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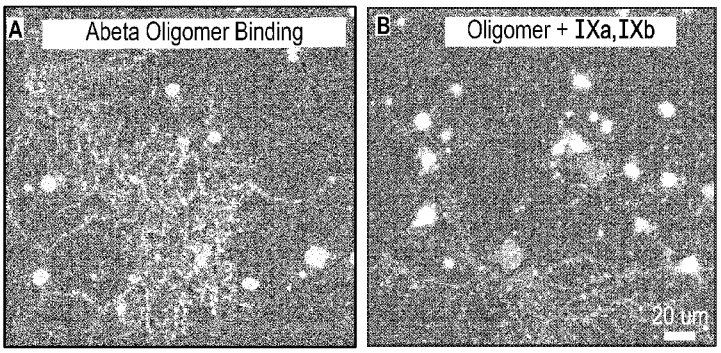

FIG. 3B

(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.

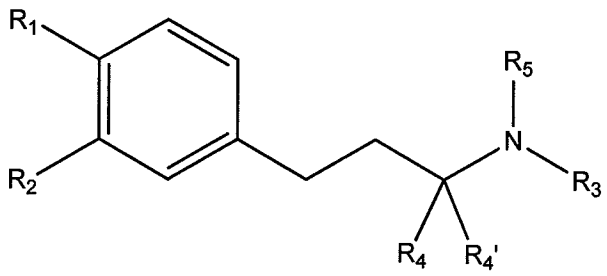
[008] The presence of soluble Aβ oligomers is believed to be to be responsible for early cognitive decline in the pre-Alzheimer's diseased brain. It is known that amyloid beta oligomers bind at neuronal synapses and that sigma-2 receptors are present in significant amounts in neurons and glia.

[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:

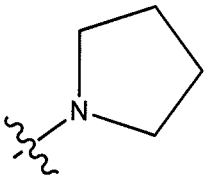


I

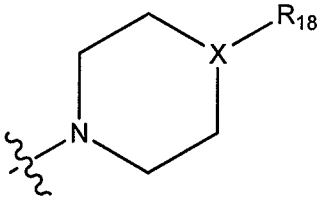
wherein

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

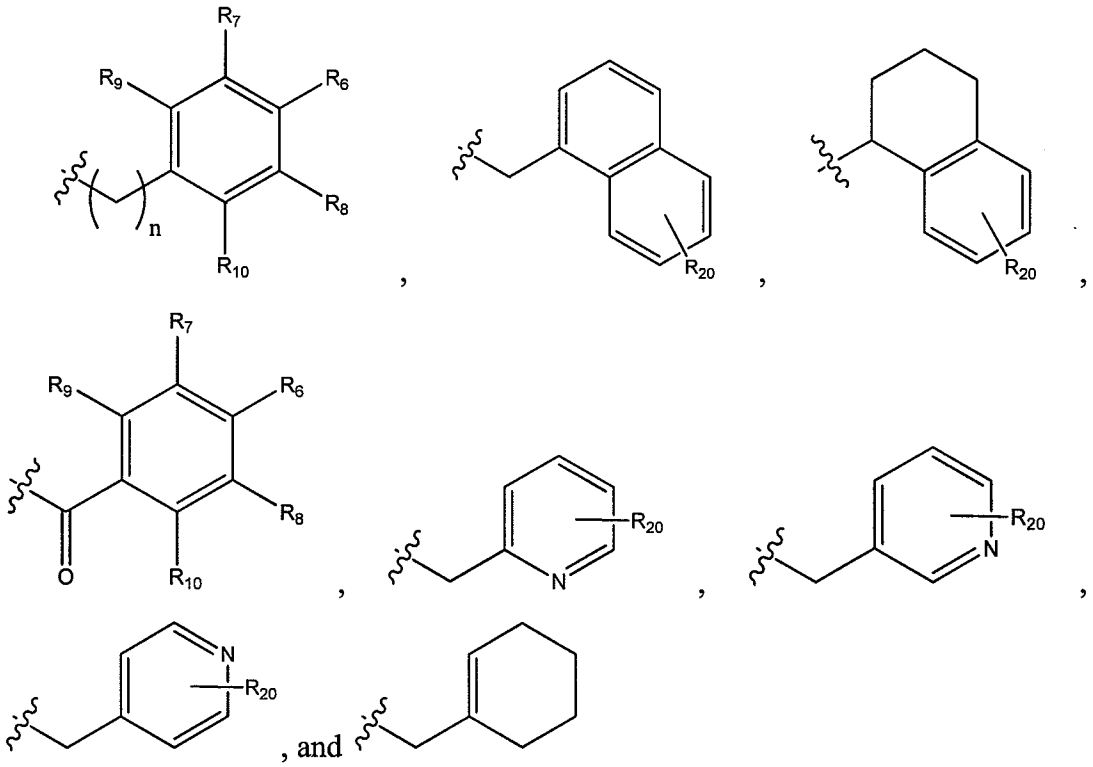


, wherein

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

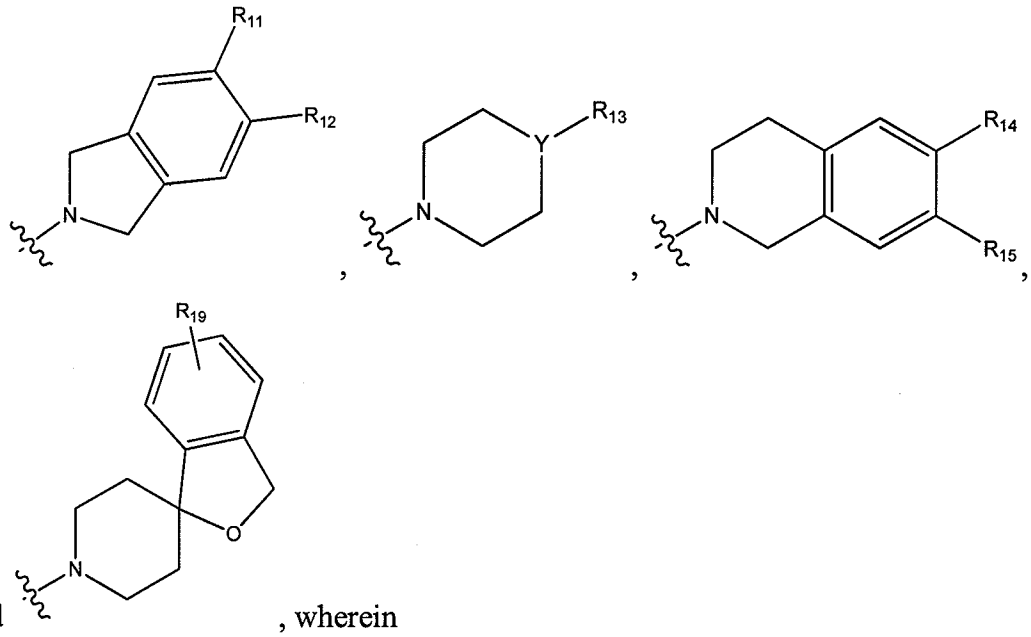
n is 1-4

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



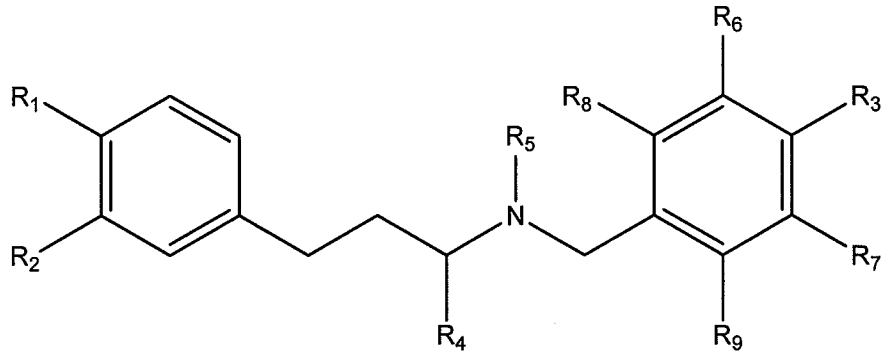
and , wherein

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 (4^{th} 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.

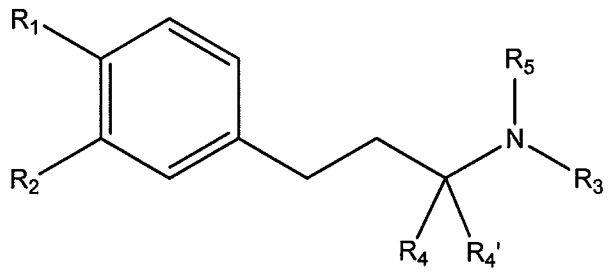
[0100] As used herein, "cognitive decline" can be any negative change in an animal's cognitive function. For example cognitive decline, includes but is not limited to, memory loss (e.g. behavioral memory loss), failure to acquire new memories, confusion, impaired judgment, personality changes, disorientation, 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

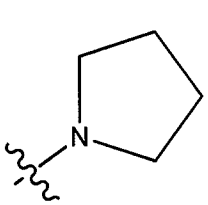


I

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



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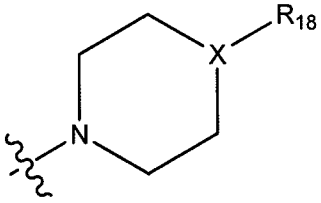
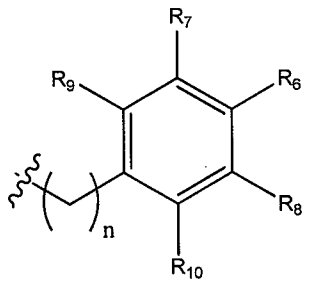


, wherein

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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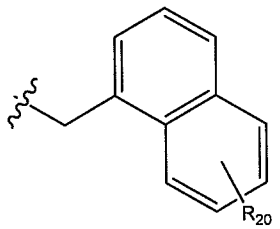

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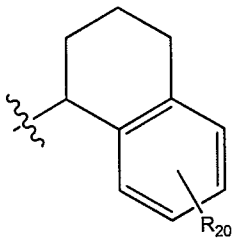

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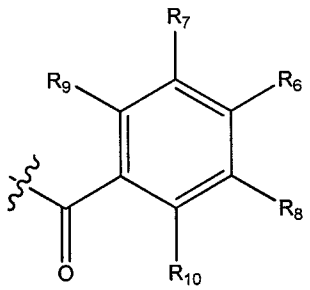

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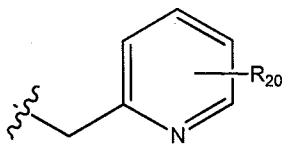

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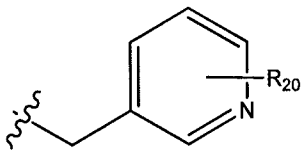

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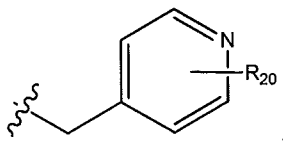

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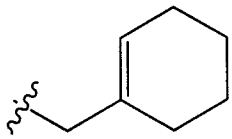


wherein

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

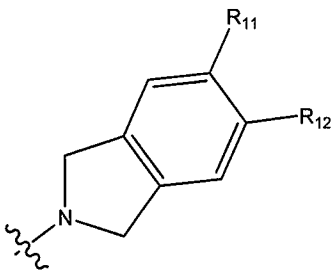
n is 1-4

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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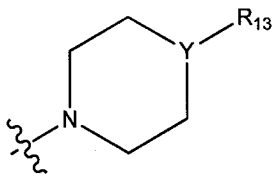

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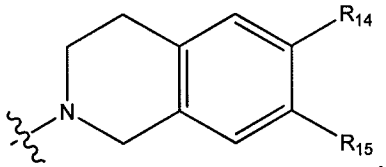

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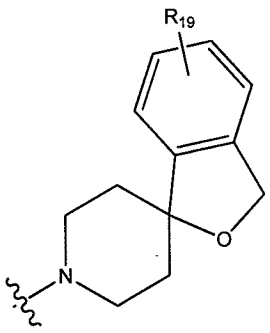


and , wherein

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

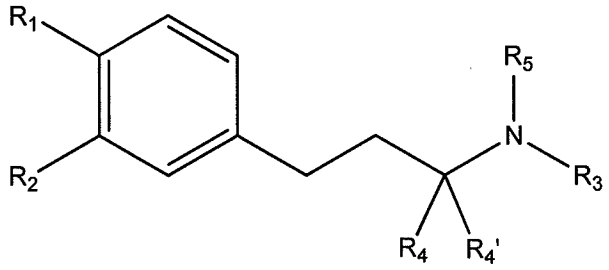
Y is CH or N;

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

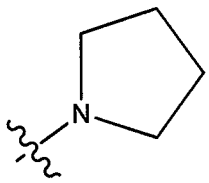


I

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, $(R_{16})(R_{17})N\text{-}C_{1-4}$ 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



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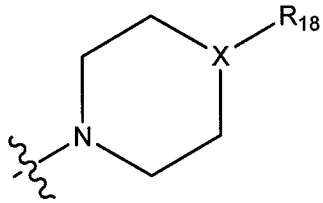
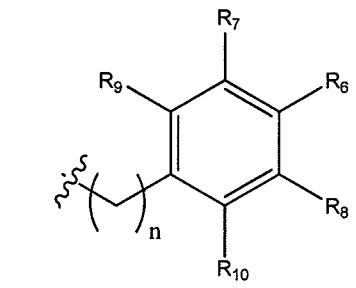
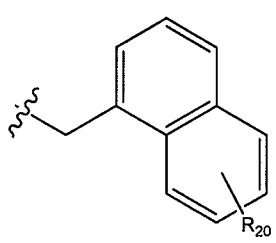
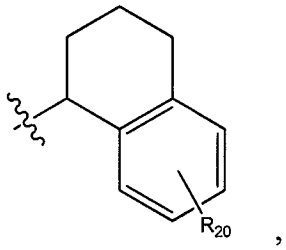
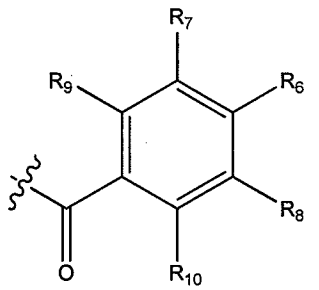
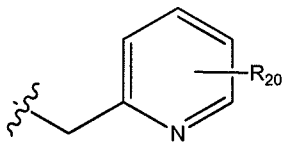
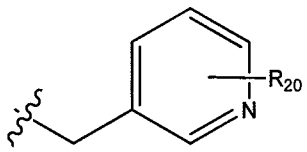
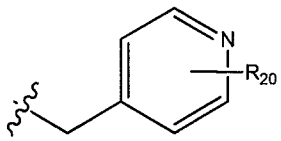
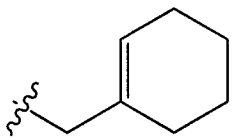


, wherein

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

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

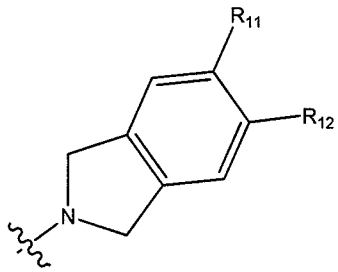
n is 1-4

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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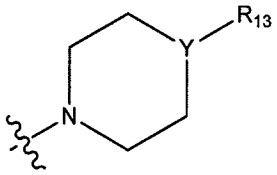

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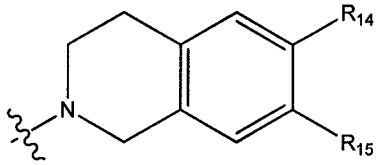

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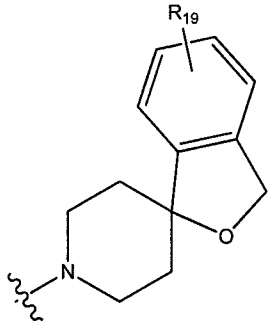


and , wherein

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

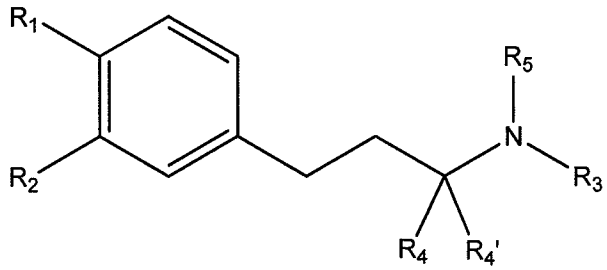
Y is CH or N;

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; 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

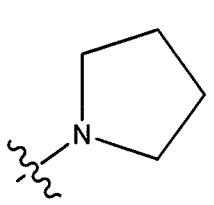


I

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



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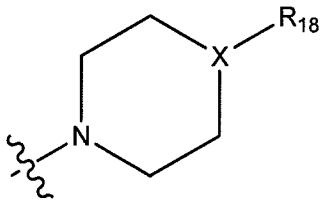


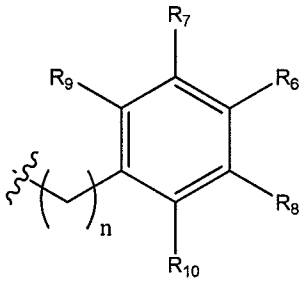
, wherein

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



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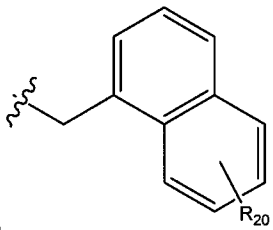

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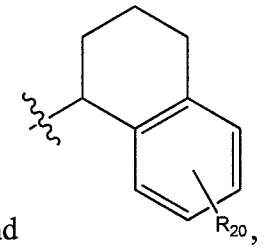


,

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

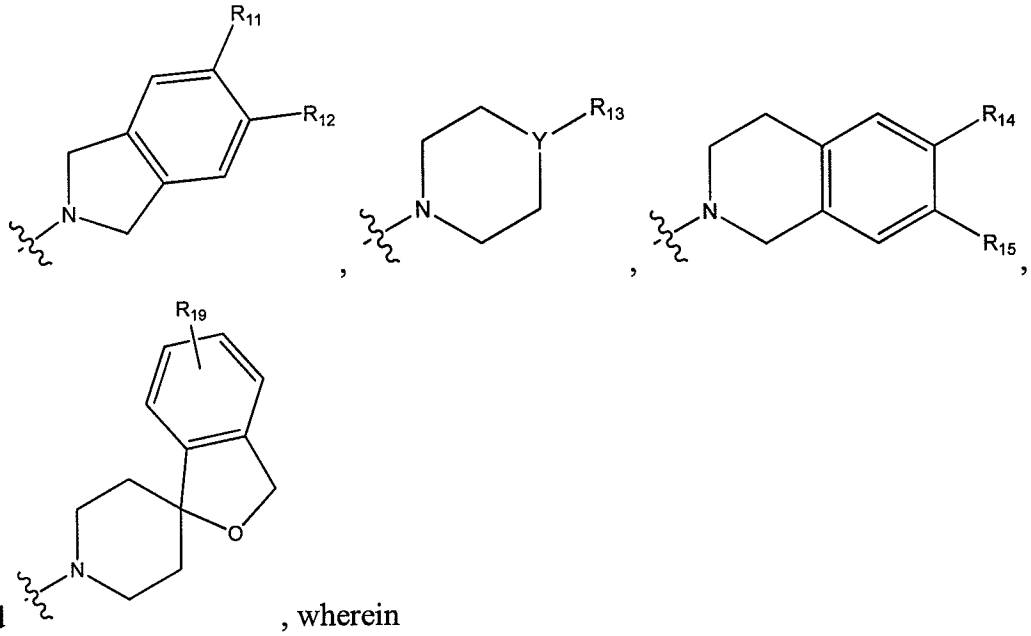
n is 1

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

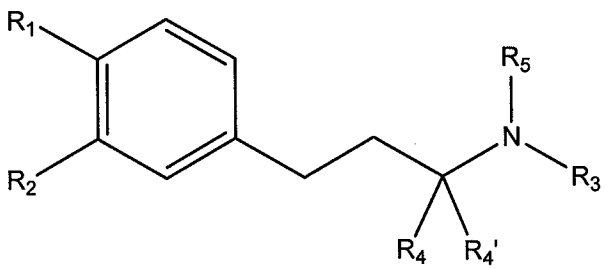
Y is CH or N;

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

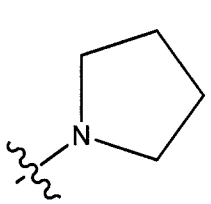


I

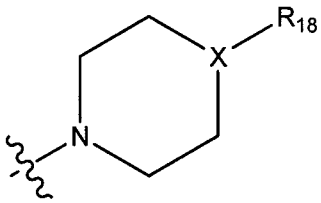
wherein

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



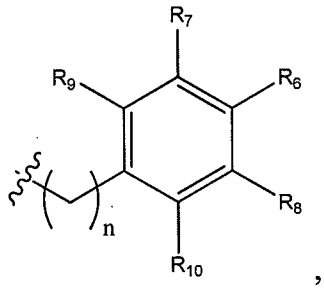
, wherein

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

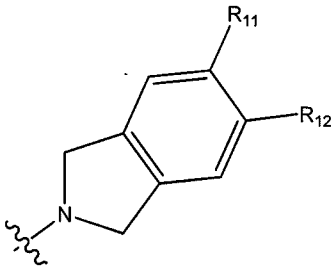
n is 1

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



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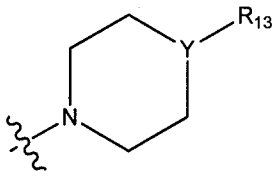

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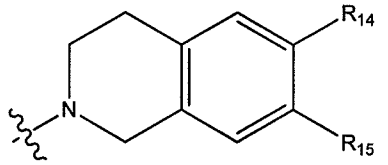

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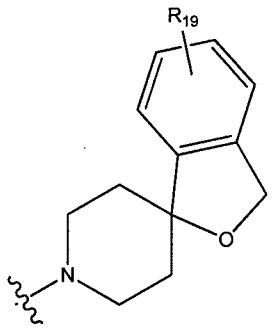


and , wherein

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

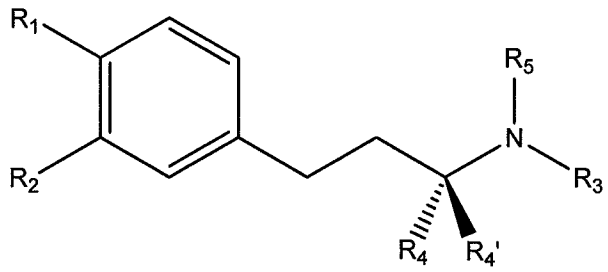
Y is CH or N;

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.

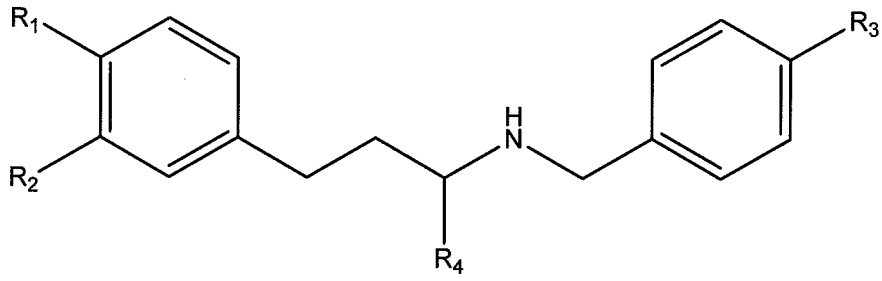
[0106] In some more specific embodiments, the sigma-2 antagonists of the present invention are those of Formula Ia



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.

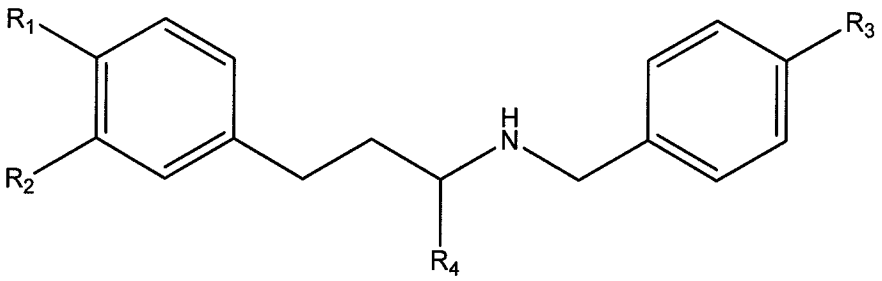
[0107] In some embodiments, the sigma-2 antagonists of the present invention are those of Formula IIa:



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.

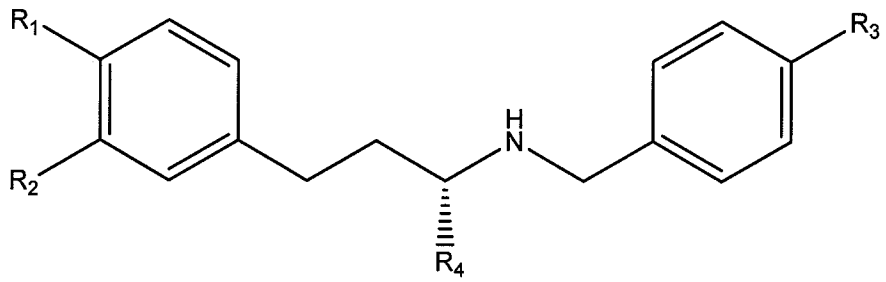
[0108] In some more specific embodiments, the sigma-2 antagonists of the present invention are those of Formula IIa.



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.

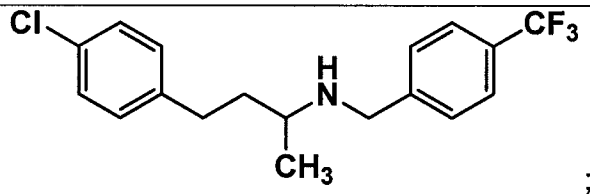
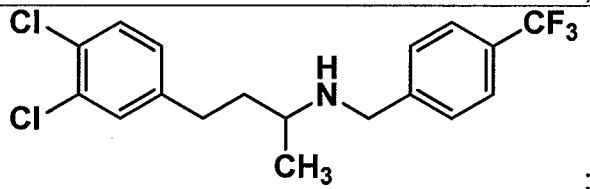
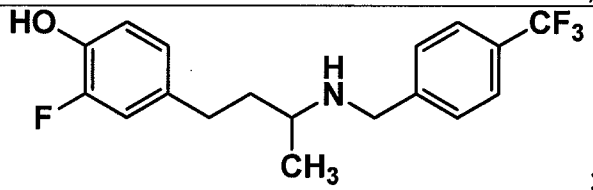
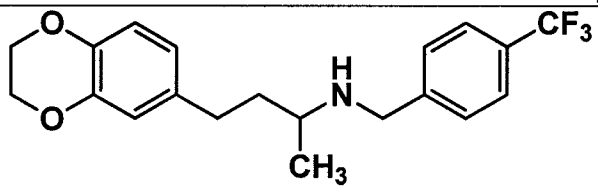
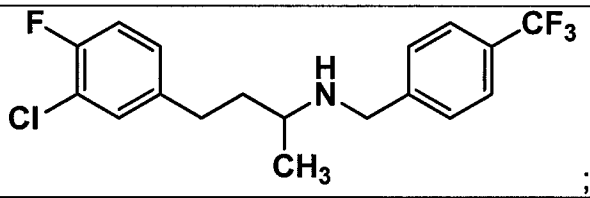
[0109] In some more specific embodiments, the sigma-2 antagonists of the present invention are those of Formula IIb

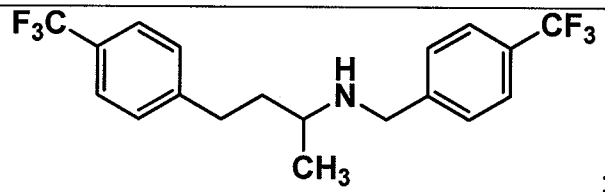


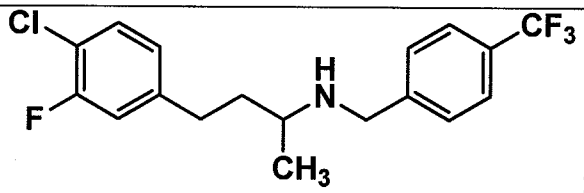
IIb

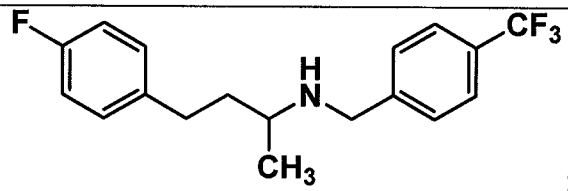
wherein R_1-R_4 are as defined above for the compounds of Formula IIa, or pharmaceutically acceptable salts thereof.

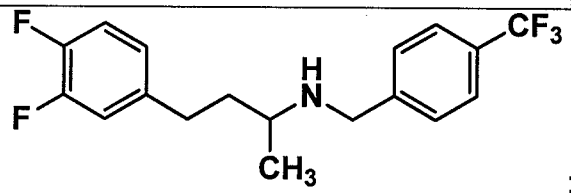
[0110] Specific exemplary compounds of the invention are set forth in the table below:

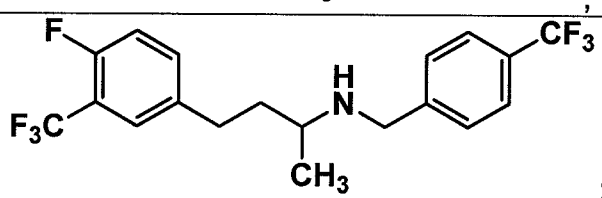
 ;
 ;
 ;
 ;
 ;

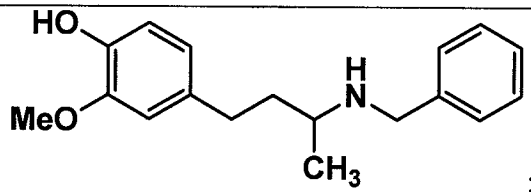

F3C
CF3
H
N
CH3
;

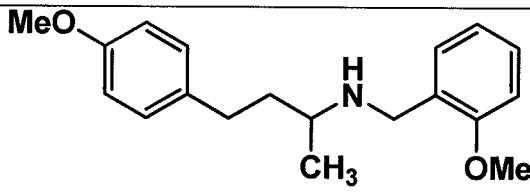

Cl
F
CF3
H
N
CH3
;

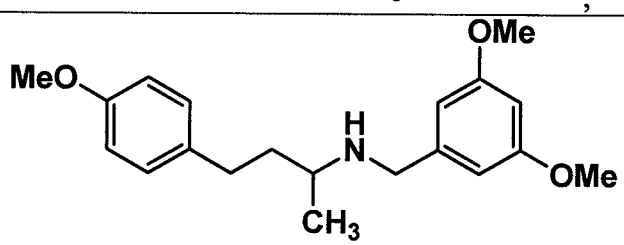

F
CF3
H
N
CH3
;

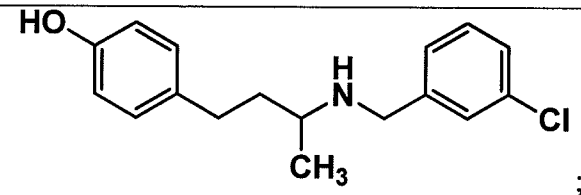

F
F
CF3
H
N
CH3
;

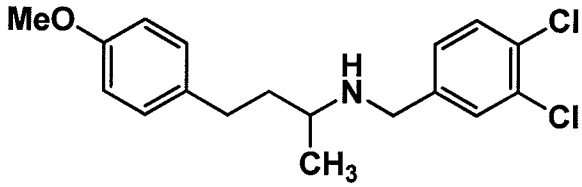

F
F3C
CF3
H
N
CH3
;


HO
MeO
H
N
CH3
;


MeO
H
N
CH3
OMe
;


OMe
MeO
H
N
OMe
CH3
;


HO
H
N
Cl
CH3
;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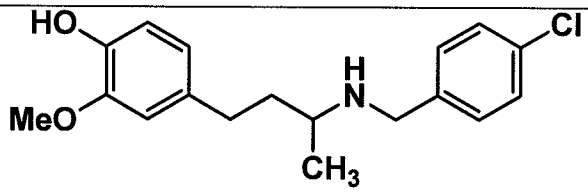
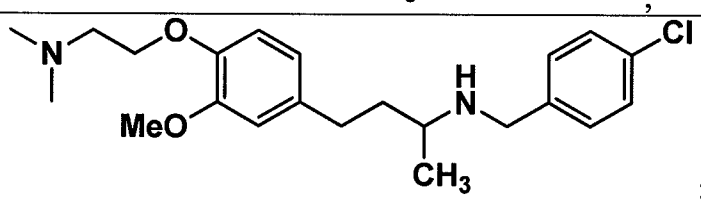
H
N
Cl
Cl
CH3
;


HO
MeO
H
N
Cl
CH3
;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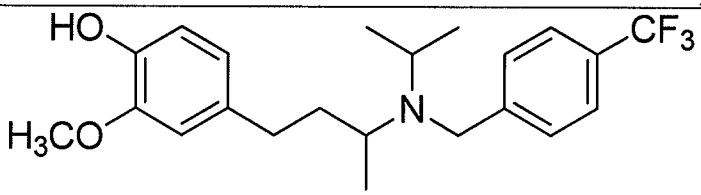
O
MeO
H
N
Cl
CH3
;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H3CO
N
CF3
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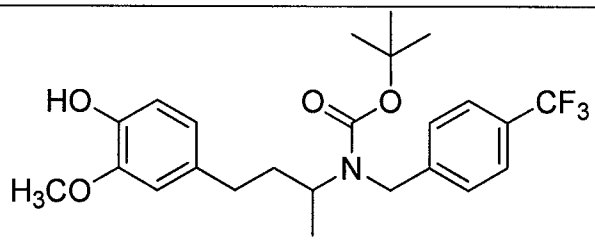
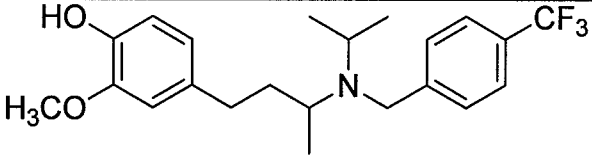

HO
H3CO
O
O
N
CF3
;


HO
H3CO
N
CF3
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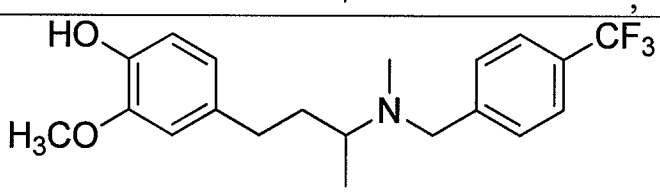
HO
H3CO
N
CF3
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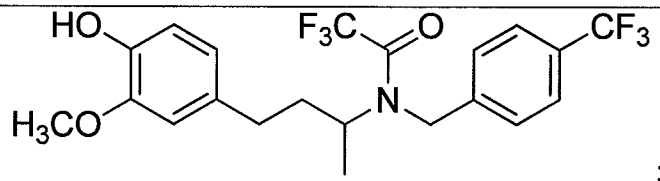
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O
H3CO
N
CF3
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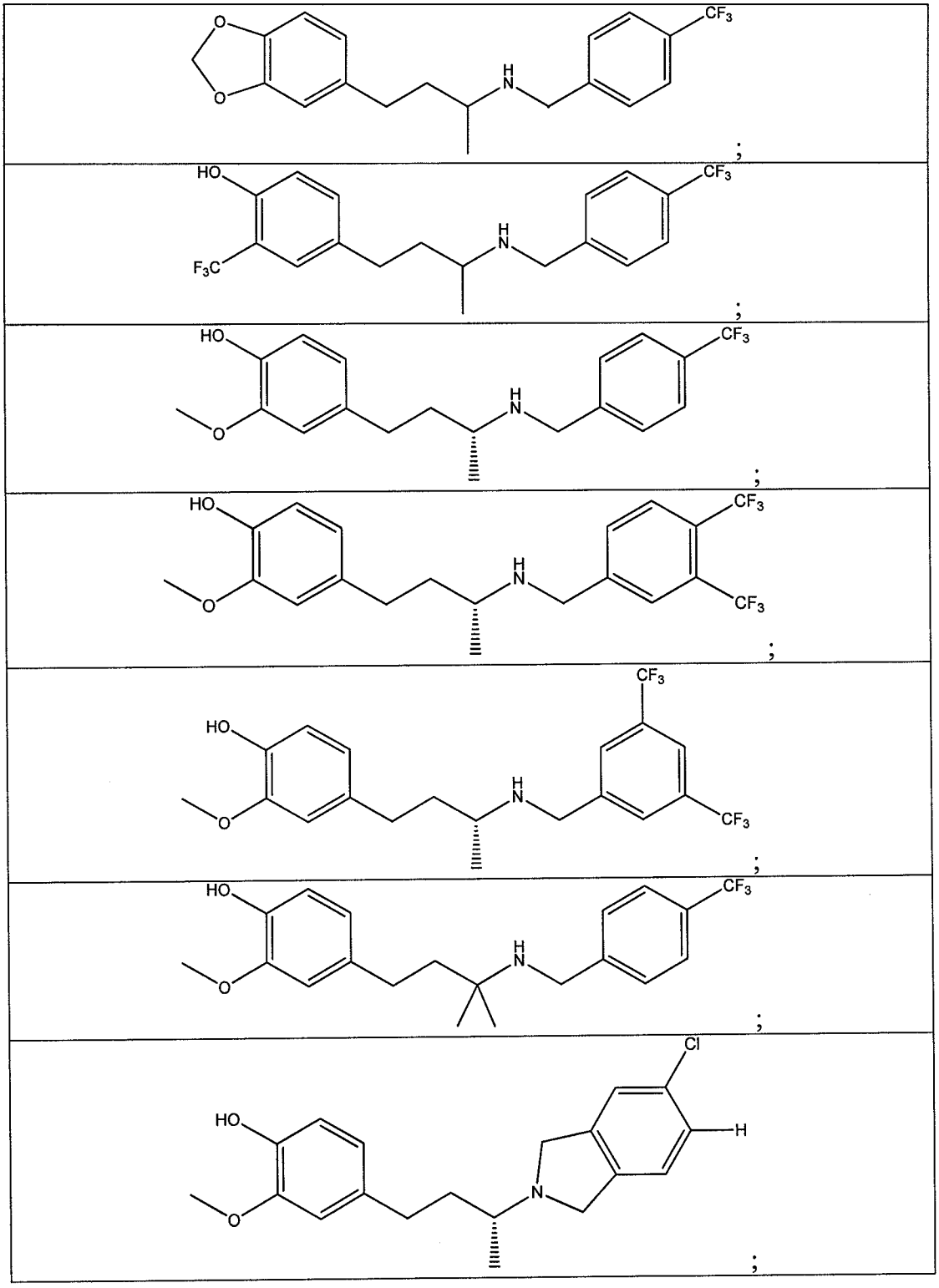

