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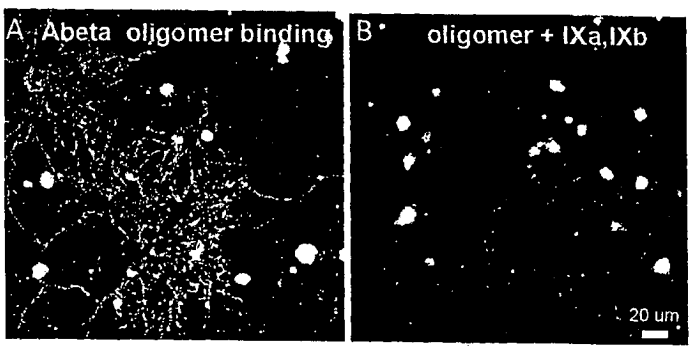
FIG. 3B



(57) Abstract: This invention relates to the use sigma-2 receptor antagonists, and of pharmaceutical compositions comprising such compounds, in methods for inhibiting Abeta- associated synapse loss or synaptic dysfunction in neuronal cells, modulating an Abeta-associated membrane trafficking change in neuronal cells, and treating cognitive decline associated with Abeta pathology and more broadly treating with such compounds and compositions neurodegenerative diseases and disorders associated with Abeta pathology. This invention also relates to methods for screening compounds for activity in inhibiting cognitive decline on the basis of their ability to bind to a sigma-2 receptor.

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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 and U.S. Provisional Patent Application No. 61/527,963, filed August 26, 2011, which applications are hereby incorporated by reference in its entirety.

Background of the Invention

[002] Overproduction and accumulation of amyloid beta is a pathologic feature of Alzheimer's disease. Human amyloid beta (Abeta) 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.

[003] 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.

[004] 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.

[005] 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.

[006] 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.

[007] One approach to development of AD therapeutics involves generation of anti-Aβ monoclonal antibodies, several of which are in various phases of clinical development including bapineuzumab (AAB-00; Janssen, Elan, Pfizer), solanezumab (LY2062430; Eli Lilly); PF-04360365 (Pfizer); MABT5102A (Genentech); GSK933776 (GlaxoSmithKline) and gantenerumab (R1450, RO4909832, Hoffman-LaRoche). However, thus far no intravenous amyloid beta specific monoclonal antibody has yet been approved for the treatment of AD. Recently, development of intravenous bapineuzumab was ended due to lack of efficacy in two late-stage trials in patients who had mild to moderate Alzheimer's disease. However, solanezumab in secondary analysis of Phase III clinical trial results was recently reported to show statistically significant slowing of cognitive decline in patients with mild AD, but not in patient's with moderate AD. One problem with this approach may be related to lack of adequate brain penetrability.

[008] There are only five medications currently FDA-approved for the treatment of Alzheimer's Disease (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 currently available data are inconclusive 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.

[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 antagonist compounds and compositions are used to treat or prevent synaptic dysfunction in a subject.

FIELD OF THE INVENTION

[010] This invention relates to the use of selective sigma-2 receptor antagonist compounds, and pharmaceutical compositions comprising them, in methods for inhibiting amyloid beta (Aβ)-associated synapse loss and synaptic dysfunction in neuronal cells. In some embodiments, the compositions are useful for modulating an Aβ-associated membrane trafficking change in neuronal cells, and treating cognitive decline associated with Aβ pathology in a patient in need thereof. In some embodiments, the compounds and compositions are used for treating neurodegenerative diseases and disorders associated with Abeta pathology. This invention also relates to methods for screening compounds for activity in inhibiting cognitive decline, on the basis of their ability to bind to and act as antagonists at a sigma-2 receptor, as well as to methods for refining such screening methods based in the first instance on whether the compounds block Aβ-induced membrane trafficking deficits, and block Aβ-induced synapse loss, but do not affect trafficking or synapse number in the absence of Aβ oligomers. The sigma-2 receptor antagonist

compound is selected from a small molecule, or an antibody or fragment thereof, selective for the sigma-2 receptor.

SUMMARY OF THE INVENTION

[011] The invention is based, in part, on the broad finding that a sigma-2 receptor antagonist, preferably one that also exhibits other aspects of a particular therapeutic phenotype, participates in inhibition and inhibits deleterious effects of soluble amyloid-beta ("Abeta", "Aβ") peptides and oligomers and other soluble species thereof on neuronal cells, as defined below, and, consequently, can be used to treat conditions, including diseases and disorders, associated with Abeta oligomer-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. Excluded are certain compounds of the prior art which were not known to be sigma-2 receptor antagonists and either (i) were known to bind to sigma-2 receptor and to reduce or eliminate Abeta induced pathologies such as a defect in membrane trafficking or synapse reduction in neuronal cells or (ii) were known to have activity against symptoms of Alzheimer's disease without implication of sigma-2 receptor interaction. 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.

[012] 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.

[013] 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.

[014] 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.

[015] 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.

[016] 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.

[017] The present invention also provides a method for screening for compounds that inhibit cognitive decline or treat a neurodegenerative disease, the method comprising selecting one or more compounds for testing on the basis of their ability to bind to a sigma-2 receptor in preference to other, non-sigma classes of CNS receptors. The sigma-2 antagonists may or may not also bind to sigma-1 receptor.

[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 potention 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.

[020] In some embodiments, an in vitro assay platform method is disclosed that is predictive of behavioral efficacy for screening a selective, sigma-2 antagonist compound for the ability to inhibit cognitive decline or to treat a neurodegenerative disease, the method comprising contacting a cell with a compound that binds and acts as an antagonist at a sigma-2 receptor and wherein said compound has each of the following 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; and

(c) it does not affect trafficking or synapse number in the absence of Abeta oligomer.

[021] The present invention also provides methods of identifying compounds that inhibit cognitive decline or treat 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 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, wherein a test compound that competitively inhibits the binding of the known ligand is identified as a sigma-2 receptor ligand. Such methods may be carried out using an animal model, which can be any animal model but it is preferably a rodent model. Any appropriate binding assay can be used to determine whether a compound binds a sigma-2 receptor (or the compound can have already been determined or even been known to do so).

BRIEF DESCRIPTION OF THE DRAWINGS

[022] 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.

[023] 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.

[024] 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.

[025] 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.

[026] 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.

[027] 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.

[028] Figure 1G shows 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.

[029] 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.

[030] 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.

[031] 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.

[032] 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.

[033] 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.

[034] 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.

[035] 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.

[036] 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.

[037] 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.

[038] 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 trangles) 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.

[039] 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.

[040] 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.

[041] 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).

[042] 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.

[043] 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.

[044] 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).

[045] 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 Figure 3A-panel B. 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).

[046] 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 (black 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.

[047] 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.

[048] 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.

[049] Figure 5A is a graph of the correlation between Sigma-2 binding affinity (from Table 2) and potency in the trafficking assay (from Table 5). Included are only compounds that were active in the trafficking assay: excluded are compounds that were also sigma 1 antagonists.

[050] Figure 5B is a graph of the same correlation between Sigma-2 binding affinity from Table 2) and potency in the trafficking assay (from Table 5) for the same compounds used to generate Fig. 5A but additionally including data points for compounds that are both sigma-2 ligands and sigma-1 antagonists (these outlier data point are clustered in the lower right hand quadrant of the graph and have not been used to calculate correlation coefficient.)

[051] Figure 5C is a graph showing the absence of a correlation between Sigma-1 binding affinity (from Table 2) and EC_{50} in the trafficking assay (from Table 5), $r^2=0.06$, $p>0.05$.

[052] Figure 5D is a graph showing the absence of correlation between Sigma 2 binding affinity (from Table 2) and maximum inhibition of Abeta in the trafficking assay (from Table 5). All data points are included in the analysis.

[053] Figure 6 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 (2nd bar, showing a significant reduction in ability of test animals to acquire new memories)) (iii) a mixture of compounds IXa and IXb, (3rd bar, showing complete (and statistically significant) inhibition of Abeta oligomer-induced memory deficit); or (iv) a mixture of compounds IXa and IXb in the absence of Abeta oligomer (4th bar, showing no effect on memory). There was no adverse behavioral effects observed. This figure demonstrates that compound mixture IXa,IXb can prevent Abeta oligomer-induced memory deficits, while have no effect on memory performance when dosed on its own.

[054] Figure 7A shows the membrane trafficking assay performed in primary hippocampal and cortical cultures used in the prevention mode where compound II, with or without threo-ifenprodil (TIF), is added before oligomers. Threo-ifenprodil (TIF) is a sigma-2 receptor ligand (Monassier et al., JPET, 322 (1):341-350, 2007) with affinity for other receptors (s2 0.9 nM, s1 59 nM, NR2B 222nM, K+ ch 88nM, etc.), that does not cause apoptosis, affect trafficking or interfere with Abeta oligomer-induced trafficking deficits when dosed alone (data not shown), therefore high affinity sigma receptor binding is not sufficient to produce therapeutic phenotype. This figure demonstrates that TIF exhibits pharmacological competition with II (and IXa,IXb; not shown) in prevention format in neurons indicating that their binding sites on sigma receptors partially overlap. This is the first demonstration of functional competition in neurons by sigma ligands demonstrating that sigma receptors participate in Abeta oligomer-induced membrane trafficking processes.

[055] Figure 7B shows the membrane trafficking assay performed in primary hippocampal and cortical cultures used in the treatment mode where compound II, with or without threo-ifenprodil (TIF), is added after oligomers. This figure demonstrates that threo-ifenprodil (TIF) exhibits pharmacological competition with compound II (and IXa,IXb; not shown) in treatment format in neurons indicating that their binding sites on sigma receptors partially overlap.

[056] Figure 7C shows membrane trafficking assay performed in primary hippocampal and cortical cultures used in the treatment mode. The data show the amount of formazan contained inintracellular vesicles in the presence of vehicle

(open square) and Abeta oligomer (open circle). The Abeta oligomer effect on membrane trafficking is attenuated by presence of Compound CF (closed squares) in a dose dependent manner. Addition of TIF significantly lowers maximum inhibition due to Compound CF, and shifts the EC50 value rightward; therefore TIF acts as a antagonist of same receptor bound by Compound CF in treatment format (compounds added after Abeta).

[057] Figure 7D shows shows membrane trafficking data in presence of vehicle (open square) and Abeta oligomer (open circle). Abeta effect is attenuated by presence of Compound II (closed squares) in a dose dependent manner. Addition of TIF significantly lowers maximum inhibition due to Compound II, and shifts EC50 value. Therefore TIF exhibits pharmacological competition with Compound II in treatment format.

[058] Figure 8A 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.

[059] Figure 8B 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.

[060] Figure 8C 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.

[061] Figure 9A 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.

[062] Figure 9B 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.

[063] Figure 10A 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.

[064] Figure 10B 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.

[065] Figure 10C 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.

[066] Figure 10D 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.

[067] Figure 11A shows the trafficking assay and trafficking deficits (reduction in vesicles compared to vehicle) due to the presence of Abeta oligomers. Addition of sigma-2 agonist siramesine blocks Abeta oligomer trafficking deficits at low concentrations, but causes cellular toxicity at high concentrations. Sigma-2 antagonist II blocks oligomer-induced trafficking deficits at all concentrations tested. This figure demonstrates that cmpd II exhibits a therapeutic phenotype, and does not behave similarly to known sigma-2 agonists.

[068] Figure 11B shows the trafficking assay and trafficking deficits (reduction in vesicles compared to vehicle) due to the presence of Abeta oligomers. Addition of sigma-2 agonist SV119 blocks Abeta oligomer trafficking deficits at

low concentrations, but causes cellular toxicity at high concentrations. Sigma-2 antagonist RHM-1 blocks oligomer-induced trafficking deficits at all concentrations tested, but does not exhibit a therapeutic phenotype since its structure is not drug-like. This figure demonstrates that cmpd II behaves similarly to known sigma-2 antagonists.

[069] Figure 12A 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.

[070] Figure 12B 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.

[071] Figure 13A shows a fluoromicrograph of Abeta oligomers binding to primary neuronal cultures 21 DIV visualized with 6E10 Abeta specific antibody immunolabeling.

[072] Figure 13B shows the same field as 13A in which neurons are selectively visualized via neuron-specific MAP2 immunolabeling.

[073] Figure 13C shows a fluoromicrograph of Abeta oligomers (visualized with 6E10 immunolabeling) binding to sister primary neuronal cultures pretreated with 78 nM anti-PGRMC1 C-terminal antibody 21 DIV. This figure demonstrates that the presence of anti-PGRMC C-terminal antibody resulted in significantly reduced Abeta oligomer binding that was 47% lower than control Abeta-only-treated cultures.

[074] Figure 13D shows the same field as 13C in which neurons are selectively visualized via neuron-specific MAP2 immunolabeling. A similar density of neurons are present in the culture as are seen in sister control cultures (Fig 13A)..

[075] Figure 13E shows a fluoromicrograph of Abeta oligomers (visualized with 6E10 immunolabeling) binding to sister primary neuronal cultures pretreated with 78 nM control antibody (non-immune IgG). This figure demonstrates that the presence of nonimmune IgG does not significantly change Abeta binding intensity from control cultures treated with Abeta only (Fig. 13A).

[076] Figure 13F shows the same field as 13E in which neurons are selectively visualized via neuron-specific MAP2 immunolabeling. A similar density of neurons are present in the culture as are seen in sister control cultures (Fig 13A).

[077] Figure 13G shows a fluoromicrograph of Abeta oligomers (visualized with 6E10 immunolabeling) binding to sister primary neuronal cultures pretreated with 78 nM anti-PGRMC1 N-terminal antibody. This figure demonstrates that the presence of 78 nM anti-PGRMC1 N-terminal antibody does not significantly change Abeta binding intensity from control cultures treated with Abeta only (Fig. 13A).

[078] Figure 13H shows the same field as 13G in which neurons are selectively visualized via neuron-specific MAP2 immunolabeling. A similar density of neurons are present in the culture as are seen in sister control cultures (Fig 13A). Scale bar = 15 microns.

[079] Figure 14A shows a quantification of the data shown in Figure 13 in a bar graph of Abeta binding intensity per neuron calculated by image processing-based quantification of the number, intensity and area of 6E10-immunolabeled

areas of the fluoromicrographs. Abeta oligomer binding intensity per neuron in the absence of antibodies (open bar labeled "Control") and with three concentrations each of C-terminal, and N-terminal specific anti-PGRMC1 antibodies, as well as nonimmune control IgG antibodies (respectively labeled bars). The C-terminal specific anti-PGRMC1 antibody is the only antibody that significantly decreased Abeta oligomer binding in a dose-dependent manner. Antibody induced changes to binding puncta number and binding area per neuron were very similar to those shown for intensity. This figure is consistent with the interpretation that a large percentage of Abeta oligomers present in these cultures bind to sigma-2 receptor.

[080] Figure 14B shows the nuclear area in the same neuronal cultures in Fig 13 and 14A. When treated with increasing concentration of antibodies, nuclear area, a measure of cellular toxicity, does not change. Therefore, the addition of antibodies does not affect the health of the neuronal cultures.

DETAILED DESCRIPTION OF THE INVENTION

[081] 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.

[082] 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

[083] 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.

[084] 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 %.

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

[086] 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.

[087] 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.

[088] The term "antagonist" refers to an entity, e.g., a compound, antibody or fragment, 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. As used herein, the term "sigma-2 receptor antagonist" is used to describe a compound that acts as a "functional antagonist" at the sigma-2 receptor in that it blocks Abeta effects, for example, Abeta oligomer-induced synaptic dysfunction, for example, as seen in an in vitro assay, such as a membrane trafficking assay, or a synapse loss assay, or Abeta oligomer mediated sigma-2 receptor activation of caspase-3, 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 to a sigma-2

receptor, or indirectly, by interfering with downstream signaling resultant from Abeta oligomer binding the sigma-2 receptor.

[089] The term "sigma-2 receptor antagonist compound" refers to a small molecule, antibody, or active binding fragment thereof, that binds to a sigma-2 receptor in a measurable amount and acts as a functional antagonist with respect to Abeta effects oligomer induced synaptic dysfunction resultant from sigma-2 receptor binding.

[090] The term "selectivity" or "selective" refers to a difference in the binding affinity of a compound (K_i) for a sigma receptor, for example, 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 K_i for a sigma-2 receptor or both a sigma-2 and a sigma-1 receptor is compared to the K_i for a non-sigma receptor. In some embodiments, the selective sigma-2 receptor antagonist, or sigma-1 receptor ligand, has at least 10-fold, 20-fold, 30-fold, 50-fold, 70-fold, 100-fold, or 500-fold higher affinity, or more, for binding to a sigma receptor compared to a non-sigma receptor as assessed by a comparison of binding dissociation constant Ki values, or IC_{50} values, or binding constant, at different receptors. Any known assay protocol can be used to assess the Ki or IC_{50} values at different receptors, for example, by monitoring the competitive displacement from receptors of a radiolabeled compound with a known dissociation constant, for example, by the method of Cheng and Prusoff (1973) (Biochem. Pharmacol. 22, 3099-3108), or specifically as provided herein. In some embodiments, the sigma-2 antagonist compound is an antibody, or active binding fragment thereof, specific for binding to a sigma-2 receptor compared to a non-sigma receptor. In the case of an antibody, or fragment, binding constants at a sigma-2 receptor, or fragment, can be calculated and compared to binding constants at a non-sigma receptor by any means known in the art, for example, by the method of Beatty et al., 1987, J Immunol Meth, 100(1-2):173-179, or the method of Chalquest, 1988, J. Clin. Microbiol. 26(12): 2561-2563. The non-sigma receptor is, for example, selected from 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.

[091] 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.

[092] The term "therapeutic phenotype" is used to describe a pattern of activity for compounds in the in vitro assays that is 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 with respect to Abeta oligomer-induced effects in a neuron, is said to have the "therapeutic phenotype" if (i) it blocks or reduces Aβ-induced membrane trafficking deficits; (ii) it blocks or reduces Aβ-induced synapse loss and (iii) it 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" and is predictive of behavioral efficacy.

[093] The term "therapeutic profile" is used to describe a compound that meets the therapeutic phenotype, and also has good brain penetrability(the ability to cross the blood brain barrier), good plasma stability and good metabolic stability.

[094] The term "drug-like properties" is used herein to describe the pharmacokinetic and stability characteristics of the sigma-2 receptor ligands upon administration; including brain penetrability, metabolic stability and/or plasma stability.

[095] "Abeta species" or "Aβ" shall include compositions comprising soluble amyloid peptide-containing components such as Abeta monomers, Abeta oligomers, or 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.

[096] "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.

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

[098] 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.

[099] 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.

[0100] 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.

[0101] 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.

[0102] The term "inhibiting trafficking deficits" refers to the ability to block soluble Ab oligomer-induced membrane trafficking deficits in a cell, preferably a neuronal cell. A compound capable of inhibiting trafficking deficits has an EC50 < 20 μM, less than 15 μM, less than 10 μM, less than 5 μM, 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, for example, as described in Example 6.

[0103] 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 of 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 e.g. methyl (C_1 alkyl), ethyl (C_2 alkyl), C_3 alkyl, C_4 alkyl, C_5 alkyl, and C_6 alkyl as well as, e.g. C_1-C_2 alkyl, C_1-C_3 alkyl, C_1-C_4 alkyl, C_2-C_3 alkyl, C_2-C_4 alkyl, C_3-C_6 alkyl, C_4-C_5 alkyl, and C_5-C_6 alkyl.

[0104] 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.

[0105] 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.

[0106] 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).

[0107] As used herein, "alkenyl" refers to an alkyl group having one or more double carbon-carbon bonds. Example alkenyl groups include, but are not limited to, ethenyl, propenyl, cyclohexenyl, and the like. The term "alkenylenyl" refers to a divalent linking alkenyl group.

[0108] As used herein, "alkynyl" refers to an alkyl group having one or more triple carbon-carbon bonds. Example alkynyl groups include, but are not limited to, ethynyl, propynyl, and the like. The term "alkynylenyl" refers to a divalent linking alkynyl group.

[0109] 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.

[0110] 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.

[0111] 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 of 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

[0112] As used herein, "heteroaryl" groups refer to an aromatic heterocycle having up to 20 ring-forming atoms and having at least one heteroatom ring member (ring-forming atom) such as sulfur, oxygen, or nitrogen. In some embodiments, the heteroaryl group has at least one or more heteroatom ring-forming atoms each independently selected from sulfur, oxygen, and nitrogen. Heteroaryl groups include monocyclic and polycyclic (e.g., having 2, 3 or 4 fused rings) systems. Examples of heteroaryl groups include without limitation, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, furyl, quinolyl, isoquinolyl, thienyl, imidazolyl, thiazolyl, indolyl, pyrryl, oxazolyl, benzofuryl, benzothienyl, benzthiazolyl, isoxazolyl, pyrazolyl, triazolyl, tetrazolyl, indazolyl, 1,2,4-thiadiazolyl, isothiazolyl, benzothienyl, purinyl, carbazolyl, benzimidazolyl, indolinyl, and the like. In some embodiments, the heteroaryl group has from 1 to about 20 carbon atoms, and in further embodiments from about 1 to about 5, from about 1 to about 4, from about 1 to about 3, from about 1 to about 2, carbon atoms as ring-forming atoms. In some embodiments, the heteroaryl group contains 3 to about 14, 3 to about 7, or 5 to 6 ring-forming atoms. In some embodiments, the heteroaryl group has 1 to about 4, 1 to about 3, or 1 to 2 heteroatoms.

[0113] As used herein, "heterocycloalkyl" refers to non-aromatic heterocycles having up to 20 ring-forming atoms including cyclized alkyl, alkenyl, and alkynyl groups where one or more of the ring-forming carbon atoms is replaced by a heteroatom such as an O, N, or S atom. Heterocycloalkyl groups can be mono or polycyclic (e.g., both fused and spiro systems). Example "heterocycloalkyl" groups include morpholino, thiomorpholino, piperazinyl, tetrahydrofuranyl,

tetrahydrothienyl, 2,3-dihydrobenzofuryl, 1,3-benzodioxole, benzo-1,4-dioxane, piperidinyl, pyrrolidinyl, isoxazolidinyl, isothiazolidinyl, pyrazolidinyl, oxazolidinyl, thiazolidinyl, imidazolidinyl, pyrrolidin-2-one-3-yl, and the like. Ring-forming carbon atoms and heteroatoms of a heterocycloalkyl group can be optionally substituted by oxo or sulfido. For example, a ring-forming S atom can be substituted by 1 or 2 oxo [i.e., form a S(O) or $S(O)_2$]. For another example, a ring-forming C atom can be substituted by oxo (i.e., form carbonyl). Also included in the definition of heterocycloalkyl are moieties that have one or more aromatic rings fused (i.e., having a bond in common with) to the nonaromatic heterocyclic ring, for example pyridinyl, thiophenyl, phthalimidyl, naphthalimidyl, and benzo derivatives of heterocycles such as indolene, isoindolene, isoindolin-1-one-3-yl, 4,5,6,7-tetrahydrothieno[2,3-c]pyridine-5-yl, 5,6-dihydrothieno[2,3-c]pyridin-7(4H)-one-5-yl, and 3,4-dihydroisoquinolin-1(2H)-one-3yl groups. Ring-forming carbon atoms and heteroatoms of the heterocycloalkyl group can be optionally substituted by oxo or sulfido. In some embodiments, the heterocycloalkyl group has from 1 to about 20 carbon atoms, and in further embodiments from about 3 to about 20 carbon atoms. In some embodiments, the heterocycloalkyl group contains 3 to about 14, 3 to about 7, or 5 to 6 ring-forming atoms. In some embodiments, the heterocycloalkyl group has 1 to about 4, 1 to about 3, or 1 to 2 heteroatoms. In some embodiments, the heterocycloalkyl group contains 0 to 3 double bonds. In some embodiments, the heterocycloalkyl group contains 0 to 2 triple bonds.

[0114] As used herein, "halo" or "halogen" includes fluoro, chloro, bromo, and iodo.

[0115] 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.

[0116] 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.

[0117] As used herein, "arylalkyl" refers to a C_{1-6} alkyl substituted by aryl and "cycloalkylalkyl" refers to C_{1-6} alkyl substituted by cycloalkyl.

[0118] As used herein, "heteroarylalkyl" refers to a C_{1-6} alkyl group substituted by a heteroaryl group, and "heterocycloalkylalkyl" refers to a C_{1-6} alkyl substituted by heterocycloalkyl.

[0119] As used herein, "amino" refers to NH_2.

[0120] As used herein, "alkylamino" refers to an amino group substituted by an alkyl group.

[0121] As used herein, "dialkylamino" refers to an amino group substituted by two alkyl groups.

[0122] As used here, C(O) refers to C(=O).

[0123] 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, in indicated.

[0124] As used herein, an "amyloid beta effect", for example, a "nonlethal amyloid beta effect", or Abeta oligomer 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*. In some embodiments, an Abeta effect is selected from Abeta oligomer-induced synaptic dysfunction, for example, as seen in an in vitro assay, such as a membrane trafficking assay, or a synapse loss assay, or Abeta oligomer mediated sigma-2 receptor activation of caspase-3, or Abeta induced neuronal dysfunction, Abeta mediated decrease in long term potentiation (LTP), or in cognitive decline in a behavioral assay, or in a patient in need thereof.

[0125] 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.

[0126] 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, assays utilize a 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.

[0127] 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.

[0128] 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.

[0129] 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.

[0130] 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.

[0131] 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 (e.g., lessen or postpone the onset of) 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.

[0132] Generally speaking, the term "tissue" refers to any aggregation of similarly specialized cells which are united in the performance of a particular function.

[0133] 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).

[0134] 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, Compounds IXa and IXb, as well as all the other compounds which are described herein, are not normally present in a subject, and, therefore, would not be considered natural ligands.

[0135] Sigma-2 Receptors

[0136] The sigma receptors are multifunctional adapter/chaperone proteins that participate in several distinct protein signaling complexes in a tissue and state-related manner. The sigma-2 receptor is expressed in brain and various peripheral tissues at low levels. (Walker et al., 1990 *Sigma receptors: biology and function.* Pharmacol. Rev. 42:355-402). Sigma-2 receptors are present in human hippocampus and cortex. The sigma-2 receptor was also previously validated as a biomarker for tumor cell proliferation. (Mach et al., *Sigma-2 receptors as potential biomarkers of proliferation in breast cancer.* Cancer Res. 57:156-161, 1997).

[0137] Sigma-2 receptors are implicated in many signaling pathways such as heme binding, Cytochrome P450 metabolism, cholesterol synthesis, progesterone signaling, apoptosis and membrane trafficking. Only a subset of sigma receptor binding sites/signaling pathways are relevant to oligomer signaling in AD. No sigma-2 receptor knock-outs are currently available and human mutations in sigma-2 sequence have not been studied in a neurodegeneration context.

[0138] A sigma-2 receptor was recently identified as the progesterone receptor membrane component 1 (PGRMC1) in rat liver by use of a photoaffinity probe WC-21, which irreversibly labels sigma-2 receptors in rat liver. Xu et al. *Identification of the PGRMC1 protein complex as the putative sigma-2 receptor binding site.* Nature Communications 2, article number 380, July 5, 2011, incorporated herein by reference. PGRMC1 (progesterone receptor membrane

component 1) was identified as the critical 25kDa component of sigma-2 receptor activity in August 2011 by Xu et al. PGRMC1 is a single transmembrane protein with no homology to sigma-1 protein; family members include PGRMC2 and neudesin. PGRMC1 contains a cytochrome b5 heme-binding domain. PGRMC1 is a single transmembrane protein with no homology to S1 protein; family members include PGRMC2 and neudesin. PGRMC1 contains a cytochrome b5 heme-binding domain. Endogenous PGRMC1 ligands include progesterone/steroids, cholesterol metabolites, glucocorticoids, and heme. PGRMC1 functions as chaperone/adapter associated with different protein complexes in different subcellular locations (Cahill 2007. *Progesterone receptor membrane component 1: an integrative review.* J. Steroid Biochem. Mol. Biol. 105:16-36). PGRMC1 binds heme with reducing activity, complexes with CYP450 proteins (regulated redox reactions), associates with PAIRBP1 and mediates progesterone block of apoptosis, and associates with Insig-1 and SCAP to induce SRE-related gene transcription in response to low cholesterol. The *C. elegans* homolog VEM1 associates with UNC-40/DCC to mediate axon guidance. PGRMC1 contains two SH2 target sequences, an SH3 target sequence, a tyrosine kinase site, two acidophilic kinase sites (CK2), and consensus binding sites for ERK1 and PDK1. PGRMC1 contains several ITAM sequences involved in membrane trafficking (vesicle transport, clathrin-dependent endocytosis of calveolin-containing pits).

[0139] Sigma-2 receptor therapeutics have reached human Phase II clinical trials for other CNS indications, but not for treatment of AD. Many of the sigma-2 receptor ligands are not very selective and have high affinity for other non-sigma CNS receptors. For example, Cyr-101/MT-210 (Cyrenaic Pharmaceuticals; Mitsubishi) is a sigma-2 receptor antagonist in phase IIa clinical trials for schizophrenia, but has multiple other receptor interactions including at 5HT2a, ADRA1, and histamine H1. Siramesine (Lundbeck, Forest Lu28179) is a sigma-2 receptor agonist that previously was in clinical trials for anxiety, but was discontinued. Sigma-1 receptor ligands are in clinical trials for various CNS indications. Cutamesine dihydrochloride (AGY SA4503, M's Science Corp.) is a sigma-1 receptor agonist that was in phase II clinical trials for stroke, and phase II

trials for depression. Anavex 2-73 is a sigma-1 receptor agonist that also acts as at muscarinic cholinergic receptors as M2/3 antagonist, M1 agonist, and is an antagonist with respect to various ion channels (NMDAR, Na+, Ca++). Anavex 2-73 entered phase IIa clinical trials for patients with AD and mild cognitive impairment. There are no previous clinical trials with highly selective sigma-2 receptor ligand therapeutics in AD.

[0140] Sigma-2 Antagonists

[0141] 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.

[0142] 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.

[0143] In some embodiments, the sigma-2 receptor antagonist of the present invention acts as a functional antagonist 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.

[0144] 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.

[0145] 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.

[0146] 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.

[0147] In some embodiments, the antagonist of the invention may bind with greater affinity to sigma-1 receptor than to a sigma-2 receptor, but must still behave as a functional neuronal antagonist with respect to blocking or inhibiting an Abeta oligomer-induced effect (Abeta effect).

[0148] 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.

[0149] 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.

[0150] 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.

[0151] 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.

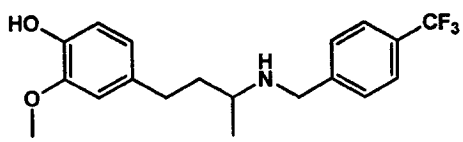
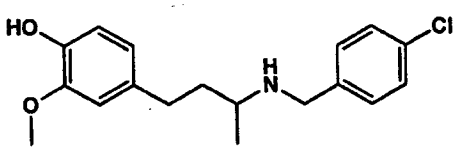
[0152] As used herein the term "plasma stability" refers to the degradation of compounds in plasma, for example, by enzymes such as hydrolases and esterases. Any of a variety of in vitro assays can be employed. Drugs are incubated in plasma over various time periods. The percent parent compound (analyte) 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.

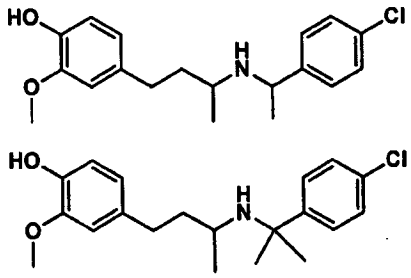
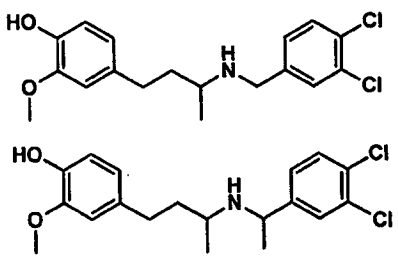
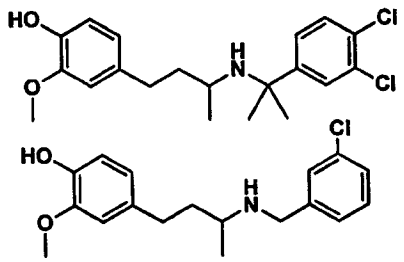
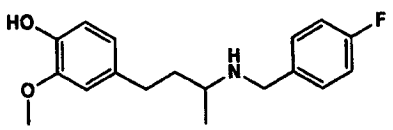
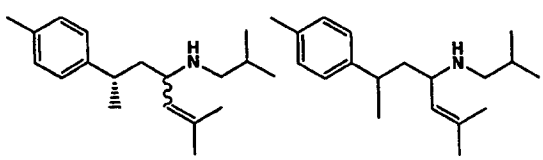
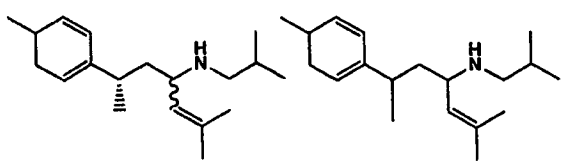
[0153] As used herein the term "metabolic stability" refers to the ability of the compound to survive first-pass metabolism (intestinal and hepatic degradation or conjugation of a drug administered orally). This can be assessed, for example, in vitro by exposure of the compounds to mouse or human hepatic microsomes. In

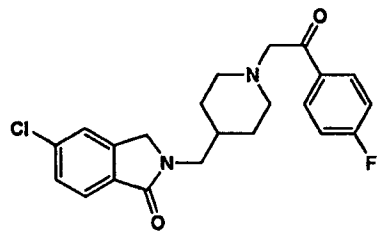
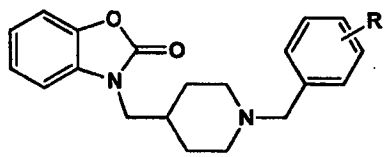
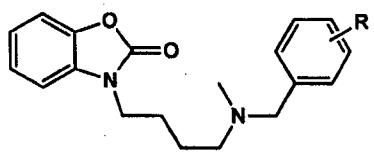
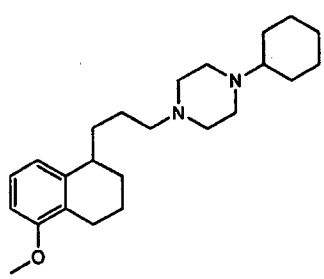
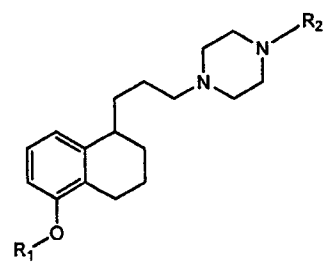
some embodiments, good metabolic stability refers to a $t_{1/2}$ > 5 min, > 10 min, > 15 minutes, > 20 minutes, and preferably > 30 min upon exposure of a compound to mouse or human hepatic microsomes. In some embodiments, good metabolic stability refers to an Intrinsic Clearance Rate (Cl_{int}) of < 300 uL/min/mg, preferably ≤ 200 uL/min/mg, and more preferably ≤ 100 uL/min/mg.

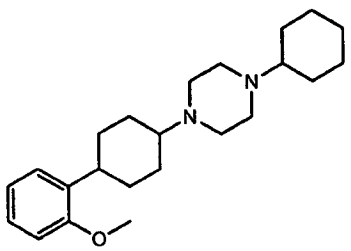
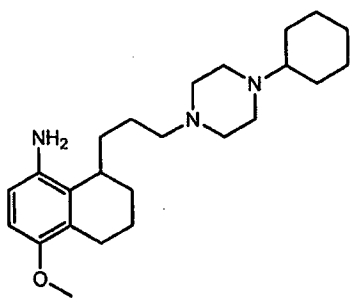
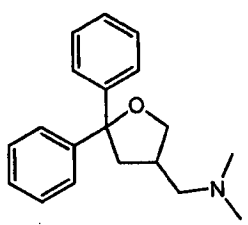
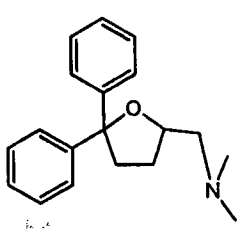
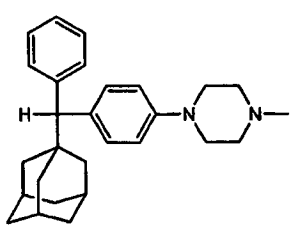
[0154] In some embodiments, excluded are certain compounds of the prior art which were not known to be sigma 2 antagonists and either (i) were known to bind to sigma 2 receptor and to reduce or eliminate Abeta induced pathologies such as a defect in membrane trafficking or synapse reduction in neuronal cells or (ii) were known to have activity against symptoms of Alzheimer's disease without implication of sigma 2 receptor interaction. In some embodiments, the compounds described in Table 1A are disclaimed with respect to compositions or methods of the disclosure, with respect to Table 1A, R, R_1, and R_2 can independently be alkyl, alkoxy, halo, halo alkyl, or halo alkoxy, and n=0-8.

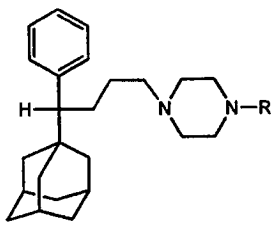
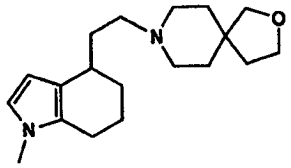
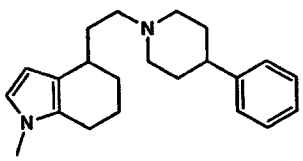
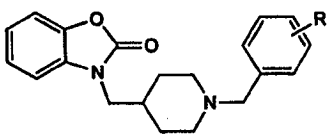
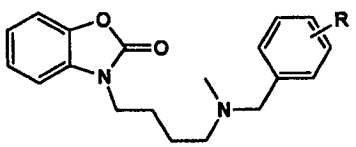
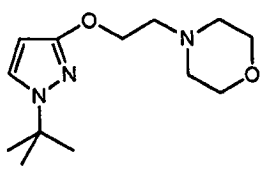
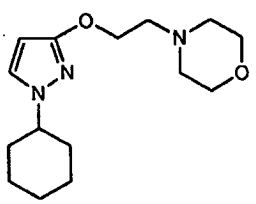
[0155] **Table 1A.** Disclaimed Compounds.

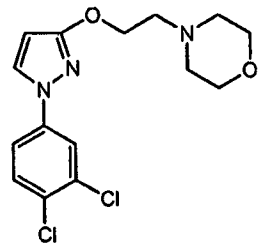
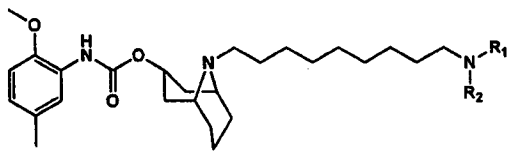
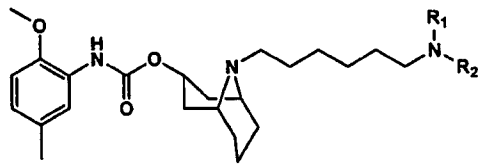
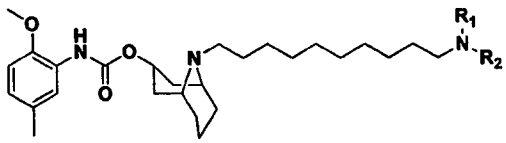
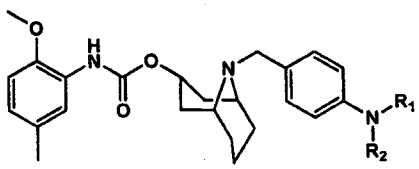
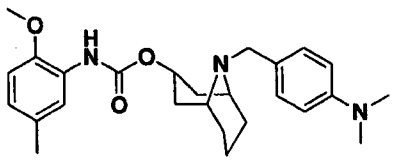
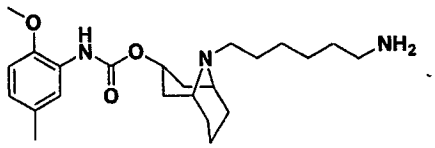
Disclaimed Compound	Reference
	CogRx Rishton, Catalano WO 2010/118055 A1 Example 2, pp. 46-47
	CogRx Rishton, Catalano WO 2010/118055 A1 Example 1, pp. 43-44

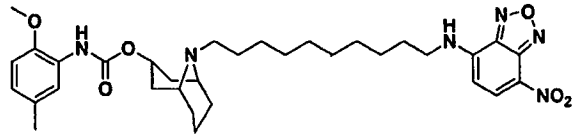
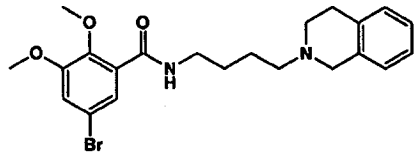
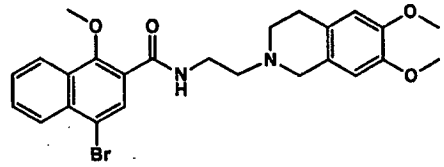
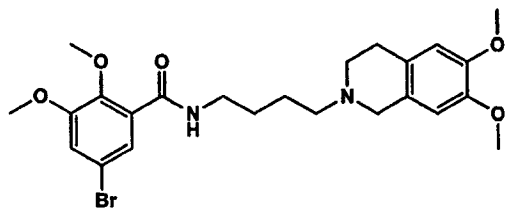
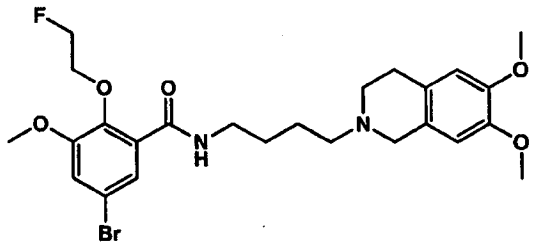
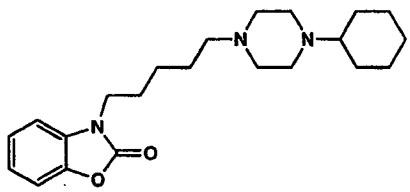
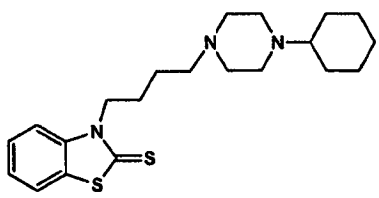
Disclaimed Compound	Reference
	CogRx Rishton, Catalano WO 2010/118055 A1 p. 32
	CogRx Rishton, Catalano WO 2010/118055 A1 p. 32
	CogRx Rishton, Catalano WO 2010/118055 A1 p. 32
	CogRx Rishton, Catalano WO 2010/118055 A1 p. 32
	CogRx Rishton, Catalano WO 2011/014880 A1 Example 1, pp. 41-42
	CogRx Rishton, Catalano WO 2011/014880 A1

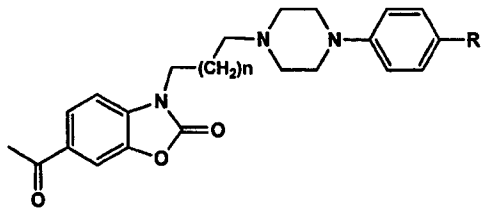
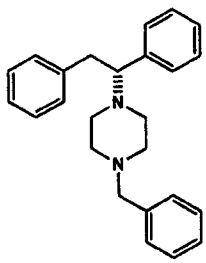
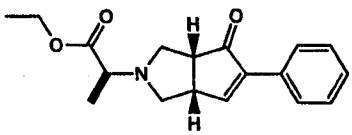
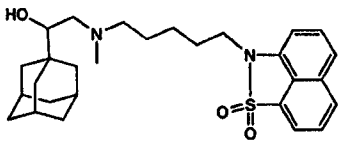
Disclaimed Compound	Reference
	Example 1, p. 42
	Rocher, Yamabe WO 2001/64670 A1 Laurini, *et al.* Bioorganic and Medicinal Chemistry Letters 20 (2010) 2954-2957
	Rocher, Yamabe WO 2001/64670 A1 Laurini, *et al.* Bioorganic and Medicinal Chemistry Letters 20 (2010) 2954-2957
	Rocher, Yamabe WO 2001/64670 A1 Laurini, *et al.* Bioorganic and Medicinal Chemistry Letters 20 (2010) 2954-2957
	Colabufo WO 2007/077543 A2
	Colabufo WO 2007/077543 A2

Disclaimed Compound	Reference
	Colabufo WO 2007/077543 A2
	Abate WO 2009/104058 A1
	Anavex US 2010/0069484 A1 Anavex WO 2008/087458 A2
	Anavex US 2010/0069484 A1 Anavex WO 2008/087458 A2
	Anavex US 2010/0069484 A1 Anavex WO 2008/087458 A2

Disclaimed Compound	Reference
	Anavex US 2010/0069484 A1 Anavex WO 2008/087458 A2
	Corbrera EP 1829862 A1 2007
	Corbrera EP 1829862 A1 2007
	Rocher, Yamabe WO 0164670 A1
	Rocher, Yamabe WO 0164670 A1
	Cuberes EP 1829867 A1 2007
	Cuberes EP 1829867 A1 2007

Disclaimed Compound	Reference
	Cuberes EP 1829867 A1 2007
	Hawkins, Mach US 2009/0176705 A1
	Hawkins, Mach US 2009/0176705 A1
	Hawkins, Mach US 2009/0176705 A1
	Hawkins, Mach US 2009/0176705 A1
	Compound WC-26 Hawkins, Mach US 2009/0176705 A1
	Compound SV-119 Hawkins, Mach US 2009/0176705 A1

Disclaimed Compound	Reference
	Compound SW-120 Hawkins, Mach US 2009/0176705 A1
	Mach US 2005/0107398 A1
	Mach US 2005/0107398 A1
	Mach US 2005/0107398 A1
	Mach US 2005/0107398 A1
	McCurdy WO 2009/026227 A2
	McCurdy JPET 333:491–500, 2010 US 2011/0280804 A1

Disclaimed Compound	Reference
	McCurdy WO 2009/026227 A2
	Mita JP 2001/26584
	Pericas WO 2007/128458 A1
	Rocher, Yamabe WO 1996/05185

[0156] In some embodiments, the compounds known as RHM-1, RHM-4, PB28, SM-21, M-14, NE100, BD1008, BD1047, fluvoxamine, PPBP, pentazocine or haloperidol are disclaimed with respect to compositions or methods of the disclosure. In certain assays, siramesine, SV-119 and WC-26 are functional sigma-2 receptor agonists. In some embodiments, the compositions and methods of the disclosure do not comprise siramesine, SV-119 or WC-26.

[0157] Sigma-2 Agonists cause Cellular Toxicity

[0158] Without being bound by theory, it is proposed that Abeta oligomers are sigma-2 receptor agonists that bind to sigma protein complexes and can cause various deleterious Abeta effects such as neuronal toxicity, aberrant trafficking and synapse loss. It is demonstrated herein that known sigma-2 receptor agonists such

as siramesine, SV-119, and WC-26, are cytotoxic to tumor cells and neurons, as exhibited by the ability to kill tumor cells and cause abnormal nuclear morphology in neurons (FIGs 9A and 9B). Sigma-2 agonists (siramesine, SV-119), although capable of blocking oligomer-induced trafficking deficits at low concentrations, cause cellular toxicity and caspase-3 activation at higher concentrations (see agonist siramesine FIG 10A and SV119 in 10B). Sigma-2 antagonists such as Compound II and IXa,IXb can block caspase-3 activation in neuronal cells caused by sigma-2 receptor agonists such as SV-119, as seen in Figure 10D. Sigma-2 antagonists block Abeta oligomer –induced trafficking deficits at all tested concentrations without causing cellular toxicity, for example, see II in FIG 11A and RHM-1 in FIG 11B.

[0159] It is herein disclosed that a high affinity, selective sigma-2 functional antagonist having the therapeutic phenotype, and good drug-like properties, can be used to treat Abeta oligomer-induced synaptic dysfunction.

[0160] In certain embodiments, the compositions of the invention comprise selective sigma-2 functional antagonists that have high binding affinity to the sigma receptors. The sigma receptors include both the sigma-1 and sigma-2 subtypes. See Hellewell, S. B. and Bowen, W. D., Brain Res. 527: 224-253 (1990); and Wu, X.-Z. et al., J. Pharmacol. Exp. Ther. 257: 351-359 (1991). A sigma receptor binding assay which quantitates the binding affinity of a putative ligand for both sigma sites (against ^{3}H-DTG, which labels both sites with about equal affinity) is disclosed by Weber et al., Proc. Natl. Acad. Sci (USA) 83: 8784-8788 (1986). Alternatively, [^{3}H]pentozocine may be used to selectively label the sigma-1 binding site in a binding assay. A mixture of [^{3}H]DTG and unlabeled (+)pentazocine is used to selectively label the sigma-2 site in a binding assay. The present invention is also directed to compositions comprising certain ligands which are selective for the sigma-1 and sigma-2 receptors and act as sigma-2 functional antagonists as well as use of these compositions to treat Abeta oligomer-induced synaptic dysfunction. The discovery of such ligands which are selective for one of the two sigma receptor subtypes may be an important factor in identifying compounds which are efficacious in treating central nervous system disorders with minimal side effects.

[0161] In some embodiments, the sigma-2 antagonist is selected from a small molecule or an antibody, or active binding fragment thereof, with high affinity for the sigma-2 receptor that has the ability to block soluble Abeta oligomer binding or Abeta oligomer-induced synaptic dysfunction.

[0162] Anti-Abeta Antibodies Several anti-Abeta monoclonal antibosies are in clinical development for the treatment of Alzheimer's disease. In some embodiments, the disclosure provides compositions comprising an sigma-2 receptor antagonist with an anti-Abeta antibody. For example, Bapineuzumab (AAB-00; Janssen, Elan, Pfizer) is an anti-β-amyloid humanized IgG_1 monoclonal antibody in Phase III clinical development for intravenous treatment of mild to moderate Alzheimer's disease. In a phase II clinical study, certain patients receiving the high dose of 2 mg/kg experienced reversible vasogenic edema. Although no significant differences were found in the primary efficacy analysis, meta-analysis showed potential treatment differences in APOE epsilon4 non-carriers. Salloway et al., 2009, *A phase 2 ascending dose trial of bapineuzumab in mild to moderate Alzheimer's disease* Neurol. 2009; 73(24):2061-70. Now undergoing phase III studies, bapineuzumab is administered at 0.5 or 1.0 mg/kg by intravenous infusion once about every 13 weeks with concurrent use of a cholinesterase inhibitor or memantidine allowed. Bapineuzumab recognizes an N-terminal epitope of Abeta: $Abeta_{1-5}$. Other anti-Aβ humanized monoclonal antibodies are in various phases of clinical development including solanezumab (LY2062430; Lilly) raised to $Abeta_{13-28}$, PF-04360365 (Pfizer) which targets $Abeta_{33-40}$; MABT5102A (Genentech); GSK933776 (GlaxoSmithKline) and gantenerumab (R1450, RO4909832, Hoffman-LaRoche). Solanezumab in secondary analysis of Phase III clinical trial results was recently reported to show statistically significant slowing of cognitive decline in patients with mild AD, but not in patient's with moderate AD. In one embodiment, a composition comprising a sigma-2 antagonist and solanezumab is used in a method for slowing cognitive decline in patients with mild AD.

[0163] It is acknowledged that peripherally administered antibodies may not have access to the tissue of interest, although passive immunization appeared to work in mice. One hypothesis was that circulating antibodies to Aβ shift the

equilibrium of the Aβ peptide from the cerebrospinal fluid to the plasma, indirectly reducing the brain's Aβ burden. Kerchner at al, 2010, *Bapineuzumab*, Expert Opin Biol Ther., 10(7):1121-1130. Alternatively, it was proposed that it may be possible that intravenously-administered antibodies may bind Aβ directly in the brain. See, e.g., Yamada et al., 2009, *Aβ immunotherapy: Intracerebral sequestration of Aβ by an anti-Ab monoclonal antibody 266 with high affinity to soluble Aβ.* J Neurosci 29(36):11393-11398. Unfortunately, thus far intravenous amyloid beta specific monoclonal antibodies have not proven particularly efficacious; for example, recently, development of intravenous bapineuzumab was ended due to lack of efficacy in two late-stage trials in patients who had mild to moderate Alzheimer's disease.

[0164] Anti-Abeta polyclonal antibodies occur naturally in pooled preparations of intravenous immunoglobulin (IVIg or IGIV), which is already FDA-approved for the treatment of other neurological conditions. At least two clinical trials using IVIg in AD are underway by Baxter and Octpharma. Kerchner et al., 2010 infra. In some embodiments, the disclosure provides methods and compositions for the treatment of cognitive decline, or Alzheimer's disease, wherein the compositions comprise a sigma-2 receptor antagonist compound and an anti-Abeta antibody and a pharmaceutically acceptable carrier.

[0165] Sigma-2 Receptor Antibodies

[0166] In some embodiments, the sigma-2 receptor antagonist compound is a sigma-2 receptor specific antibody, or active binding fragment thereof, that has the ability to block soluble Abeta oligomer binding or Abeta oligomer-induced synaptic dysfunction. In preferred embodiments, the sigma-2 antagonist antibody or immunospecific fragment thereof for use in the methods disclosed herein will not elicit a deleterious immune response in the animal to be treated, e.g., in a human. In certain embodiments, the sigma-2 antagonist antibodies or active binding fragments thereof for use in the treatment methods disclosed herein may be modified to reduce their immunogenicity using art-recognized techniques. For example, antibodies can be humanized, primatized, deimmunized, synthetic or chimeric antibodies can be

made. These types of antibodies are derived from a non-human antibody, typically a murine or primate antibody, that retains or substantially retains the antigen-binding properties of the parent antibody, but which is less immunogenic in humans. This may be achieved by various methods, for example, but not limited to, (a) grafting the entire non-human variable domains onto human constant regions to generate chimeric antibodies; (b) grafting at least a part of one or more of the non-human complementarity determining regions (CDRs) into a human framework and constant regions with or without retention of critical framework residues; (c) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues or (d) use of genetically modified mice wherein the mouse engineered to express human repertoire, for example, human immunoglobulin heavy and light chain variable domains. Such methods are disclosed in Morrison et al., Proc. Natl. Acad. Sci. 81:6851-6855 (1984); Morrison et al., Adv. Immunol. 44:65-92 (1988); Verhoeyen et al., Science 239:1534-1536 (1988); Padlan, Molec. Immun. 28:489-498 (1991); Padlan, Molec. Immun. 31:169-217 (1994), Peterson, ILAR Journal 46(3): 314-319 (2005), Lonberg, Nat. Biotechnol. 23(9): 1119-1125 (2005) and U.S. Pat. Nos. 5,585,089, 5,693,761, 5,693,762, 6,190,370, and US2012/0021409, all of which are hereby incorporated by reference in their entirety.

[0167] De-immunization can also be used to decrease the immunogenicity of an antibody. As used herein, the term "de-immunization" includes alteration of an antibody to modify T cell epitopes (see, e.g., WO9852976A1, WO0034317A2). For example, V_H and V_L sequences from the starting antibody are analyzed and a human T cell epitope "map" from each V region showing the location of epitopes in relation to complementarity-determining regions (CDRs) and other key residues within the sequence. Individual T cell epitopes from the T cell epitope map are analyzed in order to identify alternative amino acid substitutions with a low risk of altering activity of the final antibody. A range of alternative V_H and V_L sequences are designed comprising combinations of amino acid substitutions and these sequences are subsequently incorporated into a range of binding polypeptides, e.g., sigma-2 antagonist antibodies or immunospecific fragments thereof for use in the methods

disclosed herein, which are then tested for function. Typically, between 12 and 24 variant antibodies are generated and tested. Complete heavy and light chain genes comprising modified V and human C regions are then cloned into expression vectors and the subsequent plasmids introduced into cell lines for the production of whole antibody. The antibodies are then compared in appropriate biochemical and biological assays, and the optimal variant is identified.

[0168] Sigma-2 antagonist antibodies or fragments thereof for use in the methods of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies can be produced by various procedures well known in the art. For example, a sigma-2 polypeptide fragment can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum. Such adjuvants are also well known in the art.

[0169] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 2nd ed. (1988); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas Elsevier, N.Y., 563-681 (1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Thus, the term "monoclonal antibody" is not limited to

antibodies produced through hybridoma technology. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma and recombinant and phage display technology.

[0170] In some embodiments, the sigma-2 antagonists, such as monoclonal antibodies, or active binding fragments thereof, specific for the sigma-2 receptor, can be engineered to enhance the ability to cross the blood-brain barrier using any available technique known in the art. Recombinant protein therapeutics cannot generally be employed for brain delivery since they will not cross the blood brain barrier; however, techniques are known in the art for brain delivery of biologic therapeutics. For example, see Pardridge and Boado, Reengineering biopharmaceuticals for targeted delivery across the blood-brain barrier, Methods Enzymol. 2012; 503:269-292, incorporated herein by reference. Pardridge and Boado report recombinant proteins can be reengineered as BBB-penetrating IgG fusion proteins, where the IgG part is a genetically engineered monoclonal antibody (MAb) against an endogenous BBB receptor, such as the human insulin receptor (HIR) or the transferrin receptor (TfR). The IgG binds the endogenous insulin receptor or TfR to trigger transport across the BBB and acts as a molecular Trojan horse (MTH) to ferry into brain the fused protein therapeutic. The pharmacokinetic (PK) properties of the IgG fusion proteins differ from that of typical MAb drugs and resemble the PK profiles of small molecules due to rapid uptake by peripheral tissues, as well as brain. The brain uptake of the IgG fusion proteins, 2-3% of injected dose/brain, is comparable to the brain uptake of small molecules. The IgG fusion proteins have been administered chronically in mouse models, and the immune response is low titer and has no effect on the fusion protein clearance from blood or brain uptake in vivo. For example, Zhou et al used the "Trojan horse" to re-engineer an anti-Abeta amyloid antibody (AAA) as a fusion protein with a blood-brain barrier (BBB) molecular Trojan horse. The AAA was engineered as a single-chain Fv (ScFv) antibody, and the ScFv was fused to the heavy chain of a chimeric monoclonal antibody (Mab) against the mouse transferrin receptor (TfR). The cTfRMAb-ScFv protein penetrates mouse brain from blood via transport on the BBB TfR, and the brain uptake is 3.5 % of injected dose/gram brain following an

intravenous administration. Zhou et al., Receptor-mediated Abeta Amyloid Antibody Targeting to Alzheimer's Disease Mouse Brain. Mol. Pharm. 2011, Feb 7; 8(1):280-285. The BBB MTH technology enables the reengineering of a wide spectrum of recombinant protein therapeutics for targeted drug delivery to the brain.

[0171] In some embodiments, the antibodies can be engineered to enhance brain uptake by the method of Yu et al., 2011. Antibodies targeting the transferrin receptor, which is highly expressed by endothelial cells that make up the BBB, have been reported to cross the BBB by receptor-mediated transcytosis. One problem with this approach is that high affinity antibodies targeting the transferrin receptor might reduce the probability of antibody being released from the CNS vasculature. Yu et al designed antibodies with low affinity for transferrin to increase release of antibody from brain vascular endothelium and to enhance uptake and distribution to the brain. Yu reported that lower-affinity ant-TfR antibodies show increased brain uptake. In some embodiments, the anti-sigma-2 receptor antibody is a bispecific antibodywith one arm comprising a low-affinity anti-transferrin receptor antibody and the other arm comprising a high-affinity anti-sigma-2 receptor antibody or anti-PGRMC1 antibody by the method of Y. Joy Yu et al., Sci Transl Med 3, 84ra44(2011), and US2012/0171120, each of which is incorporated herein by reference. →

[0172] In some embodiments, the sigma-2 antagonist is selected from any anti-PGRMC1 antibody, or from any antibody, or fragment thereof, that is specific for binding the sigma-2 receptor and that also blocks Abeta oligomer binding or Abeta oligomer-induced synaptic dysfunction or that acts as a functional neuronal antagonist, or that blocks Abeta oligomer binding and Abeta effects.

[0173] In some embodiments, the sigma-2 receptor antibody or binding fragment thereof can be reengineered as BBB-penetrating IgG fusion protein, or conjugate, where the IgG part is a genetically engineered monoclonal antibody (MAb) against an endogenous BBB receptor, such as the human insulin receptor (HIR) or the transferrin receptor (TfR). Conjugates of anti-sigma-2 receptor antibodies, or fragments thereof, to HIR or TfR Mabs via, for example, chitosan nanoparticles, or poly-malic acid conjugates. See, e.g., Yemisci et al., Transport of

a caspase inhibitor across the blood-brain barrier by chitosan nanoparticles, Methods Enzymol., 2012; 508:243-269; and Ding et al., Inhibition of brain tumor growth by intravenous poly(b-L-malic acid) nanobioconjugate with a pH-dependent drug release. PNAS, 2010 107(42) 18143-18148, each of which is incorporated herein by reference. In some embodiments, single domain antibodies that are raised against receptors that undergo cytosis across the blood-brain barrier are employed in fusion proteins or conjugates. See for example, Abulrob et al., J Neurochem 2005, 95(4):1201-121, incorporated herein by reference.

[0174] In some embodiments, the anti-sigma-2 receptor antibody is raised to an epitope of human membrane –associated progesterone receptor component 1 (human PGRMC1) or an isoform, homolog, variant, extracellular domain or fragment thereof. For example, one protein sequence of human PGRMC1 is a 195 amino acid (aa) protein; GI:48146103:

maaedvvatg adpsdlesgg llheiftspl nllllglcif llykivrgdq paasgdsddd

eppplprlkr rdftpaelrr fdgvqdpril maingkvfdv tkgrkfygpe gpygvfagrd

asrglatfcl dkealkdeyd dlsdltaaqq etlsdwesqf tfkyhhvgkl lkegeeptvy

sdeeepkdes arknd SEQ ID NO: 1.

[0175] For example, progesterone receptor membrane component 1, isoform CRA_a [Homo sapiens], a 195 aa protein; GI:119610285:

maaedvvatg adpsdlesgg llheiftspl nllllglcif llykivrgdq paasgdsddd

eppplprlkr rdftpaelrr fdgvqdpril maingkvfdv tkgrkfygpe gpygvfagrd

asrglatfcl dkealkdeyd dlsdltaaqq etlsdwesqf tfkyhhvgkl lkegeeptvy

sdeeepkdes arknd SEQ ID NO: 2.

[0176] For example, progesterone receptor membrane component 1, isoform CRA_b [Homo sapiens], a 170 aa protein; GI:119610286:

maaedvvatg adpsdlesgg llheiftspl nllllglcif llykivrgdq paasgdsddd

eppplprlkr rdftpaelrr fdgvqdpril maingkvfdv tkgrkfygpe gpygvfagrd

asrglatfcl dkemrknqkm rvpgkmikaf sgsisifvfc kiicnsplcl

SEQ ID NO: 3.

[0177] For example, progesterone receptor membrane component 1, isoform CRA_c [Homo sapiens], a 143 aa protein; GI:119610287:

maaedvvatg adpsdlesgg llheiftspl nllllglcif llykivrgdq paasgdsddd

eppplprlkr rdftpaelrr fdgvqdpril maingkvfdv tkgrkfygpv kyhhvgkllk

egeeptvysd eeepkdesar knd SEQ ID NO: 4.

[0178] Homologs of human PGRMC1 include, e.g.,rat PGRMC1. For example a rat PGRMC1, a 243 aa protein; GI:11120720:

maaedvvatg adpseleggg llqeiftspl nllllglcif llykivrgdq pgasgdnddd

eppplprlkp rdftpaelrr ydgvqdpril maingkvfdv tkgrkfygpe gpygvfagrd

asrglatfcl dkealkdeyd dlsdltpaqq etlndwdsqf sspsstitwg kllegaeepi

vysddeeqkm rllgrvteav sgaylflyfa ksfvtfqsvf ttw SEQ ID NO: 5.

[0179] Another homolog is rat PGRMC1, a 195 aa protein; GI:38303845:

maaedvvatg adpseleggg llqeiftspl nllllglcif llykivrgdq pgasgdnddd

eppplprlkp rdftpaelrr ydgvqdpril maingkvfdv tkgrkfygpe gpygvfagrd

asrglatfcl dkealkdeyd dlsdltpaqq etlndwdsqf tfkyhhvgkl lkegeeptvy

sddeepkdea arksd SEQ ID NO: 6.

[0180] In some embodiments, the specific sigma-2 receptor antagonist compound is an anti-sigma-2 receptor antibody that blocks binding between soluble Abeta oligomers and a sigma-2 receptor. In some embodiments, the anti-sigma-2 receptor antibodies recognize an epitope corresponding to an amino acid sequence of a PGRMC1 protein. In some embodiments, the sigma-2 receptor specific

antibody may be specific for an epitope corresponding to an amino acid sequence derived from an N-terminal sequence, C-terminal sequence, internal sequence, or full length protein corresponding to PGRMC1. In embodiments, the sigma-2 receptor specific antibody may be specific for binding to one or more of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 9, or 10. In some embodiments, the specific sigma-2 receptor antagonist compound is an anti-PGRMC1 antibody recognizing the synthetic peptide: EPKDESARKND (SEQ ID NO: 7), corresponding to C terminal amino acids 185-195 of human PGRMC1. In some embodiments, sigma-2 receptor antagonist compound is not an antibody specific for residues 1-46 at the N-terminus of human PGRMC1 protein (MAAEDVVATGADPSDLESGGLLHEIFTSPLNLLLLGLCIFLLYKI (SEQ ID NO: 9), #sc-98680, Santa Cruz),

[0181] In some embodiments, the anti-sigma-2 receptor antibodies include those raised against, or in any event recognizing, any known full length PGRMC1 protein, or any variant, fragment, immunogen or epitope thereof; including an N-terminal, central fragment, or C-terminal region of PGRMC1, or homolog, immunogen or variant thereof. Isolated, purified, or synthetic proteins or peptides can be employed as immunogens. The proteins or fragments are optionally adjuvanted and or conjugated by various means known in the art to enhance immunogenicity. Synonyms for PGRMC1 include progesterone binding protein, HPR6.6; HGNC:16090, progesterone receptor membrane binding component 1, and MPR. In one embodiment, the fragment or epitope is EPKDESARKND SEQ ID NO: 7, corresponding to C terminal amino acids 185-195 of Human PGRMC1. This fragment was used to raise commercially-available goat anti-human PGRMC1 polyclonal antibodies (e.g., Abcam ab48012; Sigma-Aldrich SAB2500782; and Everest Biotech, Ltd. EB07207). Another fragment consists of residues 50-150 of human PGRMC1, taaqq etlsdwesqf tfkyhhvgkl lkegeeptvy sdeeepkdes arknd (SEQ ID NO: 10); this fragment was conjugated to KLH by means known in the art; rabbit anti-PGRMC1 polyclonal antibodies were generated; commercially available as Abcam ab88948. Other commercially-available antibodies include Santa Cruz Biotechnology sc-98680 (goat anti-human PGRMC1 polyclonal antibodies raised

against N-terminus aa 1-46, (MAAEDVVATG ADPSDLESGG LLHEIFTSPL NLLLLGLCIF LLYKIV (SEQ ID NO: 9); sc-82694 (goat anti-human PGRMC1 polyclonal antibodies raised against an internal epitope); sc-133906 (rabbit anti-human PGRMC1 polyclonal antibodies raised against synthetic PGRMC1 peptide); sc-135720 (PGRMC1 (12B7) mouse Mab); sc-271275 (PGRMC1(c-3) mouse Mab raised against N-terminal 1-46); and Sigma-Aldrich HPA002877 anti-PGRMC1 rabbit polyclonal antibodies raised against Membrane-associated progesterone receptor component 1 recombinant protein epitope signature tag (PrEST).

[0182] Other antibodies raised against sigma-1 receptor (opioid receptor, sigma-1; Oprs 1) proteins, fragments, epitopes or immunogens are employed in the examples provided herein. Such anti-sigma-1 receptor antibodies include Thermo Scientific PAS-12326 (rabbit anti-sigma-1 receptor polyclonal antibodies raised to N-terminal region of OPRS1 conjugated to KLH); Santa Cruz Biotechnology, Inc. sigma receptor (L-20) sc-16203, goat anti-human raised to an internal region of sigma-1 receptor); Santa Cruz Biothechnology, Inc. sigma receptor (FL-223) sc-20935 raised to rabbit anti-human full length sigma receptor aa 1-223; Santa Cruz Biotechnology, Inc. sigma receptor (S-18) sc-22948 goat anti-human polyclonal antibodies raised against and internal region of human sigma receptor; Santa Cruz Biotechnology, Inc. sigma receptor (B-5) sc-137075 a mouse monoclonal antibody (Mab) specific for an epitope mapping between amino acids 136-169 of an internal region of human sigma-1 receptor; and Santa Cruz Biotechnology, Inc. sigma receptor (F-5) a mouse monoclonal antibody raised against amino acids 1-223 full length human sigma-1 receptor.

[0183] The human Sigma-1 receptor is a 223 aa protein; GI:74752153:

mqwavgrrwa waalllavaa vltqvvwlwl gtqsfvfqre eiaqlarqya gldhelafsr

livelrrlhp ghvlpdeelq wvfvnaggwm gamcllhasl seyvllfgta lgsrghsgry

waeisdtiis gtfhqwregt tksevfypge tvvhgpgeat avewgpntwm veygrgvips

tlafaladtv fstqdfltlf ytlrsyargl rlelttylfg qdp SEQ ID NO: 8.

[0184] In some embodiments, any sigma-1 receptor full length protein, homolog variant, or fragment, including N-terminal, C-terminal, central regions, can be employed to raise antibodies as sigma-1 receptor antagonists by any method known in the art.

[0185] In some embodiments, the sigma-2 antagonist is a small molecule compound with high affinity for the sigma-2 receptor.

[0186] Sigma-2 Receptor Ligands for Selection as Sigma-2 Receptor Antagonists

[0187] In some embodiments, sigma-2 receptor antagonists for use in the present invention are selected from among sigma-2 receptor ligand compounds that also meet additional selection criteria. Additional criteria are used to select sigma-2 receptor antagonists for use in the present invention from among sigma-2 receptor ligands. Additional selection criteria include: acting as a functional antagonist 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; having high selectivity for one or more sigma receptors compared to any other non-sigma receptor; exhibiting high affinity at a sigma-2 receptor; and exhibiting good drug-like properties including good brain penetrability, good metabolic stability and good plasma stability. In some embodiments, the sigma-2 receptor antagonist is further selected on the basis of exhibiting one or more of the additional following properties: 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.

[0188] In some embodiments, certain sigma-2 receptor ligand compounds subject to further selection criteria are selected from compounds described herein and can be synthesized according to the methods described herein or in WO 2011/014880 (Application No. PCT/US2010/044136), WO 2010/118055 (Application No. PCT/US2010/030130), Application No. PCT/US2011/026530, and

WO2012/106426, each of which is incorporated herein by reference in its entirety. Additional options for preparing these compounds are discussed in detail below.

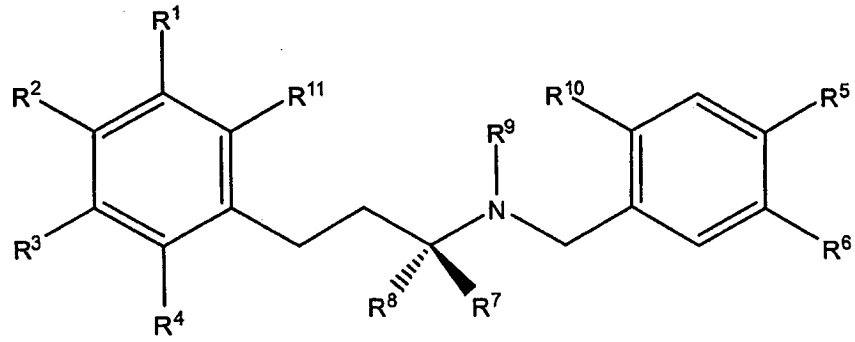
[0189] In some embodiments, the sigma-2 ligand is an optionally substituted piperazine, phenyltetrahydrofuran-N,N-dimethylmethanamine, diphenyltetrahydrofuran-N,N-dimethylmethanamine, a 4-phenylpentyl-piperazine, benzylphenyl-piperazine, indole-oxa-azaspiro-decane, piperadine-indole, phenylpiperadine-indole, pyrazole-morpholine, pyrazole-piperadine, pyrazol-N,N-diethylethanamine, pyrazole-pyrrolidine, phenyl-pyrazol-morpholine, benzamide-quinoline compound, or derivatives thereof.

[0190] In some embodiments, the sigma-2 ligand is an optionally substituted, N-(4-(3,4-dihydroisoquinolin-2(1H)-yl)butyl)benzamide, 1-cyclohexyl-4-(3-(1,2,3,4-tetrahydronaphthalen-1-yl)propyl)piperazine, (5,5-diphenyltetrahydrofuran-3-yl)methanamine, 1-(5,5-diphenyltetrahydrofuran-3-yl)-N,N-dimethylmethanamine,N,N-dimethyl-1-(5-phenyltetrahydrofuran-3-yl)methanamine, 1-(4-phenylbutyl)piperazine,1-(4-benzylphenyl)-4-methylpiperazine, cyclohexyl-4-(4-phenylcyclohexyl)piperazine, 1-cyclohexyl-4-(4-phenylcyclohexyl)piperazine, cyclohexyl-4-(3-(1,2,3,4-tetrahydronaphthalen-1-yl)propyl)piperazine, 1-cyclohexyl-4-(3-(1,2,3,4-tetrahydronaphthalen-1-yl)propyl)piperazine, 8-(2-(4,5,6,7-tetrahydro-1H-indol-4-yl)ethyl)-2-oxa-8-azaspiro[4.5]decane, 4-(2-(4-phenylpiperidin-1-yl)ethyl)-4,5,6,7-tetrahydro-1H-indole, 4-(2-(1H-pyrazol-3-yloxy)alkyl)morpholine, 4-(2-(1H-pyrazol-3-yloxy)ethyl)morpholine, 1-(2-(1H-pyrazol-3-yloxy)ethyl)piperidine,1-(2-(1H-pyrazol-3-yloxy)alkyl)piperidine, 2-(1H-pyrazol-3-yloxy)-N,N-diethylethanamine, 2-(1H-pyrazol-3-yloxy)-N,N-dialkyl-alkanamine, 3-(2-(pyrrolidin-1-yl)ethoxy)-1H-pyrazole, 3-(2-(pyrrolidin-1-yl)alkoxy)-1H-pyrazole,1-(1H-pyrazol-3-yloxy)-3-(piperazin-1-yl)propan-2-ol, 1-(1H-pyrazol-3-yloxy)-3-(piperazin-1-yl)alkan-2-ol, 1-(1-aryl-1H-pyrazol-3-yloxy)-3-(piperazin-1-yl)alkan-2-ol, 1-(1-phenyl-1H-pyrazol-3-yloxy)-3-(piperazin-1-yl)alkan-2-ol, 1-(1-aryl-1H-pyrazol-3-yloxy)-3-(piperazin-1-yl)propan-2-ol, 1-(1-heteroaryl-1H-pyrazol-3-yloxy)-3-(piperazin-1-yl)alkan-2-ol, 1-(1-(dihaloaryl)-1H-pyrazol-3-yloxy)-3-(piperazin-1-yl)alkan-2-ol, 1-(1-dihaloheteroaryl)-1H-pyrazol-3-yloxy)-3-(piperazin-1-yl)alkan-2-ol, 1-(1-(3,4-dihaloaryl)-1H-pyrazol-3-yloxy)-3-(piperidin-1-yl)alkan-2-ol,

1-(1-(3,4-dihaloheteroaryl)-1H-pyrazol-3-yloxy)-3-(piperidin-1-yl)alkan-2-ol, 1-(1-(dihaloaryl)-1H-pyrazol-3-yloxy)-3-(dialkylamino)alkan-2-ol, 1-(1-(dihaloheteroaryl)-1H-pyrazol-3-yloxy)-3-(dialkylamino)alkan-2-ol, 1-(1-(dichloroheteroaryl)-1H-pyrazol-3-yloxy)-3-(dialkylamino)alkan-2-ol, N-(4-(3,4-dihydroisoquinolin-2(1H)-yl)butyl)benzamide, N-(4-(3,4-dihydroisoquinolin-2(1H)-yl)alkyl)benzamide, N-(4-(3,4-dihydroisoquinolin-2(1H)-yl)alkyl)-2-hydroxybenzamide, N-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)benzamide, N-(4-(6,7-dialkoxy-3,4-dihydroisoquinolin-2(1H)-yl)alkyl)benzamide, N-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2-naphthamide, N-(4-(6,7-dialkoxy-3,4-dihydroisoquinolin-2(1H)-yl)alkyl)-2-naphthamide, N-(4-(3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2-naphthamide, N-(4-(3,4-dihydroisoquinolin-2(1H)-yl)alkyl)-2-naphthamide, N-(2-(3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-2,3-dimethoxybenzamide, N-(2-(3,4-dihydroisoquinolin-2(1H)-yl)alkyl)-2,3-dialkoxybenzamide, N-(2-(3,4-dihydroisoquinolin-2(1H)-yl)ethyl)benzamide, N-(2-(3,4-dihydroisoquinolin-2(1H)-yl)alkyl)benzamide, N-(2-(4-(2,3-dichlorophenyl)piperidin-1-yl)ethyl)-2-naphthamide, N-(2-(4-(2,3-dihaloaryl)piperidin-1-yl)alkyl)-2-naphthamide, 2,3-dimethoxy-N-(4-(4-phenylpiperidin-1-yl)butyl)benzamide, 2,3-dialkoxy-N-(4-(4-phenylpiperidin-1-yl)alkyl)benzamide, N-(4-(4-(2,3-dihalophenyl)piperidin-1-yl)alkyl)-2,3-dialkoxybenzamide, 5-halo-N-(4-(4-(2,3-dihalophenyl)piperidin-1-yl)alkyl)-2,3-dialkoxybenzamide (wherein the 2, 3, & 5 position halo are the same or independently F, Cl, Br, or I), N-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2-(2-fluoroethoxy)-5-iodo-3-methoxybenzamide, N-(4-(6,7-dialkoxy-3,4-dihydroisoquinolin-2(1H)-yl)alkyl)-2-(2-haloethoxy)-5-halo-3-methoxybenzamide (wherein the halo substituents are the same or independently F, Cl, Br, or I), 9-benzyl-9-azabicyclo[3.3.1]nonan-3-yl phenylcarbamate, 9-benzyl-9-azabicyclo[3.3.1]nonan-3-yl 2-alkoxyphenylcarbamate, 9-(5-phenylalkyl)-9-azabicyclo[3.3.1]nonan-3-yl 2-methoxy-5-methylphenylcarbamate, 9-alkyl-9-azabicyclo[3.3.1]nonan-3-yl phenylcarbamate, 3-(2-(4-cyclohexylpiperazin-1-yl)alkyl)benzo[d]oxazol-2(3H)-one, 6-acetyl-3-(4-(4-cyclohexylpiperazin-1-yl)alkyl)benzo[d]oxazol-2(3H)-one, 1-benzyl-4-(1,2-diphenylethyl)piperazine, 1-aryl-4-(1,2-diphenylethyl)piperazine, ethyl 2-(6-oxo-5-phenyl-3,3a,6,6a-

tetrahydrocyclopenta[c]pyrrol-2(1H)-yl)propanoate, ethyl 2-(5-alkyl-6-oxo-3,3a,6,6a-tetrahydrocyclopenta[c]pyrrol-2(1H)-yl)propanoate, 2-((3-(2H-naphtho[1,8-cd]isothiazol-2-yl)alkyl)(methyl)amino)alkanol, 2-((1-(2-(1-alkyl-1H-pyrrol-2-yl)-2-oxoethyl)piperidin-4-yl)alkyl)isoindolin-1-one, 2-((1-(2-oxoaklyl)piperidin-4-yl)alkyl)isoindolin-1-one, 1'-(4-(1-(4-haloaryl)-1H-indol-3-yl)alkyl)-3H-spiro[isobenzofuran-1,4'-piperidine], 1'-(4-(1-(4-haloheteroaryl)-1H-indol-3-yl)alkyl)-3H-spiro[isobenzofuran-1,4'-piperidine], 3-(3-alkylbut-2-alkynyl)-1,2,3,4,5,6-hexahydrobenzo[d]azocin-8-ol, pentazocine compound, or analogues or derivatives thereof.

[0191] In some embodiments, the sigma-2 ligand comprises a compound of Formula I:



I

or a pharmaceutically acceptable salt thereof, wherein:

R^1, R^2, R^3, R^4, and R^{11} are each independently selected from H, OH, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and NH(C_{1-4} alkyl);

R^5 and R^6 are each independently selected from H, C_{1-6} haloalkyl, C_{1-6} alkyl, and C_{3-7} cycloalkyl, and NH(C_{1-4} alkyl);

R^7 and R^8 are each independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, and C_{3-7} cycloalkyl;

or R^2 and R^3 together with the C atom to which they are attached form a 4- to 8-membered cycloalkyl, aryl, heteroaryl, heteroarylalkyl, or heterocycloalkyl that is

optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, and heterocycloalkyl and R^2 and R^3 are each independently selected from a bond, C, N, S, and O;

or R^9 and R^{10} together with the N and C atoms to which they are attached form a 4- to 8-membered heterocycloalkyl or heteroaryl group that is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, and heterocycloalkyl and R^9 and R^{10} are each independently selected from a bond, C, N, S, and O;

or R^9 and R^{11} together with the N and C atoms to which they are attached form a 6- to 8-membered heterocycloalkyl or heteroaryl group that is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, and heterocycloalkyl and R^9 and R^{11} are each independently selected from a bond, C, N, S, and O;

or R^1 and R^{11} together with the C atom to which they are attached form a 4-, 5-, 6- 7-or 8- membered cycloalkyl, heterocycloalkyl, aryl, or heteroaryl group that is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, and heterocycloalkyl and R^1 and R^{11} are each independently selected from a bond, C, N, S, and O;

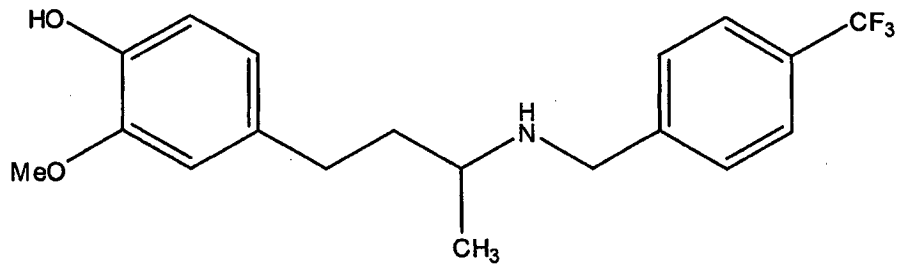
or R^1 and R^2 together with the C atom to which they are attached form a 4-, 5-, 6- 7-or 8- membered cycloalkyl, heterocycloalkyl, aryl, or heteroaryl group that is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, and heterocycloalkyl and R^1 and R^2 are each independently selected from a bond, C, N, S, and O;

or R^3 and R^4 together with the C atom to which they are attached form a 4-, 5-, 6- 7-or 8- membered cycloalkyl, heterocycloalkyl, aryl, or heteroaryl group that

is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, and heterocycloalkyl and R^3 and R^4 are each independently selected from a bond, C, N, S, and O;

wherein each of the O, C_{1-6} alkyl, C_{1-6} haloalkyl, heteroaryl, aryl, heteroaryl, heterocycloalkyl, and cycloalkyl is optionally independently substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl and heterocycloalkyl.

[0192] In some embodiments, the sigma-2 ligand comprises a racemic mixture or an enantiomer of compound II



II.

[0193] In some embodiments, the sigma-2 ligand is a compound or a pharmaceutically acceptable salt of Formula I, wherein R^2 and R^3 are independently selected from OH and C_{1-6} alkoxy.

[0194] In some embodiments, the sigma-2 ligand is a compound or a pharmaceutically acceptable salt of Formula I, wherein R^2 and R^3 are independently selected from OH and NH(C_{1-4} alkyl).

[0195] In some embodiments, the sigma-2 ligand is a compound or a pharmaceutically acceptable salt of Formula I, wherein R^2 and R^3 are independently selected from H, halo, and C_{1-6} haloalkyl.

[0196] In some embodiments, the sigma-2 ligand is a compound or a pharmaceutically acceptable salt of Formula I, wherein R^2 and R^3 are each

independently selected from OH and C_{1-6} alkoxy and R^7 and R^8 are each independently C_{1-6} alkyl. In some embodiments, R^7 and R^8 are each methyl.

[0197] In some embodiments, the sigma-2 ligand is a compound or a pharmaceutically acceptable salt of Formula I, wherein R^5 and R^6 are each independently selected from H and C_{1-6} haloalkyl.

[0198] In some embodiments, the sigma-2 ligand is a compound or a pharmaceutically acceptable salt of Formula I, wherein R^9 is H.

[0199] In some embodiments, the sigma-2 ligand is a compound or a pharmaceutically acceptable salt of Formula I, wherein R^2 and R^3 or R^3 and R^4 together with the C atom to which they are attached form a 6-membered cycloalkyl, cycloheteroalkyl, aryl or heteroaryl ring. In some embodiments R^2 and R^3 are O.

[0200] In some embodiments, the sigma-2 ligand is a compound or a pharmaceutically acceptable salt of Formula I, wherein R^7 is C_{1-6} alkyl and R^8 is H.

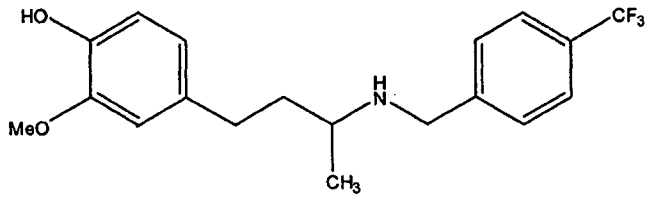
[0201] In some embodiments, the sigma-2 ligand is a compound or a pharmaceutically acceptable salt of Formula I, wherein R^7 is H and R^8 is C_{1-6} alkyl.

[0202] In some embodiments, the sigma-2 ligand is a compound or a pharmaceutically acceptable salt of Formula I, wherein R^2 and R^3 are independently selected from H, OH, halo, C_{1-6} alkoxy and C_{1-6} haloalkyl.

[0203] In some embodiments, the sigma-2 ligand is a compound or a pharmaceutically acceptable salt of Formula I, wherein R^2 and R^3 are independently selected from H, OH, Cl, F, -OMe, and $-CF_3$.

[0204] In some embodiments, the sigma-2 ligand is a compound or a pharmaceutically acceptable salt of Formula I, wherein R^2 and R^3 are independently selected from H, OH, Cl, F, -OMe, and $-CF_3$, wherein R^7 and R^8 are each independently selected from H and C_{1-6} alkyl, wherein R^9 is H, and wherein R^5 and R^6 are each independently selected from H and C_{1-6} haloalkyl.

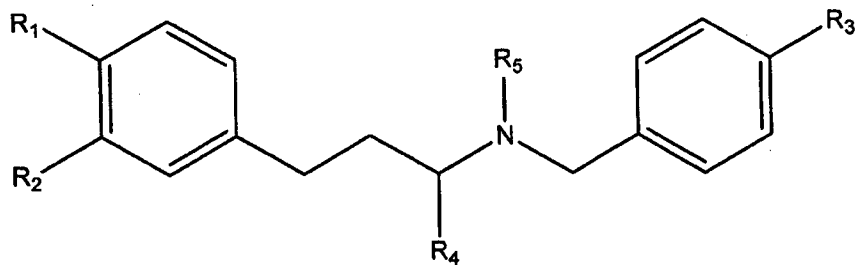
[0205] In some embodiments, the compound of Formula I is a substantially pure (+) or (-) enantiomer of :

[0206] 

Compound II,

wherein the substantially pure enantiomer comprises at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% of one enantiomer of compound II. In some embodiments, a composition comprising a substantially pure enantiomer of compound II is at least 99.5% one enantiomer, and in other embodiments, the composition comprises only one enantiomer of compound II.

[0207] In one more specific embodiment, the sigma-2 ligands of the present invention are the novel compounds represented by Formula III:



III

wherein

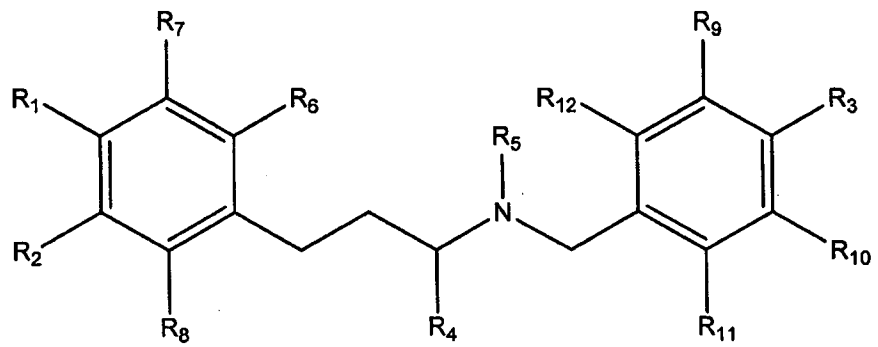
R_1 and R_2 are independently selected from H, OH, halo, CN, NO_2, NH_2, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{3-7} cycloalkyl, $NH(C_{1-4}$ alkyl), $N(C_{1-4}$ alkyl)$_2$, $NH(C_{3-7}$ cycloalkyl), $NHC(O)(C_{1-4}$ alkyl), (C_{1-4} alkyl)$_2$N-C_{1-4} methylene-O-, 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_{1-4} methylene-O- group, and wherein at least one of R1 and R2 is not H;

R_3 is 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), C(O)OH, $C(O)O(C_{1-4}$ alkyl), $C(O)(C_{1-4}$ alkyl), and $C(O)NH(C_{1-4}$ alkyl);

R_4 is 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), and $C(O)(C_{1-4}$haloalkyl).

[0208] In another more specific embodiment, the sigma-2 ligands of the present invention are the novel compounds represented by Formula IV:



IV

wherein

R_1, R_2, R_6, R_7 and R_8 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_6, R_7 and R_8 is not H;

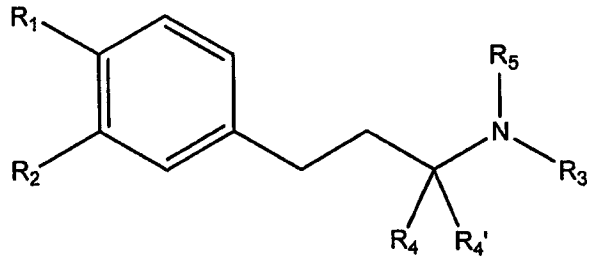
R_3 is selected from H, halo, and C_{1-6} haloalkyl;

R_9, R_{10}, R_{11}, and R_{12} are independently selected from H, C_{1-6} alkoxy and halo.

