same ring carbon atom, or alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring;

each R^{a2} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, and halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

R^{b2} is selected from H and C_1 to C_3 alkyl optionally substituted with halo, OH, or O- C_{1-3} alkyl;

n is 1 or 2;

z is 0, 1, or 2;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH; and

w is 0, 1 or 2.

[0121] For the compounds according to Formula VI, z may be selected to be 1, 2 or 3; z may be selected to be 1 or 2; or z may be selected to be 1. R^{a2} may be selected to be methyl, fluoro or chloro; or R^{a2} may be selected to be methyl. Alternatively, z may be 0.

[0122] For the compounds according to Formula VI, w may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl. Alternatively, w may be 0.

[0123] For the compounds according to Formula VI, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 . Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

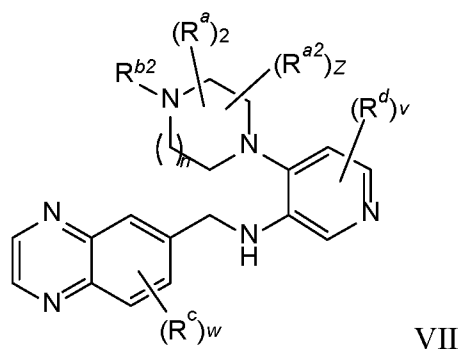
[0124] For the compounds according to Formula VI, n may be 1.

[0125] For the compounds according to Formula VI, X^a may be N.

[0126] For the compounds according to Formula VI, R^{b2} may be C_1 to C_3 alkyl, which may be unsubstituted or substituted with halo, OH, or $O-C_{1-3}$ alkyl. Alternatively, R^{b2} may be C_3 to C_6 cycloalkyl, which may be unsubstituted or substituted with halo, OH, or $O-C_{1-3}$ alkyl. For example, R^{b2} may be $-CH_3$, $-CH_2CH_3$, cyclopropyl, $-CH_2CH_2OH$, $-CH_2CH_2F$ and the like. For compounds of the Formula VI, R^{b2} may be H or methyl. Alternatively, R^{b2} may be H.

[0127] For compounds of Formula VI, each R^a is independently selected from C_1 to C_3 alkyl both of which C_1 to C_3 alkyl groups are bonded to the same ring carbon atom; or each R^a is methyl. Alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring; or two R^a taken together with the carbon to which they are attached may form a spiro-cyclopropyl group.

[0128] In other embodiments, the small molecule has a structure according to Formula VII:



or a pharmaceutically acceptable salt thereof, wherein

each R^a is independently selected from C_1 to C_3 alkyl both of which C_1 to C_3 alkyl groups are bonded to the same ring carbon atom, or alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring;

each R^{a2} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, and halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

R^{b2} is selected from H and C_1 to C_3 alkyl optionally substituted with halo, OH, or $O-C_{1-3}$ alkyl;

n is 1 or 2;

z is 0, 1, or 2;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

[0129] For the compounds according to Formula VII, z may be selected to be 1, 2 or 3; z may be selected to be 1 or 2; or z may be selected to be 1. R^{a2} may be selected to be methyl, fluoro or chloro; or R^{a2} may be selected to be methyl. Alternatively, z may be 0.

[0130] For the compounds according to Formula VII, w may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl.

Alternatively, w may be 0.

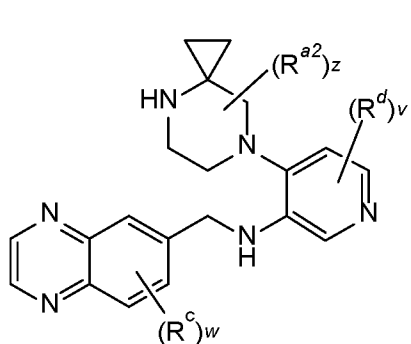
[0131] For the compounds according to Formula VII, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 . Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

[0132] For the compounds according to Formula VII, n may be 1.

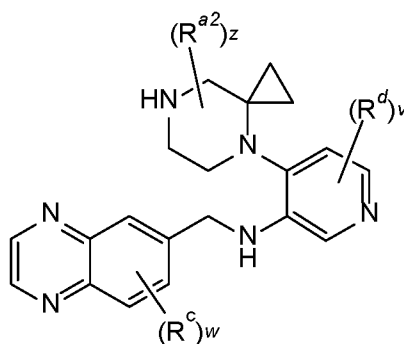
[0133] For compounds of the Formula VII, R^{b2} may be C_1 to C_3 alkyl, which may be unsubstituted or substituted with halo, OH, or $O-C_{1-3}$ alkyl. Alternatively, R^{b2} may be C_3 to C_6 cycloalkyl, which may be unsubstituted or substituted with halo, OH, or $O-C_{1-3}$ alkyl. For example, R^{b2} may be $-CH_3$, $-CH_2CH_3$, cyclopropyl, $-CH_2CH_2OH$, $-CH_2CH_2F$ and the like. For compounds of the Formula VII, R^{b2} may be H or methyl. Alternatively, R^{b2} may be H.

[0134] For compounds of Formula VII, each R^a is independently selected from C_1 to C_3 alkyl both of which C_1 to C_3 alkyl groups are bonded to the same ring carbon atom; or each R^a is methyl. Alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring; or two R^a taken together with the carbon to which they are attached may form a spiro-cyclopropyl group.

[0135] In other embodiments, the small molecule has a structure according to Formula VIIIa or VIIIb:



VIIIa or



VIIIb

or a pharmaceutically acceptable salt thereof, wherein

each R^{a2} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, and

halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

z is 0, 1, or 2;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

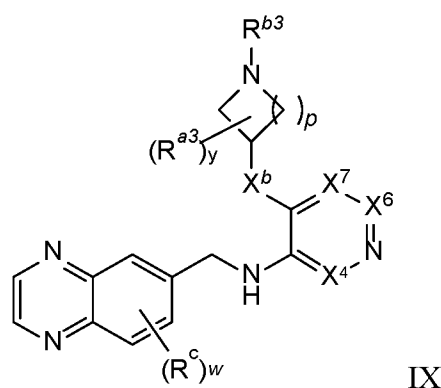
v is 0, 1 or 2.

[0136] For the compounds according to Formula VIIIa or VIIIb, z may be selected to be 1 or 2; or z may be selected to be 1. R^{a2} may be selected to be methyl, fluoro or chloro; or R^{a2} may be selected to be methyl. Alternatively, z may be 0.

[0137] For the compounds according to Formula VIIIa or VIIIb, w may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl. Alternatively, w may be 0.

[0138] For the compounds according to Formula VIIIa or VIIIb, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 . Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

[0139] In other embodiments, the small molecule has a structure according to Formula IX:



or a pharmaceutically acceptable salt thereof, wherein

X^4 is selected from CH, CR^d and N;

X^6 is selected from CH, CR^d and N;

X^7 is selected from CH, CR^d and N;

wherein 0 or 1 of X^4 , X^6 or X^7 is N;

X^b is selected from O, NH, and NCH_3 ;

each R^{a3} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b3} is C_1 to C_3 alkyl optionally substituted with halo, OH, or $O-C_{1-3}$ alkyl;

p is 1 or 2;

y is 0, 1, 2 or 3;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH; and

w is 0, 1 or 2.

[0140] For the compounds according to Formula IX, y may be selected to be 0 or 1. R^{a3} may be selected from halo or methyl; or R^{a3} may be selected to be methyl. Alternatively, y may be 0.

[0141] For the compounds according to Formula IX, w may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl. Alternatively, w may be 0.

[0142] For the compounds according to Formula IX, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 . Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

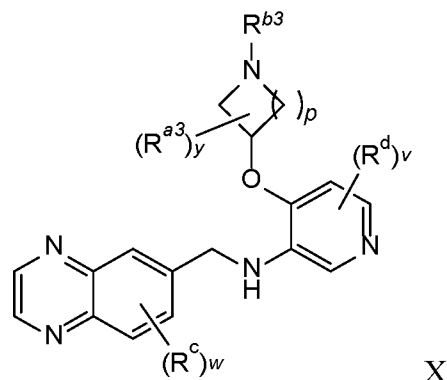
[0143] For the compounds according to Formula IX, p may be 2.

[0144] For the compounds according to Formula IX, X^b may be O. Alternatively, X^b may be NH.

[0145] For compounds of the Formula IX, R^{b3} may be C_1 to C_3 alkyl, which may be unsubstituted or substituted with halo, OH, or $O-C_{1-3}$ alkyl. For example, R^{b3} may be $-CH_3$, -

CH₂CH₃, -CH₂CH₂OH, -CH₂CH₂F and the like. For compounds of the Formula IX, R^{b3} may be methyl.

[0146] In other embodiments, the small molecule has a structure according to Formula X:



or a pharmaceutically acceptable salt thereof, wherein

each R^{a3} is independently selected from C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^{a3} attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b3} is C₁ to C₃ alkyl optionally substituted with halo, OH, or O-C₁₋₃ alkyl;

p is 1 or 2;

y is 0, 1, 2 or 3;

each R^c is independently selected from halo, C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, -CN, hydroxyl and amino;

each R^d is independently selected from halo, C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, -CN, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

[0147] For the compounds according to Formula X, *y* may be selected to be 0 or 1. R^{a3} may be selected from halo or methyl; or R^{a3} may be selected to be methyl. Alternatively, *y* may be 0.

[0148] For the compounds according to Formula X, w may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl.

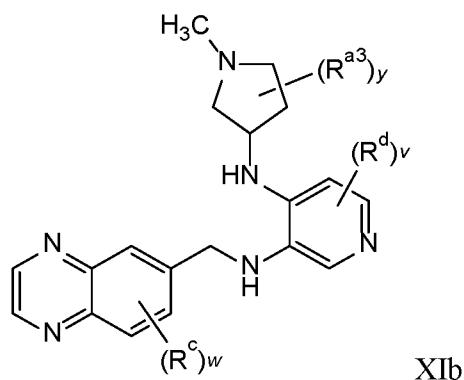
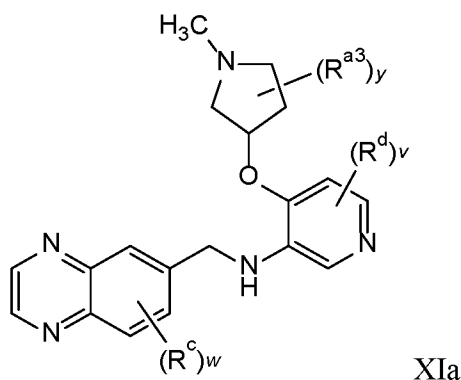
Alternatively, w may be 0.

[0149] For the compounds according to Formula X, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 . Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

[0150] For the compounds according to Formula X, p may be 2.

[0151] For compounds of the Formula X, R^{b3} may be C_1 to C_3 alkyl, which may be unsubstituted or substituted with halo, OH, or $O-C_{1-3}$ alkyl. For example, R^{b3} may be $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2OH$, $-CH_2CH_2F$ and the like. For compounds of the Formula IX, R^{b3} may be methyl.

[0152] In other embodiments, the small molecule has a structure according to Formula XIa or XIb:



or a pharmaceutically acceptable salt thereof, wherein

each R^{a3} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

y is 0, 1, 2 or 3;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken

together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

[0153] For the compounds according to Formula XIa or XIb, y may be selected to be 0 or

1. R^{a3} may be selected from halo or methyl; or R^{a3} may be selected to be methyl.

Alternatively, y may be 0.

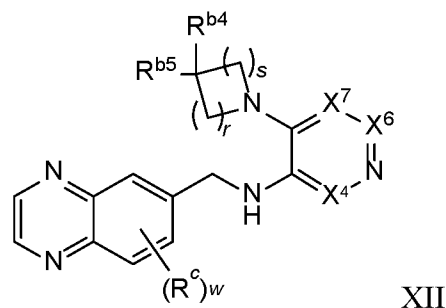
[0154] For the compounds according to Formula XIa or XIb, w may be selected from 0 or

1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl.

Alternatively, w may be 0.

[0155] For the compounds according to Formula XIa or XIb, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 . Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

[0156] In other embodiments, the small molecule has a structure according to Formula XII:



or a pharmaceutically acceptable salt thereof, wherein

X^4 is selected from CH, CR^d and N;

X^6 is selected from CH, CR^d and N;

X^7 is selected from CH, CR^d and N;

wherein 0 or 1 of X^4 , X^6 or X^7 is N;

R^{b4} is selected from OH, $-NH_2$, $-NH(C_1 \text{ to } C_3 \text{ alkyl})$, and $-N(C_1 \text{ to } C_3 \text{ alkyl})_2$;

R^{b5} is selected from H and C_1 to C_3 alkyl;

r is 1 or 2;

s is 1 or 2;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH; and

w is 0, 1 or 2.

[0157] For the compounds according to Formula XII, r and s may be independently selected from 1 or 2. In embodiments, one of r and s are selected to be 1 and the other is 2. In embodiments, both r and s are 2. In other embodiments, both r and s are 1.

[0158] For the compounds according to Formula XII, w may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl.

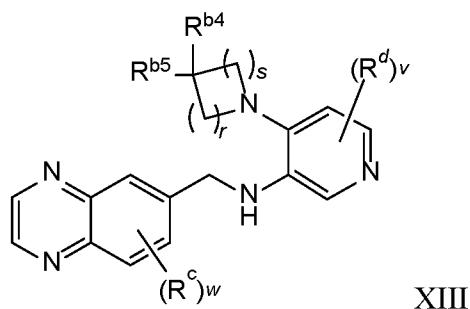
Alternatively, w may be 0.

[0159] For the compounds according to Formula XII, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 . Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

[0160] For compounds of Formula XII, R^{b4} is selected from OH, $-NH_2$, $-NH(C_1$ to C_3 alkyl), and $-N(C_1$ to C_3 alkyl) $_2$. In embodiments, R^{b4} may be $-NH_2$, $-NH(CH_3)$, $-NH(CH_2CH_3)$, $-N(CH_3)_2$, or $N(CH_2CH_3)_2$, or R^{b4} may be $-NH_2$, or $-NH(CH_3)$.

[0161] For compounds of Formula XII, R^{b5} may be C_1 to C_3 alkyl, such as CH_3 , CH_2CH_3 , $CH_2CH_2CH_3$, $CH(CH_3)_2$, and particularly CH_3 . Alternatively, R^{b5} may be H.

[0162] In other embodiments, the small molecule has a structure according to Formula XIII:



or a pharmaceutically acceptable salt thereof, wherein

R^{b4} is selected from OH, $-NH_2$, $-NH(C_1$ to C_3 alkyl), and $-N(C_1$ to C_3 alkyl) $_2$;

R^{b5} is selected from H and C_1 to C_3 alkyl;

r is 1 or 2;

s is 1 or 2;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

[0163] For the compounds according to Formula XIII, r and s may be independently selected from 1 or 2. In embodiments, one of r and s are selected to be 1 and the other is 2. In embodiments, both r and s are 2. In other embodiments, both r and s are 1.

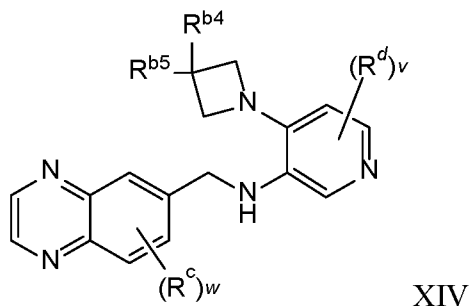
[0164] For the compounds according to Formula XIII, w may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl. Alternatively, w may be 0.

[0165] For the compounds according to Formula XIII, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 . Alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

[0166] For compounds of Formula XIII, R^{b4} is selected from OH, $-NH_2$, $-NH(C_1$ to C_3 alkyl), and $-N(C_1$ to C_3 alkyl) $_2$. In embodiments, R^{b4} may be $-NH_2$, $-NH(CH_3)$, $-NH(CH_2CH_3)$, $-N(CH_3)_2$, or $N(CH_2CH_3)_2$, or R^{b4} may be $-NH_2$, or $-NH(CH_3)$.

[0167] For compounds of Formula XIII, R^{b5} may be C_1 to C_3 alkyl, such as CH_3 , CH_2CH_3 , $CH_2CH_2CH_3$, $CH(CH_3)_2$, and particularly CH_3 . Alternatively, R^{b5} may be H.

[0168] In other embodiments, the small molecule has a structure according to Formula XIV:



or a pharmaceutically acceptable salt thereof, wherein

R^{b4} is selected from OH, $-NH_2$, $-NH(C_1 \text{ to } C_3 \text{ alkyl})$, and $-N(C_1 \text{ to } C_3 \text{ alkyl})_2$;

R^{b5} is selected from H and C_1 to C_3 alkyl;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

[0169] For the compounds according to Formula XIV, w may be selected from 0 or 1. R^c may be selected from halo or methyl; or R^c may be selected from F, Cl or methyl.

Alternatively, w may be 0.

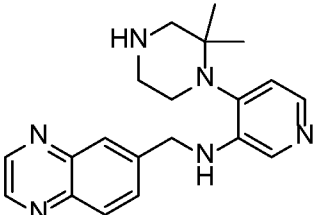
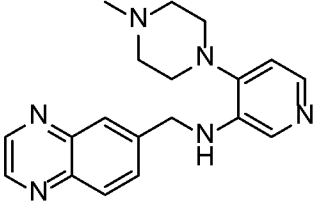
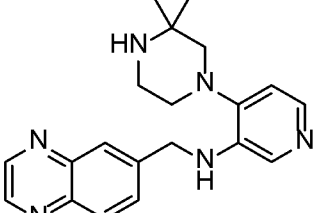
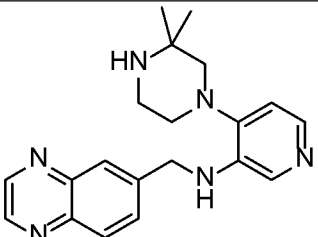
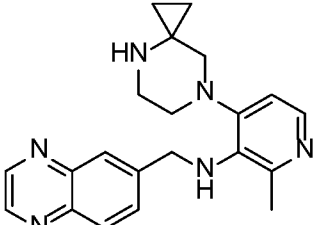
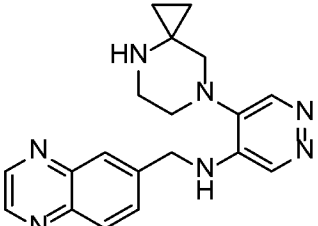
[0170] For the compounds according to Formula XIV, each R^d may be selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, and $-CHF_2$; or R^d may be selected from CH_3 , CH_2F , CHF_2 , CF_3 , F, Cl, Br, and OCH_3 , or R^d may be selected from F, Cl, and Br. Alternatively, v is 0. In other embodiments, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH.

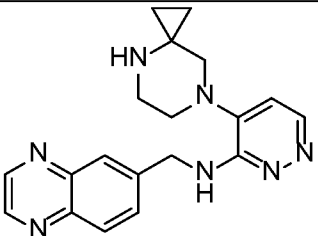
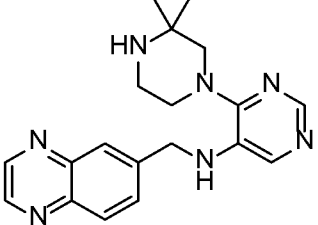
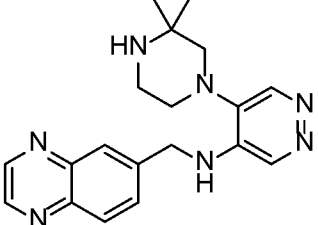
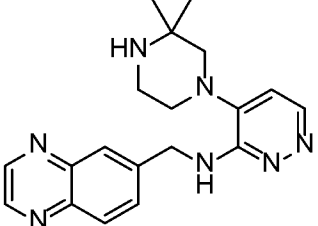
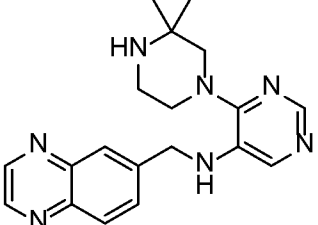
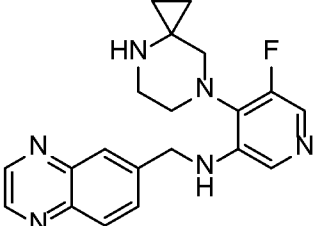
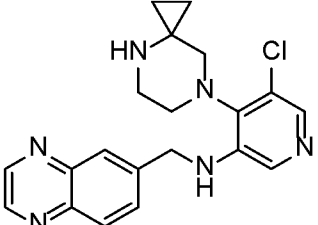
[0171] For compounds of Formula XIV, R^{b4} is selected from OH, $-NH_2$, $-NH(C_1 \text{ to } C_3 \text{ alkyl})$, and $-N(C_1 \text{ to } C_3 \text{ alkyl})_2$. In embodiments, R^{b4} may be $-NH_2$, $-NH(CH_3)$, $-NH(CH_2CH_3)$, $-N(CH_3)_2$, or $N(CH_2CH_3)_2$, or R^{b4} may be $-NH_2$, or $-NH(CH_3)$.

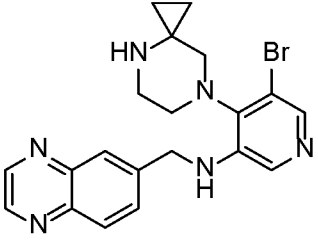
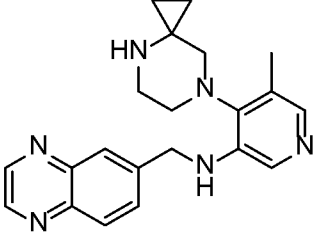
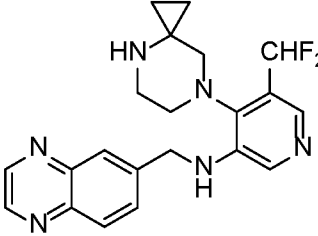
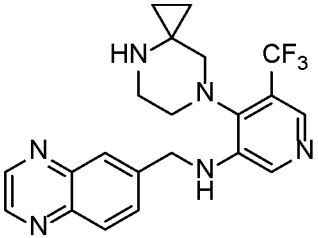
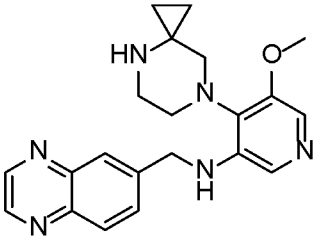
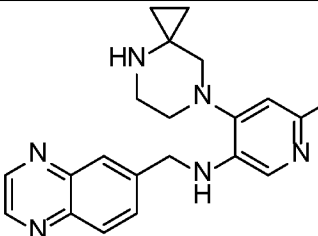
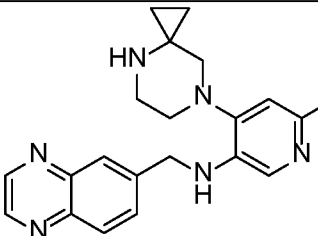
For compounds of Formula XIV, R^{b5} may be C_1 to C_3 alkyl, such as CH_3 , CH_2CH_3 , $CH_2CH_2CH_3$, $CH(CH_3)_2$. Alternatively, R^{b5} may be H.

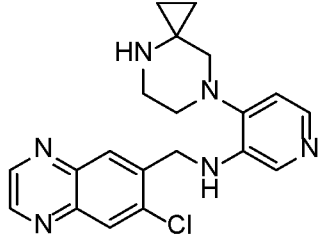
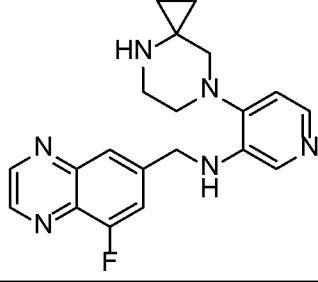
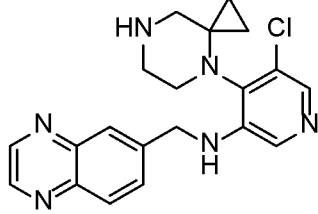
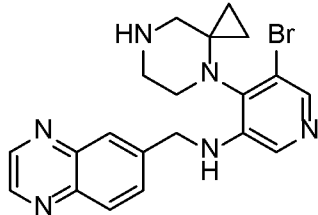
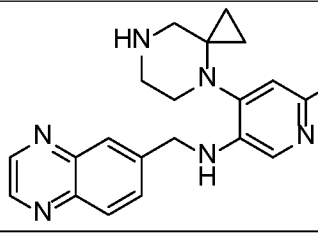
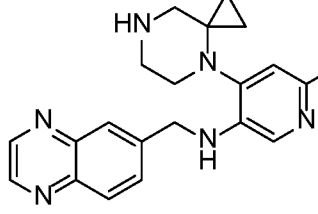
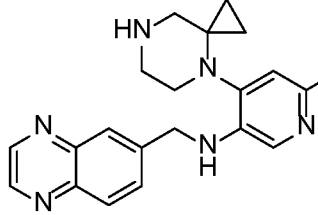
[0172] In other embodiments, the small molecule has a structure according to the compounds in Table A (or a pharmaceutically acceptable salt thereof):

Table A.

Ref. No.	Structure
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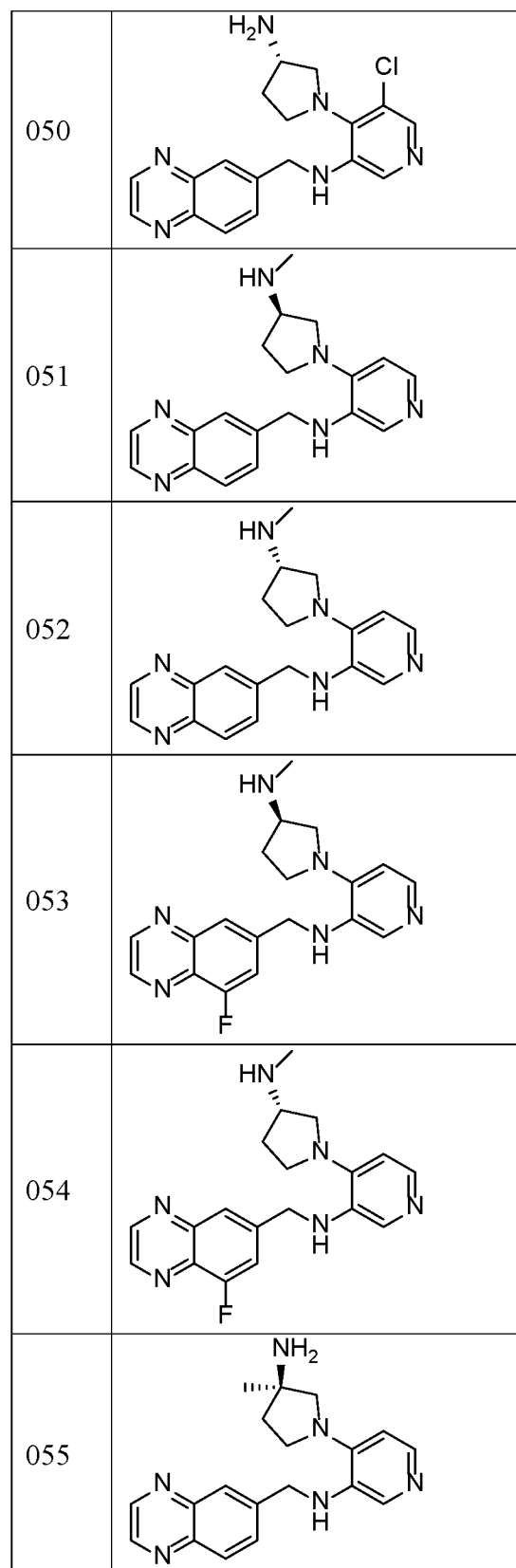
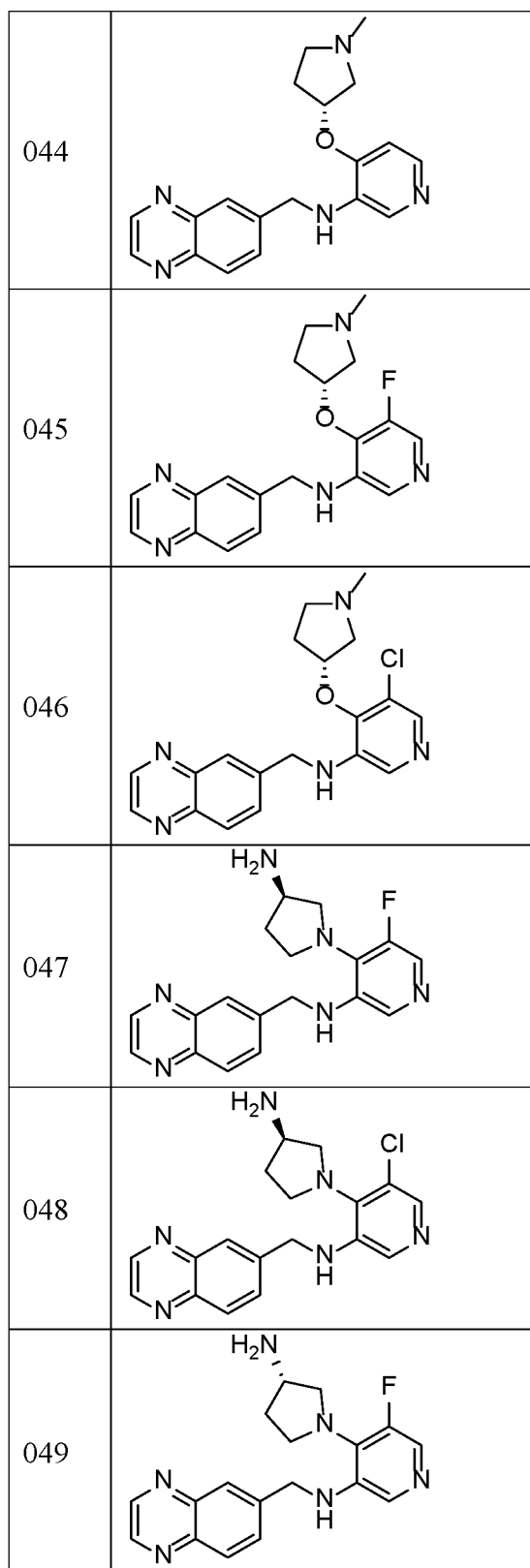
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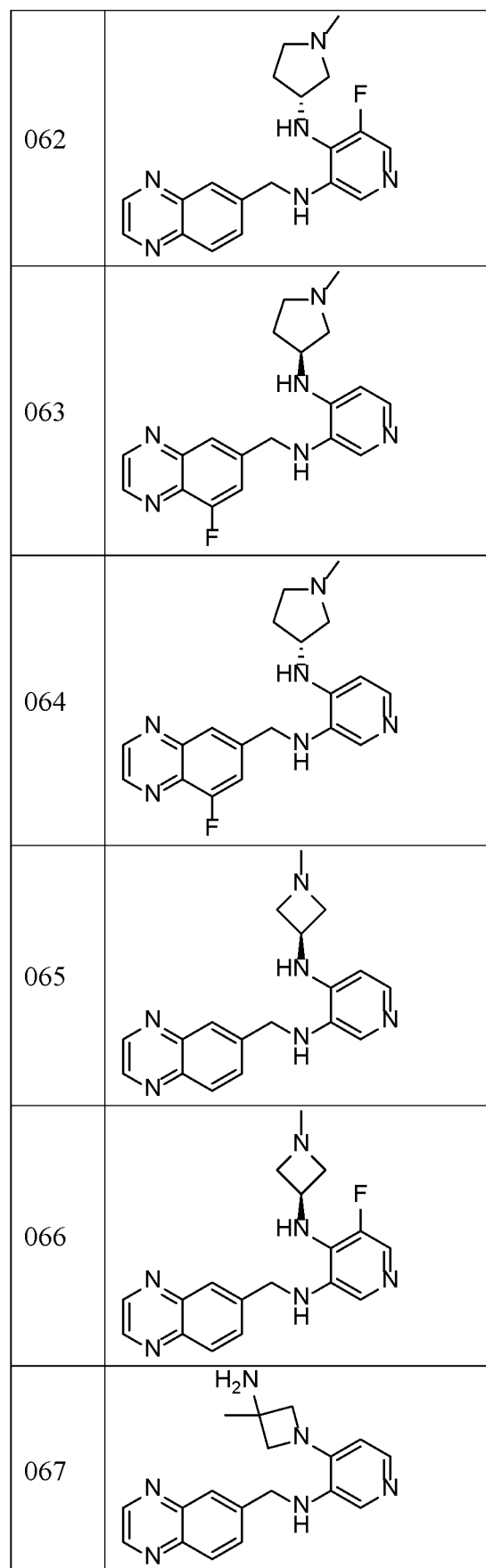
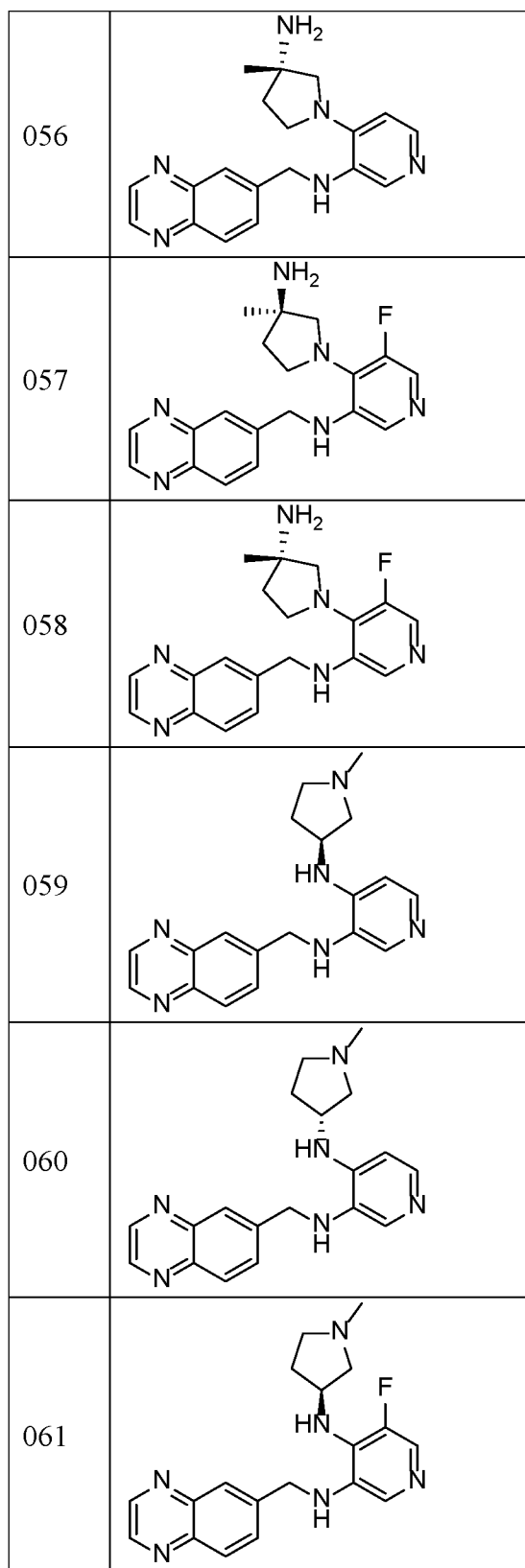
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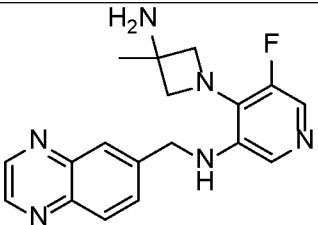
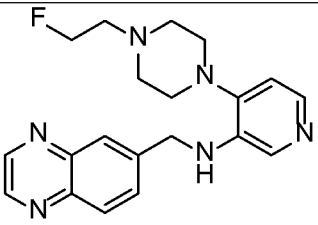
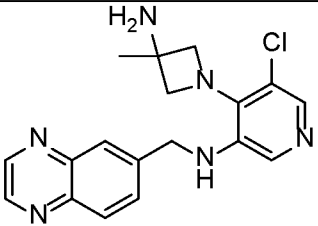
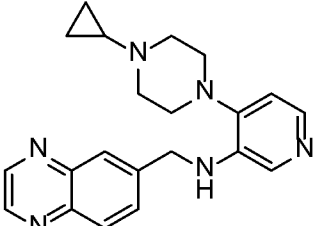
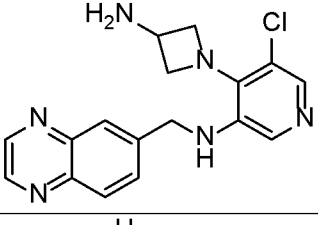
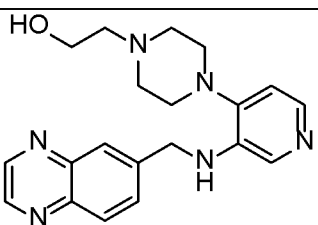
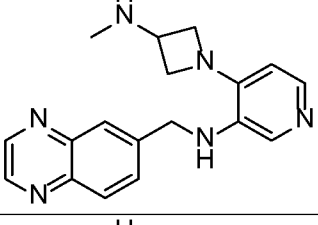
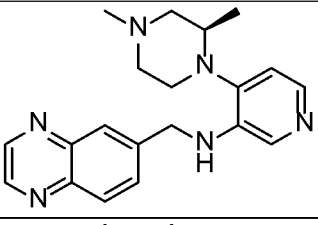
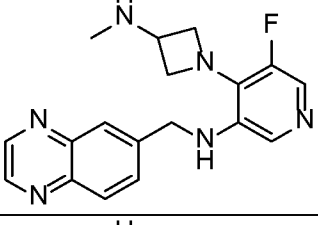
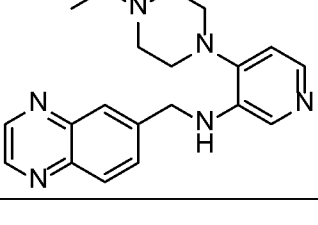
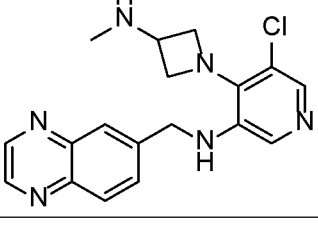
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[0173] The small molecule ligands of the Formula I-XIV and Table A may provide one or more beneficial properties, including improved penetration of the blood-brain barrier, improved partitioning into the tissue of the eye, low toxicity; and good bioavailability.

[0174] The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups and branched-chain alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 6 or fewer carbon atoms in its backbone (e.g., C₁-C₆ for straight chain, C₃-C₆ for branched chain). Alkyl groups include methyl, ethyl, propyl,

isopropyl, n-butyl, iso-butyl, tert-butyl, pentyl, isopentyl, hexyl, and the like. The term “substituted alkyl” refers to an alkyl group which has from 1 to 4 substituents independently selected from halo, amino, amido, sulfonamido, OH, OCH₃, nitro and CN.

[0175] The term "cycloalkyl" refers to saturated, carbocyclic groups having from 3 to 6 carbons in the ring. Cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

[0176] The term “bicycyl” refers to saturated carbocyclic groups having two joined ring systems, which may be fused or bridged. Bicyclic groups include bicycle[2.1.1]hexane, bicycle[2.2.1]heptane, decalin, and the like. The term “tricycyl” refers to saturated carbocyclic groups having three joined ring systems, which may be fused and/or bridged. Tricyclic groups include adamantane and the like.

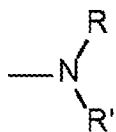
[0177] Carbocyclic refers to ring system that comprise only carbon atoms as ring atoms (i.e., the ring system does not have a heteroatom as a ring atom). Carbocyclic ring systems may be unsaturated, but preferred carbocyclic rings are not aromatic.

[0178] The term “alkenyl” refers to unsaturated aliphatic groups, including straight-chain alkenyl groups and branched-chain alkenyl groups, having at least one carbon-carbon double bond. In preferred embodiments, the alkenyl group has two to six carbon atoms (e.g., C₂-C₆ alkenyl).

[0179] As used herein, the term "halogen" or "halo" designates -F, -Cl, -Br or -I, and preferably -F, -Cl or -Br.

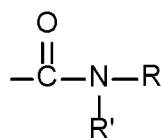
[0180] The terms "alkoxyl" or "alkoxy" as used herein refers to an alkyl group, as defined above, that is attached through an oxygen atom. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like.

[0181] The terms "amine" and "amino" refer to both unsubstituted and substituted amines, e.g., a moiety that can be represented by the general formula:



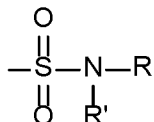
[0182] wherein R and R' are each independently selected from H and C₁-C₃ alkyl.

[0183] The terms "amido" refer to both unsubstituted and substituted amide substituents, e.g., a moiety that can be represented by the general formula:



[0184] wherein R and R' are each independently selected from H and C₁-C₃ alkyl.

[0185] The terms "sulfonamide" or "sulfonamido" refer to both unsubstituted and substituted sulfonamide substituents, e.g., a moiety that can be represented by the general formula:



[0186] wherein R and R' are each independently selected from H and C₁-C₃ alkyl.

[0187] The term "aryl" as used herein includes 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaryl" groups. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic. Accordingly, aryl includes 8- to 10-membered fused bicyclic aromatic groups that may include from zero to five heteroatoms, in which one or both rings are aromatic, for example naphthylene, quinolone, isoquinoline, benzo[b]thiophene, tetrahydronaphthylene, and the like. Each aryl group may be unsubstituted or may be substituted with 1 to 5 substituents selected from halogen, hydroxyl, amino, cyano, amido, sulfonamide, nitro, -SH, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₃-C₇ cycloalkyl, C₆-C₁₀ bicycyl, C₁-C₆ haloalkyl, C₁-C₆ perhaloalkyl, -O-(C₁-C₆ alkyl), O-(C₃-C₇ cycloalkyl), -O-(C₁-C₆ haloalkyl), -O-(C₁-C₆ perhaloalkyl), aryl, -O-aryl, -(C₁-C₆ alkyl)-aryl, -O-(C₁-C₆ alkyl)-aryl, -S-(C₁-C₆ alkyl), -S-(C₃-C₇ cycloalkyl), -S-(C₁-C₆ haloalkyl), -S-(C₁-C₆ perhaloalkyl), -S-aryl, -S-(C₁-C₆ alkyl)-aryl, heteroaryl and heterocyclyl.

[0188] The term "heterocycle" or "heterocyclyl" refer to non-aromatic heterocycles having from 1 to 3 ring heteroatoms. Preferred heterocycles are 5- and 6-membered heterocyclic groups having from 1 to 3 heteroatoms selected from the group consisting of O, N and S.

[0189] The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, and sulfur.

[0190] As used herein, the definition of each expression, e.g. alkyl, R¹, R², etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

[0191] It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

[0192] The aptamer ligands disclosed herein may exist in particular geometric or stereoisomeric forms well as mixtures thereof. Such geometric or stereoisomeric forms include, but not limited to, cis- and trans-isomers, R- and S-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group.

[0193] The compounds according to Formulas I to XIV may contain an acidic or basic functional group, and accordingly may be present in a salt form. Preferably, the salt form is a pharmaceutically acceptable salt. The term "pharmaceutically-acceptable salts" in this respect, refers to the relatively non-toxic, inorganic and organic acid and base addition salts of the compounds disclosed herein.

[0194] The compounds according to Formulas I to XIV may contain one or more basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable acids. These salts can be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, or by separately reacting a purified compound disclosed herein in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed during subsequent purification. 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, and laurylsulphonate salts and the like (*see, e.g.*, Berge et al. (1977) "Pharmaceutical Salts", J. Pharm. Sci. 66:1-19).

[0195] The pharmaceutically acceptable salts of the subject compounds include the conventional nontoxic salts or quaternary ammonium salts of the compounds, e.g., from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts include those derived from inorganic acids such as hydrochloride, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic,

propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

[0196] In other cases, the compounds according to Formulas I to XIV may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable bases. These salts can likewise be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, 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, e.g., Berge et al., supra*).

[0197] In embodiments, the aptamers provided herein bind to, or otherwise respond to the presence of, one or more compounds of Formula I - XIV provided herein, and/or bind to, or otherwise respond to, a metabolite analog or derivative of a compound of Formula I - XIV.

[0198] The specificity of the binding of an aptamer to its ligand can be defined in terms of the comparative dissociation constants (K_d) of the aptamer for its ligand as compared to the dissociation constant of the aptamer for unrelated molecules. Thus, the ligand may be considered to be a molecule that binds to the aptamer with greater affinity than to unrelated material. Typically, the K_d for the aptamer with respect to its ligand will be at least about 10-fold less than the K_d for the aptamer with unrelated molecules. In other embodiments, the K_d will be at least about 20-fold less, at least about 50-fold less, at least about 100-fold less, and at least about 200-fold less, at least about 500-fold less, at least about 1000-fold less, or at least about 10,000-fold less than the K_d for the aptamer with unrelated molecules.

[0199] Aptamers and ligands for the regulation of gene expression

[0200] In some embodiments, the aptamer and small molecules contemplated by the disclosure are used for the regulation of gene expression. Regulation of the expression of a target gene (e.g., a therapeutic transgene) is advantageous in a variety of situations. In the context of the therapeutic expression of genes, for example, techniques that enable regulated expression of transgenes in response to the presence of a small molecule can enhance safety and efficacy by allowing for the regulation of the level of target gene expression and its

timing. In a research setting, the regulation of gene expression allows a systematic investigation of different experimental conditions.

[0201] In embodiments, the sequence encoding the aptamer is part of a gene regulation cassette that provides the ability to regulate the expression level of a target gene in response to the presence or absence of a small molecule described herein. In embodiments, the gene regulation cassette further comprises a target gene. As used herein, “target gene” refers to a transgene that is expressed in response to the presence or absence of the small molecule ligands disclosed herein due to the small molecule binding to the aptamers disclosed herein. In embodiments, the target gene comprises the coding sequence for a protein (e.g., a therapeutic protein), a miRNA, or a siRNA. The target gene is heterologous to the aptamer used for the regulation of target gene expression, is heterologous to the polynucleotide cassette used for the regulation of target gene and/or is heterologous to a portion of the polynucleotide cassette used for the regulation of target gene.

[0202] When used to regulate the expression of a target gene in response to the presence/absence of a small molecule disclosed herein, the aptamers described herein can be part of a polynucleotide cassette that encodes the aptamer as part of a riboswitch. The terms “gene regulation cassette”, “regulatory cassette”, or “polynucleotide cassette” are used interchangeably herein.

[0203] In embodiments, the presence of a small molecule disclosed herein that binds to an aptamer disclosed herein leads to an increase in expression of a target gene as compared to the expression of the target gene in absence of the small molecule. In such an embodiment, the aptamer constitutes an “on” switch. In embodiments, the expression of the target gene is increased by at least 3-fold, by at least 5-fold, by at least 10-fold, by at least 15-fold, by at least 20-fold, by at least 25-fold, by at least 30-fold, by at least 40-fold, by at least 50-fold, by at least 100-fold, by at least 1000-fold, or by at least 10,000-fold in presence of the small molecule that binds to an aptamer disclosed herein as compared to in absence of the small molecule. In embodiments, the expression of the target gene is increased by between 2-fold and 10-fold, between 5-fold and 10-fold, between 5-fold and 15-fold, between 5-fold and 20-fold, between 5-fold and 25-fold, between 5-fold and 30-fold, between 10-fold and 20-fold, between 10-fold and 30-fold, between 10-fold and 40-fold, between 10-fold and 50-fold, between 10-fold and 100-fold, between 10-fold and 500-fold, between 10-fold and 1,000-fold, between 50-fold and 100-fold, between 50-fold and 500-fold, between 50-fold and 100-fold, between 50-fold and 1,000-fold, between 100-fold and 1,000-fold, or between 100-fold

and 10,000-fold in presence of the small molecule that binds to an aptamer disclosed herein as compared to in absence of the small molecule.

[0204] In embodiments, the presence of a small molecule disclosed herein that binds to an aptamer disclosed herein leads to a decrease in expression of a target gene as compared to the expression of the target gene in the absence of the small molecule. In such embodiments, the aptamer constitutes an “off” switch. In embodiments, the expression of the target gene is decreased by at least 3-fold, by at least 5-fold, by at least 10-fold, by at least 15-fold, by at least 20-fold, by at least 25-fold, by at least 30-fold, by at least 40-fold, by at least 50-fold, by at least 100-fold, by at least 1000-fold, or by at least 10,000-fold in presence of the small molecule that binds to an aptamer disclosed herein as compared to in absence of the small molecule. In one embodiment, the expression of the target gene is decreased by between 2-fold and 10-fold, between 5-fold and 10-fold, between 5-fold and 15-fold, between 5-fold and 20-fold, between 5-fold and 25-fold, between 5-fold and 30-fold, between 10-fold and 20-fold, between 10-fold and 30-fold, between 10-fold and 40-fold, between 10-fold and 50-fold, between 10-fold and 100-fold, between 10-fold and 500-fold, between 10-fold and 1,000-fold, between 50-fold and 100-fold, between 50-fold and 500-fold, between 50-fold and 100-fold, between 50-fold and 1,000-fold, between 100-fold and 1,000-fold, or between 100-fold and 10,000-fold in presence of the small molecule that binds to an aptamer disclosed herein as compared to in absence of the small molecule.

[0205] In embodiments, the aptamer is part of a riboswitch. Riboswitches are regulatory segments of an RNA polynucleotide that regulate the stability of the RNA polynucleotide and/or regulate the production of a protein from the RNA polynucleotide in response to the presence or absence of aptamer-specific ligand molecules. In embodiments, the riboswitch comprises a sensor region (e.g., the aptamer region) and an effector region that together are responsible for sensing the presence of a ligand (e.g., a small molecule) and causing an effect that leads to increased or decreased expression of the target gene. The riboswitches described herein are recombinant, utilizing polynucleotides from two or more sources. In embodiments, the sensor and effector regions are joined by a polynucleotide linker. In embodiments, the polynucleotide linker forms a RNA stem or paired region (i.e., a region of the RNA polynucleotide that is double-stranded). In embodiments, the paired region linking the aptamer to the effector region comprises all, or some of an aptamer stem (e.g., for example all, or some of the aptamer P1 stem.).

[0206] Riboswitches comprising aptamer sequences may be used, for example, to control the formation of rho-independent transcription termination hairpins leading to premature

transcription termination. Riboswitches comprising aptamer sequences may also induce structural changes in the RNA, leading to sequestration for the ribosome binding site and inhibition of translation. Alternative riboswitch structures comprising the aptamer sequences disclosed herein can further affect the splicing of mRNA in response to the presence of the small molecule ligand.

[0207] Alternative splicing riboswitch

[0208] In one embodiment, the aptamers described herein are encoded as part of a gene regulation cassette for the regulation of a target gene by aptamer/ligand mediated alternative splicing of the resulting RNA (e.g., pre-mRNA). In this context, the gene regulation cassette comprises a riboswitch comprising a sensor region (e.g., the aptamers described herein) and an effector region that together are responsible for sensing the presence of a small molecule ligand and altering splicing to an alternative exon. Splicing refers to the process by which an intronic sequence is removed from the nascent pre-messenger RNA (pre-mRNA) and the exons are joined together to form the mRNA. Splice sites are junctions between exons and introns, and are defined by different consensus sequences at the 5' and 3' ends of the intron (i.e., the splice donor and splice acceptor sites, respectively). Splicing is carried out by a large multi-component structure called the spliceosome, which is a collection of small nuclear ribonucleoproteins (snRNPs) and a diverse array of auxiliary proteins. By recognizing various cis regulatory sequences, the spliceosome defines exon/intron boundaries, removes intronic sequences, and splices together the exons into a final message (e.g., the mRNA). In the case of alternative splicing, certain exons can be included or excluded to vary the final coding message thereby changing the resulting expressed protein.

[0209] In one embodiment, the regulation of target gene expression is achieved by using any of the DNA constructs disclosed in WO2016/126747, which is hereby incorporated by reference in its entirety. In embodiments of the present disclosure, the riboswitches and polynucleotide cassettes disclosed in WO2016/126747 comprise an aptamer encoding sequence described herein in place of the aptamer sequence disclosed in WO2016/126747.

[0210] In one embodiment, the polynucleotide cassette comprises (a) a riboswitch and (b) an alternatively-spliced exon, flanked by a 5' intron and a 3' intron, wherein the riboswitch comprises (i) an effector region comprising a stem forming sequence that includes the 5' splice site sequence of the 3' intron (and sequence complementary thereto), and (ii) an aptamer disclosed herein. In embodiments, the effector region is a stem forming region that forms the P1 stem of the aptamer (*see, e.g.*, Figs. 1a and 1b). Thus, in embodiments, the

effector stem is, or comprises, the P1 stem of the aptamers disclosed herein. In other words, the effector stem comprises a first sequence (the 5' effector stem arm) that is linked to the 5' end of the aptamers disclosed herein and a second sequence (the 3' effector stem arm) that is linked to the 3' end of the aptamers disclosed herein, wherein the first or second sequence includes the 5' splice site sequence of the 3' intron and the other includes sequence complementary to the 5' splice site sequence of the 3' intron. In embodiments, the effector region stem comprises the intronic 5' splice site ("5' ss") sequence of the intron that is immediately 3' of the alternative exon, as well as the sequence complimentary to the 5' ss sequence of the 3' intron.

[0211] 5' splice site sequences are well known in the art. There is some variability among different 5' splice site sequences, and this variability is also well understood in the art. For example, Shapiro and Senapathy (Shapiro MB, Senapathy P. RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. *Nucleic Acids Res.* 1987 Sep 11;15(17):7155-74 or Zhang MQ. Statistical features of human exons and their flanking regions. *Hum Mol Genet.* 1998 May;7(5):919-32, which is incorporated in its entirety herein) describe for a variety of eukaryotes which positions of the splice site sequence have some variability, and which positions are fixed. Likewise, Zhang (Zhang MQ. Statistical features of human exons and their flanking regions. *Hum Mol Genet.* 1998 May;7(5):919-32, which is incorporated in its entirety herein) also shows which positions of the splice site sequence may have some variability, and which positions are fixed. As such, a person skilled in the art can easily recognize a splice site sequence based on the known consensus sequence and based on its location relative to the exon/intron boundary. Exemplary splice site sequences include, but are not limited to: A G G || G T G A G T; A A A || G T A A G C; G C A || G T A A G T; G A G || G T G T G G; A/C A G || G T A/G A G T; N A G || G T A/G A G T; N A G || G T A A G T; A/C A/T G || G T A N G T; and N A G/A || G T A A G T (where || denotes the exon/intron boundary and N represents A, G, C, or T).