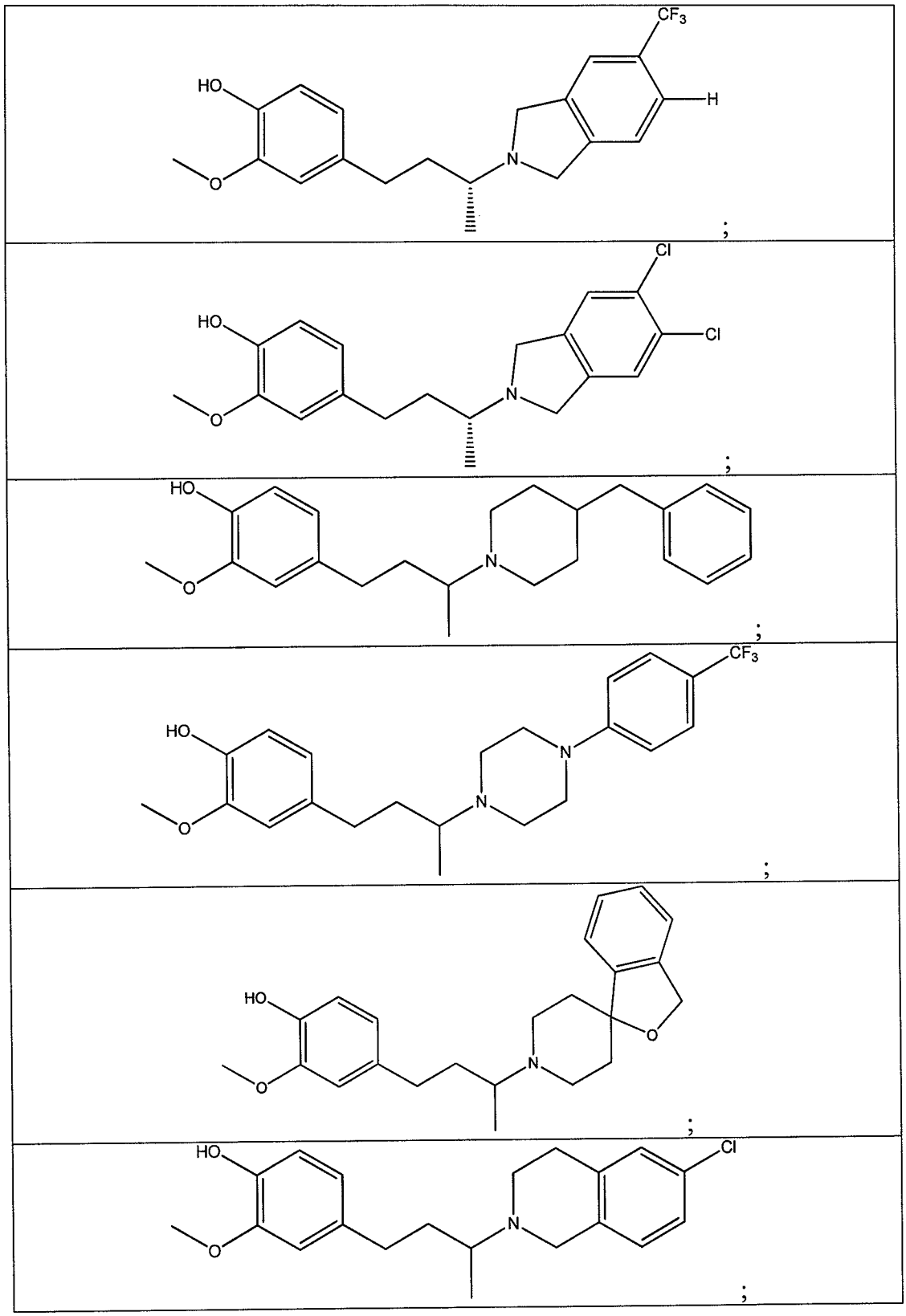

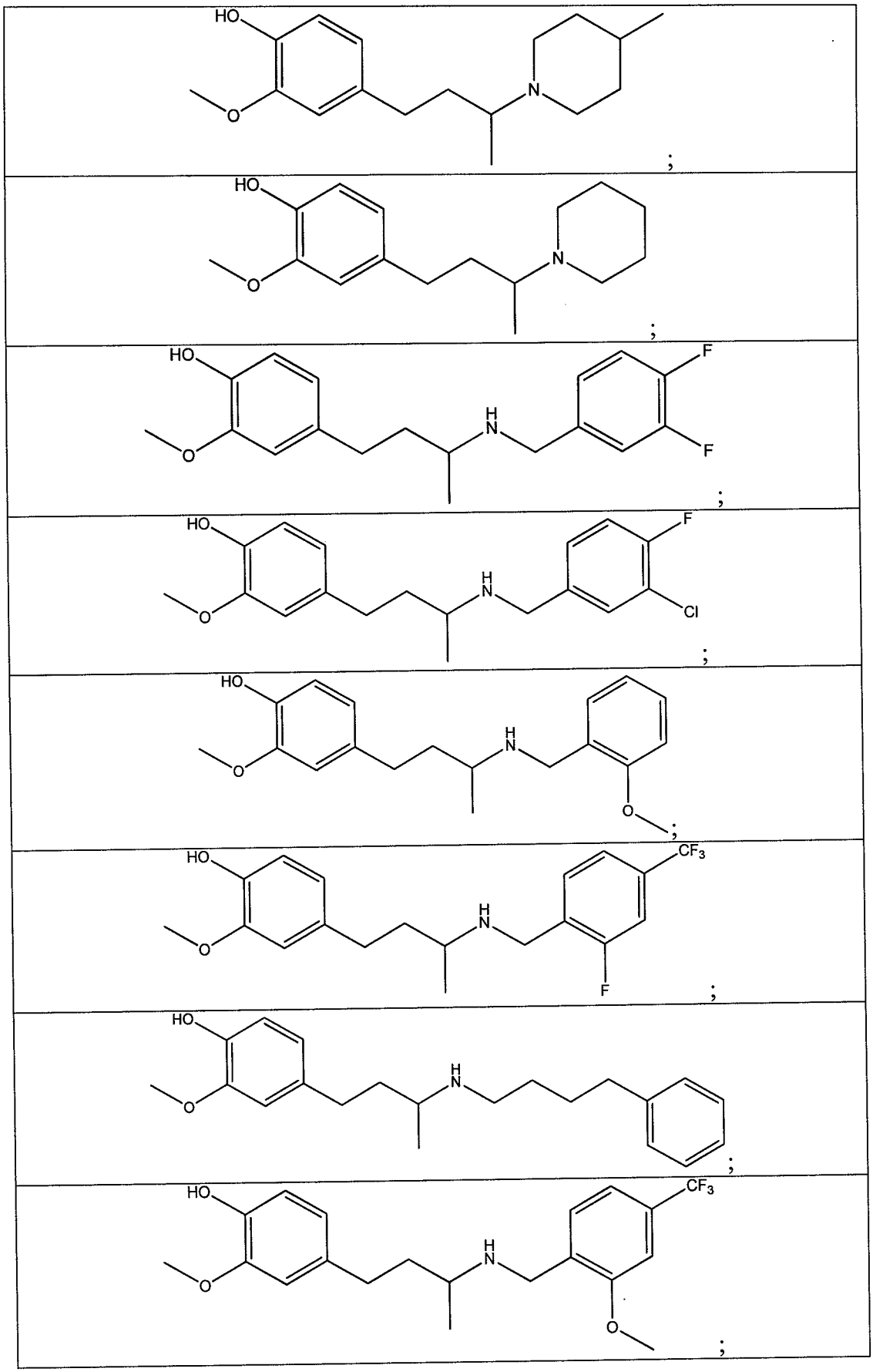

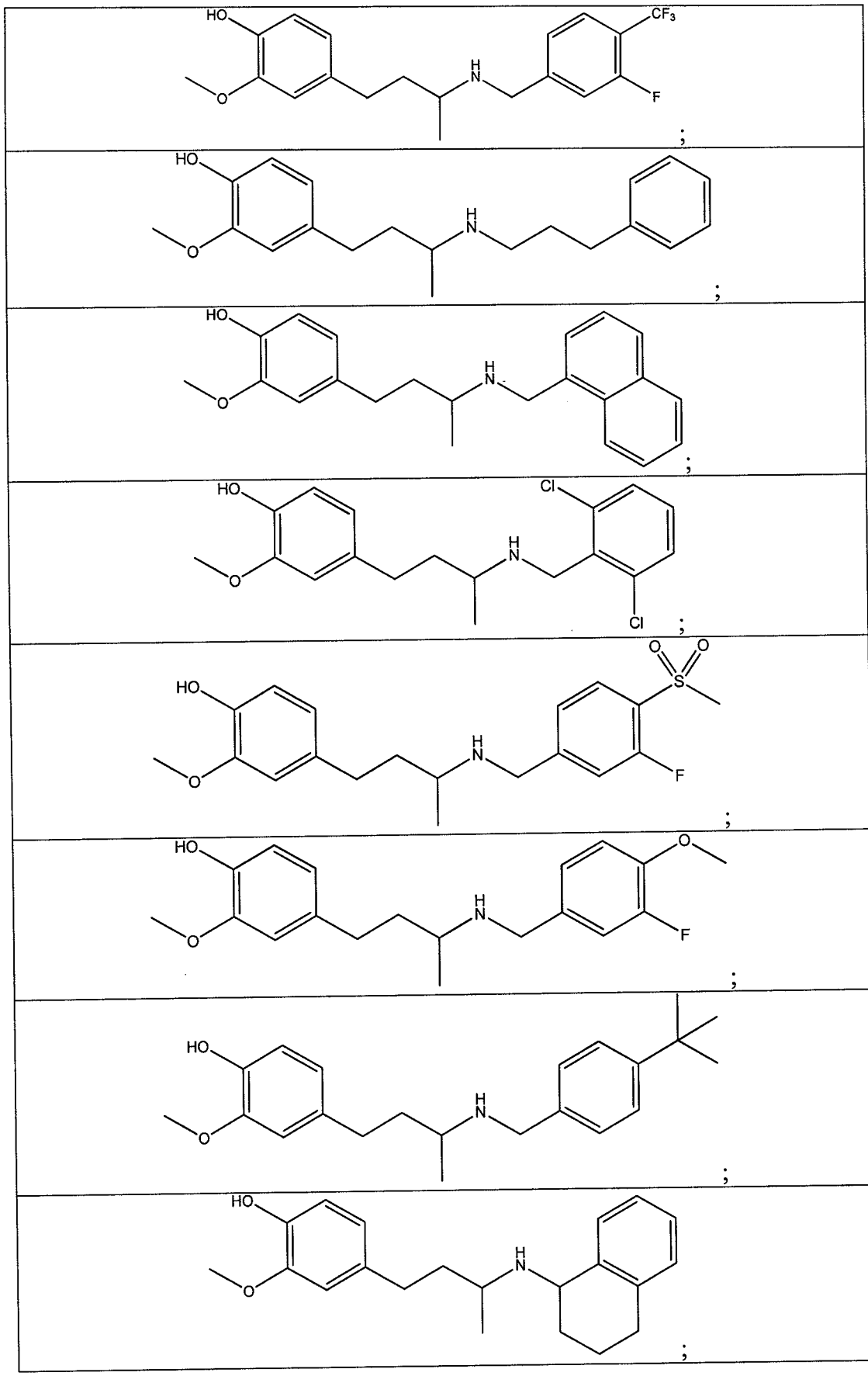

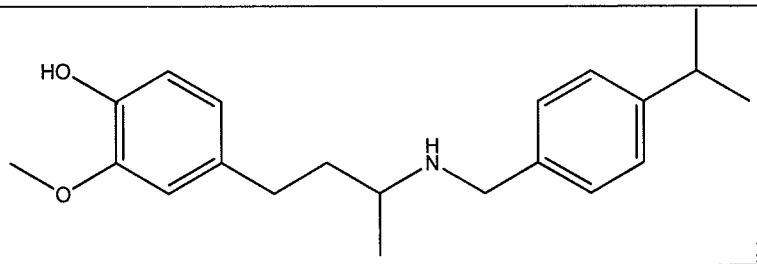
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O
H
N
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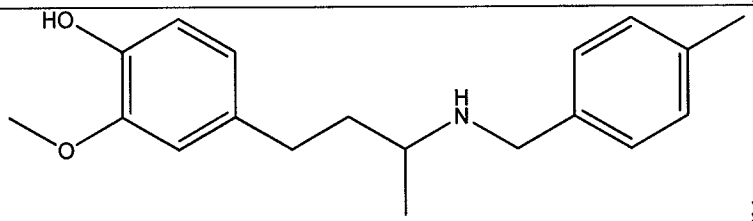
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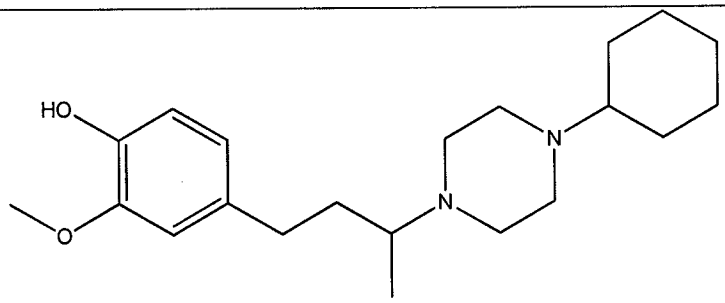
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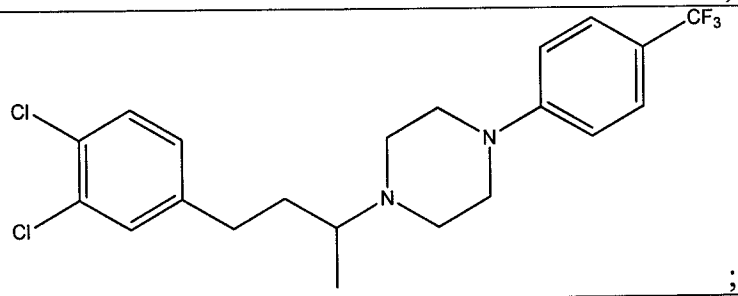
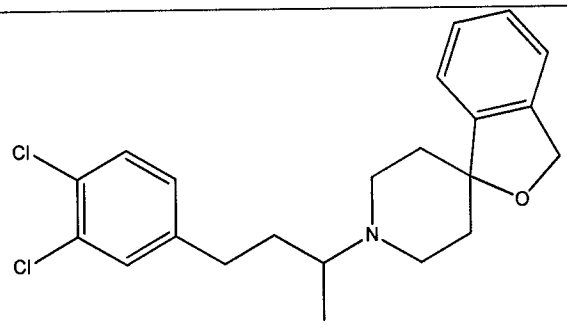

CF3
Cl
Cl
N
N
;


Cl
Cl
O
N
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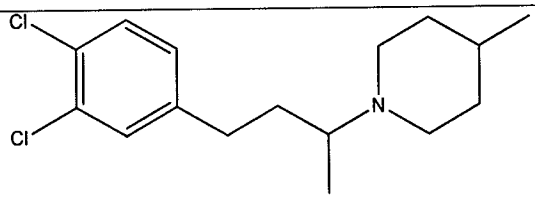
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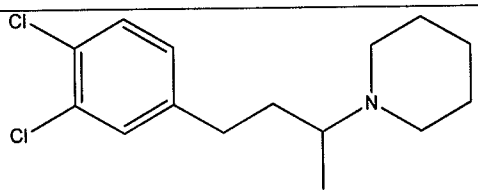
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Cl
N
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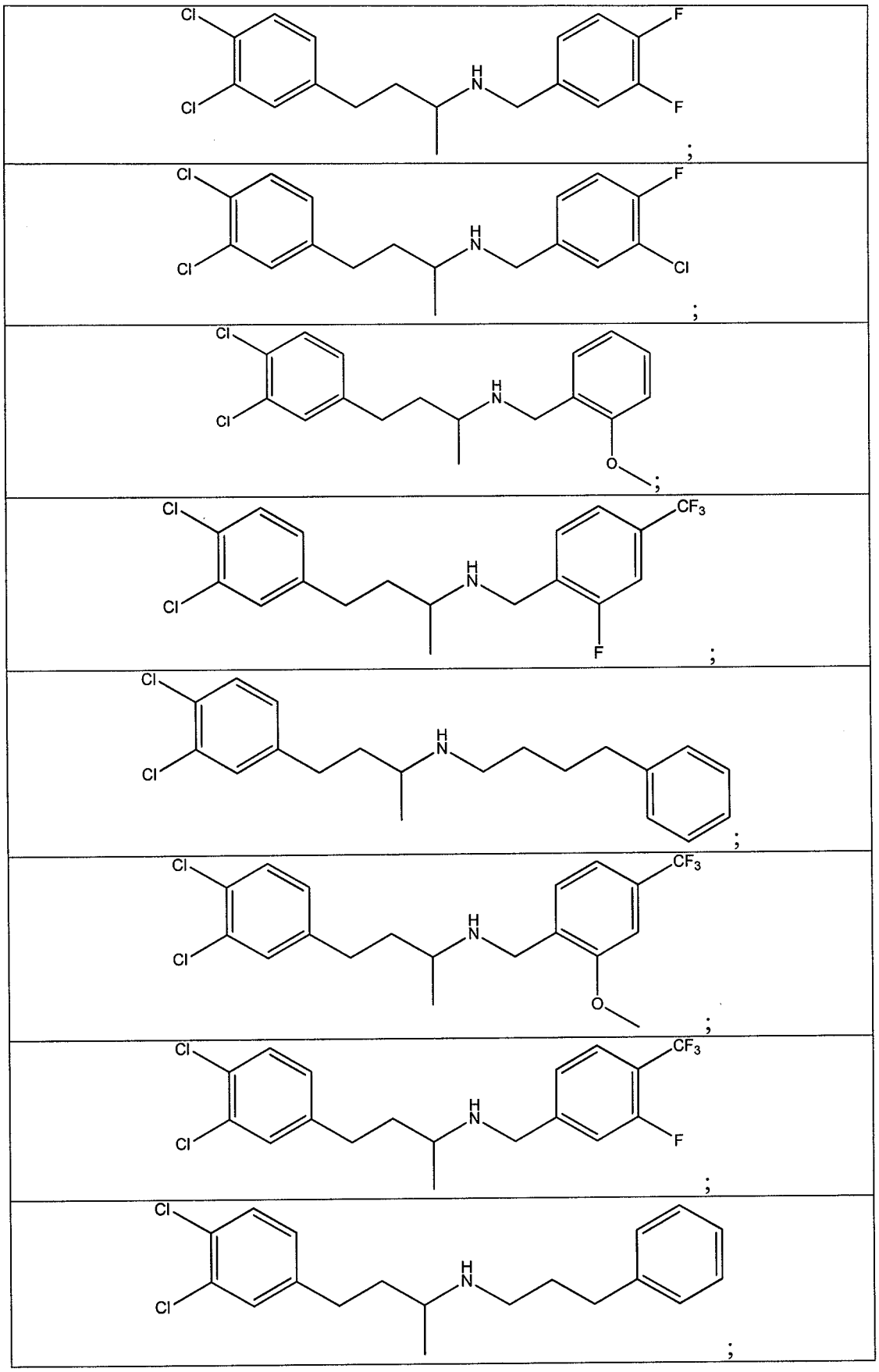

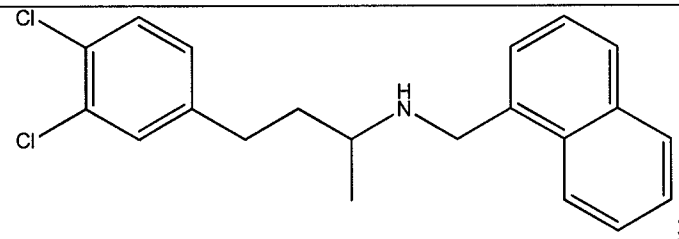
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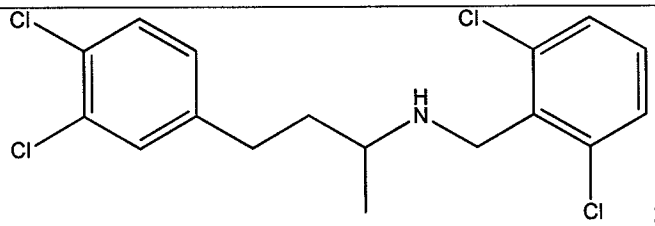
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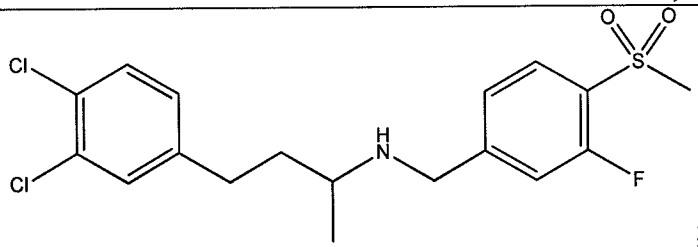
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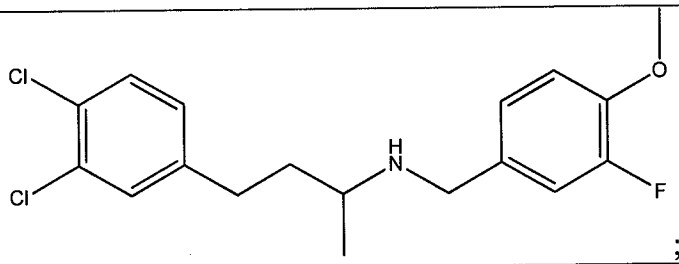
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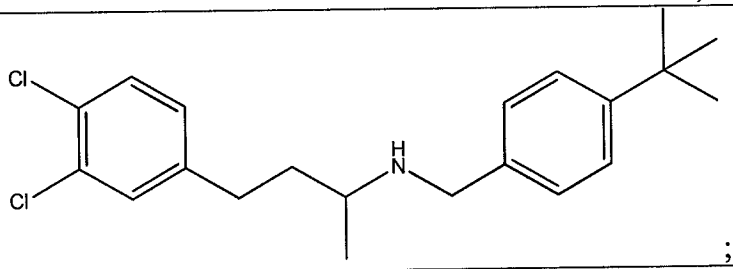
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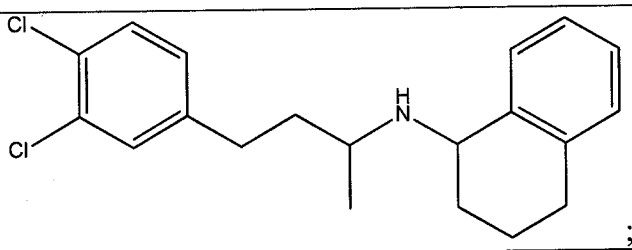
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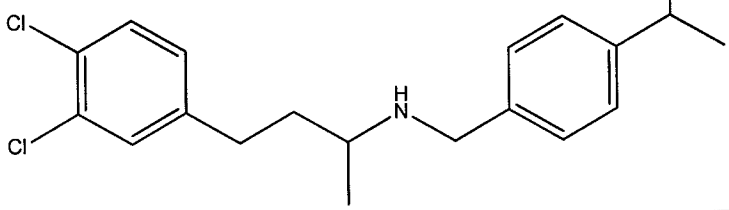
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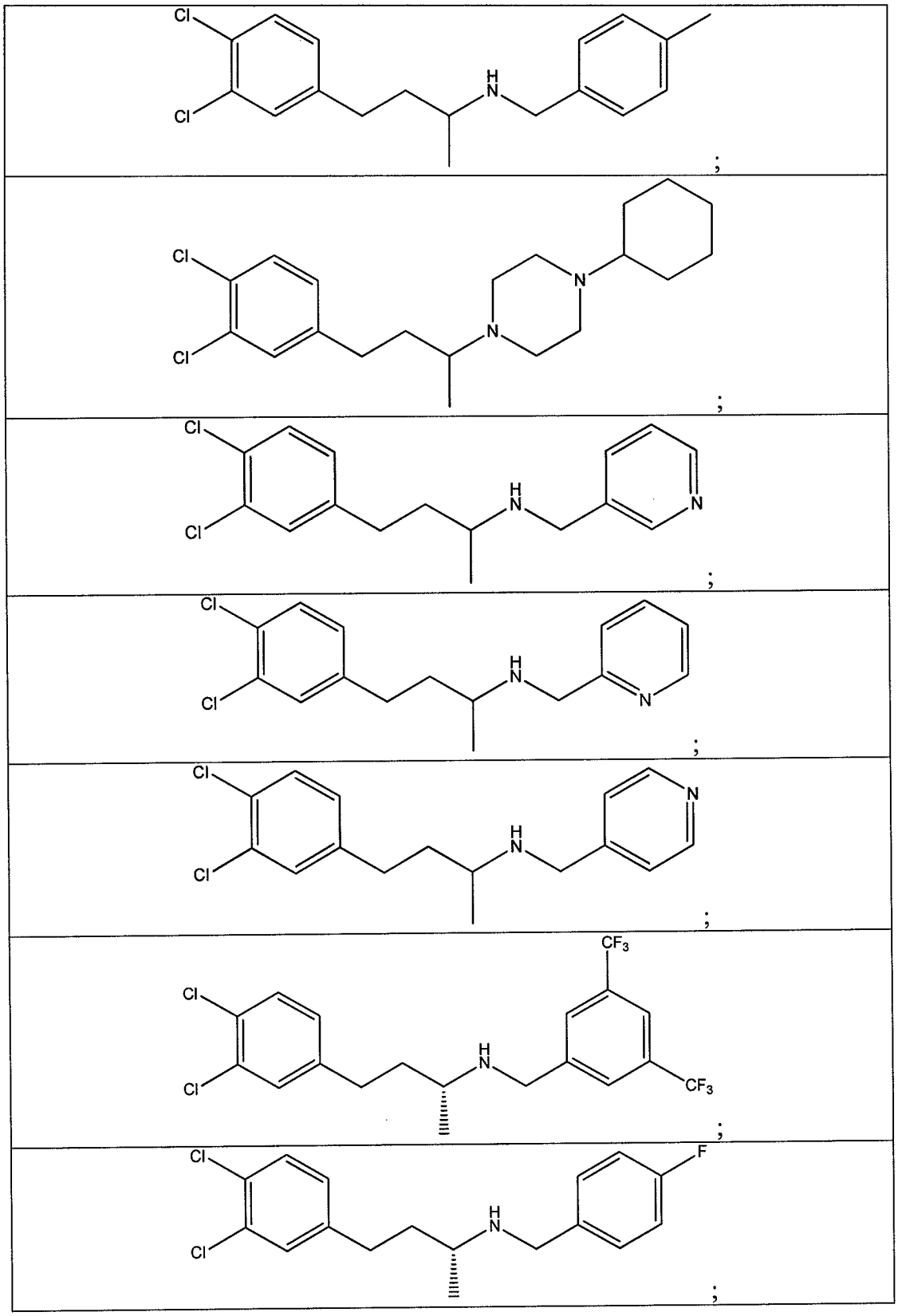
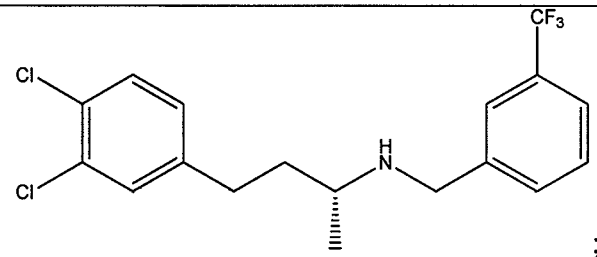
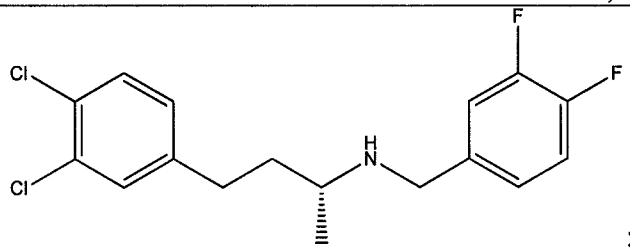
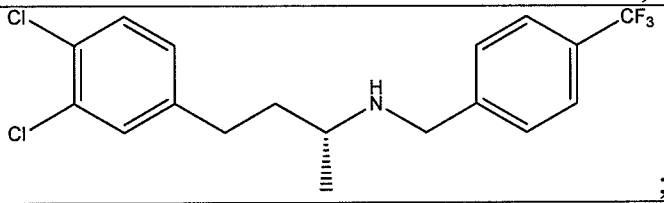
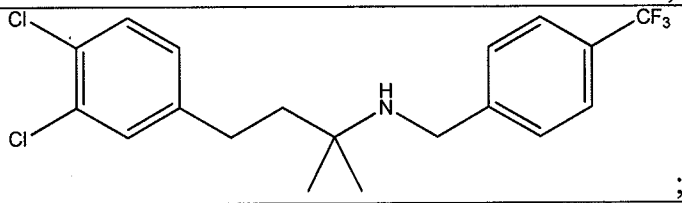
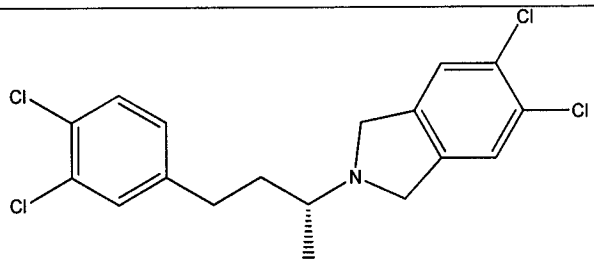


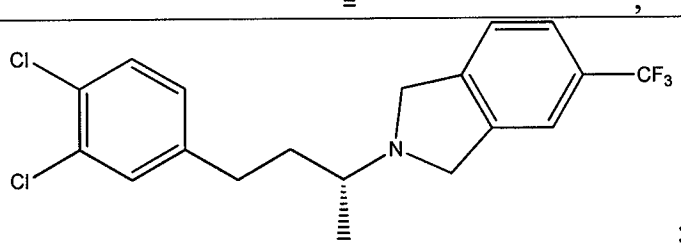

CF3
Cl
Cl
H
N
;

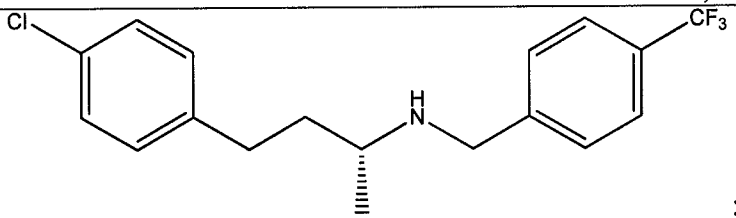

F
F
Cl
Cl
H
N
;

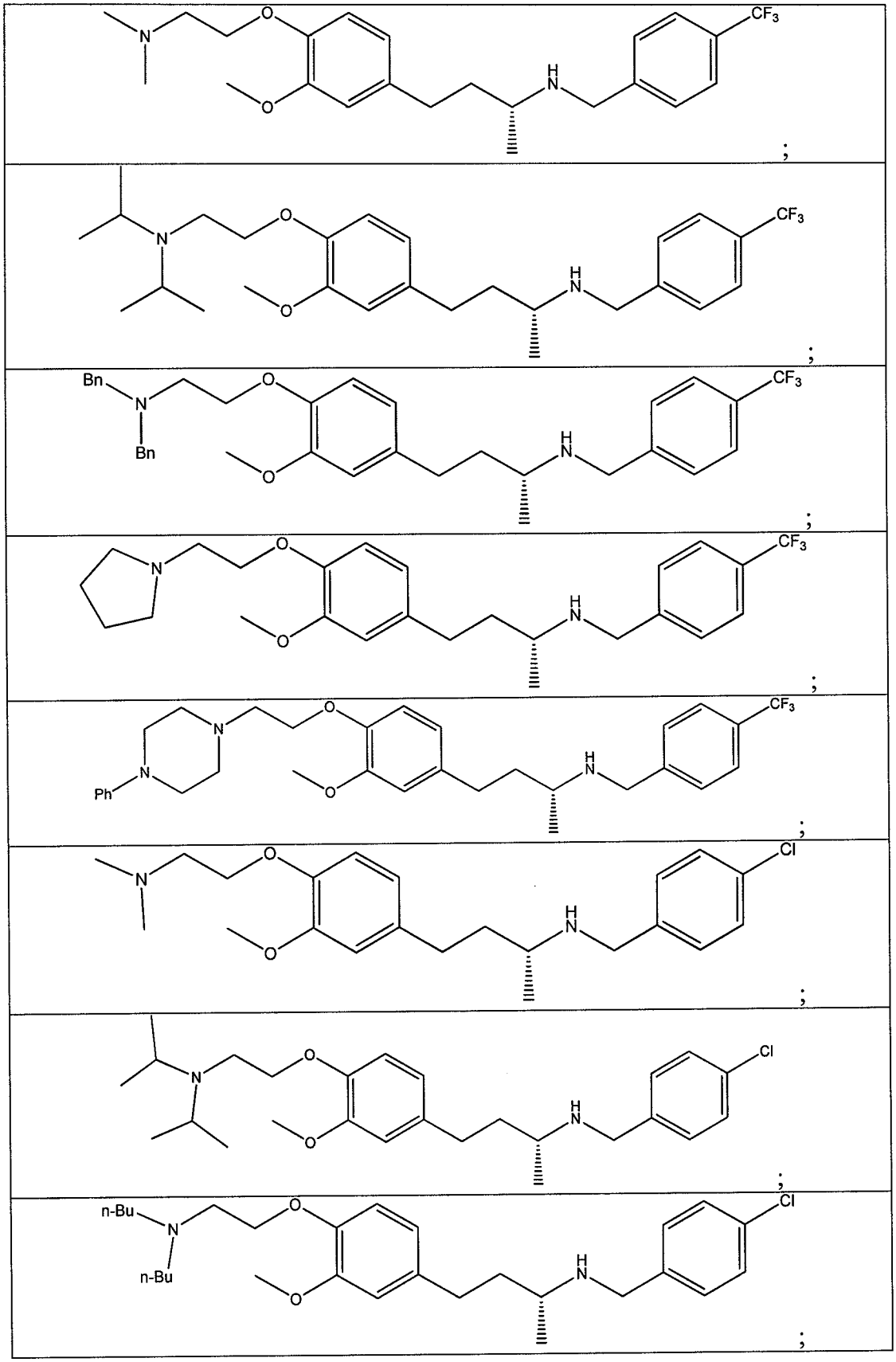

CF3
Cl
Cl
H
N
;

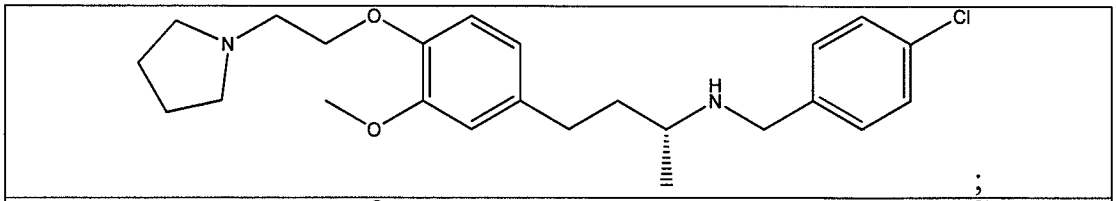

CF3
Cl
Cl
H
N
;


Cl
Cl
Cl
Cl
N
;


CF3
Cl
Cl
N
;


CF3
Cl
H
N
;

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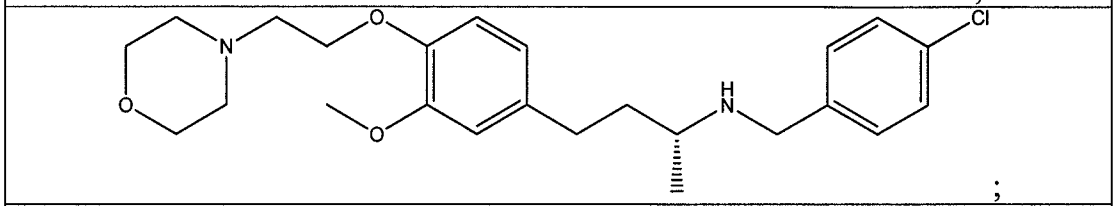
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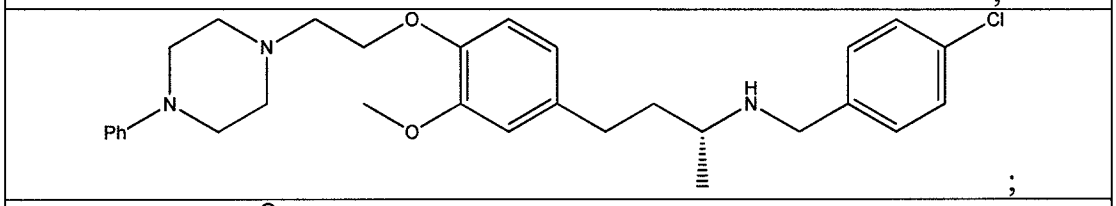
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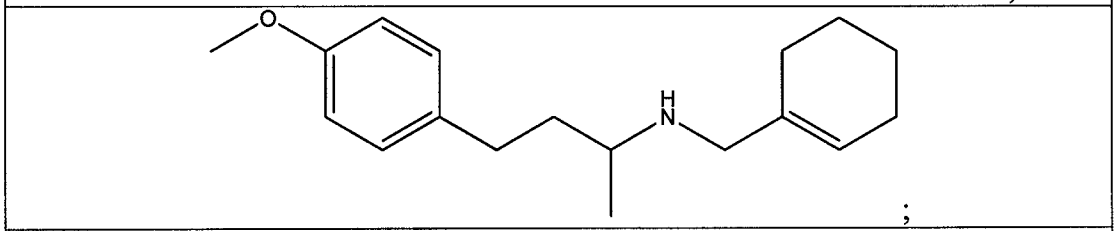
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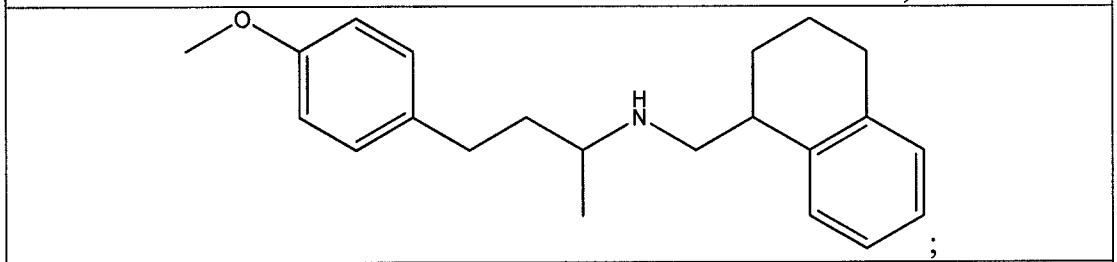
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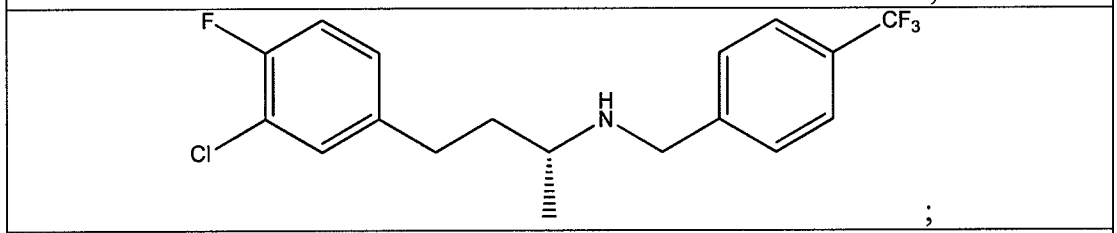
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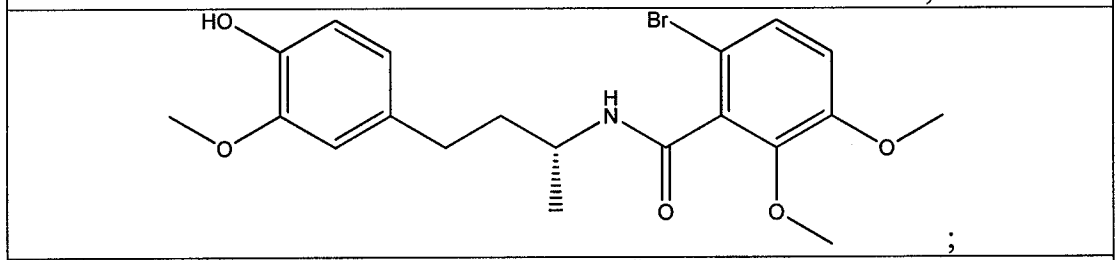
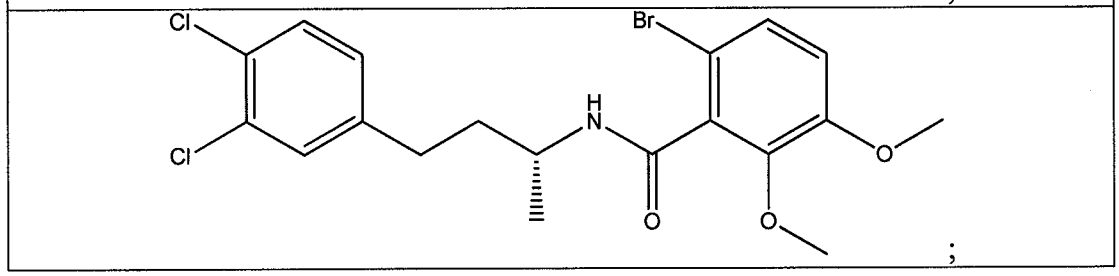

;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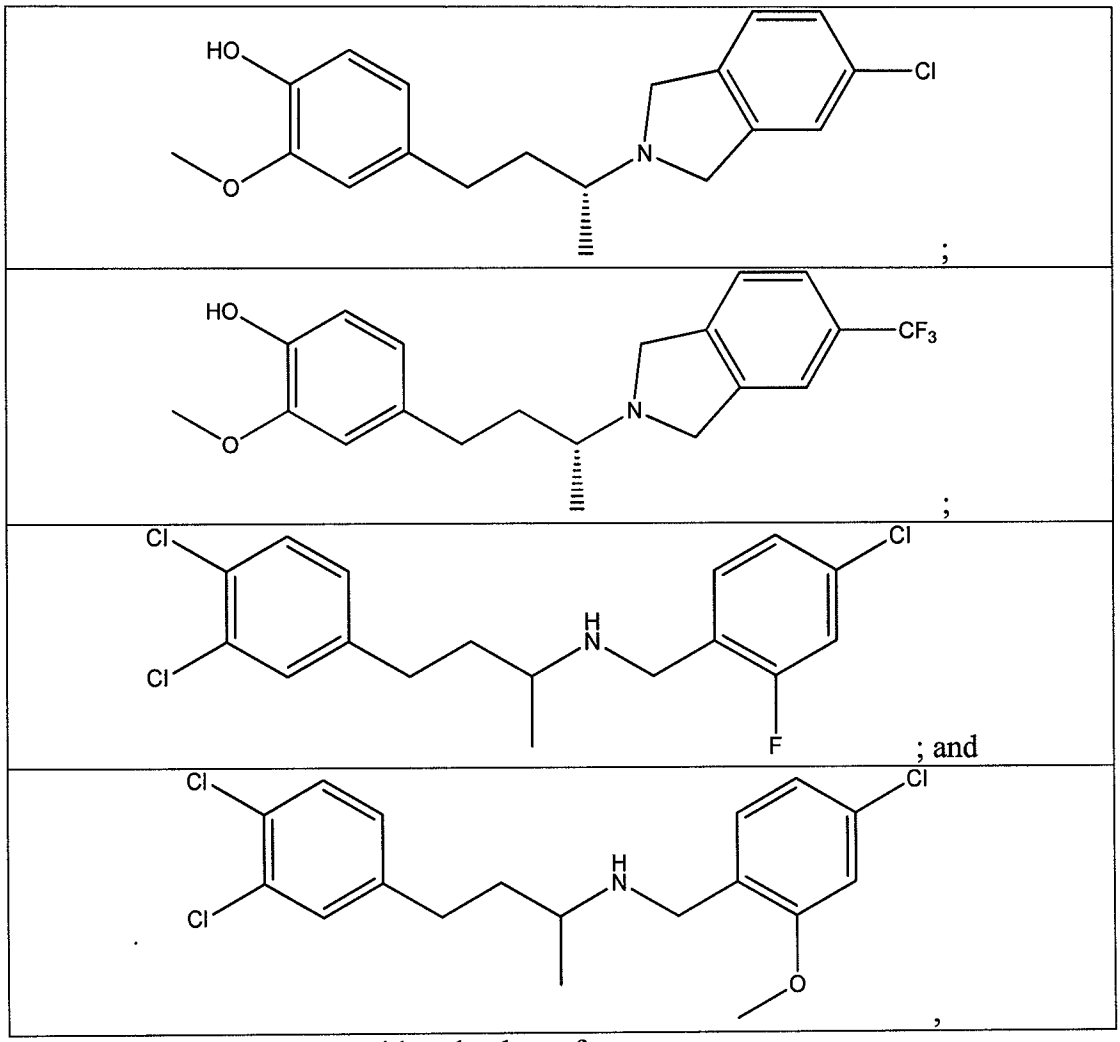
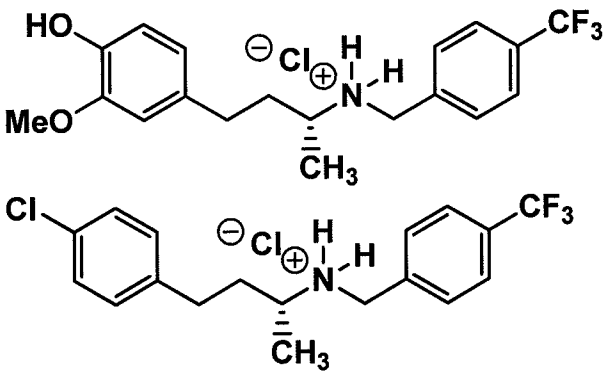
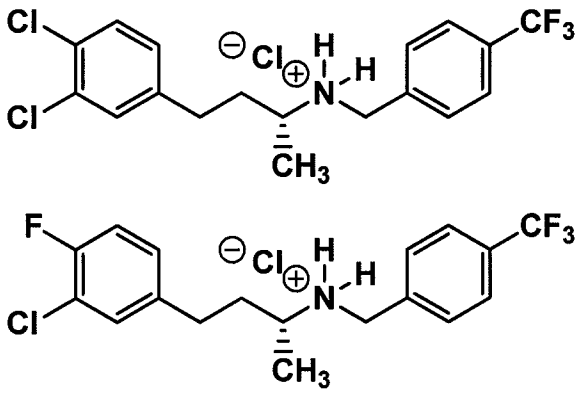


or pharmaceutically acceptable salts thereof.