R_4 is 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), C(O)(C_{1-4}haloalkyl).

[0209] In some embodiments, the sigma-2 ligands of the present invention are those of Formula Va

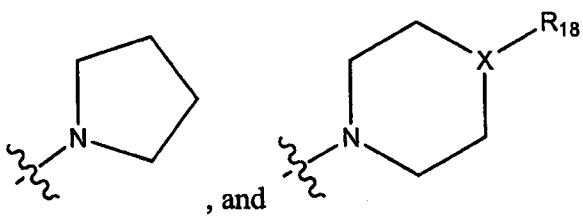


Va

wherein

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

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

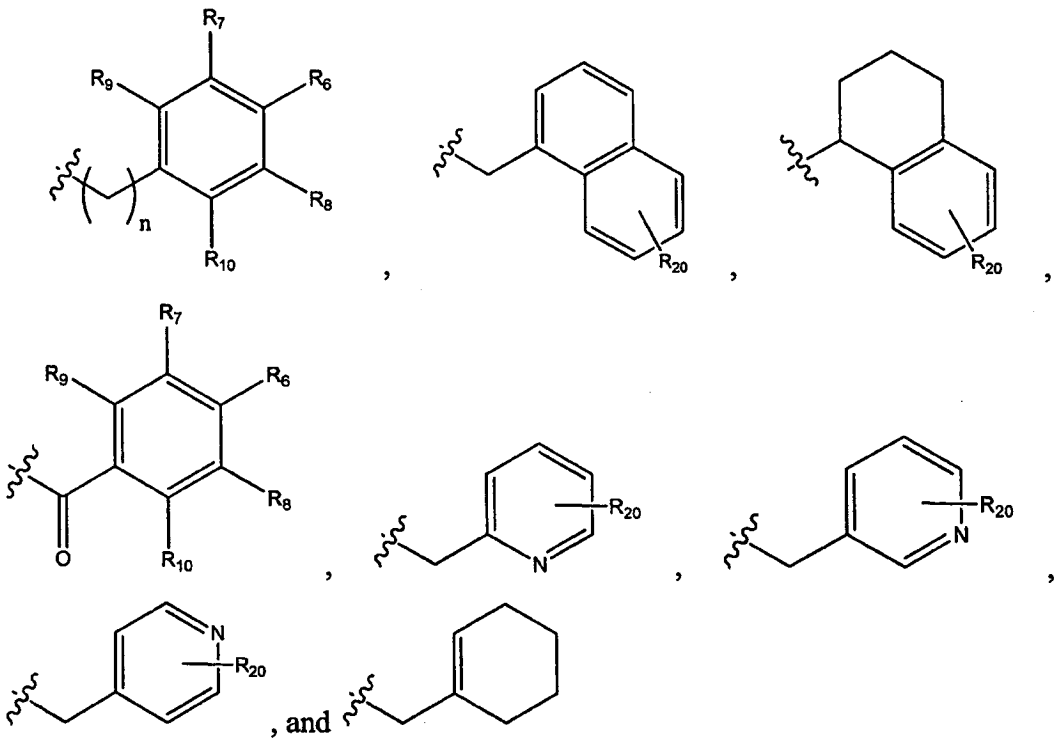


, and , wherein

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

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

R_3 is selected from



wherein

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

R_{20} is H; and

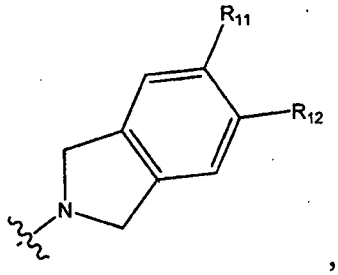
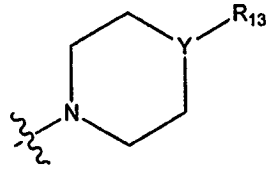
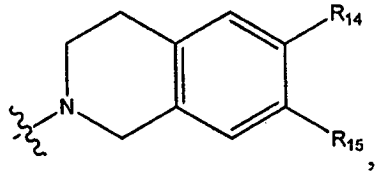
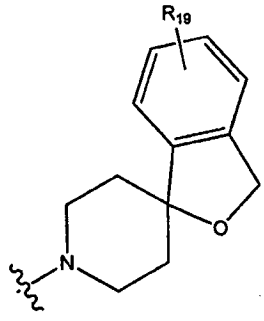
n is 1-4

R_4 is C_{1-6} alkyl;

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

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

R_3 and R_5 together with nitrogen form a ring selected from

and , wherein

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

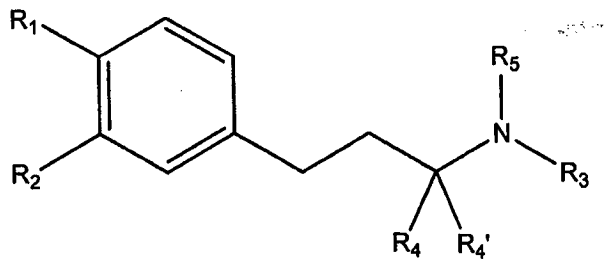
Y is CH or N;

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

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

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

[0210] In some embodiments, the sigma-2 ligands of the present invention are those of Formula Va

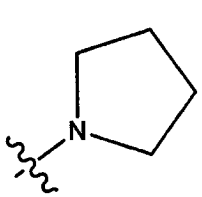


Va

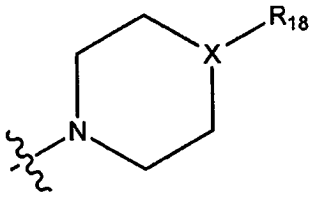
wherein

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

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



, and

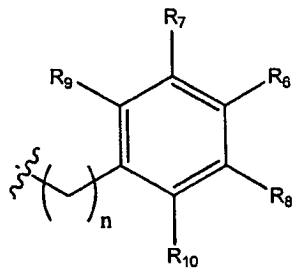


, wherein

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

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

R_3 is selected from



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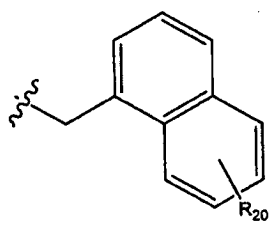

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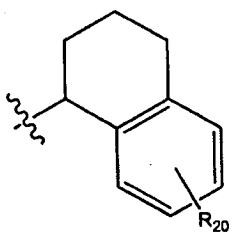

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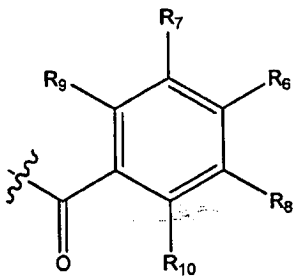

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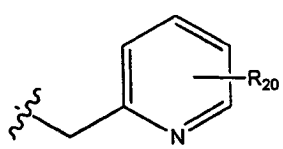

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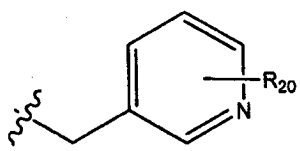

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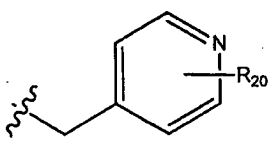

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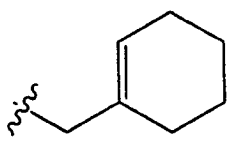


wherein

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

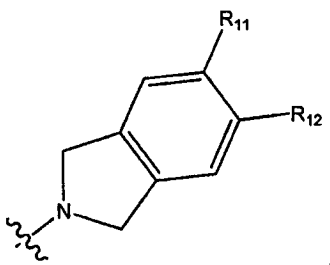
n is 1-4

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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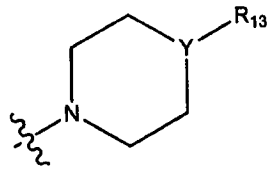

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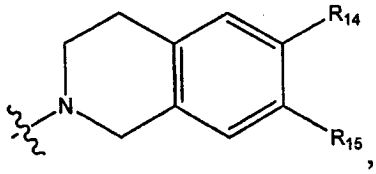

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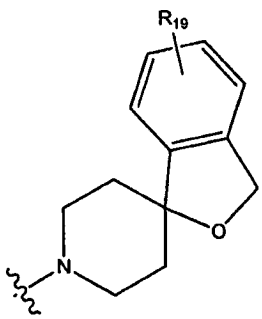
and


, wherein

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

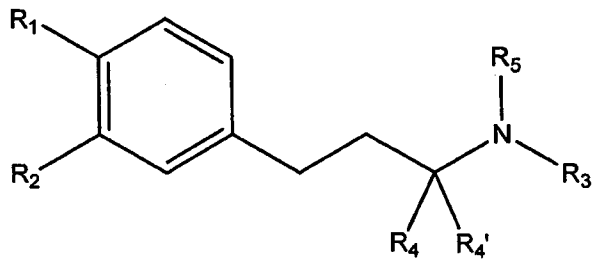
Y is CH or N;

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, or pharmaceutically acceptable salts thereof.

[0211] In some embodiments, the sigma-2 ligands of the present invention are those of Formula Va

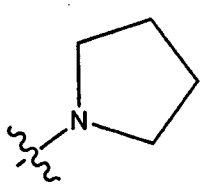


Va

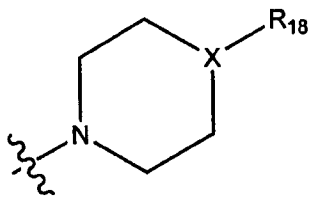
wherein

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



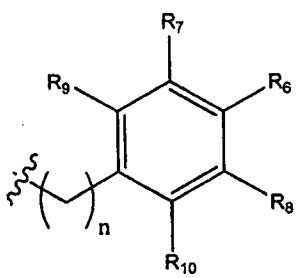
, wherein

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



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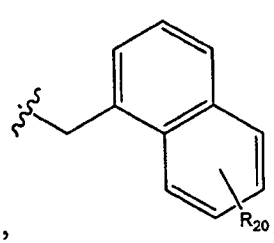

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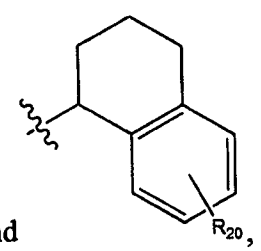


,

wherein

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

R_7 and R_8 are 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

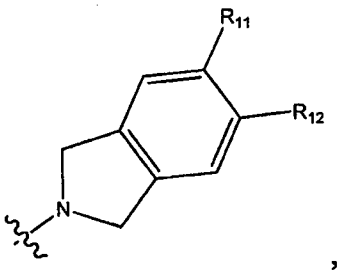
n is 1

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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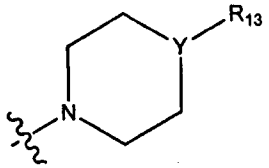

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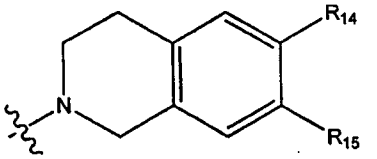

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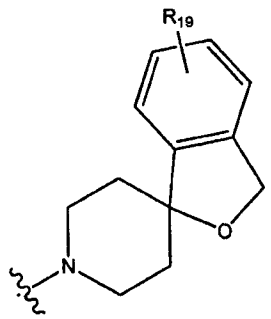


and , wherein

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

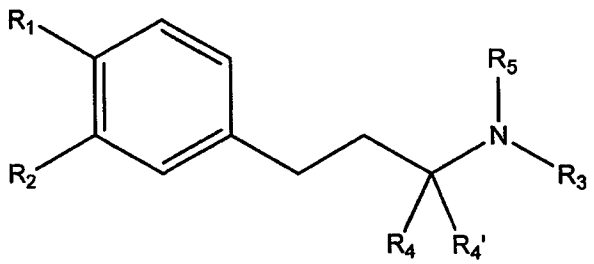
Y is CH or N;

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, or pharmaceutically acceptable salts thereof.

[0212] In some embodiments, the sigma-2 ligands of the present invention are those of Formula Va

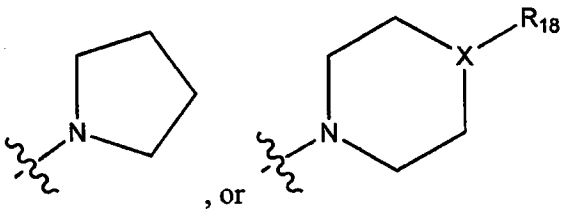


Va

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



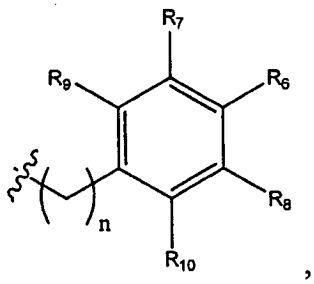
, or , wherein

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

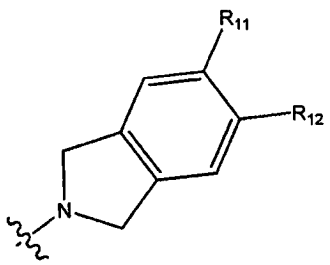
n is 1

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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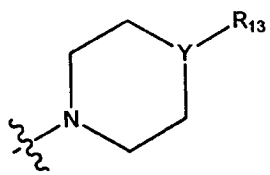
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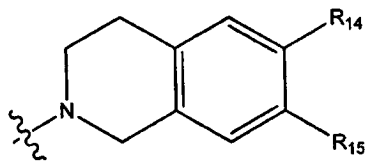
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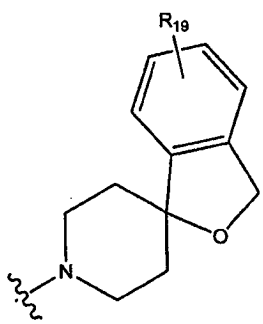

and , wherein

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

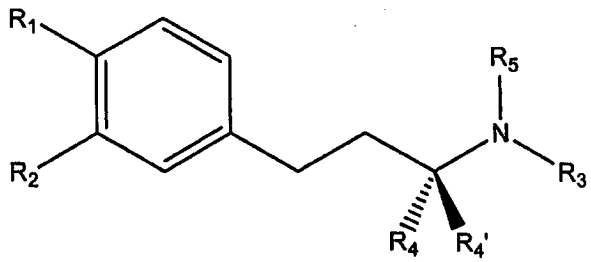
Y is CH or N;

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.

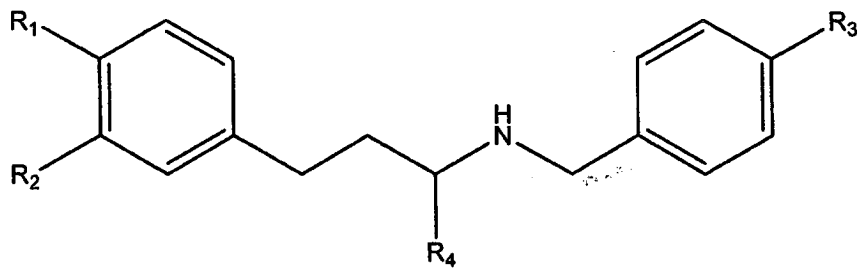
[0213] In some more specific embodiments, the sigma-2 ligands of the present invention are those of Formula Vb



Vb

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

[0214] In some embodiments, the sigma-2 ligands of the present invention are those of Formula IIIa:



IIIa

wherein

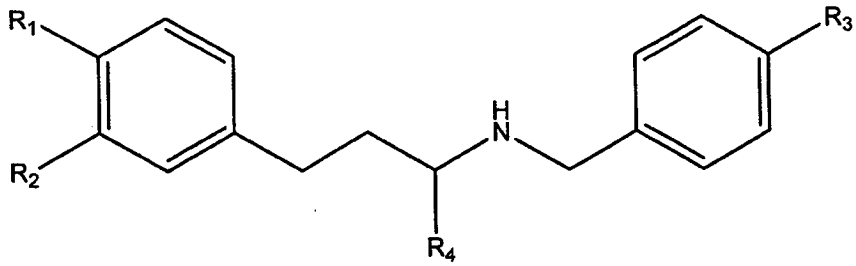
R_1 = halo, C_{1-6} haloalkyl, or OH;

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.

[0215] In some more specific embodiments, the sigma-2 ligands of the present invention are those of Formula IIIa.



IIIa

wherein

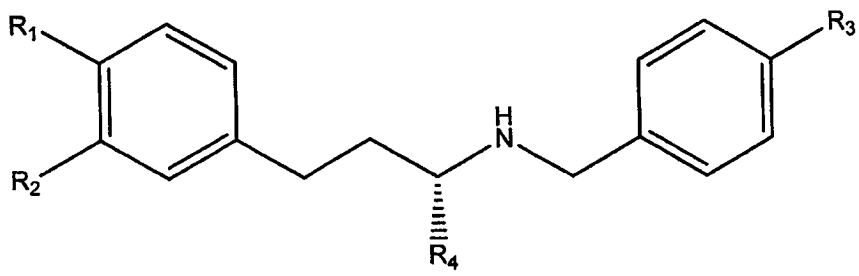
R_1 = Cl, F, CF_3, or OH;

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.

[0216] In some more specific embodiments, the sigma-2 ligands of the present invention are those of Formula IIIb



IIIc

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

[0217] Specific exemplary compounds of formulas III and IV and IIIa and IIIb as well as additional compounds are set forth in the following Table 1B.

Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb

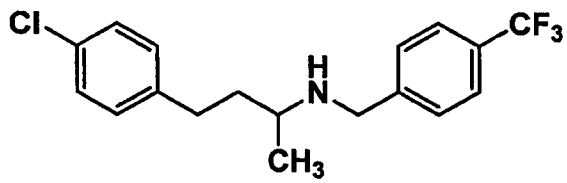
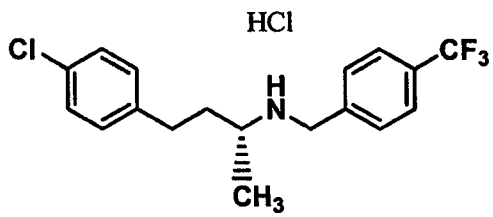
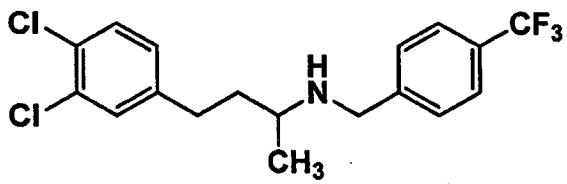
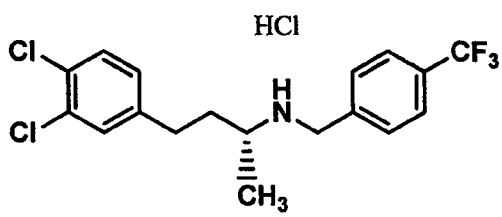
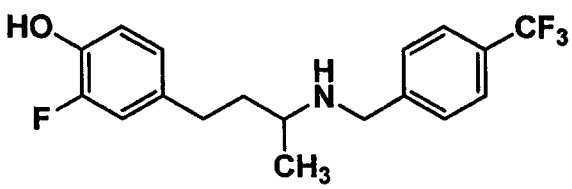
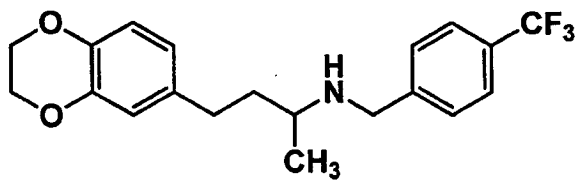
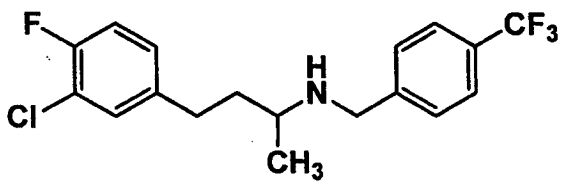
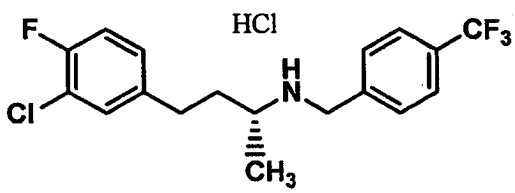
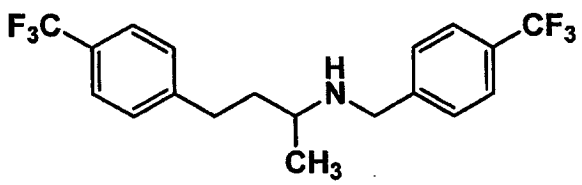






Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb





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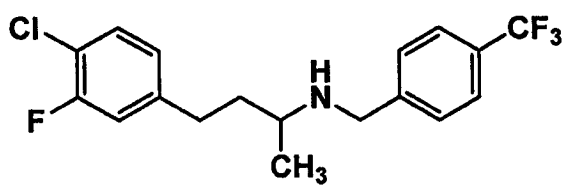
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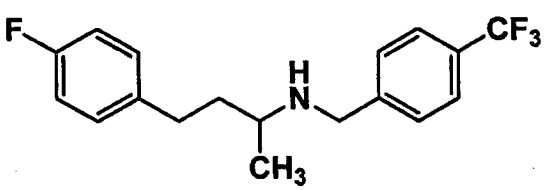


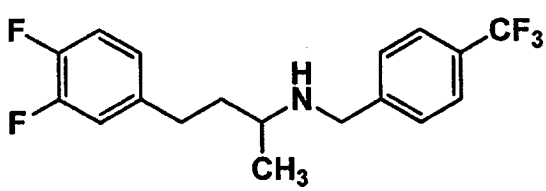
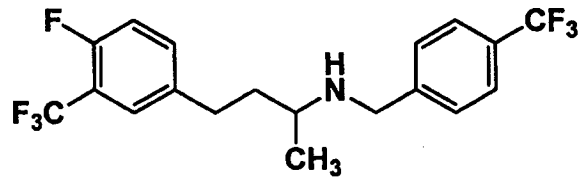
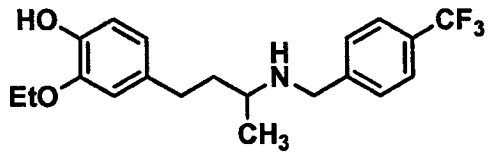
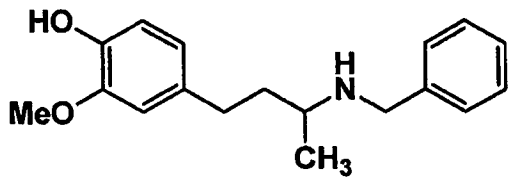
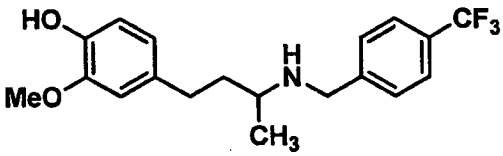


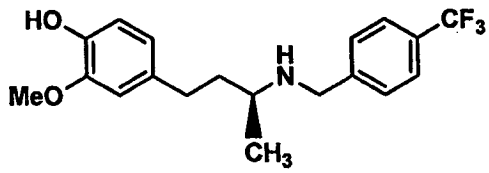
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb

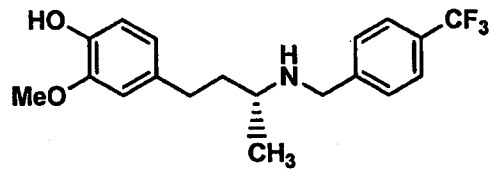












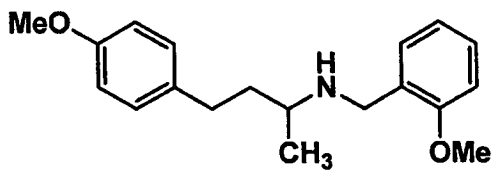
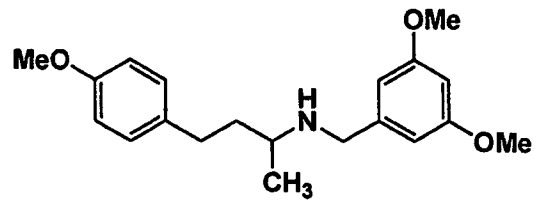
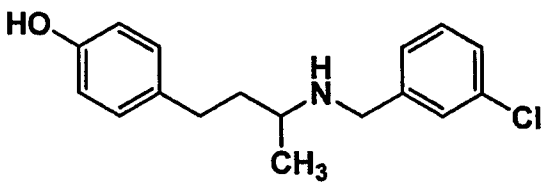
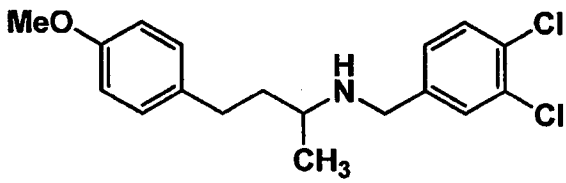
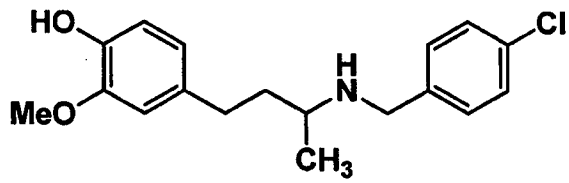


Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb









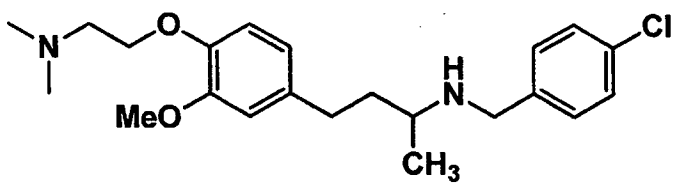
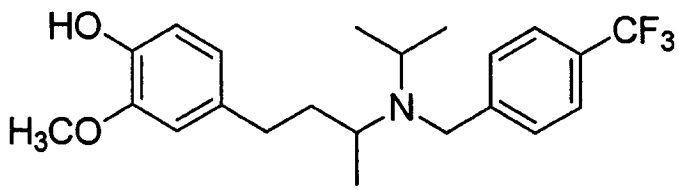
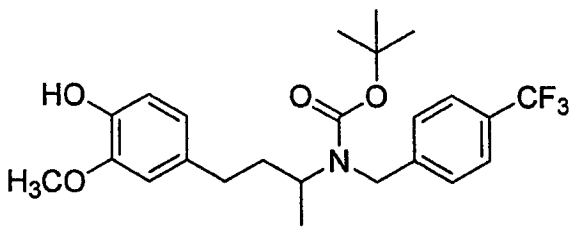


Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb





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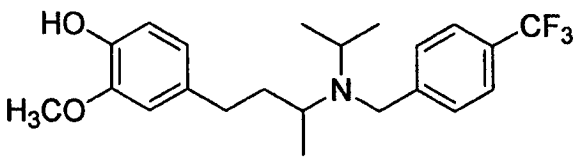

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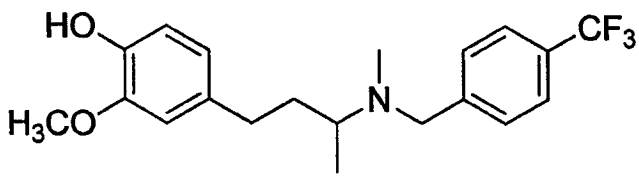

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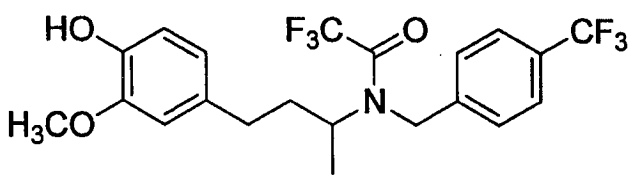

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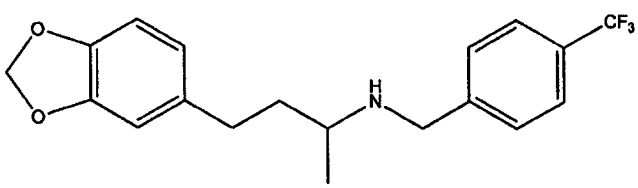

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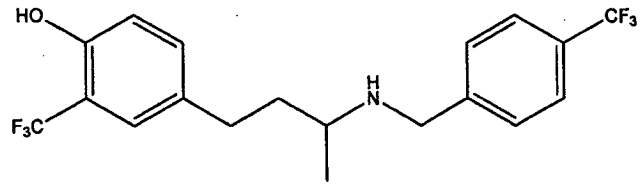
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb



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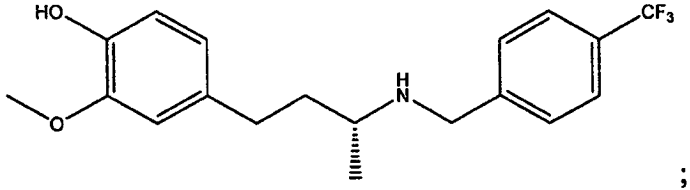

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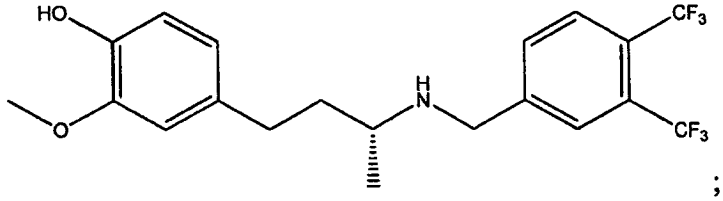

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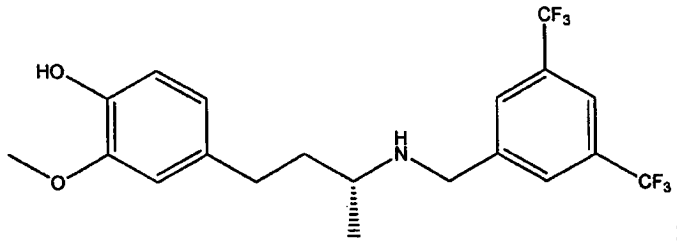

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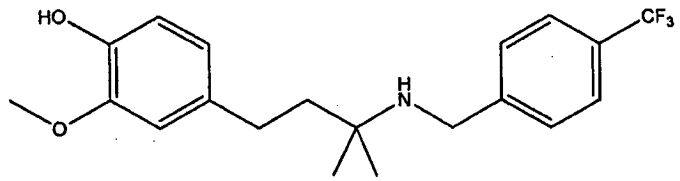

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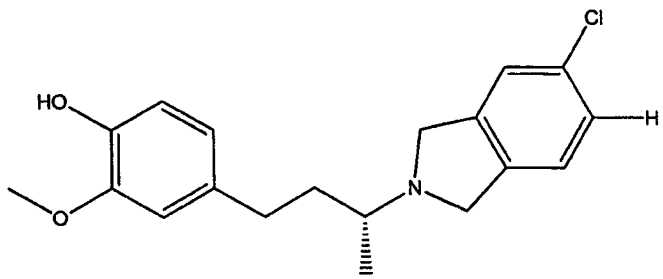

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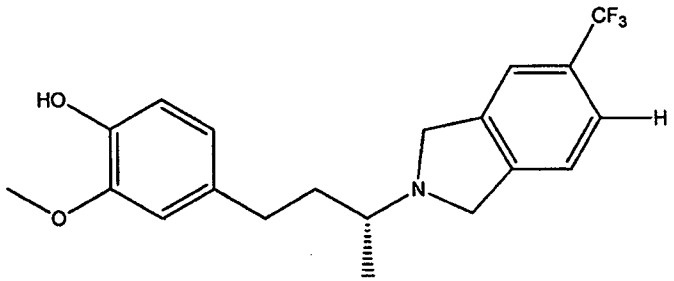
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb



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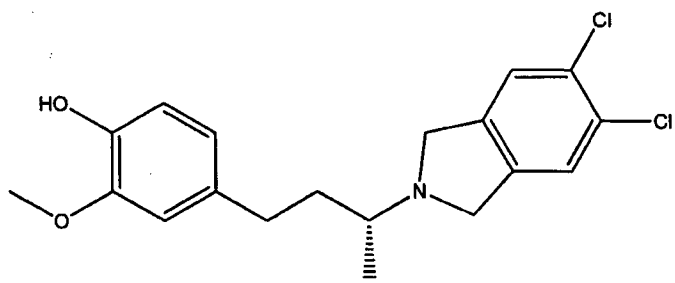

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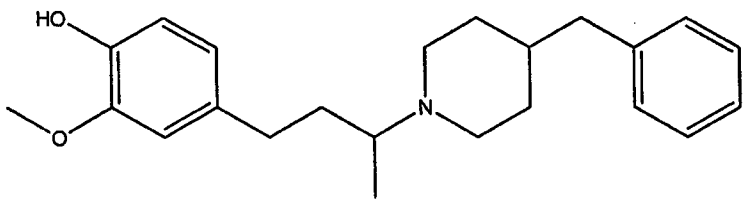

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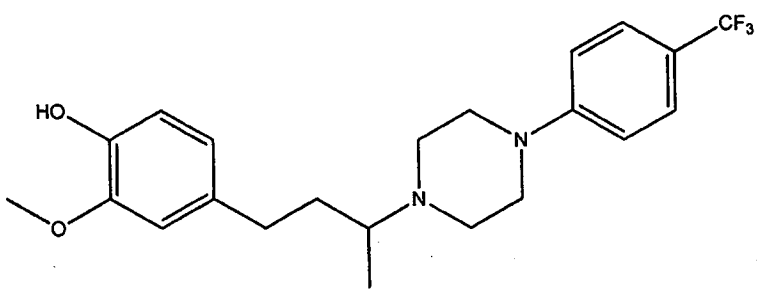

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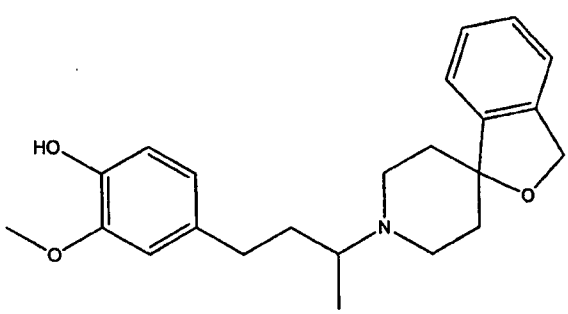

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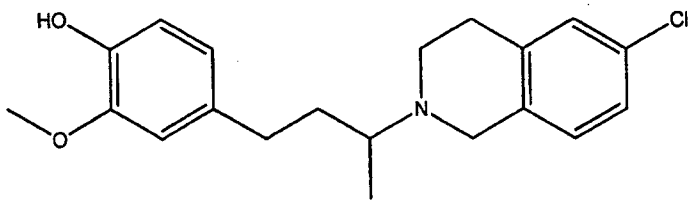
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb



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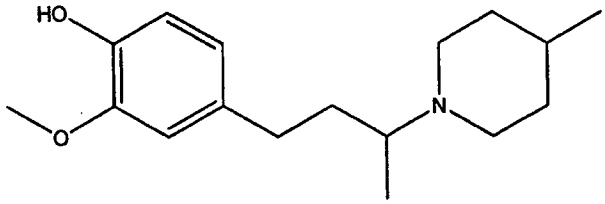

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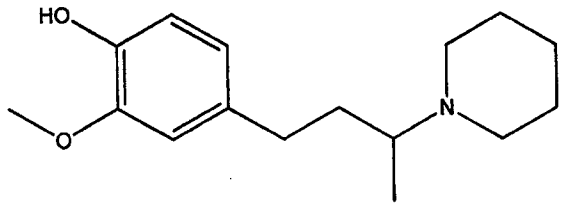

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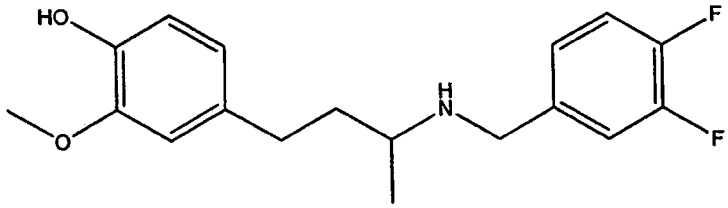

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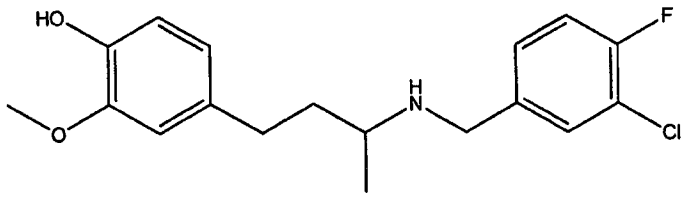

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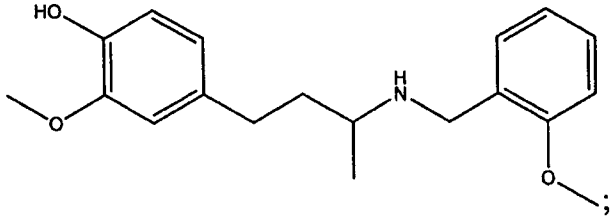

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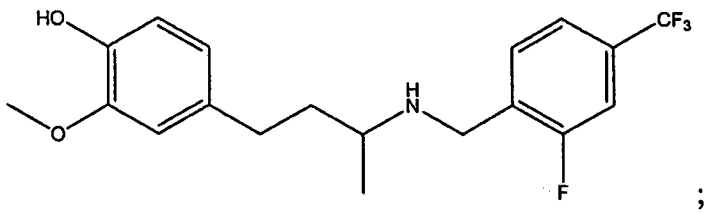
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb



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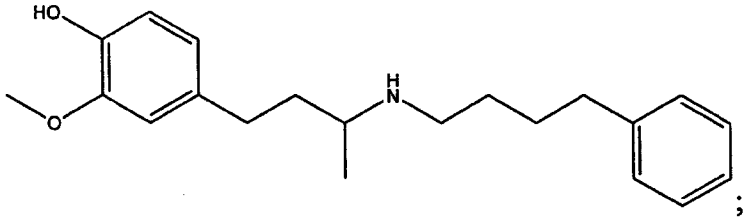

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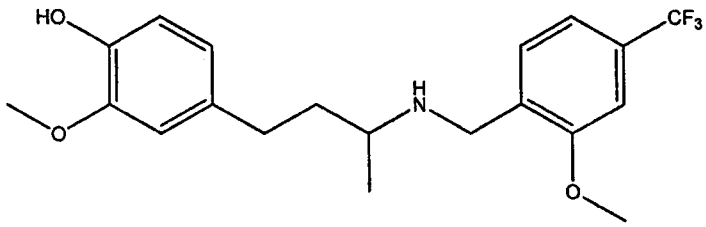

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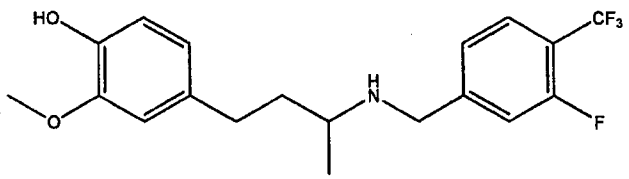

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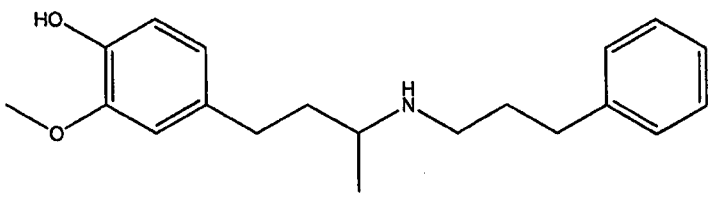

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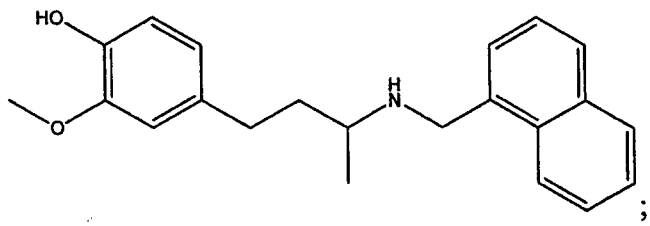

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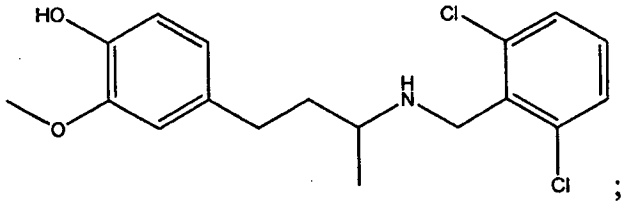
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb



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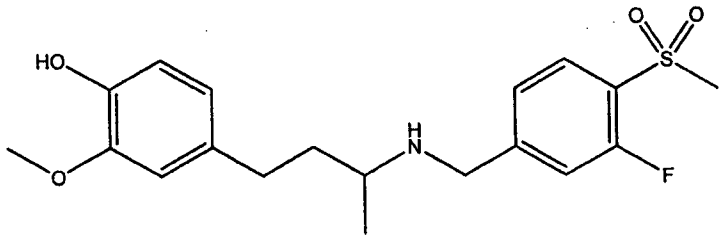

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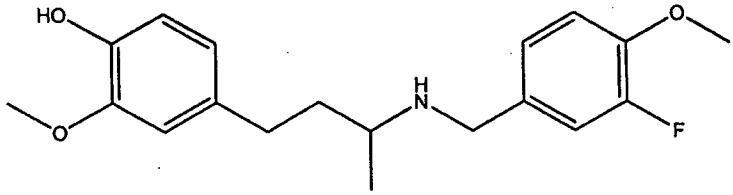

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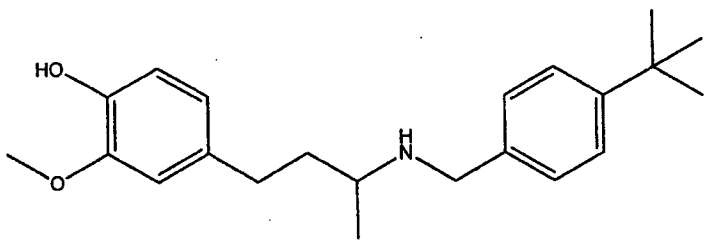

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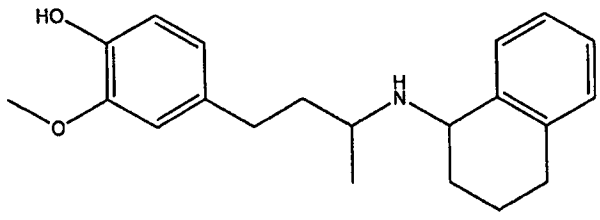

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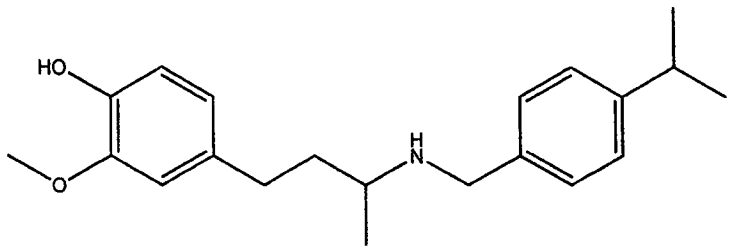
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb



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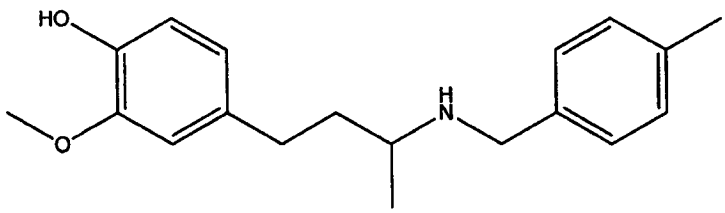

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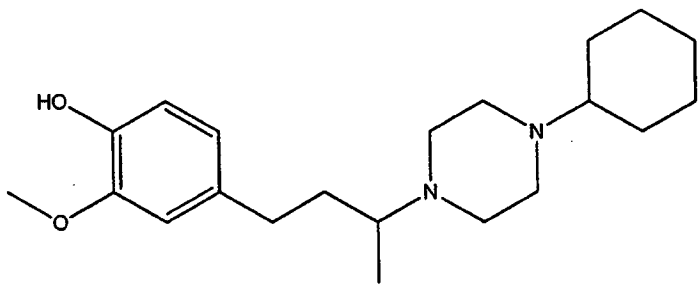

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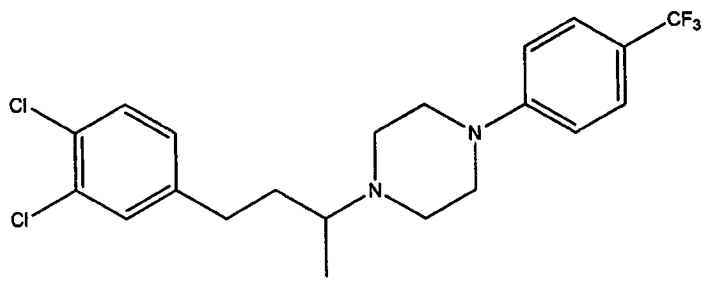

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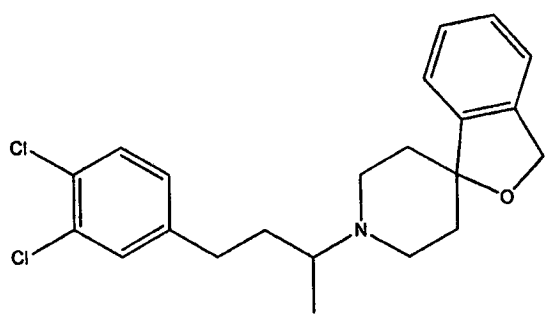

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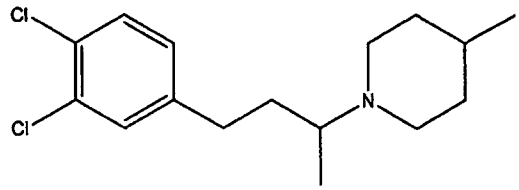
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb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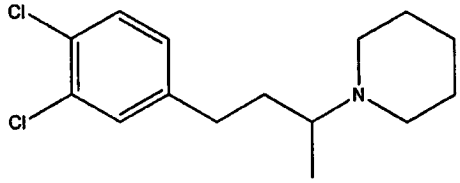
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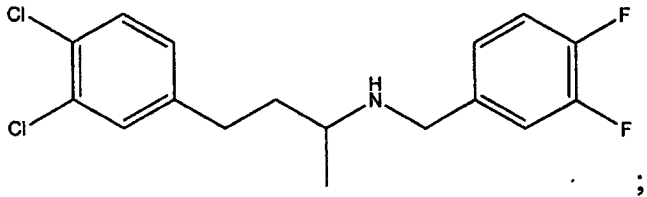
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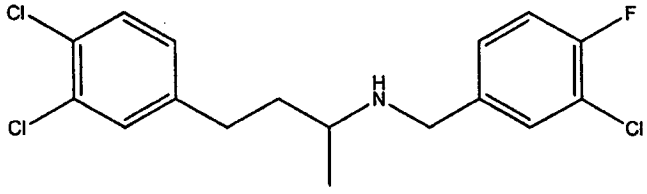
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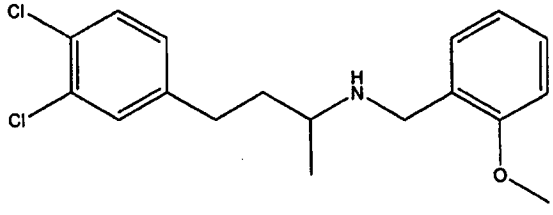
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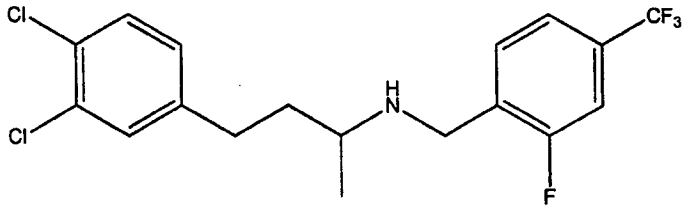
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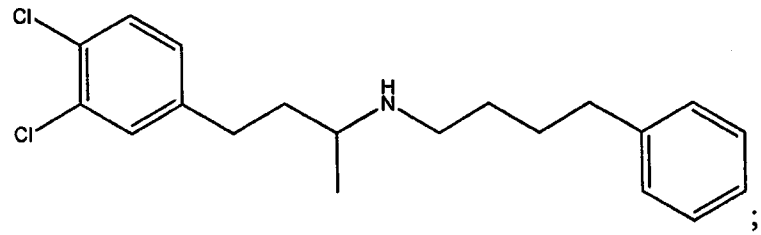
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb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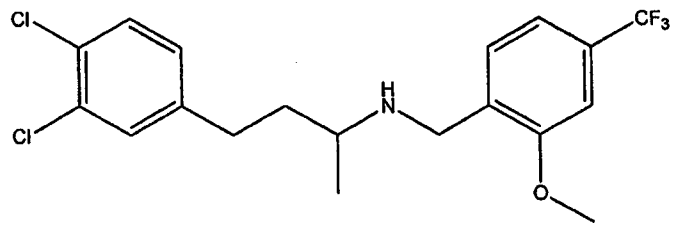
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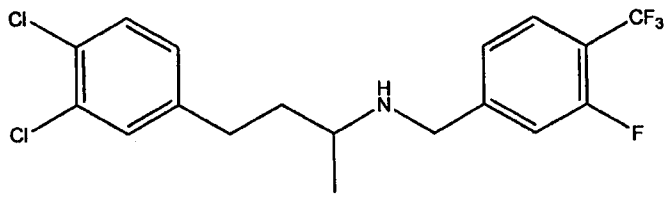
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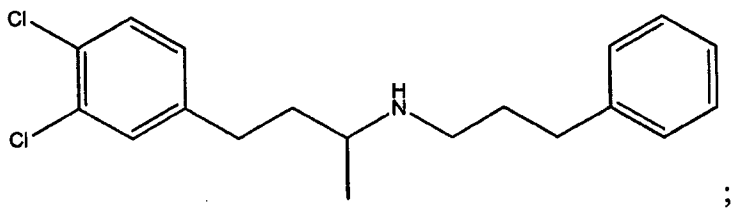
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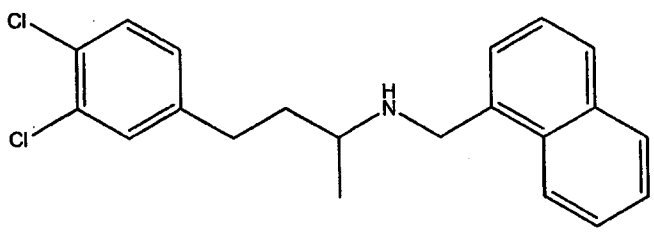
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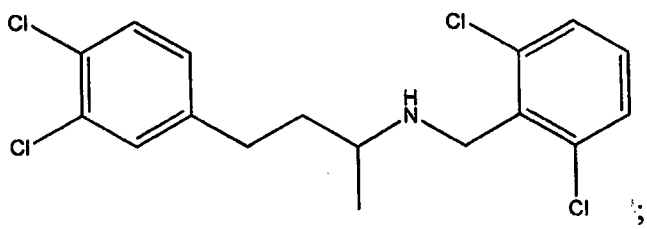
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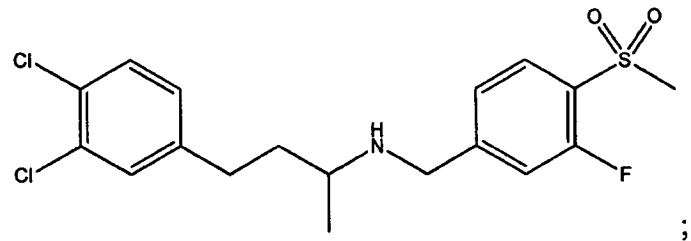
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb



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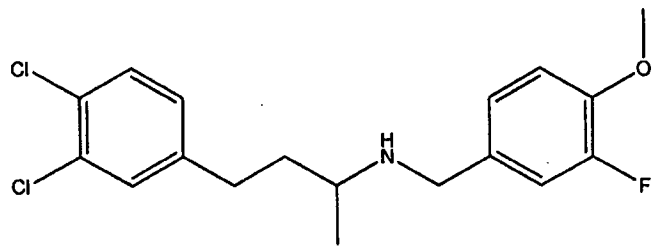

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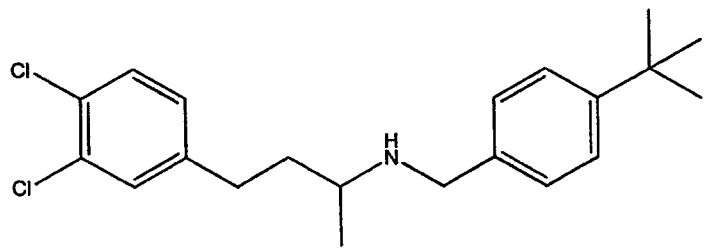

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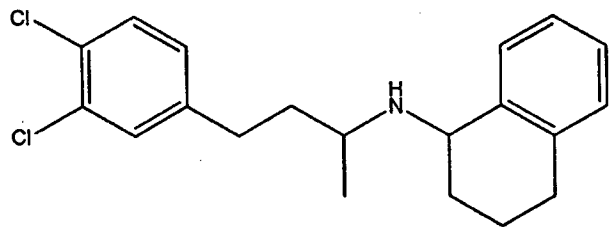

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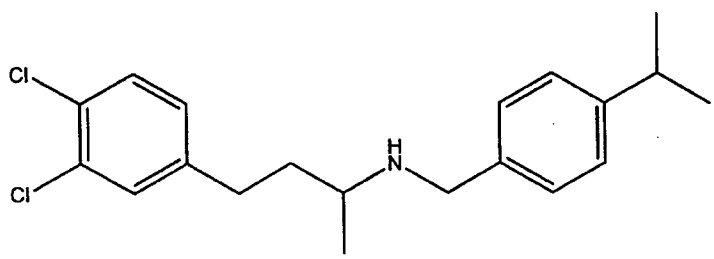

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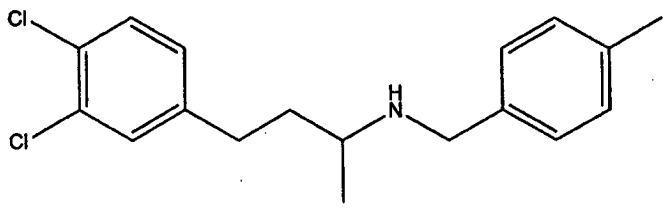
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb



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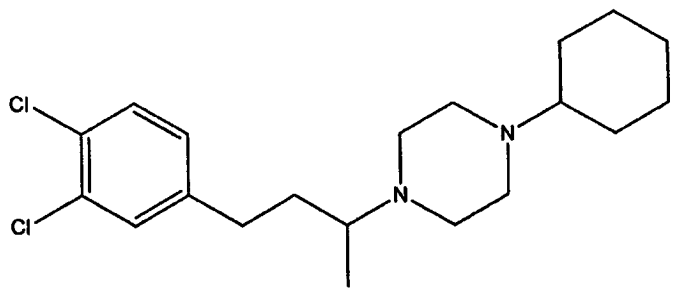

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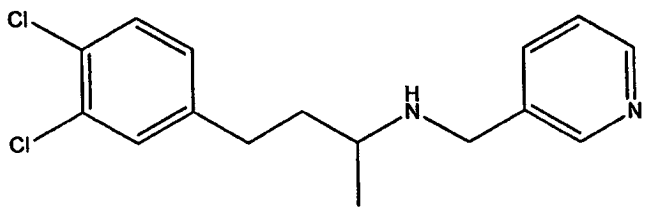

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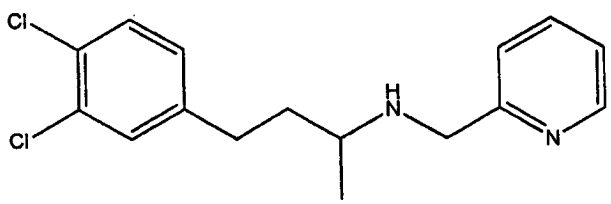

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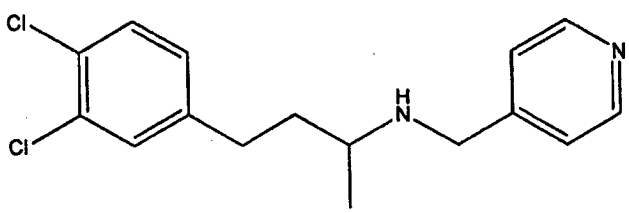

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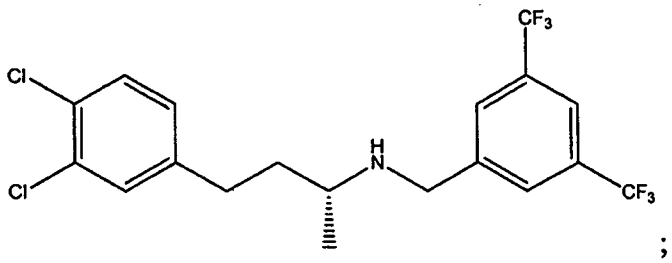
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb



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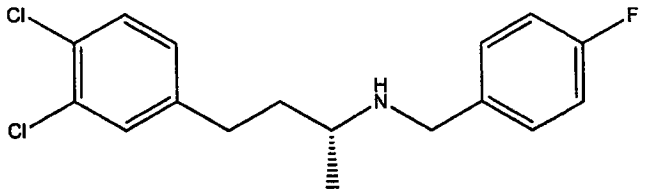

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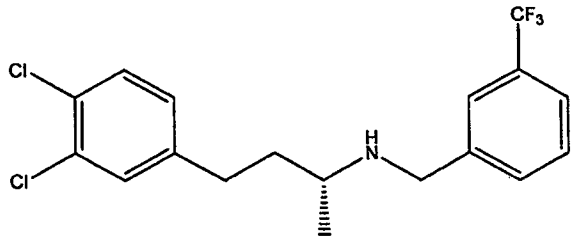

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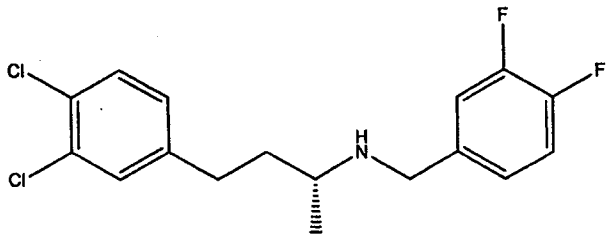

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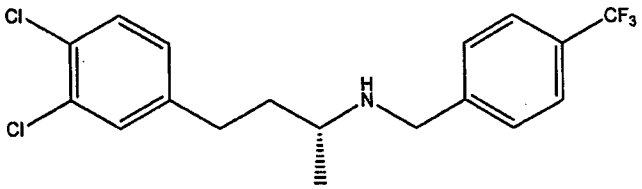

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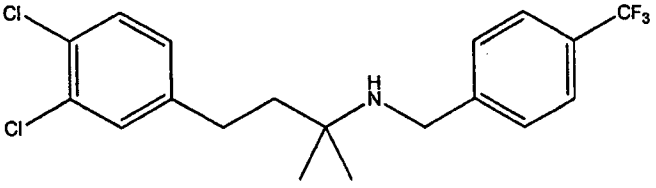

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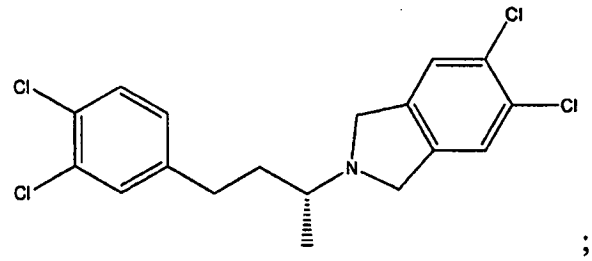
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb



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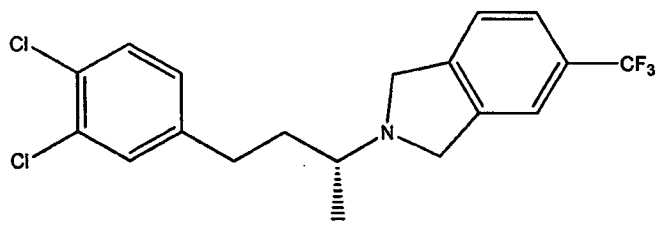

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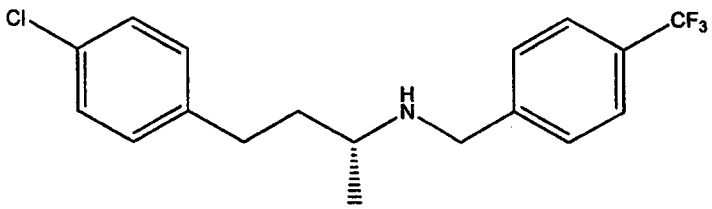

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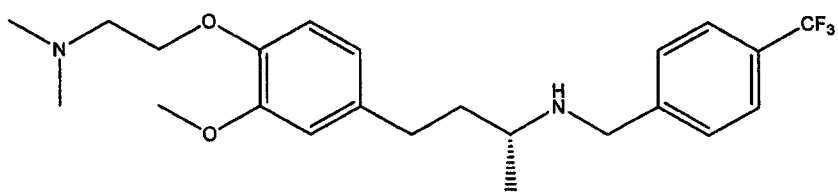

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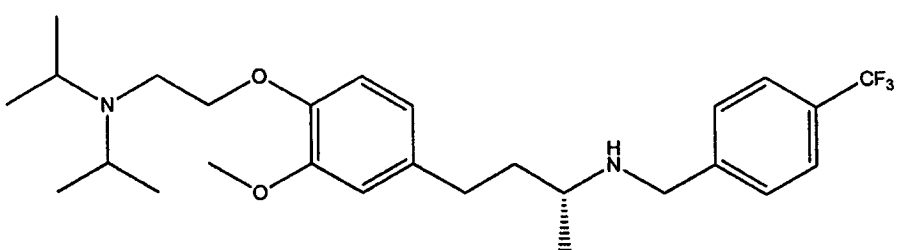

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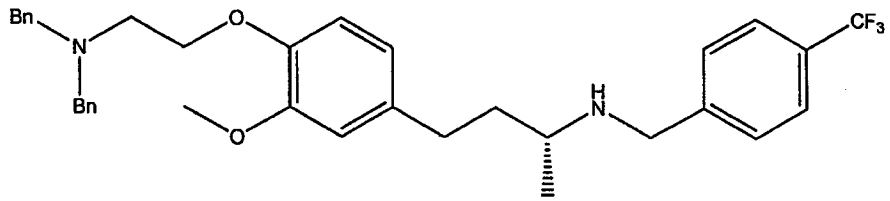
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb



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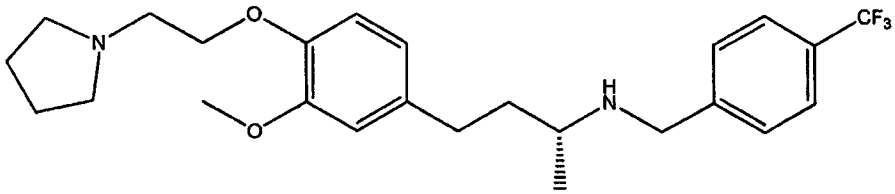

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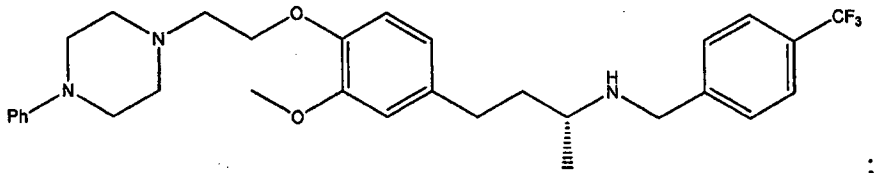

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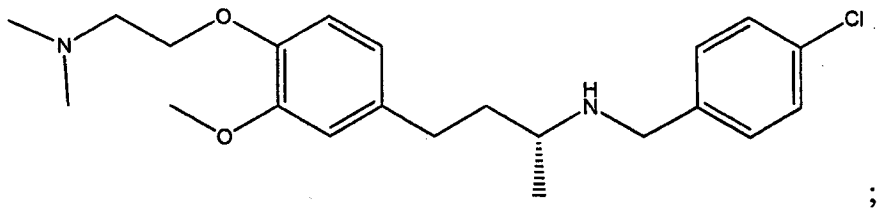

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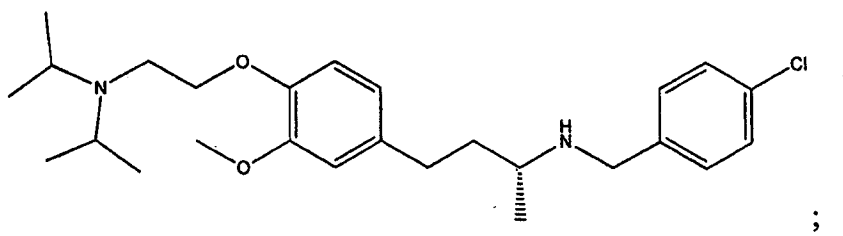

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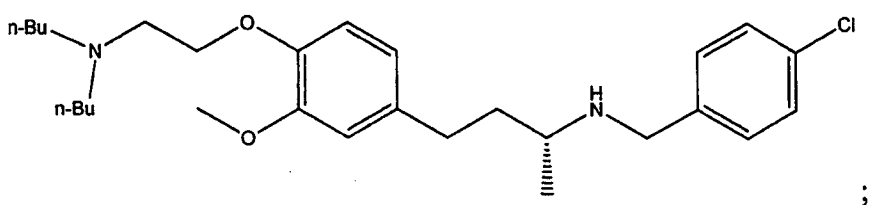

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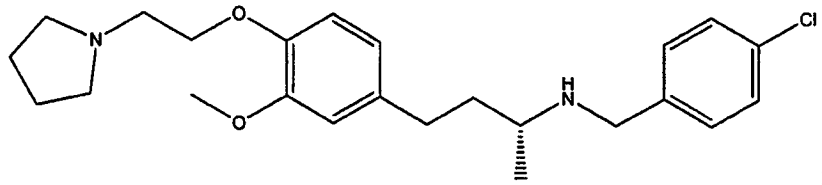
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb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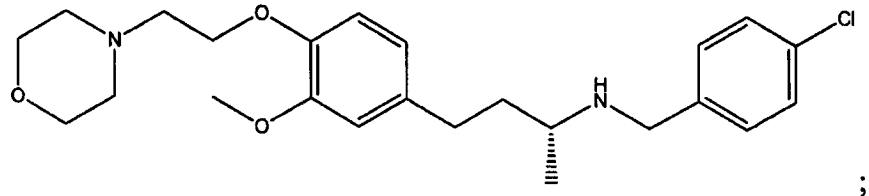
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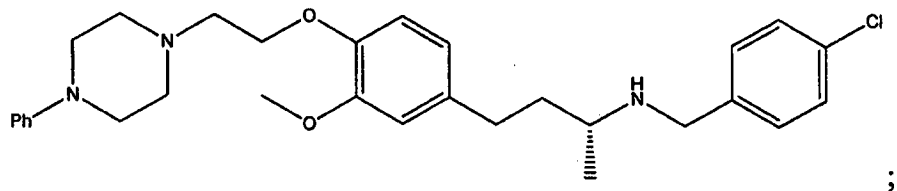
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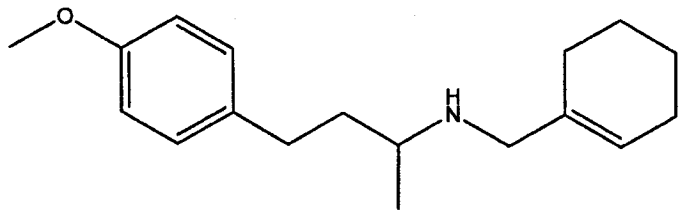
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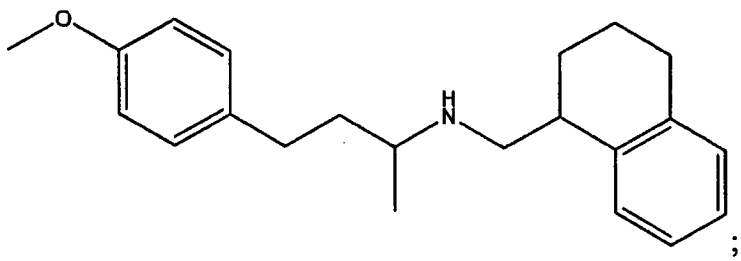
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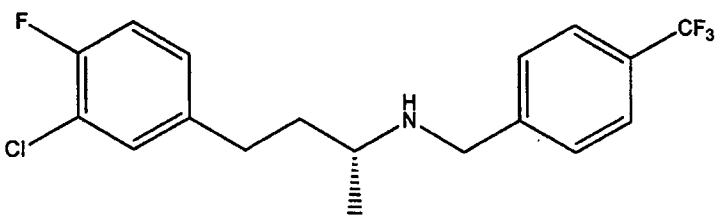
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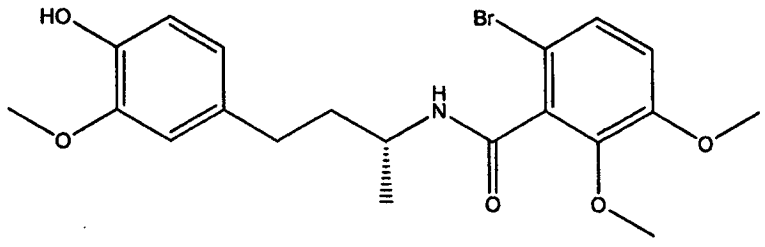
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb



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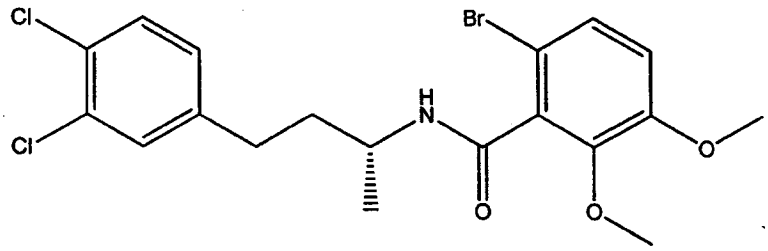

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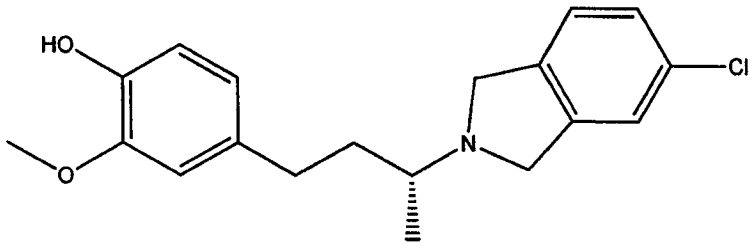

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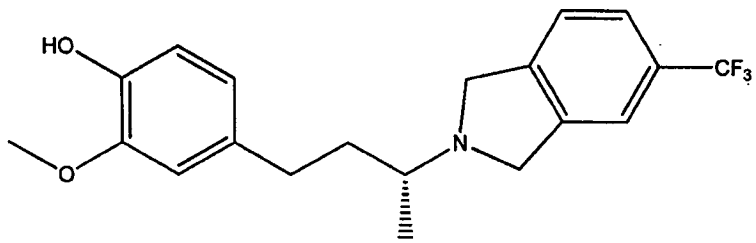

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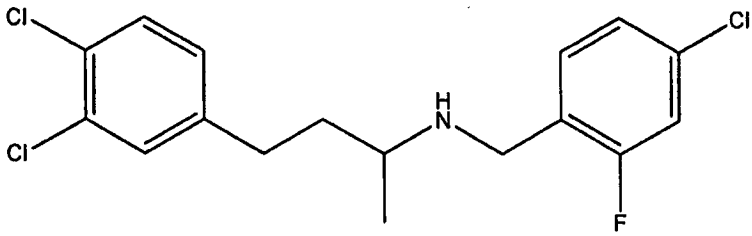
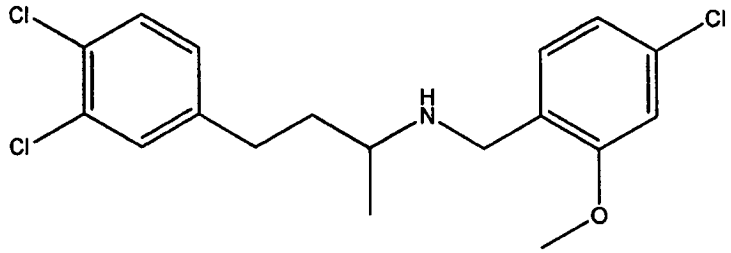


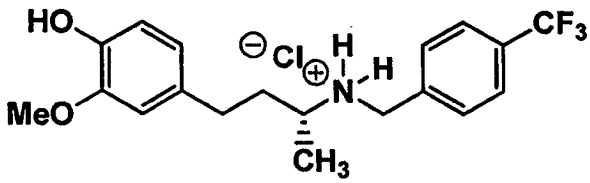
; and

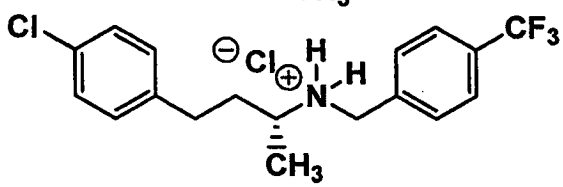
Table 1B. Exemplary Compounds of Formulas III, IIIa, IIIb, IIIc, IV, Va, and Vb

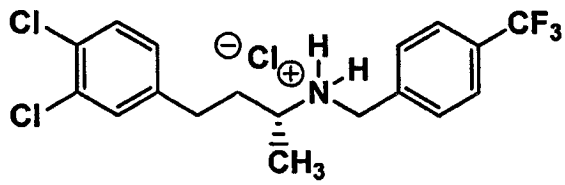


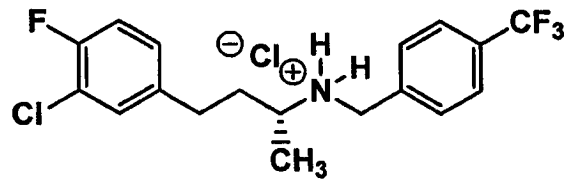
or pharmaceutically acceptable salts thereof.

[0218] Preferred salts for use in the present invention include the hydrochloride salts of the above compounds, including the following:





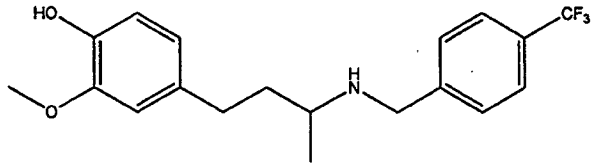




[0219] These have been synthesized in accordance with general methods provided herein and specific synthetic examples with any additional steps being well within the skill in the art. Several of these compounds have been tested in various

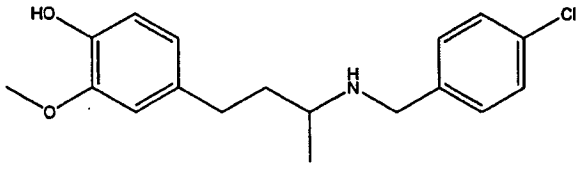
assays as detailed herein and have been found active. Tested compounds also display increased bioavailability by reference to compounds disclosed in WO 2010/110855.

[0220] Compound II has the formula:



[0221] In some embodiments, each of the general formulae above may contain a proviso to remove the compound of Formula II.

[0222] In some embodiments, each of the general formulae above may contain a proviso to remove one or more of the following compounds:



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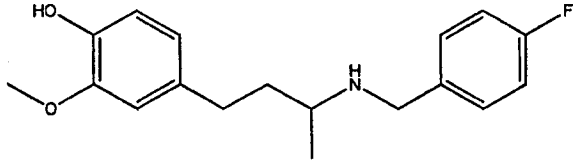

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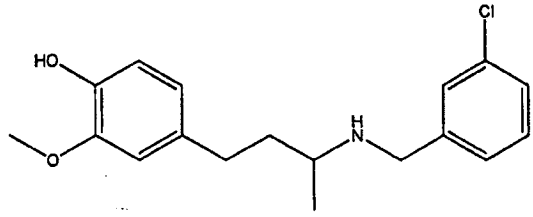

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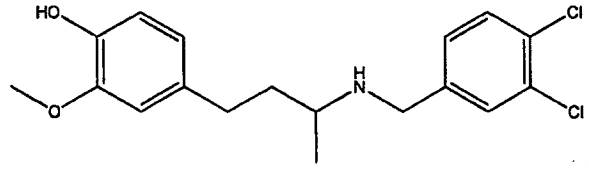

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[0223] These have been synthesized in accordance with general methods provided herein and specific synthetic examples with any additional steps being well within the skill in the art. Several of these compounds have been tested in various

assays as detailed herein and have been found active. Tested compounds also display increased bioavailability by reference to compounds disclosed in WO 2010/110855, incorporated herein by reference.

[0224] As used herein, the term "hydrogen bond acceptor group" refers to a group capable of accepting a hydrogen bond. Examples of hydrogen bond acceptor groups are known and include, but are not limited to, alkoxy groups, oxazolidin-2-one groups, -O-C(O)-N-; -C(O)-N-; -O-; the hetero atom (*e.g.* oxygen) in a cycloheteroalkyl; -N-SO_2- and the like. The groups can be bound in either direction and can be connected to another carbon or heteroatom. A hydrogen bond acceptor group can also be present in or near a hydrophobic aliphatic group. For example, a tetrahydrofuran group comprises both a hydrogen bond acceptor group and a hydrophobic aliphatic group. The oxygen present in the tetrahydrofuran ring acts as a hydrogen bond acceptor and the carbons in the tetrahydrofuran ring act as the hydrophobic aliphatic group.

[0225] As used herein, the term "hydrophobic aliphatic group" refers to a carbon chain or carbon ring. The carbon chain can be present in a cycloheteroalkyl, but the hydrophobic aliphatic group does not include the heteroatom. The tetrahydrofuran example provided above is one such example, but there are many others. In some embodiments, the hydrophobic aliphatic group is an optionally substituted C1-C6 alkyl. cycloalkyl, or C1-C6 carbons of a heterocycloalkyl. A "hydrophobic aliphatic group" is not a hydrophobic aromatic group.

[0226] As used herein, the term "positive ionizable group" refers to an atom or a group of atoms present in a structure that can be positively charged under certain conditions such as biological conditions present in solution or in a cell. In some embodiments, the positive ionizable group is a nitrogen. In some embodiments, the positive ionizable group is a nitrogen present in a cycloheteroalkyl ring. For example, in a piperazine group, the two nitrogens would be considered two positive ionizable groups. However, in some embodiments, the carbons linked to a positive ionizable group are not considered a hydrophobic aliphatic group. In some embodiments, the positive ionizable group is a nitrogen containg ring. Examples of nitrogen containing rings include, but are not limited to, piperazine,

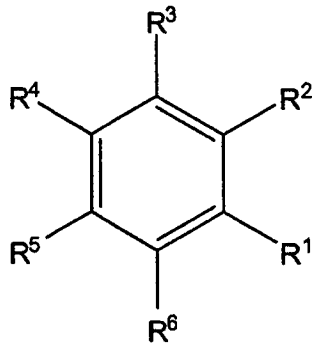
piperadine, triazinane, tetrazinane, and the like. In some embodiments with respect to the positive ionizable group, a nitrogen containing ring comprises 1, 2, 3, or 4 nitrogens. In some embodiments, the positive ionizable group is not the nitrogen present in a -N-SO_2- group

[0227] In some embodiments, a group comprises both a hydrogen bond acceptor and a positive ionizable group. For example, a morpholine group comprises both a hydrogen bond acceptor in the oxygen group and a positive ionizable group in the nitrogen.

[0228] As used herein, the term "hydrogen bond donor" refers to a group that is capable of donating a hydrogen bond. Examples of a hydrogen bond donor group include, but are not limited to, -OH, and the like.

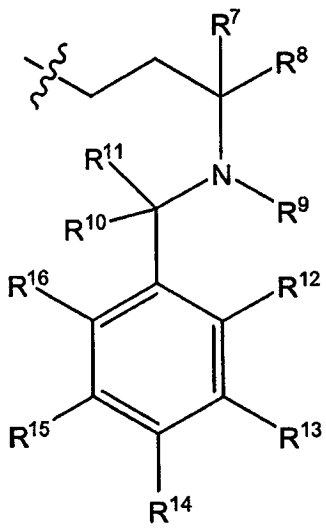
[0229] In some embodiments, the sigma-2 receptor ligand is an optionally substituted piperazine, phenyltetrahydrofuran-N,N-dimethylmethanamine diphenyltetrahydrofuran-N,N-dimethylmethanamine, a 4-phenylpentyl-piperazine, benzylphenyl-piperazine, indole-oxa-azaspiro-decane, piperadine-indole, phenylpiperadine-indole, pyrazole-morpholine, pyrazole-piperadine, pyrazol-N,N-diethylethanamine, pyrazole-pyrrolidine, phenyl-pyrazol-morpholine, benzamide-quinoline compound, or derivatives thereof.

[0230] Additionally, the sigma-2 receptor ligand can be any compound described in WO 2011/014880 (Application No. PCT/US2010/044136), WO 2010/118055 (Application No. PCT/US2010/030130), and Application No. PCT/US2011/026530, and WO 2012/106426, each of which is hereby incorporated by reference in its entirety. For example, in some embodiments, the sigma-2 ligand is a compound of Formula V:



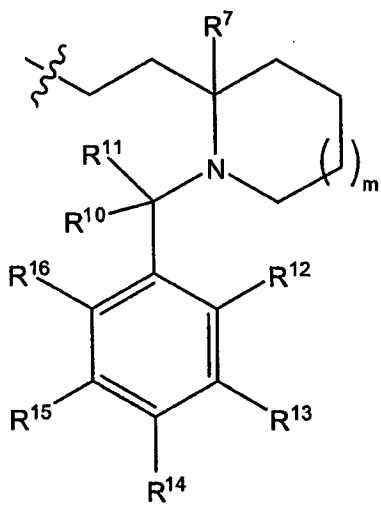
V

or pharmaceutically acceptable salts thereof wherein: R^1 is selected from (A1) and (A2):



(A1)

and



(A2)

;

wherein in a compound of Formula V, R^2, R^3, R^4, R^5, and R^6 are each, independently, selected from H, OH, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halo, CN, NO_2, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, NH_2, $NH(C_{1-4}$ alkyl), $NH(C_{3-7}$ cycloalkyl), $N(C_{1-4}$ alkyl)$_2$, $NHC(O)(C_{1-4}$ alkyl), SH, $S(C_{1-6}$ alkyl), $C(O)OR^a$, $C(O)R^b$, $C(O)NR^cR^d$, $OC(O)R^b$, $OC(O)NR^cR^d$, NR^cR^d, $NR^cC(O)R^b$, $NR^cC(O)OR^a$, $NR^cS(O)_2R^b$, $NR^cS(O)_2NR^cR^d$, $S(O)R^b$, $S(O)_2R^b$, and $S(O)_2NR^cR^d$;

wherein in a compound of Formula V R^7 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, or C_{3-7} cycloalkyl;

wherein in a compound of Formula V R^8 is C_{1-6} alkyl, C_{1-6} haloalkyl, or C_{3-7} cycloalkyl;

wherein in a compound of Formula V R^9 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, or C_{3-7} cycloalkyl;

wherein in a compound of Formula V R^{10} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, or C_{3-7} cycloalkyl;

wherein in a compound of Formula V R^{11} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, or C_{3-7} cycloalkyl;

wherein in a compound of Formula V R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are each, independently, selected from H, OH, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halo, CN, NO_2, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, NH_2, $NH(C_{1-4}$ alkyl), $NH(C_{3-7}$ cycloalkyl), $N(C_{1-4}$ alkyl)$_2$, $NHC(O)(C_{1-4}$ alkyl), SH, $S(C_{1-6}$ alkyl), $C(O)OR^{a1}$, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, $NR^{c1}S(O)_2R^{b1}$, $NR^{c1}S(O)_2NR^{c1}R^{d1}$, $S(O)R^{b1}$, $S(O)_2R^{b1}$, and $S(O)_2NR^{c1}R^{d1}$;

wherein in a compound of Formula V each R^a is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkylalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, aryl, cycloalkyl, heteroaryl and heterocycloalkyl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkylalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, aryl, cycloalkyl, heteroaryl and heterocycloalkyl is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, CN, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl and heterocycloalkyl;

wherein in a compound of Formula V each R^b is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkylalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, aryl, cycloalkyl, heteroaryl, heterocycloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl and heterocycloalkylalkyl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkylalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl,

aryl, cycloalkyl, heteroaryl, heterocycloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl and heterocycloalkylalkyl is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl and heterocycloalkyl;

wherein in a compound of Formula V R^c and R^d are independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkylalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl and heterocycloalkylalkyl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkylalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl and heterocycloalkylalkyl is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl and heterocycloalkyl;

or wherein in a compound of Formula V R^c and R^d together with the N atom to which they are attached form a 4-, 5-, 6- or 7-membered heterocycloalkyl group that is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, and heterocycloalkyl;

wherein in a compound of Formula V each R^{a1} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkylalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, aryl, cycloalkyl, heteroaryl and heterocycloalkyl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkylalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, aryl, cycloalkyl, heteroaryl and heterocycloalkyl is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, CN, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, and heterocycloalkyl;

wherein in a compound of Formula V each R^{b1} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkylalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, aryl, cycloalkyl, heteroaryl, heterocycloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl and heterocycloalkylalkyl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkylalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, aryl, cycloalkyl, heteroaryl, heterocycloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl and heterocycloalkylalkyl is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, and heterocycloalkyl;

wherein in a compound of Formula V R^{c1} and R^{d2} are independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkylalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl and heterocycloalkylalkyl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkylalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl and heterocycloalkylalkyl is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, and heterocycloalkyl;

or wherein in a compound of Formula V R^{c1} and R^{d1} together with the N atom to which they are attached form a 4-, 5-, 6- or 7-membered heterocycloalkyl group that is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, and heterocycloalkyl; and

m is 0, 1, or 2.

[0231] In some embodiments, in a compound of Formula V when R^1 is a moiety of (A1), then two of R^2, R^3, R^4, R^5, and R^6 are independently selected from OH, C_{1-6} alkoxy, and C_{1-6} haloalkoxy.

[0232] In some embodiments, in a compound of Formula V when R^1 is a moiety of (A1), then at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is other than H.

[0233] In some embodiments, in a compound of Formula V two of R^2, R^3, R^4, R^5, and R^6 are independently selected from OH, C_{1-6} alkoxy, and C_{1-6} haloalkoxy. In some further emobidments, each of the rest of R^2, R^3, R^4, R^5, and R^6 is H.

[0234] In some embodiments, in a compound of Formula V R^2, R^3, R^4, R^5, and R^6 are each, independently, selected from H, OH, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halo, CN, NO_2, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, NH_2, NH(C_{1-4} alkyl), NH(C_{3-7} cycloalkyl), N(C_{1-4} alkyl)$_2$, 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).

[0235] In some embodiments, in a compound of Formula V one of R^2, R^3, R^4, R^5, and R^6 is OH; and one of R^2, R^3, R^4, R^5, and R^6 is OH, C_{1-6} alkoxy, or C_{1-6} haloalkoxy. In some further emobidments, each of the rest of R^2, R^3, R^4, R^5, and R^6 is H.

[0236] In some embodiments, in a compound of Formula V one of R^2, R^3, R^4, R^5, and R^6 is OH; and one of R^2, R^3, R^4, R^5, and R^6 is C_{1-3} alkoxy or C_{1-3} haloalkoxy (In some further emobidments, each of the rest of R^2, R^3, R^4, R^5, and R^6 is H.). In some further embodiments, one of R^2, R^3, R^4, R^5, and R^6 is OH; and one of R^2, R^3, R^4, R^5, and R^6 is methoxy or trihalomethoxy (In some further emobidments, each of the rest of R^2, R^3, R^4, R^5, and R^6 is H.). In still further embodiments, one of R^2, R^3, R^4, R^5, and R^6 is OH; and one of R^2, R^3, R^4, R^5, and R^6 is methoxy (In some further emobidments, each of the rest of R^2, R^3, R^4, R^5, and R^6 is H.).

[0237] In some embodiments, in a compound of Formula V R^4 is OH; and R^5 is methoxy. In some further embodiments, R^4 is OH; R^5 is methoxy; and R^2, R^3, and R^6 are each H.

[0238] In some embodiments, in a compound of Formula V R^7 is H or C_{1-6} alkyl. In some further embodiments, R^7 is H or C_{1-3} alkyl.

[0239] In some embodiments, in a compound of Formula V R^7 is C_{1-3} alkyl. In some further embodiments, R^7 is methyl or ethyl. In still further embodiments, R^7 is methyl.

[0240] In some embodiments, in a compound of Formula V R^7 is H.

[0241] In some embodiments, in a compound of Formula V R^8 is C_{1-6} alkyl. In some further embodiments, R^8 is C_{1-3} alkyl. In still further embodiments, R^8 is methyl.

[0242] In some embodiments, in a compound of Formula V R^9 is H or C_{1-6} alkyl. In some further embodiments, R^9 is H or C_{1-3} alkyl.

[0243] In some embodiments, in a compound of Formula V R^9 is H.

[0244] In some embodiments, in a compound of Formula V R^9 is C_{1-3} alkyl.

[0245] In some embodiments, in a compound of Formula V R^{10} is H or C_{1-6} alkyl. In some further embodiments, R^{10} is H or C_{1-3} alkyl. In still further embodiments, R^{10} is H. In other embodiments, R^{10} is C_{1-3} alkyl.

[0246] In some embodiments, in a compound of Formula V R^{11} is H or C_{1-6} alkyl. In some further embodiments, R^{11} is H or C_{1-3} alkyl. In still further embodiments, R^{11} is H. In other embodiments, R^{11} is C_{1-3} alkyl.

[0247] In some embodiments, in a compound of Formula V at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is other than H.

[0248] In some embodiments, in a compound of Formula V R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are each, independently, selected from H, OH, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halo, CN, NO_2, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, NH_2, $NH(C_{1-4}$

alkyl), NH(C_{3-7} cycloalkyl), N(C_{1-4} alkyl)$_2$, 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).

[0249] In some embodiments, in a compound of Formula V R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are each, independently, selected from H, halo, CN, NO_2, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, C(O)O(C_{1-4} alkyl), C(O)(C_{1-4} alkyl), and C(O)NH(C_{1-4} alkyl).

[0250] In some embodiments, in a compound of Formula V at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo, CN, NO_2, C_{1-6} haloalkyl, C(O)O(C_{1-4} alkyl), C(O)(C_{1-4} alkyl), and C(O)NH(C_{1-4} alkyl).