[0212] When the aptamer binds its ligand, the effector region forms a stem and thus prevents splicing to the splice donor site at the 3' end of the alternative exon, which prevents incorporation of the alternative exon in the target gene mRNA causing increased target gene expression in the presence of the ligand. Under certain conditions (for example, when the aptamer is not bound to its ligand), the effector region is in a context that provides access to the splice donor site at the 3' end of the alternative exon, leading to inclusion of the alternative exon in the target gene mRNA thereby suppressing target gene expression. In

some embodiments, the polynucleotide cassette is placed in the target gene to regulate expression of the target gene in response to a ligand. In one embodiment, the alternatively-spliced exon comprises a stop codon that is in-frame with the target gene when the alternatively-spliced exon is spliced into the target gene mRNA.

[0213] In one embodiment, the gene regulation cassette comprises the sequences of SEQ ID NOs: 2 and 3, wherein SEQ ID NO: 2 is located 5' of an aptamer encoding sequence disclosed herein and SEQ ID NO: 3 is located 3' of the aptamer encoding sequence disclosed herein as shown below (where -X- comprises the aptamer encoding sequence). Lower case letters indicate paired stem sequence linking the aptamer to the remainder of the riboswitch. In one embodiment, the alternative exon (underlined in SEQ ID NO: 2, below) is replaced with another alternative exon sequence.

[0214] SEQ ID NO: 2 (5' of -X-) and SEQ ID NO: 3 (3' of -X-):

[0215] GTGAGTCTATGGGACCCTTGATGTTTTCTTTCCCCTTCTTTTCTATGGTT
AAGTTCATGTCATAGGAAGGGGAGAAGTAACAGGGTACACATATTGACCAAATC
AGGGTAATTTTGCATTTGTAATTTTAAAAAATGCTTTCTTCTTTTAATATACTTTTT
TGTTTATCTTATTTCTAATACTTTCCCTAATCTCTTTCTTTCAGGGCAATAATGATA
CAATGTATCATGCCGAGTAACGCTGTTTCTCTAACTTGTAGGAATGAATTCAGAT
ATTTCCAGAGAATGAAAAAAAAAATCTTCAGTAGAAGgtaatgt-X-
acattacGCACCATTTCTAAAGAATAACAGTGATAATTTCTGGGTTAAGGCAATAGCA
ATATTTCTGCATATAAATATTTCTGCATATAAATTGTAAGTATGTAAGAGGTTTC
ATATTGCTAATAGCAGCTACAATCCAGCTACCATTCTGCTTTTATTTTATGGTTGG
GATAAGGCTGGATTATTCTGAGTCCAAGCTAGGCCCTTTTGCTAATCATGTTTCAT
ACCTCTTATCTTCTCCACAG.

[0216] The alternative exon is flanked by 5' and 3' intronic sequences. The 5' and 3' intronic sequences that can be used in the gene regulation cassettes disclosed herein can be any sequence that can be spliced out of the target gene creating either the target gene mRNA or the target gene comprising the alternative exon in the mRNA, depending upon the presence or absence of a ligand that binds the aptamer. The 5' and 3' intronic sequences each have the sequences necessary for splicing to occur, i.e., splice donor, splice acceptor and branch point sequences. In one embodiment, the 5' and 3' intronic sequences of the gene regulation cassette are derived from one or more naturally occurring introns or portions thereof. In one embodiment, the 5' and 3' intronic sequences are derived from a truncated human beta-globin intron 2 (IVS2Δ), from intron 2 of the human 03-globin gene, from the SV40 mRNA intron (used in pCMV-LacZ vector from Clontech Laboratories, Inc.), from

intron 6 of human triose phosphate isomerase (TPI) gene (Nott Ajit, et al. RNA. 2003, 9:6070617), from an intron from human factor IX (Sumiko Kurachi, et al. J. Bio. Chem. 1995, 270(10), 5276), from the target gene's own endogenous intron, or from any genomic fragment or synthetic introns (Yi Lai, et al. Hum Gene Ther. 2006:17(10): 1036) that contain elements that are sufficient for regulated splicing (Thomas A. Cooper, Methods 2005 (37):331).

[0217] In one embodiment, the alternative exon and riboswitch are engineered to be in an endogenous intron of a target gene. That is, the intron (or a substantially similar intronic sequence) naturally occurs at that position of the target gene. In this case, the intronic sequence immediately upstream of the alternative exon is referred to as the 5' intron or 5' intronic sequence, and the intronic sequence immediately downstream of the alternative exon is referred to as the 3' intron or 3' intronic sequence. In this case, the endogenous intron is modified to contain a splice acceptor sequence and splice donor sequence flanking the 5' and 3' ends of the alternative exon. In one embodiment, the 5' and/or 3' introns are exogenous to the target gene.

[0218] The splice donor and splice acceptor sites in the alternative splicing gene regulation cassette can be modified to be strengthened or weakened. That is, the splice sites can be modified to be closer to the consensus for a splice donor or acceptor by standard cloning methods, site directed mutagenesis, and the like. Splice sites that are more similar to the splice consensus tend to promote splicing and are thus strengthened. Splice sites that are less similar to the splice consensus tend to hinder splicing and are thus weakened. The consensus for the splice donor of the most common class of introns (U2) is A/C A G||G T A/G A G T (where || denotes the exon/intron boundary). The consensus for the splice acceptor is C A G||G (where || denotes the exon/intron boundary). The frequency of particular nucleotides at the splice donor and acceptor sites are described in the art (*see, e.g.*, Zhang, M. Q., Hum Mol Genet. 1988. 7(5):919-932). The strength of 5' and 3' splice sites can be adjusted to modulate splicing of the alternative exon.

[0219] Additional modifications to 5' and 3' introns present in the alternative splicing gene regulation cassette that can be made to modulate splicing include modifying, deleting, and/or adding intronic splicing enhancer elements, intronic splicing suppressor elements and or splice sites, and/or modifying the branch site sequence.

[0220] In one embodiment, the 5' intron has been modified to contain a stop codon that will be in frame with the target gene. The 5' and 3' intronic sequences can also be modified to

remove cryptic splice sites, which can be identified with publicly available software (see, e.g., Kapustin, Y. et al. Nucl. Acids Res. 2011. 1-8).

[0221] The lengths of the 5' and 3' intronic sequences can be adjusted in order to, for example, meet the size requirements for viral expression constructs. In one embodiment, the 5' and/or 3' intronic sequences are about 50 to about 300 nucleotides in length. In one embodiment, the 5' and/or 3' intronic sequences are about 125 to about 240 nucleotides in length.

[0222] The stem portion of the effector region should be of a sufficient length (and GC content) to substantially prevent alternative splicing of the alternative exon upon ligand binding the aptamer, while also allowing access to the splice site when the ligand is not present in sufficient quantities. In embodiments, the stem portion of the effector region comprises a stem sequence in addition to the 5' splice site sequence of the 3' intron and its complementary sequence of the 5' splice site sequence. In embodiments, this additional stem sequence comprises a sequence from the aptamer stem. The length and sequence of the stem portion can be modified using known techniques in order to identify stems that allow acceptable background expression of the target gene when no ligand is present and acceptable expression levels of the target gene when the ligand is present. In one embodiment, the effector region stem of the riboswitch is about 7 to about 20 base pairs in length. In one embodiment, the effector region stem is 8 to 11 base pairs in length. In addition to the length of the stem, the GC base pair content of the stem can be altered to modify the stability of the stem.

[0223] In one embodiment, the alternative exon that is part of the alternative splicing gene regulation cassettes disclosed herein is a polynucleotide sequence capable of being transcribed to a pre-mRNA and alternatively spliced into the mRNA of the target gene. In one embodiment, the alternative exon contains at least one sequence that inhibits translation such that when the alternative exon is included in the target gene mRNA, expression of the target gene from that mRNA is prevented or reduced. In a preferred embodiment, the alternative exon contains a stop codon (TGA, TAA, TAG) that is in frame with the target gene when the alternative exon is included in the target gene mRNA by splicing. In embodiments, the alternative exon comprises, in addition to a stop codon, or as an alternative to a stop codon, another sequence that reduces or substantially prevents translation when the alternative exon is incorporated by splicing into the target gene mRNA including, e.g., a microRNA binding site, which leads to degradation of the mRNA. In one embodiment, the alternative exon comprises a miRNA binding sequence that results in degradation of the mRNA. In one

embodiment, the alternative exon encodes a polypeptide sequence which reduces the stability of the protein containing this polypeptide sequence. In one embodiment, the alternative exon encodes a polypeptide sequence which directs the protein containing this polypeptide sequence for degradation.

[0224] The basal or background level of splicing of the alternative exon can be optimized by altering exon splice enhancer (ESE) sequences and exon splice suppressor (ESS) sequences and/or by introducing ESE or ESS sequences into the alternative exon. Such changes to the sequence of the alternative exon can be accomplished using methods known in the art, including, but not limited to site directed mutagenesis. Alternatively, oligonucleotides of a desired sequence (e.g., comprising all or part of the alternative exon) can be obtained from commercial sources and cloned into the gene regulation cassette. Identification of ESS and ESE sequences can be accomplished by methods known in the art, including, for example using ESEfinder 3.0 (Cartegni, L. et al. ESEfinder: a web resource to identify exonic splicing enhancers. *Nucleic Acid Research*, 2003, 31(13): 3568-3571) and/or other available resources.

[0225] In one embodiment, the alternative exon is a naturally-occurring exon. In another embodiment, the alternative exon is derived from all or part of a known exon. In this context, “derived” refers to the alternative exon containing sequence that is substantially homologous to a naturally occurring exon, or a portion thereof, but may contain various mutations, such as mutations generated by altering exon splice enhancer (ESE) sequences and exon splice suppressor (ESS) sequences and/or by introducing ESE or ESS sequences into the alternative exon. “Homology” and “homologous” as used herein refer to the percent of identity between two polynucleotide sequences or between two polypeptide sequences. The correspondence between one sequence to another can be determined by techniques known in the art. For example, homology can be determined by a direct comparison of two polypeptide molecules by aligning their sequences and using readily available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s), and size determination of the digested fragments. Two polynucleotide or two polypeptide sequences are “substantially homologous” to each other when, after optimally aligned with appropriate insertions or deletions, at least about 80%, at least about 85%, at least about 90%, and at least about 95% of the nucleotides or amino acids, respectively, match over a defined length of the molecules, as determined using the methods above.

[0226] In one embodiment, the alternative exon is exogenous to the target gene, although it may be derived from a sequence originating from the organism where the target gene will be expressed. As used herein, “exogenous” means derived from a genotypically distinct entity from that of the rest of the entity to which it is compared or into which it is introduced or incorporated. For example, a polynucleotide introduced by genetic engineering techniques into a different cell type is a heterologous polynucleotide (and, when expressed, can encode a heterologous polypeptide). In one embodiment, the alternatively-spliced exon is derived from exon 2 of the human dihydrofolate reductase gene (DHFR), mutant human Wilms tumor 1 exon 5, mouse calcium/calmodulin-dependent protein kinase II delta exon 16, or SIRT1 exon 6. In embodiments, the alternatively-spliced exon is, or comprises, the modified DHFR exon 2 in SEQ ID NO: 5.

GAATGAATTCAGATATTTCCAGAGAATGAAAAAAAAAATCTTCAGTAGAAG. In embodiments, the alternatively-spliced exon is, or comprises, the modified DHFR exon 2 in SEQ ID NO: 6

GAATGAATTCAGATATTTCCAGAGAATGAAAAAAAAAATCTTCAGTAGAAG.

[0227] Aptamer-mediated cleavage by self-cleaving ribozymes

[0228] In one embodiment, the aptamer-mediated expression of the target gene is regulated by an aptamer-mediated modulation of small endonucleolytic ribozymes. A ribozyme is an RNA enzyme that catalyzes a chemical reaction. In the nucleic acids and methods disclosed herein, a ribozyme may be any small endonucleolytic ribozyme that will self-cleave in the target cell type including, but not limited to a hammerhead, hairpin, the hepatitis delta virus, the Varkud satellite, twister, twister sister, pistol or hatchet ribozyme. Accordingly, in one embodiment, provided is a riboswitch, and a gene expression cassette comprising the riboswitch that contains a ribozyme linked to an aptamer disclosed herein. WO2017/136608, which is incorporated in its entirety by reference herein, describes such riboswitches that activate ribozyme self-cleavage in the presence of aptamer ligand (“off” switch) or riboswitches that inhibit ribozyme self-cleavage in the presence of aptamer (“on” switch).

[0229] In an “off” switch scenario, aptamer/ligand binding increases the ribonuclease function of the ribozyme, leading to cleavage of the target gene RNA that contains the polynucleotide cassette, thereby reducing target gene expression. Examples of such an off switch include a polynucleotide cassette for the regulation of the expression of a target gene comprising a riboswitch that comprises a twister ribozyme linked by a stem to an aptamer,

wherein the stem linking the twister ribozyme to the aptamer attaches to the ribozyme at the location of the P3 stem of the twister ribozyme and wherein the target gene is linked to the P1 stem of the twister ribozyme (see, e.g. Figs. 1a, 1b, or 3a of WO2017/136608 and the associated text, incorporated herein by reference).

[0230] In an “on” switch scenario, aptamer/ligand binding inhibits the ribonuclease function of the ribozyme, decreasing cleavage of the target gene RNA that contains the polynucleotide cassette, thereby increasing target gene expression in the presence of ligand. Examples of an on switch include a riboswitch that comprises a twister ribozyme linked to an aptamer, wherein the aptamer is linked to the 3' or 5' end of the twister ribozyme P1 stem, wherein when the aptamer is linked to the 3' end of the twister ribozyme P1 stem, a portion of the 3' arm of the twister ribozyme P1 stem is alternatively the 5' arm of the aptamer P1 stem, and wherein when the aptamer is linked to the 5' end of the twister ribozyme P1 stem, a portion of the 5' arm of the twister ribozyme P1 stem is alternatively the 3' arm of the aptamer P1 stem (see, e.g., Figs. 6a-6b of WO2017/136608 and the associated text, incorporated herein by reference).

[0231] Aptamer modulation of polyadenylation

[0232] In embodiments, the expression of a target gene is regulated by aptamer-modulated polyadenylation. The 3' end of almost all eukaryotic mRNAs comprises a poly(A) tail—a homopolymer of 20 to 250 adenosine residues. Because addition of the poly(A) tail to mRNA protects it from degradation, expression of a gene can be influenced by modulating the polyadenylation the corresponding mRNA.

[0233] In one embodiment, the expression of the target gene is regulated through aptamer-modulated accessibility of polyadenylation sequences as described in and WO2018/156658, which is incorporated in its entirety by reference herein. In such embodiments, the riboswitch comprises an effector stem-loop and an aptamer described herein, wherein the effector stem-loop comprises a polyadenylation signal, and wherein the aptamer and effector stem-loop are linked by an alternatively shared stem arm comprising a sequence that is complementary to the unshared arm of the aptamer stem (e.g., the aptamer P1 stem) and to the unshared arm of the effector stem loop (see, e.g., Figs 1a, 1b, 2a, and 5a of WO2018/156658 and the associated text, incorporated herein by reference). In one embodiment, the effector stem-loop is positioned 3' of the aptamer such that the alternatively shared stem arm comprises all or a portion of the 3' aptamer stem arm and all or a portion of the 5' arm of the effector stem. In one embodiment, the effector stem-loop is positioned 5' of

the aptamer such that the alternatively shared stem arm comprises all or a portion of the 5' aptamer stem arm and all or a portion of the 3' arm of the effector stem. In one embodiment, the polyadenylation signal comprises AATAA or ATTAA. In one embodiment, the polyadenylation signal is AATAAA or ATTAAA. In embodiments, the polyadenylation signal is a downstream element (DSE). In one embodiment, the polyadenylation signal is an upstream sequence element (USE). In one embodiment, the polynucleotide cassette comprises two riboswitches, wherein the effector stem loop of the first riboswitch comprises all or part of the polyadenylation signal AATAAA or ATTAAA and the effector stem loop of the second riboswitch comprises all or part of the downstream element (DSE). In one embodiment, the two riboswitches each comprise aptamers that bind the same ligand. In one embodiment, the two riboswitches comprise different aptamers that bind different ligands.

[0234] In some embodiments, the riboswitch comprises a sensing region (e.g., an aptamer described herein) and an effector region comprising a binding site for the small nuclear ribonucleoprotein (snRNP) U1, which is part of the spliceosome. WO2017/136591 describes riboswitches wherein the effector region comprises a U1 snRNP binding site (and sequence complementary thereto), and is incorporated herein by reference in its entirety. When the aptamer binds its ligand, the effector region forms a stem and sequesters the U1 snRNP binding site from binding a U1 snRNP. Under certain conditions (for example, when the aptamer is not bound to its ligand), the effector region is in a context that provides access to the U1 snRNP binding site, allowing U1 snRNP to bind the mRNA and inhibit polyadenylation leading to degradation of the message. The U1 snRNP binding site can be any polynucleotide sequence that is capable of binding the U1 snRNP, thereby recruiting the U1 snRNP to the 3' UTR of a target gene and suppressing polyadenylation of the target gene message. In one embodiment, the U1 snRNP binding site is CAGGTAAGTA, (CAGGUAAGUA, when in the mRNA). In some embodiments, the U1 snRNP binding site is a variation of this consensus sequence, including for example sequences that are shorter or have one or more nucleotides changed from the consensus sequence. In one embodiment, the U1 snRNP binding site contains the sequence CAGGTAAG. In some embodiments, the binding site is encoded by the sequence selected from CAGGTAAGTA, CAGGTAAGT, and CAGGTAAG. The U1 snRNP binding site can be any 5' splice site sequence from a gene, e.g., the 5' splice site from human DHFR exon 2.

[0235] Aptamer-mediated modulation of ribonuclease cleavage

[0236] In one embodiment, the expression of the target gene is regulated through aptamer-modulated ribonuclease cleavage. Ribonucleases (RNases) recognize and cleave specific ribonuclease substrate sequences. Provided herein are recombinant DNA constructs that, when incorporated into the DNA of a target gene, provide the ability to regulate expression of the target gene by aptamer/ligand mediated ribonuclease cleavage of the resulting RNA. In some embodiments, the aptamer encoding sequence described herein is part of a construct that contains or encodes a ribonuclease substrate sequence and a riboswitch comprising an effector region and the aptamer such that when the aptamer binds a ligand, target gene expression occurs (as described in WO2018/161053, which is incorporated in its entirety by reference herein). In embodiments, an RNase P substrate sequence is linked to a riboswitch wherein the riboswitch comprises an effector region and an aptamer described herein, wherein the effector region comprises a sequence complimentary to a portion of the RNase P substrate sequence. Binding of a suitable ligand to the aptamer induces structural changes in the aptamer and effector region, altering the accessibility of the ribonuclease substrate sequence for cleavage by the ribonuclease.

[0237] In one embodiment, the aptamer sequence is located 5' to the RNase P substrate sequence and the effector region comprises all or part of the leader sequence and all or part of the 5' acceptor stem sequence of the RNase P substrate sequence. See, e.g., Figs. 1a, 1b, and 3b of WO2018/161053 and the associated text, incorporated herein by reference. In further embodiments, the acceptor stem of the RNase P substrate and the riboswitch effector region are separated by 0, 1, 2, 3, or 4 nucleotides. In other embodiments, the effector region stem includes, in addition to leader sequence (and its complement), one or more nucleotides of the acceptor stem of the RNase P substrate, and sequence complementary to the one or more nucleotides of the acceptor stem.

[0238] In one embodiment, the aptamer sequence of the polynucleotide cassette is located 3' to the RNase P substrate sequence and the effector region comprises sequence complimentary to the all or part of the 3' acceptor stem of the RNase P substrate sequence. See, e.g., Fig. 3a of WO2018/161053 and the associated text, incorporated herein by reference. In further embodiments, the effector region sequence complimentary to the 3' acceptor stem of the RNase P substrate is 1 to 7 nucleotides. In other words, the effector region stem includes 1 to 7 nucleotides of the acceptor stem and includes sequence that is complementary to this 1 to 7 nucleotides of the acceptor stem. In embodiments, the riboswitch is located 3' of the RNase P substrate so the effector region stem and the acceptor

stem of the RNase P substrate do not overlap. In embodiments, the effector region and the acceptor stem of the RNase P substrate are immediately adjacent (i.e., not overlapping). In other embodiments, the effector region and the acceptor stem of the RNase P substrate are separated by 1, 2, 3, 4, 5 or more nucleotides.

[0239] Target Gene

[0240] The aptamers and gene regulation cassettes disclosed herein can be used to regulate the expression of any target gene that can be expressed in a target cell, tissue or organism. The term “target gene” refers to a polynucleotide that is introduced into a cell and is capable of being transcribed into RNA and translated and/or expressed under appropriate conditions. Alternatively, the target gene is endogenous to the target cell and the gene regulation cassette is positioned into the target gene (for example into an existing untranslated region or intron of the endogenous target gene).

[0241] An example of a target gene is a polynucleotide encoding a therapeutic polypeptide. In one embodiment, the target gene is exogenous to the cell in which the recombinant DNA construct is to be transcribed. In another embodiment, the target gene is endogenous to the cell in which the recombinant DNA construct is to be transcribed. The target gene may be a gene encoding a protein, or a sequence encoding a non-protein coding RNA. The target gene may be, for example, a gene encoding a structural protein, an enzyme, a cell signaling protein, a mitochondrial protein, a zinc finger protein, a hormone, a transport protein, a growth factor, a cytokine, an intracellular protein, an extracellular protein, a transmembrane protein, a cytoplasmic protein, a nuclear protein, a receptor molecule, an RNA binding protein, a DNA binding protein, a transcription factor, translational machinery, a channel protein, a motor protein, a cell adhesion molecule, a mitochondrial protein, a metabolic enzyme, a kinase, a phosphatase, exchange factors, a chaperone protein, and modulators of any of these. In embodiments, the target gene encodes erythropoietin (Epo), human growth hormone (hGH), transcription activator-like effector nucleases (TALEN), human insulin, CRISPR associated protein 9 (cas9), or an immunoglobulin (or portion thereof), including, e.g., a therapeutic antibody.

[0242] In embodiments, the target gene is Cas9 or CasRx and the expression construct further comprises a sequence encoding a guide RNA (gRNA), for example a gRNA targeting PCSK9, which can be used to regulate expression of the gRNA target.

[0243] In embodiments, the target gene is PTH. In embodiments, the target gene is insulin (e.g., comprising sequence comprising the A chain, B chain and C peptide) for use in regulating insulin levels in response to a small molecule for treating diabetes.

[0244] In embodiments, the target gene is a therapeutic antibody including an anti-PCSK9 antibody, anti-VEGFR2 antibody (e.g., for ophthalmological applications), anti-amyloid A β 3-42 antibody, anti-IL-17 antibody, anti-PD1 antibody, and anti-HER2 antibody. In embodiments when the target gene is an antibody, the heavy and light chains can be expressed from a single message separated by a protein cleave site (furan, etc.) or peptide self-leaving site (e.g., 2A peptide such as T2A or P2A).

[0245] In embodiments, the target gene encodes an antibody against the SARS-CoV-2 viral proteins or antigens (such as the spike protein)(e.g., casirivimab and/or imdevimab (Regeneron), or bamlanivimab and/or etesevimab (Eli Lilly)). In embodiments, the target gene encodes all or a portion of a SARS-CoV-2 spike protein, where induction of expression produces mRNA and thus functions like an inducible mRNA vaccine (mRNA-1273, Moderna or Comirnaty, Pfizer-BioNTech).

[0246] In embodiments, the aptamers and gene regulation cassettes disclosed herein are used to regulate the expression of a target gene in eukaryotic cells for example, mammalian cells and more particularly human cells. In embodiments, the aptamers and gene regulation cassettes disclosed herein are used to regulate the expression of a target gene in the eye (including cornea and retina), central nervous system (including the brain), liver, kidney, pancreas, heart, airway, muscle, skin, lung, cartilage, testes, arteries, thymus, bone marrow, or in tumors.

[0247] In one aspect, provided are recombinant vectors and their use for the introduction of a polynucleotide comprising a target gene and a gene regulation cassette, wherein the gene regulation cassette comprises an aptamer disclosed herein. In some embodiments, the recombinant DNA constructs include additional DNA elements including DNA segments that provide for the replication of the DNA in a host cell and expression of the target gene in target cells at appropriate levels. The ordinarily skilled artisan appreciates that expression control sequences (promoters, enhancers, and the like) are selected based on their ability to promote expression of the target gene in the target cell. “Vector” means a recombinant plasmid, yeast artificial chromosome (YAC), mini chromosome, DNA mini-circle or virus (including virus derived sequences) that comprises a polynucleotide to be delivered into a host cell, either in vitro or in vivo. In one embodiment, the recombinant vector is a viral vector or a combination of multiple viral vectors.

[0248] Viral vectors for the expression of a target gene in a target cell, tissue, or organism are known in the art and include adenoviral (AV) vectors, adeno-associated virus (AAV) vectors, retroviral and lentiviral vectors, and Herpes simplex type 1 (HSV1) vectors.

[0249] Adenoviral vectors include, for example, those based on human adenovirus type 2 and human adenovirus type 5 that have been made replication defective through deletions in the E1 and E3 regions. The transcriptional cassette can be inserted into the E1 region, yielding a recombinant E1/E3-deleted AV vector. Adenoviral vectors also include helper-dependent high-capacity adenoviral vectors (also known as high-capacity, “gutless” or “guttled” vectors), which do not contain viral coding sequences. These vectors, contain the cis-acting elements needed for viral DNA replication and packaging, mainly the inverted terminal repeat sequences (ITR) and the packaging signal (CY). These helper-dependent AV vector genomes have the potential to carry from a few hundred base pairs up to approximately 36 kb of foreign DNA.

[0250] Recombinant adeno-associated virus “rAAV” vectors include any vector derived from any adeno-associated virus serotype, including, without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-7 and AAV-8, AAV-9, AAV-10, and the like. rAAV vectors can have one or more of the AAV wild-type genes deleted in whole or in part, preferably the Rep and/or Cap genes, but retain functional flanking ITR sequences. Functional ITR sequences are retained for the rescue, replication, packaging and potential chromosomal integration of the AAV genome. The ITRs need not be the wild-type nucleotide sequences, and may be altered (e.g., by the insertion, deletion or substitution of nucleotides) so long as the sequences provide for functional rescue, replication and packaging.

[0251] Alternatively, other systems such as lentiviral vectors can be used. Lentiviral-based systems can transduce nondividing as well as dividing cells making them useful for applications targeting, for examples, the nondividing cells of the CNS. Lentiviral vectors are derived from the human immunodeficiency virus and, like that virus, integrate into the host genome providing the potential for very long-term gene expression.

[0252] Polynucleotides, including plasmids, YACs, minichromosomes and minicircles, carrying the target gene containing the gene regulation cassette can also be introduced into a cell or organism by nonviral vector systems using, for example, cationic lipids, polymers, or both as carriers. Conjugated poly-L-lysine (PLL) polymer and polyethylenimine (PEI) polymer systems can also be used to deliver the vector to cells. Other methods for delivering the vector to cells includes hydrodynamic injection and electroporation and use of ultrasound, both for cell culture and for organisms. For a review of viral and non-viral delivery systems

for gene delivery see Nayerossadat, N. et al. (Adv Biomed Res. 2012; 1:27) incorporated herein by reference.

[0253] In one aspect, this disclosure provides a method of modulating the expression of a target gene (e.g., a therapeutic gene) comprising (a) inserting the polynucleotide cassette comprising an aptamer disclosed herein into the target gene, (b) introducing the target gene comprising the polynucleotide cassette into a cell, and (c) exposing the cell to a small molecule ligand that specifically binds the aptamer in an amount effective to induce expression of the target gene. In aspects, expression of the target gene in target cells confers a desired property to a cell into which it was introduced, or otherwise leads to a desired therapeutic outcome.

[0254] In one embodiment, a gene regulation cassette comprising an aptamer disclosed herein is inserted into the protein coding sequence of the target gene (rather than in the 5' or 3' untranslated regions). In one embodiment, a single gene regulation cassette comprising an aptamer disclosed herein is inserted into the target gene. In other embodiments 2, 3, 4, or more gene regulation cassettes are inserted in the target gene, wherein one or more gene regulation cassettes comprise an aptamer disclosed herein. In one embodiment, two gene regulation cassettes are inserted into the target gene, wherein one or both gene regulation cassettes comprise an aptamer disclosed herein. When multiple gene regulation cassettes are inserted into a target gene, they each can contain the same aptamer such that a single ligand can be used to modulate target gene expression. In other embodiments, multiple gene regulation cassettes are inserted into a target gene, each can contain a different aptamer so that exposure to multiple different small molecule ligands modulates target gene expression.

[0255] Methods of Treatment and Pharmaceutical Compositions

[0256] In one aspect, provided is a method of regulating the level of a therapeutic protein delivered by gene therapy. The therapeutic gene sequence containing a regulatory cassette comprising an aptamer disclosed herein is delivered to the target cells in the body, e.g., by a vector. The cell specificity of the target gene expression may be controlled by a promoter and/or other elements within the vector and/or by the capsid of the viral vector. Delivery of the vector construct containing the target gene, and the transfection of the target tissues resulting in stable transfection of the regulated target gene, is the first step in producing the therapeutic protein. However, due to an aptamer within the target gene sequence, the target gene is not expressed at significant levels, i.e., it is in the “off state” in the absence of the specific ligand that binds to the aptamer contained within in the regulatory cassette

riboswitch. Only when the aptamer specific ligand is administered is the target gene expression activated.

[0257] The delivery of the vector construct containing the target gene and the delivery of the activating ligand generally are separated in time. The delivery of the activating ligand will control when the target gene is expressed, as well as the level of protein expression. The ligand may be delivered by a number of routes including, but not limited to, intravitreal, intraocular, inhalation, subcutaneous, intramuscular, intradermal, intralesion, topical, intraperitoneal, intravenous (IV), intra-arterial, perivascular, intracerebral, intracerebroventricular, oral, sublingual, sublabial, buccal, nasal, intrathoracic, intracardiac, intrathecal, epidural, intraosseous, or intraarticular.

[0258] The timing of delivery of the ligand will depend on the requirement for activation of the target gene. For example, if the therapeutic protein encoded by the target gene is required constantly, an oral small molecule ligand may be delivered daily, or multiple times a day, to ensure continual activation of the target gene, and thus continual expression of the therapeutic protein. If the target gene has a long acting effect, the inducing ligand may be dosed less frequently, for example, once a week, every other week, once a month.

[0259] This aptamers described herein in the context of a gene regulation cassette comprising a riboswitch allow the expression of a therapeutic transgene to be controlled temporally, in a manner determined by the temporal dosing of the ligand specific to the aptamer. The expression of the therapeutic transgene only on ligand administration, increases the safety of a gene therapy treatment by allowing the target gene to be off in the absence of the ligand.

[0260] Different aptamers can be used in multiple riboswitches to allow different ligands to up-regulate or down-regulate the expression of a target gene. In certain embodiments, each therapeutic gene containing a regulatory cassette will have a specific aptamer within the cassette that will be activated by a specific small molecule. This means that each therapeutic gene can be activated only by the ligand specific to the aptamer housed within it. In these embodiments, each ligand will only activate one therapeutic gene. This allows for the possibility that several different “target genes” may be delivered to one individual and each will be activated on delivery of the specific ligand for the aptamer contained within the regulatory cassette housed in each target gene.

[0261] The aptamers disclosed herein in the context of a riboswitch allow any therapeutic protein whose gene can be delivered to the body (such as erythropoietin (EPO) or a therapeutic antibody) to be produced by the body when the activating ligand is delivered.

This method of therapeutic protein delivery may replace the manufacture of such therapeutic proteins outside of the body which are then injected or infused, e.g., antibodies used in cancer or to block inflammatory or autoimmune disease. The body containing the regulated target gene becomes the biologics manufacturing factory, which is switched on when the gene-specific ligand is administered.

[0262] In one embodiment, the target protein may be a nuclease that can target and edit a particular DNA sequence. Such nucleases include CasRx, Cas9, zinc finger containing nucleases, or TALENs. In the case of these nucleases, the nuclease protein may be required for only a short period of time that is sufficient to edit the target endogenous genes. However, if an unregulated nuclease gene is delivered to the body, this protein may be present for the rest of the life of the cell. In the case of nucleases, there is an increasing risk of off-target editing the longer the nuclease is present. Regulation of expression of such proteins has a significant safety advantage. In this case, vector containing the nuclease target gene containing a regulatory cassette could be delivered to the appropriate cells in the body. The target gene is in the “off” state in the absence of the cassette-specific ligand, so no nuclease is produced. Only when the activating ligand is administered, is the nuclease produced. When sufficient time has elapsed allowing sufficient editing to occur, the ligand will be withdrawn and not administered again. Thus the nuclease gene is thereafter in the “off” state and no further nuclease is produced and editing stops. This approach may be used to correct genetic conditions, including a number of inherited retinopathies such as LCA10 caused by mutations in CEP290 and Stargardts disease caused by mutations in ABCA4.

[0263] Administration of a regulated target gene encoding a therapeutic protein which is activated only on specific ligand administration may be used to regulate therapeutic genes to treat many different types of diseases, e.g., cancer with therapeutic antibodies, immune disorders with immune modulatory proteins or antibodies, metabolic diseases, rare diseases such as PNH with anti-C5 antibodies or antibody fragments as the regulated gene, or ocular angiogenesis with therapeutic antibodies, and dry AMD with immune modulatory proteins.

[0264] A wide variety of specific target genes, allowing for the treatment of a wide variety of specific diseases and conditions, are suitable for use as a target gene whose expression can be regulated using an aptamer/ligand described herein. For example, insulin or an insulin analog (preferably human insulin or an analog of human insulin) may be used as the target gene to treat type I diabetes, type II diabetes, or metabolic syndrome; human growth hormone may be used as the target gene to treat children with growth disorders or growth hormone-deficient adults; erythropoietin (preferably human erythropoietin) may be used as the target

gene to treat anemia due to chronic kidney disease, anemia due to myelodysplasia, or anemia due to cancer chemotherapy. Additional target genes compatibles with the aptamers and gene expression cassettes disclosed herein include, but are not limited to, cyclic nucleotide-gated cation channel alpha-3 (CNGA3) and cyclic nucleotide-gated cation channel beta-3 (CNGB3) for the treatment of achromatopsia, retinoid isomerohydrolase (RPE65) for the treatment of retinitis pigmentosa or Leber's congenital amaurosis, X-linked retinitis pigmentosa GTPase regulator (RPGR) for the treatment of X-linked retinitis pigmentosa, glutamic acid decarboxylase (GAD) including for the treatment of Parkinson's disease, regulator of nonsense transcripts 1 (UPF1) for the treatment amyotrophic lateral sclerosis, and aquaporin for the treatment of radiation-induced xerostomia and Sjogren's syndrome. Additional target genes include ArchT (archaerhodopsin from *Halorubrum* strain TP009), Jaws (cruxhalorhodopsin derived from *Haloarcula* (Halobacterium) *salinarum* (strain Shark)), iC1C2 (a variant of a C1C2 chimaera between channel rhodopsins ChR1 and ChR2 from *Chlamydomonas reinhardtii*), or Rgs9-anchor protein (R9AP), a critical component of GTPase complex that mediates the deactivation of phototransduction cascade.

[0265] The expression constructs comprising an aptamer disclosed herein may be especially suitable for treating diseases caused by single gene defects such as cystic fibrosis, hemophilia, muscular dystrophy, thalassemia, or sickle cell anemia. Thus, human β -, γ -, δ -, or ζ -globin may be used as the target gene to treat β -thalassemia or sickle cell anemia; human Factor VIII or Factor IX may be used as the target gene to treat hemophilia A or hemophilia B.

[0266] In embodiments, the expression constructs/small molecules disclosed herein may be used to treat, prevent, or lessen the severity of a viral disease. In embodiments, the disclosure provides a method for treating, preventing, or lessening the severity of COVID-19 by expressing antibodies against the SARS-CoV-2 viral proteins or antigens (e.g., spike protein) in response to administration of a small molecule ligand. In embodiments, the disclosure provides a method for preventing (or lessening the severity of) infection by SARS-CoV-2 by expressing the spike protein (or multiple serotype spike proteins) or portions thereof, using the gene regulation cassettes described herein and administering ligand. In embodiments, the target gene is an antibody against the SARS-CoV-2 viral proteins or antigens (such as the spike protein). In other embodiments, the target gene encodes all or a portion of one or more SARS-CoV-2 spike proteins, where induction of expression produces mRNA and thus functions like an inducible mRNA vaccine. In embodiments, the expression construct is part of an AAV viral genome and the AAV vector comprising the expression

construct is administered to, e.g., the muscle of a subject followed by administration of the ligand.

[0267] In embodiments, the disclosure provides a method for restoring hemocrit and a method of treating anemia by expression of Epo from a gene regulation construct described herein, wherein a vector comprising an Epo gene regulation construct is administered to the subject in need thereof followed by administration of a small molecule ligand described herein. In embodiments, the anemia is due to chronic kidney disease in the subject.

[0268] In embodiments, the disclosure provides a method for restoring hemocrit and a method of treating chronic kidney disease by expression of Epo from a gene regulation construct described herein, wherein a vector comprising an Epo gene regulation construct is administered to the subject in need thereof followed by administration of a small molecule ligand described herein.

[0269] The small molecules described herein are generally combined with one or more pharmaceutically acceptable carriers to form pharmaceutical compositions suitable for administration to a patient. Pharmaceutically acceptable carriers include solvents, binders, diluents, disintegrants, lubricants, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, generally used in the pharmaceutical arts. Pharmaceutical compositions may be in the form of tablets, pills, capsules, troches, eye drops, and the like, and are formulated to be compatible with their intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, intranasal, subcutaneous, oral, inhalation, transdermal (topical), transmucosal, and ocular.

[0270] The pharmaceutical compositions comprising compounds of I-XVI are administered to a patient in a dosing schedule such that an amount of the compound sufficient to desirably regulate the target gene is delivered to the patient. When the dosage form is a tablet, pill, or the like, preferably the pharmaceutical composition comprises from 0.1 mg to 10 g of the compound; from 0.5 mg to 5 g of the compound; from 1 mg to 1 g of the compound; from 2 mg to 750 mg of the compound; from 5 mg to 500 mg of the compound; from 10 mg to 250 mg of the compound; or from 150 mg to 300 mg of the compound.

[0271] The pharmaceutical compositions may be dosed once per day or multiple times per day (e.g., 2, 3, 4, 5, or more times per day). Alternatively, pharmaceutical compositions may be dosed less often than once per day, e.g., once every 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days, or once a month or once every few months. In some embodiments, the

pharmaceutical compositions may be administered to a patient only a small number of times, e.g., once, twice, three times, etc.

[0272] Provided herein is a method of treating a patient in need of increased expression of a therapeutic protein encoded by a target gene, the method comprising administering to the patient a pharmaceutical composition comprising a ligand, which an aptamer disclosed herein binds to or otherwise responds to, wherein the patient previously had been administered a recombinant DNA comprising the target gene, and where the target gene contains a gene regulation cassette disclosed herein that provides the ability to regulate expression of the target gene by the ligand of the aptamer. Provided herein is a pharmaceutical composition comprising a ligand, which an aptamer disclosed herein binds to or otherwise responds to, for use in a method of treating a patient in need of increased expression of a therapeutic protein encoded by a target gene, wherein the patient previously had been administered a recombinant DNA comprising the target gene, and where the target gene contains a gene regulation cassette disclosed herein that provides the ability to regulate expression of the target gene by the ligand of the aptamer.

[0273] Aptamers for detection and/or diagnostic uses

[0274] A wide range of detection and diagnostic agents can be linked to aptamers through chimerical or physical conjugation. Further, aptamers can be incorporated in biosensors, microfluidic devices and other detection platforms. In some embodiments, the aptamer is conjugated to a polyalkylene glycol moiety, including, but not limited to, polyethylene glycol (PEG), polypropylene glycol (PPG), polyoxyethylated glycerol (POG) and other polyoxyethylated polyols, polyvinyl alcohol (PVA) and other polyalkylene oxides, polyoxyethylated sorbitol, or polyoxyethylated glucose.

[0275] In some embodiments, the aptamer is conjugated to a detectable moiety, including, but not limited to, fluorescent moieties or labels, imaging agents, radioisotopic moieties, radiopaque moieties, and the like, e.g. detectable labels such as biotin, fluorophores, chromophores, spin resonance probes, nanoparticles (including, but not limited to gold, magnetic, and superparamagnetic nanoparticles), quantum dots, radiolabels. Exemplary fluorophores include fluorescent dyes (e.g. fluorescein, rhodamine, and the like) and other luminescent molecules (e.g. luminal). A fluorophore may be environmentally-sensitive such that its fluorescence changes if it is located close to one or more residues in the modified protein that undergo structural changes upon binding a substrate (e.g. dansyl probes). Exemplary radiolabels include small molecules containing atoms with one or more low

sensitivity nuclei (^{13}C , ^{15}N , ^2H , ^{125}I , ^{123}I , ^{99}Tc , ^{43}K , ^{52}Fe , ^{67}Ga , ^{68}Ga , ^{111}In and the like). Other useful moieties are known in the art.

[0276] In some embodiments, the aptamer is conjugated to a therapeutic moiety, including, but not limited to, an anti-inflammatory agent, anti-cancer agent, anti-neurodegenerative agent, anti-infective agent, or generally a therapeutic agent.

[0277] Methods for Identifying an Aptamer That Binds to a Compound

[0278] Disclosed herein are methods for identifying an aptamer that binds to a compound of Formula I-XIV, or otherwise modulates target gene expression when part of a riboswitch, in response to the addition of, or exposure to, the compound of Formula I-XIV. In one embodiment, the method comprises the steps of:

- (i) selecting a parent aptamer sequence;
- (ii) generating an aptamer library comprising sequence encoding the aptamer selected in (i), wherein one or more nucleotides in the aptamer encoding sequence are randomly mutated at one or more positions that correspond to one or more unpaired regions in the aptamer, wherein the mutated aptamer sequences are in the context of a riboswitch that controls the expression of a reporter gene;
- (iii) screening the library from (ii) for aptamers having increased regulation (e.g., higher fold induction or repression) of the target gene expression in response to a compound disclosed herein compared to the parent aptamer sequence;
- (iv) optionally repeating steps (ii) and (iii) on an aptamer identified in step (iii) rather than an aptamer selected in step (i).

[0279] The parent aptamer sequence may be a TPP aptamer, including known TPP aptamer sequence or may be a putative TPP aptamer identified by searching for homologous sequences in available databases. The parent aptamer sequence may be an aptamer sequence disclosed herein, e.g.,

CTGGGGAGTCCTTCATGCGGGGCTGAGAGGATGGAAGCAATCGACCATCGACCC
ATTGCACCTGATCCGGATCATGCCGGCGCAGGGAG (12C6-1; SEQ ID NO: 1).

[0280] The step of selecting a parent aptamer sequence can involve, for example, (i) identifying a putative TPP aptamer; (ii) inserting the aptamer into a riboswitch that modulates the expression of a target gene (for example a reporter gene); and (iii) exposing the riboswitch/target gene construct to a thiamine or TPP analog or derivative (e.g., the compounds described herein).

[0281] Putative TPP aptamers can be identified from an appropriate sequence database such as the Rfam database, which is a collection of RNA families, each represented by multiple sequence alignments, consensus secondary structures and covariance models (CMs). In embodiments, the putative TPP aptamer is identified from the Rfam TPP riboswitch family RF00059. In embodiments, the putative TPP aptamer has a sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97% at least 98% or at least 99% identical to thiC

(GUAAUGUGUCGGAGUGCCUUAGGGAUUAUUCCCCUAAAGCUGAGACCGCAUUGCGGGAUCCGUUGAACCUGAUCAGGCUAAUACCUGCGAAGGGAACACAUAUAC, SEQ ID NO: 7) or

thiM

(GUAAUGUCUCGGGGUGCCCUUCUGCGUGAAGGCUGAGAAAUACCCGUAUCACUGAUCUGGAUAAUGCCAGCGUAGGGAAGACAUAUAC, SEQ ID NO: 8).

[0282] The putative TPP aptamer can be inserted into a riboswitch using techniques known to the ordinarily skilled artisan. The responsiveness of the aptamer to the presence of TPP and one or more thiamine or TPP analogs or derivatives (e.g., the compounds described herein) can be tested in cell culture and/or in a cell-free system. In particular, the cell culture system is a eukaryotic cell culture including, e.g., a mammalian, a plant, or an insect cell culture.

[0283] In order to identify aptamers that respond to a compound described herein, one or more nucleotide positions of the sequence encoding the aptamer (i.e., the parent aptamer) are randomized. Areas of the sequence that can be randomized include J2-4; L3a; P4/J4-5 to J5-4; and L5.

[0284] The nucleotide positions for randomization can be selected based on the structure of the parent aptamer sequence. The predicted secondary structure can be obtained using available programs such as RNAfold (<http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi>) and/or by comparison to the crystal structure of a related aptamer (e.g., the *E. coli* thiM riboswitch in Edwards, TE & Ferré-D'Amaré, AR, Structure. 2006 Sep;14(9):1459-68). For example, unpaired regions of the aptamer, including loop (L) regions (e.g., L3 and/or L5) and joining (J) regions (e.g., J3-2 (joining paired regions P3 and P2), J2-4, and/or J4-5), can be identified, and one or more nucleotides in one or more unpaired regions can be randomized to generate a library of aptamers. In embodiments, one or more nucleotides adjacent to one or more unpaired regions are randomized. Additionally, one or more nucleotides in a paired (P) region can be randomized. Further, one or more

nucleotides in an unpaired or paired region can be added or deleted. The mutagenized aptamer sequences can be provided as a library of aptamer sequences in the context of a riboswitch. In embodiments, the aptamer library is provided in the context of a riboswitch as part of a gene expression cassette disclosed herein.

[0285] The aptamer encoding sequences containing one or more mutations can be tested for responsiveness to the presence of one or more compounds described herein.

[0286] Aptamers that are responsive to the desired compound, can be further mutagenized by randomizing nucleotides. The nucleotides at selected positions, for example unpaired regions, can be randomized and a library created as described above.

[0287] Reporter proteins encoded by the reporter genes used in the methods disclosed herein are proteins that can be assayed by detecting characteristics of the reporter protein, such as enzymatic activity or spectrophotometric characteristics, or indirectly, such as with antibody-based assays. Examples of reporter gene products that are readily detectable include, but are not limited to, puromycin resistance marker (pac), 3-galactosidase, luciferase, orotidine 5'-phosphate decarboxylase (URA3), arginine permease CAN1, galactokinase (GAL1), beta-galactosidase (LacZ), or chloramphenicol acetyl transferase (CAT). Other examples of detectable signals include cell surface markers, including, but not limited to CD4. Reporter genes suitable for the use in the methods for identifying aptamers disclosed herein also include fluorescent proteins (e.g., green fluorescent protein (GFP) and its derivatives), or proteins fused to a fluorescent tag. Examples of fluorescent tags and proteins include, but are not limited to, (3-F)Tyr-EGFP, A44-KR, aacuGFP1, aacuGFP2, aceGFP, aceGFP-G222E-Y220L, aceGFP-h, AcGFP1, AdRed, AdRed-C148S, aeurGFP, afraGFP, alajGFP1, alajGFP2, alajGFP3, amCyan1, amFP486, amFP495, amFP506, amFP515, amilFP484, amilFP490, amilFP497, amilFP504, amilFP512, amilFP513, amilFP593, amilFP597, anm1GFP1, anm1GFP2, anm2CP, anobCFP1, anobCFP2, anobGFP, apulFP483, AQ14, AQ143, Aquamarine, asCP562, asFP499, AsRed2, asulCP, atenFP, avGFP, avGFP454, avGFP480, avGFP509, avGFP510, avGFP514, avGFP523, AzamiGreen, Azurite, BDFP1.6, bfloGFPa1, bfloGFPc1, BFP, BFP.A5, BFP5, bsDronpa (On), ccalGFP1, ccalGFP3, ccalOFP1, ccalRFP1, ccalYFP1, cEGFP, cerFP505, Cerulean, CFP, cFP484, cfSGFP2, cgfmKate2, CGFP, cgfTagRFP, cgigGFP, cgreGFP, CheGFP1, CheGFP2, CheGFP4, Citrine, Citrine2, Clomeleon, Clover, cp-mKate, cpCitrine, cpT-Sapphire174-173, CyOFP1, CyPet, CyRFP1 (CyRFP1), d-RFP618, D10, d1EosFP (Green), d1EosFP (Red), d2EosFP (Green), d2EosFP (Red), deGFP1, deGFP2, deGFP3, deGFP4, dendFP (Green), dendFP (Red), Dendra (Green), Dendra (Red), Dendra2 (Green), Dendra2 (Red), Dendra2-

M159A (Green), Dendra2-M159A (Orange), Dendra2-T69A (Green), Dendra2-T69A (Orange), dfGFP, dimer1, dimer2, dis2RFP, dis3GFP, dKeima, dKeima570, dLanYFP, DrCBD, Dreiklang (On), Dronpa (On), Dronpa-2 (On), Dronpa-3 (On), dsFP483, DspR1, DsRed, DsRed-Express, DsRed-Express2, DsRed-Max, DsRed.M1, DsRed.T3, DsRed.T4, DsRed2, DstC1, dTFP0.1, dTFP0.2, dTG, dTomato, dVFP, E2-Crimson, E2-Orange, E2-Red/Green, EaGFP, EBFP, EBFP1.2, EBFP1.5, EBFP2, ECFP, ECFPH148D, ECGFP, eechGFP1, eechGFP2, eechGFP3, eechRFP, efasCFP, efasGFP, eforCP, EGFP, eGFP203C, eGFP205C, Emerald, Enhanced Cyan-Emitting GFP, EosFP (Green), EosFP (Red), eqFP578, eqFP611, eqFP611V124T, eqFP650, eqFP670, EYFP, EYFP-Q69K, fabdGFP, ffDronpa (On), FoldingReporterGFP, FP586, FPrfl2.3, FR-1, FusionRed, FusionRed-M, G1, G2, G3, Gamillus (On), Gamillus0.1, Gamillus0.2, Gamillus0.3, Gamillus0.4, GCaMP2, gfasGFP, GFP(S65T), GFP-151pyTyrCu, GFP-Tyr151pyz, GFPmut2, GFPmut3, GFPxm16, GFPxm161, GFPxm162, GFPxm163, GFPxm18, GFPxm181uv, GFPxm18uv, GFPxm19, GFPxm191uv, GFPxm19uv, H9, HcRed, HcRed-Tandem, HcRed7, hcriGFP, hmGFP, HriCFP, HriGFP, iFP1.4, iFP2.0, iLov, iq-EBFP2, iq-mApple, iq-mCerulean3, iq-mEmerald, iq-mKate2, iq-mVenus, iRFP670, iRFP682, iRFP702, iRFP713, iRFP720, IrisFP (Green), IrisFP (Orange), IrisFP-M159A (Green), Jred, Kaede (Green), Kaede (Red), Katushka, Katushka-9-5, Katushka2S, KCY, KCY-G4219, KCY-G4219-38L, KCY-R1, KCY-R1-158A, KCY-R1-38H, KCY-R1-38L, KFP1 (On), KikGR1 (Green), KikGR1 (Red), KillerOrange, KillerRed, KO, Kohinoor (On), laesGFP, laGFP, LanFP1, LanFP2, lanRFP-ΔS83l, LanYFP, laRFP, LSS-mKate1, LSS-mKate2, LSSmOrange, M355NA, mAmetrine, mApple, Maroon0.1, mAzamiGreen, mBanana, mBeRFP, mBlueberry1, mBlueberry2, mc1, mc2, mc3, mc4, mc5, mc6, McaG1, McaG1ea, McaG2, mCardinal, mCarmine, mcavFP, mcavGFP, mcavRFP, mcCFP, mCerulean, mCerulean.B, mCerulean.B2, mCerulean.B24, mCerulean2, mCerulean2.D3, mCerulean2.N, mCerulean2.N(T65S), mCerulean3, mCherry, mCherry2, mCitrine, mClavGR2 (Green), mClavGR2 (Red), mClover3, mCyRFP1, mECFP, meffCFP, meffGFP, meffRFP, mEGFP, meleCFP, meleRFP, mEmerald, mEos2 (Green), mEos2 (Red), mEos2-A69T (Green), mEos2-A69T (Orange), mEos3.1 (Green), mEos3.1 (Red), mEos3.2 (Green), mEos3.2 (Red), mEos4a (Green), mEos4a (Red), mEos4b (Green), mEos4b (Red), mEosFP (Green), mEosFP (Red), mEosFP-F173S (Green), mEosFP-F173S (Red), mEosFP-M159A (Green), mEYFP, MfaG1, mGarnet, mGarnet2, mGeos-C (On), mGeos-E (On), mGeos-F (On), mGeos-L (On), mGeos-M (On), mGeos-S (On), mGinger1, mGinger2, mGrape1, mGrape2, mGrape3, mHoneydew, MiCy, mIFP, miniSOG, miniSOGQ103V, miniSOG2, miRFP, miRFP670, miRFP670nano, miRFP670v1, miRFP703,

miRFP709, miRFP720, mIrisFP (Green), mIrisFP (Red), mK-GO (Early), mK-GO (Late), mKalama1, mKate, mKateM41GS158C, mKateS158A, mKateS158C, mKate2, mKeima, mKelly1, mKelly2, mKG, mKikGR (Green), mKikGR (Red), mKillerOrange, mKO, mKO2, mKOok, mLumin, mMaple (Green), mMaple (Red), mMaple2 (Green), mMaple2 (Red), mMaple3 (Green), mMaple3 (Red), mMaroon1, mmGFP, mMiCy, mmilCFP, mNectarine, mNeonGreen, mNeptune, mNeptune2, mNeptune2.5, mNeptune681, mNeptune684, Montiporasp.#20-9115, mOrange, mOrange2, moxBFP, moxCerulean3, moxDendra2 (Green), moxDendra2 (Red), moxGFP, moxMaple3 (Green), moxMaple3 (Red), moxNeonGreen, moxVenus, mPapaya, mPapaya0.7, mPlum, mPlum-E16P, mRaspberry, mRed7, mRed7Q1, mRed7Q1S1, mRed7Q1S1BM, mRFP1, mRFP1-Q66C, mRFP1-Q66S, mRFP1-Q66T, mRFP1.1, mRFP1.2, mRojoA, mRojoB, mRouge, mRtms5, mRuby, mRuby2, mRuby3, mScarlet, mScarlet-H, mScarlet-I, mStable, mStrawberry, mT-Sapphire, mTagBFP2, mTangerine, mTFP0.3, mTFP0.7 (On), mTFP1, mTFP1-Y67W, mTurquoise, mTurquoise2, muGFP, mUkG, mVenus, mVenus-Q69M, mVFP, mVFP1, mWasabi, Neptune, NijiFP (Green), NijiFP (Orange), NowGFP, obeCFP, obeGFP, obeYFP, OFP, OFPxm, oxBFP, oxCerulean, oxGFP, oxVenus, P11, P4, P4-1, P4-3E, P9, PA-GFP (On), Padron (On), Padron(star) (On), Padron0.9 (On), PAmCherry1 (On), PAmCherry2 (On), PAmCherry3 (On), PAmKate (On), PATagRFP (On), PATagRFP1297 (On), PATagRFP1314 (On), pcDronpa (Green), pcDronpa (Red), pcDronpa2 (Green), pcDronpa2 (Red), PdaC1, pdae1GFP, phiYFP, phiYFPv, pHluorin,ecliptic, pHluorin,ecliptic (acidic), pHluorin,ratiometric (acidic), pHluorin,ratiometric (alkaline), pHluorin2 (acidic), pHluorin2 (alkaline), pHuji, PlamGFP, pmeaGFP1, pmeaGFP2, pmimGFP1, pmimGFP2, Pp2FbFP, Pp2FbFPL30M, ppluGFP1, ppluGFP2, pporGFP, pporRFP, PS-CFP (Cyan), PS-CFP (Green), PS-CFP2 (Cyan), PS-CFP2 (Green), psamCFP, PSmOrange (Far-red), PSmOrange (Orange), PSmOrange2 (Far-red), PSmOrange2 (Orange), ptilGFP, R3-2+PCB, RCaMP, RDSmCherry0.1, RDSmCherry0.2, RDSmCherry0.5, RDSmCherry1, rfloGFP, rfloRFP, RFP611, RFP618, RFP630, RFP637, RFP639, roGFP1, roGFP1-R1, roGFP1-R8, roGFP2, rrenGFP, RRvT, rsCherry (On), rsCherryRev (On), rsCherryRev1.4 (On), rsEGFP (On), rsEGFP2 (On), rsFastLime (On), rsFolder (Green), rsFolder2 (Green), rsFusionRed1 (On), rsFusionRed2 (On), rsFusionRed3 (On), rsTagRFP (ON), Sandercyanin, Sapphire, sarcGFP, SBFP1, SBFP2, SCFP1, SCFP2, SCFP3A, SCFP3B, scubGFP1, scubGFP2, scubRFP, secBFP2, SEYFP, sg11, sg12, sg25, sg42, sg50, SGFP1, SGFP2, SGFP2(206A), SGFP2(E222Q), SGFP2(T65G), SHardonnay, shBFP, shBFP-N158S/L173I, ShG24, Sirius, SiriusGFP, Skylan-NS (On), Skylan-S (On), smURFP, SNIFP, SOPP, SOPP2, SOPP3,

SPOON (on), stylGFP, SuperfolderGFP, SuperfoldermTurquoise2, SuperfoldermTurquoise2ox, SuperNovaGreen, SuperNovaRed, SYFP2, T-Sapphire, TagBFP, TagCFP, TagGFP, TagGFP2, TagRFP, TagRFP-T, TagRFP657, TagRFP675, TagYFP, td-RFP611, td-RFP639, tdimer2(12), tdKatushka2, TDsmURFP, tdTomato, tKeima, Topaz, TurboGFP, TurboGFP-V197L, TurboRFP, Turquoise-GL, Ultramarine, UnaG, usGFP, Venus, VFP, vsfGFP-0, vsfGFP-9, W1C, W2, W7, WasCFP, Wi-Phy, YPet, zFP538, zoan2RFP, ZsGreen, ZsYellow1, α GFP, 10B, 22G, 5B, 6C, A1a, aacuCP, acanFP, ahyaCP, amilCP, amilCP580, amilCP586, amilCP604, apulCP584, BFPsol, Blue102, CFP4, cgigCP, CheGFP3, Clover1.5, cpasCP, Cy11.5, dClavGR1.6, dClover2, dClover2A206K, dhorGFP, dhorRFP, dPapaya0.1, Dronpa-C62S, DsRed-Timer, echFP, echiFP, EYFP-F46L, fcFP, fcomFP, Fpaagar, Fpag_frag, Fpcondchrom, Fpmann, Fpmcavgr7.7, Gamillus0.5, gdjiCP, gfasCP, GFPhal, gtenCP, hcriCP, hfriFP, KikG, LEA, mcFP497, mcFP503, mcFP506, mCherry1.5, mClavGR1, mClavGR1.1, mClavGR1.8, mClover1.5, mcRFP, meffCP, mEos2-NA, meruFP, mKate2.5, mOFP.T.12, mOFP.T.8, montFP, moxEos3.2, mPA-GFP, mPapaya0.3, mPapaya0.6, mRFP1.3, mRFP1.4, mRFP1.5, mTFP0.4, mTFP0.5, mTFP0.6, mTFP0.8, mTFP0.9, mTFP1-Y67H, mTurquoise-146G, mTurquoise-146S, mTurquoise-DR, mTurquoise-GL, mTurquoise-GV, mTurquoise-RA, mTurquoise2-G, NpR3784g, PDM1-4, psupFP, Q80R, rfloGFP2, RpBphP1, RpBphP2, RpBphP6, rrGFP, RSGFP1, RSGFP2, RSGFP3, RSGFP4, RSGFP6, RSGFP7, Rtms5, scleFP1, scleFP2, spisCP, stylCP, sympFP, TeAPC α , tPapaya0.01, Trp-lessGFP, vsGFP, Xpa, yEGFP, YFP3, zGFP, and zRFP.