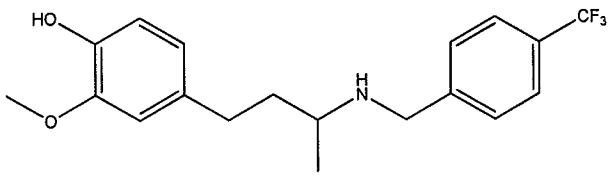
[0111] Preferred salts for use in the present invention include the hydrochloride salts of the above compounds, including the following:





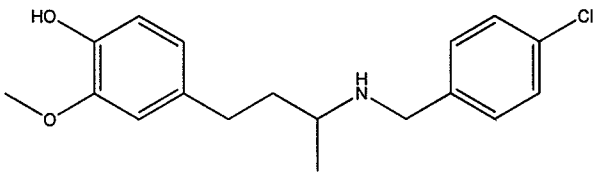
[0112] These have been synthesized in accordance with general methods provided herein and specific synthetic examples with any additional steps being well within the skill in the art. Several of these compounds have been tested in various assays as detailed herein and have been found active. Tested compounds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

[0113] Compound II has the formula

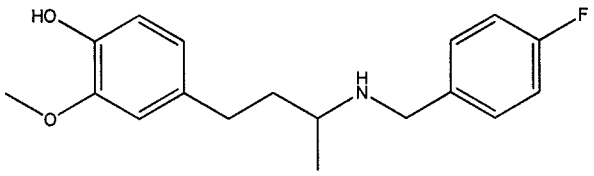


[0114] In some embodiments, each of the general formulae above may contain a proviso to remove the compound of Formula II.

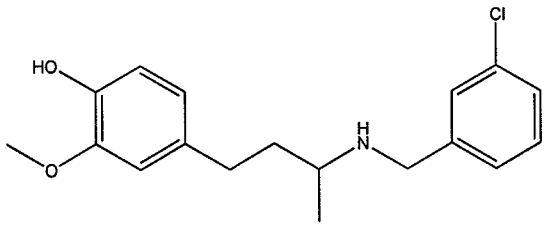
[0115] In some embodiments, each of the general formulae above may contain a proviso to remove one or more of the following compounds:



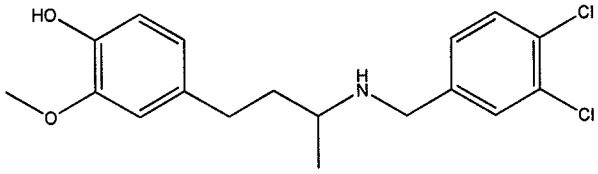
,



,

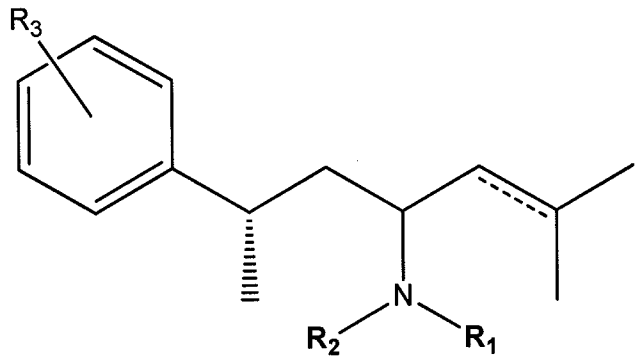


, and



.

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



VIIIa

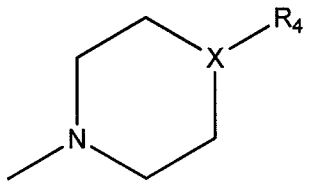
wherein:

-------- 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



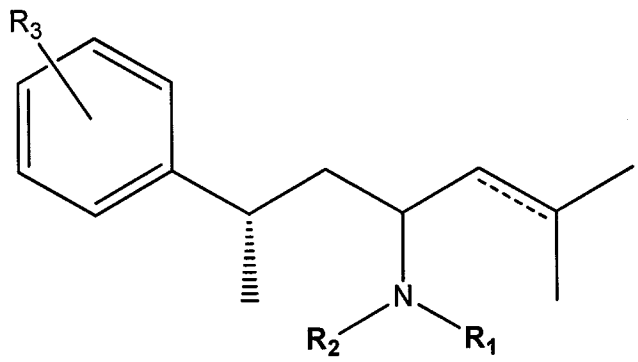
, wherein

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

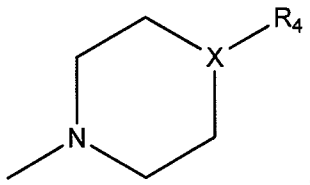
wherein:

⚌ 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



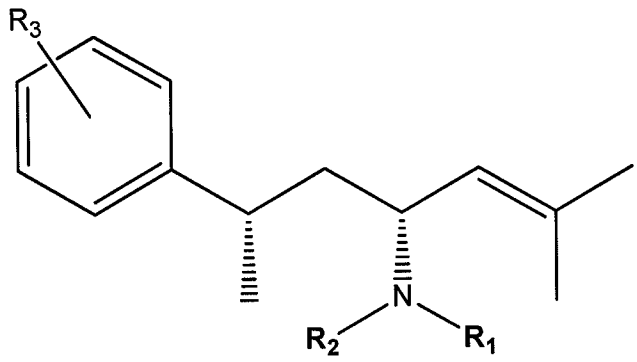
, wherein

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.

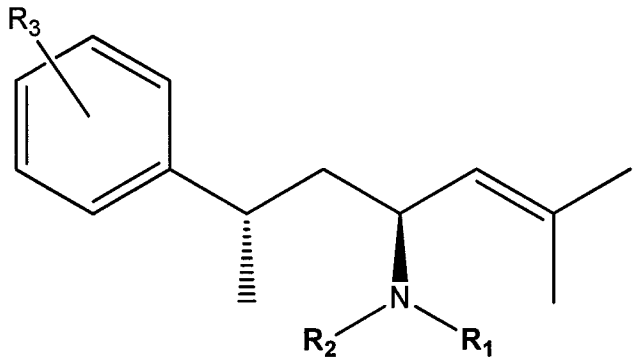
[0118] In some more specific embodiments, the sigma-2 antagonists of the present invention are those of Formula VIIIb



VIIIb

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

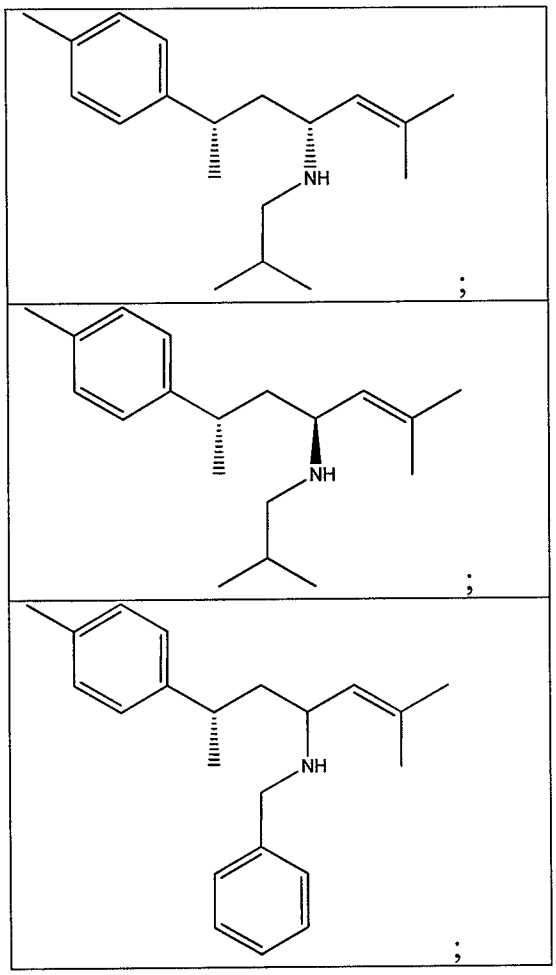
[0119] In some more specific embodiments, the sigma-2 antagonists of the present invention are those of Formula VIIIc

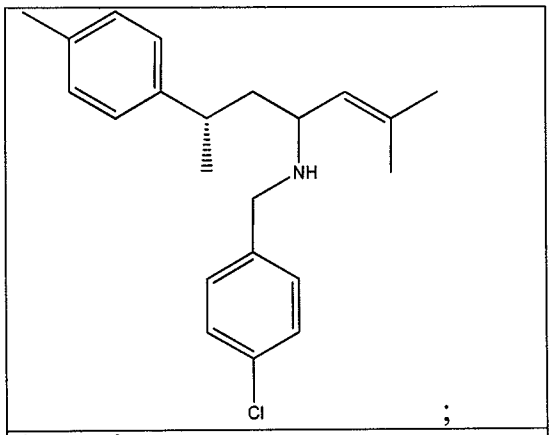


VIIIc

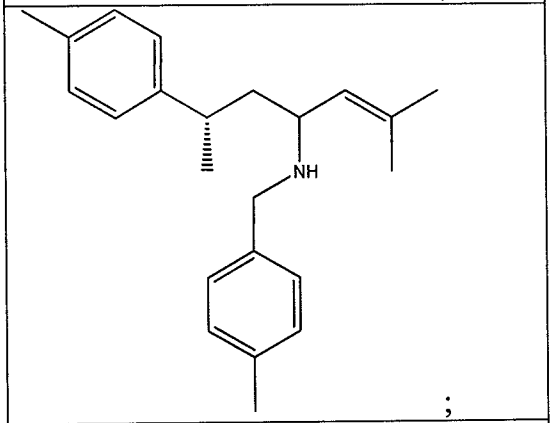
wherein R_1-R_3 are as defined above for Formula VIIIa, or pharmaceutically acceptable salts thereof.

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

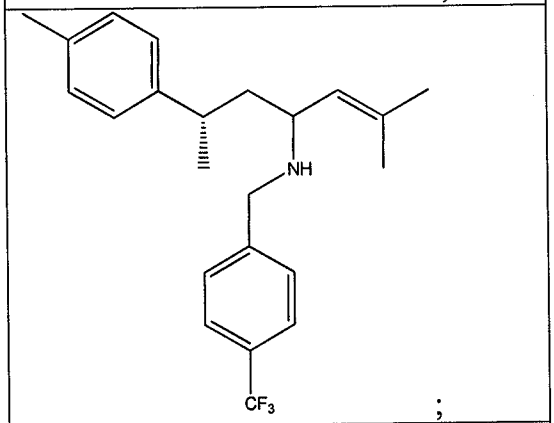



NH
Cl

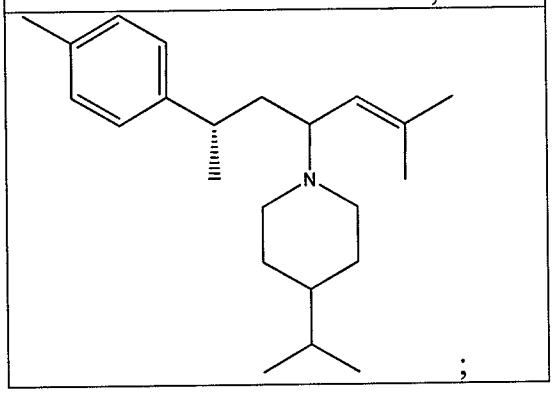
;


NH

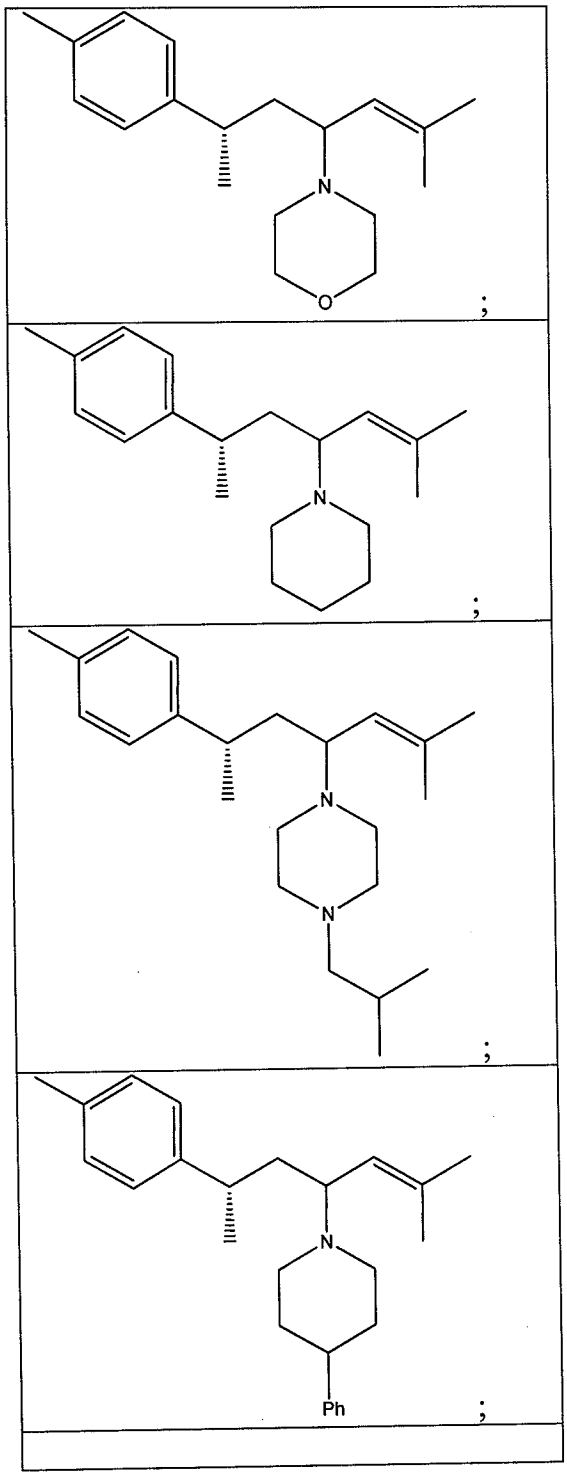
;

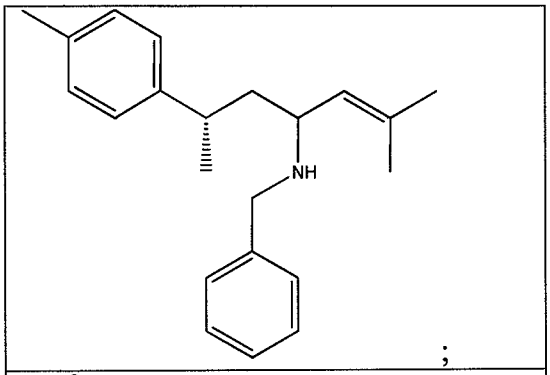

NH
CF3

;


N

;


N
O
;
N
;
N
N
;
N
Ph
;



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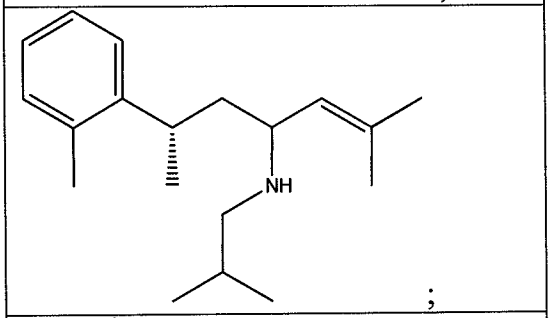

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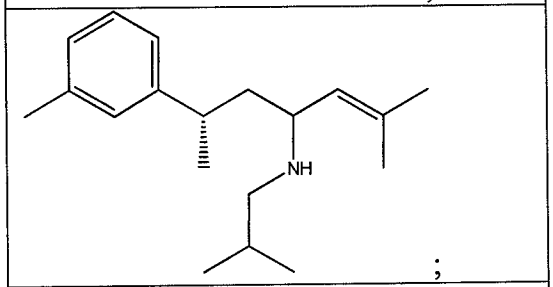

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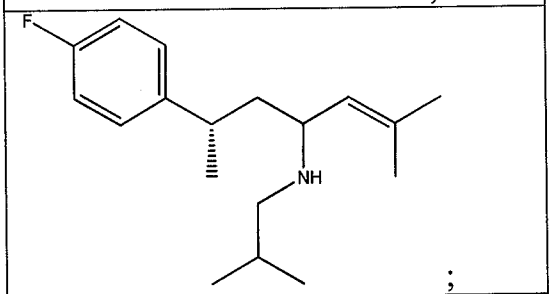
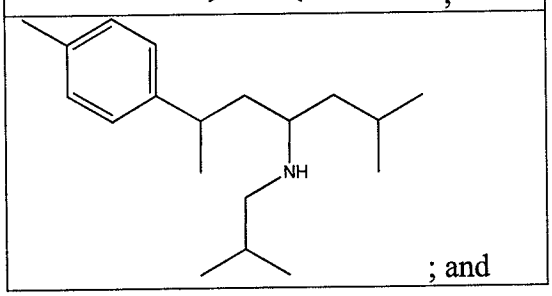
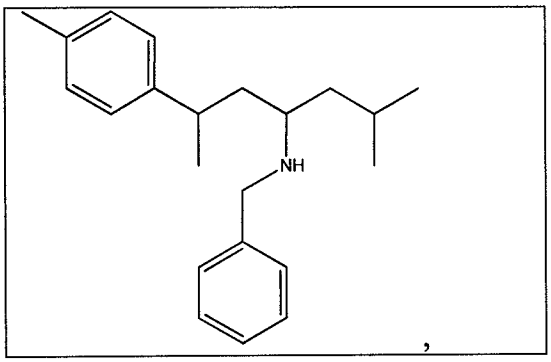


;

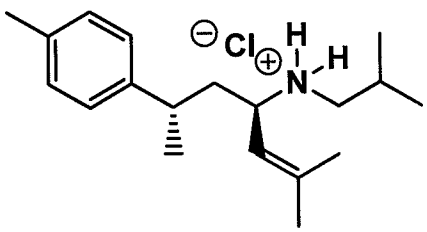


; and

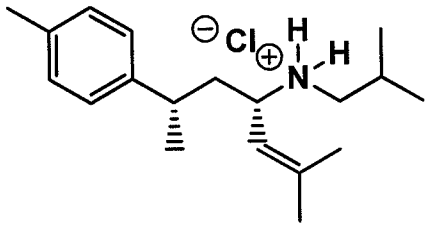


or pharmaceutically acceptable salts thereof.

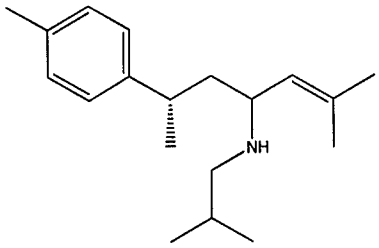
Preferred salts for use in the present invention include the hydrochloride salts of the above compounds, including the following:



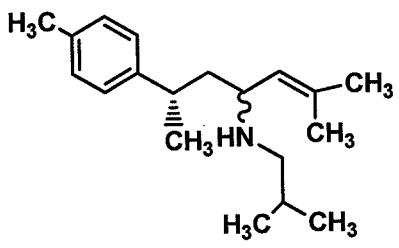
and



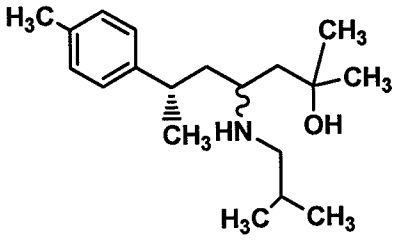
[0121] In some embodiments, each of the general formulae above may contain a proviso to remove one or more of the following compound:



[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.

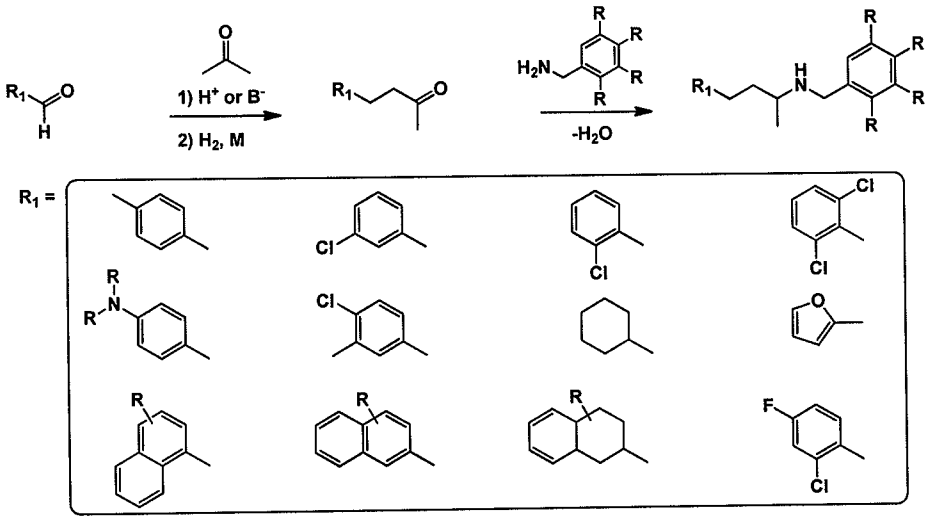
Synthesis of the Compounds of the Invention

[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

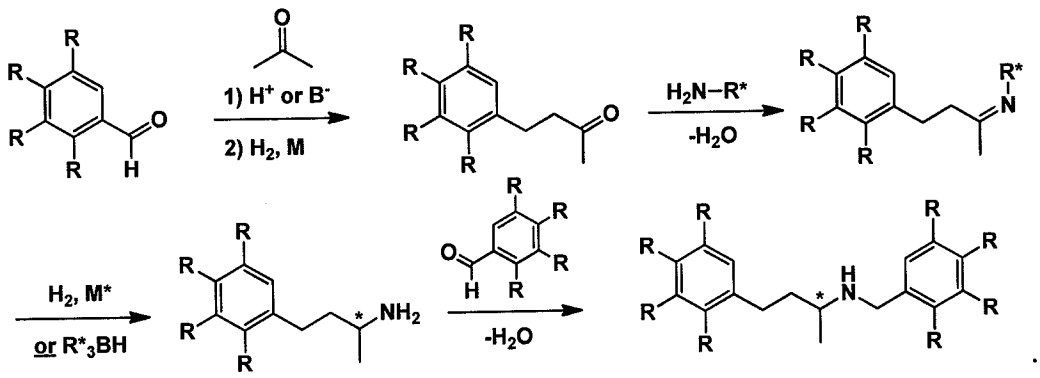
such as silica gel chromatography, HPLC on silica gel and the like, or by recrystallization. The compounds above can be synthesized via any synthetic route. For example, the compounds can be prepared according to the following scheme (Scheme 1).



Scheme 1

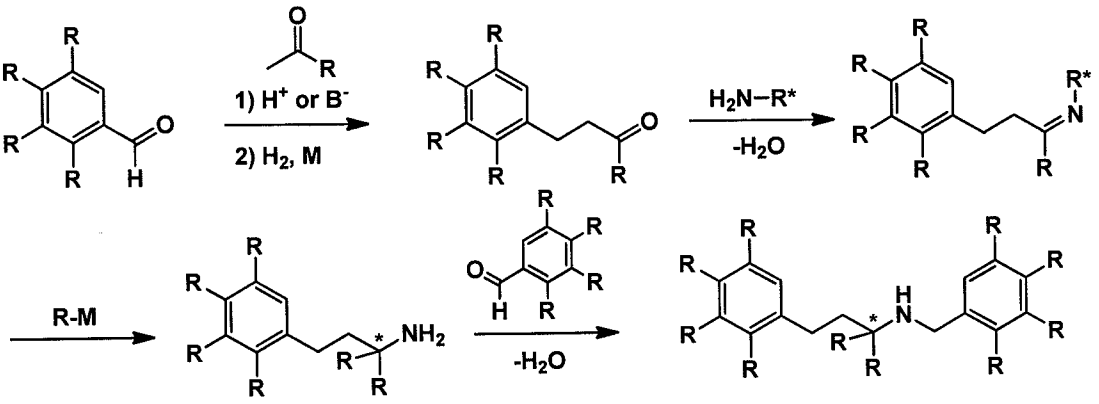
This scheme can produce a racemic mixture of the analogues described herein. Additional R1 groups can also be used to generate other analogues.

[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

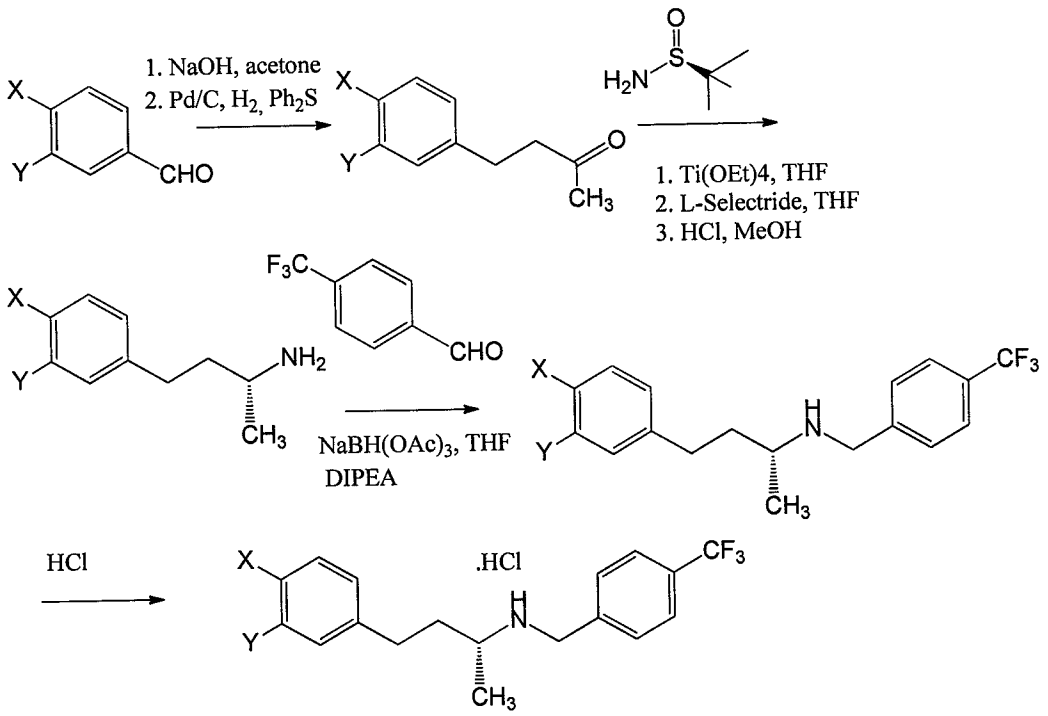
[0277] In some embodiments, the asymmetric synthesis of a compound described herein is prepared according to Scheme 3 (* indicates chiral center):



Scheme 3

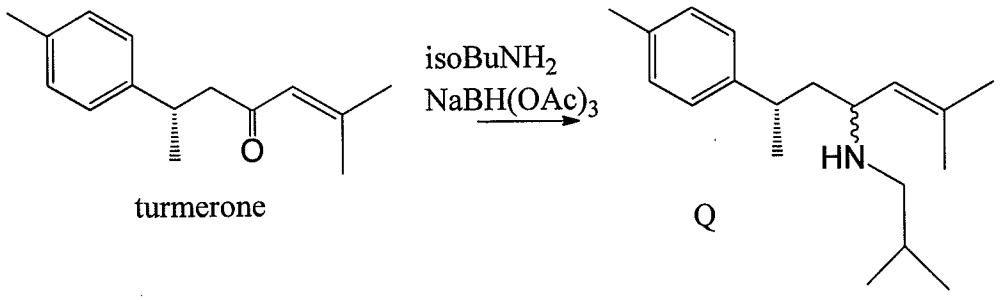
[0278] The synthetic scheme can be altered depending upon the end-product desired. The "R" groups are exemplary and can be substituted with any substituent described herein.

[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-1, [^{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.

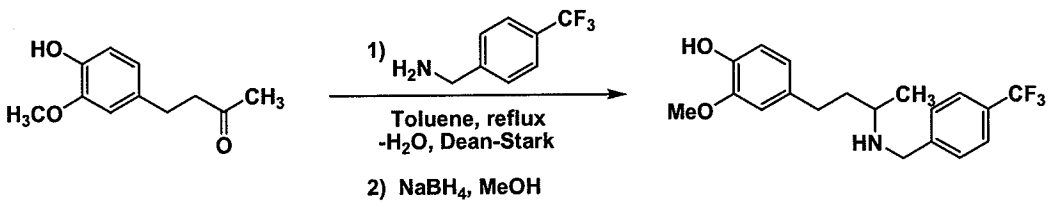
Table 13. Receptor Affinities for Orally Bioavailable Compounds.

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

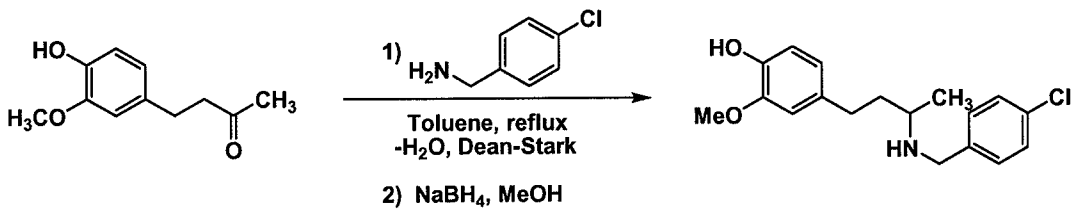
(Phenomenex Part No 00M-4423-B0_CE)

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

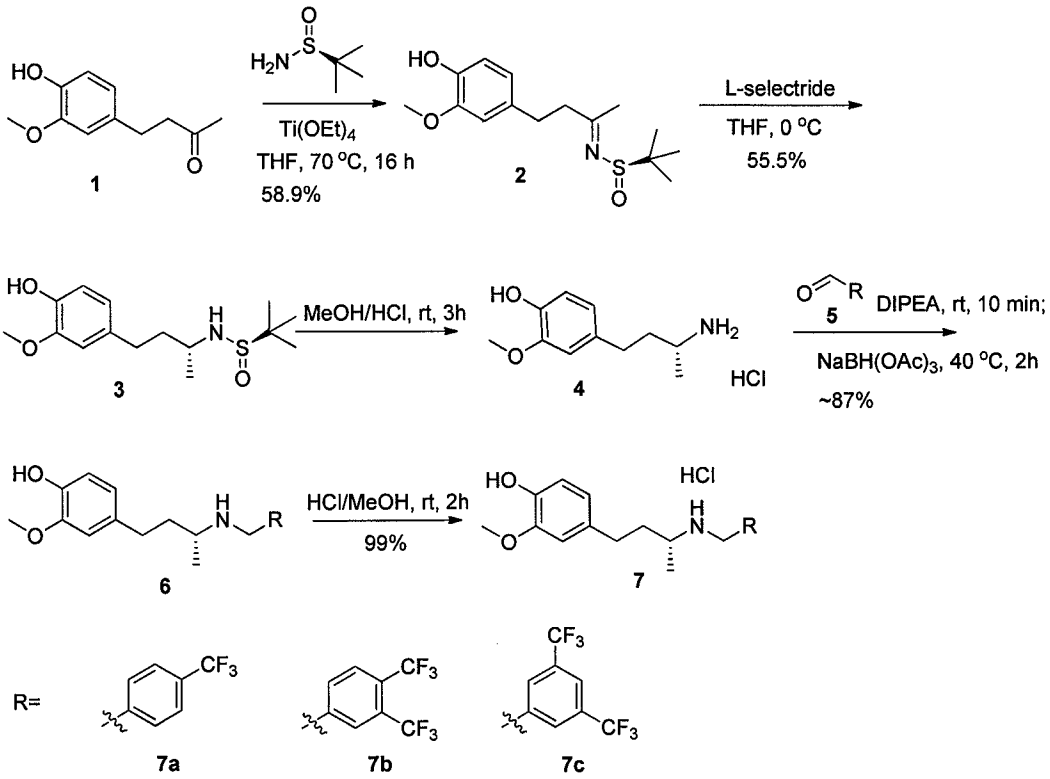
(Phenomenex Part No 00M-4423-B0_CE)

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.

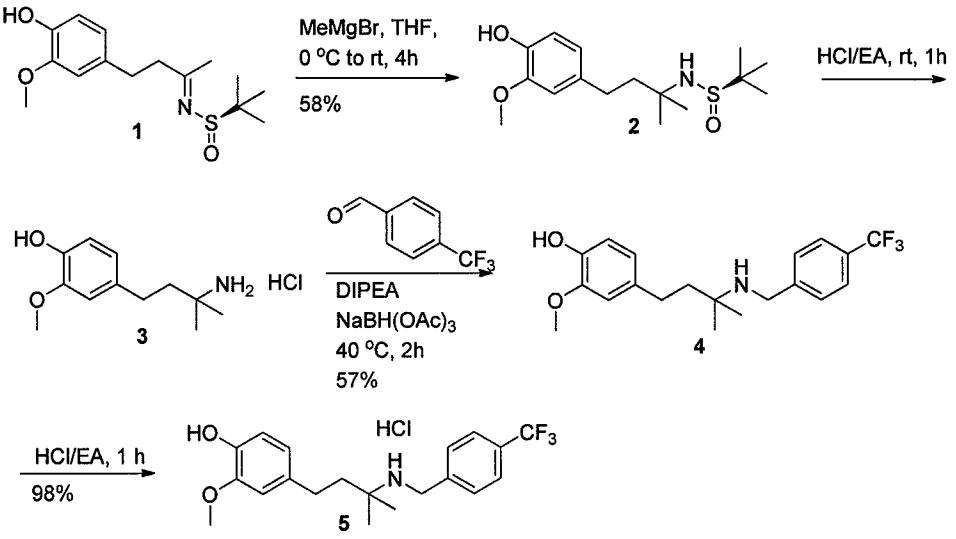
[0409] **Step3** : To a solution of **3** (7.0 g, 23.4 mmol) in MeOH (20 mL), HCl (2 M in MeOH, 20 mL) was added and the resulting solution was stirred at rt over a 3 h period. TLC analysis of the reaction mixture showed complete consumption of compound **3**. The solvent was then removed in vacuum,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

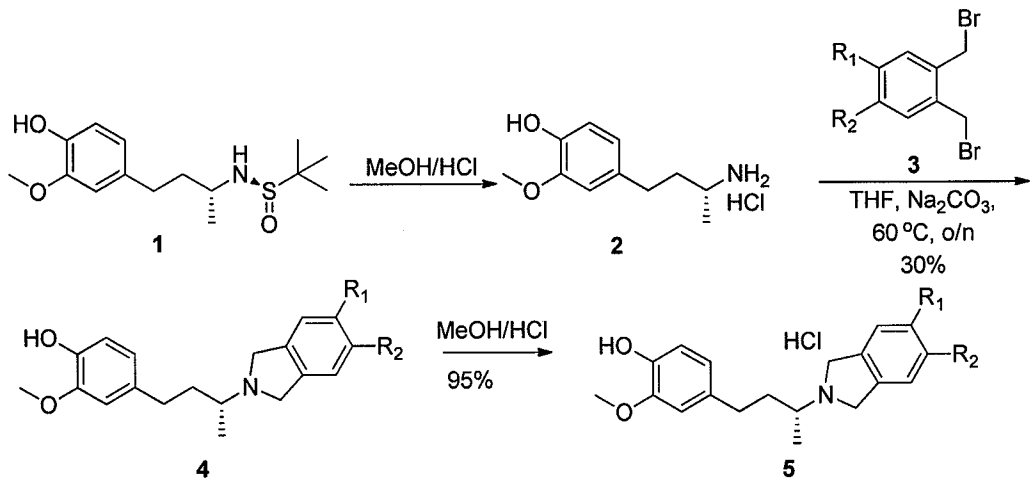
[0414] **Step2**: To a solution of **2** in EA (10 mL) at 0 °C was HCl (2 M in EA, 3 mL), and the resulting solution was stirred at rt for 1 h. Analysis of the reaction mixture by TLC showed complete consumption of **2**. Concentrated under vacuum, the crude product was directly used in next step.

[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.

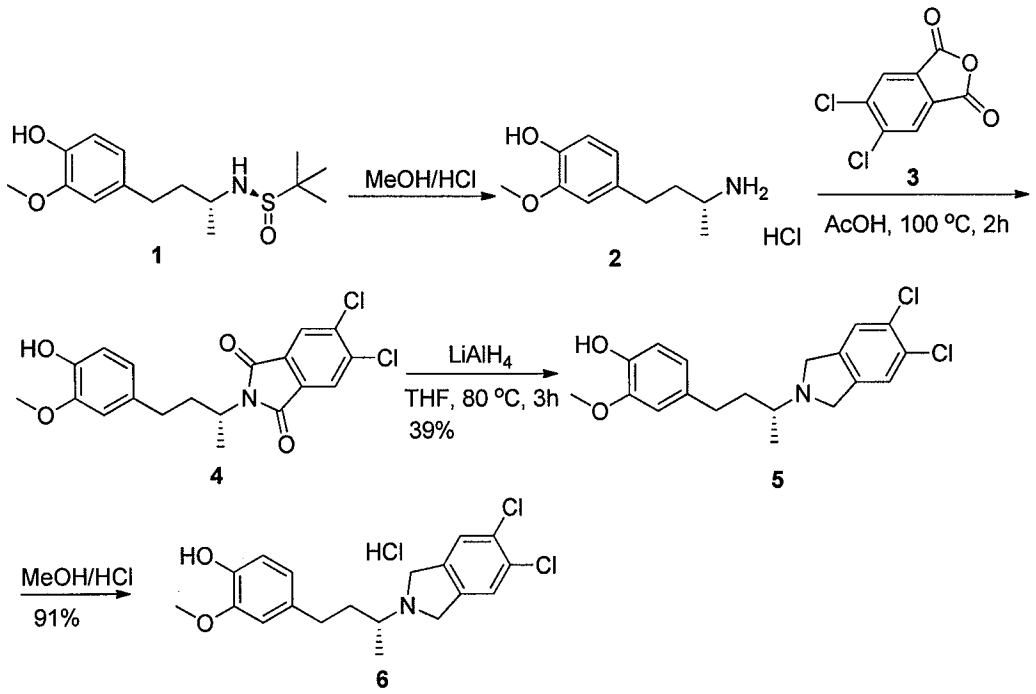
[0419] **Step2**: To a solution of **2** (0.4 g, 2 mmol) in THF (10 mL), compound **3a** (0.54 g, 2 mmol) in THF (5 mL) was added. Na_2CO_3 (0.6 g, 6 mmol) was added, and the resulting solution was stirred at 60 °C overnight. After concentration, the residue was purified by FCC to give compound **4**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

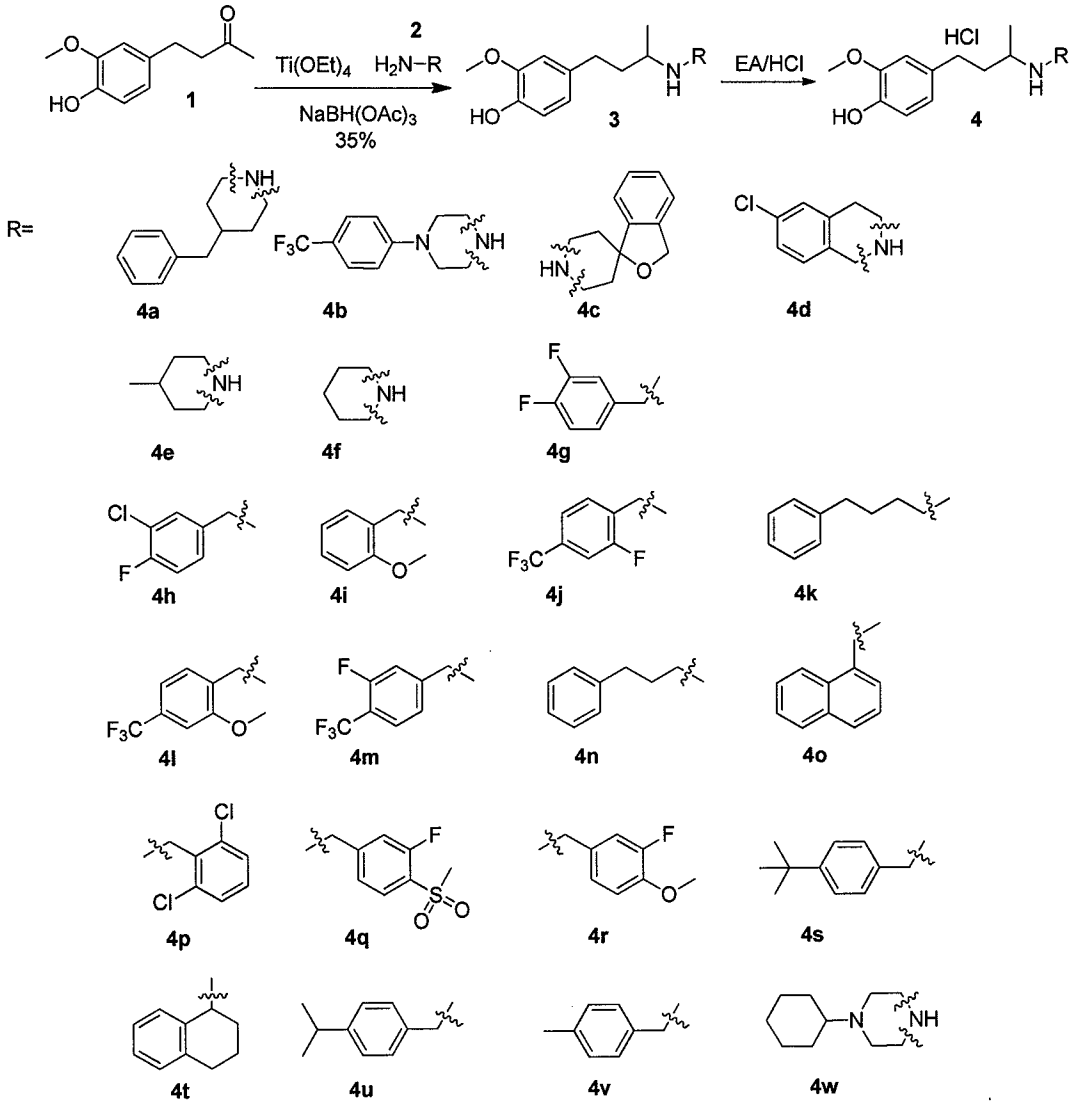
[0423] **Step2**: Compound **2** (0.2 g, 1 mmol) and **3** (0.2 g, 1 mmol) was dissolved in acetic acid (10 mL), stirred at 100 °C for 2 h. The mixture was cooled to rt and quenched by water (10 mL), extracted by EtOAc (3 x 20 mL), dried, concentrated to give the title compound **4** (0.3 g, 76%).