[0251] In some embodiments, in a compound of Formula V at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo and C_{1-6} haloalkyl. In some further embodiments, at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo and C_{1-6} haloalkyl, and each of the rest is of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is H. In yet further embodiments, one or two of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are selected from halo and C_{1-6} haloalkyl, and each of the rest is of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is H. In still further embodiments, one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo and C_{1-6} haloalkyl, and each of the rest is of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is H.

[0252] In some embodiments, in a compound of Formula V R^{14} is halo or C_{1-6} haloalkyl (In some further embodiments, each of of R^{12}, R^{13}, R^{15}, and R^{16} is H.). In some further embodiments, R^{14} is halo or C_{1-3} haloalkyl (In some further embodiments, each of of R^{12}, R^{13}, R^{15}, and R^{16} is H.). In still further embodiments, R^{14} is halo or C_1 haloalkyl (In some further embodiments, each of of R^{12}, R^{13}, R^{15}, and R^{16} is H.).

[0253] In some embodiments, in a compound of Formula V R^{14} is halo (In some further embodiments, each of of R^{12}, R^{13}, R^{15}, and R^{16} is H.). In some embodiments, R^{14} is Cl or F. In some embodiments, R^{14} is Cl. In some embodiments, R^{14} is F.

[0254] In some embodiments, in a compound of Formula V R^{14} is C_{1-6} haloalkyl (In some further embodiments, each of of R^{12}, R^{13}, R^{15}, and R^{16} is H.). In

some further embodiments, R^{14} is C_{1-3} haloalkyl. In still further embodiments, R^{14} is C_1 haloalkyl. In yet further embodiments, R^{14} is CF_3.

[0255] In some embodiments, in a compound of Formula V R^{15} is halo or C_{1-6} haloalkyl (In some further embodiments, each of of R^{12}, R^{13}, R^{14}, and R^{16} is H.). In some further embodiments, R^{15} is halo or C_{1-3} haloalkyl (In some further embodiments, each of of R^{12}, R^{13}, R^{14}, and R^{16} is H.). In still further embodiments, R^{15} is halo or C_1 haloalkyl (In some further embodiments, each of of R^{12}, R^{13}, R^{14}, and R^{16} is H.).

[0256] In some embodiments, in a compound of Formula V R^{15} is halo. In some embodiments, R^{15} is Cl or F. In some embodiments, R^{15} is Cl. In some embodiments, R^{15} is F.

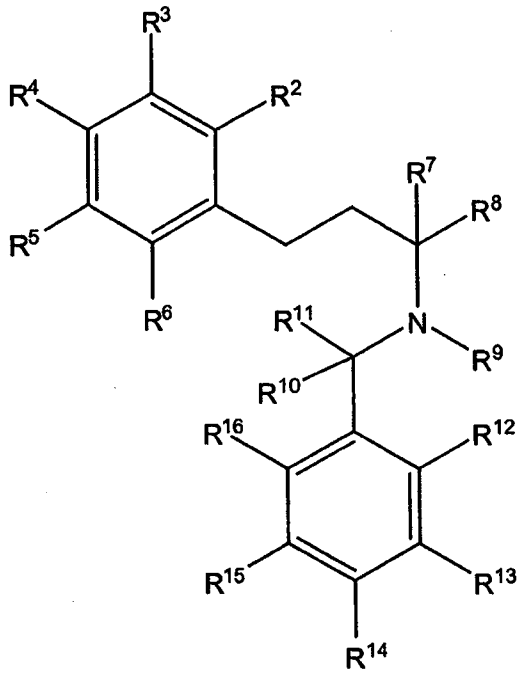
[0257] In some embodiments, in a compound of Formula V R^{15} is C_{1-6} haloalkyl. In some further embodiments, R^{15} is C_{1-3} haloalkyl. In still further embodiments, R^{15} is C_1 haloalkyl. In yet further embodiments, R^{15} is CF_3.

[0258] In some embodiments, in a compound of Formula V R^{14} and R^{15} are each independently halo or C_{1-3} haloalkyl (In some further embodiments, each of of R^{12}, R^{13}, and R^{16} is H.). In some further embodiments, R^{14} and R^{15} are each independently halo or C_1 haloalkyl.

[0259]

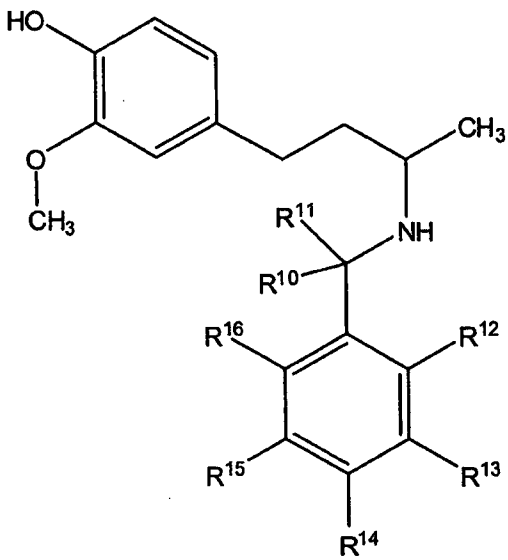
[0260] In some embodiments, in a compound of Formula V R^{14} and R^{15} are each independently halo.

[0261] In some embodiments, the compound of Formula V is a compound of Formula VI:



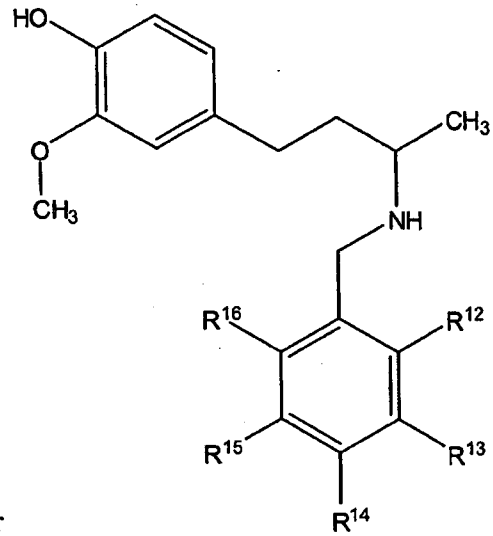
VI.

[0262] In some embodiments, the compound of Formula VI or pharmaceutically acceptable salt thereof is a compound of Formula VIa or VIb:



VIa

or



VIb

or pharmaceutically acceptable salt thereof.

[0263] In some embodiments, the compound of Formula VI is a compound of Formula VIa. In some further embodiments, R^{10} and R^{11} are each, independently, selected from H and C_{1-3} alkyl. In yet further embodiments, R^{10} and R^{11} are each, independently, selected from H and methyl. In still further embodiments, R^{10} and R^{11} are each H.

[0264] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, one of R^{10} and R^{11} is selected from H and C_{1-3} alkyl and the other is H. In some further embodiments, one of R^{10} and R^{11} is C_{1-3} alkyl. In yet further embodiments, one of R^{10} and R^{11} is methyl.

[0265] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, both of R^{10} and R^{11} are selected from C_{1-3} alkyl. In some further embodiments, both R^{10} and R^{11} are methyl.

[0266] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are each, independently, selected from H, OH, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halo, CN, NO_2, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, NH_2, NH(C_{1-4} alkyl), NH(C_{3-7} cycloalkyl), N(C_{1-4} alkyl)$_2$, 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).

[0267] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are each, independently, selected from H, halo, CN, NO_2, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, C(O)O(C_{1-4} alkyl), C(O)(C_{1-4} alkyl), and C(O)NH(C_{1-4} alkyl).

[0268] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are each, independently, selected from H, halo, CN, NO_2, C_{1-6} alkyl, C_{1-6} haloalkyl, and C_{3-7} cycloalkyl. In some further embodiments, R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are each, independently, selected from H, halo, CN, C_{1-6} alkyl, and C_{1-6} haloalkyl. In yet further embodiments, R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are each, independently, selected from H, halo, C_{1-6} alkyl, and C_{1-6} haloalkyl.

[0269] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo and C_{1-6} haloalkyl, and each of the rest is of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is H. In some further embodiments, one or two of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are selected from halo and C_{1-6} haloalkyl, and each of the rest is of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is H. In yet further embodiments, one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo and C_{1-6} haloalkyl, and each of the rest is of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is H.

[0270] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo, CN, NO_2, C_{1-6} haloalkyl, C(O)O(C_{1-4} alkyl), C(O)(C_{1-4} alkyl), and C(O)NH(C_{1-4} alkyl).

[0271] In some embodiments of the compound of Formula VIa, at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo and C_{1-6} haloalkyl.

[0272] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo and C_{1-3} haloalkyl. In some further embodiments, at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo and C_1 haloalkyl.

[0273] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, R^{14} is halo or C_{1-6} haloalkyl. In some further embodiments, R^{14} is halo or C_{1-3} haloalkyl. In still further embodiments, R^{14} is halo or C_1 haloalkyl.

[0274] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, R^{14} is halo (In some further embodiments, each of R^{12}, R^{13}, R^{15}, and R^{16} is H.). In some embodiments, R^{14} is Cl or F. In some embodiments, R^{14} is Cl. In some embodiments, R^{14} is F.

[0275] In some embodiments of the compound of Formula IIa, R^{14} is C_{1-6} haloalkyl (In some further embodiments, each of R^{12}, R^{13}, R^{15}, and R^{16} is H.). In

some further embodiments, R^{14} is C_{1-3} haloalkyl. In still further embodiments, R^{14} is C_1 haloalkyl. In yet further embodiments, R^{14} is CF_3.

[0276] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, R^{14} is halo or C_{1-6} haloalkyl and each of R^{12}, R^{13}, R^{15}, and R^{16} is H.

[0277] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, R^{15} is halo or C_{1-6} haloalkyl (In some further embodiments, each of R^{12}, R^{13}, R^{14}, and R^{16} is H.). In some further embodiments, R^{15} is halo or C_{1-3} haloalkyl. In still further embodiments, R^{15} is halo or C_1 haloalkyl.

[0278] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, R^{15} is halo. In some embodiments, R^{15} is Cl or F. In some embodiments, R^{15} is Cl. In some embodiments, R^{15} is F.

[0279] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, R^{15} is C_{1-6} haloalkyl. In some further embodiments, R^{15} is C_{1-3} haloalkyl. In still further embodiments, R^{15} is C_1 haloalkyl. In yet further embodiments, R^{15} is CF_3.

[0280] In some embodiments of the compound of Formula VIa or pharmaceutically acceptable salt thereof, R^{14} and R^{15} are each independently halo or C_{1-3} haloalkyl (In some further embodiments, each of R^{12}, R^{13}, and R^{16} is H.). In some further embodiments, R^{14} and R^{15} are each independently halo or C_1 haloalkyl. In yet further embodiments, R^{14} and R^{15} are each independently halo.

[0281] In some embodiments, the compound of Formula VI or pharmaceutically acceptable salt thereof is a compound of Formula VIb or pharmaceutically acceptable salt thereof.

[0282] In some embodiments of the compound of Formula VIb or pharmaceutically acceptable salt thereof, R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are each, independently, selected from H, OH, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halo, CN, NO_2, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, NH_2, NH(C_{1-4} alkyl), NH(C_{3-7} cycloalkyl),

$N(C_{1-4}$ alkyl$)_2$, $NHC(O)(C_{1-4}$ 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).

[0283] In some embodiments of the compound of Formula VIb or pharmaceutically acceptable salt thereof, R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are each, independently, selected from H, halo, CN, NO_2, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, $C(O)O(C_{1-4}$ alkyl), $C(O)(C_{1-4}$ alkyl), and $C(O)NH(C_{1-4}$ alkyl).

[0284] In some embodiments of the compound of Formula IIb or pharmaceutically acceptable salt thereof, R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are each, independently, selected from H, halo, CN, NO_2, C_{1-6} alkyl, C_{1-6} haloalkyl, and C_{3-7} cycloalkyl. In some further embodiments, R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are each, independently, selected from H, halo, CN, C_{1-6} alkyl, and C_{1-6} haloalkyl. In yet further embodiments, R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are each, independently, selected from H, halo, C_{1-6} alkyl, and C_{1-6} haloalkyl.

[0285] In some embodiments of the compound of Formula VIb or pharmaceutically acceptable salt thereof, at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo and C_{1-6} haloalkyl, and each of the rest is of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is H. In some further embodiments, one or two of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} are selected from halo and C_{1-6} haloalkyl, and each of the rest is of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is H. In yet further embodiments, one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo and C_{1-6} haloalkyl, and each of the rest is of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is H.

[0286] In some embodiments of the compound of Formula VIb or pharmaceutically acceptable salt thereof, at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo, CN, NO_2, C_{1-6} haloalkyl, $C(O)O(C_{1-4}$ alkyl), $C(O)(C_{1-4}$ alkyl), and $C(O)NH(C_{1-4}$ alkyl).

[0287] In some embodiments of the compound of Formula VIb, at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo and C_{1-6} haloalkyl.

[0288] In some embodiments of the compound of Formula VIb or pharmaceutically acceptable salt thereof, at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is

selected from halo and C_{1-3} haloalkyl. In some further embodiments, at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is selected from halo and C_1 haloalkyl.

[0289] In some embodiments of the compound of Formula VIb or pharmaceutically acceptable salt thereof, R^{14} is halo or C_{1-6} haloalkyl. In some further embodiments, R^{14} is halo or C_{1-3} haloalkyl. In still further embodiments, R^{14} is halo or C_1 haloalkyl.

[0290] In some embodiments of the compound of Formula VIb or pharmaceutically acceptable salt thereof, R^{14} is halo (In some further embodiments, each of R^{12}, R^{13}, R^{15}, and R^{16} is H.). In some embodiments, R^{14} is Cl or F. In some embodiments, R^{14} is Cl. In some embodiments, R^{14} is F.

[0291] In some embodiments of the compound of Formula VIb, R^{14} is C_{1-6} haloalkyl (In some further embodiments, each of R^{12}, R^{13}, R^{15}, and R^{16} is H.). In some further embodiments, R^{14} is C_{1-3} haloalkyl. In still further embodiments, R^{14} is C_1 haloalkyl. In yet further embodiments, R^{14} is CF_3.

[0292] In some embodiments of the compound of Formula VIb or pharmaceutically acceptable salt thereof, R^{14} is halo or C_{1-6} haloalkyl and each of R^{12}, R^{13}, R^{15}, and R^{16} is H.

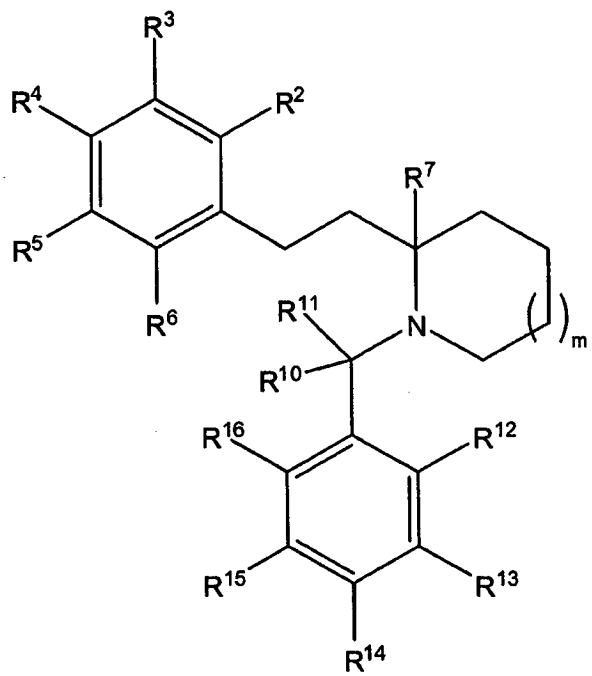
[0293] In some embodiments of the compound of Formula VIb or pharmaceutically acceptable salt thereof, R^{15} is halo or C_{1-6} haloalkyl (In some further embodiments, each of R^{12}, R^{13}, R^{14}, and R^{16} is H.). In some further embodiments, R^{15} is halo or C_{1-3} haloalkyl. In still further embodiments, R^{15} is halo or C_1 haloalkyl.

[0294] In some embodiments of the compound of Formula VIb or pharmaceutically acceptable salt thereof, R^{15} is halo. In some embodiments, R^{15} is Cl or F. In some embodiments, R^{15} is Cl. In some embodiments, R^{15} is F.

[0295] In some embodiments of the compound of Formula VIb or pharmaceutically acceptable salt thereof, R^{15} is C_{1-6} haloalkyl. In some further embodiments, R^{15} is C_{1-3} haloalkyl. In still further embodiments, R^{15} is C_1 haloalkyl. In yet further embodiments, R^{15} is CF_3.

[0296] In some embodiments of the compound of Formula VIb or pharmaceutically acceptable salt thereof, R^{14} and R^{15} are each independently halo or C_{1-3} haloalkyl (In some further embodiments, each of R^{12}, R^{13}, and R^{16} is H.). In some further embodiments, R^{14} and R^{15} are each independently halo or C_1 haloalkyl. In yet further embodiments, R^{14} and R^{15} are each independently halo.

[0297] In some embodiments, the compound of Formula V is a compound of Formula VII:



VII.

[0298] In some embodiments of compounds of Formula VII or pharmaceutically acceptable salt thereof, m is 1.

[0299] In some embodiments of compounds of Formula VII or pharmaceutically acceptable salt thereof, m is 0.

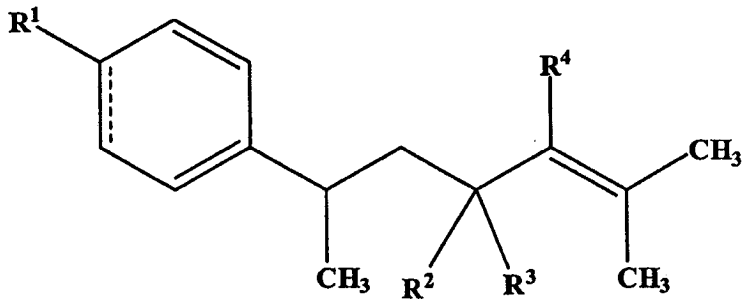
[0300] In some embodiments of compounds of Formula VII or pharmaceutically acceptable salt thereof, at least one of R^2, R^3, R^4, R^5, and R^6 is selected from OH, C_{1-6} alkoxy, and C_{1-6} haloalkoxy.

[0301] In some embodiments of compounds of Formula VII or pharmaceutically acceptable salt thereof, at least two of R^2, R^3, R^4, R^5, and R^6 are independently selected from OH, C_{1-6} alkoxy, and C_{1-6} haloalkoxy.

[0302] In some embodiments of compounds of Formula VII or pharmaceutically acceptable salt thereof, at least one of R^{12}, R^{13}, R^{14}, R^{15}, and R^{16} is other than H.

[0303] In some embodiments of the compound of Formula VII or pharmaceutically acceptable salt thereof, R^{14} and R^{15} are each independently halo or C_{1-3} haloalkyl. In some further embodiments, R^{14} and R^{15} are each independently halo or C_1 haloalkyl.

[0304] In some embodiments, the sigma-2 ligand is a a compound of Formula VIII:



VIII

or pharmaceutically acceptable salt thereof, wherein:

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

R_1 is H, CH_3, CF_3, F, Cl, Br, or $-OCF_3$;

R_2 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl is substituted by 0, 1, 2, 3, 4, or 5 substituents each independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy;

R^3 is OH or $NR_{3a}NR_{3b}$;

R_{3a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, cycloalkylalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl is substituted by 0, 1, 2, 3, 4, or 5 substituents each independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy;

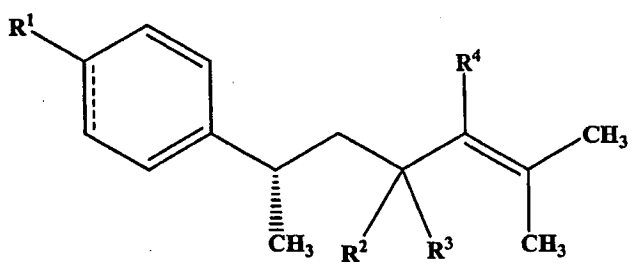
R^{3b} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, cycloalkylalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl is substituted by 0, 1, 2, 3, 4, or 5 substituents each independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy;

or R^{3a} and R^{3b} together with the N atom to which they are attached form a 4-, 5-, 6- or 7-membered heterocycloalkyl group that is substituted with 0, 1, 2, 3, 4, or 5 substituents each independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, and heterocycloalkyl; and

R^4 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl is substituted by 0, 1, 2, 3, 4, or 5 substituents each independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy.

[0305] In some embodiments of compound VIII, when ⚌ is a double bond and R^3 is OH, then at least one of R^1, R^2, and R^4 is other than H. In some embodiments, the compound of Formula VIII is other than 2-methyl-6-*p*-tolylhept-2-en-4-ol.

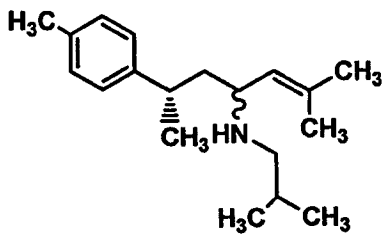
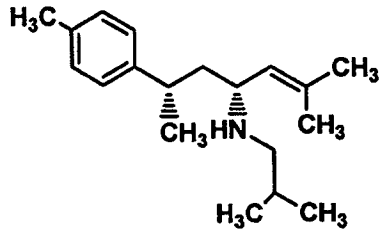
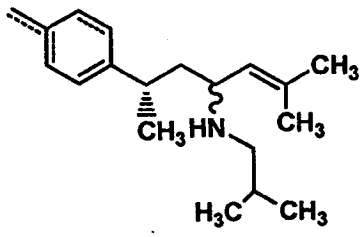
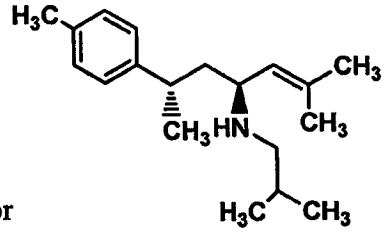
[0306] In some embodiments, the compound of the present invention or pharmaceutically acceptable salt thereof is a compound of Formula VIIIa:



VIIIa

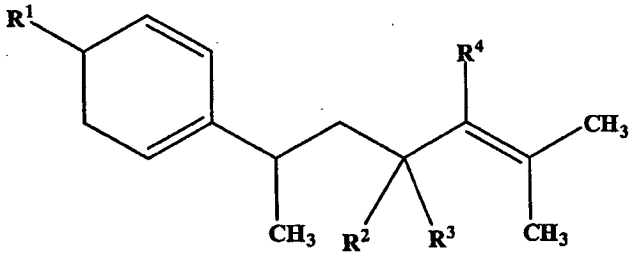
or pharmaceutically acceptable salt thereof.

[0307] In some embodiments the species of VIII are selected from one or more of the compounds:




or


[0308] In some embodiments, the compound of Formula VIIIa is other than (6*S*)-2-methyl-6-*p*-tolylhept-2-en-4-ol.

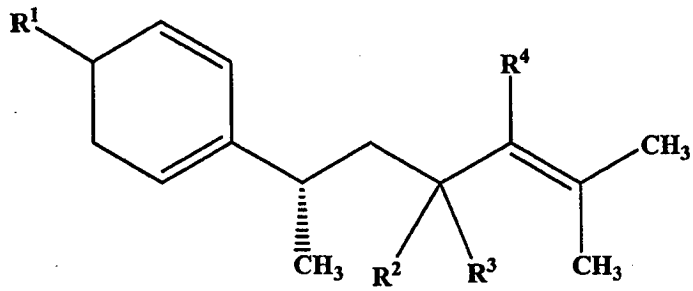
[0309] In some embodiments, the compound of the present invention or pharmaceutically acceptable salt thereof is a compound of Formula VIIIb:



VIIIb

or pharmaceutically acceptable salt thereof.

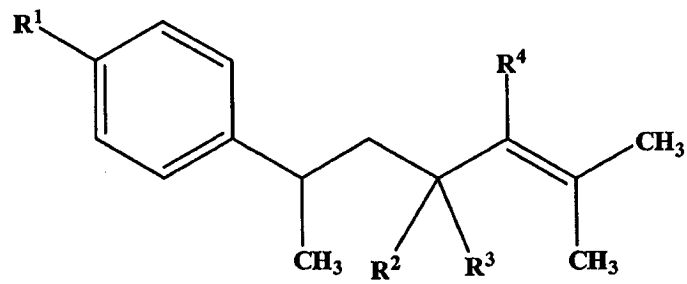
[0310] In some embodiments, the sigma-2 ligand of the present invention or pharmaceutically acceptable salt thereof is a compound of Formula VIIIc:



VIIIc

or pharmaceutically acceptable salt thereof.

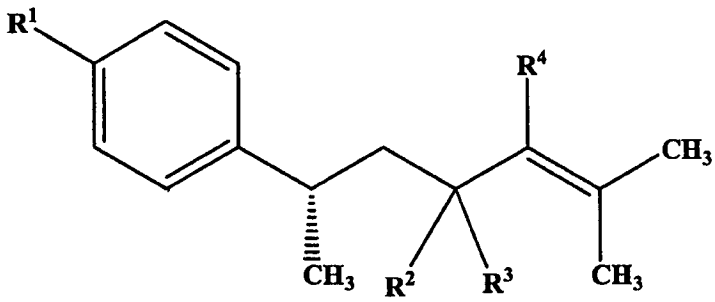
[0311] In some embodiments, the sigma-2 ligand of the present invention or pharmaceutically acceptable salt thereof is a compound of Formula VIIId:



VIIId

or pharmaceutically acceptable salt thereof.

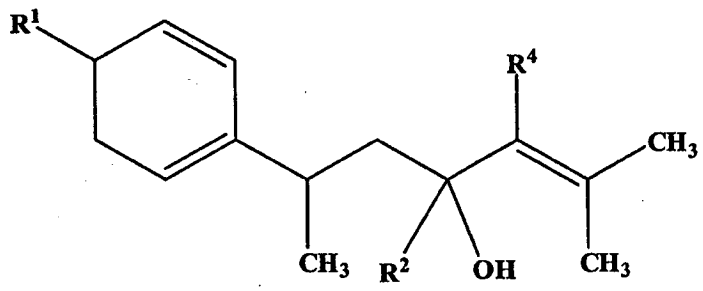
[0312] In some embodiments, the compound of the present invention or pharmaceutically acceptable salt thereof is a compound of Formula VIIIe:



VIIIe

or pharmaceutically acceptable salt thereof.

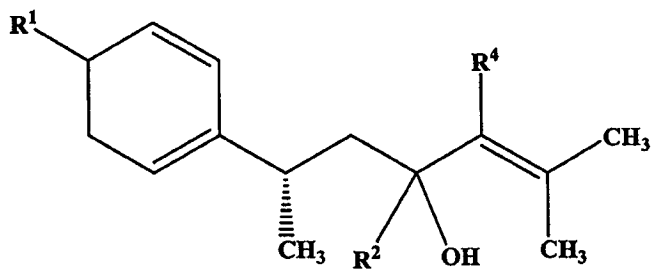
[0313] In some embodiments, the sigma-2 ligand contemplated in the present invention or pharmaceutically acceptable salt thereof is a compound of Formula VIIIf:



VIIIf

or pharmaceutically acceptable salt thereof.

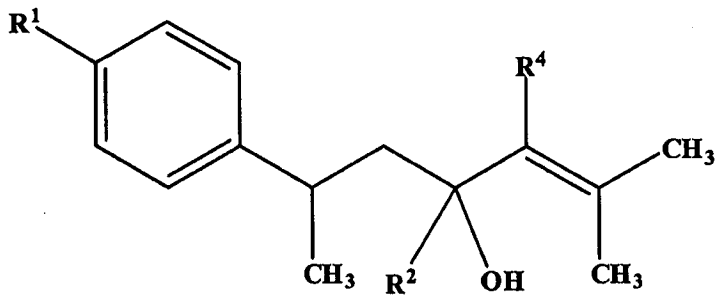
[0314] In some embodiments, the compound of the present invention or pharmaceutically acceptable salt thereof is a compound of Formula VIIIg:



VIIIg

or pharmaceutically acceptable salt thereof.

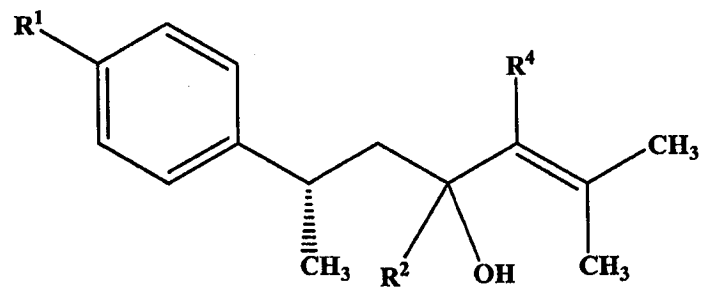
[0315] In some embodiments, the sigma-2 ligand contemplated by the present invention or pharmaceutically acceptable salt thereof is a compound of Formula VIIIh:



VIIIh

or pharmaceutically acceptable salt thereof.

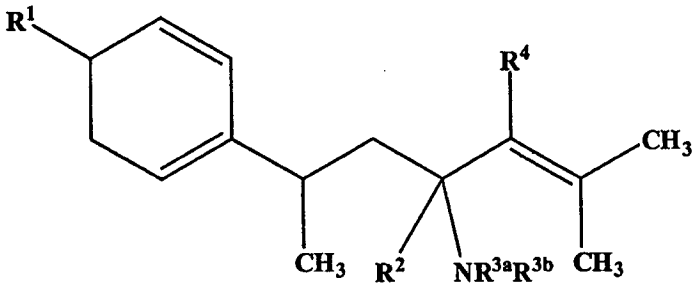
In some embodiments, the compound of the present invention or pharmaceutically acceptable salt thereof is a compound of Formula VIIIi:



VIIIi

or pharmaceutically acceptable salt thereof.

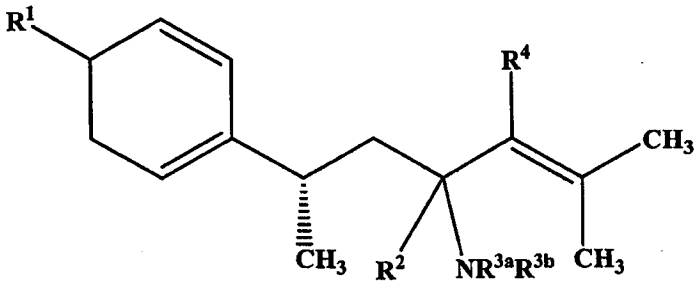
[0316] In some embodiments, the compound of the present invention or pharmaceutically acceptable salt thereof is a compound of Formula VIIIj:



VIIIj

[0317] or pharmaceutically acceptable salt thereof.

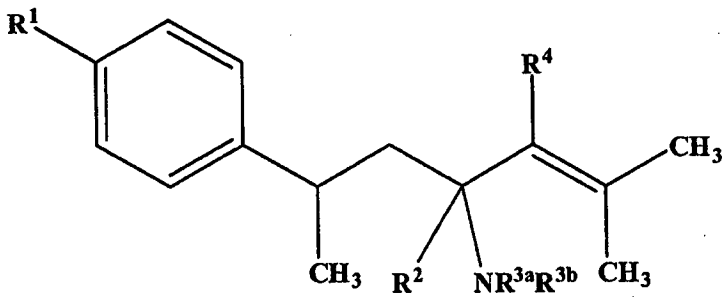
[0318] In some embodiments, a sigma-2 ligand contemplated by the present invention or pharmaceutically acceptable salt thereof is a compound of Formula VIIIk:



VIIIk

or pharmaceutically acceptable salt thereof.

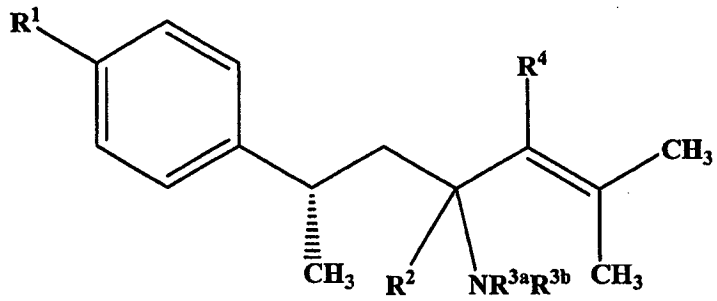
[0319] In some embodiments, the compound of the present invention or pharmaceutically acceptable salt thereof is a compound of Formula VIIIm:



VIIIm

or pharmaceutically acceptable salt thereof.

[0320] In some embodiments, the compound of the present invention or pharmaceutically acceptable salt thereof is a compound of Formula VIIIn:



VIIIn

or pharmaceutically acceptable salt thereof.

[0321] In some embodiments of the compound of VIII and subset formulae thereof, R^1 is H, CH_3, or CF_3. In some further embodiments, R^1 is CH_3 or CF_3. In yet further embodiments, R^1 is CH_3.

[0322] In some embodiments of the compound of VIII and subset formulae thereof, R^1 is H or CH_3.

[0323] In some embodiments of the compound of VIII and subset formulae thereof, R^1 is F, Cl, or Br. In other embodiments, R^1 is OCF_3.

[0324] In some embodiments of the compound of VIII and subset formulae thereof, R^2 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl. In some further embodiments, R^2 is H, C_{1-6} alkyl, or C_{3-7} cycloalkyl.

[0325] In some embodiments of the compound of VIII and subset formulae thereof, R^2 is H or C_{1-6} alkyl. In some further embodiments, R^2 is H or methyl. In yet further embodiments, R^2 is H.

[0326] In some embodiments of the compound of VIII and subset formulae thereof, R^1 is H, CH_3, or CF_3; and R^2 is H or C_{1-6} alkyl. In some further embodiments, R^1 is CH_3 or CF_3; and R^2 is H. In yet further embodiments, R^1 is CH_3; and R^2 is H.

[0327] In some embodiments of the compound of VIII and subset formulae thereof, R^{3a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, cycloalkylalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, cycloalkylalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl is substituted by 0, 1, 2, 3, 4, or 5 substituents each independently selected from halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy. In some further embodiments, R^{3a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, cycloalkylalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl.

[0328] In some embodiments of the compound of VIII and subset formulae thereof, R^{3b} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, cycloalkylalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, cycloalkylalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl is substituted by 0, 1, 2, 3, 4, or 5 substituents each independently selected from halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy. In some further embodiments, R^{3b} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, cycloalkylalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl.

[0329] In some embodiments of the compound VIII and subset formulae thereof, R^{3a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl is substituted by 0, 1, 2, 3, 4, or 5 substituents each independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy.

[0330] In some embodiments of the compound of VIII and subset formulae thereof, R^{3b} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl is substituted by 0, 1, 2, 3, 4, or 5 substituents each independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy.

[0331] In some embodiments of the compound of VIII and subset formulae thereof, R^{3a} is H; and R^{3b} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, cycloalkylalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, cycloalkylalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl is substituted by 0, 1, 2, 3, 4, or 5 substituents each independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy. In some further embodiments, R^{3a} is H;

and R^{3b} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, cycloalkylalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl, wherein each of the C_{1-6} alkyl, C_{1-6} haloalkyl, cycloalkylalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl is substituted by 0, 1, 2, 3, 4, or 5 substituents each independently selected from halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy.

[0332] In some embodiments of the compound of VIII and subset formulae thereof, R^{3a} is H; and R^{3b} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, cycloalkylalkyl, C_{3-7} cycloalkyl, arylalkyl, or C_{6-10} aryl.

[0333] In some embodiments of the compound of VIII and subset formulae thereof, R^{3a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl; and R^{3b} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl.

[0334] In some embodiments of the compound of VIII and subset formulae thereof, R^{3a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl; and R^{3b} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl;

[0335] In some embodiments of the compound of VIII and subset formulae thereof, R^{3a} is H; and R^{3b} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, or C_{3-7} cycloalkyl.

[0336] In some embodiments of the compound of VIII and subset formulae thereof, R^{3a} is H; and R^{3b} is C_{1-6} alkyl or C_{1-6} haloalkyl.

[0337] In some embodiments of the compound of VIII and subset formulae thereof, R^{3a} is H; and R^{3b} is C_{1-6} alkyl.

[0338] In some embodiments of the compound of VIII and subset formulae thereof, R^{3a} and R^{3b} together with the N atom to which they are attached form pyrrolidinyl, piperidinyl, piperazinyl, or morpholinyl, each substituted with 0, 1, 2, 3, 4, or 5 substituents each independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, and heterocycloalkyl.

[0339] In some embodiments of the compound of VIII and subset formulae thereof, R^{3a} and R^{3b} together with the N atom to which they are attached form

pyrrolidinyl, piperidinyl, piperazinyl, or morpholinyl, each substituted with 0, 1, 2, 3, 4, or 5 substituents each independently selected from OH, amino, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, phenyl, and benzyl.

[0340] In some embodiments of the compound of VIII and subset formulae thereof, R^4 is H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, or C_{6-10} aryl. In some further embodiments, R^4 is H, C_{1-6} alkyl, or C_{3-7} cycloalkyl.

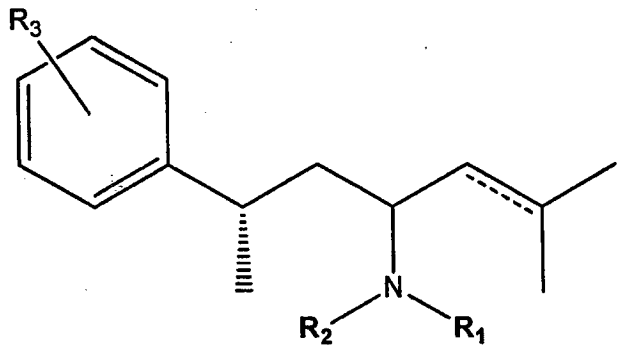
[0341] In some embodiments, R^4 is H or C_{1-6} alkyl. In some further embodiments, R^4 is H or methyl. In yet further embodiments, R^4 is H.

[0342] In some embodiments of the compound of VIII and subset formulae thereof, R^2 is H or C_{1-6} alkyl, and R^4 is H or C_{1-6} alkyl.

[0343] In some embodiments of the compound of VIII and subset formulae thereof, R^2 is H or methyl, and R^4 is H or methyl.

[0344] In some embodiments of the compound of Formula VIII and subset formulae thereof, R^1 is H, CH_3, or CF_3; R^2 is H or C_{1-6} alkyl, and R^4 is H or C_{1-6} alkyl. In some further embodiments, R^1 is CH_3 or CF_3; R^2 is H; and R^4 is H. In yet further embodiments, R^1 is CH_3; R^2 is H; and and R^4 is H.

[0345] In another embodiment, the sigma-2 ligands of the present invention are those of Formula VIIIo



VIIIo

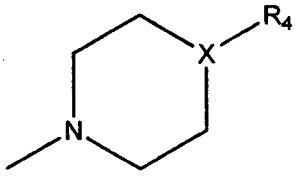
wherein:

======== 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



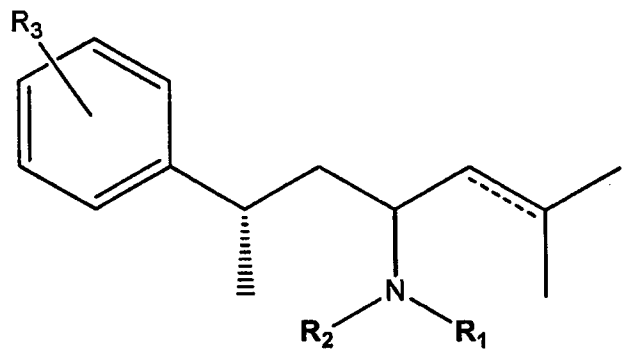
, wherein

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.

[0346] In some embodiments, the sigma-2 ligands of the present invention are those of Formula VIIIo



VIIIo

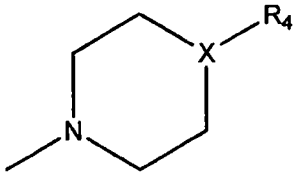
wherein:

-------- 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



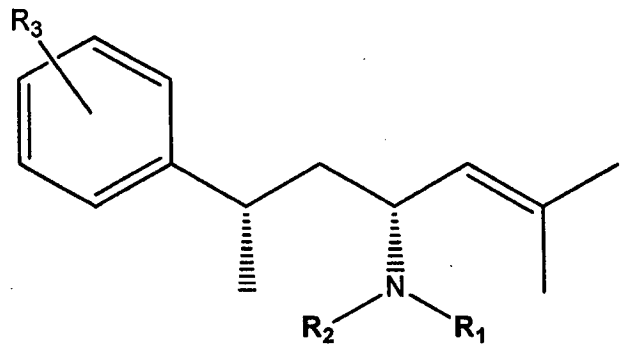
, wherein

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.

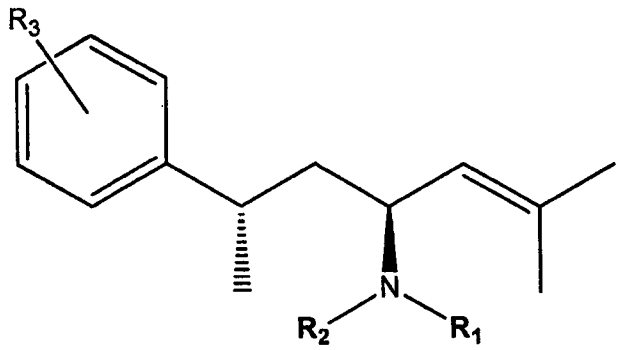
[0347] In some more specific embodiments, the sigma-2 ligands of the present invention are those of Formula VIIIp



VIIIp

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

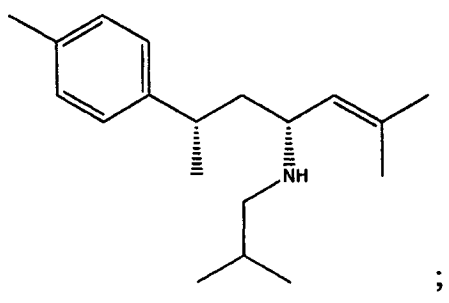
[0348] In some more specific embodiments, the sigma-2 ligands of the present invention are those of Formula VIIIq



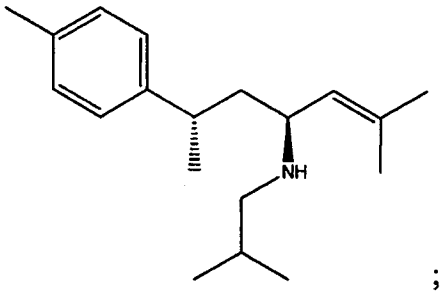
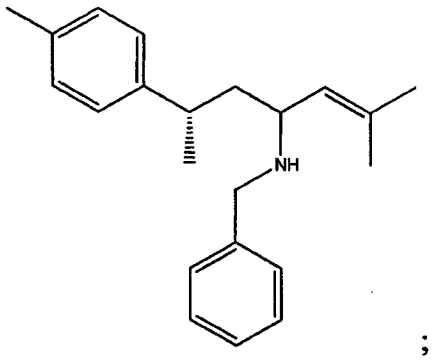
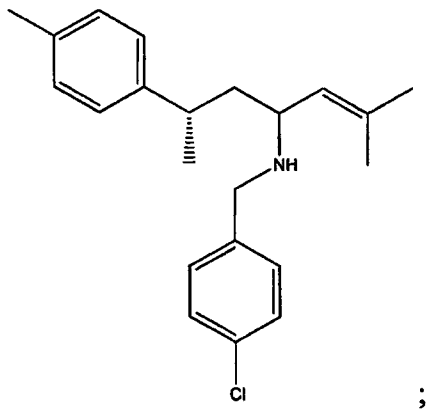
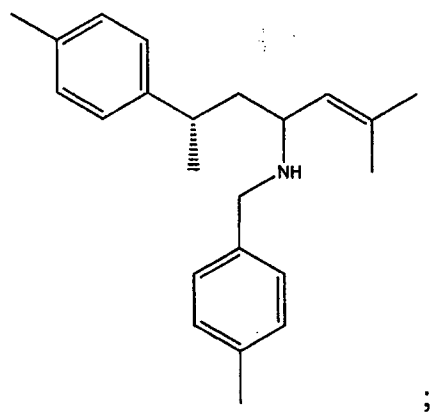
VIIIq

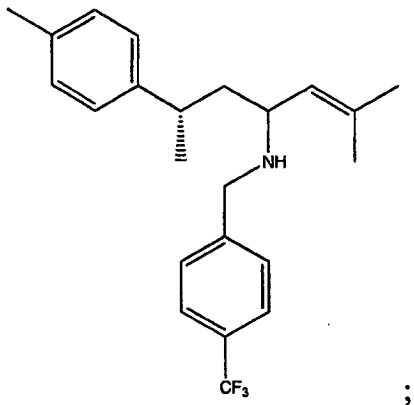
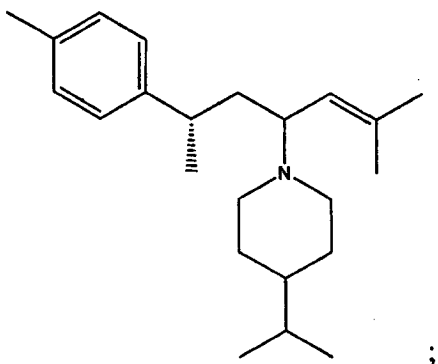
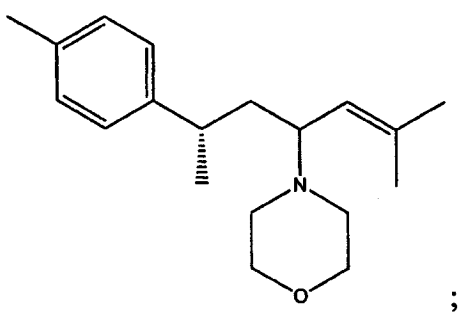
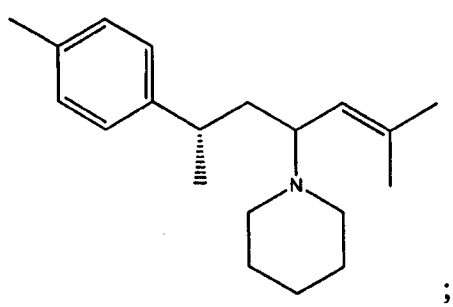
wherein R_1-R_3 are as defined above for Formula VIIIa, or pharmaceutically acceptable salts thereof.

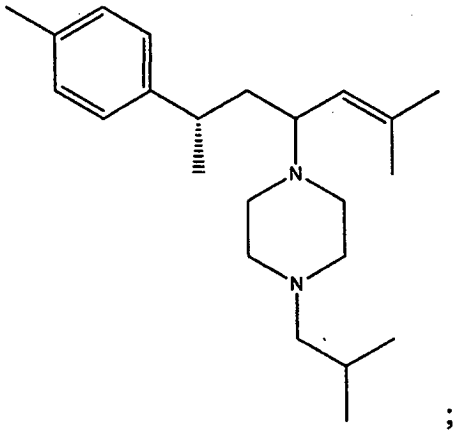
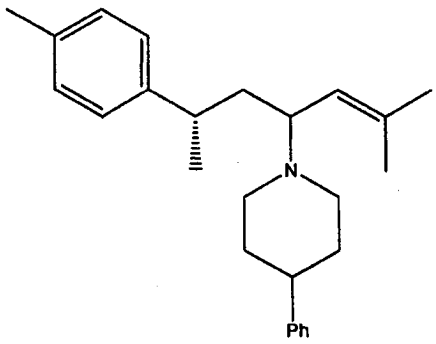
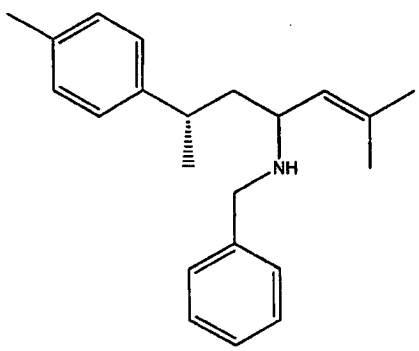
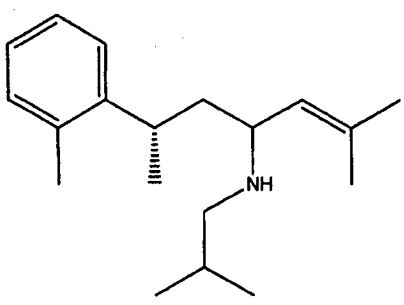
[0349] **Table 1C**: Compounds of Formula VIIIo-q Specific exemplary compounds of the invention are set forth in the table below:

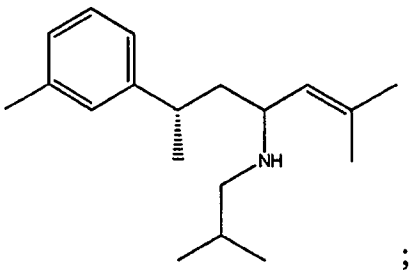
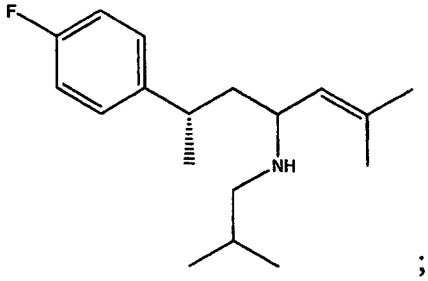
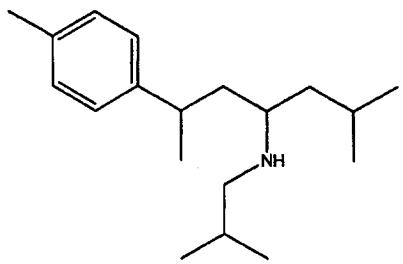
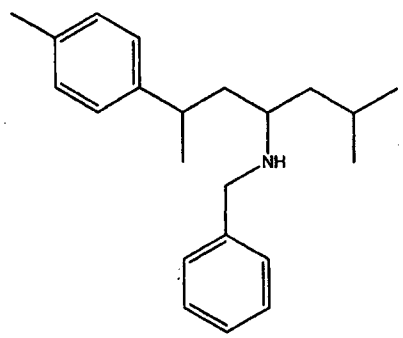
Compounds of Formulae VIIIo-q


;

Compounds of Formulae VIIIo-q
 ;
 ;
 ;
 ;

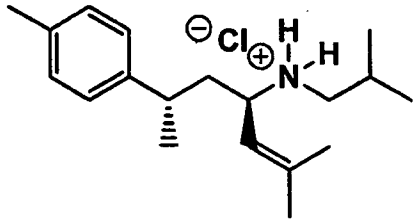
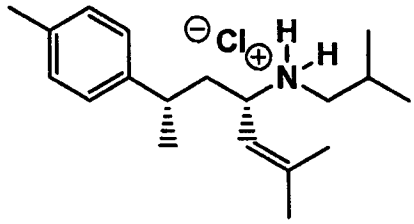
Compounds of Formulae VIIIo-q
 ;
 ;
 ;
 ;

Compounds of Formulae VIIIo-q
 ;
 ;
 ;
 ;

Compounds of Formulae VIIIo-q
 ;
 ;
 ; and
 ,

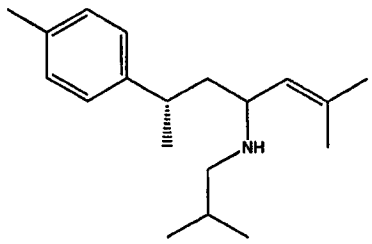
, or pharmaceutically acceptable salts thereof.

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

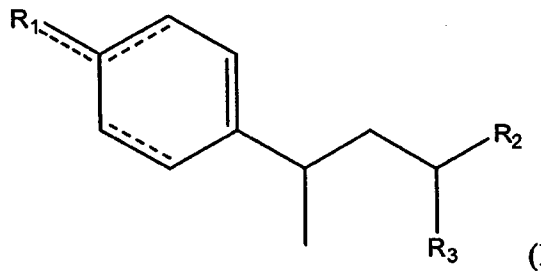



and

[0350] In some embodiments, each of the general formulae above may contain a proviso to remove one or more of the following compound:

[0351] 

[0352] In a further embodiment, the sigma-2 ligand is a compound of Formula IX:



(IX)

or pharmaceutically acceptable salt thereof, wherein:

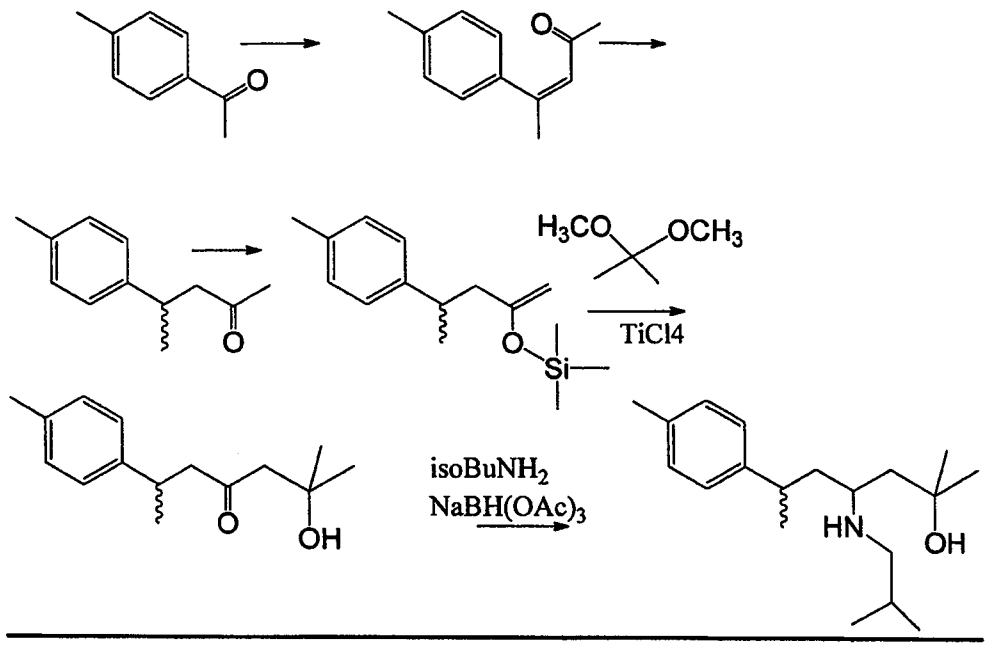
--------- is a single bond or a double bond;

R_1 is selected from CH_3, CH_2F, Cl, Br, CF_3, O-alkyl and OCF_3;

R_2 is selected from $CH_2C(CH_3)_2OH$, and $CH=C(CH_3)_2$;

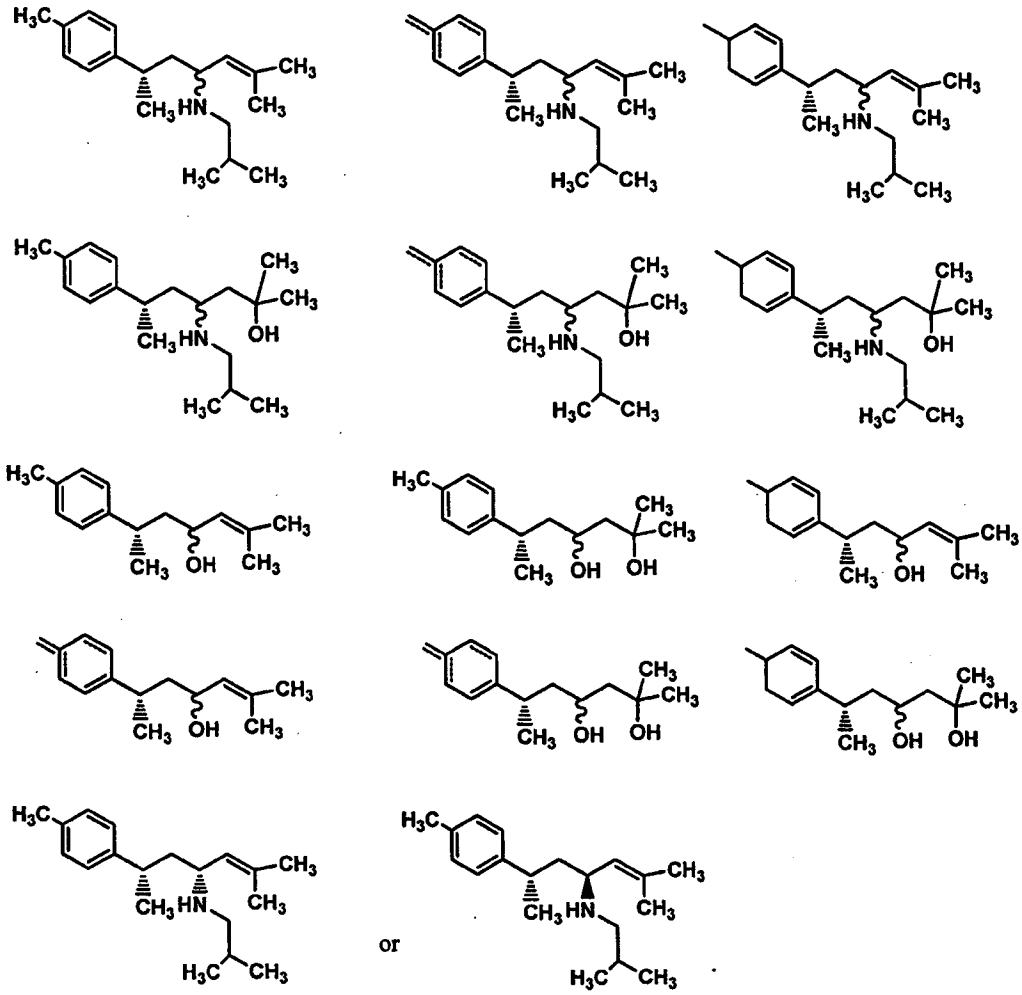
R_3 is selected from OH, or $NHCH_2CH(CH_3)_2$, or mixtures thereof.

[0353] In some embodiments, the compounds of Formula IX can be prepared by reductive amination, for example, the route shown in Scheme 8.

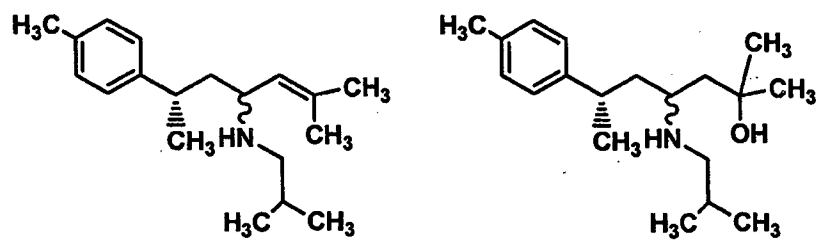


Scheme 8.

[0354] In some embodiments, the species of formula IX can be selected from:



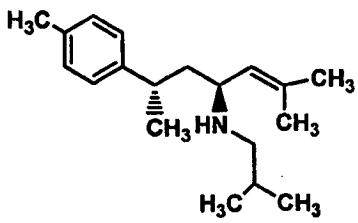
[0355] Examples of compounds of Formula IX include compounds below, which are mixtures of diastereomers, and including active aromatic amine alkenes and amino alcohol components IXa and IXb.



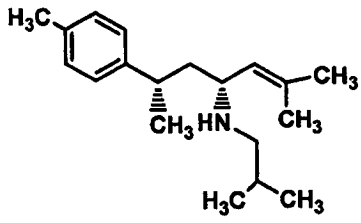
IXa IXb

[0356] In specific embodiments, the sigma-2 receptor antagonists are selected from compoundsIXa and IXb, as well as enantiomers and pharmaceutically acceptable salts.

[0357] In specific embodiments, the selective, high affinity sigma-2 receptor antagonists are selected from IXa-1 and IXa-2.



IXa-1



IXa-2

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

[0358] The present invention further encompasses salts, solvates, stereoisomers, prodrugs and active metabolites of the compounds of any of the formulae above.

[0359] 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).

[0360] In some embodiments, the sigma-2 receptor ligand compound is selected from the compounds in the Table 1D below.

[0361] **Table 1D.** Sigma-2 Receptor Ligands Hydrochloride Salt Compounds.

HCl Salt Formulation	Sigma-1 (Ki,nM)	Sigma-2 (Ki,nM)	MTTX (EC50,uM)
W	270	120	1.2
CF	180	50	12
CB	19	48	6.5
CU	5.8	1.3	3.9
DC	330	3200	10
DB	530	4000	5

[0362] The acid addition salts of the compounds of any of the formulae above 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.

[0363] 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, e.g., formula I compound or salt, with 1, 2 or 3 equivalents, preferably 1 equivalent, of acid per mole of base of a any of the formulae above compound.

[0364] 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.

[0365] 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.

[0366] 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.

[0367] 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.

[0368] Typically, a pharmaceutically acceptable salt of a compound of any of the formulae above 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 any of the formulae above and the resulting mixture evaporated to dryness (lyophilized) to obtain the acid addition salt as a solid. Alternatively, a compound of any of the formulae above 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.

[0369] 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 any of the formulae above 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.).

[0370] 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.

[0371] Compounds of any of the formulae above 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:

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

[0372] 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:

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

[0373] 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.

[0374] The present invention encompasses all individual isomers of the compounds of any of the formulae above. 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 any of the formulae above which gives rise to one set of enantiomers. Additional chiral centers may be present depending on the substituents.

[0375] 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.

[0376] 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.

[0377] 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.

[0378] 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.

[0379] 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.

[0380] 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.

[0381] 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 maybe 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.

[0382] Where applicable, compounds of any of the formulae above 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.

[0383] 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).

[0384] The absolute configuration of the stereocenters can be determined by very well known method 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).

[0385] 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).

[0386] 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.

[0387] The present invention also encompasses prodrugs of the compounds of any of the formulae above, i.e., compounds which release an active drug according to any of the formulae above *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 any of the formulae above are prepared by modifying functional groups present in the compound of any of the formulae above 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 any of the formulae above 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 any of the formulae above 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).

[0388] 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.

[0389] Unless specifically indicated, the term "active ingredient" is to be understood as referring to a compound of any of the formulae above as defined herein.

[0390] 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.

Use of the Sigma-2 Receptor Antagonists

[0391] 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 by administration of a sigm-2 receptor antagonist. 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 antagonist described herein, e.g., those encompassed by any of the formulae 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), long term depression (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.

[0392] In some embodiments, the activity/effect of one or more of Abeta oligomers on a neuron cell, amyloid aggregation and amyloid binding is the effect of Abeta oligomers on membrane trafficking or synapse number. In some embodiments, the sigma-2 antagonist inhibits the Abeta oligomer effect on membrane trafficking or synapse number or Abeta oligomer binding.

[0393] In some embodiments, the present invention provides methods of treating a proteopathic disease associated with Abeta oligomer toxicity, specifically nomlethat Abeta oligomer effects. In some embodiments, the method comprises contacting a subject with such a proteopathic disease with a sigma-2 antagonist of the present invention or a composition containing the same that binds the sigma-2 receptor.

[0394] 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.

[0395] In some embodiments, the present invention provides methods of treating one or more mild cognitive impairment (MCI), or dementia by administering a sigma-2 antagonist in accordance with the invention. In some embodiments, the present invention provides methods of treating MCI, and dementia.

[0396] In some embodiments, the present invention provides methods of treating an individual with a sigma-2 antagonist 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.

[0397] 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:

[0398] Sigma-2 receptor ligands within the formulae above, have been shown to be selective high affinity sigma-2 receptor ligands. For example Compound II exhibits K_i 9+/-4 nM at displacement of [^{3}H]DTG/300 nM (+)-pentazocine, at sigma-2 receptors in rat neocortex homogenate and Ki of 500+/- 200 nM at displacement of [3H]-((+)-pentazocine, at sigma-1 receptors in human Jurkat cell membranes. Compound IXa,IXb exhibits Ki of 54+/-22 nM at displacement of [^{3}H]DTG/300 nM (+)-pentazocine, at sigma-2 receptors in rat neocortex homogenate and Ki of 31+/12 nM at displacement of [3H]-((+)-pentazocine, at sigma-1 receptors in human Jurkat cell membranes. Similarly, Compound II exhibits K_i 59.7+/-10.4 nM at displacement of [^{3}H]DTG/500 nM (+)-pentazocine, at sigma-2 receptors in rat liver homogenate and Ki of 108.1+/- 19.9 nM at displacement of [3H]-((+)-pentazocine, at sigma-1 receptors in guinea pig brain membranes. Compound IXa,IXb exhibits Ki of 30.8+/-2.3 nM at displacement of [^{3}H]DTG/500 nM (+)-pentazocine, at sigma-2 receptors in rat liver homogenate and Ki of 6.37+/0.81 nM at displacement of [3H]-((+)-pentazocine, at sigma-1 receptors in guinea pig brain membranes

[0399] Sigma-2 receptor ligands within the formulae above, have been shown to act as sigma-2 receptor functional neuronal antagonists; for example, Compounds II, and IXa and IXb have been shown herein to inhibit synapse reduction associated with soluble Abeta oligomers 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 the formulae above have also been shown to inhibit abnormalities in membrane

trafficking. Compound II, and Compounds IXa and IXb have 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 the Formulae above, such as Compound B, 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 strong role[see this] to Abeta oligomers and other Abeta species such as assemblies in the development of amyloid pathology, such as that of early stages of Alzheimer's disease, it is anticipated that Compound II and other compounds disclosed herein 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 Formulae I, II, III, IV, V, VI and VII among those specifically disclosed above, all the compounds within Formulae I,II, III, IV, V, VI and VII are expected to be similarly active in vivo. Likewise, because of their structural similarity to compounds IXa and IXb and because there has been confirmation of the foregoing in vitro activities for compounds IXa and IXb, all the compounds within Formule VIII and IX, and especially IX, are expected to be similarly active in vivo as well.

[0400] 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 memories (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. See, for example, FIGs 4 and 8. 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.

[0401] 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, in the aforementioned fear conditioning assay, Compound II and Compound IXa, IXb significantly inhibited the effect of hippocampal Abeta oligomer exposure of wild-type mice, preserving the ability of the mice to acquire new memory.

[0402] 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. Despite its different structure, Compound IXa/IXb has similar activites in vitro and in vivo and is also a sigma-2 antagonist. Lastly, several sigma-2 antagonists also show activity in vitro despite different structures. Accordingly, combined, these results indicate that Compound II can be used to treat neurodegenerative diseases, such as Alzheimer's Disease. Other compounds within Formula I, II, III, IV, V, VI and VII have also been found to bind to sigma-2 receptor and to have similar in vitro activities as Compound II. Based on their similarity with Compound II they are expected to have similar activity in vitro and in vivo to 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 similar activities in vivo and therefore be useful for the same therapeutic indications. A number of other sigma-2 antagonist compounds within I, II, III, IV, V, VI and VII 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 abnormalities and to be similarly active in inhibiting, e,g. cognitive decline and treat Alzheimer's disease. Likewise, because compounds IXa and IXb were also shown to be active in in vitro and in vivo models, the compounds of Formula VIII and IX, and especially IX, which are structurally similar are also generally expected to have activity both in vitro and in vivo and, therefore, useful in the treatment methods of the present invention. These compounds, to the extent they have been tested are sigma-2 ligands and the remainder are expected to be sigma-2 ligands.

[0403] 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 oligomer-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 early Alzheimer's disease and mild cognitive impairment.

[0404] In some embodiments, a compound of any of the formulae above 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 effect 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 nervous system neurons).

[0405] 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 antagonist and the Abeta species wherein the sigma-2 antagonist 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 antagonist according to the invention. The present inventors have adduced biochemical assay evidence that compounds of the invention also inhibit amyloid aggregation (data not shown).

[0406] 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.

[0407] 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.

[0408] 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, III, IV, V, VI and VII 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.

Receptor Binding Assays and Compound Screening

[0409] 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 any formulae above and other compounds described as sigma-2 ligands above, wherein a test compound that competitively inhibits the binding of the known ligand is identified as a sigma-2 receptor ligand.

[0410] 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.

[0411] 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.

[0412] 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).

[0413] 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.

[0414] In some embodiments, the method further comprises determining whether a compound that binds to a sigma-2 receptor acts 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, 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.

[0415] 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.

[0416] 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.

[0417] 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.

[0418] 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.

[0419] 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 Beta as Used in the Present Disclosure

[0420] 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.

[0421] 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.

[0422] Soluble Abeta peptide is likely to play a key role during early stages of AD by perturbing synaptic dusfunction 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-dependnet 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.

[0423] 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 isincorporated 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 potention caused by exposure of neurons to Abeta oligomers.

[0424] 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.

[0425] 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.

[0426] 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.

[0427] 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.

[0428] 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.

[0429] 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.

[0430] 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.

Identification of subjects having or at risk of having Alzheimer's Disease

[0431] 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.

[0432] 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.

[0433] 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 knowndiagnostic 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

[0434] 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.

[0435] 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.

[0436] 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.

[0437] 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.

Combinations

[0438] 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.

[0439] 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 TNFalpha 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.

[0440] 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.

[0441] 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.

[0442] 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.

[0443] With respect to biologics such as monoclonal antibodies or fragments, suitable excipients will be employed to prevent aggregation and stabilize the antibody or fragment in solution with low endotoxin, generally for parenteral, for example, intravenous, administration. For example, see Formulation and Delivery Issues for Monoclonal Antibody Therapeutics, Daugherty et al., in Current Trends in Monoclonal Antibody Development and Manufacturing, Part 4, 2010, Springer, New York pp 103-129.

[0444] 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).

Routes of Administration and Unit Dosage Forms

[0445] 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. Administration of an antibody or fragment will generally be by parenteral means.

[0446] 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 12B 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 deliveryIn 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. . For example, an anti-sigma-2 receptor antibody such as an anti-PGRMC1 antibody that blocks binding of Abeta oligomers to the sigma-2 receptor can be formulated for parenteral delivery.

[0447] 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).

[0448] 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.

The antibody or antibody fragment molecules of the present invention can be formulated and administered by any of a number of routes and are administered at a concentration that is therapeutically effective in the indication or for the purpose sought. To accomplish this goal, the antibodies may be formulated using a variety of acceptable excipients known in the art. Typically, the antibodies are administered by injection, for example, intravenous injection. Methods to accomplish this administration are known to those of ordinary skill in the art. For example, Gokarn et al., 2008, J Pharm Sci 97(8):3051-3066, incorporated herein by reference, describe various high concentration antibody self buffered formulations. For example, monoclonal antibodies in self buffered formulation at e.g.,50 mg/mL mAb in 5.25% sorbitol, pH 5.0 or 60 mg/mL mAb in 5% sorbitol, 0.01% polysorbate 20, pH 5.2; or conventional buffered formulations, for example, 50 mg/mL mAb1 in 5.25% sorbitol, 25 or 50 mM acetate, glutamate or succinate, at pH 5.0; or 60 mg/mL in 10 mM acetate or glutamate, 5.25% sorbitol, 0.01% polysorbate 20, pH 5.2; other lower concentration formulations can be employed as known in the art..

[0449] Because the preferred sigma-2 receptor antagonist 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 sigma-2 antagonist compounds selected from the sigma-2 ligands and prepared for oral administration described above 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.

[0450] 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.

[0451] 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. Antibodies or fragments are typically administered parenterally, for example, intravenously.

[0452] 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.

[0453] 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.

[0454] 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.

[0455] 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 sigma-2 receptor antagonists and desired excipients for subsequent preparation of sterile solutions.

[0456] 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.

[0457] 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.

[0458] 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.

[0459] 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.

[0460] Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

[0461] 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.

[0462] 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.

[0463] 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.

[0464] 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.

[0465] 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.

[0466] 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.

[0467] 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.

[0468] 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.

[0469] 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.

[0470] 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.

[0471] Suitable examples of pharmaceutically acceptable surfactants include, but are not limited to, sodium lauryl sulphate and polysorbates.

[0472] 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.

[0473] 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.

[0474] 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.

[0475] For topical administration by inhalation the compounds according to the invention may be delivered for use in human or veterinary medicine via a nebulizer.

[0476] 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.

[0477] 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.

[0478] 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.

[0479] 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.

[0480] 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.

[0481] 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.

[0482] 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.

[0483] 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.

[0484] 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.

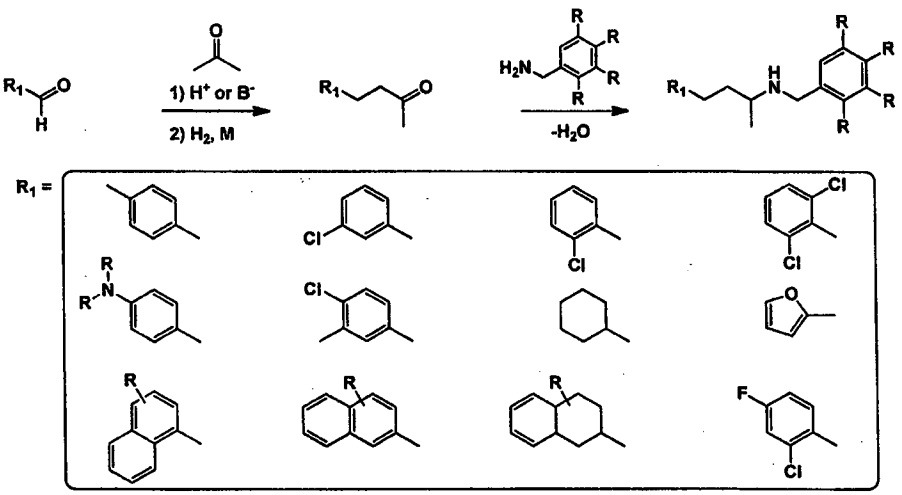
[0485] Synthesis of the Compounds of the Invention

[0486] Compounds of formulas I and II 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 groups R_{1-6}, have the meaning defined for the compounds of any of the formulae above unless otherwise stated.

[0487] 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.

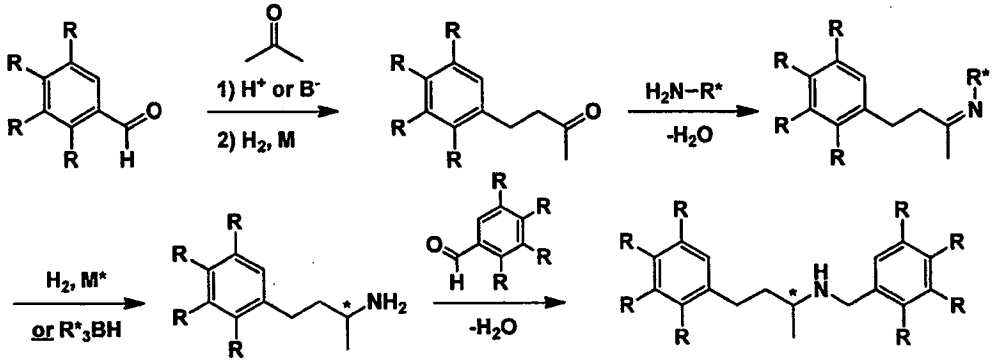
[0488] The synthesis of the target compounds is completed by removing any protecting groups which may be present in the penultimate intermediates using standard techniques, which are well-known to those skilled in the art. The deprotected final products are then purified, as necessary, using standard 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

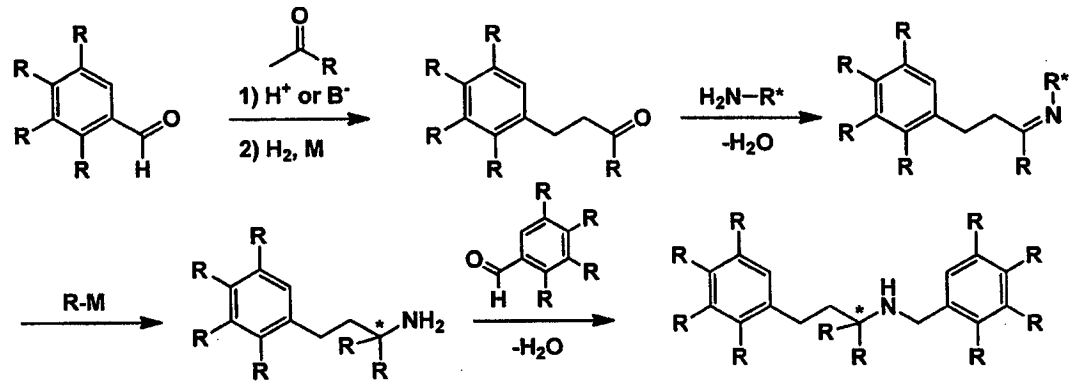
This scheme can produce a racemic mixture of the analogues described herein. Additional R1 groups can also be used to generate other analogues.

[0489] 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

[0490] In some embodiments, the asymmetric synthesis of a compound described herein is prepared according to Scheme 3 (* indicates chiral center):

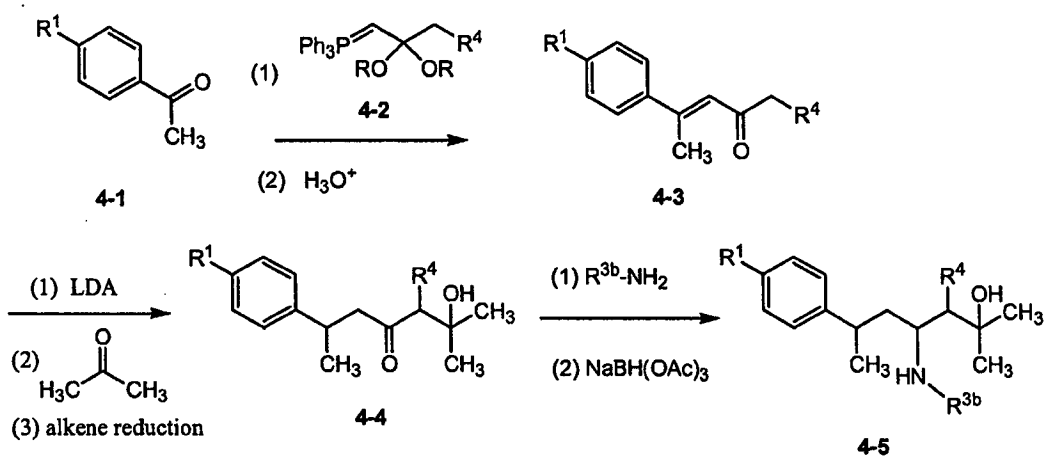


Scheme 3

[0491] 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.

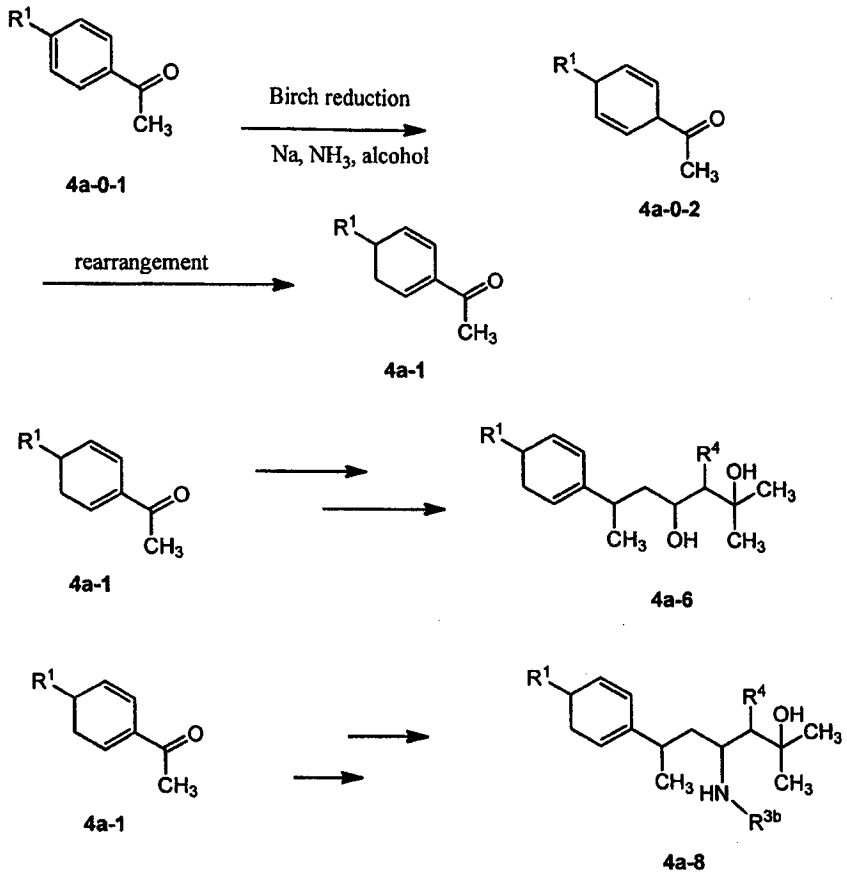
[0492] The following is a general method for preparing the compounds of Formula IX. As shown in Scheme 4, ketone 4-1 can be reacted with Wittig reagents such as 4-2 , followed by hydrolysis (for example under acidic condition) to afford ketone 4-3. The enolate of ketone 4-3 with a reagent such as LDA, and condensed with acetone followed by conjugate reduction of the alkene toto form ketone 4-4. Reductive amination of ketone 4-5 with a suitable amine $R^{3b}NH_2$ in the presence of a suitable hydride such as sodium borohydride can afford amine 4-6. Different

diastereomers of amine 4-6 can be separated by methods known to those skilled in the art such as column chromatography.



Scheme 4

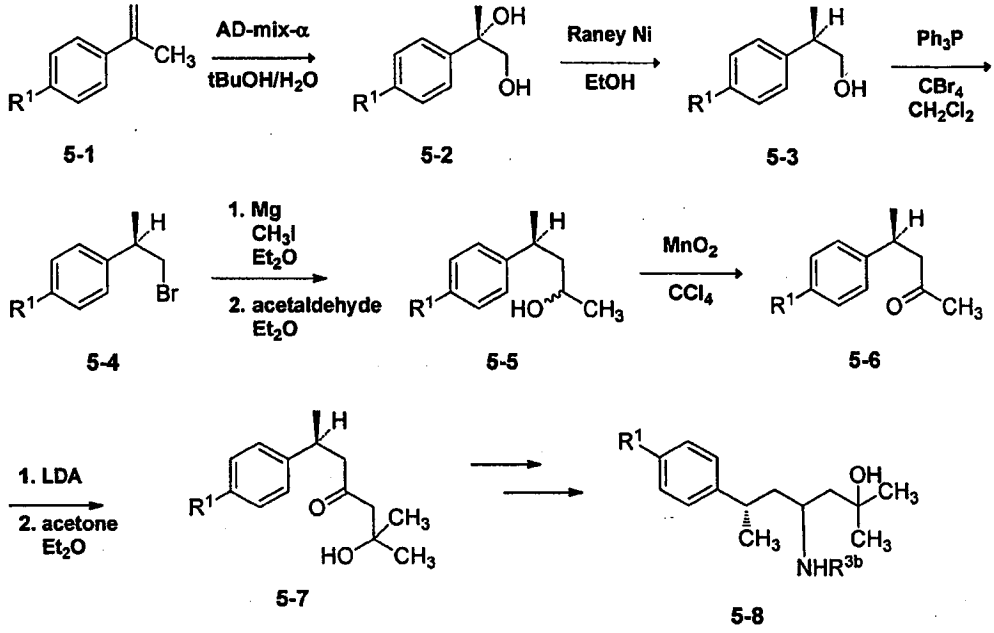
[0493] As shown in Scheme 4a, aromatic compound 4a-0-1 can be reduced to cyclohexa-1,4-diene 4a-02 under Birch reduction conditions. *See e.g.* Rabideau, P. W., "The metal-ammonia reduction of aromatic compounds", *Tetrahedron*, Volume 45, Issue 6, 1989, pages 1579-1603. Under acidic conditions (such as in the presence of catalytic amount of HCl or acetic acid), cyclohexa-1,4-diene 4a-02 can rearrange to the thermodynamically more stable cyclohexa-1,3-diene 4a-1. Cyclohexa-1,3-diene 4a-1. can be converted to alcohol 4a-6 or amine 4a-8 according to methods similar to those described in Scheme 4.



Scheme 4a

[0494] As shown Scheme 5, treatment of styrene derivative 5-1 with AD-mix-α (*See e.g.* Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; et al. *J. Org. Chem.* 1992, 57, 2771) affords diol 5-2. *See* A. Li, et. al, "Total asymmetric synthesis of (7S,9R)-(+)-bisacumol", Tetrahedron: Asymmetry (2003), 14(1), 75-78. Stereo-selective reduction of the benzylic OH of diol 5-2 with Raney nickel gives alcohol 5-3. *See id.* Both the isomer of 5-2 can be obtained based on selection of the Sharpless catalyst. Treatment of alcohol 5-3 with PPh_3 and CBr_4 in a suitable solvent such as methylene chloride affords bromide 5-4. Conversion of bromide 5-4 to the corresponding Grignard reagent in the presence of magnesium powder and CH_3I (by metal-halogen exchange), followed by reaction with acetaldehyde, provides alcohol 5-5. Different diastereomers of alcohol 5-5 can be separated by

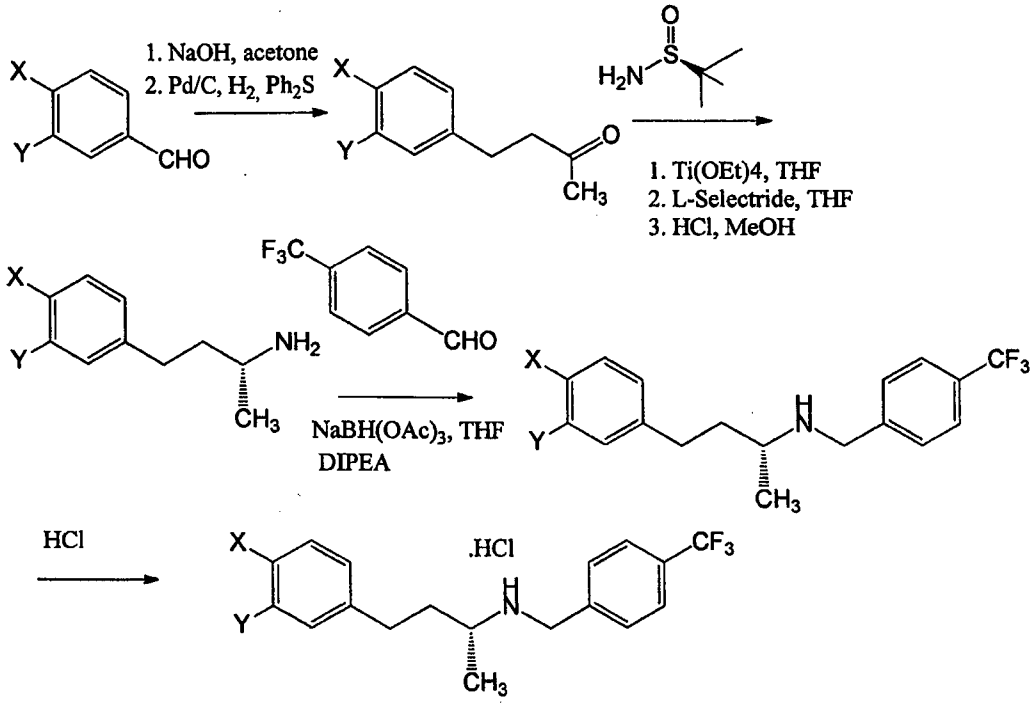
methods known to those skilled in the art such as column chromatography. *See id.* Alcohol 5-5 can be transformed into its corresponding amine compound 5-6 using similar methods to those outlined in Scheme 4. The isomers of the amine compound 5-8 can be obtained by stereoselective imine reduction.



Scheme 5

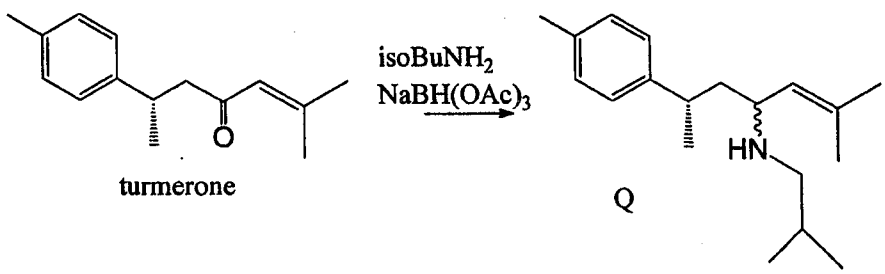
[0495] Those skilled in the art can recognize that in all of the schemes described herein, if there are functional (reactive) groups present on a substituent group such as R^1, R^2, R^3, and R^4, etc., further modification can be made if appropriate and/or desired. For example, an OH group can be converted into a better leaving group such as mesylate, which in turn is suitable for nucleophilic substitution, such as by Br. Thus, a compound of Formula I (such as compound 4-8 of Scheme 4) having a substituent which contains a functional group can be converted to another compound of Formula I having a different substituent group.

[0496] In some embodiments certain compounds of formulas I-VI are prepared, for example, by the enantioselective route shown in Scheme 6.



Scheme 6. Enantioselective synthesis of compounds of Formula I-VI.

[0497] In some embodiments, the sigma-2 antagonist is a compound of formula VIIIa. Certain compounds of various Formulas VIII can be prepared by reductive amination of corresponding ketone intermediates, for example, by the representative route shown in Scheme 7.



Scheme 7.

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

[0498] 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.

[0499] 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

[0500] 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: Isolation of beta-amyloid oligomers from human AD brain tissue.

[0501] 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 a 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).

[0502] 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.

[0503] 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.

[0504] 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.

[0505] 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.

[0506]

Example 4A: Receptor Binding Assays

[0507] 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 1E). 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.

[0508] **Table 1E.** Compound II (10 uM) inhibition of binding to known receptors.

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

[0509] Using the same protocol, the compounds for which membrane trafficking data are given in Table 5 (below) were tested for recognition of sigma-2

receptor. The results confirmed that these compounds, structurally similar to Compound II, are sigma-2 receptor ligands, i.e., preferentially bind to the sigma-2 receptor. Lastly, compounds similar to Compounds IXa and IXb, such as the compounds of Formulae VIII and IX, also are sigma-2 receptor ligands.

[0510] Competitive Radioligand Binding Assay.

[0511] 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 μM to 1 nM were used to displace 8 nM [^{3}H](+)pentazocine from endogenous receptors on Jurkat cell membranes (Ganapathy ME et al. 1991, J Pharmacol. Exp. Ther. 289:251-260). 10 μM Haloperidol was used to define non-specific binding. For Sigma-2 receptors various concentrations of test compounds from 100 μM to 1 nM were used to displace 5 nM [^{3}H] 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 μM 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).

[0512] For known prior art compounds, affinities for Sigma-1 and Sigma-2 receptors were obtained from published studies using cerebral tissue homogenates with [^{3}H](+)pentazocine to measure displacement from Sigma-1 receptors and [^{3}H] 1,3-Di-(2-tolyl)guanidine in the presence of 300 nM (+)pentazocine to measure displacement from Sigma-2 receptors. Results are shown in Table 2.