[0288] Methods for screening an aptamer library disclosed herein may include measuring the activity of the reporter gene under the control of the aptamer and/or comparing the activity of the reporter gene in presence of the thiamine or TPP analog used for the screen as compared to the activity of the reporter gene in absence of the thiamine or TPP analog used for the screen.

[0289] Articles of manufacture and kits

[0290] Also provided are kits or articles of manufacture for use in the methods described herein. In aspects, the kits comprise the compositions described herein (e.g., compositions for delivery of a vector comprising the target gene containing the gene regulation cassette) in suitable packaging. Suitable packaging for compositions (such as ocular compositions for injection) described herein are known in the art, and include, for example, vials (such as sealed vials), vessels, ampules, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. These articles of manufacture may further be sterilized and/or sealed.

[0291] Also provided are kits comprising the compositions described herein. These kits may further comprise instruction(s) on methods of using the composition, such as uses described herein. The kits described herein may further include other materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing the administration of the composition or performing any methods described herein. For example, in some embodiments, the kit comprises an rAAV for the expression of a target gene comprising a gene regulation cassette containing an aptamer sequence described herein, a pharmaceutically acceptable carrier suitable for injection, and one or more of: a buffer, a diluent, a filter, a needle, a syringe, and a package insert with instructions for performing the injections. In some embodiments, the kit is suitable for intraocular injection, intramuscular injection, intravenous injection and the like.

[0292] It is to be understood and expected that variations of the compositions of matter and methods herein disclosed can be made by one skilled in the art and it is intended that such modifications are to be included within the scope of the present disclosure. The following Examples further illustrate the invention, but should not be construed to limit the scope of the invention in any way.

[0293] All references cited herein are hereby incorporated by reference in their entirety. All nucleotide sequences provided herein are in a 5' to 3' orientation unless stated otherwise.

EXAMPLES

[0294] **Example 1: A Regulation of Target Gene Expression in Response to Small Molecule Ligands Disclosed Herein**

[0295] **Experimental Procedures:**

[0296] *Riboswitch construct:* Aptamers were synthesized by Integrated DNA Technologies, Inc. The synthesized aptamer sequence, here referred to as aptamer sequence 12C6-1, with C at 5' end and a complementary G at 3' end flanking the aptamer sequence: CTGGGGAGTCCTTCATGCGGGGCTGAGAGGATGGAAGCAATCGACCATCGACCC ATTGCACCTGATCCGGATCATGCCGGCGCAGGGAG (SEQ ID NO:1). Golden Gate cloning strategy (New England Biolabs, NEB) was used to clone the synthesized aptamer sequence into an intron-exon-intron cassette to replace the guanine aptamer in the G17 riboswitch cassette (*see* SEQ ID NO: 15 recited in WO 2016/126747, which is incorporated herein in its entirety) to generate riboswitch construct Luci-12C6-1 which contains the 12C6-1 alternate splicing gene regulation cassette (SEQ ID NO: 4).

SEQ ID NO: 4 (12C6-1 alternate splicing gene regulation cassette)

gtgagtctatgggacccttgatgttttctttcccttcttttctatggtaagttcatgcataggaaggggagaagt
aacagggtacacatatgtgaccaaatacagggaattttgcatttgtaattttaaaaaatgctttctcttttaataata
ctttttgtttatcttatttctaatactttccctaatactcttttcttcagggaataatgatacaatglatcatgceagagt
aacgctgtttctctaactttaggaatgaattcagatatttccagagaatgaaaaaaaaatcttcagtagaa
ggtaatgtCTGGGGAGTCCTTCATGCGGGGCTGAGAGGATGGAAGCAAT
CGACCATCGACCCATTGCACCTGATCCGGATCATGCCGGCGCAGGG
AGacattacgcaccattctaagaataacagtgataatttctgggttaaggcaatagcaatatttctgcatat
aaatatttctgcatataaattgtaactgatgtaagagggttcatattgctaatagcagctacaatccagctacca
tctgcttttattttatgggtgggataaggctggattattctgagtcgaagctaggcccttttgctaatacatgttcata
cctcttatcttctccacag

Caps: 12C6-1 aptamer; bold: alternative exon; underline: riboswitch stem forming sequence; Ital.: 5' intron and 3' intron

[0297] *Transfection:* Human embryonic kidney (HEK) 293 cells were plated in a 96-well flat bottom plate the day before transfection. Plasmid DNA (500 ng) was added to a tube or a 96-well U-bottom plate. Separately, TransIT-293 reagent (Mirus; 1.4 μ L) was added to 50 μ L Optimum I media (Life Technologies) and allowed to sit for 5 minutes at room temperature (RT). Then, 50 μ L of this diluted transfection reagent was added to the DNA, mixed, and incubated at RT for 20 min. Finally, 7 μ L of this solution was added to a well of cells in the 96-well plate. Four hours after transfection, medium containing transfection solution was replaced by medium with either TPP or fursultiamine as aptamer ligands.

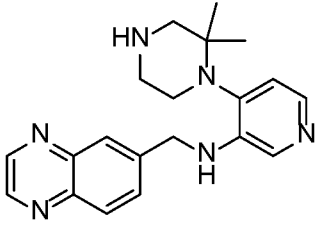
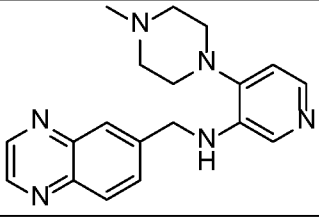
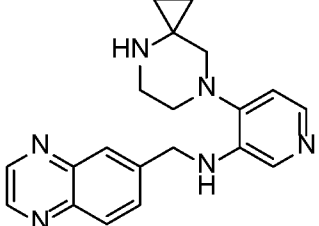
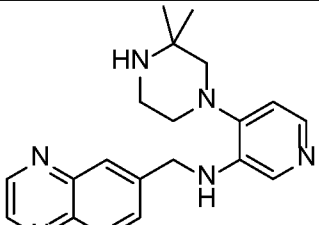
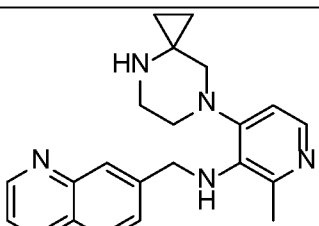
[0298] *Firefly luciferase assay of cultured cells:* Twenty-four hours after media change, plates were removed from the incubator, and equilibrated to RT for several minutes on a lab bench, then aspirated. Glo-lysis buffer (Promega, 100 μ L, RT) was added, and the plates allowed to remain at RT for at least 5 minutes. Then, the well contents were mixed by 50 μ L trituration, and 20 μ L of each sample was mixed with 20 μ L of bright-glo reagent (Promega) that had been diluted to 10% in glo-lysis buffer. 96 wells were spaced on an opaque white 384-well plate. Following a 5 min incubation at RT, luminescence was measured using a Tecan machine with 500 ms read time. The luciferase activity was expressed as mean arbitrary light units (ALU) $\pm$ S.D., and fold induction was calculated as the quotient of the

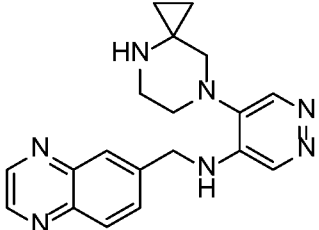
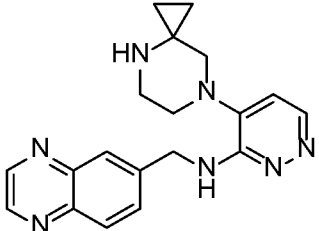
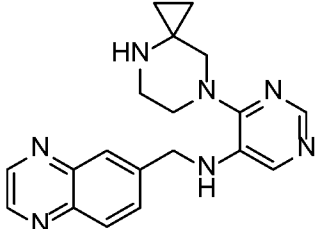
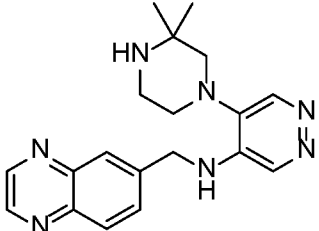
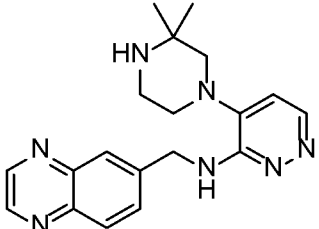
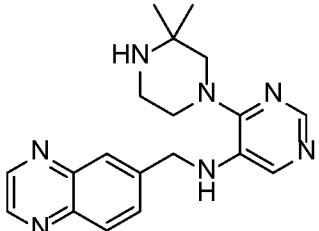
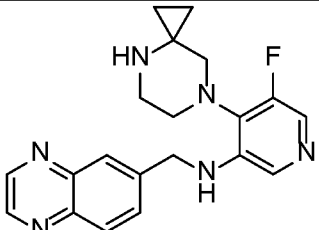
luciferase activity obtained from cells with the compound treatment divided by the luciferase activity obtained from cells without compound treatment.

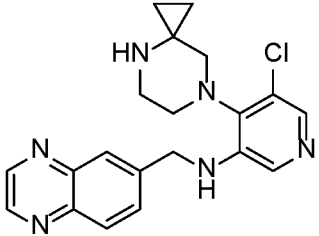
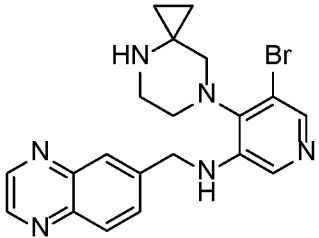
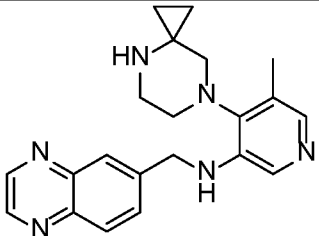
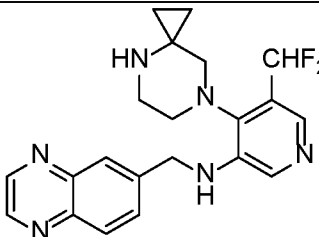
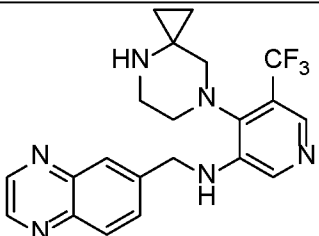
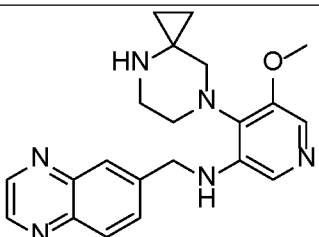
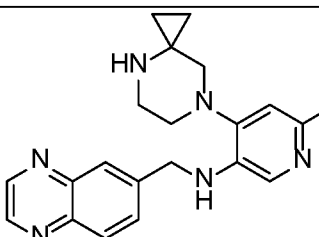
[0299] Results:

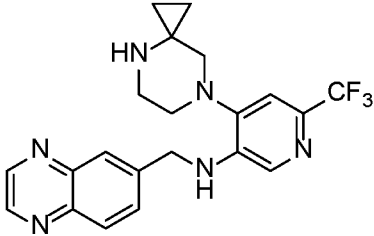
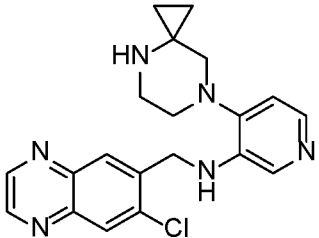
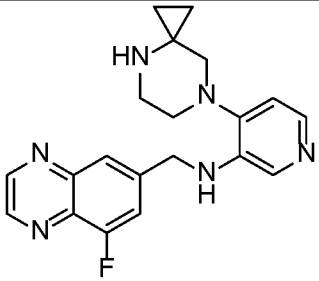
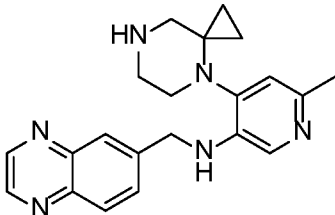
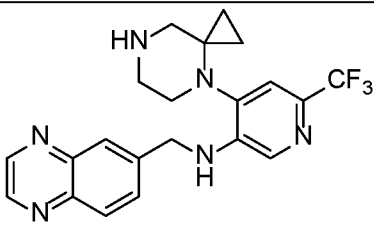
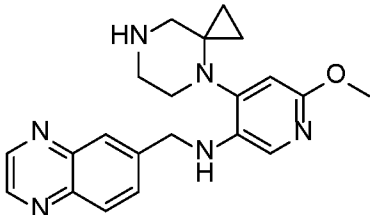
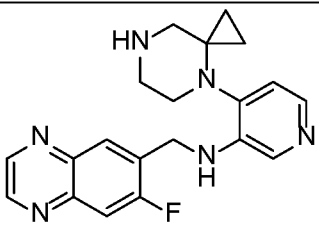
[0300] Compounds were tested against the 12C6-1 riboswitch for induction of luciferase expression in HEK293 cells. The structures for these compounds are provided in Table B, and synthesis is described herein.

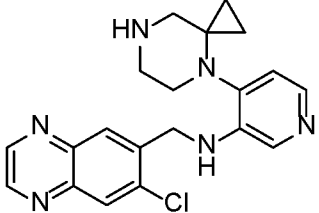
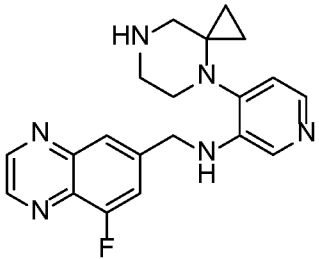
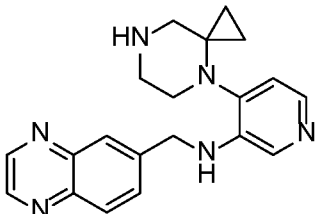
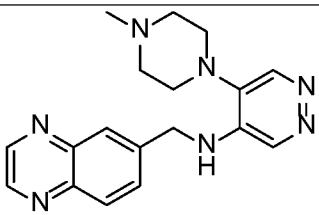
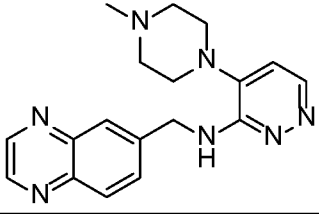
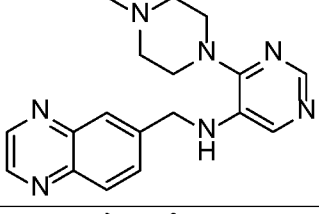
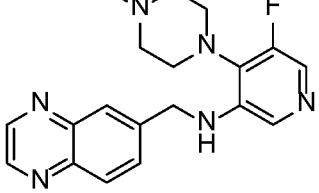
Table B.

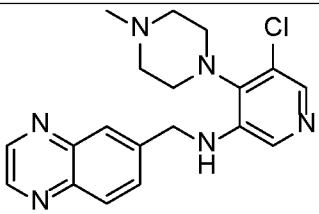
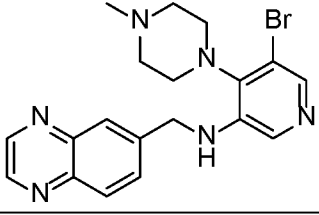
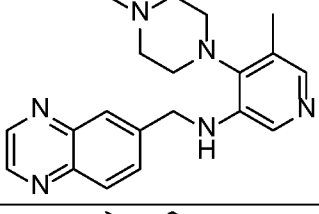
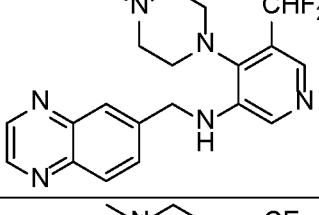
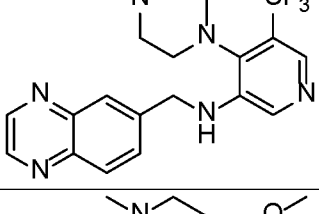
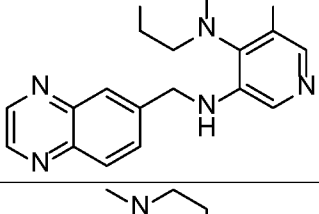
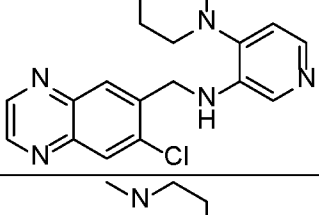
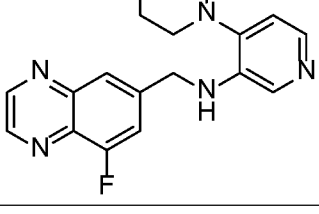
Ref. No.	Structure	Fold induction 2 μ M (HEK)
001		13.1
002		8.3
003		3.5
004		4.6
005		1.5

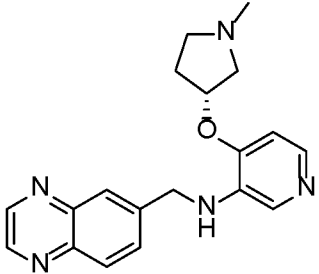
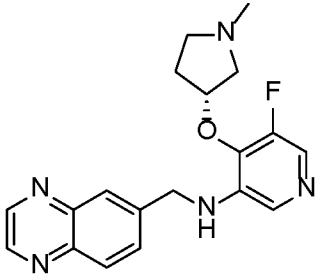
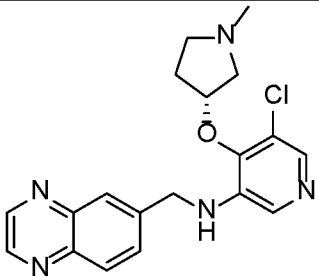
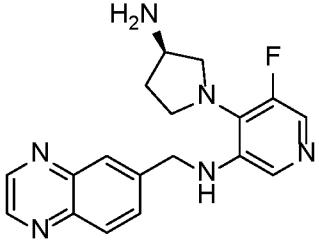
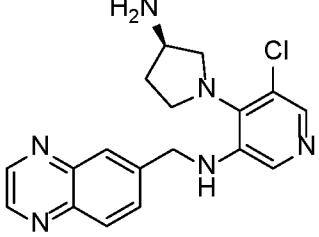
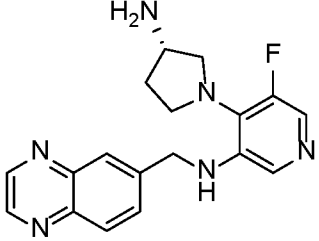
Ref. No.	Structure	Fold induction 2 μ M (HEK)
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007		1.1
008		4.0
009		1.5
010		1.6
011		7.6
012		3.8

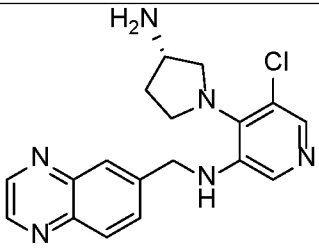
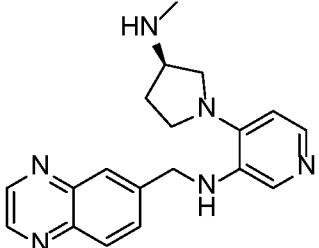
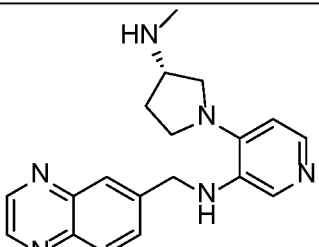
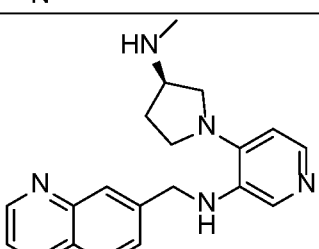
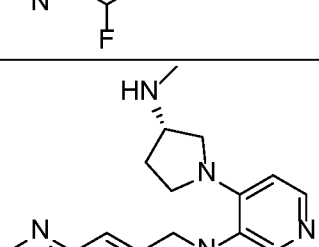
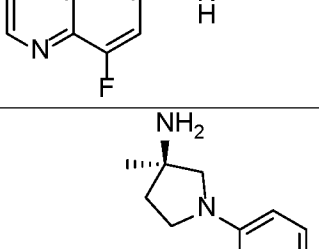
Ref. No.	Structure	Fold induction 2 μ M (HEK)
013		7.6
014		7.2
015		6.8
016		7.0
017		3.9
018		3.9
019		2.4

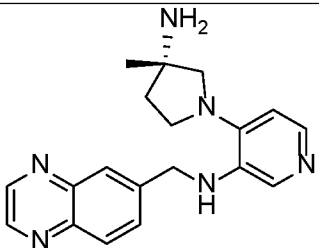
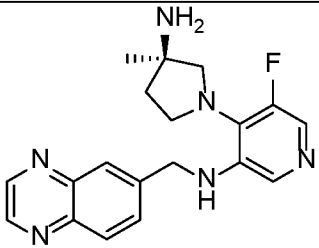
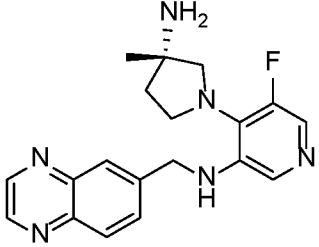
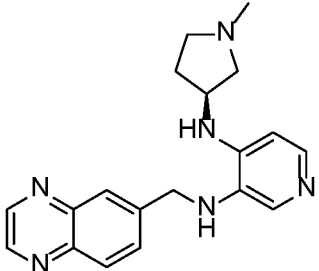
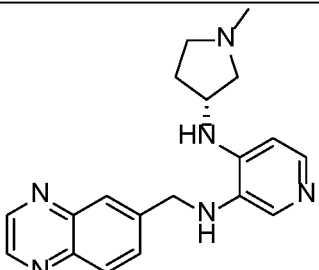
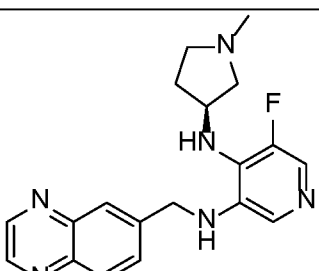
Ref. No.	Structure	Fold induction 2 μ M (HEK)
020		2.8
021		4.7
022		1.4
025		8.8
026		23.9
027		22.6
028		8.5

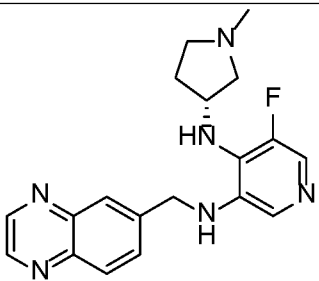
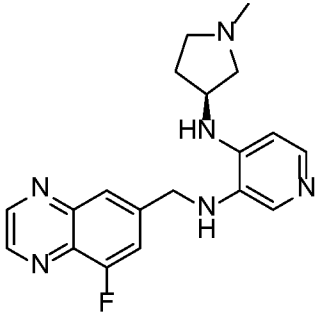
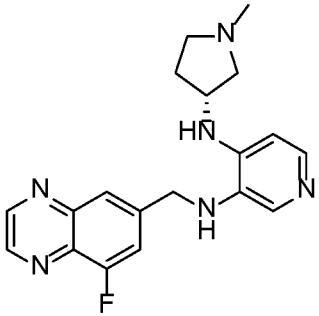
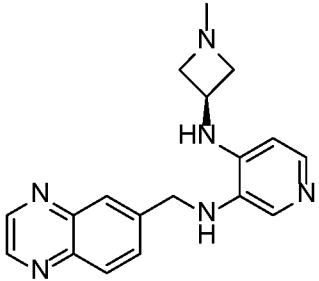
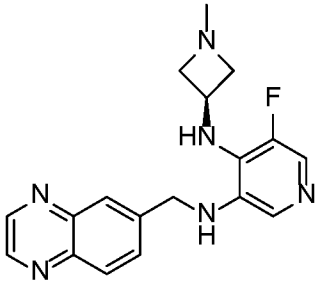
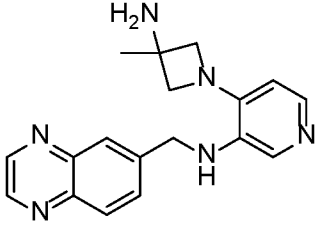
Ref. No.	Structure	Fold induction 2 μ M (HEK)
029		9.1
030		10.8
031		19.8
032		2.2
033		1.7
034		9.4
035		5.6

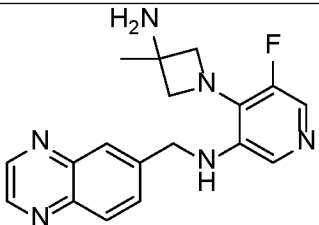
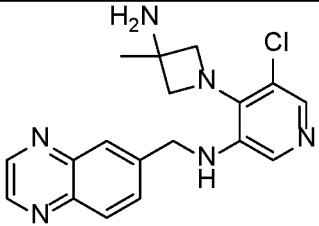
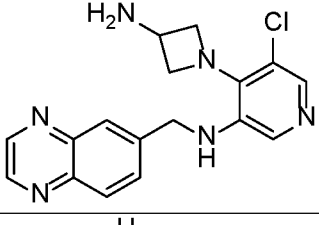
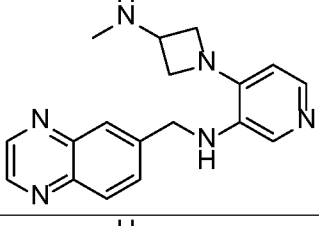
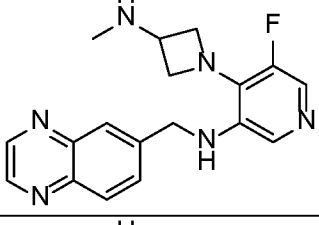
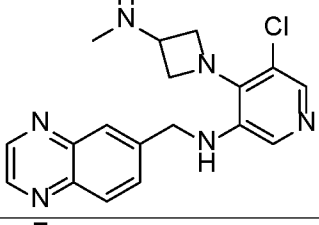
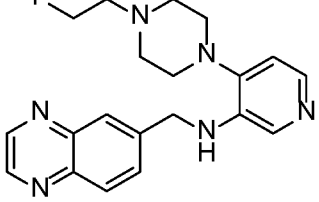
Ref. No.	Structure	Fold induction 2 μ M (HEK)
036		7.6
037		6.5
038		3.6
039		18.3
040		6.4
041		1.4
042		5.7
043		4.3

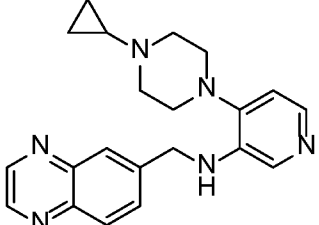
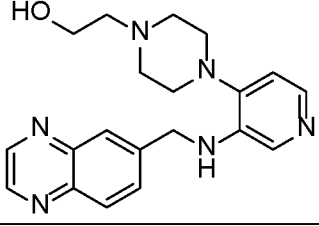
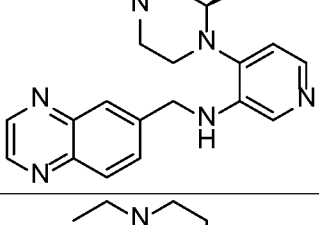
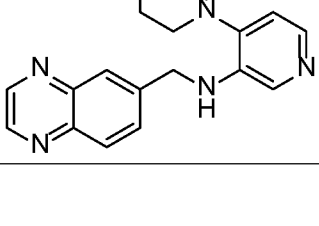
Ref. No.	Structure	Fold induction 2 μ M (HEK)
044		8.5
045		5.0
046		7.0
047		59.7
048		31.6
049		6.5

Ref. No.	Structure	Fold induction 2 μ M (HEK)
050	 <chem>N[C@@H]1CCCN1c2cc(Cl)ccc2NCCc3ccc4ncnc4c3</chem>	13.9
051	 <chem>CN[C@H]1CCCN1c2ccccc2NCCc3ccc4ncnc4c3</chem>	6.6
052	 <chem>CN[C@@H]1CCCN1c2ccccc2NCCc3ccc4ncnc4c3</chem>	1.4
053	 <chem>CN[C@H]1CCCN1c2ccccc2NCCc3cc(F)ccc4ncnc34</chem>	2.5
054	 <chem>CN[C@@H]1CCCN1c2ccccc2NCCc3cc(F)ccc4ncnc34</chem>	1.1
055	 <chem>N[C@H]1CCCN1c2ccccc2NCCc3ccc4ncnc4c3</chem>	60.3

Ref. No.	Structure	Fold induction 2 μ M (HEK)
056		4.3
057		119.3
058		9.3
059		11.5
060		21.1
061		28.1

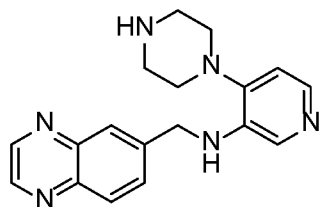
Ref. No.	Structure	Fold induction 2 μ M (HEK)
062		23.5
063		3.4
064		3.7
065		28.7
066		10.4
067		32.3

Ref. No.	Structure	Fold induction 2 μ M (HEK)
068		141.5
069		132.1
070		112.9
071		25.8
072		141.0
073		116.2
074		0.8

Ref. No.	Structure	Fold induction 2 μ M (HEK)
075		1.1
076		2.2
077		1.2
078		3.1

[0301] Comparative Compound A

[0302] The structure of Compound A is provided below:



[0303] Comp. A may be prepared as disclosed in PCT/US2020/045022.

[0304] Examples 2 to 47.

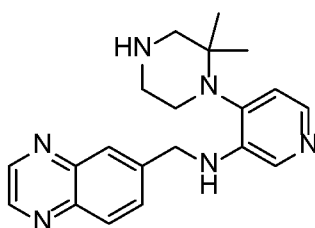
[0305] Experimental

[0306] All solvents and reagents were obtained commercially and used as received. ^1H NMR spectra were recorded on a Bruker instrument (300MHz or 400MHz) in the cited deuterated solvents. Chemical shifts are given in ppm, and coupling constants are in hertz. All final compounds were purified by flash chromatography using 220-400 mesh silica gel or reversed-phase HPLC with CH_3CN /water as the solvents. Thin-layer chromatography was

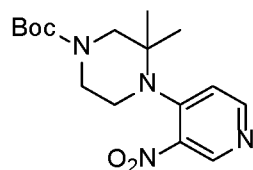
done on silica gel 60 F-254 (0.25-nm thickness) plates. Visualization was accomplished with UV light and/or 10% phosphomolybdic acid in ethanol. Nominal (low resolution) mass spectra were acquired on either a Waters LCT or an Applied Biosystems API 3000 mass spectrometer. All other LC-MS experiments were done on an Agilent 1100 HPLC coupled with an Agilent single quadrupole mass spectrometer. Compound purity was determined by a LC-MS with 230 nM and 254 nM wavelengths. All final compounds reported here have purity $\geq 95\%$.

[0307] Example 2

[0308] 4-(2,2-Dimethylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 001)

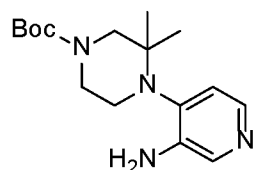


[0309] Step 1. *tert*-Butyl 3,3-dimethyl-4-(3-nitropyridin-4-yl)piperazine-1-carboxylate



[0310] A mixture of 4-chloro-3-nitropyridine (3.00 g, 18.9 mmol, 1.00 *eq*) and *tert*-butyl 3,3-dimethylpiperazine-1-carboxylate (12.2 g, 56.8 mmol, 3.00 *eq*) was stirred at 130 °C for 6 h under N₂ atmosphere. The crude product was purified by reversed-phase HPLC to give the title compound (1.30 g, 20.4%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.88 (s, 1H), 8.54 (d, *J* = 6.0 Hz, 1H), 7.52 (d, *J* = 5.6 Hz, 1H), 3.43-3.39 (m, 2H), 3.27 (s, 2H), 3.09 - 2.98 (m, 2H), 1.42 (s, 9H), 1.23 - 1.16 (m, 6H). MS (ES⁺) *m/e* 337 (M+H)⁺.

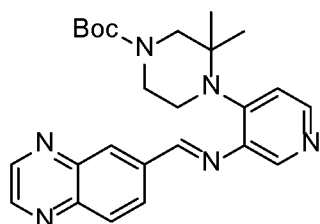
[0311] Step 2. *tert*-Butyl 4-(3-aminopyridin-4-yl)-3,3-dimethylpiperazine-1-carboxylate



[0312] To a solution of *tert*-butyl 3,3-dimethyl-4-(3-nitropyridin-4-yl)piperazine-1-carboxylate (1.30 g, 3.86 mmol, 1.00 *eq*) in MeOH (15.0 mL) was added Pt-V/C (1.01 g, 3.86 mmol, 1.00 *eq*) under N₂. The suspension was degassed under vacuum and purged with H₂

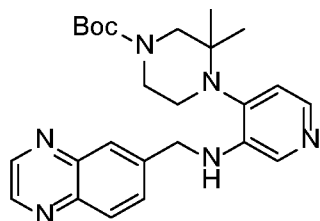
several times. The mixture was stirred under H₂ (15 psi) at 15 °C for 16 h, filtered and concentrated to give the title compound (1.20 g, crude) as a yellow oil. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.99 (s, 1H), 7.68 (d, *J* = 5.2 Hz, 1H), 6.98 (d, *J* = 5.2 Hz, 1H), 5.16 (s, 2H), 1.42 (s, 9H), 1.00 (br s, 6H). MS (ES⁺) *m/e* 307 (M+H)⁺.

[0313] Step 3. *tert*-Butyl (*E*)-3,3-dimethyl-4-(3-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)piperazine-1-carboxylate



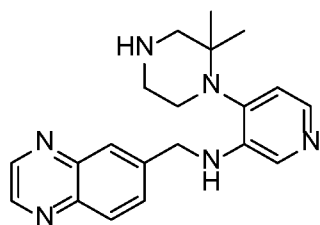
[0314] A mixture of *tert*-butyl 4-(3-aminopyridin-4-yl)-3,3-dimethylpiperazine-1-carboxylate (1.20 g, 3.92 mmol, 1.00 *eq*), quinoxaline-6-carbaldehyde (929 mg, 5.87 mmol, 1.50 *eq*), and AcOH (118mg, 1.96 mmol, 112 uL, 0.50 *eq*) in MeOH (20.0 mL) was degassed and purged with N₂ for 3 times. The mixture was stirred at 50 °C for 12 h under N₂ atmosphere. The reaction mixture was used for the next step reaction directly. MS (ES⁺) *m/e* 447 (M+H)⁺.

[0315] Step 4. *tert*-Butyl 3,3-dimethyl-4-(3-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)piperazine-1-carboxylate



[0316] To a solution of *tert*-butyl (*E*)-3,3-dimethyl-4-(3-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)piperazine-1-carboxylate (1.50 g, 3.36 mmol, 1.00 *eq*) in MeOH (20.0 mL) was added NaBH₃CN (528 mg, 8.40 mmol, 2.50 *eq*). The mixture was stirred at 25 °C for 2 h, diluted with **ethyl acetate (40.0 mL)**, washed with brine (**50.0 mL** × 3), dried over **Na₂SO₄**, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC to give the title compound (1.30 g, 86.3%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.90 (d, *J* = 1.2 Hz, 2H), 8.08 (d, *J* = 8.4 Hz, 1H), 7.99 (s, 1H), 7.89 (s, 1H), 7.79 (s, 1H), 7.73 (d, *J* = 5.2 Hz, 1H), 7.07 (d, *J* = 4.8 Hz, 1H), 6.45 (br t, *J* = 6.0 Hz, 1H), 4.69 (br d, *J* = 1.6 Hz, 2H), 4.13 - 3.87 (m, 1H), 3.81 - 3.62 (m, 1H), 3.31 - 2.95 (m, 4H), 1.44 (s, 9H), 1.24 - 0.91 (m, 6H). MS (ES⁺) *m/e* 449 (M+H)⁺.

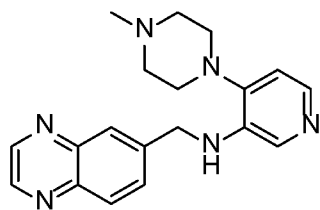
[0317] Step 5. 4-(2,2-dimethylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine



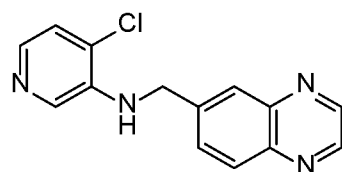
[0318] To a solution of *tert*-butyl 3,3-dimethyl-4-(3-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)piperazine-1-carboxylate (1.30 g, 2.90 mmol, 1.00 *eq*) in ethyl acetate (5.00 mL) was added 4M HCl in EtOAc (0.724 mL, 1.00 *eq*). The mixture was stirred at 15 °C for 2 h and was filtered. The filtration cake was dried *in vacuo* to provide the title compound (172 mg, 15.0%, HCl salt) as a green solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.82 (s, 2H), 8.04 (dd, *J*₁ = 1.2 Hz, *J*₂ = 8.8 Hz, 1H), 7.92 (s, 1H), 7.87 (dd, *J*₁ = 1.2 Hz, *J*₂ = 6.0 Hz, 1H), 7.83 (dd, *J*₁ = 2.0 Hz, *J*₂ = 8.8 Hz, 1H), 7.72 - 7.61 (m, 2H), 4.76 - 4.72 (m, 4H), 3.41 (br s, 2H), 3.32 - 3.26 (m, 2H), 1.31 (br s, 6H). MS (ES⁺) *m/e* 349 (M+H)⁺.

[0319] **Example 3**

[0320] 4-(4-Methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 002)



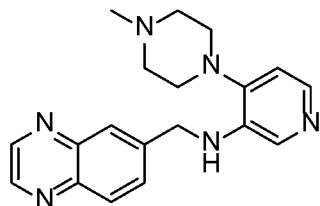
[0321] Step 1. 4-Chloro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine



[0322] To a solution of 4-chloropyridin-3-amine (15.0 g, 117 mmol, 1.00 *eq*) and quinoxaline-6-carbaldehyde (18.5 g, 117 mmol, 1.00 *eq*) in THF (150 mL) was added Ti(*i*-PrO)₄ (66.3 g, 233 mmol, 68.9 mL, 2.00 *eq*). The mixture was stirred at 50 °C for 12 h and was then diluted with EtOH (150 mL). NaBH₄ (4.38 g, 116 mmol, 1.00 *eq*) was added in several portions at 25 °C. The resulting mixture was stirred at 25 °C for 1 h and was then quenched with saturated aqueous NH₄Cl (150 mL) and extracted with ethyl acetate (150 mL × 3). The combined organic layers were washed with brine (500 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was triturated

with ethyl acetate (50.0 mL) at 25 °C for 30 min and was filtered. The cake formed from filtration was dried *in vacuo* to give the title compound (20.0 g, 57.9%) as a yellow solid. MS (ES⁺) *m/e* 271 (M+H)⁺.

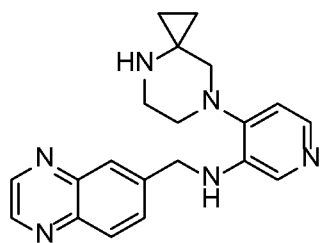
[0323] Step 2. 4-(4-Methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine



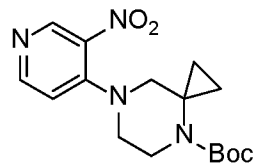
[0324] To a solution of 4-chloro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (500 mg, 1.85 mmol, 1.00 *eq*) and 1-methylpiperazine (370 mg, 3.69 mmol, 2.00 *eq*) in NMP (5.00 mL) was added DIEA (955 mg, 4.00 *eq*). The resulting mixture was heated under microwave irradiation at 180 °C for 8 h. The reaction mixture was filtered and the filtrate was purified by prep-HPLC (column: Waters Xbridge C18 150 × 50 mm × 10 μm; mobile phase: A- water (NH₄HCO₃) and B- CH₃CN; B%: 15% - 45%, 10 min) to give the title compound (198 mg, 26.7%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.90 (s, 2H), 8.08 (d, *J* = 8.4 Hz, 1H), 7.99 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.77 (d, *J* = 5.2 Hz, 1H), 7.70 (s, 1H), 6.87 (d, *J* = 5.2 Hz, 1H), 5.65 (br t, *J* = 6.0 Hz, 1H), 4.68 (br d, *J* = 6.0 Hz, 2H), 2.98 (br s, 4H), 2.58 (br s, 4H), 2.26 (s, 3H). MS (ES⁺) *m/e* 335 (M+H)⁺.

[0325] Example 4

[0326] *N*-(Quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine (Comp. 003)



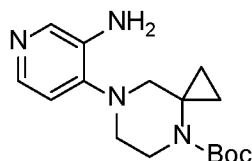
[0327] Step 1. *tert*-Butyl 7-(3-nitropyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



[0328] To a mixture of *tert*-butyl 4,7-diazaspiro[2.5]octane-4-carboxylate (3.50 g, 16.5 mmol, 1.00 *eq*) and 4-chloro-3-nitropyridine (2.61 g, 16.5 mmol, 1.00 *eq*) in *i*-PrOH (35.0 mL) was added DIEA (10.1 mL, 3.50 *eq*) at 25 °C. The reaction mixture was stirred at 80 °C

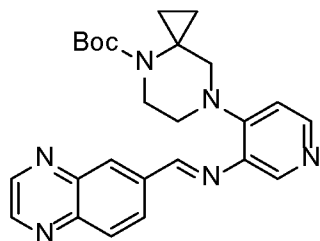
for 5 h and was concentrated under reduced pressure to give the title compound (5.00 g, 90.7%) as a yellow solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.76 (s, 1H), 8.35 (d, $J = 6.0$ Hz, 1H), 7.21 (d, $J = 6.0$ Hz, 1H), 3.58 - 3.55 (m, 2H), 3.28 - 3.25 (m, 2H), 3.08 (s, 2H), 1.38 (s, 9H), 0.96 - 0.90 (m, 2H), 0.84 - 0.79 (m, 2H). MS (ES^+) m/e 335 ($\text{M}+\text{H}$) $^+$.

[0329] Step 2. *tert*-butyl 7-(3-aminopyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



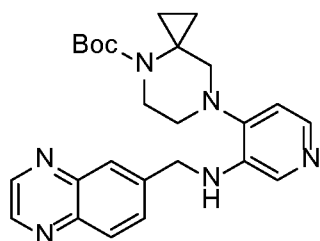
[0330] To a solution of *tert*-butyl 7-(3-nitropyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (5.50 g, 16.0 mmol, 1.00 eq) in MeOH (55.0 mL) was added Pt-V/C (3.00 g) under Ar gas. The suspension was degassed under vacuum and purged with H_2 gas several times and was stirred under H_2 (50 psi) at 25 °C for 12 h. The mixture was filtered and the filtrate was concentrated under reduced pressure to give the title compound (5.00 g, crude) as an off-white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.93 (s, 1H), 7.78 (d, $J = 5.2$ Hz, 1H), 6.86 (d, $J = 5.2$ Hz, 1H), 4.90 (br s, 2H), 3.12 (q, $J = 7.2$ Hz, 2H), 2.94 (br s, 2H), 2.81 (s, 2H), 1.41 (s, 9H), 0.92 (br s, 2H), 0.82 (s, 2H). MS (ES^+) m/e 305 ($\text{M}+\text{H}$) $^+$.

[0331] Step 3. *tert*-Butyl (*E*)-7-(3-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



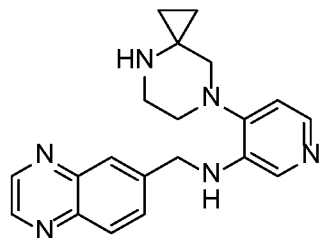
[0332] To a mixture of quinoxaline-6-carbaldehyde (2.60 g, 16.4 mmol, 1.00 eq) and *tert*-butyl 7-(3-aminopyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (5.00 g, 16.4 mmol, 1.00 eq) in THF (50.0 mL) was added $\text{Ti}(i\text{-PrO})_4$ (9.34 g, 32.9 mmol, 2.00 eq). The reaction mixture was stirred at 50 °C for 12 h and was used for the next step reaction without workup and purification. MS (ES^+) m/e 445 ($\text{M}+\text{H}$) $^+$.

[0333] Step 4. *tert*-Butyl 7-(3-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



[0334] To the reaction mixture obtained from the previous step was added MeOH (70.0 mL) followed by NaBH₄ (715 mg, 18.9 mmol, 1.20 eq) in portions at 25 °C. The mixture was stirred at 25 °C for 2 h, quenched with aqueous NH₄Cl (100 mL), concentrated under reduced pressure to remove MeOH, and was extracted with ethyl acetate (100 mL × 3). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated to give a crude product which was purified by prep-HPLC (column: Phenomenex luna C18 150 × 40 mm × 15 μm; mobile phase: A- water (trifluoroacetic acid), B-ACN; B%: 15% - 45%, 10 min) to give the title compound (5.00 g, 71.1%, 2-step) as a brown solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.91 (d, *J* = 1.6 Hz, 2H), 8.08 (d, *J* = 8.8 Hz, 1H), 8.00 (s, 1H), 7.88 (dd, *J*₁ = 1.6 Hz, *J*₂ = 8.8 Hz, 1H), 7.79 - 7.73 (m, 2H), 6.87 (d, *J* = 5.2 Hz, 1H), 5.61 (br t, *J* = 6.0 Hz, 1H), 4.69 (br d, *J* = 6.0 Hz, 2H), 3.69 (br s, 2H), 2.95 (br s, 2H), 2.82 (br s, 2H), 1.43 (s, 9H), 0.95 (br s, 2H), 0.86 (br s, 2H). MS (ES⁺) *m/e* 447 (M+H)⁺.

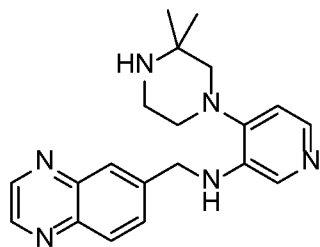
[0335] Step 5. *N*-(Quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine



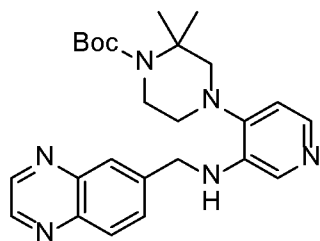
[0336] To a solution of *tert*-butyl 7-(3-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (400 mg, 0.896 mmol, 1.00 eq) in DCM (2.60 mL) was added TFA (1.30 mL, 17.6 mmol, 19.6 eq). The mixture was stirred at 20 °C for 2 h and was concentrated under reduced pressure to remove solvents. The crude product was triturated with MTBE (5.00 mL) at 20 °C for 2 h to give the title compound (174 mg, 42.3%) as a brown solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.88-8.77 (m, 2H), 8.20 (s, 1H), 8.06 (s, 1H), 8.01 (d, *J* = 5.20 Hz, 1H), 7.92 (s, 1H), 6.98 (d, *J* = 5.20 Hz, 1H), 5.46 (br t, *J* = 6.40 Hz, 1H), 4.73 (t, *J* = 6.40 Hz, 2H), 3.30-3.22 (m, 1H), 3.19 (br dd, *J* = 2.40, 12.40 Hz, 1H), 3.16-3.08 (m, 2H), 3.07-3.00 (m, 1H), 2.72 (br dd, *J* = 9.20, 11.20 Hz, 2H), 0.94 (d, *J* = 6.00 Hz, 3H). MS (ES⁺) *m/e* 369 (M+H)⁺.

