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(54) Title: PHENOTHIAZINES FOR TREATING MITOCHONDRIAL DISEASE

(57) Abstract: The present disclosure describes phenothiazine compounds substituted by a bicyclic nitrogen-containing heterocyclic ring, and compositions and methods thereof. Such compounds can be useful in treating mitochondrial diseases in a subject, such as a human.

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PHENOTHIAZINES FOR TREATING MITOCHONDRIAL DISEASE

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority to US provisional appl. no. 63/374,512, filed September 2, 2022, which is incorporated by reference herein in its entirety for all purposes.

BACKGROUND

[0002] Ferroptosis is an iron-dependent form of regulated cell death that is believed to be caused by the toxic buildup of lipid peroxides on cellular membranes. Studies have linked ferroptosis to various pathological conditions and diseases, such as neurodegenerative diseases.

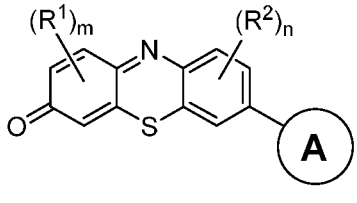
[0003] One class of ferroptosis inhibitors includes radical-trapping antioxidants, which can trap chain-carrying radicals and thereby block propagation of the radical chain reactions during lipid peroxidation. For example, α-tocopherol and ubiquinol are two naturally occurring radical-trapping antioxidants that are capable of inhibiting ferroptosis. Ferrostatin-1 and liproxstatin-1 are radical-trapping antioxidants that were identified from screenings as potent ferroptosis inhibitors.

[0004] Phenothiazines have been described as potentially useful for treating diseases with decreased mitochondrial function, such as neurodegenerative diseases. See, US Patents 10,472,340 and 10,745,366. For instance, in vitro evaluation of such compounds suggests that certain phenothiazines may be useful in increasing frataxin levels in Freidreich's ataxia cells. See, Khdour, et al. Med. Chem. Commun. 2018, 9, 1491.

[0005] There is a need to develop ferroptosis inhibitors that have favorable physicochemical and pharmacokinetic properties in order to treat diseases associated with excessive ferroptosis.

BRIEF SUMMARY

[0006] In some embodiments, the compound of the present disclosure is a compound of Formula I:



(I),

or a pharmaceutically acceptable salt thereof,

wherein

each R^1 is independently -F, -Cl, -Br, -I, -OR^a, -SR^a, -NR^aR^b, -NO_2, -CN, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl, wherein the alkyl, alkenyl, or alkynyl is substituted with 0, 1, 2, or 3 groups independently selected from -F, -Cl, -Br, -I, -OR^a, -SR^a, -NR^aR^b, oxo, -NO_2, and -CN;

each R^2 is independently -F, -Cl, -Br, -I, -OH, -OR^a, -SR^a, -NR^aR^b, -CN, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl, wherein the alkyl, alkenyl, or alkynyl is substituted with 0, 1, 2, or 3 groups independently selected from -F, -Cl, -Br, -I, -OR^a, -SR^a, -NR^aR^b, oxo, -NO_2, and -CN;

each R^a is independently H or C_{1-6} alkyl;

each R^b is independently H or C_{1-6} alkyl;

the subscript m is 0, 1, 2 or 3;

the subscript n is 0, 1, 2, or 3; and

A is a bicyclic nitrogen-containing heterocyclic ring.

[0007] In some embodiments, a pharmaceutical composition comprises a compound of the present disclosure or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

[0008] In some embodiments, a method of inhibiting ferroptosis in a cell comprises administering to the cell an effective amount of a compound of the present disclosure or pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the present disclosure.

[0009] In some embodiments, a method of treating a mitochondrial disease of the present disclosure comprises administering to a subject in need thereof a therapeutically effective amount of a compound of the present disclosure or pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the present disclosure.

DETAILED DESCRIPTION

I. GENERAL

[0010] The present disclosure describes phenothiazine compounds substituted by a bicyclic nitrogen-containing heterocyclic ring, and compositions and methods thereof. Such compounds can be useful in treating mitochondrial diseases in a subject, such as a human.

II. DEFINITIONS

[0011] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

[0012] "About" when referring to a value includes the stated value +/- 10% of the stated value. For example, about 50% includes a range of from 45% to 55%, while about 20 molar equivalents includes a range of from 18 to 22 molar equivalents. Accordingly, when referring to a range, "about" refers to each of the stated values +/- 10% of the stated value of each end of the range. For instance, a ratio of from about 1 to about 3 (weight/weight) includes a range of from 0.9 to 3.3.

[0013] "Alkyl" is a linear or branched saturated monovalent or divalent hydrocarbon. For example, an alkyl group can have 1 to 10 carbon atoms (i.e., C_{1-10} alkyl) or 1 to 8 carbon atoms (i.e., C_{1-8} alkyl) or 1 to 6 carbon atoms (i.e., C_{1-6} alkyl) or 1 to 4 carbon atoms (i.e., (C_{1-4} alkyl). Examples of alkyl groups include, but are not limited to, methyl (Me, $-CH_3$), ethyl (Et, $-CH_2CH_3$), 1-propyl (*n*-Pr, *n*-propyl, $-CH_2CH_2CH_3$), 2-propyl (*i*-Pr, *i*-propyl, $-CH(CH_3)_2$), 1-butyl (*n*-Bu, *n*-butyl, $-CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (*i*-Bu, *i*-butyl, $-CH_2CH(CH_3)_2$), 2-butyl (*s*-Bu, *s*-butyl, $-CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (*t*-Bu, *t*-butyl, $-C(CH_3)_3$), 1-pentyl (*n*-pentyl, $-CH_2CH_2CH_2CH_2CH_3$), 2-pentyl ($-CH(CH_3)CH_2CH_2CH_3$), 3-pentyl ($-CH(CH_2CH_3)_2$), 2-methyl-2-butyl ($-C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl ($-CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl ($-CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl ($-CH_2CH(CH_3)CH_2CH_3$), 1-hexyl ($-CH_2CH_2CH_2CH_2CH_2CH_3$), 2-hexyl ($-CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl ($-CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-methyl-2-pentyl ($-C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl ($-CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl ($-CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl ($-C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl ($-CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl ($-C(CH_3)_2CH(CH_3)_2$), 3,3-dimethyl-2-butyl ($-CH(CH_3)C(CH_3)_3$, and octyl ($-(CH_2)_7CH_3$).

[0014] "Alkenyl" refers to a straight chain or branched hydrocarbon having at least 2 carbon atoms and at least one double bond. Alkenyl can include any number of carbons, such as C_2, C_{2-3}, C_{2-4}, C_{2-5}, C_{2-6}, C_{2-7}, C_{2-8}, C_{2-9}, C_{2-10}, C_3, C_{3-4}, C_{3-5}, C_{3-6}, C_4, C_{4-5}, C_{4-6}, C_5, C_{5-6}, and C_6. Alkenyl groups can have any suitable number of double bonds, including, but not limited to, 1, 2, 3, 4, 5 or more. Examples of alkenyl groups include, but are not limited to, vinyl (ethenyl), propenyl, isopropenyl, 1-butenyl, 2-butenyl, isobutenyl, butadienyl, 1-pentenyl, 2-pentenyl, isopentenyl, 1,3-pentadienyl, 1,4-pentadienyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,5-hexadienyl, 2,4-hexadienyl, or 1,3,5-hexatrienyl. Alkenyl groups can be substituted or unsubstituted.

[0015] "Alkynyl" refers to either a straight chain or branched hydrocarbon having at least 2 carbon atoms and at least one triple bond. Alkynyl can include any number of carbons, such as C_2, C_{2-3}, C_{2-4}, C_{2-5}, C_{2-6}, C_{2-7}, C_{2-8}, C_{2-9}, C_{2-10}, C_3, C_{3-4}, C_{3-5}, C_{3-6}, C_4, C_{4-5}, C_{4-6}, C_5, C_{5-6}, and C_6. Examples of alkynyl groups include, but are not limited to, acetylenyl, propynyl, 1-butynyl, 2-butynyl, butadiynyl, 1-pentynyl, 2-pentynyl, isopentynyl, 1,3-pentadiynyl, 1,4-pentadiynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 1,3-hexadiynyl, 1,4-hexadiynyl, 1,5-hexadiynyl, 2,4-hexadiynyl, or 1,3,5-hexatriynyl. Alkynyl groups can be substituted or unsubstituted.

[0016] "Cycloalkyl" refers to a single saturated or partially unsaturated all carbon ring having 3 to 20 annular carbon atoms (i.e., C_{3-20} cycloalkyl), for example from 3 to 12 annular atoms, for example from 3 to 10 annular atoms, or 3 to 8 annular atoms, or 3 to 6 annular atoms, or 3 to 5 annular atoms, or 3 to 4 annular atoms. The term "cycloalkyl" also includes multiple condensed, saturated and partially unsaturated all carbon ring systems (e.g., ring systems comprising 2, 3 or 4 carbocyclic rings). Accordingly, cycloalkyl includes multicyclic carbocycles such as a bicyclic carbocycles (e.g., bicyclic carbocycles having about 6 to 12 annular carbon atoms such as bicyclo[3.1.0]hexane and bicyclo[2.1.1]hexane), and polycyclic carbocycles (e.g. tricyclic and tetracyclic carbocycles with up to about 20 annular carbon atoms). The rings of a multiple condensed ring system can be connected to each other via fused, spiro and bridged bonds when allowed by valency requirements. Non-limiting examples of monocyclic cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl and 1-cyclohex-3-enyl.

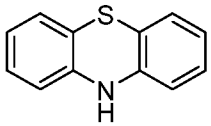
[0017] "Heterocyclyl" or "heterocycle" or "heterocycloalkyl" as used herein refers to a single saturated or partially unsaturated non-aromatic ring or a non-aromatic multiple ring system that has at least one heteroatom in the ring (i.e., at least one annular heteroatom selected from oxygen, nitrogen, and sulfur). Unless otherwise specified, a heterocyclyl group has from 3 to about 20 annular atoms, for example from 3 to 12 annular atoms, for example from 3 to 10 annular atoms, or 3 to 8 annular atoms, or 3 to 6 annular atoms, or 3 to 5 annular atoms, or 4 to 6 annular atoms, or 4 to 5 annular atoms. Thus, the term includes single saturated or partially unsaturated rings (e.g., 3, 4, 5, 6 or 7-membered rings) having from about 1 to 6 annular carbon atoms and from about 1 to 3 annular heteroatoms selected from the group consisting of oxygen, nitrogen and sulfur in the ring. The rings of the multiple condensed ring (e.g. bicyclic heterocyclyl) system can be connected to each other via fused, spiro and bridged bonds when allowed by valency requirements. Heterocycles include, but are not limited to, azetidine, aziridine, imidazolidine, morpholine, oxirane (epoxide), oxetane, thietane, piperazine, piperidine, pyrazolidine, piperidine, pyrrolidine, pyrrolidinone, tetrahydrofuran, tetrahydrothiophene, dihydropyridine, tetrahydropyridine, quinuclidine,, 2-oxa-6-azaspiro[3.3]heptan-6-yl, 6-oxa-1-azaspiro[3.3]heptan-1-yl, 2-thia-6-azaspiro[3.3]heptan-6-yl, 2,6-diazaspiro[3.3]heptan-2-yl, 2-azabicyclo[3.1.0]hexan-2-yl, 3-azabicyclo[3.1.0]hexanyl, 2-azabicyclo[2.1.1]hexanyl, 2-azabicyclo[2.2.1]heptan-2-yl, 4-azaspiro[2.4]heptanyl, 5-azaspiro[2.4]heptanyl, and the like. The heterocycle can be unsubstituted or substituted.

[0018] A "bicyclic nitrogen-containing heterocyclic ring" as used herein is a bicyclic heterocycle that has at least one nitrogen within the ring. The bicyclic nitrogen-containing heterocyclic ring can be bridged, spiro, or fused. Exemplary bicyclic nitrogen-containing heterocycles include 3,8-diazabicyclo[3.2.1]octane, 2,5-dimethyl-2,5-diazabicyclo[2.2.2]octane, 3,9-diazabicyclo[3.3.1]nonane, 2,6-diazaspiro[3.3]heptane, and 2,5-dimethyloctahydro-1H-pyrrolo[3,4-c]pyridine. The bicyclic nitrogen-containing heterocyclic ring can be unsubstituted or substituted.

[0019] "Heteroaryl" as used herein refers to a single aromatic ring that has at least one atom other than carbon in the ring, wherein the atom is selected from the group consisting of oxygen, nitrogen and sulfur; "heteroaryl" also includes multiple condensed ring systems that have at least one such aromatic ring, which multiple condensed ring systems are further described below. Thus, "heteroaryl" includes single aromatic rings of from about 1 to 6 carbon atoms and about 1-4 heteroatoms selected from the group consisting of oxygen,

nitrogen and sulfur. The sulfur and nitrogen atoms may also be present in an oxidized form provided the ring is aromatic. Exemplary heteroaryl ring systems include but are not limited to pyridyl, pyrimidinyl, oxazolyl or furyl. "Heteroaryl" also includes multiple condensed ring systems (e.g., ring systems comprising 2, 3 or 4 rings) wherein a heteroaryl group, as defined above, is condensed with one or more rings selected from heteroaryls (to form for example 1,8-naphthyridinyl), heterocycles, (to form for example 1,2,3,4-tetrahydro-1,8-naphthyridinyl), carbocycles (to form for example 5,6,7,8-tetrahydroquinolyl) and aryls (to form for example indazolyl) to form the multiple condensed ring system. Thus, a heteroaryl (a single aromatic ring or multiple condensed ring system) has about 1-20 carbon atoms and about 1-6 heteroatoms within the heteroaryl ring. Such multiple condensed ring systems may be optionally substituted with one or more (e.g., 1, 2, 3 or 4) oxo groups on the carbocycle or heterocycle portions of the condensed ring. The rings of the multiple condensed ring system can be connected to each other via fused, spiro and bridged bonds when allowed by valency requirements. It is to be understood that the individual rings of the multiple condensed ring system may be connected in any order relative to one another. It is to be understood that the point of attachment for a heteroaryl or heteroaryl multiple condensed ring system can be at any suitable atom of the heteroaryl or heteroaryl multiple condensed ring system including a carbon atom and a heteroatom (e.g., a nitrogen). It also to be understood that when a reference is made to a certain atom-range membered heteroaryl (e.g., a 5 to 10 membered heteroaryl), the atom range is for the total ring atoms of the heteroaryl and includes carbon atoms and heteroatoms. For example, a 5-membered heteroaryl would include a thiazolyl and a 10-membered heteroaryl would include a quinolinyl. Exemplary heteroaryls include but are not limited to pyridyl, pyrrolyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrazolyl, thienyl, indolyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, furyl, oxadiazolyl, thiadiazolyl, quinolyl, isoquinolyl, benzothiazolyl, benzoxazolyl, indazolyl, quinoxalyl, quinazolyl, 5,6,7,8-tetrahydroisoquinolinyl benzofuranyl, benzimidazolyl, thianaphthenyl, pyrrolo[2,3-b]pyridinyl, quinazolinyl-4(3H)-one, phenothiazinyl, and triazolyl. The heteroaryl can be substituted or unsubstituted.

[0020] "Phenothiazine" as used herein refers to 10*H*-phenothiazine, having the structure:



,

and tautomers thereof.

[0021] "Tautomer" refers to alternate forms of a compound that differ in the position of a proton, such as enol-keto and imine-enamine tautomers, or the tautomeric forms of heteroaryl groups containing a ring atom attached to both a ring -NH- and a ring =N- such as pyrazoles, imidazoles, benzimidazoles, triazoles, and tetrazoles.

[0022] A "compound of the present disclosure" includes compounds disclosed herein, for example a compound of the present disclosure includes compounds of Formula I and II, including the compounds of the Examples.

[0023] "Pharmaceutically acceptable" or "physiologically acceptable" refer to compounds, salts, compositions, dosage forms and other materials which are useful in preparing a pharmaceutical composition that is suitable for veterinary or human pharmaceutical use.

[0024] "Pharmaceutically effective amount" refers to an amount of the compound of the present disclosure in a formulation or combination thereof, that provides the desired therapeutic or pharmaceutical result.

[0025] "Pharmaceutically acceptable excipient" includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans or domestic animals.

[0026] "Treatment" or "treat" or "treating" as used herein refers to an approach for obtaining beneficial or desired results. For purposes of the present disclosure, beneficial or desired results include, but are not limited to, alleviation of a symptom and/or diminishment of the extent of a symptom and/or preventing a worsening of a symptom associated with a disease or condition. In one embodiment, "treatment" or "treating" includes one or more of the following: a) inhibiting the disease or condition (*e.g.*, decreasing one or more symptoms resulting from the disease or condition, and/or diminishing the extent of the disease or condition); b) slowing or arresting the development of one or more symptoms associated with the disease or condition (*e.g.*, stabilizing the disease or condition, delaying the worsening or progression of the disease or condition); and c) relieving the disease or condition, *e.g.*, causing the regression of clinical symptoms, ameliorating the disease state, delaying the progression of the disease, increasing the quality of life, and/or prolonging survival.

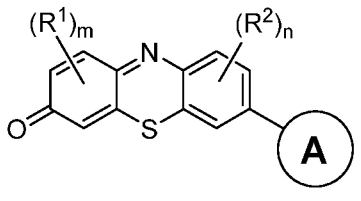
[0027] "Therapeutically effective amount" or "effective amount" as used herein refers to an amount that is effective to elicit the desired biological or medical response, including the amount of the compound that, when administered to a subject for treating a disease, is sufficient to effect such treatment for the disease. The effective amount will vary depending on the compound, the disease, and its severity and the age, weight, etc., of the subject to be treated. The effective amount can include a range of amounts. As is understood in the art, an effective amount may be in one or more doses, *i.e.*, a single dose or multiple doses may be required to achieve the desired treatment endpoint. An effective amount may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable or beneficial result may be or is achieved. Suitable doses of any co-administered compounds may optionally be lowered due to the combined action (*e.g.*, additive or synergistic effects) of the compounds.

[0028] "Subject" is any mammal, such as a mouse, a rat, a dog, a cat, including veterinary animals, such as a goat, a pig, a horse, a cow, or a donkey, and primates, such as non-human primates, e.g., a cynomolgous monkey, rhesus monkey, or chimpanzee, as well as humans. In some embodiments, the subject is a human. In some embodiments, a subject is a patient.

III. COMPOUNDS

[0029] The compounds of the present disclosure are substituted phenothiazines. Such compounds are generally useful in inhibiting ferroptosis, thereby inhibiting cell death associated with certain mitochondrial diseases. Without wishing to be bound by theory, the compound of the present disclosure, such as a compound of Formula I, possesses a bicyclic nitrogen-containing heterocyclic ring that is believed to sufficiently disrupt the crystal packing of the flat phenothiazine ring, thereby affording superior properties such as improved solubility, permeability, and bioavailability compared to compounds in the art.

[0030] In some embodiments, the compound of the present disclosure is a compound of Formula I:



(I),

or a pharmaceutically acceptable salt thereof,

wherein

each R^1 is independently -F, -Cl, -Br, -I, $-OR^a$, $-SR^a$, $-NR^aR^b$, $-NO_2$, -CN, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl, wherein the alkyl, alkenyl, or alkynyl is substituted with 0, 1, 2, or 3 groups independently selected from -F, -Cl, -Br, -I, $-OR^a$, $-SR^a$, $-NR^aR^b$, oxo, $-NO_2$, and -CN;

each R^2 is independently -F, -Cl, -Br, -I, -OH, $-OR^a$, $-SR^a$, $-NR^aR^b$, -CN, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl, wherein the alkyl, alkenyl, or alkynyl is substituted with 0, 1, 2, or 3 groups independently selected from -F, -Cl, -Br, -I, $-OR^a$, $-SR^a$, $-NR^aR^b$, oxo, $-NO_2$, and -CN;

each R^a is independently H or C_{1-6} alkyl;

each R^b is independently H or C_{1-6} alkyl;

the subscript m is 0, 1, 2 or 3;

the subscript n is 0, 1, 2, or 3; and

is a bicyclic nitrogen-containing heterocyclic ring.

[0031] In some embodiments of the compound or pharmaceutically acceptable salt thereof, each R^1 is independently -F, -Cl, -Br, -I, $-OR^a$, $-SR^a$, $-NR^aR^b$, -CN, or C_{1-6} alkyl.

[0032] In some embodiments of the compound or pharmaceutically acceptable salt thereof, each R^2 is independently -F, -Cl, -Br, -I, $-OR^a$, $-SR^a$, $-NR^aR^b$, -CN, or C_{1-6} alkyl.

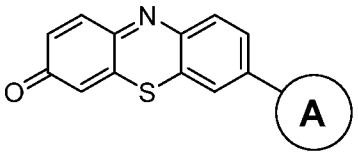
[0033] In some embodiments of the compound or pharmaceutically acceptable salt thereof, each R^a is independently H or C_{1-3} alkyl.

[0034] In some embodiments of the compound or pharmaceutically acceptable salt thereof, each R^b is independently H or C_{1-3} alkyl.

[0035] In some embodiments of the compound or pharmaceutically acceptable salt thereof, the subscript m is 0 or 1.

[0036] In some embodiments of the compound or pharmaceutically acceptable salt thereof, the subscript n is 0 or 1.

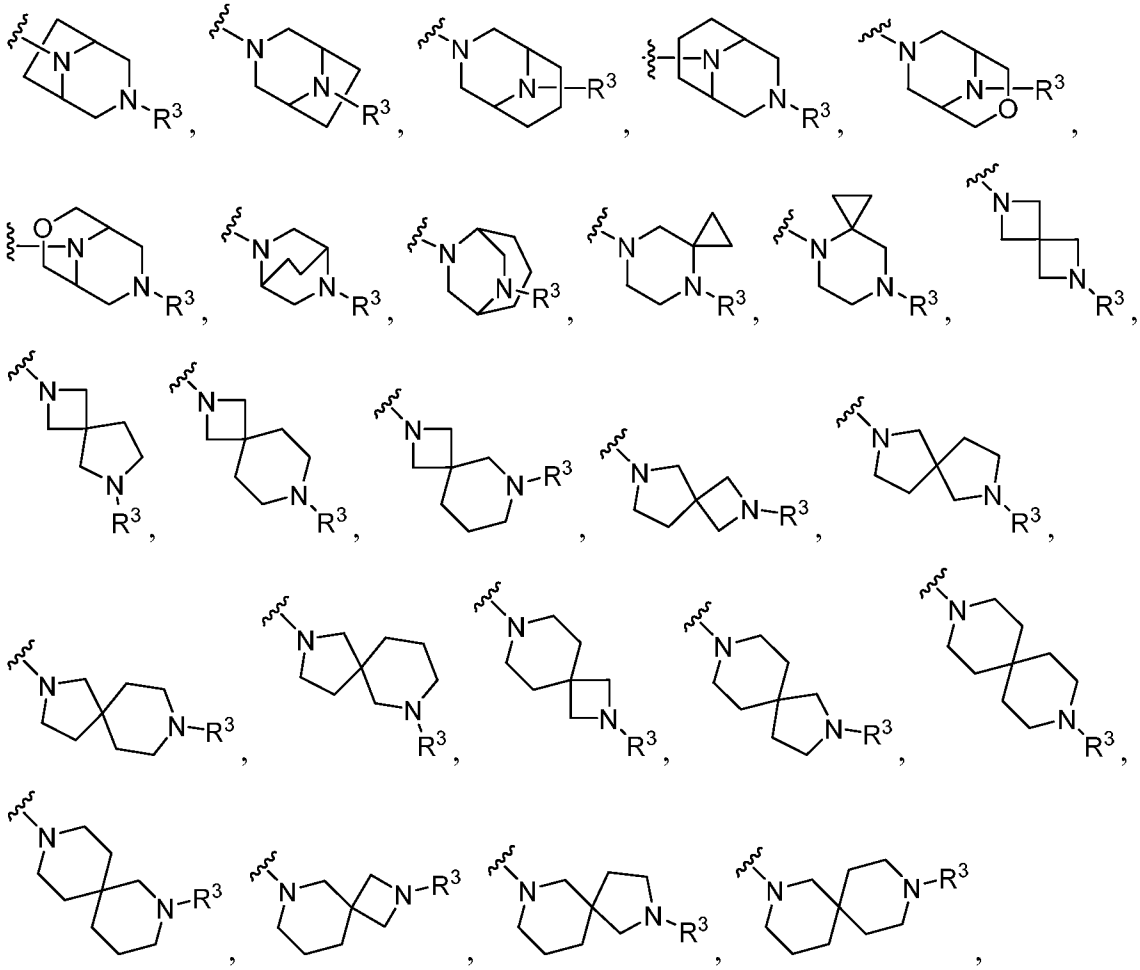
[0037] In some embodiments of the compound or pharmaceutically acceptable salt thereof, the compound has the structure of Formula II:

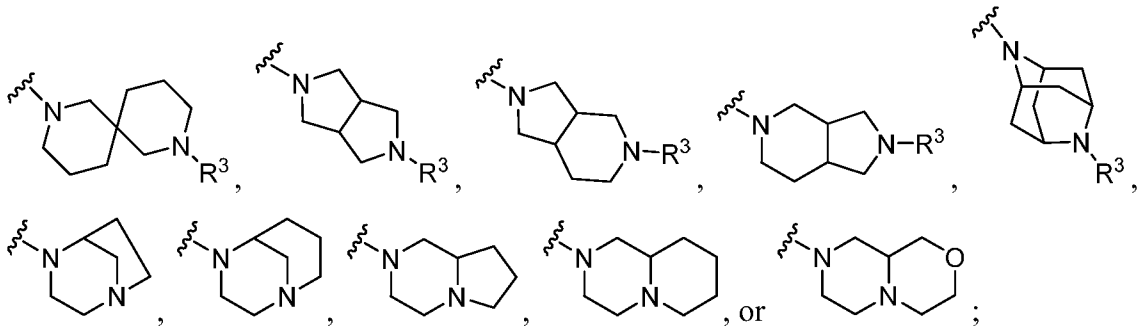

(II).

[0038] In some embodiments of the compound or pharmaceutically acceptable salt thereof, A is a bridged bicyclic nitrogen-containing heterocyclic ring.

[0039] In some embodiments of the compound or pharmaceutically acceptable salt thereof, A is attached to the phenothiazine through a nitrogen.

[0040] In some embodiments of the compound or pharmaceutically acceptable salt thereof, A has the structure:





wherein

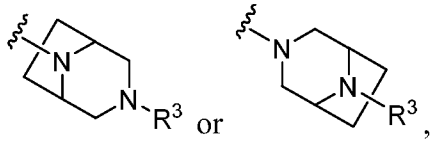
R^3 is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-8} cycloalkyl, 4- to 8-membered heterocyclyl, phenyl, or 5- to 10-membered heteroaryl, wherein the alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, phenyl, or heteroaryl is substituted with 0, 1, 2, or 3 groups independently selected from -F, -Cl, -Br, -I, $-OR^{3a}$, $-SR^{3a}$, $-NR^{3a}R^{3b}$, oxo, $-NO_2$, and –CN;

each R^{3a} is independently H or C_{1-6} alkyl; and

each R^{3b} is independently H or C_{1-6} alkyl.

[0041] In some embodiments of the compound or pharmaceutically acceptable salt thereof,

has the structure:



wherein

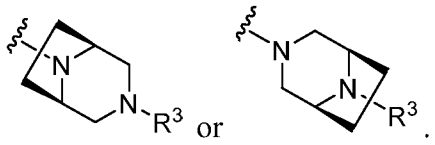
R^3 is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-8} cycloalkyl, 4- to 8-membered heterocyclyl, phenyl, or 5- to 10-membered heteroaryl, wherein the alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, phenyl, or heteroaryl is substituted with 0, 1, 2, or 3 groups independently selected from -F, -Cl, -Br, -I, $-OR^{3a}$, $-SR^{3a}$, $-NR^{3a}R^{3b}$, oxo, $-NO_2$, and –CN;

each R^{3a} is independently H or C_{1-6} alkyl; and

each R^{3b} is independently H or C_{1-6} alkyl.

[0042] In some embodiments of the compound or pharmaceutically acceptable salt thereof,

has the structure:

 or .

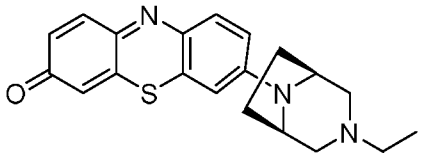
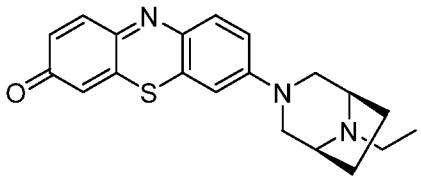
[0043] In some embodiments of the compound or pharmaceutically acceptable salt thereof, the heterocyclyl has 1, 2, or 3 atoms selected from N, O, and S.

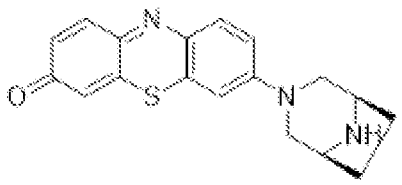
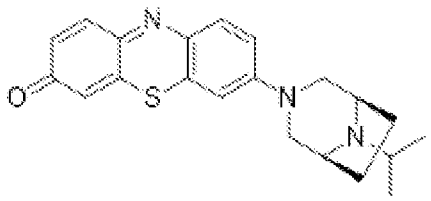
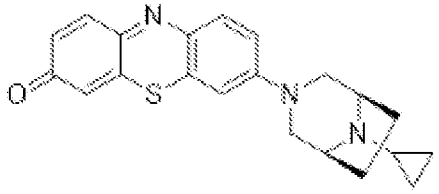
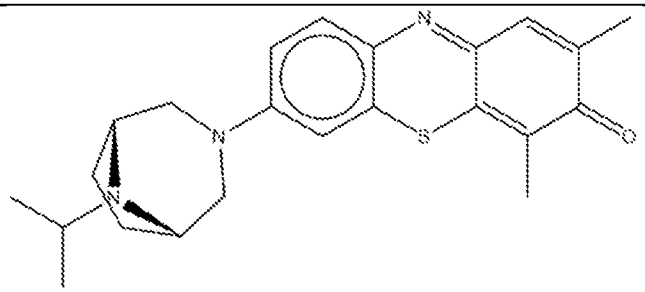
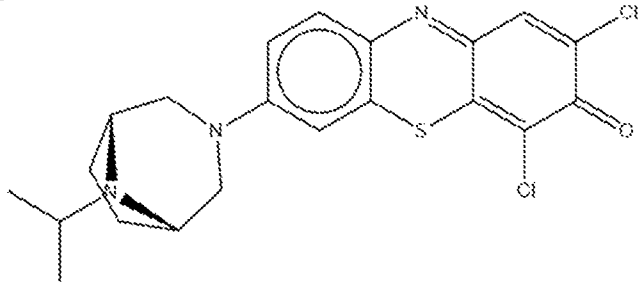
[0044] In some embodiments of the compound or pharmaceutically acceptable salt thereof, the heteroaryl has 1, 2, or 3 atoms selected from N, O, and S.

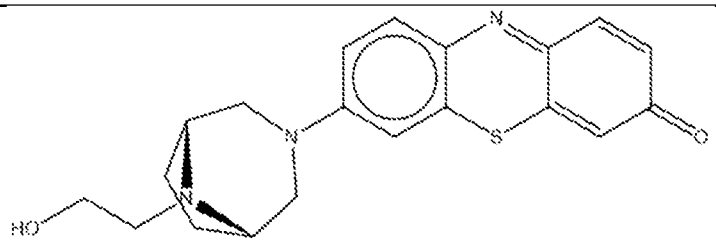
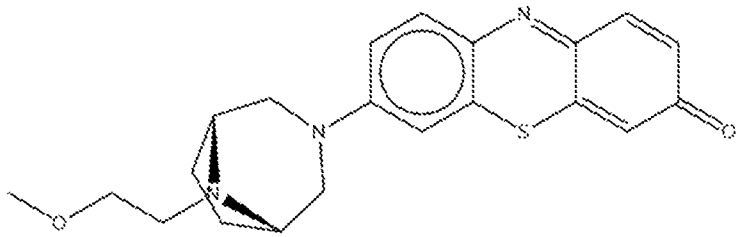
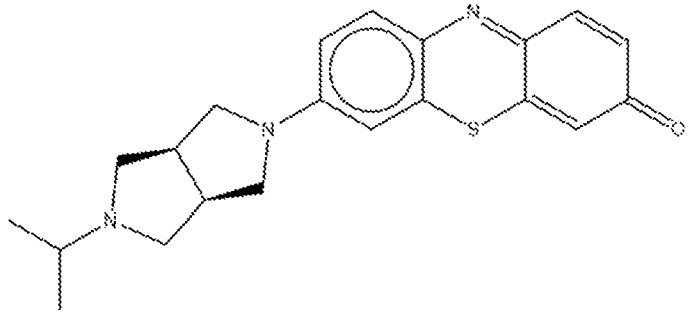
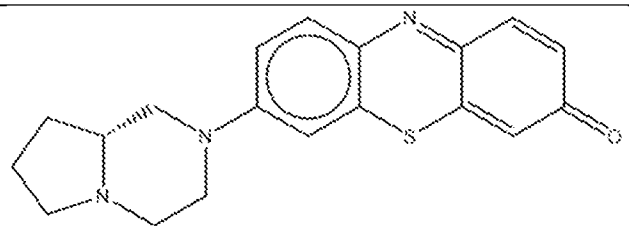
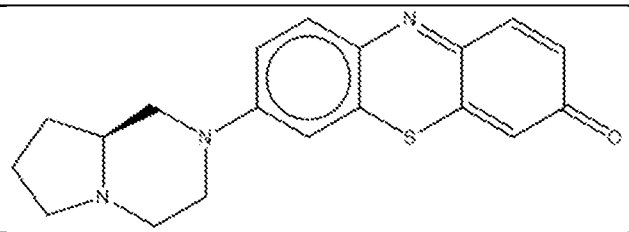
[0045] In some embodiments of the compound or pharmaceutically acceptable salt thereof, R^3 is H, C_{1-6} alkyl, or C_{3-8} cycloalkyl, wherein the alkyl or cycloalkyl is substituted with 0, 1, 2, or 3 groups independently selected from -F, -Cl, -Br, -I, $-OR^{3a}$, $-SR^{3a}$, $-NR^{3a}R^{3b}$, oxo, $-NO_2$, and –CN. In some embodiments of the compound or pharmaceutically acceptable salt thereof, R^3 is H, C_{1-6} alkyl, or C_{3-8} cycloalkyl. In some embodiments, R^3 is H, C_{1-3} alkyl, or C_{3-6} cycloalkyl. In some embodiments, R^3 is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, or cyclopropyl. In some embodiments, R^3 is H, CH_2CH_3, $CH(CH_3)_2$, or cyclopropyl.

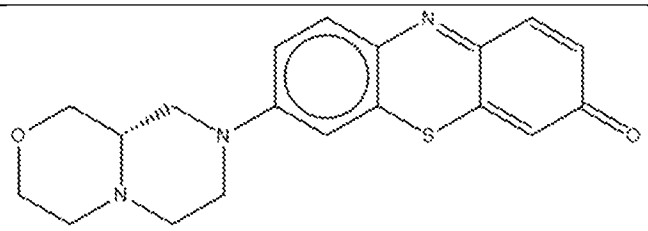
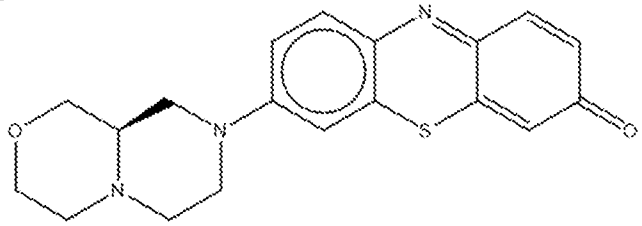
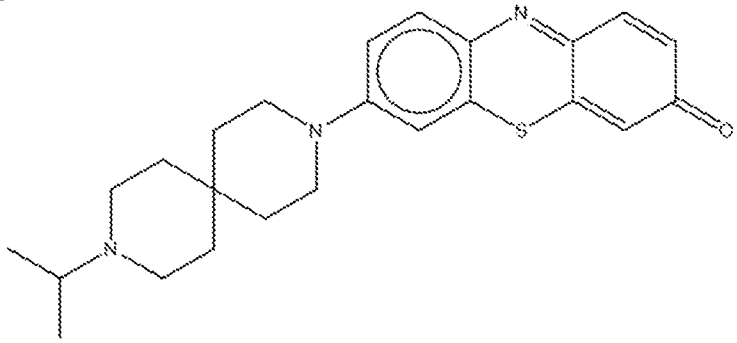
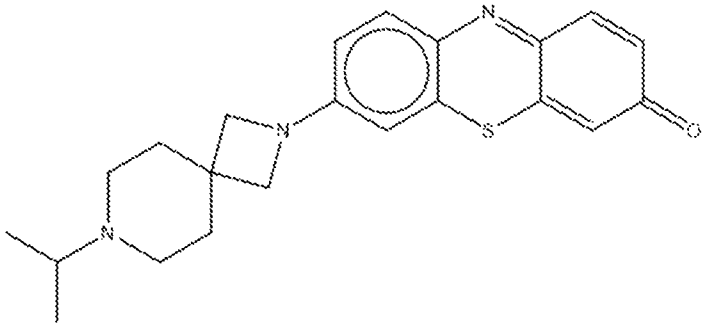
[0046] In some embodiments of a compound of the present disclosure or pharmaceutically acceptable salt thereof, the compound has a structure as shown in Table 1.

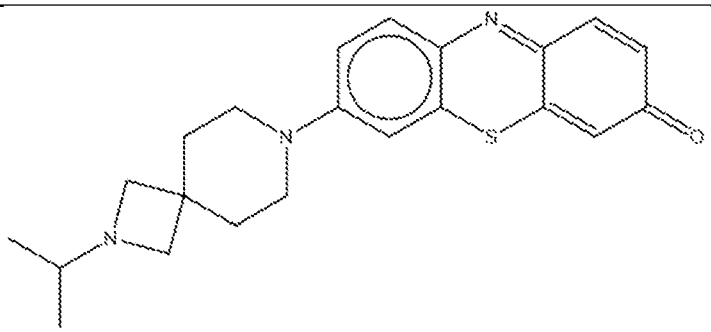
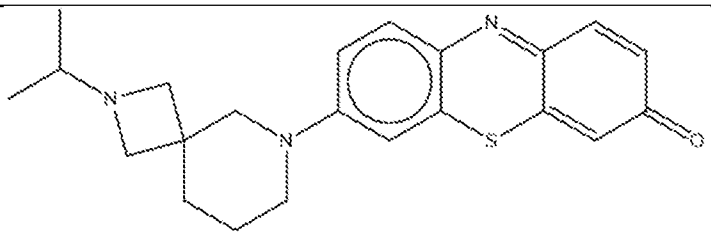
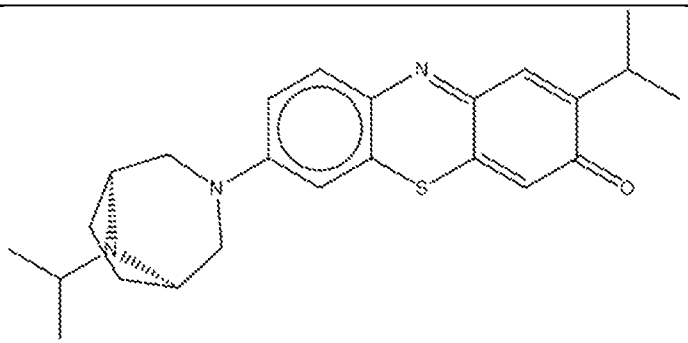
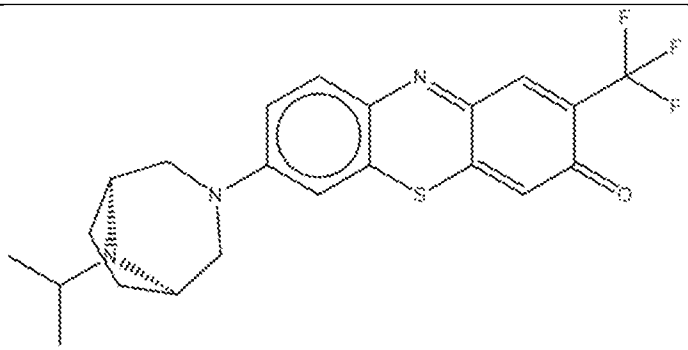
Table 1. Compounds

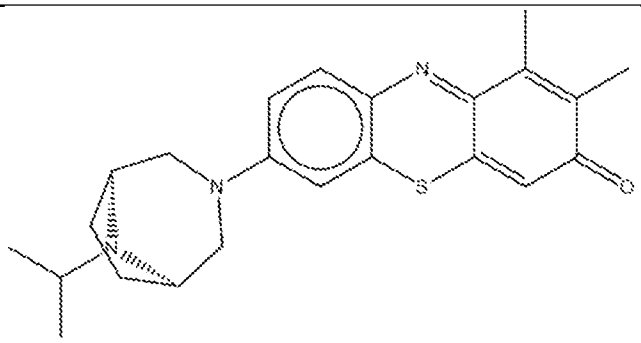
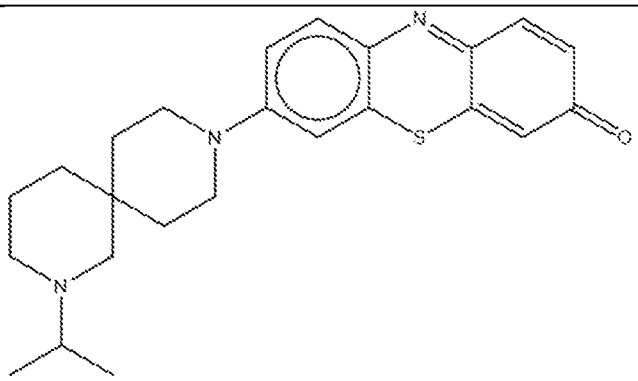
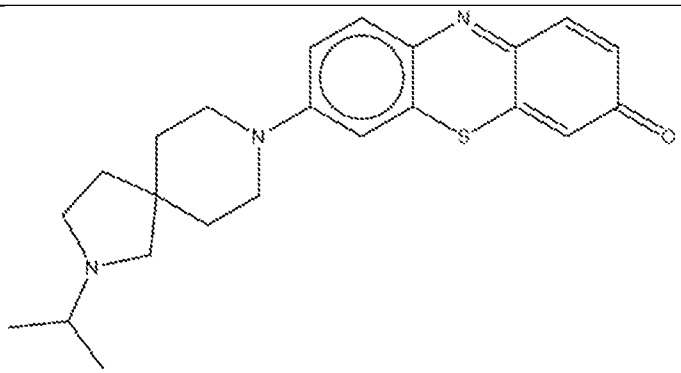
Compound	Structure/Name
1	 7-((1R,5S)-3-ethyl-3,8-diazabicyclo[3.2.1]octan-8-yl)-3H-phenothiazin-3-one
2	 7-((1R,5S)-8-ethyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-3H-phenothiazin-3-one

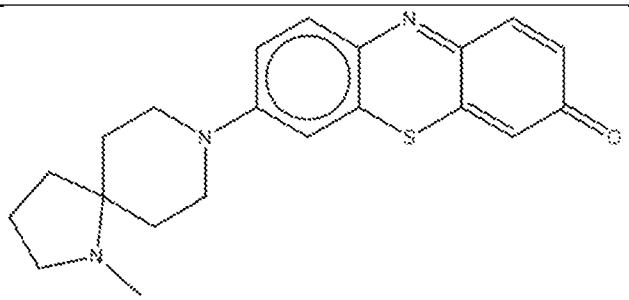
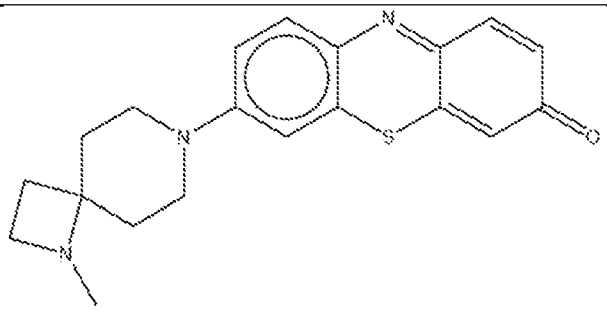
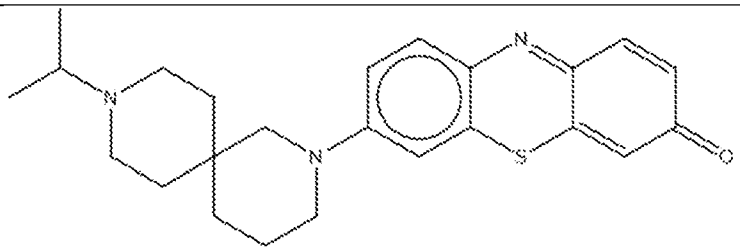
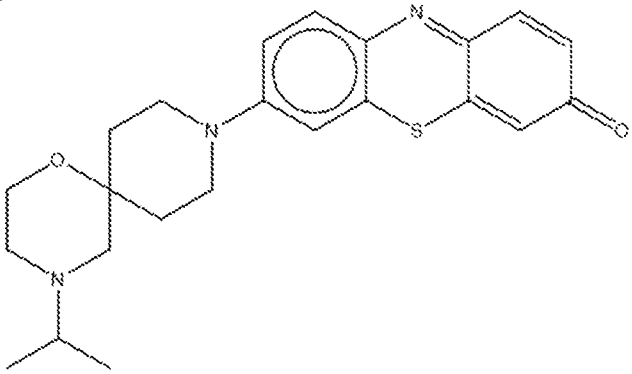
3	 7-((1R,5S)-3,8-diazabicyclo[3.2.1]octan-3-yl)-3H-phenothiazin-3-one
4	 7-((1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-3H-phenothiazin-3-one
5	 7-((1R,5S)-8-cyclopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-3H-phenothiazin-3-one
6	 7-((1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-2,4-dimethyl-3H-phenothiazin-3-one
7	 2,4-dichloro-7-((1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-3H-phenothiazin-3-one

