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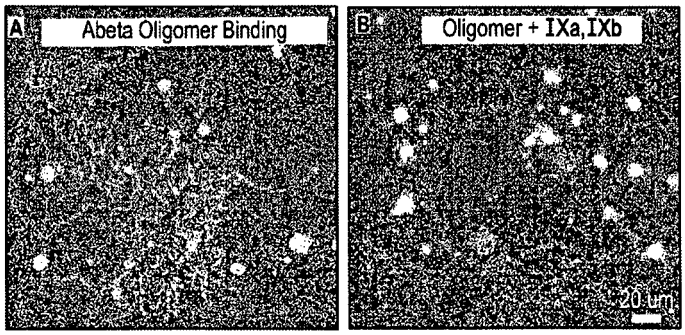


FIG. 3B

(57) Abstract: This invention relates to novel diarylamino compounds that bind to the sigma-2 receptor, to pharmaceutical compositions comprising such compounds, and to methods for inhibiting or restoring synapse loss in neuronal cells, modulating a membrane trafficking change in neuronal cells, and treating cognitive decline and neurodegenerative diseases and disorders therewith.

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COMPOSITIONS AND METHODS FOR TREATING NEURODEGENERATIVE DISEASE

[001] This application is being filed on 27 August 2012, as a PCT International Patent application in the name of Cognition Therapeutics, Inc., a U.S. national corporation, applicant for the designation of all countries except the US, and Susan M. Catalano, Gilbert Rishton and Nicholas J. Izzo, Jr., citizens of the U.S., applicants for the designation of the US only, and claims priority to U.S. Provisional Patent Application Serial No. 61/527,584, filed August 25, 2011, which application is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[002] This invention relates to novel diarylamino compounds that bind to the sigma-2 receptor, to pharmaceutical compositions comprising such compounds, and to methods for inhibiting or restoring synapse loss in neuronal cells, modulating a membrane trafficking change in neuronal cells, and treating cognitive decline and neurodegenerative diseases and disorders therewith.

BACKGROUND OF THE INVENTION

[003] There are five medications currently FDA-approved for the treatment of AD. Four are cholinesterase inhibitors: tacrine (Cognex®; Sciele), donepezil (Aricept®; Pfizer), rivastigmine (Exelon®; Novartis), and galantamine (Razadyne®; Ortho-McNeil-Janssen). Donepezil, rivastigmine, and galantamine are successors to tacrine, a first generation compound rarely prescribed because of the potential for hepatotoxicity; they are roughly equally efficacious at providing symptomatic improvement of cognition and function at all stages of AD. The fifth approved medication is memantine (Namenda®; Forest), a low-affinity, use dependent *N*-methyl-D-aspartate glutamate receptor antagonist that offers similar benefits, but only in moderate to severe AD. The clinical effects of these compounds are small and impermanent, and there are currently no conclusive data to support their use as disease modifying agents. See, e.g., Kerchner et al, 2010, *Bapineuzumab*, Expert Opin Biol Ther., 10(7):1121-1130. Clearly, alternative approaches to treatment of AD are required.

[004] Human amyloid beta (Abeta OR Aβ) is the main component of insoluble amyloid plaques-deposits found in the brain of patients with Alzheimer's disease. The plaques are composed of fibrillar aggregates of Abeta. Amyloid beta fibrils have been associated with the advanced stages of Alzheimer's disease.

[005] The cognitive hallmark of early Alzheimer's disease (AD) is an extraordinary inability to form new memories. Early memory loss is considered a synapse failure caused by soluble Aβ oligomers. These oligomers block long-term potentiation, a classic experimental paradigm for synaptic plasticity, and they are strikingly elevated in AD brain tissue and transgenic AD models. It has been hypothesized that early memory loss stems from synapse failure before neuron death and that synapse failure derives from actions of soluble Aβ oligomers rather than fibrils. Lacor et al., *Synaptic targeting by Alzheimer's-related amyloid β oligomers*, J. Neurosci. 2004, 24(45):10191-10200.

[006] Abeta is a cleavage product of an integral membrane protein, amyloid precursor protein (APP), found concentrated in the synapses of neurons. Soluble forms of Abeta are present in the brains and tissues of Alzheimer's patients, and their presence correlates with disease progression. Yu et al., 2009, *Structural characterization of a soluble amyloid beta-peptide oligomer,* Biochemistry, 48(9):1870-1877. Soluble amyloid β oligomers have been demonstrated to induce changes in neuronal synapses that block learning and memory.

[007] Smaller, soluble Aβ oligomers interfere with a number of signaling pathways critical for normal synaptic plasticity, ultimately resulting in spine and synapse loss. Selkoe et al., 2008, *Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior*, Behav Brain Res 192(1): 106-113. Alzheimer's begins and persists as a synaptic plasticity disease.

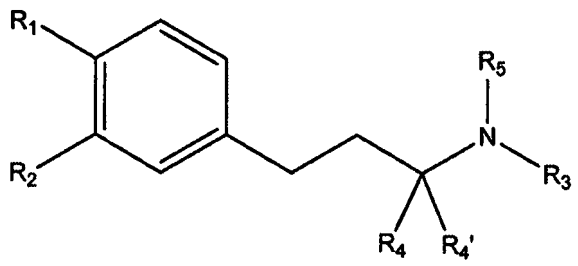
[008] The presence of soluble Aβ oligomers is believed to be to be responsible for early cognitive decline in the pre-Alzheimer's diseased brain. It is known that amyloid beta oligomers bind at neuronal synapses and that sigma-2 receptors are present in significant amounts in neurons and glia.

[009] The present invention is based, in part, on the broad finding that sigma-2 receptor antagonists, meeting certain requirements, inhibit the deleterious effects of soluble Aβ oligomers. In some embodiments, sigma-2 receptor antagonists and compositions are used to treat or prevent synaptic dysfunction in a subject.

SUMMARY OF THE INVENTION

[010] The invention is based, in part, on the broad finding that a sigma-2 antagonist, preferably one that also exhibits other aspects of a particular therapeutic phenotype, participates in inhibition and inhibits effects of amyloid beta ("Abeta" or "Aβ") peptides and oligomers and other soluble species thereof, as defined below, and, consequently, can be used to treat conditions, including diseases and disorders, associated with Abeta-induced pathology, such as Alzheimer's disease. Soluble Abeta oligomers behave like reversible pharmacological ligands that bind to specific receptors and interfere with signaling pathways critical for normal synaptic plasticity, ultimately resulting in spine and synapse loss. It has been discovered that compounds that bind to the sigma-2 receptor and that behave as functional neuronal antagonists exhibit pharmacological competition with Abeta oligomers. Sigma-2 antagonist compounds as described herein thus can decrease or prevent Abeta oligomer effects such as Abeta induced cellular toxicity. The present invention also encompasses methods for inhibiting effects of Abeta oligomers or other soluble Abeta species on a neuronal cell and more generally amyloid beta pathologies comprising contacting the cell with a sigma-2 antagonist according to the present invention. In some embodiments, methods are provided for treating early stages of Alzheimer's disease comprising administering a therapeutically effective amount of a sigma-2 functional antagonist.

[011] In one embodiment, the sigma-2 antagonists of the present invention are the novel compounds represented by Formula I:

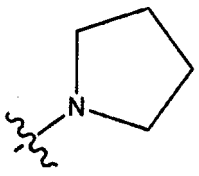


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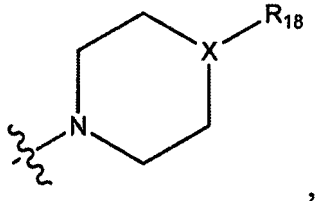
wherein

R_1 and R_2 are independently selected from H, OH, halo, CN, NO_2, NH_2, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{3-7} cycloalkyl, $NH(C_{1-4}$ alkyl), $N(C_{1-4}$ alkyl)$_2$, $NH(C_{3-7}$ cycloalkyl), $NHC(O)(C_{1-4}$ alkyl), $(R_{16})(R_{17})N$-C_{1-4} alkylene-O-, SH, $S(C_{1-6}$ alkyl), C(O)OH, $C(O)O(C_{1-4}$ alkyl), C(O) (C_{1-4} alkyl), and $C(O)NH(C_{1-4}$ alkyl), or R1 and R2 are linked together to form a –O-C_{1-2} methylene-O- group, wherein

R_{16} and R_{17} are independently H, C_{1-4} alkyl, or benzyl, or R_{16} and R_{17} together with nitrogen form a ring selected from



, and

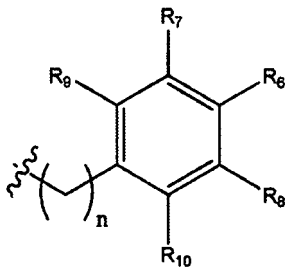
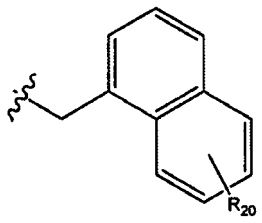
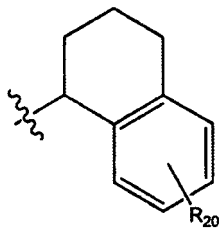
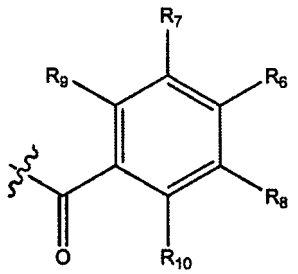
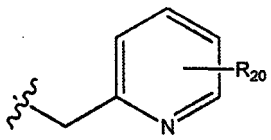
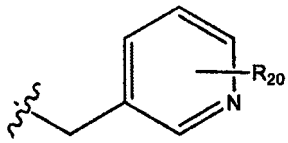
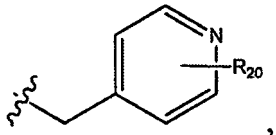
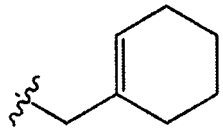


, wherein

X is CH_2, N, or O and R_{18} is absent or is H, unsubstituted phenyl or phenyl substituted with OH, halo, CN, NO_2, NH_2, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy; and

wherein at least one of R_1 and R_2 is not H;

R_3 is selected from

wherein

R_6, R_7, R_8, R_9, R_{10}, and R_{20} are independently selected from H, OH, halo, CN, NO_2, NH_2, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{3-7} cycloalkyl, $NH(C_{1-4}$ alkyl), $N(C_{1-4}$ alkyl)$_2$, $NH(C_{3-7}$ cycloalkyl), $NHC(O)(C_{1-4}$alkyl), SH, $S(C_{1-6}$ alkyl), $S(O)_2$- C_{1-6} alkyl, C(O)OH, $C(O)O(C_{1-4}$ alkyl), $C(O)(C_{1-4}$ alkyl), and $C(O)NH(C_{1-4}$ alkyl); and

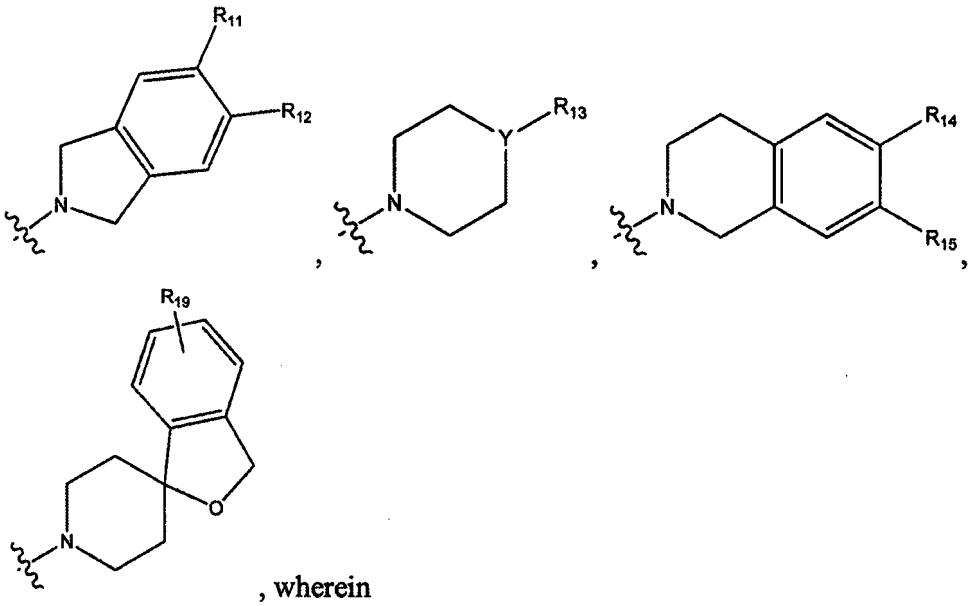
n is 1-4

R_4 is C_{1-6} alkyl;

$R_{4'}$ is H or C_{1-6} alkyl; and

R_5 is H, C_{1-6} alkyl, and $C(O)O(C_{1-4}$ alkyl), $C(O)(C_{1-4}$ alkyl), or $C(O)(C_{1-4}$haloalkyl); or

R_3 and R_5 together with nitrogen form a ring selected from



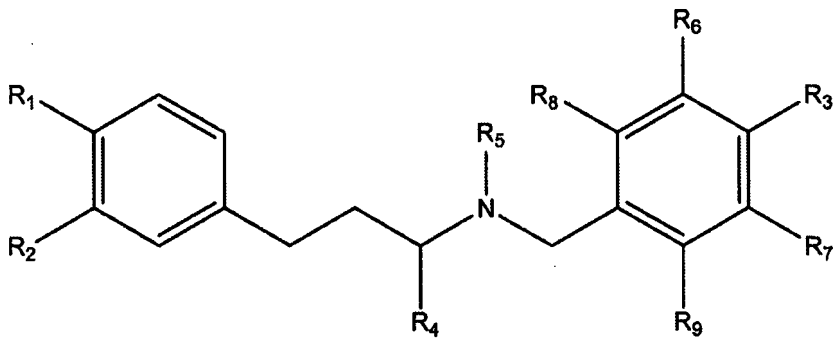
and , wherein

R_{11}, R_{12}, R_{14}, R_{15}, and R_{19} are independently selected from H, OH, halo, CN, NO_2, NH_2, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, and

Y is CH, N, or O; and

R_{13}.is absent or is H, C_{1-6} alkyl, C_{3-6} cycloalkyl, unsubstituted phenyl or phenyl substituted with OH, halo, CN, NO_2, NH_2, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, or unsubstituted benzyl, or benzyl substituted with OH, halo, CN, NO_2, NH_2, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, or pharmaceutically acceptable salts thereof.

[012] In another more specific embodiment, the sigma-2 antagonists of the present invention are the novel compounds represented by Formula II:



II

wherein

R_1 and R_2 are independently selected from H, OH, halo, CN, NO_2, NH_2, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{3-7} cycloalkyl, $NH(C_{1-4}$ alkyl), $NH(C_{1-4}$ alkyl$)_2$, $NH(C_{3-7}$ cycloalkyl), $NHC(O)(C_{1-4}$ alkyl), SH, $S(C_{1-6}$ alkyl), $C(O)OH$, $C(O)O(C_{1-4}$ alkyl), $C(O)(C_{1-4}$ alkyl), and $C(O)NH(C_{1-4}$ alkyl), or R1 and R2 are linked together to form a $-O-C_{1-4}$ methylene-O-, and wherein at least one of R_1, R_2, R_4, R_5 and R_6 is not H;

R_3 is selected from H, halo, and C_{1-6} haloalkyl;

$R_4 = C_{1-6}$ alkyl;

R_5 is H, C_{1-6} alkyl, and $C(O)O(C_{1-4}$ alkyl), $C(O)(C_{1-4}$ alkyl), $C(O)(C_{1-4}$haloalkyl); and

R_6, R_7, R_8, and R_9 are independently selected from H, OH, halo, CN, NO_2, NH_2, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, or pharmaceutically acceptable salts thereof.

[013] In some embodiments, the sigma-2 antagonists of the present invention bind to a sigma-2 receptor and inhibit the binding of Aβ oligomers to neurons, and particularly to synapses. In some embodiments, the sigma-2 antagonist competes with Aβ oligomer binding to neurons and specifically synapses, or otherwise disrupts the ability of Aβ oligomer to bind to neurons, such as by interfering with Aβ oligomer formation or binding to Aβ oligomer or possibly interfering with the ability of Aβ oligomer to set in motion signal transduction mechanisms attendant to its binding to neurons. In certain embodiments, the sigma-2 antagonists thus inhibit a non-lethal Aβ pathologic effect ("non-lethal Aβ pathology" or "non-lethal amyloid beta pathology), including a defect in membrane trafficking, synaptic dysfunction, a memory and learning defect in an animal, reduction in synapse number, change in dendritic spine length or spine morphology, or a defect in long term potentiation (LTP), among others. In other words, the present inventors observed that the sigma-2 antagonists of the invention that are active in other assays as illustrated herein, possess an ability to restore neurons to a normal state or interfere with Aβ oligomer –induced synaptic dysfunction. Without

being bound by theory, sigma-2 antagonists of the invention interfere with one or more of Aβ oligomer structure, Aβ oligomer binding to neurons or Aβ oligomer-induced molecular signaling mechanisms which is useful in counteracting the nonlethal effects of Aβ oligomers and in treating early stages of soluble Aβ oligomer -associated pathologies.

[014] In one embodiment, the sigma-2 antagonists of the present invention are functional neuronal antagonists and are used in a method of inhibiting synapse loss in a neuronal cell, the loss being associated with exposure of the cell to one or more Abeta oligomers or other Abeta complexes or, more generally, Abeta species including Abeta peptides in monomeric or oligomeric or otherwise soluble complexed form (as defined below), the method comprising contacting said cell with an amount of one or more sigma-2 antagonists in an amount effective to avert or reduce said loss or to partially or completely restore synapse number in said cell to pre-exposure levels.

[015] In another embodiment, the sigma-2 antagonists of the present invention are used in a method for modulating a membrane trafficking change in a neuronal cell, said change being associated with exposure of said cell to one or more Abeta species, the method comprising contacting said cell with an amount of one or more sigma-2 antagonists in an amount effective to avert or reduce said membrane trafficking change, or have it remain at or closer to levels observed prior to exposure of said cell to said Abeta species.

[016] In another embodiment, the sigma-2 antagonists of the present invention are used in a method for treating cognitive decline comprising administering to a subject one or more of the sigma-2 antagonists of the present invention.

[017] In yet another embodiment, the sigma-2 antagonists of the present invention are functional neuronal sigma-2 antagonists used in a method for treating a cognitive decline or neurodegenerative disorder or a defect in synapse function and/or number comprising administering to a subject one or more of the sigma-2 antagonists of the present invention.

[018] In some embodiments, the disclosure provides compositions and methods comprising sigma-2 receptor antagonists for inhibiting amyloid beta oligomer-induced synaptic dysfunction of a neuronal cell; and for inhibiting suppression of hippocampal long term potentiation caused by exposure of neurons to Abeta oligomers.

[019] The present invention provides a method of identifying a compound that inhibits cognitive decline or treats a neurodegenerative disease, the method comprising

contacting a cell with a compound that binds to a sigma-2 receptor and determining whether said compound has at least one of the following additional properties:

(a) it inhibits synapse loss in a central neuron, said loss being associated with exposure of the neuron to Abeta oligomer;

(b) it inhibits membrane trafficking abnormalities in a central neuron, the abnormalities being associated with exposure of said cell to one or more Abeta oligomers;

(c) it inhibits Abeta oligomer-mediated cognitive effects in an animal model of Alzheimer's disease; or

(d) it inhibits hippocampal-based spatial learning and memory decline in an animal model of Alzheimer's disease.

Such inhibition of nonlethal amyloid beta pathologies includes methods of inhibiting cognitive decline, inhibiting synapse loss in a neuronal cell, and inhibiting a membrane trafficking change in a neuronal cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[020] Figure 1A is a photomicrograph showing primary hippocampal and cortical cultures maintained in vitro for 21 days with intracellular vesicles containing formazan resulting from endocytosis and chemical reduction of cargo tetrazolium salt dye in the membrane trafficking assay.

[021] Figure 1B is a photomicrograph showing sister cultures with extracellular formazan crystals formed outside of the cellular membrane of neurons and glia upon exocytosis of formazan wherein the cell has been exposed to Abeta oligomer in the membrane trafficking assay. This figure shows that human Abeta 1-42 oligomers alter the phenotype of the cargo dye product formazan (intracellular vesicles vs. extracellular crystals) and therefore causes cellular membrane trafficking deficits.

[022] Figure 1C is a photomicrograph showing intracellular vesicles, wherein the cell has been exposed to both Abeta oligomer and to compound II, a selective, high affinity sigma-2 antagonist compound according to the invention. This figure shows that compound II is able to block the membrane trafficking deficits produced by Abeta oligomers, and restores the membrane trafficking phenotype to normal.

[023] Figure 1D shows quantification of the membrane trafficking assay where the y-axis represents the amount of formazan product contained in the intracellular vesicles at a given point in time after administration of the cargo tetrazolium salt dye, normalized to vehicle-treated values. Red circles represent Abeta oligomer-treated cultures, blue squares represent vehicle-treated control cultures and black or gray squares represent values from cultures treated with various concentrations of cpd II + Abeta, and cpd IXa,IXb +Abeta, when compounds are added before Abeta oligomers (prevention). The concentration log of the compounds is used in the abscissa. This figure shows that the compounds inhibit Abeta oligomer effects on membrane trafficking in a dose-dependent manner.

[024] Figure 1E shows membrane trafficking assay dose-response curves in the same type of plot as Figure 1D but when compounds are added after Abeta oligomers (treatment). The concentration log of the compounds is used in the abscissa. This figure shows that the compounds inhibit Abeta oligomer effects on membrane trafficking in a dose-dependent manner.

[025] Figure 1F shows a membrane trafficking assay in the same type of plot as Figure 1D in the presence of various concentrations of synthetic Abeta oligomer alone (EC50 820nM), and with various concentrations of compound II,

and resulting vesicles (as % vehicle) at each concentration. A rightward shift in the EC 50 (Schild slope = -0.75) was exhibited by the presence of increasing concentrations of compound II. This figure demonstrates that cpd II pharmacologically competes with oligomers for access to molecular targets that mediate membrane trafficking, and therefore the presence of compound II made synthetic Abeta oligomers less synaptotoxic.

[026] Figure 1G shows a membrane trafficking assay in the same type of plot as Figure 1D in the presence of various concentrations of synthetic Abeta oligomer alone, and with various concentrations of compound mixture IXa,IXb, and resulting vesicles (as % vehicle) at each concentration. A rightward shift in the EC 50 (Schild slope = -0.51) was exhibited by the presence of increasing concentrations of compound mixture IXa,IXb. This figure demonstrates that cpd mixture IXa,IXb pharmacologically competes with oligomers for access to molecular targets that mediate membrane trafficking, and therefore the presence of compound mixture IXa,IXb made synthetic Abeta oligomers less synaptotoxic.

[027] Figure 1H shows a membrane trafficking assay in the same type of plot as Figure 1D in the presence of various concentrations of Abeta oligomers derived from human Alzheimer's patients alone, and with various concentrations of compound II, and resulting vesicles (as % vehicle) at each concentration. A rightward shift in the EC 50 was exhibited by the presence of increasing concentrations of compound II. This figure demonstrates that cpd II pharmacologically competes with oligomers for access to molecular targets that mediate membrane trafficking, and therefore the presence of compound II made human Alzheimer's disease-relevant Abeta oligomers less synaptotoxic.

[028] Figure 1I shows a membrane trafficking assay in the same type of plot as Figure 1D in the presence of various concentrations of Abeta oligomers derived from human Alzheimer's patients alone, and with various concentrations of compound mixture IXa,IXb, and resulting vesicles (as % vehicle) at each concentration. A rightward shift in the EC 50 was exhibited by the presence of increasing concentrations of compound mixture IXa,IXb. This figure demonstrates that cpd mixture IXa,IXb pharmacologically competes with oligomers for access to

molecular targets that mediate membrane trafficking, and therefore the presence of compound mixture IXa,IXb made human Alzheimer's disease-relevant Abeta oligomers less synaptotoxic.

[029] Figure 1J shows a membrane trafficking assay in the same type of plot as Figure 1D in the presence of various concentrations of synthetic Abeta oligomer alone, and with various concentrations of compound CF, and resulting vesicles (as % vehicle) at each concentration. A rightward shift in the EC 50 was exhibited by the presence of increasing concentrations of compound CF. This figure demonstrates that cpd CF pharmacologically competes with oligomers for access to molecular targets that mediate membrane trafficking, and therefore the presence of compound CF made synthetic Abeta oligomers less synaptotoxic.

[030] Figure 1K shows a membrane trafficking assay in the same type of plot as Figure 1D in the presence of various concentrations of synthetic Abeta oligomer alone, and with various concentrations of compound W, and resulting vesicles (as % vehicle) at each concentration. A rightward shift in the EC 50 was exhibited by the presence of increasing concentrations of compound W. This figure demonstrates that cpd W pharmacologically competes with oligomers for access to molecular targets that mediate membrane trafficking, and therefore the presence of compound W made synthetic Abeta oligomers less synaptotoxic.

[031] Figure 1L shows membrane trafficking assay results using Abeta oligomers isolated from Alzheimer's disease patients. Compound CF (20 microMolar concentration) exhibited pharmacological competition with Abeta oligomers isolated from AD patients for access to molecular targets that mediate membrane trafficking and therefore the presence of compound CF made human Alzheimer's disease-relevant Abeta oligomers less synaptotoxic.

[032] Figure 1M is a bar graph of trafficking assay results with percent formazan-filled vesicles of a neuron identified (and quantitated) in the presence of (i) vehicle alone (1st bar); (ii) an Abeta oligomer preparation from human Alzheimer's disease patient brains (2nd bar, significantly reduced compared to 1st bar); (ii) compound II as disclosed herein plus Abeta oligomer (3rd bar, significantly higher than the 2nd bar); and (iv) compound II without Abeta oligomer (4th bar, not

significantly different from the first bar). This figure demonstrates that compound II blocks the membrane trafficking deficits produced by human Alzheimer's disease-relevant Abeta oligomers, and restores the membrane trafficking phenotype to normal, but does not affect membrane trafficking when dosed on its own in the absence of Abeta oligomers.

[033] Figure 1N is a bar graph identical in type to that of Figure J but depicting data generated using an Abeta oligomer preparation isolated from age-matched histologically normal human brains. This figure demonstrates that Abeta oligomers derived from normal human brain do not significantly affect membrane trafficking, and that cpd II does not further affect membrane trafficking in the presence or absence of such oligomers.

[034] Figure 2A is a plot of pharmacokinetic data in which the concentration of compound II obtained in plasma (left ordinate, ng/mL) upon a single subcutaneous (open triangles) and intravenous (i.v.) (open circles) administration of Compound II and in brain (right ordinate, ng/g) upon a single i.v. administration (filled circles) of Compound II. Compound II was known to be subject to first pass metabolism and thus was dosed subcutaneously; nevertheless Compound II was highly brain penetrant following acute dosing. This figure demonstrates that cpd II is highly brain penetrant upon acute subcutaneous dosing.

[035] Figure 2B is a plot of pharmacokinetic data in which the concentration of compound II obtained in plasma (left ordinate) upon once daily subcutaneous administration for 5 days of different amounts of Compound II (0.5 mg/kg/day: downward pointing filled triangles; 0.35 mg/kg/day: upward pointing filled triangles; and 0.1 mg/day filled squares) and in brain (right ordinate) upon subcutaneous administration of the same amounts (respectively downward pointing open triangle, upward pointing open triangle and open square) of Compound II. Compound II was known to be subject to first pass metabolism and thus was dosed subcutaneously; nevertheless Compound II was highly brain penetrant following chronic dosing. This figure demonstrates that cpd II is highly brain penetrant upon chronic subcutaneous dosing.

[036] Figure 2C is a plot of pharmacokinetic data in which the concentration of compound CB obtained following single acute oral dosing obtained in plasma (left ordinate, closed triangles) and in brain (right ordinate, open triangles) upon single acute oral administration of Compound CB (10 mg/kg/day). Compound CB was highly brain penetrant following acute oral dosing and exhibits 50% bioavailability with a plasma half-life of 3.5 hours. This figure demonstrates that cpd CB is highly brain penetrant upon acute oral dosing.

[037] Figure 2D shows is a plot of pharmacokinetic data in which the concentration of compound CB obtained following chronic once daily oral dosing for 5 days obtained in plasma (left ordinate, closed triangles) and in brain (right ordinate, open triangles) upon once daily oral administration of Compound CB (10 mg/kg/day, upright triangles) or 30 mg/kg/day (inverted triangles). Compound CB was highly brain penetrant following chronic oral dosing and exhibits a brain/plasma ratio of 3 at up to 5 days of once daily oral administration. This figure demonstrates that cpd CB is highly brain penetrant upon chronic oral dosing.

[038] Figure 3A-Panel A is a fluoromicrograph of primary hippocampal and cortical cultures maintained in vitro for 21 days exposed to Abeta oligomer in the absence of Compound IXa,IXb; Abeta (visualized with monoclonal antibody 6E10 immunolabeling) is bound to cellular membranes including neuronal postsynaptic spines at synapses.

[039] Figure 3A-Panel B is the same field of view as seen in Figure 3A-Panel A showing the number of synapses (visualized with synaptophysin immunolabeling) are reduced in the presence of Abeta oligomers compared to a negative control (not shown).

[040] Figure 3A-Panel C is a lower magnification fluoromicrograph of primary hippocampal and cortical cultures maintained in vitro for 21 days exposed to Abeta oligomer in the absence of Compound IXa,IXb; Abeta (visualized with monoclonal antibody 6E10 immunolabeling) is bound to cellular membranes including neuronal postsynaptic spines at synapses.

[041] Figure 3A-Panel D shows sister cultures of primary hippocampal and cortical cultures maintained in vitro for 21 days exposed to Abeta oligomer in the presence of Compound IXa,IXb; the amount of Abeta bound to cellular membranes including neuronal postsynaptic spines is visibly reduced.

[042] Figure 3B-Panel A is a fluoromicrograph of sister cultures of primary hippocampal and cortical cultures maintained in vitro for 21 days exposed to Abeta oligomer in the presence of Compound IXa,IXb; the amount of Abeta bound to cellular membranes including neuronal postsynaptic spines is visibly reduced. This figure demonstrates that the presence of Compound IXa,IXb (i) significantly reduced the amount of Abeta oligomer bound to cellular membranes including neuronal postsynaptic spines. Similar protection was seen in the presence of Compound II (data not shown).

[043] Figure 3B-Panel B is the same field of view as seen in Figure 3A-Panel C showing the number of synapses (visualized with synaptophysin immunolabeling) are restored in the presence of Compound IXa,IXb with increased synaptophysin visualization compared to FIG. 3B. This figure demonstrates that compound mixture IXa,IXb significantly blocks Abeta oligomer-induced synaptic loss. Similar protection was seen in the presence of Compound II (data not shown).

[044] Figure 3C is a quantification of the data shown in Figure 3A-Panels A-D in a bar graph of a synapse loss assay experiment. Synapse loss provides the closest correlate to cognitive function. In the synapse loss assay, Abeta oligomers caused an 18.2% synapse loss vs. vehicle in vitro. The presence of compound II or compound mixture IXa,IXb completely eliminated this synaptic regression. No effect was seen when the compounds were dosed in vehicle alone, without Abeta oligomers. Specifically, synapse count was calculated by image processing-based quantification of the number, intensity and area of synaptophysin-immunolabeled areas of the fluoromicrographs expressed as percent of negative control (vehicle) in neurons exposed to vehicle alone (first bar); vehicle and Compound IXa,IXb or Vehicle and Compound II (second and third bars, respectively, showing no effect on synapse number by Compounds); Abeta oligomer (fourth bar showing significant reduction in synapse count compared to first bar) and Abeta oligomer in the

presence of either Compounds IXa,IXb or II (fifth and sixth bars) showing no reduction in synapse number compared to first bar. This figure demonstrates that the compounds IXa,IXb and II exhibited protective effects and blocked Abeta oligomer-induced reduction in synapse number.

[045] Figure 3D is a quantification of the data shown in Figure 3A-Panels A-D in a bar graph of Abeta binding intensity calculated by image processing-based quantification of the number, intensity and area of 6E10-immunolabeled areas of the fluoromicrographs when Abeta alone is added to vehicle (first bar graph) and their significant reduction in the co-presence of Abeta and either Compound II or Compound mixture IXa,IXb. This figure demonstrates that compounds IXa,IXb and II lower the amount of Abeta bound to cellular membranes.

[046] Figure 4 is a bar graph of memory performance measured by percent freezing behavior in an in vivo fear conditioning assay measured at baseline training and 24 hours post-training for mice administered vehicle alone (first bar), vehicle plus Abeta oligomer (second bar) Compound II plus Abeta oligomer (third bar) and Compound II alone (fourth bar) and at 24 hours after administration of vehicle alone (first bar), vehicle plus Abeta oligomer (second, significantly reduced, bar), Compound II plus Abeta oligomer (third bar) and Compound II alone. Abeta oligomers (single 200 nanoMolar intrahippocampal injection) produced significant deficits in memory formation in 3-4 month old male wt C57BL/6 mice (N=16) compared to vehicle (N=18). Compound II (single 2 microMolar intrahippocanpal injection one hour prior to oligomers) eliminated memory deficits (N=11) produced by Abeta oligomers. There was no effect of compounds alone and no adverse behavioral effects were observed. This figure demonstrates that compound II can prevent Abeta oligomer-induced memory deficits, while have no effect on memory performance when dosed on its own.

[047] Figure 5 is the same type of bar graph as Figure 4 showing memory performance measured by freezing behavior in the same contextual fear conditioning assay as that which gave rise to Figure 4 when animals were treated with (i) vehicle alone (first bar) (ii) Abeta oligomers (2^{nd} bar, showing a significant reduction in ability of test animals to acquire new memories)) (iii) a mixture of compounds IXa

and IXb, (3rd bar, showing complete (and statistically significant) inhibition of Abeta oligomer-induced memory deficit); or (iv) a mixture of compounds IXa and IXb in the absence of Abeta oligomer (4th bar, showing no effect on memory). There was no adverse behavioral effects observed. This figure demonstrates that compound mixture IXa,IXb can prevent Abeta oligomer-induced memory deficits, while have no effect on memory performance when dosed on its own.

[048] Figure 6A shows autoradiographic binding of [^{3}H]-(+)-pentazocine (a sigma-1 receptor ligand) in (left panel) human frontal cortex tissue sections from normal patients, Lewy Body Dementia (DLB) patients, or Alzheimer's Disease (AD) patients, where BS is specific binding, and BNS is non-specific binding; and (right panel) shows a graph of average specific binding for [^{3}H]pentazocine from the autoradiography experiments from the control (normal), DLB, or AD patients. The sigma-1 receptor is statistically lower in Alzheimer's disease brains compared to control age-matched brains in parallel with the degree of neuronal loss seen in AD . This figure demonstrates that sigma-1 receptor expression may remain constant in Alzheimer's disease brains.

[049] Figure 6B shows autoradiographic binding of [^{125}I]-RHM-4 (a sigma-2 receptor ligand) in (left panel) adjacent human frontal cortex tissue sections from normal patients, Lewy Body Dementia (DLB) patients, or Alzheimer's Disease (AD) patients; and (right panel) shows a graph of average specific binding for [^{125}I]RHM-4 from the autoradiography experiments from the control (normal), DLB, or AD patients. The sigma-2 receptor is not statistically lower in Alzheimer's disease and Lewy Body Dementia brains compared to control age-matched brains despite the neuronal loss seen in these diseases This figure demonstrates that sigma-2 receptor expression on surviving neurons and/or glia may be upregulated in DLB and Alzheimer's disease brains.

[050] Figure 6C shows (left panel) displacement of 18.4 nM [^{3}H]-RHM-1 (a sigma-2 receptor ligand) in monkey frontal cortex, monkey hippocampus or human temporal cortex by sigma-2 ligands and (right panel) a graph of binding density of [^{3}H]-RHM-1 with and without 1 uM each of siramesine and compounds IXa,IXb and II. This figure demonstrates that Compounds II and mixture IXa,IXb

competitively displace known radiolabeled sigma-2 ligands such as [^{3}H]-RHM-1 from the sigma-2 receptor in monkey and human brain tissue sections, and therefore both of these compounds bind to sigma-2 receptors.

[051] Figure 7A shows tumor cell cytotoxicity of sigma-2 receptor agonists as cell viability in MTS assay in SKOV-3 human ovarian cancer cell line treated with sigma compounds for 48 hours. Sigma-2 agonists (siramesine, SV-119, WC-26) kill tumor cells. Sigma-2 antagonists (RHM-1, IXa,IXb and II) do so only at a much higher concentration in the absence of agonists. This figure demonstrates that cpds II and IXa,IXb behave similarly to known sigma-2 antagonists in this assay, and therefore implies that they are sigma-2 antagonists in tumor cells.

[052] Figure 7B shows neuronal cell cytotoxicity of sigma-2 receptor agonists as nuclear intensity variation in neuronal cultures with sigma-2 compounds after 24 hours. Sigma-2 agonists (siramesine, SV-119, WC-26) cause abnormal nuclear morphology in neurons; Sigma-2 antagonists (RHM-1, IXa,IXb and II) do not. This figure demonstrates that cpds II and IXa,IXb behave similarly to known sigma-2 antagonists in this assay, and therefore implies that they are sigma-2 antagonists in primary hippocampal and cortical cells.

[053] Figure 8A shows caspase-3 activity in SKOV-3 hyman ovarian cancer cells induced by sigma-2 agonist siramesine whereas the sigma-2 receptor antagonists RHM-1, compounds II and IXa,IXb did not induce caspase-3 activity. Abeta oligomers cause low levels of caspase-3 activation and lead to LTD. High levels of oligomers and caspase-3 lead to cell death. Sigma-2 receptor agonists (SV-119, siramesine) activate caspase-3 in tumor cells and neurons; sigma-2 antagonists do not (Figures 10A and 10B). This figure demonstrates that cpds II and IXa,IXb behave similarly to known sigma-2 antagonists in this assay, and therefore implies that they are sigma-2 antagonists in tumor cells.

[054] Figure 8B shows caspase-3 activity in neurons induced by sigma-2 agonist siramesine whereas the sigma-2 receptor antagonists RHM-1, compounds II and IXa,IXb did not induce caspase-3 activity. This figure demonstrates that cpds II and IXa,IXb behave similarly to known sigma-2 antagonists in this assay, and

therefore implies that they are sigma-2 antagonists in primary hippocampal and cortical cells.

[055] Figure 8C shows caspase-3 activation in SKOV-3 human ovarian tumor cells by sigma-2 receptor agonist SV-119. Sigma-2 receptor antagonists compounds IXa,IXb and II, RHM-1 do not block caspase-3 activation caused by sigma-2 receptor agonist SV-119 in tumor cells. This figure demonstrates that cpds II and IXa,IXb behave similarly to known sigma-2 antagonists in this assay, and therefore implies that they are sigma-2 antagonists in tumor cells.

[056] Figure 8D shows caspase-3 activation in neuronal cultures by sigma-2 receptor agonist SV-119 after 24 hours at various concentrations of agonist. This figure demonstrates that Sigma-2 receptor antagonists compounds IXa,IXb and II, but not RHM-1, blocked caspase-3 activation caused by sigma-2 receptor agonist SV-119 in primary hippocampal and cortical cells.

[057] Figure 9A shows memory performance measured by percent freezing behavior in an in vivo fear conditioning assay measured at 24 hours post-training at 1-3 minutes in a 15 month old male transgenic Alzheimer's disease mouse model following oral administration of sigma-2 receptor antagonist compounds at various doses for 5.5 months. A significant improvement of memory deficits occurred in transgenic animals that were treated with 10 and 30 mg/kg/day of CB ($p<0.05$) and 30 mg/kg/day of CF ($p<0.005$) compared to Tg animals treated with vehicle (Mann-Whitney U test). This figure demonstrates that cmpds CB and CF reverse established memory deficits in transgenic Alzheimer's mice following chronic long-term administration.

Figure 9B shows a bar graph of behavioral data for 9-month old female transgenic (Tg) Alzheimer's disease mice that exhibited significant memory deficits in the Y-maze (% alternation) when treated p.o. for 39 days with vehicle vs. vehicle treated non-trangenic littermates (i.e., vehicle treated Tg mice performed at chance, vehicle-treated non-Tg litter mates performed significantly better than chance-see asterisk and line next to each bar). Treatment of Tg animals with Cpd. CF at 30 mg/kG/day orally improved the deficits. No adverse behavioral effects were observed. This

figure demonstrates that cmpd CF reverses established memory deficits in transgenic Alzheimer's mice following chronic short-term administration.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[058] Before the compounds, compositions and methods of the invention are described in detail, it is to be understood that this invention is not limited to the particular processes, compositions, or methodologies described, as these may vary. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. 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. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described.

[059] It is further appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, can also be provided in combination in a single embodiment. Conversely, various features of the invention which are, for brevity, described in the context of a single embodiment, can also be provided separately or in any suitable subcombination.

Definitions

[060] The singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to a "cell" is a reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth.

[061] As used herein, the term "about" means plus or minus 10 % of a given value. For example, "about 50 %" means in the range of 45 % – 55 %.

[062] "Sigma-2 ligand" means a compound that binds to the sigma-2 receptor and includes agonists, antagonists, partial agonists, inverse agonists and simply competitors for other ligands of this receptor or protein.

[063] The term "agonist" refers to a compound, the presence of which results in a biological activity of a receptor that is the same as the biological activity resulting from the presence of a naturally occurring ligand for the receptor.

[064] The term "partial agonist" refers to a compound the presence of which results in a biological activity of a receptor that is of the same type as that resulting from the presence of a naturally occurring ligand for the receptor, but of a lower magnitude.

[065] The term "antagonist" refers to an entity, e.g., a compound, the presence of which results in a decrease in the magnitude of a biological activity of a receptor. In certain embodiments, the presence of an antagonist results in complete inhibition of a biological activity of a receptor. A "functional antagonist" at the sigma-2 receptor is one that blocks Abeta oligomer-induced synaptic dysfunction, for example, as seen in an in vitro assay, such as a membrane trafficking assay, or in a behavioral assay, or in a patient in need thereof. The functional antagonist may act directly by inhibiting binding of, for example, an Abeta oligomer, or indirectly, by interfering with downstream signaling resultant from Abeta oligomer binding the sigma-2 receptor.

[066] The term "selectivity" or "selective" refers to a difference in the binding affinity K_i for a sigma-2 receptor compared to a non-sigma receptor. The sigma-2 antagonists possess high selectivity for a sigma receptor in synaptic neurons. The Ki for a sigma-2 receptor or both a sigma-2 and a sigma-1 receptor is compared to the Ki for a non-sigma receptor. In one aspect, the sigma-2 or sigma-1/sigma-2 selective ligand is at least 10-fold, 20-fold, 30-fold, 50-fold, 70-fold, 100-fold, 500-fold higher affinity, or more selective, for a sigma receptor compared to a non-sigma receptor. The non-sigma receptor is, for example, a muscarinic M1-M4 receptor, serotonin (5-HT) receptor, alpha adrenergic receptor, beta adrenergic

receptor, opioid receptor, serotonin transporter, dopamine transporter, adrenergic transporter, dopamine receptor, or NMDA receptor.

[067] In the present application, the term "high affinity" is intended to mean a compound which exhibits a K_i value of less than 600 nM, 500 nM, 400 nM, 300 nM, 200 nM, less than 150 nM, less than 100 nM, less than 80 nM, less than 60 nM, or preferably less than 50 nM in a sigma receptor binding assay, for example against [^{3}H]-DTG, as disclosed by Weber et al., Proc. Natl. Acad. Sci (USA) 83: 8784-8788 (1986), incorporated herein by reference, which measures the binding affinity of compounds toward both the sigma-1 and sigma-2 receptor sites. Especially preferred sigma ligands exhibit Ki values of less than about 150 nM, preferably less than 100 nM, less than about 60 nM, less than about 10 nM, or less than about 1 nM against [^{3}H]-DTG.

[068] "Abeta species" or "Aβ" shall include compositions comprising soluble amyloid peptide-containing components such as Abeta monomers, Abeta oligomers, complexes of Abeta peptide (in monomeric, dimeric or polymeric form) with other soluble peptides or proteins as well as other soluble Abeta assemblies, including any processed product of amyloid precursor protein. Soluble Aβ oligomers are known to be neurotoxic. Even $A\beta_{1-42}$ dimers are known to impair synaptic plasticity in mouse hippocampal slices. In one theory known in the art, native $A\beta_{1-42}$ monomers are considered neuroprotective, and self-association of Aβ monomers into soluble Abeta oligomers is required for neurotoxicity. However, certain Aβ mutant monomers (arctic mutation (E22G) are reported to be associated with familial AD. See, for example, Giuffrida et al., *β-Amyloid monomers are neuroprotective.* J. Neurosci. 2009 29(34):10582-10587. Nonlimiting examples of preparations comprising Abeta species are disclosed in U.S. patent application serial number 13/021,872; U.S. Patent Publication 2010/0240868; International Patent Application WO/2004/067561; International Patent Application WO/2010/011947; U.S. Patent Publication 20070098721; U.S. Patent Publication 20100209346; International Patent Application WO/2007/005359; U.S. Patent Publication 20080044356; U.S. Patent Publication 20070218491; WO/2007/126473; U.S. Patent Publication 20050074763; International Patent Application WO/2007/126473,

International Patent Application WO/2009/048631, and U.S. Patent Publication 20080044406, each of which is incorporated herein by reference.

[069] "Administering," when used in conjunction with the compounds of the present invention, means to administer a compound directly into or onto a target tissue or to administer a compound systemically or locally to a patient or other subject..

[070] The term "animal" as used herein includes, but is not limited to, humans and non-human vertebrates such as wild, domestic and farm animals.

[071] As used herein, the terms "subject," "individual," and "patient," are used interchangeably and refer to any animal, including mammals, mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, primates, non-human primates, humans, and the like.

[072] As used herein, the term "contacting" refers to the bringing together or combining of molecules (or of a molecule with a higher order structure such as a cell or cell membrane) such that they are within a distance that allows for intermolecular interactions such as the non-covalent interaction between two peptides or one protein and another protein or other molecule, such as a small molecule. In some embodiments, contacting occurs in a solution in which the combined or contacted molecules are mixed in a common solvent and are allowed to freely associate. In some embodiments, the contacting can occur at or otherwise within a cell or in a cell-free environment. In some embodiments, the cell-free environment is the lysate produced from a cell. In some embodiments, a cell lysate may be a whole-cell lysate, nuclear lysate, cytoplasm lysate, and combinations thereof. In some embodiments, the cell-free lysate is lysate obtained from a nuclear extraction and isolation wherein the nuclei of a cell population are removed from the cells and then lysed. In some embodiments, the nuclei are not lysed, but are still considered to be a cell-free environment. The molecules can be brought together by mixing such as vortexing, shaking, and the like.

[073] The term "improves" is used to convey that the present invention changes either the characteristics and/or the physical attributes of the tissue to which

it is being provided, applied or administered. The term "improves" may also be used in conjunction with a disease state such that when a disease state is "improved" the symptoms or physical characteristics associated with the disease state are diminished, reduced, eliminated, delayed or averted.

[074] The term "inhibiting" includes the blockade, aversion of a certain result or process or the restoration of the converse result or process. In terms of prophylaxis or treatment, by administration of a compound of the present invention, "inhibiting" includes protecting against (partially or wholly) or delaying the onset of symptoms, alleviating symptoms, or protecting against, diminishing or eliminating a disease, condition or disorder.

[075] The term "inhibiting trafficking deficits" refers to the ability to block soluble Aβ oligomer-induced membrane trafficking deficits in a cell, preferably a neuronal cell. A compound capable of inhibiting trafficking deficits has an EC50 < 20 uM, less than 15 uM, less than 10 uM, less than 5 uM, and preferably less than 1 μMin the membrane trafficking assay, and further is capable of at least 50%, preferably at least 60%, and more preferably at least 70% maximum inhibition of the Abeta oligomer effects of soluble Abeta oligomer-induced membrane trafficking deficits

[076] At various places in the present specification, substituents of compounds of the invention are disclosed in groups or in ranges. It is specifically intended that embodiments the invention include each and every individual subcombination of the members of such groups and ranges. For example, the term "C_{1-6} alkyl" is specifically intended to individually disclose methyl (C_1 alkyl), ethyl (C_2 alkyl), C_3 alkyl, C_4 alkyl, C_5 alkyl, and C_6 alkyl.

[077] For compounds of the invention in which a variable appears more than once, each variable can be a different moiety selected from the Markush group defining the variable. For example, where a structure is described having two R groups that are simultaneously present on the same compound, then the two R groups can represent different moieties selected from the Markush group defined for R.

[078] The term "n-membered" where n is an integer typically describes the number of ring-forming atoms in a moiety where the number of ring-forming atoms is n. For example, pyridine is an example of a 6-membered heteroaryl ring and thiophene is an example of a 5-membered heteroaryl group.

[079] As used herein, the term "alkyl" is meant to refer to a saturated hydrocarbon group which is straight-chained or branched. Example alkyl groups include, but are not limited to, methyl (Me), ethyl (Et), propyl (*e.g.*, n-propyl and isopropyl), butyl (*e.g.*, n-butyl, isobutyl, t-butyl), pentyl (e.g., n-pentyl, isopentyl, neopentyl), and the like. An alkyl group can contain from 1 to about 20, from 2 to about 20, from 1 to about 10, from 1 to about 8, from 1 to about 6, from 1 to about 4, or from 1 to about 3 carbon atoms. The term "alkylene" refers to a divalent alkyl linking group. An example of alkylene is methylene (CH_2).

[080] As used herein, "haloalkyl" refers to an alkyl group having one or more halogen substituents. Example haloalkyl groups include, but are not limited to, CF_3, C_2F_5, CHF_2, CCl_3, $CHCl_2$, C_2Cl_5, CH_2CF_3, and the like.

[081] As used herein, "aryl" refers to monocyclic or polycyclic (e.g., having 2, 3 or 4 fused rings) aromatic hydrocarbons such as, for example, phenyl, naphthyl, anthracenyl, phenanthrenyl, indanyl, indenyl, and the like. In some embodiments, aryl groups have from 6 to about 20 carbon atoms. In some embodiments, aryl groups have from 6 to about 10 carbon atoms.

[082] As used herein, "cycloalkyl" refers to non-aromatic cyclic hydrocarbons including cyclized alkyl, alkenyl, and alkynyl groups that contain up to 20 ring-forming carbon atoms. Cycloalkyl groups can include mono- or polycyclic (e.g., having 2, 3 or 4 fused rings) ring systems as well as spiro ring systems. A cycloalkyl group can contain from 3 to about 15, from 3 to about 10, from 3 to about 8, from 3 to about 6, from 4 to about 6, from 3 to about 5, or from 5 to about 6 ring-forming carbon atoms. Ring-forming carbon atoms of a cycloalkyl group can be optionally substituted by oxo or sulfido. Example cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclopentenyl, cyclohexenyl, cyclohexadienyl, cycloheptatrienyl,

norbornyl, norpinyl, norcarnyl, adamantyl, and the like. Also included in the definition of cycloalkyl are moieties that have one or more aromatic rings fused (i.e., having a bond in common with) to the cycloalkyl ring, for example, benzo or thienyl derivatives of pentane, pentene, hexane, and the like (e.g., 2,3-dihydro-1H-indene-1-yl, or 1H-inden-2(3H)-one-1-yl). Preferably, "cycloalkyl" refers to cyclized alkyl groups that contain up to 20 ring-forming carbon atoms. Examples of cycloalkyl preferably include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, adamantyl, and the like.

[083] As used herein, "halo" or "halogen" includes fluoro, chloro, bromo, and iodo.

[084] As used herein, "alkoxy" refers to an -O-alkyl group. Example alkoxy groups include methoxy, ethoxy, propoxy (*e.g.*, n-propoxy and isopropoxy), t-butoxy, and the like.

[085] As used herein, "haloalkoxy" refers to an –O-haloalkyl group. An example haloalkoxy group is OCF_3. As used herein, "trihalomethoxy" refers to a methoxy group having three halogen substituents. Examples of trihalomethoxy groups include, but are not limited to, $-OCF_3$, $-OCClF_2$, $-OCCl_3$, and the like.

[086] As used herein, "amino" refers to NH_2.

[087] As used herein, "alkylamino" refers to an amino group substituted by an alkyl group.

[088] As used herein, "dialkylamino" refers to an amino group substituted by two alkyl groups.

[089] As used here, C(O) refers to C(=O).

[090] As used herein, the term "optionally substituted" means that substitution is optional and therefore includes both unsubstituted and substituted atoms and moieties. A "substituted" atom or moiety indicates that any hydrogen on the designated atom or moiety can be replaced with a selection from the indicated substituent group, provided that the normal valence of the designated atom or

moiety is not exceeded, and that the substitution results in a stable compound. For example, if a methyl group (i.e., CH_3) is optionally substituted, then 3 hydrogen atoms on the carbon atom can be replaced with substituent groups as indicated.

[091] As used herein, a "nonlethal amyloid beta effect" refers to an effect, particularly a nonlethal effect, on a cell that is contacted with an Abeta species. For example, it has been found that when a neuronal cell is contacted with a soluble Amyloid-beta ("Abeta") oligomer, the oligomers bind to a subset of synapses on a subset of neuronal cells in vitro. This binding can be quantified in an assay measuring Abeta oligomer binding in vitro for example. Another documented effect of Abeta species is a reduction in synapse number, which has been reported to be about 18% in the human hippocampus (Scheff et al, 2007) and can be quantified (for example, in an assay measuring synapse number). As another example, it has been found that, when a neuronal cell is contacted with an Amyloid-beta ("Abeta") oligomer, membrane trafficking is modulated and alteration of membrane trafficking ensues. This abnormality can be visualized with many assays, including but not limited to, an MTT assay. For example, yellow tetrazolium salts are endocytosed by cells and the salts are reduced to insoluble purple formazan by enzymes located within vesicles in the endosomal pathway. The level of purple formazan is a reflection of the number of actively metabolizing cells in culture, and reduction in the amount of formazan is taken as a measure of cell death or metabolic toxicity in culture. When cells that are contacted with a yellow tetrazolium salt are observed through a microscope, the purple formazan is first visible in intracellular vesicles that fill the cell. Over time, the vesicles are exocytosed and the formazan precipitates as needle-shaped crystals on the outer surface of the plasma membrane as the insoluble formazan is exposed to the aqueous media environment. Still other effects of Abeta species include cognitive decline, such as a decline in the ability to form new memories and memory loss which can be measured in assays using animal models *in vivo*.

[092] In some embodiments, a test compound is said to be effective to treat cognitive decline or a disease associated therewith when it can inhibit an effect associated with soluble Abeta oligomer species on a neuronal cell more than about

10%, preferably more than 15%, and preferably more than 20% as compared to a negative control. In some embodiments, a test agent is said to be effective when it can inhibit a processed product of amyloid precursor protein-mediated effect more than about 10%, preferably more than 15%, and preferably more than 20% as compared to a positive control. For example, as shown in the Examples below, inhibition of Abeta oligomer binding by only 18% inhibits synapse reduction completely. For example, see FIGs 3C and 3D. Although the present specification focuses on inhibition of nonlethal effects of Abeta species, such as abnormalities in neuronal metabolism and synapse number reduction, these are shown to correlate with cognitive function and are furthermore expected, over time, to result in reduction (compared to untreated subjects) of downstream measurable symptoms of amyloid pathology, notably clinical symptoms such as 1) fibril or plaque accumulation measured by amyloid imaging agents such as fluorbetapir, PittB or any other imaging agent, 2) synapse loss or cell death as measured by glucose hypometabolism detected with FDG-PET, or 3) changes in protein expression or metabolite amount in the brain or body detectable by imaging or protein/metabolite detection in cerebrospinal fluid, brain biopsies or plasma obtained from patients by ELISA, (such as changes in levels and or ratios of Abeta 42, phosphorylated tau, total tau measured by ELISA, or patterns of protein expression changes detectable in an ELISA panel (see reference: Wyss-Coray T. et al. Modeling of pathological traits in Alzheimer's disease based on systemic extracellular signaling proteome. *Mol Cell Proteomics* 2011 Jul 8, which is hereby incorporated by reference in its entirety), 4) cerebral vascular abnormalities as measured by the presence of vascular edema or microhemorrhage detectable by MRI and any other symptoms detectable by imaging techniques, and 5) cognitive loss as measured by any administered cognitive test such as ADAS-Cog, MMSE, CBIC or any other cognitive testing instrument.

[093] As used herein, the term "a neuronal cell" can be used to refer to a single cell or to a population of cells. In some embodiments, the neuronal cell is a primary neuronal cell. In some embodiments, the neuronal cell is an immortalized or transformed neuronal cell or a stem cell. A primary neuronal cell is a neuronal cell that cannot differentiate into other types of neuronal cells, such as glia cells. A stem cell is one that can differentiate into neurons and other types of neuronal cells

such as glia. In some embodiments, the composition comprising at least one neuronal cell is free of glia cells. In some embodiments, the composition comprises less than about 30%, 25%, 20%, 15%, 10%, 5%, or 1% of glia cells, which are known to internalize and accumulate Abeta. The primary neuronal cell can be derived from any area of the brain of an animal. In some embodiments, the neuronal cell is a hippocampal or cortical cell. The presence of glia cells can be determined by any method. In some embodiments, glia cells are detected by the presence of GFAP and neurons can be detected by staining positively with antibodies directed against MAP2.

[094] The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are generally regarded as safe and nontoxic. In particular, pharmaceutically acceptable carriers, diluents or other excipients used in the pharmaceutical compositions of this invention are physiologically tolerable, compatible with other ingredients, and do not typically produce an allergic or similar untoward reaction (for example, gastric upset, dizziness and the like) when administered to a patient. Preferably, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly in humans. The phrase "pharmaceutically acceptable salt(s)", as used herein, includes those salts of compounds of the invention that are safe and effective for use in mammals and that possess the desired biological activity. Pharmaceutically acceptable salts include salts of acidic or basic groups present in compounds of the invention or in compounds identified pursuant to the methods of the invention. Pharmaceutically acceptable acid addition salts include, but are not limited to, hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, tartrate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzensulfonate, p-toluenesulfonate and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Certain compounds of the invention can form pharmaceutically acceptable salts with various amino acids. Suitable base salts

include, but are not limited to, aluminum, calcium, lithium, magnesium, potassium, sodium, zinc, iron and diethanolamine salts. Pharmaceutically acceptable base addition salts are also formed with amines, such as organic amines. Examples of suitable amines are N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine.

[095] As used herein, the term "therapeutic" means an agent utilized to treat, combat, ameliorate, protect against or improve an unwanted condition or disease of a subject.

[096] As used herein, the term "effective amount" refers to an amount that results in measurable inhibition of at least one symptom or parameter of a specific disorder or pathological process. For example, an amount of a sigma-2 ligand of the present invention that provides a measurably lower synapse reduction in the presence of Abeta oligomer qualifies as an effective amount because it reduces a pathological process even if no clinical symptoms of amyloid pathology are altered, at least immediately.

[097] A "therapeutically effective amount" or "effective amount" of a compound or composition of the invention is a predetermined amount which confers a therapeutic effect on the treated subject, at a reasonable benefit/risk ratio applicable to any medical treatment. The therapeutic effect may be objective (i.e., measurable by some test or marker) or subjective (i.e., subject gives an indication of or feels an effect or physician observes a change). An effective amount of a compound of the invention may broadly range from about 0.01 mg/Kg to about 500 mg/Kg, about 0.1 mg/Kg to about 400 mg/Kg, about 1 mg/Kg to about 300 mg/Kg, about 0.05 to about 20 mg/Kg, about 0.1 mg/Kg to about 10 mg/Kg, or about 10 mg/Kg to about 100 mg/Kg. The effect contemplated herein includes both medical therapeutic and/or prophylactic treatment, as appropriate. The specific dose of a compound administered according to this invention to obtain therapeutic and/or prophylactic effects will, of course, be determined by the particular circumstances surrounding the case, including, for example, the compound administered, the route of administration, the co-administration of other active ingredients, the condition

being treated, the activity of the specific compound employed, the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed and the duration of the treatment;. The effective amount administered will be determined by the physician in the light of the foregoing relevant circumstances and the exercise of sound medical judgment. A therapeutically effective amount of a compound of this invention is typically an amount such that when it is administered in a physiologically tolerable excipient composition, it is sufficient to achieve an effective systemic concentration or local concentration in the tissue. The total daily dose of the compounds of this invention administered to a human or other animal in single or in divided doses can be in amounts, for example, from 0.01 mg/Kg to about 500 mg/Kg, about 0.1 mg/Kg to about 400 mg/Kg, about 1 mg/Kg to about 300 mg/Kg, about 10 mg/Kg to about 100 mg/Kg, or more usually from 0.1 to 25 mg/kg body weight per day. Single dose compositions may contain such amounts or submultiples thereof to make up the daily dose. In general, treatment regimens according to the present invention comprise administration to a patient in need of such treatment will usually include from about 1 mg to about 5000 mg, 10 mg to about 2000 mg of the compound(s), 20 to 1000 mg, preferably 20 to 500 mg and most preferably about 50 mg, of this invention per day in single or multiple doses.

[098] The terms "treat", "treated", or "treating" as used herein refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to protect against (partially or wholly)or slow down (lessen) an undesired physiological condition, disorder or disease, or to obtain beneficial or desired clinical results such as partial or total restoration or inhibition in decline of a parameter, value, function or result that had or would become abnormal. For the purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent or vigor or rate of development of the condition, disorder or disease; stabilization (*i.e.*, not worsening) of the state of the condition, disorder or disease; delay in onset or slowing of the progression of the condition, disorder or disease; amelioration of the condition, disorder or disease state; and remission (whether partial or total), whether or not it

translates to immediate lessening of actual clinical symptoms, or enhancement or improvement of the condition, disorder or disease. Treatment seeks to elicit a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment.

[099] Generally speaking, the term "tissue" refers to any aggregation of similarly specialized cells which are united in the performance of a particular function.

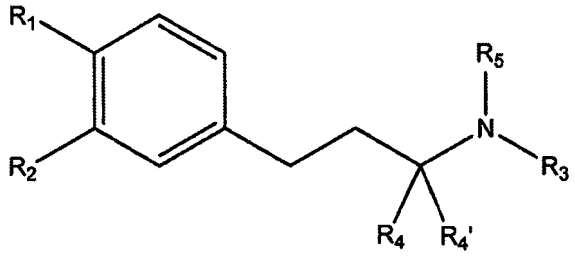
[0100] As used herein, "cognitive decline" can be any negative change in an animal's cognitive function. For example cognitive decline, includes but is not limited to, memory loss (e.g. behavioral memory loss), failure to acquire new memories, confusion, impaired judgment, personality changes, disorientation, or any combination thereof. A compound that is effective to treat cognitive decline can be thus effective by restoring long term neuronal potentiation (LTP) or long term neuronal depression (LTD) or a balance of synaptic plasticity measured electrophysiologically; inhibiting, treating, and/or abatement of neurodegeneration; inhibiting, treating, and/or abatement of general amyloidosis; inhibiting, treating, abatement of one or more of amyloid production, amyloid assembly, amyloid aggregation, and amyloid oligomer binding; inhibiting, treating, and/or abatement of a nonlethal effect of one or more of Abeta species on a neuron cell (such as synapse loss or dysfunction and abnormal membrane trafficking); and any combination thereof. Additionally, that compound can also be effective in treating Abeta related neurodegenerative diseases and disorders including, but not limited to dementia, including but not limited to Alzheimer's Disease (AD) including mild Alzheimer's disease, Down's syndrome, vascular dementia (cerebral amyloid angiopathy and stroke), dementia with Lewy bodies, HIV dementia, Mild Cognitive Impairment (MCI); Age-Associated Memory Impairment (AAMI); Age-Related Cognitive Decline (ARCD), preclinical Alzheimer's Disease (PCAD); and Cognitive Impairment No Dementia (CIND).As used herein, the term "natural ligand" refers to a ligand present in a subject that can bind to a protein, receptor, membrane lipid or other binding partner *in vivo* or that is replicated *in vitro*. The natural ligand can be

synthetic in origin, but must also be present naturally and without human intervention in the subject. For example, Abeta oligomers are known to exist in human subjects. Therefore the Abeta oligomers found in a subject would be considered natural ligands. The binding of Abeta oligomers to a binding partner can be replicated *in vitro* using recombinant or synthetic techniques, but the Abeta oligomer would still be considered a natural ligand regardless of how the Abeta oligomer is prepared or manufactured. A synthetic small molecule that can also bind to the same binding partner is not a natural ligand if it does not exist in a subject. For example, Compound II, which is described herein, is not normally present in a subject, and, therefore, would not be considered a natural ligand.

Novel Compounds of the Invention

[0101] The compounds described herein can be synthesized according to the methods described herein or as described in WO 2011/014880 (Application No. PCT/US2010/044136), WO 2010/118055 (Application No. PCT/US2010/030130), Application No. PCT/US2011/026530, and WO 2012/106426 (Application No. PCT/US2012/023483), each of which are hereby incorporated by reference in their entireties. Additional options for preparing these compounds are discussed in detail below.

[0102] In some embodiments, the sigma-2 antagonists of the present invention are those of Formula I

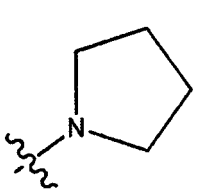
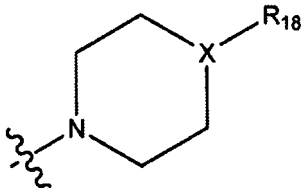


I

wherein

R_1 and R_2 are independently selected from H, OH, halo, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, $(R_{16})(R_{17})N\text{-}C_{1-4}$ alkylene-O-, or R1 and R2 are linked together to form a $-O\text{-}C_{1-2}$ methylene-O- group, wherein

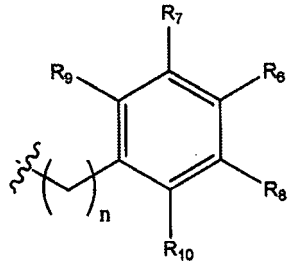
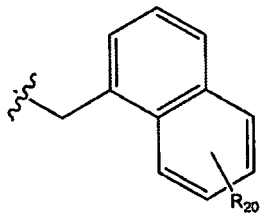
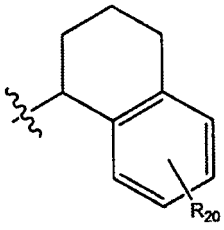
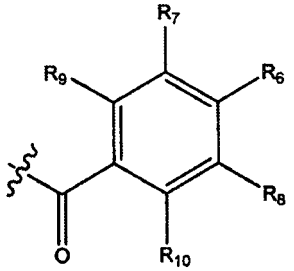
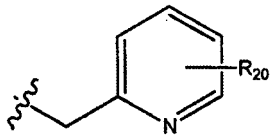
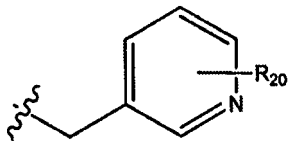
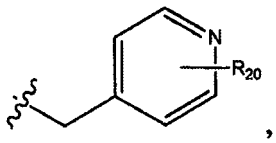
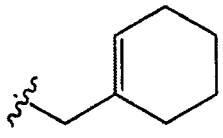
R_{16} and R_{17} are independently C_{1-4} alkyl or benzyl, or R_{16} and R_{17} together with nitrogen form a ring selected from


, and

, wherein

X is N or O and R_{18} is H or unsubstituted phenyl; and

wherein at least one of R_1 and R_2 is not H;

R_3 is selected from


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, and


wherein

R_6, R_7, R_8, R_9, and R_{10}, are independently selected from H, halo, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, and $S(O)_2$- C_{1-6} alkyl;

R_{20} is H; and

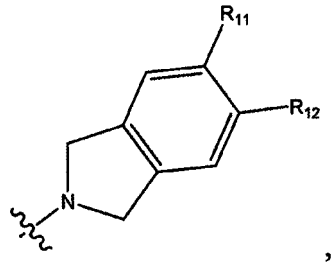
n is 1-4

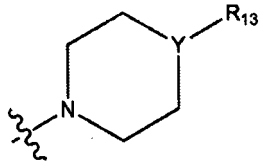
R_4 is C_{1-6} alkyl;

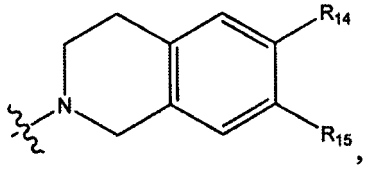
$R_{4'}$ is H or C_{1-6} alkyl; and

R_5 is H, C_{1-6} alkyl, and C(O)O(C_{1-4} alkyl), C(O)(C_{1-4} alkyl), or C(O)(C_{1-4}haloalkyl); or

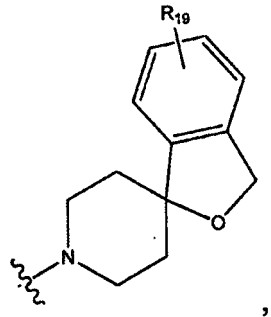
R_3 and R_5 together with nitrogen form a ring selected from



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and , wherein

R_{11} and R_{12}, are independently selected from H, halo, and C_{1-6} haloalkyl, and

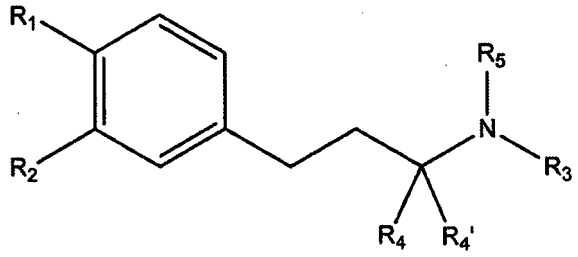
Y is CH or N;

R_{13}.is H, C_{1-6} alkyl, C_{3-6} cycloalkyl, unsubstituted phenyl or phenyl substituted with C_{1-6} haloalkyl, or unsubstituted benzyl

R_{14} and R_{15} are independently selected from H and halo;

R_{19} is H, or pharmaceutically acceptable salts thereof.

[0103] In some embodiments, the sigma-2 antagonists of the present invention are those of Formula I

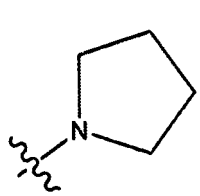
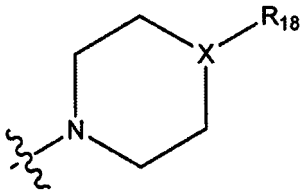


I

wherein

R_1 and R_2 are independently selected from H, OH, halo, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, $(R_{16})(R_{17})N$-C_{1-4} alkylene-O-, or R1 and R2 are linked together to form a –O-C_{1-2} methylene-O- group, wherein

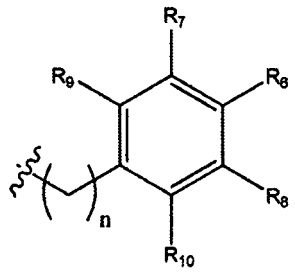
R_{16} and R_{17} are independently C_{1-4} alkyl or benzyl, or R_{16} and R_{17} together with nitrogen form a ring selected from

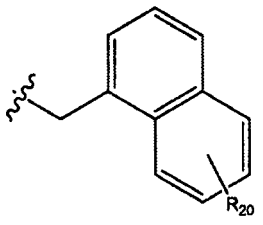

, and

, wherein

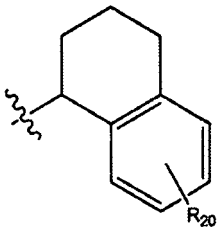
X is N or O and R_{18} is absent or is H or unsubstituted phenyl; and

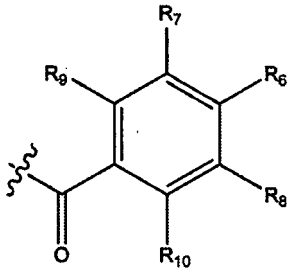
wherein at least one of R_1 and R_2 is not H;

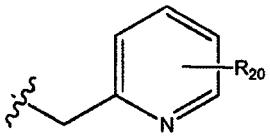
R_3 is selected from

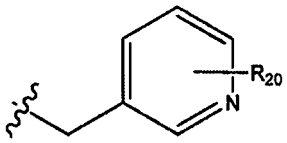


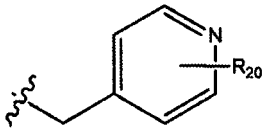
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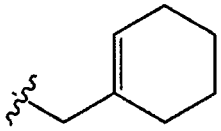
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, and


wherein

R_6, R_7, R_8, R_9, and R_{10}, are independently selected from H, halo, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, and $S(O)_2$- C_{1-6} alkyl;

R_{20} is H; and

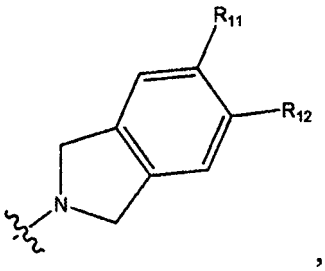
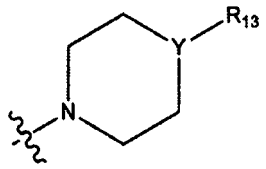
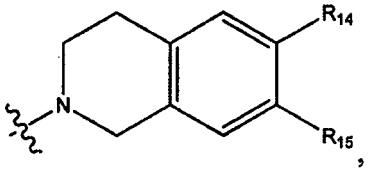
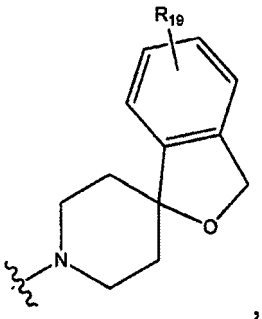
n is 1-4

R_4 is C_{1-6} alkyl;

$R_{4'}$ is H or C_{1-6} alkyl; and

R_5 is H, C_{1-6} alkyl, and C(O)O(C_{1-4} alkyl), C(O)(C_{1-4} alkyl), or C(O)(C_{1-4}haloalkyl); or

R_3 and R_5 together with nitrogen form a ring selected from

and , wherein

R_{11} and R_{12}, are independently selected from H, halo, and C_{1-6} haloalkyl, and

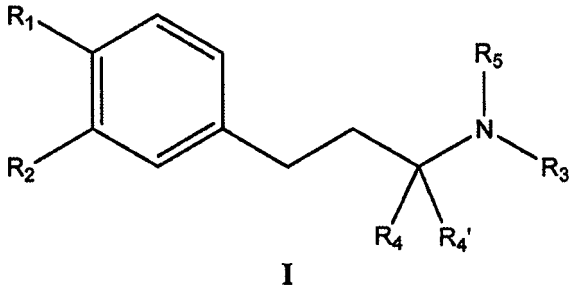
Y is CH or N;

R_{13}.is H, C_{1-6} alkyl, C_{3-6} cycloalkyl, unsubstituted phenyl or phenyl substituted with C_{1-6} haloalkyl, or unsubstituted benzyl

R_{14} and R_{15} are independently selected from H and halo; and

R_{19} is H, or pharmaceutically acceptable salts thereof.

[0104] In some embodiments, the sigma-2 antagonists of the present invention are those of Formula I

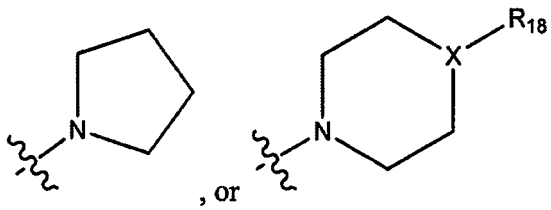


I

wherein

R_1 is selected from OH, OMe, F, Cl, CF_3, $(R_{16})(R_{17})$N-ethylene-O-, wherein

R_{16} and R_{17} are each methyl, isopropyl, n-butyl or benzyl, or R_{16} and R_{17} together with nitrogen form a ring selected from



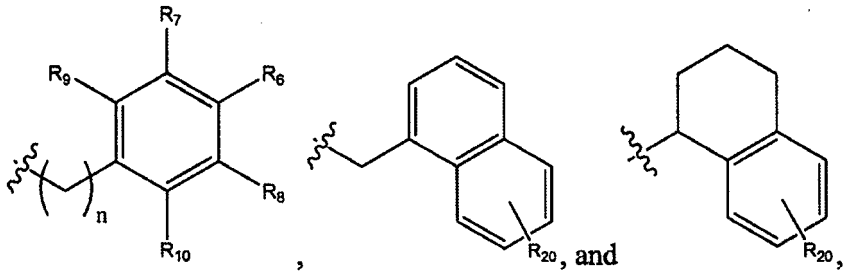
, or , wherein

X is N or O and R_{18} absent or is unsubstituted phenyl; and

R_2 is H, Cl, F, CF_3, OMe, OCF_3 or

R_1 and R_2 are linked together to form a –O-C_{1-2} methylene-O- group

R_3 is selected from



, , and ,

wherein

R_6 is H, F, Cl, Me, isopropyl, t-butyl, OMe, CF_3, or $S(O)_2Me$,

R_7 and R_8 are independently H, OMe, F, Cl, or CF_3,

R_9, and R_{10} are independently selected from H, OMe, F, and Cl,

R_{20} is H; and

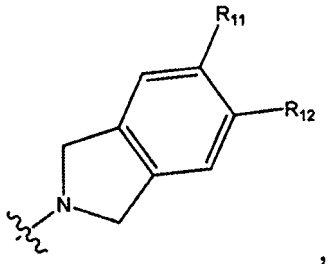
n is 1

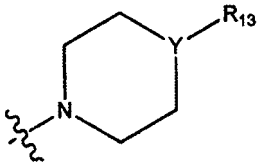
R_4 is Me;

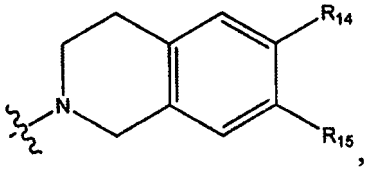
$R_{4'}$ is H or Me; and

R_5 is H; or

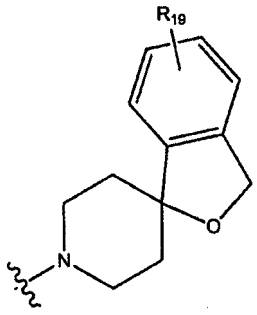
R_3 and R_5 together with nitrogen form a ring selected from



,


,


,



and , wherein

R_{11} and R_{12}, are independently selected from H, Cl, and CF_3, and

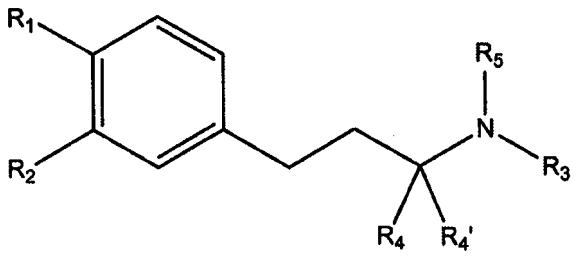
Y is CH or N;

R_{13}.is H, Me, cyclohexyl, unsubstituted phenyl or phenyl substituted with CF_3, or unsubstituted benzyl

R_{14} and R_{15} are independently selected from H and Cl; and

R_{19} is H, or pharmaceutically acceptable salts thereof.

[0105] In some embodiments, the sigma-2 antagonists of the present invention are those of Formula I

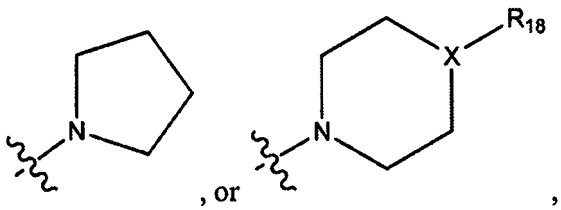


I

wherein

R_1 is selected from OH, OMe, F, Cl, CF_3, $(R_{16})(R_{17})$N-ethylene-O-, wherein

R_{16} and R_{17} are each methyl, isopropyl, n-butyl or benzyl, or R_{16} and R_{17} together with nitrogen form a ring selected from



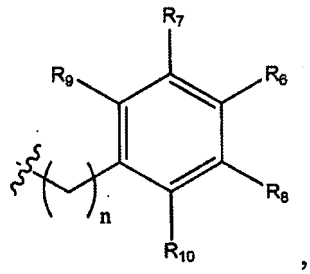
, or , wherein

X is N or O and R_{18} absent or is unsubstituted phenyl; and

R_2 is H, Cl, F, CF_3, OMe, OCF_3 or

R_1 and R_2 are linked together to form a $-O-C_{1-2}$ methylene-O- group

R_3 is selected from



,

wherein

R_6 is H, F, Cl, Me, isopropyl, t-butyl, OMe, CF_3, or $S(O)_2Me$,

R_7 and R_8 are indenpendently H, OMe, F, Cl, or CF_3,

R_9, and R_{10} are independently selected from H, OMe, F, and Cl, and

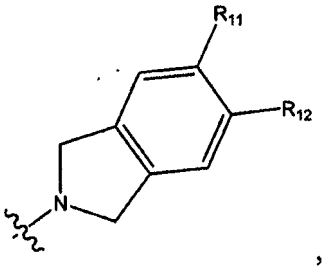
n is 1

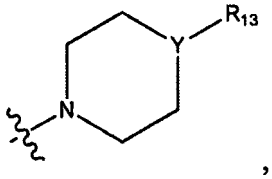
R_4 is Me;

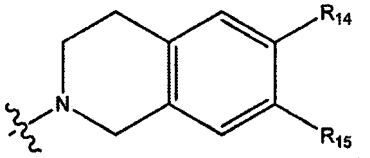
$R_{4'}$ is H; and

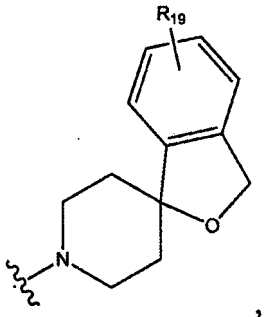
R_5 is H; or

R_3 and R_5 together with nitrogen form a ring selected from



,


,


,


and , wherein

R_{11} and R_{12}, are independently selected from H, Cl, and CF_3, and

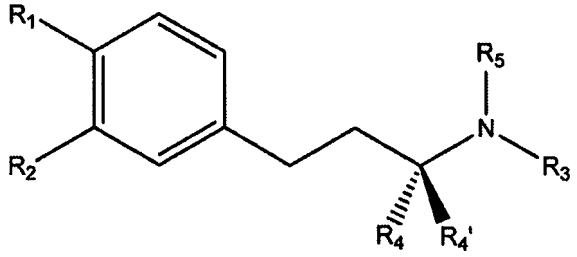
Y is CH or N;

R_{13}.is H, Me, cyclohexyl, unsubstituted phenyl or phenyl substituted with CF_3, or unsubstituted benzyl

R_{14} and R_{15} are independently selected from H and Cl; and

R_{19} is H, or pharmaceutically acceptable salts thereof.

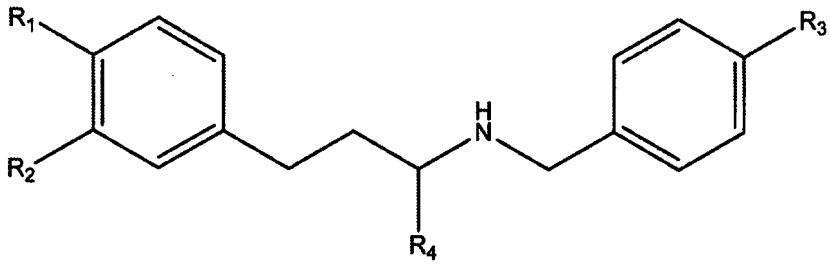
[0106] In some more specific embodiments, the sigma-2 antagonists of the present invention are those of Formula Ia



Ia

wherein $R_{4'}$ is H and the remaining groups are as defined above for the compounds of Formula I, or pharmaceutically acceptable salts thereof.

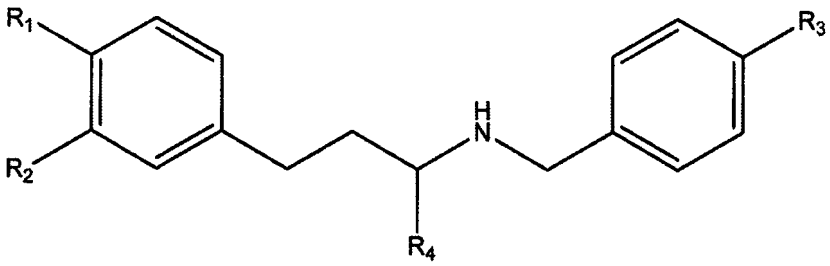
[0107] In some embodiments, the sigma-2 antagonists of the present invention are those of Formula IIa:



IIa

wherein
R_1 = halo, C_{1-6} haloalkyl, or OH;
R_2 = H, halo or C_{1-6} haloalkyl, or R_1 and R_2 are linked together to form a -O-methylene-O- group;
R_3 = C_{1-6} haloalkyl; and
R_4 = C_{1-6} alkyl, or pharmaceutically acceptable salts thereof.

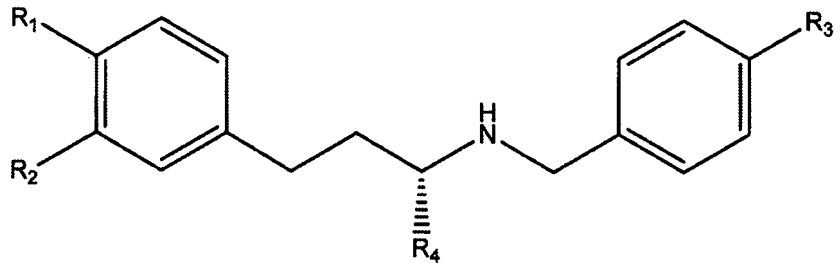
[0108] In some more specific embodiments, the sigma-2 antagonists of the present invention are those of Formula IIa.



IIa

wherein
R_1 = Cl, F, CF_3, or OH;
R_2 = H, Cl, F, CF_3, or R_1 and R_2 are linked together to form a -O-ethylene-O- group;
R_3 = CF_3; and
R_4 = methyl, or pharmaceutically acceptable salts thereof.

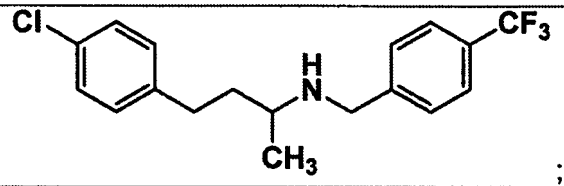
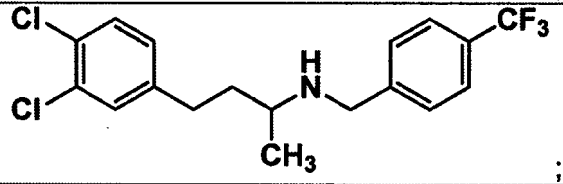
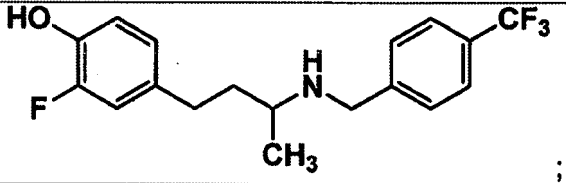
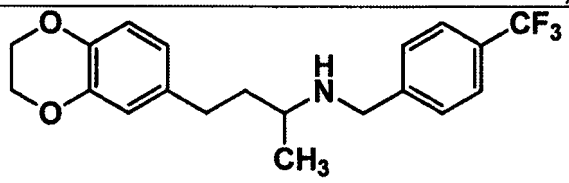
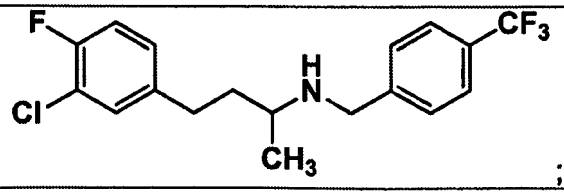
[0109] In some more specific embodiments, the sigma-2 antagonists of the present invention are those of Formula IIb

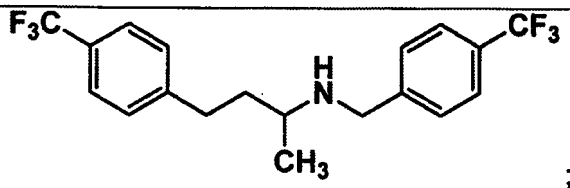


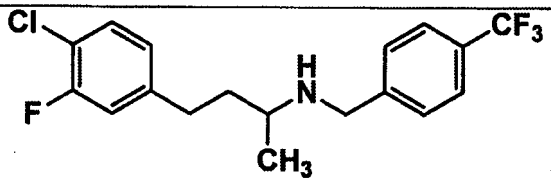
IIb

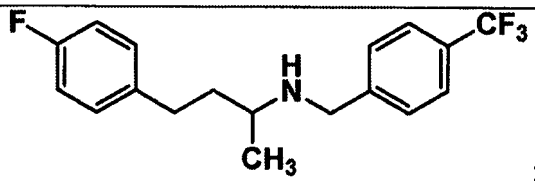
wherein R_1-R_4 are as defined above for the compounds of Formula IIa, or pharmaceutically acceptable salts thereof.

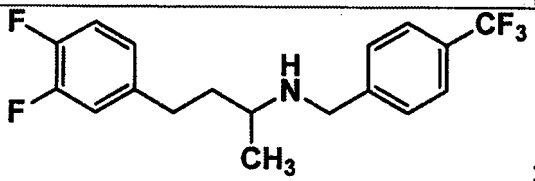
[0110] Specific exemplary compounds of the invention are set forth in the table below:

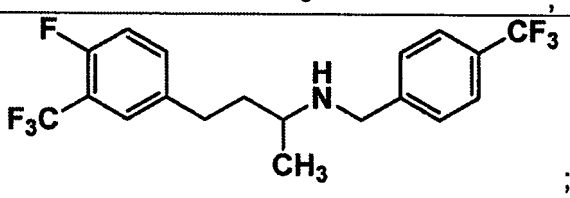
 ;
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 ;
 ;

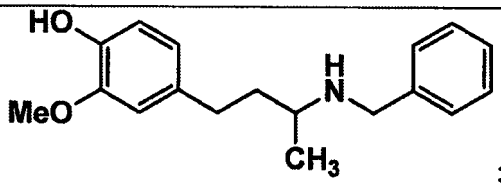

F3C
CF3
H
N
CH3
;

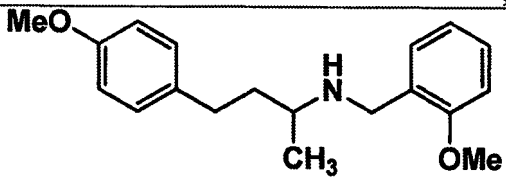

Cl
F
CF3
H
N
CH3
;

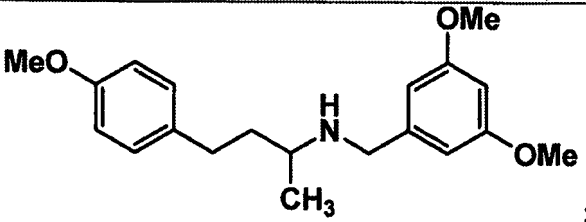

F
CF3
H
N
CH3
;

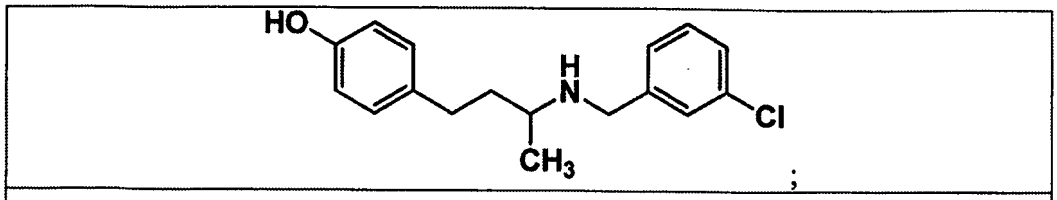

F
F
CF3
H
N
CH3
;

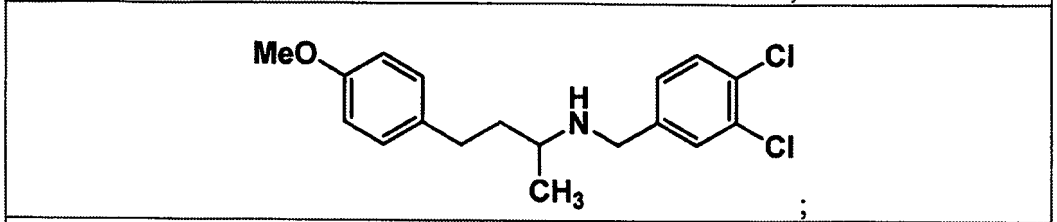

F
F3C
CF3
H
N
CH3
;

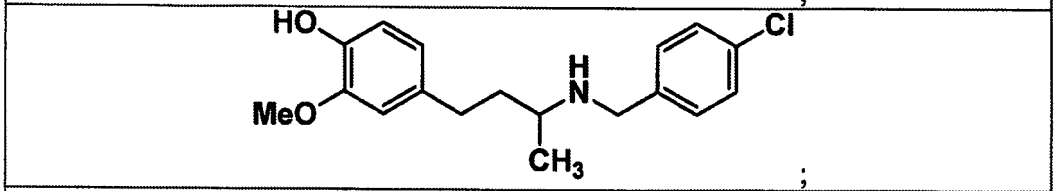

HO
MeO
H
N
CH3
;

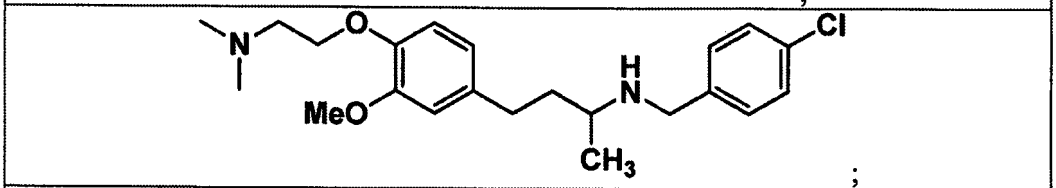

MeO
H
N
CH3
OMe
;

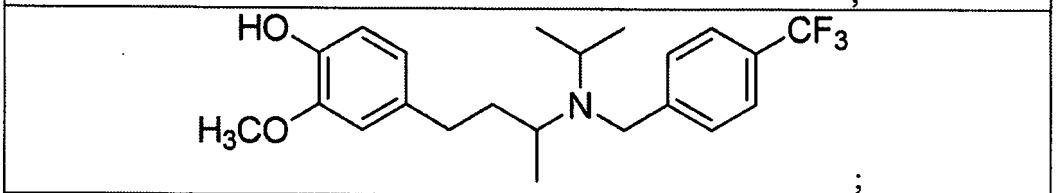

MeO
OMe
H
N
OMe
CH3
;

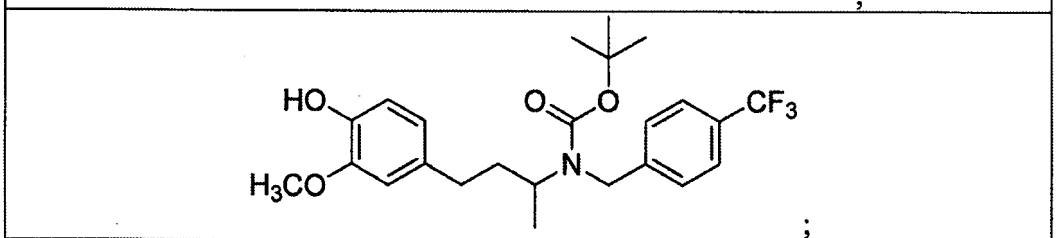

HO
H
N
Cl
CH3
;

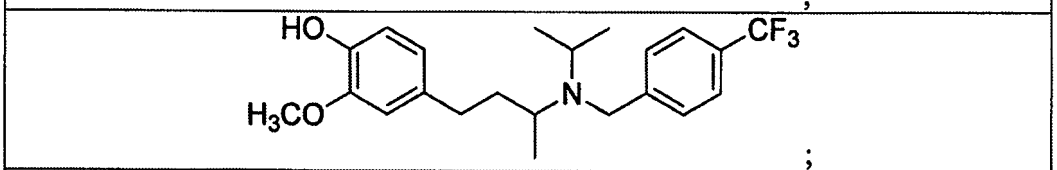

MeO
H
N
Cl
Cl
CH3
;

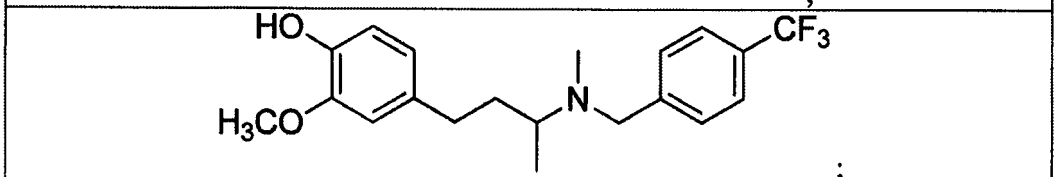

HO
MeO
H
N
Cl
CH3
;

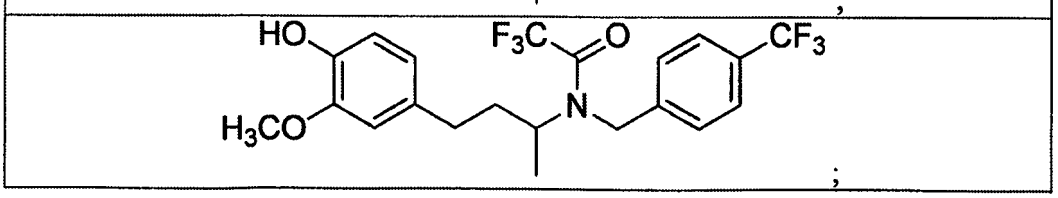

N
O
MeO
H
N
Cl
CH3
;

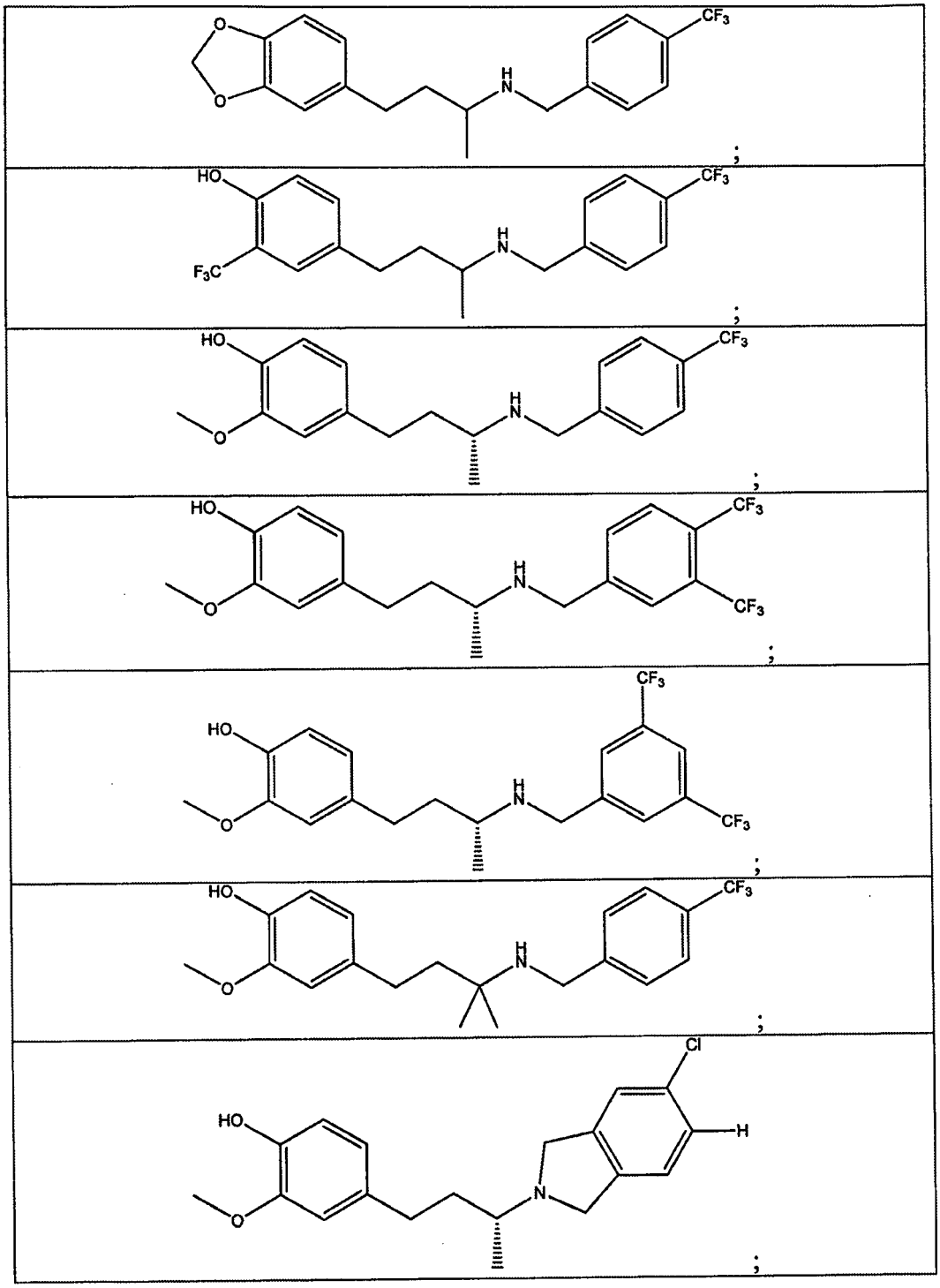

HO
H3CO
N
CF3
;

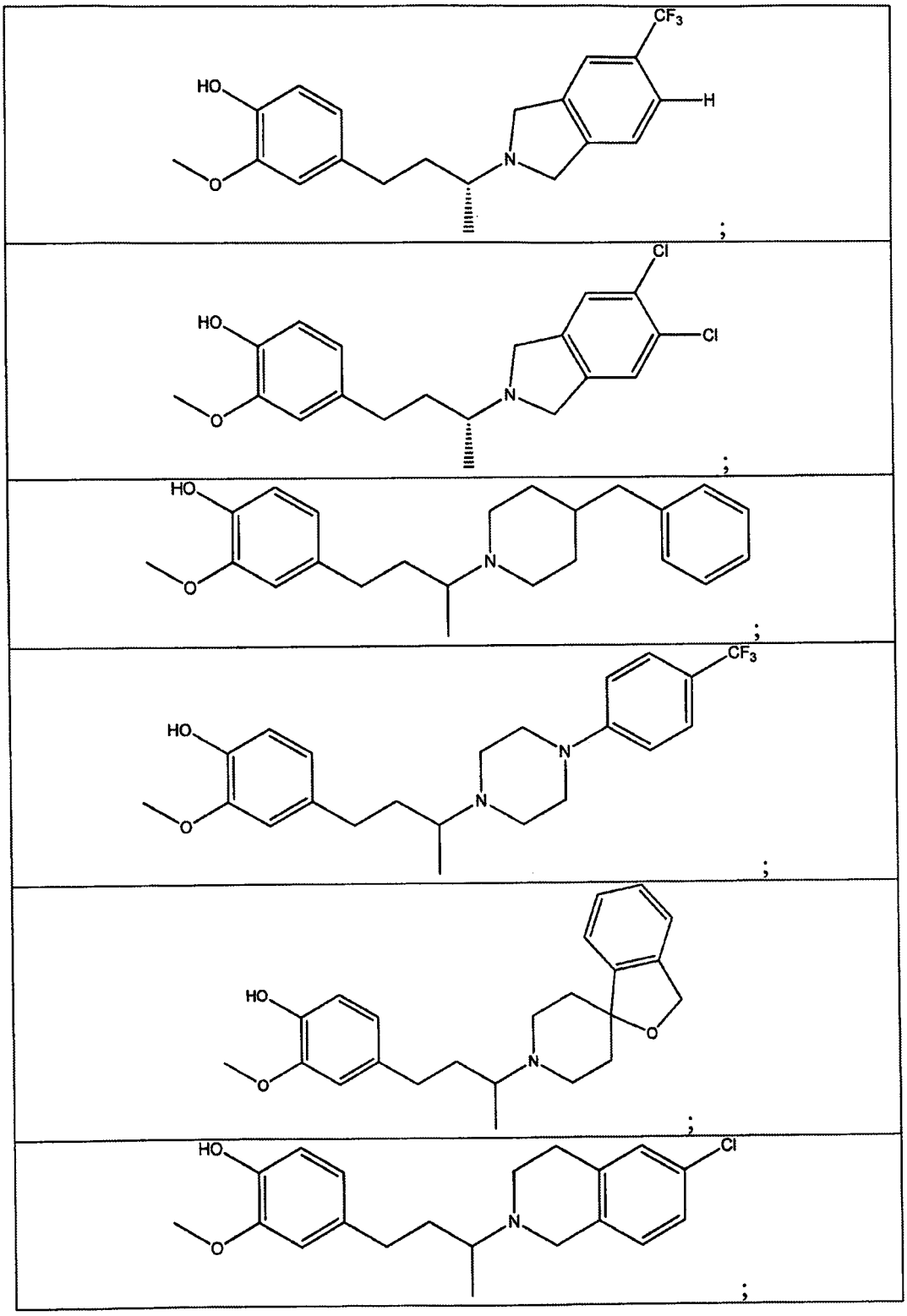

HO
H3CO
O
O
N
CF3
;

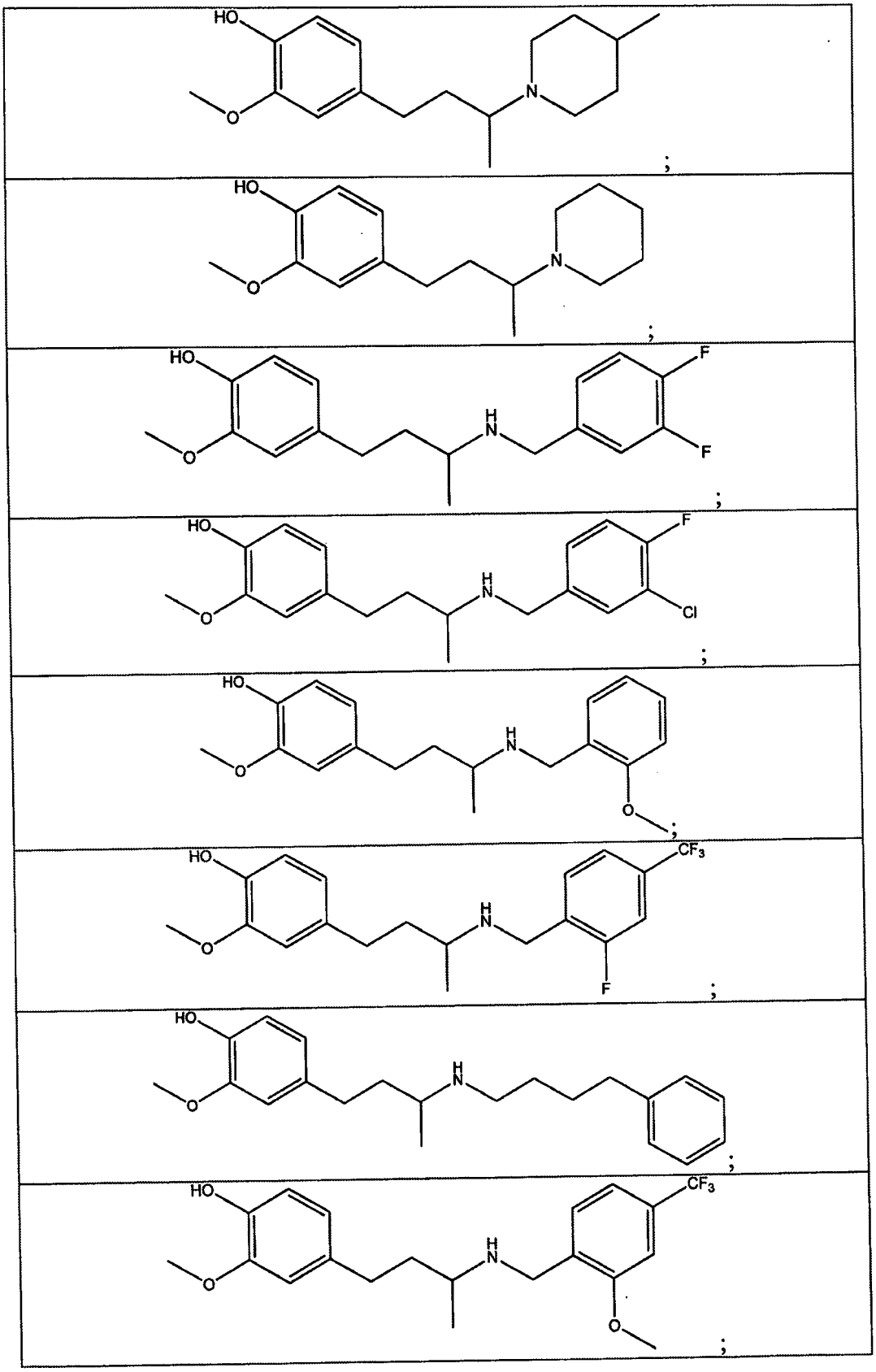

HO
H3CO
N
CF3
;

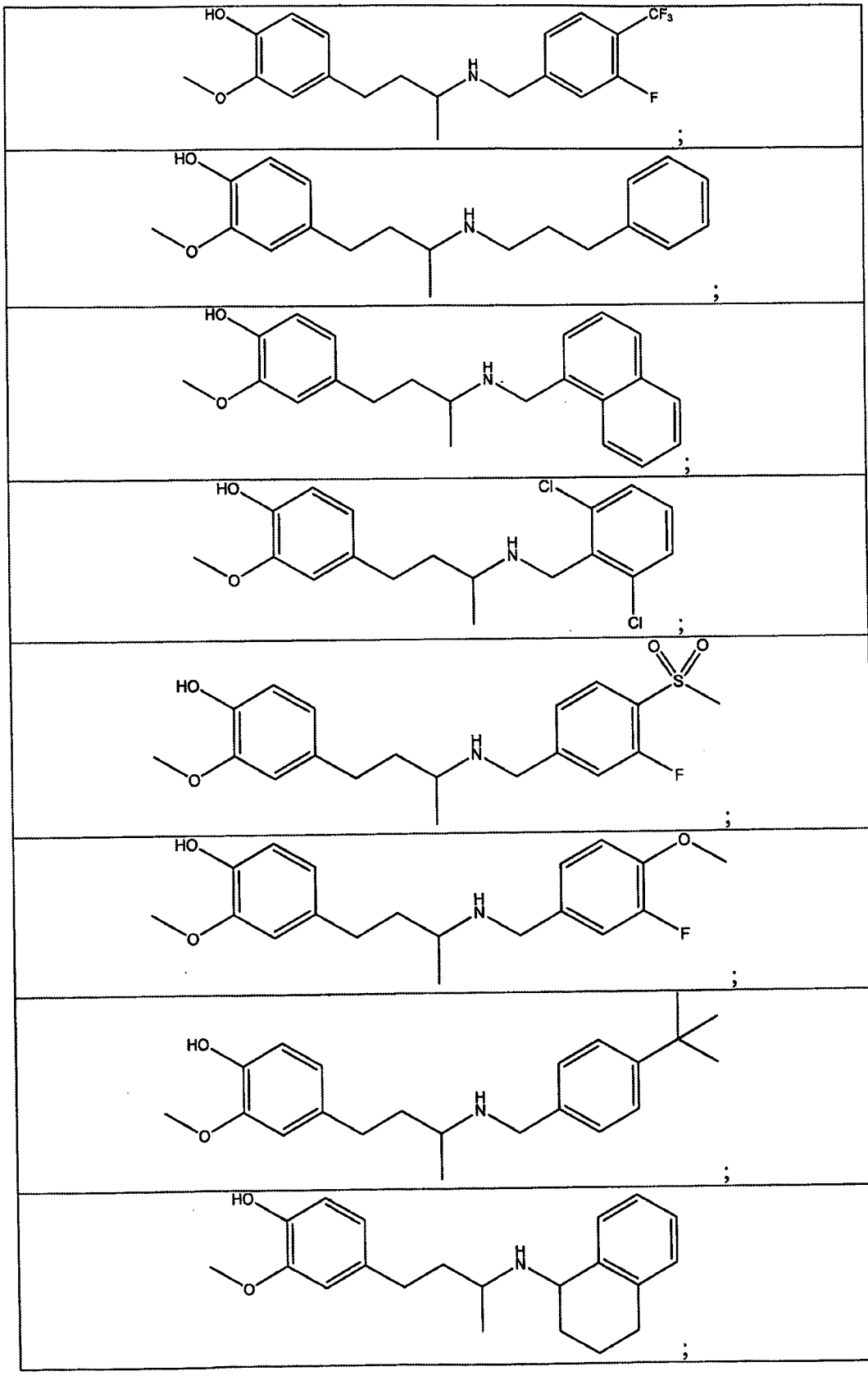

HO
H3CO
N
CF3
;

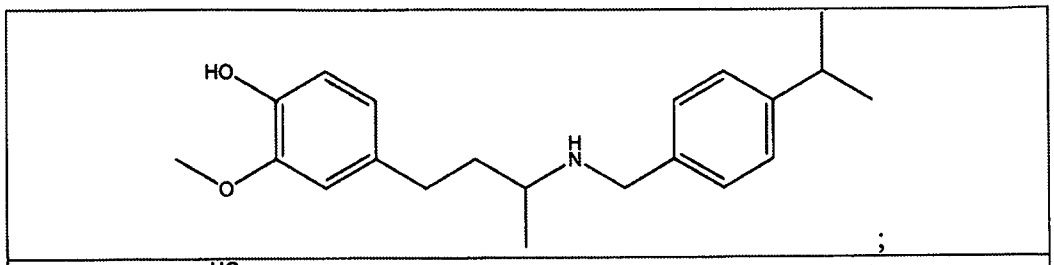

HO
H3CO
F3C
O
N
CF3
;

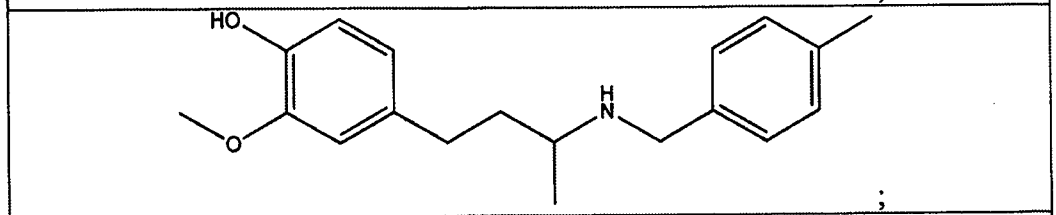


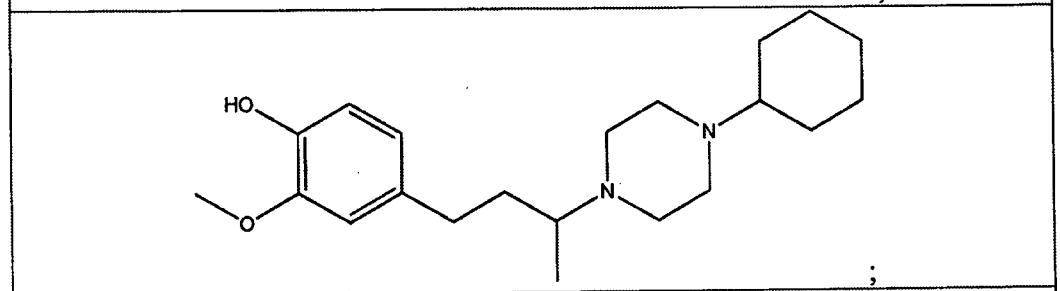


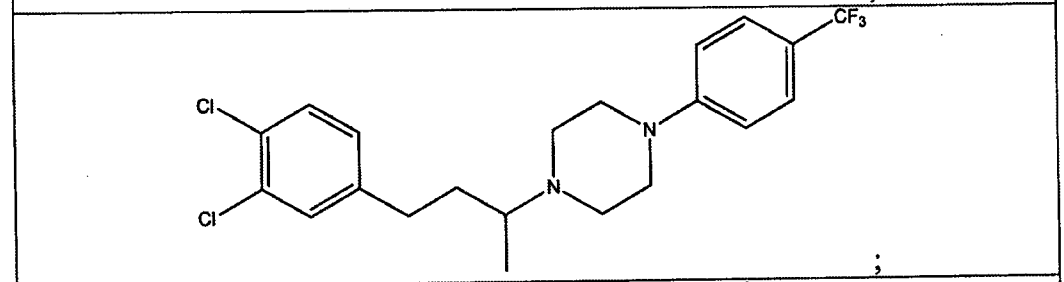


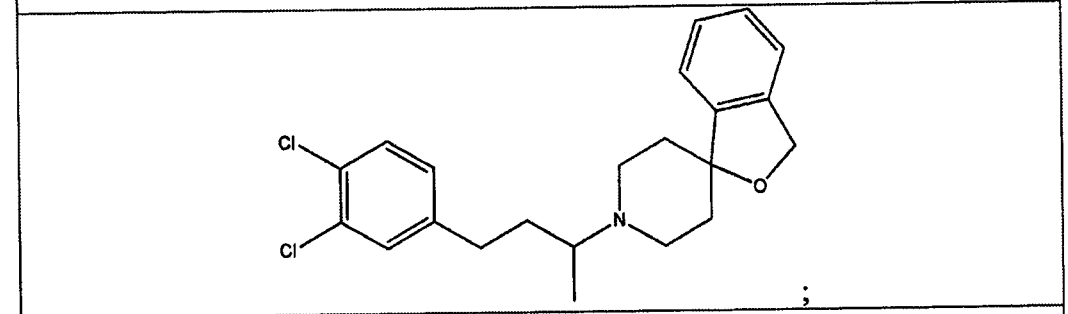


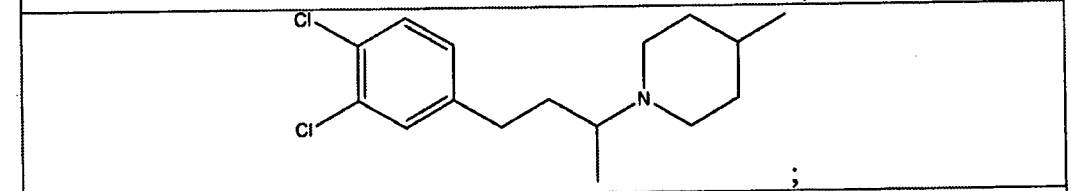

HO
O
H
N
;

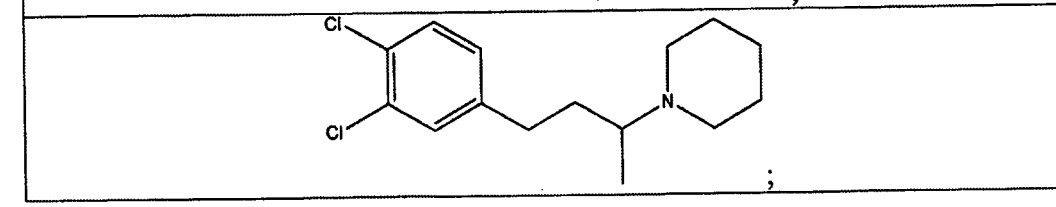

HO
O
H
N
;

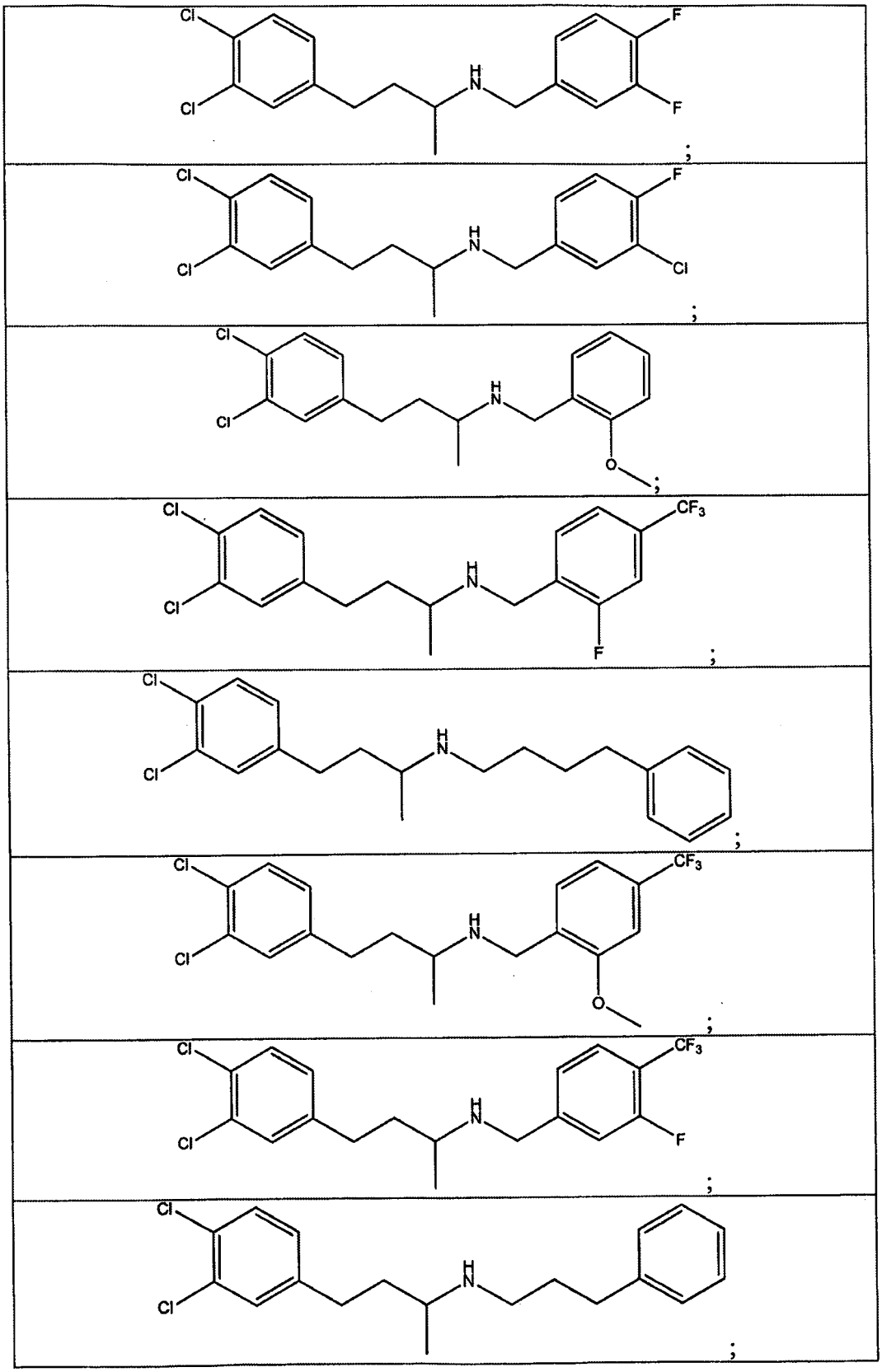

HO
O
N
N
;

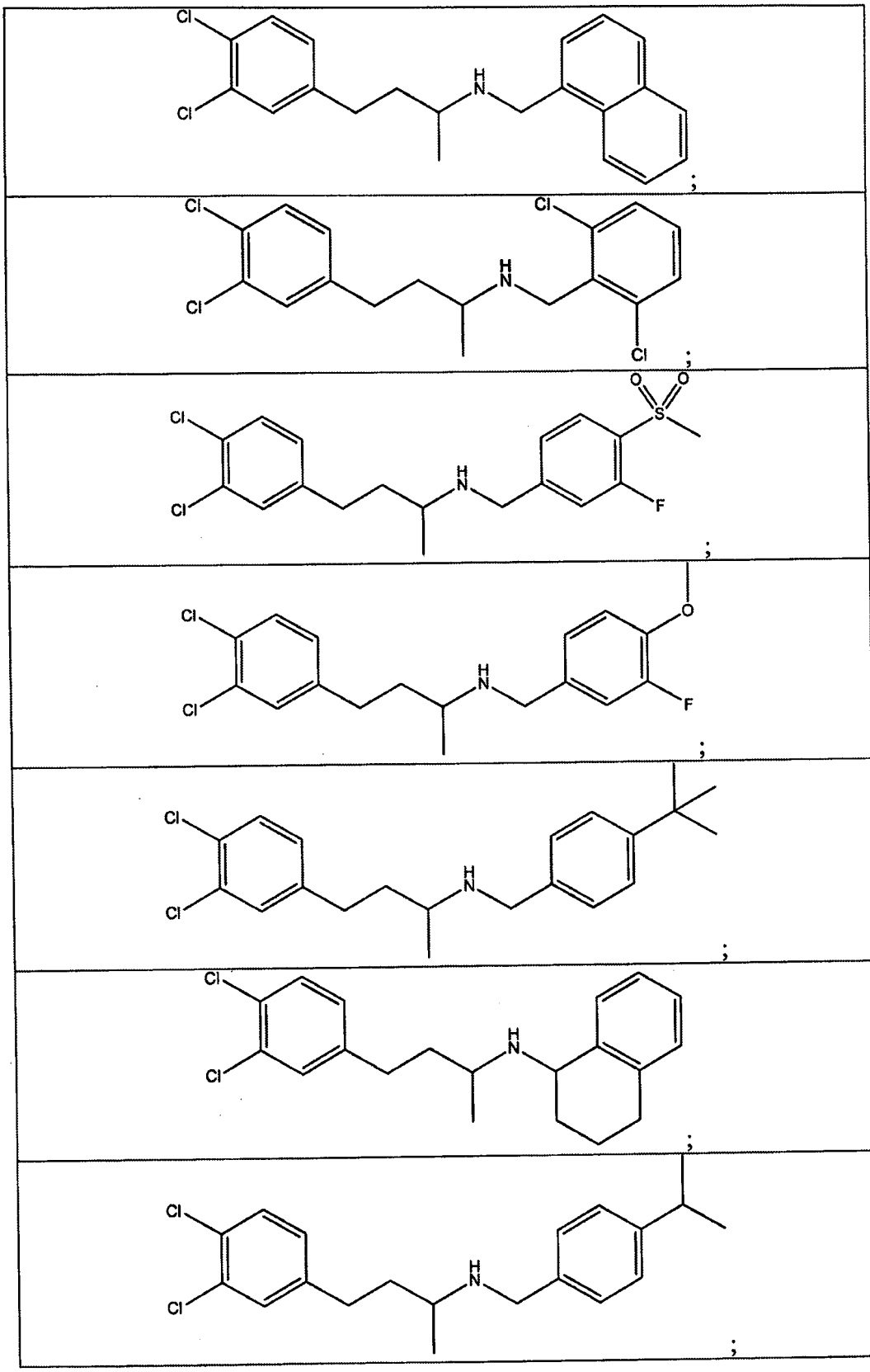

CF3
Cl
Cl
N
N
;

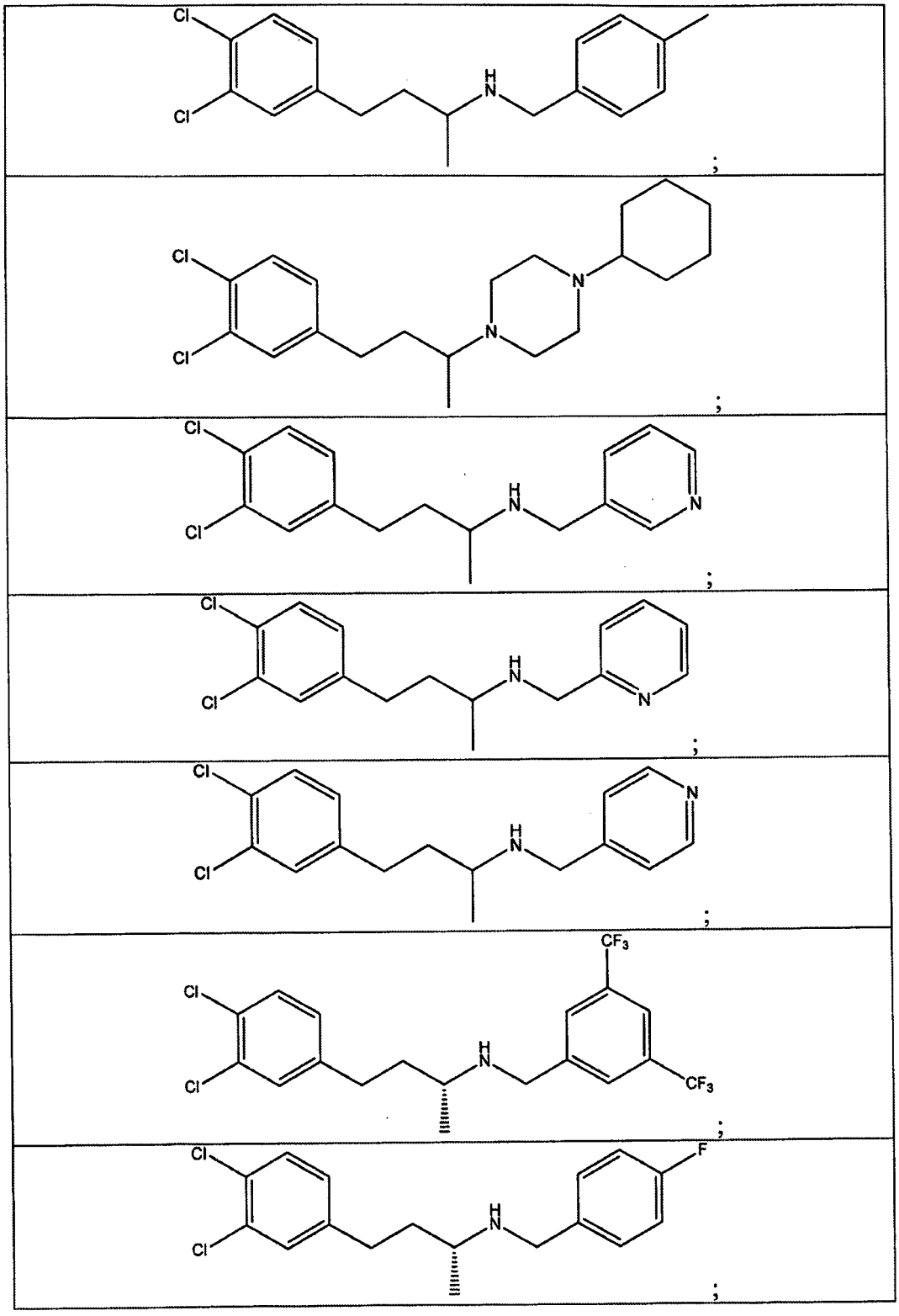

Cl
Cl
O
N
;

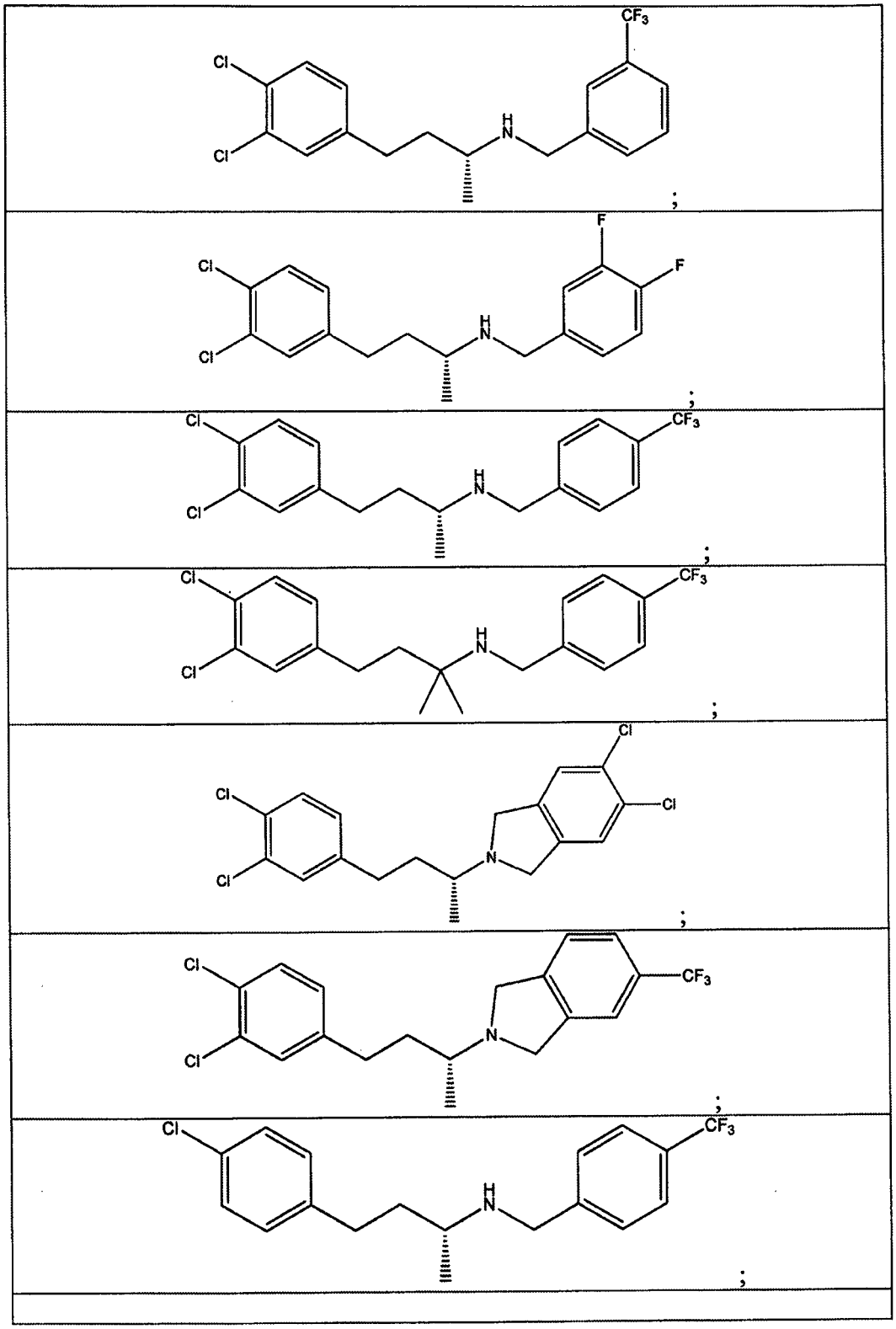

Cl
Cl
N
;

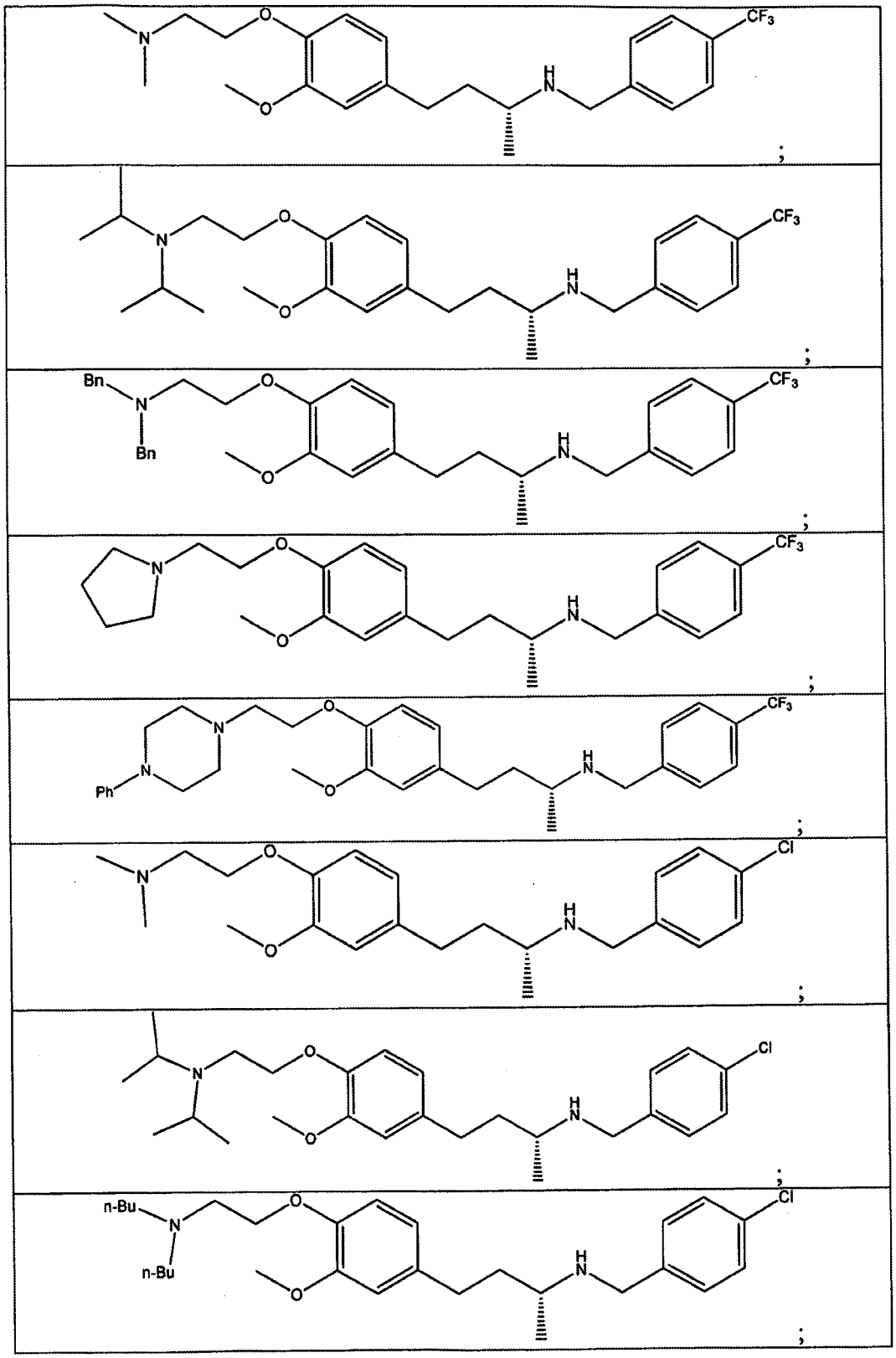

Cl
Cl
N
;

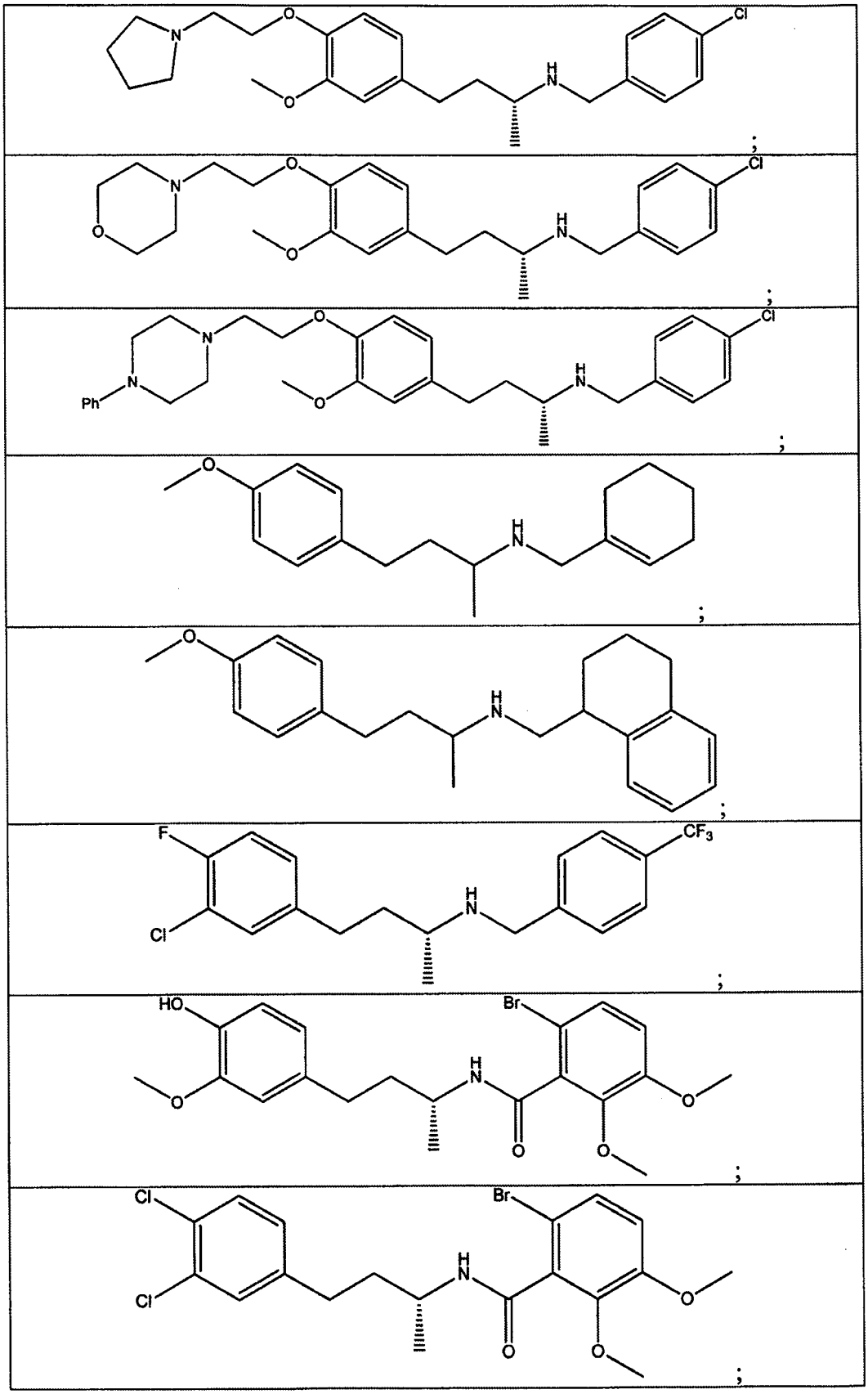


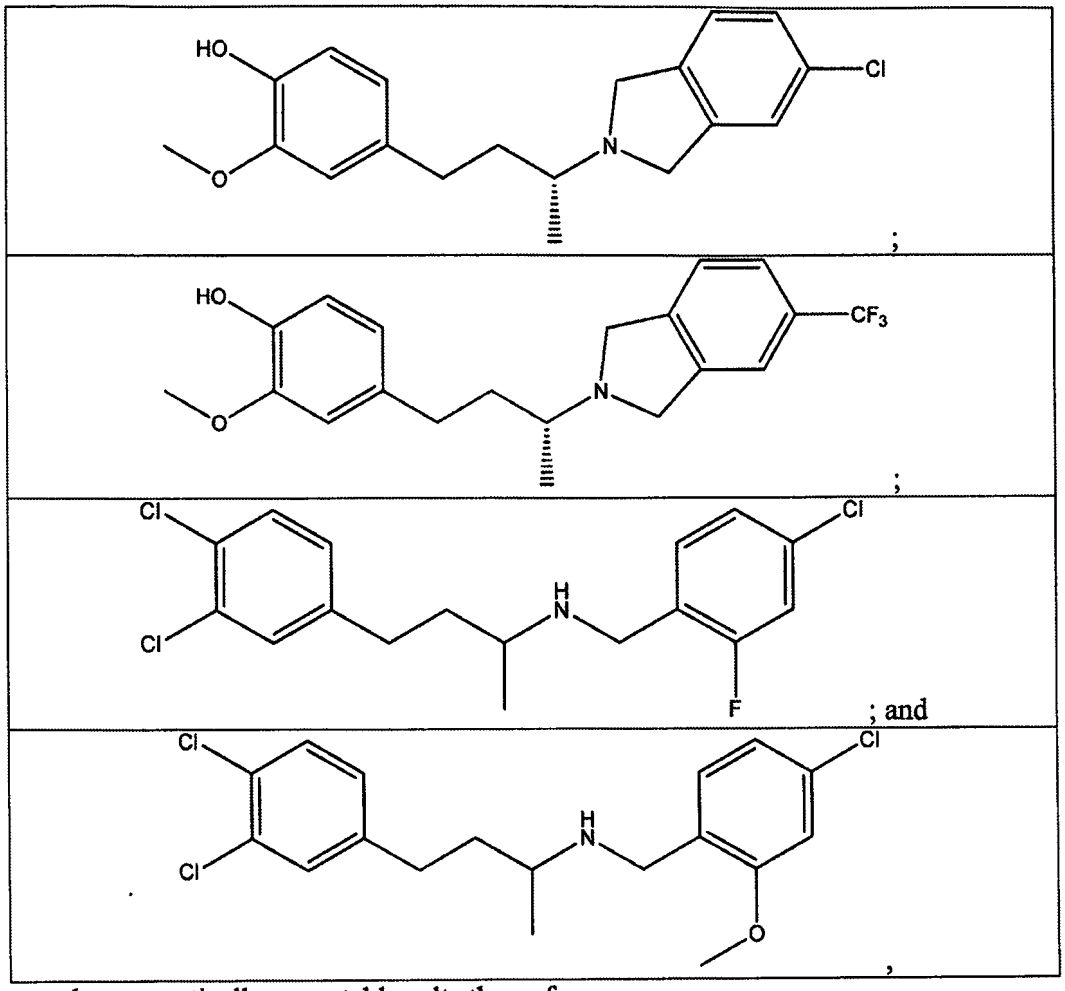






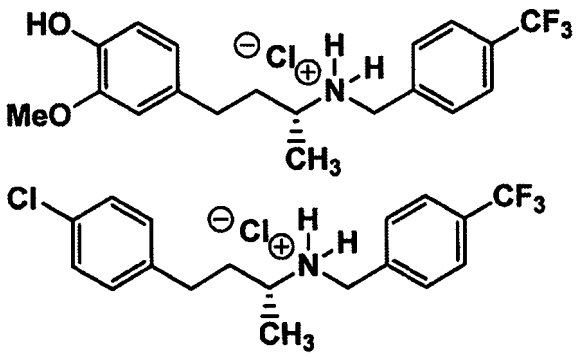


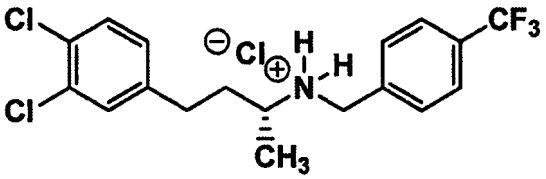


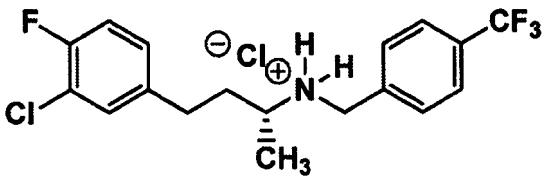


or pharmaceutically acceptable salts thereof.

[0111] Preferred salts for use in the present invention include the hydrochloride salts of the above compounds, including the following:

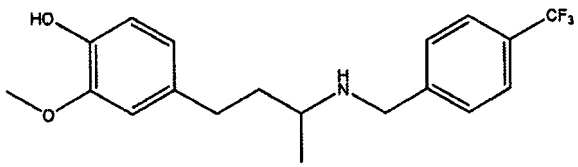






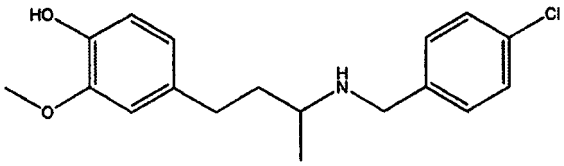
[0112] These have been synthesized in accordance with general methods provided herein and specific synthetic examples with any additional steps being well within the skill in the art. Several of these compounds have been tested in various assays as detailed herein and have been found active. Tested compounds also display increased bioavailability by reference to compounds disclosed in WO 2010/110855.

[0113] Compound II has the formula

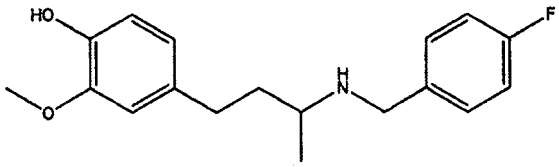


[0114] In some embodiments, each of the general formulae above may contain a proviso to remove the compound of Formula II.

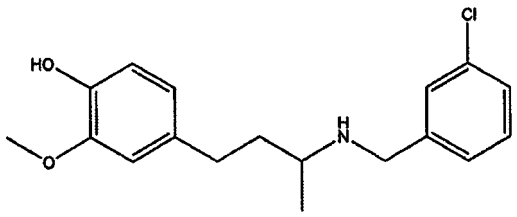
[0115] In some embodiments, each of the general formulae above may contain a proviso to remove one or more of the following compounds:



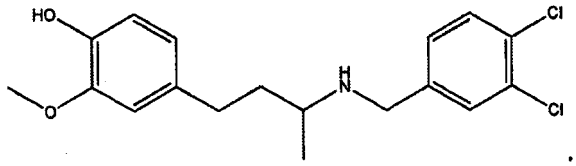
,



,

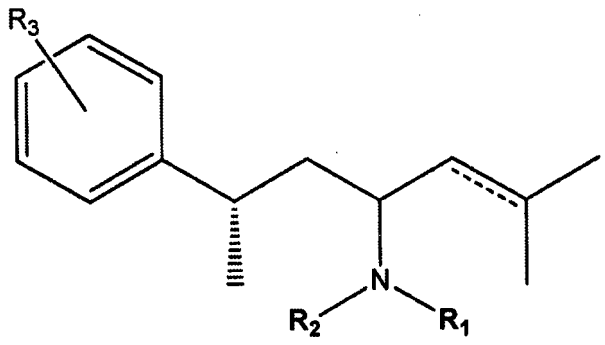


, and



.

[0116] In another embodiment, the sigma-2 antagonists of the present invention are those of Formula VIIIa



VIIIa

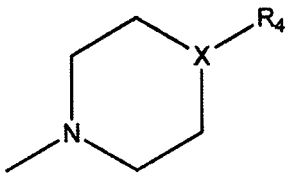
wherein:

-------- is a single bond or a double bond;

R_1 is C_{1-6} alkyl, C_{1-6} haloalkyl, unsubstituted benzyl or benzyl substituted with halo, C_{1-6} alkyl, or C_{1-6} haloalkyl;

R_2 is H, or

R_1 and R_2 together with nitrogen form the ring



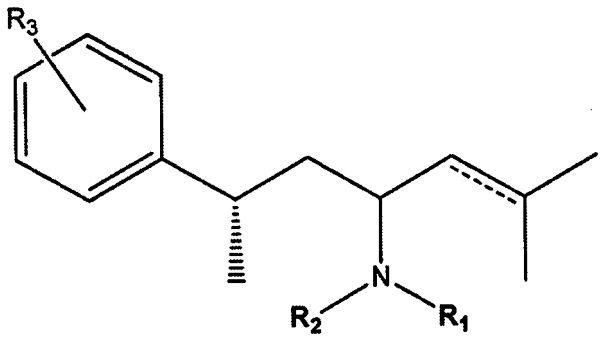
, wherein

X is CH, N, or O, and

R_4 is absent, or is H, C_{1-6} alkyl, or unsubstituted phenyl or phenyl substituted with halo, C_{1-6} alkyl, or C_{1-6} haloalkyl; and

R_3 is C_{1-4} alkyl, halo, or C_{1-6} haloalkoxy, or pharmaceutically acceptable salts thereof.

[0117] In some embodiments, the sigma-2 antagonists of the present invention are those of Formula VIIIa



VIIIa

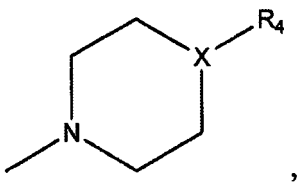
wherein:

======== is a single bond or a double bond;

R_1 is isobutyl, benzyl or benzyl substituted with chloro, methyl, or CF_3;

R_2 is H, or

R_1 and R_2 together with nitrogen form the ring



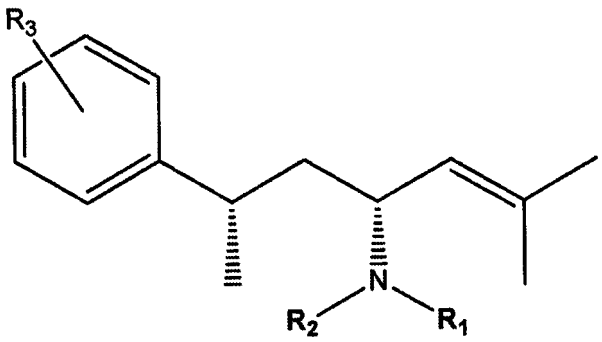
, wherein

X is CH, N, or O, and

R_4 is absent, or is H, isopropyl, or unsubstituted phenyl; and

R_3 is ortho-Me, meta-Me, para-Me, para-F, or para-OCF_3, or pharmaceutically acceptable salts thereof.

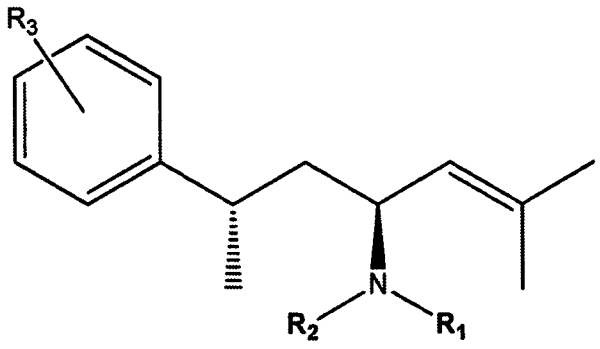
[0118] In some more specific embodiments, the sigma-2 antagonists of the present invention are those of Formula VIIIb



VIIIb

wherein R_1-R_3 are as defined above for Formula VIIIa, or pharmaceutically acceptable salts thereof.

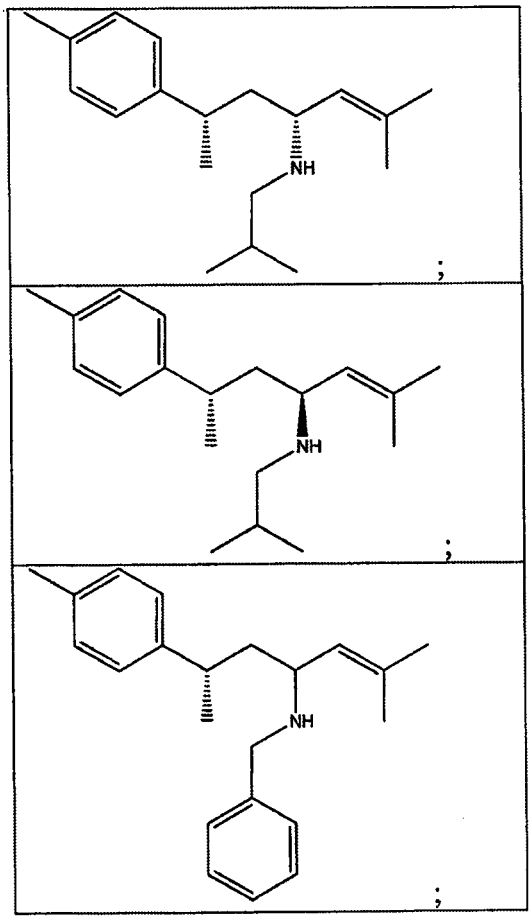
[0119] In some more specific embodiments, the sigma-2 antagonists of the present invention are those of Formula VIIIc

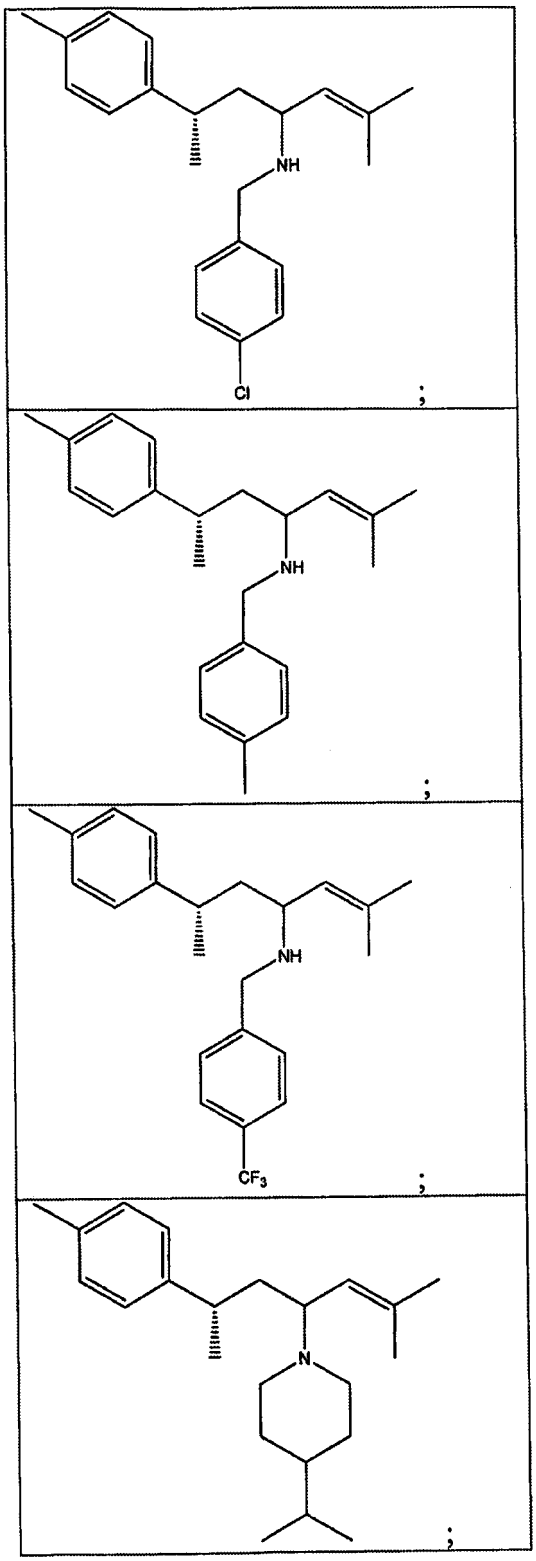


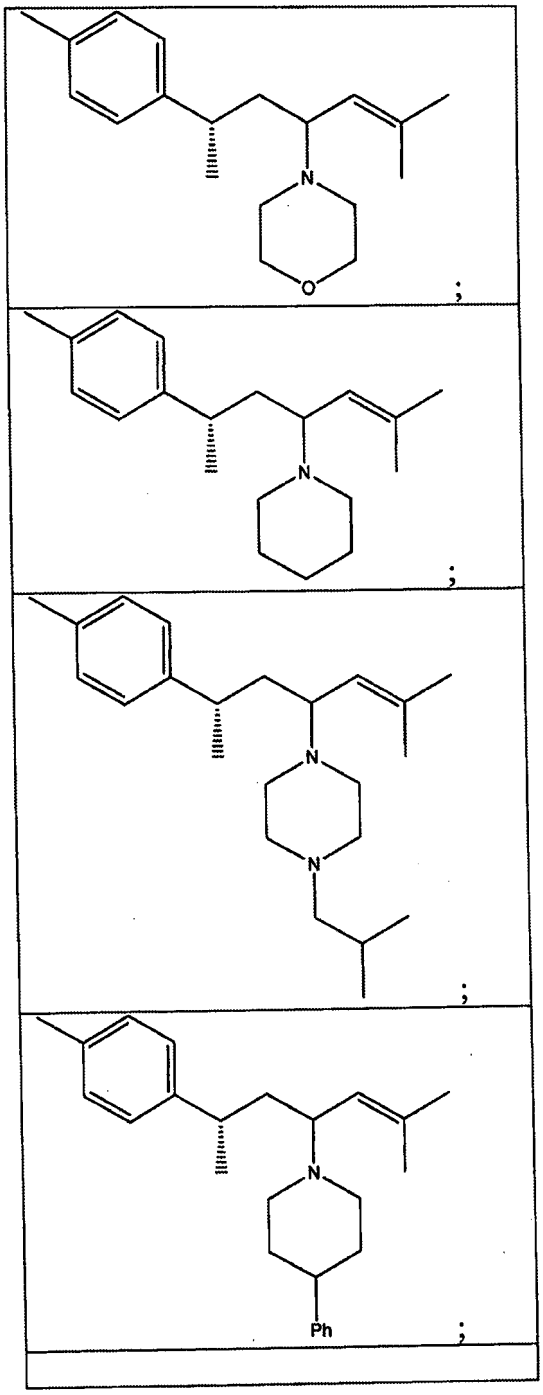
VIIIc

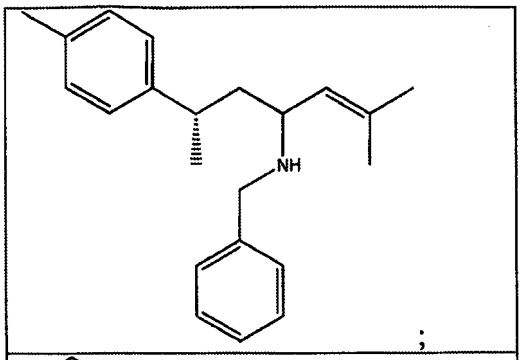
wherein R_1-R_3 are as defined above for Formula VIIIa, or pharmaceutically acceptable salts thereof.

[0120] Specific exemplary compounds of the invention are set forth in the table below:

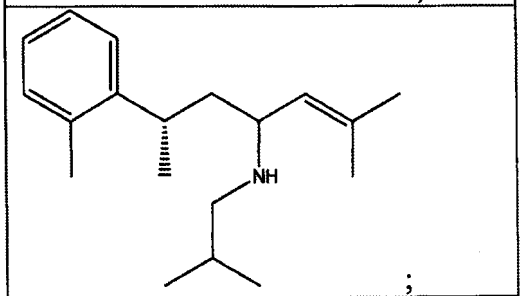



NH
Cl
;
NH
;
NH
CF3
;
N
;

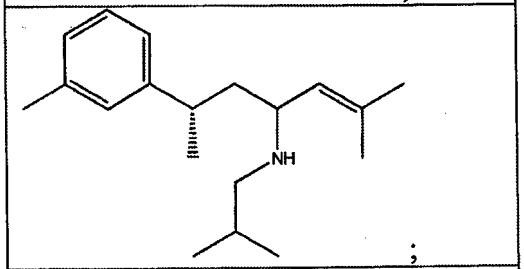

N
O
;
N
;
N
N
;
N
Ph
;



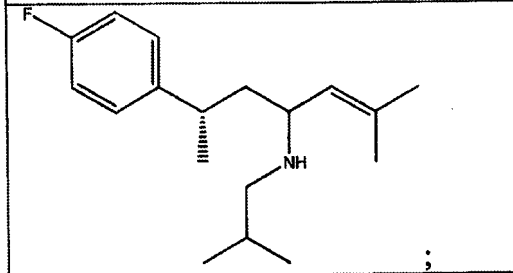
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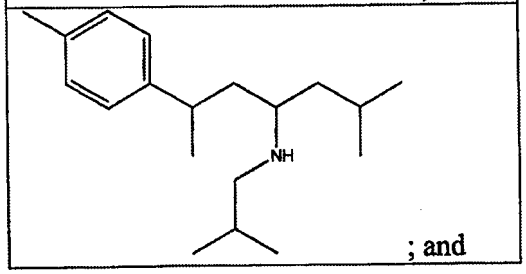
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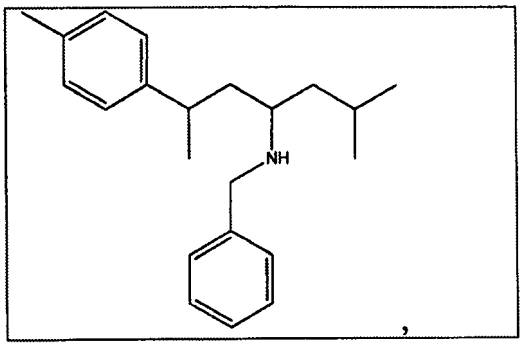
;



;

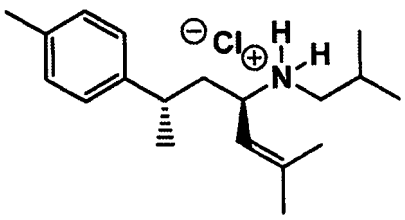


; and

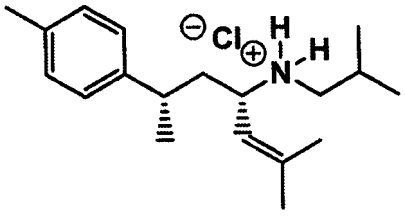


or pharmaceutically acceptable salts thereof.

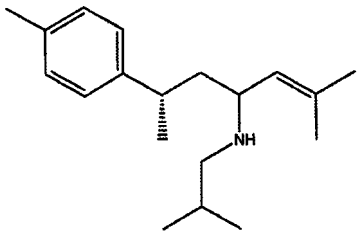
Preferred salts for use in the present invention include the hydrochloride salts of the above compounds, including the following:



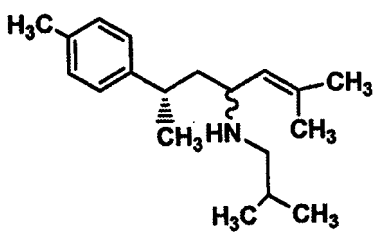
and



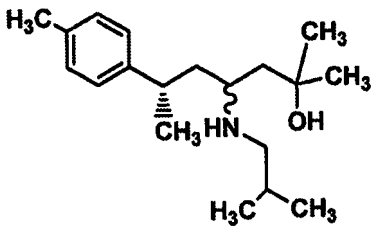
[0121] In some embodiments, each of the general formulae above may contain a proviso to remove one or more of the following compound:



[0122] Additionally disclosed are compounds of Formula IXa and IXb which are mixtures of diastereomers.



IXa



IXb

[0123] Sigma-2 Antagonists

[0124] While not being bound by theory, it is proposed that the sigma-2 receptor is a receptor for Abeta oligomer in neurons. Various receptors have been proposed in the literature for soluble Abeta oligomers including prion protein, insulin receptor, beta adrenergic receptor and RAGE (receptor for advanced glycation end products). Laurén, J. et al, 2009, Nature, 457(7233): 1128-1132; Townsend, M. et al, J. Biol. Chem. 2007, 282:33305-33312; Sturchler, E. et al, 2008, J. Neurosci. 28(20):5149-5158. Indeed many investigators believe that Abeta oligomer may bind to more than one receptor protein. Without being bound by theory, on the basis of evidence presented herein, the present inventors postulate an additional receptor for Abeta oligomer located (not necessarily exclusively) in neurons.

[0125] Without being bound by theory, Abeta oligomers are sigma receptor agonists that bind to sigma protein complexes and cause aberrant trafficking and synapse loss. It is demonstrated herein that high affinity sigma-2 ligands that antagonize this interaction and/or sigma receptor function in neurons will compete with Abeta oligomers and return neuronal responses to normal. Such ligands are considered functional sigma-2 receptor antagonists and are referred to as such or more simply as sigma-2 receptor antagonists or as sigma-2 antagonists.