[0513] **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	27
Compound IXa	89	21
Compound IXb	190	23
Compound W	270	120
Compound AC	23	240
Compound AE	16	35
Compound AF	8	110

Compound	Sigma 1 Binding Ki (nM)	Sigma 2 Binding Ki (nM)
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	Sigma 1 Binding Ki (nM)	Sigma 2 Binding Ki (nM)
Compound CF	180	50
Compound CG	360	3200
Compound CJ	44	810
Compound CL	190	5,000
Compound CO	130	7,200
Compound CR	3.5	16
Compound CS	78	85
Compound DH	23	8.3
Compound DR	330	3,200
Other Compounds	Sigma 1 Binding Ki (nM) (Type of Activity)	Sigma 2 Binding Ki (nM)
BD1047	0.9 (antagonist)	47
DTG	88 (agonist)	35
Haloperidol	5 (antagonist)	110
Ifenprodil	26	4.9
Mach-14	12,900	8
NE 100	1.1 (antagonist)	170

Compound	Sigma 1 Binding Ki (nM)	Sigma 2 Binding Ki (nM)
PB 28	15	0.8
PRE-84	2.2 (agonist)	13,091
SM-21	1050 (antagonist)	145
threo-ifenprodil	59	0.9 (agonist)*
Sertraline	8.6 (antagonist)	170
PPBP	0.8 (agonist)	1
BD1008	2.2 (antagonist)	8
Fluvoxamine	13 (agonist)	710
BD1063	8.8 (antagonist)	625
SFK10047	597 (agonist)	39,740
siramesine	19	0.19 (agonist)

*Monassier et al., JPET, 322 (1):341-350, 2007.

[0514] Competitive Radioligand Binding Assay 2.

[0515] 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.

[0516] 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.

[0517] 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.

[0518] 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 compund.

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 4B. Anti-receptor antibody-mediated reduction of oligomer binding to receptor

[0519] As described herein, progesterone receptor membrane component 1 (PGRMC1) was recently identified as the critical 25kDa component of sigma-2 receptor activity by Xu et al.2011. Specifically, PGRMC1 was identified in rat liver by use of a photoaffinity probe WC-21, which irreversibly labels sigma-2 receptors in rat liver. Xu et al. *Identification of the PGRMC1 protein complex as the putative sigma-2 receptor binding site.* Nature Communications 2, article number 380, July 5, 2011, incorporated herein by reference. Therefore, monoclonal antibodies specific for various C-terminal or N-terminal amino acid sequences of human PGRMC1 were employed in these experiments.

[0520] The ability of receptor antibodies to affect Abeta oligomer binding are tested using the following general assay procedure. Positive control: 6E10 antibody (Covance) (recognizes the N-terminus of all Abeta species, and will reduce Abeta binding to neurons by virtue of high affinity binding to oligomer in solution

prior to receptor binding). Neurons in culture were prepared as for the membrane trafficking assay. Negative control: non-immune IgG. Methods:

1. Aspirate plate to 10uL volume
2. Add 30ul of specified concentration of receptor monoclonal antibody (mAb), 6E10 or non-immune IgG for 30 minutes at 37°C
3. Add 10uL of Abeta oligomers at specified concentration; incubate for 1 hour at 37°C
4. Fix and wash plate 3 times with PBS
5. Block plate for one hour using blocking compound (1L PBS, 50mL normal goat serum, 50mL 10% TritonX)
6. Aspirate plate to 10uL
7. Add 25uL primary detection antibodies 4G8 and MAP2; seal and refrigerated over night.
8. Wash plate 3 times with blocking compound (aspirate to 10uL, wash with 60uL block)
9. Add 25uL of secondary antibodies (Alexa fluor 647 goat anti mouse, Alexa flour 488 goat anti rabbit, Alexa flour 646 goat anti chicken); let sit for an hour at room temperature.
10. Aspirate plate to 10uL, wash with 60uL PBS, aspirate plate to 10uL add 60uL Dapi, aspirate plate to 10uL wash with 60uL PBS.
11. Cover with aluminum seal
12. Scan and analyze images with proprietary Cellomics Arrayscan protocol.

[0521] Specific Antibody Blocking Experiment.

[0522] Antibodies recognizing the synthetic peptide: C-EPKDESARKND (SEQ ID NO: 7), corresponding to C terminal amino acids 185-195 of human PGRMC1 (#EB07207, Everest Biosciences), or residues 1-46 at the N-terminus of human PGRMC1 protein (MAAEDVVATGADPSDLESGGLLHEIFTSPLNLLLLGLCIFLLYKI (SEQ ID NO: 9), #sc-98680, Santa Cruz), or nonimmune control IgG were employed in these experiments. Each antibody was applied to neurons for 30 minutes at 38 nM (5x), 58 nM (7.5x) or 77 nM (10x) Final Assay Concentrations.

[0523] Abeta 1-42 oligomers were then added at 500 nM total Abeta concentration and allowed to bind to neurons for an additional 15 minutes. Cultures were then fixed and immunolabeled for bound Abeta species using 6E10 antibody. Oligomers bound to postsynaptic membranes in a characteristic punctate pattern were quantified via automated image processing. Neurons were identified via MAP2 immunolabeling. Quantitative measures of neuron health such as the average nuclear area were quantified via image processing and results are shown in Figures 13A to 13H.

[0524] There was a dose-dependent reduction of the intensity of Abeta oligomer binding with neurons incubated with C-terminal antibody (FIG. 13C), compared to control Abeta (FIG. 13A), but not with N-terminal antibody (FIG. 13G) or nonimmune IgG (FIG. 13E). This reduction in intensity appears to consist of a reduction in the number and area of oligomer-positive puncta. Whether this reduction in oligomer binding intensity is due to competition for binding epitopes between oligomer and antibody, or an antibody-mediated reduction in surface PGRMC1 protein expression is not known. In some embodiments, anti-sigma-2 receptor antibodies and anti-PGRMC1 antibodies that block binding between soluble Abeta oligomers and a sigma-2 receptor are considered to be sigma-2 receptor antagonist compounds.

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

[0525] 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.

[0526] 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 were with a low dose (0.1 mg/kg/day) and a high dose (0.5 mg/kg/day) of compound II. 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.

[0527] 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.

[0528] 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).

[0529] 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.

[0530] 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).

[0531] 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.

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

[0532] Sigma-2 ligands selected 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 /exocytosis assay (MTT assay). The results are indicated in Table 5 below. The rationale for this assay was as follows:

[0533] 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).

[0534] 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.

[0535] 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.

[0536] The methods used to generate the results were as follows in the Membrane Trafficking/Exocytosis (MTT) assay.

[0537] 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).

[0538] 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.

[0539] 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.

[0540] 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.

[0541] Based on these results, selective, high affinity sigma-2 receptor antagonist compounds as disclosed herein are that effective for inhibiting Abeta oligomer toxicity are promising as therapeutic and (in very early stages) prophylactic modalities for amyloid beta oligomer toxicity related cognitive decline such as that seen in Alzheimer's disease. Saturable competitive binding to Abeta oligomers could not be demonstrated in these experiments because toxicity limits the upper concentrations.

[0542] 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,IX, 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 non linear 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 possibile mechanism of action is that the sigma-2 receptor compounds act as competitive receptor antagonists.

[0543] 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 μM	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

[0544] As shown in Table 4 above, the rightward shift in the dose response curve of % vesicles against Abeta oligomer concentration for 20 μM of enantiomer versus 0 μM of enantiomer (i.e., Abeta oligomer alone) is significantly more pronounced for the (+) enantiomer at higher concentrations of Abeta oligomer.

Experimental controls:

[0545] 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.

[0546] Various different Abeta oligomer preparations were demonstrated to cause an Abeta effect in the membrane trafficking assay, including notably oligomer preparations isolated from the brain of Alzheimer's disease patients.

[0547] 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. 1J) produce a statistically significant inhibition of membrane trafficking compared to vehicle (first bar, Fig. 1J). 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).

[0548] 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 compositions of the invention comprising a sigma-2 receptor antagonist compound.

[0549] 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. Compound II has no significant effect on membrane trafficking when dosed on its own (black diagonal bar, Fig. 1J).

[0550] 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. 1K, second bar), and do not produce significant deficits in membrane trafficking vs. vehicle (Fig. 1K, first bar). Under these conditions, Compound II has no effect when dosed with oligomers or alone (Fig 1K, third and 4th bar respectively. Again, data are averaged (n=3 except for second bar, wherein n=5).

[0551] 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

[0552] 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

[0553] 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 μM concentration on neurons would also be rejected.

[0554] 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:

[0555] 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.

[0556] Selected compounds within the Formulas of the invention, including Formulas III-IV, VIIIq and VIIIo as indicated in the Table 5 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**	**Max Inhibition of Abeta (%)**
Compound II (three different batches: racemic mixture, (+) isomer and (-) isomer)	2.2 uM 5.6 uM 10.9 uM	68 (78) (64)
Compound A	3.4 uM	78
Compound B	5.5 uM	84
Compound C	5.4 uM	93
Compound D	8.9 uM	58
Compound E	8.2 uM	68

Sigma-2 Receptor Ligand	EC_{50} in inhibiting amyloid beta effect in Cell Measured by Membrane Trafficking Assay	Max Inhibition of Abeta (%)
Compound F	2.6 uM	69
Compound G	5.8 uM	92
Compound H	2.2 uM	-
Compound I	3.4 uM	100
Compound J	3.9 uM	97
Compound K	14 uM	13
Compound L	2.4 uM	34
Compound M	0.6 uM	60
Compound N	5.2 uM	46

Sigma-2 Receptor Ligand	**EC_{50} in inhibiting amyloid beta effect in Cell Measured by Membrane Trafficking Assay**	**Max Inhibition of Abeta (%)**
Compound O	2.7 uM	27
Compound P	20.0 uM (19.5 uM)	43 (73)
Compound Q	0.5 uM	82
Compound R	6.7 μM	55
Compound R'	39 μM (inactive)	38
Compound S	5.4 μM	100
Compound S'	>30 μM (inactive)	0
Compound T	7.7 μM	45
Compound IXa	4.9 μM	76

Sigma-2 Receptor Ligand	**EC_{50} in inhibiting amyloid beta effect in Cell Measured by Membrane Trafficking Assay**	**Max Inhibition of Abeta (%)**
Compound IXb	6.9 μM	97
Compound AC	2.4 μM	-
Compound AD	0.7 μM	-
Compound AG	6.1 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	-
Compound CT	3 μM	-

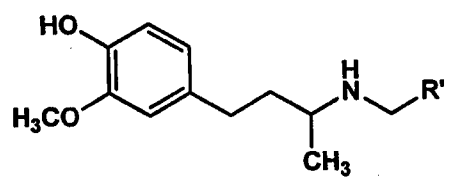
Sigma-2 Receptor Ligand	EC_{50} in inhibiting amyloid beta effect in Cell Measured by Membrane Trafficking Assay	Max Inhibition of Abeta (%)
Compound CW	2.5 μM	-
Compound CX	1.3 μM	-
Compound CY	14 μM	-
Compound DE	>20.0 μM	-
Compound	**EC_{50} in inhibiting amyloid beta effect in Cell Measured by Membrane Trafficking Assay**	**Max Inhibition of Abeta (%)**
BD1047	11	100
DTG	N/A	23
Haloperidol	6.2	100
Ifenprodil	1.3	38
Mach-14	1	80
NE 100	9.1	98
PB 28	2.2	84
PRE-84	N/A	38

Sigma-2 Receptor Ligand	EC_{50} in inhibiting amyloid beta effect in Cell Measured by Membrane Trafficking Assay	Max Inhibition of Abeta (%)
SM-21	11	65
threo-ifenprodil	N/A	39

[0557] 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.

[0558] Table 6A shows membrane trafficking EC_{50} data for certain additional compounds.

Table 6A: Additional Membrane Trafficking Data.



Entry	R'	Membrane Trafficking EC_{50} (μM)
1	4-CF3-Ph-	2.2
2	4-Cl-Ph-	12

3	Ph-	20
4	isoBu-	>30
5	H-	30

Correlation Between Trafficking Assay and Sigma Receptor Binding Data

[0559] Binding affinity of compounds to Sigma-1 or Sigma-2 receptors (from Table 2) and their EC_{50} and maximum effect in the membrane trafficking assay (from Table 5) were analyzed using Spotfire software to discover correlations between receptor binding and assay activity. The goodness of fit between the log of the EC_{50} in the MTTX was calculated vs Log sigma-1 and sigma-2 binding Ki, between the max inhibition of Abeta vs Log sigma-1 and sigma-2 binding Ki and for Log EC_{50} in the trafficking assay vs the ratio of sigma-1 binding Ki to sigma-2 binding Ki. All calculations were performed for 4 groupings of compounds: A) bolded compound plus known prior art compounds listed in Table 5, B) only bolded compounds from Table 5, C) only reference compounds from Table 5, and D) All compounds from Group A except sigma-1 antagonists (NE-100, Haloperidol, BD1047, SM21).

Table 6B: Correlation between sigma binding affinity and trafficking assay activity.

Y Axis	X Axis	R^2 (P value) All 19 compounds	R^2 (P Value) Table 5 Bolded Compounds	R^2 (P Value) 10 Ref Compounds	R^2 (P Value) W/O sigma-1 antagonists

MTTX log(EC 50)	Vs Log S1 Ki	0.06 (ns)	0.01 (ns)	0.02 (ns)	0.02 (ns)
	Vs Log S2 Ki	0.15 (ns)	0.70 (< 0.001)	0.27 (ns)	0.78 (< 0.001)
Max inhibition of Abeta	Vs Log S1 Ki	0.00 (ns)	0.14 (ns)	0.06 (ns)	0.00 (ns)
	Vs Log S2 Ki	0.00 (ns)	0.00 (ns)	0.00 (ns)	0.00 (ns)
MTTX log(EC 50)	S1/S2	0.01 (ns)	0.11 (ns)	0.04 (ns)	0.01 (ns)

[0560] As can be seen in Table 6B, the highest correlation for the bolded compounds alone, was between their Log (EC_{50}) in the MTTX assay and the Log (K_i) for Sigma-2 binding (R^2 = 0.70, P < 0.001). No other comparisons were statistically significant. This same correlation was not statistically significant when the reference compounds were added into the analysis (R^2 = 0.15, P> 0.05) and the reference compounds alone did not show a statistically significant correlation between these parameters ((R^2 = 0.27, P> 0.05).

[0561] Graphs of a different representation of this correlation are also shown in Figures 5A-5D.

[0562] Of the known prior art compounds, Mach-14 is highly selective for Sigma-2 receptors (Sigma-1 Ki = 12,900nM, Sigma-2 Ki = 9 nM) and it inhibited the Abeta effect in the trafficking assay by 80%. In contrast, PRE-84 is highly specific for Sigma-1 receptors (Sigma-1 Ki = 2.2 nM, Sigma-2 Ki = 13,091 nM) and was a poor inhibitor in the trafficking assay (max inhibition 38%). This result is consistent with the theory that Sigma-2 receptor binding, rather than sigma-1

receptor binding, is associated with reversing the effects of Abeta in the trafficking assay. Results of this assay provide further support for the development of the therapeutic phenotype.

[0563] When the bolded compounds from Table 5, along with known prior art compounds PB 28, Haloperidol and Mach 14 are graphed (Figure 5A) there is a strong correlation between Sigma-2 binding affinity and potency in the trafficking assay (R^2 = 0.79, P<0.001). In comparison, there is no significant correlation between binding to Sigma-1 receptors and potency in the MTTX assay (R^2 = 0.02, P = 0.18) (Figure 5C). This result shows a strong relationship between binding to Sigma-2 receptors and inhibition of Abeta effects in trafficking and appears to indicate a poor relationship between binding to Sigma-1 receptors and inhibition of Abeta.

[0564] Three other known prior art compounds (BD1047, NE100 and SM-21) all were more potent in the membrane trafficking MTTX assay than could be accounted for by their Sigma-2 binding affinity alone. Haloperidol, PB 28 and Mach-14 demonstrated a close correlation between Sigma-2 binding and potency in the trafficking assay.

[0565] PRE-084 is inactive in the trafficking assay and this is consistent with the observation that it is a potent Sigma-1 agonist and is not potent at the sigma-2 receptor.

[0566] Two bolded compounds, R' and S', were inactive in the trafficking assay despite their substantial affinity for the sigma-2 receptor. In some embodiments, compounds R' and S' do not meet the therapeutic profile.

[0567] Additionally, a mixture of compounds IXa and IXb synergistically inhibit 100% of oligomer effects on membrane trafficking with a reproducible EC_{50} of 5.2 μM +/- 1.1 (Figure 7). Similarly, additional compounds will be tested in the assay reported in this Example. These will also be selected from Formula I, II, III-VII, and IX as well as compounds encompassed by the other formulae (e.g., Formula XIII) and other compounds described as sigma-2 ligands described above.

[0568] Selected compounds in Table 2 were dosed in the membrane trafficking assay and were shown to block the Abeta oligomer-induced membrane trafficking abnormalities with the indicated EC_{50}. Accordingly, the compounds in Table 2 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-2receptor ligands can be used as candidate compounds to block the Abeta oligomer induced membrane trafficking abnormalities. As the compounds embraced by the above formulae are expected to also be sigma-2 ligands, and will therefore also be useful in blocking the Abeta oligomer induced acceleration of exocytosis.

Example 7. Pharmacokinetic and Metabolic Stability Studies.

[0569] 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 7A 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.

[0570] The results for half-life in microsomes are shown in Table 7A and 7B.

[0571] **Table 7A**: Compound Mouse Microsome Stability.

Compound	$t_{1/2}$ in microsomes of mice
II	16
A	33
B	55
C	10
D	2

Compound	$t_{1/2}$ in microsomes of mice
E	46
F	72
G	42
H	24
I	33
J	47

[0572] 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 bioavalability after oral administration for these compounds. The same compounds have been tested by the membrane trafficking assay described above and their activity as refered to herein.

[0573] 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.

[0574] 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

[0575] 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:

[0576] 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.

[0577] 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

[0578] 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

[0579] 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).

[0580] 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.

[0581] 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

[0582] 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.

[0583] Brain sample collection from animals

[0584] Immediately after blood sampling, animals were decapitated and the whole brains were quickly removed, rinsed with cold saline (0.9% NaCl, g/mL), surface 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 -20 °C until further processing.

Linearity in Brain samples

[0585] 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).

[0586] 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 penetratrability

[0587] 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

[0588] 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

[0589] 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.

[0590] 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.

[0591] AUC_{last}: area under the curve from the time of dosing to the time of last observation that was greater than the limit of quantitation.

[0592] AUC_{INF}: Area under the curve from the time of dosing extrapolated to infinity.

[0593] C_{max} - 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.

[0594] T_{max} - Time at maximum observed plasma concentration (Cmax) noted in minutes after administration of drug.

[0595] $T_{1/2}$ - Terminal elimination half-life from both IV and non-IV dosing.

[0596] 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.

[0597] The results showed that the tested compounds II and B were highly bioavailable and highly brain penetrability 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.

[0598] The results for compound B and those for Compound II acute administration are shown in Table 8.

[0599] **Table 8.** 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	

[0600] As shown in this table, the oral bioavailability of Compound B and its other PK parameters are improved over Compound II.

[0601] 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.

[0602] 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).

[0603] 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 9.

[0604] **Table 9**. 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

[0605] 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.

[0606] 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.

[0607] 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.

[0608] 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, 3B, 3C and 3D. 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.

[0609] 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.

[0610] 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:

[0611] 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

[0612] Pre-adsorption of anti-Abeta antibody 6E10 with oligomer preparation inhibits synapse binding in a dose dependent manner (at 7.84×10^{-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

[0613] 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

[0614] 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

[0615] 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).

Example 9: Fear Conditioning Assay

[0616] 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.

[0617] 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 6, 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.

[0618] 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.

[0619] 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.

[0620] 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 ofthc 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 cortexand the hippocampus (dentate gyrus, hippocampal CA I and CA3).

[0621] Autoradiography at sigma-1 and sigma-2 receptors is shown at FIGs 8A and 8B, respectively. FIG. 8C 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. 8A, 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. 8B 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. 8C 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.

[0622] 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 FIGs. 9A. Data from FIG. 9A is shown in Table 10.

Table 10. 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

[0623] , 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, as shown in FIG 9B, 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.

[0624] 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.

[0625] 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. 10A and 10B. Sigma-2 receptor antagonist RHM-1 inhibits the activation in tumor cells (FIG 10A), but was not able to block activation by agonist SV-119 in neurons in this experiment (FIG 10B). 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.

[0626] 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. 10A, 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. 10B 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.

[0627] 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 11A.

Table 11A. 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

[0628] 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.

[0629] Functional neuronal phenotype for several Compound II analogs, predicted to have oral bioavailability, with in vitro assay characterization, is shown in Table 11B.

Table 11B. 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	II	2.2	500	9	100%	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
affinity at sigma-2						
	II (+) isomer	5.6	100	80	47%	Antagonist
	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

Selectivity	Compound	Inhibition Abeta oligomer-induced Membrane Trafficking EC50(uM)	S1 binding Ki (nM)	S2 binding Ki (nM)	Block synapse loss	Functional Neuronal Phenotype
	X	6.1	3.5	16	100%	Antagonist
	E	8.2	2	3.6	34%	Antagonist
	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

Therapeutic Phenotype

[0630] Table 11C shows known prior art compounds with high affinity for either sigma-2 or sigma-1 receptors. Several sigma-2 ligands fall into three

functional neuronal phenotypes: antagonists (block Abeta signaling); agonists (block Abeta signaling with U-shaped dose-response curve and toxicity at high doses; and inactive (no effect in neuronal cultures). The known prior art sigma-1 receptor ligands fall into two categories: antagonists (block A beta signaling) and inactive (no effect in neuronal cultures). Most of the prior art compounds suffer from low selectivity in that they have significant affinity to other, non-sigma, receptors. Several of the prior art compounds may not be able to penetrate the blood brain barrier (BBB) and are likely substrates for oxidative metabolism, and thus would not fit the therapeutic profile.

Table 11C. Compounds-Selective Sigma-2 and Sigma-1 Receptor Ligands.

Selectivity	**Compound**	**Inhibition Abeta oligomer-induced Membrane Trafficking EC50 (uM)**	**S1 binding Ki (nM)**	**S2 binding Ki (nM)**	**Functional Neuronal Phenotype**
Higher Affinity at Sigma-2	PB 28	1	15	0.8	Antagonist
	Siramesine	1.3	19	0.19	Agonist
	WC-26	1.6	1,400	3	Agonist
	SM-21	2.2	1050	145	Antagonist
	SV119	2.6	1,400	8	Agonist
	M-14	6.2	12,900	8	Antagonist
	Ifenprodil	>20	26	4.9	Inactive
	threo-ifenprodil	>20	59	0.9	Inactive

Selectivity	Compound	Inhibition Abeta oligomer-induced Membrane Trafficking EC50 (uM)	S1 binding Ki (nM)	S2 binding Ki (nM)	Functional Neuronal Phenotype
	DTG	>20	88	35	Inactive
Higher Affinity at Sigma-1	NE 100	1.3	1.1	170	Antagonist
	BD1008	1.5	2.2	8	Antagonist
	BD1047	2.4	0.9	47	Antagonist
	Fluvoxamine	2.5	13	710	Antagonist
	Pentazocine	7.6	3	>5,000	Antagonist
	PPBP	10.9	0.8	1	Antagonist
	Haloperidol	11	5	110	Antagonist
	PRE-84	>30	2.2	13,091	Inactive
	BD1063	>30	8.8	625	Inactive
	SKF 10,047	>30	149	>10,000	Inactive

Although several clinical compounds have the desired functional phenotype, they do not meet the desired therapeutic profile. Known prior art compounds with the desired antagonist functional neuronal phenotype, but that fail the criteria for therapeutic profile, either by being non-selective, or by failing to cross the BBB, or by being predicted to be an oxidative substrate and having metabolic instability, are shown in Table 11D.

Table 11 D. Characterization of Certain Known Compounds.

Cpd	Membrane trafficking IC50 (uM)	Functional Neuronal Phenotype	Sigma-1 Receptor Ki (nM)	Sigma-2 Ki (nM) Receptor	Selectivity other activities	Drug-like properties
PB 28	1	Antagonist	15	0.8	Not Reported	Oxidative substr.
SM-21	2.2	Antagonist	1050	145	Muscarinic antag	Oxidative substr.
M-14	6.2	Antagonist	12,900	8	D3	Not BBB, Ox Sub.
NE 100	1.3	Antagonist	1.1	170	Not Reported	Oxidative substr.
BD1008	1.5	Antagonist	2.2	8	Not reported	Oxidative substr.
BD1047	2.4	Antagonist	0.9	47	D2, β_3-AR	Oxidative substr.
Fluvoxamine	2.5	Antagonist	13	710	5HT, DA, AR transporters	Yes

Cpd	Membrane trafficking IC50 (uM)	Functional Neuronal Phenotype	Sigma-1 Receptor Ki (nM)	Sigma-2 Ki (nM) Receptor	Selectivity other activities	Drug-like properties
(+) Pentazocine	7.6	Antagonist	3	>5,000	NMDAR, PCP	Yes
PPBP	10.9	Antagonist	0.8	1	Not reported	Oxidative substr.
Haloperidol	11	Antagonist	5	110	D2, D3, D4, 5HT2A, a1B, a2A,a2B, a2C, NMDAR, Ach, GluR, Ca2+	Yes

In addition, many clinical compounds that bind to the sigma-2 and or sigma-1 receptors are not highly selective as illustrated in Table 11E. In fact, 10-fold, 20-fold, and particularly100-fold selectivity for sigma receptors compared to other non-sigma receptors is rare.

Table 11E. Clinical Compound Selectivity.

Drug	Therapeutic Effect	Sigma Affinity (S1/S2) nM	Other Targets
Fluoxetine	SSRI antidepressant	75 / 260	5-HT2C (33nM), 2A (141 nM), muscarinic M1-M4(500 nM), serotonin transporter (1-270 nM)

Drug	Therapeutic Effect	Sigma Affinity (S1/S2) nM	Other Targets
Fluvoxamine	SSRI antidepressant	13 / 710	Dopamine transporter (1.5 nM), Serotonin (12 nM), Adrenergic transporter (299 nM)
Pentazocine	Opioid analgesic	1.2 / 5,000	NMDAR2B (2.5 uM), 2A (2.7 uM), PCP (2.9 uM)
Opipramol	Antidepressant, anxyolytic	7/56	5-HT2A,B,C (120 nM), alpha 1A,1B(200 nM)
Siramesine	Antidepressant, anxyolytic	19 / 0.19	D2 (800 nM), alpha 1A,1B (300 nM)
Cutamesine	Antidepressant, stroke	17 / >1800	VAChT(50nM), muscarinic M1, 2, D2 (2uM), alpha1 AR, 5-HT1a/2, H1 (2uM)
Anavex 1-41	Alzheimer's	7/>10,000	Muscarinic M1-M4 (18-114 nM), Na+ ch2 (>10uM)
Anavex 2-73	Alzheimer's	860/>20,000	Muscarinic M1-M4 (3-5 uM), NMDAR2B (8 uM), Na+ ch2

Drug	Therapeutic Effect	Sigma Affinity (S1/S2) nM	Other Targets
			(5uM)
II	Alzheimer's	500/9	D3 (4uM), Mu opioid (4 uM), Na+ch2 (2 uM)
IXa,IXb	Alzheimer's	31/54	DA transporter (1.5 uM)

Example 14: In vitro Toxicity.

[0631] 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 12.

[0632] **Table 12.**

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.

[0633] In vitro toxicity for Compound II was tested in a number of standard assays.Testing in vitro tox 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 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.

[0634] 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.

[0635] 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.

[0636] 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 12, 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.

are shown in the Table 13.

Table 13. 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

[0637] 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.

[0638] 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 14.

Table 14. Receptor Affinities for Orally Bioavailable Compounds.

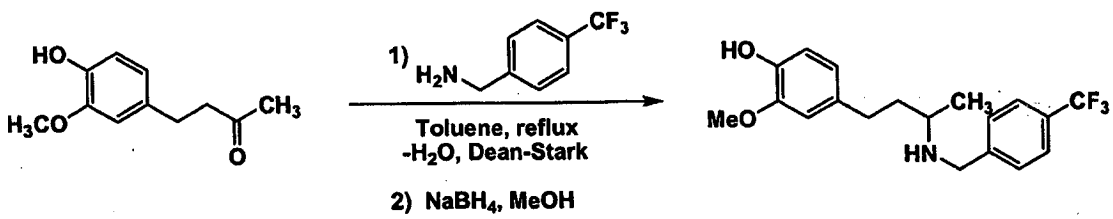
Drug	Therapeutic Effect	Sigma Receptor Affinity (sigma-1/sigma-2) (Ki, nM)	Other Receptor Affinities (Ki, nM)
CB	Alzheimer's	19/48	Muscarinic M1 (1.5 uM), M2 (1.5 uM), M3 (1.8 uM)
			kappa opioid (1.5 uM)
			Ca++ ch - L-type (860 nM)
			Transporters: NE (1.4 uM), DA (220 nM), 5-HT (970 nM)
CF	Alzheimer's	180/50	Muscarinic M1 (1.1 uM), M2 (2.5 uM), M3 (3.7 uM)

Drug	Therapeutic Effect	Sigma Receptor Affinity (sigma-1/sigma-2) (Ki, nM)	Other Receptor Affinities (Ki, nM)
			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)

[0639] 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.

Synthetic Examples.

Synthesis Example 1: Synthesis of compounds by reductive amination.



[0640] 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^+).

[0641] 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

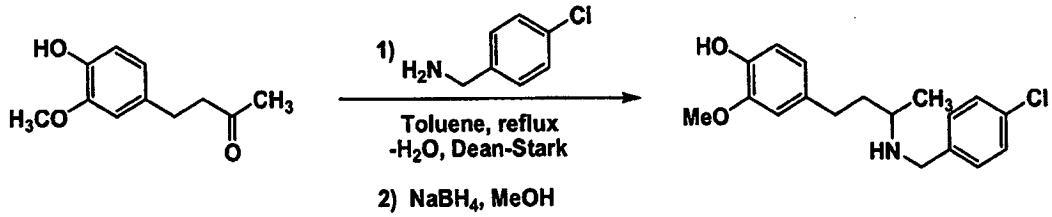
(Phenomenex Part No 00M-4423-B0_CE)

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

[0642] 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.



[0643] 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.

[0644] 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

[0645] 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

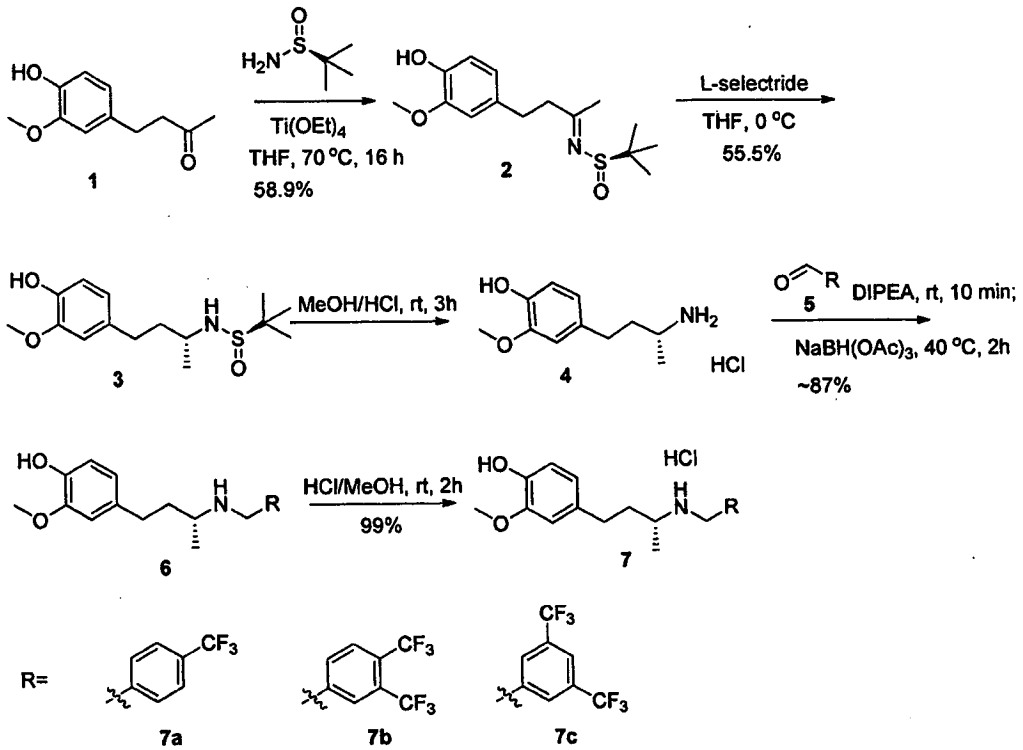
(Phenomenex Part No 00M-4423-B0_CE)

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

[0646] 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 9

[0647] **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%).

[0648] **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.

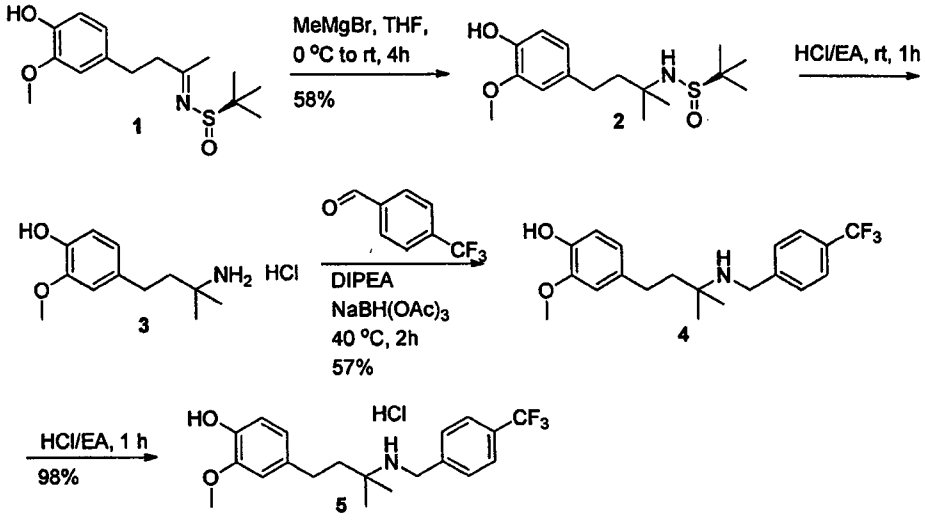
[0649] **Step3** : To a solution of **3** (7.0 g, 23.4 mmol) in MeOH (20 mL), HCl (2 M in MeOH, 20 mL) was added and the resulting solution was stirred at rt over a 3 h period. TLC analysis of the reaction mixture showed complete consumption of compound **3**. The solvent was then removed in vacuum, and the resulting residue **4** was used directly for the next step.

[0650] **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%).

[0651] **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.

[0652] m/z (ESI+) (M+H)+: 7a [354.2]; 7b [422.2]; 7c [422.2].

Synthesis Example 4



Scheme 10

[0653] **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%).

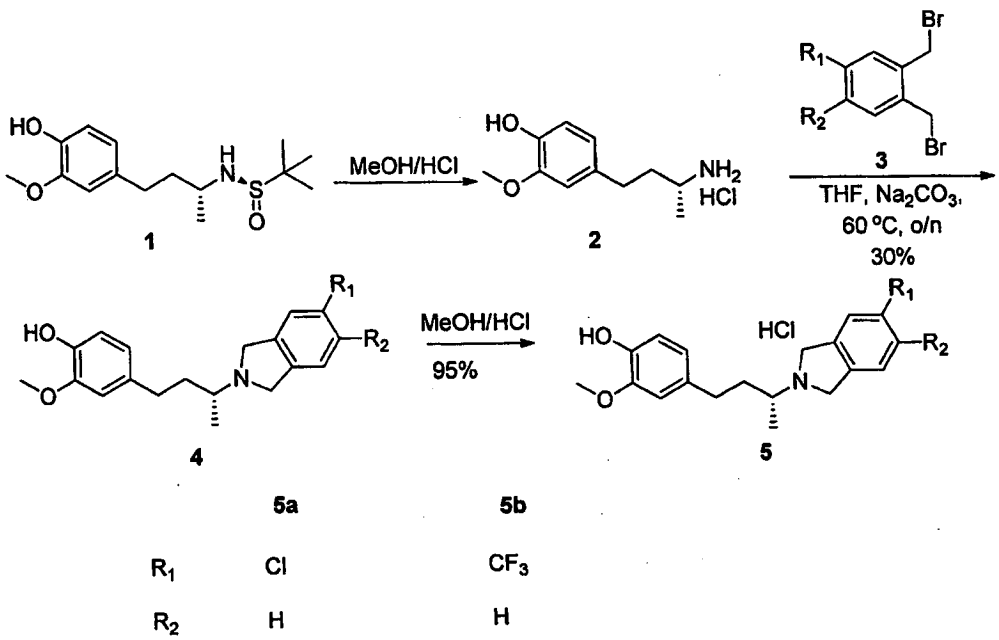
[0654] **Step2**: To a solution of **2** in EA (10 mL) at 0 °C was HCl (2 M in EA, 3 mL), and the resulting solution was stirred at rt for 1 h. Analysis of the reaction mixture by TLC showed complete consumption of **2**. Concentrated under vacuum, the crude product was directly used in next step.

[0655] **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%).

[0656] **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%).

[0657] m/z (ESI+) (M+H)+: **5** [368.2].

Synthesis Example 5



Scheme 11

[0658] **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.

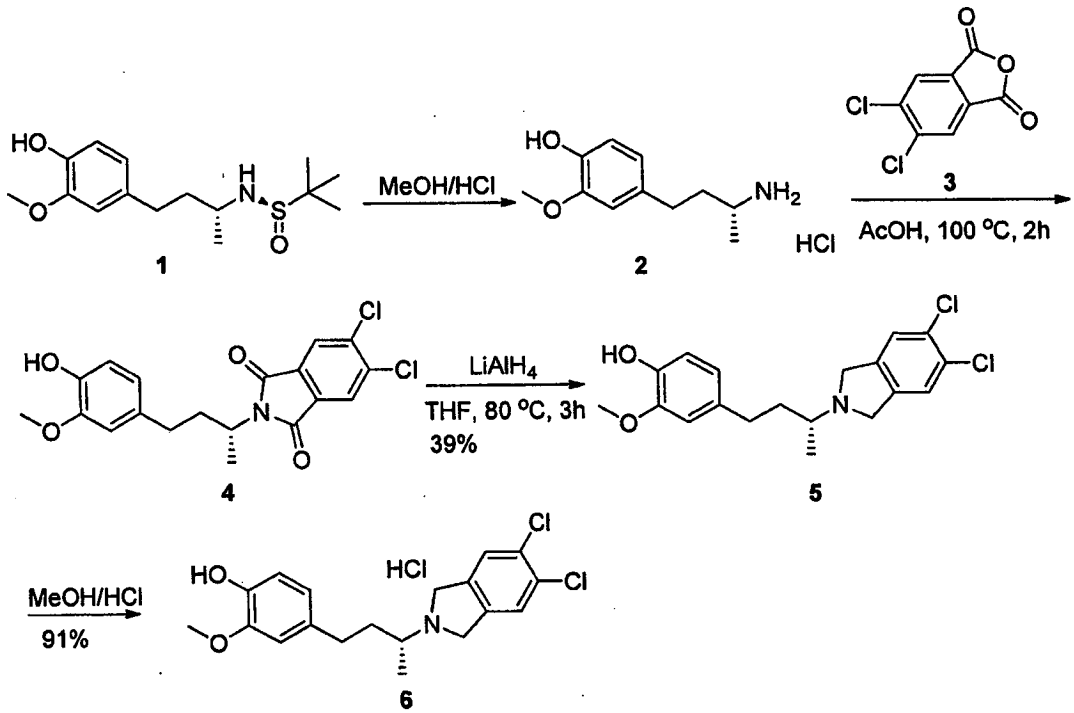
[0659] **Step2**: To a solution of **2** (0.4 g, 2 mmol) in THF (10 mL), compound **3a** (0.54 g, 2 mmol) in THF (5 mL) was added. Na_2CO_3 (0.6 g, 6 mmol) was added, and the resulting solution was stirred at 60 °C overnight. After concentration, the residue was purified by FCC to give compound **4** (0.2 g, 30%).

[0660] **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.

[0661] m/z (ESI+) (M+H)+: **5a** [332.1]; **5b** [366.1].

Synthesis Example 6



Scheme 12

[0662] **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.

[0663] **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

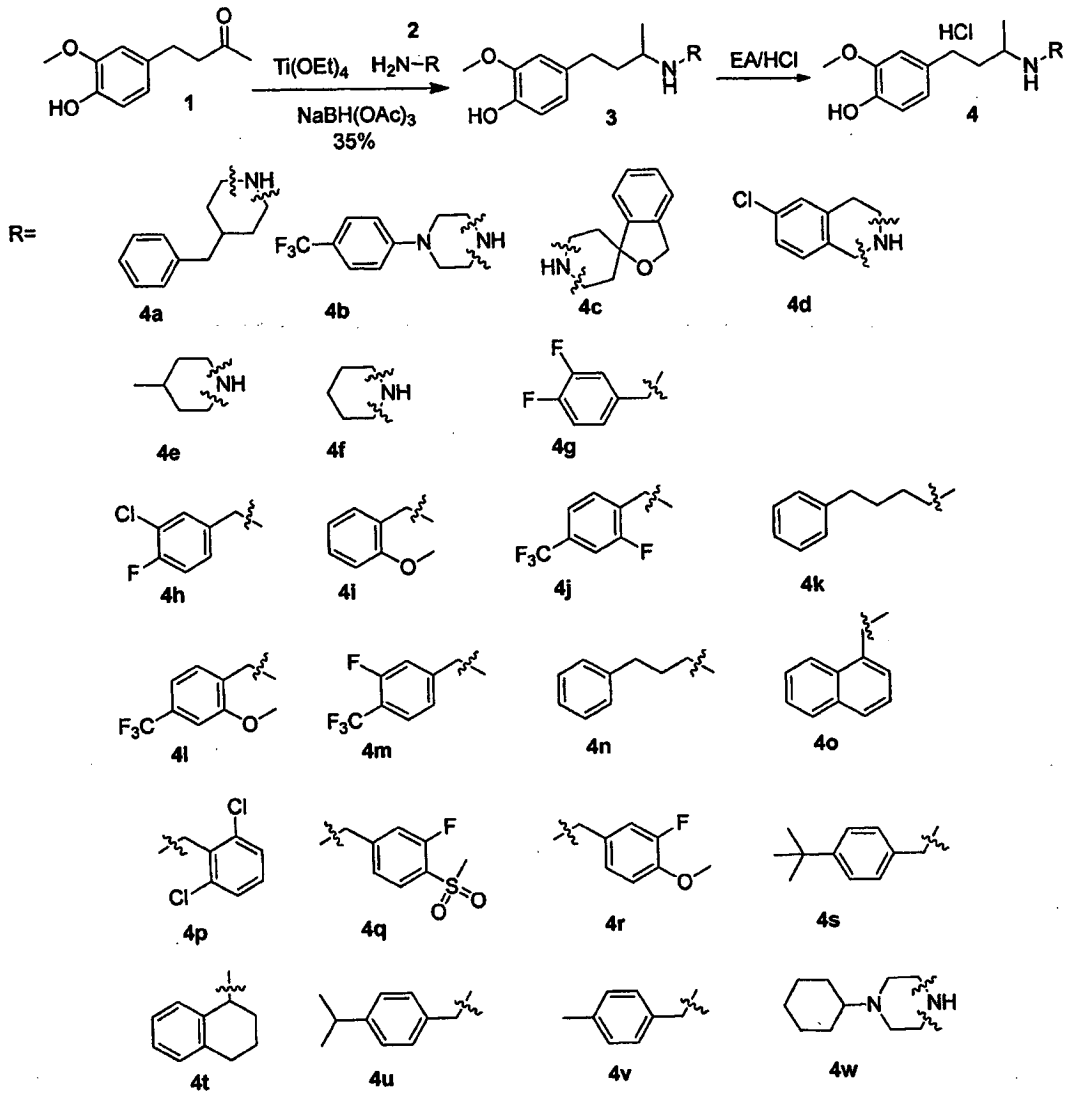
rt and quenched by water (10 mL), extracted by EtOAc (3 x 20 mL), dried, concentrated to give the title compound **4** (0.3 g, 76%).

[0664] **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%).

[0665] **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%).

[0666] m/z (ESI+) (M+H)+: **6** [366.2].

Synthesis Example 7



Scheme 13

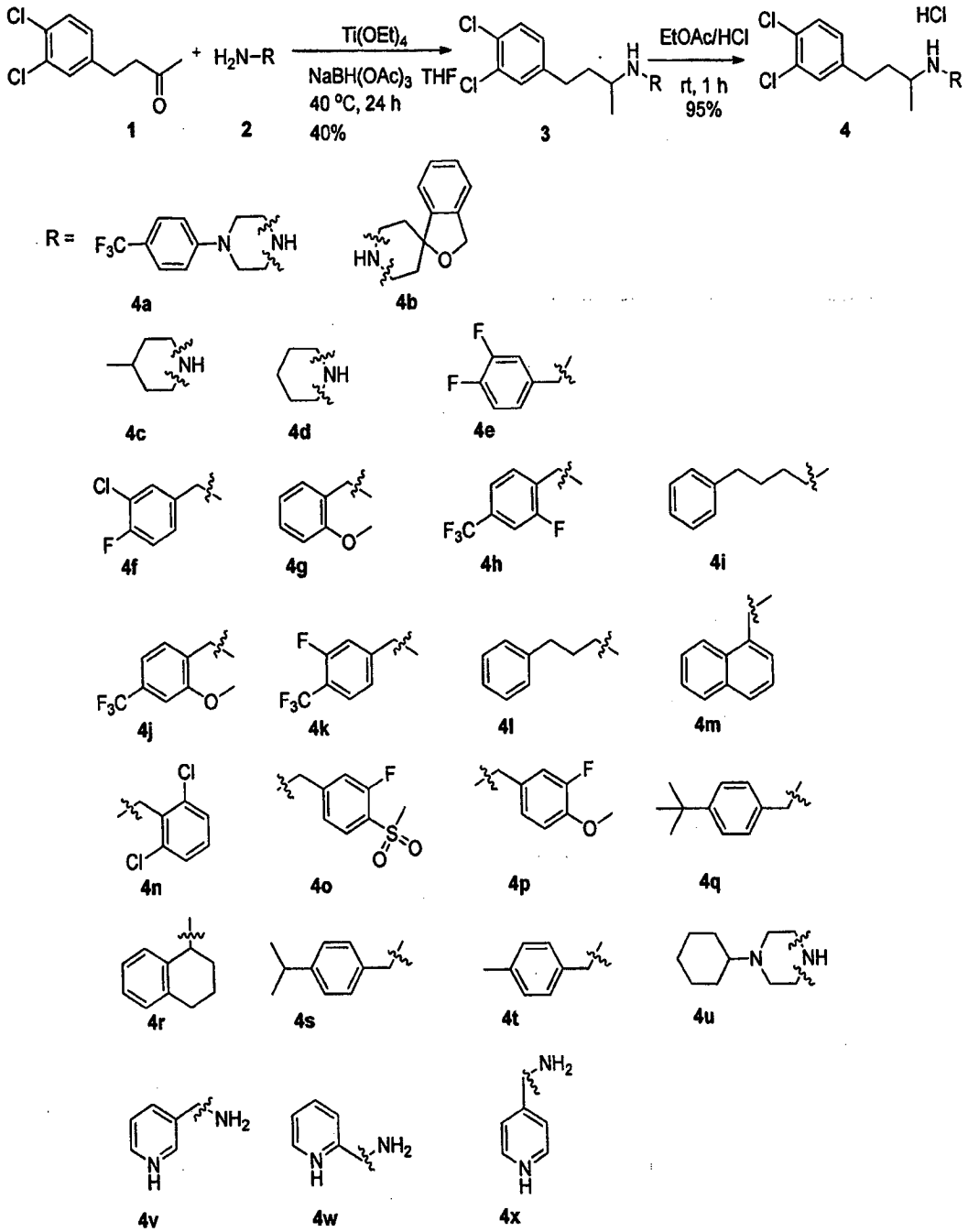
[0667] **Step1:** To a solution of **1** (0.278 g, 1.43 mmol) in THF (20 mL) was added $Ti(OEt)_4$ (2.1 g, 9.2 mmol) and (4-benzylpiperidine (0.34 g, 1.3 mmol). The mixture was stirred at 40 °C for one day, quenched by ice water, extracted with ethyl acetate (3 x 20 mL). After being concentrated *in vacuo*, the crude product was purified by column chromatography (PE/EA:1/1) to give **3** (205 mg, 35%).

[0668] **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.

[0669] 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 14

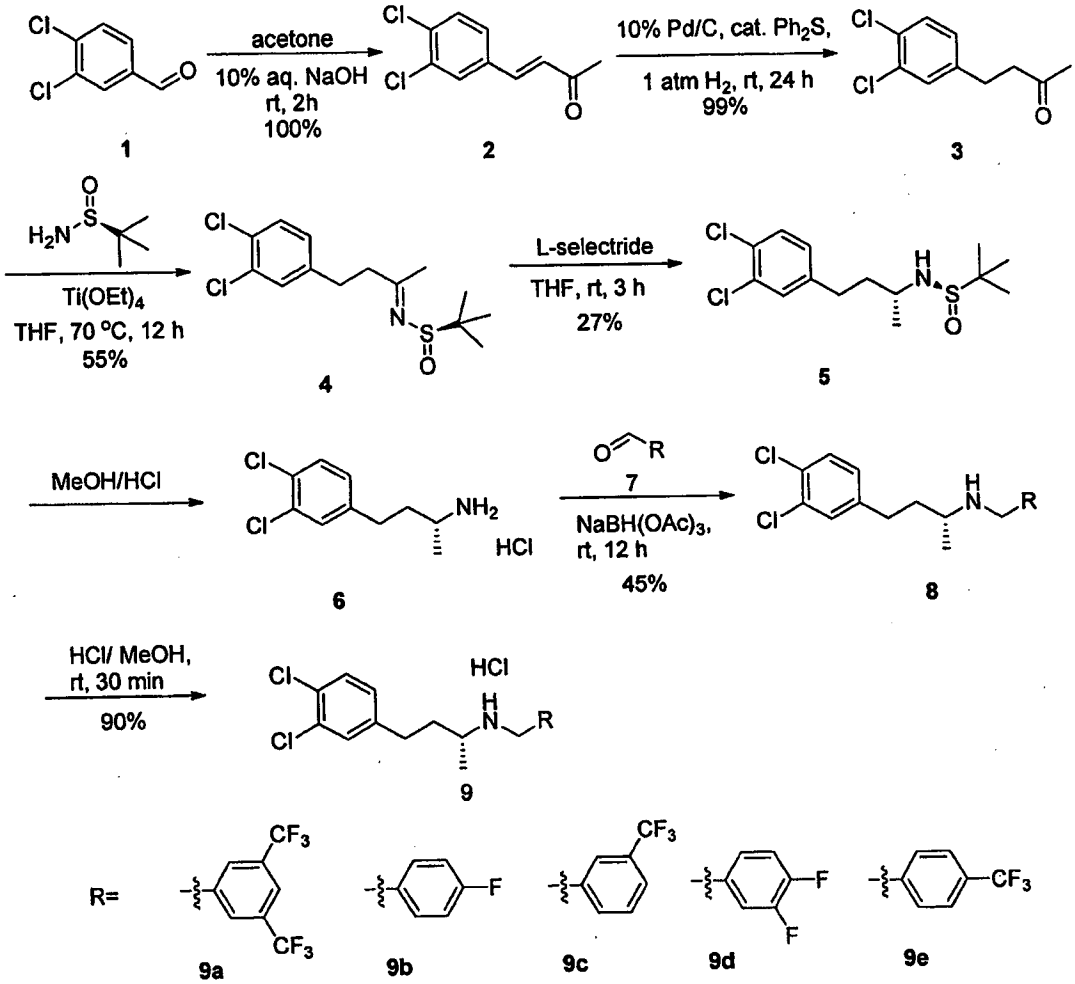
[0670] **Step1:** To a solution of **1** (0.31 g, 1.43 mmol) in THF (20 mL) was added $Ti(OEt)_4$ (0.595 g, 2.58 mmol) and N-(4-trifluoromethylphenyl)-piperazine **2**

(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%).

[0671] **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.

[0672] 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 15

[0673] **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%).

[0674] **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%).

[0675] **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%).

[0676] 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%).

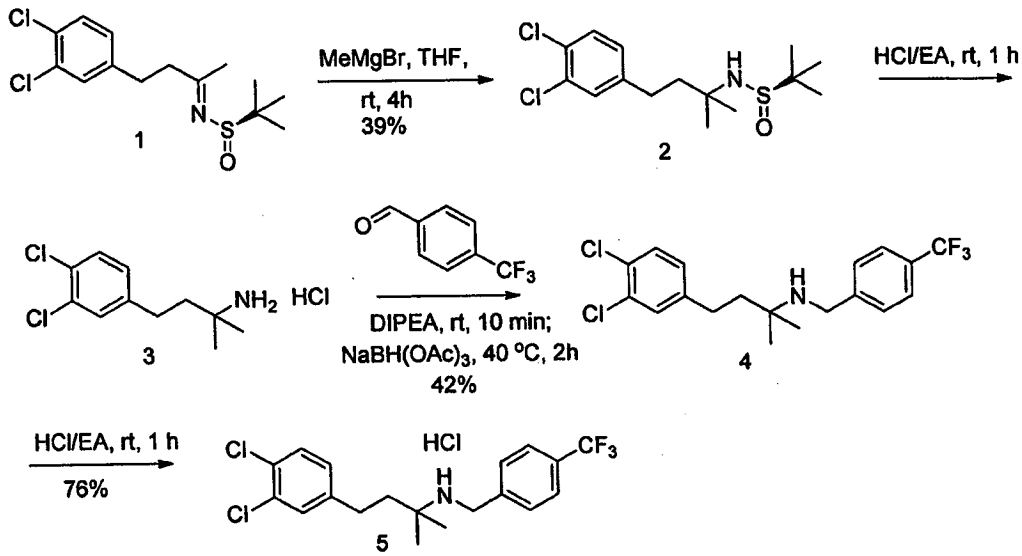
[0677] **Step5 :** To a solution of compound **5** (5 g, 15.5 mmol) in MeOH (10 mL), HCl (2 M in MeOH, 10 mL) was added and the resulting solution was stirred at rt for 3 h. TLC analysis of the reaction mixture showed complete consumption of compound **5**. The solvent was removed in vacuum, and the crude **6** (3.95 g, 100%) was used directly for next step without further purification.

[0678] **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%).

[0679] **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.

[0680] m/z (ESI+) (M+H)+: **9a**[444.2]; **9b** [326.25]; **9c** [376.2]; **9d** [344.2]; **9e** [376.1].

Synthesis Example 10



Scheme 16

[0681] **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%).

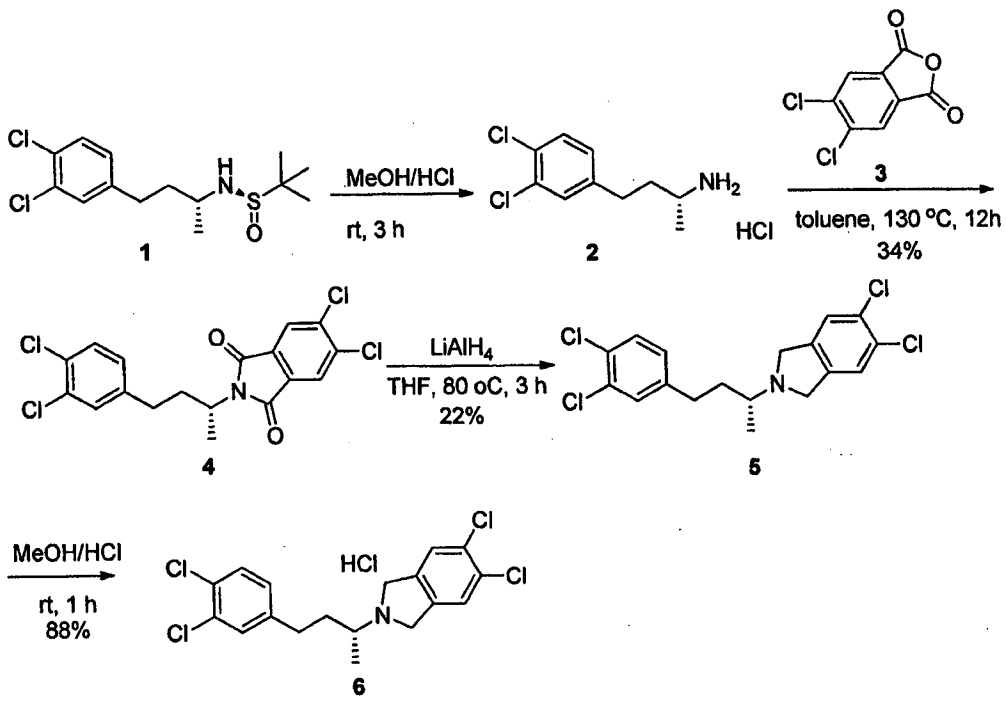
[0682] **Step2**: To a solution of compound **2** (0.6 g, 1.8 mmol) in ethyl acetate (10 mL), HCl (2 M in MeOH, 3 mL) was added and the resulting solution was stirred at rt for 1 h. TLC analysis of the reaction mixture showed complete consumption of compound **2**. The solvent was then removed in vacuum, and the crude compound **3** was used directly for next step.

[0683] **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%).

[0684] **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%).

[0685] m/z (ESI+) (M+H)+: **5** [390.14].

Synthesis Example 11



Scheme 17

[0686] **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.

[0687] **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%).

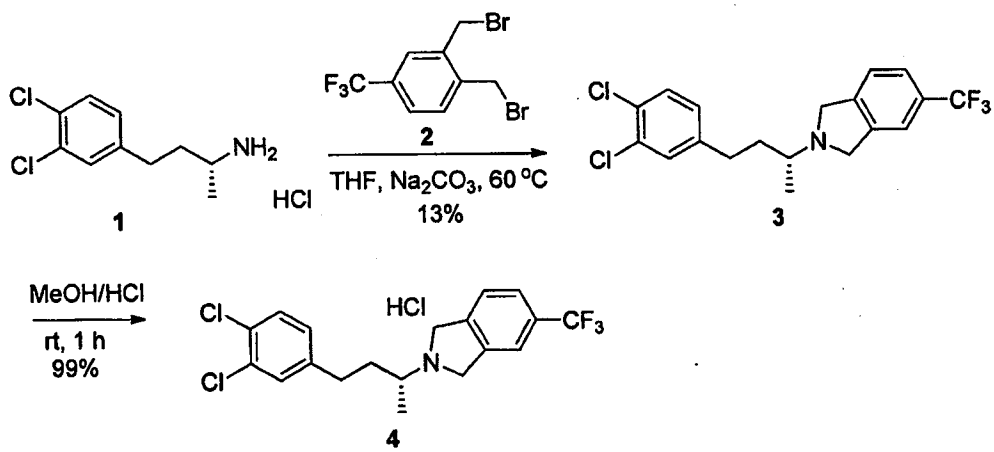
[0688] **Step3**: To a solution of compound **4** (0.78 g, 1.87 mmol) in THF (20 mL) was added LAH (0.36 g, 9.1 mmol). The mixture was stirred at 80℃ for 3h.

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%).

[0689] **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%).

[0690] m/z (ESI+) (M+H)+: **6** [390.0].

Synthesis Example 12



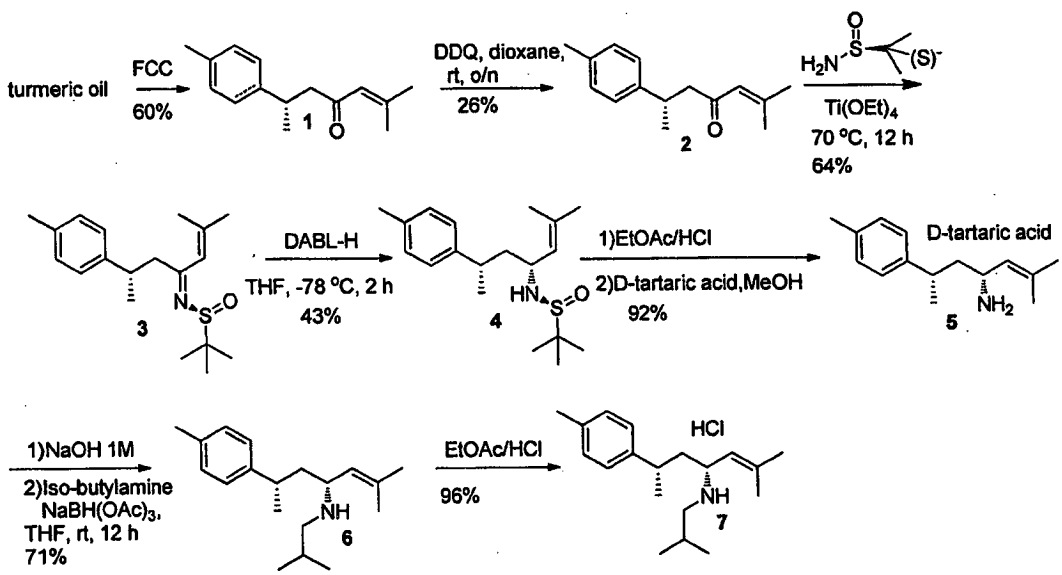
Scheme 18

[0691] **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%).

[0692] **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%).

[0693] m/z (ESI+) (M+H)+: **4** [388.1].

Synthesis Example 13



Scheme 19

[0694] **Step1:** Turmeric oil (100 g) was purified by column chromatography (PE:EA/100:1) to provide crude product **1** (60 g).

[0695] **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%).

[0696] **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%).

[0697] **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.

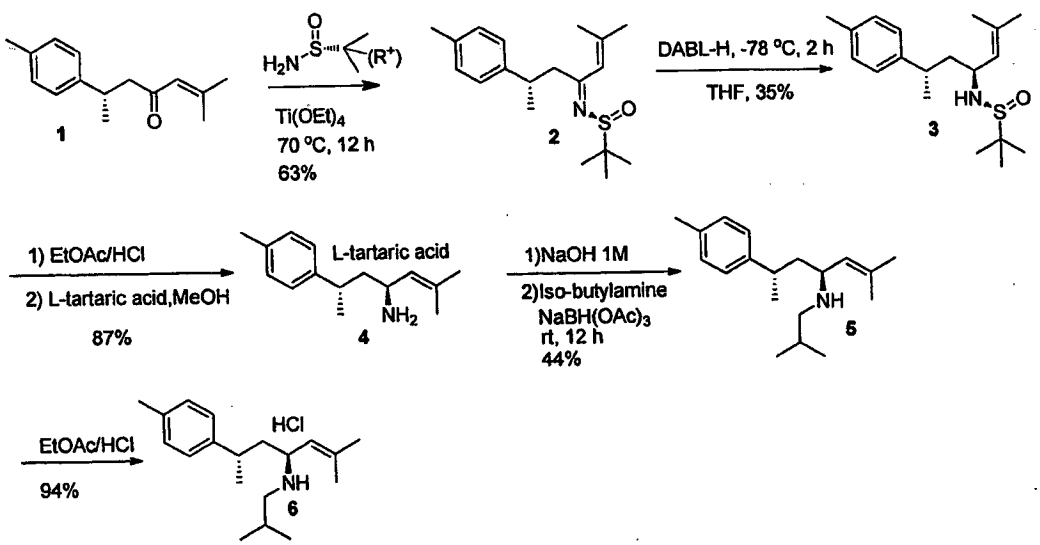
[0698] **Step 6**: A solution of compound **5** (1.7 g, 4.6 mmol) in water (20 mL) was adjusted to pH 9-10 by 1M NaOH. The product was extracted with ethyl acetate

(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%).

[0699] **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%).

[0700] m/z (ESI+) (M+H)+: 7 [274.20].

Synthesis Example 14



Scheme 20

[0701] **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%).

[0702] **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%).

[0703] **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%).

[0704] **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%).

[0705] **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%).

[0706] m/z (ESI+) (M+H)+: **6** [274.20]

[0707] 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.

[0708] 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.

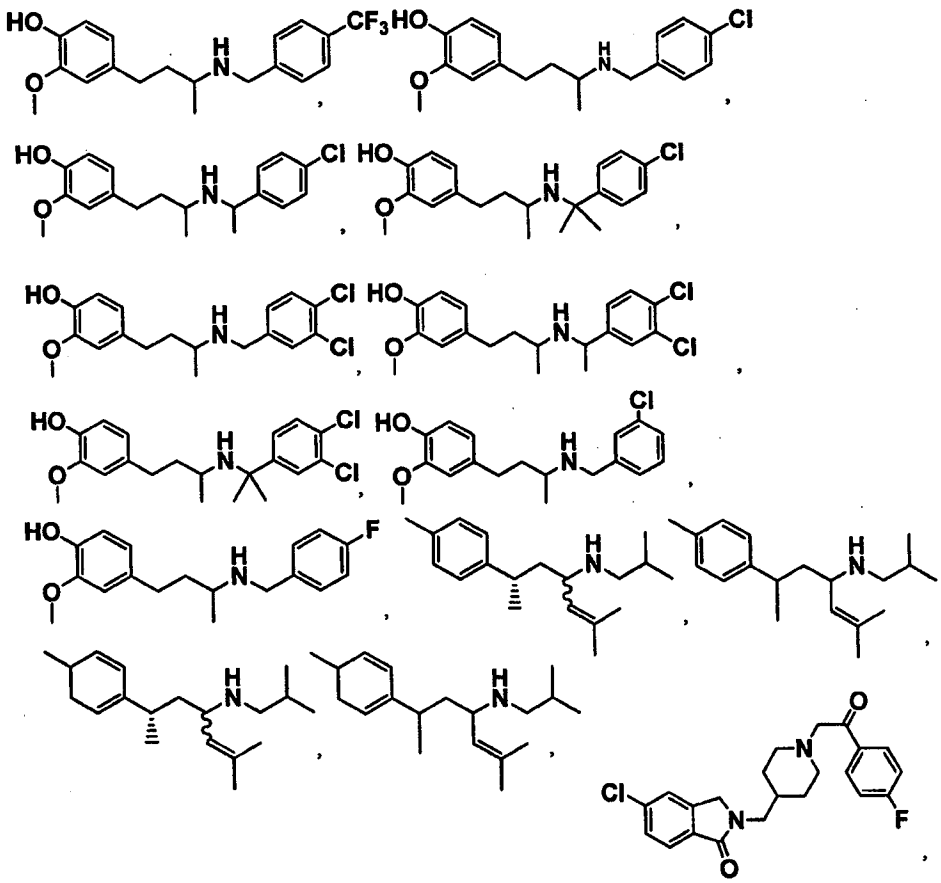
CLAIMS

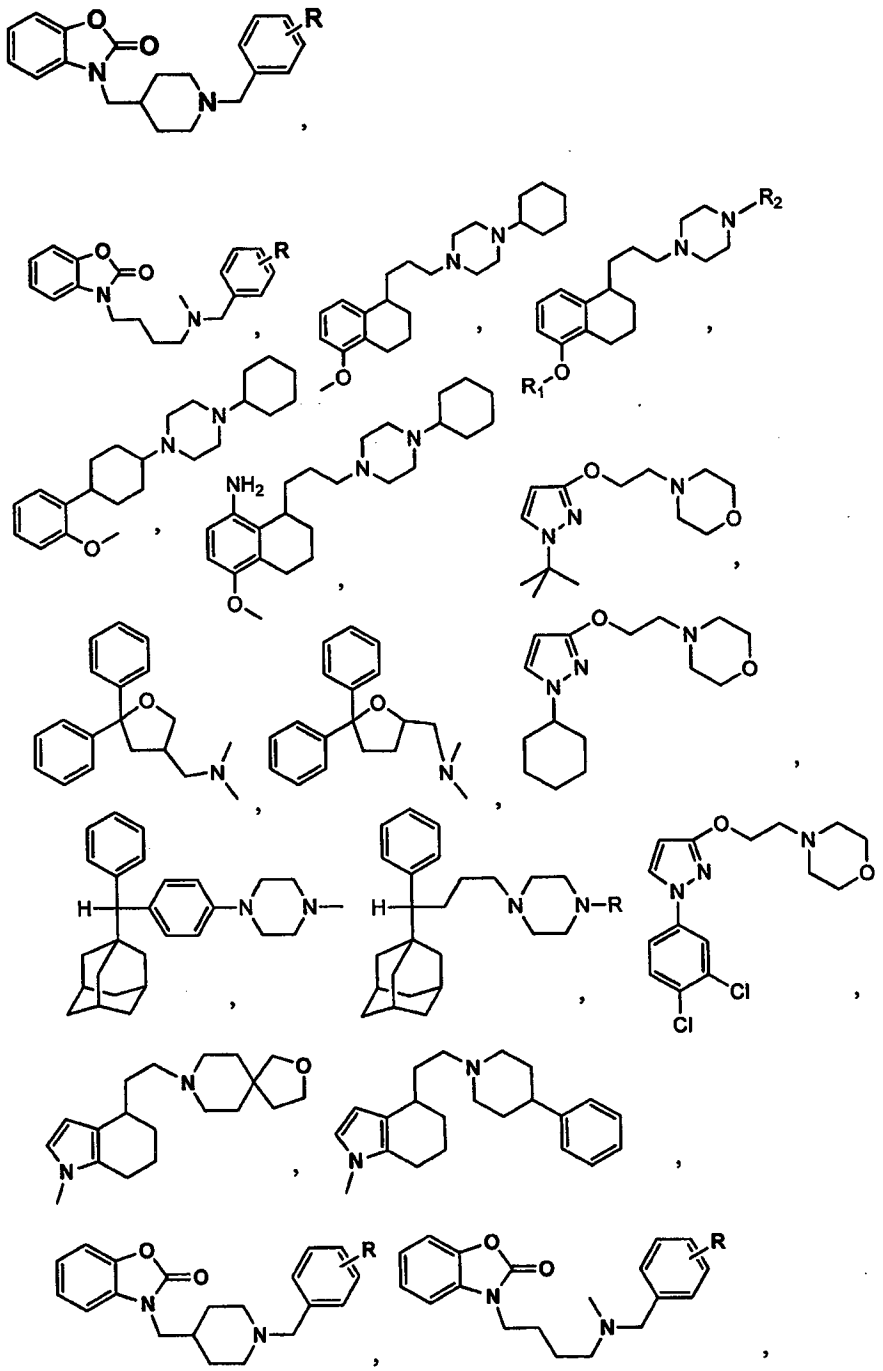
1. A method/use for inhibiting an amyloid beta effect on a neuronal cell comprising administering an effective amount of a composition comprising

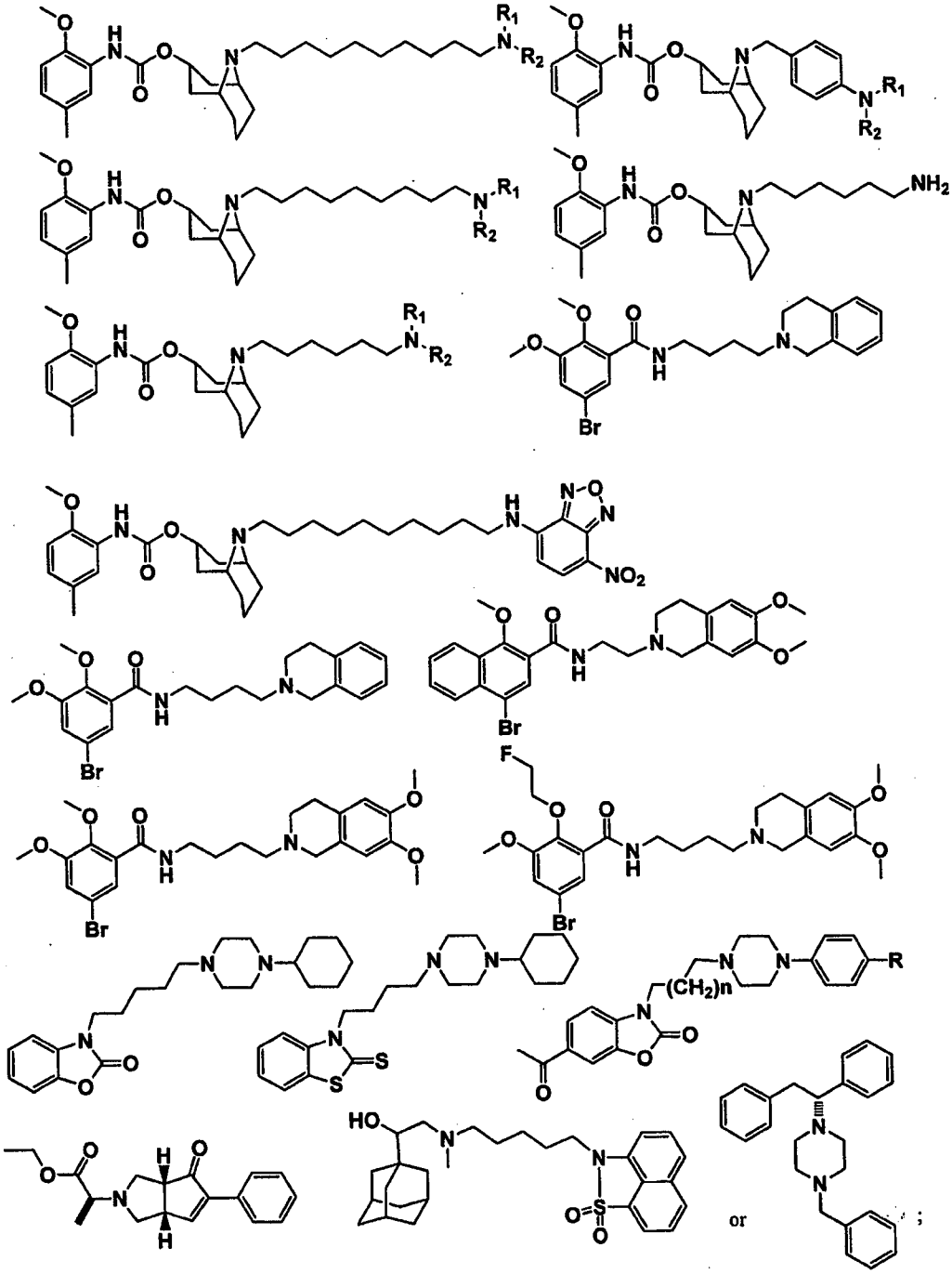
a selective sigma-2 receptor antagonist compound in an amount effective to inhibit amyloid beta oligomer binding in said cell; and

a pharmaceutically acceptable carrier.

2. The method/use of claim 1 wherein the compound is not







where R, R_1, and R_2 are independently C_{1-6} alkyl, alkoxy, halo, halo alkyl, or halo alkoxy, and n=0 to 8.

3. The method/use of claim 1 or claim 2, 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.

4. The method/use of any one of claims 1to 3, 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.

5. The method/use of any one of claims 1 to 4, wherein the compound is administered in an amount effective to inhibit a soluble amyloid beta oligomer-mediated cognitive effect.

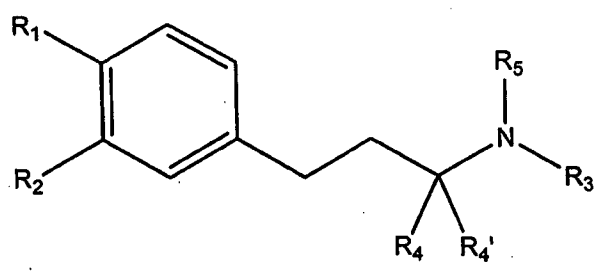
6. The method/use of claim 5, wherein the cognitive effect is cognitive decline as tested in an animal model of cognitive decline.

7. The method/use of claim 6 wherein the cognitive decline is a decline in learning as tested by a fear conditioning assay.

8. The method/use of claim 6 wherein the cognitive decline is a decline in spatial learning and memory as tested by a Morris water maze test.

9. The method/use of claim 6, wherein the cognitive decline is hippocampal-based spatial learning and memory decline as tested in a transgenic animal model of Alzheimer's disease.

10. The method/use of any one of claims 1-9 wherein the compound is of Formula VIIIq:

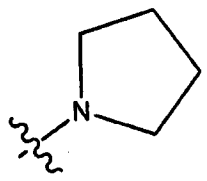


(VIIIq)

wherein

R_1 and R_2 are independently selected from H, OH, halo, C_{1-6} alkoxy, C_{1-6} haloalkoxy, C_{1-6} haloalkyl, $(R_{16})(R_{17})N$-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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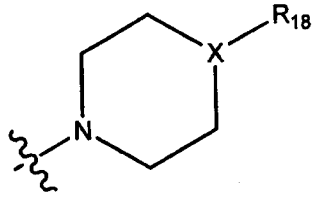
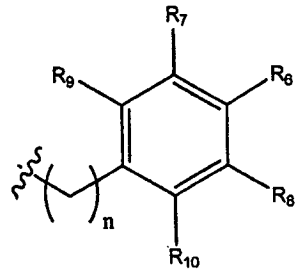
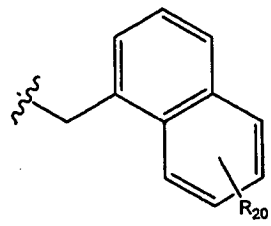
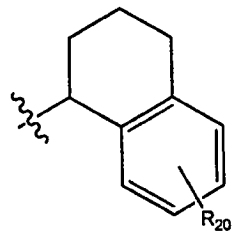
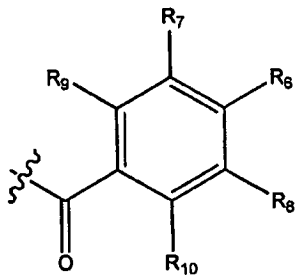
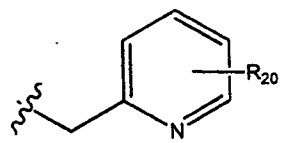
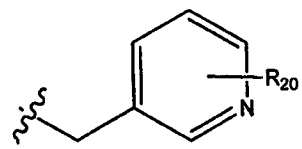
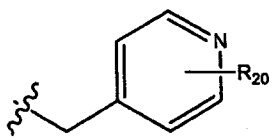
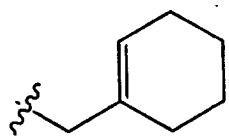


, wherein

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

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

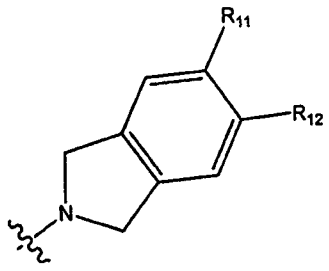
n is 1-4

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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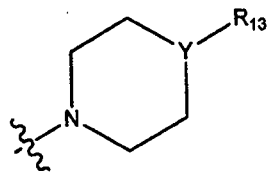

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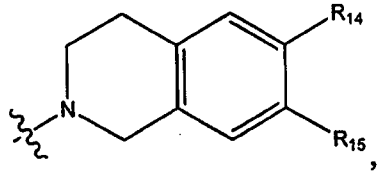

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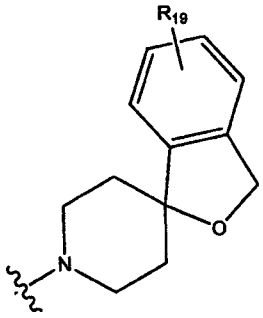


, wherein

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

Y is CH or N;

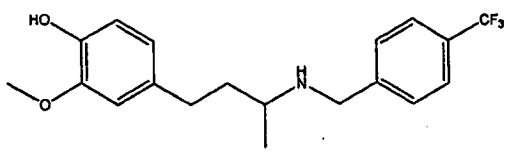
R_{13}.is H, C_{1-6} alkyl, C_{3-6} cycloalkyl, unsubstituted phenyl or phenyl substituted with C_{1-6} haloalkyl, or unsubstituted benzyl