[0337] Example 5

[0338] 4-(3,3-Dimethylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 004)

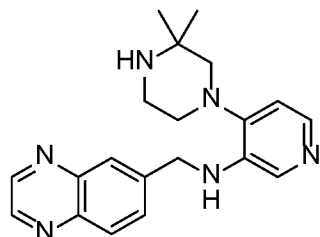


[0339] Step 1. *tert*-Butyl 2,2-dimethyl-4-(3-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)piperazine-1-carboxylate



[0340] To a solution of 4-chloro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (595 mg, 2.78 mmol, 1.50 *eq*) in NMP (5.00 mL) was added DIEA (717 mg, 5.55 mmol, 967 μ L, 3.00 *eq*) and *tert*-butyl 2,2-dimethylpiperazine-1-carboxylate (500 mg, 1.85 mmol, 1.00 *eq*). The mixture was heated at 180 °C for 16 h under microwave and was purified by reversed-phase HPLC to give the title compound (320 mg, 38.6%) as a brown oil. ^1H NMR (400 MHz, DMSO- d_6) δ 9.57 (br s, 1H), 8.93 (s, 2H), 8.16 - 7.98 (m, 3H), 7.94 - 7.89 (m, 1H), 7.84 - 7.59 (m, 1H), 7.43 - 7.11 (m, 1H), 6.87 - 6.21 (m, 1H), 4.89 - 4.58 (m, 2H), 3.79 - 3.55 (m, 4H), 1.52 - 1.34 (m, 13H), 1.05 (t, J = 7.2 Hz, 2H). MS (ES^+) m/e 449 ($\text{M}+\text{H}$) $^+$.

[0341] Step 2. 4-(3,3-Dimethylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine

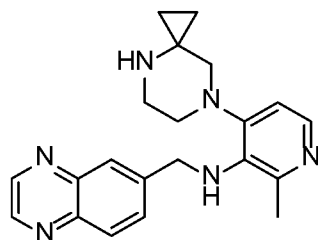


[0342] To a solution of *tert*-butyl 2,2-dimethyl-4-(3-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)piperazine-1-carboxylate (220 mg, 0.490 mmol, 1.00 *eq*) in ethyl acetate (1.10 mL) was added 4M HCl in ethyl acetate (1.23 mL, 10.0 *eq*). The mixture was stirred at 25 °C for 2 h and was then concentrated under reduced pressure to remove the

solvents. The crude product was purified by reversed-phase HPLC to give the title compound (52.9 mg, 52.9%) as a brown solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.91 (s, 2H), 8.09 (d, $J = 8.4$ Hz, 1H), 8.02 (s, 1H), 7.89 (dd, $J_1 = 1.6$ Hz, $J_2 = 8.8$ Hz, 1H), 7.79 (d, $J = 5.2$ Hz, 1H), 7.75 (s, 1H), 6.82 (d, $J = 4.8$ Hz, 1H), 5.43 (br t, $J = 6.0$ Hz, 1H), 4.69 (br d, $J = 6.0$ Hz, 2H), 2.98 (br s, 2H), 2.81 (br s, 2H), 2.64 (s, 2H), 1.15 (s, 6H). MS (ES^+) m/e 349 ($\text{M}+\text{H}$) $^+$.

[0343] Example 6

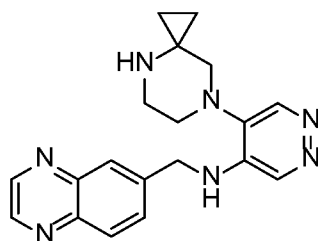
[0344] 2-Methyl-*N*-(quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine (Comp. 005)



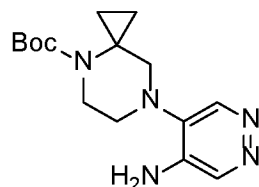
[0345] The title compound was synthesized following a similar procedure described for the preparation of Comp. 003 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.86 (q, $J = 2.0$ Hz, 2H), 7.96 (d, $J = 8.4$ Hz, 1H), 7.86 (d, $J = 1.2$ Hz, 1H), 7.78 (dd, $J = 2.0, 8.4$ Hz, 1H), 7.68 (d, $J = 5.6$ Hz, 1H), 6.63 (d, $J = 5.2$ Hz, 1H), 5.12 (t, $J = 7.6$ Hz, 1H), 4.69 (d, $J = 7.2$ Hz, 2H), 3.05-2.90 (m, 4H), 2.84 (s, 2H), 2.28 (s, 3H), 0.54 (s, 4H). MS (ES^+) m/e 361 ($\text{M}+\text{H}$) $^+$.

[0346] Example 7

[0347] *N*-(Quinoxalin-6-ylmethyl)-5-(4,7-diazaspiro[2.5]octan-7-yl)pyridazin-4-amine (Comp. 006)

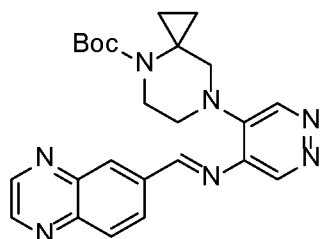


[0348] Step 1. *tert*-Butyl 7-(5-aminopyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



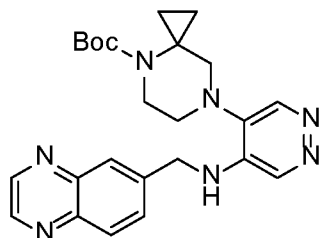
[0349] A mixture of 5-chloropyridazin-4-amine (1.00 g, 7.72 mmol, 1.00 eq) and *tert*-butyl 4,7-diazaspiro[2.5]octane-4-carboxylate (4.92 g, 23.1 mmol, 3.00 eq) was stirred at 120 °C for 10 h. The crude product was purified by reversed-phase HPLC to provide the title compound (0.700 g, 29.7%) as a yellow oil. MS (ES⁺) m/e 306 (M+H)⁺.

[0350] Step 2. *tert*-Butyl (*E*)-7-(5-((quinoxalin-6-ylmethylene)amino)pyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



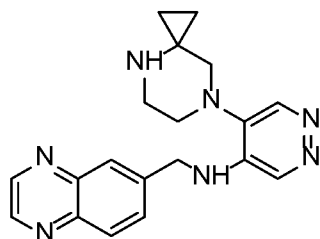
[0351] To a solution of *tert*-butyl 7-(5-aminopyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (0.300 g, 982 μmol, 1.00 eq) and quinoxaline-6-carbaldehyde (466 mg, 2.95 mmol, 3.00 eq) in toluene (10.0 mL) was added AcOH (177 mg, 2.95 mmol, 168 μL, 3.00 eq). The mixture was stirred at 130 °C for 16 h. The reaction mixture was concentrated under reduced pressure to give the title compound (0.500 g, crude) as a brown oil which was used directly for the next step reaction without further purification. MS (ES⁺) m/e 446 (M+H)⁺.

[0352] Step 3. *tert*-Butyl 7-(5-((quinoxalin-6-ylmethyl)amino)pyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



[0353] To a solution of *tert*-butyl (*E*)-7-(5-((quinoxalin-6-ylmethylene)amino)pyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (0.400 g, 897 μmol, 1.00 eq) in MeOH (4.00 mL) was added NaBH₃CN (56.4 mg, 0.897 mmol, 1.00 eq) and AcOH (53.9 mg, 0.897 mmol, 51.4 μL, 1.00 eq). The mixture was stirred at 25 °C for 10 h. The reaction mixture was quenched with saturated NH₄Cl solution (50.0 mL), neutralized with NaHCO₃ to pH = 8-9, and extracted with DCM (30.0 mL × 3). The combined organic layers were washed with brine (30.0 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC to provide the title compound (0.141 g, 35.1%) as a yellow oil. ¹H NMR (400 MHz, DMSO-*d*₆) δ MS (ES⁺) m/e 448 (M+H)⁺.

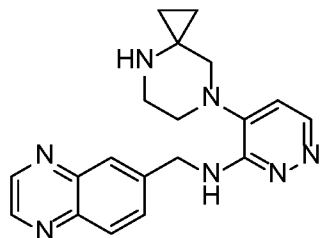
[0354] Step 4. *N*-(Quinoxalin-6-ylmethyl)-5-(4,7-diazaspiro[2.5]octan-7-yl)pyridazin-4-amine



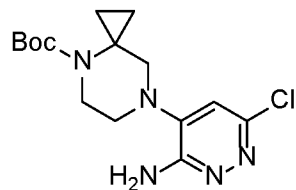
[0355] To a solution of *tert*-butyl 7-(5-((quinoxalin-6-ylmethyl)amino)pyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (0.141 g, 0.315 mmol, 1.00 eq) in MeOH (2.00 mL) was added 4M HCl in MeOH (28.2 mL, 358 eq). The mixture was stirred at 25 °C for 2 h and was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC to provide the title compound (0.009 g, 8.1%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.98 (br s, 2 H), 8.95 (s, 2 H), 8.81 (s, 1 H), 8.78 (s, 1 H), 8.14 (d, *J* = 8.8 Hz, 1 H), 8.09 (d, *J* = 1.2 Hz, 1 H), 7.93 (dd, *J* = 8.8, 1.88 Hz, 1 H), 5.16 - 5.27 (m, 1 H), 5.08 (br d, *J* = 6.4 Hz, 2 H), 3.31 (br s, 2 H), 3.24 (br s, 2 H), 2.52 (br s, 1 H), 2.51 - 2.53 (m, 1 H), 1.16 - 1.27 (m, 2 H), 0.83 - 0.93 (m, 2 H). MS (ES⁺) *m/e* 348 (M+H)⁺.

[0356] Example 8

[0357] *N*-(Quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridazin-3-amine (Comp. 007)



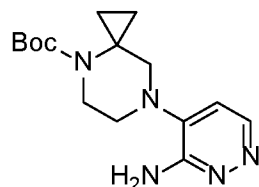
[0358] Step 1. *tert*-Butyl 7-(3-amino-6-chloropyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



[0359] To a solution of 4-bromo-6-chloropyridazin-3-amine (1.50 g, 7.20 mmol, 1.00 eq) and *tert*-butyl 4,7-diazaspiro[2.5]octane-4-carboxylate (1.60 g, 7.56 mmol, 1.05 eq) in DMA (2.00 mL) was added K₂CO₃ (2.98 g, 21.5 mmol, 3.00 eq). The mixture was stirred at 100 °C for 16 h. The reaction mixture was filtered, and the filtrate was diluted with water 20.0 mL

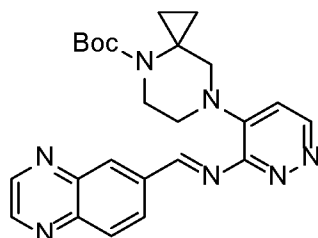
and extracted with DCM (50.0 mL $\times$ 3). The combined organic layers were washed with brine (20.0 mL $\times$ 2), dried over Na₂SO₄, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, petroleum ether/ethyl acetate = 10/1 to 3/1) to provide the title compound (2.14 g, 87.4%) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 6.72 (s, 1 H), 5.01 (br s, 2 H), 3.05 (br t, *J* = 4.8 Hz, 2 H), 3.02 (s, 2 H), 2.94 (s, 2 H), 1.07 - 1.14 (m, 2 H), 0.84 (s, 2 H). MS (ES⁺) *m/e* 340 (M+H)⁺.

[0360] Step 2. *tert*-Butyl 7-(3-aminopyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



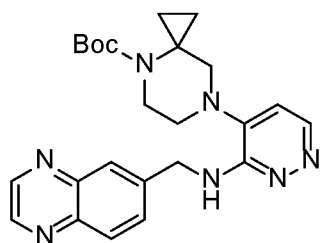
[0361] To a solution of *tert*-butyl 7-(3-amino-6-chloropyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (2.00 g, 5.89 mmol, 1.00 eq) in MeOH (20.0 mL) was added 10% Pd/C (1.00 g) under N₂. The suspension was degassed under vacuum and purged with H₂ several times. The mixture was stirred under H₂ (50 psi) at 25 °C for 10 h, filtered and concentrated under reduced pressure to give the title compound (1.50 g, 83.4%) as a white solid which was used directly for the next step reaction without further purification. MS (ES⁺) *m/e* 306 (M+H)⁺.

[0362] Step 3. *tert*-Butyl (*E*)-7-(3-((quinoxalin-6-ylmethylene)amino)pyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



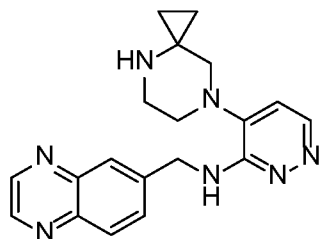
[0363] A mixture of *tert*-butyl 7-(3-aminopyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (1.50 g, 4.91 mmol, 1.00 eq), quinoxaline-6-carbaldehyde (815 mg, 5.16 mmol, 1.05 eq) and AcOH (589 mg, 9.82 mmol, 562 μ L, 2.00 eq) in toluene (15.0 mL) was stirred at 130 °C for 10 h. The reaction mixture concentrated under reduced pressure to give the title compound (2.00 g, crude) as a black solid that was used directly to the next step reaction without further purification. MS (ES⁺) *m/e* 446 (M+H)⁺.

[0364] Step 4. *tert*-Butyl 7-(3-((quinoxalin-6-ylmethyl)amino)pyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



[0365] To a solution of *tert*-butyl (*E*)-7-(3-((quinoxalin-6-ylmethylene)amino)pyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (2.00 g, 4.49 mmol, 1.00 eq) and AcOH (134 mg, 2.24 mmol, 128 μ L, 0.50 eq) in MeOH (10.0 mL) was added NaBH₃CN (282 mg, 4.49 mmol, 1.00 eq) in portions at 25 °C. The mixture was stirred at 40 °C for 10 h, quenched by addition of saturated NH₄Cl solution (50.0 mL), neutralized with NaHCO₃ to pH = 8-9, and extracted with DCM (30.0 mL $\times$ 3). The combined organic layers were washed with brine (30.0 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC to provide the title compound (1.30 g, 64.7%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ MS (ES⁺) *m/e* 448 (M+H)⁺.

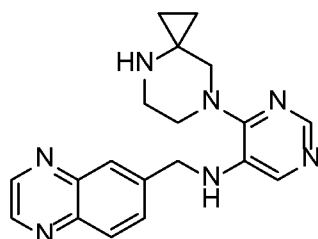
[0366] Step 5. *N*-(Quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridazin-3-amine hydrochloride



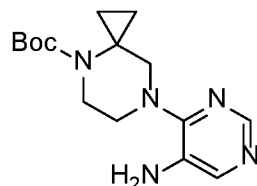
[0367] To a solution of *tert*-butyl 7-(3-((quinoxalin-6-ylmethyl)amino)pyridazin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (1.20 g, 2.68 mmol, 1.00 eq) in MeOH (13.0 mL) was added 4M HCl in MeOH (12.0 mL, 17.9 eq). The mixture was stirred at 25 °C for 0.5 h and was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC to give the title compound (0.655 g, 70.1%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.40 (br s, 2 H), 8.92 (s, 2 H), 8.74 (d, *J* = 6.0 Hz, 1 H) 8.16 (br s, 1 H), 8.08 (d, *J* = 8.8 Hz, 1 H), 8.00 (s, 1 H), 7.91 (dd, *J* = 8.8, 1.6 Hz, 1 H), 7.40 (d, *J* = 6.4 Hz, 1 H), 4.84 (br d, *J* = 5.6 Hz, 2 H), 3.76 (br s, 2 H), 3.50 - 3.65 (m, 4 H), 1.18 - 1.25 (m, 2 H), 0.84 - 0.91 (m, 2 H). MS (ES⁺) *m/e* 348 (M+H)⁺.

[0368] Example 9

[0369] *N*-(Quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyrimidin-5-amine (Comp. 008)

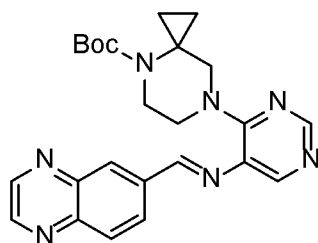


[0370] Step 1. *tert*-Butyl 7-(5-aminopyrimidin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



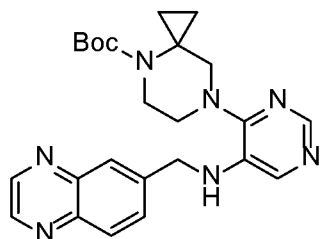
[0371] To a solution of 4-chloropyrimidin-5-amine (3.00 g, 23.1 mmol, 1.00 *eq*) and *tert*-butyl 4,7-diazaspiro[2.5]octane-4-carboxylate (4.92 g, 23.2 mmol, 1.00 *eq*) in ACN (10.0 mL) was added DIEA (8.98 g, 69.5 mmol, 12.1 mL, 3.00 *eq*). The mixture was stirred at 100 °C for 16 h and was concentrated under reduced pressure to give a residue. The residue was diluted with water (20.0 mL) and extracted with DCM (20.0 mL $\times$ 3). The combined organic layers were washed with brine (10.0 mL), dried over Na₂SO₄, and concentrated under reduced pressure to give a crude product which was purified by column chromatography (SiO₂, petroleum ether/ethyl acetate = 10/1 to 5/1) to afford the title compound (6.00 g, 84.8%) as a yellow oil. MS (ES⁺) *m/e* 306 (M+H)⁺.

[0372] Step 2. *tert*-Butyl (*E*)-7-(5-((quinoxalin-6-ylmethylene)amino)pyrimidin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



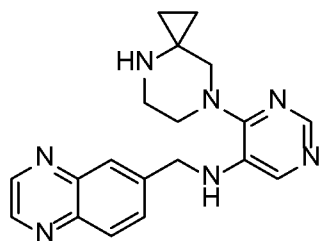
[0373] A mixture of *tert*-butyl 7-(5-aminopyrimidin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (2.00 g, 6.55 mmol, 1.00 *eq*), quinoxaline-6-carbaldehyde (1.09 g, 6.88 mmol, 1.05 *eq*) and AcOH (786 mg, 13.1 mmol, 749 μ L, 2.00 *eq*) in toluene (20.0 mL) was stirred at 130 °C for 12 h and was then concentrated under reduced pressure to give the title compound (2.90 g, 99.4%) as a yellow oil which was used directly for the next step reaction without further purification. MS (ES⁺) *m/e* 446 (M+H)⁺.

[0374] Step 3. *tert*-Butyl 7-(5-((quinoxalin-6-ylmethyl)amino)pyrimidin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



[0375] To a solution of *tert*-butyl (*E*)-7-(5-((quinoxalin-6-ylmethylene)amino)pyrimidin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (2.90 g, 6.51 mmol, 1.00 *eq*), AcOH (195 mg, 3.25 mmol, 186 μ L, 0.500 *eq*) in MeOH (30.0 mL) was added NaBH₃CN (818 mg, 13.0 mmol, 2.00 *eq*) in portions at 25 °C. The mixture was stirred at 40 °C for 12 h. Na₂CO₃ was added to adjust the pH to 8-9. The mixture was extracted with DCM (30.0 mL $\times$ 3). The combined organic layers were washed with brine (30.0 mL), dried over Na₂SO₄, and concentrated under reduced pressure to give a residue which was purified by reversed-phase HPLC to provide the title compound (2.03 g, 69.7%) as a yellow solid. MS (ES⁺) *m/e* 448 (M+H)⁺.

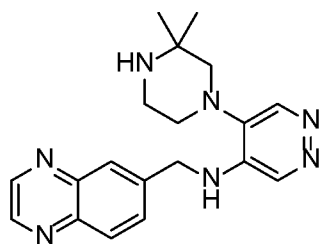
[0376] Step 4. *N*-(Quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyrimidin-5-amine



[0377] To a solution of *tert*-butyl 7-(5-((quinoxalin-6-ylmethyl)amino)pyrimidin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (1.50 g, 3.35 mmol, 1.00 *eq*) in MeOH (5.0 mL) was added HCl/MeOH (4.00 M, 18.8 mL, 22.4 *eq*). The mixture was stirred at 25 °C for 2 h and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC to provide the title compound (0.956 g, 82.1%) as a brown solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.41 (br s, 2 H), 8.93 (q, *J* = 2.0 Hz, 2 H), 8.54 (d, *J* = 1.2 Hz, 1 H), 8.05 - 8.13 (m, 2 H), 7.92 (dd, *J* = 8.8, 1.75 Hz, 1 H), 7.73 (s, 1 H) 6.88 (br s, 1 H), 4.66 (br s, 2 H), 4.20 (br s, 2 H), 4.00 (s, 2 H), 3.43 (br s, 2 H), 2.07 (s, 1 H), 1.11 - 1.22 (m, 2 H), 0.85 - 0.96 (m, 2 H). MS (ES⁺) *m/e* 348 (M+H)⁺.

[0378] Example 10

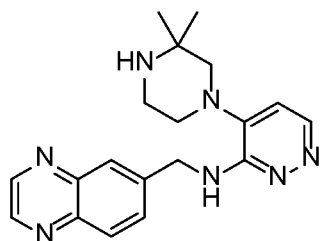
[0379] 5-(3,3-Dimethylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridazin-4-amine
(Comp. 009)



[0380] The title compound was synthesized following a similar procedure described for the preparation of Comp. 006 with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.55 (br s, 2 H), 8.98 - 9.06 (m, 1 H), 8.95 (s, 2 H), 8.85 (s, 1 H), 8.79 (s, 1 H), 8.10 - 8.16 (m, 2 H), 7.97 (br d, *J* = 8.4 Hz, 1 H), 5.10 (br d, *J* = 5.6 Hz, 2 H), 3.56 - 3.68 (m, 2 H), 3.20 (br s, 2 H), 3.07 (br d, *J* = 4.0 Hz, 2 H), 1.49 (s, 6 H). MS (ES⁺) *m/e* 350 (M+H)⁺.

[0381] Example 11

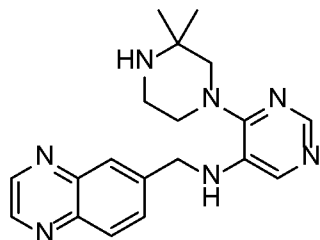
[0382] 4-(3,3-Dimethylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridazin-3-amine
(Comp. 010)



[0383] The title compound was synthesized following a similar procedure described for the preparation of Comp. 007 with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.97 (br s, 2 H), 8.92 (s, 2 H), 8.75 (d, *J* = 6.0 Hz, 1 H), 8.34 (br s, 1 H), 8.08 (d, *J* = 8.8 Hz, 1 H), 8.02 (s, 1 H), 7.94 (d, *J* = 8.8 Hz, 1 H), 7.44 (d, *J* = 6.0 Hz, 1 H), 4.87 (br d, *J* = 5.6 Hz, 2 H), 3.62 (br s, 4 H), 3.44 (s, 2 H), 1.48 (s, 6 H). MS (ES⁺) *m/e* 350 (M+H)⁺.

[0384] Example 12

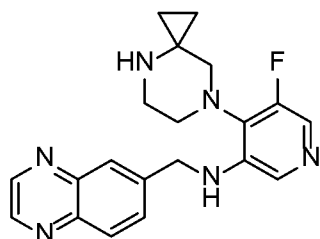
[0385] 4-(3,3-Dimethylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyrimidin-5-amine
(Comp. 011)



[0386] The title compound was synthesized following a similar procedure described for the preparation of Comp. 008 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 9.97 (br s, 2 H), 8.93 (q, $J = 2.0$ Hz, 2 H), 8.53 (s, 1 H), 8.08 - 8.14 (m, 2 H), 7.94 (dd, $J = 8.8, 1.6$ Hz, 1 H), 7.71 - 7.71 (m, 1 H), 7.71 (s, 1 H), 6.92 (br s, 1 H), 4.67 (br s, 2 H), 4.11 (br s, 2 H), 3.86 (s, 2 H) 3.46 (br s, 2 H), 1.41 (s, 6 H). MS (ES^+) m/e 350 ($\text{M}+\text{H}$) $^+$.

[0387] Example 13

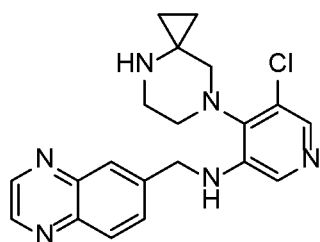
[0388] 5-Fluoro-*N*-(quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine (Comp. 012)



[0389] The title compound was synthesized following a similar procedure described for the preparation of Comp. 003 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 10.25 (br s, 2H), 8.93 (s, 2H), 8.32 (d, $J = 4.8$ Hz, 1H), 8.13-8.05 (m, 2H), 7.91 (br d, $J = 8.8$ Hz, 1H), 7.83 (s, 1H), 7.12-6.91 (m, 1H), 4.82 (br s, 2H), 3.64-3.41 (m, 6H), 1.21 (br s, 2H), 0.87 (s, 2H). MS (ES^+) m/e 365 ($\text{M}+\text{H}$) $^+$.

[0390] Example 14

[0391] 5-Chloro-*N*-(quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine (Comp. 013)

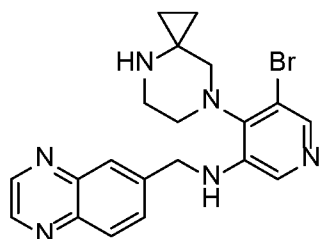


[0392] The title compound was synthesized following a similar procedure described for the preparation of Comp. 003 with appropriate starting materials and intermediates. ^1H NMR

(400 MHz, DMSO- d_6) δ 10.0 (d, J = 1.6 Hz, 2H), 8.92 (q, J = 2.0 Hz, 2H), 8.24 (s, 1H), 8.08 (d, J = 8.8 Hz, 2H), 7.98 (dd, J = 2.0, 8.8 Hz, 1H), 7.92 (s, 1H), 4.88 (s, 2H), 3.58 (br t, J = 5.6 Hz, 2H), 3.46 - 3.18 (m, 6H), 2.38 - 2.04 (m, 2H). MS (ES⁺) m/e 381 (M+H)⁺.

[0393] Example 15

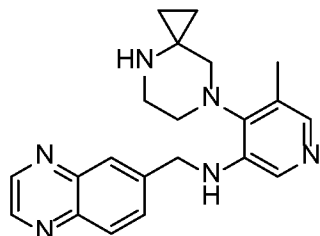
[0394] 5-Bromo-*N*-(quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine (Comp. 014)



[0395] The title compound was synthesized following a similar procedure described for the preparation of Comp. 003 with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO- d_6) δ 10.08 (br s, 2H), 8.93 (q, J = 2.0 Hz, 2H), 8.17 (s, 1H), 8.11-8.06 (m, 2H), 7.93-7.89 (m, 2H), 6.88 (br s, 1H), 4.81 (s, 2H), 3.87-3.10 (m, 6H), 1.23 (br s, 2H), 0.86 (s, 2H). MS (ES⁺) m/e 425 (M+H)⁺.

[0396] Example 16

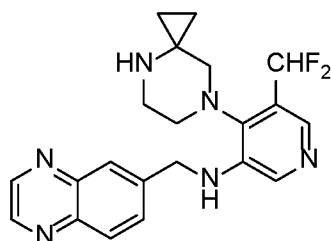
[0397] 5-Methyl-*N*-(quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine (Comp. 015)



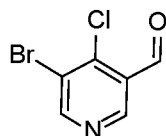
[0398] The title compound was synthesized following a similar procedure described for the preparation of Comp. 003 with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO- d_6) δ 15.57-15.07 (m, 1H), 10.06 (br s, 2H), 8.93 (s, 2H), 8.11 (d, J = 8.4 Hz, 1H), 8.05 (s, 1H), 7.98 (s, 1H), 7.90 (dd, J = 1.6, 8.8 Hz, 1H), 7.85 (s, 1H), 6.74 (br t, J = 6.0 Hz, 1H), 4.81 (br d, J = 5.6 Hz, 2H), 3.77-3.28 (m, 6H), 2.40 (s, 3H), 1.22 (br s, 2H), 0.86 (s, 2H). MS (ES⁺) m/e 361 (M+H)⁺.

[0399] Example 17

[0400] 5-(Difluoromethyl)-*N*-(quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine (Comp. 016)

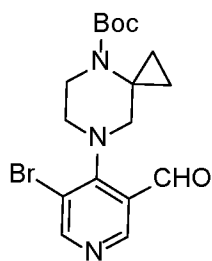


[0401] Step 1. 5-Bromo-4-chloronicotinaldehyde



[0402] To a solution of 3,5-dibromo-4-chloropyridine (18.0 g, 66.3 mmol, 1.00 *eq*) in tetrahydrofuran (180 mL) was added *i*-PrMgCl (2.00 M, 36.5 mL, 1.10 *eq*). The mixture was stirred at 0 °C for 1 h. DMF (9.70 g, 132 mmol, 10.2 mL, 2.00 *eq*) was added to the mixture at 0 °C and the reaction mixture was stirred at 20 °C for 3 h, quenched with saturated NH₄Cl aqueous solution (100 mL), and extracted with ethyl acetate (200 mL × 3). The combined organic layers were washed with brine (150 mL) and concentrated under reduced pressure to give a residue which was purified by column chromatography (SiO₂, petroleum ether/ethyl acetate=20/1 to 5/1) to give the title compound (12.5 g, 85.4%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 10.48 (s, 1H), 8.94 (d, *J* = 3.2 Hz, 2H).

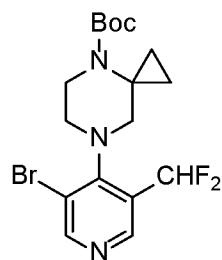
[0403] Step 2. *tert*-Butyl 7-(3-bromo-5-formylpyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



[0404] To a solution of 5-bromo-4-chloronicotinaldehyde (3.00 g, 13.6 mmol, 1.00 *eq*) and *tert*-butyl 4,7-diazaspiro[2.5]octane-4-carboxylate (3.09 g, 14.5 mmol, 1.07 *eq*) in CH₃CN (30.0 mL) was added DIEA (4.40 g, 34.0 mmol, 5.93 mL, 2.50 *eq*). The mixture was stirred at 80 °C for 16 h, diluted with H₂O (30.0 mL), and extracted with ethyl acetate (50.0 mL × 3). The combined organic layers were washed with brine (50.0 mL × 3), dried over Na₂SO₄, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, petroleum ether/ethyl acetate = 10/1 to 1/1) to give the title compound (3.70 g, 53.1%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.10 (s,

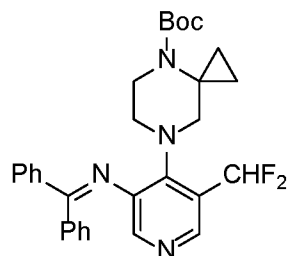
1H), 8.64 (s, 2H), 3.84-3.66 (m, 2H), 3.52-3.24 (m, 2H), 3.11 (s, 2H), 1.44 (s, 9H), 1.03-0.95 (m, 2H), 0.73-0.64 (m, 2H). MS (ES⁺) m/e 398 (M+H)⁺.

[0405] Step 3. *tert*-Butyl 7-(3-bromo-5-(difluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



[0406] To a solution of *tert*-butyl 7-(3-bromo-5-formylpyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (5.22 g, 13.1 mmol, 1.00 *eq*) in dichloromethane (50.0 mL) was added DAST (10.6 g, 65.8 mmol, 8.70 mL, 5.00 *eq*) at -70 °C. The mixture was stirred at 25 °C for 12 h. Saturated NaHCO₃ aqueous solution was added to adjust the pH to ~7. The mixture was extracted with ethyl acetate (50.0 mL×3). The combined organic layers were dried with Na₂SO₄ and concentrated under reduced pressure to give a residue which was purified by column chromatography (SiO₂, dichloromethane/methanol = 10/1 to 2/1) to provide the title compound (4.20 g, 75.9%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.66 (dd, *J* = 2.8, 12.8 Hz, 2H), 7.16-6.83 (m, 1H), 3.97-2.27 (m, 6H), 1.44 (d, *J* = 2.4 Hz, 9H), 0.99 (s, 2H), 0.74 (s, 2H). MS (ES⁺) m/e 432 (M+H)⁺.

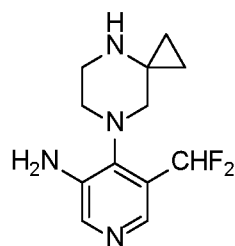
[0407] Step 4. *tert*-Butyl 7-(3-(difluoromethyl)-5-((diphenylmethylene)amino)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



[0408] To a solution of *tert*-butyl 7-(3-bromo-5-(difluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (4.20 g, 10.0 mmol, 1.00 *eq*) in toluene (40.0 mL) was added diphenylmethanimine (2.18 g, 12.1 mmol, 2.02 mL, 1.20 *eq*), (1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one-palladium (288 mg, 502 μmol, 0.05 *eq*), BINAP (625 mg, 1.00 mmol, 0.10 *eq*) and *t*-BuONa (2.00 M, 10.0 mL, 2.00 *eq*). The mixture was stirred at 100 °C for 12 h and was then concentrated under reduced pressure to provide the title compound as a

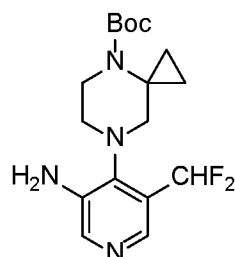
brown oil which was used for the next step reaction without purification. MS (ES^+) m/e 519 ($\text{M}+\text{H}$) $^+$.

[0409] Step 5. 5-(Difluoromethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine



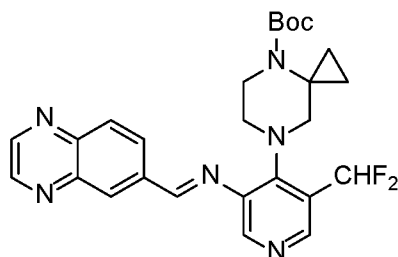
[0410] To a solution of *tert*-butyl 7-(3-(difluoromethyl)-5-((diphenylmethylene)amino)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (5.20 g, 10.0 mmol, 1.00 *eq*) in THF (50.0 mL) was added aqueous HCl solution (12 M, 8.36 mL, 10.0 *eq*). The mixture was stirred at 25 °C for 2 h and saturated Na_2CO_3 aqueous solution was added to adjust pH to 9. The mixture was extracted with EtOAc and combined organic layers were dried over Na_2SO_4 and concentrated to provide the title compound (2.55 g, crude) as a brown oil. MS (ES^+) m/e 255 ($\text{M}+\text{H}$) $^+$.

[0411] Step 6. *tert*-Butyl 7-(3-amino-5-(difluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



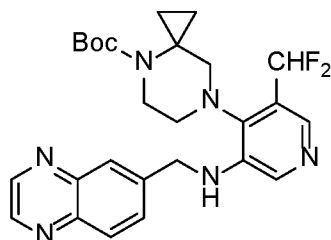
[0412] To a solution of 5-(difluoromethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine (2.55 g, 10.03 mmol, 1.00 *eq*) in THF (30.0 mL) was added Boc_2O (2.19 g, 10.0 mmol, 2.30 mL, 1.00 *eq*). The mixture was stirred at 20 °C for 2 h and was filtered. The filtrate was concentrated under reduced pressure to give a residue which was purified by column chromatography (SiO_2 , petroleum ether/ethyl acetate = 5/1 to 1/2) to provide the title compound (2.30 g, 63.0%). MS (ES^+) m/e 355 ($\text{M}+\text{H}$) $^+$.

[0413] Step 7. *tert*-Butyl (*E*)-7-(3-(difluoromethyl)-5-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



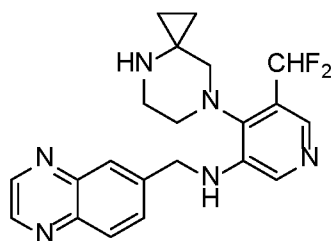
[0414] To a solution of *tert*-butyl 7-(3-amino-5-(difluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (2.16 g, 6.09 mmol, 1.00 *eq*) in toluene (20.0 mL) was added quinoxaline-6-carbaldehyde (963 mg, 6.09 mmol, 1.00 *eq*) and AcOH (366 mg, 6.09 mmol, 348 μ L, 1.00 *eq*). The mixture was stirred at 130 °C for 12 h and was concentrated under reduced pressure to give the title compound as a yellow oil which was used in the next step reaction without purification.

[0415] Step 8. *tert*-Butyl 7-(3-(difluoromethyl)-5-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



[0416] To a solution of *tert*-butyl (*E*)-7-(3-(difluoromethyl)-5-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (1.00 g, 2.02 mmol, 1.00 *eq*) in MeOH (10.0 mL) was added AcOH (121 mg, 2.02 mmol, 115 μ L, 1.00 *eq*) and NaBH₃CN (254 mg, 4.04 mmol, 2.00 *eq*). The mixture was stirred at 25 °C for 2 h and was concentrated under reduced pressure to give a residue which was purified by reversed-phase HPLC to the title compound (380 mg, 37.8%) as a yellow solid. MS (ES⁺) *m/e* 497 (M+H)⁺.

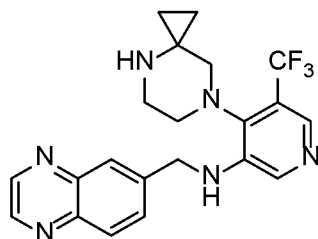
[0417] Step 9. 5-(Difluoromethyl)-*N*-(quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine



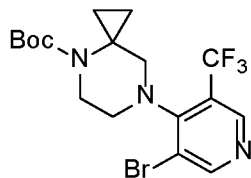
[0418] A mixture of *tert*-butyl 7-(3-(difluoromethyl)-5-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (380 mg, 0.765 mmol, 1.00 *eq*), MeOH (2.00 mL), and HCl/MeOH (12.0 M, 2.00 mL, 31.3 *eq*) was stirred at 20 °C for 15 min. The mixture was concentrated under reduced pressure to give a residue which was purified by reversed-phase HPLC to provide the title compound (204 mg, 56.1%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.99 (dd, *J* = 1.9, 7.6 Hz, 2H), 8.30 (s, 1H), 8.21 (d, *J* = 8.8 Hz, 1H), 8.12 (d, *J* = 15.9 Hz, 2H), 8.03 (dd, *J* = 1.6, 8.8 Hz, 1H), 7.34 (t, *J* = 54.1 Hz, 1H), 4.96 (s, 2H), 3.77 (s, 2H), 3.66 (s, 2H), 3.54 (s, 2H), 1.30-1.19 (m, 2H), 1.14-1.03 (m, 2H). MS (ES⁺) *m/e* 397 (M+H)⁺.

[0419] Example 18

[0420] *N*-(Quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)-5-(trifluoromethyl)pyridin-3-amine (Comp. 017)

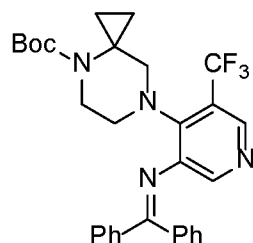


[0421] Step 1. *tert*-Butyl 7-(3-bromo-5-(trifluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



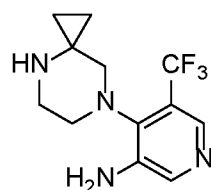
[0422] A mixture of 3-bromo-4-chloro-5-(trifluoromethyl)pyridine (1.00 g, 3.84 mmol 1.00 *eq*) and *tert*-butyl 4,7-diazaspiro[2.5]octane-4-carboxylate (1.06 g, 4.99 mmol, 1.30 *eq*) was stirred at 80 °C for 12 hr. The crude product was purified by column chromatography (SiO₂, petroleum ether/ethyl acetate=100/1 to 0/1) to provide the title compound (800 mg, 46.5 % yield). MS (ES⁺) *m/e* 437 (M+H)⁺.

[0423] Step 2. *tert*-Butyl 7-(3-((diphenylmethylene)amino)-5-(trifluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



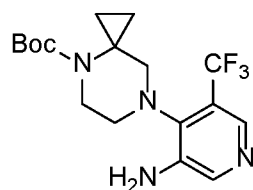
[0424] To a solution of *tert*-butyl 7-(3-bromo-5-(trifluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (800 mg, 1.83 mmol, 1.00 *eq*) in toluene (8.00 mL) was added diphenylmethanimine (498 mg, 2.75 mmol, 461 μ L, 1.50 *eq*), BINAP (114 mg, 183 μ mol, 0.10 *eq*), Pd₂(dba)₃ (167 mg, 183 μ mol, 0.10 *eq*) and *t*-BuONa (352 mg, 3.67 mmol, 2.00 *eq*). The mixture was stirred at 100 °C for 2 h and was concentrated under reduced pressure to give the title compound (880 mg, 89.4%) as a brown oil that was used directly for next step reaction without purification. MS (ES⁺) *m/e* 537 (M+H)⁺.

[0425] Step 3. 4-(4,7-Diazaspiro[2.5]octan-7-yl)-5-(trifluoromethyl)pyridin-3-amine



[0426] To a solution of *tert*-butyl 7-(3-((diphenylmethylene)amino)-5-(trifluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (800 mg, 1.64 mmol, 1.00 *eq*) in tetrahydrofuran (8.00 mL) was added HCl (12 M, 2.00 mL, 14.6 *eq*). The mixture was stirred at 25 °C for 12 h, diluted EtOAc, and washed with H₂O (50.0 mL $\times$ 3). The combined aqueous layers were concentrated to provide the title compound (400 mg, crude) as a brown oil. MS (ES⁺) *m/e* 273 (M+H)⁺.

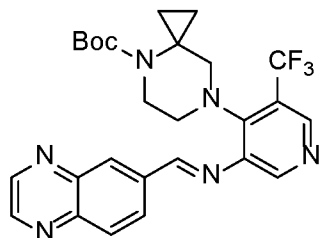
[0427] Step 4. *tert*-Butyl 7-(3-amino-5-(trifluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



[0428] To a solution of 4-(4,7-diazaspiro[2.5]octan-7-yl)-5-(trifluoromethyl)pyridin-3-amine (400 mg, 1.47 mmol, 1.00 *eq*) in H₂O (10.0 mL) was added Na₂CO₃ (155 mg, 1.47 mmol, 1.00 *eq*) and (Boc)₂O (320 mg, 1.47 mmol, 337 μ L, 1.00 *eq*). The mixture was stirred

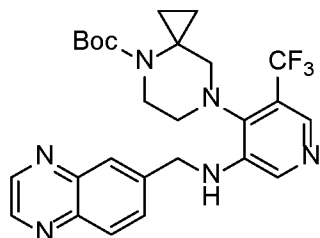
at 25 °C for 2 h and was then extracted with ethyl acetate (50.0 mL $\times$ 3). The combined organic layers were washed with brine (50.0 mL $\times$ 3), dried over Na₂SO₄, and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO₂, petroleum ether/ethyl acetate = 1/1, R_f = 0.30) to give the title compound (400 mg, 70.8%) as a yellow oil. ¹H NMR (400 MHz, DMSO-*d*₆) δ MS (ES⁺) m/e 373 (M+H)⁺.

[0429] Step 5. *tert*-Butyl (*E*)-7-(3-((quinoxalin-6-ylmethylene)amino)-5-(trifluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



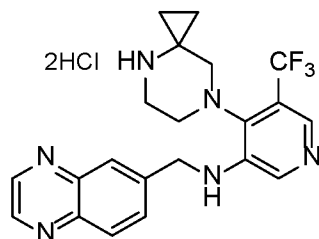
[0430] To a solution of *tert*-butyl 7-(3-amino-5-(trifluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (400 mg, 1.07 mmol, 1.00 *eq*) in toluene (4.00 mL) was added quinoxaline-6-carbaldehyde (254 mg, 1.61 mmol, 1.50 *eq*) and AcOH (64.5 mg, 1.07 mmol, 61.4 μ L, 1.00 *eq*). The mixture was stirred at 130 °C for 12 h and was concentrated under reduced pressure to give the title compound (640 mg, crude) as a brown oil. MS (ES⁺) m/e 513 (M+H)⁺.

[0431] Step 6. *tert*-Butyl 7-(3-((quinoxalin-6-ylmethyl)amino)-5-(trifluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate



[0432] To a solution of *tert*-butyl (*E*)-7-(3-((quinoxalin-6-ylmethylene)amino)-5-(trifluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (640 mg, 1.25 mmol, 1.00 *eq*) in methanol (6.00 mL) was added NaBH₃CN (154 mg, 2.50 mmol, 2.00 *eq*) and AcOH (74.9 mg, 1.25 mmol, 71.4 μ L, 1.00 *eq*). The mixture was stirred at 25 °C for 12 h and was concentrated under reduced pressure to give a residue. The crude product was purified by reversed-phase HPLC to give the title compound (210 mg, 32.1%) as a yellow solid. MS (ES⁺) m/e 512 (M+H)⁺.

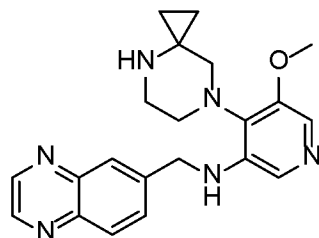
[0433] Step 7. *N*-(Quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)-5-(trifluoromethyl)pyridin-3-amine



[0434] To a solution of *tert*-butyl 7-(3-((quinoxalin-6-ylmethyl)amino)-5-(trifluoromethyl)pyridin-4-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (200 mg, 388 μ mol, 1.00 *eq*) in methanol (20.0 mL) was added HCl/MeOH (4 M, 10.0 mL, 102 *eq*). The mixture was stirred at 25 °C for 2 h and was concentrated under reduced pressure to give a residue which was purified by reversed-phase HPLC to provide the title compound (144 mg, 75.5%) as a yellow solid. ^1H NMR (400 MHz, MeOD- d_4) δ 8.93 (q, J = 2.0 Hz, 1H), 8.47 (s, 1H), 8.24 (s, 1H), 8.19 (d, J = 8.8 Hz, 1H), 8.10 (d, J = 0.8 Hz, 1H), 8.00 (dd, J = 8.8, 2.0 Hz, 1H), 4.99 (s, 2H), 3.65-3.84 (m, 4H), 3.57 (br s, 2H), 1.28-1.20 (m, 2H), 1.13-1.05 (m, 2H). MS (ES^+) m/e 415 ($\text{M}+\text{H}$) $^+$.

[0435] Example 19

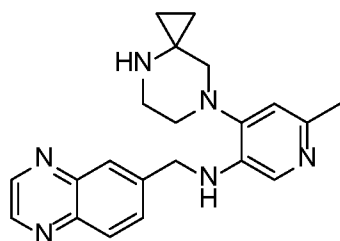
[0436] 5-Methoxy-*N*-(quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine (Comp. 018)



[0437] The title compound was synthesized following a similar procedure described for the preparation of Comp. 003 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 9.93 (br s, 2H), 8.95 - 8.91 (m, 2H), 8.10 (d, J = 8.6 Hz, 1H), 8.03 (s, 1H), 7.97 (s, 1H), 7.89 (dd, J = 1.9, 8.8 Hz, 1H), 7.76 (s, 1H), 6.91 (br s, 1H), 4.80 (br d, J = 4.3 Hz, 2H), 3.93 (s, 3H), 3.52 (br s, 2H), 3.36 (br s, 4H), 1.18 (s, 2H), 0.84 (s, 2H). MS (ES^+) m/e 377 ($\text{M}+\text{H}$) $^+$.

[0438] Example 20

[0439] 6-Methyl-*N*-(quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine (Comp. 019)

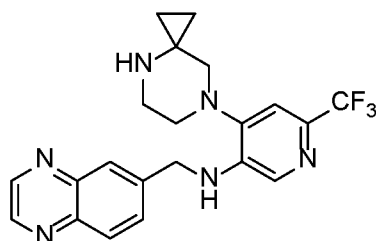


[0440] The title compound was synthesized following a similar procedure described for the preparation of Comp. 003 with appropriate starting materials and intermediates.

^1H NMR (400 MHz, DMSO- d_6) δ 15.03 (br s, 1H), 10.34 (br s, 2H), 8.94 (s, 2H), 8.10 (d, J = 8.8 Hz, 1H), 8.03 (s, 1H), 7.91 (dd, J = 1.6, 8.8 Hz, 1H), 7.55 (br d, J = 2.8 Hz, 1H), 7.26 (s, 1H), 6.54 (br d, J = 0.8 Hz, 1H), 4.74 (s, 2H), 3.66 - 3.53 (m, 4H), 3.50 (s, 2H), 2.48 (s, 3H), 1.26 - 1.20 (m, 2H), 0.90 - 0.84 (m, 2H). MS (ES $^+$) m/e 361 (M+H) $^+$.

[0441] Example 21

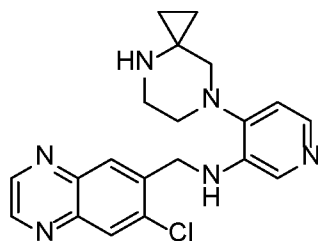
[0442] *N*-(Quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)-6-(trifluoromethyl)pyridin-3-amine (Comp. 020)



[0443] The title compound was synthesized following a similar procedure described for the preparation of Comp. 003 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 10.00 (br s, 2H), 9.04 - 8.83 (m, 2H), 8.10 (d, J = 8.8 Hz, 1H), 8.03 (s, 1H), 7.91 (dd, J = 2.0, 8.8 Hz, 1H), 7.87 (s, 1H), 7.23 (s, 1H), 6.73 - 6.29 (m, 1H), 4.79 (s, 2H), 3.53 (br s, 2H), 3.33 - 3.13 (m, 4H), 1.29 - 1.14 (m, 2H), 0.98 - 0.84 (m, 2H). MS (ES $^+$) m/e 415 (M+H) $^+$.

[0444] Example 22

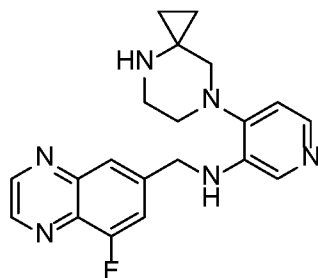
[0445] *N*-((7-Chloroquinoxalin-6-yl)methyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine (Comp. 021)



[0446] The title compound was synthesized following a similar procedure described for the preparation of Comp. 003 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 15.21-14.83 (m, 1H), 10.20 (br s, 2H), 8.96 (dd, J = 1.6, 11.6 Hz, 2H), 8.31 (s, 1H), 8.13 (d, J = 6.0 Hz, 1H), 7.90 (d, J = 16.4 Hz, 2H), 7.40 (d, J = 6.4 Hz, 1H), 6.64 (br t, J = 5.6 Hz, 1H), 4.75 (br d, J = 5.2 Hz, 2H), 3.71-3.41 (m, 6H), 1.27-1.14 (m, 2H), 0.93-0.79 (m, 2H). MS (ES^+) m/e 381 ($\text{M}+\text{H}$) $^+$.

[0447] Example 23

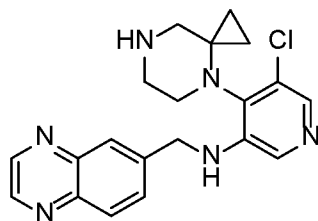
[0448] *N*-((8-Fluoroquinoxalin-6-yl)methyl)-4-(4,7-diazaspiro[2.5]octan-7-yl)pyridin-3-amine (Comp. 022)



[0449] The title compound was synthesized following a similar procedure described for the preparation of Comp. 003 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 14.97-14.64 (m, 1H), 10.10 (br s, 2H), 9.00 (dd, J = 1.6, 14.6 Hz, 2H), 8.09 (d, J = 6.4 Hz, 1H), 7.91 (s, 1H), 7.83-7.75 (m, 2H), 7.35 (d, J = 6.4 Hz, 1H), 6.71 (br t, J = 6.0 Hz, 1H), 4.74 (br d, J = 5.6 Hz, 2H), 3.58 (br s, 4H), 3.49 (s, 2H), 1.23-1.17 (m, 2H), 0.92 - 0.86 (m, 2H). MS (ES^+) m/e 365 ($\text{M}+\text{H}$) $^+$.

[0450] Example 24

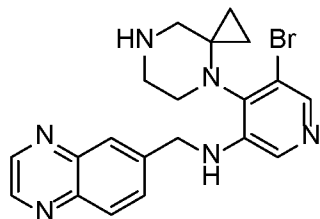
[0451] 5-Chloro-*N*-(quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-4-yl)pyridin-3-amine (Comp. 023)



[0452] The title compound was synthesized following a similar procedure described for the preparation of Comp. 001 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, Methanol- d_4) δ 8.98 (s, 2H), 8.24 - 8.10 (m, 3H), 8.00 (d, J = 8.8 Hz, 1H), 7.84 (s, 1H), 4.88 (br s, 2H), 4.12 (ddd, J = 2.8, 7.2, 13.2 Hz, 1H), 3.94 (ddd, J = 2.4, 7.2, 13.2 Hz, 1H), 3.70 (br d, J = 12.8 Hz, 1H), 3.68 - 3.62 (m, 1H), 3.56 (br d, J = 12.8 Hz, 1H), 3.48 - 3.38 (m, 1H), 1.24 - 1.06 (m, 3H), 1.04 - 0.96 (m, 1H). MS (ES^+) m/e 381 ($\text{M}+\text{H}$) $^+$.