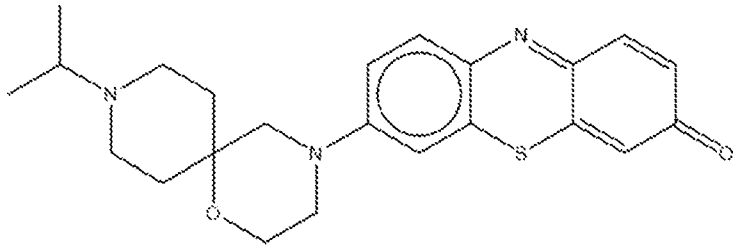
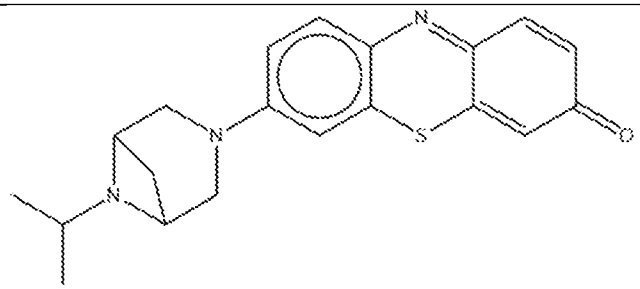
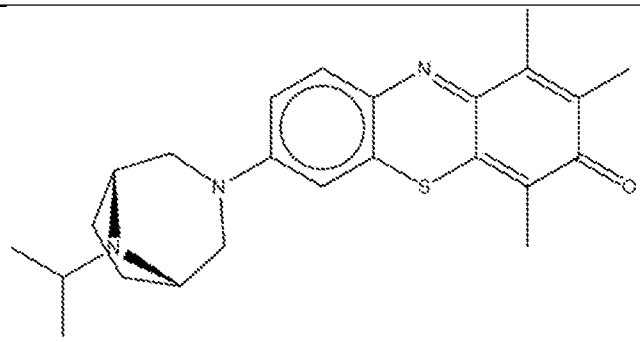
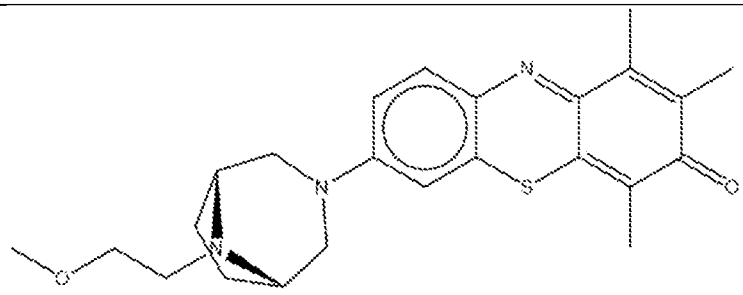
8	 7-((1R,5S)-8-(2-hydroxyethyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-3H-phenothiazin-3-one
9	 7-((1R,5S)-8-(2-methoxyethyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-3H-phenothiazin-3-one
10	 7-((3aR,6aS)-5-isopropylhexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)-3H-phenothiazin-3-one
11	 (R)-7-(hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl)-3H-phenothiazin-3-one
12	

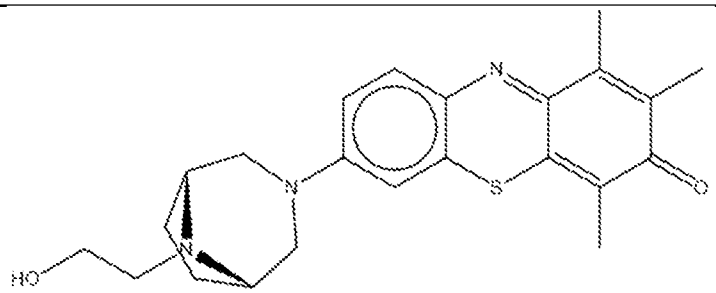
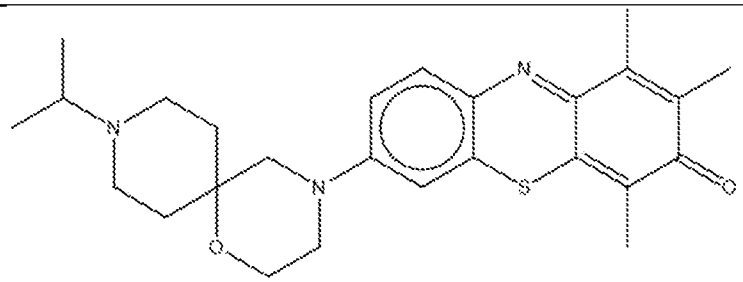
	(S)-7-(hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl)-3H-phenothiazin-3-one
13	 (S)-7-(hexahydropyrazino[2,1-c][1,4]oxazin-8(1H)-yl)-3H-phenothiazin-3-one
14	 (R)-7-(hexahydropyrazino[2,1-c][1,4]oxazin-8(1H)-yl)-3H-phenothiazin-3-one
15	 7-(9-isopropyl-3,9-diazaspiro[5.5]undecan-3-yl)-3H-phenothiazin-3-one
16	 7-(7-isopropyl-2,7-diazaspiro[3.5]nonan-2-yl)-3H-phenothiazin-3-one

17	 7-(2-isopropyl-2,7-diazaspiro[3.5]nonan-7-yl)-3H-phenothiazin-3-one
18	 7-(2-isopropyl-2,6-diazaspiro[3.5]nonan-6-yl)-3H-phenothiazin-3-one
19	 2-isopropyl-7-((1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-3H-phenothiazin-3-one
20	 7-((1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-2-(trifluoromethyl)-3H-phenothiazin-3-one

21	 7-((1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-1,2-dimethyl-3H-phenothiazin-3-one
22	 7-(2-isopropyl-2,9-diazaspiro[5.5]undecan-9-yl)-3H-phenothiazin-3-one
23	 7-(2-isopropyl-2,8-diazaspiro[4.5]decan-8-yl)-3H-phenothiazin-3-one

24	 7-(1-methyl-1,8-diazaspiro[4.5]decan-8-yl)-3H-phenothiazin-3-one
25	 7-(1-methyl-1,7-diazaspiro[3.5]nonan-7-yl)-3H-phenothiazin-3-one
26	 7-(9-isopropyl-2,9-diazaspiro[5.5]undecan-2-yl)-3H-phenothiazin-3-one
27	 7-(4-isopropyl-1-oxa-4,9-diazaspiro[5.5]undecan-9-yl)-3H-phenothiazin-3-one

28	 7-(9-isopropyl-1-oxa-4,9-diazaspiro[5.5]undecan-4-yl)-3H-phenothiazin-3-one
29	 7-(6-isopropyl-3,6-diazabicyclo[3.1.1]heptan-3-yl)-3H-phenothiazin-3-one
30	 7-((1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-1,2,4-trimethyl-3H-phenothiazin-3-one
31	 7-((1R,5S)-8-(2-methoxyethyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-1,2,4-trimethyl-3H-phenothiazin-3-one

32	 7-((1R,5S)-8-(2-hydroxyethyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-1,2,4-trimethyl-3H-phenothiazin-3-one
33	 7-(9-isopropyl-1-oxa-4,9-diazaspiro[5.5]undecan-4-yl)-1,2,4-trimethyl-3H-phenothiazin-3-one

[0047] As known in the art, stereoisomers refer to compounds that differ in the chirality of one or more stereocenters. Stereoisomers include enantiomers and diastereomers. The compounds may exist in stereoisomeric form if they possess one or more asymmetric centers or a double bond with asymmetric substitution and, therefore, can be produced as individual stereoisomers or as mixtures. Unless otherwise indicated, the description is intended to include individual stereoisomers as well as mixtures. The methods for the determination of stereochemistry and the separation of stereoisomers are well-known in the art (see, *e.g.*, Chapter 4 of Advanced Organic Chemistry, 4th ed., J. March, John Wiley and Sons, New York, 1992).

[0048] The compounds described herein may be prepared and/or formulated as pharmaceutically acceptable salts or when appropriate as a free base. Pharmaceutically acceptable salts are non-toxic salts of a free base form of a compound that possess the desired pharmacological activity of the free base. These salts may be derived from inorganic or organic acids or bases. For example, a compound that contains a basic nitrogen may be prepared as a pharmaceutically acceptable salt by contacting the compound with an inorganic or organic acid. Non-limiting examples of pharmaceutically acceptable salts include sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, phosphates, monohydrogen-phosphates,

dihydrogenphosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrates, caproates, heptanoates, propiolates, oxalates, malonates, succinates, suberates, sebacates, fumarates, maleates, butyne-1,4-dioates, hexyne-1,6-dioates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, sulfonates, methylsulfonates, propylsulfonates, besylates, xylenesulfonates, naphthalene-1-sulfonates, naphthalene-2-sulfonates, phenylacetates, phenylpropionates, phenylbutyrates, citrates, lactates, γ-hydroxybutyrates, glycolates, tartrates, and mandelates. Lists of other suitable pharmaceutically acceptable salts are found in Remington: The Science and Practice of Pharmacy, 21st Edition, Lippincott Wiliams and Wilkins, Philadelphia, Pa., 2006.

[0049] Examples of pharmaceutically acceptable salts of the compounds disclosed herein also include salts derived from an appropriate base, such as an alkali metal (for example, sodium, potassium), an alkaline earth metal (for example, magnesium), ammonium and NX_4^+ (wherein X is C_1–C_4 alkyl). Also included are base addition salts, such as sodium or potassium salts.

[0050] The compounds of the present disclosure generally possess sufficient solubility to permit dissolution in biological media, such as blood or plasma. Kinetic solubility assays can be assessed by diluting a solution of the compound of the present disclosure prepared in DMSO into aqueous buffer, simulated intestinal fluid (SIF), or simulated gastric fluid (SGF). See, United States Pharmacopeia, USP40-NF35 (2017). In some embodiments, a compound of Formula I, or a pharmaceutically acceptable salt thereof, has a solubility of greater than about 5 μM, such as greater than about 10 μM, greater than about 20 μM, greater than about 30 μM, greater than about 40 μM, greater than about 50 μM, greater than about 100 μM, greater than about 200 μM, or greater than about 300 μM. In some embodiments, a compound of Formula I, or a pharmaceutically acceptable salt thereof, has a solubility of from about 5 μM to about 300 μM, such as from about 10 μM to about 200 μM or from about 20 μM to about 200 μM.

IV. COMPOSITIONS

[0051] In some embodiments, the pharmaceutical composition of the present invention is a pharmaceutical composition comprising a compound or pharmaceutically acceptable salt as described herein, and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition comprises a therapeutically effective amount of a compound of

Formula I or pharmaceutically acceptable salt as described herein, and a pharmaceutically acceptable excipient.

[0052] The compound can be administered by any useful route and means, such as by oral or parenteral (*e.g.,* intravenous) administration. Therapeutically effective amounts of the compound may include from about 0.00001 mg/kg body weight per day to about 10 mg/kg body weight per day, such as from about 0.0001 mg/kg body weight per day to about 10 mg/kg body weight per day, or such as from about 0.001 mg/kg body weight per day to about 1 mg/kg body weight per day, or such as from about 0.01 mg/kg body weight per day to about 1 mg/kg body weight per day, or such as from about 0.05 mg/kg body weight per day to about 0.5 mg/kg body weight per day, or such as from about 0. 3 mg to about 30 mg per day, or such as from about 30 mg to about 300 mg per day.

A. Formulation

[0053] For preparing pharmaceutical compositions from the compound or pharmaceutically acceptable salt of the present invention, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, cachets, and dispersible granules. A solid carrier can be one or more substances, which may also act as diluents, binders, preservatives, disintegrating agents, or an encapsulating material. Details on techniques for formulation and administration are well described in the scientific and patent literature, see, e.g., the latest edition of Remington's Pharmaceutical Sciences, Maack Publishing Co, Easton PA ("Remington's").

[0054] In powders, the carrier is a finely divided solid, which is in a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain from 5% or 10% to 70% of the conjugates of the present invention.

[0055] Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water/propylene glycol solutions. For parenteral injection, liquid preparations can be formulated in solution in aqueous polyethylene glycol solution.

[0056] Aqueous solutions suitable for oral use can be prepared by dissolving the compound or pharmaceutically acceptable salt of the present invention in water and adding suitable colorants, flavors, stabilizers, and thickening agents as desired. Aqueous suspensions suitable

for oral use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethylene oxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol (e.g., polyoxyethylene sorbitol mono-oleate), or a condensation product of ethylene oxide with a partial ester derived from fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan mono-oleate). The aqueous suspension can also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose, aspartame or saccharin. Formulations can be adjusted for osmolality.

[0057] Also included are solid form preparations, which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

[0058] Oil suspensions can be formulated by suspending the compound or pharmaceutically acceptable salt of the present invention in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin; or a mixture of these. The oil suspensions can contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents can be added to provide a palatable oral preparation, such as glycerol, sorbitol or sucrose. These formulations can be preserved by the addition of an antioxidant such as ascorbic acid. As an example of an injectable oil vehicle, see Minto, J. Pharmacol. Exp. Ther. 281 :93-102, 1997. The pharmaceutical formulations of the invention can also be in the form of oil-in- water emulsions. The oily phase can be a vegetable oil or a mineral oil, described above, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan mono-oleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan mono-oleate. The emulsion can

also contain sweetening agents and flavoring agents, as in the formulation of syrups and elixirs. Such formulations can also contain a demulcent, a preservative, or a coloring agent.

[0059] The compositions of the present invention can also be delivered as microspheres for slow release in the body. For example, microspheres can be formulated for administration via intradermal injection of drug-containing microspheres, which slowly release subcutaneously (see Rao, J. Biomater Sci. Polym. Ed. 7:623-645, 1995; as biodegradable and injectable gel formulations (see, e.g., Gao Pharm. Res. 12:857-863, 1995); or, as microspheres for oral administration (see, e.g., Eyles, J. Pharm. Pharmacol. 49:669-674, 1997). Both transdermal and intradermal routes afford constant delivery for weeks or months.

[0060] In another embodiment, the compositions of the present invention can be formulated for parenteral administration into a body cavity. The formulations for administration will commonly comprise a solution of the compositions of the present invention dissolved in a pharmaceutically acceptable carrier. Among the acceptable vehicles and solvents that can be employed are water and Ringer's solution, an isotonic sodium chloride. In addition, sterile fixed oils can conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid can likewise be used in the preparation of injectables. These solutions are sterile and generally free of undesirable matter. These formulations may be sterilized by conventional, well known sterilization techniques. The formulations may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents, e.g., sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of the compositions of the present invention in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight, and the like, in accordance with the particular mode of administration selected and the patient's needs. For IV, intratumoral, or intravitreal administration, the formulation can be a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally-acceptable diluent or solvent, such as a solution of 1,3-butanediol.

[0061] In another embodiment, the formulations of the compositions of the present invention can be delivered by the use of liposomes which fuse with the cellular membrane or are endocytosed, i.e., by employing ligands attached to the liposome, or attached directly to the oligonucleotide, that bind to surface membrane protein receptors of the cell resulting in endocytosis. By using liposomes, particularly where the liposome surface carries ligands specific for target cells, or are otherwise preferentially directed to a specific organ, one can focus the delivery of the compositions of the present invention into the target cells in vivo. (See, e.g., Al-Muhammed, J. Microencapsul. 13:293-306, 1996; Chonn, Curr. Opin. Biotechnol. 6:698-708, 1995; Ostro, Am. J. Hosp. Pharm. 46: 1576-1587, 1989).

[0062] Lipid-based drug delivery systems include lipid solutions, lipid emulsions, lipid dispersions, self-emulsifying drug delivery systems (SEDDS) and self-microemulsifying drug delivery systems (SMEDDS). In particular, SEDDS and SMEDDS are isotropic mixtures of lipids, surfactants and co-surfactants that can disperse spontaneously in aqueous media and form fine emulsions (SEDDS) or microemulsions (SMEDDS). Lipids useful in the formulations of the present invention include any natural or synthetic lipids including, but not limited to, sesame seed oil, olive oil, castor oil, peanut oil, fatty acid esters, glycerol esters, Labrafil®, Labrasol®, Cremophor®, Solutol®, Tween®, Capryol®, Capmul®, Captex®, and Peceol®.

B. Administration

[0063] The compound or pharmaceutically acceptable salt and compositions of the present invention can be delivered by any suitable means, including oral, parenteral and topical methods.

[0064] A compound or composition of the present disclosure may be administered to an individual in accordance with an effective dosing regimen for a desired period of time or duration, such as at least about one month, at least about 2 months, at least about 3 months, at least about 6 months, or at least about 12 months or longer. In one variation, the compound is administered on a daily or intermittent schedule for the duration of the individual's life.

[0065] The dosage or dosing frequency of a compound or composition of the present disclosure may be adjusted over the course of the treatment, based on the judgment of the administering physician.

[0066] The compound or composition may be administered to an individual (*e.g.,* a human) in an effective amount. In some embodiments, the compound is administered once daily.

[0067] The pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the compounds and compositions of the present invention. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules.

[0068] The compounds and compositions of the present invention can be co-administered with other agents. Co-administration includes administering the compound or composition of the present invention within 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, or 24 hours of the other agent. Co-administration also includes administering simultaneously, approximately simultaneously (e.g., within about 1, 5, 10, 15, 20, or 30 minutes of each other), or sequentially in any order. Moreover, the compounds and compositions of the present invention can each be administered once a day, or two, three, or more times per day so as to provide the preferred dosage level per day.

[0069] In some embodiments, co-administration can be accomplished by co-formulation, i.e., preparing a single pharmaceutical composition including the compounds and compositions of the present invention and any other agent. Alternatively, the various components can be formulated separately.

[0070] The compounds and compositions of the present invention, and any other agents, can be present in any suitable amount, and can depend on various factors including, but not limited to, weight and age of the subject, state of the disease, etc. Suitable dosage ranges include from about 0.1 mg to about 10,000 mg, or about 1 mg to about 1000 mg, or about 10 mg to about 750 mg, or about 25 mg to about 500 mg, or about 50 mg to about 250 mg. Suitable dosages also include about 1 mg, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 mg. The composition can also contain other compatible therapeutic agents. The compounds described herein can be used in combination with one another, with other active agents known to be useful in modulating ferroptosis, or with adjunctive agents that may not be effective alone, but may contribute to the efficacy of the active agent.

V. METHODS AND/OR USES

A. Methods of Reducing Expression and/or Activity with Phenothiazines

[0071] Ferroptosis is an iron-dependent form of cell death that occurs as a consequence of lipid reactive oxygen species (ROS) production. Cells undergoing ferroptosis exhibit subtle morphological features, including smaller-than-normal mitochondria with increased density. The presence of ferroptosis can be confirmed by looking at whether cell death is prevented by inhibitors, and by measuring lipid peroxides. Dysregulation of mitochondrial metabolism is considered a biochemical feature of diseases, such as neurodegenerative diseases, linked to ferroptosis. Accordingly, it is believed that certain mitochondrial diseases can be treated by inhibiting ferroptosis.

[0072] Cellular assays for measuring ferroptosis, such as those that use RAS-selective lethal (RSL) compounds, such as RSL3, to induce ferroptosis, are known in the art. See, e.g., Dixon, S. J. et al. Cell 2012, vol. 149 (5), pages 1060-1072; Chen, X. et al. Frontiers in Cell and Development Biology January 2021, vol. 9, article 637162; and references cited therein, as well as the biological assay described in Example 2 herein.

[0073] As described in Example 2 herein, compounds or compositions of the present disclosure are useful for inhibiting ferroptosis in a cell. Accordingly, in some embodiments, a method of inhibiting ferroptosis in a cell comprises administering to the cell an effective amount of a compound of the present disclosure or pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the present disclosure. In some embodiments, the method comprises administering the compound of the present disclosure or pharmaceutically acceptable salt thereof in vitro, ex vivo, or in vivo.

[0074] The compounds or compositions of the present disclosure are believed to be useful for inhibiting ferroptosis in the treatment of mitochondrial diseases. Accordingly, in some embodiments, a method of treating a mitochondrial disease in a subject in need thereof comprises administering to the subject a therapeutically effective amount of a compound of the present disclosure or pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the present disclosure.

[0075] In some embodiments, a method of the present disclosure is a method of reducing a Miro1 and/or Miro2 level in a cell, comprising contacting the cell with an effective amount of a compound of Formula (I), or pharmaceutically acceptable salt thereof.

[0076] In some embodiments, reduction of a Miro1 and/or Miro2 level in a method as described herein is a reduction in the amount of a Miro1 and/or Miro2 nucleic acid, e.g., RNA and/or DNA, and/or a reduction in the activity of a Miro1 and/or Miro2 protein. In some embodiments, the reduction of a Miro1 and/or Miro2 level is a reduction in the amount of a Miro1 and/or Miro2 DNA as determined by any assay method, including assays known in the art and the assays described in the present disclosure. In some embodiments, the reduction of a Miro1 and/or Miro2 level is a reduction in the amount of a Miro1 and/or Miro2 RNA as determined by any assay method, including assays known in the art and the assays described in the present disclosure.

[0077] In some embodiments, reduction of a Miro1 and/or Miro2 level in a method as described herein is a reduction in the amount of a Miro1 and/or Miro2 protein and/or a reduction in the activity of a Miro1 and/or Miro2 protein. In some embodiments, the reduction of a Miro1 and/or Miro2 level is a reduction in the amount of a Miro1 and/or Miro2 protein as determined by any assay method, including assays known in the art and the assays described in the present disclosure, that results in a reduction in the Miro1 and/or Miro2 activity.

[0078] Any suitable cell can be used in a method of reducing, or downregulating, Miro1 and/or Miro2 level described herein. Cultured cells may be derived from a subject (e.g., a patient) or control samples; and may be modified to generate genetically-modified cells, in vitro differentiated cells, cells exposed to a candidate therapeutic agent; and the like. In some embodiments, the cell is a skin cell. In some embodiments, the cell is a muscle cell. For example, the muscle cell can be a cardiac cell, that is, a cardiomyocyte. In some embodiments, the cell is a renal cell. In some embodiments, the cell is a liver cell. In some embodiments, the cell is a neuronal cell. The method can be performed in a cell *in vitro*, *ex vivo*, or *in vivo*. In some embodiments, the reducing Miro1 and/or Miro2 level is *in vitro* or *ex vivo*. In some embodiments, the reducing Miro1 and/or Miro2 level is *in vivo*.

[0079] Any suitable biological sample comprising cells can be used in the methods described herein. The methods can be performed with a biological sample obtained from a subject, including without limitation biological samples such as fibroblasts, such as skin fibroblasts, peripheral blood lymphocytes, iPSCs, and the like.

[0080] A Miro1 and/or Miro2 level measured in a method described herein can be compared to a control Miro1 and/or Miro2 level by any method known in the art. See, for

example, the ELISA assay described in Hsieh C-H, et al. Cell Metab. 2019; 1131–1140. See also, the Miro2 assays described in Cao, Y. et al. Circulation Research 2019; 125(8):728-743; Oeding, S. J. et al Journal of Cell Science 2018; 131(17): jcs219469; and Furnish, M. et al. Molecular Cancer Research 2022; 20(4): 607-621.

[0081] In some embodiments, a compound of the present disclosure, e.g., a compound of Formula (I) or pharmaceutically acceptable salt thereof, reduces the level of Miro1 and/or Miro2 to a normal range. For example, in some embodiments, a Miro1 and/or Miro2 normal range can be the range observed between untreated or naïve healthy fibroblast or iPSC DA neuron cells (top of the range) and mitochondrial stressor-challenged healthy fibroblast or iPSC DA neuron cells (bottom of the range).

[0082] In some embodiments, a compound of Formula (I) or pharmaceutically acceptable salt thereof reduces, or downregulates, the level of Miro1 and/or Miro2 to within about 50%, about 40%, about 30%, or about 20% relative to a control level of Miro1 and/or Miro2. In some embodiments, the control level of Miro1 and/or Miro2 is measured in a control cell from a control subject that does not have or is not suspected of having a disease or disorder mediated by an aberrant Miro1 and/or Miro2 level. For example, a compound of Formula (I) or pharmaceutically acceptable salt thereof can downregulate the Miro1 and/or Miro2 level in a neuronal cell from a Parkinson's disease patient to within about 50% relative to a control level of Miro1 and/or Miro2 in a control neuronal cell from an age-matched patient that does not have or is not suspected of having Parkinson's disease.

[0083] The level of Miro1 in a cell after contacting with the compound of Formula (I) or pharmaceutically acceptable salt thereof can be higher or lower than the control level of Miro1 in the control cell. In some embodiments, the level of Miro1 in a cell after contacting with the compound of Formula (I) or pharmaceutically acceptable salt thereof is about 20%, about 30%, about 40%, or about 50% higher than the control level of Miro1 in the control cell. In some embodiments, the level of Miro1 in a cell after contacting with the Compound of Formula (I) or pharmaceutically acceptable salt thereofis from about 20% to about 50% higher than the control level of Miro1 in the control cell. In some embodiments, the level of Miro1 in a cell after contacting with the compound of Formula (I) or pharmaceutically acceptable salt thereofis about 20%, about 30%, about 40%, or about 50% lower than the control level of Miro1 in the control cell. In some embodiments, the level of Miro1 in a cell after contacting with the Compound of Formula (I) or pharmaceutically acceptable salt

thereofis from about 20% to about 50% lower than the control level of Miro1 in the control cell.

[0084] Any suitable concentration of a Compound of Formula (I) or pharmaceutically acceptable salt thereof in a cell can be used to effect reducing the Miro1 level in the cell to a desired level. In some embodiments, the concentration of Compound of Formula (I) or pharmaceutically acceptable salt thereof in the cell can be from about 1 nM to about 100 μM, such as from about 1 nM to about 10 μM, from about 1 nM to about 1 μM, from about 10 nM to about 100 μM, from about 10 nM to about 10 μM, from about 10 nM to about 1 μM, from about 100 nM to about 100 μM, from about 100 nM to about 10 μM, from about 100 nM to about 1 μM, from about 1 μM to about 100 μM, or from about 1 μM to about 10 μM.

[0085] In some embodiments, a compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 20% or more, for example, about 30% or more, about 40% or more, or about 50% or more, about 60% or more, about 70% or more, or about 80% or more, e.g. about 90%, about 95%, or about 100%, relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 20% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 25% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 30% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 35% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 40% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 45% relative to an untreated

control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 50% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 55% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 60% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 70% relative to an untreated control not contacted with the Miro1 reducer and/or Miro1-reducing agent. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 80% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 90% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 95% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof.

[0086] In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 20% to about 100% relative to an untreated control not contacted with the Miro1 reducer and/or Miro1-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 20% to about 90% relative to an untreated control not contacted with the Miro1 reducer and/or Miro1-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 20% to about 80% relative to an untreated control not contacted with the Miro1 reducer and/or Miro1-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof

reduces the level or biological activity of Miro1 by about 20% to about 70% relative to an untreated control not contacted with the Miro1 reducer and/or Miro1-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 20% to about 60% relative to an untreated control not contacted with the Miro1 reducer and/or Miro1-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 20% to about 50% relative to an untreated control not contacted with the Miro1 reducer and/or Miro1-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 20% to about 40% relative to an untreated control not contacted with the Miro1 reducer and/or Miro1-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 30% to about 50% relative to an untreated control not contacted with the Miro1 reducer and/or Miro1-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 by about 40% to about 60% relative to an untreated control not contacted with the Miro1 reducer and/or Miro1-reducing agent.

[0087] The level of Miro2 in a cell after contacting with the compound of Formula (I) or pharmaceutically acceptable salt thereof can be higher or lower than the control level of Miro2 in the control cell. In some embodiments, the level of Miro2 in a cell after contacting with the compound of Formula (I) or pharmaceutically acceptable salt thereof is about 20%, about 30%, about 40%, or about 50% higher than the control level of Miro2 in the control cell. In some embodiments, the level of Miro2 in a cell after contacting with the Compound of Formula (I) or pharmaceutically acceptable salt thereofis from about 20% to about 50% higher than the control level of Miro2 in the control cell. In some embodiments, the level of Miro2 in a cell after contacting with the Compound of Formula (I) or pharmaceutically acceptable salt thereofis about 20%, about 30%, about 40%, or about 50% lower than the control level of Miro2 in the control cell. In some embodiments, the level of Miro2 in a cell after contacting with the Compound of Formula (I) or pharmaceutically acceptable salt thereofis from about 20% to about 50% lower than the control level of Miro2 in the control cell.

[0088] Any suitable concentration of a Compound of Formula (I) or pharmaceutically acceptable salt thereof in a cell can be used to effect reducing the Miro2 level in the cell to a

desired level. In some embodiments, the concentration of Compound of Formula (I) or pharmaceutically acceptable salt thereof in the cell can be from about 1 nM to about 100 μM, such as from about 1 nM to about 10 μM, from about 1 nM to about 1 μM, from about 10 nM to about 100 μM, from about 10 nM to about 10 μM, from about 10 nM to about 1 μM, from about 100 nM to about 100 μM, from about 100 nM to about 10 μM, from about 100 nM to about 1 μM, from about 1 μM to about 100 μM, or from about 1 μM to about 10 μM.

[0089] In some embodiments, a compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 20% or more, for example, about 30% or more, about 40% or more, or about 50% or more, about 60% or more, about 70% or more, or about 80% or more, e.g. about 90%, about 95%, or about 100%, relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 20% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 25% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 30% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 35% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 40% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 45% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 50% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically

acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 55% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 60% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 70% relative to an untreated control not contacted with the Miro2 reducer and/or Miro2-reducing agent. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 80% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 90% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof. In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 95% relative to an untreated control not contacted with the compound of Formula (I) or pharmaceutically acceptable salt thereof.

[0090] In some embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 20% to about 100% relative to an untreated control not contacted with the Miro2 reducer and/or Miro2-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 20% to about 90% relative to an untreated control not contacted with the Miro2 reducer and/or Miro2-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 20% to about 80% relative to an untreated control not contacted with the Miro2 reducer and/or Miro2-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 20% to about 70% relative to an untreated control not contacted with the Miro2 reducer and/or Miro2-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 20% to about 60% relative to an

untreated control not contacted with the Miro2 reducer and/or Miro2-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 20% to about 50% relative to an untreated control not contacted with the Miro2 reducer and/or Miro2-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 20% to about 40% relative to an untreated control not contacted with the Miro2 reducer and/or Miro2-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 30% to about 50% relative to an untreated control not contacted with the Miro2 reducer and/or Miro2-reducing agent. In embodiments, a Compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro2 by about 40% to about 60% relative to an untreated control not contacted with the Miro2 reducer and/or Miro2-reducing agent.

[0091] In some embodiments, a compound of Formula (I) or pharmaceutically acceptable salt thereof reduces the level or biological activity of Miro1 and Miro2 as described abovea.

B. Methods and/or Uses of Treatment with Phenothiazines

[0092] The phenothiazine compounds of the present disclosure are capable of acting as ferroptosis inhibitors, and are believed to be useful for treatment of diseases or disorders associated with mitochondrial dysfunction.

[0093] Mitochondrial dysfunction contributes to various disease states.
Some mitochondrial diseases are due to mutations or deletions in the mitochondrial genome. If a threshold proportion of mitochondria in the cell is defective, and if a threshold proportion of such cells within a tissue have defective mitochondria, symptoms of tissue or organ dysfunction can result. Practically any tissue can be affected, and a large variety of symptoms may be present, depending on the extent to which different tissues are involved. Some examples of mitochondrial diseases include Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, Friedreich's ataxia (FRDA), Leber's Hereditary Optic Neuropathy (LHON), mitochondrial myopathy, encephalopathy, lactacidosis, and stroke (MELAS), Myoclonus Epilepsy Associated with Ragged-Red Fibers (MERRF) syndrome, Maternally Inherited Diabetes and Deafness (MIDD), and respiratory chain disorders. Mitochondrial diseases can involve children who manifest the signs and

symptoms of accelerated aging, including neurodegenerative diseases, stroke, blindness, hearing or balance impairment, diabetes, and heart failure.

[0094] Friedreich's ataxia is an autosomal recessive neurodegenerative and cardiodegenerative disorder caused by decreased levels of the protein Frataxin. The disease causes the progressive loss of voluntary motor coordination (ataxia) and cardiac complications. Symptoms typically begin in childhood, and the disease progressively worsens as the patient grows older; patients eventually become wheelchair-bound due to motor disabilities.

[0095] Leber's Hereditary Optic Neuropathy (LHON) is a disease characterized by blindness which occurs on average between 27 and 34 years of age. Other symptoms may also occur, such as cardiac abnormalities and neurological complications.

[0096] Mitochondrial myopathy, encephalopathy, lactacidosis, and stroke (MELAS) can manifest itself in infants, children, or young adults. Strokes, accompanied by vomiting and seizures, are one of the most serious symptoms; it is postulated that the metabolic impairment of mitochondria in certain areas of the brain is responsible for cell death and neurological lesions, rather than the impairment of blood flow as occurs in ischemic stroke.

[0097] Myoclonus Epilepsy Associated with Ragged-Red Fibers (MERRF) syndrome is one of a group of rare muscular disorders that are called mitochondrial encephalomyopathies. Mitochondrial encephalomyopathies are disorders in which a defect in the genetic material arises from a part of the cell structure that releases energy (mitochondria). This can cause a dysfunction of the brain and muscles (encephalomyopathies). The most characteristic symptom of MERRF syndrome is myoclonic seizures that are usually sudden, brief, jerking, spasms that can affect the limbs or the entire body, difficulty speaking (dysarthria), optic atrophy, short stature, hearing impairment, dementia, and involuntary jerking of the eyes (nystagmus) may also occur.

[0098] Leigh's disease is a rare inherited neurometabolic disorder characterized by degeneration of the central nervous system where the symptoms usually begin between the ages of 3 months to 2 years and progress rapidly. In most children, the first signs may be poor sucking ability and loss of head control and motor skills. These symptoms may be accompanied by loss of appetite, vomiting, irritability, continuous crying, and seizures. As the disorder progresses, symptoms may also include generalized weakness, lack of muscle

tone, and episodes of lactic acidosis, which can lead to impairment of respiratory and kidney function. Heart problems may also occur.

[0099] Maternally Inherited Diabetes and Deafness (MIDD) is caused by a mutation in mitochondrial DNA (3243 tRNA). The diabetes is a non insulin dependent type that usually presents before the age of 40 years; it is due to a defect in beta cell function with normal insulin sensitivity. The associated deafness is sensorineural and develops in most of the diabetic subjects. In keeping with other mitochondrial disorders, MIDD may have other multi-organ features: for example, elevated serum lactate, neuromuscular and cardiac problems, pigmented retinopathy, and nephropathy with proteinuria.

[0100] Co-Enzyme Q10 Deficiency is a respiratory chain disorder, with syndromes such as myopathy with exercise intolerance and recurrent myoglobin in the urine manifested by ataxia, seizures or mental retardation and leading to renal failure, childhood-onset cerebellar ataxia and cerebellar atrophy; and infantile encephalomyopathy associated with nephrosis. Biochemical measurement of muscle homogenates of patients with CoQ10 deficiency showed severely decreased activities of respiratory chain complexes I and II+III, while complex IV (COX) was moderately decreased.

[0101] Complex I Deficiency or NADH dehydrogenase NADH-CoQ reductase deficiency is a respiratory chain disorder, with symptoms classified by three major forms: (1) fatal infantile multisystem disorder, characterized by developmental delay, muscle weakness, heart disease, congenital lactic acidosis, and respiratory failure; (2) myopathy beginning in childhood or in adult life, manifesting as exercise intolerance or weakness; and (3) mitochondrial encephalomyopathy (including MELAS), which may begin in childhood or adult life and consists of variable combinations of symptoms and signs, including ophthalmoplegia, seizures, dementia, ataxia, hearing impairment, pigmentary retinopathy, sensory neuropathy, and uncontrollable movements.

[0102] Complex II Deficiency or Succinate dehydrogenase deficiency is a respiratory chain disorder with symptoms including encephalomyopathy and various manifestations, including failure to thrive, developmental delay, hyoptonia, lethargy, respiratory failure, ataxia, myoclonus and lactic acidosis.

[0103] Complex III Deficiency or Ubiquinone-cytochrome C oxidoreductase deficiency is a respiratory chain disorder with symptoms categorized in four major forms: (1) fatal infantile encephalomyopathy, congenital lactic acidosis, hypotonia, dystrophic posturing,

seizures, and coma; (2) encephalomyopathies of later onset (childhood to adult life): various combinations of weakness, short stature, ataxia, dementia, hearing impairment, sensory neuropathy, pigmentary retinopathy, and pyramidal signs; (3) myopathy, with exercise intolerance evolving into fixed weakness; and (4) infantile histiocytoid cardiomyopathy.

[0104] Complex IV Deficiency or Cytochrome C oxidase deficiency is a respiratory chain disorder with symptoms categorized in two major forms: (1) encephalomyopathy, which is typically normal for the first 6 to 12 months of life and then show developmental regression, ataxia, lactic acidosis, optic atrophy, ophthalmoplegia, nystagmus, dystonia, pyramidal signs, respiratory problems and frequent seizures; and (2) myopathy with two main variants: (a) fatal infantile myopathy—may begin soon after birth and accompanied by hypotonia, weakness, lactic acidosis, ragged-red fibers, respiratory failure, and kidney problems: and (b) benign infantile myopathy—may begin soon after birth and accompanied by hypotonia, weakness, lactic acidosis, ragged-red fibers, respiratory problems, but (if the child survives) followed by spontaneous improvement.

[0105] Complex V Deficiency or ATP synthase deficiency is a respiratory chain disorder including symptoms such as slow, progressive myopathy.

[0106] CPEO or Chronic Progressive External Ophthalmoplegia Syndrome is a respiratory chain disorder including symptoms such as visual myopathy, retinitis pigmentosa, or dysfunction of the central nervous system.

[0107] Kearns-Sayre Syndrome (KSS) is a mitochondrial disease characterized by a triad of features including: (1) typical onset in persons younger than age 20 years; (2) chronic, progressive, external ophthalmoplegia; and (3) pigmentary degeneration of the retina. In addition, KSS may include cardiac conduction defects, cerebellar ataxia, and raised cerebrospinal fluid (CSF) protein levels (e.g., >100 mg/dL). Additional features associated with KSS may include myopathy, dystonia, endocrine abnormalities (e.g., diabetes, growth retardation or short stature, and hypoparathyroidism), bilateral sensorineural deafness, dementia, cataracts, and proximal renal tubular acidosis.

[0108] In addition to congenital disorders involving inherited defective mitochondria, acquired mitochondrial dysfunction contributes to diseases, particularly neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), or Huntington's disease. The incidence of somatic mutations in mitochondrial DNA rises exponentially with age; diminished respiratory chain activity is found universally in

aging people. Mitochondrial dysfunction is also implicated in excitoxic, neuronal injury, such as that associated with cerebral vascular accidents, seizures and ischemia.

[0109] Parkinson's disease has been associated with mitochondrial dysfunction since 1983, when 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was found to cause parkinsonian-like symptoms in intravenous drug users. When MPTP penetrates the blood-brain barrier, the compound is bio-transformed into its toxic form 1-methyl-4-phenylpyridinium (MPP+) by glial monoamine oxidase (MAO). MPP+ specifically interferes with the activity of respiratory chain (RC) complex I (NADH: Ubiquinone oxidoreductase) in dopaminergic (DA) neurons, causing selective neurodegeneration in both human and mouse substantia nigra (SN). In post-mortem studies, varying degrees of Complex I and complex II (succinate dehydrogenase, SDH) deficiency have been found in individual SN neurons from PD patients (~60% Complex I and ~65% Complex II deficiency).

[0110] Mitochondria may mediate, drive, or contribute to a variety of Alzheimer's disease (AD) pathologies. Amyloid-β (Aβ) may induce AD mitochondrial dysfunction. Alternatively, data indicate mitochondrial dysfunction exists independent of Aβ, potentially lies upstream of Aβ deposition, and suggest a primary mitochondrial cascade hypothesis that assumes mitochondrial pathology hierarchically supersedes Aβ pathology. Mitochondria, therefore, appear at least to mediate or possibly even initiate pathologic molecular cascades in AD.

[0111] Amyotrophic lateral sclerosis (ALS) is caused by selective degeneration of motor neurons in the brain and spinal cord, which may be mediated by mitochondrial dysfunction. Studies suggest that ferroptosis mediates selective motor neuron death in amyotrophic lateral sclerosis. See, Wang, T. et al. Cell Death Differ 29, 1187–1198 (2022).

[0112] Compounds of the disclosure or pharmaceutically acceptable salts thereof are also useful for methods of aiding the treatment of a disease, a disorder, and/or a health condition associated with a mitochondrial disease, such as Parkinson's disease. As used herein, a "method of aiding" generally refers to methods of assisting in performing or practicing a method disclosed herein, for example, methods of assisting in (i) performing, (ii) practicing, and/or (iii) making a determination concerning the detection, classification, treatment regiment, or nature, of a mitochondrial disease (e.g., Parkinson's disease), a disease, a disorder, and/or a health condition.

[0113] Accordingly, in some embodiments, a method of aiding in the treatment of a mitochondrial disease in a subject in need thereof comprises administering to the subject a

therapeutically effective amount of a compound of the present disclosure or pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the present disclosure. In some embodiments, the method of aiding in the treatment comprises administering a pharmaceutical composition of the compound of Formula I as described in Section IV above.

[0114] In some embodiments, a use of the present disclosure comprises a compound of the present disclosure or pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the present disclosure for the manufacture of a medicament for aiding in the treatment of a mitochondrial disease in a subject in need thereof. In some embodiments, the use comprises a pharmaceutical composition of the compound of Formula I as described in Section IV above.

[0115] In some embodiments, a compound of the present disclosure or pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the present disclosure is for use in aiding in the treatment of a mitochondrial disease in a subject in need thereof. In some embodiments, the compound for use comprises a pharmaceutical composition of the compound of Formula I as described in Section IV above.

[0116] In some embodiments, a use of the present disclosure comprises a compound of the present disclosure or pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the present disclosure for the manufacture of a medicament for treating a mitochondrial disease in a subject in need thereof. In some embodiments, the use comprises a pharmaceutical composition of the compound of Formula I as described in Section IV above.

[0117] In some embodiments, a compound of the present disclosure or pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the present disclosure is for use in treating a mitochondrial disease in a subject in need thereof. In some embodiments, the compound for use comprises a pharmaceutical composition of the compound of Formula I as described in Section IV above.

[0118] Kits that comprise a compound of the present disclosure, or pharmaceutically acceptable salt thereof, or a pharmaceutical composition containing any of the above, are also included in the present disclosure. In some embodiments, a kit further includes instructions for use. In some embodiments, a kit includes a compound of the disclosure, or a pharmaceutically acceptable salt thereof, and a label and/or instructions for use of the compounds in the treatment of the indications, such as the diseases or conditions, described herein. In some embodiments,kits comprising a compound of the present disclosure, or a

pharmaceutically acceptable salt thereof, in combination with one or more (*e.g.,* one, two, three, four, one or two, or one to three, or one to four) additional therapeutic agents are provided.

[0119] Provided herein are also articles of manufacture that include a compound of the present disclosure or a pharmaceutically acceptable salt thereof in a suitable container. The container may be a vial, jar, ampoule, preloaded syringe, and intravenous bag.

VI. EXAMPLES

[0120] The following examples are provided to further aid in understanding the embodiments disclosed in the application, and presuppose an understanding of conventional methods well known to those persons having ordinary skill in the art to which the examples pertain. The particular materials and conditions described hereunder are intended to exemplify particular aspects of embodiments disclosed herein and should not be construed to limit the reasonable scope thereof.

[0121] Many general references providing commonly known chemical synthetic schemes and conditions useful for synthesizing the disclosed compounds are available (see, *e.g.*, Smith, March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 7th edition, Wiley-Interscience, 2013.)

[0122] Compounds as described herein can be purified by any of the means known in the art, including chromatographic means, such as high performance liquid chromatography (HPLC), preparative thin layer chromatography, flash column chromatography and ion exchange chromatography. Any suitable stationary phase can be used, including normal and reversed phases as well as ionic resins. For example, disclosed compounds can be purified via silica gel chromatography. *See, e.g.,* Introduction to Modern Liquid Chromatography, 2nd ed., ed. L. R. Snyder and J. J. Kirkland, John Wiley and Sons, 1979; and Thin Layer Chromatography, E. Stahl (ed.), Springer-Verlag, New York, 1969.

[0123] Compounds were characterized using standard instrumentation methods. Identification of the compound was carried out by hydrogen nuclear magnetic resonance spectrum (^{1}H-NMR) and mass spectrum (MS). ^{1}H-NMR was measured at 400 MHz, unless otherwise specified. In some cases, exchangeable hydrogen could not be clearly observed depending on the compound and measurement conditions. The designation br. or broad, used herein, refers to a broad signal. HPLC preparative chromatography was carried out by a

commercially available ODS column in a gradient mode using water/methanol (containing formic acid) as eluents, unless otherwise specified.

[0124] The Examples provided herein describe the synthesis of compounds disclosed herein as well as intermediates used to prepare the compounds. It is to be understood that individual steps described herein may be combined. It is also to be understood that separate batches of a compound may be combined and then carried forth in the next synthetic step.

[0125] Representative syntheses of compounds of the present disclosure are described in schemes below, and the particular examples that follow.

[0126] **Abbreviations**. Certain abbreviations and acronyms are used in describing the experimental details. Although most of these would be understood by one skilled in the art, contains a list of many of these abbreviations and acronyms.