[0126] In some embodiments, the sigma-2 receptor antagonist compounds of the present invention act as functional antagonists in a neuronal cell with respect to inhibiting soluble Aβ oligomer induced synapse loss, and inhibiting soluble Aβ oligomer induced deficits in a membrane trafficking assay; exhibiting high affinity at a sigma-2 receptor; as well as having high selectivity for one or more sigma receptors compared to any other non-sigma receptor; and exhibiting good drug-like properties.

[0127] In some embodiments, a sigma-2 receptor functional antagonist meeting certain in vitro assay criteria detailed herein will exhibit behavioral efficacy, or be predicted to have behavioral efficacy, in one or more relevant animal behavioral models as disclosed in this specification. In some embodiments, behavioral efficacy is determined at 10 mg/kg p.o., or less.

[0128] In some embodiments, the disclosure provides an in vitro assay platform predictive of behavioral efficacy for high affinity sigma-2 receptor ligands. In accordance with the in vitro assay platform, the ligand binds with high affinity to a sigma-2 receptor; acts as a functional antagonist with respect to Abeta oligomer-induced effects in a neuron; inhibits Abeta oligomer-induced synapse loss in a central neuron or reduces Abeta oligomer binding to neurons to inhibit synapse loss; and does not affect trafficking or synapse number in the absence of Abeta oligomer. This pattern of activity in the in vitro assays is termed the "therapeutic phenotype". The ability of a sigma-2 receptor antagonist to block Abeta oligomer effects in mature neurons without affecting normal function in the absence of Abeta oligomers meets the criteria for the therapeutic phenotype. It is now disclosed that a selective sigma-2 antagonist having a therapeutic phenotype, can block Abeta oligomer-induced synaptic dysfunction.

[0129] In some embodiments, high affinity, selective sigma-2 antagonists having the therapeutic phenotype that also possess the following characteristics are suitable as a therapeutic candidates for treating Abeta oligomer induced synaptic dysfunction in a patient in need thereof: high affinity at sigma receptors; high selectivity for sigma receptors compared to other non-sigma CNS receptors; higher affinity for a sigma-2 receptor, or comparable affinity, for example within an order of magnitude, at sigma-2 and sigma-1 receptors; selectivity for sigma receptors as opposed to other receptors relevant in the central nervous system and good drug-like properties. Drug-like properties include acceptable brain penetrability(the ability to cross the blood brain barrier), good stability in plasma and good metabolic stability, for example, as measured by exposure to liver microsomes. Without being bound by theory, high affinity sigma-2 receptor antagonists compete with Abeta oligomers, and/or stop pathological sigma receptor signaling, that leads to Alzheimer's disease.

[0130] In some embodiments, a sigma-2 antagonist having the therapeutic phenotype that also possesses the following characteristics is suitable as a therapeutic candidate for treating Abeta oligomer induced synaptic dysfunction in a patient in need thereof: high affinity at sigma receptors; high selectivity for sigma receptors compared to other non-sigma CNS receptors; high affinity for a sigma-2 receptor, or comparable affinity at sigma-2 and sigma-1 receptors; and good drug-

like properties. Drug-like properties include high brain penetrability, plasma stability, and metabolic stability.

[0131] In some embodiments, in the binding activity studies, an IC_{50} or Ki value of at most about 600 nM, 500 nM, 400 nM, 300 nM, 200 nM, 150 nM, 100 nM, preferably at most about 75 nM, preferably at most about 60 nM, preferably at most about 40 nM, more preferably at most 10 nM, most preferably at most 1 nM indicates a high binding affinity with respect to the sigma receptor binding sites.

[0132] In some embodiments, a sigma-2 receptor antagonist with high affinity (preferably Ki less than about 600 nM, 500 nM, 400 nM, 300 nM, 200 nM, 150 nM, 100 nM, 70 nM, 60 nM, 50 nM, 30 nM, or 10 nM) at sigma-2 receptors that have greater than about 20-fold, 30-fold, 50-fold, 70-fold, or preferably greater than 100-fold selectivity for sigma receptors compared to other non-sigma CNS or target receptors, and have good drug-like properties including brain penetrability and good metabolic and/or plasma stability, and that possess the therapeutic phenotype, are predicted to have behavioral efficacy and can be used to treat Abeta oligomer-induced synaptic dysfunction in a patient in need thereof.

[0133] As used herein the term "brain penetrability" refers to the ability of a drug, antibody or fragment, to cross the blood-brain barrier. In some embodiments, an animal pharmacokinetic (pK) study, for example, a mouse pharmacokinetic/blood-brain barrier study can be used to determine or predict brain penetrability. In some embodiments various concentrations of drug can be administered, for example at 3, 10 and 30 mg/kg, for example p.o. for 5 days and various pK properties are measured, e.g., in an animal model. In some embodiments, dose related plasma and brain levels are determined. In some embodiments, brain Cmax > 100, 300, 600, 1000, 1300, 1600, or 1900 ng/mL. In some embodiments good brain penetrability is defined as a brain/plasma ratio of > 0.1, > 0.3, > 0.5, > 0.7, > 0.8 , >0.9, preferably >1, and more preferably > 2, >5, or > 10. In other embodiments, good brain penetrability is defined as greater than about 0.1%, 1%, 5%, greater than about 10%, and preferably greater than about 15% of an administered dose crossing the BBB after a predetermined period of time. In certain embodiments, the dose is administered orally (p.o.). In other embodiments,

the dose is administered intravenously (i.v.), prior to measuring pK properties. Assays and brain penetrability are described in Example 7 for and data for compound II are shown in FIGs 2A and 2B, Compound II was known to be subject to first pass metabolism and thus was dosed subcutaneously; nevertheless Compound II was highly brain penetrant following both acute and chronic dosing. Brain/plasma ratio for compound II was >8.

[0134] As used herein the term "plasma stability" refers to the degradation of compounds in plasma, for example, by enzymes such as hydrolases and esterases. Any of a variety of in vitro assays can be employed. Drugs are incubated in plasma over various time periods. The percent parent compound (analyte) remaining at each time point reflects plasma stability. Poor stability characteristics can tend to have low bioavailability. Good plasma stability can be defined as greater than 50% analyte remaining after 30 min, greater than 50% analyte remaining after 45 minutes, and preferably greater than 50% analyte remaining after 60 minutes.

As used herein the term "metabolic stability" refers to the ability of the compound to survive first-pass metabolism (intestinal and hepatic degradation or conjugation of a drug administered orally). This can be assessed, for example, in vitro by exposure of the compounds to mouse or human hepatic microsomes. In some embodiments, good metabolic stability refers to a $t_{1/2}$ > 5 min, > 10 min, > 15 minutes, > 20 minutes, and preferably > 30 min upon exposure of a compound to mouse or human hepatic microsomes. In some embodiments, good metabolic stability refers to an Intrinsic Clearance Rate (Cl_{int}) of < 300 uL/min/mg, preferably $\leq$ 200 uL/min/mg, and more preferably $\leq$ 100 uL/min/mg.

Salts, solvates, stereoisomers, derivatives, prodrugs and active metabolites of the novel compounds of the invention.

[0135] The present invention further encompasses salts, solvates, stereoisomers, prodrugs and active metabolites of the compounds of formula I.

[0136] The term "salts" can include acid addition salts or addition salts of free bases. Preferably, the salts are pharmaceutically acceptable. Examples of acids which may be employed to form pharmaceutically acceptable acid addition salts include, but are not limited to, salts derived from nontoxic inorganic acids such as

nitric, phosphoric, sulfuric, or hydrobromic, hydroiodic, hydrofluoric, phosphorous, as well as salts derived from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxyl alkanoic acids, alkanedioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, and acetic, maleic, succinic, or citric acids. Non-limiting examples of such salts include napadisylate, besylate, sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, trifluoroacetate, propionate, caprylate, isobutyrate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, phthalate, benzenesulfonate, toluenesulfonate, phenylacetate, citrate, lactate, maleate, tartrate, methanesulfonate, and the like. Also contemplated are salts of amino acids such as arginate and the like and gluconate, galacturonate (see, for example, Berge, *et al.* "Pharmaceutical Salts," *J. Pharma. Sci.* 1977;66:1).

[0137] The acid addition salts of the compounds of formula I may be prepared by contacting the free base form with a sufficient amount of the desired acid to produce the salt in the conventional manner. The free base form may be regenerated by contacting the salt form with a base and isolating the free base in the conventional manner. The free base forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free base for purposes of the present invention.

[0138] Also included are both total and partial salts, that is to say salts with 1, 2 or 3, preferably 2, equivalents of base per mole of acid of a formula I compound or salt, with 1, 2 or 3 equivalents, preferably 1 equivalent, of acid per mole of base of a formula I compound.

[0139] For the purposes of isolation or purification it is also possible to use pharmaceutically unacceptable salts. However, only the pharmaceutically acceptable, non-toxic salts are used therapeutically and they are therefore preferred.

[0140] Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine.

[0141] The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid.

[0142] Compounds of the invention may have both a basic and an acidic center and may therefore be in the form of zwitterions or internal salts.

[0143] Typically, a pharmaceutically acceptable salt of a compound of formula I may be readily prepared by using a desired acid or base as appropriate. The salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent. For example, an aqueous solution of an acid such as hydrochloric acid may be added to an aqueous suspension of a compound of formula I and the resulting mixture evaporated to dryness (lyophilized) to obtain the acid addition salt as a solid. Alternatively, a compound of formula I may be dissolved in a suitable solvent, for example an alcohol such as isopropanol, and the acid may be added in the same solvent or another suitable solvent. The resulting acid addition salt may then be precipitated directly, or by addition of a less polar solvent such as diisopropyl ether or hexane, and isolated by filtration.

[0144] Those skilled in the art of organic chemistry will appreciate that many organic compounds can form complexes with solvents in which they are reacted or from which they are precipitated or crystallized. These complexes are known as "solvates". For example, a complex with water is known as a "hydrate". Solvates of the compound of the invention are within the scope of the invention. The salts of the compound of formula I may form solvates (*e.g.*, hydrates) and the invention also includes all such solvates. The meaning of the word "solvates" is

well known to those skilled in the art as a compound formed by interaction of a solvent and a solute (i.e., solvation). Techniques for the preparation of solvates are well established in the art (see, for example, Brittain. *Polymorphism in Pharmaceutical solids.* Marcel Decker, New York, 1999.).

[0145] The present invention also encompasses N-oxides of the compounds of formulas I. The term "N-oxide" means that for heterocycles containing an otherwise unsubstituted sp^2 N atom, the N atom may bear a covalently bound O atom, i.e., -N→O. Examples of such N-oxide substituted heterocycles include pyridyl N-oxides, pyrimidyl N-oxides, pyrazinyl N-oxides and pyrazolyl N-oxides.

[0146] Compounds of formula I may have one or more chiral centers and, depending on the nature of individual substituents, they can also have geometrical isomers. Isomers that differ in the arrangement of their atoms in space are termed "stereoisomers". Stereoisomers that are not mirror images of one another are termed "diastereomers" and those that are non-superimposable mirror images of each other are termed "enantiomers". When a compound has a chiral center, a pair of enantiomers is possible. An enantiomer can be characterized by the absolute configuration of its asymmetric center and is described by the R--and S-sequencing rules of Cahn and Prelog, or by the manner in which the molecule rotates the plane of polarized light and designated as dextrorotatory or levorotatory (i.e., as (+) or (-)-isomer respectively). A chiral compound can exist as either an individual enantiomer or as a mixture of enantiomers. A mixture containing equal proportions of the enantiomers is called a "racemic mixture". A mixture containing unequal portions of the enantiomers is described as having an "enantiomeric excess" (*ee*) of either the R or S compound. The excess of one enantiomer in a mixture is often described with a % enantiomeric excess (% *ee*) value determined by the formula:

$$\% ee = (R) - (S) / (R) + (S)$$

[0147] The ratio of enantiomers can also be defined by "optical purity" wherein the degree at which the mixture of enantiomers rotates plane polarized light is compared to the individual optically pure R and S compounds. Optical purity can be determined using the following formula:

$$\text{Optical purity} = \text{enant.}_{major} / (\text{enant.}_{major} + \text{enant.}_{minor})$$

[0148] The compounds can also be a substantially pure (+) or (-) enantiomer of the compounds described herein. In some embodiments, a composition comprising a substantially pure enantiomer comprises at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% of one enantiomer. In some embodiments, a composition comprising a substantially pure enantiomer is at least 99.5% one enantiomer. In some embodiments, the composition comprises only one enantiomer of a compound described herein.

[0149] The present invention encompasses all individual isomers of the compounds of formula I. The description or naming of a particular compound in the specification and claims is intended to include both individual enantiomers and mixtures, racemic or otherwise, thereof. Methods for the determination of stereochemistry and the resolution or stereotactic synthesis of stereoisomers are well-known in the art. Specifically, there is a chiral center shown in the compounds of the general formulas I and II which gives rise to one set of enantiomers. Additional chiral centers may be present depending on the substituents.

[0150] For many applications, it is preferred to carry out stereoselective syntheses and/or to subject the reaction product to appropriate purification steps so as to produce substantially optically pure materials. Suitable stereoselective synthetic procedures for producing optically pure materials are well known in the art, as are procedures for purifying racemic mixtures into optically pure fractions. Those of skill in the art will further recognize that invention compounds may exist in polymorphic forms wherein a compound is capable of crystallizing in different forms. Suitable methods for identifying and separating polymorphisms are known in the art.

[0151] Diastereomers differ in both physical properties and chemical reactivity. A mixture of diastereomers can be separated into enantiomeric pairs based on solubility, fractional crystallization or chromatographic properties, e.g., thin layer chromatography, column chromatography or HPLC.

[0152] Purification of complex mixtures of diastereomers into enantiomers typically requires two steps. In a first step, the mixture of diastereomers is resolved into enantiomeric pairs, as described above. In a second step, enantiomeric pairs are further purified into compositions enriched for one or the other enantiomer or, more preferably resolved into compositions comprising pure enantiomers. Resolution of enantiomers typically requires reaction or molecular interaction with a chiral agent, e.g., solvent or column matrix. Resolution may be achieved, for example, by converting the mixture of enantiomers, e.g., a racemic mixture, into a mixture of diastereomers by reaction with a pure enantiomer of a second agent, i.e., a resolving agent. The two resulting diastereomeric products can then be separated. The separated diastereomers are then reconverted to the pure enantiomers by reversing the initial chemical transformation.

[0153] Resolution of enantiomers can also be accomplished by differences in their non-covalent binding to a chiral substance, e.g., by chromatography on homochiral adsorbants. The noncovalent binding between enantiomers and the chromatographic adsorbant establishes diastereomeric complexes, leading to differential partitioning in the mobile and bound states in the chromatographic system. The two enantiomers therefore move through the chromatographic system, e.g., column, at different rates, allowing for their separation.

[0154] Chiral resolving columns are well known in the art and are commercially available (e.g., from MetaChem Technologies Inc., a division of ANSYS Technologies, Inc., Lake Forest, CA). Enantiomers can be analyzed and purified using, for example, chiral stationary phases (CSPs) for HPLC. Chiral HPLC columns typically contain one form of an enantiomeric compound immobilized to the surface of a silica packing material.

[0155] D-phenylglycine and L-leucine are examples of Type I CSPs and use combinations of π- π interactions, hydrogen bonds, dipole-dipole interactions, and steric interactions to achieve chiral recognition. To be resolved on a Type I column, analyte enantiomers must contain functionality complementary to that of the CSP so that the analyte undergoes essential interactions with the CSP. The sample should preferably contain one of the following functional groups: π -acid or π -base,

hydrogen bond donor and/or acceptor, or an amide dipole. Derivatization is sometimes used to add the interactive sites to those compounds lacking them. The most common derivatives involve the formation of amides from amines and carboxylic acids.

[0156] The MetaChiral ODM™ is an example of a type II CSP. The primary mechanisms for the formation of solute-CSP complexes is through attractive interactions, but inclusion complexes also play an important role. Hydrogen bonding, π - π interactions, and dipole stacking are important for chiral resolution on the MetaChiral™ ODM. Derivatization may be necessary when the solute molecule does not contain the groups required for solute-column interactions. Derivatization, usually to benzylamides, may be required for some strongly polar molecules like amines and carboxylic acids, which would otherwise interact strongly with the stationary phase through non-specific-stereo interactions.

[0157] Where applicable, compounds of formula I, or II can be separated into diastereomeric pairs by, for example, separation by column chromatography or TLC on silica gel. These diastereomeric pairs are referred to herein as diastereomer with upper TLC Rf; and diastereomer with lower TLC Rf. The diastereomers can further be enriched for a particular enantiomer or resolved into a single enantiomer using methods well known in the art, such as those described herein.

[0158] The relative configuration of the diastereomeric pairs can be deduced by the application of theoretical models or rules (e.g. Cram's rule, the Felkin-Ahn model) or using more reliable three-dimensional models generated by computational chemistry programs . In many instances, these methods are able to predict which diastereomer is the energetically favored product of a chemical transformation. As an alternative, the relative configuration of the diastereomeric pairs can be indirectly determined by discovering the absolute configurations of a single enantiomer in one (or both) of the diastereomeric pair(s).

[0159] The absolute configuration of the stereocenters can be determined by very well-known methods to those skilled in the art (e.g. X-Ray diffraction, circular dichroism). Determination of the absolute configuration can be useful also to

confirm the predictability of theoretical models and can be helpful to extend the use of these models to similar molecules prepared by reactions with analogous mechanisms (e.g. ketone reductions and reductive amination of ketones by hydrides).

[0160] The present invention may also encompass stereoisomers of the Z-E type, and mixtures thereof due to R_2-R_3 substituents to the double bond not directly linked to the ring. Additional Z-E stereoisomers are encountered when m is not 1 and m and n are different. The Cahn-Ingold-Prelog priority rules are applied to determine whether the stereoisomers due to the respective position in the plane of the double bond of the doubly bonded substituents are Z or E. The stereoisomer is designated as *Z* (*zusammen* = together) if the 2 groups of highest priority lie on the same side of a reference plane passing through the C=C bond. The other stereoisomer is designated as *E* (*entgegen* = opposite).

[0161] Mixture of stereoisomers of E-Z type can be separated (and/or characterized) in their components using classical method of purification that are based on the different chemico-physical properties of these compounds. Included in these method are fractional crystallization, chromatography carried out by low, medium or high pressure techniques, fractional distillation and any other method very well known to those skilled in the art.

[0162] The present invention also encompasses prodrugs of the compounds of formula I or II, i.e., compounds which release an active drug according to formula I or II *in vivo* when administered to a mammalian subject. A prodrug is a pharmacologically active or more typically an inactive compound that is converted into a pharmacologically active agent by a metabolic transformation. Prodrugs of a compound of formula I are prepared by modifying functional groups present in the compound of formula I in such a way that the modifications may be cleaved *in vivo* to release the parent compound. *In vivo*, a prodrug readily undergoes chemical changes under physiological conditions (*e.g.*, are hydrolyzed or acted on by naturally occurring enzyme(s)) resulting in liberation of the pharmacologically active agent. Prodrugs include compounds of formula I or II wherein a hydroxy, amino, or carboxy group is bonded to any group that may be cleaved *in vivo* to

regenerate the free hydroxyl, amino or carboxy group, respectively. Examples of prodrugs include, but are not limited to esters (*e.g.*, acetate, formate, and benzoate derivatives) of compounds of formula I or any other derivative which upon being brought to the physiological pH or through enzyme action is converted to the active parent drug. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described in the art (see, for example, Bundgaard. Design of Prodrugs. Elsevier, 1985).

[0163] Prodrugs may be administered in the same manner as the active ingredient to which they convert or they may be delivered in a reservoir form, *e.g.*, a transdermal patch or other reservoir which is adapted to permit (by provision of an enzyme or other appropriate reagent) conversion of a prodrug to the active ingredient slowly over time, and delivery of the active ingredient to the patient.

[0164] Unless specifically indicated, the term "active ingredient" is to be understood as referring to a compound of formula I as defined herein.

[0165] The present invention also encompasses metabolites. "Metabolite" of a compound disclosed herein is a derivative of a compound which is formed when the compound is metabolized. The term "active metabolite" refers to a biologically active derivative of a compound which is formed when the compound is metabolized. The term "metabolized" refers to the sum of the processes by which a particular substance is changed in the living body. In brief, all compounds present in the body are manipulated by enzymes within the body in order to derive energy and/or to remove them from the body. Specific enzymes produce specific structural alterations to the compound. For example, cytochrome P450 catalyzes a variety of oxidative and reductive reactions while uridine diphosphate glucuronyltransferases catalyze the transfer of an activated glucuronic-acid molecule to aromatic alcohols, aliphatic alcohols, carboxylic acids, amines and free sulphydryl groups. Further information on metabolism may be obtained from *The Pharmacological Basis of Therapeutics*, 9th Edition, McGraw-Hill (1996), pages 11-17.Metabolites of the compounds disclosed herein can be identified either by administration of compounds to a host and analysis of tissue samples from the host, or by incubation of

compounds with hepatic cells *in vitro* and analysis of the resulting compounds. Both methods are well known in the art.

Sigma-2 Receptor Compositions

[0166] In some embodiments, the present invention provides compositions such as binding assay mixtures comprising a sigma-2 receptor, including compositions comprising a sigma-2 receptor and a sigma-2 ligand compound according to Formula I or II, including without limitation individual compounds described specifically herein.

[0167] In some embodiments, the sigma-2 receptor is complexed with a sigma-2 ligand compound. In some embodiments, the composition comprising a sigma-2 receptor is an isolated composition. As used herein, the term "isolated composition" in reference to a sigma-2 receptor refers to a sigma-2 receptor that is cell-free or otherwise removed from its native environment. In some embodiments, the native environment is a cell that has not been lysed or otherwise disrupted. The isolation of a sigma-2 receptor from a cell can be done by routine and known methods, such as separation of the various cell components and testing each for the presence of the sigma-2 receptor (using, by way of nonlimiting example, a competitive radioligand method. Thus an isolated sigma-2 receptor can be present in the cytoplasm or in various subcompartments of a cell, such as mitochondria or endoplasmic reticulum, endosome or lysosome or in a lipid raft, which may be the physical location of the sigma-2 receptor in its native environment. Lipid rafts are generally small (10–200 nm), heterogeneous and highly dynamic assemblies that are enriched in specific components, such as, but not limited to, cholesterol and sphingolipids. Other components of a lipid raft include but are not limited to glutamate receptor (e.g. ionotropic (cation-specific ion channels) and/or metabotropic (G-protein-coupled), mGluR5), cholesterol, lipids, BACE, γ-secretase, full-length APP (amyloid precursor protein), gangliosides (e.g. Ganglioside GM1), cellular prion protein, transmembrane proteins identified amyloid-β precursor-like protein 1 (APLP1), transmembrane protein 30B (TMEM30B), α7 nicotinic acetylcholine receptor (nAChRα7) , advanced glycation end products receptor (RAGE), N-methyl-D-aspartate receptors (NMDARs), nerve growth factor receptors

(NGFRs) (e.g., TrkA and p75 neurotrophin receptor), insulin receptor subunits, or any combination thereof. See Rushworth *et al.*, International Journal of Alzheimer's Disease, Volume 2011, Article ID 603052, 14 pages, which is hereby incorporated by reference in its entirety. The lipid raft, can be isolated from a cell and the isolated lipid raft can contain the sigma-2 receptor.

[0168] In some embodiments, the sigma-2 receptor is exposed to Abeta oligomer or other Abeta species such that it will be associated or in complex with an amyloid-beta oligomer. In some embodiments, the amyloid-beta oligomers has been isolated from a cell. In some embodiments, the amyloid-beta oligomer has been synthetically made or prepared *in vitro*. Non-limiting examples of amyloid-beta oligomers and species are described herein.

[0169] In some embodiments, the composition comprising a sigma-2 receptor additionally comprises other receptors or a panel thereof. Examples are: glutamate receptor (*e.g.* ionotropic (cation-specific ion channels) and/or metabotropic (G-protein-coupled), known as mGluR5), $\alpha 7$ nicotinic acetylcholine receptor (nAChR$\alpha 7$), advanced glycation end products receptor (RAGE), N-methyl-D-aspartate receptors (NMDARs), nerve growth factor receptors (NGFRs) (*e.g.*, TrkA and p75 neurotrophin receptor), insulin receptor subunits, or any combination thereof. Other possible components of the composition include cholesterol, lipids, BACE, γ-secretase, full-length APP (amyloid precursor protein), gangliosides (*e.g.* Ganglioside GM1), cellular prion protein, transmembrane proteins, amyloid-β precursor-like protein 1 (APLP1), transmembrane protein 30B (TMEM30B),.

[0170] The compositions comprising a sigma-2 receptor, a sigma-2 ligand, and a lipid raft or a protein, lipid, cholesterol, or other component from a lipid raft are prepared by, e.g. isolating a sigma-2 receptor from a cell and contacting the sigma-2 receptor with the sigma-2 ligand. In some embodiments, the sigma-2 ligand and the sigma-2 receptor are contacted under conditions sufficient to form a complex. In some embodiments, the complex is formed in the presence of amyloid beta oligomers.

[0171] Various receptors have been proposed in the literature for Abeta oligomer including prion protein, insulin receptor, beta adrenergic receptor and RAGE (receptor for advanced glycation end products). Laurén, J. et al, 2009, Nature, 457(7233): 1128-1132; Townsend, M. et al, J. Biol. Chem. 2007, 282:33305-33312; Sturchler, E. et al, 2008, J. Neurosci. 28(20):5149-5158. Indeed many investigators believe that Abeta oligomer may bind to more than one receptor protein. Krafft GA, Klein WL *Neuropharmacology* (2010) Sep-Oct;59(4-5):230-42. On the basis of evidence presented herein and also in the co-pending commonly assigned application filed on even date herewith, the present inventors postulate an additional receptor for Abeta oligomer located (not necessarily exclusively) in neurons. While not being bound by theory, it is proposed that sigma-2 receptor is a receptor for Abeta oligomer in neurons. In some embodiments, the present invention provides compositions comprising an Abeta oligomer receptor expressed in neurons, and an Abeta oligomer. Such compositions can additionally comprise one or more neuronal proteins that are not an Abeta oligomer receptor. In some embodiments, the Abeta oligomer receptor is a sigma-2 receptor. In some embodiments, the sigma-2 receptor is an activated receptor. In some embodiments, the sigma-2 receptor is an inactive or desensitized receptor. In some embodiments, the compositions comprise a lipid raft protein. Examples of lipid raft proteins are described herein but the examples are non-limiting. A neuronal protein is a protein that is specifically expressed in a neuronal cell or in any event in the central nervous system. In some embodiments, the neuronal protein is specifically expressed in the brain. In some embodiments, a neuronal protein is a protein that is expressed in the neuron and no other tissue or cell type. In some embodiments, a neuronal protein is a protein that is expressed in the neuron and no other tissue or cell type other than the testes. In some embodiments, an additional protein may facilitate deleterious effects of Abeta oligomer in neurons.

[0172] The foregoing compositions containing a sigma-2 receptor can be employed in assays for identifying further compounds that bind to this receptor and are thus potentially active in protecting against, reducing or reversing synapse loss or membrane trafficking abnormalities and further active in inhibiting cognitive decline and treating MCI and Alzheimer's disease. For example such assays would

include but are not limited to assays in which the displacement of a labeled sigma-2 ligand by an unlabeled candidate sigma-2 ligand can be measured. Such competitive binding assays to identify active compounds have been used for many decades in the pharmaceutical industry and are known to one skilled in the art.

Use of the Compounds of the Invention

[0173] In some embodiments, the present invention provides methods of inhibiting synapse number decline or membrane trafficking abnormalities associated with exposure of a neuronal cell to Abeta species. The present invention also provides methods for treating cognitive decline and/or a neurodegenerative disease, e.g. Alzheimer's disease or mild cognitive impairment (MCI) in a patient comprising administering to the patient a sigma-2 ligand described herein, or a pharmaceutically acceptable salt thereof. In some embodiments, the method of inhibiting, or treating, cognitive decline and/or a neurodegenerative disease, e.g. Alzheimer's disease comprises inhibiting, or treating one or more symptoms of cognitive decline selected from the group consisting of memory loss, confusion, impaired judgment, personality changes, disorientation, and loss of language skills. In some embodiments, the method comprises inhibiting, or treating, diseases or disorders or conditions mediated by or associated with Abeta oligomers (see paragraph 002). In some embodiments, the method of inhibiting, or treating, cognitive decline and/or a neurodegenerative disease, e.g. Alzheimer's disease, comprises one or more of: (i) restoration of long term potentiation (LTP), LTD or synaptic plasticity detectable by electrophysiological measurements or any of the other negative changes in cognitive function as mentioned in the definition of the term above; and/or (ii) inhibiting, or treating, neurodegeneration; and/or (iii) inhibiting, or treating, general amyloidosis; and/or (iv) inhibiting, or treating, one or more of amyloid production, amyloid assembly, amyloid aggregation, and amyloid oligomer binding, and amyloid deposition; and/or (v) inhibiting, treating, and/or abating an effect, notably a nonlethal effect, of one or more of Abeta oligomers on a neuron cell. In some embodiments, the method of inhibiting, treating, and/or abating cognitive decline and/or a neurodegenerative disease, e.g. Alzheimer's disease comprises inhibiting, treating, and/or abating one or more of amyloid production, amyloid assembly, the activity/effect of one or more of Abeta oligomers on a neuron

cell, amyloid aggregation, amyloid binding, and amyloid deposition. In some embodiments, the method of inhibiting, treating, and/or abating cognitive decline and/or a neurodegenerative disease, e.g. Alzheimer's disease comprises inhibiting, treating, and/or abating one or more of the activity/effect of one or more of Abeta oligomers on a neuron cell.

[0174] In some embodiments, the activity/effect of one or more of Abeta oligomers on a neuron cell, amyloid aggregation, amyloid binding, and amyloid deposition is the effect of Abeta oligomers on membrane trafficking or synapse number. In some embodiments, the sigma-2 ligand inhibits the Abeta oligomer effect on membrane trafficking or synapse number or Abeta oligomer binding.

[0175] In some embodiments, the present invention provides methods of treating a proteopathic disease. In some embodiments, the method comprises contacting a subject with proteopathic disease with a sigma-2 ligand of the present invention or a composition containing the same that binds the sigma-2 receptor.

[0176] In some embodiments, the proteopathic disease is a CNS proteopathy, characterized by an increase in Abeta protein, such as MCI, Down's Syndrome, macular degeneration or Alzheimer's disease, and the like.

[0177] In some embodiments, the present invention provides methods of treating one or more mild cognitive impairment (MCI), or dementia by administering a sigma-2 ligand in accordance with the invention. In some embodiments, the present invention provides methods of treating MCI, and dementia.

[0178] In some embodiments, the present invention provides methods of treating an individual with a sigma-2 ligand according to the invention to restore partially or totally the subject's cells to a normal phenotype in terms of functions affected adversely by Abeta species, such as Abeta oligomers. Examples are synaptic number reduction and membrane trafficking abnormalities, which can be measured by various methods including assays described herein. The normal phenotype can be, for example, normal membrane trafficking. In some embodiments, the normal phenotype is normal cognitive ability. The "normal"

phenotype can be determined by comparing a subject's results with a sample of normal subjects. The sample may be as small as 1 subject or 1 sample or may be more than 10 samples or subjects and the norm is an average that is calculated based upon a plurality of subjects.

[0179] In some embodiments, the method comprises administering to a subject afflicted with cognitive decline or with a neurodegenerative disease a compound or composition that binds a sigma-2 protein and inhibits a beta-amyloid pathology. In some embodiments, the beta-amyloid pathology is a membrane trafficking defect, a decrease in synapse number, a decrease in dendritic spine number, a change in dendritic spine morphology, a change in LTP, a change in LTD, , a defect in measures of memory and learning in an animal, or any combination thereof, and the like. The foregoing uses result from evidence adduced by the inventors as follows:

[0180] Compounds within Formula I and II, specifically Compound II has been shown herein to inhibit synapse reduction associated with Abeta in neuronal cells and, when added before or after Abeta oligomer introduction, to inhibit abnormalities in membrane trafficking in neuronal cells (e.g., using the MTT assay described below) attending exposure of such cells to Abeta oligomers in synthetic preparations or in preparations isolated from Alzheimer's human brains (the latter being substantially more potent in mediating amyloid pathologies in vitro). Other compounds within Formula I and II have also been shown to inhibit abnormalities in membrane trafficking. Compound II has also been shown herein to inhibit cognitive deficits exhibited in transgenic and induced animal models of Alzheimer's disease as described herein, which correlate with cognitive decline and memory loss. Compound II as well as other compounds within Formula I, such as Compound B and II have also been shown in pharmacokinetic studies to be systemically absorbed and to cross the blood brain barrier and to be bioavalable. As a result of these properties, and given the state of the art which ascribes a large role to Abeta oligomers and Abeta assemblies in the development of amyloid pathology, such as that of early stages of Alzheimer's disease, it is anticipated that Compound II will be active in treatment of and protection against mild cognitive impairment and in the

treatment (as defined herein) of Alzheimer's disease. Furthermore, because of their structural similarity to Compound II and because there has been confirmation of the foregoing in vitro activities for Compound II, pharmacokinetic properties and sigma-2 ligand status for a representative number of other compounds within Formulas I and II, among those specifically disclosed above, all the compounds within Formula I and II are expected to be similarly active in vivo.

[0181] Compound II Behavioral Efficacy: Abeta oligomer-induced memory deficits in mouse fear conditioning is a model established in the laboratory of Dr. Ottavio Arancio of Columbia University (Puzzo '08). Several pharmaceutical companies use this same model in their discovery efforts. Contextual fear conditioning is an accepted model of associative memory formation which correlates to human cognitive function and specifically the creation of new (Delgado '06). Abeta oligomers are injected into the hippocampus of wild-type animals immediately before conditioning training and memory is assessed via freezing behavior after 24 hours. Details are provided in Example 9. Therein, Compound II was able to completely eliminate memory deficits in the mice without inhibiting memory when dosed alone or causing any behavioral or motor toxicities. This model system was chosen because intrahippocampal administration of oligomers allows rapid comparative assessment of compound activity and off-target toxicity. The results are shown graphically in Figure 4.

[0182] Compound II was also tested in vivo in two transgenic Alzheimer's models to show the compound's effect in reversing Abeta oligomer-associated memory loss. Specifically, Compound II restored the ability of two different mutant mouse models which on aging progressively develop cognitive decline characterized by memory loss, to remember skills acquired prior to the onset of the memory loss. In addition Compound II significantly inhibited the effect of hippocampal Abeta oligomer exposure of wild-type mice, preserving the ability of the mice to acquire new memory.

[0183] These behavioral studies collectively demonstrated that Compound II causes improvement in learning and memory in two different behavioral tasks, with two different models of Alzheimer's disease, in both genders and following short or

long-term administration and demonstrate that the in vitro assays correlate with in vivo activity. Accordingly, combined with the data showing that Compound II binds to the sigma-2 receptor and that it inhibits Abeta-associated pathologies in vitro, these results indicate that Compound II can be used to treat neurodegenerative diseases, such as Alzheimer's Disease. Other compounds within Formula I and II have also been found to bind to sigma-2 receptor and to have the same in vitro activity as Compound II. Based on their similarity with Compound II and with the pharmacophore of Abeta oligomer, which Compound II mimics, they are expected to have the same activity in vitro and in vivo as Compound II. Indeed, to the extent these compounds have been tested in vitro, they have the same type of activity as Compound II and are therefore expected to have the same activities in vivo and the same therapeutic indications. A number of other sigma-2 ligand compounds within Formula I or II were or will be tested in the synapse reduction and/or membrane trafficking assay described herein and are expected to be active in inhibiting Abeta oligomer-associated synapse loss and in inhibiting Abeta oligomer-associated membrane trafficking aberrations and to be similarly active in inhibiting cognitive decline and treat Alzheimer's disease.

[0184] The foregoing conclusions are based on the following background for Alzheimer's disease and mild cognitive impairment. As discussed herein, evidence suggests that Abeta oligomer-mediated reduction in neuronal surface receptor expression mediated by membrane trafficking are the basis for oligomer inhibition of electrophysiological measures of synaptic plasticity (LTP) and thus learning and memory (See Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, Sisodia S, Malinow R. APP processing and synaptic function. Neuron. 2003 Mar 27;37(6):925-37; and Hsieh H, Boehm J, Sato C, Iwatsubo T, Tomita T, Sisodia S, Malinow R. AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. Neuron. 2006 Dec 7;52(5):831-43). Measuring membrane trafficking rate changes induced by oligomers via formazan morphological shifts has been used in cell lines to discover Abeta oligomer-blocking drugs [Maezawa I, Hong HS, Wu HC, Battina SK, Rana S, Iwamoto T, Radke GA, Pettersson E, Martin GM, Hua DH, Jin LW. A novel tricyclic pyrone compound ameliorates cell death associated with intracellular amyloid-beta oligomeric complexes. J Neurochem.

2006 Jul;98(1):57-67; Liu Y, Schubert D. Cytotoxic amyloid peptides inhibit cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction by enhancing MTT formazan exocytosis. J Neurochem. 1997 Dec;69(6):2285-93; Liu Y, Dargusch R, Banh C, Miller CA, Schubert D. Detecting bioactive amyloid beta peptide species in Alzheimer's disease. J Neurochem. 2004 Nov;91(3):648-56; Liu Y, Schubert D. Treating Alzheimer's disease by inactivating bioactive amyloid beta peptide. Curr Alzheimer Res. 2006 Apr;3(2):129-35; Rana S, Hong HS, Barrigan L, Jin LW, Hua DH. Syntheses of tricyclic pyrones and pyridinones and protection of Abeta-peptide induced MC65 neuronal cell death. Bioorg Med Chem Lett. 2009 Feb 1;19(3):670-4. Epub 2008 Dec 24; and Hong HS, Maezawa I, Budamagunta M, Rana S, Shi A, Vassar R, Liu R, Lam KS, Cheng RH, Hua DH, Voss JC, Jin LW. Candidate anti-Abeta fluorene compounds selected from analogs of amyloid imaging agents. Neurobiol Aging. 2008 Nov 18. (Epub ahead of print)] that lower Abeta brain levels in rodents in vivo [Hong HS, Rana S, Barrigan L, Shi A, Zhang Y, Zhou F, Jin LW, Hua DH. Inhibition of Alzheimer's amyloid toxicity with a tricyclic pyrone molecule in vitro and in vivo. J Neurochem. 2009 Feb;108(4):1097-1108]. Accordingly, the foregoing tests have established relevance in identifying compounds to treat Alzheimer's disease and mild cognitive impairment.

[0185] In some embodiments, a compound has an IC_{50} value of less than 100μM, 50 μM, 20 μM, 15 μM, 10 μM, 5 μM, 1 μM, 500 nM, 100 nM, 50 nM, or 10 nM with respect to inhibition of one or more of the effects of Abeta oligomers on neurons (such as neurons in the brain), amyloid assembly or disruption thereof, and amyloid (including amyloid oligomer) binding, and amyloid deposition. In some embodiments, the compound has an IC_{50} value of less than 100μM, 50 μM, 20 μM, 15 μM, 10 μM, 5 μM, 1 μM, 500 nM, 100 nM, 50 nM, or 10 nM with respect to inhibition of the activity/effect of Abeta species such as oligomers on neurons (such as central neurons).

[0186] In some embodiments, percentage inhibition by the compound of the invention of one or more of the effects of Abeta species such as oligomers on neurons (such as neurons in the brain), such as amyloid (including amyloid

oligomer) binding to synapses, and abnormalities in membrane trafficking mediated by Abeta oligomer was measured at a concentration of from 10 nM to 10 μM. In some embodiments, the percentage inhibition measured is about 1% to about 20%, about 20% to about 50%, about 1% to about 50%, or about 1% to about 80%. Inhibition can be assessed for example by quantifying synapse number of a neuron prior to and after exposure to an amyloid beta species or quantifying the number of synapses in the presence of both of a sigma-2 ligand and the Abeta species wherein the sigma-2 ligand is simultaneous with, or precedes or follows, Abeta species exposure. As another example, inhibition can be assessed by determining membrane trafficking and comparing one or more parameters that measure exocytosis rate and extent, endocytosis rate and extent, or other indicators of cell metabolism in the presence and absence of an Abeta species and in the presence and absence of a sigma-2 ligand according to the invention. The present inventors have adduced biochemical assay evidence that compounds of the invention also inhibit amyloid aggregation.

[0187] In some embodiments, the compounds described herein bind specifically to a sigma-2 receptor. A compound that binds specifically to a specific receptor refers to a compound that has a preference for one receptor over another. For example, although a compound may be capable of binding both sigma-1 and sigma-2 receptor, a compound can be said to be specific for a sigma-2 receptor when it binds with a binding affinity that is at least 10% greater than to the sigma-1 receptor. In some embodiments, the specificity is at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, or 1000% greater for one binding partner (e.g. receptor) than a second binding partner.

[0188] In some embodiments, the present invention provides methods of measuring beta-amyloid-associated cognitive decline in an animal using a labeled sigma-2 ligand. In some embodiments, the method comprises contacting the animal with a labeled sigma-2 ligand according to the invention and measuring sigma-2 activity or expression. In some embodiments, the method comprises comparing the sigma-2 activity or expression in the animal with an animal known to have beta-amyloid induced cognitive decline. If the activity or expression is the same as the

animal known to have beta-amyloid induced cognitive decline the animal is said to have the same level of cognitive decline. The animals can be ranked according the similarities in known activity or expression of various stages of beta amyloid induced cognitive decline. Any of the sigma-2 ligands described herein can be labeled so that the labeled sigma-2 ligand can be used in vivo.

[0189] In determining whether a compound of any of the formulae above and other compounds described as sigma-2 antagonists above is effective in treating the various conditions described herein, *in vitro* assays can be used. The *in vitro* assays have been correlated with an *in vivo* effect using Compound II For example, if a compound of formulae III-IV which bears structural similarity to compound II is active, for example, in the *in vitro* assays described herein, it can also be used *in vivo* to treat or ameliorate the conditions described herein including inhibiting or restoring synapse loss, modulating a membrane trafficking change in neuronal cells, protecting against or restoring memory loss, and treating cognitive decline conditions, diseases and disorders such as MCI and Alzheimer's disease. The assays are based, in part, on the amyloid beta oligomers and their function in binding to neurons at the synapses and the effect that amyloid beta oligomers have on neurons *in vitro*. In some embodiments, an Abeta oligomer receptor in neurons which the present inventors believe includes a sigma-2 protein is contacted with an amyloid beta assembly as described herein and a compound according to Formula I, II or VIII that binds to the sigma-2 protein will inhibit the binding of the amyloid beta assembly to the receptor. In competitive radioligand binding assays the present inventors have shown that the present compounds are specific for the sigma-2 receptor. The inventors have also shown that the compounds of the invention inhibit binding of Abeta oligomers to their heretofore unidentified receptor on the surface of neurons. In some embodiments, methods are provided to determine a compound of any above formula's sigma-2 ligand efficacy in neuronal signaling. In some embodiments, the method comprises contacting a cell, such as but not limited to, a primary neuron, with a sigma-2 ligand and measuring neuronal function. In some embodiments, the cell is contacted *in vitro*. In some embodiments the cell is contacted *in vivo*. The neuronal activity can be signaling activity, electrical activity, the production or release of synaptic proteins, and the like. A sigma-2 antagonist

that enhances or restores the signaling is identified as a compound that is effective in modulating neuronal activity. In some embodiments, the cell is derived from a pathological sample. In some embodiments, the cell is derived from a subject having a neurodegenerative disease. In some embodiments, the neurodegenerative disease is MCI or Alzheimer's Disease, especially mild Alzheimer's disease.

[0190] Embodiments, of amyloid beta assemblies and methods of using the assemblies are described herein and below and in WO 2011/014880 (Application No. PCT/US2010/044136), WO 2010/118055 (Application No. PCT/US2010/030130), and Application No. PCT/US2011/026530, each of which is hereby incorporated by reference in its entirety. Other assays that can be used, such as a membrane trafficking assay and/or a fear condition assay can also be used. These methods are described herein and in WO 2011/014880 (Application No. PCT/US2010/044136), WO 2010/118055 (Application No. PCT/US2010/030130), and Application No. PCT/US2011/026530, each of which is hereby incorporated by reference in its entirety.

Receptor Binding Assays and Compound Screening

[0191] The present invention also provides methods of identifying another compound that inhibits cognitive decline or treats a neurodegenerative disease. In some embodiments, the method comprises contacting a cell with a compound that binds a sigma-2 receptor. In some embodiments, the method comprises determining if the compound inhibits beta-amyloid pathology, wherein a compound that inhibits beta-amyloid pathology is identified as a compound that binds a sigma-2 receptor and that inhibits cognitive decline or treats a neurodegenerative disease. In some embodiments, the method also comprises identifying an additional compound that binds a sigma-2 receptor. In some embodiments, a method of identifying a compound that binds to a sigma-2 receptor comprises a competitive binding assay wherein a test compound is contacted with a sigma-2 receptor in the presence of a known sigma-2 ligand, such as the compounds of the invention, wherein a test compound that competitively inhibits the binding of the known ligand is identified as a sigma-2 receptor ligand.

[0192] Methods of determining whether a compound can bind to a sigma-2 receptor are known and any method can be used. For example, testing was performed by a contract research organization. can be used to determine if a compound binds to Sigma-2. Various assays can be performed to determine if a compound binds to a Sigma-2 receptor. In some embodiments, cells, such as but not limited to, human embryonic kidney (HEK293), Jurkat cells, or Chinese hamster ovary (CHO) cells that stably express homogeneous populations of human receptors, including but not limited to sigma-2 receptor are used. In other cases, tissue sources of sigma-2 receptors such as rodent neocortical membranes are used. An example of this is described in the Examples section herein.

[0193] In some embodiments, a test compound is contacted with the cell or cell membrane to determine if the test compound can bind to the sigma-2 receptor. In some embodiments, the test compound is dissolved in a carrier or vehicle, such as but not limited to, dimethyl sulfoxide. In some embodiments, the cells are cultured until confluent. In some embodiments, upon confluence, the cells can be detached by gentle scraping. In some embodiments, the cells are detached by trypsinization, or any other suitable detachment means.

[0194] In some embodiments, the binding of the test compound to the sigma-2 receptor can be determined by, for example, a competitive radioligand binding assay. Radioligand binding assays can be carried out on intact cells stably expressing human receptors or a tissue source. The detached cells or tissue can, for example, be washed, centrifuged, and/or resuspended in a buffer. The test compound can be radiolabeled according to any method including, but not limited to, those described herein. The radioligand can be used at a fixed concentration of 0.1 μCi in the absence and presence of various concentrations (the range can be, for example, 10^{10}-10^{3}M OR 10^{11}-10^{4}M of competing drugs. The drugs can be added to the tissue or cells (~ e.g., 50,000 cells) in a buffer and allowed to incubate. Nonspecific binding can be determined in the presence of broad spectrum activators or inhibitors or functional agonists or antagonists for each receptor subtype (for example, for sigma receptors, in the presence of e.g., 10 μM of an appropriate ligand for each receptor). Reactions can be terminated by rapid filtration, which can be

followed by washes with ice-cold buffer twice. Radioactivity on the dried filter discs can be measured using any method, including but not limited to, a liquid scintillation analyzer. The displacement curves can be plotted and the Ki values of the test ligands for the receptor subtypes cam be determined using, for example, GraphPad Prism (GraphPad Software Inc., San Diego, CA). The percentage specific binding can be determined by dividing the difference between total bound (disintegrations per minute) and nonspecific bound (disintegrations per minute) by the total bound (disintegrations per minute).

[0195] In some embodiments, for binding studies in cell lines or tissues sources, varying concentrations of each drug were added in duplicate within each experiment, and the individual IC_{50} values were determined using, for example, GraphPad Prism software. The Ki value of each ligand can be determined according to the equation described by Cheng and Prusoff (1973), and final data can presented as pKi ± S.E.M., where in some embodiments, the number of tests is about 1-6.

[0196] In some embodiments, the method further comprises determining whether a compound that binds to a sigma-2 receptor acts as an antagonist at a sigma-2 receptor by inhibiting soluble Aβ oligomer induced neurotoxicity with respect to inhibiting soluble Aβ oligomer induced synapse loss, and inhibiting soluble Aβ oligomer induced deficits in a membrane trafficking assay. In some embodiments the method further determining that the sigma-2 receptor antagonist does not affect trafficking or synapse number in the absence of Abeta oligomer; does not induce caspase-3 activity in a neuronal cell; inhibits induction of caspase-3 activity by a sigma-2 receptor agonist; and/or decreases or protects against neuronal toxicity in a neuronal cell caused by a sigma-2 receptor agonist.

[0197] The testing can also include a functional assay to determine the effect of the test compound on the function of the binding partner, which can be, but is not limited to sigma-2 receptor. A variety of standard assay technologies can be used. For example, methods can be used to measure functional agonist-like or antagonist-like activity of compounds in living cells or tissues. Methods include, but are not limited to, TR-FRET to determine cAMP concentration and IP1 levels, real time fluorescence to monitor calcium flux, cellular dielectric spectroscopy to measure

impedance modulation, ileum contraction, or tumor cell apoptosis. The specificity of the test compound can also be determined by, for example, determining if the compound binds to Sigma-1 receptor, Sigma-2 receptor, neither, or both. A method for determining if a test compound binds to a Sigma-1 receptor is described in Ganapathy, M.E et al.(1999) J. Pharmacol. Exp. Ther., 289: 251-260, which is hereby incorporated by reference in its entirety. A method for determining if a test compound binds to a Sigma-1 receptor is described in Bowen, W.D et al.(1993) Mol. Neuropharmacol., 3: 117-126, which is hereby incorporated by reference in its entirety, and also Xu, J. et al, Nature Communications, 2011, 2:380 DOI:10.1038/ncomms 1386 which is also hereby incorporated by reference here in its entirety.

[0198] In various embodiments, the disclosure provides assay protocols for identification of a selective, high affinity sigma-2 receptor ligands that can act as a functional antagonist at a sigma-2 receptor by inhibiting soluble Aβ oligomer-induced neurotoxicity with respect to inhibiting soluble Aβ oligomer induced synapse loss, that inhibits soluble Aβ oligomer induced deficits in a membrane trafficking assay, that does not affect trafficking or synapse number in the absence of Abeta oligomer; and that exhibits good drug like properties as described herein such that the selective, high affinity sigma-2 receptor antagonist compound thus identified can be used to treat soluble Aβ oligomer-induced synaptic dysfunction in vivo.

[0199] In some embodiments, screening methods are provided for identifying compounds that will be active in abating or protecting against nonlethal Abeta oligomer toxicity would substantially benefit from incorporating as a screening criterion an ability of a test compound to bind to sigma-2 receptor, assessed for example by its ability to displace known ligands or by any other method. In addition, the test compound should be subjected to at least one in vitro test that can assess the ability of the compound to block or to abate nonlethal deleterious effects of Abeta oligomers on neurons, such as the membrane trafficking assay or the synapse number or oligomer binding assay described herein or an in vivo assay assessing treatment of cognitive decline, such as those described herein.

[0200] In some embodiments, the present invention provides methods of determining whether a subject should be treated with a sigma-2 antagonist, wherein the subject is suspected of having cognitive decline or a neurodegenerative disease or other condition, disease or disorder described herein. In some embodiments, the method comprises contacting a sample derived from the patient with a sigma-2 antagonist and determining whether the sigma-2 modulating compound inhibits or ameliorates a beta-amyloid pathology present in the sample, wherein a sample that shows inhibition or amelioration of the beta-amyloid pathology present in the sample indicates that the subject should be treated with a sigma-2 antagonist.

[0201] Additionally, the present invention includes methods to identify sigma-2 antagonists that inhibit an Aβ oligomer induced reduction in synapse number, and the like. In some embodiments, the methods can be used to identify sigma-2 antagonists for treating a beta-amyloid pathology. In some embodiments, the methods are used to determine the efficacy of a treatment to treat a beta-amyloid pathology. In some embodiments, the beta-amyloid pathology is a defect in membrane trafficking, synaptic dysfunction, memory and learning defect in an animal, reduction in synapse number, change in dendritic spine length or spine morphology, a defect in LTP, or an increase in the phosphorylation of Tau protein.

Amyloid Amyloid Beta as Used in the Present Disclosure

[0202] Human amyloid β is the cleavage product of an integral membrane protein, amyloid precursor protein (APP), found concentrated in the synapses of neurons. Amyloid β self-associates to form metastable, oligomeric assemblies. At higher concentrations, Abeta will polymerize and assemble into linear-shaped fibrils, facilitated by lower pH. It is not presently clear whether fibrils are formed from oligomers. Amyloid β oligomers have been demonstrated to cause Alzheimer's disease in animal models by inducing changes in neuronal synapses that block learning and memory, and amyloid β fibrils have long been associated with the advanced stages Alzheimer's disease in animals and humans. In fact, the modern working hypothesis for Alzheimer's disease, and one that has gained a lot of support, is that Abeta assemblies and notably Abeta oligomers are at the center of early pathology associated with Alzheimer's as well as of pathologies associated

with less grave dementias, such as MCI and mild AD. Cleary, James P. *et al.* "Natural oligomers of the amyloid-β protein specifically disrupt cognitive function." *Nature Neuroscience* Vol. 8 (2005): 79 – 84; Klyubin, I. *et al.* "Amyloid beta protein dimer-containing human CSF disrupts synaptic plasticity: prevention by systemic passive immunization." *J Neurosci.* Vol. 28 (2008): 4231-4237. However, very little is known about how oligomers form and the structural state of the oligomer. For example, the number of amyloid β subunits that associate to form the oligomer is currently unknown, as is the structural form of the oligomers, or which residues are exposed. There is evidence to suggest that more than one structural state of oligomer is neuroactive. Reed, Jess D. *et al.* "MALDI-TOF mass spectrometry of oligomeric food polyphenols." *Phytochemistry* 66:18 (September 2005): 2248-2263; Cleary, James P. *et al.* "Natural oligomers of the amyloid-β protein specifically disrupt cognitive function." *Nature Neuroscience* Vol. 8 (2005): 79 – 84.

[0203] Amyloid β has affinity for many proteins found in the brain, including ApoE and ApoJ. However, it is unclear whether chaperones or other proteins form associations with the protein that can affect its final structural state and/or its neuroactivity.

[0204] Soluble Abeta peptide is likely to play a key role during early stages of AD by perturbing synaptic dysfunction and cognitive processes. For example, Origlia et al. showed soluble Abeta (Abeta 42) impairs long term potentiation (LTP) in the entorhinal cortex through neuronal receptor for advanced glycation end products (RAGE)-mediated activation of p38MAPK. Origlia et al. 2008, Receptor for advanced glycation end product-dependent activation of p38 mitogen-activated protein kinase contributes to amyloid-beta-mediated cortical synaptic dysfunction. J. Neuroscience 28(13):3521-3530, incorporated herein by reference.

[0205] Synaptic dysfunction is involved in early stages of Alzheimer's disease. Amyloid beta peptides have been shown to alter synaptic function. Puzzo et al reported that a synthetic fibrillar form of Abeta impairs the late protein synthesis dependent phase of LTP without affecting the early protein synthesis phase. The report is consistent with earlier reports that Abeta oligomers are highly toxic to cells and involved in synaptic dysfunction. Puzzo et al., 2006, Curr Alzheimer's Res

3(3):179-183, which is incorporated herein by reference. Abeta has been found to markedly impair hippocampal long-term potentiation(LTP) by various second messenger cascades including a nitric oxide cascade. NO/cGMP/cGK/CREB. Puzzo et al., J Neurosci. 2005, In some embodiments, the disclosure provides compositions and methods comprising sigma-2 receptor antagonists for inhibiting amyloid beta oligomer-induced synaptic dysfunction of a neuronal cell; and inhibiting suppression of hippocampal long term potentiation caused by exposure of neurons to Abeta oligomers.

[0206] Any form of amyloid β may be used in the practice of the screening methods and of the assays according to the invention, including amyloid β monomers, oligomers, fibrils, as well as amyloid β associated with proteins ("protein complexes") and more generally amyloid β assemblies. For example, screening methods can employ various forms of soluble amyloid β oligomers as disclosed, for example, in U.S. patent application serial number 13/021,872; U.S. Patent Publication 2010/0240868; International Patent Application WO/2004/067561; International Patent Application WO/2010/011947; U.S. Patent Publication 20070098721; U.S. Patent Publication 20100209346; International Patent Application WO/2007/005359; U.S. Patent Publication 20080044356; U.S. Patent Publication 20070218491; WO/2007/126473; U.S. Patent Publication 20050074763; International Patent Application WO/2007/126473, International Patent Application WO/2009/048631, and U.S. Patent Publication 20080044406,U.S. Patent No. 7,902,328 and U.S. Patent No. 6,218,506, each of which is incorporated herein by reference.

[0207] Amyloid β forms, including monomers or oligomers of amyloid β may be obtained from any source. For example, in some embodiments, commercially available amyloid β monomers and/or amyloid β oligomers may be used in the aqueous solution, and in other embodiments, amyloid β monomers and/or amyloid β oligomers that are used in the aqueous protein solution can be isolated and purified by the skilled artisan using any number of known techniques. In general, the amyloid β monomers and/or amyloid β oligomers used in the preparation of the aqueous solution of proteins and amyloid β of various

embodiments may be soluble in the aqueous solution. Therefore, both the proteins of the aqueous solution and the amyloid β may be soluble.

[0208] The amyloid β added may be of any isoform. For example, in some embodiments, the amyloid β monomers may be amyloid β 1-42, and in other embodiments the amyloid β monomers may be amyloid β 1-40. In still other embodiments, the amyloid β may be amyloid β 1-39 or amyloid β 1-41. Hence, the amyloid β of various embodiments may encompass any C-terminal isoform of amyloid β. Yet other embodiments include amyloid β in which the N-terminus has been frayed, and in some embodiments, the N-terminus of any of amyloid β C-terminal isomers described above may be amino acid 2, 3, 4, 5, or 6. For example, amyloid β 1-42 may encompass amyloid β 2-42, amyloid β 3-42, amyloid β 4-42, or amyloid β 5-42 and mixtures thereof, and similarly, amyloid β 1-40 may encompass amyloid β 2-40, amyloid β 3-40, amyloid β 4-40, or amyloid β 5-40.

[0209] The amyloid β forms used in various embodiments may be wild type, i.e. having an amino acid sequence that is identical to the amino acid sequence of amyloid β synthesized *in vivo* by the majority of the population, or in some embodiments, the amyloid β may be a mutant amyloid β. Embodiments are not limited to any particular variety of mutant amyloid β. For example, in some embodiments, the amyloid β introduced into the aqueous solution may include a known mutation, such as, for example, amyloid β having the "Dutch" (E22Q) mutation or the "Arctic" (E22G) mutation. Such mutated monomers may include naturally occurring mutations such as, for example, forms of amyloid β isolated from populations of individuals that are predisposed to, for example, Alzheimer's disease, familial forms of amyloid β. In other embodiments, mutant amyloid β monomers may be synthetically produced by using molecular techniques to produce an amyloid β mutant with a specific mutation. In still other embodiments, mutant amyloid β monomers may include previously unidentified mutations such as, for example, those mutants found in randomly generated amyloid β mutants. The term "amyloid β" as used herein is meant to encompass both wild type forms of amyloid β as well as any of the mutant forms of amyloid β.

[0210] In some embodiments, the amyloid β in the aqueous protein solution may be of a single isoform. In other embodiments, various C-terminal isoforms of amyloid β and/or various N-terminal isoforms of amyloid β may be combined to form amyloid β mixtures that can be provided in the aqueous protein solution. In yet other embodiments, the amyloid β may be derived from amyloid precursor protein (APP) that is added to the protein containing aqueous solution and is cleaved *in situ*, and such embodiments, various isoforms of amyloid β may be contained within the solution. Fraying of the N-terminus and/or removal of C-terminal amino acids may occur within the aqueous solution after amyloid β has been added. Therefore, aqueous solutions prepared as described herein may include a variety of amyloid β isoforms even when a single isoform is initially added to the solution.

[0211] The amyloid β monomers added to the aqueous solution may be isolated from a natural source such as living tissue, and in other embodiments, the amyloid β may be derived from a synthetic source such as transgenic mice or cultured cells. In some embodiments, the amyloid β forms, including monomers, oligomers, or combinations thereof are isolated from normal subjects and/or patients that have been diagnosed with cognitive decline or diseases associated therewith, such as, but not limited to, Alzheimer's disease. In some embodiments, the amyloid β monomers, oligomers, or combinations thereof are Abeta assemblies that have been isolated from normal subjects or diseased patients. In some embodiments, the Abeta assemblies are high molecular weight, e.g. greater than 100KDa. In some embodiments, the Abeta assemblies are intermediate molecular weight, e.g. 10 to 100KDa. In some embodiments, the Abeta assemblies are less than 10 kDa.

[0212] The amyloid β oligomers of some embodiments may be composed of any number of amyloid β monomers consistent with the commonly used definition of "oligomer." For example, in some embodiments, amyloid β oligomers may include from about 2 to about 300, about 2 to about 250, about 2 to about 200 amyloid β monomers, and in other embodiments, amyloid β oligomers may be composed from about 2 to about 150, about 2 to about 100, about 2 to about 50, or about 2 to about 25, amyloid β monomers. In some embodiments, the amyloid β oligomers may include 2 or more monomers. The amyloid β oligomers of various

embodiments may be distinguished from amyloid β fibrils and amyloid β protofibrils based on the confirmation of the monomers. In particular, the amyloid β monomers of amyloid β oligomers are generally globular consisting of β-pleated sheets whereas secondary structure of the amyloid β monomers of fibrils and protofibrils is parallel β-sheets.

[0213] Identification of subjects having or at risk of having Alzheimer's Disease

[0214] Alzheimer's disease (AD) is defined histologically by the presence of extracellular β-amyloid (Aβ) plaques and intraneuronal neurofibrillary tangles in the cerebral cortex. Various diagnostic and prognostic biomarkers are known in the art, such as magnetic resonance imaging, single photon emission tomography, FDG PET, PiB PET, CSF tau and Abeta analysis, as well as available data on their diagnostic accuracy are discussed in Alves et al., 2012, Alzheimer's disease: a clinical practice-oriented review, Frontiers in Neurology, April, 2012, vol 3, Article 63, 1-20, which is incorporated herein by reference.

[0215] The diagnosis of dementia, along with the prediction of who will develop dementia, has been assisted by magnetic resonance imaging and positron emission tomography (PET) by using [(18)F]fluorodeoxyglucose (FDG). These techniques are not specific for AD. See, e.g.,Vallabhajosula S. *Positron emission tomography radiopharmaceuticals for imaging brain Beta-amyloid.* Semin Nucl Med. 2011 Jul;41(4):283-99. Another PET ligand recently FDA approved for imaging moderate to frequent amyloid neuritic plaques in patients with cognitive impairment is Florbetapir F 18 injection, (4-((1E)-2-(6-{2-(2-(2-(18F)fluoroethoxy)ethoxy)ethoxy}pyridin-3-yl)ethenyl)-N- methylbenzenamine, AMYVID®, Lilly). Florbetapir binds specifically to fibrillar Abeta, but not to neurofibrillary tangles. See,e.g., Choi SR, et al., *Correlation of amyloid PET ligand florbetapir F 18 binding with Aβ aggregation and neuritic plaque deposition in postmortem brain tissue.* Alzheimer Dis Assoc Disord. 2012 Jan;26(1):8-16. The PET ligand florbetapir suffers from low specificity with respect to qualitative visual assessment of the PET scans. Camus et al., 2012, Eur J Nucl Med Mol Imaging 39:621-631. However, many people with neuritic plaques seem cognitively normal.

[0216] CSF markers for Alzheimer's disease include total tau, phosphor-tau and Abeta42. See, for example, Andreasen , Sjogren and Blennow, World J Biol Psyciatry, 2003, 4(4): 147-155, which is incorporated herein by reference. Reduced CSF levels of the 42 amino acid form of Abeta (Abeta42) and increased CSF levels of total tau in AD have been found in numerous studies. In addition, there are known genetic markers for mutations in the APP gene useful in the identification of subjects at risk for developing AD. See, for example, Goate et al., Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease, Nature, 349, 704-706, 1991, which is incorporated herein by reference. In embodiments, any known diagnostic or prognostic method can be employed to identify a subject having or at risk of having Alzheimer's disease.

Pharmaceutical Compositions Comprising a Sigma-2 Receptor Antagonist

[0217] The sigma-2 receptor antagonist compounds, antibodies, or fragments, identified by means of the present invention can be administered in the form of pharmaceutical compositions. These compositions can be prepared in a manner well known in the pharmaceutical art, and can be administered by a variety of routes, depending upon whether local or systemic treatment is desired and upon the area to be treated.

[0218] Thus, another embodiment of the present invention comprises pharmaceutical compositions comprising a pharmaceutically acceptable excipient or diluent and a therapeutically effective amount of a sigma-2 receptor antagonist compound of the invention, including an enantiomer, diastereomer, N-oxide or pharmaceutically acceptable salt thereof.

[0219] While it is possible that a compound may be administered as the bulk substance, it is preferable to present the active ingredient in a pharmaceutical formulation, *e.g.*, wherein the active agent is in admixture with a pharmaceutically acceptable carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

[0220] Accordingly, in one aspect, the present invention provides a pharmaceutical composition comprising at least one compound, antibody or

fragment, of any of the formulae above and other compounds described as sigma-2 receptor antagonists above described above or a pharmaceutically acceptable derivative (*e.g.*, a salt or solvate) thereof, and, optionally, a pharmaceutically acceptable carrier. In particular, the invention provides a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of any of the formulae above or a pharmaceutically acceptable derivative thereof, and, optionally, a pharmaceutically acceptable carrier.

Pharmaceutical Compositions Comprising a Compound of the Invention

[0221] The compounds or compositions (*e.g.* sigma-2 antagonists) of the present invention can be administered in the form of pharmaceutical compositions. These compositions can be prepared in a manner well known in the pharmaceutical art, and can be administered by a variety of routes, depending upon whether local or systemic treatment is desired and upon the area to be treated.

[0222] Thus, another embodiment of the present invention comprises pharmaceutical compositions comprising a pharmaceutically acceptable excipient or diluent and a therapeutically effective amount of a compound of the invention, or an enantiomer, diastereomer, N-oxide or pharmaceutically acceptable salt thereof.

[0223] While it is possible that a compound may be administered as the bulk substance, it is preferable to present the active ingredient in a pharmaceutical formulation, *e.g.*, wherein the agent is in admixture with a pharmaceutically acceptable carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

[0224] Accordingly, in one aspect, the present invention provides a pharmaceutical composition comprising at least one compound of formula I or II or a pharmaceutically acceptable derivative (*e.g.*, a salt or solvate) thereof, and, optionally, a pharmaceutically acceptable carrier. In particular, the invention provides a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of formula I or a pharmaceutically acceptable derivative thereof, and, optionally, a pharmaceutically acceptable carrier.

[0225] When combined in the same formulation it will be appreciated that the two compounds must be stable and compatible with each other and the other components of the formulation. When formulated separately they may be provided in any convenient formulation, conveniently in such manner as are known for such compounds in the art.

[0226] The compounds of the invention may be formulated for administration in any convenient way for use in human or veterinary medicine and the invention therefore includes within its scope pharmaceutical compositions comprising a compound of the invention adapted for use in human or veterinary medicine. Such compositions may be presented for use in a conventional manner with the aid of one or more suitable carriers. Acceptable carriers for therapeutic use are well-known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). The choice of pharmaceutical carrier can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, in addition to, the carrier any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), and/or solubilizing agent(s).

[0227] Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, ascorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

[0228] The compounds of the invention may be milled using known milling procedures such as wet milling to obtain a particle size appropriate for tablet formation and for other formulation types. Finely divided (nanoparticulate) preparations of the compounds of the invention may be prepared by processes known in the art, for example see WO 02/00196 (SmithKline Beecham).

Combinations

[0229] For the compositions and methods of the invention, a compound of any of the formulae above and other compounds described as sigma-2 receptor

antagonists above described above may be used in combination with other therapies and/or active agents.

[0230] In some embodiments, the sigma-2 antagonist compound can be combined with one or more of a cholinesterase inhibitor, an N-methyl-D-aspartate (NMDA) glutamate receptor antagonist, a beta-amyloid specific antibody, a beta-secretase 1 (BACE1, beta-site amyloid precursor protein cleaving enzyme 1) inhibitor, a tumor necrosis factor alpha (TNF alpha) modulator, an intravenous immunoglobulin (IVIG), or a prion protein antagonist. In some embodiments the sigma-2 receptor antagonist is combined with a cholinesterase inhibitor selected from tacrine (COGNEX®; Sciele), donepezil (ARICEPT®; Pfizer), rivastigmine (EXELON®; Novartis), or galantamine (RAZADYNE®; Ortho-McNeil-Janssen). In some embodiments, the sigma-2 receptor antagonist is combined with a TNF-alpha modulator that is perispinal etanercept (ENBREL®, Amgen/Pfizer). In some embodiments, the sigma-2 receptor antagonist is combined with a beta-amyloid specific antibody selected from bapineuzumab (Pfizer), solanezumab (Lilly), PF-04360365 (Pfizer), GSK933776 (GlaxoSmithKline), Gammagard (Baxter) or Octagam (Octapharma). In some embodiments, the sigma-2 receptor antagonist is combined with an NMDA receptor antagonist that is memantine (NAMENDA®; Forest). In some embodiments, the BACE1 inhibitor is MK-8931 (Merck). In some embodiments, the sigma-2 receptor antagonist is combined with IVIG as described in Magga et al., J Neuroinflam 2010, 7:90, Human intravenous immunoglobulin provides protection against Ab toxicity by multiple mechanisms in a mouse model of Alzheimer's disease, and Whaley et al., 2011, Human Vaccines 7:3, 349-356, Emerging antibody products and Nicotiana manufacturing; each of which is incorporated herein by reference. In some embodiments, the sigma-2 receptor antagonist is combined with a prion protein antagonist as disclosed in Strittmatter et al., US 2010/0291090, which is incorporated herein by reference.

[0231] Accordingly, the present invention provides, in a further aspect, a pharmaceutical composition comprising at least one compound of any of the formulae above or a pharmaceutically acceptable derivative thereof, a second active agent, and, optionally a pharmaceutically acceptable carrier.

[0232] When combined in the same formulation it will be appreciated that the two compounds, antibodies or fragments must be stable and compatible with each other and the other components of the formulation. When formulated separately they may be provided in any convenient formulation, conveniently in such manner as are known for such compounds in the art.

Routes of Administration and Unit Dosage Forms

[0233] The routes for administration (delivery) include, but are not limited to, one or more of: oral (*e.g.*, as a tablet, capsule, or as an ingestible solution), topical, mucosal (*e.g.*, as a nasal spray or aerosol for inhalation), parenteral (*e.g.*, by an injectable form), gastrointestinal, intraspinal, intraperitoneal, intramuscular, intravenous, intracerebroventricular, or other depot administration etc.

[0234] Therefore, the compositions of the invention include those in a form especially formulated for, the mode of administration. In certain embodiments, the pharmaceutical compositions of the invention are formulated in a form that is suitable for oral delivery. For example compound CB and compound CF are sigma-2 receptor antagonist compounds that are orally bioavailable in animal models and have been administered orally once per day and shown efficacy in a fear conditioning model, see for example Figure 9B Orally bioavailable compounds as described herein can be prepared in an oral formulation. In some embodiments, the sigma-2 antagonist compound is an orally bioavailable compound, suitable for oral delivery. ,In other embodiments, the pharmaceutical compositions of the invention are formulated in a form that is suitable for parenteral delivery. In some embodiments, the sigma-2 receptor antagonist compound is an antibody or fragment thereof, wherein the antibody or fragment is formulated in a parenteral composition.

[0235] The compounds of the invention may be formulated for administration in any convenient way for use in human or veterinary medicine and the invention therefore includes within its scope pharmaceutical compositions comprising a compound of the invention adapted for use in human or veterinary medicine. Such compositions may be presented for use in a conventional manner with the aid of one or more suitable carriers. Acceptable carriers for therapeutic use

are well-known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). The choice of pharmaceutical carrier can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, in addition to, the carrier any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), and/or solubilizing agent(s).

[0236] There may be different composition/formulation requirements depending on the different delivery systems. It is to be understood that not all of the compounds need to be administered by the same route. Likewise, if the composition comprises more than one active component, then those components may be administered by different routes. By way of example, the pharmaceutical composition of the present invention may be formulated to be delivered using a mini-pump or by a mucosal route, for example, as a nasal spray or aerosol for inhalation or ingestible solution, or parenterally in which the composition is formulated by an injectable form, for delivery, by, for example, an intravenous, intramuscular or subcutaneous route. Alternatively, the formulation may be designed to be delivered by multiple routes.

[0237] Because the compounds of the invention cross the blood brain barrier they can be administered in a variety of methods including for example systemic (e.g., by iv, SC, oral, mucosal, transdermal route) or localized methods (e.g., intracranially). Where the compound of the invention is to be delivered mucosally through the gastrointestinal mucosa, it should be able to remain stable during transit though the gastrointestinal tract; for example, it should be resistant to proteolytic degradation, stable at acid pH and resistant to the detergent effects of bile. For example, the compound of Formula I or II may be coated with an enteric coating layer. The enteric coating layer material may be dispersed or dissolved in either water or in a suitable organic solvent. As enteric coating layer polymers, one or more, separately or in combination, of the following can be used; *e.g.*, solutions or dispersions of methacrylic acid copolymers, cellulose acetate phthalate, cellulose acetate butyrate, hydroxypropyl methylcellulose phthalate, hydroxypropyl

methylcellulose acetate succinate, polyvinyl acetate phthalate, cellulose acetate trimellitate, carboxymethylethylcellulose, shellac or other suitable enteric coating layer polymer(s). For environmental reasons, an aqueous coating process may be preferred. In such aqueous processes methacrylic acid copolymers are most preferred.

[0238] Where appropriate, the pharmaceutical compositions can be administered by inhalation, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavoring or coloring agents, or they can be injected parenterally, for example intravenously, intramuscularly or subcutaneously. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges, which can be formulated in a conventional manner.

[0239] Where the composition of the invention is to be administered parenterally, such administration includes without limitation: intravenously, intraarterially, intrathecally, intraventricularly, intracranially, intramuscularly or subcutaneously administering the compound of the invention; and/or by using infusion techniques.

[0240] Pharmaceutical compositions suitable for injection or infusion may be in the form of a sterile aqueous solution, a dispersion or a sterile powder that contains the active ingredient, adjusted, if necessary, for preparation of such a sterile solution or dispersion suitable for infusion or injection. This preparation may optionally be encapsulated into liposomes. In all cases, the final preparation must be sterile, liquid, and stable under production and storage conditions. To improve storage stability, such preparations may also contain a preservative to prevent the growth of microorganisms. Prevention of the action of micro-organisms can be achieved by the addition of various antibacterial and antifungal agents, *e.g.*, paraben, chlorobutanol, or acsorbic acid. In many cases isotonic substances are recommended, *e.g.*, sugars, buffers and sodium chloride to assure osmotic pressure similar to those of body fluids, particularly blood. Prolonged absorption of such

injectable mixtures can be achieved by introduction of absorption-delaying agents, such as aluminum monostearate or gelatin.

[0241] Dispersions can be prepared in a liquid carrier or intermediate, such as glycerin, liquid polyethylene glycols, triacetin oils, and mixtures thereof. The liquid carrier or intermediate can be a solvent or liquid dispersive medium that contains, for example, water, ethanol, a polyol (*e.g.*, glycerol, propylene glycol or the like), vegetable oils, non-toxic glycerine esters and suitable mixtures thereof. Suitable flowability may be maintained, by generation of liposomes, administration of a suitable particle size in the case of dispersions, or by the addition of surfactants.

[0242] For parenteral administration, the compound is best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

[0243] Sterile injectable solutions can be prepared by mixing a compound of formulas I, with an appropriate solvent and one or more of the aforementioned carriers, followed by sterile filtering. In the case of sterile powders suitable for use in the preparation of sterile injectable solutions, preferable preparation methods include drying in vacuum and lyophilization, which provide powdery mixtures of the aldosterone receptor antagonists and desired excipients for subsequent preparation of sterile solutions.

[0244] The compounds according to the invention may be formulated for use in human or veterinary medicine by injection (*e.g.*, by intravenous bolus injection or infusion or via intramuscular, subcutaneous or intrathecal routes) and may be presented in unit dose form, in ampoules, or other unit-dose containers, or in multi-dose containers, if necessary with an added preservative. The compositions for injection may be in the form of suspensions, solutions, or emulsions, in oily or aqueous vehicles, and may contain formulatory agents such as suspending,

stabilizing, solubilizing and/or dispersing agents. Alternatively the active ingredient may be in sterile powder form for reconstitution with a suitable vehicle, *e.g.*, sterile, pyrogen-free water, before use.

[0245] The compounds of the invention can be administered in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, for immediate-, delayed-, modified-, sustained-, pulsed-or controlled-release applications.

[0246] The compounds of the invention may also be presented for human or veterinary use in a form suitable for oral or buccal administration, for example in the form of solutions, gels, syrups, or suspensions, or a dry powder for reconstitution with water or other suitable vehicle before use. Solid compositions such as tablets, capsules, lozenges, pastilles, pills, boluses, powder, pastes, granules, bullets or premix preparations may also be used. Solid and liquid compositions for oral use may be prepared according to methods well-known in the art. Such compositions may also contain one or more pharmaceutically acceptable carriers and excipients which may be in solid or liquid form.

[0247] The tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycolate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC), sucrose, gelatin and acacia.

[0248] Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

[0249] The compositions may be administered orally, in the form of rapid or controlled release tablets, microparticles, mini tablets, capsules, sachets, and oral solutions or suspensions, or powders for the preparation thereof. Oral preparations may optionally include various standard pharmaceutical carriers and excipients, such as binders, fillers, buffers, lubricants, glidants, dyes, disintegrants, odorants, sweeteners, surfactants, mold release agents, antiadhesive agents and coatings.

Some excipients may have multiple roles in the compositions, *e.g.*, act as both binders and disintegrants.

[0250] Examples of pharmaceutically acceptable disintegrants for oral compositions useful in the present invention include, but are not limited to, starch, pre-gelatinized starch, sodium starch glycolate, sodium carboxymethylcellulose, croscarmellose sodium, microcrystalline cellulose, alginates, resins, surfactants, effervescent compositions, aqueous aluminum silicates and cross-linked polyvinylpyrrolidone.

[0251] Examples of pharmaceutically acceptable binders for oral compositions useful herein include, but are not limited to, acacia; cellulose derivatives, such as methylcellulose, carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxypropylcellulose or hydroxyethylcellulose; gelatin, glucose, dextrose, xylitol, polymethacrylates, polyvinylpyrrolidone, sorbitol, starch, pre-gelatinized starch, tragacanth, xanthine resin, alginates, magnesium–aluminum silicate, polyethylene glycol or bentonite.

[0252] Examples of pharmaceutically acceptable fillers for oral compositions include, but are not limited to, lactose, anhydrolactose, lactose monohydrate, sucrose, dextrose, mannitol, sorbitol, starch, cellulose (particularly microcrystalline cellulose), dihydro- or anhydro-calcium phosphate, calcium carbonate and calcium sulphate.

[0253] Examples of pharmaceutically acceptable lubricants useful in the compositions of the invention include, but are not limited to, magnesium stearate, talc, polyethylene glycol, polymers of ethylene oxide, sodium lauryl sulphate, magnesium lauryl sulphate, sodium oleate, sodium stearyl fumarate, and colloidal silicon dioxide.

[0254] Examples of suitable pharmaceutically acceptable odorants for the oral compositions include, but are not limited to, synthetic aromas and natural aromatic oils such as extracts of oils, flowers, fruits (*e.g.*, banana, apple, sour cherry, peach) and combinations thereof, and similar aromas. Their use depends on many

factors, the most important being the organoleptic acceptability for the population that will be taking the pharmaceutical compositions.

[0255] Examples of suitable pharmaceutically acceptable dyes for the oral compositions include, but are not limited to, synthetic and natural dyes such as titanium dioxide, beta-carotene and extracts of grapefruit peel.

[0256] Examples of useful pharmaceutically acceptable coatings for the oral compositions, typically used to facilitate swallowing, modify the release properties, improve the appearance, and/or mask the taste of the compositions include, but are not limited to, hydroxypropylmethylcellulose, hydroxypropylcellulose and acrylate-methacrylate copolymers.

[0257] Suitable examples of pharmaceutically acceptable sweeteners for the oral compositions include, but are not limited to, aspartame, saccharin, saccharin sodium, sodium cyclamate, xylitol, mannitol, sorbitol, lactose and sucrose.

[0258] Suitable examples of pharmaceutically acceptable buffers include, but are not limited to, citric acid, sodium citrate, sodium bicarbonate, dibasic sodium phosphate, magnesium oxide, calcium carbonate and magnesium hydroxide.

[0259] Suitable examples of pharmaceutically acceptable surfactants include, but are not limited to, sodium lauryl sulphate and polysorbates.

[0260] Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the agent may be combined with various sweetening or flavoring agents, coloring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

[0261] As indicated, the compounds of the present invention can be administered intranasally or by inhalation and is conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurized

container, pump, spray or nebulizer with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134AT) or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA), carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container, pump, spray or nebulizer may contain a solution or suspension of the active compound, *e.g.*, using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, *e.g.*, sorbitan trioleate.

[0262] Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of the compound and a suitable powder base such as lactose or starch.

[0263] For topical administration by inhalation the compounds according to the invention may be delivered for use in human or veterinary medicine via a nebulizer.

[0264] The pharmaceutical compositions of the invention may contain from 0.01 to 99% weight per volume of the active material. For topical administration, for example, the composition will generally contain from 0.01-10%, more preferably 0.01-1% of the active material.

[0265] The compounds can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

[0266] The pharmaceutical composition or unit dosage form of the present invention may be administered according to a dosage and administration regimen defined by routine testing in the light of the guidelines given above in order to obtain optimal activity while minimizing toxicity or side effects for a particular patient. However, such fine tuning of the therapeutic regimen is routine in the light of the guidelines given herein.

[0267] The dosage of the compounds of the present invention may vary according to a variety of factors such as underlying disease conditions, the individual's condition, weight, sex and age, and the mode of administration. An effective amount for treating a disorder can easily be determined by empirical methods known to those of ordinary skill in the art, for example by establishing a matrix of dosages and frequencies of administration and comparing a group of experimental units or subjects at each point in the matrix. The exact amount to be administered to a patient will vary depending on the state and severity of the disorder and the physical condition of the patient. A measurable amelioration of any symptom or parameter can be determined by a person skilled in the art or reported by the patient to the physician. It will be understood that any clinically or statistically significant attenuation or amelioration of any symptom or parameter of urinary tract disorders is within the scope of the invention. Clinically significant attenuation or amelioration means perceptible to the patient and/or to the physician.

[0268] The amount of the compound to be administered can range between about 0.01 and about 25 mg/kg/day, usually between about 0.1 and about 10 mg/kg/day and most often between 0.2 and about 5 mg/kg/day. It will be understood that the pharmaceutical formulations of the present invention need not necessarily contain the entire amount of the compound that is effective in treating the disorder, as such effective amounts can be reached by administration of a plurality of divided doses of such pharmaceutical formulations.

[0269] In a preferred embodiment of the present invention, the compounds I are formulated in capsules or tablets, usually containing 10 to 200 mg of the compounds of the invention, and are preferably administered to a patient at a total daily dose of 10 to 300 mg, preferably 20 to 150 mg and most preferably about 50 mg.

[0270] A pharmaceutical composition for parenteral administration contains from about 0.01% to about 100% by weight of the active compound of the present invention, based upon 100% weight of total pharmaceutical composition.

[0271] Generally, transdermal dosage forms contain from about 0.01% to about 100% by weight of the active compound versus 100% total weight of the dosage form.

[0272] The pharmaceutical composition or unit dosage form may be administered in a single daily dose, or the total daily dosage may be administered in divided doses. In addition, co-administration or sequential administration of another compound for the treatment of the disorder may be desirable. To this purpose, the combined active principles are formulated into a simple dosage unit.

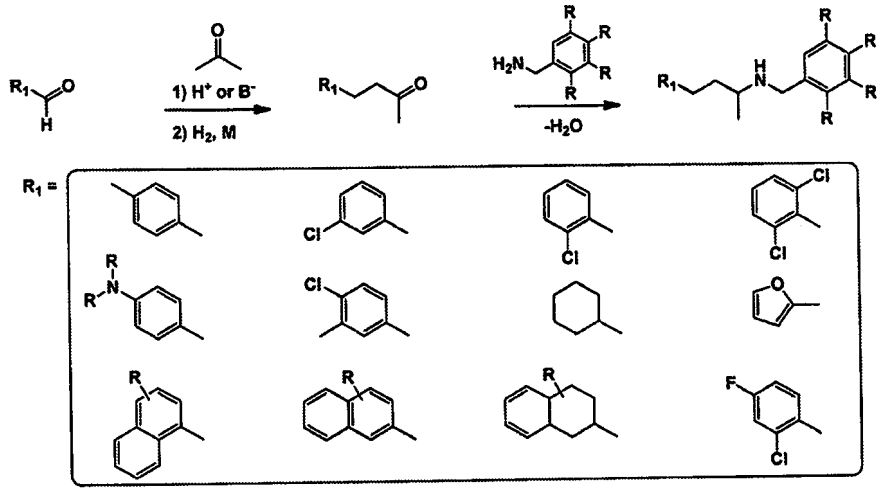
Synthesis of the Compounds of the Invention

[0273] Compounds of formulae I, II, and VIII and enantiomers, diastereomers, N-oxides, and pharmaceutically acceptable salts thereof may be prepared by the general methods outlined hereinafter, said methods constituting a further aspect of the invention. In the following description, the R groups have the meaning defined for the compounds of the above formulae unless otherwise stated.

[0274] It will be appreciated by those skilled in the art that it may be desirable to use protected derivatives of intermediates used in the preparation of the compounds I. Protection and deprotection of functional groups may be performed by methods known in the art (see, for example, Green and Wuts *Protective Groups in Organic Synthesis.* John Wiley and Sons, New York, 1999.). Hydroxy or amino groups may be protected with any hydroxy or amino protecting group. The amino protecting groups may be removed by conventional techniques. For example, acyl groups, such as alkanoyl, alkoxycarbonyl and aroyl groups, may be removed by solvolysis, *e.g.*, by hydrolysis under acidic or basic conditions. Arylmethoxycarbonyl groups (*e.g.*, benzyloxycarbonyl) may be cleaved by hydrogenolysis in the presence of a catalyst such as palladium-on-charcoal.

[0275] The synthesis of the target compounds is completed by removing any protecting groups which may be present in the penultimate intermediates using standard techniques, which are well-known to those skilled in the art. The deprotected final products are then purified, as necessary, using standard techniques

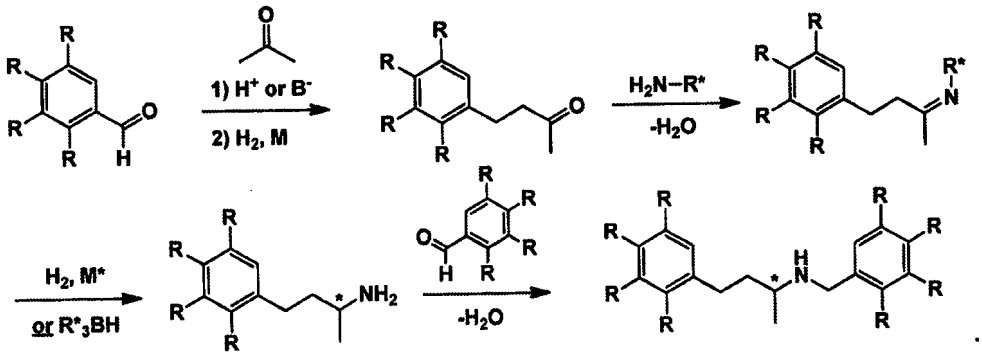
such as silica gel chromatography, HPLC on silica gel and the like, or by recrystallization. The compounds above can be synthesized via any synthetic route. For example, the compounds can be prepared according to the following scheme (Scheme 1).



Scheme 1

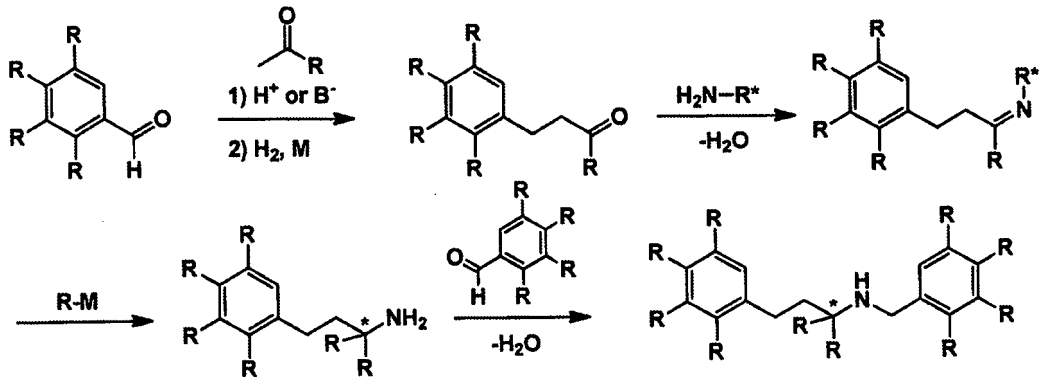
This scheme can produce a racemic mixture of the analogues described herein. Additional R1 groups can also be used to generate other analogues.

[0276] In some embodiments, the synthesis is performed asymmetrically in order to produce a substantially pure or pure enantiomer of one of an analogue. In some embodiments, the asymmetric synthesis of a compound described herein is prepared according to Scheme 2 (* indicates chiral center):



Scheme 2

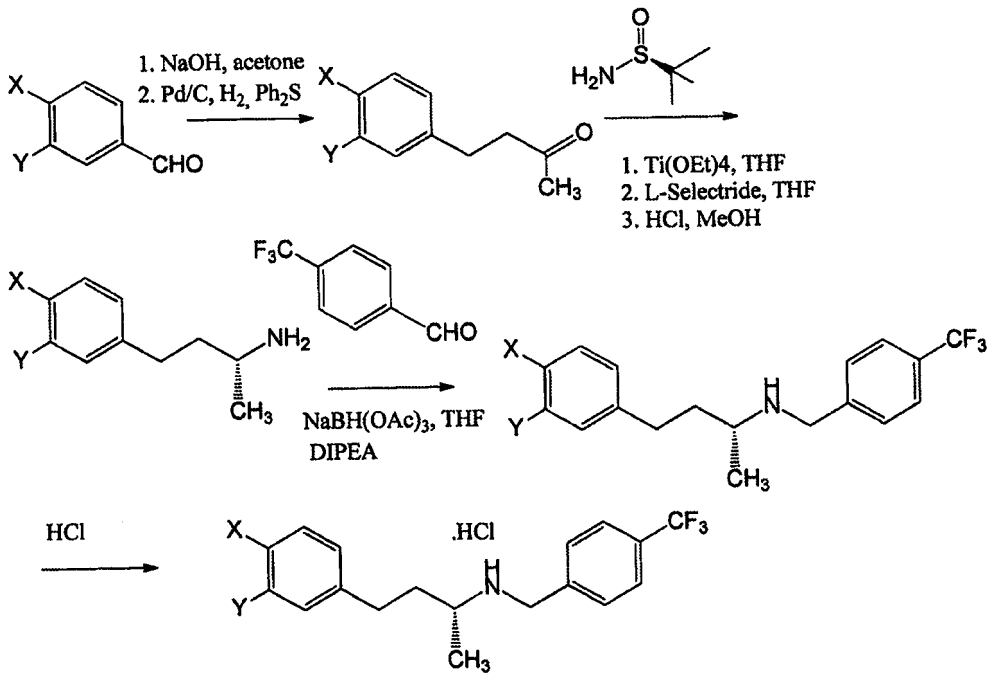
[0277] In some embodiments, the asymmetric synthesis of a compound described herein is prepared according to Scheme 3 (* indicates chiral center):



Scheme 3

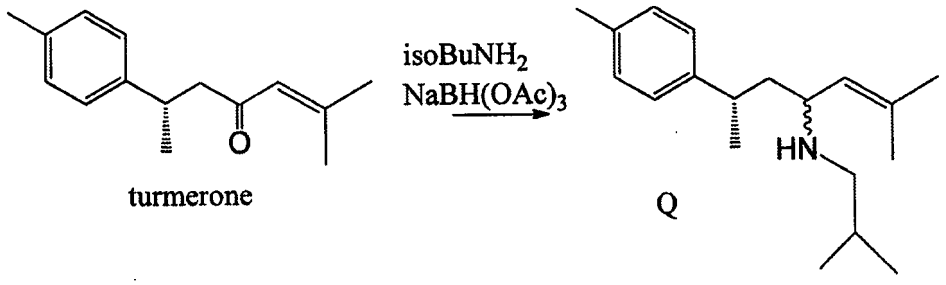
[0278] The synthetic scheme can be altered depending upon the end-product desired. The "R" groups are exemplary and can be substituted with any substituent described herein.

[0279] In some embodiments certain compounds of formulas I and II are prepared, for example, by the enantioselective route shown in Scheme 4.



Scheme 4

[0280] In some embodiments, the sigma-2 antagonist is a compound of formula VIII. Certain compounds of Formula VIII can be prepared by reductive amination of corresponding ketone intermediates, for example, by the representative route shown in Scheme 5.



turmerone Q

Scheme 5

WORKING AND SYNTHESIS EXAMPLES

Examples 1 and 2 describe Abeta oligomer preparations that could be used for experiments such as those described herein. The particular preparations used in the membrane trafficking and oligomer bindin/synapse reduction assays as well as those used in the in vivo assays described below are each described in the example to which they pertain.

Example 1: Preparation of Amyloid β Oligomers

[0281] The conditions in which amyloid β may oligomerize in nervous tissue, a milieu of aqueous-soluble proteins with which it may associate, were re-created to identify the more disease-relevant structural state of amyloid β oligomers and fibrils. Aqueous soluble proteins were prepared from rat brain by ultracentrifugation. Specifically, 5 volumes of TBS buffer (20mM Tris-HCL, pH 7.5, 34mM NaCl and a complete protease inhibitor cocktail (Santa Cruz) per gram of brain tissue was added to the rat brain tissue on ice. Dounce homogenization was then carried out with a tight-fitting pestle. The homogenized brain tissues were then

centrifuged at 150,000 x g for 1 hour at 4° C (40,000 rpm Ty65). The infranatant (between floating myelin and a half cm above the pellet) was then removed and aliquots were frozen at -75° C. The pellets were then resuspended in TBS to the original volume and frozen in aliquots at -75° C. Synthetic, monomeric human amyloid β 1-42 was added to this mixture to provide a final concentration of 1.5uM amyloid β, and the solution was incubated for 24 hours at 4° C. Centrifugation of the mixture at 5,800g for 10 minutes was performed to remove fibrillar assemblies and then Immunoprecipitation was performed using 6E10 conjugated agarose spin columns (Pierce Chemical Company) for 24 hours at 4° C. The eluted amyloid β oligomers were then subject to MALDI -Tof mass spectroscopic analysis to identify the contents of the sample, FIG. 1.

[0282] The amyloid β self-associated in the protein containing solution to form subunit assemblies of 22,599 Da, 5 subunit pentamers and 31,950 Da, 7 subunit, 7mers. Another peak at 49,291 Da may represent 12 subunit, 12mers, although this would not appear to be an accurate molecular weight for amyloid β 12mers. Notably, no peaks are observed at either 4518 Da or 9036 Da which would represent amyloid β monomers and dimers. However, peaks at 9,882 Da and 14,731 Da could represent amyloid β dimers associated with a 786 Da (or 2 x 393 Da) lipids or proteins and amyloid β trimers associated with 3 x 393 Da lipids or proteins, respectively. In addition, the presence of a peak at 19,686 Da is indicative of an assembly state possibly involving a trimer complex and a rat amyloid β fragment of 4954 Da. Accordingly these data may reflect the association of small lipids or proteins with dimers and trimers of amyloid β which may direct the assembly of conformational states unique to physiological systems.

Example 2: Preparation of beta-amyloid oligomers

[0283] A solution of 1.5uM monomeric human amyloid β 1-42 in a mixture of rat brain soluble proteins was incubated for 24 hours at 4° C as described in Example 1. This solution was then treated with tri-fluoro ethanol (TFE) prior to taking the spectra. In TFE, assembled protein structures and non-covalently bound protein complexes dissociate into denatured proteins, and the peaks associated with assembled oligomers are expected to disappear. The majority of protein peaks

observed in Example 1 disappeared including the 9822 Da, 14,731 Da, 31,950 Da, and 49,291 Da peaks identified above. However, an abundant peak is observed at 4518 Da which represents amyloid β monomer peak. A peak at 4954.7 is apparent which may represent a longer Abeta fragment similar to amyloid β 1-46. An additional peak is observed at 7086 Da which was not present in the preparation described in Example 1, which may represent amyloid β monomers associated with a 2550 Da covalently bound protein.

Example 3: Methods Employed in Assays of the Invention

[0284] TBS soluble extracts: Samples of post-mortem brain tissue from human patients characterized via histopathological analysis as Braak Stage V/VI Alzheimer's disease (AD) were obtained from Rhode Island Hospital brain tissue bank. Age and gender matched AD and normal tissue specimens were diluted to 0.15gm tissue/ml in 20mM Tris-HCL,137mM NaCl, pH 7.6 containing 1mM EDTA and 1mg/ml complete protease inhibitor cocktail (Sigma P8340) and homogenized. Ultracentrifugation of the tissue homogenates was performed at 105,000g for 1 hour in a Beckman Optima XL-80K Ultracentrifuge. The resulting TBS soluble fractions were immunodepleted using protein-A and protein-G agarose columns (Pierce Chemical) and then size fractionated with Amicon Ultra 3, 10 & 100 kDa NMWCO filters (Millipore Corporation).

[0285] Immunoprecipitation: Size fractionated and immunodepleted TBS soluble extracts were concentrated to approximately 200ul in the appropriate NMWCO Amicon Ultra filters. The concentrated TBS soluble extracts were diluted up to 400ul with TBS sample buffer (Pierce Chemical) and centrifuged for 10 minutes at 5,800 g to remove fibrils. The resulting supernatant was then immunoprecipitated with 6E10-conjugated agarose beads overnight at 4 °C followed by antigen elution using high osmotic strength Gentle elution buffers (Pierce Chemical) to isolate Abeta containing protein species.

[0286] MALDI-mass spectrometry: Immunoisolated beta amyloid was subjected to mass spectroscopic analysis using an Applied Biosystems (ABI) Voyager DE-Pro MALDI-Tof instrument. Samples were analyzed using various

matrix types such as α-Cyano-4-hydroxycinnamic acid (CHCA), Sinapic acid (SA), or 6-Aza-2-thiothymine (ATT) depending on the target molecular weight range of the analysis. The instrument was run in a linear-positive ion mode along with a variable extraction delay. Non- accumulated spectra represented 100 shots of a "hot spot" per acquisition while accumulated spectra were represented by 12 separate areas of each spot with 200 laser shots per acquisition.

[0287] Data analysis: Data acquisition and analysis was performed using Voyager's Data Explorer software package. Standard processing of the mass spectra included smoothing of the spectrum and baseline subtraction functions in addition to variations in the signal to noise ratio.

[0288] ELISA for Ab quantification: Immunoprecipitated TBS soluble fractions were analyzed for both "total" Abeta and Abeta oligomer concentration using a modified sandwich ELISA technique. Briefly, 6E10 and 4G8 coated Nunc MaxiSorp 96-well plates were incubated with Abeta containing samples and then probed with a Biotinylated 4G8 detection antibody. Incubation with Streptavidin-HRP (Rockland) followed by development of a Tetramethyl benzidine (TMB) substrate allowed for colorimetric detection (OD 450) of Abeta on a BioTEk Synergy HT plate reader. Monomeric Abeta 1-42 was used for generation of a standard curve and along with GEN 5 software allowed for quantification of Abeta levels in the immuno-precipitated samples. [WHAT WAS DEEMED ACCEPTABLE?]

[0289] Example 4: Receptor Binding Assay Compound II interacted with several receptors by blocking the binding or action of their agonists or antagonists. Compound II was tested to see whether it interacted directly with known cellular receptor or signaling proteins. Compound II (10μM) was tested for its ability to displace binding of known agonists or antagonists of a given human receptor that was overexpressed in cell lines or isolated from tissue. It was also tested for its ability to block downstream signaling induced by agonists or antagonists of a given human receptor. Compound II was tested for action at 100 known receptors, and Compound II showed activity >50% (assay window) at only 5 of these receptors (Table A). This indicates that Compound II is highly specific and active at only a

small subset of CNS-relevant receptors. It binds the sigma-2 receptor with the highest affinity and is therefore a sigma-2 ligand.

Table A. Compound II (10 uM) inhibition of binding to known receptors	% Inhibition of Control	SEM % Control
sigma 2 (agonist radioligand)	89	0.6
mu (MOP) (h) (agonist radioligand)	60	1.4
Na+ channel (site 2) (antagonist radioligand)	54	4.7
D3 (h) (agonist effect)	66	4.0
alpha 1A (h) (antagonist effect)	56	1.1

[0290] Using the same protocol, the compounds of Table 2 (below) were tested for recognition of sigma-2 receptor. The results confirmed that these compounds, structurally similar to Compound II, are indeed sigma-2 ligands, i.e., preferentially bind to the sigma-2 receptor.

Competitive Radioligand Binding Assay

[0291] Radioligand binding assays for Sigma-1 receptors and Sigma-2 receptors were carried out by a commercial contract research organization. For Sigma-1 binding, various concentrations of test compounds from 100 uM to 1 nM were used to displace 8 nM [3H](+)pentazocine from endogenous receptors on Jurkat cell membranes (Ganapathy ME et al. 1991, J Pharmacol. Exp. Ther. 289:251-260). 10 uM Haloperidol was used to define non-specific binding. For Sigma-2 receptors various concentrations of test compounds from 100 uM to 1 nM were used to displace 5 nM [3H] 1,3-Di-(2-tolyl)guanidine from endogenous receptors on membranes from rat cerebral cortex in the presence of 300 nM

(+)pentazocine to mask Sigma-1 receptors. (Bowen WD, et al. 1993, Mol. Neuropharmcol 3:117-126). 10 uM Haloperidol was used to define non-specific binding. Reactions were terminated by rapid filtration through Whatman GF/C filters using a Brandel 12R cell harvester followed by two washes with ice-cold buffer. Radioactivity on the dried filter discs was measured using a liquid scintillation analyzer (Tri-Carb 2900TR; PerkinElmer Life and Analytical Sciences). The displacement curves were plotted and the Ki values of the test ligands for the receptor subtypes were determined using GraphPad Prism (GraphPad Software Inc., San Diego, CA). The percentage specific binding was determined by dividing the difference between total bound (disintegrations per minute) and nonspecific bound (disintegrations per minute) by the total bound (disintegrations per minute).

[0292] For reference compounds, affinity for Sigma-1 and Sigma-2 receptors were obtained from published studies using cerebral tissue homogenates with [3H](+)pentazocine to measure displacement from Sigma-1 receptors and [3H] 1,3-Di-(2-tolyl)guanidine in the presence of 300 nM (+)pentazocine to measure displacement from Sigma-2 receptors

[0293] Results are shown in Table 2.

[0294] **Table 2.** Sigma-2 and Sigma-1 Receptor Affinity.

Compound	**Sigma 1 Binding Ki (nM)**	**Sigma 2 Binding Ki (nM)**
II (three different batches: racemic mixture, (+) isomer and (-) isomer)	500 100 46	9 52 63
Compound A	47	16
Compound B	47	16
Compound E	1890 (no substantial affinity to sigma-1 receptor)	16
Compound P	320	110
Compound R'	26	27
Compound S'	31	37
Compound W	270	120
Compound AC	23	240
Compound AE	16	35

Compound	Sigma 1 Binding Ki (nM)	Sigma 2 Binding Ki (nM)
Compound AF	8	110
Compound AH	23	50
Compound AI	250	130
Compound AL	3100	690
Compound AX	620	440
Compound AY	5	23
Compound AZ	34	340
Compound BB	0.72	5.2
Compound BC	4.2	13
Compound BD	2.1	19
Compound BE	7.4	14
Compound BH	4	7.4
Compound BJ	6.2	25
Compound BP	53	8.9
Compound BT	1	4
Compound CB	19	48
Compound CC	12	3.9
Compound CD	56	2.7
Compound CE	33	2.2
Compound CF	180	50
Compound (S)-CG	360	3200
Compound (R)-CG	680	-
Compound CJ	44	810
Compound (S)-CL	190	5000
Compound (R)-CL	830	>10000
Compound CO	130	7200
Compound CR	3.5	16
Compound CS	78	85
Compound DH	23	8.3
Compound DR	330	3200

Competitive Radioligand Binding Assay 2

[0295] The affinity of candidate sigma-2 ligand compounds at sigma-1 and sigma-2 receptors was also determined by displacement of different known labeled sigma-2 or sigma-1 ligands. Filtration assays were conducted according the previously published procedure (Xu, et al., 2005). Test compounds were dissolved in *N,N*-Dimethylformamide (DMF), dimethyl sulfoxide (DMSO) or ethanol and then diluted in 50 mM Tris-HCl pH 7.4 buffer containing 150 mM NaCl and 100 mM EDTA. Membrane homogenates were made from guinea pig brain for sigma-1

binding assay and rat liver for sigma-2 binding assay. Membrane homogenates were diluted with 50 mM Tris-HCl buffer, pH 8.0 and incubated at 25°C in a total volume of 150 uL in 96 well plates with the radioligand and test compounds with concentrations ranging from 0.1 nM to 10 uM. After incubation was completed, the reactions were terminated by the addition of 150 uL of ice-cold wash buffer (10 mM Tris HCl, 150 mM NaCl, pH 7.4) using a 96 channel transfer pipette (Fisher Scientific, Pittsburgh,PA) and the samples harvested and filtered rapidly through 96 well fiber glass filter plate (Millipore, Billerica, MA) that had been presoaked with 100 uL of 50 mM Tris-HCl buffer. Each filter was washed four times with 200 uL of ice-cold wash buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4). A Wallac 1450 MicroBeta liquid scintillation counter (Perkin Elmer, Boston, MA) was used to quantitate the bound radioactivity.

[0296] The sigma-1 receptor binding assays were conducted using guinea pig brain membrane homogenates (~300 ug protein) and ~5 nM [^{3}H](+)-pentazocine (34.9 Ci/mmol, Perkin Elmer, Boston, MA), incubation time was 90 min at room temperature. Nonspecific binding was determined from samples that contained 10 μM of cold haloperidol.

[0297] The sigma-2 receptor binding assays were conducted using rat liver membrane homogenates (~300 ug protein) and ~2 nM sigma-2 highly selective radioligand [^{3}H]RHM-1 only (no other blockers) (America Radiolabeled Chemicals Inc. St. Louis, MO), ~10 nM [^{3}H]DTG (58.1 Ci/mmol, Perkin Elmer, Boston, MA) or ~10 nM [^{3}H]Haloperidol (America Radiolabeled Chemicals Inc., St. Louis, MO) in the presence of 1uM (+)-pentazocine to block sigma-1 sites, incubation times were 6 minutes for [^{3}H]RHM-1, 120 min for [^{3}H]DTG and [^{3}H]haloperidol at room temperature. Nonspecific binding was determined from samples that contained 10uM of cold haloperidol.

[0298] Data from the competitive inhibition experiments were modeled using nonlinear regression analysis to determine the concentration of inhibitor that inhibits 50% of the specific binding of the radioligand (IC_{50} value). The binding affinity, *Ki* values was calculated using the method of Cheng and Prusoff. The Kd value used for [^{3}H](+)-pentazocine in guinea pig brain was 7.89 nM, for [^{3}H]RHM-1

and [^{3}H]DTG in rat liver were 0.66 nM and 30.73 nM respectively. The standard compound haloperidol was used for quality assurance. Affinity data at sigma-1 and sigma-2 receptor for compound IXa,IXb and compound II are shown in Table 3. Therefore, any sigma-2 receptor binding assay known in the art can be employed to determine the Ki or IC50 of a candidate compound.

Table 3. Sigma-2 and Sigma-1 Receptor Affinity for Candidate Sigma-2 Ligands in Competitive Radioligand Binding Assay 2.

No	Compound	Sigma-1 (Ki, nM) ±mean SEM	Sigma-2 (Ki, nM) ±mean SEM
1	IXa,IXb	6.37± 0.81	30.8± 2.3
2	II	108.1 ±19.9	59.7± 10.4

Example 5: Memory Loss in Transgenic Mice: Morris Swim Test

[0299] Compound II was tested to determine if it could reverse memory loss seen in older transgenic mouse models of Alzheimer's disease, where oligomers build up with age. For this study hAPP mice expressing human APP751 Swedish (670/671) and London (717) mutations under the control of the murine Thy-1 promoter were chosen.] These mice exhibit an age-dependent increase in the amount of Abeta, with plaques developing beginning at 3-6 months and exhibit established cognitive deficits by 8 month of age. In this study, rather than preventing deficits from occurring, deficits that were already established were treated. These studies were performed pursuant to a service contract by scientists who were blind to the experimental conditions. The compound was infused at 0.5 and 0.1 mg/kg/day for one month in 8 month old female mice via subcutaneous minipump and cognitive performance was tested in the Morris water maze, a test of hippocampal-based spatial learning and memory. This mouse model does not

exhibit neuronal loss so the restoration of memory cannot be attributed to aversion of apoptosis.

[0300] The swim speed was analyzed as part of the Morris measurements to determine if there were any motor or motivational deficits. Our vehicle is a 5% DMSO/5% Solutol, 90% saline mixture. The transgenic animals treated with a low dose (0.1 mg/kg/day) and a high dose (0.5 mg/kg/day) of compound. The average of three daily trials on each of four consecutive days were determined. We could detect no significant motor deficits or abnormal behaviors of any kind, and lost only one animal from the transgenic vehicle group during the course of the study, below expected mortality levels at this age. In addition we maintained a sentinel group of animals that had periodic blood draws to monitor plasma levels of compound, and these showed very little change from the plasma levels seen in the preliminary PK study.

[0301] Escape latency measurements from the Morris water test were taken. On the second day of testing a significant difference between wild-type and transgenic animals was observed, with the wild-type learning faster than transgenics. On this day a significant improvement in transgenic performance at the higher compound dose vs. vehicle was also observed. Therefore, it is concluded that Compound II administered at 0.5 mg/kg/day is capable of improving cognitive performance in transgenic models of AD.

[0302] Abeta 42 oligomers caused an 18% decrease in synapse number; 100% of this loss is eliminated by Compound II and its enantiomer. Similar to compound II, several other sigma-2 receptor antagonists also block synapse loss. Known prior art Sigma-2 receptor ligands NE-100 and haloperidol completely eliminated synapse loss, while SM-21, a selective Sigma 1 ligand was only weakly active in eliminating synapse loss (20% recovery).

[0303] A mixture of Compounds IXa and IXb was also tested using a similar assay. The mixture of compounds IXa and IXb (1 mg/kg/day, N=8 or 10 mg/kg/day, N=8) or vehicle (5% DMSO/5% Solutol/90% saline, N=15) was systemically administered via subcutaneous dosing (Alzet minipump) to 9 month old male

hAPPSL transgenic mice (N=8) or nontransgenic littermates (N=6) for 20 days and spatial learning and memory of these mice were evaluated in the Morris water maze. During the final four days of treatment, mice were tested to find the hidden platform in three trials/day. A computerized tracking system automatically quantified escape latency, or swim length.

[0304] There was no significant difference in the performance of transgenic animals vs. nontransgenic animals on any day of the test (analysis restricted to these 2 groups; two-way (genotype and time) ANOVA with repeated measures followed by Bonferroni's post-hoc test). A similar analysis, restricted to the transgenic groups (treatment and time), showed that transgenic animals treated with 10 mg/kg/day of a mixture of Compounds IXa and IXb performed significantly better than vehicle-treated transgenic animals on the second and fourth day of testing ($p<0.05$, analyzed by Student's t-test). Nnontransgenic vehicle-treated animals performed significantly better than transgenic vehicle-treated animals on the first and second day of testing. Treatment with the mixture of compounds IXa and IXb significantly improved transgenic animal performance compared to vehicle treatment on the first (both doses) second (10 mg/kg/day dose) and fourth (10 mg/kg/day dose) days of testing ($p < 0.05$; swim length).

[0305] This demonstrates that a mixture of compounds IXa and IXb is capable of reversing established behavioral deficits in learning and memory in aged transgenic animals in a dose-dependent manner.

[0306] Based on the results with Compound II, the structural similarity among them and the fact that compounds of the invention disclosed herein are sigma-2 ligands and are or are expected to be active in the membrane trafficking assay and are or expected to be active in the oligomer binding and synapse reduction assay, it is expected that compounds within Formula I and II will act similarly in this memory loss test. Similarly, it is expected that the compounds of Formula VIII will act similarly to the compounds IXa and IXb.

Example 6: Inhibition of Abeta Oligomer Effect on Neuronal Cells by Membrane Trafficking Assay

[0307] Sigma-2 ligands from Table 2 above were tested for their ability to inhibit an amyloid beta effect on the cells. The sigma-2 ligands were able to inhibit the amyloid beta effect as measured by a membrane trafficking assay. The results are indicated in Table 5 below. The rationale for this assay was as follows:

[0308] Since synaptic and memory deficits, and not widespread cell death, predominate at the earliest stages of Alzheimer's disease, assays that measure these changes are particularly well suited to discovering small molecule inhibitors of oligomer activity. The MTT assay is frequently used as a measure of toxicity in cultures. Yellow tetrazolium salts are endocytosed by cells and reduced to insoluble purple formazan in the endosomal pathway. The level of purple formazan is a reflection of the number of actively metabolizing cells in culture, and reduction in the amount of formazan is taken as a measure of cell death or metabolic toxicity in culture. When observed through a microscope, the purple formazan is first visible in intracellular vesicles that fill the cell. Over time, the vesicles are exocytosed and the formazan precipitates as needle-shaped crystals on the outer surface of the plasma membrane as the insoluble formazan is exposed to the aqueous media environment. Liu and Schubert ('97) discovered that cells respond to sublethal levels of Abeta oligomers by selectively accelerating the exocytosis rate of reduced formazan, while leaving endocytosis rate unaffected. The inventors have replicated these observations in mature primary neurons in culture and quantified these morphological shifts via automated microscopy and image processing. Under these circumstances, there is no overall change in the total amount of reduced formazan, simply a shift in its morphology reflective of changes in rate of its formation and/or expulsion from the cell. The inventors have confirmed previous findings that this assay is sensitive to low levels of oligomers that do not cause cell death (Liu and Schubert '04, Hong et al., '07). Indeed, low amounts of oligomers that lead to inhibition of LTP do not lead to cell death (Tong et al., '04) and are not expected to change total amounts of formazan in culture (or in brain slices).

[0309] Evidence adduced by other investigators suggests that Abeta oligomer-mediated reduction in neuronal surface receptor expression mediated by

membrane trafficking is the basis for oligomer inhibition of electrophysiological measures of synaptic plasticity (LTP) and thus learning and memory (Kamenetz et al., '03, Hseih et al., '06). Measuring membrane trafficking rate changes induced by oligomers via formazan morphological shifts has been used in cell lines to discover Abeta oligomer-blocking drugs (Maezawa et al., '06, Liu and Schubert '97, '04,'06, Rana et al., '09, Hong et al., '08) that lower Abeta brain levels in rodents in vivo (Hong et al., '09). Similar procedures for exocytosis assays/MTT assays can be found in the literature. See e.g., Liu Y, et. al., Detecting bioactive amyloid beta peptide species in Alzheimer's disease. J Neurochem. 2004 Nov;91(3):648-56; Liu Y, and Schubert D. "Cytotoxic amyloid peptides inhibit cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction by enhancing MTT formazan exocytosis." J Neurochem. 1997 Dec;69(6):2285-93; and Liu Y, and Schubert D. "Treating Alzheimer's disease by inactivating bioactive amyloid beta peptide" Curr. Alzheimer Res. 2006 Apr;3(2):129-35. Therefore the approach is valid.

[0310] The present exocytosis assay was adapted for use with mature primary neuronal cultures grown for 3 weeks in vitro. See WO/2011/106785, incorporated by reference in its entirety. Abeta oligomers cause a dose-dependent decrease in the amount of intracellular vesicles (puncta) filled with reduced purple formazan as measured via image processing using a Cellomics VTI automated microscopy system. Compare for example Figure 1A (a photomicrograph for a cultured neuronal cell exposed to vehicle alone showing vesicles filled with formazan) with Figure 1B (a photomicrograph of a neuronal cell exposed to vehicle plus Abeta oligomer showing considerably fewer vesicles filled with formazan and instead exocytosed formazan which when encountering the extracellular environment precipitates into crystals). Increasing the amount of Abeta oligomers eventually results in overt toxicity. Thus, the concentration of neuroactive Abeta oligomers used in the assay is much lower than that causing cell death. The inventors confirmed that the assay is operative by showing that the effects of Abeta oligomer are blocked upon addition of anti-Abeta antibody but antibody alone has no effect on its own (data not shown). When configured in this manner, the assay is able to detect compounds that inhibit nonlethal effects of Abeta oligomer whether

these compounds act via disruption of oligomers, inhibition of oligomer binding to neurons, or counteraction of signal transduction mechanisms of action initiated by oligomer binding.

[0311] The methods used to generate the results were as follows in the Membrane Trafficking/Exocytosis (MTT) assay.

[0312] Primary hippocampal neurons from E18 Sprague-Dawley rat embryos were plated at optimized concentrations in 384 well plates in NB media (Invitrogen). Neurons were maintained in cultures for 3 weeks, with twice weekly feeding of NB media with N_2 supplement (Invitrogen). These neurons express the full complement of synaptic proteins characteristic of neurons in the mature brain, and exhibit a complex network of activity-dependent electrical signaling. Neurons and glia in such cultures have molecular signaling networks exhibiting excellent registration with intact brain circuitry, and for this reason have been used for over two decades as a model system for learning and memory (See e.g. Kaech S, Banker G. Culturing hippocampal neurons. Nat Protoc. 2006;1(5):2406-15. Epub 2007 Jan 11; See also Craig AM, Graf ER, Linhoff MW. How to build a central synapse: clues from cell culture. Trends Neurosci. 2006 Jan;29(1):8-20. Epub 2005 Dec 7. Review).

[0313] A test compound was added to cells at concentrations ranging from 100uM to 0.001 nM followed by addition of vehicle or Abeta oligomer preparations (3 μM total Abeta protein concentration), and incubated for 1 to 24 hr at 37 °C in 5% CO_2. MTT reagent (3-(4,5-dimethylthizaol-2yl)-2,5diphenyl tetrazolium bromide) (Roche Molecular Biochemicals) was reconstituted in phosphate buffered saline to 5mg/mL. 10 μL of MTT labeling reagent is added to each well and incubated at 37 °C for 1h, then imaged. Exocytosis was assessed by automated microscopy and image processing to quantify the amount of endocytosed and exocytosed formazan.

[0314] Each assay plate was formatted so that compounds are tested with and without Abeta oligomer on each plate. This design eliminates toxic or metabolically active compounds early on in the screening cascade (at the level of the primary screen). Reduced formazan was first visible in intracellular vesicles. Eventual formazan exocytosis was accelerated via Abeta oligomers. Figure 1A and

1B are examples of photomicrographs of neurons, the first of intracellular vesicles where formazan is first seen and the second of a neuron covered with insoluble purple dye that has been extruded via exocytosis. The dye precipitated in the aqueous environment of the culture and formed needle-shaped crystals on the surface of the neuron.

In the presence of 15 micromolar Compound II, the membrane traffic changes captured in Figure 1B are blocked (see Fig. 1C) and the cell in Fig. 1C is indistinguishable from a vehicle-treated neuron. Furthermore, this effect of Compound II appears to be independent of whether Compound II is added before or after exposure of the cells to Abeta oligomer, which indicates a therapeutic as well as a prophylactic effect. See Fig. 1D, a plot (dose response curve) of membrane trafficking changes expressed as percent vesicles seen on image processing versus the log of Compound II concentration in the presence of various amounts of Abeta oligomer added before (Fig. 1D) or after (Fig. 1E) addition of various amounts of Compounds II or a mixture of IXa, IXb. Abeta oligomer alone is indicated by the circle at bottom left of Figs. 1D and 1E. Vehicle alone is indicated by filled squares. When added before oligomers (prevention mode) compound II blocks oligomer effects with EC_{50} = 2.2 uM and compound IXa,IXb blocks oligomer effects with EC_{50} = 4.9 uM. When added after oligomers (treatment mode), compound II blocks oligomer effects with EC_{50} = 4.1 uM and compound IXa,IXb blocks oligomer effects with EC_{50} = 2.0 uM. In either case, Compound II or a mixture of IXa, IXb each blocks membrane trafficking effects of Abeta oligomer seen in this assay. Ascending doses of selective, high affinity sigma-2 receptor antagonist compounds from two structurally distinct series (II and IXa,IXb) stop oligomer effects and make the cultures look more like vehicle-treated cultures.

[0315] Figure 1E is a similar dose response plot of percent vesicles filled with formazan but against Abeta concentration in the presence of various amounts of Compound II (0 indicated by diamonds, 1.1 uM indicated by downward pointing triangles, 3.2 uM indicated by upward pointing triangles and 9.7 uM indicated by filled squares). The resulting curve shifts to the right for increasing amounts of Compound II, indicating competition between Abeta oligomer and Compound II for

the same target, which in turn mediates the membrane trafficking changes caused by Abeta oligomer in this assay. Increasing amounts of Compound II decrease the apparent potency (and hence the toxicity) of Abeta oligomer, a result which the present inventors believe has not been shown prior to the present invention. Indeed, based on these results, compounds such as the ones of the present invention countering Abeta oligomer toxicity are promising as therapeutic and (in very early stages) prophylactic modalities for amyloid toxicity related cognitive decline such as that seen in Alzheimer's disease.

[0316] Synthetic Abeta oligomers were dosed in the membrane trafficking assay as seen in the Figures 1F and 1G, where it exhibited an EC50 of 820nM. Each concentration of Abeta was tested against several concentrations of each selective high affinity sigma-2 receptor antagonist compound drug candidates II and IXa,IXb, which each caused a rightward shift in the EC_{50} by almost two orders of magnitude. When the data were fitted to classical linear and nonlinear models, the data were linear with a Schild analysis (Hill slope nH of 1), which indicates that the sigma-2 receptor compound compounds exhibit true pharmacological competition between oligomers and compound for targets that mediate membrane trafficking. Abeta oligomers derived from Alzheimer's patient's brains were dosed against these compounds as shown in Figs. 1J and 1K, and also a rightward shift was also exhibited by compound exposure. Specifically, at effective doses, compound II and IXa,IXb exhibit pharmacological competition with both synthetic (Fig. 1F,G, Schild slope = -0.75, -0.51) and human Alzheimer's patient-derived (Fig. 1J, 1K) oligomers. The net effect of this is that these two selective high affinity sigma-2 receptor antagonist compound candidate drugs effectively make Abeta oligomers less synaptotoxic, and these are the only therapeutics to date we're aware of that have demonstrated this property. Without being bound by theory, the simplest possible mechanism of action is that the sigma-2 receptor compounds act as competitive receptor antagonists.

[0317] In a related experiment, a rightward shift in dose response curves (% vesicles against Abeta oligomer concentration) was observed based on the effect of 0 or 20 μM of Compound II enantiomers: see Table 4 below. The (+) enantiomer was shown to be more effective at higher concentrations of Abeta oligomer.

Table 4

Compound	EC50 against Abeta at Concentration 0 uM	EC50 against Abeta at Concentration 20 uM	Curve shift (fold)	EC 50 in screening assay using single concentration of Abeta oligomer
(+) enantiomer of Cpd II	1.19	1.46	2.86	5.6 uM
(-) enantiomer of Cpd II	1.05	2.82	1.22	10.9 uM

[0318] As shown in Table 4 above, the rightward shift in the dose response curve of % vesicles against Abeta oligomer concentration for 20 uM of enantiomer versus 0uM of enantiomer (i.e., Abeta oligomer alone) is significantly more pronounced for the (+) enantiomer at higher concentrations of Abeta oligomer (see also Figures 1F and 1G).

Experimental controls:

[0319] Abeta 1-42 oligomers made according to published methods were used as positive controls. [*See e.g.* Dahlgren et al., "Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability" J Biol Chem. 2002 Aug 30;277(35):32046-53. Epub 2002 Jun 10.; LeVine H 3rd. "Alzheimer's beta-peptide oligomer formation at physiologic concentrations" Anal Biochem. 2004 Dec 1;335(1):81-90; Shrestha et.al, "Amyloid beta peptide adversely affects spine number and motility in hippocampal neurons" Mol Cell Neurosci. 2006 Nov;33(3):274-82. Epub 2006 Sep 8; Puzzo et al., "Amyloid-beta peptide inhibits activation of the nitric oxide/cGMP/cAMP-responsive element-binding protein pathway during hippocampal synaptic plasticity" J Neurosci. 2005 Jul 20;25(29):6887-97; Barghorn et al., "Globular amyloid beta-peptide oligomer - a homogenous and stable neuropathological protein in Alzheimer's disease" J Neurochem. 2005 Nov;95(3):834-47. Epub 2005 Aug 31; Johansson et al., Physiochemical characterization of the Alzheimer's disease-related peptides A beta 1-42 Arctic and A beta 1-42wt. FEBS J. 2006 Jun;2 73(12):2618-30] as well as brain-derived Abeta oligomers (*See e.g.* Walsh et al., Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo.

Nature (2002). 416, 535-539; Lesne et al., A specific amyloid-beta protein assembly in the brain impairs memory. Nature. 2006 Mar 16;440(7082):352-7; Shankar et al, Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med. 2008 Aug;14(8):837-42. Epub 2008 Jun 22). It should be noted that any Abeta oligomer preparation can be used in this assay or as a control, including preparations described in the patent literature, cited above and incorporated by reference in their entirety.

[0320] This was shown in a membrane trafficking assay using different Abeta oligomer preparations, including notably oligomer preparations isolated from the brain of Alzheimer's disease patients. Although potencies of various Abeta oligomer preparations differ (for example native Alzheimer's isolates are more potent than any of the synthetic preparations tested—data not shown), the results are qualitatively the same: pathologies mediated by oligomers are countered by Compound II and therefore other compounds of the invention. Oligomers were isolated from postmortem human hippocampus or prefrontal cortex without the use of detergents and inhibited membrane trafficking in a dose-dependent manner with a Kd of 6 pMolar. Human Alzheimer's disease patient-derived Abeta oligomers (137 pM, second bar Fig. 1H) produce a statistically significant inhibition of membrane trafficking compared to vehicle (first bar, Fig. 1H). Compound II (third bar) eliminates the membrane trafficking deficits induced by AD brain-derived Abeta oligomers, but does not affect trafficking when dosed in the absence of Abeta (fourth, hatched, bar). The data are averaged from 3 experiments (n=3).

[0321] In the presence of Compound II at an excess (15 uM, third bar Fig 1J) shown in the black bar, oligomer-induced membrane trafficking deficits are completely eliminated. CT0109 has no significant effect on membrane trafficking when dosed on its own (black diagonal bar, Fig 1J).

[0322] In contrast, oligomers isolated from the same postmortem brain areas taken from cognitively normal age-matched individuals are generally present at lower concentrations per gram weight of tissue, 90 pM as opposed to 137 pM, (Fig. 1F , second bar), and do not produce significant deficits in membrane trafficking vs. vehicle (Fig. 1F, first bar). Under these conditions, Compound II has no effect when

dosed with oligomers or alone (Fig 1F, third and 4th bar respectively. Again, data are averaged (n=3 except for second bar, wherein n=5).

[0323] Negative controls include vehicle-treated neurons as well as neurons treated with supraphysiological, 28 μM, concentrations of memantine. Memantine produces 50% inhibition of oligomer effects at this dose. These controls, on each plate, serve as normalization tools to calibrate assay performance on a plate-by-plate basis.

Primary neuronal cultures

[0324] Optimal cell density is determined based on cellular response to Abeta oligomers using the exocytosis assay as a readout, and immunohistochemical analysis of the relative proportion of glia to neurons in the cultures. Cultures are monitored on a weekly basis with immunohistochemistry and image processing-based quantification to monitor the percentage of the cultures that are neurons vs. glia (Glial cells). Cultures containing more than 20% glia (positive for GFAP) vs. neurons (staining positively with (chicken polyclonal) antibodies (Millipore) directed against MAP2 at 1:5000 (concentration variable)) at the screening age of 21 days in vitro (21 DIV) are rejected.

Abeta Oligomer preparations

[0325] Human amyloid peptide 1-42 was obtained from a number of commercial vendors such as California Peptide, with lot-choice contingent upon quality control analysis. Quality controls of oligomer preparations consist of Westerns to determine oligomer size ranges and relative concentrations, and the MTT assay to confirm exocytosis acceleration without toxicity. Toxicity was monitored in each image-based assay via quantification of nuclear morphology visualized with the DNA binding blue dye DAPI (Invitrogen). Nuclei that are fragmented are considered to be in late stage apoptosis (Majno and Joris '95) and the test would be rejected. Peptide lots producing unusual peptide size ranges or significant toxicity at a standard 1.5 uM concentration on neurons would also be rejected.

[0326] Plate-based controls –The assay optimization was considered complete when reformatted plates achieve a minimum of statistically significant two-fold separation between vehicle and Abeta oligomer-treated neurons ($p<0.01$, Student's t-test, unequal variance) on a routine basis, with no more than 10% CV between plates.

Statistical software and Analysis:

[0327] Data handling and analysis were accomplished by Cellomics VTI image analysis software and STORE automated database software. Because of the low dynamic range and neuronal well-to-well variability after three weeks in culture, statistical comparisons are made via pairwise Tukey-Kramer analysis to determine the significance of the separation between compound + Abeta oligomers from Abeta alone, and between compound alone from vehicle. The ability of mature primary neurons to more closely approximate the electrophysiologically mediated signal transduction network of the adult brain justifies this screening strategy. Power analysis was set for a number of replicate screening wells that minimized false negatives (e.g. N=4). Test compounds of the invention significantly reverse the effects of Abeta oligomers on membrane trafficking but do not affect neuronal metabolism themselves.

[0328] Compounds within Formula I, II and VIII as indicated in the table below were dosed in the MTT assay described herein prior to Abeta oligomer addition and were shown to block the Abeta oligomer-induced membrane trafficking deficits with the indicated EC_{50}. Specifically, these results indicate that compounds block/abate the activity/effect of Abeta oligomer on membrane trafficking of neuron cells at micromolar concentrations.

Table 5: Sigma-2 Receptor Ligands and Ability to inhibit amyloid oligomer effects on membrane trafficking:

Sigma-2 Receptor Ligand	**EC_{50} in inhibiting amyloid beta effect in Cell Measured by Membrane Trafficking Assay**
Compound A	3.4 μM
Compound B	5.5 μM
Compound C	5.4 μM

Compound D	8.9 μM
Compound E	8.2 μM
Compound F	2.6 μM
Compound G	5.8 μM
Compound H	2.2 μM
Compound I	3.4 μM
Compound J	3.9 μM
Compound K	14 μM
Compound L	2.4 μM
Compound M	0.6 μM
Compound N	5.2 μM
Compound O	2.7 μM
Compound P	20.0 μM
Compound Q	0.5 μM
Compound R	6.7 μM
Compound R'	39 μM (inactive)
Compound S	5.4 μM
Compound S'	>30 μM (inactive)
Compound T	7.7 μM
Compound AC	2.4 μM
Compound AD	0.7 μM
Compound AG	6.1 μM (1.3 μM)
Compound BA	< 1.0 μM
Compound BT	0.4 μM
Compound BY	0.8 μM
Compound CA	1.9 μM
Compound CB	18.2 μM
Compound CR	1 μM
Compound CS	6.9 μM (3 μM)
Compound CV	2.5 μM
Compound CX	1.3 μM
Compound CY	14 μM
Compound DE	> 20 μM
Compound DN	> 20 μM

[0329] The compounds in Table 5 were shown to block the Abeta oligomer-induced acceleration of exocytosis with the indicated EC_{50}. Accordingly, the compounds in Table 5 significantly blocked Abeta oligomer-mediated changes in membrane trafficking. These results indicate that compounds block/abate the activity/effect of Abeta oligomer on neuron cells and that sigma-2 ligands can be used to block the Abeta oligomer induced membrane trafficking abnormalities.

Example 7: Pharmacokinetic Studies and Metabolic Stability Studies

[0330] A first pharmacokinetic study was performed in microsomes of mice by a commercial contract research organization. The studies were performed according to Obach, R.S et al.(1997) J. Pharmacol. Exp. Ther., 283: 46-58, which is hereby incorporated by reference. The half-life of the compounds in Table 2 that were tested ranged from 2-72 minutes and the half-life of the remaining compounds is expected to be in about the same range.

[0331] The results for half-life in microsomes are shown in Table 6:

Table 6: Compound Mouse Microsome Stability.

Compound	t1/2 in microsomes of mice
II	16
A	33
B	55
C	10
D	2
E	46
F	72
G	42
H	24
I	33
J	47

[0332] The results indicate that several of the compounds tested had a substantially longer half-life in mouse liver microsomes than Compound II. This result portends greater bioavailability after oral administration for these compounds. The same compounds have been tested by the membrane trafficking assay described above and their activity has been reported.

[0333] The rate of intrinsic clearance of Compound II was rapid, suggesting substantial first pass metabolism. In order to improve pharmacokinetic properties, additional compounds were designed to enhance metabolic stability and improve drug-like properties. Microsomal stability experiments and plasma stability experiments were performed to determine metabolic and hepatic stability of candidate compounds.

[0334] A second PK study was conducted in vivo and involved measuring plasma levels and brain levels for test compounds administered by various routes and in an acute or chronic manner, as follows:

HPLC-MS Optimization

[0335] A solution of each test compound was prepared and infused into the TSQ Quantum spectrometer (Fisher Thermo Scientific) source via syringe pump at a constant rate. Full scan MS (mass spectroscopy) analysis was conducted and total ion current chromatograms and corresponding mass spectra were generated for each test compound in both positive and negative ionization modes. The precursor ions for MS/MS were selected from either the positive or the negative mass spectrum, as a function of the respective ion abundance. In addition, product ion MS/MS analysis was performed in order to determine the appropriate selected fragmentation reaction for use in quantitative analysis. The final reaction monitoring parameters were chosen to maximize the ability to quantify the test compound when present within a complex mixture of components. Following identification of the specific SRM transition to be used for each test compound, the detection parameters were optimized using the automated protocol in the TSQ Quantum Compound Optimization workspace. Finally, the chromatographic conditions to be used for LC-MS analysis were identified by injection and separation of the analyte on a suitable LC column and adjustment of the gradient conditions as necessary.

Formulation for IV dosing:

[0336] The solubility of the test compound in phosphate-buffered saline, pH 7.4 (PBS) was first evaluated by visual inspection. PBS was used as the vehicle if the compound was soluble at the target concentration. (Other vehicles that are compatible with IV dosing may be evaluated if the compound is not completely soluble in PBS. Such vehicles include DMSO, polyethylene glycol (PEG 400), Solutol HS 15, and Cremophor EL among others.) In the experiments reported here a single bolus, 10 mg/kg, of Compound II was administered IV

[0337] Formulation for PO dosing: The solubility of the test compound in PBS was first evaluated. PBS was used as the vehicle if the compound is soluble at

the target concentration. (DMSO/Solutol HS 15/PBS (5/5/90, v/v/v), or DMSO/1 % methylcellulose (5/95, v/v) may be used if the test compound is not completely soluble in PBS at the respective concentration.)

Linearity in Plasma

[0338] Aliquots of plasma were spiked with the test compounds at the specified concentrations. The spiked samples were processed using acetonitrile precipitation and analyzed by HPLC-MS or HPLC-MS/MS. A calibration curve of peak area versus concentration was constructed. The reportable linear range of the assay was determined, along with the lower limit of quantitation (LLQ).

Quantitative Bioanalysis of Plasma Samples

[0339] The plasma samples were processed using acetonitrile precipitation and analyzed by HPLC-MS or HPLC-MS/MS. A plasma calibration curve was generated. Aliquots of drug-free plasma were spiked with the test compound at the specified concentration levels. The spiked plasma samples were processed together with the unknown plasma samples using the same procedure. The processed plasma samples (dried extracts) were typically stored frozen (-20 °C) until the HPLC-MS or HPLC-MS/MS analysis. The dried extracts were reconstituted into a suitable solvent and after centrifugation were analyzed by HPLC-MS or HPLC-MS/MS. Peak areas were recorded, and the concentrations of the test compound in the unknown plasma samples were determined using the respective calibration curve. The reportable linear range of the assay was determined, along with the lower limit of quantitation (LLQ).

[0340] Animals used in the study were male C57BL/6 mice weighing 20-30 g each or male Sprague-Dawley rats weighing 180-250 g. Three animals were treated for each administration condition and each time point, so that each animal was subjected to only one blood draw. Subcutaneous compound administration was accomplished by intraperitoneal injection. Per oral administration was accomplished by gastric gavage. Intravenous administration was accomplished via jugular catheter.

[0341] Following compound administration at various concentrations, plasma samples were collected at 10, 30, 60, 120, 240, 360, 480 and 1440 min.

Plasma Sample Collection from Mice and Rats

[0342] Animals were sedated under general inhalant anesthesia (3 % isoflurane) for blood collection by cardiac puncture (mice) or jugular catheter (rats). Blood aliquots (300-400 μL) were collected in tubes coated with lithium heparin, mixed gently, then kept on ice and centrifuged at 2,500 ×g for 15 minutes at 4°C, within 1 hour of collection. The plasma was then harvested and kept frozen at -20 °C until further processing.

Animal Dosing Design - In vivo PK - Non cannulated, nonfasted animals

Group 1: SC, n=3 animals per time point (24 animals total) or

IV, n=3 animals per time point (24 animals total)

Group 2: PO, n=3 animals per time point (24 animals total)

Group 3: Control animals (for drug-free blood), n=5 mice

Each animal was subject to one blood draw and one brain collection.

[0343] Brain sample collection from animals

[0344] Immediately after blood sampling, animals were decapitated and the whole brains were quickly removed, rinsed with cold saline (0.9% NaCl, g/mL), surfacc vasculature ruptured, blotted dry with gauze, weighted, kept on ice until further processing within one hour of collection. Each brain was homogenized in 1.5 mL cold phosphate buffered saline, pH 7.4 (mice =1.5 mL, rats =), for 10 seconds on ice using the Power Gen 125. The brain homogenate from each brain was then stored at -20oC until further processing.

Linearity in Brain samples

[0345] Aliquots of brain homogenate were spiked with the test compound at the specified concentrations. To each brain aliquot an equal volume of chilled 26%

(g/mL) neutral Dextran (average molecular Weight 65,000-85,000 from Sigma, catalog number D-1390) solution was added to obtain a final Dextran concentration of 13%. The homogenate was centrifuged at 54000 x g for 15 minutes at 4 °C. The supernatants were subsequently processed using acetonitrile precipitation and analyzed by HPLC-MS/MS. A calibration curve of peak are versus concentration was constructed. The reportable linear range of the assay was determined, along with the lower limit of quantitation (LLQ).

[0346] Quantitative analysis of Brain Samples

To each brain homogenate aliquot an equal volume of chilled 26% (g/mL) neutral Dextran (average molecular Weight 65,000-85,000 from Sigma, catalog number D-1390) solution was added to obtain a final Dextran concentration of 13%. The homogenate was centrifuged at 54000 x g for 15 minutes at 4oC. The supernatants were subsequently processed using acetonitrile precipitation and analyzed by HPLC-MS/MS. A brain calibration curve was generated. Aliquots of drug-free brain homogenate were spiked with the test compound at specified concentration levels. The spiked brain homogenate samples were processed together with the unknown brain homogenate samples using the same procedure. The processed brain samples were stored at -20oC until the LC-MS/MS analysis, at which time peak areas were recorded, and the concentrations of test compound in the unknown brain samples were determined using the respective calibration curve. The reportable linear range of the assay was determined along with the lower limit of quantitation (LLQ).

Brain penetration

[0347] The concentrations of the test compound in brain (ng/g tissue) and in plasma (ng/mL) as well as the ratio of the brain concentration and the plasma concentration at each time point were determined by LC-MS/MS and reported as described above.

Pharmacokinetics

[0348] Plots of plasma concentration of compound versus time were constructed. The fundamental pharmacokinetic parameters of compound after oral and SC dosing (AUClast, AUCINF, T1/2, Tmax, and Cmax) were obtained from the

non-compartmental analysis (NCA) of the plasma data using WinNonlin (Pharsight). Noncompartmental analysis does not require the assumption of a specific compartmental model for either drug or metabolite. NCA allows the application of the trapezoidal rule for measurements of the area under a plasma concentration-time curve (Gabrielsson, J. and Weiner, D. Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications. Swedish Pharmaceutical Press. 1997).

Definitions of Terms Reported

[0349] Area Under the Curve (AUC) - Measure of the total amount of unchanged drug that reaches the systemic circulation. The area under the curve was a geometric measurement that was calculated by plotting concentration versus time and summing the incremental areas of each trapezoid.

[0350] WinNonlin has two computational methods for calculation of the area: the linear trapezoidal method and the linear-log trapezoidal method. Because the linear trapezoidal method may give biased results on the descending part of the concentration-time curve and overestimate the AUC, WinNonlin provides the linear-log option for calculation of AUC. By default, the log-linear trapezoidal method was used to measure the post-Tmax area for the remainder of the plasma concentration-time curve.

[0351] AUClast: area under the curve from the time of dosing to the time of last observation that was greater than the limit of quantitation.

[0352] AUCINF: Area under the curve from the time of dosing extrapolated to infinity.

[0353] Cmax - Maximum plasma drug concentration obtained after oral or non-IV administration of a drug between the time of doing and the final observed time point.

[0354] Tmax - Time at maximum observed plasma concentration (Cmax) noted in minutes after administration of drug.

[0355] T1/2 - Terminal elimination half-life from both IV and non-IV dosing.

[0356] where lambda Z (z) is the first order rate constant associated with the terminal (log-linear) portion of the plasma concentration-time curve. z was estimated by linear regression of time versus log concentration.

[0357] The results showed that the compounds are highly bioavailable and highly brain penetrant when they are administered at doses ranging from 0.1 to 0.5 mg/kg acutely or chronically (daily over 5 days). The results for acute administration of Compound II are shown in Figure 2A. Figure 2A is a graph wherein plasma levels of compound are shown on the left y-axis in units of ng/mL. Brain levels are shown on the right y-axis in green in units of ng/g. The x axis shows the time following bolus IV or SC administration at time zero. Following acute IV administration at 10mg/kg i.v., Compound II reached a high brain concentration and at 180 minutes post-dosing still had a concentration of 171 ng/g (57x the efficacious brain dose in vivo, shown by the open diamond). A similar pattern followed acute SC administration. Compound B showed the same level of bioavailability on parenteral administration but was substantially more bioavailable by oral route. Both compounds tested were found to be clearly highly BBB-penetrant, and Compound II had a brain/plasma ratio of 8 at 3 hours.

[0358] The results for compound B and those for Compound II acute administration are shown in Table 7.

[0359] **Table 7**. Pharmacokinetics for Compounds B and II in Mice.

Parameter	Compound B - 10 mg/kg		Compound II – 10 mg/kg	
	PO Plasma	SC Plasma	PO Plasma	SC Plasma
T_{max} (min)	30	120	NC	30
C_{max} (ng/mL)	599.3	607.7	NC	279
$t_{1/2}$ (min)	210	218	NC	100
AUC_{all} ((ng*hr)/mL)	2,851.1	5,242.3	NC	384.7

F_{rel} (%)	54.4		NC	

[0360] As shown in this table, the oral bioavailability of Compound B and its other PK parameters are improved over Compound II.

[0361] Dosing over a range of 0.1, 0.35 and 0.5 mg/kg gave relatively stable plasma levels of Compound II in chronic administration over the course of 5 days, with good brain exposure and similar brain/plasma ratios as the acute setting. The results are shown in Fig. 2B. Figure 2B is a plot of pharmacokinetic data obtained in plasma (left ordinate) upon once daily subcutaneous administration of different amounts of Compound II (0.5 mg/kg/day: downward pointing filled triangles; 0.35 mg/kg/day: upward pointing filled triangles; and 0.1 mg/day filled squares) and in brain (right ordinate) upon SC administration of the same amounts (respectively downward pointing open triangle, upward pointing open triangle and open square) of Compound II.

[0362] Brain level measurements for Compound B showed a lower peak concentration than Compound II but at a higher sustained level. At the three-hour point, the amount of Compound II in the brain is almost 40x higher for PO and 60x higher for SC than the 50 ng/mL efficacious dose determined for Compound II. However, the three-hour time point for Compound II is still >3x higher for SC than the efficacious dose for that compound (data not shown).

[0363] A mixture of Compounds IXa and IXb, when dosed at 1 mg/kg intravenously in mice, displayed a half-life of 2.7 hours. However, when the compound was dosed at 5 mg/kg orally, it displayed negligible amounts of drug in the plasma. A subsequent study (Table 6) of the mixture of Compounds IXa and IXb plus drug standards in mouse hepatic microsomes measured a half-life for the mixture of 8.7 minutes and an intrinsic clearance of 267 microL/min/mg indicating that the mixture is susceptible to first pass metabolism. In general, CNS active compounds tend to have a low to moderate intrinsic clearance rate ($CL_{int} \leq 100$ microL/min/mg) (Wager et al.,'10). Human hepatic microsomal stability data of 13 CNS active drugs gave an average half-life of 51±29 minutes (Orbach '99). Thus, reasonable goals for the improvement of Compound II to first pass metabolism

would be a $T_{1/2}$ > 30 minutes and a $CL_{int} \leq 100$ microL/min/mg. Compounds IXa,IXb exhibited comparable hepatic stability compared to certain known drugs, as shown in Table 8.

[0364] **Table 8**. Mouse hepatic microsome data for Compounds IXa and IXb and select CNS drug standards. CL'int = intrinsic clearance. Experiments were performed by a contract research organization and run as standards with Compound II.

Drug	Mode of action	Microsomal concentration (mg/mL)	T1/2 (min)	CL' INT (uL/min/mg)
Mixture of Compounds IXa and IXb	Candidate compound	0.3	8.7 +/- 0.1	267
Imipramine	antidepressant	0.3	11.5	200
Propranolol	Beta blocker	0.3	16.4 +/- 0.5	141
Terfenadine	antihistamine	14.6	8.7 +/- 1.1	159
Verapamil	Ca++ channel blocker	0.3	11.4 +/- 0.5	204

Example 8: Abeta 1-42 Oligomer Binding and Synapse Loss Assay

[0365] In this assay, Abeta oligomers were brought in contact with mature primary neurons in culture and their binding was determined by immunohistochemistry (anti-Abeta antibody) and quantified by image processing. The amount of Abeta in neuronal dendrites is assessed by counting the number of labeled puncta on the neuritis. Abeta oligomers are known to bind, saturably (Kd approximately 400 nM; Laurén 2009) and with high affinity to a subset of postsynaptic neurons present on a significant percentage (30 to 50%) of hippocampal neurons in primary cultures (Lacor et al, 2004; Lambert et al, 2007) and this correlates well with observations of Abeta binding in brains from

Alzheimer's patients (Lambert et al, 2007). This labeling is associated with synapses, co-localizing with the post-synaptic scaffold protein PSD-95 (Lacor et al., '04). Abeta oligomers are also known to mediate synapse loss, reported as 18% in human hippocampal neurons in brain slices (Schef et al , 2007) and to inhibit long term potentiation (LTP). The number of synapses can also be quantified in this assay by immunofluorochemistry. Similar procedures for binding assays can be found in the literature. *See e.g.*, Look GC, et. al. Discovery of ADDL--targeting small molecule drugs for Alzheimer's disease. Curr Alzheimer Res. 2007 Dec;4(5):562-7. Review.

[0366] Measurement of the amount of Abeta bound to the surface of neurons can be used as a secondary screen to identify compounds acting via one or more of the following mechanisms: blocking Abeta effects by interference with Abeta oligomer binding to neuronal surface or by effecting alterations to the oligomers themselves (inverse agonism or oligomer dissociation) or alteration of the surface receptors that the oligomers bind to (allosteric modulation or classical receptor antagonism) It can also distinguish these compounds from compounds acting on downstream signaling events. Accordingly, this assay is relevant to disease states characterized by Abeta oligomer nonlethal effects on neurons and forms part of a screening cascade employed by the present inventors to identify clinically relevant compounds. Importantly, one of the compounds disclosed here, Compound II, has been active in membrane trafficking assay and in this binding/synapse loss assay and has been proved also active in two different transgenic models for Alzheimer's disease and in an induced model as well. Accordingly, this as well as the membrane trafficking assay is useful in identifying clinically relevant compounds and appears to have predictive value for in vivo results. The predictive validity of this assay is being confirmed by demonstrating its ability to predict compound properties using compounds outside of the scope of the present invention.

[0367] Primary hippocampal neuronal culture was established as in the membrane trafficking assay above. Compound II (at concentrations of 10^{-8} to 30 micromolar) was added and any other compound to be tested in the future (at concentrations of 10^{-8} to 30 micromolar) were added to a plate followed by an addition of Abeta 1-42 oligomer containing preparation at a concentration to reach

saturation binding. Pretreatment with compounds to be tested lasted for 1 hr and addition of Abeta oligomers or no oligomer (vehicle alone) in a final concentration of 70 ul was followed by incubation for an additional 23 hrs.

[0368] The plates were fixed with 3.7% paraformaldehyde in phosphate buffered saline for 15 min. The plates were then washed 3x with PBS for 5 min each. The plates were blocked at RT for 1 hr in 5% goat serum and 0.5% Triton X-100 in PBS. Primary antibodies (anti-MAP 2 polyclonal, Millipore #AB5622 and anti-Beta Amyloid 6E10 monoclonal, Covance #SIG-39300, at 1 microgram/ml, and rabbit polyclonal anti-synaptophysin, Anaspec, at 0.2 microgram/ml) were diluted 1:1000 in 5% goat serum with PBS. Primary antibodies were incubated overnight at 4 °C. The plates were then washed 3x with PBS for 5 min each. Secondary antibodies (Alex Flor 488 polyclonal, Invitrogen #A11008 and Alexa Flor 647 monoclonal, Invitrogen #A21235) were diluted 1:1000 in 5% goat serum with PBS. Secondary antibodies were incubated at RT for 1 hr. The plates were washed once with PBS. DAPI (4',6-diamidino-2-phenylindole, Invitrogen) was then applied at 0.03 ug/ul and incubated at RT for 5 min, then washed with PBS. The results show that, as expected, Abeta oligomer, prepared as detailed below and dosed at 3 or 1 μM depending on the preparation used, bound to neurons at synapses, as was revealed by a red dye. In humans with early Alzheimer's disease, the number of synapses in the hippocampus has been shown to be reduced by 18% compared to age-matched cognitively normal individuals (Scheff et al., '07) and this result could also be visualized on this assay by 20% regression of fluorescent puncta and therefore of the number of synapses. In the co-presence of Compound II (15 uM), however, the Abeta binding was reduced to essentially control levels, and the green fluorescence was unaffected indicating an undiminished synapse number. See figures 3A to 3F. In Figure 3A-panelA, Abeta 42 oligomers bind to postsynaptic spines; Figure 3A-panel B shows presynaptic spines are labeled with synaptophysin in primary neurons (21 DIV). Figure 3A-Panels C and D shows the post-synaptic spines and synapses, respectively, at essentially control levels when IXa,IXb have been added to the culture. As shown quantitatively in the bar diagram of Figure 3C, Abeta 42 oligomers added alone caused a 20% decrease in the density of synaptophysin puncta (as calculated) after 24 hrs (fourth bar) compared to vehicle

alone (first bar). This loss was reversed by either Compound II or IXa,IXb (fifth or sixth bars) and this result was statistically significant. In the absence of Abeta oligomer, neither Compound IXa,IXb nor Compound II affects synaptic number (hatched bar) and it remains at levels comparable to control (vehicle alone). Scale bar = 20 um. $p<0.001$ ANOVA . Figure 3D ($p<0.001$ ANOVA) is also a bar diagram and shows that the Abeta binding intensity as calculated by the Abeta puncta is reduced by 18% in the presence of Compound II or IXa,IXb, yet this decrease is sufficient to permit synapse count to reach control levels in the presence of this compound.

[0369] Additionally, punctate synaptic Abeta oligomer binding is reduced by 38% in the presence of a mixture of Compounds IXa and IXb in a concentration-dependent manner, with an IC_{50} of 1.2 μM (data not shown). A histogram of puncta intensity reveals that the normal bimodal binding population (neurons with bright puncta and a population with less bright puncta) is left-shifted in the presence of drug (data not shown). Partial inhibition of Abeta oligomer binding has been reported to restore 100% of LTP function (Strittmatter SM et al., Cellular Prion Protein Mediates Impairment of Synaptic Plasticity by Amyloid-Beta Oligomers *Nature* (2009) 457 (7233:1128-32)). Further, as shown in Figure 3C, Abeta oligomer (fourth bar) caused a 20% decrease in the density of synaptophysin puncta after 24 hrs compared to vehicle-treated (first bar), which was reversed by 5 μM of the mixture of Compounds IXa and IXb (fifth bar). In the absence of Abeta (second bar), the mixture of Compounds IXa and IXb do not affect synaptic number. Abeta oligomers cause an 18.2% decrease in synapse number; 100% of this loss is eliminated by 5 μM of compound IXa,IXb or II (Fig 3C). The mixture of Compounds IXa and IXb cause a 17.7% decrease in the intensity of Abeta labeled puncta (Fig 3D) with an IC_{50} of 1.21 uM.

[0370] Nuclei, visualized with DAPI, exhibited a normal morphology, indicating an absence of neurodegeneration. The procedure will be repeated with additional test compounds selected from among those encompassed by Formula I-IX, as well as other compounds described as sigma-2 ligands above.

Abeta oligomer preparations:

[0371] Human amyloid peptide 1-42 was obtained from California Peptide, with lot-choice contingent upon quality control analysis. Abeta 1-42 oligomers were made according to published methods as described above. [*See e.g.* Dahlgren et al., "Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability" J Biol Chem. 2002 Aug 30;277(35):32046-53. Epub 2002 Jun 10.; LeVine H 3rd. "Alzheimer's beta-peptide oligomer formation at physiologic concentrations" Anal Biochem. 2004 Dec 1;335(1):81-90; Shrestha et.al, "Amyloid beta peptide adversely affects spine number and motility in hippocampal neurons" Mol Cell Neurosci. 2006 Nov;33(3):274-82. Epub 2006 Sep 8; Puzzo et al., "Amyloid-beta peptide inhibits activation of the nitric oxide/cGMP/cAMP-responsive element-binding protein pathway during hippocampal synaptic plasticity" J Neurosci. 2005 Jul 20;25(29):6887-97; Barghorn et al., "Globular amyloid beta-peptide oligomer - a homogenous and stable neuropathological protein in Alzheimer's disease" J Neurochem. 2005 Nov;95(3):834-47. Epub 2005 Aug 31; Johansson et al., Physiochemical characterization of the Alzheimer's disease-related peptides A beta 1-42 Arctic and A beta 1-42wt. FEBS J. 2006 Jun;2 73(12):2618-30] as well as brain-derived Abeta oligomers (*See e.g.* Walsh et al., Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature (2002). 416, 535-539; Lesne et al., A specific amyloid-beta protein assembly in the brain impairs memory. Nature. 2006 Mar 16;440(7082):352-7; Shankar et al, Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med. 2008 Aug;14(8):837-42. Epub 2008 Jun 22). Quality controls of oligomer preparations consist of Westerns to determine oligomer size ranges and relative concentrations, and the MTT assay to confirm exocytosis acceleration without toxicity. Toxicity was monitored in each image-based assay via quantification of nuclear morphology visualized with the DNA binding dye DAPI (Invitrogen). Nuclei that were fragmented are considered to be in late stage apoptosis and the test rejected (Majno and Joris Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol 1995;146:3–16). Peptide lots producing unusual peptide size ranges or significant toxicity at standard concentrations on neurons would be rejected.

Controls

[0372] Pre-adsorption of anti-Abeta antibody 6E10 with oligomer preparation inhibits synapse binding in a dose dependent manner (at 7.84 x 10^{-6}) and is used as a positive control. The antibody was used at 1:1000 (1 microgram/ml). For the synapse loss assay, the NMDA antagonist dizocilpine (MK-801) is used as the positive control at 80 uM.

Image Processing

[0373] Images were captured and analyzed with the Cellomics VTI automated microscope platform, using the Neuronal Profiling algorithm. For statistical analysis, a Tukey-Kramer pair-wise comparison with unequal variance was used.

Western blots

[0374] Samples containing Abeta 1-42 were diluted (1:5) in non-reducing lane marker sample buffer (Pierce #1859594). A 30 microliter (μL) sample was loaded onto an eighteen well precast 4-15% Tris-HCl gel (BIORAD #345-0028). Electrophoresis was performed in a BIO-RAD Criterian precast gel system using Tris-Glycine buffer at 125 volt (V) for 90 minutes. The gels were blotted onto 0.2 μM nitrocellulose membranes in Tris-Glycine/10% methanol buffer at 30V for 120 minutes. The membranes were boiled for 5 minutes in a PBS solution and blocked over night with TBS/5% milk solution at 4 °C. The membrane was probed with 6E10-HRP (Covance #SIG-39345) diluted to 10 μg/mL in TBS/1% milk solution for one hour at room temperature. Membrane was washed three times for 40 minutes each with a solution of TBS/0.05% tween-20 and developed with ECL reagent (BIO-RAD #162-0112) for 5 minutes. Image acquisition was performed on an Alpha Innotech FluorChem Q quantitative imaging system and analyzed with AlphaView Q software.

Activity

[0375] Compound II was shown and compounds selected from those specifically disclosed herein are expected to be shown to partially block binding of

the Abeta oligomer ligand to neurons by about 25% according to the binding assay (using imaging processing algorithm).

[0376]

Example 9: Fear Conditioning Assay

[0377] Compound II was tested in an animal model of a memory-dependent behavioral task known as fear conditioning. The study protocol was designed based on published protocols (*See e.g.* Puzzo D, Privitera L, Leznik E, Fà M, Staniszewski A, Palmeri A, Arancio O. Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. J Neurosci. 2008 Dec 31;28(53):14537-45.). The formation of contextual memories is dependent upon the integrity of medial temporal lobe structures such as the hippocampus. In this assay mice were trained to remember that a particular salient context (conditioned stimulus; CS) is associated with an aversive event, in this case a mild foot shock (the unconditioned stimulus, US). Animals that show good learning will express an increase in freezing behavior when placed back into the same context. This freezing is absent in a novel context. Increased freezing in the context indicates strong hippocampal-dependent memory formation in animals. Memory tested in Fear Conditioning is sensitive to elevations of soluble Aβ. Compound II was effective at stopping Abeta oligomer mediated effects on membrane trafficking. When administered to animals prior to Abeta oligomer administration, Compound II blocked oligomer effects on memory in a dose-dependent manner. The compound completely blocked oligomer-mediated memory deficits at the 2 pmol dose.

[0378] Indeed, as shown in Figure 4, Compound II completely eliminated Abeta oligomer-induced deficits in memory (black bar) but did not affect memory when dosed alone (hatched bar). The effect of Abeta oligomer alone is shown by the red bar. Additionally, as shown in Figure 5, a mixture of Compounds IXa and IXb provided a similar result. This behavioral efficacy demonstrates that the membrane trafficking assay is able to predict which compounds will be efficacious in treating the behavioral memory loss caused by oligomers. The fear condition model for memory was performed as described herein. No adverse behavioral

changes were observed at any dose. Accordingly, there is a correlation between the performance of this compound in the membrane trafficking assay and its performance in the fear conditioning assay, the latter being an indicator of memory loss. It is anticipated that the compounds listed in Table2 will be active in the fear conditioning assay and therefore will be shown to be efficacious in treating memory loss. The correlation between the performance of a compound in the fear condition model and its usefulness in treating memory loss has been established in the literature. (Delgado MR, Olsson A, Phelps EA. "Extending animal models of fear conditioning to humans" *Biol. Psychol.* 2006 Jul;73(1):39-48).

Example 10. Autoradiography studies with Rat, Rhesus monkey and Human postmortem brain samples.

[0379] Autoradiography imaging studies for the neurological and pharmacological profiling of the sigma-2 and sigma-1 receptor ligands were conducted by a modification of the protocol previously reported by Xu et al., 2010. Xu, J., Hassanzadeh B, Chu W, Tu Z, Vangveravong S, .Tones LA, Leudtke RR, Perlmutter JS, Mintun MA, Mach RH. *[^{3}H]4-(Dimethylamino)-N-[4-(4-(2-methoxyphenyl)piperazin- 1-yl)butyl]benzamide, a selective radioligand for dopamine D(3) receptors. II. Quantitative analysis of dopamine D3 and D2 receptor density ratio in the caudate-putamen.* Synapse 64: 449-459(2010), which is incorporated herein by reference. Labeled RHM-1 was obtained by the method of Xu J, Tu Z, Jones LA, Wheeler KT, Mach RH. *[^{3}H]N-[4-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1 H)-yl)butyl]-2-methoxy-5-methylbenzamide: a Novel Sigma-2 Receptor Probe.* Eur. J. Pharmacol. 525: 8-17 (2005), which is incorporated herein by reference.

[0380] Brain sections in 20 μM thickness from rats, rhesus monkeys and postmortem human brains were cut using with a Microm cryotome and mounted on superfrost plus glass slides (Fisher Scientific, Pittsburgh, PA)., and serial sections through the brain regions of cerebral cortex and hippocampus were used in this study. Brain section were incubated with 5 nM [^{3}H](+)-Pentazocine for sigma-1 receptor profiling, 4 nM [^{3}H]RHM-1 only for sigma-2 receptor characterization, 10 nM [^{3}H]DTG and [^{3}H]Haloperidol in the presence of sigma-1 receptor block (+)-

Pentazocine to image the sigma-2 receptor distribution; after incubation with the radioligands for 30 minutes, the brain sections containing glass slides were rinsed 5 times at one minute each time with ice-cold buffer.

[0381] Slides were dried and made conductive by coating with a copper foil tape on the free side and then placed in the gas chamber [mixture of argon and triethylamine (Sigma-Aldrich, USA)] of a gaseous detector, the Beta Imager 2000Z Digital Beta Imaging System (Biospace, France). After the gas is well mixed and a homogenous state is reached, further exposure for 24 hours to 48 hours until high quality images are observed. [^{3}H]Microscale (American Radiolabeled Chemicals, Inc., St. Louis, MO) was counted at the same time as a reference for total radioactivity quantitative analysis, i.e., to convert the cpm/mm2 to *nCi/mg* tissue. Quantitative analysis was performed with the program Beta-Image Plus (BioSpace, France) for the anatomical regions of interest (ROI), i.e., to obtain the quantitative radioactivity uptake (cpm/nlln2) in the regions of cortex and hippocampus. The binding density was normalized to fmol/mg tissue based on the specific activities of the corresponding radioligands and calibration curve from the standard [^{3}H]Microscale. A series of dilutions of candidate compounds (10 nM, 100 nM, 1,000 nM and 10,000 nM) were tested for competing the binding sites using the quantitative autoradiography, for those four radioligands, [^{3}H](+)-Pentazocine, [^{3}H]RHM-l, [^{3}H]DTG and [^{3}H]Haloperidol, then the specific binding (% control) was analyzed to derive the binding affinity in the regions of the cortex and the hippocampus (dentate gyrus, hippocampal CA I and CA3).

[0382] Autoradiography at sigma-1 and sigma-2 receptors is shown at FIGs 8A and 8B, respectively. FIG. 6C shows (A) [^{3}H]-(+)-Pentazocine (a sigma-1 receptor ligand) autoradiography in human frontal cortex slices from normal patients, Lewy Body Dementia (DLB) patients, or Alzheimer's Disease (AD) patients and (B) a graph of specific binding compared to control. As shown in FIG. 6A, sigma-1 receptors are statistically downregulated in Alzheimer's disease and possibly DLB compared to normal control. This finding confirms that of Mishina et al. who reported low density of sigma-1 receptors in early Alzheimer's disease. Mishina et al., 2008, *Low density of sigma1 receptors in early Alzheimer's disease.* Ann. Nucl Med 22: 151-156. FIG. 6B shows (A) [^{125}I]-RHM-4 (a sigma-2 receptor

ligand) autoradiography in human frontal cortex slices from normal patients, Lewy Body Dementia (DLB) patients, or Alzheimer's Disease (AD) patients, and (B) a graph of specific binding compared to control. Sigma-2 receptors are not statistically downregulated in AD. FIG. 6C shows (A) displacement of 18.4 nM [^{3}H]-RHM-1 in monkey frontal cortex, monkey hippocampus or human temporal cortex by sigma-2 ligands and (B) a graph of binding density of [^{3}H]-RHM-1 with and without 1 μM each of siramesine and compounds IXA,IXB and J. Siramesine and compounds IXA,IXB and II partially displace [^{3}H]-RHM-1 in the target tissues.

Example 11. MTS assay: Determination of agonist or antagonist activity of various sigma-2 ligands.

[0383] The cytotoxicity of compounds shown below was determined using the CellTiter96 Aqueous One Solution Assay (Promega, Madison, WI). Briefly, MDA-MB-435 or MDA-MB231or SKOV-3 cells were seeded in a 96-well plate at a density of 2000 cells/well on the day prior to treatment with sigma-2 receptor selective ligands. After a 24 hour treatment, the CellTiter 96 AQueous One Solution Reagent was added to each well, and the plate incubated for 2 hours at 37°C. The plate was read at 490 nm in a Victor3 plate reader (PerkinElmer Life and Analytical Sciences, Shelton, CT). The EC^{50} value, defined as the concentration of the sigma ligand required to inhibit cell viability by 50% relative to untreated cells, was determined from the dose response curve for each cell line. Siramesine is accepted as an agonist. The agonists and antagonists of the sigma-2 ligands were defined as the following: If the EC50s of a sigma-2 ligand was less than 2 fold of EC50 of siramesine, this sigma-2 ligand is considered as an agonist. If the EC50 of a sigma-2 ligand was between 2 and 10 fold of EC50 of siramesine, this sigma-2 ligand was considered as a partial agonist. If the EC50 of a sigma-2 ligand is larger than 10 fold of EC50 of siramesine, this sigma-2 ligand is considered as an antagonist. The sigma-2 ligands used for the studies are: agonists (siramesine and SV 119), partial agonist (WC26), antagonist (RHM-1), and candidate compounds (II and IXa,IXb). Results are shown in FIG. 9A. Data from FIG. 7A is shown in Table 9.

Table 9. IC_{50} values for TumorCell Viability assay.