[0453] Example 25

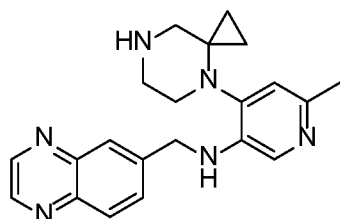
[0454] 5-Bromo-*N*-(quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-4-yl)pyridin-3-amine (Comp. 024)



[0455] The title compound was synthesized following a similar procedure described for the preparation of Comp. 001 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 9.99 (br s, 1H), 9.50 (br d, $J = 6.0$ Hz, 1H), 8.93 (q, $J = 2.0$ Hz, 2H), 8.21 (s, 1H), 8.14-8.04 (m, 2H), 7.99-7.71 (m, 2H), 6.71-6.47 (m, 1H), 4.91-4.62 (m, 2H), 4.06-3.84 (m, 2H), 3.68-3.56 (m, 1H), 3.53-3.38 (m, 1H), 3.32-3.19 (m, 1H), 3.13 (br d, $J = 12.4$ Hz, 1H), 1.04-0.88 (m, 2H), 0.78-0.67 (m, 2H). MS (ES^+) m/e 425 ($\text{M}+\text{H}$) $^+$.

[0456] Example 26

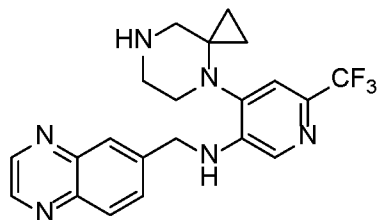
[0457] 6-Methyl-*N*-(quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-4-yl)pyridin-3-amine (Comp. 025)



[0458] The title compound was synthesized following a similar procedure described for the preparation of Comp. 001 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 14.94 - 14.82 (m, 1H), 10.55 - 9.34 (m, 2H), 8.92 (d, $J = 0.8$ Hz, 2H), 8.08 (d, $J = 8.4$ Hz, 1H), 8.03 (s, 1H), 7.89 (dd, $J = 2.0, 8.8$ Hz, 1H), 7.49 (d, $J = 6.0$ Hz, 1H), 7.35 (s, 1H), 6.48 (br s, 1H), 4.62 (br s, 2H), 4.00 (br s, 2H), 3.22 - 2.84 (m, 2H), 2.52 - 2.50 (m, 3H), 1.13 (br s, 2H), 1.03 - 0.53 (m, 2H). MS (ES^+) m/e 361 ($\text{M}+\text{H}$) $^+$.

[0459] Example 27

[0460] *N*-(Quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-4-yl)-6-(trifluoromethyl)pyridin-3-amine (Comp. 026)

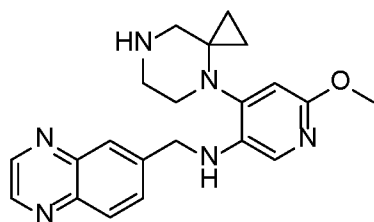


[0461] The title compound was synthesized following a similar procedure described for the preparation of Comp. 001 with appropriate starting materials and intermediates.

¹H NMR (400 MHz, DMSO-*d*₆) δ 9.50 - 9.26 (m, 2H), 8.95-8.88 (m, 2H), 8.09 (d, *J* = 8.4 Hz, 1H), 8.04 (s, 1H), 7.90 (dd, *J* = 1.6, 8.8 Hz, 1H), 7.84 (s, 1H), 7.26 (s, 1H), 6.46 (br s, 1H), 4.71 (br s, 2H), 3.59 (br s, 2H), 3.37 (br s, 2H), 3.15 (br s, 2H), 0.94 (br s, 2H), 0.56 (br s, 2H). MS (ES⁺) *m/e* 415 (M+H)⁺.

[0462] Example 28

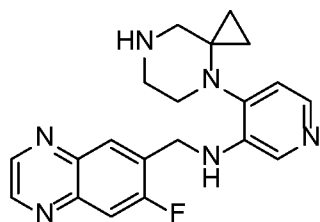
[0463] 6-Methoxy-*N*-(quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-4-yl)pyridin-3-amine (Comp. 027)



[0464] The title compound was synthesized following a similar procedure described for the preparation of Comp. 001 with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.62 (br d, *J* = 2.4 Hz, 2H), 8.94 (q, *J* = 2.0 Hz, 2H), 8.08 (d, *J* = 8.4 Hz, 1H), 8.04 (s, 1H), 7.88 (dd, *J* = 2.4, 8.4 Hz, 1H), 7.18 (s, 1H), 6.66 (s, 1H), 6.22 - 5.42 (m, 1H), 4.54 (s, 2H), 4.04 - 3.85 (m, 5H), 3.13 (br d, *J* = 5.2 Hz, 2H), 2.54 (br s, 2H), 1.12 (br s, 2H), 0.84 (br s, 2H). MS (ES⁺) *m/e* 377 (M+H)⁺.

[0465] Example 29

[0466] *N*-((7-Fluoroquinoxalin-6-yl)methyl)-4-(4,7-diazaspiro[2.5]octan-4-yl)pyridin-3-amine (Comp. 028)

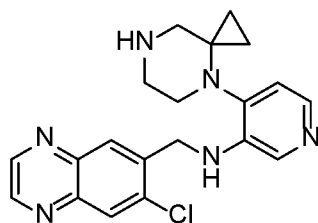


[0467] The title compound was synthesized following a similar procedure described for the preparation of Comp. 001 with appropriate starting materials and intermediates. ¹H NMR

(400 MHz, DMSO- d_6) δ 14.87-14.54 (m, 1H), 9.82-9.44 (m, 2H), 8.94 (dd, J = 2.0, 14.6 Hz, 2H), 8.07 (d, J = 6.4 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.96 (d, J = 10.8 Hz, 1H), 7.85 (s, 1H), 7.50 (d, J = 6.4 Hz, 1H), 6.50 (br t, J = 5.6 Hz, 1H), 4.66 (br d, J = 4.4 Hz, 2H), 4.08-3.83 (m, 2H), 3.54-3.52 (m, 2H), 3.10 (br dd, J = 2.4, 5.3 Hz, 2H), 1.14 (br s, 2H), 0.99-0.73 (m, 2H). MS (ES⁺) m/e 365 (M+H)⁺.

[0468] Example 30

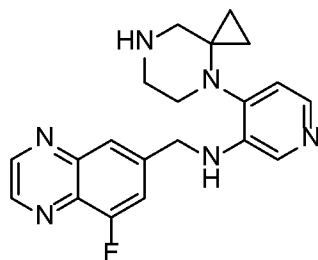
[0469] *N*-((7-Chloroquinoxalin-6-yl)methyl)-4-(4,7-diazaspiro[2.5]octan-4-yl)pyridin-3-amine (Comp. 029)



[0470] The title compound was synthesized following a similar procedure described for the preparation of Comp. 001 with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO- d_6) δ 14.71-14.44 (m, 1H), 9.81-9.24 (m, 2H), 8.97 (dd, J = 1.8, 9.6 Hz, 2H), 8.31 (s, 1H), 8.09 (d, J = 6.4 Hz, 1H), 7.99 (s, 1H), 7.80 (s, 1H), 7.52 (d, J = 6.4 Hz, 1H), 6.51 (t, J = 5.4 Hz, 1H), 4.64 (br d, J = 3.2 Hz, 2H), 4.09-3.81 (m, 2H), 3.43-2.93 (m, 4H), 1.14 (br s, 2H), 1.04-0.65 (m, 2H). MS (ES⁺) m/e 381 (M+H)⁺.

[0471] Example 31

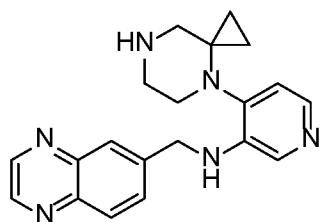
[0472] *N*-((8-Fluoroquinoxalin-6-yl)methyl)-4-(4,7-diazaspiro[2.5]octan-4-yl)pyridin-3-amine (Comp. 030)



[0473] The title compound was synthesized following a similar procedure described for the preparation of Comp. 001 with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO- d_6) δ 14.73 (br d, J = 2.4 Hz, 1H), 9.70 (br d, J = 4.0 Hz, 2H), 9.00 (dd, J = 1.6, 16.0 Hz, 2H), 8.03 (d, J = 6.4 Hz, 1H), 7.93 (s, 1H), 7.81-7.71 (m, 2H), 7.47 (d, J = 6.4 Hz, 1H), 6.65 (br t, J = 5.6 Hz, 1H), 4.64 (br d, J = 4.4 Hz, 2H), 4.00 (br d, J = 4.0 Hz, 2H), 3.65 (br s, 2H), 3.18-2.96 (m, 2H), 1.14 (br s, 2H), 1.01-0.62 (m, 2H). MS (ES⁺) m/e 365 (M+H)⁺.

[0474] Example 32

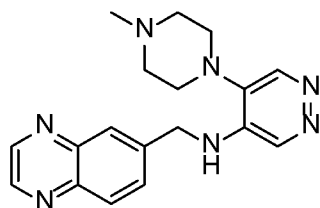
[0475] *N*-(Quinoxalin-6-ylmethyl)-4-(4,7-diazaspiro[2.5]octan-4-yl)pyridin-3-amine
(Comp. 031)



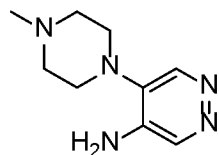
[0476] The title compound was synthesized following a similar procedure described for the preparation of Comp. 001 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 14.82 (br d, J = 2.4 Hz, 1H), 10.13-9.41 (m, 2H), 8.93 (s, 2H), 8.13-7.98 (m, 3H), 7.90 (dd, J = 1.6, 8.8 Hz, 1H), 7.72 (s, 1H), 7.47 (d, J = 6.4 Hz, 1H), 6.66 (br s, 1H), 4.65 (br s, 2H), 4.06-3.84 (m, 2H), 3.58-2.73 (m, 4H), 1.14 (br s, 2H), 1.00 - 0.58 (m, 2H). MS (ES^+) m/e 347 ($\text{M}+\text{H}$) $^+$.

[0477] Example 33

[0478] 5-(4-Methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridazin-4-amine (Comp. 032)

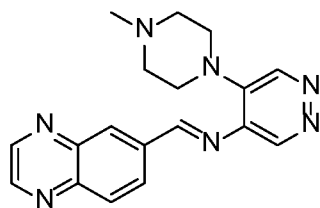


[0479] Step 1. 5-(4-Methylpiperazin-1-yl)pyridazin-4-amine



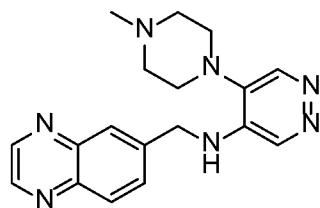
[0480] A mixture of 5-(4-methylpiperazin-1-yl)pyridazin-4-amine (1.28 g, 12.7 mmol, 1.41 mL, 1.10 eq), 5-chloropyridazin-4-amine (1.50 g, 11.58 mmol, 1.00 eq) and DIEA (14.9 g, 115 mmol, 20.1 mL, 10.0 eq) was stirred at 130 °C for 16 h. The reaction mixture was filtered. The filtrate was diluted with water (20.0 mL) and extracted with DCM (50.0 mL $\times$ 3). The combined organic layers were washed with brine (20.0 mL $\times$ 2), dried over Na_2SO_4 , and concentrated under reduced pressure to give a residue which was purified by reversed-phase HPLC to provide the title compound (1.50 g, 67.0%) as a yellow oil. MS (ES^+) m/e 194 ($\text{M}+\text{H}$) $^+$.

[0481] Step 2. (*E*)-*N*-(5-(4-Methylpiperazin-1-yl)pyridazin-4-yl)-1-(quinoxalin-6-yl)methanimine



[0482] A mixture of 5-(4-methylpiperazin-1-yl)pyridazin-4-amine (0.827 g, 4.28 mmol, 1.00 eq), (*E*)-*N*-(5-(4-methylpiperazin-1-yl)pyridazin-4-yl)-1-(quinoxalin-6-yl)methanimine (744 mg, 4.71 mmol, 1.10 eq) and AcOH (514 mg, 8.56 mmol, 490 μ L, 2.00 eq) in toluene (10.0 mL) was stirred at 130 $^{\circ}$ C for 10 h and was concentrated under reduced pressure to give the title compound (1.50 g, crude) as a black solid which was used directly to the next step reaction without purification. MS (ES^+) m/e 334 ($\text{M}+\text{H}$) $^+$.

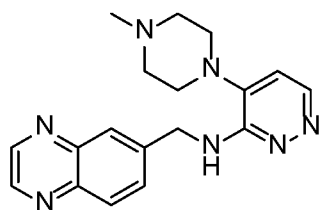
[0483] Step 3. 5-(4-Methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridazin-4-amine



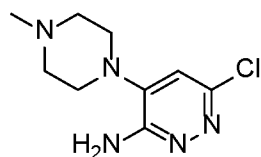
[0484] To a solution of (*E*)-*N*-(5-(4-methylpiperazin-1-yl)pyridazin-4-yl)-1-(quinoxalin-6-yl)methanimine (1.30 g, 3.90 mmol, 1.00 eq) and AcOH (234 mg, 3.90 mmol, 223 μ L, 1.00 eq) in MeOH (13.0 mL) was added NaBH_3CN (367 mg, 5.85 mmol, 1.50 eq) in portions at 25 $^{\circ}$ C. The mixture was stirred at 40 $^{\circ}$ C for 12 h and was quenched by addition of saturated NH_4Cl solution (50.0 mL). NaHCO_3 was added to adjust the pH = 8-9. The mixture was extracted with DCM (30.0 mL $\times$ 3). The combined organic layers were washed with brine (30.0 mL), dried over Na_2SO_4 , and concentrated under reduced pressure to give a residue which was purified by prep-HPLC to provide the title compound (0.156 g, 31.2%) as a yellow solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.59 (br d, J = 1.2 Hz, 1 H), 9.15 (br s, 1 H), 8.94 (s, 2 H), 8.84 (s, 1 H), 8.77 (s, 1 H), 8.08 - 8.16 (m, 2 H), 7.98 (dd, J = 8.8, 2.0 Hz, 1 H), 5.09 (br d, J = 6.4 Hz, 2 H), 3.42 - 3.61 (m, 6 H), 3.23 - 3.38 (m, 2 H), 2.81 (d, J = 4.4 Hz, 3 H). MS (ES^+) m/e 336 ($\text{M}+\text{H}$) $^+$.

[0485] Example 34

[0486] 4-(4-Methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridazin-3-amine (Comp. 033)

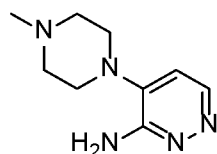


[0487] Step 1. 6-Chloro-4-(4-methylpiperazin-1-yl)pyridazin-3-amine



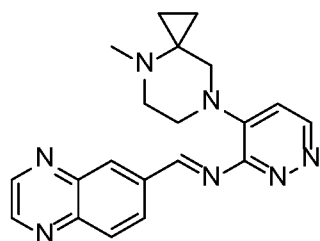
[0488] To a solution of 4-bromo-6-chloropyridazin-3-amine (1.50 g, 7.20 mmol, 1.00 eq) and 6-chloro-4-(4-methylpiperazin-1-yl)pyridazin-3-amine (792 mg, 7.92 mmol, 878 μ L, 1.10 eq) in DMA (2.00 mL) was added K_2CO_3 (2.98 g, 21.6 mmol, 3.00 eq). The mixture was stirred at 100 °C for 16 h and was filtered. The filtrate was diluted with water (20.0 mL) and extracted with DCM (50.0 mL $\times$ 3). The combined organic layers were washed with brine (20.0 mL $\times$ 2), dried over Na_2SO_4 , and concentrated under reduced pressure to give a residue which was purified by reversed-phase HPLC to provide the title compound (1.00 g, 61.0%) as a yellow oil. MS (ES^+) m/e 228 ($M+H$) $^+$.

[0489] Step 2. 4-(4-Methylpiperazin-1-yl)pyridazin-3-amine



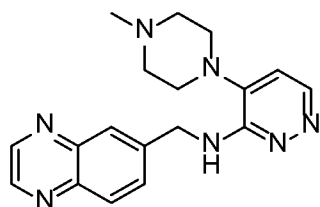
[0490] To a solution of 6-chloro-4-(4-methylpiperazin-1-yl)pyridazin-3-amine (0.50 g, 2.20 mmol, 1.00 eq) in MeOH (10.0 mL) was added 10% Pd/C (0.250 g) under N_2 . The suspension was degassed under vacuum and purged with H_2 several times. The mixture was stirred under H_2 (50.0 psi) at 25 °C for 10 h and was filtered. The filtrate was concentrated under reduced pressure to give the title compound (0.400 g, 94.3%) as a white solid. ^1H NMR (400 MHz, $DMSO-d_6$) δ 6.74 (s, 1 H), 5.05 (br s, 2 H), 3.10 (br s, 4 H), 2.59 (br s, 4 H), 2.37 (s, 3 H). MS (ES^+) m/e 194 ($M+H$) $^+$.

[0491] Step 3. (*E*)-*N*-(4-(4-Methyl-4,7-diazaspiro[2.5]octan-7-yl)pyridazin-3-yl)-1-(quinoxalin-6-yl)methanimine



[0492] A mixture of 4-(4-methylpiperazin-1-yl)pyridazin-3-amine (0.400 g, 2.07 mmol, 1.00 eq), quinoxaline-6-carbaldehyde (360 mg, 2.28 mmol, 1.10 eq) and AcOH (248 mg, 4.14 mmol, 236 μ L, 2.00 eq) in toluene (15.0 mL) was stirred at 130 °C for 10 h and was then concentrated under reduced pressure to give the title compound (0.600 g, crude) as a black solid that was used directly for the next step reaction without further purification. MS (ES⁺) m/e 334 (M+H)⁺.

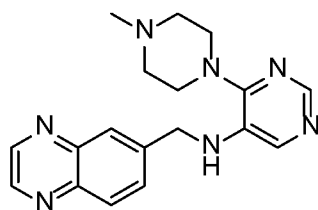
[0493] Step 4. 4-(4-Methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridazin-3-amine



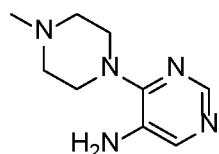
[0494] To a solution of (*E*)-*N*-(4-(4-methylpiperazin-1-yl)pyridazin-3-yl)-1-(quinoxalin-6-yl)methanimine (0.60 g, 1.80 mmol, 1.00 eq) and AcOH (108 mg, 1.80 mmol, 103 μ L, 1.00 eq) in MeOH (8.00 mL) was added NaBH₃CN (113 mg, 1.80 mmol, 1.00 eq) in portions at 25 °C. The mixture was stirred at 40 °C for 12 h, quenched by addition of saturated NH₄Cl solution (50.0 mL). NaHCO₃ was added to adjust the pH = 8-9, and the mixture was extracted with DCM (30.0 mL $\times$ 3). The combined organic layers were washed with brine (30.0 mL), dried over Na₂SO₄, and concentrated under reduced pressure to give a residue which was purified by prep-HPLC to provide the title compound (0.049 g, 24.5%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.82 (br d, *J* = 4.0 Hz, 1 H), 8.92 (s, 2 H), 8.78 (d, *J* = 6.0 Hz, 1 H), 8.27 (br s, 1 H), 8.08 (d, *J* = 8.8 Hz, 1 H), 8.02 (s, 1 H), 7.92 (dd, *J* = 8.8, 2.0 Hz, 1 H), 7.48 (d, *J* = 6.0 Hz, 1 H), 4.84 (br s, 2 H), 4.69 - 4.71 (m, 1 H), 3.96 - 3.97 (m, 1 H), 3.95 (br d, *J* = 12.4 Hz, 1 H), 3.50 - 3.62 (m, 4 H), 3.34 - 3.47 (m, 2 H), 2.81 (br d, *J* = 4.0 Hz, 3 H). MS (ES⁺) m/e 336 (M+H)⁺.

[0495] **Example 35**

[0496] 4-(4-Methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyrimidin-5-amine (Comp. 034)

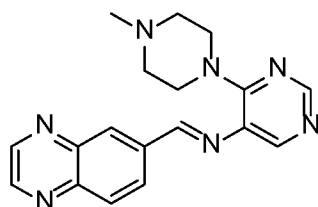


[0497] Step 1. 4-(4-Methylpiperazin-1-yl)pyrimidin-5-amine



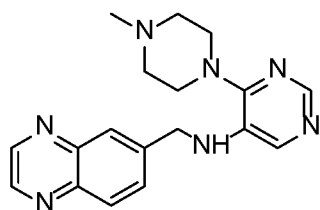
[0498] To a solution of 4-chloropyrimidin-5-amine (1.00 g, 7.72 mmol, 1.00 eq) and 1-methylpiperazine (773 mg, 7.72 mmol, 856 μ L, 1.00 eq) in ACN (2.00 mL) was added DIEA (2.99 g, 23.1 mmol, 4.03 mL, 3.00 eq). The mixture was stirred at 100 °C for 16 h, concentrated under reduced pressure, diluted with H₂O (20.0 mL), and extracted with DCM (20.0 mL $\times$ 3). The combined organic layers were washed with brine (10.0 mL), dried over Na₂SO₄, and concentrated under reduced pressure to give a residue which was purified by column chromatography (SiO₂, petroleum ether/ethyl acetate = 10/1 to 5/1) to provide the title compound (0.50 g, 33.5%) as a yellow solid. MS (ES⁺) m/e 192 (M+H)⁺.

[0499] Step 2. (*E*)-*N*-(4-(4-Methylpiperazin-1-yl)pyrimidin-5-yl)-1-(quinoxalin-6-yl)methanimine



[0500] A mixture of 4-(4-methylpiperazin-1-yl)pyrimidin-5-amine (0.500 g, 2.59 mmol, 1.00 eq), quinoxaline-6-carbaldehyde (450 mg, 2.85 mmol, 1.10 eq) and AcOH (155 mg, 2.59 mmol, 148 μ L, 1.00 eq) in toluene (15.0 mL) was stirred at 130 °C for 9 h and was concentrated under reduced pressure to give the title compound (0.50 g, crude) as a black oil which was used directly for the next step reaction without purification. MS (ES⁺) m/e 334 (M+H)⁺.

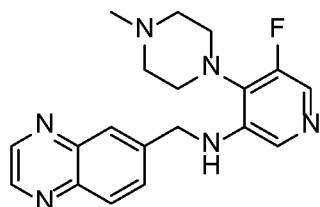
[0501] Step 3. 4-(4-Methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyrimidin-5-amine



[0502] To a solution of (*E*)-*N*-(4-(4-methylpiperazin-1-yl)pyrimidin-5-yl)-1-(quinoxalin-6-yl)methanimine (0.50 g, 1.50 mmol, 1.00 eq) and NaBH₃CN (188 mg, 3.00 mmol, 2.00 eq) in MeOH (5.00 mL) was added AcOH (90.0 mg, 749 μ mol, 85.8 μ L, 1.00 eq) in portions at 25 °C. The mixture was stirred at 40 °C for 12 h, quenched by addition saturated NH₄Cl solution (50.0 mL). NaHCO₃ was added to adjust the pH = 8-9. The mixture was extracted with DCM (30.0 mL $\times$ 3). The combined organic layers were washed with brine (30.0 mL), dried over Na₂SO₄, and concentrated under reduced pressure to give a residue that was purified by prep-HPLC to provide the title compound (0.20 g, 39.6%) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.91 (d, *J* = 1.2 Hz, 2 H), 8.12 (s, 1 H), 8.08 (d, *J* = 8.8 Hz, 1 H), 8.01 (s, 1 H), 7.88 (dd, *J* = 8.8, 1.6 Hz, 1 H), 7.68 (s, 1 H), 5.77 (t, *J* = 6.0 Hz, 1 H), 4.63 (d, *J* = 6.0 Hz, 2 H), 3.32 - 3.35 (m, 2 H), 2.51 - 2.56 (m, 4 H), 2.24 (s, 3 H). MS (ES⁺) *m/e* 336 (M+H)⁺.

[0503] Example 36

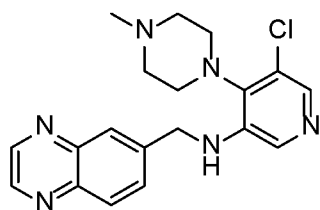
[0504] 5-Fluoro-4-(4-methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 035)



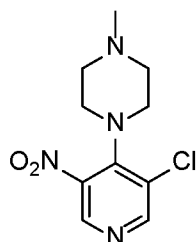
[0505] The title compound was synthesized following a similar procedure described for the preparation of Comp. 036 with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.89 (s, 2H), 8.23 (dd, *J*₁ = 0.8 Hz, *J*₂ = 5.2 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 1H), 8.07 (d, *J* = 1.2 Hz, 1H), 7.96 (dd, *J*₁ = 2.0 Hz, *J*₂ = 8.8 Hz, 1H), 7.82 (d, *J* = 0.8 Hz, 1H), 4.89 (s, 2H), 3.78-3.60 (m, 8H), 3.02 (s, 3H). MS (ES⁺) *m/e* 353 (M+H)⁺.

[0506] Example 37

[0507] 5-Chloro-4-(4-methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 036)

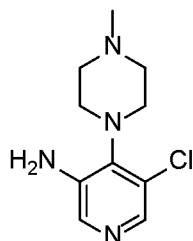


[0508] Step 1. 1-(3-Chloro-5-nitropyridin-4-yl)-4-methylpiperazine



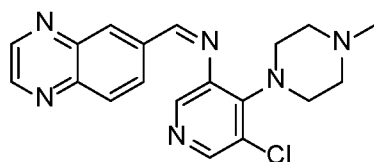
[0509] To a solution of 3,4-dichloro-5-nitropyridine (2.00 g, 10.36 mmol, 1.00 *eq*) in ACN (20 mL) was added K₂CO₃ (3.58 g, 25.9 mmol, 2.50 *eq*) and 1-methylpiperazine (1.04 g, 10.3 mmol, 1.15 mL, 1.00 *eq*). The mixture was stirred at 80 °C for 4 h, poured into H₂O (20 mL), and extracted with ethyl acetate (20 mL × 3). The combined organic phases were dried over Na₂SO₄ and concentrated under reduced pressure to give the title compound (2.77 g, crude) as a yellow solid. MS (ES⁺) *m/e* 257 (M+H)⁺.

[0510] Step 2. 5-Chloro-4-(4-methylpiperazin-1-yl)pyridin-3-amine



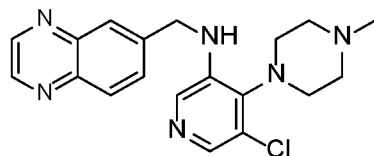
[0511] To a solution of 1-(3-chloro-5-nitropyridin-4-yl)-4-methylpiperazine (1.00 g, 3.90 mmol, 1.00 *eq*) in THF (10 mL) was added Pt-V/C (1.02 g, 3.90 mmol, 1.00 *eq*) and NH₃•H₂O (1.46 g, 11.6 mmol, 1.61 mL, 28.0% purity, 3.00 *eq*). The mixture was stirred at 50 °C for 12 h under H₂ (50 psi), filtered, and concentrated to give the title compound (0.93 g, crude) as a brown solid. MS (ES⁺) *m/e* 227 (M+H)⁺.

[0512] Step 3. (Z)-N-(5-Chloro-4-(4-methylpiperazin-1-yl)pyridin-3-yl)-1-(quinoxalin-6-yl)methanimine



[0513] To a solution of 5-chloro-4-(4-methylpiperazin-1-yl)pyridin-3-amine (0.93 g, 4.13 mmol, 1.00 *eq*) in toluene (10.0 mL) was added AcOH (347 mg, 5.78 mmol, 330 μ L, 1.40 *eq*) and quinoxaline-6-carbaldehyde (652 mg, 4.13 mmol, 1.00 *eq*). The mixture was stirred at 130 °C for 12 h and was concentrated to give the title compound (1.64 g, crude) as a yellow solid. MS (ES^+) *m/e* 367 ($\text{M}+\text{H}^+$).

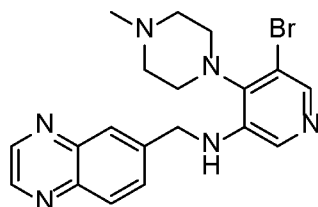
[0514] Step 4. 5-Chloro-4-(4-methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine



[0515] To a solution of (*Z*)-*N*-(5-chloro-4-(4-methylpiperazin-1-yl)pyridin-3-yl)-1-(quinoxalin-6-yl)methanimine (1.64 g, 4.47 mmol, 1.00 *eq*) in MeOH (15 mL) was added NaBH_3CN (309 mg, 4.92 mmol, 1.10 *eq*) and AcOH (268 mg, 4.47 mmol, 255 μ L, 1.00 *eq*). The mixture was stirred at 25 °C for 4 h, quenched with NaHCO_3 (1 mL), and concentrated to give a residue. The residue was purified by prep-HPLC to provide the title compound (0.59 g, 39.2%) as a brown solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.42 (br d, $J = 1.2$ Hz, 1H), 8.94 (d, $J = 1.2$ Hz, 2H), 8.16 (s, 1H), 8.16 - 8.06 (m, 2H), 7.98 (dd, $J = 1.6, 8.8$ Hz, 1H), 7.80 (s, 1H), 7.56 - 7.26 (m, 1H), 4.80 (s, 2H), 3.80 - 3.68 (m, 4H), 3.48 (br d, $J = 8.4$ Hz, 2H), 3.30 (br d, $J = 9.6$ Hz, 2H), 2.80 (br d, $J = 4.4$ Hz, 3H), 2.08 (s, 1H). MS (ES^+) *m/e* 369 ($\text{M}+\text{H}^+$).

[0516] Example 38

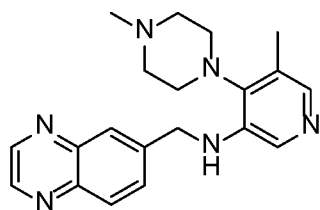
[0517] 5-Bromo-4-(4-methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 037)



[0518] The title compound was synthesized following a similar procedure described for the preparation of Comp. 036 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.08 (br s, 1H), 8.92 (q, $J = 1.6$ Hz, 2H), 8.08 (dd, $J = 4.4, 13.2$ Hz, 3H), 7.92 (dd, $J = 2.0, 8.4$ Hz, 1H), 7.86 (s, 1H), 7.45-7.08 (m, 1H), 4.80 (s, 2H), 3.89-3.63 (m, 4H), 3.57-3.40 (m, 2H), 3.38-3.15 (m, 2H), 2.84 (d, $J = 4.4$ Hz, 3H). MS (ES^+) *m/e* 415 ($\text{M}+\text{H}^+$).

Example 39

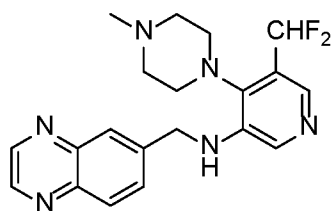
[0519] 5-Methyl-4-(4-methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 038)



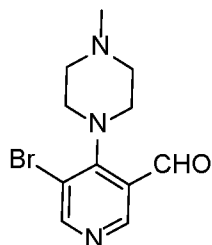
[0520] The title compound was synthesized following a similar procedure described for the preparation of Comp. 036 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.90 (q, $J = 2.0$ Hz, 2H), 8.07 (d, $J = 8.4$ Hz, 1H), 7.98 (s, 1H), 7.86 (dd, $J = 1.6, 8.8$ Hz, 1H), 7.66 (s, 1H), 7.55 (s, 1H), 5.92 (t, $J = 6.4$ Hz, 1H), 4.69 (d, $J = 6.4$ Hz, 2H), 3.32 (s, 4H), 2.26 (s, 3H), 2.24 (s, 3H). MS (ES^+) m/e 349 ($\text{M}+\text{H}^+$).

[0521] Example 40

[0522] 5-(Difluoromethyl)-4-(4-methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 039)



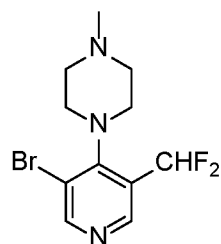
[0523] Step 1. 5-Bromo-4-(4-methylpiperazin-1-yl)nicotinaldehyde



[0524] To a solution of 5-bromo-4-chloronicotinaldehyde (3.00 g, 13.6 mmol, 1.00 *eq*) and 1-methylpiperazine (1.46 g, 14.5 mmol, 1.62 mL, 1.07 *eq*) in CH_3CN (30.0 mL) was added DIEA (4.40 g, 34.0 mmol, 5.93 mL, 2.50 *eq*). The mixture was stirred at 80 °C for 16 h and was diluted with H_2O (30.0 mL) and extracted with ethyl acetate (50.0 mL $\times$ 3). The combined organic layers were washed with brine (50.0 mL $\times$ 3), dried over Na_2SO_4 , and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2 , petroleum ether/ethyl acetate = 10/1 to 1/1) to provide the title

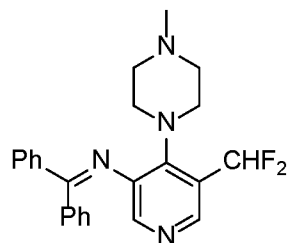
compound (3.70 g, 95.6%) as a yellow solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.11 (s, 1H), 8.65 (d, $J = 4.6$ Hz, 2H), 3.48 (s, 4H), 2.66 (s, 4H), 2.40 (s, 3H). MS (ES^+) m/e 286 ($\text{M}+\text{H}$) $^+$.

[0525] Step 2. 1-(3-Bromo-5-(difluoromethyl)pyridin-4-yl)-4-methylpiperazine



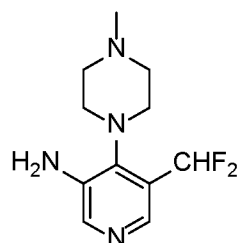
[0526] To a solution of 5-bromo-4-(4-methylpiperazin-1-yl)nicotinaldehyde (3.69 g, 12.9 mmol, 1.00 *eq*) in DCM (35.0 mL) was added DAST (10.4 g, 64.9 mmol, 8.58 mL, 5.00 *eq*) dropwise at -70 °C. The reaction mixture was stirred at 25 °C for 16 h and was neutralized with saturated NaHCO_3 solution to pH ~ 7 . The mixture was extracted with EtOAc (50 mL $\times 3$), dried with Na_2SO_4 , and concentrated under reduced pressure to give a residue which was purified by column chromatography (SiO_2 , dichloromethane/methanol = 10/1 to 2/1) to provide the title compound (0.88 g, 18.4%) as a yellow solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.72 (d, $J = 14.3$ Hz, 2H), 7.15-6.81 (m, 1H), 3.57-3.03 (m, 4H), 2.58 (s, 4H), 2.38 (s, 3H). MS (ES^+) m/e 306 ($\text{M}+\text{H}$) $^+$.

[0527] Step 2. *N*-(5-(Difluoromethyl)-4-(4-methylpiperazin-1-yl)pyridin-3-yl)-1,1-diphenylmethanimine



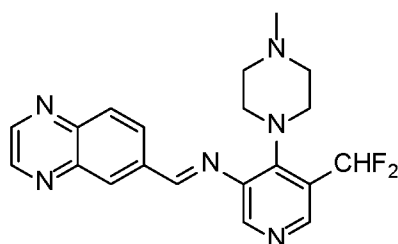
[0528] To a solution of 1-(3-bromo-5-(difluoromethyl)pyridin-4-yl)-4-methylpiperazine (800 mg, 2.61 mmol, 1.00 *eq*) and diphenylmethanimine (568 mg, 3.14 mmol, 526 μL , 1.20 *eq*) in toluene (8.00 mL) was added $\text{Pd}(\text{dba})_2$ (75.1 mg, 130 μmol , 0.05 *eq*), *t*-BuONa (2.00 M, 2.61 mL, 2.00 *eq*), and BINAP (162 mg, 261 μmol , 0.10 *eq*) under N_2 atmosphere. The suspension was degassed and purged with N_2 three times. The mixture was stirred under N_2 at 100 °C for 12 h and was concentrated under reduced pressure to provide the title compound (1.06 g, crude) as a brown oil which was used in the next step reaction without purification. MS (ES^+) m/e 407 ($\text{M}+\text{H}$) $^+$.

[0529] Step 3. 5-(Difluoromethyl)-4-(4-methylpiperazin-1-yl)pyridin-3-amine



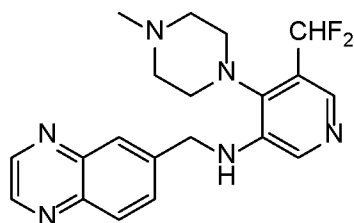
[0530] To a solution of *N*-(5-(difluoromethyl)-4-(4-methylpiperazin-1-yl)pyridin-3-yl)-1,1-diphenylmethanimine (1.00 g, 2.46 mmol, 1.00 *eq*) in THF (10.0 mL) was added HCl (12.0 M, 2.05 mL, 10.0 *eq*) at 25 °C. The mixture was stirred at 25 °C for 2 h and was concentrated under reduced pressure to remove THF. The mixture was extracted with EtOAc (20.0 mL×2). The combined organic layers were washed with brine (20.0 mL), dried over Na₂SO₄, and concentrated under reduced pressure to give the residue which was purified by reversed-phase HPLC to provide the title compound (350 mg, 58.7%) as a brown solid. MS (ES⁺) *m/e* 243 (M+H)⁺.

[0531] Step 4. (*E*)-*N*-(5-(Difluoromethyl)-4-(4-methylpiperazin-1-yl)pyridin-3-yl)-1-(quinoxalin-6-yl)methanimine



[0532] To a solution of 5-(difluoromethyl)-4-(4-methylpiperazin-1-yl)pyridin-3-amine (350 mg, 1.44 mmol, 1.00 *eq*) and quinoxaline-6-carbaldehyde (228 mg, 1.44 mmol, 1.00 *eq*) in toluene (5.00 mL) was added AcOH (86.7 mg, 1.44 mmol, 82.7 μL, 1.00 *eq*). The mixture was stirred at 130 °C for 2 h and was concentrated under reduced pressure to provide the title compound (552 mg, crude) as a brown oil.

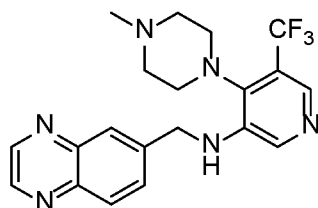
[0533] Step 5. 5-(Difluoromethyl)-4-(4-methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine



[0534] To a solution of (*E*)-*N*-(5-(difluoromethyl)-4-(4-methylpiperazin-1-yl)pyridin-3-yl)-1-(quinoxalin-6-yl)methanimine (552 mg, 1.44 mmol, 1.00 *eq*) in MeOH (5.00 mL) was added NaBH₃CN (90.7 mg, 1.44 mmol, 1.00 *eq*) and AcOH (86.6 mg, 1.44 mmol, 82.6 μ L, 1.00 *eq*). The mixture was stirred at 25 °C for 1.5 h and was concentrated under reduced pressure to give a residue. The residue was purified by reversed-phase HPLC to provide the title compound (106 mg, 19.0%) as a green solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.20-8.85 (m, 2H), 8.31 (s, 1H), 8.26-8.18 (m, 2H), 8.15-8.02 (m, 2H), 7.36 (t, *J* = 54.0 Hz, 1H), 4.99 (s, 3H), 3.96-3.79 (m, 2H), 3.76-3.52 (m, 6H), 3.06 (s, 3H). MS (ES⁺) *m/e* 385 (M+H)⁺.

[0535] Example 41

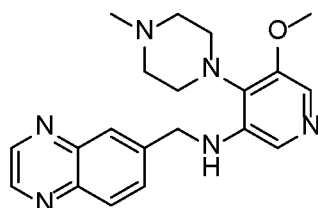
[0536] 4-(4-Methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)-5-(trifluoromethyl)pyridin-3-amine (Comp. 040)



[0537] The title compound was synthesized following a similar procedure described for the preparation of Comp. 036 with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.96-8.87 (m, 2H), 8.45 (s, 1H), 8.20-8.15(m, 2H), 8.11 (d, *J* = 1.2 Hz, 1H), 8.00 (dd, *J* = 8.8, 2.0 Hz, 1H), 4.99 (s, 2H), 3.92-3.60 (m, 8H), 3.06 (s, 3H). MS (ES⁺) *m/e* 403 (M+H)⁺.

[0538] Example 42

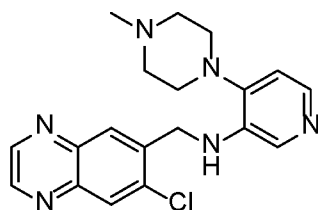
[0539] 5-Methoxy-4-(4-methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 041)



[0540] The title compound was synthesized following a similar procedure described for the preparation of Comp. 036 with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.90 (q, *J* = 2.0 Hz, 2H), 8.07 (d, *J* = 8.4 Hz, 1H), 7.96 (s, 1H), 7.85 (dd, *J* = 1.6, 8.4 Hz, 1H), 7.62 (s, 1H), 7.55 (s, 1H), 6.09 (t, *J* = 6.4 Hz, 1H), 4.70 (d, *J* = 6.4 Hz, 2H), 3.81 (s, 3H), 3.31 (s, 8H), 2.24 (s, 3H). MS (ES⁺) *m/e* 365 (M+H)⁺.

[0541] Example 43

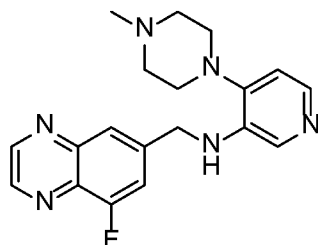
[0542] *N*-((7-Chloroquinoxalin-6-yl)methyl)-4-(4-methylpiperazin-1-yl)pyridin-3-amine
(Comp. 042)



[0543] The title compound was synthesized following a similar procedure described for the preparation of Comp. 036 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 15.21-14.80 (m, 1H), 11.73-1.44 (m, 1H), 8.96 (dd, $J = 1.6, 13.2$ Hz, 2H), 8.32 (s, 1H), 8.14 (d, $J = 6.4$ Hz, 1H), 7.95-7.83 (m, 2H), 7.46 (d, $J = 6.4$ Hz, 1H), 6.78 (br t, $J = 5.6$ Hz, 1H), 4.76 (br d, $J = 5.2$ Hz, 2H), 3.93-3.77 (m, 2H), 3.57 (br d, $J = 8.0$ Hz, 2H), 3.48-3.32 (m, 4H), 2.82 (d, $J = 4.0$ Hz, 3H). MS (ES^+) m/e 369 ($\text{M}+\text{H}$) $^+$.

[0544] Example 44

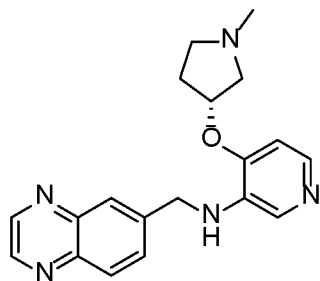
[0545] *N*-((8-Fluoroquinoxalin-6-yl)methyl)-4-(4-methylpiperazin-1-yl)pyridin-3-amine
(Comp. 043)



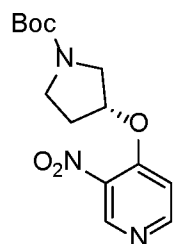
[0546] The title compound was synthesized following a similar procedure described for the preparation of Comp. 036 with appropriate starting materials and intermediates. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.99 (dd, $J = 1.6, 14.6$ Hz, 2H), 8.09 (d, $J = 6.0$ Hz, 1H), 7.93 (s, 1H), 7.87-7.73 (m, 2H), 7.41 (d, $J = 6.4$ Hz, 1H), 6.86 (br t, $J = 5.6$ Hz, 1H), 4.75 (br d, $J = 5.6$ Hz, 2H), 4.06-3.90 (m, 4H), 3.88-3.77 (m, 2H), 3.64-3.52 (m, 2H), 3.49-3.35 (m, 4H), 2.83 (br s, 3H). MS (ES^+) m/e 353 ($\text{M}+\text{H}$) $^+$.

[0547] Example 45

[0548] (*R*)-4-((1-Methylpyrrolidin-3-yl)oxy)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine
(Comp. 044)

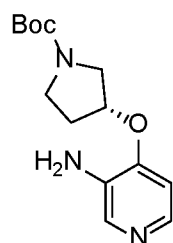


[0549] Step 1. *tert*-Butyl (R)-3-((3-nitropyridin-4-yl)oxy)pyrrolidine-1-carboxylate



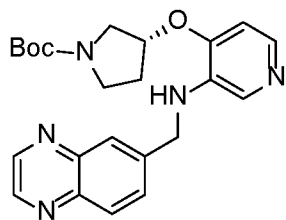
[0550] To a solution of *tert*-butyl (R)-3-hydroxypyrrolidine-1-carboxylate (6.50 g, 34.7 mmol, 1.10 *eq*) in THF (30.0 mL) was added *t*-BuOK (1 M, 37.8 mL, 1.20 *eq*) at 25 °C. The mixture was stirred at 25 °C for 1 h, then 4-chloro-3-nitropyridine (5.00 g, 31.5 mmol, 1.00 *eq*) in THF (25.0 mL) was added. The mixture was stirred at 25 °C for 2 h, quenched with NH₄Cl (saturated solution, 500 mL), and extracted with EtOAc (250 mL × 3). The combined organic layers were washed with brine (450 mL), dried over Na₂SO₄, and concentrated under reduced pressure to give a residue which was purified by column chromatography (SiO₂, petroleum ether: etOAc = 10: 1 ~ 2: 1) to provide the title compound (5.09 g, 52.2%) as a brown oil. MS (ES⁺) *m/e* 310 (M+H)⁺.

[0551] Step 2. *tert*-Butyl (R)-3-((3-aminopyridin-4-yl)oxy)pyrrolidine-1-carboxylate



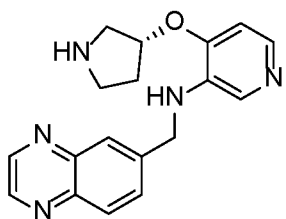
[0552] To a solution of *tert*-butyl (R)-3-((3-nitropyridin-4-yl)oxy)pyrrolidine-1-carboxylate (5.00 g, 16.2 mmol, 1.00 *eq*) in MeOH (50.0 mL) was added Pt-V/C (4.22 g, 16.2 mmol, 1.00 *eq*) under N₂ atmosphere. The mixture was degassed and purged with H₂ for 3 times and stirred at 25 °C for 12 h and filtered. The filtrate was concentrated to provide the title compound (4.6 g, crude) as a brown oil. MS (ES⁺) *m/e* 280 (M+H)⁺.

[0553] Step 3. *tert*-Butyl (R)-3-((3-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)oxy)pyrrolidine-1-carboxylate



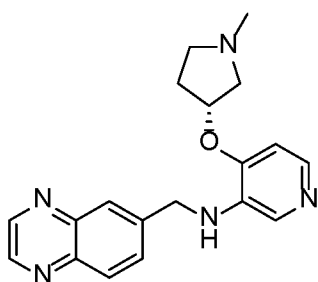
[0554] To a mixture of *tert*-butyl (*R*)-3-((3-aminopyridin-4-yl)oxy)pyrrolidine-1-carboxylate (2.00 g, 7.16 mmol, 1.00 eq) and quinoxaline-6-carbaldehyde (2.26 g, 14.3 mmol, 2.00 eq) in MeOH (10.0 mL) and AcOH (0.50 mL) was added borane-2-methylpyridine (1.53 g, 14.3 mmol, 2.00 eq). The mixture was stirred at 25 °C for 12 h and was concentrated. The crude product was purified by reversed-phase HPLC to provide the title compound (0.422 g, 14.0%) as a yellow oil. MS (ES⁺) *m/e* 422 (M+H)⁺.

[0555] Step 4. (*R*)-4-(pyrrolidin-3-yloxy)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine



[0556] To a solution of HCl/EtOAc (4 M, 3.00 mL, 12.6 eq) in EtOAc (3.00 mL) was added *tert*-butyl (*R*)-3-((3-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)oxy)pyrrolidine-1-carboxylate (0.40 g, 949 μmol, 1.00 eq) in EtOAc (2.00 mL). The mixture was stirred at 25 °C for 0.5 h and was concentrated under reduced pressure to provide the title compound (259 mg, 84.9%) as a brown solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.81 (s, 2H), 8.03 - 7.91 (m, 3H), 7.83 (d, *J* = 8.8 Hz, 1H), 7.59 (s, 1H), 7.33 (d, *J* = 6.4 Hz, 1H), 5.61 (br d, *J* = 2.4 Hz, 1H), 4.71 (s, 1H), 3.85 - 3.77 (m, 1H), 3.75 - 3.66 (m, 1H), 3.59 (t, *J* = 7.6 Hz, 2H), 2.52 - 2.43 (m, 2H). MS (ES⁺) *m/e* 322 (M+H)⁺.

[0557] Step 5. (*R*)-4-((1-methylpyrrolidin-3-yl)oxy)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine

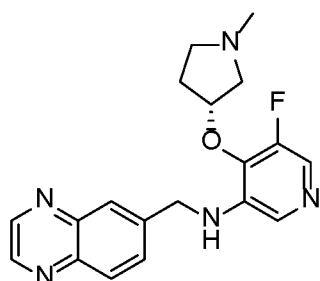


[0558] To a solution of (*R*)-4-(pyrrolidin-3-yloxy)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (0.30 g, 933 μmol, 1.00 eq) and (CHO)_n (318 mg, 9.33 mmol, 10.0 eq) in MeOH (5.00

mL) was added NaBH₃CN (176 mg, 2.80 mmol, 3.00 eq). The mixture was stirred at 25 °C for 1h, concentrated, and purified by prep-HPLC to provide the title compound (151.62 mg, 47.8%) as a brown solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 15.12 (br s, 1H), 11.90 (br s, 1H), 8.92 (s, 2H), 8.16-8.05 (m, 3H), 8.04-7.89 (m, 2H), 7.83 (s, 1H), 7.59-7.44 (m, 1H), 5.70 (br s, 1H), 4.87-4.59 (m, 2H), 4.12-3.89 (m, 1H), 3.85-3.64 (m, 1H), 3.63-3.39 (m, 1H), 3.28-3.24 (m, 1H), 3.05-2.82 (m, 3H), 2.71-2.57 (m, 1H), 2.44-2.04 (m, 1H). MS (ES⁺) *m/e* 336 (M+H)⁺.

[0559] Example 46

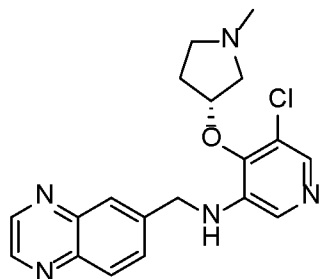
[0560] (*R*)-5-fluoro-4-((1-methylpyrrolidin-3-yl)oxy)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 045)



[0561] The title compound was synthesized following a similar procedure described for the preparation of Comp. 044 with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.96-8.85 (m, 2H), 8.14-8.10 (m, 2H), 8.02 (br s, 1H), 7.89 (br d, *J* = 8.4 Hz, 1H), 7.66 (br s, 1H), 5.98-5.83 (m, 1H), 4.85-4.80 (m, 2H), 4.34-4.14 (m, 1H), 4.05-3.87 (m, 1H), 3.78-3.53 (m, 1H), 3.46-3.29 (m, 1H), 3.12-3.03 (m, 3H), 2.90-2.78 (m, 1H), 2.64-2.46 (m, 1H). MS (ES⁺) *m/e* 354 (M+H)⁺.

[0562] Example 47

[0563] (*R*)-5-chloro-4-((1-methylpyrrolidin-3-yl)oxy)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 046)

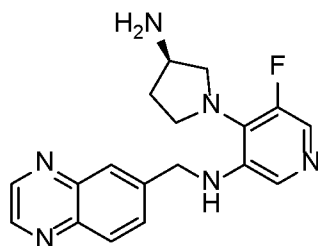


[0564] The title compound was synthesized following a similar procedure described for the preparation of Comp. 044 with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.4 (br s, 1H), 8.92 (d, *J* = 2.4 Hz, 2H), 8.14 (s, 1H), 8.10 - 8.04

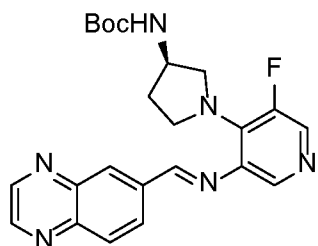
(m, 2H), 8.02 (s, 1H), 7.94 (s, 1H), 5.62 (br s, 1H), 4.78 (s, 2H), 3.92 (br d, $J = 11.2$ Hz, 2H), 3.42 (br d, $J = 5.2$ Hz, 1H), 3.22 (br d, $J = 4.4$ Hz, 1H), 3.02 - 2.84 (m, 3H), 2.64 (br dd, $J = 8.8, 14.8$ Hz, 1H), 2.46 - 2.22 (m, 1H). MS (ES⁺) m/e 370 (M+H)⁺.

[0565] Example 48

[0566] (*R*)-4-(3-aminopyrrolidin-1-yl)-5-fluoro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 047)

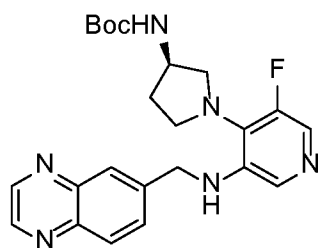


[0567] Step 1. *tert*-Butyl (*R,E*)-(1-(3-fluoro-5-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate



[0568] To a solution of *tert*-butyl (*R*)-(1-(3-amino-5-fluoropyridin-4-yl)pyrrolidin-3-yl)carbamate (500 mg, 1.69 mmol, 1.00 *eq*) and quinoxaline-6-carbaldehyde (266 mg, 1.69 mmol, 1.00 *eq*) in toluene (10.0 mL) was added AcOH (202 mg, 3.37 mmol, 193 μ L, 2.00 *eq*). The reaction mixture was concentrated under reduced pressure to give a yellow solid which was used in the next step without further purification. MS (ES⁺) m/e 437.2 (M+H)⁺.