Table 2. List of abbreviations and acronyms.

Abbreviation	Meaning
ACN	acetonitrile
Bn	benzyl
Boc	tert-butoxycarbonyl
Bu	butyl
DCE	1,2-dichloroethane
DCM	dichloromethane
DIPEA	N,N-diisopropylethylamine
DMSO	dimethylsulfoxide
DMF	dimethylformamide
ESI	electron spray ionization
Et	ethyl
HPLC	high performance liquid chromatography
HPLCMS	high performance liquid chromatography-mass spectrometry
LC	liquid chromatography
LCMS	liquid chromatography-mass spectrometry
Me	methyl
m/z	mass to charge ratio
MS or ms	mass spectrum

Pyr	pyridine
RT	retention time
TEA	triethylamine
TES	triethylsilane
TFA	trifluoroacetic acid
THF	tetrahydrofuran
δ	parts per million referenced to residual non-deuterated solvent peak

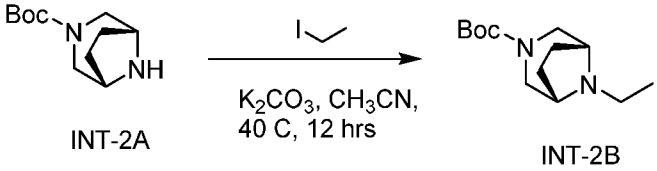
Example 1. Compound Synthesis

[0127] HPLCMS Method A: The column was an Xbridge Shield RP18 2.1*50mm, (5 um particles). UV detection was by diode array (DAD). The MS mode was positive electrospray ionization. The MS range was 100-1000. Mobile phase A was 10 mM ammonium bicarbonate in water. Mobile phase B was HPLC grade acetonitrile. The gradient was 5-95% B in 2.05 min. The flow rate was 1.0 mL/min.

[0128] HPLCMS Method B: The column was a ZORBAX Eclipse XDB-C18 2.1*30 mm, (3.5 um particles). UV detection was by diode array (DAD). The MS mode was positive electrospray ionization. The MS range was 100-1000. Mobile phase A was 0.037% trifluoroacetic acid in water. Mobile phase B was 0.018% trifluoroacetic acid in HPLC grade acetonitrile. The gradient was 5-95% B in 2.20 min. The flow rate was 1.0 mL/min.

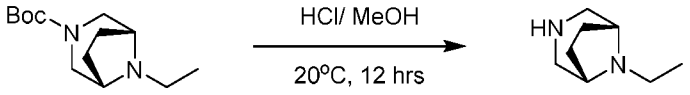
[0129] Preparative HPLC Method: The column was a GromSil 80 Si NP-1 5µm, 250mm x 4.6mm column. The mobile phase was heptane/ethanol with a gradient of 5%-95% ethanol over 15 min.

Synthesis of Intermediate 8-ethyl-3,8-diazabicyclo[3.2.1]octane (INT-2)



[0130] To a mixture of INT-2A (20 g, 94.21 mmol) and K_2CO_3 (26.04 g, 188.42 mmol) in acetonitrile (200 mL) was added ethyl iodide (14.69 g, 94.21 mmol) at 15°C. The mixture was stirred at 40°C for 12 hours under N_2. LCMS indicated starting material was consumed, and desired product was detected. The reaction mixture was cooled to 15°C. The mixture

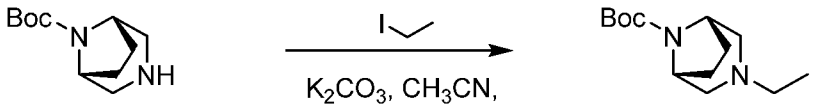
was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate) to give INT-2B (14 g, 58.25 mmol, 62% yield) as a white solid. HPLCMS Method A (ESI+): m/z 241.2 (MH+), RT 0.765 min.



INT-2B INT-2

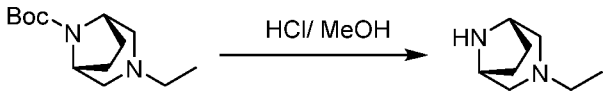
[0131] To a mixture of INT-2B (14 g, 58.25 mmol) in methanol (100 mL) was added HCl/MeOH (4 M, 87.50 mL) at 20°C. The mixture was stirred at 20°C for 12 hrs. The reaction mixture was concentrated under reduced pressure to give crude 8-ethyl-3,8-diazabicyclo[3.2.1]octane (INT-2) dihydrochloride (10 g, 46.92 mmol, 80% yield) as a yellow solid.

Synthesis of Intermediate 3-ethyl-3,8-diazabicyclo[3.2.1]octane (INT-3)



INT-3A INT-3B

[0132] To a mixture of INT-3A (20 g, 94.21 mmol) and K_2CO_3 (26.04 g, 188.42 mmol) in acetonitrile (200 mL) was added ethyl iodide (14.69 g, 94.21 mmol) at 15°C. The mixture was stirred at 40°C for 12 hours under N_2. LCMS indicated starting material was consumed, and desired product was detected. The reaction mixture was cooled to 15°C. The mixture was filtered, and the filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (ethyl acetate) to give INT-3B (14 g, 58.25 mmol, 62% yield) as a white solid. HPLCMS Method A (ESI+): m/z 241.2 (MH+), RT 0.853 min.

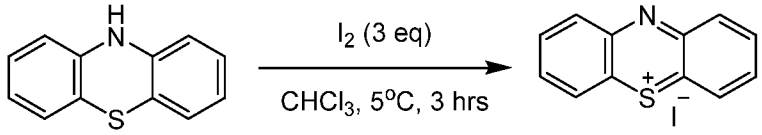


INT-3B INT-3

[0133] To a mixture of INT-3B (14 g, 58.25 mmol) in methanol (100 mL) was added HCl/MeOH (4 M, 87.50 mL) at 20°C. The mixture was stirred at 20°C for 12 hrs. The reaction mixture was concentrated under reduced pressure to give crude 3-ethyl-3,8-

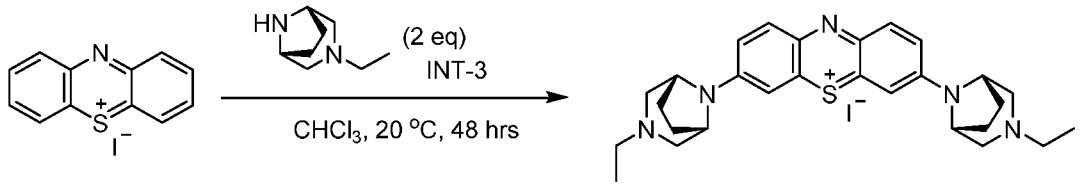
diazabicyclo[3.2.1]octane (INT-3) dihydrochloride (10 g, 46.92 mmol, 80% yield) as a yellow solid.

Synthesis of 7-((1R,5S)-3-ethyl-3,8-diazabicyclo[3.2.1]octan-8-yl)-3H-phenothiazin-3-one (Compound 1)



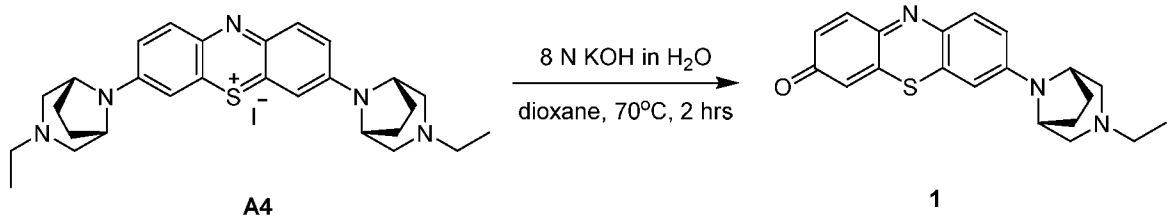
A1 **A2**

[0134] To a mixture of **A1** (10 g, 50.18 mmol) in $CHCl_3$ (500 mL) was added dropwise a mixture of iodine (38.21 g, 150 mmol) in $CHCl_3$ (750 mL) at 5°C for 1 hr. The mixture was stirred at 5°C for 3 hours. The mixture was filtered, and the filter cake was washed with $CHCl_3$ (200 mL). The filter cake was dried by high vacuum to give crude **A2** (10 g) as a dark blue solid. ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 7.30-7.76 (m, 3H), 7.85-8.19 (m, 4H), 8.36-8.63 (m, 1H).



A2 A4

[0135] To a mixture of **A2** (7.6 g, 23.37 mmol) and 3-ethyl-3,8-diazabicyclo[3.2.1]octane dihydrochloride (9.96 g, 46.75 mmol, 2 *eq*) in $CHCl_3$ (450 mL) was added N-ethyl-N-isopropylpropan-2-amine (15.10 g, 116.86 mmol, 5 *eq*) at 20°C. The mixture was stirred at 20°C for 48 hrs. The mixture was concentrated under reduced pressure, and the residue was washed with methyl tert-butyl ether (100 mL) and dried by high vacuum to give crude **A4** (9 g) as a blue solid. HPLCMS Method B (ESI+): m/z 474.3 (M+), RT 0.176 min.

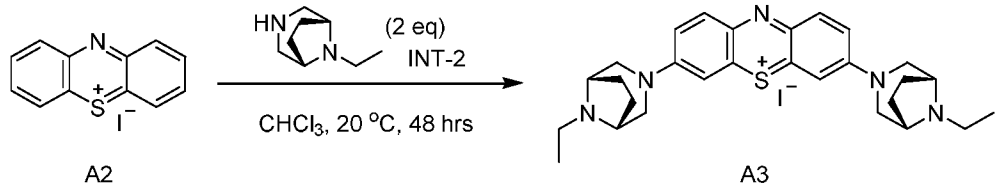


A4 **1**

[0136] To a mixture of crude **A4** (9 g, 14.96 mmol) in dioxane (150 mL) was added potassium hydroxide (8 N, 150 mL) at 20°C. The mixture was stirred at 70°C for 2 hrs. The mixture was cooled to 15°C and concentrated under reduced pressure, and the residue was

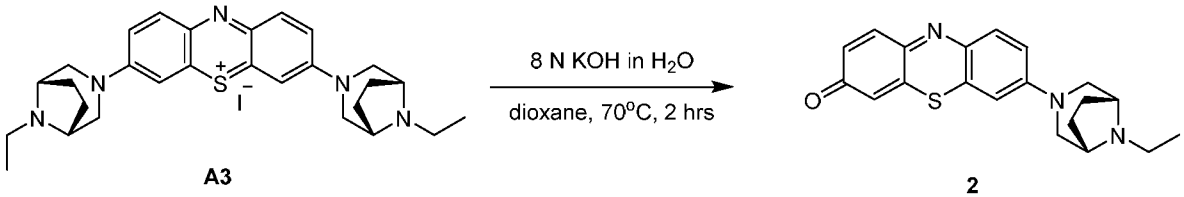
extracted with ethyl acetate (300 mL). The organic layer was concentrated under reduced pressure, and the crude product was purified by column chromatography on silica gel (ethyl acetate: methanol = 1: 1) three times, and then purified twice by normal-phase preparative HPLC to give after lyophilization Compound 1 (55 mg) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (t, *J*=7.28 Hz, 3H), 1.98-2.03 (m, 2H), 2.07-2.15 (m, 2H), 2.30-2.41 (m, 4H), 2.72 (dd, *J*=10.79, 2.26 Hz, 2H), 4.34 (br d, *J*=2.01 Hz, 2H), 6.67 (dd, *J*=12.80, 2.76 Hz, 2H), 6.85 (ddd, *J*=11.54, 9.29, 2.26 Hz, 2H), 7.56 (d, *J*=10.04 Hz, 1H), 7.69 (d, *J*=9.03 Hz, 1H). HPLCMS Method A (ESI+): m/z 352.1 (MH+), RT 2.621 min.

Synthesis of 7-((1R,5S)-8-ethyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-3H-phenothiazin-3-one (Compound 2)



A2 A3

[0137] To a mixture of **A2** (7.6 g, 23.37 mmol) and 8-ethyl-3,8-diazabicyclo[3.2.1]octane dihydrochloride (9.96 g, 46.75 mmol, 2 *eq*) in $CHCl_3$ (450 mL) was added N-ethyl-N-isopropylpropan-2-amine (15.10 g, 116.86 mmol, 5 *eq*) at 20 °C. The mixture was stirred at 20 °C for 48 hours. The mixture was concentrated under reduced pressure, and the residue was washed with methyl tert-butyl ether (100 mL) and dried by high vacuum to give crude **A3** (9 g) as a blue solid. HPLCMS Method B (ESI+): m/z 474.3 (M+), RT 0.461 min.

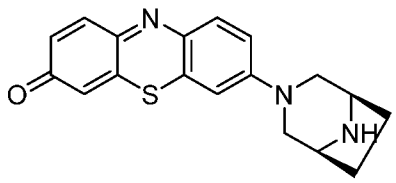


A3 **2**

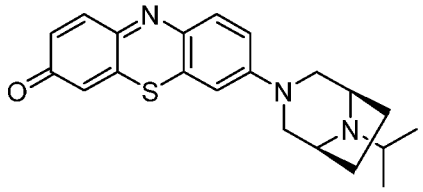
[0138] To a mixture of crude **A3** (9 g, 14.96 mmol) in dioxane (150 mL) was added potassium hydroxide (8 N, 150 mL) at 20°C. The mixture was stirred at 70°C for 2 hrs. The mixture was cooled to 15°C and concentrated under reduced pressure to remove most of the organic solvents. The residue was extracted with ethyl acetate (300 mL). The organic layer was concentrated under reduced pressure, and the crude product was purified by column chromatography on silica gel (from ethyl acetate to ethyl acetate: methanol = 1: 1) three times, and then purified twice by normal-phase preparative HPLC to give after lyophilization Compound 2 (60 mg) as a brown solid. ^{1}H NMR (400MHz, $CDCl_3$) δ 1.15 (t, *J*=7.28 Hz, 3H), 1.70 (d, *J*=7.53 Hz, 2H), 2.00-2.07 (m, 2H), 2.45-2.55 (m, 2H), 3.26 (dd, *J*=11.04, 2.01

Hz, 2H), 3.46 (br d, *J*=1.51 Hz, 2H), 3.49-3.55 (m, 2H), 6.68 (t, *J*=2.51 Hz, 2H), 6.84 (dd, *J*=9.79, 2.26 Hz, 1H), 6.92 (dd, *J*=9.03, 3.01 Hz, 1H), 7.55 (d, *J*=9.54 Hz, 1H), 7.70 (d, *J*=9.03 Hz, 1H). HPLCMS Method A (ESI+): m/z 352.1 (MH+), RT 2.352 min.

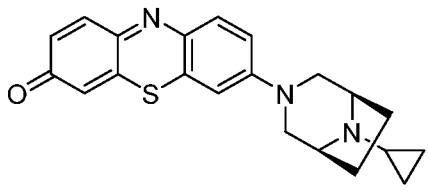
[0139] The following compounds were prepared by similar methods.



[0140] Compound 3: ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 1.70 (d, *J*=7.53 Hz, 2H), 2.00-2.07, 3.26 (dd, *J*=11.04, 2.01 Hz, 2H), 3.46 (br d, *J*=1.51 Hz, 2H), 3.49-3.55 (m, 2H), 6.68 (t, *J*=2.51 Hz, 2H), 6.84 (dd, *J*=9.79, 2.26 Hz, 1H), 6.92 (dd, *J*=9.03, 3.01 Hz, 1H), 7.55 (d, *J*=9.54 Hz, 1H), 7.70 (d, *J*=9.03 Hz, 1H). HPLCMS Method A (ESI+): m/z 324.1 (MH+), RT 1.852 min.

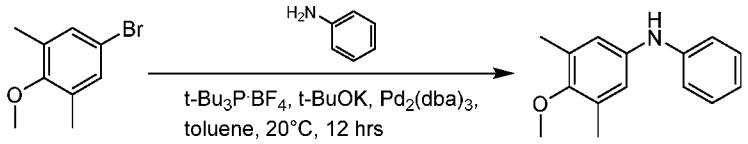


[0141] Compound 4: ^{1}H NMR (400MHz, $CDCl_3$) δ 1.15 (d, *J*=7.28 Hz, 6H), 1.70 (d, *J*=7.53 Hz, 2H), 2.00-2.07 (m, 2H), 2.60-2.70 (m, 1H), 3.26 (dd, *J*=11.04, 2.01 Hz, 2H), 3.46 (br d, *J*=1.51 Hz, 2H), 3.49-3.55 (m, 2H), 6.68 (t, *J*=2.51 Hz, 2H), 6.84 (dd, *J*=9.79, 2.26 Hz, 1H), 6.92 (dd, *J*=9.03, 3.01 Hz, 1H), 7.55 (d, *J*=9.54 Hz, 1H), 7.70 (d, *J*=9.03 Hz, 1H). HPLCMS Method A (ESI+): m/z 366.1 (MH+), RT 2.492 min.



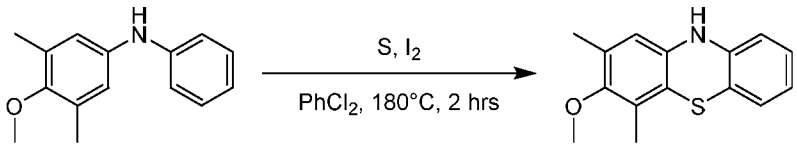
[0142] Compound 5: ^{1}H NMR (600 MHz, CD_2Cl_2) δ 7.66 (d, *J* = 9.2 Hz, 1H), 7.51 (d, *J* = 9.7 Hz, 1H), 6.93 (dd, *J* = 2.8, 9.2 Hz, 1H), 6.74 (dd, *J* =2.1, 9.8 Hz, 1H), 6.72 (d, *J* = 2.6 Hz, 1H), 6.61 (d, *J* = 2.2 Hz, 1H), 3.55 - 3.47 (m, 4H), 3.16 (br d, *J* = 10.6 Hz, 2H), 2.12 - 2.05 (m, 2H), 1.90 (br s, 1H), 1.71 (br d, *J* = 7.6 Hz, 2H), 0.52 - 0.43 (m, 4H) HPLCMS Method A (ESI+): m/z 364.1 (MH+), RT 2.411 min.

Synthesis of 7-((1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-2,4-dimethyl-3H-phenothiazin-3-one (Compound 6)



6-1 6-2

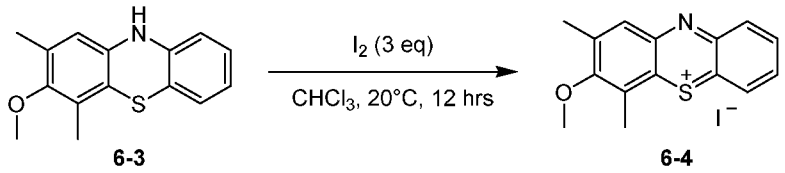
[0143] A mixture of aniline (5.85 g, 62.77 mmol), Intermediate 6-1 (9 g, 41.84 mmol), tri-tert-butylphosphonium tetrafluoroborate (728.40 mg, 2.51 mmol, 0.06 eq), potassium tert-butoxide (1 M, 54.40 mL), $Pd_2(dba)_3$ (1.15 g, 1.26 mmol) in toluene (100 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 20°C for 12 hrs under N_2 atmosphere. LCMS showed the starting material was consumed. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was then purified by column chromatography on silica gel eluted with petroleum ether/ethyl acetate (from 1% to 20%) to give Intermediate 6-2 (5.81 g, 25.56 mmol, 61.09% yield) as a yellow oil: **LCMS (ESI+)**: 0.565 min, m/z 228.2 [10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm, 5 um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min. 10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.]



6-2 6-3

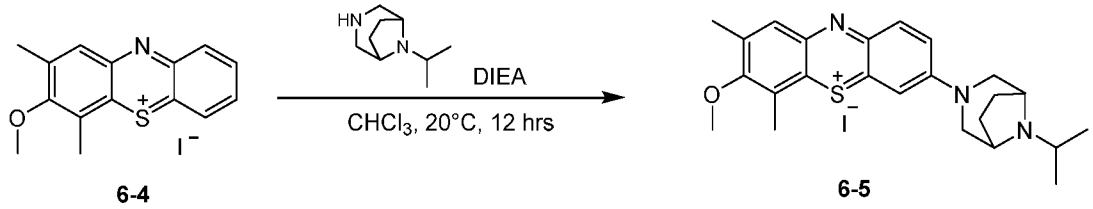
[0144] A mixture of Intermediate 6-2 (4 g, 17.60 mmol, 1 eq), I_2 (446.65 mg, 1.76 mmol, 0.1 eq) and S (1.20 g, 37.42 mmol, 2.13 eq) in $PhCl_2$ (200 mL) was stirred at 180°C for 2 hrs. LCMS showed the starting material was consumed completely and a major peak with desired ms was detected. The reaction mixture was concentrated under reduced pressure to give a residue. It was then purified by column chromatography on silica gel eluted with petroleum ether/ethyl acetate (from 1% to 10%) to give Intermediate 6-3 (2 g, 7.78 mmol, 44.1% yield) as yellow solid: **LCMS (ESI+)**: 2.200 min, m/z 258.2 [5-95AB_3.5min: LC/MS (The column was a Luna C18 30*2.0mm, 3um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The

gradient was 5-95% B in 3.50 min .5% B in 0.01 min, 5-95% B (0.01-2.50 min) with a hold at 95% B for 0.50 min, 95-5% B (3.00 -3.01 min) with a hold at 5% B for 0.49 min. The flow rate was 1 mL/min (0.01-3.00min)-1.2 mL/min (3.01-3.50min).]



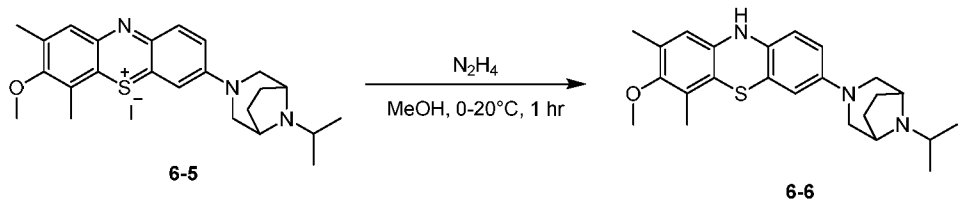
6-3 6-4

[0145] To a solution of Intermediate 6-3 (1 g, 3.89 mmol) in $CHCl_3$ (10 mL) was added dropwise a mixture of I_2 (2.96 g, 11.66 mmol) in $CHCl_3$ (200 ml) at 0°C for 0.5 hr. The mixture was stirred at 12°C for 12 hrs. TLC showed the reaction was completed and a major new spot was detected. The mixture was filtered and the filter cake was washed with $CHCl_3$ (200 mL). The filter cake was dried under vacuum to give Intermediate 6-4 (1.7 g) as a black solid.



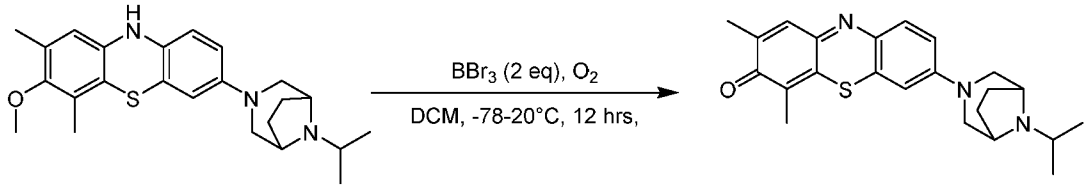
6-4 6-5

[0146] To a solution of Intermediate 6-4 (1.7 g, 2.23 mmol, 1 *eq*) and 8-isopropyl-3,8-diazabicyclo[3.2.1]octane (686.50 mg, 4.45 mmol, 2 *eq*) in $CHCl_3$ (25 mL) was added DIEA (575.18 mg, 4.45 mmol, 2 *eq*) in $CHCl_3$ (5 mL) at 0°C. The mixture was stirred at 20°C for 12 hrs. LCMS showed the starting material was consumed completely and a major peak with desired ms was detected. The reaction mixture was filtered and the filter cake was washed with DCM (30 mL) and dried in vacuum to give Intermediate 6-5 (0.7 g, 1.31 mmol, 58.75% yield, iodide salt) as a black solid: **LCMS (ESI+)**: 1.092 min, m/z 408.3 [5-95AB_3.5min: LC/MS (The column was a Luna C18 30*2.0mm, 3um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min .5% B in 0.01 min, 5-95% B (0.01-2.50 min) with a hold at 95% B for 0.50 min, 95-5% B (3.00 -3.01 min) with a hold at 5% B for 0.49 min. The flow rate was 1 mL/min (0.01-3.00 min)-1.2 mL/min (3.01-3.50min).]



6-5 6-6

[0147] To a solution of Intermediate 6-5 (300 mg, 560.24 μmol, 1 *eq*, I) in ethanol (2 mL) and MeOH (1 mL) was added $N_2H_4 \cdot H_2O$ (280 mg, 5.60 mmol, 10 *eq*) at 0°C. The resulting mixture was stirred at 20°C for 1 hr. LCMS showed a major peak with desired ms was detected. The reaction mixture was poured into aqueous $NaHCO_3$ (20 mL) and extracted with DCM (3 × 10 mL). The organic phases were combined, washed with brine (20 mL), dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give Intermediate 6-6 (150 mg, 366.22 μmol, 65.37% yield) as a purple solid: **LCMS (ESI+)**: 1.555 min, m/z 410.2 [5-95AB_3.5min: LC/MS (The column used for chromatography was a Halo C18 5μm, 3.0*30mm. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min .5% B in 0.01 min, 5-95% B (0.01-2.50 min), with a hold at 95% B for 0.50 min.95-5% B (3.0-3.01min), with a hold at 5% B for 0.49 min. The flow rate was 1.0 mL/min (0.01-3.01min） 1.2 mL/min （3.02-3.50 min） .] ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.93 (s, 1H), 6.59 - 6.44 (m, 2H), 6.42 (s, 1H), 6.33 (s, 1H), 3.53 (s, 3H), 3.32 (s, 2H), 3.14 (br d, *J* = 10.0 Hz, 2H), 2.71 (br d, *J* = 10.1 Hz, 2H), 2.59 - 2.52 (m, 1H), 2.07 (s, 6H), 1.77 (br d, *J* = 3.8 Hz, 2H), 1.61 (br d, *J* = 6.8 Hz, 2H), 1.01 (br d, *J* = 5.9 Hz, 6H).

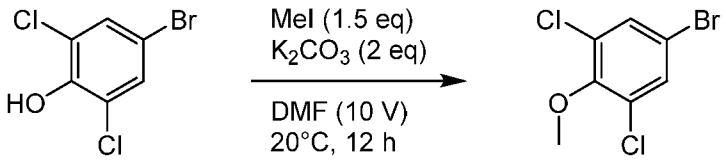


6-6 Compound 6

[0148] To a solution of Intermediate 6-6 (100 mg, 244.15 μmol, 1 *eq*) in DCM (1 mL) was added BBr_3 (122.3 mg, 488 μmol, 2 *eq*) at -78°C. The mixture was stirred at 20°C for 12 hrs under oxygen balloon. The reaction mixture was quenched with methanol (0.1 mL) dropwise at -78°C. The mixture was then poured into aqueous $NaHCO_3$ (20 mL) and extracted with DCM (3 × 20 mL). The organic phases were combined, washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give a crude product. The residue was then purified by flash chromatography on silica gel eluted with DCM/MeOH (0% to 20%) to give the crude product (20 mg), which was then triturated with

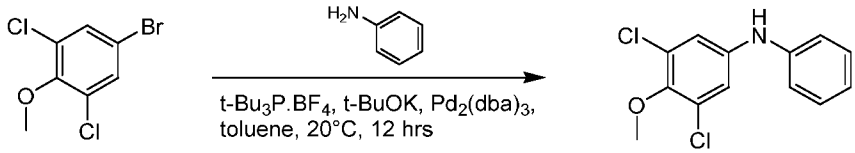
petroleum ether/acetonitrile (0.5 mL/0.5 mL) to give Compound 6 (2.9 mg, 6.73 μmol, 2.76% yield, 91.3% purity) as a purple solid: ^{1}H NMR (400 MHz, CHLOROFORM-d) δ = 7.71 (d, *J* = 9.2 Hz, 1H), 7.43 (d, *J* = 1.1 Hz, 1H), 6.93 (dd, *J* = 2.8, 9.1 Hz, 1H), 6.80 (d, *J* = 2.7 Hz, 1H), 3.84 - 3.60 (m, 2H), 3.47 (br d, *J* = 10.6 Hz, 2H), 3.41 - 3.18 (m, 2H), 2.82 - 2.58 (m, 1H), 2.21 (d, *J* = 0.7 Hz, 3H), 2.14 (s, 3H), 2.10 - 1.98 (m, 2H), 1.86 - 1.71 (m, 2H), 1.39 - 1.01 (m, 6H).

Synthesis of 2,4-dichloro-7-((1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-3H-phenothiazin-3-one (Compound 7)



7-1 **7-2**

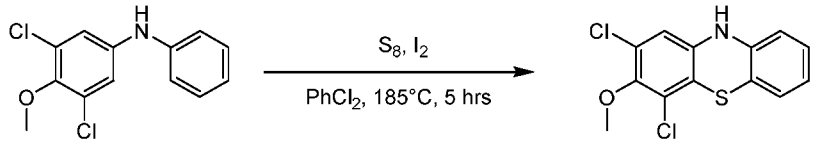
[0149] To a solution of 4-bromo-2,6-dichloro-phenol (Intermediate 7-1, 5 g, 20.67 mmol, 1 *eq*) in DMF (60 mL) was added MeI (4.40 g, 31.00 mmol, 1.93 mL, 1.5 *eq*) and K2CO3 (5.71 g, 41.34 mmol, 2 *eq*) at 20°C. The mixture was stirred at 20 °C for 12hr. LC-MS showed Reactant 1 was consumed completely and one main peak with desired mass was detected. The reaction mixture was diluted with water 300 mL and extracted with MTBE 300 mL (3 x 100 mL). The combined organic layers were washed with brine 150 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 7-2 (4 g, 15.63 mmol, 75.62% yield) as white solid: ^{1}H NMR (400 MHz, CHLOROFORM-d) δ = 7.46 (s, 2H), 3.90 (s, 3H).



7-2 **7-3**

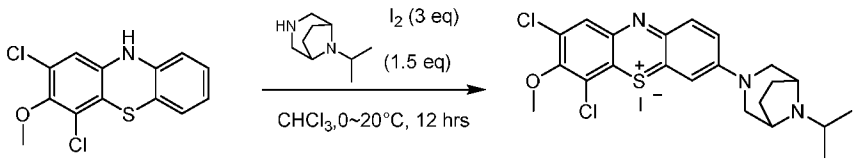
[0150] To a mixture of 5-bromo-1,3-dichloro-2-methoxy-benzene (Intermediate 7-2, 3 g, 11.72 mmol, 1 *eq*), aniline (1.64 g, 17.58 mmol, 1.60 mL, 1.5 *eq*), $Pd_2(dba)_3$ (322.03 mg, 351.67 μmol, 0.03 *eq*) and tritert-butylphosphonium;tetrafluoroborate (204.06 mg, 703.33 μmol, 0.06 *eq*) in toluene (30 mL) was added t-BuOK (in THF) (1 M, 15.24 mL, 1.3 *eq*) at 20°C. The mixture was stirred at 20°C for 12 hrs. The reaction mixture was filtered and the filter cake was washed with toluene. The filtrate was concentrated under reduced pressure to

give a residue which was then purified by column chromatography on silica gel eluted with petroleum ether/ethyl acetate (0 to 3%) to give 3,5-dichloro-4-methoxy-N-phenyl-aniline (Intermediate 7-3, 2 g, 7.09 mmol, 60.45% yield, 95% purity) as a yellow solid: Rf=0.58 (petroleum ether/ethyl acetate= 10:1); **^{1}H NMR:** 400 MHz, $CDCl_3$ δ = 7.32 (t, *J* = 7.9 Hz, 2H), 7.12 - 7.00 (m, 3H), 6.97 (s, 2H), 5.61 (br s, 1H), 3.87 (s, 3H).



7-3 7-4

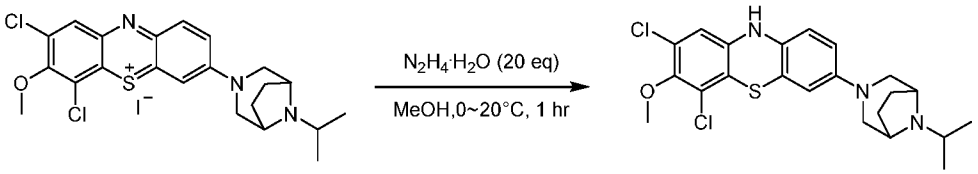
[0151] To a solution of Intermediate 7-3 (1 g, 3.58 mmol), I_2 (272.61 mg, 1.07 mmol, 216.36 µL) in $PhCl_2$ (10 mL) was added S_8 (229.67 mg, 7.16 mmol) at 20°C. The mixture was stirred at 185°C for 5 hrs. The reaction mixture was concentrated under reduced pressure to give a residue, which was purified by column chromatography (SiO_2, Petroleum ether / Ethyl acetate = 30 / 1 to 15 / 1) to give Intermediate 7-4 (1 g, 42.82% yield, 96% purity) as a blue solid: **^{1}H NMR:** 400 MHz, DMSO-d6 δ = 8.73 (s, 1H), 7.05 - 6.93 (m, 2H), 6.78 (br dd, *J* = 1.1, 7.5 Hz, 1H), 6.65 (s, 1H), 6.61 (dd, *J* = 1.0, 7.9 Hz, 1H), 3.70 (s, 3H).



7-4 7-5

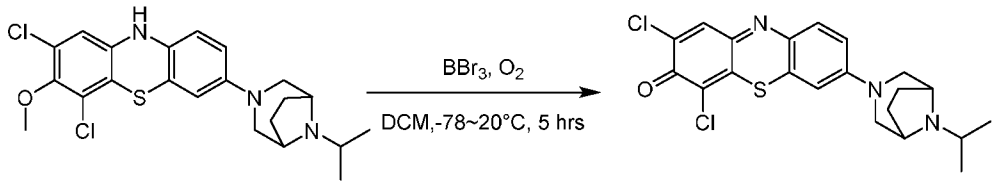
[0152] To a solution of 8-isopropyl-3,8-diazabicyclo[3.2.1]octane (372.46 mg, 2.41 mmol) in $CHCl_3$ (5 mL) was added Intermediate 7-4 (0.5 g, 1.61 mmol) and I_2 (1.23 g, 4.83 mmol) at 0°C. The mixture was stirred at 20°C for 12 hrs. LCMS showed the starting material was consumed, and 52% product with desired ms was detected. The solvent filtered and the filter-cake was dried under reduced pressure to give a crude product. It was purified by re-crystallization from DCM (15 mL × 5). The solvent filtered and the filter-cake was dried under reduced pressure to give Intermediate 7-5 (2.2 g, 1.20 mmol, 29.70% yield, 50.6% purity, 4 I^-) as a blue solid: **LCMS (ESI+)**: 0.477 min, m/z 448.2 [10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm, 5um. Detection methods are diode array (DAD) MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-

100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.]



7-5 7-6

[0153] To a solution of Intermediate 7-5 (2 g, 2.31 mmol) in MeOH (8 mL) was added dropwise hydrazine (1.48 g, 46.28 mmol) at 0°C. The mixture was stirred at 20°C for 1 hr. LCMS showed the starting material was consumed, and desired compound was detected. The reaction mixture was concentrated under reduced pressure to give a residue. It was purified by column chromatography (SiO_2, Petroleum ether / Ethyl acetate = 30 / 1 to 15 / 1) to give Intermediate 7-6 (900 mg, 1.64 mmol, 70.80% yield, 82% purity) as a blue solid: **LCMS (ESI+)**:1.731 min, m/z 450.2 [5-95AB_3.5min: LC/MS (The column was a Luna C18 30*2.0mm, 3um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min .5% B in 0.01 min, 5-95% B (0.01-2.50 min) with a hold at 95% B for 0.50 min, 95-5% B (3.00 -3.01 min) with a hold at 5% B for 0.49 min. The flow rate was 1 mL/min (0.01-3.00min)-1.2 mL/min (3.01-3.50 min).]

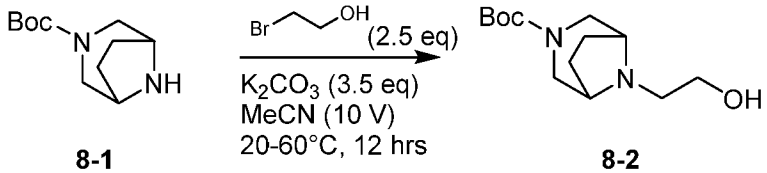


7-6 Compound 7

[0154] To a solution of Intermediate 7-6 (0.2 g, 364.10 μmol) in DCM (1 mL) was added dropwise BBr_3 (182.43 mg, 728.20 μmol) at -78°C. The mixture was stirred at 20°C for 5 hrs under oxygen balloon (15 psi). LCMS showed the starting material was consumed, and 56% product with desired ms was detected. Then MeOH (2 mL) was added dropwise into reaction at -78°C, and the reaction mixture was concentrated under reduced pressure to give a residue. It was then diluted with DCM (20 mL), and poured into aqueous saturated $NaHCO_3$ (30 mL). The aqueous phase was then extracted with DCM (2 mL × 3). The combined organic layers were washed with brine (5 mL × 2), dried over Na_2SO_4, filtered and concentrated under

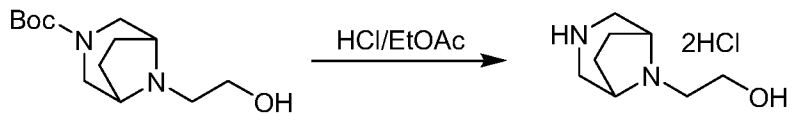
reduced pressure to give a crude product. It was purified by prep-TLC (SiO_2, Dichloromethane: Methanol = 8 / 1) to give residue. It was further purified by prep-TLC (SiO_2, Dichloromethane: Methanol = 8 / 1) to give Compound 7 (51.2 mg, 114.57 μmol, 31.47% yield, 97.2% purity) as dark purple solid: **^{1}H NMR:** 400 MHz, CHLOROFORM-d δ = 7.84 (s, 1H), 7.78 (d, J = 9.4 Hz, 1H), 7.04 (dd, *J* = 2.7, 9.3 Hz, 1H), 6.87 (d, *J* = 2.5 Hz, 1H), 3.73 (br s, 2H), 3.52 (br d, *J* = 11.2 Hz, 2H), 3.36 (br d, *J* = 10.4 Hz, 2H), 2.73 - 2.64 (m, 1H), 2.10 - 1.99 (m, 2H), 1.76 (br d, *J* = 7.7 Hz, 2H), 1.17 (d, *J* = 6.1 Hz, 6H); **LCMS (ESI+):**3.267 min, m/z 434.0 [5_95CD_6min-220-254-ELSD : LC/MS (The gradient was 5% B in 0.40min and 5-95% B at 0.40-3.40 min ,hold on 95% B for 0.45min, and then 95-5% B in 0.01min, the flow rate was 0.8 ml/min. Mobile phase A was H2O + 10mM NH4HCO3, mobile phase B was Acetonitrile. The column used for chromatography was a Xbridge C18 2.1*50mm column (5um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection .MS mode was positive electrospray ionization.MS range was 100-1000.]

Synthesis of 7-((1R,5S)-8-(2-hydroxyethyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-3H-phenothiazin-3-one (Compound 8)



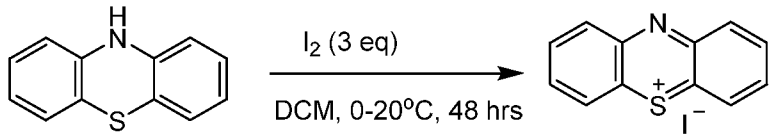
8-1 **8-2**

[0155] To a solution of Intermediate 8-1 (10 g, 47.11 mmol, 1 *eq*) and 2-bromoethanol (14.72 g, 117.76 mmol, 8.35 mL, 2.5 *eq*) in AcCN (100 mL) was added K_2CO_3 (22.79 g, 164.87 mmol, 3.5 *eq*) at 20°C. The mixture was stirred at 60°C for 12 hours. TLC (Ethyl acetate: Methanol=2:1, Rf =0.25) showed the reaction worked well. The mixture was filtered and the filter cake was washed with ethyl acetate (100 mL). The filtrate was concentrated under reduced pressure to give a crude product. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=0 to 100%, Ethyl acetate/methanol=0 to 30%) to give Intermediate 8-2 (17 g, 46.42 mmol, 98.55% yield, 70% purity) as yellow oil: **^{1}H NMR** (400 MHz, CHLOROFORM-d) δ = 3.63 (t, *J* = 5.3 Hz, 3H), 3.26 (br d, *J* = 18.6 Hz, 2H), 3.19 - 3.00 (m, 2H), 2.60 - 2.51 (m, 2H), 1.96 - 1.84 (m, 2H), 1.70 (br dd, *J* = 8.0, 15.7 Hz, 2H), 1.51 - 1.41 (m, 9H).



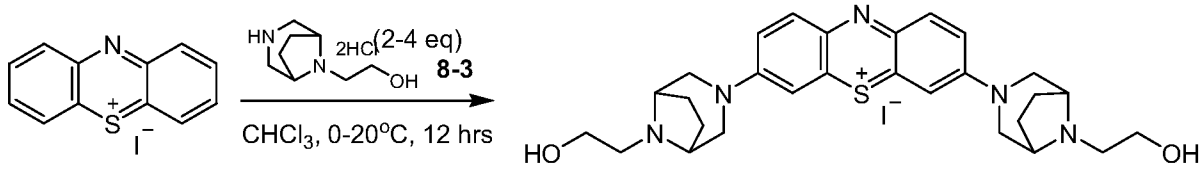
8-2 8-3

[0156] To a solution of Intermediate 8-2 (17 g, 66.32 mmol, 1 *eq*) in ETHYL ACETATE (100 mL) was added HCl/EtOAc (4 M, 40 mL, 2.41 *eq*) at 20°C. The mixture was stirred at 20°C for 2 hours. TLC (ethyl acetate: methanol=3:1, Rf=0) showed the reaction worked. The mixture was concentrated under reduced pressure to give the product. The crude product was triturated with ethyl acetate: dichloromethane (5/1, 20 mL) at 20°C for 10 min and then filtered. The solid was dried in high vacuum to give Intermediate 8-3 (10 g, 43.64 mmol, 65.80% yield, 2HCl) as white solid: **^{1}H NMR** (METHANOL-d4, 400 MHz) δ (ppm) 4.43 (br s, 2H), 3.92-4.02 (m, 2H), 3.83 (d, J = 13.9 Hz, 2H), 3.48-3.62 (m, 2H), 3.27 (dt, J = 3.3, 1.6 Hz, 2H), 2.44-2.56 (m, 2H), 2.22-2.37 (m, 2H).



8-4 8-5

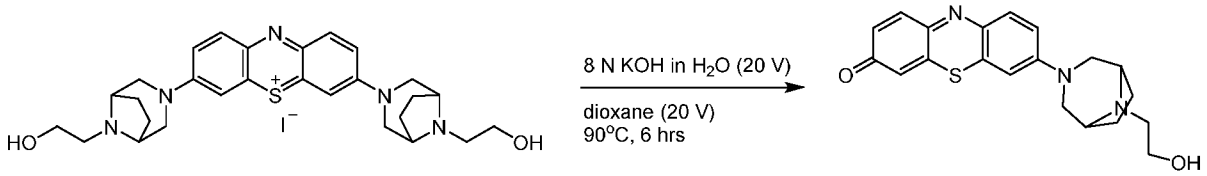
[0157] To a solution of Intermediate 8-4 (25 g, 125.46 mmol, 1 *eq*) in DCM (500 mL) was added a solution of I_2 (95.53 g, 376.37 mmol, 75.81 mL, 3 *eq*) in DCM (2000 mL) dropwise at 0°C. The mixture was stirred at 20 °C for 48 hrs. LC-MS showed starting material was consumed completely and one main peak with desired mass was detected. The mixture was filtered and the cake was washed with DCM (20 x 200 mL). Intermediate 8-5 (180 g, 229.17 mmol, 91.34% yield, 90% purity) was obtained as black solid: **1H NMR** (400 MHz, DMSO-d6) δ = 8.05 (br s, 2H), 7.95 - 7.82 (m, 2H), 7.77 - 7.65 (m, 2H), 7.65 - 7.46 (m, 2H).



8-5 8-6

[0158] To a solution of Intermediate 8-3 (12.99 g, 56.67 mmol, 4 *eq*, 2HCl) and Intermediate 8-5 (10 g, 14.17 mmol, 1 *eq*) in $CHCl_3$ (250 mL) was added DIPEA (18.31 g, 141.67 mmol, 24.68 mL, 10 *eq*) dropwise at 0°C. The mixture was stirred at 20°C

for 12 hours. LCMS showed the reaction worked. The mixture was concentrated under reduced pressure, and the residue was washed with methyl tert-butyl ether (100 mL) and dried by high vacuum to give Intermediate 8-6 (20 g, 12.63 mmol, 89.13% yield, 40% purity) as blue solid: **LCMS** (ESI+): m/z 506 (M^+), RT: 0.677min [5-95CD_2min: LC/MS (The column used for chromatography was Xbridge C18 2.1*50mm,5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 10 mM Ammonium bicarbonate in water, and mobile phase B was HPLC grade acetonitrile.The gradient was 5-95% B in 1.50 min. 5% B in 0.01 min,5-95% B (0.01-0.70 min) ,95%B(0.70-1.16min), 95-5% B (1.16-1.50 min) . The flow rate was 1.5mL/min.]