[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

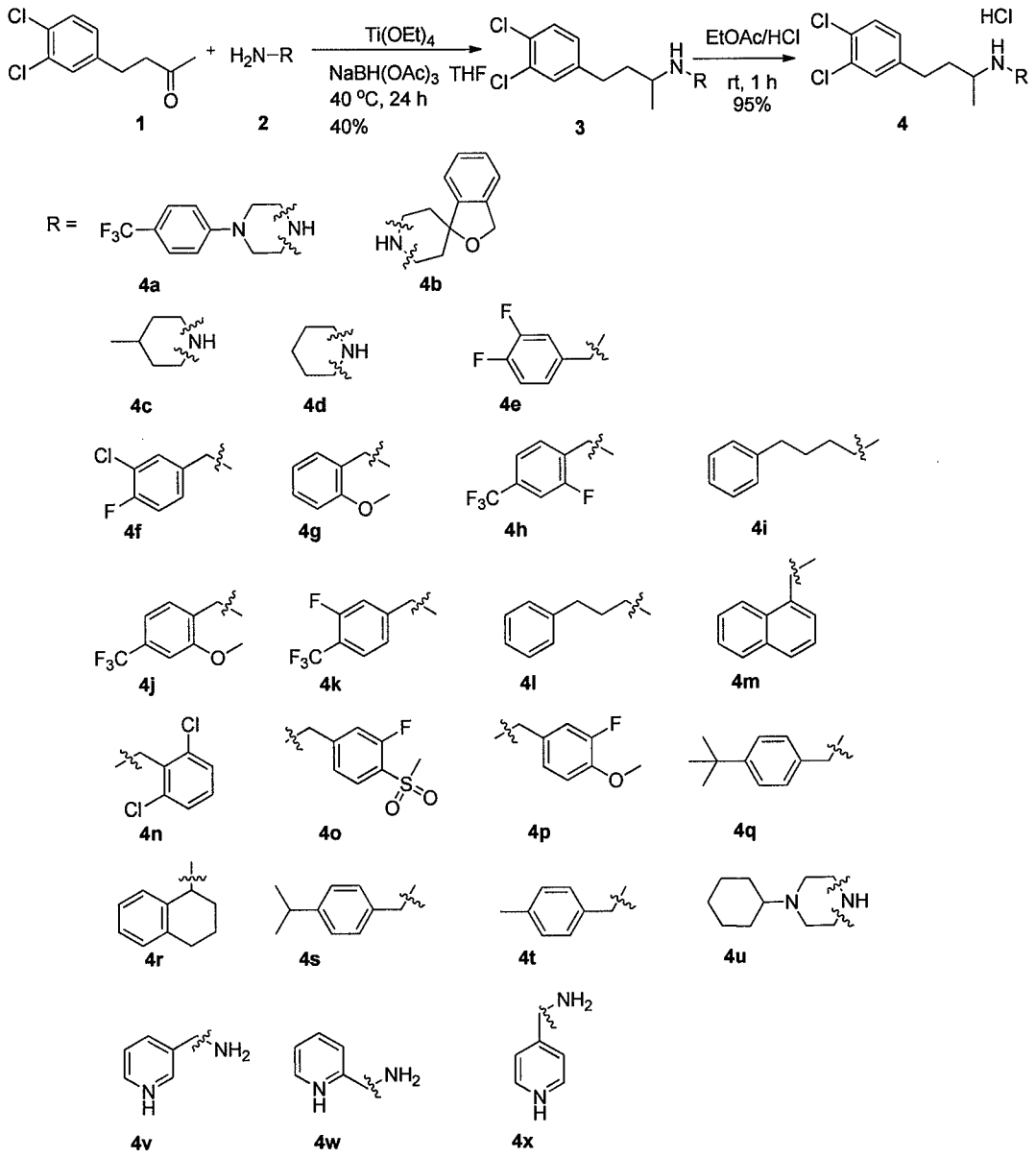
[0427] **Step1:** To a solution of **1** (0.278 g, 1.43 mmol) in THF (20 mL) was added $Ti(OEt)_4$ (2.1 g, 9.2 mmol) and (4-benzylpiperidine (0.34 g, 1.3 mmol). The mixture was stirred at 40 °C for one day, quenched by ice water, extracted with ethyl acetate (3 x 20 mL). After being concentrated *in 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



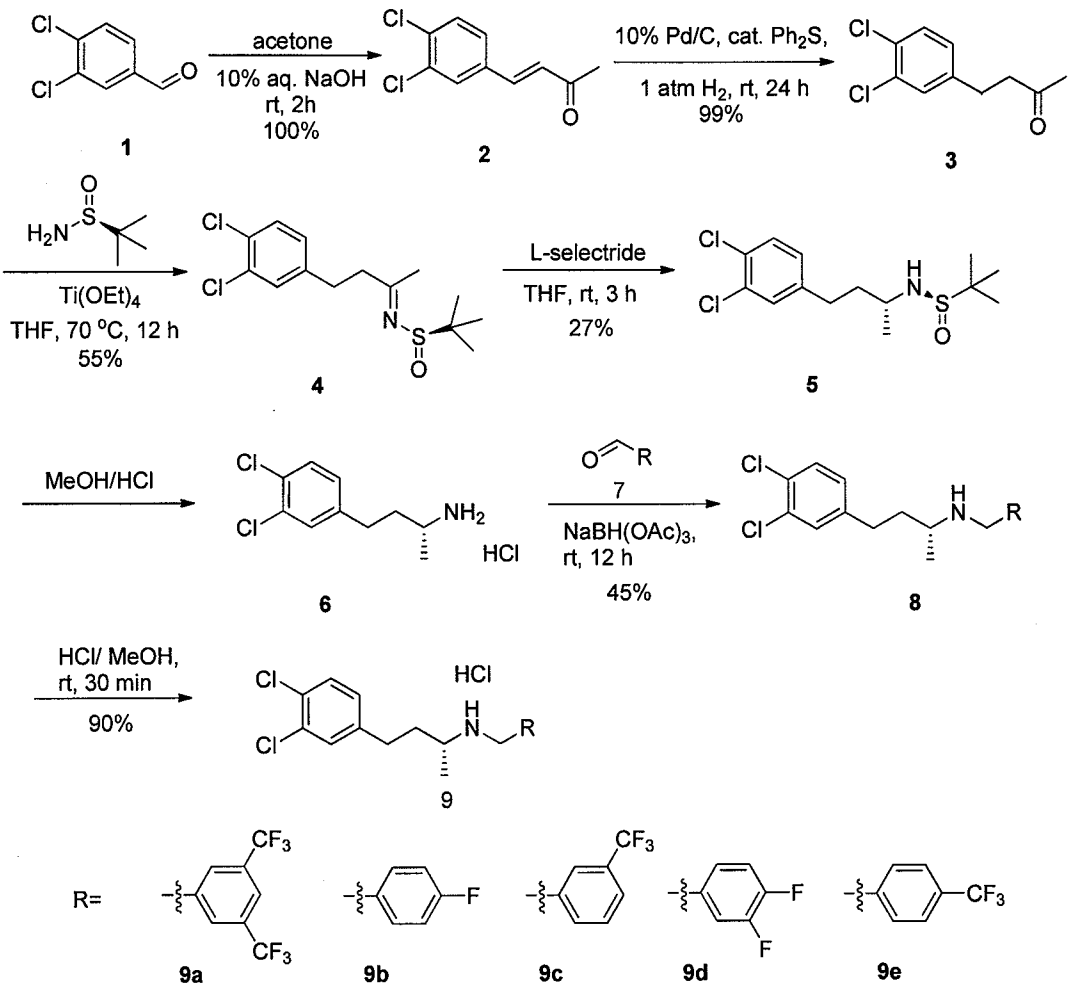
Scheme 8

[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.

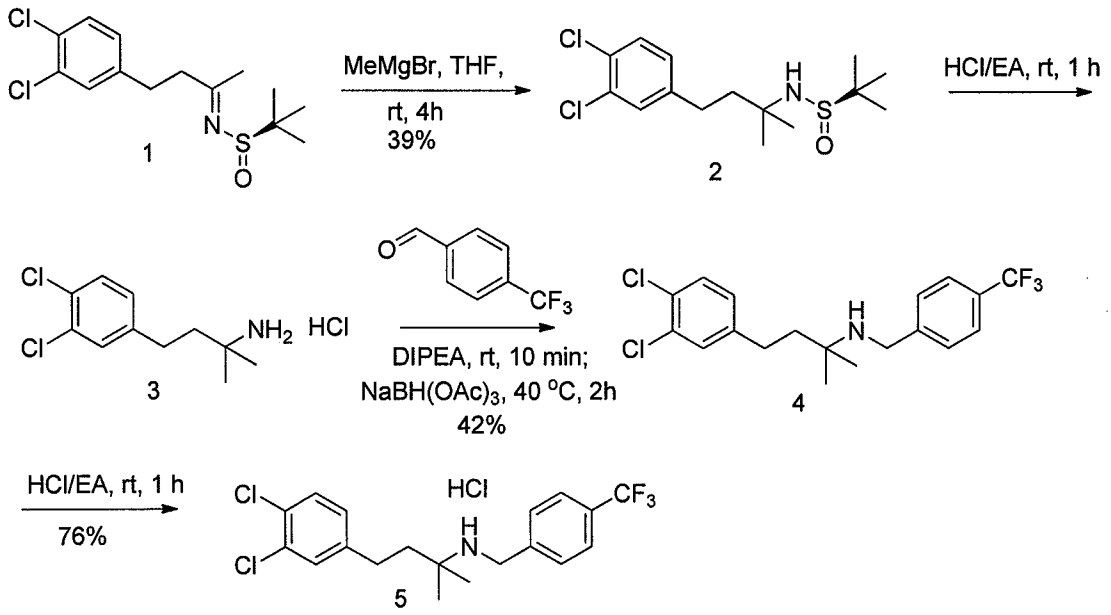
[0438] **Step6 :** To a solution of **6** (0.6 g, 2.4 mmol) in THF (10 mL), DIPEA (0.4 g, 3.1 mmol) and 3-trifluoromethylbenzaldehyde (0.41 g, 2.4 mmol) were added subsequently. The resulting solution was stirred at rt for 10 min. $NaBH(OAc)_3$ (1.0 g, 4.7 mmol) was added and the mixture was stirred 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%).

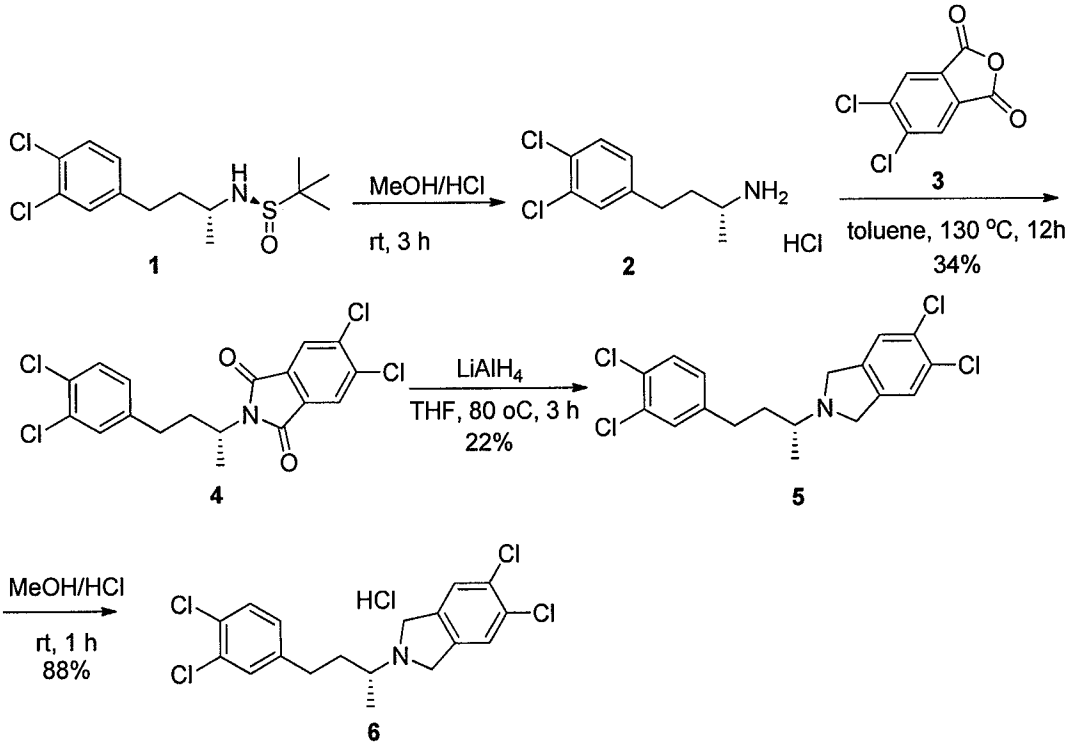
[0442] **Step2**: To a solution of compound **2** (0.6 g, 1.8 mmol) in ethyl acetate (10 mL), HCl (2 M in MeOH, 3 mL) was added and the resulting solution was stirred at rt for 1 h. TLC analysis of the reaction mixture showed complete consumption of compound **2**. The solvent was then removed in vacuum,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

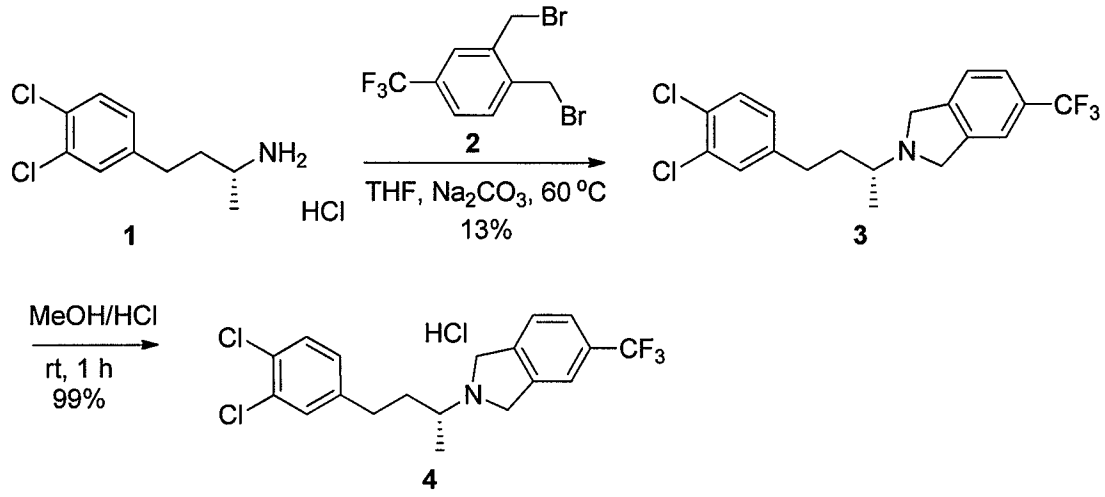
[0448] **Step3**: To a solution of compound **4** (0.78 g, 1.87 mmol) in THF (20 mL) was added LAH (0.36 g, 9.1 mmol). The mixture was stirred at 80℃ for 3h.

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



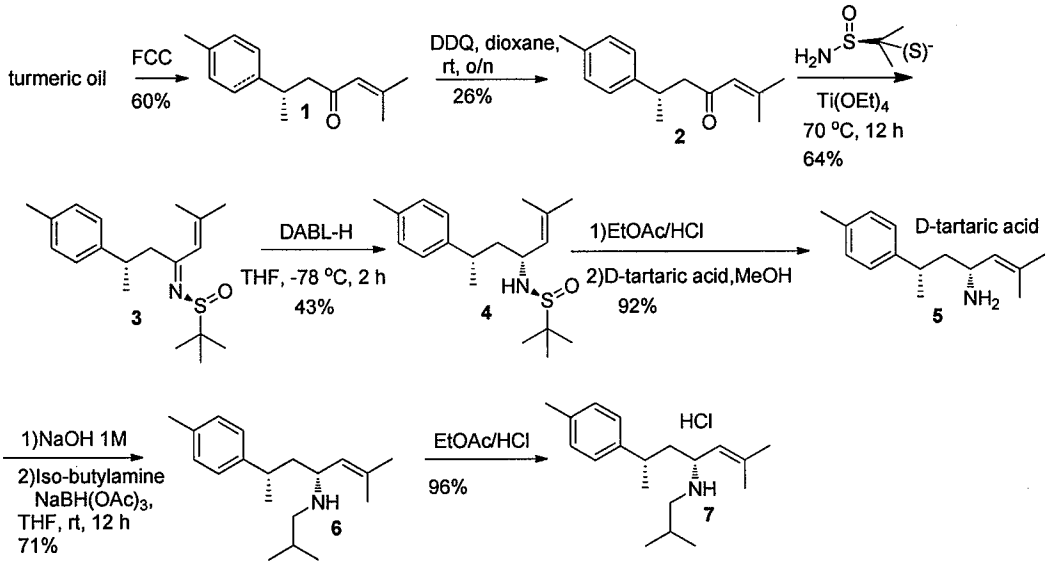
Scheme 12

[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%).

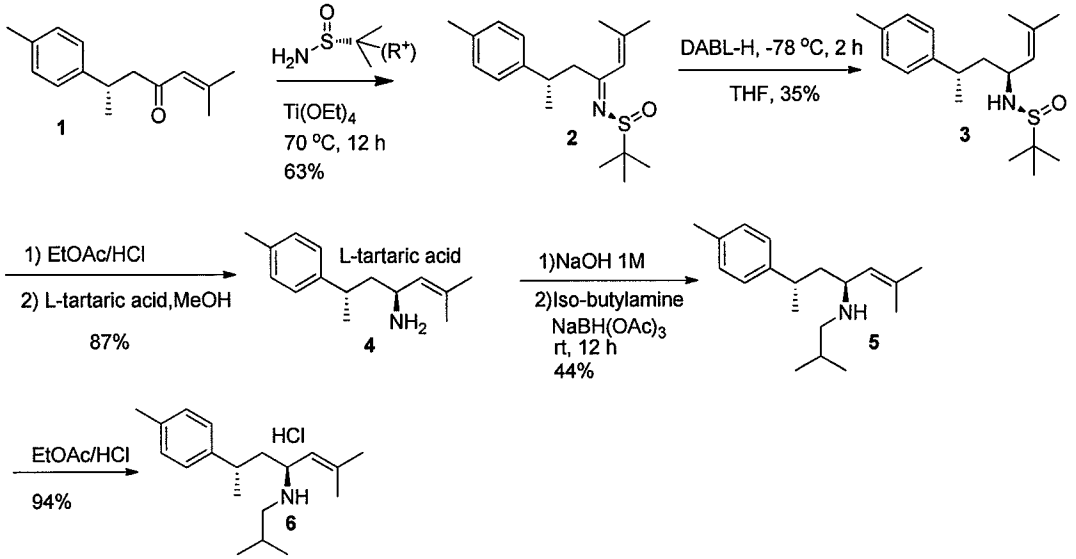
[0457] **Step 5:** To a solution of compound **4** (1.6 g, 5.0 mmol) in ethyl acetate (10 mL) was added HCl-ethyl acetate (2 N, 10 mL), and the resulting solution was stirred at room temperature for 3 h. TLC analysis of the reaction mixture showed complete consumption of compound **3**. The solvent was 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%).

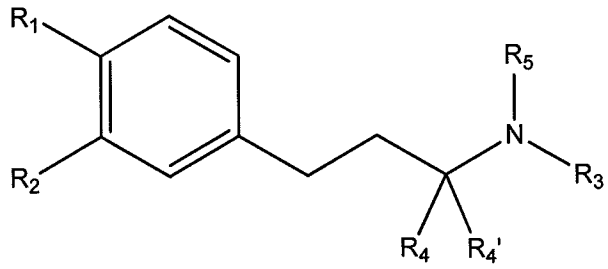
[0466] m/z (ESI+) (M+H)+: **6** [274.20]

[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:

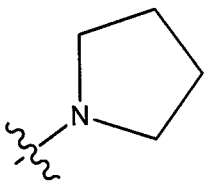


I

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, $(R_{16})(R_{17})N$-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



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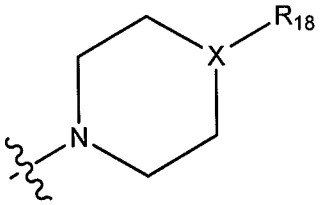
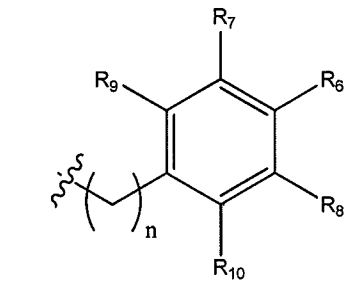


, wherein

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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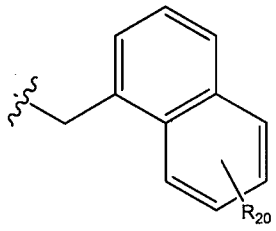

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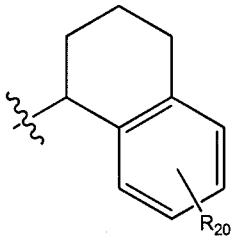

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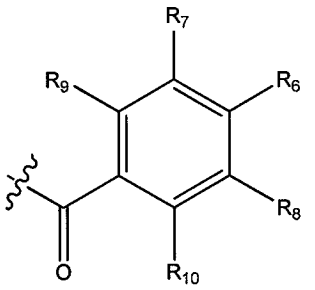

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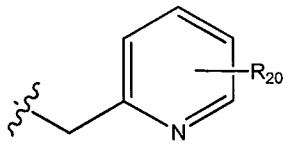

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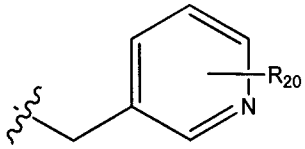

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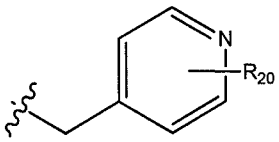

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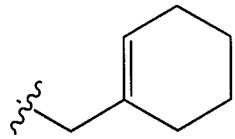


wherein

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

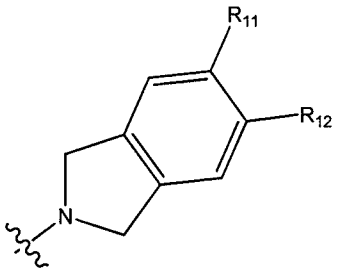
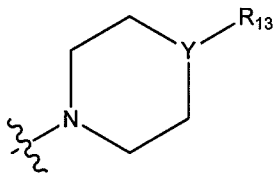
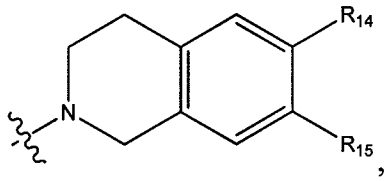
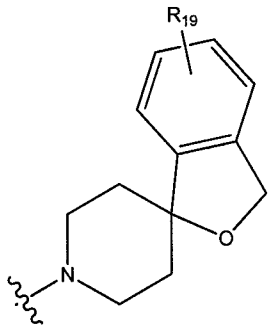
n is 1-4

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;

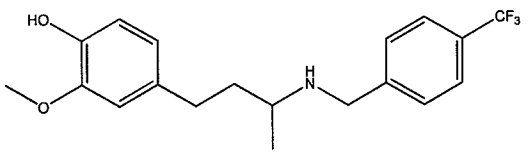
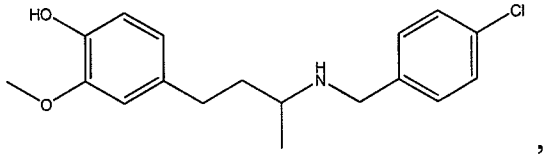
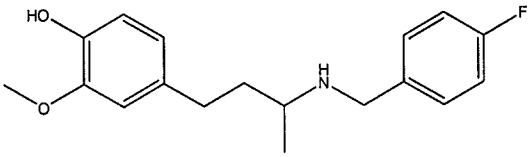
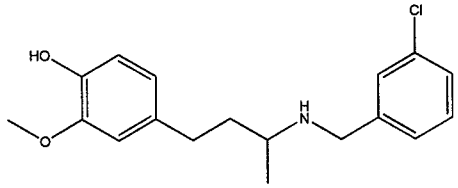
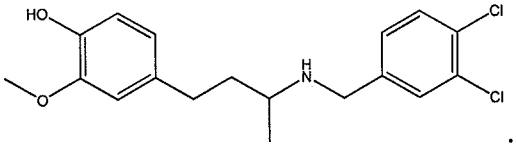
R_{13}.is H, C_{1-6} alkyl, C_{3-6} cycloalkyl, unsubstituted phenyl or phenyl substituted with C_{1-6} haloalkyl, or unsubstituted benzyl

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

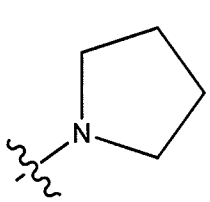






, and .

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, $(R_{16})(R_{17})N$-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



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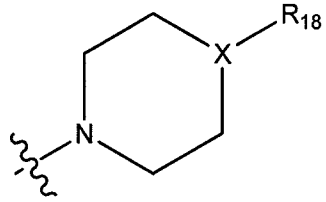
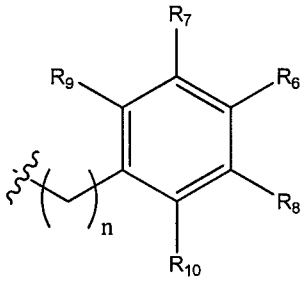


, wherein

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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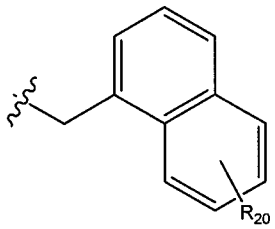

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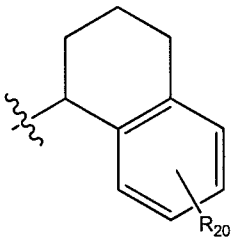

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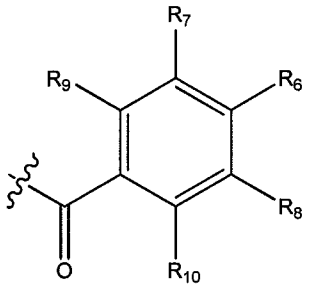

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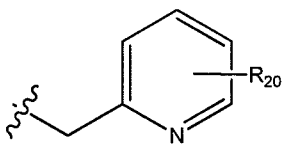

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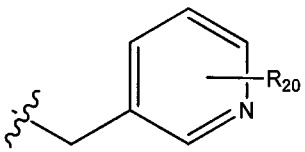

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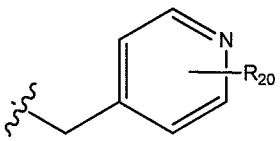

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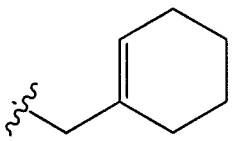


wherein

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

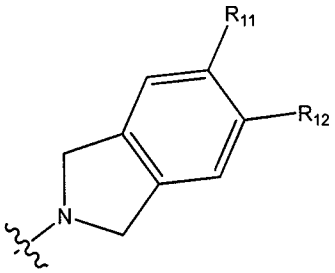
n is 1-4

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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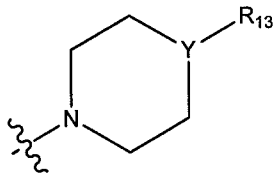

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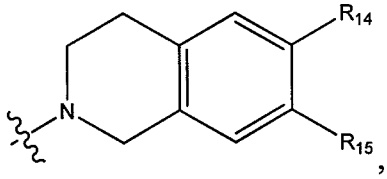

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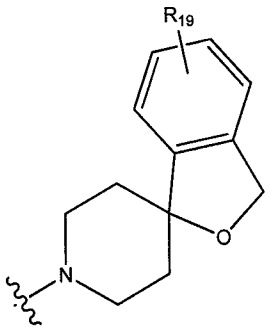


and , wherein

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; and

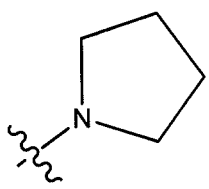
R_{19} is H, and

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



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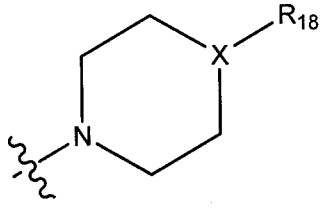


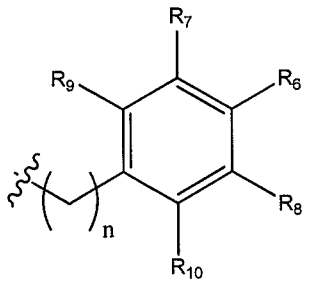
, wherein

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



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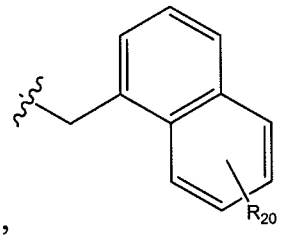
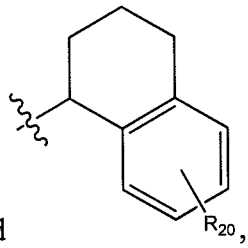


, and


,

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

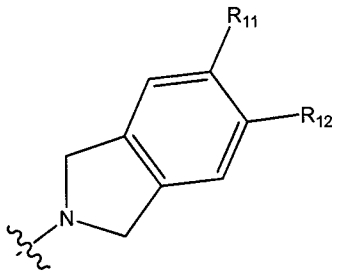
n is 1

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



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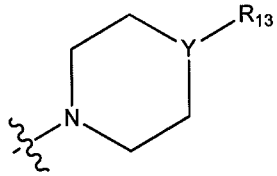

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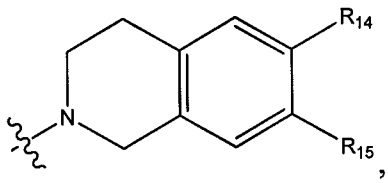

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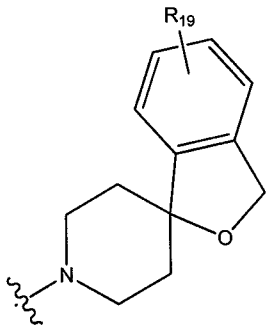


and , wherein

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

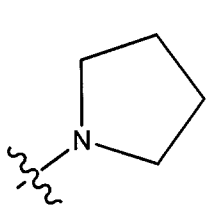
R_{19} is H, and

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



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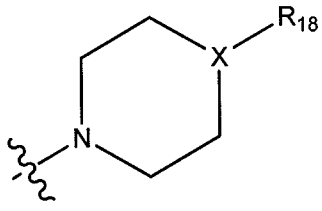


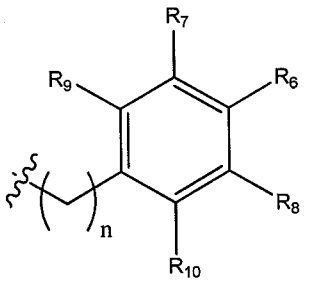
, wherein

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

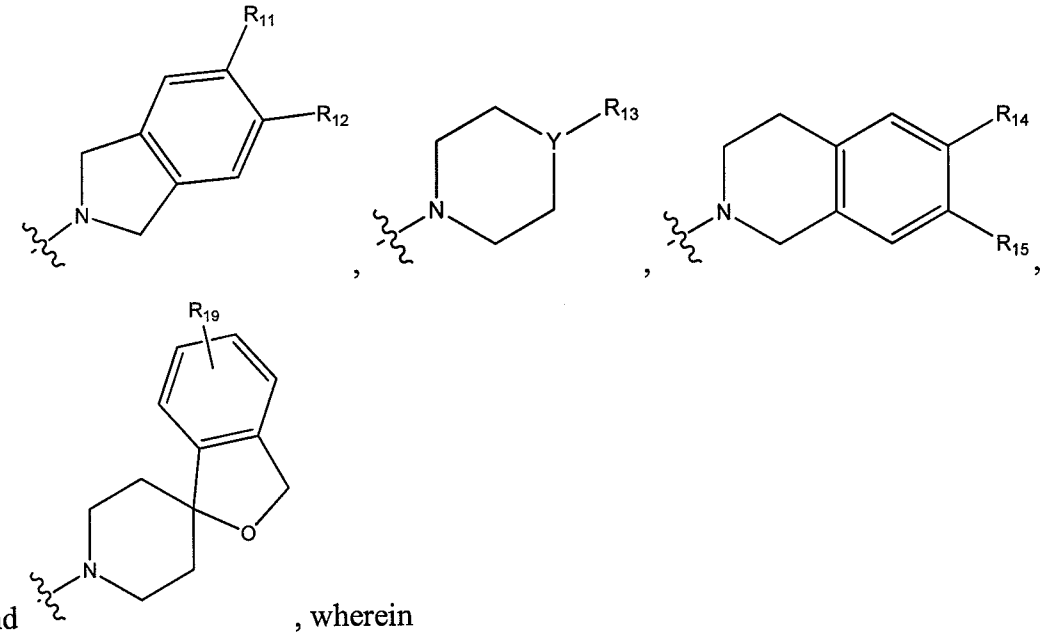
n is 1

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;

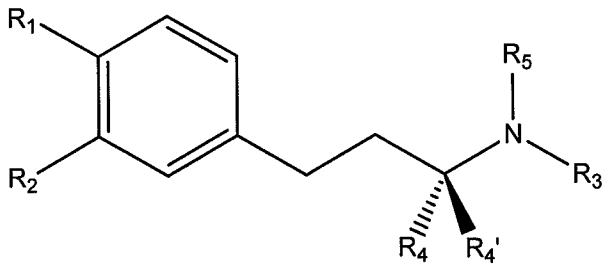
R_{13}.is H, Me, cyclohexyl, unsubstituted phenyl or phenyl substituted with CF_3, or unsubstituted benzyl

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

R_{19} is H, and

pharmaceutically acceptable salts thereof.

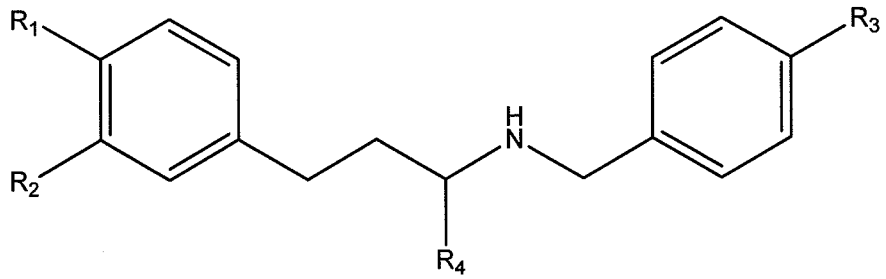
5. The compound of claim 1 that is a compound of Formula Ia



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



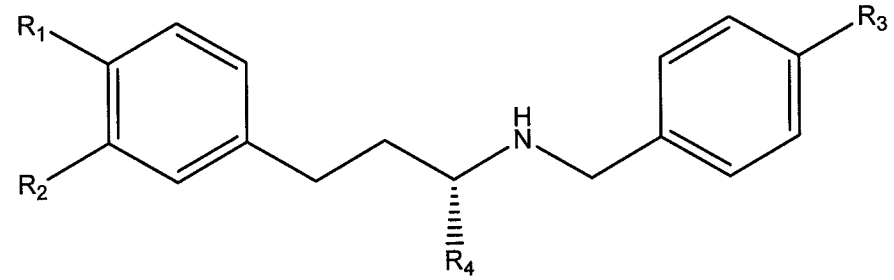
IIa

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.

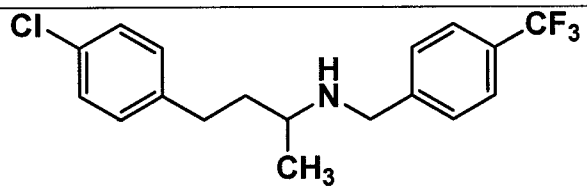
8. The compound of claim 6 that is a compound of Formula IIb



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: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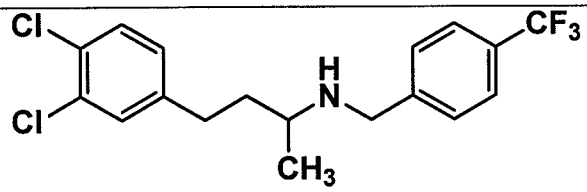
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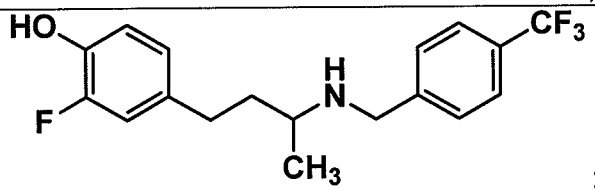
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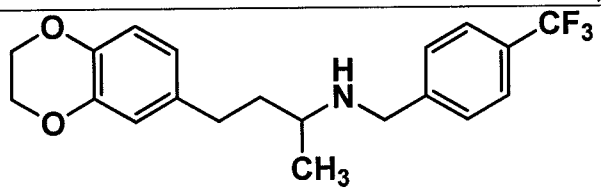
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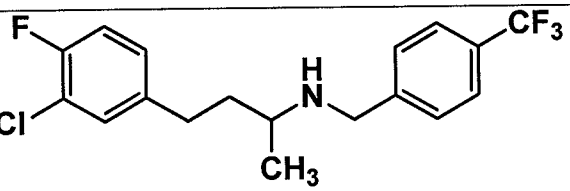
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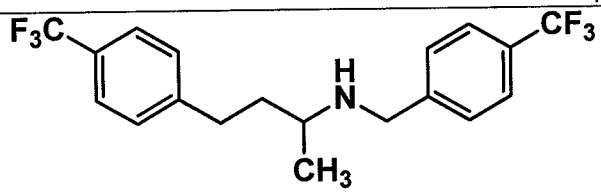
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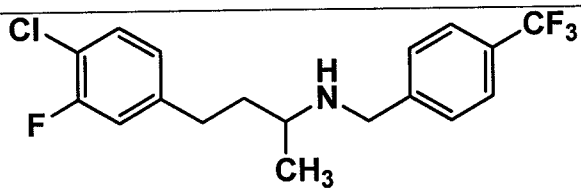
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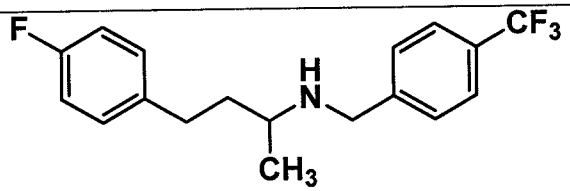
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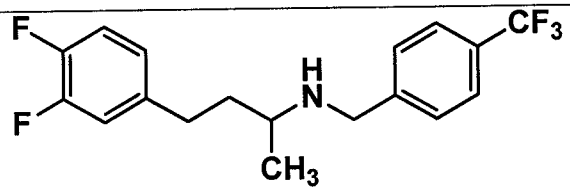
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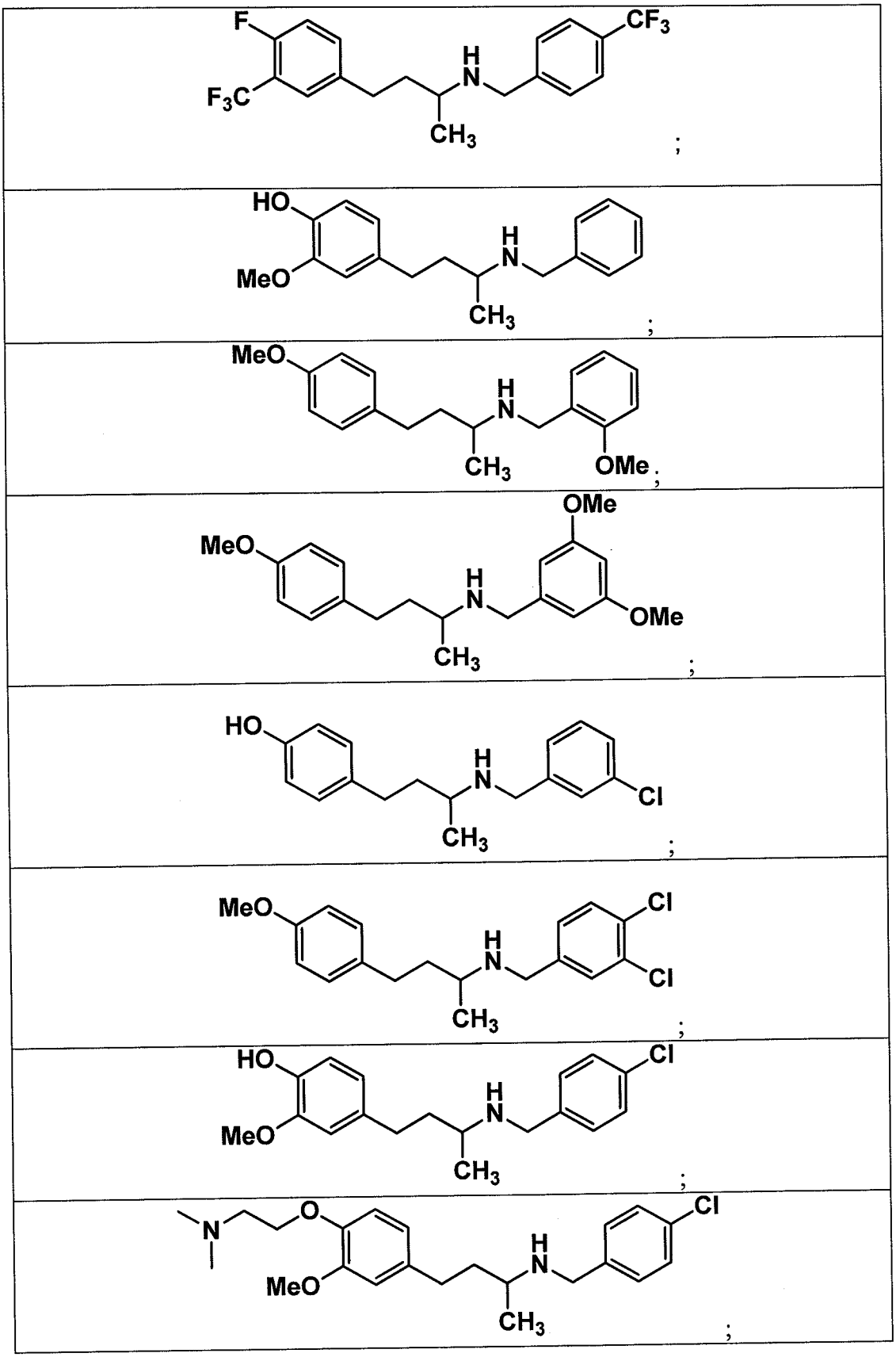