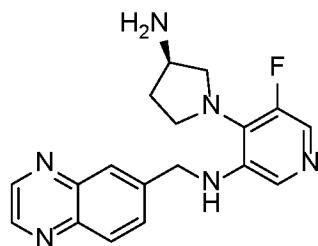
[0569] Step 2. *tert*-Butyl (*R*)-(1-(3-fluoro-5-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate



[0570] To a solution of *tert*-butyl (*R,E*)-(1-(3-fluoro-5-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate (736 mg, 1.69 mmol, 1.00 *eq*) in methanol (6.00 mL) was added NaBH₃CN (211 mg, 3.37 mmol, 2.00 *eq*) and AcOH (50.6

mg, 844 μmol , 48.2 μL , 0.500 *eq*). The mixture was stirred at 25 °C for 12 h, concentrated under reduced pressure to remove methanol, diluted with H₂O (20.0 mL), and extracted with ethyl acetate (20.0 mL $\times$ 2). The combined organic layers were washed with brine (50.0 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue which was purified by prep-HPLC (column: Waters Xbridge 150*25mm* 5 μm ; mobile phase: [water (ammonia hydroxide v/v)-ACN];gradient:25%-55% B over 10 min) to give the title compound (280 mg, 37.5%) as a yellow solid. which was confirmed by HPLC (EW49030-20-P1H3) and HNMR (EW49030-20-P1C1). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.95 (s, 2H), 8.19-8.02 (m, 2H), 7.93 (dd, *J* = 1.2, 8.6 Hz, 1H), 7.75-7.59 (m, 2H), 7.49-7.21 (m, 1H), 6.55-6.38 (m, 1H), 4.75 (br d, *J* = 6.4 Hz, 2H), 4.33-4.15 (m, 1H), 3.50-3.46 (m, 2H), 3.17-3.04 (m, 2H), 2.35-2.22 (m, 1H), 1.89-1.80 (m, 1H), 1.44 (s, 9H). MS (ES⁺) *m/e* 439.1 (M+H)⁺.

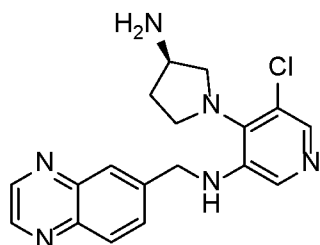
[0571] Step 3. (*R*)-4-(3-Aminopyrrolidin-1-yl)-5-fluoro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine



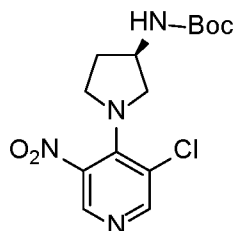
[0572] A mixture of *tert*-butyl (*R*)-(1-(3-fluoro-5-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate (250 mg, 570 μmol , 1.00 *eq*) and HCl/MeOH (2 M, 3.00 mL, 10.5 *eq*) in methanol (3.00 mL) was degassed and purged with N₂ for 3 times, and then the mixture was stirred at 25 °C for 12 h under N₂ atmosphere. The reaction mixture was concentrated to give a residue which was purified by prep-HPLC (column: Phenomenex luna C18 150*25mm* 10 μm ;mobile phase: [water(HCl)-ACN];gradient:1%-15% B over 10 min) to the title compound (143 mg, 50.6%) as a yellow solid. ¹H NMR (400 MHz, MeOD) δ 8.93 (s, 2H), 8.24-8.10 (m, 3H), 8.01 (dd, *J* = 2.0, 8.8 Hz, 1H), 7.71 (s, 1H), 4.84 (d, *J* = 6.0 Hz, 2H), 4.30-4.08 (m, 3H), 3.91-3.81 (m, 1H), 3.67-3.55 (m, 1H), 2.66-2.50 (m, 1H), 2.37-2.23 (m, 1H). MS (ES⁺) *m/e* 339.0 (M+H)⁺.

[0573] Example 49

[0574] (*R*)-4-(3-aminopyrrolidin-1-yl)-5-chloro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 048)

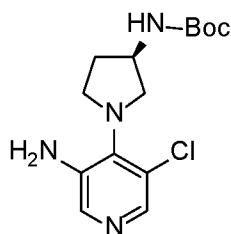


[0575] Step 1. *tert*-Butyl (*R*)-(1-(3-chloro-5-nitropyridin-4-yl)pyrrolidin-3-yl)carbamate



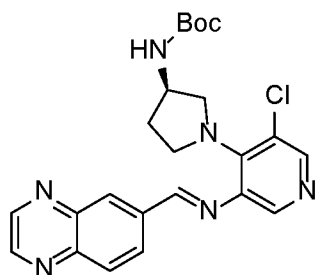
[0576] To a solution of *tert*-butyl (*R*)-pyrrolidin-3-ylcarbamate (965 mg, 5.18 mmol, 1.00 eq) in ACN (10.0 mL) was added K₂CO₃ (1.79 g, 13.0 mmol, 2.50 eq) and 3,4-dichloro-5-nitropyridine (1.00 g, 5.18 mmol, 1.00 eq). The mixture was stirred at 60 °C for 5 h, diluted with water (100 mL), and extracted with ethyl acetate (100 mL × 3). The combined organic phase was dried over Na₂SO₄, filtered and concentrated under reduced pressure to give the title compound (1.70 g, crude) as yellow oil, which was used directly for the next step reaction without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ . MS (ES⁺) *m/e* 343.1 (M+H)⁺.

[0577] Step 2. *tert*-Butyl (*R*)-(1-(3-amino-5-chloropyridin-4-yl)pyrrolidin-3-yl)carbamate



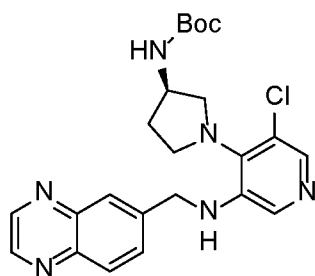
[0578] The reaction was carried out via flow chemistry. A mixture of *tert*-butyl (*R*)-(1-(3-chloro-5-nitropyridin-4-yl)pyrrolidin-3-yl)carbamate (1.80 g, 5.25 mmol, 1.00 eq) in THF (18.0 mL) was stirred at 20 °C until it became a clear solution. The following conditions were used for the flow chemistry: H₂ back pressure - 1.5 MPa, the flow rate of H₂ - 30 ml/min, fixed bed (1% Pt/C, 3 g) temperature 55 °C. The solution was pumped into the reactor at a flow rate of 0.4 mL/min. After 0.75 h, the reaction was complete. The reaction mixture was concentrated under reduced pressure to give the title compound (1.60 g, crude) as a brown oil which was used directly for the next step without further purification. MS (ES⁺) *m/e* 313.0 (M+H)⁺.

[0579] Step 3. *tert*-Butyl (*R*)-(1-(3-amino-5-chloropyridin-4-yl)pyrrolidin-3-yl)carbamate



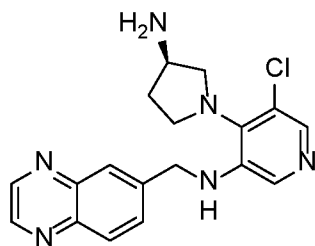
[0580] A mixture of quinoxaline-6-carbaldehyde (1.21 g, 7.67 mmol, 1.50 eq), *tert*-butyl (*R*)-(1-(3-amino-5-chloropyridin-4-yl)pyrrolidin-3-yl)carbamate (1.60 g, 5.12 mmol, 1.00 eq), and AcOH (768 mg, 12.8 mmol, 732 μ L, 2.50 eq) in toluene (20.0 mL) was stirred at 120 °C for 12 h. The mixture was then concentrated under reduced pressure to afford the title compound (2.40 g, crude) as a yellow oil which was used directly for the next step reaction without further purification. MS (ES^+) m/e 453.2 ($\text{M}+\text{H}$) $^+$.

[0581] Step 4. *tert*-Butyl (*R*)-(1-(3-chloro-5-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate



[0582] To a solution of *tert*-butyl (*R,E*)-(1-(3-chloro-5-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate (2.40 g, 5.30 mmol, 1.00 eq) and NaBH_3CN (499 mg, 7.95 mmol, 1.50 eq) in MeOH (20.0 mL) was added AcOH (636 mg, 10.6 mmol, 607 μ L, 2.00 eq) in portions at 25 °C. The mixture was stirred at 25 °C for 12 h, quenched with sat. Na_2CO_3 (100 mL), and extracted with ethyl acetate (100 mL $\times$ 3). The combined organic phase was dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a crude product which was purified by reversed-phase HPLC (0.1% FA condition) and lyophilized to give the title compound (200 mg, 8.30%) as a yellow solid. MS (ES^+) m/e 455.1 ($\text{M}+\text{H}$) $^+$.

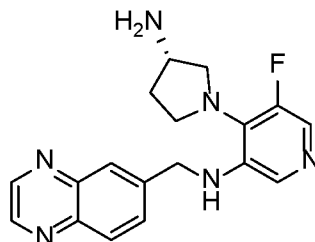
[0583] Step 5. (*R*)-4-(3-Aminopyrrolidin-1-yl)-5-chloro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine



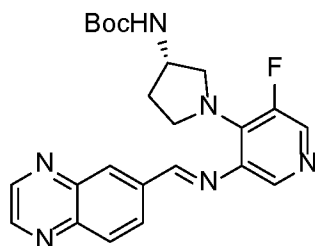
[0584] A solution of *tert*-butyl (*R*)-(1-(3-chloro-5-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate (300 mg, 659 μ mol, 1.00 eq) in HCl/MeOH (2 M, 7.50 mL, 22.8 eq) was stirred at 25 °C for 2 h. The mixture was concentrated under reduced pressure to give a crude product which was purified by prep-HPLC (column: Phenomenex luna C18 150 $\times$ 25 mm $\times$ 10 μ m; mobile phase: [water (HCl) - ACN]; gradient: 1% - 20% B over 10 min) and lyophilized to give the title compound (52.8 mg, 22.3%) as a yellow solid. ^1H NMR (400 MHz, DMSO- d_6) δ 8.90 (s, 2H), 8.18 - 8.12 (m, 2H), 8.08 (s, 1H), 7.97 (d, J = 8.8 Hz, 1H), 7.81 (s, 1H), 4.91 (br s, 1H), 4.84 (br s, 1H), 4.24 - 4.08 (m, 2H), 4.00 - 3.88 (m, 1H), 3.74 - 3.57 (m, 2H), 2.69-2.58 (m, 1H), 2.35 - 2.23 (m, 1H). MS (ES^+) m/e 355.0 ($\text{M}+\text{H}$) $^+$.

[0585] Example 50

[0586] (*S*)-4-(3-aminopyrrolidin-1-yl)-5-fluoro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 049)



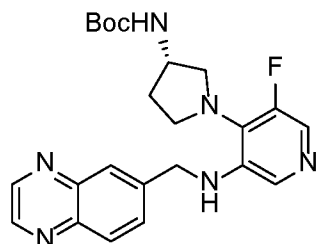
[0587] Step 1. *tert*-Butyl (*S,E*)-(1-(3-fluoro-5-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate



[0588] To a solution of *tert*-butyl (*S*)-(1-(3-amino-5-fluoropyridin-4-yl)pyrrolidin-3-yl)carbamate (500.00 mg, 1.69 mmol, 1 eq) and quinoxaline-6-carbaldehyde (266 mg, 1.69 mmol, 1.00 eq) in toluene (10.0 mL) was added AcOH (202 mg, 3.37 mmol, 193 μ L, 2.00 eq). The mixture was stirred at 130 °C for 12 h. The reaction mixture was concentrated under

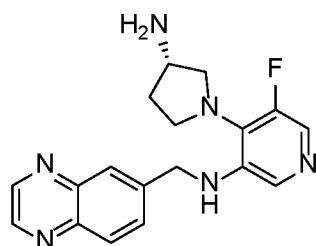
reduced pressure to give a residue (736 mg, crude) which was used to the next step reaction without purification. MS (ES^+) m/e 437.2 ($M+H$) $^+$.

[0589] Step 2. *tert*-Butyl (*S*)-(1-(3-fluoro-5-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate



[0590] To a solution of *tert*-butyl (*S,E*)-(1-(3-fluoro-5-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate (736 mg, 1.69 mmol, 1.00 *eq*) in methanol (7.00 mL) was added $NaBH_3CN$ (211 mg, 3.37 mmol, 2.00 *eq*) and AcOH (50.6 mg, 843 μ mol, 48.2 μ L, 0.500 *eq*). The mixture was stirred at 25 $^{\circ}C$ for 12 h. The reaction mixture was concentrated under reduced pressure to remove methanol, diluted with H_2O (20.0 mL), and extracted with ethyl acetate (20.0 mL $\times$ 2). The combined organic layers were washed with brine (50.0 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Waters Xbridge 150*25mm* 5 μ m; mobile phase: [water (ammonia hydroxide v/v)-ACN];gradient:25%-55% B over 10 min) to afford the title compound (260 mg, 34.9%) as a yellow solid. ^1H NMR (400 MHz, $DMSO-d_6$) δ 8.95 (d, J = 1.2 Hz, 2H), 8.17-8.02 (m, 2H), 7.93 (dd, J = 1.6, 8.8 Hz, 1H), 7.75-7.60 (m, 2H), 7.43-7.23 (m, 1H), 6.54-6.42 (m, 1H), 4.75 (br d, J = 6.4 Hz, 2H), 4.33-4.16 (m, 1H), 3.52-3.46 (m, 2H), 3.19-3.04 (m, 2H), 2.29 (dt, J = 7.8, 13.2 Hz, 1H), 1.91-1.76 (m, 1H), 1.44 (s, 9H). MS (ES^+) m/e 439.1 ($M+H$) $^+$.

[0591] Step 3. (*S*)-4-(3-Aminopyrrolidin-1-yl)-5-fluoro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine

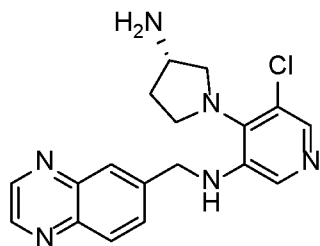


[0592] A mixture of *tert*-butyl (*S*)-(1-(3-fluoro-5-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate (260 mg, 592.93 μ mol, 1 *eq*), HCl/MeOH (2 M, 3 mL, 10.12 *eq*), and MeOH (3 mL) was degassed and purged with N_2 for 3 times. The reaction mixture was stirred at 25 $^{\circ}C$ for 12 h under N_2 atmosphere and was then concentrated under reduced

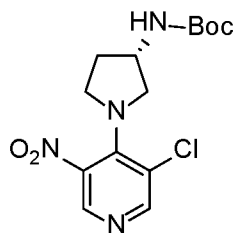
pressure to give a residue which was purified by prep-HPLC (column: Phenomenex luna C18 150*25mm* 10μm; mobile phase: [water(HCl)-ACN]; gradient: 1%-15% B over 10 min) to give the title compound (76.0 mg, 37.8%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.94 (s, 2H), 8.20-8.12 (m, 3H), 8.02 (dd, *J* = 2.0, 8.6 Hz, 1H), 7.72-7.68 (m, 1H), 4.84 (d, *J* = 6.8 Hz, 2H), 4.28-4.20 (m, 1H), 4.19-4.10 (m, 2H), 3.92-3.81 (m, 1H), 3.64-3.53 (m, 1H), 2.65-2.49 (m, 1H), 2.36-2.24 (m, 1H). MS (ES⁺) *m/e* 339.0 (M+H)⁺.

[0593] Example 51

[0594] (*S*)-4-(3-Aminopyrrolidin-1-yl)-5-chloro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 050)

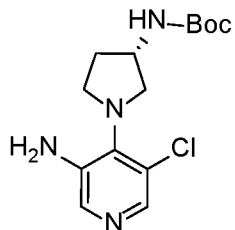


[0595] Step 1. *tert*-Butyl (*S*)-(1-(3-chloro-5-nitropyridin-4-yl)pyrrolidin-3-yl)carbamate



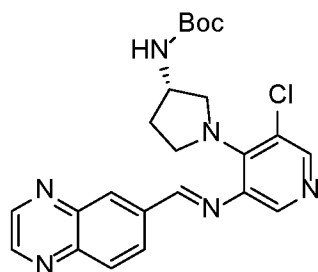
[0596] To a solution of 3,4-dichloro-5-nitropyridine (1.50 g, 7.77 mmol, 1.00 eq) in ACN (20.0 mL) was added K₂CO₃ (2.15 g, 15.6 mmol, 2.00 eq) and *tert*-butyl (*S*)-pyrrolidin-3-ylcarbamate (1.45 g, 7.77 mmol, 1.00 eq). The mixture was stirred at 60 °C for 5 h, cooled to room temperature, diluted with water (100 mL) and extracted with DCM (200 mL × 3). The combined organic layers were washed with brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure to give the title compound (3.10 g, crude) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.56 (s, 1H), 8.37 (s, 1H), 4.93 (br d, *J* = 6.4 Hz, 1H), 3.83 (br d, *J* = 4.8 Hz, 1H), 3.73-3.62 (m, 1H), 3.61-3.48 (m, 1H), 3.39 (br dd, *J* = 4.0, 10.8 Hz, 1H), 1.47-1.43 (m, 9H).

[0597] Step 2. *tert*-Butyl (*S*)-(1-(3-amino-5-chloropyridin-4-yl)pyrrolidin-3-yl)carbamate



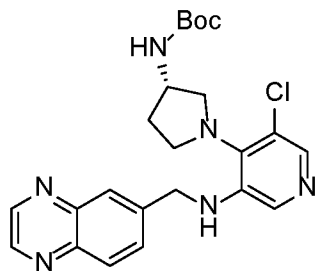
[0598] The reaction was carried out via flow chemistry. A mixture of *tert*-butyl (*S*)-(1-(3-chloro-5-nitropyridin-4-yl)pyrrolidin-3-yl)carbamate (3.10 g, 9.04 mmol, 1.00 eq) in THF (18.0 mL) was stirred at 20 °C until it became a clear solution. The following conditions were used for the flow chemistry: H₂ back pressure - 1.5 MPa, the flow rate of H₂ - 30 ml/min, fixed bed (1% Pt/C, 3 g) temperature 55 °C. The solution was pumped into the reactor at a flow rate of 0.4 mL/min. After 1.3 h, the reaction was complete. The reaction mixture was concentrated under reduced pressure to give the title compound (2.50 g, 88.4%) as a brown oil which was used directly for the next step reaction without purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ . MS (ES⁺) *m/e* 313.0 (M+H)⁺.

[0599] Step 3. *tert*-Butyl (*S,E*)-(1-(3-chloro-5-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate



[0600] To a solution of *tert*-butyl (*S*)-(1-(3-amino-5-chloropyridin-4-yl)pyrrolidin-3-yl)carbamate (2.50 g, 7.99 mmol, 1.00 eq) in toluene (25 mL) was added AcOH (1.20 g, 20.0 mmol, 1.14 mL, 2.50 eq) and quinoxaline-6-carbaldehyde (1.26 g, 7.99 mmol, 1.00 eq). The mixture was stirred at 130 °C for 12 h and concentrated to provide the title compound (3.61 g, crude) as a brown oil which was used directly for the next step reaction without purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ . MS (ES⁺) *m/e* 451.2 (M+H)⁺.

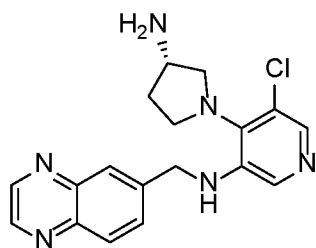
[0601] Step 4. *tert*-Butyl (*S,E*)-(1-(3-chloro-5-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate



[0602] To a solution of *tert*-butyl (*S,E*)-(1-(3-chloro-5-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate (3.60 g, 7.95 mmol, 1.00 eq) in MeOH (40.0 mL) was added NaBH₃CN (1.25 g, 19.9 mmol, 2.50 eq) and AcOH (1.19 g, 19.9 mmol, 1.14 mL, 2.50 eq). The mixture was stirred at 25 °C for 2 h, quenched with

NaHCO₃, concentrated under reduced pressure to move MeOH, and extracted with EtOAc 150 mL (50.0 mL × 3). The combined organic layers were washed with brine (50.0 mL × 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by reversed-phase HPLC (0.1% FA condition) and lyophilized to give the title compound (0.60 g, 16.6%) as a brown oil. ¹H NMR (400 MHz, DMSO-*d*₆) δ . MS (ES⁺) *m/e* 455.1 (M+H)⁺.

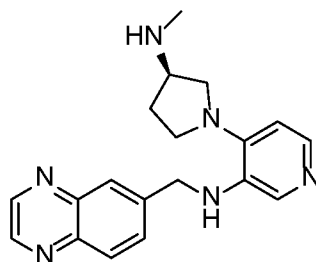
[0603] Step 5. (*S*)-4-(3-Aminopyrrolidin-1-yl)-5-chloro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine



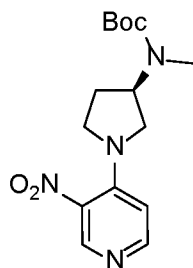
[0604] To a solution of *tert*-butyl (*S*)-(1-(3-chloro-5-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate (0.50 g, 1.10 mmol, 1.00 eq) was added HCl/MeOH (2.00 M, 550 μ L, 1.00 eq). The mixture was stirred at 25 °C for 1 h and was concentrated under reduce pressure to move MeOH. The crude product was purified by reversed-phase HPLC (column: Phenomenex luna C18 150 × 25mm × 10 μ m; mobile phase: [water(HCl)-ACN]; gradient: 1%-20% B over 10 min) and lyophilized to give the title compound (120 mg, 30.8%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.92 (s, 2H), 8.71 (br s, 3H), 8.16 (s, 1H), 8.12-8.06 (m, 2H), 7.97 (d, *J* = 9.2 Hz, 1H), 7.80 (s, 1H), 4.80 (s, 2H), 4.02 (br s, 1H), 3.95-3.85 (m, 2H), 3.55 (br d, *J* = 10.4 Hz, 1H), 3.35 (dt, *J* = 5.6, 9.2 Hz, 1H), 2.45-2.32 (m, 1H), 2.28-2.16 (m, 1H). MS (ES⁺) *m/e* 355.0 (M+H)⁺.

[0605] Example 52

[0606] (*R*)-4-(3-(methylamino)pyrrolidin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 051)

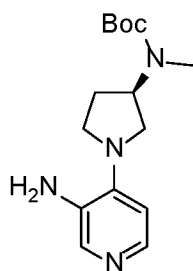


[0607] Step 1. *tert*-Butyl (*R*)-methyl(1-(3-nitropyridin-4-yl)pyrrolidin-3-yl)carbamate



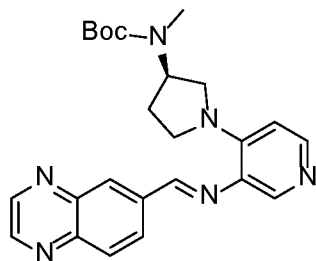
[0608] To a solution of 4-chloro-3-nitropyridine (1.58 g, 9.99 mmol, 1.00 eq) and *tert*-butyl (*R*)-methyl(pyrrolidin-3-yl)carbamate (2.00 g, 9.99 mmol, 1.00 eq) in ACN (15.0 mL) was added K₂CO₃ (2.76 g, 20.0 mmol, 2.00 eq). The mixture was stirred at 60 °C for 5 h and was concentrated under reduced pressure to give a residue. To the residue was added water (100 mL) and the resulting solution was extracted with DCM (200 mL × 3). The combined organic layers were washed with brine (100 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give the title compound (2.00 g, crude) as a yellow oil. MS (ES⁺) *m/e* 323.1 (M+H)⁺.

[0609] Step 2. *tert*-Butyl (*R*)-(1-(3-aminopyridin-4-yl)pyrrolidin-3-yl)(methyl)carbamate



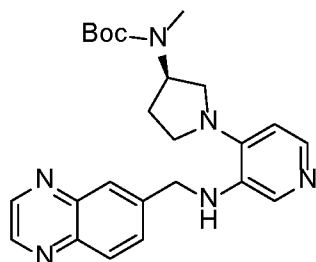
[0610] The reaction was carried out via flow chemistry. A mixture of *tert*-butyl (*R*)-methyl(1-(3-nitropyridin-4-yl)pyrrolidin-3-yl)carbamate (2.00 g, 6.20 mmol, 1.00 eq) in MeOH (20 mL) was stirred at 20 °C until it became a clear solution. The following conditions were used for the flow chemistry: H₂ back pressure - 1.5 MPa, the flow rate of H₂ - 30 ml/min, fixed bed (1% Pt/C, 3 g) temperature 55 °C. The solution was pumped into the reactor at a flow rate of 0.4 mL/min. After 0.83 h the reaction was complete. The reaction mixture was concentrated under reduced pressure to give the title compound (1.80 g, 99.2%) as a brown oil which was used directly without purification for the next step. MS (ES⁺) *m/e* 293.1 (M+H)⁺.

[0611] Step 2. *tert*-Butyl (*R,E*)-methyl(1-(3-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate



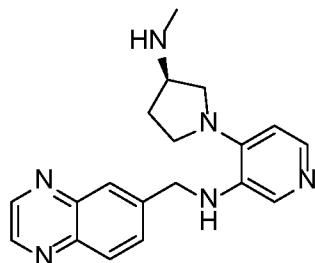
[0612] To a solution of *tert*-butyl (*R*)-(1-(3-aminopyridin-4-yl)pyrrolidin-3-yl)(methyl)carbamate (600 mg, 2.05 mmol, 1.00 *eq*) in toluene (10.0 mL) was added AcOH (308 mg, 5.13 mmol, 294 μ L, 2.50 *eq*) and quinoxaline-6-carbaldehyde (325 mg, 2.05 mmol, 1.00 *eq*). The mixture was stirred at 130 °C for 12 and was concentrated under reduced pressure to give the title compound (880 mg, 99.1%) as a brown oil which was used directly without purification for the next step reaction. MS (ES^+) m/e 433.3 ($M+H$) $^+$.

[0613] Step 3. *tert*-Butyl (*R*)-methyl(1-(3-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate



[0614] To a solution of *tert*-butyl (*R,E*)-methyl(1-(3-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate (850 mg, 1.97 mmol, 1.00 *eq*) in MeOH (10.0 mL) was added $NaBH_3CN$ (309 mg, 4.91 mmol, 2.50 *eq*) and AcOH (295 mg, 4.91 mmol, 281 μ L, 2.50 *eq*). The mixture was stirred at 25 °C for 12h, quenched by saturated $NaHCO_3$ (50.0 mL), adjusted to pH 7, concentrated under reduce to remove MeOH, and extracted with EtOAc 150mL (50.0 mL $\times$ 3). The combined organic layers were washed with brine (50.0 mL $\times$ 2), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. The residue was purified by reversed-phase HPLC (column: Phenomenex luna C18 150 $\times$ 40 mm $\times$ 15 μ m; mobile phase: [water (FA) - ACN]; gradient: 13%-43% B over 15 min) and lyophilized to give the title compound (330 mg, 36.7%) as a brown oil. MS (ES^+) m/e 435.1 ($M+H$) $^+$.

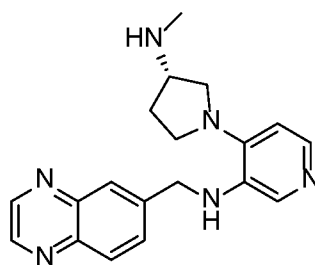
[0615] Step 4. (*R*)-4-(3-(Methylamino)pyrrolidin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine



[0616] A solution of *tert*-butyl (*R*)-methyl(1-(3-((quinoxalin-6-yl)methyl)amino)pyridin-4-yl)pyrrolidin-3-yl)carbamate (330 mg, 759 μ mol, 1.00 eq) in HCl/MeOH (3 mL) was stirred at 25 °C for 12 h. The reaction mixture was concentrated under reduced pressure to afford the title compound (280 mg, 99.4%) as a brown solid. ^1H NMR (400 MHz, DMSO- d_6) δ 14.15 (br s, 1H), 9.82-9.60 (m, 2H), 8.93 (s, 2H), 8.17-8.05 (m, 2H), 8.03-7.92 (m, 2H), 7.58 (br d, J = 4.4 Hz, 1H), 7.00 (d, J = 6.4 Hz, 1H), 6.87-6.65 (m, 1H), 4.32-4.19 (m, 1H), 3.98-3.86 (m, 3H), 3.42 (td, J = 6.8, 10.2 Hz, 1H), 2.65-2.57 (m, 3H), 2.33 (br d, J = 4.0 Hz, 2H). MS (ES^+) m/e 335.1 ($\text{M}+\text{H}$) $^+$.

[0617] Example 53

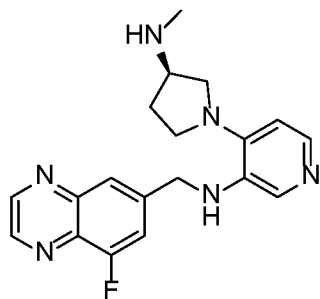
[0618] (*S*)-4-(3-(methylamino)pyrrolidin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 052)



[0619] The title compound was synthesized following a similar procedure described for the preparation of Example 52 (Comp. 051) with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 14.21-13.48 (m, 1H), 9.23-9.01 (m, 2H), 8.93 (s, 2H), 8.13-8.07 (m, 2H), 8.00 (d, J = 6.4 Hz, 1H), 7.93 (dd, J = 1.6, 8.8 Hz, 1H), 7.62 (s, 1H), 6.95 (d, J = 6.8 Hz, 1H), 6.20 (br s, 1H), 4.63 (br s, 2H), 4.14-4.04 (m, 1H), 4.01-3.85 (m, 3H), 3.72-3.62 (m, 1H), 2.69 (br s, 3H), 2.43-2.31 (m, 1H), 2.30-2.18 (m, 1H). MS (ES^+) m/e 335.1 ($\text{M}+\text{H}$) $^+$.

[0620] Example 54

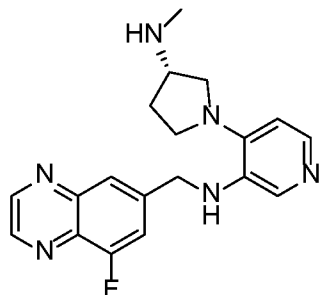
[0621] (*R*)-*N*-((8-fluoroquinoxalin-6-yl)methyl)-4-(3-(methylamino)pyrrolidin-1-yl)pyridin-3-amine (Comp. 053)



[0622] The title compound was synthesized following a similar procedure described for the preparation of Example 52 (Comp. 051) with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 13.98 (br s, 1H), 9.57 (br s, 2H), 9.00 (dd, $J = 1.6, 16.0$ Hz, 2H), 8.02-7.94 (m, 2H), 7.87 (dd, $J = 1.2, 11.2$ Hz, 1H), 7.60 (s, 1H), 7.00 (d, $J = 6.4$ Hz, 1H), 6.75 (br s, 1H), 4.64 (br d, $J = 2.4$ Hz, 2H), 4.29-4.19 (m, 1H), 3.97-3.88 (m, 3H), 3.44 (td, $J = 6.8, 10.4$ Hz, 1H), 2.63 (t, $J = 5.2$ Hz, 3H), 2.33 (br d, $J = 4.4$ Hz, 2H). MS (ES^+) m/e 353.0 ($\text{M}+\text{H}$) $^+$.

[0623] Example 55

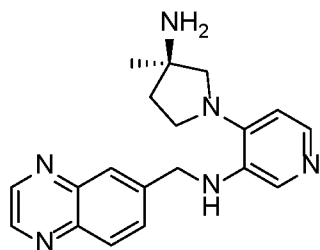
[0624] (*S*)-*N*-((8-fluoroquinoxalin-6-yl)methyl)-4-(3-(methylamino)pyrrolidin-1-yl)pyridin-3-amine (Comp. 054)



[0625] The title compound was synthesized following a similar procedure described for the preparation of Example 52 (Comp. 051) with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 14.28-13.02 (m, 1H), 9.11 (br d, $J = 2.0$ Hz, 2H), 9.00 (dd, $J = 1.6, 15.2$ Hz, 2H), 8.04-7.98 (m, 1H), 7.97 (s, 1H), 7.79 (dd, $J = 1.2, 11.0$ Hz, 1H), 7.61 (s, 1H), 6.95 (d, $J = 6.4$ Hz, 1H), 6.21 (br s, 1H), 4.61 (br d, $J = 3.6$ Hz, 2H), 4.18-4.02 (m, 1H), 4.01-3.87 (m, 3H), 3.68 (ddd, $J = 5.6, 8.0, 10.4$ Hz, 1H), 2.69 (br s, 3H), 2.42-2.31 (m, 1H), 2.25 (td, $J = 6.0, 12.0$ Hz, 1H). MS (ES^+) m/e 353.0 ($\text{M}+\text{H}$) $^+$.

[0626] Example 56

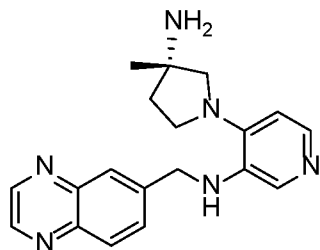
[0627] (*R*)-4-(3-amino-3-methylpyrrolidin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 055)



[0628] The title compound was synthesized following a similar procedure described for the preparation of Example 52 (Comp. 051) with appropriate starting materials and intermediates. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 13.89 (br s, 1H), 8.99-8.85 (m, 2H), 8.68 (br s, 3H), 8.14-8.07 (m, 2H), 8.01-7.93 (m, 2H), 7.57 (br d, $J = 3.2$ Hz, 1H), 6.97 (d, $J = 6.8$ Hz, 1H), 6.71 (br s, 1H), 4.72-4.54 (m, 2H), 4.43-4.29 (m, 1H), 3.85-3.79 (m, 1H), 3.76-3.71 (m, 1H), 3.38-3.29 (m, 1H), 2.35-2.26 (m, 1H), 2.20-2.10 (m, 1H), 1.53 (s, 3H). MS (ES^+) m/e 335.1 ($\text{M}+\text{H}$) $^+$.

[0629] Example 57

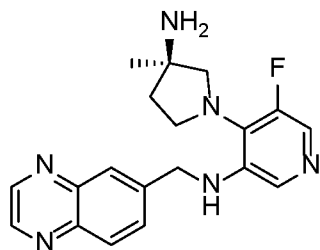
[0630] (*S*)-4-(3-amino-3-methylpyrrolidin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 056)



[0631] The title compound was synthesized following a similar procedure described for the preparation of Example 52 (Comp. 051) with appropriate starting materials and intermediates. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 13.96 (br s, 1H), 8.93 (s, 2H), 8.71 (br s, 3H), 8.15-8.08 (m, 2H), 8.02-7.94 (m, 2H), 7.57 (br d, $J = 4.0$ Hz, 1H), 6.97 (d, $J = 6.4$ Hz, 1H), 6.75 (br d, $J = 2.4$ Hz, 1H), 4.74-4.58 (m, 2H), 4.46-4.34 (m, 1H), 3.85-3.80 (m, 1H), 3.74 (s, 1H), 3.50 (dt, $J = 3.2, 9.6$ Hz, 1H), 2.36 - 2.26 (m, 1H), 2.14 (td, $J = 8.8, 13.2$ Hz, 1H), 1.54 (s, 3H). MS (ES^+) m/e 374.2 ($\text{M}+\text{H}$) $^+$.

[0632] Example 58

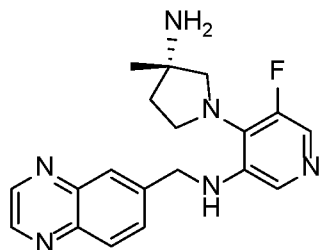
[0633] (*R*)-4-(3-amino-3-methylpyrrolidin-1-yl)-5-fluoro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 057)



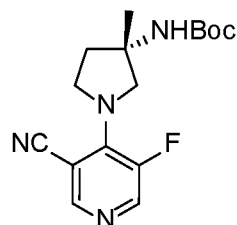
[0634] The title compound was synthesized following a similar procedure described for the preparation of Example 52 (Comp. 051) with appropriate starting materials and intermediates. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.93 (s, 2H), 8.24 - 8.09 (m, 3H), 8.02 (dd, $J = 2.0, 8.8$ Hz, 1H), 7.68 (d, $J = 0.8$ Hz, 1H), 4.83 (d, $J = 12.0$ Hz, 2H), 4.38 - 4.32 (m, 1H), 3.94 (d, $J = 4.0$ Hz, 2H), 3.60 - 3.53 (m, 1H), 2.48 - 2.37 (m, 1H), 2.37 - 2.24 (m, 1H), 1.64 (s, 3H). MS (ES^+) m/e 353.0 ($\text{M}+\text{H}$) $^+$.

[0635] Example 59

[0636] (*S*)-4-(3-amino-3-methylpyrrolidin-1-yl)-5-fluoro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 058)

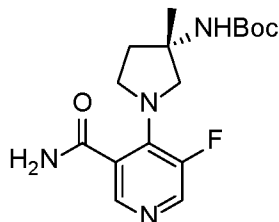


[0637] Step 1. *tert*-Butyl (*S*)-(1-(3-cyano-5-fluoropyridin-4-yl)-3-methylpyrrolidin-3-yl)carbamate



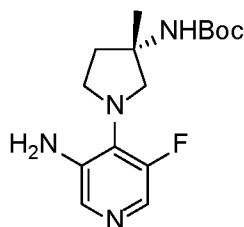
[0638] To a solution of 4-chloro-5-fluoronicotinonitrile (750 mg, 4.79 mmol, 1.00 *eq*) and *tert*-butyl (*S*)-(3-methylpyrrolidin-3-yl)carbamate (959 mg, 4.79 mmol, 1.00 *eq*) in MeCN (7.5 mL) was added DIEA (1.86 g, 14.3 mmol, 2.50 mL, 3.00 *eq*) and the mixture was stirred at 80 °C for 12 h. The reaction mixture was purified by prep. HPLC (column: Waters Xbridge BEH C18 250*50mm*10um; mobile phase: [water(NH_4HCO_3)-ACN]; gradient: 20%-50% B over 20 min) to give the title compound (900 mg, 58.6%) as a yellow solid. (ES^+) m/e 321.3 ($\text{M}+\text{H}$) $^+$.

[0639] Step 2. *tert*-Butyl (*S*)-(1-(3-carbamoyl-5-fluoropyridin-4-yl)-3-methylpyrrolidin-3-yl)carbamate



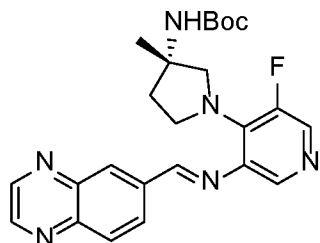
[0640] To a solution of *tert*-butyl (*S*)-(1-(3-cyano-5-fluoropyridin-4-yl)-3-methylpyrrolidin-3-yl)carbamate (800 mg, 2.50 mmol, 1.00 *eq*) in MeOH (9 mL) was added a solution of Na₂CO₃ (540 mg, 5.09 mmol, 2.04 *eq*) in H₂O (9 mL) at 25 °C. H₂O₂ (3.00 g, 26.46 mmol, 2.54 mL, 30% purity, 10.6 *eq*) was added at 0 °C and the mixture was stirred at 25 °C for 60 h. The reaction mixture was quenched by addition of aqueous Na₂SO₃ (50 mL) at 0 °C and was stirred for 30 min, diluted with H₂O (50 mL) and extracted with EtOAc (50 mL × 2). The organic layer was collected and concentrated to provide a residue which was purified by column chromatography (SiO₂, Petroleum ether/ Ethyl acetate = 1/ 1 to 1/ 1, TLC: PE /EA = 1/1, R_f = 0.79 and 0.1) to give the title compound (500 mg, 59.1%) as a white solid. MS (ES⁺) *m/e* 321.2 (M+H)⁺.

[0641] Step 3. *tert*-Butyl (*S*)-(1-(3-amino-5-fluoropyridin-4-yl)-3-methylpyrrolidin-3-yl)carbamate



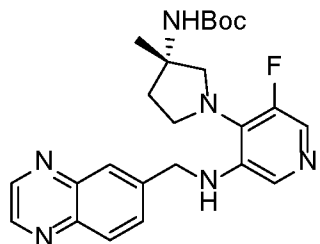
[0642] To a solution of *tert*-butyl (*S*)-(1-(3-carbamoyl-5-fluoropyridin-4-yl)-3-methylpyrrolidin-3-yl)carbamate (450 mg, 1.33 mmol, 1.00 *eq*) in ACN (2 mL) and H₂O (2 mL) was added NaOH (159 mg, 3.99 mmol, 3.00 *eq*) and NaClO (1.98 g, 2.66 mmol, 1.64 mL, 10% purity, 2.00 *eq*). The mixture was stirred at 60 °C for 2 h, poured into ice-water (w/w = 1/1) (50 mL) and stirred for additional 20 min. The reaction mixture was extracted with ethyl acetate (50 mL × 2). The combined organic phases were washed with brine (50 mL × 2), dried with anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give the title compound (430 mg, crude) as a yellow oil which was used for the next step reaction without purification. MS (ES⁺) *m/e* 311.2 (M+H)⁺.

[0643] Step 4. *tert*-Butyl (S,E)-(1-(3-fluoro-5-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)-3-methylpyrrolidin-3-yl)carbamate



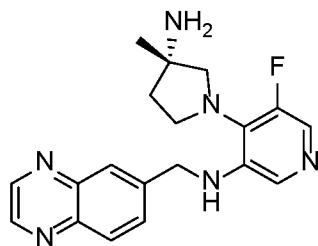
[0644] To a solution of *tert*-butyl (S)-1-(3-amino-5-fluoropyridin-4-yl)-3-methylpyrrolidin-3-yl)carbamate (400 mg, 1.29 mmol, 1.00 *eq*) and quinoxaline-6-carbaldehyde (203 mg, 1.29 mmol, 1.00 *eq*) in toluene (5 mL) was added AcOH (154 mg, 2.58 mmol, 147 μ L, 2.00 *eq*) and the mixture was stirred at 130 °C for 12 under N₂ atmosphere. The reaction mixture was then concentrated under reduced pressure to afford the title compound (580 mg, crude) as a brown oil which was used for the next step reaction without purification.

[0645] Step 5. *tert*-Butyl (S)-1-(3-fluoro-5-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)-3-methylpyrrolidin-3-yl)carbamate



[0646] To a solution of *tert*-butyl (S,E)-1-(3-fluoro-5-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)-3-methylpyrrolidin-3-yl)carbamate (580 mg, 1.29 mmol, 1.00 *eq*) in MeOH (6 mL) was added NaBH₃CN (161 mg, 2.57 mmol, 2.00 *eq*) and AcOH (38.6 mg, 643.7 μ mol, 36.8 μ L, 0.50 *eq*). The mixture was stirred at 25 °C for 12 h, concentrated under reduced pressure to remove MeOH, diluted with H₂O (10 mL), and extracted with ethyl acetate (10 mL $\times$ 2). The combined organic layers were washed with brine (20 mL), dried over Na₂SO₄, and concentrated under reduced pressure to give a residue which was purified by prep-HPLC (column: Phenomenex luna C18 150 * 40mm x 15 μ m; mobile phase: [water(FA)-ACN]; gradient: 12%-42% B over 15 min) to give the title compound (140 mg, 24.0%) as a yellow solid. MS (ES⁺) *m/e* 453.4 (M+H)⁺.

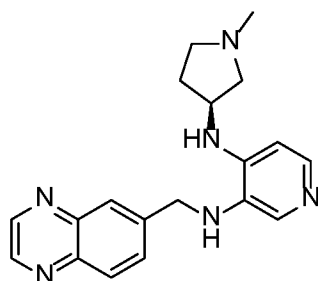
[0647] Step 6. (S)-4-(3-Amino-3-methylpyrrolidin-1-yl)-5-fluoro-N-(quinoxalin-6-ylmethyl)pyridin-3-amine



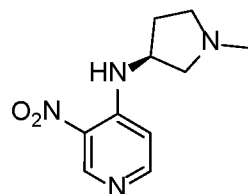
[0648] To a solution of *tert*-butyl (*S*)-(1-(3-fluoro-5-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)-3-methylpyrrolidin-3-yl)carbamate (140 mg, 309 μ mol, 1.00 eq) in MeOH (1.00 mL) was added HCl/MeOH (2 M, 1.65 mL, 10.6 eq). The reaction was stirred at 25 °C for 12 h and was concentrated under reduced pressure to provide a residue which was purified by prep-HPLC (column: Phenomenex luna C18 150 x 25mm x 10um; mobile phase: [water (HCl)-ACN]; gradient: 1%-20% B over 10 min) to give the title compound (80.0 mg, 73.2%) as a yellow solid. ^1H NMR (400 MHz, DMSO- d_6) δ 8.92 (s, 2H), 8.18 - 8.11 (m, 3H), 8.00 (dd, J = 2.0, 8.8 Hz, 1H), 7.68 (s, 1H), 4.82 (br d, J = 12.0 Hz, 2H), 4.37 - 4.30 (m, 1H), 3.99 - 3.86 (m, 2H), 3.60 - 3.54 (m, 1H), 2.48 - 2.36 (m, 1H), 2.36 - 2.23 (m, 1H), 1.64 (s, 3H). MS (ES^+) m/e 351.3 ($\text{M}+\text{H}$) $^+$.

[0649] Example 60

[0650] (*S*)- N^4 -(1-methylpyrrolidin-3-yl)- N^3 -(quinoxalin-6-ylmethyl)pyridine-3,4-diamine (Comp. 059)

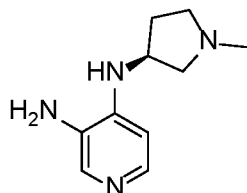


[0651] Step 1. (*S*)-*N*-(1-Methylpyrrolidin-3-yl)-3-nitropyridin-4-amine



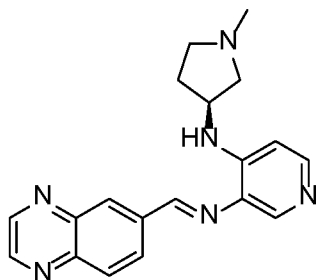
[0652] To a solution of 4-chloro-3-nitropyridine (0.80 g, 5.05 mmol, 1.00 eq) and (*S*)-1-methylpyrrolidin-3-amine (505 mg, 5.05 mmol, 1.00 eq) in ACN (10.0 mL) was added K_2CO_3 (1.05 g, 7.57 mmol, 1.50 eq). The mixture was stirred at 60 °C for 2 h, diluted with DCM (200mL) and filtered. The mother liquid was concentrated under reduced pressure to give the title compound (650 mg, 58.0%) as a yellow oil. MS (ES^+) m/e 223.3 ($\text{M}+\text{H}$) $^+$.

[0653] Step 2. (*S*)-N4-(1-Methylpyrrolidin-3-yl)pyridine-3,4-diamine



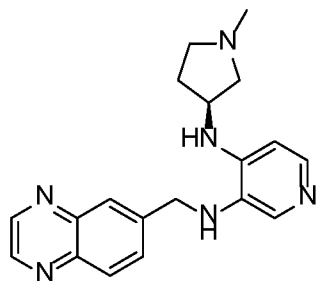
[0654] To a solution of (*S*)-N-(1-methylpyrrolidin-3-yl)-3-nitropyridin-4-amine (0.60 g, 2.70 mmol, 1.00 eq) in THF (10.0 mL) was added 10% Pd/C (287 mg, 270 μ mol, 0.10 eq) under N₂. The suspension was degassed under vacuum and purged with H₂ for several times. The mixture was stirred under H₂ (50 psi) at 40 °C for 12 h and was filtered. The filtrate was concentrated under reduced pressure to give the title compound (0.6 g, crude) as a yellow oil which was used directly for the next step reaction without purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.61 (s, 1H), 7.56 (d, *J* = 5.2 Hz, 1H), 6.29 (d, *J* = 5.2 Hz, 1H), 5.28 (br d, *J* = 6.8 Hz, 1H), 4.64 (s, 2H), 3.97 - 3.87 (m, 1H), 2.70 (dd, *J* = 6.8, 9.2 Hz, 1H), 2.60 (dt, *J* = 5.2, 8.0 Hz, 1H), 2.43 - 2.32 (m, 2H), 2.29 - 2.17 (m, 4H), 1.67 - 1.56 (m, 1H).

[0655] Step 3. (*S,E*)-N-(1-Methylpyrrolidin-3-yl)-3-((quinoxalin-6-ylmethylene)amino)pyridin-4-amine



[0656] A solution of (*S*)-N4-(1-methylpyrrolidin-3-yl)pyridine-3,4-diamine (0.30 g, 1.56 mmol, 1.00 eq), quinoxaline-6-carbaldehyde (292 mg, 1.87 mmol, 1.20 eq), and AcOH (234 mg, 3.90 mmol, 223 μ L, 2.50 eq) in toluene (10.0 mL) was stirred at 120 °C for 12 h with a Dean-Stark water trap. The mixture was then concentrated under reduced pressure to give the title compound (0.52 g, crude) as a yellow oil which was used directly for the next step reaction without purification. MS (ES⁺) *m/e* 333.3 (M+H)⁺.

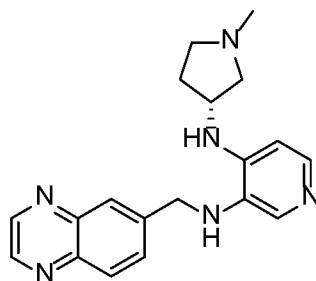
[0657] Step 4. (*S*)-N4-(1-Methylpyrrolidin-3-yl)-N3-(quinoxalin-6-ylmethyl)pyridine-3,4-diamine



[0658] To a solution of (S,E)-N-(1-methylpyrrolidin-3-yl)-3-((quinoxalin-6-ylmethylene)amino)pyridin-4-amine (0.52 g, 1.56 mmol, 1.00 eq) and AcOH (141 mg, 2.35 mmol, 134 μ L, 1.50 eq) in MeOH (10.0 mL) was added NaBH₃CN (197 mg, 3.13 mmol, 2.00 eq) in portions at 25 °C. The mixture was stirred at 25 °C for 3 h. Then the mixture was quenched with saturated Na₂CO₃ (100 mL), filtered and concentrated under vacuum to give a crude product which was purified by reversed-phase HPLC (0.1% FA condition) and lyophilized to the title compound (300 mg, 57.3%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.94 - 8.89 (m, 2H), 8.09 (d, *J* = 8.8 Hz, 1H), 8.04 (s, 1H), 7.88 (dd, *J* = 2.0, 8.8 Hz, 1H), 7.60 (d, *J* = 5.2 Hz, 1H), 7.48 (s, 1H), 6.35 (d, *J* = 5.2 Hz, 1H), 5.69 (t, *J* = 5.6 Hz, 1H), 5.57 (d, *J* = 6.4 Hz, 1H), 4.61 (d, *J* = 5.6 Hz, 2H), 4.05 - 3.87 (m, 1H), 2.75 - 2.61 (m, 2H), 2.46 (d, *J* = 4.4 Hz, 1H), 2.40 - 2.23 (m, 5H), 1.72 - 1.61 (m, 1H). MS (ES⁺) *m/e* 335.2 (M+H)⁺.

[0659] Example 61

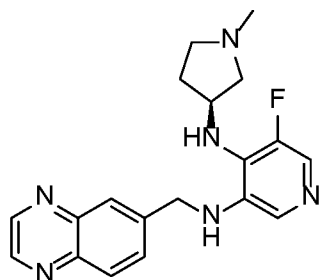
[0660] (*R*)-N⁴-(1-methylpyrrolidin-3-yl)-N³-(quinoxalin-6-ylmethyl)pyridine-3,4-diamine (Comp. 060)



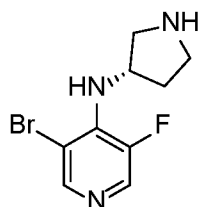
[0661] The title compound was synthesized following a similar procedure described for the preparation of Example 60 (Comp. 059) with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.47 (br s, 1H), 10.32 (br s, 1H), 8.94 (s, 2H), 8.13 (d, *J* = 8.8 Hz, 1H), 8.08 (s, 1H), 7.96 (br d, *J* = 6.4 Hz, 1H), 7.88 (dd, *J* = 1.6, 8.8 Hz, 1H), 7.67 - 7.29 (m, 2H), 6.89 (d, *J* = 6.8 Hz, 1H), 6.70 - 6.43 (m, 1H), 4.73 (br d, *J* = 4.4 Hz, 2H), 4.66 - 4.41 (m, 1H), 4.18 - 3.72 (m, 2H), 3.28 - 3.07 (m, 2H), 2.92 (br s, 3H), 2.48 - 2.36 (m, 1H), 2.24 - 1.98 (m, 1H). MS (ES⁺) *m/e* 335.2 (M+H)⁺.

[0662] Example 62

[0663] (*S*)-5-fluoro-*N*⁴-(1-methylpyrrolidin-3-yl)-*N*³-(quinoxalin-6-ylmethyl)pyridine-3,4-diamine (Comp. 061)

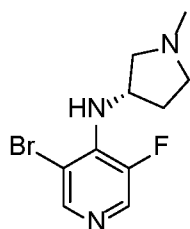


[0664] Step 1. (*S*)-3-Bromo-5-fluoro-*N*-(pyrrolidin-3-yl)pyridin-4-amine



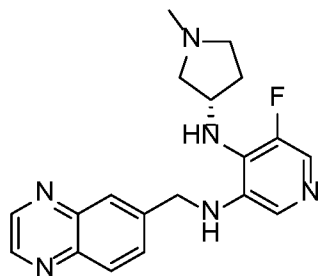
[0665] To a solution of *tert*-butyl (*S*)-3-((3-bromo-5-fluoropyridin-4-yl)amino)pyrrolidine-1-carboxylate (4.00 g, 11.1 mmol, 1.00 *eq*) in MeOH (5 mL) was added HCl/MeOH (2 M, 50 mL, 9.01 *eq*). The reaction mixture was stirred at 25 °C for 12 h and was then concentrated under reduced pressure to give the title compound (3.29 g, crude) as a yellow solid. MS (ES⁺) *m/e* 260 (M+H)⁺.