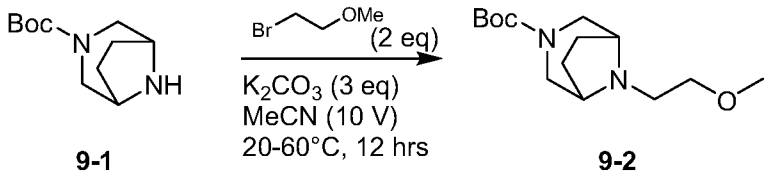


8-6 Compound 8

[0159] To a solution of Intermediate 8-6 (20 g, 12.63 mmol, 1 *eq*) in dioxane (200 mL) was added a solution of KOH (8 M, 200 mL, 126.72 *eq*) at 0°C under N_2. The mixture was stirred at 90°C for 6 hours. LCMS showed starting material consumed and desired Mass was detected. The mixture was extracted with ethyl acetate (3 x 500 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product. The residue was purified by column chromatography (SiO_2, polarity gradient: Petroleum ether/Ethyl acetate=100% to 0%, ethyl acetate/dichloromethane =80% to 90%, dichloromethane/methyl alcohol=95%). The residue was triturated with ethyl acetate (10 mL) at 20°C for 10 min and then filtered. The solid was dried in high vacuum to give Compound 8 (241 mg, 649.29 μmol, 5.14% yield, 99% purity) as dark purple solid: **LCMS (ESI+)**: 2.184 min, m/z 368 (M+H). LC/MS (The gradient was 5%B in 0.40min and 5-95% B at 0.40-3.40 min ,hold on 95% B for 0.45min, and then 95-5%B in 0.01min, the flow rate was 0.8 ml/min. Mobile phase A was H_2O+10 mM NH_4HCO_3, mobile phase B was Acetonitrile. The column used for chromatography was a Xbridge Shield RP18 2.1*50mm column (5um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection as well as positive electrospray ionization.MS range was 100-1000.) **^{1}H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 7.64 (d, J = 9.1 Hz, 1H), 7.49 (d, J = 9.9 Hz, 1H), 6.85 (dd, J = 9.3, 2.8 Hz, 1H), 6.78 (dd, J = 9.8, 2.1 Hz, 1H), 6.62 (t, J = 2.1 Hz, 2H), 3.58 (br

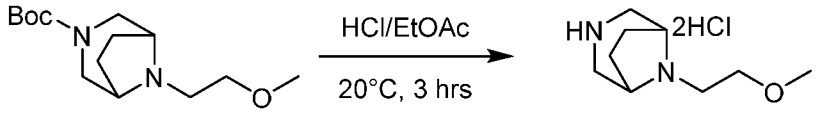
t, J = 5.1 Hz, 2H), 3.45-3.53 (m, 2H), 3.36 (br s, 2H), 3.16 (br d, J = 10.9 Hz, 2H), 2.86-3.02 (m, 1H), 2.54 (t, J = 5.3 Hz, 2H), 1.85-2.02 (m, 2H), 1.59-1.78 (m, 2H).

Synthesis of 7-((1R,5S)-8-(2-methoxyethyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-3H-phenothiazin-3-one (Compound 9)



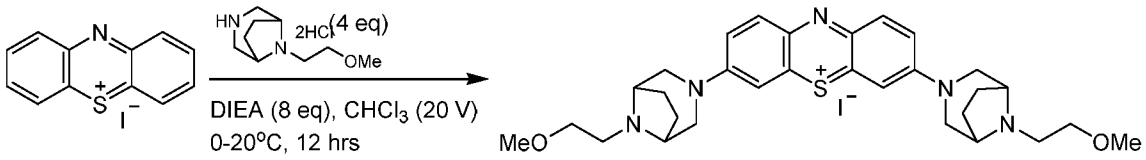
9-1 9-2

[0160] To a solution of Intermediate 9-1 (5 g, 23.55 mmol, 1 eq) and 1-bromo-2-methoxy-ethane (6.55 g, 47.11 mmol, 4.43 mL, 2 eq) in AcCN (50 mL) was added K_2CO_3 (9.77 g, 70.66 mmol, 3 eq) at 20°C. The mixture was stirred at 60°C for 12 hours. One addition vial in 200 mg scale and one additional vial in 1 g scale were set up as described. TLC (ethyl acetate: methanol=3:1, R_f=0.3, I_2) showed the reaction worked well. The mixture was combined with other reaction mixture together. The mixture was filtered and the filter cake was washed with ethyl acetate (100 mL). The filtrate was concentrated under reduced pressure to give Intermediate 9-2 (7.9 g, 26.30 mmol, 90.04% yield, 90% purity) as colorless oil: **^{1}H NMR** (400 MHz, CHLOROFORM-d) δ = 3.75 - 3.66 (m, 1H), 3.62 - 3.54 (m, 1H), 3.51 (t, J = 5.8 Hz, 2H), 3.36 (s, 3H), 3.28 - 3.15 (m, 2H), 3.14 - 2.98 (m, 2H), 2.54 (t, J = 5.8 Hz, 2H), 1.90 (br dd, J = 3.3, 6.6 Hz, 2H), 1.71 - 1.55 (m, 2H), 1.45 (s, 9H).



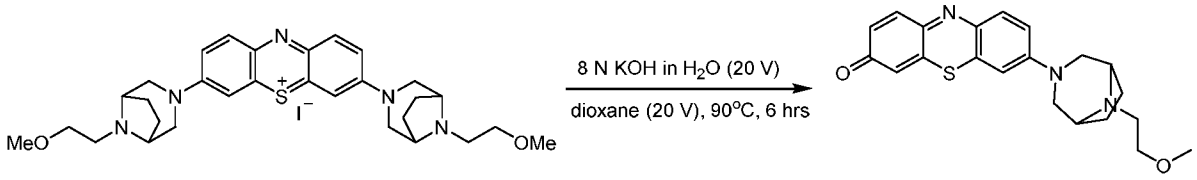
9-2 9-3

[0161] A solution of Intermediate 9-2 (5.9 g, 19.64 mmol, 1 eq, purity 90%) in HCl/ethyl acetate (20 mL) was stirred at 20°C for 3 hours. TLC (ethyl acetate: methanol=3:1, R_f=0.3, I_2) showed the reaction worked well. The mixture was concentrated under reduced pressure to give Intermediate 9-3 (5.5 g, 18.09 mmol, 92.13% yield, 80% purity, 2HCl) as off white solid: **^{1}H NMR** (400 MHz, METHANOL-d4) δ = 4.41 (br s, 2H), 3.89 - 3.81 (m, 4H), 3.57 (br d, J = 14.5 Hz, 2H), 3.42 (s, 3H), 3.41 - 3.34 (m, 2H), 2.57 - 2.46 (m, 2H), 2.34 - 2.26 (m, 2H).



8-5 9-4

[0162] To a solution of Intermediate 8-5 (4.5 g, 5.74 mmol, 1 eq, purity 90%) and Intermediate 9-3 (6.20 g, 22.95 mmol, 4 eq, purity 80%, 2HCl) in $CHCL_3$ (90 mL) was added DIEA (5.93 g, 45.90 mmol, 7.99 mL, 8 eq) dropwise at 0°C under N_2. The mixture was stirred at 20°C for 12 hours. LCMS showed the reaction worked well. The mixture was concentrated under reduced pressure to give the crude. The crude product was triturated with tert-butyl methyl ether (50 mL), filtered and the filter cake was washed with tert-butyl methyl ether (50 mL). The filter cake was dried by high vacuum to give crude Intermediate 9-4 (18 g, 3.54 mmol, 61.64% yield, 13% purity) as blue solid which was used without further purification.

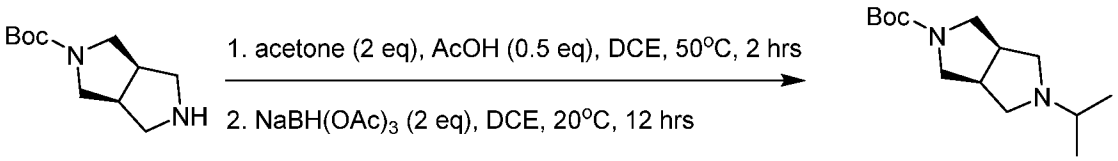


9-4 Compound 9

[0163] To a solution of Intermediate 9-4 (5.00 g, 982.41 μmol, 1 eq, purity 13%) in dioxane (100 mL) was added KOH (8 M, 100 mL, 814.32 eq) at 20°C. The mixture was stirred at 90°C for 5 hours. One additional vial in 1.5 g scale and one additional vial in 5 g scale were set up as described above. LCMS showed the reaction worked well. The mixture was combined with other reaction mixture together. The mixture was extracted with ethyl acetate (3 x 100 mL). The combined organic phase was washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the crude. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate=0 to 100%, Ethyl acetate/dichloromethane=0 to 20%, dichloromethane/methanol=0 to 5%) and the desired eluent was concentrated under reduced pressure to give the crude. The crude was triturated with tret-butyl methyl ether (5 mL), filtered and the filter cake was washed with petroleum ether (30 mL), filtered and the filter cake was dried by high vacuum to give Compound 9 (300 mg, 783.24 μmol, 34.66% yield, 99.6% purity) as dark purple solid: 1H NMR (400 MHz, CHLOROFORM-d) δ = 7.71 (d, J = 9.3 Hz, 1H), 7.56 (d, J = 9.9 Hz, 1H),

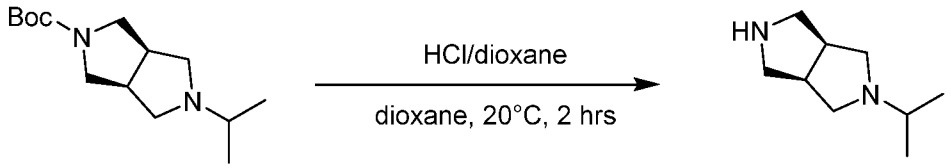
6.92 (dd, J = 2.7, 9.2 Hz, 1H), 6.85 (dd, J = 2.2, 9.8 Hz, 1H), 6.69 (d, J = 2.1 Hz, 2H), 3.58 (br t, J = 5.4 Hz, 2H), 3.51 (br d, J = 10.8 Hz, 4H), 3.39 (s, 3H), 3.33 (br d, J = 9.8 Hz, 2H), 2.67 (br t, J = 5.1 Hz, 2H), 2.12 - 2.01 (m, 2H), 1.78 - 1.67 (m, 2H); **LCMS (ESI+):** m/z 382.1 (M+1), RT: 1.701 min, 5_95AB_6min-220-254-ELSD： LC/MS(The gradient was 5%B in 0.40min and 5-95% B in 2.60 min , hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Kinetex C18 2.1*50mm,5um. Detection methods are diode array (DAD), and evaporative light scattering detection (ELSD) . MS mode was positive electrospray ionization.MS range was 100-1000.

Synthesis of 7-((3aR,6aS)-5-isopropylhexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)-3H-phenothiazin-3-one (Compound 10)



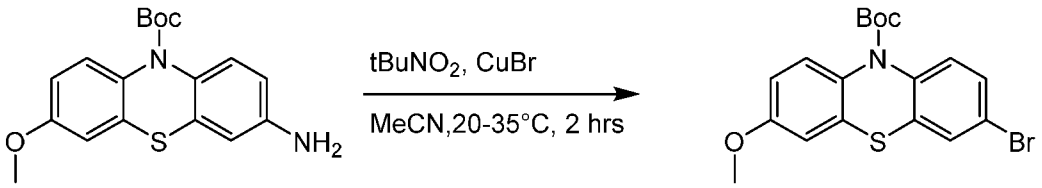
10-1 10-2

[0164] To a solution of Intermediate 10-1 (3 g, 14.13 mmol) in DCE (30 mL) was added CH_3COOH (424.32 mg, 7.07 mmol, 404.50 µL) and acetone (1.64 g, 28.26 mmol, 2.08 mL). The mixture was stirred at 50°C at 2 hrs. Then was added $NaBH(OAc)_3$ (5.99 g, 28.26 mmol) at 20°C. The mixture was stirred at 20 °C for 12 hrs. An additional four reactions were set up as described above. The combined reaction mixture was poured into saturated aqueous $NaHCO_3$ (50 mL) and extracted with DCM (2 × 50 mL). The organic phase was separated, washed with brine (2 × 50 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 10-2 (14 g, 44.03 mmol, 62.31% yield) as a yellow oil. The crude product was used into the next step without further purification: **^{1}H NMR:** 400MHz CHLOROFORM-d δ = 3.48 (br s, 2H), 3.27 (br d, J = 1.5 Hz, 2H), 2.91 (br d, J = 8.4 Hz, 2H), 2.80 (br s, 2H), 2.43 - 2.32 (m, 1H), 2.28 (br d, J = 6.0 Hz, 2H), 1.46 (s, 9H), 1.08 (d, J = 6.4 Hz, 6H).



10-2 10-3

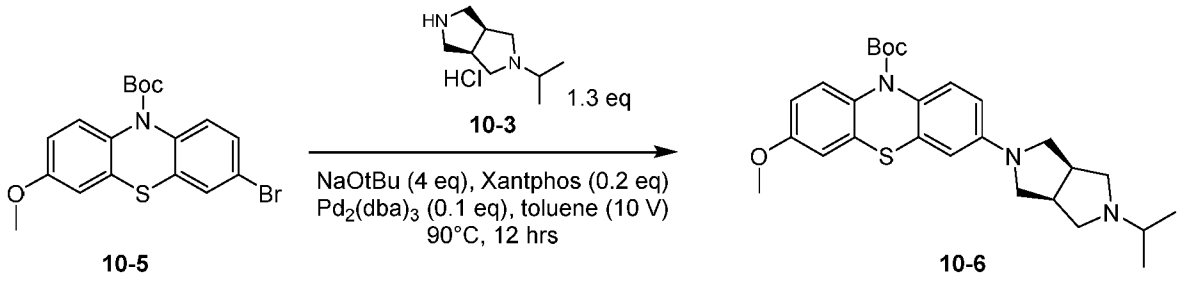
[0165] To a solution of Intermediate 10-2 (2.50 g, 7.86 mmol) in dioxane (5 mL) was added HCl/dioxane (4 M, 25 mL). The mixture was stirred at 20°C for 2 hrs. Additional four reactions were set up as described above. The combined reaction mixture was concentrated under reduced pressure to give a residue. The crude product was triturated with acetonitrile (100 mL) to give Intermediate 10-3 (10.4 g, 36.62 mmol, 2 HCl salt) as white solid: **^{1}H NMR:** 400MHz DMSO-d6) δ = 11.31 (br d, J = 2.0 Hz, 1H), 10.57 - 9.16 (m, 2H), 3.74 - 3.65 (m, 1H), 3.59 (br dd, J = 4.6, 10.6 Hz, 1H), 3.41 (br d, J = 4.6 Hz, 2H), 3.35 - 3.09 (m, 7H), 1.30 (dd, J = 6.5, 15.9 Hz, 6H); **LCMS (ESI+)**:0.099 min, m/z 155.3, 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm, 5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.



10-4 10-5

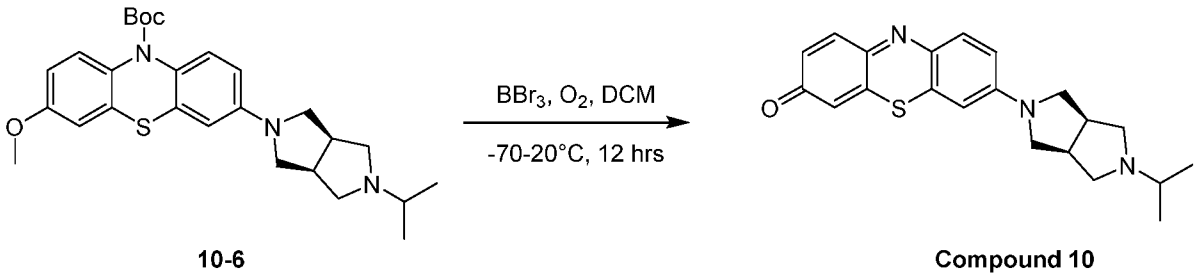
[0166] To a solution of dibromocopper (7.00 g, 31.36 mmol, 1.47 mL, 1.2 *eq*) and tert-butyl nitrite (4.04 g, 39.20 mmol, 4.66 mL, 1.5 *eq*) in ACN (100 mL) was stirred at 20°C for 10 mins. Then Intermediate 10-4 (10 g, 26.13 mmol, 1 *eq*) was added to the mixture at 20°C. The mixture was stirred at 35°C for 2 hours. One additional vial in 1 g scale was set up as described above. LCMS showed the reaction worked. The mixture wa10-4s diluted with water (100 mL) and ethyl acetate (100 mL). The mixture was extracted with ethyl acetate (3 x 100 mL), washed with brine (100 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=6%) to give Intermediate 10-5 (4.7 g, 10.36 mmol, 36.04% yield, 90% purity) as yellow solid: **1H NMR** (DMSO-d6, 400 MHz) δ

(ppm) 7.69 (d, J = 2.1 Hz, 1H), 7.39-7.57 (m, 3H), 7.02 (d, J = 2.9 Hz, 1H), 6.91 (dd, J = 8.9, 2.8 Hz, 1H), 3.76 (s, 3H), 1.42 (s, 9H).



10-5 10-6

[0167] To a solution of Intermediate 10-5 (180 mg, 440.84 μmol, 1 *eq*) and Intermediate 10-3 (130.19 mg, 573.10 μmol, 1.3 *eq*, 2HCl) in toluene (10 mL) was added Xantphos (51.02 mg, 88.17 μmol, 0.2 *eq*), $Pd_2(dba)_3$ (40.37 mg, 44.08 μmol, 0.1 *eq*) and tBuONa (169.46 mg, 1.76 mmol, 4 *eq*) at 20°C under N_2. The mixture was stirred at 90°C for 12 hours under N_2. One additional vial in 0.02 g scale was set up as described above. LCMS showed the reaction worked. The mixture was poured into water (20 mL), extracted with ethyl acetate (3 x 10 ml), washed with brine (10 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the product. The mixture was purified by prep-TLC (petroleum ether: ethyl acetate=3:1, Rf=0.39) to give Intermediate 10-6 (70 mg, 145.33 μmol, 29.97% yield) as yellow solid: **LCMS (ESI+)**: 0.484 min, m/z 482 (M^+), 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm,5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.

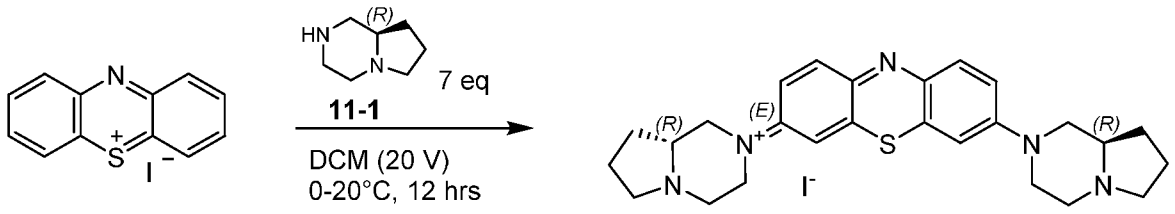


10-6 Compound 10

[0168] To a solution of Intermediate 10-6 (70 mg, 145.33 μmol, 1 *eq*) in DCM (5 mL) was added BBr_3 (182.05 mg, 726.67 μmol, 70.02 μL, 5 *eq*) dropwise at -78°C under N_2. The mixture was stirred at 20°C for 12 hours under O_2 (15 psi). LCMS showed the reaction

worked. The mixture was quenched with MeOH (20 mL) at -40°C and then adjust to PH=8 with saturated sodium bicarbonate aqueous solution (20 mL). The mixture was extracted with dichloromethane (3 x 20 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product. The product was purified by column chromatography (SiO_2, polarity gradient: petroleum ether/Ethyl acetate=100% to 0%, ethyl acetate/dichloromethane =80% to 90%, dichloromethane/methyl alcohol=95%). And then purified by prep-TLC (dichloromethane: methanol=2:1). The residue was triturated with ethyl acetate (10 mL) at 20°C for 10 min and then filtered. The solid was dried in high vacuum to give Compound 10 (10 mg, 24.35 μmol, 16.76% yield, 89% purity) as dark purple solid: **1H NMR** (DMSO+D_2O-d6, 400 MHz) δ (ppm) 7.71 (d, J = 9.0 Hz, 1H), 7.59 (d, J = 9.6 Hz, 1H), 6.92 (dd, J = 9.3, 2.0 Hz, 1H), 6.86 (br d, J = 2.1 Hz, 1H), 6.66-6.80 (m, 2H), 5.63 (s, 1H), 3.65 (br dd, J = 10.3, 6.6 Hz, 2H), 3.48 (br d, J = 11.3 Hz, 2H), 3.27-3.41 (m, 2H), 2.97-3.25 (m, 5H), 1.18 (br d, J = 6.4 Hz, 6H); **LCMS (ESI+)**: 2.564 min, m/z 366 (M+H), 5_95CD_6min-220-254-ELSD : LC/MS(The gradient was 5%B in 0.40min and 5-95% B at 0.40-3.40 min ,hold on 95% B for 0.45min, and then 95-5%B in 0.01min, the flow rate was 0.8 ml/min. Mobile phase A was H2O+10mM NH4HCO3, mobile phase B was Acetonitrile. The column used for chromatography was a Xbridge C18 2.1*50mm column (5um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection .MS mode was positive electrospray ionization. MS range was 100-1000.

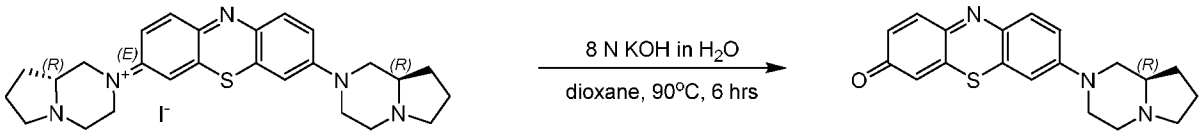
Synthesis of (R)-7-(hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl)-3H-phenothiazin-3-one (Compound 11)



8-5 **11-2**

[0169] To a solution of Intermediate 8-5 (0.5 g, 707.33 μmol, 1 *eq*) in DCM (5 mL) was added Intermediate 11-1 (624.85 mg, 4.95 mmol, 7 *eq*) at 0°C dropwise slowly. The mixture was stirred at 20°C for 12 hours. One additional vial in 0.2 g scale were set up as described above. LCMS showed starting material consumed and 22% of desired mass was detected. The mixture was concentrated under reduced pressure to give the crude product. The crude

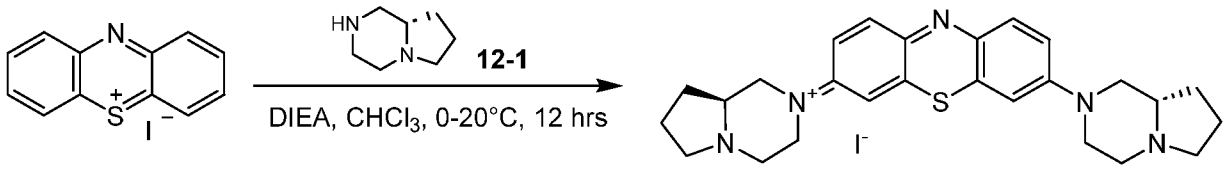
product was triturated with methyl tert-butyl ether (10 mL) at 20°C for 10 min and then filtered to give Intermediate 11-2 (1 g, 383.59 μmol, 36.15% yield, 22% purity) as blue solid: **LCMS** (ESI+): m/z 446 (M^+), RT: 0.70min (Description: Mobile Phase: 0.04% TFA in water(solvent A) and mobile phase B was 0.02% TFA in HPLC grade acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over 2.5 minutes and holding at 95% for 0.5 minutes at a flow rate of 1 mL/min(0.01-3.00min), Column:Agilent Poroshell SB-C18 3.0*30mm,4.0um Wavelength: UV 220nm&254nm Column temperature: 40°C; MS ionization: ESI).



11-2 **Compound 11**

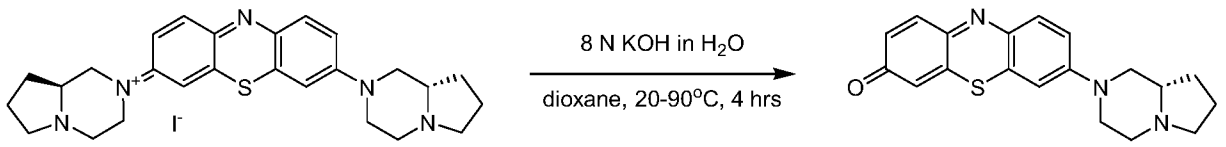
[0170] A solution of Intermediate 11-2 (1 g, 1.74 mmol, 1 *eq*) in dioxane (10 mL) and KOH (8 M, 10 mL, 45.88 *eq*) was stirred at 90°C for 6 hours. LCMS showed starting material consumed and major peak with desired mass was detected. The mixture was extracted with ethyl acetate (3 x 10 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the product. The residue was purified by column chromatography (SiO_2, polarity gradient: petroleum ether/ethyl acetate=100% to 0%, ethyl acetate/ dichloromethane=80% to 90%, dichloromethane/methyl alcohol=95%). The claret red eluent was collected and concentrated under reduced pressure. The residue was triturated with ethyl acetate (10 mL) at 20°C for 10 min and then filtered to give Compound 11 (22 mg, 63.89 μmol, 3.66% yield, 98% purity) as dark purple solid: **1H NMR** (DMSO-d6, 400 MHz) δ (ppm) 7.69 (d, J = 9.0 Hz, 1H), 7.50-7.62 (m, 1H), 7.21-7.31 (m, 2H), 6.67-6.80 (m, 2H), 4.23 (br d, J = 11.6 Hz, 1H), 4.07 (br d, J = 12.1 Hz, 1H), 2.98-3.13 (m, 3H), 2.71 (br t, J = 11.4 Hz, 1H), 2.16-2.26 (m, 1H), 2.09 (q, J = 8.9 Hz, 1H), 1.94-2.05 (m, 1H), 1.81-1.92 (m, 1H), 1.64-1.80 (m, 2H), 1.39 (qd, J = 10.8, 7.1 Hz, 1H); **LCMS (ESI+)**: 2.468 min, m/z 338 (M+H), LC/MS (The gradient was 5%B in 0.40min and 5-95% B at 0.40-3.40 min ,hold on 95% B for 0.45min, and then 95-5%B in 0.01min, the flow rate was 0.8 ml/min. Mobile phase A was H_2O+10 mM NH_4HCO_3, mobile phase B was Acetonitrile. The column used for chromatography was a Xbridge Shield RP18 2.1*50mm column (5um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection as well as positive electrospray ionization.MS range was 100-1000.)

Synthesis of (S)-7-(hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl)-3H-phenothiazin-3-one (Compound 12)



8-5 12-2

[0171] To a mixture of Intermediate 8-5 (0.5 g, 708.34 μmol, 4 I salt) in $CHCl_3$ (10 mL) was added dropwise a solution of Intermediate 12-1 (846.27 mg, 4.25 mmol, 2 HCl) and N,N-diisopropylethylamine (1.37 g, 10.63 mmol) in $CHCl_3$ (10 mL) at 0°C. The mixture was stirred at 20°C for 12 hrs. The mixture was concentrated under vacuum to give a residue. The residue was purified by column chromatography (SiO2, dichloromethane: methyl alcohol = 5/1 to 3/1) to give Intermediate 12-2 (360 mg, 62.8% purity, I salt) as a dark solid: **LCMS (ESI+)**:1.030 min, m/z 446.2, 5-95AB_3.5min: LC/MS (The column used for chromatography was a Halo C18 5μm, 3.0*30mm. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min .5% B in 0.01 min, 5-95% B (0.01-2.50 min), with a hold at 95% B for 0.50 min.95-5% B (3.0-3.01min), with a hold at 5% B for0.49 min. The flow rate was 1.0 mL/min (0.01-3.01min) 1.2 mL/min (3.02-3.50 min).

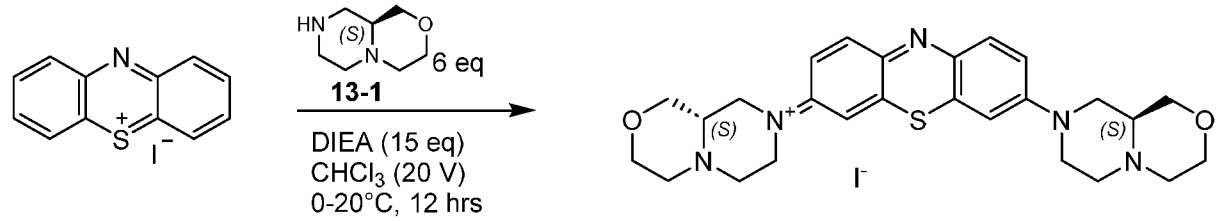


12-2 Compound 12

[0172] To a solution of Intermediate 12-2 (300 mg, 421.83 μmol) in dioxane (15 mL) was added dropwise aqueous KOH (8 M, 3 mL) at 20°C. The mixture was stirred at 90°C for 4 hrs. LCMS showed the starting material was consumed, and 57% product with desired was detected. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, dichloromethane: methyl alcohol = 20/1 to 10/1) to give the crude product. It was then triturated with ethyl acetate (5 mL) to

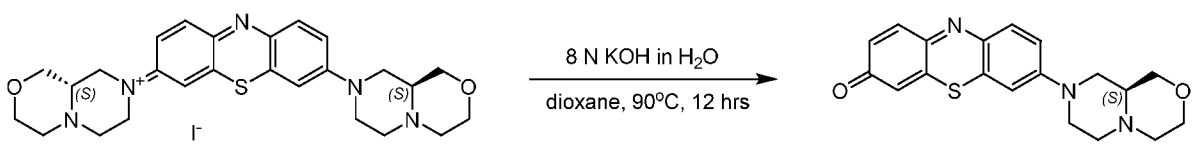
give Compound 12 (22.8 mg, 99.1% purity) as a purple solid: **^{1}H NMR:** 400MHz CHLOROFORM-d δ = 7.65 (d, *J* = 9.3 Hz, 1H), 7.49 (d, *J* = 9.8 Hz, 1H), 6.96 (br dd, *J* = 2.1, 9.1 Hz, 1H), 6.78 (dd, *J* = 1.9, 9.8 Hz, 1H), 6.73 (d, *J* = 2.3 Hz, 1H), 6.62 (d, *J* = 1.9 Hz, 1H), 3.99 - 3.92 (m, 1H), 3.82 - 3.82 (m, 1H), 3.20 - 3.04 (m, 3H), 2.86 - 2.63 (m, 1H), 2.42 - 2.26 (m, 1H), 2.22 - 2.03 (m, 2H), 1.87-1.50 (m, 4H), **LCMS (ESI+)**:1.612 min, m/z 338.1, 5_95AB_6min-220-254-ELSD : LC/MS (The gradient was 5% B in 0.40 min and 5-95% B in 2.60 min, hold on 95% B in 1.00min, and then 95-5% B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Luna C18 50*2.0mm column (5 um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection. MS mode was positive electrospray ionization. MS range was 100-1000.

Synthesis of (S)-7-(hexahydropyrazino[2,1-c][1,4]oxazin-8(1H)-yl)-3H-phenothiazin-3-one (Compound 13)



8-5 **13-2**

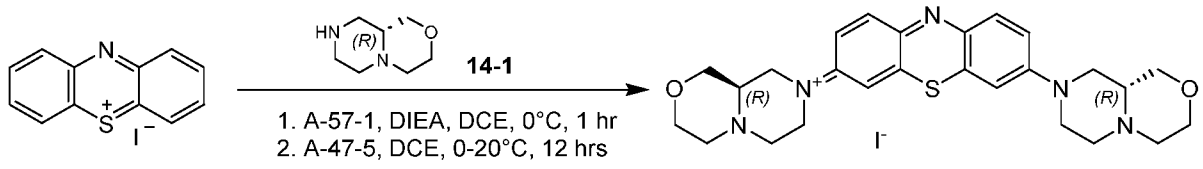
[0173] A mixture of Intermediate 13-1 (1 g, 4.65 mmol, 6 *eq*) and DIEA (1.50 g, 11.62 mmol, 2.02 mL, 15 *eq*) in DCM (20 mL) was stirred for 30 min at 20°C, then added to a mixture of Intermediate 8-5 (607.65 mg, 774.76 μmol, 1 *eq*, I4) in DCM (20 mL) dropwise at 0°C. The resulting mixture was stirred for 12 h at 20°C. One additional vial in 0.5 g scale was set up as described above. The mixtures were combined and concentrated under reduced pressure to give the crude residue, which was triturated with MEBT (100 mL) for 30 min and the solvent was removed. Intermediate 13-2 (2.5 g, 412.86 μmol, crude) was obtained as a blue solid and used directly.



13-2 **Compound 13**

[0174] To a solution of Intermediate 13-2 (2.5 g, 412.86 μmol, 1 *eq*) in dioxane (30 mL) was added a aqueous solution of KOH (8 M, 29.64 mL, 574.35 *eq*)at 20°C. The mixture was stirred at 90°C for 12 h. One additional vial in 0.5 g scale was set up as described above. The dioxane layer was separated and the aqueous layer was extracted with EtOAc (3 X 20 mL). The organic layer was combined and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, DCM: MeOH = 95:5 to 90:10) and the desired eluent was concentrated under reduced pressure to give the crude product, which was triturated with EtOAc (4 mL) and filtered. The filter cake was collected and dried by high vacuum, then diluted withe MeCN (1 mL) and lyophilized to give Compound 13 (31.5 mg, 85.92 μmol, two steps 7.4% yield, 96.4 % purity) as black purple solid: **^{1}H NMR** (400 MHz, CHLOROFORM-d) δ = 7.74 (d, J = 9.1 Hz, 1H), 7.57 (d, J = 9.9 Hz, 1H), 7.01 (dd, J = 2.5, 9.1 Hz, 1H), 6.87 (dd, J = 1.9, 9.8 Hz, 1H), 6.78 (d, J = 2.5 Hz, 1H), 6.71 (d, J = 1.9 Hz, 1H), 3.97 - 3.80 (m, 3H), 3.74 (br t, J = 10.1 Hz, 1H), 3.65 (br d, J = 11.9 Hz, 1H), 3.49 - 3.28 (m, 1H), 3.28 - 3.07 (m, 1H), 3.04 - 2.84 (m, 1H), 2.84 - 2.57 (m, 2H), 2.56 - 2.30 (m, 3H), 2.02 (s, 1H); **LCMS (ESI+)**:2.264 min, m/z 354, LC/MS(The gradient was 5%B in 0.40min and 5-95% B in 2.60 min , hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Kinetex C18 2.1*50mm, 5 um. Detection methods are diode array (DAD), and evaporative light scattering detection (ELSD). MS mode was positive electrospray ionization.MS range was 100-1000.)

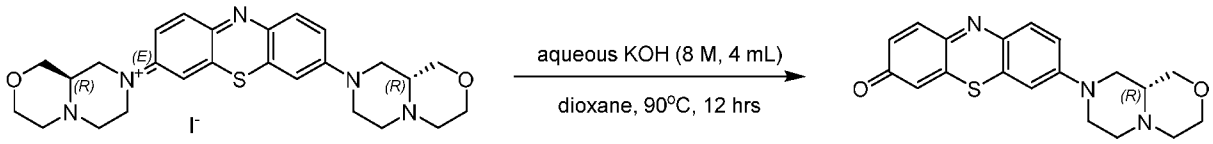
Synthesis of (R)-7-(hexahydropyrazino[2,1-c][1,4]oxazin-8(1H)-yl)-3H-phenothiazin-3-one (Compound 14)



8-5 14-2

[0175] To a solution of Intermediate 14-1 (2.44 g, 11.33 mmol, 2 HCl) in 1, 2-dichloroethane (20 mL) was added N,N-diisopropylethylamine (2.75 g, 21.3 mmol) at 0°C, then the mixture was stirred at 0°C for 1 hr. The mixture was added dropwise to a solution of Intermediate 8-5 (1 g, 1.42 mmol) in 1, 2-dichloroethane (20 mL) at 0°C. The resulting

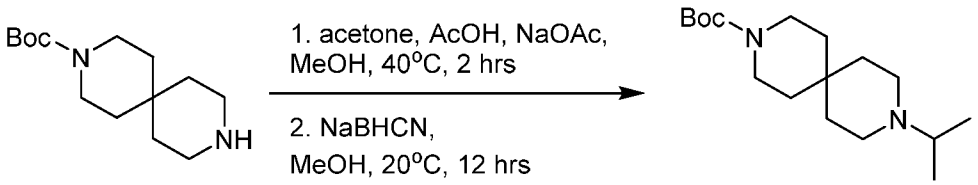
mixture was stirred at 20°C for 12 hrs. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, DCM: MeOH = 2:1 to 1:1) to give Intermediate 14-2 (800 mg, 81% purity, I) as a blue solid: LCMS (ESI+): RT: 0.832 min, m/z 478.2 (5-95AB_3.5min: LC/MS (The column used for chromatography was a Halo C18 5μm, 3.0*30mm. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min. 5% B in 0.01 min, 5-95% B (0.01-2.50 min), with a hold at 95% B for 0.50 min.95-5% B (3.0-3.01min), with a hold at 5% B for 0.49 min. The flow rate was 1.0 mL/min (0.01-3.01 min) 1.2 mL/min (3.02-3.50 min).)



14-2 Compound 14

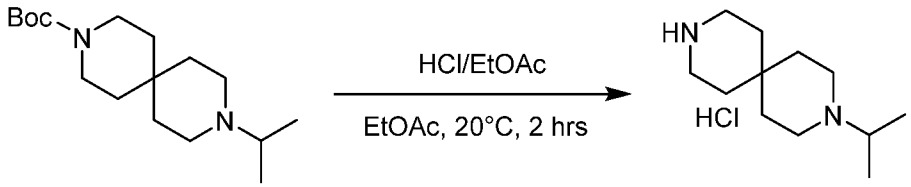
[0176] To a solution of Intermediate 14-2 (500 mg, 668.83 μmol, I) in dioxane (5 mL) was added aqueous KOH (8 M, 4 mL). The mixture was stirred at 90°C for 12 hrs. LCMS showed the starting material was consumed. The mixture was extracted with CH_2Cl_2 (5 mL × 2). The combined organic phase was washed with brine (2 mL × 2), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by column chromatography (SiO_2, DCM: MeOH = 20/1 to 10/1) to give a crude product. It was then purified by trituration with ethyl acetate (3 mL) to give Compound 14 (30.5 mg, 97.3% purity) as a purple solid: **^{1}H NMR:** 400MHz CHLOROFORM-d δ = 7.74 (d, *J* = 9.3 Hz, 1H), 7.57 (d, *J* = 9.8 Hz, 1H), 7.01 (dd, *J* = 2.8, 9.2 Hz, 1H), 6.87 (dd, *J* = 2.2, 9.9 Hz, 1H), 6.78 (d, *J* = 2.8 Hz, 1H), 6.71 (d, *J* = 2.1 Hz, 1H), 3.95 - 3.80 (m, 3H), 3.79 - 3.70 (m, 1H), 3.67 (br d, *J* = 1.5 Hz, 1H), 3.35 (br t, *J* = 10.6 Hz, 1H), 3.24 - 3.15 (m, 1H), 2.98 - 2.87 (m, 1H), 2.79 - 2.65 (m, 2H), 2.53 - 2.35 (m, 3H); **LCMS (ESI+)**: m/z 354.0 $(M+H)^+$, RT=1.611 min, 5_95AB_6min-220-254-ELSD : LC/MS (The gradient was 5% B in 0.40 min and 5-95% B in 2.60 min, hold on 95% B in 1.00min, and then 95-5% B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Kinetex C18 2.1*50mm, 5um. Detection methods are diode array (DAD), and evaporative light scattering detection (ELSD). MS mode was positive electrospray ionization. MS range was 100-1000.)

Synthesis of 7-(9-isopropyl-3,9-diazaspiro[5.5]undecan-3-yl)-3H-phenothiazin-3-one (Compound 15)



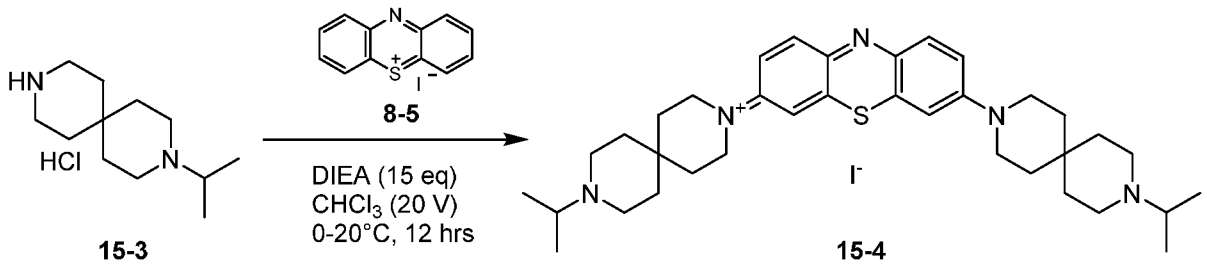
15-1 15-2

[0177] A mixture of Intermediate 15-1 (3 g, 10.32 mmol), acetone (3.00 g, 51.58 mmol), AcOH (619.44 mg, 10.32 mmol) and NaOAc (3.38 g, 41.26 mmol) in MeOH (30 mL) was stirred at 40°C for 2 hrs. After cooling to 20°C, $NaBH_3CN$ (1.30 g, 20.63 mmol) was then added to the reaction mixture and stirred at 20°C for another 12 hrs. The reaction mixture concentrated under reduced pressure to give a residue. It was then diluted with DCM (50 mL) and was poured into aqueous $NaHCO_3$ solution (50 mL). After partition, the aqueous phase was extracted with DCM (3 × 30 mL). The organic phases were combined, washed with brine (2 × 50 mL), dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give Intermediate 15-2 (3 g, 98.10% yield) as white solid: **^{1}H NMR:** 400MHz $CDCl_3$ δ 3.33 (br d, 4H, J=4.4 Hz), 2.98 (td, 1H, J=6.5, 13.2 Hz), 2.72 (br s, 4H), 1.69 (br t, 4H, J=5.1 Hz), 1.40 (s, 13H), 1.17 (d, 6H, J=6.5 Hz).

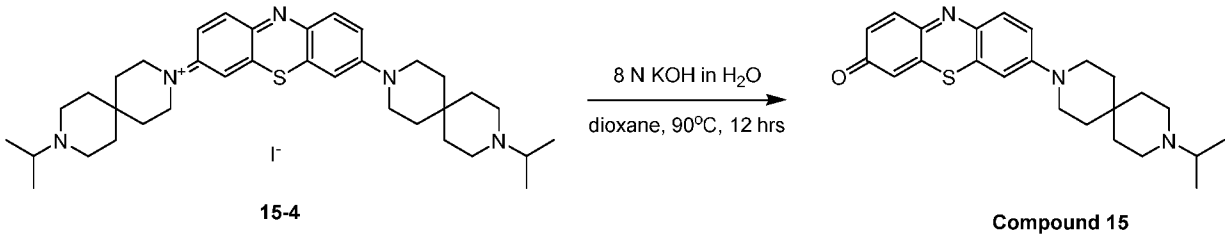


15-2 15-3

[0178] To a mixture of Intermediate 15-2 (3 g, 10.12 mmol) in EtOAc (60 mL) was added HCl/EtOAc (4 M, 60 mL) at 20°C. The mixture was stirred at 20°C for 2 hrs. The reaction mixture was concentrated under reduced pressure to give Intermediate 15-3 (3g, 99.09% yield) as a white solid and used for the next step directly: **^{1}H NMR:** 400MHz MeOD δ 3.53 (td, 1H, J=6.6, 13.4 Hz), 3.39 (br d, 1H, J=2.0 Hz), 3.36 (br d, 1H, J=1.9 Hz), 3.1-3.3 (m, 6H), 2.06 (br d, 2H, J=14.1 Hz), 1.9-2.0 (m, 2H), 1.8-1.9 (m, 2H), 1.7-1.7 (m, 2H), 1.39 (d, 6H, J=6.6 Hz).



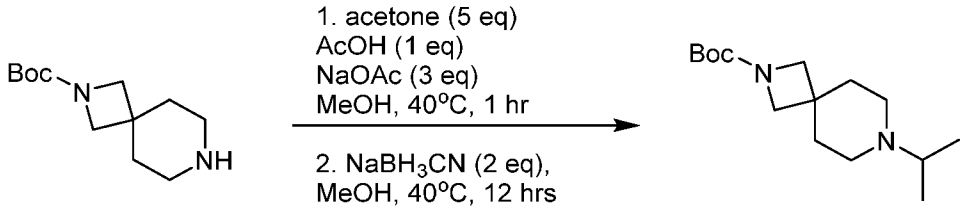
[0179] To a mixture of Intermediate 8-5 (1 g, 1.41 mmol) in $CHCl_3$ (20 mL) was added DIEA (2.73 g, 21.13 mmol) in portions at 0°C. The mixture was stirred at 0°C for 10 min. Then the mixture was added to Intermediate 15-3 (2.28 g, 8.45 mmol) in $CHCl_3$ (20 mL) and the resulting mixture was stirred at 20 °C for 3 hrs under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure to give a residue. It was then purified by column chromatography on silica gel with CH_2Cl_2/MeOH (0-7%) to give Intermediate 15-4 (700 mg, 84.67% yield) as a blue solid: **LCMS (ESI+)**: 1.088 min, m/z 586.3, 5-95AB_3.5min: LC/MS (The column used for chromatography was a Halo C18 5μm, 3.0*30mm. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min .5% B in 0.01 min, 5-95% B (0.01-2.50 min), with a hold at 95% B for 0.50 min.95-5% B (3.0-3.01min), with a hold at 5% B for 0.49 min. The flow rate was 1.0 mL/min (0.01-3.01min), 1.2 mL/min (3.02-3.50 min).)