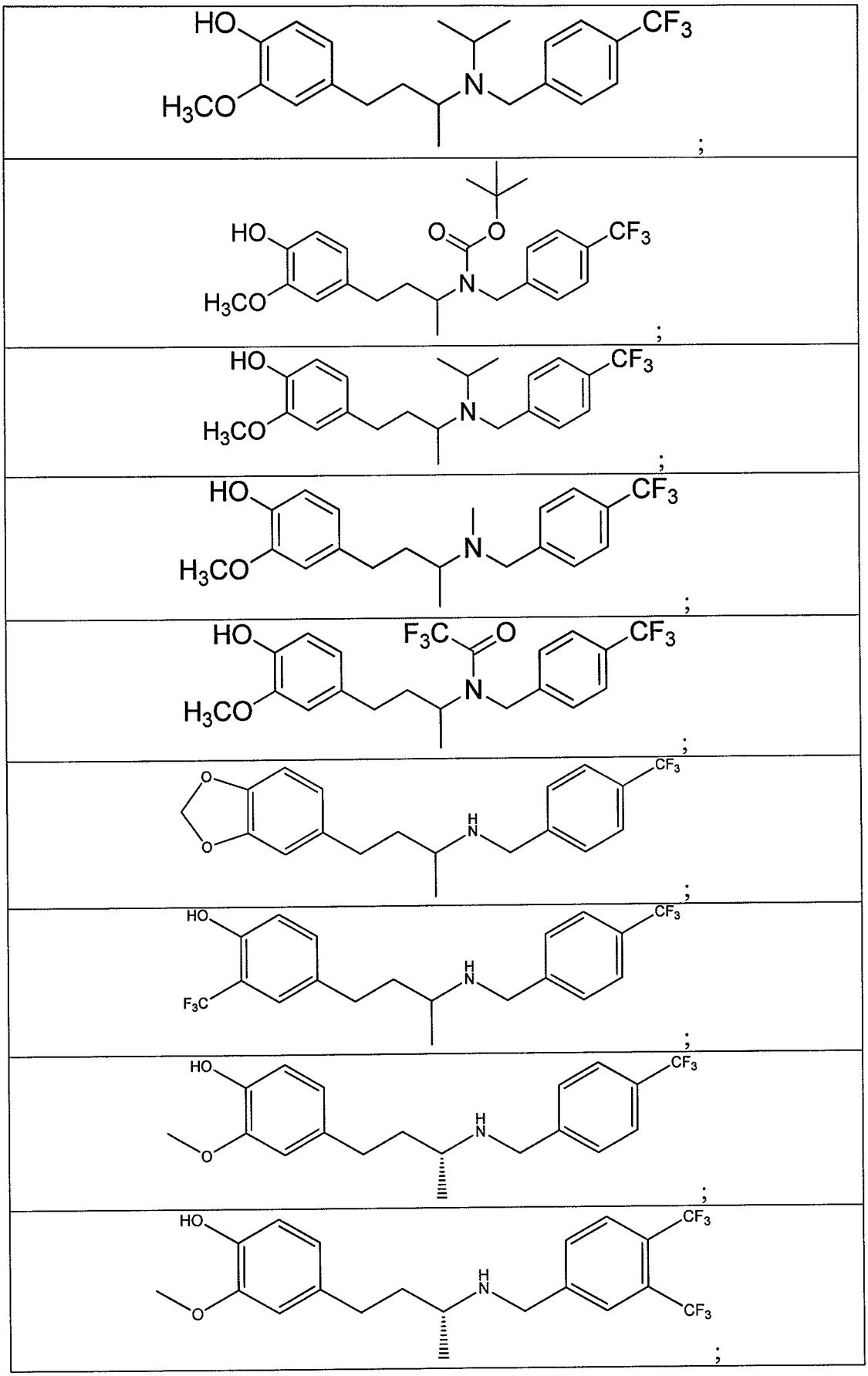

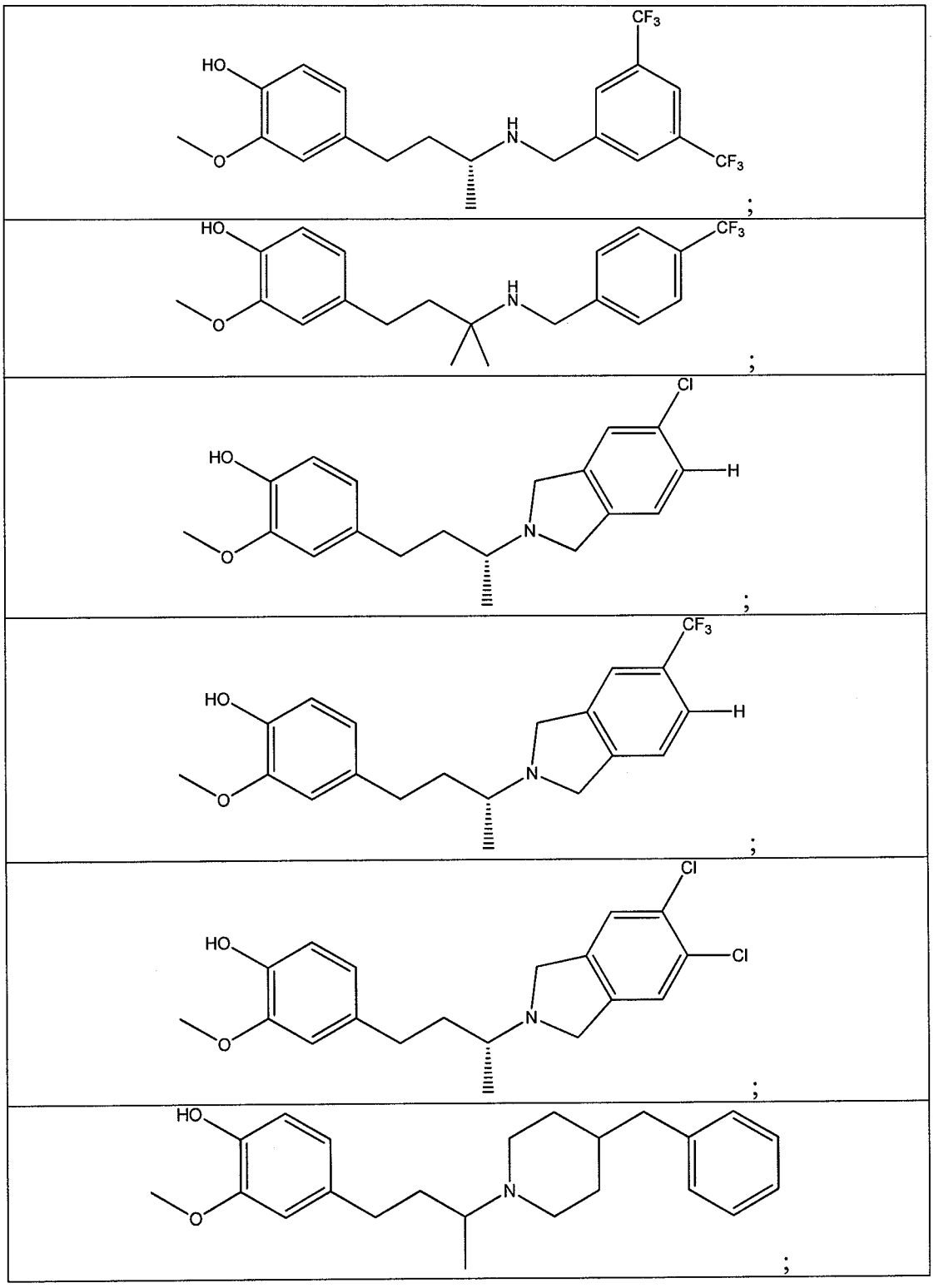

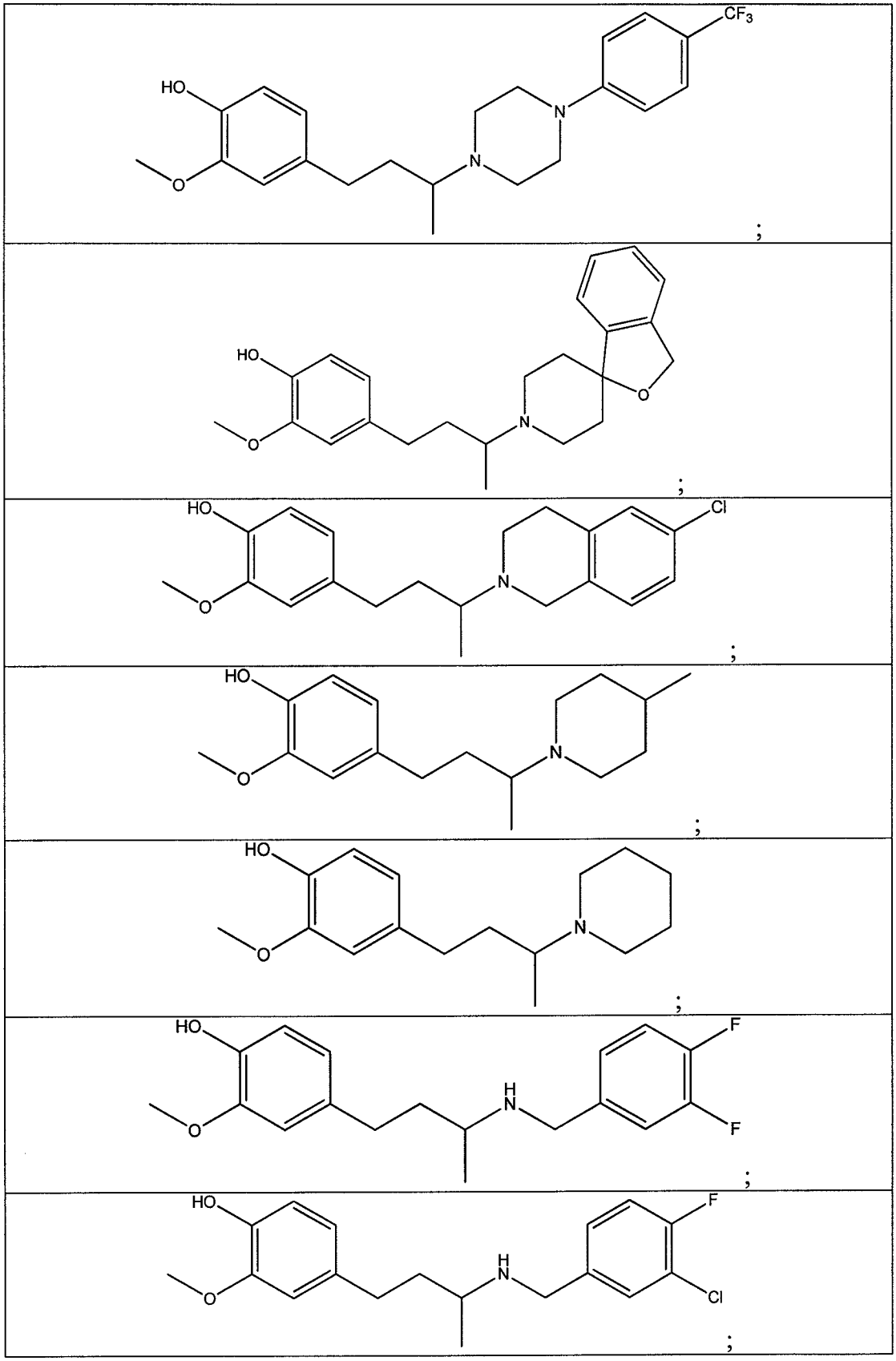

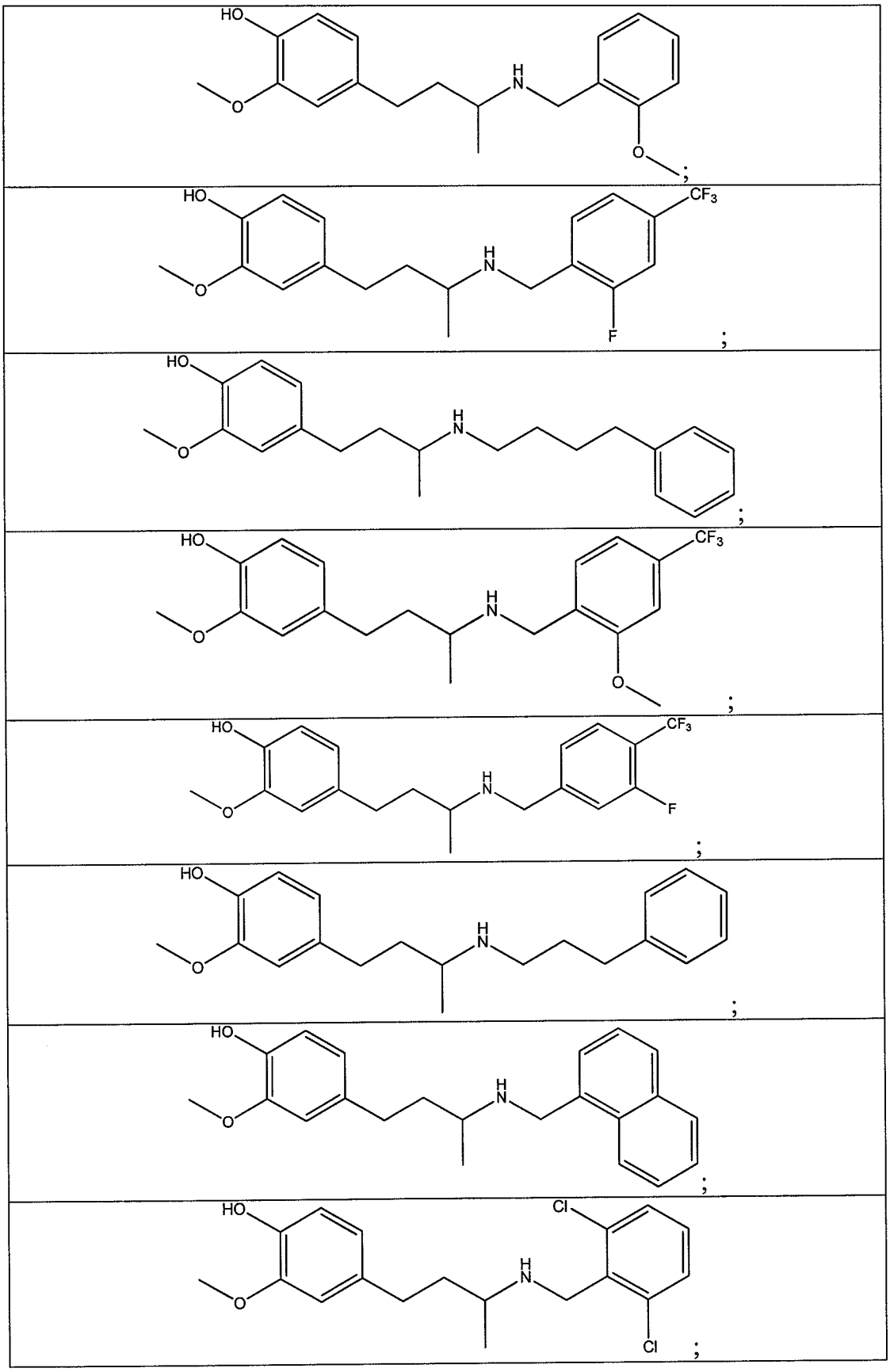

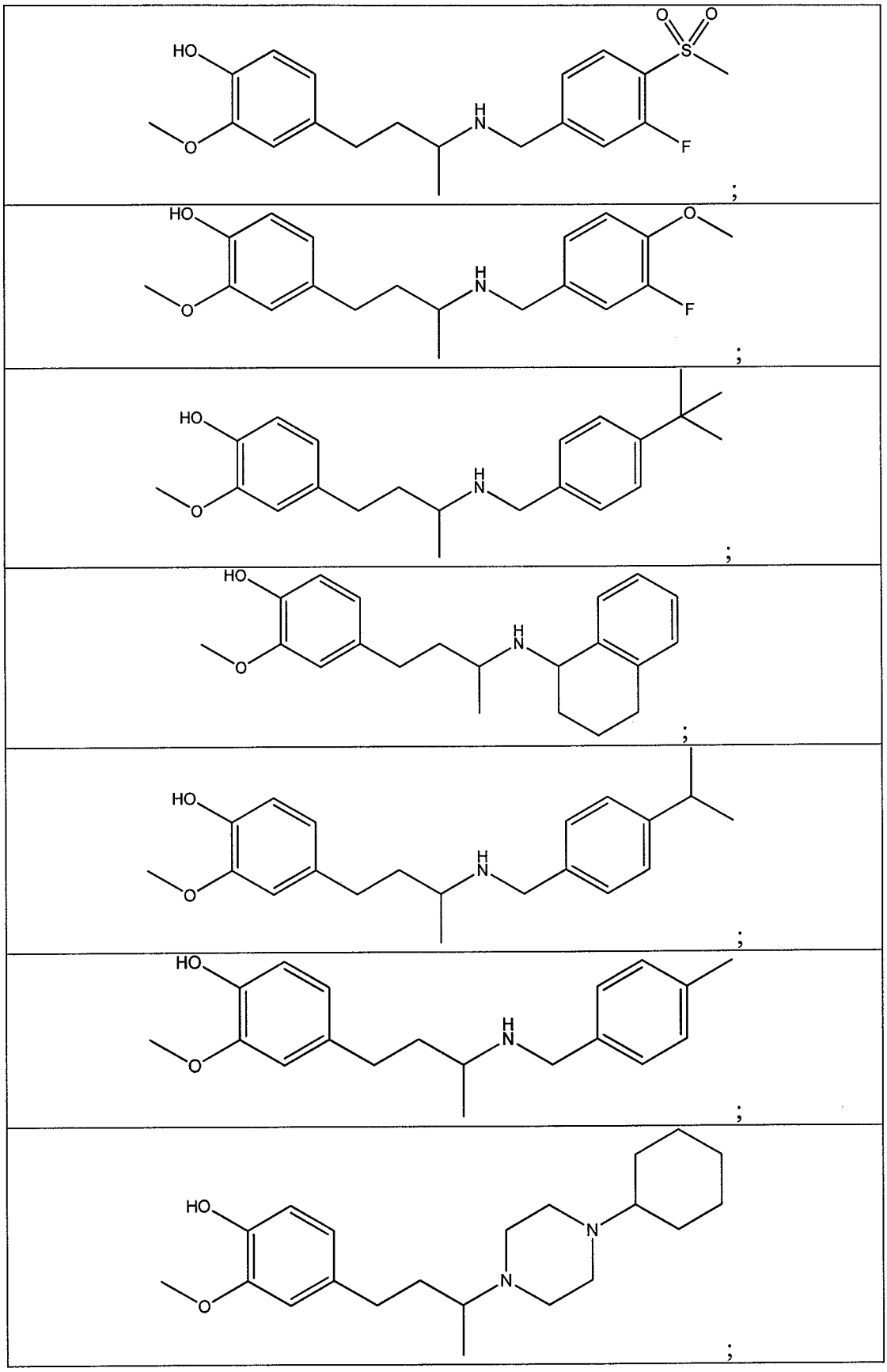

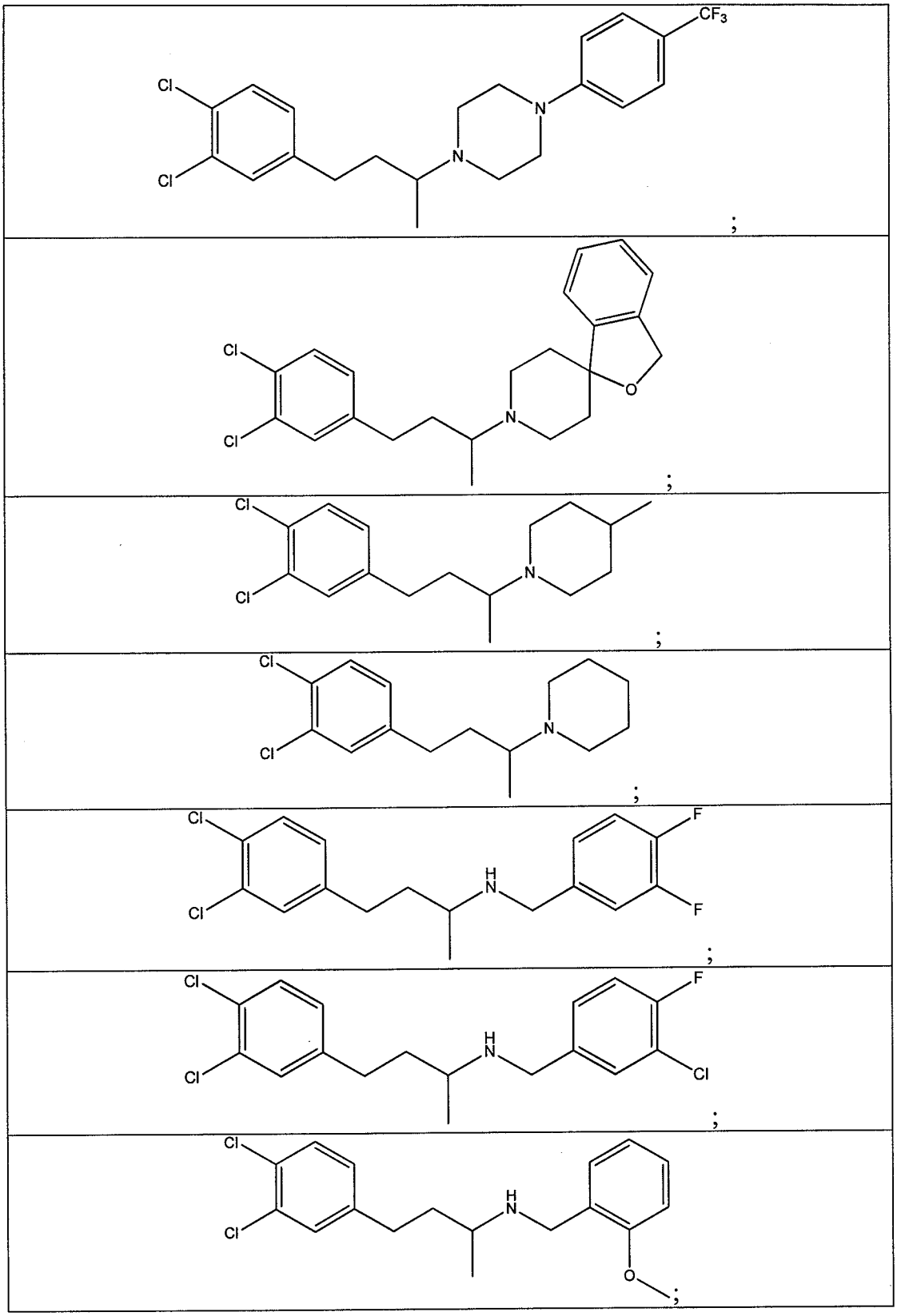

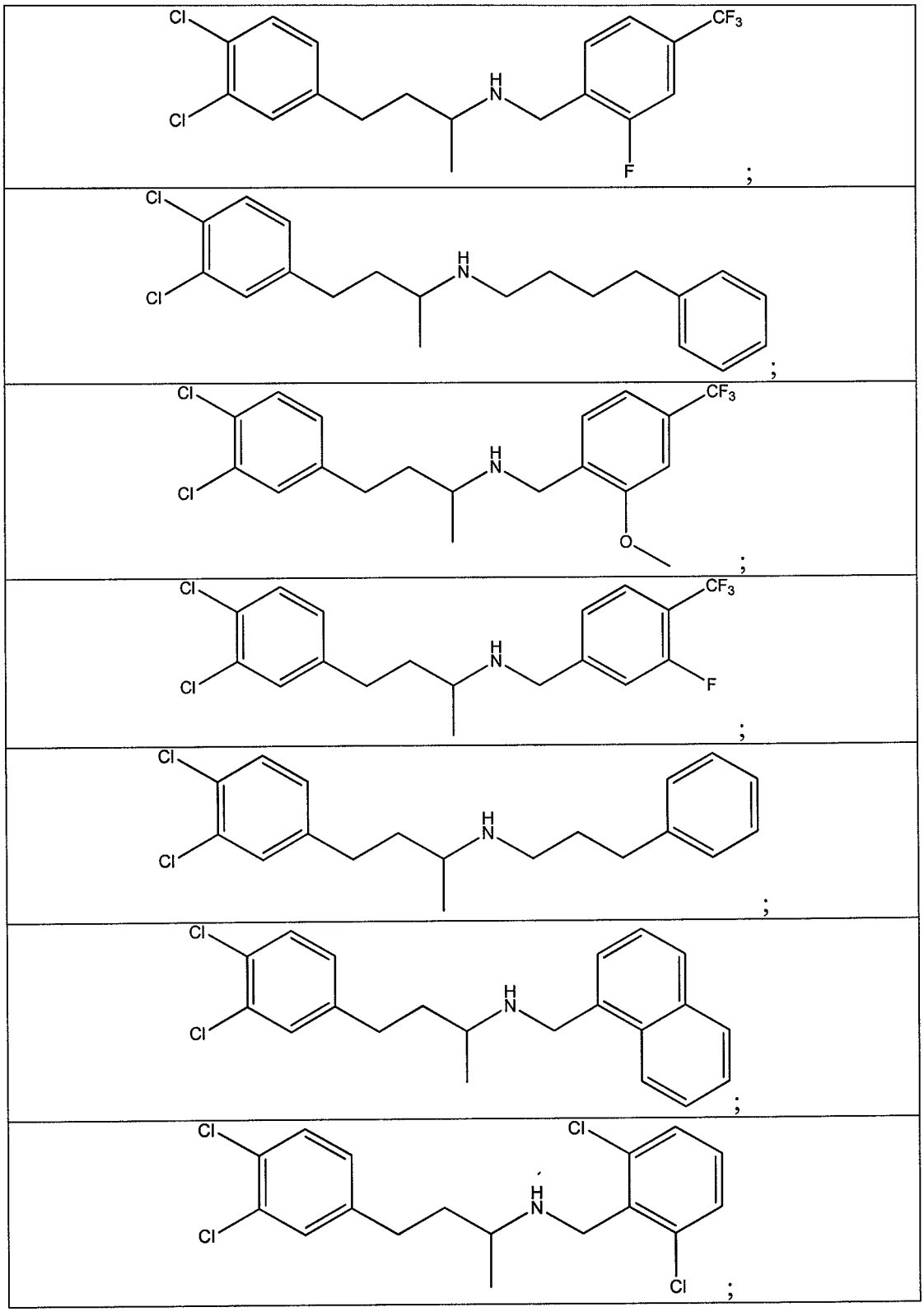

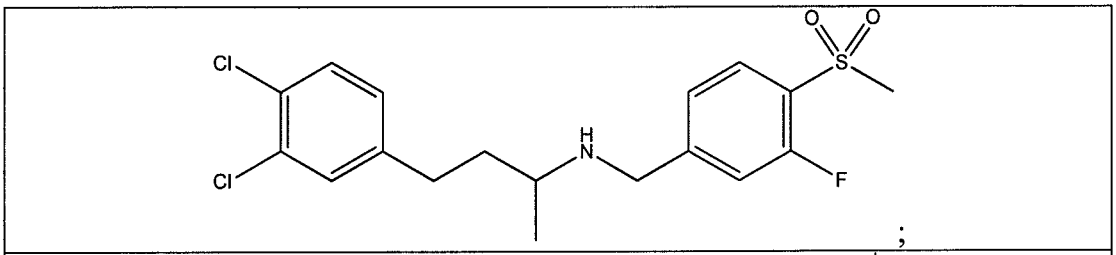
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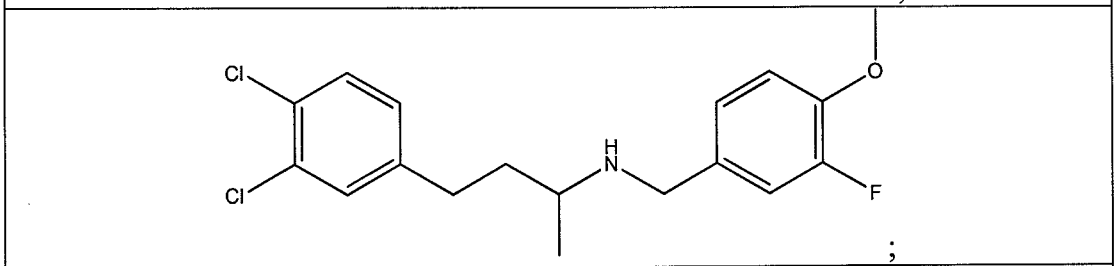
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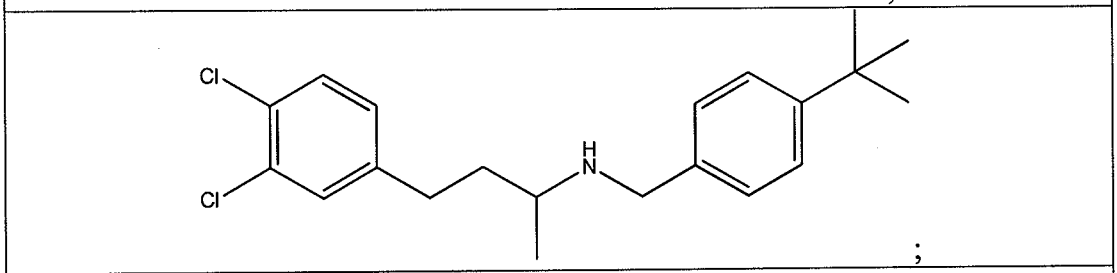
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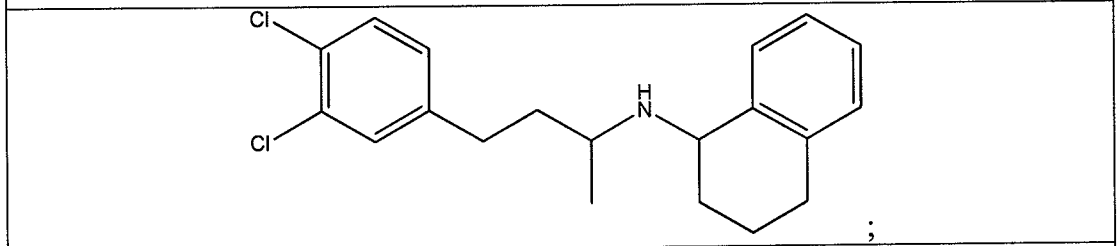
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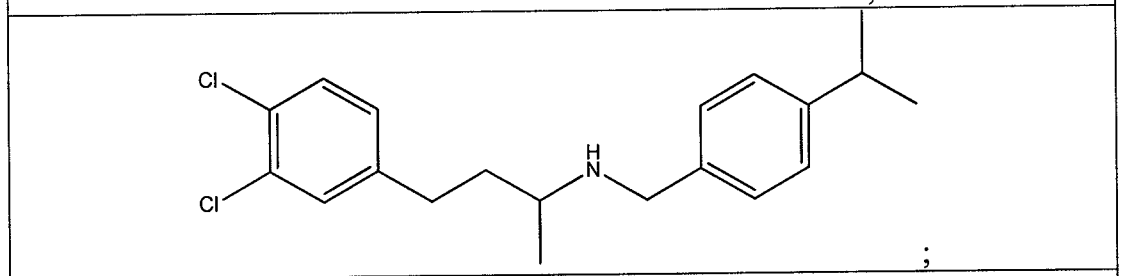
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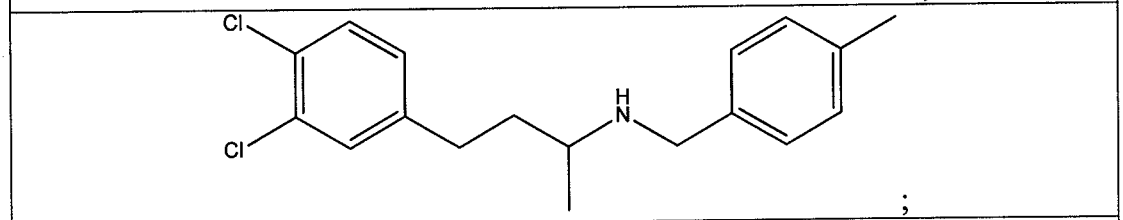
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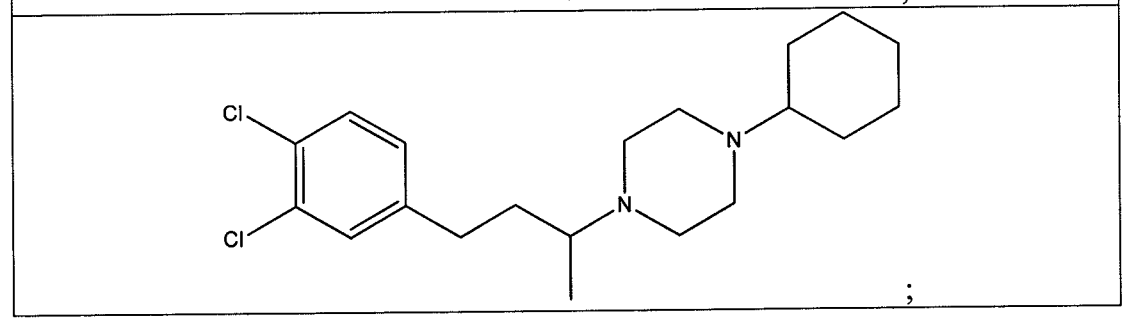
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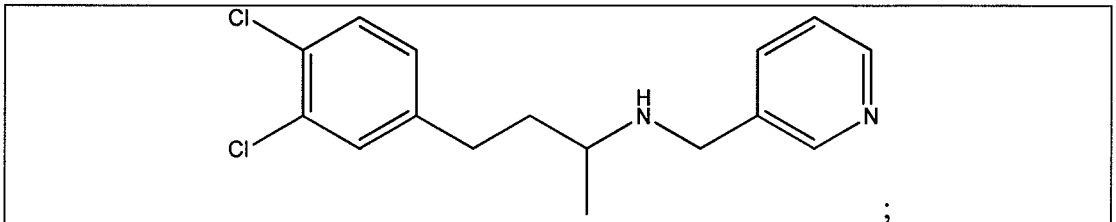
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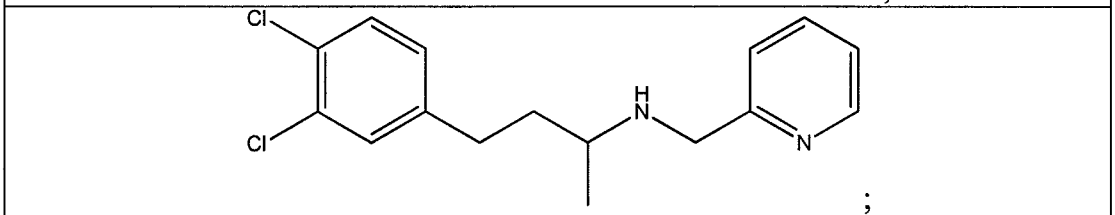
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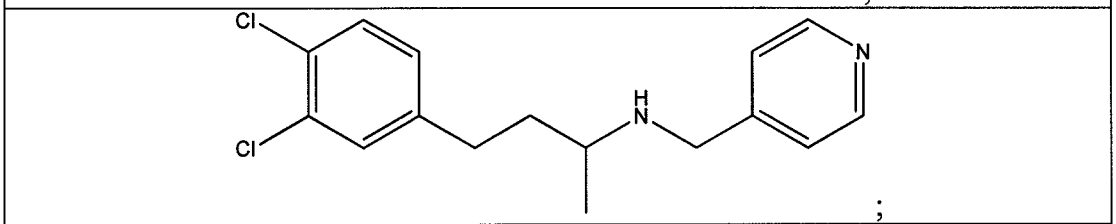
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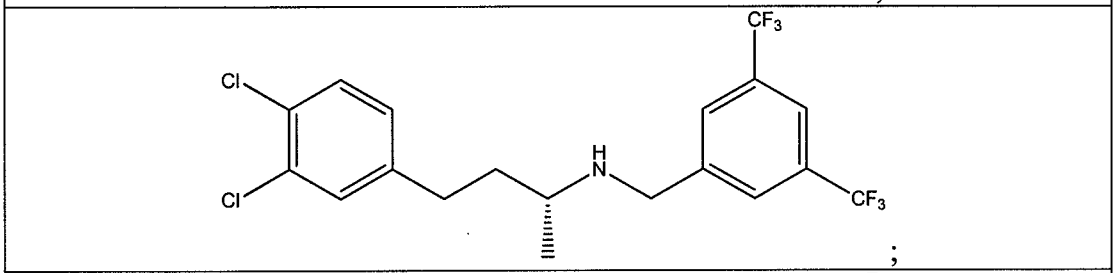
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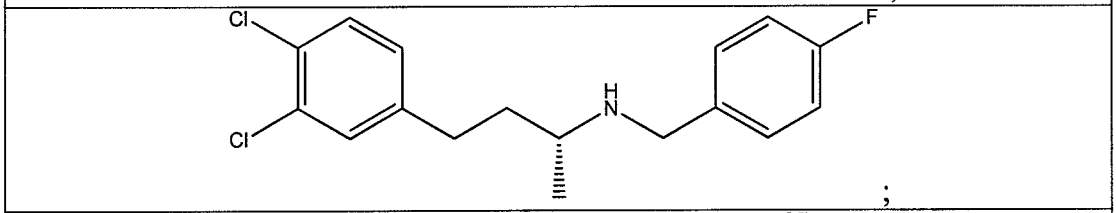
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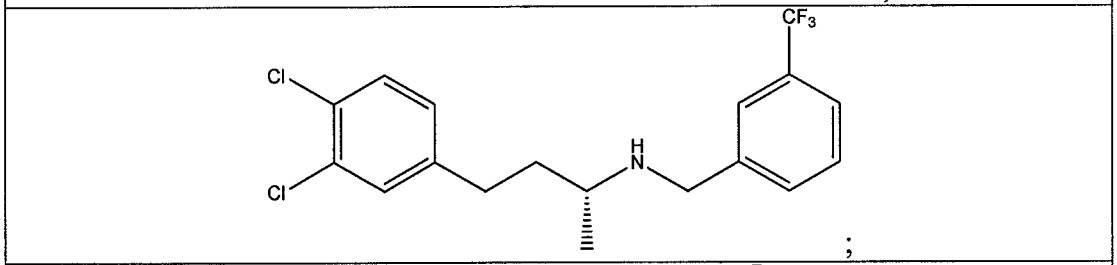
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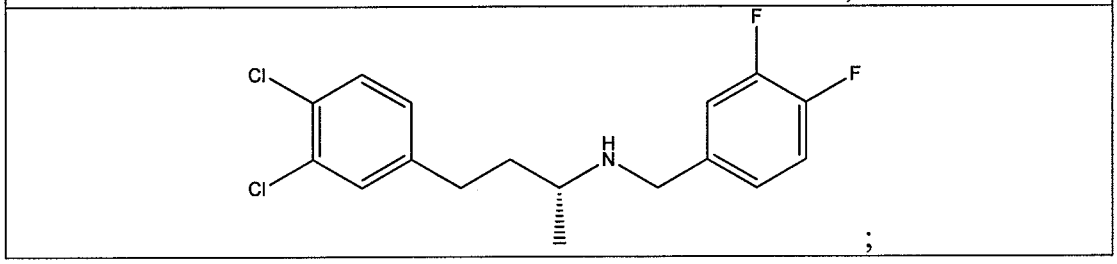
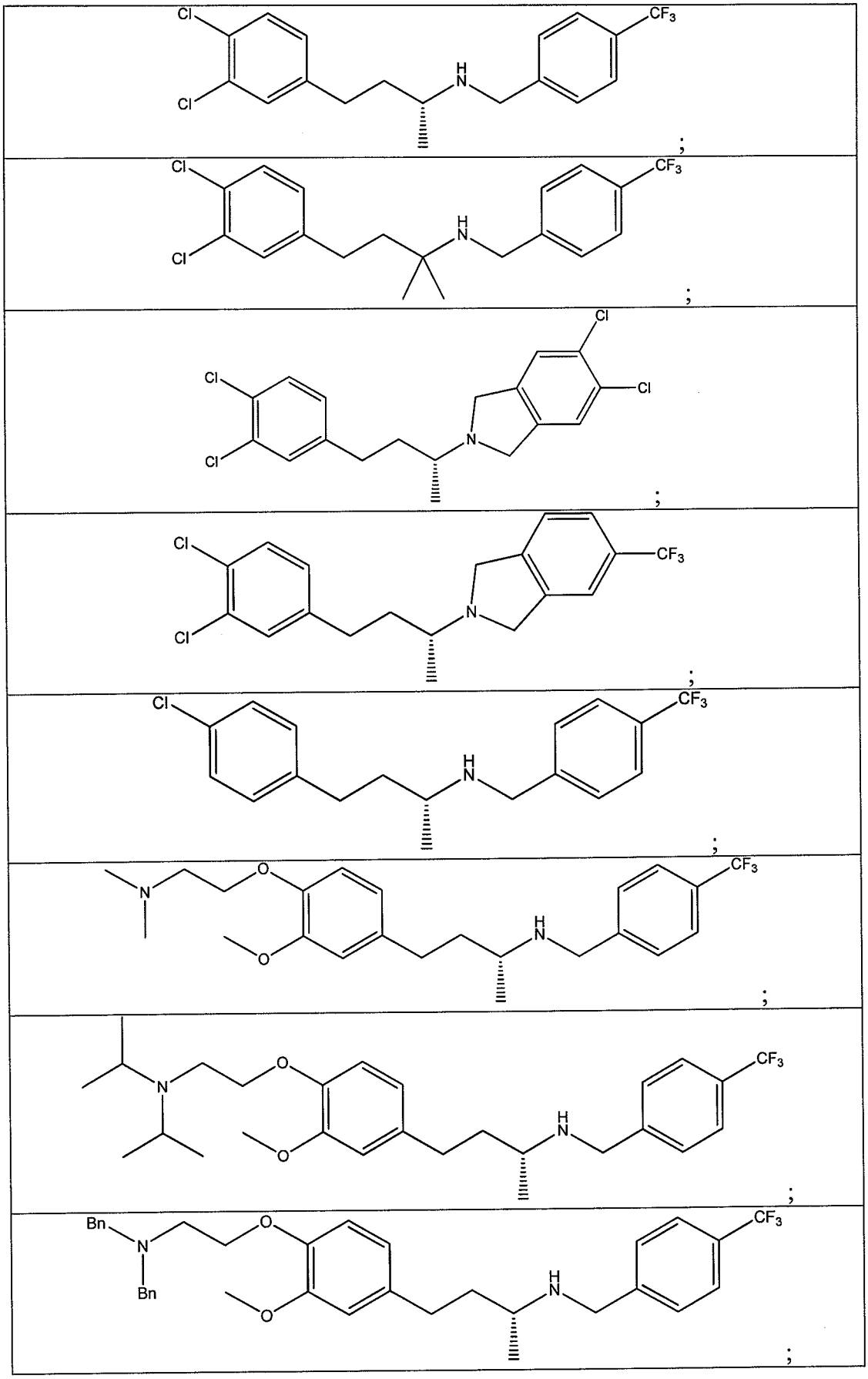
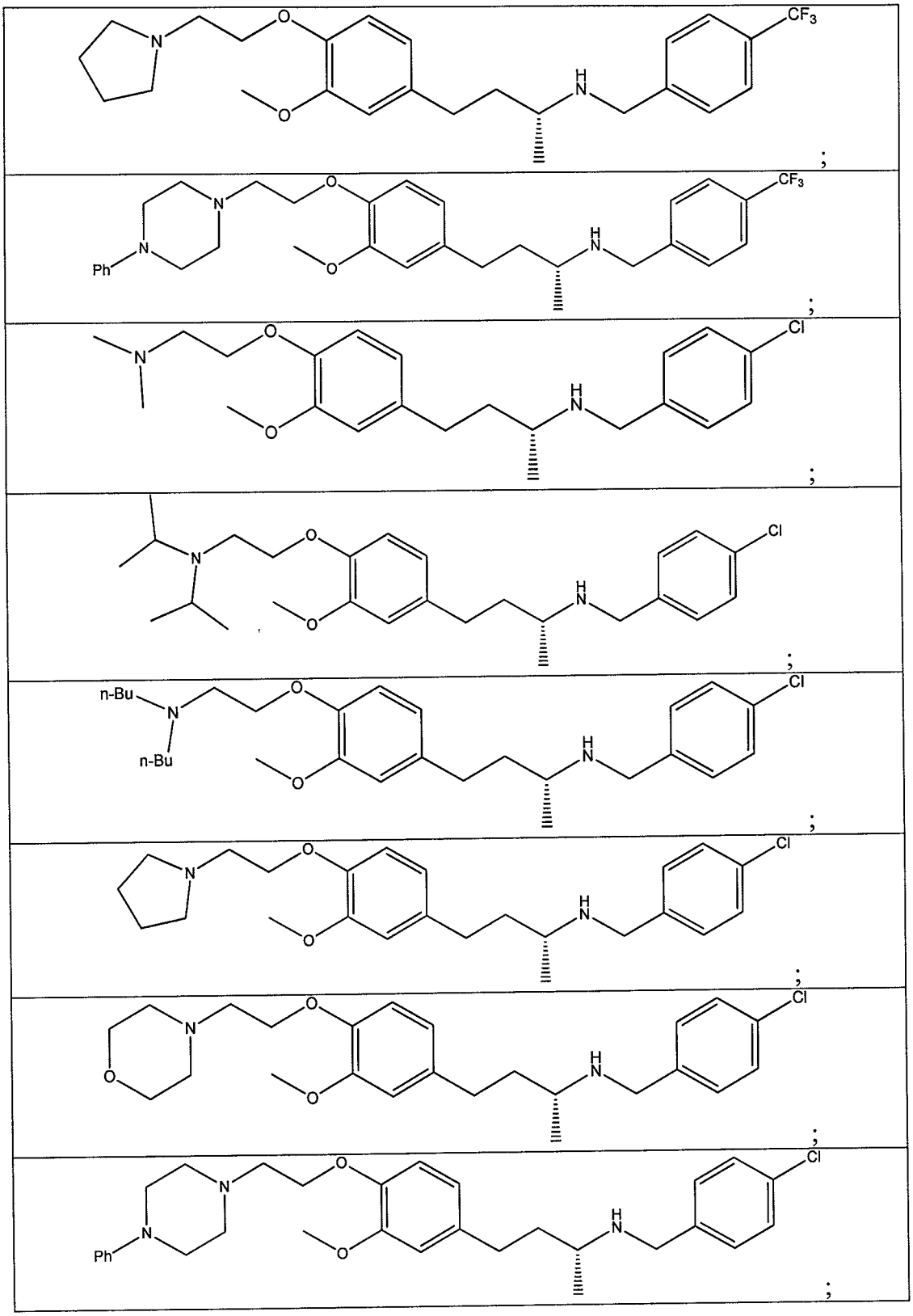
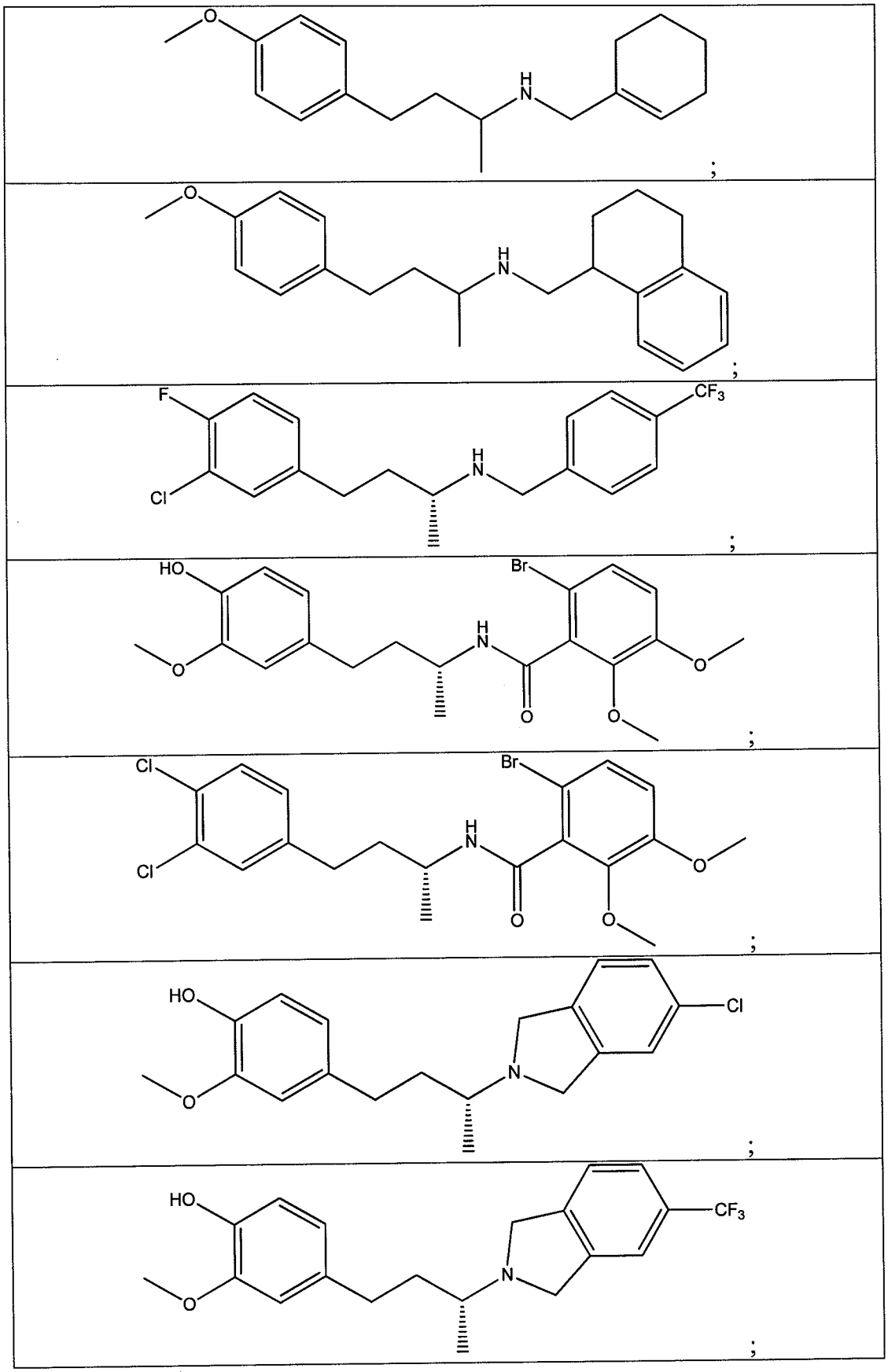
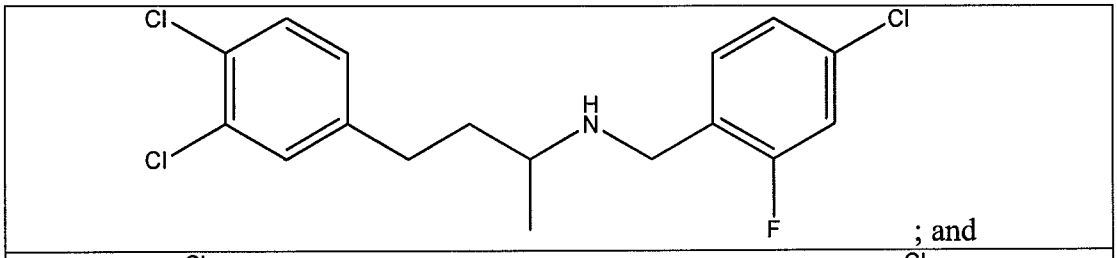
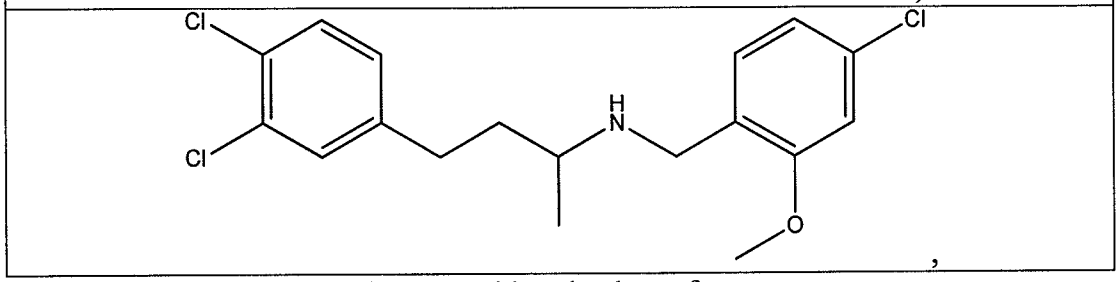