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

R_{19} is H, and

pharmaceutically acceptable salts thereof,

with the proviso that the following racemic mixtures of compounds are excluded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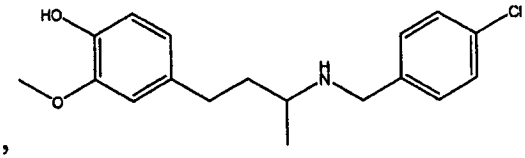
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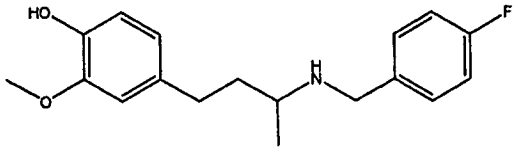
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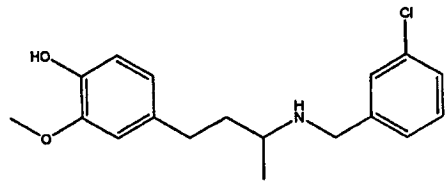
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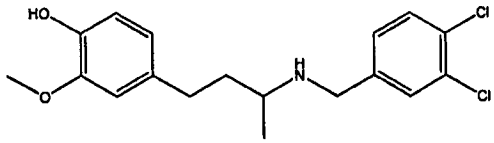
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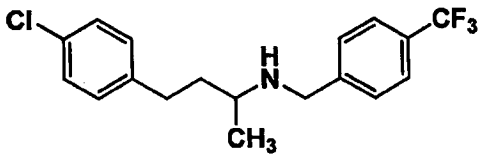
11. The method/use of any one of claims 1- 10 wherein said compound is selected from one of the following compounds, or a pharmaceutically acceptable salt thereof: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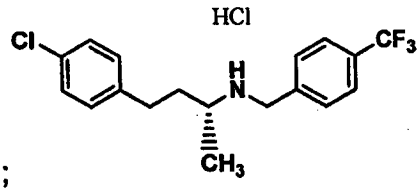
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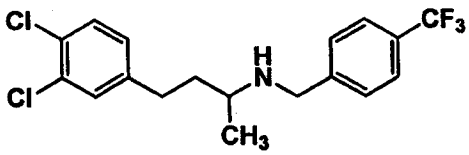
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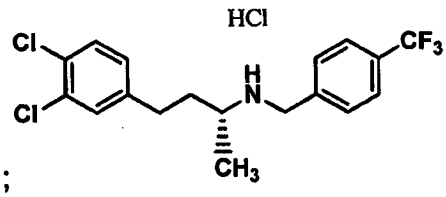
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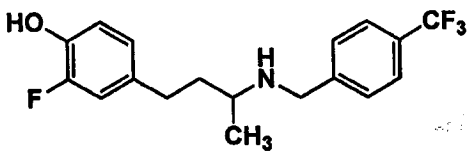
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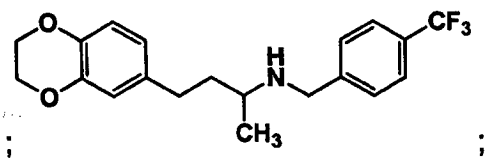
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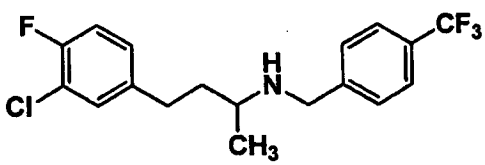
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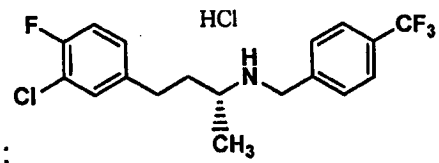
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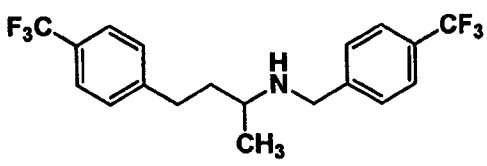
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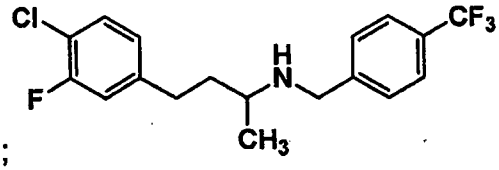
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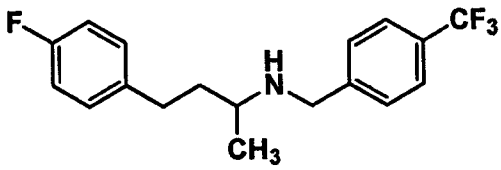
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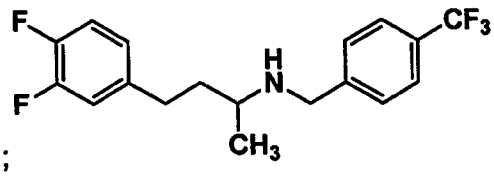
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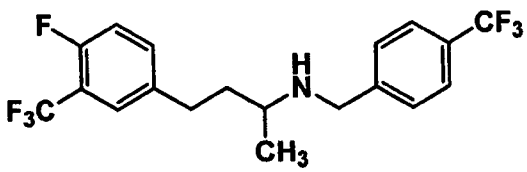
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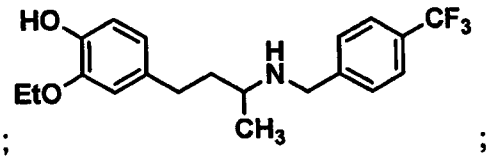
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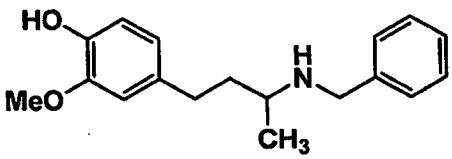
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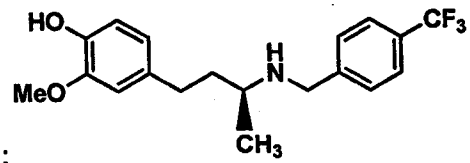
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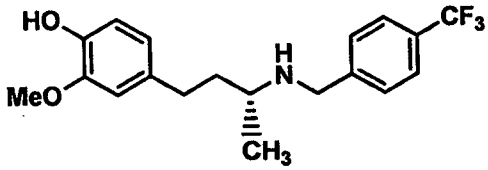
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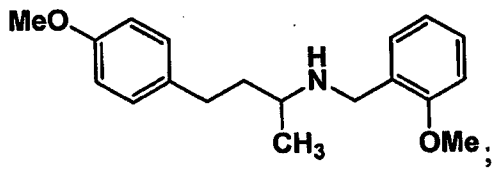
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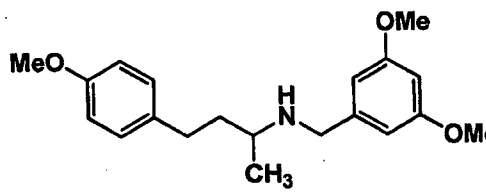
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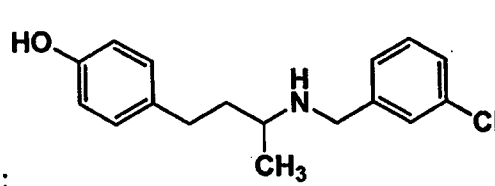
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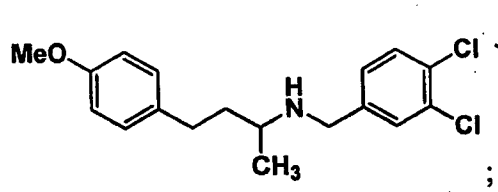
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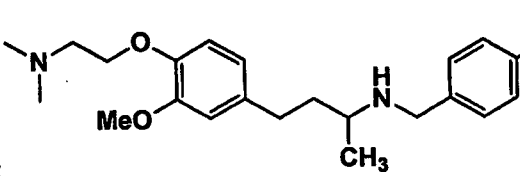
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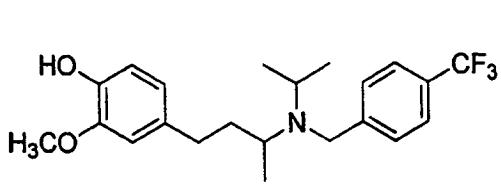
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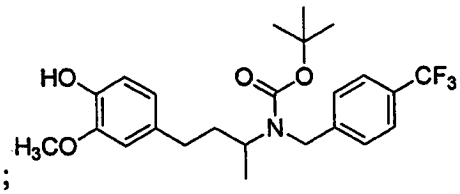
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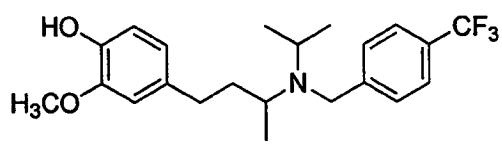
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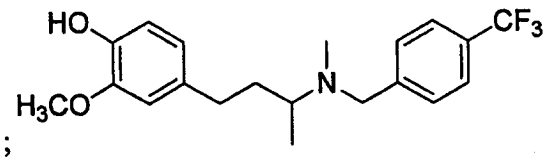
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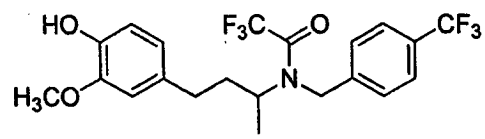
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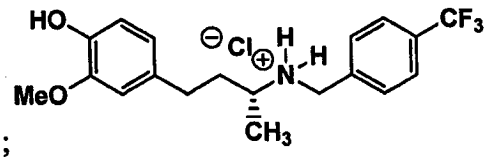
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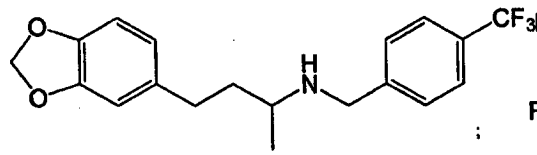
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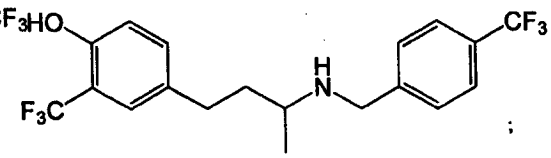
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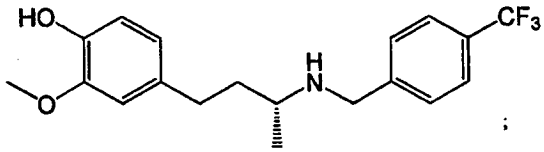
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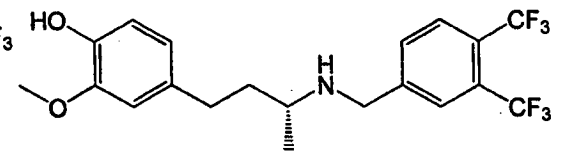
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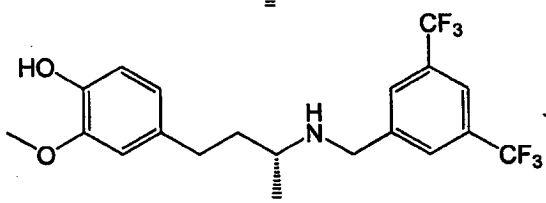
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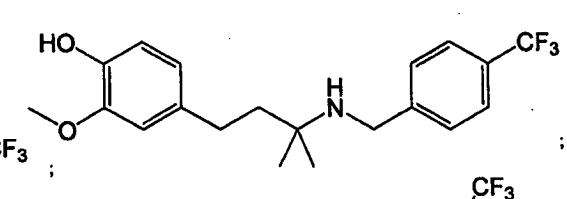
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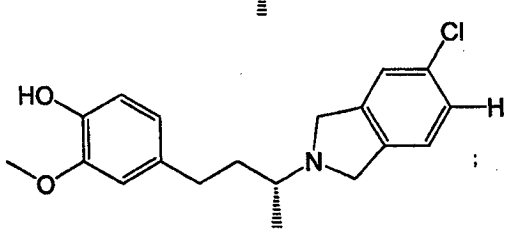
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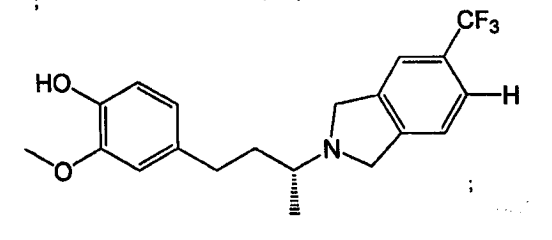
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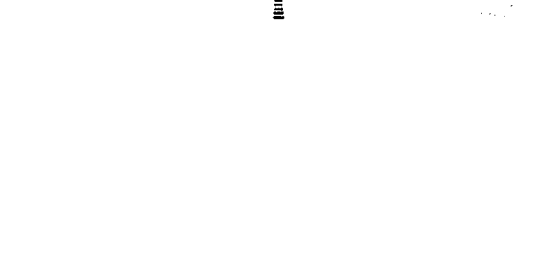
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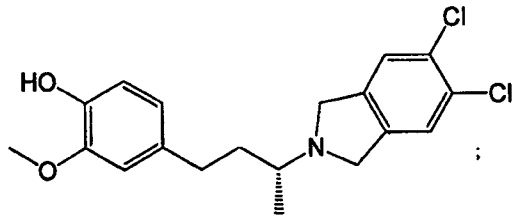
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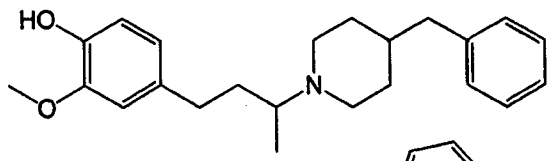
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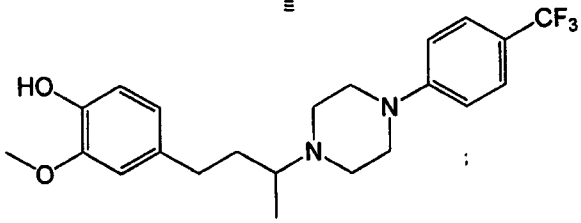
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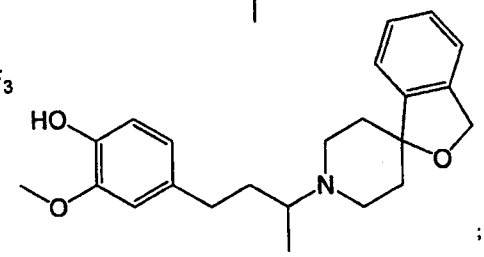
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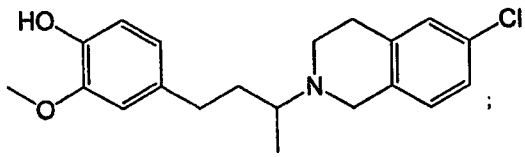
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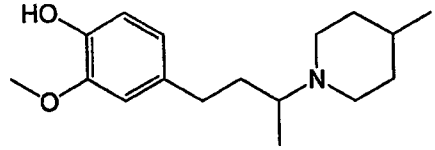
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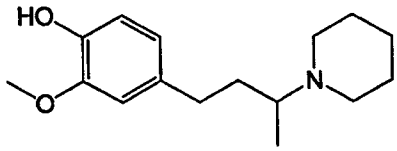
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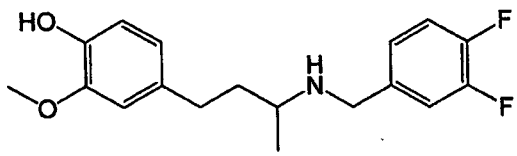
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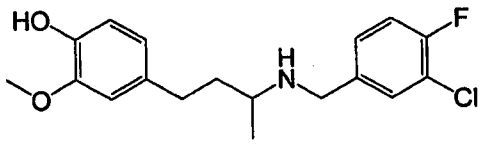
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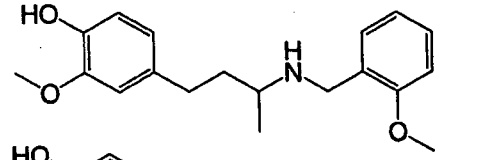
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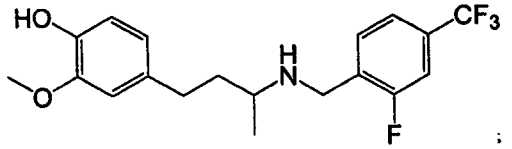
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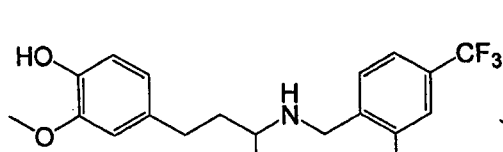
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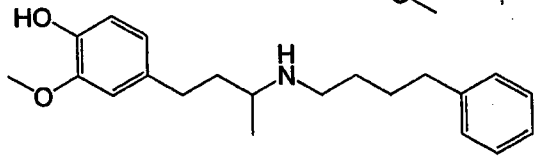
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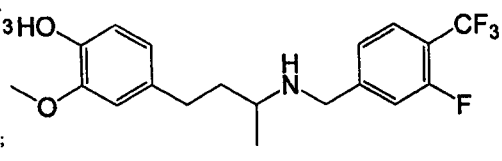
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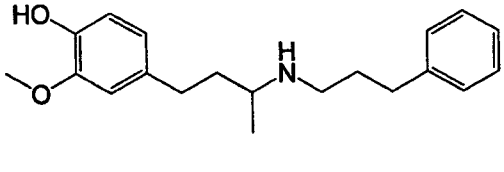
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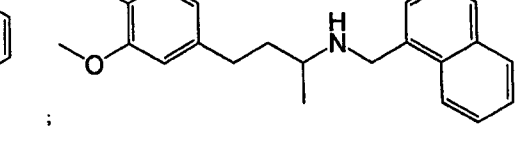
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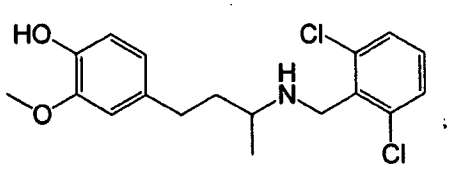
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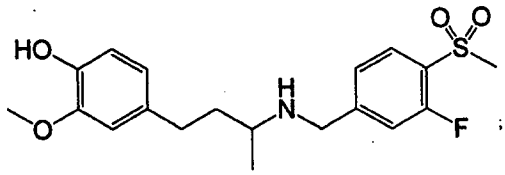
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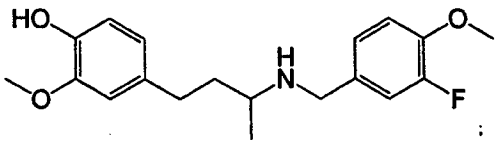
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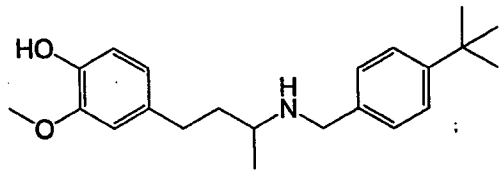
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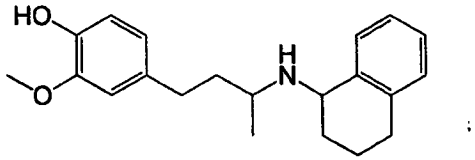
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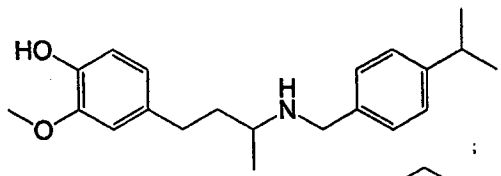
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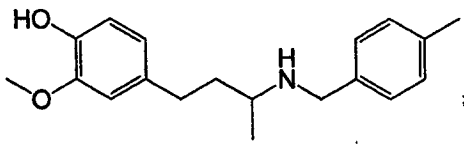
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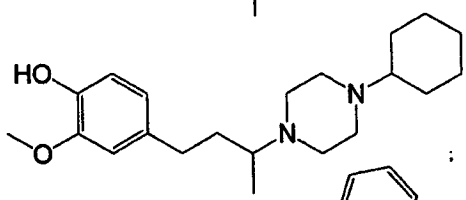
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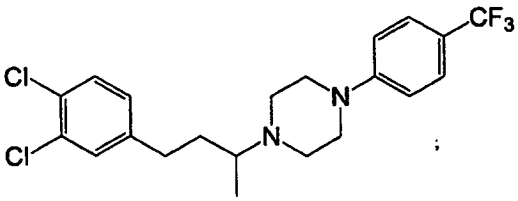
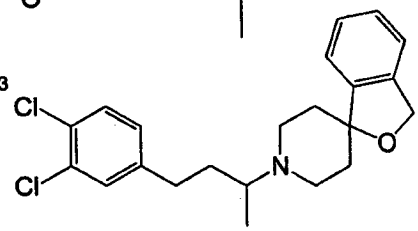
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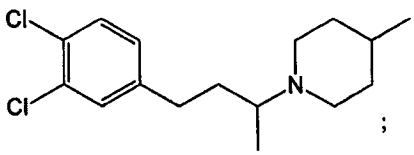
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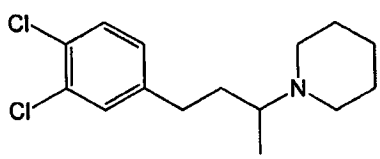
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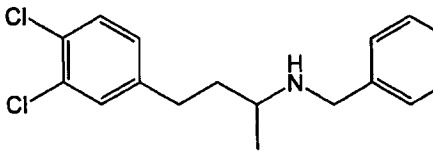
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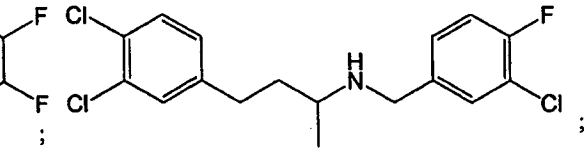
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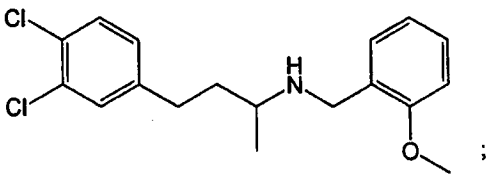
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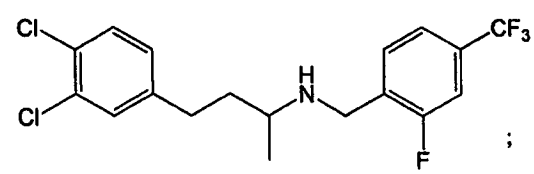
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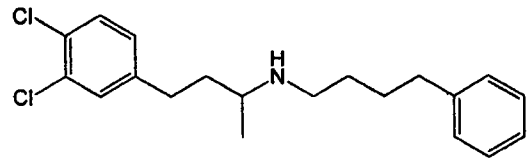
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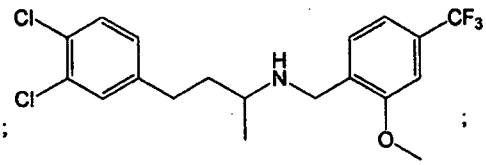
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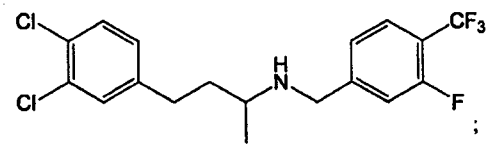
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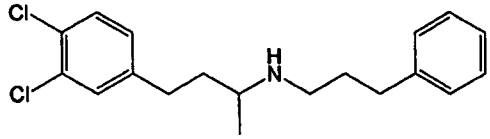
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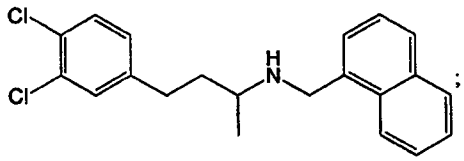
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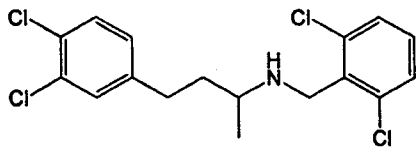
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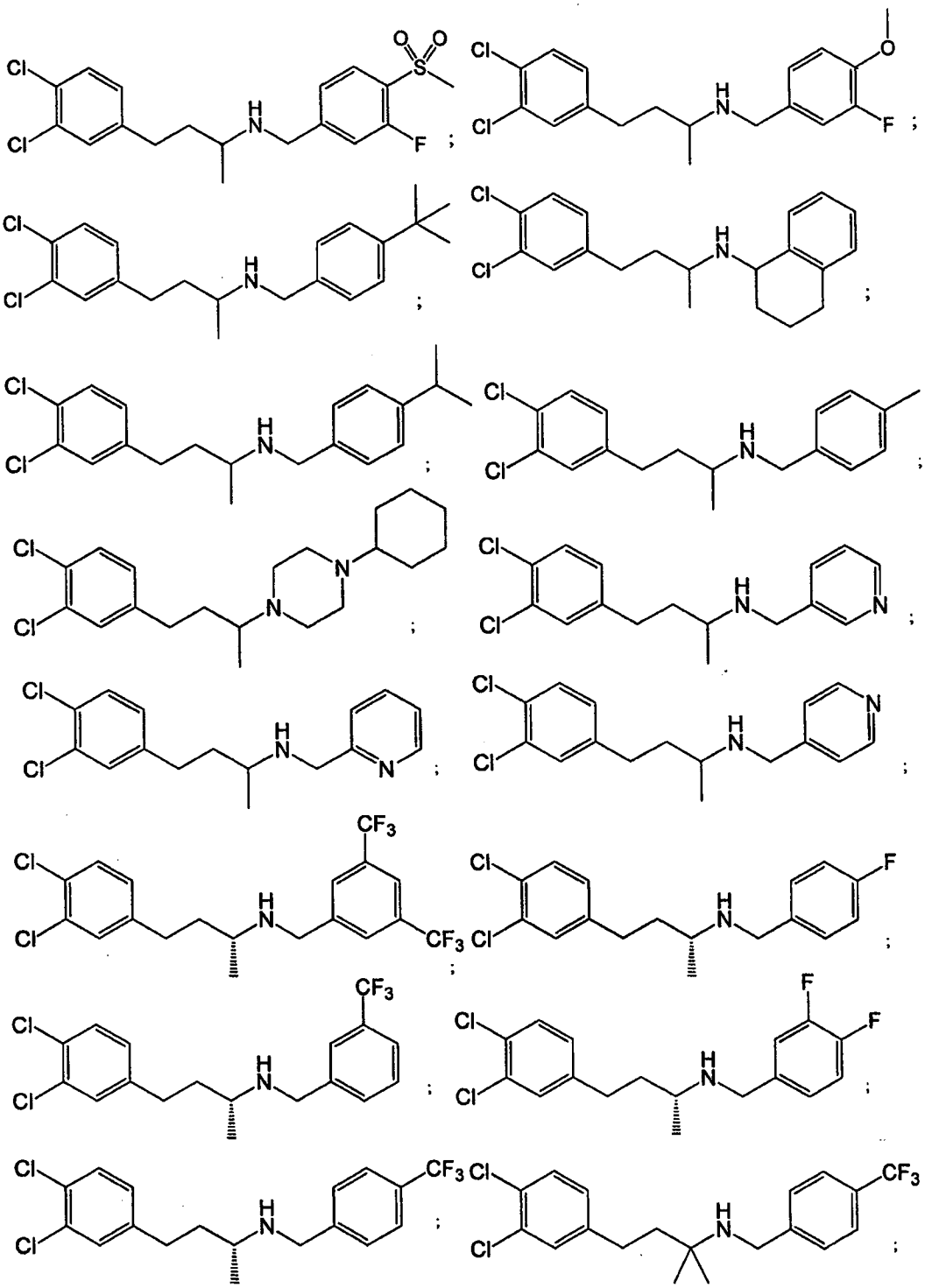

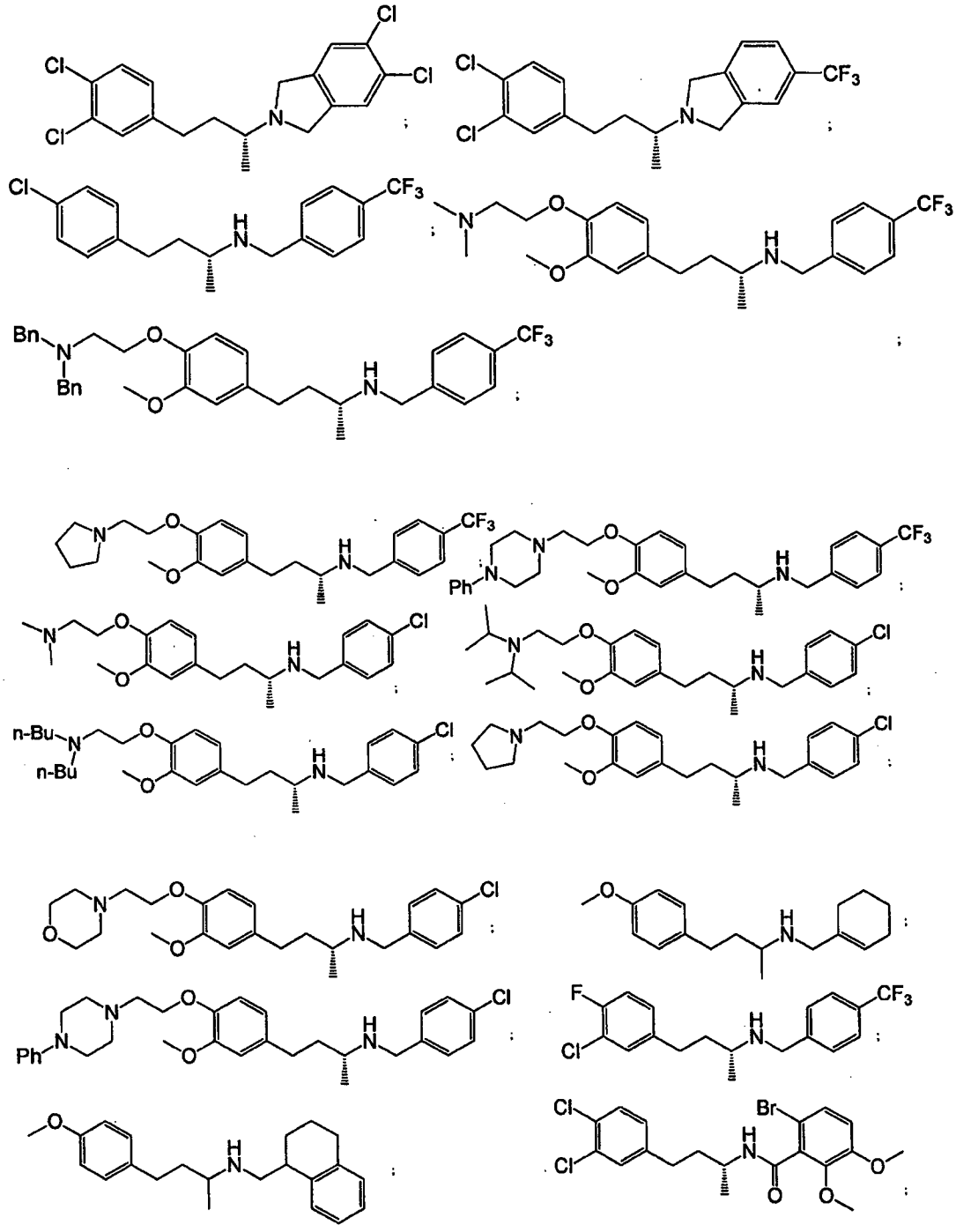

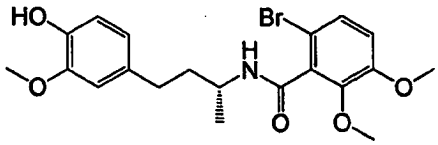
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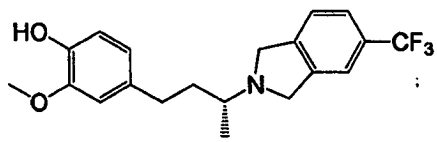
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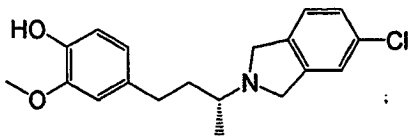
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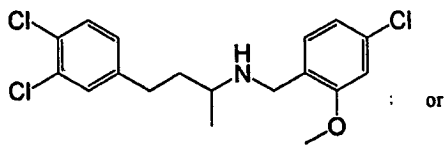
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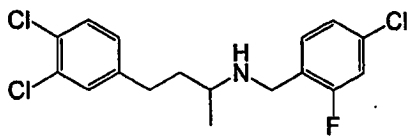
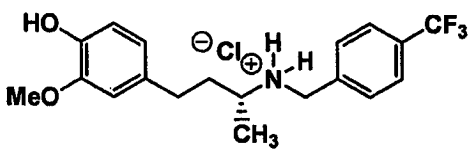


12. The method/use of claim 11 wherein the compound is selected from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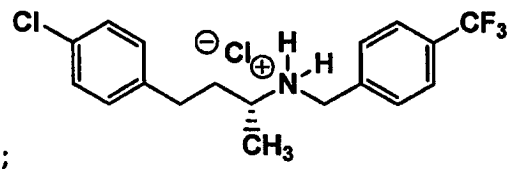
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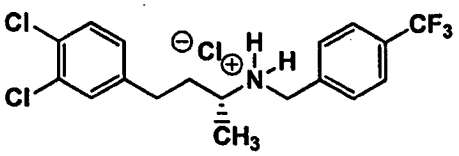
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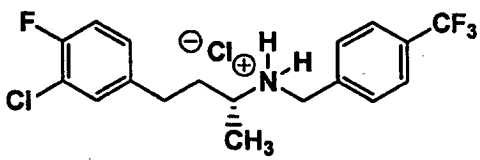
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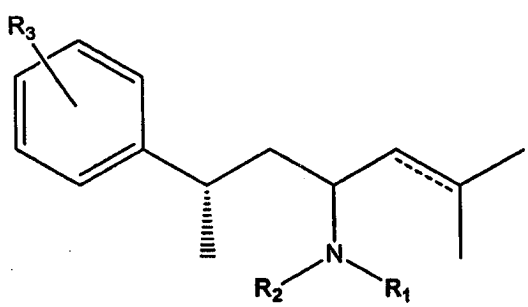
13. The method/use of claim 2 wherein the compound is of Formula VIIIo



VIIIo

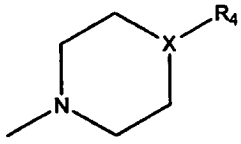
wherein:

$\cdots\cdots$ 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



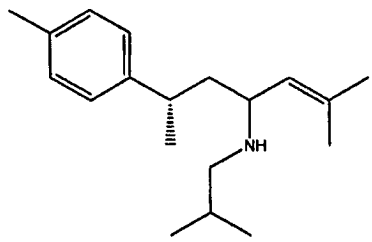
, wherein

X is CH, N, or O, and

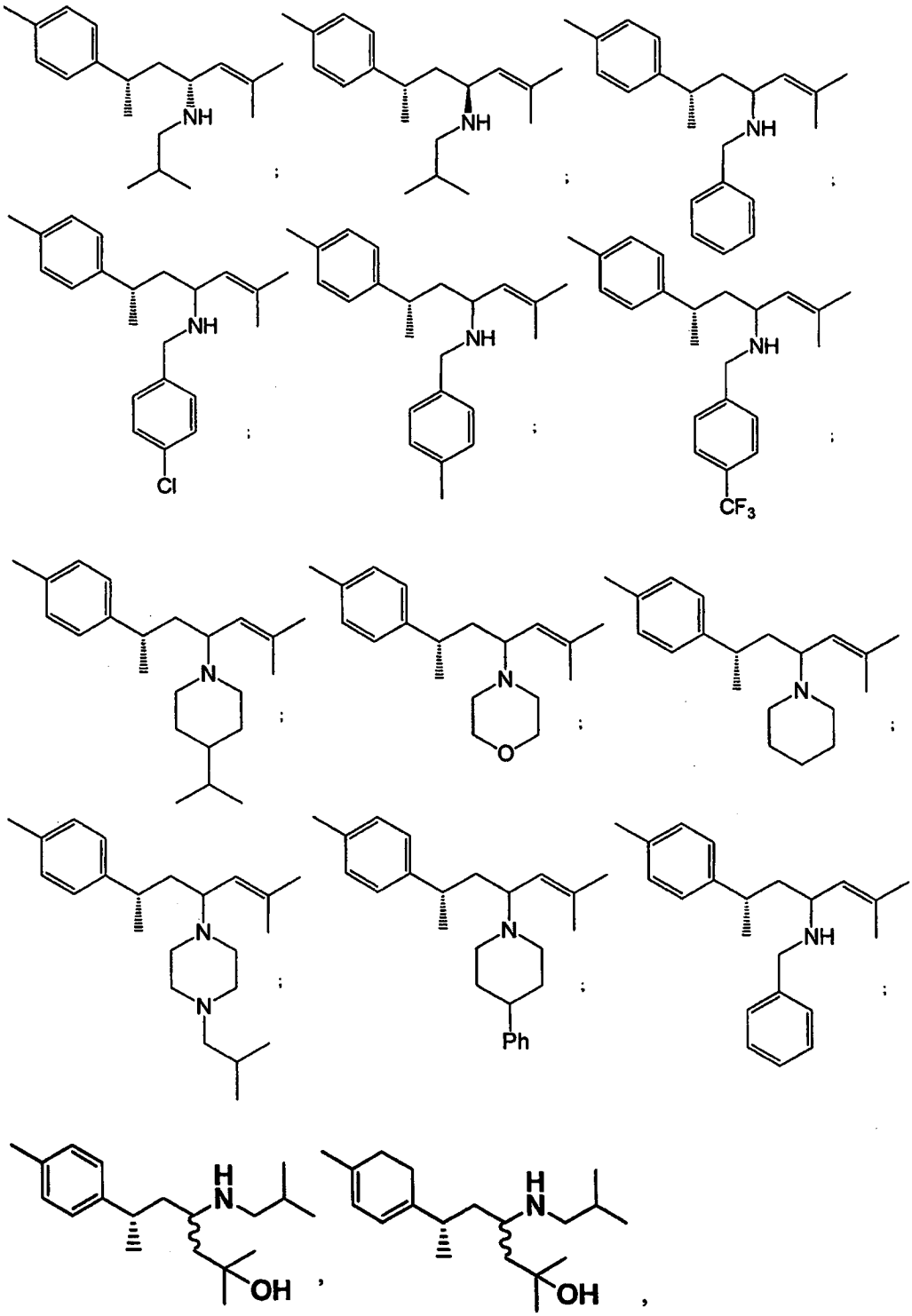
R_4 is absent, or is H, C_{1-6} alkyl, or unsubstituted phenyl or phenyl substituted with halo, C_{1-6} alkyl, or C_{1-6} haloalkyl; and

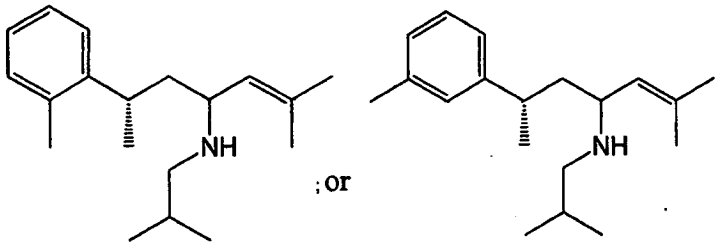
R_3 is C_{1-4} alkyl, C_{1-6} haloalkoxy, or halo, and

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



14. The method/use of claim 13 wherein said compound is selected from one of the following compounds, or a pharmaceutically acceptable salt thereof:





15. The method/use of claim 1 wherein said compound is selected from an antibody, or active binding fragment thereof, wherein the antibody or fragment is specific for a sigma-2 receptor.

16. The method/use of claim 15 wherein the antibody or fragment is specific for an epitope of an antigen present in one or more of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 9, or SEQ ID NO: 10.

17. The method/use of any one of claims 1-16 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.

18. The method/use of any one of claims 1-16 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.

19. The method/use of any one of claims 1-16 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.

20. The method/use of any one of claims 1-16 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.

21. The method/use of any one of claims 1-16 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.

22. The method/use of any one of claims 1-21 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.

23. The method/use for identifying a selective, high affinity sigma-2 antagonist compound comprising:

selecting a compound for testing on the basis of its ability to selectively bind 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;

contacting a neuronal cell with the compound; and

determining whether said compound has one or more of the following additional properties:

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

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

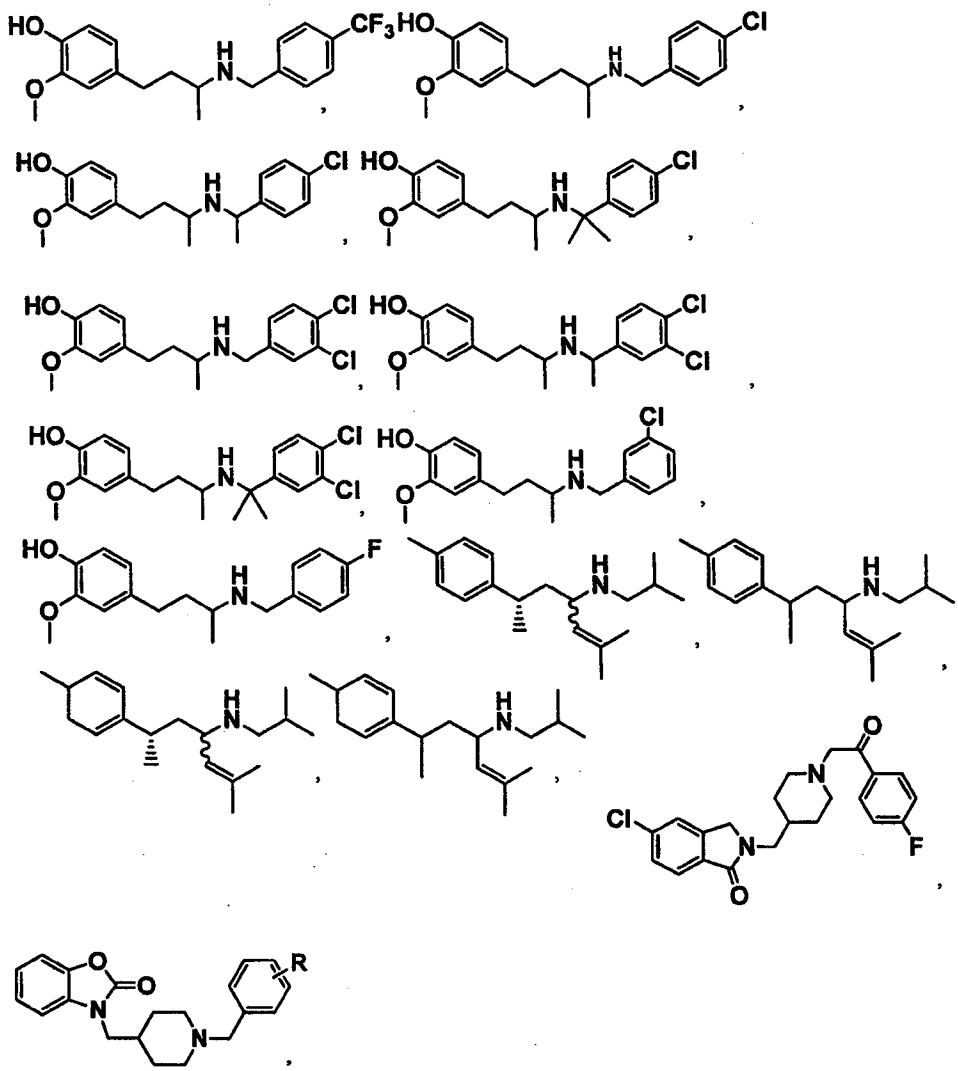
(c) it fails to affect trafficking or synapse number in the absence of amyloid beta oligomers.

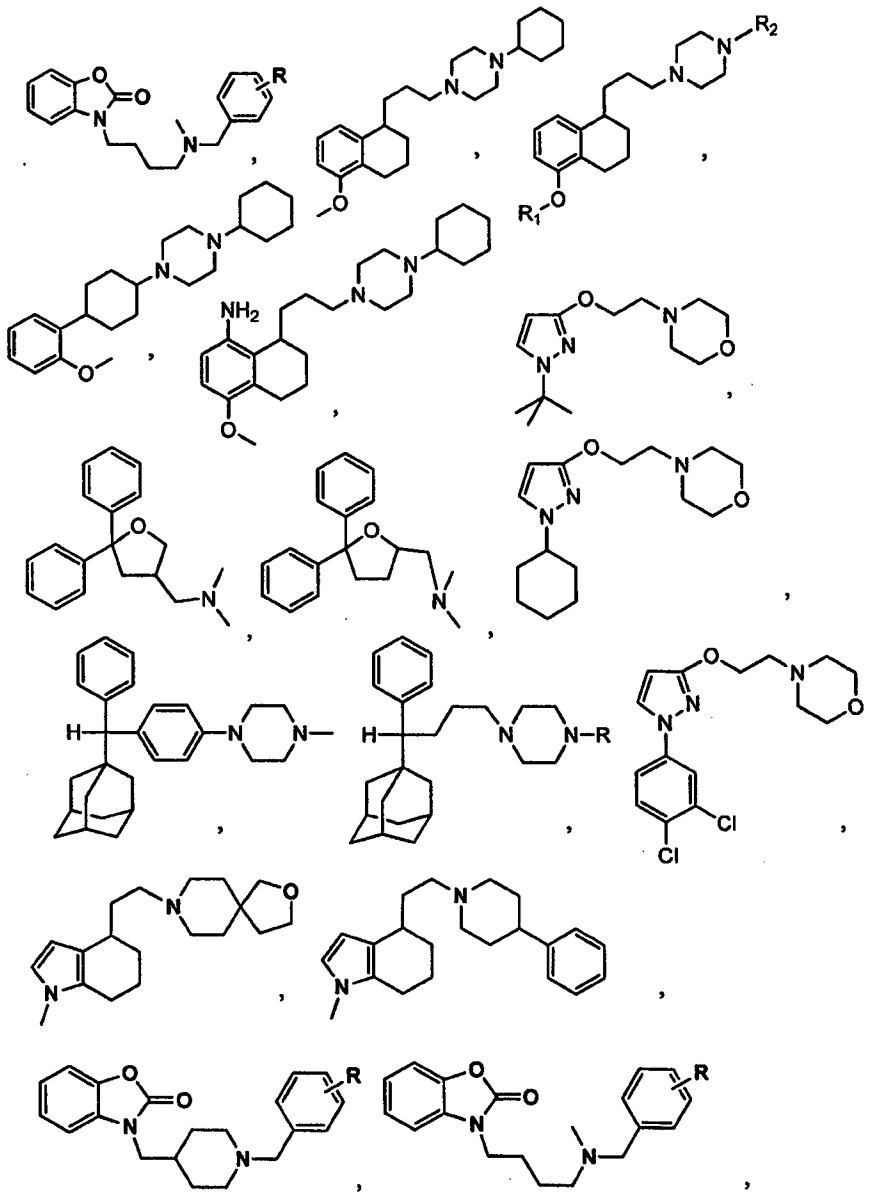
24. A composition for inhibiting an amyloid beta effect on a neuronal cell comprising

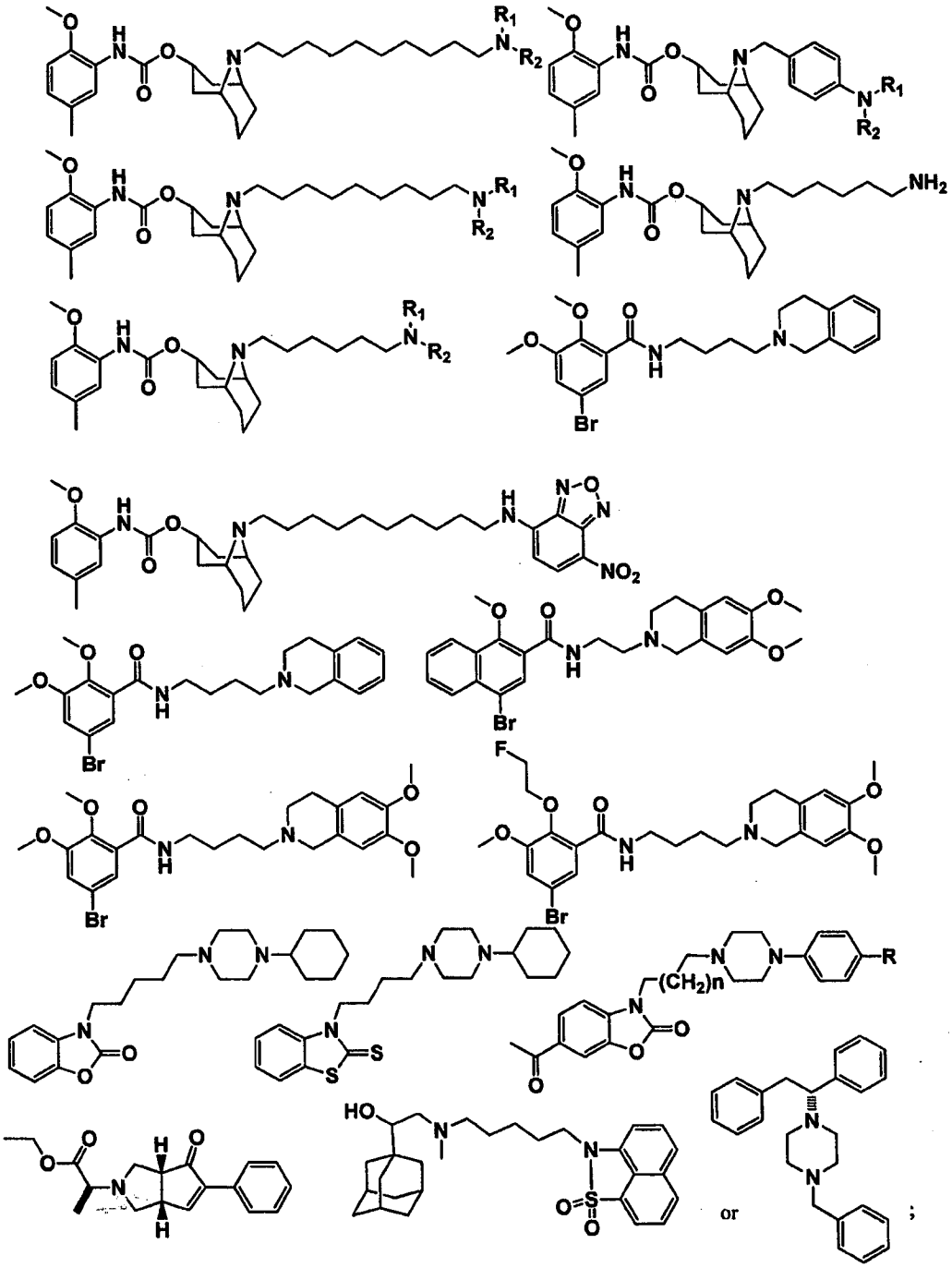
a selective, sigma-2 receptor antagonist compound in an amount effective to inhibit amyloid beta oligomer binding in said cell; and

a pharmaceutically acceptable carrier.

25. The composition of claim 24 comprising a sigma-2 receptor antagonist wherein the compound is not

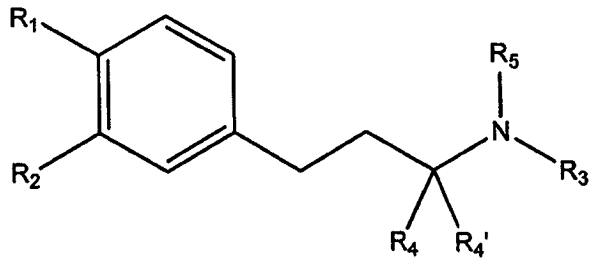






where R, R_1, and R_2 are independently C_{1-6} alkyl, alkoxy, halo, halo alkyl, or halo alkoxy, and n=0 to 8.

26. The composition of claim 24 or claim 25 wherein the compound is of Formula VIIIq:

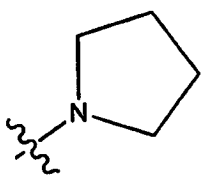


(VIIIq)

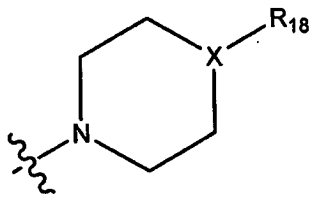
wherein

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

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



, and

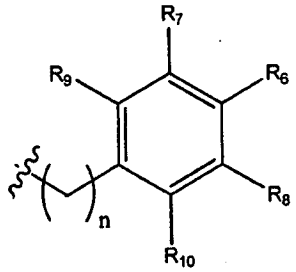
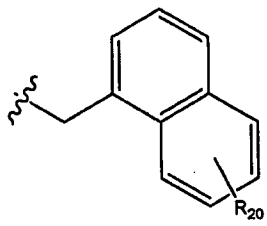
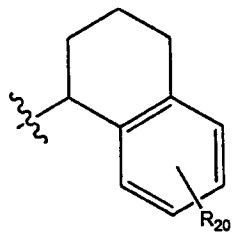
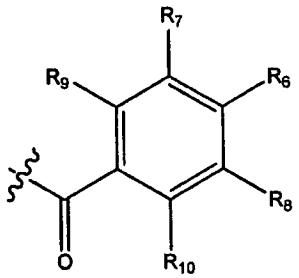
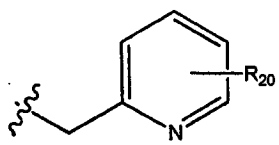
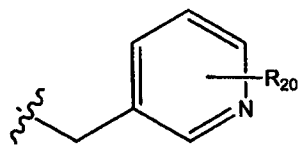
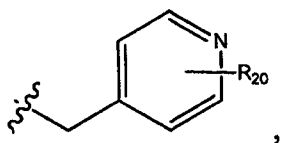
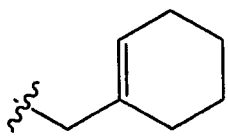


, wherein

X is N or O and R_{18} is 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

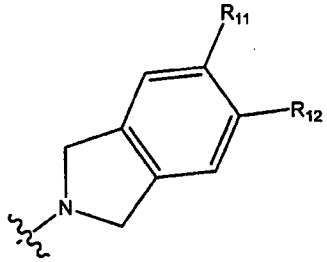
n is 1-4

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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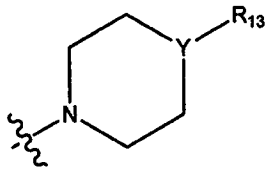

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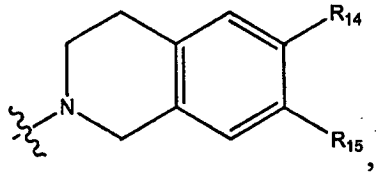

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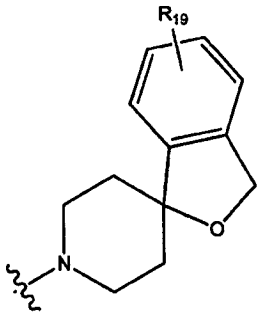


and , wherein

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

Y is CH or N;

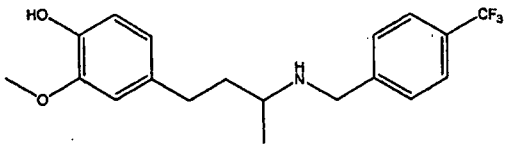
R_{13}.is H, C_{1-6} alkyl, C_{3-6} cycloalkyl, unsubstituted phenyl or phenyl substituted with C_{1-6} haloalkyl, or unsubstituted benzyl

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

R_{19} is H, and

pharmaceutically acceptable salts thereof,

with the proviso that the following racemic mixtures of compounds are excluded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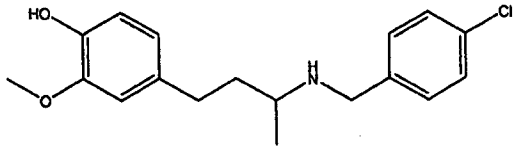
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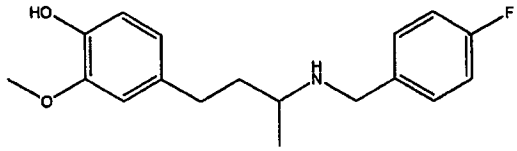
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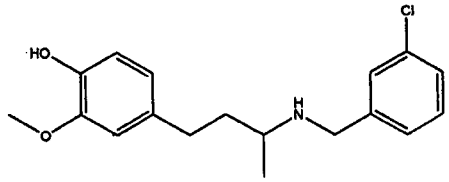
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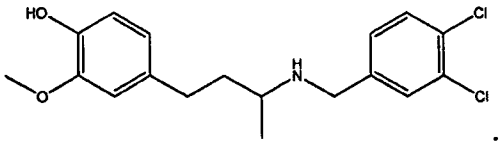
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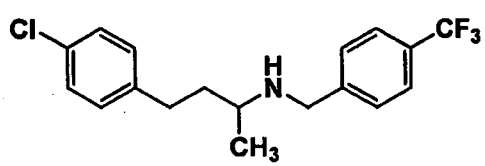
27. The composition of any one of claims 24-26 wherein said compound is selected from one of the following compounds, or a pharmaceutically acceptable salt thereof: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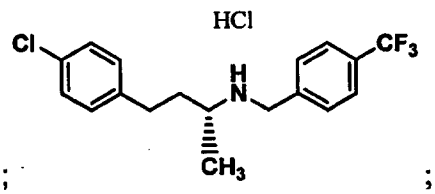
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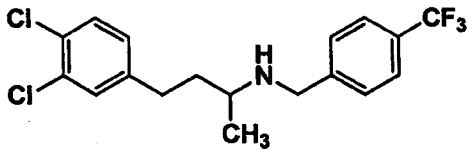
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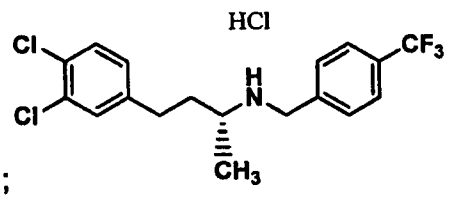
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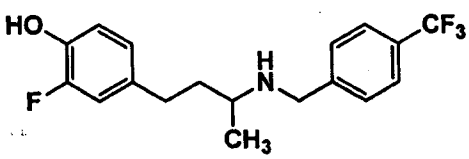
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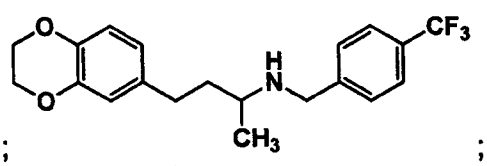
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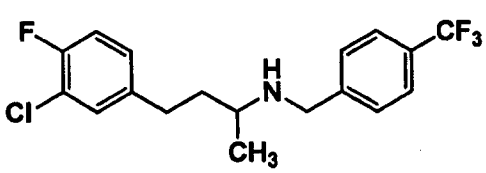
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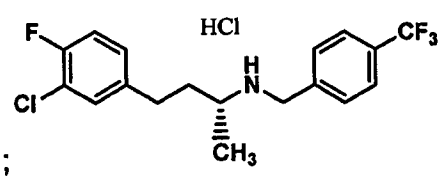
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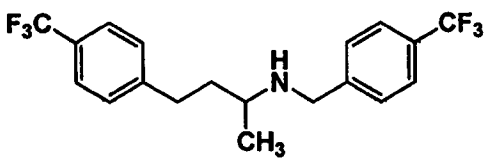
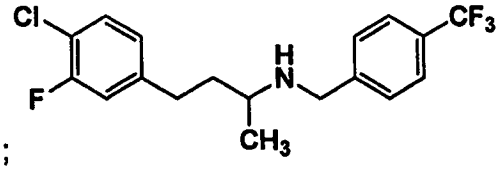
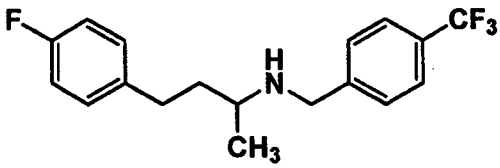
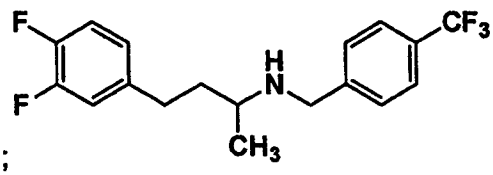
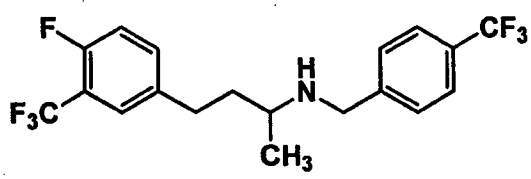
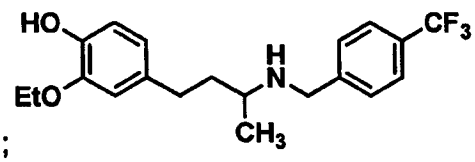
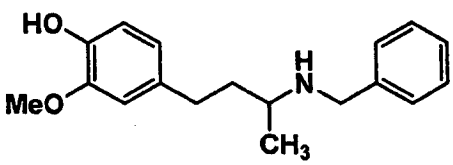
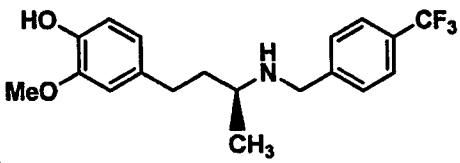
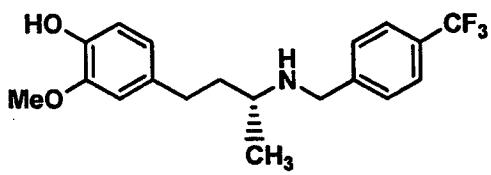
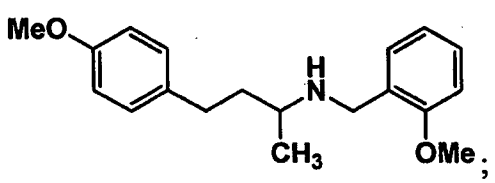
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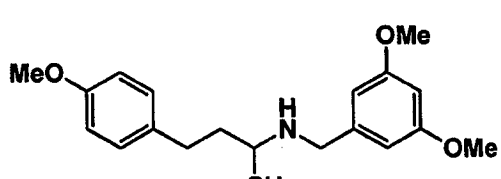
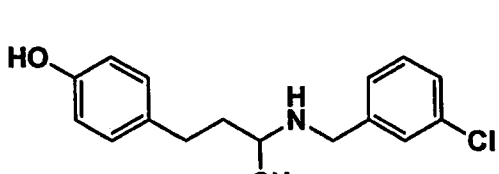
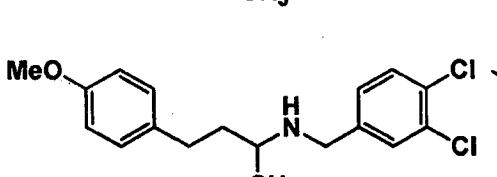
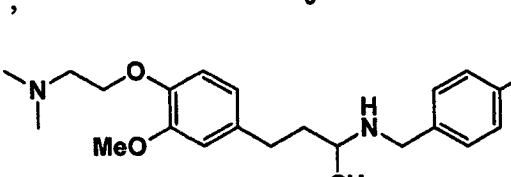
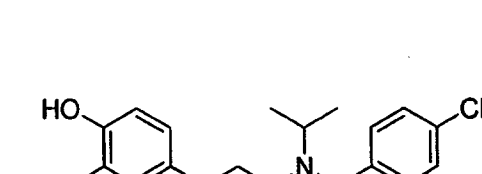
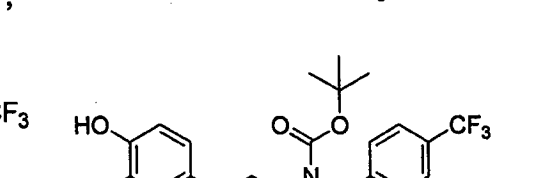
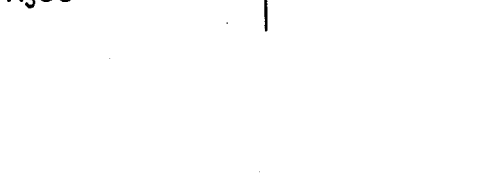
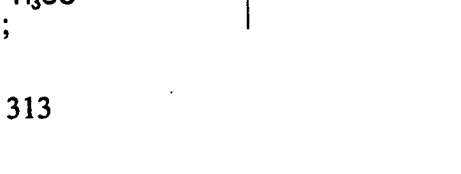

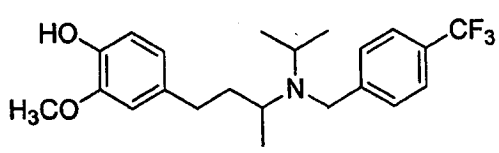
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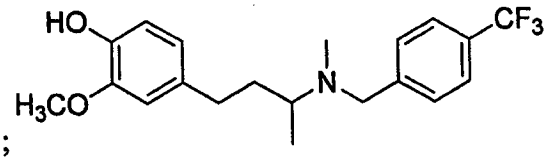
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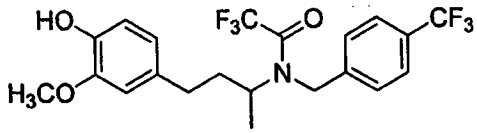
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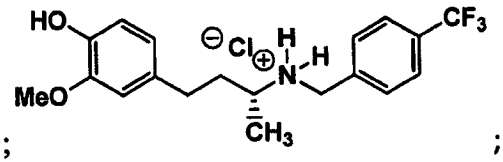
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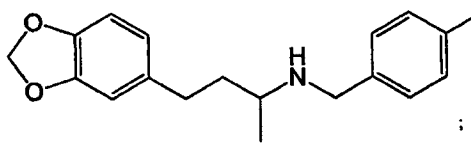
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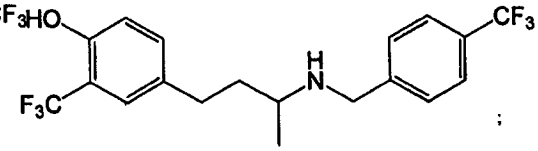
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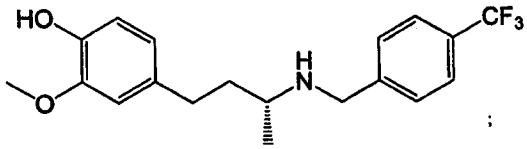
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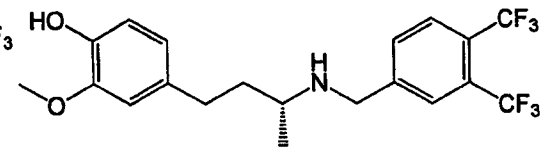
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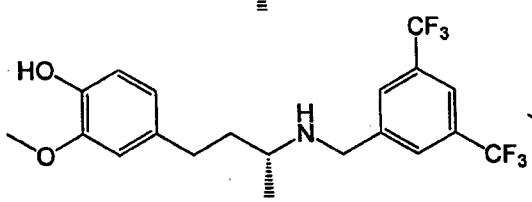
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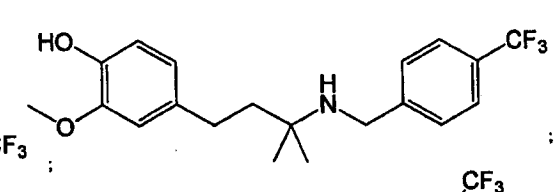
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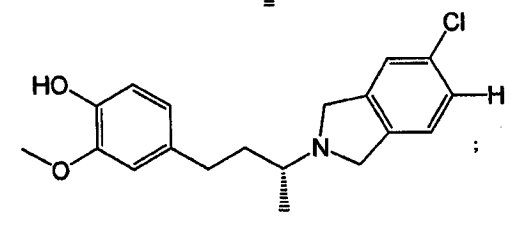
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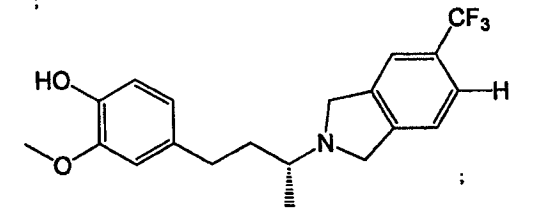
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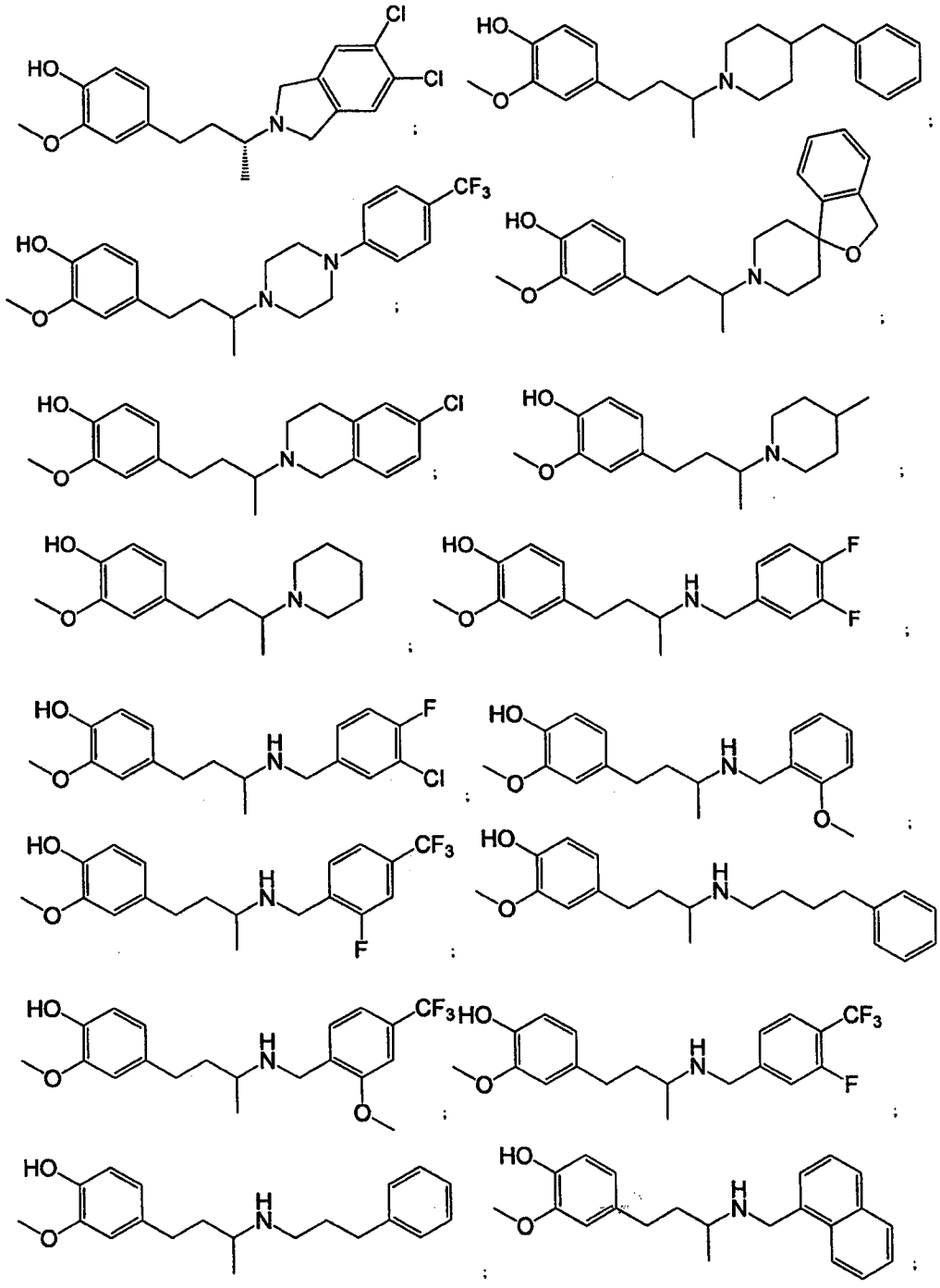

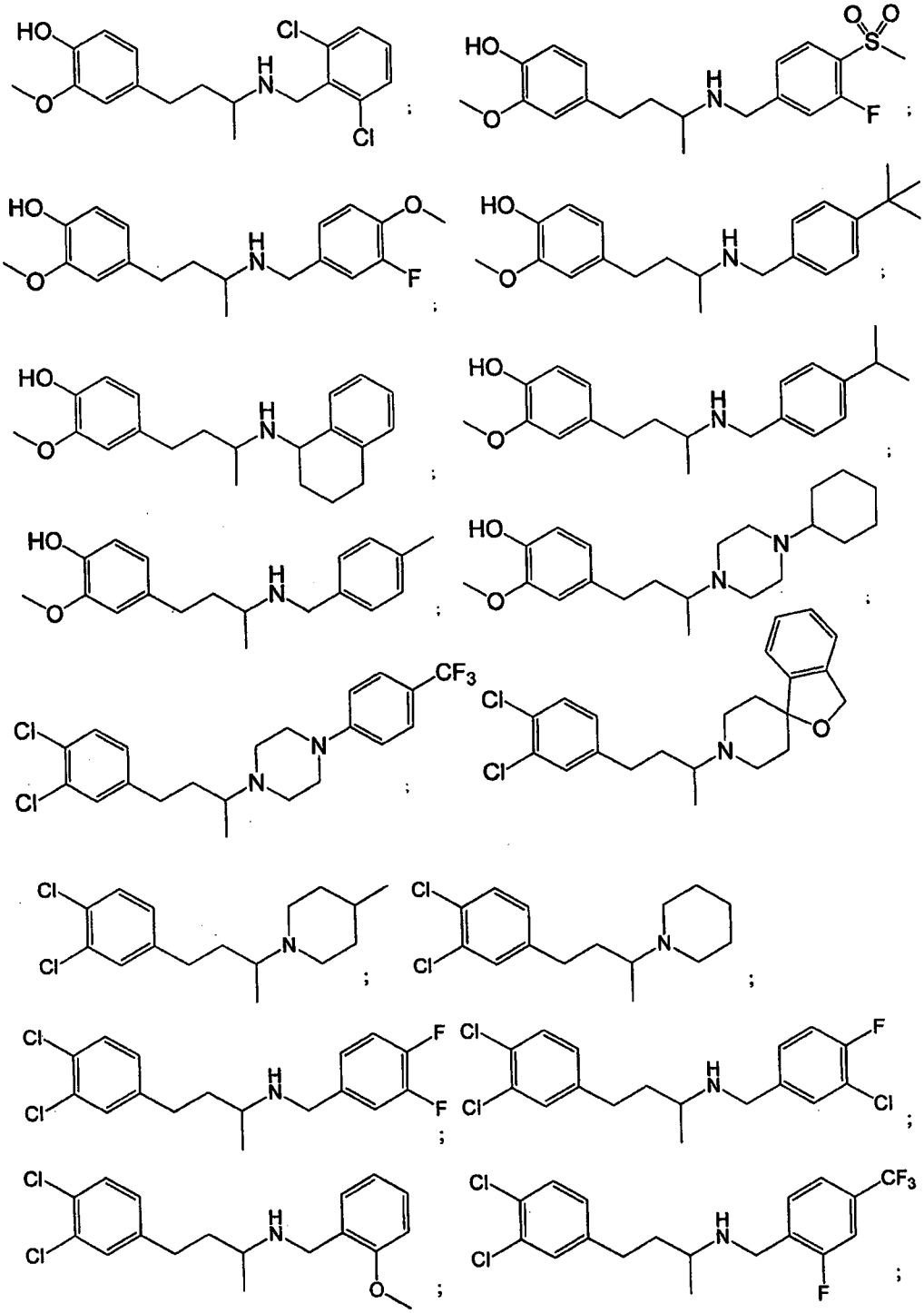

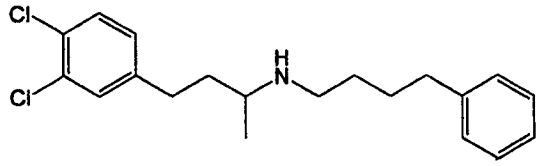
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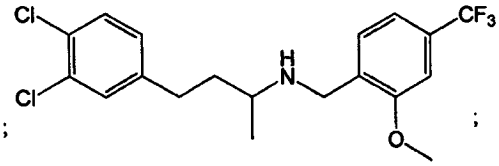
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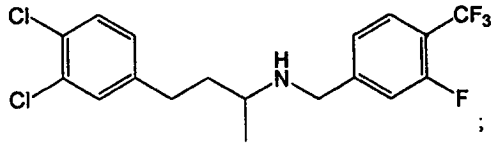
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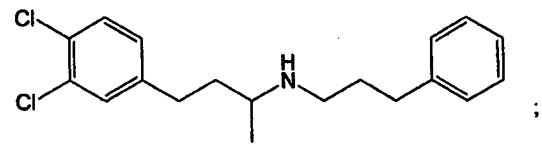
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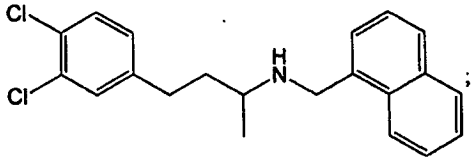
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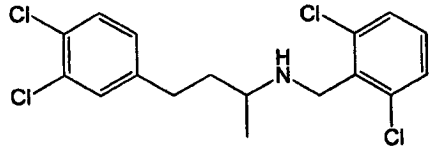
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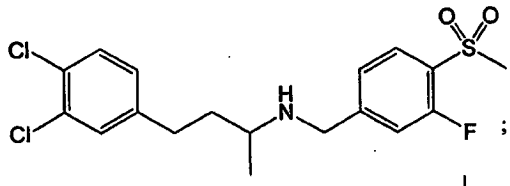
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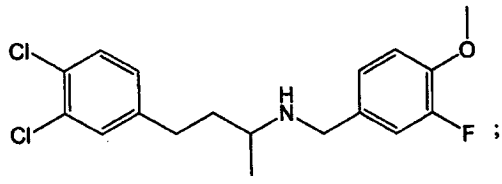
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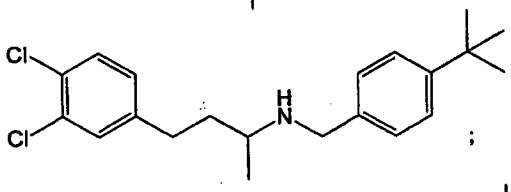
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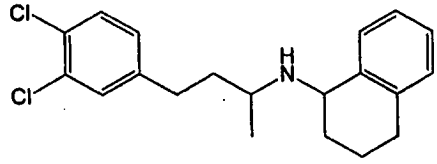
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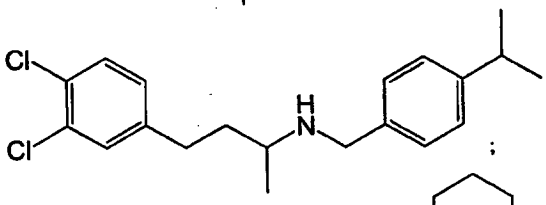
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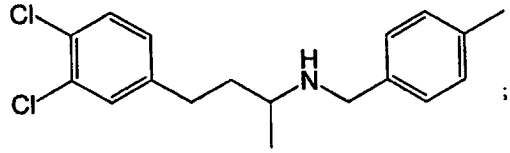
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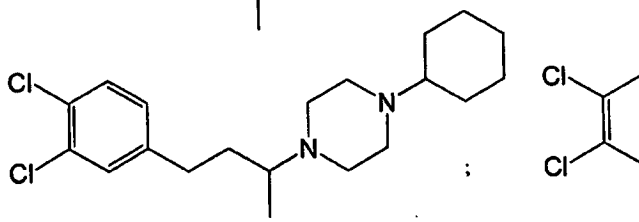
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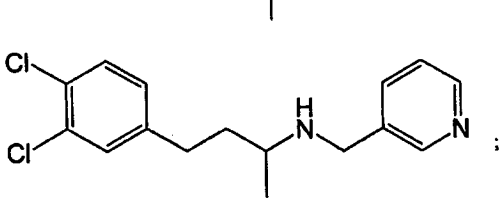
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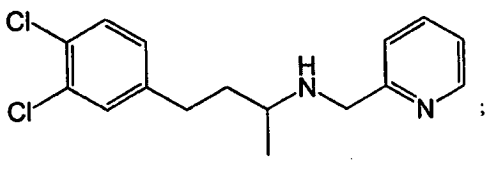
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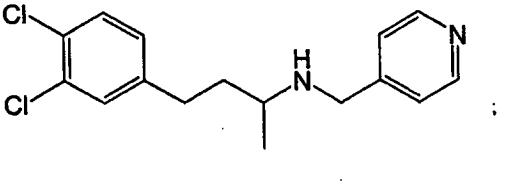
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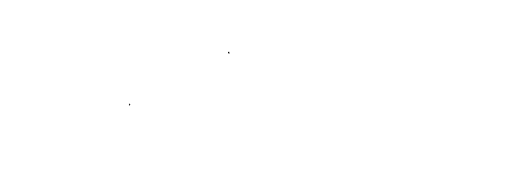
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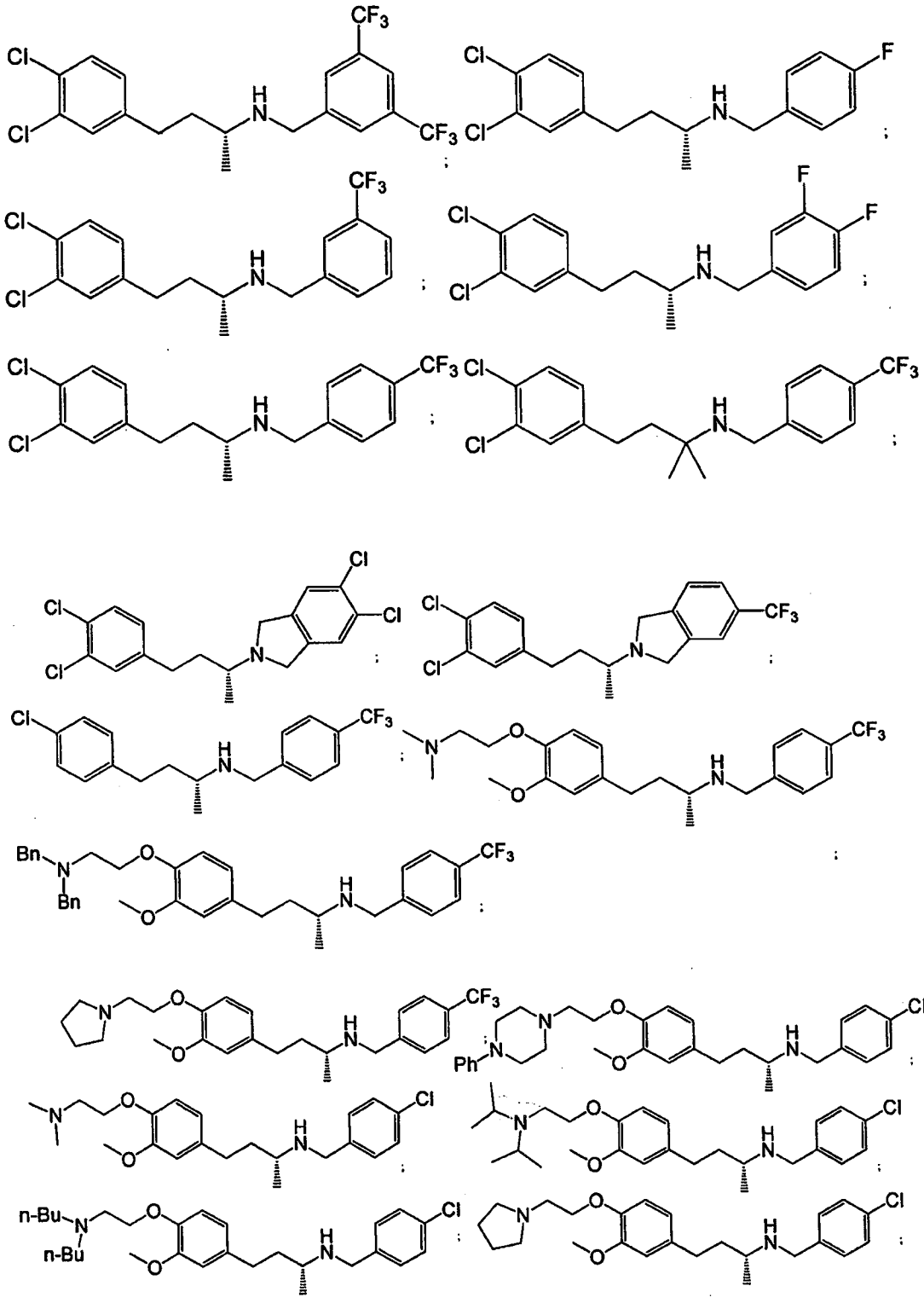
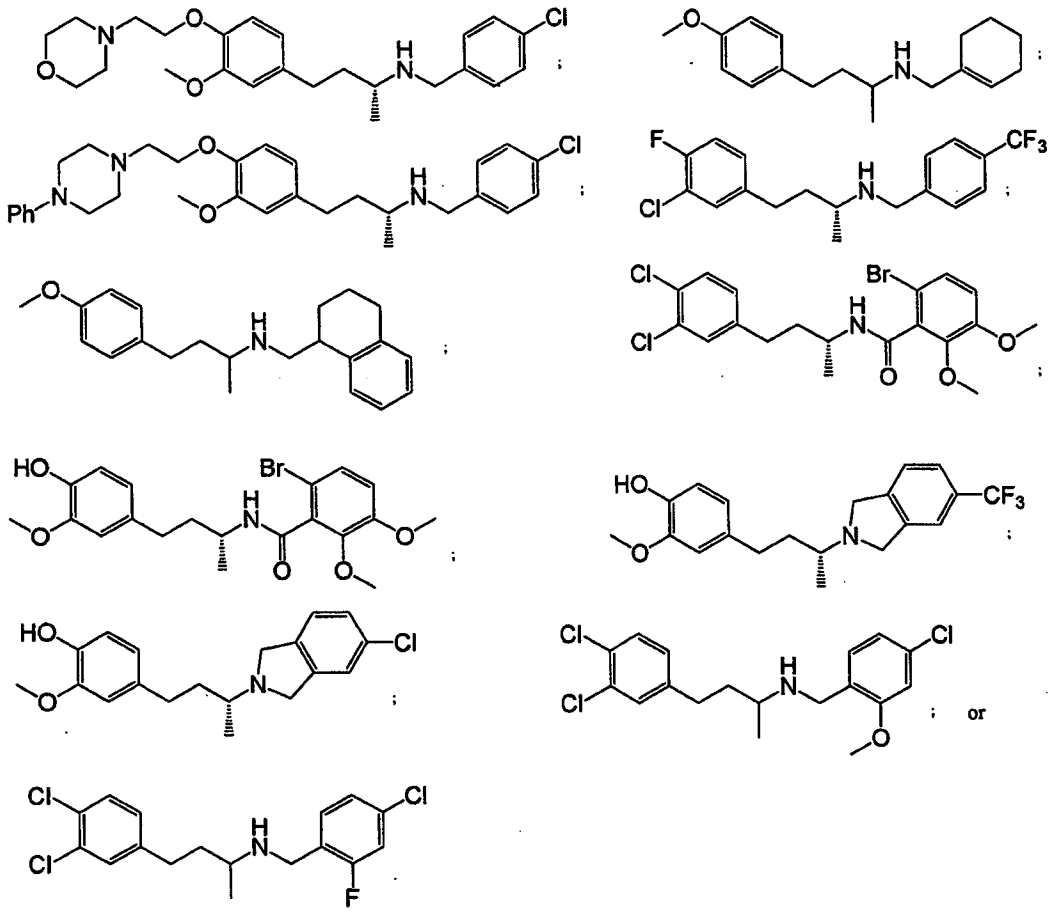
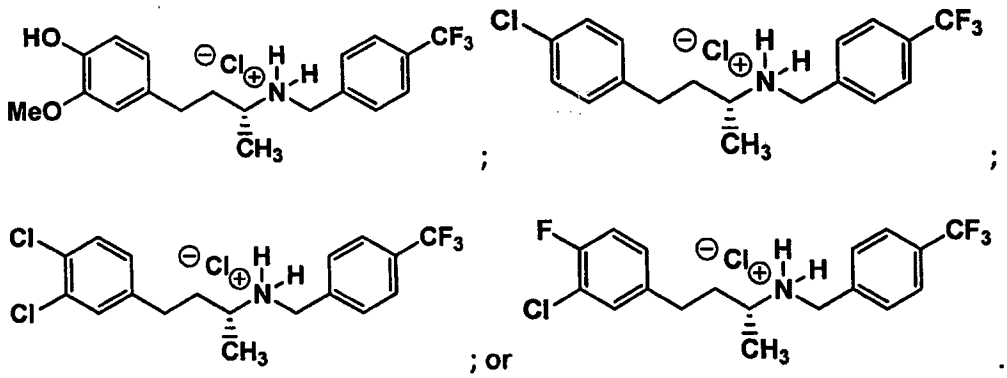
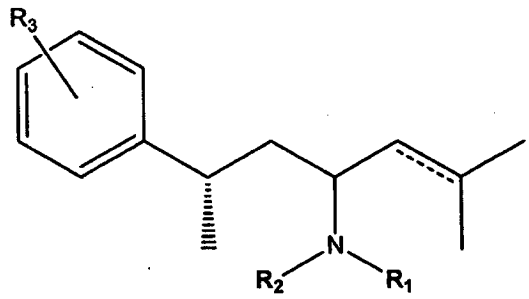




28. The composition of claim 27 wherein the compound is selected from



29. The composition of claim 24 or claim 25 wherein the compound is of Formula VIIIo



VIIIo

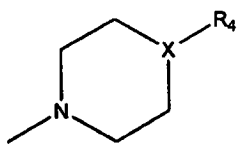
wherein:

-------- 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



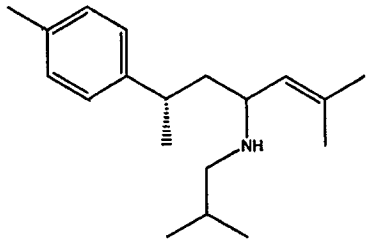
, wherein

X is CH, N, or O, and

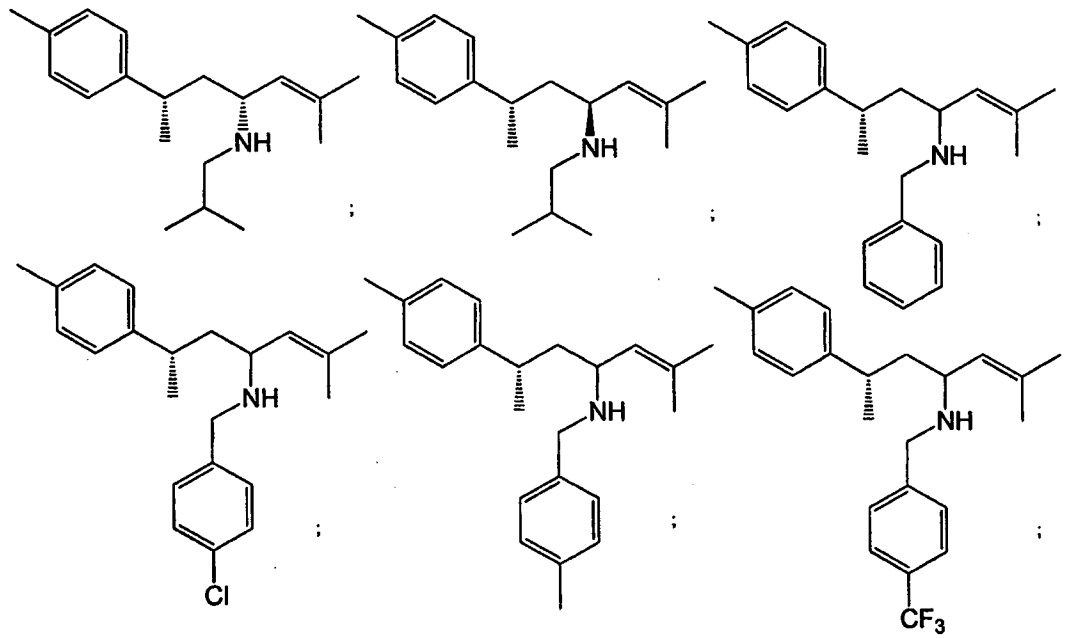
R_4 is absent, or is H, C_{1-6} alkyl, or unsubstituted phenyl or phenyl substituted with halo, C_{1-6} alkyl, or C_{1-6} haloalkyl; and

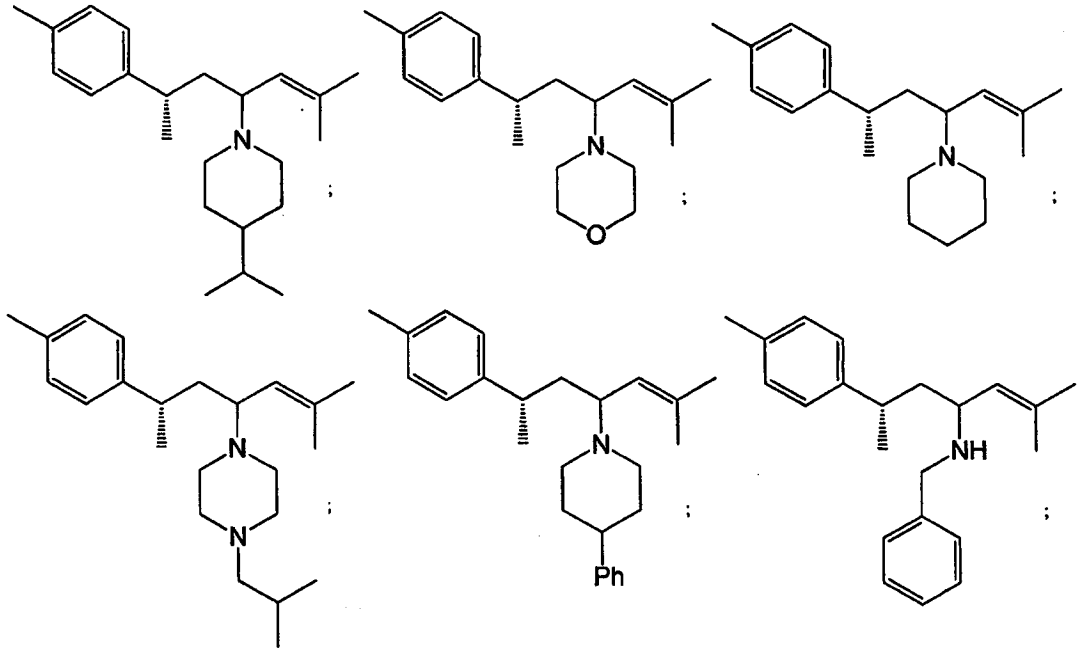
R_3 is C_{1-4} alkyl, C_{1-6} haloalkoxy, or halo, and

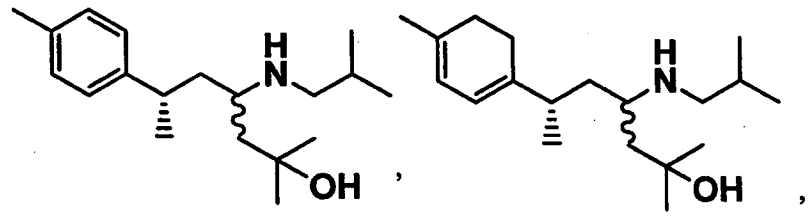
pharmaceutically acceptable salts thereof, with the proviso that the following racemic mixture of compounds is excluded:

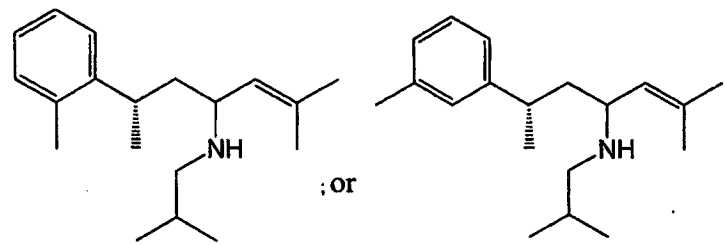


30. The composition of claim 29 wherein said compound is selected from one of the following compounds, or a pharmaceutically acceptable salt thereof:





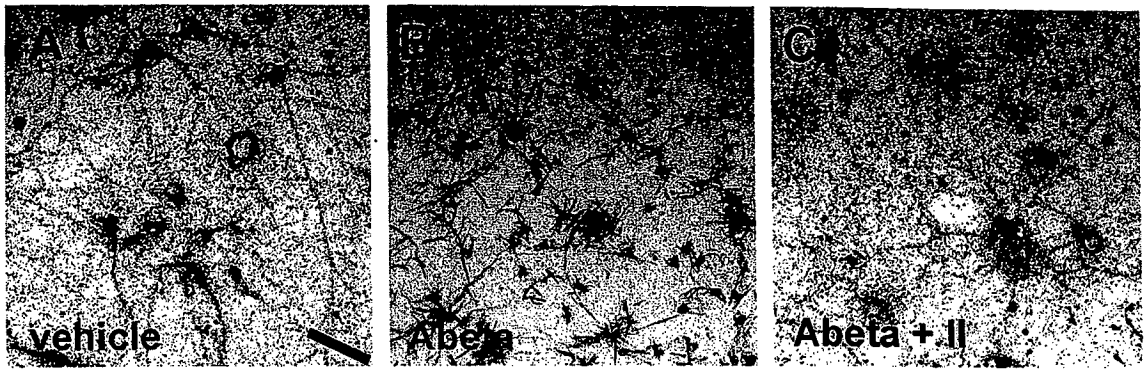




31. The composition of claim 24 wherein said compound is selected from an antibody, or active binding fragment thereof, wherein the antibody or fragment is specific for a sigma-2 receptor.

32. The composition of claim 31 wherein the antibody or fragment is specific for an epitope of an antigen present in one or more of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 9, or SEQ ID NO: 10.