Compound	IC_{50}, 48 hrs. (uM)	Action
RHM-1	203 ± 13	Antagonist
Siramesine	11.8 ± 2.7	Full agonist
SV-119	21.7 ± 2.9	Full agonist
WC-26	65.6 ± 6.3	Partial agonist
IXa,IXb	169 ± 9	Antagonist
II	150 ± 12	Antagonist

[0384] Neuronal cultures were treated with various concentrations of sigma compounds for 24 hours and nuclear intensity compared to vehicle was measured. Sigma-2 agonists (siramesine, SV-119, WC-26) caused significant abnormal nuclear morphology in neurons in contrast to sigma-2 antagonists (RHM-1, IXa,IXb and II) which did not decrease nuclear intensity at the test concentrations. Therefore, sigma-2 receptor agonists were cytotoxic to the neuronal and cancer cells; however sigma-2 receptor antagonists were not toxic and further blocked the cytotoxicity caused by sigma-2 receptor agonists.

Example 12. Caspase-3 assays. Determination of agonist or antagonist activity of sigma-2 ligands.

[0385] As described herein, Xu et al. identified PGRMC1 protein complex as the putative sigma-2 receptor binding site. Xu et al., 2011. Nature Commun. 2, article number 380, incorporated herein by reference. Sigma-2 receptor agonists can induce Caspase-3-dependent cell death. Xu et al 2011 disclose functional assays to examine the ability of the PGRMC1 to regulate caspase-3 activation by sigma-2 receptor agonist WC-26.

[0386] Abeta oligomers cause low levels of caspase-3 activation and lead to LTD. High levels of Abeta oligomers and caspase-3 activation lead to cell death. Li et al., 2010; Olsen and Sheng 2012. It was demonstrated herein that sigma-2 receptor agonists (SV-119, siramesine) activate caspase-3 in tumor cells and

neurons; see, for example, FIGS. 8A and 8B. Sigma-2 receptor antagonist RHM-1 inhibits the activation in tumor cells (FIG 8A), but was not able to block activation by agonist SV-119 in neurons in this experiment (FIG 8B). Test compounds II and IXa,IXb (all of which are sigma 2 receptor antagonists as shown below) were able to inhibit caspase-3 activation in tumor cells and block sigma-2 receptor agonist SV-119 activation of caspase-3 in neurons. Therefore, the test compounds II and IXa,IXb acted as sigma-2 receptor antagonists in caspase-3 assays in tumor cells and neurons, as demonstrated in this example.

[0387] The activation of endogenous caspase-3 by sigma-2 receptor ligands was measured using the Caspase-3 Colorimetric Activity Assay Kit (Milipore, Billerica, MA) according to the manufacture's protocol. Briefly, MDA-MB 435 or MDA-MB23I cells were plated at 0.5 x 10^6 cells 100 mm dish. 24 hours after plating, sigma-2 ligands were added to the culture dishes to induce caspase 3 activation. The final concentration of the sigma-2 ligand was its EC50. 24 hours after treatment, cells were harvested, lysed in 300 uL of Cell Lysis Buffer, and centrifuged for 5 minutes at 10,000 x g. Supernatant was collected and incubated with caspase-3 substrate, DEVD-pNA, for 2 hours at 37°C. The protein concentration was determined using Dc protein assay kit (Bio-Rad, Hercules, CAl. The resulting free pNA was measured using a Victor^3 microplate reader (PerkinEliner Life and Analytical Sciences, Shelton, CT) at 405 nm. The ligands tested included: sigma-2 agonists (siramesine, SV119, WC26), and sigma-2 antagonist, RHMWU-I-102 (RHM-1), and candidate compounds (II and IXa,IXb). The ligands which activated caspase 3 were considered as agonists, whereas the ligands which did not activate caspase 3 were considered antagonists. As shown in FIG. 8A, the sigma-2 agonist siramesine induced caspase-3 activity, whereas sigma-2 antagonists RHM-1, and candidate compounds II and IXa,IXb did not induce caspase-3 activity. FIG. 8B shows activation of caspase-3 by sigma-2 agonist SV-119, that is blocked by compounds IXa,IXb and II. Compounds IXa,IXb and II behaved like sigma-2 antagonists in both cancer cells and neurons.

Example 13. Therapeutic Phenotype.

[0388] In some embodiments, the disclosure provides an in vitro assay platform predictive of behavioral efficacy. A compound that (1) selectively binds with high affinity to a sigma-2 receptor; and (2) acts as a functional antagonist in a neuron and is predicted to have behavioral efficacy if: it blocks Aβ-induced membrane trafficking deficits; blocks Aβ-induced synapse loss and does not affect trafficking or synapse number in the absence of Abeta oligomer. This pattern of activity in the in vitro assays is termed the "therapeutic phenotype". The ability of a sigma-2 receptor antagonist to block Abeta oligomer effects in mature neurons without affecting normal function in the absence of Abeta oligomers is one criteria for the therapeutic phenotype. Compounds that affect trafficking or synapse number in the absence of oligomers are not behaviorally efficacious. Only those compounds that selectively block oligomers without affecting normal trafficking or altering synapse number are behaviorally efficacious in preventing and treating Abeta oligomer-induced memory loss. In one embodiment, the in vitro assay platform can predict behavioral efficacy. This pattern of activity in the platform assays is therefore a therapeutic phenotype.

For example, see Table 10A.

Table 10A. Therapeutic Phenotype.

Compound	Block Aβ-induced membrane trafficking deficits EC50(uM)	Block Aβ-induced synapse loss	Assay effects in the absence of Aβ	Behavioral efficiency
II	2.2	++	No	Yes
Z	6.1	+++	Yes	No
Z'	4.3	+++	Yes	No

IXa+IXb	4.9	+++	No	Yes

[0389] In summary, sigma-2 antagonists with high affinity (preferably Ki less than about 600 nM, 500 nM, 400 nM, 300 nM, 200 nM, 150 nM, 100 nM, or 70 nM) at sigma-2 receptors that have greater than about 20-fold, 30-fold, 50-fold, 70-fold, or preferably greater than 100-fold selectivity for sigma receptors compared to other non-sigma CNS or target receptors, have good drug-like properties including brain penetrability and good metabolic and/or plasma stability, and that possess the therapeutic phenotype, are predicted to have behavioral efficacy and can be used to treat Abeta oligomer-induced synaptic dysfunction in a patient in need thereof.

[0390] Functional neuronal phenotype for several Compound II analogs, predicted to have oral bioavailability, with in vitro assay characterization, is shown in Table 10B.

Table 10B. Functional Neuronal Phenotype

Selectivity	**Compound**	**Inhibition Abeta oligomer-induced Membrane Trafficking EC50(uM)**	**S1 binding Ki (nM)**	**S2 binding Ki (nM)**	**Block synapse loss**	**Functional Neuronal Phenotype**
Higher affinity at sigma-2	II	2.2	500	9	100%	Antagonist
	II (+) isomer	5.6	100	80	47%	Antagonist

Selectivity	Compound	Inhibition Abeta oligomer-induced Membrane Trafficking EC50(uM)	S1 binding Ki (nM)	S2 binding Ki (nM)	Block synapse loss	Functional Neuronal Phenotype
	W	8.7	110	36	43%	Antagonist
	S'	>20	25	8	0%	Inactive
	P	>20	320	110	0%	Inactive
Higher affinity at sigma-1	A	3.4	3	13	100%	Antagonist
	B	5.5	1.3	3.9	100%	Antagonist
	X	6.1	3.5	16	100%	Antagonist
	E	8.2	2	3.6	34%	Antagonist

Selectivity	Compound	Inhibition Abeta oligomer-induced Membrane Trafficking EC50(uM)	S1 binding Ki (nM)	S2 binding Ki (nM)	Block synapse loss	Functional Neuronal Phenotype
	II (-) isomer	10.9	46	63	0%	Antagonist
Comparable affinity at sigma-2 and sigma-1	Y	4.3	78	85	100%	Antagonist
	R'	>20	11	16	33%	Inactive

Example 14: In vitro Toxicity.

[0391] Representative sigma-2 antagonists II and IXa,IXb did not induce neuronal or glial toxicity with acute or chronic dosing in vitro. The sigma-2 receptor antagonists eliminated or reduced Abeta oligomer-induced changes in membrane trafficking. No significant effect of compounds on membrane trafficking occurred when dosed without oligomers. There was no toxicity relative to neuron number, glial number, nuclear size, nuclear morphology, neurite length, cytoskeletal morphology when tested to 10 times the EC50 concentration (up to 50 μM II or IXa,IXb) for three days. See Table 11.

[0392] **Table 11.**

Compound	IXa, IXb	II
EC50 (uM)	4.9	2.2
Max Inhib. of Abeta (Conc)	100% (14)	85% (10)
Calculated Ki*	0.58	0.26
Cpd alone at Ki	+ 9%	+1%

*Km for Abeta = 0.4 uM; assay concentr. 3 μM total Abeta.

[0393] In vitro toxicity for Compound II was tested in a number of standard assays. Testing in vitro toxicity studies reveals there is no genotoxicity at 10 μM (AMES, micronucleus, bacterial cytotox). HepG2 toxicity of 66% at 10 μM (100-fold above affinity at receptor x) may be due to compound lipophilicity or receptor overexpression in HepG2 tumor cell line. Partial inhibition (46-73%) of CYP 450 enzymes 2D6, 3A4, and 2C19 occurred at 10 uM. Moderate hERG inhibition (24%) was seen at 100nM. Compound II exhibited very weak (IC50>30uM) activity at PGP.

Example 15: Separation and Activities of Enantiomers of Compound II in the Membrane Trafficking Assay.

[0394] Compound II was separated into its (+) and (-) enantiomers. The racemic mixture was applied to a chiral column CHIRALPAK AD-H (amylose tris (3,5-dimethylphenylcarbamate) coated on silica-gel; 4.6X250mm). The sample was injected into the column in a 15 microliterl volume. The eluent was Hexane/EtOH/TEA (95/5/0.1) with a flow rate of 1 ml/min at 25 degrees Celsius. The two enantiomers were separated in distinct peaks. The (+) enantiomer eluted in a first peak at approximately 16 minutes and the (-) enantiomer eluted in a second peak eluting at approximately 20 minutes. The enantiomers were at least 98% pure. The (+) enantiomer had a specific rotation of +10.1 (c 1.80 in MeOH) and the (-)

enantiomer had a specific rotation of -7.2(c 1.80 in MeOH). The (+) enantiomer was more potent in the membrane trafficking assay described in Example 6 than the (-) enantiomer. In one sample, the (+) enantiomer had an EC50 of 5.6 and the (-) enantiomer has an EC50 of 10.9 μM in inhibiting amyloid beta induced deficits in the membrane trafficking assay.

Example 16. Behavioral Efficacy of Orally Available Compounds-Improvement of Memory Deficits in Transgenic Alzheimer's Mouse Model.

[0395] Male hAPP Swe/Ldn transgenic (Tg) mice were utilized as a TG model of AD. Transgenic mice that were treated with vehicle, 10 or 30 mg/kg/day of CB or CF for 5.5 months p.o., as well as non-transgenic vehicle-treated littermates were subjected to a standard fear conditioning paradigm. Vehicle-treated 9 month old male hAPP Swe/Ldn transgenic (Tg) mice that were treated p.o. for 5.5 months with vehicle exhibited significant memory deficits vs. vehicle-treated non-transgenic littermates in contextual fear conditioning.

[0396] When the animals were tested for associative memory 24 hours after training, two-way (genotype and time) ANOVA with repeated measures did not detect a significant difference in total freezing time between transgenic and nontransgenic vehicle-treated mice. However, the more sensitive analysis of freezing behavior during individual timed intervals indicates that transgenic mice performed significantly worse during the 1-3- minute interval compared to the non-transgenic vehicle-treated animals (Mann-Whitney U test, $p<0.05$). During this interval, transgenic animals that were treated with 10 and 30 mg/kg/day of CB ($p<0.05$) and 30 mg/kg/day of CF ($p<0.005$) significantly improved performance compared to vehicle (Mann-Whitney U test). Results are shown in Figure 9, both doses of CB significantly reversed memory deficits in AD mice; and the higher dose of CF revered memory deficits in AD mice. Treatment of Tg animals with CB at 10 and 30 mg/kg/day or CF at 30 mg/kg/day improves the deficits at measured brain concentrations of 394 ± 287, 793 ± 325, or 331 ± 373 nM respectively (AVG ± S.D.).Brain/trough plasma and brain/peak plasma ratios for orally available compounds.

Results are shown in the Table 12.

Table 12. Brain/trough plasma and brain/peak plasma ratios for orally available compounds.

Compound	Dose (p.o.)	Brain/Trough Plasma Ratio	Brain/Peak Plasma Ratio
CF	30 mg/kg	13	0.5
CF	10 mg/kg	11	0.2
CB	30 mg/kg	14	0.4
CB	10 mg/kg	17	0.7

[0397] Therefore, both compounds CB and CF are orally bioavailable, capable of achieving significant brain penetration and reversing established memory deficits in aged transgenic Alzheimer's mouse models animals following chronic long-term administration. No adverse behavioral effects observed.

[0398] Both CB and CF are selective, high affinity sigma-2 receptor antagonist compounds. Both CB and CF bind to the sigma-2 and sigma-1 receptors with high affinity as shown in Table 14. Counterscreening was performed against a panel of 40 brain receptors and results indicated that CB and CF are highly selective for sigma receptors, as shown in the Table 13.

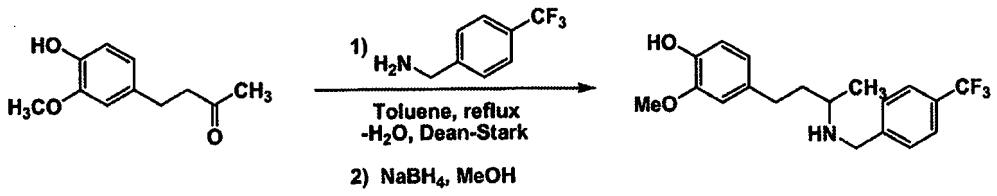
Table 13. Receptor Affinities for Orally Bioavailable Compounds.

Drug	Therapeutic Effect	Sigma Receptor Affinity (sigma-1/sigma-2) (Ki, nM)	Other Receptor Affinities (Ki, nM)
CB	Alzheimer's	19/48	Muscarinic M1 (1.5 uM), M2 (1.5 uM), M3 (1.8 uM)
			kappa opioid (1.5 uM)
			Ca++ ch - L-type (860 nM)

Drug	Therapeutic Effect	Sigma Receptor Affinity (sigma-1/sigma-2) (Ki, nM)	Other Receptor Affinities (Ki, nM)
			Transporters: NE (1.4 uM), DA (220 nM), 5-HT (970 nM)
CF	Alzheimer's	180/50	Muscarinic M1 (1.1 uM), M2 (2.5 uM), M3 (3.7 uM)
			kappa opioid (6.1 uM)
			Ca++ ch - L-type (2.5 uM)
			Transporters: NE (1.9 uM), DA (940 nM), 5-HT (3.2 uM)

[0399] CB at a 10 mg/kg/day dose results in compound brain levels that are at or above the Ki for sigma and dopamine transporters, 30 mg/kg/day dose hits those plus Ca++ ch and 5-HT transporter. Subsequent studies can be used to determine the minimum effective dose of this compound. CF at the 30 mg/kg/day dose results in compound brain levels that are selective for sigma receptors only, therefore its affinity at sigma receptors accounts for its behavioral efficacy at these brain concentrations.

Synthesis Example 1: Synthesis of compounds by reductive amination.



[0400] Vanillylacetone (5.00 g, 25.7 mmol) was dissolved in toluene (250 mL) and 4-trifluoromethylbenzylamine (4.73 g, 27.0 mmol) was added. The

mixture was maintained under an atmosphere of nitrogen and heated at reflux with removal of water by Dean-Stark distillation for 16 hours. At this time the Dean-Stark trap was removed and the reaction mixture was cooled to 0°C on an ice bath. A solution of sodium borohydride (5 g) in methanol (100 mL) was added portion-wise over 30 minutes with vigorous stirring. When the addition was complete the mixture was heated at reflux for 16 hours. At this time the reaction mixture was cooled to room temperature and poured into saturated aqueous sodium bicarbonate solution (300 mL). The resulting mixture was concentrated by rotary evaporation and the aqueous residue was partitioned between water and chloroform. The chloroform layer was dried over anhydrous sodium sulfate and then filtered and concentrated. The product was then purified using silica gel column chromatography employing a mobile phase of 5% ammonia-methanol in chloroform. Product-containing fractions were combined and concentrated then dried under high vacuum overnight to provide a light brown oil (6.72 g, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.57 (d, J = 7.8 Hz, 2H), 7.43 (d, J = 7.9 Hz, 2H), 6.82 (d, J = 7.3 Hz, 1H), 6.65 (m, 2H), 5.16-4.42 (br s, 2H), 3.90 (d, J = 13.7 Hz, 1H), 3.84 (s, 3H), 3.80 (d, J = 13.7 Hz, 1H), 2.76-2.70 (m, 1H), 2.67-2.55 (m, 2H), 1.84-1.77 (m, 1H), 1.69-1.63 (m, 1H), 1.17 (d, J = 6.3 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 146.7, 144.6, 143.9, 134.0, 129.1, 128.4, 127.5, 125.4, 125.3, 123.2, 120.8, 114.6, 111.0, 55.7, 52.1, 50.6, 38.8, 32.0, 20.1. MS (CI) *m/z* 353 (M^+).

[0401] The chemical shift measure by ^{1}H NMR may vary, for example, up to 0.2 ppm. The chemical shift measure by ^{13}H NMR may vary, for example, up to 0.5 ppm. The analytical Mass Spectrum may have an experimental error of +/- 0.3.

Purity Determination

The purity of the product was measured by HPLC. The major peak of retention time of 2.22 minutes indicating greater than about 80%, 85%, 90, or 95% of purity. The HPLC conditions used are as follows.

HPLC conditions:

Mobile Phase A: 13.3 mM ammonium formate/6.7 mM formic acid in water

Mobile Phase B: 6 mM ammonium formate/3 mM formic acid in water/CH_3CN (1/9, v/v)

Column: Synergi Fusion-RP 100A Mercury, 2 x 20 mm, 2.5 micron

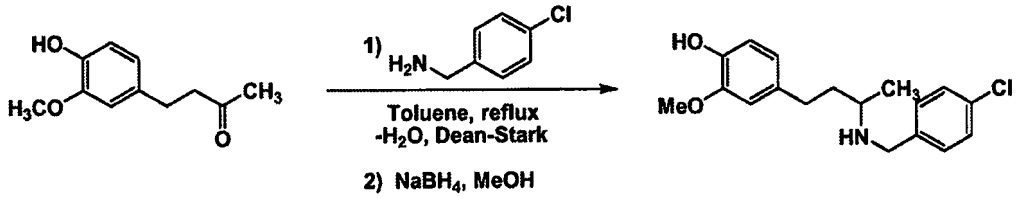
(Phenomenex Part No 00M-4423-B0_CE)

Gradient Program: RT = 2.22 minutes

Time, minute	% Phase B	Flow rate, ml/min
0	100	0.5
1	100	0.5
2.5	40	0.5
3.4	40	0.5
3.5	100	0.5
4.5	100	0.5

[0402] The purity of the product was also measure by ^1H NMR indicating it to be a single compound of a purity of greater than 90% or 95%. The synthesis described herein can be modified depending upon the final-product to be synthesized.

Synthesis Example 2: Synthesis of compounds by reductive amination.



[0403] Vanillylacetone (5.00 g, 25.7 mmol) was dissolved in toluene (250 mL) and 4-chlorobenzylamine (4.73 g, 27.0 mmol) was added. The mixture was maintained under an atmosphere of nitrogen and heated at reflux with removal of water by Dean-Stark distillation for 16 hours. At this time the Dean-Stark trap was

removed and the reaction mixture was cooled to 0°C on an ice bath. A solution of sodium borohydride (5 g) in methanol (100 mL) was added portion-wise over 30 minutes with vigorous stirring. When the addition was complete the mixture was heated at reflux for 16 hours. At this time the reaction mixture was cooled to room temperature and poured into saturated aqueous sodium bicarbonate solution (300 mL). The resulting mixture was concentrated by rotary evaporation and the aqueous residue was partitioned between water and chloroform. The chloroform layer was dried over anhydrous sodium sulfate and then filtered and concentrated. The product was then purified using silica gel column chromatography employing a mobile phase of 5% ammonia-methanol in chloroform. Product-containing fractions were combined and concentrated then dried under high vacuum overnight to provide a light brown oil (6.16 g, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.30-7.24 (m, 4H), 6.81 (d, J = 7.8 Hz, 1H), 6.66-6.62 (m, 2H), 4.25 (br s, 2H), 3.82 (s, 3H), 3.82 (d, J = 13.2 Hz, 1H), 3.72 (d, J = 13.2 Hz, 1H), 2.73 (m, 1H), 2.66-2.51 (m, 1H), 1.86-1.78 (m, 1H), 1.72-1.63 (m, 1H), 1.62-1.51 (m, 1H), 1.17 (d, J = 6.3 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 146.6, 143.8, 133.9 132.8, 129.9, 129.7, 128.6, 120.8, 114.5, 110.9, 55.8, 51.9, 50.2, 38.5, 31.9, 31.6, 29.7, 26.9, 22.6, 19.9. MS(MH^+): *m/z* 320.

[0404] The chemical shift measure by ^{1}H NMR may vary, for example, up to 0.2 ppm. The chemical shift measure by ^{13}H NMR may vary, for example, up to 0.5 ppm. The analytical Mass Spectrum may have an experimental error of +/- 0.3.

Purity Determination

[0405] The purity of the product was measured by HPLC. The major peak of retention time of 2.22 minutes indicating greater than about 80%, 85%, 90, or 95% of purity. The HPLC conditions used are as follows.

HPLC conditions:

Mobile Phase A: 13.3 mM ammonium formate/6.7 mM formic acid in water

Mobile Phase B: 6 mM ammonium formate/3 mM formic acid in water/CH_3CN (1/9, v/v)

Column: Synergi Fusion-RP 100A Mercury, 2 x 20 mm, 2.5 micron

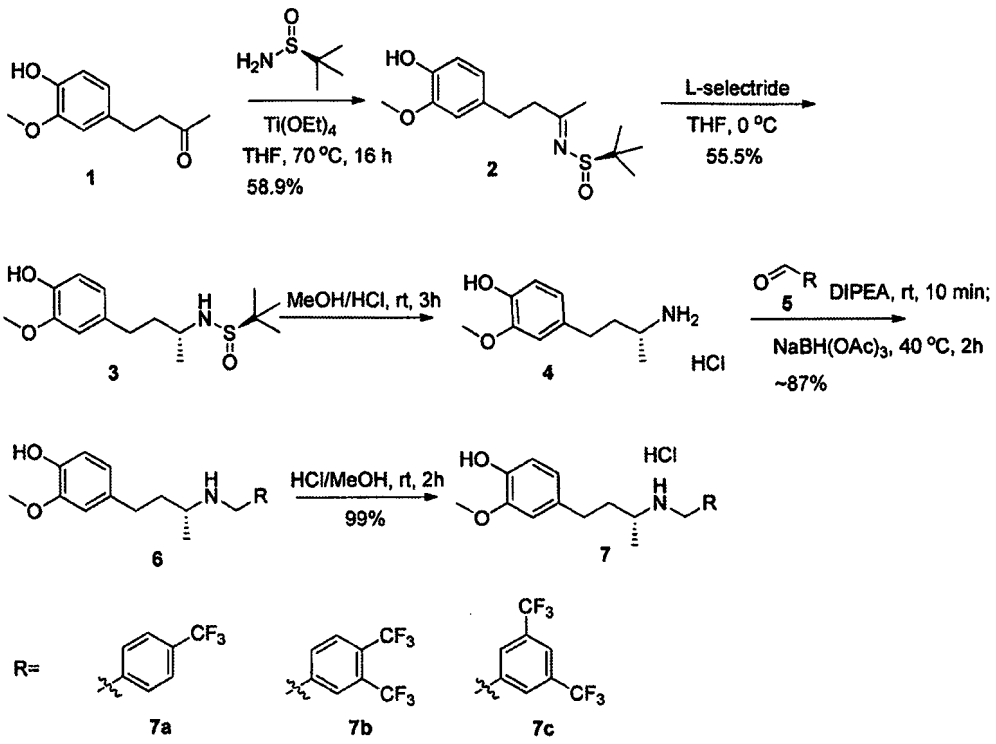
(Phenomenex Part No 00M-4423-B0_CE)

Gradient Program: RT = 2.22 minutes

Time, minute	% Phase B	Flow rate, ml/min
0	100	0.5
1	100	0.5
2.5	40	0.5
3.4	40	0.5
3.5	100	0.5
4.5	100	0.5

[0406] The purity of the product was also measure by ^1H NMR indicating it to be a single compound of a purity of greater than 90% or 95%. The synthesis described herein can be modified depending upon the final-product to be synthesized.

Synthesis Example 3



Scheme 1

[0407] **Step1:** To a solution of 4-(4-hydroxy-3-methoxy-phenyl)-butan-2-one (38.8 g, 200 mmol) in THF (600 mL) was added $Ti(OEt)_4$ (136.9 g, 600 mmol) and (S)-(-)-*tert*-butylsulfinamide (29 g, 240 mmol). The mixture was stirred at 70 °C for 16 h, quenched by ice water, extracted with EA (3 x 300 mL), dried over Na_2SO_4, concentrated to obtain a crude product, which was purified by column chromatography (PE/EA:3/1) to give the title compound **2** (35 g, 59%).

[0408] **Step2**: To a solution of compound **2** (18 g, 60 mmol) in THF (180 mL) was added L-Selectride (180 mL, 1.0 M in THF, 180 mmol) at 0 °C. The reaction was allowed to warm to rt over a 3 h period. Analysis of the reaction mixture by TLC showed complete consumption of the starting imine **2**. The solution was then quenched by adding water and extracted by EA (3 x 200 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under vacuum to give a residue, which was purified by column chromatography (PE/EA:2/1) to provide product. The product continued purified by recrystallization with PE/EA(1:1) to got product **3** (9.9 g, 55%). The ee value was determined by HPLC.

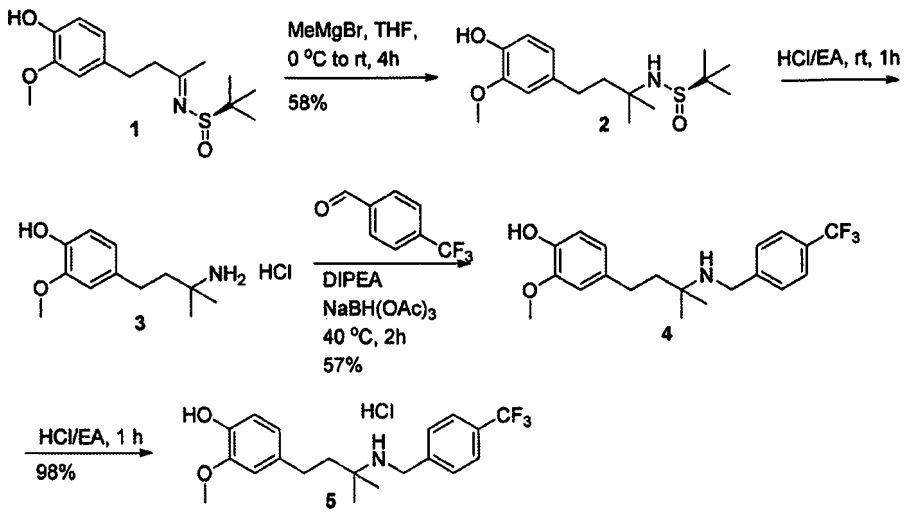
[0409] **Step3** : To a solution of **3** (7.0 g, 23.4 mmol) in MeOH (20 mL), HCl (2 M in MeOH, 20 mL) was added and the resulting solution was stirred at rt over a 3 h period. TLC analysis of the reaction mixture showed complete consumption of compound **3**. The solvent was then removed in vacuum, and the resulting residue **4** was used directly for the next step.

[0410] **Step4** : To a solution of the crude compound **4** (5.4 g, 23.4 mmol) in THF (100 mL) were added DIPEA (4.53 g, 35.1 mmol) and 4-trifluoromethylbenzaldehyde **5** (4.28 g, 24.6 mmol). The resulting solution was stirred at rt for 10 min. Then $NaBH(OAc)_3$ (14.9 g, 70.2 mmol) was added and the mixture was stirred at 40 °C for 2 h. The mixture was quenched by water at 0 °C, filtered and extracted by EtOAc. The organic layer was washed by brine, dried over sodium sulfate, filtered and the filtrate was concentrated under reduced pressure to afford a residue. The residue was purified by column chromatography (PE/EA =1:2) to give product **6** (7.0 g, 87%).

[0411] **Step5** : To a solution of **6** (1.0 g, 2.8 mmol) in MeOH (5 mL), HCl (2 M in MeOH, 20 mL) was added and the resulting solution was stirred at rt for 30 min. The solvent was removed to give the product **7a** (1.1 g, 99%) as white solid. Compounds **7b** and **7c** were similarly made by substituting compound **5** with the appropriate benzaldehyde.

[0412] m/z (ESI+) (M+H)+: 7a [354.2]; 7b [422.2]; 7c [422.2].

Synthesis Example 4



Scheme 4

[0413] **Step1:** To a solution of methylmagnesium bromide in THF (5 mL) was added a solution of **1** (1.0 g, 3.3 mmol) in THF (5 mL) at 0 °C. The mixture was stirred at rt for 4 h, quenched by adding ice-water, extracted with ethyl acetate (3 x 30 mL), dried by vacuum to afford a crude product, which was purified by column chromatography (PE/EA:3/1) to give compound **2** (0.6 g, 58%).

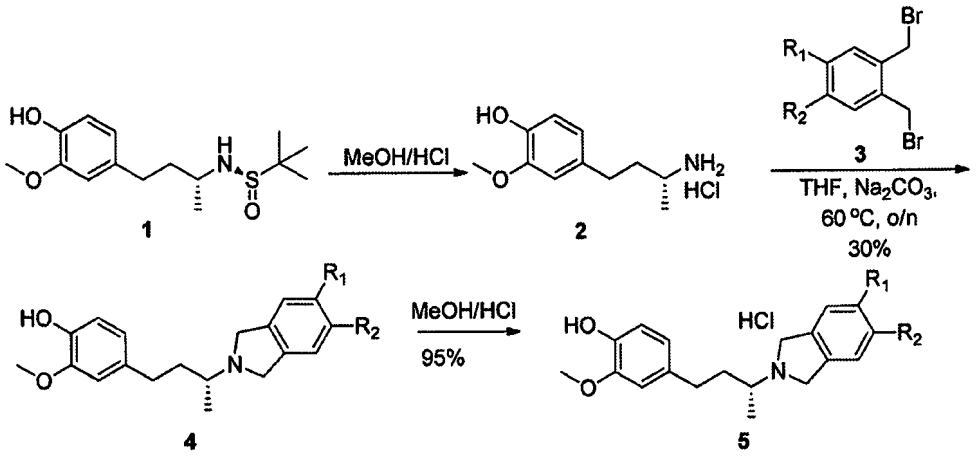
[0414] **Step2**: To a solution of **2** in EA (10 mL) at 0 °C was HCl (2 M in EA, 3 mL), and the resulting solution was stirred at rt for 1 h. Analysis of the reaction mixture by TLC showed complete consumption of **2**. Concentrated under vacuum, the crude product was directly used in next step.

[0415] **Step3** : To a solution of **3** (0.4 g, 1.9 mmol) in THF (20 mL), DIPEA (0.6 g, 4.6 mmol) and trifluoromethylbenzaldehyde (0.4 g, 2.3 mmol) were added subsequently. The resulting solution was stirred at rt for 10 min. Sodium triacetoxylboronhydride (1.63g, 7.7mmol) was added and the mixture was stirred at 40 °C for 2 h. The mixture was quenched by water at 0 °C, filtered and extracted by ethyl acetate (3 x 40 mL). The organic layer was washed by brine, dried over sodium sulfate, filtered and the filtrate was concentrated under reduced pressure to afford a residue. The residue was purified by column chromatography (PE/EA =1:1) to give **4** (0.4 g, 57%).

[0416] **Step4**: To a solution of **4** in EA (10 mL), HCl (2 M in MeOH, 2 mL) was added and the resulting solution was stirred at rt for 1 h. After concentrated by vacuum, the residue was washed with ethyl acetate to afford **5** (0.4 g, 98%).

[0417] m/z (ESI+) (M+H)+: **5** [368.2].

Synthesis Example 5



	5a	5b
R_1	Cl	CF_3
R_2	H	H

Scheme 5

[0418] **Step1**: To a solution of compound **1** (2 g, 6 mmol) in MeOH (30 mL), HCl (2 M in MeOH, 30 mL) was added and the resulting solution was stirred at rt for 3 h. TLC analysis of the reaction mixture showed complete consumption of compound **1**. The solvent was then removed in vacuum, and it was used directly for next step.

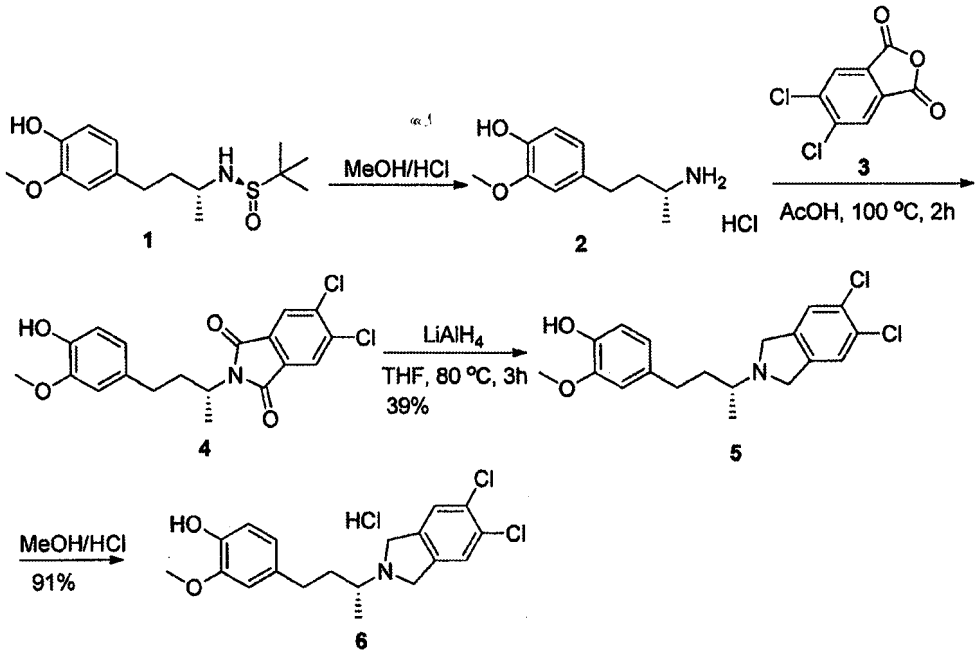
[0419] **Step2**: To a solution of **2** (0.4 g, 2 mmol) in THF (10 mL), compound **3a** (0.54 g, 2 mmol) in THF (5 mL) was added. Na_2CO_3 (0.6 g, 6 mmol) was added, and the resulting solution was stirred at 60 °C overnight. After concentration, the residue was purified by FCC to give compound **4** (0.2 g, 30%).

[0420] **Step3**: To a solution of **4** in EA (5 mL), HCl (2 M in MeOH, 3 mL) was added and the resulting solution was stirred at rt for 1 h. After being

concentrated *in vacuo*, the residue was washed by ethyl acetate to give compound **5** (0.2 g, 95%). Compound **5b** was similarly made by substituting compound **3** with the appropriate dibenzyl bromide.

[0421] m/z (ESI+) (M+H)+: **5a** [332.1]; **5b** [366.1].

Synthesis Example 6



Scheme 6

[0422] **Step1:** To a solution of compound **1** (0.4 g, 1.3 mmol) in MeOH (10 mL), HCl (2 M in MeOH, 10 mL) was added and the resulting solution was stirred at rt for 3 h. TLC analysis of the reaction mixture showed complete consumption of compound **1**. The solvent was removed in vacuum, and it was used directly for next step.

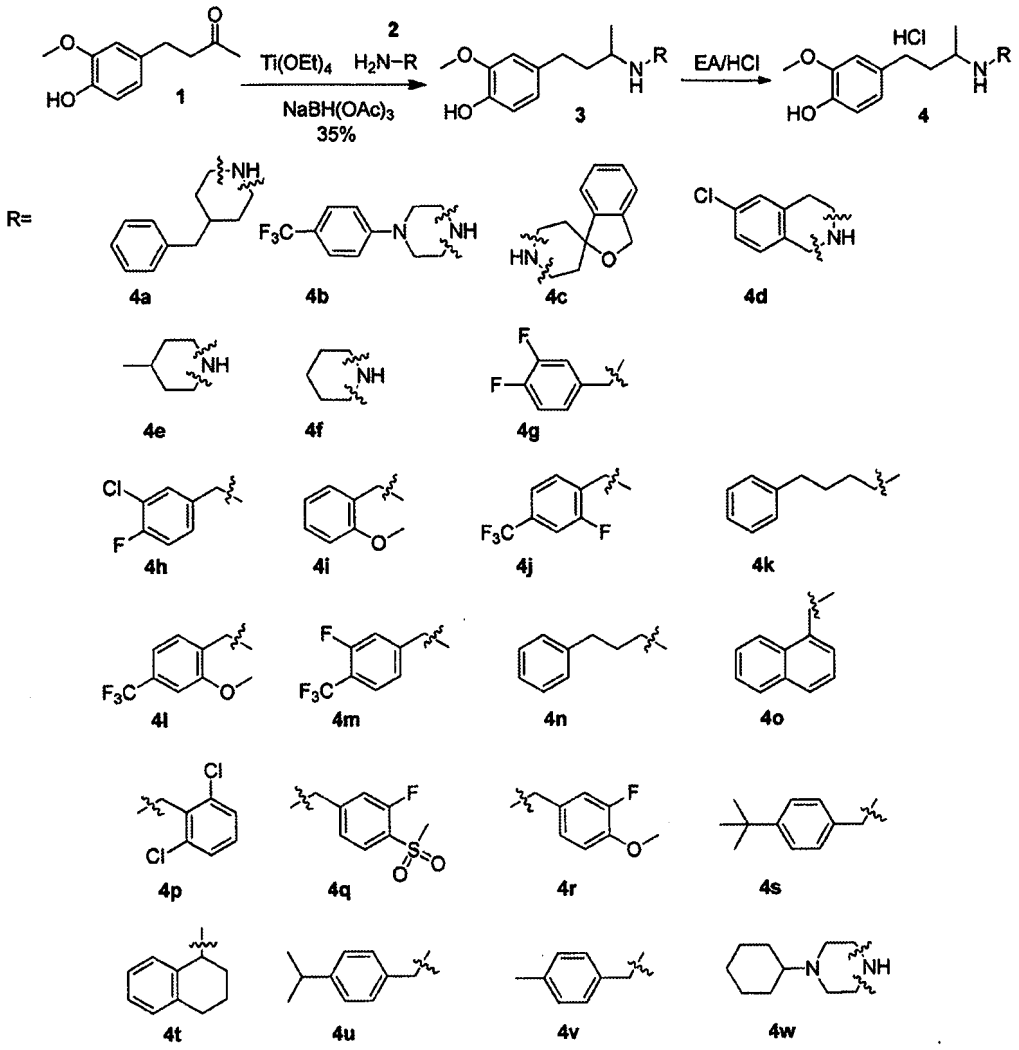
[0423] **Step2**: Compound **2** (0.2 g, 1 mmol) and **3** (0.2 g, 1 mmol) was dissolved in acetic acid (10 mL), stirred at 100 °C for 2 h. The mixture was cooled to rt and quenched by water (10 mL), extracted by EtOAc (3 x 20 mL), dried, concentrated to give the title compound **4** (0.3 g, 76%).

[0424] **Step3**: To a solution of **4** (0.3 g, 0.7 mmol) in THF (10 mL) was added LAH (0.1 g, 3.5 mmol). The mixture was stirred at 80 °C for 3 h. The mixture was quenched by water (0.1 mL), 15% of NaOH (0.1 mL) and water (0.3 mL), filtered, concentrated. The crude product was purified by column chromatography (PE/EA = 5:1) to give compound **5** (0.1 g, 39%).

[0425] **Step4**: To a solution of **5** in ethyl acetate (5 mL), HCl (2 M in MeOH, 3 mL) was added and the resulting solution was stirred at rt for 1 h. The reaction was concentrated by vacuum to afford the title compound **6** (0.16 g, 91%).

[0426] m/z (ESI+) (M+H)+: **6** [366.2].

Synthesis Example 7



Scheme 7

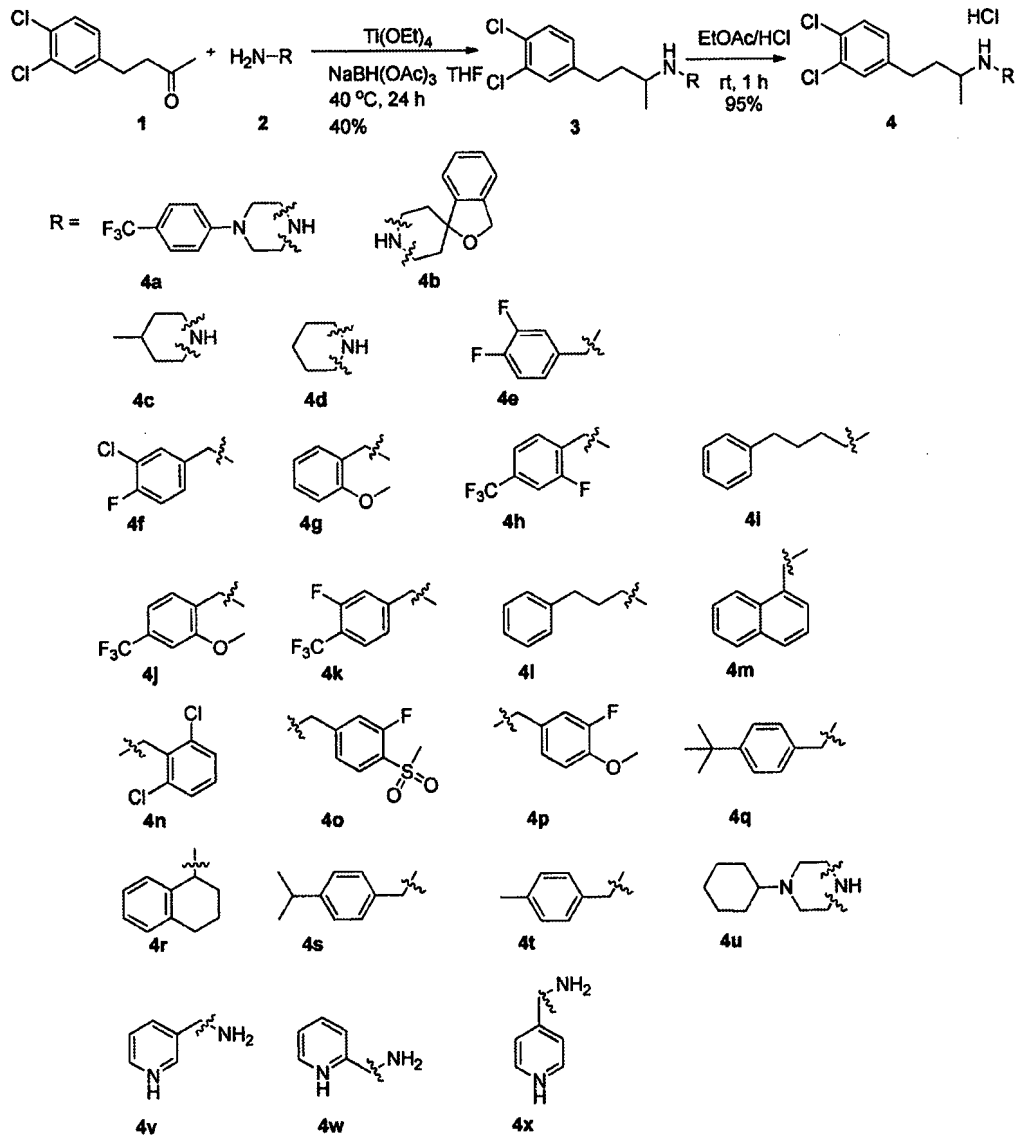
[0427] **Step1:** To a solution of **1** (0.278 g, 1.43 mmol) in THF (20 mL) was added $Ti(OEt)_4$ (2.1 g, 9.2 mmol) and (4-benzylpiperidine (0.34 g, 1.3 mmol). The mixture was stirred at 40 °C for one day, quenched by ice water, extracted with ethyl acetate (3 x 20 mL). After being concentrated *in vacuo*, the crude product was purified by column chromatography (PE/EA:1/1) to give **3** (205 mg, 35%).

[0428] **Step2**: To a solution of **3** (0.2 g, 0.47 mmol) in ethyl acetate (5 mL), HCl (2 M in MeOH, 3 mL) was added and the resulting solution was stirred at rt for 1 h. The reaction was concentrated by vacuum to get **4a** (0.2 g, 95%). Compounds

4b-4w were similarly made by substituting amine compound **2** with the appropriate amine.

[0429] m/z (ESI+) (M+H)+: **4a** [354.3]; **4b** [409]; **4c** [368.2]; **4d** [346.1]; **4e** [278.50]; **4f** [264.05]; **4g** [322.10]; **4h** [338.05]; **4i** [316.15]; **4j** [372.10]; **4k** [328.25]; **4l** [384.15]; **4m** [372.10]; **4n** [314.10]; **4o** [336.15]; **4p** [354.10]; **4q** [382.20]; **4r** [334.15]; **4s** [342.15]; **4t** [326.15]; **4u** [328.20]; **4v** [300.10]; **4w** [347.6].

Synthesis Example 8



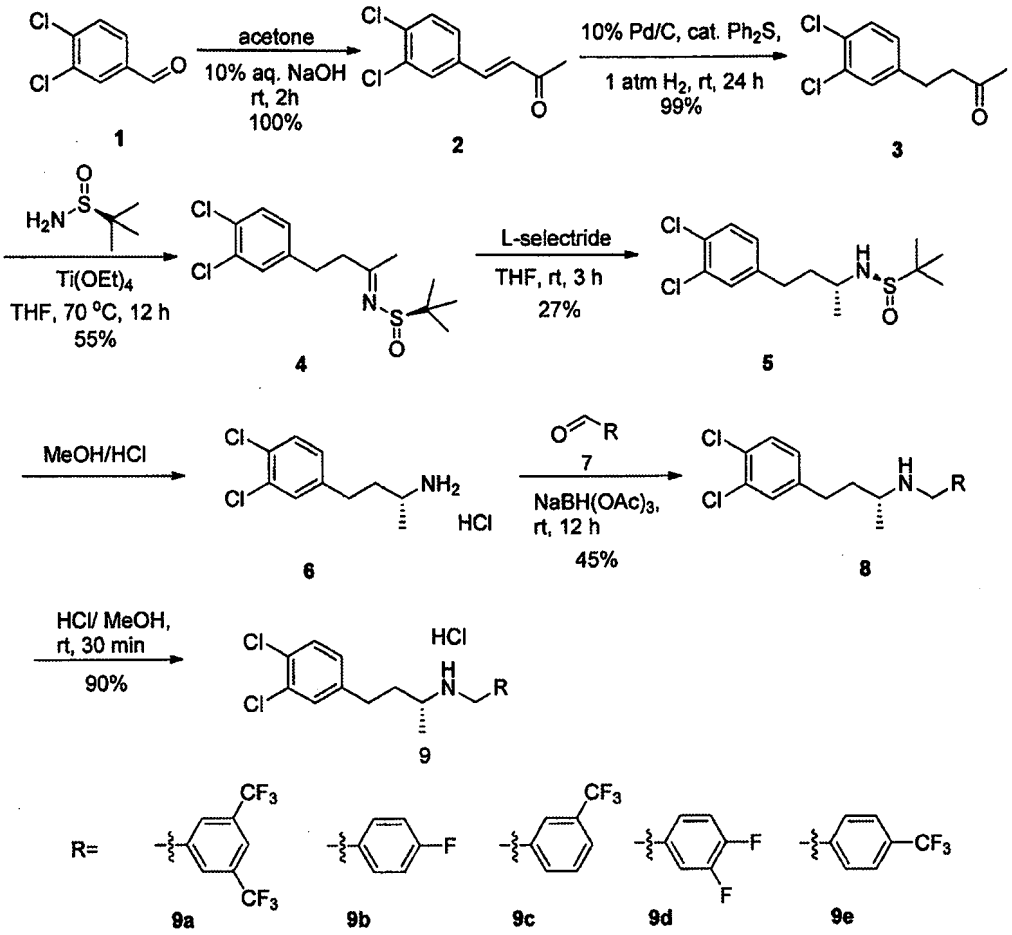
Scheme 8

[0430] **Step1:** To a solution of **1** (0.31 g, 1.43 mmol) in THF (20 mL) was added $Ti(OEt)_4$ (0.595 g, 2.58 mmol) and N-(4-trifluoromethylphenyl)-piperazine **2** (0.3 g, 1.3 mmol). The mixture was stirred at 40℃ for 24h, quenched by adding ice-water, extracted with ethyl acetate (3 x 20 mL), dried. Purification by column chromatography (PE/EA:1/1) gave product **3** (0.25 g, 41%).

[0431] **Step2**: To a solution of compound **3** (0.25 g, 0.58 mmol) in ethyl acetate (5 mL) was added MeOH-HCl (2 N, 4 mL). The mixture was stirred at room temperature for 1h. Concentration *in vacuo* gave compound **4** (0.25 g, 95%). Compounds **4b-4x** were similarly made by substituting amine compound **2** with the appropriate amine.

[0432] m/z (ESI+) (M+H)+: **4a** [431.2]; **4b** [390.2]; **4c** [300.05]; **4d** [286.00]; **4e** [344.05]; **4f** [362.00]; **4g** [338.05]; **4h** [394.10]; **4i** [350.05]; **4j** [406.05]; **4k** [394.15]; **4l** [336.05]; **4m** [358.05]; **4n** [378.05]; **4o** [445.20]; **4p** [356.10]; **4q** [364.10]; **4r** [348.05]; 4s [350.10]; **4t** [322.10]; **4u** [369.2]; **4v** [309.00]; **4w** [308.95]; **4x** [309.00].

Synthesis Example 9



Scheme 9

[0433] **Step1:** To a solution of **1** (3.5 g, 20 mmol) in acetone (20 mL) and ethanol (2 mL) was added aqueous NaOH (10%, 15 mL) and water (80 mL). The mixture was stirred at rt for 2 h, extracted with EA (3 x 50 mL). The organic layers were dried and concentrated to give **2** (4.3 g, 100%).

[0434] **Step2:** To a solution of **2** (4.3 g, 20 mmol) in MeOH (50 mL) was added diphenylsulfide (0.15 mL) and Pd/C (10%, 0.43 g). The mixture was vigorously stirred at 25 °C under 1 atm of hydrogen for 24 h. The reaction mixture was filtered through a pad of Celite, washed with methanol, and the filtrate was concentrated to provide **3** (4.3 g, 99%).

[0435] **Step3:** To a solution of **3** (10 g, 46 mmol) in THF (100 mL) was added $Ti(OEt)_4$ (21 g, 92 mmol), and (S)-(-)-*tert*-butylsulfinamide (6.1 g, 50 mmol).

The mixture was stirred at 70 °C for 12 h, quenched by ice water, extracted with ethyl acetate (3 x 250 mL). After being concentrated by vacuum, the crude product was purified by column chromatography (PE/EA:10/1) to afford compound **4** (8.1 g, 55%).

[0436] Step4: To a solution of compound 4 (3.3 g, 10 mmol) in THF (30 mL) was added L-Selectride (33 mL, 1.0 M in THF, 33 mmol) at 0 °C. The reaction was allowed to warm to rt over a 3 h period. Analysis of the reaction mixture by TLC showed complete consumption of the starting imine **4**. The solution was quenched by water and extracted by ethyl acetate (3 x 30 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated under vacuum to give a residue, which was purified by column chromatography (PE/EA:2/1) to provide product **5** (0.9 g, 27%).

[0437] **Step5 :** To a solution of compound **5** (5 g, 15.5 mmol) in MeOH (10 mL), HCl (2 M in MeOH, 10 mL) was added and the resulting solution was stirred at rt for 3 h. TLC analysis of the reaction mixture showed complete consumption of compound **5**. The solvent was removed in vacuum, and the crude **6** (3.95 g, 100%) was used directly for next step without further purification.

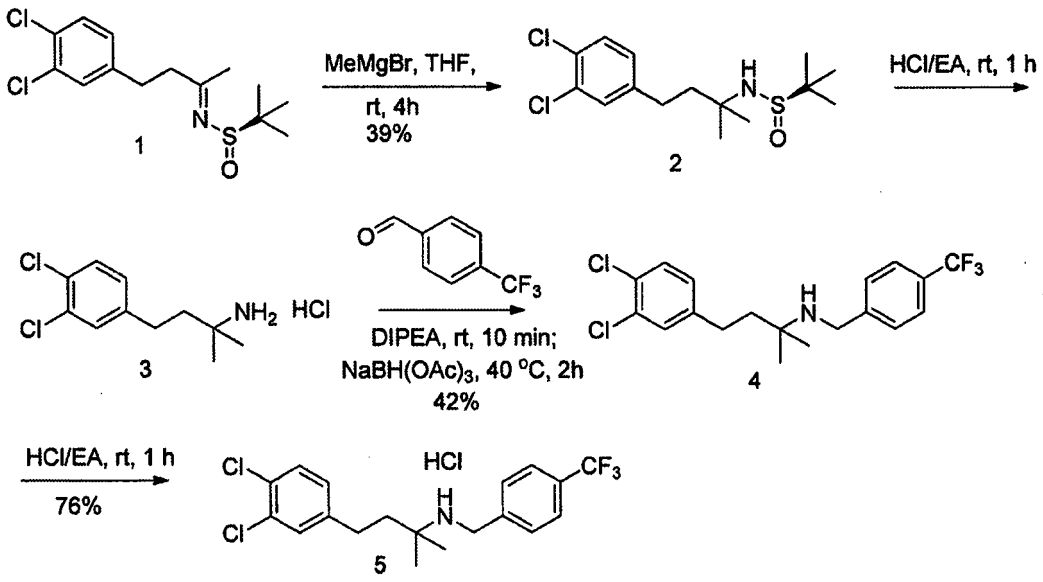
[0438] **Step6 :** To a solution of **6** (0.6 g, 2.4 mmol) in THF (10 mL), DIPEA (0.4 g, 3.1 mmol) and 3-trifluoromethylbenzaldehyde (0.41 g, 2.4 mmol) were added subsequently. The resulting solution was stirred at rt for 10 min. $NaBH(OAc)_3$ (1.0 g, 4.7 mmol) was added and the mixture was stirred for 12 h. The mixture was quenched by water at 0 °C, filtered and extracted by EtOAc (3 x 30mL). The organic layer was washed by brine, dried over sodium sulfate, filtered and the filtrate was concentrated under reduced pressure to afford a residue. The residue was purified by column chromatography (PE/EA =1:1) to give compound **8** (0.4 g, 45%).

[0439] **Step7 :** To a solution of **8** (0.4 g, 1.08 mmol) in MeOH (5 mL), HCl (2 M in MeOH, 4 mL) was added and the resulting solution was stirred at rt for 0.5

h. The reaction was concentrated to give amine **9a** (0.4 g, 90%). Compounds **9b-9e** were similarly made by substituting compound **7** with the appropriate benzaldehyde.

[0440] m/z (ESI+) (M+H)+: **9a**[444.2]; **9b** [326.25]; **9c** [376.2]; **9d** [344.2]; **9e** [376.1].

Synthesis Example 10



Scheme 10

[0441] **Step1:** To a solution of methylmagnesium bromide in THF (3 M, 15 mL) was added a solution of **1** (1.5 g, 4.6 mmol) in THF (20 mL) at 0 °C. The mixture was stirred at rt for 4 h, quenched by ice water, extracted with ethyl acetate (3 x 30 mL). After being concentrated, the crude product was purified by column chromatography (PE/EA: 3/1) to afford compound **2** (0.6 g, 39%).

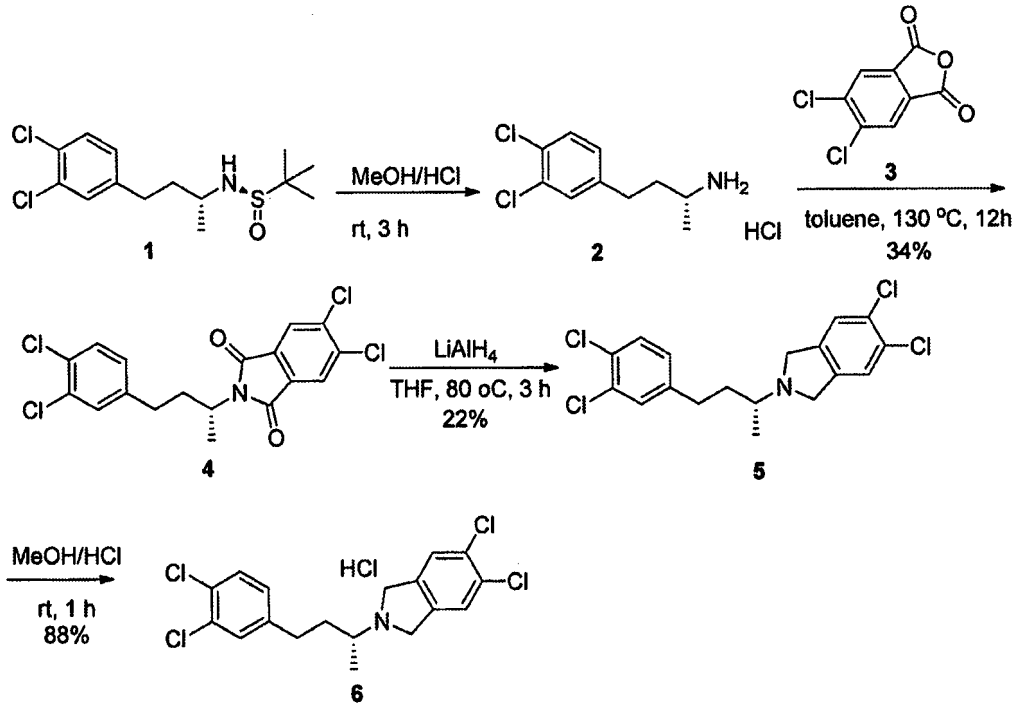
[0442] **Step2**: To a solution of compound **2** (0.6 g, 1.8 mmol) in ethyl acetate (10 mL), HCl (2 M in MeOH, 3 mL) was added and the resulting solution was stirred at rt for 1 h. TLC analysis of the reaction mixture showed complete consumption of compound **2**. The solvent was then removed in vacuum, and the crude compound **3** was used directly for next step.

[0443] **Step3** : To a solution of **3** (0.43 g, 1.8 mmol) in THF (20 mL), DIPEA (0.54 g, 4.0 mmol) and 4-trifluoromethylbenzaldehyde (0.36 g, 2.0 mmol) were added sequentially. The resulting solution was stirred at rt for 10 min. $NaBH(OAc)_3$ (1.57 g, 7.4 mmol) was added and the mixture was stirred at 40 °C for 2 h. The mixture was quenched by water at 0 °C, filtered and extracted by EtOAc (3 x 30 mL). The organic layers were washed by brine, dried over sodium sulfate, filtered and the filtrate was concentrated under reduced pressure to afford a residue, which was purified by column chromatography (PE/EA =3:1) to give compound **4** (0.3 g, 43%).

[0444] **Step4**: To a solution of **4** (0.3 g, 0.8 mmol) in ethyl acetate (10 mL), HCl (2 M in MeOH, 2 mL) was added and the resulting solution was stirred at rt for 1 h. The precipitate was filtered to obtain compound **5** (0.25 g, 76%).

[0445] m/z (ESI+) (M+H)+: **5** [390.14].

Synthesis Example 11



Scheme 11

[0446] **Step1:** A mixture of compound **1** (1.75 g, 5.43 mmol) in MeOH-HCl (2 M, 10 mL) was stirred at rt for 3h. The reaction mixture was concentrated in vacuo to give a crude **2**, which was used for next step without further purification.

[0447] **Step2**: A solution of compound amine **2** (1.4 g, 5.43 mmol) and anhydride **3** (1.18 g, 5.43 mmol) in toluene (12 mL) was heated at 130℃ for 12 h.

The mixture was cooled to rt and water (10 mL) was added, extracted by EtOAc (3 x 20 mL), dried, concentrated. The crude product was purified by column chromatography (PE/EA =10:1) to give product **4** (0.78 g, 34%).

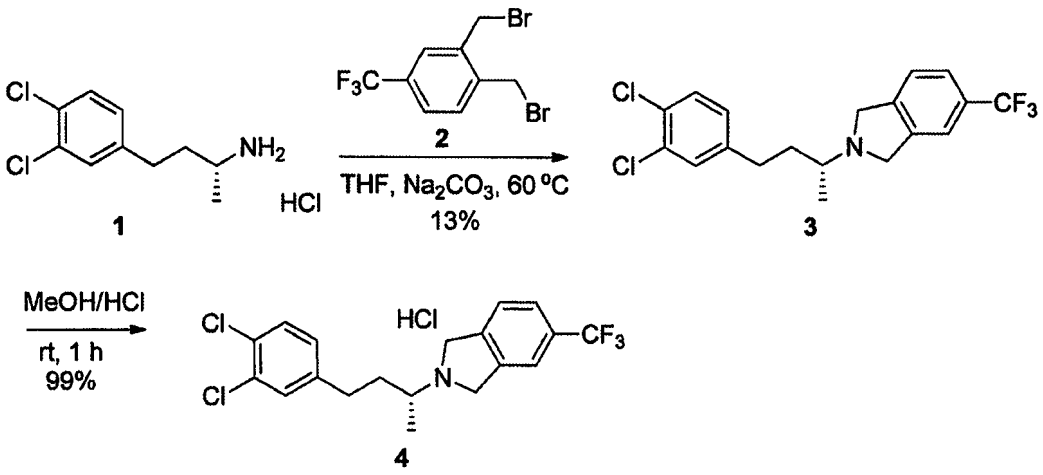
[0448] **Step3**: To a solution of compound **4** (0.78 g, 1.87 mmol) in THF (20 mL) was added LAH (0.36 g, 9.1 mmol). The mixture was stirred at 80℃ for 3h.

The cooled mixture was quenched by water (3.46 mL), 15% of NaOH (3.46 mL) and water (13.5 mL). The reaction mixture was filtered, concentrated. The crude product was purified by column chromatography (PE/EA =5:1) to give product **5** (0.16 g, 23%).

[0449] **Step4**: Compound 5 (0.3 g, 0.8 mmol) was dissolved in ethyl acetate (5 mL), MeOH-HCl (2N, 3 mL) was added. The mixture was stirred at room temperature for 1h, concentrated to give compound **6** (0.16g, 89%).

[0450] m/z (ESI+) (M+H)+: **6** [390.0].

Synthesis Example 12



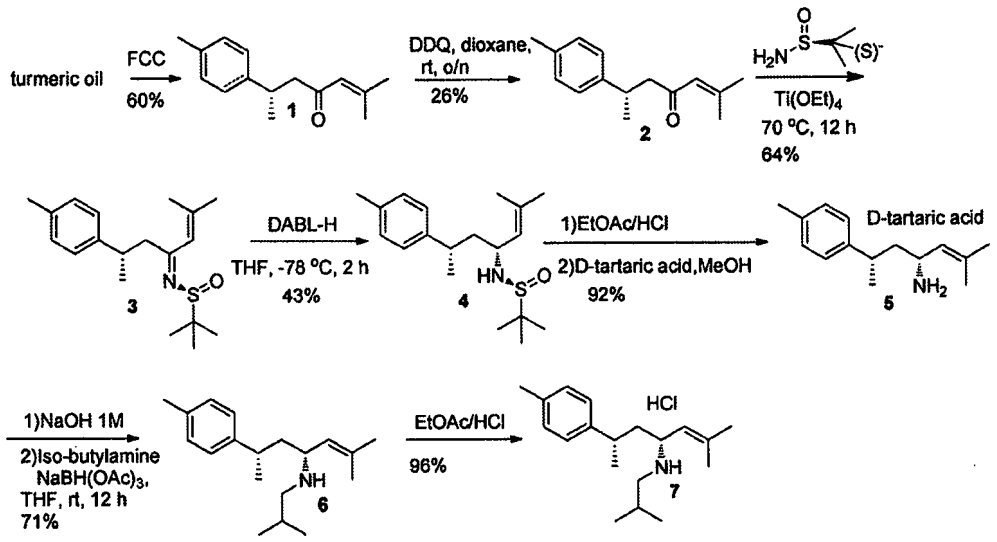
Scheme 12

[0451] **Step1**: To a solution of compound **1** (0.4 g, 2 mmol) in DMF (6 mL) was added dibromide **2** (0.6 g, 2 mmol). The resulting solution was stirred at 80°C overnight, concentrated, purified by preparative-HPLC to give compound **3** (0.1 g, 13%).

[0452] **Step2**: Compound **3** (0.1 g, 0.2 mmol) was dissolved in ethyl acetate (5 mL), MeOH-HCl (2 N, 3 mL) was added. The mixture was stirred at room temperature for 1h. The mixture was filtered to give compound **4** (0.11 g, 99%).

[0453] m/z (ESI+) (M+H)+: **4** [388.1].

Synthesis Example 13



Scheme 13

[0454] **Step1:** Turmeric oil (100 g) was purified by column chromatography (PE:EA/100:1) to provide crude product **1** (60 g).

[0455] **Step 2:** The crude product **1** (60 g) was dissolved in dioxane (200 mL), DDQ (81.7g, 360 mmol) was added. The mixture was stirred at rt overnight, then quenched by water (500 mL), filtered through a pad of Celite. The filtrate was extracted by ethyl acetate (3 x 200mL). The organic layers were dried, concentrated and purified by column chromatography (PE:EA/30:1) to provide compound 2 (15.6 g, 26%).

[0456] **Step 3:** To a solution of **2** (7.4 g, 34.2 mmol) in $Ti(OEt)_4$ (23.4 g, 102.6 mmol) was added (*S*)-(-)-2-methyl-2-propanesulfinamide. The mixture was stirred at 70℃ for 12h, quenched by ic- water, extracted with ethyl acetate (3 x 100 mL), and dried to give a residue, which was purified by column chromatography (PE/EA:5/1) to give product **3** (7.1 g, 64%).

Step 4: To a solution of compound **3** (7.0 g, 21.9 mmol) in THF (70 mL) at -78 °C was added DIBAL-H (22 mL, 1.5 M in THF, 33 mmol). The resulting solution was stirred at -78 °C for 2h. Analysis of the reaction mixture by TLC showed complete consumption of the starting imine to give sulfinamide compound **4**. The solution was quenched by water and extracted by ethyl acetate (3 x 200mL). The combined

organic layers were washed with brine, dried over Na_2SO_4, and concentrated to furnish an orange oil. The crude product was subjected to column chromatography (PE:EA/3:1) to provide product **4** (3.1 g, 43%).

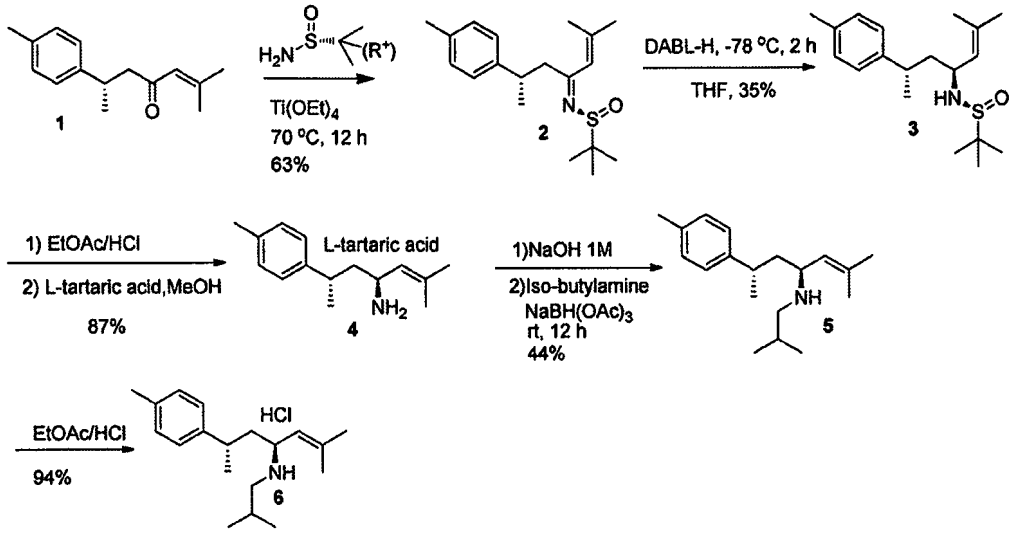
[0457] **Step 5:** To a solution of compound **4** (1.6 g, 5.0 mmol) in ethyl acetate (10 mL) was added HCl-ethyl acetate (2 N, 10 mL), and the resulting solution was stirred at room temperature for 3 h. TLC analysis of the reaction mixture showed complete consumption of compound **3**. The solvent was removed in vacuum. The residue was dissolved in water (10 mL), and pH was adjusted to 9-10 by a saturation aqueous solution of K_2CO_3, extracted by ethyl acetate (3 x 20mL), dried, and concentrated to give a free amine. The free amine (1.1 g, 5.0 mmol) was dissolved in methanol (15 mL). D-tartaric acid (0.75 g, 5.0 mmol) was added to the solution,. The mixture was stirred under reflux for 1h. The solution was slowly cooled to rt. The formed crystals were filtered to give product **5** (1.7 g, 93%). Mp. 172 -174 °C. The absolute stereochemistry of the compound **5** was determined by X-ray crystallography.

[0458] **Step 6**: A solution of compound **5** (1.7 g, 4.6 mmol) in water (20 mL) was adjusted to pH 9-10 by 1M NaOH. The product was extracted with ethyl acetate (3 x 20 mL). The organic layers were dried, concentrated to give a free amine. The free amine was dissolved in THF (10 mL), iso-butylamine (0.40 g, 5.5 mmol) and $NaBH(OAc)_3$ (3.90 g, 18.4 mmol) was added. The mixture was stirred at rt for 12h, quenched with water, extracted by ethyl acetate (3 x 30mL). The organic layers were dried, concentrated and purified by column chromatography (PE:EA/3:1) to provide product **6** (0.9 g, 72%).

[0459] **Step7**: To a solution of compound **6** (0.9 g, 3.2 mmol) in ethyl acetate (10 mL) was added ethyl acetate-HCl (2 N, 5 mL). The mixture stirred at room temperature for 1h. Ethyl acetate was removed in vacuo to afford compound **7** (0.95 g, 96%).

[0460] m/z (ESI+) (M+H)+: **7** [274.20].

Synthesis Example 14



Scheme 14

[0461] **Step1:** To a solution of **1** (7.4 g, 34.2 mmol) in $Ti(OEt)_4$ (23.4 g, 102.6 mmol) was added (*R*)-(+)-2-methyl-2-propanesulfinamide. The mixture was stirred at 70 °C for 12 h, quenched by ice-water, extracted with ethyl acetate (3 x 100 mL), dried. Purified by column chromatography (PE/EA:5/1) to afford product **2** (6.9 g, 63%).

[0462] **Step2**: Compound **2** (7.0 g, 21.9 mmol) was dissolved in THF (70 mL) and cooled to -78 °C. To the vessel was then added DIBAL-H (22 mL, 1.5 M in THF, 33 mmol), and the resulting solution was stirred at -78°C for 2 h. Analysis of the reaction mixture by TLC showed complete consumption of the starting imine to give sulfinamide compound **3**. The solution was then quenched by water and extracted by ethyl acetate (3 x 200mL).The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under vacuum to furnish an orange oil. The crude product was subjected to column chromatography (PE:EA/3:1) to provide product 3 (2.5 g, 36%).

[0463] **Step3:** To a solution of **3** (2.5 g, 7.8 mmol) in ethyl acetate (10 mL), 2M HCl in ethyl acetate (10 mL) was added and the resulting solution was stirred at room temperature for 3 h. TLC analysis of the reaction mixture showed complete consumption of compound **3**. The solvent was removed with vacuum. The residue was dissolved in water (10 mL), whose pH was adjusted to 9-10 by adding saturated

K_2CO_3. The mixture was extracted by ethyl acetate (3 x 20 mL), dried, concentrated to get free amine **4**. The free amine **4** was dissolved in methanol (15 mL), L-trataric acid (1.17 g, 7.8 mmol) was added. The mixture was stirred under reflux for 1 h, cooled to rt, filtered to get crystalline salt **4** (2.5 g, 87%).

[0464] **Step4**: L-trataric acid salt **4** (2.5 g, 6.8 mmol) was dissolved in water (20 mL), whose pH was adjusted to 9-10 by adding 1 M NaOH. The mixture was then extracted by ethyl acetate (3 x 50mL), dried, concentrated to get free amine **4**. The free amine **4** was redissolved in THF, iso-butylamine (0.60 g, 8.2 mmol) and $NaBH(OAc)_3$ (5.85 g, 27.6 mmol) was added. The mixture was stirred at rt for 12 h, quenched by water, extracted by ethyl acetate (3 x 30mL). The organic layer was dried, concentrated and purified by column chromatography (PE:EA/3:1) to provide product **5** (0.83 g, 45%).

[0465] **Step5**: To a solution of **5** (0.83 g, 3.0 mmol) in ethyl acetate (10 mL), HCl (2 M in ethyl acetate, 5 mL) was added and the resulting solution was stirred at rt for 1 h. The solvent was removed to give the product **6** (0.88 g, 94%).

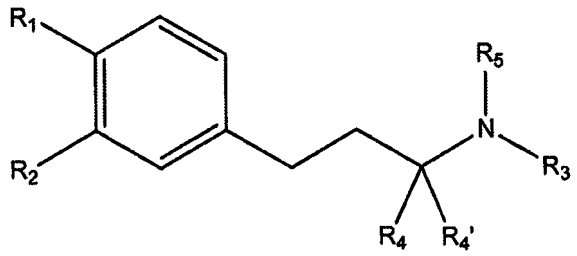
[0466] m/z (ESI+) (M+H)+: **6** [274.20]

[0467] All features disclosed in the specification, including the abstract and drawings, and all the steps in any method or process disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive. Each feature disclosed in the specification, including abstract and drawings, can be replaced by alternative features serving the same, equivalent or similar purpose, unless expressly stated otherwise. Thus, unless expressly stated otherwise, each feature disclosed is one example only of a generic series of equivalent or similar features. Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

[0468] All publications mentioned herein are incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

CLAIMS

1. A compound of Formula I:

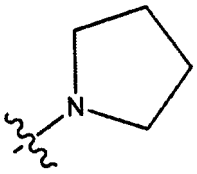


I

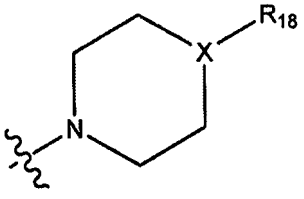
wherein

R_1 and R_2 are independently selected from H, OH, halo, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, $(R_{16})(R_{17})N\text{-}C_{1-4}$ alkylene-O-, or R1 and R2 are linked together to form a $-O\text{-}C_{1-2}$ methylene-O- group, wherein

R_{16} and R_{17} are independently C_{1-4} alkyl or benzyl, or R_{16} and R_{17} together with nitrogen form a ring selected from



, and

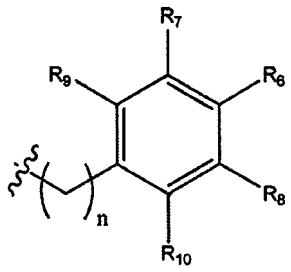
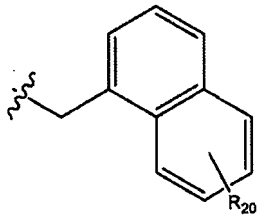
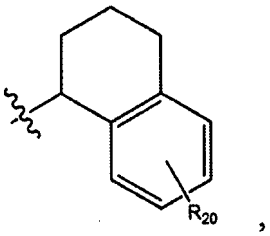
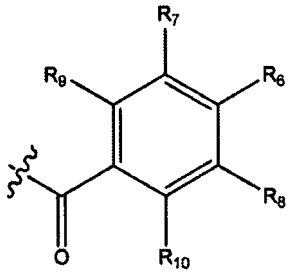
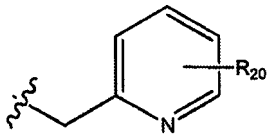
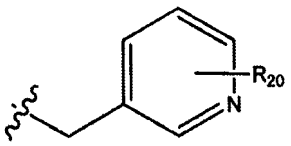
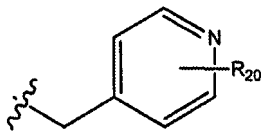
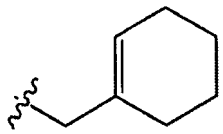


, wherein

X is N or O and R_{18} is H or unsubstituted phenyl; and

wherein at least one of R_1 and R_2 is not H;

R_3 is selected from

wherein

R_6, R_7, R_8, R_9, and R_{10}, are independently selected from H, halo, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, and $S(O)_2$- C_{1-6} alkyl;

R_{20} is H; and

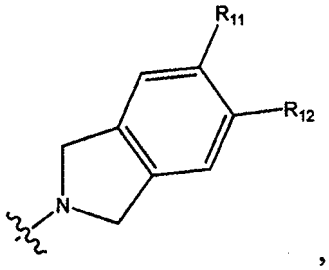
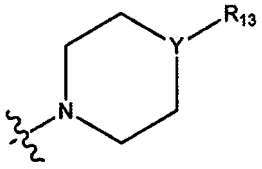
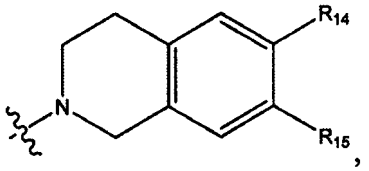
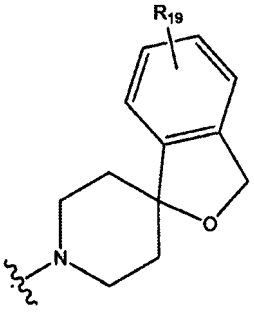
n is 1-4

R_4 is C_{1-6} alkyl;

$R_{4'}$ is H or C_{1-6} alkyl; and

R_5 is H, C_{1-6} alkyl, and C(O)O(C_{1-4} alkyl), C(O)(C_{1-4} alkyl), or C(O)(C_{1-4}haloalkyl); or

R_3 and R_5 together with nitrogen form a ring selected from

and , wherein

R_{11} and R_{12}, are independently selected from H, halo, and C_{1-6} haloalkyl, and

Y is CH or N;

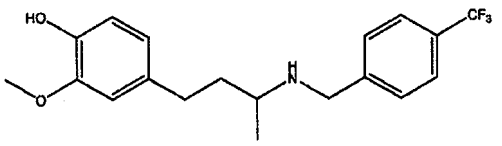
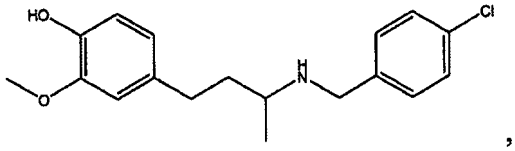
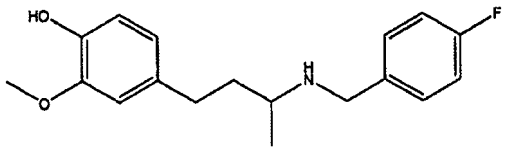
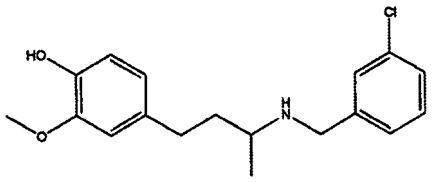
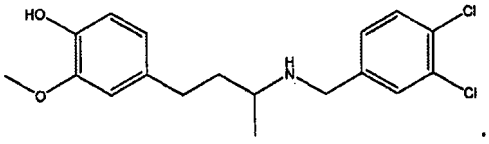
R_{13}.is H, C_{1-6} alkyl, C_{3-6} cycloalkyl, unsubstituted phenyl or phenyl substituted with C_{1-6} haloalkyl, or unsubstituted benzyl

R_{14} and R_{15} are independently selected from H and halo;

R_{19} is H, and

pharmaceutically acceptable salts thereof,

with the proviso that the following racemic mixtures of compounds are excluded

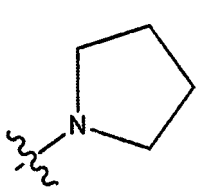






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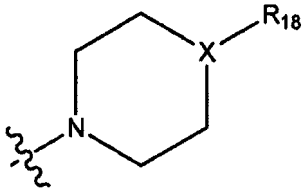
2. The compound of claim 1 wherein

R_1 and R_2 are independently selected from H, OH, halo, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, $(R_{16})(R_{17})N\text{-}C_{1-4}$ alkylene-O-, or R1 and R2 are linked together to form a $-O\text{-}C_{1-2}$ methylene-O- group, wherein

R_{16} and R_{17} are independently C_{1-4} alkyl or benzyl, or R_{16} and R_{17} together with nitrogen form a ring selected from



, and

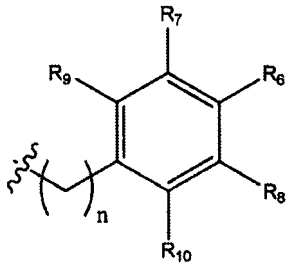


, wherein

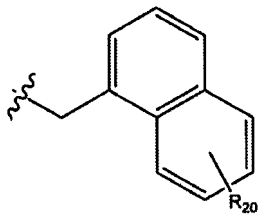
X is N or O and R_{18} is absent or is H or unsubstituted phenyl; and

wherein at least one of R_1 and R_2 is not H;

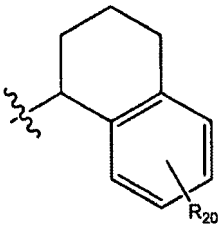
R_3 is selected from



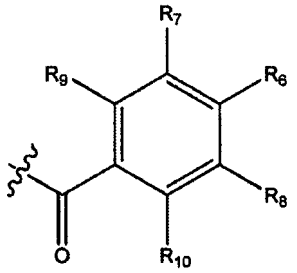
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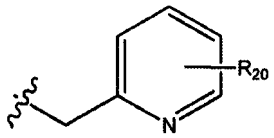
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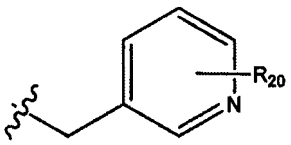
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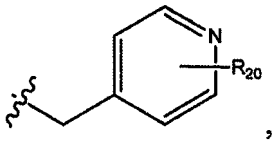
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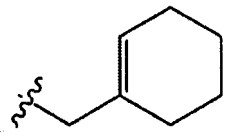
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,



, and



wherein

R_6, R_7, R_8, R_9, and R_{10}, are independently selected from H, halo, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, and $S(O)_2\text{-}$ C_{1-6} alkyl;

R_{20} is H; and

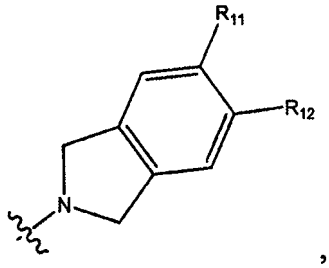
n is 1-4

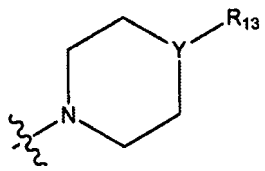
R_4 is C_{1-6} alkyl;

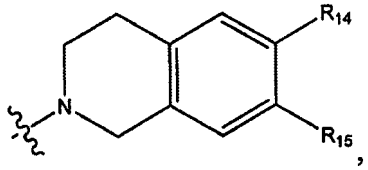
$R_{4'}$ is H or C_{1-6} alkyl; and

R_5 is H, C_{1-6} alkyl, and C(O)O(C_{1-4} alkyl), C(O)(C_{1-4} alkyl), or C(O)(C_{1-4}haloalkyl); or

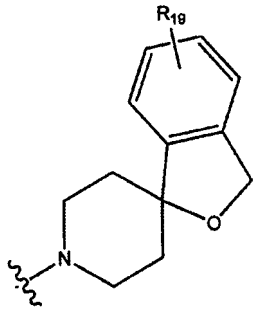
R_3 and R_5 together with nitrogen form a ring selected from



,


,


,



and , wherein

R_{11} and R_{12}, are independently selected from H, halo, and C_{1-6} haloalkyl, and

Y is CH or N;

R_{13}.is H, C_{1-6} alkyl, C_{3-6} cycloalkyl, unsubstituted phenyl or phenyl substituted with C_{1-6} haloalkyl, or unsubstituted benzyl

R_{14} and R_{15} are independently selected from H and halo; and

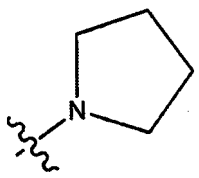
R_{19} is H, and

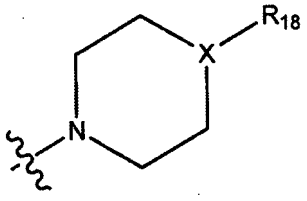
pharmaceutically acceptable salts thereof.

3. The compound of claim 1, wherein

R_1 is selected from OH, OMe, F, Cl, CF_3, (R_{16})(R_{17})N-ethylene-O-, wherein

R_{16} and R_{17} are each methyl, isopropyl, n-butyl or benzyl, or R_{16} and R_{17} together with nitrogen form a ring selected from



, or


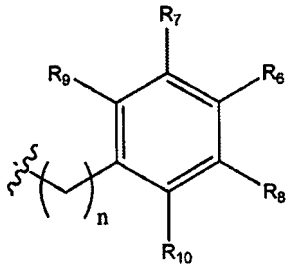
, wherein

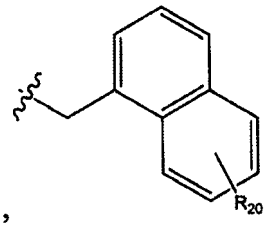
X is N or O and R_{18} absent or is unsubstituted phenyl; and

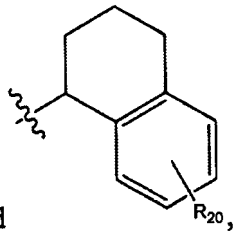
R_2 is H, Cl, F, CF_3, OMe, OCF_3 or

R_1 and R_2 are linked together to form a –O-C_{1-2} methylene-O- group

R_3 is selected from



,


, and


,

wherein

R_6 is H, F, Cl, Me, isopropyl, t-butyl, OMe, CF_3, or $S(O)_2Me$,

R_7 and R_8 are indenpendently H, OMe, F, Cl, or CF_3,

R_9, and R_{10} are independently selected from H, OMe, F, and Cl,

R_{20} is H; and

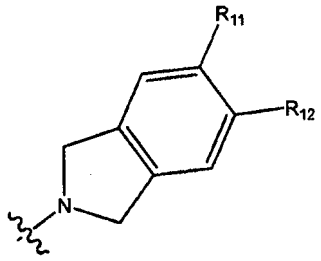
n is 1

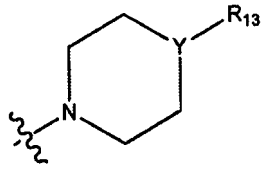
R_4 is Me;

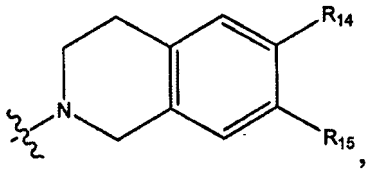
$R_{4'}$ is H or Me; and

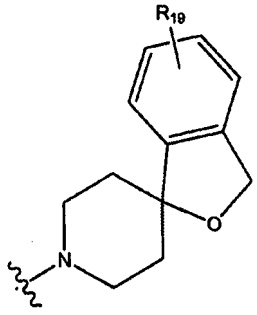
R_5 is H; or

R_3 and R_5 together with nitrogen form a ring selected from



,


,


,


and , wherein

R_{11} and R_{12}, are independently selected from H, Cl, and CF_3, and

Y is CH or N;

R_{13}.is H, Me, cyclohexyl, unsubstituted phenyl or phenyl substituted with CF_3, or unsubstituted benzyl

R_{14} and R_{15} are independently selected from H and Cl; and

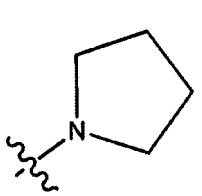
R_{19} is H, and

pharmaceutically acceptable salts thereof.

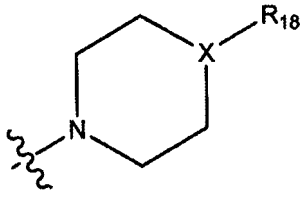
4. The compound of claim 1, wherein

R_1 is selected from OH, OMe, F, Cl, CF_3, $(R_{16})(R_{17})$N-ethylene-O-, wherein

R_{16} and R_{17} are each methyl, isopropyl, n-butyl or benzyl, or R_{16} and R_{17} together with nitrogen form a ring selected from



, or



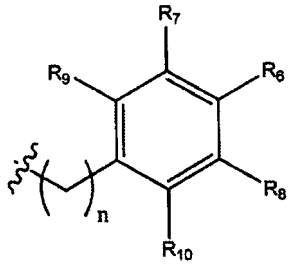
, wherein

X is N or O and R_{18} absent or is unsubstituted phenyl; and

R_2 is H, Cl, F, CF_3, OMe, OCF_3 or

R_1 and R_2 are linked together to form a –O-C_{1-2} methylene-O- group

R_3 is selected from



,

wherein

R_6 is H, F, Cl, Me, isopropyl, t-butyl, OMe, CF_3, or $S(O)_2Me$,

R_7 and R_8 are indenpendently H, OMe, F, Cl, or CF_3,

R_9, and R_{10} are independently selected from H, OMe, F, and Cl, and

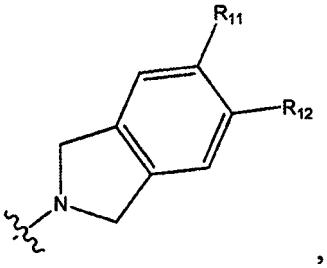
n is 1

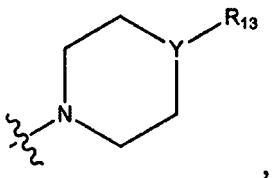
R_4 is Me;

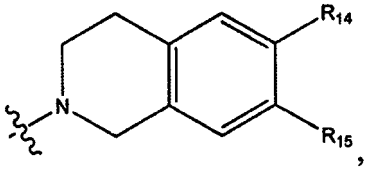
$R_{4'}$ is H; and

R_5 is H; or

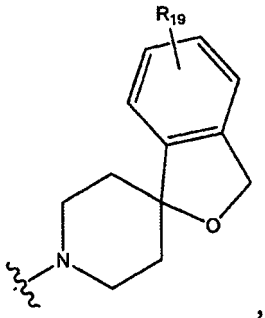
R_3 and R_5 together with nitrogen form a ring selected from



,


,


,



and , wherein

R_{11} and R_{12}, are independently selected from H, Cl, and CF_3, and

Y is CH or N;

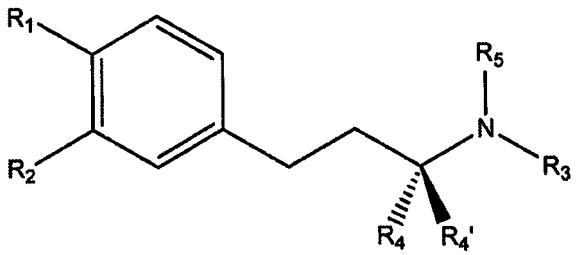
R_{13}.is H, Me, cyclohexyl, unsubstituted phenyl or phenyl substituted with CF_3, or unsubstituted benzyl

R_{14} and R_{15} are independently selected from H and Cl; and

R_{19} is H, and

pharmaceutically acceptable salts thereof.

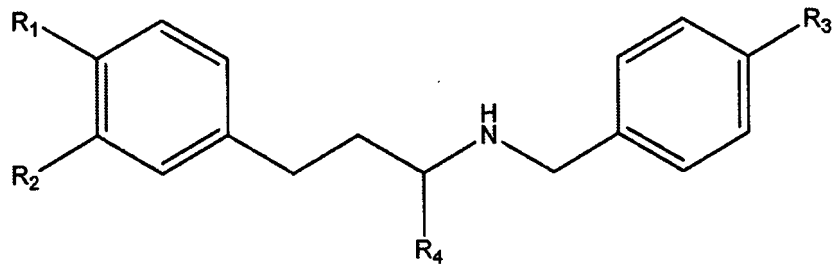
5. The compound of claim 1 that is a compound of Formula Ia



Ia

wherein $R_{4'}$ is H and the remaining groups are as defined in claim 1, and pharmaceutically acceptable salts thereof.

6. A compound of Formula IIa



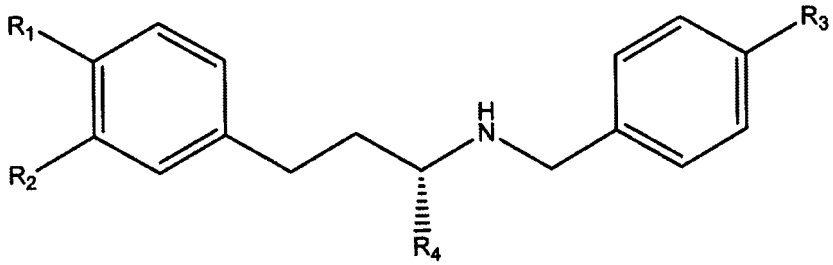
IIa

wherein
R_1 = halo, C_{1-6} haloalkyl, or OH;
R_2 = H, halo or C_{1-6} haloalkyl, or R_1 and R_2 are linked together to form a -O-methylene-O- group;
R_3 = C_{1-6} haloalkyl; and
R_4 = C_{1-6} alkyl, or pharmaceutically acceptable salts thereof.

7. The compound of claim 6, wherein

R_1 = Cl, F, CF_3, or OH;
R_2 = H, Cl, F, CF_3, or R_1 and R_2 are linked together to form a -O-ethylene-O- group;
R_3 = CF_3; and
R_4 = methyl, and pharmaceutically acceptable salts thereof.

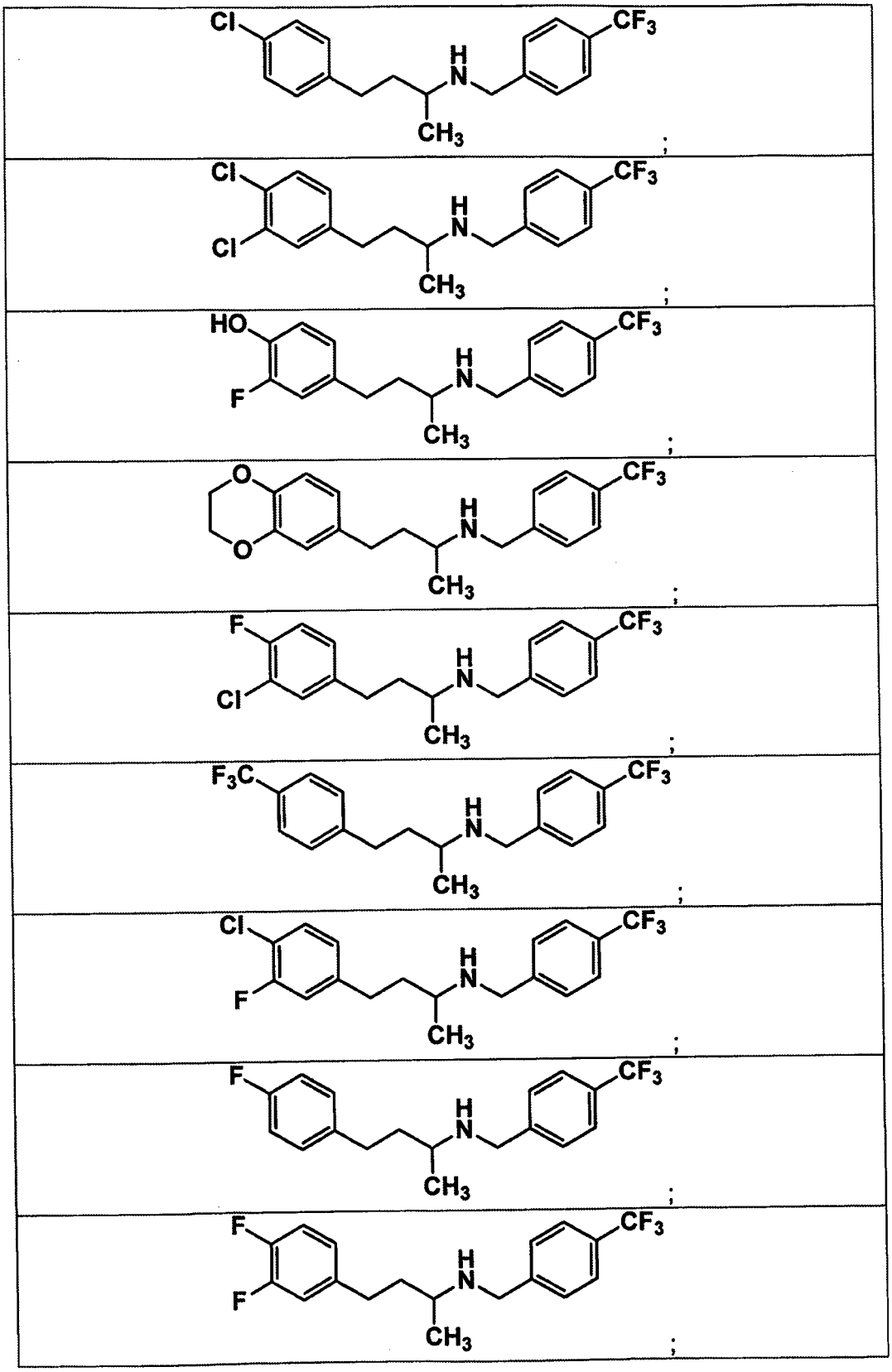
8. The compound of claim 6 that is a compound of Formula IIb

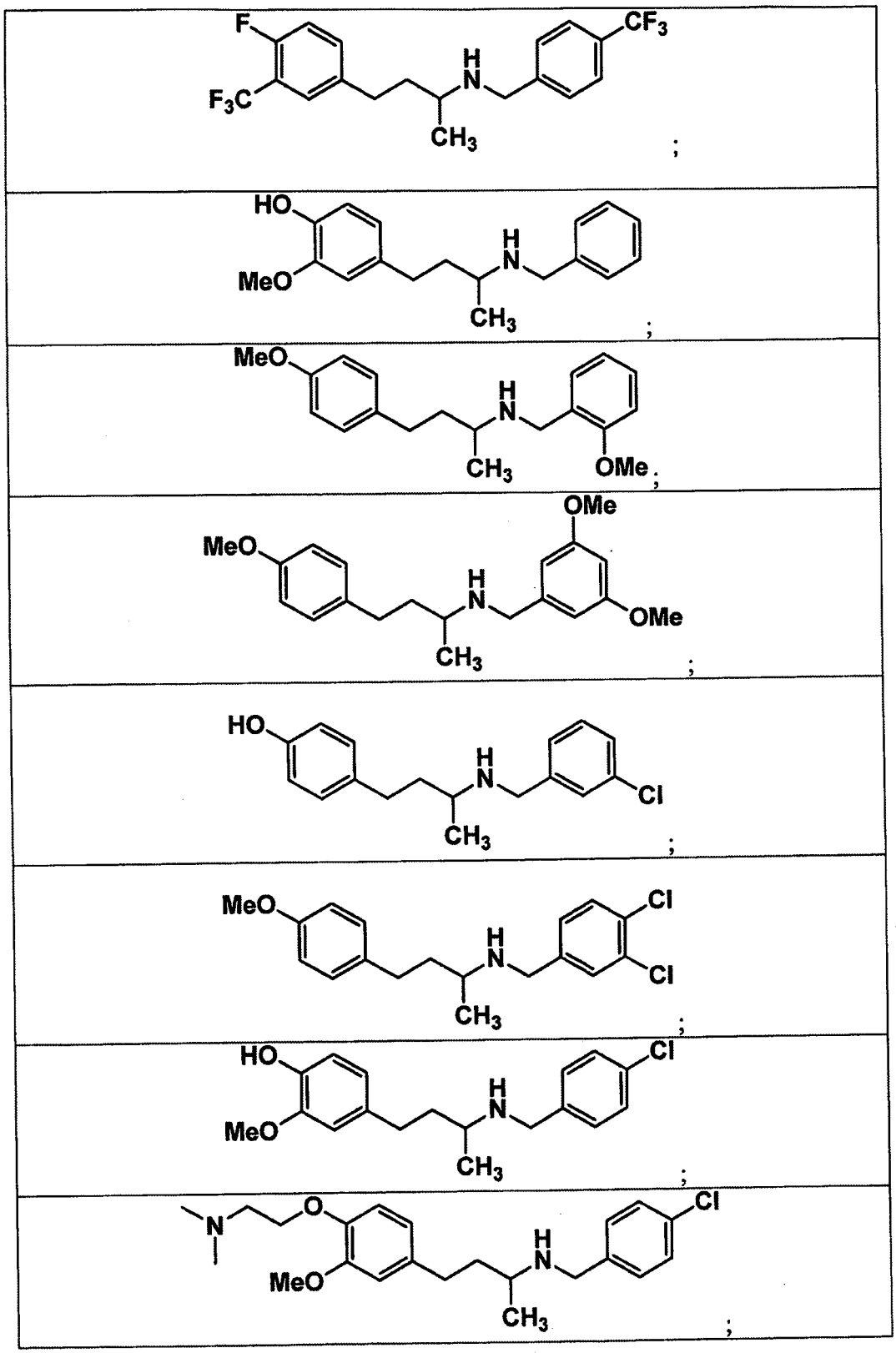


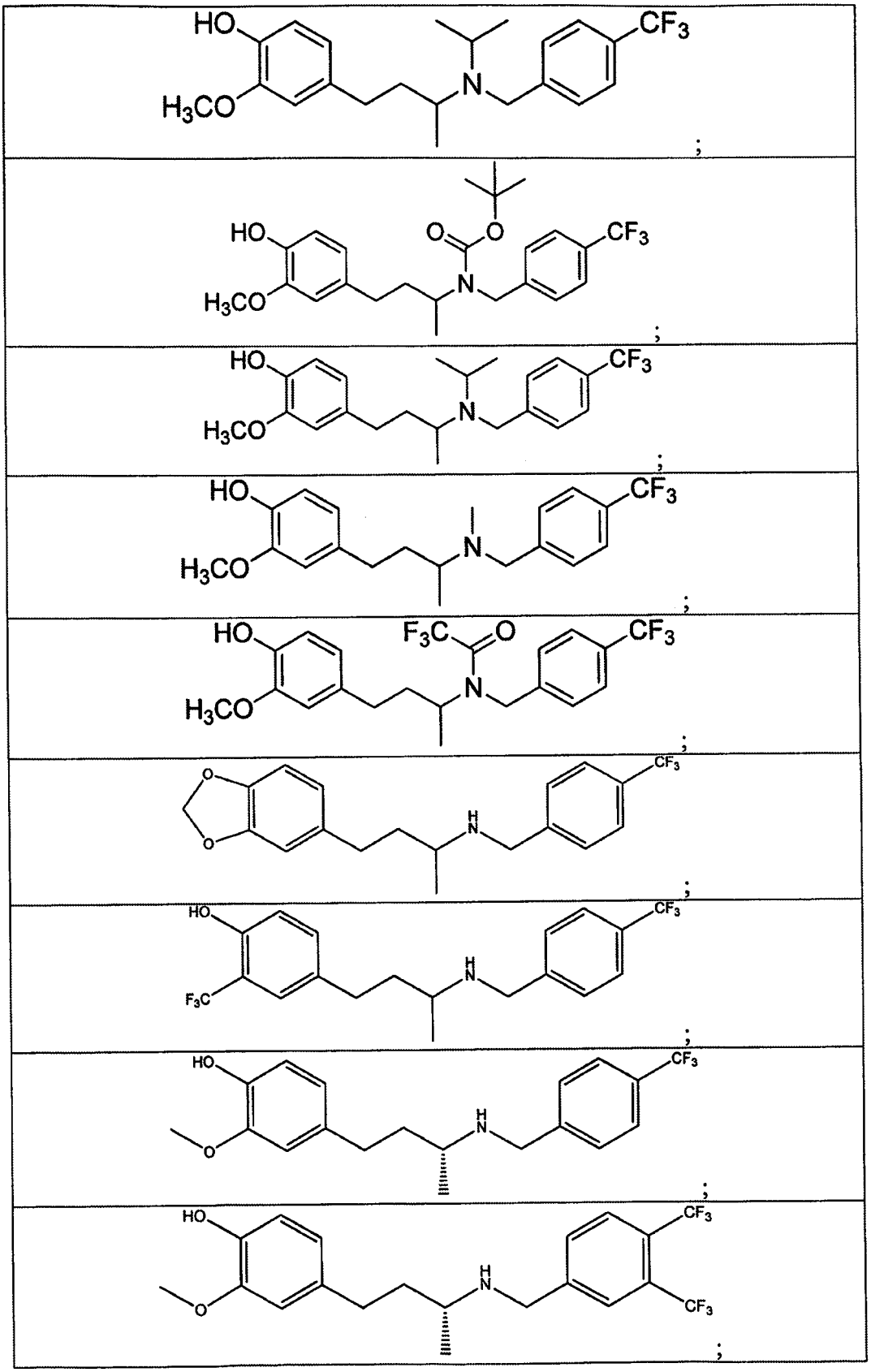
IIb

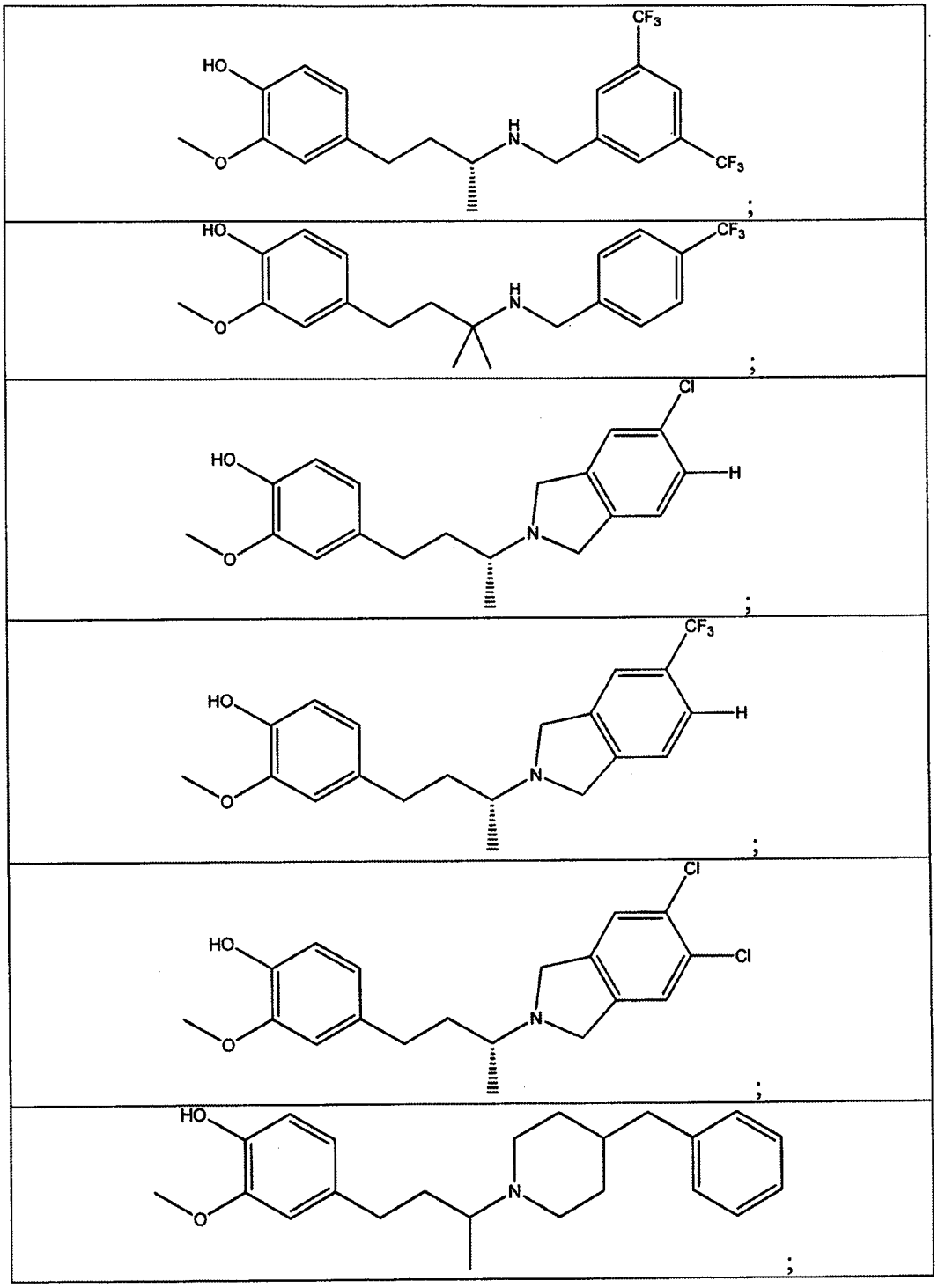
wherein R_1-R_4 are as defined in claim 6, and pharmaceutically acceptable salts thereof.

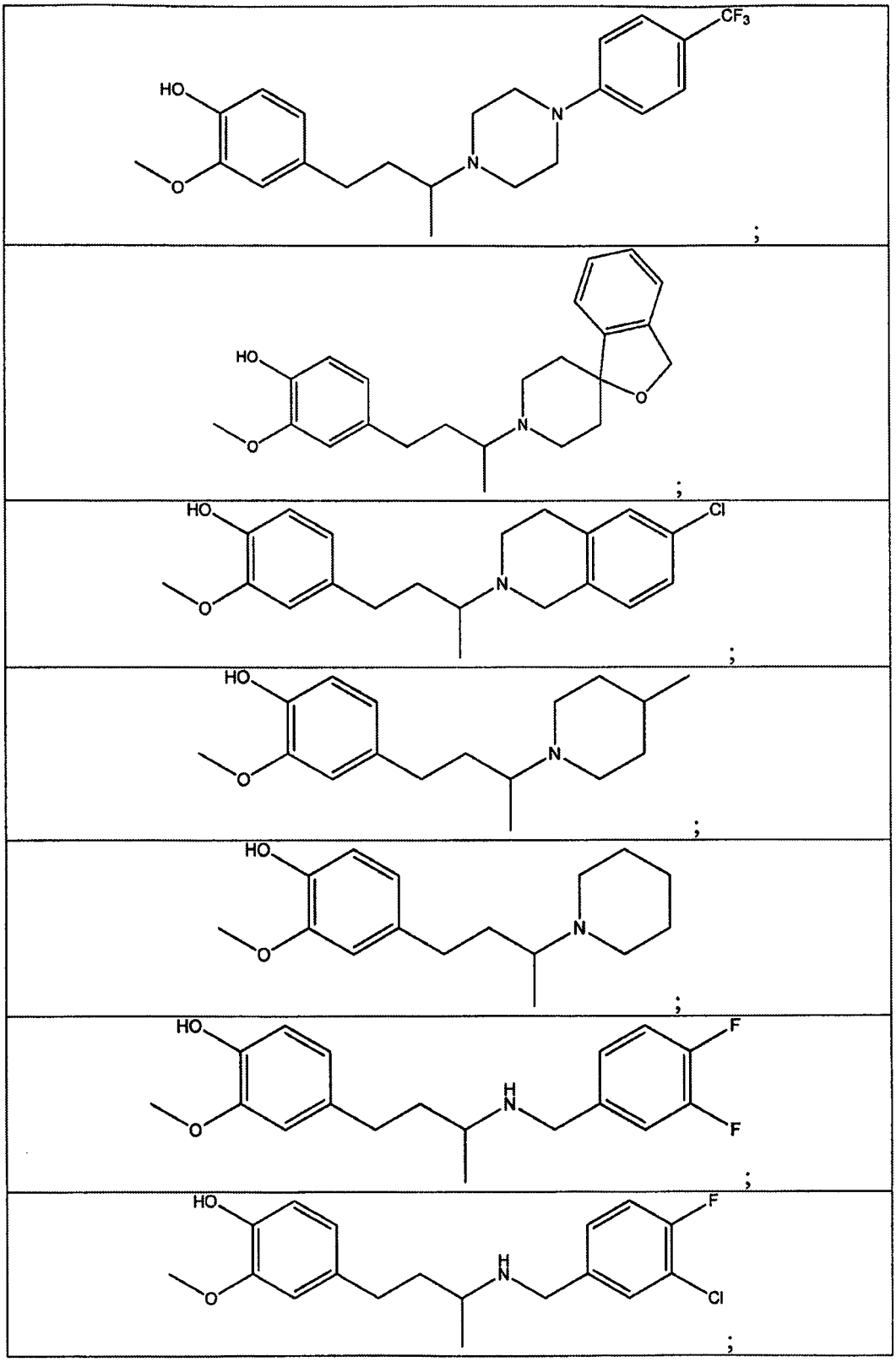
9. A compound selected from the group consisting of:

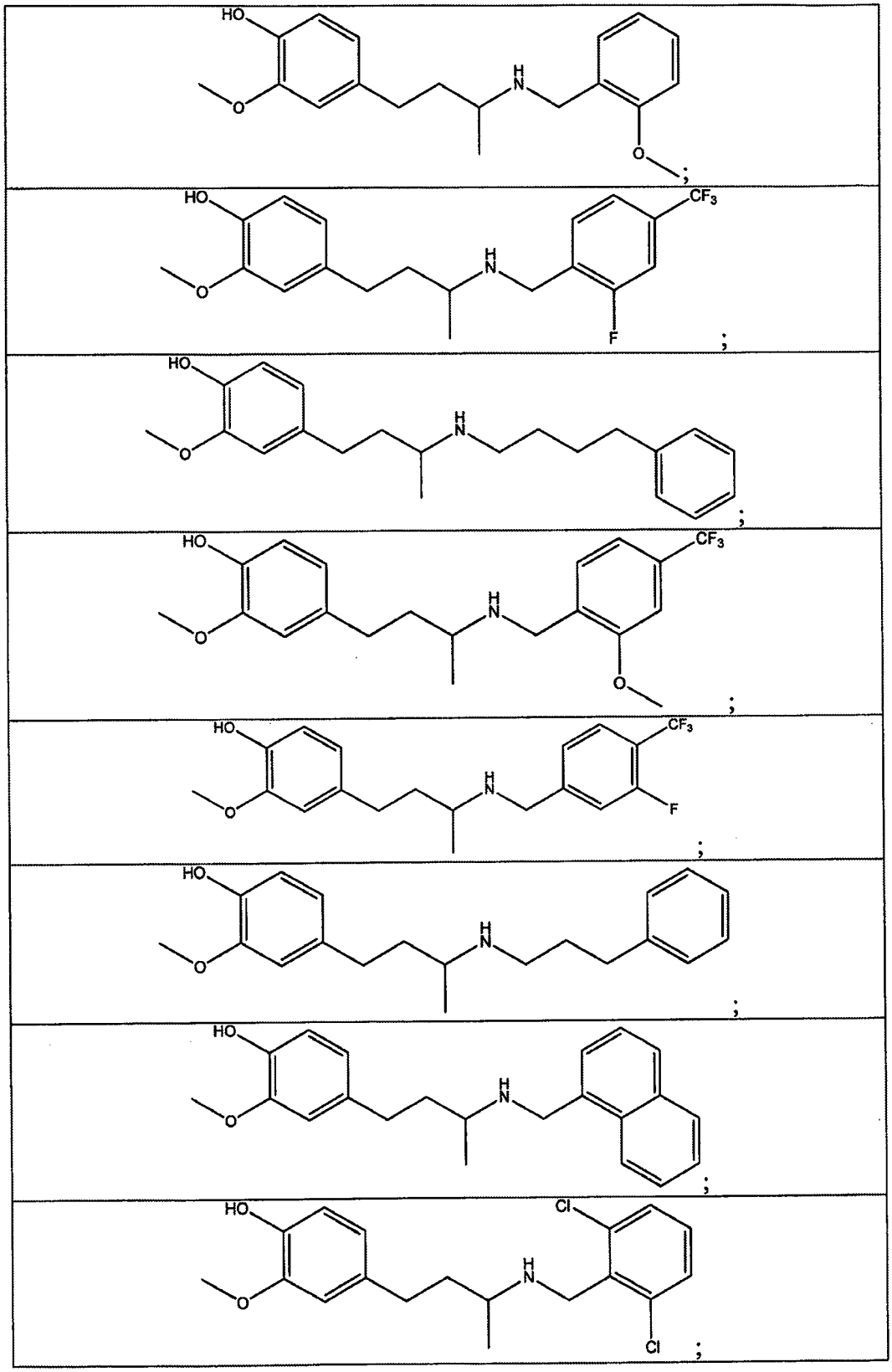


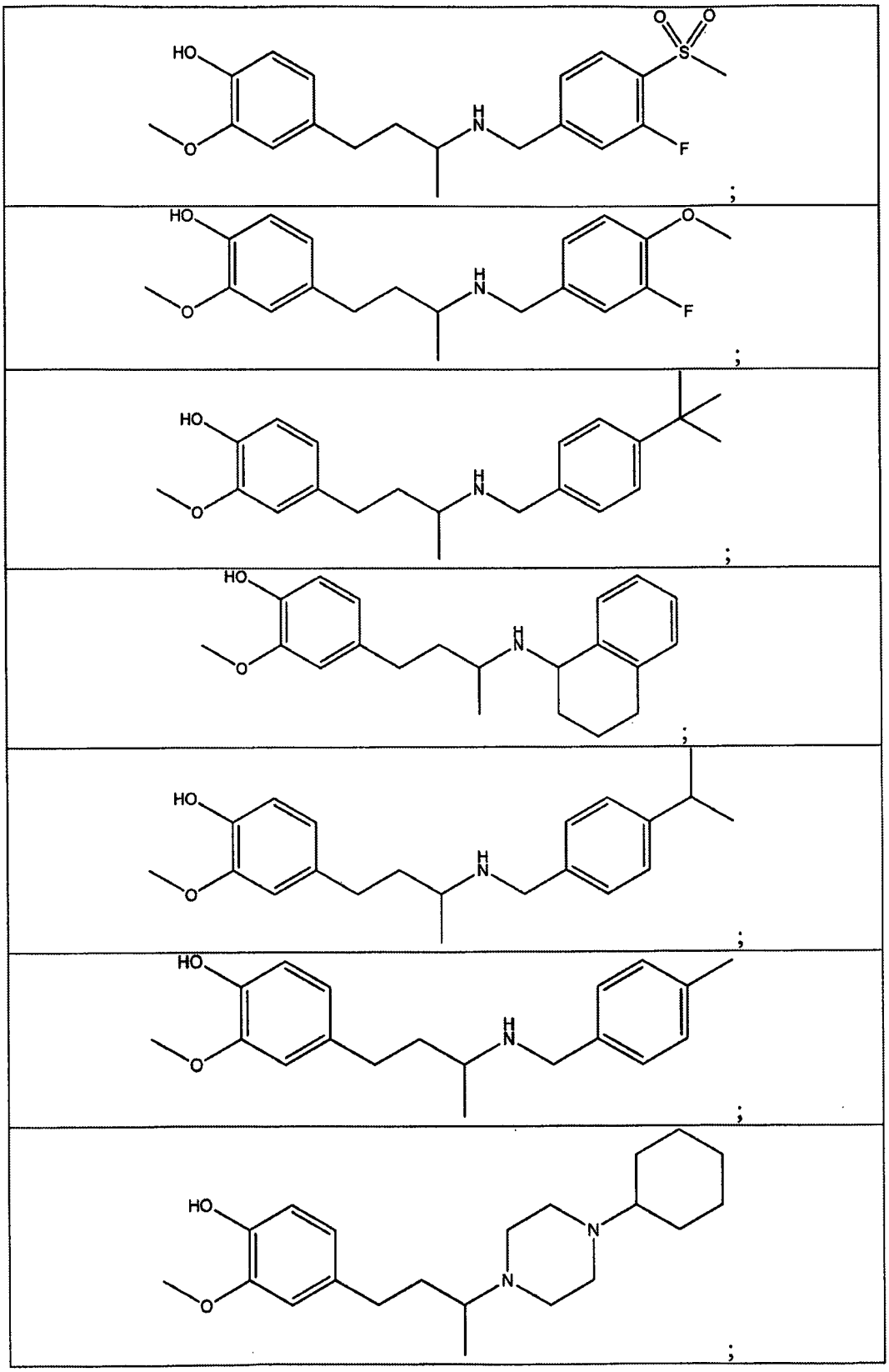







HO
O
N
N
CF3
;
HO
O
N
O
;
HO
O
N
Cl
;
HO
O
N
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HO
O
N
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HO
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H
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F
F
;
HO
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H
N
F
Cl
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;

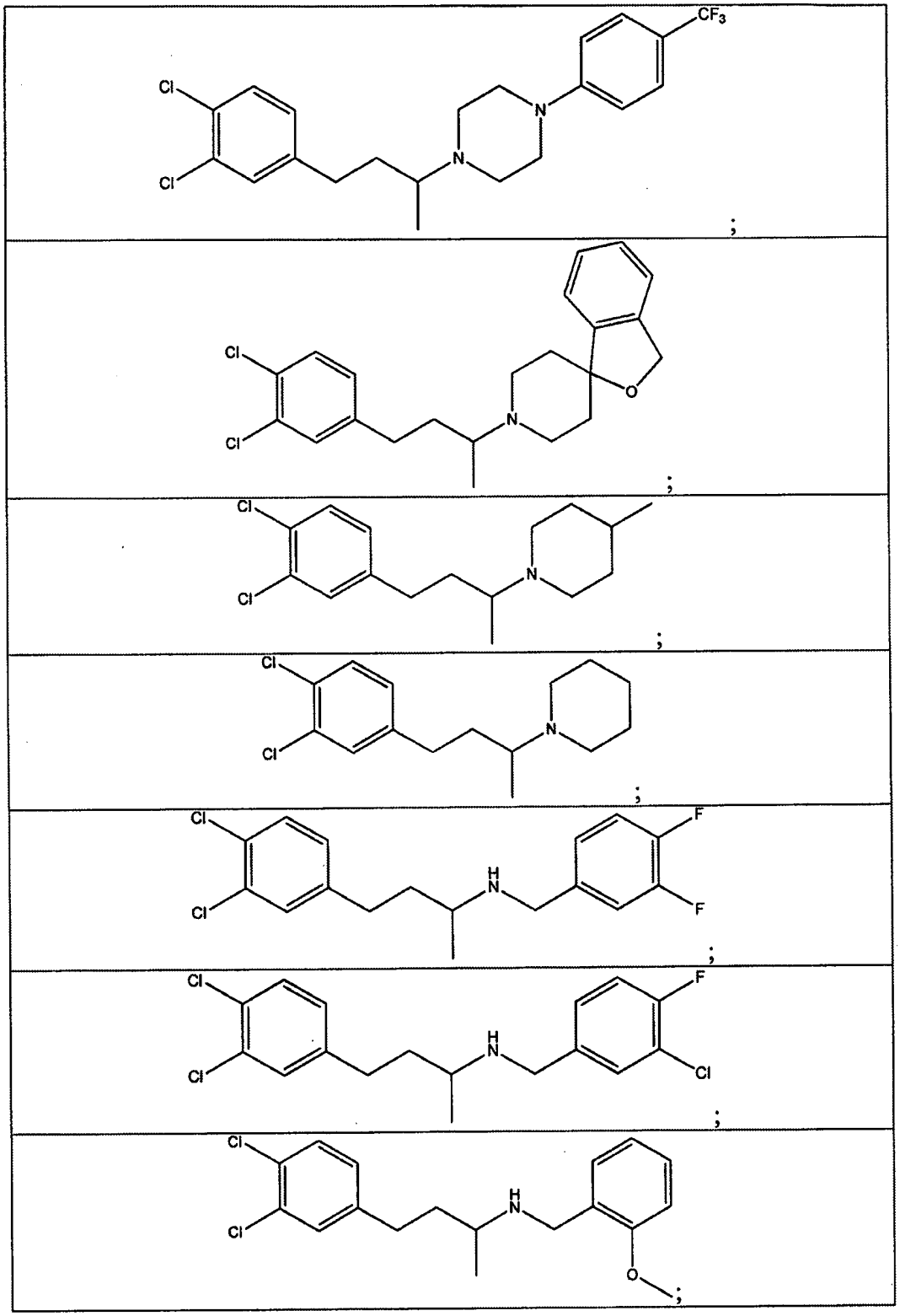
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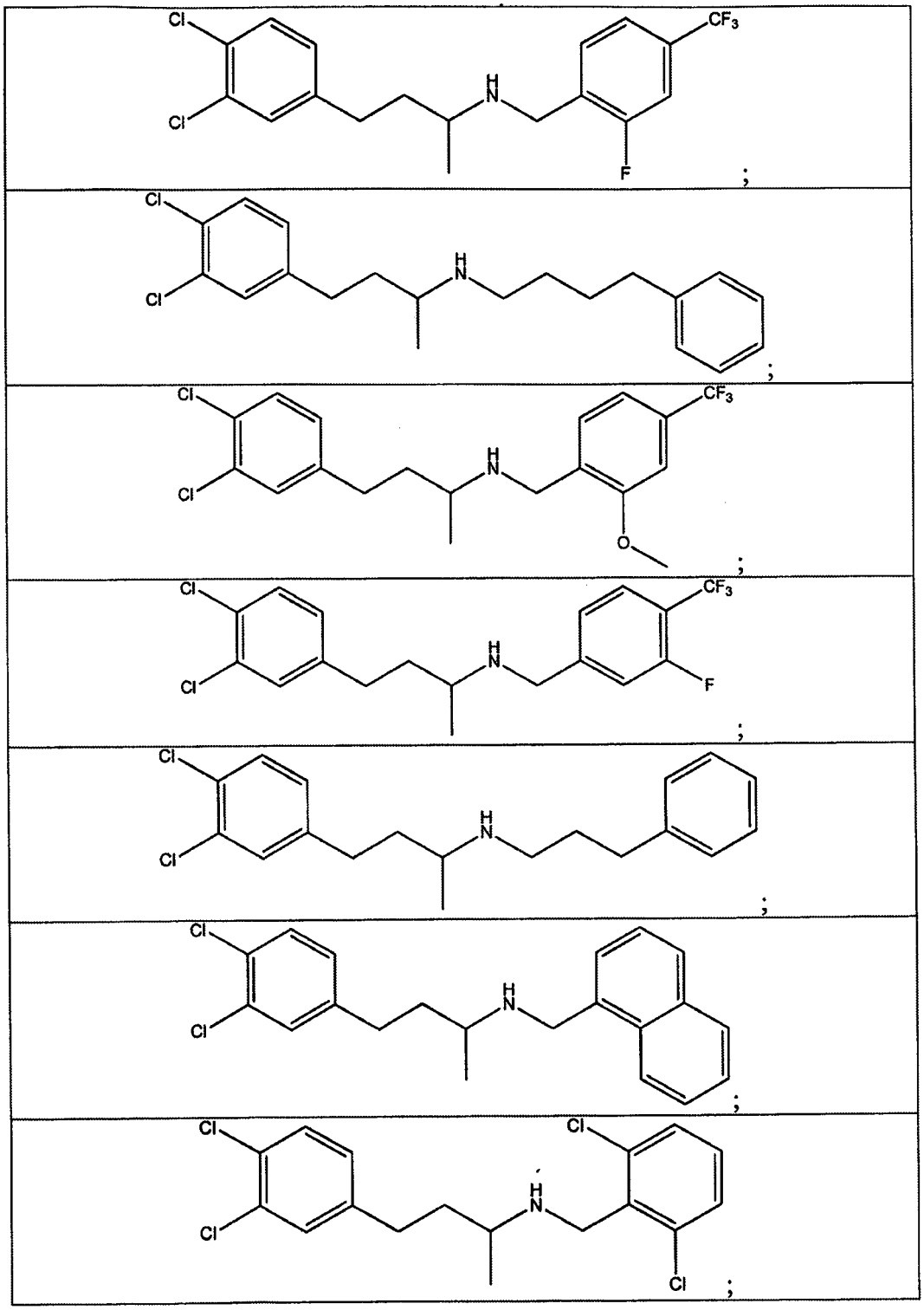
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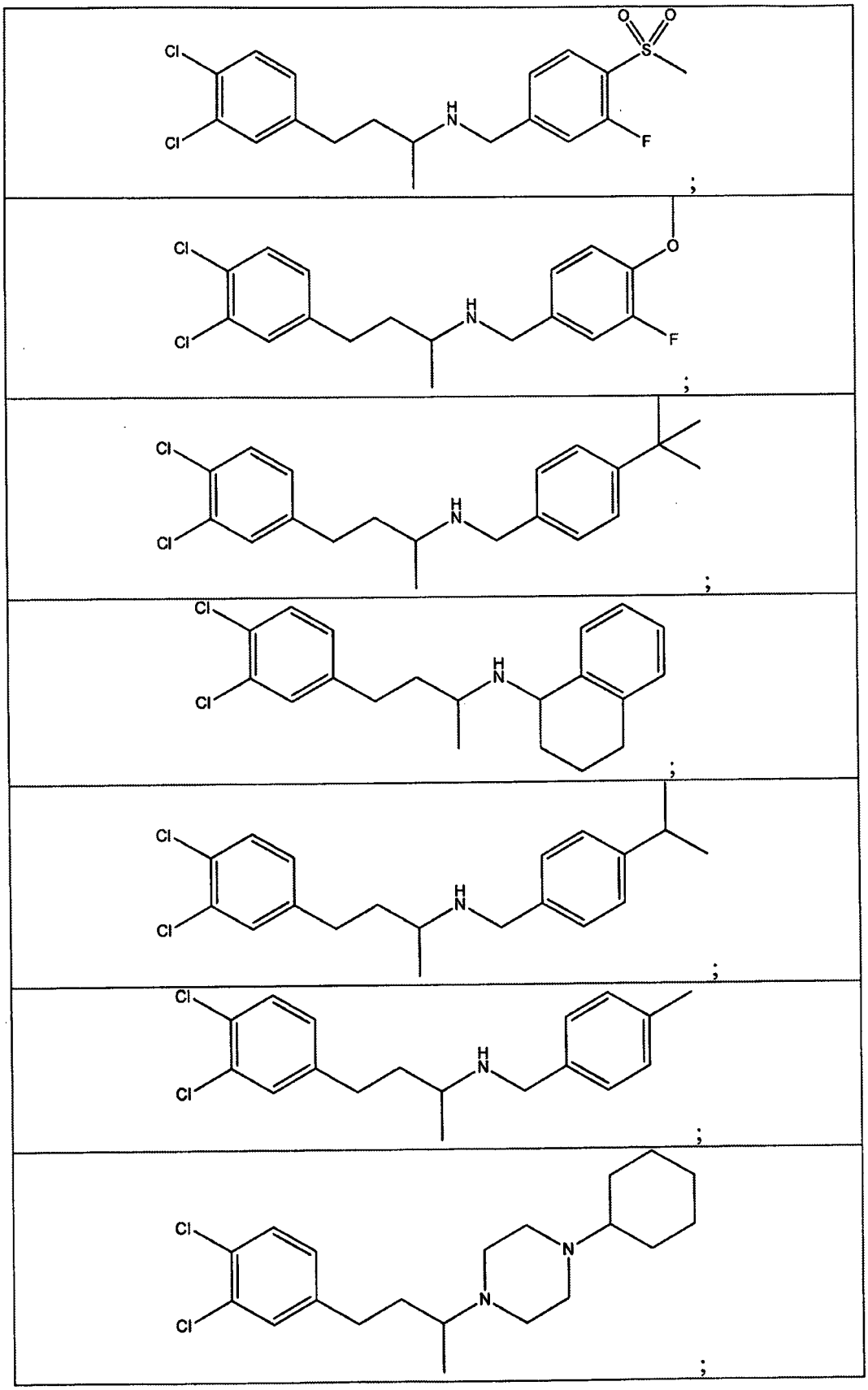
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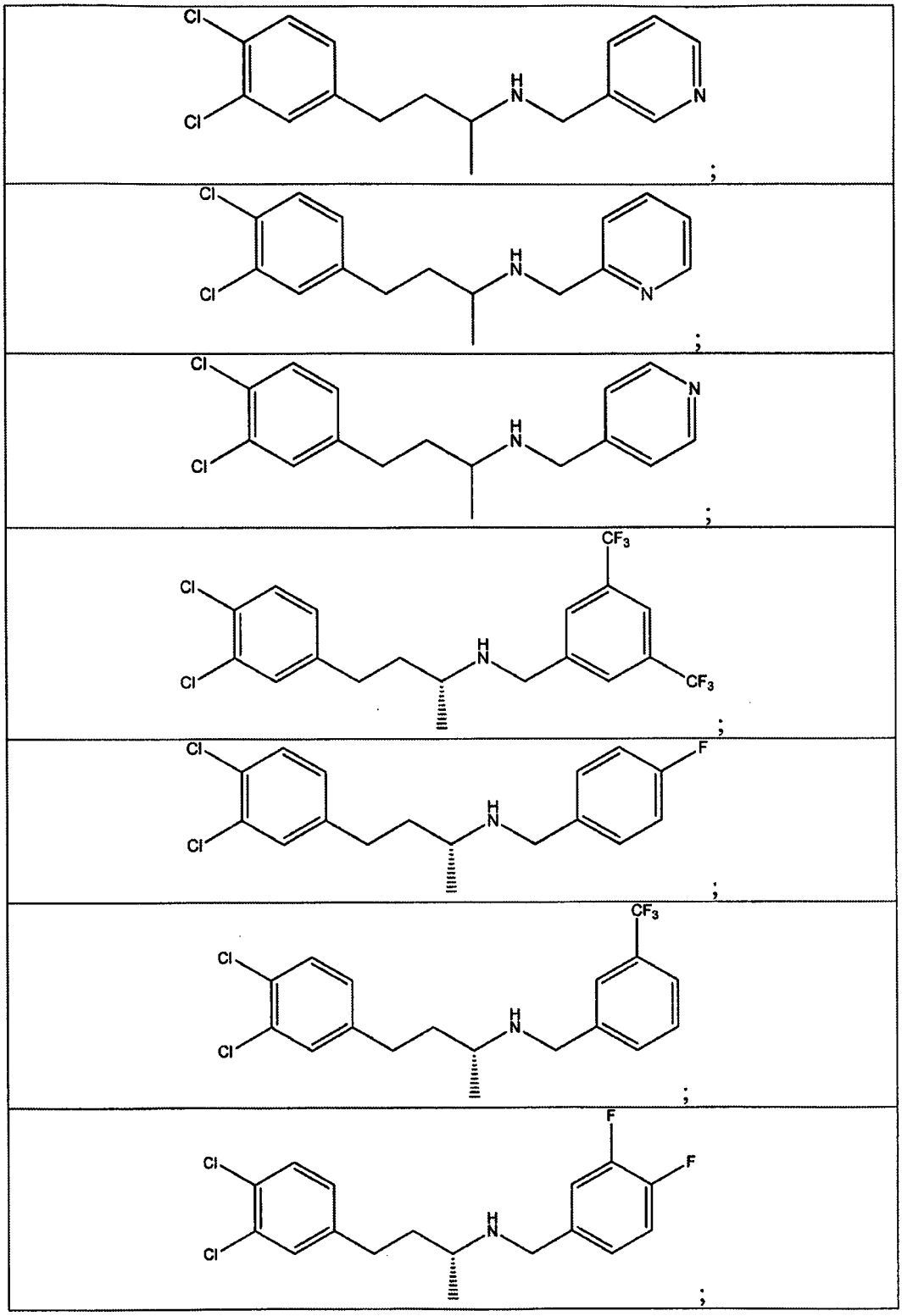
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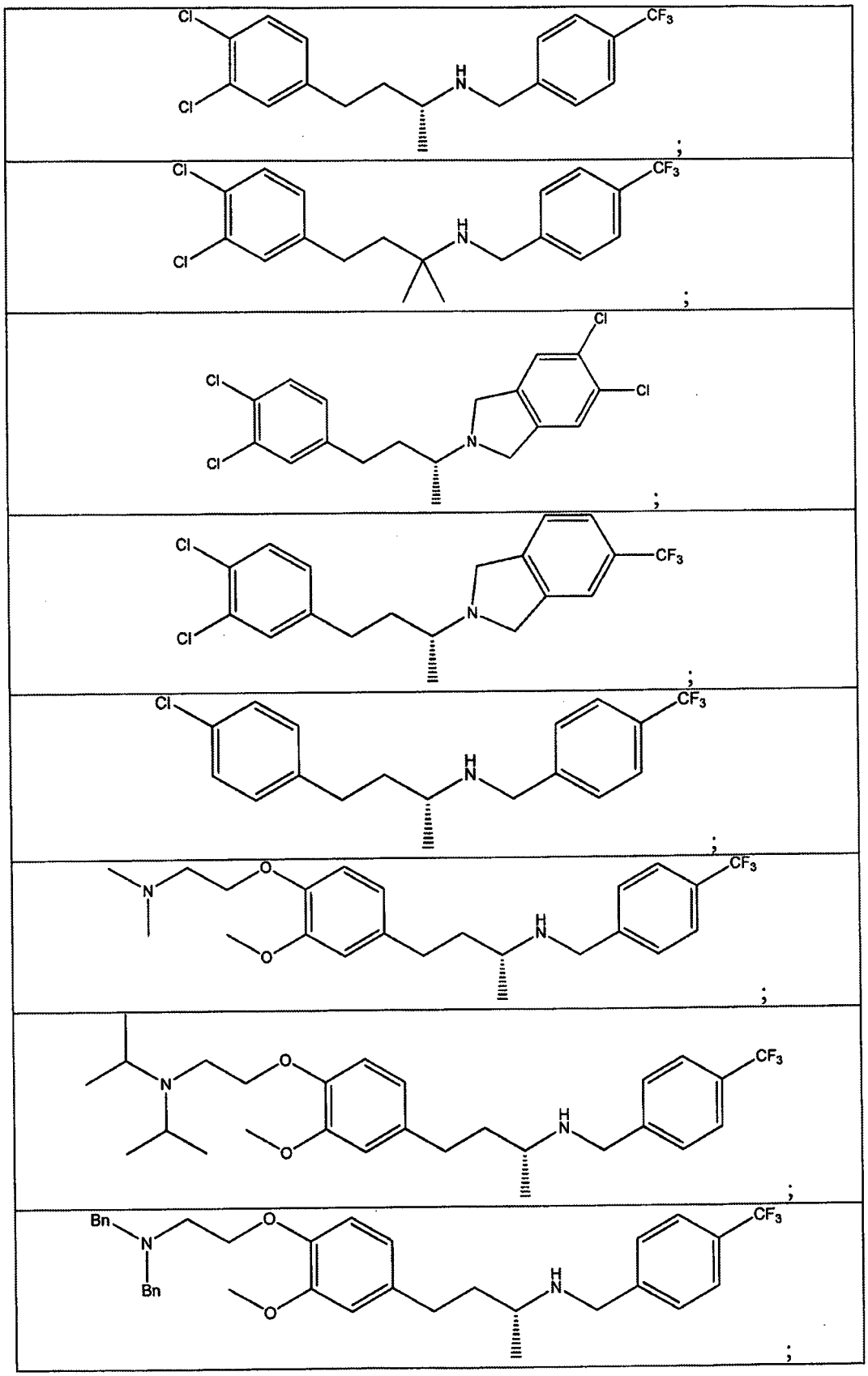


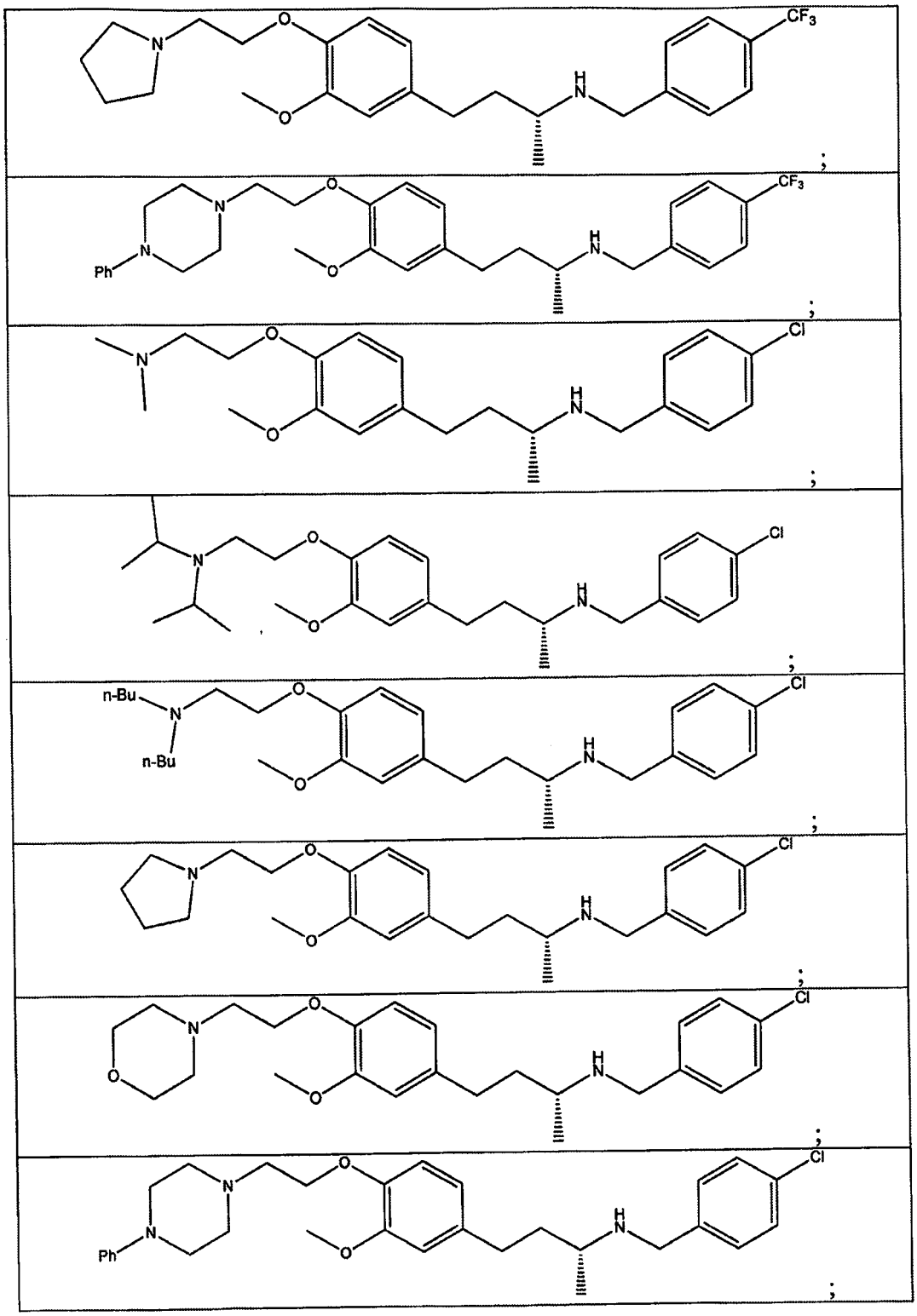


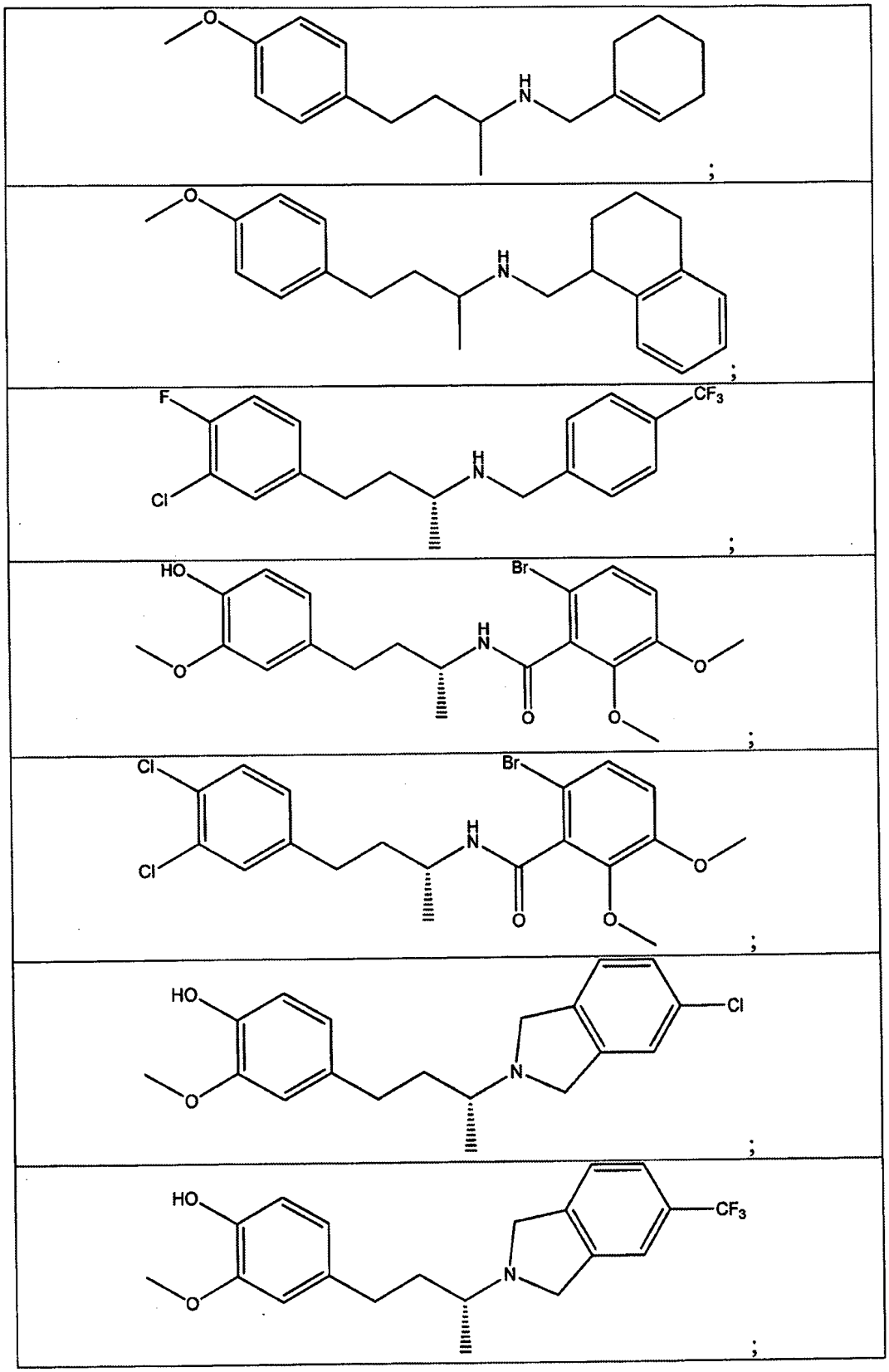


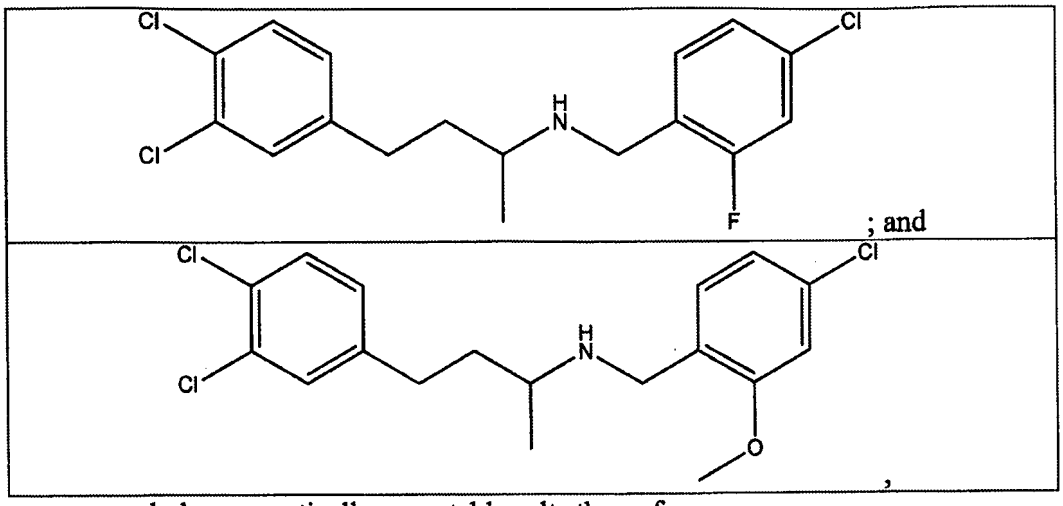
207





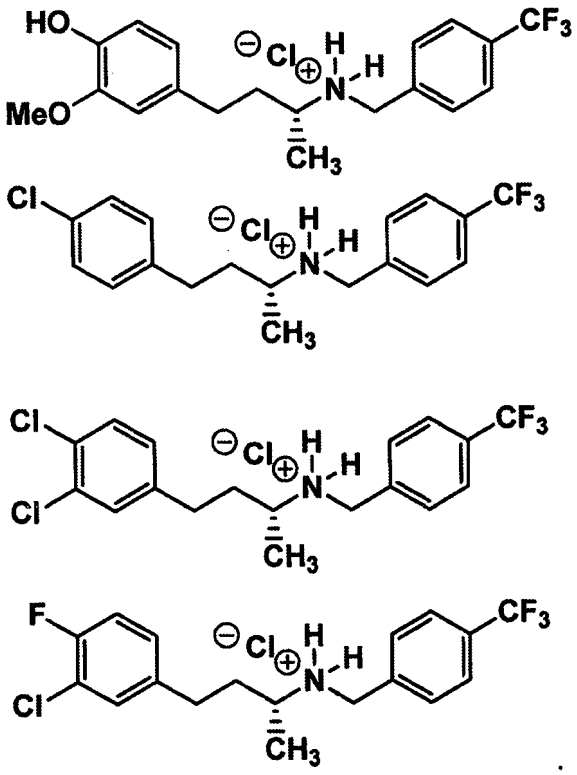




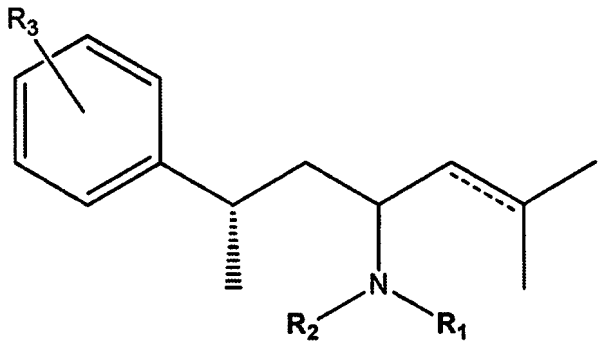


and pharmaceutically acceptable salts thereof.

10. A compound of claim 9 selected from



11. A compound of Formula VIIIa



VIIIa

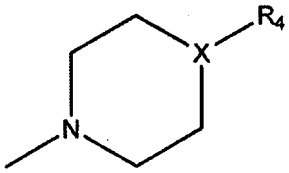
wherein:

-------- is a single bond or a double bond;

R_1 is C_{1-6} alkyl, C_{1-6} haloalkyl, unsubstituted benzyl or benzyl substituted with halo, C_{1-6} alkyl, or C_{1-6} haloalkyl;

R_2 is H, or

R_1 and R_2 together with nitrogen form the ring



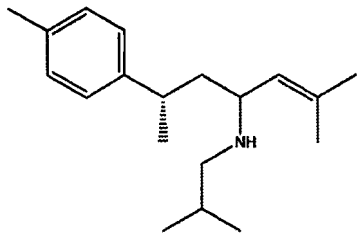
, wherein

X is CH, N, or O, and

R_4 is absent, or is H, C_{1-6} alkyl, or unsubstituted phenyl or phenyl substituted with halo, C_{1-6} alkyl, or C_{1-6} haloalkyl; and

R_3 is C_{1-4} alkyl, halo, or C_{1-6} haloalkoxy, and

pharmaceutically acceptable salts thereof, with the proviso that the following racemic mixture of compounds is excluded:



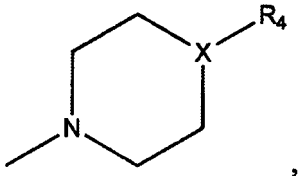
12. The compound of claim 11 wherein

-------- is a single bond or a double bond;

R_1 is isobutyl, benzyl or benzyl substituted with chloro, methyl, or CF_3;

R_2 is H, or

R_1 and R_2 together with nitrogen form the ring



, wherein

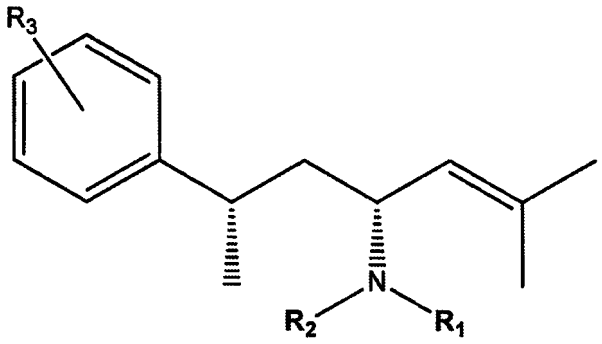
X is CH, N, or O, and

R_4 is absent, or is H, isopropyl, or unsubstituted phenyl; and

R_3 is ortho-Me, meta-Me, para-Me, para-F, para-OCF_3 and

pharmaceutically acceptable salts thereof.

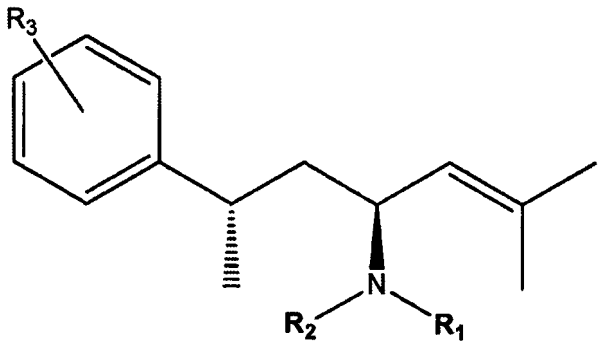
13. The compound of claim 11 that has the Formula VIIIb



VIIIb

wherein R_1-R_3 are as defined in claim 11, and pharmaceutically acceptable salts thereof.

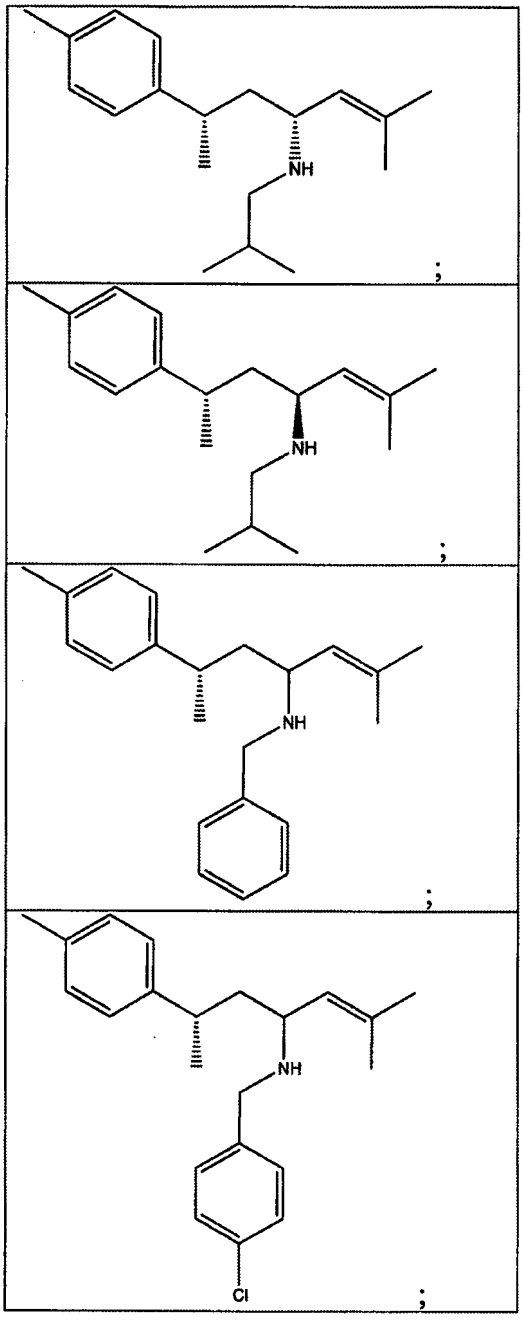
14. The compound of claim 11 that has the Formula VIIIc

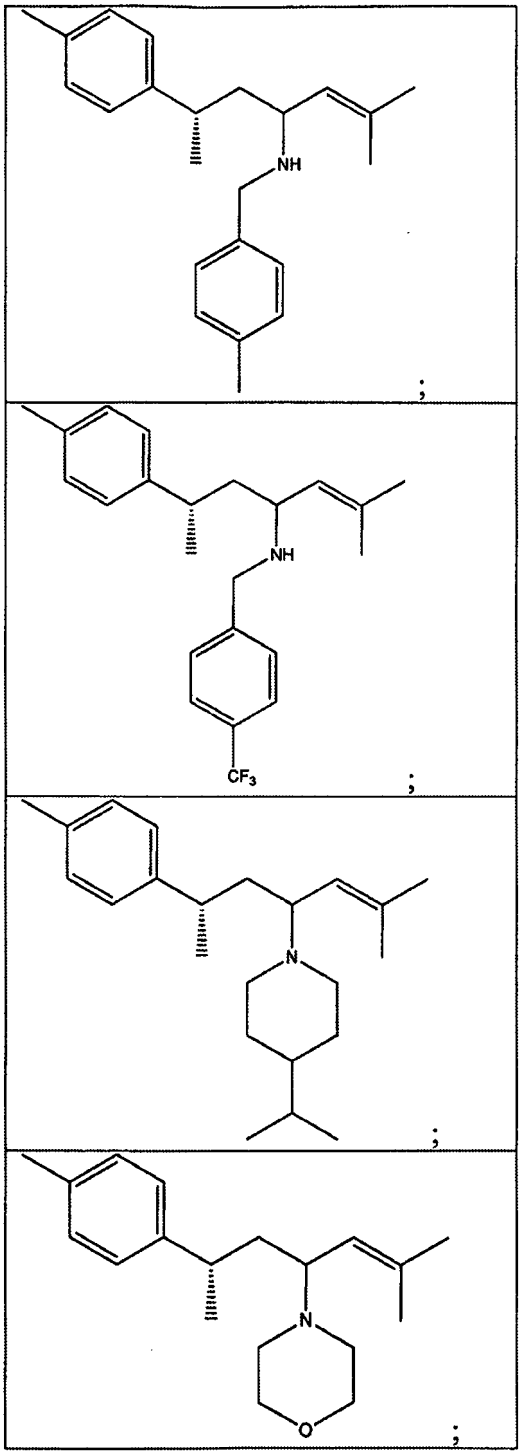


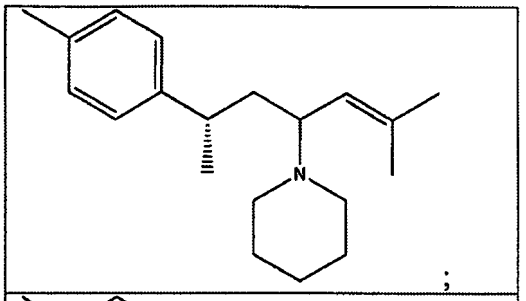
VIIIc

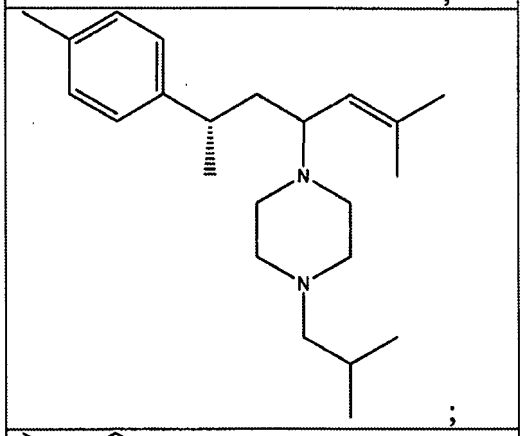
wherein R_1-R_3 are as defined in claim 11, and pharmaceutically acceptable salts thereof.

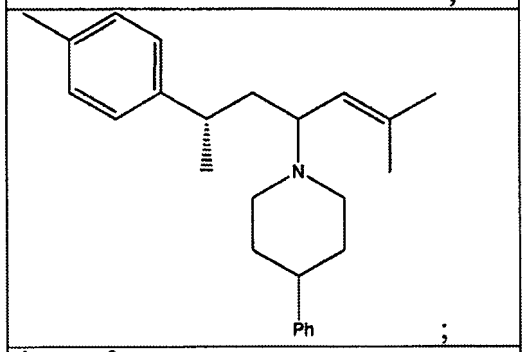
15. A compound selected from the group consisting of:

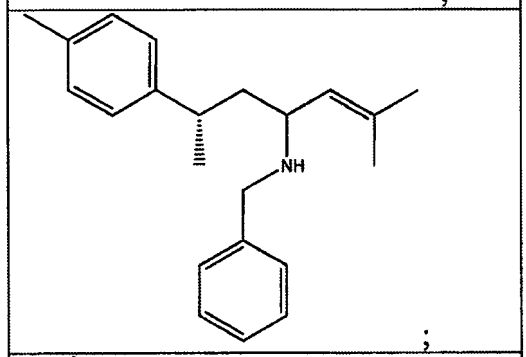


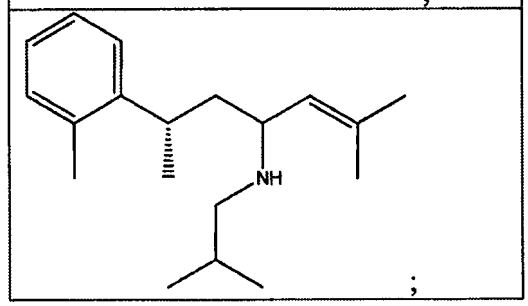

NH
;
NH
CF3
;
N
;
N
O
;

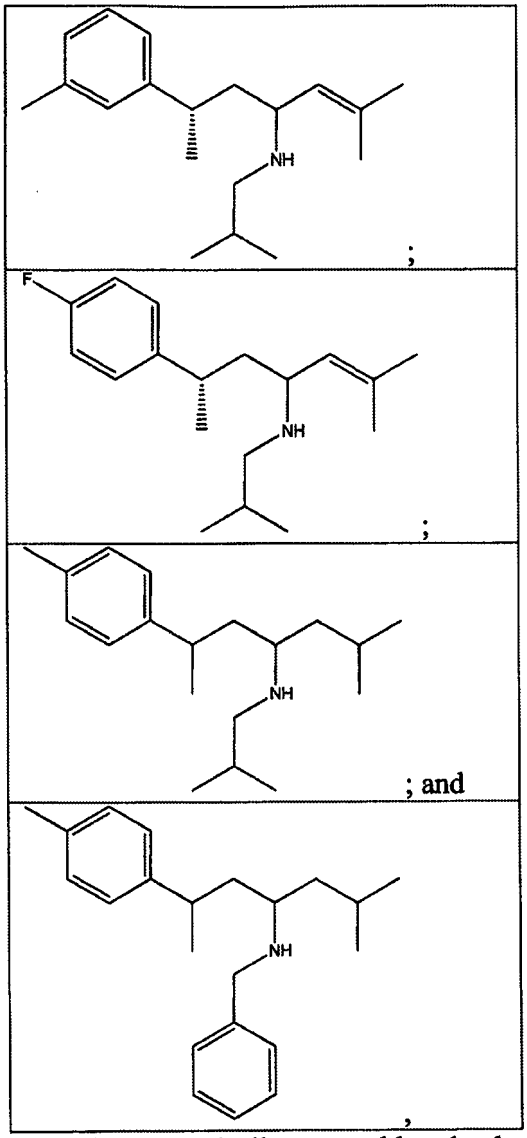

;


;


;

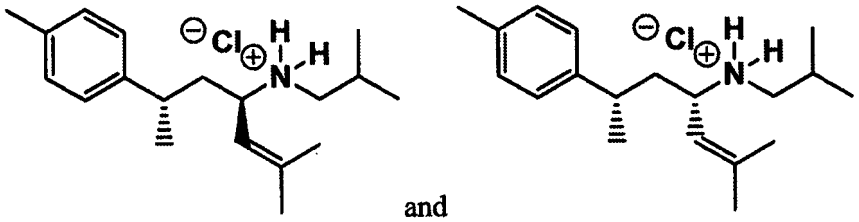

;


;



and pharmaceutically acceptable salts thereof.

16. The compound of claim 15 selected from



and

.

17. A method/use for inhibiting an amyloid beta effect on a neuronal cell comprising administering an effective amount of a composition comprising

A compound of any one of claims 1-16 in an amount effective to inhibit amyloid beta oligomer binding in said cell; and

a pharmaceutically acceptable carrier.

18. The method/use of claim 17, wherein the compound is administered in an amount also effective to inhibit membrane trafficking deficits in said cell, said membrane trafficking effects being associated with exposure of said cell to soluble amyloid beta oligomers.

19. The method/use of any one of claims 17 and 18, wherein the compound is in an amount effective to inhibit both the oligomer binding and synapse loss associated with exposure of the cell to soluble amyloid beta oligomer in said cell.

20. The method/use of any one of claims 17 to 19, wherein the compound is administered in an amount effective to inhibit a soluble amyloid beta oligomer-mediated cognitive effect.

21. The method/use of claim 20, wherein the cognitive effect is cognitive decline as tested in an animal model of cognitive decline.

22. The method/use of claim 21 wherein the cognitive decline is a decline in learning as tested by a fear conditioning assay.

23 The method/use of claim 21 wherein the cognitive decline is a decline in spatial learning and memory as tested by a Morris water maze test.

24. The method/use of claim 21, wherein the cognitive decline is hippocampal-based spatial learning and memory decline as tested in a transgenic animal model of Alzheimer's disease.

25. The method/use of claim 17 for inhibiting amyloid beta oligomer-induced synaptic dysfunction of a neuronal cell; comprising contacting the cell with the composition comprising a sigma-2 receptor antagonist compound in an amount effective to inhibit amyloid beta oligomer binding in said cell, said dysfunction being associated with exposure of the cells to soluble amyloid beta oligomer.