;





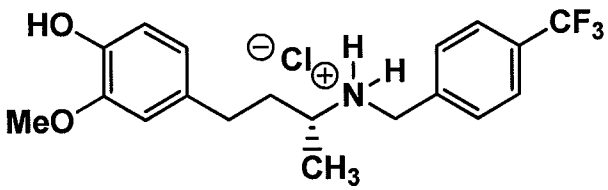


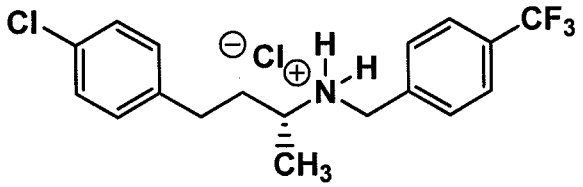

; and

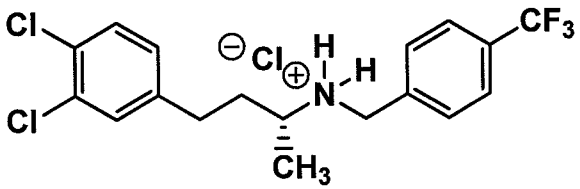

,

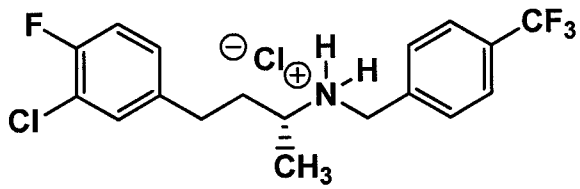
and pharmaceutically acceptable salts thereof.

10. A compound of claim 9 selected from

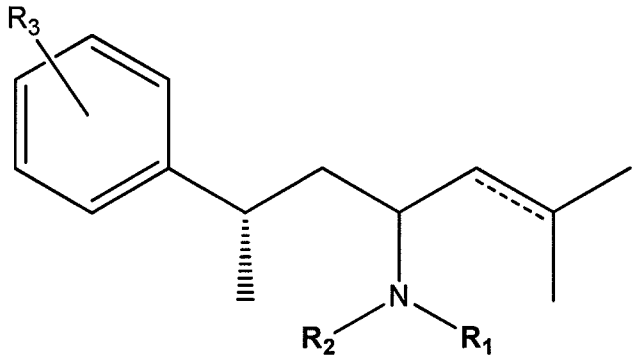








11. A compound of Formula VIIIa



VIIIa

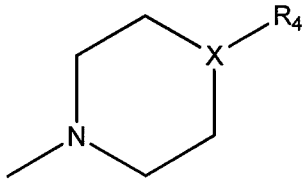
wherein:

⚌ 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



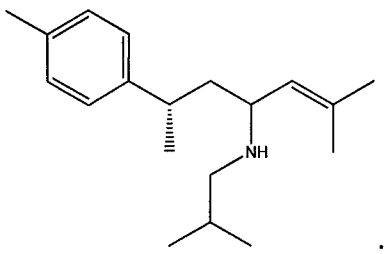
, wherein

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:



.

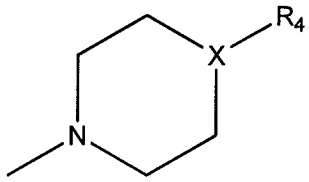
12. The compound of claim 11 wherein

⚌ 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

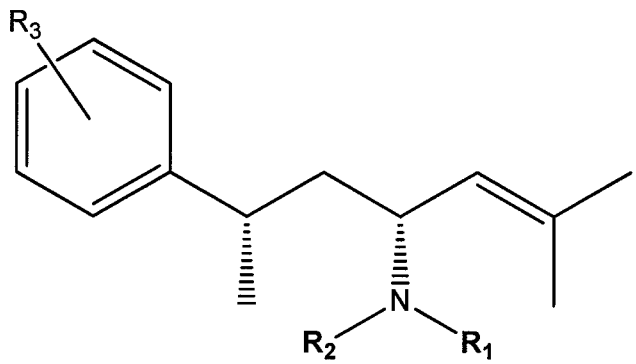
X is CH, N, or O, and

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.

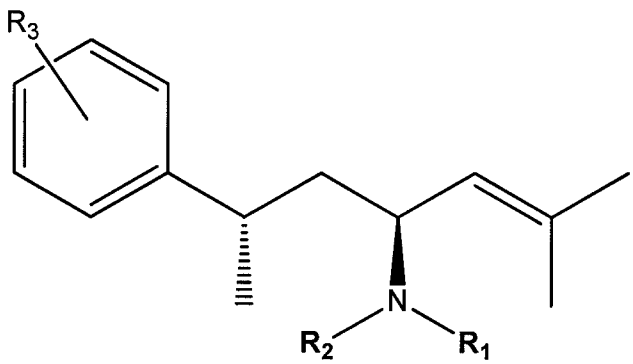
13. The compound of claim 11 that has the Formula VIIIb



VIIIb

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

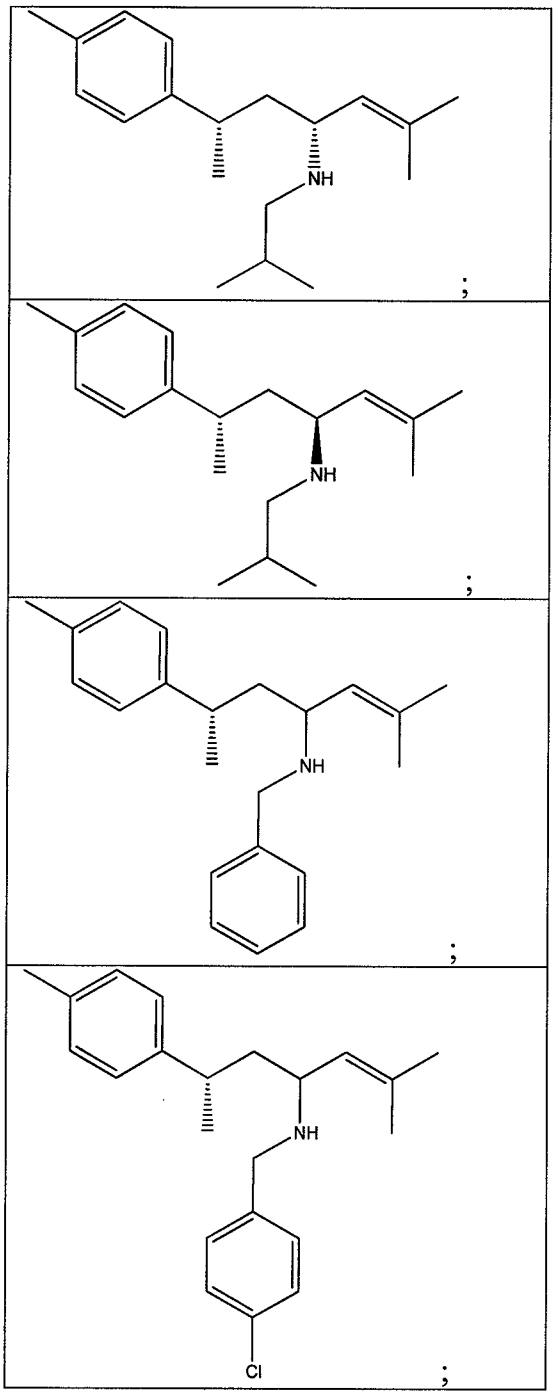
14. The compound of claim 11 that has the Formula VIIIc

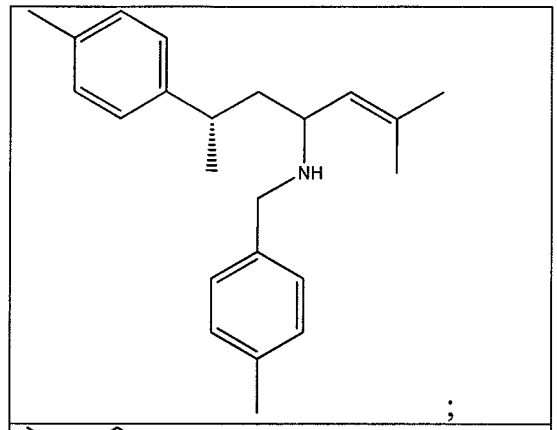


VIIIc

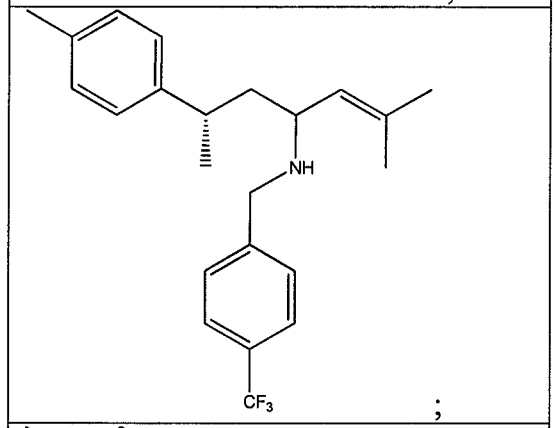
wherein R_1-R_3 are as defined in claim 11, and pharmaceutically acceptable salts thereof.

15. A compound selected from the group consisting of:

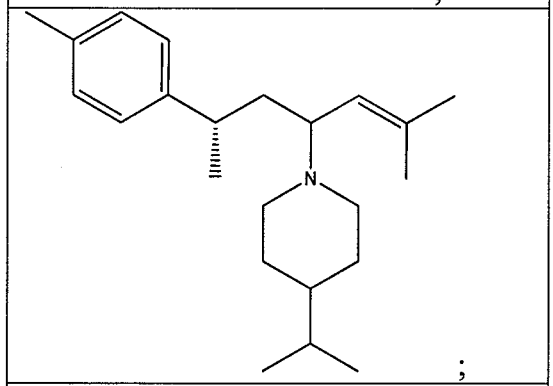



NH

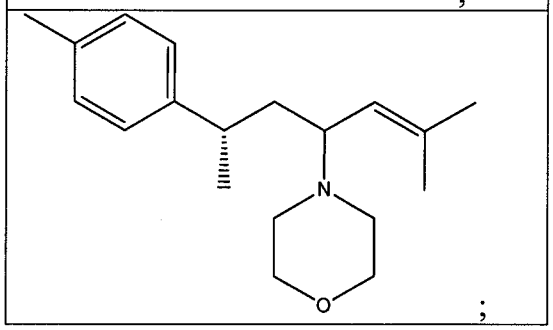
;


NH
CF3

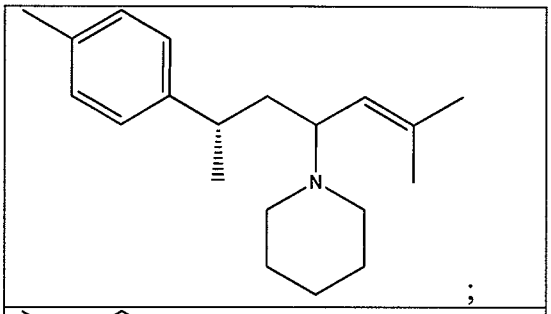
;

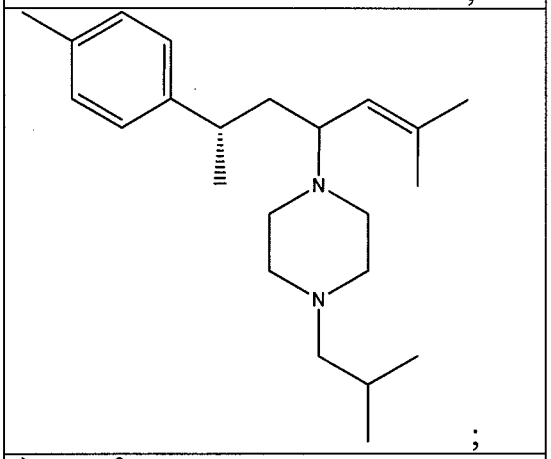

N

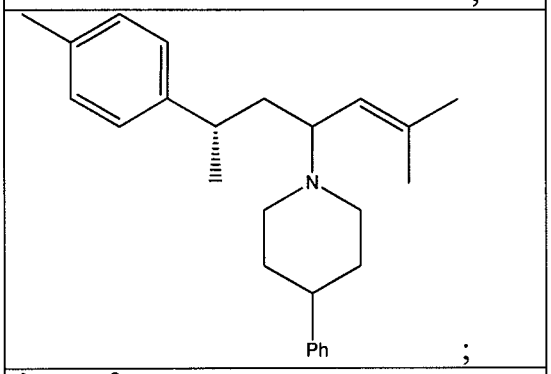
;

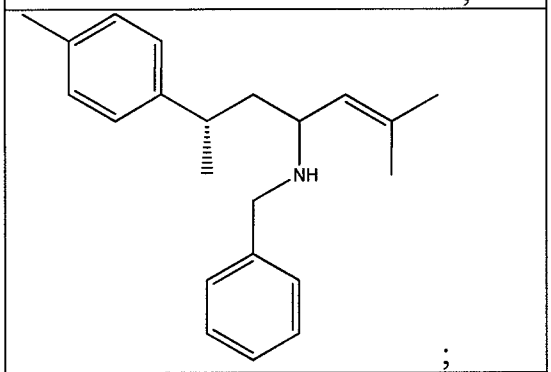

N
O

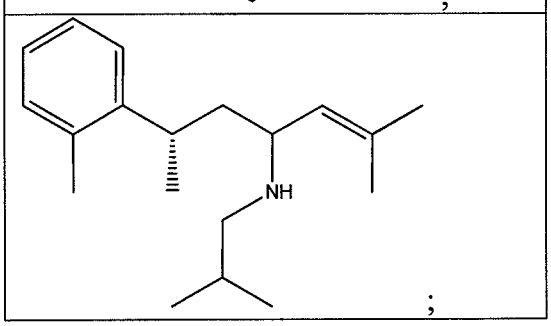
;

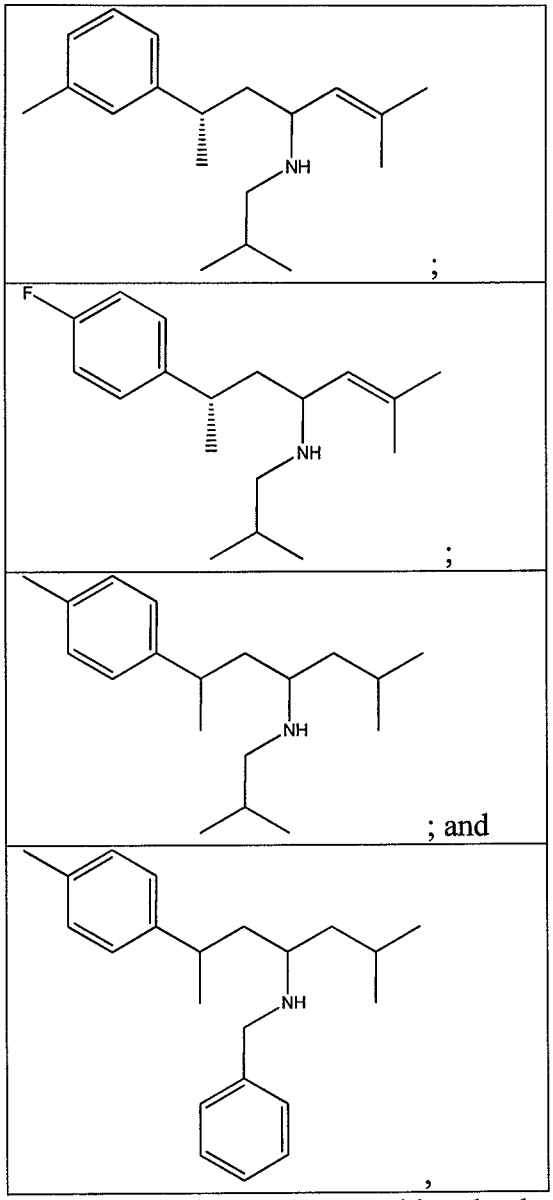

;


;


;

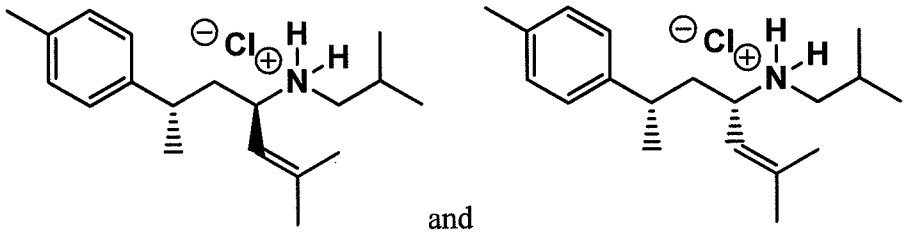

;


;



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.

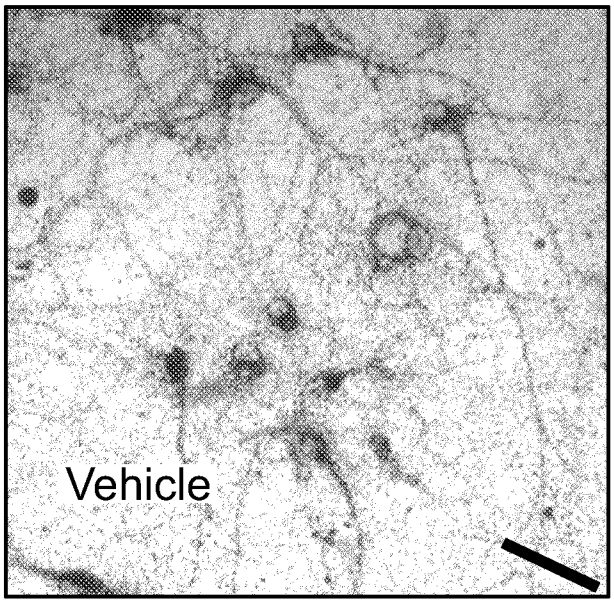


FIG. 1A

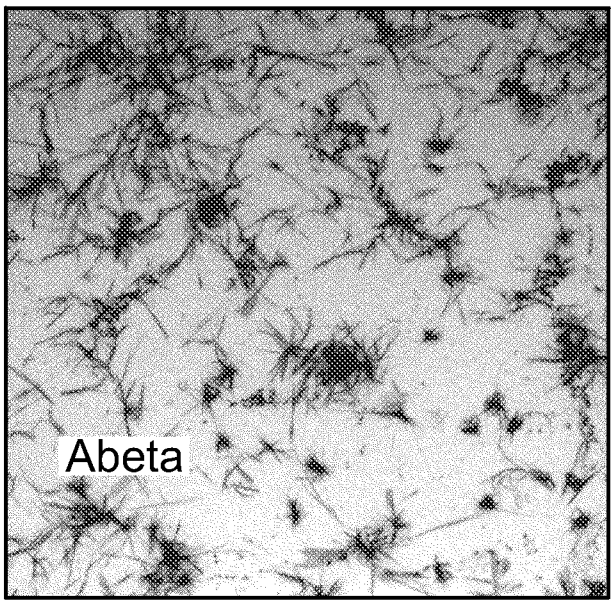


FIG. 1B

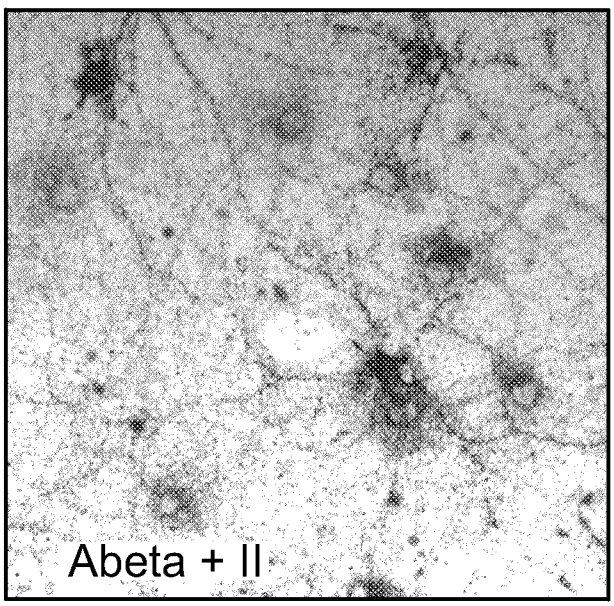


FIG. 1C

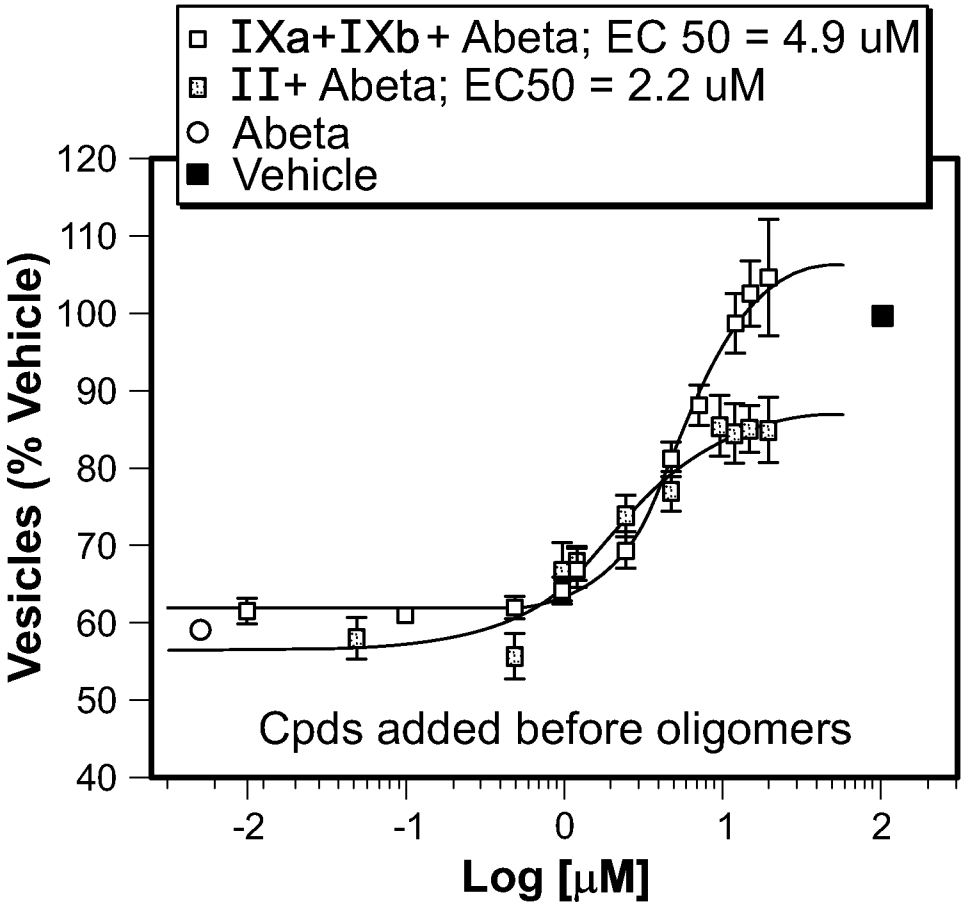

IXa+IXb + Abeta; EC 50 = 4.9 uM
II + Abeta; EC50 = 2.2 uM
Abeta
Vehicle
Vesicles (% Vehicle)
120
110
100
90
80
70
60
50
40
-2
-1
0
1
2
Log [μM]
Cpds added before oligomers

FIG. 1D

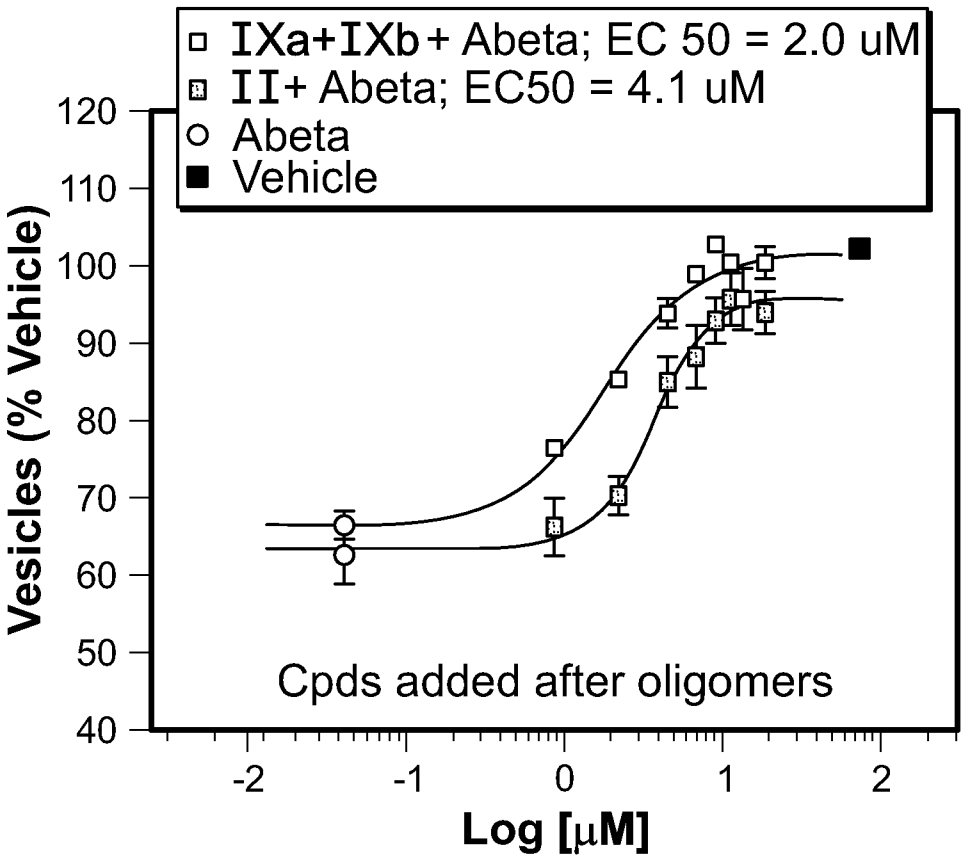

IXa+IXb + Abeta; EC 50 = 2.0 uM
II + Abeta; EC50 = 4.1 uM
Abeta
Vehicle
Vesicles (% Vehicle)
120
110
100
90
80
70
60
50
40
-2
-1
0
1
2
Log [μM]
Cpds added after oligomers

FIG. 1E

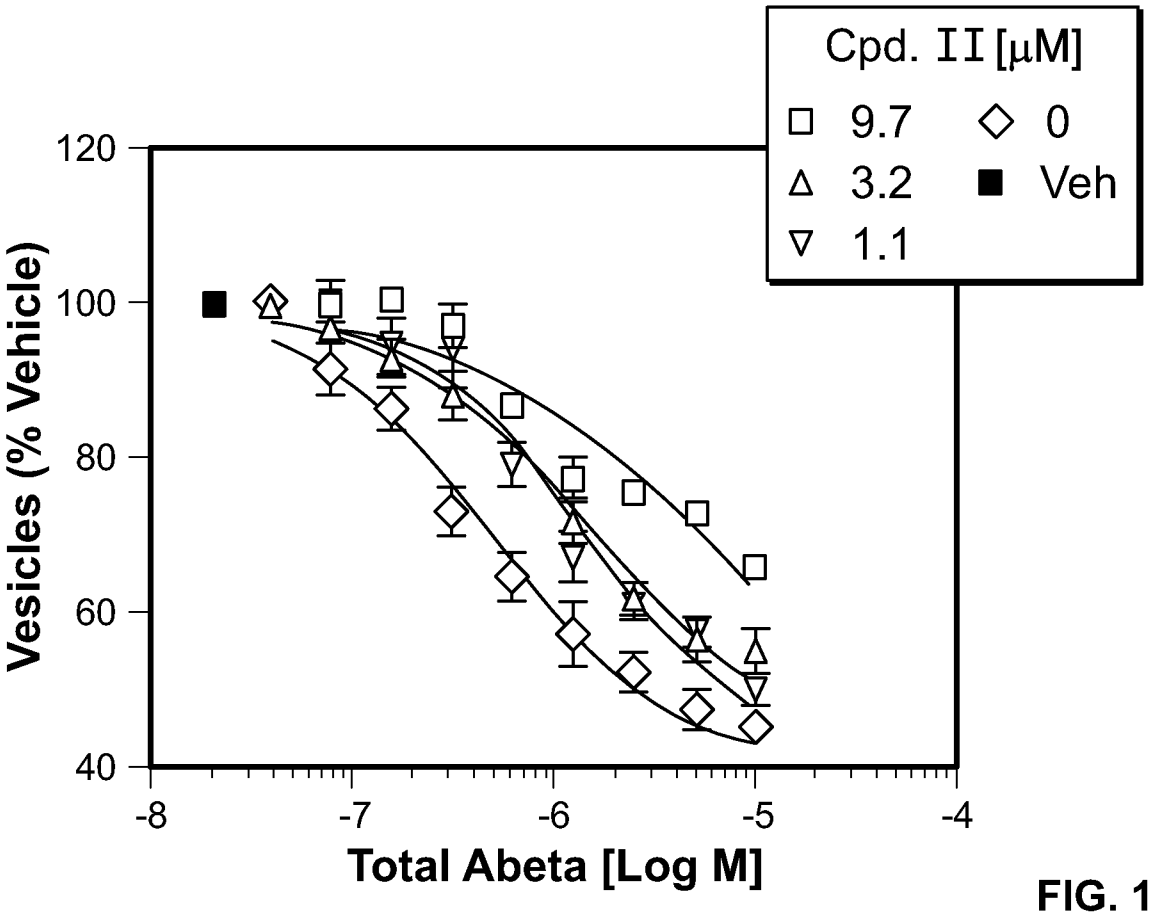

Cpd. II [μM]
9.7
0
3.2
Veh
1.1
120
100
80
60
40
Vesicles (% Vehicle)
-8
-7
-6
-5
-4
Total Abeta [Log M]

FIG. 1F

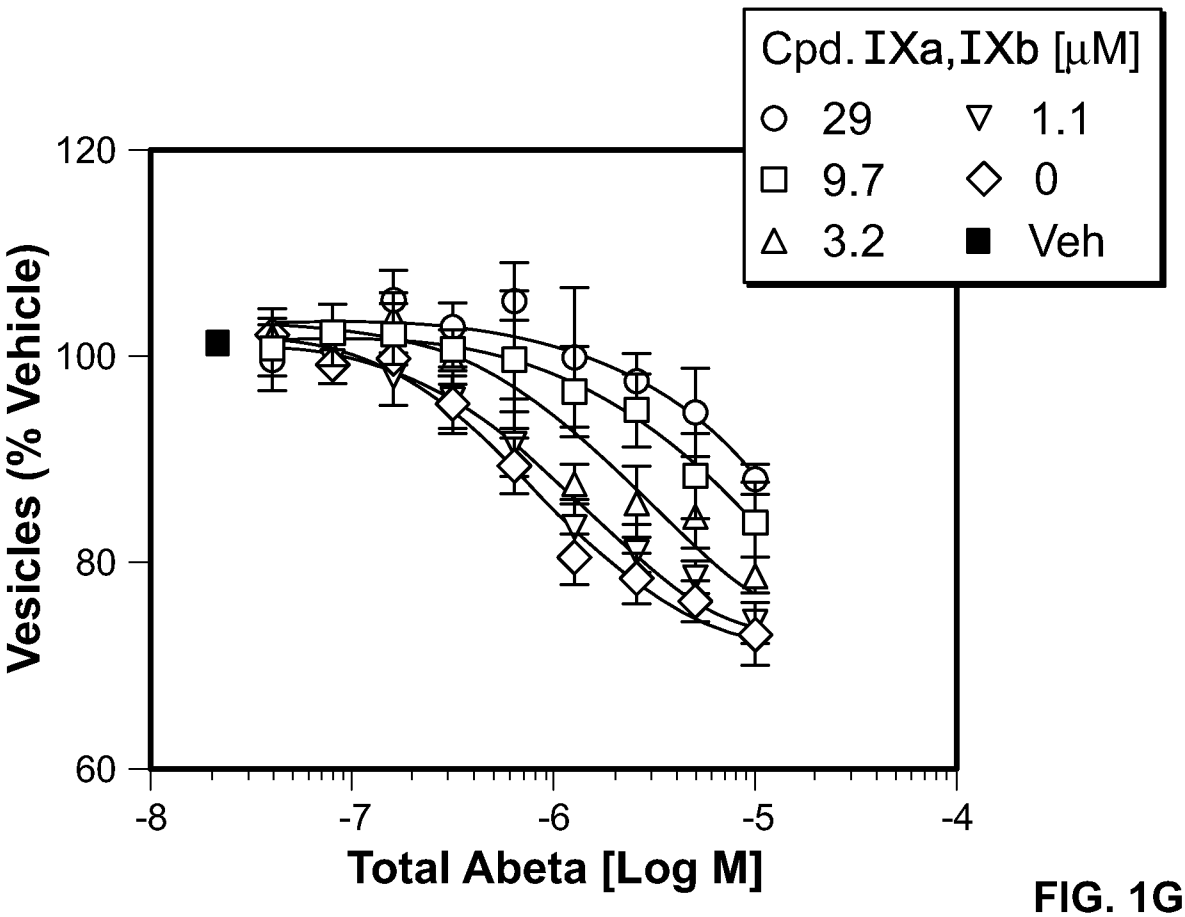

Cpd. IXa,IXb [μM]
29
1.1
9.7
0
3.2
Veh
120
100
80
60
Vesicles (% Vehicle)
-8
-7
-6
-5
-4
Total Abeta [Log M]

FIG. 1G

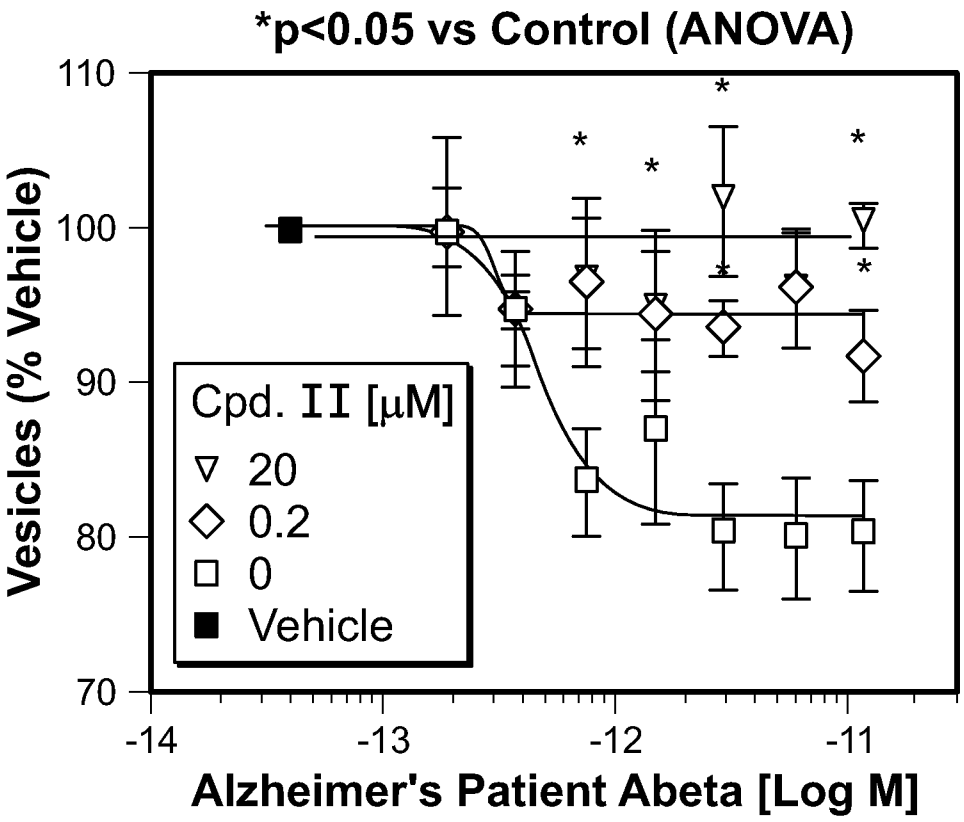

*p<0.05 vs Control (ANOVA)
Vesicles (% Vehicle)
110
100
90
80
70
-14
-13
-12
-11
Alzheimer's Patient Abeta [Log M]
Cpd. II [μM]
20
0.2
0
Vehicle
*