[0180] To a mixture of Intermediate 15-4 (500 mg, 851.94 μmol) in dioxane (10 mL) was added aqueous KOH (8 N, 10 mL, 80.00 mmol) and then the mixture was stirred at 90°C for 12 hrs under nitrogen atmosphere. The reaction mixture was partitioned and the aqueous phase was extracted with DCM (3 × 10 mL). The organic phases were combined, washed with brine (2 × 10 mL), dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give a crude product. It was then purified by column chromatography on silica gel eluted with Dichloromethane/Methanol (from 1% to 15%) to give Compound 15 (3.8 mg, 1.09% yield) as a dark purple solid: **LCMS (ESI+)**: 1.676 min, m/z 408.1, 5_95AB_6min-220-254-ELSD : LC/MS (The gradient was 5%B in 0.40min and 5-95% B in

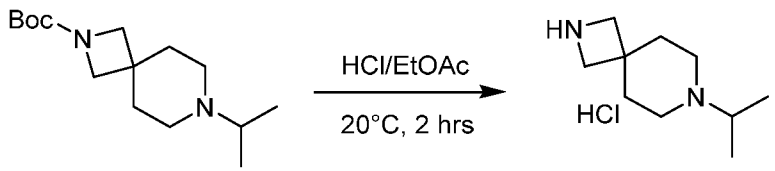
2.60 min , hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Kinetex C18 2.1×50mm, 5um. Detection methods are diode array (DAD), and evaporative light scattering detection (ELSD). MS mode was positive electrospray ionization.MS range was 100-1000; **^{1}H NMR:** 400MHz MeOD δ 7.76 (d, 1H, J=9.4 Hz), 7.68 (d, 1H, J=9.6 Hz), 7.25 (dd, 1H, J=2.7, 9.4 Hz), 7.14 (d, 1H, J=2.6 Hz), 6.85 (dd, 1H, J=2.2, 9.7 Hz), 6.73 (d, 1H, J=2.3 Hz), 3.6-3.7 (m, 4H), 3.4-3.4 (m, 1H), 3.15 (br s, 4H), 1.8-2.0 (m, 4H), 1.6-1.8 (m, 4H), 1.34 (d, 6H, J=6.6 Hz).

Synthesis of 7-(7-isopropyl-2,7-diazaspiro[3.5]nonan-2-yl)-3H-phenothiazin-3-one (Compound 16)



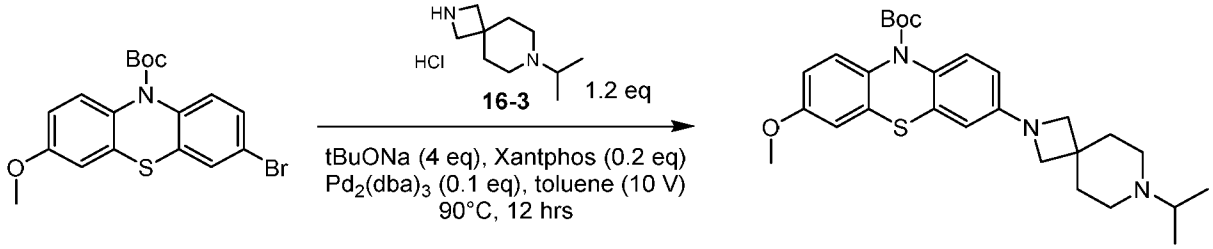
16-1 **16-2**

[0181] To a solution of Intermediate 16-1 (4.2 g, 18.56 mmol, 1 eq) and Acetone (5.39 g, 92.79 mmol, 6.82 mL, 5 eq) in MeOH (50 mL) was added NaOAc (4.57 g, 55.67 mmol, 3 eq) and AcOH (1.11 g, 18.56 mmol, 1.06 mL, 1 eq) at 20°C. The mixture was stirred at 40°C for 1 hour. Then $NaBH_3CN$ (3.50 g, 55.67 mmol, 3 eq) was added to the mixture at 40°C. The mixture was stirred at 40°C for 12 hours. LCMS showed the reaction worked well. The mixture was diluted with water (50 mL), extracted with ethyl acetate (3 x 30 mL). The combined organic phase was washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 16-2 (5.6 g, 17.74 mmol, 95.56% yield, 85% purity) as off white solid: **^{1}H NMR** (400 MHz, CHLOROFORM-d) δ = 3.67 (s, 4H), 3.07 (td, J = 6.4, 12.8 Hz, 1H), 2.90 - 2.57 (m, 4H), 2.00 (br t, J = 4.9 Hz, 4H), 1.44 (s, 9H), 1.22 (d, J = 6.6 Hz, 6H).



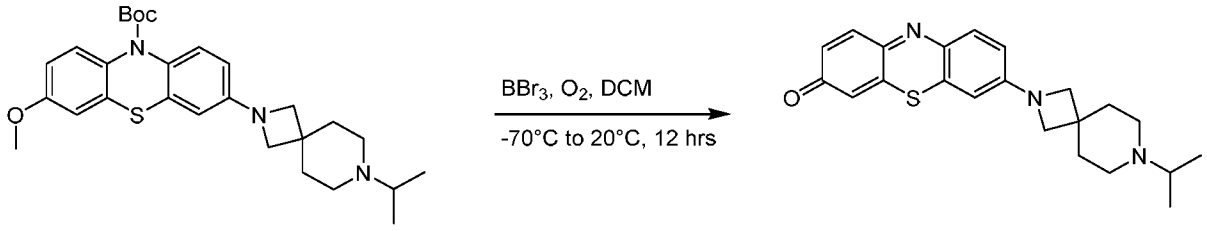
16-2 16-3

[0182] A solution of Intermediate 16-2 (5.6 g, 17.74 mmol, 1 eq, purity 90%) in HCl/EtOAc (100 mL, 4 M) was stirred for 2 hours at 20°C. LCMS showed the reaction worked well. The mixture was concentrated under reduced pressure to give Intermediate 16-3 (5 g, 17.62 mmol, 99.35% yield, 85% purity, 2HCl) as off white solid: **^{1}H NMR** (400 MHz, METHANOL-d4) δ = 4.05 (s, 2H), 3.91 (s, 2H), 3.56 - 3.50 (m, 1H), 3.50 - 3.43 (m, 2H), 3.04 (br t, J = 12.5 Hz, 2H), 2.43 - 2.31 (m, 2H), 2.12 (dt, J = 4.0, 14.0 Hz, 2H), 1.38 (d, J = 6.6 Hz, 6H).



10-5 16-4

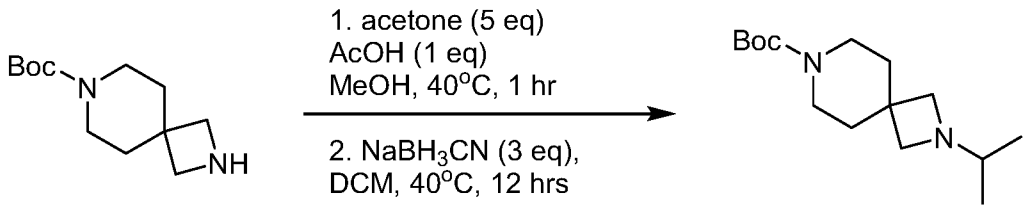
[0183] To a solution of Intermediate 10-5 (0.5 g, 1.22 mmol, 1 *eq*) and Intermediate 16-3 (354.44 mg, 1.47 mmol, 1.2 *eq*, 2HCl) in toluene (10 mL) was added t-BuONa (470.72 mg, 4.90 mmol, 4 *eq*), Xantphos (141.71 mg, 244.91 μmol, 0.2 *eq*) and $Pd_2(dba)_3$ (112.14 mg, 122.46 μmol, 0.1 *eq*) at 20°C under N_2. The mixture was stirred at 90°C for 12 hours under N_2. LCMS showed the reaction worked. The mixture was diluted with ethyl acetate (10 mL) and water (10 mL). The mixture was extracted with ethyl acetate (3 x 10 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product. The product was purified by preparative TLC (dichloromethane: methanol=2:1, Rf =0.3) to give Intermediate 16-4 (0.2 g, 403.49 μmol, 32.95% yield) as yellow solid: **^{1}H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 7.38 (br d, J = 8.8 Hz, 1H), 7.31 (d, J = 8.7 Hz, 1H), 6.85 (d, J = 2.7 Hz, 1H), 6.79 (dd, J = 8.9, 2.7 Hz, 1H), 6.37 (d, J = 2.5 Hz, 1H), 6.32 (dd, J = 8.7, 2.5 Hz, 1H), 3.79 (s, 3H), 3.58 (s, 4H), 2.76-2.93 (m, 1H), 2.54 (br s, 4H), 1.88 (br s, 4H), 1.47 (s, 9H), 1.09 (br d, J = 6.4 Hz, 6H).



16-4 Compound 16

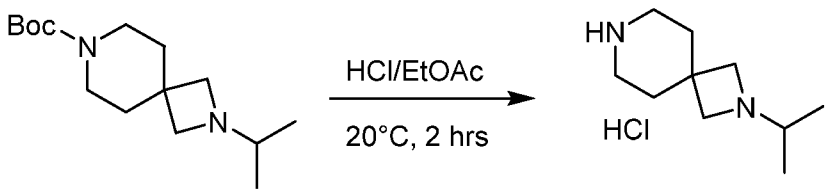
[0184] To a solution of Intermediate 16-4 (0.2 g, 403.49 μmol, 1 *eq*) in DCM (5 mL) was added BBr_3 (505.42 mg, 2.02 mmol, 194.39 μL, 5 *eq*) dropwise at -78°C under N_2. The mixture was stirred at 20°C for 12 hours under O_2 (15 psi). LCMS showed the reaction worked. The mixture was quenched with MeOH (20 mL) at -40°C and then adjust to pH=8 with saturated sodium bicarbonate aqueous solution (20 mL). The mixture was extracted with dichloromethane (3 x 20 mL). The combined organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product. The product was purified by column chromatography (SiO_2, polarity gradient: Petroleum ether/Ethyl acetate=100% to 0%, ethyl acetate/dichloromethane =80% to 90%, dichloromethane /methyl alcohol=95%) to give the crude. And the crude product was purified by preparative TLC (dichloromethane: methanol=2:1). Then the residue was triturated with ethyl acetate/dichloromethane/ methanol=5/1/1(10 mL) at 20°C for 10 min and then filtered. The cake was dried by high vacuum to give Compound 16 (34.2 mg, 87.41 μmol, 21.66% yield, 97% purity) as dark purple solid: **^{1}H NMR** (METHANOL-d4, 400 MHz) δ (ppm) 7.80 (d, J = 9.0 Hz, 1H), 7.71 (d, J = 9.8 Hz, 1H), 6.88 (dd, J = 9.8, 2.3 Hz, 1H), 6.73-6.83 (m, 2H), 6.69 (d, J = 2.4 Hz, 1H), 3.90-4.20 (m, 4H), 3.42-3.69 (m, 3H), 2.95-3.21 (m, 2H), 2.23-2.47 (m, 2H), 1.94-2.21 (m, 2H), 1.40 (br d, J = 6.5 Hz, 6H); **LCMS (ESI+)**: 2.450 min, m/z 380 (M+H) LC/MS (The gradient was 5%B in 0.40min and 5-95% B at 0.40-3.40 min ,hold on 95% B for 0.45min, and then 95-5%B in 0.01min, the flow rate was 0.8 ml/min. Mobile phase A was H_2O+10 mM NH_4HCO_3, mobile phase B was Acetonitrile. The column used for chromatography was a Xbridge Shield RP18 2.1*50mm column (5um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection as well as positive electrospray ionization.MS range was 100-1000.)

Synthesis of 7-(2-isopropyl-2,7-diazaspiro[3.5]nonan-7-yl)-3H-phenothiazin-3-one (Compound 17)



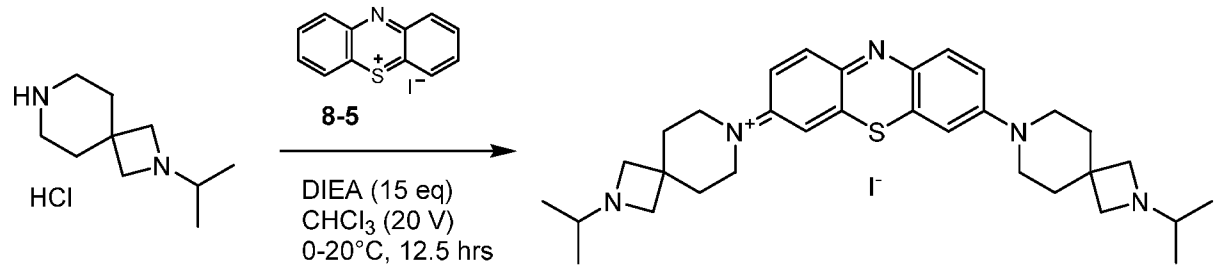
17-1 **17-2**

[0185] To a solution of Intermediate 17-1 (1.5 g, 6.63 mmol, 1 *eq*) and acetone (1.92 g, 33.14 mmol, 2.44 mL, 5 *eq*) in MeOH (30 mL) was added NaOAc (1.63 g, 19.88 mmol, 3 *eq*) and HOAc (398.02 mg, 6.63 mmol, 379.43 µL, 1 *eq*) at 20°C. The mixture was stirred at 40°C for 1 hour. Then $NaBH_3CN$ (1.25 g, 19.88 mmol, 3 *eq*) was added to the mixture at 20°C. The mixture was stirred at 40°C for 12 hours. LC-MS showed starting material was consumed completely and one main peak with desired mass was detected. The mixture was diluted with water (50 mL) and ethyl acetate (50 mL). The mixture was extracted with ethyl acetate (3 x 30 mL). The organic layer was washed with brine (10 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 17-2 (1.3 g, 4.84 mmol, 73.08% yield) was obtained as colorless oil: **LCMS** (ESI+): m/z 269 (M+H), RT: 0.352 min, 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm,5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.)



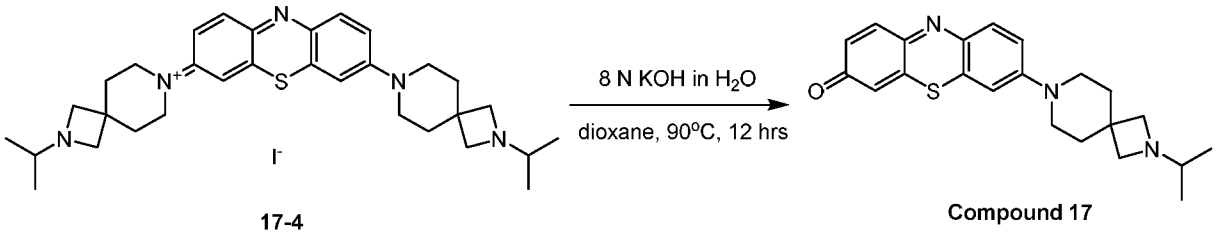
17-2 **17-3**

[0186] To a solution of Intermediate 17-2 (2 g, 7.45 mmol, 1 *eq*) in ETHYL ACETATE (10 mL) was added HCl/EtOAc (4 M, 10 mL, 5.37 *eq*) at 20°C. The mixture was stirred at 20°C for 2 hours. LCMS showed starting material consumed. The mixture was concentrated under reduced pressure to give Intermediate 17-3 (2.4 g, 6.97 mmol, 93.47% yield, 70% purity, 2HCl) as white solid: **1H NMR** (METHANOL-d4, 400 MHz) δ (ppm) 4.05-4.13 (m, 2H), 3.98-4.05 (m, 2H), 3.51 (spt, J = 6.5 Hz, 1H), 3.23-3.28 (m, 2H), 3.11-3.20 (m, 2H), 2.19-2.28 (m, 2H), 2.04-2.13 (m, 2H), 1.25-1.30 (m, 6H).



17-3 17-4

[0187] To a solution of Intermediate 17-3 (1.84 g, 7.64 mmol, 6 *eq*, 2HCl) in $CHCl_3$ (10 mL) was added DIPEA (2.47 g, 19.10 mmol, 3.33 mL, 15 *eq*) dropwise at 0°C under N_2. The mixture was stirred at 20°C for 0.5 hours. Then the mixture was added to a mixture of Intermediate 8-5 (0.9 g, 1.27 mmol, 1 *eq*) in $CHCl_3$ (10 mL) dropwise at 0°C. The mixture was stirred at 20°C for 12 hours. The mixture was concentrated under reduced pressure, and the residue was washed with methyl tert-butyl ether (10 mL) and dried by high vacuum to give Intermediate 17-4 (2 g, 790.64 μmol, 62.10% yield, 26% purity) as blue gum: **LCMS (ESI+)**: 0.960 min, m/z 530 (M^+), 5-95AB_3.5min: LC/MS (The column was a Luna C18 30*2.0mm,3um . Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min .5% B in 0.01 min, 5-95% B (0.01-2.50 min) with a hold at 95% B for 0.50 min, 95-5% B (3.00 -3.01 min) with a hold at 5% B for 0.49 min. The flow rate was 1 mL/min(0.01-3.00min)-1.2 mL/min(3.01-3.50min).)

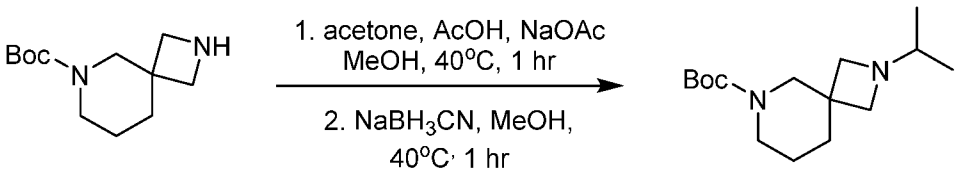


17-4 Compound 17

[0188] A solution of Intermediate 17-4 (769.23 mg, 304.09 μmol, 1 *eq*) in dioxane (10 mL) and KOH (8 M, 6.32 mL, 166.14 *eq*) was stirred at 90°C for 12 hours. LCMS showed the reaction worked. The mixture was extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine (20 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the product. The residue was purified by column chromatography (SiO_2, polarity gradient: petroleum ether/ethyl acetate=100% to 0%, ethyl acetate/dichloromethane=80% to 90%, dichloromethane/methyl alcohol=95%). The residue was triturated with ethyl acetate (10 mL) at 20°C for 10 min and then filtered to give

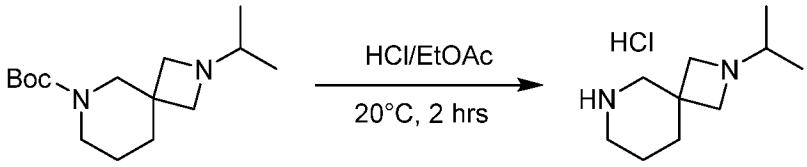
Compound 17 (12.6 mg, 32.20 μmol, 10.59% yield, 97% purity) as dark purple solid: **1H NMR** (DMSO-d6, 400 MHz) δ (ppm) 7.67 (d, J = 8.9 Hz, 1H), 7.56 (d, J = 10.1 Hz, 1H), 7.20-7.30 (m, 2H), 6.69-6.76 (m, 2H), 3.66-4.03 (m, 2H), 3.53 (br s, 5H), 1.62-2.07 (m, 5H), 0.77-1.20 (m, 7H); **1H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 7.72 (d, J = 9.1 Hz, 1H), 7.56 (d, J = 9.8 Hz, 1H), 7.00 (dd, J = 9.1, 2.6 Hz, 1H), 6.86 (dd, J = 9.8, 2.0 Hz, 1H), 6.77 (d, J = 2.5 Hz, 1H), 6.70 (d, J = 2.0 Hz, 1H), 3.36-3.72 (m, 7H), 1.87-2.28 (m, 5H), 1.26 (br s, 7H); **LCMS (ESI+)**: 1.634 min, m/z 380 (M+H), 5_95AB_6min-220-254-ELSD : LC/MS(The gradient was 5%B in 0.40min and 5-95% B in 2.60 min , hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Luna C18 50*2.0mm column (5um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection .MS mode was positive electrospray ionization. MS range was 100-1000.

Synthesis of 7-(2-isopropyl-2,6-diazaspiro[3.5]nonan-6-yl)-3H-phenothiazin-3-one (Compound 18)



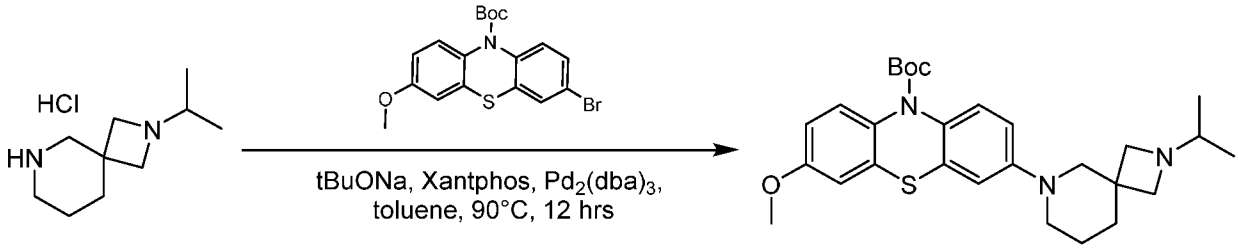
18-1 **18-2**

[0189] A mixture of Intermediate 18-1 (1.5 g, 6.63 mmol), acetone (1.92 g, 33.14 mmol), NaOAc (1.63 g, 19.88 mmol) and AcOH (398.02 mg, 6.63 mmol) in MeOH (15 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 40°C for 1 hr under N_2 atmosphere. Then to the mixture was added $NaBH_3CN$ (833.03 mg, 13.26 mmol). The reaction mixture was stirred at 40°C for 1 hr. The reaction mixture was concentrated under reduced pressure to remove MeOH. The residue was diluted with water (20 mL) and extracted with DCM (10 mL × 3). The combined organic layers were washed with brine (20 mL × 3), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 18-2 (1.41 g, crude) as a yellow oil: **^{1}H NMR:** 400 MHz, CHLOROFORM-d δ 3.4-3.5 (m, 2H), 3.3-3.3 (m, 2H), 3.19 (br d, 2H, *J*=5.6 Hz), 2.5-2.6 (m, 1H), 2.1-2.3 (m, 1H), 1.7-1.9 (m, 3H), 1.4-1.6 (m, 13H), 1.26 (s, 1H), 1.13 (br s, 6H).



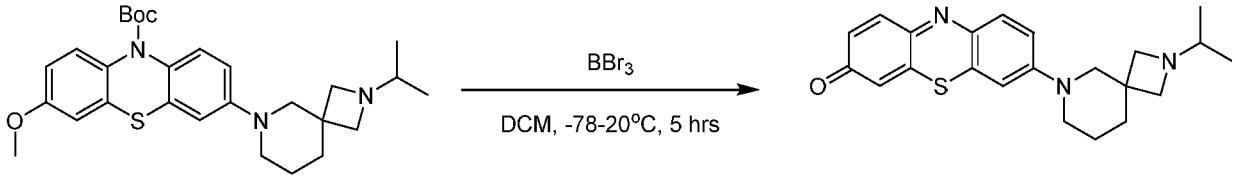
18-2 18-3

[0190] To a solution of Intermediate 18-2 (1.1 g, 4.10 mmol) in EtOAc (1 mL) was added HCl/EtOAc (4 M, 10 mL). The mixture was stirred at 20°C for 2 hrs. The reaction mixture was concentrated under reduced pressure to give Intermediate 18-3 (0.9 g, crude, 2 HCl) as a white solid: **^{1}H NMR:** 400 MHz, DMSO-d6 δ 11.6-11.9 (m, 1H), 9.5-9.7 (m, 2H), 3.9-4.0 (m, 1H), 3.8-3.9 (m, 1H), 3.69 (br dd, 1H, *J*=6.6, 10.2 Hz), 3.2-3.3 (m, 1H), 2.9-2.9 (m, 2H), 1.8-1.9 (m, 1H), 1.7-1.8 (m, 2H), 1.6-1.7 (m, 2H), 1.14 (br s, 6H).



18-3 18-4

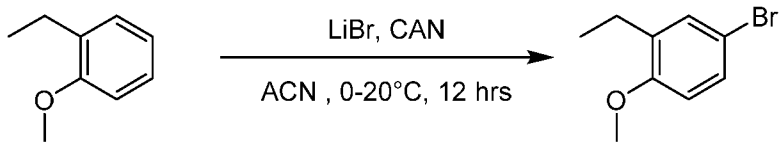
[0191] To a mixture of Intermediate 18-3 (88.61 mg, 367.37 μmol, 2HCl) in toluene (2 mL) was added tert-butyl 3-bromo-7-methoxy-phenothiazine-10-carboxylate (0.12 g, 293.90 μmol), t-BuONa (141.22 mg, 1.47 mmol), Xantphos (42.51 mg, 73.47 μmol) and $Pd_2(dba)_3$ (33.64 mg, 36.74 μmol) at 20°C. The mixture was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 90°C for 12 hrs under N_2 atmosphere. Additional three reactions were set up as described above. The four parallel reaction mixtures were combined and purified. The reaction mixture was filtered and the filtrate was concentrated to give a crude product. It was purified by silica gel chromatography eluted with Dichloromethane: Methanol = 10 / 1 to 7 / 1 to give Intermediate 18-4 (171 mg, 20.39% yield, 56% purity) as a blue solid: **LCMS (ESI+)**:1.830 min, m/z 496.4, 5-95AB_3.5min: LC/MS (The column was a Luna C18 30*2.0mm,3um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min. 5% B in 0.01 min, 5-95% B (0.01-2.50 min) with a hold at 95% B for 0.50 min, 95-5% B (3.00 -3.01 min) with a hold at 5% B for 0.49 min. The flow rate was 1 mL/min(0.01-3.00min)-1.2 mL/min (3.01-3.50min).)



18-4 Compound 18

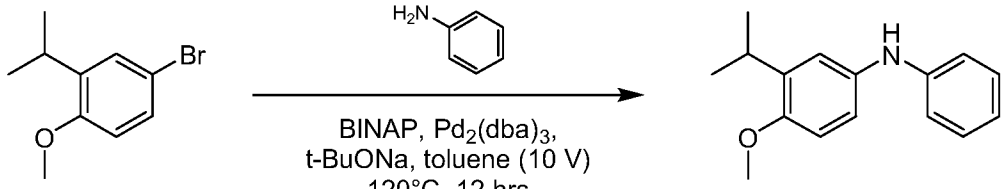
[0192] To a mixture of Intermediate 18-4 (0.07 g, 141.22 μmol) in DCM (0.5 mL) was added BBr_3 (707.58 mg, 2.82 mmol) at -78°C, and then the mixture was stirred at 20°C for 5 hrs under O_2. Additional one reaction was set up as described above. The reaction mixtures from two reactions were combined and purified. The combined reaction was poured into cold MeOH (2 mL), then was concentrated under reduced pressure to give a residue, which was then diluted with DCM (20 mL), and poured into aqueous saturated $NaHCO_3$ (30 mL). The aqueous phase was then extracted with DCM (20 mL × 3). The combined organic layers were washed with brine (5 mL × 2), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Dichloromethane: Methanol = 15 / 1 to 10 / 1) to give crude product. It was further purified by prep-TLC (SiO_2, Dichloromethane: Methanol=8/1, visible light, brown color) to give Compound 18 (24.3 mg, 21.63% yield, 95.4% purity) as a dark purple solid: **^{1}H NMR:** 400 MHz, CHLOROFORM-d δ = 7.71 (d, *J* = 9.2 Hz, 1H), 7.57 (d, *J* = 9.9 Hz, 1H), 7.11 (br d, *J* = 9.4 Hz, 1H), 6.92 - 6.82 (m, 2H), 6.70 (s, 1H), 3.61 (s, 2H), 3.38 (br s, 2H), 3.21 (br d, *J* = 5.6 Hz, 2H), 2.86 (br d, *J* = 6.4 Hz, 2H), 2.44 - 2.32 (m, 1H), 1.77 (br d, *J* = 5.6 Hz, 2H), 1.70 (br d, *J* = 4.3 Hz, 2H), 0.96 (br d, *J* = 5.9 Hz, 6H); **LCMS (ESI+)**:1.660 min, m/z 380.1, 5_95AB_6min-220-254-ELSD：LC/MS (The gradient was 5% B in 0.40 min and 5-95% B in 2.60 min, hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Kinetex C18 2.1*50mm,5um. Detection methods are diode array (DAD), and evaporative light scattering detection (ELSD). MS mode was positive electrospray ionization. MS range was 100-1000.)

Synthesis of 2-isopropyl-7-((1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-3H-phenothiazin-3-one (Compound 19)



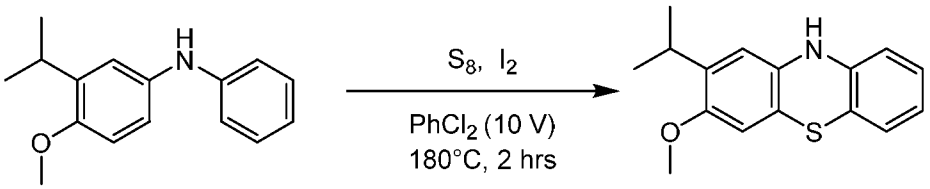
19-1 19-2

[0193] To a solution of LiBr (6.36 g, 73.23 mmol) in ACN (100 mL) was added CAN (40.14 g, 73.23 mmol) at 0°C. The mixture was stirred at 0°C for 1 hr. The flask was flushed with N_2 and a solution of Intermediate 19-1 (10 g, 66.57 mmol) in ACN (30 mL) was added slowly and the reaction was stirred at 20°C for 12 hrs. The mixture was filtered and concentrated under reduced pressure to give a residue. It was purified by column chromatography (SiO_2, Petroleum ether / Ethyl acetate = 10 / 1 to 5 / 1) to give Intermediate 19-2 (13.5 g, 85.86% yield, 97% purity) as yellow oil: **^{1}H NMR:** 400 MHz, DMSO-d6 δ = 7.35 - 7.26 (m, 2H), 6.91 (d, *J* = 8.7 Hz, 1H), 3.78 (s, 3H), 3.20 (td, *J* = 6.9, 13.8 Hz, 1H), 1.14 (d, *J* = 6.9 Hz, 6H).



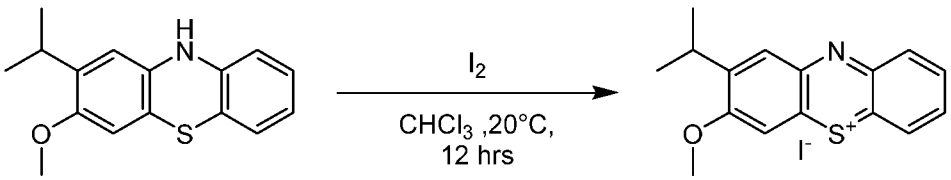
19-2 19-3

[0194] To a mixture of Intermediate 19-2 (10 g, 42.34 mmol) in toluene (100 mL) was added aniline (5.91 g, 63.51 mmol), t-BuONa (12.21 g, 127.01 mmol), BINAP (7.91 g, 12.70 mmol) and $Pd_2(dba)_3$ (5.82 g, 6.35 mmol). The mixture was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 120°C for 12 hrs under N_2 atmosphere. TLC showed the starting material was consumed. The reaction mixture was concentrated under reduced pressure to give a residue. It was purified by column chromatography (SiO_2, Petroleum ether / Ethyl acetate = 30 / 1 to 15 / 1) to give Intermediate 19-3 (8 g, 21.66% yield, 83% purity) as a pink solid: **^{1}H NMR:** 400MHz CHLOROFORM-d δ = 7.25 - 7.19 (m, 2H), 7.19 - 7.12 (m, 2H), 7.02 (br d, *J* = 2.4 Hz, 1H), 6.93 (br d, *J* = 7.5 Hz, 2H), 6.87 - 6.79 (m, 2H), 5.98 - 5.59 (m, 1H), 3.83 (s, 3H), 3.32 (td, *J* = 6.9, 13.9 Hz, 1H), 1.21 (d, *J* = 7.0 Hz, 6H).



19-3 19-4

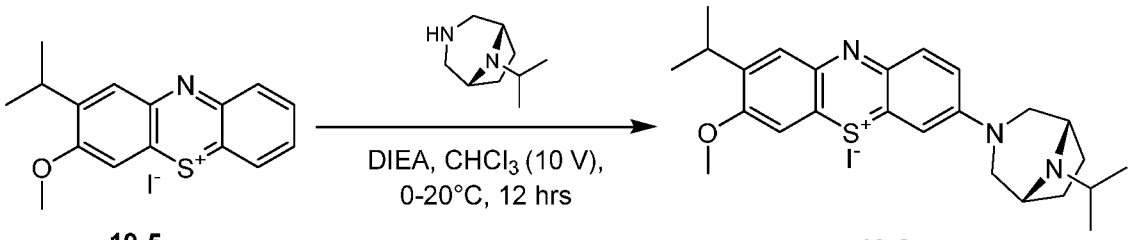
[0195] To a solution of Intermediate 19-3 (5 g, 17.20 mmol) in $PhCl_2$ (80 mL) was added I_2 (436.46 mg, 1.72 mmol) and S_8 (1.10 g, 34.39 mmol) at 20°C. The mixture was stirred at 180°C for 2 hrs under nitrogen. LCMS showed the starting material was consumed, and 27% product with desired ms was detected. The reaction mixture was concentrated under reduced pressure to give a residue. It was purified by column chromatography (SiO_2, Petroleum ether / Ethyl acetate = 15/1 to 10/1) to give Intermediate 19-4 (3.2 g, 55.54% yield, 81% purity) as a blue solid: **LCMS (ESI+)**: RT=2.352 min, m/z 272.2, 5-95AB_3.5min: LC/MS (The column was a Luna C18 30*2.0mm, 3um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min. 5% B in 0.01 min, 5-95% B (0.01-2.50 min) with a hold at 95% B for 0.50 min, 95-5% B (3.00 -3.01 min) with a hold at 5% B for 0.49 min. The flow rate was 1 mL/min (0.01-3.00 min)-1.2 mL/min (3.01-3.50 min).



19-4 19-5

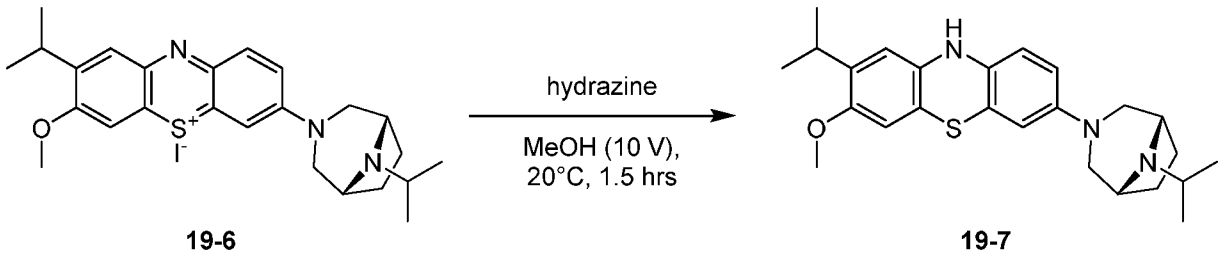
[0196] To a solution of Intermediate 19-4 (1.5 g, 5.20 mmol) in $CHCl_3$ (800 mL) was added I_2 (3.96 g, 15.59 mmol) at 20°C. The mixture was stirred at 20°C for 12 hrs. LCMS showed the starting material was consumed, and 36% product with desired ms was detected. The reaction mixture was filtered and the filter cake was washed with DCM (20 mL × 2). The filter cake was dried under reduced pressure to give Intermediate 19-5 (3 g, 74.22% yield,62% purity, 4 I^-) as a blue solid: **LCMS (ESI+)**: RT=1.331 min, m/z 270.2, 5-95AB_3.5min: LC/MS (The column was a Luna C18 30*2.0mm,3um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min. 5% B in 0.01 min, 5-95% B (0.01-2.50 min) with a hold at 95% B for 0.50 min, 95-5% B (3.00 -3.01 min) with a hold

at 5% B for 0.49 min. The flow rate was 1 mL/min (0.01-3.00min)-1.2 mL/min (3.01-3.50 min).)



19-5 19-6

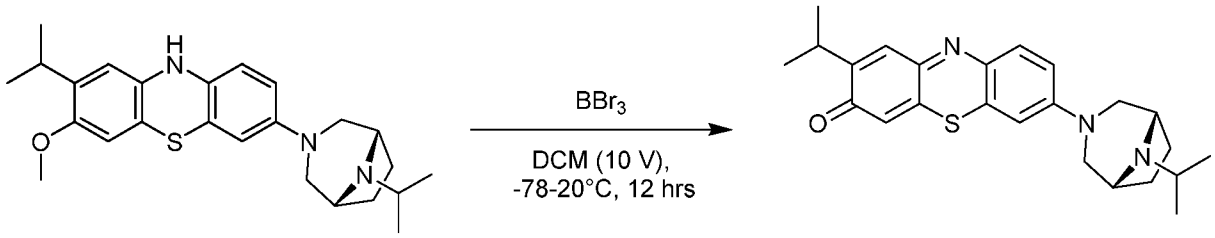
[0197] To a solution of Intermediate 19-5 (1.5 g, 1.25 mmol, 4 I^-) in $CHCl_3$ (15 mL) was added dropwise (1R, 5S)-8-isopropyl-3, 8-diazabicyclo [3.2.1] octane (386.63 mg, 2.51 mmol) and DIEA (323.94 mg, 2.51 mmol) at 0°C. The mixture was stirred at 20°C for 12 hrs. LCMS showed the starting material was consumed, and 96% product with desired MS was detected. The reaction mixture was filtered and the filter cake was washed with DCM (20 mL × 2). The filter cake was dried under reduced pressure to give Intermediate 19-6 (700 mg, 99% purity) as a blue solid: **^{1}H NMR:** 400 MHz, CHLOROFORM-d δ = 8.23 - 8.16 (m, 1H), 8.14 (s, 1H), 7.91 (d, *J* = 2.8 Hz, 1H), 7.72 - 7.62 (m, 1H), 4.20 (s, 3H), 3.99 - 3.78 (m, 3H), 2.67 (s, 1H), 2.16 - 2.09 (m, 5H), 1.35 (d, *J* = 6.8 Hz, 6H), 1.28 - 1.23 (m, 6H); **LCMS (ESI+)**:RT = 1.278 min, m/z 422.3, 5-95AB_3.5min: LC/MS (The column was a Luna C18 30*2.0mm,3um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min. 5% B in 0.01 min, 5-95% B (0.01-2.50 min) with a hold at 95% B for 0.50 min, 95-5% B (3.00 -3.01 min) with a hold at 5% B for 0.49 min. The flow rate was 1 mL/min(0.01-3.00 min)-1.2 mL/min (3.01-3.50min).)



19-6 19-7

[0198] To a solution of Intermediate 19-6 (700 mg, 1.65 mmol) in MeOH (10 mL) was added hydrazine (827.53 mg, 16.53 mmol) at 20°C. The mixture was stirred at 20°C for 1.5 hrs. LCMS showed the starting material was consumed, and 78% product with desired ms was detected. The reaction mixture was concentrated under reduced pressure to give Intermediate 19-7 (180 mg, 22.36% yield, 81% purity) as a yellow solid which was used

directly to the next step without further purification: **^{1}H NMR:** 400 MHz, CHLOROFORM-d δ = 6.54 (br d, *J* = 8.4 Hz, 2H), 6.50 (br s, 1H), 6.46 (br s, 1H), 5.48 (br s, 1H), 3.75 (s, 3H), 3.59 (br s, 2H), 3.28 - 3.18 (m, 1H), 3.18 - 3.09 (m, 2H), 3.05 - 2.93 (m, 2H), 2.70 - 2.59 (m, 1H), 1.99 - 1.87 (m, 2H), 1.77 (br d, *J* = 7.0 Hz, 2H), 1.18 - 1.09 (m, 12H); **LCMS (ESI+)**: RT=0.467 min, m/z 422.3, 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm,5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.)

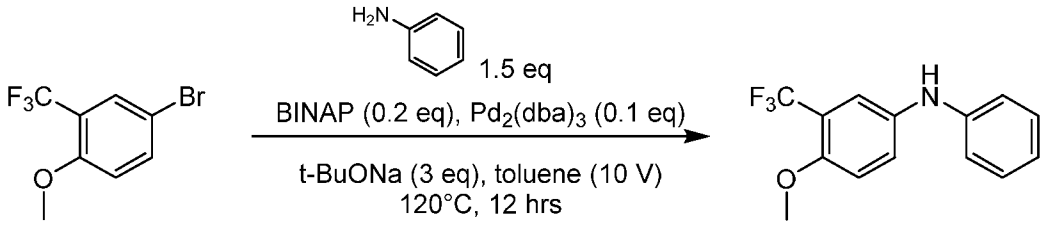


19-7 **Compound 19**

[0199] To a solution of Intermediate 19-7 (100 mg, 205.38 μmol) in DCM (1 mL) was added dropwise BBr_3 (514.51 mg, 2.05 mmol) at -78°C. The mixture was stirred at 20°C for 12 hrs. LCMS showed the starting material was consumed, and 28.5% product with desired Ms was detected. The reaction was poured into cold MeOH (2 mL), then was concentrated under reduced pressure to give a residue. It was then diluted with DCM (10 mL), and poured into aqueous saturated $NaHCO_3$ (10 mL). The aqueous phase was then extracted with DCM (20 mL × 3). The combined organic layers were washed with brine (5 mL × 2), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. It was purified by column chromatography (SiO_2, Dichloromethane: Methanol = 15/1 to 10/1) to give crude product. It was purified by prep-TLC (SiO_2, Dichloromethane: Methanol = 8 / 1) to give Compound 19 (31.2 mg, 97.9% purity) as a dark purple solid: **^{1}H NMR:** 400 MHz, CHLOROFORM-d δ = 7.71 (d, *J* = 9.3 Hz, 1H), 7.41 (s, 1H), 6.93 (dd, *J* = 2.7, 9.2 Hz, 1H), 6.75 - 6.65 (m, 2H), 3.69 (br s, 2H), 3.44 (br d. *J* = 11.3 Hz, 2H), 3.31 (br s, 1H), 3.31 - 3.23 (m, 3H), 2.71 - 2.63 (m, 1H), 2.06 - 1.96 (m, 2H), 1.73 (br d, *J* = 7.4 Hz, 2H), 1.22 (d, *J* = 6.8 Hz, 6H), 1.15 (d, *J* = 6.0 Hz, 6H); **LCMS (ESI+)**:RT = 2.095 min, m/z 408.2, 5_95AB_6min-220-254-ELSD : LC/MS (The gradient was 5% B in 0.40 min and 5-95% B in 2.60 min, hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was

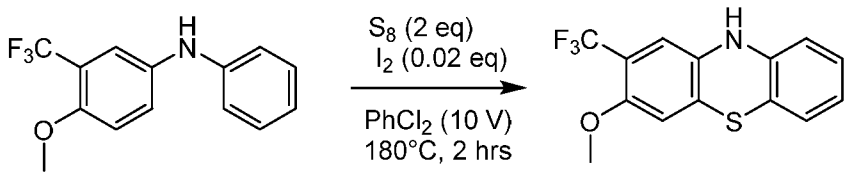
0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Luna C18 50*2.0mm column (5um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection MS mode was positive electrospray ionization. MS range was 100-1000.)

Synthesis of 7-((1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-2-(trifluoromethyl)-3H-phenothiazin-3-one (Compound 20)



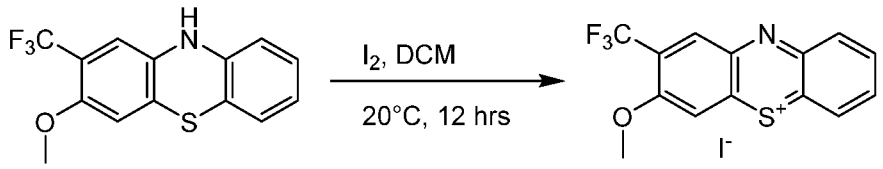
20-1 **20-2**

[0200] To a solution of Intermediate 20-1 (5 g, 19.61 mmol, 1 eq) and aniline (2.74 g, 29.41 mmol, 2.68 mL, 1.5 eq) in toluene (100 mL) was added [1-(2-diphenylphosphanyl-1-naphthyl)-2-naphthyl]-diphenyl-phosphane (3.66 g, 5.88 mmol, 0.3 eq) and sodium;2-methylpropan-2-olate (5.65 g, 58.82 mmol, 3 eq) at 20°C. The mixture was degassed and purged with N_2 for 3 times. Then (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one;palladium (2.69 g, 2.94 mmol, 0.15 eq) was added to the mixture. The mixture was degassed and purged with N_2 for 3 times and stirred at 120°C for 12 hours under N_2. LCMS showed the reaction worked well. The mixture was filtered and the filter cake was washed with ethyl acetate (200 mL), and the filtrate was concentrated under reduced pressure to give the crude. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=0 to 10%) to give Intermediate 20-2 (5 g, 16.84 mmol, 85.89% yield, 90% purity) as yellow oil: **^{1}H NMR** (400 MHz, DMSO-d6) δ 3.83 (s, 3H), 6.80 (t, J=7.32 Hz, 1H), 6.97 (br dd, J=8.50, 0.88 Hz, 2H), 7.14-7.29 (m, 4H), 7.34 (dd, J=8.88, 2.63 Hz, 1H), 8.14 (s, 1H).