26. The method/use of claim 17 for inhibiting suppression of long term potentiation in a subject comprising administering to the subject in need thereof a therapeutically effective amount of the composition comprising a sigma-2 receptor antagonist compound.

27. The method/use of claim 17 for inhibiting cognitive decline in a subject exhibiting, or at risk of exhibiting, cognitive decline, comprising administering a therapeutically effective amount of the composition comprising a sigma-2 receptor antagonist compound to the subject.

28. The method/use of claim 17 for inhibiting cognitive decline in a subject associated with an amyloid beta oligomer effect on central neurons comprising administering a therapeutically effective amount of the composition comprising a sigma-2 receptor antagonist compound to a subject afflicted with said cognitive decline.

29. The method/use of claim 17 for the treatment of mild cognitive impairment in Alzheimer's disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the composition comprising a sigma-2 receptor antagonist compound.

30. The method/use of any one of claims 25-27 wherein the sigma-2 antagonist compound has one or more of the following additional properties:

(a) it selectively binds to a sigma-2 receptor with at least 10-fold, 20-fold, 50-fold, or 100-fold greater affinity compared to one or more non-sigma CNS receptors, wherein the compound binds to a sigma-2 receptor with a K_i less than 200 nM, 150 nM, 100 nM or 60 nM

(b) it inhibits Abeta oligomer binding to or synapse loss in neuronal cells said loss being associated with exposure of the cells to Abeta oligomer;

(c) it inhibits membrane trafficking abnormalities in a central neuron, the abnormalities being associated with exposure of said cell to one or more Abeta oligomers;

(d) it fails to affect trafficking or synapse number in central neurons in the absence of amyloid beta oligomers.

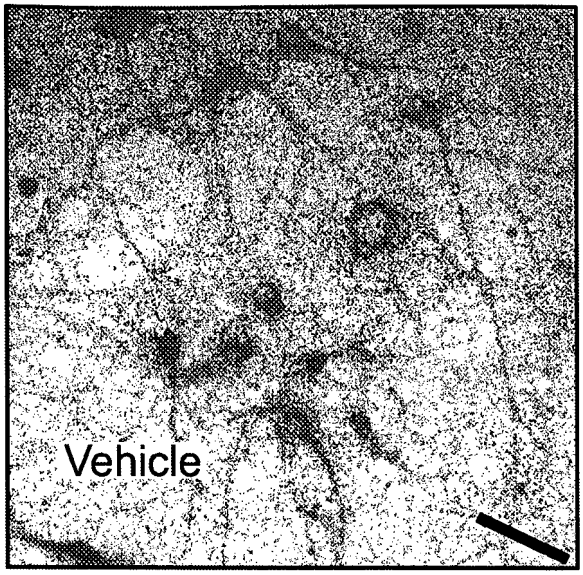


FIG. 1A

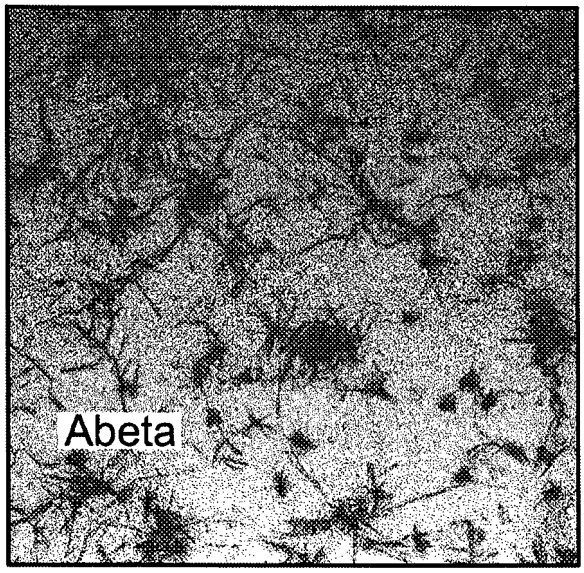


FIG. 1B

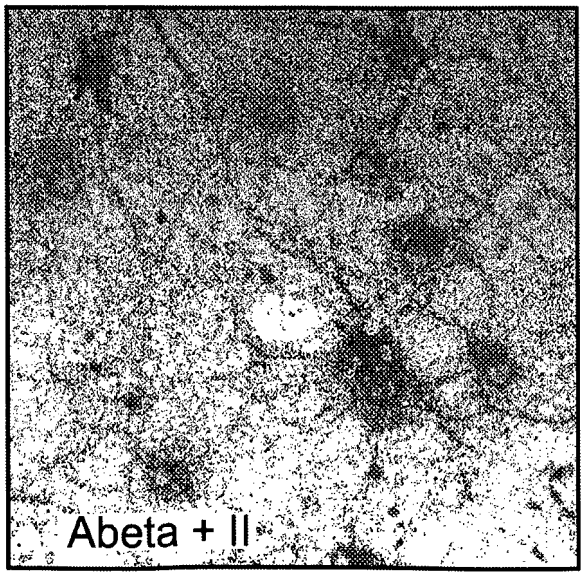


FIG. 1C

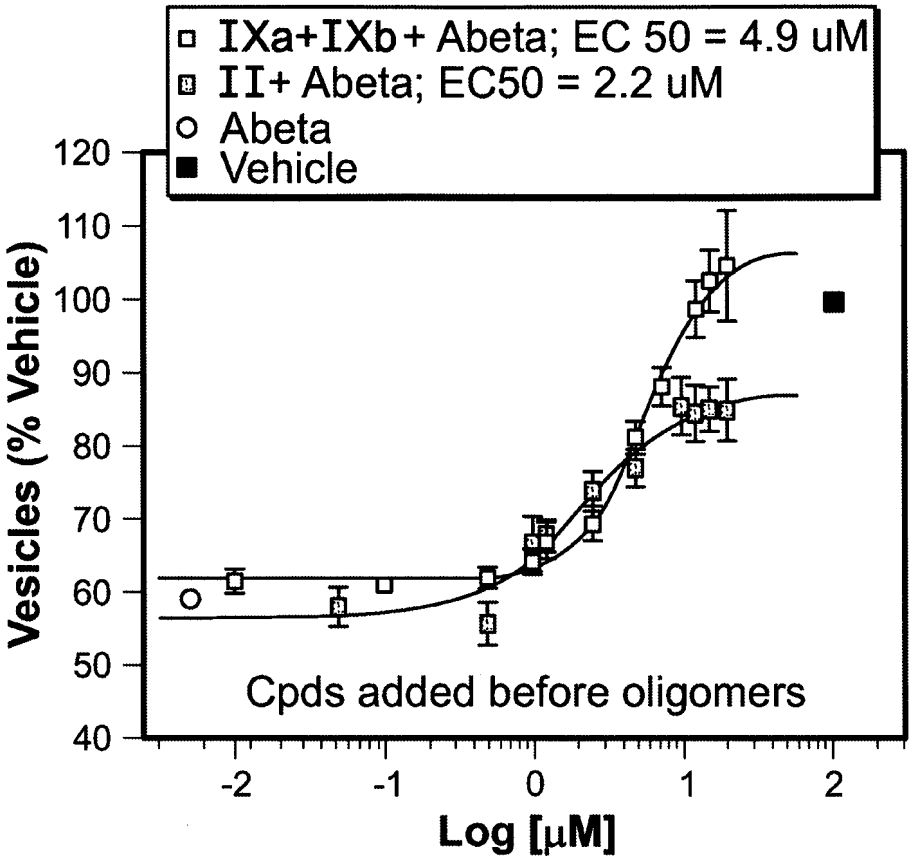

IXa+IXb+ Abeta; EC 50 = 4.9 uM
II+ Abeta; EC50 = 2.2 uM
Abeta
Vehicle
Vesicles (% Vehicle)
120
110
100
90
80
70
60
50
40
Cpds added before oligomers
-2
-1
0
1
2
Log [μM]

FIG. 1D

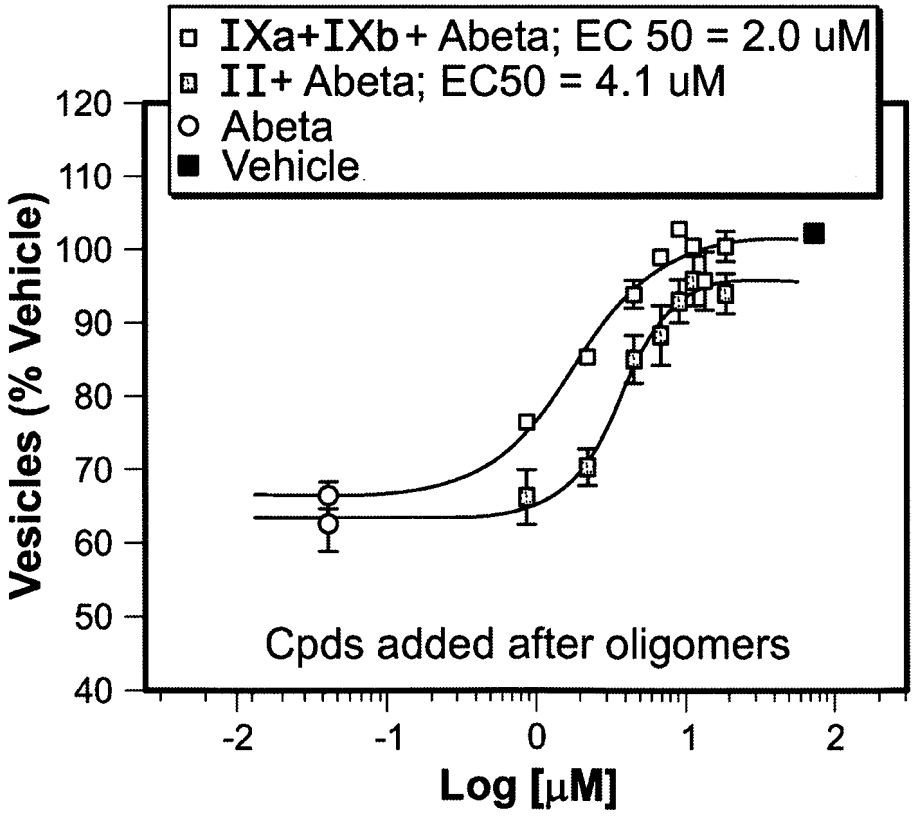

IXa+IXb+ Abeta; EC 50 = 2.0 uM
II+ Abeta; EC50 = 4.1 uM
Abeta
Vehicle
Vesicles (% Vehicle)
120
110
100
90
80
70
60
50
40
Cpds added after oligomers
-2
-1
0
1
2
Log [μM]

FIG. 1E

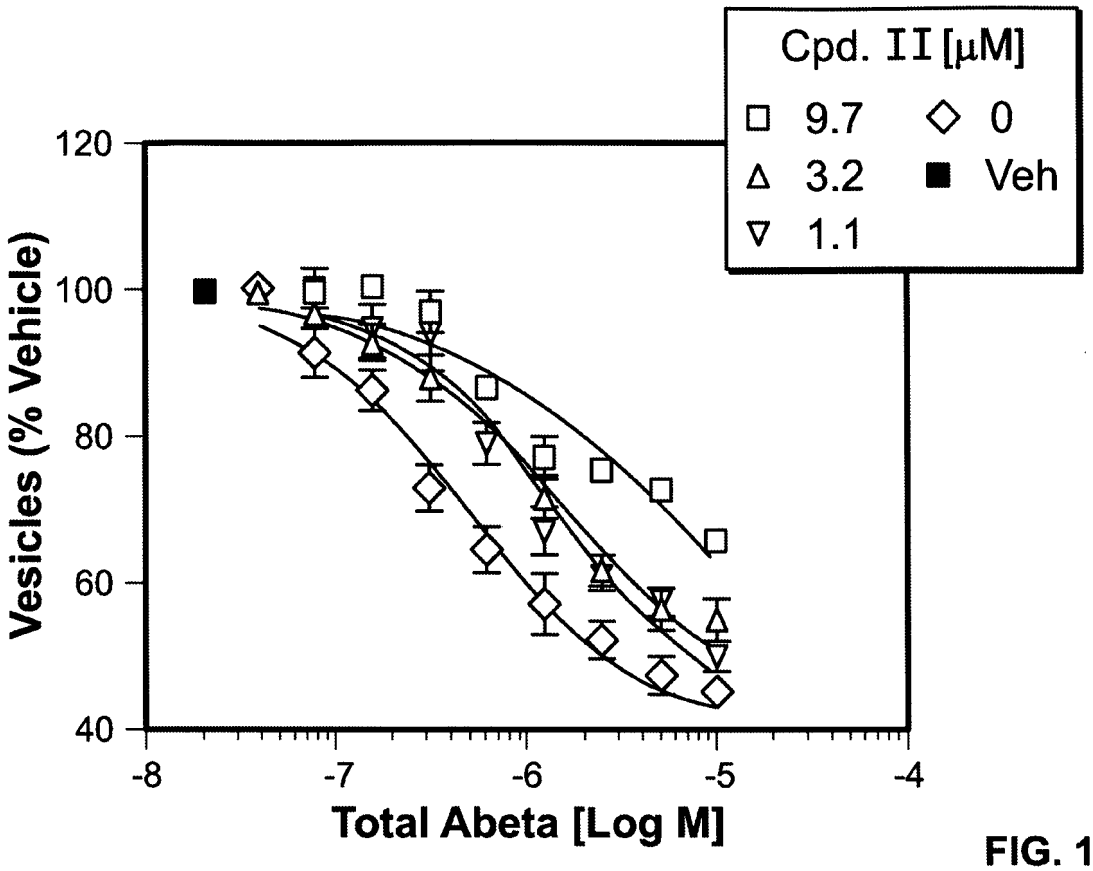

Cpd. II [μM]
9.7
0
3.2
Veh
1.1
120
100
80
60
40
Vesicles (% Vehicle)
-8
-7
-6
-5
-4
Total Abeta [Log M]

FIG. 1F

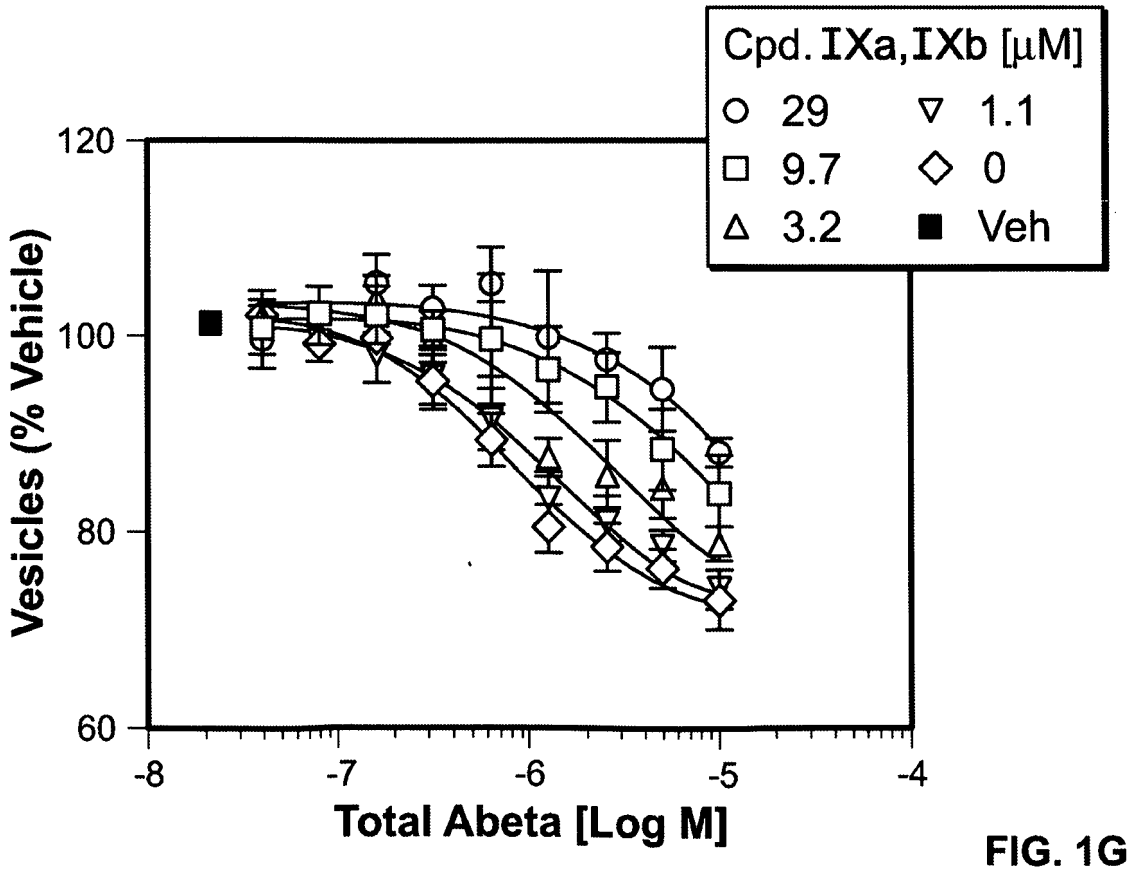

Cpd. IXa,IXb [μM]
29
1.1
9.7
0
3.2
Veh
120
100
80
60
Vesicles (% Vehicle)
-8
-7
-6
-5
-4
Total Abeta [Log M]

FIG. 1G

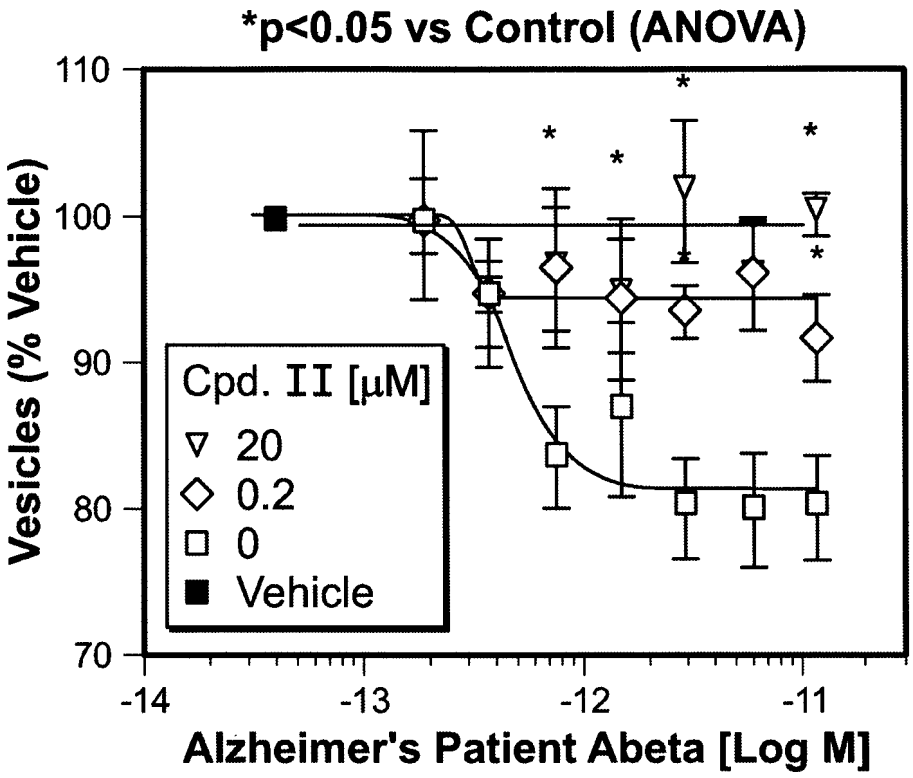

*p<0.05 vs Control (ANOVA)
Vesicles (% Vehicle)
110
100
90
80
70
-14
-13
-12
-11
Alzheimer's Patient Abeta [Log M]
Cpd. II [μM]
20
0.2
0
Vehicle
*

FIG. 1H

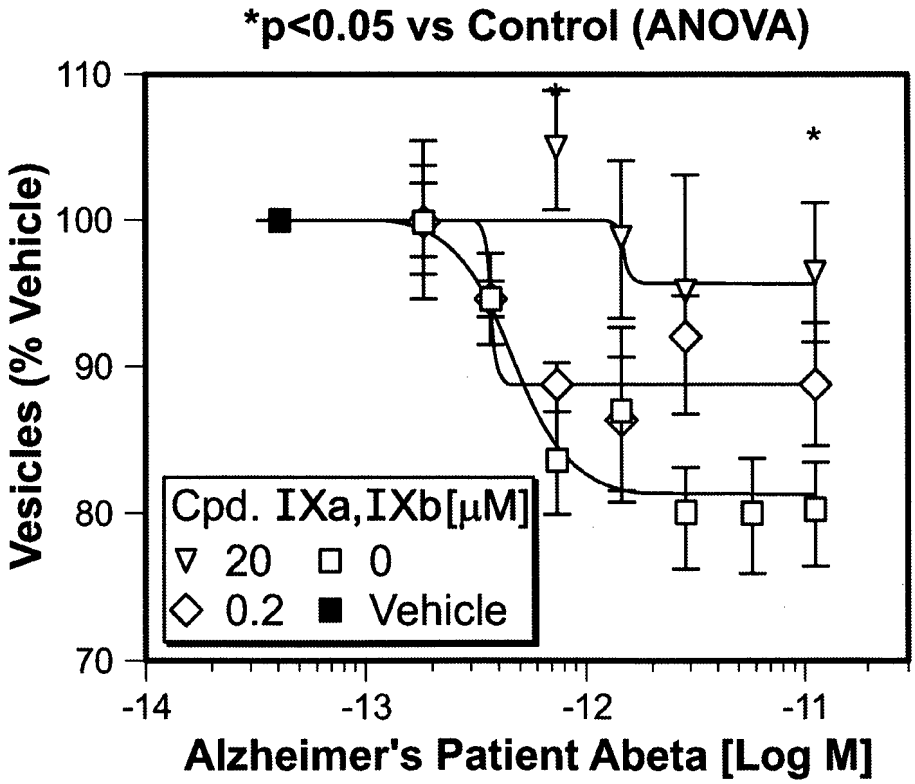

*p<0.05 vs Control (ANOVA)
Vesicles (% Vehicle)
110
100
90
80
70
-14
-13
-12
-11
Alzheimer's Patient Abeta [Log M]
Cpd. IXa,IXb[μM]
20
0
0.2
Vehicle
*

FIG. 1I

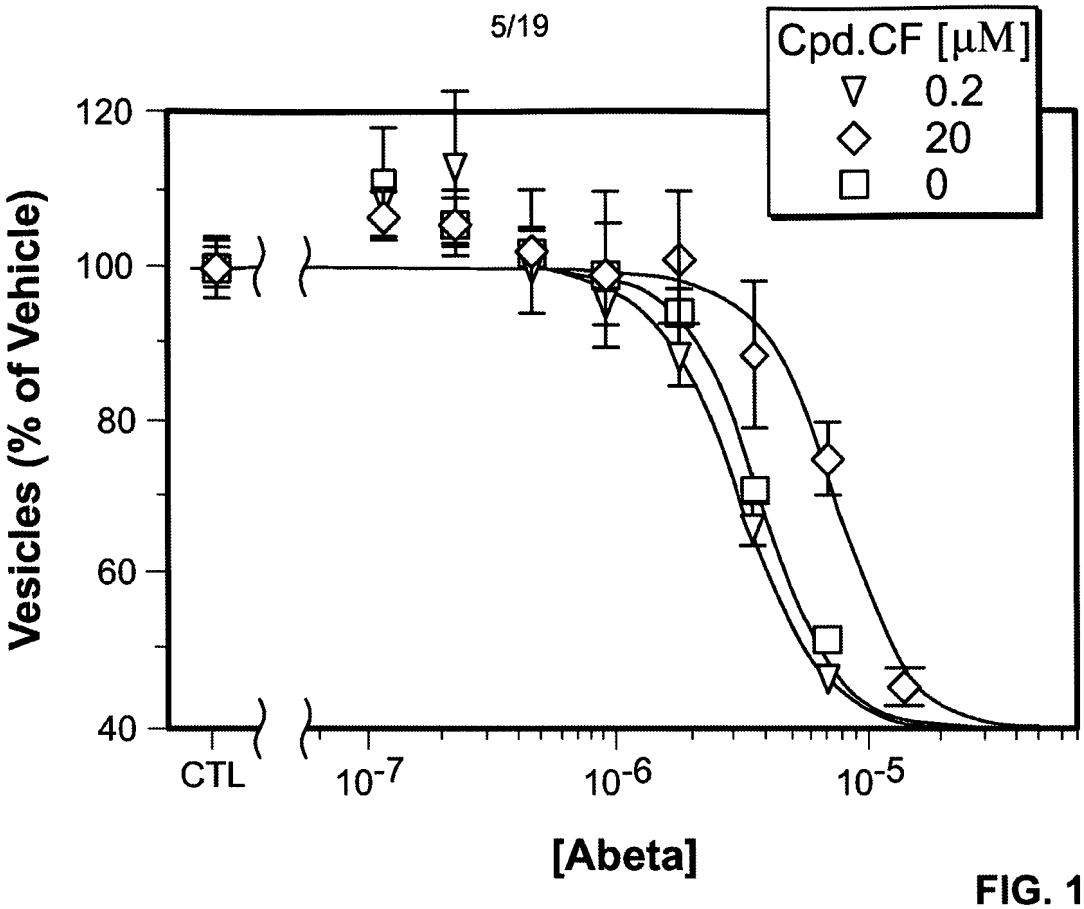

Cpd.CF [μM]
0.2
20
0
120
100
80
60
40
Vesicles (% of Vehicle)
CTL
10-7
10-6
10-5
[Abeta]

FIG. 1J

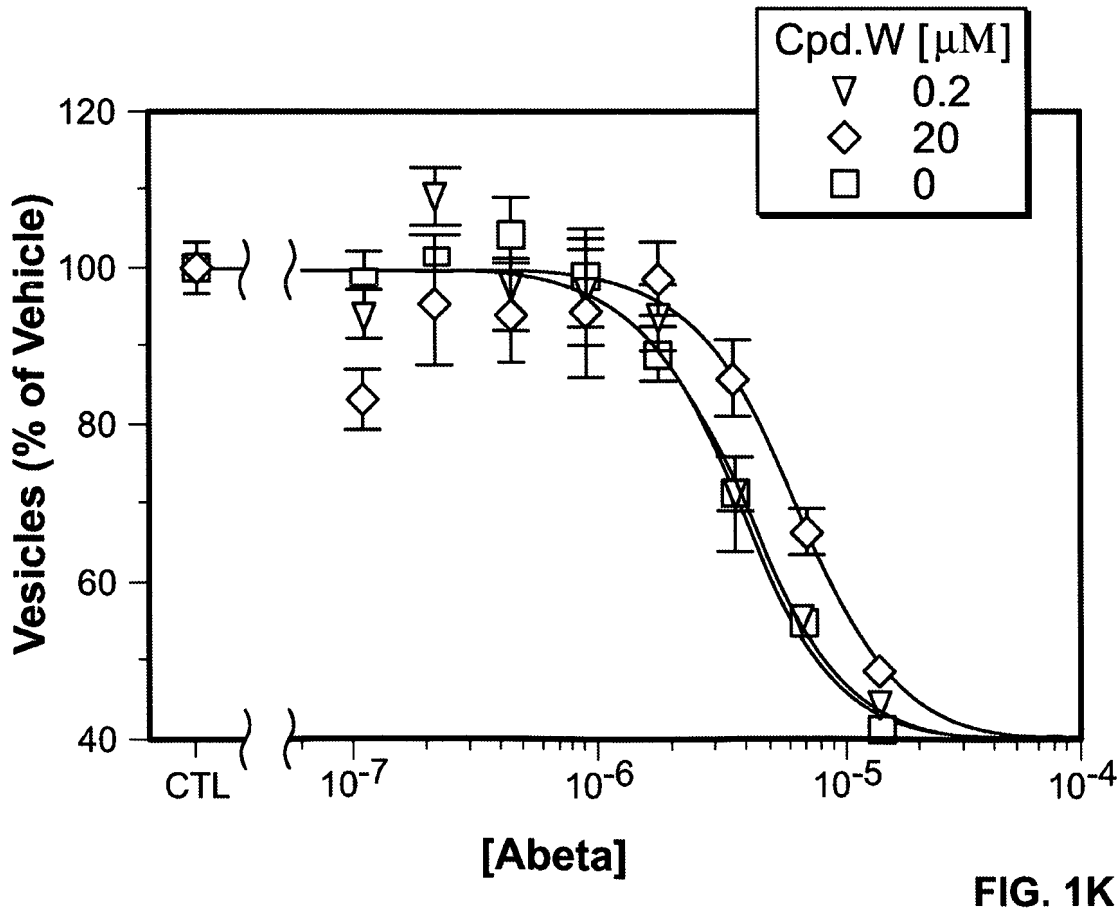

Cpd.W [μM]
0.2
20
0
120
100
80
60
40
Vesicles (% of Vehicle)
CTL
10-7
10-6
10-5
10-4
[Abeta]

FIG. 1K

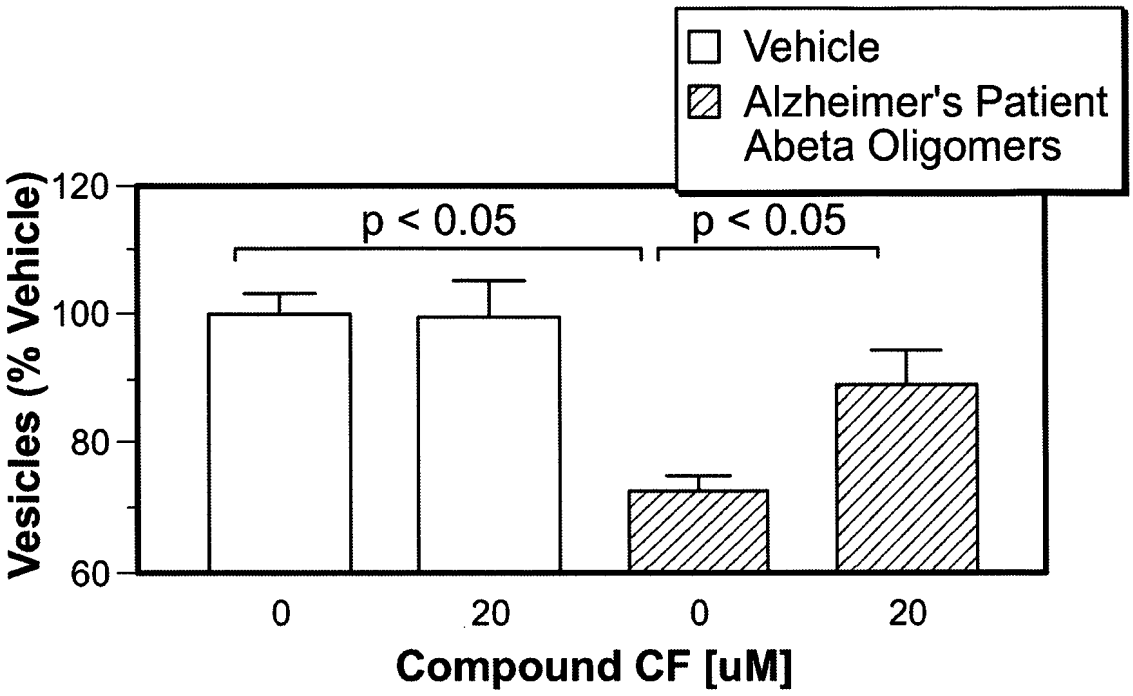

Vehicle
Alzheimer's Patient Abeta Oligomers
p < 0.05
p < 0.05
Vesicles (% Vehicle)
120
100
80
60
0
20
0
20
Compound CF [uM]

FIG. 1L

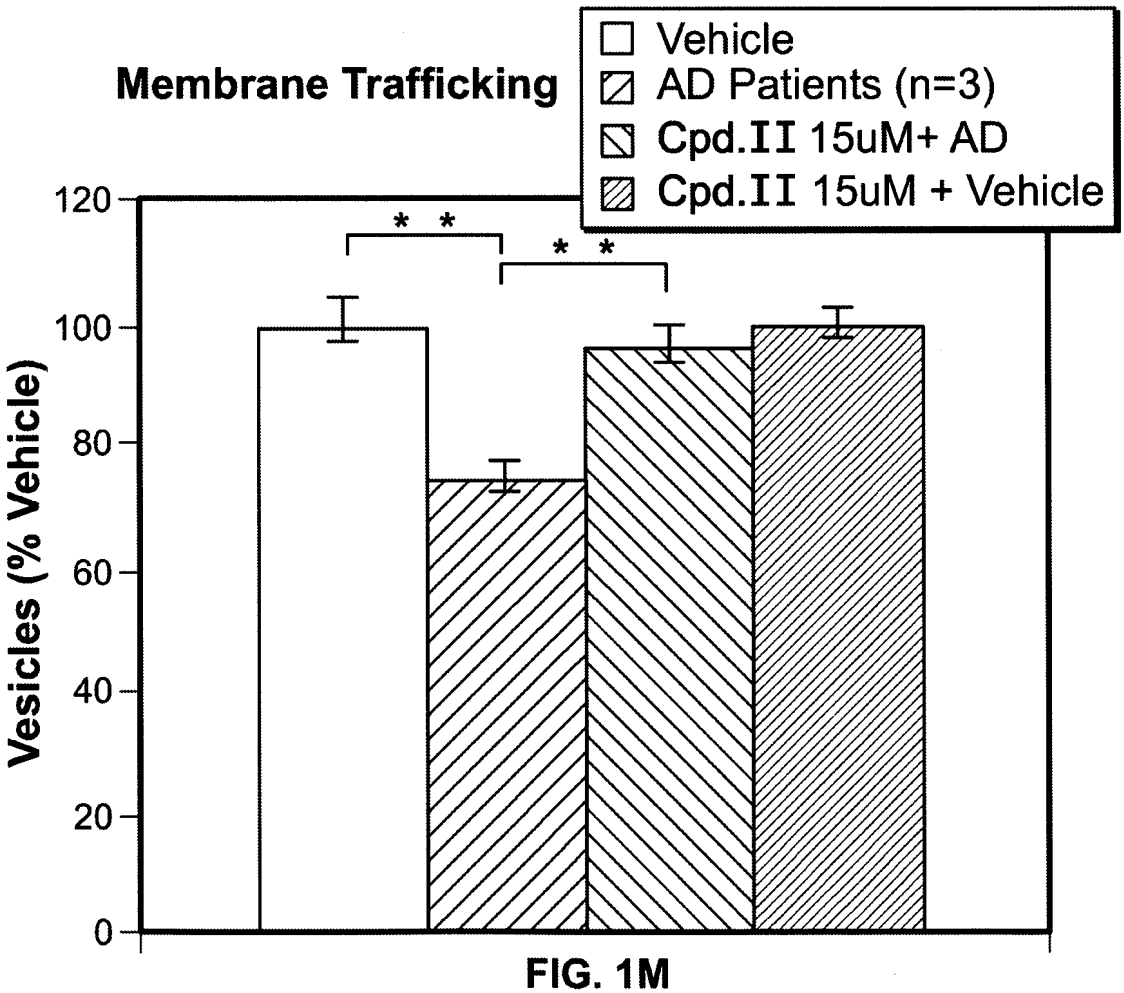

Membrane Trafficking
Vehicle
AD Patients (n=3)
Cpd.II 15uM+ AD
Cpd.II 15uM + Vehicle
* *
* *
Vesicles (% Vehicle)
120
100
80
60
40
20
0

FIG. 1M

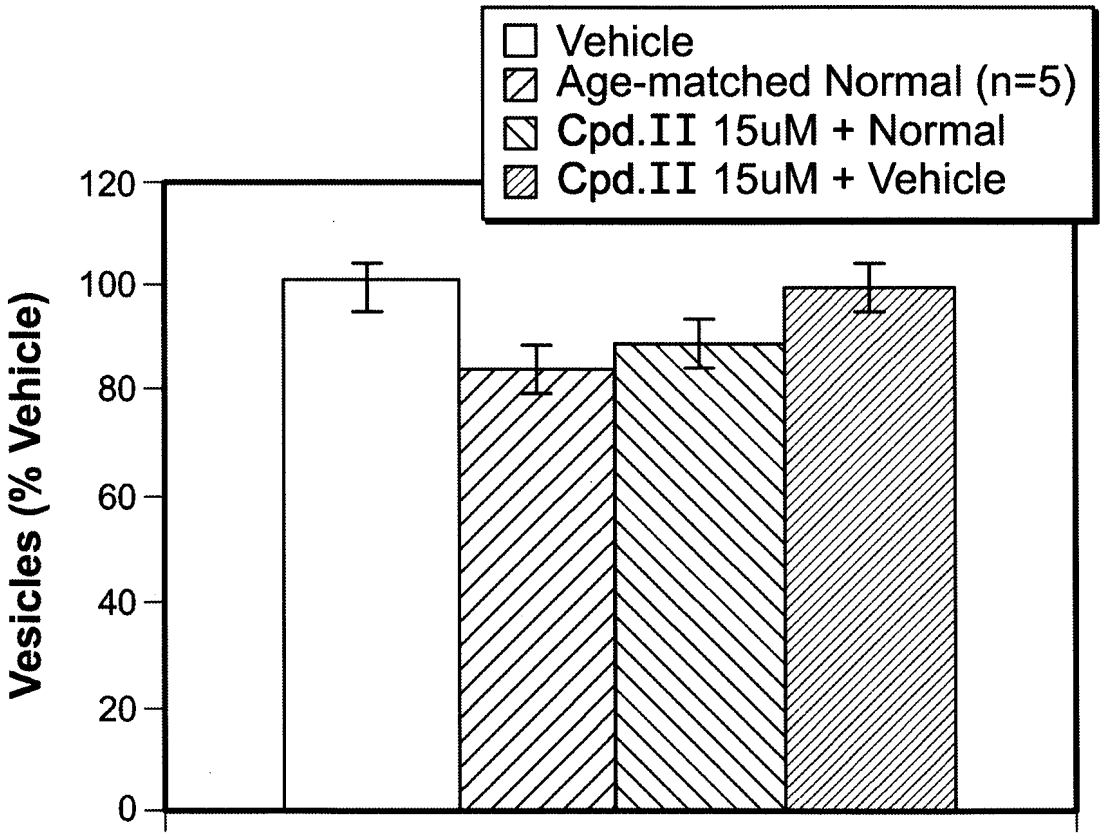

Vehicle
Age-matched Normal (n=5)
Cpd.II 15uM + Normal
Cpd.II 15uM + Vehicle
Vesicles (% Vehicle)
120
100
80
60
40
20
0

FIG. 1N

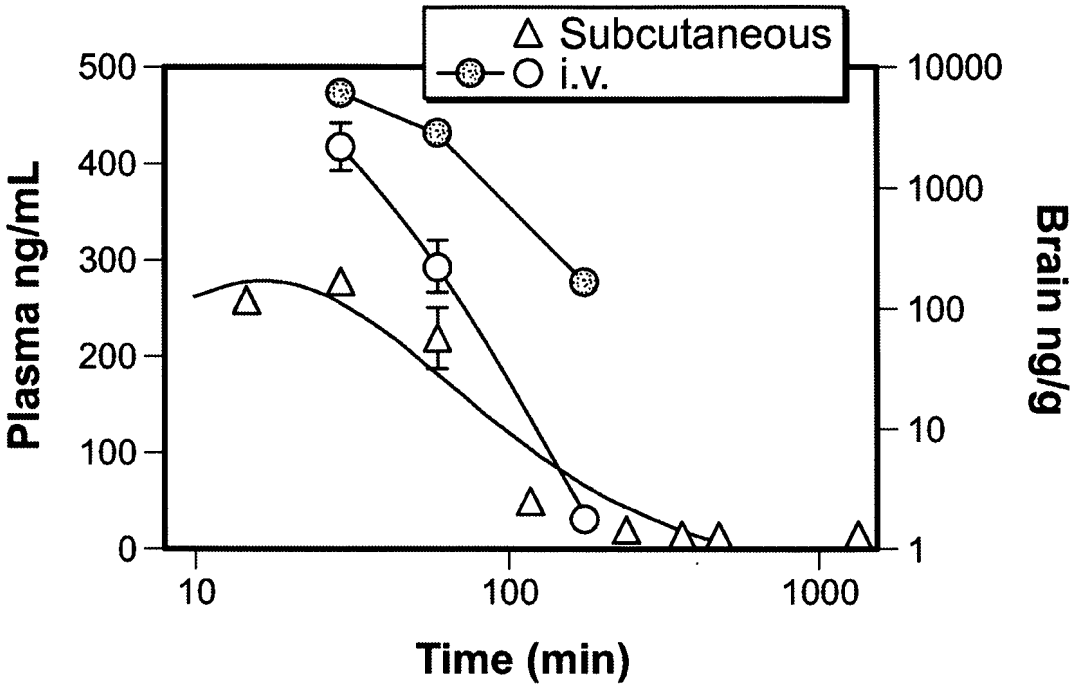

Subcutaneous
i.v.
Plasma ng/mL
Brain ng/g
500
400
300
200
100
0
10000
1000
100
10
1
10
100
1000
Time (min)

FIG. 2A

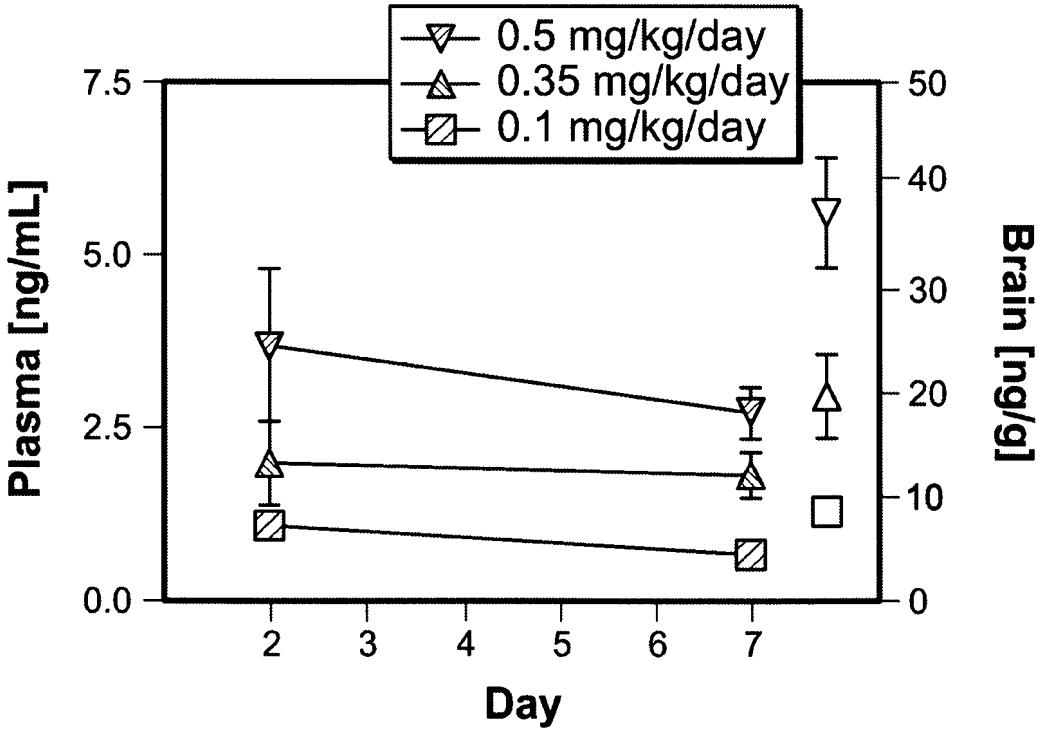

0.5 mg/kg/day
0.35 mg/kg/day
0.1 mg/kg/day
Plasma [ng/mL]
Brain [ng/g]
7.5
5.0
2.5
0.0
50
40
30
20
10
0
2
3
4
5
6
7
Day

FIG. 2B

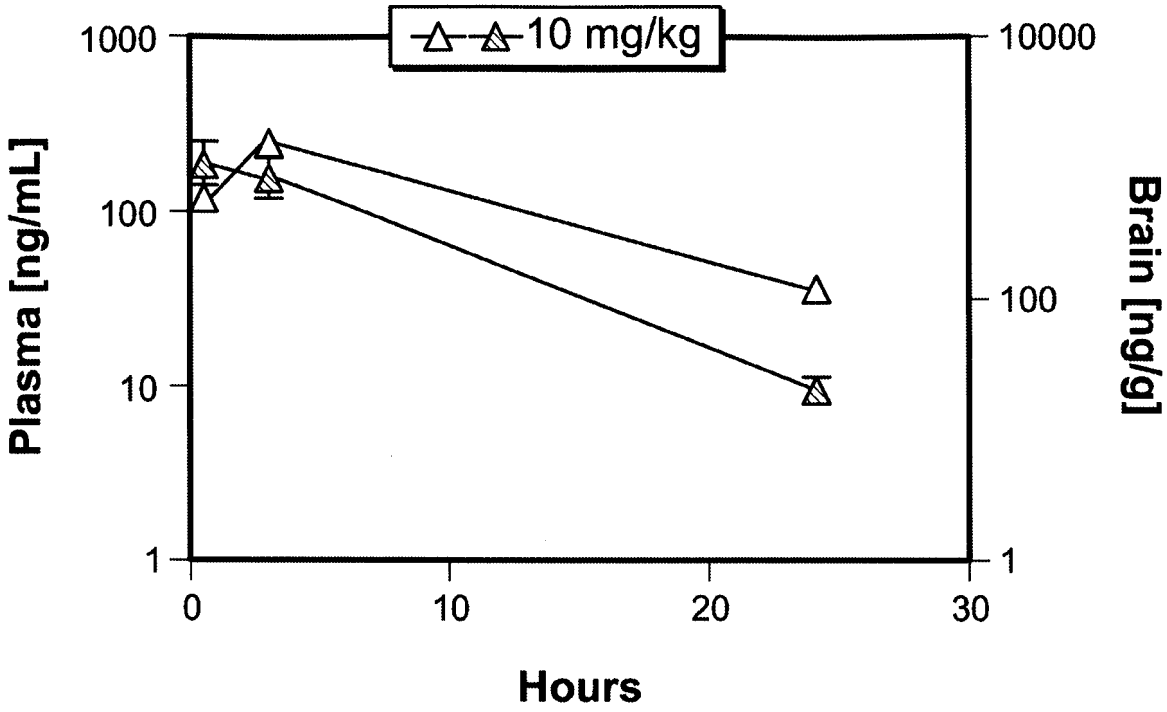

10 mg/kg
1000
100
10
1
10000
100
1
0
10
20
30
Plasma [ng/mL]
Brain [ng/g]
Hours

FIG. 2C

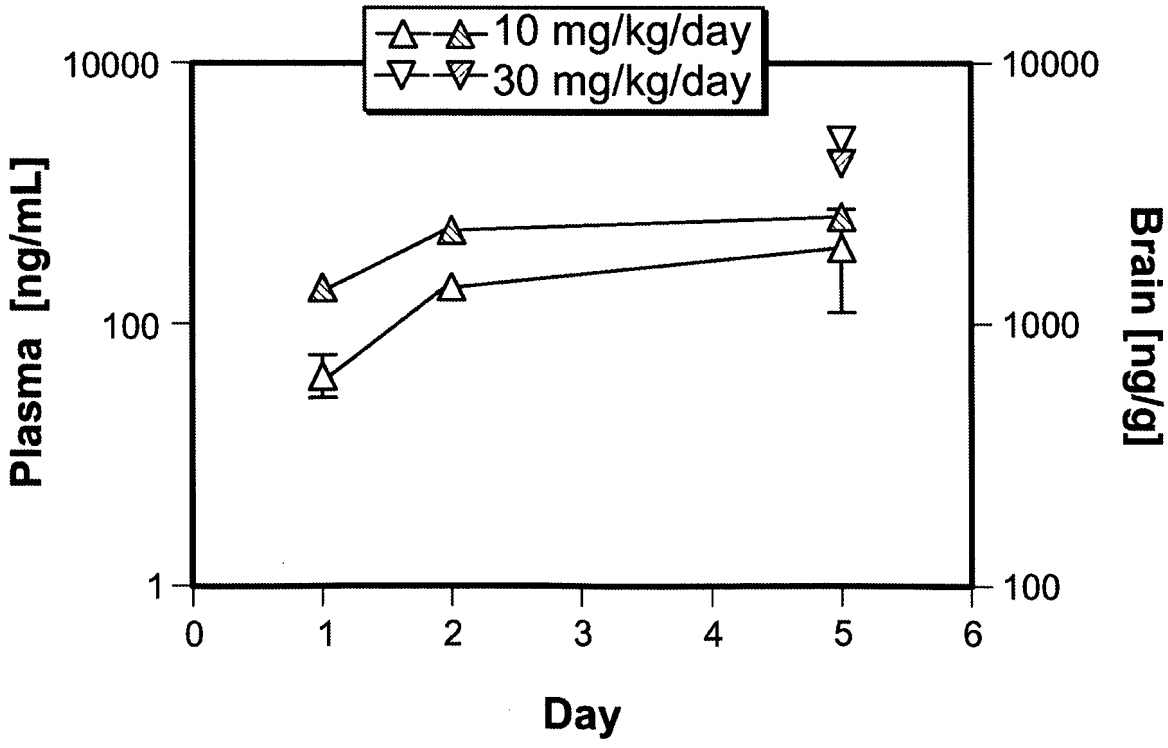

10 mg/kg/day
30 mg/kg/day
10000
100
1
10000
1000
100
0
1
2
3
4
5
6
Plasma [ng/mL]
Brain [ng/g]
Day

FIG. 2D

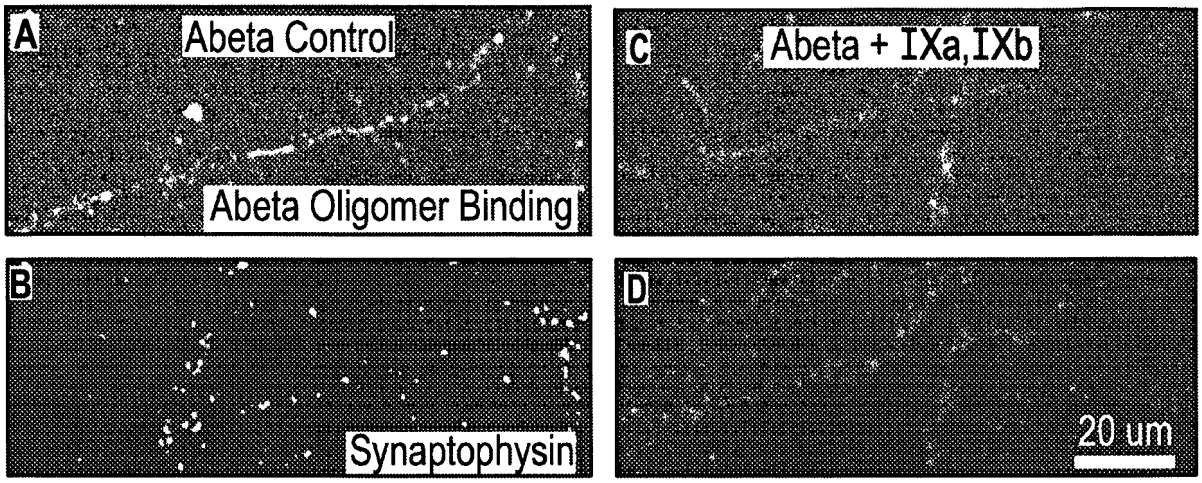

A
Abeta Control
Abeta Oligomer Binding
C
Abeta + IXa,IXb
B
Synaptophysin
D
20 um

FIG. 3A

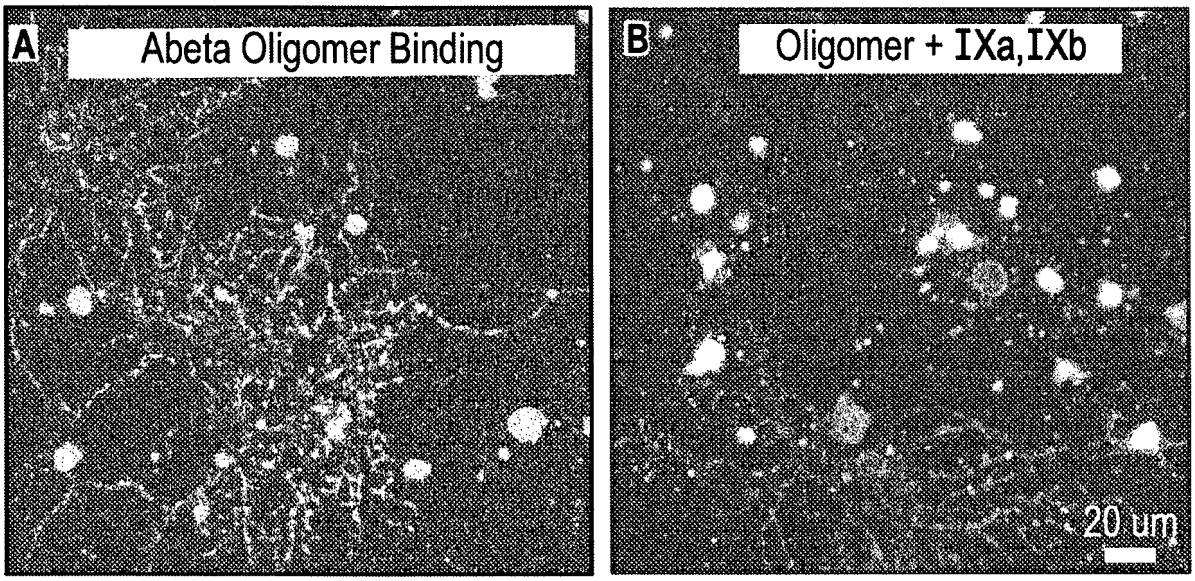

A
Abeta Oligomer Binding
B
Oligomer + IXa,IXb
20 um

FIG. 3B

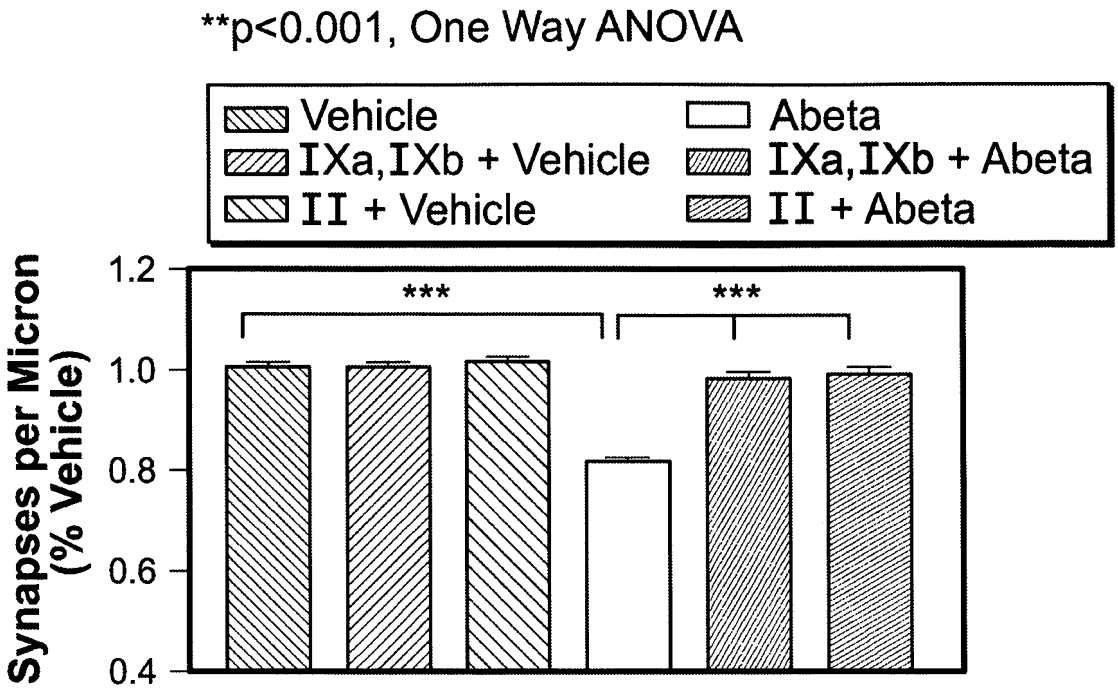

**p<0.001, One Way ANOVA
Vehicle
IXa,IXb + Vehicle
II + Vehicle
Abeta
IXa,IXb + Abeta
II + Abeta
Synapses per Micron (% Vehicle)
1.2
1.0
0.8
0.6
0.4
***
***

FIG. 3C

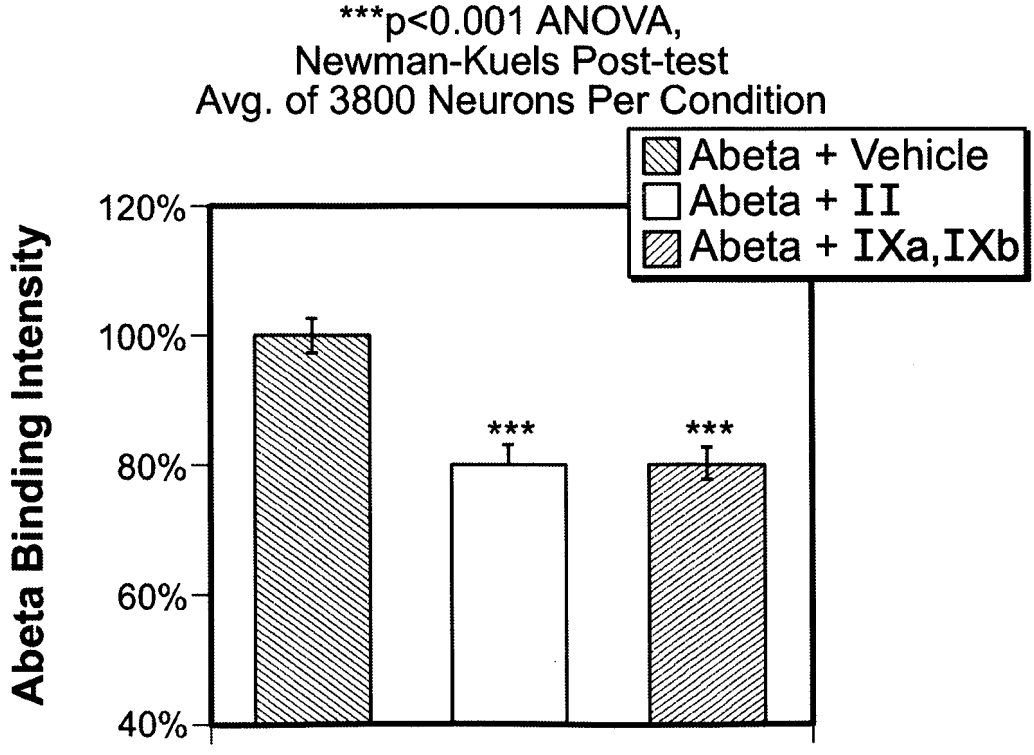

***p<0.001 ANOVA,
Newman-Kuels Post-test
Avg. of 3800 Neurons Per Condition
Abeta + Vehicle
Abeta + II
Abeta + IXa,IXb
Abeta Binding Intensity
120%
100%
80%
60%
40%
***
***

FIG. 3D

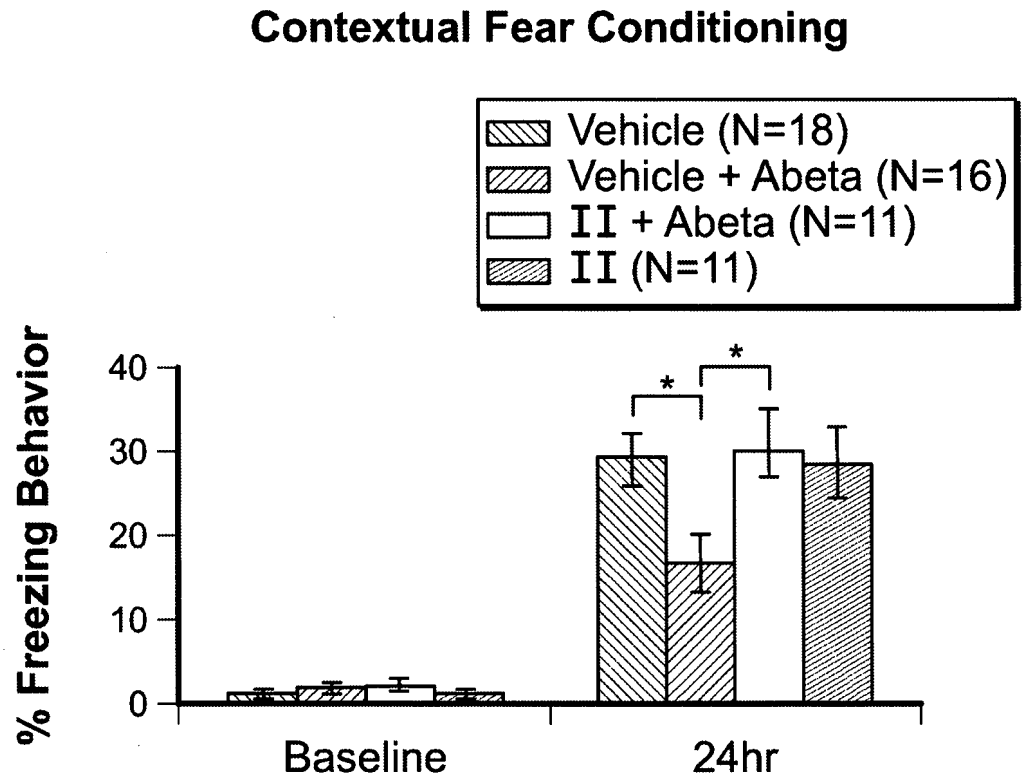

Contextual Fear Conditioning
Vehicle (N=18)
Vehicle + Abeta (N=16)
II + Abeta (N=11)
II (N=11)
% Freezing Behavior
40
30
20
10
0
*
*
Baseline
24hr

FIG. 4

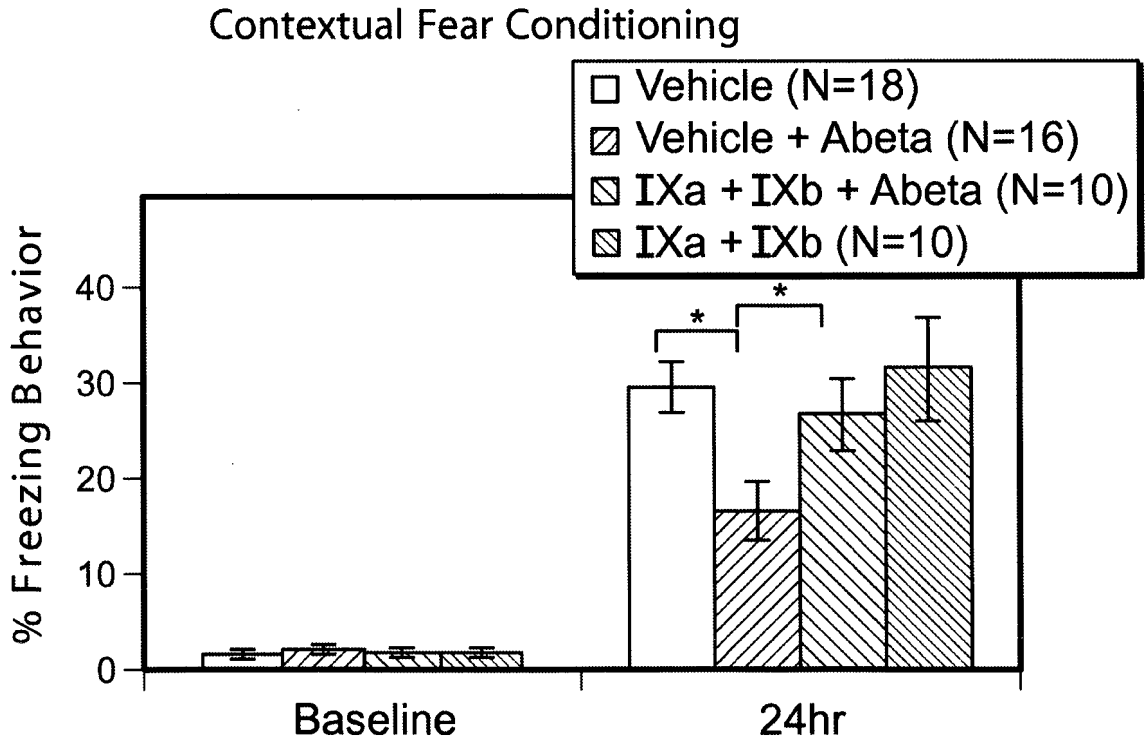

Contextual Fear Conditioning
Vehicle (N=18)
Vehicle + Abeta (N=16)
IXa + IXb + Abeta (N=10)
IXa + IXb (N=10)
% Freezing Behavior
0
10
20
30
40
*
*
Baseline
24hr

FIG. 5

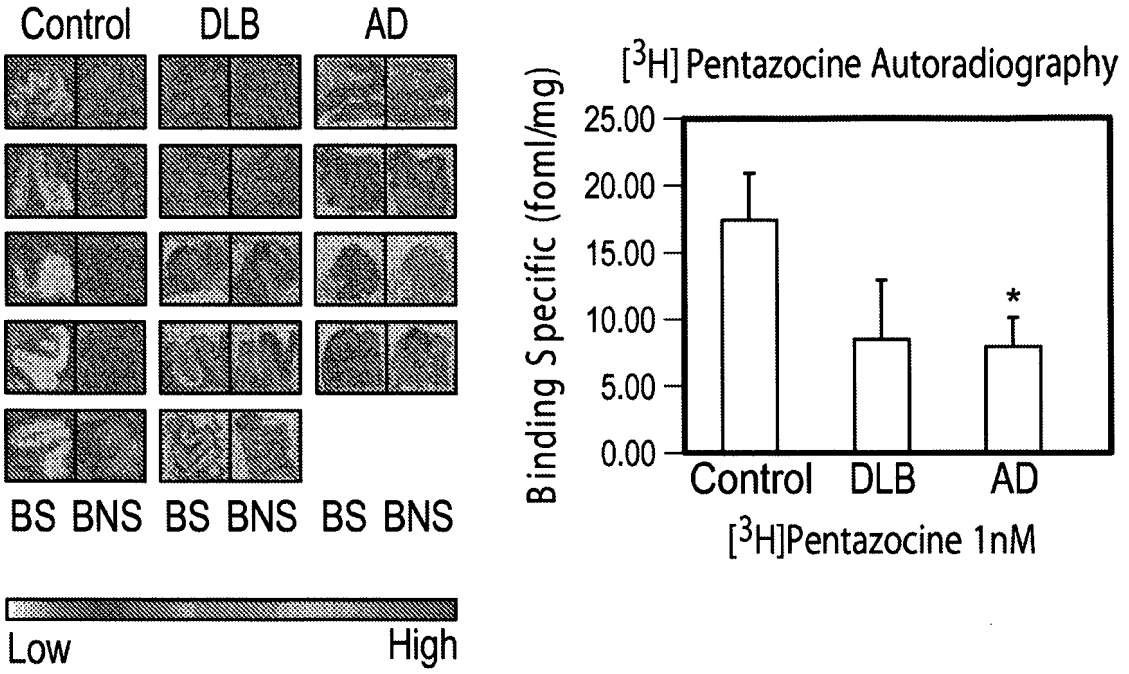

Control
DLB
AD
BS BNS BS BNS BS BNS
Low
High
[3H] Pentazocine Autoradiography
Binding Specific (fomI/mg)
25.00
20.00
15.00
10.00
5.00
0.00
*
Control DLB AD
[3H]Pentazocine 1nM

FIG. 6A

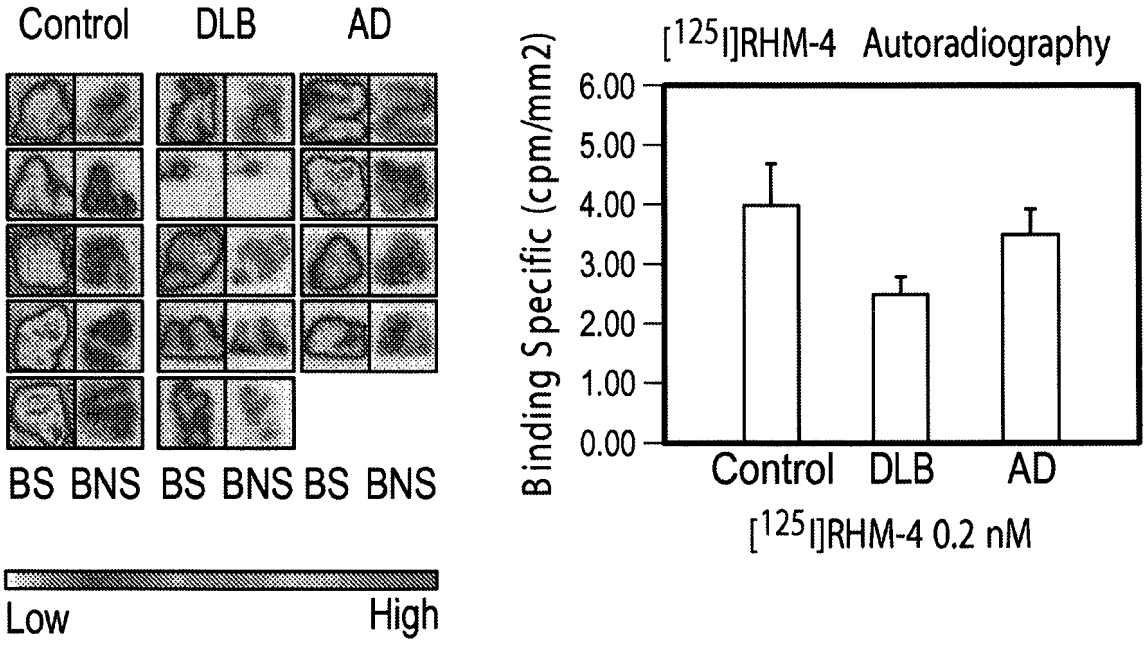
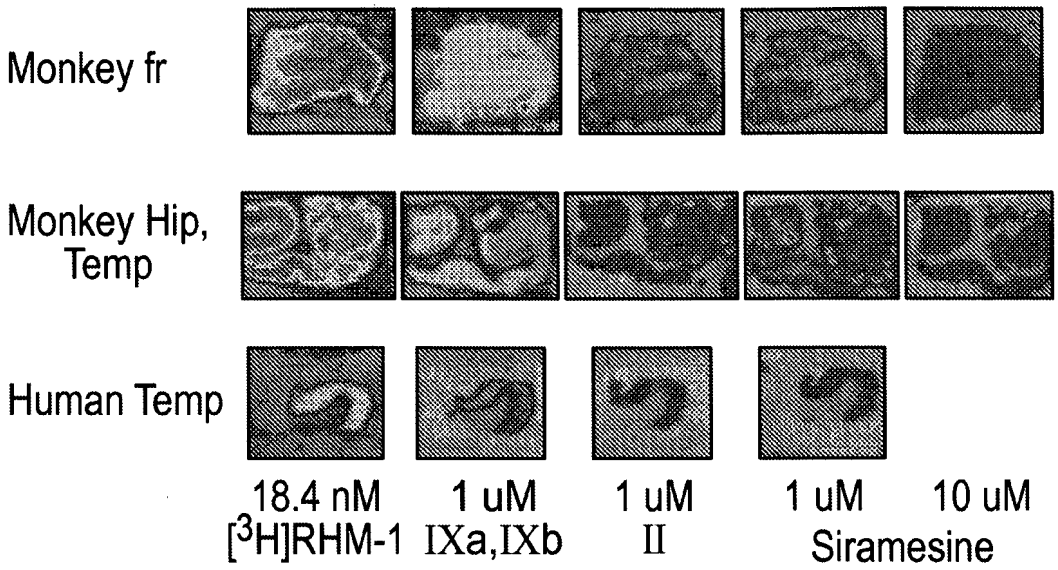

Control
DLB
AD
BS BNS BS BNS BS BNS
Low
High
[125I]RHM-4 Autoradiography
Binding Specific (cpm/mm2)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Control DLB AD
[125I]RHM-4 0.2 nM

FIG. 6B


Monkey fr
Monkey Hip, Temp
Human Temp
18.4 nM [3H]RHM-1
1 uM IXa,IXb
1 uM II
1 uM
10 uM Siramesine

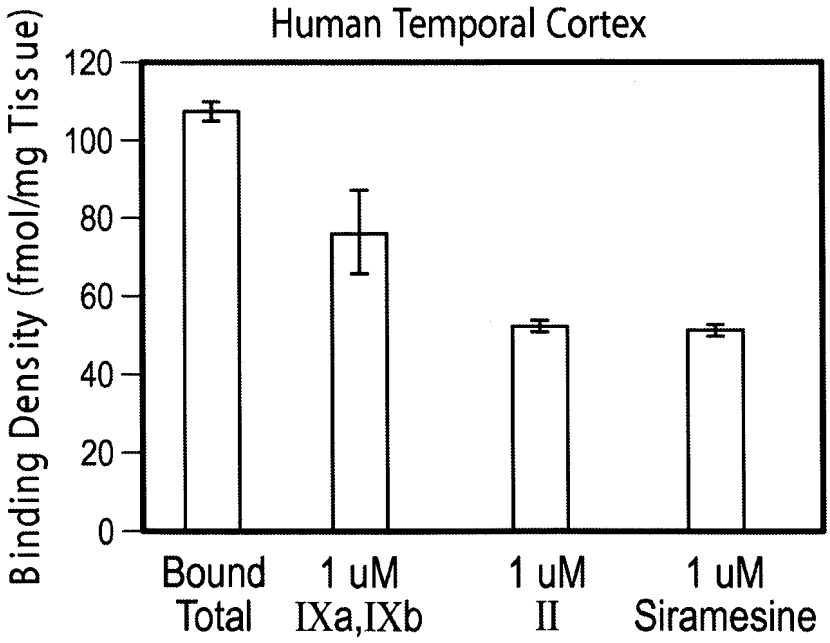

Human Temporal Cortex
Binding Density (fmol/mg Tissue)
120
100
80
60
40
20
0
Bound Total
1 uM IXa,IXb
1 uM II
1 uM Siramesine

FIG. 6C

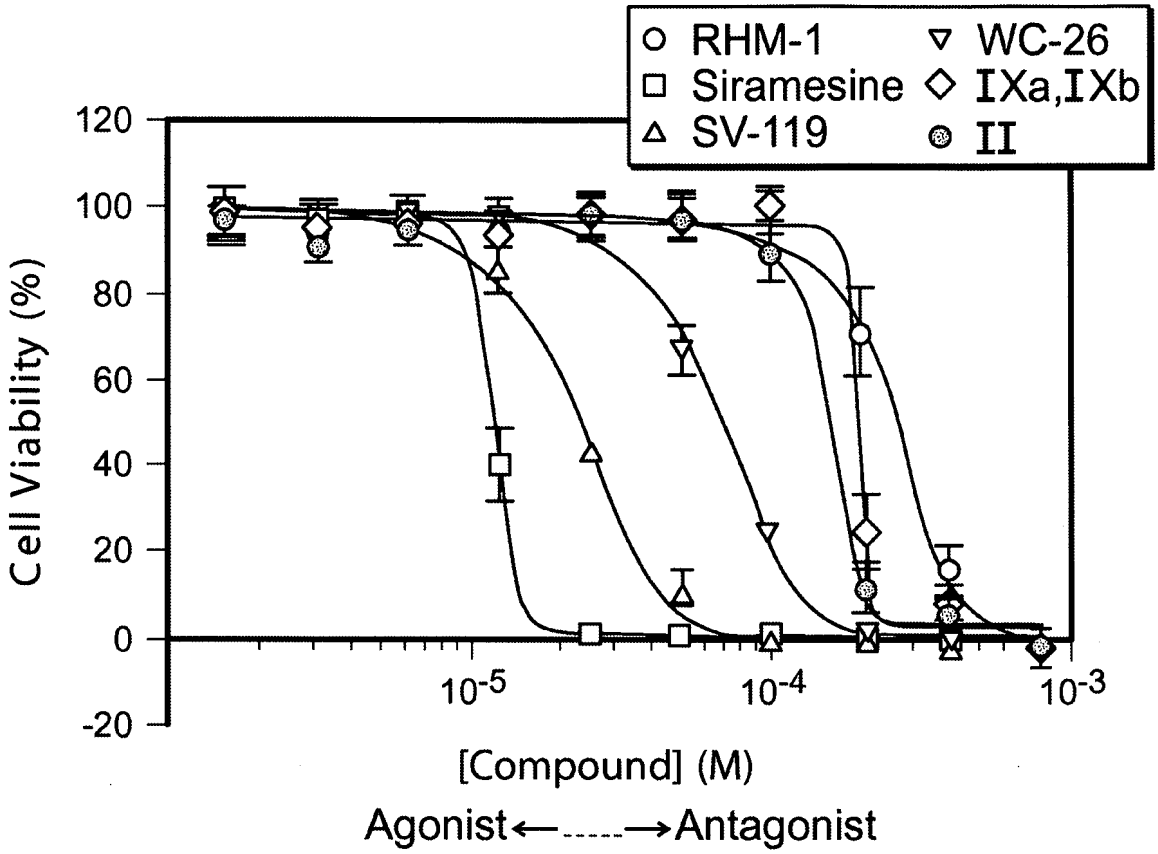

RHM-1
Siramesine
SV-119
WC-26
IXa,IXb
II
120
100
80
60
40
20
0
-20
Cell Viability (%)
10^{-5}
10^{-4}
10^{-3}
[Compound] (M)
Agonist←-----→Antagonist

FIG. 7A

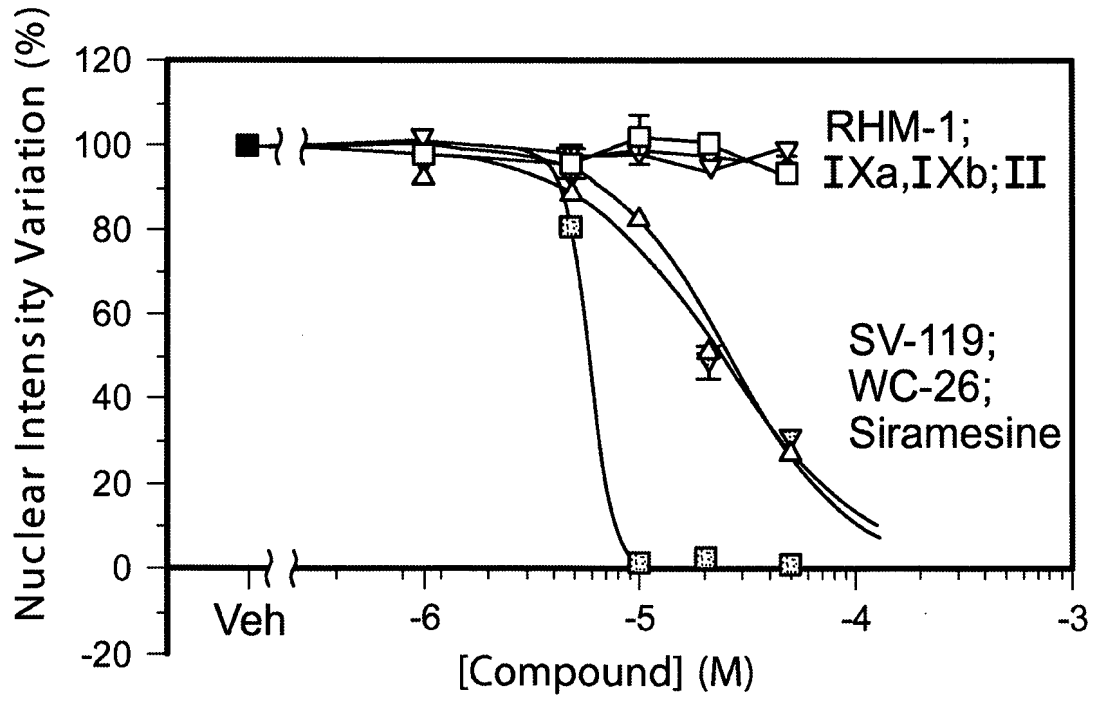

120
100
80
60
40
20
0
-20
Nuclear Intensity Variation (%)
RHM-1;
IXa,IXb;II
SV-119;
WC-26;
Siramesine
Veh
-6
-5
-4
-3
[Compound] (M)

FIG. 7B

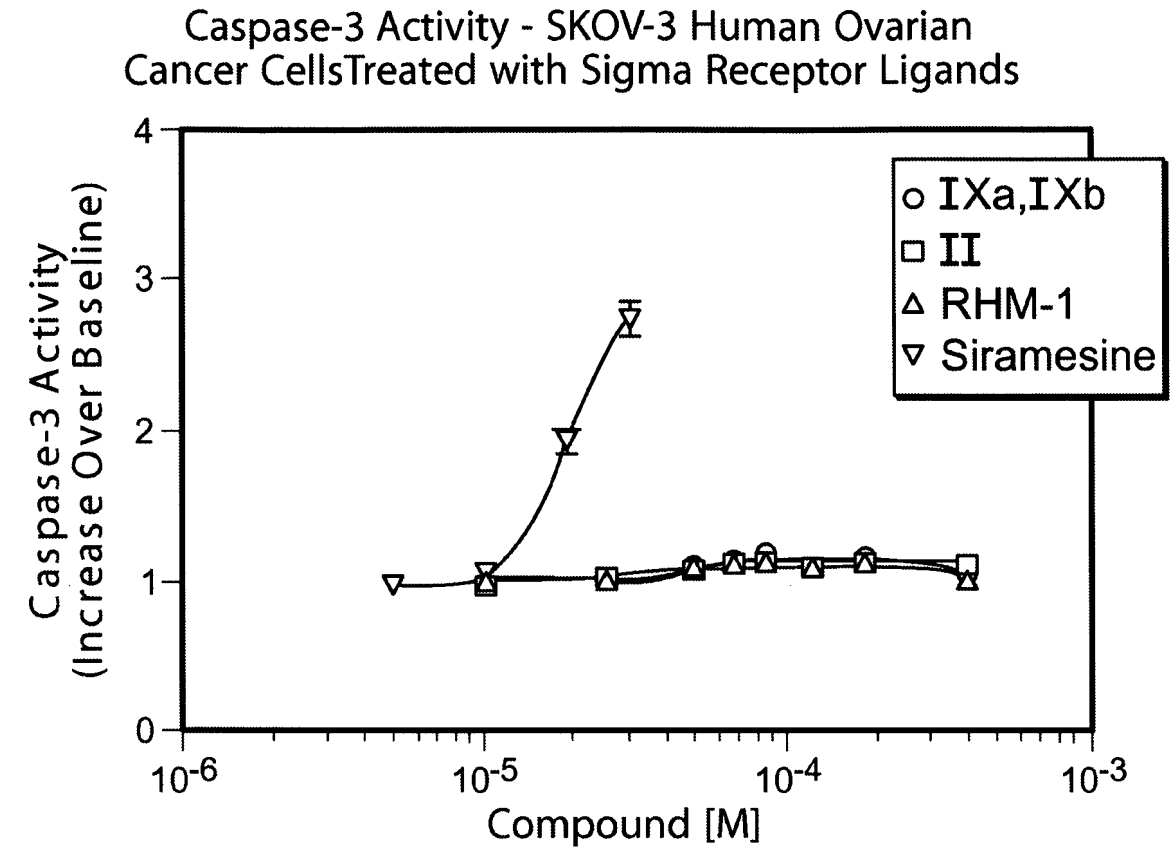


FIG. 10A

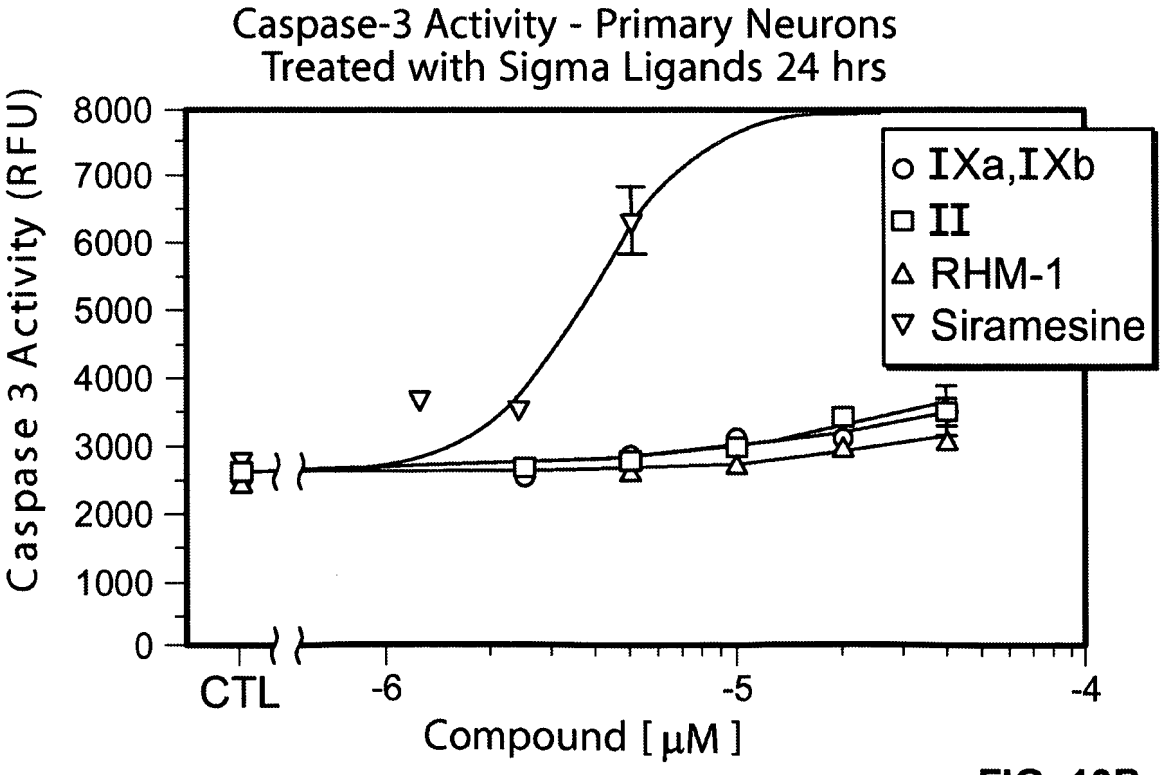


FIG. 10B

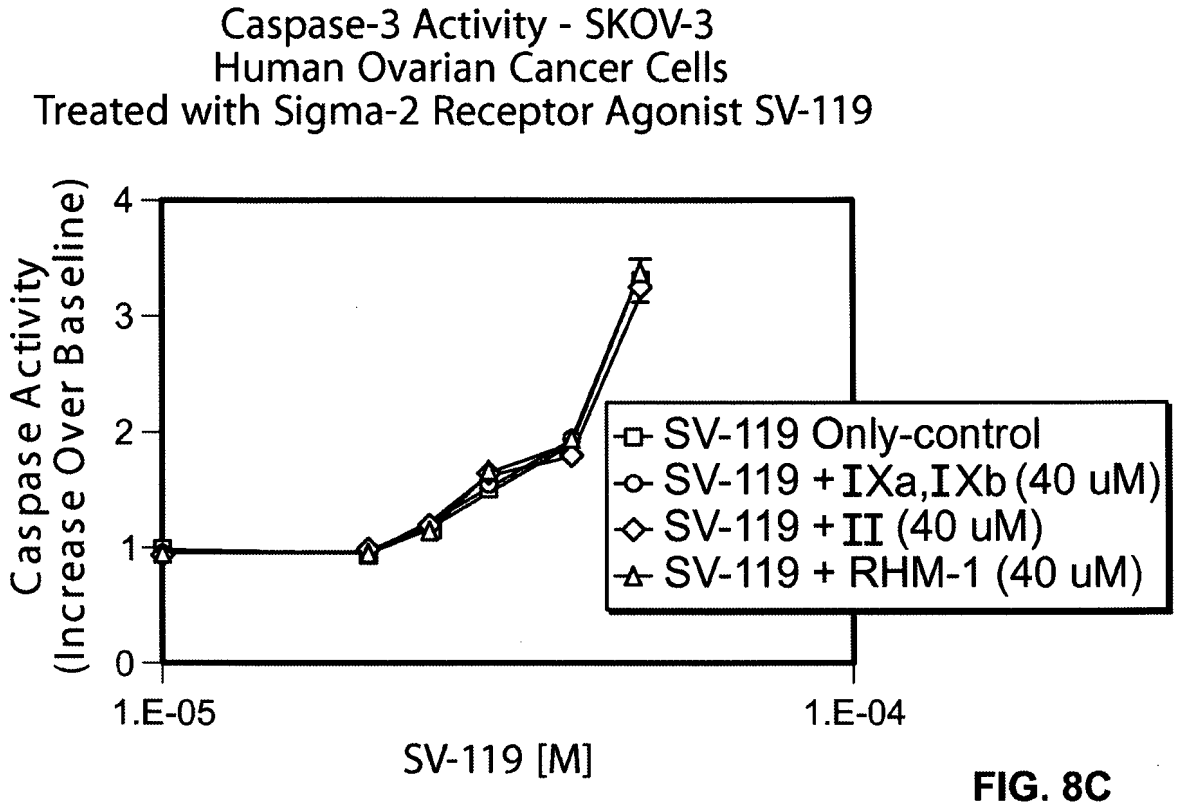

Caspase-3 Activity - SKOV-3
Human Ovarian Cancer Cells
Treated with Sigma-2 Receptor Agonist SV-119
Caspase Activity
(Increase Over Baseline)
4
3
2
1
0
1.E-05
1.E-04
SV-119 [M]
SV-119 Only-control
SV-119 + IXa,IXb (40 uM)
SV-119 + II (40 uM)
SV-119 + RHM-1 (40 uM)

FIG. 8C

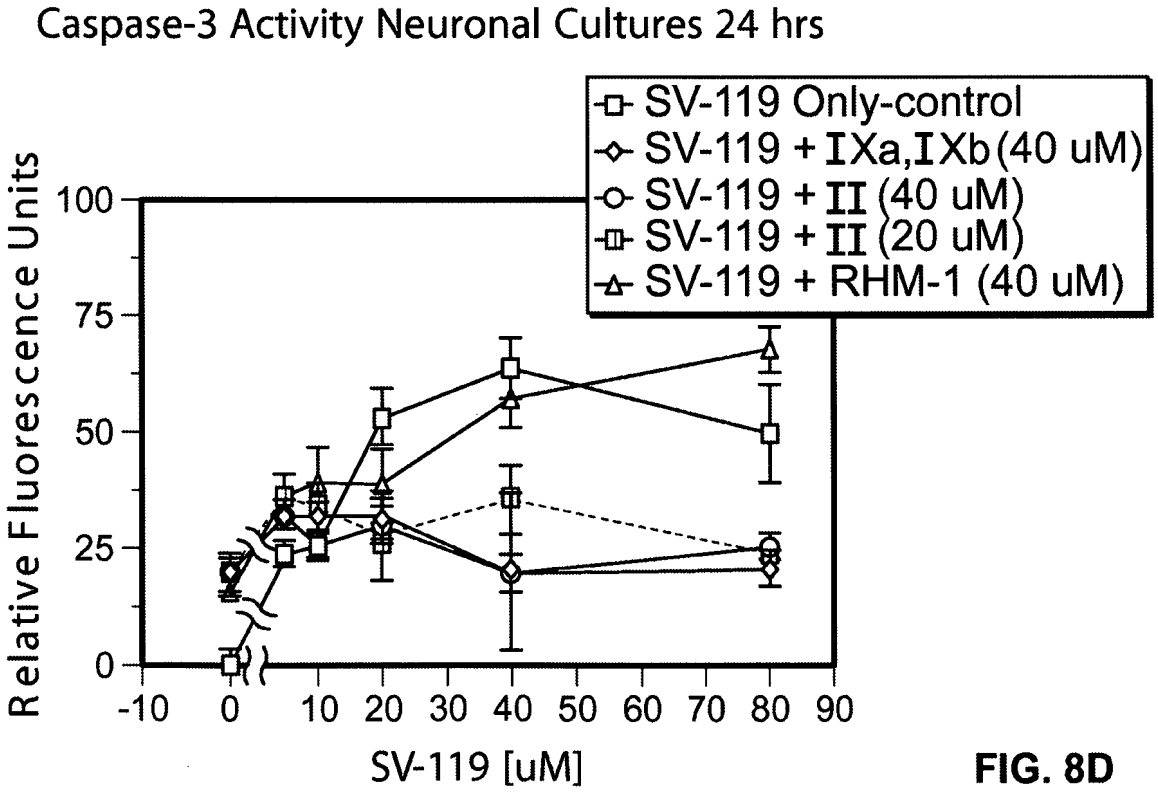

Caspase-3 Activity Neuronal Cultures 24 hrs
Relative Fluorescence Units
100
75
50
25
0
-10
0
10
20
30
40
50
60
70
80
90
SV-119 [uM]
SV-119 Only-control
SV-119 + IXa,IXb (40 uM)
SV-119 + II (40 uM)
SV-119 + II (20 uM)
SV-119 + RHM-1 (40 uM)

FIG. 8D

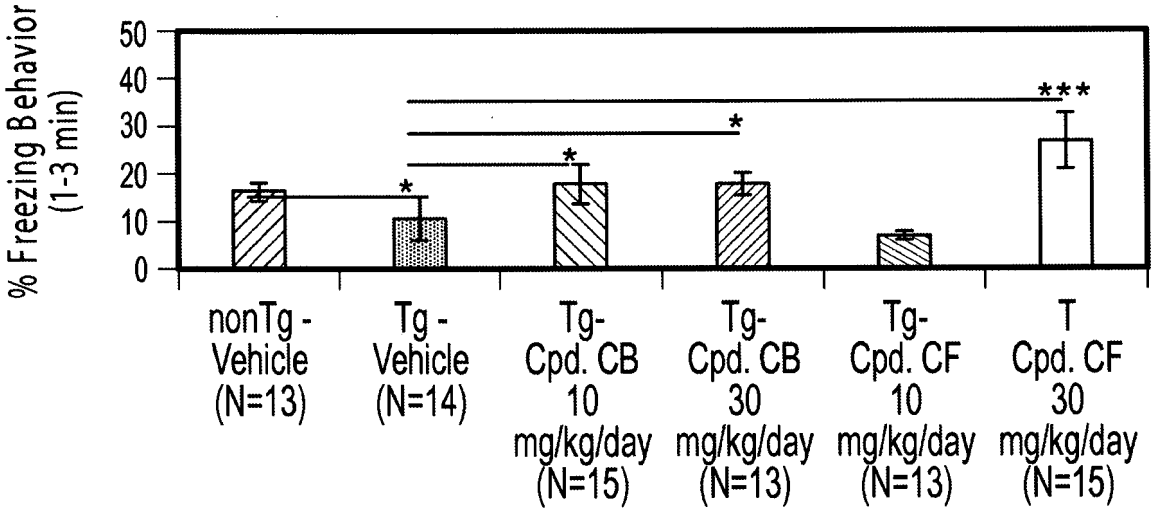

% Freezing Behavior (1-3 min)
50
40
30
20
10
0
*
*
*
***
nonTg - Vehicle (N=13)
Tg - Vehicle (N=14)
Tg- Cpd. CB 10 mg/kg/day (N=15)
Tg- Cpd. CB 30 mg/kg/day (N=13)
Tg- Cpd. CF 10 mg/kg/day (N=13)
T Cpd. CF 30 mg/kg/day (N=15)

FIG. 9A

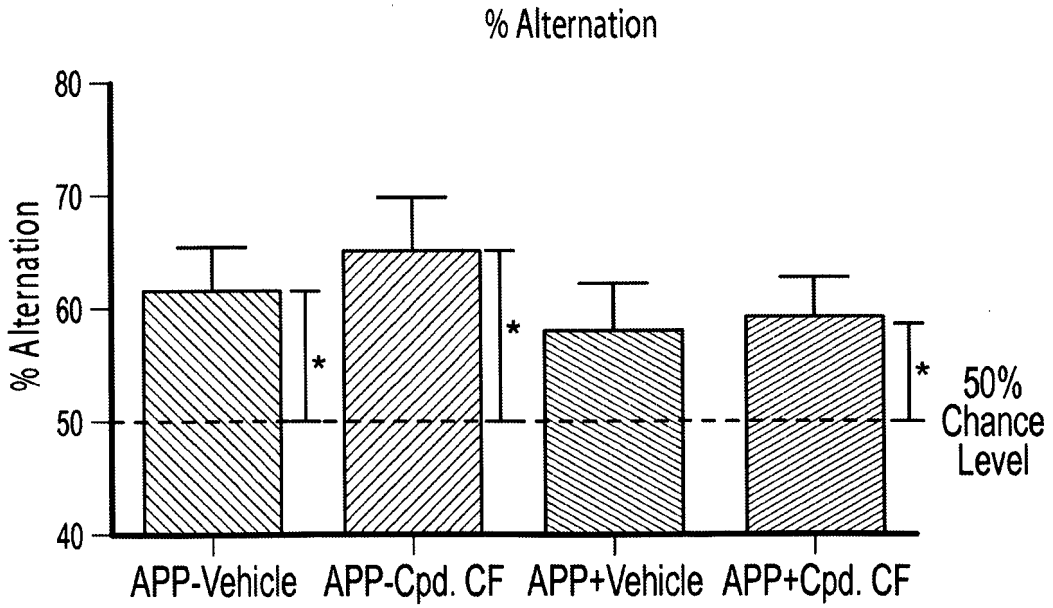

% Alternation
% Alternation
80
70
60
50
40
*
*
*
50% Chance Level
APP-Vehicle
APP-Cpd. CF
APP+Vehicle
APP+Cpd. CF

FIG. 9B

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(43)申请公布日 2014.09.17

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(22)申请日 2012.08.27

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PCT/US2012/052572 2012.08.27

(87)PCT国际申请的公布数据
WO2013/029057 EN 2013.02.28

(71)申请人 考格尼申治疗股份有限公司
地址 美国宾夕法尼亚州

(72)发明人 S.M. 卡塔拉诺 G. 里什顿
N.J. 伊佐

(74)专利代理机构 北京尚诚知识产权代理有限公司 11322
代理人 龙淳

(51)Int.Cl.
A61K 31/135(2006.01)

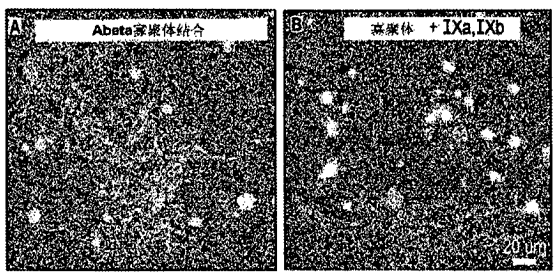
权利要求书31页 说明书116页 附图24页
按照条约第19条修改的权利要求书35页

(54)发明名称

用于治疗神经退行性疾病的组合物和方法

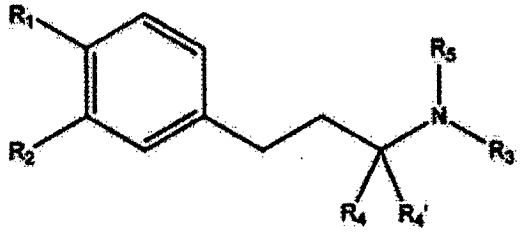
(57)摘要

本发明涉及新的与 σ-2 受体结合的二芳基氨基化合物，涉及包括这样的化合物的药物组合物，并涉及用其抑制或恢复神经元细胞中的突触丧失、调节神经元细胞中的膜运输变化以及治疗认知衰退和神经退行性疾病和病症的方法。



CN 104053435 A

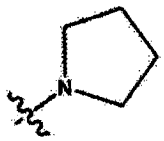
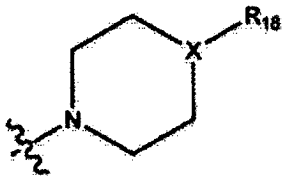
1. 一种式 I 的化合物及其药学上可接受的盐：



I

其中

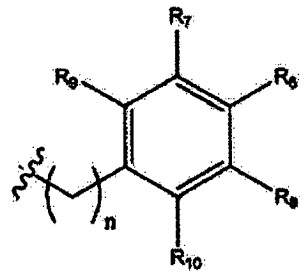
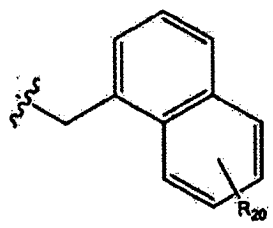
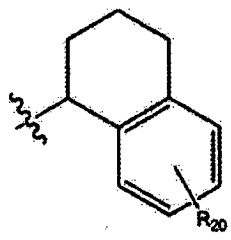
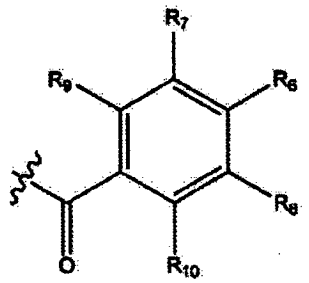
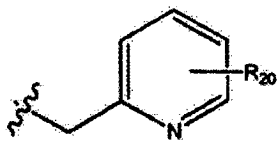
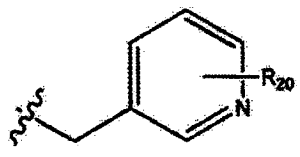
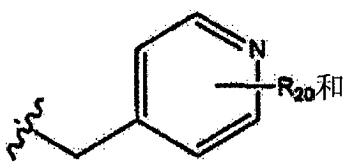
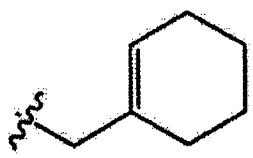
R_1 和 R_2 独立选自 H、OH、卤素、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、$(R_{16})(R_{17})N-C_{1-4}$ 亚烷基 -O-，或 R_1 和 R_2 连接在一起形成 $-O-C_{1-2}$ 亚甲基 -O- 基，其中

R_{16} 和 R_{17} 独立地为 C_{1-4} 烷基或苄基，或 R_{16} 和 R_{17} 与氮一起形成选自  和  的环，其中

X 是 N 或 O 且 R_{18} 是 H 或未被取代的苯基；且

其中 R_1 和 R_2 的至少一个不是 H；

R_3 选自

 ，  ，  ，  ，  ，  ，  和 

其中

R_6、R_7、R_8、R_9 和 R_{10} 独立选自 H、卤素、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基和 $S(O)_2$-C_{1-6} 烷基；

R_{20} 是 H；且

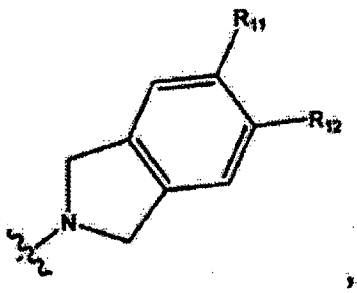
n 是 1-4

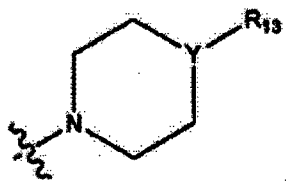
R_4 是 C_{1-6} 烷基；

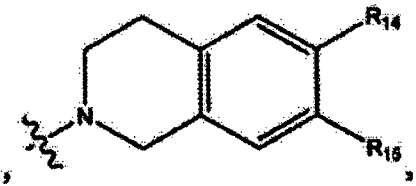
$R_{4'}$ 是 H 或 C_{1-6} 烷基；且

R_5 是 H、C_{1-6} 烷基和 C(O)O(C_{1-4} 烷基)、C(O)(C_{1-4} 烷基) 或 C(O)(C_{1-4} 卤代烷基)；或

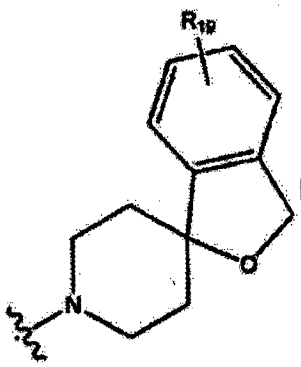
R_3 和 R_5 与氮一起形成选自



，


，


，和



的环，其中

R_{11} 和 R_{12} 独立选自 H、卤素和 C_{1-6} 卤代烷基，且

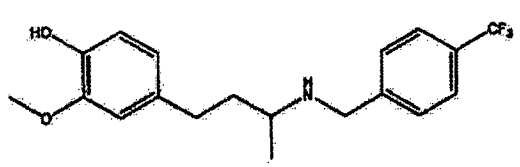
Y 是 CH 或 N；

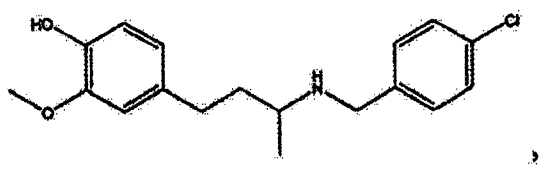
R_{13} 是 H、C_{1-6} 烷基、C_{3-6} 环烷基、未被取代的苯基或被 C_{1-6} 卤代烷基取代的苯基、或未被取代的苄基；

R_{14} 和 R_{15} 独立选自 H 和卤素；

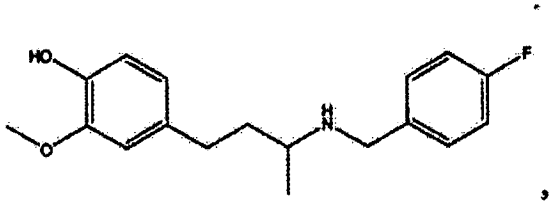
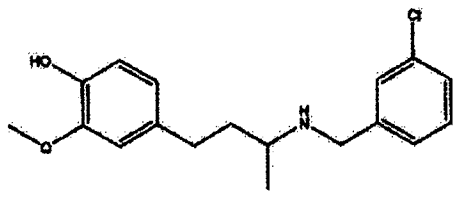
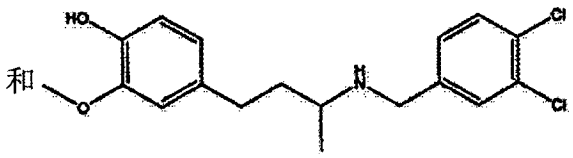
R_{19} 是 H，

条件是排除以下化合物的外消旋混合物



，


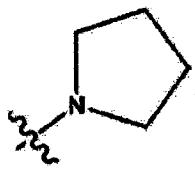
，

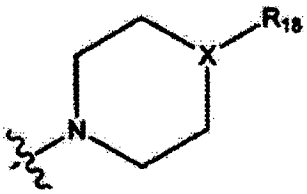




和

2. 如权利要求 1 所述的化合物及其药学上可接受的盐，其中

R_1 和 R_2 独立选自 H、OH、卤素、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、$(R_{16})(R_{17})N-C_{1-4}$ 亚烷基 -O-，或 R_1 和 R_2 连接在一起形成 $-O-C_{1-2}$ 亚甲基 -O- 基，其中

R_{16} 和 R_{17} 独立地为 C_{1-4} 烷基或苄基，或 R_{16} 和 R_{17} 与氮一起形成选自  和

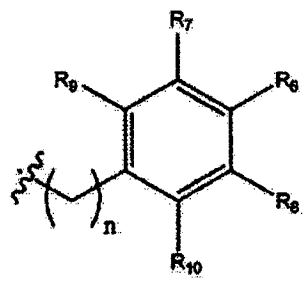
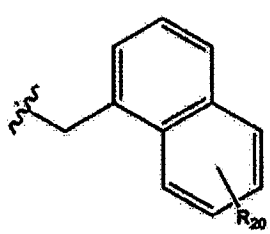
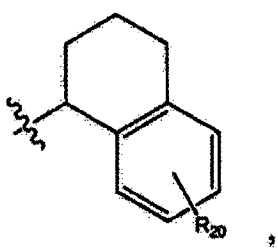
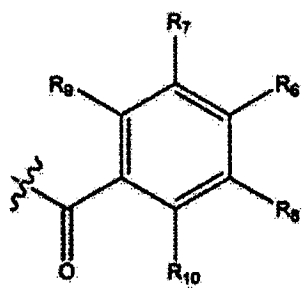
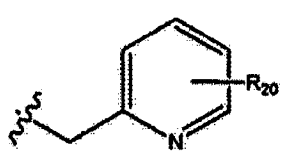
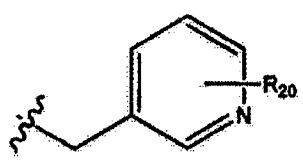


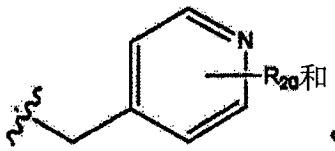
的环，其中

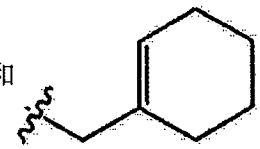
X 是 N 或 O 且 R_{18} 不存在或者是 H 或未被取代的苯基；且

其中 R_1 和 R_2 的至少一个不是 H；

R_3 选自



和


其中

R_6、R_7、R_8、R_9 和 R_{10} 独立选自 H、卤素、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基和 $S(O)_2$-C_{1-6} 烷基；

R_{20} 是 H；且

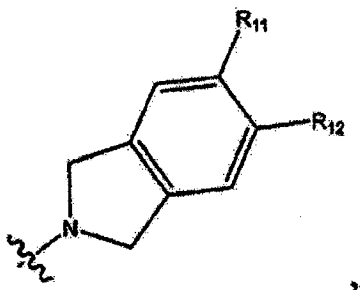
n 是 1-4

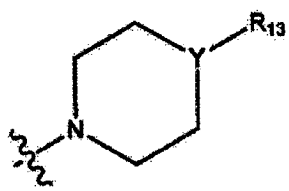
R_4 是 C_{1-6} 烷基；

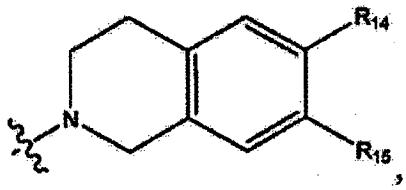
$R_{4'}$ 是 H 或 C_{1-6} 烷基；且

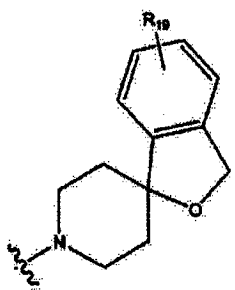
R_5 是 H、C_{1-6} 烷基和 C(O)O(C_{1-4} 烷基)、C(O)(C_{1-4} 烷基) 或 C(O)(C_{1-4} 卤代烷基)；或

R_3 和 R_5 与氮一起形成选自



，


，


，和


的环，其中

R_{11} 和 R_{12} 独立选自 H、卤素和 C_{1-6} 卤代烷基，且

Y 是 CH 或 N；

R_{13} 是 H、C_{1-6} 烷基、C_{3-6} 环烷基、未被取代的苯基或被 C_{1-6} 卤代烷基取代的苯基、或未被取代的苄基；

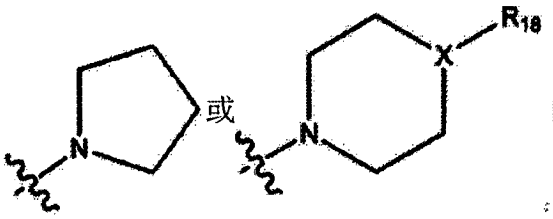
R_{14} 和 R_{15} 独立选自 H 和卤素；且

R_{19} 是 H。

3. 如权利要求 1 所述的化合物及其药学上可接受的盐，其中

R_1 选自 OH、OMe、F、Cl、CF_3、(R_{16})(R_{17})N- 亚乙基 -O-，其中

R_{16} 和 R_{17} 各自是甲基、异丙基、正丁基或苄基，或 R_{16} 和 R_{17} 与氮一起形成选自


或

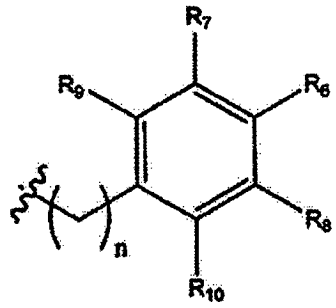
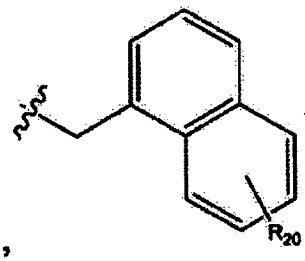
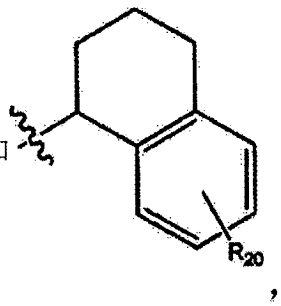
的环，其中

X 是 N 或 O 且 R_{18} 不存在或是未被取代的苯基；且

R_2 是 H、Cl、F、CF_3、OMe、OCF_3 或

R_1 和 R_2 连接在一起形成 -O-C_{1-2} 亚甲基 -O- 基

R_3 选自


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和

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其中

R_6 是 H、F、Cl、Me、异丙基、叔丁基、OMe、CF_3、或 $S(O)_2Me$，

R_7 和 R_8 独立为 H、OMe、F、Cl 或 CF_3；

R_9 和 R_{10} 独立选自 H、OMe、F 和 Cl，

R_{20} 是 H；且

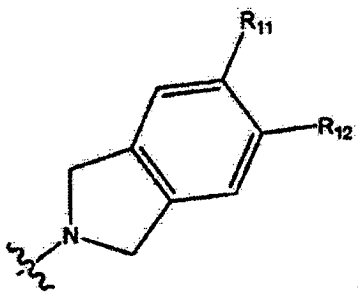
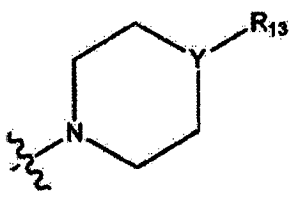
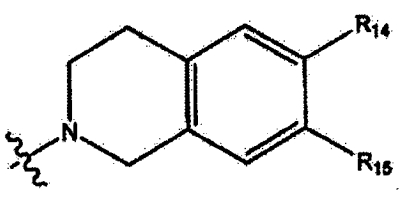
n 是 1

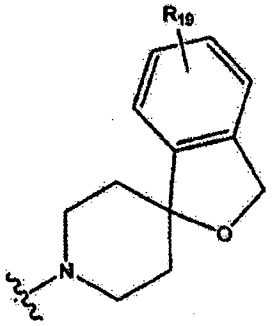
R_4 是 Me；

$R_{4'}$ 是 H 或 Me；且

R_5 是 H；或

R_3 和 R_5 与氮一起形成选自


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和



的环，其中

R_{11} 和 R_{12} 独立选自 H、Cl 和 CF_3，且

Y 是 CH 或 N；

R_{13} 是 H、Me、环已基、未被取代的苯基或被 CF_3 取代的苯基、或未被取代的苄基；

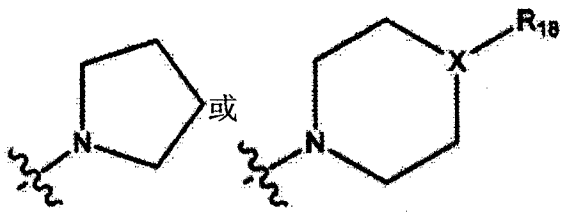
R_{14} 和 R_{15} 独立选自 H 和 Cl；且

R_{19} 是 H。

4. 如权利要求 1 所述的化合物及其药学上可接受的盐，其中

R_1 选自 OH、OMe、F、Cl、CF_3、$(R_{16})(R_{17})N-$ 亚乙基 -O-，其中

R_{16} 和 R_{17} 各自是甲基、异丙基、正丁基或苄基，或 R_{16} 和 R_{17} 与氮一起形成选自



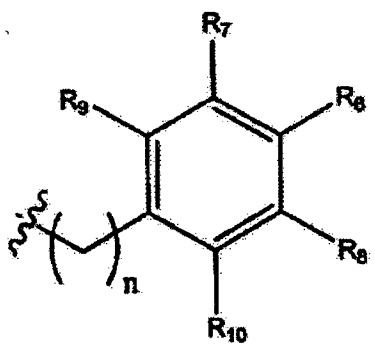
的环，其中

X 是 N 或 O 且 R_{18} 不存在或是未被取代的苯基；且

R_2 是 H、Cl、F、CF_3、OMe、OCF_3 或

R_1 和 R_2 连接在一起形成 $-O-C_{1-2}$ 亚甲基 -O- 基

R_3 选自



其中

R_6 是 H、F、Cl、Me、异丙基、叔丁基、OMe、CF_3、或 $S(O)_2Me$，

R_7 和 R_8 独立地为 H、OMe、F、Cl、或 CF_3；

R_9 和 R_{10} 独立选自 H、OMe、F、和 Cl，且

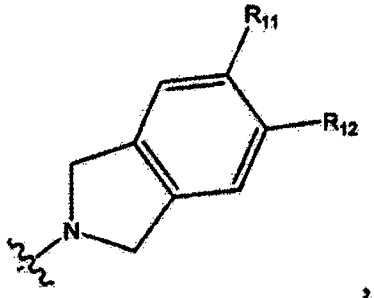
n 是 1

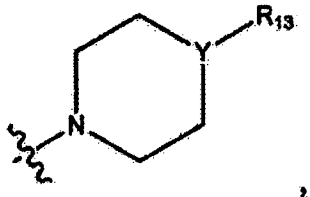
R_4 是 Me；

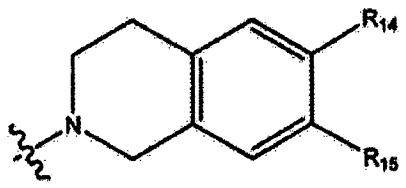
$R_{4'}$ 是 H；且

R_5 是 H ;或

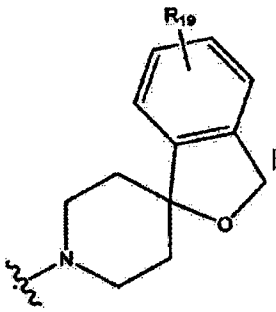
R_3 和 R_5 与氮一起形成选自



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和



的环，其中

R_{11} 和 R_{12} 独立选自 H、Cl、和 CF_3，且

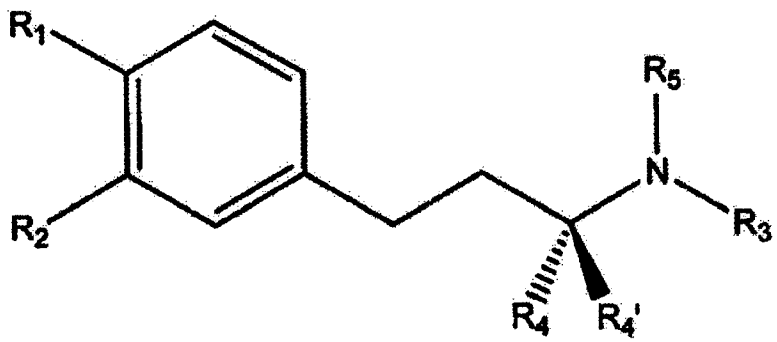
Y 是 CH 或 N ;

R_{13} 是 H、Me、环己基、未被取代的苯基或被 CF_3 取代的苯基、或未被取代的苄基 ;

R_{14} 和 R_{15} 独立选自 H 和 Cl ;且

R_{19} 是 H。

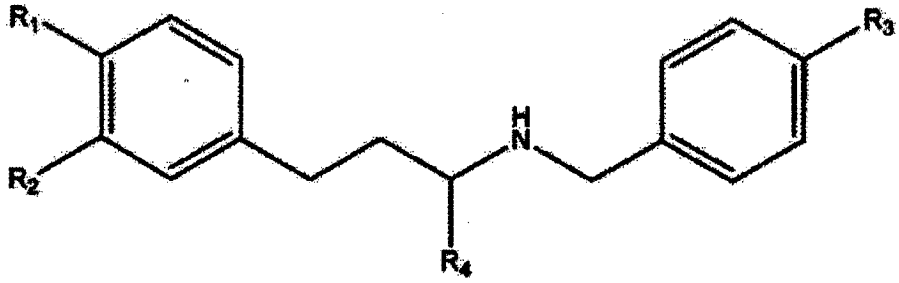
5. 如权利要求 1 所述的化合物及其药学上可接受的盐，所述化合物为式 Ia 的化合物



Ia

其中 $R_{4'}$ 是 H 且剩余基团如权利要求 1 中所限定。

6. 一种式 IIa 的化合物或其药学上可接受的盐 :



IIa

其中

R_1 = 卤素、C_{1-6} 卤代烷基或 OH；

R_2 = H、卤素或 C_{1-6} 卤代烷基，或 R_1 和 R_2 连接在一起形成 -O- 亚甲基 -O- 基；

R_3 = C_{1-6} 卤代烷基；且

R_4 = C_{1-6} 烷基。

7. 如权利要求 6 所述的化合物及其药学上可接受的盐，其中

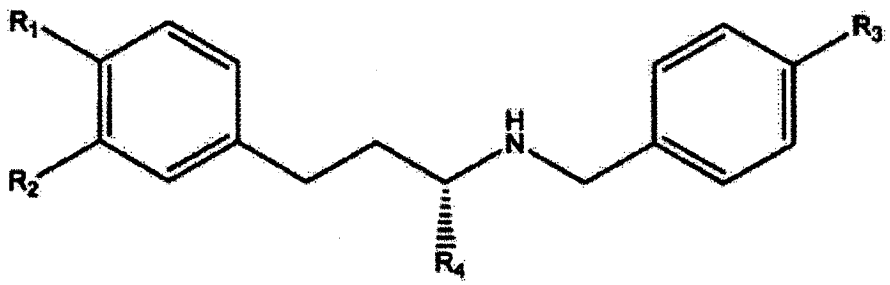
R_1 = Cl、F、CF_3、或 OH；

R_2 = H、Cl、F、CF_3，或 R_1 和 R_2 连接在一起形成 -O- 亚乙基 -O- 基；

R_3 = CF_3；且

R_4 = 甲基。

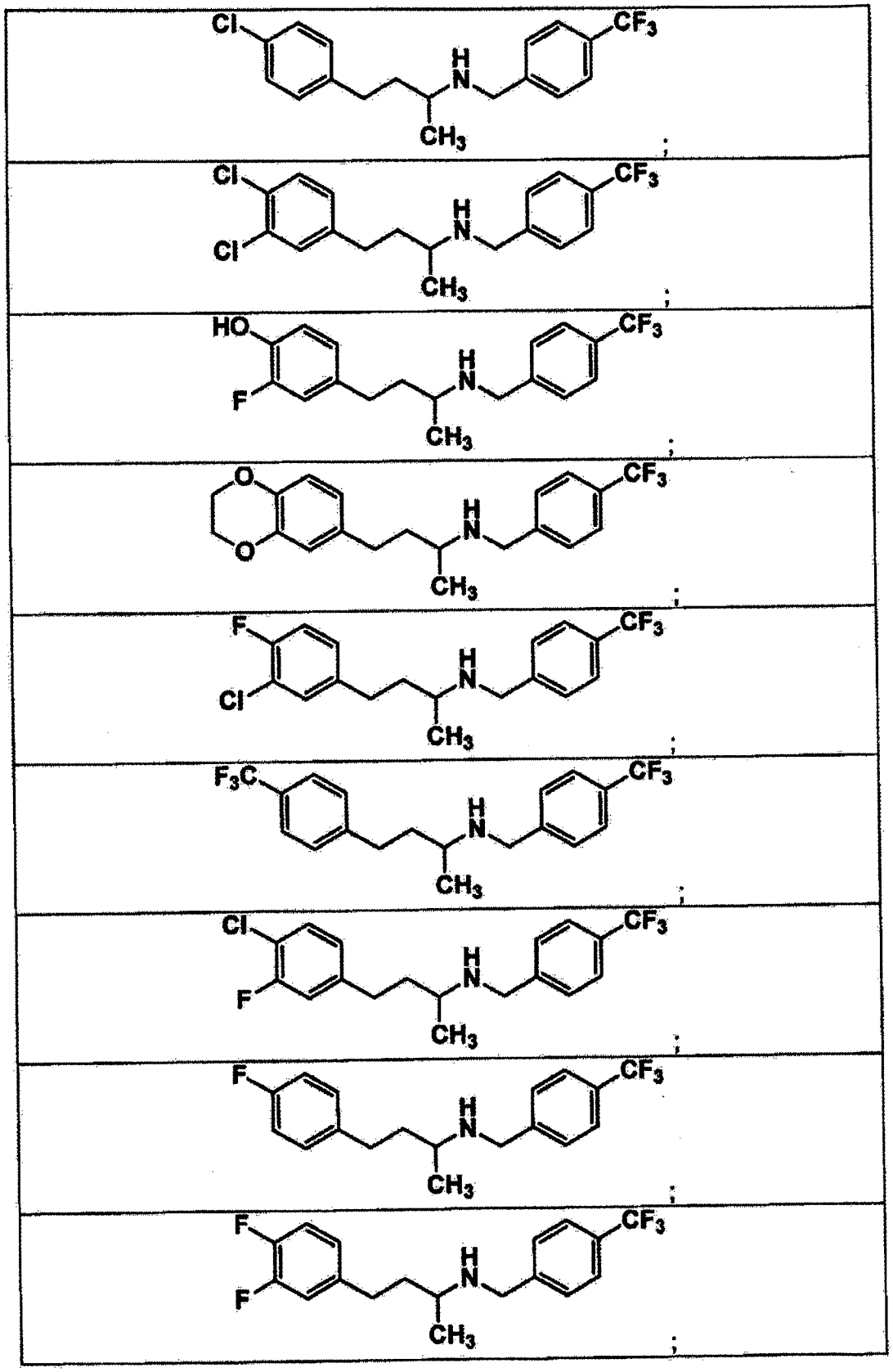
8. 如权利要求 6 所述的化合物及其药学上可接受的盐，所述化合物为式 IIb 的化合物

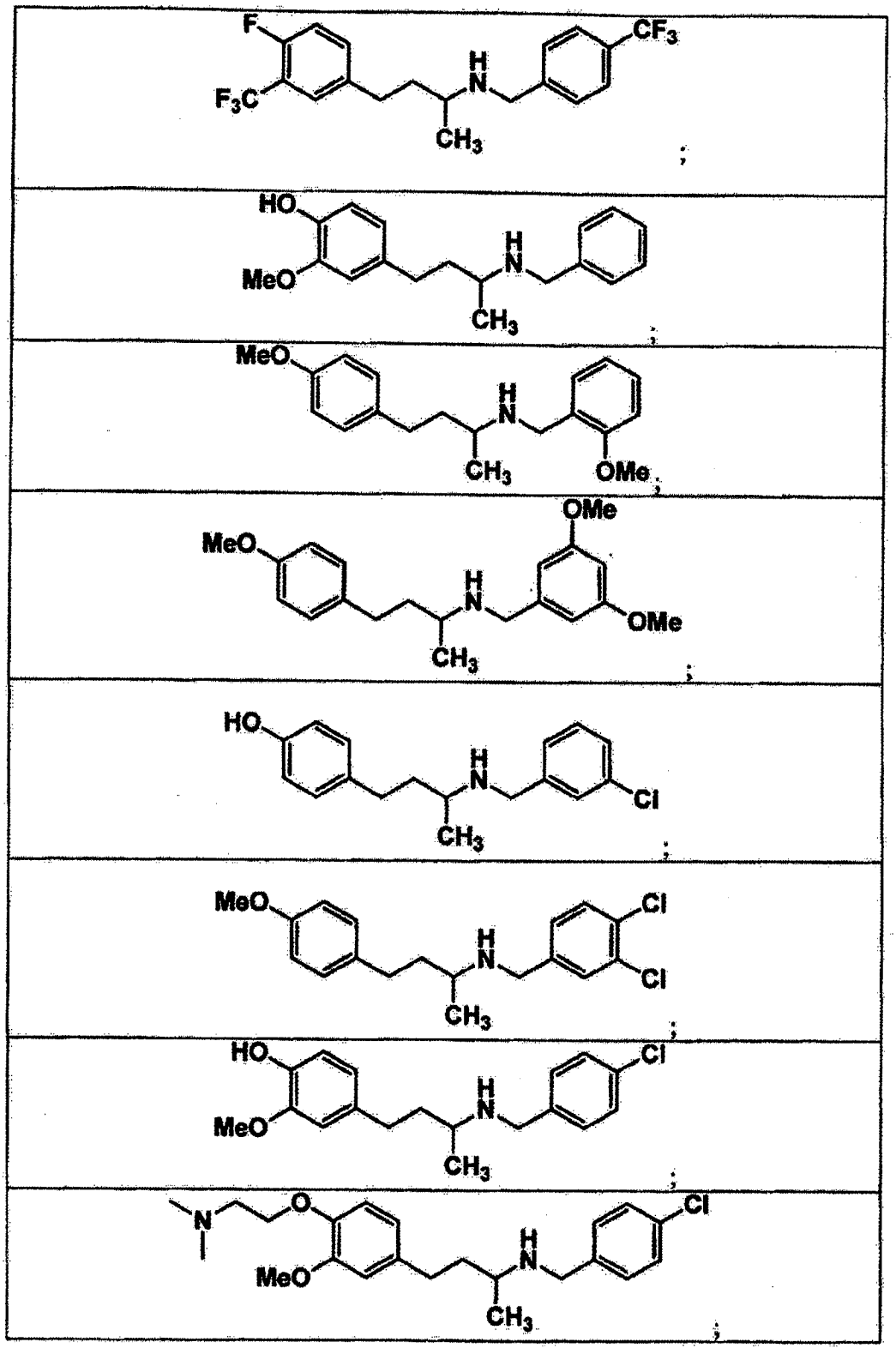


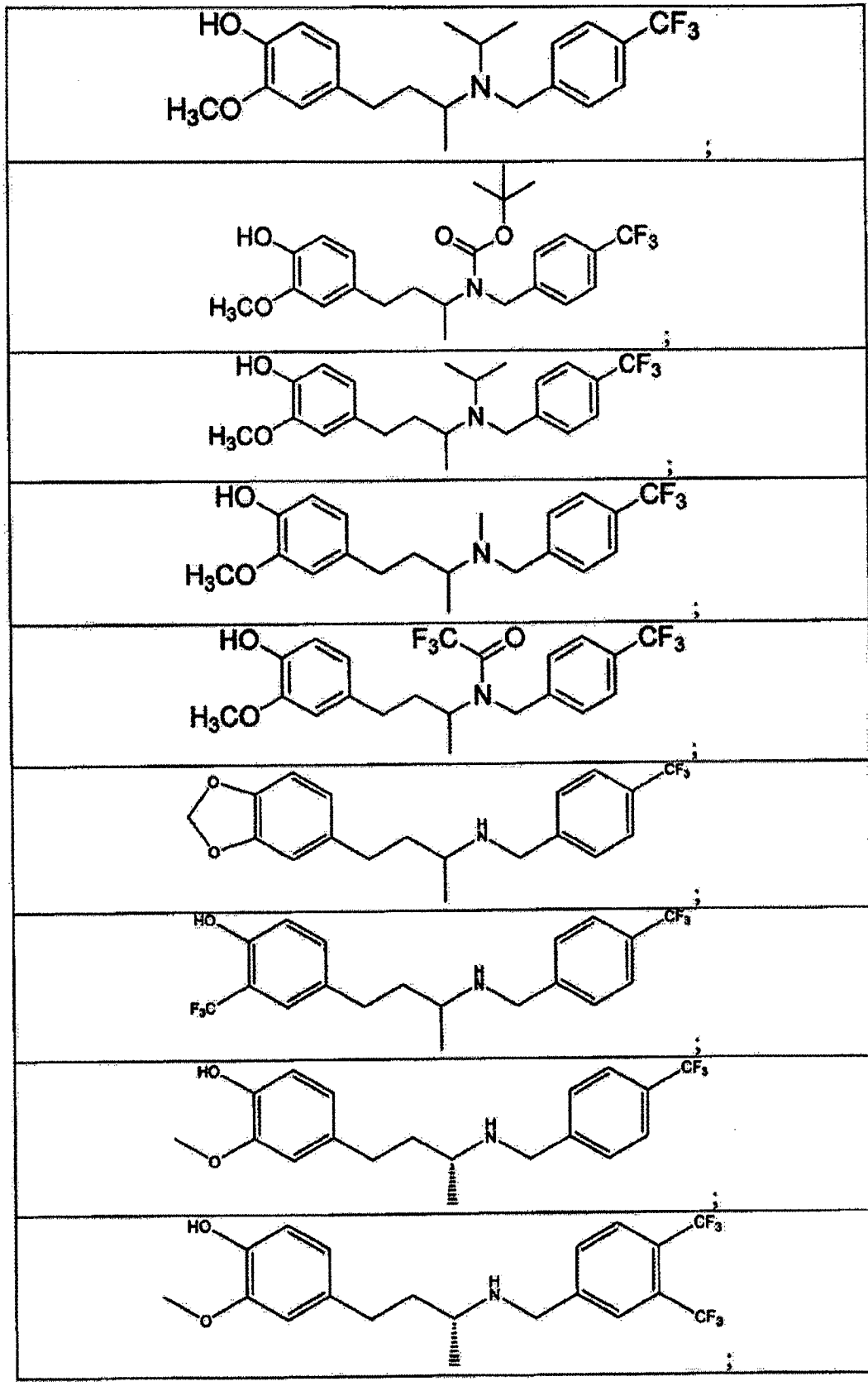
IIb

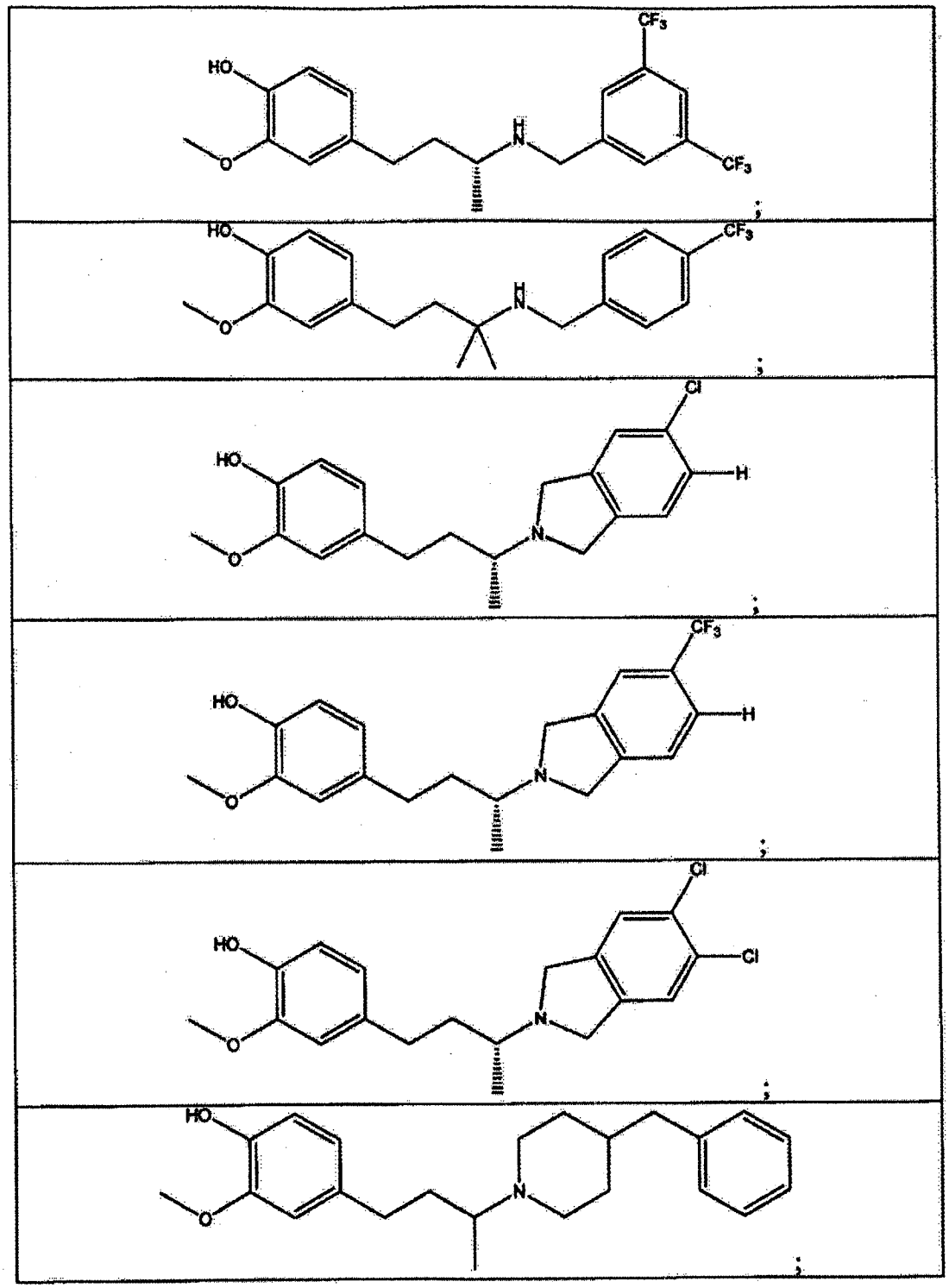
其中 R_1-R_4 如权利要求 6 中所限定。

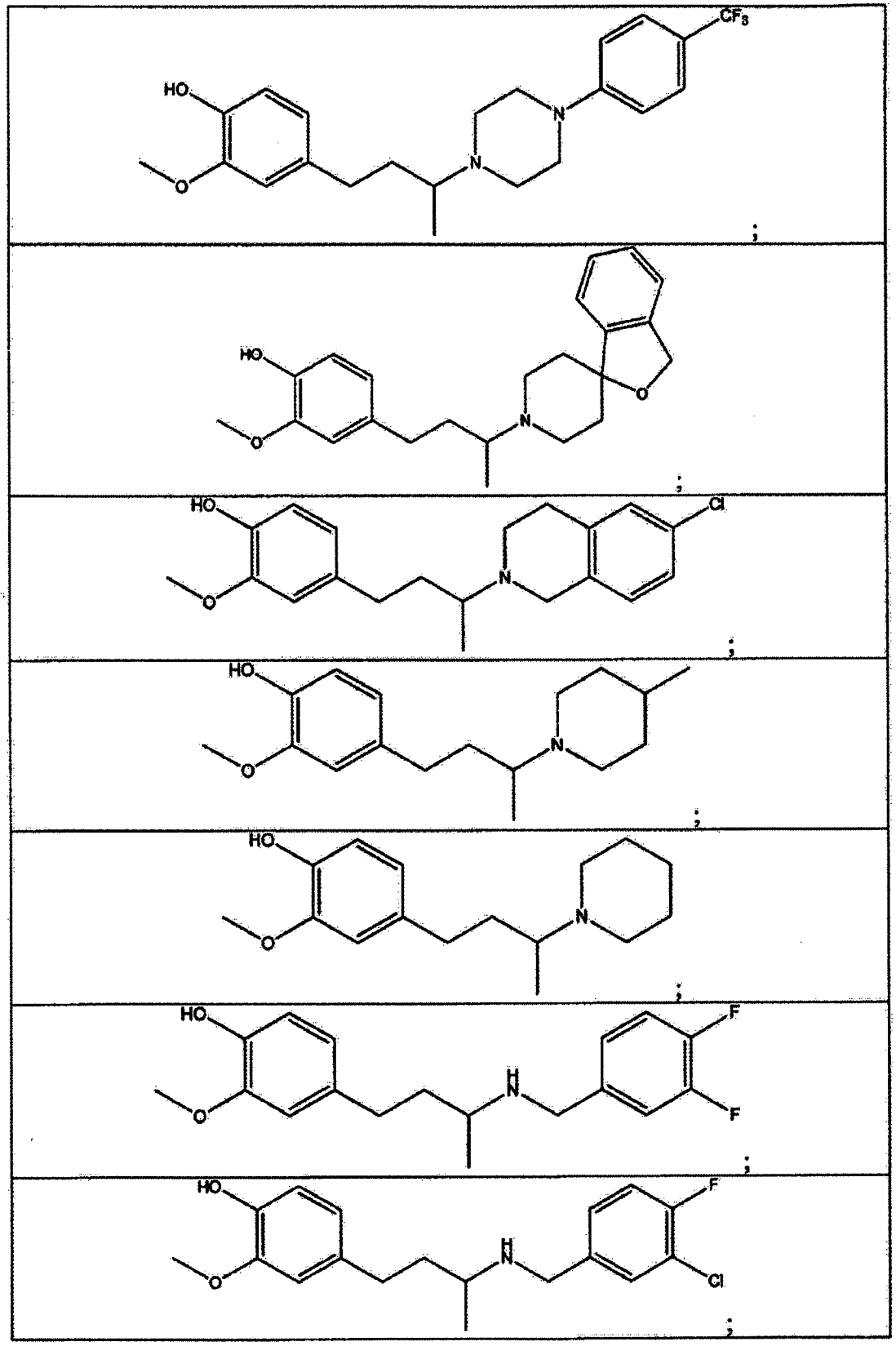
9. 一种化合物，选自

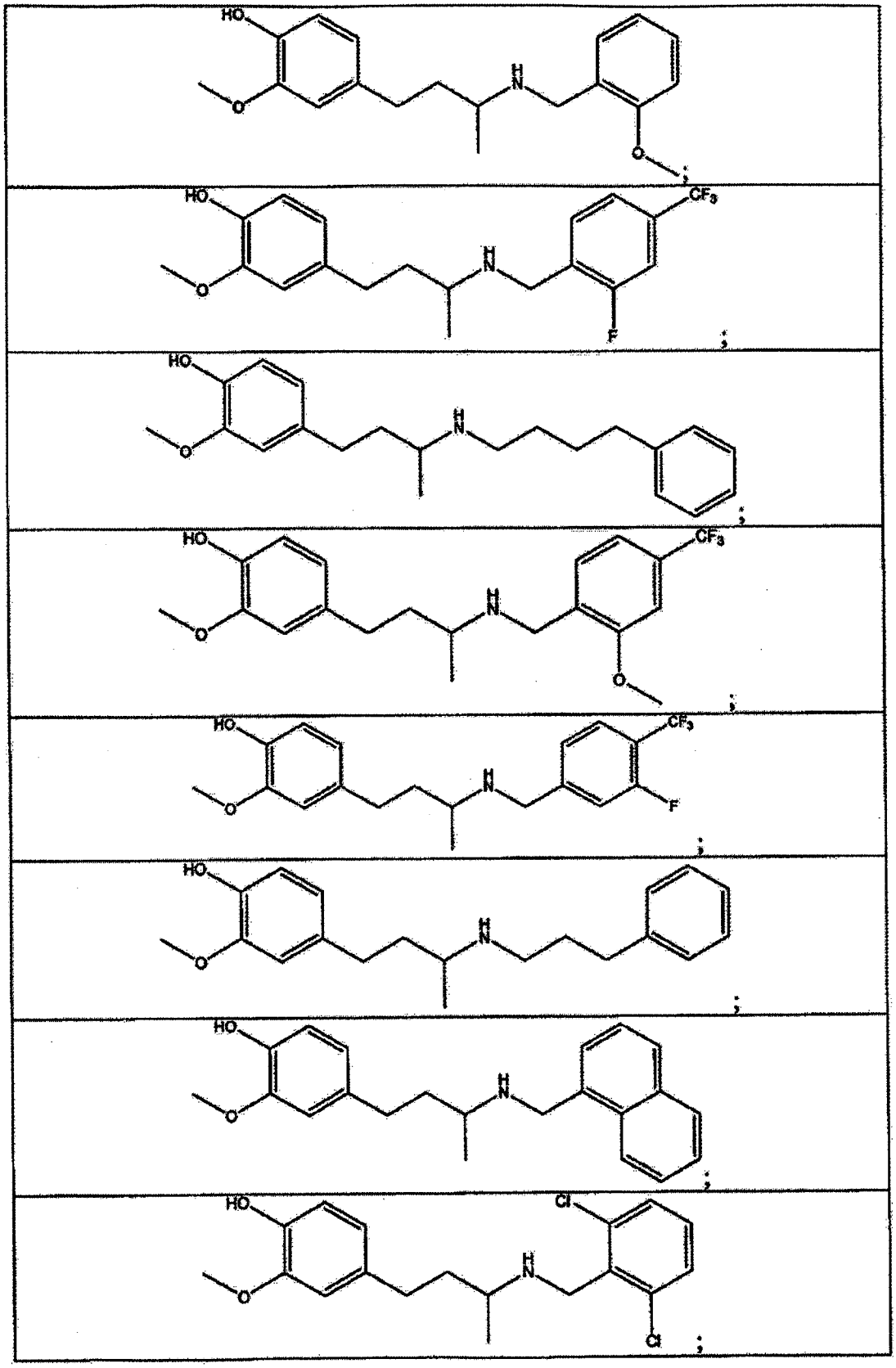


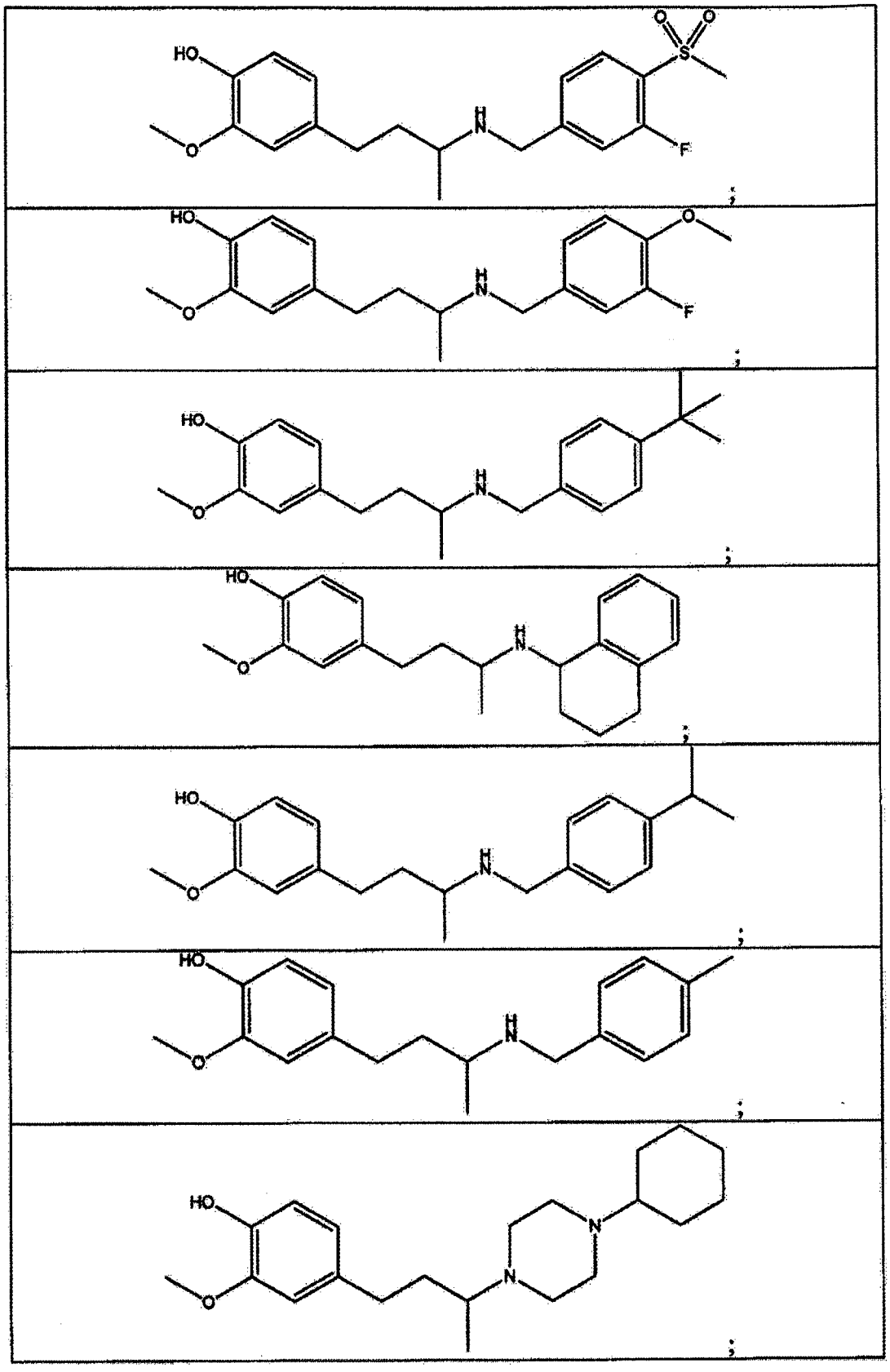


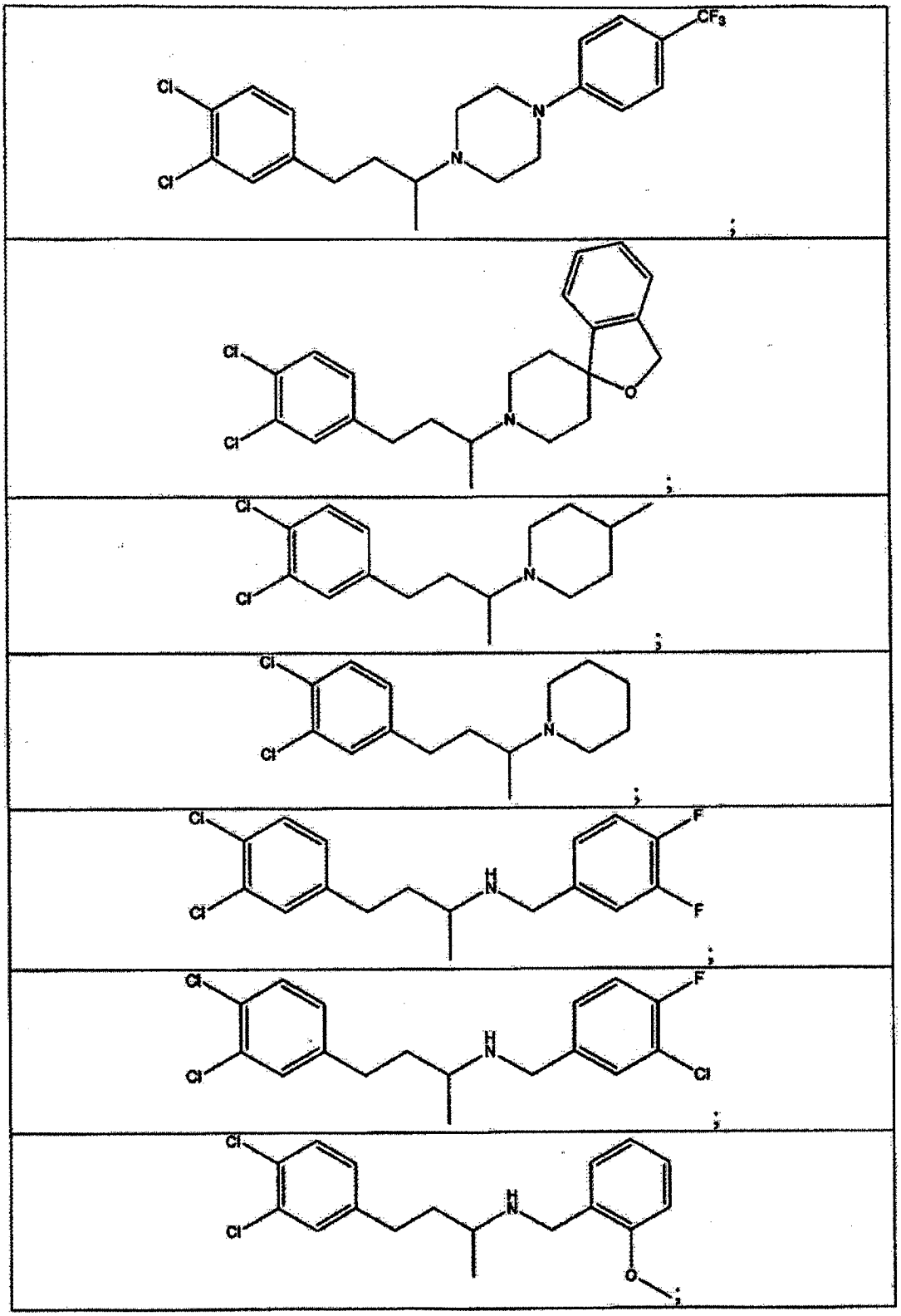


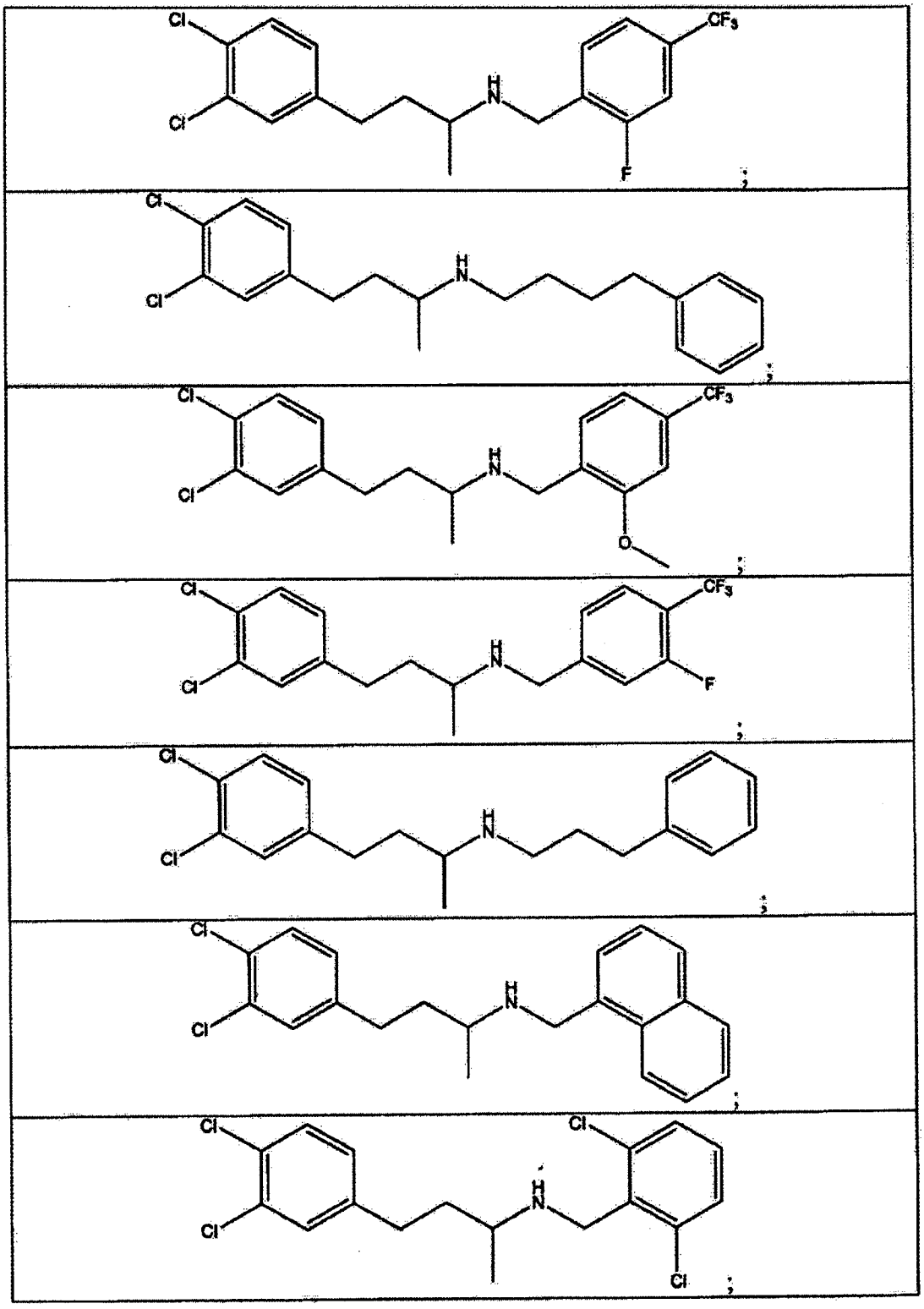


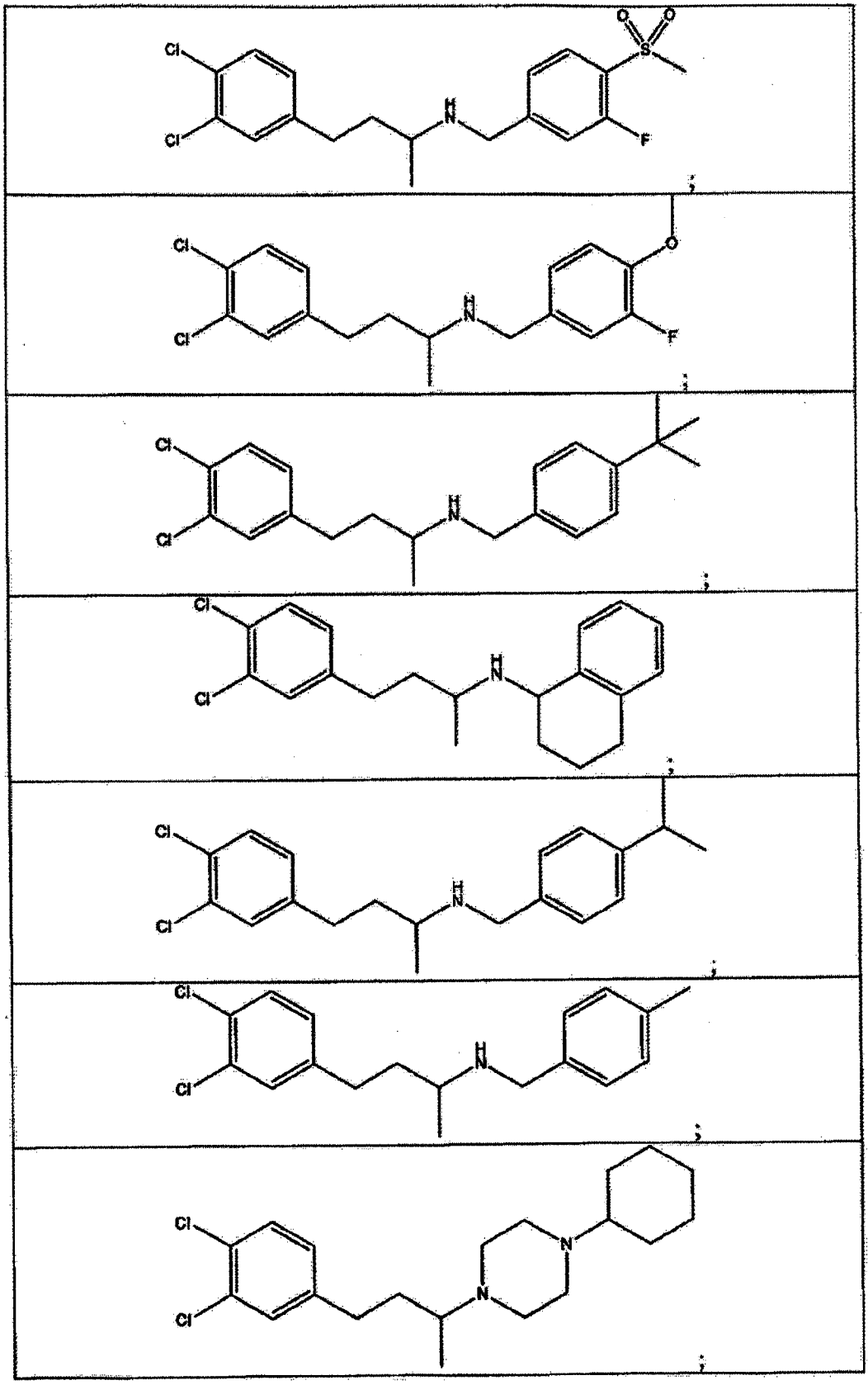


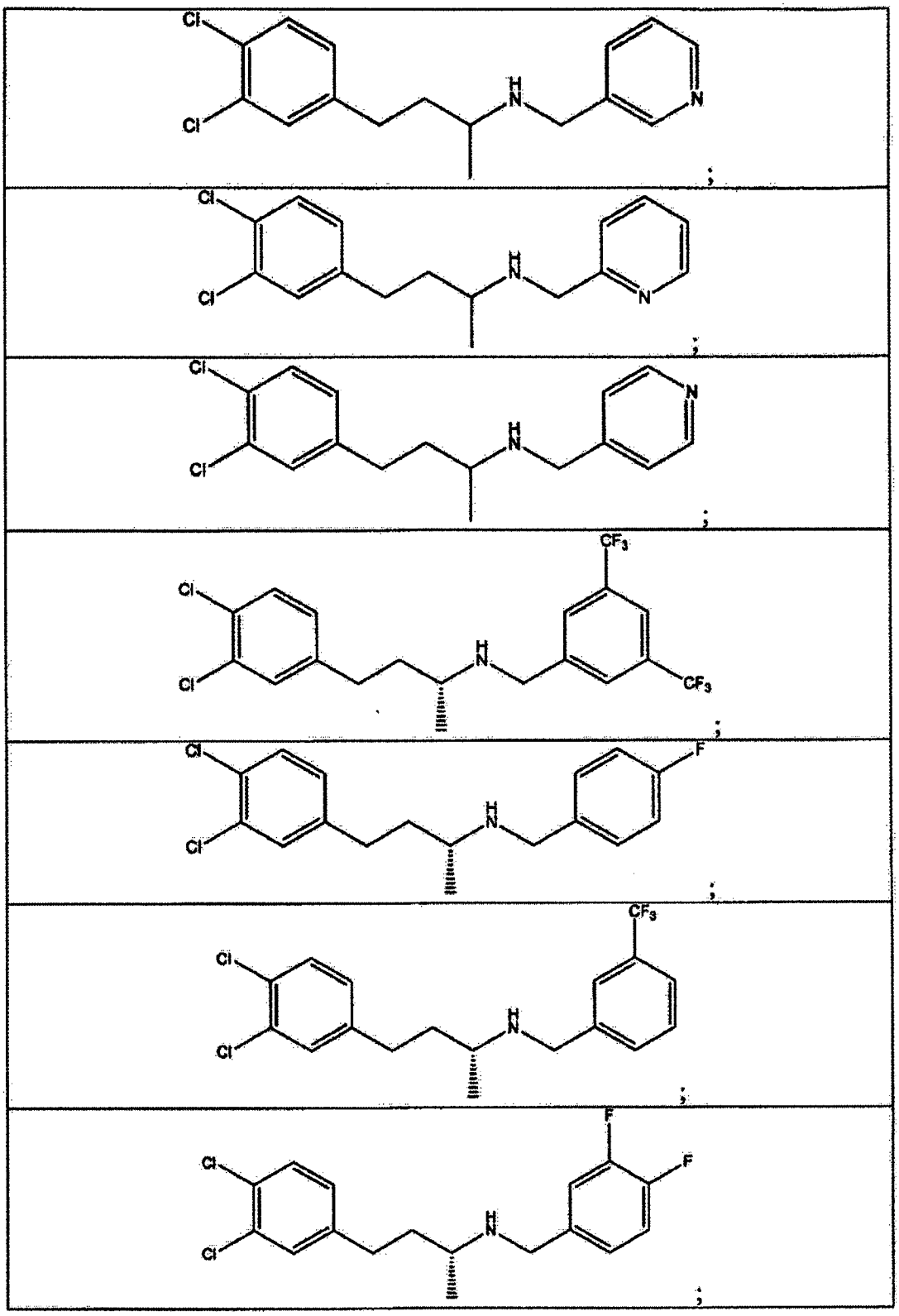


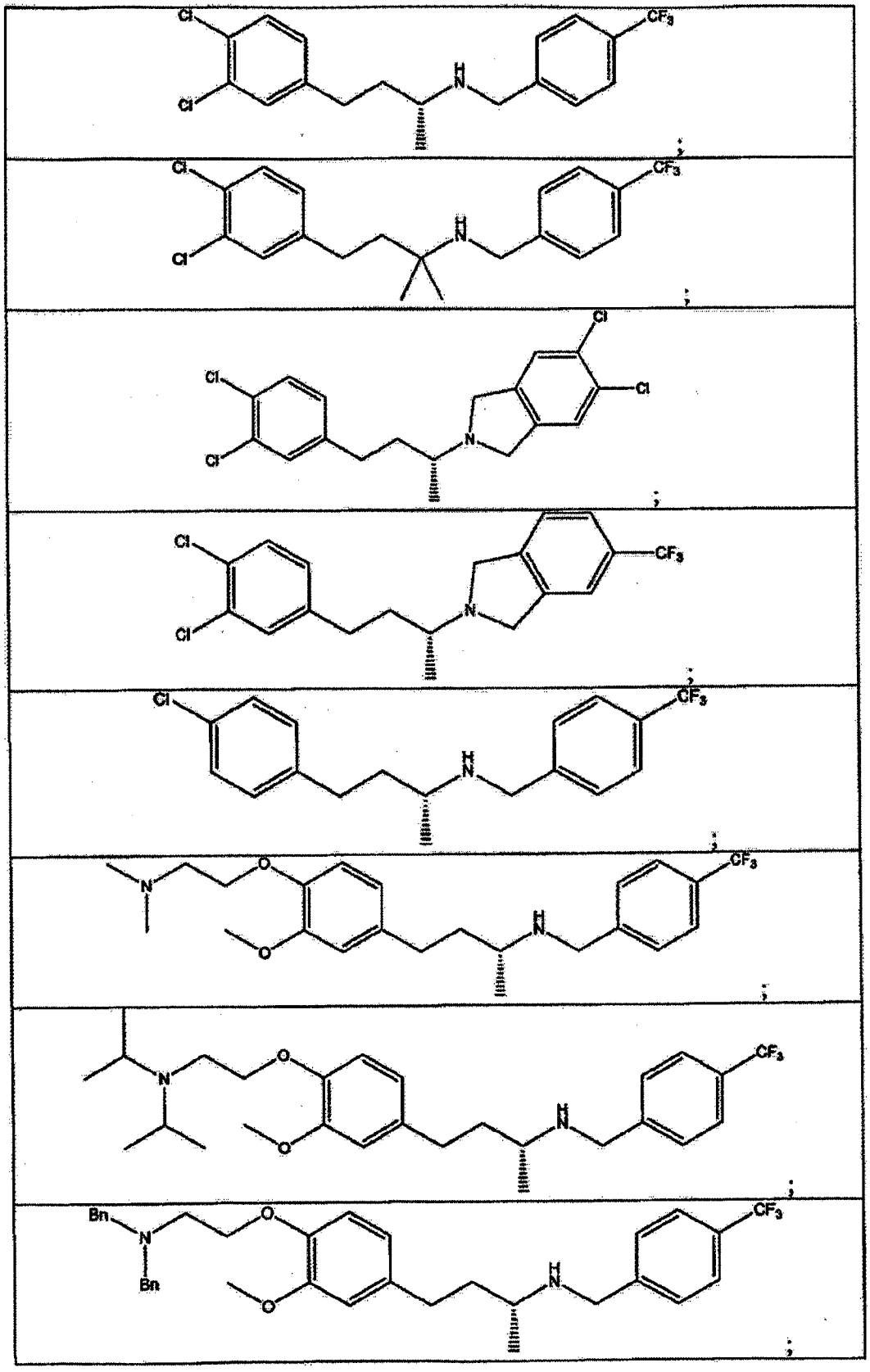


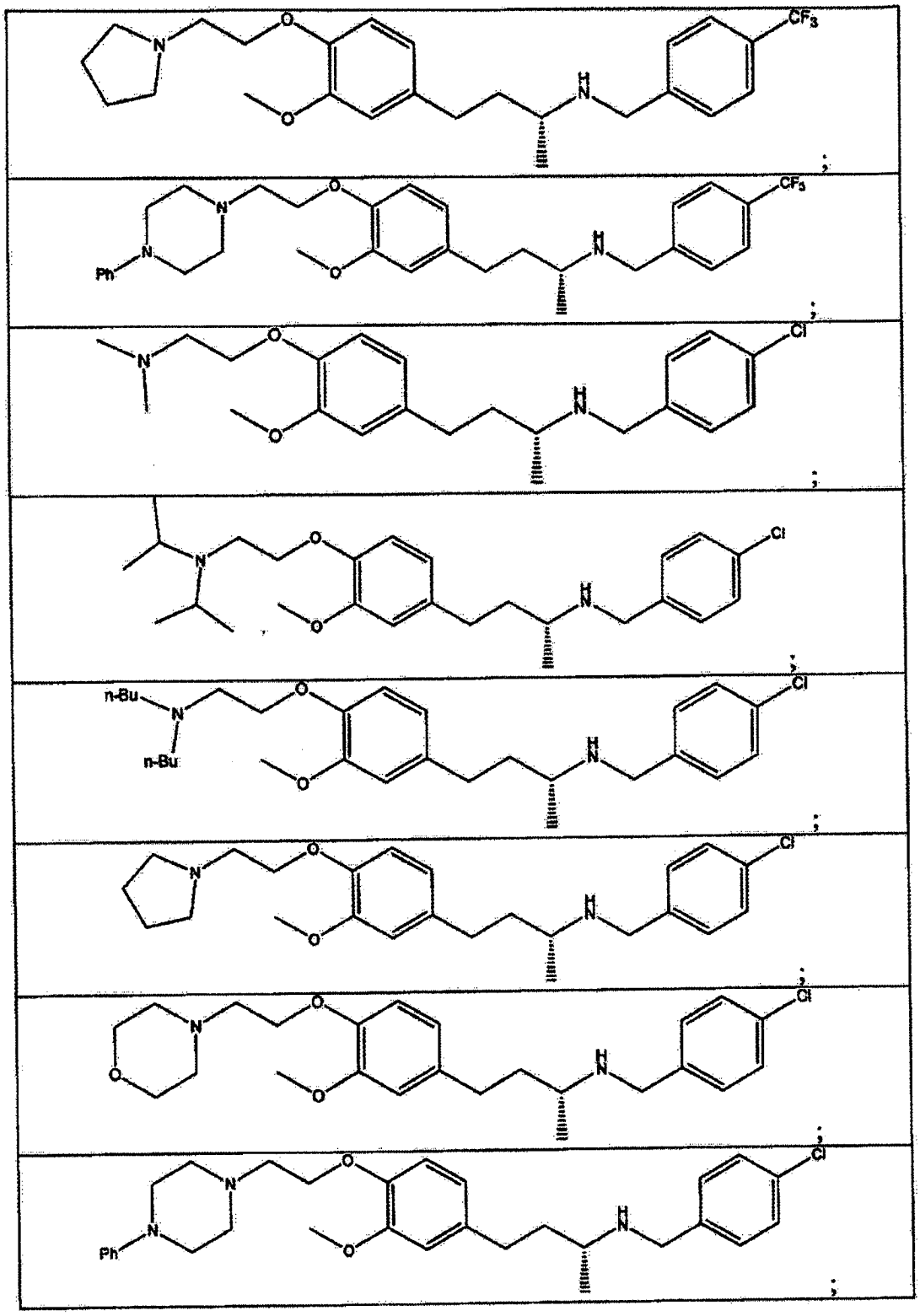


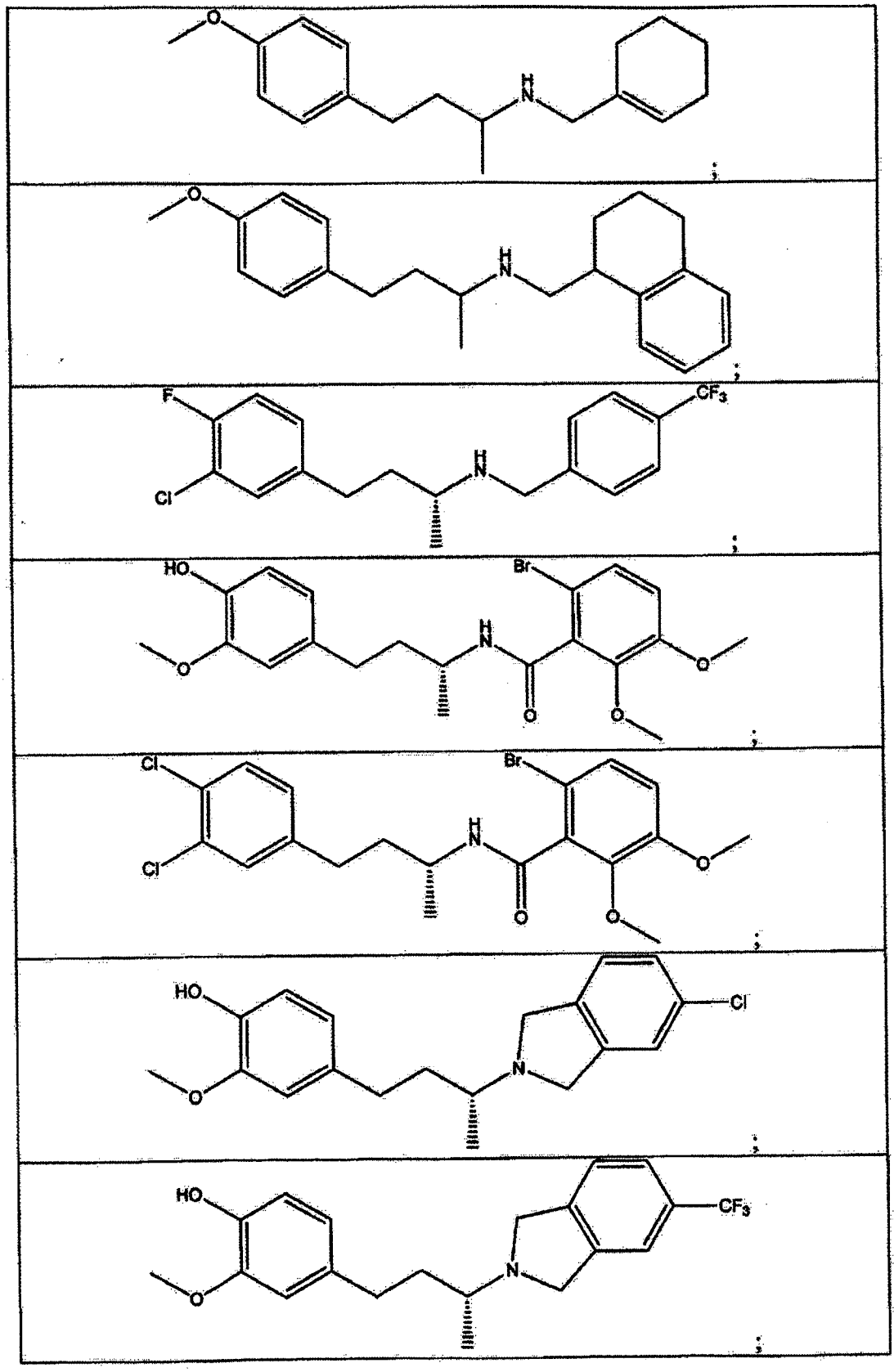


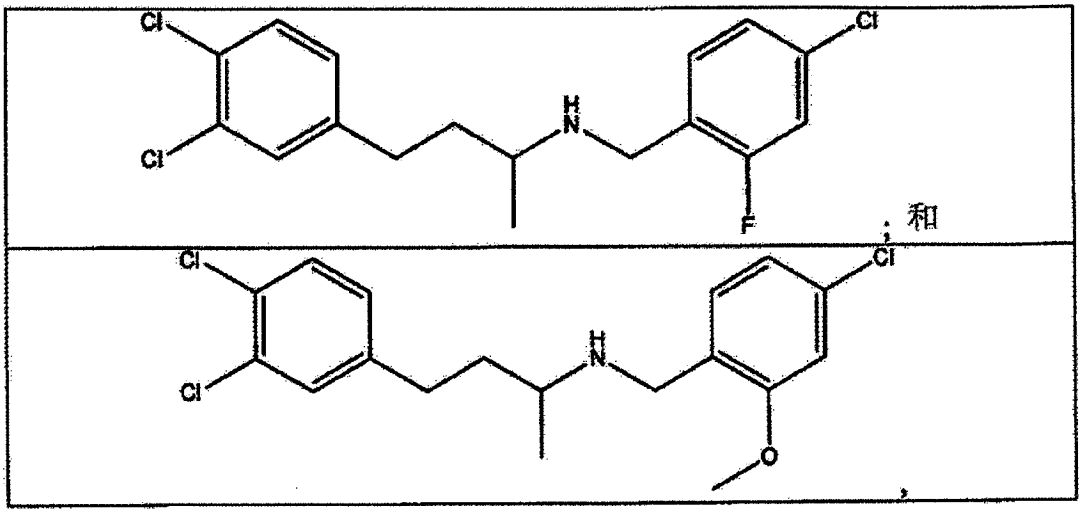






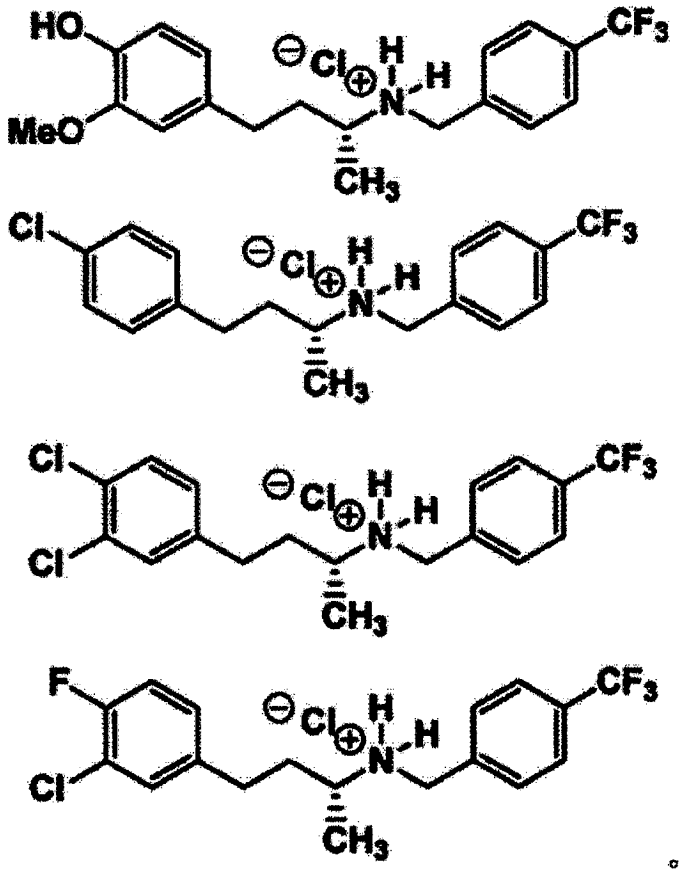




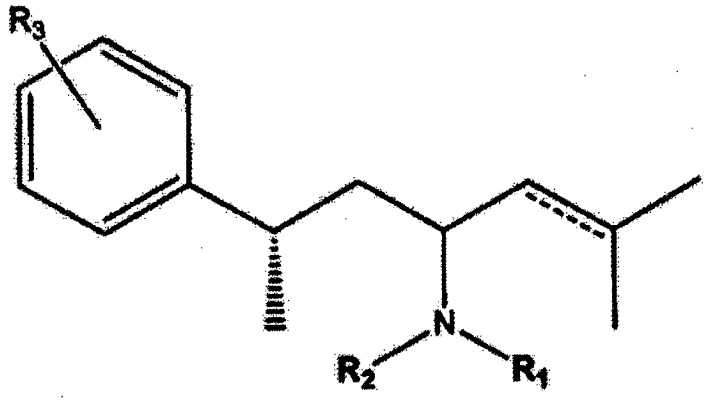


及其药学上可接受的盐。

10. 如权利要求 9 所述的化合物，选自



11. 一种式 VIIIa 的化合物及其药学上可接受的盐



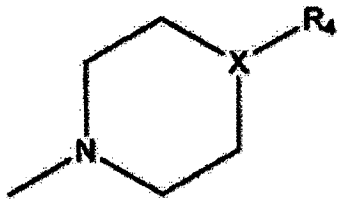
VIIIa

其中：

是单键或双键；

R_1 是 C_{1-6} 烷基、C_{1-6} 卤代烷基、未被取代的苄基或被卤素、C_{1-6} 烷基或 C_{1-6} 卤代烷基取代的苄基；

R_2 是 H，或

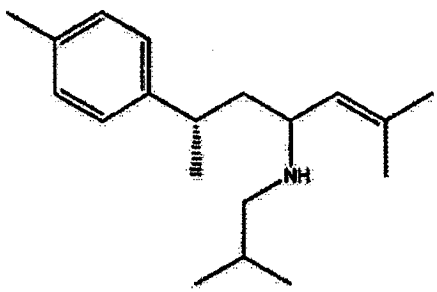
R_1 和 R_2 与氮一起形成环  其中

X 是 CH、N 或 O，且

R_4 不存在，或是 H、C_{1-6} 烷基、或未被取代的苯基或被卤素、C_{1-6} 烷基、或 C_{1-6} 卤代烷基取代的苯基；且

R_3 是 C_{1-4} 烷基、卤素、或 C_{1-6} 卤代烷氧基，

条件是排除以下化合物的外消旋混合物：

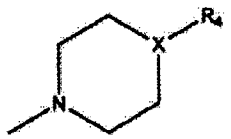


12. 如权利要求 11 所述的化合物及其药学上可接受的盐，其中

是单键或双键；

R_1 是异丁基、苄基、或被氯、甲基或 CF_3 取代的苄基；

R_2 是 H，或

R_1 和 R_2 与氮一起形成环  其中

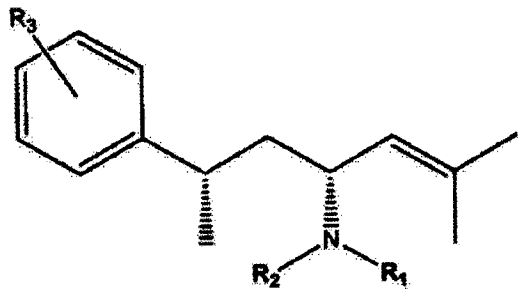
，

X 是 CH、N、或 O，且

R_4 不存在，或是 H、异丙基、或未被取代的苯基；且

R_3 是邻 -Me、间 -Me、对 -Me、对 -F、对 $-OCF_3$。

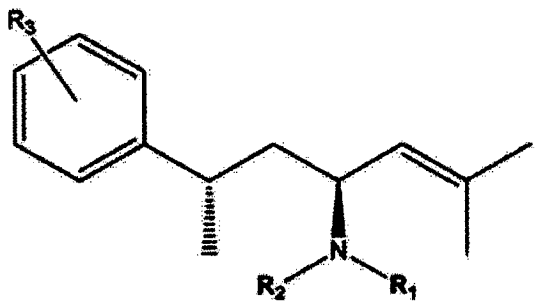
13. 如权利要求 11 所述的化合物及其药学上可接受的盐，所述化合物具有式 VIIIb