FIG. 1A FIG. 1B FIG. 1C



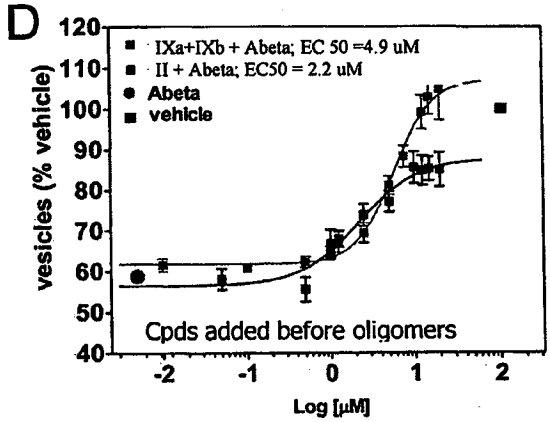


FIG. 1D

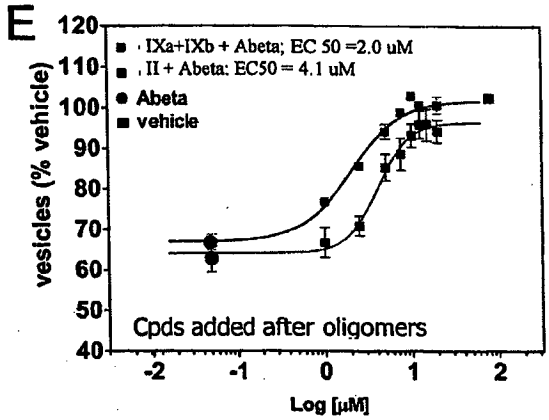


FIG. 1E

FIG. 1F

FIG. 1G

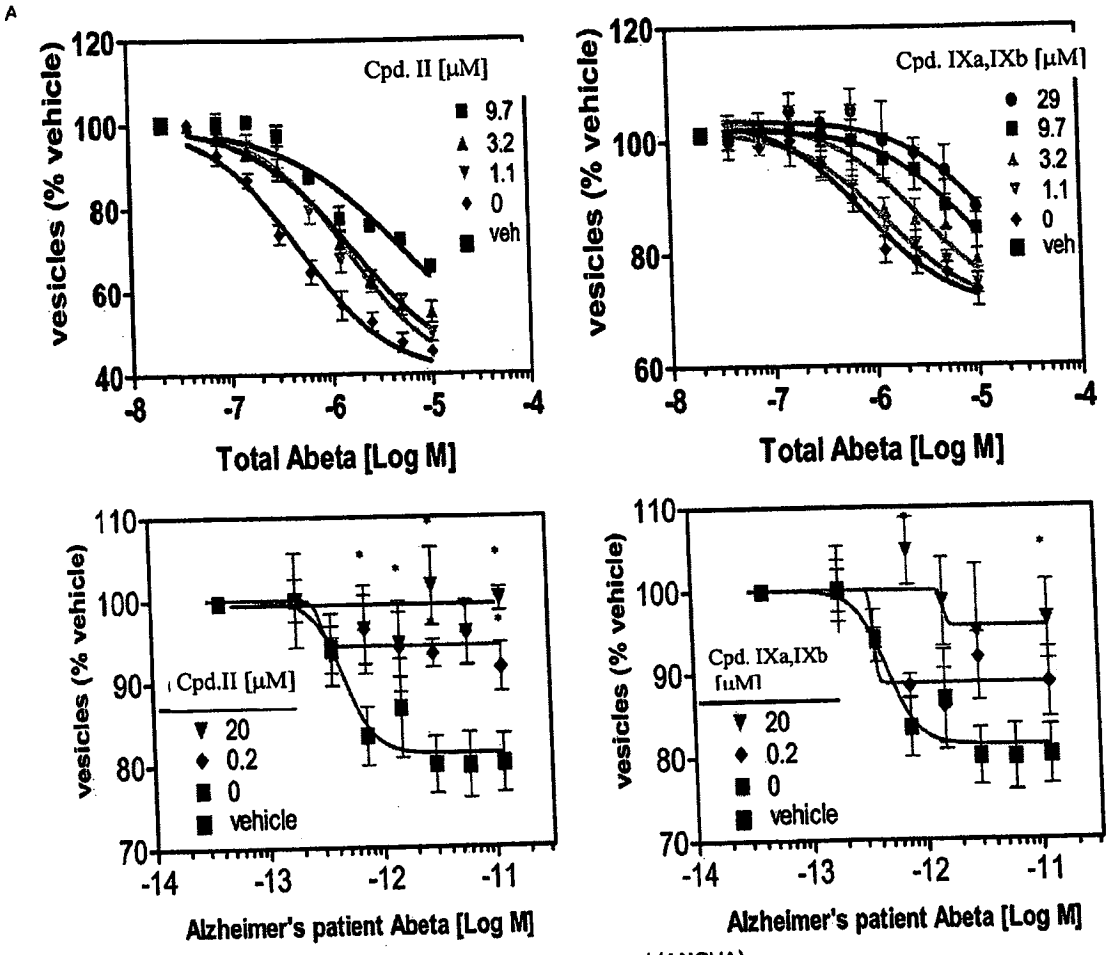


FIG. 1H

FIG. 1I

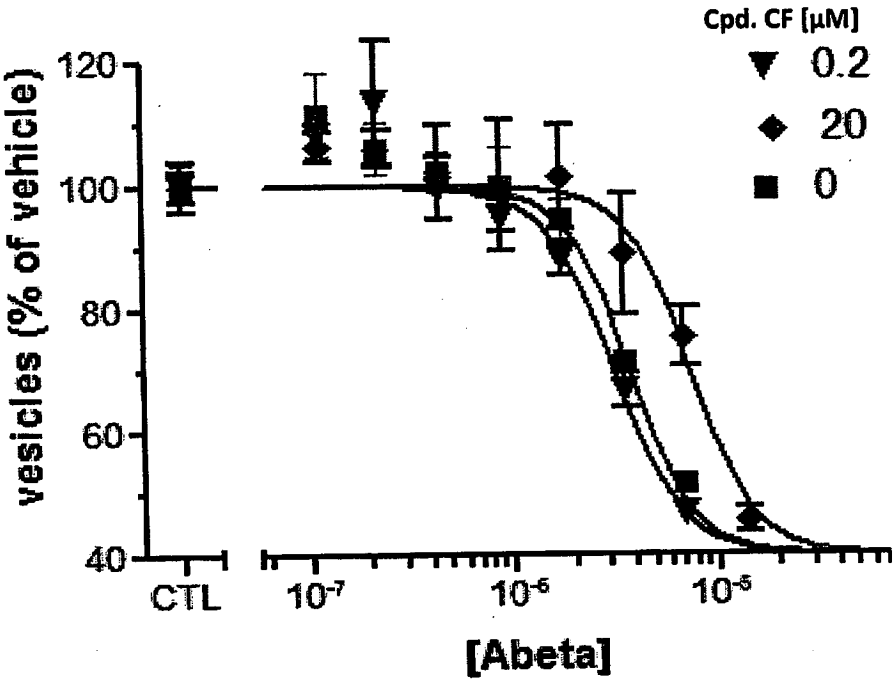

Cpd. CF [µM]
0.2
20
0
vesicles (% of vehicle)
120
100
80
60
40
CTL
10-7
10-6
10-5
[Abeta]

FIG.1J

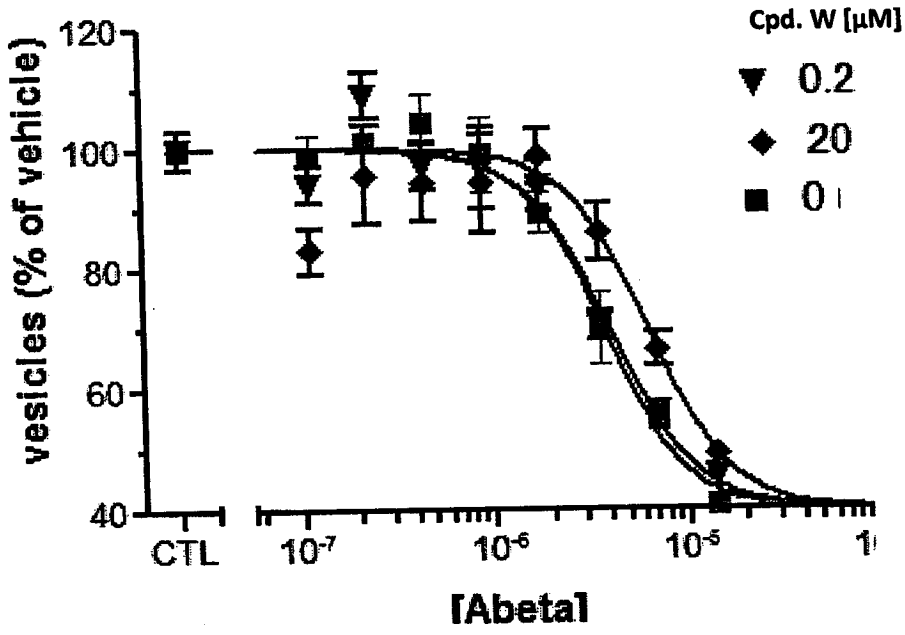

Cpd. W [µM]
0.2
20
0
vesicles (% of vehicle)
120
100
80
60
40
CTL
10-7
10-6
10-5
[Abeta]

FIG 1K

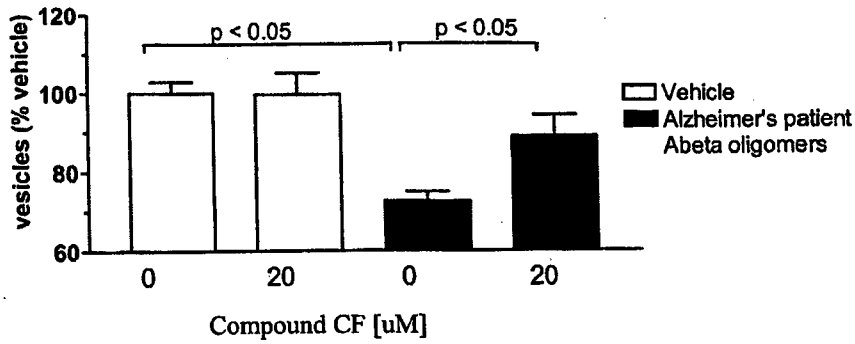

vesicles (% vehicle)
120
100
80
60
p < 0.05
p < 0.05
0
20
0
20
Compound CF [uM]
Vehicle
Alzheimer's patient
Abeta oligomers

FIG. 1L

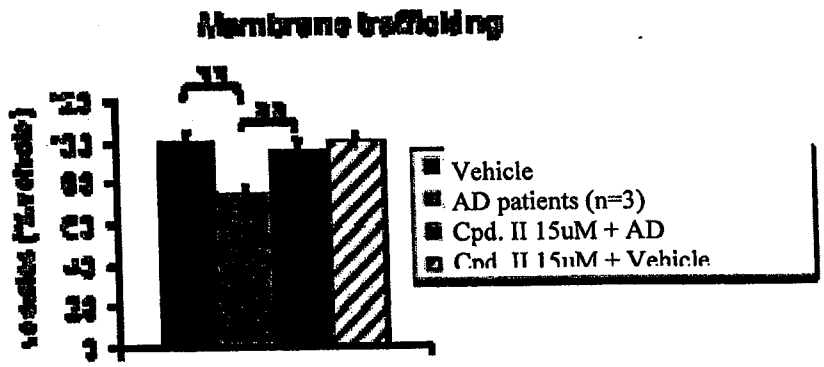

Membrane trafficking
Vehicle
AD patients (n=3)
Cpd. II 15uM + AD
Cpd. II 15uM + Vehicle

FIG 1M

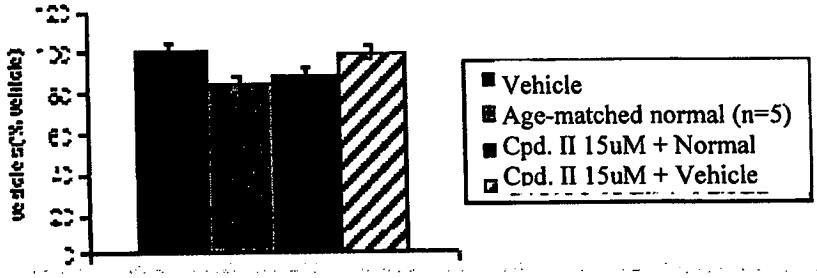

Vehicle
Age-matched normal (n=5)
Cpd. II 15uM + Normal
Cpd. II 15uM + Vehicle

FIG 1N

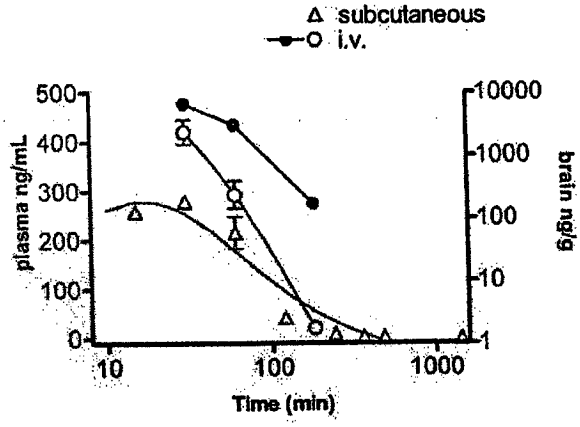

subcutaneous
i.v.
plasma ng/mL
brain ng/g
Time (min)

FIG 2A

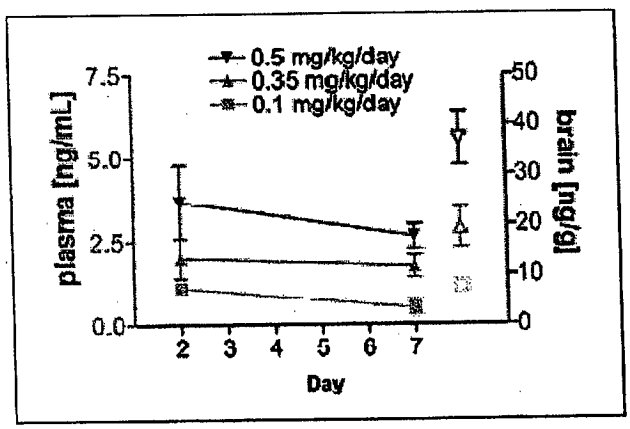

0.5 mg/kg/day
0.35 mg/kg/day
0.1 mg/kg/day
plasma [ng/mL]
brain [ng/g]
Day

FIG 2B

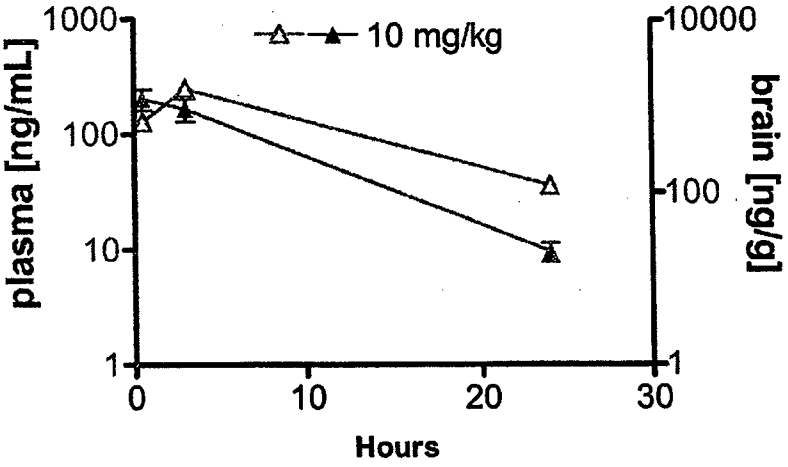

10 mg/kg
plasma [ng/mL]
brain [ng/g]
1000
100
10
1
10000
100
1
0
10
20
30
Hours

FIG. 2C

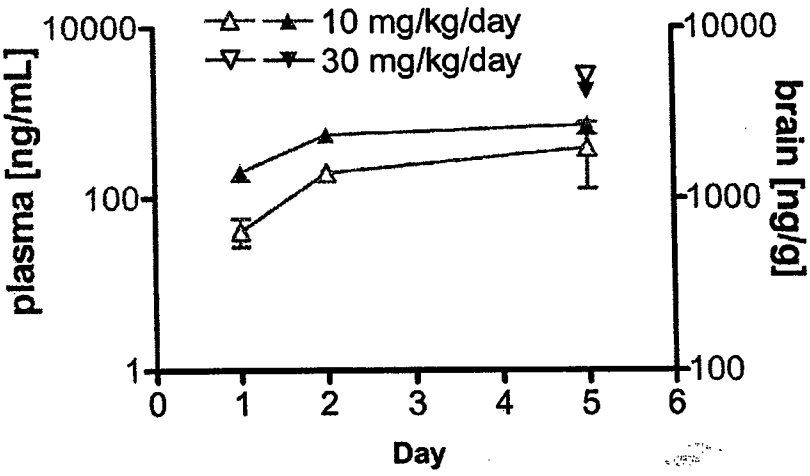

10 mg/kg/day
30 mg/kg/day
plasma [ng/mL]
brain [ng/g]
10000
100
1
10000
1000
100
0
1
2
3
4
5
6
Day

FIG 2D

FIG. 3A

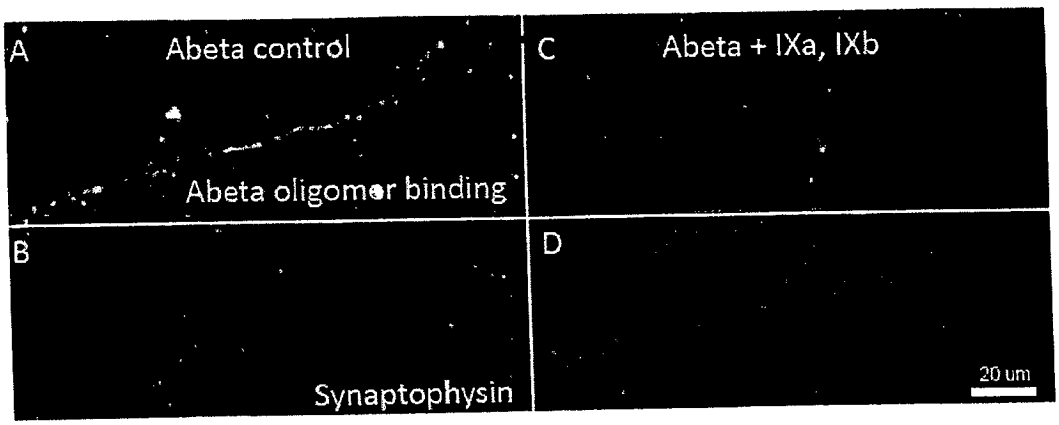
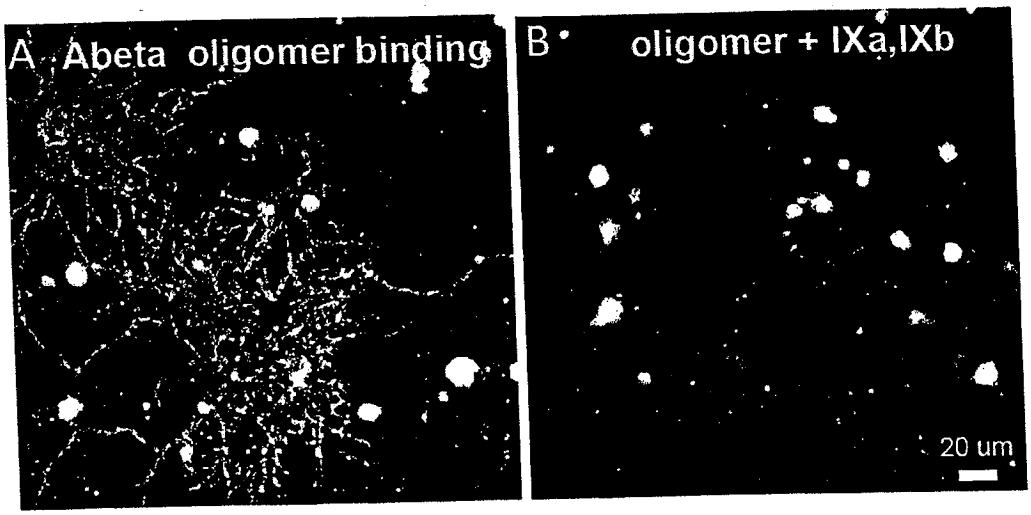

A
Abeta control
Abeta oligomer binding
C
Abeta + IXa, IXb
B
Synaptophysin
D
20 um

FIG. 3B


A Abeta oligomer binding
B oligomer + IXa,IXb
20 um

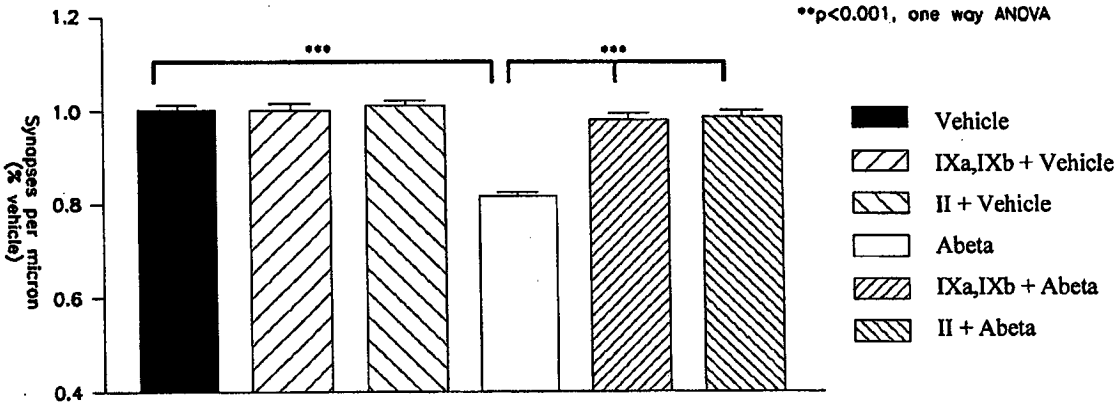

**p<0.001, one way ANOVA
Synapses per micron (% vehicle)
1.2
1.0
0.8
0.6
0.4
***
***
Vehicle
IXa,IXb + Vehicle
II + Vehicle
Abeta
IXa,IXb + Abeta
II + Abeta

FIG. 3C

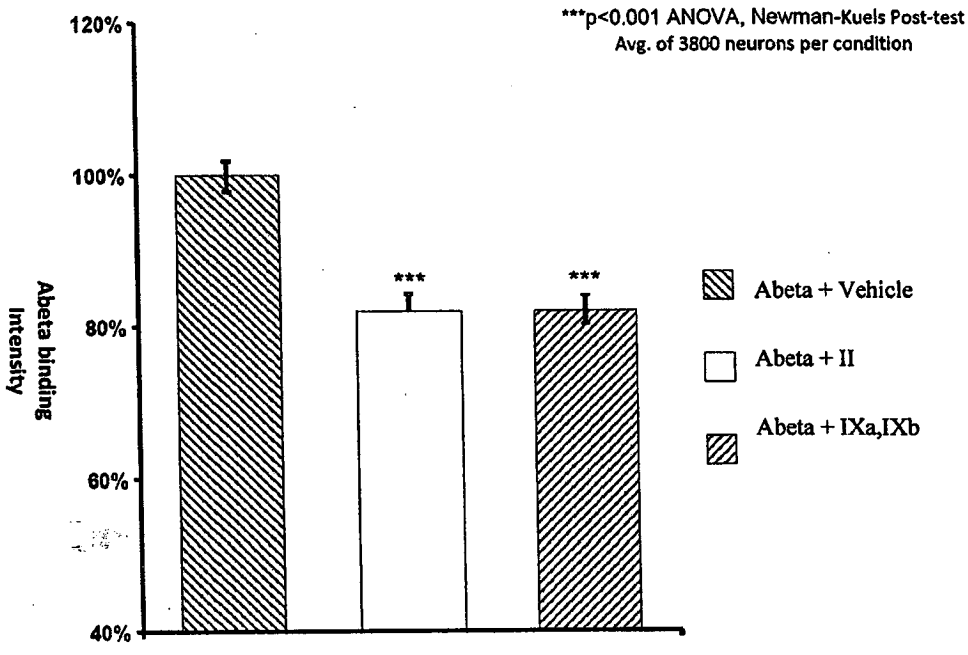

***p<0.001 ANOVA, Newman-Kuels Post-test
Avg. of 3800 neurons per condition
Abeta binding Intensity
120%
100%
80%
60%
40%
***
***
Abeta + Vehicle
Abeta + II
Abeta + IXa,IXb

FIG. 3D

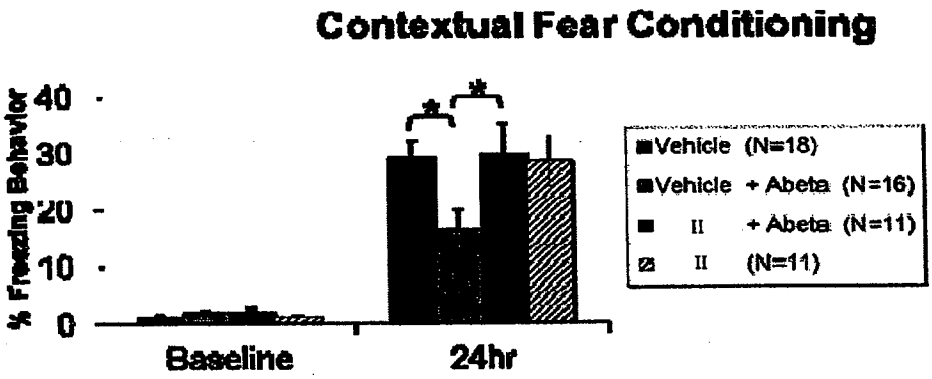

Contextual Fear Conditioning
% Freezing Behavior
40
30
20
10
0
Baseline
24hr
Vehicle (N=18)
Vehicle + Abeta (N=16)
II + Abeta (N=11)
II (N=11)

FIG 4

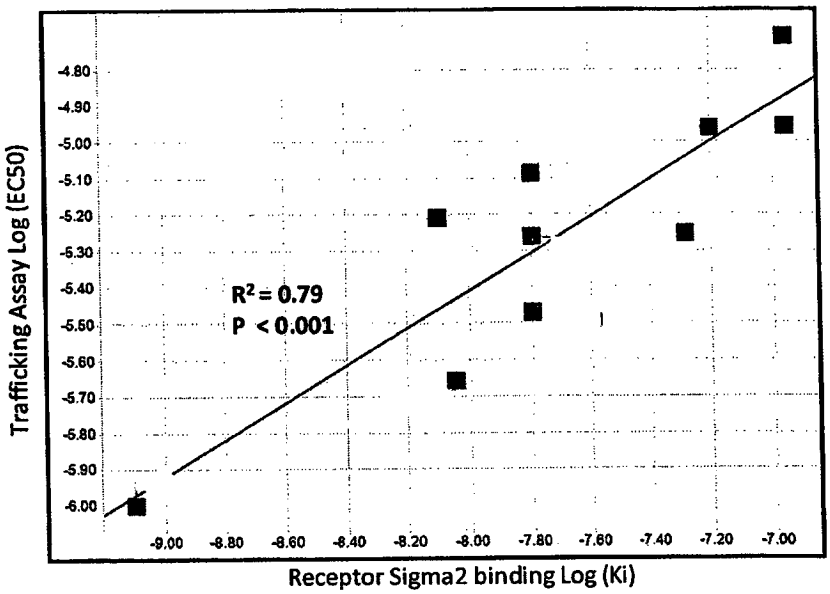

Trafficking Assay Log (EC50)
-4.80
-4.90
-5.00
-5.10
-5.20
-5.30
-5.40
-5.50
-5.60
-5.70
-5.80
-5.90
-6.00
R² = 0.79
P < 0.001
-9.00 -8.80 -8.60 -8.40 -8.20 -8.00 -7.80 -7.60 -7.40 -7.20 -7.00
Receptor Sigma2 binding Log (Ki)

FIG 5A

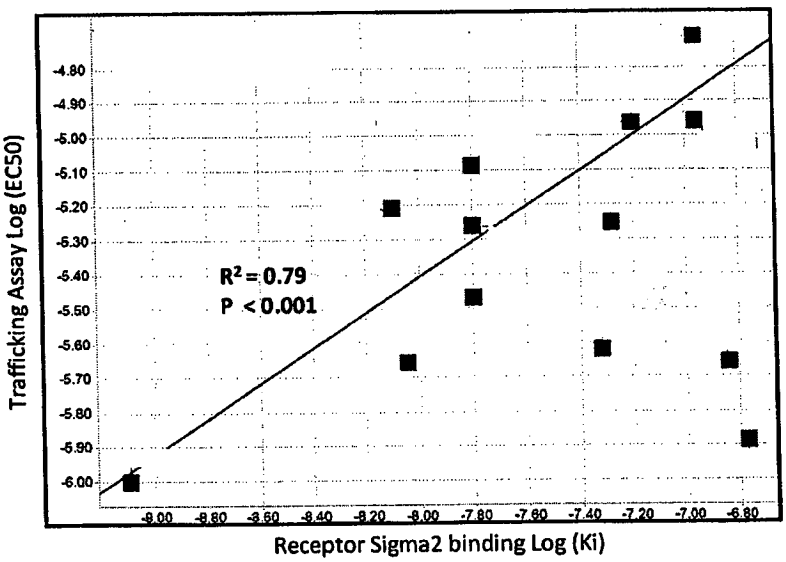

Trafficking Assay Log (EC50)
-4.80
-4.90
-5.00
-5.10
-5.20
-5.30
-5.40
-5.50
-5.60
-5.70
-5.80
-5.90
-6.00
R² = 0.79
P < 0.001
-9.00 -8.80 -8.60 -8.40 -8.20 -8.00 -7.80 -7.60 -7.40 -7.20 -7.00 -6.80
Receptor Sigma2 binding Log (Ki)

FIG 5B

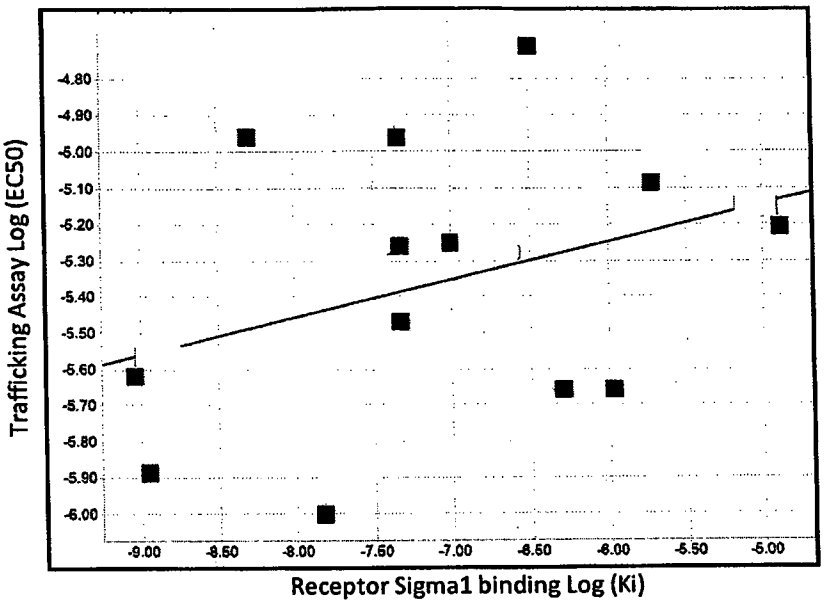

Trafficking Assay Log (EC50)
-4.80
-4.90
-5.00
-5.10
-5.20
-5.30
-5.40
-5.50
-5.60
-5.70
-5.80
-5.90
-6.00
-9.00
-8.50
-8.00
-7.50
-7.00
-6.50
-6.00
-5.50
-5.00
Receptor Sigma1 binding Log (Ki)

FIG 5C

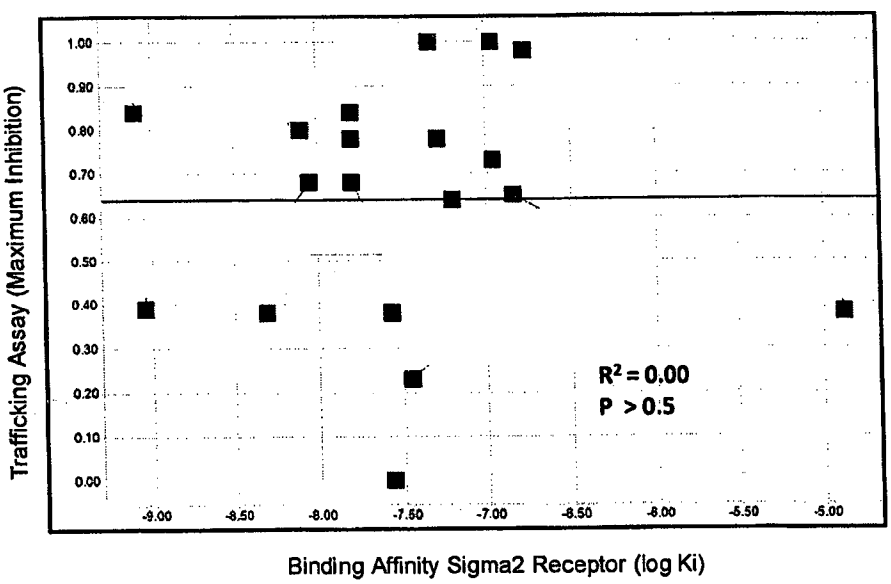

Trafficking Assay (Maximum Inhibition)
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
-9.00
-8.50
-8.00
-7.50
-7.00
-6.50
-6.00
-5.50
-5.00
R² = 0.00
P > 0.5
Binding Affinity Sigma2 Receptor (log Ki)

FIG 5D

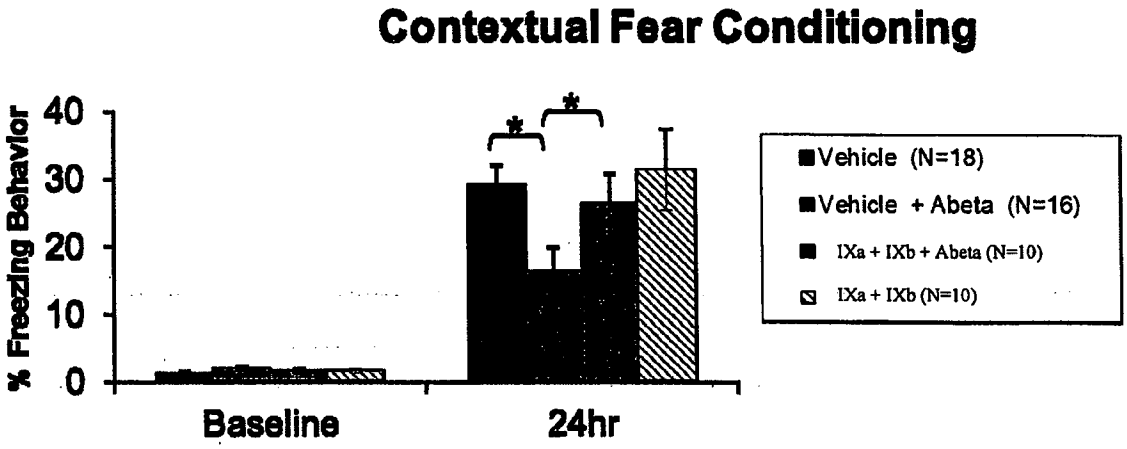

Contextual Fear Conditioning
% Freezing Behavior
40
30
20
10
0
Baseline
24hr
*
*
Vehicle (N=18)
Vehicle + Abeta (N=16)
IXa + IXb + Abeta (N=10)
IXa + IXb (N=10)

FIG 6

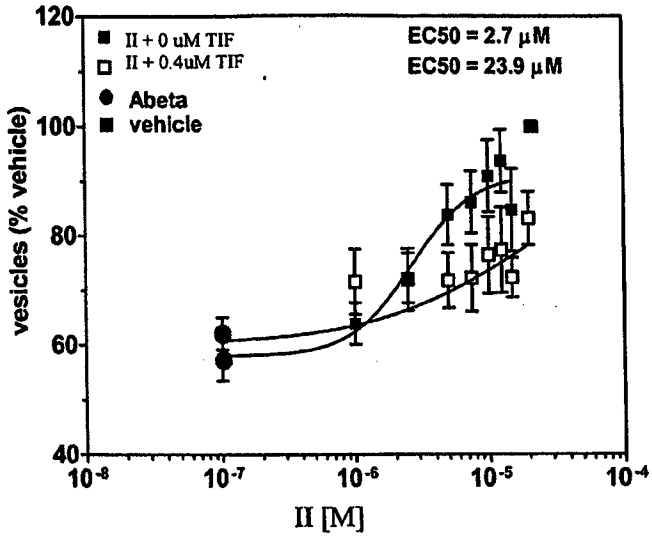

II + 0 uM TIF
II + 0.4uM TIF
Abeta
vehicle
EC50 = 2.7 µM
EC50 = 23.9 µM
vesicles (% vehicle)
120
100
80
60
40
10-8
10-7
10-6
10-5
10-4
II [M]

FIG 7A

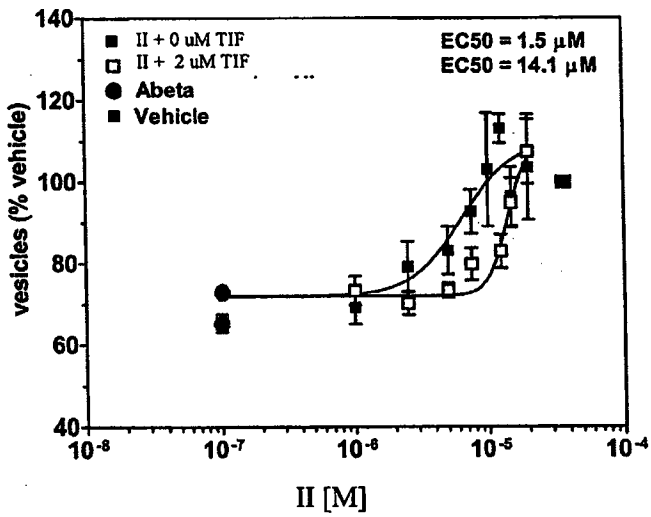
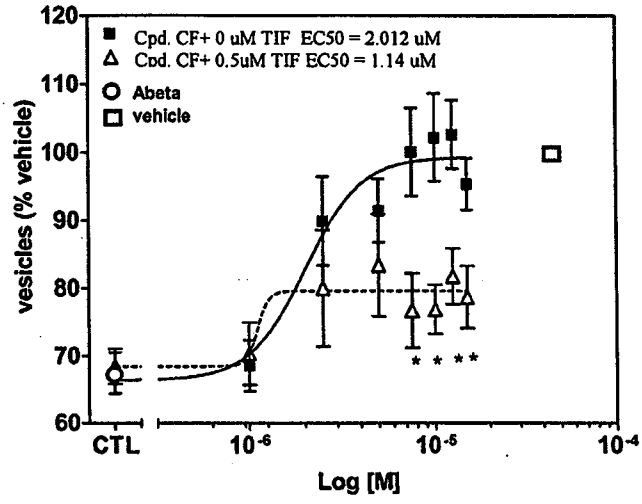
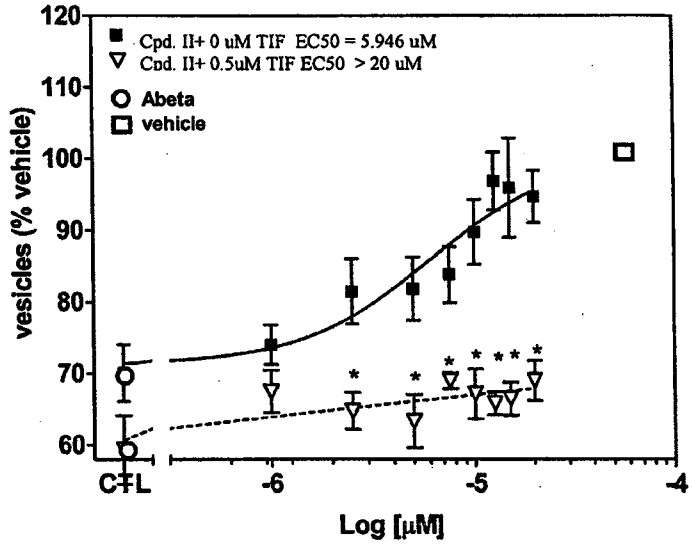

II + 0 uM TIF
II + 2 uM TIF
Abeta
Vehicle
EC50 = 1.5 µM
EC50 = 14.1 µM
vesicles (% vehicle)
140
120
100
80
60
40
10-8
10-7
10-6
10-5
10-4
II [M]

FIG 7B



	% inhibition of Abeta	t-test
Cntrl	100%	
0.5 TIF	34%	P< 0.05

FIG. 7C



	% inhibition of Abeta	t-test
Cntrl	100%	
0.5 TIF	17%	P< 0.05

FIG. 7D

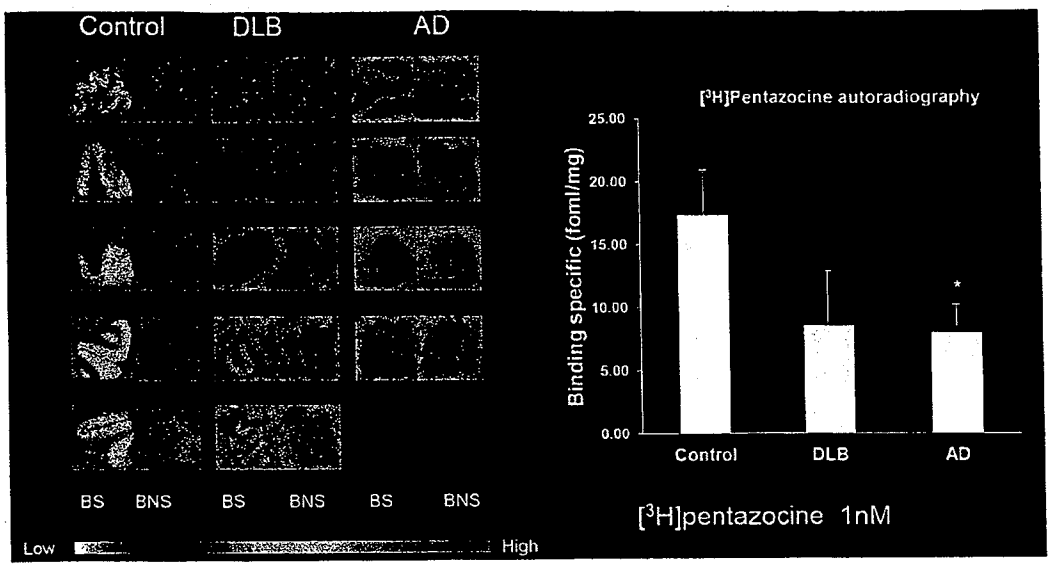

Control
DLB
AD
BS BNS BS BNS BS BNS
Low
High
[3H]Pentazocine autoradiography
Binding specific (fomI/mg)
25.00
20.00
15.00
10.00
5.00
0.00
Control
DLB
AD
*
[3H]pentazocine 1nM

FIG. 8A

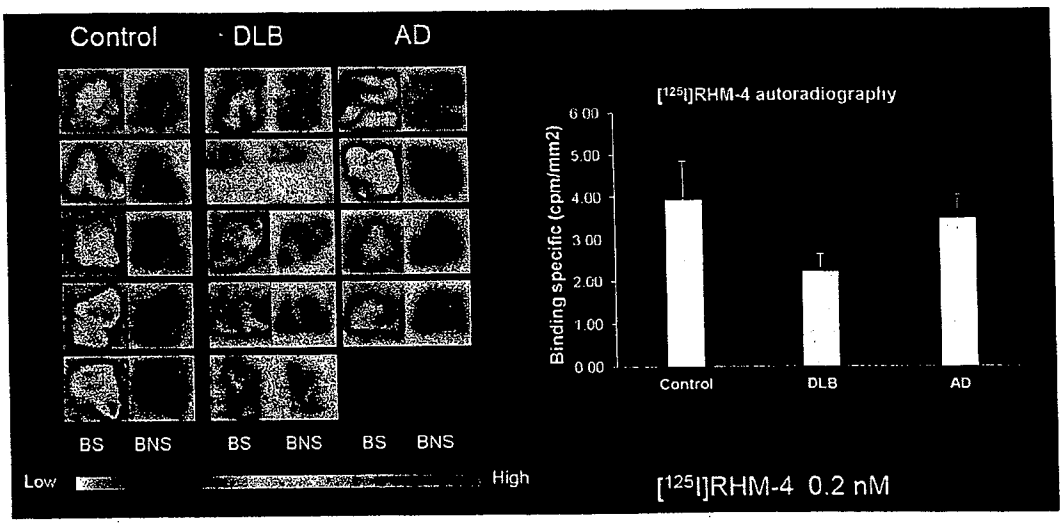

Control
DLB
AD
BS BNS BS BNS BS BNS
Low
High
[125I]RHM-4 autoradiography
Binding specific (cpm/mm2)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Control
DLB
AD
[125I]RHM-4 0.2 nM

FIG. 8B

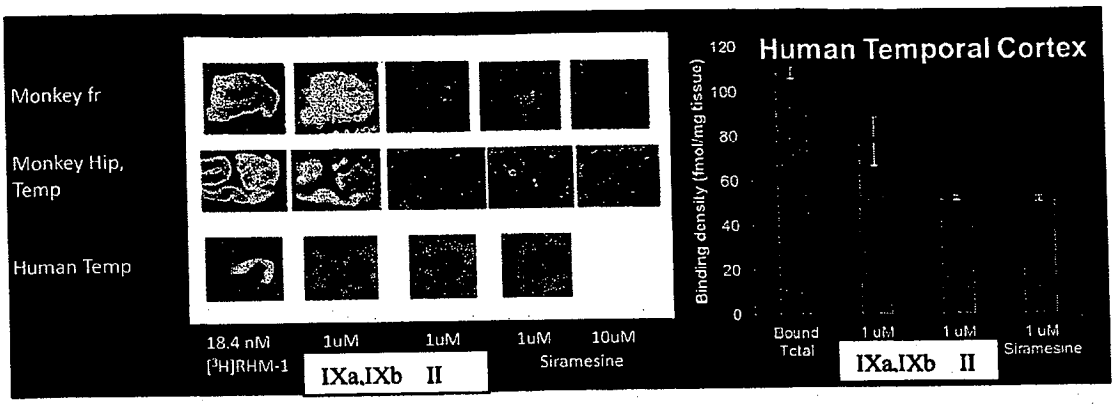
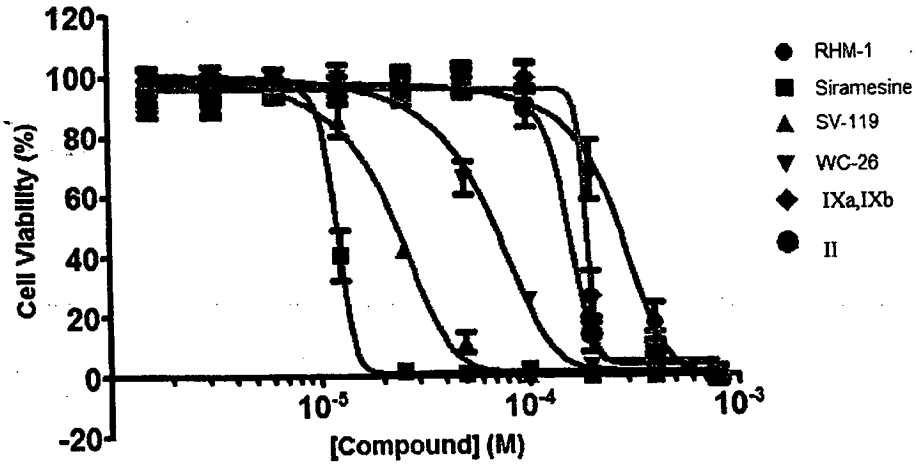

Monkey fr
Monkey Hip, Temp
Human Temp
18.4 nM [3H]RHM-1
1uM
1uM
IXa,IXb II
1uM
10uM
Siramesine
Human Temporal Cortex
Binding density (fmol/mg tissue)
120
100
80
60
40
20
0
Bound Total
1 uM
IXa,IXb II
1 uM
1 uM
Siramesine

FIG. 8C



Agonist ←------→ Antagonist
FIG 9A

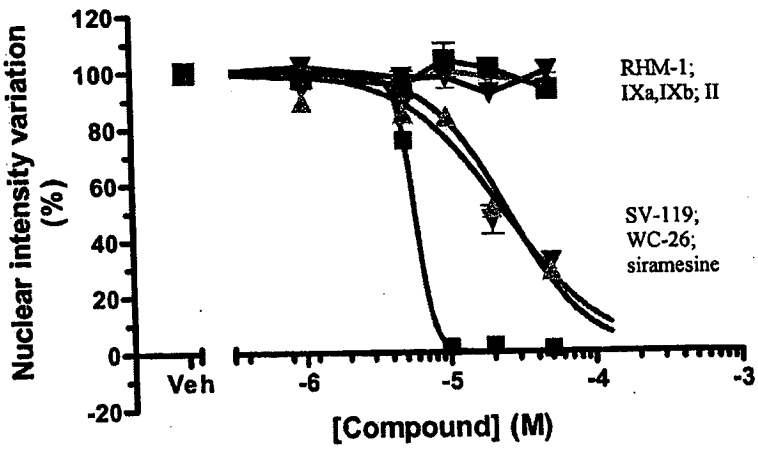


FIG 9B

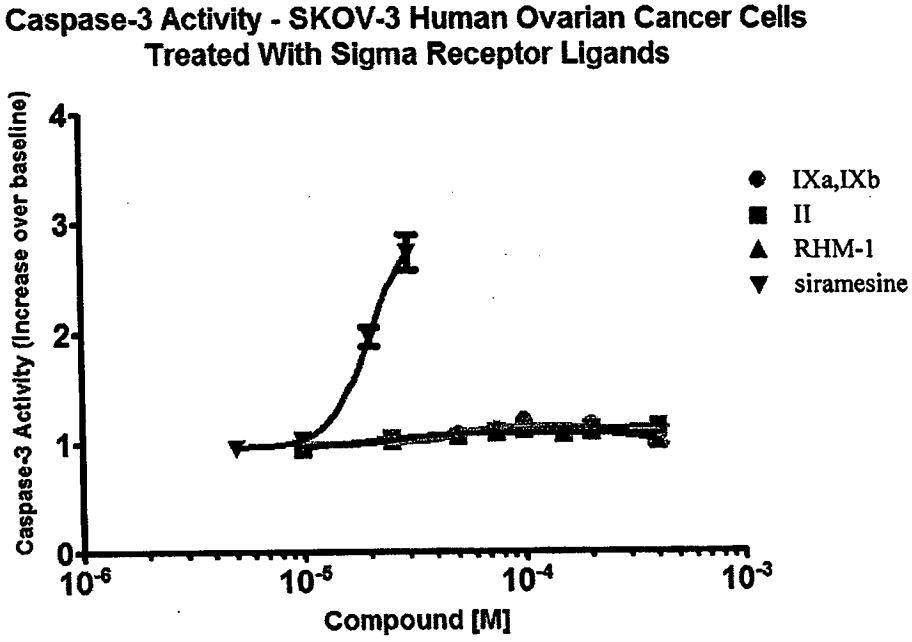


FIG. 10A

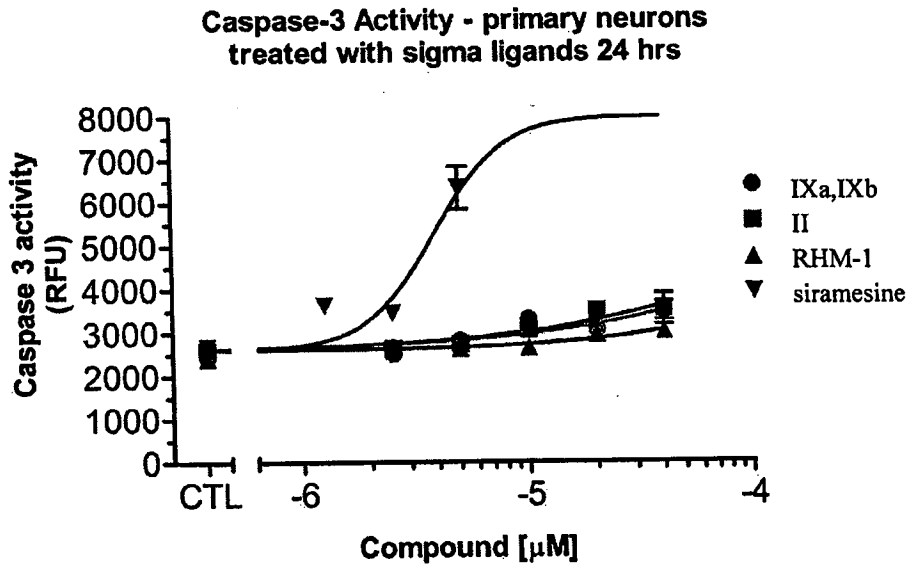


FIG. 10B

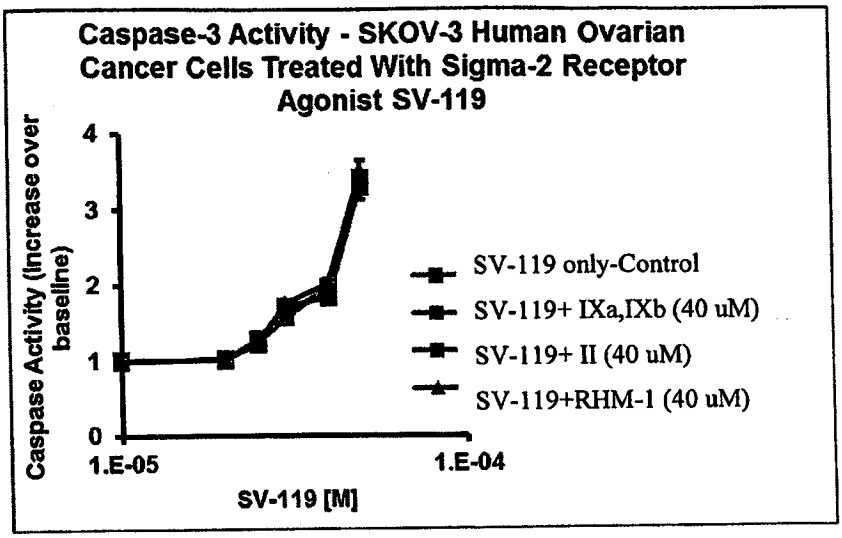


FIG. 10C

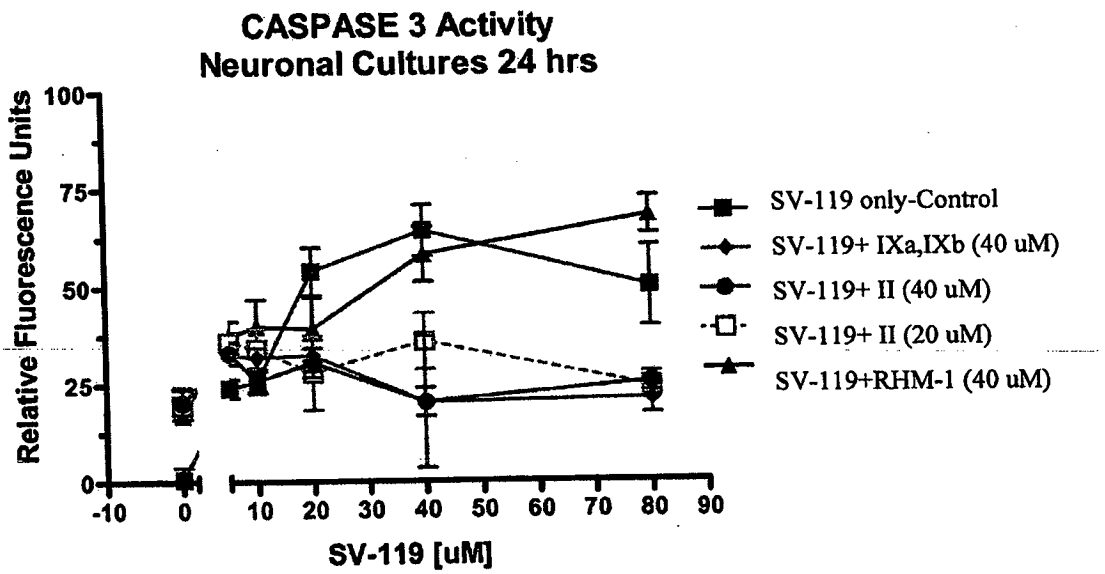


FIG. 10D

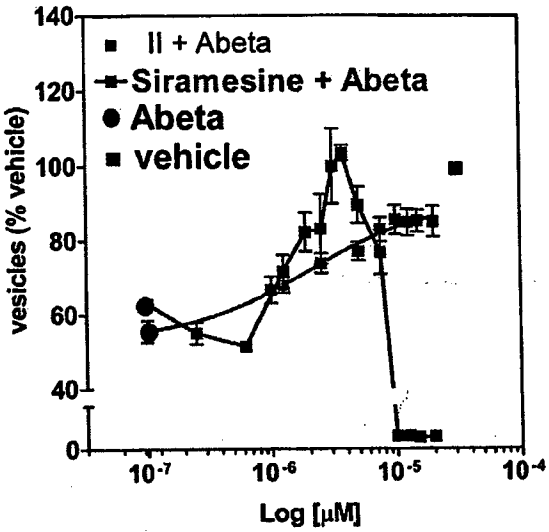


FIG. 11A

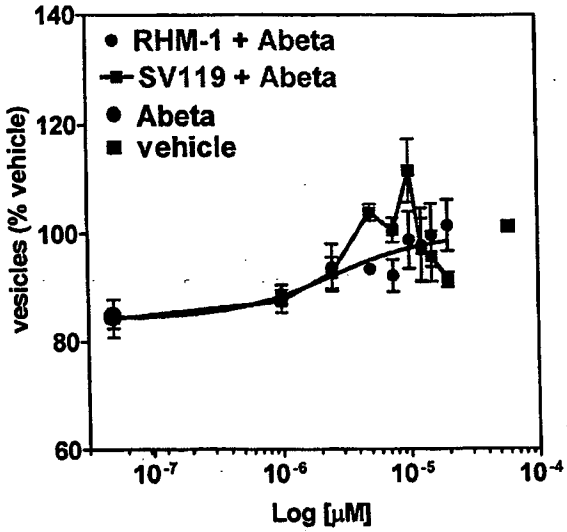


FIG 11B

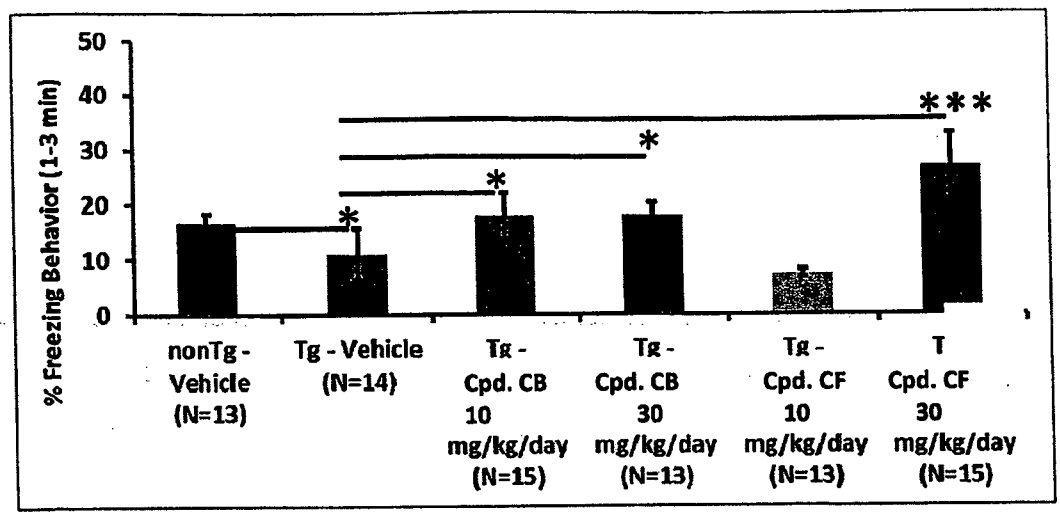


FIG. 12A

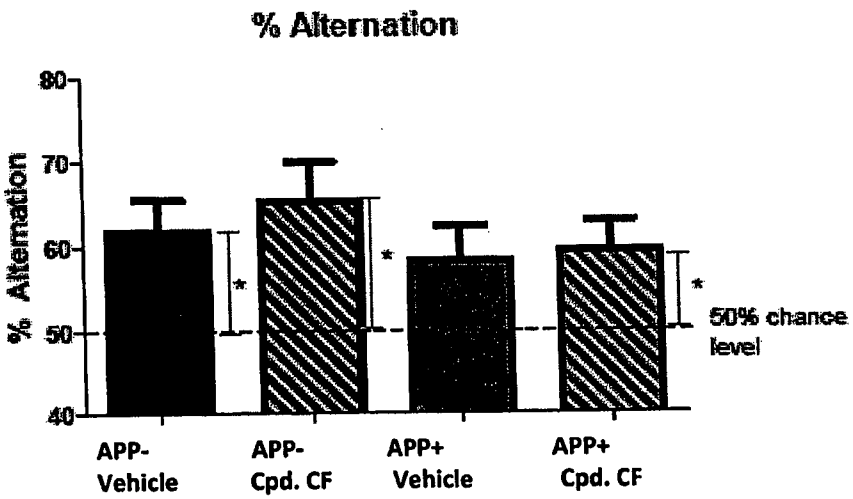


FIG. 12B

FIG. 13A FIG. 13C FIG. 13E FIG. 13G

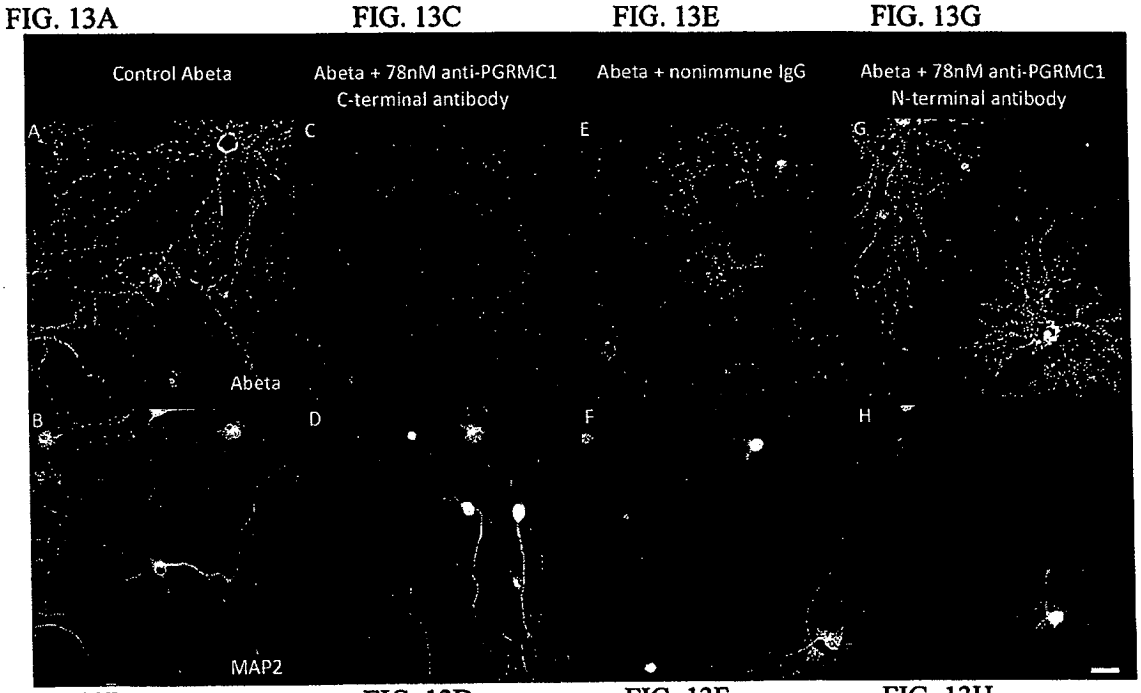


FIG. 13B FIG. 13D FIG. 13F FIG. 13H

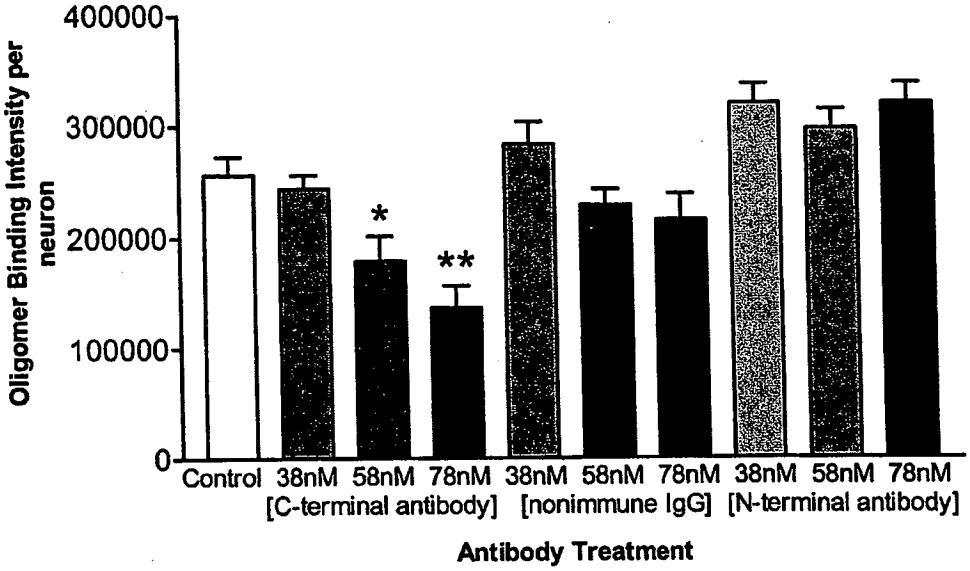


FIG. 14A

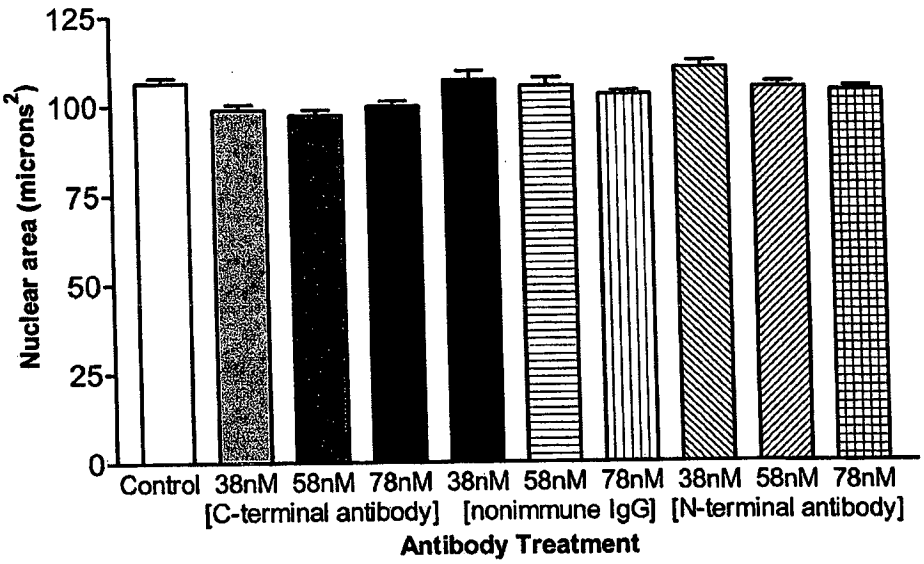


FIG. 14B

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(71)申请人 考格尼申治疗股份有限公司
地址 美国宾夕法尼亚州
(72)发明人 S.M. 卡塔拉诺 G. 里什顿 N.J. 伊佐
(74)专利代理机构 北京尚诚知识产权代理有限公司 11322
代理人 龙淳
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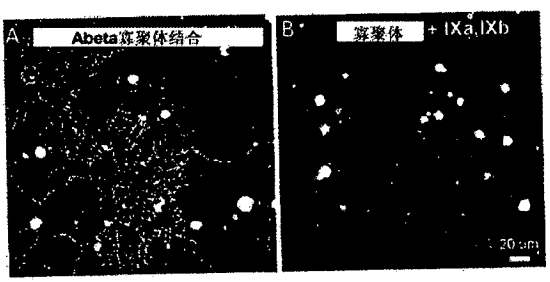
(54)发明名称

用于治疗神经退行性疾病的组合物和方法

(57)摘要

本发明涉及在用于抑制神经元细胞中Abeta相关的突触损失或突触功能障碍、调节神经元细胞中Abeta相关的膜运输改变以及治疗与Abeta病理相关的认知衰退的方法中使用σ-2受体拮抗剂以及包括这样的化合物的药物组合物，更宽泛地为使用这样的化合物和组合物来治疗与Abeta病理相关的神经退行性疾病和病症。本发明还涉及基于化合物与σ-2受体结合的能力来筛选其在抑制认知衰退中的活性的方法。



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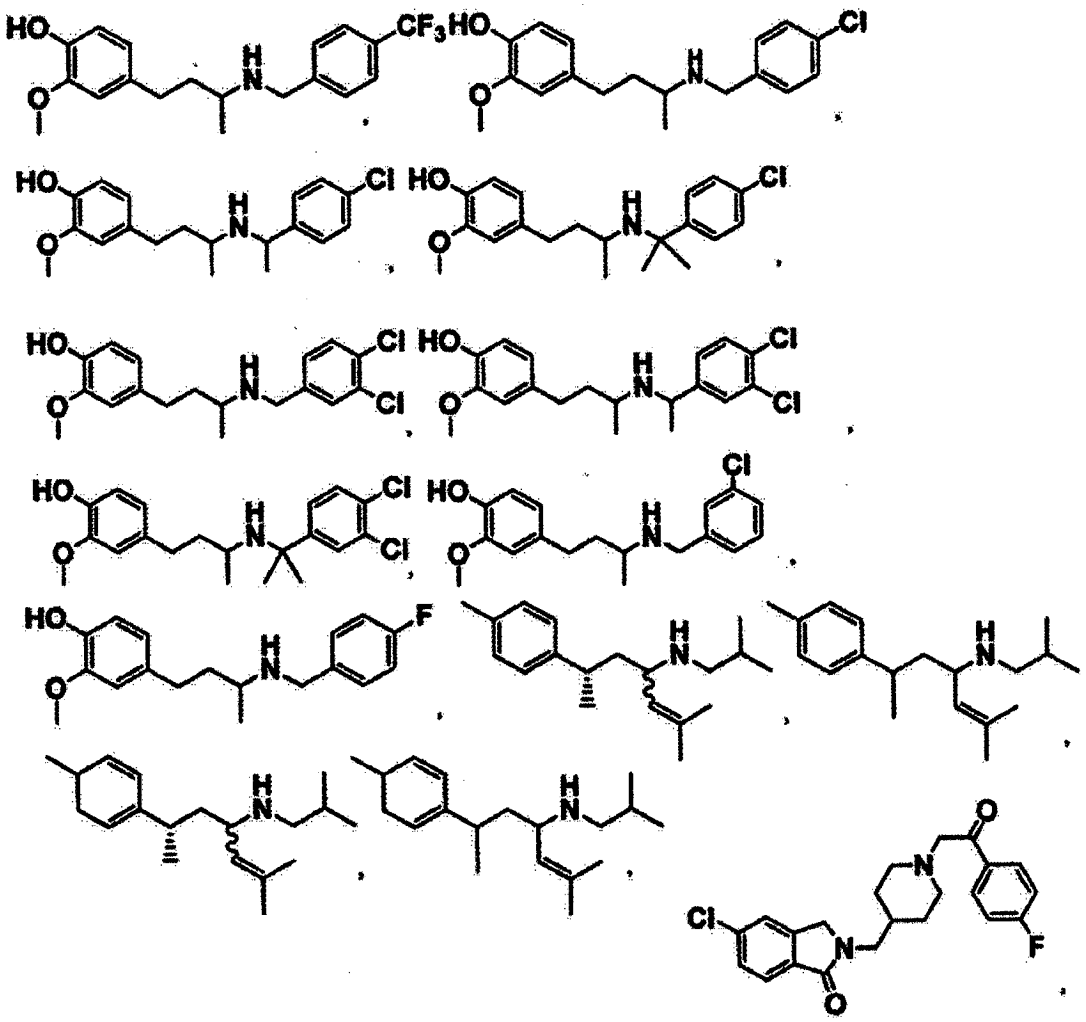
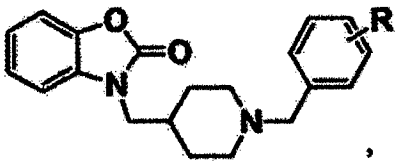
1. 用于抑制 β 淀粉样蛋白对神经元细胞的作用的方法 / 用途，包括施用有效量的组合物，所述组合物包括：

选择性 σ -2 受体拮抗剂，其含量为能够有效抑制所述细胞中的 β 淀粉样蛋白寡聚体结合；以及

药学上可接受的载体。

2. 根据权利要求 1 所述的方法 / 用途，其中化合物不是





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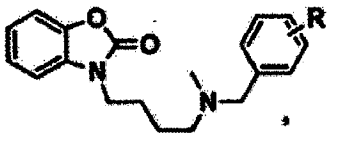

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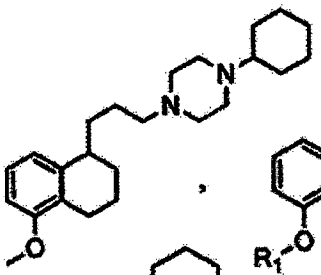

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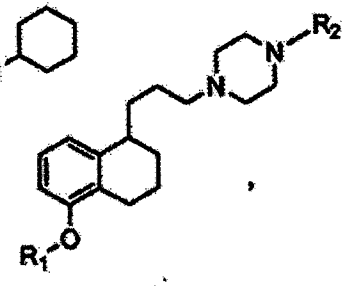

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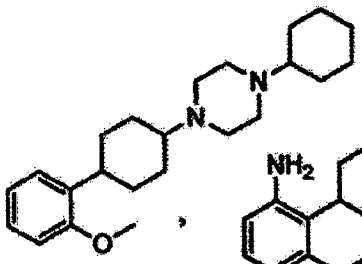

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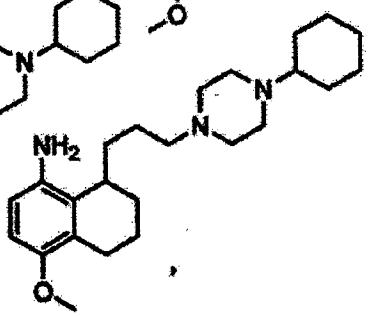

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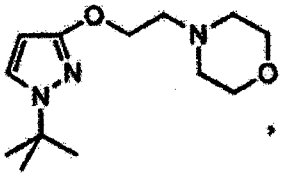

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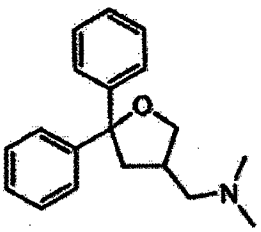

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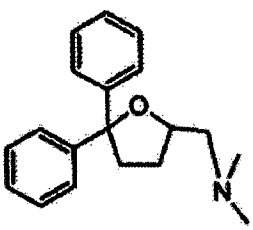

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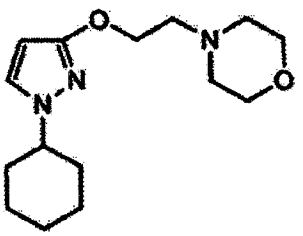

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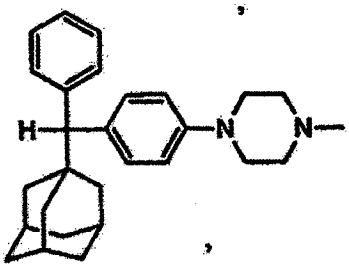

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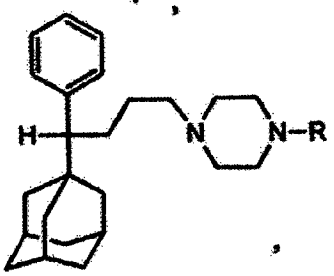

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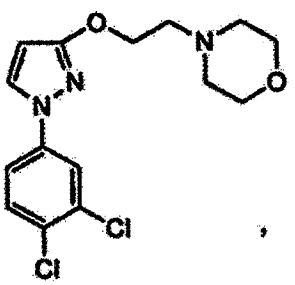

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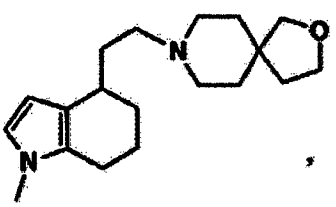

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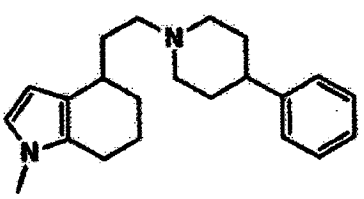

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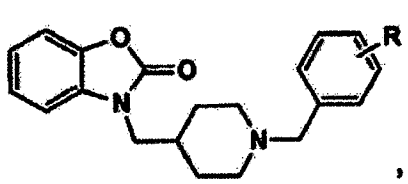

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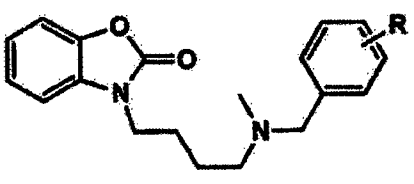

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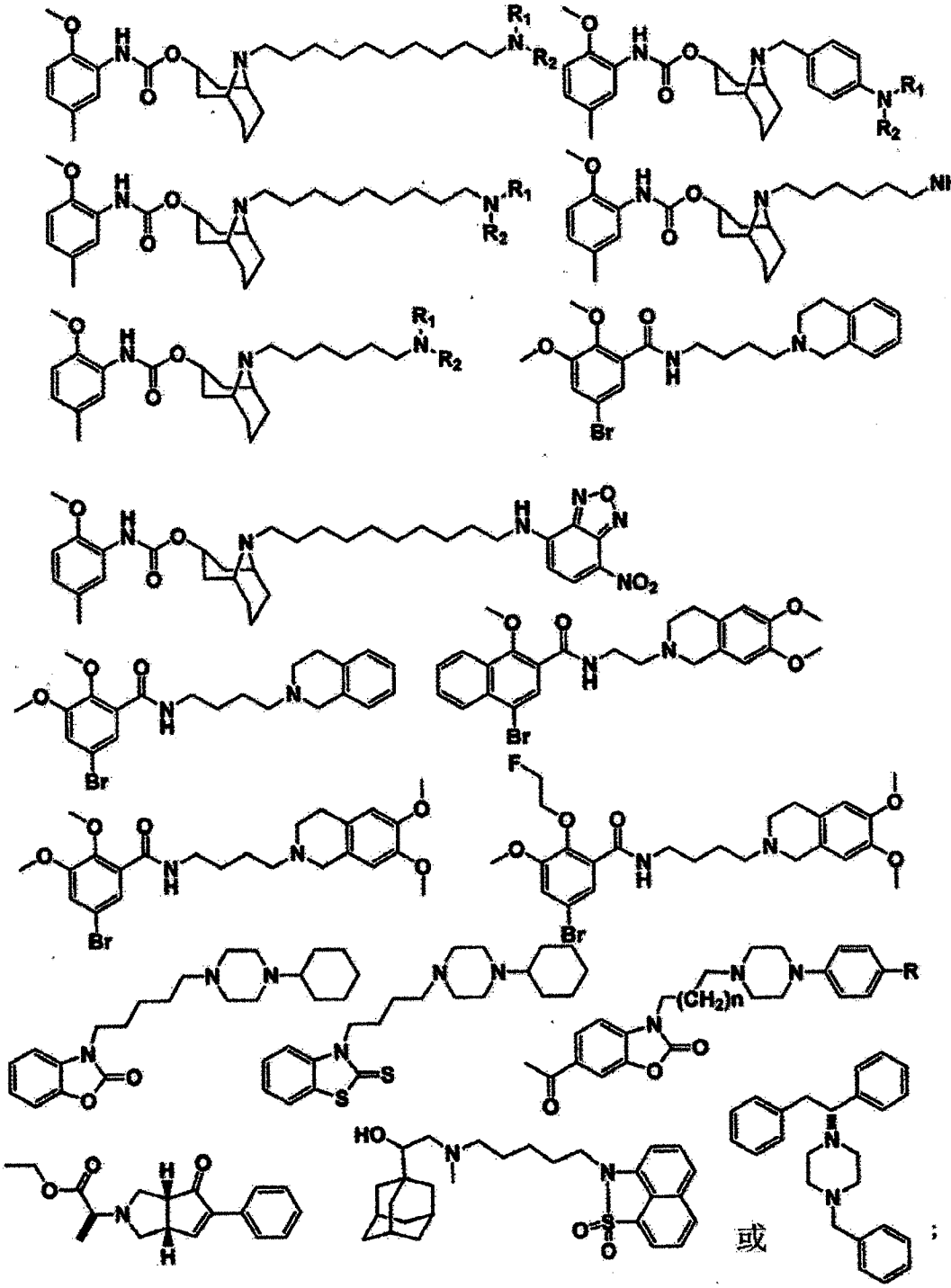


其中 R、R_1 和 R_2 独立地为 C_{1-6} 烷基、烷氧基、卤素、卤代烷基或卤代烷氧基，且 n = 0 ～ 8。

3. 根据权利要求 1 或 2 所述的方法 / 用途，其中所述化合物以还能够有效抑制所述细胞中的膜运输缺陷的量进行施用，所述膜运输效应与所述细胞暴露于可溶 β 淀粉样蛋白寡聚体相关。

4. 根据权利要求 1 ～ 3 中任一项所述的方法 / 用途，其中所述化合物的含量为能够有效抑制所述细胞中与所述细胞暴露于可溶 β 淀粉样蛋白寡聚体相关的寡聚体结合和突触

损失两者。

5. 根据权利要求 1 ～ 4 中任一项所述的方法 / 用途，其中所述化合物以能够有效抑制可溶 β 淀粉样蛋白寡聚体介导的认知效应的量进行施用。

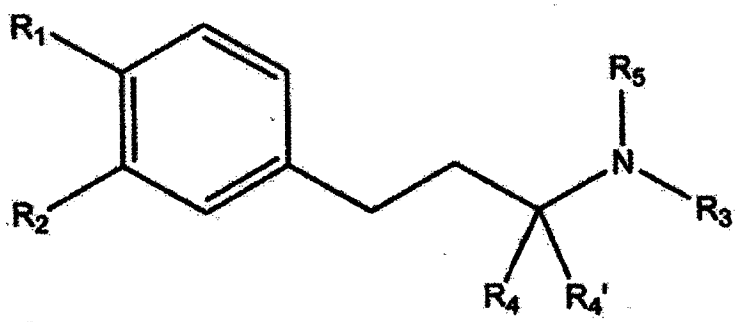
6. 根据权利要求 5 所述的方法 / 用途，其中所述认知效应是在认知衰退的动物模型中测试的认知衰退。

7. 根据权利要求 6 所述的方法 / 用途，其中所述认知衰退是经由条件性恐惧检定所测试的学习衰退。

8. 根据权利要求 6 所述的方法 / 用途，其中所述认知衰退是经由 Morris 水迷宫测试所测试的空间学习和记忆衰退。

9. 根据权利要求 6 所述的方法 / 用途，其中所述认知衰退是在阿尔茨海默病的转基因动物模型中测试的基于海马的空间学习和记忆衰退。

10. 根据权利要求 1 ～ 9 中任一项所述的方法 / 用途，其中所述化合物是式 VIIIq 及其药学上可接受的盐：

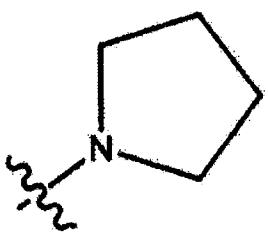


（VIIIq）

其中

R_1 和 R_2 独立地选自 H、OH、卤素、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、C_{1-6} 卤代烷基、$(R_{16})(R_{17})N-C_{1-4}$ 亚烷基 -O-，或者 R1 和 R2 连接在一起形成 $-O-C_{1-2}$ 亚甲基 -O- 基团，其中

R_{16} 和 R_{17} 独立地为 C_{1-4} 烷基或苄基，或者 R_{16} 和 R_{17} 与氮一起形成选自



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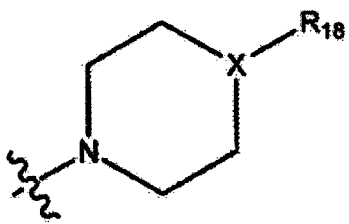
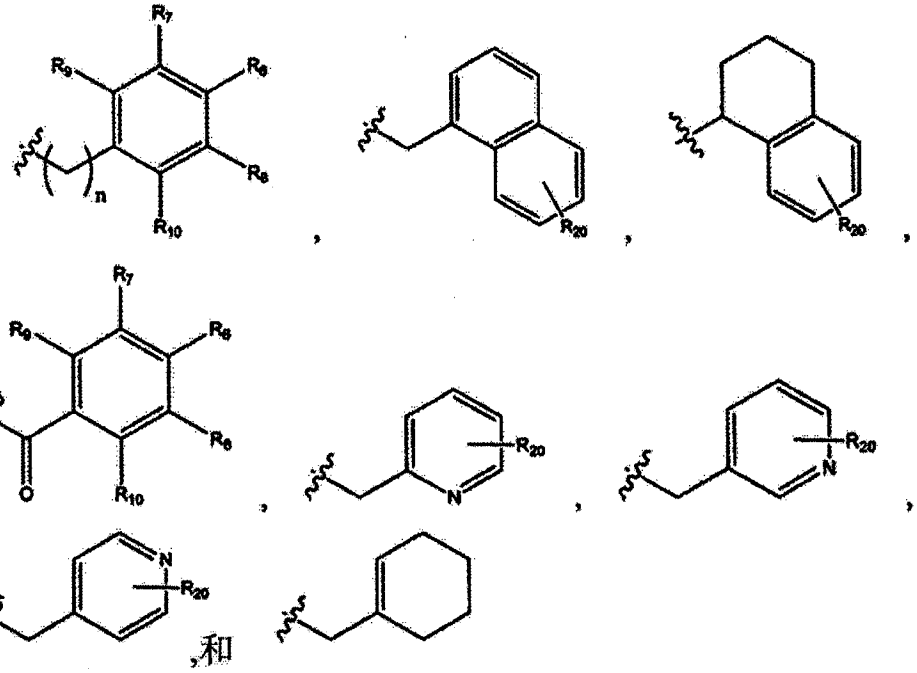


的环，其中

X 是 N 或 O，且 R_{18} 为 H 或未被取代的苯基；且

其中 R_1 和 R_2 中的至少一个不是 H；

R_3 选自



其中

R_6、R_7、R_8、R_9 和 R_{10} 独立地选自 H、卤素、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基和 $S(O)_2$-C_{1-6} 烷基；

R_{20} 为 H；且

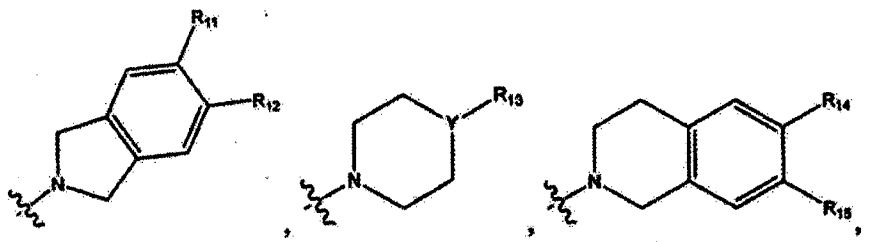
n 为 1 ～ 4

R_4 为 C_{1-6} 烷基；

$R_{4'}$ 为 H 或 C_{1-6} 烷基；且

R_5 为 H、C_{1-6} 烷基和 C(O)O(C_{1-4} 烷基)、C(O)(C_{1-4} 烷基) 或 C(O)(C_{1-4} 卤代烷基)；或

R_3 和 R_5 与氮一起形成选自



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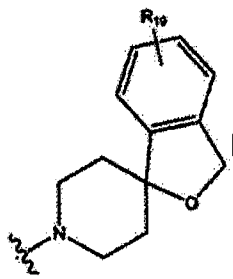


的环，其中

R_{11} 和 R_{12} 独立地选自 H、卤素和 C_{1-6} 卤代烷基，且

Y 为 CH 或 N；

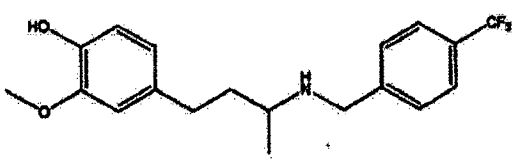
R_{13} 为 H、C_{1-6} 烷基、C_{3-6} 环烷基、未被取代的苯基或被 C_{1-6} 卤代烷基取代的苯基、或未被

取代的苄基；

R_{14} 和 R_{15} 独立地选自 H 和卤素；

R_{19} 为 H，且

条件是排除以下化合物的外消旋混合物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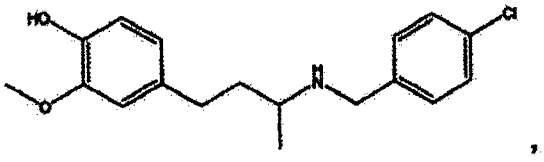
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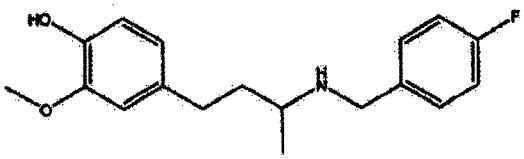
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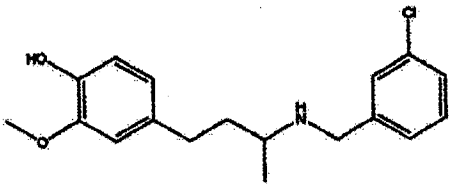
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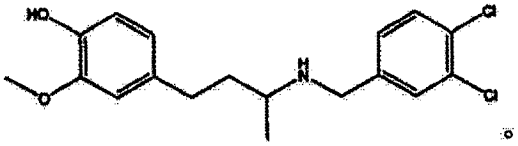
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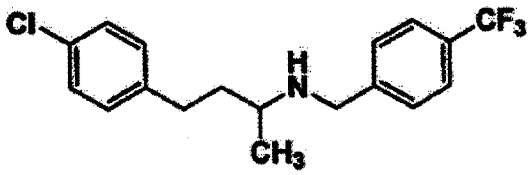
11. 根据权利要求 1 ～ 10 中任一项所述的方法 / 用途，其中所述化合物是选自以下化合物中的一种或其药学上可接受的盐：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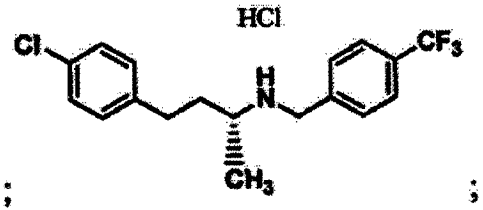
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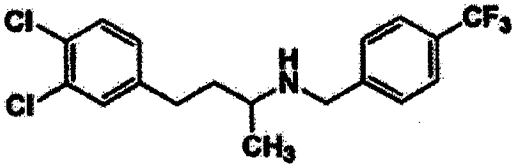
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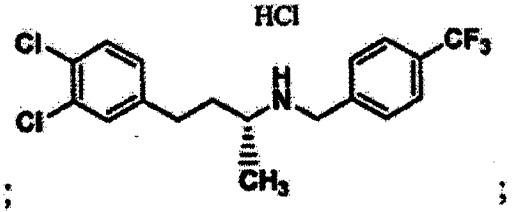
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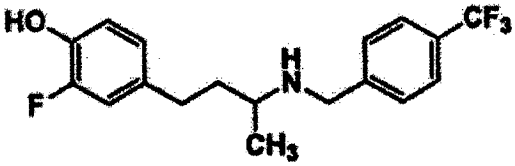
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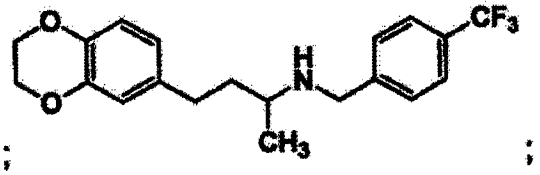
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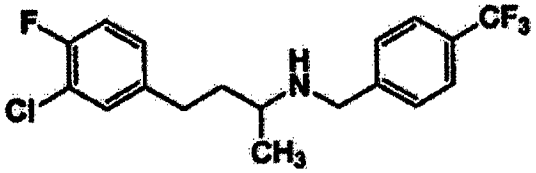
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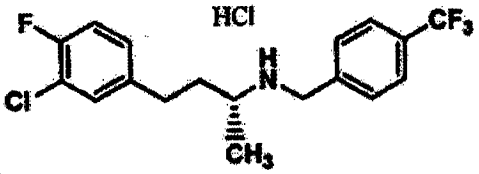
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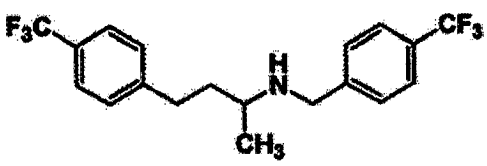
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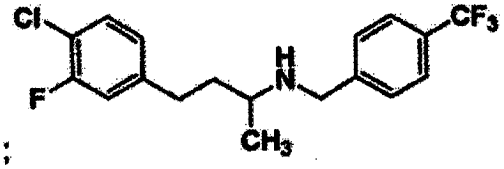
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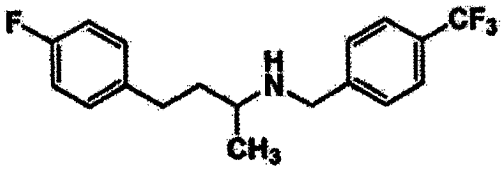
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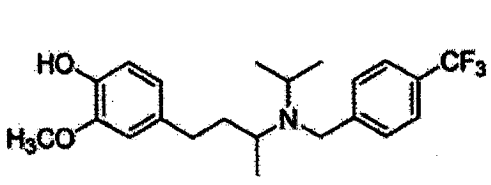
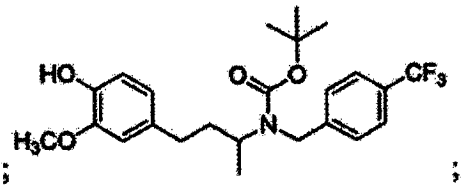
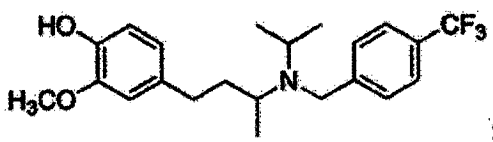
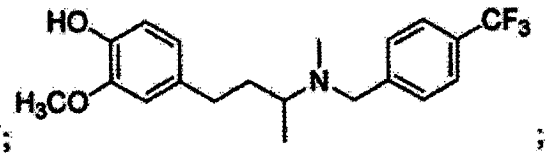
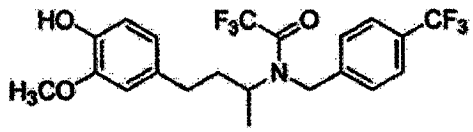
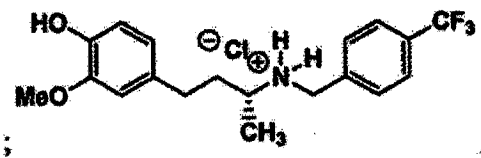
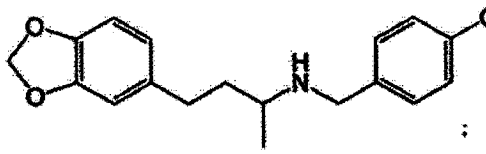
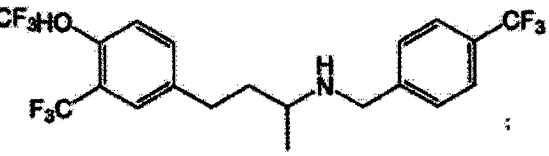
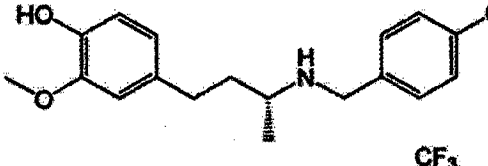
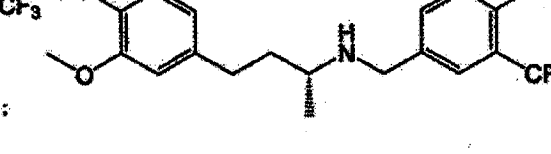
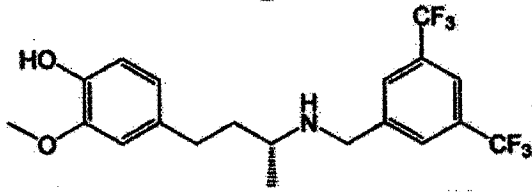
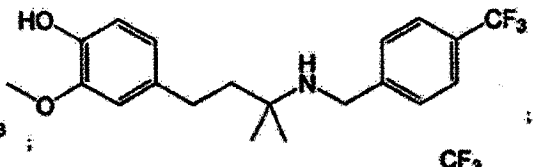
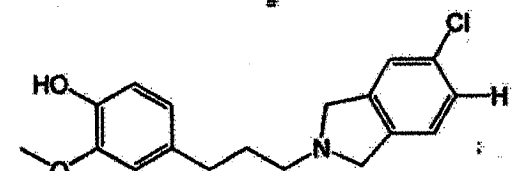
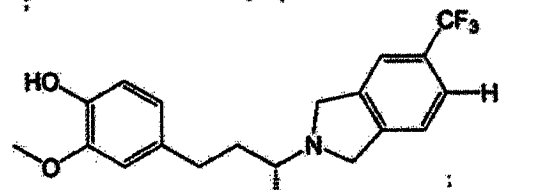
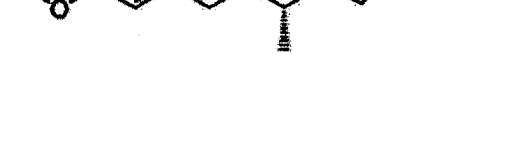