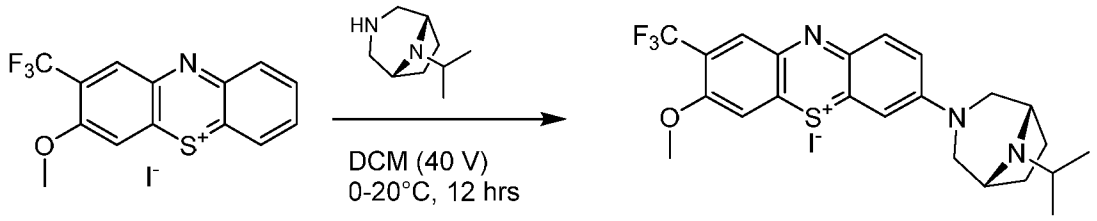
20-2 **20-3**

[0201] To a solution of Intermediate 20-2 (0.9 g, 3.03 mmol, 1 eq) in 1,2-dichlorobenzene (12 mL) was added sulfur (242.97 mg, 7.58 mmol, 2.5 eq) and I_2 (153.85 mg, 606.18 μmol, 122.11 μL, 0.2 eq) at 20°C. The mixture was stirred at 200°C for 2 hours. One additional vial in 3 g was set up as described above. The mixture was combined the other mixture together. The mixture was cooled to 20°C and petroleum ether (100 mL) was added to the mixture. The mixture was filtered and filter cake was washed with petroleum ether (50 mL). The filtrate was concentrated under reduced pressure to give the crude. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate=0 to 5%) to give Intermediate 20-3 (1.3 g, 3.94 mmol, 29.99% yield, 90% purity) as yellow solid: **^{1}H NMR** (400 MHz, METHANOL-d4) δ 3.79 (s, 3H), 6.61 (dd, J=8.00, 1.13 Hz, 1H), 6.73-6.79 (m, 2H), 6.84 (s, 1H), 6.88 (dd, J=7.69, 1.31 Hz, 1H), 6.93-7.00 (m, 1H).



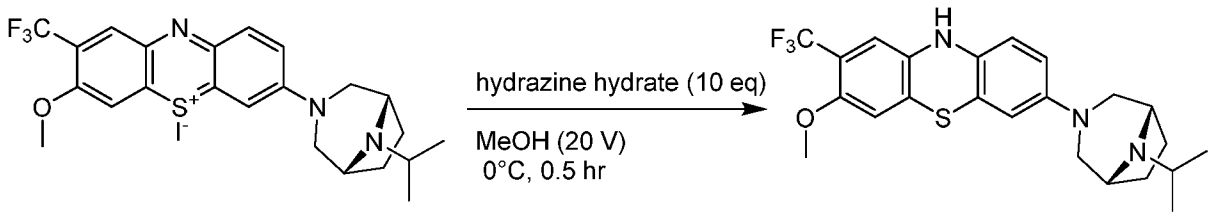
20-3 **20-4**

[0202] To a solution of Intermediate 20-3 (500 mg, 1.51 mmol, 1 *eq*, purity 90%) in DCM (20 mL) was added a solution of I_2 (1.15 g, 4.54 mmol, 914.70 μL, 3 *eq*) in DCM (200 mL) dropwise at 0°C. The mixture was stirred at 20°C for 12 hours. The mixture was concentrated under reduced pressure to give the crude. The crude product was triturated with tert-butyl methyl ether (50 mL), filtered and the filter cake was washed with tert-butyl methyl ether (50 mL). The filter cake was dried by high vacuum to give Intermediate 20-4 (1.6 g, 796.12 μmol, 52.60% yield, 40% purity) as blue solid: **LCMS(ESI+):** m/z 296.0(M+H), RT: 0.364 min, 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm,5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.)



20-4 **20-5**

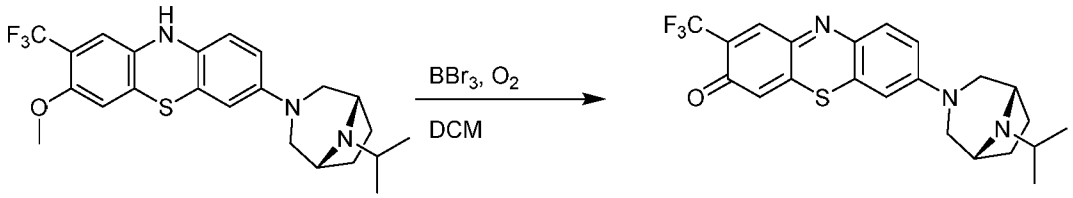
[0203] To a solution of Intermediate 20-4 (1.5 g, 746.36 μmol, 1 eq, purity 40%) in DCM (30 mL) was added a solution of (1S,5R)-8-isopropyl-3,8-diazabicyclo[3.2.1]octane (383.76 mg, 2.24 mmol, 3 eq, purity 90%) in DCM (30 mL) dropwise slowly at 0°C under N_2. The mixture was stirred at 20°C for 12 hours under N_2. The mixture was concentrated under reduced pressure to give the crude. The crude was triturated with tert-butyl methyl ether (50 mL), filtered and the filter cake was washed with tert-butyl methyl ether (50 mL). The filter cake was dried by high vacuum to give Intermediate 20-5 (2.1 g, 364.95 μmol, 48.90% yield, 10% purity) as blue solid: **LCMS (ESI+):** m/z 448.2(M+H), RT: 0.349 min, 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm,5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.)



20-5 **20-6**

[0204] To a solution of Intermediate 20-5 (2.1 g, 3.65 mmol, 1 eq, purity 10%) in MeOH (42 mL) was added hydrazine hydrate (1.83 g, 36.49 mmol, 1.77 mL, 10 eq) at 0°C. The mixture was stirred at 0°C for 30 minutes. LCMS showed the reaction worked well. The mixture was diluted with water (40 mL), extracted with dichloromethane (3 x 30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the crude Intermediate 20-6 (0.7 g, 934.31 μmol, 25.60% yield, 60% purity) as brown solid: **LCMS (ESI+):** m/z 450(M+H), RT: 0.446 min, 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30 mm, 5 um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in

water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.)

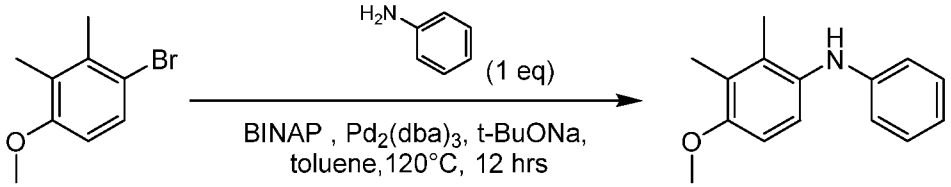


20-6 Compound 20

[0205] To a solution of Intermediate 20-6 (500 mg, 667.36 μmol, 1 eq, purity 60%) in DCM (50 mL) was added BBr_3 (1.67 g, 6.67 mmol, 643.04 μL, 10 eq) dropwise at 0°C under N_2. The mixture was stirred at 20°C under oxygen (21.35 mg, 667.36 μmol, 1 eq) (15 psi) for 12 hours. LCMS showed the reaction worked well. The mixture was quenched with methanol (30 mL) slowly at 0°C, then diluted with saturated aqueous $NaHCO_3$ (50 mL). The mixture was extracted with dichloromethane (3 x 50 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the crude. The crude was purified by column chromatography (SiO_2, dichloromethane/methanol=0 to 5%) and concentrated under reduced pressure to give the crude product. The crude product was triturated with a mixture of ethyl acetate and methyl tert-butyl ether (5 mL) (the ratio is 1/5), filtered and the filter cake was washed with tert-butyl ether (10 mL). The cake was collected and dried by high vacuum to give Compound 20 (30 mg, 67.34 μmol, 10.09% yield, 97.3% purity) as dark purple solid: **^{1}H NMR** (400 MHz, CHLOROFORM-d) δ 1.22 (br d, J=16.51 Hz, 6H), 1.79 (br s, 2H), 1.99-2.20 (m, 2H), 2.55-2.88 (m, 1H), 3.29-3.67 (m, 4H), 3.68-3.95 (m, 2H), 6.60-6.81 (m, 2H), 6.90-7.05 (m, 1H), 7.67-7.81 (m, 1H), 7.94 (br s, 1H); **^{19}F NMR** (377 MHz, CHLOROFORM-d) δ -65.50 (br s, 3F); **^{1}H NMR** (400 MHz, DMSO-d6) δ 0.97-1.13 (m, 6H), 1.56 (br d, J=4.38 Hz, 2H), 1.86 (br s, 2H), 2.57-2.68 (m, 1H), 3.22 (br d, J=10.38 Hz, 2H), 3.53-3.66 (m, 2H), 3.73 (br d, J=11.13 Hz, 2H), 6.75-6.89 (m, 1H), 7.23 (br s, 2H), 7.74 (br d, J=9.13 Hz, 1H), 7.93 (s, 1H); **LCMS (ESI+):** m/z 434 (M+1), RT: 2.008 min, 5_95AB_6min-220-254-ELSD : LC/MS (The gradient was 5%B in 0.40min and 5-95% B in 2.60 min , hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Kinetex C18 2.1*50mm,5um. Detection methods are diode array (DAD), and evaporative

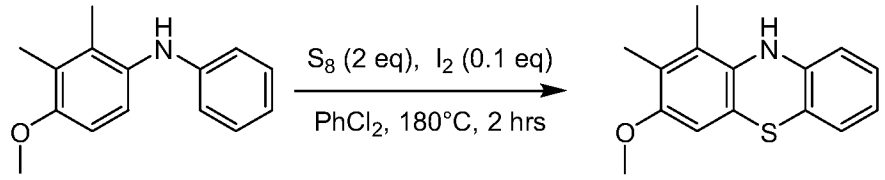
light scattering detection (ELSD). MS mode was positive electrospray ionization.MS range was 100-1000.) prep-HPLC (column: Waters Xbridge BEH C18 100*30mm*10um; liquid phase: [A-10mM NH4HCO3 in H2O; B-ACN]B%: 20%-50%,8min]).

Synthesis of 7-((1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-1,2-dimethyl-3H-phenothiazin-3-one (Compound 21)



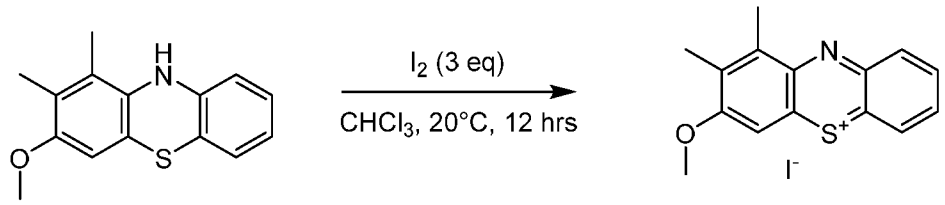
21-1 21-2

[0206] To a mixture of Intermediate 21-1 (10 g, 46.49 mmol) in toluene (130 mL) was added aniline (4.33 g, 46.49 mmol, 4.24 mL), BINAP (8.68 g, 13.95 mmol), $Pd_2(dba)_3$ (6.39 g, 6.97 mmol) and t-BuONa (13.40 g, 139.48 mmol) at 20°C. The mixture was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 120°C for 12 hrs under N_2 atmosphere. LCMS showed the starting material was consumed, and 16.8% product with desired Ms was detected. The reaction mixture was filtered and the filtrate was concentrated to give a crude product. It was purified by silica gel chromatography eluted with Petroleum ether / Ethyl acetate = 50 / 1 to 20 / 1 to give Intermediate 21-2 (6 g, 54.50% yield, 92.8% purity) as a pink solid: **^{1}H NMR:** 400MHz CHLOROFORM-d δ = 7.22 - 7.02 (m, 3H), 6.86 (d, *J* = 8.8 Hz, 1H), 6.80 - 6.70 (m, 2H), 6.69 - 6.59 (m, 2H), 5.27 (br s, 1H), 3.87 - 3.78 (m, 4H), 2.20 (d, *J* = 13.6 Hz, 6H); **LCMS (ESI+)**:2.421 min, m/z 228.2, 5-95CD_3.5min: LC/MS (The column used for chromatography was Gemini C18 5um 50×2.0mm, 5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 10 mM Ammonium bicarbonate in water, and mobile phase B was HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min .5% B in 0.01 min, 5-95% B (0.01-2.50 min) with a hold at 95% B for 0.50 min, 95-5% B (3.00 - 3.01 min) with a hold at 5% B for 0.49 min. The flow rate was 1 mL/min(0.01-3.00min)-1.2 mL/min(3.01-3.50min).)



21-2 21-3

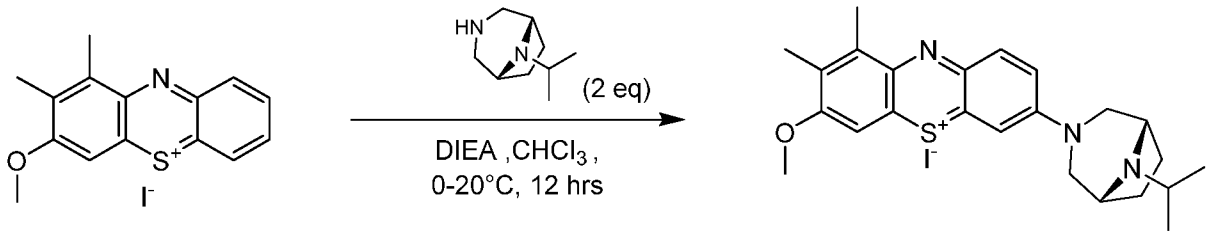
[0207] To a solution of Intermediate 21-2 (3.5 g, 14.78 mmol) and I_2 (375.19 mg, 1.48 mmol) in $PhCl_2$ (20 mL) was added S_8 (948.13 mg, 29.57 mmol) at 20°C. The mixture was stirred at 180°C for 2 hrs. LCMS showed the starting material was consumed, and 49.1% product with desired Ms was detected. The reaction mixture was concentrated under reduced pressure to give a residue. It was purified by silica gel chromatography eluted with Petroleum ether / Ethyl acetate = 30 / 1 to 15 / 1) to give Intermediate 21-3 (3 g, 64.59% yield, 86% purity) as a white solid: **^{1}H NMR:** 400MHz CHLOROFORM-d δ = 7.08 - 6.98 (m, 2H), 6.87 - 6.80 (m, 1H), 6.68 - 6.61 (m, 1H), 6.50 (s, 1H), 5.81 (br s, 1H), 3.75 (s, 3H), 2.16 (d, *J* = 13.9 Hz, 6H); **LCMS (ESI+)**:2.252 min, m/z 258.2, 5-95AB_3.5min: LC/MS (The column was a Luna C18 30*2.0mm,3um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min. 5% B in 0.01 min, 5-95% B (0.01-2.50 min) with a hold at 95% B for 0.50 min, 95-5% B (3.00 -3.01 min) with a hold at 5% B for 0.49 min. The flow rate was 1 mL/min(0.01-3.00min)-1.2 mL/min (3.01-3.50min).)



21-3 21-4

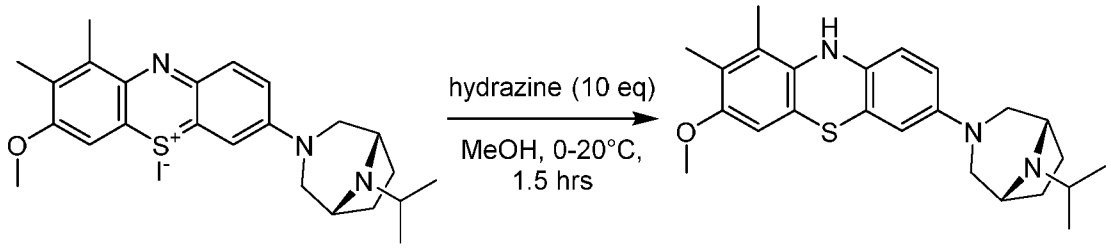
[0208] A mixture of Intermediate 21-3 (3 g, 11.66 mmol) and I_2 (8.88 g, 34.97 mmol) in $CHCl_3$ (1800 mL) was stirred at 20°C for 12 hrs. LCMS showed the starting material was consumed, and desired compound was detected. The reaction mixture was filtered and the filter cake was washed with DCM (20 mL × 2). The filter cake was dried under reduced pressure to give Intermediate 21-4 (6 g, 53.90% yield, 70.5% purity, 4 I^-) as a blue solid: **^{1}H NMR:** 400 MHz, DMSO-d_6 δ = 7.91 (br d, *J* = 9.3 Hz, 1H), 7.79 - 7.67 (m, 1H), 7.60 - 7.52 (m, 2H), 6.86 (s, 1H), 3.17 (s, 3H), 2.45 (s, 3H), 2.12 - 2.07 (m, 3H); **LCMS (ESI+)**:1.238 min, m/z 256.2, 5-95AB_3.5min: LC/MS (The column was a Luna C18 30*2.0mm,3um.

Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 5-95% B in 3.50 min. 5% B in 0.01 min, 5-95% B (0.01-2.50 min) with a hold at 95% B for 0.50 min, 95-5% B (3.00 -3.01 min) with a hold at 5% B for 0.49 min. The flow rate was 1 mL/min(0.01-3.00min)-1.2 mL/min (3.01-3.50min).)



21-4 **21-5**

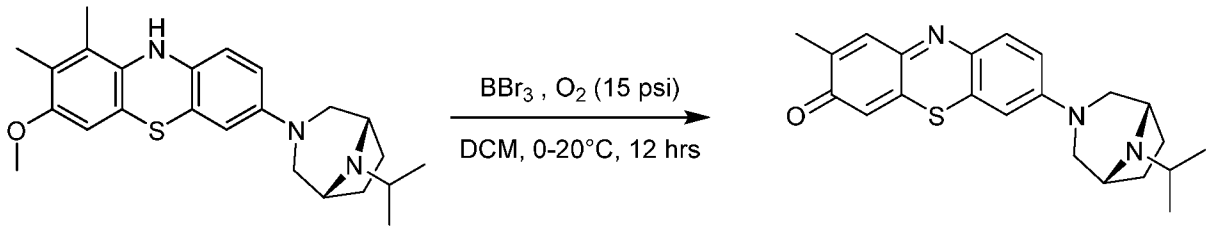
[0209] To a mixture of Intermediate 21-4 (4 g, 4.19 mmol, 4 I^-) in $CHCl_3$ (50 mL) was added dropwise a mixture of (1R,5S)-8-isopropyl-3,8-diazabicyclo-[3.2.1]-octane (1.29 g, 8.38 mmol) and DIEA (1.08 g, 8.38 mmol) in $CHCl_3$ (5 mL) at 0°C. The reaction mixture was filtered and the filter cake was washed with DCM (20 mL × 2). The filter cake was dried under reduced pressure to give Intermediate 21-5 (1.7 g, 75.79% yield, 97.8% purity, I^-) as a blue solid: **LCMS (ESI+)**:0.355 min, m/z 408.3, 10-100AB_1MIN:LC/MS (The column used for chromatography was a Halo C18 3.0*30mm,5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.)



21-5 **21-6**

[0210] To a solution of Intermediate 21-5 (700 mg, 1.31 mmol, I^-) in MeOH (10 mL) was added hydrazine (418.91 mg, 13.07 mmol) at 0°C. The mixture was stirred at 20°C for 1.5 hrs under N_2. LCMS showed the starting material was consumed, and 66% product with desired ms was detected. The reaction was poured into ice water (10 mL), and extracted with

DCM (5 mL × 2). The combined organic layers were washed with brine (5 mL × 2), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 21-6 (300 mg, 48.19% yield, 66% purity) as a yellow solid: **LCMS (ESI+)**: 0.451 min, m/z 408.3, 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm,5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.)

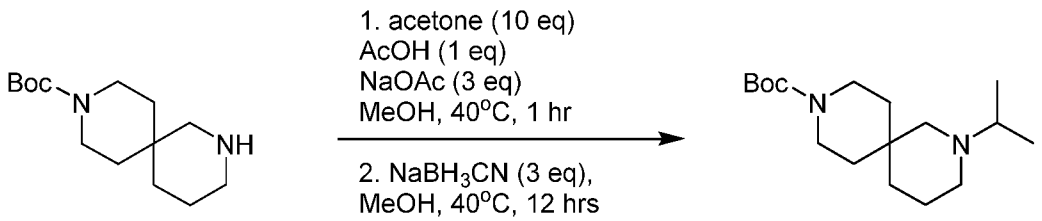


21-6 **Compound 21**

[0211] To a solution of Intermediate 21-6 (200 mg, 419.94 μmol) in DCM (3 mL) was added dropwise BBr_3 (841.63 mg, 3.36 mmol) at 0°C under N_2. The mixture was stirred at 20°C for 12 hrs under O_2 (15 psi). LCMS showed the starting material was consumed, and 55% product with desired ms was detected. MeOH (2 mL) was dropwise into reaction mixture at 0°C. The mixture was concentrated under reduced pressure to give a residue. It was then diluted with DCM (20 mL), and poured into aqueous saturated $NaHCO_3$ (30 mL). The aqueous phase was then extracted with DCM (20 mL × 3). The combined organic layers were washed with brine (5 mL × 2), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, Dichloromethane: Methanol = 15 / 1 to 10 / 1) to give crude product. It was then further purified by prep-TLC (SiO_2, Dichloromethane : Methanol = 8 / 1) to give Compound 21 (12.8 mg, 16.53% yield, 94.4% purity) as a dark purple solid: **^{1}H NMR:** 400 MHz, CHLOROFORM-d δ = 7.73 (br d, *J* = 9.0 Hz, 1H), 6.90 (br d, *J* = 8.5 Hz, 1H), 6.67 (br d, *J* = 6.0 Hz, 2H), 3.71 (br s, 2H), 3.45 (br d, *J* = 10.9 Hz, 2H), 3.29 (br s, 2H), 2.70 (br s, 1H), 2.49 (s, 3H), 2.18 (s, 3H), 2.10 - 1.93 (m, 2H), 1.77 (br s, 2H), 1.18 (br s, 6H); **LCMS (ESI+)**:2.105 min, m/z 394.2, 5_95AB_6min-220-254-ELSD : LC/MS (The gradient was 5%B in 0.40min and 5-95% B in 2.60 min , hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for

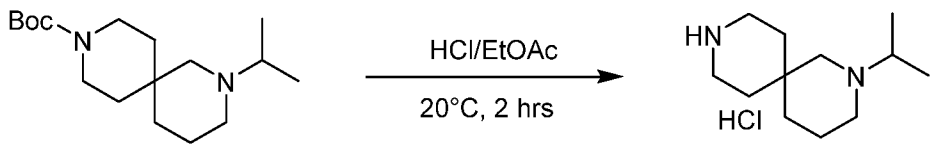
chromatography was a Luna C18 50*2.0mm column (5um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection .MS mode was positive electrospray ionization. MS range was 100-1000.)

Synthesis of 7-(2-isopropyl-2,9-diazaspiro[5.5]undecan-9-yl)-3H-phenothiazin-3-one (Compound 22)



22-1 **22-2**

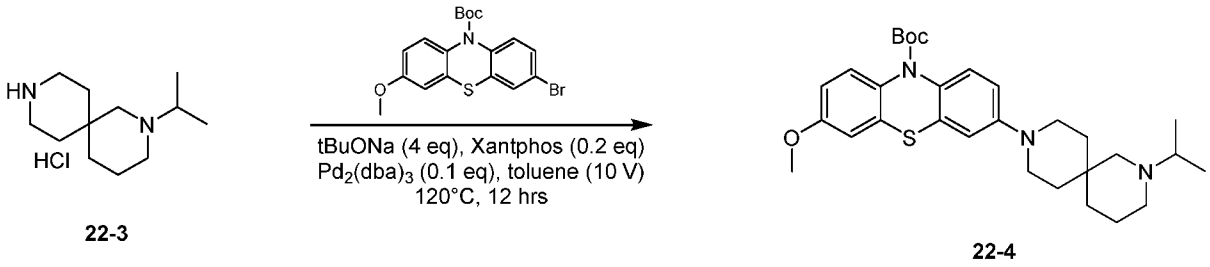
[0212] To a solution of Intermediate 22-1 (0.2 g, 786.26 μmol, 1 *eq*) in MeOH (5 mL) was added acetone (456.65 mg, 7.86 mmol, 578.04 μL, 10 *eq*), NaOAc (193.50 mg, 2.36 mmol, 3 *eq*) and HOAc (47.22 mg, 786.26 μmol, 45.01 μL, 1 *eq*) at 20°C. The mixture was stirred at 40°C for 1 hour. Then $NaBH_3CN$ (148.23 mg, 2.36 mmol, 3 *eq*) was added to the mixture at 20°C. The mixture was stirred at 40°C for 12 hours. The mixture was diluted with ethyl acetate (15 mL) and water (15 mL). The mixture was extracted with ethyl acetate (3 x 15 mL). The organic layer was washed with brine (15 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 22-2 (0.2 g, 674.66 μmol, 85.81% yield) as yellow solid: **^{1}H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 3.57-3.71 (m, 2H), 3.49 (dt, J = 13.2, 6.5 Hz, 1H), 3.15-3.32 (m, 2H), 2.01-2.11 (m, 3H), 1.46 (s, 9H), 1.39 (br d, J = 6.4 Hz, 6H).



22-2 **22-3**

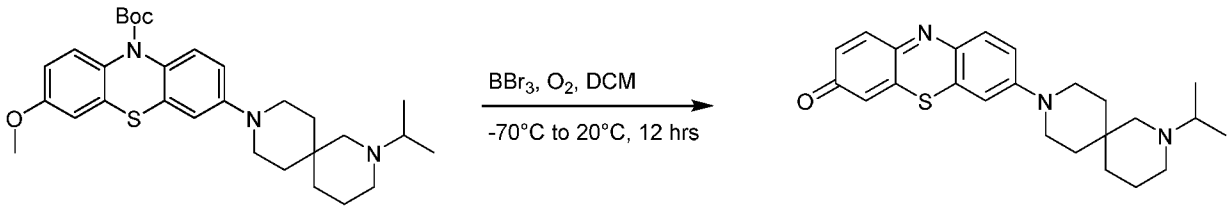
[0213] To a solution of Intermediate 22-2 (1.4 g, 4.72 mmol, 1 *eq*) in EtOAc (10 mL) was added HCl/EtOAc (4 M, 10 mL, 8.47 *eq*) at 20°C. The mixture was stirred at 20°C for 2 hours. LCMS showed the reaction worked. The mixture was concentrated under reduced pressure to give Intermediate 22-3 (1.2 g, 4.46 mmol, 94.37% yield, 2HCl) as white solid: **^{1}H NMR** (METHANOL-d4, 400 MHz) δ (ppm) 3.56 (dt, J = 13.4, 6.7 Hz, 1H), 3.34-3.45 (m,

2H), 3.31 (s, 5H), 3.00 (td, J = 12.1, 4.3 Hz, 1H), 2.90 (d, J = 12.9 Hz, 1H), 1.85-2.20 (m, 5H), 1.65-1.84 (m, 2H), 1.41 (dd, J = 8.4, 6.7 Hz, 6H).



22-3 22-4

[0214] To a solution of tert-butyl 3-bromo-7-methoxy-phenothiazine-10-carboxylate (0.3 g, 734.74 μmol, 1 *eq*) in toluene (10 mL) was added Intermediate 22-3 (296.75 mg, 1.10 mmol, 1.5 *eq*, 2HCl), t-BuONa (282.44 mg, 2.94 mmol, 4 *eq*), Xantphos (85.03 mg, 146.95 μmol, 0.2 *eq*) and $Pd_2(dba)_3$ (67.28 mg, 73.47 μmol, 0.1 *eq*) at 20°C under N_2. The mixture was stirred at 120°C for 12 hours. One additional vial in 50 mg scale was set up as described above. The combined reaction mixture was diluted with ethyl acetate (10 mL) and water (10 mL). The mixture was extracted with ethyl acetate (3 x 10 ml). The combined organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the product. The crude product was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=0%:100%, ethyl acetate/methanol=90%) to give Intermediate 22-4 (0.2 g, 381.88 μmol, 44.81% yield) as yellow solid: **^{1}H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 7.40 (br d, J = 8.9 Hz, 1H), 7.35 (br d, J = 8.7 Hz, 1H), 6.84-6.88 (m, 2H), 6.80 (td, J = 9.3, 2.7 Hz, 2H), 3.79 (s, 3H), 3.15 (br d, J = 7.2 Hz, 2H), 3.03-3.12 (m, 2H), 2.68-2.78 (m, 1H), 2.47 (br s, 2H), 2.26 (br s, 2H), 1.66-1.75 (m, 2H), 1.56-1.66 (m, 4H), 1.48 (s, 9H), 1.30-1.40 (m, 2H), 0.99 (d, J = 6.6 Hz, 6H).

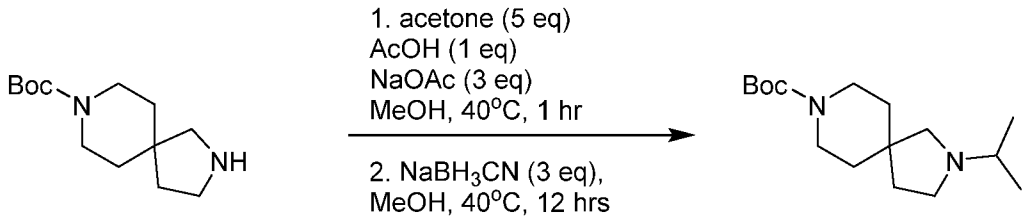


22-4 Compound 22

[0215] To a solution of Intermediate 22-4 (0.1 g, 190.94 μmol, 1 *eq*) in DCM (5 mL) was added BBr_3 (143.50 mg, 572.82 μmol, 55.19 μL, 3 *eq*) dropwise at -78°C. The mixture was stirred at 20°C for 12 hours under O_2 (15 psi). LCMS showed the reaction worked well. The mixture was quenched with methanol (10 mL) at -40°C. The mixture was adjust to pH=8 with saturated sodium carbonate aqueous solution (10 mL). The mixture was extracted with dichloromethane (3 x 10 mL). The combined organic layer was dried with Na_2SO_4, filtered

and concentrated under reduced pressure to give the crude product. The product was purified by column chromatography (SiO_2, polarity gradient: Petroleum ether/Ethyl acetate=100% to 0%, ethyl acetate/dichloromethane =80% to 90%, dichloromethane/methyl alcohol=95%). And then the crude was purified by prep-TLC (dichloromethane: methanol=2:1) to give a residue. The residue was triturated with ethyl acetate (10 mL) at 20°C for 10 min and then filtered. Th cake was dried by high vacuum to give Compound 22 (5.5 mg, 13.13 μmol, 6.88% yield, 97.3% purity) as dark purple solid: **^{1}H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 7.70 (d, J = 9.1 Hz, 1H), 7.57 (d, J = 9.8 Hz, 1H), 6.99 (dd, J = 9.3, 2.6 Hz, 1H), 6.85 (dd, J = 9.8, 2.1 Hz, 1H), 6.76 (d, J = 2.5 Hz, 1H), 6.69 (d, J = 2.0 Hz, 1H), 3.33-3.57 (m, 4H), 2.73 (dt, J = 13.1, 6.5 Hz, 1H), 2.47 (br s, 2H), 2.29 (br s, 2H), 1.75 (br dd, J = 10.3, 3.4 Hz, 2H), 1.59-1.67 (m, 4H), 1.39 (br d, J = 6.3 Hz, 2H), 1.00 (d, J = 6.5 Hz, 6H); **LCMS (ESI+)**: 1.763 min, m/z 408 (M+H), 5_95AB_6min-220-254-ELSD : LC/MS (The gradient was 5%B in 0.40min and 5-95% B in 2.60 min , hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Luna C18 50*2.0mm column (5um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection .MS mode was positive electrospray ionization. MS range was 100-1000.)

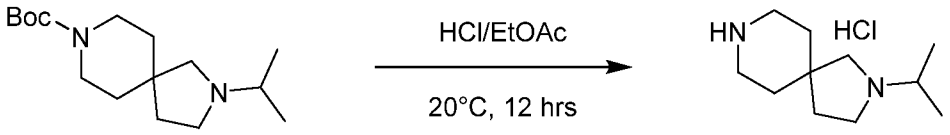
Synthesis of 7-(2-isopropyl-2,8-diazaspiro[4.5]decan-8-yl)-3H-phenothiazin-3-one (Compound 23)



23-1 **23-2**

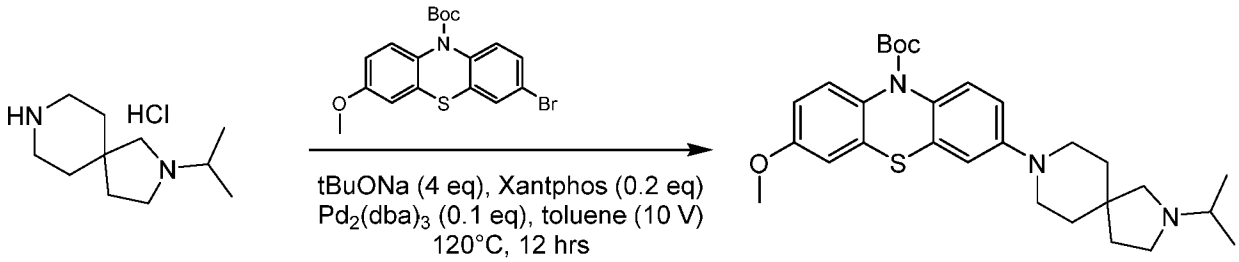
[0216] To a solution of Intermediate 23-1 (1.8 g, 7.49 mmol, 1 *eq*) in MeOH (20 mL) was added acetone (2.17 g, 37.45 mmol, 2.75 mL, 5 *eq*), HOAc (449.75 mg, 7.49 mmol, 428.74 μL, 1 *eq*) and NaOAc (1.84 g, 22.47 mmol, 3 *eq*) inorder at 20°C. The mixture was stirred at 40°C for 1 hr. Then $NaBH_3CN$ (1.41 g, 22.47 mmol, 3 *eq*) was added to the mixture at 20°C. The mixture was stirred at 40°C for 12 hours. The mixture was diluted with ethyl acetate (20 mL) and water (20 mL). The mixture was extracted with ethyl acetate (3 x 20 mL). The

combined organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 23-2 (1.6 g, 5.67 mmol, 75.64% yield) as yellow solid: **^{1}H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 3.42-3.52 (m, 2H), 3.27-3.37 (m, 2H), 3.02 (br s, 2H), 2.82 (td, J = 12.4, 5.9 Hz, 3H), 1.85 (t, J = 7.1 Hz, 2H), 1.59 (t, J = 5.6 Hz, 4H), 1.46 (s, 9H), 1.24-1.29 (m, 6H).



23-2 **23-3**

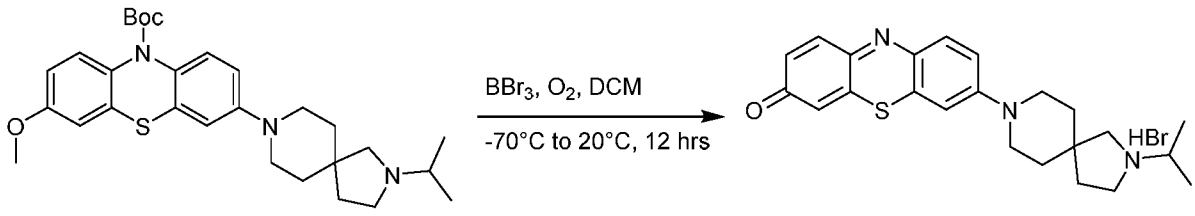
[0217] To a solution of Intermediate 23-2 (1.6 g, 5.67 mmol, 1 *eq*) in EtOAc (10 mL) was added HCl/EtOAc (4 M, 5 mL, 3.53 *eq*) at 0°C. The mixture was stirred at 20°C for 12 hours. The mixture was concentrated under reduced pressure to give Intermediate 23-3 (1.4 g, 5.49 mmol, 96.82% yield, 2HCl) as white solid: **^{1}H NMR** (METHANOL-d4, 400 MHz) δ (ppm) 3.73 (ddd, J = 11.7, 7.7, 4.1 Hz, 1H), 3.61-3.68 (m, 1H), 3.47 (dt, J = 13.0, 6.5 Hz, 1H), 3.33-3.38 (m, 1H), 3.18-3.28 (m, 4H), 3.10 (d, J = 12.1 Hz, 1H), 2.11-2.21 (m, 1H), 2.01-2.08 (m, 1H), 1.87-2.00 (m, 4H), 1.42 (d, J = 6.5 Hz, 6H).



23-3 **23-4**

[0218] To a solution of tert-butyl 3-bromo-7-methoxy-phenothiazine-10-carboxylate (0.3 g, 734.74 μmol, 1 *eq*) in toluene (10 mL) was added Intermediate 23-3 (281.29 mg, 1.10 mmol, 1.5 *eq*, 2HCl), NaOBu-t (282.44 mg, 2.94 mmol, 4 *eq*), Xantphos (85.03 mg, 146.95 μmol, 0.2 *eq*) and $Pd_2(dba)_3$ (67.28 mg, 73.47 μmol, 0.1 *eq*) at 20°C under N_2. The mixture was stirred at 120°C for 12 hours. One additional vial in 50 mg scale was set up as described above. The mixtures were combined and diluted with water (10 mL) and ethyl acetate (10 mL). The mixture was extracted with ethyl acetate (3 x 10 mL). The combined organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=0%:100%, ethyl acetate/methanol=90%) to give Intermediate 23-4 (0.1 g,

196.19 μmol, 23.02% yield) as yellow solid: **LCMS (ESI+)**: 0.451 min, m/z 410 (M+1), 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm,5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.)

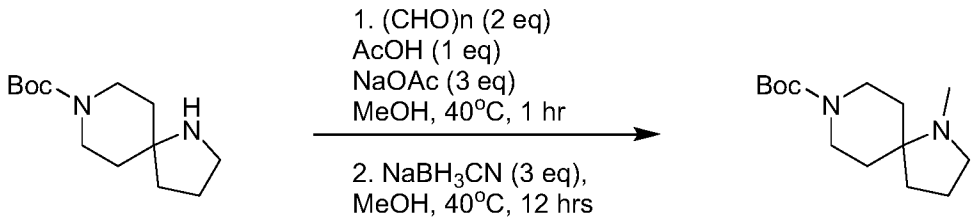


23-4 **Compound 23**

[0219] To a solution of Intermediate 23-4 (80 mg, 156.95 μmol, 1 *eq*) in DCM (5 mL) was added BBr_3 (196.60 mg, 784.77 μmol, 75.62 μL, 5 *eq*) dropwise at -70°C under N_2. The mixture was stirred at 20°C for 12 hours under oxygen (15 psi). LCMS showed the reaction worked. One additional vial in 20 mg scale was set up as described above. The mixtures were combined and quenched with methanol (10 mL) at -40°C. The mixture was adjust to pH=8 with saturated sodium bicarbonate aqueous solution (20 mL). The mixture was extracted with dichloromethane (3 x 20 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product. The residue was purified by column chromatography (SiO_2, polarity gradient: petroleum ether/ethyl acetate=100% to 0%, ethyl acetate/dichloromethane=80% to 90%, dichloromethane/methyl alcohol=95%). The residue was triturated with ethyl acetate (10 mL) at 20°C for 10 min and then filtered. The solid was dried in high vacuum to give Compound 23 (20 mg, 40.26 μmol, 20.52% yield, 95.5% purity, HBr salt) as dark purple solid: **^{1}H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 11.77-12.00 (m, 1H), 7.66 (d, J = 9.1 Hz, 1H), 7.49 (d, J = 9.9 Hz, 1H), 6.93 (dd, J = 9.1, 2.4 Hz, 1H), 6.78 (dd, J = 9.9, 1.9 Hz, 1H), 6.70 (d, J = 2.4 Hz, 1H), 6.62 (d, J = 1.8 Hz, 1H), 3.72-3.88 (m, 1H), 3.62 (br dd, J = 11.5, 6.1 Hz, 1H), 3.26-3.51 (m, 5H), 2.89-3.00 (m, 1H), 2.60 (br dd, J = 11.3, 8.3 Hz, 1H), 2.26 (dt, J = 13.4, 8.4 Hz, 1H), 2.02-2.15 (m, 2H), 1.90-1.99 (m, 1H), 1.67-1.80 (m, 2H), 1.48 (br d, J = 7.1 Hz, 6H); **LCMS (ESI+)**: 1.725 min, m/z 394 (M+H), 5_95AB_6min-220-254-ELSD : LC/MS (The gradient was 5%B in 0.40min and 5-95% B in 2.60 min , hold on 95% B in 1.00min, and then 95-5%B

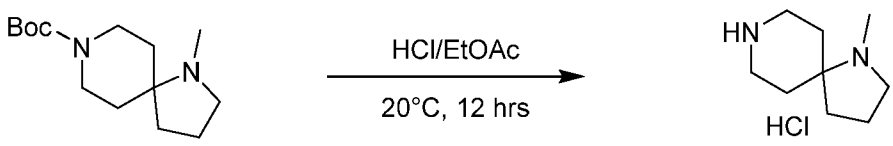
in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Luna C18 50*2.0mm column (5um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection .MS mode was positive electrospray ionization. MS range was 100-1000.)

Synthesis of 7-(1-methyl-1,8-diazaspiro[4.5]decan-8-yl)-3H-phenothiazin-3-one (Compound 24)



24-1 **24-2**

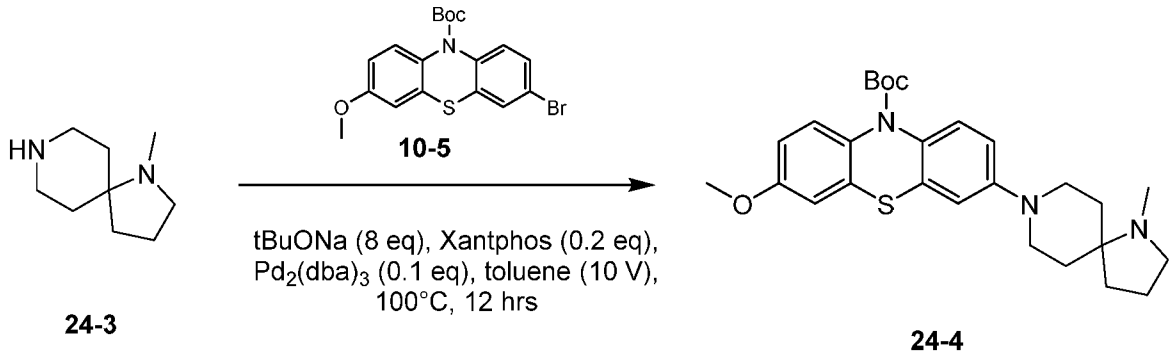
[0220] To a solution of Intermediate 24-1 (1.2 g, 4.99 mmol, 1 *eq*) in MeOH (10 mL) was added formaldehyde (299.83 mg, 9.99 mmol, 275.08 µL, 2 *eq*), HOAc (299.83 mg, 4.99 mmol, 285.83 µL, 1 *eq*) and NaOAc (1.23 g, 14.98 mmol, 3 *eq*) at 20°C. The mixture was stirred at 40°C for 1 hour. Then $NaBH_3CN$ (941.29 mg, 14.98 mmol, 3 *eq*) was added to the mixture at 20°C. The mixture was stirred at 40°C for 12 hours. The mixture was diluted with ethyl acetate (20 mL) and water (20 mL). The mixture was extracted with ethyl acetate (3 x 15 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 24-2 (1.2 g, 4.72 mmol, 94.49% yield) as yellow solid: **^{1}H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 4.32 (s, 2H), 2.90 (br s, 2H), 2.75 (br t, *J* = 12.4 Hz, 2H), 2.36 (s, 3H), 1.78-1.92 (m, 4H), 1.64 (td, *J* = 12.8, 4.8 Hz, 2H), 1.46 (s, 9H), 1.34 (br d, *J* = 11.0 Hz, 2H).



24-2 **24-3**

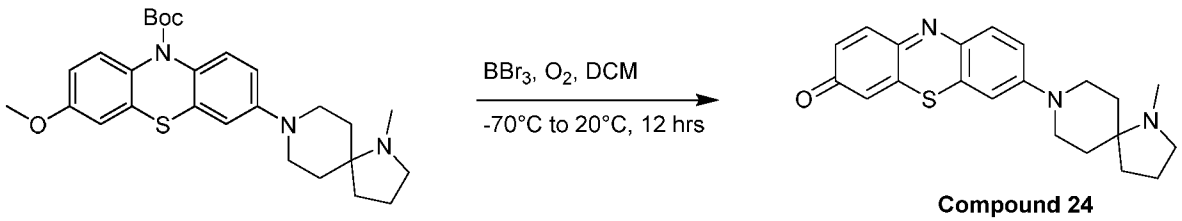
[0221] To a solution of Intermediate 24-2 (1.2 g, 4.72 mmol, 1 *eq*) in EtOAc (10 mL) was added HCl/EtOAc (4 M, 5 mL, 4.24 *eq*) at 0°C. The mixture was stirred at 20°C for 12 hours. LCMS showed the reaction worked. The mixture was concentrated under reduced pressure to give the product Intermediate 24-3 (1 g, 4.40 mmol, 93.31% yield, 2HCl) as white

solid: **^{1}H NMR** (METHANOL-d4, 400 MHz) δ (ppm) 3.74 (td, J = 7.8, 4.6 Hz, 1H), 3.50-3.61 (m, 2H), 3.10-3.30 (m, 3H), 2.87 (s, 3H), 2.45-2.56 (m, 1H), 2.36 (td, J = 13.5, 4.7 Hz, 1H), 2.04-2.25 (m, 6H).