VIIIb

其中 R_1-R_3 如权利要求 11 中所限定。

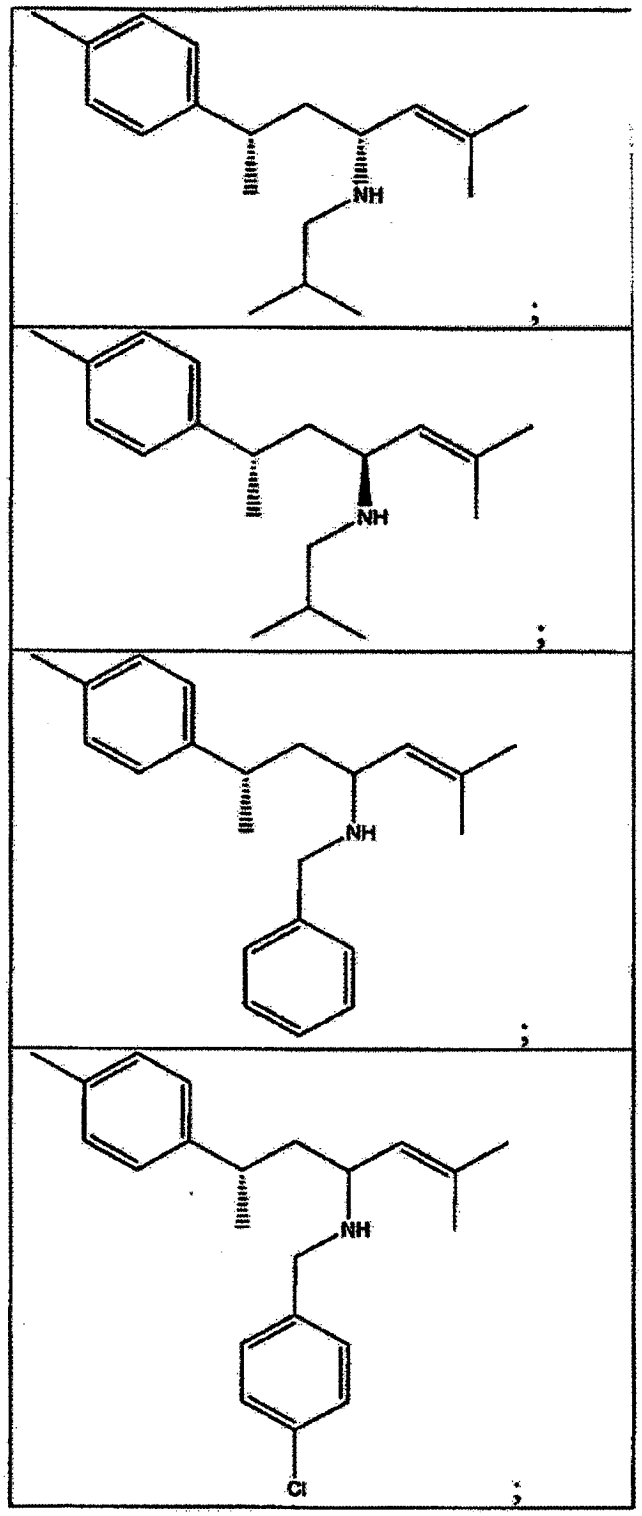
14. 如权利要求 11 所述的化合物及其药学上可接受的盐，所述化合物具有式 VIIIc

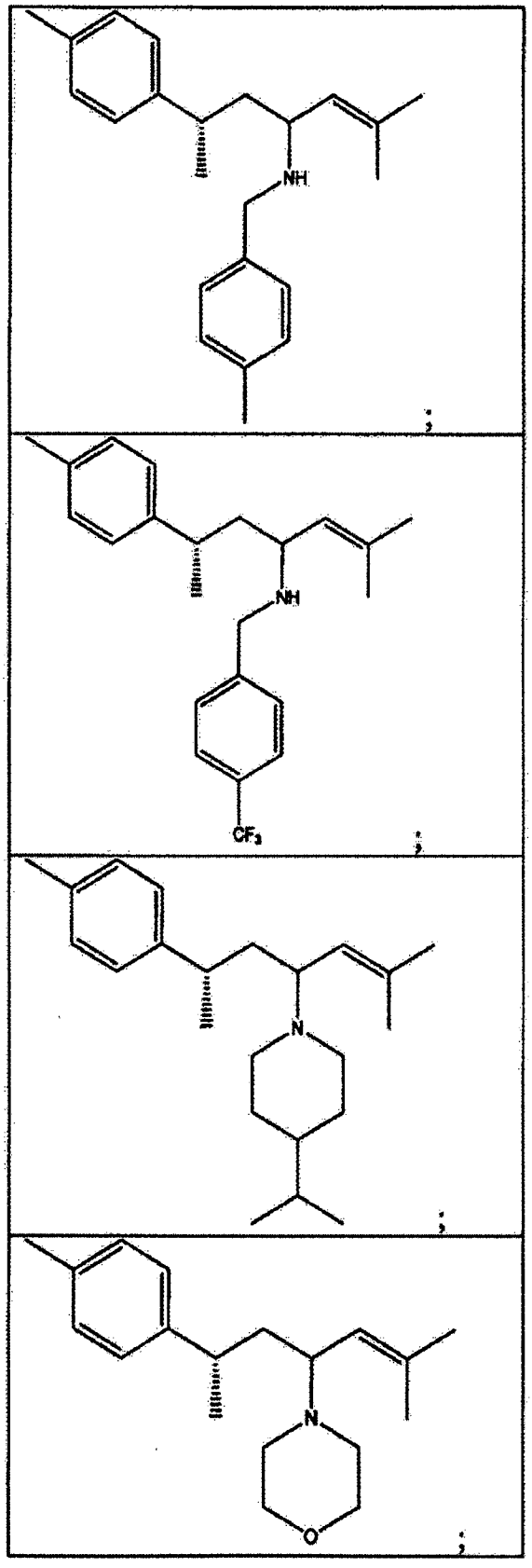


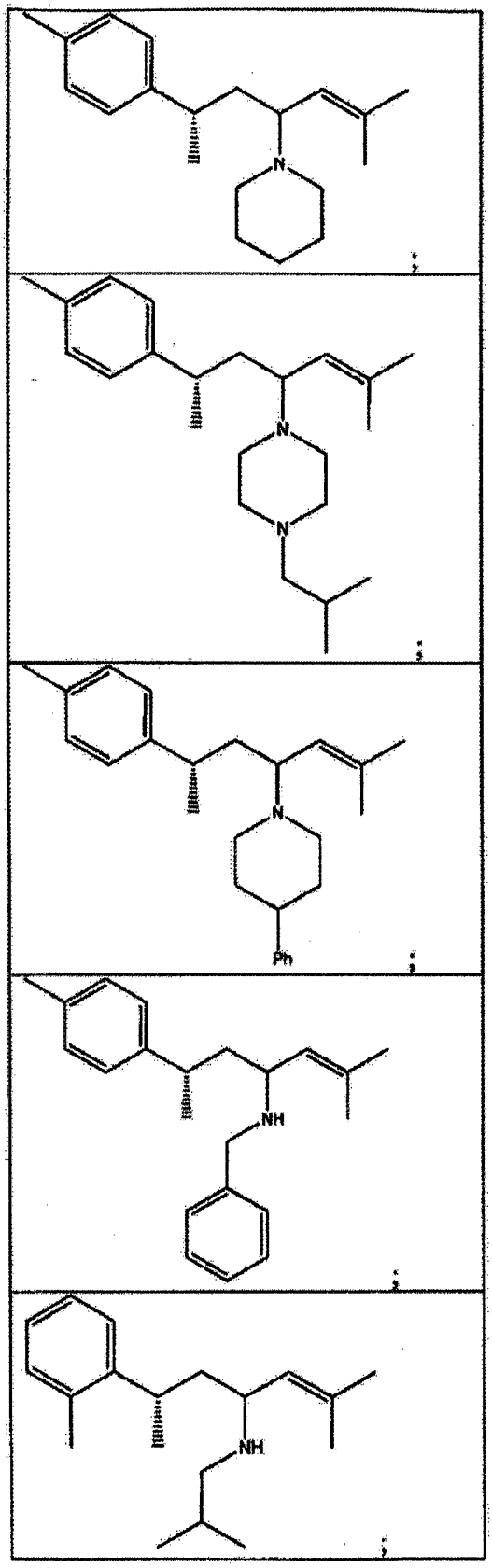
VIIIc

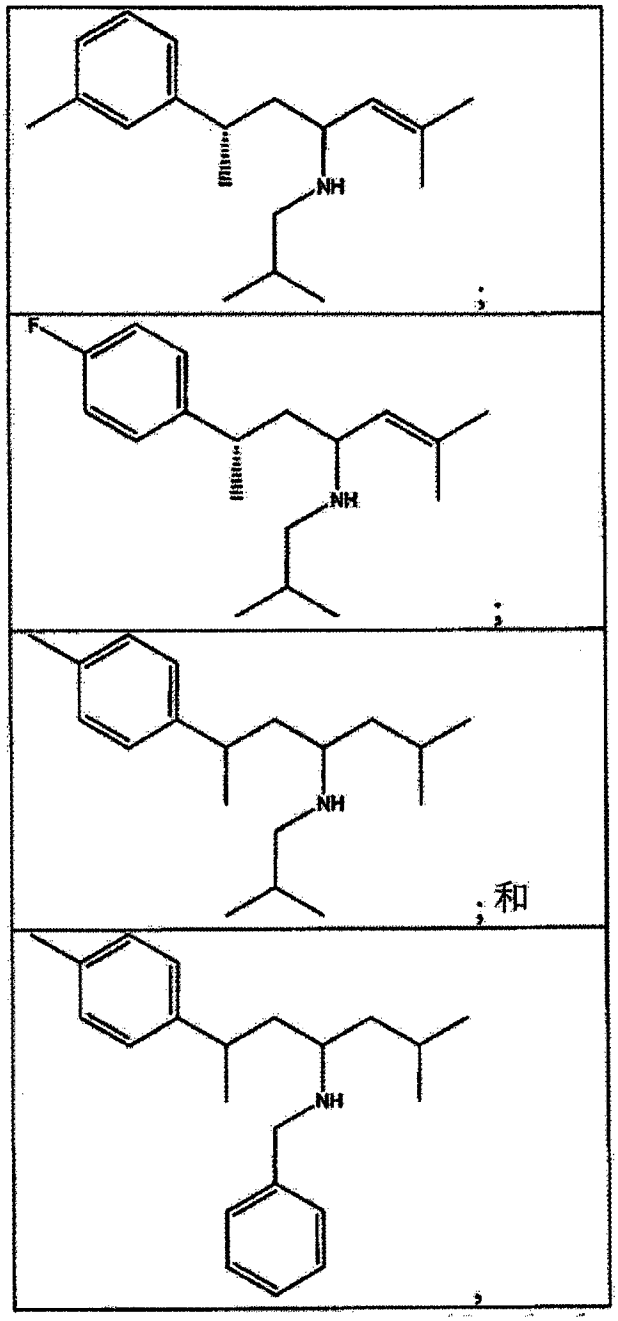
其中 R_1-R_3 如权利要求 11 中所限定。

15. 一种化合物，选自


NH
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NH
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NH
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NH
Cl
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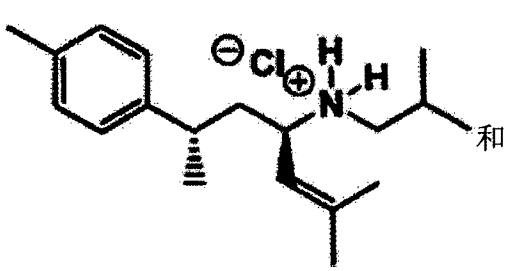

NH
NH
CF3
N
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O


N
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NH
NH

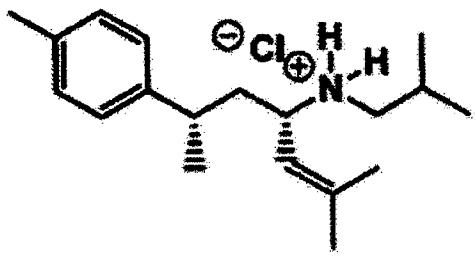


及其药学上可接受的盐。

16. 如权利要求 15 所述的化合物，选自



和



。

17. 一种用于抑制神经元细胞上的 β 淀粉样蛋白效应的方法 / 用途，包括施用有效量的组合物，所述组合物包括，

如权利要求 1-16 中任一项所述的化合物，含量为有效抑制 β 淀粉样蛋白寡聚体在所述细胞中的结合；以及

药学上可接受的载体。

18. 如权利要求 17 所述的方法 / 用途，其中以在所述细胞中还有效抑制膜运输缺陷的量施用所述化合物，所述膜运输效应与所述细胞暴露于可溶 β 淀粉样蛋白寡聚体相关联。

19. 如权利要求 17 和 18 中任一项所述的方法 / 用途，其中所述化合物的含量为在所述细胞中同时有效抑制与所述细胞暴露于可溶 β 淀粉样蛋白寡聚体相关联的寡聚体结合和突触丧失。

20. 如权利要求 17 ～ 19 中任一项所述的方法 / 用途，其中以有效抑制可溶 β 淀粉样蛋白寡聚体所介导的认知效应的量施用所述化合物。

21. 如权利要求 20 所述的方法 / 用途，其中所述认知效应是在认知衰退的动物模型中所测试的认知衰退。

22. 如权利要求 21 所述的方法 / 用途，其中所述认知衰退是通过条件性恐惧检定所测试的学习衰退。

23. 如权利要求 21 所述的方法 / 用途，其中所述认知衰退是通过 Morris 水迷宫测试所测试的空间学习和记忆的衰退。

24. 如权利要求 21 所述的方法 / 用途，其中所述认知衰退是在阿尔茨海默病的转基因动物模型中所测试的基于海马的空间学习和记忆的衰退。

25. 如权利要求 17 所述的方法 / 用途，其用于抑制神经元细胞的 β 淀粉样蛋白寡聚体所诱导的突触功能障碍，包括使所述细胞与包括含量为在所述细胞中有效抑制 β 淀粉样蛋白寡聚体结合的 σ-2 受体拮抗剂化合物的组合物接触，所述功能障碍与所述细胞暴露于可溶 β 淀粉样蛋白寡聚体相关联。

26. 如权利要求 17 所述的方法 / 用途，其用于抑制受试者中长时程增强效应的压制，包括对需要的受试者施用治疗有效量的包括 σ-2 受体拮抗剂化合物的组合物。

27. 如权利要求 17 所述的方法 / 用途，其用于抑制表现出认知衰退或具有表现出认知衰退的风险的受试者中的认知衰退，包括对所述受试者施用治疗有效量的包括 σ-2 受体拮抗剂化合物的组合物。

28. 如权利要求 17 所述的方法 / 用途，其用于抑制受试者中与中枢神经元上的 β 淀粉样蛋白寡聚体效应相关联的认知衰退，包括对遭受所述认知衰退的受试者施用治疗有效量的包括 σ-2 受体拮抗剂化合物的组合物。

29. 如权利要求 17 所述的方法 / 用途，其用于治疗需要的受试者中的阿尔茨海默病中的轻度认知障碍，包括对所述受试者施用治疗有效量的包括 σ-2 受体拮抗剂化合物的组合物。

30. 如权利要求 25 ～ 27 中任一项所述的方法 / 用途，其中所述 σ-2 拮抗剂化合物具有一个或多个以下额外特性：

(a) 相比于一种或多种非 σ CNS 受体，其以高出至少 10 倍、20 倍、50 倍或 100 倍的亲和性与 σ-2 受体选择性结合，其中所述化合物以小于 200nM、150nM、100nM 或 60nM 的 K_i 与 σ-2 受体结合；

(b) 其抑制 Abeta 寡聚体结合至神经元细胞或神经元细胞中的突触丧失，所述丧失与

所述细胞暴露于 Abeta 寡聚体相关联；

(c) 其抑制中枢神经元中的膜运输异常，所述异常与所述细胞暴露于一种或多种 Abeta 寡聚体相关联；

(d) 在不存在 β 淀粉样蛋白寡聚体的情况下，其不能影响中枢神经元中的运输或突触数目。

用于治疗神经退行性疾病的组合物和方法

[0001] 本申请于2012年8月27日提交为PCT国际专利申请，以美国公司Cognition Therapeutics, Inc.的名义作为除美国之外的所有指定国的申请人，以美国公民Susan M. Catalano、Gilbert Rishton和Nicholas J. Izzo, Jr.仅作为美国指定的申请人，并要求2011年8月25日提交的美国临时专利申请序列号61/527,584的优先权，该申请整体并入本文以供参考。

技术领域

[0002] 本发明涉及新的与σ-2受体结合的二芳基氨基化合物，涉及包括这样的化合物的药物组合物，并涉及用其抑制或恢复神经元细胞中的突触丧失、调节神经元细胞中的膜运输变化以及治疗认知衰退和神经退行性疾病和病症的方法。

背景技术

[0003] 存在五种目前FDA批准用于治疗AD的药物。四种是胆碱酯酶抑制剂：他克林（Cognex®; Sciele）、多奈哌齐（Aricept®; Pfizer）、卡巴拉汀（Exelon®; Novartis）和加兰他敏（Razadyne®; Ortho-McNeil-Janssen）。多奈哌齐、卡巴拉汀和加兰他敏是他克林（第一代化合物，因其肝毒性潜能很少开进药方）的后继者，它们在AD的所有阶段在提供认知和功能的症状改进方面大略同等有效。第五个批准的药物是美金刚（Namenda®; Forest），其是低亲和性、使用依赖的N-甲基-D-天冬氨酸谷氨酸受体拮抗剂，提供相似益处，但对严重AD仅适度。这些化合物的临床效果较小且是非永久性的，并且目前没有结论性的数据支持其作为疾病调修剂的用途。参见例如Kerchner等人，2010, Bapineuzumab, Expert Opin Biol Ther., 10(7):1121-1130。明显地，需要另外可选的治疗AD的方法。

[0004] 人β淀粉样蛋白（Abeta或Aβ）是在患有阿尔茨海默病的患者大脑中发现的不溶性淀粉样斑块沉积的主要成分。该斑块由Abeta的纤维状聚集物组成。β淀粉样蛋白原纤维与阿尔茨海默病的晚期相关联。

[0005] 早期阿尔茨海默病（AD）的认知特点是特别不能形成新记忆。早期记忆丧失被视为是由可溶Aβ寡聚体引起的突触障碍。这些寡聚体阻断长时程增强效应（对突触可塑性的经典实验范式），并且它们在AD大脑组织和转基因AD模型中显著升高。已推测，早期记忆丧失源自神经元死亡之前的突触障碍，并且突触障碍源自可溶Aβ寡聚体的作用而不是原纤维的作用。Lacor等人，Synaptic targeting by Alzheimer's-related amyloid β oligomers, J. Neurosci. 2004, 24(45):10191-10200。

[0006] Abeta是整合膜蛋白淀粉样前体蛋白（APP）的剪切产物，被发现集中于神经元的突触中。Abeta的可溶形式存在于阿尔茨海默患者的大脑和组织中，且其存在与疾病进展相关。Yu等人，2009, Structural characterization of a soluble amyloid beta-peptide oligomer, Biochemistry, 48(9):1870-1877。可溶β淀粉样蛋白寡聚体已证实为引起阻断学

习和记忆的神经元突触的改变。

[0007] 较小的可溶 A β 寡聚体干扰大量对正常突触可塑性而言关键的信号传导通路，最终导致棘 (spine) 和突触丧失。Selkoe 等人，2008, Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior, Behav Brain Res192(1):106-113。阿尔茨海默病作为突触可塑性疾病开始和持续。

[0008] 可溶 A β 寡聚体的存在被认为是患有前阿尔茨海默病的大脑中早期认知衰退的原因。已知 β 淀粉样蛋白寡聚体结合于神经元突触且 σ-2 受体大量存在于神经元和神经胶质。

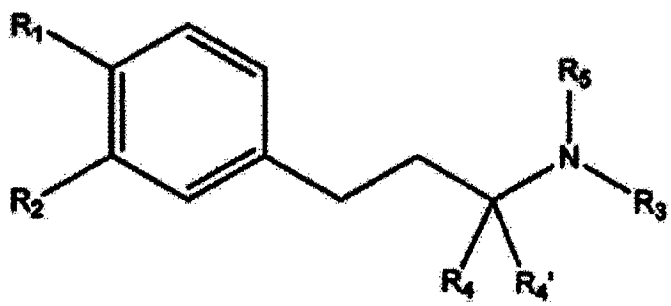
[0009] 本发明部分基于如下宽泛的发现，即满足某些条件的 σ-2 受体拮抗剂抑制可溶 A β 寡聚体的有害作用。在一些实施方式中，使用 σ-2 受体拮抗剂和组合物来治疗或预防受试者中的突触功能障碍。

发明内容

[0010] 本发明部分基于以下宽泛的发现，即 σ-2 拮抗剂，优选还表现出特定治疗表型的其它方面的一种拮抗剂，如下所述参与抑制并且抑制 β 淀粉样蛋白（“Abeta”或“A β”）肽和寡聚体及其其它可溶种类的作用，因此可以用于治疗与 Abeta- 诱导的病理学例如阿尔茨海默病相关的包括疾病和障碍的病症。可溶 Abeta 寡聚体表现为与特异受体结合并干扰对正常突触可塑性而言关键的信号传导通路的可逆药理学配体，最终导致棘和突触丧失。已发现，与 σ-2 受体结合且表现为功能性神经元拮抗剂的化合物表现出与 Abeta 寡聚体的药理学竞争。因此，本文所述的 σ-2 拮抗剂化合物可以降低或防止 Abeta 寡聚体作用例如 Abeta 诱导的细胞毒性。本发明还包括用于抑制 Abeta 寡聚体或其它可溶 Abeta 种类对神经元细胞的作用，且更广泛而言，β 淀粉样蛋白病理学的方法，包括使细胞与根据本发明的 σ-2 拮抗剂接触。在一些实施方式中，提供用于治疗阿尔茨海默病早期的方法，包括施用治疗有效量的 σ-2 功能性拮抗剂。

[0011] 在一个实施方式中，本发明的 σ-2 拮抗剂是由式 I 表示的新化合物或其药学上可接受的盐：

[0012]



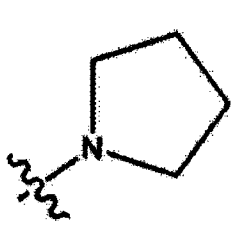
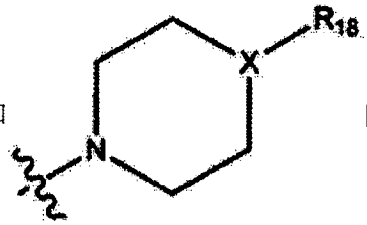
I

[0013] 其中

[0014] R_1 和 R_2 独立选自 H、OH、卤素、CN、NO_2、NH_2、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、C_{3-7} 环烷基、NH(C_{1-4} 烷基)、N(C_{1-4} 烷基)$_2$、NH(C_{3-7} 环烷基)、NHC(O)(C_{1-4} 烷基)、(R_{16})(R_{17})N-C_{1-4} 亚烷基 -O-、SH、S(C_{1-6} 烷基)、C(O)OH、C(O)O(C_{1-4} 烷基)、C(O)(C_{1-4} 烷

基）和 C(O)NH(C_{1-4} 烷基），或者 R_1 和 R_2 连接在一起形成 -O-C_{1-2} 亚甲基 -O- 基团，其中

[0015] R_{16} 和 R_{17} 独立为 H、C_{1-4} 烷基或苄基，或者 R_{16} 和 R_{17} 与氮一起形成选自

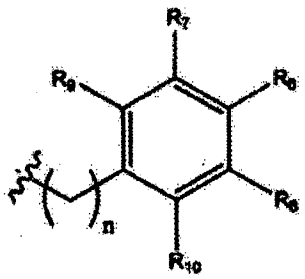
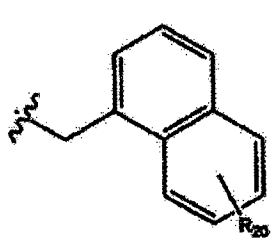
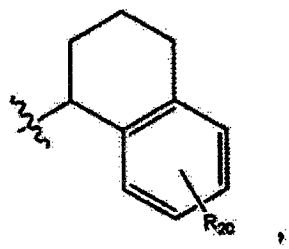
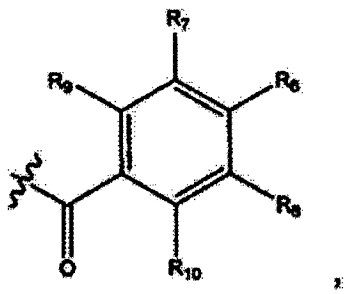
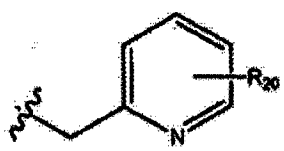
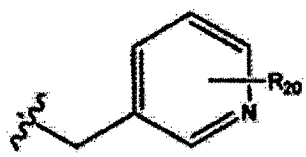
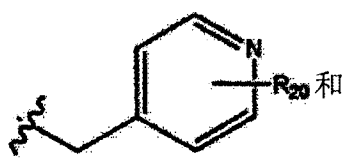
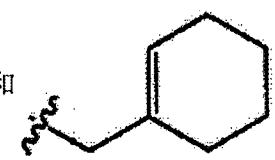

和

的环，其中

[0016] X 是 CH_2、N 或 O 且 R_{18} 不存在或者是 H、未被取代的苯基或被 OH、卤素、CN、NO_2、NH_2、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基取代的苯基；并且

[0017] 其中 R_1 和 R_2 的至少一者不是 H；

[0018] R_3 选自

[0019]


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和


[0020] 其中

[0021] R_6、R_7、R_8、R_9、R_{10} 和 R_{20} 独立选自 H、OH、卤素、CN、NO_2、NH_2、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、C_{3-7} 环烷基、NH(C_{1-4} 烷基）、N(C_{1-4} 烷基）$_2$、NH(C_{3-7} 环烷基）、NHC(O)(C_{1-4} 烷基）、SH、S(C_{1-6} 烷基）、S(O)$_2$-C_{1-6} 烷基、C(O)OH、C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）和 C(O)NH(C_{1-4} 烷基）；并且

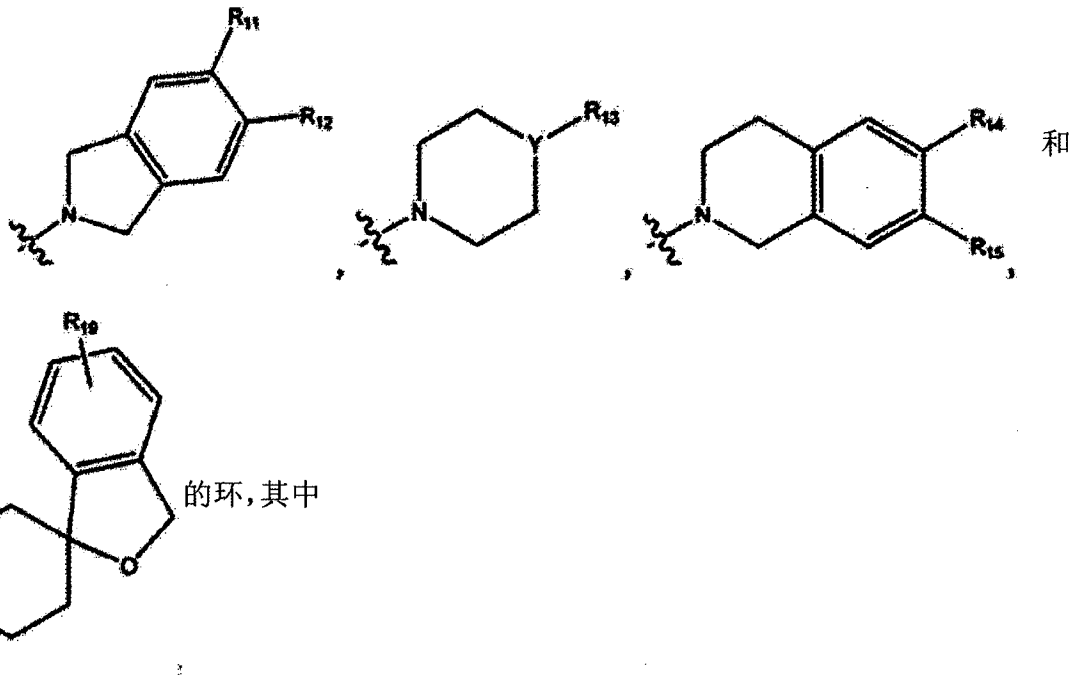
[0022] n 是 1-4

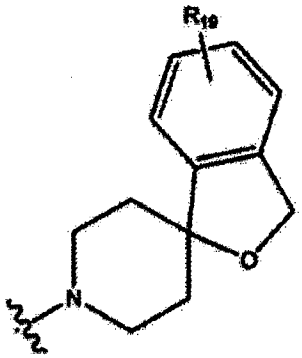
[0023] R_4 是 C_{1-6} 烷基；

[0024] $R_{4'}$ 是 H 或 C_{1-6} 烷基；并且

[0025] R_5 是 H、C_{1-6} 烷基和 C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）或 C(O)(C_{1-4} 卤代烷基）；或

[0026] R_3 和 R_5 与氮一起形成选自

[0027]  和

 的环，其中

[0028] R_{11}、R_{12}、R_{14}、R_{15} 和 R_{19} 独立选自 H、OH、卤素、CN、NO_2、NH_2、

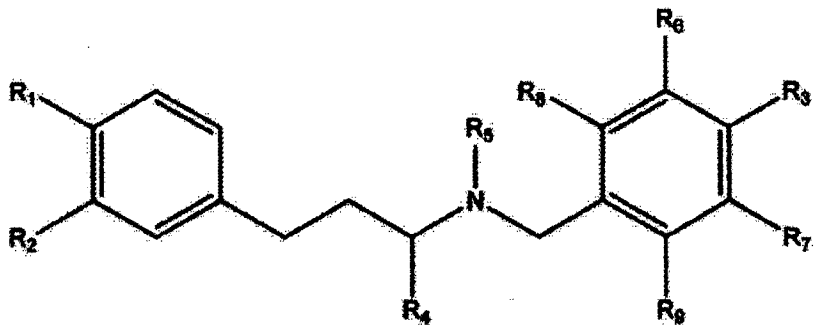
[0029] C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基，并且

[0030] Y 是 CH、N 或 O；并且

[0031] R_{13} 不存在或是 H、C_{1-6} 烷基、C_{3-6} 环烷基、未被取代的苯基或被 OH、卤素、CN、NO_2、NH_2、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基取代的苯基、或未被取代的苄基或被 OH、卤素、CN、NO_2、NH_2、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基取代的苄基。

[0032] 在另一更具体的实施方式中，本发明的 σ-2 拮抗剂是由式 II 表示的新化合物或其药学上可接受的盐：

[0033]



II

[0034] 其中

[0035] R_1 和 R_2 独立选自 H、OH、卤素、CN、NO_2、NH_2、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、C_{3-7} 环烷基、NH(C_{1-4} 烷基)、N(C_{1-4} 烷基)$_2$、NH(C_{3-7} 环烷基)、NHC(O)(C_{1-4} 烷基)、SH、S(C_{1-6} 烷基)、C(O)OH、C(O)O(C_{1-4} 烷基)、C(O)(C_{1-4} 烷基) 和 C(O)NH(C_{1-4} 烷基)，或 R_1 和 R_2 连接在一起形成 -O-C_{1-4} 亚甲基-O-，并且其中 R_1、R_2、R_4、R_5 和 R_6 的至少一者不是 H；

[0036] R_3 选自 H、卤素和 C_{1-6} 卤代烷基；

[0037] R_4 = C_{1-6} 烷基；

[0038] R_5 是 H、C_{1-6} 烷基和 C(O)O(C_{1-4} 烷基)、C(O)(C_{1-4} 烷基)、C(O)(C_{1-4} 卤代烷基)；且

[0039] R_6、R_7、R_8 和 R_9 独立选自 H、OH、卤素、CN、NO_2、NH_2、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基。

[0040] 在一些实施方式中，本发明的 σ-2 拮抗剂与 σ-2 受体结合并且抑制 A β 寡聚体与神经元特别是突触的结合。在一些实施方式中，σ-2 拮抗剂与 A β 寡聚体竞争结合至神经元特别是突触，或以其他方式中断 A β 寡聚体结合神经元的能力，例如通过干扰 A β 寡聚体形成或结合至 A β 寡聚体或可能地干扰 A β 寡聚体在伴随结合至神经元时开始运动信号转导机制的能力。在某些实施方式中，σ-2 拮抗剂由此抑制非致死 A β 病理效应（“非致死 A β 病理”或“非致死 β 淀粉样蛋白病理”），除其它之外包括膜运输缺陷、突触功能障碍、动物中的记忆和学习缺陷、突触数目的减少、树突棘长度或棘形态的改变、或长时程增强效应 (LTP) 缺陷。换句话说，本发明者观察到，本文说明的在其它检定中具有活性的本发明的 σ-2 拮抗剂具有将神经元恢复到正常状态或干扰 A β 寡聚体诱导的突触功能障碍的能力。不拘泥于理论，本发明的 σ-2 拮抗剂干扰 A β 寡聚体结构、A β 寡聚体与神经元的结合或 A β 寡聚体诱导的分子信号转导机制中的一种或多种，这可用于对抗 A β 寡聚体的非致死效应以及治疗早期阶段的可溶 A β 寡聚体相关的病理。

[0041] 在一个实施方式中，本发明的 σ-2 拮抗剂是功能性神经元拮抗剂且用于抑制神经元细胞中的突触丧失的方法中，该丧失与细胞暴露于一种或多种 Abeta 寡聚体或其它 Abeta 复合物，或更广泛而言，包括单体或寡聚或其它可溶复合形式的 Abeta 肽（如下所述）的 Abeta 种类相关，该方法包括使所述细胞与一定量的一种或多种 σ-2 拮抗剂接触，该一定量可有效避免或减少所述丧失或使所述细胞中的突触数目部分或完全恢复到暴露前的水平。

[0042] 在另一实施方式中，本发明的 σ-2 拮抗剂用于调节神经元细胞中膜运输变化的方法中，所述变化与所述细胞暴露于一种或多种 Abeta 种类相关，该方法包括使所述细胞与一定量的一种或多种 σ-2 拮抗剂接触，该一定量可有效避免或减少所述膜运输变化，或使其保持在或接近所述细胞暴露于所述 Abeta 种类之前所观察到的水平。

[0043] 在另一实施方式中，本发明的 σ-2 拮抗剂用于治疗认知衰退的方法中，包括对受试者施用一种或多种本发明的 σ-2 拮抗剂。

[0044] 在又一实施方式中，本发明的 σ-2 拮抗剂是功能性神经元 σ-2 拮抗剂，其用于治疗认知衰退或神经退行性病症或突触功能和 / 或数量缺陷的方法中，该方法包括对受试者施用一种或多种本发明的 σ-2 拮抗剂。

[0045] 在一些实施方式中，本公开内容提供用于抑制 β 淀粉样蛋白寡聚体诱导的神经元细胞的突触功能障碍，以及用于抑制由神经元暴露于 Abeta 寡聚体所引起的海马长时程增强效应的阻抑的包括 σ-2 受体拮抗剂的组合物和方法。

[0046] 本发明提供对抑制认知衰退或治疗神经退行性疾病的化合物进行鉴定的方法，该方法包括

[0047] 使细胞与结合至 σ-2 受体的化合物接触，并确定所述化合物是否具有至少一种以下附加特性：

[0048] (a) 其抑制中枢神经元中的突触丧失，所述丧失与神经元暴露于 Abeta 寡聚体相关；

[0049] (b) 其抑制中枢神经元中的膜运输异常，该异常与所述细胞暴露于一种或多种 Abeta 寡聚体相关；

[0050] (c) 其抑制阿尔茨海默病动物模型中 Abeta 寡聚体介导的认知效应；或

[0051] (d) 其抑制阿尔茨海默病动物模型中基于海马的空间学习和记忆衰退。

[0052] 这样的对非致死 β 淀粉样蛋白病理的抑制包括抑制认知衰退、抑制神经元细胞中的突触丧失、和抑制神经元细胞中的膜运输变化的方法。

附图说明

[0053] 图 1A 是示出在膜运输检定中与含有甲臜的胞内囊泡在体外保持 21 天的初级海马和皮层培养物的显微照片，其中甲臜得自货物 (cargo) 四唑盐染料的内吞作用和化学还原。

[0054] 图 1B 是示出具有胞外甲臜晶体的同批培养物的显微图，其中胞外甲臜晶体在甲臜胞吐时在神经元和胶质细胞细胞膜的外部形成，且其中细胞在膜运输检定中已经暴露于 Abeta 寡聚体。该图示出人 Abeta1 ～ 42 寡聚体改变货物染料产物甲臜的表型（胞内囊泡 vs. 胞外晶体），因此造成细胞膜运输缺陷。

[0055] 图 1C 是示出胞内囊泡的显微图，其中细胞已经暴露于 Abeta 寡聚体和化合物 II 二者，化合物 II 为本发明的选择性高亲和性 σ -2 拮抗剂化合物。该图示出化合物 II 能够阻抑由 Abeta 寡聚体引起的膜运输缺陷，并将膜运输表型恢复至正常。

[0056] 图 1D 示出膜运输检定的定量结果，其中 y 轴表示在施用货物四唑盐染料后在指定时间包含在胞内囊泡中的甲臜产物的量（归一化为媒介物处理的值）。红色的圈表示 Abeta 寡聚体处理的培养物，蓝色方块表示媒介物处理的对照培养物，且黑色或灰色方块表示用多种浓度的 cpd II+Abeta 和 cpd IXa、IXb+Abeta 处理的培养物给出的值（当化合物在 Abeta 寡聚体之前添加时（预防））。在横坐标中使用化合物的对数浓度。该图示出化合物以剂量依赖的方式抑制 Abeta 寡聚体对膜运输的作用。

[0057] 图 1E 示出与图 1D 的图类型相同但化合物在 Abeta 寡聚体之后添加（治疗）的膜运输检定剂量响应曲线。在横坐标中使用化合物的对数浓度。该图示出化合物以剂量依赖的方式抑制 Abeta 寡聚体对膜运输的作用。

[0058] 图 1F 示出在多种浓度的单独合成 Abeta 寡聚体 (EC50820nM) 的存在下并具有多种浓度的化合物 II 和在各个浓度的所得囊泡（作为%媒介物）的与图 1D 的图类型相同的膜运输检定。通过递增浓度的化合物 II 的存在，表现出 EC50 的右移 (Schild 斜率 = -0.75)。该图表明，cpd II 在药理学上与寡聚体竞争靠近介导膜运输的分子靶标，因此化合物 II 的存在使得合成 Abeta 寡聚体具有更小的突触毒性。

[0059] 图 1G 示出在多种浓度的单独合成 Abeta 寡聚体的存在下并具有多种浓度的化合物混合物 IXa、IXb 和在各个浓度的所得囊泡（作为%媒介物）的与图 1D 的图类型相同的膜运输检定。通过递增浓度的化合物混合物 IXa、IXb 的存在，表现出 EC50 的右移 (Schild 斜率 = -0.51)。该图表明，cpd 混合物 IXa、IXb 在药理学上与寡聚体竞争靠近介导膜运输的分子靶标，因此化合物混合物 IXa、IXb 的存在使得合成 Abeta 寡聚体具有更小的突触毒

性。

[0060] 图 1H 示出在多种浓度的单独的得自人类阿尔茨海默病患者的 Abeta 寡聚体的存在下并具有多种浓度的化合物 II 和在各个浓度的所得囊泡（作为%媒介物）的与图 1D 的图类型相同的膜运输检定。通过递增浓度的化合物 II 的存在，表现出 EC50 的右移。该图表明，cpd II 在药理学上与寡聚体竞争靠近介导膜运输的分子靶标，因此化合物 II 的存在使得人类阿尔茨海默病相关的 Abeta 寡聚体具有更小的突触毒性。

[0061] 图 1I 示出在多种浓度的单独的得自人类阿尔茨海默病患者的 Abeta 寡聚体的存在下并具有多种浓度的化合物混合物 IXa、IXb 和在各个浓度的所得囊泡（作为%媒介物）的与图 1D 的图类型相同的膜运输检定。通过递增浓度的化合物混合物 IXa、IXb 的存在，表现出 EC50 的右移。该图表明，cpd 混合物 IXa、IXb 在药理学上与寡聚体竞争靠近介导膜运输的分子靶标，因此化合物混合物 IXa、IXb 的存在使得人类阿尔茨海默病相关的 Abeta 寡聚体具有更小的突触毒性。

[0062] 图 1J 示出在多种浓度的单独合成 Abeta 寡聚体的存在下并具有多种浓度的化合物 CF 和在各个浓度的所得囊泡（作为%媒介物）的与图 1D 的图类型相同的膜运输检定。通过递增浓度的化合物 CF 的存在，表现出 EC50 的右移。该图表明，cpd CF 在药理学上与寡聚体竞争靠近介导膜运输的分子靶标，因此化合物 CF 的存在使得合成 Abeta 寡聚体具有更小的突触毒性。

[0063] 图 1K 示出在多种浓度的单独合成 Abeta 寡聚体的存在下并具有多种浓度的化合物 W 和在各个浓度的所得囊泡（作为%媒介物）的与图 1D 的图类型相同的膜运输检定。通过递增浓度的化合物 W 的存在，表现出 EC50 的右移。该图表明，cpd W 在药理学上与寡聚体竞争靠近介导膜运输的分子靶标，因此化合物 W 的存在使得合成 Abeta 寡聚体具有更小的突触毒性。

[0064] 图 1L 示出使用从阿尔茨海默病患者中分离的 Abeta 寡聚体的膜运输检定结果。化合物 CF(20 微摩尔浓度）表现出与分离自 AD 患者的 Abeta 寡聚体在药理学上竞争靠近介导膜运输的分子靶标，因此化合物 CF 的存在使得人类阿尔茨海默病相关的 Abeta 寡聚体具有更小的突触毒性。

[0065] 图 1M 是显示在以下物质的存在下所识别（和定量）神经元的甲臜填充囊泡的百分比的运输检定结果的条形图，即在 (i) 单独媒介物（第一条）；(ii) 来自人阿尔茨海默病患者脑的 Abeta 寡聚体制剂（第二条，与第一条相比为显著降低）；(ii) 本文公开的化合物 II+Abeta 寡聚体（第三条，比第二条显著更高）；以及 (iv) 化合物 II 而无 Abeta 寡聚体（第四条，与第一条没有显著不同）的存在下。该图表明，化合物 II 阻抑由人类阿尔茨海默病相关的 Abeta 寡聚体所引起的膜运输缺陷，并将膜运输表型恢复至正常，但是当不存在 Abeta 寡聚体而仅有其自身给药时不影响膜运输。

[0066] 图 1N 是与图 J 类型相同但是示出使用从年龄匹配的组织学正常的人脑中分离的 Abeta 寡聚体制剂而产生的数据的条形图。该图表明，得自正常人脑的 Abeta 寡聚体并不显著影响膜运输，且 cpd II 在存在或不存在该寡聚体时不会进一步影响膜运输。

[0067] 图 2A 是药代动力学数据的图，其中示出单一皮下（空心三角）和静脉 (i.v.)（空心圆圈）施用化合物 II 时从血浆中（左纵坐标，ng/mL) 中得到的以及在单一 i.v. 施用化合物 II（填充圆圈）时在脑中（右纵坐标，ng/g) 得到的化合物 II 的浓度。已知化合物 II

经过首过代谢，因此皮下给药；然而，在急性给药后，化合物 II 为高度脑穿透性。该图表明，cpd II 在急性皮下给药时为高度脑穿透性。

[0068] 图 2B 是药代动力学数据的图，其中示出在每天一次皮下施用不同量的化合物 II(0.5mg/kg/ 天：填充的下三角；0.35mg/kg/ 天：填充的上三角；以及 0.1mg/ 天填充的正方形）并持续 5 天时在血浆（左纵坐标）中得到的以及在皮下施用相同量（分别是空心下三角、空心上三角和空心正方形）的化合物 II 时在大脑（右纵坐标）中得到的化合物 II 浓度。已知化合物 II 经过首过代谢，因此皮下给药；然而，在慢性给药后，化合物 II 为高度脑穿透性。该图表明，cpd II 在慢性皮下给药时为高度脑穿透性。

[0069] 图 2C 是药代动力学数据的图，其中示出在化合物 CB 的单一急性经口给药 (10mg/kg/ 天）时在血浆（左纵坐标，实心三角）和脑（右纵坐标，空心三角）中得到的在单一急性经口给药后获得的化合物 CB 的浓度。化合物 CB 在急性经口给药后为高度脑穿透性并表现出 50％的生物可得性，血浆半衰期为 3.5 小时。该图表明，cpd CB 在急性经口给药时为高度脑穿透性。

[0070] 图 2D 是药代动力学数据的图，其中示出在每天一次经口施用化合物 CB 时 (10mg/kg/ 天，直立的三角）或 30mg/kg/ 天（反转的三角）时在慢性每天一次经口给药并持续 5 天后获得的在血浆（左纵坐标，实心三角）和脑（右纵坐标，空心三角）中得到的化合物 CB 的浓度。化合物 CB 在慢性经口给药后为高度脑穿透性并在高达 5 天的每天一次经口给药时表现出脑 / 血浆比为 3。该图表明，cpd CB 在慢性经口给药时为高度脑穿透性。

[0071] 图 3A- 板块 A 是不存在化合物 IXa、IXb 时暴露于 Abeta 寡聚体体外保持 21 天的初级海马和皮层培养物的荧光显微图；Abeta（经单克隆抗体 6E10 免疫标记而可视化）与突触处的包括神经元突触后棘的细胞膜结合。

[0072] 图 3A- 板块 B 处于与图 3A- 板块 A 中所见相同的视野，示出与阴性对照（未示出）相比，突触数量（经突触素免疫标记而可视化）在 Abeta 寡聚体的存在下减少。

[0073] 图 3A- 板块 C 是不存在化合物 IXa、IXb 时暴露于 Abeta 寡聚体体外保持 21 天的初级海马和皮层培养物的较低分辨率的荧光显微图；Abeta（经单克隆抗体 6E10 免疫标记而可视化）与突触处的包括神经元突触后棘的细胞膜结合。

[0074] 图 3A- 板块 D 示出存在化合物 IXa、IXb 时暴露于 Abeta 寡聚体体外保持 21 天的初级海马和皮层培养物的同批次培养物；与包括神经元突触后棘的细胞膜结合的 Abeta 量在视觉上减少。

[0075] 图 3B- 板块 A 是存在化合物 IXa、IXb 时暴露于 Abeta 寡聚体体外保持 21 天的初级海马和皮层培养物的同批次培养物的荧光显微图；与包括神经元突触后棘的细胞膜结合的 Abeta 量在视觉上减少。该图表明，化合物 IXa、IXb 的存在 (i) 使得与包括神经元突触后棘的细胞膜结合的 Abeta 寡聚体的含量显著减少。在化合物 II 的存在下可以见到相似的保护作用（数据未示出）。

[0076] 图 3B- 板块 B 处于与图 3A- 板块 C 中所见相同的视野，示出突触数量（经突触素免疫标记而可视化）在化合物 IXa、IXb 的存在下恢复，其与图 3B 相比具有增加的可视突触素。该图表明，化合物混合物 IXa、IXb 显著地阻断 Abeta 寡聚体引发的突触丧失。在化合物 II 的存在下可以见到相似的保护作用（数据未示出）。

[0077] 图 3C 是以突触丧失检定实验的条形图对图 3A- 板块 A ～ D 所示数据的定量结果。

突触丧失提供对于认知功能的最紧密相关。在突触丧失检定中，与媒介物相比，Abeta 寡聚体在体外引起 18.2%突触丧失。化合物 II 或化合物混合物 IXa、IXb 的存在完全消除该突触衰退。当化合物仅以媒介物而不含 Abeta 寡聚体给药时，不起作用。具体而言，通过对神经元的荧光显微图的突触素 - 免疫标记区域的数量、强度和面积的基于图像操作的定量来计算突触计数，其表示为与阴性对照（媒介物）的百分比，其中神经元暴露于单独媒介物（第一条）、媒介物与化合物 IXa、IXb 或媒介物与化合物 II（分别为第二条和第三条，示出化合物对突触数量没有作用）、Abeta 寡聚体（第四条，示出与第一条相比有显著的突触计数降低）以及化合物 IXa、IXb 或 II 存在下的 Abeta 寡聚体（第五条和第六条，示出与第一条相比没有突触数量的降低）。该图表明，化合物 IXa、IXb 和 II 表现出保护性作用并阻断 Abeta 寡聚体引发的突触数量减少。

[0078] 图 3D 是以 Abeta 结合强度的条形图对图 3A- 板块 A ～ D 中所示数据的定量结果，通过在将 Abeta 单独添加至媒介物（第一条）以及在 Abeta 与化合物 II 或化合物混合物 IXa、IXb 共存下它们显著降低时的荧光显微图的 6E10 免疫标记区的数量、强度和面积的基于图像处理的定量来计算 Abeta 结合强度。该图表明，化合物 IXa、IXb 和 II 降低与细胞膜结合的 Abeta 的量。

[0079] 图 4 是记忆表现的条形图，记忆表现通过体内条件性恐惧检定中僵立行为的百分比而测量，僵立行为在基线训练和训练后 24 小时对施用单独媒介物（第一条）、媒介物 +Abeta 寡聚体（第二条）、化合物 II+Abeta 寡聚体（第三条）和单独化合物 II（第四条）的小鼠并且在施用单独媒介物（第一条）、媒介物 +Abeta 寡聚体（第二个显著降低的条）、化合物 II+Abeta 寡聚体（第三条）和单独化合物 II 之后 24 小时时进行测量。与媒介物 (N = 18) 相比，Abeta 寡聚体（单一的 200 纳摩尔海马内注射）在 3 ～ 4 月龄雄性野生型 C57BL/6 小鼠 (N = 16) 中产生记忆形成的显著缺陷。化合物 II（在寡聚体一小时前单次 2 微摩尔海马内注射）消除由 Abeta 寡聚体引起的记忆缺陷 (N = 11)。单独化合物没有作用，且没有观察到不利的行为效应。该图表明，化合物 II 可以预防 Abeta 寡聚体引发的记忆缺陷，而当仅其自身给药时对记忆表现没有作用。

[0080] 图 5 是与图 4 相同类型的条形图，示出当动物用 (i) 单独媒介物（第一条）、(ii) Abeta 寡聚体（第二条，示出测试动物获取新记忆的能力有显著降低）、(iii) 化合物 IXa 和 IXb 的混合物（第三条，示出对 Abeta 寡聚体引发的记忆缺陷的完全（且统计学显著的）抑制）、或 (iv) 化合物 IXa 和 IXb 的混合物（不存在 Abeta 寡聚体）（第四条，示出对记忆无作用）处理时，通过在与产生图 4 相同的情境条件性恐惧检定中的僵立行为而测量的记忆表现。没有观察到不利的行为作用。该图表明，化合物混合物 IXa、IXb 可以预防 Abeta 寡聚体引发的记忆缺陷，而当仅其自身给药时对记忆表现没有作用。

[0081] 图 6A 示出来自正常患者、路易体痴呆症 (DLB) 患者或阿尔茨海默病 (AD) 患者的人额皮层组织切片中（左板块）的 [^{3}H]-(+)- 喷他佐辛（σ -1 受体配体）的放射自显影结合，其中 BS 是特异性结合，且 BNS 是非特异性结合；（右板块）示出来自对照（正常）、DLB 或 AD 患者的放射自显影实验的 [^{3}H] 喷他佐辛的平均特异性结合的图。与年龄匹配的与 AD 中神经元丧失度平行的对照脑相比，阿尔茨海默病脑中的 σ -1 受体在统计学上更低。该图表明，σ -1 受体表达可以在阿尔茨海默病脑中保持恒定。

[0082] 图 6B 示出来自正常患者、路易体痴呆症 (DLB) 患者或阿尔茨海默病 (AD) 患者的

相邻人额皮层组织切片中（左板块）的 [^{125}I]-RHM-4（σ-2 受体配体）的放射自显影结合；（右板块）示出来自对照（正常）、DLB 或 AD 患者的放射自显影实验的 [^{125}I]RHM-4 的平均特异性结合的图。与年龄匹配的对照脑相比，阿尔茨海默病和路易体痴呆症脑中的 σ-2 受体没有在统计学上更低，尽管在这些疾病中看到神经元损失。该图表明，成活的神经元和/或神经胶质中的 σ-2 受体表达可能在 DLB 和阿尔茨海默病脑中上调。

[0083] 图 6C 示出（左板块）在猴的额皮层、猴的海马或人颞叶皮层中 σ-2 配体对 18.4nM[^{3}H]-RHM-1（一种 σ-2 受体配体）的替代，以及（右板块）具有和不具有 1μM 西拉美新与化合物 IXa、IXb 和 II 中各一组时的 [^{3}H]-RHM-1 的结合密度图。该图表明，化合物 II 和 IXa、IXb 混合物竞争性地替代已知的放射性标记的 σ-2 配体例如猴和人脑组织切片中 σ-2 受体的 [^{3}H]-RHM-1，因此两种化合物均与 σ-2 受体结合。

[0084] 图 7A 示出在用 σ 化合物处理 48 小时的 SKOV-3 人卵巢癌细胞系中，MTS 检定中 σ-2 受体激动剂对细胞成活性的肿瘤细胞细胞毒性。σ-2 激动剂（西拉美辛、SV-119、WC-26）杀死肿瘤细胞。σ-2 拮抗剂（RHM-1、IXa 与 IXb、和 II）仅在不存在激动剂时在高得多的浓度下杀死肿瘤细胞。该图表明，cpd II 和 IXa、IXb 在该检定中表现与已知的 σ-2 拮抗剂相似，因此暗示着它们是肿瘤细胞中的 σ-2 拮抗剂。

[0085] 图 7B 示出在用 σ-2 化合物处理 24 小时后的神经元培养物中 σ-2 受体激动剂对细胞核强度变化的神经元细胞细胞毒性。σ-2 激动剂（西拉美辛、SV-119、WC-26）造成神经元中的异常细胞核形态。σ-2 拮抗剂（RHM-1、IXa 与 IXb、和 II）不会如此。该图表明，cpd II 和 IXa、IXb 在该检定中表现与已知的 σ-2 拮抗剂相似，因此暗示着它们是初级海马和皮层细胞中的 σ-2 拮抗剂。

[0086] 图 8A 示出 SKOV-3 人卵巢癌细胞中由 σ-2 激动剂西拉美新引发的半胱氨酸天冬氨酸酶 -3 活性，而 σ-2 受体拮抗剂 RHM-1、化合物 II、和 IXa 与 IXb 不引起半胱氨酸天冬氨酸酶 -3 活性。Abeta 寡聚体造成低水平的半胱氨酸天冬氨酸酶 -3 激活并引起 LTD。高水平的寡聚体和半胱氨酸天冬氨酸酶 -3 引起细胞死亡。σ-2 受体激动剂（SV-119、西拉美新）激活肿瘤细胞和神经元中的半胱氨酸天冬氨酸酶 -3；σ-2 拮抗剂则不这样（图 10A 和 10B）。该图表明，cpd II 和 IXa、IXb 在该检定中表现与已知的 σ-2 拮抗剂相似，因此暗示着它们是肿瘤细胞中的 σ-2 拮抗剂。

[0087] 图 8B 示出神经元中由 σ-2 激动剂西拉美新引发的半胱氨酸天冬氨酸酶 -3 活性，而 σ-2 受体拮抗剂 RHM-1、化合物 II、和 IXa 与 IXb 不引起半胱氨酸天冬氨酸酶 -3 活性。该图表明，cpd II 和 IXa、IXb 在该检定中表现与已知的 σ-2 拮抗剂相似，因此暗示着它们是初级海马和皮层细胞中的 σ-2 拮抗剂。

[0088] 图 8C 示出 SKOV-3 人卵巢肿瘤细胞中由 σ-2 受体激动剂 SV-119 引发的半胱氨酸天冬氨酸酶 -3 激活。σ-2 受体拮抗剂化合物 IXa 与 IXb 以及 II、RHM-1 不阻断肿瘤细胞中由 σ-2 受体激动剂 SV-119 引起的半胱氨酸天冬氨酸酶 -3 激活。该图表明，cpd II 和 IXa、IXb 在该检定中表现与已知的 σ-2 拮抗剂相似，因此暗示着它们是肿瘤细胞中的 σ-2 拮抗剂。

[0089] 图 8D 示出在多种浓度激动剂下 24 小时后神经元培养物中由 σ-2 受体激动剂 SV-119 引起的半胱氨酸天冬氨酸酶 -3 激活。该图表明，σ-2 受体拮抗剂化合物 IXa、IXb 和 II 阻断而 RHM-1 不阻断初级海马和皮层细胞中由 σ-2 受体激动剂 SV-119 引起的半胱

氨酸天冬氨酸酶 -3 激活。

[0090] 图 9A 示出通过体内条件性恐惧检定中僵立行为的百分比而测量的记忆表现，其中在经口施用多种剂量的 σ -2 受体拮抗剂化合物 5.5 个月后，在 15 月龄雄性转基因阿尔茨海默病小鼠模型中，在训练 24 小时后以 1 ～ 3 分钟测量僵立行为的百分比。与用媒介物处理的 Tg 动物相比 (Mann-Whitney U 测试)，记忆缺陷的显著改善发生在用 10 和 30mg/kg/天的 CB($p<0.05$) 和 30mg/kg/ 天的 CF($p<0.005$) 处理的转基因动物中。该图表明，化合物 CB 和 CF 在慢性长期给药后逆转转基因阿尔茨海默病小鼠中已建立的记忆缺陷。

[0091] 图 9B 示出 9 月龄雌性转基因 (Tg) 阿尔茨海默病小鼠的行为数据的条形图，该小鼠在用媒介物 p.o. 处理 39 天时，相比于媒介物处理的非转基因同窝仔，在 Y- 迷宫 (%交替) 中表现出显著的记忆缺陷 (即，媒介物处理的 Tg 小鼠随机表现 (at chance)，媒介物处理的非 Tg 同窝仔表现得显著更优于机率可见 (chance-see) 的紧邻于条形柱的星号和线)。用 Cpd. CF 以 30mg/kg/ 天口服处理 Tg 动物改善缺陷。没有观察到不利的行为效应。该图表明 cmpd CF 在慢性短期给药后逆转转基因阿尔茨海默病小鼠中已建立的记忆缺陷。

具体实施方式

[0092] 定义

[0093] 在详细描述本发明的化合物、组合物和方法之前，应理解本发明不限于所描述的具体操作、组合物或方法论，因为这些可以变化。还应理解，本说明书使用的术语仅出于描述具体的版本或实施方式的目的，而不意在限制本发明的范围，本发明的范围将由所附权利要求限定。除非另外限定，本文使用的所有技术和科学术语具有与本领域普通技术人员通常理解的相同的含义。尽管可以将任何与本文描述类似或等同的方法和材料用于本发明实施方式的实施或测试中，但现在将描述优选的方法、装置和材料。

[0094] 还应理解的是为清楚起见而描述于分开实施方式的上下文中的本发明的某些特征还可以在单个实施方式中组合提供。相反地，为简洁起见而描述于单个实施方式的上下文中的本发明的各种特征还可以单独或以任何合适的亚组合提供。

[0095] 定义

[0096] 单数形式“一”、“一个”和“该”包括复数提及，除非上下文另外有清楚规定。因此，例如，对“细胞”的提及是对一个或多个细胞以及本领域技术人员已知的其等同物的提及，等等。

[0097] 本文使用的术语“约”是指给定数值加减 10%。例如，“约 50%”是指 45% -55% 的范围。

[0098] “σ -2 配体”是指与 σ -2 受体结合的化合物，包括激动剂、拮抗剂、部分激动剂、反向激动剂和简单地该受体或蛋白的其它配体的竞争者。

[0099] 术语“激动剂”是指其存在引起受体的如下生物学活性的化合物，该生物学活性与由该受体的天然出现的配体的存在所引起的生物学活性相同。

[0100] 术语“部分激动剂”是指其存在引起受体的如下生物学活性的化合物，该生物学活性的类型与由该受体的天然出现的配体的存在所引起的生物学活性相同，但量级更低。

[0101] 术语“拮抗剂”是指其存在引起受体的生物学活性量级下降的实体，例如化合物。在某些实施方式中，拮抗剂的存在导致受体的生物学活性被完全抑制。σ -2 受体的“功能

性拮抗剂”是例如如在体外检定例如膜运输检定中或在行为检定中或在需要其的患者中所示地阻断 Abeta 寡聚体诱导的突触功能障碍的拮抗剂。功能性拮抗剂可以通过抑制例如 Abeta 寡聚体的结合而直接起作用，或通过干扰由 Abeta 寡聚体结合 σ-2 受体引起的下游信号转导而间接起作用。

[0102] 术语“选择性”或“选择性的”是指相比于非 σ 受体，对 σ-2 受体的结合亲和性 K_i 的差异。σ-2 拮抗剂对突触神经元中的 σ 受体具有高选择性。将对 σ-2 受体的 K_i 或对 σ-2 和 σ-1 受体两者的 K_i 与对非 σ 受体的 K_i 进行比较。在一方面，σ-2 或 σ-1/σ-2 选择性配体对 σ 受体的亲和性相比于对非 σ 受体高至少 10 倍、20 倍、30 倍、50 倍、70 倍、100 倍、500 倍，或更有选择性。非 σ 受体是，例如毒蕈碱 M1-M4 受体、血清素 (5-HT) 受体、α 肾上腺素受体、β 肾上腺素受体、阿片样受体、血清素转运体、多巴胺转运体、肾上腺素转运体、多巴胺受体或 NMDA 受体。

[0103] 在本申请中，术语“高亲和性”意指在 σ 受体结合检定中，例如对于 [^{3}H]-DTG，化合物表现出小于 600nM、500nM、400nM、300nM、200nM，小于 150nM，小于 100nM，小于 80nM，小于 60nM，或优选小于 50nM 的 K_i 值，如 Weber 等人，Proc. Natl. Acad. Sci (USA) 83:8784-8788 (1986) 中公开，其引入本文以供参考，其中测量了化合物对 σ-1 和 σ-2 受体位点两者的结合亲和性。特别优选的 σ 配体对于 [^{3}H]-DTG 表现出小于约 150nM、优选小于 100nM、小于约 60nM、小于约 10nM、或小于约 1nM 的 K_i 值。

[0104] “Abeta 种类”或“Aβ”应包括组合物，该组合物包括含有可溶淀粉样蛋白肽的成分，例如 Abeta 单体、Abeta 寡聚体、Abeta 肽（单体、二聚或聚合形式）与其它可溶肽或蛋白的复合物以及其它可溶 Abeta 组装体 (assembly)，包括淀粉样蛋白前体蛋白的任何加工的产物。已知可溶 Aβ 寡聚体是神经毒性的。甚至已知 $Aβ_{1-42}$ 二聚体破坏小鼠海马切片中的突触可塑性。在本领域已知的一个理论中，认为原生 $Aβ_{1-42}$ 单体是神经保护性的，且神经毒性需要 Aβ 单体自缔合为可溶 Abeta 寡聚体。然而，某些 Aβ 突变体单体（北极突变 (E22G)）被报道为与家族 AD 相关。参见例如 Giuffrida 等人，β-Amyloid monomers are neuroprotective. J. Neurosci. 200929 (34) :10582-10587。包括 Abeta 种类的制剂的非限制性实例公开于美国专利申请序列号第 13/021,872 号；美国专利公开第 2010/0240868 号；国际专利申请第 WO/2004/067561 号；国际专利申请第 WO/2010/011947 号；美国专利公开第 20070098721 号；美国专利公开第 20100209346 号；国际专利申请第 WO/2007/005359 号；美国专利公开第 20080044356 号；美国专利公开第 20070218491 号；WO/2007/126473；美国专利公开第 20050074763 号；国际专利申请第 WO/2007/126473 号，国际专利申请第 WO/2009/048631 号，和美国专利公开第 20080044406 号，其各自引入本文以作参考。

[0105] 当结合本发明的化合物使用时，“施用”是指将化合物直接施用到目标组织中或目标组织上或将化合物全身或局部地施用到患者或其它受试者。

[0106] 本文使用的术语“动物”包括但不限于人类和非人类脊椎动物，例如野生动物、家畜和农畜。

[0107] 本文使用的术语“受试者”、“个体”和“患者”可互换使用，且是指何动物，包括哺乳动物、小鼠、大鼠、其他啮齿动物、兔、狗、猫、猪、牛、羊、马、灵长类动物、非人灵长类动物、人等。

[0108] 本文使用的术语“使……接触”是指使分子（或具有更高级结构的分子例如细胞

或细胞膜）在一起或结合，使得它们处于允许分子间相互作用例如两个肽或一个蛋白与另一蛋白或其它分子（例如小分子）之间的非共价相互作用的距离内。在一些实施方式中，接触发生在所结合或所接触的分子在共同溶剂中混合并允许自由缔合的溶液中。一些实施方式中，接触可以在细胞处或细胞内或在无细胞的环境中发生。在一些实施方式中，无细胞的环境是由细胞产生的裂解物。在一些实施方式中，细胞裂解物可以是全细胞裂解物、核裂解物、细胞质裂解物及其组合。在一些实施方式中，无细胞的裂解物是从核提取和分离得到的裂解物，其中将细胞群的核从细胞中去除然后裂解。在一些实施方式中，不将核裂解，但仍视为是无细胞的环境。可以通过混合例如涡旋、振摇等使分子在一起。

[0109] 术语“改善”被用于表示本发明使得提供、应用或施用了本发明的组织的特性和/或身体属性改变。术语“改善”也可以结合疾病状态使用，这样当疾病状态被“改善”时，与疾病状态相关的症状或身体特性减弱、减小、消除、延迟或避免。

[0110] 术语“抑制”包括阻断、避免某个结果或过程或恢复相反的结果或过程。在预防或治疗方面，通过施用本发明的化合物，“抑制”包括（部分或全部地）保护免受或延迟症状的发作、缓解症状，或保护免受、减弱或消除疾病、病症或障碍。

[0111] 术语“抑制运输不足”是指阻断细胞优选神经元细胞中可溶 A β 寡聚体诱导的膜运输不足的能力。能够抑制运输不足的化合物在膜运输检定中的 EC50<20uM、小于 15uM、小于 10uM、小于 5uM 且优选小于 1 μ M，且还能够至少 50%、优选至少 60%、且更优选至少 70%地最大抑制可溶 Abeta 寡聚体诱导的膜运输不足的 Abeta 寡聚体效应。

[0112] 在本说明书的各处，本发明化合物的取代基公开为组或范围。具体意图是，本发明的实施方式包括这样的组和范围中的成员的各个和每个各自独立的亚组合。例如，术语“C_{1-6} 烷基”具体意在各自独立地公开甲基（C_1 烷基）、乙基（C_2 烷基）、C_3 烷基、C_4 烷基、C_5 烷基和 C_6 烷基。

[0113] 对于其中变量出现多于一次的本发明的化合物，各个变量可以是选自限定变量的马库什群组的不同组成成分。例如，当结构被描述为具有两个同时存在于同一化合物上的 R 基团时，则这两个 R 基团可以表示选自针对 R 进行限定的马库什群组的不同组成成分。

[0114] 其中 n 是整数的术语“n- 元”通常描述组成成分中成环原子的数目，其中成环原子的数目是 n。例如，吡啶是 6- 元杂芳环的实例，而噻吩是 5- 元杂芳基的实例。

[0115] 本文使用的术语“烷基”意指直链或支链的饱和烃基。实例烷基包括，但不限于，甲基 (Me)、乙基 (Et)、丙基（例如正丙基和异丙基）、丁基（例如正丁基、异丁基、叔丁基）、戊基（例如正戊基、异戊基、新戊基）等。烷基可以含有 1 至约 20、2 至约 20、1 至约 10、1 至约 8、1 至约 6、1 至约 4，或 1 至约 3 个碳原子。术语“亚烷基”指二价烷基连接基团。亚烷基的实例是亚甲基 (CH_2)。

[0116] 本文使用的“卤代烷基”是指具有一个或多个卤素取代基的烷基。实例卤代烷基包括，但不限于，CF_3、C_2F_5、CHF_2、CCl_3、$CHCl_2$、C_2Cl_5、CH_2CF_3 等。

[0117] 本文使用的“芳基”是指单环或多环（例如具有 2、3 或 4 个稠环）的芳香烃，例如苯基、萘基、蒽基、菲基、二氢茚基、茚基等。在一些实施方式中，芳基具有 6 至约 20 个碳原子。在一些实施方式中，芳基具有 6 至约 10 个碳原子。

[0118] 本文使用的“环烷基”是指非芳香族环烃，包括环化的烷基、烯基和炔基，其含有最多 20 个成环碳原子。环烷基可以包括单环或多环（例如具有 2、3 或 4 个稠环）的环体系

以及螺环体系。环烷基可以含有 3 至约 15、3 至约 10、3 至约 8、3 至约 6、4 至约 6、3 至约 5 或 5 至约 6 个成环碳原子。环烷基的成环碳原子可以任选地被氧 (oxo) 或硫 (sulfido) 取代。实例环烷基包括，但不限于，环丙基、环丁基、环戊基、环己基、环庚基、环戊烯基、环己烯基、环己二烯基、环庚三烯基、降冰片基、降蒎基 (norpinyl)、降莰基 (norcarnyl)、金刚烷基等。环烷基的定义中还包括的是具有一个或更多个稠合（即具有共用的键）到环烷基环的芳香环的组成成分，例如，戊烷、戊烯、己烷等的苯并或噻吩基衍生物（例如 2,3- 二氢 -1H- 茚 -1- 基，或 1H- 茚 -2(3H)- 酮 -1- 基）。优选地，“环烷基”是指含有最高 20 个成环碳原子的环化烷基。环烷基的实例优选地包括环丙基、环丁基、环戊基、环己基、环庚基、金刚烷基等。

[0119] 本文使用的“卤代”或“卤素”包括氟、氯、溴和碘。

[0120] 本文使用的“烷氧基”是指 -O- 烷基。实例烷氧基包括甲氧基、乙氧基、丙氧基（例如正丙氧基和异丙氧基）、叔丁氧基等。

[0121] 本文使用的“卤代烷氧基”是指 -O- 卤代烷基。实例卤代烷氧基是 OCF_3。本文使用的“三卤代甲氧基”是指具有三个卤素取代基的甲氧基。三卤代甲氧基的实例包括，但不限于 $-OCF_3$、$-OCClF_2$、$-OCCl_3$ 等。

[0122] 本文使用的“氨基”是指 NH_2。

[0123] 本文使用的“烷基氨基”是指被烷基取代的氨基。

[0124] 本文使用的“二烷基氨基”是指被两个烷基取代的氨基。

[0125] 本文使用的 C(O) 是指 C(= O)。

[0126] 本文使用的术语“任选地取代”意指取代是任选的，并且因此包括未被取代的和被取代的原子和组成成分两者。“取代的”原子或组成成分表明指定的原子或组成成分上任意的氢可以被来自所指明的取代基的选择代替，条件是不超过指定原子或组成成分的正常价态，并且取代产生稳定的化合物。例如，如果甲基（即 CH_3）被任选地取代，则碳原子上的 3 个氢原子可以被所指明的取代基代替。

[0127] 本文使用的“非致死 β 淀粉样蛋白效应”是指与 Abeta 种类接触的细胞的效应，特别是非致死效应。例如，已发现当神经元细胞与可溶 β 淀粉样蛋白（“Abeta”）寡聚体接触时，寡聚体在体外与神经元细胞的子集上的突触子集结合。该结合可以在例如测量体外 Abeta 寡聚体结合的检定中被定量。Abeta 种类的另一文献记载的效应是突触数目的减少，其已被报道在人海马中为约 18% (Scheff 等人，2007) 且可以被定量（例如，在测量突触数目的检定中）。作为另一例子，已发现，当神经元细胞与 β 淀粉样蛋白（“Abeta”）寡聚体接触时，膜运输被调节且继而发生膜运输的改变。该异常可以用许多检定包括但不限于 MTT 检定进行可视化。例如，黄色四唑盐被细胞内吞，且盐被位于内体途径中的囊泡内的酶还原为不溶性紫色甲臜。紫色甲臜的水平反映出培养物中的活性代谢细胞的数量，并且甲臜量的减少被认为是对培养物中的细胞死亡或代谢毒性的衡量。当通过显微镜观察与黄色四唑盐接触的细胞时，首先在填充细胞的胞内囊泡中可见紫色甲臜。随着时间的推移，囊泡被胞吐，并且当不可溶甲臜暴露于水性介质环境时，甲臜在质膜外表面上沉淀为针状结晶。Abeta 种类的其它效应包括认知衰退，例如形成新记忆的能力衰退和记忆丧失，其可以使用动物模型在体内检定中进行测量。

[0128] 在一些实施方式中，当与阴性对照相比，测试化合物可以将与可溶 Abeta 寡聚体

种类对于神经元细胞相关的效应抑制大于约 10%、优选大于 15%且优选大于 20%时，其被认为可有效治疗认知衰退或与其相关的疾病。在一些实施方式中，当与阳性对照相比，测试剂可以将淀粉样蛋白前体蛋白的加工产物所介导的效应抑制大于约 10%、优选大于 15%且优选大于 20%时，其被认为是有效的。例如，如以下实施例中所示，将 Abeta 寡聚体结合仅抑制 18%可完全抑制突触减少。例如，参见图 3C 和 3D。尽管本说明书关注对 Abeta 种类非致死效应例如神经元代谢的异常和突触数目减少的抑制，但显示这些与认知功能相关，而且随时间推移预期可引起淀粉样蛋白病理的下游可测症状的减少（与未治疗的受试者相比），尤其是临床症状，例如 1) 通过淀粉样蛋白成像剂例如 fluorbetapir、PittB 或任何其它成像剂而测量的原纤维或斑块聚集，2) 通过经 FDG-PET 检测的葡萄糖代谢减退而测量的突触丧失或细胞死亡，或 3) 通过经由 ELISA 的对得自患者的脑脊液、脑组织活检或血浆进行的成像或蛋白 / 代谢物检测而可检测的大脑或身体内的蛋白表达或代谢物量的改变（例如经 ELISA 测量的 Abeta42、磷酸化 tau、全部 tau 的水平或比率的改变，或 ELISA 板中可检测的蛋白表达改变的模式）（参见文献 :Wyss-Coray T. 等人 . Modeling of pathological traits in Alzheimer's disease based on systemic extracellular signaling proteome. Mol Cell Proteomics2011 年 7 月 8 日，其全部引入本文以供参考），4) 通过血管水肿的存在来测量的脑血管异常或通过 MRI 可检测的微出血和任何其它通过成像技术可检测的症状，以及 5) 通过任何施用的认知测试例如 ADAS-Cog、MMSE、CBIC 或任何其它认知测试仪器测量的认知丧失。

[0129] 本文使用的术语“神经元细胞”可以用来指单个细胞或细胞群。在一些实施方式中，神经元细胞是初级神经元细胞。在一些实施方式中，神经元细胞是永生化或转化的神经元细胞或干细胞。初级神经元细胞是不能分化成其他类型的神经元细胞的神经元细胞，例如神经胶质细胞。干细胞是可以分化成神经元和其它类型的神经元细胞例如神经胶质的细胞。在一些实施方式中，包含至少一种神经元细胞的组合物没有神经胶质细胞。在一些实施方式中，组合物包括少于约 30%、25%、20%、15%、10%、5%、或 1%的神经胶质细胞，已知其内化和聚集 Abeta。初级神经元细胞可以源自动物脑的任何区域。在一些实施方式中，神经元细胞是海马细胞或皮质细胞。可以通过任何方法确定神经胶质细胞的存在。在一些实施方式中，通过 GFAP 的存在检测神经胶质细胞，并且可以通过针对 MAP2 用抗体阳性染色来检测神经元。

[0130] 短语“药学上可接受的”是指通常被认为安全无毒的分子实体和组合物。具体地，本发明的药物组合物中使用的药学上可接受的载体、稀释剂或其它赋形剂是生理学上可耐受的，与其它成分相容的，且在对患者施用时通常不产生过敏或类似不宜反应（例如胃不适、眩晕等）。优选地，本文使用的术语“药学上可接受的”是指被联邦管制机构或州政府批准或列于美国药典或其它通常认可的用于动物尤其是人类的药典中。本文使用的短语“药学上可接受的盐”包括本发明化合物的用于哺乳动物安全有效且具有期望的生物学活性的盐。药学上可接受的盐包括本发明化合物中或根据本发明的方法识别的化合物中存在的酸基或碱基的盐。药学上可接受的酸加成盐包括，但不限于，盐酸盐、氢溴酸盐、氢碘酸盐、硝酸盐、硫酸盐、硫酸氢盐、磷酸盐、酸式磷酸盐、异烟酸盐、乙酸盐、乳酸盐、水杨酸盐、柠檬酸盐、酒石酸盐、泛酸盐、酒石酸氢盐、抗坏血酸盐、琥珀酸盐、马来酸盐、龙胆酸盐 (gentisinate)、富马酸盐、葡糖酸盐、葡糖二酸盐 (glucaronate)、糖酸盐、甲酸盐、苯甲酸

盐、谷氨酸盐、甲磺酸盐、乙磺酸盐、苯磺酸盐、对甲苯磺酸盐和双羟萘酸盐（即 1,1'- 亚甲基 - 双 -(2- 羟基 -3- 萘甲酸盐））。本发明的某些化合物可以与多种氨基酸形成药学上可接受的盐。合适的碱盐包括，但不限于，铝、钙、锂、镁、钾、钠、锌、铁和二乙醇胺盐。药学上可接受的碱加成盐也与胺例如有机胺形成。合适的胺的实例是 N, N'- 二苄基乙二胺、氯普鲁卡因、胆碱、二乙醇胺、二环己胺、乙二胺、N- 甲基葡糖胺和普鲁卡因。

[0131] 本文使用的术语“治疗的”是指用于治疗、对抗、改良、保护免受或改善受试者的不希望的病症或疾病的试剂。

[0132] 本文使用的术语“有效量”是指对具体的病症或病理过程的至少一种症状或参数引起可测量的抑制的量。例如，在 Abeta 寡聚体的存在下提供可测量的较低的突触减少的本发明的 σ -2 配体的量被认为是有效量，因为其减弱病理过程，即使淀粉样蛋白病理的临床症状没有被改变，至少没有被立即改变。

[0133] 本发明的化合物或组合物的“治疗有效量”或“有效量”是以适用于任何医疗的合理收益 / 风险比，对被治疗的受试者赋予治疗效果的预定量。治疗效果可以是客观的（即通过一些测试或标记物可测量的）或主观的（即受试者给出表示或感觉到效果或医师观察到变化）。本发明的化合物的有效量可以广泛地为约 0.01mg/Kg 至约 500mg/Kg、约 0.1mg/Kg 至约 400mg/Kg、约 1mg/Kg 至约 300mg/Kg、约 0.05 至约 20mg/Kg、约 0.1mg/Kg 至约 10mg/Kg、或约 10mg/Kg 至约 100mg/Kg。本文考虑的效果适当地包括医学治疗和 / 或预防处理。根据本发明施用以获得治疗和 / 或预防效果的化合物的具体剂量当然根据案例周围的具体环境确定，包括例如施用的化合物、施用途径、其它有效成分的共同施用、受治疗的病症、所采用的具体化合物的活性、所采用的具体组合物、患者的年龄、体重、一般健康状况、性别和饮食；施用时间、施用途径、所采用的具体化合物的排泄速率以及治疗时长。施用的有效量由医师鉴于前述相关情况并运用合理的医学判断来确定。本发明的化合物的治疗有效量通常是如下量，使得当其以生理上可耐受的赋形剂组合物施用时，足以在实现有效全身浓度或组织局部浓度。以单剂量或分剂量施用至人或其它动物的本发明化合物的每日总剂量的量可以是例如 0.01mg/Kg 至约 500mg/Kg、约 0.1mg/Kg 至约 400mg/Kg、约 1mg/Kg 至约 300mg/Kg、约 10mg/Kg 至约 100mg/Kg、或更通常约 0.1 ～ 25mg/kg 体重 / 天。单剂量组合物可以含有这样的量或其因数以组成日剂量。通常，根据本发明的治疗方案包括对需要这样的治疗的患者以单剂量或多剂量每天施用约 1mg 至约 5000mg、10mg 至约 2000mg 的化合物，20 ～ 1000mg，优选 20 ～ 500mg 且最优选约 50mg 的本发明化合物。

[0134] 本文使用的术语“治疗”是指治疗处理和预防或防护措施两者，其中目的是保护免受（部分或全部）或减慢（减轻）不期望的生理病症、障碍或疾病，或获得有益的或期望的临床结果，例如部分或完全恢复或抑制已经变得或将会变得异常的参数、值、功能或结果的减退。对于本发明的目的，有益的或期望的临床结果包括，但不限于，减轻症状；减小病症、障碍或疾病的发展程度或活力或速度；稳定（即不恶化）病症、障碍或疾病的状态；延迟病症、障碍或疾病的发作或减慢病症、障碍或疾病的进展；改良病症、障碍或疾病状态；以及缓解（无论是部分还是全部），不论其是否转化为实际的临床症状的立即减轻，或者增强或改善病症、障碍或疾病。治疗寻求引起临床上显著的响应而无过度水平的副作用。治疗还包括与如果不接受治疗的预期存活相比延长存活。

[0135] 一般而言，术语“组织”是指统一执行特定功能的相似的专门细胞的任何集合。

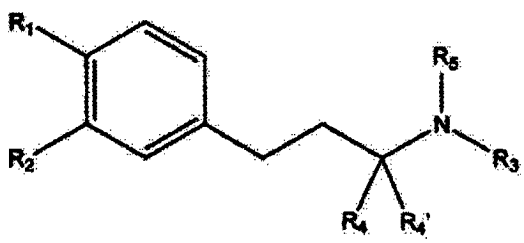
[0136] 本文使用的“认知衰退”可以是动物认知功能的任何负面变化。例如，认知衰退，包括但不限于，记忆丧失（例如行为记忆丧失）、无法获得新记忆、思维混乱、判断受损、人格改变、定向障碍、或其任意组合。因此，可有效治疗认知衰退的化合物可以通过以下而有效：恢复长时程神经元增强效应（LTP）或长时程神经元抑制效应（LTD）或电生理学测量的突触可塑性的平衡；抑制、治疗、和/或减轻神经退行性；抑制、治疗、和/或减轻一般淀粉样变性；抑制、治疗、减轻淀粉样蛋白产生、淀粉样蛋白组装、淀粉样蛋白聚集和淀粉样蛋白寡聚体结合中的一种或多种；抑制、治疗、和/或减轻一种或多种 Abeta 种类对神经元细胞的非致死效应（例如突触丧失或功能障碍和异常膜运输）；以及其任意组合。此外，该化合物也可以有效地治疗 Abeta 相关的神经退行性疾病和病症，包括但不限于：痴呆，其包括但不限于包括轻度阿尔茨海默病的阿尔茨海默病（AD）、唐氏综合征、血管性痴呆（脑淀粉样血管病和中风）、路易体痴呆、HIV 痴呆、轻度认知障碍（MCI）；年龄相关的记忆损害（AAMI）；年龄相关的认知衰退（ARCD）、临床前阿尔茨海默病（PCAD）；和非痴呆认知障碍（CIND）。本文使用的术语“天然配体”是指存在于受试者中的可以与蛋白质、受体、膜脂或体内的或在体外复制的其他结合配偶体结合的配体。天然配体可以是合成来源的，但也须在受试者中天然存在而无人为干预。例如，已知 Abeta 寡聚体在人类受试者中存在。因此，受试者中发现的 Abeta 寡聚体将被视为天然配体。Abeta 寡聚体与结合配偶体的结合可以使用重组或合成技术在体外进行复制，但不管 Abeta 寡聚体如何制备或制造，Abeta 寡聚体仍将被认为是天然配体。也可以与相同的结合配偶体结合的合成小分子如果在受试者中不存在，则其不是天然配体。例如，本文描述的化合物 II 通常不存在于受试者中，因此将不会被认为是天然配体。

[0137] 本发明的新化合物

[0138] 本文描述的化合物可以根据本文所述的方法或记载于 WO2011/014880（申请号 PCT/US2010/044136）、WO2010/118055（申请号 PCT/US2010/030130）、申请号 PCT/US2011/026530 和 WO2012/106426（申请号 PCT/US2012/023483）（其各自全部引入本文以供参考）中的方法进行合成。制备这些化合物的其它选择在下面详述。

[0139] 在一些实施方式中，本发明的 σ-2 拮抗剂是式 I 的化合物或其药学上可接受的盐：

[0140]

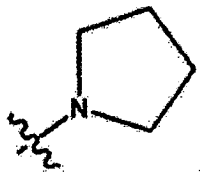
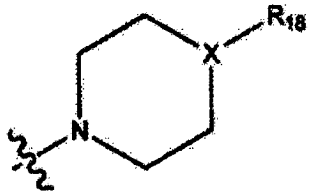


I

[0141] 其中

[0142] R_1 和 R_2 独立选自 H、OH、卤素、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、$(R_{16})(R_{17})N-C_{1-4}$ 亚烷基 -O-，或 R_1 和 R_2 连接在一起形成 $-O-C_{1-2}$ 亚甲基 -O- 基，其中

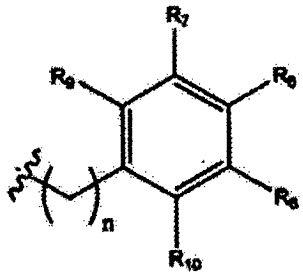
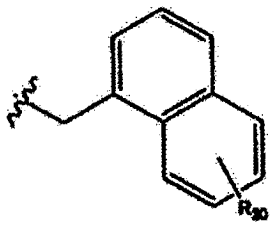
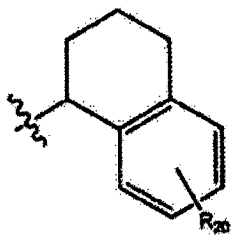
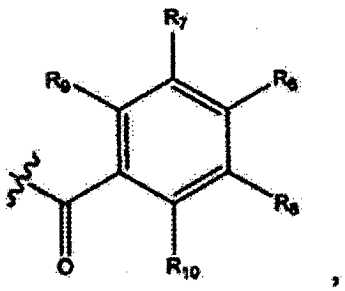
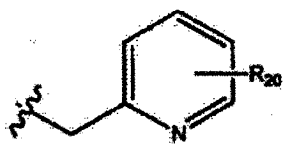
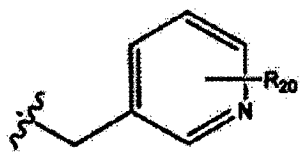
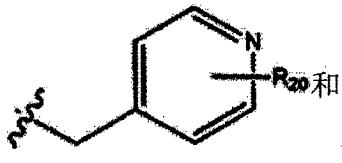
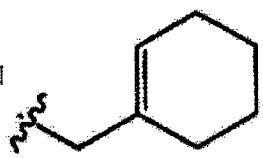
[0143] R_{16} 和 R_{17} 独立为 C_{1-4} 烷基或苄基，或 R_{16} 和 R_{17} 与氮一起形成选

[0144] 自  和  的环，其中

[0145] X 是 N 或 O 且 R_{18} 是 H 或未被取代的苯基；且

[0146] 其中 R_1 和 R_2 的至少一个不是 H；

[0147] R_3 选自

[0148]  ,  ,  ,  ,  ,  ,  和 

[0149] 其中

[0150] R_6、R_7、R_8、R_9 和 R_{10} 独立选自 H、卤素、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基和 $S(O)_2$-C_{1-6} 烷基；

[0151] R_{20} 是 H；且

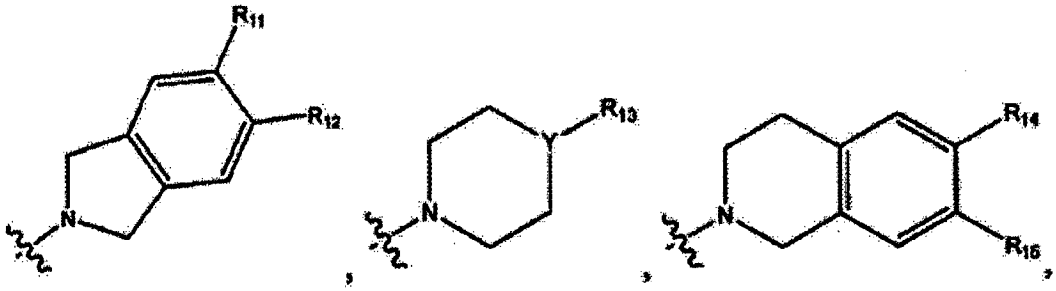
[0152] n 是 1-4

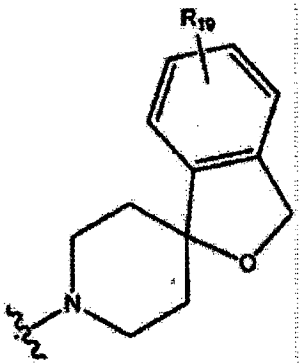
[0153] R_4 是 C_{1-6} 烷基；

[0154] $R_{4'}$ 是 H 或 C_{1-6} 烷基；且

[0155] R_5 是 H、C_{1-6} 烷基和 C(O)O(C_{1-4} 烷基)、C(O)(C_{1-4} 烷基) 或 C(O)(C_{1-4} 卤代烷基)；或

[0156] R_3 和 R_5 与氮一起形成选自

[0157] 

和  的环，其中

[0158] R_{11} 和 R_{12} 独立选自 H、卤素、和 C_{1-6} 卤代烷基，且

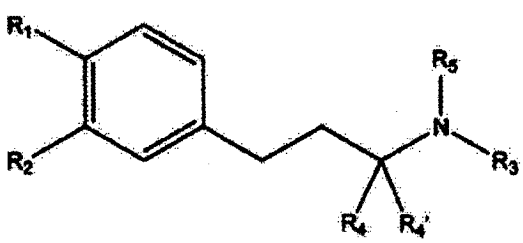
[0159] Y 是 CH 或 N；

[0160] R_{13} 是 H、C_{1-6} 烷基、C_{3-6} 环烷基、未被取代的苯基或被 C_{1-6} 卤代烷基取代的苯基、或未被取代的苄基；

[0161] R_{14} 和 R_{15} 独立选自 H 和卤素；

[0162] R_{19} 是 H。

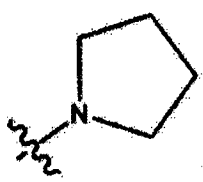
[0163] 在一些实施方式中，本发明的 σ-2 拮抗剂是式 I 的化合物或其药学上可接受的盐：

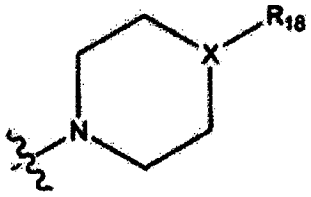
[0164] 

I

[0165] 其中

[0166] R_1 和 R_2 独立选自 H、OH、卤素、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、$(R_{16})(R_{17})N-C_{1-4}$ 亚烷基 -O-，或 R_1 和 R_2 连接在一起形成 $-O-C_{1-2}$ 亚甲基 -O- 基，其中

[0167] R_{16} 和 R_{17} 独立为 C_{1-4} 烷基或苄基，或 R_{16} 和 R_{17} 与氮一起形成选自  和



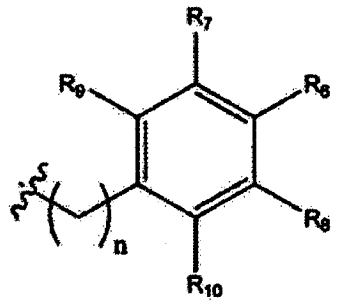
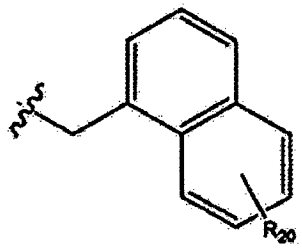
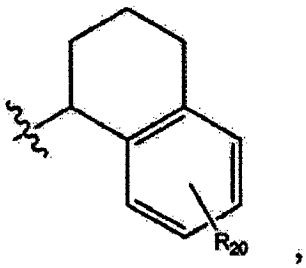
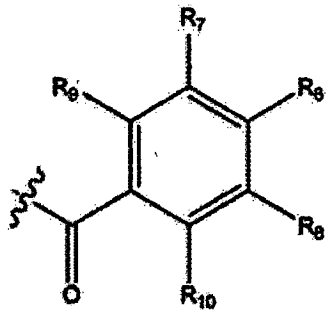
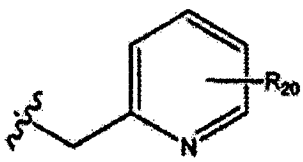
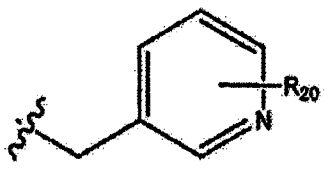
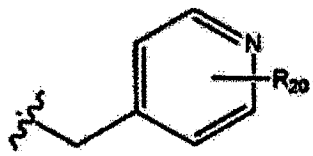
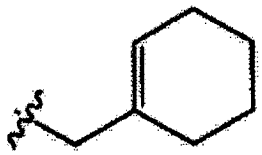
的环，其中

[0168] X 是 N 或 O 且 R_{18} 不存在或者是 H 或未被取代的苯基；且

[0169] 其中 R_1 和 R_2 的至少一个不是 H；

[0170] R_3 选自

[0171]


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和


[0172] 其中

[0173] R_6、R_7、R_8、R_9 和 R_{10} 独立选自 H、卤素、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基和 $S(O)_2$-C_{1-6} 烷基；

[0174] R_{20} 是 H；且

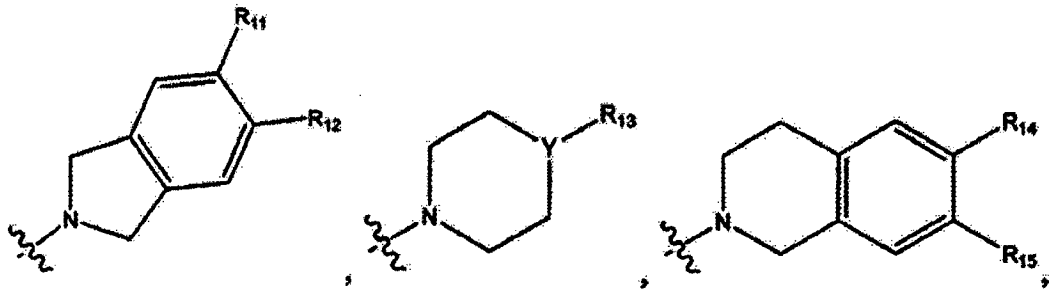
[0175] n 是 1-4

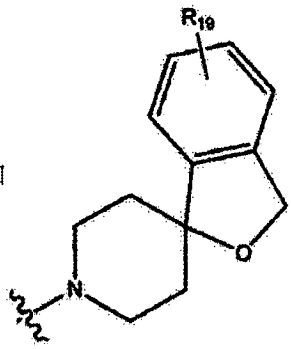
[0176] R_4 是 C_{1-6} 烷基；

[0177] $R_{4'}$ 是 H 或 C_{1-6} 烷基；且

[0178] R_5 是 H、C_{1-6} 烷基和 C(O)O(C_{1-4} 烷基)、C(O)(C_{1-4} 烷基) 或 C(O)(C_{1-4} 卤代烷基)；或

[0179] R_3 和 R_5 与氮一起形成选自

[0180] 

和  的环，其中

[0181] R_{11} 和 R_{12} 独立选自 H、卤素、和 C_{1-6} 卤代烷基，且

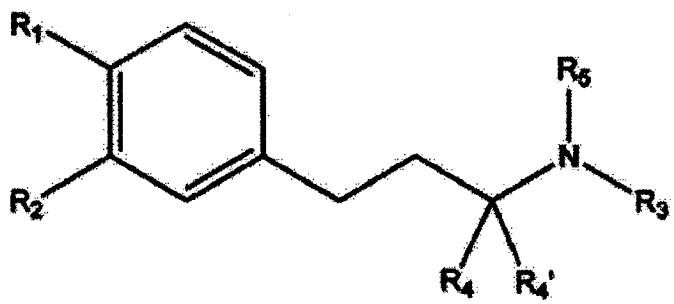
[0182] Y 是 CH 或 N；

[0183] R_{13} 是 H、C_{1-6} 烷基、C_{3-6} 环烷基、未被取代的苯基或被 C_{1-6} 卤代烷基取代的苯基、或未被取代的苄基；

[0184] R_{14} 和 R_{15} 独立选自 H 和卤素；且

[0185] R_{19} 是 H。

[0186] 在一些实施方式中，本发明的 σ-2 拮抗剂是式 I 的化合物或其药学上可接受的盐：

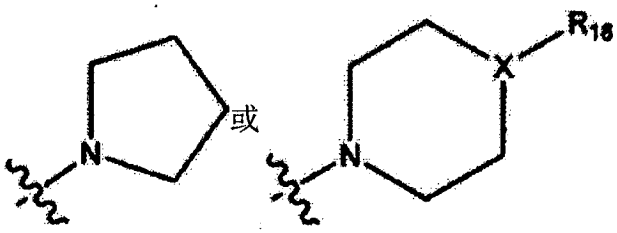
[0187] 

I

[0188] 其中

[0189] R_1 选自 OH、OMe、F、Cl、CF_3、$(R_{16})(R_{17})N$- 亚乙基 -O-，其中

[0190] R_{16} 和 R_{17} 各自是甲基、异丙基、正丁基或苄基，或 R_{16} 和 R_{17} 与氮一起形成选自



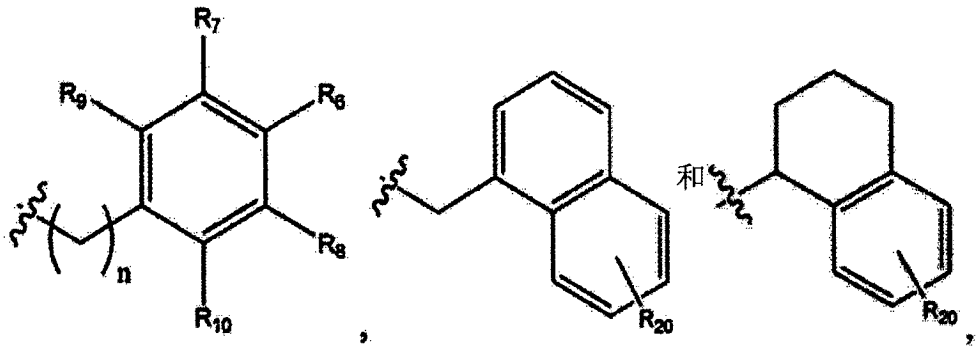
的环，其中

[0191] X 是 N 或 O 且 R_{18} 不存在或是未被取代的苯基；且

[0192] R_2 是 H、Cl、F、CF_3、OMe、OCF_3 或

[0193] R_1 和 R_2 连接在一起形成 -O-C_{1-2} 亚甲基 -O- 基

[0194] R_3 选自

[0195] 

[0196] 其中

[0197] R_6 是 H、F、Cl、Me、异丙基、叔丁基、OMe、CF_3、或 $S(O)_2Me$，

[0198] R_7 和 R_8 独立地为 H、OMe、F、Cl、或 CF_3；

[0199] R_9 和 R_{10} 独立选自 H、OMe、F、和 Cl，

[0200] R_{20} 是 H；且

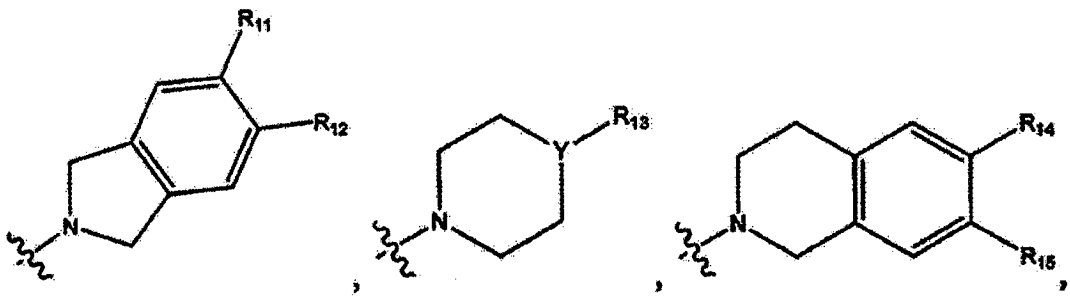
[0201] n 是 1

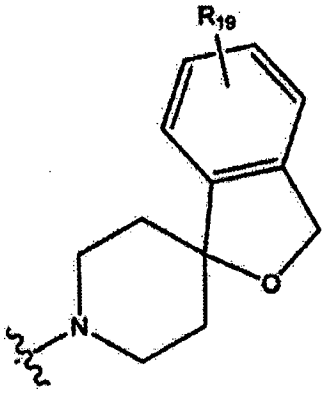
[0202] R_4 是 Me；

[0203] $R_{4'}$ 是 H 或 Me；且

[0204] R_5 是 H；或

[0205] R_3 和 R_5 与氮一起形成选自

[0206] 

和  的环，其中

[0207] R_{11} 和 R_{12} 独立选自 H、Cl、和 CF_3，且

[0208] Y 是 CH 或 N；

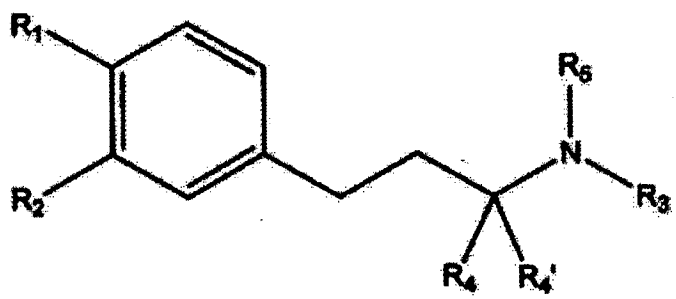
[0209] R_{13} 是 H、Me、环己基、未被取代的苯基或被 CF_3 取代的苯基、或未被取代的苄基

[0210] R_{14} 和 R_{15} 独立选自 H 和 Cl；且

[0211] R_{19} 是 H。

[0212] 在一些实施方式中，本发明的 σ-2 拮抗剂是式 I 的化合物或其药学上可接受的盐：

[0213]

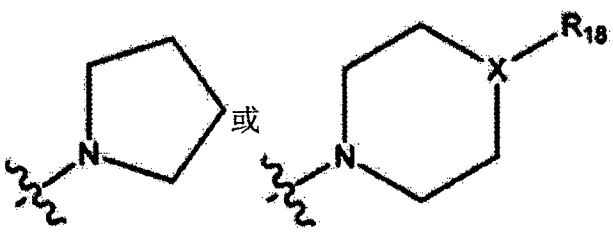


I

[0214] 其中

[0215] R_1 选自 OH、OMe、F、Cl、CF_3、$(R_{16})(R_{17})N$- 亚乙基 -O-，其中

[0216] R_{16} 和 R_{17} 各自是甲基、异丙基、正丁基或苄基，或 R_{16} 和 R_{17} 与氮一起形成选自

 或 的环，其中

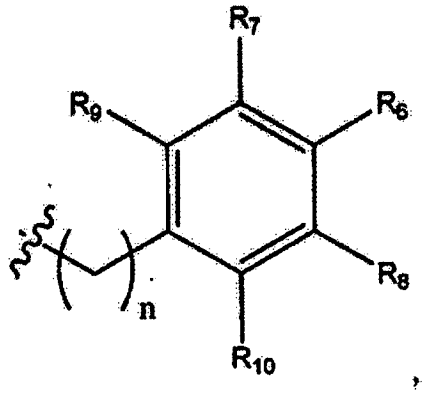
[0217] X 是 N 或 O 且 R_{18} 不存在或是未被取代的苯基；且

[0218] R_2 是 H、Cl、F、CF_3、OMe、OCF_3 或

[0219] R_1 和 R_2 连接在一起形成 -O-C_{1-2} 亚甲基 -O- 基

[0220] R_3 选自

[0221]



[0222] 其中

[0223] R_6 是 H、F、Cl、Me、异丙基、叔丁基、OMe、CF_3、或 $S(O)_2Me$，

[0224] R_7 和 R_8 独立地为 H、OMe、F、Cl、或 CF_3，

[0225] R_9 和 R_{10} 独立选自 H、OMe、F、和 Cl，且

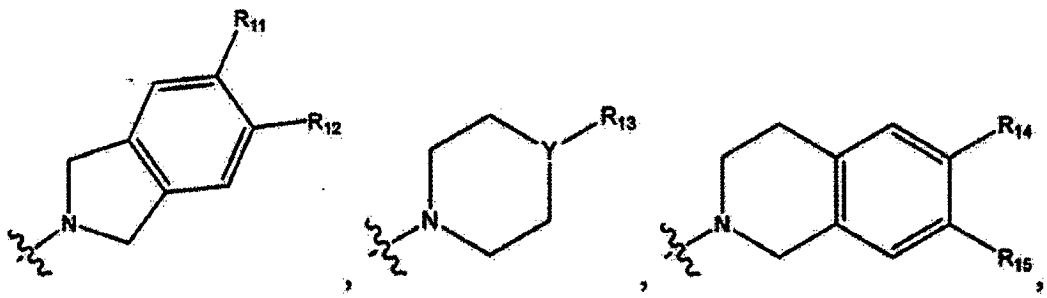
[0226] n 是 1

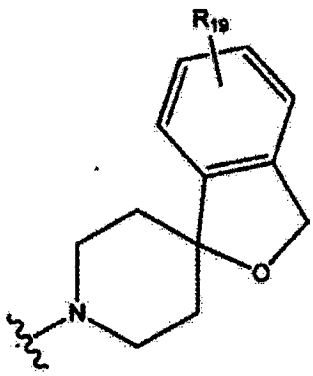
[0227] R_4 是 Me；

[0228] $R_{4'}$ 是 H；且

[0229] R_5 是 H；或

[0230] R_3 和 R_5 与氮一起形成选自

[0231] 

和  的环，其中

[0232] R_{11} 和 R_{12} 独立选自 H、Cl、和 CF_3，且

[0233] Y 是 CH 或 N；

[0234] R_{13} 是 H、Me、环己基、未被取代的苯基或被 CF_3 取代的苯基、或未被取代的苄基

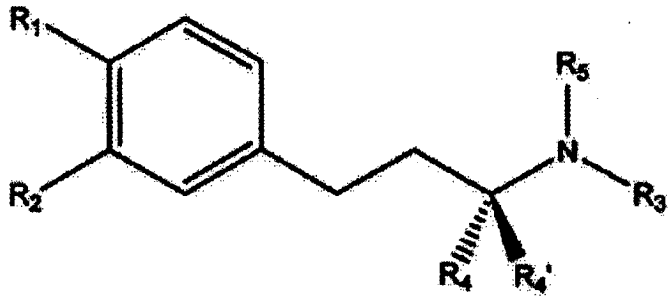
[0235] R_{14} 和 R_{15} 独立选自 H 和 Cl；且

[0236] R_{19} 是 H。

[0237] 在一些更加具体的实施方式中，本发明的 σ-2 拮抗剂是式 Ia 的化合物或其药学

上可接受的盐：

[0238]

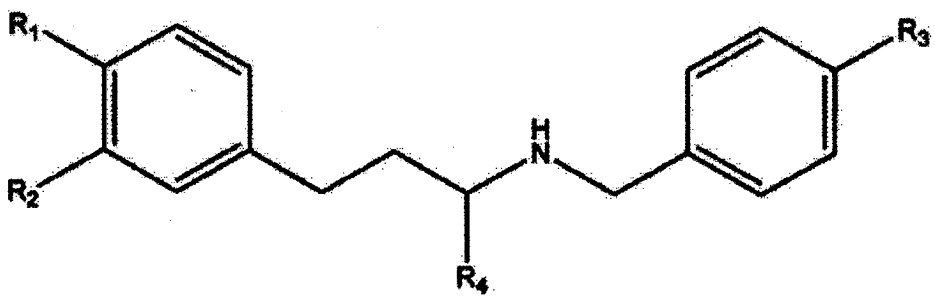


Ia

[0239] 其中 $R_{4'}$ 是 H 且剩余基团如以上针对式 I 的化合物所限定。

[0240] 在一些实施方式中，本发明的 σ-2 拮抗剂是式 IIa 的化合物或其药学上可接受的盐：

[0241]



IIa

[0242] 其中

[0243] R_1 = 卤素、C_{1-6} 卤代烷基或 OH；

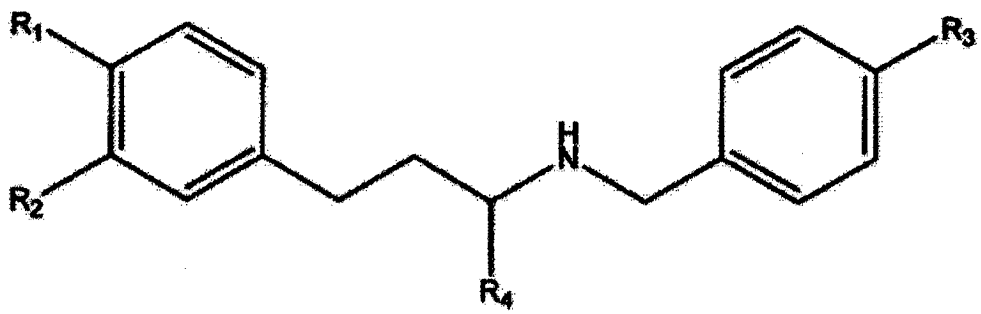
[0244] R_2 = H、卤素或 C_{1-6} 卤代烷基，或 R_1 和 R_2 连接在一起形成 -O- 亚甲基 -O- 基；

[0245] R_3 = C_{1-6} 卤代烷基；且

[0246] R_4 = C_{1-6} 烷基。

[0247] 在一些更加具体的实施方式中，本发明的 σ-2 拮抗剂是式 IIa 的化合物或其药学上可接受的盐：

[0248]



IIa

[0249] 其中

[0250] R_1 = Cl、F、CF_3、或 OH；

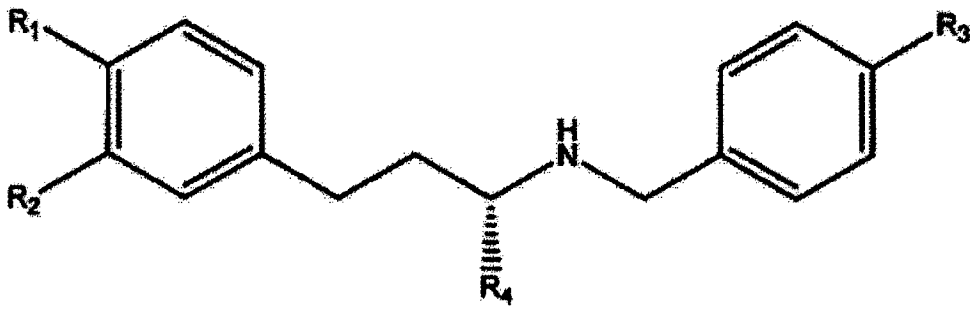
[0251] R_2 = H、Cl、F、CF_3，或 R_1 和 R_2 连接在一起形成 -O- 亚乙基 -O- 基；

[0252] R_3 = CF_3；且

[0253] R_4 = 甲基。

[0254] 在一些更加具体的实施方式中，本发明的 σ-2 拮抗剂是式 IIb 的化合物或其药学上可接受的盐：

[0255]

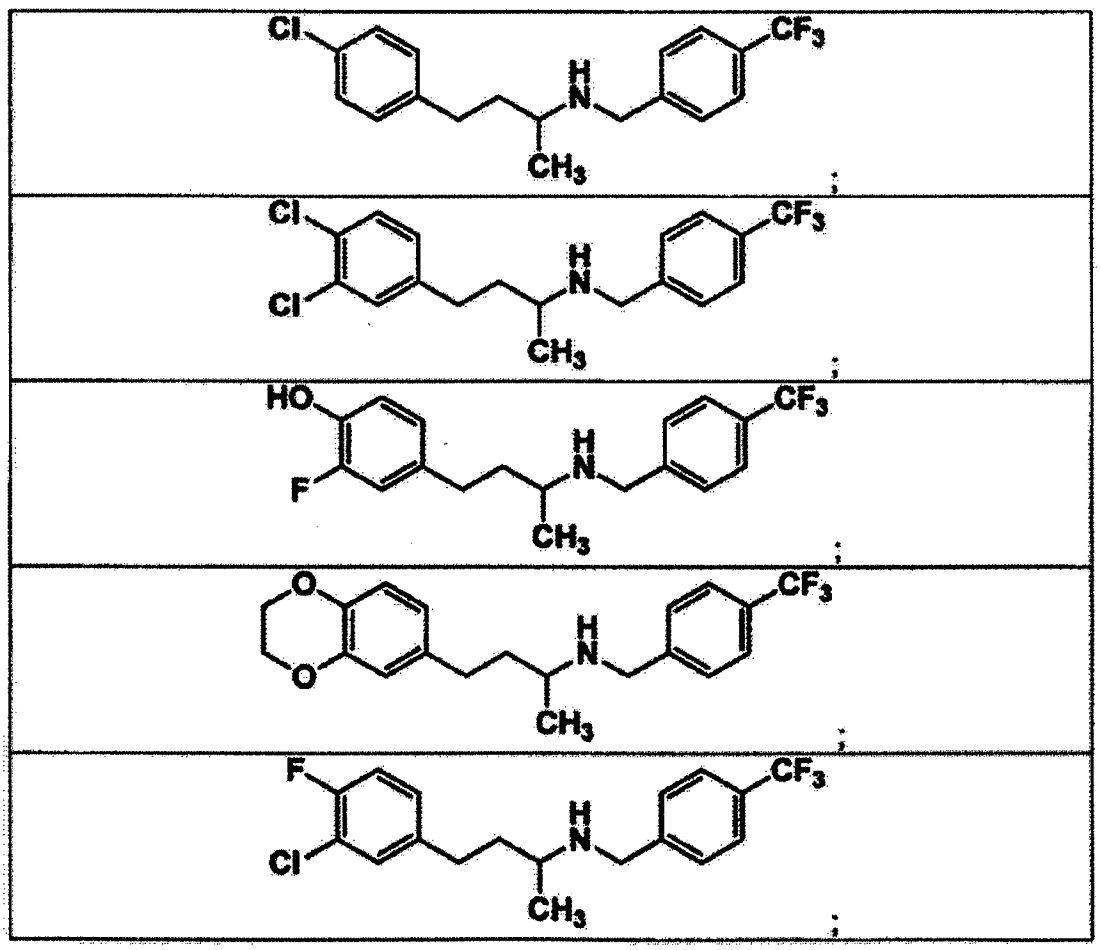


IIb

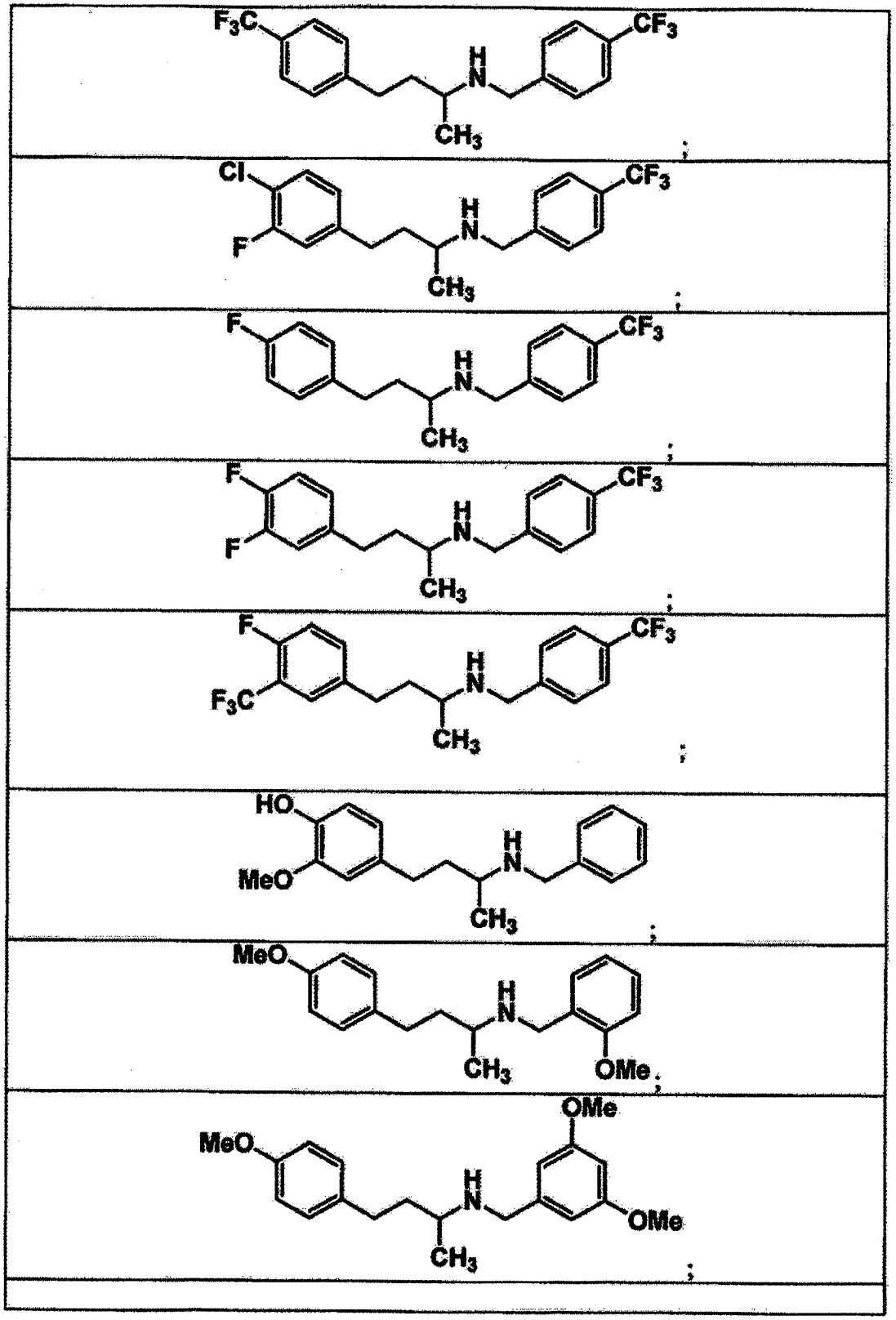
[0256] 其中 R_1-R_4 如以上针对式 IIa 的化合物所限定。

[0257] 本发明的具体示例性化合物在下表中给出：

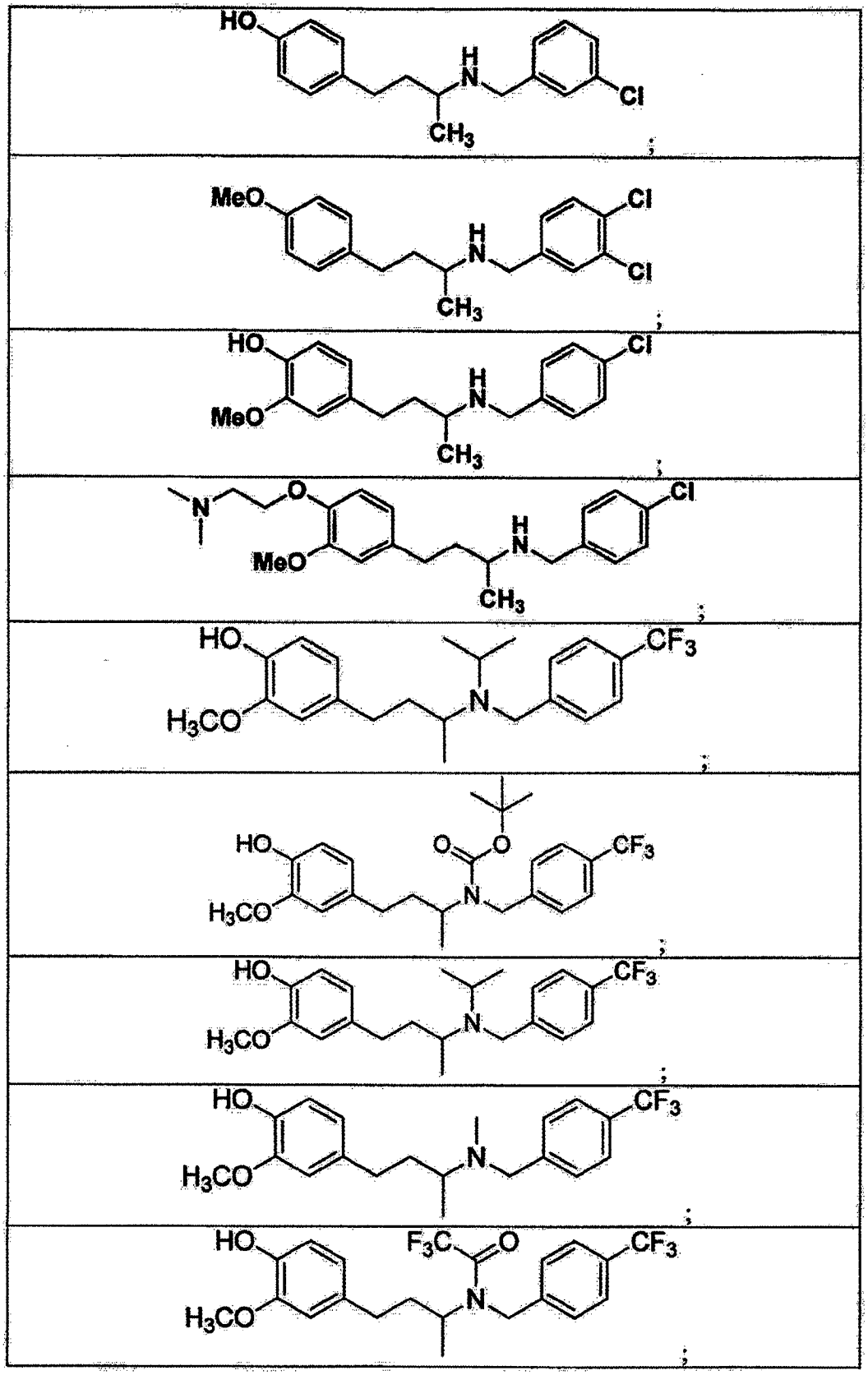
[0258]



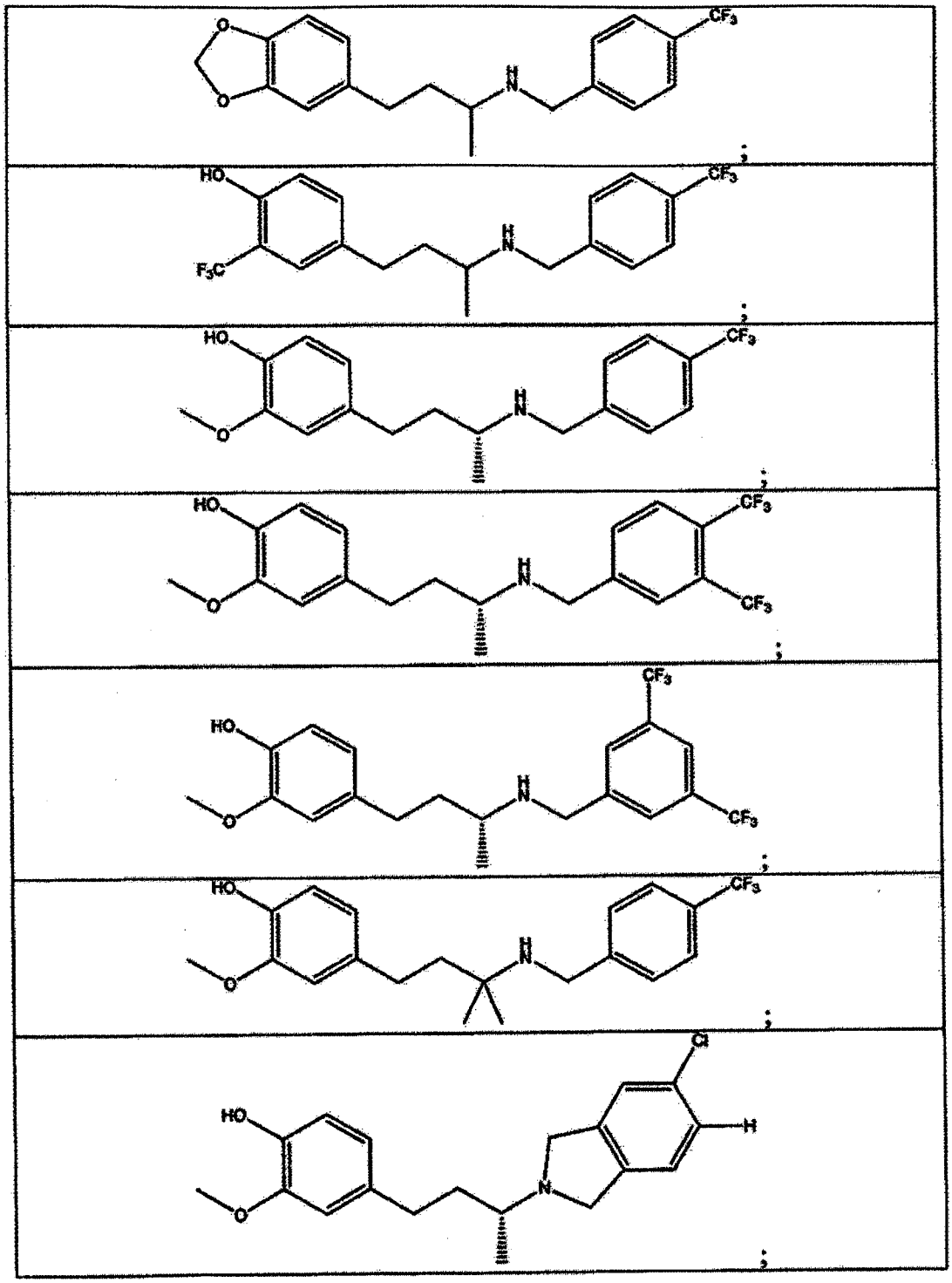
[0259]



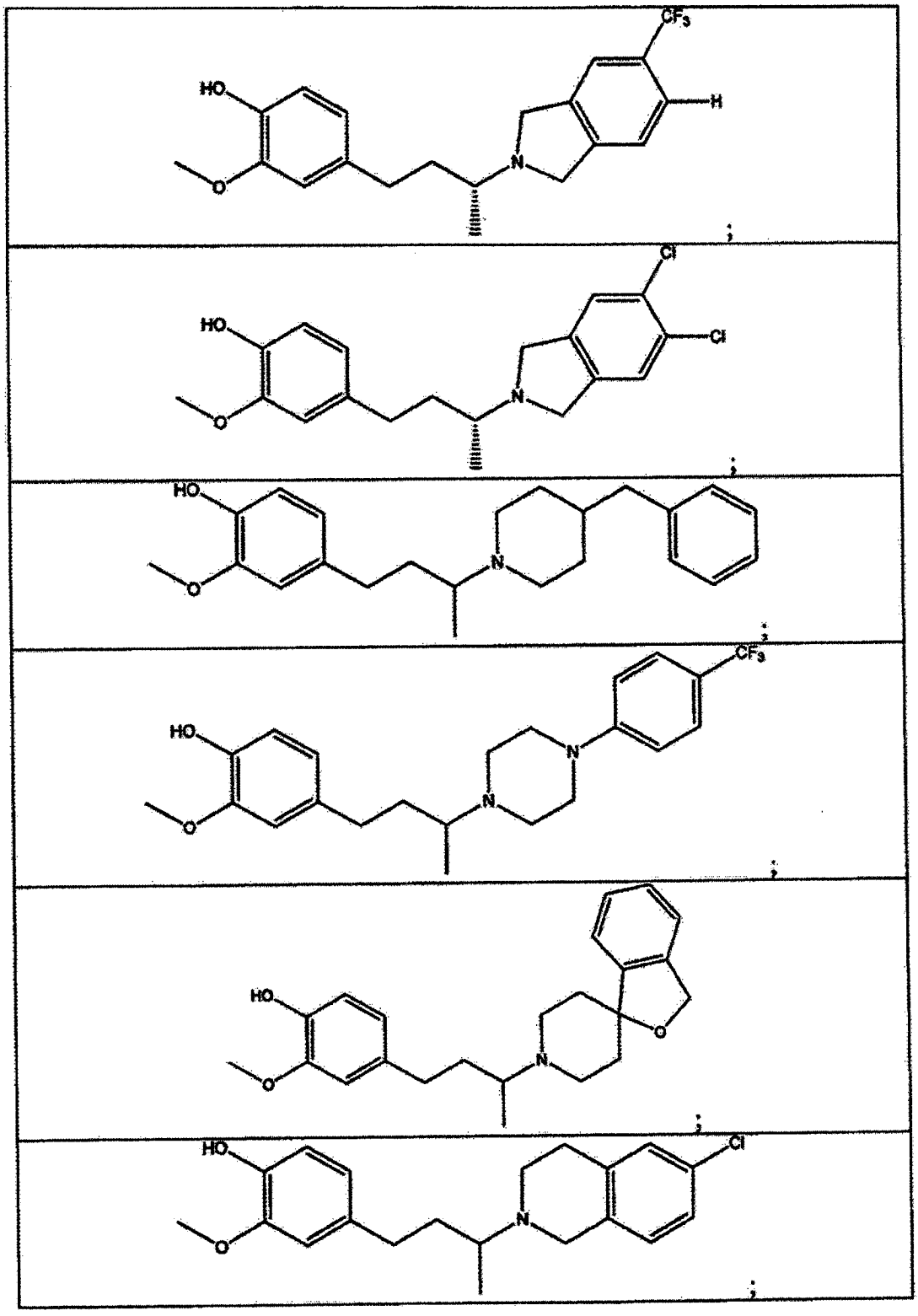
[0260]



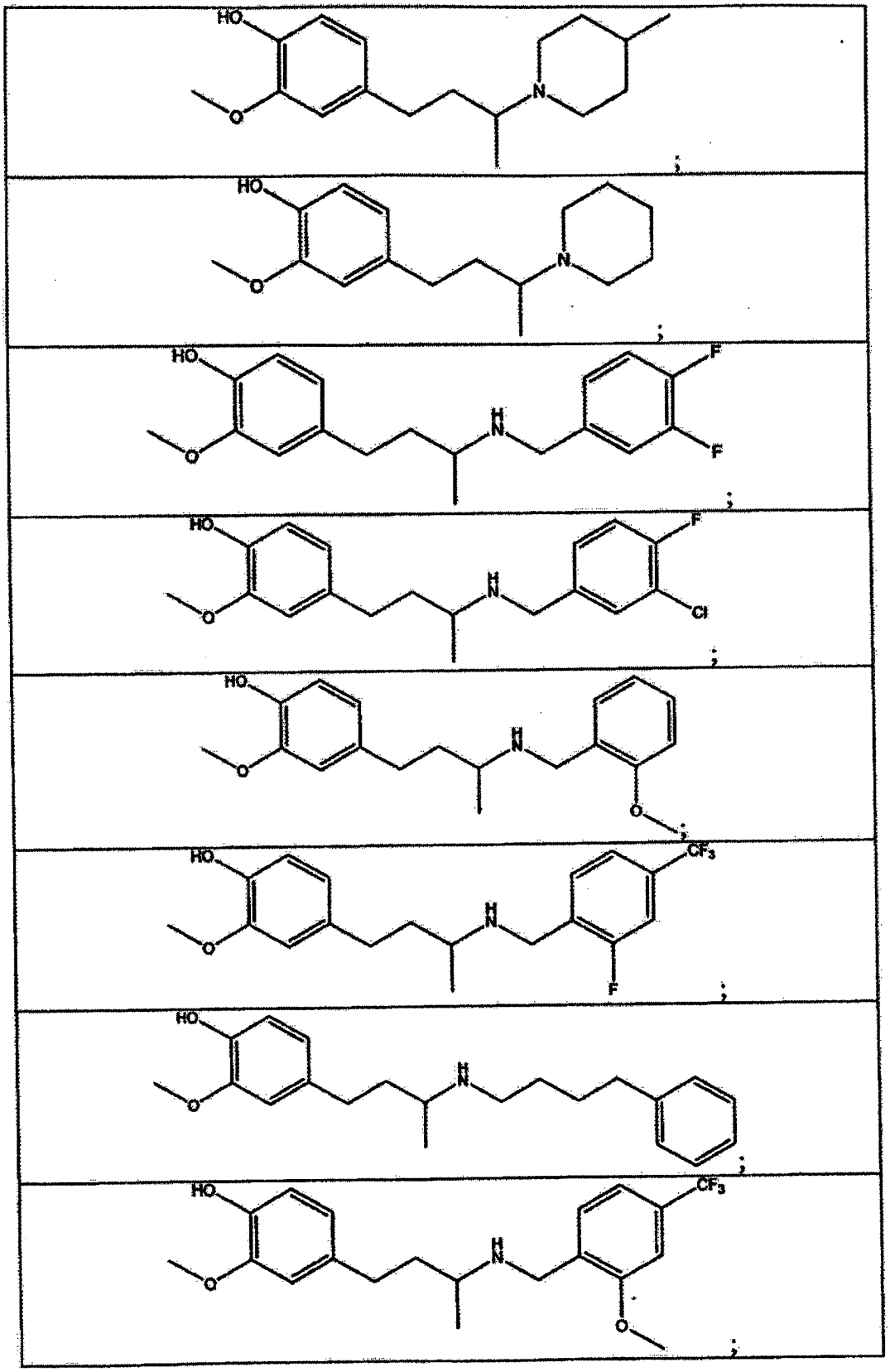
[0261]



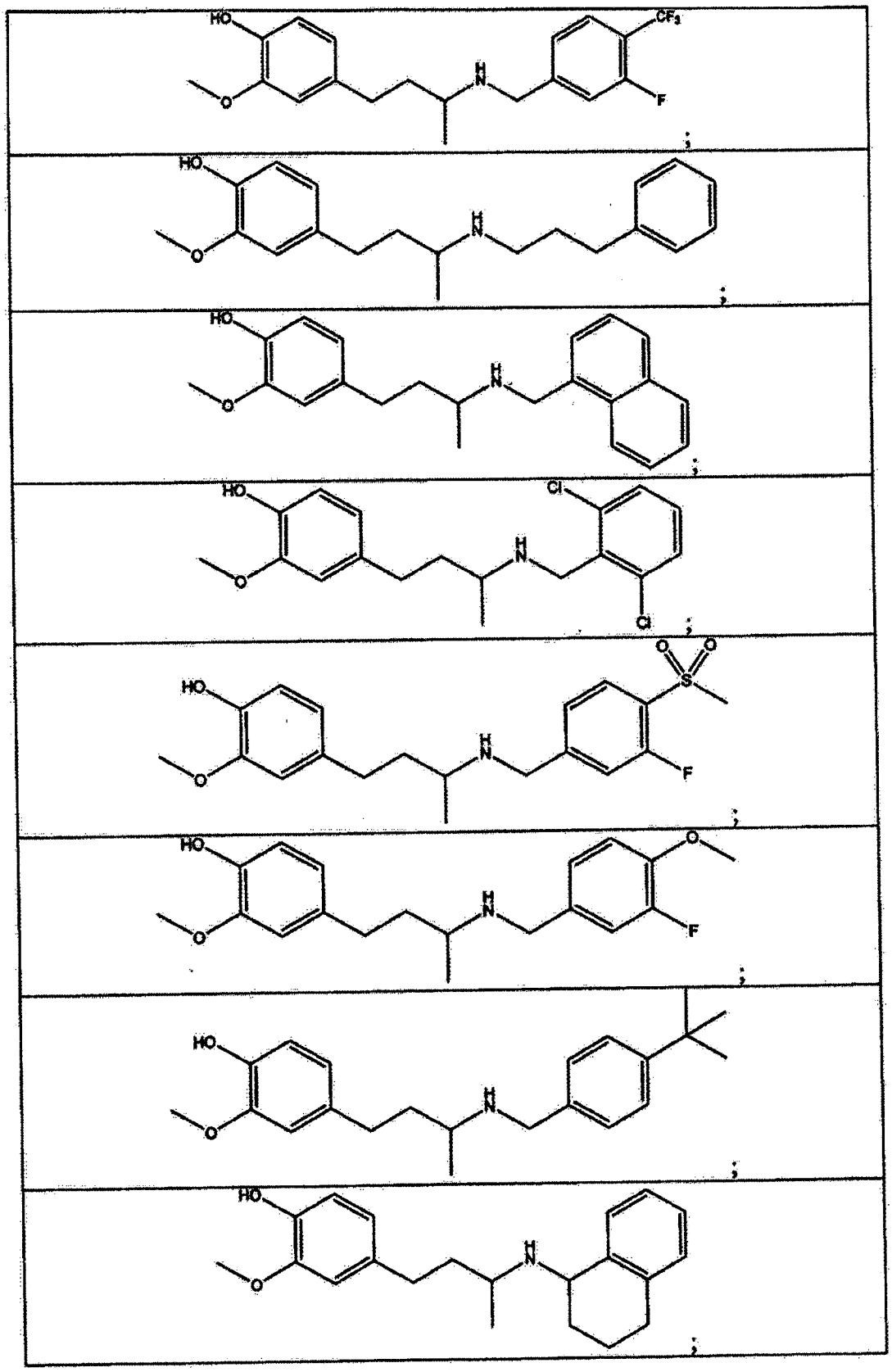
[0262]



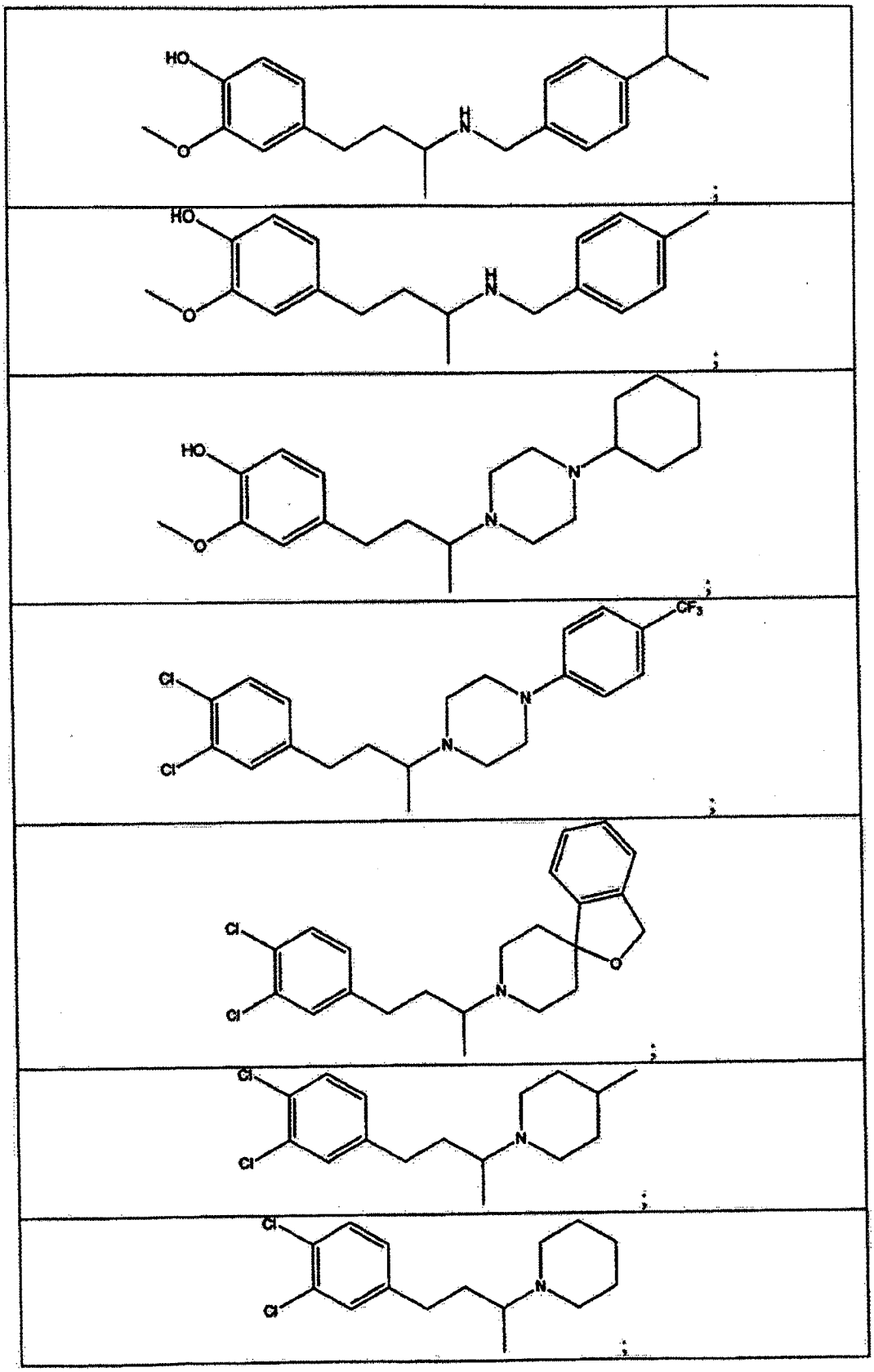
[0263]



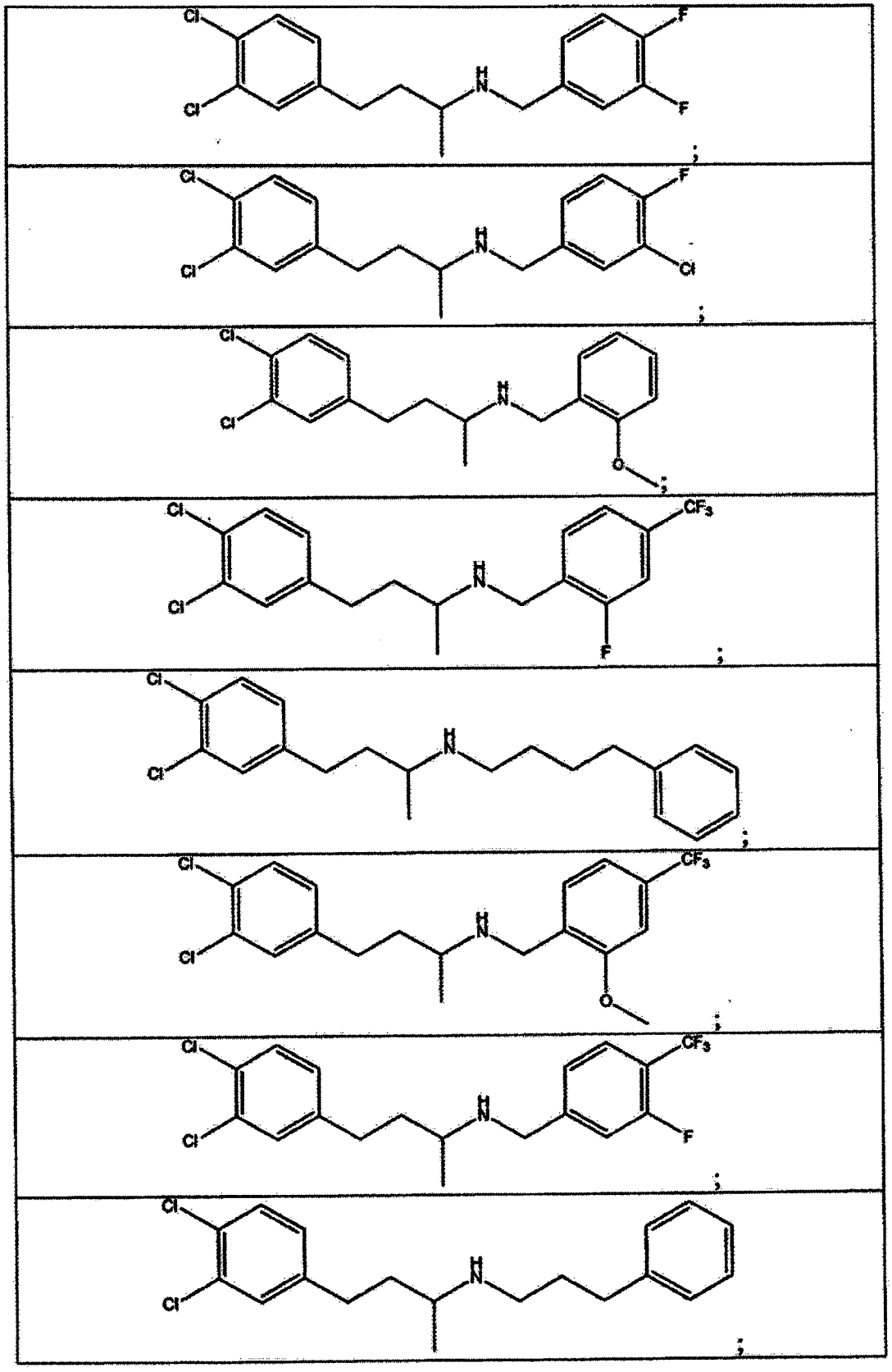
[0264]



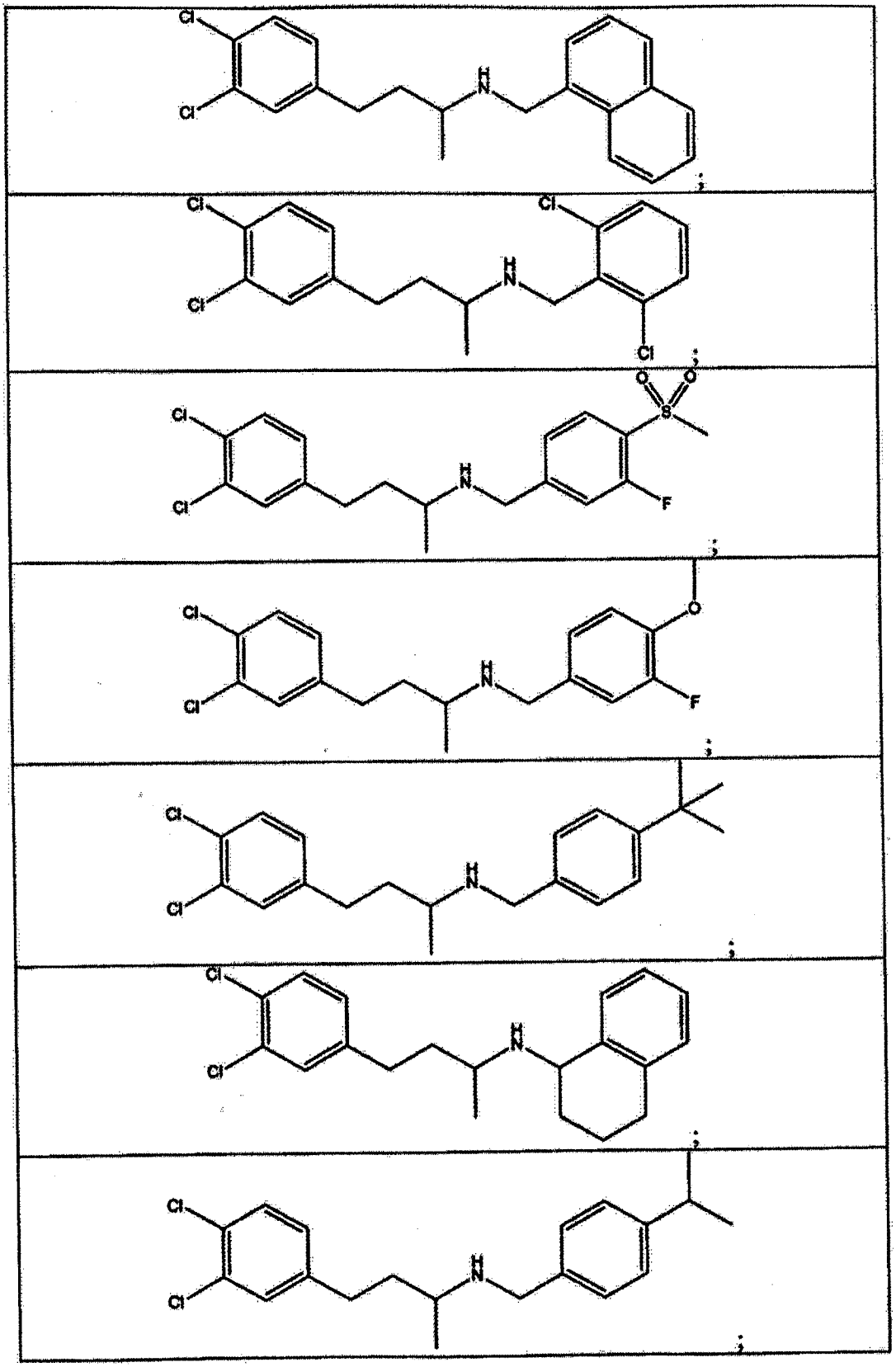
[0265]



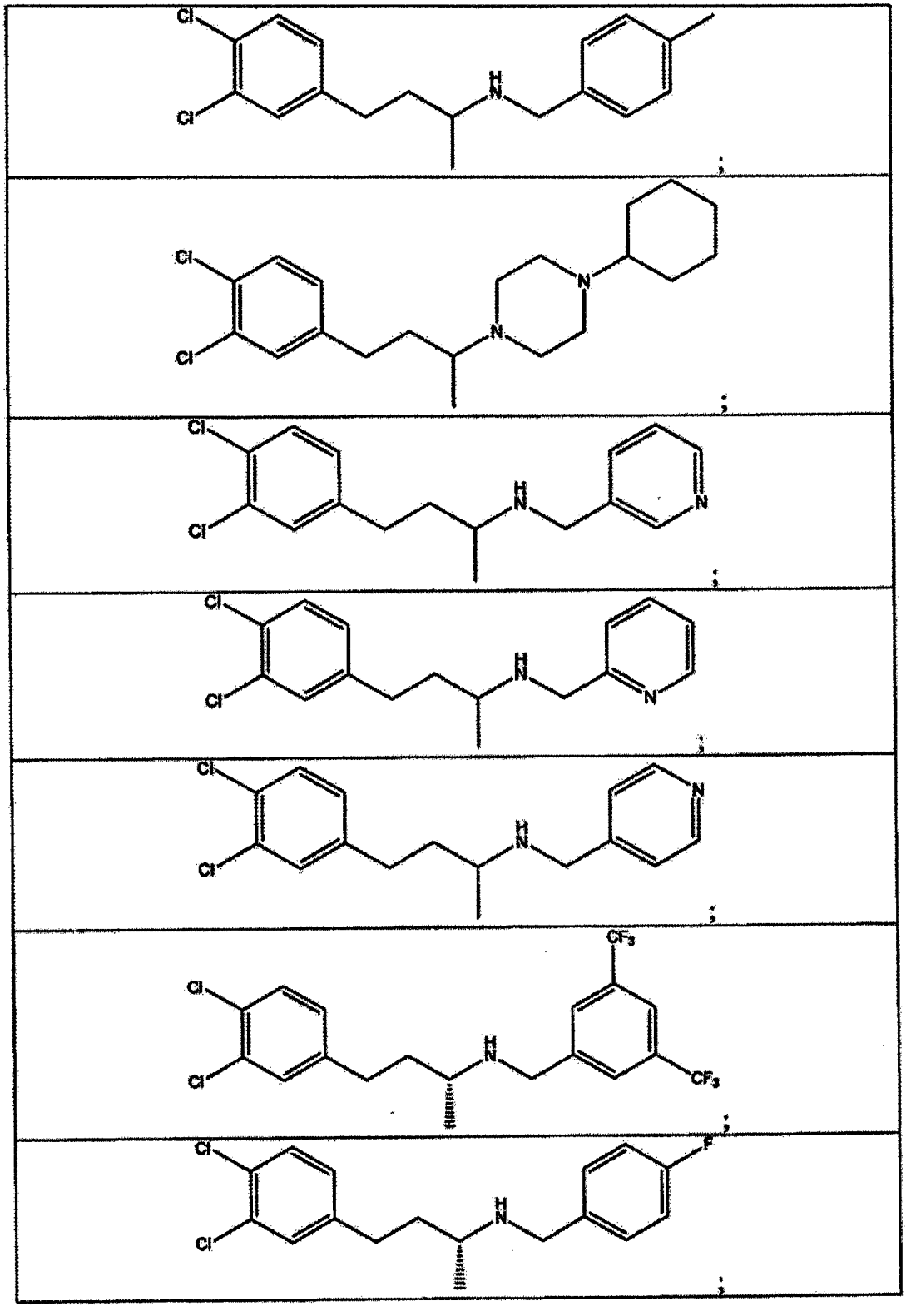
[0266]