FIG. 1H

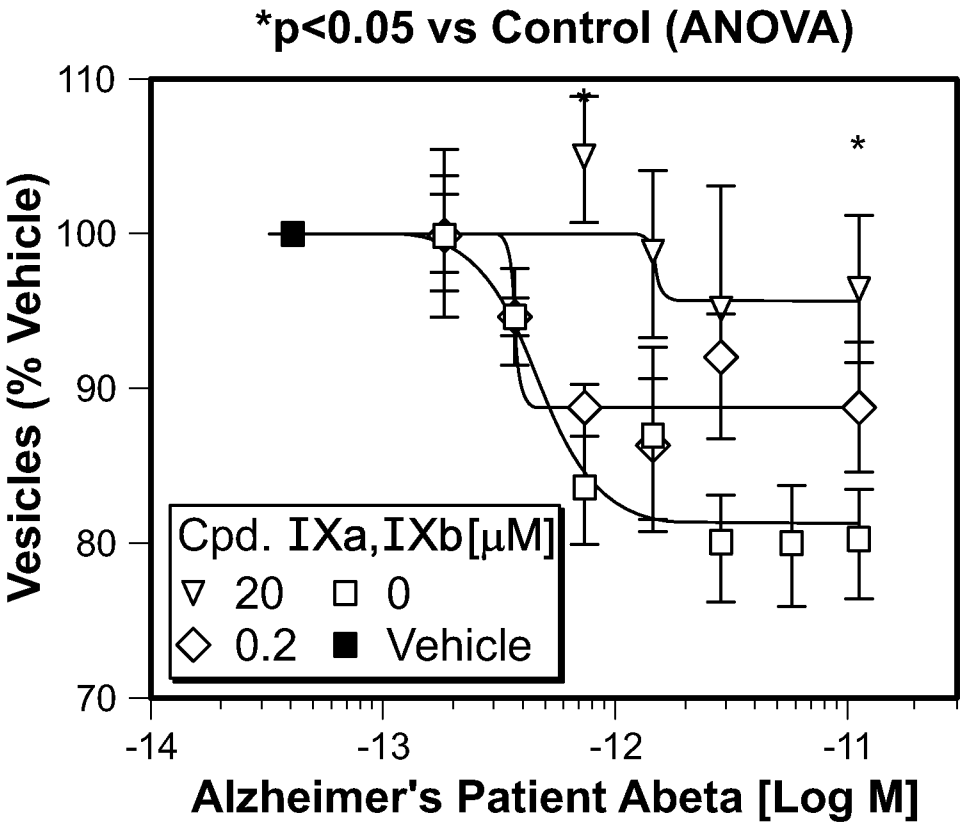

*p<0.05 vs Control (ANOVA)
Vesicles (% Vehicle)
110
100
90
80
70
-14
-13
-12
-11
Alzheimer's Patient Abeta [Log M]
Cpd. IXa,IXb[μM]
20
0
0.2
Vehicle
*

FIG. 1I

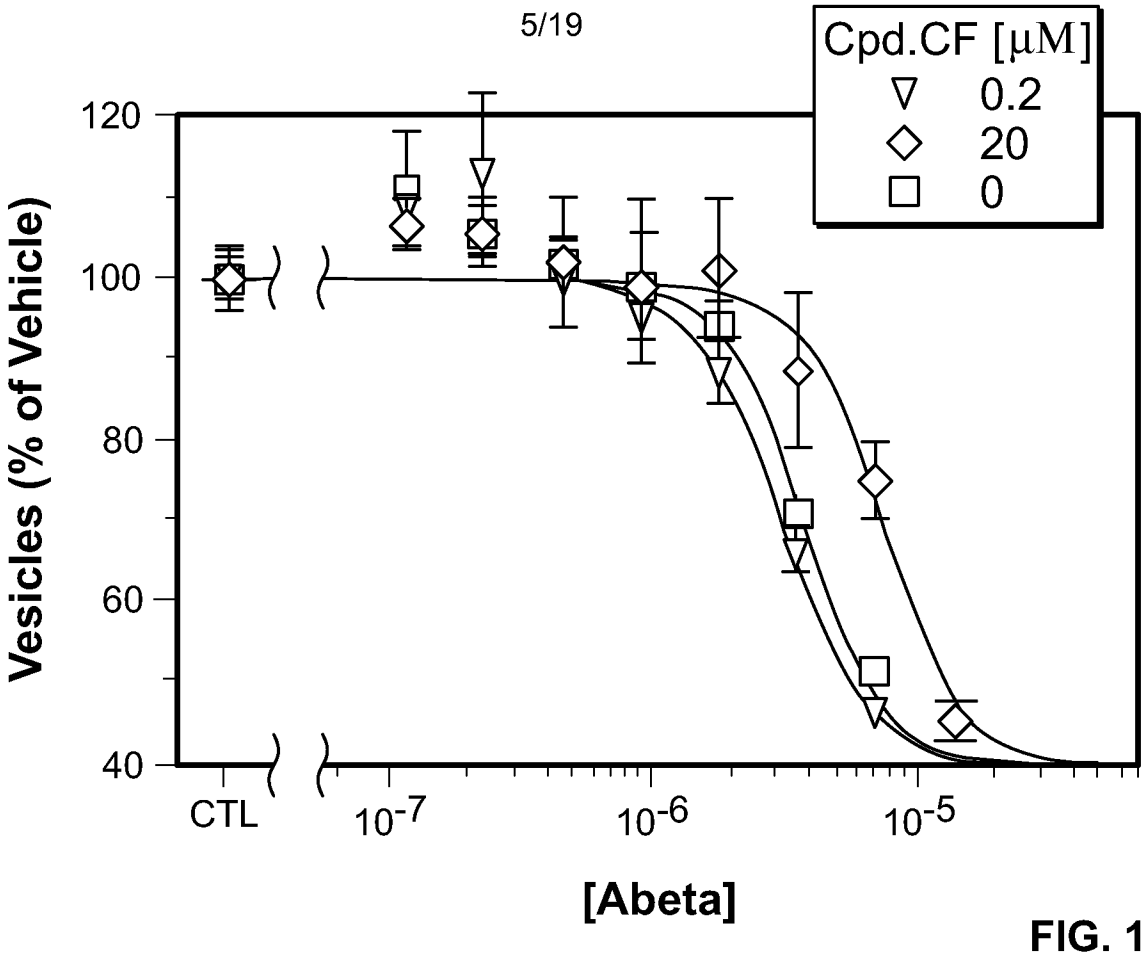

Cpd.CF [μM]
0.2
20
0
120
100
80
60
40
Vesicles (% of Vehicle)
CTL
10-7
10-6
10-5
[Abeta]

FIG. 1J

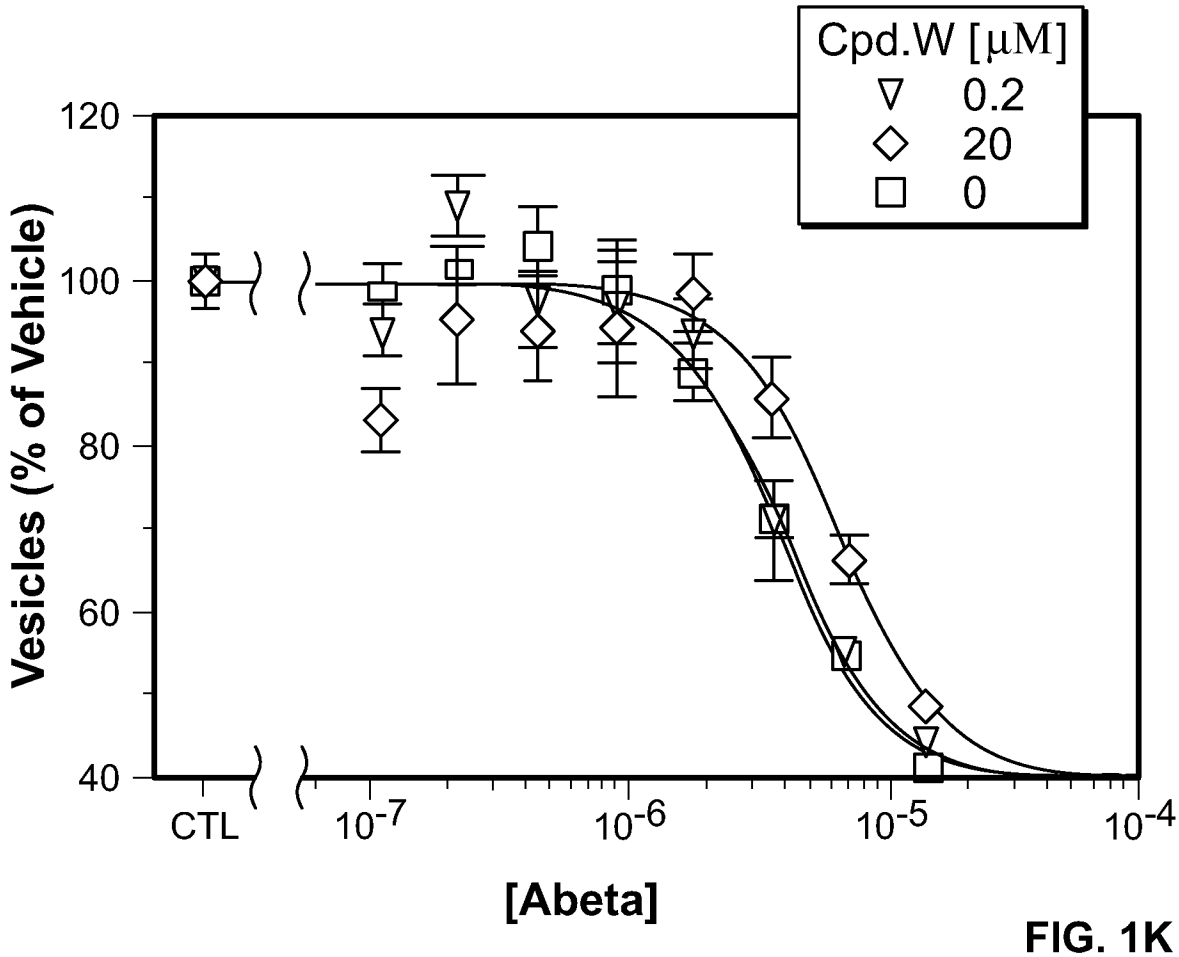

Cpd.W [μM]
0.2
20
0
120
100
80
60
40
Vesicles (% of Vehicle)
CTL
10-7
10-6
10-5
10-4
[Abeta]

FIG. 1K

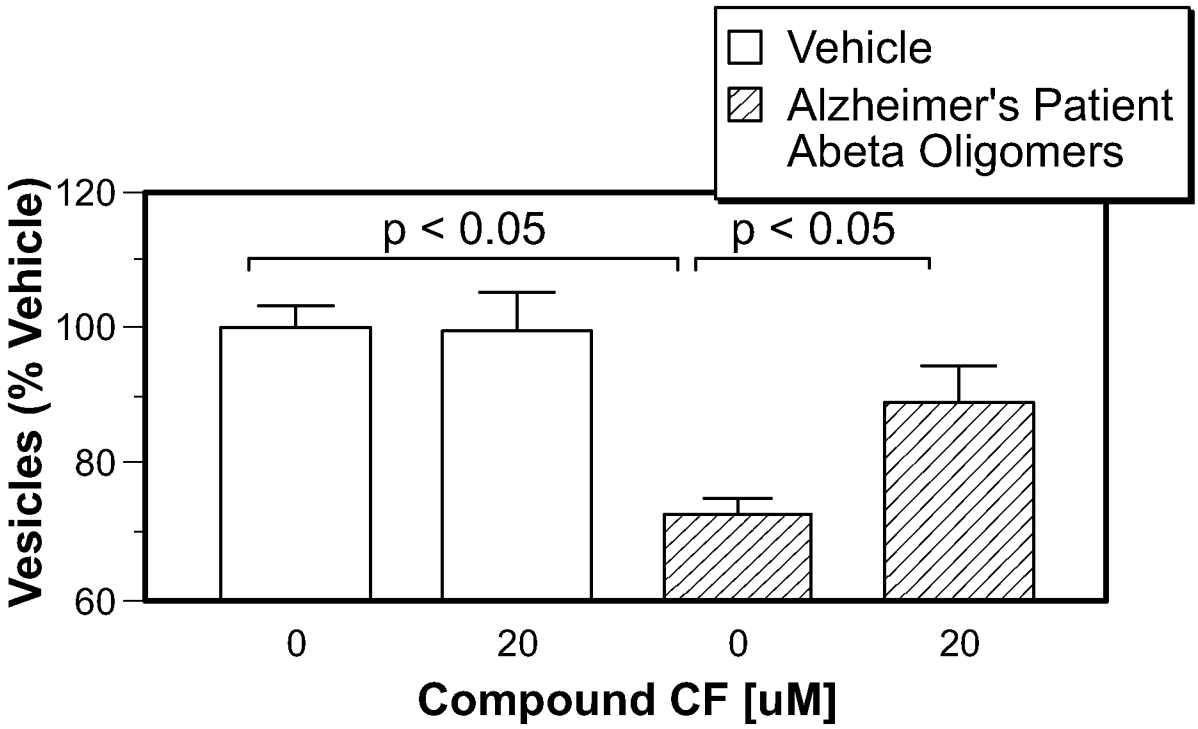

Vehicle
Alzheimer's Patient Abeta Oligomers
p < 0.05
p < 0.05
Vesicles (% Vehicle)
120
100
80
60
0
20
0
20
Compound CF [uM]

FIG. 1L

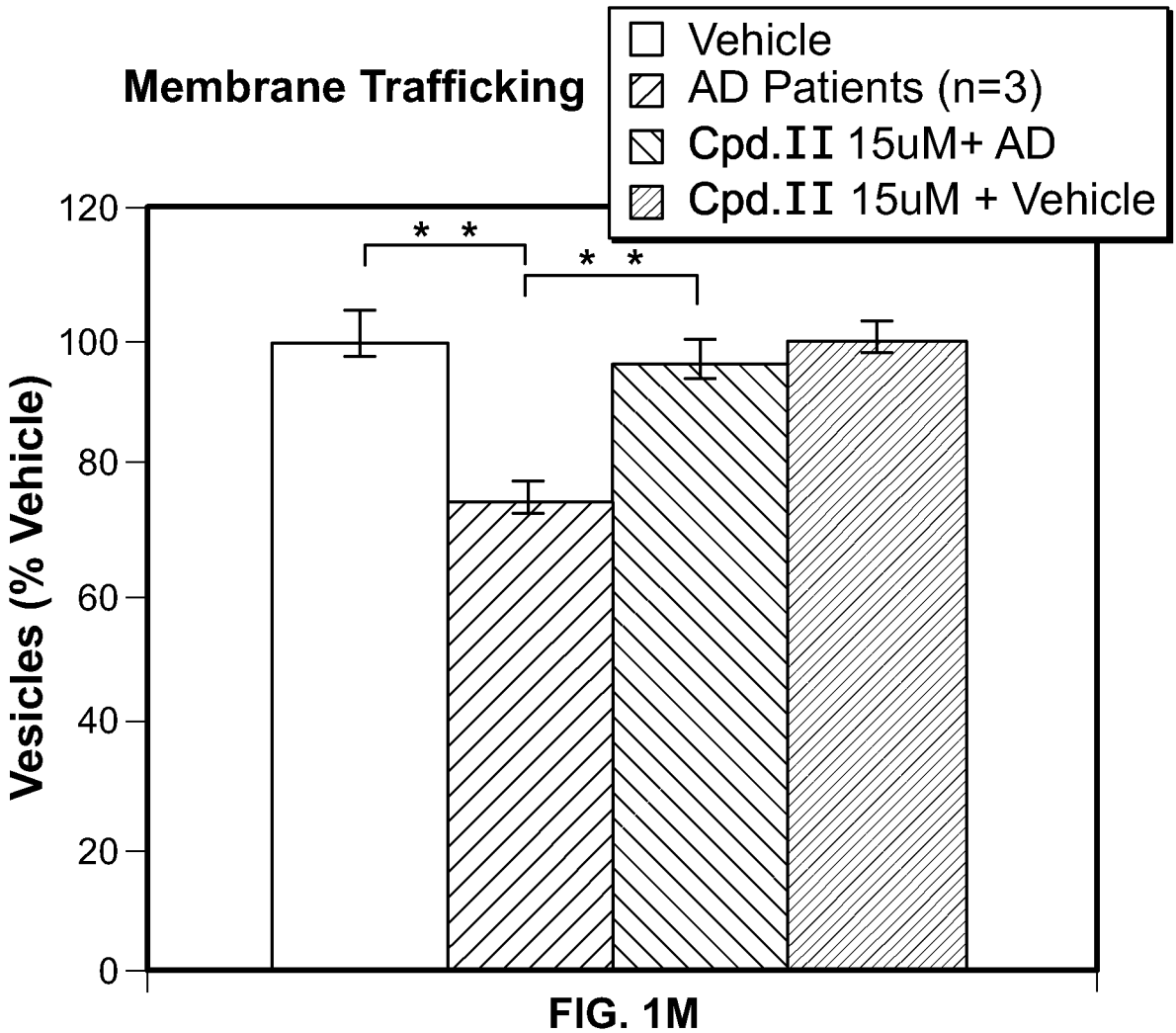

Membrane Trafficking
Vehicle
AD Patients (n=3)
Cpd.II 15uM+ AD
Cpd.II 15uM + Vehicle
* *
* *
Vesicles (% Vehicle)
120
100
80
60
40
20
0

FIG. 1M

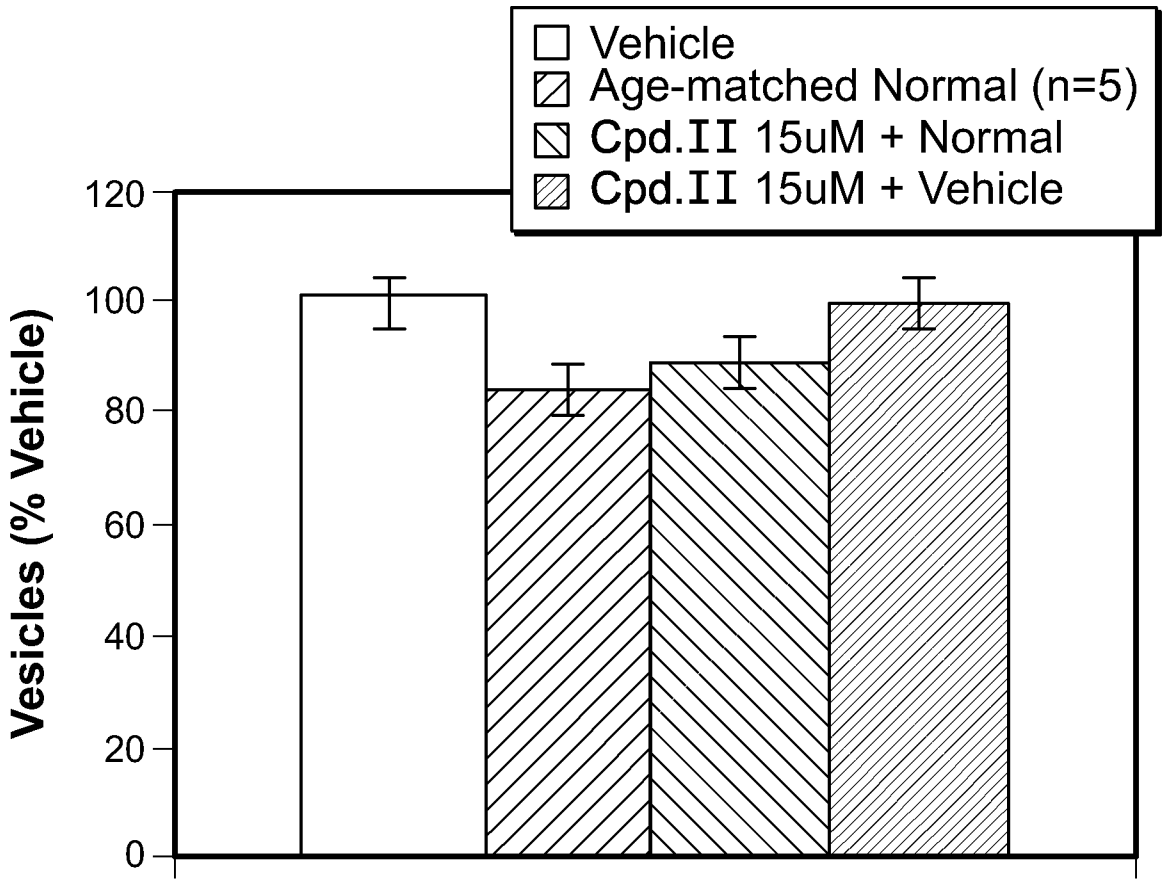

Vehicle
Age-matched Normal (n=5)
Cpd.II 15uM + Normal
Cpd.II 15uM + Vehicle
Vesicles (% Vehicle)
120
100
80
60
40
20
0

FIG. 1N

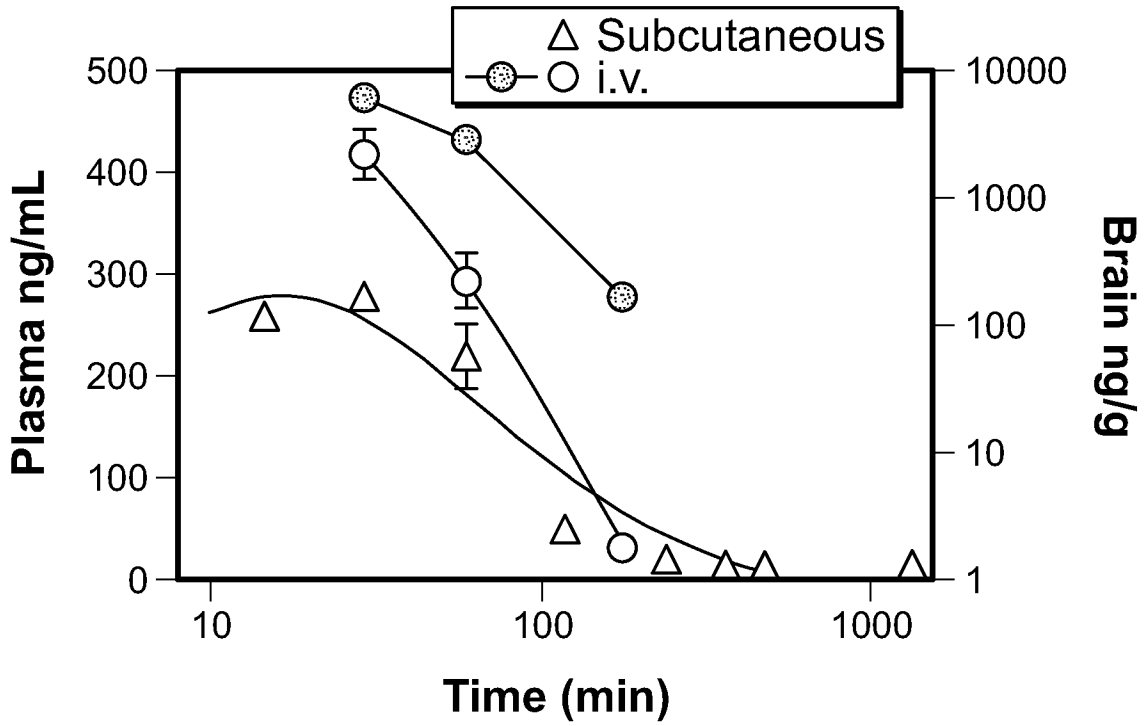

Subcutaneous
i.v.
Plasma ng/mL
Brain ng/g
Time (min)
500
400
300
200
100
0
10000
1000
100
10
1
10
100
1000

FIG. 2A

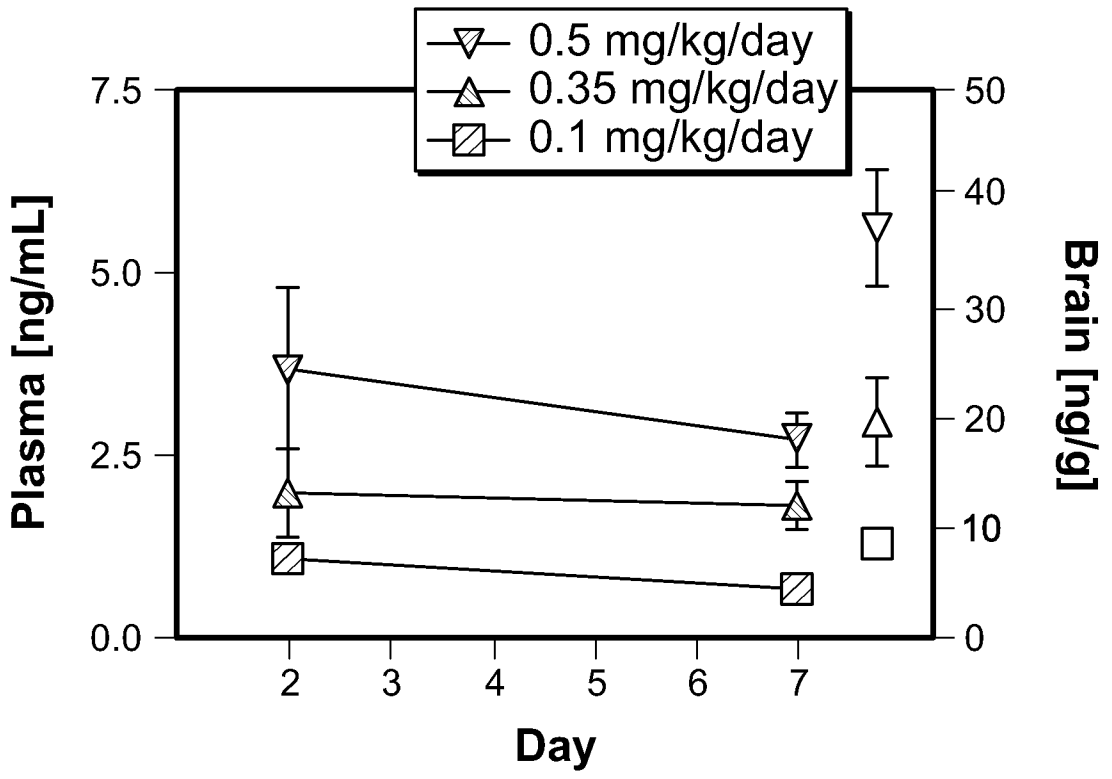

0.5 mg/kg/day
0.35 mg/kg/day
0.1 mg/kg/day
Plasma [ng/mL]
Brain [ng/g]
Day
7.5
5.0
2.5
0.0
50
40
30
20
10
0
2
3
4
5
6
7

FIG. 2B

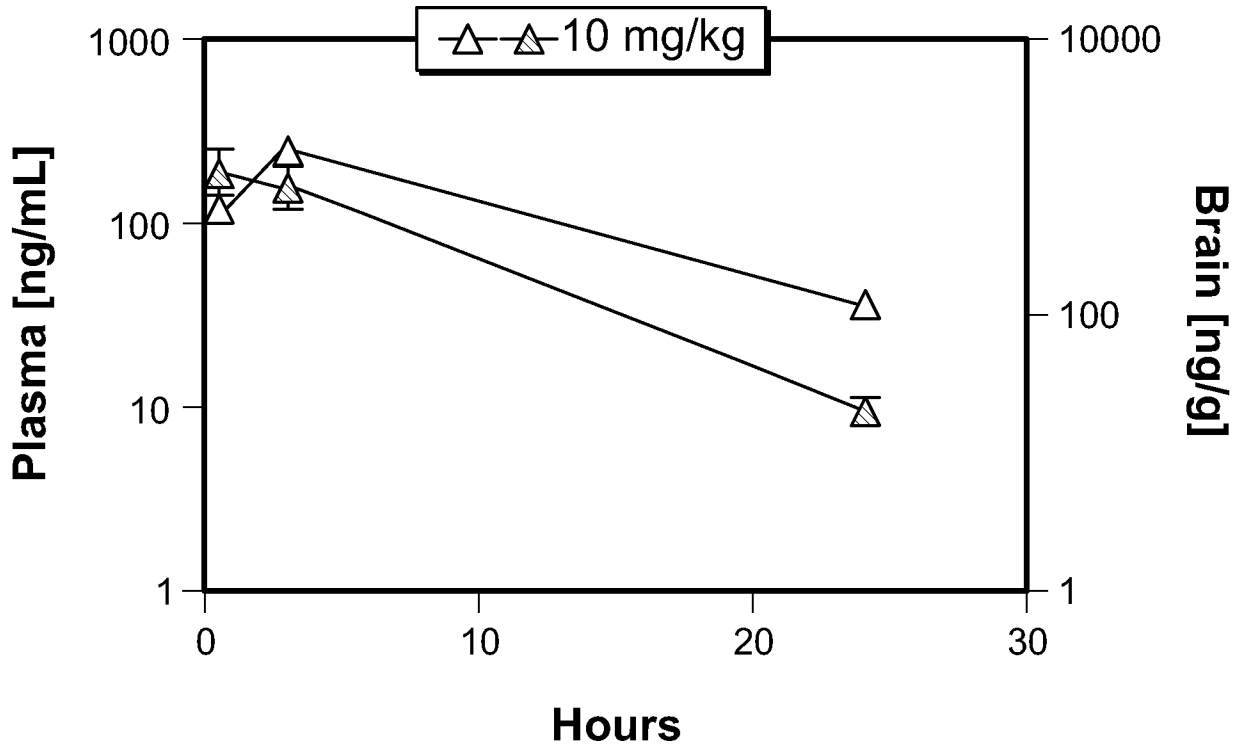

10 mg/kg
1000
100
10
1
10000
100
1
0
10
20
30
Plasma [ng/mL]
Brain [ng/g]
Hours

FIG. 2C

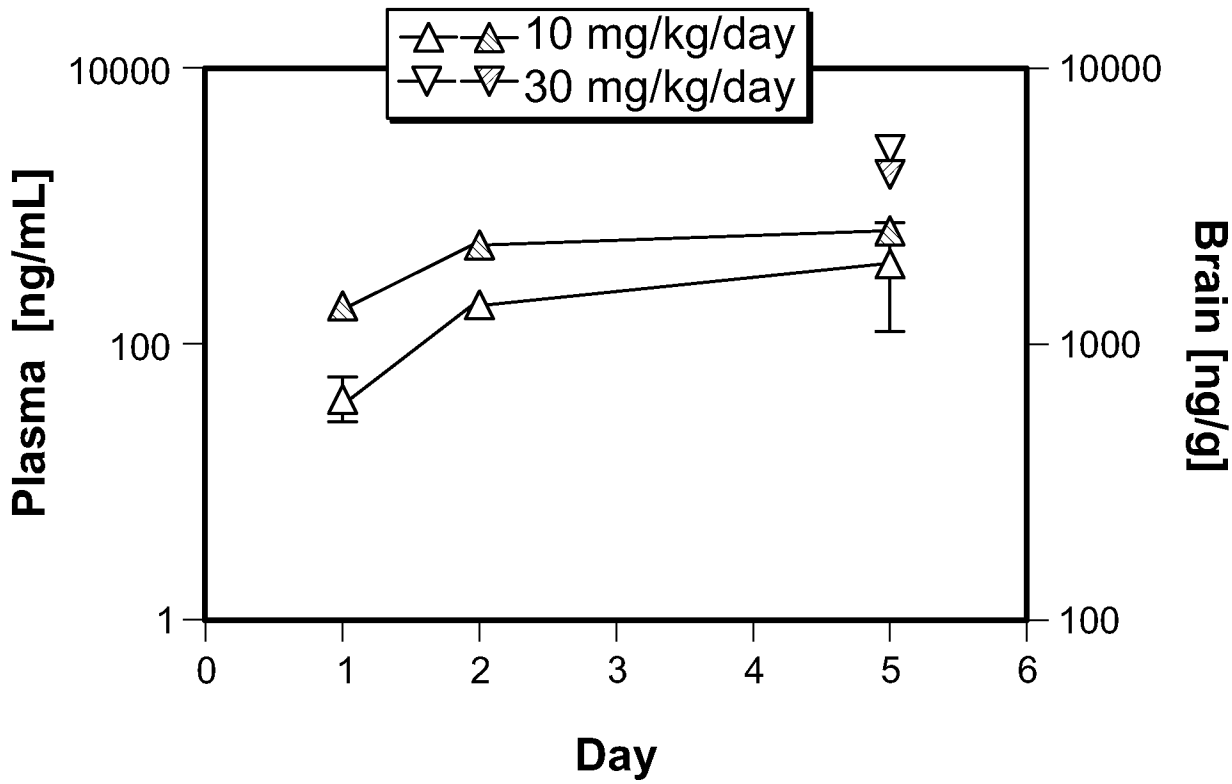

10 mg/kg/day
30 mg/kg/day
10000
100
1
10000
1000
100
0
1
2
3
4
5
6
Plasma [ng/mL]
Brain [ng/g]
Day

FIG. 2D

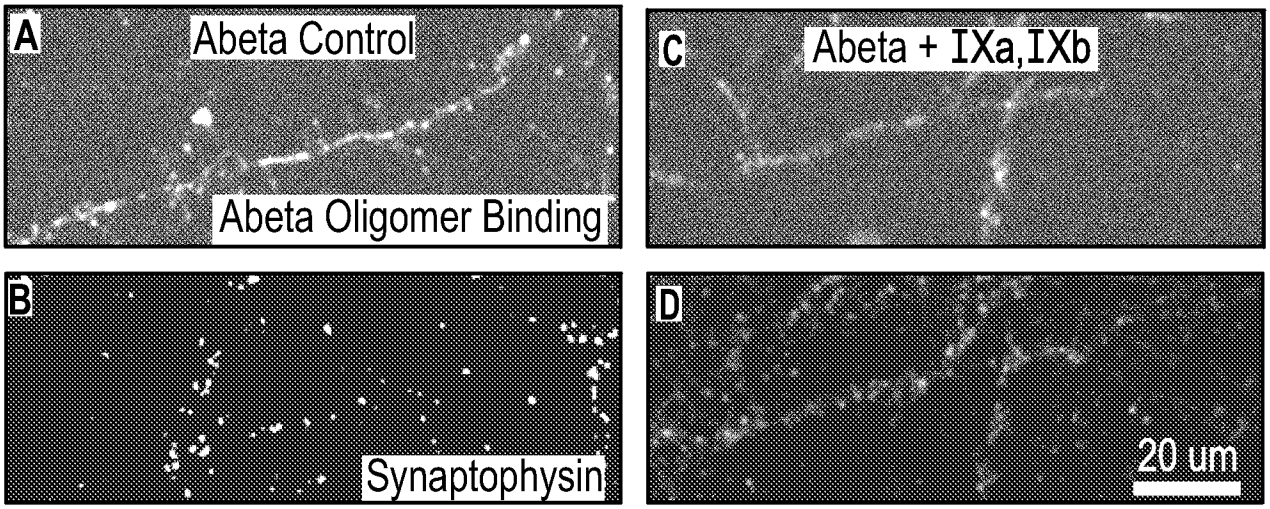

A
Abeta Control
Abeta Oligomer Binding
C
Abeta + IXa,IXb
B
Synaptophysin
D
20 um

FIG. 3A

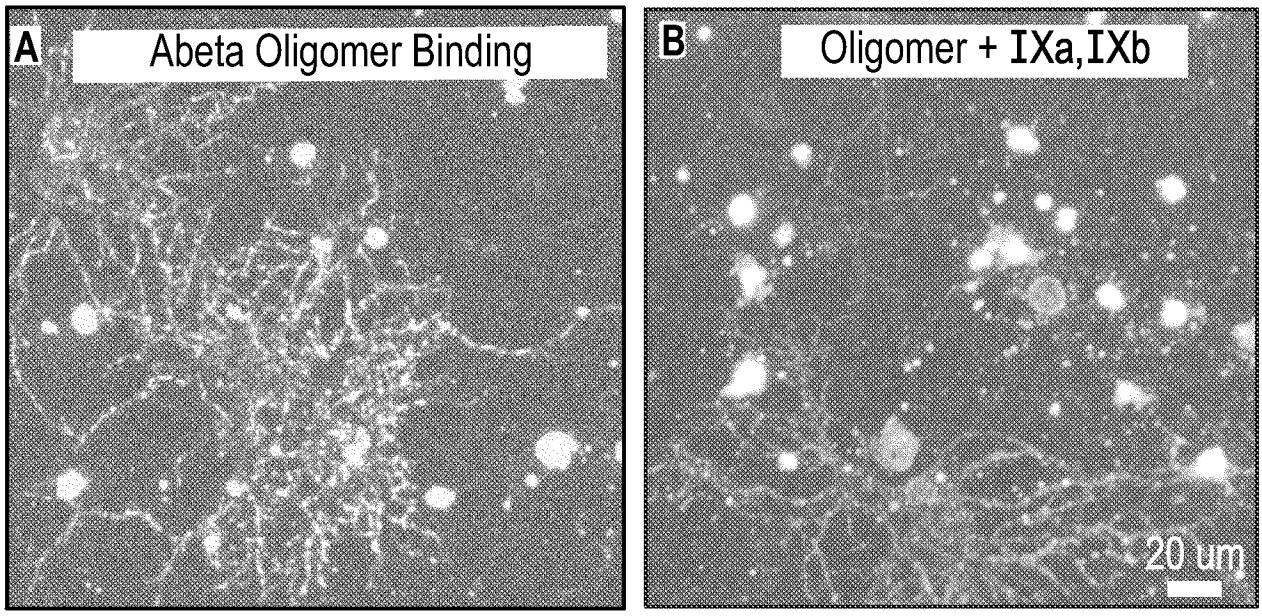

A
Abeta Oligomer Binding
B
Oligomer + IXa,IXb
20 um

FIG. 3B

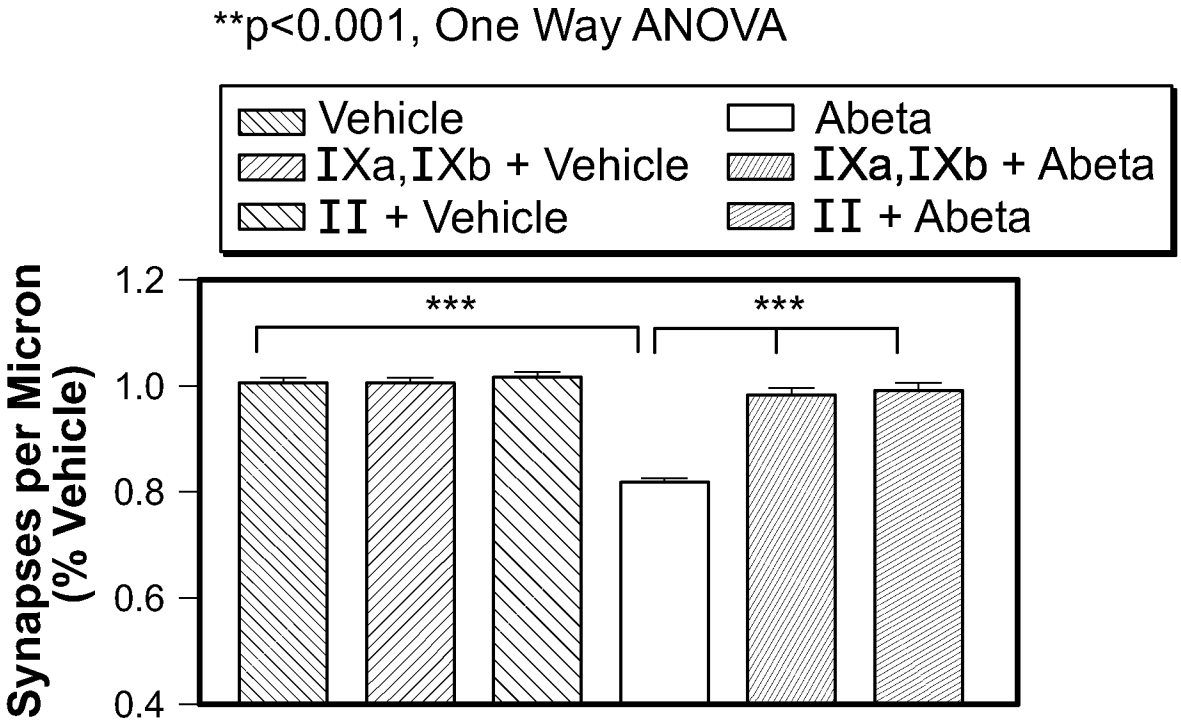

**p<0.001, One Way ANOVA
Vehicle
IXa,IXb + Vehicle
II + Vehicle
Abeta
IXa,IXb + Abeta
II + Abeta
Synapses per Micron (% Vehicle)
1.2
1.0
0.8
0.6
0.4
***
***

FIG. 3C

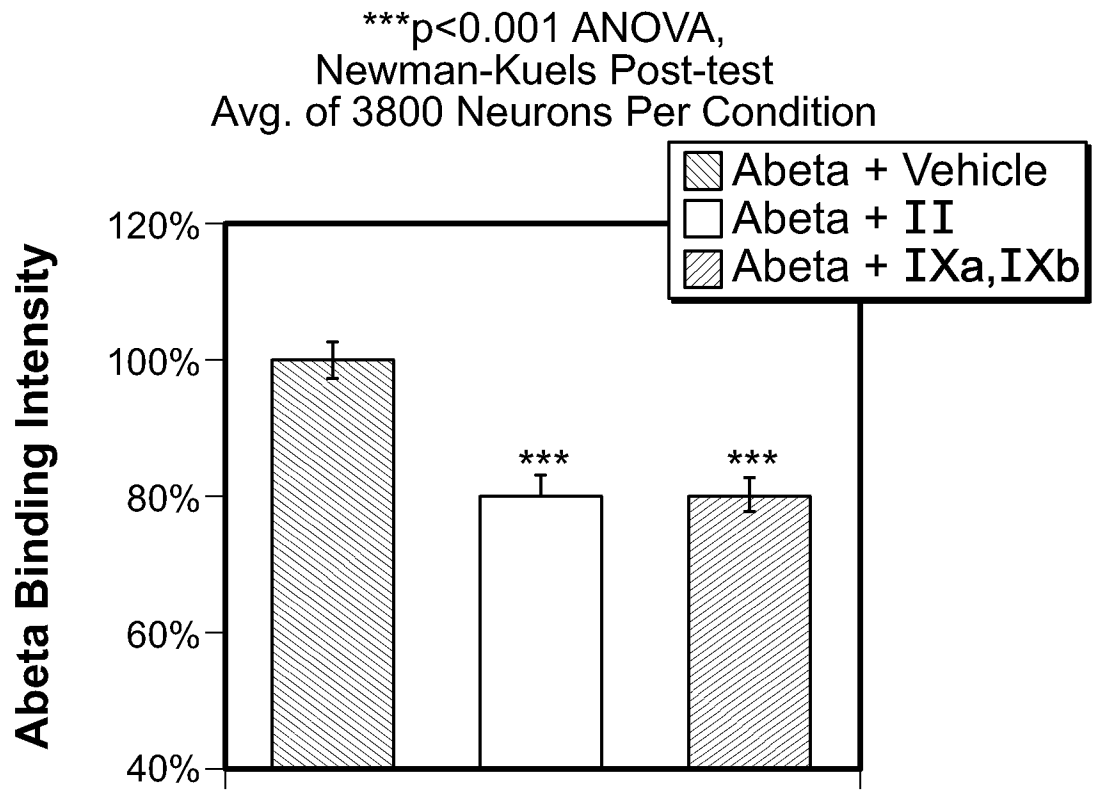

***p<0.001 ANOVA,
Newman-Kuels Post-test
Avg. of 3800 Neurons Per Condition
Abeta + Vehicle
Abeta + II
Abeta + IXa,IXb
Abeta Binding Intensity
120%
100%
80%
60%
40%
***
***