[0666] Step 2. (*S*)-3-Bromo-5-fluoro-*N*-(1-methylpyrrolidin-3-yl)pyridin-4-amine



[0667] To a solution of (*S*)-3-bromo-5-fluoro-*N*-(pyrrolidin-3-yl)pyridin-4-amine (3.29 g, 12.6 mmol, 1.00 *eq*), HCHO (3.08 g, 37.9 mmol, 2.83 mL, 3.00 *eq*) and AcOH (7.60 g, 126 mmol, 7.24 mL, 10.0 *eq*) in MeOH (50 mL) was added a solution of 2-methylpyridine borane complex (1.49 g, 13.9 mmol, 1.10 *eq*) in MeOH (50 mL). The reaction was stirred at 25 °C for 20 min and concentrated under reduced pressure to remove the solvent. The crude product was purified by reversed-phase HPLC (0.1% FA condition) to give the title compound (2.50 g, 72.1%) as a colorless oil. MS (ES⁺) *m/e* 274 (M+H)⁺.

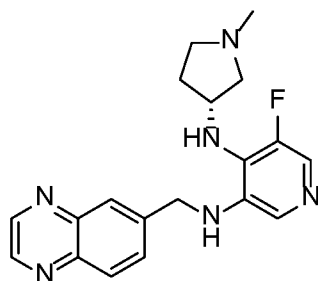
[0668] Step 3. (*S*)-5-Fluoro-*N*⁴-(1-methylpyrrolidin-3-yl)-*N*³-(quinoxalin-6-ylmethyl)pyridine-3,4-diamine



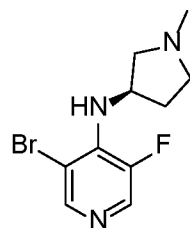
[0669] To a solution of (*S*)-3-bromo-5-fluoro-*N*-(1-methylpyrrolidin-3-yl)pyridin-4-amine (200 mg, 729 μ mol, 1.00 *eq*) and quinoxalin-6-ylmethanamine (232 mg, 1.46 mmol, 2.00 *eq*) in dioxane (5 mL) was added Cs_2CO_3 (594 mg, 1.82 mmol, 2.50 *eq*) and BrettPhos Pd G3 (66.1 mg, 72.9 μ mol, 0.10 *eq*). The mixture was stirred at 100 °C for 1 h under N_2 and was partitioned between H_2O (20 mL) and EtOAc (20 mL). The organic phase was separated, washed with brine (30 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Phenomenex luna C18 150 x 25mm x 10 μ m; mobile phase: [water(TFA)-ACN]; gradient: 5%-25% B over 18 min) to give the title compound (31.2 mg, 9.14%) as a yellow solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.88 (s, 2H), 8.13 - 8.06 (m, 3H), 7.92 (dd, J = 2.0, 8.8 Hz, 1H), 7.51 (d, J = 0.8 Hz, 1H), 5.06 (br s, 1H), 4.76 (s, 2H), 3.79 (br s, 4H), 3.02 (s, 3H), 2.70 (br s, 1H), 2.41 - 2.37 (m, 1H). MS (ES^+) m/e 353.2 ($\text{M}+\text{H}$) $^+$.

[0670] Example 63

[0671] (*R*)-5-fluoro- N^4 -(1-methylpyrrolidin-3-yl)- N^3 -(quinoxalin-6-ylmethyl)pyridine-3,4-diamine (Comp. 062)



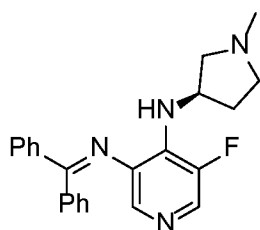
[0672] Step 1. (*R*)-3-Bromo-5-fluoro-*N*-(1-methylpyrrolidin-3-yl)pyridin-4-amine



[0673] To a solution of 3-bromo-4-chloro-5-fluoropyridine (4.20 g, 19.9 mmol, 1.00 *eq*) and (*R*)-1-methylpyrrolidin-3-amine (2.00 g, 19.9 mmol, 1.00 *eq*) in DMSO (50 mL) was

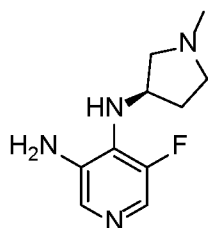
added DIEA (5.16 g, 39.9 mmol, 6.95 mL, 2.00 *eq*) and CsF (6.06 g, 39.9 mmol, 2.00 *eq*). The mixture was stirred at 140 °C for 12 h, poured into ice-water (w/w = 1/1) (500 mL), stirred for 20 min and extracted with ethyl acetate (500 mL x 2). The combined organic layers were washed with brine (500 mL x 2), dried with anhydrous Na₂SO₄, filtered and concentrated to provide a residue. The residue was purified by column chromatography (SiO₂, petroleum ether/ ethyl acetate = 10/ 1 to 1/ 1, TLC: Petroleum ether/ Ethyl acetate = 1/ 1, product R_f = 0.43) to afford the title compound (1.30 g, 23.7%) as a yellow oil. MS (ES⁺) m/e 274.1 (M+H)⁺.

[0674] Step 2. (*R*)-3-((Diphenylmethylene)amino)-5-fluoro-*N*-(1-methylpyrrolidin-3-yl)pyridin-4-amine



[0675] To a solution of (*R*)-3-bromo-5-fluoro-*N*-(1-methylpyrrolidin-3-yl)pyridin-4-amine (1.25 g, 4.56 mmol, 1.00 *eq*) and diphenylmethanimine (991 mg, 5.47 mmol, 918 μ L, 1.20 *eq*) in toluene (10 mL) was added Pd₂(dba)₃ (208 mg, 227 μ mol, 0.05 *eq*), BINAP (283 mg, 455 μ mol, 0.1 *eq*) and t-BuONa (1.10 g, 11.4 mmol, 2.50 *eq*). The mixture was stirred at 110 °C for 12 h, poured into water (100 mL) and extracted with ethyl acetate (100 mL x 2). The combined organic layers were washed with brine (100 mL), dried with anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to provide a residue which was purified by reversed-phase HPLC (0.1% FA condition) to give the title compound (0.24 g, 14.0%) as a yellow oil. MS (ES⁺) m/e 375.1 (M+H)⁺.

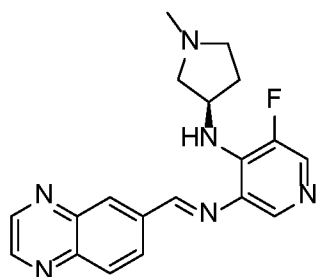
[0676] Step 3. (*R*)-5-Fluoro-N4-(1-methylpyrrolidin-3-yl)pyridine-3,4-diamine



[0677] To a solution of (*R*)-3-((diphenylmethylene)amino)-5-fluoro-*N*-(1-methylpyrrolidin-3-yl)pyridin-4-amine (195 mg, 521 μ mol, 1.00 *eq*) in MeOH (5 mL) was added NH₂OH•HCl (54.3 mg, 781 μ mol, 1.50 *eq*) and AcONa (128 mg, 1.56 mmol, 3.00 *eq*). The reaction was stirred at 25 °C for 2 h and was then concentrated under reduced pressure to

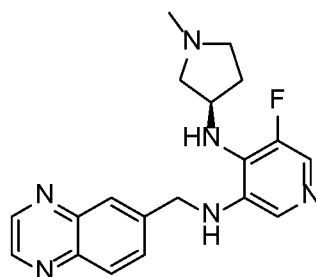
remove the solvent. The residue was purified by prep-HPLC (column: Waters Atlantis T3 150 x 30mm x 5um; mobile phase: [water(FA)-ACN]; gradient: 1%-10% B over 9 min) to give the title compound (109 mg, 441 μ mol, 84.8% yield, HCl) as a yellow oil. MS (ES^+) m/e 211.1 ($M+H$)⁺.

[0678] Step 4. (R,E)-3-Fluoro-N-(1-methylpyrrolidin-3-yl)-5-((quinoxalin-6-ylmethylene)amino)pyridin-4-amine



[0679] To a solution of (R)-5-fluoro-N4-(1-methylpyrrolidin-3-yl)pyridine-3,4-diamine (100 mg, 475 μ mol, 1.00 eq) and quinoxaline-6-carbaldehyde (75.2 mg, 475.6 μ mol, 1.00 eq) in toluene (5 mL) was added AcOH (57.1 mg, 951.2 μ mol, 54.4 μ L, 2.00 eq) and the mixture was stirred at 110 °C for 12 h and was concentrated under reduced pressure to remove solvent to afford the title compound (166 mg, 99.6%) as a yellow oil. MS (ES^+) m/e 351.2 ($M+H$)⁺.

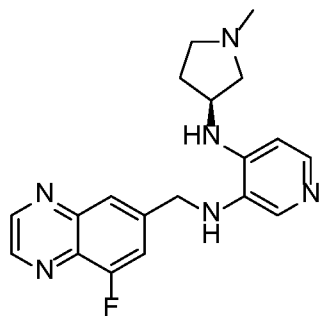
[0680] Step 5. (R)-5-Fluoro-N4-(1-methylpyrrolidin-3-yl)-N3-(quinoxalin-6-ylmethyl)pyridine-3,4-diamine



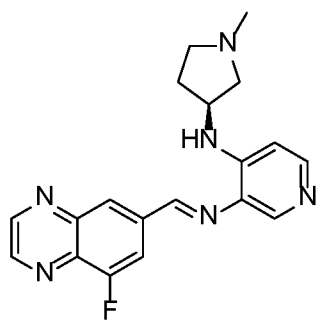
[0681] To a solution of (R,E)-3-fluoro-N-(1-methylpyrrolidin-3-yl)-5-((quinoxalin-6-ylmethylene)amino)pyridin-4-amine (166 mg, 473 μ mol, 1.00 eq) and AcOH (14.2 mg, 236 μ mol, 13.5 μ L, 0.50 eq) in MeOH (5 mL) was added NaBH₃CN (59.5 mg, 947 μ mol, 2.00 eq). The reaction was stirred at 25 °C for 5 h and was concentrated under reduced pressure to remove the solvent. The residue was purified by prep-HPLC (column: Phenomenex luna C18 150 x 25mm x 10um; mobile phase: [water(TFA)-ACN]; gradient: 0%-25% B over 18 min) to give the title compound (25.5 mg, 11.5%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.89 (s, 2H), 8.16 - 8.06 (m, 3H), 7.93 (dd, *J* = 2.0, 8.8 Hz, 1H), 7.53 (s, 1H), 5.05 (br s, 1H), 4.77 (s, 2H), 3.92 (br s, 2H), 3.60 - 3.43 (m, 2H), 3.02 (s, 3H), 2.76 (br s, 1H), 2.38 (br s, 1H). MS (ES^+) m/e 353.1 ($M+H$)⁺.

[0682] Example 64

[0683] (*S*)-N³-(((8-fluoroquinoxalin-6-yl)methyl)-N⁴-(1-methylpyrrolidin-3-yl)pyridine-3,4-diamine (Comp. 063)

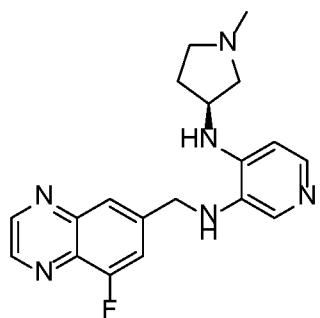


[0684] Step 1. (*S,E*)-3-(((8-Fluoroquinoxalin-6-yl)methylene)amino)-*N*-(1-methylpyrrolidin-3-yl)pyridin-4-amine



[0685] A solution of (*S*)-N⁴-(1-methylpyrrolidin-3-yl)pyridine-3,4-diamine (560 mg, 2.91 mmol, 1.00 eq), 8-fluoroquinoxaline-6-carbaldehyde (616 mg, 3.50 mmol, 1.20 eq), and AcOH (262 mg, 4.37 mmol, 250 μ L, 1.50 eq) in toluene (10.0 mL) was stirred at 120 °C for 2 h with a Dean-Stark water trap. The mixture was concentrated under reduced pressure to give the title compound (1.10 g, crude) as a yellow oil which was used directly for the next step reaction without purification. MS (ES^+) m/e 351.2 ($\text{M}+\text{H}^+$).

[0686] Step 2. (*S*)-N³-(((8-Fluoroquinoxalin-6-yl)methyl)-N⁴-(1-methylpyrrolidin-3-yl)pyridine-3,4-diamine

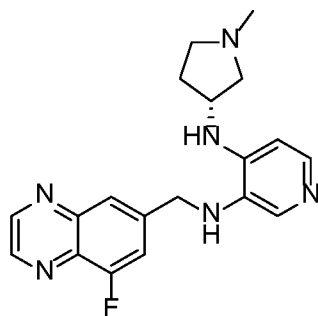


[0687] To a mixture of (*S,E*)-3-(((8-fluoroquinoxalin-6-yl)methylene)amino)-*N*-(1-methylpyrrolidin-3-yl)pyridin-4-amine (1.05 g, 3.00 mmol, 1.00 eq) and NaBH_3CN (377 mg,

5.99 mmol, 2.00 eq) in MeOH (15.0 mL) was added AcOH (270 mg, 4.49 mmol, 257 μ L, 1.50 eq) in portions at 25 °C. The mixture was stirred at 25 °C for 18 h, quenched with saturated Na₂CO₃ (100 mL), filtered and concentrated under reduced pressure to give a residue which was purified by reversed-phase HPLC (0.1% FA condition) and lyophilized to give a yellow solid which was further purified by prep-HPLC (column: Phenomenex luna C18 150 $\times$ 40 mm $\times$ 15 μ m; mobile phase: [water (TFA) - ACN]; gradient: 0% - 22% B over 15 min) to give the title compound (174.93 mg, 12.34% yield) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.50 (br s, 1H), 10.40 (br s, 1H), 9.02 (d, *J* = 1.6 Hz, 1H), 8.99 (d, *J* = 1.6 Hz, 1H), 7.97 (br d, *J* = 6.4 Hz, 1H), 7.94 (s, 1H), 7.77 - 7.70 (m, 1H), 7.67 - 7.32 (m, 2H), 6.90 (d, *J* = 6.4 Hz, 1H), 6.62 (br s, 1H), 4.82 - 4.42 (m, 3H), 4.22 - 4.01 (m, 1H), 3.88 - 3.58 (m, 2H), 3.25 - 3.07 (m, 2H), 2.92 (br s, 3H), 2.78 - 2.53 (m, 1H), 2.29 - 2.04 (m, 1H). MS (ES⁺) *m/e* 353.0 (M+H)⁺.

[0688] Example 65

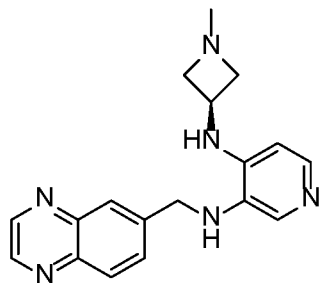
[0689] (*R*)-N³-((8-fluoroquinoxalin-6-yl)methyl)-N⁴-(1-methylpyrrolidin-3-yl)pyridine-3,4-diamine (Comp. 064)



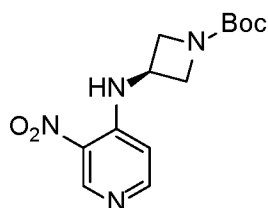
[0690] The title compound was synthesized following a similar procedure described for the preparation of Example 64 (Comp. 063) with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.87 - 13.21 (m, 1H), 10.72 - 10.15 (m, 1H), 9.02 (d, *J* = 1.2 Hz, 1H), 8.99 (d, *J* = 1.6 Hz, 1H), 7.97 (br d, *J* = 6.4 Hz, 1H), 7.94 (s, 1H), 7.74 (br d, *J* = 10.4 Hz, 1H), 7.67 - 7.38 (m, 1H), 6.90 (d, *J* = 6.8 Hz, 1H), 6.63 (br d, *J* = 2.8 Hz, 1H), 4.71 (br d, *J* = 3.6 Hz, 2H), 4.67 - 4.40 (m, 1H), 4.23 - 3.76 (m, 2H), 3.32 - 3.12 (m, 2H), 2.92 (br s, 3H), 2.79 - 2.60 (m, 1H), 2.30 - 2.08 (m, 1H). MS (ES⁺) *m/e* 353.1 (M+H)⁺.

[0691] Example 66

[0692] N⁴-(1-methylazetidin-3-yl)-N³-(quinoxalin-6-ylmethyl)pyridine-3,4-diamine (Comp. 065)

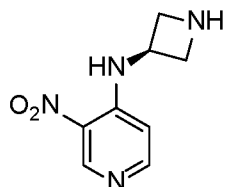


[0693] Step 1. *tert*-Butyl 3-((3-nitropyridin-4-yl)amino)azetidine-1-carboxylate



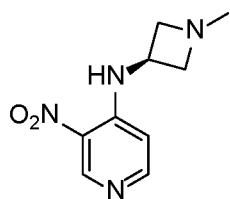
[0694] To a solution of 4-chloro-3-nitropyridine (920 mg, 5.81 mmol, 1.00 *eq*) and *tert*-butyl 3-aminoazetidine-1-carboxylate (1.00 g, 5.81 mmol, 1.00 *eq*) in ACN (20.0 mL) was added K₂CO₃ (1.61 g, 11.6 mmol, 2.00 *eq*). The mixture was stirred at 60 °C for 5 h, diluted with water (100 mL) and extracted with DCM (200 mL × 3). The combined organic layers were washed with brine (100 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give the title compound (1.5 g, crude) as a brown oil which was used directly to the next step reaction without further purification.

[0695] Step 2. N-(Azetidin-3-yl)-3-nitropyridin-4-amine



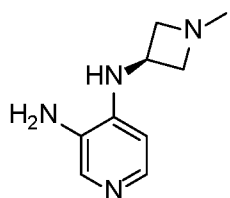
[0696] To a solution of *tert*-butyl 3-((3-nitropyridin-4-yl)amino)azetidine-1-carboxylate (1.10 g, 3.74 mmol, 1.00 *eq*) in DCM (10.0 mL) was added TFA (7.68 g, 67.3 mmol, 5.00 mL, 18.0 *eq*) at 20 °C. The mixture was stirred at 20 °C for 1 h and was concentrated under vacuum to give a yellow oil which was basified with by treatment with basic resin (10 g) and filtered. The filtrate was concentrated under reduced pressure to give the title compound (0.7 g, 96.4%) as a yellow oil which was used directly for the next step reaction. MS (ES⁺) *m/e* 195.2 (M+H)⁺.

[0697] Step 3. N-(1-Methylazetidin-3-yl)-3-nitropyridin-4-amine



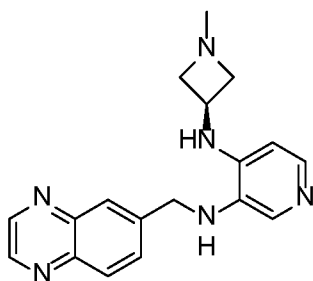
[0698] To a solution of N-(azetidin-3-yl)-3-nitropyridin-4-amine (1.00 g, 5.15 mmol, 1.00 *eq*) in MeOH (10.0 mL) was added NaBH₃CN (647 mg, 10.3 mmol, 2.00 *eq*) and HCHO (1.55 g, 51.5 mmol, 1.42 mL, 10.0 *eq*). The mixture was stirred at 25 °C for 2 h and filtered. The filtrate was concentrated under reduced pressure to give the title compound (400 mg, 37.3%) as a brown oil which was used directly for the next step reaction without purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.05-9.01 (m, 1H), 8.32-8.25 (m, 2H), 6.84 (d, *J* = 6.0 Hz, 1H), 4.29-4.22 (m, 1H), 3.70-3.64 (m, 2H), 3.02 (t, *J* = 6.8 Hz, 2H), 2.26 (s, 3H).

[0699] Step 4. N4-(1-methylazetidin-3-yl)pyridine-3,4-diamine



[0700] To a solution of N-(1-methylazetidin-3-yl)-3-nitropyridin-4-amine (0.40 g, 1.92 mmol, 1.00 *eq*) in MeOH (5.00 mL) and THF (5.00 mL) was added 28% NH₃.H₂O (481 mg, 3.84 mmol, 528 μL, 2.00 *eq*) and 10% Pd/C (204 mg, 192 μmol, 0.10 *eq*) under N₂. The suspension was degassed under vacuum and purged with H₂ for several times. The mixture was stirred under H₂ (50 psi) at 40°C for 12 h and filtered. The filtrate was concentrated under reduced pressure to give the title compound (0.35 g, crude) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.64 (s, 1H), 7.55 (d, *J* = 5.2 Hz, 1H), 6.18 (d, *J* = 5.2 Hz, 1H), 5.63 (br d, *J* = 6.4 Hz, 1H), 4.62 (s, 2H), 3.99 - 3.89 (m, 1H), 3.69 - 3.60 (m, 2H), 2.86 - 2.77 (m, 2H), 2.25 (s, 3H).

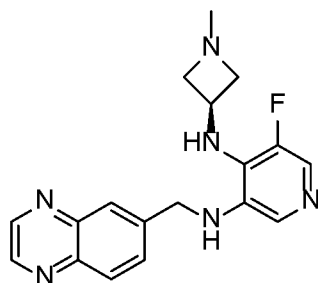
[0701] Step 5. N4-(1-Methylazetidin-3-yl)-N3-(quinoxalin-6-ylmethyl)pyridine-3,4-diamine



[0702] To a solution of quinoxaline-6-carbaldehyde (244 mg, 1.54 mmol, 1.10 eq), N⁴-(1-methylazetidin-3-yl)pyridine-3,4-diamine (0.25 g, 1.40 mmol, 1.00 eq) in THF (10 mL) was added Ti(i-PrO)₄ (797 mg, 2.81 mmol, 828 μ L, 2.00 eq). The mixture was stirred at 50 °C for 2 h and cooled to 20 °C. NaBH₄ (0.07 g, 1.85 mmol, 1.32 eq) was added in portions and the resulting mixture was stirred at 20 °C for 2 h. The reaction mixture was quenched by addition of saturated NH₄Cl (5.00 mL) at 20 °C, diluted with H₂O (20.0 mL) and extracted with DCM/MeOH (5:1, 25 mL $\times$ 4). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue which was purified by prep-HPLC (column: Waters Xbridge 150 $\times$ 25 mm $\times$ 5 μ m; mobile phase: [water (ammonia hydroxide v/v) - ACN]; gradient: 13% - 43% B over 10 min) and lyophilized to give the title compound (77.82 mg, 17.3%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.93 - 8.89 (m, 2H), 8.09 (d, *J* = 8.8 Hz, 1H), 8.05 (s, 1H), 7.89 (dd, *J* = 2.0, 8.8 Hz, 1H), 7.59 (d, *J* = 5.2 Hz, 1H), 7.51 (s, 1H), 6.24 (d, *J* = 5.2 Hz, 1H), 5.92 (d, *J* = 6.0 Hz, 1H), 5.60 (t, *J* = 5.6 Hz, 1H), 4.62 (d, *J* = 5.6 Hz, 2H), 4.06 - 3.91 (m, 1H), 3.68 (t, *J* = 7.2 Hz, 2H), 2.97 - 2.78 (m, 2H), 2.27 (s, 3H). MS (ES⁺) *m/e* 351.1 (M+H)⁺.

[0703] **Example 67**

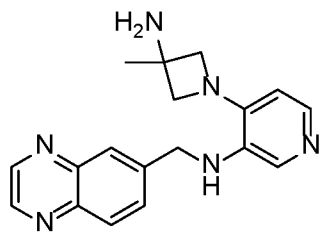
[0704] 5-fluoro-N⁴-(1-methylazetidin-3-yl)-N³-(quinoxalin-6-ylmethyl)pyridine-3,4-diamine (Comp. 066)



[0705] The title compound was synthesized following a similar procedure described for the preparation of Example 66 (Comp. 65) with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.41 (s, 1H), 8.93 (s, 2H), 8.22 - 8.04 (m, 2H), 7.97 - 7.83 (m, 1H), 7.34 (s, 1H), 6.87 (s, 1H), 5.00 - 4.71 (m, 3H), 4.63 - 4.13 (m, 4H), 2.93 (s, 3H). MS (ES⁺) *m/e* 339.1 (M+H)⁺.

[0706] **Example 68**

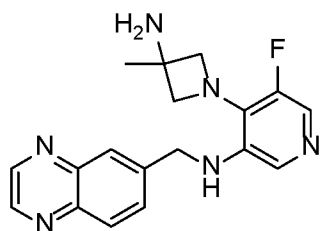
[0707] 4-(3-amino-3-methylazetidin-1-yl)-N-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 067)



[0708] The title compound was synthesized following a similar procedure described for the preparation of Example 49 (Comp. 048) with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 13.80 (br d, $J = 5.6$ Hz, 1H), 8.93 (s, 2H), 8.73 (br s, 2H), 8.13-8.06 (m, 2H), 7.97 (d, $J = 6.4$ Hz, 1H), 7.90 (dd, $J = 1.6, 8.8$ Hz, 1H), 7.57 (s, 1H), 6.70 (d, $J = 6.4$ Hz, 1H), 6.06 (br s, 1H), 4.61 (br d, $J = 3.6$ Hz, 2H), 4.54-4.45 (m, 4H), 1.66 (s, 3H). MS (ES^+) m/e 321.1 ($\text{M}+\text{H}^+$).

[0709] Example 69

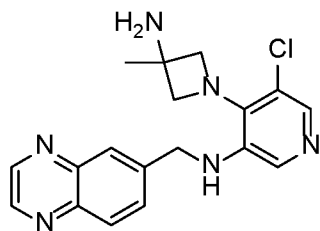
[0710] 4-(3-amino-3-methylazetidin-1-yl)-5-fluoro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 068)



[0711] The title compound was synthesized following a similar procedure described for the preparation of Example 59 (Comp. 058) with appropriate starting materials and intermediates. ^1H NMR (400 MHz, DMSO- d_6) δ 8.91 - 8.87 (m, 2H), 8.15 - 8.08 (m, 3H), 7.93 (dd, $J = 2.0, 8.8$ Hz, 1H), 7.59 (s, 1H), 4.88 - 4.86 (m, 2H), 4.81 - 4.75 (m, 2H), 4.63 (s, 3H), 1.74 (s, 3H). MS (ES^+) m/e 339.2 ($\text{M}+\text{H}^+$).

[0712] Example 70

[0713] 4-(3-amino-3-methylazetidin-1-yl)-5-chloro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 069)

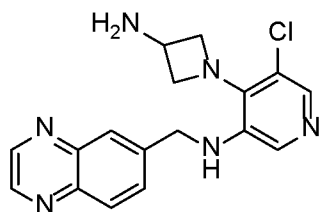


[0714] The title compound was synthesized following a similar procedure described for the preparation of Example 49 (Comp. 048) with appropriate starting materials and

intermediates. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.90 (s, 2H), 8.16 - 8.05 (m, 3H), 7.92 (dd, $J = 2.0, 8.8$ Hz, 1H), 7.60 (s, 1H), 5.05 - 4.97 (m, 2H), 4.97 - 4.92 (m, 2H), 4.60 (s, 2H), 1.72 (s, 3H). MS (ES^+) m/e 355.0 ($\text{M}+\text{H}$) $^+$.

[0715] Example 71

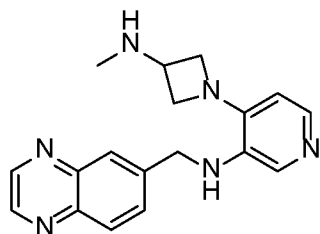
[0716] 4-(3-aminoazetidin-1-yl)-5-chloro-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 070)



[0717] The title compound was synthesized following a similar procedure described for the preparation of Example 49 (Comp. 048) with appropriate starting materials and intermediates. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.94 (s, 2H), 8.72 (br s, 2H), 8.17 (s, 1H), 8.13-8.06 (m, 2H), 7.91 (dd, $J = 2.0, 8.4$ Hz, 1H), 7.52 (s, 1H), 6.31 (br t, $J = 5.2$ Hz, 1H), 5.19-5.06 (m, 2H), 4.87 (dd, $J = 4.8, 10.4$ Hz, 2H), 4.58 (br d, $J = 4.8$ Hz, 2H), 4.05 (br s, 1H). MS (ES^+) m/e 341.1 ($\text{M}+\text{H}$) $^+$.

[0718] Example 72

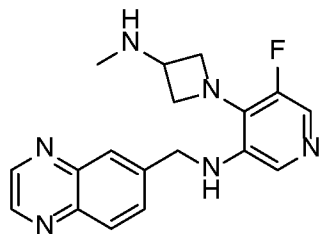
[0719] 4-(3-(methylamino)azetidin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 071)



[0720] The title compound was synthesized following a similar procedure described for the preparation of Example 49 (Comp. 048) with appropriate starting materials and intermediates. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.53-9.26 (m, 1H), 8.94 (s, 2H), 8.11 (d, $J = 8.8$ Hz, 1H), 8.07 (d, $J = 1.2$ Hz, 1H), 7.99-7.95 (m, 1H), 7.90 (dd, $J = 2.0, 8.7$ Hz, 1H), 7.53 (s, 1H), 6.67 (d, $J = 6.8$ Hz, 1H), 6.06 (t, $J = 5.6$ Hz, 1H), 4.76 (dd, $J = 8.4, 10.4$ Hz, 2H), 4.62 (d, $J = 5.2$ Hz, 2H), 4.52 (dd, $J = 4.8, 10.6$ Hz, 2H), 4.26-4.16 (m, 1H), 2.66 (s, 3H). MS (ES^+) m/e 321.2 ($\text{M}+\text{H}$) $^+$.

[0721] Example 73

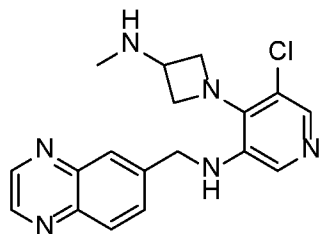
[0722] 5-fluoro-4-(3-(methylamino)azetidin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 072)



[0723] The title compound was synthesized following a similar procedure described for the preparation of Example 59 (Comp. 058) with appropriate starting materials and intermediates. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.91 - 8.86 (m, 2H), 8.15 - 8.08 (m, 3H), 7.93 (dd, $J = 2.0, 8.8$ Hz, 1H), 7.58 (s, 1H), 5.11 - 5.07 (m, 2H), 4.89 - 4.82 (m, 2H), 4.63 (s, 2H), 4.29 - 4.18 (m, 1H), 2.80 (s, 3H). MS (ES^+) m/e 339.1 ($\text{M}+\text{H}$) $^+$.

[0724] Example 74

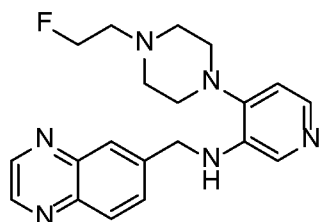
[0725] 5-chloro-4-(3-(methyamino)azetidin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 073)



[0726] The title compound was synthesized following a similar procedure described for the preparation of Example 49 (Comp. 048) with appropriate starting materials and intermediates. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.42-9.04 (m, 2H), 8.94 (s, 2H), 8.15-8.00 (m, 3H), 7.89 (dd, $J = 1.6, 8.8$ Hz, 1H), 7.57 (s, 1H), 6.02 (br s, 1H), 5.12-4.90 (m, 2H), 4.85-4.72 (m, 2H), 4.57 (br d, $J = 4.4$ Hz, 2H), 4.04 (br s, 1H), 2.65 (s, 3H). MS (ES^+) m/e 355.0 ($\text{M}+\text{H}$) $^+$.

[0727] Example 75

[0728] 4-(4-(2-fluoroethyl)piperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 074)

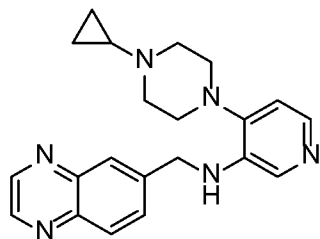


[0729] The title compound was synthesized following a similar procedure described for the preparation of Example 60 (Comp. 059) with appropriate starting materials and intermediates. ^1H NMR (400 MHz, D_2O) δ 9.08 (s, 2H), 8.31 (d, $J = 8.6$ Hz, 1H), 8.15-8.23

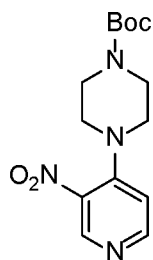
(m, 2H), 8.11 (dd, $J = 8.8, 1.6$ Hz, 1H), 7.88 (s, 1H), 7.60 (d, $J = 6.4$ Hz, 1H), 5.15-5.20 (m, 1H), 5.03-5.09 (m, 2H), 4.99-5.02 (m, 2H), 4.17-4.33 (m, 2H), 4.00-4.14 (m, 2H), 3.89-3.95 (m, 1H), 3.83-3.88 (m, 1H), 3.68-3.81 (m, 2H), 3.47-3.65 (m, 2H). MS (ES⁺) m/e 367.3 (M+H)⁺.

[0730] Example 76

[0731] 4-(4-cyclopropylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine
(Comp. 075)

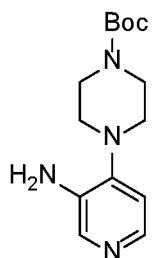


[0732] Step 1. *tert*-Butyl 4-(3-nitropyridin-4-yl)piperazine-1-carboxylate



[0733] To a mixture of 4-chloro-3-nitropyridine (500 g, 3.15 mol, 1.00 eq) and *tert*-butyl piperazine-1-carboxylate (587.4 g, 3.15 mol, 1.00 eq) in *i*-PrOH (4.00 L) was added DIEA (1.02 kg, 7.88 mol, 1.37 L, 2.50 eq) at 10 °C dropwise over 30 min. The reaction mixture was heated to 85 °C and stirred for 8 h, diluted with MTBE (4.00 L) and stirred for additional 30 min. The suspension was filtered and solid was washed with MTBE (2.00 L × 2) to give the title compound (3.00 kg, 77.13%) as yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.79 (s, 1H), 8.38 (d, $J = 6.4$ Hz, 1H), 7.17 (d, $J = 6.4$ Hz, 1H), 3.55 - 3.39 (m, 4H), 3.32 - 3.19 (m, 4H), 1.42 (s, 9H). MS (ES⁺) m/e 309.1 (M+H)⁺.

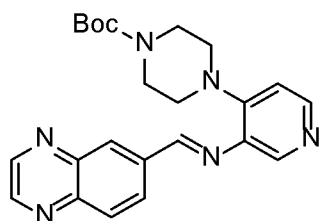
Step 2. *tert*-Butyl 4-(3-aminopyridin-4-yl)piperazine-1-carboxylate



[0734] To a solution of *tert*-butyl 4-(3-nitropyridin-4-yl)piperazine-1-carboxylate (400 g, 1.30 mol, 1.00 eq) in MeOH (7.00 L) was added Pt-V/C (80.0 g) in portions at 25 °C. The

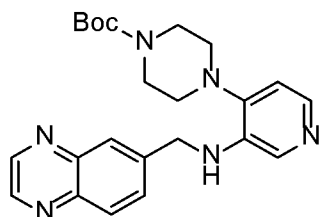
mixture was stirred at 25 °C for 16 h under H₂ (150 Psi) and was filtered. The filtrate was concentrated under reduced pressure to give the crude product which was triturated with EtOH/H₂O (1/10, 5.00 L) at 25 °C for 1 h to give the title compound (2.00 kg, 74.1%) as white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.94 (s, 1H), 7.74 (d, *J* = 5.2 Hz, 1H), 6.76 (d, *J* = 5.2 Hz, 1H), 4.84 (s, 2H), 3.59 - 3.41 (m, 4H), 2.92 - 2.73 (m, 4H), 1.42 (s, 9H). MS (ES⁺) *m/e* 279.1 (M+H)⁺.

[0735] Step 3. *tert*-Butyl (E)-4-(3-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)piperazine-1-carboxylate



[0736] To a mixture of *tert*-butyl 4-(3-aminopyridin-4-yl)piperazine-1-carboxylate (500 g, 1.80 mol, 1.00 *eq*) in MeOH (3.00 L) was added AcOH (53.9 g, 898 mmol, 51.4 mL, 0.50 *eq*) dropwise over 30 min. The mixture was stirred at 25 °C for 1 h followed by addition of quinoxaline-6-carbaldehyde (284 g, 1.80 mol 1.00 *eq*) and the reaction mixture was warmed to 45 °C and stirred for 7 h. The mixture was filtered at 45 °C and washed with MeOH (1.00 L × 2) and the filtration cake was dried in vacuum to give the title compound (2.00 kg, 66.5%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.03 (dd, *J* = 2.0, 8.4 Hz, 2H), 8.89 (s, 1H), 8.58 (d, *J* = 1.6 Hz, 1H), 8.47 (dd, *J* = 2.0, 8.8 Hz, 1H), 8.29 - 8.19 (m, 2H), 8.14 (s, 1H), 6.93 (d, *J* = 5.6 Hz, 1H), 3.46 (br d, *J* = 5.2 Hz, 4H), 3.33 - 3.29 (m, 4H), 1.40 (s, 9H). MS (ES⁺) *m/e* 419.2 (M+H)⁺.

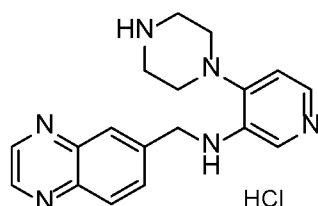
[0737] Step 4. *tert*-Butyl 4-(3-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)piperazine-1-carboxylate



[0738] To a mixture of *tert*-butyl (E)-4-(3-((quinoxalin-6-ylmethylene)amino)pyridin-4-yl)piperazine-1-carboxylate (350 g, 836.3 mmol, 1.00 *eq*) in MeOH (300 mL) and EtOH (1500 mL) was added NaBH₄ (32.3 g, 853.2 mmol, 1.02 *eq*) in portions at 30 °C. The mixture was stirred at 30 °C for 4 h, quenched with *aq.* NH₄Cl (10.0 L), and filtered. The solid was washed with water (2.00 L × 2), dried in oven under N₂ atmosphere and triturated with

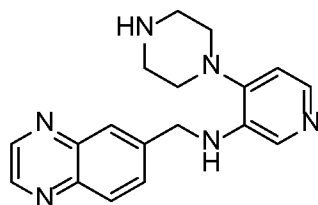
EtOAc (2.00 L) at 25 °C for 1 h to give the title compound (700 g, 38.0%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.91 (s, 2H), 8.09 (d, *J* = 8.8 Hz, 1H), 8.02 (s, 1H), 7.90 (dd, *J* = 0.8, 8.8 Hz, 1H), 7.79 (d, *J* = 5.2 Hz, 1H), 7.73 (s, 1H), 6.88 (d, *J* = 5.2 Hz, 1H), 5.86 (br t, *J* = 6.0 Hz, 1H), 4.70 (br d, *J* = 5.6 Hz, 2H), 3.59 (br s, 4H), 2.93 (br s, 4H), 1.44 (s, 9H). MS (ES⁺) *m/e* 421.1 (M+H)⁺.

[0739] Step 5. 4-(Piperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine HCl salt



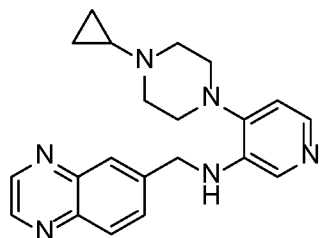
[0740] To a mixture of *tert*-butyl 4-(3-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)piperazine-1-carboxylate (175 g, 416.2 mmol, 1.00 eq) in MeOH (1.00 L) was added HCl/MeOH (4 M, 1.04 L, 10.0 eq) at 25 °C and the mixture was stirred at 25 °C for 0.5 h, filtered and washed with MeOH (1.00 L × 2). The cake was dried in vacuum to give the product. The product was then dissolved in H₂O (2.00 L) and the mixture was stirred at 45 °C for 1 hr and was lyophilized to the title compound (603.85 g, 84.4%, 3HCl) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 15.21 (br s, 1H), 9.88 (br s, 2H), 8.92 (s, 2H), 8.16 - 8.00 (m, 3H), 7.92 (br d, *J* = 8.8 Hz, 1H), 7.77 (s, 1H), 7.39 (d, *J* = 6.4 Hz, 1H), 6.94 (br s, 1H), 4.77 (br s, 2H), 3.50 (br s, 4H), 3.41 (br s, 4H). MS (ES⁺) *m/e* 321.1 (M+H)⁺.

[0741] Step 6. 4-(Piperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine



[0742] To a mixture of 4-(piperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine HCl salt (26.2 g, 60.9 mmol, 1.00 eq, 3 HCl) in H₂O (200 mL) was added Na₂CO₃ (12.9 g, 121.9 mmol, 2.00 eq) at 15 °C. The mixture was stirred at 15 °C for 0.5 h and was extracted with DCM/MeOH (10/1, 500 mL × 3). The combined organic layers were dried with anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to provide the title compound (18.97 g, 95.86%) as a light yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 15.21 (s, 1H), 9.89 (s, 2H), 8.92 (s, 2H), 8.19 - 8.00 (m, 3H), 7.92 (dd, *J*₁ = 1.6 Hz, *J*₂ = 8.8 Hz, 1H), 7.77 (s, 1H), 7.39 (d, *J* = 6.4 Hz, 1H), 6.95 (s, 1H), 4.77 (s, 2H), 3.51 (t, *J* = 4.4 Hz, 4H), 3.41 (t, *J* = 4.4 Hz, 4H). MS (ES⁺) *m/e* 321.1 (M+H)⁺.

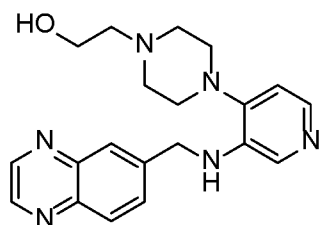
[0743] Step 7. 4-(4-Cyclopropylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine



[0744] To a solution of 4-(piperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (400 mg, 1.25 mmol, 1.00 eq) in THF (2.00 mL) and MeOH (2.00 mL) was added (1-ethoxycyclopropoxy)trimethylsilane (870 mg, 4.99 mmol, 1.00 mL, 4.00 eq), NaBH₃CN (235 mg, 3.75 mmol, 3.00 eq) and AcOH (239 mg, 4.00 mmol, 228 μ L, 3.20 eq). The mixture was stirred at 50 °C for 12 h and concentrated under reduced pressure to give a residue. The crude product was purified by reversed-phase HPLC (column: Phenomenex luna C18 (250 x 70mm, 10 μ m); mobile phase: [water (HCl)-ACN]; gradient: 1%-15% B over 20 min) to provide the title compound (100 mg, 22.2%) as a brown solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.58 (s, 1H), 9.74 (s, 2H), 8.92-8.87 (m, 3H), 8.76-8.74 (dd, *J* = 1.60, 8.40, 1H), 8.60 (s, 2H), 8.21-8.19 (d, *J* = 6.60, 2H), 7.76 (s, 1H), 5.59 (s, 1H), 4.67-4.64 (m, 1H), 5.59 (s, 1H), 4.44 (s, 4H), 4.33-4.30 (m, 2H), 3.72 (s, 1H), 2.09-2.06 (m, 2H), 1.66-1.64 (d, *J* = 8.20, 2H). MS (ES⁺) *m/e* 361.2 (M+H)⁺.

[0745] **Example 77**

[0746] 2-(4-(3-((quinoxalin-6-ylmethyl)amino)pyridin-4-yl)piperazin-1-yl)ethan-1-ol (Comp. 076)

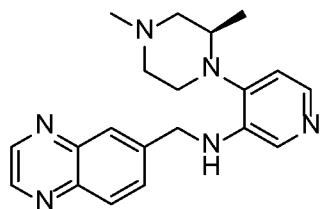


[0747] To a solution of 4-(piperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (250 mg, 780 μ mol, 1.00 eq) and 1,4-dioxane-2,5-diol (93.7 mg, 780 μ mol, 1.00 eq) in MeOH (4.00 mL) was added NaBH₃CN (98.1 mg, 1.56 mmol, 2.00 eq). The mixture was stirred at 25 °C for 2 h and was concentrated under reduced pressure to remove MeOH. The crude product was purified by reversed-phase HPLC (column: Waters X bridge 150 x 25mm x 5 μ m; mobile phase: [water (ammonia hydroxide v/v)-ACN]; gradient: 3%-33% B over 10 min) and lyophilized to give the title compound (150 mg, 52.8%) as a yellow solid. ¹H NMR (400

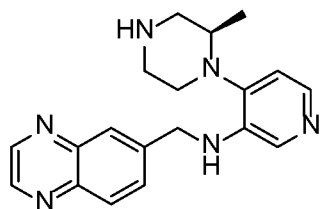
MHz, DMSO- d_6) δ 8.90 (s, 2H), 8.08 (d, J = 8.4 Hz, 1H), 8.00 (s, 1H), 7.88 (dd, J = 1.8, 8.4 Hz, 1H), 7.77 (d, J = 5.2 Hz, 1H), 7.69 (s, 1H), 6.87 (d, J = 5.2 Hz, 1H), 5.66 (t, J = 6.0 Hz, 1H), 4.68 (d, J = 6.0 Hz, 2H), 4.44 (t, J = 5.2 Hz, 1H), 3.55 (q, J = 6.0 Hz, 2H), 2.98 (br s, 4H), 2.68 (br s, 4H), 2.49-2.46 (m, 2H). MS (ES⁺) m/e 365.1 (M+H)⁺.

[0748] Example 78

[0749] (*R*)-4-(2,4-dimethylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 077)

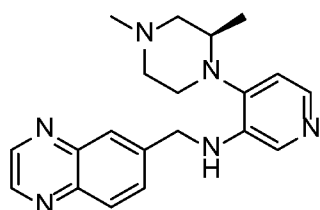


[0750] Step 1. (*R*)-4-(2-Methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine



[0751] The title compound was synthesized following a similar procedure described for the preparation of Example 76 (Comp. 075) with appropriate starting materials and intermediates. ¹H NMR (400 MHz, DMSO- d_6) δ 8.89 (s, 2H), 8.07 (d, J = 8.4 Hz, 1H), 7.97 (s, 1H), 7.86 (dd, J = 1.6, 8.4 Hz, 1H), 7.80 - 7.73 (m, 2H), 6.95 (d, J = 5.2 Hz, 1H), 6.01 (br t, J = 6.0 Hz, 1H), 4.83 - 4.59 (m, 2H), 3.20 (br s, 1H), 3.05 - 2.91 (m, 3H), 2.91 - 2.81 (m, 1H), 0.82 (d, J = 6.4 Hz, 3H). MS (ES⁺) m/e 335.2 (M+H)⁺.

[0752] Step 2. (*R*)-4-(2,4-dimethylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine

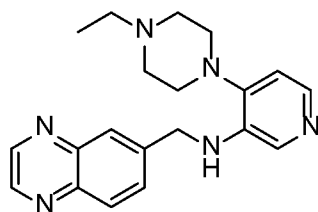


[0753] The reaction was carried out via flow chemistry. While (*R*)-4-(2-methylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (1.00 g, 2.99 mmol, 1.00 eq), TEA (907 mg, 8.97 mmol, 1.25 mL, 3.00 eq), AcOH (1.80 g, 29.9 mmol, 1.71 mL, 10.0 eq) and formaldehyde (269 mg, 8.97 mmol, 247 μ L, 3.00 eq) in MeOH (10.0 mL) was introduced into the flow chemistry instrument at a flow rate of 18.5 mL / min, a solution of 2-

methylpyridine borane complex (3.20 g, 29.9 mmol, 10.0 eq) in MeOH (10.0 mL) was introduced at the same flow rate at the same time. The reaction was carried out at 25°C for 3 h and the reaction solution was then collected and was quenched by addition aqueous NH₄Cl solution (10.0 mL) and concentrated under reduce pressure to give a residue. The residue was purified by prep-HPLC (HCl condition) to afford the title compound (83.1 mg, 7.98%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.87-8.93 (m, 2H), 8.07 (d, *J* = 8.6 Hz, 1H), 7.97 (d, *J* = 1.2 Hz, 2.0 H), 7.86 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.74-7.81 (m, 2H), 6.99 (d, *J* = 4.8 Hz, 1H), 5.95 (br s, 1H), 4.58-4.80 (m, 2H), 2.99-3.10 (m, 1H), 2.75-2.84 (m, 1H), 2.56-2.68 (m, 2H), 2.37-2.47 (m, 1H), 2.24 (s, 3H), 2.01-2.14 (m, 1H), 0.86 (d, *J* = 6.4 Hz, 3H). MS (ES⁺) *m/e* 349.1 (M+H)⁺.

[0754] Example 79

[0755] 4-(4-ethylpiperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (Comp. 078)



[0756] To a solution of 4-(piperazin-1-yl)-*N*-(quinoxalin-6-ylmethyl)pyridin-3-amine (250 mg, 780 μmol, 1 eq) in MeOH (2.00 mL) was added acetaldehyde (85.9 mg, 780 μmol, 109 μL, 1.00 eq) and NaBH₃CN (98.1 mg, 1.56 mmol, 2.00 eq). The mixture was stirred at 25 °C for 3 h, concentrates under reduces pressure to remove MeOH. The crude product was purified by reversed-phase HPLC (column: Waters X bridge 150 × 25 mm × 5 um; mobile phase: [water (ammonia hydroxide v/v)-ACN]; gradient: 10%-40% B over 10 min) and lyophilized to give the title compound (150 mg, 55.2%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.92-8.87 (m, 2H), 8.08 (d, *J* = 8.6 Hz, 1H), 8.00 (s, 1H), 7.88 (dd, *J* = 1.6, 8.6 Hz, 1H), 7.77 (d, *J* = 5.2 Hz, 1H), 7.69 (s, 1H), 6.87 (d, *J* = 5.2 Hz, 1H), 5.66 (t, *J* = 6.0 Hz, 1H), 4.68 (d, *J* = 6.0 Hz, 2H), 2.99 (br s, 4H), 2.62 (br s, 4H), 2.41 (q, *J* = 7.2 Hz, 2H), 1.05 (t, *J* = 7.2 Hz, 3H). MS (ES⁺) *m/e* 349.0 (M+H)⁺.

[0757] Example 80

[0758] Compounds were tested for the exposure levels when dosed orally at 100 mg/kg in CD-1 mice.

[0759] CD-1 mice were treated with the compounds via oral administration. The oral administration was by gavage with a methyl cellulose/Tween 80 formulation. Blood samples were collected at the 2 hour timepoint post-dose and were transferred into EDTA-K3 anti-

coagulant tubes. The blood plasma samples were obtained by centrifugation at 4000g for 5 min and were kept at -75 ± 15 °C until bioanalysis. The compound concentrations in the plasma samples were assayed using LC-MS/MS. Quality control samples (prepared with CD-1 mouse plasma) were included in each analysis to ensure assay performance.

[0760] For the tissue collection, the animal was fully exsanguinated prior to the tissue collection. The following procedure was followed: open the cavitas thoracis, expose the heart, catheterize from left ventricular, make an incision at the right atrial appendage, and perfuse (10 mL saline) from the left ventricular. The fluid outflows from the right atrial appendage. The fresh tissues were then collected, put in an appropriate tube, quickly frozen in liquid nitrogen and kept at -75 ± 15 °C until analysis. The tissue samples were homogenized at a ratio of 1:4 with PBS (W/V, 1:4) and the compound concentration was determined using LC-MS/MS. Quality control samples (prepared with CD-1 mouse tissue homogenate) were included in each analysis to ensure assay performance.

[0761] The exposure levels for selected compounds are reported in Table C.

Table C

Compound exposure levels in CD-1 mice when dosed at 100 mg/kg orally.					
Compound	Plasma conc. (ng/mL)	Brain conc. (ng/g)	Eye conc. (ng/g)	B/P ratio	E/P ratio
Comp. A	1145.1	183.48	2402.4	0.2	2.1
002	1243	7017	4197	5.6	3.4
003	2050	6453	5565	3.1	2.7
005	1830	4333	10550	2.4	5.8
012	6537	10517	5308	1.6	0.8
013	8713	11883	4462	1.4	0.5
015	4270	8233	11350	1.9	2.7
017	4490	6483	2408	1.4	0.5
020	2917	6083	3163	2.1	1.1
021	1490	6767	7967	4.5	5.3
042	810	4538	3978	5.6	4.9
043	624	2167	3065	3.5	4.9
055	1108	54.5	890	0.05	0.8
057	1509	372	1720	0.25	1.1
058	1143	251	1225	0.22	1.1
059	771	38.5	806	0.05	1.0
060	302	46.9	465	0.16	1.5
061	936	873	2865	0.93	3.1
062	255	466	1620	1.8	6.4
063	865	36.7	622	0.04	0.72
064	590	38	616	0.06	1.0
065	245	19.6	131	0.08	0.53
066	1507	411	5912	0.27	3.9
067	1079	91	1628	0.08	1.5
068	1607	811	3360	0.5	2.1

072	474	674	349	1.4	0.74
074	248	441	893	1.8	3.6
075	358	1351	675	3.8	1.9
076	1480	262	3535	0.18	2.4
077	835	1915	1953	2.3	2.3
078	1328	6400	4468	4.8	3.4

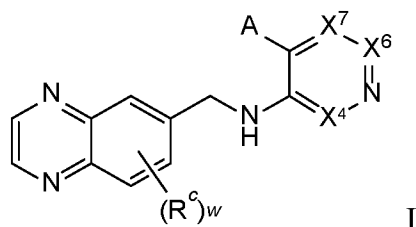
Table D. Sequences.