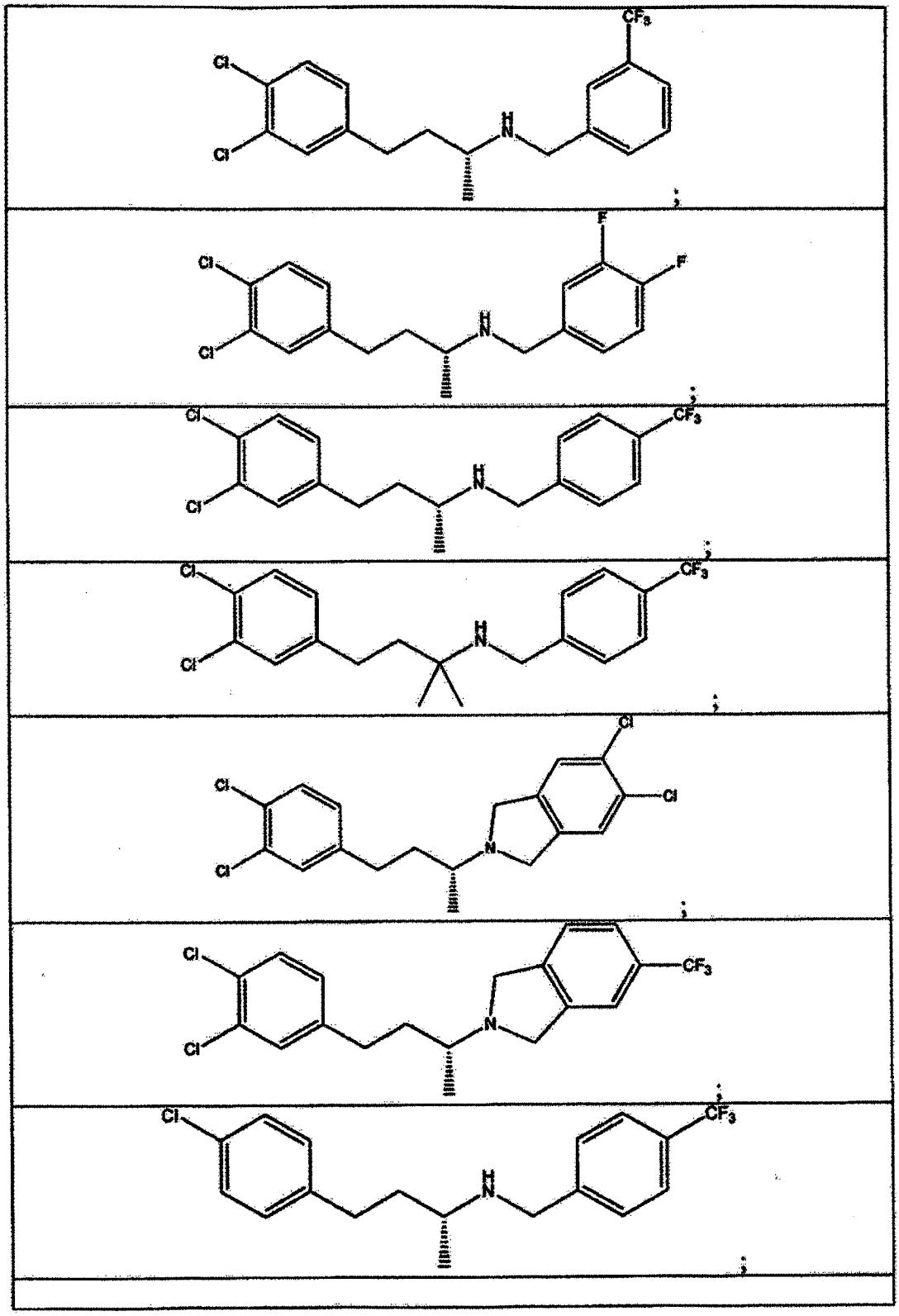
[0267]



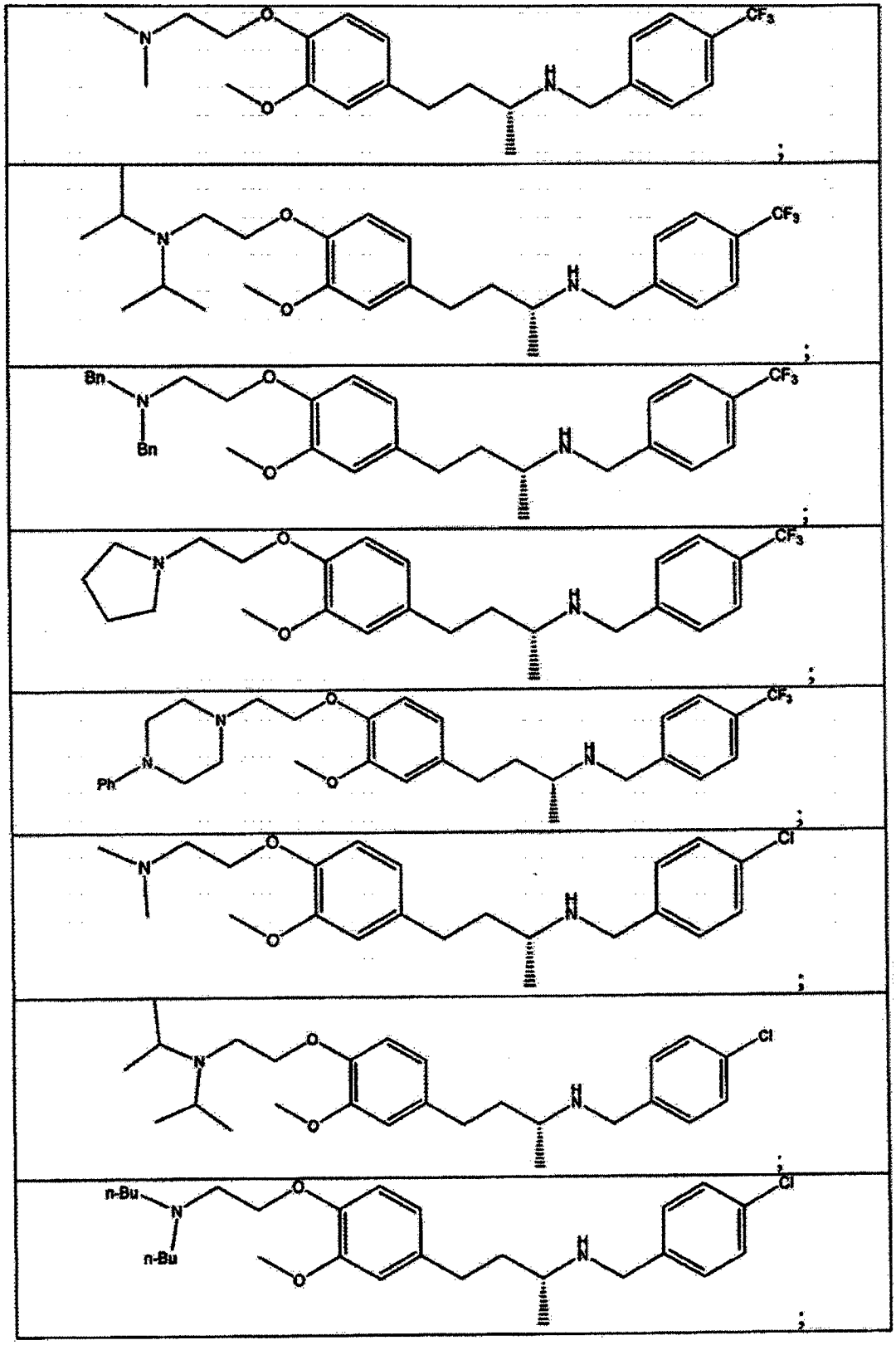
[0268]



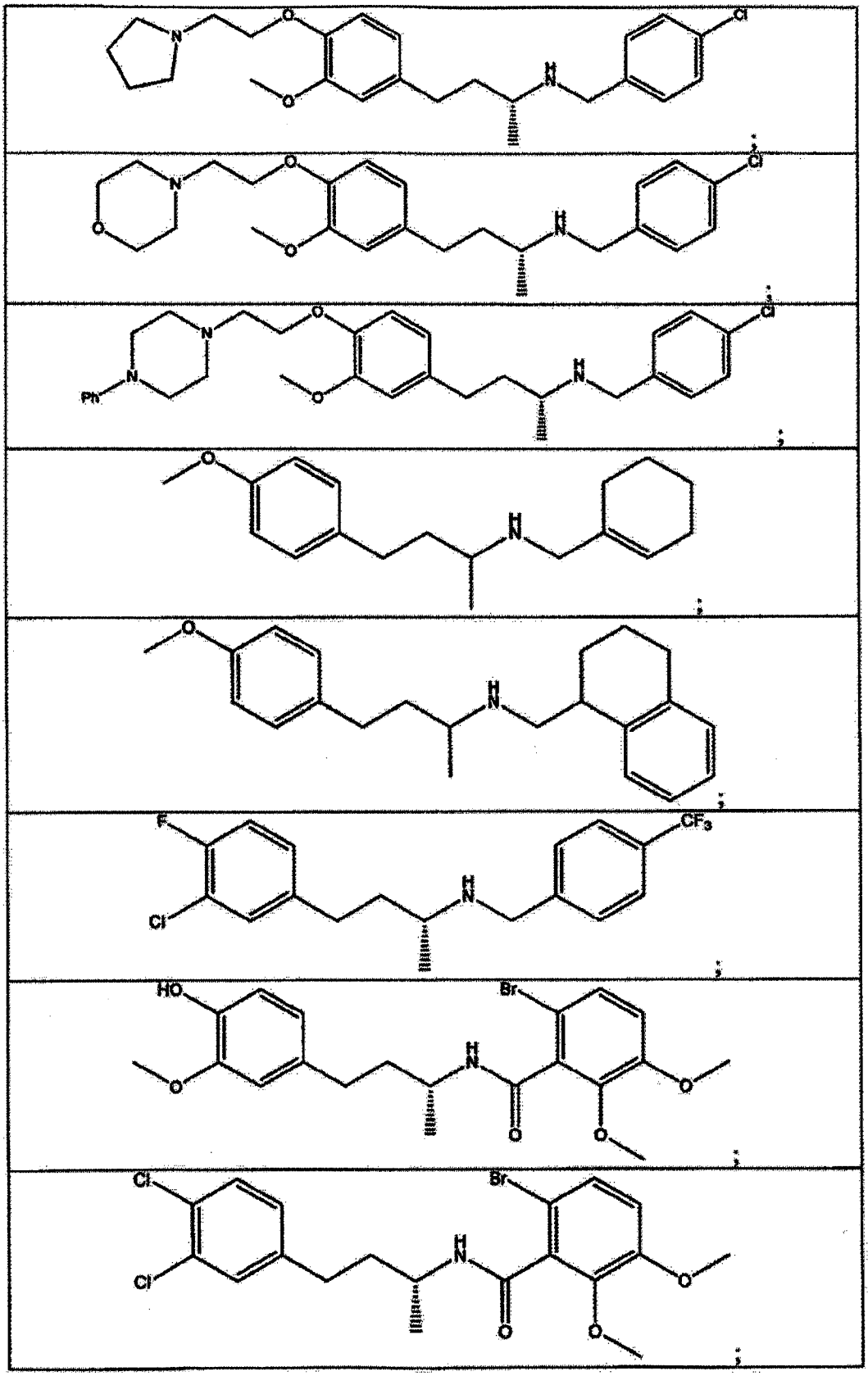
[0269]

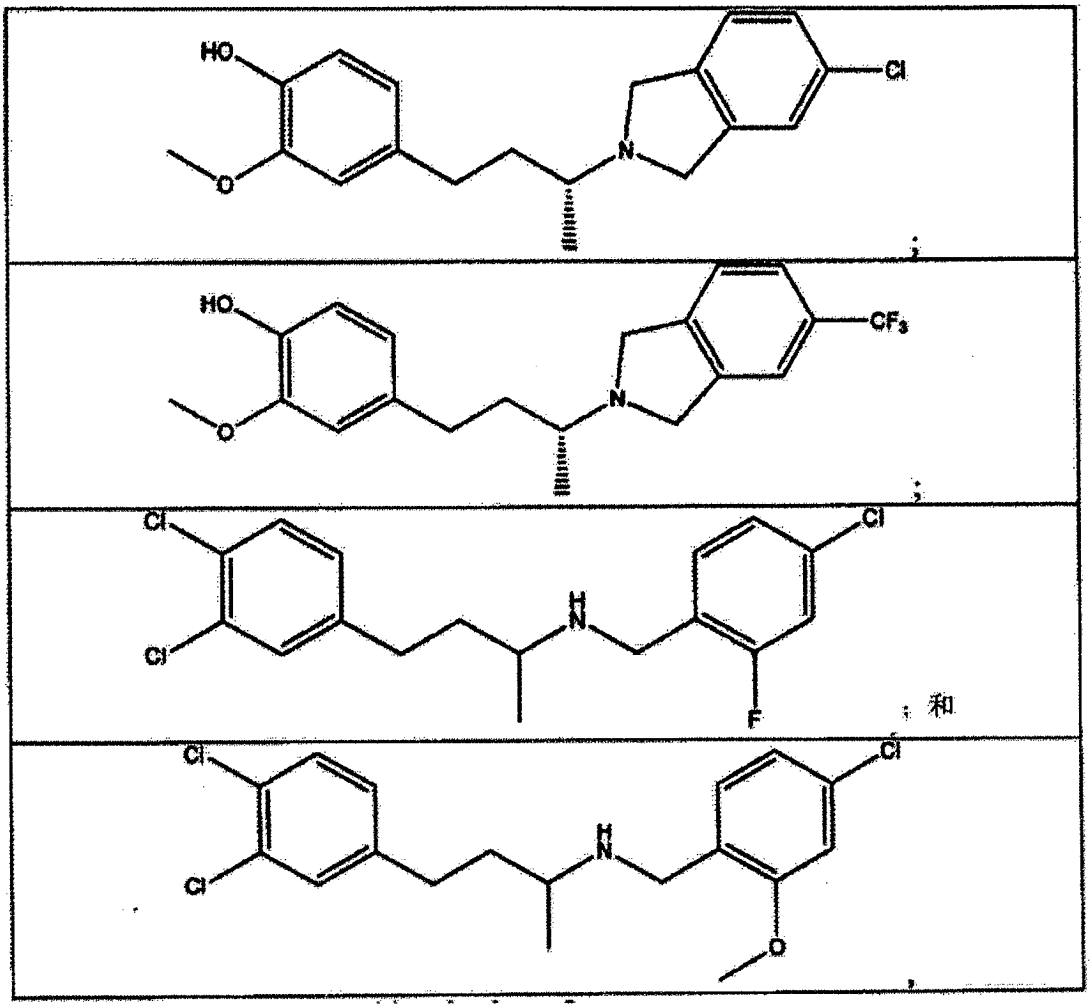


[0270]



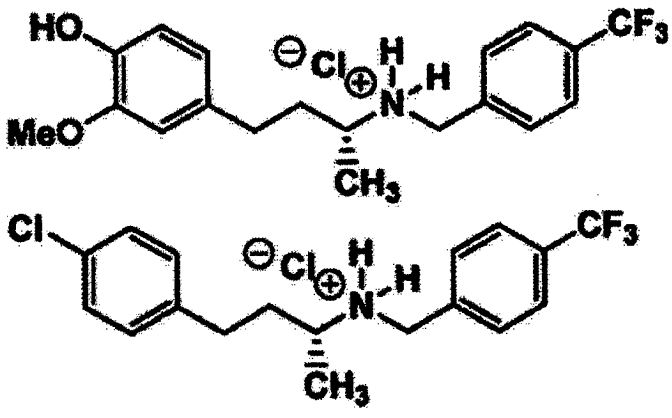
[0271]



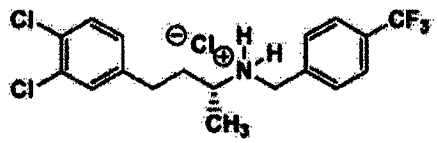
[0272] 

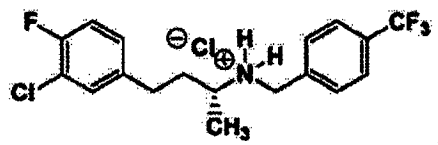
[0273] 或其药学上可接受的盐。

[0274] 用于本发明的优选的盐包括上述化合物的盐酸盐，包括以下：

[0275] 

[0276]

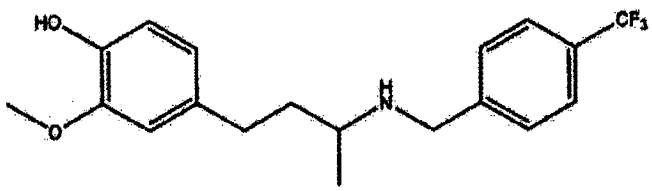




[0277] 这些已根据本文提供的一般方法合成，且具有任何附加步骤的具体合成实例在本领域技术内熟知。数个这些化合物已在如本文所详述的多个检定中进行检测，且已被发现为具有活性。所测试的化合物参考 WO2010/110855 中公开的化合物还显示出增加的生物利用度。

[0278] 化合物 II 具有下式：

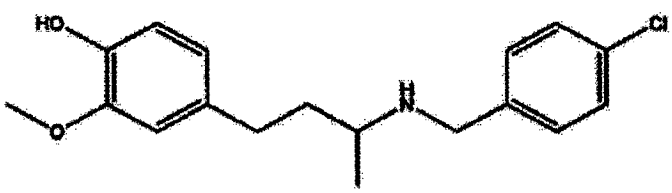
[0279]

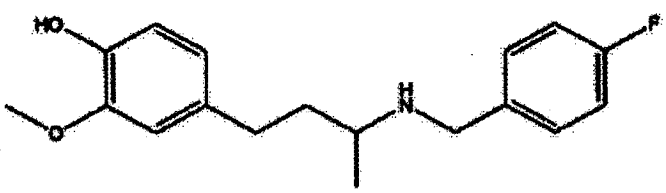


[0280] 在一些实施方式中，上述的各个通式可以含有去除式 II 的化合物的条件。

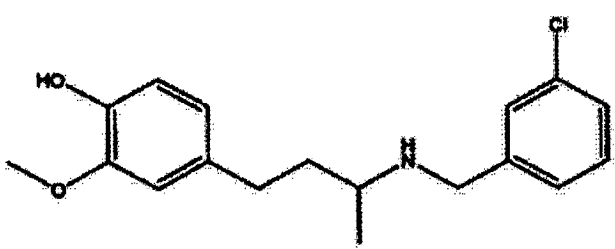
[0281] 在一些实施方式中，上述的各个通式可以含有去除以下化合物的一种或多种的条件：

[0282]

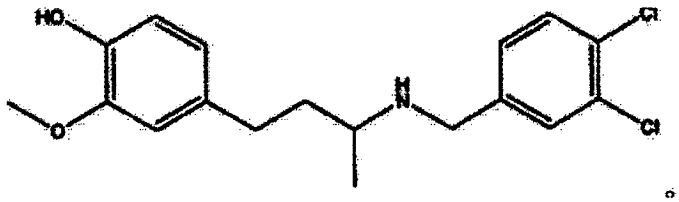




和

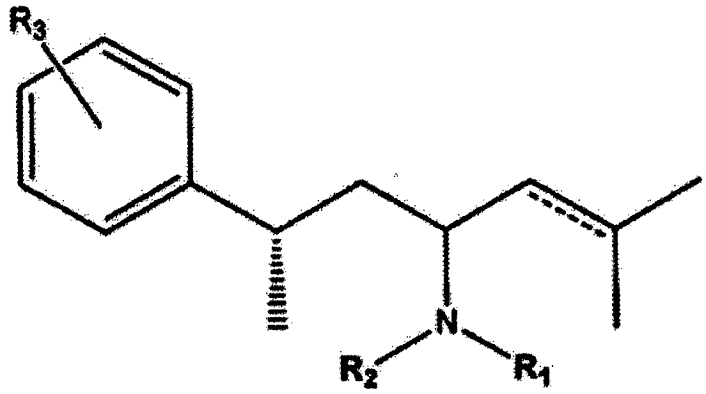


[0283]



[0284] 在另一实施方式中，本发明的 σ-2 拮抗剂是式 VIIIa 的化合物或其药学上可接受的盐：

[0285]



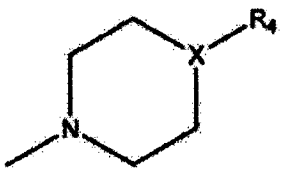
VIIIa

[0286] 其中：

[0287] ---------- 是单键或双键；

[0288] R_1 是 C_{1-6} 烷基、C_{1-6} 卤代烷基、未被取代的苄基或被卤素、C_{1-6} 烷基、或 C_{1-6} 卤代烷基取代的苄基；

[0289] R_2 是 H，或

[0290] R_1 和 R_2 与氮一起形成环  其中

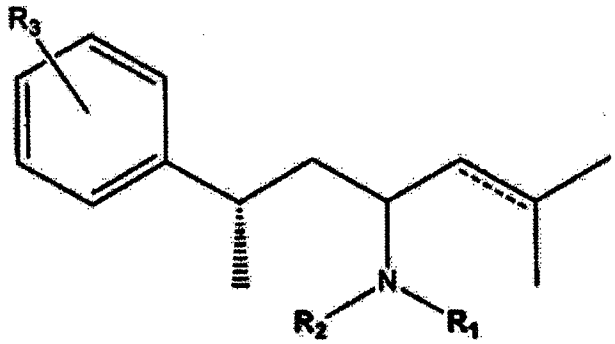
[0291] X 是 CH、N、或 O，且

[0292] R_4 不存在，或是 H、C_{1-6} 烷基、或未被取代的苯基或被卤素、C_{1-6} 烷基、或 C_{1-6} 卤代烷基取代的苯基；且

[0293] R_3 是 C_{1-4} 烷基、卤素、或 C_{1-6} 卤代烷氧基。

[0294] 在一些实施方式中，本发明的 σ-2 拮抗剂是式 VIIIa 的化合物或其药学上可接受的盐：

[0295]



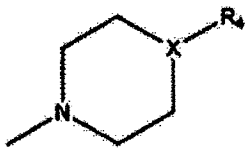
VIIIa

[0296] 其中：

[0297] ---------- 是单键或双键；

[0298] R_1 是异丁基、苄基、或被氯、甲基或 CF_3 取代的苄基；

[0299] R_2 是 H，或

[0300] R_1 和 R_2 与氮一起形成环  其中

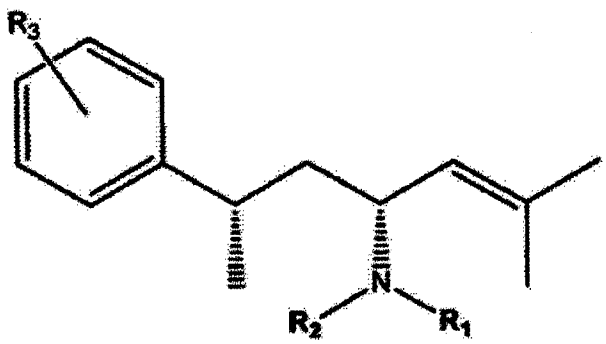
[0301] X 是 CH、N、或 O，且

[0302] R_4 不存在，或是 H、异丙基、或未被取代的苯基；且

[0303] R_3 是邻 -Me、间 -Me、对 -Me、对 -F、或对 $-OCF_3$。

[0304] 在一些更加具体的实施方式中，本发明的 σ-2 拮抗剂是式 VIIIb 的化合物或其药学上可接受的盐：

[0305]

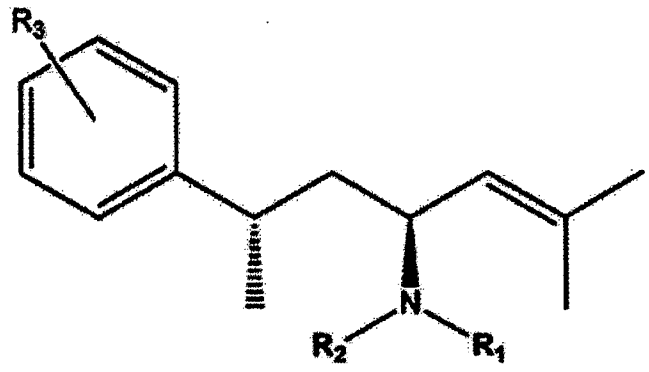


VIIIb

[0306] 其中 R_1-R_3 如以上针对式 VIIIa 所限定。

[0307] 在一些更加具体的实施方式中，本发明的 σ-2 拮抗剂是式 VIIIc 的化合物或其药学上可接受的盐：

[0308]

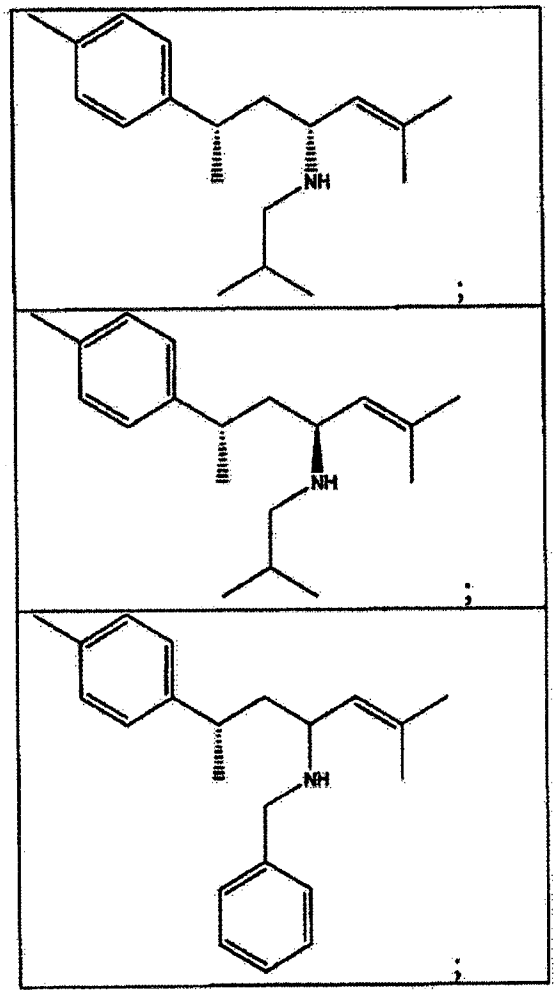


VIIIc

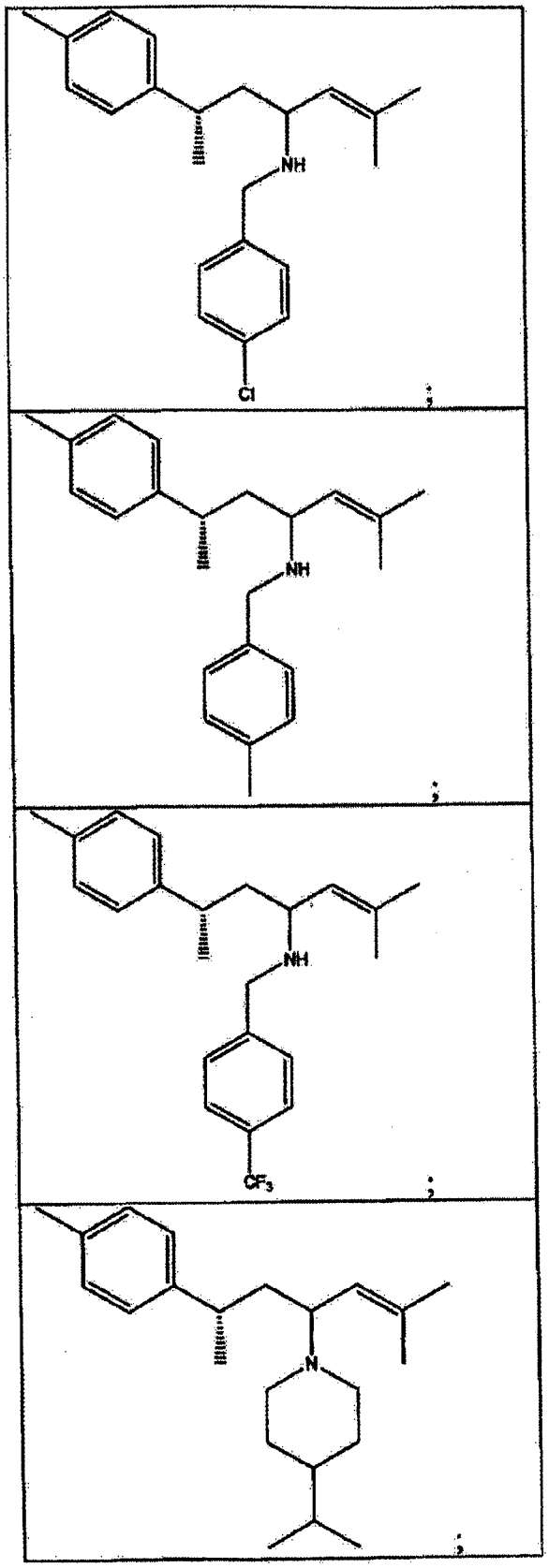
[0309] 其中 R_1-R_3 如以上针对式 VIIIa 所限定。

[0310] 本发明的具体示例性化合物在下表中给出：

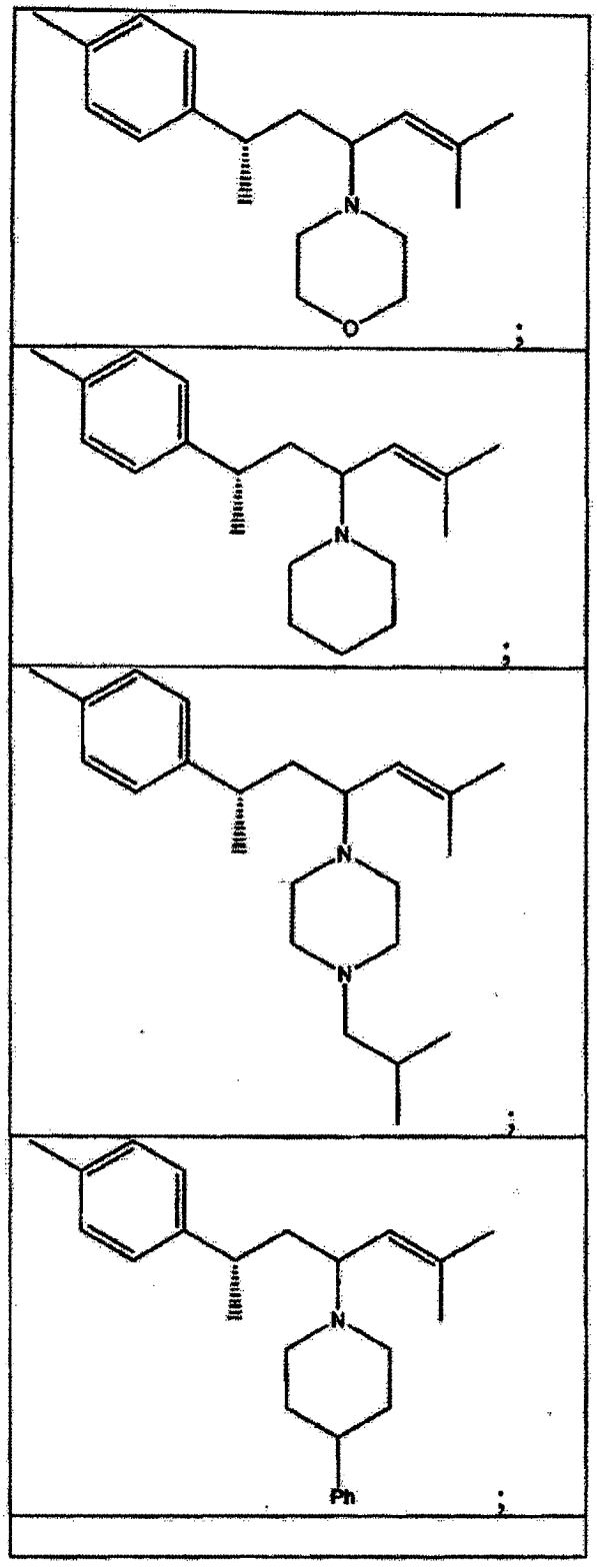
[0311]

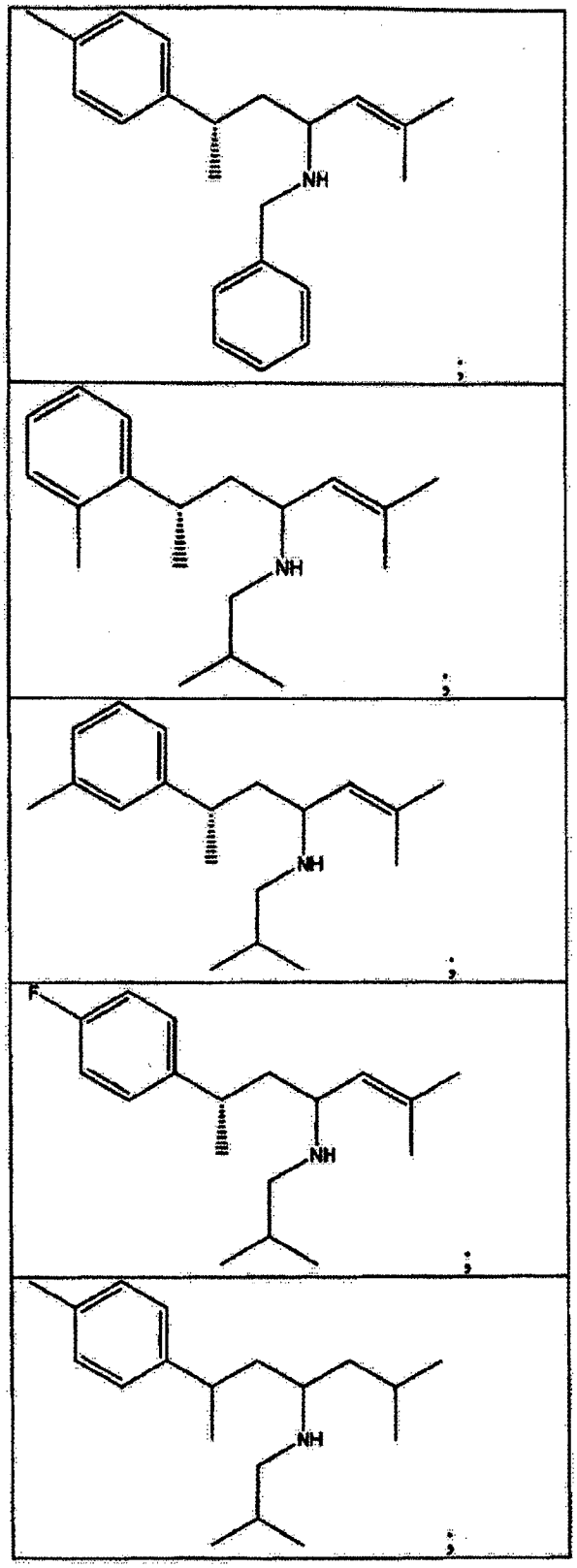


[0312]

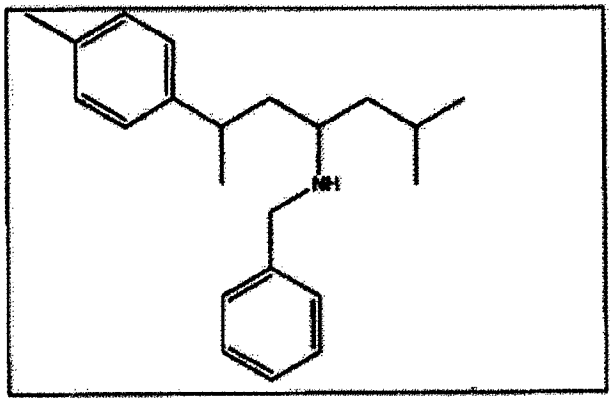


[0313]



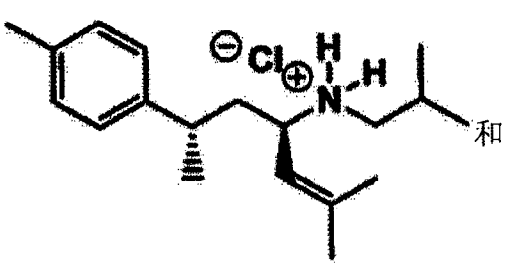
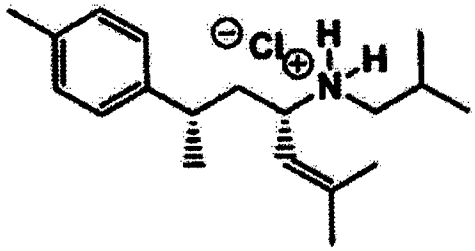
[0314] 

和

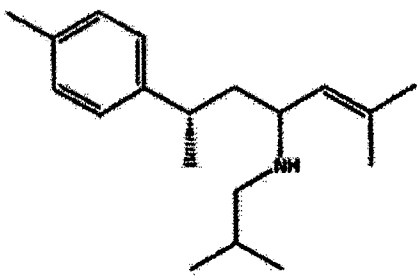
[0315]  ,

[0316] 或其药学上可接受的盐。

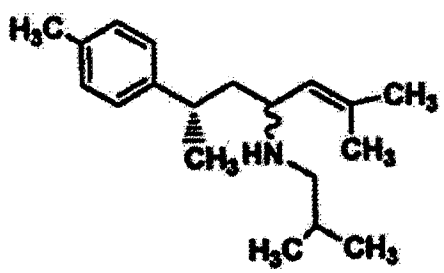
[0317] 用于本发明的优选的盐包括上述化合物的盐酸盐，包括以下：

[0318]  和  。

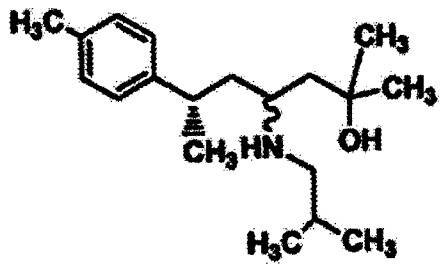
[0319] 在一些实施方式中，各个上述通式可以含有去除以下化合物的一种或多种的条件：

[0320] 

[0321] 另外公开的是式 IXa 和 IXb 的化合物，其为非对映体的混合物。

[0322] 

IXa



IXb

[0323] σ-2 拮抗剂

[0324] 不受理论的约束，提议 σ-2 受体是神经元中 Abeta 寡聚体的受体。文献中

已提议可溶 Abeta 寡聚体的多种受体，包括朊蛋白、胰岛素受体、β 肾上腺素能受体和 RAGE（晚期糖化终产物的受体）。Laurén, J. 等人，2009, Nature, 457(7233):1128-1132；Townsend, M. 等人，J. Biol. Chem. 2007, 282:33305-33312；Sturchler, E. 等人，2008, J. Neurosci. 28(20):5149-5158。实际上，很多研究者相信，Abeta 寡聚体可以与多于一种的受体蛋白结合。不受理论的约束，基于本文呈现的证据，本发明的发明者假定出（不必须排他地）位于神经元中的 Abeta 寡聚体的其他受体。

[0325] 不受理论的约束，Abeta 寡聚体是与 σ 蛋白复合物结合并造成异常运输和突触丧失的 σ 受体激动剂。本文表明，在神经元的该相互作用和 / 或 σ 受体功能中起拮抗作用的高亲和性 σ-2 配体将与 Abeta 寡聚体竞争并使神经元应答回复至正常。这些配体被认为是功能性 σ-2 受体拮抗剂并如此称谓或更简单地称为 σ-2 受体拮抗剂或 σ-2 拮抗剂。

[0326] 在一些实施方式中，本发明的 σ-2 受体拮抗剂化合物对于以下方面作为神经细胞中的功能性拮抗剂：抑制可溶 Aβ 寡聚体诱导的突触丧失和抑制可溶 Aβ 寡聚体诱导的膜运输检定中的缺陷；表现出在 σ-2 受体处的高亲和性；以及具有与任何其他非 σ 受体相比的对于一种或多种 σ 受体的高选择性；以及表现出良好的药物样特性。

[0327] 在一些实施方式中，如在本说明书中公开的，本文详述的满足某些体外检定标准的 σ-2 受体功能性拮抗剂将在一种或多种相关的动物行为模型中表现出行为效力，或被预测具有行为效力。在一些实施方式中，行为效力确定在 10mg/kg 口服 (p.o.) 或更低。

[0328] 在一些实施方式中，本公开内容提供预测高亲和性 σ-2 受体配体的行为效力的体外检定平台。根据该体外检定平台，配体以高亲和性与 σ-2 受体结合；作为与神经元中 Abeta 寡聚体诱导效应相关的功能性拮抗剂；抑制中枢神经元中 Abeta 寡聚体诱导的突触丧失或降低 Abeta 寡聚体对神经元的结合以抑制突触丧失；以及在不存在 Abeta 寡聚体时不影响运输或突触数量。在体外检定中的这种活性模式被称为“治疗表型”(therapeutic phenotype)。σ-2 受体拮抗剂阻抑成熟神经元中的 Abeta 寡聚体效应而在不存在 Abeta 寡聚体时不影响正常功能的能力满足治疗表型的标准。现在公开了具有治疗表型的选择性 σ-2 拮抗剂能够够阻抑 Abeta 寡聚体诱导的突触功能障碍。

[0329] 在一些实施方式中，具有治疗表型且同时具有以下特征的高亲和性的选择性 σ-2 拮抗剂适合用作用于治疗有需求的患者中的 Abeta 寡聚体诱导的突触功能障碍的治疗候选物：在 σ 受体处的高亲和性；相比于其他非 σ CNS 受体，对于 σ 受体的高选择性；对于 σ-2 受体有更高的亲和性或可比的亲和性，例如在 σ-2 和 σ-1 受体处为一个数量级内；对于 σ 受体的与其他中枢神经系统中的相关受体相反的选择性，以及良好的药物样特性。药物样特性包括可接受的脑穿透性（穿越脑血屏障的能力）、良好的血浆中稳定性和良好的代谢稳定性，例如通过对肝脏微粒体的暴露而测量的。不受理论的约束，高亲和性的 σ-2 受体拮抗剂与 Abeta 寡聚体竞争，和 / 或停止病理 σ 受体信号转导，引起阿尔茨海默病。

[0330] 在一些实施方式中，具有治疗表型且同时具有以下特征的 σ-2 拮抗剂适合用作用于治疗有需求的患者中的 Abeta 寡聚体诱导的突触功能障碍的治疗候选物：在 σ 受体处的高亲和性；相比于其他非 σ CNS 受体，对于 σ 受体的高选择性；对 σ-2 受体的高亲和性或在 σ-2 和 σ-1 受体处可比的亲和性；以及良好的药物样特性。药物样特性包括高的脑穿透性、血浆稳定性和代谢稳定性。

[0331] 在一些实施方式中，在结合活性研究中，至多约 600nM、500nM、400nM、300nM、

200nM、150nM、100nM，优选为至多约 75nM，优选为至多约 60nM，优选为至多约 40nM，更优选为至多 10nM，最优选为至多 1nM 的 IC_{50} 或 Ki 值表明对于 σ 受体结合位点的高结合亲和性。

[0332] 在一些实施方式中，相比于其他非 σ CNS 或靶标受体，对 σ 受体具有高于约 20 倍、30 倍、50 倍、70 倍或优选高于 100 倍选择性的、且具有良好的药物样特性（包括脑穿透性和良好的代谢和 / 或血浆稳定性）并且具有治疗表型的在 σ-2 受体处具有高亲和性（优选 Ki 小于约 600nM、500nM、400nM、300nM、200nM、150nM、100nM、70nM、60nM、50nM、30nM 或 10nM）的 σ-2 受体拮抗剂被预测为具有行为效力并且可以用于在需要其的患者中治疗 Abeta 寡聚体诱导的突触功能障碍。

[0333] 本文所用的术语“脑穿透性”是指药物、抗体或片段穿越血脑屏障的能力。在一些实施方式中，动物药物代谢动力学 (pK) 研究例如小鼠药物代谢动力学 / 血脑屏障研究可以用于确定或预测脑穿透性。在一些实施方式中，多个浓度的药物可以例如以 3、10 和 30mg/kg 例如 p.o. 施用 5 天，并且多个 pK 特性在例如动物模型中测量。在一些实施方式中，确定剂量相关的血浆和脑中含量。在一些实施方式中，脑 Cmax>100、300、600、1000、1300、1600 或 1900ng/mL。在一些实施方式中，良好的脑穿透性被定义为，脑 / 血浆比为 >0.1、>0.3、>0.5、>0.7、>0.8、>0.9、优选 >1、更优选 >2、>5 或 >10。在其他实施方式中，良好的脑穿透性被定义为，在预定时间段后高于约 0.1%、1%、5%、高于约 10%、优选高于约 15% 的所施用剂量透过 BBB。在某些实施方式中，剂量为经口施用 (p.o.)。在其他实施方式中，剂量为经静脉施用 (i.v.)，在测量 pK 特性之前。检定和脑穿透性在实施例 7 中描述，且化合物 II 的数据显示在图 2A 和 2B 中，已知化合物 II 进行首过代谢，因此皮下给药；尽管如此，化合物 II 在急性和慢性给药后均为高度脑穿透性。化合物 II 的脑 / 血浆比 >8。

[0334] 本文所用的术语“血浆稳定性”是指化合物在血浆中例如通过酶（如水解酶和酯酶）的降解。可以采用多种体外检定中的任一种。药物在血浆中孵育不同时间段。在各个时间点剩余的百分比母化合物（分析物）反映出血浆稳定性。较差的血浆稳定性特征可能趋于具有较低的生物利用度。良好的血浆稳定性可以定义为，在 30 分钟后剩余高于 50% 分析物，在 45 分钟后剩余高于 50% 分析物，且优选为在 60 分钟后剩余高于 50% 分析物。

[0335] 本文所用的术语“代谢稳定性”是指化合物在首过代谢（口服药物的肠或肝降解或结合）后有活性的能力。这可以例如在体外通过将化合物暴露于小鼠或人的肝微粒体而评测。在一些实施方式中，良好的代谢稳定性是指化合物暴露于小鼠或人肝微粒体时，$t_{1/2}$>5min、>10min、>15 分钟、>20 分钟、优选为 >30 分钟。在一些实施方式中，良好的代谢稳定性是指内在清除率 (Cl_{int})<300uL/min/mg，优选为≤ 200uL/min/mg，更优选为≤ 100uL/min/mg。

[0336] 本发明的新化合物的盐、溶剂化物、立体异构体、衍生物、前药和活性代谢物

[0337] 本发明还包括式 I 化合物的盐、溶剂化物、立体异构体、前药和活性代谢物。

[0338] 术语“盐”可以包括游离碱的酸加成盐或加成盐。优选地，盐为药学上可接受的。可以用于形成药学上可接受的酸加成盐的酸的实例包括，但不限于得自无毒无机酸以及得自无毒有机酸的盐，无机酸为例如硝酸、磷酸、硫酸或氢溴酸、氢碘酸、氢氟酸、亚磷酸，有机酸为例如脂肪族单羧酸和二羧酸、苯基取代的链烷酸、羟基链烷酸、链烷双酸、芳香酸、脂肪族和芳香族磺酸、和醋酸、马来酸、琥珀酸或柠檬酸。这些盐的非限制性实例包括萘二磺酸盐、苯磺酸盐、硫酸盐、焦硫酸盐、硫酸氢盐、亚硫酸盐、亚硫酸氢盐、硝酸盐、磷酸盐、磷酸一

氢盐、磷酸二氢盐、偏磷酸盐、焦磷酸盐、氯化物、溴化物、碘化物、醋酸盐、三氟醋酸盐、丙酸盐、辛酸盐、异丁酸盐、草酸盐、丙二酸盐、琥珀酸盐、辛二酸盐、癸二酸盐、富马酸盐、马来酸盐、扁桃酸盐、苯甲酸盐、氯苯甲酸盐、甲基苯甲酸盐、二硝基苯甲酸盐、邻苯二甲酸盐、苯磺酸盐、甲苯磺酸盐、苯乙酸盐、柠檬酸盐、乳酸盐、马来酸盐、酒石酸盐、甲烷磺酸盐等。也包括氨基酸的盐例如精氨酸盐等以及葡糖酸盐、半乳糖醛酸盐（参见，例如 Berge 等人“Pharmaceutical Salts,”J. Pharma. Sci. 1977 ;66:1）。

[0339] 式 I 化合物的酸加成盐可以通过使游离碱形式与足量的所需酸接触而制备，以传统方式产生盐。这些游离碱形式可以通过使盐形式与碱接触并以传统方式分离游离碱而再生。这些游离碱形式在某些物理特性例如在极性溶剂中的溶解度方面不同于它们各自的盐形式，但除此以外，这些盐对于本发明目的而言等同于它们各自的游离碱。

[0340] 还包括全盐 (total salt) 和偏盐 (partial salt)，即相对于每摩尔的式 I 化合物的酸具有 1、2 或 3 优选为 2 当量的碱的盐，或者相对于每摩尔的式 I 化合物的碱具有 1、2 或 3 当量优选为 1 当量的酸的盐。

[0341] 出于分离或纯化的目的，也可以使用药学上不可接受的盐。然而，仅有药学可接受的非毒性盐为治疗使用，因此它们是优选的。

[0342] 药学可接受的碱加成盐是与金属或胺形成的，例如碱金属和碱土金属或有机胺。用作阳离子的金属的实例为钠、钾、镁、钙等。合适的胺的实例为 N, N'- 二苄基乙二胺、氯普鲁卡因、胆碱、二乙醇胺、二环己胺、乙二胺、N- 甲基葡糖胺和普鲁卡因。

[0343] 所述酸性化合物的碱加成盐通过使游离酸形式与足量的所需碱接触而制备，以传统方式生产盐。游离酸形式可以通过使盐形式与酸接触并分离游离酸而再生。

[0344] 本发明的化合物可以同时具有碱性和酸性中心，因此可以处于两性离子或内盐的形式。

[0345] 通常而言，式 I 化合物的药学可接受盐可以容易地通过使用合适的所需酸或碱而制备。盐可以从溶液中沉淀并通过过滤而收集或者可以通过蒸发溶剂而回收。例如，酸例如盐酸的水溶液可以被加至式 I 化合物的水性混悬液中，并将所得的混合物蒸发至干（冻干）而获得固体的酸加成盐。或者，式 I 化合物可以溶解在合适的溶剂例如醇（如异丙醇）中，且酸可以加到同一溶剂或另一合适溶剂中。所得的酸加成盐可以在之后直接沉淀，或通过添加极性较小的溶剂例如二异丙醚或己烷而沉淀，并通过过滤而分离。

[0346] 有机化学领域的技术人员将认识到，很多有机化合物可以与溶剂（有机化合物在该溶剂中反应，或从其中沉淀或结晶）形成复合物。这些复合物被称为“溶剂化物”。例如，与水的复合物被称为“水合物”。本发明化合物的溶剂化物在本发明的范围内。式 I 化合物的盐可以形成溶剂化物（例如，水合物），且本发明也包括所有这些溶剂化物。词语“溶剂化物”的意思对于本领域技术人员熟知为通过溶剂和溶质的相互作用（即，溶剂化作用）形成的化合物。用于制备溶剂化物的技术在领域内已良好建立（参见，例如 Brittain. Polymorphism in Pharmaceutical solids. Marcel Decker, New Youk, 1999）。

[0347] 本发明也包括式 I 化合物的 N 氧化物。术语“N 氧化物”是指，对于含有另外未取代的 sp^2N 原子的杂环，N 原子可以具有共价结合的 O 原子，即 -N → O。这些 N 氧化物取代的杂环的实例包括吡啶基 N 氧化物、嘧啶基 N 氧化物、吡嗪基 N 氧化物和吡唑基 N 氧化物。

[0348] 式 I 的化合物可以具有一个或多个手性中心，且基于单独取代基的性质，它们也

可以具有几何异构体。在空间上原子排布不同的异构体被称为“立体异构体”。不是互为镜像的立体异构体被称为“非对映体”，且相互为彼此的非重叠镜像的那些被称为“对映体”。当化合物具有手性中心时，一对对映体是可能的。对映体可以通过其非对称中心的绝对构型而特征化，并且通过 Cahn 和 Prelog 的 R—和 S- 序列法则或者通过分子旋转偏振光的平面的方式而描述，并命名为右旋或左旋（即，分别为 (+) 或 (-) 异构体）。手性化合物可以以单独对映体或对映体的混合物存在。含有等比例对映体的混合物称为“外消旋混合物”。含有非等比例对映体的混合物被描述为具有“对映体过量”(ee) 的 R 或 S 化合物。混合物中一种对映体的过量程度经常用下式确定的%对映体过量 (% ee) 值描述：

[0349] % ee = (R)-(S)/(R)+(S)

[0350] 对映体的比率也可以通过“光学纯度”来定义，其中将对映体混合物旋转平面偏振光的度数与单独光学纯的 R 和 S 化合物做比较。光学纯度可以使用下式确定：

[0351] 光学纯度＝对映体$_{主要}$/(对映体$_{主要}$+对映体$_{次要}$)。

[0352] 化合物也可以是本文所述化合物的基本纯的 (+) 或 (-) 对映体。在一些实施方式中，含有基本纯的对映体的组合物包含至少 90、91、92、93、94、95、96、97、98 或 99%的一种对映体。在一些实施方式中，含有基本纯的对映体的组合物为至少 99.5%的一种对映体。在一些实施方式中，组合物仅包含本文所述化合物的一种对映体。

[0353] 本发明包括式 I 化合物的所有单个异构体。说明书和权利要求书中特定化合物的描述或命名意在包括单独对映体及其混合物、外消旋物或其他。用于确定立体异构体的立体化学和拆分 (resolution) 或立体定位合成的方法为本领域熟知。具体而言，在通式 I 和 II 的化合物中显示有手性中心，其产生一组对映体。其他手性中心可以视取代基情况而存在。

[0354] 对于很多应用，优选执行立体选择性合成和 / 或使反应产物进行合适的纯化步骤，从而来制备基本光学纯的材料。用于制备光学纯的材料的合适的立体选择性合成程序为领域内熟知，用于将外消旋混合物纯化为光学纯的级分的程序也如此。本领域技术人员还将意识到，本发明化合物可以以多晶型存在，其中化合物能够以不同形式结晶。用于识别和分离多晶型的适当方法为本领域已知。

[0355] 非对映体在物理特性和化学反应性上均有区别。非对映体的混合物可以基于溶解度、分级结晶或色谱特性例如薄层层析、柱层析或 HPLC 而分离成对映体对。

[0356] 将非对映体的复杂混合物纯化为对映体通常需要两个步骤。在第一个步骤中，将非对映体的混合物拆分成对映体对，如上所述。在第二个步骤中，对映体对进一步被纯化为富集了一种或另一种对映体的组成物，更优选拆分为含有纯对映体的组成物。对映体的拆分通常需要与手性试剂例如溶剂或柱基质进行反应或分子相互作用。可以例如通过与第二试剂（即，拆分剂）的纯对映体的反应，将对映体混合物（例如外消旋混合物）转化为非对映体的混合物而达到拆分。之后可以分离所得的两种非对映异构产品。分离的非对映体之后可以通过逆转最初的化学转化而再转化为纯的对映体。

[0357] 对映体的拆分也可以通过它们与手性物质的非共价结合上的差异而实现，例如，通过在纯手性吸附剂上的层析。对映体与层析吸附剂之间的非共价结合产生非对映异构复合物，引起层析体系中移动和结合状态中的差异分区。因此，两种对映体以不同速率经层析体系（例如柱）移动，实现它们的分离。

[0358] 手性拆分柱在领域内熟知，并市售可得（例如，从 MetaChem Technologies Inc.，a division of ANSYS Technologies, Inc.，Lake Forest, CA）。对映体可以使用例如用于 HPLC 的手性固定相 (CSP) 而分析和纯化。手性 HPLC 柱通常含有固定至二氧化硅填充料表面的一种形式的对映体化合物。

[0359] D- 苯基甘氨酸和 L- 亮氨酸是 I 型 CSP 的实例并利用 π - π 相互作用、氢键、偶极 - 偶极相互作用和空间相互作用的组合来实现手性识别。为在 I 型柱上拆分，分析物对映体必须含有与 CSP 互补的官能性，从而分析物与 CSP 进行必要的相互作用。样本应该优选包含一种以下官能团：π - 酸或 π - 碱、氢键供体和 / 或受体、或酰胺偶极。有时利用衍生化作用将作用位点加至缺乏其的化合物。最常见的衍生化涉及由胺和羧酸形成酰胺。

[0360] MetaChiral ODM™ 是 II 型 CSP 的实例。形成溶质 -CSP 复合物的主要机制是通过吸引相互作用，但是包含配合体也起重要作用。氢键、π - π 相互作用和偶极叠合对于 MetaChiral™ ODM 上的手性拆分较为重要。当溶质分子不包含溶质 - 柱相互作用所需的基团时，衍生化可能是有必要的。通常对苯甲胺的衍生对于一些强极性分子例如胺和羧酸是所需的，否则，它们会通过非特异性的立体相互作用与固定相强烈反应。

[0361] 当适用时，式 I 或 II 的化合物可以通过例如柱层析分离或硅胶 TLC 分离而分离成非对映体对。这些非对映体对在本文中提及为具有高 TLC Rf 的非对映体；和具有低 TLC Rf 的非对映体。非对映体可以使用本领域熟知的方法（例如本文描述的那些）而进一步富集特定对映体，或拆分为单一对映体。

[0362] 非对映体对的相对构型可以通过理论模型或法则（例如 Cram 法则、Felkin-Ahn 模型）的应用或使用计算化学程序产生的更可靠的三维模型来进行推导。在很多例子中，这些方法能够预测哪个非对映体是化学转换的能量上有利的产物。作为可替换方法，非对映体对的相对构型可以通过发现一种（或两种）非对映体对中的单个对映体的绝对构型而间接确定。

[0363] 立体中心的绝对构型可以通过本领域技术人员非常熟知的方法（例如，X 射线衍射、圆二色性）而确定。绝对构型的确定也可用于证实理论模型的可预测性，并且可用于拓展这些模型对于经由相似机制反应而制备的相似分子的用途（例如，经由氢化物的酮还原或酮的还原胺化）。

[0364] 本发明还可以包括由于不直接与环连接的双键上的 R_2 ～ R_3 取代基而产生 Z-E 型的立体异构体及其混合物。当 m 不是 1 且 m 和 n 不同时，会遇到其他 Z-E 立体异构体。应用 Cahn-Ingold-Prelog 优选法则来确定由于双键连接的取代基的双键平面中的各个位置引起的立体异构体是否为 Z 或 E。如果最高优先的 2 个基团位于经过 C ＝ C 键的参考平面的同一侧，则立体异构体被命名为 Z(zusammen ＝一起）。另一种立体异构体被命名为 E(entgegen ＝相反）。

[0365] E-Z 型的立体异构体的混合物可以使用基于这些化合物的不同化学物理特性的典型纯化方法而分离成（和 / 或表征）其成分。包括在这些方法中的是分级结晶，通过低、中或高压技术执行的层析，分级蒸馏和任何其他本领域技术人员非常熟知的方法。

[0366] 本发明也包括式 I 或 II 的化合物的前药，即，当施用至哺乳动物受试者时，在体内释放式 I 或 II 的活性药物的化合物。前药是药学上有活性的化合物，或者更典型地为通过代谢转化而转化成药学活性剂的非活性化合物。以修饰可以被体内切割从而释放母体化合

物的方式，通过修饰式 I 化合物中存在的官能团来制备式 I 化合物的前药。在体内，前药容易在生理条件下经历化学变化（例如，被水解或受到天然存在的酶的作用），引起药学活性剂的释放。前药包括式 I 或式 II 的化合物，其中羟基、氨基或羧基与任何可以在体内被切割从而分别再生出游离羟基、氨基或羧基的基团结合。前药的实例包括但不限于式 I 化合物的酯（例如，乙酸酯、甲酸酯和苯甲酸酯衍生物）或任何其他在接触到生理 pH 时或通过酶作用被转化为活性母体药物的衍生物。选择和制备合适的前药衍生物的常规步骤在本领域中记载（参见，例如，Bundgaard. Design of Prodrugs. Elsevier, 1985）。

[0367] 前药可以以与它们所转化的活性成分相同的方式进行施用，或者它们可以以储库形式例如经皮贴剂或其他适用于（通过提供酶或其他合适试剂）将前药随时间缓慢地转化为活性成分并将活性成分运送至患者的储库而进行运送。

[0368] 除非特别指出，术语“活性成分”被理解为指代本文定义的式 I 的化合物。

[0369] 本发明也包括代谢物。本文公开化合物的“代谢物”是在化合物代谢时形成的化合物的衍生物。术语“活性代谢物”是指当化合物代谢时形成的化合物的生物学活性衍生物。术语“代谢的”是指特定物质在生命体内变化的过程总和。简单而言，身体内存在的所有化合物被体内的酶操纵，以从中获取能量和 / 或将其从身体中除去。特定酶对化合物引起特定结构变化。例如，细胞色素 P450 催化多种氧化和还原反应，而尿苷二磷酸葡糖醛酸转移酶催化活化的葡糖醛酸分子向芳香醇、脂肪醇、羧酸、胺和游离巯基的转移。其他关于代谢的信息可以从 The Pharmacological Basis of Therapeurics，第九版，McGraw-Hill (1996)，11 ～ 17 页中获得。可以通过将化合物施用至宿主并分析宿主的组织样本、或通过化合物与肝细胞的体外孵育并分析所得化合物来识别本文公开的化合物的代谢物。两种方法均为本领域熟知。

[0370] σ-2 受体组合物

[0371] 在一些实施方式中，本发明提供组合物，例如包括 σ-2 受体的结合检定混合物，其包括含有 σ-2 受体和式 I 或式 II 的 σ-2 配体化合物（包括但不限于本文特别描述的个别化合物）的组合物。

[0372] 在一些实施方式中，σ-2 受体与 σ-2 配体化合物复合。在一些实施方式中，包括 σ-2 受体的组合物是分离的组合物。本文关于 σ-2 受体所用的术语“分离的组合物”是指无细胞或者以其他方式从其天然环境移出的 σ-2 受体。在一些实施方式中，天然环境是未被裂解或未以其他方式破坏的细胞。从细胞中分离 σ-2 受体可以通过常规已知的方法完成，例如分离各种细胞组分并各自测试其中 σ-2 受体的存在（通过非限制性实例的方式，使用竞争性放射性配体方法）。因此，分离的 σ-2 受体可以存在于细胞质中或细胞的各种亚区室中，例如线粒体或内质网、内体或溶酶体、或脂筏中，这可以是 σ-2 受体在其天然环境中的物理位置。脂筏是富含特定组分例如但不限于胆固醇和鞘脂的通常较小 (10-200nm)、异质且高度动态的组件。脂筏的其它组分包括但不限于，谷氨酸受体（如离子型（阳离子特异的离子通道）和 / 或代谢型 (G 蛋白偶联），mGluR5)、胆固醇、脂质、BACE、γ- 分泌酶、全长 APP（淀粉样蛋白前体蛋白）、神经节苷脂（例如神经节苷脂 GM1)、细胞朊蛋白、跨膜蛋白识别的淀粉样蛋白 -β 前体样蛋白 1 (APLP1)、跨膜蛋白 30B (TMEM30B)、α7 烟碱型乙酰胆碱受体 (nAChRα7)、晚期糖基化终产物受体 (RAGE)、N- 甲基 -D- 天冬氨酸受体 (NMDAR)、神经生长因子受体 (NGFR)（例如 TrkA 和 p75 神经营养因子受体）、胰岛素受

体亚单元、或其任何组合。参见 Rushworth 等人，International Journal of Alzheimer's Disease，第 2011 卷，Article ID603052，14 页，其全部引入本文以供参考。脂筏可以从细胞中分离且分离的脂筏可以含有 σ-2 受体。

[0373] 在一些实施方式中，将 σ-2 受体暴露于 Abeta 寡聚体或其它 Abeta 种类，使得其与 β 淀粉样蛋白寡聚体缔合或复合。在一些实施方式中，β 淀粉样蛋白寡聚体已从细胞中分离。在一些实施方式中，以合成方式制备或在体外制备 β 淀粉样蛋白寡聚体。在本文中描述 β 淀粉样蛋白寡聚体和种类的非限制性实例。

[0374] 在一些实施方式中，包括 σ-2 受体的组合物额外包括其它受体或其组 (panel)。实例为：谷氨酸受体（如离子型（阳离子特异的离子通道）和/或代谢型 (G 蛋白偶联)，已知 GluR5)、α 7 烟碱型乙酰胆碱受体 (nAChR α 7)、晚期糖基化终产物受体 (RAGE)、N-甲基-D-天冬氨酸受体 (NMDAR)、神经生长因子受体 (NGFR)（例如 TrkA 和 p75 神经营养因子受体）、胰岛素受体亚单元、或其任何组合。组合物的其它可能组分包括胆固醇、脂质、BACE、γ-分泌酶、全长 APP（淀粉样蛋白前体蛋白）、神经节苷脂（例如神经节苷脂 GM1)、细胞朊蛋白、跨膜蛋白、β 淀粉样蛋白前体样蛋白 1(APLP1)、跨膜蛋白 30B(TMEM30B)。

[0375] 包括 σ-2 受体、σ-2 配体和脂筏或者来自脂筏的蛋白、脂质、胆固醇或其它组分的组合物通过例如从细胞中分离 σ-2 受体并将 σ-2 受体与 σ-2 配体接触来制备。在一些实施方式中，σ-2 配体和 σ-2 受体在足以形成复合物的条件下接触。在一些实施方式中，复合物在 β 淀粉样蛋白寡聚体的存在下形成。

[0376] 文献中已提议 Abeta 寡聚体的多种受体，包括朊蛋白、胰岛素受体、β 肾上腺素能受体和 RAGE（晚期糖化终产物的受体）。Laurén, J. 等人，2009, Nature, 457(7233):1128-1132；Townsend, M. 等人，J. Biol. Chem. 2007, 282:33305-33312；Sturchler, E. 等人，2008, J. Neurosci. 28(20):5149-5158。实际上，很多研究者相信，Abeta 寡聚体可以与多于一种的受体蛋白结合。Krafft GA, Klein WL Neuropharmacology (2010) Sep-Oct ;59(4-5):230-42。基于本文呈现的以及在与本申请同日提交的共同待决的共同转让申请中的证据，本发明的发明者假定出（不必须排他地）位于神经元中的 Abeta 寡聚体的其他受体。不受理论的约束，提出 σ-2 受体是神经元中 Abeta 寡聚体的受体。在一些实施方式中，本发明提供组合物，其包括神经元中表达的 Abeta 寡聚体受体以及 Abeta 寡聚体。这样的组合物还可以包括不是 Abeta 寡聚体受体的一种或多种神经元蛋白。在一些实施方式中，Abeta 寡聚体受体是 σ-2 受体。在一些实施方式中，σ-2 受体是活化受体。在一些实施方式中，σ-2 受体是不活化或失敏受体。在一些实施方式中，组合物包括脂筏蛋白。脂筏蛋白的实例在本文描述但实例是非限制性的。神经元蛋白是在神经元细胞中或任何情况下在中枢神经系统中特异表达的蛋白。在一些实施方式中，神经元蛋白在脑中特异表达。在一些实施方式中，神经元蛋白是在神经元中而不在其它组织或细胞类型中表达的蛋白。在一些实施方式中，神经元蛋白是在神经元中而不在睾丸以外的其它组织或细胞类型中表达的蛋白。在一些实施方式中，额外的蛋白可以促进 Abeta 寡聚体在神经元中的有害作用。

[0377] 前述含有 σ-2 受体的组合物可以在用于识别与该受体结合的其它化合物的检定中采用，这样的化合物因此在使免受、减少或逆转突触丧失或膜运输异常中具有潜在活性，进一步在抑制认知衰退和治疗 MCI 和阿尔茨海默病中具有活性。例如，这样的检定包括但不限于可以测量标记的 σ-2 配体被未标记的候选 σ-2 配体取代的检定。这样的用于识别

活性化合物的竞争性结合检定已在制药产业使用了许多年，且为本领域技术人员所知晓。

[0378] 本发明化合物的用途

[0379] 在一些实施方式中，本发明提供抑制与神经元细胞暴露于 Abeta 种类相关的突触数量减少或膜运输异常的方法。本发明也提供在患者中治疗认知衰退和 / 或神经退行性疾病例如阿尔茨海默病或轻度认知障碍 (MCI) 的方法，包括向患者施用本文记载的 σ -2 配体或其药学可接受的盐。在一些实施方式中，抑制或治疗认知衰退和 / 或神经退行性疾病 (例如阿尔茨海默病) 的方法包括抑制或治疗一种或多种选自记忆丧失、思维混乱、判断力受损、人格改变、定向障碍和语言技巧丧失中的认知衰退症状。在一些实施方式中，方法包括抑制或治疗由 Abeta 寡聚体介导的或与 Abeta 寡聚体相关的疾病或障碍或病症 (参见段落 002)。在一些实施方式中，抑制或治疗认知衰退和 / 或神经退行性疾病例如阿尔茨海默病的方法包括以下的一种或多种 :(i) 恢复可经电生理学测量检测的长时程增强效应 (LTP)、LTD 或突触可塑性或者任何在以上术语定义中提及的认知功能的其它负面变化 ; 和 / 或 (ii) 抑制或治疗神经退行性变 ;和 / 或 (iii) 抑制或治疗一般的淀粉样变性 ;和 / 或 (iv) 抑制或治疗淀粉样蛋白产生、淀粉样蛋白组装、淀粉样蛋白聚集和淀粉样蛋白寡聚体结合及淀粉样蛋白沉积中的一种或多种 ;和 / 或 (v) 抑制、治疗和 / 或减轻一种或多种 Abeta 寡聚体对神经元细胞的作用，特别是非致死作用。在一些实施方式中，抑制、治疗和 / 或减轻认知衰退和 / 或神经退行性疾病例如阿尔茨海默病的方法包括抑制、治疗和 / 或减轻淀粉样蛋白产生、淀粉样蛋白组装、一种或多种 Abeta 寡聚体对神经元细胞的活性 / 作用、淀粉样蛋白积聚、淀粉样蛋白结合和淀粉样蛋白沉积中的一种或多种。在一些实施方式中，抑制、治疗和 / 或减轻认知衰退和 / 或神经退行性疾病例如阿尔茨海默病的方法包括抑制、治疗和 / 或减轻一种或多种 Abeta 寡聚体对神经元细胞的一种或多种活性 / 作用。

[0380] 在一些实施方式中，一种或多种 Abeta 寡聚体对神经元细胞、淀粉样蛋白积聚、淀粉样蛋白结合和淀粉样蛋白沉积的活性 / 作用是 Abeta 寡聚体对膜运输或突触数量的作用。在一些实施方式中，σ -2 配体抑制 Abeta 寡聚体对膜运输或突触数量的作用或 Abeta 寡聚体结合。

[0381] 在一些实施方式中，本发明提供治疗蛋白质构象病 (proteopathic disease) 的方法。在一些实施方式中，方法包括使具有蛋白质构象病的受试者与本发明的结合 σ -2 受体的 σ -2 配体或含有该配体的组合物接触。

[0382] 在一些实施方式中，蛋白质构象病是 CNS 蛋白质构象病，特征为 Abeta 蛋白增加，例如 MCI、唐氏综合症、黄斑变性或阿尔茨海默病等。

[0383] 在一些实施方式中，本发明提供通过施用本发明的 σ -2 配体来治疗一种或多种轻度认知障碍 (MCI) 或痴呆的方法。在一些实施方式中，本发明提供治疗 MCI 和痴呆的方法。

[0384] 在一些实施方式中，本发明提供用本发明的 σ -2 配体治疗个体，以在受到 Abeta 种类例如 Abeta 寡聚体不利影响的功能方面部分地或全部地将受试者的细胞修复到正常表型的方法。实例是可以通过多种方法包括本文所述检定而测量的突触数量减少和膜运输异常。正常的表型可以是例如正常的膜运输。在一些实施方式中，正常的表型是正常的认知能力。“正常”表型可以通过比较受试者的结果与正常受试者的样本的结果而确定。样本可以少至 1 个受试者或 1 个样本，或者可以是多于 10 个样本或受试者，且标准是基于多个

受试者计算的均值。

[0385] 在一些实施方式中，方法包括向受到认知衰退或神经退行性疾病折磨的受试者施用与 σ-2 蛋白结合并抑制 β 淀粉样蛋白病理的化合物或组合物。在一些实施方式中，β 淀粉样蛋白病理是膜运输缺陷、突触数量的减少、树突脊数量的减少、树突脊形态的变化、LTP 的变化、LTD 的变化、动物的记忆和学习措施中的缺陷或其任意组合等。以上用途得自发明者举出的以下证据：

[0386] 已在本文显示式 I 和式 II 内的化合物具体为化合物 II 抑制神经细胞中与 Abeta 相关的突触减少，并且当在引入 Abeta 寡聚体之前或之后添加化合物时，其对伴随着这些细胞暴露于合成制剂或从阿尔茨海默病人脑中分离的制剂（后者在体外介导淀粉样蛋白病理中更有潜力得多）中的 Abeta 寡聚体时的神经元细胞中的膜运输异常（例如，使用下述的 MTT 检定）产生抑制。在式 I 和式 II 内的其他化合物也显示为抑制膜运输中的异常。化合物 II 也在本文中示出为抑制在如本文所述的转基因和诱导的阿尔茨海默病动物模型中展现的认知缺陷，其与认知衰退和记忆丧失有关。化合物 II 以及式 I 内的其他化合物例如化合物 B 和 II 也已经在药物代谢动力学研究中示出为全身性吸收并穿越血脑屏障并为生物可得。由于这些特性，并鉴于大部分作用被归因于淀粉样蛋白病理（例如阿尔茨海默病早期）的发展中的 Abeta 寡聚体和 Abeta 组装体的技术现状，预期化合物 II 将在轻度认知障碍的治疗和防止中以及在阿尔茨海默病的治疗（如本文所述）中有活性。此外，因为与化合物 II 的结构相似性，并且因为已经证实化合物 II 的以上体外活性、在以上具体公开的化合物当中的代表性数量的式 I 和式 II 内的其他化合物的药物代谢动力学特性和 σ-2 配体状态，预期式 I 和式 II 内的所有化合物在体内相似地有活性。

[0387] 化合物 II 行为效力：小鼠条件性恐惧中 Abeta 寡聚体诱导的记忆缺陷是在哥伦比亚大学 Ottavio Arancio 博士的实验室中建立的模型 (Puzzo'08)。一些药物公司在他们的发现工作中使用该同一模型。情境条件性恐惧是与人认知功能尤其是新记忆创建相关的联想记忆形成的公认模型 (Delgado' 06)。将 Abeta 寡聚体在临条件训练前注射到野生型动物的海马中，在 24 小时后通过僵立行为对记忆进行评估。详情提供在实施例 9 中。其中，化合物 II 能够完全地消除小鼠中的记忆缺陷而不抑制记忆（当单独给药时）或造成任何行为或行动毒性。选择该模型体系是因为寡聚体的海马内施用使得可以快速比较评估化合物活性和脱靶毒性。结果以图表显示在图 4 中。

[0388] 化合物 II 也在两个转基因阿尔茨海默病模型中进行体内测试，以示出化合物在逆转 Abeta 寡聚体相关的记忆丧失中的作用。具体而言，化合物 II 修复两个不同突变小鼠模型的能力（该突变小鼠模型随着年龄增长而逐渐发展出以记忆丧失为特征的认知衰退），以记起记忆丧失发生前获取的技能。此外，化合物 II 显著抑制野生型小鼠的海马 Abeta 寡聚体暴露的效应，保留小鼠获取新记忆的能力。

[0389] 这些行为研究统一证明了，使用两个不同的阿尔茨海默病模型，以两种性别，并按照短期或长期给药，化合物 II 引起两个不同行为任务中学习和记忆的改善，并且表明了体外检定与体内活性相关。因此，与显示化合物 II 结合 σ-2 受体以及其在体外抑制 Abeta 相关的病理的数据相结合，这些结果表明化合物 II 可以用于治疗神经退行性疾病例如阿尔茨海默病。式 I 和式 II 内的其他化合物也被发现与 σ-2 受体结合并具有如化合物 II 的相似体外活性。基于它们与化合物 II 的相似性，并且具有化合物 II 类似的 Abeta 寡聚体

的药效团，预计它们具有如化合物 II 的相似体外活性和体内活性。确实，截止这些化合物已经在体外进行了测试的程度，它们具有如化合物 II 的同类活性，因此预计它们具有相似的体内活性，因此可用于相同的治疗症状。多种在式 I 或式 II 内的其他 σ-2 配体化合物已经或将在本文所述的突触减少和 / 或膜运输检定中进行测试，并预期在抑制 Abeta 寡聚体相关的突触丧失中以及在抑制 Abeta 寡聚体相关的膜运输异常中具有活性，并且在抑制认知衰退和治疗阿尔茨海默病中具有相似活性。

[0390] 前述结论基于以下阿尔茨海默病和轻度认知障碍的背景。如本文所讨论的，证据表明，由 Abeta 寡聚体介导的经膜运输介导的神经元表面受体表达减少是突触可塑性 (LTP) 的电生理测量的寡聚体抑制以及因此的学习和记忆的基础（参见Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, Sisodia S, Malinow R. APP processing and synaptic function. Neuron. 2003Mar27 ;37(6):925-37 ； 和 Hsieh H, Boehm J, Sato C, Iwatsubo T, Tomita T, Sisodia S, Malinow R. AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. Neuron. 2006Dec7 ;52(5):831-43)。经甲臜形态转变测量由寡聚体引起的膜运输速率变化已经用于细胞系中，以发现 Abeta 寡聚体-阻抑药物 [Maezawa I, Hong HS, Wu HC, Battina SK, Rana S, Iwamoto T, Radke GA, Pettersson E, Martin GM, Hua DH, Jin LW. A novel tricyclic pyrone compound ameliorates cell death associated with intracellular amyloid-beta oligomeric complexes. J Neurochem. 2006Jul ;98(1):57-67 ;Liu Y, Schubert D. Cytotoxic amyloid peptides inhibit cellular3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT)reduction by enhancing MTT formazan exocytosis. J Neurochem. 1997Dec ;69(6):2285-93 ;Liu Y, Dargusch R, Banh C, Miller CA, Schubert D. Detecting bioactive amyloid beta peptide species in Alzheimer's disease. J Neurochem. 2004Nov ;91(3):648-56 ;Liu Y, Schubert D. Treating Alzheimer's disease by inactivating bioactive amyloid beta peptide. Curr Alzheimer Res. 2006Apr ;3(2):129-35 ;Rana S, Hong HS, Barrigan L, Jin LW, Hua DH. Syntheses of tricyclic pyrones and pyridinones and protection of Abeta-peptide induced MC65neuronal cell death. Bioorg Med Chem Lett. 2009Feb 1 ;19(3):670-4. Epub2008Dec24 ;和Hong HS, Maezawa I, Budamagunta M, Rana S, Shi A, Vassar R, Liu R, Lam KS, Cheng RH, Hua DH, Voss JC, Jin LW. Candidate anti-Abeta fluorene compounds selected from analogs of amyloid imaging agents. Neurobiol Aging. 2008Nov18. (Epub ahead of print)]，这样的药物降低啮齿动物体内的 Abeta 脑含量 [Hong HS, Rana S, Barrigan L, Shi A, Zhang Y, Zhou F, Jin LW, Hua DH. Inhibition of Alzheimer's amyloid toxicity with a tricyclic pyrone molecule in vitro and in vivo. J Neurochem. 2009Feb ;108(4):1097-1108]。因此，上述测试已经在识别化合物以治疗阿尔茨海默病和轻度认知障碍中建立相关性。

[0391] 在一些实施方式中，关于抑制 Abeta 寡聚体对神经元（例如脑中的神经元）、淀粉样蛋白组装或其破坏以及淀粉样蛋白（包括淀粉样蛋白寡聚体）结合和淀粉样蛋白沉积的一种或多种作用，化合物具有小于 100μM、50μM、20μM、15μM、10μM、5μM、1μM、500nM、100nM、50nM 或 10nM 的 IC_{50} 值。在一些实施方式中，关于抑制 Abeta 种类例如寡聚体对神经元（例如中枢神经元）的活性 / 作用，化合物具有小于 100μM、50μM、20μM、15μM、10μM、

5μM、1μM、500nM、100nM、50nM 或 10nM 的 IC_{50} 值。

[0392] 在一些实施方式中，本发明化合物对 Abeta 种类例如寡聚体对神经元（例如脑中神经元）的一种或多种作用（例如淀粉样蛋白（包括淀粉样蛋白寡聚体）与突触的结合以及由 Abeta 寡聚体介导的膜运输异常）的抑制百分比在 10nM 至 10μM 的浓度进行测量。在一些实施方式中，所测量的抑制百分比为约 1%至约 20%、约 20%至约 50%、约 1%至约 50%、或约 1%至约 80%。抑制可以例如通过在暴露于 β 淀粉样蛋白种类之前和之后定量神经元的突触数量或者在同时存在 σ-2 配体和 Abeta 种类时定量突触数量而评测，其中 σ-2 配体与 Abeta 种类暴露同时、或在之前或在之后。作为另一个实例，抑制可以通过存在和不存在 Abeta 种类以及存在和不存在本发明 σ-2 配体时确定膜运输并比较测量胞吐速率和程度、内吞速率和程度或其他细胞代谢指标的一个或多个参数而进行评测。本发明的发明者已举出生化检定证据表明本发明的化合物也抑制淀粉样蛋白聚集。

[0393] 在一些实施方式中，本文所述的化合物与 σ-2 受体特异性地结合。与特定受体特异性结合的化合物是指相对于另一受体偏好一种受体的化合物。例如，尽管化合物对 σ-1 和 σ-2 受体都能结合，但以高出 σ-1 受体至少 10%的亲和性结合时，可以说化合物是对 σ-2 受体特异性的。在一些实施方式中，对于一种结合配偶体（例如受体）的特异性相对于第二结合配偶体高出至少 10、20、30、40、50、60、70、80、90、100、200、300、400、500 或 1000%。

[0394] 在一些实施方式中，本发明提供使用标记的 σ-2 配体在动物中测量 β 淀粉样蛋白相关的认知衰退的方法。在一些实施方式中，方法包括使动物与本发明的标记 σ-2 配体接触并测量 σ-2 活性或表达。在一些实施方式中，方法包括将动物中的 σ-2 活性或表达与已知具有 β 淀粉样蛋白诱导的认知衰退的动物进行比较。如果活性或表达与已知具有 β 淀粉样蛋白诱导的认知衰退的动物相同，称该动物具有相同水平的认知衰退。动物可以根据在多个 β 淀粉样蛋白诱导的认知衰退阶段中的已知活性或表达中的相似度来分级。本文所述的任意 σ-2 配体可以被标记，从而标记的 σ-2 配体可以在体内使用。

[0395] 在确定任意上述通式的化合物和上述描述为 σ-2 拮抗剂的其它化合物是否可有效治疗本文所述的各种病症时，可以使用体外检定。体外检定已与使用化合物 II 的体内效果相关联。例如，如果与化合物 II 具有结构相似性的式 III-IV 的化合物例如在本文所述的体外检定中具有活性，则也可将其用于体内治疗或改善本文所述的病症，包括抑制或恢复突触丧失、调节神经元细胞中的膜运输变化、使免受记忆丧失或恢复、以及治疗认知衰退病症、疾病和障碍例如 MCI 和阿尔茨海默病。检定部分基于 β 淀粉样蛋白寡聚体及其在突触处与神经元结合的功能以及 β 淀粉样蛋白寡聚体在体外对神经元的作用。在一些实施方式中，本发明者认为包括 σ-2 蛋白的神经元中的 Abeta 寡聚体受体与本文所述的 β 淀粉样蛋白组装体结合，且与 σ-2 蛋白结合的式 I、II 或 VIII 的化合物会抑制 β 淀粉样蛋白组装体与受体的结合。在竞争性放射性配体结合检定中，本发明者已显示本发明的化合物对于 σ-2 受体具有特异性。发明者也已显示，本发明的化合物抑制 Abeta 寡聚体与其迄今未识别的受体在神经元表面上的结合。在一些实施方式中，提供方法来确定任何上式的化合物在神经元信号传导中的 σ-2 配体功效。在一些实施方式中，方法包括使细胞（例如但不限于初级神经元）与 σ-2 配体结合，以及测定神经元功能。在一些实施方式中，在体外接触细胞。在一些实施方式中，在体内接触细胞。神经元活性可以是信号传导活性、电活

性、突触蛋白的产生或释放等。增强或恢复信号传导的 σ-2 拮抗剂被识别为可有效调节神经元活性的化合物。在一些实施方式中，细胞源自病理学样品。在一些实施方式中，细胞源自具有神经退行性疾病的受试者。在一些实施方式中，神经退行性疾病是 MCI 或阿尔茨海默病，尤其是轻度阿尔茨海默病。

[0396] β 淀粉样蛋白组装体和组装体使用方法的实施方式在此处和以下以及 WO2011/014880（申请号 PCT/US2010/044136）、WO2010/118055（申请号 PCT/US2010/030130）和申请号 PCT/US2011/026530 中描述，其各自全部引入本文以供参考。还可以使用可使用的其它检定，例如膜运输检定和/或条件性恐惧检定。这些方法在本文及 WO2011/014880（申请号 PCT/US2010/044136）、WO2010/118055（申请号 PCT/US2010/030130）和申请号 PCT/US2011/026530 中描述，其各自全部引入本文以供参考。

[0397] 受体结合检定和化合物筛选

[0398] 本发明还提供对抑制认知衰退或治疗神经退行性疾病的另一化合物进行鉴定的方法。在一些实施方式中，方法包括使细胞与结合 σ-2 受体的化合物接触。在一些实施方式中，方法包括确定化合物是否抑制 β 淀粉样蛋白病理学，其中抑制 β 淀粉样蛋白病理学的化合物被鉴定为结合 σ-2 受体且抑制认知衰退或治疗神经退行性疾病的化合物。在一些实施方式中，方法还包括鉴定结合 σ-2 受体的其它化合物。在一些实施方式中，鉴定与 σ-2 受体结合的化合物的方法包括竞争性结合检定，其中使测试化合物在已知 σ-2 配体例如本发明化合物的存在下与 σ-2 受体接触，其中竞争性地抑制已知配体的结合的测试化合物被识别为 σ-2 受体配体。

[0399] 确定化合物是否可与 σ-2 受体结合的方法是已知的，且可以使用任何方法。例如，通过合同研究组织进行的测试可以用于确定化合物是否与 σ-2 结合。可以进行各种检定以确定化合物是否与 σ-2 受体结合。在一些实施方式中，使用稳定地表达人受体包括但不限于 σ-2 受体的均质群体的细胞，例如但不限于人胚肾（HEK293）、Jurkat 细胞、或中国仓鼠卵巢（CHO）细胞。在其它情况下，使用 σ-2 受体的组织来源，例如啮齿动物新皮质膜。其实例描述于本文的实施例部分。

[0400] 在一些实施方式中，使测试化合物与细胞或细胞膜接触以确定测试化合物是否可与 σ-2 受体结合。在一些实施方式中，将测试化合物溶解于载体或媒介物，例如但不限于二甲亚砜。在一些实施方式中，培养细胞直到汇合（confluent）。在一些实施方式中，在汇合时，可以通过柔和的刮擦使细胞脱离。在一些实施方式中，通过胰蛋白酶化或任何其它合适的脱附方式使细胞脱离。

[0401] 在一些实施方式中，可以通过例如竞争性放射性配体结合检定来确定测试化合物与 σ-2 受体的结合。放射性配体结合检定可以在稳定表达人受体的完整细胞或组织来源上进行。例如，可以在缓冲剂中洗涤、离心和/或重悬脱离的细胞或组织。可以根据任何方法包括但不限于本文所述的那些对测试化合物进行放射性标记。可以在不存在和存在各种浓度（范围可以是例如 10^{-10}-10^{-3}M 或 10^{-11}-10^{-4}M）的竞争药物的情况下以 0.1 μCi 的固定浓度使用放射性配体。可以将药物添加到缓冲液中的组织或细胞（～例如 50,000 细胞）并使其孵育。可以在存在各种受体亚型的广谱活化剂或抑制剂或功能性激动剂或拮抗剂的情况下确定非特异结合（例如，针对 σ 受体，对各受体存在例如 10 μM 合适配体）。可以通过快速过滤（其后可以用冰冷的缓冲液洗涤两次）来终止反应。可以使用任何方法包括但不

限于液体闪烁分析仪来测量干燥滤片上的放射性。可以绘制取代曲线，并且可以使用例如GraphPad Prism(GraphPad Software Inc.，San Diego，CA)来确定测试配体对于受体亚型的Ki值。可以通过将总结合（每分钟衰变数）与非特异结合（每分钟衰变数）之间的差异除以总结合（每分钟衰变数）来确定特异结合百分比。

[0402] 在一些实施方式中，对于细胞系或组织来源中的结合研究，在各个实验内添加变化浓度的各个药物，一式两份，并且使用例如GraphPad Prism软件确定各自的IC_{50}值。可以根据Cheng and Prusoff(1973)描述的等式确定各个配体的Ki值，且最终数据可以呈现为pKi±S.E.M.，其中在一些实施方式中，测试次数为约1～6。

[0403] 在一些实施方式中，方法还包括确定与σ-2受体结合的化合物是否在σ-2受体处通过抑制可溶Aβ寡聚体诱导的神经毒性（关于抑制可溶Aβ寡聚体诱导的突触丧失和抑制可溶Aβ寡聚体诱导的膜运输检定不足）起到拮抗剂作用。在一些实施方式中，方法还包括确定σ-2受体拮抗剂在不存在Abeta寡聚体的情况下不影响运输或突触数目；不诱导神经元细胞中的caspase-3活性；抑制经σ-2受体激动剂对caspase-3活性的诱导；且/或降低或免受由σ-2受体激动剂引起的神经元细胞中的神经元毒性。

[0404] 测试还可包括确定测试化合物对结合配偶体（其可以是但不限于σ-2受体）功能的作用的功能性检定。可以使用多种标准检定技术。例如，可以使用方法来测量化合物在活细胞或组织中的功能性的类激动剂或类拮抗剂的活性。方法包括但不限于，确定cAMP浓度和IP1水平的TR-FRET，监测钙流量的实时荧光，测量阻抗调制、回肠收缩、肿瘤细胞凋亡的细胞介电谱。还可以通过例如确定化合物是否与σ-1受体、σ-2受体、两者均不或两者均结合来确定测试化合物的特异性。用于确定测试化合物是否与σ-1受体结合的方法描述于Ganapathy，M.E等人(1999)J.Pharmacol.Exp.Ther.，289:251-260，其全部引入本文以供参考。用于确定测试化合物是否与σ-1受体结合的方法描述于Bowen，W.D等人(1993)Mol.Neuropharmacol.，3:117-126，其全部引入本文以供参考，以及Xu，J.等人，Nature Communications，2011，2:380DOI:10.1038/ncomms1386，其也全部引入本文以供参考。

[0405] 在各个实施方式中，本公开内容提供用于鉴定选择性、高亲和性σ-2受体配体的检定方案，该σ-2受体配体在抑制可溶Aβ寡聚体诱导的突触丧失方面通过抑制可溶Aβ寡聚体诱导的神经毒性在σ-2受体处用作功能性拮抗剂，抑制膜运输检定中可溶Aβ寡聚体诱导的不足，在不存在Abeta寡聚体的情况下不影响运输或突触数目，并且显示出本文所述的较好的药物样特性，使得由此鉴定的选择性、高亲和性σ-2受体拮抗剂化合物可以用于在体内治疗可溶Aβ寡聚体诱导的突触功能障碍。

[0406] 在一些实施方式中，提供用于鉴定对减轻或使免受非致死Abeta寡聚体毒性具有活性的化合物的筛选方法，其基本上受益于将测试化合物与σ-2受体结合的能力引入为筛选标准，该能力通过例如其取代已知配体的能力或通过任何其它方法来评估。此外，测试化合物应进行体外测试或体内检定中的至少一种，其中体外测试可以评估化合物阻断或减轻Abeta寡聚体对神经元的非致死有害作用的能力（例如本文所述的膜运输检定或突触数目或寡聚体结合检定），体内检定评估对认知衰退的治疗（例如本文所述的那些）。

[0407] 在一些实施方式中，本发明提供确定受试者是否应受σ-2拮抗剂治疗的方法，其中受试者疑似具有认知衰退或神经退行性疾病或本文所述的其它病症、疾病或障碍。在一些实施方式中，方法包括使源自患者的样品与σ-2拮抗剂接触，以及确定σ-2调节化合物

是否抑制或改善样品中存在的 β 淀粉样蛋白病理学，其中显示样品中存在的 β 淀粉样蛋白病理学被抑制或改善的样品指示受试者应受 σ-2 拮抗剂治疗。

[0408] 另外，本发明包括鉴定抑制 A β 寡聚体诱导的突触数目减少等的 σ-2 拮抗剂的方法。在一些实施方式中，可以使用方法来鉴定用于治疗 β 淀粉样蛋白病理的 σ-2 拮抗剂。在一些实施方式中，使用方法来确定治疗 β 淀粉样蛋白病理的疗法功效。在一些实施方式中，β 淀粉样蛋白病理是膜运输缺陷、突触功能障碍、动物内的记忆和学习缺陷、突触数目减少、树突棘长度或棘形态的改变、LTP 缺陷、或 Tau 蛋白磷酸化的增加。

[0409] 用于本公开内容的淀粉样的 β 淀粉样蛋白

[0410] 人 β 淀粉样蛋白是发现集中于神经元突触中的整合膜蛋白淀粉样蛋白前体蛋白 (APP) 的切割产物。β 淀粉样蛋白自缔合形成亚稳的寡聚组装体 (assembly)。在较高浓度下，Abeta 会聚合并组装成线形原纤维，这通过较低 pH 得到促进。目前不清楚的是原纤维是否由寡聚体形成。β 淀粉样蛋白寡聚体已证实为在动物模型中通过诱导阻断学习和记忆的神经元突触的改变而引起阿尔茨海默病，且 β 淀粉样蛋白原纤维早已与动物和人中的晚期阿尔茨海默病相关联。事实上，现代一种广受支持的阿尔茨海默病作用假说是，Abeta 组装体尤其是 Abeta 寡聚体处于与阿尔茨海默病相关的早期病理学以及与较不严重的痴呆（例如 MCT 和轻度 AD）相关的病理学的中心。明显地，James P. 等人，"Natural oligomers of the amyloid-β protein specifically disrupt cognitive function."Nature Neuroscience Vol.8(2005):79-84;Klyubin,I. 等人，"Amyloid beta protein dimer-containing human CSF disrupts synaptic plasticity:prevention by systemic passive immunization."J Neurosci.Vol.28(2008):4231-4237。然而，对于寡聚体如何形成以及寡聚体的结构状态知之甚少。例如，缔合形成寡聚体的 β 淀粉样蛋白亚单位的数目目前是未知的，寡聚体的结构形式或暴露哪些残基也如此。有证据表明多于一种结构状态的寡聚体具有神经活性。Reed,Jess D. 等人，"MALDI-TOF mass spectrometry of oligomeric food polyphenols."Phytochemistry66:18(September2005):2248-2263；Cleary,James P. 等 人，"Natural oligomers of the amyloid-β protein specifically disrupt cognitive function."Nature Neuroscience Vol.8(2005):79-84。

[0411] β 淀粉样蛋白对大脑内发现的许多蛋白包括 ApoE 和 ApoJ 具有亲和性。然而，不清楚分子伴侣或其它蛋白是否与可影响其最终结构状态和/或其神经活性的蛋白缔合。

[0412] 可溶 Abeta 肽很可能通过扰乱突触功能障碍和认知过程而在 AD 的早期起关键作用。例如，Origlia 等人显示，可溶 Abeta(Abeta42) 在内嗅皮质中通过晚期糖基化终产物 (RAGE) 所介导的 p38MAPK 活化的神经元受体而损害长时程增强效应 (LTP)。Origlia 等 人，2008,Receptor for advanced glycation end product-dependent activation of p38mitogen-activated protein kinase contributes to amyloid-beta-mediated cortical synaptic dysfunction.J.Neuroscience28(13):3521-3530，其引入本文以供参考。

[0413] 在阿尔茨海默病的早期涉及突触功能障碍。已显示 β 淀粉样蛋白肽改变突触功能。Puzzo 等人报道，Abeta 的合成纤维状形式损害 LTP 的晚期蛋白合成依赖阶段，而不影响早期蛋白合成阶段。该报道与 Abeta 寡聚体对细胞具有高度毒性且涉及到突触功能障碍中的先前报道一致。Puzzo 等人，2006,Curr Alzheimer's Res3(3):179-183，其引入本文以

供参考。已发现 Abeta 通过多种第二信使级联包括一氧化氮级联而显著损害海马长时程增强效应 (LTP)。NO/cGMP/cGK/CREB. Puzzo 等人，J Neurosci. 2005。在一些实施方式中，本公开内容提供包含 σ-2 受体拮抗剂的用于抑制 β 淀粉样蛋白寡聚体诱导的神经元细胞突触功能障碍；和抑制由神经元暴露于 Abeta 寡聚体而引起的海马长时程增强效应压抑的组合物以及方法。

[0414] 可以将任何形式的 β 淀粉样蛋白用于实施本发明的筛选方法和检定，包括 β 淀粉样蛋白单体、寡聚体、原纤维、以及与蛋白缔合的 β 淀粉样蛋白（“蛋白复合物”）且更通常为 β 淀粉样蛋白组装体。例如，筛选方法可以采用各种形式的可溶 β 淀粉样蛋白寡聚体，例如公开于 U. S. 专利申请序列号第 13/021, 872 号；U. S. 专利公开 2010/0240868；国际专利申请 WO/2004/067561；国际专利申请 WO/2010/011947；U. S. 专利公开 20070098721；U. S. 专利公开 20100209346；国际专利申请 WO/2007/005359；U. S. 专利公开 20080044356；U. S. 专利公开 20070218491；WO/2007/126473；U. S. 专利公开 20050074763；国际专利申请 WO/2007/126473、国际专利申请 WO/2009/048631 和 U. S. 专利公开 20080044406、U. S. 专利第 7, 902, 328 号以及 U. S. 专利第 6, 218, 506 号，其各自引入本文以供参考。

[0415] β 淀粉样蛋白形式，包括 β 淀粉样蛋白的单体或寡聚体可以得自任何来源。例如，在一些实施方式中，市售的 β 淀粉样蛋白单体和 / 或 β 淀粉样蛋白寡聚体可以以水溶液使用，且在其它实施方式中，以蛋白水溶液使用的 β 淀粉样蛋白单体和 / 或 β 淀粉样蛋白寡聚体可以由技术人员使用任何数量的已知技术进行分离和纯化。通常，各个实施方式的以蛋白和 β 淀粉样蛋白的水溶液制剂使用的 β 淀粉样蛋白单体和 / 或 β 淀粉样蛋白寡聚体可溶于水溶液中。因此，水溶液的蛋白和 β 淀粉样蛋白均可以是可溶的。

[0416] 添加的 β 淀粉样蛋白可以是任何亚型。例如，在一些实施方式中，β 淀粉样蛋白单体可以是 β 淀粉样蛋白 1-42，且在其它实施方式中，β 淀粉样蛋白单体可以是 β 淀粉样蛋白 1-40。在其它实施方式中，β 淀粉样蛋白可以是 β 淀粉样蛋白 1-39 或 β 淀粉样蛋白 1-41。因此，各个实施方式的 β 淀粉样蛋白可以包括 β 淀粉样蛋白的任何 C 端亚型。其它实施方式包括 N 端已缺损的 β 淀粉样蛋白，且在一些实施方式中，任何上述 β 淀粉样蛋白 C 端异构体的 N 端可以是氨基酸 2、3、4、5、或 6。例如，β 淀粉样蛋白 1-42 可以包括 β 淀粉样蛋白 2-42、β 淀粉样蛋白 3-42、β 淀粉样蛋白 4-42 或 β 淀粉样蛋白 5-42 及其混合物，且类似地，β 淀粉样蛋白 1-40 可包括 β 淀粉样蛋白 2-40、β 淀粉样蛋白 3-40、β 淀粉样蛋白 4-40 或 β 淀粉样蛋白 5-40。

[0417] 各个实施方式中使用的 β 淀粉样蛋白形式可以是野生型，即具有与大多数种群体内合成的 β 淀粉样蛋白的氨基酸序列等同的氨基酸序列，或者在一些实施方式中，β 淀粉样蛋白可以是突变 β 淀粉样蛋白。实施方式不限于任何特定种类的突变 β 淀粉样蛋白。例如，在一些实施方式中，引入水溶液中的 β 淀粉样蛋白可以包括已知的突变，例如具有“Dutch”(E22Q) 突变或“Arctic”(E22G) 突变的 β 淀粉样蛋白。这样的突变单体可以包括天然出现的突变，例如从易患例如阿尔茨海默病的个体的群体分离的 β 淀粉样蛋白形式，β 淀粉样蛋白的家族形式。在其它实施方式中，可以通过使用分子技术合成制备突变 β 淀粉样蛋白单体，以制备具有特定突变的 β 淀粉样蛋白突变体。在其它实施方式中，突变 β 淀粉样蛋白单体可以包括之前未识别的突变，例如，在随机产生的 β 淀粉样蛋白突变体中发现的那些突变体。本文使用的术语“β 淀粉样蛋白”意在包括 β 淀粉样蛋白的野生型形

式以及 β 淀粉样蛋白的任何突变形式。

[0418] 在一些实施方式中，蛋白水溶液中的 β 淀粉样蛋白可以是单一亚型。在其它实施方式中，β 淀粉样蛋白的各种C端亚型和/或 β 淀粉样蛋白的各种N端亚型可以组合形成 β 淀粉样蛋白混合物，其可以以蛋白水溶液提供。在其它实施方式中，β 淀粉样蛋白可以源自被添加到含蛋白的水溶液中且原位切割的淀粉样蛋白前体蛋白 (APP)，以及这样的实施方式，β 淀粉样蛋白的各种亚型可以包含在该溶液中。在添加 β 淀粉样蛋白之后，在水溶液内可出现N端氨基酸缺损 (fraying) 和/或C端氨基酸移除。因此，即使在初始向溶液中添加单一亚型的情况下，如本文所述制备的水溶液也可以包括多种 β 淀粉样蛋白亚型。

[0419] 添加到水溶液中的 β 淀粉样蛋白单体可以从自然来源例如活组织中分离，且在其它实施方式中，β 淀粉样蛋白可以源自合成来源例如转基因小鼠或经培养的细胞。在一些实施方式中，β 淀粉样蛋白形式包括单体、寡聚体或其组合从正常受试者和/或被诊断为具有认知衰退或与其相关的疾病（例如但不限于阿尔茨海默病）的患者中分离。在一些实施方式中，β 淀粉样蛋白单体、寡聚体、或其组合是已从正常受试者或疾病患者中分离的Abeta 组装体。在一些实施方式中，Abeta 组装体具有高分子量，例如大于 100KDa。在一些实施方式中，Abeta 组装体具有中等分子量，例如 10 ～ 100KDa。在一些实施方式中，Abeta 组装体小于 10kDa。

[0420] 一些实施方式的 β 淀粉样蛋白寡聚体可以由与“寡聚体”的常用定义一致的任何数目的 β 淀粉样蛋白单体组成。例如，在一些实施方式中，β 淀粉样蛋白寡聚体可以包括约 2 至约 300、约 2 至约 250、约 2 至约 200 个 β 淀粉样蛋白单体，且在在其它实施方式中，β 淀粉样蛋白寡聚体可以由约 2 至约 150、约 2 至约 100、约 2 至约 50、或约 2 至约 25 个 β 淀粉样蛋白单体组成。在一些实施方式中，β 淀粉样蛋白寡聚体可以包括 2 个或更多个单体。各个实施方式的 β 淀粉样蛋白寡聚体可以基于单体的确认而与 β 淀粉样蛋白原纤维和 β 淀粉样蛋白初原纤维区别开。具体地，β 淀粉样蛋白寡聚体的 β 淀粉样蛋白单体通常是由 β-折叠片组成的球状，而原纤维和初原纤维的 β 淀粉样蛋白单体的二级结构是平行 β-片。

[0421] 患有阿尔茨海默病或存在患病风险的受试者的识别

[0422] 通过大脑皮质中细胞外 β 淀粉样蛋白 (Aβ) 斑块和神经元内神经原纤维缠结的存在来从组织学上定义阿尔茨海默病 (AD)。各种诊断和预后用生物标记物在本领域内已知，例如磁共振成像、单光子发射断层摄像术、FDG PET、PiB PET、CSF tau，且 Abeta 分析以及关于其诊断准确性的可得数据讨论于 Alves 等人，2012, Alzheimer's disease:a clinical practice-oriented review, Frontiers in Neurology, April, 2012, vol3, Article63, 1-20，其引入本文以供参考。

[0423] 痴呆诊断以及对将患痴呆个体的预测借助于磁共振成像和通过使用 [(18)F] 氟脱氧葡萄糖 (FDG) 的正电子发射断层摄像术 (PET)。这些技术不特定用于 AD。参见，例如 Vallabhajosula S. Positron emission tomography radiopharmaceuticals for imaging brain Beta-amyloid. Semin Nucl Med. 2011Jul ;41(4):283-99。最近FDA批准用于对认知障碍患者中的中度至重度淀粉样蛋白神经斑成像的另一PET配体是Florbetapir F18注射剂，(4-((1E)-2-(6-{2-(2-(2-(18F) 氟乙氧基）乙氧基）乙氧基} 吡啶-3-基）乙烯基)-N-甲基苯胺，AMYVID®, Lilly)。Florbetapir与纤维状Abeta特异结合，但不与神经原纤维

缠结特异结合。参见例如,Choi SR等人,Correlation of amyloid PET ligand florbetapir F18binding with A β aggregation and neuritic plaque deposition in postmortem brain tissue. Alzheimer Dis Assoc Disord. 2012Jan ;26(1):8-16。PET 配体 Florbetapir 的问题是在PET 扫描的定性视觉评估上具有较低特异性。Camus 等人,2012, Eur J Nucl Med Mol Imaging39:621-631。然而,许多具有神经斑的人看似认知正常。

[0424] 阿尔茨海默病的CSF 标记物包括总 tau、磷 -tau 和 Abeta42。参见例如,Andreasen, Sjogren and Blennow, World J Biol Psyciatry, 2003, 4(4):147-155,其引入本文以供参考。已经在许多研究中发现,在 AD 中,Abeta 的 42 氨基酸形式 (Abeta42) 的 CSF 水平降低且总 tau 的 CSF 水平增高。此外,对于 APP 基因中的可用于鉴定具有 AD 患病风险的受试者的突变,存在已知的遗传标记物。参见例如,Goate 等人,Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease, Nature, 349, 704-706, 1991,其引入本文以供参考。在实施方式中,可以采用任何已知的诊断或预后方法来鉴定患有阿尔茨海默病或存在阿尔茨海默病患病风险的受试者。

[0425] 包括 σ -2 受体拮抗剂的药物组合物

[0426] 通过本发明识别的 σ -2 受体拮抗剂化合物、抗体或片段可以以药物组合物的形式施用。这些组合物可以以制药领域公知的方式制备,且可以通过多种途径施用,取决于是否需要局部或全身治疗以及待治疗的区域。

[0427] 因此,本发明的另一实施方式包括药物组合物,该药物组合物包括药学上可接受的赋形剂或稀释剂以及治疗有效量的本发明的 σ -2 受体拮抗剂化合物(包括其对映异构体、非对映体、N- 氧化物或药学上可接受的盐)。

[0428] 尽管化合物可以作为散装物质 (bulk substance) 施用,但优选以药物制剂提供活性成分,例如,其中活性剂与药学上可接受的载体混合,该药学上可接受的载体关于既定的给药途径和标准的制药实践进行选择。

[0429] 因此,一方面,本发明提供一种药物组合物,其包括任何上式的至少一种化合物、抗体或片段,以及以上描述为 σ -2 受体拮抗剂的其它化合物,或其药学上可接受的衍生物(例如盐或溶剂化物),以及任选地,药学上可接受的载体。具体地,本发明提供一种药物组全物,其包括治疗有效量的任何上式的至少一种化合物或其药学上可接受的衍生物,以及任选地,药学上可接受的载体。

[0430] 包括本发明化合物的药物组合物

[0431] 本发明的化合物或组合物(例如 σ -2 拮抗剂)可以以药物组合物的形式施用。这些组合物可以以制药领域公知的方式制备,且可以通过多种途径施用,取决于是否需要局部或全身治疗以及待治疗的区域。

[0432] 因此,本发明的另一实施方式包括一种药物组合物,其包括药学上可接受的赋形剂或稀释剂以及治疗有效量的本发明化合物或其对映异构体、非对映体、N- 氧化物或药学上可接受的盐。

[0433] 尽管化合物可以作为散装物质 (bulk substance) 施用,但优选以药物制剂提供活性成分,例如,其中活性剂与药学上可接受的载体混合,该药学上可接受的载体关于既定的给药途径和标准的制药实践进行选择。

[0434] 因此,一方面,本发明提供一种药物组合物,其包括式 I 或 II 的至少一种化合物或

其药学上可接受的衍生物（例如盐或溶剂化物），以及任选地，药学上可接受的载体。具体地，本发明提供一种药物组全物，其包括治疗有效量的式 I 的至少一种化合物或其药学上可接受的衍生物，以及任选地，药学上可接受的载体。

[0435] 当组合到同一制剂中时，应理解，两种化合物须稳定且互相相容以及与制剂的其它组分相容。当单独配制时，它们可以以任何便利的制剂提供，便利的是以本领域中对于这样的化合物为已知的方式。

[0436] 本发明的化合物可以配制成以用于人或兽药的任何便利方式施用，因此本发明的范围内包括含有适用于人或兽药的本发明化合物的药物组合物。这样的组合物可以借助一种或多种合适载体以常规方式进行使用。用于治疗用途的可接受的载体在制药领域内公知，且描述于例如 Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985)。选择药物载体可以关于既定的给药途径和标准的制药实践进行选择。除载体之外，药物组合物还可以包括任何合适的粘合剂、润滑剂、助悬剂、涂覆剂和 / 或增溶剂。

[0437] 可以在药物组合物中提供防腐剂、稳定剂、染料以及甚至调味剂。防腐剂的实例包括苯甲酸钠、抗坏血酸和对羟基苯甲酸酯。还可使用抗氧化剂和助悬剂。

[0438] 本发明的化合物可以使用已知的研磨步骤例如湿磨进行研磨，以得到适合于片剂形成和其他制剂类型的粒径。本发明化合物的细分（纳米颗粒）制剂可以通过本领域中已知的方法来制备，例如参见 WO02/00196 (SmithKline Beecham)。

[0439] 组合

[0440] 对于本发明的组合物和方法，任何上式的化合物和以上描述为 σ-2 受体拮抗剂的其它化合物可以与其它治疗剂和 / 或活性剂组合使用。

[0441] 在一些实施方式中，σ-2 拮抗剂化合物可以与胆碱酯酶抑制剂、N- 甲基 -D- 天冬氨酸 (NMDA) 谷氨酸受体拮抗剂、β 淀粉样蛋白特异抗体、β 分泌酶 1 (BACE1，β 位淀粉样蛋白前体蛋白切割酶 1) 抑制剂、肿瘤坏死因子 α (TNFα) 调节剂、静脉注射免疫球蛋白 (IVIG)、或朊蛋白拮抗剂中的一种或多种结合。在一些实施方式中，σ-2 受体拮抗剂与选自他克林（COGNEX®; Sciel)，多奈哌齐（ARICEPT®; Pfizer)，利斯的明（EXELON®; Novartis)，或加兰他敏（RAZADYNE®; Ortho-McNeil-Janssen) 的胆碱酯酶抑制剂结合。在一些实施方式中，σ-2 受体拮抗剂与作为髓周依那西普（ENBREL®, Amgen/Pfizer) 的 TNFα 调节剂组合。在一些实施方式中，σ-2 受体拮抗剂与选自巴匹珠单克隆抗体 (Pfizer)、茄尼醇单克隆抗体 (Lilly)、PF-04360365 (Pfizer)、GSK933776 (GlaxoSmithKline)、Gammagard (Baxter) 或 Octagam (Octapharma) 的 β 淀粉样蛋白特异性抗体组合。在一些实施方式中，σ-2 受体拮抗剂与作为美金刚（NAMENDA®; Forest) 的 NMDA 受体拮抗剂组合。在一些实施方式中，BACE1 抑制剂是 MK-8931 (Merck)。在一些实施方式中，σ-2 受体拮抗剂与 IVIG 结合，如描述于 Magga 等人，J Neuroinflam2010, 7:90, Human intravenous immunoglobulin provides protection against Ab toxicity by multiple mechanisms in a mouse model of Alzheimer's disease, 以及 Whaley 等人，2011, Human Vaccines7:3, 349-356, Emerging antibody products and Nicotiana manufacturing ;其各自引入本文以供参考。在一些实施方式中，σ-2 受体拮抗

剂与朊蛋白拮抗剂结合，如公开于 Strittmatter 等人，US2010/0291090，其引入本文以供参考。

[0442] 因此，另一方面，本发明提供一种药物组合物，其包括任何上式的至少一种化合物或其药学上可接受的衍生物、第二活性剂、以及任选地，药学上可接受的载体。

[0443] 当组合到同一制剂中时，应理解，两种化合物、抗体或片段须稳定且互相相容以及与制剂的其它组分相容。当单独配制时，它们可以以任何便利的制剂提供，便利的是以本领域中对于这样的化合物为已知的方式。

[0444] 给药途径和单位剂型

[0445] 给药（输送）途径包括但不限于以下一种或多种：口服（例如作为片剂、胶囊或作为可摄入的溶液）、局部、粘膜（例如作为吸入用鼻喷剂或气雾剂）、肠胃外（例如通过可注射形式）、胃肠、脊柱内、腹膜内、肌内、静脉内、脑室内、或其它储库 (depot) 施用等。

[0446] 因此，本发明的组合物包括特别是配制用于给药模式的形式的组合物。在某些实施方式中，本发明的药物组合物配制成适用于口服输送的形式。例如，化合物 CB 和化合物 CF 是在动物模型中口服可生物利用且已在条件性恐惧模型中每天口服一次并显示功效的 σ-2 受体拮抗剂化合物，参见例如图 9B。本文所述的口服可生物利用的化合物可以以口服制剂制备。在一些实施方式中，σ-2 拮抗剂化合物是口服可生物利用的化合物，适于口服递送。在其它实施方式中，本发明的药物组合物配制成适用于肠胃外输送的形式。在一些实施方式中，σ-2 受体拮抗剂化合物是其抗体或片段，其中抗体或片段配制成肠胃外组合物。

[0447] 本发明的化合物可以配制成以用于人或兽药的任何便利方式施用，因此本发明的范围内包括含有适用于人或兽药的本发明化合物的药物组合物。这样的组合物可以借助一种或多种合适载体以常规方式进行使用。用于治疗用途的可接受的载体在制药领域内公知，且描述于例如 Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985)。选择药物载体可以关于既定的给药途径和标准的制药实践进行选择。除载体之外，药物组合物还可以包括任何合适的粘合剂、润滑剂、助悬剂、涂覆剂和 / 或增溶剂。

[0448] 根据不同的输送体系可以有不同的组成 / 配方要求。应理解，并不是所有的化合物都需要通过相同的途径施用。同样地，如果组合物包括多于一种活性组分，则这些组分可以通过不同途径施用。通过实例的方式，本发明的药物组合物可以配制成使用微型泵，或通过粘膜途径（例如作为吸入用鼻喷剂或气雾剂或者可摄入溶液），或肠胃外（其中组合物配制成通过可注射形式例如通过静脉内、肌内或皮下途径进行输送）进行输送。或者，制剂可以设计成通过多个途径进行输送。

[0449] 因为本发明的化合物穿过血脑屏障，因此它们可以以多种方法进行施用，包括，例如全身（例如通过 iv、SC、口服、粘膜、经皮途径）或局部方法（例如颅内）。在本发明的化合物经由胃肠粘膜进行粘膜输送时，其应能够在经由胃肠道的运输过程中保持稳定；例如，其应耐受蛋白降解，在酸 pH 下稳定，且耐受胆汁的净化 (detergent) 作用。例如，式 I 或 II 的化合物可以由肠溶包衣层包衣。肠溶包衣层材料可以分散或溶解于水或合适有机溶剂中。作为肠溶包衣层聚合物，可以单独或组合使用以下的一种或多种：例如甲基丙烯酸共聚物、邻苯二甲酸乙酸纤维素、乙酸丁酸纤维素、羟丙基甲基纤维素邻苯二甲酸酯、醋酸羟丙基甲基纤维素琥珀酸酯、聚乙酸乙烯邻苯二甲酸酯、乙酸纤维素偏苯三酸酯、羧甲基乙基纤

维素、虫胶或其它合适的肠溶包衣层聚合物的溶液或分散体。由于环境原因，优选水性包衣工艺。在这样的水性工艺中，最优选甲基丙烯酸共聚物。

[0450] 合适时，药物组合物可通过吸入，通过使用皮肤贴剂，以含有赋形剂如淀粉或乳糖的片剂形式或者单独或与赋形剂混合的胶囊或胚珠 (ovule) 形式或者含有调味剂或着色剂的酏剂、溶液或混悬液形式口服施用，或者它们可以经肠胃外注射，例如静脉内、肌内或皮下。对于颊 (buccal) 或舌下给药，组合物可以以片剂或锭剂的形式施用，其可以以常规方式进行配制。

[0451] 在本发明的组合物经胃肠外施用时，这样的施用包括但不限于：静脉内、动脉内、鞘内、心室内、颅内、肌内或皮下施用本发明的化合物；和 / 或通过使用输注技术。

[0452] 适用于注射或输注的药物组合物可以是无菌水溶液、分散液或无菌粉末形式，其含有（若需要则进行调整）用于这样的适用于输注或注射的无菌溶液或分散液制剂的活性成分。该制剂可任选地包入脂质体中。在所有情况下，最终制剂在制备和储存条件下须无菌、液态且稳定。为提高储存稳定性，这样的制剂还可含有防腐剂以防止微生物生长。对微生物作用的防止可通过添加各种抗细菌剂和抗真菌剂来实现，例如对羟基苯甲酸酯类 (paraben)、氯丁醇、或抗坏血酸。在很多情况下，推荐等渗物质例如糖、缓冲液和氯化钠，以保证与体液特别是血液相似的渗透压。对这样的可注射混合物的延长吸收可通过引入吸收延迟剂例如单硬脂酸铝或明胶来实现。

[0453] 分散体可以在液体载体或中间体（例如甘油、液体聚乙二醇、三醋酸甘油及其混合物）中制备。液体载体或中间体可以是溶剂或液体分散介质，其含有例如水、乙醇、多元醇（例如丙三醇、丙二醇等）、植物油、无毒性甘油酯及其合适混合物。合适的流动性可以通过生成脂质体、在分散液的情况下施用合适的粒径或通过添加表面活性剂来得到保持。

[0454] 对于肠胃外施用，最好以无菌水溶液的形式使用化合物，该无菌水溶液可含有其它物质，例如足够的盐或葡萄糖以使溶液与血液等渗。若需要，水溶液应被适当缓冲（优选到 3 ～ 9 的 pH）。在无菌条件下制备合适的肠胃外制剂可通过本领域技术人员熟知的标准制药技术来容易地完成。

[0455] 无菌注射液可以通过使式 I 的化合物与合适的溶剂以及一种或多种前述载体混合，随后无菌过滤来制备。在适用于制备无菌注射液的无菌粉末的情况下，制备方法优选包括在真空下干燥以及冻干，这提供醛固酮受体拮抗剂和所需赋形剂的粉状混合物以便随后制备无菌溶液。

[0456] 根据本发明的化合物可以配制成通过注射（例如通过静脉内推注或输注或经肌内、皮下或鞘内途径）用于人或兽药且可以以单位剂型提供于安剖瓶中或其他单位剂量的容器或多剂量容器中，若需要添加防腐剂。注射用组合物可以是混悬液、溶液或乳液的形式，处于油性或水性媒介物中，且可以含有配制剂例如助悬剂、稳定剂、增溶剂和 / 或分散剂。或者，活性成分可以是用于在使用前与合适的媒介物例如无菌无热原的水重构的无菌粉末形式。

[0457] 本发明的化合物可以以片剂、胶囊、胚珠 (ovules)、酏剂、溶液或混悬液的形式施用，用于速释、延释、调释、缓释、脉冲释放或控释应用。

[0458] 本发明的化合物还可以以适用于口服或颊服的形式提供供人或兽医使用，例如以溶液、凝胶、糖浆或混悬液形式，或在使用前与水或其它合适的媒介物重构的干燥粉末形

式。还可以使用固体组合物例如片剂、胶囊、锭剂、软锭剂、丸剂、大丸剂、粉末、糊剂、颗粒、药筒(bullet)或预混制剂。用于口服用途的固体和液体组合物可以根据领域内熟知的方法制备。这样的组合物还可以含有固体或液体形式的一种或多种药学上可接受的载体和赋形剂。

[0459] 片剂可含有赋形剂例如微晶纤维素、乳糖、柠檬酸钠、碳酸钙、二碱式磷酸钙和甘氨酸,崩解剂例如淀粉(优选玉米、马铃薯或木薯淀粉)、羟基乙酸淀粉钠、交联羧甲基纤维素钠和某些复合硅酸盐,以及制粒粘合剂例如聚乙烯吡咯烷酮、羟丙甲基纤维素(HPMC)、羟丙基纤维素(HPC)、蔗糖、明胶和阿拉伯胶。

[0460] 另外,可以包括润滑剂例如硬脂酸镁、硬脂酸、山嵛酸甘油酯和滑石。

[0461] 组合物可以口服施用,以速释或控释的片剂、微粒、微型片剂、胶囊、袋(sachet)、以及口服溶液或混悬液、用于其制备的粉末的形式。口服制剂可以任选地包括各种标准的制药载体和赋形剂,例如粘合剂、填充剂、缓冲剂、润滑剂、助流剂、染料、崩解剂、增味剂(odorant)、甜味剂、表面活性剂、脱模剂、防附着剂和包衣。

[0462] 一些赋形剂在组合物中可以具有多种作用,例如同时起粘合剂和崩解剂的作用。

[0463] 可用于本发明的口服组合物的药学上可接受的崩解剂的实例包括但不限于淀粉、预糊化淀粉、羟基乙酸淀粉钠、羧甲基纤维素钠、交联羧甲基纤维素钠、微晶纤维素、藻酸盐、树脂、表面活性剂、泡腾组合物、水性硅酸铝和交联聚乙烯吡咯烷酮。

[0464] 本文可用的口服组合物的药学上可接受的粘合剂的实例包括但不限于,阿拉伯胶;纤维素衍生物,例如甲基纤维素、羧甲基纤维素、羟丙甲基纤维素、羟丙基纤维素、或羟乙基纤维素;明胶、葡萄糖、右旋糖、木糖醇、聚甲基丙烯酸酯、聚乙烯吡咯烷酮、山梨醇、淀粉、预糊化淀粉、黄蓍胶、黄嘌呤树脂、藻酸盐、镁铝硅酸盐、聚乙二醇或膨润土。

[0465] 口服组合物的药学上可接受的填充剂的实例包括但不限于,乳糖、脱水乳糖(anhydrolactose)、乳糖单水合物、蔗糖、右旋糖、甘露糖醇、山梨醇、淀粉、纤维素(特别是微晶纤维素)、磷酸二氢钙或脱水磷酸钙、碳酸钙和硫酸钙。

[0466] 可用于本发明的组合物的药学上可接受的润滑剂的实例包括但不限于,硬脂酸镁、滑石、聚乙二醇、环氧乙烷的聚合物、月桂基硫酸钠、月桂基硫酸镁、油酸钠、硬脂酰富马酸钠、和胶体二氧化硅。

[0467] 口服组合物的药学上可接受的合适增味剂的实例包括但不限于,合成香料和天然芳香油例如油、花、果(例如香蕉、苹果、酸樱桃、桃)以及其组合的提取物,以及类似的香料。它们的使用取决于很多因素,最重要的是将要服用该药物组合物的群体的感官可接受性。

[0468] 口服组合物的药学上可接受的合适染料的实例包括但不限于合成和天然染料,例如二氧化钛、β-胡萝卜素和葡萄柚皮提取物。

[0469] 通常用于促进吞咽、改变释放性质、改善外观和/或掩盖组合物的味道的用于口服组合物的药学上可接受的有用包衣的实例包括但不限于羟丙甲基纤维素、羟丙基纤维素和丙烯酸酯-甲基丙烯酸酯共聚物。

[0470] 口服组合物的药学上可接受的甜味剂的合适实例包括但不限于阿斯巴甜、糖精、糖精钠、环己基氨基磺酸钠、木糖醇、甘露糖醇、山梨醇、乳糖和蔗糖。

[0471] 药学上可接受的缓冲剂的合适实例包括但不限于,柠檬酸、柠檬酸钠、碳酸氢钠、

二碱式磷酸钠、氧化镁、碳酸钙和氢氧化镁。

[0472] 药学上可接受的表面活性剂的合适实例包括但不限于月桂基硫酸钠和聚山梨醇酯。

[0473] 相似类型的固体组合物也可用作明胶胶囊中的填充剂。鉴于此，优选的赋形剂包括乳糖、淀粉、纤维素、奶糖或高分子量聚乙二醇。对于水性混悬液和/或酏剂，试剂可以与各种甜味剂或调味剂、着色物质或染料，与乳化剂和/或助悬剂，且与稀释剂例如水、乙醇、聚乙二醇和甘油，及其组合进行组合。

[0474] 如所示，本发明的化合物可以经鼻内或通过吸入进行施用，且以干粉吸入剂或气雾喷雾剂形式从加压容器、泵、喷雾器或雾化器使用合适的抛射剂例如二氯二氟甲烷、三氯氟甲烷、二氯四氟乙烷、氢氟烷烃例如1,1,1,2-四氟乙烷(HFA134AT)或1,1,1,2,3,3,3-七氟丙烷(HFA227EA)、二氧化碳或其它合适的气体进行便利的输送。在加压气雾剂的情况下，可以通过设置输送计量的量的阀来确定剂量单位。加压容器、泵、喷雾器或雾化器可以含有活性化合物的溶液或混悬液，例如使用乙醇和抛射剂的混合物作为溶剂，其可额外含有润滑剂例如山梨醇三油酸酯。

[0475] 用于吸入器或吹入器的胶囊和药筒(例如，由明胶制成)可以配制为含有化合物和合适粉末基底例如乳糖或淀粉的粉末混合物。

[0476] 对于通过吸入进行的局部施用，可以经雾化器输送用于人或兽药的本发明化合物。

[0477] 本发明的药物组合物可以含有0.01～99wt%/v活性材料。对于局部施用，例如，组合物通常含有0.01-10%、更优选0.01-1%的活性材料。

[0478] 化合物还可以以脂质体输送体系的形式进行施用，例如小单层囊泡、大单层囊泡和多层囊泡。脂质体可以由多种磷脂例如胆固醇、硬脂酰胺或磷脂酰胆碱形成。

[0479] 本发明的药物组合物或单位剂型可以根据通过鉴于以上指导而进行的常规测试所限定的剂量和施用方案来进行施用，以便获得最佳活性，同时使对于特定患者的毒性或副作用最小化。然而，治疗方案的这样的微调鉴于本文所给出的指导是常规的。

[0480] 本发明化合物的剂量可以根据多种因素变化，例如潜在的疾病状态，个体的状态、体重、性别和年龄，以及给药模式。用于治疗病症的有效量可以通过本领域普通技术人员已知的经验方法来容易地确定，例如通过建立剂量和施用频率的矩阵并比较矩阵中各点处的一组实验单元或受试者。施用于患者的精确量会根据病症的状态和严重程度以及患者的身体状态而变化。任何症状或参数的可测量的改善可以通过本领域技术人员确定或通过患者向医师报告。应理解，尿道病症的任何症状或参数的任何临床或统计学显著的减轻或改善都在本发明的范围内。临床显著的减轻或改善是指对于患者和/或医师可感知。

[0481] 待施用的化合物的量的范围可以为约0.01至约25mg/kg/天，通常约0.1～约10mg/kg/天，且最通常约0.2～约5mg/kg/天。应理解，本发明的药物制剂并不必然需要含有可有效治疗病症的全部量的化合物，因为这样的有效量可以通过施用多个分开剂量的这样的药物制剂来达到。

[0482] 在本发明的优选实施方式中，化合物I配制成胶囊或片剂，通常含有10～200mg本发明的化合物，且优选以10～300mg、优选20～150mg且更优选约50mg的每日总剂量施用给患者。

[0483] 用于肠胃外给药的药物组合物基于 100wt%的总药物组合物含有约 0.01wt%至约 100wt%的本发明的活性化合物。

[0484] 通常，经皮剂型相对于 100wt%的总剂型含有约 0.01wt%至约 100wt%的活性化合物。

[0485] 药物组合物或单位剂型可以以单个日剂量施用，或者总的日剂量可以以分开剂量施用。另外，共同施用或相继施用用于治疗病症的另一化合物可以是理想的。为此目的，组合的活性成分配制到简单的剂量单位中。

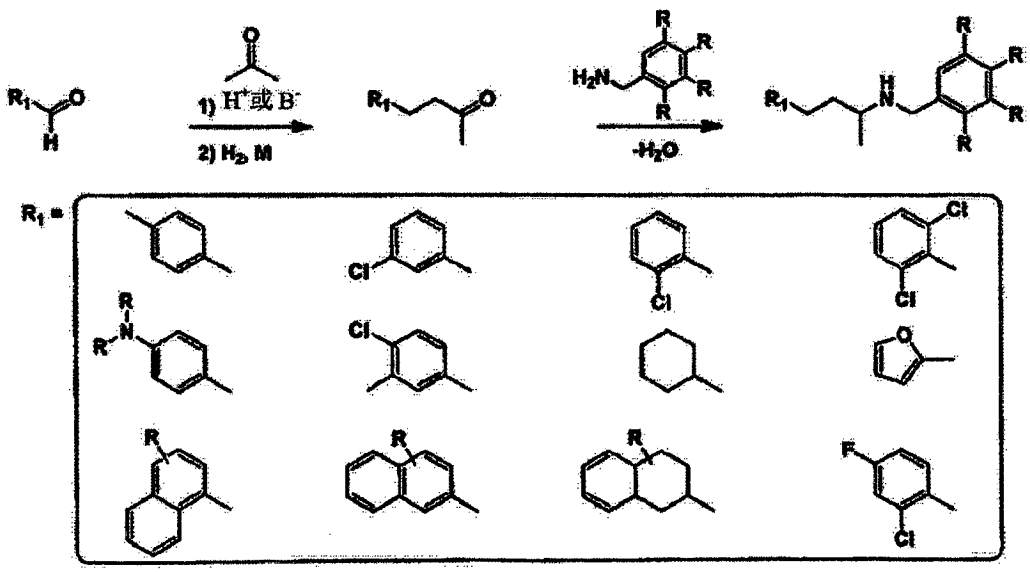
[0486] 本发明化合物的合成

[0487] 式 I、II 和 VIII 的化合物及其对映异构体、非对映体、N-氧化物和药学上可接受的盐可以通过下文概述的一般方法来制备，该方法构成本发明的又一方面。在以下描述中，R 基团具有针对上述各式的化合物所限定的含义，除非另外说明。

[0488] 本领域技术人员应理解，理想的是使用化合物 I 的制备中所使用的中间体的受保护衍生物。官能团的保护和去保护可以通过领域内已知的方法进行（参见例如，Green and Wuts Protective Groups in Organic Synthesis. John Wiley and Sons, New York, 1999）。羟基或氨基可以用任何羟基或氨基保护基团进行保护。氨基保护基团可以通过常规技术移除。例如，酰基，例如烷酰基、烷氧基羰基和芳酰基，可以通过溶剂分解作用例如通过酸性或碱性条件下的水解来移除。芳基甲氧羰基（例如苄氧羰基）可以在催化剂例如钯炭催化剂（palladium-on-charcoal）的存在下通过氢解作用来切割。

[0489] 目标化合物的合成通过使用本领域技术人员熟知的标准技术移除任何可存在于倒数第二个中间体的保护基来完成。然后使用标准技术例如硅胶层析、硅胶上的 HPLC 等，或通过重结晶来按需纯化去保护的终产物。上述化合物可以经任何合成路线来合成。例如，可以通过以下方案（方案 1）来制备化合物。

[0490]



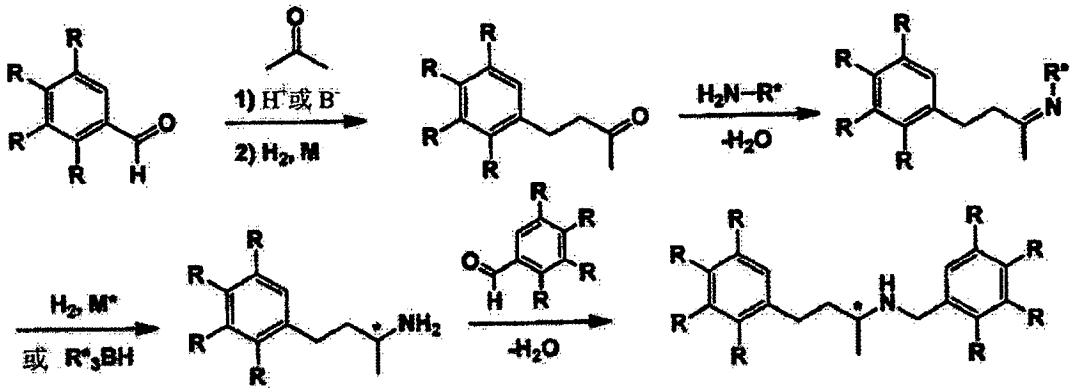
方案 1

[0491] 该方案可以产生本文描述的类似物的外消旋混合物。

[0492] 另外的 R_1 基团还可以用于产生其它类似物。

[0493] 在一些实施方式中，不对称地进行合成，以便产生一个类似物的基本上纯或纯的对映异构体。在一些实施方式中，本文所述化合物的不对称合成根据方案 2(* 表示手性中心）制备：

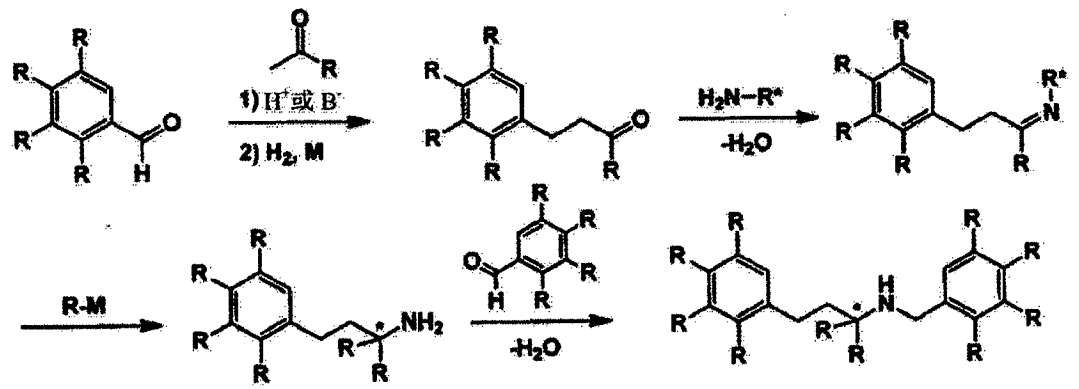
[0494]



方案 2

[0495] 在一些实施方式中，本文所述化合物的不对称合成根据方案 3(* 表示手性中心）制备：

[0496]

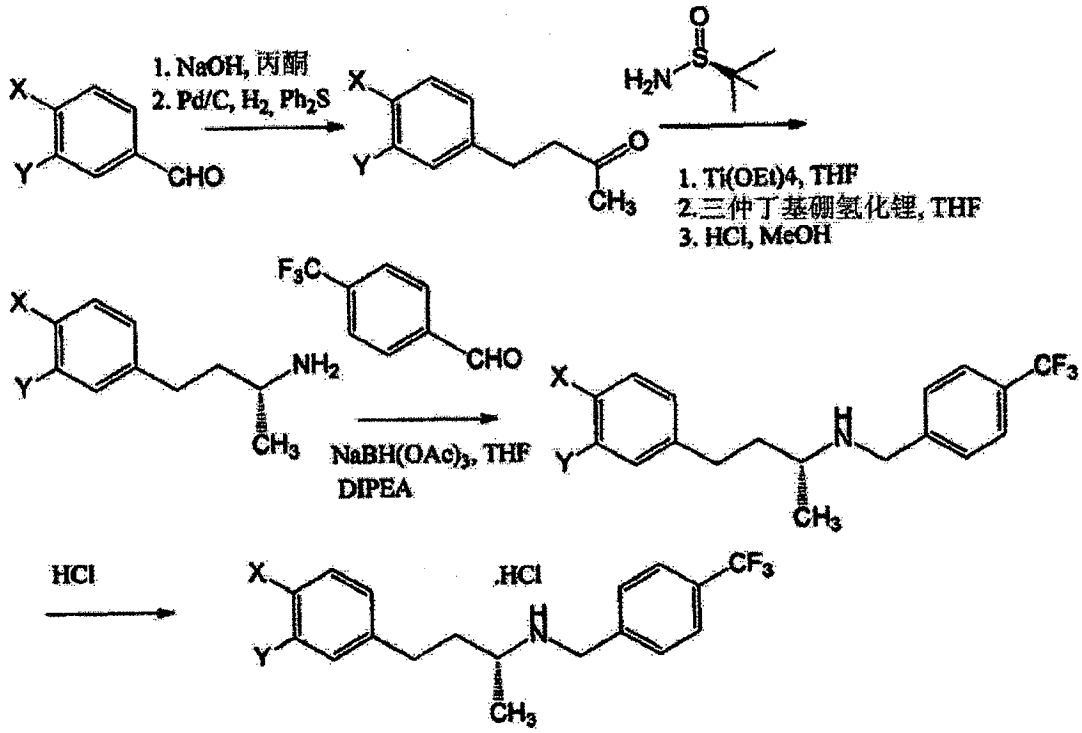


方案 3

[0497] 合成方案可以根据所需的终产物而改变。“R”基团是例示性的且可以被本文所述的任何取代基取代。

[0498] 在一些实施方式中，式 I 和 II 的某些化合物例如通过方案 4 所示的对映选择性路线制备。

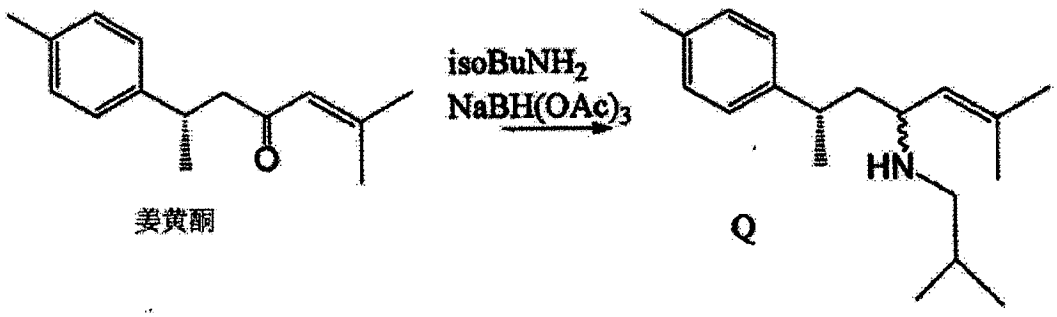
[0499]



方案 4

[0500] 在一些实施方式中，σ-2 拮抗剂是式 VIII 的化合物。式 VIII 的某些化合物可以通过相应酮中间体的还原氨化来制备，例如通过方案 5 所示的代表性路线。

[0501]



方案 5

[0502] 工作例和合成例

[0503] 实施例 1 和 2 描述了可用于例如本文所述的实验的 Abeta 寡聚体制剂。用于膜运输和寡聚体结合 / 突触减少检定的具体制剂以及用于下面所述的体内检定的具体制剂各自在其所属的实施例中进行描述。

[0504] 实施例 1：β 淀粉样蛋白寡聚体的制备

[0505] β 淀粉样蛋白在神经组织中可以寡聚的条件，一种水性可溶蛋白经其可缔合的环境被再创造以识别 β 淀粉样蛋白寡聚体和原纤维的更多疾病相关的结构状态。水性可溶蛋白从大鼠大脑通过超速离心制备。具体地，将 5 体积 TBS 缓冲液 (20mM Tris-HCL，pH7.5，34mM NaCl) 和完整的蛋白酶抑制剂混合物 (Santa Cruz) (/ 每克大脑组织) 添加到冰上的大

鼠大脑组织。然后使用紧定杵 (tight-fitting pestle) 进行 Dounce 均化。然后以 150,000x g 在 4℃对均化的大脑组织离心 1 小时 (40,000rpm Ty65)。次层清液 (infranatant,在漂浮的髓磷脂之间,在球粒之上半厘米) 然后被移除,并在 -75℃冷冻等分试样。然后将球粒于 TBS 中重悬至原始体积,然后在 -75℃等份冷冻。将合成的单体的人 β 淀粉样蛋白 1-42 添加到该混合物以提供终浓度 1.5uM 的 β 淀粉样蛋白,并且将溶液在 4℃孵育 24 小时。以 5,800g 对混合物进行 10 分钟离心,以移除纤维状组装体,然后在 4℃使用 6E10 结合的琼脂糖离心柱 (Pierce Chemical Company) 进行免疫沉淀 24 小时。然后将洗脱的 β 淀粉样蛋白寡聚体进行 MALDI-Tof 质谱分析,以识别样品的含量,图 1。

[0506] β 淀粉样蛋白在含蛋白溶液中自缔合以形成 22,599Da 的 5 亚单位五聚体和 31,950Da 的 7 亚单位 7 聚体的亚单位组装体。49,291Da 处的另一峰可表示 12 亚单位 12 聚体,尽管这不会显示为 β 淀粉样蛋白 12 聚物的精确分子量。明显地,在代表 β 淀粉样蛋白单体和二聚体的 4518Da 或 9036Da 处未观察到峰。然而,9,882Da 和 14,731Da 处的峰可分别表示与 786Da(或 2x393Da) 脂质或蛋白缔合的 β 淀粉样蛋白二聚体以及与 3x393Da 脂质或蛋白缔合的 β 淀粉样蛋白三聚体。此外,19,686Da 处的峰的存在指示出可能涉及到三聚体复合物和 4954Da 的大鼠 β 淀粉样蛋白片段的组装状态。因此,这些数据可以反映小脂质或蛋白与 β 淀粉样蛋白的二聚体和三聚体的缔合,这可能指导生理学体系独特的构象状态的组装。

[0507] 实施例 2 :β 淀粉样蛋白寡聚体的制备

[0508] 如实施例 1 中所述将 1.5uM 单体人 β 淀粉样蛋白 1-42 于大鼠大脑可溶蛋白的混合物中的溶液在 4℃孵育 24 小时。然后在进行光谱之前用三氟乙醇 (TFE) 处理该溶液。在 TFE 中,组装的蛋白结构和非共价结合的蛋白复合物解离为变性蛋白,且预期与组装的寡聚体相关的峰消失。大多数在实施例 1 中观察到的蛋白峰消失,包括如上识别的 9822Da、14,731Da、31,950Da 和 49,291Da 峰。然而,在 4518Da 观察到高丰度峰,其表示 β 淀粉样蛋白单体峰。4954.7 处的峰很明显,其可表示与 β 淀粉样蛋白 1-46 相似的较长的 Abeta 片段。在 7086Da 观察到额外的峰,其在实施例 1 描述的制备中不存在,可能代表与 2550Da 共价结合的蛋白相缔合的 β 淀粉样蛋白单体。

[0509] 实施例 3 :本发明的检定中采用的方法

[0510] TBS 可溶提取物 :经组织病理学分析特征为 Braak Stage V/VI 阿尔茨海默病 (AD) 的人患者的死后脑组织的样品得自 Rhode Island Hospital 脑组织库。年龄和性别匹配的 AD 和正常组织样本稀释到含有 1mM EDTA 和 1mg/ml 完整蛋白酶抑制剂混合物 (Sigma P8340) 的 20mM Tris-HCL,137mM NaCl,pH7.6 中 0.15gm 组织 /ml,且均化。在 Beckman Optima XL-80K 超速离心器中以 105,000g 对组织匀浆进行超速离心 1 小时。将所得的 TBS 可溶部分使用蛋白 -A 和蛋白 -G 琼脂糖柱 (Pierce Chemical) 免疫耗竭,然后用 Amicon Ultra3,10&100kDa NMWCO 过滤器 (Millipore Corporation) 进行大小分级。

[0511] 免疫沉淀 :将大小分级且免疫耗竭的 TBS 可溶提取物在合适的 NMWCO Amicon Ultra 过滤器中浓缩到约 200ul。将浓缩的 TBS 可溶提取物用 TBS 样品缓冲液 (Pierce Chemical) 稀释到 400ul,并以 5,800g 离心 10 分钟以移除原纤维。然后将所得的上清液用 6E10- 结合的琼脂糖珠在 4℃免疫沉淀过夜,随后使用高渗强度 Gentle 洗脱缓冲液 (Pierce Chemical) 进行抗原洗脱以分离含有 Abeta 的蛋白种类。

[0512] MALDI-质谱:使用Applied Biosystems(ABI)Voyager DE-Pro MALDI-Tof仪器对免疫分离的β淀粉样蛋白进行质谱分析。基于分析的目标分子量范围,使用多种基质类型例如α-氰基-4-羟基肉桂酸(CHCA)、芥子酸(SA)或6-氮杂-2-硫代胸腺嘧啶(ATT)来分析样本。仪器以线性正离子模式以及可变提取延迟来运行。非累积谱表示每次获取的100次"热点"击中(shot)而累积谱表示每次获取的200激光击中中各次击中的12个分离区域。

[0513] 数据分析:使用Voyager数据探测软件包来进行数据获取和分析。质谱的标准操作包括光谱的平滑和扣减基线功能以及信号噪音比的变化。

[0514] 对Ab定量的ELISA:使用改进的夹心ELISA技术对免疫沉淀的TBS可溶部分分析"总"Abeta和Abeta寡聚体浓度。简单来说,将6E10和4G8涂覆的Nunc MaxiSorp96孔板用含有Abeta的样品进行孵育,然后用生物素化的4G8检测抗体进行探测。用链霉亲和素-HRP(Rockland)孵育,然后形成四甲基联苯胺(TMB)基质,允许在BioTEk Synergy HT读板仪上对Abeta进行比色检测(OD450)。使用单体Abeta1-42来产生标准曲线,并且连同GEN5软件一起实现对免疫沉淀的样品中的Abeta水平进行定量。[什么被认为是可接受的?]