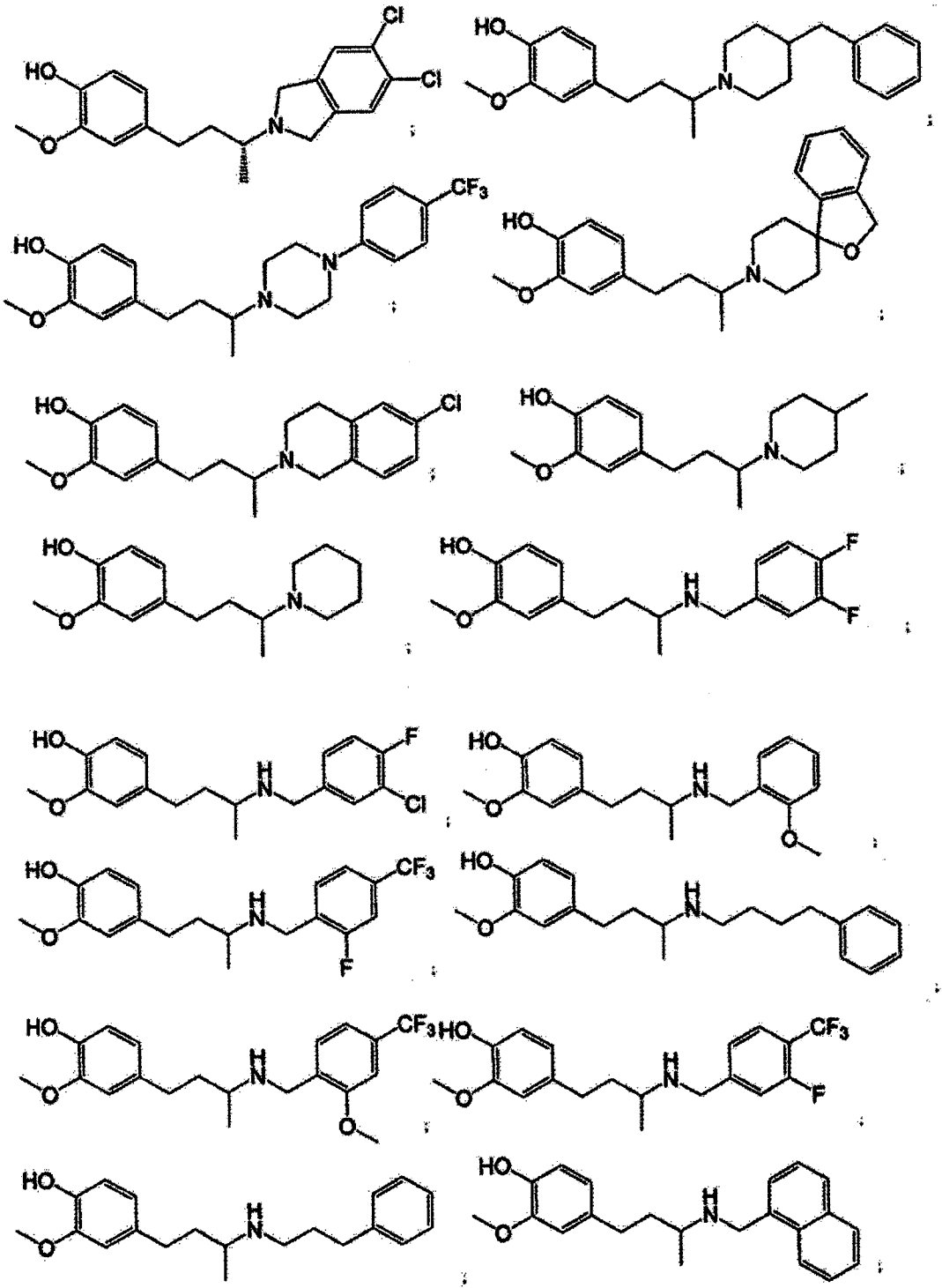

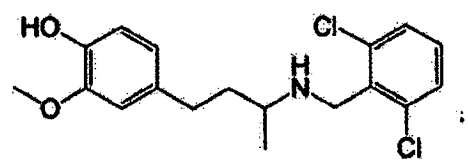
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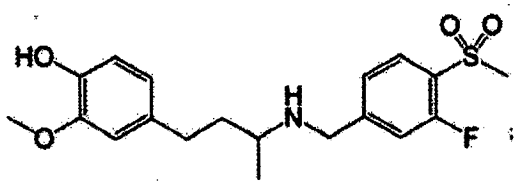
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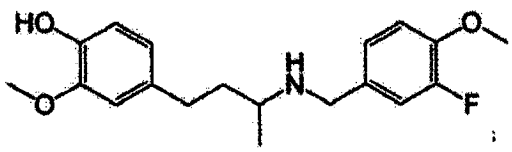
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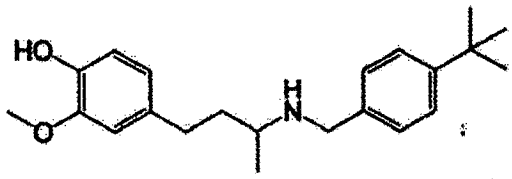
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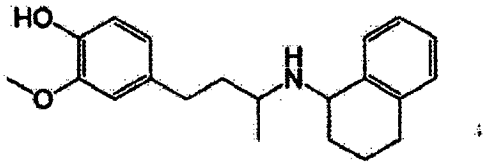
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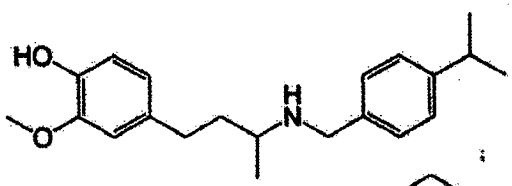
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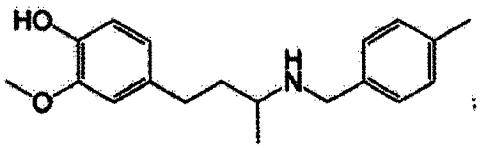
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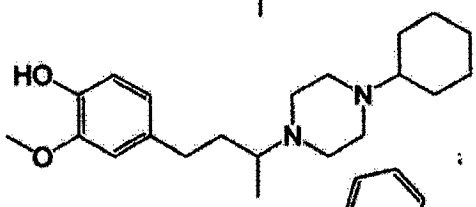
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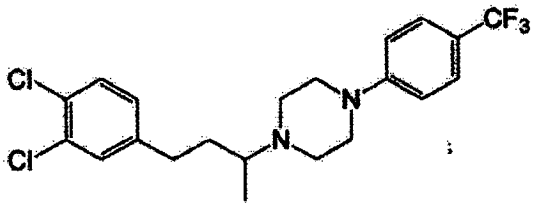
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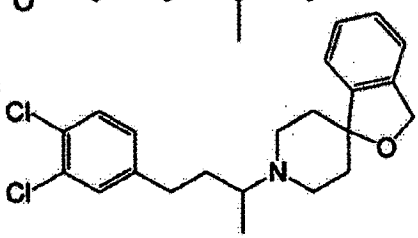
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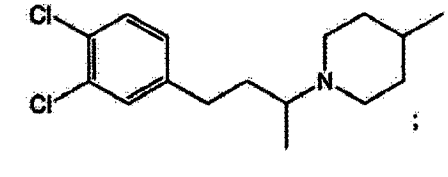
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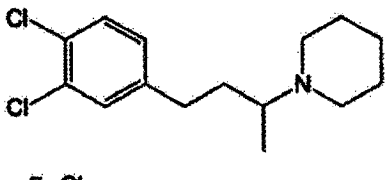
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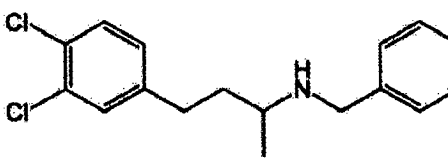
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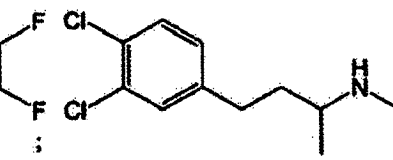
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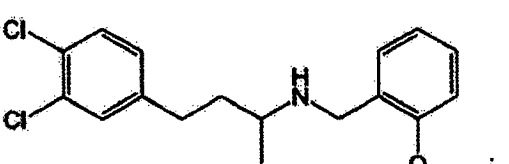
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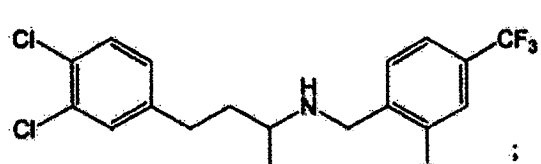
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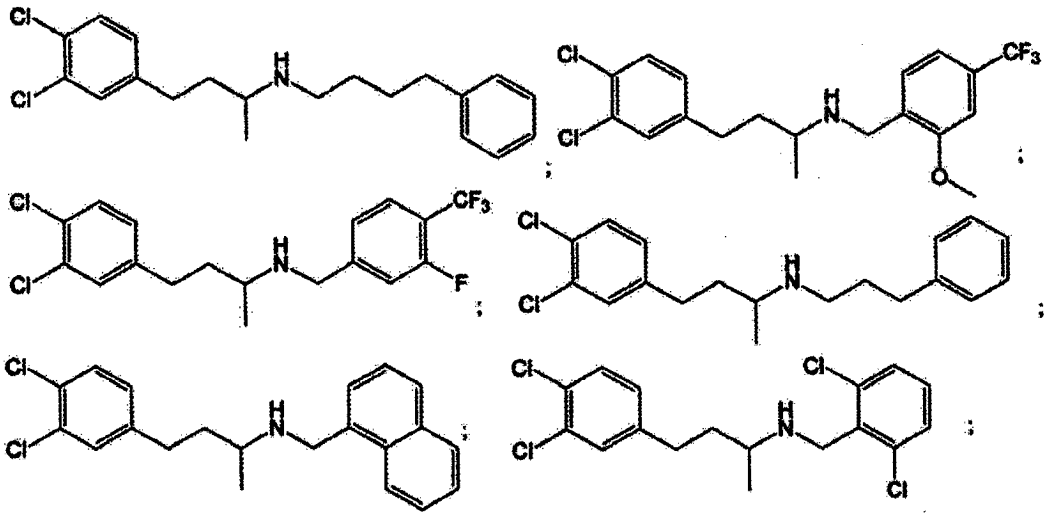

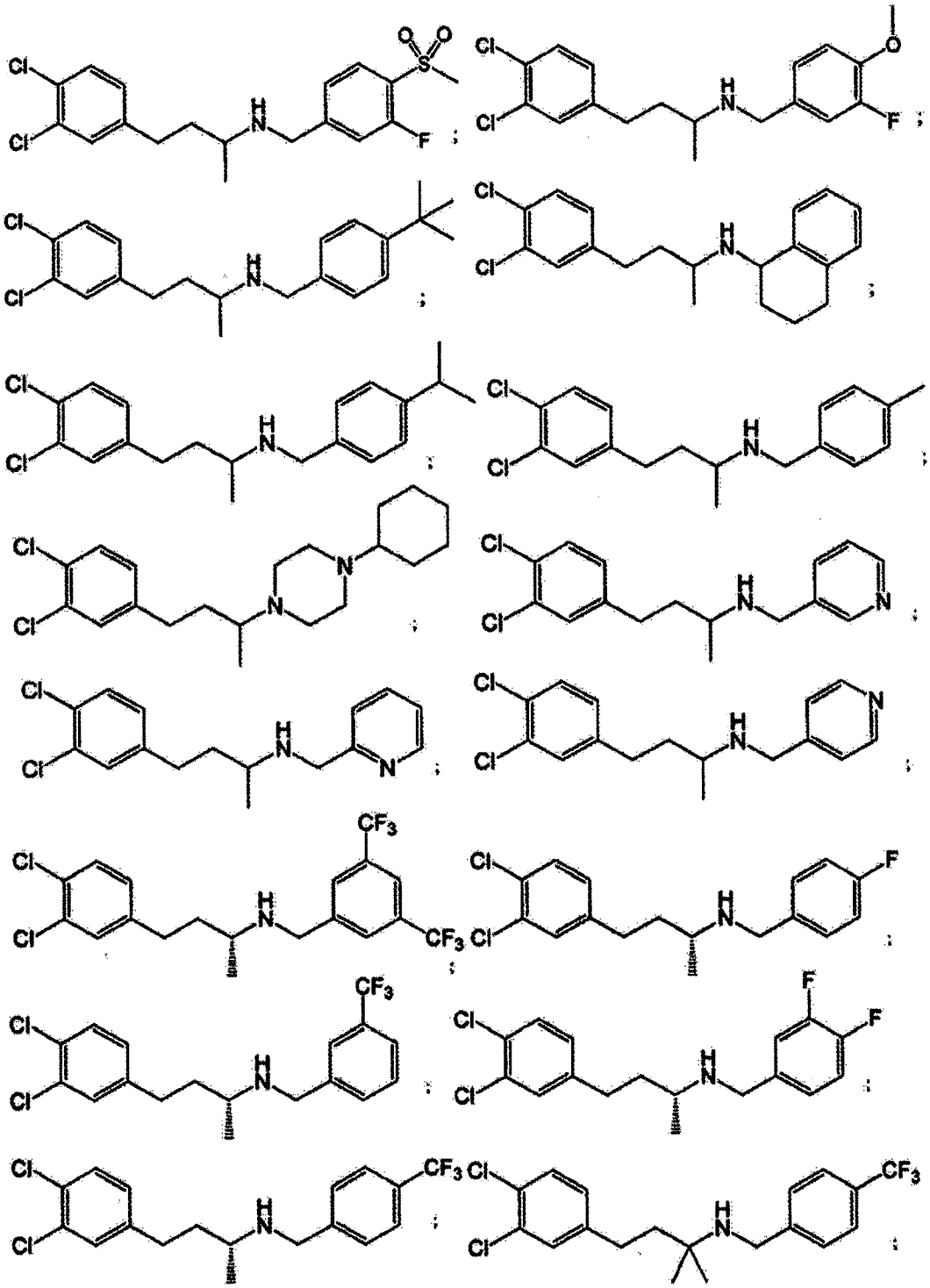

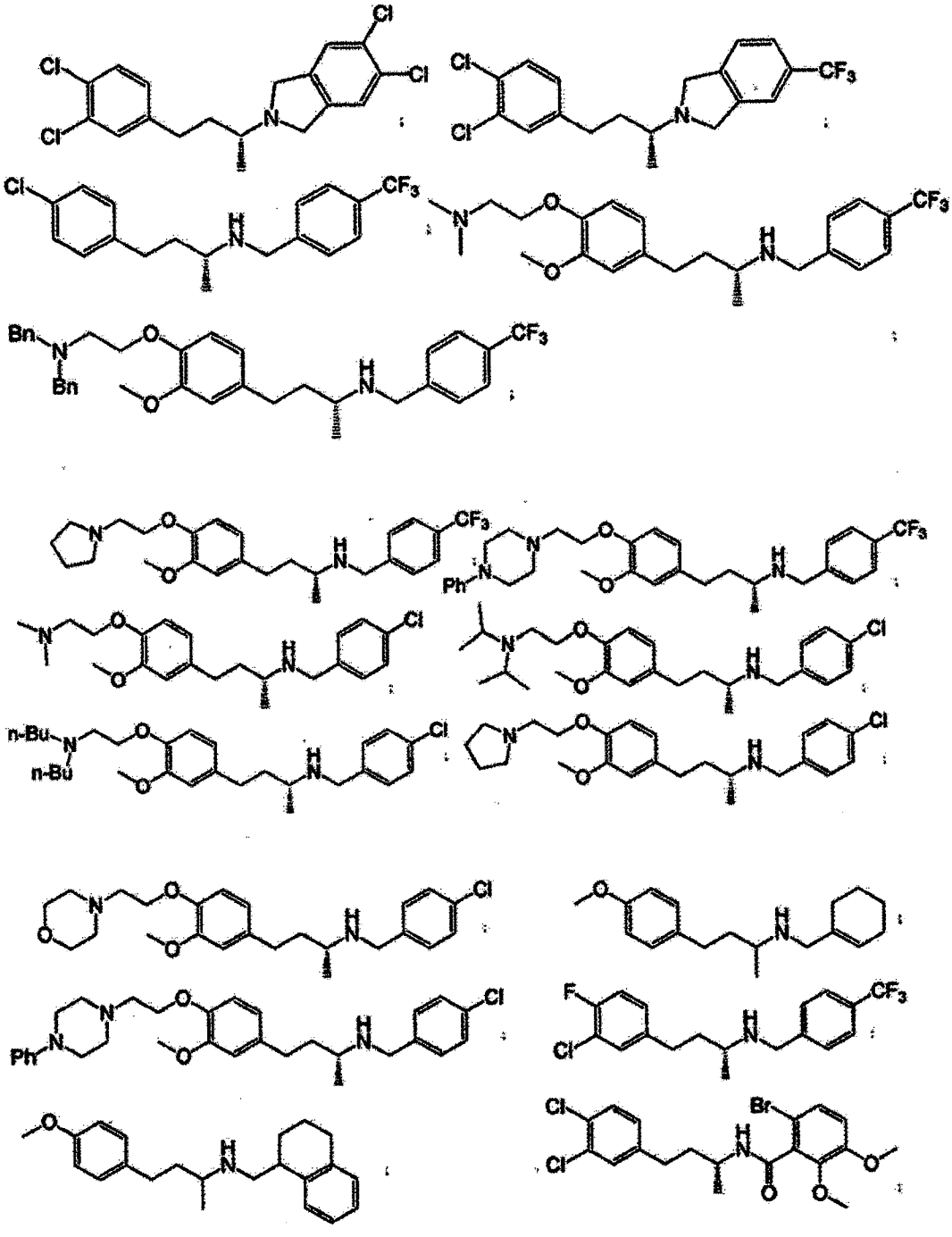

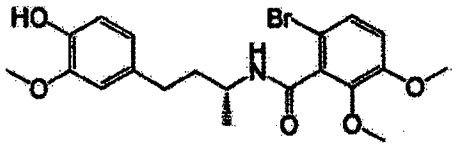
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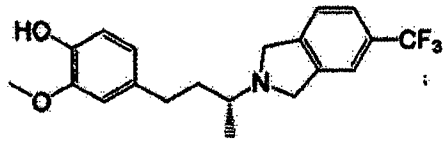
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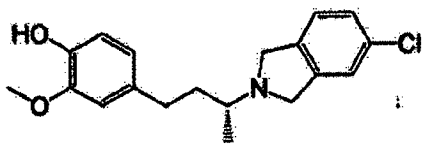
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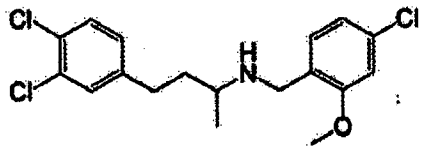
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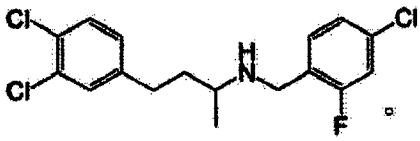
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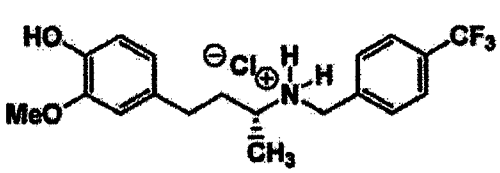
12. 根据权利要求 11 所述的方法 / 用途，其中所述化合物选自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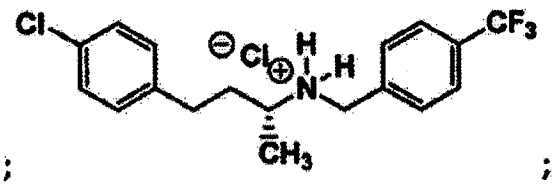
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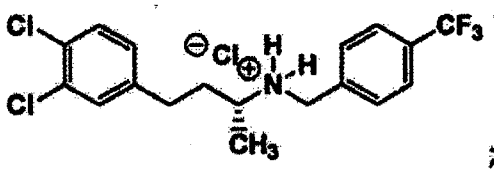
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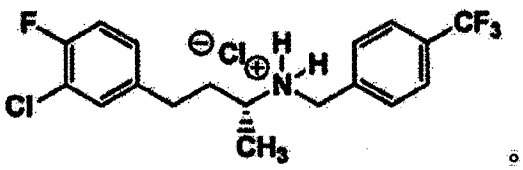
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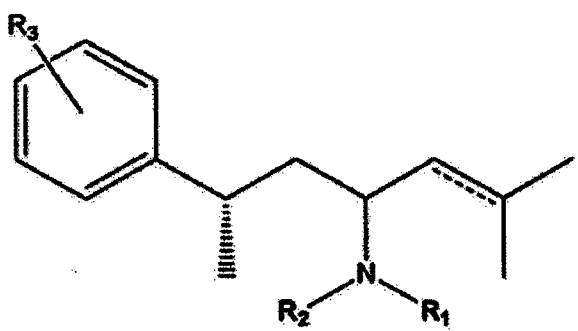
13. 根据权利要求 2 所述的方法 / 用途，其中所述化合物是式 VIIIo 及其药学上可接受的盐



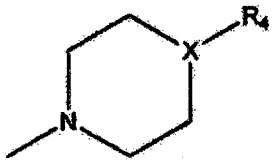
VIIIo

其中，

为单键或双键；

R_1 为 C_{1-6} 烷基、C_{1-6} 卤代烷基、未被取代的苄基或被卤素、C_{1-6} 烷基或 C_{1-6} 卤代烷基取代的苄基；

R_2 为 H，或者

R_1 和 R_2 与氮一起形成环  其中

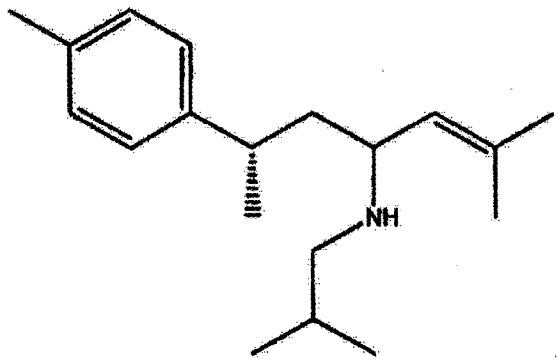
，

X 为 CH、N 或 O，且

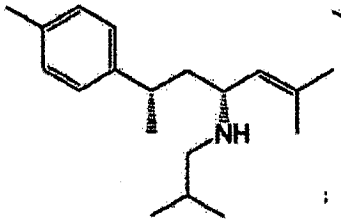
R_4 不存在或为 H、C_{1-6} 烷基或未被取代的苯基或被卤素、C_{1-6} 烷基或 C_{1-6} 卤代烷基取代的苯基；且

R_3 为 C_{1-4} 烷基、C_{1-6} 卤代烷氧基或卤素，

条件是排除以下化合物的外消旋混合物：



14. 根据权利要求 13 所述的方法 / 用途，其中所述化合物是选自以下化合物中的一种或其药学上可接受的盐：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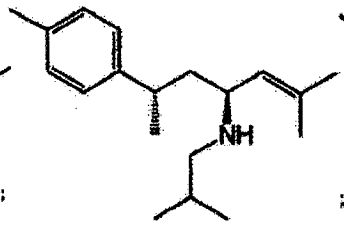
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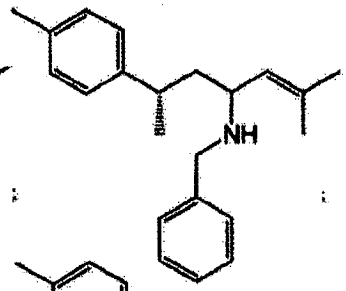
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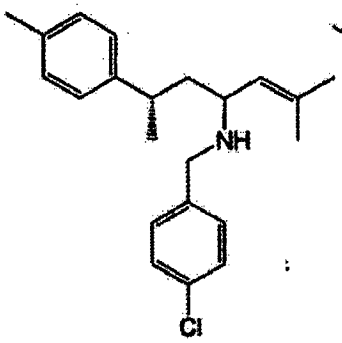
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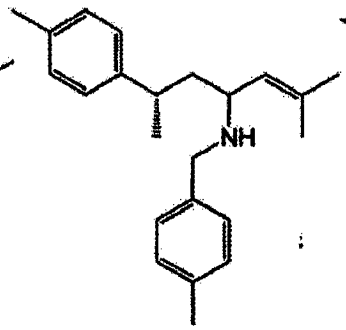
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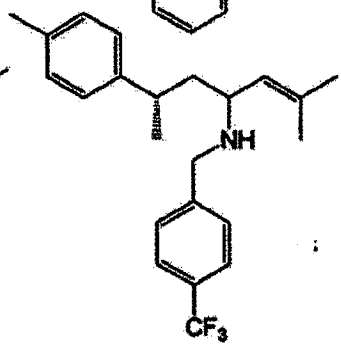
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CF3
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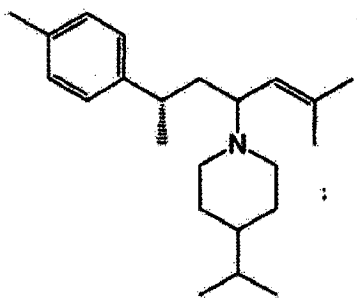
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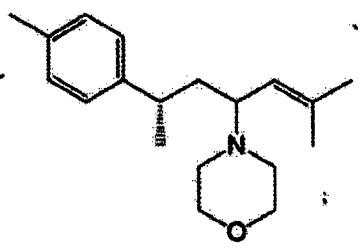
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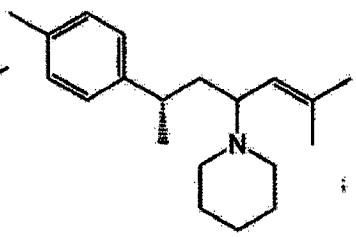
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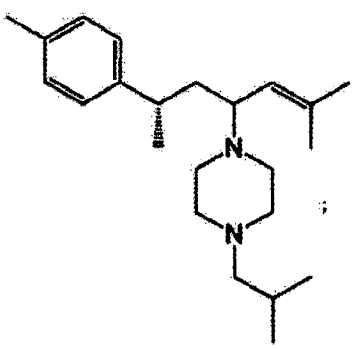
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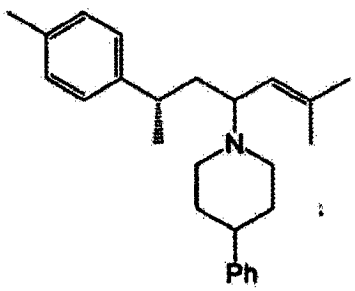
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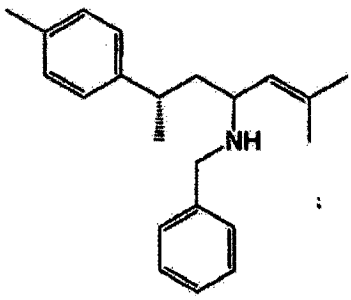
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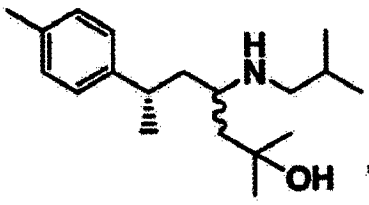
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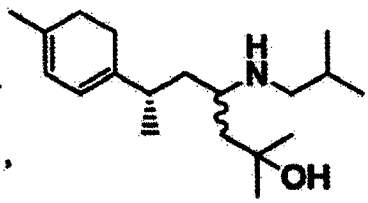
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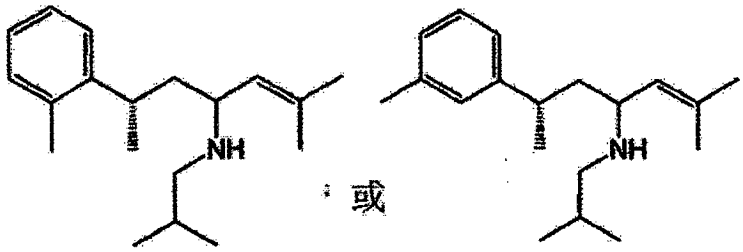


15. 根据权利要求 1 所述的方法 / 用途，其中所述化合物选自抗体或其活性结合片段，其中所述抗体或片段对 σ-2 受体具有特异性。

16. 根据权利要求 15 所述的方法 / 用途，其中所述抗体或片段对存在于 SEQ ID NO:1、SEQ ID NO:2、SEQ ID NO:3、SEQ ID NO:4、SEQ ID NO:5、SEQ ID NO:6、SEQ ID NO:7、SEQ ID NO:9 或 SEQ ID NO:10 中的一种或多种中的抗原的表位具有特异性。

17. 根据权利要求 1 ～ 16 中任一项所述的方法 / 用途，用于抑制神经元细胞的 β 淀粉样蛋白寡聚体诱导的突触功能障碍，包括使所述细胞与包括 σ-2 受体拮抗剂化合物的组合物接触，其中所述组合物的含量为能够有效抑制所述细胞中的 β 淀粉样蛋白寡聚体结合，所述功能障碍与所述细胞暴露于可溶 β 淀粉样蛋白寡聚体相关。

18. 根据权利要求 1 ～ 16 中任一项所述的方法 / 用途，用于抑制在受试者中对长时程增强效应的压制，包括对需要的受试者施用治疗有效量的包括 σ-2 受体拮抗剂化合物的组合物。

19. 根据权利要求 1 ～ 16 中任一项所述的方法 / 用途，用于在表现认知衰退或存在表现认知衰退风险的受试者中抑制认知衰退，包括对所述受试者施用治疗有效量的包括 σ-2 受体拮抗剂化合物的组合物。

20. 根据权利要求 1 ～ 16 中任一项所述的方法 / 用途，用于在受试者中抑制与 β 淀粉样蛋白寡聚体对中枢神经元的作用相关的认知衰退，包括对遭受所述认知衰退的受试者施用治疗有效量的包括 σ-2 受体拮抗剂化合物的组合物。

21. 根据权利要求 1 ～ 16 中任一项所述的方法 / 用途，用于在需要的受试者中治疗阿尔茨海默病中的轻度认知障碍，包括对所述受试者施用治疗有效量的包括 σ-2 受体拮抗剂化合物的组合物。

22. 根据权利要求 1 ～ 21 中任一项所述的方法 / 用途，其中所述 σ-2 拮抗剂化合物具有一种或多种以下附加特性：

(a) 其以比一种或多种非 σ CNS 受体高出至少 10 倍、20 倍、50 倍或 100 倍的亲和性与 σ-2 受体选择性结合，其中所述化合物以小于 200nM、150nM、100nM 或 60nM 的 K_i 与 σ-2 受体结合；

(b) 其抑制 Abeta 寡聚体与神经元细胞的结合或抑制神经元细胞中的突触损失，其中所述损失与所述细胞暴露于 Abeta 寡聚体相关；

(c) 其在中枢神经元中抑制膜运输异常，所述异常与所述细胞暴露于一种或多种 Abeta 寡聚体相关；

(d) 在不存在 β 淀粉样蛋白寡聚体时，其不影响中枢神经元中的运输或突触数目。

23. 用于识别选择性的、高亲和性 σ-2 拮抗剂化合物的方法 / 用途，包括：

基于以比一种或多种非 σ CNS 受体高出至少 10 倍、20 倍、50 倍或 100 倍的亲和性与

σ-2 受体选择性结合的能力，选择测试用化合物，其中所述化合物以小于 200nM、150nM、100nM 或 60nM 的 K_i 与 σ-2 受体结合；

使神经元细胞与所述化合物接触，以及

确定所述化合物是否具有一种或多种以下附加特性：

(a) 其抑制中枢神经元中的 β 淀粉样蛋白寡聚体结合或突触损失，其中所述损失与所述神经元暴露于 β 淀粉样蛋白寡聚体相关；

(b) 其在中枢神经元中抑制膜运输异常，所述异常与所述细胞暴露于一种或多种 β 淀粉样蛋白寡聚体相关；以及

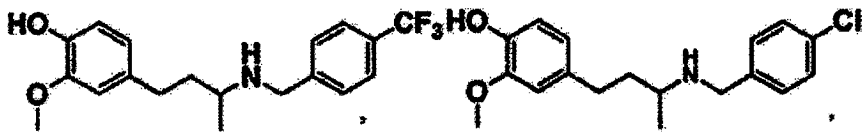
(c) 在不存在 β 淀粉样蛋白寡聚体时，其不影响运输或突触数目。

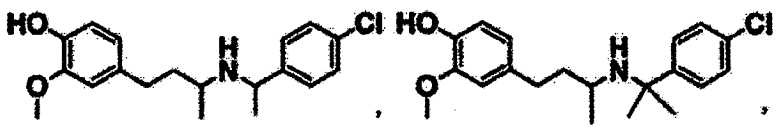
24. 一种用于抑制 β 淀粉样蛋白对神经元细胞的作用的组合物，包括

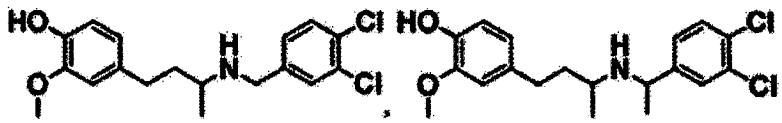
选择性 σ-2 受体拮抗剂化合物，其含量为能够有效抑制所述细胞中的 β 淀粉样蛋白寡聚体结合；以及

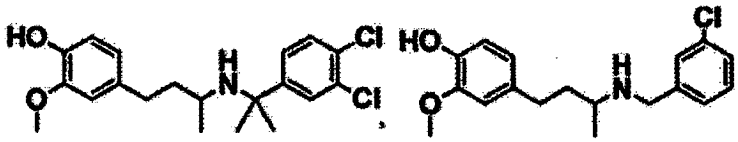
药学上可接受的载体。

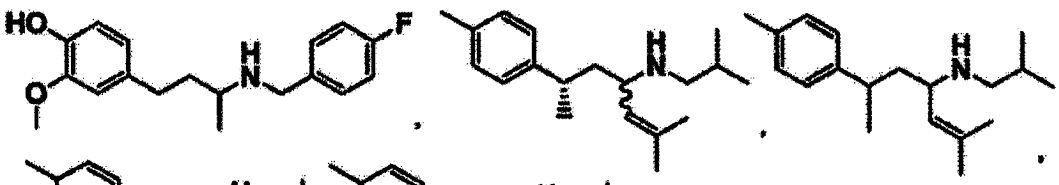
25. 根据权利要求 24 所述的组合物，包括 σ-2 受体拮抗剂，其中所述化合物不是

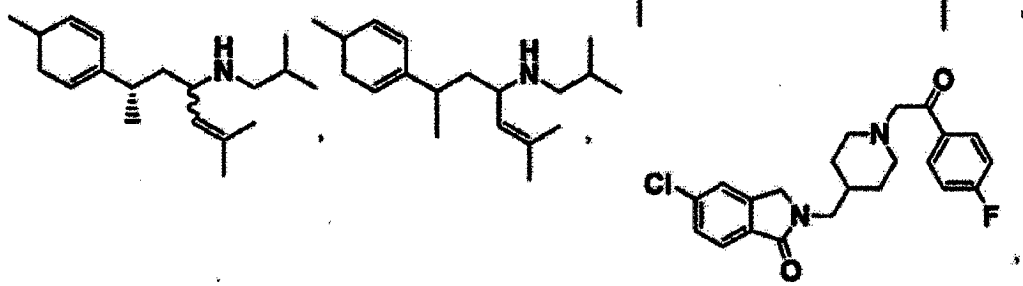


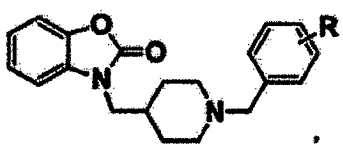


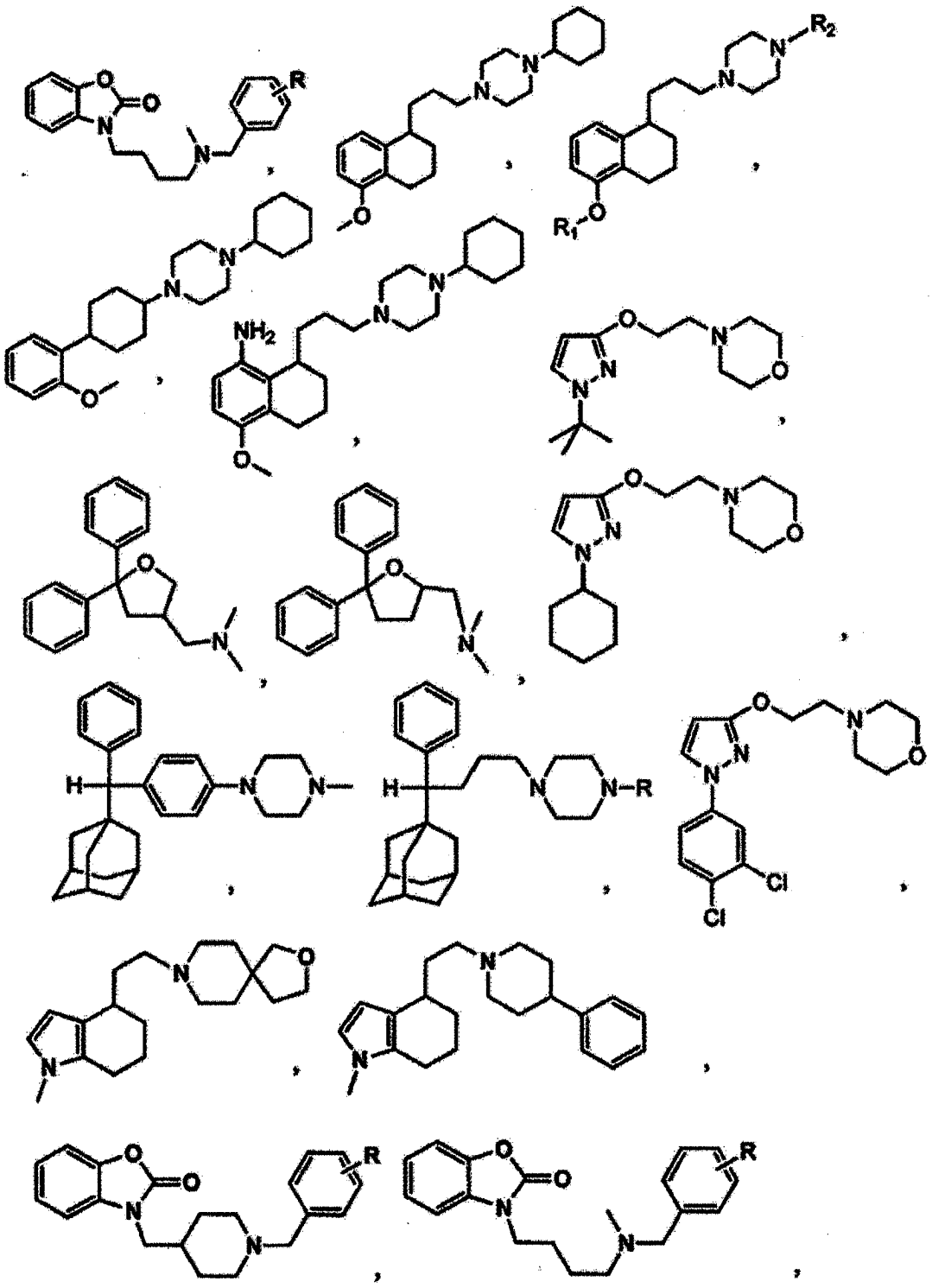


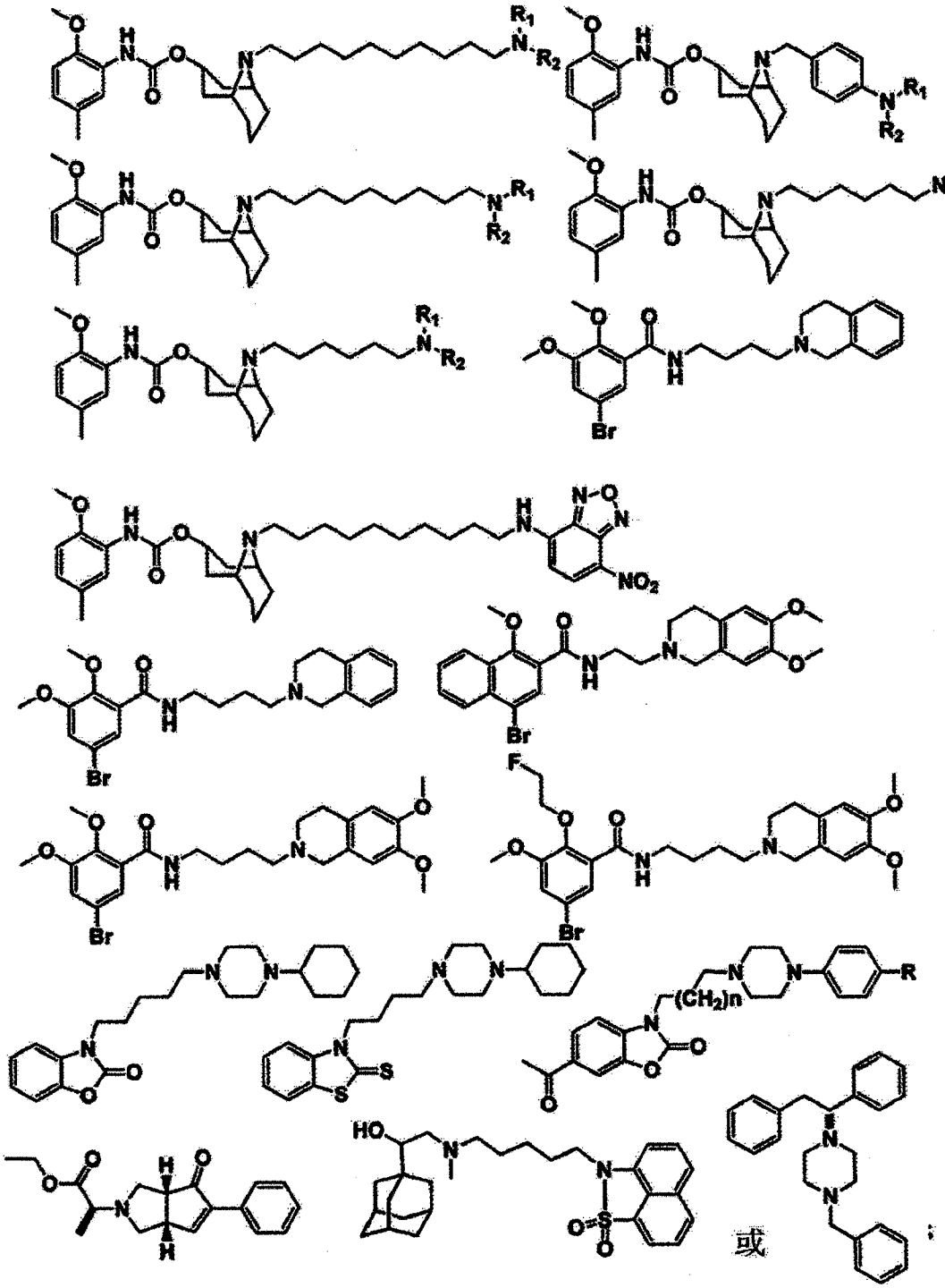






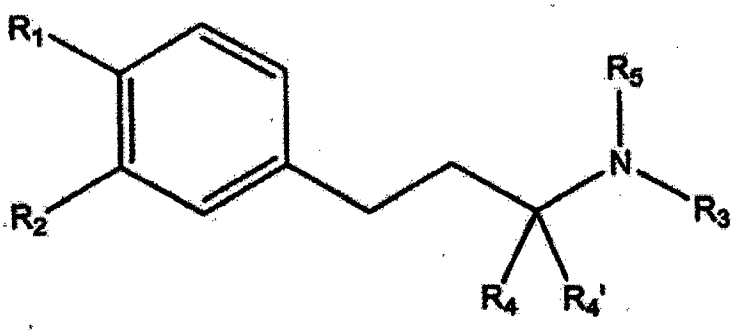






其中 R、R_1 和 R_2 独立地为 C_{1-6} 烷基、烷氧基、卤素、卤代烷基或卤代烷氧基，且 n = 0 ～ 8。

26. 根据权利要求 24 或权利要求 25 所述的组合物，其中所述化合物是式 VIIIq 及其药学上可接受的盐：

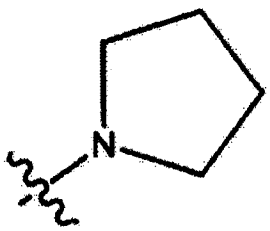


(VIIIq)

其中

R_1 和 R_2 独立地选自 H、OH、卤素、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、C_{1-6} 卤代烷基、$(R_{16})(R_{17})N-C_{1-4}$ 亚烷基 -O-，或者 R1 和 R2 连接在一起形成 $-O-C_{1-2}$ 亚甲基 -O- 基团，其中

R_{16} 和 R_{17} 独立地为 C_{1-4} 烷基或苄基，或者 R_{16} 和 R_{17} 与氮一起形成选自



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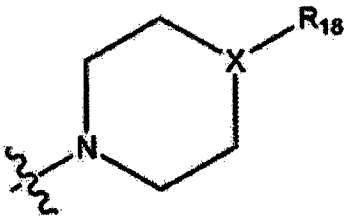
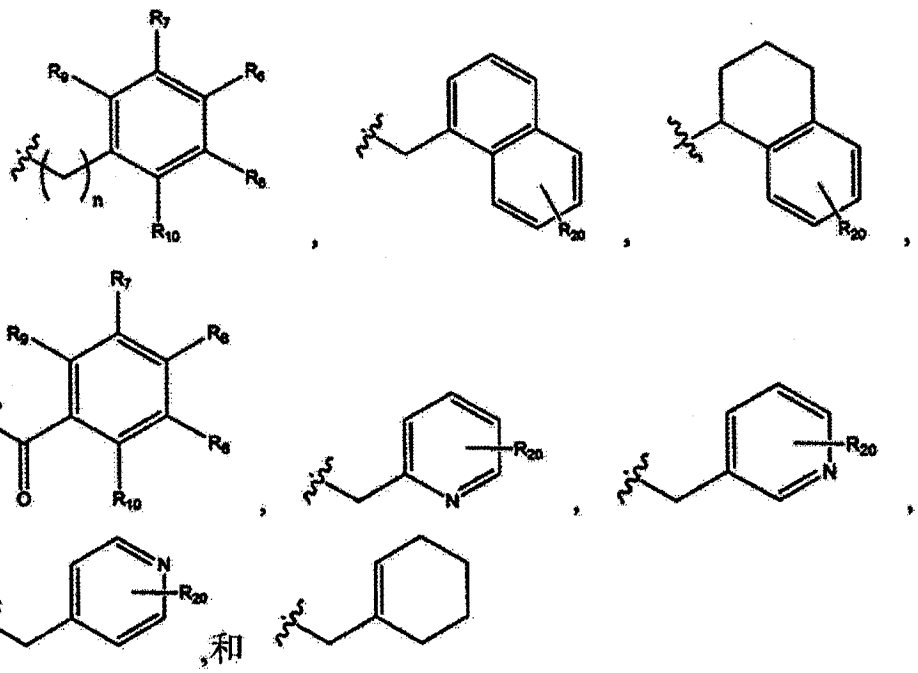


的环，其中

X 是 N 或 O，且 R_{18} 为 H 或未被取代的苯基；且

其中 R_1 和 R_2 中的至少一个不是 H；

R_3 选自



其中

R_6、R_7、R_8、R_9 和 R_{10} 独立地选自 H、卤素、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基和 $S(O)_2-C_{1-6}$ 烷基；

R_{20} 为 H；且

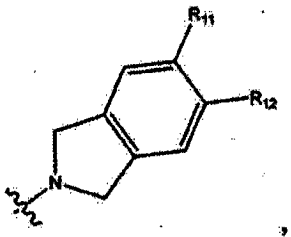
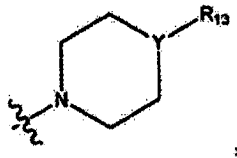
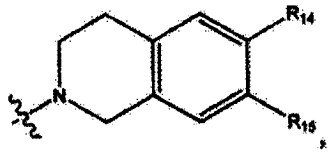
n 为 1 ～ 4

R_4 为 C_{1-6} 烷基；

$R_{4'}$ 为 H 或 C_{1-6} 烷基；且

R_5 为 H、C_{1-6} 烷基和 C(O)O(C_{1-4} 烷基)、C(O)(C_{1-4} 烷基) 或 C(O)(C_{1-4} 卤代烷基)；或

R_3 和 R_5 与氮一起形成选自

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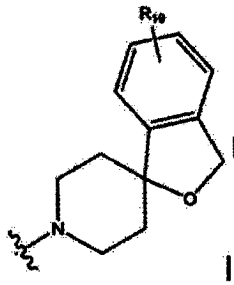


的环，其中

I

R_{11} 和 R_{12} 独立地选自 H、卤素和 C_{1-6} 卤代烷基，且

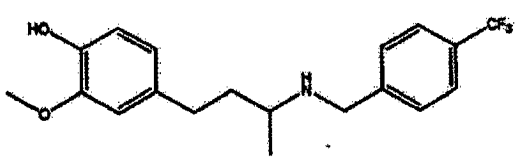
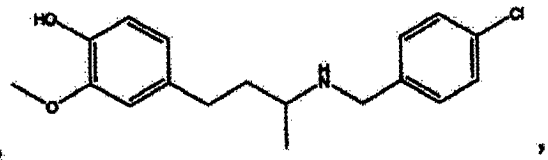
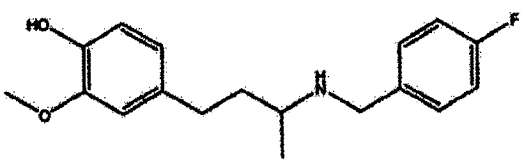
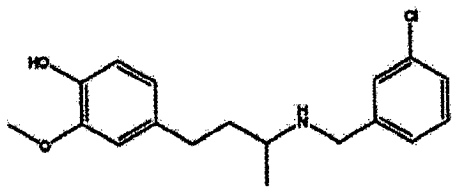
Y 为 CH 或 N；

R_{13} 为 H、C_{1-6} 烷基、C_{3-6} 环烷基、未被取代的苯基或被 C_{1-6} 卤代烷基取代的苯基、或未被取代的苄基；

R_{14} 和 R_{15} 独立地选自 H 和卤素；

R_{19} 为 H，

条件是排除以下化合物的外消旋混合物

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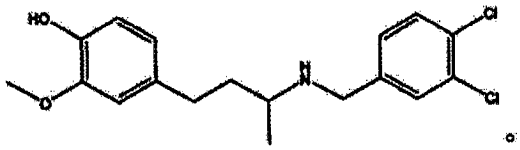

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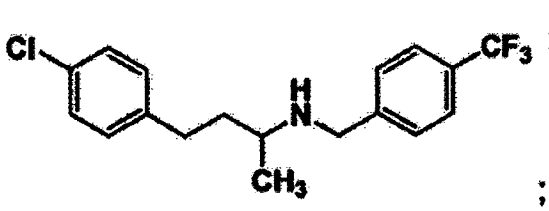
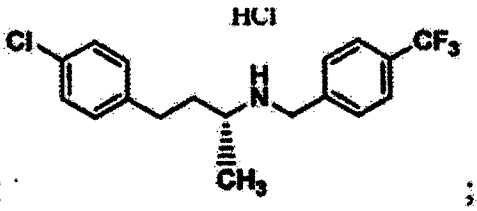
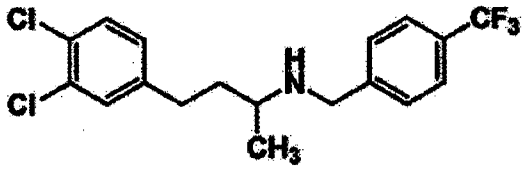
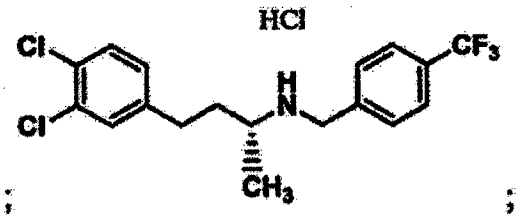
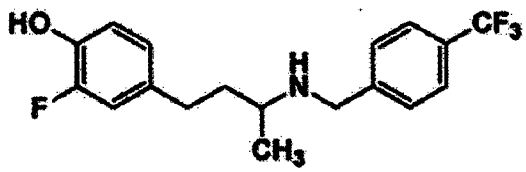
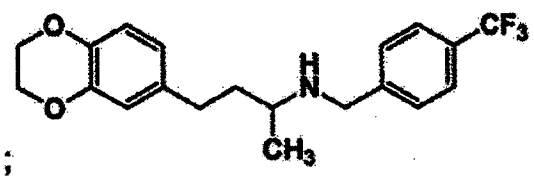
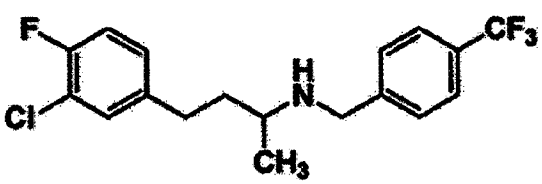
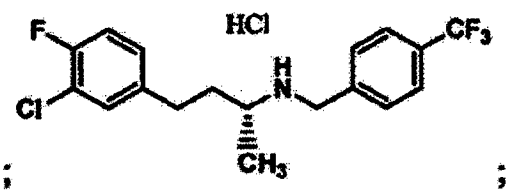
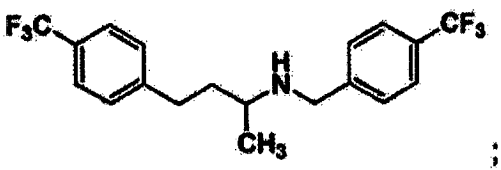
27. 根据权利要求 24 ～ 26 中任一项所述的组合物，其中所述化合物是选自以下化合物中的一种或其药学上可接受的盐：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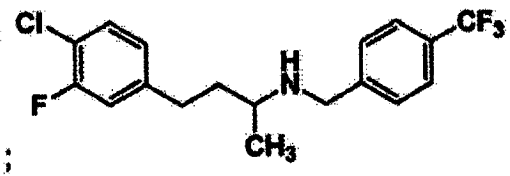
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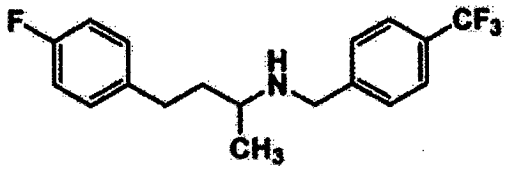
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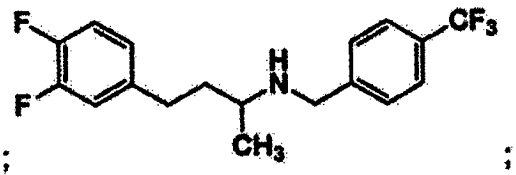
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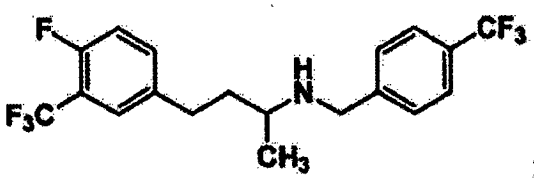
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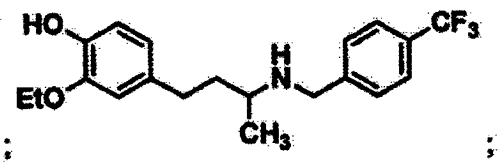
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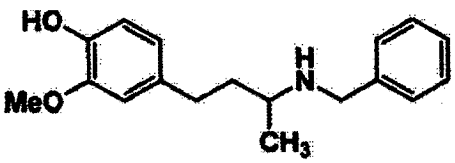
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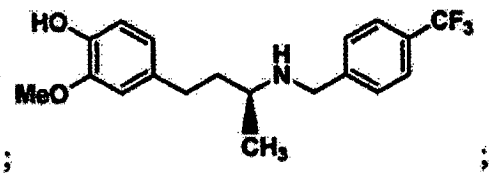
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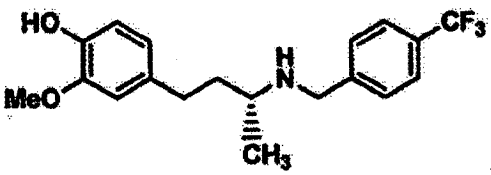
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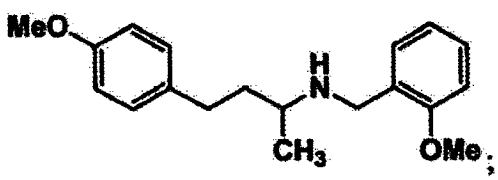
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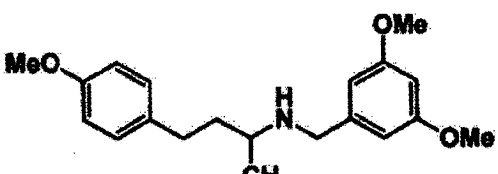
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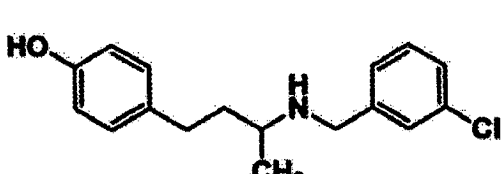
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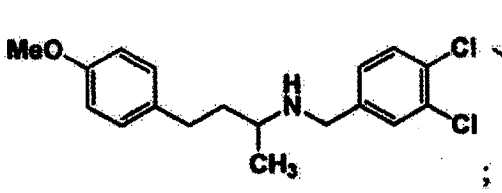
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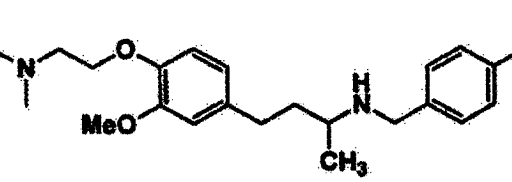
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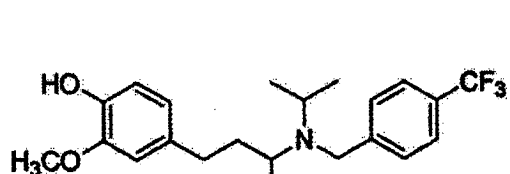
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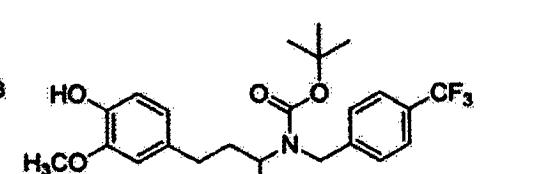
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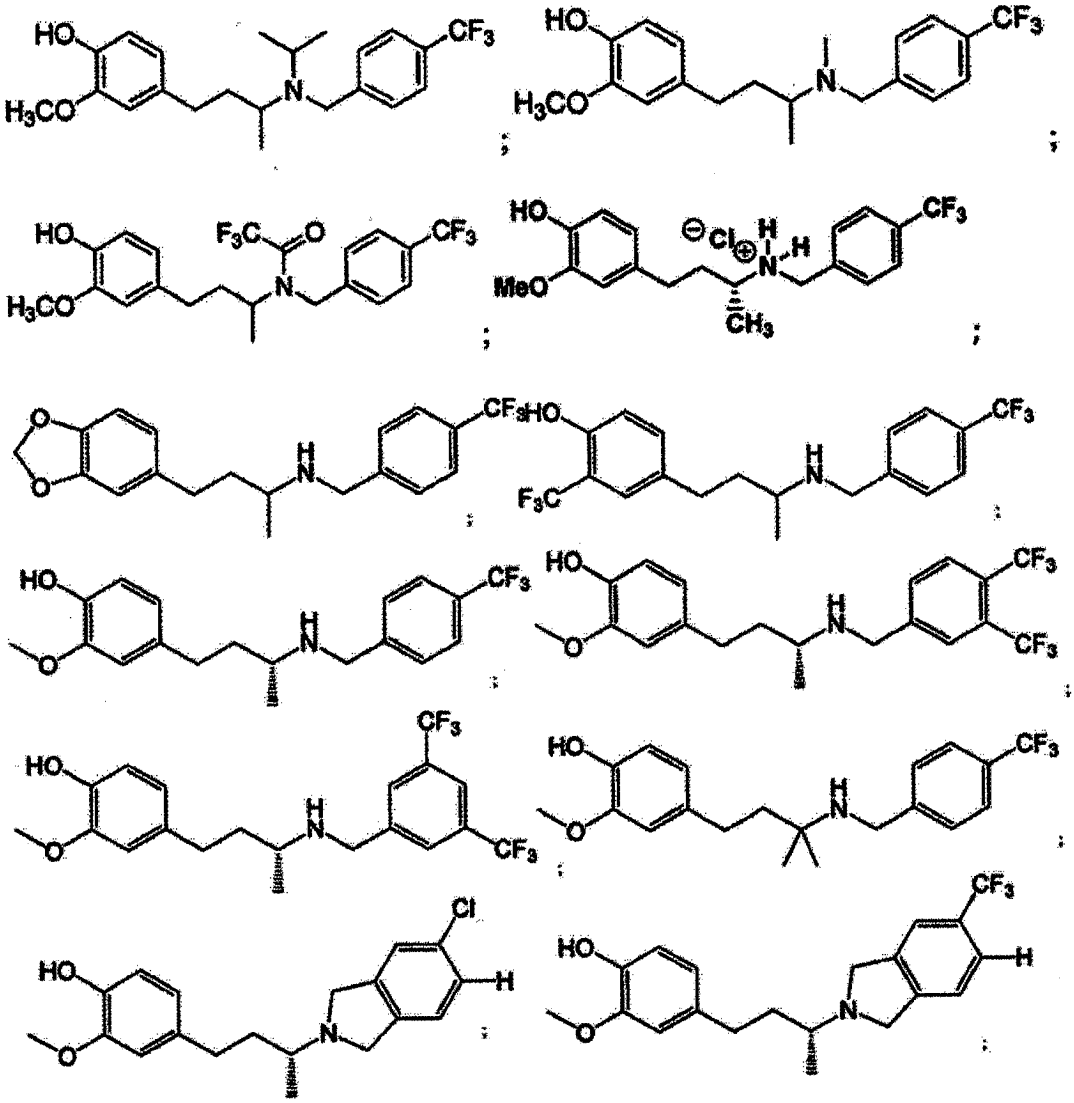

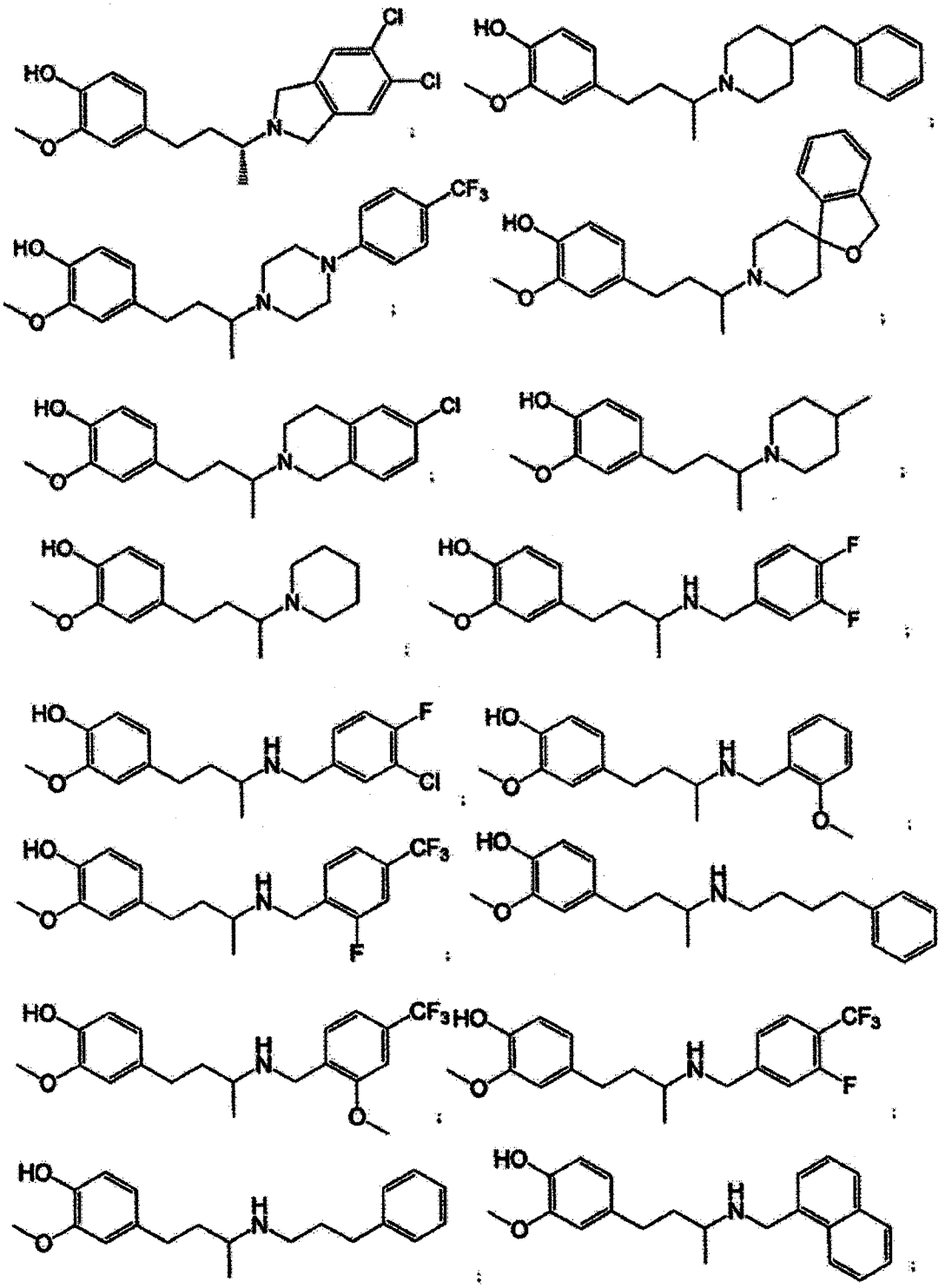

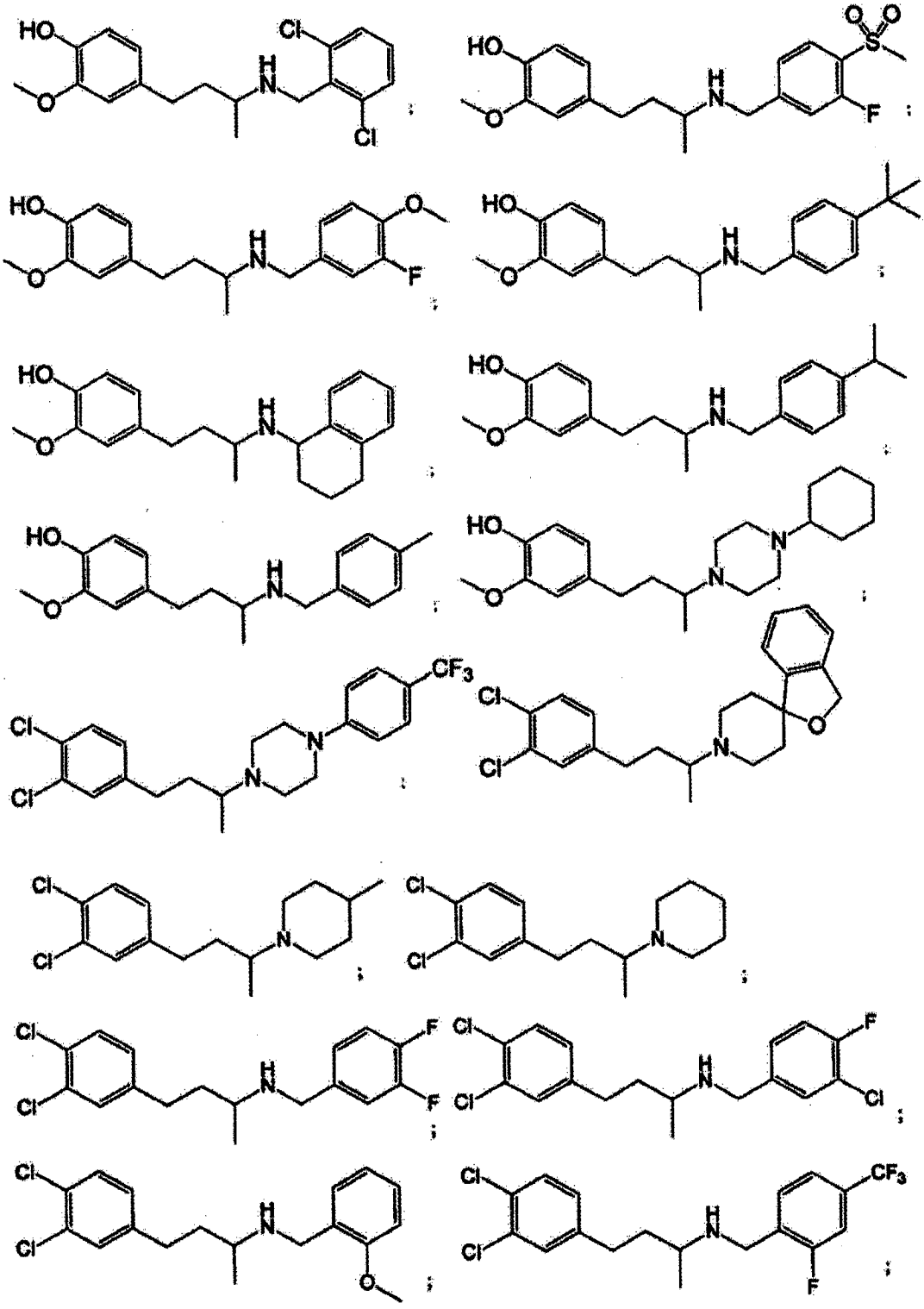

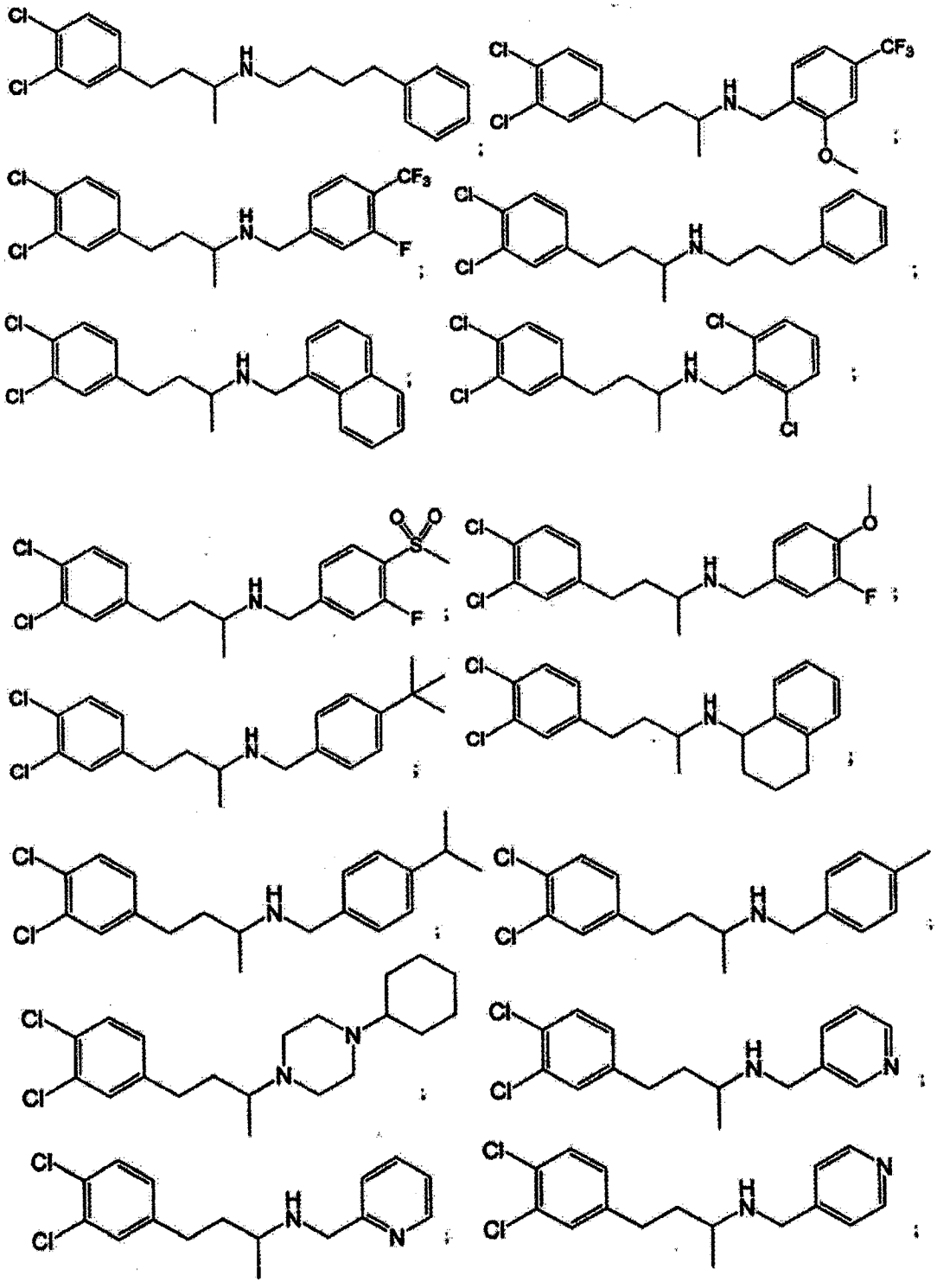

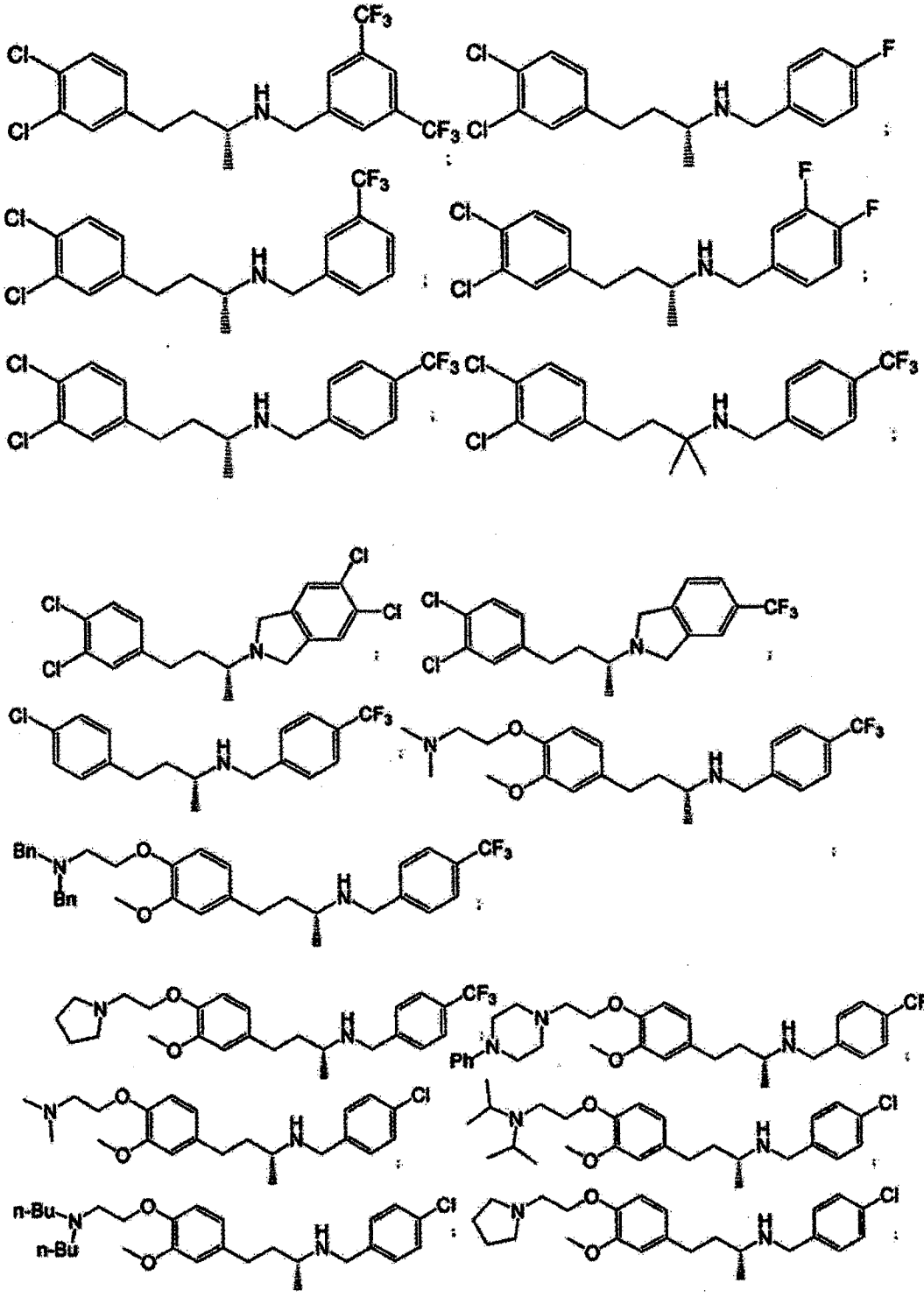

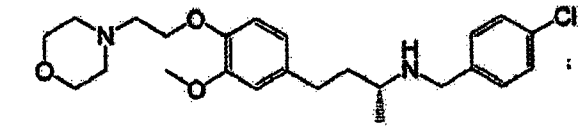
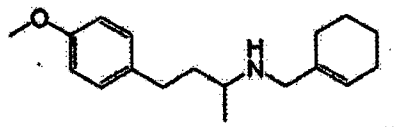
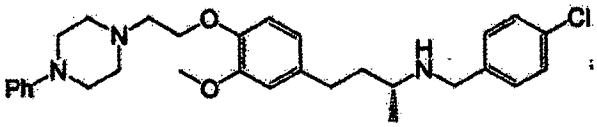
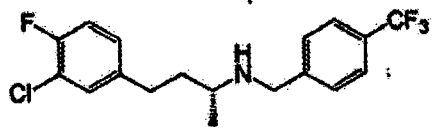
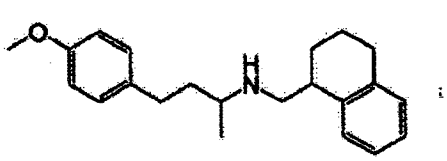
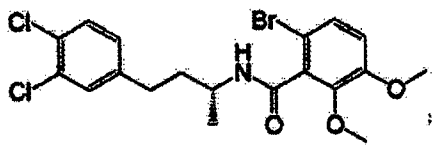
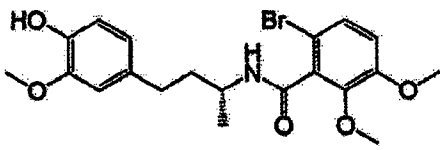
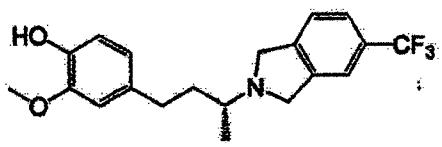
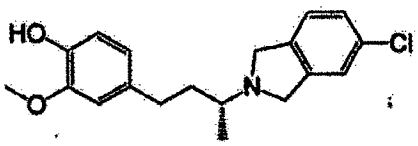
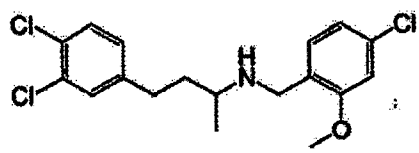
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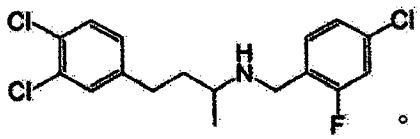
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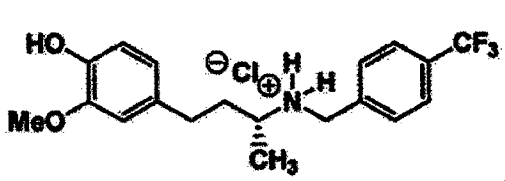
28. 根据权利要求 27 所述的组合物，其中所述化合物选自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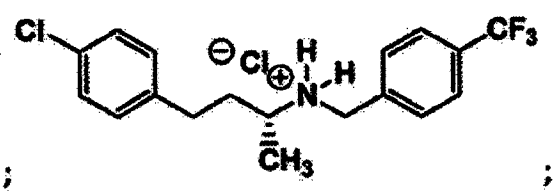
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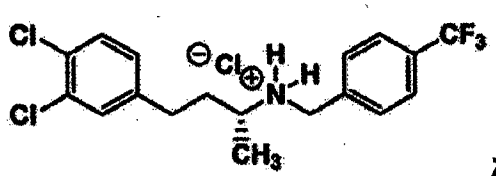
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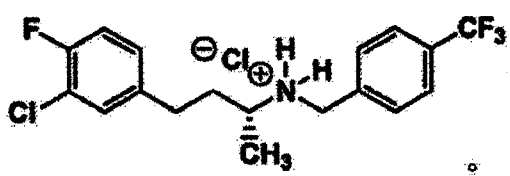
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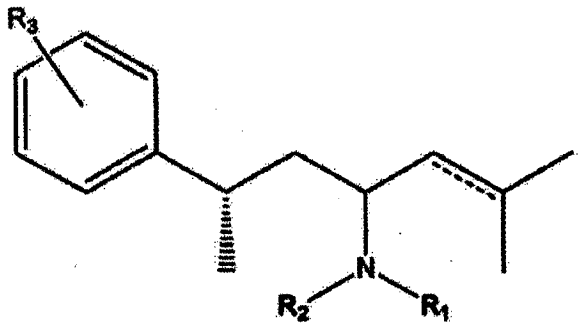
29. 根据权利要求 24 或权利要求 25 所述的组合物，其中所述化合物是式 VIIIo 及其药学上可接受的盐



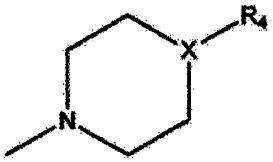
VIIIo

其中，

为单键或双键；

R_1 为 C_{1-6} 烷基、C_{1-6} 卤代烷基、未被取代的苄基或被卤素、C_{1-6} 烷基或 C_{1-6} 卤代烷基取代的苄基；

R_2 为 H，或者

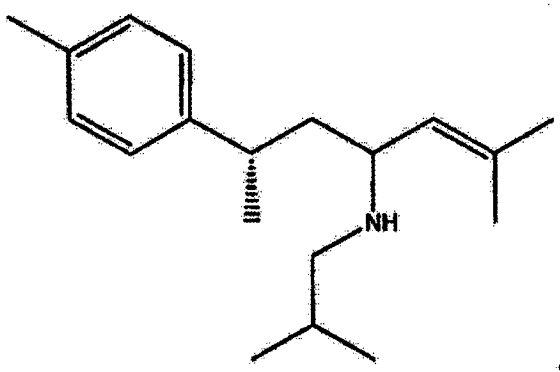
R_1 和 R_2 与氮一起形成环  其中

X 为 CH、N 或 O，且

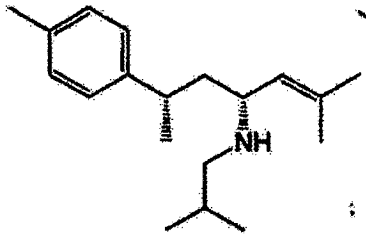
R_4 不存在或为 H、C_{1-6} 烷基或未被取代的苯基或被卤素、C_{1-6} 烷基或 C_{1-6} 卤代烷基取代的苯基；且

R_3 为 C_{1-4} 烷基、C_{1-6} 卤代烷氧基或卤素，

条件是排除以下化合物的外消旋混合物：



30. 根据权利要求 29 所述的组合物，其中所述化合物是选自以下化合物中的一种或其药学上可接受的盐：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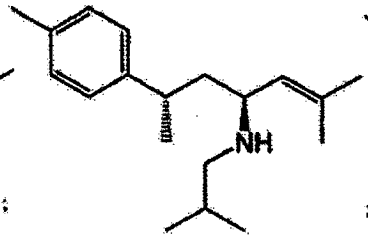
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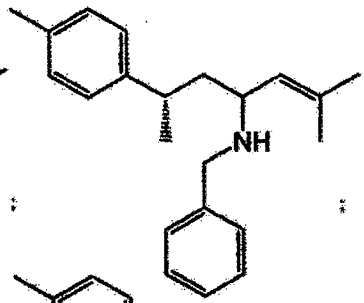
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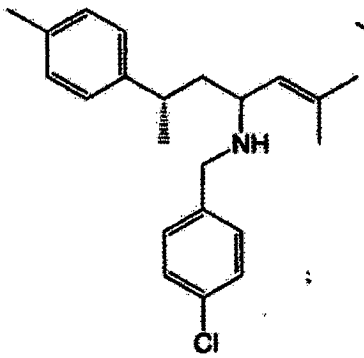
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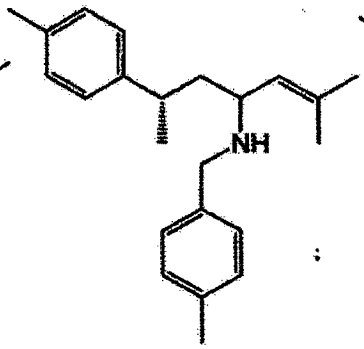
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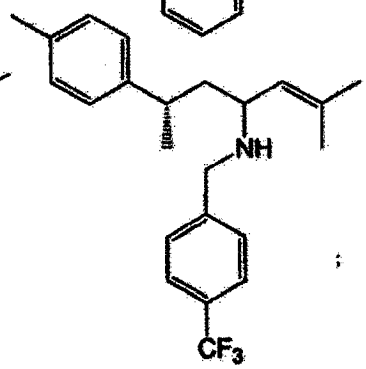
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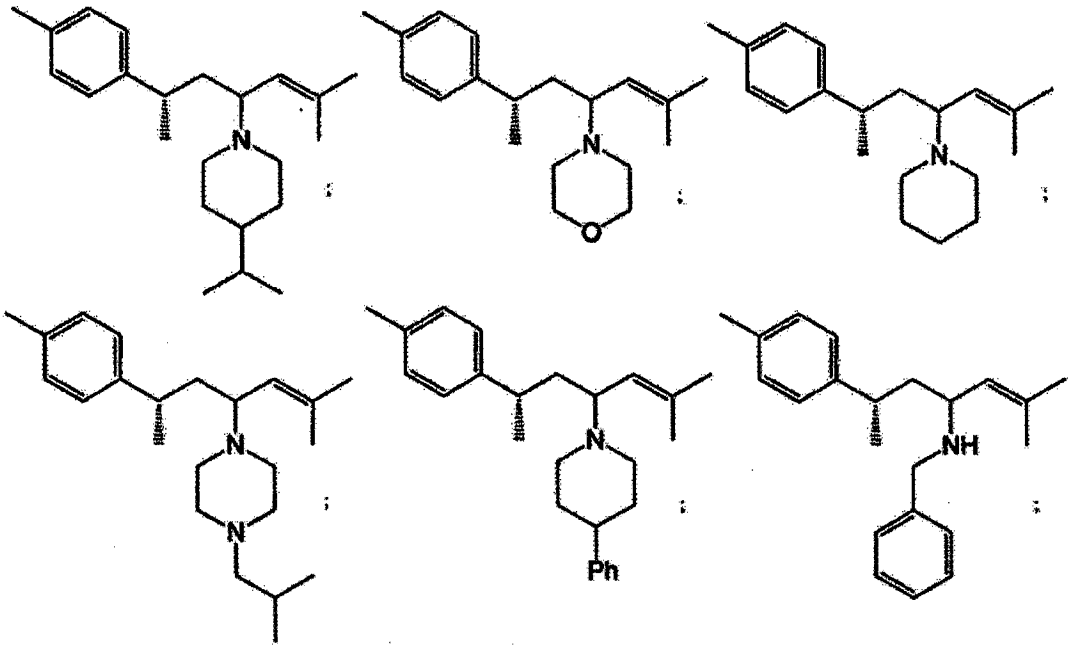
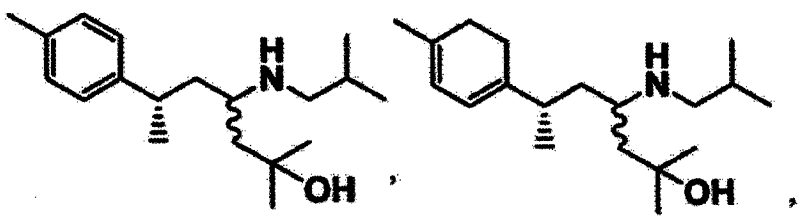
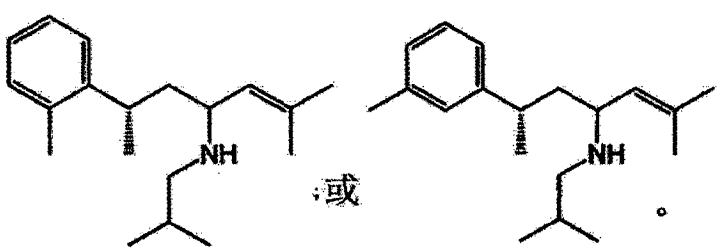