SEQ ID NO:	Description	Sequence
1	12C6-1 aptamer	CTGGGGAGTCCTTCATGCGGGGCTGAGAGG ATGGAAGCAATCGACCATCGACCCATTGCA CCTGATCCGGATCATGCCGGGCGCAGGGAG
2, 3	Embodiment of the alternative splicing gene regulation cassette -X- represents an aptamer encoding sequence disclosed herein; alternative exon is underlined SEQ ID NO: 2 contains the sequence 5' of the -X- SEQ ID NO: 3 contains the sequence 3' of the -X-	GTGAGTCTATGGGACCCTTGATGTTTTCTTT CCCCTTCTTTTCTATGGTTAAGTTCATGTCA TAGGAAGGGGAGAAGTAACAGGGGTACACA TATTGACCAAATCAGGGTAATTTTGCATTTG TAATTTTAAAAAATGCTTTCTTCTTTTAATA TACTTTTTTGTATCTTATTTCTAATACTTT CCCTAATCTCTTTCTTTCAGGGCAATAATGA TACAATGTATCATGCCGAGTAACGCTGTTTC TCTAACTTGTAGGAATGAATTCAGATATTTTC <u>CAGAGAATGAAAAAAAAAATCTTCAGTAGA</u> <u>AG</u> gtaatgt-X- acattacGCACCATTTCTAAAGAATAACAGTGAT AATTTCTGGGTTAAGGCAATAGCAATATTT CTGCATATAAATATTTCTGCATATAAATTGT AACTGATGTAAGAGGTTTCATATTGCTAAT AGCAGCTACAATCCAGCTACCATTCTGCTTT TATTTTATGGTTGGGATAAAGGCTGGATTATT CTGAGTCCAAGCTAGGCCCTTTTGCTAATCA TGTTTCATACCTCTTATCTTCTCCACAG
4	12C6-1 alternate splicing gene regulation cassette Caps: 12C6-1 aptamer; Bold: alt. exon; Underline: riboswitch stem forming sequence; Ital.: 5' intron and 3' intron	<i>gtgagtctatgggacccttgatgttttcttcccccttctttctatggttaagt</i> <i>tcatgtcataggaaggggagaagtaacaggggtacacatattgacca</i> <i>aalcagggtaatgttgcatttgaattttaaaaaatgctttcttctttaata</i> <i>tactttttgtttatcttatttctaatactttccctaactcttcttccaggga</i> <i>ataatgatacaatgtatcatgccgagtaacgctgtttcttaactttag</i> gaatgaattcagatatttccagagaatgaaaaaaaaaatcttcagta gaaggtaatgt <u>CTGGGGAGTCCTTCATGCGGGGC</u> TGAGAGGATGGAAGCAATCGACCATCGACC CATTGCACCTGATCCGGATCATGCCGGGCGC <u>AGGGAG</u> <u>Gacattacgcaccattctaaagaataacagtataatttc</u> <i>tgggttaaggcaatagcaatatttctgcatataaatatttctgcatataa</i> <i>attgtaactgatgtaagaggttcatattgctaatagcagctacaatcc</i> <i>agctaccattctgcttttattttatggttgggataaggctggattatttctga</i> <i>gtccaagctaggcccttttgctaatacatgttcataccttattcttctccc</i> <i>acag</i>

5	Modified DHFR exon 2	GAATGAATTCAGATATTTCCAGAGAATGAA AAAAAATCTTCAGTAGAAG
6	Modified DHFR exon 2	GAATGAATTCAGATATTTCCAGAGAATGAA AAAAAATCTTCAGTAGAAG
7	thiC	GUAAUGUGUCGGAGUGCCUAGGGGAUUUAU UCCCCUAAAGCUGAGACCGCAUUGCGGGA UCCGUUGAACCUGAUCAGGCUAAUACCUG CGAAGGGAACACAUUAC
8	thiM	GUAAUGUCUCGGGGUGCCCUUCUGCGUGA AGGCUGAGAAAUACCCGUUACACCUGAUC UGGAUAAUGCCAGCGUAGGGAAGACAUUA C

We claim:

1. A compound having the structure according to Formula I:



or a pharmaceutically acceptable salt thereof, wherein

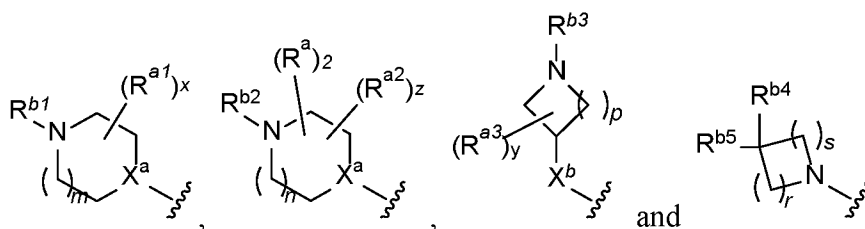
X^4 is selected from CH, CR^d and N;

X^6 is selected from CH, CR^d and N;

X^7 is selected from CH, CR^d and N;

wherein 0 or 1 of X^4 , X^6 or X^7 is N;

A is selected from the group consisting of:



X^a is selected from N and CH;

X^b is selected from O, NH, and NCH_3 ;

each R^{a1} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a1} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

each R^a is independently selected from C_1 to C_3 alkyl both of which C_1 to C_3 alkyl groups are bonded to the same ring carbon atom, or alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring;

each R^{a2} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, and halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

each R^{a3} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the

same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b1} is C_1 to C_3 alkyl or C_3 to C_6 cycloalkyl, each optionally substituted with halo, OH, or O- C_{1-3} alkyl;

R^{b2} is selected from H, C_1 to C_3 alkyl optionally substituted with halo, OH, or O- C_{1-3} alkyl;

R^{b3} is C_1 to C_3 alkyl optionally substituted with halo, OH, or O- C_{1-3} alkyl;

R^{b4} is selected from OH, $-NH_2$, $-NH(C_1 \text{ to } C_3 \text{ alkyl})$, and $-N(C_1 \text{ to } C_3 \text{ alkyl})_2$;

R^{b5} is selected from H and C_1 to C_3 alkyl;

m is 1 or 2; n is 1 or 2; p is 1 or 2; r is 1 or 2; s is 1 or 2;

x is 0, 1, 2 or 3;

y is 0, 1, 2 or 3;

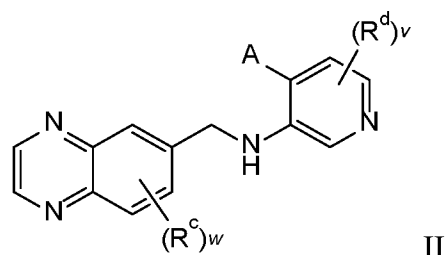
z is 0, 1, or 2;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH; and

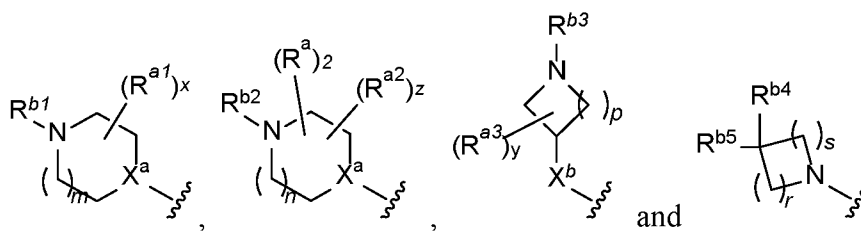
w is 0, 1 or 2.

2. The compound of claim 1, having the structure according to Formula II



or a pharmaceutically acceptable salt thereof, wherein

A is selected from the group consisting of:



X^a is selected from N and CH;

X^b is selected from O, NH, and NCH₃;

each R^{a1} is independently selected from C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, halo, hydroxyl and amino; or additionally or alternatively, two R^{a1} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

each R^a is independently selected from C₁ to C₃ alkyl both of which C₁ to C₃ alkyl groups are bonded to the same ring carbon atom, or alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring;

each R^{a2} is independently selected from C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, and halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

each R^{a3} is independently selected from C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b1} is C₁ to C₃ alkyl or C₃ to C₆ cycloalkyl, each optionally substituted with halo, OH, or O-C₁₋₃ alkyl;

R^{b2} is selected from H, C₁ to C₃ alkyl optionally substituted with halo, OH, or O-C₁₋₃ alkyl;

R^{b3} is C₁ to C₃ alkyl optionally substituted with halo, OH, or O-C₁₋₃ alkyl;

R^{b4} is selected from OH, -NH₂, -NH(C₁ to C₃ alkyl), and -N(C₁ to C₃ alkyl)₂;

R^{b5} is selected from H and C₁ to C₃ alkyl;

m is 1 or 2; n is 1 or 2; p is 1 or 2; r is 1 or 2; s is 1 or 2;

x is 0, 1, 2 or 3;

y is 0, 1, 2 or 3;

z is 0, 1, or 2;

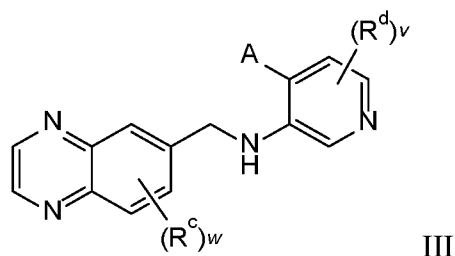
each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

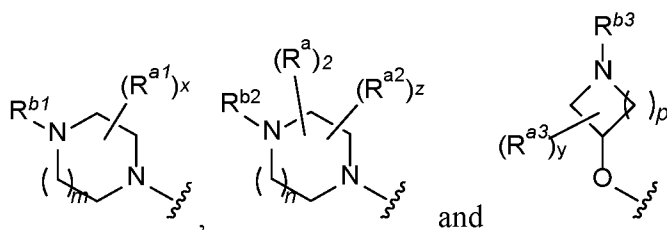
v is 0, 1 or 2.

3. The compound of claim 54, having the structure according to Formula III:



or a pharmaceutically acceptable salt thereof, wherein

A is selected from the group consisting of:



each R^{a1} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a1} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

each R^a is independently selected from C_1 to C_3 alkyl both of which C_1 to C_3 alkyl groups are bonded to the same ring carbon atom, or alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring;

each R^{a2} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, and halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

each R^{a3} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b1} is C_1 to C_3 alkyl or C_3 to C_6 cycloalkyl, each optionally substituted with halo, OH, or $O-C_{1-3}$ alkyl;

R^{b2} is selected from H, C_1 to C_3 alkyl optionally substituted with halo, OH, or $O-C_{1-3}$ alkyl;

R^{b3} is C_1 to C_3 alkyl optionally substituted with halo, OH, or $O-C_{1-3}$ alkyl;

m is 1 or 2; n is 1 or 2; p is 1 or 2;

x is 0, 1, 2 or 3;

y is 0, 1, 2 or 3;

z is 0, 1, or 2;

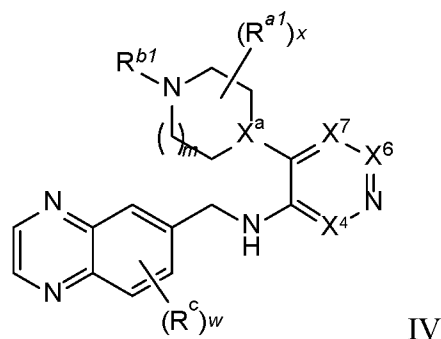
each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

4. The compound of claim 1, having the structure according to Formula IV:



or a pharmaceutically acceptable salt thereof, wherein

X^4 is selected from CH, CR^d and N;

X^6 is selected from CH, CR^d and N;

X^7 is selected from CH, CR^d and N;

wherein 0 or 1 of X^4 , X^6 or X^7 is N;

X^a is selected from N and CH;

each R^{a1} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a1} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b1} is C_1 to C_3 alkyl or C_3 to C_6 cycloalkyl, each optionally substituted with halo, OH, or $O-C_{1-3}$ alkyl;

m is 1 or 2;

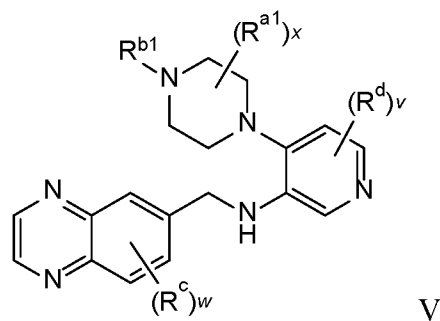
x is 0, 1, 2 or 3;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH; and

w is 0, 1 or 2.

5. The compound of claim 1, having the structure according to Formula V:



or a pharmaceutically acceptable salt thereof, wherein

each R^{a1} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a1} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b1} is C_1 to C_3 alkyl or C_3 to C_6 cycloalkyl, each optionally substituted with halo, OH, or $O-C_{1-3}$ alkyl;

x is 0, 1, 2 or 3;

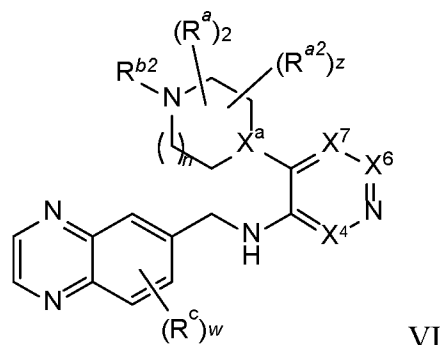
each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

6. The compound of claim 1, having the structure according to Formula VI:



or a pharmaceutically acceptable salt thereof, wherein

X⁴ is selected from CH, CR^d and N;

X⁶ is selected from CH, CR^d and N;

X⁷ is selected from CH, CR^d and N;

wherein 0 or 1 of X⁴, X⁶ or X⁷ is N;

X^a is selected from N and CH;

each R^a is independently selected from C₁ to C₃ alkyl both of which C₁ to C₃ alkyl groups are bonded to the same ring carbon atom, or alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring;

each R^{a2} is independently selected from C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, and halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

R^{b2} is selected from H and C₁ to C₃ alkyl optionally substituted with halo, OH, or O-C₁₋₃ alkyl;

n is 1 or 2;

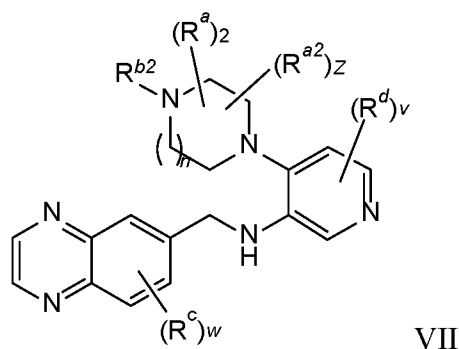
z is 0, 1, or 2;

each R^c is independently selected from halo, C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, -CN, hydroxyl and amino;

each R^d is independently selected from halo, C₁ to C₃ alkyl, -OCH₃, -CF₃, -CH₂F, -CHF₂, -CN, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH; and

w is 0, 1 or 2.

7. The compound of claim 1, having the structure according to Formula VII:



or a pharmaceutically acceptable salt thereof, wherein

each R^a is independently selected from C_1 to C_3 alkyl both of which C_1 to C_3 alkyl groups are bonded to the same ring carbon atom, or alternatively two R^a attached to the same ring carbon atom form a 3- to 5-membered carbocyclic ring;

each R^{a2} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, and halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

R^{b2} is selected from H and C_1 to C_3 alkyl optionally substituted with halo, OH, or $O-C_{1-3}$ alkyl;

n is 1 or 2;

z is 0, 1, or 2;

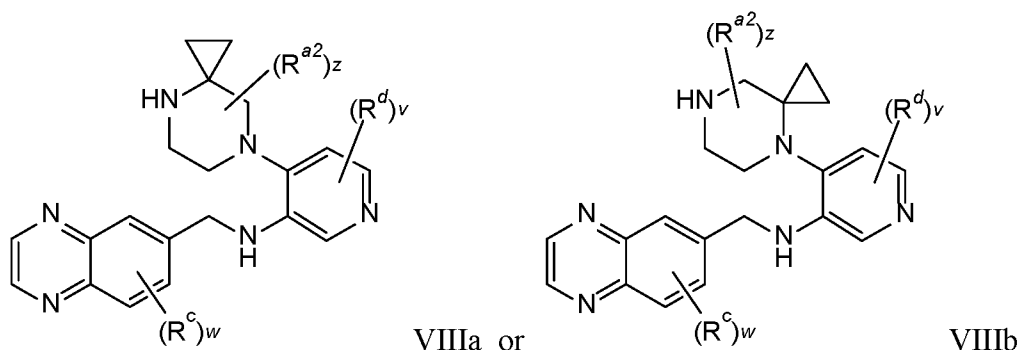
each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

8. The compound of claim 1, having the structure according to Formula VIIIa or VIIIb:



or a pharmaceutically acceptable salt thereof, wherein

each R^{a2} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, and halo; or additionally or alternatively, two R^{a2} attached to the same carbon atom form an oxo group;

z is 0, 1, or 2;

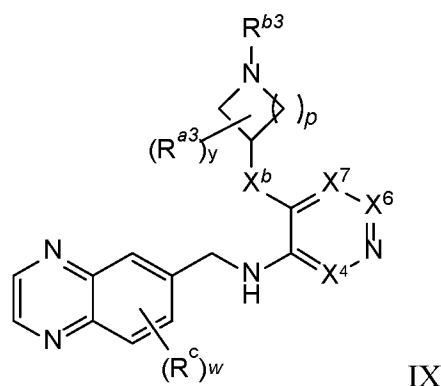
each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

9. The compound of claim 1, having the structure according to Formula IX:



or a pharmaceutically acceptable salt thereof, wherein

X^4 is selected from CH, CR^d and N;

X^6 is selected from CH, CR^d and N;

X^7 is selected from CH, CR^d and N;

wherein 0 or 1 of X^4 , X^6 or X^7 is N;

X^b is selected from O, NH, and NCH_3 ;

each R^{a3} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b3} is C_1 to C_3 alkyl optionally substituted with halo, OH, or $O-C_{1-3}$ alkyl;

p is 1 or 2;

y is 0, 1, 2 or 3;

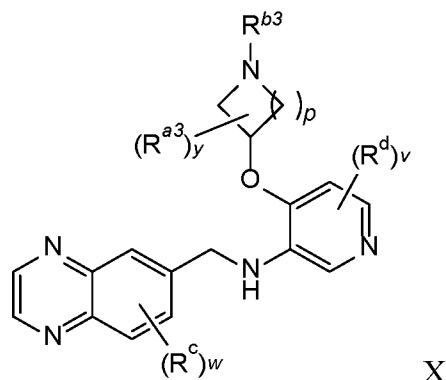
each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken

together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH; and

w is 0, 1 or 2.

10. The compound of claim 1, having the structure according to Formula X:



or a pharmaceutically acceptable salt thereof, wherein

each R^{a3} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

R^{b3} is C_1 to C_3 alkyl optionally substituted with halo, OH, or $O-C_{1-3}$ alkyl;

p is 1 or 2;

y is 0, 1, 2 or 3;

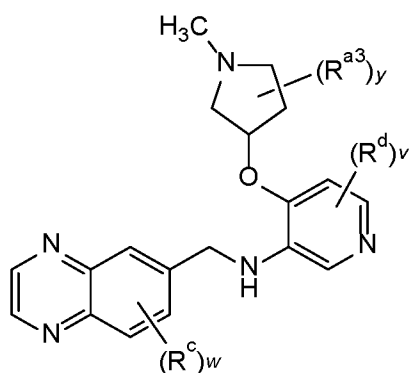
each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

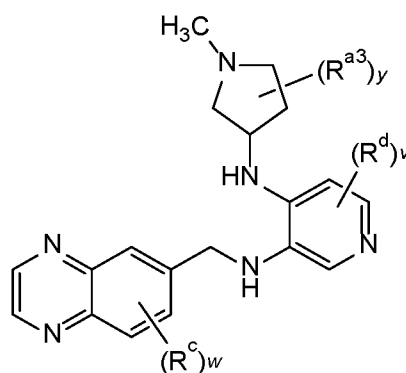
w is 0, 1 or 2; and

v is 0, 1 or 2.

11. The compound of claim 1, having the structure according to Formula XIa or XIb:



XIa



XIb

or a pharmaceutically acceptable salt thereof, wherein

each R^{a3} is independently selected from C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, halo, hydroxyl and amino; or additionally or alternatively, two R^{a3} attached to the same carbon atom form an oxo group, or a 3- to 5-membered carbocyclic ring; or two R^a attached to different carbon atoms form a 4- to 6- membered carbocyclic ring or a 4- to 6- membered heterocyclic ring having 1 or 2 heteroatoms selected from O and NH;

y is 0, 1, 2 or 3;

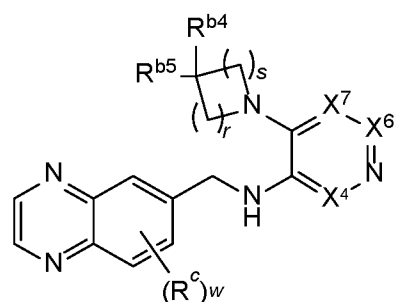
each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

12. The compound of claim 1, having the structure according to Formula XII:



XII

or a pharmaceutically acceptable salt thereof, wherein

X^4 is selected from CH, CR^d and N;

X^6 is selected from CH, CR^d and N;

X^7 is selected from CH, CR^d and N;

wherein 0 or 1 of X^4 , X^6 or X^7 is N;

R^{b4} is selected from OH, $-NH_2$, $-NH(C_1 \text{ to } C_3 \text{ alkyl})$, and $-N(C_1 \text{ to } C_3 \text{ alkyl})_2$;

R^{b5} is selected from H and $C_1 \text{ to } C_3 \text{ alkyl}$;

r is 1 or 2;

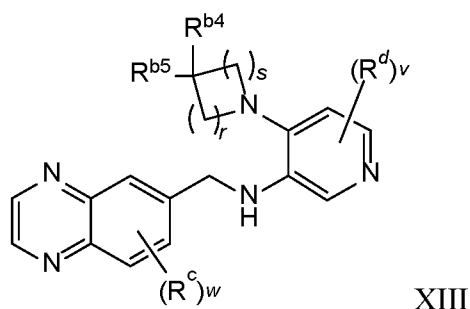
s is 1 or 2;

each R^c is independently selected from halo, $C_1 \text{ to } C_3 \text{ alkyl}$, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, $C_1 \text{ to } C_3 \text{ alkyl}$, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH; and

w is 0, 1 or 2.

13. The compound of claim 1, having the structure according to Formula XIII:



or a pharmaceutically acceptable salt thereof, wherein

R^{b4} is selected from OH, $-NH_2$, $-NH(C_1 \text{ to } C_3 \text{ alkyl})$, and $-N(C_1 \text{ to } C_3 \text{ alkyl})_2$;

R^{b5} is selected from H and $C_1 \text{ to } C_3 \text{ alkyl}$;

r is 1 or 2;

s is 1 or 2;

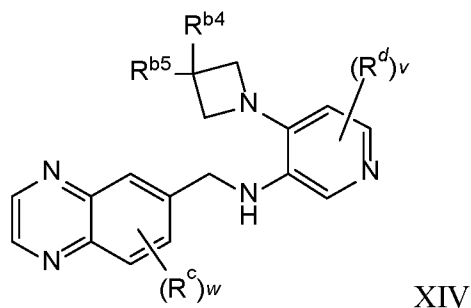
each R^c is independently selected from halo, $C_1 \text{ to } C_3 \text{ alkyl}$, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, $C_1 \text{ to } C_3 \text{ alkyl}$, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

w is 0, 1 or 2; and

v is 0, 1 or 2.

14. The compound of claim 1, having the structure according to Formula XIV:



or a pharmaceutically acceptable salt thereof, wherein

R^{b4} is selected from OH, $-NH_2$, $-NH(C_1 \text{ to } C_3 \text{ alkyl})$, and $-N(C_1 \text{ to } C_3 \text{ alkyl})_2$;

R^{b5} is selected from H and C_1 to C_3 alkyl;

each R^c is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino;

each R^d is independently selected from halo, C_1 to C_3 alkyl, $-OCH_3$, $-CF_3$, $-CH_2F$, $-CHF_2$, $-CN$, hydroxyl and amino; alternatively, two R^d on adjacent ring positions may be taken together to form a 5- or 6-membered aromatic ring having from 0 to 2 heteroatoms selected from O, S, N and NH;

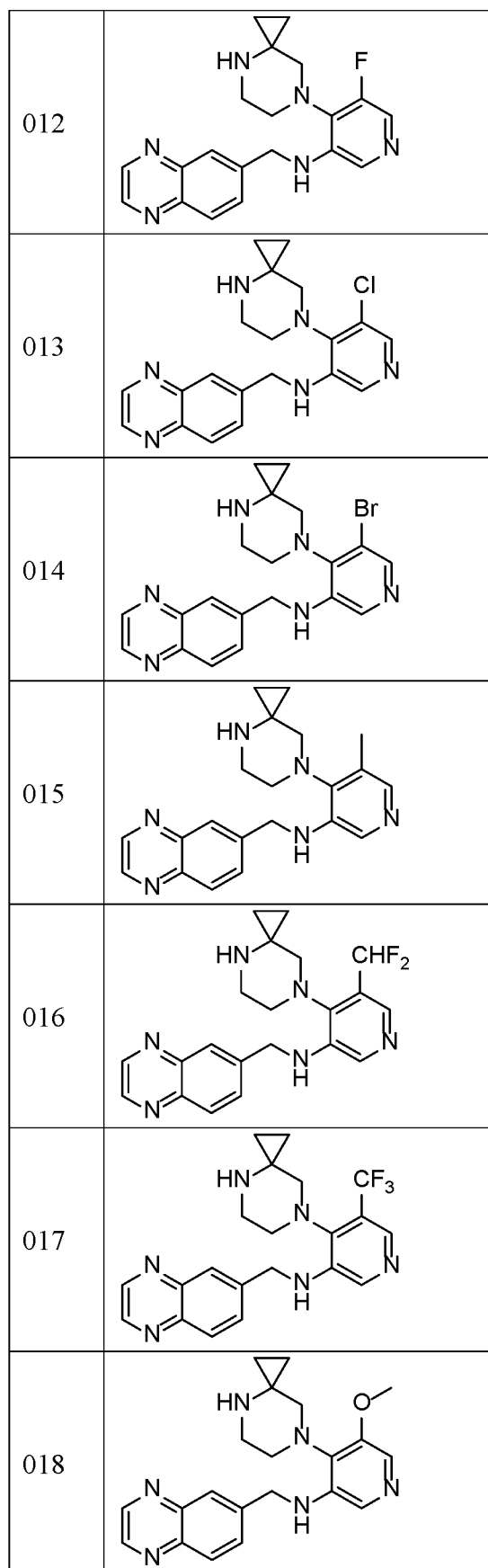
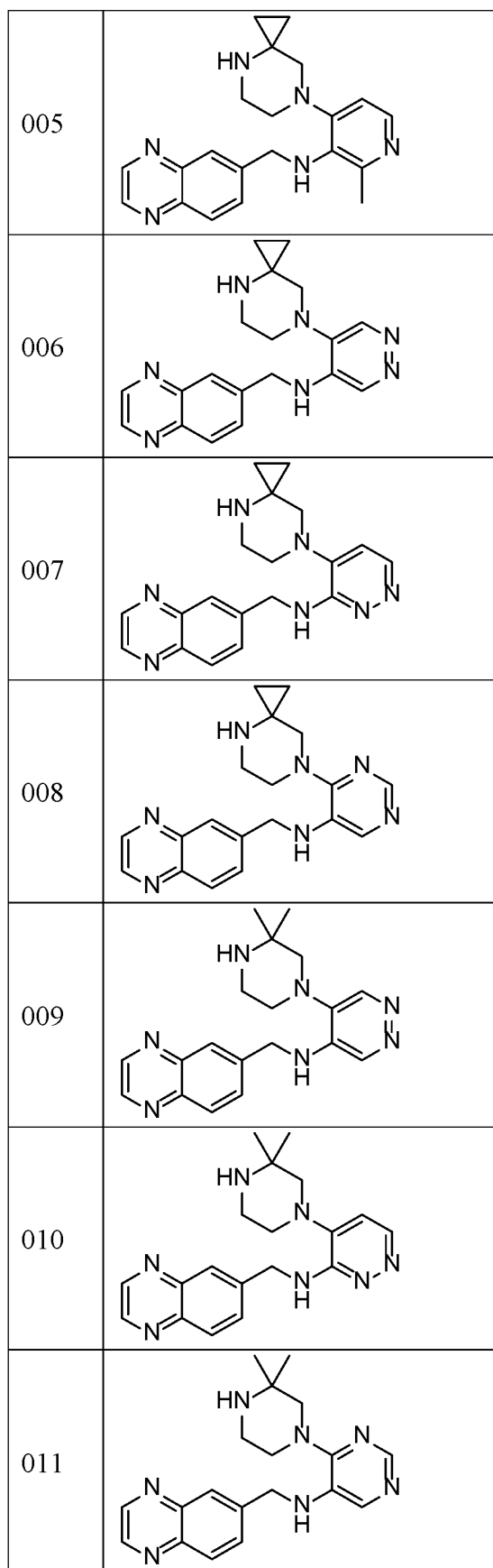
w is 0, 1 or 2; and

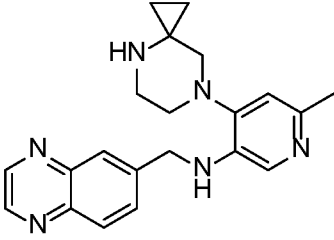
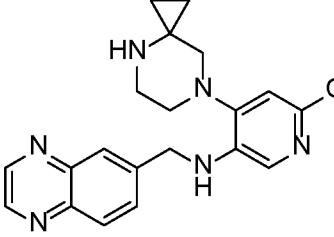
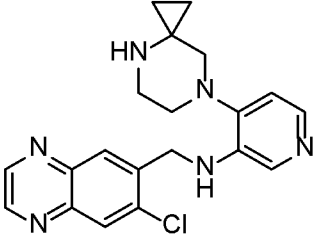
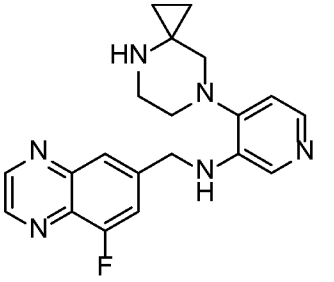
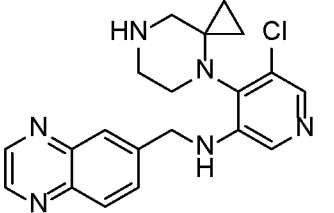
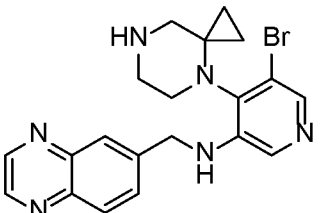
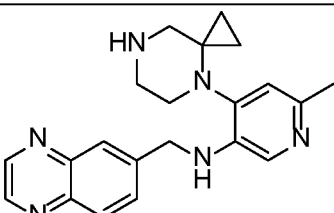
v is 0, 1 or 2.

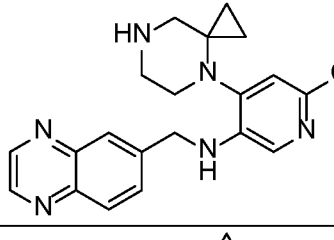
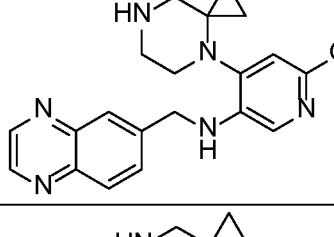
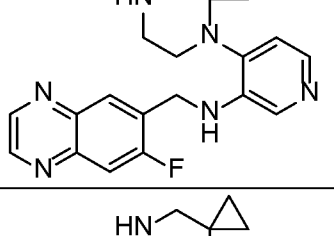
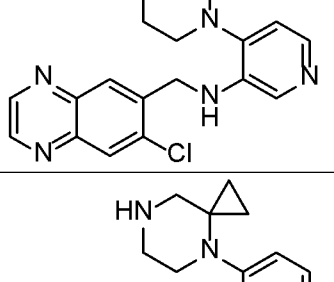
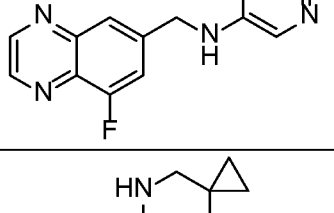
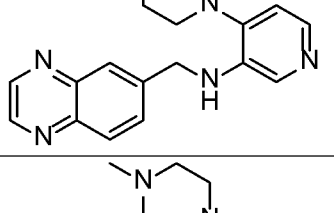
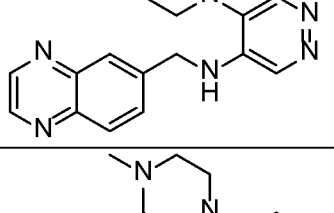
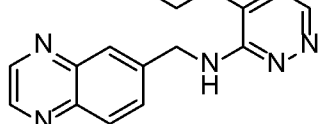
15. The compound of claim 1, having the structure according to one of the compounds of Table A or a pharmaceutically acceptable salt thereof:

Table A.

Ref. No.	Structure
001	
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004	

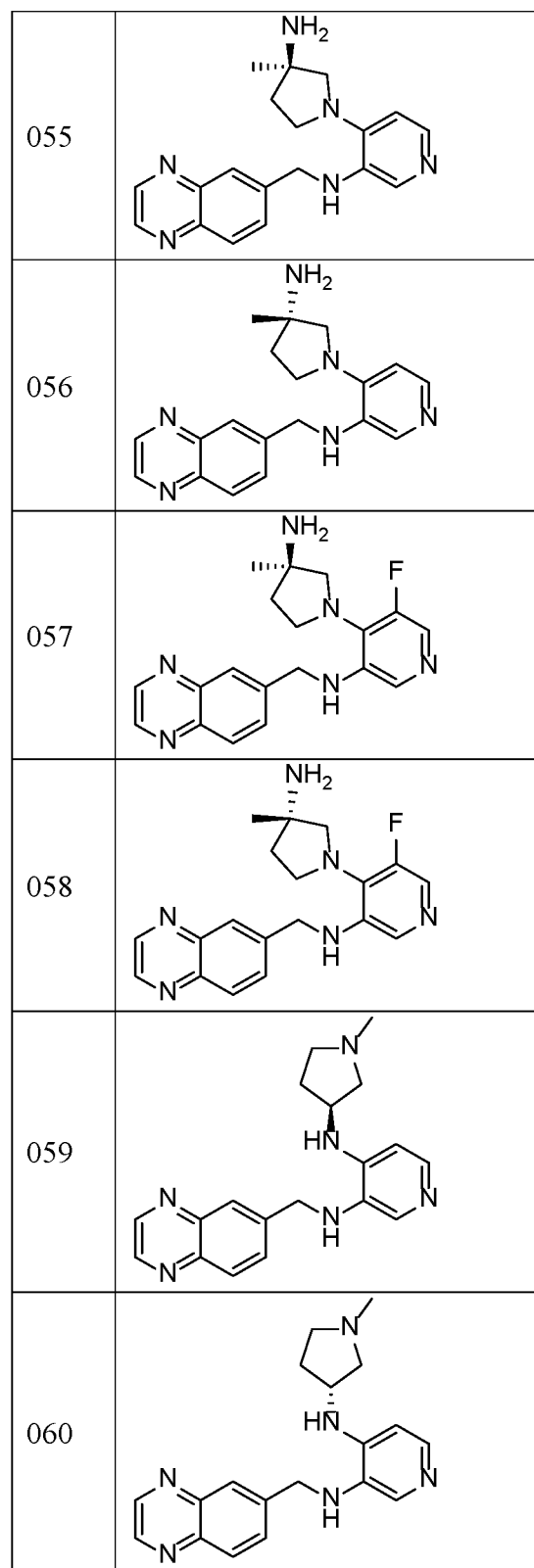
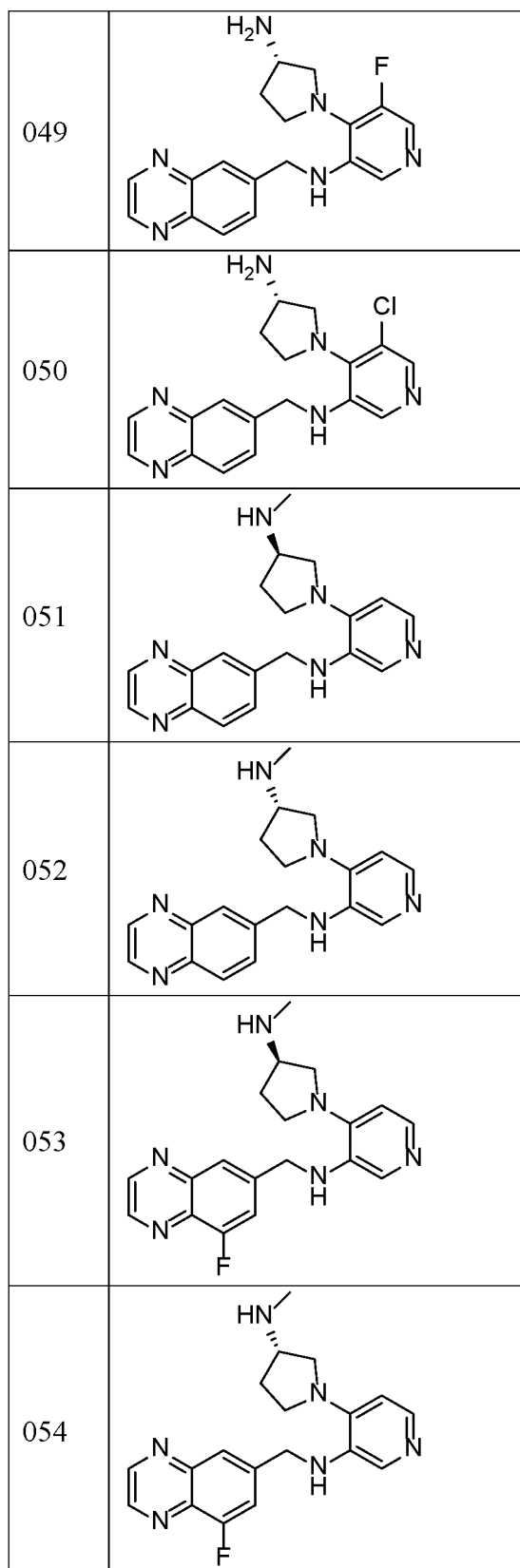


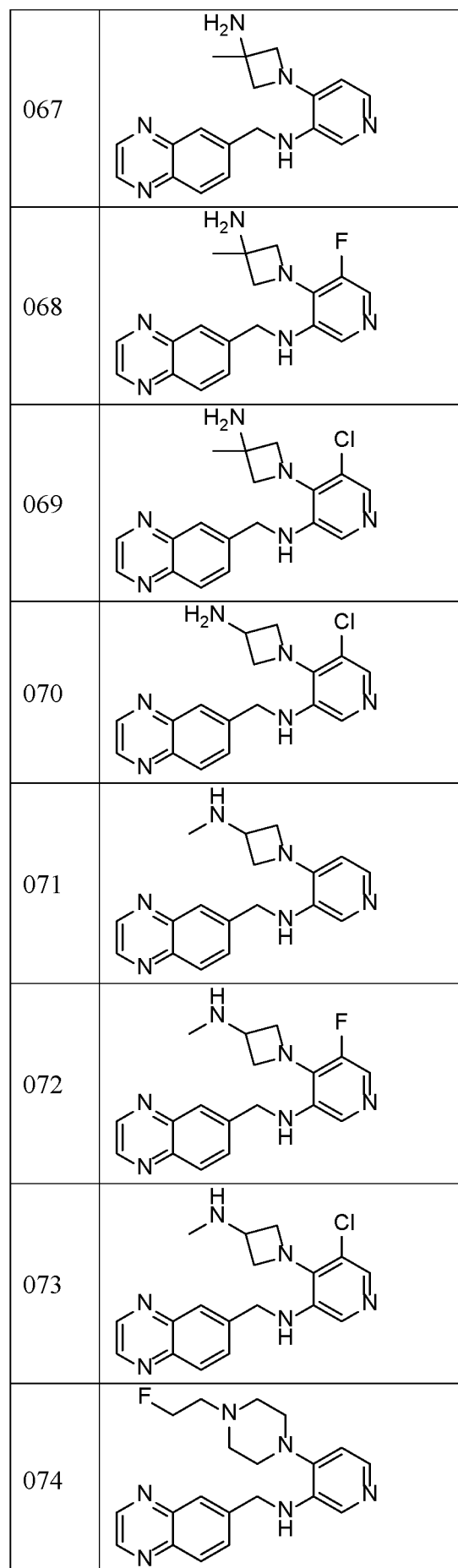
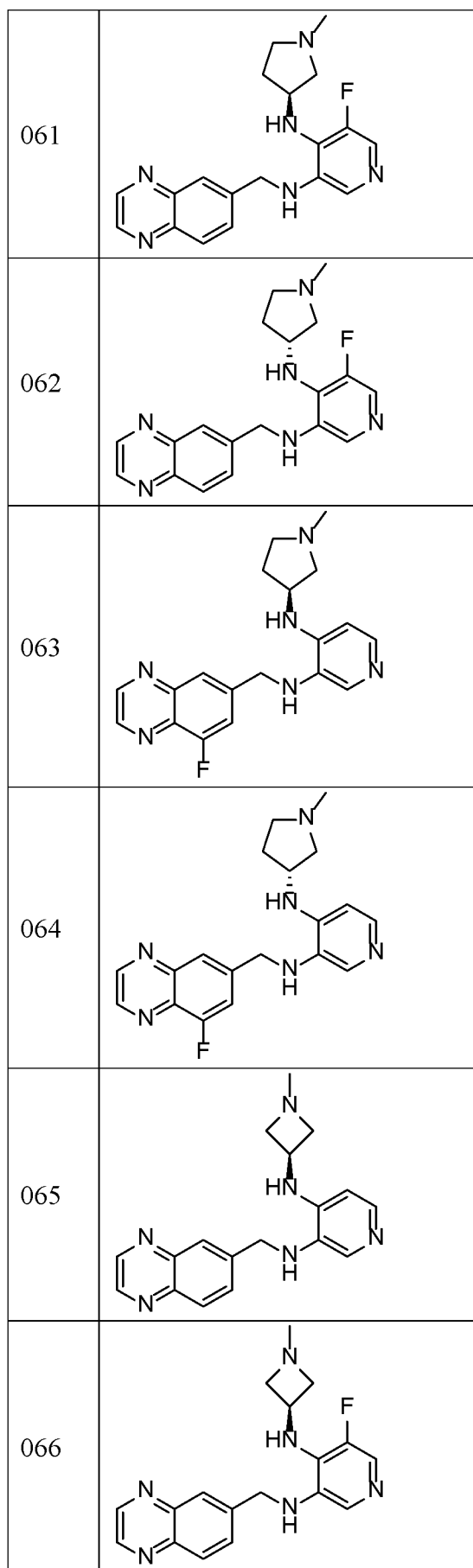
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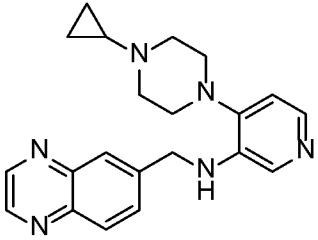
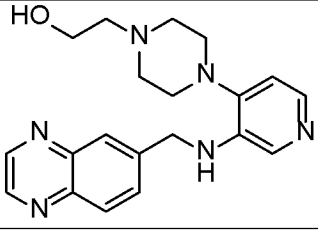
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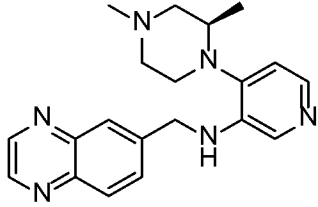
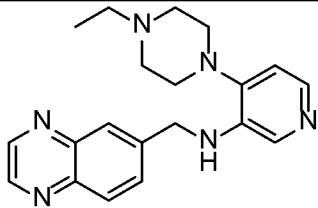
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Figure 1A

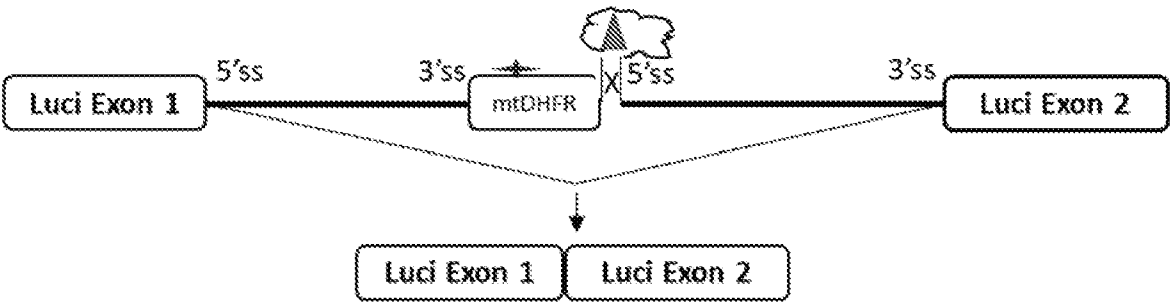


Figure 1B

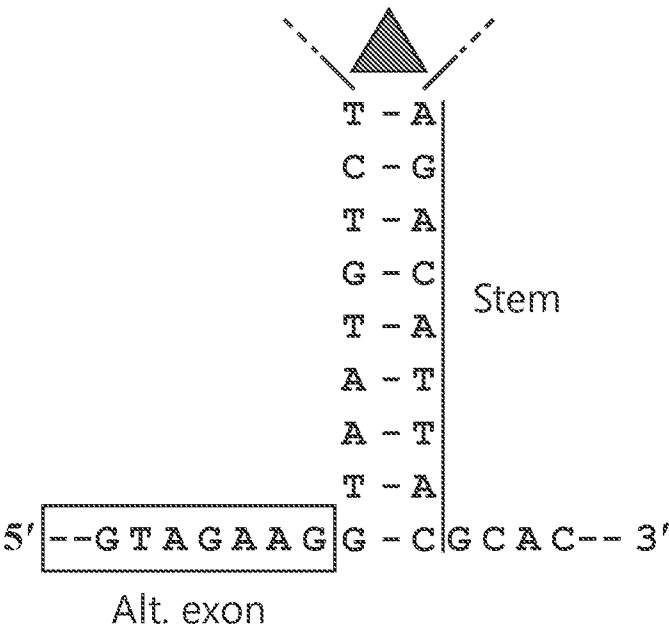
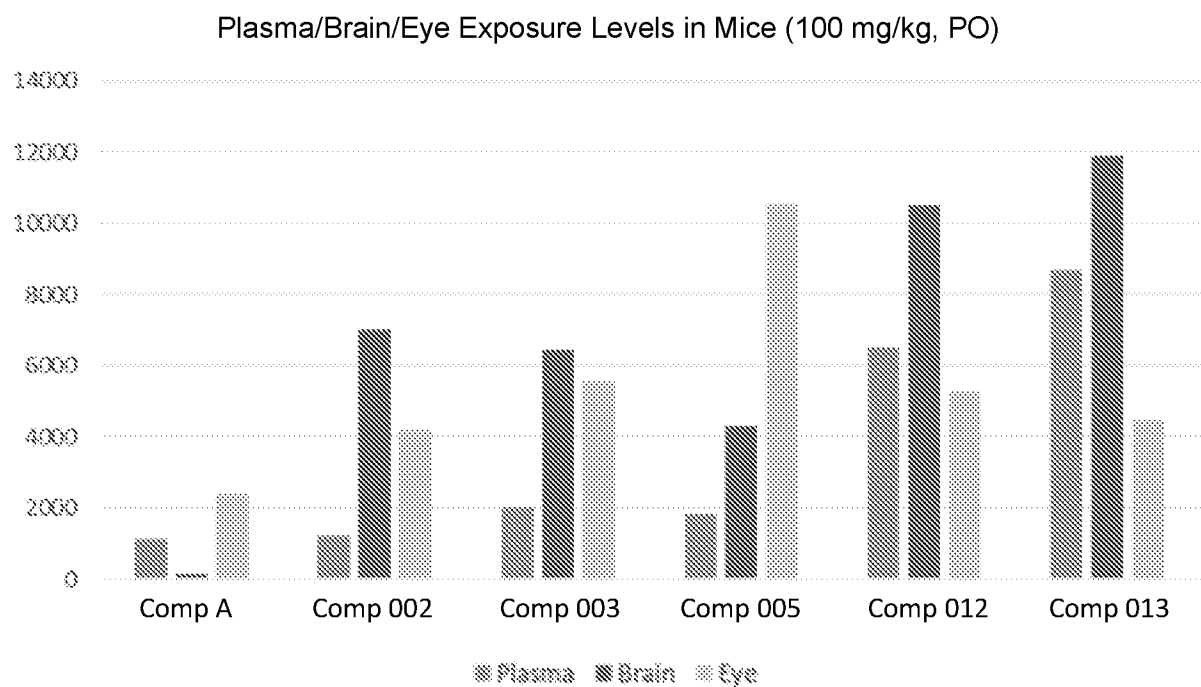
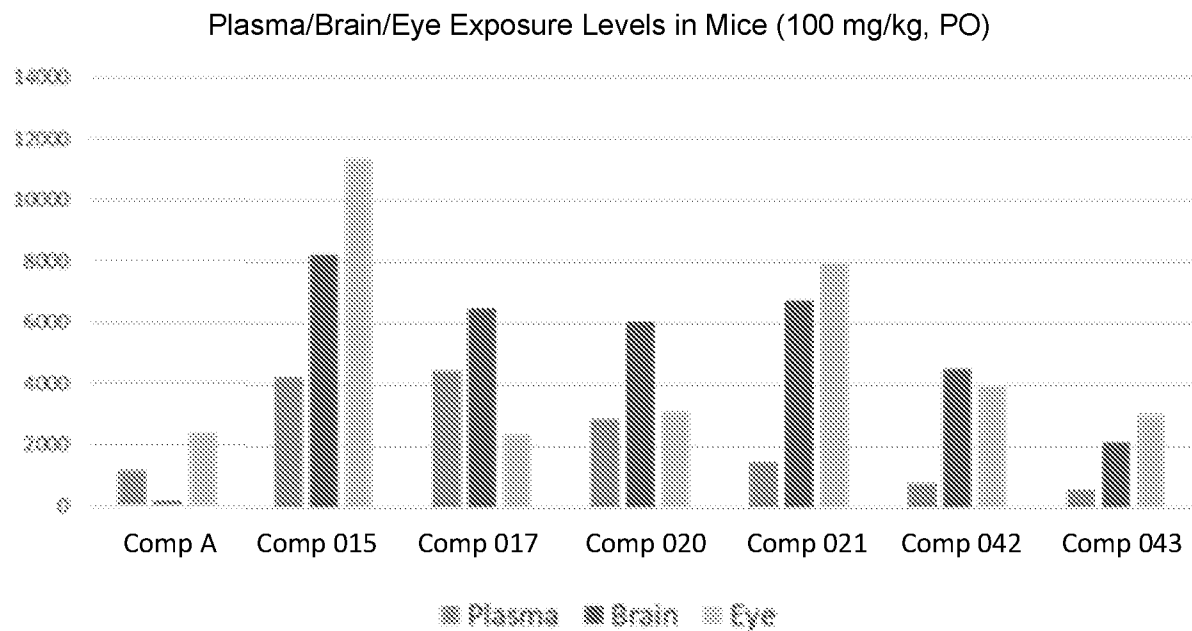


Figure 2A



	Comp. A	Comp 002	Comp 003	Comp 005	Comp 012	Comp 013
Plasma	1145.1	1243	2050	1830	6537	8713
Brain	183.48	7017	6453	4333	10517	11883
Eye	2402.4	4197	5565	10550	5308	4462
B/P ratio	0.2	5.6	3.1	2.4	1.6	1.4
E/P ratio	2.1	3.4	2.7	5.8	0.8	0.5
Fold Activation	58.1	8.3	3.5	1.5	3.8	7.6

Figure 2B



	Comp. A	Comp 015	Comp 017	Comp 020	Comp 021	Comp 042	Comp 043
Plasma	1145.1	4270	4490	2917	1490	810	624
Brain	183.48	8233	6483	6083	6767	4538	2167
Eye	2402.4	11350	2408	3163	7967	3978	3065
B/P ratio	0.2	1.9	1.4	2.1	4.5	5.6	3.5
E/P ratio	2.1	2.7	0.5	1.1	5.3	4.9	4.9
Fold Activation	58.1	6.8	3.9	2.8	4.7	5.7	4.3

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/000314

A. CLASSIFICATION OF SUBJECT MATTER		
INV. C07D401/12 C07D403/12 C07D401/14 C07D403/14 A61P31/12 A61K31/498		
ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) C07D		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, CHEM ABS Data, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2021/026245 A1 (UNIV NORTH CAROLINA CHAPEL HILL [US]) 11 February 2021 (2021-02-11) cited in the application claims 6,12,18,22-35 -----	1 - 15
A	Zeller Meredith J ET AL: "SHAPE-enabled fragment-based ligand discovery for RNA", , 13 May 2022 (2022-05-13), XP093207935, DOI: 10.1073/pnas.2122660119/-/DCSupplemental Retrieved from the Internet: URL:https://www.pnas.org/doi/pdf/10.1073/p nas.2122660119 the whole document ----- -/-	1 - 15
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 24 September 2024		Date of mailing of the international search report 09/10/2024
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Schuemacher, Anne

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2024/000314

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SURESH BLESSY M. ET AL: "Fragment-Based Approaches to Identify RNA Binders", JOURNAL OF MEDICINAL CHEMISTRY, vol. 66, no. 10, 25 May 2023 (2023-05-25), pages 6523-6541, XP093207947, US ISSN: 0022-2623, DOI: 10.1021/acs.jmedchem.3c00034 Retrieved from the Internet: URL:https://pubs.acs.org/doi/pdf/10.1021/acs.jmedchem.3c00034> figure 9; compound 38 -----	1 - 15
X,P	WO 2023/111686 A2 (MEIRAGTX UK II LTD [GB]) 22 June 2023 (2023-06-22) claims 44-61 -----	1 - 15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2024/000314

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2024/000314

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