[0515] 实施例4:受体结合检定

[0516] 化合物II与数种受体通过阻断其激动剂或拮抗剂的结合或作用而相互作用。测试化合物II以观察其是否与已知的细胞受体或细胞信号蛋白直接相互作用。测试化合物II(10μM)对给定人受体的已知激动剂或拮抗剂的结合的取代能力,该人受体在细胞系中过表达或从组织中分离。还测试其阻断由给定人受体的激动剂或拮抗剂所诱导的下游细胞信号传导的能力。测试化合物II在100个已知受体处的作用,并且化合物II仅在这些受体中的5个处显示出活性>50%(检定窗)(表A)。这表明化合物II仅在小子集的CNS相关受体处具有高度特异性和活性。其以最高亲和性结合σ-2受体,因此是σ-2配体。

[0517]

表A. 化合物II(10uM)对已知受体结合的抑制	%对照抑制	SEM%对照
σ-2(激动剂放射性配体)	89	0.6
mu(MOP)(h)(激动剂放射性配体)	60	1.4
Na+通道(位点2)(拮抗剂放射性配体)	54	4.7
D3(h)(激动剂作用)	66	4.0
α1A(h)(拮抗剂作用)	56	1.1

[0518] 使用相同的方案,测试表2(以下)的化合物对σ-2受体的识别。结果证实与化合物II结构相似的这些化合物的确是σ-2配体,即优先与σ-2受体结合。

[0519] 竞争性放射性配体结合检定

[0520] 通过商业合同研究组织进行σ-1受体和σ-2受体的放射性配体结合检定。对于σ-1结合,使用测试化合物从100uM至1nM的各个浓度,来取代来自Jurkat细胞膜

上的内源性受体的 8nM[3H](+) 喷他佐辛 (Ganapathy ME 等人,1991,J Pharmacol. Exp. Ther. 289:251-260)。使用 10uM 氟哌啶醇来确定非特异结合。对于 σ-2 受体,在存在 300nM(+) 喷他佐辛掩盖 σ-1 受体的情况下,使用测试化合物从 100uM 至 1nM 的各个浓度,来取代来自大鼠大脑皮质的膜上的内源性受体的 5nM[3H]1,3-二-(2-甲苯基)胍 (Bowen WD 等人,1993,Mol. Neuropharmcol3:117-126)。使用 10uM 氟哌啶醇来确定非特异结合。使用 Brandel12R 细胞收集器,通过经 Whatman GF/C 过滤器的快滤来终止反应,随后用冰冷的缓冲液洗涤两次。使用液体闪烁分析仪 (Tri-Carb2900TR ;PerkinElmer Life and Analytical Sciences) 来测量干燥滤片上的放射性。绘制取代曲线,并且使用 GraphPad Prism(GraphPad Software Inc.,San Diego,CA) 来确定测试配体对受体亚型的 K_i 值。通过将总结合(每分钟衰变数)与非特异结合(每分钟衰变数)之间的差除以总结合(每分钟衰变数)来确定特异结合百分比。

[0521] 对于参考化合物,对 σ-1 和 σ-2 受体的亲和性从使用脑组织匀浆的公开研究中得到,其中用 [3H](+) 喷他佐辛测量 σ-1 受体处的取代,且用 [3H]1,3-二-(2-甲苯基)胍在 300nM(+) 喷他佐辛的存在下测量 σ-2 受体处的取代。

[0522] 结果显示于表 2 中。

[0523] 表 2σ-2 和 σ-1 受体亲和性

[0524]

化合物	σ-1 结合 Ki（nM）	σ-2 结合 Ki（nM）
II（三个不同的批次：外消旋混合物，(+)异构体和(-)异构体）	500 100 46	9 52 63
化合物 A	47	16
化合物 B	47	16
化合物 E	1890（对 σ-1 受体无实质亲和性）	16
化合物 P	320	110
化合物 R'	26	27
化合物 S'	31	37
化合物 W	270	120
化合物 AC	23	240
化合物 AE	16	35
化合物 AF	8	110
化合物 AH	23	50
化合物 AI	250	130
化合物 AL	3100	690
化合物 AX	620	440
化合物 AY	5	23
化合物 AZ	34	340
化合物 BB	0.72	5.2
化合物 BC	4.2	13
化合物 BD	2.1	19
化合物 BE	7.4	14
化合物 BH	4	7.4
化合物 BJ	6.2	25
化合物 BP	53	8.9
化合物 BT	1	4
化合物 CB	19	48
化合物 CC	12	3.9
化合物 CD	56	2.7
化合物 CE	33	2.2
化合物 CF	180	50

[0525]

化合物(S)-CG	360	3200
化合物(R)-CG	680	-
化合物 CJ	44	810
化合物(S)-CL	190	5000
化合物(R)-CL	830	>10000
化合物 CO	130	7200
化合物 CR	3.5	16
化合物 CS	78	85
化合物 DH	23	8.3
化合物 DR	330	3200

[0526] 竞争性放射性配体结合检定 2

[0527] 候选 σ-2 配体化合物在 σ-1 和 σ-2 受体处的亲和性还通过对不同的已知的标记 σ-2 或 σ-1 配体的取代来确定。过滤检定根据之前公开的步骤 (Xu 等人,2005) 进行。将测试化合物溶解于 N,N- 二甲基甲酰胺 (DMF)、二甲亚砜 (DMSO) 或乙醇中,然后在含有 150mM NaCl 和 100mM EDTA 的 50mM Tris-HCl pH7.4 缓冲液中稀释。由豚鼠大脑制备用于 σ-1 结合检定的膜匀浆且由大鼠肝脏制备用于 σ-2 结合检定的膜匀浆。用 50mM Tris-HCl 缓冲液 (pH8.0) 稀释膜匀浆,并在 25℃在 96 孔板中在 150uL 的总体积中与浓度范围为 0.1nM 至 10uM 的放射性配体和测试化合物进行孵育。在孵育完成之后,使用 96 通道移液管 (Fisher Scientific, Pittsburgh, PA) 通过添加 150uL 冰冷的洗涤缓冲液 (10mM Tris HCl, 150mM NaCl, pH7.4) 来终止反应,收集样品并通过已用 100uL 的 50mM Tris-HCl 缓冲液预浸的 96 孔纤维玻璃滤板 (Millipore, Billerica, MA) 迅速过滤。将各个过滤器用 200uL 的冰冷洗涤缓冲液 (10mM Tris-HCl, 150mM NaCl, pH7.4) 洗涤四次。使用 Wallac1450MicroBeta 液体闪烁计数仪 (Perkin Elmer, Boston, MA) 来对结合放射性进行定量。

[0528] 使用豚鼠大脑膜匀浆 (～ 300ug 蛋白) 和～ 5nM[^{3}H](+)- 喷他佐辛 (34.9Ci/mmol, Perkin Elmer, Boston, MA) 进行 σ-1 受体结合检定,孵育时间在室温为 90min。非特异结合根据含有 10μM 的冷氟哌啶醇的样品确定。

[0529] 使用大鼠肝脏膜匀浆 (～ 300ug 蛋白) 和仅～ 2nM σ-2 高度选择性的放射性配体 [^{3}H]RHM-1 (无其它阻断剂) (America Radiolabeled Chemicals Inc. St. Louis, MO)、～ 10nM[^{3}H]DTG(58.1Ci/mmol, Perkin Elmer, Boston, MA) 或～ 10nM[^{3}H] 氟哌啶醇 (America Radiolabeled Chemicals Inc., St. Louis, MO) 在阻断 σ-1 位点的 1uM(+)- 喷他佐辛的存在下进行 σ-2 受体结合检定,孵育时间在室温对于 [^{3}H]RHM-1 为 6 分钟,对于 [^{3}H]DTG 和 [^{3}H] 氟哌啶醇为 120min。非特异结合根据含有 10μM 的冷氟哌啶醇的样品确定。

[0530] 使用非线性回归分析对来自竞争性抑制实验的数据建模,以确定抑制放射性配体的特异结合的 50% 的抑制剂浓度 (IC_{50} 值)。结合亲和性 Ki 值使用 Cheng 和 Prusoff 的方法进行计算。豚鼠大脑中用于 [^{3}H](+)- 喷他佐辛的 Kd 值为 7.89nM,大鼠肝脏中用于 [^{3}H]RHM-1 和 [^{3}H]DTG 的 Kd 值分别为 0.66nM 和 30.73nM。使用标准化合物氟哌啶醇以保证质量。化合物 IXa、IXb 和化合物 II 在 σ-1 和 σ-2 受体处的亲和性数据显示于表 3 中。因

此，可以采用本领域已知的任何 σ-2 受体结合检定来确定候选化合物的 Ki 或 IC50。

[0531] 表 3. 竞争性放射性配体结合检定 2 中候选 σ-2 配体的 σ-2 和 σ-1 受体亲和性

[0532]

编号	化合物	σ-1 (Ki，nM)±平均 SEM	σ-2 (Ki，nM)±平均 SEM
1	IXa、IXb	6.37±0.81	30.8±2.3
2	II	108.1±19.9	59.7±10.4

[0533] 实施例 5：转基因小鼠中的记忆丧失：Morris 游泳测试

[0534] 测试化合物 II 以确定其是否可逆转阿尔茨海默病的较老转基因小鼠模型（其中寡聚体随着年龄堆积）中所见的记忆丧失。对于该研究，选择在鼠 Thy-1 启动子的控制下表达人 APP751Swedish(670/671) 和 London(717) 突变的 hAPP 小鼠。这些小鼠的 Abeta 量表现出年龄依赖的增加，且在 3-6 个月开始形成斑，且在 8 个月年龄时表现出确立的认知障碍。在该研究中，不是防止出现障碍，而是治疗已确立的障碍。这些研究按照不知道实验条件的科学家的服务合同而进行。在 8 个月大的雌性小鼠中经皮下微型泵将化合物以 0.5 和 0.1mg/kg/ 天输注一个月，并且在 Morris 水迷宫（一种海马类空间学习和记忆的测试）中测试认知表现。该小鼠模型不表现神经元丧失，因此记忆的恢复不能归因于凋亡的避免。

[0535] 将游泳速度作为 Morris 测量的一部分进行分析，以确定是否存在任何的运动或动机缺陷。媒介物是 5% DMSO/5% Solutol、90% 盐水混合物。经低剂量 (0.1mg/kg/ 天）和高剂量 (0.5mg/kg/ 天）的化合物处理转基因动物。确定连续四天中的每天所进行的三个每日试验的平均值。可检测到没有显著的运动缺陷或任何种类的异常行为，且在研究的过程中仅损失了转基因媒介物组的一只动物，低于在该年龄预期的死亡水平。另外，我们维持有定期抽血以监测化合物的血浆水平的哨兵 (sentinel) 动物组，且这些显示在初步 PK 研究中所见的血浆水平几乎没有改变。

[0536] 进行 Morris 水迷宫测试的逃避潜伏期测量。在测试的第二天，观察到野生型与转基因动物之间的显著差异，野生型学得比转基因型更快。这天，还观察到较高化合物剂量 vs 媒介物在转基因表现上有显著改善。因此，得到结论以 0.5mg/kg/ 天施用的化合物 II 能够改善 AD 转基因模型中的认知表现。

[0537] Abeta42 寡聚体引起突触数目降低 18%；该丧失的 100% 被化合物 II 及其对映体消除。与化合物 II 相似，数个其它 σ-2 受体拮抗剂也阻抑突触丧失。已知的现有技术 σ-2 受体配体 NE-100 和氟哌啶醇完全消除突触丧失，而作为选择性 σ-1 配体的 SM-21 在消除突触丧失中仅具有较弱活性 (20% 恢复）。

[0538] 也使用类似检定对化合物 IXa 和 IXb 的混合物进行测试。化合物 IXa 和 IXb 的混合物 (1mg/kg/ 天，N = 8 或 10mg/kg/ 天，N = 8) 或媒介物 (5% DMSO/5% Solutol/90% 盐水，N = 15) 经皮下给药 (Alzet 微型泵）全身性施用至 9 月龄雄性 hAPPSL 转基因小鼠 (N = 8) 或非转基因同窝仔 (N = 6)，持续 20 天，并且在 Morris 水迷宫中评价这些小鼠的空间学习和记忆。在治疗的最后四天，以 3 次试验 / 天测试小鼠寻找隐蔽平台。计算机化的跟踪系统自动对逃避潜伏期或游泳长度进行定量。

[0539] 在测试的任一天，转基因动物 vs. 非转基因动物的表现中均没有显著差异（分析

限于这 2 组；重复测量的双因素（基因型和时间）ANOVA，随后进行 Bonferroni 事后检验）。限于转基因组（处理和时间）的相似分析显示，经 10mg/kg/ 天的化合物 IXa 和 IXb 的混合物处理的转基因动物比经媒介物处理的转基因动物在测试的第二和第四天表现得显著更好（$p<0.05$，经 student t 检验分析）。非转基因的媒介物处理的动物比转基因的媒介物处理的动物在测试的第一和第二天表现得显著更好。用化合物 IXa 和 IXb 的混合物进行处理与媒介物处理相比在测试的第一天（两种剂量）、第二天（10mg/kg/ 天剂量）和第四天（10mg/kg/ 天剂量）显著改善转基因动物表现（$p<0.05$；游泳长度）。

[0540] 这表明，化合物 IXa 和 IXb 的混合物能够以剂量依赖的方式在老龄转基因动物中逆转学习记忆中的已建立的行为缺陷。

[0541] 基于化合物 II 的结果、化合物间的结构相似性和本文公开的本发明化合物是 σ-2 配体且在膜运输检定中具有或预期具有活性且在寡聚体结合和突触减少检定中具有或预期具有活性的事实，预期式 I 和 II 内的化合物在该记忆丧失测试中相似地起作用。类似地，预期式 VIII 的化合物会与化合物 IXa 和 IXb 相似地起作用。

[0542] 实施例 6：在膜运输检定中抑制 Abeta 寡聚体对神经元细胞的作用

[0543] 测试以上表 2 的 σ-2 配体抑制 β 淀粉样蛋白对细胞的效应的能力。如通过膜运输检定所测量，σ-2 配体能够抑制 β 淀粉样蛋白效应。结果显示于下表 5。该检定的原理如下：

[0544] 由于突触和记忆缺陷而非广泛的细胞死亡在阿尔茨海默病的最早阶段占主导地位，测量这些变化的检定尤其适合发现寡聚体活性的小分子抑制剂。MTT 检定多被用作培养物中毒性的测量方式。黄色四唑盐被细胞内吞，且在内体通路中被还原为不溶紫色甲臜。紫色甲臜的水平反映出培养物中的活性代谢细胞的数量，并且甲臜量的减少被视为对培养物中细胞死亡或代谢毒性的衡量。当通过显微镜观察时，首先在填充细胞的胞内囊泡中可见紫色甲臜。随着时间的推移，囊泡被胞吐，并且当不可溶甲臜暴露于水性介质环境时，甲臜在质膜外表面上沉淀为针状结晶。Liu 和 Schubert('97) 发现，细胞通过选择性加速还原甲臜的胞吐速率来应答亚致死水平的 Abeta 寡聚体，而不影响内吞速率。发明人已在培养的成熟初级神经元中重复了这些观察，并通过自动显微镜术和成像处理对这些形态变化进行了定量。在这些情况下，还原甲臜的总量没有总体改变，仅仅是反映其形成和 / 或从细胞中排出的速率变化的形态学具有变化。发明人已证实先前的发现，即该检定对不引起细胞死亡的低水平寡聚体敏感 (Liu 和 Schubert' 04, Hong 等人，' 07)。确实，引起 LTP 抑制的低量寡聚体不导致细胞死亡 (Tong 等人，' 04)，且预期不改变培养物中（或脑片中）的甲臜的总量。

[0545] 其他研究者举出的证据表明，经膜运输介导的神经元表面受体表达中由 Abeta 寡聚体介导的减少是突触可塑性 (LTP) 以及因此的学习和记忆的电生理学测量的寡聚体抑制的基础 (Kamenetz 等人，' 03, Hseih 等人，' 06)。经甲臜形态学变化测量由寡聚体诱导的膜运输速率变化已被用于细胞系中，以发现阻抑 Abeta 寡聚体的药物 (Maezawa et al.，' 06, Liu and Schubert' 97，' 04，' 06, Rana 等人，' 09, Hong 等人，' 08)，其在体内降低啮齿动物的 Abeta 脑水平 (Hong 等人，' 09)。用于胞吐检定 /MTT 检定的相似步骤可以在文献中找到。参见例如 Liu Y 等人，Detecting bioactive amyloid beta peptide species in Alzheimer's disease. J Neurochem. 2004Nov ;91(3) :648-56 ;Liu Y

和 Schubert D. "Cytotoxic amyloid peptides inhibit cellular3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT)reduction by enhancing MTT formazan exocytosis. "J Neurochem. 1997Dec ;69(6):2285-93 ;以及 Liu Y 和 Schubert D. "Treating Alzheimer's disease by inactivating bioactive amyloid beta peptide"Curr. Alzheimer Res. 2006Apr ;3(2):129-35。因此,该方法是有效的。

[0546] 本发明的胞吐检定适用于在体外生长 3 周的成熟初级神经元培养物。参见 WO/2011/106785,以其全部引入本文以供参考。如经使用 Cellomics VTI 自动化显微镜系统通过图像处理所测量,Abeta 寡聚体导致填充有还原紫色甲臜的胞内囊泡(点(puncta))量呈剂量依赖性的减少。将例如图 1A(暴露于单独的媒介物的培养神经元细胞的显微照片,显示填充有甲臜的囊泡)与图 1B(暴露于媒介物 +Abeta 寡聚体的神经元细胞的显微照片,显示显著较少的填充有甲臜的囊泡,反而具有在遇到胞内环境时析出为晶体的胞吐甲臜)进行比较。增加 Abeta 寡聚体的量最终导致明显的毒性。因此,用于检定中的神经活性 Abeta 寡聚体的浓度比导致细胞死亡的浓度低得多。发明人通过示出 Abeta 寡聚体的效应在添加抗 Abeta 抗体时被阻断但仅抗体自身无效应(数据未示出),证实该检定是有效的。当以这种方式配置时,检定能够检测阻抑 Abeta 寡聚体的非致死效应的化合物是否通过破坏寡聚体、抑制寡聚体结合神经元、或对抗由寡聚体结合引发的作用的信号传导机制来起作用。

[0547] 用于产生结果的方法如下示于膜运输 / 胞吐 (MTT) 检定。

[0548] 对来自 E18Sprague-Dawley 大鼠胚胎的初级海马神经元以优化浓度在 384 孔板中在 NB 介质 (Invitrogen) 中进行铺板。将神经元保持在培养物中 3 周,每周供应两次具有 N_2 补充物 (Invitrogen) 的 NB 介质。这些神经元在成熟脑中表达神经元特有的突触蛋白的全补体,并表现出活性依赖的电信号传导的复杂网络。这样的培养物中的神经元和神经胶质具有表现出与完整脑回路优异配准的分子信号网络,为此已被用作学习和记忆的模型体系多于 20 年(参见例如 Kaech S, Banker G. Culturing hippocampal neurons. Nat Protoc. 2006 ;1(5):2406-15. Epub2007Jan11 ; 还 参 见 Craig AM, Graf ER, Linhoff MW. How to build a central synapse:clues from cell culture. Trends Neurosci. 2006Jan ; 29(1):8-20. Epub2005Dec7. Review)。

[0549] 以范围为 100uM 至 0.001nM 的浓度向细胞添加测试化合物,随后添加媒介物或 Abeta 寡聚体制剂 (3μM 总 Abeta 蛋白浓度),并将其在 37℃在 5% CO_2 中孵育 1 ~ 24hr。将 MTT 试剂 (3-(4,5- 二甲基噻唑 -2- 基)-2,5 二苯基溴化四唑)(Roche Molecular Biochemicals) 在磷酸盐缓冲盐水中重构为 5mg/mL。将 10μL MTT 标记试剂添加到各孔中并在 37℃孵育 1h,然后成像。通过自动显微术和图像处理评价胞吐,以对胞吞和胞吐的甲臜的量进行定量。

[0550] 将各个检定板板式设置为在各个板上在具有和不具有 Abeta 寡聚体的情况下测试化合物。该设计消除筛选级联早期的毒性或代谢活性的化合物(在初步筛选的水平)。首先在胞内囊泡中可见还原的甲臜。最终的甲臜胞吐经 Abeta 寡聚体加速。图 1A 和 1B 是神经元的显微照片的实例,第一个是先看见甲臜的胞内囊泡,且第二个是覆盖有经胞吐挤出的不溶紫色染料的神经元。染料在培养物的水性环境中沉淀且在神经元的表面上形成针状结晶。

[0551] 在 15 微摩尔化合物 II 的存在下，图 1B 中拍摄的膜运输变化被阻抑（参见图 1C）且图 1C 中的细胞与经媒介物处理的神经元不可区分。而且，化合物 II 的该作用看似与化合物 II 是在细胞暴露于 Abeta 寡聚体之前或之后添加无关，这表明治疗以及预防效果。参见图 1D，膜运输变化的图（剂量应答曲线）表示为，在添加多种含量的化合物 II 或 IXa 与 IXb 的混合物之前（图 1D）或之后（图 1E）添加的多种含量的 Abeta 寡聚体的存在下，在图像处理中见到的囊泡百分比 vs. log（化合物 II 浓度）。单独的 Abeta 寡聚体由图 1D 和 1E 的左下角的圆圈表示。单独的媒介物由填充的正方形表示。当在寡聚体之前添加时（预防模式），化合物 II 以 EC_{50} = 2.2uM 阻抑寡聚体效应，且化合物 IXa、IXb 以 EC_{50} = 4.9uM 阻抑寡聚体效应。当在寡聚体之后添加时（治疗模式），化合物 II 以 EC_{50} = 4.1uM 阻抑寡聚体效应，且化合物 IXa、IXb 以 EC_{50} = 2.0uM 阻抑寡聚体效应。在任一种情况中，化合物 II 或 IXa 与 IXb 的混合物各自阻抑该检定中所见的 Abeta 寡聚体的膜运输效应。上升剂量的来自两个结构不同的系列（II 和 IXa、IXb）的选择性、高亲和性 σ-2 受体拮抗剂化合物阻止寡聚体效应，且使培养物看起来更像经媒介物处理的培养物。

[0552] 图 1E 是在不同量的化合物 II（0 由菱形表示，1.1uM 由下三角表示，3.2uM 由上三角表示且 9.7uM 由填充的正方形表示）的存在下，填充有甲臜的囊泡百分比相对于 Abeta 浓度的相似剂量响应曲线。所得曲线针对增加量的化合物 II 向右移动，表明 Abeta 寡聚体和化合物 II 对相同目标存在竞争，该目标介导由 Abeta 寡聚体在该检定中引起的膜运输变化。增加量的化合物 II 降低 Abeta 寡聚体的明显效力（因此降低其毒性），本发明的发明人相信该结果在本发明之前未被显示过。确实，基于这些结果，对抗 Abeta 寡聚体毒性的化合物例如本发明的化合物有希望作为淀粉样蛋白毒性相关的认知衰退（例如阿尔茨海默病中所见）的治疗和（在非常早的阶段）预防方式。

[0553] 如图 1F 和 1G 中所见，在膜运输检定中用合成 Abeta 寡聚体进行给药，其显示出 820nM 的 EC_{50}。各个浓度的 Abeta 针对各个选择性高亲和性 σ-2 受体拮抗剂化合物药物候选物 II 和 IXa、IXb 的数种浓度（其各自引起 EC_{50} 向右移动几乎两个数量级）进行测试。当将数据拟合到经典的线性和非线性模型时，数据经 Schild 分析（斜率 nH 为 1）成线性，这表明 σ-2 受体化合物表现出寡聚体与化合物之间针对介导膜运输的靶标的真实药理学竞争。对抗这些化合物给药源自阿尔茨海默病患者大脑的 Abeta 寡聚体，如图 1J 和 1K 中所示，也通过化合物暴露而显示出右移。具体地，在有效剂量下，化合物 II 和 IXa、IXb 表现出与合成寡聚体（图 1F、G，Schild 斜率 = -0.75，-0.51）和源自人阿尔茨海默病患者（图 1J、1K）的寡聚体两者的药理学竞争。其净效应是这两种选择性高亲和性 σ-2 受体拮抗剂化合物候选药物有效地使 Abeta 寡聚体的突触毒性更小，且这些是目前为止我们所知的已证实该特征的仅有的疗法。不拘泥于理论，最简单可能的作用机制是 σ-2 受体化合物起到竞争性受体拮抗剂的作用。

[0554] 在相关的实验中，基于 0 或 20 μM 化合物 II 对映异构体的效应，观察到剂量响应曲线（%囊泡 vs Abeta 寡聚体浓度）向右移动：参见下表 4。显示 (+) 对映异构体在更高浓度的 Abeta 寡聚体下更有效。

[0555] 表 4

[0556]

化合物	针对 0 uM 浓度 Abeta 的 EC_{50}	针对 20 μM 浓度 Abeta 的 EC_{50}	曲线移动（倍）	筛选检定中使用单一浓度的 Abeta 寡聚体的 EC_{50}
化合物 II 的(+)对映异构体	1.19	1.46	2.86	5.6 uM
化合物 II 的(-)对映异构体	1.05	2.82	1.22	10.9 uM

[0557] 如以上表 4 所示，在较高浓度的 Abeta 寡聚体下，示为％囊泡针对 Abeta 寡聚体浓度的剂量应答曲线对于 20uM 对映异构体 vs. 0uM 对映异构体（即，仅 Abeta 寡聚体）的右移在 (+) 对映异构体的情况下显著地更明显（也参见图 1F 和图 1G）。

[0558] 实验对照：

[0559] 根据公开方法制备的 Abeta1-42 寡聚体被用作阳性对照。[参见例如 Dahlgren 等人，"Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability"J Biol Chem. 2002Aug30 ;277(35):32046-53. Epub2002Jun10. ;LeVine H3rd. "Alzheimer's beta-peptide oligomer formation at physiologic concentrations"Anal Biochem. 2004Dec 1 ;335(1):81-90 ;Shrestha 等人，"Amyloid beta peptide adversely affects spine number and motility in hippocampal neurons"Mol Cell Neurosci. 2006Nov ;33(3):274-82. Epub2006Sep8 ; Puzzo 等人，"Amyloid-beta peptide inhibits activation of the nitric oxide/cGMP/cAMP-responsive element-binding protein pathway during hippocampal synaptic plasticity"J Neurosci. 2005Jul20 ;25(29):6887-97 ;Barghorn 等人，"Globular amyloid beta-peptide oligomer-a homogenous and stable neuropathological protein in Alzheimer's disease"J Neurochem. 2005Nov ;95(3):834-47. Epub2005Aug31 ;Johansson 等人，Physiochemical characterization of the Alzheimer's disease-related peptides A beta1-42Arctic and A beta 1-42wt. FEBS J. 2006Jun ;273(12):2618-30] 以及源自大脑的 Abeta 寡聚体（参见例如 Walsh 等人，Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature(2002). 416, 535-539 ;Lesne 等人，A specific amyloid-beta protein assembly in the brain impairs memory. Nature. 2006Mar16 ;440(7082):352-7 ;Shankar 等人，Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med. 2008Aug ;14(8):837-42. Epub2008Jun22）。应注意，任何 Abeta 寡聚体制剂可以用于该检定或作为对照，包括以上引用的专利文献中描述的制剂，这些专利文献全部引入本文以供参考。

[0560] 这在使用不同 Abeta 寡聚体制剂（特别包括从阿尔茨海默病患者的大脑中分离的寡聚体制剂）的膜运输检定中显示。尽管各种 Abeta 寡聚体制剂的效力不同（例如，天然阿尔茨海默病的分离物比任何所测试的合成制剂更有效力－数据未显示），但结果在性质上相同：由寡聚体介导的病理学受化合物 II 以及因此本发明的其它化合物对抗。寡聚体在

不使用洗涤剂的情况下从死后人海马或前额皮质分离，且以剂量依赖的方式以6pM的Kd抑制膜运输。源自人阿尔茨海默病患者的Abeta寡聚体（137pM，第二条，图1H）相比于媒介物（第一条，图1H）对膜运输产生统计学显著的抑制。化合物II（第三条）消除了由源自AD脑的Abeta寡聚体所诱导的膜运输缺陷，但在不存在Abeta的情况下给药时不影响运输（第四阴影条）。对3次实验（n = 3）的数据取平均值。

[0561] 在黑条所示的过量化合物II的存在下（15uM，第三条，图1J），寡聚体诱导的膜运输缺陷被完全消除。CT0109当以其自身给药时对膜运输没有显著作用（黑色斜条，图1J）。

[0562] 相比之下，从相同死后大脑区域（取自认知正常的年龄匹配的个体）分离的寡聚体通常相对于每克组织重量以较低浓度存在，为90pMvs.137pM（图1F，第二条），且相比于媒介物（图1F，第一条）不产生显著的膜运输缺陷。在这些条件下，化合物II在与寡聚体一起给药或单独给药时没有作用（图1F，分别是第三和第四条）。对数据也取平均值（n = 3，除了其中n = 5的第二条）。

[0563] 阴性对照包括经媒介物处理的神经元以及经超生理浓度28μM的美金刚处理的神经元。美金刚以该剂量对寡聚体效应产生50％抑制。这些对照在各个板上起到标准化工具的作用，以基于逐个板校准检定性能。

[0564] 初级神经元培养物

[0565] 使用胞吐检定作为读出，基于对Abeta寡聚体的细胞应答、以及培养物中胶质细胞与神经元的相对比例的免疫组织化学分析来确定最佳细胞密度。每周用免疫组化和基于图像处理的定量法对培养物进行监测，以监测培养物的神经元vs.神经胶质（神经胶质细胞）的百分比。不接受在体外21天筛选年龄（21DIV）时含有的神经胶质（GFAP阳性）相比于神经元（用针对MAP2的（鸡多克隆）抗体（Millipore）以1:5000（浓度可变）染色为阳性）高于20％的培养物。

[0566] Abeta寡聚体制剂

[0567] 从若干商业供应商例如California Pepride获得人淀粉样蛋白肽1～42，在质量控制分析中具有批次选择偶然事件。寡聚体制剂的质量控制由确定寡聚体的尺寸范围和相对浓度的Western以及确认胞吐加速且无毒性的MTT检定组成。通过经DNA结合蓝色染料DAPI（Invitrogen）可视的核形态的定量在各个基于图像的检定中监控毒性。片段化的细胞核被认为处于凋亡晚期（Majno和Joris'95）且不接受测试。产生不寻常的肽尺寸范围或在1.5uM的标准浓度对神经元显著毒性的肽批次也不被接受。

[0568] 基于板的控制—当重新排布的板在常规基础上在经媒介物与经Abeta寡聚体处理的神经元之间达到最小统计学显著的两倍分离（$p<0.01$，Student检验，不等方差），且各板间不多于10％ CV时，检定优化被视为完成。

[0569] 统计学软件和分析：

[0570] 通过Cellomics VTI图像分析软件和STORE自动化数据库软件完成数据处理和分析。因为在培养三周后较低的动态范围和神经元孔间的可变性，通过成对Tukey-Kramer分析进行统计学比较，以确定化合物+Abeta寡聚体与单独的Abeta以及单独的化合物与媒介物的分离显著性。成熟初级神经元更密切地接近成人大脑的电生理介导的信号传导网络的能力证实该筛选策略合理。对大量重复筛选孔设置检定力（power）分析，其使假阴性最小化（例如N = 4）。本发明的测试化合物显著逆转Abeta寡聚体对膜运输的作用且不影响神

经元代谢自身。

[0571] 将下表所示的式 I、II 和 VIII 内的化合物在本文所述的 MTT 检定中在添加 Abeta 寡聚体之前进行给药，并且显示为以所示的 EC_{50} 阻断 Abeta 寡聚体诱导的膜运输缺陷。具体地，这些结果表明化合物以微摩尔浓度阻抑 / 减轻 Abeta 寡聚体对神经元细胞的膜运输的活性 / 效应。

[0572] 表 5：σ -2 受体配体和抑制淀粉样蛋白寡聚体对膜运输的效应的能力：

[0573]

σ-2 受体配体	通过膜运输检定测量的抑制细胞中 β 淀粉样蛋白效应的 EC_{50}
化合物 A	3.4 μM
化合物 B	5.5 μM
化合物 C	5.4 μM
化合物 D	8.9 μM
化合物 E	8.2 μM
化合物 F	2.6 μM
化合物 G	5.8 μM
化合物 H	2.2 μM
化合物 I	3.4 μM
化合物 J	3.9 μM
化合物 K	14 μM

[0574]

化合物 L	2.4 μM
化合物 M	0.6 μM
化合物 N	5.2 μM
化合物 O	2.7 μM
化合物 P	20.0 μM
化合物 Q	0.5 μM
化合物 R	6.7 μM
化合物 R'	39 μM（无活性）
化合物 S	5.4 μM
化合物 S'	>30 μM（无活性）
化合物 T	7.7 μM
化合物 AC	2.4 μM
化合物 AD	0.7 μM
化合物 AG	6.1 μM（1.3 μM）
化合物 BA	<1.0 μM
化合物 BT	0.4 μM
化合物 BY	0.8 μM
化合物 CA	1.9 μM
化合物 CB	18.2 μM
化合物 CR	1 μM
化合物 CS	6.9 μM（3 μM）
化合物 CV	2.5 μM
化合物 CX	1.3 μM
化合物 CY	14 μM
化合物 DE	>20 μM
化合物 DN	>20 μM

[0575] 表 5 中的化合物显示为以所示 EC_{50} 阻抑 Abeta 寡聚体诱导的胞吐加速。因此，表 5 中的化合物显著阻抑 Abeta 寡聚体介导的膜运输变化。这些结果表明化合物阻抑 / 减轻 Abeta 寡聚体对神经元细胞的活性 / 效应，以及 σ-2 配体可以用于阻抑 Abeta 寡聚体诱导的膜运输异常。

[0576] 实施例 7 :药代动力学研究和代谢稳定性研究

[0577] 第一药代动力学研究由商业合同研究组织在小鼠的微粒体中进行。研究根据 Obach, R. S 等人 (1997) J. Pharmacol. Exp. Ther., 283:46-58 进行，其引入本文以供参考。所测试的表 2 中化合物的半衰期的范围为 2 ～ 72 分钟且剩余化合物的半衰期预期为处于大约相同的范围。

[0578] 微粒体中半衰期的结果显示于表 6 中 :

[0579] 表 6 :化合物的小鼠微粒体稳定性

[0580]

化合物	小鼠微粒体中 t1/2
II	16
A	33
B	55
C	10
D	2
E	46
F	72
G	42
H	24
I	33
J	47

[0581] 结果表明,所测试的数个化合物相比化合物 II 在小鼠肝脏微粒体中具有实质更长的半衰期。该结果预示这些化合物在口服后有更大的生物利用度。相同的化合物已通过上述的膜运输检定进行测试且报告其活性。

[0582] 化合物 II 的内在清除速率较快,表明基本上首过代谢。为改善药代动力学性质,设计其它化合物以提高代谢稳定性并改善药物样特性。进行微粒体稳定性实验和血浆稳定性实验以确定候选化合物的代谢和肝稳定性。

[0583] 第二 PK 研究在体内进行,且涉及测量通过各种途径以急性或慢性方式施用的测试化合物的血浆水平和大脑水平,如下 :

[0584] HPLC-MS 优化

[0585] 制备各个测试化合物的溶液且经注射泵以恒定速度输入到 TSQ Quantum 分光仪 (Fisher Thermo Scientific) 源中。进行全扫描 MS(质谱) 分析,并对于各个测试化合物以正电离化和负电离化模式产生总离子流色谱和对应的质谱。从正或负质谱中选择用于 MS/MS 的前体离子,作为各离子丰度的函数。另外,进行产物离子 MS/MS 分析,以确定用于定量分析的合适的选定的碎裂反应。选择终反应监测参数,使得对存在于组分的复杂混合物中的测试化合物进行定量的能力最大化。在识别待用于各个测试化合物的具体 SRM 转化之后,使用自动化方案在 TSQ Quantum Compound Optimization 工作区中优化检测参数。最后,待用于 LC-MS 分析的色谱条件通过在合适的 LC 柱上注射和分离分析物且按需调节梯度条件而确定。

[0586] 用于 IV 给药的制剂：

[0587] 测试化合物在磷酸盐缓冲盐水 pH7.4(PBS) 中的溶解性首先通过视觉检查评价。如果化合物在目标浓度下可溶，则将 PBS 用作媒介物。（如果化合物在 PBS 中不完全溶解，则可以评价与 IV 给药相容的其它媒介物。这样的媒介物除其它之外包括 DMSO、聚乙二醇 (PEG400)、Solutol HS15 和 Cremophor EL)。在本文报道的实验中，IV 施用单次推注的 10mg/kg 化合物 II。

[0588] 用于 PO 给药的制剂：首先评价测试化合物在 PBS 中的溶解性。如果化合物在目标浓度下可溶，则将 PBS 用作媒介物。（如果测试化合物在各浓度下不能完全溶解于 PBS，则可以使用 DMSO/Solutol HS15/PBS(5/5/90，v/v/v) 或 DMSO/1％甲基纤维素 (5/95，v/v))。

[0589] 血浆中的线性

[0590] 在等份的血浆中掺入指定浓度的测试化合物。使用乙腈沉淀处理所掺的样品，并通过 HPLC-MS 或 HPLC-MS/MS 分析。构建峰面积对于浓度的校准曲线。确定该检定的可报道的线性范围，以及定量下限 (LLQ)。

[0591] 血浆样品的定量生物分析

[0592] 使用乙腈沉淀处理血浆样品，并通过 HPLC-MS 或 HPLC-MS/MS 分析。生成血浆校准曲线。在等份的无药血浆中掺入指定浓度水平的测试化合物。将所掺的血浆样品与未知的血浆样品一起使用相同的步骤进行处理。处理的血浆样品（干燥提取物）通常被冷冻储存 (-20℃）直至进行 HPLC-MS 或 HPLC-MS/MS 分析。将干燥提取物重构到合适的溶剂中且在离心后通过 HPLC-MS 或 HPLC-MS/MS 分析。记录峰面积，且使用各校准曲线确定未知血浆样品中的测试化合物的浓度。确定该检定的可报道的线性范围，以及定量下限 (LLQ)。

[0593] 用于研究的动物是各自称量为 20-30g 的雄性 C57BL/6 小鼠或称量为 180-250g 的雄性 Sprague-Dawley 大鼠。以各个施用条件和各个时间点处理三只动物，使得各只动物进行仅一种血液抽取。通过腹腔内注射完成皮下化合物施用。通过灌胃完成每次口腔施用。经颈静脉导管完成静脉内施用。

[0594] 在以各种浓度施用化合物之后，在 10、30、60、120、240、360、480 和 1440min 收集血浆样品。

[0595] 从小鼠和大鼠收集血浆样品

[0596] 在一般吸入麻醉 (3％异氟烷）下使动物镇静，以通过心脏穿刺（小鼠）或颈静脉导管（大鼠）收集血液。在收集的 1 小时内，在涂覆有肝素锂的管中收集等份血液 (300-400μL)，轻轻地混合，然后保持于冰上且以 2,500xg 在 4℃离心 15 分钟。然后将血浆收集并冷冻保持于 -20℃直到进一步处理。

[0597] 动物给药设计 - 体内 PK- 非插管、非禁食的动物

[0598] 组 1：SC，每个时间点 n ＝ 3 动物（总计 24 只动物）或

[0599] IV，每个时间点 n ＝ 3 动物（总计 24 只动物）

[0600] 组 2：PO，每个时间点 n ＝ 3 动物（总计 24 只动物）

[0601] 组 3：对照动物（对于无药血液），n ＝ 5 小鼠

[0602] 各个动物进行一次抽血和一次脑收集。

[0603] 从动物中收集大脑样品

[0604] 紧接血液取样后，将动物断颈，并快速取出整个脑，用冷盐水 (0.9％ NaCl，g/mL)

清洗，使表面脉管系统断裂，用纱布吸干，称重，保持在冰上直至在收集一个小时内的进一步处理。使用 Power Gen125，将各个脑在 1.5mL 冷的磷酸缓冲盐水 pH7.4（小鼠＝ 1.5mL，大鼠＝）中于冰上匀浆 10 秒钟。之后将来自各个脑的脑匀浆物储存在 -20℃直至进一步处理。

[0605] 大脑样品中的线性

[0606] 在等份的大脑匀浆中掺入指定浓度的测试化合物。向各个大脑等份物中，添加等体积的冷 26％ (g/mL) 中性右旋糖酐（来自 Sigma，平均分子量 65,000-85,000，目录号 D-1390）溶液以得到 13％的最终右旋糖酐浓度。将匀浆在 4℃以 54000x g 离心 15 分钟。上清液随后使用乙腈沉淀处理并通过 HPLC-MS/MS 分析。构建峰面积对于浓度的校准曲线。确定该检定的可报道的线性范围，以及定量下限 (LLQ)。

[0607] 大脑样品的定量分析

[0608] 向各个大脑匀浆等份物中，添加等体积的冷 26％ (g/mL) 中性右旋糖酐（来自 Sigma，平均分子量 65,000-85,000，目录号 D-1390）溶液以得到 13％的最终右旋糖酐浓度。将匀浆在 4℃以 54000x g 离心 15 分钟。上清液随后使用乙腈沉淀处理并通过 HPLC-MS/MS 分析。生成脑校准曲线。在无药大脑匀浆等份物中掺入指定浓度水平的测试化合物。将所掺的大脑匀浆样品与未知的脑匀浆样品一起使用相同的步骤进行处理。将处理的大脑样品在 -20℃储存直到 LC-MS/MS 分析，分析时记录峰面积，并使用各校准曲线确定未知大脑样品中的测试化合物的浓度。确定该检定的可报道的线性范围，以及定量下限 (LLQ)。

[0609] 大脑渗透

[0610] 通过 LC-MS/MS 确定各时间大脑中 (ng/g 组织）和血浆中 (ng/mL) 测试化合物的浓度以及大脑浓度与血浆浓度的比率，并如上所述进行报告。

[0611] 药代动力学

[0612] 构建化合物的血浆浓度相对于时间的图。口服和 SC 给药后化合物的基本药代动力学参数 (AUClast、AUCINF、T1/2、Tmax 和 Cmax) 得自使用 WinNonlin(Pharsight) 的血浆数据的非房室分析 (NCA)。非房室分析不需要假定用于药物或代谢物的特定的房室模型。NCA 使得可以应用梯形法则来测量血浆浓度 - 时间曲线下的面积 (Gabrielsson, J. 和 Weiner, D. Pharmacokinetic and Pharmacodynamic Data Analysis:Concepts and Applications. Swedish Pharmaceutical Press. 1997)。

[0613] 所报道的术语的定义

[0614] 曲线下面积 (AUC)- 达到全身循环的未变化药物的总量的量度。曲线下面积是几何测量，其通过通过用浓度 vs. 时间作图并对各个梯形的增量面积求和来计算。

[0615] WinNonlin 具有两种用于计算面积的计算方法：线性梯形法和线性对数梯形法。因为线性梯形法对浓度 - 时间曲线的下降部分可能提供有偏结果并高估 AUC，因此 WinNonlin 对 AUC 的计算提供线性对数选项。默认使用对数线性梯形法来对血浆浓度 - 时间曲线的剩余部分测量 Tmax 后面积。

[0616] AUClast：高于定量限制的从给药时间到最后观察时间的曲线下面积。

[0617] AUCINF：从给药时间外推到无限的曲线下面积。

[0618] Cmax- 在给药时间与最后观察时间点之间得到的经口或非 IV 药物施用的最大血浆药物浓度。

[0619] Tmax- 在药物施用之后以分钟计的在最大观察血浆浓度 (Cmax) 时的时间。

[0620] T1/2-IV 和非 IV 给药的末端消除半衰期。

[0621] 其中 λZ(z) 是与血浆浓度 - 时间曲线的末端（对数线性）部分相关的一级速率常数。Z 通过时间相对于对数浓度的线性回归而估计。

[0622] 结果显示，当以范围为 0.1 ～ 0.5mg/kg 的剂量急性或慢性（在 5 天内每天）施用时，化合物生物利用度高且大脑渗透性高。急性施用化合物 II 的结果显示于图 2A 中。图 2A 是其中化合物的血浆水平以 ng/mL 为单位显示于左边 y 轴的图。大脑水平以绿色以 ng/g 为单位显示于右边 y 轴。X 轴显示在零时推注 IV 或 SC 施用之后的时间。在以 10mg/kgi.v. 急性 IV 施用之后，化合物 II 达到脑中高浓度且在给药后 180 分钟仍具有 171ng/g 的浓度（体内有效脑剂量的 57x，通过空心菱形表示）。在急性 SC 给药后为相似的模式。化合物 B 在肠胃外施用时显示相同水平的生物利用度，但通过口腔途径生物利用度基本上更大。两种所测试的化合物均发现为具有明显较高的 BBB 渗透性，且化合物 II 在 3 小时的大脑 / 血浆比率为 8。

[0623] 化合物 B 的结果和化合物 II 急性给药的结果显示于表 7 中。

[0624] 表 7. 化合物 B 和 II 在小鼠中的药物代谢动力学

[0625]

参数	化合物 B- 10 mg /kg		化合物 II-10 mg/kg	
	PO 血浆	SC 血浆	PO 血浆	SC 血浆
T_{max}（min）	30	120	NC	30
C_{max}（ng/mL）	599.3	607.7	NC	279
$t_{1/2}$（min）	210	218	NC	100
AUC_{all} ((ng*hr)/mL)	2,851.1	5,242.3	NC	384.7
F_{rel}（%）	54.4		NC	

[0626] 如该表中所示，化合物 B 的口服生物利用度及它的其它 PK 参数相对于化合物 II 有改善。

[0627] 在 5 天的时程内以慢性施用，0.1、0.35 和 0.5mg/kg 范围内的给药提供化合物 II 相对稳定的血浆水平，脑暴露较好且具有与急性设定相似的脑 / 血浆比。结果显示于图 2B 中。图 2B 是在每天一次皮下施用不同量的化合物 II(0.5mg/kg/ 天 ：填充的下三角 ；0.35mg/kg/ 天 ：填充的上三角 ；以及 0.1mg/ 天填充的正方形）时在血浆（左纵坐标）中得到的以及在 SC 施用相同量（分别是空心下三角、空心上三角和空心正方形）的化合物 II 时在大脑（右纵坐标）中得到的药代动力学数据的图。

[0628] 化合物 B 的脑水平测量显示出比化合物 II 更低的峰浓度，但更高的持续水平。在三小时点处，大脑中化合物 II 的量相比于针对化合物 II 确定的 50ng/mL 有效剂量，对 PO 几乎高 40x 且对 SC 高 60x。然而，化合物 II 的三小时时间点相比该化合物的有效剂量仍高 >3x（对于 SC)（数据未显示）。

[0629] 当以 1mg/kg 在小鼠中静脉给药时，化合物 IXa 和 IXb 的混合物显示出 2.7 小时的半衰期。然而，当化合物以 5mg/kg 口服给药时，其在血浆中显示出可忽略的药物量。对化合物 IXa 和 IXb 的混合物 + 药物标准品在小鼠肝微粒体中随后的研究（表 6）测量出混

合物的半衰期为 8.7 分钟且内在清除率为 267 微升 /min/mg，表明混合物易受首过代谢影响。通常，CNS 活性化合物倾向于具有低至中度的内在清除率 (CL_{int} ≤ 100 微升 /min/mg)(Wager 等人，'10)。13 种 CNS 活性药物的人肝微粒体稳定性数据给出 51±29 分钟的平均半衰期 (Orbach'99)。因此，对于化合物 II 首过代谢的改善的合理目标将是 $T_{1/2}$>30 分钟且 CL_{int} ≤ 100 微升 /min/mg。化合物 IXa、IXb 相比于某些已知药物表现出可比较的肝稳定性，如图 8 中所示。

[0630] 表 8. 化合物 IXa 和 IXb 以及选择 CNS 药物标准物的小鼠肝微粒体数据。CL'int = 内在清除率。通过合同研究组织进行实验，并以化合物 II 为标准物进行。

[0631]

药物	作用模式	微粒体浓度 (mg/mL)	$T_{1/2}$ (min)	CL'INT (uL/min/mg)

[0632]

化合物 IXa 和 IXb 的混合物	候选化合物	0.3	8.7 +/- 0.1	267
丙咪嗪	抗抑郁剂	0.3	11.5	200
心得安	Beta 阻断剂	0.3	16.4 +/- 0.5	141
特非那定	抗阻胺剂	14.6	8.7 +/- 1.1	159
维拉帕米	Ca++通道阻断剂	0.3	11.4 +/- 0.5	204

[0633] 实施例 8 :Abeta1-42 寡聚体结合和突触丧失检定

[0634] 在该检定中，使 Abeta 寡聚体与培养中的成熟初级神经元接触，且通过免疫组化 (抗 Abeta 抗体) 确定其结合并通过图像处理进行定量。神经元树突中的 Abeta 的量通过对神经炎上标记点的数目进行计数来评价。已知 Abeta 寡聚体与原代培养中占显著百分数 (30 ～ 50％) 的海马神经元上所存在的突触后神经元的子集饱和地 (Kd 约 400nM ; Laurén2009) 且高亲和性地结合 (Lacor 等人，2004 ;Lambert 等人，2007)，且这与阿尔茨海默病患者大脑中的 Abeta 结合的观察结果很好地相关 (Lambert 等人，2007)。该标记与突触相关联，与突触后支架蛋白 PSD-95 共定位 (Lacor 等人，'04)。还已知 Abeta 寡聚体介导突触丧失 (报道在脑片的人海马神经元中为 18％ (Schef 等人，2007)) 且抑制长时程增强效应 (LTP)。突触数目也可以在该检定中通过免疫荧光化学 (immunofluorochemistry) 进行定量。用于结合检定的相似步骤可在文献中找到。参见例如，Look GC 等人 . Discovery of ADDL-targeting small molecule drugs for Alzheimer's disease. Curr Alzheimer Res. 2007Dec ;4(5) :562-7. Review.

[0635] 对结合于神经元表面的 Abeta 的量的测量可以用作对识别经一种或多种以下机制起作用的化合物的第二筛选 :通过干扰 Abeta 寡聚体与神经元表面的结合或通过影响寡聚体自身的变化 (反向激动或寡聚体解离) 或寡聚体结合的表面受体的变化 (变构调节或经典受体拮抗) 来阻断 Abeta 效应。其还可以将这些化合物与在下游信号传导事件中起作用的化合物区分开。因此，该检定与以 Abeta 寡聚体对神经元的非致死作用为特征的疾病

状态相关，且形成本发明的发明人所采用的筛选级联的一部分，从而识别临床相关的化合物。重要地，此处公开的化合物之一即化合物 II 在膜运输检定且在该结合 / 突触丧失检定中具有活性，且已证明还在两种不同的阿尔茨海默病转基因模型中且在诱导的模型中也具有活性。因此，这以及膜运输检定可用于识别临床相关的化合物且似乎对于体内结果具有预测价值。该检定的预测有效性通过使用本发明范围外的化合物证实其预测化合物性质的能力来确认。

[0636] 初级海马神经元培养物在以上膜运输检定中确立。向板添加化合物 II（以 10^{-8} ～ 30 微摩尔的浓度），并添加任何其它未来将检测的化合物（以 10^{-8} ～ 30 微摩尔的浓度），随后以达到饱和结合的浓度添加含有 Abeta1-42 寡聚体的制剂。用待测试的化合物进行的预处理持续 1 小时并以 70uL 的终浓度添加 Abeta 寡聚体或不添加寡聚体（仅媒介物），之后再孵育 23 小时。

[0637] 用 3.7％多聚甲醛于磷酸盐缓冲盐水中的溶液将板固定 15min。然后用 PBS 洗涤板 3x，每次 5min。在室温下于 PBS 的 5％山羊血清和 0.5％ Triton X-100 中封闭板 1 小时。用 PBS 中的 5％山羊血清将一抗 (MAP2 多克隆抗体，Millopore#AB5622 和 β 淀粉样蛋白 6E10 单克隆抗体，Covance#SIG-39300，1 微克 /ml，以及突触素兔多克隆抗体，Anaspe，0.2 微克 / ml) 稀释 1:1000。将一抗在 4℃孵育过夜。然后用 PBS 洗涤板 3x，每次 5min。用 PBS 中的 5％山羊血清将二抗 (Alex Flor488 多克隆抗体，Invitrogen#A11008 和 Alexa Flor647 单克隆抗体，Invitrogen#A21235) 稀释 1:1000。将二抗在室温孵育 1 小时。用 PBS 将板洗涤一次。然后以 0.03ug/ul 施用 DAPI (4'，6- 二脒基 -2- 苯基吲哚，Invitrogen) 且将其在室温孵育 5min，然后用 PBS 洗涤。结果显示，如预期，如下详述制备且根据所使用的制剂以 3 或 1μM 给药的 Abeta 寡聚体与突触处的神经元结合，如由红色染料所显示。在具有早期阿尔茨海默病的人中，与年龄匹配的认知正常的个体相比，海马中的突触数量显示为减少 18％ (Scheff 等人，'07)，且该结果也可以在该检定中可视为荧光点进而突触数量的 20％衰退。然而，在化合物 II (15uM) 的共存下，Abeta 结合基本上减少到对照水平，且绿色荧光未受影响，表明突触数量未减少。参见图 3A ～ 3F。在图 3A- 板块 A 中，Abeta42 寡聚体与突触后棘结合，图 3A- 板块 B 显示，突触前棘经初级神经元中的突触素标记 (21DIV)。图 3A- 板块 C 和 D 分别示出当将 IXa、IXb 加入培养物中时处于基本上对照水平的突触后棘和突触。如在图 3C 的条形图中所定量显示，相比于单独的媒介物（第一条），单独添加的 Abeta42 寡聚体在 24 小时后（第四条）引起突触素点密度降低 20％（如计算）。该丧失被化合物 II 或 IXa、IXb（第五或第六条）逆转，且该结果统计学显著。在不存在 Abeta 寡聚体的情况下，化合物 IXa、IXb 或化合物 II 均不影响突触数目（阴影条）且其保持在与对照（单独的媒介物）可比较的水平。比例尺＝ 20urn。p<0.001ANOVA。图 3D (p<0.001ANOVA) 也是条形图且显示通过 Abeta 点计算的 Abeta 结合强度在化合物 II 或 IXa、IXb 的存在下减少 18％，而该降低足以允许突触计数在该化合物的存在下达到对照水平。

[0638] 此外，在化合物 IXa 和 IXb 的混合物的存在下，点状突触 Abeta 寡聚体结合以浓度依赖方式减少 38％，IC_{50} 为 1.2μM（数据未示出）。点密度的柱状图显示，正常双峰结合群（具有亮点的神经元和具有不太亮的点的群）在药物存在下左移（数据未示出）。已经报道对 Abeta 寡聚体结合的部分抑制重获 100％的 LTP 功能 (Strittmatter SM 等人，Cellular Prion Protein Mediates Impairment of Synaptic Plasticity by Amyloid-Beta Oligomers

Nature(2009)457(7233:1128-32))。此外,如图 3C 所示,Abeta 寡聚体(第四条)在 24 小时后与媒介物处理(第一条)相比引起突触素点密度的 20%降低,其被 5μM 的化合物 IXa 和 IXb 的混合物逆转(第五条)。不存在 Abeta 时(第二条),化合物 IXa 和 IXb 的混合物不影响突触数量。Abeta 寡聚体造成突触数量的 18.2%降低;100%的该丧失被 5μM 的化合物 IXa、IXb 或 II 消除(图 3C)。化合物 IXa 和 IXb 的混合物以 1.21uM 的 IC_{50} 引起 Abeta 标记点密度的 17.7%降低(图 3D)。

[0639] 用 DAPI 可视化的细胞核表现出正常的形态,表明不存在神经退行。该过程将使用选自被式 I ～ IX 包括的那些、以及其他描述为以上 σ-2 配体的化合物的其他测试化合物进行重复。

[0640] Abeta 寡聚体制剂:

[0641] 人淀粉样蛋白肽 1 ～ 42 得自 California Peptide,在质量控制分析中存在批次选择偶然事件。根据上述的公开方法来制备 Abeta1 ～ 42 寡聚体。[参见,例如 Dahlgren 等 人,"Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability"J Biol Chem. 2002Aug30;277(35):32046-53. Epub2002Jun10.;LeVine H3rd."Alzheimer's beta-peptide oligomer formation at physiologic concentrations"Anal Biochem. 2004Dec1;335(1):81-90;Shrestha 等 人,"Amyloid beta peptide adversely affects spine number and motility in hippocampal neurons"Mol Cell Neurosci. 2006Nov;33(3):274-82. Epub2006Sep8; Puzzo 等 人,"Amyloid-beta peptide inhibits activation of the nitric oxide/cGMP/cAMP-responsive element-binding protein pathway during hippocampal synaptic plasticity"J Neurosci. 2005Jul20;25(29):6887-97;Barghorn 等人,"Globular amyloid beta-peptide oligomer-a homogenous and stable neuropathological protein in Alzheimer's disease"J Neurochem. 2005Nov;95(3):834-47. Epub2005Aug31;Johansson 等 人,Physiochemical characterization of the Alzheimer's disease-related peptides A betal-42Arctic and A beta 1-42wt. FEBS J. 2006Jun;273(12):2618-30] 以及得自脑的 Abeta 寡聚体(参见,例如 Walsh 等人,Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature(2002). 416,535-539;Lesne 等 人,A specific amyloid-beta protein assembly in the brain impairs memory. Nature. 2006Mar16;440(7082):352-7;Shankar 等人,Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med. 2008Aug;14(8):837-42. Epub2008Jun22)。寡聚体制剂的质量控制由确定寡聚体尺寸范围和相对浓度的蛋白印迹以及证实胞吐加速而没有毒性的 MTT 检定构成。毒性在各个基于图像的检定中经由对用 DNA 结合染料 DAPI(Invitrogen)可视化的细胞核形态进行定量而进行监控。片段化的细胞核被认为处于晚期细胞凋亡,因此去掉检测(Majno and Joris Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol1995;146:3-16)。产生不正常肽尺寸范围或在标准浓度下对神经元产生显著毒性的肽批次将被拒绝。

[0642] 对照

[0643] 用寡聚体制剂对 Abeta 抗体 6E10 预吸收以剂量依赖的方式($7.84x10^{-6}$)抑制突触

结合，并用作阳性对照。抗体以 1:1000(1 微克 /ml) 使用。对于突触丧失检定，NMDA 拮抗剂地佐环平 (MK-801) 以 80uM 用作阳性对照。

[0644] 图像处理

[0645] 拍摄图像并用 Cellomics VTI 自动显微镜平台运用神经元图谱化算法进行分析。对于统计分析，使用不等方差的 Tukey-Kramer 成对比较。

[0646] 蛋白免疫印迹

[0647] 将含有 Abeta1 ～ 42 的样本在非还原性泳道标记物上样缓冲液 (Pierce#1859594) 中稀释 (1:5)。将 30 微升 (μL) 样本装载到十八个孔的预制 4 ～ 15％ Tris-HCl 凝胶 (BIORAD#345-0028) 中。在 BIO-RAD Criterian 预制凝胶系统中使用 Tris- 甘氨酸缓冲液在 125 伏特 (V) 进行 90 分钟的电泳。在 Tris- 甘氨酸 /10％甲醇缓冲液中，在 30V 下，经 120 分钟将凝胶转印到 0.2μM 硝酸纤维素膜上。膜在 PBS 溶液中煮 5 分钟并用 TBS/5％牛奶溶液在 4℃封闭过夜。在 TBS/1％牛奶溶液中用稀释至 10μg/mL 的 6E10-HRP(Covance#SIG-39345) 于室温对膜探测 1 小时。膜用 TBS/0.05％吐温 -20 的溶液洗涤三次，每次 40 分钟，并用 ECL 试剂 (BIO-RAD#162-0112) 显影 5 分钟。在 Alpha Innotech FluorChem Q 定量成像系统中进行图像获取，并用 AlphaView Q 软件进行分析。

[0648] 活性

[0649] 示出化合物 II，根据结合检定（使用成像处理算法），预计选自本文具体公开的那些的化合物显示为部分地阻断 Abeta 寡聚体配体对神经元的结合（约 25％）。

[0650] 实施例 9 :条件性恐惧检定

[0651] 在已知为条件性恐惧的记忆依赖行为任务的动物模型中测试化合物 II。基于所公开的操作指南来设计研究操作指南（参见，例如 Puzzo D, Privitera L, Leznik E, FàM, Staniszewski A, Palmeri A, Arancio O. Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. J Neurosci. 2008Dec31 ; 28(53):14537-45.）。情境记忆的形成依赖于颞叶内侧结构例如海马的完整性。在该检定中，将小鼠训练成记住特定的主要情境（条件性刺激 ;CS) 与厌恶事件（在该情况下为温和足部电击）(非条件性刺激，US) 相关。示出良好学习的动物在放回相同情境时将表现出僵立行为增加。该僵立不存在于新的情境中。在情境中僵立的增加表明动物中强烈的海马依赖的记忆形成。在条件性恐惧中测试的记忆对可溶 Aβ 的升高较为敏感。化合物 II 可有效停止 Abeta 寡聚体介导的对膜运输的作用。当在 Abeta 寡聚体施用前对动物给药时，化合物 II 以剂量依赖的方式阻断寡聚体对记忆的作用。化合物在 2pmol 的剂量完全阻断寡聚体介导的记忆缺陷。

[0652] 确实，如图 4 所示，化合物 II 完全消除 Abeta 寡聚体引发的记忆缺陷（黑条）而当单独给药时不影响记忆（阴影条）。单独 Abeta 寡聚体的作用通过红条示出。此外，如图 5 所示，化合物 IXa 和 IXb 的混合物提供相似的结果。该行为效力表明，膜运输检定能够预测哪些化合物将有效治疗由寡聚体引起的行为记忆丧失。如本文所述的那样进行对于记忆的恐惧条件模型。在任何剂量均没有观察到不利的行为变化。因此，在该化合物在膜运输检定中的性能与其在条件性恐惧检定中的性能之间具有相关性，后者是记忆丧失的指示。预测表 2 中所列的化合物将在条件性恐惧检定中有活性，因此将示出对治疗记忆丧失有效。化合物在恐惧条件模型中的性能与其在治疗记忆丧失中的有用性之间的相关性

已经在文献中确定。(Delgado MR, Olsson A, Phelps EA. "Extending animal models of fear conditioning to humans"Biol. Psychol. 2006Jul ;73(1):39-48)。

[0653] 实施例 10. 对大鼠、猕猴和人死后脑组织的放射自显影研究

[0654] 对于 σ-2 和 σ-1 受体配体的神经学和药理学图谱的放射自显影成像研究通过修改先前由 Xu 等人 (2010) 报道的操作指南来进行, Xu, J., Hassanzadeh B, Chu W, Tu Z, Vangveravong S, . Tones LA, Leudtke RR, Perlmutter JS, Mintun MA, Mach RH. [^{3}H]4-(Dimethylamino)-N-[4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl]benzamide, a selective radioligand for dopamine D(3) receptors. II. Quantitative analysis of dopamine D3and D2receptor density ratio in the caudate-putamen. Synapse64:449-459(2010), 其并入本文以作参考。标记的 RHM-1 通过 Xu J, Tu Z, Jones LA, Wheeler KT, Mach RH. [^{3}H]N-[4-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1H)-yl)butyl]-2-methoxy-5-methylbenzamide:a Novel Sigma-2Receptor Probe. Eur. J. Pharmacol. 525:8-17(2005) 的方法得到, 其并入本文以作参考。

[0655] 来自大鼠、猕猴和死后人脑的 20μM 厚的脑切片使用 Microm 冷冻切片机进行切片, 并装载至 superfrost plus 载玻片 (Fisher Scientific, Pittsburgh, PA) 上, 并在该研究中使用经过大脑皮层和海马的脑区的系列切片。将脑切片与 5nM[^{3}H](+)-喷他佐辛 (对于 σ-1 受体图谱化)、仅 4nM[^{3}H]RHM-1 (对于 σ-2 受体特征化)、在 σ-1 受体阻断剂 (+)-喷他佐辛的存在下与 10nM[^{3}H]DTG 和 [^{3}H] 氟哌啶醇 (对 σ-2 受体分布进行成像) 一起孵育; 在与放射性配体孵育 30 分钟后, 含有脑切片的载玻片用冰冷缓冲液冲洗 5 次, 每次一分钟。

[0656] 将玻片干燥, 并通过在自由侧覆盖铜箔带而使其导电, 然后将其放置在气体检测器 Beta Imager2000Z Digital Beta 成像系统 (Biospace, France) 的气体箱 [氩气和三乙胺的混合物 (Sigma-Aldrich, USA)] 中。在气体良好混合并达到均质状态时, 再暴露 24 小时至 48 小时直至观察到高质量图像。在相同时间对 [^{3}H] 微尺度 (American Radiolabeled Chemicals, Inc., St. Louis, MO) 计数, 作为对总放射性定量分析的参考, 即, 将 cpm/mm2 转化为 nCi/mg 组织。对感兴趣的解剖区 (ROI) 用 Beta-Image Plus 程序 (BioSpace, France) 进行定量分析, 即, 在皮层和海马区获得定量放射性摄取 (cpm/nlln2)。基于相应放射性配体的特定活性以及标准 [^{3}H] 微尺度的校准曲线, 将结合密度标准化为 fmol/mg 组织。使用定量放射自显影, 对于四种放射性配体 [^{3}H](+)-喷他佐辛、[^{3}H]RHM-1、[^{3}H]DTG 和 [^{3}H] 氟哌啶醇, 对一系列候选化合物的稀释物 (10nM、100nM、1,000nM 和 10,000nM) 测试其对结合位点的竞争, 之后分析特异性结合 (%对照) 来得到在皮层和海马区 (齿状回、海马 CA I 和 CA3) 的结合亲和性。

[0657] 在 σ-1 和 σ-2 受体处的放射自显影分别示于图 8A 和 8B 中。图 6C 示出 (A) 在正常患者、路易体痴呆症 (DLB) 患者或阿尔茨海默病 (AD) 患者的人额叶皮层切片中的 [^{3}H]-(+)-喷他佐辛 (σ-1 受体配体) 放射自显影, 以及 (B) 与对照相比的特异性结合的图。如图 6A 所示, 与正常对照相比, σ-1 受体在阿尔茨海默病和可能的 DLB 中统计学地下调。该发现证实了 Mishina 等人的发现, Mishina 等人报道在早期阿尔茨海默病中的低密度 σ-1 受体。Mishina 等人, 2008, Low density of sigmal receptors in early Alzheimer's disease. Ann. Nucl Med22:151-156。图 6B 示出 (A) 在正常患者、路易体痴呆症 (DLB) 患者或阿尔茨海默病 (AD) 患者的人额叶皮层切片中的 [^{125}I]-RHM-4 (σ-2 受体配体) 放射

自显影，以及 (B) 与对照相比的特异性结合的图。σ-2 受体在 AD 中没有统计学下调。图 6C 示出 (A) 在猴的额叶皮层、猴的海马或人颞叶皮层中 σ-2 配体对 18.4nM[^{3}H]-RHM-1 的替代，以及 (B) 具有以及不具有 1μM 西拉美新和化合物 IXA、IXB 以及 J 中每一组情况下的 [^{3}H]-RHM-1 结合密度图。西拉美新以及化合物 IXA、IXB 和 II 部分替代靶标组织中的 [^{3}H]-RHM-1。

[0658] 实施例 11. MTS 检定：确定多种 σ-2 配体的激动剂或拮抗剂活性

[0659] 以下示出的化合物的细胞毒性使用 CellTiter96Aqueous One Solution 检定 (Promega, Madison, WI) 来确定。简单而言，将 MDA-MB-435 或 MDA-MB231 或 SKOV-3 细胞以 2000 细胞 / 孔的密度在用 σ-2 受体选择性配体处理前的一天接种在 96 孔板中。在 24 小时处理后，向各个孔加入 CellTiter96Aqueous One Solution 试剂，且板在 37℃孵育 2 小时。在 Victor3 酶标仪 (PerkinElmer Life and Analytical Sciences, Shelton, CT) 中于 490nm 对板读数。定义为相对于未处理细胞抑制 50％细胞成活所需的 σ 配体浓度的 EC^{50} 值，从对于各个细胞系的剂量应答曲线中确定。西拉美新被接收作为激动剂。σ-2 配体的激动剂和拮抗剂被定义如下：如果 σ-2 配体的 EC50 小于西拉美新的 EC50 的 2 倍，则该 σ-2 配体被认为是激动剂。如果 σ-2 配体的 EC50 为西拉美新的 EC50 的 2 ～ 10 倍，则该 σ-2 配体被认为是部分激动剂。如果 σ-2 配体的 EC50 大于西拉美新 EC50 的 10 倍，则该 σ-2 配体被认为是拮抗剂。用于研究的 σ-2 配体是：激动剂 (西拉美新和 SV119)、部分激动剂 (WC26)、拮抗剂 (RHM-1) 和候选化合物 (II 和 IXa、IXb)。结果显示在图 9A 中。图 7A 的数据显示在表 9 中。

[0660] 表 10. 肿瘤细胞成活检定的 IC_{50} 值

[0661]

化合物	IC_{50}，48 小时 (uM)	作用
RHM-1	203±13	拮抗剂
西拉美新	11.8±2.7	完全激动剂
SV-119	21.7±2.9	完全激动剂
WC-26	65.6±6.3	部分激动剂

[0662]

IXa、IXb	169±9	拮抗剂
II	150±12	拮抗剂

[0663] 神经元培养物用多种浓度的 σ 化合物处理 24 小时，并测量相对于媒介物的细胞核密度。与在测试浓度不引起细胞核密度降低的 σ-2 拮抗剂 (RHM-1、IXa 及 IXb 和 II) 相反，σ-2 激动剂 (西拉美新、SV-119、WC-26) 造成神经元中显著异常的细胞核形态。因此，σ-2 受体激动剂对神经元和癌细胞有细胞毒性；然而，σ-2 受体拮抗剂无毒性且还阻断由 σ-2 受体激动剂引起的细胞毒性。

[0664] 实施例 12. 半胱氨酸天冬氨酸酶 -3 检定. 确定 σ-2 配体的激动剂或拮抗剂活性。

[0665] 如本文所述，Xu 等人识别出 PGRMC1 蛋白复合物为假定的 σ-2 受体结合位点。Xu 等人，2011. Nature Commun. 2, article number380，并入本文以作参考。σ-2 受体激动剂可以引发半胱氨酸天冬氨酸酶 -3 依赖的细胞死亡。Xu 等人 (2011) 公开了检测 PGRMC1 通过 σ-2 受体激动剂 WC-26 来调节半胱氨酸天冬氨酸酶 -3 活化的能力的功能性检定。

[0666] Abeta 寡聚体引起低水平的半胱氨酸天冬氨酸酶 -3 活化，并引起 LTD。高水平的 Abeta 寡聚体和半胱氨酸天冬氨酸酶活化造成细胞死亡。Li 等人，2010 ；Olsen and Sheng2012。本文已表明，σ -2 受体激动剂 (SV-119、西拉美新) 激活肿瘤细胞和神经元中的半胱氨酸天冬氨酸酶 -3 ；参见，例如图 8A 和 8B。σ -2 受体拮抗剂 RHM-1 抑制肿瘤细胞中的激活 (图 8A)，但是在该实验中无法在神经元中阻断激动剂 SV-119 引起的激活 (图 8B)。测试化合物 II 和 IXa、IXb(所有这些为如下所示的 σ -2 受体拮抗剂) 能够抑制肿瘤细胞中的半胱氨酸天冬氨酸酶 -3 激活，并阻断神经元中半胱氨酸天冬氨酸酶 -3 的 σ -2 受体激动剂 SV-119 激活。因此，如在此实施例中证实的，测试化合物 II 和 IXa、IXb 作为肿瘤细胞和神经元的半胱氨酸天冬氨酸酶 -3 检定中的 σ -2 受体拮抗剂。

[0667] 根据制造商的操作指南，使用半胱氨酸天冬氨酸酶 -3 比色活性检定试剂盒 (Milipore, Billerica, MA) 来测量内源半胱氨酸天冬氨酸酶 -3 经由 σ -2 受体配体的激活。简单而言，将 MDA-MB435 或 MDA-MB23I 细胞以 $0.5x10^6$ 细胞 /100mm 皿进行铺板。在铺板后 24 小时，将 σ -2 配体加到培养皿中，以引发半胱氨酸天冬氨酸酶 3 的激活。σ -2 配体的最终浓度为其 EC50。在处理 24 小时后，收获细胞，在 300uL 的细胞裂解缓冲液中裂解，并在 10,000x g 离心 5 分钟。收集上清液，并与半胱氨酸天冬氨酸酶 -3 底物 DEVD-pNA 在 37℃孵育 2 小时。使用 Dc 蛋白检定试剂盒 (Bio-Rad, Hercules, CA1) 确定蛋白浓度。使用 $Victor^3$ 酶标仪 (PerkinEliner Life and Analytical Sciences, Shelton, CT) 在 405nm 测量所得的游离 pNA。检测的配体包括 ：σ -2 激动剂 (西拉美新、SV119、WC26) 和 σ -2 拮抗剂 RHMWU-I-102(RHM-1) 以及候选化合物 (II 和 IXa、IXb)。激活半胱氨酸天冬氨酸酶 3 的配体被认为是激动剂，而不激活半胱氨酸天冬氨酸酶 3 的配体被认为是拮抗剂。如图 8A 所示，σ -2 激动剂西拉美新引发半胱氨酸天冬氨酸酶 -3 活性，而 σ -2 拮抗剂 RHM-1 和候选化合物 II 和 IXa、IXb 不引发半胱氨酸天冬氨酸酶 -3 活性。图 8B 示出经由 σ -2 激动剂 SV-119 的半胱氨酸天冬氨酸酶 -3 激活被化合物 IXa、IXb 和 II 阻断。化合物 IXa、IXb 和 II 在癌细胞和神经元中表现类似于 σ -2 拮抗剂。

[0668] 实施例 13. 治疗表型

[0669] 在一些实施方式中，本公开内容提供可预测行为效力的体外检定平台。对于 (1) 选择性地以高亲和性与 σ -2 受体结合 ；且 (2) 在神经元中作为功能性拮抗剂的化合物，如果其阻抑 Aβ 引发的膜运输缺陷、阻抑 Aβ - 引发的突触丧失且在不存在 Abeta 寡聚体时不影响运输或突触数量，则被预测为具有行为效力。在体外检定中的这种活性形式被称为“治疗表型”。σ -2 受体拮抗剂阻断成熟神经元中 Abeta 寡聚体效应而在不存在 Abeta 寡聚体时不影响正常功能的能力是治疗表型的一个标准。当不存在寡聚体时影响运输或突触数量的化合物不是行为上有效的。仅有选择性地阻断寡聚体且不影响正常运输或改变突触数量的那些化合物，在预防和治疗 Abeta 寡聚体引起的记忆丧失中是行为上有效的。在一个实施方式中，体外检定平台可以预测行为效力。由此，在该平台检定中的活性形式是治疗表型。

[0670] 例如，参见表 10A。

[0671] 表 10A. 治疗表型

[0672]

化合物	阻断 Aβ 引发的膜运输缺陷 EC50（uM）	阻断 Aβ 引发的突触丧失	不存在 Aβ 时的检定效果	行为效力
II	2.2	++	否	是
Z	6.1	+++	是	否
Z'	4.3	+++	是	否
IXa+IXb	4.9	+++	否	是

[0673] 总之，在 σ-2 受体处具有高亲和性（优选 Ki 小于约 600nM、500nM、400nM、300nM、200nM、150nM、100nM 或 70nM），且相比于其他非 σ CNS 或靶标受体对 σ 受体具有高于约 20 倍、30 倍、50 倍、70 倍或优选高于 100 倍选择性，具有良好的药物样特性（包括脑穿透性和良好的代谢和 / 或血浆稳定性）并具有治疗表型的 σ-2 拮抗剂，被预测为具有行为效力且可以用于在有需求的患者中治疗 Abeta 寡聚体引发的突触功能障碍。

[0674] 通过体外检定表征预测为具有经口生物可得性的一些化合物 II 类似物的功能性神经元表型示于表 10B 中。

[0675] 表 10B. 功能性神经元表型

[0676]

选择性	化合物	抑制 Abeta 寡聚体引发的膜运输 EC50（uM）	S1 结合 Ki（nM）	S2 结合 Ki（nM）	阻抑突触丧失	功能性神经元表型
在 σ-2 处的更高亲和性	II	2.2	500	9	100%	拮抗剂
	II（+）异构体	5.6	100	80	47%	拮抗剂
	W	8.7	110	36	43%	拮抗剂
	S'	>20	25	8	0%	无活性
	P	>20	320	110	0%	无活性
在 σ-1 处的更高亲和性	A	3.4	3	13	100%	拮抗剂
	B	5.5	1.3	3.9	100%	拮抗剂
	X	6.1	3.5	16	100%	拮抗剂
	E	8.2	2	3.6	34%	拮抗剂
	II（-）异构体	10.9	46	63	0%	拮抗剂

[0677]

在 σ-2 和 σ-1 处可比较的亲和性	Y	4.3	78	85	100%	拮抗剂
	R'	>20	11	16	33%	无活性

[0678] 实施例 14 :体外毒性

[0679] 代表性 σ -2 拮抗剂 II 和 IXa、IXb 在急性或慢性体外给药时不引起神经元或胶质细胞毒性。σ -2 受体拮抗剂消除或降低膜运输中 Abeta 寡聚体引发的变化。当不存在寡聚体而给药时,化合物对膜运输没出现显著作用。当以 EC50 浓度 (高达 50 μ M II 或 IXa、IXb) 在三天测试至 10 次时,相对于神经元数量、胶质细胞数量、细胞核尺寸、细胞核形态、神经突长度、细胞骨架形态没有毒性。参见表 11。

[0680] 表 11

[0681]

化合物	IXa、IXb	II
EC50(uM)	4.9	2.2
Abeta 的最大抑制 (浓度)	100% (14)	85% (10)
计算的 Ki*	0.58	0.26
Ki 时的单独 Cpd	+9%	+1%

[0682] *Abeta 的 Km = 0.4uM ;检定浓度 3 μ M 总 Abeta。

[0683] 在大量标准检定中测试化合物 II 的体外毒性。体外毒性研究中的测试表明,在 10 μ M 没有基因毒性 (AMES,微核,细菌细胞毒性)。在 10 μ M 的 66%的 HepG2 毒性 (在受体 x 处 100 倍以上的亲和性) 可能由 HepG2 肿瘤细胞系中的化合物亲油性或受体过表达引起。CYP450 酶 2D6、3A4 和 2C19 的部分抑制 (46 ～ 73%) 发生在 10uM。在 100nM 下观察到中度 hERG 抑制 (24%)。化合物 II 在 PGP 表现出非常弱 (IC50>30uM) 的活性。

[0684] 实施例 15 :化合物 II 对映异构体在膜运输检定中的分离和活性

[0685] 将化合物 II 分离成其 (+) 和 (-) 对映异构体。将外消旋混合物施用至手性柱 CHIRALPAK AD-H(直链淀粉三 (3,5- 二甲基苯基氨基甲酸酯) 涂覆在硅胶上 ;4.6X250mm)。将 15 微升体积的样本注射到柱中。洗提液为己烷 /EtOH/TEA(95/5/0.1),25 摄氏度,流速为 1ml/min。两种对映异构体在不同的峰分离。(+) 对映异构体在约 16 分钟的第一个峰洗提出,且 (-) 对映异构体在约 20 分钟的第二个峰洗提出。对映异构体为至少 98%纯。(+) 对映异构体具有 +10.1 的比旋度 (c1.80 于 MeOH 中),且 (-) 对映异构体具有 -7.2 的比旋度 (c1.80 于 MeOH 中)。在实施例 6 所述的膜运输检定中,(+) 对映异构体比 (-) 对映异构体更有效力。在一个样本中,在抑制膜运输检定中的 β 淀粉样蛋白引发的缺陷中,(+) 对映异构体的 EC50 为 5.6,且 (-) 对映异构体的 EC50 为 10.9 μ M。

[0686] 实施例 16. 经口可得化合物的行为效力 - 改善转基因阿尔茨海默病小鼠模型中的记忆缺陷。

[0687] 将雄性 hAPP Swe/Ldn 转基因 (Tg) 小鼠用作 AD 的 TG 模型。用媒介物、10 或 30mg/kg/ 天的 CB 或 CF 经 p.o. 处理 5.5 个月的转基因小鼠，以及媒介物处理的非转基因同窝幼崽进行标准条件性恐惧范例。在情境条件性恐惧中，用媒介物 p.o. 处理 5.5 个月的媒介物处理的 9 月龄雄性 hAPP Swe/Ldn 转基因 (Tg) 小鼠表现出显著的记忆缺陷 vs. 媒介物处理的非转基因同窝幼崽。

[0688] 当在训练 24 小时后对动物测试其关联记忆时，使用反复测量的双因素（基因型和时间）ANOVA 并没有检测出转基因和非转基因媒介物处理小鼠之间的总僵立时间的显著差异。然而，在各个定时的时间间隔中对僵立行为的更加敏感的分析表明，相比于媒介物处理的非转基因动物，转基因小鼠在 1 ～ 3 分钟时间间隔中表现得更差 (Mann-Whitney U 测试，$p<0.05$)。在该时间间隔中，与媒介物相比，用 10 和 30mg/kg/ 天的 CB ($p<0.05$) 和 30mg/kg/ 天的 CF ($p<0.005$) 处理的转基因动物显著改善表现 (Mann-Whitney U 测试)。结果显示在图 9 中，两种剂量的 CB 显著地逆转 AD 小鼠中的记忆缺陷；且更高剂量的 CF 逆转 AD 小鼠中的记忆缺陷。用 10 和 30mg/kg/ 天的 CB 或 30mg/kg/ 天的 CF 处理 Tg 动物，分别在 394±287、793±325 或 331±373nM(AVG±S.D.) 的测试脑浓度下改善缺陷。对于经口可得化合物的脑 / 谷血浆和脑 / 峰血浆的比例，结果示于表 12 中。

[0689] 表 12. 对于经口可得化合物的脑 / 谷血浆和脑 / 峰血浆比例

[0690]

化合物	剂量 (p.o.)	脑 / 谷血浆比	脑 / 峰血浆比
CF	30mg/kg	13	0.5
CF	10mg/kg	11	0.2
CB	30mg/kg	14	0.4
CB	10mg/kg	17	0.7

[0691]

[0692] 因此，化合物 CB 和 CF 均为经口生物可得，能够实现显著的脑穿透并在按照慢性长时程给药的老年转基因阿尔茨海默病小鼠模型动物中逆转建立的记忆缺陷。没有观察到不利的行为效应。

[0693] CB 和 CF 均为选择性的高亲和性 σ-2 受体拮抗剂化合物。CB 和 CF 均以高亲和性与 σ-2 以及 σ-1 受体结合，如表 14 所示。进行针对一组 40 种脑受体的反筛选，且结果表明 CB 和 CF 对于 σ 受体为高度选择性，如表 13 中所示。

[0694] 表 13. 对于经口生物可得化合物的受体亲和性

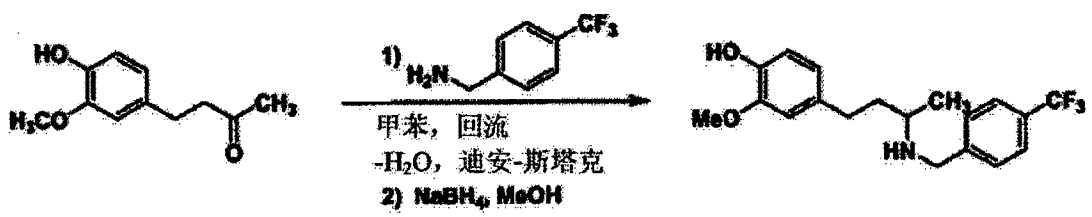
[0695]

药物	治疗作用	σ受体亲和性(σ-1/σ-2)（Ki，nM）	其他受体亲和性（Ki，nM）
CB	阿尔茨海默病	19/48	毒蕈碱 M1（1.5 uM）、M2（1.5 uM）、M3（1.8 uM）
			Kappa 型阿片（1.5 uM）
			Ca++ch-L-型（860 nM）
			转运体：NE（1.4 uM）、DA（220 nM）、5-HT（970 nM）
CF	阿尔茨海默病	180/50	毒蕈碱 M1（1.1 uM）、M2（2.5 uM）、M3（3.7 uM）
			Kappa 型阿片（6.1 uM）
			Ca++ch-L-型（2.5 nM）
			转运体：NE（1.9 uM）、DA（940 nM）、5-HT（3.2 uM）

[0696] 10mg/kg/天剂量的CB对σ和多巴胺转运体引起处在或高于Ki的脑部化合物水平，30mg/kg/天剂量则击中那些加上Ca++ch和5-HT转运体。接下来的研究可用来确定该化合物的最小有效剂量。剂量在30mg/kg/天的CF引起仅对σ受体有选择性的脑部化合物水平，因此其在σ受体处的亲和性解释了其在这些脑浓度下的行为效力。

[0697] 合成例1：通过还原胺化而合成化合物

[0698]



[0699] 将姜酮(5.00g，25.7mmol)溶于甲苯(250mL)并且添加4-三氟甲基苄基胺(4.73g，27.0mmol)。将混合物保持在氮氛下并在回流下加热16小时，通过Dean-Stark蒸馏去除水。这时，移除Dean-Stark阱并将反应混合物在冰浴上冷却到0℃。在大力搅拌下在30分钟内分批添加硼氢化钠(5g)于甲醇(100mL)中的溶液。当完成添加时，将混合物在回流下加热16小时。这时，将反应混合物冷却到室温并倒入饱和碳酸氢钠水溶液(300mL)。将所得混合物通过旋转蒸发浓缩并且水性残余物在水与氯仿之间分隔开。使氯仿层在无水硫酸钠上干燥，然后过滤和浓缩。然后使用硅胶柱层析采用5％氨-甲醇于氯仿中的溶液的流动相来纯化产物。将含有产物的部分合并和浓缩，然后在高真空下干燥过夜，以提供浅棕色油(6.72g，74％)。^{1}H NMR(500MHz，$CDCl_3$) δ：7.57(d，J = 7.8Hz，2H)，7.43(d，J = 7.9Hz，2H)，6.82(d，J = 7.3Hz，1H)，6.65(m，2H)，5.16-4.42(br s，2H)，3.90(d，J =

13.7Hz, 1H), 3.84(s, 3H), 3.80(d, J = 13.7Hz, 1H), 2.76-2.70(m, 1H), 2.67-2.55(m, 2H), 1.84-1.77(m, 1H), 1.69-1.63(m, 1H), 1.17(d, J = 6.3Hz, 3H). ^{13}CNMR(125MHz, $CDCl_3$) δ:146.7, 144.6, 143.9, 134.0, 129.1, 128.4, 127.5, 125.4, 125.3, 123.2, 120.8, 114.6, 111.0, 55.7, 52.1, 50.6, 38.8, 32.0, 20.1. MS(CI)m/z353(M^+)。

[0700] 经 ^{1}H NMR 的化学位移测量可能变化例如高至 0.2ppm。经 ^{13}HNMR 的化学位移测量可能变化例如高至 0.5ppm。分析质谱可能具有 +/-0.3 的实验误差。

[0701] 纯度确定

[0702] 通过 HPLC 测量产物的纯度。保留时间为 2.22 分钟的主峰表明纯度大于约 80%、85%、90%、或 95%。所使用的 HPLC 条件如下。

[0703] HPLC 条件：

[0704] 流动相 A：13.3mM 甲酸铵 /6.7mM 甲酸于水中的溶液

[0705] 流动相 B：6mM 甲酸铵 /3mM 甲酸于水中的溶液 /CH_3CN(1/9, v/v)

[0706] 柱：Synergi Fusion-RP100A Mercury, 2x20mm, 2.5 微米

[0707] (Phenomenex Part No00M-4423-B0_CE)

[0708] 梯度程序：RT = 2.22 分钟

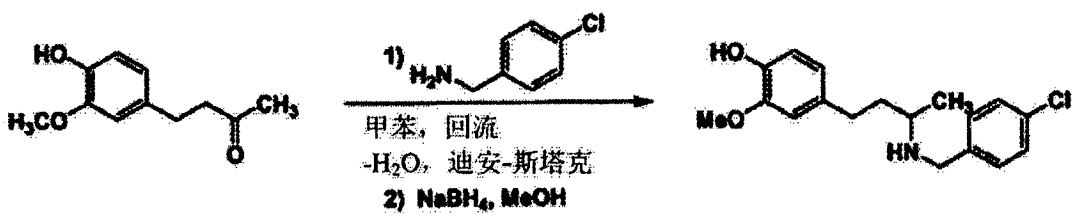
[0709]

时间，分钟	%相 B	流速，ml/min
0	100	0.5
1	100	0.5
2.5	40	0.5
3.4	40	0.5
3.5	100	0.5
4.5	100	0.5

[0710] 还通过 ^{1}H NMR 测量产物的纯度，表明其是纯度大于 90%或 95%的单一化合物。本文所述的合成可以根据待合成的终产物进行修改。

[0711] 合成例 2：通过还原胺化而合成化合物

[0712]



[0713] 将姜酮 (5.00g, 25.7mmol) 溶于甲苯 (250mL) 并添加 4-氯苄胺 (4.73g, 27.0mmol)。将混合物保持在氮氛下并在回流下加热 16 小时，通过 Dean-Stark 蒸馏去除水。这时，移除 Dean-Stark 阱并将反应混合物在冰浴上冷却到 0℃。在大力搅拌下在 30 分

钟内分批添加硼氢化钠 (5g) 于甲醇 (100mL) 中的溶液。当完成添加时，将混合物在回流下加热 16 小时。这时，将反应混合物冷却到室温并倒入饱和碳酸氢钠水溶液 (300mL)。将所得混合物通过旋转蒸发浓缩并且水性残余物在水与氯仿之间分隔开。使氯仿层在无水硫酸钠上干燥，然后过滤和浓缩。然后使用硅胶柱层析采用 5％氨 - 甲醇于氯仿中的溶液的流动相来纯化产物。将含有产物的部分合并和浓缩，然后在高真空下干燥过夜，以提供浅棕色油 (6.16g, 75％)。^{1}H NMR(500MHz, $CDCl_3$) δ :7.30-7.24(m, 4H), 6.81(d, J = 7.8Hz, 1H), 6.66-6.62(m, 2H), 4.25(br s, 2H), 3.82(s, 3H), 3.82(d, J = 13.2Hz, 1H), 3.72(d, J = 13.2Hz, 1H), 2.73(m, 1H), 2.66-2.51(m, 1H), 1.86-1.78(m, 1H), 1.72-1.63(m, 1H), 1.62-1.51(m, 1H), 1.17(d, J = 6.3Hz, 3H). ^{13}C NMR(125MHz, $CDCl_3$) δ :146.6, 143.8, 133.9132.8, 129.9, 129.7, 128.6, 120.8, 114.5, 110.9, 55.8, 51.9, 50.2, 38.5, 31.9, 31.6, 29.7, 26.9, 22.6, 19.9。MS(MH^+) :m/z320。

[0714] 经 ^{1}H NMR 的化学位移测量可能变化例如高至 0.2ppm。经 ^{13}HNMR 的化学位移测量可能变化例如高至 0.5ppm。分析质谱可能具有 +/-0.3 的实验误差。

[0715] 纯度确定

[0716] 通过 HPLC 测量产物的纯度。保留时间为 2.22 分钟的主峰表明纯度大于约 80％、85％、90％、或 95％。所使用的 HPLC 条件如下。

[0717] HPLC 条件：

[0718] 流动相 A :13.3mM 甲酸铵 /6.7mM 甲酸于水中的溶液

[0719] 流动相 B :6mM 甲酸铵 /3mM 甲酸于水中的溶液 /CH_3CN(1/9, v/v)

[0720] 柱 :Synergi Fusion-RP100A Mercury, 2x20mm, 2.5 微米

[0721] (Phenomenex Part No00M-4423-B0_CE)

[0722] 梯度程序 :RT = 2.22 分钟

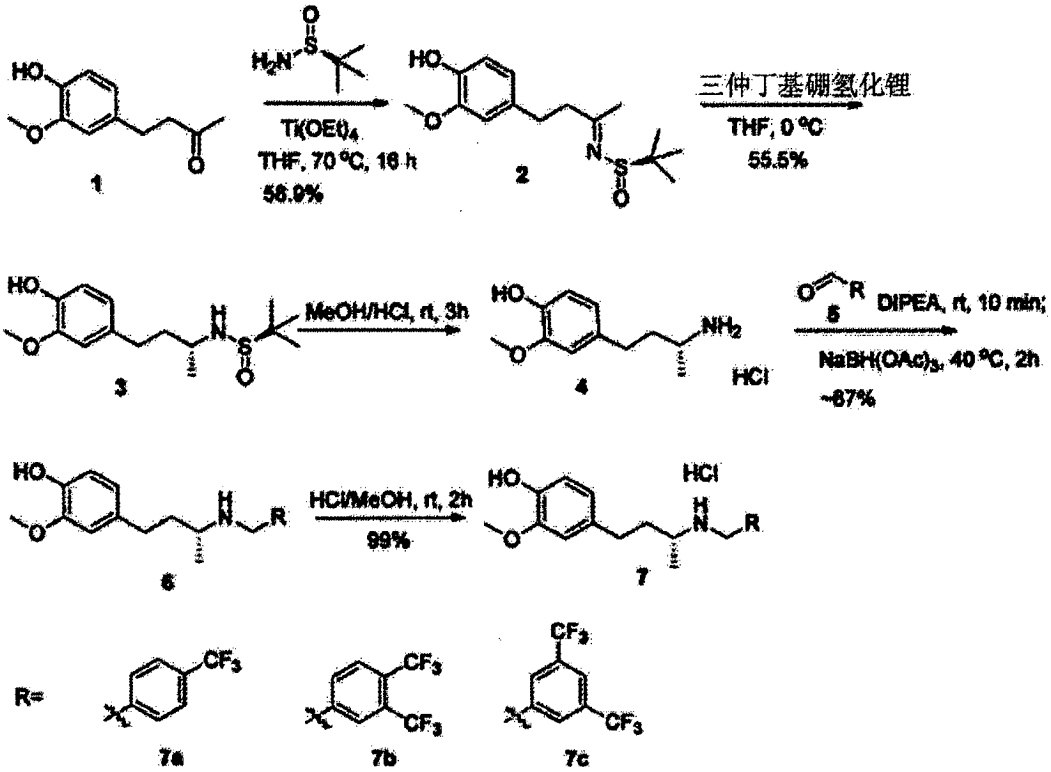
[0723]

时间，分钟	％相 B	流速，ml/min
0	100	0.5
1	100	0.5
2.5	40	0.5
3.4	40	0.5
3.5	100	0.5
4.5	100	0.5

[0724] 还通过 ^{1}H NMR 测量产物的纯度，表明其是纯度大于 90％或 95％的单一化合物。本文所述的合成可以根据待合成的终产物进行修改。

[0725] 合成例 3

[0726]



方案 1

[0727] 步骤 1：向 4-(4- 羟基 -3- 甲氧基 - 苯基)- 丁 -2- 酮 (38.8g,200mmol) 于 THF(600mL) 的溶液中添加 $Ti(OEt)_4$(136.9g,600mmol) 和 (S)-(-)- 叔丁基亚磺酰胺 (29g, 240mmol)。将混合物在 70℃搅拌 16h，通过冰水猝灭，用 EA(3x300mL) 萃取，在 Na_2SO_4 上干燥，浓缩以得到粗产物，其通过柱层析 (PE/EA:3/1) 纯化以得到标题化合物 2(35g,59％)。

[0728] 步骤 2：在 0℃向化合物 2(18g,60mmol) 于 THF(180mL) 的溶液中添加三仲丁基硼氢化锂 (L-selectride)(180mL，THF 中 1.0M,180mmol)。使反应在 3h 时间内温热至室温。经 TLC 对反应混合物进行分析显示起始亚胺 2 的完全消耗。然后通过添加水使溶液猝灭，并通过 EA(3x200mL) 萃取。将合并的有机层用盐水洗涤，在 Na_2SO_4 上干燥，并在真空下浓缩，以得到残余物，其通过柱层析 (PE/EA:2/1) 纯化以提供产物。继续用 PE/EA(1:1) 通过重结晶纯化产物以得到产物 3(9.9g,55％)。通过 HPLC 确定 ee 值。

[0729] 步骤 3：向 3(7.0g,23.4mmol) 于 MeOH(20mL) 的溶液中添加 HCl(MeOH 中 2M,20mL)，并将所得溶液在室温下搅拌 3h 时间。对反应混合物的 TLC 分析显示化合物 3 的完全消耗。然后在真空下去除溶剂，并将所得残余物 4 直接用于下一步骤。

[0730] 步骤 4：向粗化合物 4(5.4g,23.4mmol) 于 THF(100mL) 的溶液中添加 DIPEA(4.53g,35.1mmol) 和 4- 三氟甲基苯甲醛 5(4.28g,24.6mmol)。将所得溶液在室温搅拌 10min。然后，添加 $NaBH(OAc)_3$(14.9g,70.2mmol)，并将混合物在 40℃搅拌 2h。使混合物经 0℃水猝灭，过滤并通过 EtOAc 萃取。将有机层通过盐水洗涤，在硫酸钠上干燥，过滤，并在减压下浓缩滤液以提供残余物。将残余物通过柱层析 (PE/EA ＝ 1:2) 纯化以得到产物 6(7.0g,87％)。

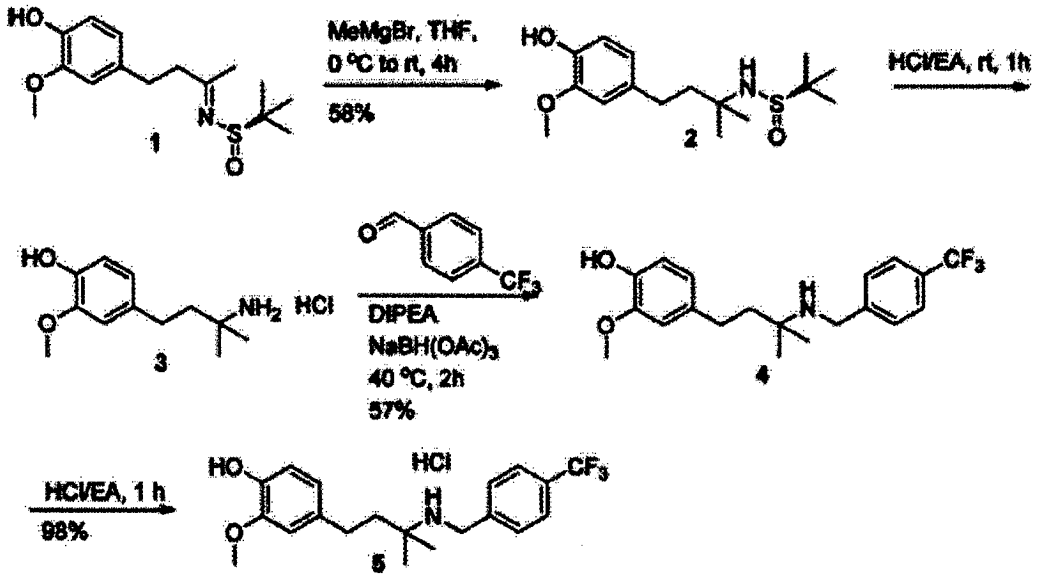
[0731] 步骤 5：向 6(1.0g,2.8mmol) 于 MeOH(5mL) 的溶液中添加 HCl(MeOH 中 2M,20mL)，然后将所得溶液在室温搅拌 30min。去除溶剂以得到产物 7a(1.1g,99％)，其为白色固体。

化合物 7b 和 7c 通过用合适的苯甲醛替换化合物 5 来相似地制备。

[0732] m/z(ESI+)(M+H)+:7a[354.2];7b[422.2];7c[422.2]。

[0733] 合成例 4

[0734]



[0735] 步骤 1:在 0℃向甲基溴化镁于 THF(5mL)的溶液中添加 1(1.0g,3.3mmol)于 THF(5mL)的溶液。将混合物在室温搅拌 4h,通过添加冰水猝灭,用乙酸乙酯(3x30mL)萃取,通过真空干燥以提供粗产物,其通过柱层析(PE/EA:3/1)纯化以得到化合物 2(0.6g,58%)。

[0736] 步骤 2:在 0℃向 2 于 EA(10mL)的溶液中添加 HCl(EA 中 2M,3mL),所得溶液在室温下搅拌 1 小时。经 TLC 对反应混合物的分析显示 2 被完全消耗。真空浓缩,将粗产物直接用于下一步骤。

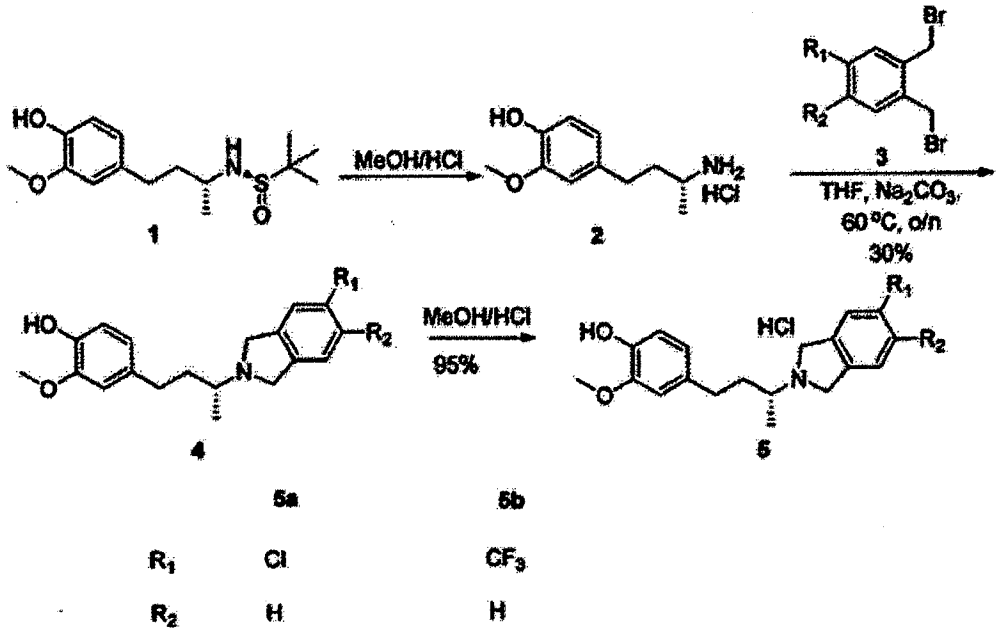
[0737] 步骤 3:随后向 3(0.4g,1.9mmol)于 THF(20mL)的溶液添加 DIPEA(0.6g,4.6mmol)和三氟甲基苯甲醛(0.4g,2.3mmol)。将所得溶液在室温搅拌 10min。添加三乙酰氧基硼氢化钠(1.63g,7.7mmol),并将混合物在 40℃搅拌 2h。使混合物经 0℃水猝灭,过滤,通过乙酸乙酯(3x40mL)萃取。将有机层用盐水洗涤,在硫酸钠上干燥,过滤,并在减压下浓缩滤液以提供残余物。将残余物通过柱层析(PE/EA = 1:1)纯化以得到 4(0.4g,57%)。

[0738] 步骤 4:向 4 于 EA(10mL)的溶液中添加 HCl(MeOH 中 2M,2mL),并将所得溶液在室温下搅拌 1h。在真空浓缩之后,将残余物用乙酸乙酯洗涤以提供 5(0.4g,98%)。

[0739] m/z(ESI+)(M+H)+:5[368.2]。

[0740] 合成例 5

[0741]



	5a	5b
R_1	Cl	CF_3
R_2	H	H

方案 5

[0742] 步骤 1 :向化合物 1(2g,6mmol) 于 MeOH(30mL) 的溶液添加 HCl(MeOH 中 2M,30mL),并将所得溶液在室温搅拌 3h。对反应混合物的 TLC 分析显示化合物 1 被完全消耗。然后在真空下去除溶剂,将其直接用于下一步骤。

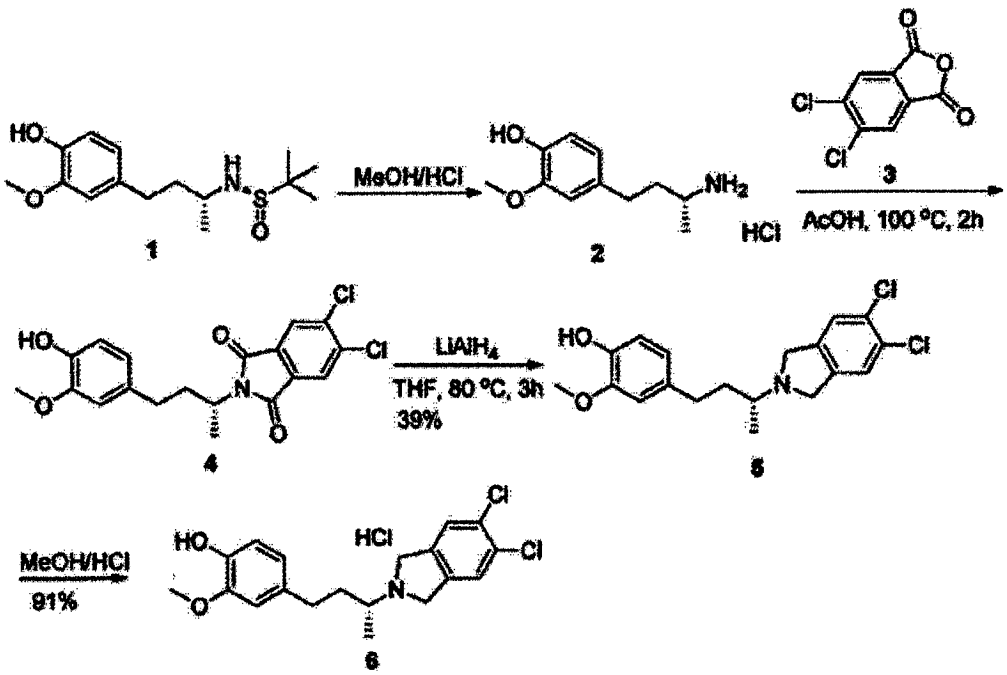
[0743] 步骤 2 :向 2(0.4g,2mmol) 于 THF(10mL) 的溶液添加化合物 3a(0.54g,2mmol) 于 THF(5mL) 的溶液。添加 Na_2CO_3(0.6g,6mmol),并将所得溶液在 60℃搅拌过夜。在浓缩之后,将残余物通过 FCC 纯化以得到化合物 4(0.2g,30%)。

[0744] 步骤 3 :向 4 于 EA(5mL) 的溶液添加 HCl(MeOH 中 2M,3mL),并将所得溶液在室温搅拌 1h。在真空内浓缩之后,将残余物通过乙酸乙酯洗涤以得到化合物 5(0.2g,95%)。化合物 5b 通过用合适的二苄基溴替换化合物 3 来相似地制备。

[0745] m/z(ESI+)(M+H)+ :5a[332.1] ;5b[366.1]。

[0746] 合成例 6

[0747]



[0748] 步骤 1 :向化合物 1(0.4g,1.3mmol) 于 MeOH(10mL) 的溶液中添加 HCl(MeOH 中 2M,10mL),并将所得溶液在室温搅拌 3h。对反应混合物的 TLC 分析显示化合物 1 被完全消耗。在真空中去除溶剂,并将其直接用于下一步骤。

[0749] 步骤 2 :将化合物 2(0.2g,1mmol) 和 3(0.2g,1mmol) 溶于乙酸 (10mL),在 100℃搅拌 2h。将混合物冷却到室温,经水 (10mL) 猝灭,通过 EtOAc(3x20mL) 萃取,干燥,浓缩,以得到标题化合物 4(0.3g,76%)。

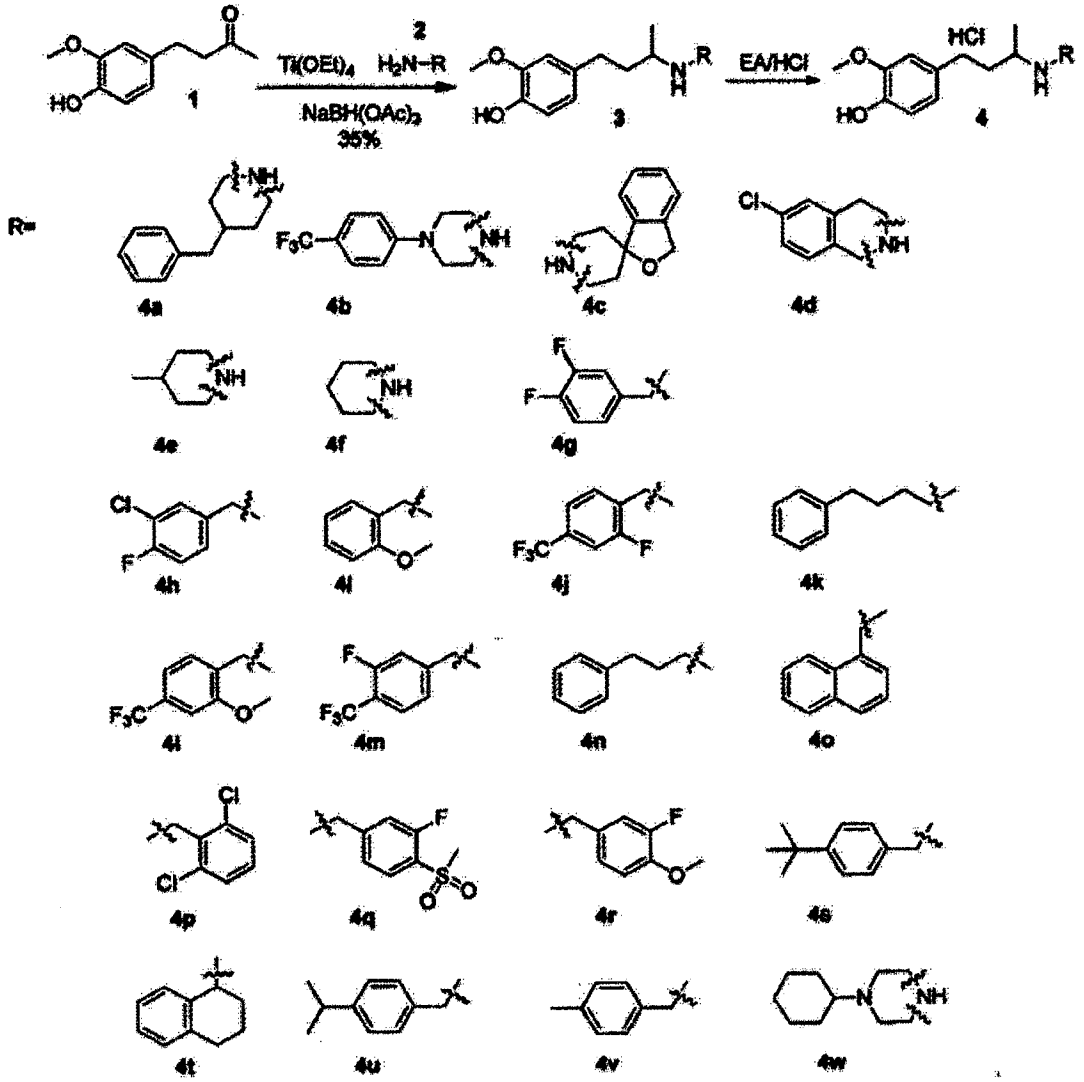
[0750] 步骤 3 :向 4(0.3g,0.7mmol) 于 THF(10mL) 的溶液添加 LAH(0.1g,3.5mmol)。将混合物在 80℃搅拌 3h。使混合物经水 (0.1mL)、15% NaOH(0.1mL) 和水 (0.3mL) 猝灭,过滤,浓缩。将粗产物通过柱层析 (PE/EA = 5:1) 纯化以得到化合物 5(0.1g,39%)。

[0751] 步骤 4 :向 5 于乙酸乙酯 (5mL) 的溶液添加 HCl(MeOH 中 2M,3mL) 并将所得溶液在室温搅拌 1h。将反应通过真空浓缩以提供标题化合物 6(0.16g,91%)。

[0752] m/z(ESI+) (M+H)+ :6[366.2]。

[0753] 合成例 7

[0754]



方案 7

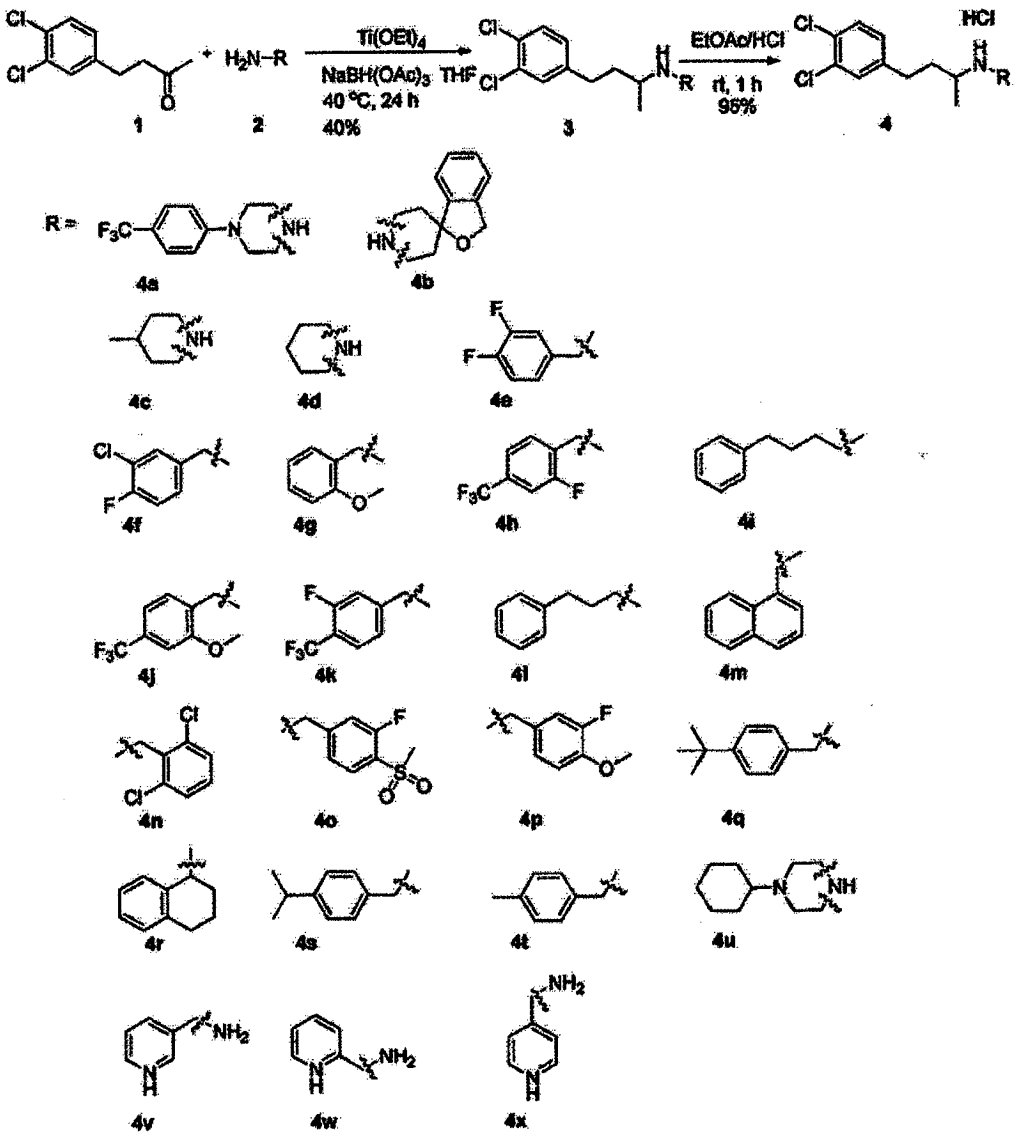
[0755] 步骤 1：向 1(0.278g,1.43mmol) 于 THF(20mL) 的溶液添加 $Ti(OEt)_4$(2.1g,9.2mmol) 和 4- 苄基哌啶 (0.34g,1.3mmol)。将混合物在 40℃搅拌一天，经冰水猝灭，用乙酸乙酯 (3x20mL) 萃取。在真空内浓缩之后，将粗产物通过柱层析 (PE/EA:1/1) 纯化以得到 3(205mg,35％)。

[0756] 步骤 2：向 3(0.2g,0.47mmol) 于乙酸乙酯 (5mL) 的溶液添加 HCl(MeOH 中 2M,3mL) 并将所得溶液在室温搅拌 1h。将反应通过真空浓缩以得到 4a(0.2g,95％)。化合物 4b-4w 通过用合适的胺替换胺化合物 2 来相似地制备。

[0757] m/z(ESI+)(M+H)+：4a[354.3]；4b[409]；4c[368.2]；4d[346.1]；4e[278.50]；4f[264.05]；4g[322.10]；4h[338.05]；4i[316.15]；4j[372.10]；4k[328.25]；4l[384.15]；4m[372.10]；4n[314.10]；4o[336.15]；4p[354.10]；4q[382.20]；4r[334.15]；4s[342.15]；4t[326.15]；4u[328.20]；4v[300.10]；4w[347.6]。

[0758] 合成例 8

[0759]



方案 8

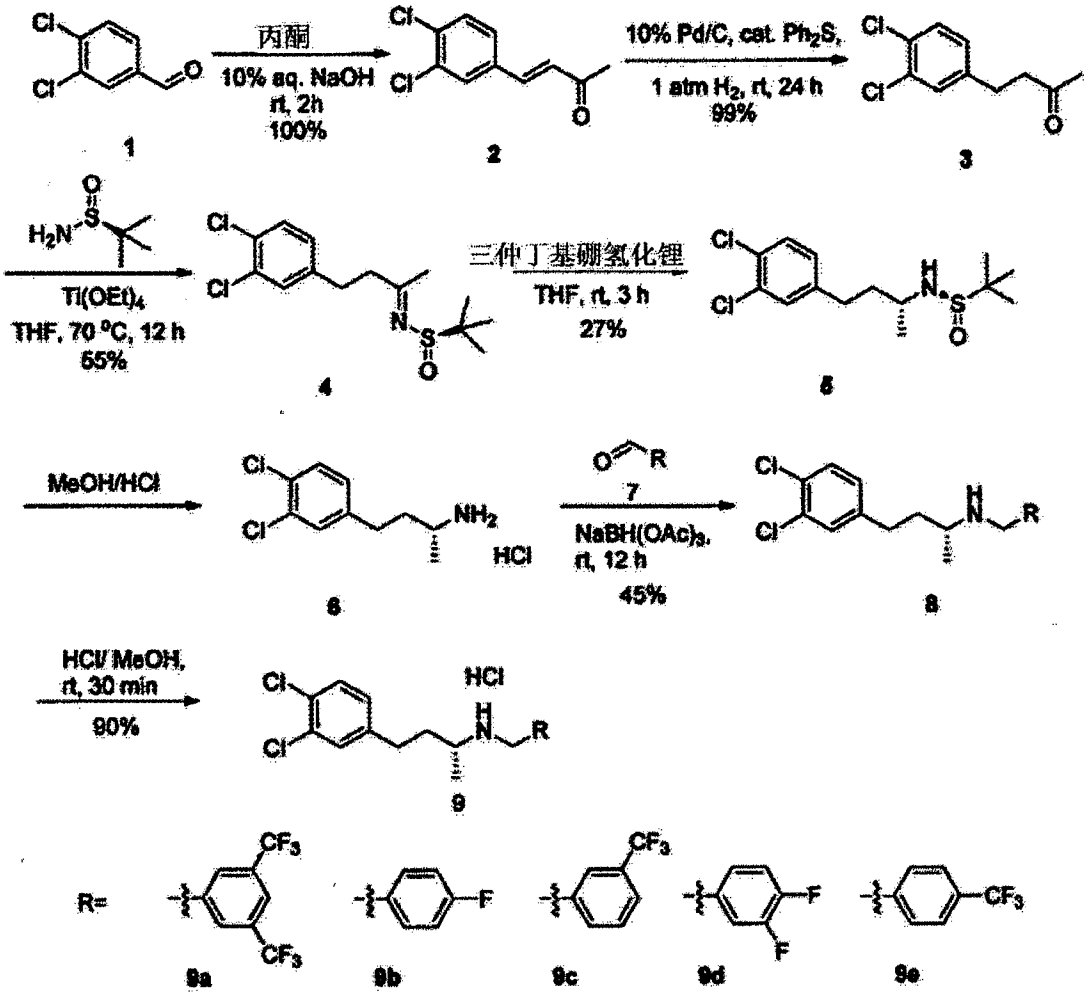
[0760] 步骤 1：向 1(0.31g,1.43mmol) 于 THF(20mL) 的溶液添加 $Ti(OEt)_4$(0.595g, 2.58mmol) 和 N-(4- 三氟甲基苯基)- 哌嗪 2(0.3g,1.3mmol)。将混合物在 40℃搅拌 24h，通过添加冰水猝灭，用乙酸乙酯 (3x20mL) 萃取，干燥。通过柱层析 (PE/EA:1/1) 的纯化得到产物 3(0.25g,41%)。

[0761] 步骤 2：向化合物 3(0.25g,0.58mmol) 于乙酸乙酯 (5mL) 的溶液添加 MeOH-HCl(2N,4mL)。将混合物在室温搅拌 1 h。真空内的浓缩得到化合物 4(0.25g,95%)。化合物 4b-4x 通过用合适的胺替换胺化合物 2 来相似地制备。

[0762] m/z(ESI+)(M+H)+ :4a[431.2] ;4b[390.2] ;4c[300.05] ;4d[286.00] ;4e[344.05] ;4f[362.00] ;4g[338.05] ;4h[394.10] ;4i[350.05] ;4j[406.05] ;4k[394.15] ;4l[336.05] ;4m[358.05] ;4n[378.05] ;4o[445.20] ;4p[356.10] ;4q[364.10] ;4r[348.05] ;4s[350.10] ;4t[322.10] ;4u[369.2] ;4v[309.00] ;4w[308.95] ;4x[309.00]。

[0763] 合成例 9

[0764]



方案 9

[0765] 步骤 1：向 1(3.5g,20mmol) 于丙酮 (20mL) 和乙醇 (2mL) 的溶液添加 NaOH 水溶液 (10%,15mL) 和水 (80mL)。将混合物在室温搅拌 2h,用 EA(3x50mL) 萃取。将有机层干燥并浓缩以得到 2(4.3g,100%)。

[0766] 步骤 2：向 2(4.3g,20mmol) 于 MeOH(50mL) 的溶液添加二苯硫醚 (0.15mL) 和 Pd/C(10%,0.43g)。将混合物在 25℃在 1atm 氢下大力搅拌 24h。将反应混合物经硅藻土 (Celite) 垫过滤,用甲醇洗涤,并将滤液浓缩以提供 3(4.3g,99%)。

[0767] 步骤 3：向 3(10g,46mmol) 于 THF(100mL) 的溶液添加 $Ti(OEt)_4$(21g,92mmol) 和 (S)-(-)-叔丁基亚磺酰胺 (6.1g,50mmol)。将混合物在 70℃搅拌 12h,经冰水猝灭,用乙酸乙酯 (3x250mL) 萃取。在真空浓缩之后,将粗产物通过柱层析 (PE/EA:10/1) 纯化以提供化合物 4(8.1g,55%)。

[0768] 步骤 4：在 0℃向化合物 4(3.3g,10mmol) 于 THF(30mL) 的溶液添加三仲丁基硼氢化锂 (33mL,THF 中 1.0M,33mmol)。使反应在 3h 时间内温热至室温。经 TLC 对反应混合物的分析显示起始亚胺 4 被完全消耗。将溶液通过水猝灭,并通过乙酸乙酯 (3x30mL) 萃取。将合并的有机层用盐水洗涤,在 Na_2SO_4 上干燥,并在真空下浓缩以得到残余物,其通过柱层

析 (PE/EA:2/1) 纯化以提供产物 5(0.9g,27%)。

[0769] 步骤 5:向化合物 5(5g,15.5mmol) 于 MeOH(10mL) 的溶液添加 HCl(MeOH 中 2M,10mL) 并将所得溶液在室温搅拌 3h。反应混合物的 TLC 分析显示化合物 5 被完全消耗。在真空中去除溶剂,并将粗 6(3.95g,100%) 直接用于下一步骤而不经进一步纯化。

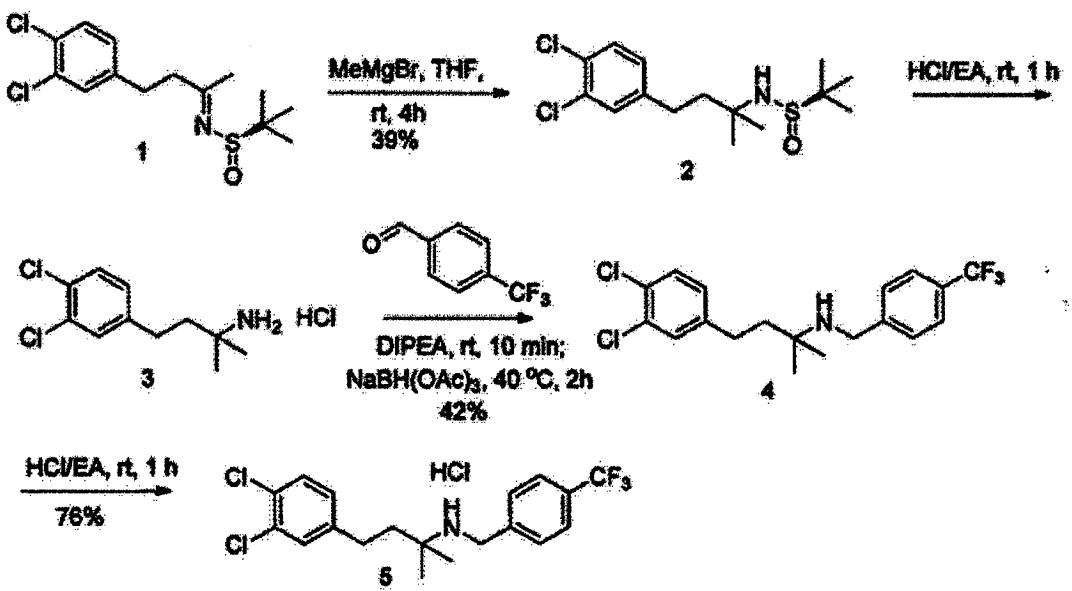
[0770] 步骤 6:随后向 6(0.6g,2.4mmol) 于 THF(10mL) 的溶液添加 DIPEA(0.4g,3.1mmol) 和 3-三氟甲基苯甲醛 (0.41g,2.4mmol)。将所得溶液在室温搅拌 10min。添加 $NaBH(OAc)_3$(1.0g,4.7mmol) 并将混合物搅拌 12h。将混合物经 0℃水猝灭,过滤并通过 EtOAc(3x30mL) 萃取。将有机层用盐水洗涤,在硫酸钠上干燥,过滤,并在减压下浓缩滤液以提供残余物。将残余物通过柱层析 (PE/EA = 1:1) 纯化以得到化合物 8(0.4g,45%)。

[0771] 步骤 7:向 8(0.4g,1.08mmol) 于 MeOH(5mL) 的溶液添加 HCl(MeOH 中 2M,4mL) 并将所得溶液在室温搅拌 0.5h。将反应浓缩以得到胺 9a(0.4g,90%)。化合物 9b-9e 通过用合适的苯甲醛替换化合物 7 来相似地制备。

[0772] m/z(ESI+)(M+H)+:9a[444.2];9b[326.25];9c[376.2];9d[344.2];9e[376.1]。

[0773] 合成例 10

[0774]



方案 10

[0775] 步骤 1:在 0℃向甲基溴化镁于 THF(3M,15mL) 的溶液添加 1(1.5g,4.6mmol) 于 THF(20mL) 的溶液。将混合物在室温搅拌 4h,经冰水猝灭,用乙酸乙酯 (3x30mL) 萃取。在浓缩之后,将粗产物通过柱层析 (PE/EA:3/1) 纯化以提供化合物 2(0.6g,39%)。

[0776] 步骤 2:向化合物 2(0.6g,1.8mmol) 于乙酸乙酯 (10mL) 的溶液添加 HCl(MeOH 中 2M,3mL) 并将所得溶液在室温搅拌 1h。对反应混合物的 TLC 分析显示出化合物 2 被完全消耗。然后在真空中去除溶剂,并将粗化合物 3 直接用于下一步骤。

[0777] 步骤 3:向 3(0.43g,1.8mmol) 于 THF(20mL) 的溶液相继添加 DIPEA(0.54g,4.0mmol) 和 4-三氟甲基苯甲醛 (0.36g,2.0mmol)。将所得溶液在室温搅拌 10min。添加 $NaBH(OAc)_3$(1.57g,7.4mmol) 并将混合物在 40℃搅拌 2h。将混合物经 0℃水猝灭,过滤,通过 EtOAc(3x30mL) 萃取。将有机层通过盐水洗涤,在硫酸钠上干燥,过滤,并将滤液在减压

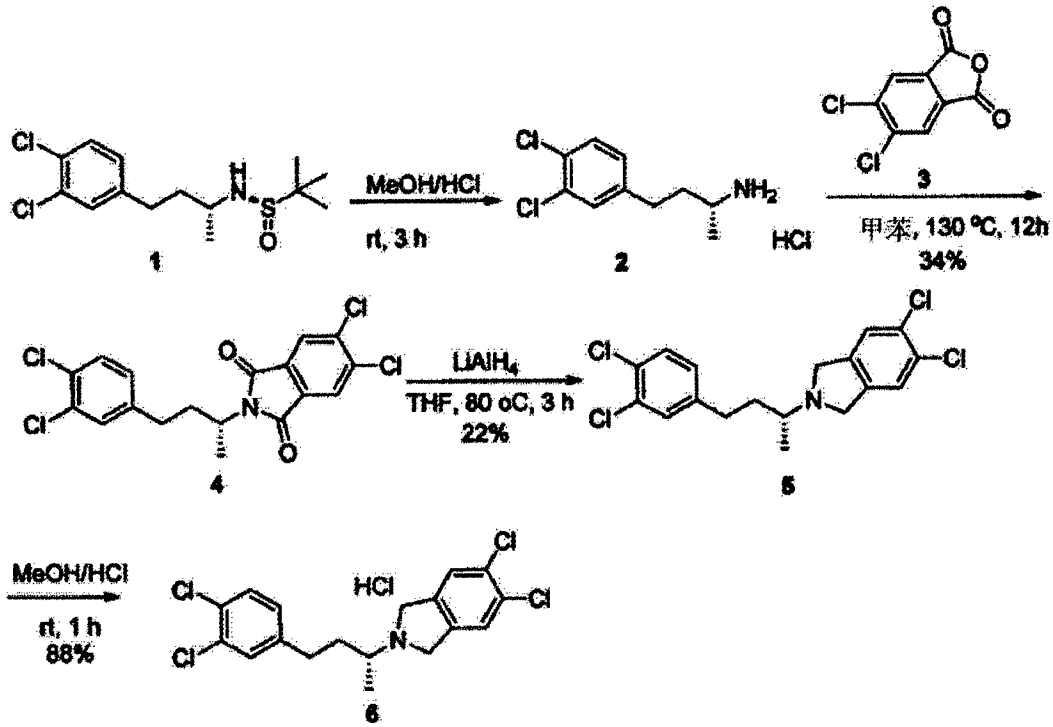
下浓缩以提供残余物，其通过柱层析 (PE/EA = 3:1) 纯化以得到化合物 4(0.3g，43%)。

[0778] 步骤 4：向 4(0.3g，0.8mmol) 于乙酸乙酯 (10mL) 的溶液添加 HCl(MeOH 中 2M，2mL) 并将所得溶液在室温搅拌 1h。将析出物过滤，得到化合物 5(0.25g，76%)。

[0779] m/z(ESI+) (M+H)+：5[390.14]。

[0780] 合成例 11

[0781]



方案 11

[0782] 步骤 1：将化合物 1(1.75g，5.43mmol) 于 MeOH-HCl(2M，10mL) 的混合物在室温搅拌 3h。将反应混合物在真空内浓缩，得到粗 2，其用于下一步骤而不经进一步纯化。

[0783] 步骤 2：将化合物胺 2(1.4g，5.43mmol) 和酐 3(1.18g，5.43mmol) 于甲苯 (12mL) 中的溶液在 130℃加热 12h。

[0784] 将混合物冷却到室温并添加水 (10mL)，通过 EtOAc(3x20mL) 萃取，干燥，浓缩。将粗产物通过柱层析 (PE/EA = 10:1) 纯化得到产物 4(0.78g，34%)。

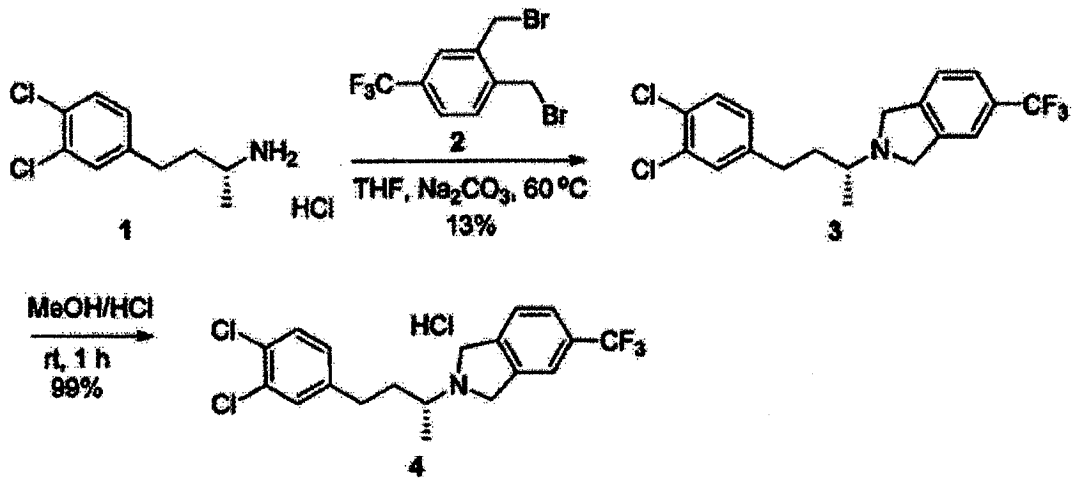
[0785] 步骤 3：向化合物 4(0.78g，1.87mmol) 于 THF(20mL) 中的溶液中添加 LAH(0.36g，9.1mmol)。将混合物在 80℃搅拌 3h。将冷却的混合物通过水 (3.46mL)、15% NaOH(3.46mL) 和水 (13.5mL) 猝灭。将反应混合物过滤，浓缩。将粗产物通过柱层析 (PE/EA = 5:1) 纯化得到产物 5(0.16g，23%)。

[0786] 步骤 4：将化合物 5(0.3g，0.8mmol) 溶于乙酸乙酯 (5mL)，添加 MeOH-HCl(2N，3mL)。将混合物在室温搅拌 1 h，浓缩以得到化合物 6(0.16g，89%)。

[0787] m/z(ESI+) (M+H)+：6[390.0]。

[0788] 合成例 12

[0789]



方案 12

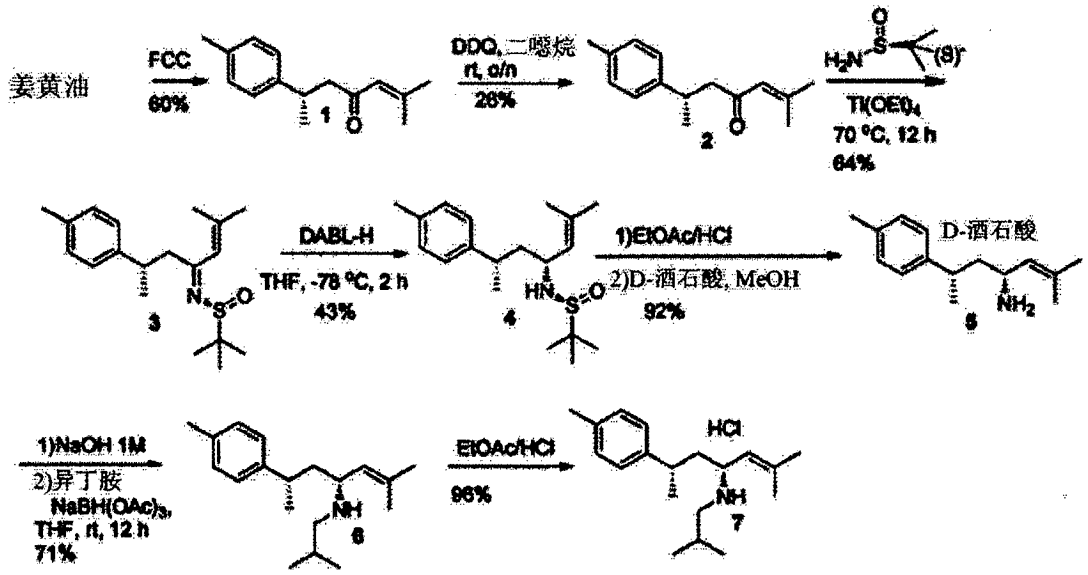
[0790] 步骤 1：向化合物 1(0.4g,2mmol) 于 DMF(6mL) 中的溶液中添加二溴化物 2(0.6g,2mmol)。将所得溶液在 80℃搅拌过夜，浓缩，通过制备型 HPLC 纯化，得到化合物 3(0.1g,13%)。

[0791] 步骤 2：将化合物 3(0.1g,0.2mmol) 溶于乙酸乙酯 (5mL)，添加 MeOH-HCl(2N,3mL)。将混合物在室温搅拌 1 h。将混合物过滤以得到化合物 4(0.11g,99%)。

[0792] m/z(ESI+)(M+H)+：4[388.1]。

[0793] 合成例 13

[0794]



方案 13

[0795] 步骤 1：将姜黄油 (100g) 通过柱层析 (PE:EA/100:1) 纯化以提供粗产物 1(60g)。

[0796] 步骤 2：将粗产物 1(60g) 溶于二噁烷 (200mL)，添加 DDQ(81.7g,360mmol)。将混合物在室温搅拌过夜，然后经水 (500mL) 猝灭，通过硅藻土垫过滤。将滤液通过乙酸乙酯 (3x200mL) 萃取。将有机层干燥，浓缩并通过柱层析 (PE:EA/30:1) 纯化以提供化合物 2(15.6g,26%)。

[0797] 步骤 3：向 2(7.4g,34.2mmol) 于 $Ti(OEt)_4$(23.4g,102.6mmol) 的溶液中添加 (S)-(-)-2- 甲基 -2- 丙亚磺酰胺。将混合物在 70℃搅拌 12h，经冰水猝灭，用乙酸乙酯 (3x100mL) 萃取，并干燥以得到残余物，其通过柱层析 (PE/EA:5/1) 纯化得到产物 3(7.1g，64％)。

[0798] 步骤 4：在 -78℃向化合物 3(7.0g,21.9mmol) 于 THF(70mL) 的溶液中添加 DIBAL-H(22mL，THF 中 1.5M,33mmol)。将所得溶液在 -78℃搅拌 2h。经 TLC 对反应混合物的分析显示出起始亚胺的完全消耗，得到亚磺酰胺化合物 4。将溶液经水猝灭，并通过乙酸乙酯 (3x200mL) 萃取。将合并的有机层用盐水洗涤，在 Na_2SO_4 上干燥，并浓缩以提供橙色油。使粗产物进行柱层析 (PE:EA/3:1) 以提供产物 4(3.1g,43％)。

[0799] 步骤 5：向化合物 4(1.6g,5.0mmol) 于乙酸乙酯 (10mL) 的溶液中添加 HCl- 乙酸乙酯 (2N,10mL)，并将所得溶液在室温搅拌 3h。对反应混合物的 TLC 分析显示出化合物 3 被完全消耗。在真空中去除溶剂。将残余物溶于水 (10mL)，并将 pH 通过 K_2CO_3 的饱和水溶液调整为 9-10，通过乙酸乙酯 (3x20mL) 萃取，干燥，并浓缩以得到游离胺。将游离胺 (1.1g，5.0mmol) 溶于甲醇 (15mL)。将 D- 酒石酸 (0.75g,5.0mmol) 添加到溶液。将混合物在回流下搅拌 1 h。将溶液缓慢冷却到室温。将所形成的结晶过滤，得到产物 5(1.7g,93％)。Mp. 172-174℃。通过 X- 射线结晶学确定化合物 5 的绝对立体化学。

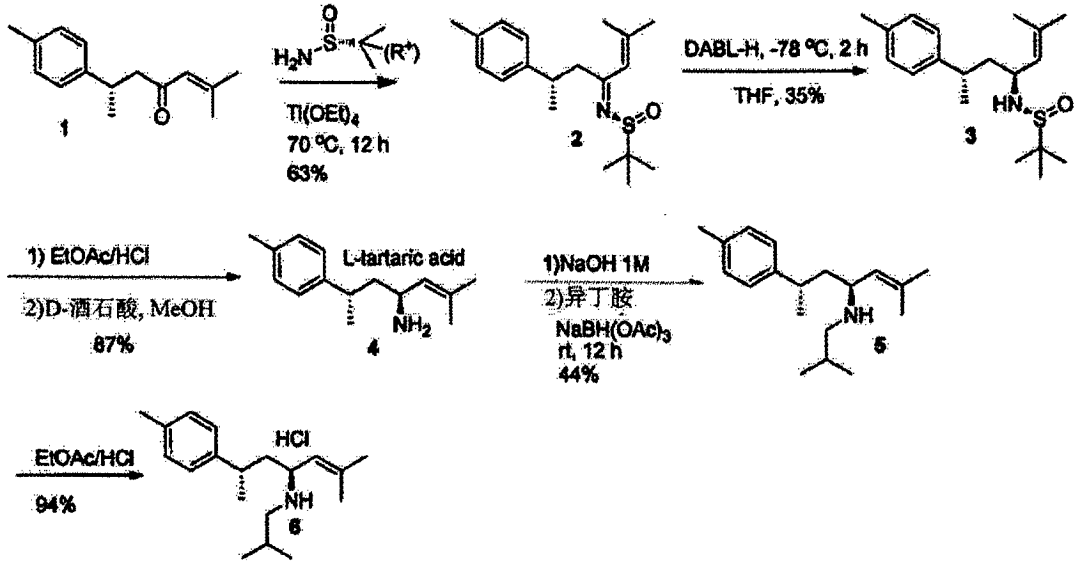
[0800] 步骤 6：通过 1M NaOH 将化合物 5(1.7g,4.6mmol) 于水 (20mL) 中的溶液调节为 pH9-10。将产物用乙酸乙酯 (3x20mL) 萃取。将有机层干燥，浓缩，以得到游离胺。将游离胺溶于 THF(10mL) 中，添加异丁胺 (0.40g,5.5mmol) 和 $NaBH(OAc)_3$(3.90g,18.4mmol)。将混合物在室温搅拌 12h，用水猝灭，通过乙酸乙酯 (3x30mL) 萃取。将有机层干燥，浓缩，并通过柱层析 (PE:EA/3:1) 纯化以提供产物 6(0.9g,72％)。

[0801] 步骤 7：向化合物 6(0.9g,3.2mmol) 于乙酸乙酯 (10mL) 的溶液中添加乙酸乙酯 -HCl(2N,5mL)。将混合物在室温搅拌 1h。在真空中去除乙酸乙酯，得到化合物 7(0.95g，96％)。

[0802] m/z(ESI+)(M+H)+:7[274.20]。

[0803] 合成例 14

[0804]



方案 14

[0805] 步骤 1：向 1(7.4g,34.2mmol) 于 $Ti(OEt)_4$(23.4g,102.6mmol) 的溶液中添加 (R)-(+)-2- 甲基 -2- 丙亚磺酰胺。将混合物在 70℃搅拌 12h，经冰水猝灭，用乙酸乙酯 (3x100mL) 萃取，干燥。通过柱层析 (PE/EA:5/1) 纯化，得到产物 2(6.9g,63%)。

[0806] 步骤 2：将化合物 2(7.0g,21.9mmol) 溶于 THF(70mL) 并冷却到 -78℃。然后向容器中添加 DIBAL-H(22mL，THF 中 1.5M,33mmol)，并将所得溶液在 -78℃搅拌 2h。经 TLC 对反应混合物的分析显示出起始亚胺的完全消耗，得到亚磺酰胺化合物 3。然后将溶液经水猝灭并通过乙酸乙酯 (3x200mL) 萃取。将合并的有机层用盐水洗涤，在 Na_2SO_4 上干燥，并在真空下浓缩以提供橙色油。使粗产物进行柱层析 (PE:EA/3:1) 以提供产物 3(2.5g,36%)。

[0807] 步骤 3：向 3(2.5g,7.8mmol) 于乙酸乙酯 (10mL) 的溶液中添加 2M HCl 于乙酸乙酯 (10mL) 中的溶液，并将所得溶液在室温搅拌 3h。对反应混合物的 TLC 分析显示出化合物 3 被完全消耗。真空去除溶剂。将残余物溶于水 (10mL) 中，通过添加饱和 K_2CO_3 将其 pH 调节为 9-10。将混合物通过乙酸乙酯 (3x20mL) 萃取，干燥，浓缩，以得到游离胺 4。将游离胺 4 溶于甲醇 (15mL)，添加 L- 酒石酸 (1.17g,7.8mmol)。将混合物在回流下搅拌 1h，冷却到室温，过滤以得到结晶盐 4(2.5g,87%)。

[0808] 步骤 4：将 L- 酒石酸盐 4(2.5g,6.8mmol) 溶于水 (20mL)，通过添加 1M NaOH 将其 pH 调节为 9-10。然后将混合物通过乙酸乙酯 (3x50mL) 萃取，干燥，浓缩以得到游离胺 4。将游离胺 4 再溶解于 THF，添加异丁胺 (0.60g,8.2mmol) 和 $NaBH(OAc)_3$(5.85g,27.6mmol)。将混合物在室温搅拌 12h，经水猝灭，通过乙酸乙酯 (3x30mL) 萃取。将有机层干燥，浓缩，并通过柱层析 (PE:EA/3:1) 纯化以提供产物 5(0.83g,45%)。

[0809] 步骤 5：向 5(0.83g,3.0mmol) 于乙酸乙酯 (10mL) 的溶液中添加 HCl(乙酸乙酯中 2M,5mL)，并将所得溶液在室温搅拌 1h。去除溶剂以得到产物 6(0.88g,94%)。

[0810] m/z(ESI+) (M+H)+ :6[274.20]

[0811] 所有在说明书（包括摘要和附图）中公开的特征、以及所有在任意所公开方法或工序中的步骤可以以任意组合进行结合，除其中至少一些特征和 / 或步骤相互排斥的组合之外。在说明书（包括摘要和附图）中公开的各个特征可以用提供相同、等同或相似目的

的可替代特征进行替换，除非明确指出相反情况。因此，除非明确指出相反情况，所公开的各个特征仅是通用系列的等同或相似特征的一个实例。基于上述说明，除本文记载的那些外的本发明的多种修改对于本领域技术人员将是明显的。这些修改也意在落入所附权利要求的范围内。

[0812] 所有本文提及的公开物全部并入本文以作参考。本文中没有事物构成对本发明由于先前发明而没有早于这些公开的资格的承认。

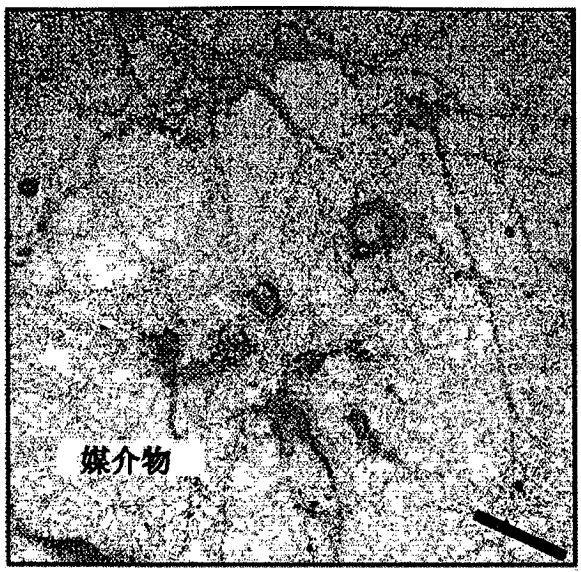


图 1A

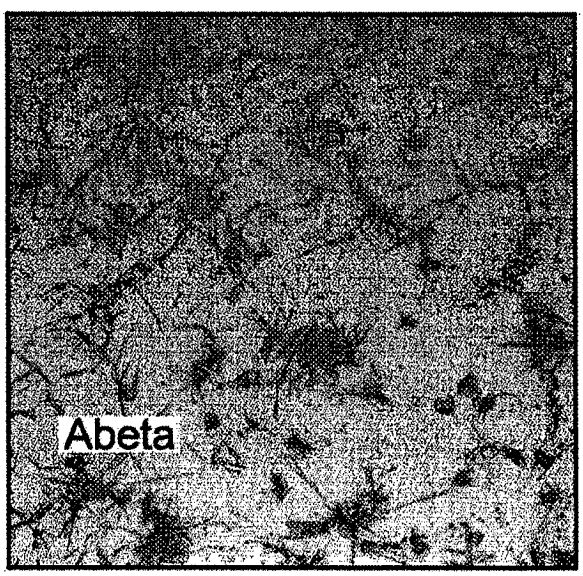


图 1B

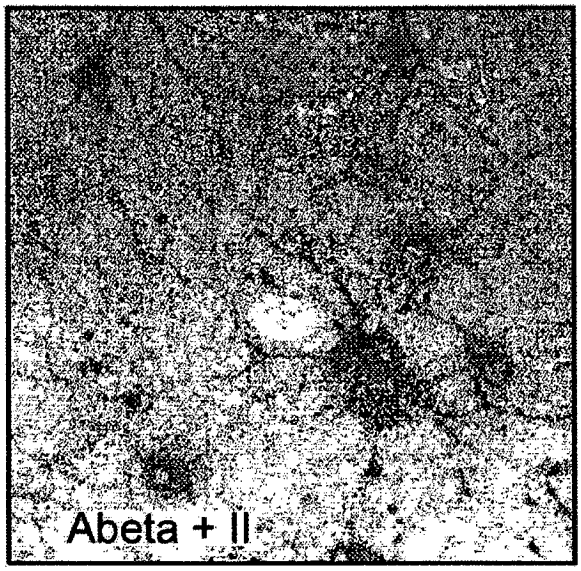

Abeta + II

图 1C

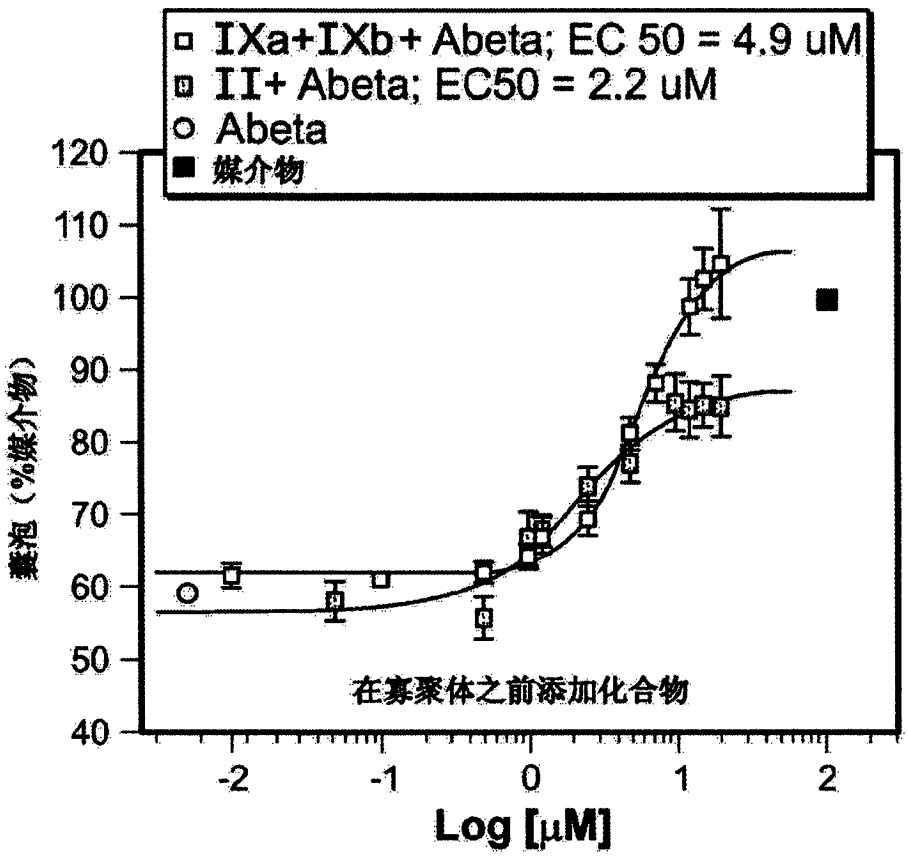

IXa+IXb+ Abeta; EC 50 = 4.9 uM
II+ Abeta; EC50 = 2.2 uM
Abeta
媒介物
120
110
100
90
80
70
60
50
40
囊泡（%媒介物）
在寡聚体之前添加化合物
-2
-1
0
1
2
Log [μM]

图 1D

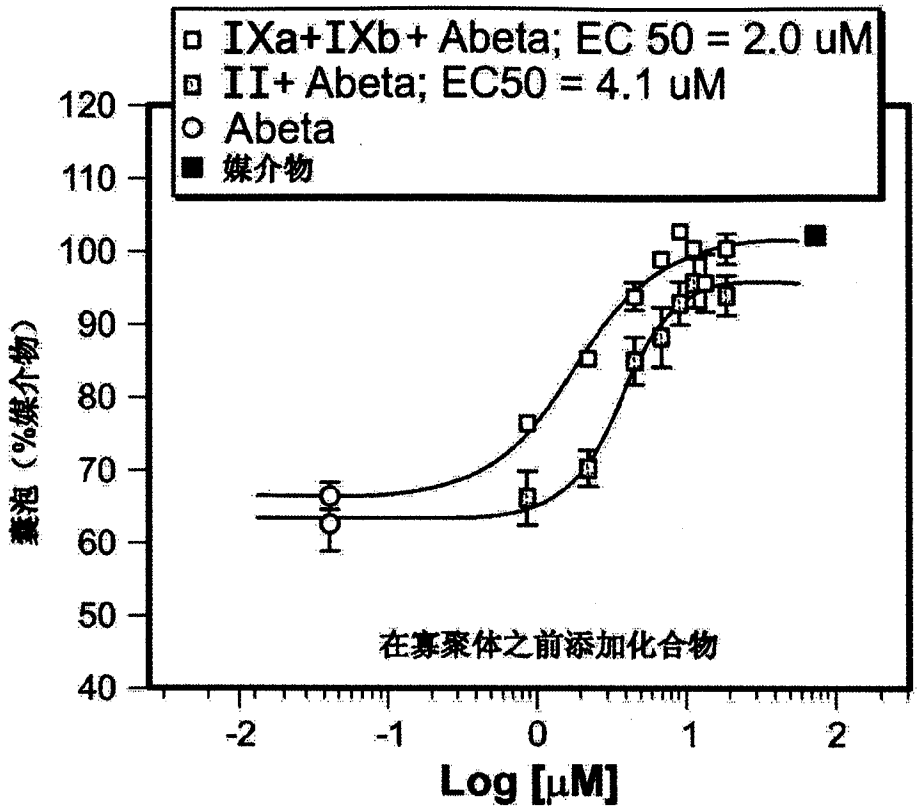

IXa+IXb+ Abeta; EC 50 = 2.0 uM
II+ Abeta; EC50 = 4.1 uM
Abeta
媒介物
120
110
100
90
80
70
60
50
40
囊泡（%媒介物）
在寡聚体之前添加化合物
-2
-1
0
1
2
Log [μM]

图 1E

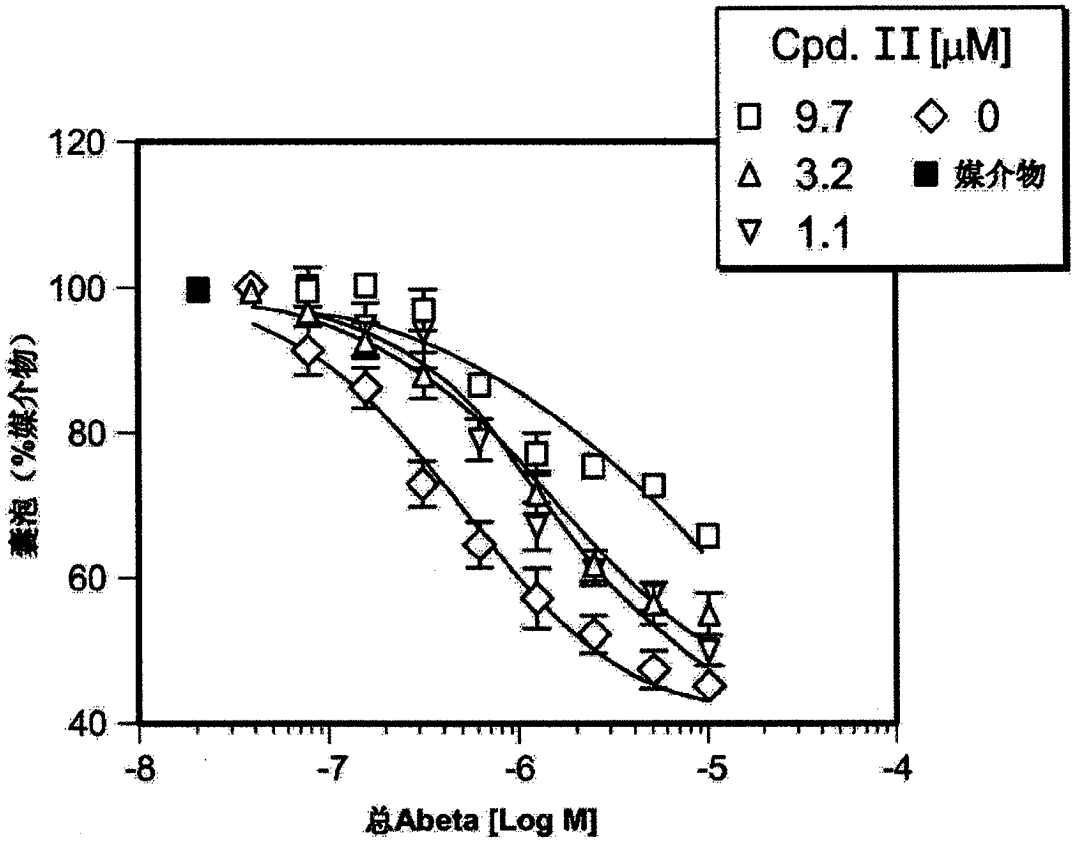


图 1F

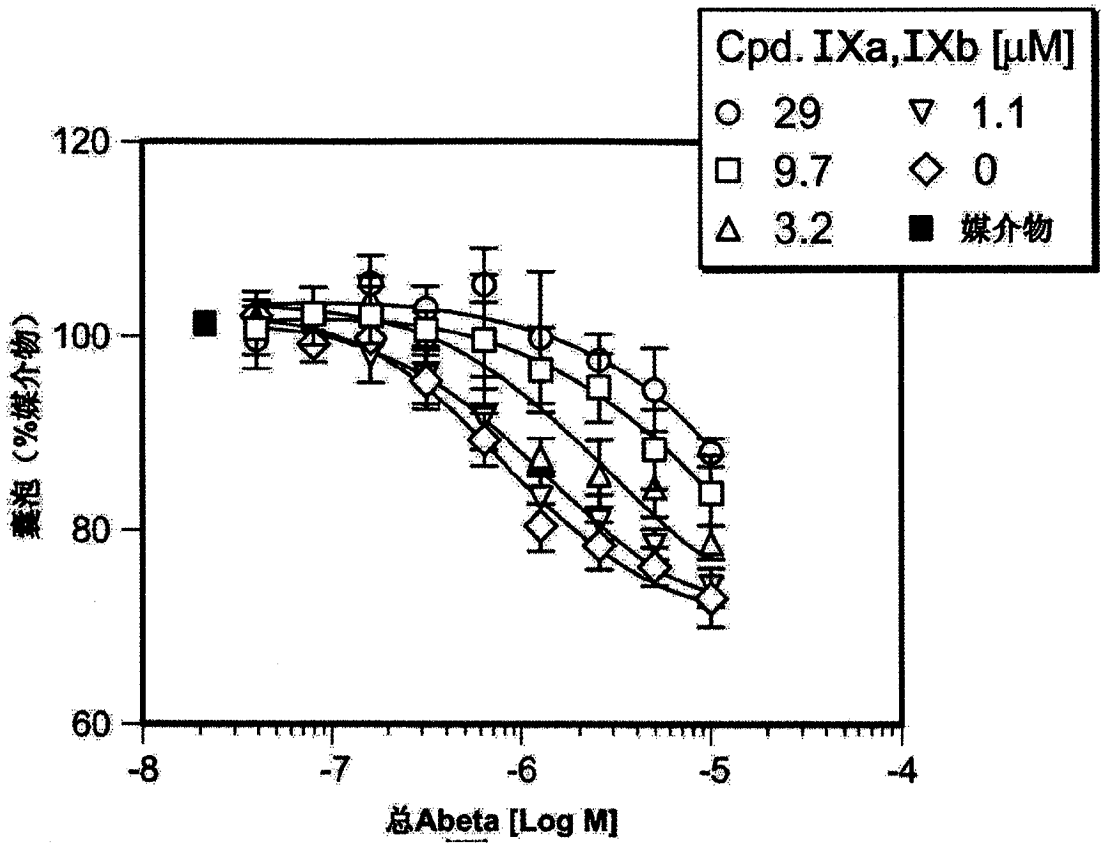

Cpd. IXa,IXb [μM]
29
1.1
9.7
0
3.2
媒介物
120
100
80
60
囊泡（%媒介物）
-8
-7
-6
-5
-4
总Abeta [Log M]

图 1G

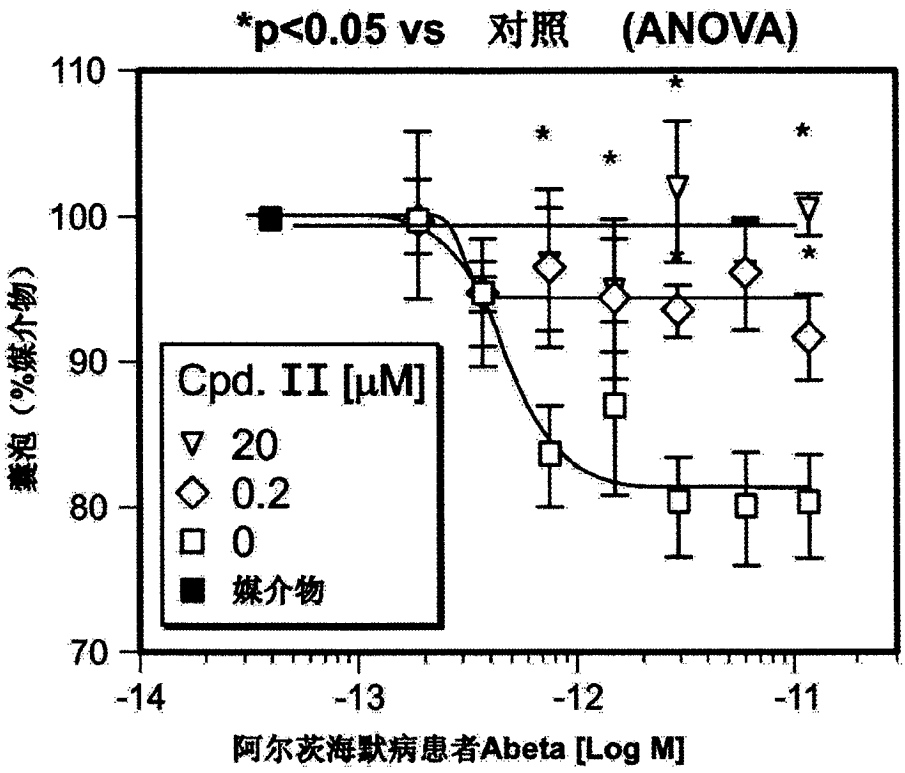

*p<0.05 vs 对照 (ANOVA)
110
100
90
80
70
-14
-13
-12
-11
囊泡（%媒介物）
阿尔茨海默病患者Abeta [Log M]
Cpd. II [μM]
20
0.2
0
媒介物

图 1H

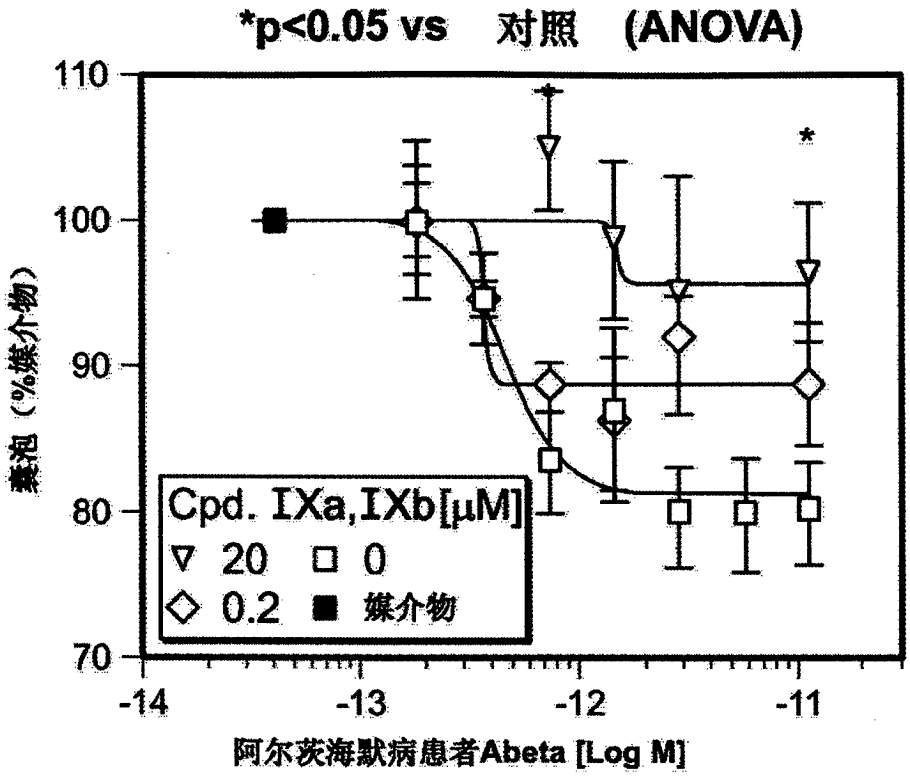

*p<0.05 vs 对照 (ANOVA)
110
100
90
80
70
-14
-13
-12
-11
*
Cpd. IXa,IXb[μM]
20
0
0.2
媒介物
囊泡（%媒介物）
阿尔茨海默病患者Abeta [Log M]

图 1I

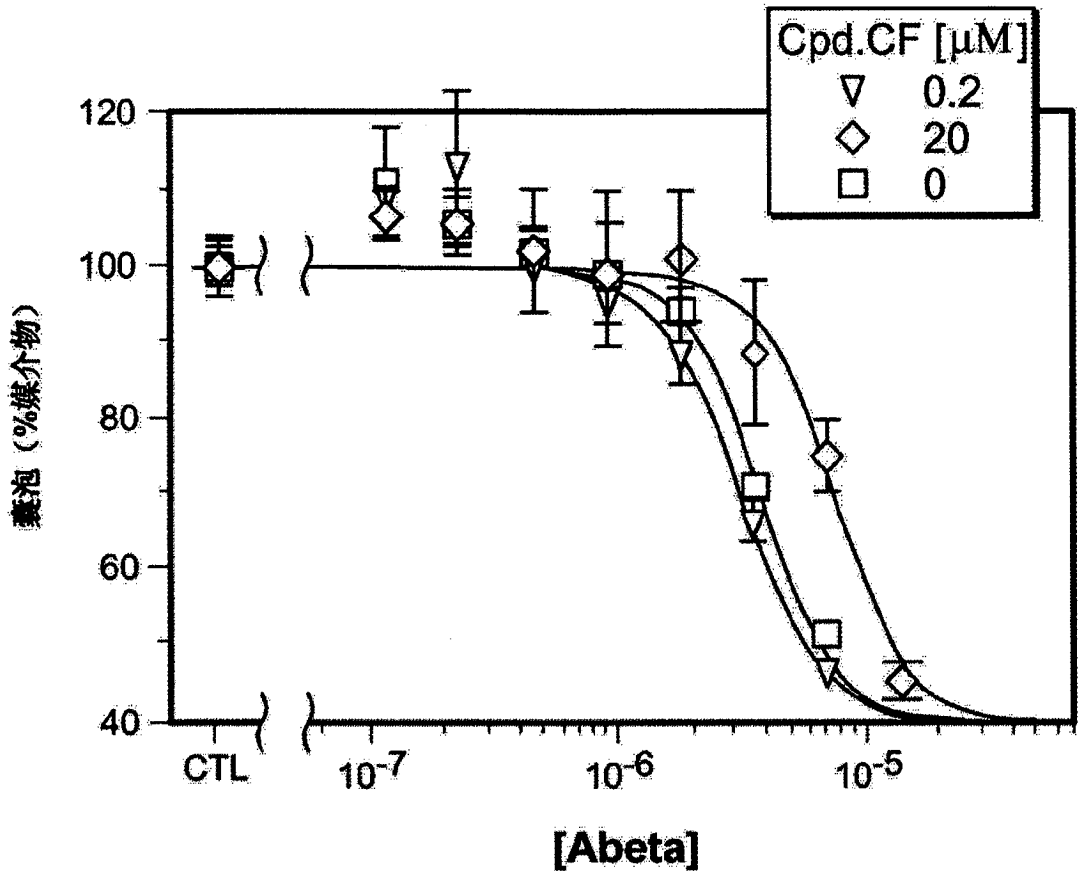


图 1J

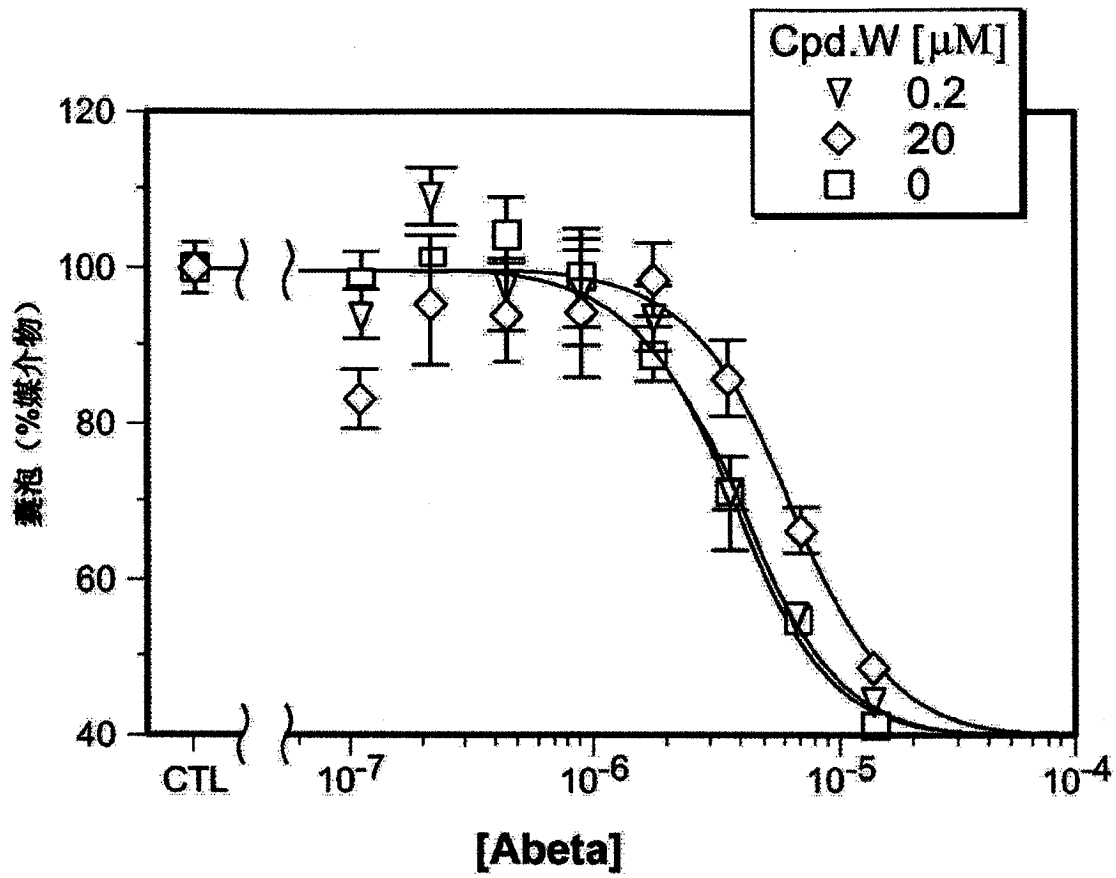


图 1K

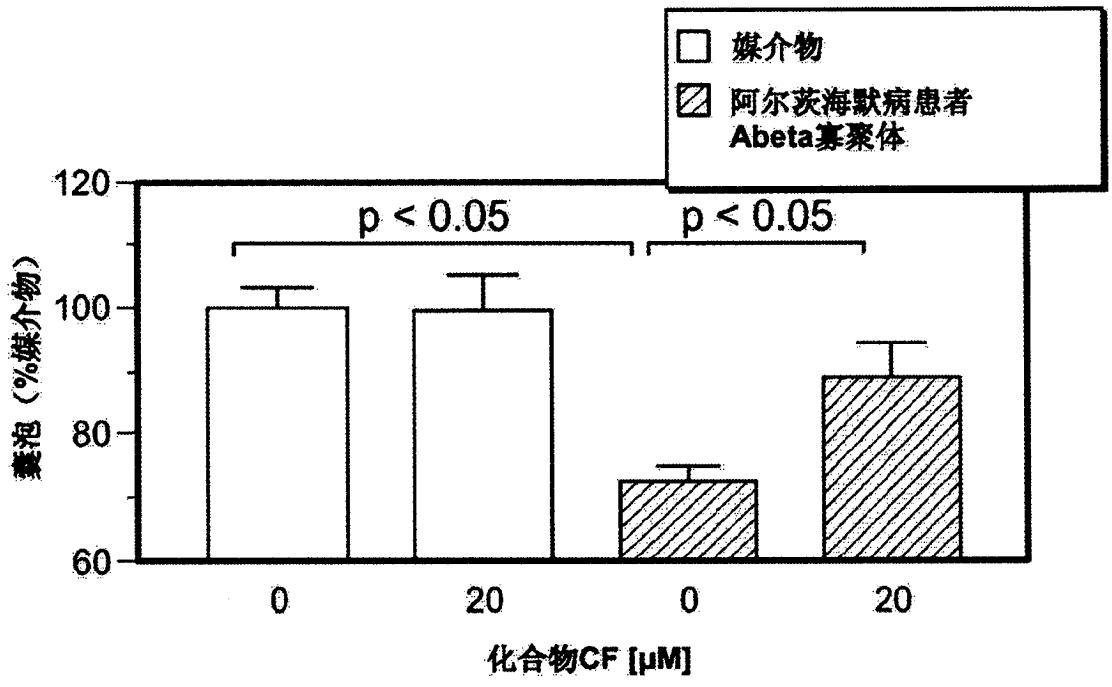

媒介物
阿尔茨海默病患者
Abeta寡聚体
p < 0.05
p < 0.05
120
100
80
60
囊泡（%媒介物）
0
20
0
20
化合物CF [μM]

图 1L

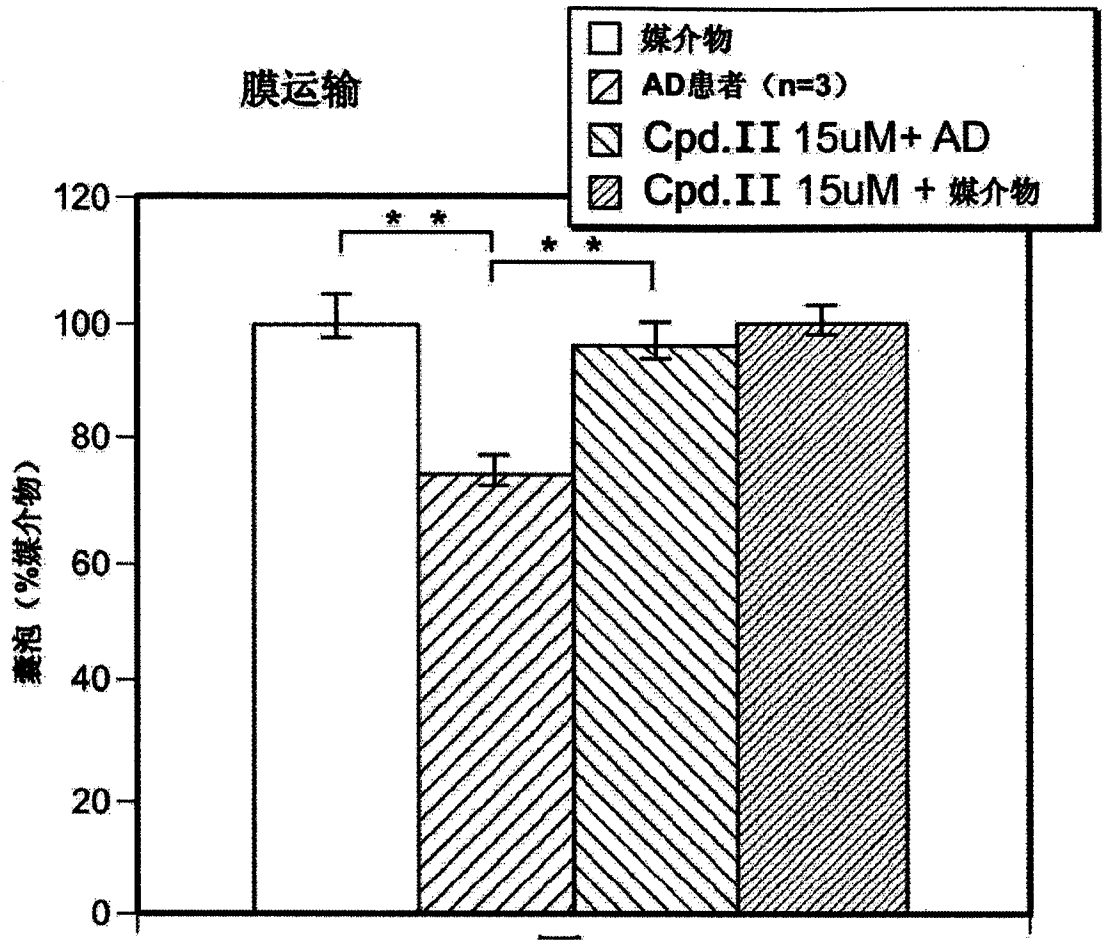

膜运输
媒介物
AD患者（n=3）
Cpd.II 15uM+ AD
Cpd.II 15uM + 媒介物
120
100
80
60
40
20
0
囊泡（%媒介物）
* *
* *

图 1M

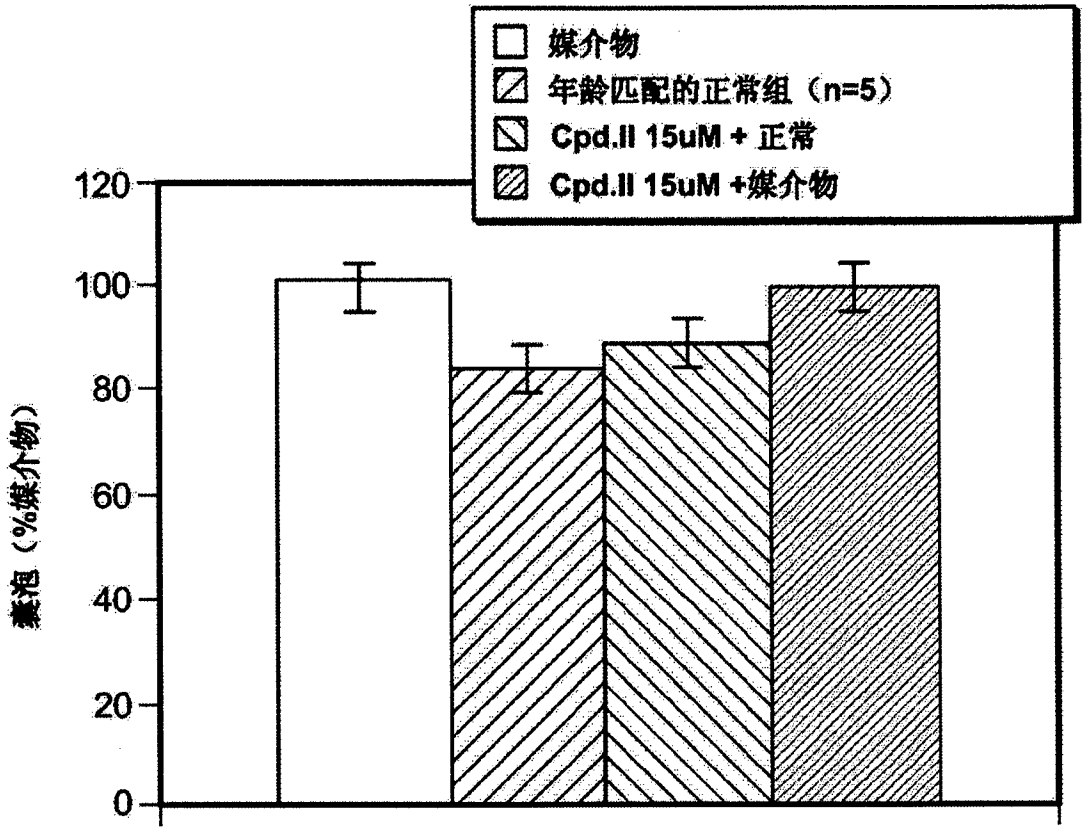

媒介物
年龄匹配的正常组（n=5）
Cpd.II 15uM + 正常
Cpd.II 15uM +媒介物
120
100
80
60
40
20
0
囊泡（%媒介物）

图 1N

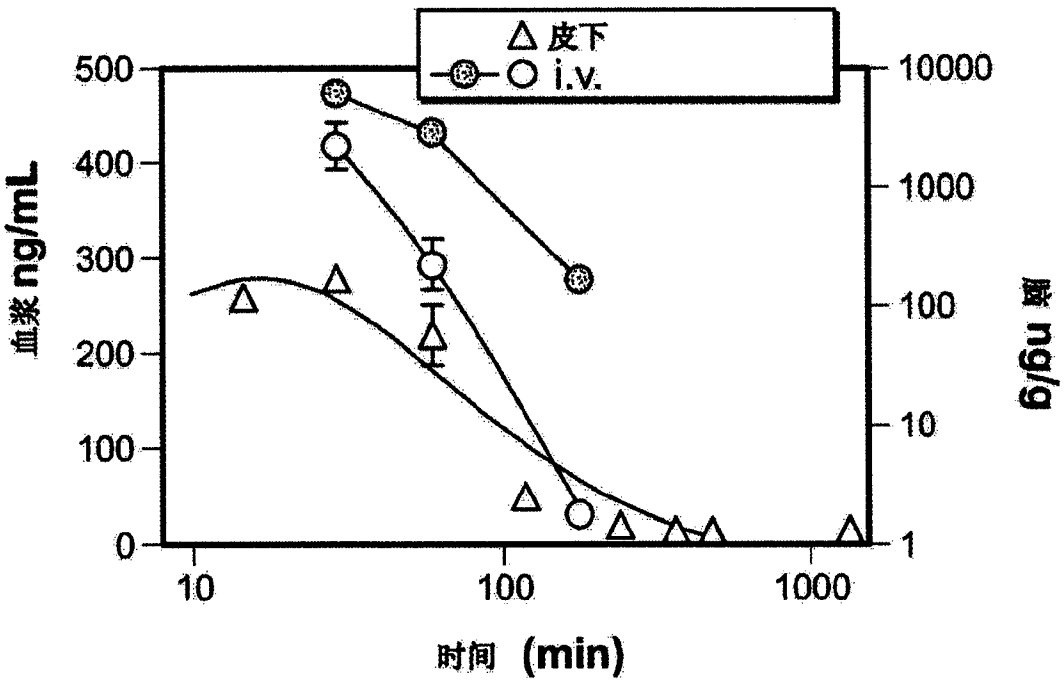

皮下
i.v.
500
400
300
200
100
0
血浆 ng/mL
10000
1000
100
10
1
10
100
1000
时间 (min)