FIG. 3D

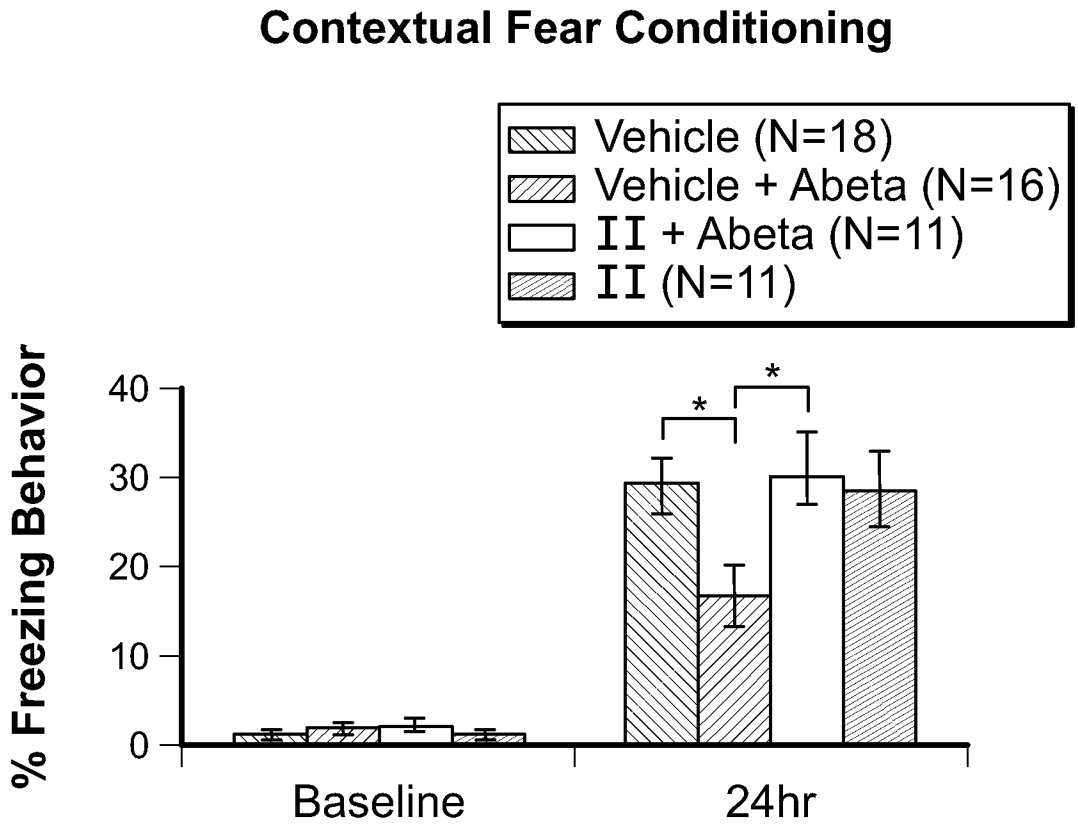

Contextual Fear Conditioning
Vehicle (N=18)
Vehicle + Abeta (N=16)
II + Abeta (N=11)
II (N=11)
% Freezing Behavior
40
30
20
10
0
*
*
Baseline
24hr

FIG. 4

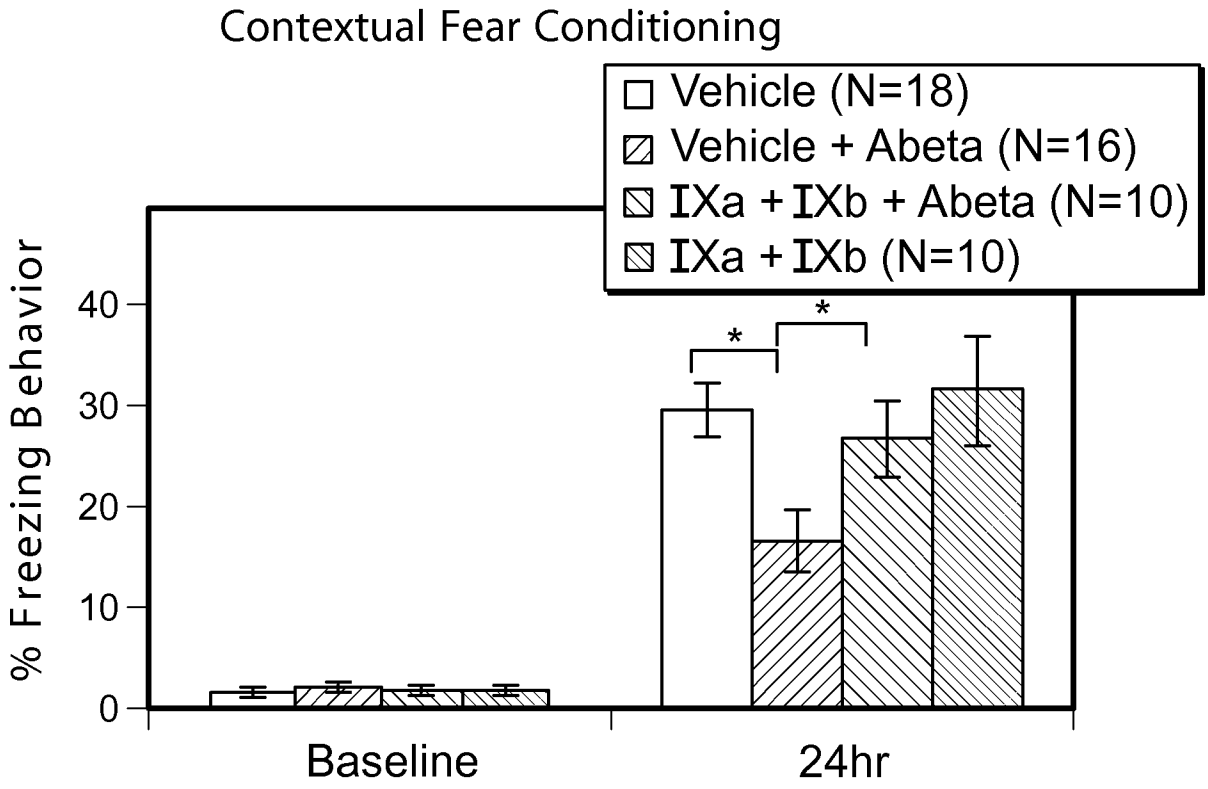

Contextual Fear Conditioning
Vehicle (N=18)
Vehicle + Abeta (N=16)
IXa + IXb + Abeta (N=10)
IXa + IXb (N=10)
% Freezing Behavior
0
10
20
30
40
*
*
Baseline
24hr

FIG. 5

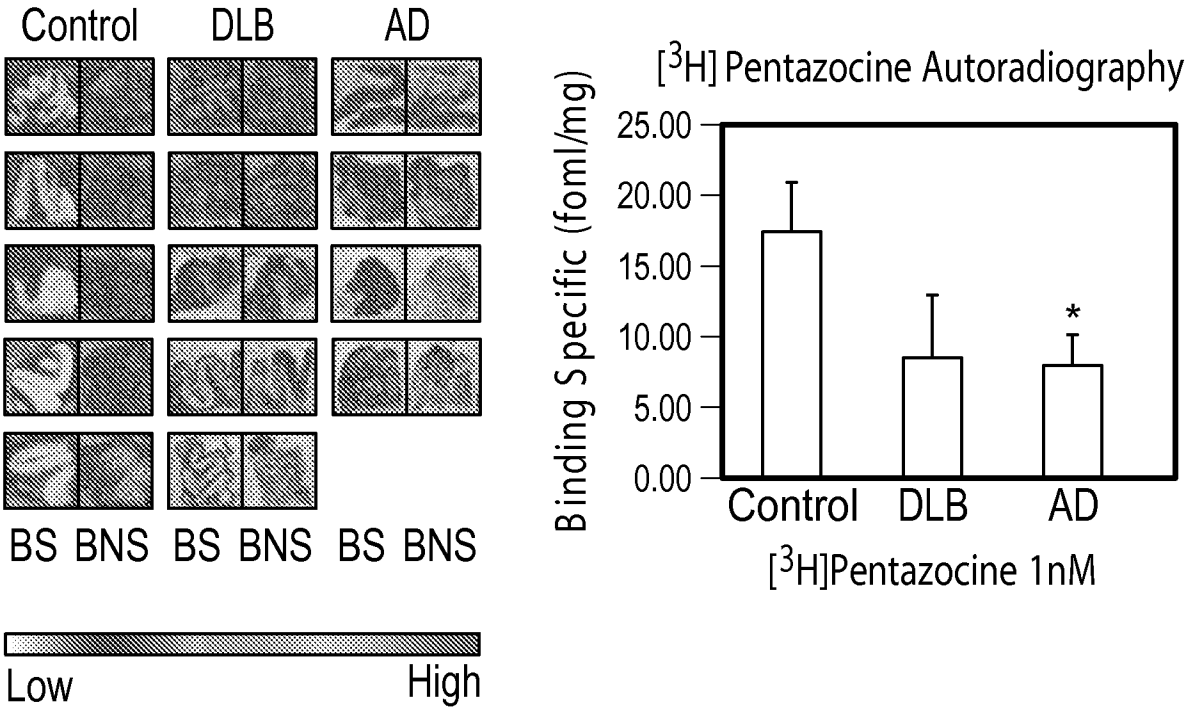

Control
DLB
AD
BS BNS BS BNS BS BNS
Low
High
[3H] Pentazocine Autoradiography
Binding Specific (foml/mg)
25.00
20.00
15.00
10.00
5.00
0.00
*
Control DLB AD
[3H]Pentazocine 1nM

FIG. 6A

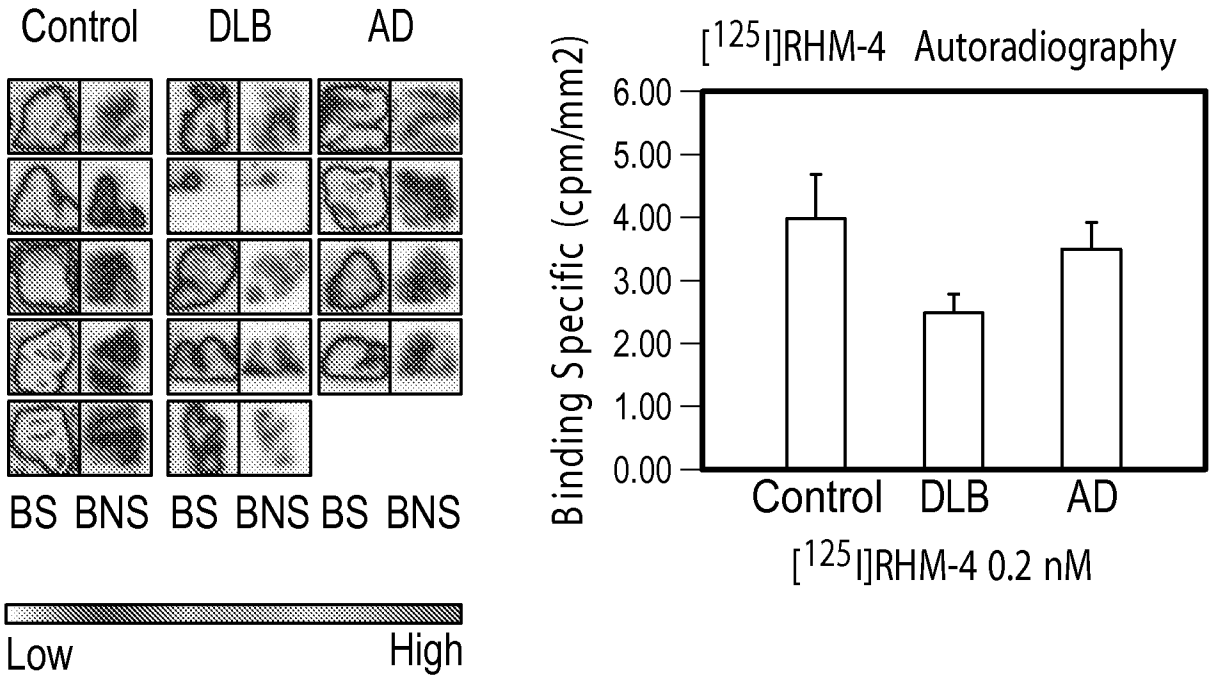
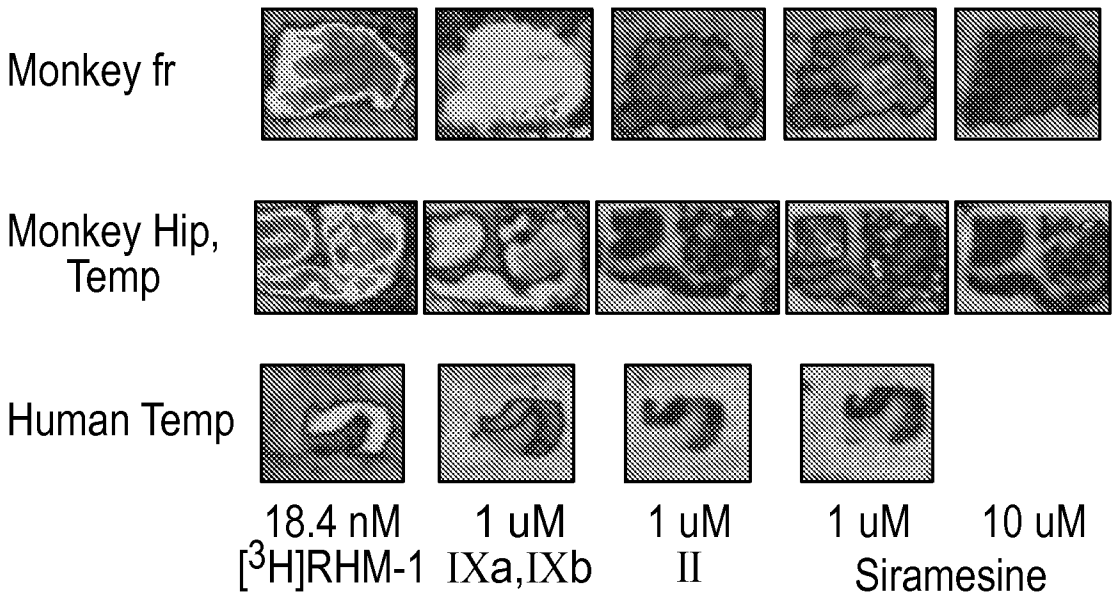

Control
DLB
AD
BS BNS BS BNS BS BNS
Low
High
[125I]RHM-4 Autoradiography
Binding Specific (cpm/mm2)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Control DLB AD
[125I]RHM-4 0.2 nM

FIG. 6B


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

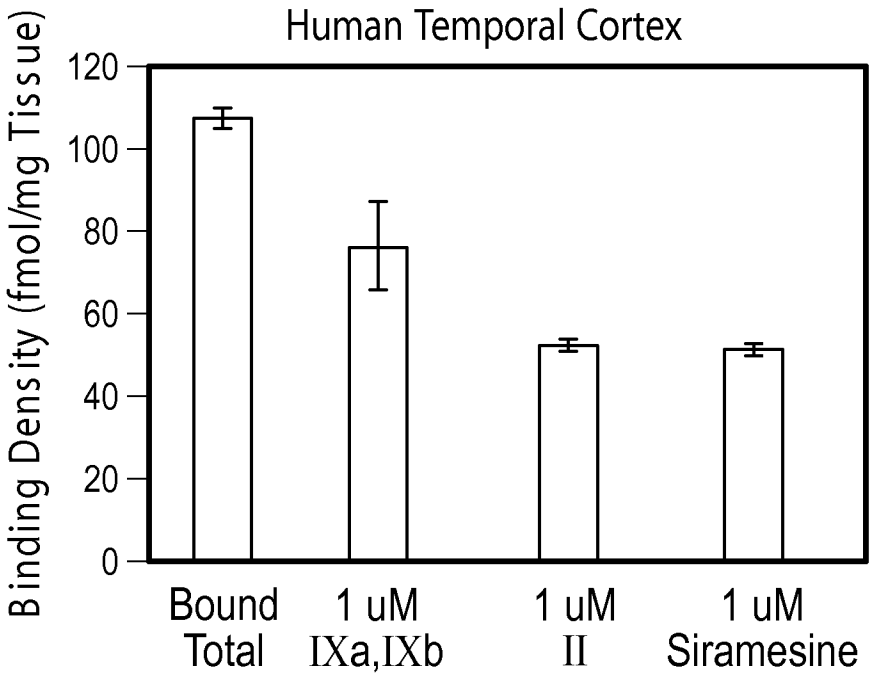

Human Temporal Cortex
Binding Density (fmol/mg Tissue)
120
100
80
60
40
20
0
Bound Total
1 uM IXa,IXb
1 uM II
1 uM Siramesine

FIG. 6C

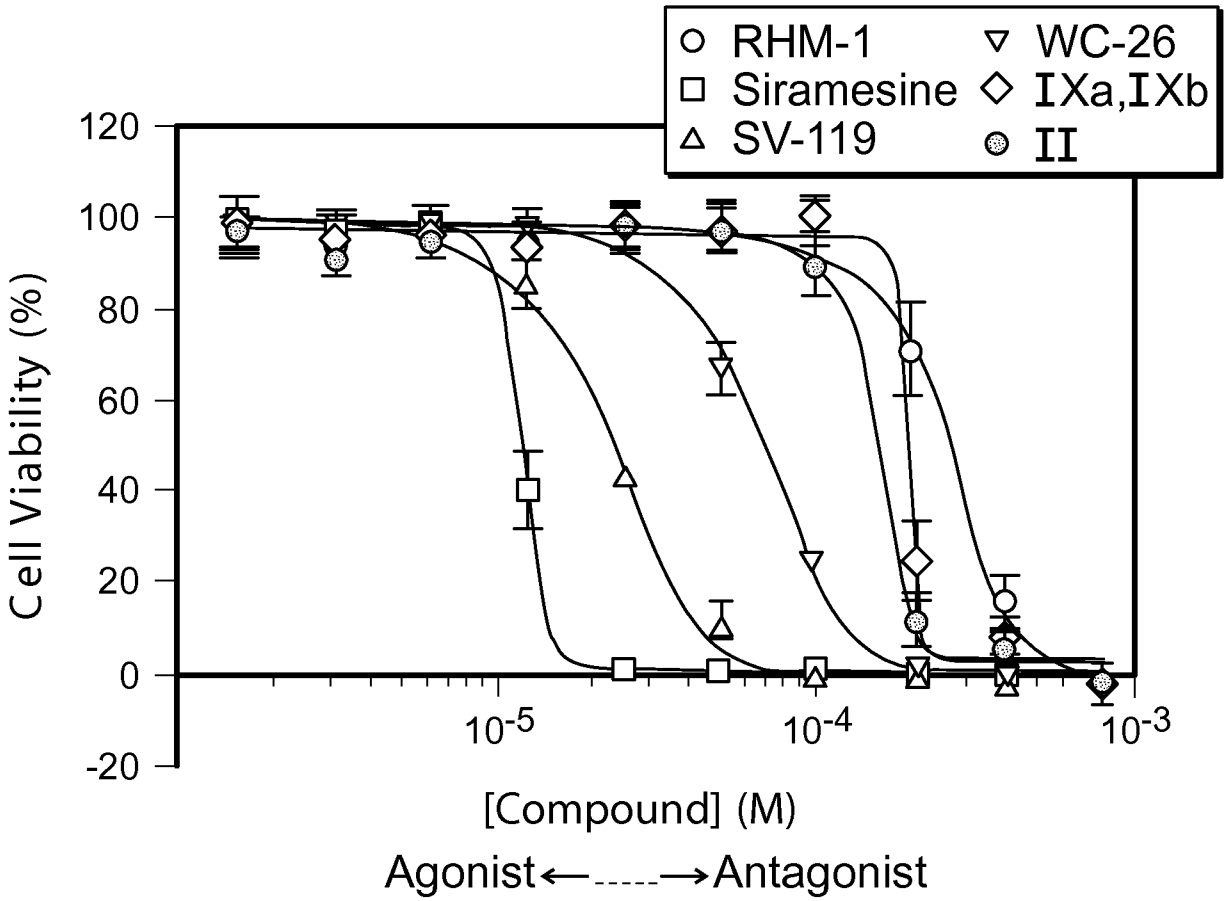

RHM-1
Siramesine
SV-119
WC-26
IXa,IXb
II
Cell Viability (%)
120
100
80
60
40
20
0
-20
10^{-5}
10^{-4}
10^{-3}
[Compound] (M)
Agonist←----→Antagonist

FIG. 7A

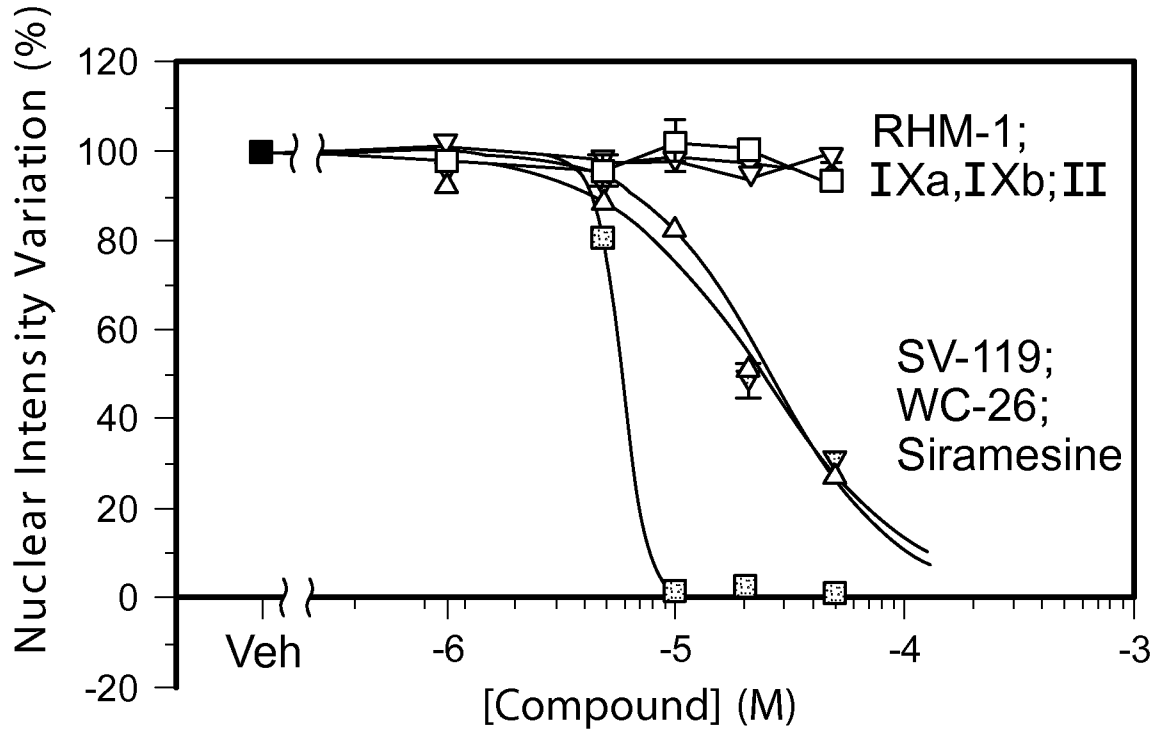

Nuclear Intensity Variation (%)
120
100
80
60
40
20
0
-20
RHM-1;
IXa,IXb;II
SV-119;
WC-26;
Siramesine
Veh
-6
-5
-4
-3
[Compound] (M)

FIG. 7B

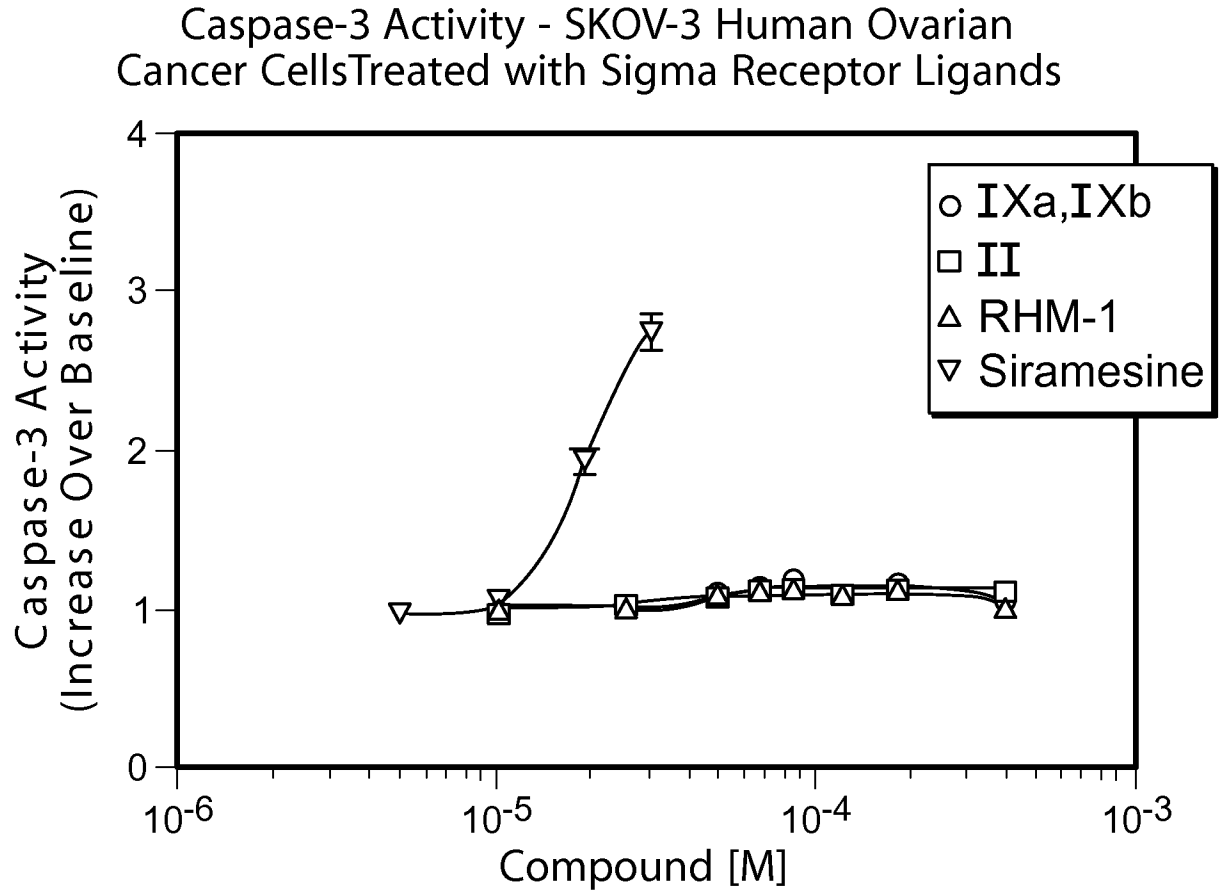


FIG. 10A

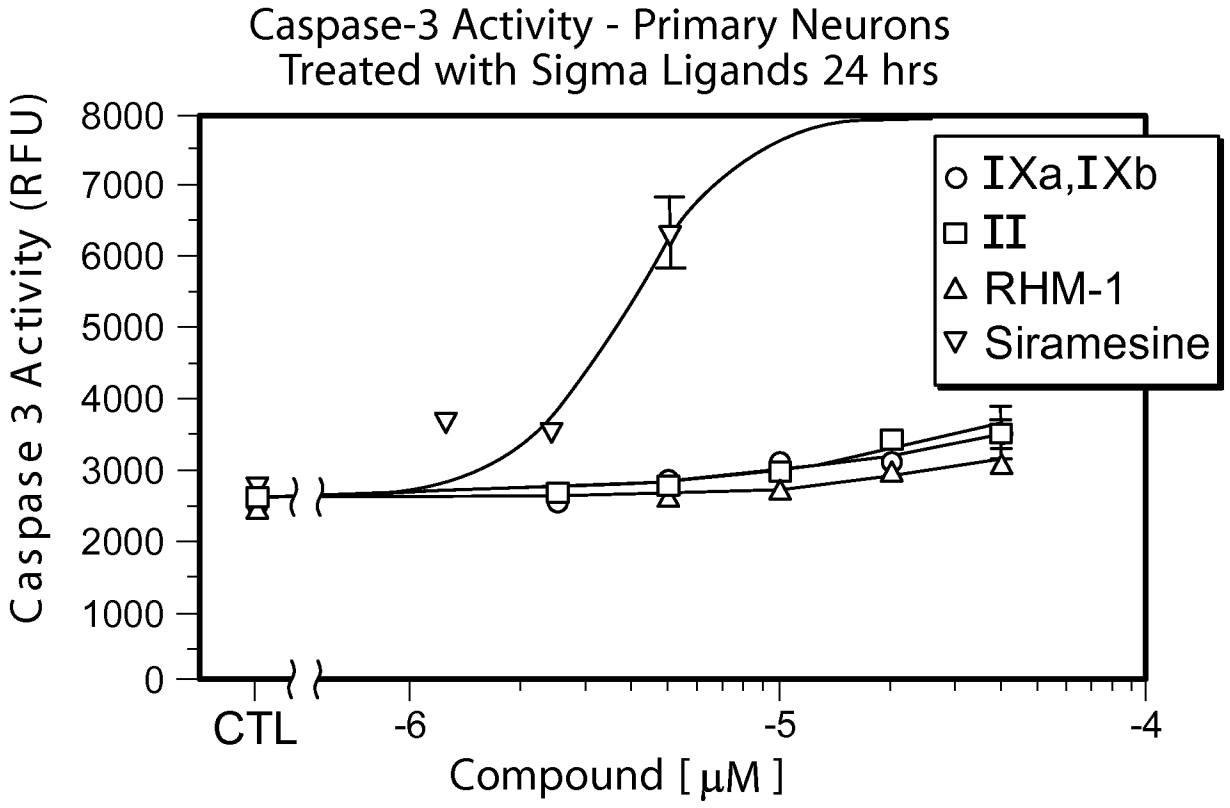


FIG. 10B

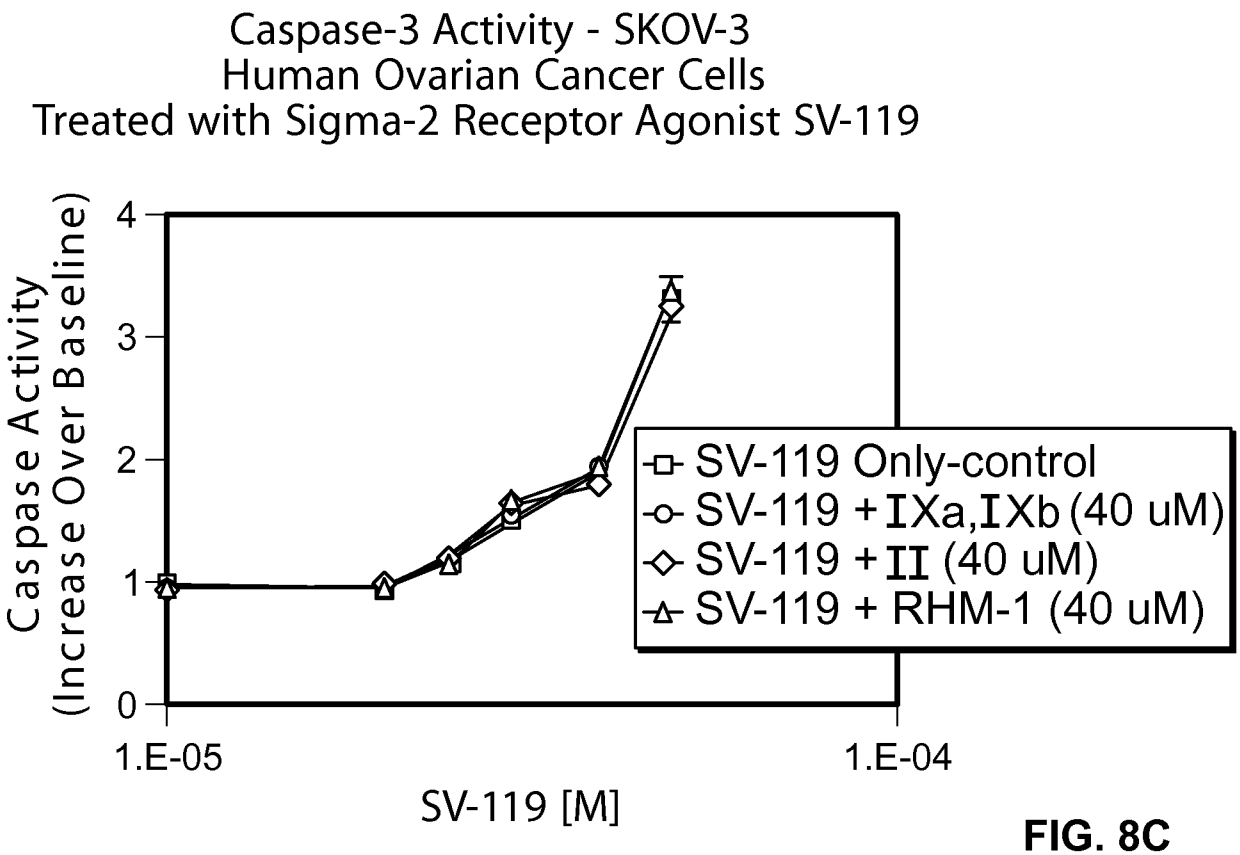

Caspase-3 Activity - SKOV-3
Human Ovarian Cancer Cells
Treated with Sigma-2 Receptor Agonist SV-119
Caspase Activity
(Increase Over Baseline)
4
3
2
1
0
1.E-05
1.E-04
SV-119 [M]
SV-119 Only-control
SV-119 + IXa,IXb (40 uM)
SV-119 + II (40 uM)
SV-119 + RHM-1 (40 uM)

FIG. 8C

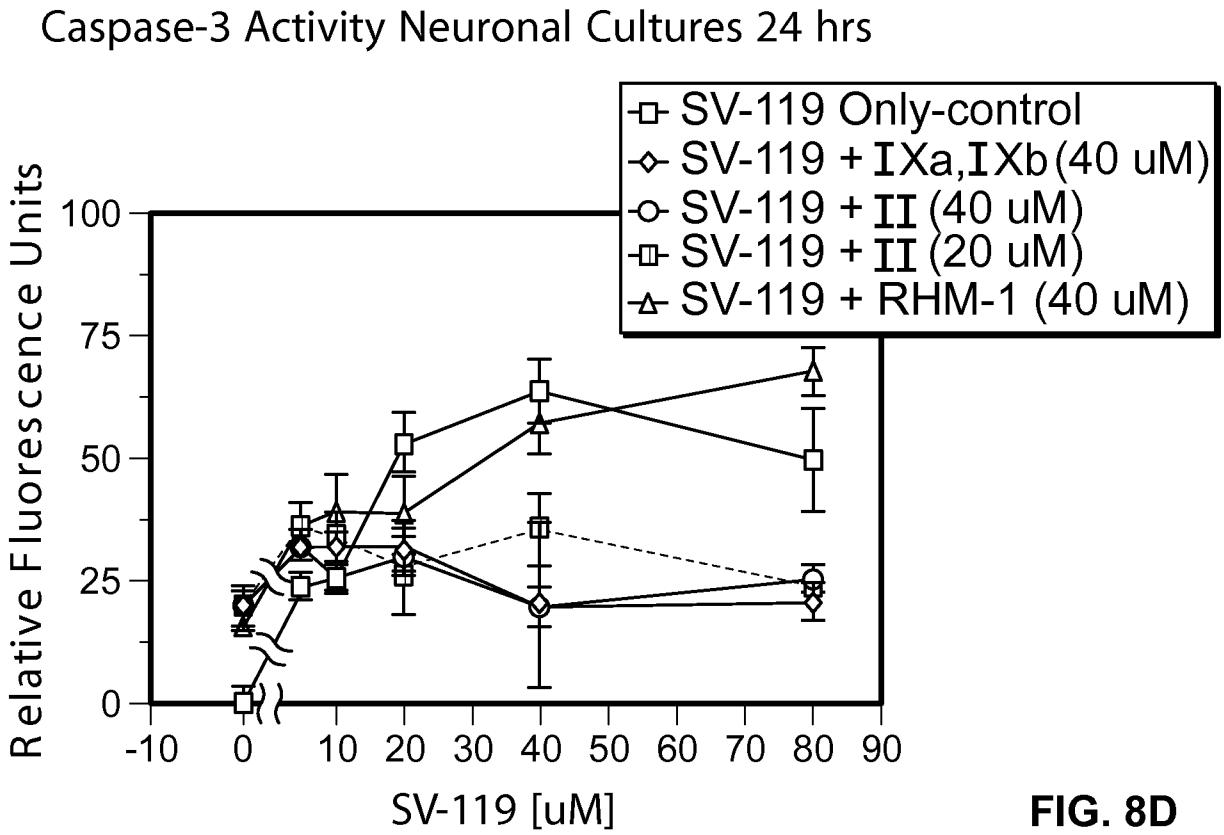

Caspase-3 Activity Neuronal Cultures 24 hrs
Relative Fluorescence Units
100
75
50
25
0
-10
0
10
20
30
40
50
60
70
80
90
SV-119 [uM]
SV-119 Only-control
SV-119 + IXa,IXb (40 uM)
SV-119 + II (40 uM)
SV-119 + II (20 uM)
SV-119 + RHM-1 (40 uM)

FIG. 8D

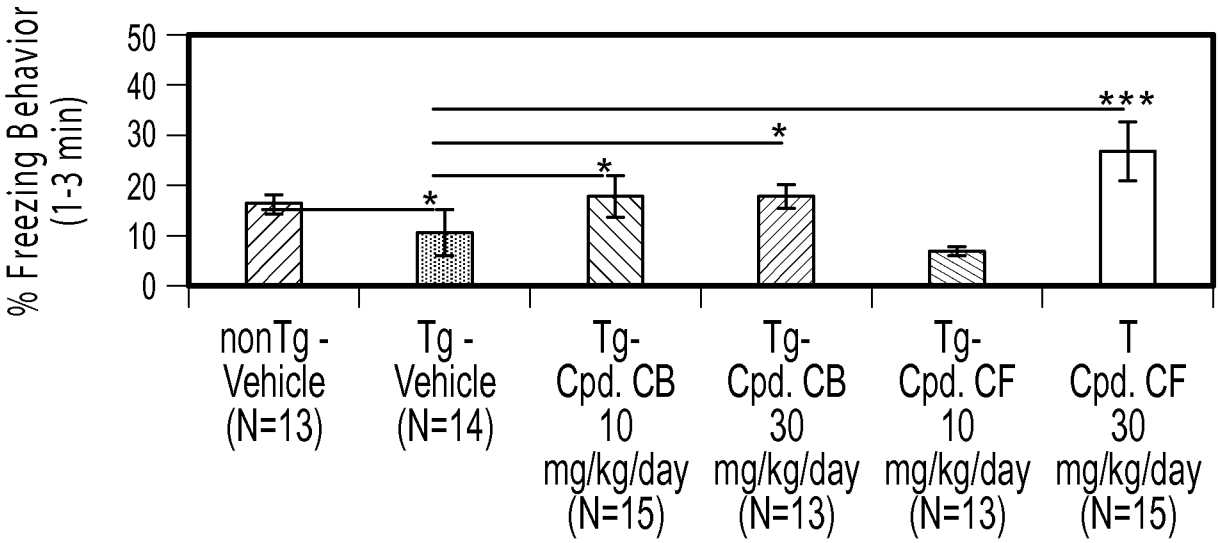

% Freezing Behavior (1-3 min)
50
40
30
20
10
0
*
*
*
***
nonTg - Vehicle (N=13)
Tg - Vehicle (N=14)
Tg- Cpd. CB 10 mg/kg/day (N=15)
Tg- Cpd. CB 30 mg/kg/day (N=13)
Tg- Cpd. CF 10 mg/kg/day (N=13)
T Cpd. CF 30 mg/kg/day (N=15)

FIG. 9A

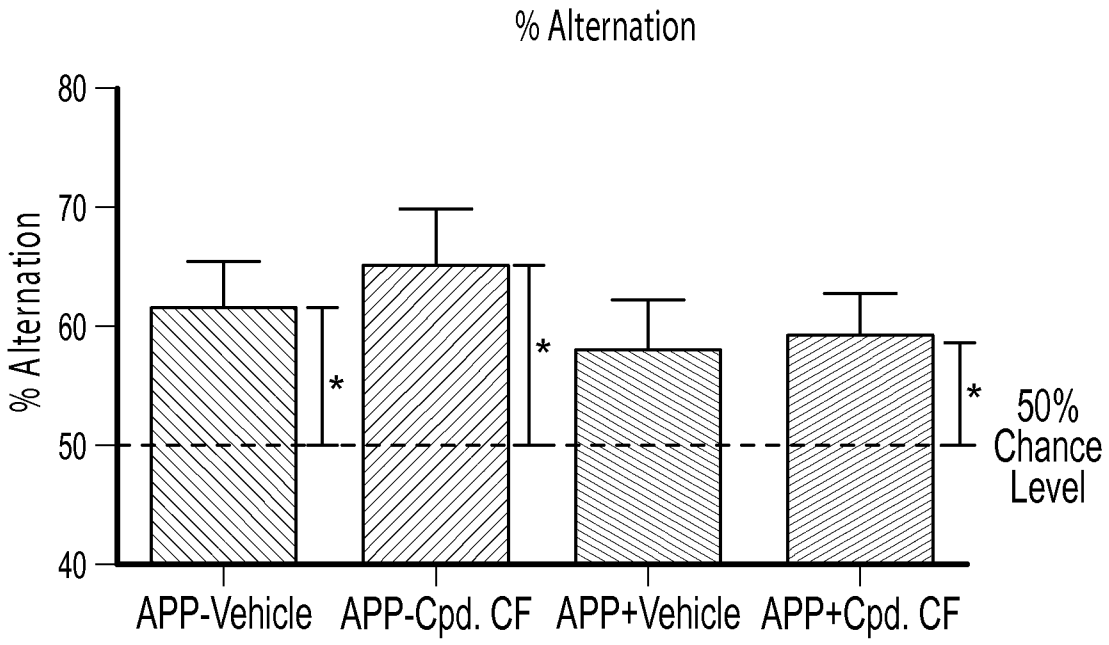

% Alternation
% Alternation
80
70
60
50
40
*
*
*
50% Chance Level
APP-Vehicle
APP-Cpd. CF
APP+Vehicle
APP+Cpd. CF

FIG. 9B