24-3 **24-4**

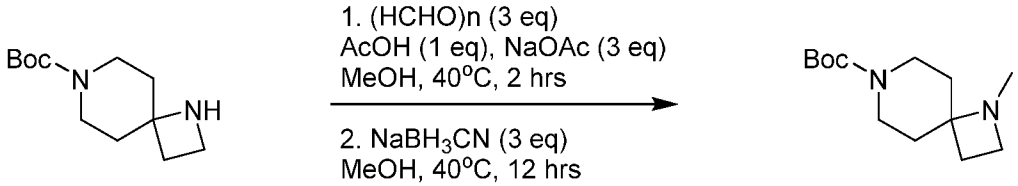
[0222] To a solution of Intermediate 10-5 (0.1 g, 244.91 μmol, 1 eq) in toluene (10 mL) was added Intermediate 24-3 (83.46 mg, 541.04 μmol, 2.21 eq, free), NaOBu-t (188.30 mg, 1.96 mmol, 8 eq), Xantphos (28.34 mg, 48.98 μmol, 0.2 eq) and $Pd_2(dba)_3$ (22.43 mg, 24.49 μmol, 0.1 *eq*) at 20°C under N_2. The mixture was charged with N_2 for 3 times. One additional vial in 50 mg scale and three additional vials in 100 mg scale were set up as described above. The mixtures were stirred at 100°C for 12 hours under N_2. The combined reaction mixture was diluted with ethyl acetate (10 mL) and water (10 mL). The mixture was extracted with ethyl acetate (3 x 10 mL). The combined with organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product. The crude product was purified by prep-HPLC under formic acid system. The solution was adjusted to pH=7 with solution of $NaHCO_3$(15 mL). The mixture was extracted with ethyl acetate (3 x 50 mL). The combined with organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 24-4 (0.2 g, 415.24 μmol, 48.44% yield) as yellow solid: **LCMS** (ESI+): m/z 482 (M+1), RT: 0.438 min, 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm,5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min .)



24-4 Compound 24

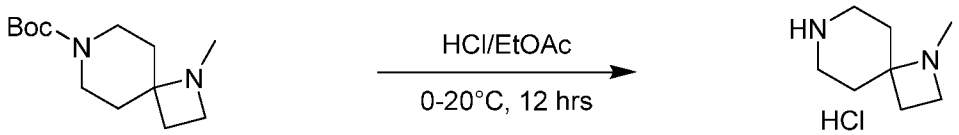
[0223] To a solution of Intermediate 24-4 (0.2 g, 415.24 μmol, 1 *eq*) in DCM (3 mL) was added BBr_3 (520.14 mg, 2.08 mmol, 200.05 μL, 5 *eq*) dropwise at -78°C under N_2. The mixture was stirred at 20°C for 12 hours under OXYGEN (15 psi). LC-MS showed starting material was consumed completely and one main peak with desired mass was detected. The mixture was poured into cold water (10 mL) at 0°C and then adjust to pH=8 with solution of Na_2CO_3(about 20 mL). The mixture was extracted with dichloromethane (3 x 15 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give a crude product. The product was purified by prep-TLC (dichloromethane: methanol=2:1) to give Compound 24 (10.1 mg, 26.53 μmol, 6.39% yield, 96% purity) as dark purple solid: **^{1}H NMR** (DMSO-d6, 400 MHz) δ (ppm) 7.67 (br d, J = 9.4 Hz, 1H), 7.56 (d, J = 10.3 Hz, 1H), 7.18-7.29 (m, 2H), 6.67-6.78 (m, 2H), 4.15 (br d, J = 13.5 Hz, 2H), 3.05 (br t, J = 12.9 Hz, 2H), 2.72-2.86 (m, 2H), 2.23 (br s, 3H), 1.74-1.91 (m, 4H), 1.67 (br t, J = 11.0 Hz, 2H), 1.39 (br d, J = 11.5 Hz, 2H); **LCMS (ESI+)**: 1.674 min, m/z 366.1 (M+H), 5_95AB_6min-220-254 : LC/MS (The gradient was 5%B in 0.40min and 5-95% B in 2.60 min, hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Luna C18 50*2.0mm column (5um particles). Detection methods are diode array (DAD) detection. MS mode was positive electrospray ionization. MS range was 100-1000.)

Synthesis of 7-(1-methyl-1,7-diazaspiro[3.5]nonan-7-yl)-3H-phenothiazin-3-one (Compound 25)



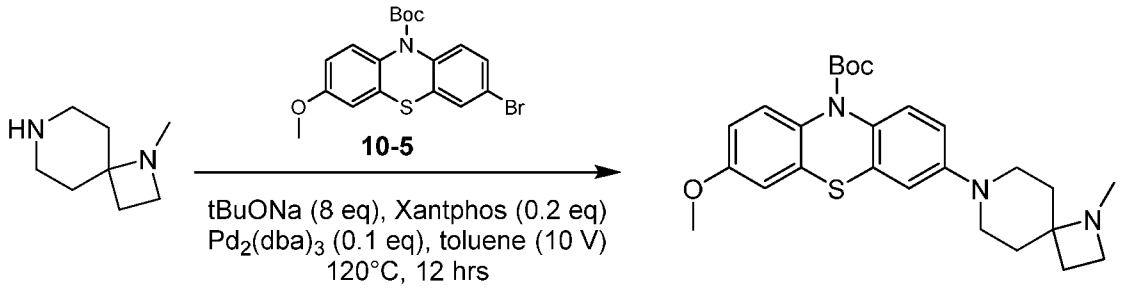
25-1 25-2

[0224] A mixture of Intermediate 25-1 (1.8 g, 7.95 mmol, 1 eq), HCHO (716.44 mg, 23.86 mmol, 657.28 µL, 3 eq), AcOH (477.63 mg, 7.95 mmol, 455.32 µL, 1 eq), NaOAc (1.96 g, 23.86 mmol, 3 eq) in anhydrous MeOH (40 mL) was stirred for 2 hours at 40°C. Then $NaBH_3CN$ (1.50 g, 23.86 mmol, 3 eq) was added to above mixture in portions at 20°C, then the mixture was stirred at 40°C for 12 hrs. The mixture was diluted with water (100 mL), extracted with ethyl acetate (4 x 60 mL). The combined organic phases were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give crude product Intermediate 25-1 (1 g, 3.95 mmol, 49.70% yield, 95% purity) as a yellow oil: **^{1}H NMR** (400 MHz, CHLOROFORM-d) δ ppm 4.37 (s, 2 H), 4.04 - 4.20 (m, 2 H), 3.52 - 3.64 (m, 2 H), 2.77 (br t, J=12.44 Hz, 2 H), 2.42 (s, 3 H), 2.21 (t, J=7.50 Hz, 2 H), 1.94 (br d, J=11.01 Hz, 2 H), 1.72 (td, J=12.57, 4.63 Hz, 2 H), 1.47 (s, 9 H).



25-2 **25-3**

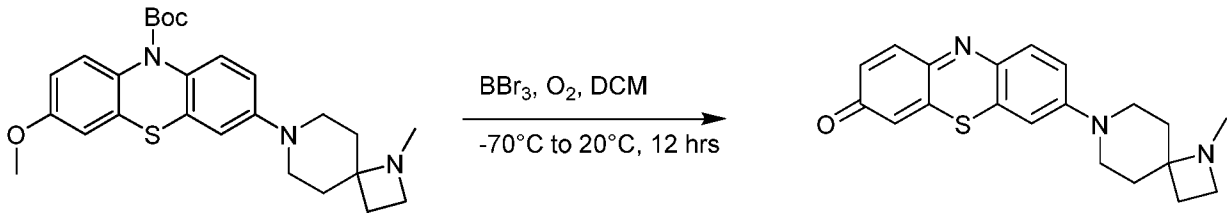
[0225] To a mixture of Intermediate 25-2 (1 g, 3.95 mmol, 1 eq) in EtOAc (10 mL) was added HCl/EtOAc (10 mL, 4 mol/L) dropwise at 0°C. The mixture was stirred for 12 hours at 20°C. The mixture was concentrated under reduced pressure to give Intermediate 25-3 (1.1 g, crude, HCl salt) as a white solid: **^{1}H NMR** (400 MHz, DEUTERIUM OXIDE) δ ppm 4.23 - 4.34 (m, 1 H), 3.89 (q, J=9.84 Hz, 1 H), 3.46 - 3.63 (m, 2 H), 3.14 (q, J=12.76 Hz, 2 H), 2.81 (s, 3 H), 2.39 - 2.64 (m, 4 H), 2.16 - 2.30 (m, 2 H).



25-3 **25-4**

[0226] To a solution of Intermediate 10-5 (0.1 g, 244.91 µmol, 1 *eq*) in toluene (10 mL) was added Intermediate 25-3 (103.03 mg, 734.74 µmol, 3 *eq, free*), t-BuONa (188.30 mg, 1.96 mmol, 8 *eq*), $Pd_2(dba)_3$ (22.43 mg, 24.49 µmol, 0.1 *eq*) and Xantphos (28.34 mg, 48.98 µmol, 0.2 *eq*) inorder at 20°C. The mixture was charged with N_2 for 3 times. Then the mixture was stirred at 120°C for 12 hours under N_2. One additional vial in 50 mg scale and three additional vials in 100 mg scale were set up as described above. LC-MS showed

starting material was consumed completely and one main peak with desired mass was detected. The mixtures were combined for work up together. Then the mixture was diluted with ethyl acetate (20 mL) and water (20 mL). The mixture was extracted with ethyl acetate (3 x 20 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product. The crude product was purified by prep-HPLC (under FA system). The solution was extracted with ethyl acetate (3 x 500 mL). The combined organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 25-4 (0.17 g, 327.19 μmol, 33.40% yield, 90% purity) as yellow solid: **LCMS** (ESI+): m/z 468 (M+1), RT: 0.438 min, 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm,5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.)

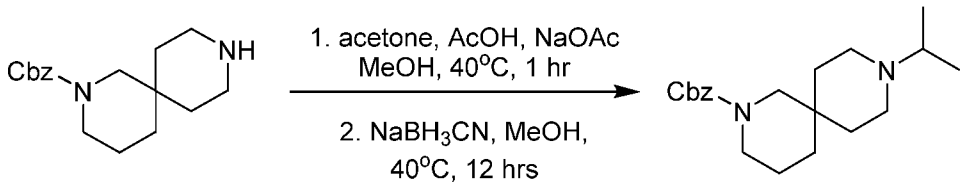


25-4 **Compound 25**

[0227] To a solution of Intermediate 25-4 (0.17 g, 363.54 μmol, 1 *eq*) in DCM (10 mL) was added BBr_3 (455.38 mg, 1.82 mmol, 175.15 μL, 5 *eq*) at -70°C dropwise under N_2. The mixture was stirred at 20°C for 12 hours under O_2 (15 psi). LC-MS showed starting material was consumed completely and one main peak with desired mass was detected. The mixture was poured into cold water (15 mL) slowly. The mixture was extracted with dichloromethane (2 x 5 mL). The aqueous phase was adjust to pH=8 with solution of Na_2CO_3 (15 mL). The mixture was extracted with dichloromethane (3 x 15 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product. The product was purified by prep-TLC (dichloromethane: methanol=2:1, Rf=0.05) to give Compound 25 (7 mg, 18.82 μmol, 5.18% yield, 94.5% purity) as dark purple solid: **^{1}H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 7.72 (d, J = 9.1 Hz, 1H), 7.57 (d, J = 9.9 Hz, 1H), 7.02 (dd, J = 9.3, 2.8 Hz, 1H), 6.86 (dd, J = 9.8, 2.2 Hz, 1H), 6.79 (d, J = 2.8 Hz, 1H), 6.70 (d, J = 2.1 Hz, 1H), 3.94 (br d, J = 13.4 Hz, 2H), 3.28 (t, J

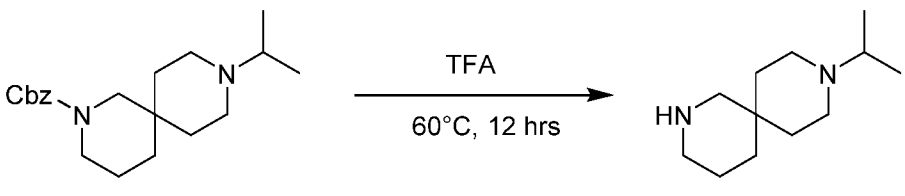
= 7.0 Hz, 2H), 3.04 (td, J = 12.9, 2.4 Hz, 2H), 2.24 (s, 3H), 2.07 (t, J = 7.0 Hz, 2H), 1.86-1.96 (m, 2H), 1.77 (td, J = 12.6, 4.1 Hz, 2H); **LCMS (ESI+)**: 1.654 min, m/z 352 (M+H), 5_95AB_6 min-220-254 : LC/MS (The gradient was 5%B in 0.40min and 5-95% B in 2.60 min, hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Luna C18 50*2.0mm column (5um particles). Detection methods are diode array (DAD) detection. MS mode was positive electrospray ionization. MS range was 100-1000.)

Synthesis of 7-(9-isopropyl-2,9-diazaspiro[5.5]undecan-2-yl)-3H-phenothiazin-3-one (Compound 26)



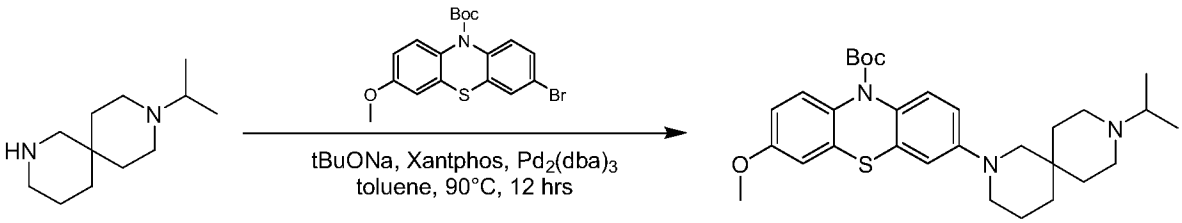
26-1 **26-2**

[0228] A mixture of Intermediate 26-1 (0.6 g, 2.08 mmol), acetone (604.18 g, 10.40 mmol), NaOAc (512.01 mg, 6.24 mmol) and AcOH (124.94 mg, 2.08 mmol) in MeOH (10 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 40°C for 1 hr under N_2 atmosphere. Then to the mixture was added $NaBH_3CN$ (392.23 mg, 6.24 mmol). The reaction mixture was stirred at 40°C for 12 hrs. The reaction mixture was concentrated under reduced pressure to remove MeOH. The residue was diluted with water (20 mL) and extracted with DCM (3 × 10 mL). The combined organic layers were washed with brine (3 × 20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 26-2 (0.7 g, crude) as a yellow oil: **^{1}H NMR:** ET75755-21-P1A1 400 MHz, CHLOROFORM-d δ 7.3-7.4 (m, 5H), 5.12 (s, 2H), 3.1-3.6 (m, 5H), 3.0-3.1 (m, 1H), 2.8-2.9 (m, 1H), 2.6-2.8 (m, 1H), 2.4-2.6 (m, 1H), 1.8-2.0 (m, 1H), 1.7-1.8 (m, 2H), 1.58 (br s, 5H), 1.3-1.4 (m, 3H), 1.0-1.2 (m, 3H).



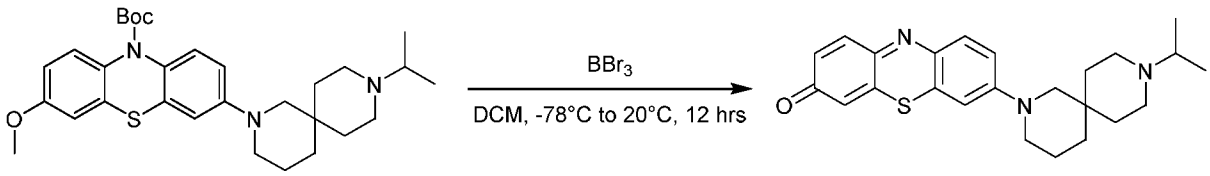
26-2 **26-3**

[0229] A mixture of Intermediate 26-2 (0.7 g, 2.12 mmol) in TFA (2 mL) was stirred at 60°C for 12 hrs. The reaction mixture was concentrated under reduced pressure to give the crude product which was then neutralized with AMBERJET 4400 OH strongly basic anionic exchanger resin (CAS: 9017-79-2) to give Intermediate 26-3 (0.4 g, crude) as a white solid: **^{1}H NMR:** 400 MHz, DMSO-d6 δ 2.8-3.0 (m, 5H), 2.5-2.6 (m, 3H), 1.4-1.7 (m, 8H), 1.03 (d, 6H, J=6.5 Hz).



26-3 **26-4**

[0230] To a mixture of Intermediate 26-3 (49.41 mg, 159.19 μmol, 1.3 *eq*), tert-butyl 3-bromo-7-methoxy-phenothiazine-10-carboxylate (50 mg, 122.46 μmol, 1 *eq*), tBuONa (35.31 mg, 367.37 μmol, 3 *eq*), XantPhos (14.17 mg, 24.49 μmol, 0.2 *eq*) in toluene (10 mL) was added $Pd_2(dba)_3$ (11.21 mg, 12.25 μmol, 0.1 *eq*) at 20°C. The mixture was stirred at 90°C for 12 hrs under nitrogen atmosphere. Additional five reactions were set up as described above. The reaction mixtures were combined and filtered through celite. The filtrate was concentrated under reduced pressure to give a residue which was purified by column chromatography on silica gel eluted with petroleum ether/ethyl acetate (100:1 to 1:1) and then DCM/MeOH (1:0 to 0:1) to give Intermediate 26-4 (200 mg, 145.11 μmol, 23.70% yield, 38% purity) as a blue solid which was used without further purification.

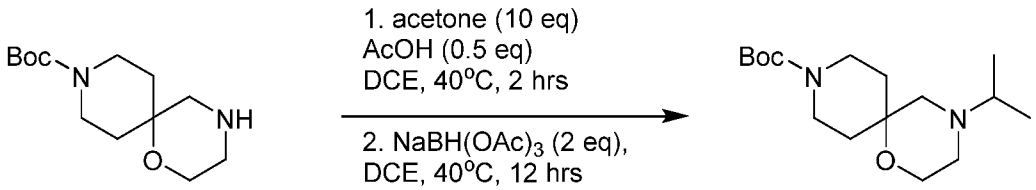


26-4 **Compound 26**

[0231] To a solution of Intermediate 26-4 (200 mg, 381.88 μmol, 1 *eq*) in DCM (40 mL) was added BBr_3 (287.01 mg, 1.15 mmol, 110.39 μL, 3 *eq*) at -78°C. The mixture was stirred at 20°C for 12 hrs. LCMS showed the starting material was consumed completely and a major peak with desired ms was detected. The reaction mixture was poured into saturated aqueous $NaHCO_3$ (20 mL) and extracted with DCM (3 × 10 mL). The organic phases were combined, washed with brine (10 mL), dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give a crude product. It was then purified by prep-

TLC (DCM/MeOH=100/1 to 1/1). The crude product further purified by prep-TLC purification for another three times. Compound 26 (1.5 mg, 3.42 μmol, 92.8% purity) was obtained as a brown solid: **^{1}H NMR:** ^{1}H NMR (400 MHz, CHLOROFORM-d) δ = 7.70 (d, *J* = 9.1 Hz, 1H), 7.57 (d, *J* = 9.9 Hz, 1H), 7.01 (br d, *J* = 8.4 Hz, 1H), 6.91 - 6.79 (m, 2H), 6.69 (d, *J* = 1.9 Hz, 1H), 4.08 (quin, *J* = 7.3 Hz, 1H), 3.44 (br t, *J* = 5.3 Hz, 2H), 3.33 (s, 2H), 3.28 - 2.67 (m, 4H), 1.66 (br d, *J* = 5.8 Hz, 3H), 1.34 - 1.30 (m, 5H), 1.07 (s, 6H); **LCMS (ESI+)**: 1.657 min, m/z 408.1, 5_95AB_6min-220-254-ELSD : LC/MS (The gradient was 5%B in 0.40min and 5-95% B in 2.60 min, hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Kinetex C18 2.1*50mm,5um. Detection methods are diode array (DAD), and evaporative light scattering detection (ELSD). MS mode was positive electrospray ionization. MS range was 100-1000.)

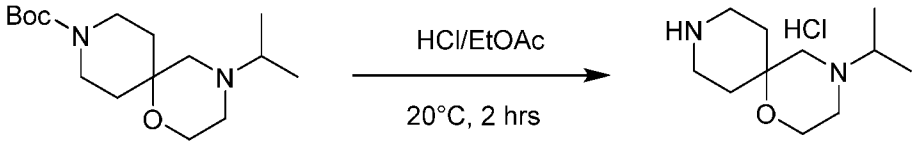
Synthesis of 7-(4-isopropyl-1-oxa-4,9-diazaspiro[5.5]undecan-9-yl)-3H-phenothiazin-3-one (Compound 27)



27-1 **27-2**

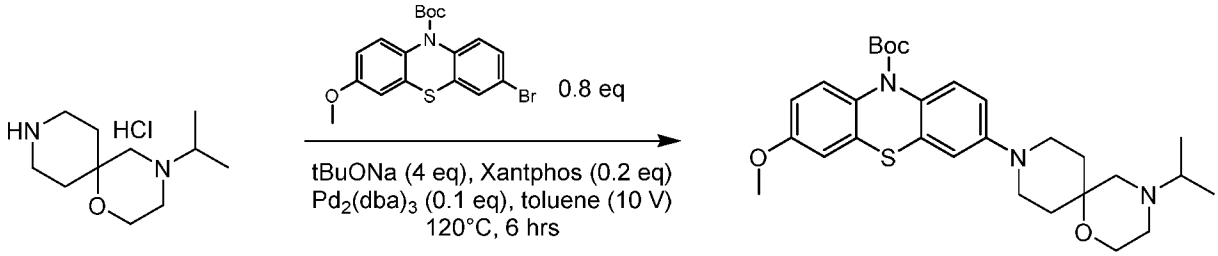
[0232] To a solution of Intermediate 27-1 (0.5 g, 1.95 mmol, 1 eq) in MeOH (10 mL) was added acetone (1.13 g, 19.51 mmol, 1.43 mL, 10 eq) , AcOH (117.13 mg, 1.95 mmol, 111.66 μL, 1 eq) and NaOAc (480.03 mg, 5.85 mmol, 3 eq) in order at 20°C, and the mixture was stirred at 40°C for 1 h. $NaBH_3CN$ (367.73 mg, 5.85 mmol, 3 eq) was added to the mixture at 20°C, then the mixture was stirred at 40°C for 12 hours. The mixture was diluted with water (30 mL), extracted with ethyl acetate (3 x 20 mL). The combined organic phase was washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 27-2 (0.6 g, 1.61 mmol, 82.46% yield, 80% purity) as yellow solid: 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm, 5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile

phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.



27-2 27-3

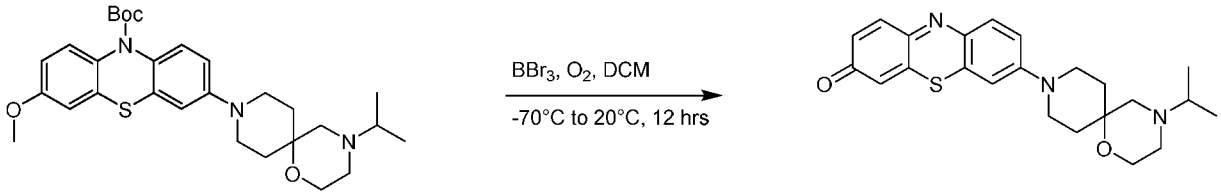
[0233] Intermediate 27-2 (600 mg, 1.61 mmol, 1 eq) was added to HCl/EtOAc (4 M, 4.00 mL, 9.95 eq) at 20°C, and the mixture was stirred at 2 hours at 20°C. LCMS showed the reaction worked well. The mixture was concentrated under reduced pressure to give Intermediate 27-3 (0.5 g, 1.49 mmol, 92.69% yield, 70% purity, 2HCl) as white solid: 10-100CD_1min: LC/MS (The column used for chromatography was a Xbridge C18 3.0*30 mm (5um particles). Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 10 mM Ammonium bicarbonate in water, and mobile phase B was HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min. 10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min (0.00-0.90 min).



27-3 27-4

[0234] To a solution of Intermediate 27-3 (218.55 mg, 930.94 μmol, 1.27 eq, HCl) in toluene (5 mL) was added tert-butyl 3-bromo-7-methoxy-phenothiazine-10-carboxylate (300 mg, 734.74 μmol, 1 eq), tBuONa (353.05 mg, 3.67 mmol, 5 eq), Xantphos (42.51 mg, 73.47 μmol, 0.1 eq) and $Pd_2(dba)_3$ (67.28 mg, 73.47 μmol, 0.1 eq) in order at 20°C, then the mixture was stirred at 120°C for 6 hours under N_2. LCMS showed the reaction worked well. The reaction was filtered through pad of celite and the cake was washed with ethyl acetate (3 x 10 mL). The filtrate was concentrated under reduced pressure to give the crude. The crude was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 100: 25) to give Intermediate 27-4 (250 mg, 456.53 μmol, 62.14% yield, 96% purity) was obtained as white solid: ^1H NMR (400 MHz, CHLOROFORM-d) δ = 7.37 (br dd, J = 8.9, 18.9 Hz, 2H), 6.90 -

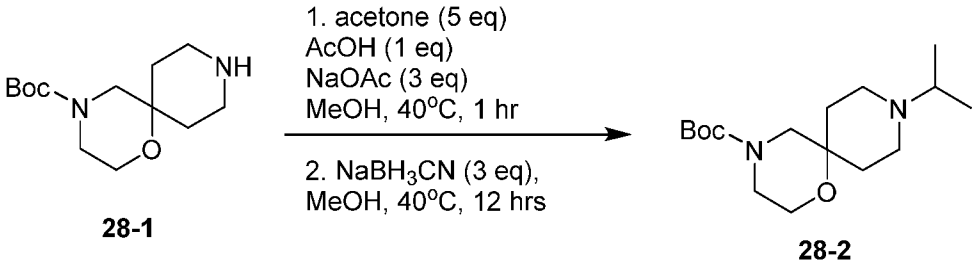
6.74 (m, 4H), 3.81 - 3.72 (m, 5H), 3.29 (br d, J = 12.1 Hz, 2H), 3.17 - 3.08 (m, 2H), 2.66 - 2.55 (m, 1H), 2.49 (br d, J = 4.8 Hz, 2H), 2.32 (s, 2H), 2.10 - 2.00 (m, 2H), 1.69 - 1.61 (m, 2H), 1.51 - 1.42 (m, 9H), 1.00 (d, J = 6.5 Hz, 6H).



27-4 Compound 27

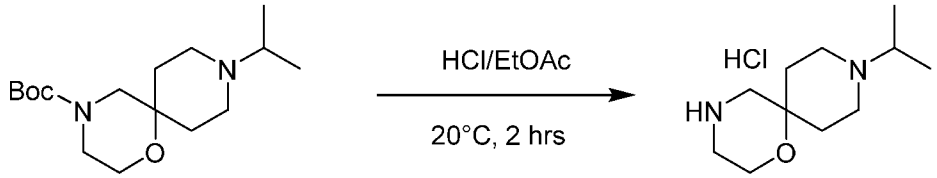
[0235] To a solution of Intermediate 27-4 (0.2 g, 380.44 μmol, 1 eq) in DCM (10 mL) was added BBr_3 (953.10 mg, 3.80 mmol, 366.58 μL, 10 eq) at -78°C dropwise under N_2. then the mixture was warmed up to 20°C and stirred 20°C for 12 hours under O_2 at 15 psi. LCMS showed the reaction worked well. The reaction was quenched by 2 mL of methanol at -78°C under N_2, then poured into ice water (30 mL) and extracted by diethyl ether (3 × 20 mL). The organic phases were combined and concentrated under reduced pressure to give the crude. The crude was purified by column chromatography (SiO_2, DCM: MeOH= 10:1) and prep-TLC (SiO_2, EtOAc: MeOH = 5:1) to give Compound 27 (5 mg, 11.44 μmol, 3.01% yield, 93.7% purity) as dark purple solid: **^{1}H NMR** (400 MHz, DMSO-d6) δ = 7.70 - 7.64 (m, 1H), 7.56 (d, J = 10.1 Hz, 1H), 7.25 - 7.17 (m, 2H), 6.77 - 6.64 (m, 2H), 3.77 (br d, J = 13.3 Hz, 2H), 3.68 - 3.58 (m, 2H), 3.38 - 3.33 (m, 2H), 2.62 - 2.55 (m, 1H), 2.43 - 2.37 (m, 2H), 2.29 (s, 2H), 2.00 - 1.86 (m, 2H), 1.65 - 1.51 (m, 2H), 0.94 (d, J = 6.5 Hz, 6H); **LCMS** (ESI+): m/z 410.7 $[M+H]^+$, Rt: 1.737 min, 5_95AB_6min-220-254-ELSD : LC/MS (The gradient was 5%B in 0.40min and 5-95% B in 2.60 min , hold on 95% B in 1.00 min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Luna C18 50*2.0mm column (5um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection .MS mode was positive electrospray ionization. MS range was 100-1000.)

Synthesis of 7-(9-isopropyl-1-oxa-4,9-diazaspiro[5.5]undecan-4-yl)-3H-phenothiazin-3-one (Compound 28)



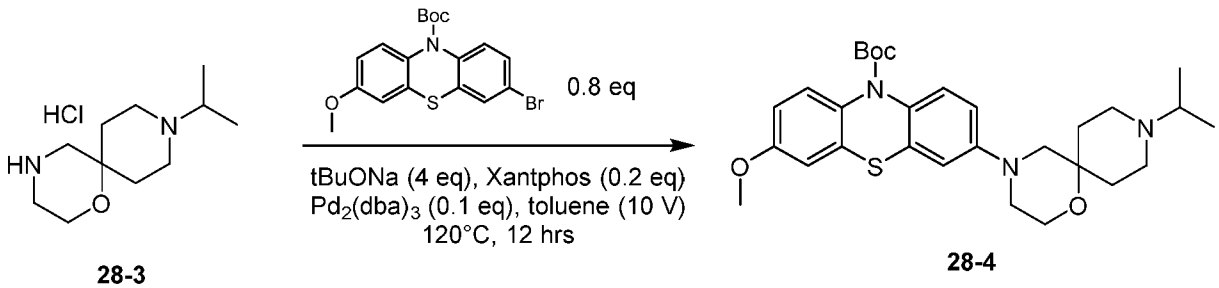
28-1 28-2

[0236] To a solution of Intermediate 28-1 (1.2 g, 4.10 mmol, 1 *eq*, HCl) in MeOH (15 mL) was added acetone (1.19 g, 20.49 mmol, 1.51 mL, 5.00 *eq*), HOAc (246.11 mg, 4.10 mmol, 234.62 µL, 1 *eq*) and NaOAc (1.01 g, 12.30 mmol, 3 *eq*) in order at 20°C. The mixture was stirred at 40°C for 1 hr. Then $NaBH_3CN$ (772.64 mg, 12.30 mmol, 3 *eq*) was added to the mixture at 20°C. The mixture was stirred at 40°C for 12 hours. LCMS showed the reaction worked well. The mixture was diluted with ethyl acetate (20 mL) and water (20 mL). The mixture was extracted with ethyl acetate (3 x 20 mL). The combined organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 28-2 (1.2 g, 4.02 mmol, 98.12% yield) as yellow gum: **^{1}H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 3.60-3.69 (m, 2H), 3.38-3.49 (m, 3H), 3.34 (s, 2H), 3.19 (br d, J = 10.4 Hz, 2H), 3.00-3.12 (m, 2H), 1.98-2.16 (m, 4H), 1.43-1.54 (m, 9H), 1.40 (d, J = 6.8 Hz, 6H).



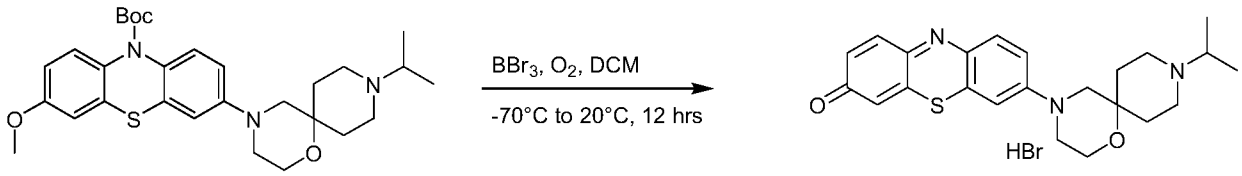
28-2 28-3

[0237] To a solution of Intermediate 28-2 (1.4 g, 4.69 mmol, 1 *eq*) in EtOAc (10 mL) was added HCl/EtOAc (4 M, 10 mL, 8.53 *eq*) at 20°C. The mixture was stirred at 20°C for 2 hours. LCMS showed the reaction worked well. The mixture was concentrated under reduced pressure to give Intermediate 28-3 (1.2 g, 4.42 mmol, 94.31% yield, 2HCl) as white solid: **1H NMR** (METHANOL-d4, 400 MHz) δ (ppm) 3.90-4.00 (m, 2H), 3.48-3.58 (m, 1H), 3.34-3.43 (m, 2H), 3.23 (br d, J = 4.9 Hz, 2H), 3.20 (s, 2H), 2.37 (br d, J = 14.3 Hz, 2H), 1.90-2.06 (m, 2H), 1.55 (s, 2H), 1.39 (d, J = 6.8 Hz, 6H).



28-3 28-4

[0238] To a solution of tert-butyl 3-bromo-7-methoxy-phenothiazine-10-carboxylate (0.3 g, 734.74 μmol, 1 *eq*) in toluene (15 mL) was added Intermediate 28-3 (298.92 mg, 1.10 mmol, 1.5 *eq*, 2HCl), t-BuONa (282.44 mg, 2.94 mmol, 4 *eq*), Xantphos (85.03 mg, 146.95 μmol, 0.2 *eq*) and $Pd_2(dba)_3$ (67.28 mg, 73.47 μmol, 0.1 *eq*) at 20°C under N_2. The mixture was stirred at 120°C for 12 hours. LCMS showed the reaction worked well. The mixture was diluted with ethyl acetate (10 mL) and water (10 mL). The mixture was extracted with ethyl acetate (3 x 10 ml). The combined organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the product. The crude product was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=0% to 100%, ethyl acetate/methanol=90%) to give Intermediate 28-4 (0.2 g, 380.44 μmol, 44.64% yield) as yellow solid: **^{1}H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 7.39 (dd, *J* = 8.7, 5.4 Hz, 2H), 6.73-6.91 (m, 4H), 3.75-3.85 (m, 5H), 3.24-3.35 (m, 1H), 3.06-3.12 (m, 2H), 2.96 (br s, 2H), 2.80-2.92 (m, 2H), 2.09-2.22 (m, 2H), 1.95-2.08 (m, 4H), 1.44-1.53 (m, 9H), 1.21-1.31 (m, 6H).

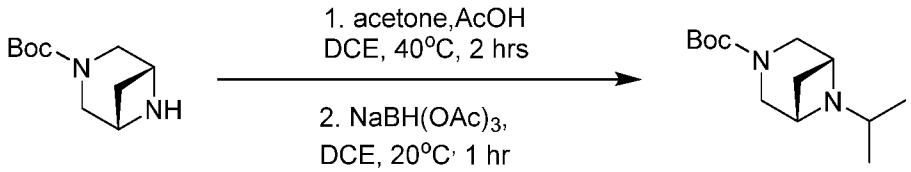


28-4 **Compound 28**

[0239] To a solution of Intermediate 28-4 (0.2 g, 380.44 μmol, 1 *eq*) in DCM (5 mL) was added BBr_3 (476.55 mg, 1.90 mmol, 183.29 μL, 5 *eq*) dropwise at -78°C under N_2. The mixture was stirred at 20°C for 12 hours under O_2 (15 psi). LCMS showed the reaction worked well. The mixture was quenched with methanol (5 mL) and then adjust to pH=8 with saturated sodium bicarbonate aqueous solution (15 mL). The mixture was extracted with dichloromethane (3 x 20 mL). The combined organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure to give the product. The residue was purified by column chromatography (SiO_2, DCM: MeOH = 95:5 to 90:10**)** and the desired eluent was concentrated under reduced pressure to give the crude. The crude was purified by prep-TLC (dichloromethane: methanol=2:1, Rf=0.24) and then triturated with ethyl acetate (4 mL) and filtered. The cake was collected and dried by high vacuum to give Compound 28 (5.1 mg, 11.83 μmol, 3.11% yield, 95% purity) as dark purple solid: **^{1}H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 11.97-12.19 (m, 1H), 7.76 (d, *J* = 9.1 Hz, 1H), 7.58 (d, *J* = 9.9 Hz, 1H), 6.96-7.07 (m, 1H), 6.88 (br d, *J* = 9.8 Hz, 1H), 6.70-6.82 (m, 2H), 3.80-3.93 (m, 2H), 3.33-3.53 (m, 5H), 3.26 (br d, *J* = 11.0 Hz, 2H), 3.01-3.16 (m, 2H), 2.54-2.69 (m, 2H), 2.20 (br d,

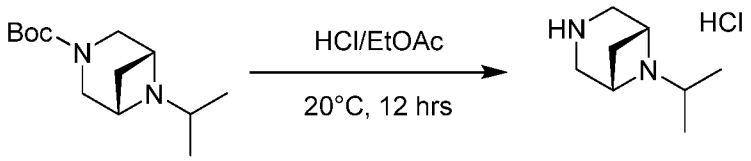
J = 13.8 Hz, 2H), 1.47 (br d, *J* = 6.6 Hz, 6H); **LCMS (ESI+)**: 1.634 min, m/z 380 (M+H), 5_95AB_6min-220-254-ELSD：LC/MS (The gradient was 5%B in 0.40min and 5-95% B in 2.60 min , hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Luna C18 50*2.0mm column (5um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection .MS mode was positive electrospray ionization. MS range was 100-1000.)

Synthesis of 7-(6-isopropyl-3,6-diazabicyclo[3.1.1]heptan-3-yl)-3H-phenothiazin-3-one (Compound 29)



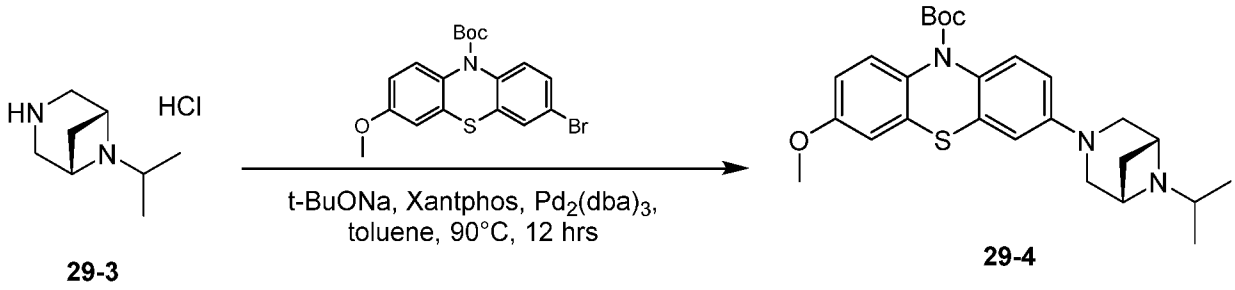
29-1 **29-2**

[0240] A mixture of Intermediate 29-1 (0.5 g, 1.84 mmol, 2HCl), acetone (214.17 mg, 3.69 mmol) and AcOH (55.36 mg, 921.89 μmol) in DCE (12 mL) was stirred at 40°C for 2 hrs. After cooling to 20°C, $NaBH(OAc)_3$ (781.54 mg, 3.69 mmol) was added into the reaction. The resulting mixture was stirred at 20°C for 1 hr. Additional reaction was set up as described above. The reaction mixtures were combined and purified. The combined reaction mixture was poured into saturated aqueous $NaHCO_3$ to adjust the pH~9. After partition, the aqueous phase was extracted with DCM (15 mL × 2). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (10 mL), brine (10 mL × 2), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give compound Intermediate 28-2 (0.8 g, 90.27% yield, 100% purity) which was was used into the next step without further purification: **^{1}H NMR:** 400 MHz, CHLOROFORM-d δ = 3.74 (s, 2H), 3.65 (br s, 1H), 3.58 (br dd, *J* = 7.4, 12.7 Hz, 2H), 3.40 - 3.29 (m, 2H), 2.80 - 2.70 (m, 1H), 2.54 (br d, *J* = 7.4 Hz, 1H), 1.50 (s, 9H), 0.98 (d, *J* = 6.0 Hz, 6H).



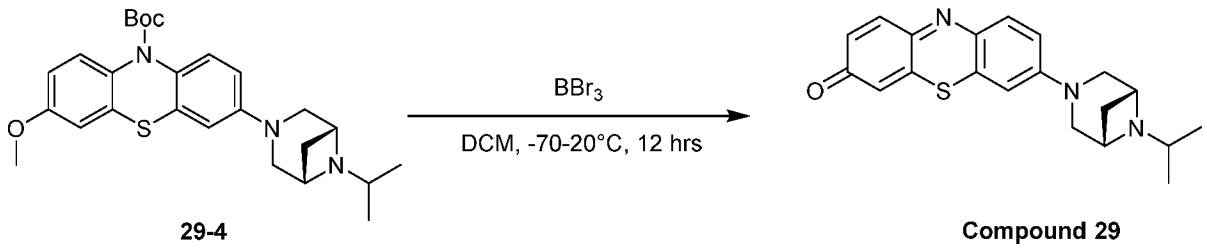
29-2 29-3

[0241] To a solution of Intermediate 29-2 (0.6 g, 2.50 mmol) in EtOAc (2 mL) was added HCl/EtOAc (4 M, 7.50 mL) at 0°C. The resulting mixture was stirred at 20°C for 12 hrs. The product was concentrated under reduced pressure to give Intermediate 29-3 (72.28% yield, 100% purity, 2 HCl) as a white solid: **^{1}H NMR:** 400 MHz, METHANOL-d_4 δ = 4.64 (br t, *J* = 6.0 Hz, 2H), 4.25 (d, *J* = 14.3 Hz, 1H), 4.01 - 3.91 (m, 3H), 3.88 (s, 1H), 3.78 - 3.68 (m, 1H), 3.44 (td, *J* = 6.5, 12.6 Hz, 1H), 3.22 - 3.06 (m, 1H), 2.36 - 2.22 (m, 1H), 1.42 (d, *J* = 6.5 Hz, 3H), 1.31 (d, *J* = 6.4 Hz, 3H).



29-3 29-4

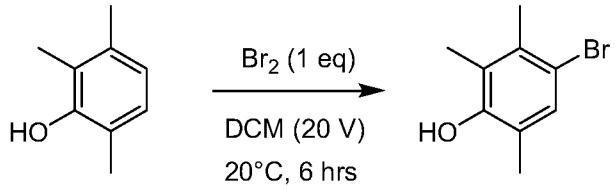
[0242] To a mixture of Intermediate 29-3 (65.25 mg, 306.14 μmol, 2 HCl) in toluene (5 mL) was added tert-butyl 3-bromo-7-methoxy-phenothiazine-10-carboxylate (0.1 g, 244.91 μmol), t-BuONa (117.68 mg, 1.22 mmol), Xantphos (35.43 mg, 61.23 μmol) and $Pd_2(dba)_3$ (28.03 mg, 30.61 μmol) at 20°C. The mixture was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 90°C for 12 hrs under N_2 atmosphere. LCMS showed the starting material was consumed, and 66% product with desired ms was detected. The reaction mixtures from ET72982-133,137 and ET72982-138 were combined and purified as details in ET72982-138. The reaction mixture was filtered and the filtrate was concentrated to give a crude. The crude product was purified by silica gel chromatography eluted with Dichloromethane: Methanol = 10 / 1 to 7 / 1 to give Intermediate 29-4 (180 mg, 30.18% yield, 60% purity) as a yellow solid: **^{1}H NMR:** 400 MHz, CHLOROFORM-d δ = 7.40 (br d, *J* = 8.8 Hz, 2H), 7.24 (br d, *J* = 7.8 Hz, 2H), 6.98 (br s, 1H), 6.87 (d, *J* = 2.7 Hz, 1H), 6.79 (dd, *J* = 2.8, 8.8 Hz, 1H), 3.85 (br d, *J* = 4.7 Hz, 2H), 3.79 (s, 3H), 3.52 (br d, *J* = 11.1 Hz, 2H), 3.50 (s, 3H), 3.28 (br d, *J* = 10.8 Hz, 2H), 2.60 (br d, *J* = 5.9 Hz, 2H), 1.49 (s, 9H), 0.96 (d, *J* = 5.9 Hz, 6H).



29-4 Compound 29

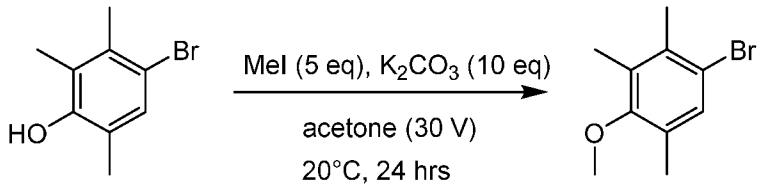
[0243] To a mixture of Intermediate 29-4 (0.08 g, 102.65 μmol) in DCM (1 mL) was added BBr_3 (514.31 mg, 2.05 mmol) at -70°C, and then the mixture was stirred at 20°C for 12 hrs under O_2. LCMS showed the starting material was consumed, and 31% product with desired ms was detected. The reaction was poured into cold MeOH (2 mL), then was concentrated under reduced pressure to give a residue. It was then diluted with DCM (10 mL), and poured into aqueous saturated $NaHCO_3$ (10 mL). The aqueous phase was then extracted with DCM (20 mL × 3). The combined organic layers were washed with brine (5 mL × 2), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Dichloromethane: Methanol = 15 / 1 to 10 / 1) to give crude product. It was purified by prep-TLC (SiO2, Dichloromethane: Methanol = 8 / 1) to give Compound 29 (30.5 mg, 38.42% yield, 97.7% purity) as a brown solid: **^{1}H NMR:** ET72982-144-P1B 400 MHz, CHLOROFORM-d δ = 7.77 (d, *J* = 9.1 Hz, 1H), 7.57 (d, *J* = 9.8 Hz, 1H), 6.92 (dd, *J* = 2.5, 9.3 Hz, 1H), 6.84 (dd, *J* = 1.9, 9.8 Hz, 1H), 6.76 - 6.58 (m, 2H), 3.89 (br d, *J* = 5.5 Hz, 2H), 3.69 (br d, *J* = 11.5 Hz, 2H), 3.43 (br d, *J* = 11.5 Hz, 2H), 2.75 - 2.58 (m, 2H), 1.60 (br d, *J* = 8.9 Hz, 1H), 1.02 (d, *J* = 6.0 Hz, 6H); **LCMS** (ESI+):1.649 min, m/z 352.1, 5_95AB_6min-220-254-ELSD : LC/MS (The gradient was 5%B in 0.40min and 5-95% B in 2.60 min , hold on 95% B in 1.00min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Kinetex C18 2.1*50mm,5um. Detection methods are diode array (DAD), and evaporative light scattering detection (ELSD) . MS mode was positive electrospray ionization.MS range was 100-1000.)