图 2A

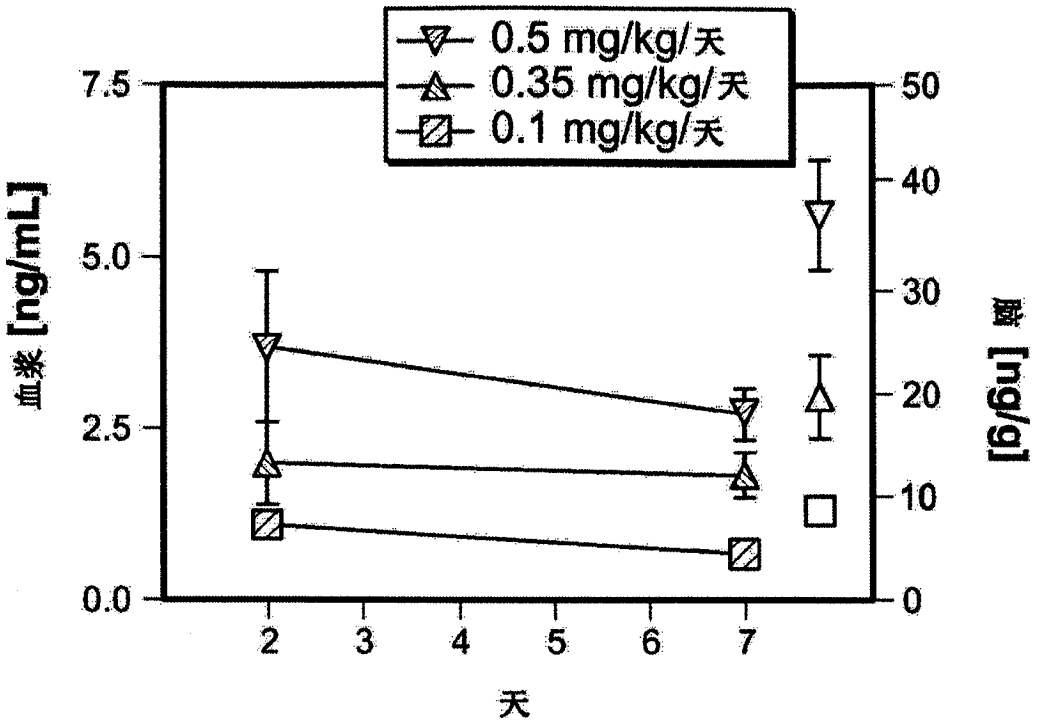

0.5 mg/kg/天
0.35 mg/kg/天
0.1 mg/kg/天
血浆 [ng/mL]
脑 [ng/g]
7.5
5.0
2.5
0.0
50
40
30
20
10
0
2
3
4
5
6
7
天

图 2B

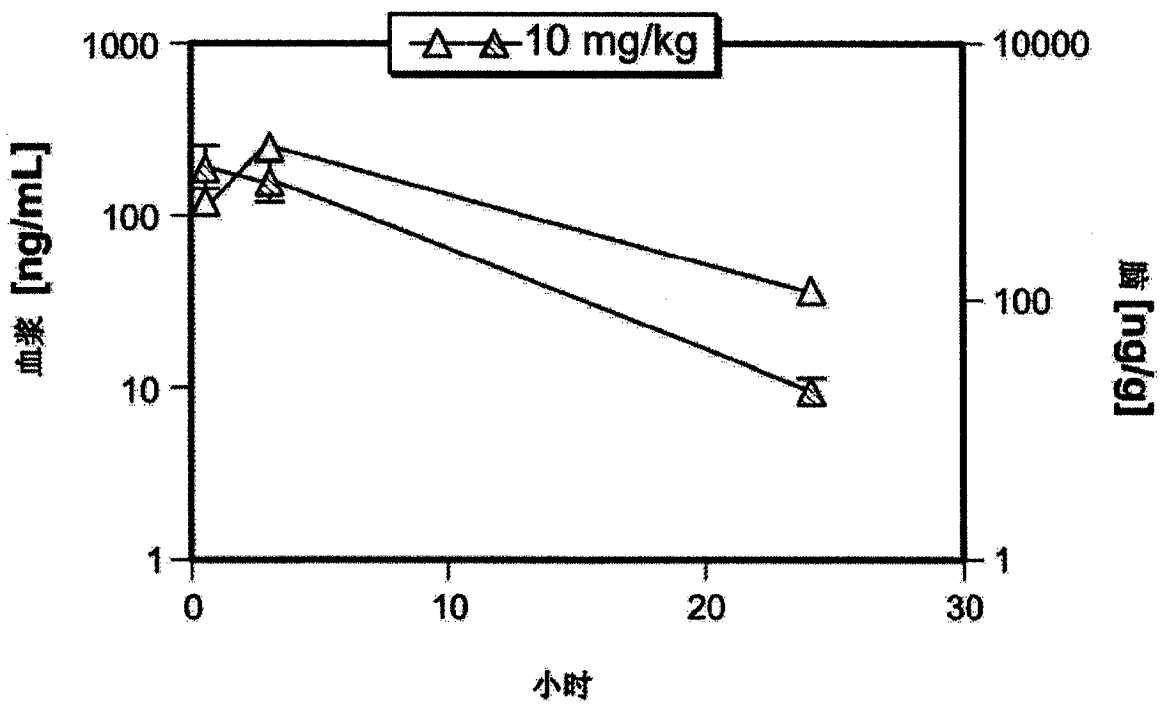

10 mg/kg
血浆 [ng/mL]
脑 [ng/g]
1000
100
10
1
10000
100
1
0
10
20
30
小时

图 2C

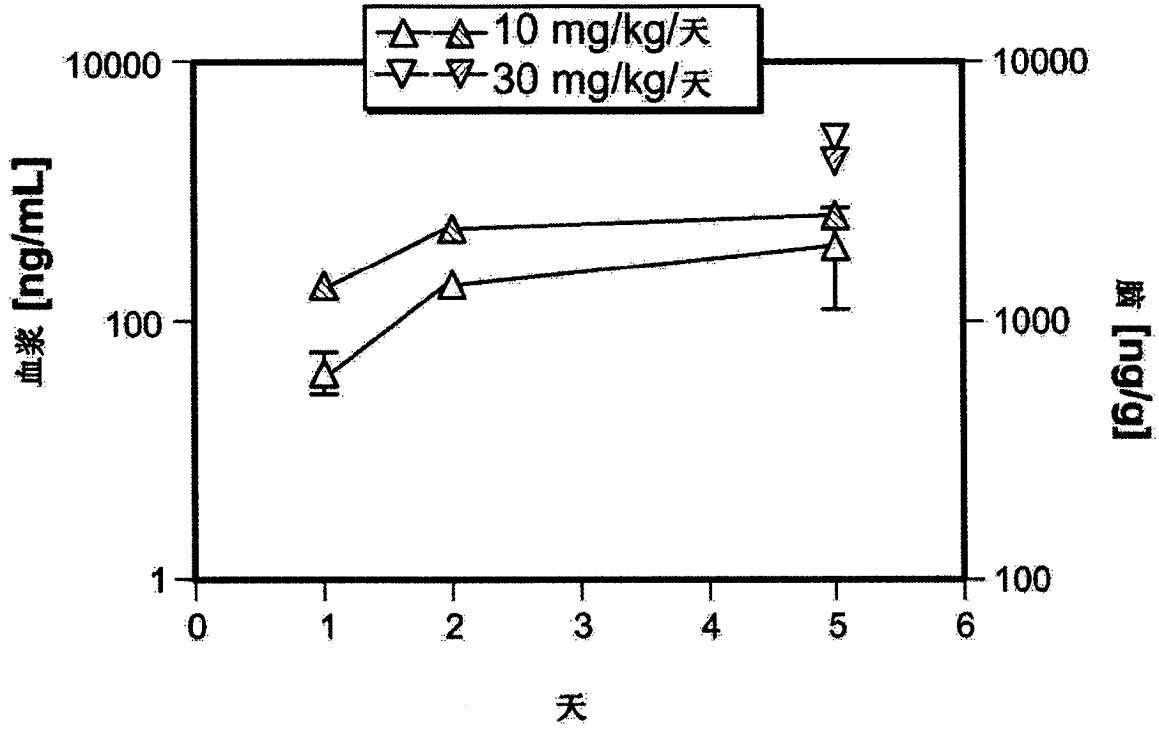

10 mg/kg/天
30 mg/kg/天
10000
100
1
血浆 [ng/mL]
10000
1000
100
脑 [ng/g]
0
1
2
3
4
5
6
天

图 2D

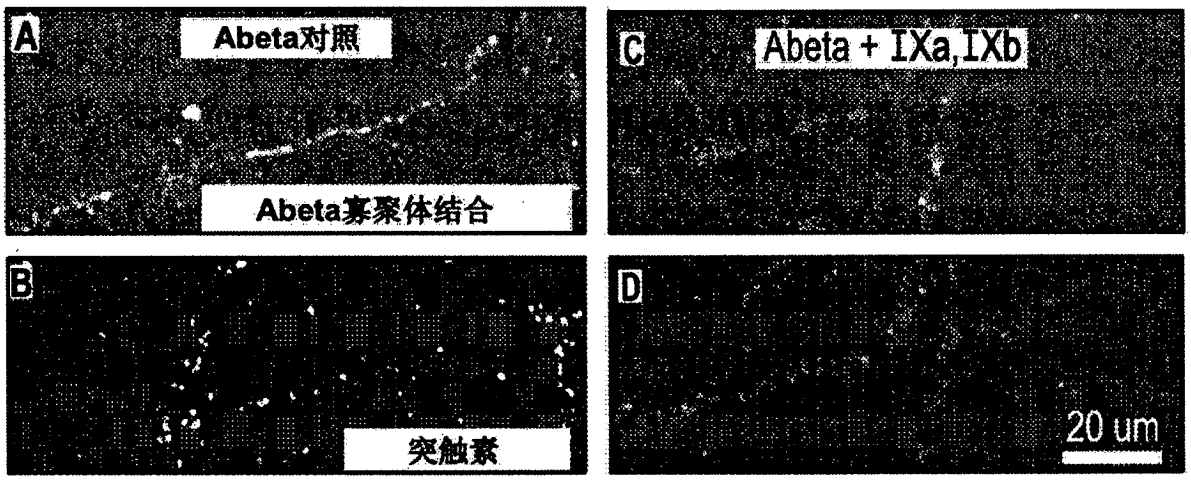

A
Abeta对照
Abeta寡聚体结合
C
Abeta + IXa,IXb
B
突触素
D
20 um

图 3A

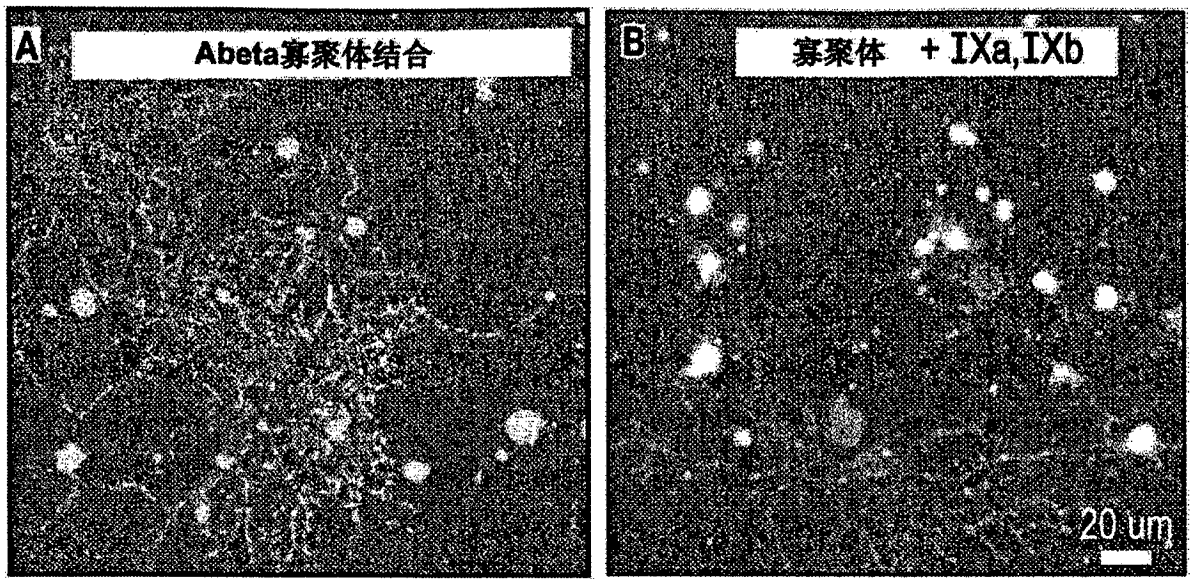

A
Abeta寡聚体结合
B
寡聚体 + IXa,IXb
20 um

图 3B

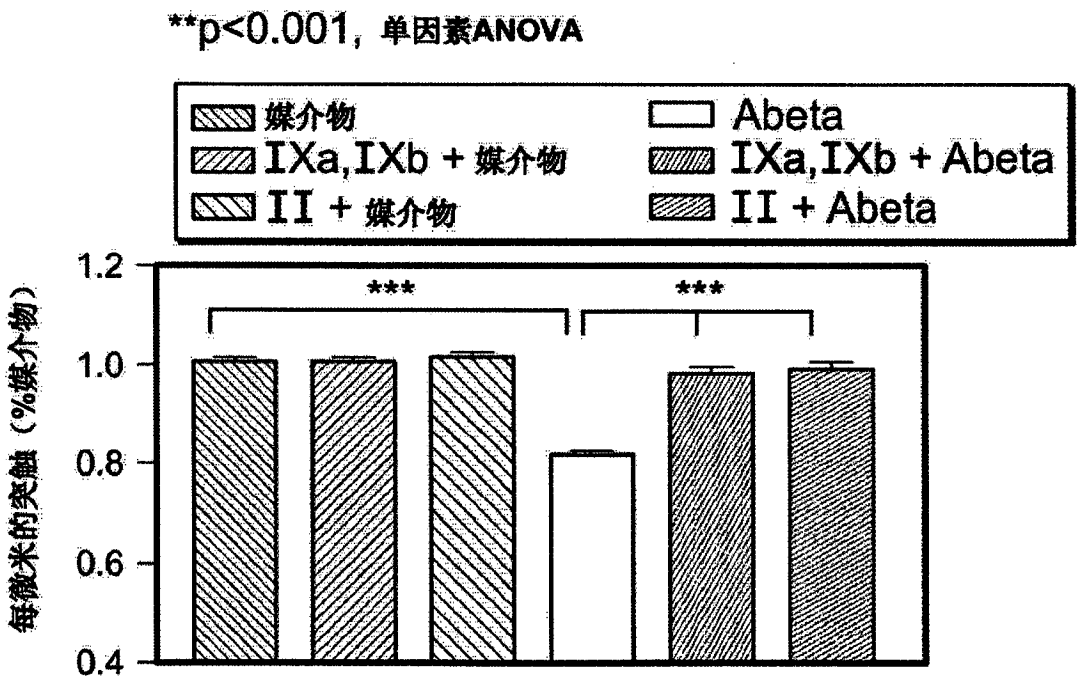

**p<0.001, 单因素ANOVA
媒介物
IXa,IXb + 媒介物
II + 媒介物
Abeta
IXa,IXb + Abeta
II + Abeta
每微米的突触（%媒介物）
1.2
1.0
0.8
0.6
0.4
***
***

图 3C

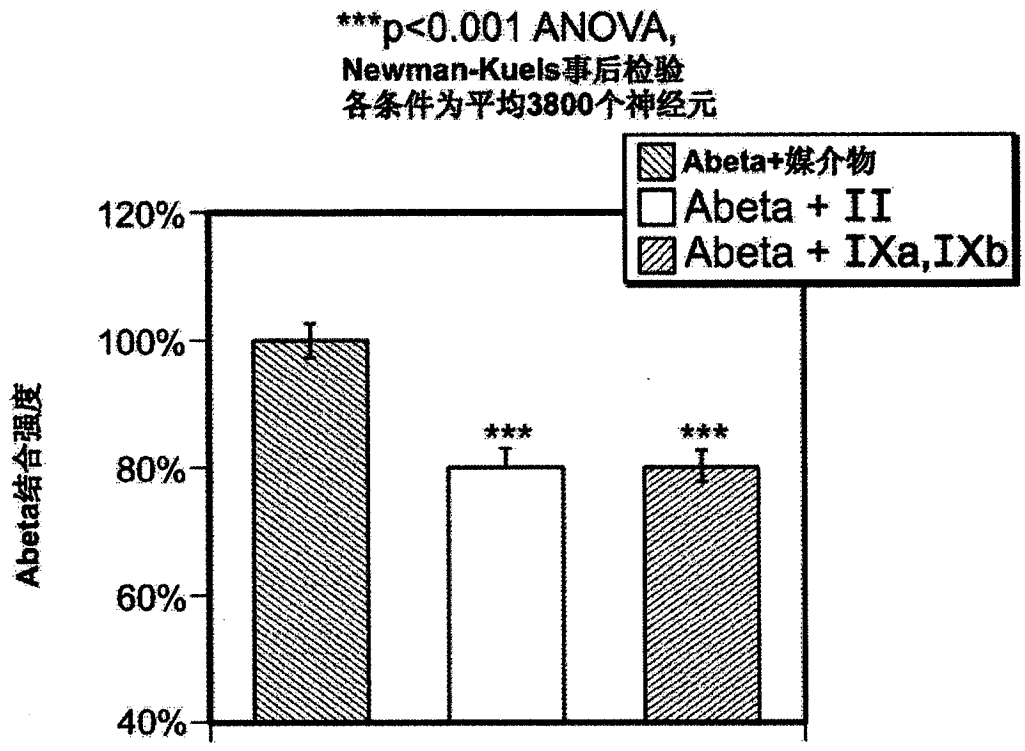

***p<0.001 ANOVA,
Newman-Kuels事后检验
各条件为平均3800个神经元
Abeta+媒介物
Abeta + II
Abeta + IXa,IXb
120%
100%
80%
60%
40%
Abeta结合强度
***
***

图 3D

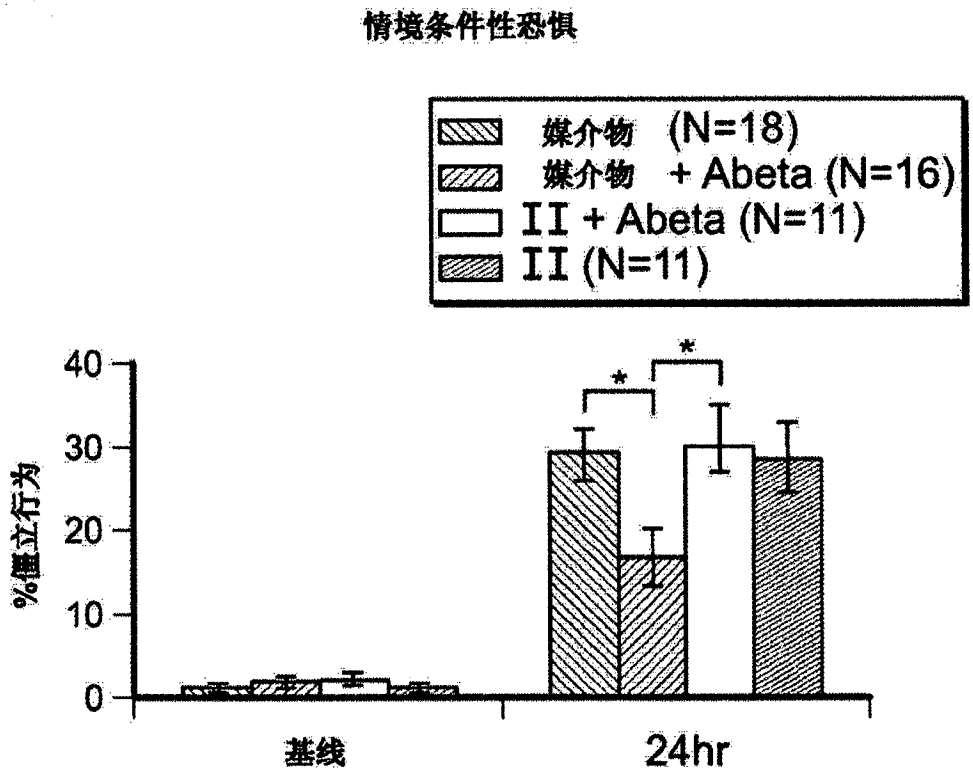

情境条件性恐惧
媒介物 (N=18)
媒介物 + Abeta (N=16)
II + Abeta (N=11)
II (N=11)
40
30
20
10
0
%僵立行为
*
*
基线
24hr

图 4

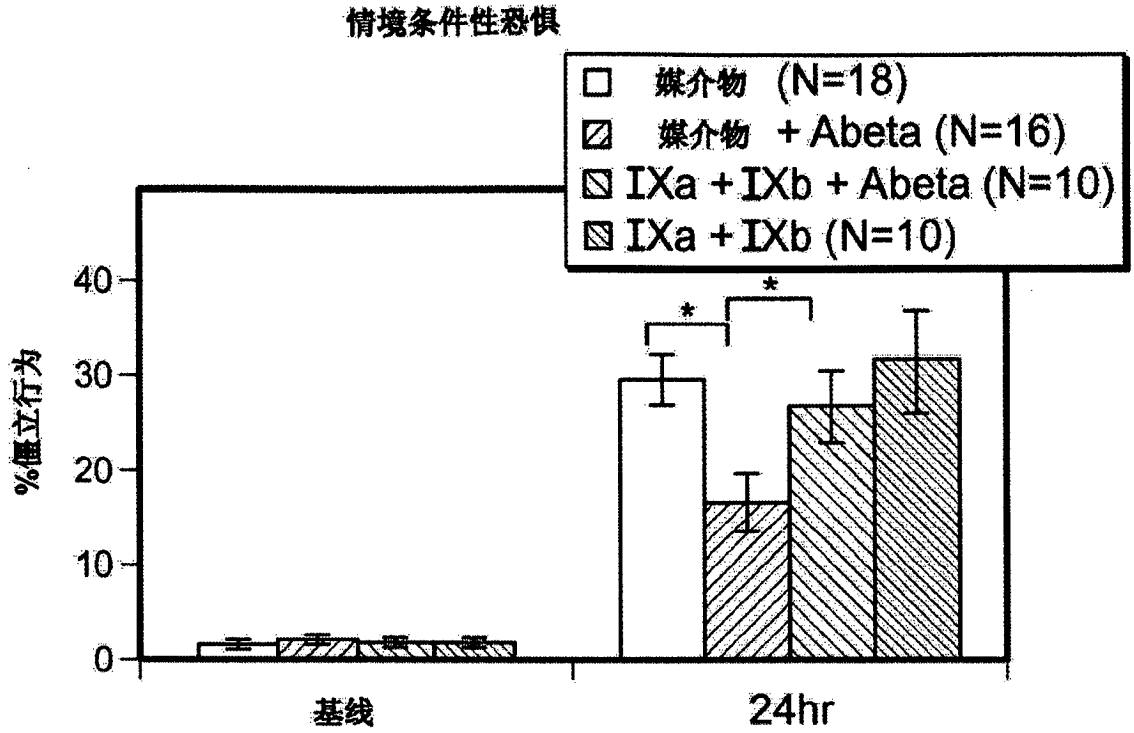
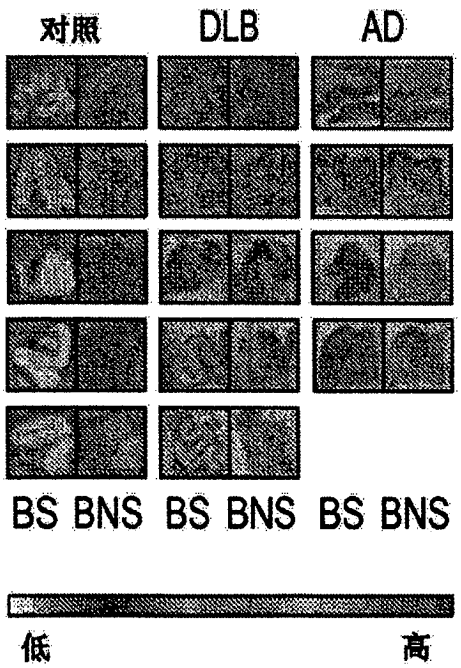

情境条件性恐惧
媒介物 (N=18)
媒介物 + Abeta (N=16)
IXa + IXb + Abeta (N=10)
IXa + IXb (N=10)
%僵立行为
40
30
20
10
0
*
*
基线
24hr

图 5


对照
DLB
AD
BS BNS BS BNS BS BNS
低
高

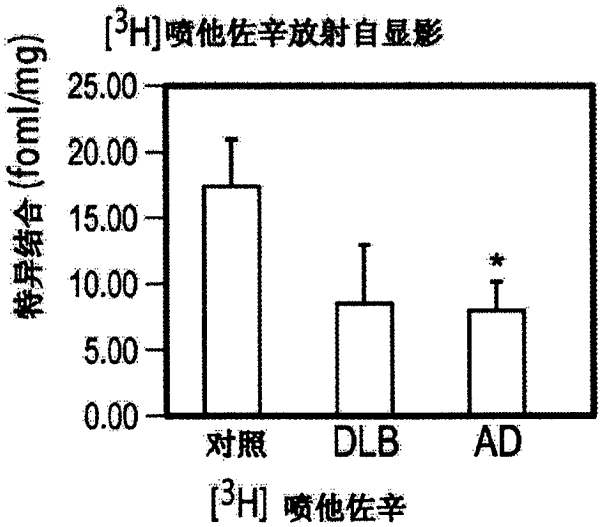

[3H]喷他佐辛放射自显影
特异结合 (fomI/mg)
25.00
20.00
15.00
10.00
5.00
0.00
*
对照
DLB
AD
[3H] 喷他佐辛

图 6A

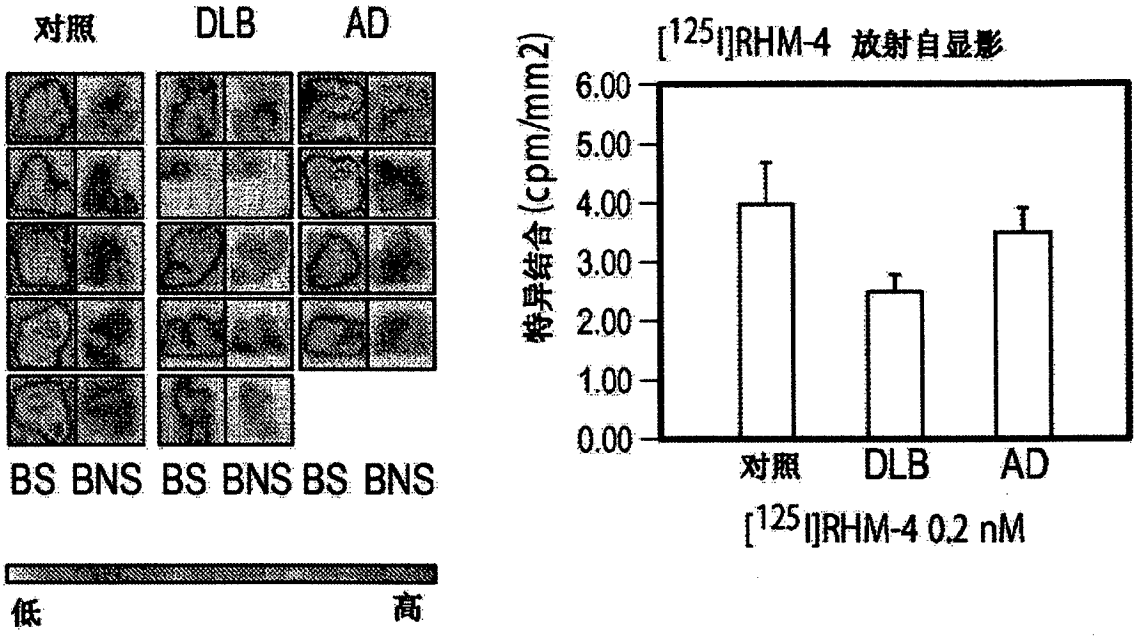
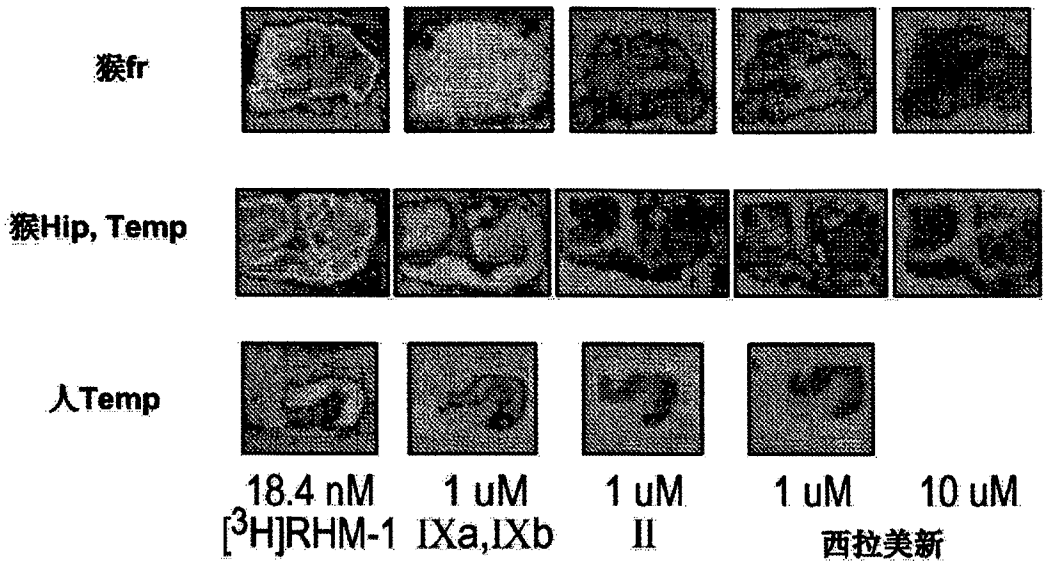

对照
DLB
AD
BS BNS BS BNS BS BNS
低
高
[125I]RHM-4 放射自显影
特异结合 (cpm/mm2)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
对照
DLB
AD
[125I]RHM-4 0.2 nM

图 6B


猴fr
猴Hip, Temp
人Temp
18.4 nM
[3H]RHM-1
1 uM
IXa,IXb
1 uM
II
1 uM
西拉美新
10 uM

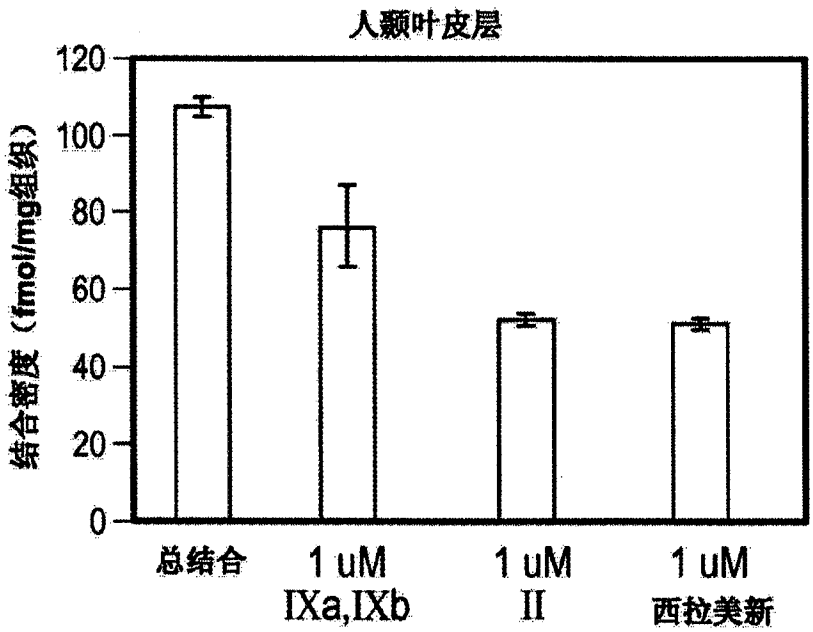

人颞叶皮层
结合密度（fmol/mg组织）
120
100
80
60
40
20
0
总结合
1 uM
IXa,IXb
1 uM
II
1 uM
西拉美新

图 6C

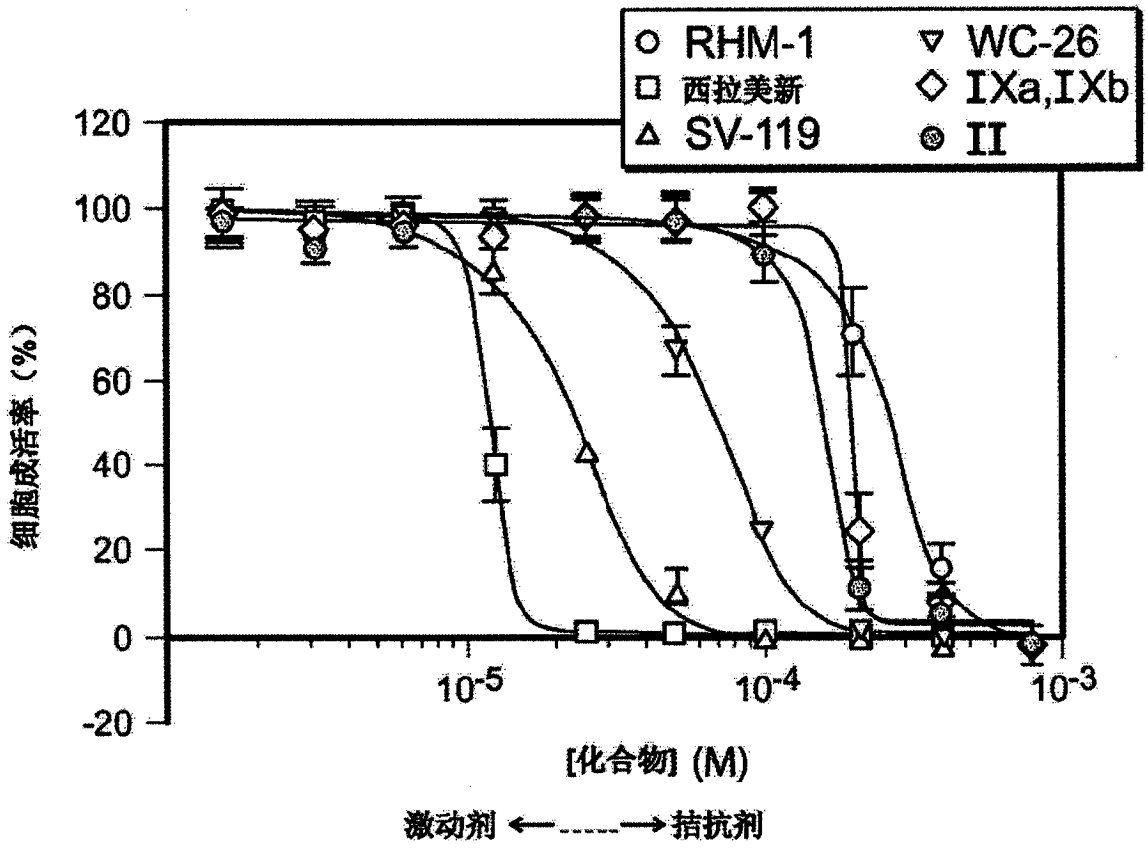

RHM-1
西拉美新
SV-119
WC-26
IXa,IXb
II
120
100
80
60
40
20
0
-20
细胞成活率（%）
10^{-5}
10^{-4}
10^{-3}
[化合物] (M)
激动剂 ←-----→ 拮抗剂

图 7A

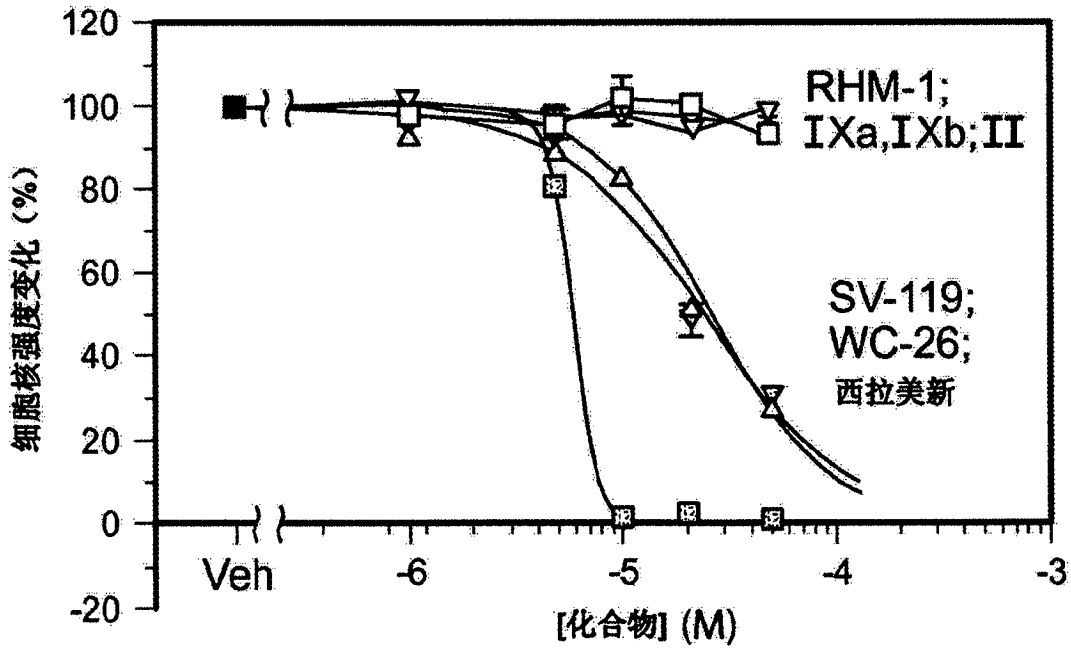

120
100
80
60
40
20
0
-20
细胞核强度变化（%）
RHM-1;
IXa,IXb;II
SV-119;
WC-26;
西拉美新
Veh
-6
-5
-4
-3
[化合物] (M)

图 7B

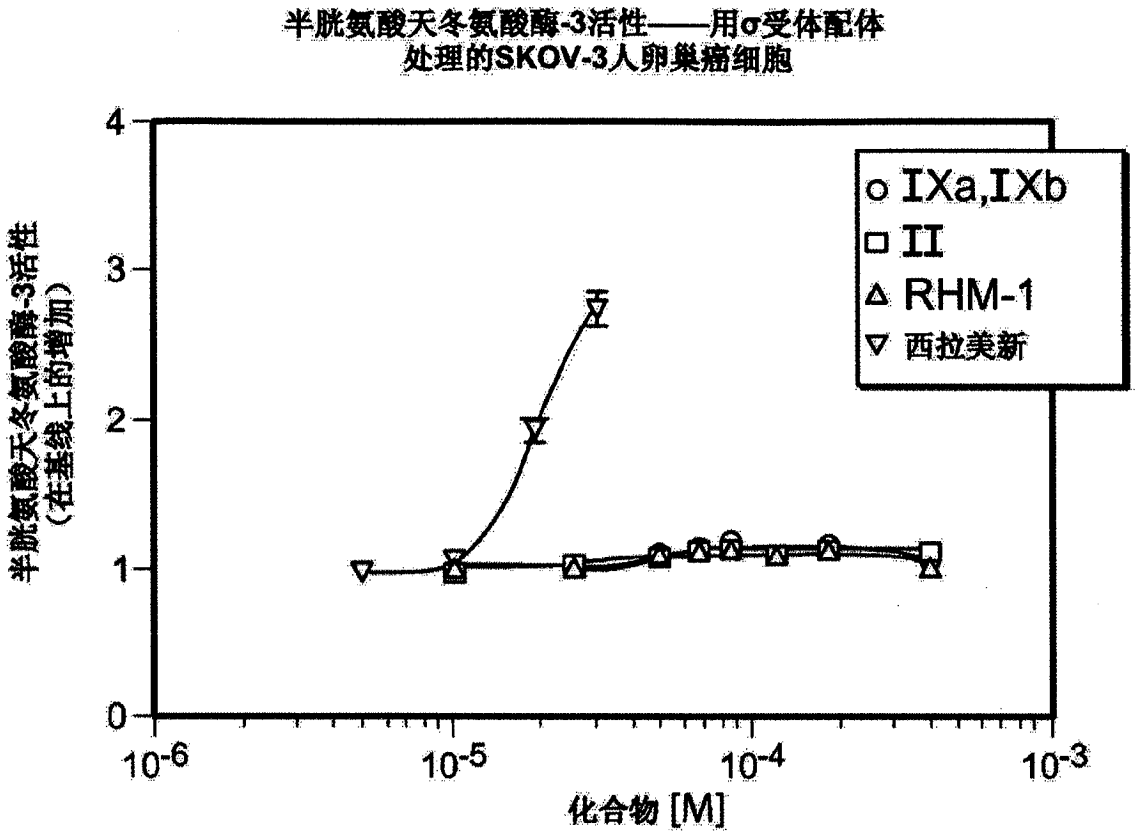

半胱氨酸天冬氨酸酶-3活性——用σ受体配体
处理的SKOV-3人卵巢癌细胞
半胱氨酸天冬氨酸酶-3活性
(在基线上的增加)
4
3
2
1
0
10-6
10-5
10-4
10-3
化合物 [M]
IXa,IXb
II
RHM-1
西拉美新

图 10A

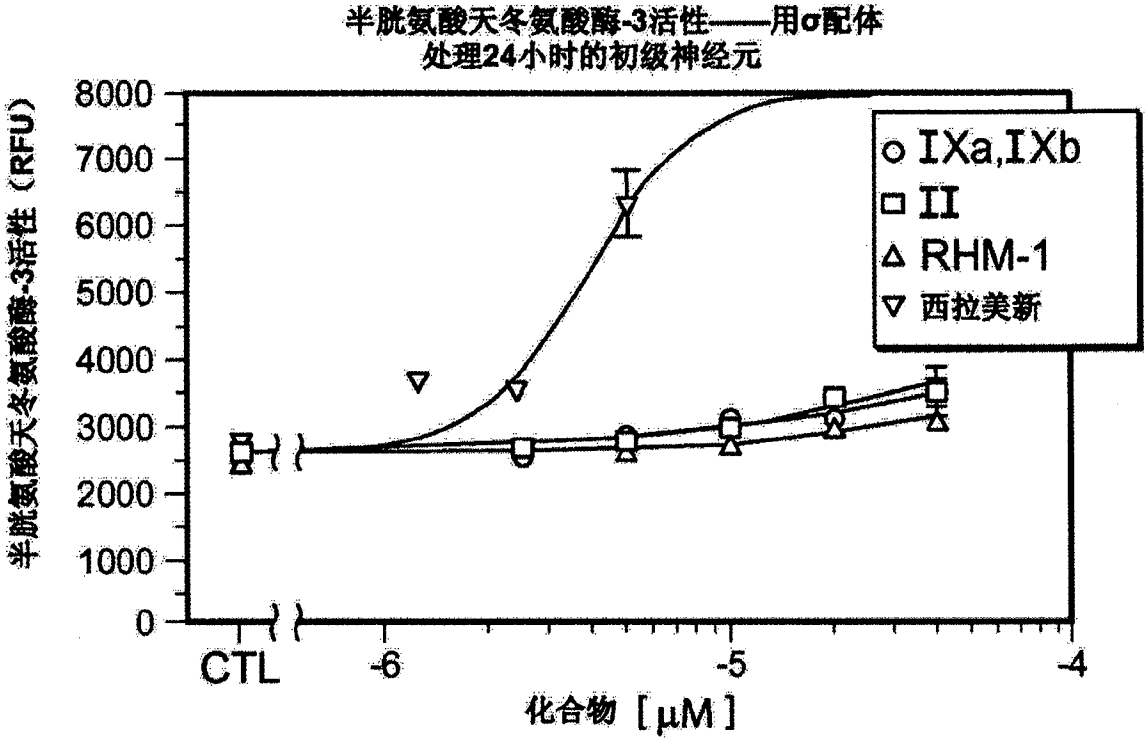

半胱氨酸天冬氨酸酶-3活性——用σ配体
处理24小时的初级神经元
半胱氨酸天冬氨酸酶-3活性（RFU）
8000
7000
6000
5000
4000
3000
2000
1000
0
CTL
-6
-5
-4
化合物［μM］
IXa,IXb
II
RHM-1
西拉美新

图 10B

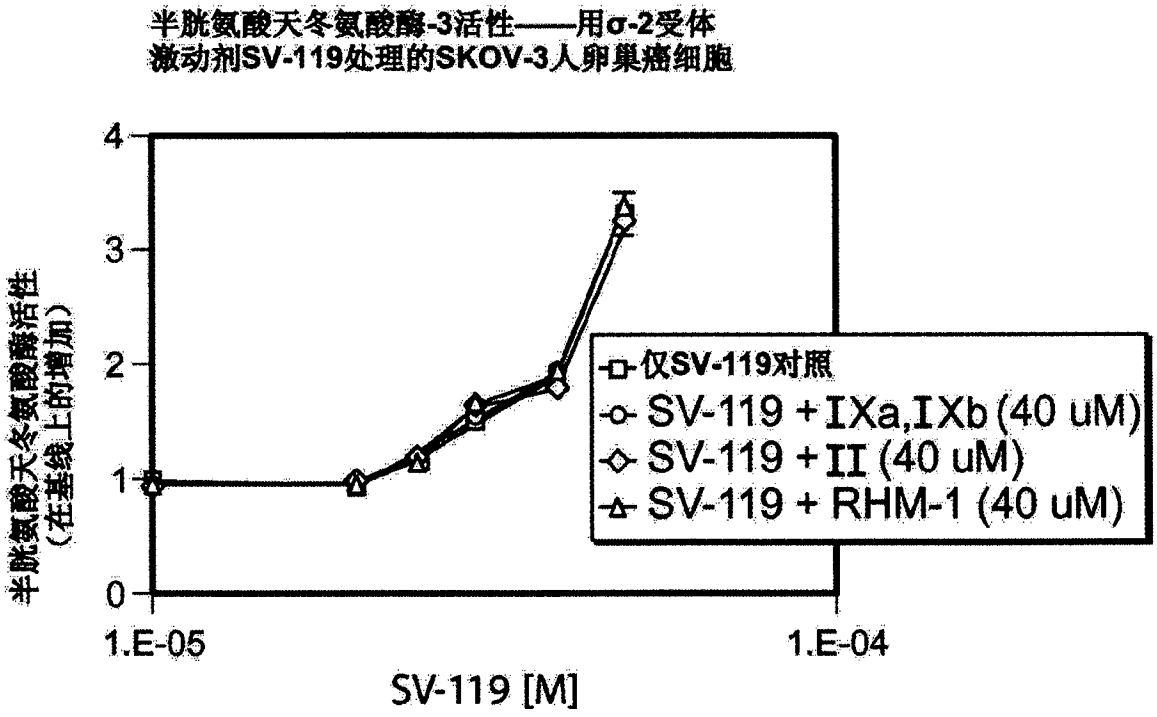

半胱氨酸天冬氨酸酶-3活性——用σ-2受体
激动剂SV-119处理的SKOV-3人卵巢癌细胞
半胱氨酸天冬氨酸酶活性
（在基线上的增加）
4
3
2
1
0
1.E-05
1.E-04
SV-119 [M]
仅SV-119对照
SV-119 + IXa,IXb (40 uM)
SV-119 + II (40 uM)
SV-119 + RHM-1 (40 uM)

图 8C

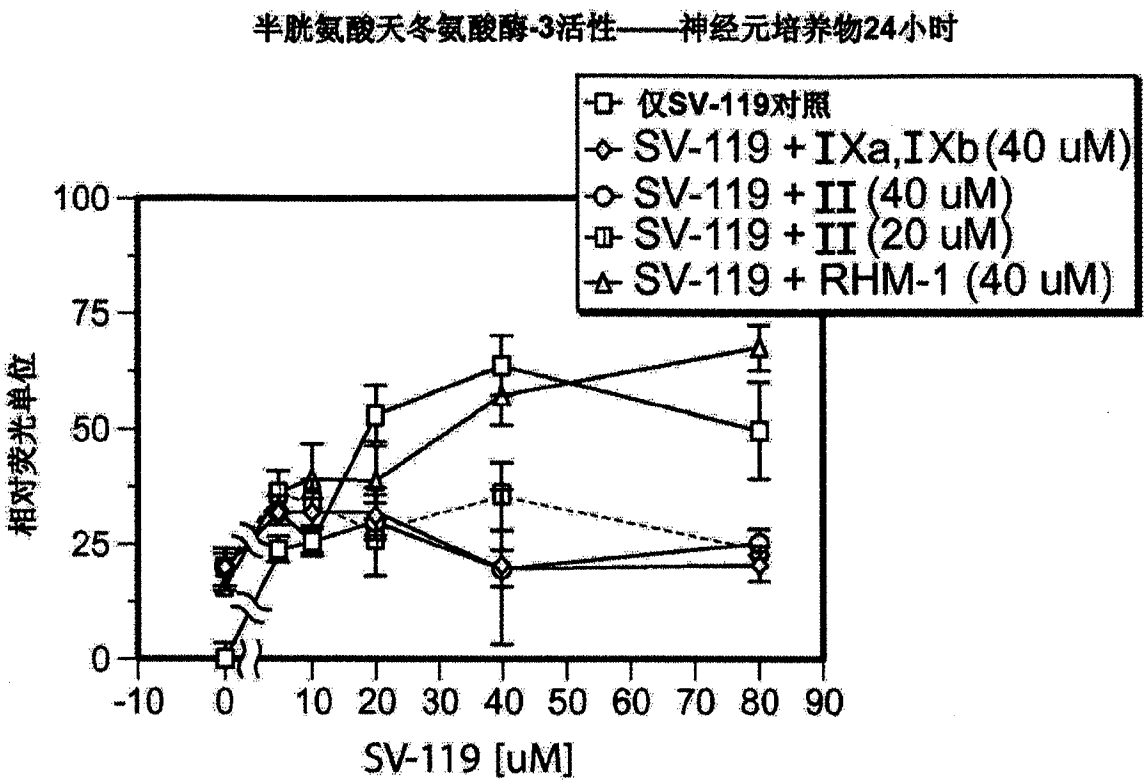

半胱氨酸天冬氨酸酶-3活性——神经元培养物24小时
仅SV-119对照
SV-119 + IXa,IXb (40 uM)
SV-119 + II (40 uM)
SV-119 + II (20 uM)
SV-119 + RHM-1 (40 uM)
相对荧光单位
100
75
50
25
0
-10
0
10
20
30
40
50
60
70
80
90
SV-119 [uM]

图 8D

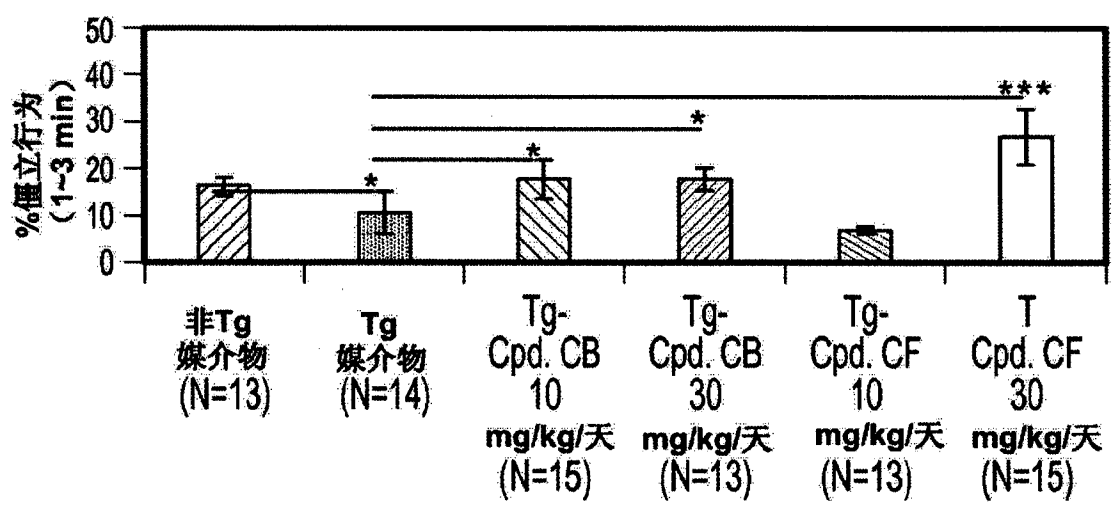

%僵立行为 (1~3 min)
50
40
30
20
10
0
*
*
*
***
非Tg 媒介物 (N=13)
Tg 媒介物 (N=14)
Tg-Cpd. CB 10 mg/kg/天 (N=15)
Tg-Cpd. CB 30 mg/kg/天 (N=13)
Tg-Cpd. CF 10 mg/kg/天 (N=13)
T Cpd. CF 30 mg/kg/天 (N=15)

图 9A

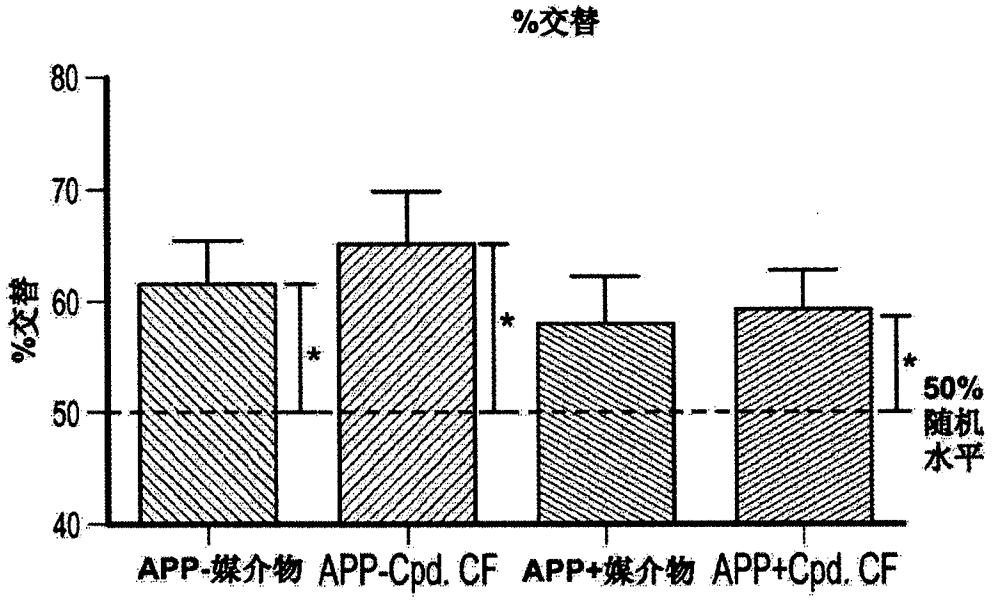
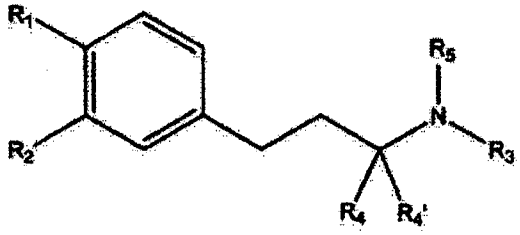


图 9B

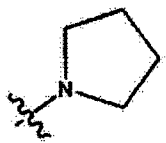
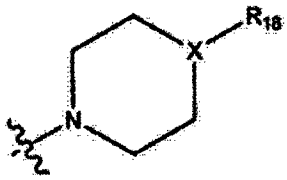
1. 一种式 I 的化合物及其药学上可接受的盐：



I

其中

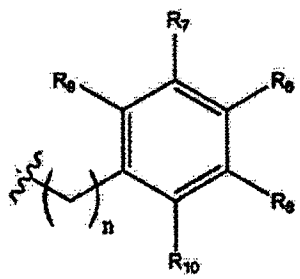
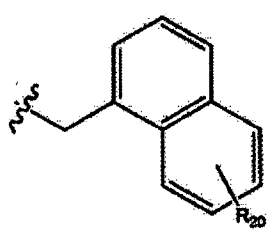
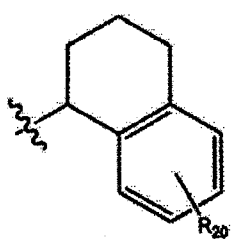
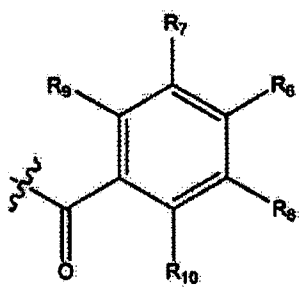
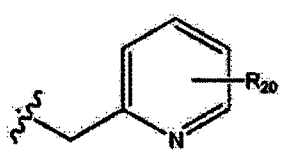
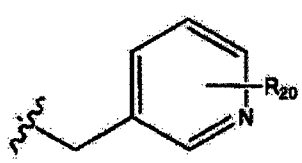
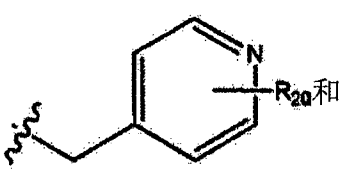
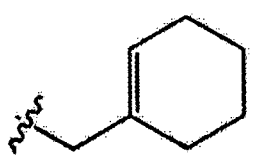
R_1 和 R_2 独立选自 H、OH、卤素、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、$(R_{16})(R_{17})N-C_{1-4}$ 亚烷基 -O-，或 R_1 和 R_2 连接在一起形成 -O- 亚乙基 -O- 基，其中

R_{16} 和 R_{17} 独立地为 C_{1-4} 烷基或苄基，或 R_{16} 和 R_{17} 与氮一起形成选自  和  的环，其中

X 是 N 或 O 且 R_{18} 是 H 或未被取代的苯基；且

其中 R_1 和 R_2 的至少一个不是 H；

R_3 选自

 ，  ，  ，  ，  ，  ，  和 

其中

R_6、R_7、R_8、R_9 和 R_{10} 独立选自 H、卤素、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基和 $S(O)_2$-C_{1-6} 烷基；

R_{20} 是 H；且

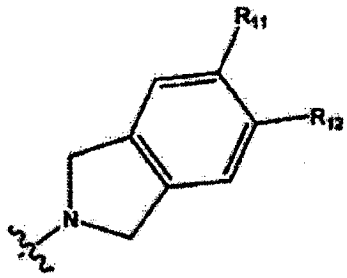
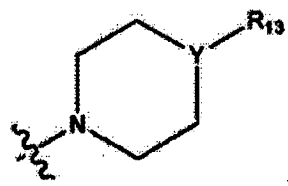
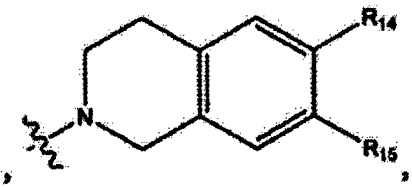
n 是 1-4

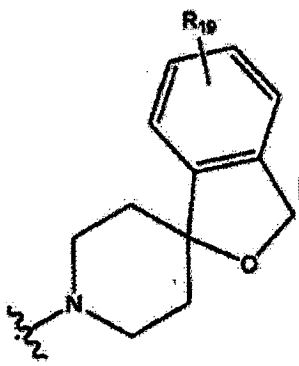
R_4 是 C_{1-6} 烷基；

$R_{4'}$ 是 H 或 C_{1-6} 烷基；且

R_5 是 H、C_{1-6} 烷基和 C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）或 C(O)(C_{1-4} 卤代烷基）；或

R_3 和 R_5 与氮一起形成选自




和


的环，其中

R_{11} 和 R_{12} 独立选自 H、卤素和 C_{1-6} 卤代烷基，且

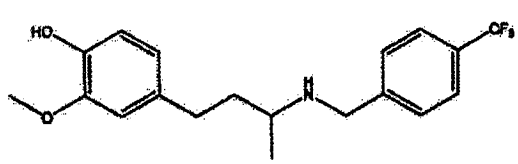
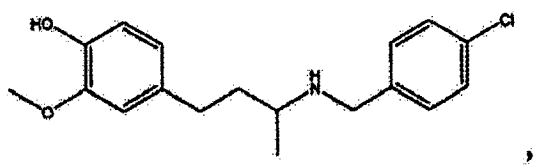
Y 是 CH 或 N；

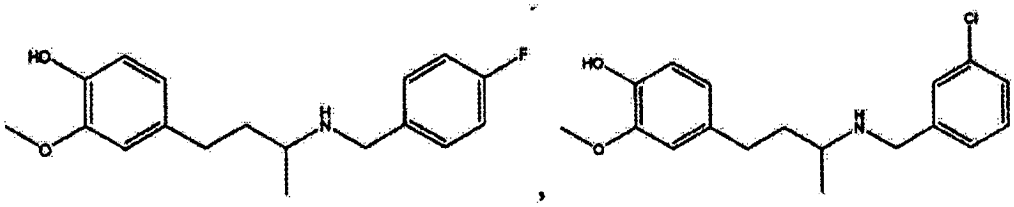
R_{13} 是 H、C_{1-6} 烷基、C_{3-6} 环烷基、未被取代的苯基或被 C_{1-6} 卤代烷基取代的苯基、或未被取代的苄基；

R_{14} 和 R_{15} 独立选自 H 和卤素；

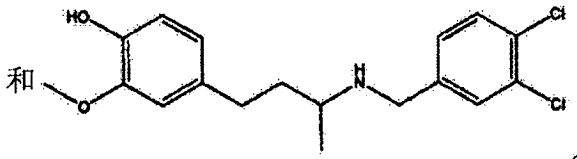
R_{19} 是 H，

条件是排除以下化合物的外消旋混合物



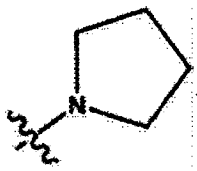
和

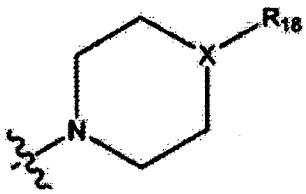


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2. 如权利要求 1 所述的化合物及其药学上可接受的盐，其中

R_1 和 R_2 独立选自 H、OH、卤素、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、$(R_{16})(R_{17})N-C_{1-4}$ 亚烷基 -O-，或 R_1 和 R_2 连接在一起形成 -O- 亚乙基 -O- 基，其中

R_{16} 和 R_{17} 独立地为 C_{1-4} 烷基或苄基，或 R_{16} 和 R_{17} 与氮一起形成选自  和

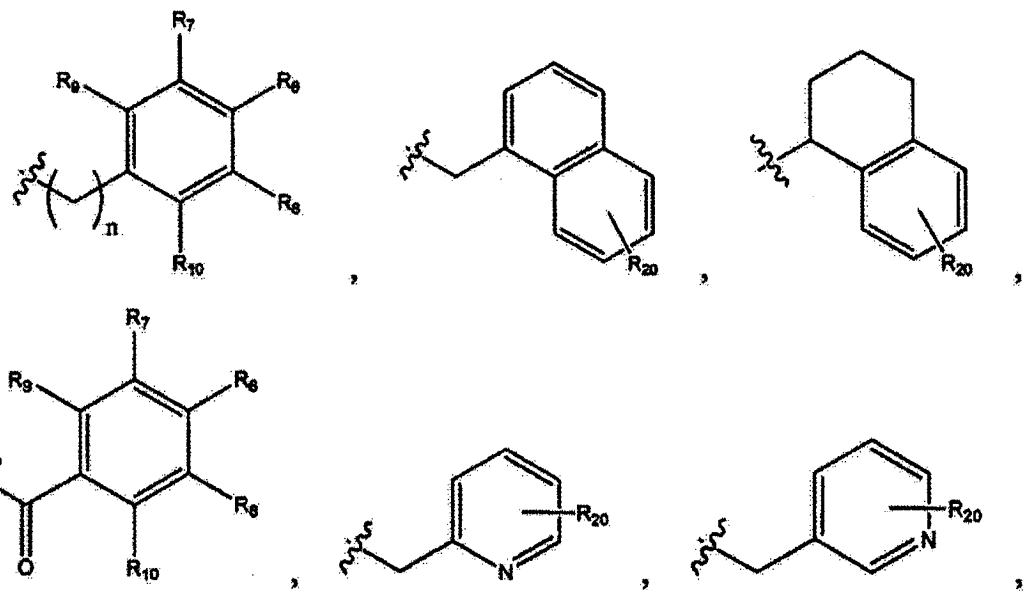


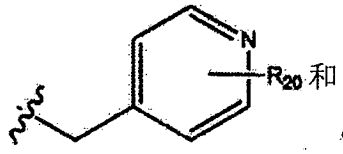
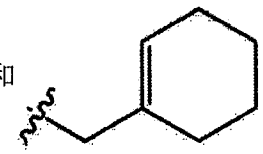
的环，其中

X 是 N 或 O 且 R_{18} 不存在或者是 H 或未被取代的苯基；且

其中 R_1 和 R_2 的至少一个不是 H；

R_3 选自




和


其中

R_6、R_7、R_8、R_9 和 R_{10} 独立选自 H、卤素、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基和 $S(O)_2$-C_{1-6} 烷基；

R_{20} 是 H；且

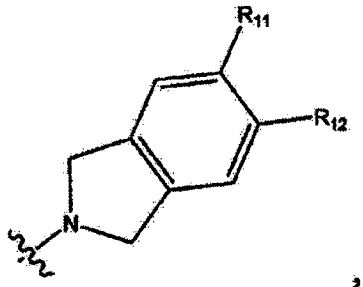
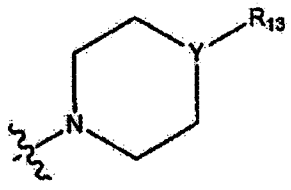
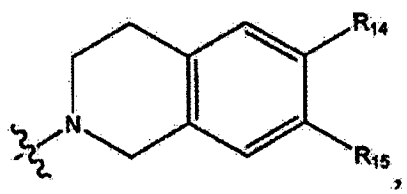
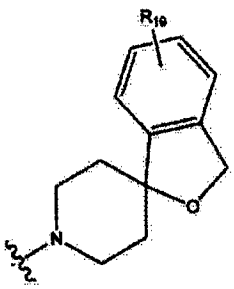
n 是 1-4

R_4 是 C_{1-6} 烷基；

$R_{4'}$ 是 H 或 C_{1-6} 烷基；且

R_5 是 H、C_{1-6} 烷基和 C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）或 C(O)(C_{1-4} 卤代烷基）；或

R_3 和 R_5 与氮一起形成选自


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和

的环，其中

R_{11} 和 R_{12} 独立选自 H、卤素和 C_{1-6} 卤代烷基，且

Y 是 CH 或 N；

R_{13} 是 H、C_{1-6} 烷基、C_{3-6} 环烷基、未被取代的苯基或被 C_{1-6} 卤代烷基取代的苯基、或未被取代的苄基；

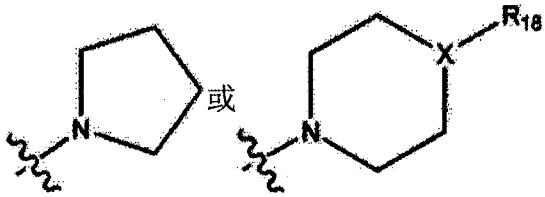
R_{14} 和 R_{15} 独立选自 H 和卤素；且

R_{19} 是 H。

3. 如权利要求 1 所述的化合物及其药学上可接受的盐，其中

R_1 选自 OH、OMe、F、Cl、CF_3、(R_{16})(R_{17})N- 亚乙基 -O-，其中

R_{16} 和 R_{17} 各自是甲基、异丙基、正丁基或苄基，或 R_{16} 和 R_{17} 与氮一起形成选自


或

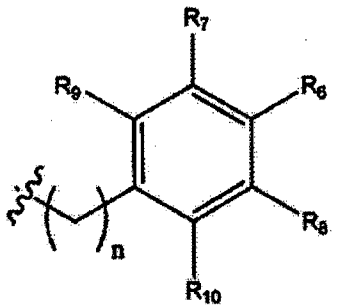
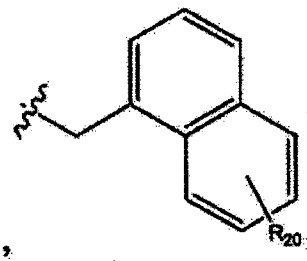
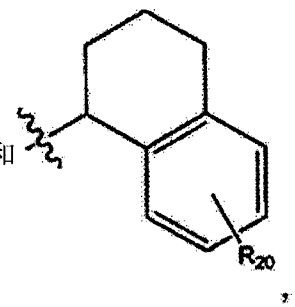
的环，其中

X 是 N 或 O 且 R_{18} 不存在或是未被取代的苯基；且

R_2 是 H、Cl、F、CF_3、OMe、OCF_3 或

R_1 和 R_2 连接在一起形成 -O- 亚乙基 -O- 基

R_3 选自


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和

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其中

R_6 是 H、F、Cl、Me、异丙基、叔丁基、OMe、CF_3、或 $S(O)_2Me$，

R_7 和 R_8 独立为 H、OMe、F、Cl 或 CF_3；

R_9 和 R_{10} 独立选自 H、OMe、F 和 Cl，

R_{20} 是 H；且

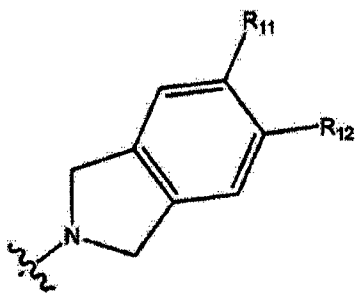
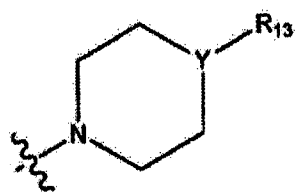
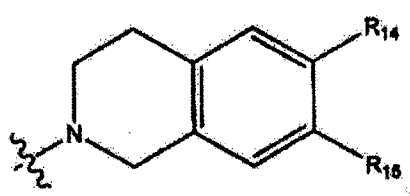
n 是 1

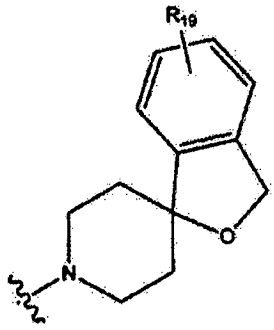
R_4 是 Me；

$R_{4'}$ 是 H 或 Me；且

R_5 是 H；或

R_3 和 R_5 与氮一起形成选自


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和



的环，其中

R_{11} 和 R_{12} 独立选自 H、Cl 和 CF_3，且

Y 是 CH 或 N；

R_{13} 是 H、Me、环己基、未被取代的苯基或被 CF_3 取代的苯基、或未被取代的苄基；

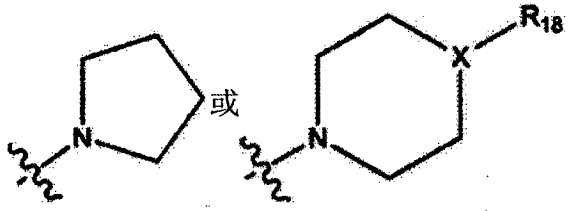
R_{14} 和 R_{15} 独立选自 H 和 Cl；且

R_{19} 是 H。

4. 如权利要求 1 所述的化合物及其药学上可接受的盐，其中

R_1 选自 OH、OMe、F、Cl、CF_3、$(R_{16})(R_{17})N$- 亚乙基 -O-，其中

R_{16} 和 R_{17} 各自是甲基、异丙基、正丁基或苄基，或 R_{16} 和 R_{17} 与氮一起形成选自



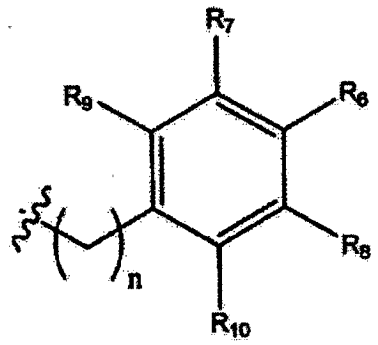
的环，其中

X 是 N 或 O 且 R_{18} 不存在或是未被取代的苯基；且

R_2 是 H、Cl、F、CF_3、OMe、OCF_3 或

R_1 和 R_2 连接在一起形成 -O- 亚乙基 -O- 基

R_3 选自



其中

R_6 是 H、F、Cl、Me、异丙基、叔丁基、OMe、CF_3、或 $S(O)_2Me$，

R_7 和 R_8 独立地为 H、OMe、F、Cl、或 CF_3；

R_9 和 R_{10} 独立选自 H、OMe、F、和 Cl，且

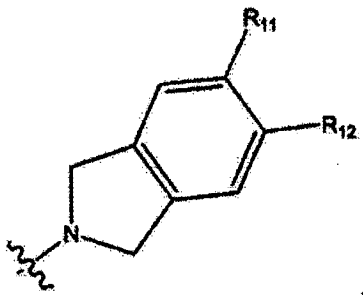
n 是 1

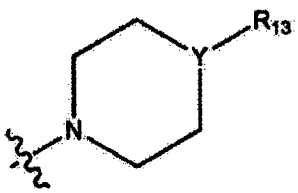
R_4 是 Me；

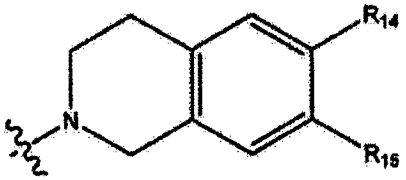
$R_{4'}$ 是 H；且

R_5 是 H；或

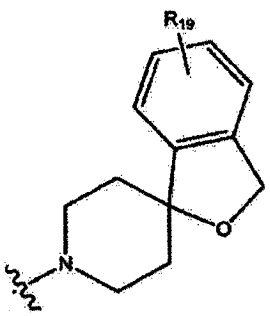
R_3 和 R_5 与氮一起形成选自



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和



的环，其中

R_{11} 和 R_{12} 独立选自 H、Cl、和 CF_3，且

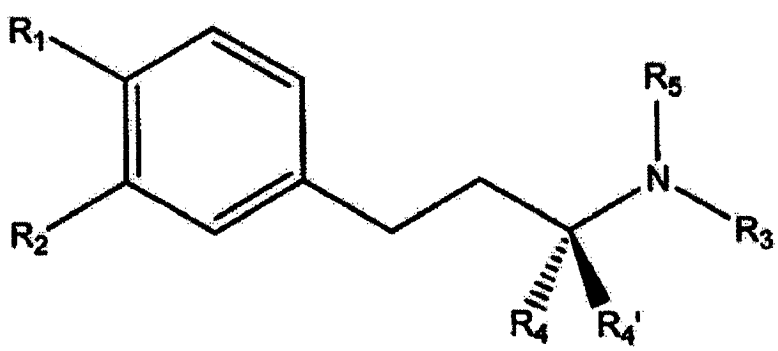
Y 是 CH 或 N；

R_{13} 是 H、Me、环己基、未被取代的苯基或被 CF_3 取代的苯基、或未被取代的苄基；

R_{14} 和 R_{15} 独立选自 H 和 Cl；且

R_{19} 是 H。

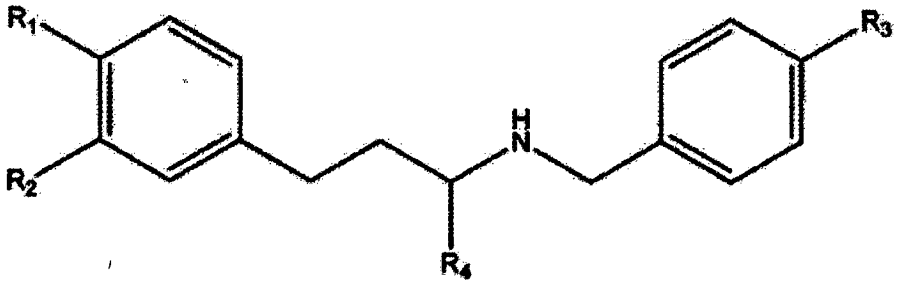
5. 如权利要求 1 所述的化合物及其药学上可接受的盐，所述化合物为式 Ia 的化合物



Ia

其中 $R_{4'}$ 是 H 且剩余基团如权利要求 1 中所限定。

6. 一种式 IIa 的化合物或其药学上可接受的盐：



IIa

其中

R_1 ＝卤素、C_{1-6} 卤代烷基或 OH；

R_2 ＝ H、卤素或 C_{1-6} 卤代烷基，或 R_1 和 R_2 连接在一起形成 -O- 亚乙基 -O- 基；

R_3 ＝ C_{1-6} 卤代烷基；且

R_4 ＝ C_{1-6} 烷基。

7. 如权利要求 6 所述的化合物及其药学上可接受的盐，其中

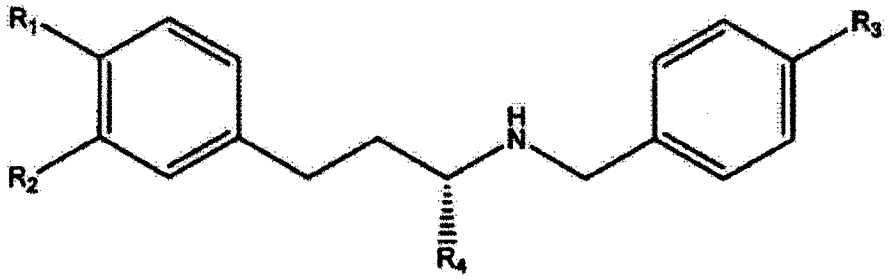
R_1 ＝ Cl、F、CF_3、或 OH；

R_2 ＝ H、Cl、F、CF_3，或 R_1 和 R_2 连接在一起形成 -O- 亚乙基 -O- 基；

R_3 ＝ CF_3；且

R_4 ＝甲基。

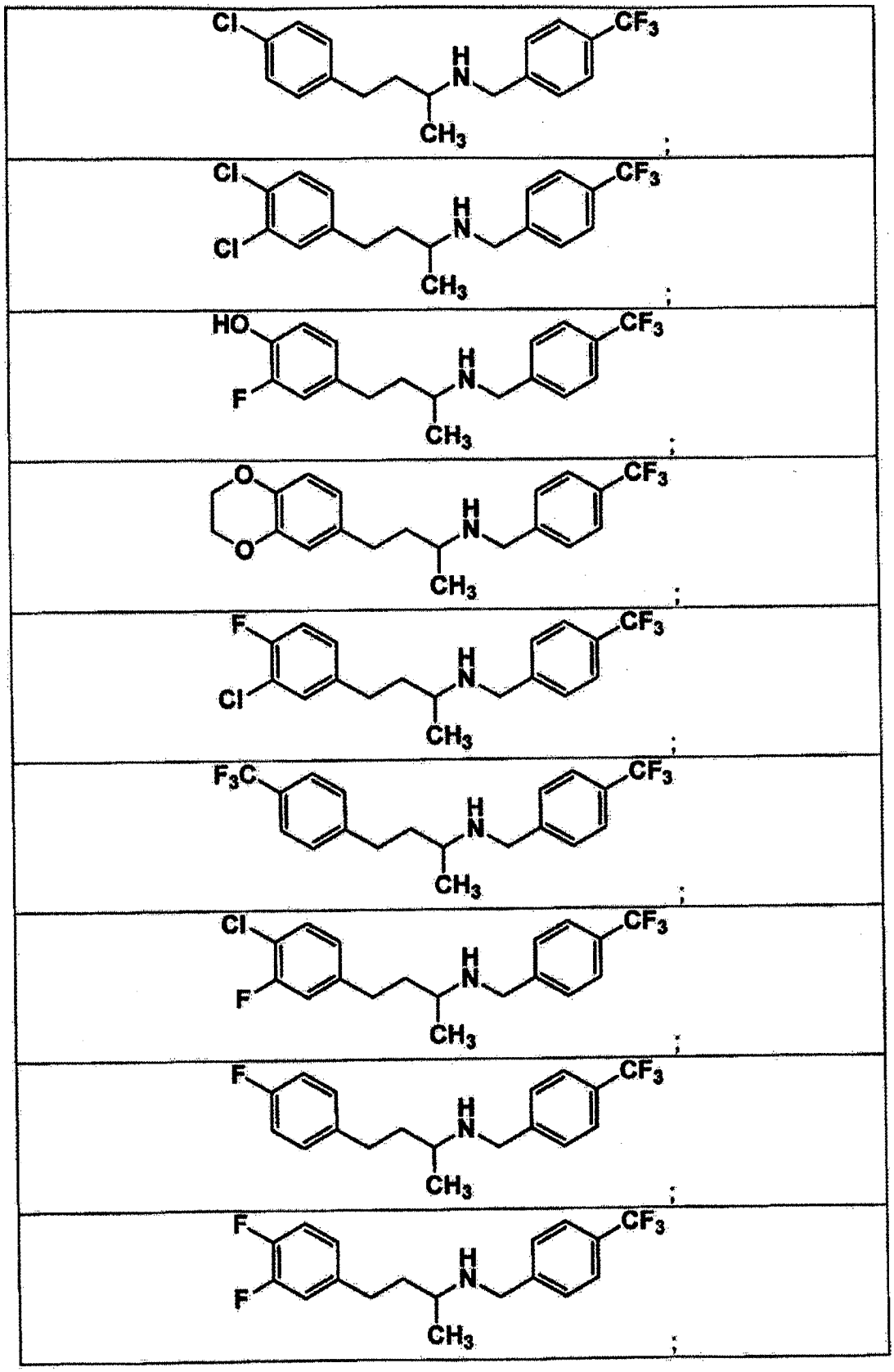
8. 如权利要求 6 所述的化合物及其药学上可接受的盐，所述化合物为式 IIb 的化合物

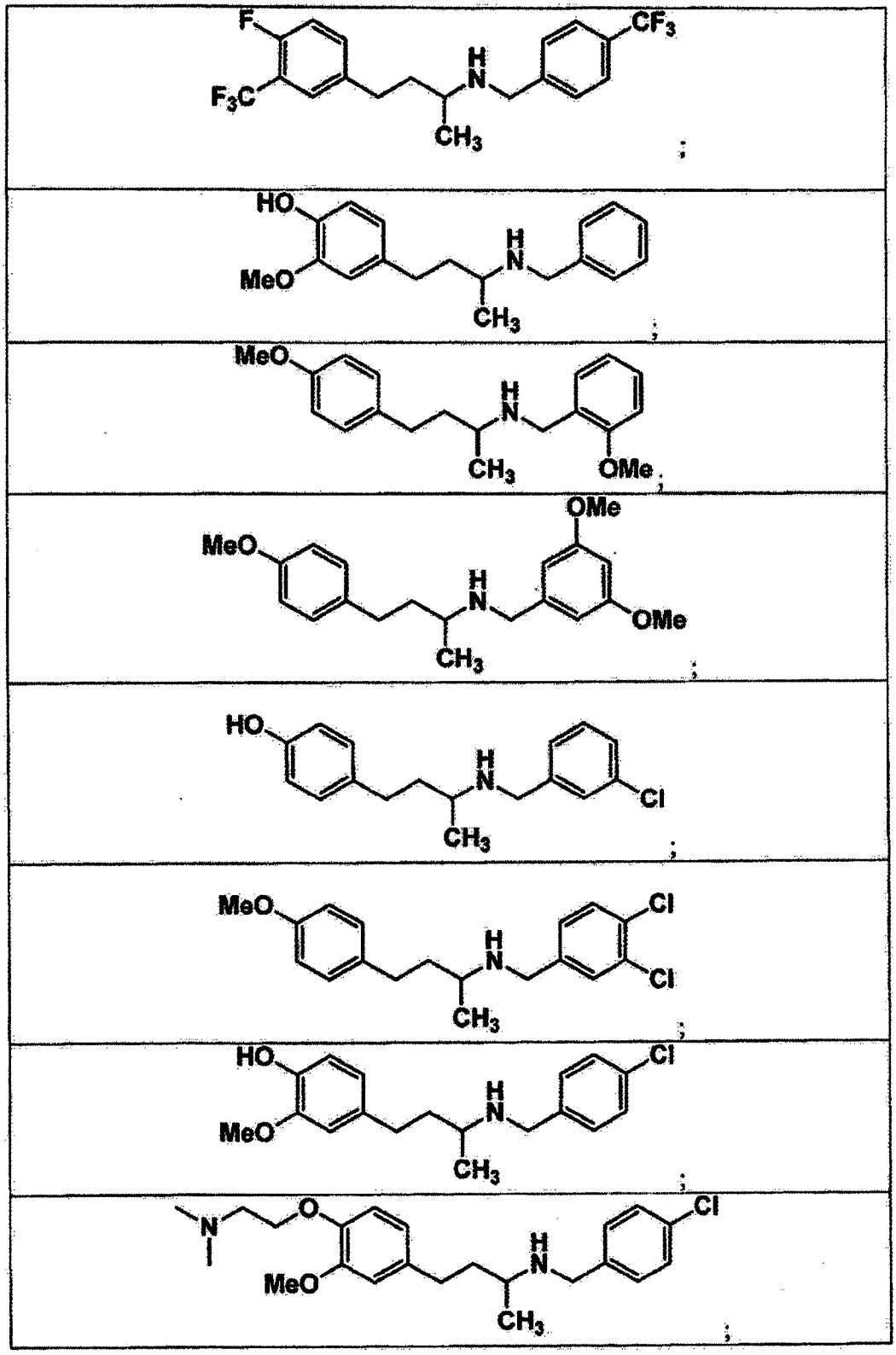


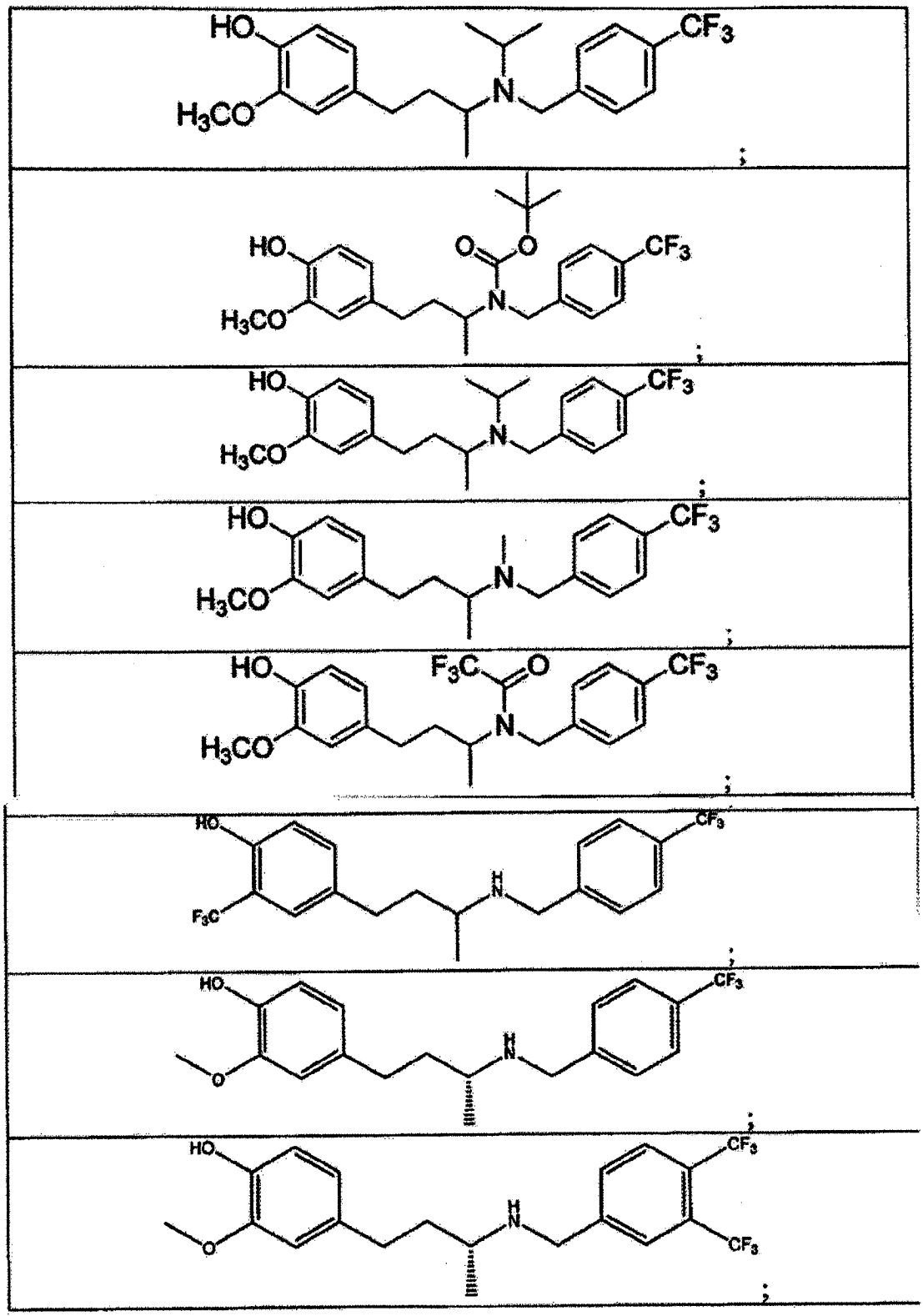
IIb

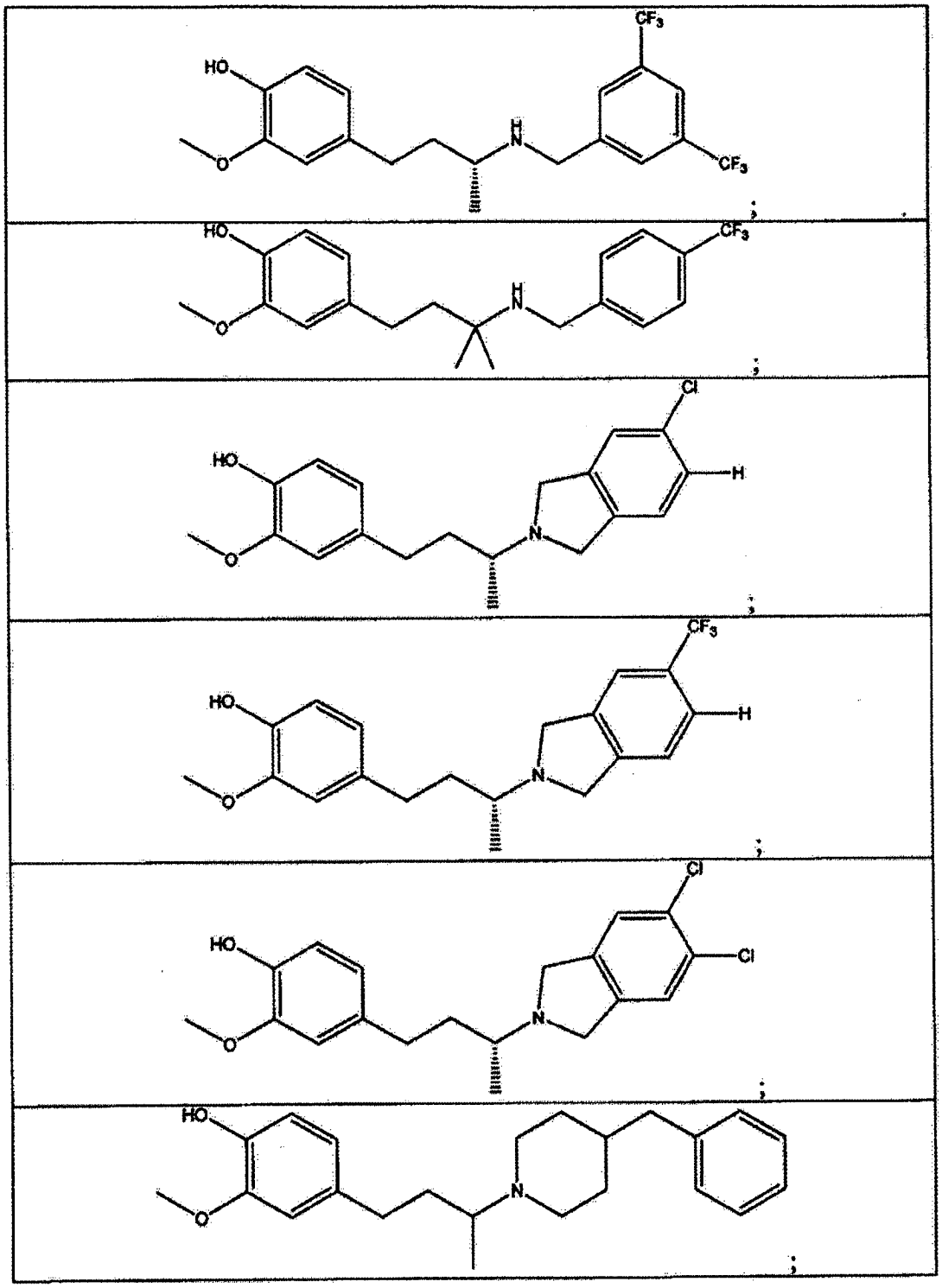
其中 R_1-R_4 如权利要求 6 中所限定。

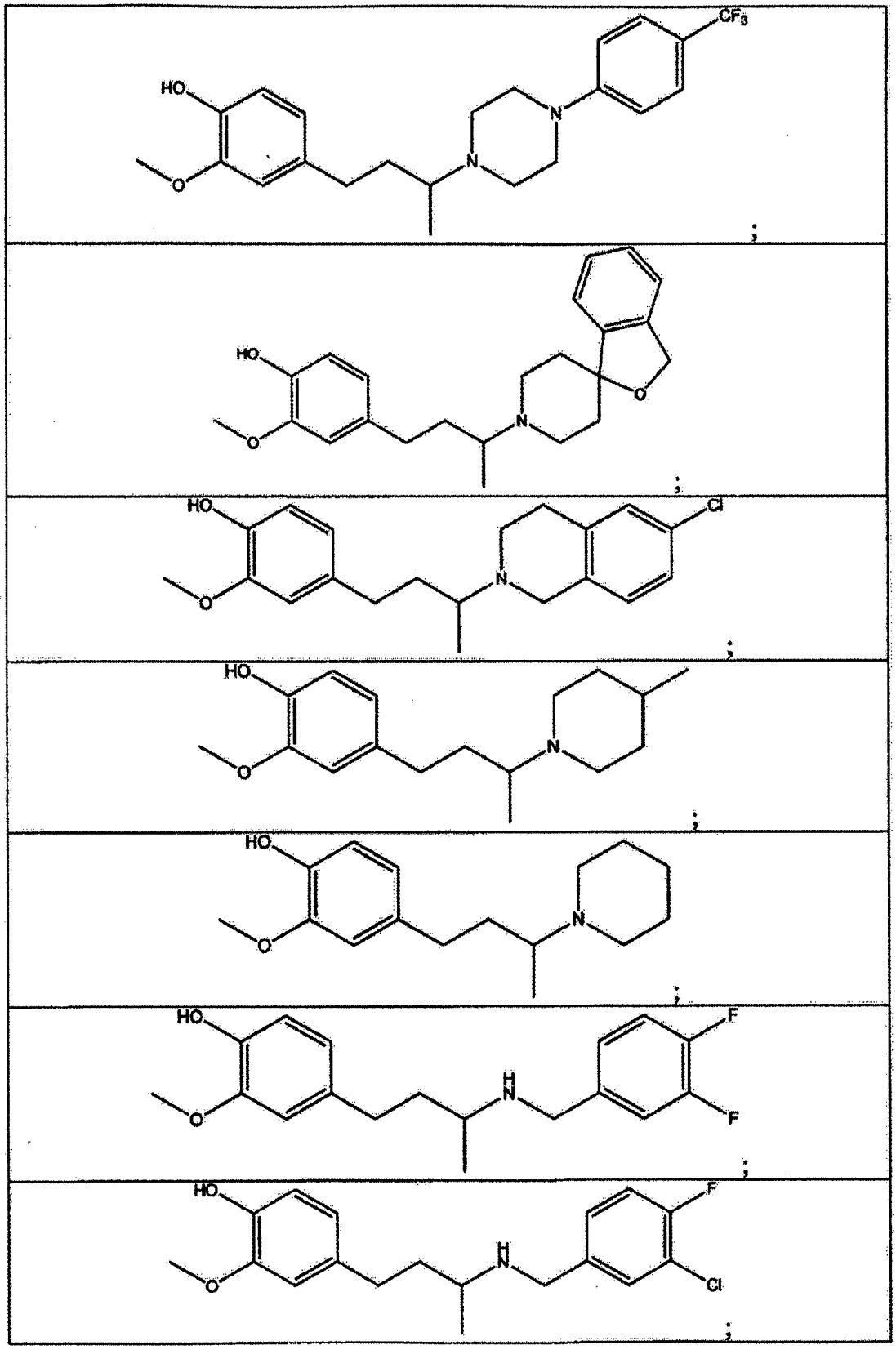
9. 一种化合物，选自

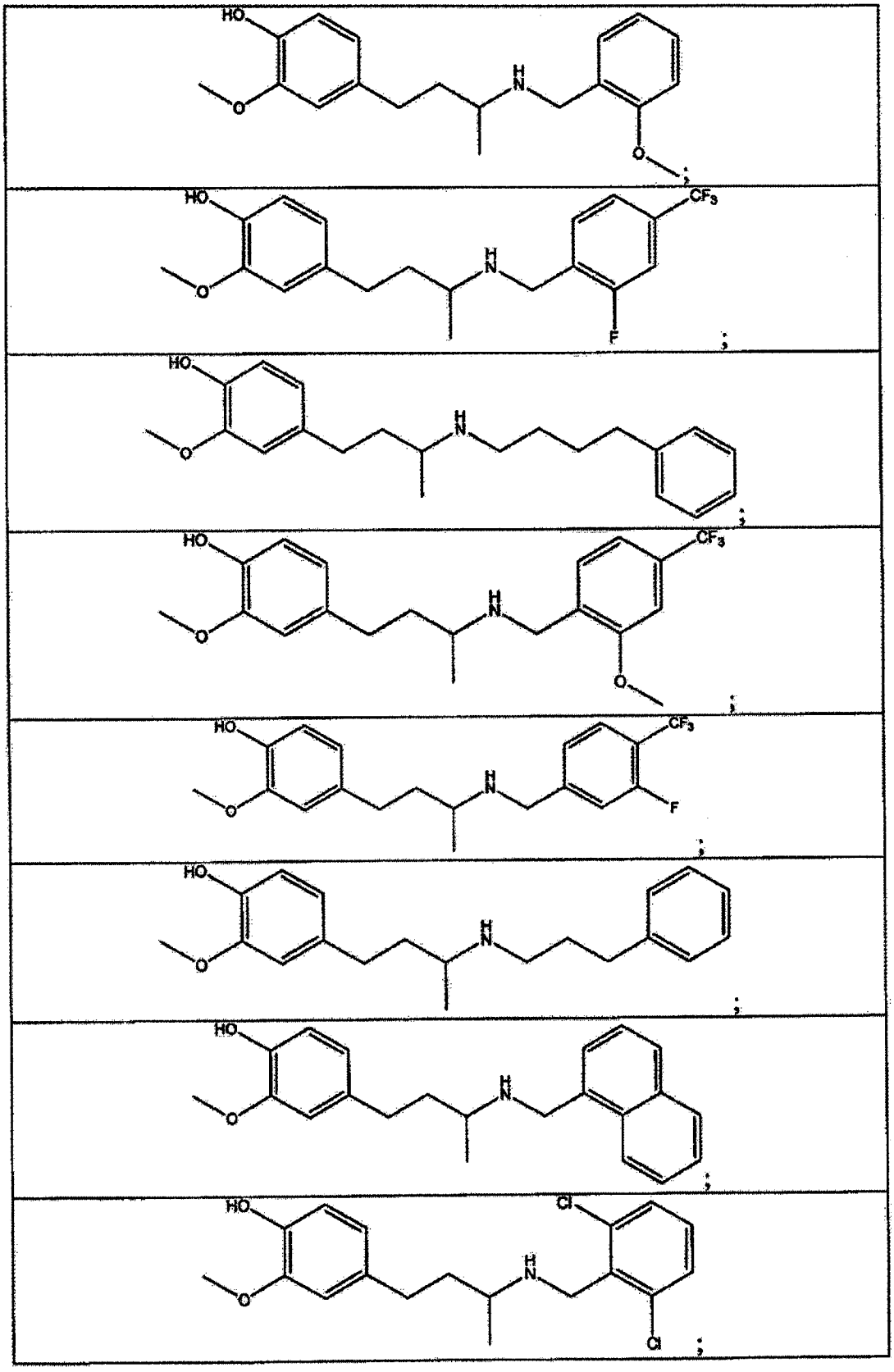


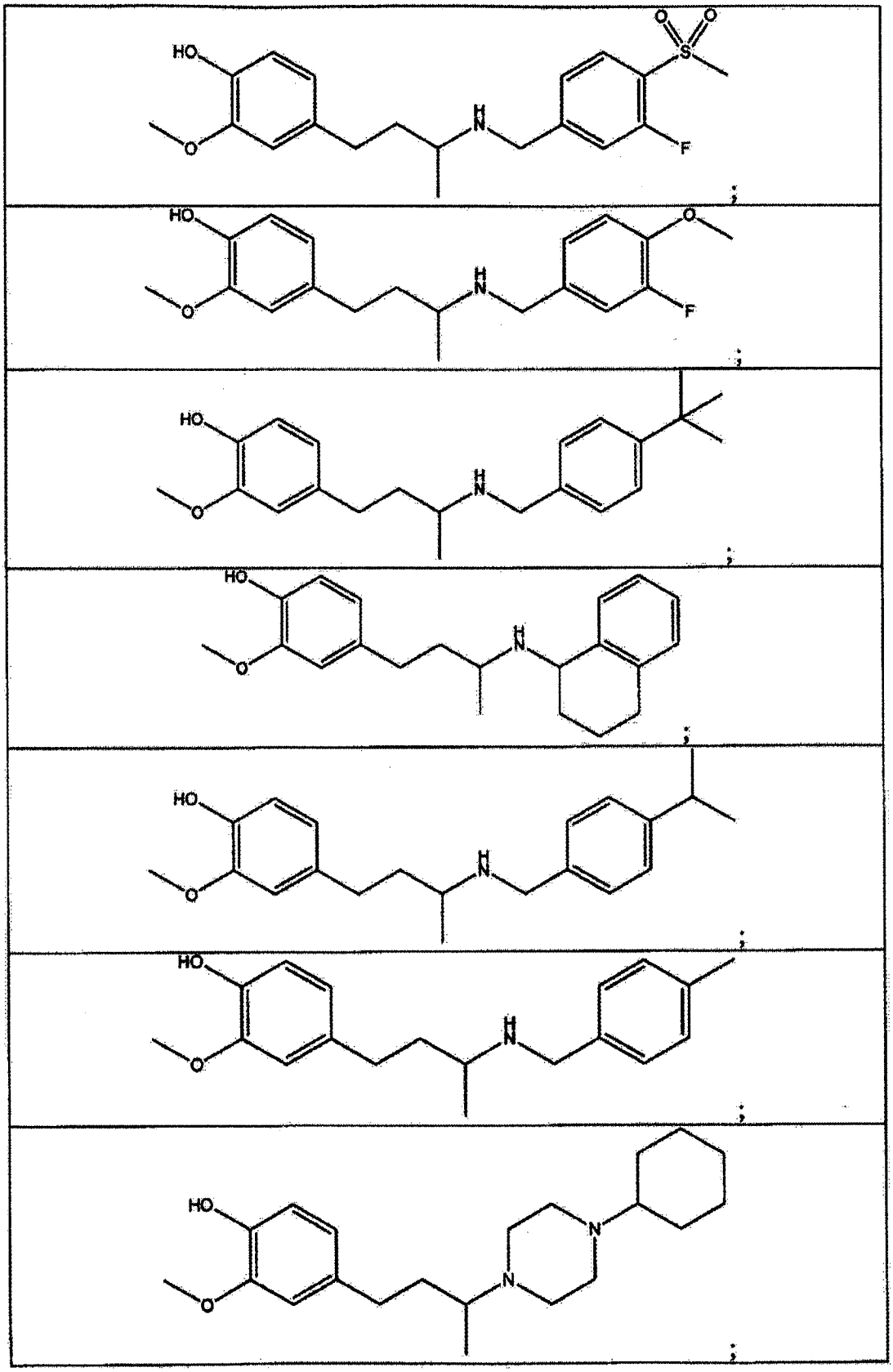


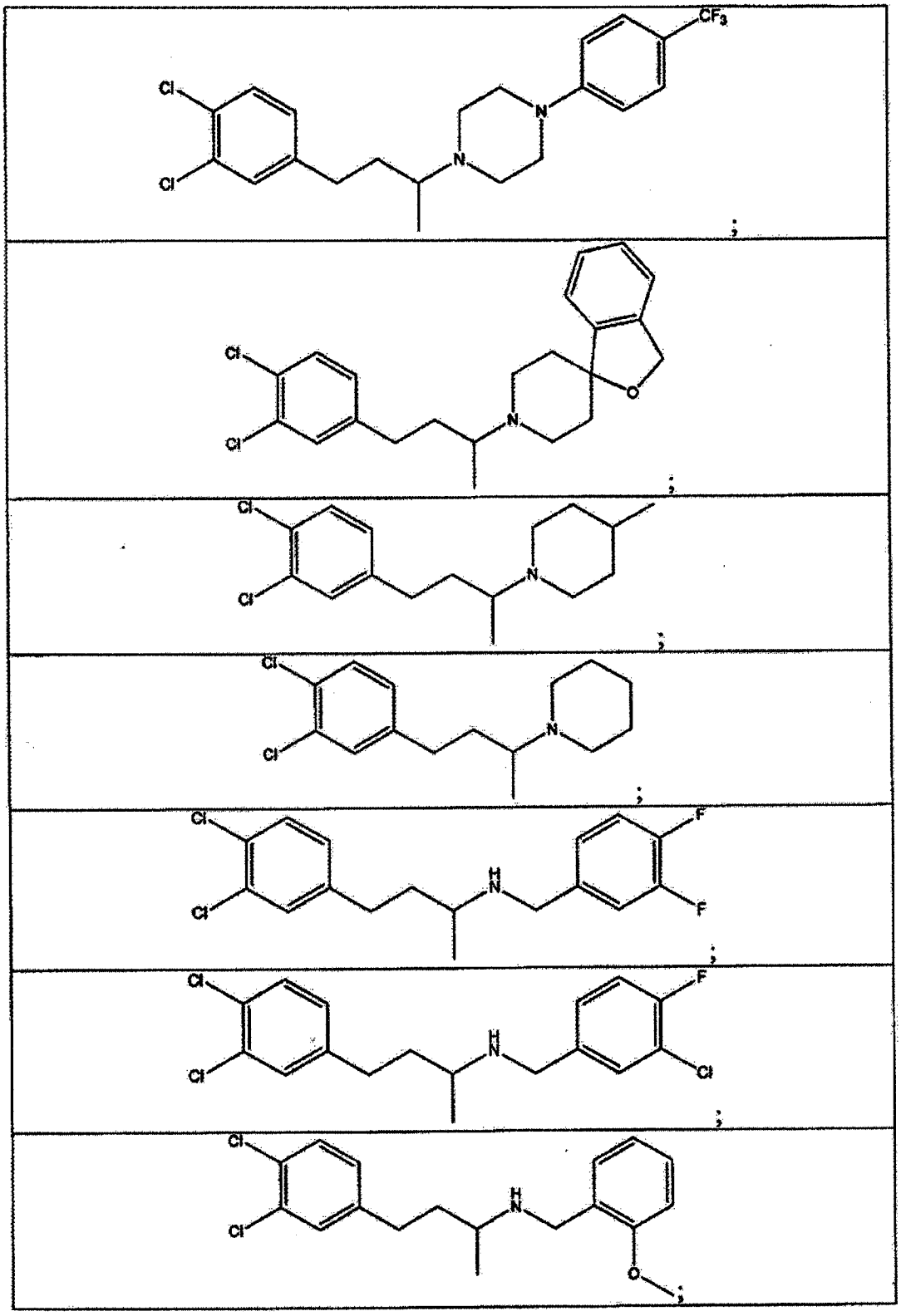


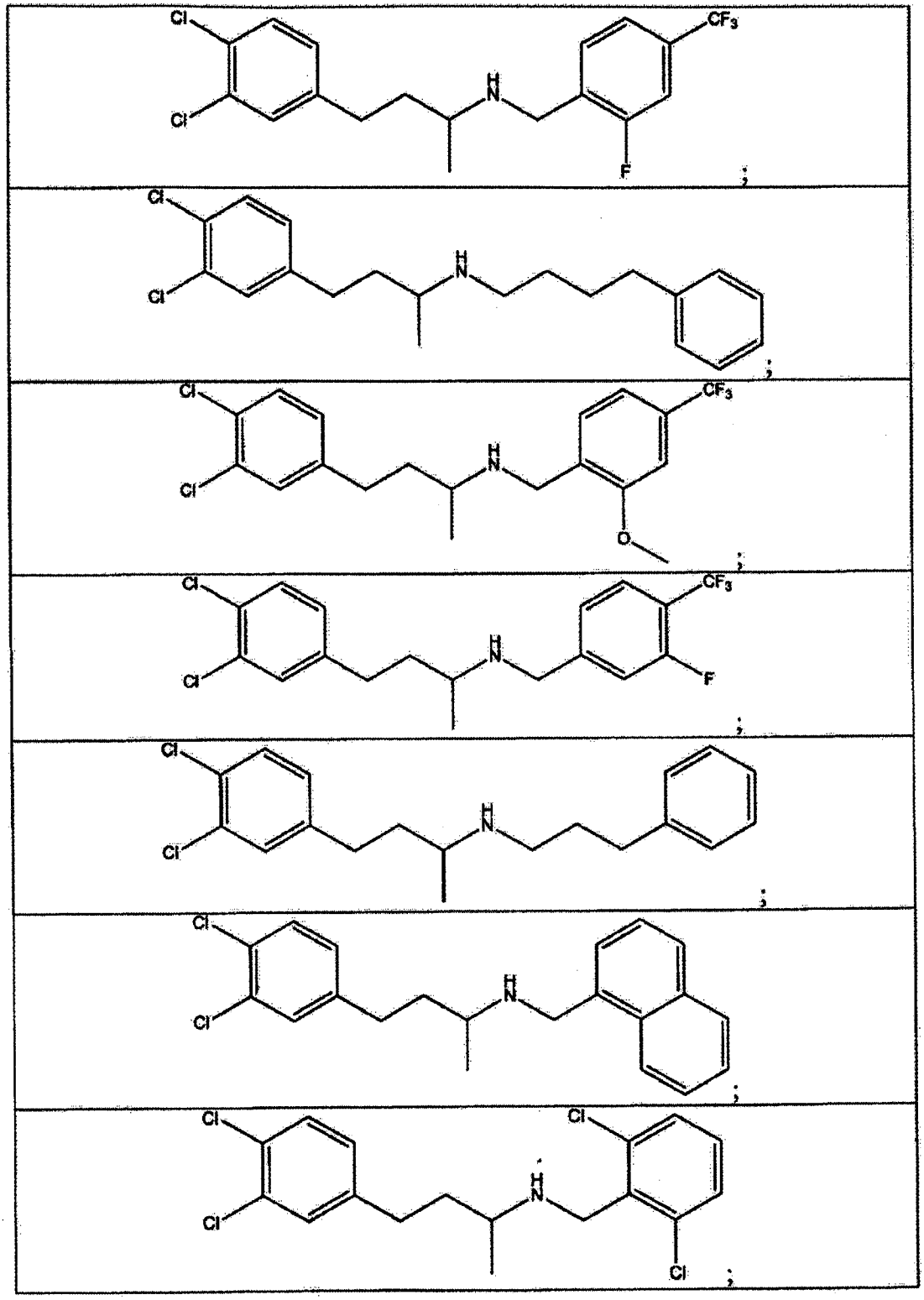


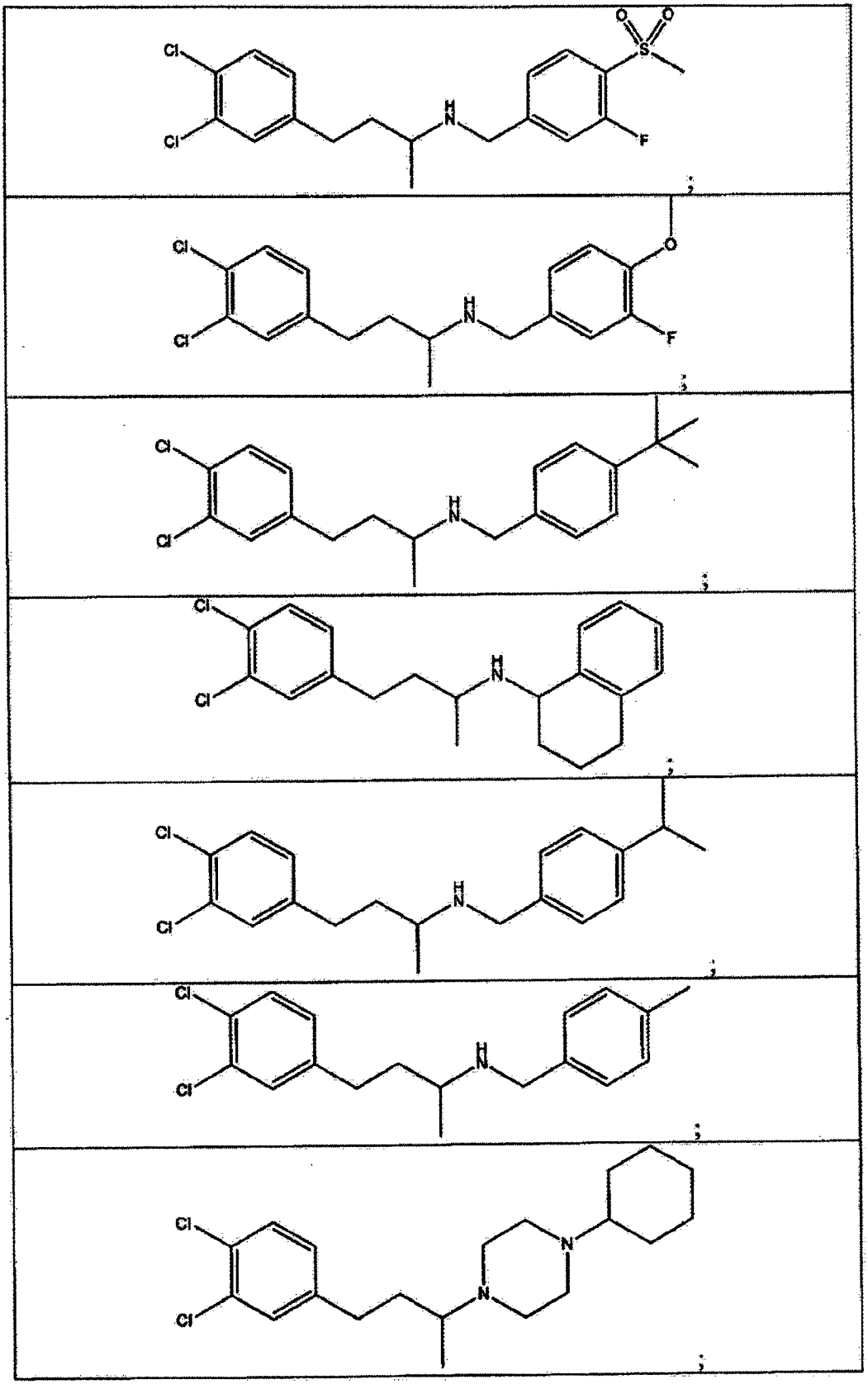


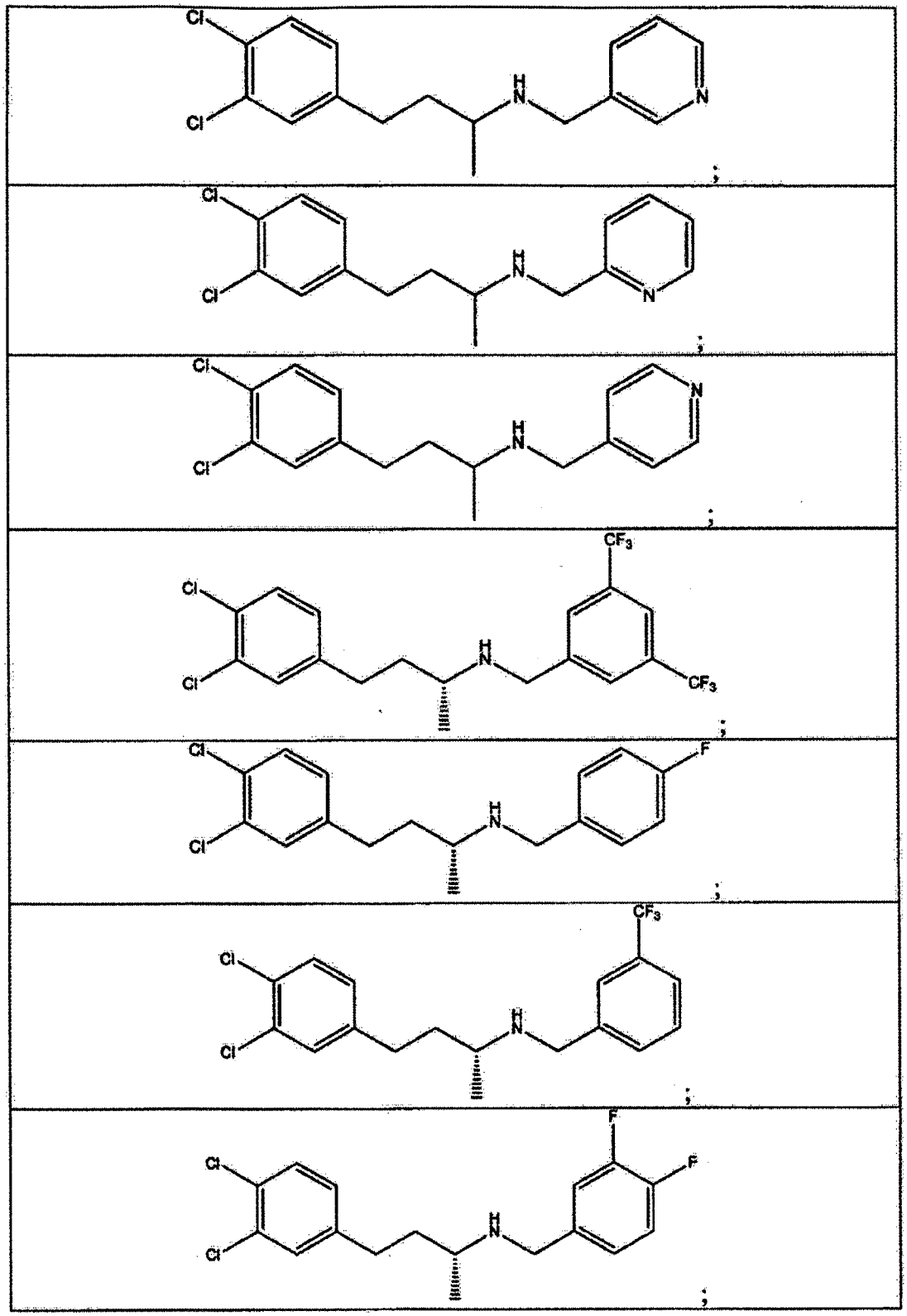


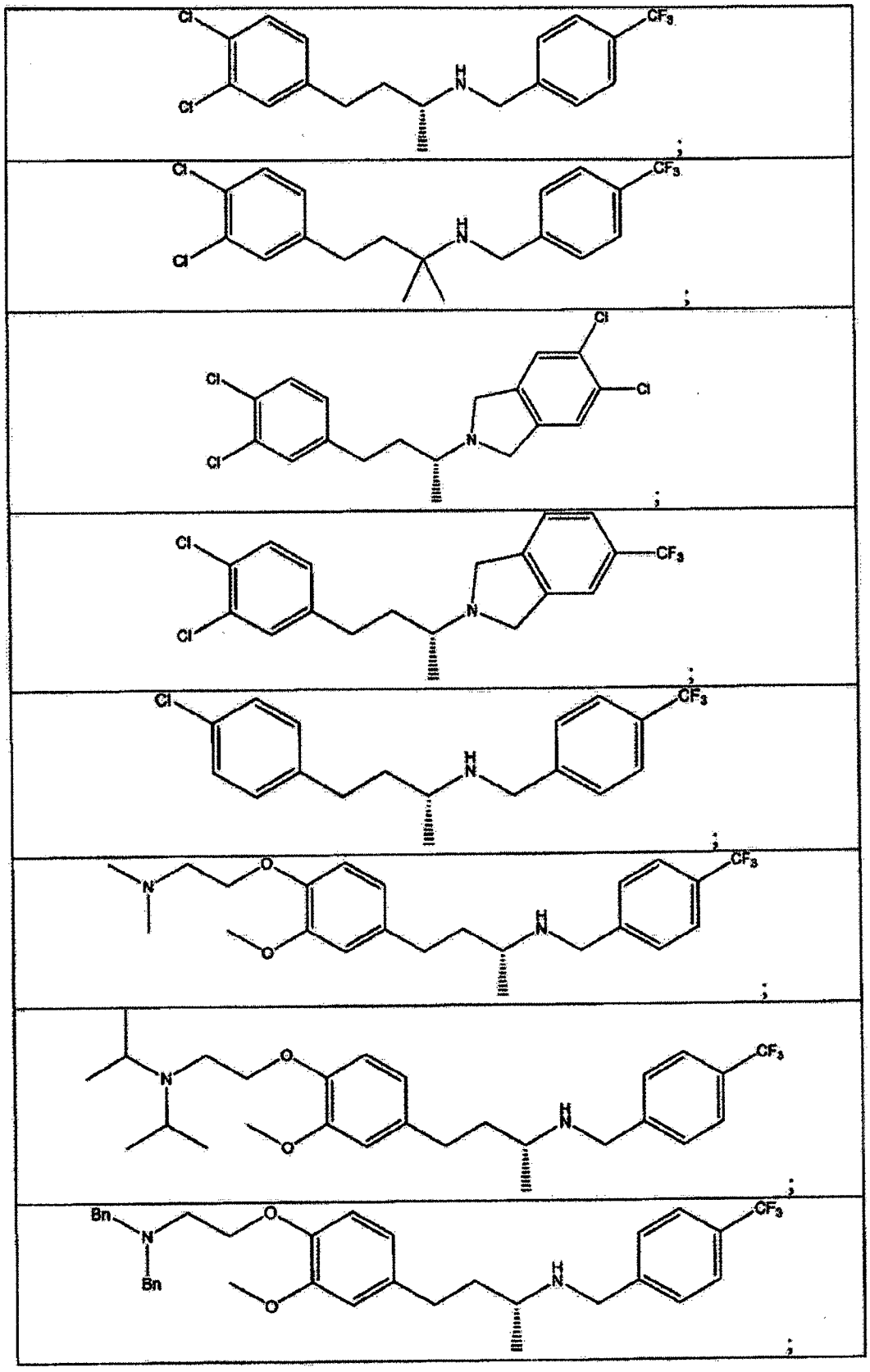


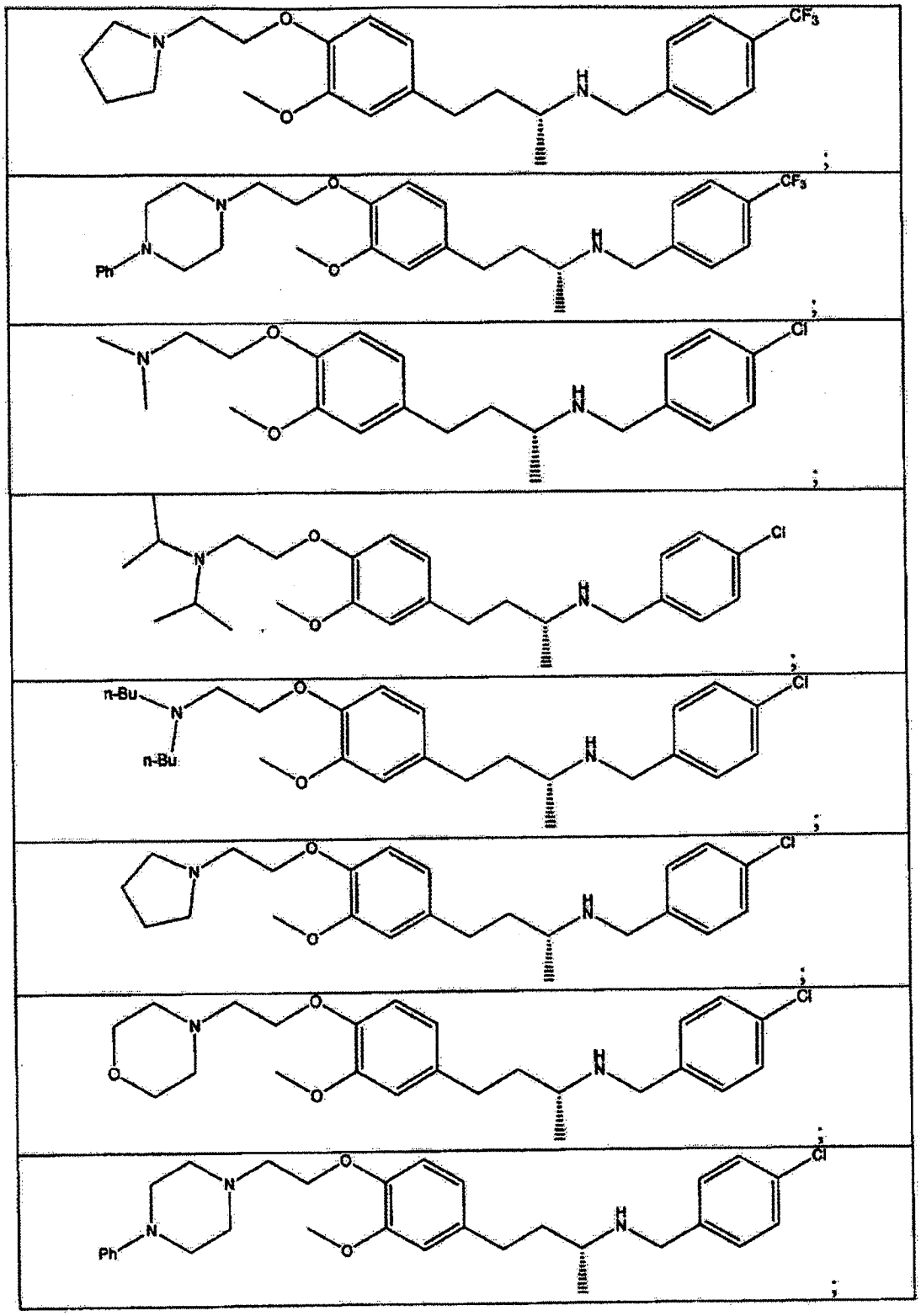


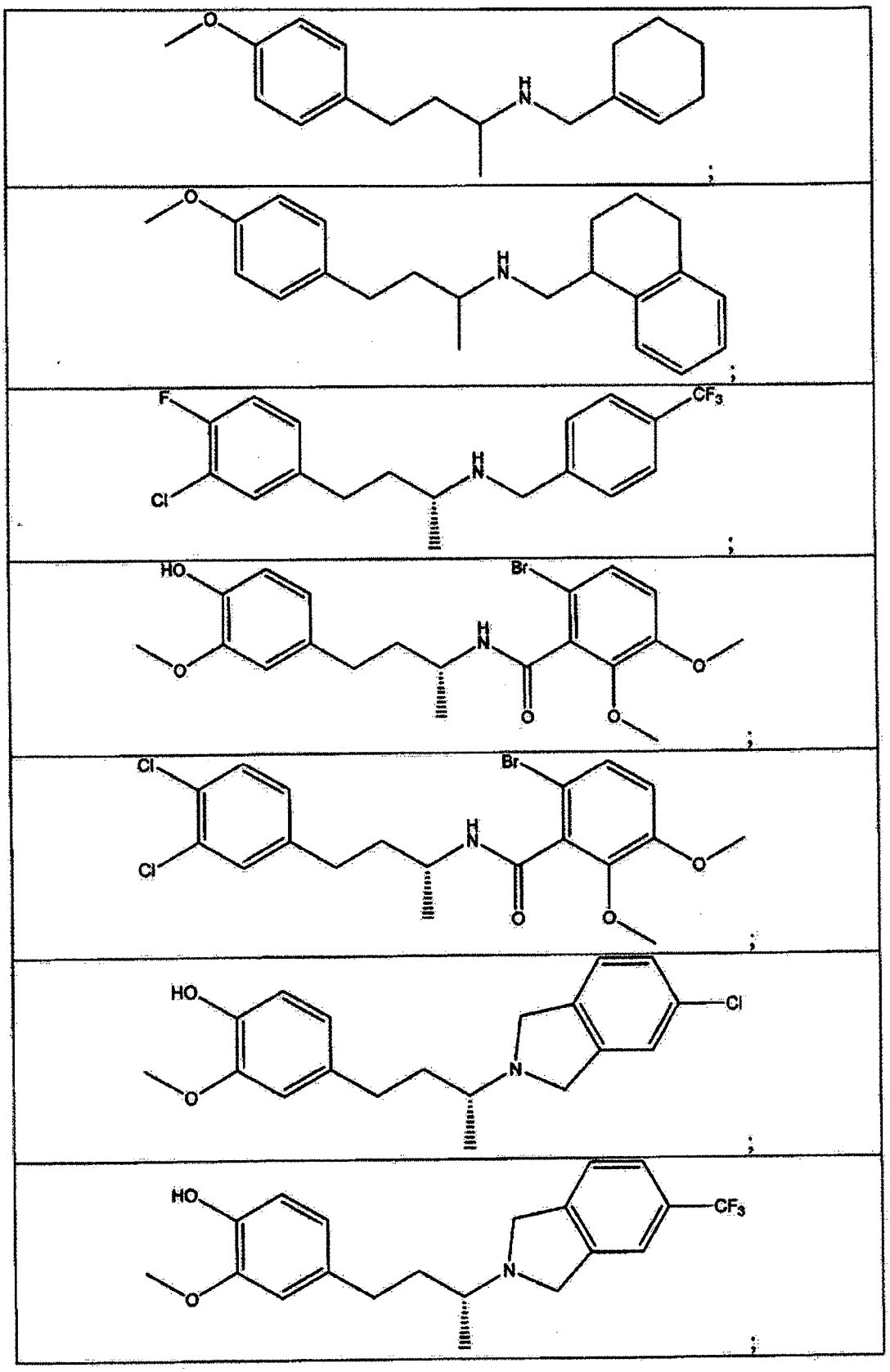


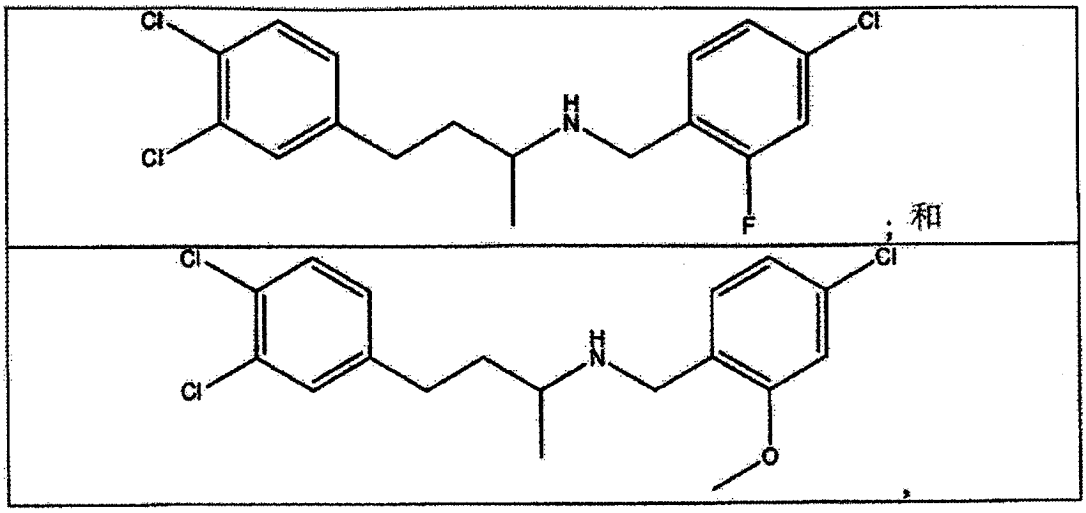








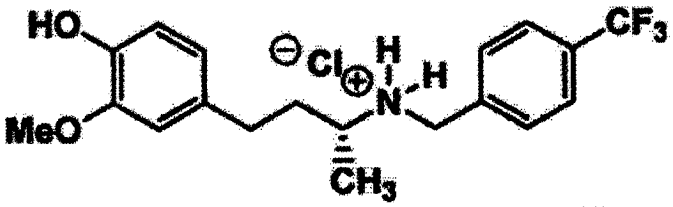


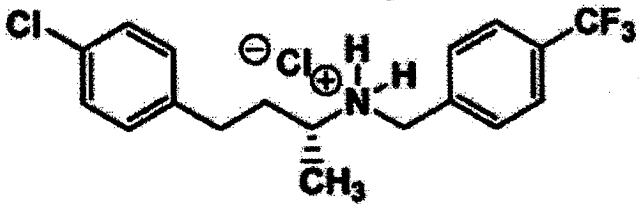


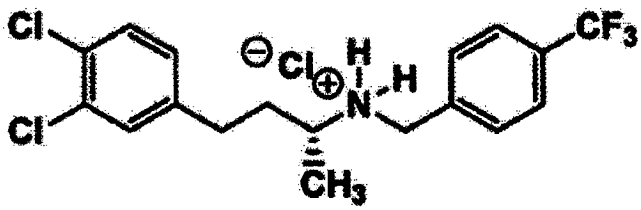
；和

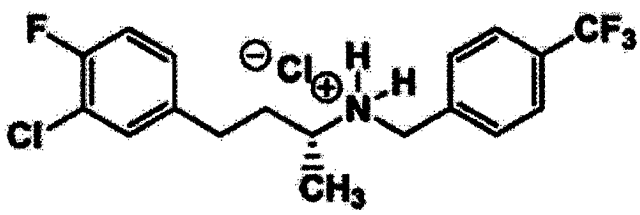
及其药学上可接受的盐。

10. 如权利要求 9 所述的化合物，选自

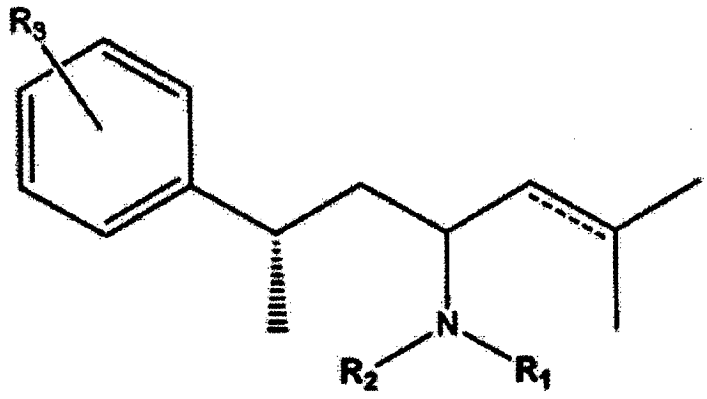






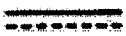


11. 一种式 VIIIa 的化合物及其药学上可接受的盐



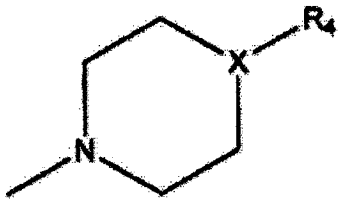
VIIIa

其中：

 是单键或双键；

R_1 是 C_{1-6} 烷基、C_{1-6} 卤代烷基、未被取代的苄基或被卤素、C_{1-6} 烷基或 C_{1-6} 卤代烷基取代的苄基；

R_2 是 H，或

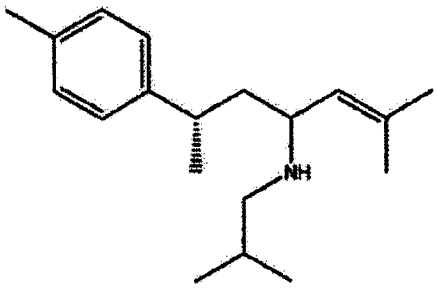
R_1 和 R_2 与氮一起形成环  其中

X 是 CH、N 或 O，且

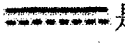
R_4 不存在，或是 H、C_{1-6} 烷基、或未被取代的苯基或被卤素、C_{1-6} 烷基、或 C_{1-6} 卤代烷基取代的苯基；且

R_3 是 C_{1-4} 烷基、卤素、或 C_{1-6} 卤代烷氧基，

条件是排除以下化合物的外消旋混合物：

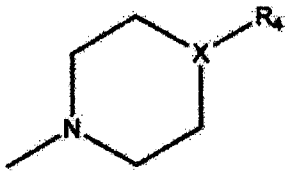


12. 如权利要求 11 所述的化合物及其药学上可接受的盐，其中

 是单键或双键；

R_1 是异丁基、苄基、或被氯、甲基或 CF_3 取代的苄基；

R_2 是 H，或

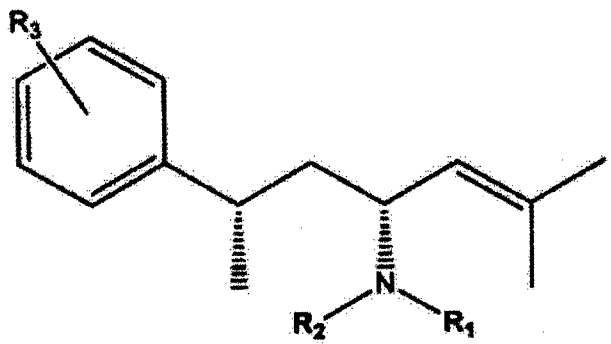
R_1 和 R_2 与氮一起形成环  其中

X 是 CH、N、或 O，且

R_4 不存在，或是 H、异丙基、或未被取代的苯基；且

R_3 是邻 -Me、间 -Me、对 -Me、对 -F、对 $-OCF_3$。

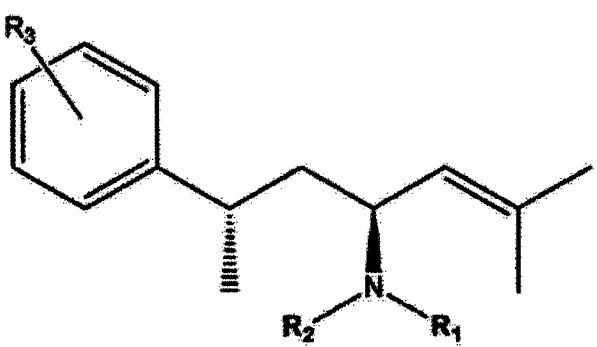
13. 如权利要求 11 所述的化合物及其药学上可接受的盐，所述化合物具有式 VIIIb



VIIIb

其中 R_1-R_3 如权利要求 11 中所限定。

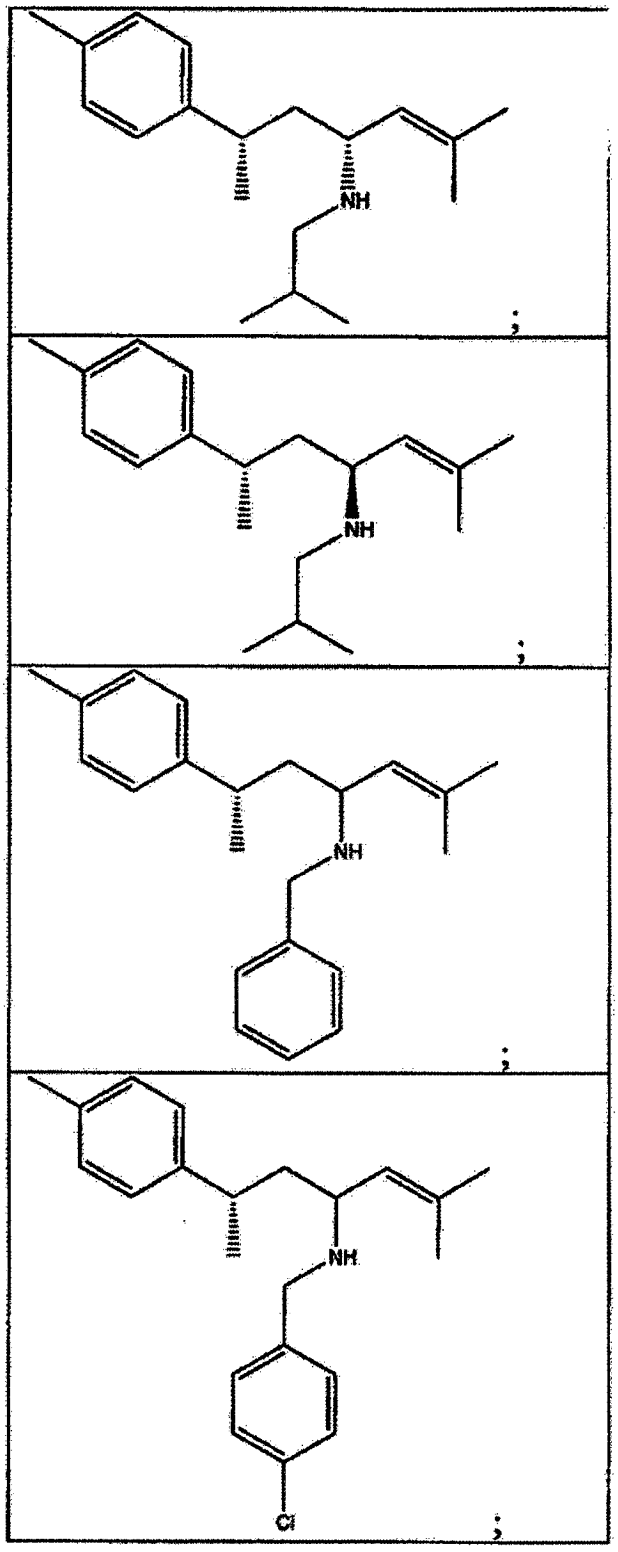
14. 如权利要求 11 所述的化合物及其药学上可接受的盐，所述化合物具有式 VIIIc

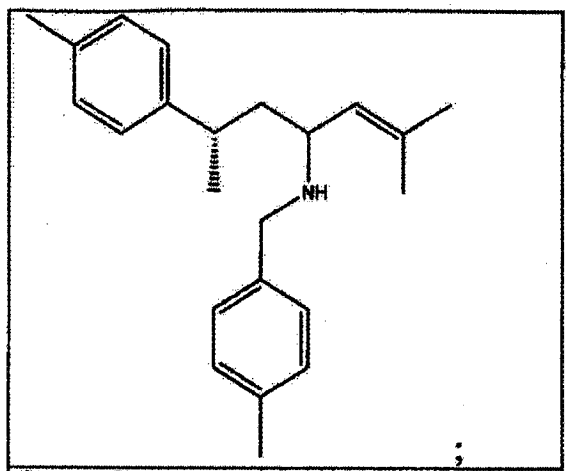


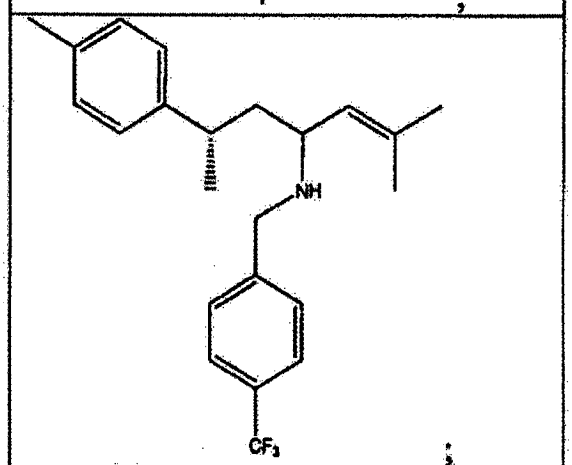
VIIIc

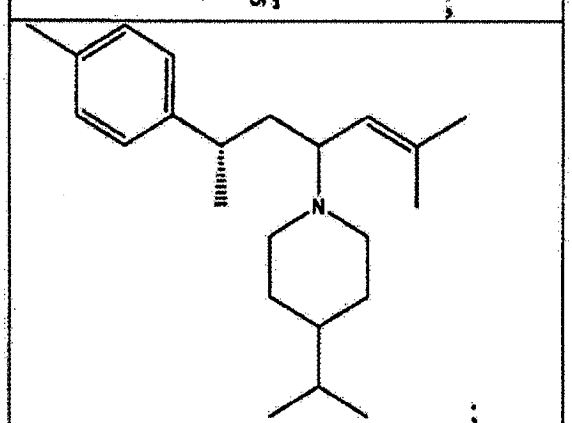
其中 R_1-R_3 如权利要求 11 中所限定。

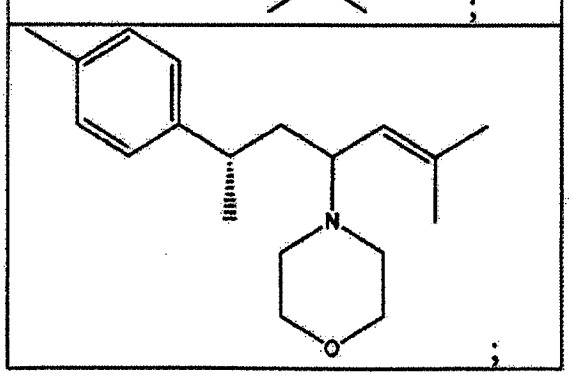
15. 一种化合物，选自

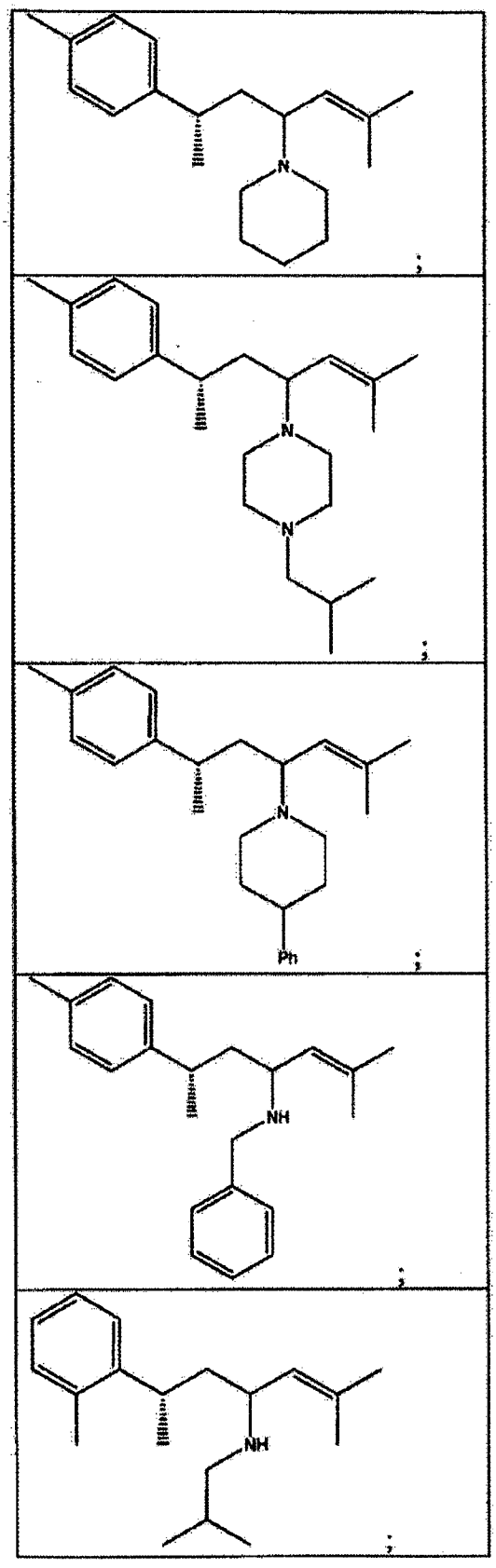

NH
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NH
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NH
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NH
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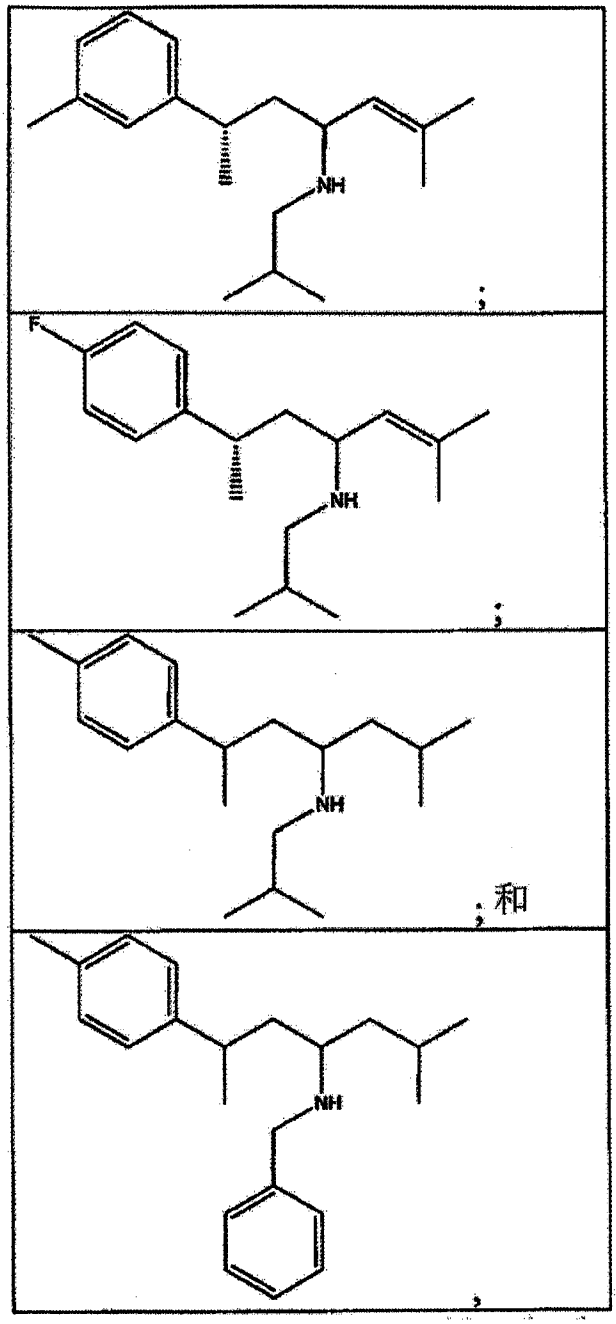

NH


NH
CF3


N

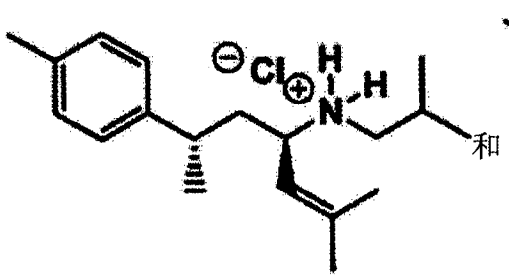

N
O


N
N
N
Ph
NH
NH

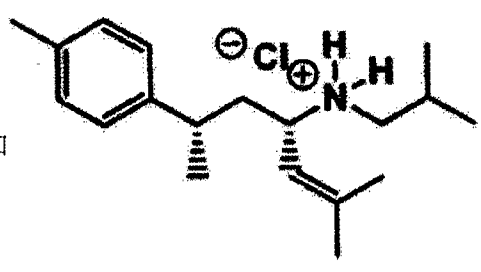


及其药学上可接受的盐。

16. 如权利要求 15 所述的化合物，选自



和



。

17. 一种用于抑制神经元细胞上的 β 淀粉样蛋白效应的方法 / 用途，包括施用有效量的组合物，所述组合物包括，

如权利要求 1-16 中任一项所述的化合物，含量为有效抑制 β 淀粉样蛋白寡聚体在所述细胞中的结合；以及

药学上可接受的载体。

18. 如权利要求 17 所述的方法 / 用途，其中以在所述细胞中还有效抑制膜运输缺陷的量施用所述化合物，所述膜运输效应与所述细胞暴露于可溶 β 淀粉样蛋白寡聚体相关联。

19. 如权利要求 17 和 18 中任一项所述的方法 / 用途，其中所述化合物的含量为在所述细胞中同时有效抑制与所述细胞暴露于可溶 β 淀粉样蛋白寡聚体相关联的寡聚体结合和突触丧失。

20. 如权利要求 17 ～ 19 中任一项所述的方法 / 用途，其中以有效抑制可溶 β 淀粉样蛋白寡聚体所介导的认知效应的量施用所述化合物。

21. 如权利要求 20 所述的方法 / 用途，其中所述认知效应是在认知衰退的动物模型中所测试的认知衰退。

22. 如权利要求 21 所述的方法 / 用途，其中所述认知衰退是通过条件性恐惧检定所测试的学习衰退。

23. 如权利要求 21 所述的方法 / 用途，其中所述认知衰退是通过 Morris 水迷宫测试所测试的空间学习和记忆的衰退。

24. 如权利要求 21 所述的方法 / 用途，其中所述认知衰退是在阿尔茨海默病的转基因动物模型中所测试的基于海马的空间学习和记忆的衰退。

25. 如权利要求 17 所述的方法 / 用途，其用于抑制神经元细胞的 β 淀粉样蛋白寡聚体所诱导的突触功能障碍，包括使所述细胞与包括含量为在所述细胞中有效抑制 β 淀粉样蛋白寡聚体结合的 σ-2 受体拮抗剂化合物的组合物接触，所述功能障碍与所述细胞暴露于可溶 β 淀粉样蛋白寡聚体相关联。

26. 如权利要求 17 所述的方法 / 用途，其用于抑制受试者中长时程增强效应的压制，包括对需要的受试者施用治疗有效量的包括 σ-2 受体拮抗剂化合物的组合物。

27. 如权利要求 17 所述的方法 / 用途，其用于抑制表现出认知衰退或具有表现出认知衰退的风险的受试者中的认知衰退，包括对所述受试者施用治疗有效量的包括 σ-2 受体拮抗剂化合物的组合物。

28. 如权利要求 17 所述的方法 / 用途，其用于抑制受试者中与中枢神经元上的 β 淀粉样蛋白寡聚体效应相关联的认知衰退，包括对遭受所述认知衰退的受试者施用治疗有效量的包括 σ-2 受体拮抗剂化合物的组合物。

29. 如权利要求 17 所述的方法 / 用途，其用于治疗需要的受试者中的阿尔茨海默病中的轻度认知障碍，包括对所述受试者施用治疗有效量的包括 σ-2 受体拮抗剂化合物的组合物。

30. 如权利要求 25 ～ 27 中任一项所述的方法 / 用途，其中所述 σ-2 拮抗剂化合物具有一个或多个以下额外特性：

(a) 相比于一种或多种非 σ CNS 受体，其以高出至少 10 倍、20 倍、50 倍或 100 倍的亲和性与 σ-2 受体选择性结合，其中所述化合物以小于 200nM、150nM、100nM 或 60nM 的 K_i 与 σ-2 受体结合；

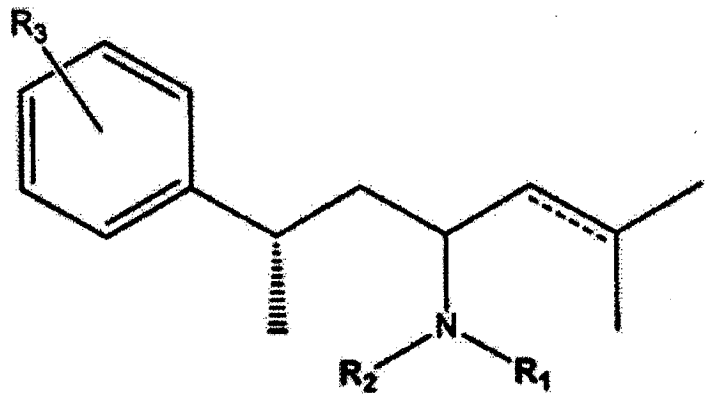
(b) 其抑制 Abeta 寡聚体结合至神经元细胞或神经元细胞中的突触丧失，所述丧失与

所述细胞暴露于 Abeta 寡聚体相关联；

(c) 其抑制中枢神经元中的膜运输异常，所述异常与所述细胞暴露于一种或多种 Abeta 寡聚体相关联；

(d) 在不存在 β 淀粉样蛋白寡聚体的情况下，其不能影响中枢神经元中的运输或突触数目。

31. 如权利要求 11 所述的化合物及其药学上可接受的盐，其根据式 VIIIa



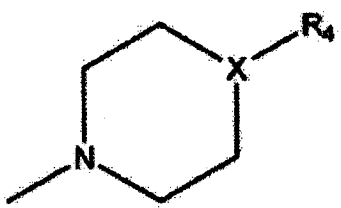
VIIIa

其中：

是单键或双键；

R_1 是 C_{1-6} 烷基、C_{1-6} 卤代烷基、未被取代的苄基或被卤素、C_{1-6} 烷基或 C_{1-6} 卤代烷基取代的苄基；

R_2 是 H，或

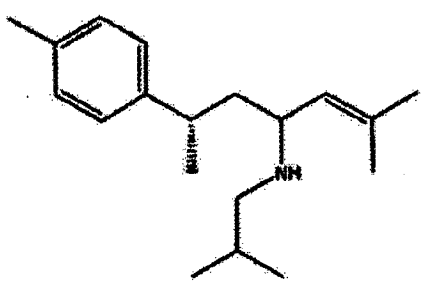
R_1 和 R_2 与氮一起形成环 

其中

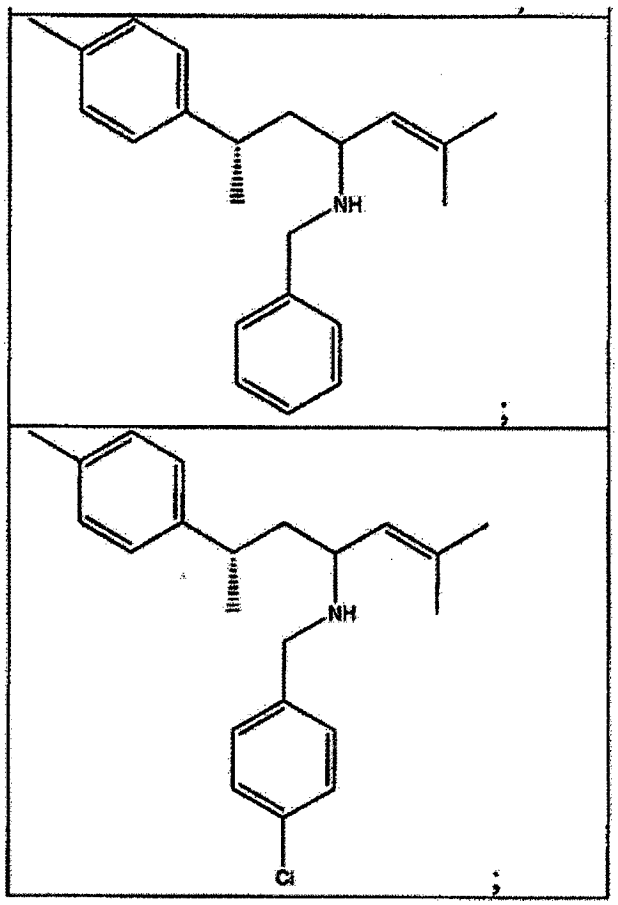
X 是 CH、N、或 O，且

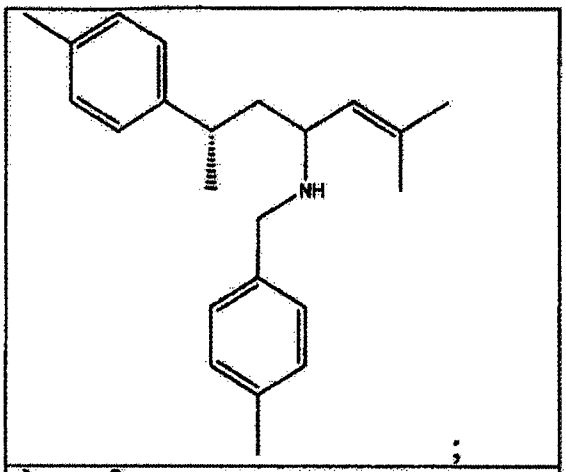
R_4 不存在，或是 H、C_{1-6} 烷基、或未被取代的苯基或被卤素、C_{1-6} 烷基、或 C_{1-6} 卤代烷基取代的苯基；且

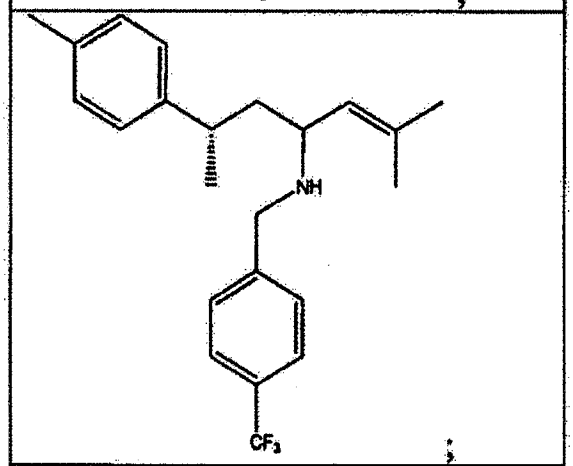
R_3 是 C_{1-4} 烷基、卤素、或 C_{1-6} 卤代烷氧基，条件是排除以下化合物的外消旋混合物及其拆分得到的单一化合物

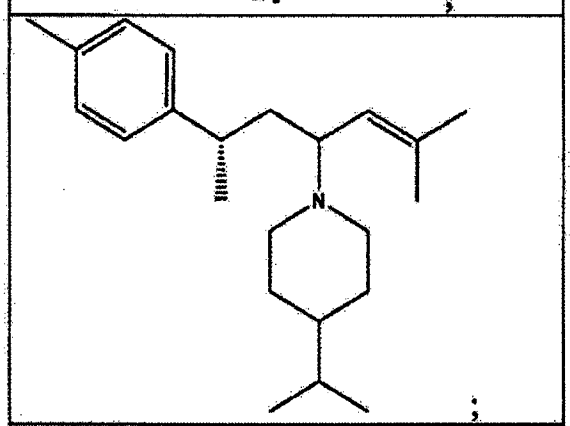


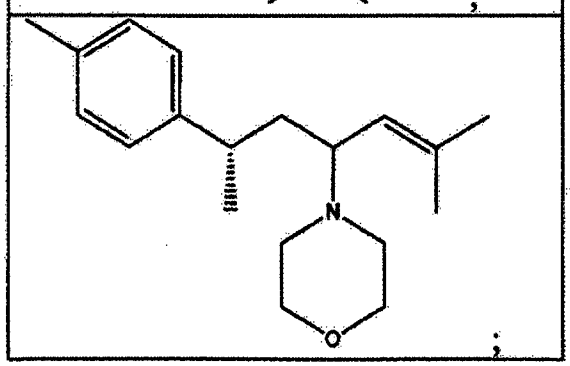
32. 如权利要求 15 所述的化合物，选自

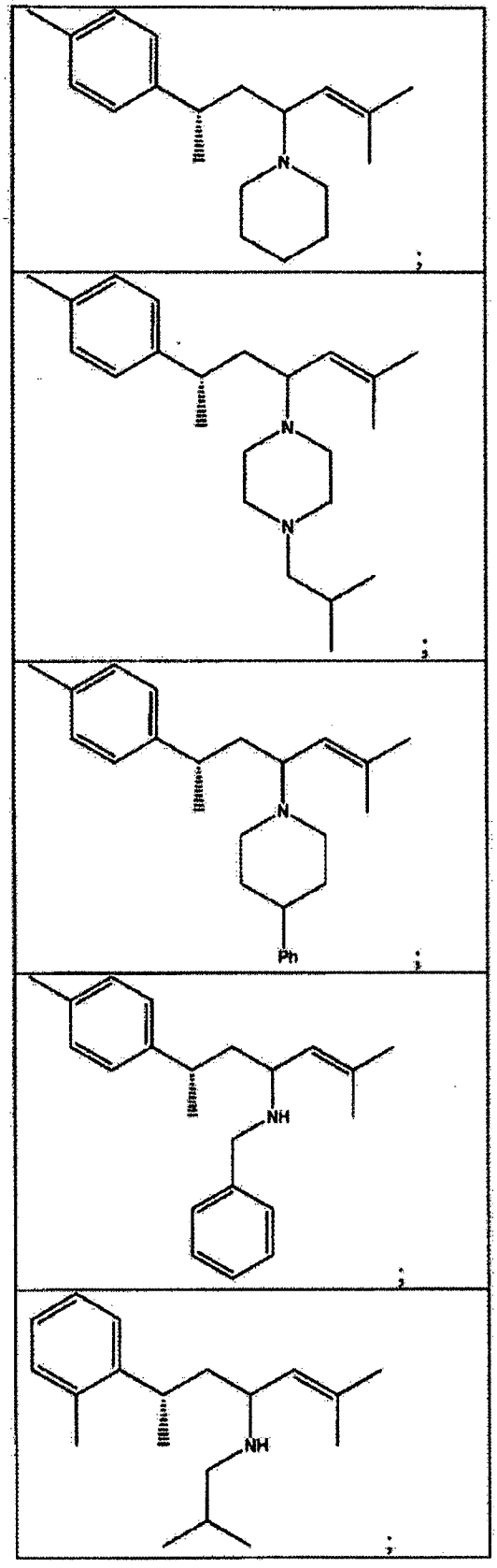

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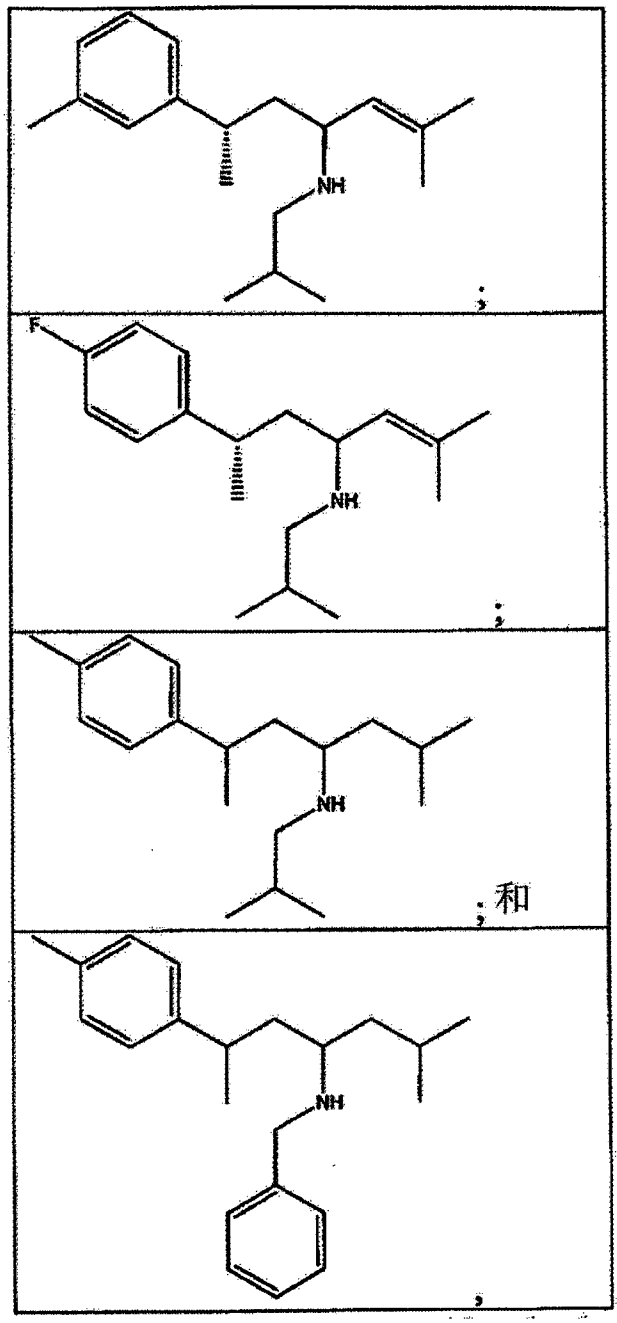

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及其药学上可接受的盐。