Synthesis of 7-((1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octan-3-yl)-1,2,4-trimethyl-3H-phenothiazin-3-one (Compound 30)



30-1 30-2

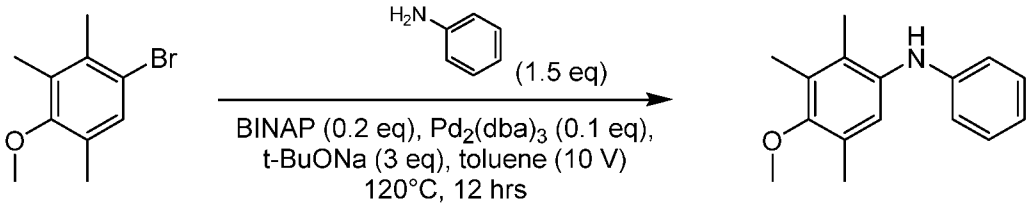
[0244] To a solution of Intermediate 30-1 18 g, 132.17 mmol, 1 eq) in DCM (260 mL) was added a solution of Br_2 (21.12 g, 132.17 mmol, 6.81 mL, 1 eq) in DCM (100 mL) dropwise at 20°C. The mixture was stirred at 20°C for 6 hrs. TLC (UV, petroleum ether: ethyl acetate = 5:1, R_f = 0.47) showed the reaction worked well, the starting material was consumed and one new spot was generated. The mixture was quenched with saturated Na_2SO_3 (aqueous, 500 mL). The layers was separated and the aqueous layer was extracted with ethyl acetate (2 x 300 mL). One additional vial in 5 g scale was dissolved in ethyl acetate (200 mL) and combined. The combined organic phase was washed with saturated $NaHCO_3$ (aqueous, 2 x 300 mL), brine (200 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to remove the solvent. Intermediate 30-2 (35 g, 162.73 mmol, 96.19% yield, 100% purity) was obtained as a light orange solid: **^{1}H NMR** (400 MHz, CHLOROFORM-d) δ ppm 7.20 (s, 1 H), 2.35 (s, 3 H), 2.22 (d, J=11.01 Hz, 6 H); **^{1}H NMR** (400 MHz, METHANOL-d4) δ ppm 7.10 (s, 1 H), 2.29 (s, 3 H), 2.17 (d, J=18.89 Hz, 6 H).



30-2 30-3

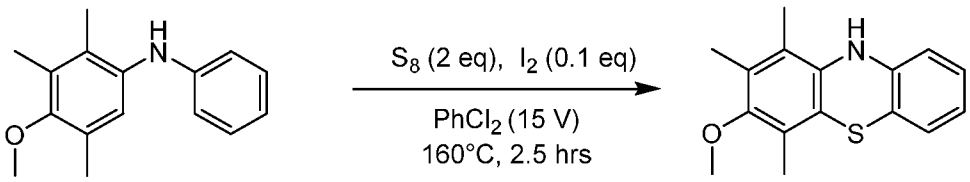
[0245] To a solution of Intermediate 30-2 (35 g, 162.73 mmol, 1 eq) in Acetone (350 mL) was added K_2CO_3 (67.47 g, 488.18 mmol, 3 eq) and then MeI (46.19 g, 325.45 mmol, 20.26 mL, 2 eq) dropwise at 20°C. The mixture was stirred at 20°C for 12 hrs. TLC (PMA, petroleum ether: ethyl acetate = 7:1, R_f = 0.79) showed about 50% of starting material was remained, then acetone (700 mL), K_2CO_3 (157.43 g, 1.14 mol, 7 eq) was added to the mixture at 20°C. Then MeI (69.29 g, 488.18 mmol, 30.39 mL, 3 eq) was added to above mixture dropwise at 20°C. The mixture was stirred for 12 hrs at 20°C. TLC showed the reaction worked well, the starting material was consumed, one new spot was generated. The mixture was filtered, the filtrate was concentrated under reduced pressure to remove acetone. Then the residue was diluted with ethyl acetate (300 mL) and water (500 mL). The layers was separated, the aqueous layer was extracted with ethyl acetate (2 x 300 mL). The combined

organic phases were washed with brine (300 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give compound Intermediate 30-3 (36.2 g, 150.10 mmol, 92.24% yield, 95% purity) as an orange oil: **^{1}H NMR** (400 MHz, CHLOROFORM-d) δ ppm 7.25 (s, 1 H), 3.68 (s, 3 H), 2.34 (s, 3 H), 2.25 (d, J=7.25 Hz, 6 H).



30-3 **30-4**

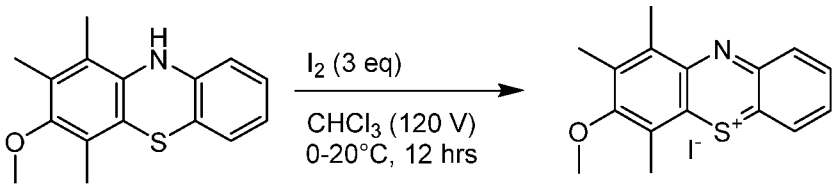
[0246] A mixture of Intermediate 30-3 (24 g, 99.51 mmol, 1 eq), ANILINE (13.90 g, 149.27 mmol, 13.60 mL, 1.5 eq), BINAP (12.39 g, 19.90 mmol, 0.2 eq), $Pd_2(dba)_3$ (9.11 g, 9.95 mmol, 0.1 eq) and t-BuONa (28.69 g, 298.54 mmol, 3 eq) in toluene (240 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 120°C for 12 hrs under N_2 atmosphere. One additional vial in 2 g scale and one additional vial in 10 g scale were set up as described above. These three reactions were combined and extracted with ethyl acetate (300 mL * 3). The combined organic layers were washed with brine 300 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 0-2%) to give Intermediate 30-4 (33.6 g, 139.23 mmol, 93.27% yield, 100% purity) as an orange oil: **^{1}H NMR** (400 MHz, METHANOL-d4) δ ppm 7.04 - 7.13 (m, 2 H), 6.84 (s, 1 H), 6.58 - 6.73 (m, 3 H), 3.68 (s, 3 H), 2.21 (d, J=5.25 Hz, 6 H), 2.08 (s, 3 H).



30-4 **30-5**

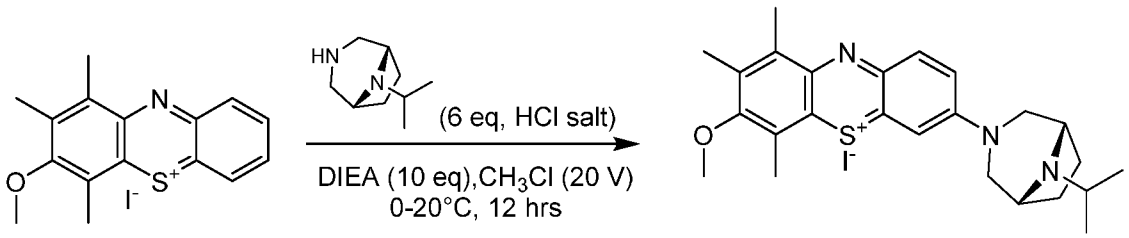
[0247] To a mixture of Intermediate 30-4 (13.8 g, 57.18 mmol, 1 eq) in 1,2-dichlorobenzene (200 mL) was added sublimed sulfur (3.67 g, 114.37 mmol, 2 eq) and I_2 (1.45 g, 5.72 mmol, 1.15 mL, 0.1 eq) in order at 20°C. Then the mixture was heated to 160°C and stirred for 2.5 hrs at 160°C. TLC showed the reaction worked, a new spot was detected, about 10% of 4-methoxy-2,3,5-trimethyl-N-phenyl-aniline remained. The reaction mixture was cooled to 20°C, one additional vial in 5 g scale and one additional vial in 13.8 g scale was set up as described. These three reactions were combined and diluted with

petroleum ether (1000 mL), filtered. The filter cake was washed with ethyl acetate (400 mL), the filtrate was concentrated under reduced pressure to give crude product. The crude product was purified by column chromatography (SiO_2, Petroleum ether in Ethyl acetate = 3%) to give Intermediate 30-5 (14.3 g, 50.06 mmol, 37.09% yield, 95% purity) as a light orange solid: **^{1}H NMR** (400 MHz, DMSO-d6) δ ppm 7.49 (s, 1 H), 6.92 - 7.03 (m, 3 H), 6.75 (ddd, J=7.88, 6.13, 2.25 Hz, 1 H), 3.50 (s, 3 H), 2.10 (d, J=2.63 Hz, 6 H), 2.07 (s, 3 H).



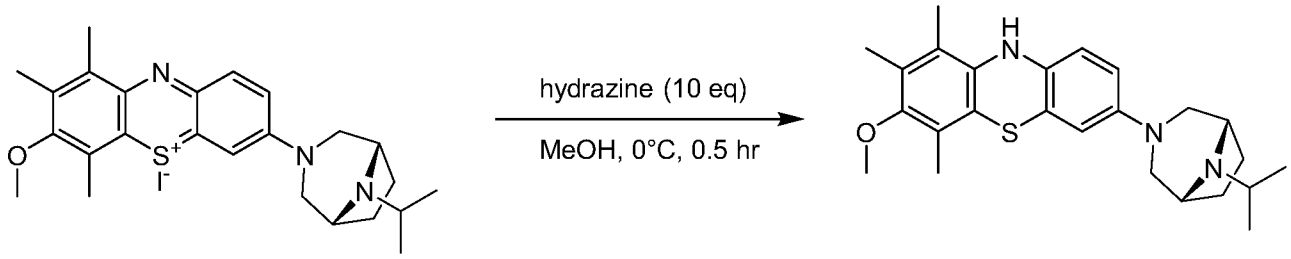
30-5 **30-6**

[0248] To a mixture of Intermediate 30-5 (1 g, 3.50 mmol, 1 *eq*) in $CHCl_3$ (10 mL) was added a solution of I_2 (2.67 g, 10.50 mmol, 2.12 mL, 3 *eq*) in $CHCl_3$ (100 mL) dropwise at 0°C under N_2. The mixture was stirred for 12 hrs at 20°C under N_2. The mixture was concentrated under reduced pressure to remove solvent and give a residue. The residue was triturated with methyl tert-butyl ether (30 mL) and dried in high vacuo. Intermediate 30-6 (3.17 g, crude) was obtained as dark-purple solid and used for next step directly.



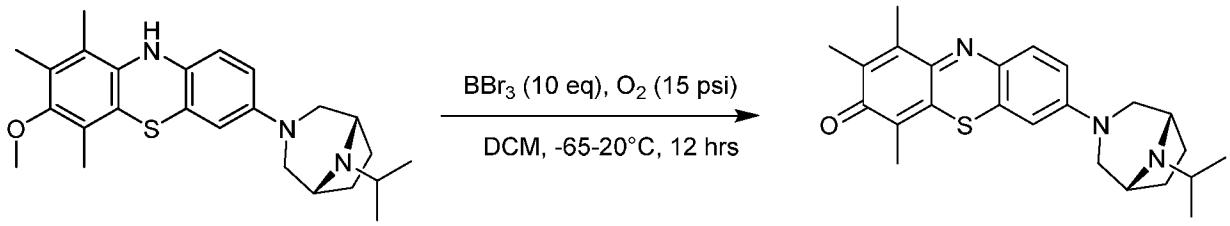
30-6 **30-7**

[0249] To a mixture of Intermediate 30-6 (1.2 g, 1.54 mmol, 1 *eq*) in $CHCl_3$ (12 mL) was added a solution of (1R,5S)-8-isopropyl-3,8-diazabicyclo[3.2.1]octane (2.47 g, 9.25 mmol, 6 *eq*, 2HCl salt) and DIEA (1.99 g, 15.42 mmol, 2.69 mL, 10 *eq*) in $CHCl_3$ (50 mL) dropwise at 0°C under N_2. The mixture was stirred for 12 hrs at 20°C under N_2. LCMS showed the reaction worked, one new peak with desired product was detected. The mixture was concentrated under reduced pressure to give a residue. The residue was triturated with tert-butyl methyl ether (15 mL), filtered. The filter cake was dried in high vacuo to give Intermediate 30-7 (4.7 g, crude) as a black-purple solid which was used for next step directly.



30-7 30-8

[0250] To a mixture of Intermediate 30-7 (4.7 g, 8.55 mmol, 1 *eq*) in MeOH (100 mL) was added $NH_2NH_2 \cdot H_2O$ (4.28 g, 85.53 mmol, 4.15 mL, 10 *eq*) dropwise at 0°C. Then the mixture was stirred for 30 min at 0°C. LCMS showed the starting material was consumed. The mixture was diluted with water (100 mL), extracted with ethyl acetate (2 x 50 mL). The combined organic phases were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give Intermediate 30-8 (600 mg, crude) as purple solid and used for next step directly.

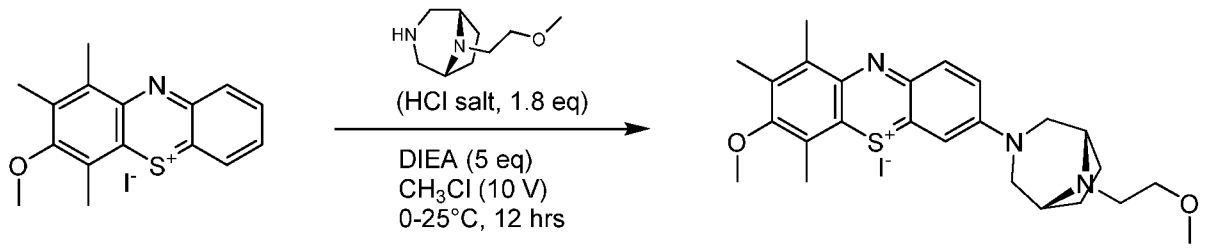


30-8 Compound 30

[0251] To a mixture of Intermediate 30-8 (500 mg, crude) in DCM (40 mL) was added BBr_3 (2.96 g, 11.80 mmol, 1.14 mL, 10 eq) dropwise at -65°C under N_2. Then the mixture was degassed and purged with O_2 for 3 times, and then the mixture was stirred at 20°C for 12 hrs under O_2 atmosphere (15 psi). LCMS showed the starting material was consumed. The mixture was added into ice-water (30 mL), adjusted pH to 8 with Na_2CO_3, extracted with DCM (3 x 30 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give crude product 0.5 g. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=100% to 0%), and then purified by prep-TLC for two times to give Compound 30 (78.5 mg, 192.60 μmol, 15.70% yield) as dark purple solid: **^{1}H NMR** (CHLOROFORM-d, 400 MHz) δ (ppm) 7.73 (d, J = 9.1 Hz, 1H), 6.90 (dd, J = 9.1, 2.9 Hz, 1H), 6.77 (d, J = 2.6 Hz, 1H), 3.70 (br s, 2H), 3.44 (br d, J = 11.0 Hz, 2H), 3.26 (br d, J = 9.1 Hz, 2H), 2.65-2.76 (m, 1H), 2.50 (s, 3H), 2.21 (s, 3H), 2.13 (s, 3H), 2.00 (br d, J = 3.9 Hz, 2H), 1.76 (br d, J = 6.8 Hz, 2H), 1.16 (br d, J = 4.3 Hz, 6H); **LCMS (ESI+)**: 2.346 min, m/z 408 (M+H), 5_95AB_6min-220-254 : LC/MS (The gradient was 5%B in 0.40min and 5-95% B in 2.60 min, hold on 95% B in 1.00min, and then

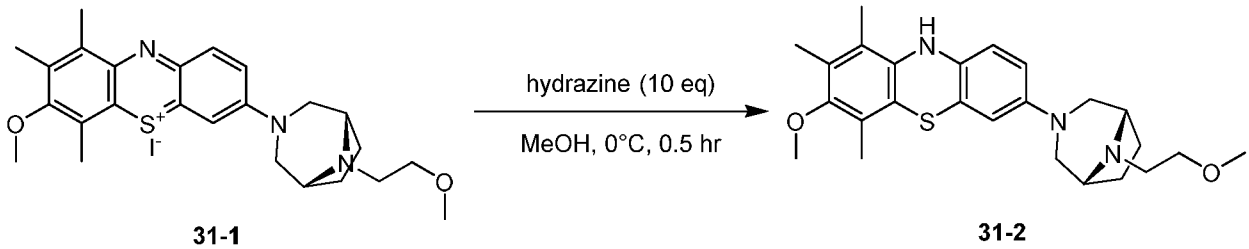
95-5%B in 0.01min, the flow rate was 1.0 ml/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Luna C18 50*2.0mm column (5um particles). Detection methods are diode array (DAD) detection .MS mode was positive electrospray ionization. MS range was 100-1000.)

Synthesis of 7-((1R,5S)-8-(2-methoxyethyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-1,2,4-trimethyl-3H-phenothiazin-3-one (Compound 31)



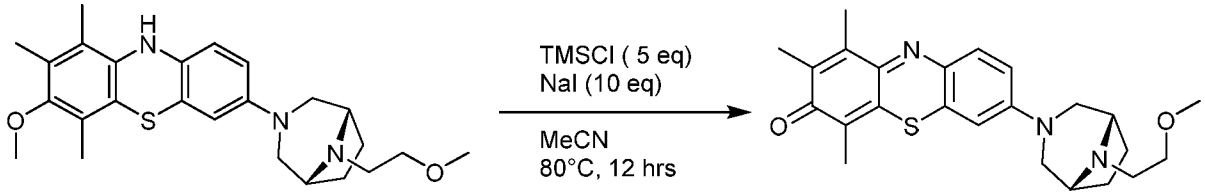
30-6 31-1

[0252] To a solution of Intermediate 30-6 (2 g, 2.57 mmol, 1 *eq*) in $CHCl_3$ (20 mL) was added a solution of DIEA (1.66 g, 12.85 mmol, 2.24 mL, 5 *eq*) and (1R,5S)-8-(2-methoxyethyl)-3,8-diazabicyclo[3.2.1]octane (1.41 g, 4.63 mmol, 1.8 *eq*, 2HCl) in $CHCl_3$ (20 mL) dropwise at 0°C. The mixture was stirred at 25 °C for 12 hrs. LCMS showed the starting material was consumed. One additional vial in 0.3 g scale was set up as described above and concentrated under reduced pressure to give the crude product. The residue was washed with methyl tert-butyl ether 50 mL, filtered and the filter cake was dried in high vacuo to give crude Intermediate 31-1 (6 g, crude) as dark-purple solid and used for next step directly: **LCMS (ESI+)**: 0.348 min, m/z 438 (M+), 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm,5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.)



31-1 31-2

[0253] To a mixture of Intermediate 31-1 (6 g, 10.61 mmol, 1 *eq*) in MeOH (120 mL) was added $NH_2NH_2 \cdot H_2O$ (5.31 g, 106.10 mmol, 5.15 mL, 10 *eq*) dropwise at 0°C. Then the mixture was stirred for 30 min at 0°C. LCMS showed the starting material was consumed. The mixture was diluted with water (200 mL), extracted with ethyl acetate (3 x 100 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Ethyl acetate in Petroleum ether = 0-100%) to give crude Intermediate 31-2 (0.66 g, crude) as purple solid: **LCMS (ESI+)**: 0.451 min, m/z 440 (M+H), 10-100AB_1MIN: LC/MS (The column used for chromatography was a Halo C18 3.0*30mm,5um. Detection methods are diode array (DAD). MS mode was positive electrospray ionization. MS range was 50-2000. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade acetonitrile. The gradient was 10-100% B in 0.90 min .10% B in 0.01 min, 10-100% B (0.01-0.50 min) with a hold at 100% B for 0.40 min. The flow rate was 2.0 mL/min.)

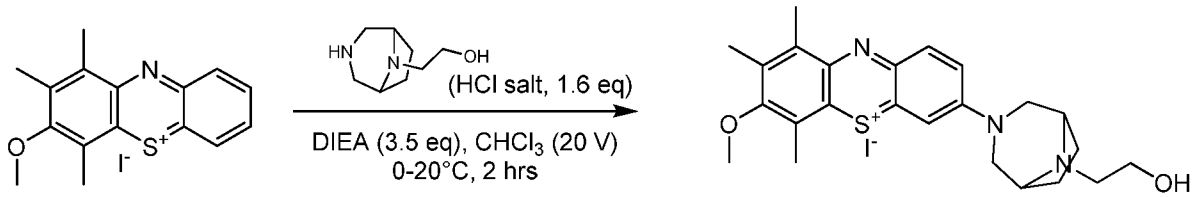


31-2 **Compound 31**

[0254] To a mixture of Intermediate 31-2 (0.66 g, 1.50 mmol, 1 eq) in MeCN (20 mL) was added TMSCl (815.53 mg, 7.51 mmol, 952.73 µL, 5 eq) and NaI (2.25 g, 15.01 mmol, 10 eq) at 25°C under N_2. The mixture was changed with N_2 for 3 times. Then the mixture was stirred at 80°C for 12 hrs under N_2. The mixture was added into water (60 mL), adjusted pH to 8 with Na_2CO_3, extracted with DCM (3 x 20 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography for twice (SiO_2, Ethyl acetate in Petroleum ether = 0 to 100%, MeOH in DCM = 0-10%) to give crude product. The crude product was triturated with ethyl acetate: MeOH = 20: 1 (20 mL), filtered and the filter cake was dried in high vacuo to give Compound 31 (98 mg, 226.05 µmol, 67.83% yield, 97.7% purity) as a brick-red solid: **^{1}H NMR** (400 MHz, DMSO-d6) δ ppm 7.66 (d, J=9.01 Hz, 1 H), 7.02 - 7.12 (m, 2 H), 3.61 (br d, J=11.63 Hz, 2 H), 3.47 (br t, J=5.94 Hz, 2 H), 3.43 (br s, 2 H), 3.27 (s, 3 H), 3.05 (br d, J=10.88 Hz, 2 H), 2.56 (br s, 2 H), 2.41 (s, 3 H), 2.07 (s, 3 H), 1.99 (s, 3 H), 1.86 - 1.95 (m, 2 H), 1.59 (br d, J=7.13 Hz, 2 H); **LCMS (ESI+)**: 2.253 min, m/z 424 (M+H),

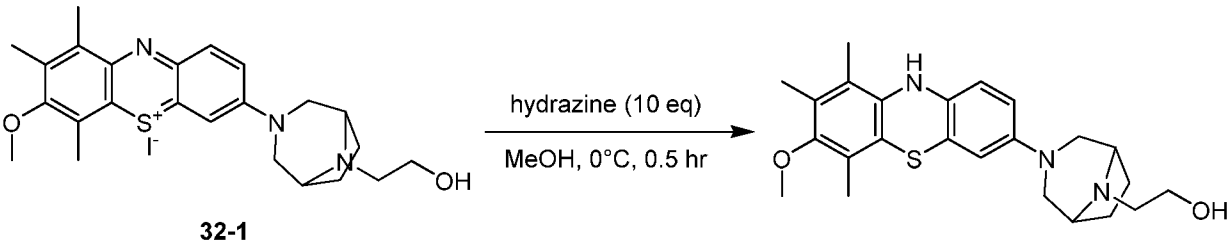
5_95AB_6 min-220-254-ELSD : LC/MS (The gradient was 5% B in 0.40 min and 5-95% B in 2.60 min, hold on 95% B in 1.00 min, and then 95-5% B in 0.01 min, the flow rate was 1.0 mL/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Kinetex C18 2.1*50 mm, 5 um. Detection methods are diode array (DAD), and evaporative light scattering detection (ELSD). MS mode was positive electrospray ionization. MS range was 100-1000.)

Synthesis of 7-((1R,5S)-8-(2-hydroxyethyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-1,2,4-trimethyl-3H-phenothiazin-3-one (Compound 32)



30-6 32-1

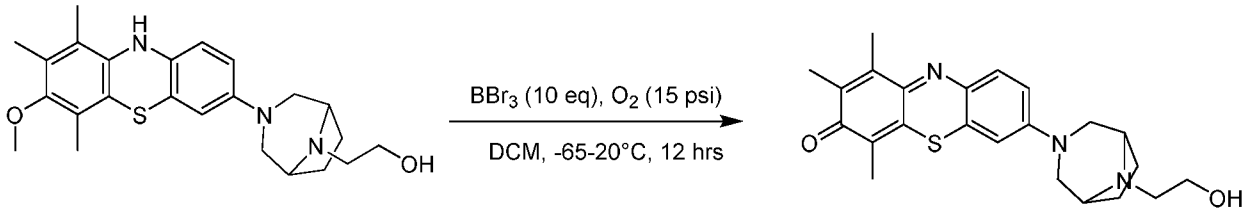
[0255] To a solution of Intermediate 30-6 (1.3 g, 1.67 mmol, 1 *eq*) in $CHCl_3$ (13 mL) was added a solution of DIEA (755.85 mg, 5.85 mmol, 1.02 mL, 3.5 *eq*) and 2-(3,8-diazabicyclo[3.2.1]octan-8-yl)ethanol (612.64 mg, 2.67 mmol, 1.6 *eq*, 2HCl salt) in $CHCl_3$ (13 mL) dropwise at 0°C. The mixture was stirred for 2 hrs at 20°C. The mixture was concentrated under reduced pressure to give a residue. The residue was triturated with methyl tert-butyl ether 15 mL, filtered. The filter cake was dried in high vacuo to give Intermediate 32-1 (2.4 g, crude) as a dark-purple solid.



32-1 32-2

[0256] To a solution of Intermediate 32-1 (3.4 g, 6.17 mmol, 1 *eq*) in MeOH (70 mL) was added $NH_2NH_2 \cdot H_2O$ (3.09 g, 61.65 mmol, 2.99 mL, 10 *eq*) dropwise at 0°C. The mixture was stirred for 30 min at 0°C. The mixture was diluted with water (150 mL), extracted with ethyl acetate (3 x 50 mL). The combined organic phases were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. Crude

product Intermediate 32-2 (720 mg, 1.69 mmol, 27.44% yield) was obtained as dark-purple solid and used for next step directly: **^{1}H NMR** (400 MHz, METHANOL-d4) δ ppm 6.73 (br d, J=7.63 Hz, 1 H), 6.45 - 6.59 (m, 2 H), 3.70 (t, J=6.13 Hz, 2 H), 3.56 - 3.60 (m, 3 H), 3.41 (br s, 2 H), 3.24 (br d, J=11.13 Hz, 2 H), 2.83 - 2.95 (m, 2 H), 2.60 (t, J=6.13 Hz, 2 H), 2.09 - 2.19 (m, 10 H), 1.99 (br d, J=5.75 Hz, 1 H), 1.77 - 1.83 (m, 2 H).

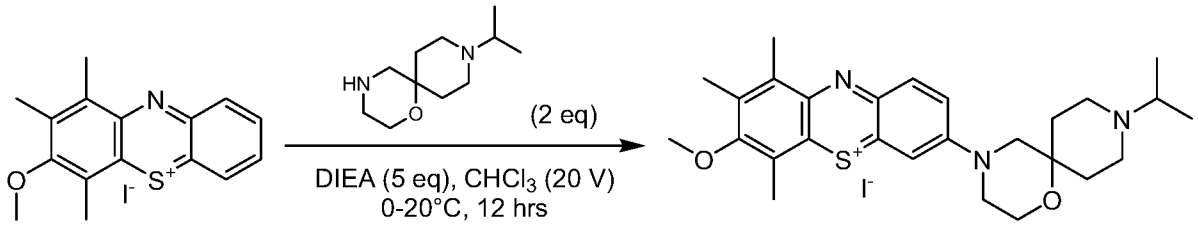


32-2

Compound 32

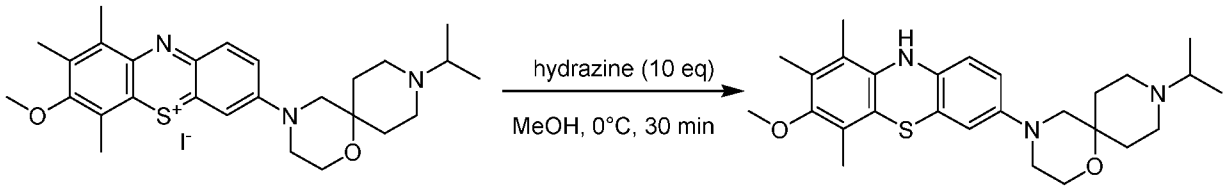
[0257] To a solution of Intermediate 32-2 (620 mg, 1.46 mmol, 1 eq) in DCM (20 mL) was added BBr_3 (3.65 g, 14.57 mmol, 1.40 mL, 10 eq) dropwise at -65°C under N_2. Then the mixture was degassed and purged with O_2 for 3 times, and then the mixture was stirred at 20°C for 12 hrs under O_2 atmosphere (15 psi). One additional vial in 100 mg scale was set up as described above. These two reactions were combined and added into ice-water (100 mL), adjusted pH>8 with Na_2CO_3, extracted with DCM (3 x 20 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 0-100%) and DCM: MeOH = 5-15%) and give a crude product, which was triturated with ethyl acetate 20 mL. Compound 32 (166 mg, 389.12 μmol, 23.03% yield, 96% purity) was obtained as a brick red solid: **^{1}H NMR** (400 MHz, METHANOL-d4) δ ppm 7.67 (d, J=9.13 Hz, 1 H), 7.05 (dd, J=9.07, 2.69 Hz, 1 H), 6.96 (d, J=2.50 Hz, 1 H), 3.74 (t, J=5.82 Hz, 2 H), 3.65 (br d, J=10.88 Hz, 2 H), 3.56 (br s, 2 H), 3.22 (br d, J=10.51 Hz, 2 H), 2.67 (t, J=5.82 Hz, 2 H), 2.46 (s, 3 H), 2.12 (s, 3 H), 2.06 - 2.11 (m, 2 H), 2.03 (s, 3 H), 1.72 - 1.84 (m, 2 H); **LCMS (ESI+)**: 2.149 min, m/z 410 (M+H), 5_95AB_6 min-220-254-ELSD : LC/MS (The gradient was 5% B in 0.40 min and 5-95% B in 2.60 min, hold on 95% B in 1.00 min, and then 95-5% B in 0.01 min, the flow rate was 1.0 mL/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Kinetex C18 2.1*50 mm, 5 um. Detection methods are diode array (DAD), and evaporative light scattering detection (ELSD). MS mode was positive electrospray ionization. MS range was 100-1000.)

Synthesis of 7-(9-isopropyl-1-oxa-4,9-diazaspiro[5.5]undecan-4-yl)-1,2,4-trimethyl-3H-phenothiazin-3-one (Compound 33)



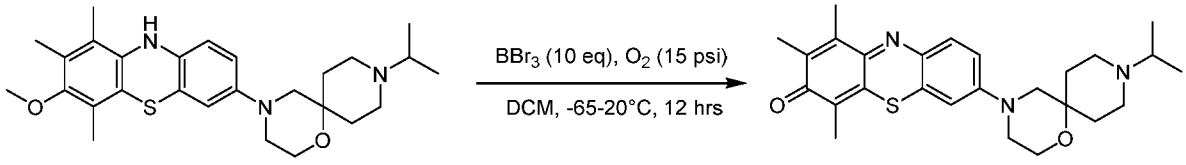
30-6 33-1

[0258] To a solution of Intermediate 30-6 (2 g, 2.57 mmol, 1 *eq*) in $CHCl_2$ (20 mL) was added a solution of 9-isopropyl-1-oxa-4,9-diazaspiro[5.5]undecane (1.02 g, 5.14 mmol, 2 *eq*) and DIEA (1.66 g, 12.85 mmol, 2.24 mL, 5 *eq*) in $CHCl_3$ (20 mL) dropwise at 0°C. The mixture was stirred for 12 hrs at 20°C. LCMS showed the reaction worked well. The mixture was concentrated under reduced pressure to give a residue. The residue was triturated with methyl tert-butyl ether (35 mL), filtered and the filter cake was dried in high vacuo to give Intermediate 33-1 (4.8 g, crude) as a dark solid.



33-1 33-2

[0259] To a solution of Intermediate 33-1 (4.8 g, crude) in MeOH (96 mL) was added $NH_2NH_2 \cdot H_2O$ (4.05 g, 80.87 mmol, 3.92 mL, 10 *eq*) dropwise at 0°C. The mixture was stirred for 30 min at 0°C. One addition vial in 0.6 g scale was set up as described above. The combined mixtures were diluted with water (200 mL), extracted with ethyl acetate (3 x 100 mL). The combined organic phases were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue, which was combined and purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 0-100% and then DCM: MeOH = 0-15%) to give Intermediate 33-2 (500 mg, 1.07 mmol) as a dark-purple solid: **^{1}H NMR** (400 MHz, METHANOL-d4) δ ppm 6.77 (d, J=8.25 Hz, 1 H), 6.59 - 6.66 (m, 2 H), 3.80 - 3.84 (m, 2 H), 3.58 (s, 3 H), 3.38 - 3.45 (m, 6 H), 3.29 (br s, 3 H), 2.99 - 3.05 (m, 1 H), 2.89 - 2.97 (m, 4 H), 1.90 (br d, J=13.38 Hz, 6 H), 1.68 - 1.75 (m, 2 H), 1.20 (d, J=6.63 Hz, 6 H).



33-2 Compound 33

[0260] To a mixture of Intermediate 33-2 (440 mg, 940.84 μmol, 1 *eq*) in DCM (20 mL) was added BBr_3 (2.36 g, 9.41 mmol, 906.55 μL, 10 *eq*) dropwise at -65°C under N_2. Then the mixture was degassed and purged with O_2 for 3 times, and then the mixture was stirred at 20°C for 12 hrs under O_2 atmosphere (15 Psi). LCMS showed the reaction worked well. The mixture was added into ice-water (100 mL) dropwise, adjusted pH > 8 with Na_2CO_3. The mixture was extracted with DCM (3 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 0-100%, DCM: MeOH = 0-15%). Then the crude product was triturated with ethyl acetate: DCM: MeOH = 10 mL: 1 mL: 0.5 mL for 20 min. Then the mixture was filtered and the filter cake was dried in high vacuo to give crude product (100 mg, 205.04 μmol, 92.6% purity) as a dark-purple solid. Then the solid (100 mg, 205.04 μmol, 1 *eq*) was dissolved in EtOAc (10 mL), DCM (2 mL) and MeOH (2 mL) and stirred for 1 hr at 20°C. The mixture was filtered, the filter cake was dried in high vacuo to give Compound 33 (48 mg, 101.39 μmol, 49.45% yield, 95.4% purity) as a brick-red solid: **^{1}H NMR** (400 MHz, METHANOL-d4) δ ppm 7.67 (d, J=9.01 Hz, 1 H), 7.12 (dd, J=8.94, 2.44 Hz, 1 H), 7.05 (d, J=2.25 Hz, 1 H), 3.92 (br t, J=4.88 Hz, 2 H), 3.52 - 3.60 (m, 1 H), 3.43 - 3.49 (m, 2 H), 3.40 (s, 2 H), 3.33 - 3.38 (m, 2 H), 3.19 - 3.29 (m, 2 H), 2.44 (s, 3 H), 2.34 (br d, J=12.63 Hz, 2 H), 2.10 (s, 3 H), 2.00 (s, 3 H), 1.83 - 1.97 (m, 2 H), 1.40 (d, J=6.63 Hz, 6 H); **LCMS (ESI+)**: 2.335 min, m/z 452 (M+H), 5_95AB_6 min-220-254-ELSD : LC/MS (The gradient was 5% B in 0.40 min and 5-95% B in 2.60 min, hold on 95% B in 1.00 min, and then 95-5% B in 0.01 min, the flow rate was 1.0 mL/min. Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile. The column used for chromatography was a Kinetex C18 2.1*50 mm, 5 um. Detection methods are diode array (DAD), and evaporative light scattering detection (ELSD). MS mode was positive electrospray ionization. MS range was 100-1000.)

Example 2. Biological Data

[0261] Human fibroblast survival assay. All fibroblasts were obtained from the National Institute of Neurological Disorders and Stroke (NINDS) cell line repository housed at Rutgers University. Compounds were tested in at least one of the following Parkinson's disease subject-derived fibroblast lines: Line ND29802 (LRRK2 G2019S mutation carrier), Line ND40996 (SNCA A53T mutation carrier), or Line ND34263 (GBA N370S mutation carrier).

[0262] Human fibroblasts were seeded into 96-well plates on Day 0. On Day 1, fibroblasts were challenged with RSL3 (a ferroptosis activator, CAS No. 1219810-16-8) alone or in co-treatment with test compound administered in dose response. On Day 2, fibroblasts were fixed and stained by the nuclear stain 4', 6-diamidino-2-phenylindole (DAPI), and the number of DAPI-positive nuclei were counted for each condition using the Yokogawa CQ1 imaging platform to quantify the EC_{50} for cell survival. Exemplary results are shown in Table 3 below.

Table 3. Compound Activity

Compound	EC_{50} (nM)
1	2.2
2	1.4
3	0.9
4	2.9
5	1.2

[0263] Alternatively, compounds were tested in the following survival assay. Human fibroblasts were maintained and passaged with standard protocols used by the field. Fibroblasts are split and plated at 3K / well in 96-well culture plates. After 24 hours, cells were treated with either DMSO (vehicle), 75nM RSL3 (challenge), or 75nM RSL3 with 100nM, 33nM, or 5nM doses of each compound. Each condition was performed in quadruplicate wells. After 24 hours, images of the cells were captured using the Sartorius IncuCyte SX5 imaging system and cell confluency was calculated using the built-in confluency calculation software. To determine % Cell Viability Rescue, the DMSO (vehicle) cell confluency was first set to 100% and the 75nM RSL3 (challenge) cell confluency was set

to 0%. The data shown represents the cell confluency of each condition (the average of quadruplicate wells) normalized to that scale.

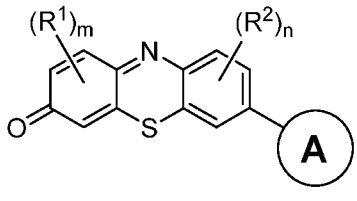
Table 4. Cell Viability of Human Fibroblasts with Compound after RSL3 Challenge

% Cell Viability Rescue from RSL3 Challenge			
Compound	**100nM**	**33nM**	**5nM**
6	90	92	0
8	92	90	91
9	89	90	0
10	89	91	55
11	90	92	42
12	91	90	28
13	91	92	0
14	92	86	31
15	92	86	43
16	91	87	62
17	91	91	47
19	93	75	27
20	97	100	48
21	91	69	29
22	91	94	0
23	93	96	54
28	90	91	83
30	71	0	0
31	98	103	0
32	90	114	42
33	91	109	19

[0264] Although the foregoing invention has been described in some detail by way of illustration and Example for purposes of clarity of understanding, one of skill in the art will appreciate that certain changes and modifications may be practiced within the scope of the appended claims. In addition, each reference provided herein is incorporated by reference in its entirety to the same extent as if each reference was individually incorporated by reference. Where a conflict exists between the instant application and a reference provided herein, the instant application shall dominate.

WHAT IS CLAIMED IS:

1. A compound of Formula I:



(I),

or a pharmaceutically acceptable salt thereof,

wherein

each R^1 is independently -F, -Cl, -Br, -I, -OR^a, -SR^a, -NR^aR^b, -NO_2, -CN, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl, wherein the alkyl, alkenyl, or alkynyl is substituted with 0, 1, 2, or 3 groups independently selected from -F, -Cl, -Br, -I, -OR^a, -SR^a, -NR^aR^b, oxo, -NO_2, and -CN;

each R^2 is independently -F, -Cl, -Br, -I, -OH, -OR^a, -SR^a, -NR^aR^b, -CN, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl, wherein the alkyl, alkenyl, or alkynyl is substituted with 0, 1, 2, or 3 groups independently selected from -F, -Cl, -Br, -I, -OR^a, -SR^a, -NR^aR^b, oxo, -NO_2, and -CN;

each R^a is independently H or C_{1-6} alkyl;

each R^b is independently H or C_{1-6} alkyl;

the subscript m is 0, 1, 2 or 3;

the subscript n is 0, 1, 2, or 3; and

is a bicyclic nitrogen-containing heterocyclic ring.

2. The compound of claim 1, or pharmaceutically acceptable salt thereof, wherein each R^1 is independently -F, -Cl, -Br, -I, -OR^a, -SR^a, -NR^aR^b, -CN, or C_{1-6} alkyl.

3. The compound of claim 1 or 2, or pharmaceutically acceptable salt thereof, wherein each R^2 is independently -F, -Cl, -Br, -I, -OR^a, -SR^a, -NR^aR^b, -CN, or C_{1-6} alkyl.

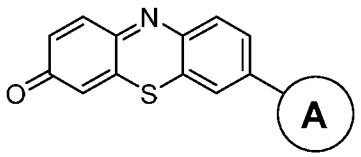
4. The compound of any one of claims 1 to 3, or pharmaceutically acceptable salt thereof, wherein each R^a is independently H or C_{1-3} alkyl.

5. The compound of any one of claims 1 to 4, or pharmaceutically acceptable salt thereof, wherein each R^b is independently H or C_{1-3} alkyl.

6. The compound of any one of claims 1 to 5, or pharmaceutically acceptable salt thereof, wherein the subscript m is 0 or 1.

7. The compound of any one of claims 1 to 6, or pharmaceutically acceptable salt thereof, wherein the subscript n is 0 or 1.

8. The compound of any one of claims 1 to 7, or pharmaceutically acceptable salt thereof, having the structure of Formula II:



(II).

9. The compound of any one of claims 1 to 8, or pharmaceutically acceptable salt thereof, wherein

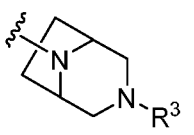
is a bridged bicyclic nitrogen-containing heterocyclic ring.

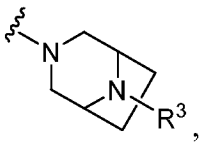
10. The compound of any one of claims 1 to 9, or pharmaceutically acceptable salt thereof, wherein

is attached to the phenothiazine through a nitrogen.

11. The compound of any one of claims 1 to 10, or pharmaceutically acceptable salt thereof, wherein

has the structure:



or


,

wherein

R^3 is H, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl, C_{3-8} cycloalkyl, 4- to 8-membered heterocyclyl, phenyl, or 5- to 10-membered heteroaryl, wherein the alkyl,

alkenyl, alkynyl, cycloalkyl, heterocyclyl, phenyl, or heteroaryl is substituted with 0, 1, 2, or 3 groups independently selected from -F, -Cl, -Br, -I, -OR^{3a}, -SR^{3a}, -$NR^{3a}R^{3b}$, oxo, -NO_2, and –CN;

each R^{3a} is independently H or C_{1-6} alkyl; and

each R^{3b} is independently H or C_{1-6} alkyl.

12. The compound of any one of claims 1 to 11, or pharmaceutically acceptable salt thereof, wherein the compound has a structure as shown in Table 1.

13. A pharmaceutical composition comprising a compound of any one of claims 1 to 12, or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

14. A method of inhibiting ferroptosis in a cell, comprising administering to the cell an effective amount of a compound of any one of claims 1 to 12, or pharmaceutically acceptable salt thereof, or a pharmaceutical composition of claim 13.

15. A method of treating a mitochondrial disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a compound of any one of claims 1 to 12, or pharmaceutically acceptable salt thereof, or a pharmaceutical composition of claim 13.

16. The method of claim 15, wherein the mitochondrial disease is Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, Friedreich's ataxia (FRDA), Leber's Hereditary Optic Neuropathy (LHON), mitochondrial myopathy, encephalopathy, lactacidosis, and stroke (MELAS), Myoclonus Epilepsy Associated with Ragged-Red Fibers (MERRF) syndrome, Maternally Inherited Diabetes and Deafness (MIDD), or a respiratory chain disorder.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/73312

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

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

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

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

3. ☒ Claims Nos.: 4-16
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/73312

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61K 31/395, A61K 31/425, A61K 31/407 (2023.01)
ADD. A61K 31/33 (2023.01)

CPC - INV. A61K 31/395, A61K 31/425, A61K 31/407

ADD. A61K 31/33

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)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/0238543 A1 (HURT, et.al.) 20 September 2012 (20.09.2012) para [0066];[0072]	1-3
A	US 3,885,034 A (RISSE, et.al.) 20 May 1975 (20.05.1975) ENTIRE DOCUMENT	1-3
A	Cignarella G., et.al. Bicyclic homologs of piperazine. IX. Synthesis and pharmacological properties of phenothiazine and of 10,11-dihydrodibenzocycloheptene derivatives of 3,8-diazabicyclo[3.2.1]octanes in J Med Chem. 1969, Vol. 12(5), pp. 836-839. ENTIRE DOCUMENT	1-3

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

Date of the actual completion of the international search	Date of mailing of the international search report
31 OCTOBER 2023 (31.10.2023)	JAN 04 2024
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300