或

31. 根据权利要求 24 所述的组合物，其中所述化合物选自抗体或其活性结合片段，其中所述抗体或片段对 σ-2 受体具有特异性。

32. 根据权利要求 31 所述的组合物，其中所述抗体或片段对存在于 SEQ ID NO:1、SEQ ID NO:2、SEQ ID NO:3、SEQ ID NO:4、SEQ ID NO:5、SEQ ID NO:6、SEQ ID NO:9 或 SEQ ID NO:10 中的一种或多种中的抗原的表位具有特异性。

用于治疗神经退行性疾病的组合物和方法

[0001] 本申请于2012年8月27日提交为PCT国际专利申请，以美国公司Cognition Therapeutics, Inc.的名义作为除美国之外的所有指定国的申请人，以美国公民Susan M. Catalano、Gilbert Rishton和Nicholas J. Izzo, Jr.作为仅仅美国指定的申请人，并要求2011年8月25日提交的美国临时专利申请序列号61/527,584以及2011年8月26日提交的美国临时专利申请61/527,963的优先权，这些申请全部并入本文以供参考。

技术领域

[0002] 本发明涉及选择性σ-2受体拮抗剂化合物、包含它们的药物组合物在用于抑制神经元细胞中β淀粉样蛋白(Aβ)相关的突触损失和突触功能障碍的方法中的用途。在一些实施方式中，组合物可用于调节神经元细胞中Aβ相关的膜运输变化，以及用于在需要的患者中治疗与Aβ病理相关的认知衰退。在一些实施方式中，化合物和组合物用于治疗与Abeta病理相关的神经退行性疾病和病症。本发明还涉及基于化合物结合σ-2受体并用作为σ-2受体拮抗剂的能力来筛选其在抑制认知衰退中的活性的方法，以及涉及首先基于化合物是否阻断Aβ诱导的膜运输缺陷、且阻断Aβ诱导的突触损失但在不存在Aβ寡聚体时不影响运输或突触数目来完善这些筛选方法的方法。σ-2受体拮抗剂化合物选自对σ-2受体有选择性的小分子、或抗体或其片段。

背景技术

[0003] β淀粉样蛋白的过度产生和积聚是阿尔茨海默病的病理特征。人β淀粉样蛋白(Abeta)是在患有阿尔茨海默病的患者脑中发现的不可溶淀粉样蛋白斑沉积的主要成分。该斑由Abeta的纤维状聚集物组成。β淀粉样蛋白原纤维与阿尔茨海默病的晚期相关联。

[0004] 早期阿尔茨海默病(AD)的认知标志是特别不能形成新记忆。早期记忆丧失被认为是由可溶Aβ寡聚体引起的突触障碍。这些寡聚体阻断长时程增强效应(突触可塑性的经典实验范式)，并且它们在AD脑组织和转基因AD模型中显著升高。已假设，早期记忆丧失源自神经元死亡之前的突触障碍，并且突触障碍源自可溶Aβ寡聚体的作用而不是原纤维的作用。Lacor等人，Synaptic targeting by Alzheimer's-related amyloid β oligomers, J. Neurosci. 2004, 24(45):10191-10200。

[0005] Abeta是整合膜蛋白即淀粉样蛋白前体蛋白(APP)的剪切产物，被发现集中于神经元的突触中。Abeta的可溶形式存在于阿尔茨海默患者的脑和组织中，且其存在与疾病进展相关。Yu等人，2009, Structural characterization of a soluble amyloid beta-peptide oligomer, Biochemistry, 48(9):1870-1877。可溶β淀粉样蛋白寡聚体已证实为引起阻断学习和记忆的神经元突触的改变。

[0006] 较小的可溶Aβ寡聚体干扰大量对正常突触可塑性而言关键的信号传导通路，最终导致棘(spine)和突触损失。Selkoe等人，2008, Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior, Behav Brain Res192(1):106-113。阿尔茨海默病作为突触可塑性疾病开始并持续。

[0007] 可溶 A β 寡聚体的存在被认为是前兆阿尔茨海默病脑中的早期认知衰退的原因。已知 β 淀粉样蛋白寡聚体在神经元突触处结合且 σ-2 受体大量存在于神经元和神经胶质。

[0008] 发展 AD 疗法的一个方法涉及 Aβ 单克隆抗体的生成，其中一些处于临床发展的各个阶段，包括巴匹珠单克隆抗体 (bapineuzumab) (AAB-00 ;Janssen, Elan, Pfizer) ；茄尼醇单克隆抗体 (solanezumab) (LY2062430 ;Eli Lilly) ;PF-04360365 (Pfizer) ；MABT5102A (Genentech) ;GSK933776 (GlaxoSmithKline) 和更汀芦单克隆抗体 (gantenerumab) (R1450, RO4909832, Hoffman-LaRoche)。然而，迄今还没有批准静脉内 β 淀粉样蛋白特异性单克隆抗体用于治疗 AD。近来，静脉内巴匹珠单克隆抗体的开发已终止，因为在患有轻度到中度阿尔茨海默病的患者中的两个晚期试验中缺乏效力。然而，近来报道，茄尼醇单克隆抗体在 III 期临床试验结果的二次分析中在患有轻度 AD 的患者而非患有中度 AD 的患者中显示出统计显著的认知衰退的减缓。这种方法的一个问题可能与缺乏充分的脑渗透性有关。

[0009] 目前仅有五种 FDA 批准用于治疗阿尔茨海默病 (AD) 的药物。四种是胆碱酯酶抑制剂：他克林 (**COGNEX®**; Sciele)、多奈哌齐 (**ARICEPT®**; Pfizer)、卡巴拉汀 (**EXELON®**; Novartis) 和加兰他敏 (**RAZADYNE®**; Ortho-McNeil-Janssen)。多奈哌齐、卡巴拉汀和加兰他敏是他克林 (第一代化合物，因其潜在的肝毒性而很少开进药方) 的后继者；它们在 AD 的所有阶段提供认知和功能的症状改进方面大略同等有效。第五个批准的药物是美金刚 (**NAMENDA®**; Forest)，其是低亲和性、用途依赖的 N- 甲基 -D- 天冬氨酸谷氨酸受体拮抗剂，提供相似益处，但仅限于中度到严重 AD。这些化合物的临床效果较小且非永久性的，并且目前没有结论性的数据支持其作为疾病调节剂的用途。参见例如 Kerchner 等人，2010, Bapineuzumab, Expert Opin Biol Ther., 10(7):1121-1130。明显地，需要另外可选的治疗 AD 的方法。

[0010] 本发明部分基于如下宽泛的发现，即满足某些条件的 σ-2 受体拮抗剂抑制可溶 A β 寡聚体的有害作用。在一些实施方式中，使用 σ-2 受体拮抗剂化合物和组合物来治疗或预防受试者中的突触功能障碍。

发明内容

[0011] 本发明部分基于以下宽泛的发现，即 σ-2 受体拮抗剂，优选为还表现出特定治疗表型的其它方面的拮抗剂，如下所述参与抑制并且抑制可溶 β 淀粉样蛋白 (“Abeta”、“A β”) 肽和寡聚体及其其它可溶物对神经元细胞的作用，因此可以用于治疗与 Abeta 寡聚体诱导的病理例如阿尔茨海默病相关的包括疾病和障碍的病症。可溶 Abeta 寡聚体表现为与特异性受体结合并干扰对正常突触可塑性而言关键的信号传导通路的可逆药理学配体，最终导致棘和突触损失。已发现，与 σ-2 受体结合且表现为功能性神经元拮抗剂的化合物表现出与 Abeta 寡聚体的药理学竞争。因此，本文所述的 σ-2 拮抗剂化合物可以降低或防止 Abeta 寡聚体作用例如 Abeta 诱导的细胞毒性。本发明排除某些现有技术化合物，这些化合物不知是 σ-2 受体拮抗剂且要么 (i) 已知与 σ-2 受体结合并降低或消除 Abeta 诱导的病理例如神经元细胞中膜运输的缺陷或突触减少；要么 (ii) 已知针对阿尔茨海默

病的症状有活性而没有提示 σ-2 受体相互作用。本发明还包括用于抑制 Abeta 寡聚体或其它可溶 Abeta 种类对神经元细胞的作用、更广泛而言 β 淀粉样蛋白病理的方法，包括使细胞与根据本发明的 σ-2 拮抗剂接触。在一些实施方式中，提供方法以用于治疗早期阶段的阿尔茨海默病，包括施用治疗有效量的 σ-2 功能性拮抗剂。

[0012] 在一些实施方式中，本发明的 σ-2 拮抗剂与 σ-2 受体结合并且抑制 Aβ 寡聚体与神经元特别是突触的结合。在一些实施方式中，σ-2 拮抗剂与结合神经元特别是突触的 Aβ 寡聚体竞争，或者破坏 Aβ 寡聚体结合神经元的能力，例如通过干扰 Aβ 寡聚体形成或结合至 Aβ 寡聚体或可能地伴随其与神经元的结合干扰 Aβ 寡聚体介入运动信号传导机制的能力。在某些实施方式中，σ-2 拮抗剂由此抑制非致死 Aβ 病理作用（“非致死 Aβ 病理”或“非致死 β 淀粉样蛋白病理”），除其它之外包括膜运输缺陷、突触功能障碍、动物中的记忆和学习缺陷、突触数目的减少、树突棘长度或棘形态的改变、或长时程增强效应 (LTP) 缺陷。换句话说，本发明者观察到，在本文说明的其它检定中具有活性的本发明的 σ-2 拮抗剂具有将神经元恢复到正常状态或干扰 Aβ 寡聚体诱导的突触功能障碍的能力。不拘泥于理论，本发明的 σ-2 拮抗剂干扰 Aβ 寡聚体结构、Aβ 寡聚体与神经元的结合或 Aβ 寡聚体诱导的分子信号传导机制中的一种或多种，这可用于对抗 Aβ 寡聚体的非致死作用以及治疗早期阶段的可溶 Aβ 寡聚体相关的病理。

[0013] 在一个实施方式中，本发明的 σ-2 拮抗剂是功能性神经元拮抗剂并可以用在抑制神经元细胞中的突触损失的方法中，该丧失与细胞暴露于一种或多种 Abeta 寡聚体或其它 Abeta 复合物，或更广泛而言，包括单体或寡聚或可溶复合形式的 Abeta 肽（如下所述）的 Abeta 种类相关，该方法包括使所述细胞与一定量的一种或多种 σ-2 拮抗剂接触，该一定量可有效避免或减少所述丧失或将所述细胞中的突触数目部分或完全恢复到暴露前的水平。

[0014] 在另一实施方式中，将本发明的 σ-2 拮抗剂用于调节神经元细胞中膜运输变化的方法，所述变化与所述细胞暴露于一种或多种 Abeta 种类相关，该方法包括使所述细胞与一定量的一种或多种 σ-2 拮抗剂接触，该一定量可有效避免或减少所述膜运输变化，或使其保持在或接近将所述细胞暴露于所述 Abeta 种类之前观察到的水平。

[0015] 在另一实施方式中，将本发明的 σ-2 拮抗剂用于治疗认知衰退的方法，包括对受试者施用一种或多种本发明的 σ-2 拮抗剂。

[0016] 在又一实施方式中，本发明的 σ-2 拮抗剂是功能性神经元 σ-2 拮抗剂，其被用于治疗认知衰退或神经退行性病症或突触功能和 / 或数目缺陷的方法，该方法包括对受试者施用一种或多种本发明的 σ-2 拮抗剂。

[0017] 本发明还提供一种用于筛选抑制认知衰退或治疗神经退行性疾病的化合物的方法，该方法包括基于相对于其他非 σ 类 CNS 受体而偏好与 σ-2 受体结合的能力来选择一种或多种测试用化合物。该 σ-2 拮抗剂可以或者可以不与 σ-1 受体结合。

[0018] 在一些实施方式中，本公开内容提供用于抑制神经元细胞的 β 淀粉样蛋白寡聚体诱导的突触功能障碍，以及用于抑制由神经元暴露于 Abeta 寡聚体所引起的海马长时程增强效应的压制的包括 σ-2 受体拮抗剂的组合物和方法。

[0019] 本发明提供一种对抑制认知衰退或治疗神经退行性疾病的化合物进行识别的方法，该方法包括

[0020] 使细胞与结合 σ-2 受体的化合物接触，并确定所述化合物是否具有至少一种以下附加特性：

[0021] (a) 其抑制中枢神经元中的突触损失，所述丧失与神经元暴露于 Abeta 寡聚体相关；

[0022] (b) 其抑制中枢神经元中的膜运输异常，该异常与所述细胞暴露于一种或多种 Abeta 寡聚体相关；

[0023] (c) 其抑制阿尔茨海默病动物模型中 Abeta 寡聚体介导的认知效应；或

[0024] (d) 其抑制阿尔茨海默病动物模型中基于海马的空间学习和记忆衰退。

[0025] 在一些实施方式中，公开体外检定平台法，其可预测用于筛选选择性 σ-2 拮抗剂化合物的抑制认知衰退或治疗神经退行性疾病的能力的行为效力，该方法包括使细胞与结合 σ-2 受体并用作为 σ-2 受体拮抗剂的化合物接触，其中所述化合物具有各个以下特性：

[0026] (a) 其抑制中枢神经元中的突触损失，所述丧失与神经元暴露于 Abeta 寡聚体相关；

[0027] (b) 其抑制中枢神经元中的膜运输异常，该异常与所述细胞暴露于一种或多种 Abeta 寡聚体相关；以及

[0028] (c) 其在不存在 Abeta 寡聚体的情况下不影响运输或突触数目。

[0029] 本发明还提供一种对抑制认知衰退或治疗神经退行性疾病的化合物进行识别的方法。在一些实施方式中，该方法包括使细胞与结合 σ-2 受体的化合物接触。在一些实施方式中，该方法还包括识别结合 σ-2 受体的其他化合物。在一些实施方式中，对结合 σ-2 受体的化合物进行识别的方法包括竞争性结合检定，其中测试化合物与 σ-2 受体在已知 σ-2 配体的存在下接触，其中竞争性地抑制已知配体的结合的测试化合物被识别为 σ-2 受体配体。这些方法可以使用动物模型进行，动物模型可以是任何动物模型但优选为啮齿动物模型。任何合适的结合检定可以用于确定化合物是否与 σ-2 受体结合（或者该化合物已经被确定或甚至已知这样做）。

附图说明

[0030] 图 1A 是示出膜运输检定中在体外保持 21 天的具有含有甲臘的胞内囊泡的初级海马和皮质培养物的显微照片，其中甲臘得自货物 (cargo) 四唑盐染料的胞吞以及化学还原。

[0031] 图 1B 是示出具有胞外甲臘结晶的姐妹培养物的显微照片，其中胞外甲臘结晶在甲臘胞吐时在神经元和胶质细胞的细胞膜的外部形成，且其中细胞已经在膜运输检定中暴露于 Abeta 寡聚体。该图示出人 Abeta1 ～ 42 寡聚体改变货物染料产物甲臘的表型（胞内囊泡相对于胞外结晶），因此引起细胞膜运输缺陷。

[0032] 图 1C 是示出胞内囊泡的显微照片，其中细胞已经同时暴露于 Abeta 寡聚体和化合物 II，化合物 II 为本发明的选择性的、高亲和性 σ-2 拮抗剂化合物。该图示出化合物 II 能够阻断由 Abeta 寡聚体生成的膜运输缺陷，并将膜运输表型恢复至正常。

[0033] 图 1D 示出膜运输检定的定量，其中 y 轴表示在施用货物四唑盐染料后在给定时间点包含在胞内囊泡中的甲臜产物的标准化为媒介物处理值的量。红色圆圈表示 Abeta 寡聚体处理的培养物，蓝色方块表示媒介物处理的对照培养物，且黑色或灰色方块表示用多种浓度的 cpdII+Abeta 和 cpd IXa、IXb+Abeta 处理的培养物的值（当化合物在 Abeta 寡聚体之前添加时（预防））。在横坐标中使用化合物的对数浓度。该图示出，化合物以剂量依赖的方式抑制 Abeta 寡聚体对膜运输的作用。

[0034] 图 1E 示出膜运输检定剂量响应曲线，其为与图 1D 相同类型的图中但化合物在 Abeta 寡聚体之后添加（治疗）。在横坐标中使用化合物的对数浓度。该图示出，化合物以剂量依赖的方式抑制 Abeta 寡聚体对膜运输的作用。

[0035] 图 1F 示出在多种浓度的单独的合成 Abeta 寡聚体 (EC50820nM) 的存在下的如图 1D 的相同类型的图的膜运输检定，其具有多种浓度的化合物 II 和各个浓度处的所得囊泡（%媒介物）。通过递增浓度的化合物 II 的存在，表现出 EC50 的右移 (Schild 斜率 = -0.75)。该图表明，cpdII 在药理学上与寡聚体竞争对介导膜运输的分子靶标的接近，因此化合物 II 的存在使得合成 Abeta 寡聚体具有更小的突触毒性。

[0036] 图 1G 示出在多种浓度的单独的合成 Abeta 寡聚体的存在下的如图 1D 的相同类型的图的膜运输检定，其具有多种浓度的化合物混合物 IXa、IXb 和各个浓度处的所得囊泡（%媒介物）。通过递增浓度的化合物混合物 IXa、IXb 的存在，表现出 EC50 的右移 (Schild 斜率 = -0.51)。该图表明，cpd 混合物 IXa、IXb 在药理学上与寡聚体竞争对介导膜运输的分子靶标的接近，因此化合物混合物 IXa、IXb 的存在使得合成 Abeta 寡聚体具有更小的突触毒性。

[0037] 图 1H 示出在多种浓度的单独的得自人阿尔茨海默病患者的 Abeta 寡聚体的存在下的如图 1D 的相同类型的图的膜运输检定，其具有多种浓度的化合物 II 和各个浓度处的所得囊泡（%媒介物）。通过递增浓度的化合物 II 的存在，表现出 EC50 的右移。该图表明，cpd II 在药理学上与寡聚体竞争对介导膜运输的分子靶标的接近，因此化合物 II 的存在使得人阿尔茨海默病相关的 Abeta 寡聚体具有更小的突触毒性。

[0038] 图 1I 示出在多种浓度的单独的得自人阿尔茨海默病患者的 Abeta 寡聚体的存在下的如图 1D 的相同类型的图的膜运输检定，其具有多种浓度的化合物混合物 IXa、IXb 和各个浓度处的所得囊泡（%媒介物）。通过递增浓度的化合物混合物 IXa、IXb 的存在，表现出 EC50 的右移。该图表明，cpd 混合物 IXa、IXb 在药理学上与寡聚体竞争对介导膜运输的分子靶标的接近，因此化合物混合物 IXa、IXb 的存在使得人阿尔茨海默病相关的 Abeta 寡聚体具有更小的突触毒性。

[0039] 图 1J 示出在多种浓度的单独的合成 Abeta 寡聚体的存在下的如图 1D 的相同类型的图的膜运输检定，其具有多种浓度的化合物 CF 和各个浓度处的所得囊泡（%媒介物）。通过递增浓度的化合物 CF 的存在，表现出 EC50 的右移。该图表明，cpd CF 在药理学上与寡聚体竞争对介导膜运输的分子靶标的接近，因此化合物 CF 的存在使得合成 Abeta 寡聚体具有更小的突触毒性。

[0040] 图 1K 示出在多种浓度的单独的合成 Abeta 寡聚体的存在下的如图 1D 的相同类型的图的膜运输检定，其具有多种浓度的化合物 W 和各个浓度处的所得囊泡（%媒介物）。通过递增浓度的化合物 W 的存在，表现出 EC50 的右移。该图表明，cpd W 在药理学上与寡聚体

竞争对介导膜运输的分子靶标的接近，因此化合物 W 的存在使得合成 Abeta 寡聚体具有更小的突触毒性。

[0041] 图 1L 示出使用从阿尔茨海默病患者中分离的 Abeta 寡聚体的膜运输检定结果。化合物 CF(20 微摩尔浓度）表现出与从 AD 患者分离的 Abeta 寡聚体在药理学上竞争对介导膜运输的分子靶标的接近，因此化合物 CF 的存在使得人阿尔茨海默病相关的 Abeta 寡聚体具有更小的突触毒性。

[0042] 图 1M 是具有所识别（并定量）的神经元的甲腈填充囊泡的百分比的运输检定结果的条形图，在 (i) 单独的媒介物（第一条）；(ii) 来自人阿尔茨海默病患者脑的 Abeta 寡聚体制剂（第二条，与第一条相比显著降低）；(ii) 本文公开的化合物 II+Abeta 寡聚体（第三条，比第二条显著更高）；以及 (iv) 化合物 II 而无 Abeta 寡聚体（第四条，与第一条没有显著差异）的存在下。该图表明，化合物 II 阻断由人阿尔茨海默病相关 Abeta 寡聚体生成的膜运输缺陷，并将膜运输表型恢复至正常，但是在不存在 Abeta 寡聚体而以其自身给药时不影响膜运输。

[0043] 图 1N 是与图 J 类型相同但是示出使用从年龄匹配的组织学正常的人脑中分离的 Abeta 寡聚体制剂产生的数据的条形图。该图表明，得自正常人脑的 Abeta 寡聚体不显著影响膜运输，且 cpd II 在存在或不存在这样的寡聚体时不会进一步影响膜运输。

[0044] 图 2A 是药代动力学数据的图，其中示出在单一皮下（空心三角）和静脉 (i.v.)（空心圆圈）施用化合物 II 时血浆中（左纵坐标，ng/mL) 中得到的以及在单一 i.v. 施用化合物 II（实心圆圈）时脑中（右纵坐标，ng/g) 得到的化合物 II 的浓度。已知化合物 II 经受首过代谢，因此皮下给药；然而，在急性给药后，化合物 II 具有高度脑渗透性。该图表明，cpd II 在急性皮下给药时具有高度脑渗透性。

[0045] 图 2B 是药代动力学数据的图，其中示出在每天一次皮下施用不同量的化合物 II(0.5mg/kg/ 天：实心下三角；0.35mg/kg/ 天：实心上三角；以及 0.1mg/ 天：实心方块）并持续 5 天时血浆中（左纵坐标）中得到的以及在皮下施用相同量（分别为空心下三角、空心上三角和空心方块）的化合物 II 时脑中（右纵坐标）得到的化合物 II 的浓度。已知化合物 II 经受首过代谢，因此皮下给药；然而，在慢性给药后，化合物 II 具有高度脑渗透性。该图表明，cpd II 在慢性皮下给药时具有高度脑渗透性。

[0046] 图 2C 是药代动力学数据的图，其中示出在单一急性口服化合物 CB(10mg/kg/ 天）时在血浆（左纵坐标，实心三角）和脑（右纵坐标，空心三角）中得到的在单一急性口服给药后获得的化合物 CB 的浓度。化合物 CB 在急性口服给药后具有高度脑渗透性并表现出血浆半衰期为 3.5 小时的 50％生物利用度。该图表明，cpd CB 在急性口服给药时具有高度脑渗透性。

[0047] 图 2D 是药代动力学数据的图，其中示出在每天一次口服施用化合物 CB(10mg/kg/ 天，上三角，或 30mg/kg/ 天，倒三角）时在血浆（左纵坐标，实心三角）和脑（右纵坐标，空心三角）中得到的在慢性每天一次口服给药并持续 5 天之后获得的化合物 CB 的浓度。化合物 CB 在慢性口服给药后具有高度脑渗透性并在高达 5 天的每天一次口服给药时表现出 3 的脑 / 血浆比率。该图表明，cpd CB 在慢性口服给药时具有高度脑渗透性。

[0048] 图 3A- 板块 A 是在不存在化合物 IXa、IXb 的情况下暴露于 Abeta 寡聚体的体外保持 21 天的初级海马和皮质培养物的荧光显微图；Abeta（用单克隆抗体 6E10 免疫标记而可

视化）与包括神经元突触后棘的细胞膜在突触处结合。

[0049] 图 3A- 板块 B 是图 3A- 板块 A 中所见的相同视野，示出与阴性对照（未示出）相比，突触数目（用突触素免疫标记而可视化）在 Abeta 寡聚体的存在下减少。

[0050] 图 3A- 板块 C 是在不存在化合物 IXa、IXb 的情况下暴露于 Abeta 寡聚体的体外保持 21 天的初级海马和皮质培养物的较低分辨率荧光显微图；Abeta（用单克隆抗体 6E10 免疫标记而可视化）与包括神经元突触后棘的细胞膜在突触处结合。

[0051] 图 3A- 板块 D 示出在存在化合物 IXa、IXb 的情况下暴露于 Abeta 寡聚体的体外保持 21 天的初级海马和皮质培养物的姐妹培养物；与包括神经元突触后棘的细胞膜结合的 Abeta 的量在视觉上减少。

[0052] 图 3B- 板块 A 是在存在化合物 IXa、IXb 的情况下暴露于 Abeta 寡聚体的体外保持 21 天的初级海马和皮质培养物的姐妹培养物的荧光显微图；与包括神经元突触后棘的细胞膜结合的 Abeta 的量在视觉上减少。该图表明，化合物 IXa、IXb 的存在 (i) 显著地减少与包括神经元突触后棘的细胞膜结合的 Abeta 寡聚体的量。在化合物 II 的存在下观察到相似的保护（数据未示出）。

[0053] 图 3B- 板块 B 是如图 3A- 板块 C 中见到的相同视野，示出在化合物 IXa、IXb 的存在下，突触数目（用突触素免疫标记而可视化）恢复，与图 3A- 板块 B 相比，具有增加的可视突触素。该图表明，化合物混合物 IXa、IXb 显著地阻断 Abeta 寡聚体诱导的突触损失。在化合物 II 的存在下观察到相似的保护（数据未示出）。

[0054] 图 3C 是以突触损失检定实验的条形图对示于图 3A- 板块 A ～ D 中的数据的定量。突触损失提供与认知功能的最紧密相关。在突触损失检定中，相对于媒介物，Abeta 寡聚体在体外引起 18.2% 突触损失。化合物 II 或化合物混合物 IXa、IXb 的存在完全消除该突触消退。当化合物以单独的媒介物而不具有 Abeta 寡聚体给药时，没有观察到作用。具体而言，通过对荧光显微图的突触素免疫标记区的数目、强度和面积进行基于图像处理的定量来计算突触计数，表达为暴露于单独的媒介物（黑色第一条）、媒介物与化合物 IXa、IXb 或媒介物与化合物 II（分别为第二条和第三条，示出化合物对突触数目没有作用）、Abeta 寡聚体（第四条，示出与第一条相比显著的突触计数降低）以及在化合物 IXa、IXb 或 II 存在下的 Abeta 寡聚体（第五和第六条，示出与第一条相比没有突触数目的降低）的神经元中的阴性对照（媒介物）的百分比。该图表明，化合物 IXa、IXb 和 II 表现出保护性作用并阻断 Abeta 寡聚体诱导的突触数目减少。

[0055] 图 3D 是以 Abeta 结合强度的条形图对示于图 3A- 板块 A ～ D 中的数据的定量，Abeta 结合强度通过对在将 Abeta 单独添加至媒介物（第一条图）时的荧光显微图的 6E10 免疫标记区的数目、强度和面积以及在 Abeta 与化合物 II 或化合物混合物 IXa、IXb 的共存下它们的显著降低进行基于图像处理的定量来计算。该图表明，化合物 IXa、IXb 和 II 降低与细胞膜结合的 Abeta 的量。

[0056] 图 4 是记忆表现的条形图，记忆表现通过体内条件性恐惧检定中僵立行为的百分比而测量，僵立行为的百分比在基线训练和训练后 24 小时对施用单独的媒介物（第一条）、媒介物 +Abeta 寡聚体（第二条）、化合物 II+Abeta 寡聚体（第三条）和单独的化合物 II（第四条）的小鼠进行测量并且在施用单独的媒介物（第一条）、媒介物 +Abeta 寡聚体（第二个显著降低的条）、化合物 II+Abeta 寡聚体（第三条）和单独的化合物 II 之后 24 小时时进

行测量。与媒介物 (N = 18) 相比,Abeta 寡聚体 (单一的 200 纳摩尔海马内注射) 在 3 ～ 4 个月大的雄性 wt C57BL/6 小鼠 (N = 16) 中产生显著的记忆形成缺陷。化合物 II(在寡聚体之前一小时单一的 2 微摩尔海马内注射) 消除由 Abeta 寡聚体生成的记忆缺陷 (N = 11)。单独的化合物没有作用,且没有观察到不利的行为效应。该图表明,化合物 II 可以预防 Abeta 寡聚体诱导的记忆缺陷,而当其自身给药时对记忆表现没有作用。

[0057] 图 5A 是 σ -2 结合亲和性 (表 2) 与运输检定中的效力 (表 5) 之间的相关性的图。仅包括在运输检定中有活性的化合物 ;排除也是 σ -1 拮抗剂的化合物。

[0058] 图 5B 是对于用来产生图 5A 的化合物的 σ -2 结合亲和性 (表 2) 与运输检定中的效力 (表 5) 之间的同一相关性的图,但额外包括同时为 σ -2 配体和 σ -1 拮抗剂的化合物的数据点 (这些离群数据点集中在图的右下象限,且没有被用来计算相关系数)。

[0059] 图 5C 是示出不存在 σ -1 结合亲和性 (表 2) 与运输检定中的 EC_{50}(表 5) 之间的相关性的图, $r^2 = 0.06$, $p>0.05$。

[0060] 图 5D 是示出不存在 σ -2 结合亲和性 (表 2) 与运输检定中的 Abeta 的最大抑制 (表 5) 之间的相关性的图。分析中包括所有数据点。

[0061] 图 6 是与图 4 相同类型的条形图,示出当动物用 (i) 单独的媒介物 (第一条)、(ii) Abeta 寡聚体 (第二条,示出测试动物获取新记忆的能力显著降低)、(iii) 化合物 IXa 和 IXb 的混合物 (第三条,示出对 Abeta 寡聚体诱导的记忆缺陷的完全 (且统计学显著的) 抑制)、或 (iv) 化合物 IXa 和 IXb 的混合物 (不存在 Abeta 寡聚体)(第四条,示出对记忆无作用) 处理时,通过与产生图 4 相同的情境条件性恐惧检定中的僵立行为而测量的记忆表现。没有观察到不利的行为作用。该图表明,化合物混合物 IXa、IXb 可以预防 Abeta 寡聚体诱导的记忆缺陷,而当以其自身给药时对记忆表现没有作用。

[0062] 图 7A 示出在预防模式中使用的在初级海马和皮质培养物中进行的膜运输检定,其中在寡聚体前加入化合物 II,具有或不具有苏 - 艾芬地尔 (TIF)。苏 - 艾芬地尔 (TIF) 是对其他受体具有亲和性 (s20.9nM, s159nM, NR2B222nM, K+ch88nM 等) 的 σ -2 受体配体 (Monassier 等人, JPET,322(1):341-350,2007),当单独给药时其不引起细胞凋亡、影响运输或干扰 Abeta 寡聚体诱导的运输缺陷 (数据未示出),因而高亲和性 σ 受体结合不足以产生治疗表型。该图表明,TIF 在神经元中在预防形式中表现出与 II(以及 IXa、IXb ;未示出) 的药理学竞争,表明它们在 σ 受体上的结合位点部分重叠。这是神经元中 σ 配体的功能性竞争的第一个证明,表明 σ 受体参与 Abeta 寡聚体诱导的膜运输过程。

[0063] 图 7B 示出在治疗模式中使用的在初级海马和皮质培养物中进行的膜运输检定,其中在寡聚体后加入化合物 II,具有或不具有苏 - 艾芬地尔 (TIF)。该图表明,苏 - 艾芬地尔 (TIF) 在神经元中在治疗形式中表现出与化合物 II(以及 IXa、IXb ;未示出) 的药理学竞争,表明它们在 σ 受体上的结合位点部分重叠。

[0064] 图 7C 示出在治疗模式中使用的在初级海马和皮质培养物中进行的膜运输检定。数据示出在存在媒介物 (空心方块) 和 Abeta 寡聚体 (空心圆圈) 时包含在胞内囊泡中的甲腈的量。Abeta 寡聚体对膜运输的作用通过化合物 CF(实心方块) 的存在以剂量依赖的方式减轻。TIF 的添加显著地降低由化合物 CF 引起的最大抑制,并使 EC50 值右移 ;因此 TIF 在治疗形式中 (在 Abeta 后添加化合物) 用作为化合物 CF 结合的同一受体的拮抗剂。

[0065] 图 7D 示出在存在媒介物 (空心方块) 和 Abeta 寡聚体 (空心圆圈) 时的膜运输

数据。Abeta 作用通过化合物 II（实心方块）的存在以剂量依赖的方式减轻。TIF 的添加显著地降低由化合物 II 引起的最大抑制，并使 EC50 值移动。因此 TIF 在治疗形式中表现出与化合物 II 的药理学竞争。

[0066] 图 8A 示出来自正常患者、路易体痴呆 (DLB) 患者或阿尔茨海默病 (AD) 患者的人额皮质组织切片中（左板块）的 [^{3}H]-(+)-戊唑辛（σ-1 受体配体）的放射自显影结合，其中 BS 是特异性结合，且 BNS 是非特异性结合；（右板块）示出来自对照（正常）、DLB 或 AD 患者的放射自显影实验的 [^{3}H] 戊唑辛的平均特异性结合图。与年龄匹配的与 AD 中所见的神经元损失度平行的对照脑相比，阿尔茨海默病脑中的 σ-1 受体在统计学上更低。该图表明，σ-1 受体表达可以在阿尔茨海默病脑中保持恒定。

[0067] 图 8B 示出来自正常患者、路易体痴呆 (DLB) 患者或阿尔茨海默病 (AD) 患者的相邻人额皮质组织切片中（左板块）的 [^{125}I]-RHM-4（σ-2 受体配体）的放射自显影结合；（右板块）示出来自对照（正常）、DLB 或 AD 患者的放射自显影实验的 [^{125}I]-RHM-4 的平均特异性结合图。与年龄匹配的对照脑相比，阿尔茨海默病和路易体痴呆脑中的 σ-2 受体在统计学上没有更低，尽管在这些疾病中看到神经元损失。该图表明，存活的神经元和/或胶质细胞上的 σ-2 受体表达在 DLB 和阿尔茨海默病脑中可能上调。

[0068] 图 8C 示出（左板块）在猴额皮质、猴海马或人颞皮质中 σ-2 配体对 18.4nM [^{3}H]-RHM-1 的替代，以及（右板块）具有和不具有 1μM 西拉美新与化合物 IXa、IXb 和 II 中的每一个的 [^{3}H]-RHM-1 的结合密度图。该图表明，化合物 II 和混合物 IXa、IXb 在猴和人脑组织切片中从 σ-2 受体竞争性地替代已知的放射性标记的 σ-2 配体例如 [^{3}H]-RHM-1，因此两些化合物均与 σ-2 受体结合。

[0069] 图 9A 示出在用 σ 化合物处理 48 小时的 SKOV-3 人卵巢癌细胞系中，作为 MTS 检定中细胞活力的 σ-2 受体激动剂的肿瘤细胞细胞毒性。σ-2 激动剂（西拉美辛、SV-119、WC-26）杀死肿瘤细胞。σ-2 拮抗剂 (RHM-1、IXa、IXb 和 II) 仅在不存在激动剂时在高得多的浓度下杀死肿瘤细胞。该图表明，cpd II 和 IXa、IXb 在该检定中表现得与已知 σ-2 拮抗剂相似，因此表明它们是肿瘤细胞中的 σ-2 拮抗剂。

[0070] 图 9B 示出在用 σ-2 化合物处理 24 小时的神经元培养物中作为细胞核强度变化的 σ-2 受体激动剂的神经元细胞细胞毒性。σ-2 激动剂（西拉美辛、SV-119、WC-26）引起神经元中的异常细胞核形态。σ-2 拮抗剂 (RHM-1、IXa、IXb 和 II) 不会如此。该图表明，cpd II 和 IXa、IXb 在该检定中表现得与已知 σ-2 拮抗剂相似，因此表明它们是初级海马和皮质细胞中的 σ-2 拮抗剂。

[0071] 图 10A 示出 SKOV-3 人卵巢癌细胞中由 σ-2 激动剂西拉美新诱导的 caspase-3 活性，而 σ-2 受体拮抗剂 RHM-1、化合物 II 和 IXa、IXb 不诱导 caspase-3 活性。Abeta 寡聚体引起低水平的 caspase-3 活化并引起 LTD。高水平的寡聚体和 caspase-3 引起细胞死亡。σ-2 受体激动剂 (SV-119、西拉美新）使肿瘤细胞和神经元中的 caspase-3 活化；σ-2 拮抗剂则不（图 10A 和 10B）。该图表明，cpd II 和 IXa、IXb 在该检定中表现得与已知 σ-2 拮抗剂相似，因此表明它们是肿瘤细胞中的 σ-2 拮抗剂。

[0072] 图 10B 示出神经元中由 σ-2 激动剂西拉美新诱导的 caspase-3 活性，而 σ-2 受体拮抗剂 RHM-1、化合物 II 和 IXa、IXb 不诱导 caspase-3 活性。该图表明，cpd II 和 IXa、IXb 在该检定中表现得与已知 σ-2 拮抗剂相似，因此表明它们是初级海马和皮质细胞中的

σ-2 拮抗剂。

[0073] 图 10C 示出 SKOV-3 人卵巢癌细胞中由 σ-2 激动剂 SV-119 诱导的 caspase-3 活化。σ-2 受体拮抗剂化合物 IXa、IXb 和 II、RHM-1 不阻断肿瘤细胞中由 σ-2 受体激动剂 SV-119 引起的 caspase-3 活化。该图表明，cpd II 和 IXa、IXb 在该检定中表现得与已知 σ-2 拮抗剂相似，因此表明它们是肿瘤细胞中的 σ-2 拮抗剂。

[0074] 图 10D 示出在多种浓度的激动剂 24 小时后神经元培养中由 σ-2 受体激动剂 SV-119 引起的 caspase-3 活化。该图表明，σ-2 受体拮抗剂化合物 IXa、IXb 和 II 而非 RHM-1 阻断初级海马和皮质细胞中由 σ-2 受体激动剂 SV-119 引起的 caspase-3 活化。

[0075] 图 11A 示出运输检定以及由 Abeta 寡聚体的存在而引起的运输缺陷（与媒介物相比，囊泡减少）。σ-2 激动剂西拉美新的添加在低浓度阻断 Abeta 寡聚体运输缺陷，但是在高浓度引起细胞毒性。σ-2 拮抗剂 II 在所有的测试浓度阻断寡聚体诱导的运输缺陷。该图表明，化合物 II 表现出治疗表型，并且表现得与已知 σ-2 激动剂不相似。

[0076] 图 11B 示出运输检定以及由 Abeta 寡聚体的存在而引起的运输缺陷（与媒介物相比，囊泡减少）。σ-2 激动剂 SV119 的添加在低浓度阻断 Abeta 寡聚体运输缺陷，但是在高浓度引起细胞毒性。σ-2 拮抗剂 RHM-1 在所有的测试浓度阻断寡聚体诱导的运输缺陷，但是不表现出治疗表型，因为其结构不是药物样。该图表明，化合物 II 表现得与已知 σ-2 拮抗剂相似。

[0077] 图 12A 示出通过体内条件性恐惧检定中的僵立行为的百分比而测量的记忆表现，其中僵立行为的百分比在口服施用各种剂量的 σ-2 受体拮抗剂化合物 5.5 个月后，在 15 个月大的雄性转基因阿尔茨海默病小鼠模型中，在以 1 ～ 3 分钟训练 24 小时后测量。与用媒介物处理的 Tg 动物相比 (Mann-Whitney U 检测)，在用 10 和 30mg/kg/ 天的 CB($p<0.05$) 和 30mg/kg/ 天的 CF($p<0.005$) 处理的转基因动物中发生记忆缺陷的显著改善。该图表明，化合物 CB 和 CF 在转基因阿尔茨海默病小鼠中在慢性长期给药后逆转已建立的记忆缺陷。

[0078] 图 12B 示出 9 个月大的雌性转基因 (Tg) 阿尔茨海默病小鼠的行为数据的条形图，该小鼠在用媒介物 p.o. 处理 39 天时，相对于媒介物处理的非转基因同窝仔，在 Y- 迷宫（% 交替）中表现出显著的记忆缺陷（即，媒介物处理的 Tg 小鼠偶尔表现，媒介物处理的非 Tg 同窝仔表现得显著好于紧挨着各个条的机率可见 (chance-see) 的星号和线）。用化合物 CF 以 30mg/kg/ 天口服处理 Tg 动物改善缺陷。没有观察到不利的行为效应。该图表明化合物 CF 在转基因阿尔茨海默病小鼠中在慢性短期给药后逆转已建立的记忆缺陷。

[0079] 图 13A 示出用 6E10Abeta 特异性抗体免疫标记而可视化的 Abeta 寡聚体与初级神经元培养物 21DIV 的结合的荧光显微图。

[0080] 图 13B 示出与 13A 相同的视野，其中神经元经神经元特异性 MAP2 免疫标记而选择性地可视化。

[0081] 图 13C 示出 Abeta 寡聚体（用 6E10 免疫标记而可视化）与用 78nMPGRMC1C 端抗体 21DIV 预处理的姐妹初级神经元培养物的结合的荧光显微图。该图表明，PGRMC C 端抗体的存在引起显著降低的 Abeta 寡聚体结合，比仅 Abeta 处理的对照培养物低 47%。

[0082] 图 13D 示出与 13C 相同的视野，其中神经元经神经元特异性 MAP2 免疫标记而选择性地可视化。如同在姐妹对照培养物中所见（图 13A），在培养物中存在相似密度的神经元。

[0083] 图 13E 示出 Abeta 寡聚体（用 6E10 免疫标记而可视化）与用 78nM 对照抗体（非

免疫 IgG) 预处理的姐妹初级神经元培养物的结合的荧光显微图。该图表明，与仅用 Abeta 处理的对照培养物（图 13A) 相比，非免疫 IgG 的存在不显著改变 Abeta 结合强度。

[0084] 图 13F 示出与 13E 相同的视野，其中神经元经神经元特异性 MAP2 免疫标记而选择性地可视化。如同在姐妹对照培养物中所见（图 13A)，在培养物中存在相似密度的神经元。

[0085] 图 13G 示出 Abeta 寡聚体（用 6E10 免疫标记而可视化）与用 78nMPGRMC1N 端抗体预处理的姐妹初级神经元培养物的结合的荧光显微图。该图表明，与仅用 Abeta 处理的对照培养物（图 13A) 相比，78nM PGRMC1N 端抗体的存在不显著改变 Abeta 结合强度。

[0086] 图 13H 示出与 13G 相同的视野，其中神经元经神经元特异性 MAP2 免疫标记而选择性地可视化。如同在姐妹对照培养物中所见（图 13A)，在培养物中存在相似密度的神经元。化合尺＝ 15 微米。

[0087] 图 14A 以相对于每个神经元的 Abeta 结合强度的条形图示出图 13 中所示数据的定量，其中相对于每个神经元的 Abeta 结合强度通过对荧光显微图的 6E10 免疫标记区的数目、强度和面积进行基于图像处理的定量而进行计算。在不存在抗体的情况下（空心条标记的“对照”)、以及在 C 端和 N 端特异性 PGRMC1 抗体以及非免疫对照 IgG 抗体（分别为标记条）中每一种的三种浓度下，相对于每个神经元的 Abeta 寡聚体结合强度。C 端特异性 PGRMC1 抗体是以剂量依赖的方式显著地降低 Abeta 寡聚体结合的唯一抗体。抗体诱导的相对于每个神经元的结合点数目和结合面积的变化与针对强度所示的那些非常相似。该图与如下解释一致，即这些培养物中存在的较高百分比的 Abeta 寡聚体与 σ-2 受体结合。

[0088] 图 14B 示出在图 13 和 14A 中相同的神经元培养物中的细胞核面积。当用递增浓度的抗体处理时，作为细胞毒性的量度的细胞核面积没有改变。因此，抗体的添加不影响神经元培养物的健康状况。

具体实施方式

[0089] 在详细描述本发明的化合物、组合物和方法之前，应理解本发明不限于所描述的具体操作、组合物或方法论，因为这些可以变化。还应理解，本说明书使用的术语仅出于描述具体的版本 (versions) 或实施方式的目的，而不意在限制本发明的范围，本发明的范围将由所附权利要求限定。除非另外限定，本文使用的所有技术和科学术语具有与本领域普通技术人员通常理解相同的含义。尽管可以将任何与本文描述类似或等同的方法和材料用于本发明实施方式的实施和测试，但现在将描述优选的方法、装置和材料。

[0090] 还应理解的是为清楚起见而描述于单独的实施方式中的本发明的某些特征还可以在单个实施方式中组合提供。相反地，为简洁起见而描述于单个实施方式中的本发明的各种特征还可以单独或以任何合适的亚组合提供。

[0091] 定义

[0092] 单数形式“一”、“一个”和“该”包括复数，除非上下文另外有清楚规定。因此，例如，关于“细胞”是指一个或多个细胞及其本领域技术人员已知的等同物，等等。

[0093] 本文使用的术语“约”是指给定数值加减 10%。例如，“约 50%”是指 45%～ 55% 的范围。

[0094] “σ-2 配体”是指与 σ-2 受体结合的化合物，且包括激动剂、拮抗剂、部分激动剂、反向激动剂和简单地该受体或蛋白的其它配体的竞争者。

[0095] 术语“激动剂”是指其存在引起受体的如下生物学活性的化合物，该生物学活性与由该受体的天然出现的配体的存在所引起的生物学活性相同。

[0096] 术语“部分激动剂”是指其存在引起受体的如下生物学活性的化合物，该生物学活性的类型与由该受体的天然出现的配体的存在所引起的生物学活性相同，但量级更低。

[0097] 术语“拮抗剂”是指其存在引起受体的生物学活性的量级下降的实体，例如化合物、抗体或片段。在某些实施方式中，拮抗剂的存在导致受体的生物学活性的完全抑制。本文所用的术语“σ-2受体拮抗剂”用来描述用作为σ-2受体的“功能性拮抗剂”的化合物，其阻断Abeta作用，例如，Abeta寡聚体诱导的突触功能障碍，例如可见于体外检定中，例如膜运输检定或突触损失检定，或Abeta寡聚体介导的caspase-3的σ-2受体活化，或行为检定中，或有需要的患者中。功能性拮抗剂可以通过抑制例如Abeta寡聚体与σ-2受体的结合而直接作用，或者通过干扰Abeta寡聚体与σ-2受体结合引起的下游信号传导而间接作用。

[0098] 术语“σ-2受体拮抗剂化合物”是指小分子、抗体或其活性结合片段，其以可测量的量与σ-2受体结合并针对由σ-2受体结合引起的Abeta作用寡聚体诱导的突触功能障碍充当功能性拮抗剂。

[0099] 术语“选择性”或“选择性的”是指化合物对σ受体例如σ-2受体相比于对非σ受体的结合亲和性(Ki)差异。σ-2拮抗剂对突触神经元中的σ受体具有高选择性。对σ-2受体的Ki或对σ-2和σ-1受体两者的Ki与对非σ受体的Ki进行比较。在一些实施方式中，选择性的σ-2受体拮抗剂或σ-1受体配体对与σ受体结合相比于与非σ受体结合具有至少10倍、20倍、30倍、50倍、70倍、100倍或500倍或更高的亲和性，通过比较不同受体处的结合离解常数Ki值、或IC_{50}值或结合常数而评定。任何已知的检定方案均可以用来评定不同受体处的Ki或IC_{50}值，例如通过监测具有已知离解常数的放射性化合物从受体的竞争性置换，例如通过Cheng and Prusoff(1973)(Biochem. Pharmacol. 22, 3099-3108)的方法或本文具体提供的方法。在一些实施方式中，σ-2拮抗剂化合物是相比于非σ受体对σ-2受体具有结合特异性的抗体或其活性结合片段。在抗体或片段的情况下，σ-2受体处的结合常数可以通过本领域已知的任何方式进行计算并与非σ受体处的结合常数进行比较，例如通过Beatty等人，1987, J Immunol Meth, 100(1-2):173-179的方法或Chalquest, 1988, J. Clin. Microbiol. 26(12):2561-2563的方法。非σ受体例如选自毒蕈碱M1-M4受体、五羟色胺(5-HT)受体、α肾上腺素受体、β肾上腺素受体、阿片受体、五羟色胺转运体、多巴胺转运体、肾上腺素转运体、多巴胺受体或NMDA受体。

[0100] 在本申请中，术语“高亲和性”意在指在σ受体结合检定中表现出小于600nM、500nM、400nM、300nM、200nM、小于150nM、小于100nM、小于80nM、小于60nM、或优选小于50nM的Ki值的化合物，例如相对于[^{3}H]-DTG，如Weber等人，Proc. Natl. Acad. Sci(USA)83:8784-8788(1986)中公开，其并入本文以供参考，其测量了化合物对于σ-1和σ-2受体位点两者的结合亲和性。特别优选的σ配体相对于[^{3}H]-DTG表现出小于约150nM、优选小于100nM、小于约60nM、小于约10nM、或小于约1nM的Ki值。

[0101] 术语“治疗表型”用来描述化合物在预测行为效力的体外检定中的活性模式。(1)选择性地以高亲和性与σ-2受体结合且(2)在神经元中针对Abeta寡聚体诱导的作用充当功能性拮抗剂的化合物被认为具有“治疗表型”，如果(i)其阻断或减少Aβ诱导的膜运

输缺陷；(ii) 阻断或减少 A β 诱导的突触损失且 (iii) 在不存在 Abeta 寡聚体时不影响运输或突触数目。在体外检定中的活性模式被称为“治疗表型”并可以预测行为效力。

[0102] 术语“治疗分布 (profile)”用来描述满足治疗表型、并具有较好的脑渗透性（跨越血脑屏障的能力）、较好的血浆稳定性和较好的代谢稳定性的化合物。

[0103] 术语“药物样特性”在本文中用来描述 σ-2 受体配体在施用时的药代动力学和稳定性特性，包括脑渗透性、代谢稳定性和 / 或血浆稳定性。

[0104] “Abeta 种类”或“ A β ”包括组合物，该组合物包括含有可溶淀粉样蛋白肽的成分，例如 Abeta 单体、Abeta 寡聚体、或 Abeta 肽（单体、二聚或多聚形式）与其它可溶肽或蛋白的复合物以及其它可溶 Abeta 组装 (assemblies)，包括淀粉样蛋白前体蛋白的任何加工产物。已知可溶 A β 寡聚体具有神经毒性。甚至已知 $A\beta_{1-42}$ 二聚体破坏小鼠海马切片中的突触可塑性。在本领域已知的一个理论中，认为天然 $A\beta_{1-42}$ 单体具有神经保护性，且神经毒性需要 A β 单体自缔合为可溶 Abeta 寡聚体。然而，某些 A β 突变单体（北极型突变 (E22G) 被报道为与家族 AD 相关。参见例如 Giuffrida 等人，β-Amyloid monomers are neuroprotective. J. Neurosci. 200929(34):10582-10587。包括 Abeta 种类的制剂的非限制性实例公开于美国专利申请第 13/021,872 号；美国专利公开第 2010/0240868 号；国际专利申请第 WO/2004/067561 号；国际专利申请第 WO/2010/011947 号；美国专利公开第 20070098721 号；美国专利公开第 20100209346 号；国际专利申请第 WO/2007/005359 号；美国专利公开第 20080044356 号；美国专利公开第 20070218491 号；WO/2007/126473；美国专利公开第 20050074763 号；国际专利申请第 WO/2007/126473 号，国际专利申请第 WO/2009/048631 号，和美国专利公开第 20080044406 号，其各自引入本文以作参考。

[0105] 结合本发明的化合物使用的“施用”是指将化合物直接施用于目标组织中或目标组织上或将化合物全身或局部地施用于患者或其它受试者。

[0106] 本文使用的术语“动物”包括但不限于人和非人脊椎动物，例如野生动物、实验动物、家畜和农畜动物以及宠物。

[0107] 本文使用的术语“受试者”、“个体”和“患者”可互换使用，且是指何动物，包括哺乳动物、小鼠、大鼠、其他啮齿动物、兔、狗、猫、猪、牛、羊、马、灵长类动物、非人灵长类动物、人等。

[0108] 本文使用的术语“使……接触”是指使分子（或具有更高级结构的分子例如细胞或细胞膜）在一起或结合，使得它们处于允许分子间相互作用例如两个肽或一个蛋白与另一蛋白或其它分子之间的非共价相互作用的距离内。在一些实施方式中，接触发生在溶液中，其中所结合或所接触的分子在普通溶剂中混合并使得自由缔合。在一些实施方式中，接触可以发生在细胞处或在细胞内或在无细胞环境中。在一些实施方式中，无细胞环境是由细胞产生的裂解液。在一些实施方式中，细胞裂解液可以是全细胞裂解液、核裂解液、细胞质裂解液及其组合。在一些实施方式中，无细胞裂解液是得自核提取和分离的裂解液，其中将细胞群的核从细胞中去除然后裂解。在一些实施方式中，核不裂解，但仍认为是无细胞环境。可以通过混合例如涡旋、振摇等使分子在一起。

[0109] 术语“改善”被用于表示本发明改变了提供、应用或施用本发明的组织的特性和 / 或物理属性。术语“改善”还可结合疾病状态使用，使得当“改善”疾病状态时，与疾病状态相关的症状或物理特性减弱、减少、消除、延迟或避免。

[0110] 术语“抑制”包括阻断、避免某个结果或过程，或恢复到相反的结果或过程。在预防或治疗方面，通过施用本发明的化合物，“抑制”包括使免受（部分或全部）或延迟症状的发作、缓解症状，或使免受、减弱或消除疾病、病症或障碍。

[0111] 术语“抑制运输缺陷”是指阻断细胞、优选神经元细胞中可溶 Ab 寡聚体诱导的膜运输缺陷的能力。能够抑制运输缺陷的化合物在膜运输检定中的 EC50<20 μM、小于 15 μM、小于 10 μM、小于 5 μM 且优选小于 1 μ M，且还能够至少 50%、优选至少 60%、且更优选至少 70%地最大抑制可溶 Abeta 寡聚体诱导的膜运输缺陷的 Abeta 寡聚体作用，例如如实施例 6 中所述。

[0112] 在本说明书的多个地方，本发明化合物的取代基以组或范围公开。具体意图是，本发明的实施方式包括这样的组和范围的成员的各个和每个各自独立的亚组合。例如，术语“C_{1-6} 烷基”具体意在各自独立地公开例如甲基 (C_1 烷基）、乙基 (C_2 烷基）、C_3 烷基、C_4 烷基、C_5 烷基和 C_6 烷基，以及例如 C_1-C_2 烷基、C_1-C_3 烷基、C_1-C_4 烷基、C_2-C_3 烷基、C_2-C_4 烷基、C_3-C_6 烷基、C_4-C_5 烷基和 C_5-C_6 烷基。

[0113] 对于其中变量出现多于一次的本发明的化合物，各个变量可以是选自限定变量的马库什基团的不同组成部分。例如，当结构被描述为具有两个同时存在于同一化合物上的 R 基团时，则这两个 R 基团可以表示选自针对 R 所限定的马库什基团的不同组成部分。

[0114] 其中 n 是整数的术语“n- 元”通常描述组成部分中成环原子的数目，其中成环原子的数目是 n。例如，吡啶是 6 元杂芳环的实例，而噻吩是 5 元杂芳基的实例。

[0115] 本文使用的术语“烷基”意指直链或支链的饱和烃基。实例烷基包括但不限于甲基 (Me)、乙基 (Et)、丙基（例如正丙基和异丙基）、丁基（例如正丁基、异丁基、叔丁基）、戊基（例如正戊基、异戊基、新戊基）等。烷基可以含有 1 至约 20、2 至约 20、1 至约 10、1 至约 8、1 至约 6、1 至约 4、或 1 至约 3 个碳原子。术语“亚烷基”指二价烷基连接基团。亚烷基的实例是亚甲基 (CH_2)。

[0116] 本文使用的“烯基”是指具有一个或多个碳碳双键的烷基。实例烯基包括但不限于乙烯基、丙烯基、环己烯基等。术语“亚烯基”是指二价连接烯基。

[0117] 本文使用的“炔基”是指具有一个或多个碳碳三键的烷基。实例炔基包括但不限于乙炔基、丙炔基等。术语“亚炔基”是指二价连接炔基。

[0118] 本文使用的“卤代烷基”是指具有一个或更多个卤素取代基的烷基。实例卤代烷基包括但不限于 CF_3、C_2F_5、CHF_2、CCl_3、$CHCl_2$、C_2Cl_5、CH_2CF_3 等。

[0119] 本文使用的“芳基”是指单环或多环（例如具有 2、3 或 4 个稠环）的芳族烃，例如苯基、萘基、蒽基、菲基、二氢茚基、茚基等。在一些实施方式中，芳基具有 6 至约 20 个碳原子。在一些实施方式中，芳基具有 6 至约 10 个碳原子。

[0120] 本文使用的“环烷基”是指非芳族环烃，包括环化的烷基、烯基和炔基，其含有高达 20 个成环碳原子。环烷基可以包括单环或多环（例如具有 2、3 或 4 个稠环）的环体系以及螺环体系。环烷基可以含有 3 至约 15、3 至约 10、3 至约 8、3 至约 6、4 至约 6、3 至约 5 或 5 至约 6 个成环碳原子。环烷基的成环碳原子可以任选地被氧 (oxo) 或硫 (sulfido) 取代。环烷基的实例包括但不限于环丙基、环丁基、环戊基、环己基、环庚基、环戊烯基、环己烯基、环己二烯基、环庚三烯基、降冰片基、降蒎基 (norpinyl)、降莰基 (norcarnyl)、金刚烷基等。环烷基的定义中还包括的是具有一个或更多个稠合（即具有共用的键）到环烷

基环的芳族环的组成部分，例如，戊烷、戊烯、己烷等的苯并或噻吩基衍生物（例如 2,3- 二氢 -1H- 茚 -1- 基，或 1H- 茚 -2(3H)- 酮 -1- 基）。优选地，“环烷基”是指含有高达 20 个成环碳原子的环化的烷基。环烷基的实例优选地包括环丙基、环丁基、环戊基、环己基、环庚基、金刚烷基等。

[0121] 本文使用的“杂芳基”是指具有高达 20 个成环原子并具有至少一个杂原子环成员（成环原子）例如硫、氧或氮的芳族杂环。在一些实施方式中，杂芳基具有至少一个或多个杂原子成环原子，其各自独立地选自硫、氧和氮。杂芳基包括单环或多环（例如，具有 2、3 或 4 个稠环）体系。杂芳基的实例包括而不限于吡啶基、嘧啶基、吡嗪基、哒嗪基、三嗪基、呋喃基、喹啉基、异喹啉基、噻吩基、咪唑基、噻唑基、吲哚基、吡咯基、噁唑基、苯并呋喃基、苯并噻吩基、苯并噻唑基、异噁唑基、吡唑基、三唑基、四唑基、吲唑基、1,2,4- 噻二唑基、异噻唑基、苯并噻吩基、嘌呤基、咔唑基、苯并咪唑基、二氢吲哚基等。在一些实施方式中，杂芳基具有 1 至约 20 个碳原子，且在其他实施方式中为约 1 至约 5 个、约 1 至约 4 个、约 1 至约 3 个、约 1 至约 2 个作为成环原子的碳原子。在一些实施方式中，杂芳基包含 3 至约 14 个、3 至约 7 个或 5 至 6 个成环原子。在一些实施方式中，杂芳基具有 1 至约 4 个、1 至约 3 个、或 1 至 2 个杂原子。

[0122] 本文使用的“杂环烷基”是指具有高达 20 个成环原子的非芳族杂环，包括环化的烷基、烯基和炔基，其中一个或多个成环碳原子被杂原子例如 O、N 或 S 原子取代。杂环烷基可以是单环或多环（例如，稠环和螺环体系）。实例“杂环烷基”包括吗啉基、硫代吗啉基、哌嗪基、四氢呋喃基、四氢噻吩基、2,3- 四氢苯并呋喃基、1,3- 苯并间二氧杂环戊烯、苯并 -1,4- 二噁烷、哌啶基、吡咯烷基、异噁唑烷基、异噻唑烷基、吡唑烷基、噁唑烷基、噻唑烷基、咪唑烷基、吡咯烷 -2- 酮 -3- 基等。杂环烷基的成环碳原子和杂原子可以任选地被氧或硫取代。例如，成环 S 原子可以被 1 或 2 个氧取代 [即，形成 S(O) 或 $S(O)_2$]。再如，成环 C 原子可以被氧取代（即，形成羰基）。杂环烷基的定义中还包括的是具有一个或多个稠合（即具有共用的键）到非芳族杂环的芳族环的组成部分，例如，吡啶基、噻吩基、酞酰亚胺基、萘酰亚胺基和杂环的苯并衍生物例如吲哚基 (indolene)、异吲哚基、异二氢吲哚 -1- 酮 -3- 基、4,5,6,7- 四氢噻吩并 [2,3-c] 吡啶 -5- 基、5,6- 二氢噻吩并 [2,3-c] 吡啶 -7(4H)- 酮 -5- 基和 3,4- 二氢异喹啉 -1(2H)- 酮 -3 基。杂环烷基的成环碳原子和杂原子可以任选地被氧或硫取代。在一些实施方式中，杂环烷基具有 1 至约 20 个碳原子，且在其他实施方式中为约 3 至约 20 个碳原子。在一些实施方式中，杂环烷基包含约 3 至约 14 个、3 至约 7 个、或 5 至 6 个成环原子。在一些实施方式中，杂环烷基具有 1 至约 4 个、1 至约 3 个、或 1 至 2 个杂原子。在一些实施方式中，杂环烷基包含 0 至 3 个双键。在一些实施方式中，杂环烷基包含 0 至 2 个三键。

[0123] 本文使用的“卤代”或“卤素”包括氟、氯、溴和碘。

[0124] 本文使用的“烷氧基”是指 -O- 烷基。实例烷氧基包括甲氧基、乙氧基、丙氧基（例如正丙氧基和异丙氧基）、叔丁氧基等。

[0125] 本文使用的“卤代烷氧基”是指 -O- 卤代烷基。实例卤代烷氧基是 OCF_3。本文使用的“三卤代甲氧基”是指具有三个卤素取代基的甲氧基。三卤代甲氧基的实例包括但不限于 $-OCF_3$、$-OCClF_2$、$-OCCl_3$ 等。

[0126] 本文使用的“芳烷基”是指被芳基取代的 C_{1-6} 烷基，且“环烷基烷基”是指被环烷基

取代的 C_{1-6} 烷基。

[0127] 本文使用的“杂芳烷基”是指被杂芳基取代的 C_{1-6} 烷基，且“杂环烷基烷基”是指被杂环烷基取代的 C_{1-6} 烷基。

[0128] 本文使用的“氨基”是指 NH_2。

[0129] 本文使用的“烷基氨基”是指被烷基取代的氨基。

[0130] 本文使用的“二烷基氨基”是指被两个烷基取代的氨基。

[0131] 本文使用的 C(O) 是指 C(= O)。

[0132] 本文使用的术语“任选地取代”意指取代是任选的，并且因此包括未被取代的和取代的原子和组成部分两者。“取代的”原子或组成部分表明指定的原子或组成部分上的任意氢可以被来自指明的取代基的选择代替，条件是不超过指定原子或组成部分的正常价态，并且取代产生稳定的化合物。例如，如果甲基（即 CH_3）被任选地取代，则碳原子上的 3 个氢原子可以被所指明的取代基代替。

[0133] 本文使用的“β 淀粉样蛋白作用”，例如“非致死 β 淀粉样蛋白作用”或 Abeta 寡聚体作用是指与 Abeta 种类接触的细胞上的作用，特别是非致死作用。例如，已发现当神经元细胞与可溶 β 淀粉样蛋白（“Abeta”）寡聚体接触时，寡聚体在体外与神经元细胞的子集上的突触子集结合。该结合可以在例如测量体外 Abeta 寡聚体结合的检定中被定量。Abeta 种类的另一文献记载的作用是突触数目的减少，其已被报道在人海马中为约 18%（Scheff 等人，2007）且可以被定量（例如，在测量突触数目的检定中）。作为另一例子，已发现，当神经元细胞与 β 淀粉样蛋白（“Abeta”）寡聚体接触时，调节膜运输且继而发生膜运输的改变。该异常可以用许多检定包括但不限于 MTT 检定进行可视化。例如，黄色四唑盐被细胞胞吞，且盐被位于胞内体途径的囊泡内的酶还原为不可溶紫色甲臜。紫色甲臜的水平反映出培养中的活性代谢细胞的数目，并且甲臜量的减少被认为是对培养中的细胞死亡或代谢毒性的测量。当通过显微镜观察与黄色四唑盐接触的细胞时，首先在填充细胞的细胞内囊泡中可见紫色甲臜。随着时间的推移，囊泡被胞吐，并且在不可溶甲臜暴露于水性介质环境时，甲臜作为针状结晶在质膜外表面上析出。Abeta 种类的其它作用包括认知衰退例如形成新记忆的能力衰退和记忆丧失，其可以在使用动物模型的体内检定中进行测量。在一些实施方式中，Abeta 作用选自 Abeta 寡聚体诱导的突触功能障碍，例如可见于体外检定中，例如膜运输检定或突触损失检定，或 Abeta 寡聚体介导的 caspase-3 的 σ-2 受体活化，或 Abeta 诱导的神经元功能障碍，Abeta 介导的长时程增强效应（LTP）下降，或行为检定中的认知衰退中，或在有需要的患者中。

[0134] 在一些实施方式中，当与阴性对照相比，测试化合物可以将神经元细胞上的与可溶 Abeta 寡聚体种类相关的作用抑制大于约 10%、优选大于 15% 且优选大于 20% 时，其被认为可有效治疗认知衰退或与其相关的疾病。在一些实施方式中，当与阳性对照相比，测试剂可以将淀粉样蛋白前体蛋白的加工产物所介导的作用抑制大于约 10%、优选大于 15% 且优选大于 20% 时，其被认为是有效的。例如，如以下实施例中所示，将 Abeta 寡聚体结合仅抑制 18% 可完全抑制突触减少。例如，参见图 3C 和 3D。尽管本说明书关注对 Abeta 种类的非致死作用（例如神经元代谢的异常和突触数目减少）的抑制，但这些显示为与认知功能相关，而且随时间推移期望可引起淀粉样蛋白病理的下游可测量症状的减少，尤其是临床症状例如 1）通过淀粉样蛋白成像剂例如 fluorbetapir、PittB 或任何其它成像剂

而测量的原纤维或斑块积聚，2) 通过用 FDG-PET 检测的葡萄糖代谢减退而测量的突触损失或细胞死亡，或 3) 通过得自患者的脑脊液、脑组织活检或血浆中的经 ELISA 的成像或蛋白 / 代谢物检测而可检测的脑或身体内的蛋白表达或代谢物量的改变（例如经 ELISA 测量的 Ab_et_a42、磷酸化 t_{au}、全部 t_{au} 的水平或比率的改变，或 ELISA 板中可检测的蛋白表达改变的模式）（参见文献 :Wyss-Coray T. 等人 . Modeling of pathological traits in Alzheimer's disease based on systemic extracellular signaling proteome. Mol Cell Proteomics2011Jul8，其全部引入本文以供参考），4) 通过血管水肿的存在来测量的脑血管异常或通过 MRI 可检测的微出血和任何其它通过成像技术可检测的症状，以及 5) 通过任何施用的认知测试例如 ADAS-Cog、MMSE、CBIC 或任何其它认知测试仪器测量的认知丧失。

[0135] 本文使用的术语“神经元细胞”可以用来指单个细胞或细胞群。在一些实施方式中，神经元细胞是初级 (primary) 神经元细胞。在一些实施方式中，神经元细胞是永生化或转化的神经元细胞或干细胞。初级神经元细胞是不能分化成其它类型的神经元细胞例如神经胶质细胞的神经元细胞。干细胞是可以分化成神经元和其它类型的神经元细胞例如神经胶质的细胞。在一些实施方式中，检定使用包括至少一种神经元细胞而没有神经胶质细胞的组合物。在一些实施方式中，组合物包括少于约 30%、25%、20%、15%、10%、5%、或 1% 的神经胶质细胞，已知其内化和积聚 Abeta。初级神经元细胞可以源自动物脑的任何区域。在一些实施方式中，神经元细胞是海马或皮质细胞。可以通过任何方法确定神经胶质细胞的存在。在一些实施方式中，通过 GFAP 的存在检测神经胶质细胞，并且可以通过针对 MAP2 用抗体阳性染色来检测神经元。

[0136] 短语“药学上可接受的”是指通常被认为安全无毒性的分子实体和组合物。具体地，本发明的药物组合物中使用的药学上可接受的载体、稀释剂或其它赋形剂是生理学上可耐受的，与其它成分相容的，且在对患者施用时通常不产生过敏或相似不宜反应（例如胃不适、眩晕等）。优选地，本文使用的术语“药学上可接受的”是指被联邦管制机构或州政府批准或列于美国药典或其它通常认可的用于动物、更优选人的药典中。本文使用的短语“药学上可接受的盐”包括本发明化合物的用于哺乳动物安全有效且具有期望的生物学活性的盐。药学上可接受的盐包括本发明化合物中或根据本发明的方法识别的化合物中存在的酸性基团或碱性基团的盐。药学上可接受的酸加成盐包括但不限于盐酸盐、氢溴酸盐、氢碘酸盐、硝酸盐、硫酸盐、硫酸氢盐、磷酸盐、酸式磷酸盐、异烟酸盐、乙酸盐、乳酸盐、水杨酸盐、柠檬酸盐、酒石酸盐、泛酸盐、酒石酸氢盐、抗坏血酸盐、琥珀酸盐、马来酸盐、龙胆酸盐 (gentisinate)、富马酸盐、葡糖酸盐、葡糖醛酸盐 (glucaronate)、糖酸盐、甲酸盐、苯甲酸盐、谷氨酸盐、甲磺酸盐、乙磺酸盐、苯磺酸盐、对甲苯磺酸盐和双羟萘酸盐（即 1,1'- 亚甲基 - 双 -(2- 羟基 -3- 萘甲酸盐））。本发明的某些化合物可以与各种氨基酸形成药学上可接受的盐。合适的碱盐包括但不限于铝、钙、锂、镁、钾、钠、锌、铁和二乙醇胺盐。药学上可接受的碱加成盐也与胺例如有机胺形成。合适的胺的实例是 N, N'- 二苄基乙二胺、氯普鲁卡因、胆碱、二乙醇胺、二环己胺、乙二胺、N- 甲基葡糖胺和普鲁卡因。

[0137] 本文使用的术语“治疗性”是指用于治疗、反抗、改良、使免受或改善受试者的不希望的病症或疾病的试剂。

[0138] 本文使用的术语“有效量”是指对具体的病症或病理过程的至少一种症状或参数引起可测量的抑制的量。例如，在 Abeta 寡聚体的存在下提供可测量的较低的突触减少的

本发明的 σ-2 配体的量被认为是有效量，因为其减少病理过程，即使没有淀粉样蛋白病理的临床症状被改变，至少没有被立即改变。

[0139] 本发明的化合物或组合物的“治疗有效量”或“有效量”是以适用于任何医疗的合理收益 / 风险比，对被治疗的受试者赋予治疗效果的预定量。治疗效果可以是客观的（即通过一些测试或标记物可测量的）或主观的（即受试者给出表示或感觉到效果或医师观察到变化）。本发明的化合物的有效量可以广泛地为约 0.01mg/Kg 至约 500mg/Kg、约 0.1mg/Kg 至约 400mg/Kg、约 1mg/Kg 至约 300mg/Kg、约 0.05 至约 20mg/Kg、约 0.1mg/Kg 至约 10mg/Kg、或约 10mg/Kg 至约 100mg/Kg。本文考虑的效果适当地包括医疗和 / 或预防处理。根据本发明施用以获得治疗和 / 或预防效果的化合物的具体剂量当然根据案例周围的具体情况确定，包括例如施用的化合物、施用途径、其它有效成分的共同施用、经治疗的病症、所采用的具体化合物的活性、所采用的具体组合物、患者的年龄、体重、一般健康状况、性别和饮食、施用时间、施用途径、所采用的具体化合物的排泄速率以及治疗时长。施用的有效量由医师鉴于前述相关情况并运用合理的医学判断来确定。本发明化合物的治疗有效量通常是如下量，使得当其以生理学上可耐受的赋形剂组合物施用时，足以实现有效全身浓度或组织局部浓度。以单剂量或分开剂量施用至人或其它动物的本发明的化合物的日总剂量的量可以是例如 0.01mg/Kg 至约 500mg/Kg、约 0.1mg/Kg 至约 400mg/Kg、约 1mg/Kg 至约 300mg/Kg、约 10mg/Kg 至约 100mg/Kg、或更通常约 0.1 ～ 25mg/kg 体重 / 天。单剂量组合物可以含有这样的量或其因数以组成日剂量。通常，根据本发明的治疗方案包括对需要这样的治疗的患者以单剂量或多剂量每天施用约 1mg 至约 5000mg、10mg 至约 2000mg、20 ～ 1000mg、优选 20 ～ 500mg 且最优选约 50mg 本发明的化合物。

[0140] 本文使用的术语“治疗”是指治疗处理和预防或防护措施两者，其中目的是使免受（部分或全部）或减缓（减轻或延迟发作）不期望的生理病症、障碍或疾病，或获得有益的或期望的临床结果，例如部分或完全恢复或抑制已经或将会变得异常的参数、值、功能或结果的减退。对于本发明的目的，有益的或期望的临床结果包括但不限于减轻症状；减弱病症、障碍或疾病的发展程度或活力或速度；稳定（即不恶化）病症、障碍或疾病的状态；延迟病症、障碍或疾病的发作或减缓病症、障碍或疾病的进展；改良病症、障碍或疾病状态；以及缓解（无论是部分还是全部），不论其是否转化为实际的临床症状的立即减轻，或增强或改善病症、障碍或疾病。治疗寻求引起临床上显著的响应而无过度水平的副作用。治疗还包括与如果不接受治疗的预期存活相比延长存活。

[0141] 通常而言，术语“组织”是指统一执行特定功能的相似的专门细胞的集合。

[0142] 本文使用的“认知衰退”可以是动物认知功能的任何负面变化。例如，认知衰退包括但不限于记忆丧失（例如行为记忆丧失）、无法获得新记忆、混乱、判断受损、人格改变、定向障碍、或其任意组合。因此，可有效治疗认知衰退的化合物可以通过以下而有效：恢复长时程神经元增强 (LTP) 或长时程神经元抑制 (LTD) 或电生理测量的突触可塑性的平衡；抑制、治疗、和 / 或减轻神经退行；抑制、治疗、和 / 或减轻一般淀粉样变性；抑制、治疗、减轻淀粉样蛋白产生、淀粉样蛋白组装、淀粉样蛋白聚集、和淀粉样蛋白寡聚体结合中的一种或多种；抑制、治疗、和 / 或减轻一种或多种 Abeta 种类对神经元细胞的非致死作用（例如突触损失或功能障碍和异常膜运输）；以及其任意组合。此外，该化合物还可以有效地治疗 Abeta 相关的神经退行性疾病和病症，包括但不限于痴呆，包括但不限于阿尔茨海默病

(AD)，包括轻度阿尔茨海默病、唐氏综合征、血管性痴呆（脑淀粉样血管病和中风）、路易体痴呆、HIV 痴呆、轻度认知障碍 (MCI)、年龄相关的记忆受损 (AAMI)、年龄相关的认知衰退 (ARCD)、临床前阿尔茨海默病 (PCAD) 和非痴呆认知障碍 (CIND)。

[0143] 本文使用的术语“天然配体”是指存在于受试者中的可以与蛋白质、受体、膜脂或体内或体外复制的其他结合配偶体结合的配体。天然配体可以是合成来源的，但也须在受试者中天然存在而无人为干预。例如，已知 Abeta 寡聚体在人受试者中存在。因此，受试者中发现的 Abeta 寡聚体将被视为天然配体。Abeta 寡聚体与结合配偶体的结合可以使用重组或合成技术在体外进行复制，但不管 Abeta 寡聚体如何制备或制造，Abeta 寡聚体仍被认为是天然配体。也可以与同一结合配偶体结合的合成小分子如果在受试者中不存在，则其不是天然配体。例如，本文描述的化合物 II、化合物 IXa 和 IXb 以及所有其他化合物通常不存在于受试者中，因此将不会被认为是天然配体。

[0144] σ-2 受体

[0145] σ 受体是以组织和状态相关的方式参与数个不同的蛋白信号传导复合体的多功能衔接子 / 分子伴侣蛋白。σ-2 受体以低水平在脑和多种外周组织中表达。(Walker 等人，1990Sigma receptors:biology and function. Pharmacol. Rev. 42:355-402)。σ-2 受体存在于人的海马和皮质中。σ-2 受体还在先前被确认为肿瘤细胞增殖的生物标记物。(Mach 等人，Sigma-2receptors as potential biomarkers of proliferation in breast cancer. Cancer Res. 57:156-161, 1997)。

[0146] σ- 受体涉及到很多信号传导通路中，例如血红素结合、细胞色素 P450 代谢、胆固醇合成、孕酮信号传导、细胞凋亡和膜运输。仅有一个子集的 σ 受体结合位点 / 信号传导通路与 AD 中的寡聚体信号传导相关。σ-2 受体基因敲除目前尚不可得，且 σ-2 序列中的人突变还没有在神经退行情况下进行过研究。

[0147] 近来，通过使用可逆地标记大鼠肝脏中的 σ-2 受体的光亲和性探针 WC-21，σ-2 受体被识别为大鼠肝脏中的孕酮受体膜成分 1(PGRMC1)。Xu 等人 . Identification of the PGRMC1 protein complex as the putative sigma-2receptor binding site. Nature Communications2, 文章数第 380，2011 年 7 月 5 日，其并入本文以供参考。PGRMC1（孕酮受体膜成分 1）在 2011 年 8 月被 Xu 等人识别为 σ-2 受体活性的关键性 25kDa 成分。PGRMC1 是单跨膜蛋白，与 σ-1 蛋白没有同源性；家族成员包括 PGRMC2 和 neudesin。PGRMC1 包含细胞色素 b5 血红素结合域。PGRMC1 是与 S1 蛋白没有同源性的单跨膜蛋白；家族成员包括 PGRMC2 和 neudesin。PGRMC1 包含细胞色素 b5 血红素结合域。内源性 PGRMC1 配体包括孕酮 / 类固醇、胆固醇代谢物、糖皮质激素和血红素。PGRMC1 用作为与不同亚细胞位置中的不同蛋白复合物相关的分子伴侣 / 衔接子 (Cahill2007. Progesterone receptor membrane componentl:an integrative review. J. Steroid Biochem. Mol. Biol. 105:16-36)。PGRMC1 以降低的活性与血红色结合，与 CYP450 蛋白复合（调节的氧化还原反应），与 PAIRBP1 缔合并介导细胞凋亡的孕酮阻断，与 Insig-1 和 SCAP 缔合以诱导应答于低胆固醇的 SRE 相关的基因转录。秀丽隐杆线虫 (C. elegans) 同源物 VEM1 与 UNC-40/DCC 缔合以介导轴突导向。PGRMC1 包含两个 SH2 靶序列、SH3 靶序列、酪氨酸激酶位点、两个嗜酸激酶位点 (CK2) 和对于 ERK1 和 PDK1 的一致性结合位点。PGRMC1 包含数个参与膜运输（囊泡转运、含有 calveolin 的窝的网格蛋白依赖的胞吞）的 ITAM 序列。

[0148] σ-2 受体疗法对于其他 CNS 适应征已经达到了人 II 期临床试验，但对于 AD 的治疗并未如此。很多 σ-2 受体配体不是非常有选择性，并且对其他非 σ CNS 受体具有高亲和性。例如，Cyr-101/MT-210(Cyrenaic Pharmaceuticals ;Mitsubishi) 是精神分裂症 IIa 期临床试验中的 σ-2 受体拮抗剂，但是具有多种其他受体相互作用包括在 5HT2a、ADRA1 和组胺 H1 处。希拉美新 (Lundbeck, Forest Lu28179) 是先前用于焦虑临床试验但没有继续的 σ-2 受体激动剂。σ-1 受体配体用于针对多种 CNS 适应征的临床试验。库他美新二盐酸盐 (AGY SA4503, M' s Sience Corp.) 是用于中风 II 期临床试验以及抑郁 II 期试验的 σ-1 受体激动剂。Anavex2-73 是 σ-1 受体激动剂，其还在毒蕈碱胆碱能受体处用作为 M2/3 拮抗剂、M1 激动剂，并且是相对于多种离子通道 (NMDAR, Na+, Ca++) 的拮抗剂。Anavex2-73 进入了具有 AD 和轻度认知障碍的患者的 IIa 期临床试验。之前没有在 AD 中进行高度选择性 σ-2 受体配体疗法的临床试验。

[0149] σ-2 拮抗剂

[0150] 不拘泥于理论，提出 σ-2 受体是神经元中 Abeta 寡聚体的受体。文献中针对可溶 Abeta 寡聚体已提出多种受体，包括朊蛋白、胰岛素受体、β 肾上腺素能受体和 RAGE(糖基化终末产物的受体)。Laurén, J. 等人，2009, Nature, 457(7233):1128-1132 ; Townsend, M. 等人，J. Biol. Chem. 2007, 282:33305-33312 ;Sturchler, E. 等人，2008, J. Neurosci. 28(20):5149-5158。很多研究者确实相信，Abeta 寡聚体可以与多于一种的受体蛋白结合。不拘泥于理论，基于本文呈现的证据，本发明的发明者假定出位于（不必须专门地）神经元中的 Abeta 寡聚体的其他受体。

[0151] 不拘泥于理论，Abeta 寡聚体是与 σ 蛋白复合物结合并引起异常运输和突触损失的 σ 受体激动剂。本文证实，在神经元的该相互作用和 / 或 σ 受体功能中起拮抗作用的高亲和性 σ-2 配体会与 Abeta 寡聚体竞争并将神经元应答回复至正常。这样的配体被认为是功能性 σ-2 受体拮抗剂并如此称谓或更简单地称为 σ-2 受体拮抗剂或 σ-2 拮抗剂。

[0152] 在一些实施方式中，本发明的 σ-2 受体拮抗剂用作为神经元细胞中的功能性拮抗剂，有关于抑制可溶 Aβ 寡聚体诱导的突触损失和抑制膜运输检定中的可溶 Aβ 寡聚体诱导的缺陷 ;表现出在 σ-2 受体处的高亲和性 ;以及与任何其他非 σ 受体相比对于一种或多种 σ 受体具有高选择性 ;以及表现出较好的药物样特性。

[0153] 在一些实施方式中，本文详述的满足某些体外检定标准的 σ-2 受体功能性拮抗剂在本说明书中公开的一种或多种相关动物行为模型中表现出行为效力，或被预测具有行为效力。在一些实施方式中，在 10mg/kg p.o. 或更低确定行为效力。

[0154] 在一些实施方式中，本公开内容提供可预测高亲和性 σ-2 受体配体的行为效力的体外检定平台。根据该体外检定平台，配体以高亲和性与 σ-2 受体结合 ;用作为相对于神经元中 Abeta 寡聚体诱导的作用的功能性拮抗剂 ;抑制中枢神经元中 Abeta 寡聚体诱导的突触损失或降低 Abeta 寡聚体与神经元的结合以抑制突触损失 ;以及在不存在 Abeta 寡聚体时不影响运输或突触数目。体外检定中的该活性模式被称为“治疗表型”。σ-2 受体拮抗剂阻断成熟神经元中的 Abeta 寡聚体作用而在不存在 Abeta 寡聚体时不影响正常功能的能力满足治疗表型的标准。如今公开了具有治疗表型的选择性 σ-2 拮抗剂能够够阻断 Abeta 寡聚体诱导的突触功能障碍。

[0155] 在一些实施方式中，具有治疗表型且同时具有以下特征的高亲和性的选择性 σ-2

拮抗剂适合作为用于在有需要的患者中治疗 Abeta 寡聚体诱导的突触功能障碍的治疗候选物：在 σ 受体处的高亲和性；与其他非 σ CNS 受体相比，对 σ 受体的高选择性；对 σ-2 受体的更高的亲和性或可比的亲和性，例如在 σ-2 和 σ-1 受体处为一个数量级内；对 σ 受体的与中枢神经系统中相关的其它受体相反的选择性，以及较好的药物样特性。药物样特性包括可接受的脑渗透性（跨越血脑屏障的能力）、较好的血浆稳定性和较好的代谢稳定性，例如通过对肝脏微粒体的暴露而测量的。不拘泥于理论，高亲和性 σ-2 受体拮抗剂与 Abeta 寡聚体竞争，且 / 或停止引起阿尔茨海默病的病理 σ 受体信号传导。

[0156] 在一些实施方式中，本发明的拮抗剂可以以比 σ-2 受体更高的亲和性与 σ-1 受体结合，但是必须仍关于阻断或抑制 Abeta 寡聚体诱导的作用（Abeta 作用）表现为功能性神经元拮抗剂。

[0157] 在一些实施方式中，具有治疗表型且具有以下特征的 σ-2 拮抗剂适合作为用于在有需要的患者中治疗 Abeta 寡聚体诱导的突触功能障碍的治疗候选物：在 σ 受体处的高亲和性；相比于其他非 σ CNS 受体，对 σ 受体的高亲和性；对 σ-2 受体的高亲和性或在 σ-2 和 σ-1 受体处可比的亲和性；以及较好的药物样特性。药物样特性包括较高的脑渗透性、血浆稳定性和代谢稳定性。

[0158] 在一些实施方式中，在结合活性研究中，至多约 600nM、500nM、400nM、300nM、200nM、150nM、100nM、优选为至多约 75nM、优选为至多约 60nM、优选为至多约 40nM、最优选为至多 10nM、最优选为至多 1nM 的 IC_{50} 或 Ki 值表明关于 σ 受体结合位点的高结合亲和性。

[0159] 在一些实施方式中，相比于其他非 σ CNS 或靶标受体对 σ 受体具有高于约 20 倍、30 倍、50 倍、70 倍或优选高于 100 倍的选择性、具有较好的药物样特性包括脑渗透性和较好的代谢和 / 或血浆稳定性且具有治疗表型的在 σ-2 受体处具有高亲和性（优选 Ki 小于约 600nM、500nM、400nM、300nM、200nM、150nM、100nM、70nM、60nM、50nM、30nM 或 10nM）的 σ-2 受体拮抗剂被预测为具有行为效力并且可以用于在有需要的患者中治疗 Abeta 寡聚体诱导的突触功能障碍。

[0160] 本文使用的术语“脑渗透性”是指药物、抗体或片段跨越血脑屏障的能力。在一些实施方式中，动物药代动力学 (pK) 研究例如小鼠药代动力学 / 血脑屏障研究可以用于确定或预测脑渗透性。在一些实施方式中，可以施用多个浓度的药物，例如以 3、10 和 30mg/kg，例如 p.o. 施用 5 天，并且测量多个 pK 特性，例如在动物模型中。在一些实施方式中，确定剂量相关的血浆和脑中水平。在一些实施方式中，脑 Cmax>100、300、600、1000、1300、1600 或 1900ng/mL。在一些实施方式中，较好的脑渗透性被定义为，脑 / 血浆比率为 >0.1、>0.3、>0.5、>0.7、>0.8、>0.9、优选 >1、且更优选 >2、>5 或 >10。在其他实施方式中，较好的脑渗透性被定义为，在预定时间段后高于约 0.1%、1%、5%、高于约 10%、且优选高于约 15% 的所施用剂量跨越 BBB。在某些实施方式中，剂量为经口服施用 (p.o.)。在其他实施方式中，在测量 pK 特性之前，剂量为经静脉施用 (i.v.)。检定和脑渗透性在实施例 7 中描述，且化合物 II 的数据显示在图 2A 和 2B 中，已知化合物 II 经受首过代谢，因此皮下给药；尽管如此，化合物 II 在急性和慢性给药后均具有高脑渗透性。化合物 II 的脑 / 血浆比率为 >8。

[0161] 本文使用的术语“血浆稳定性”是指化合物在血浆中例如通过酶例如水解酶和酯酶的降解。可以采用多种体外检定中的任一种。药物在血浆中孵育多种时间段。在各个时间点剩余的百分比母体化合物（分析物）反映出血浆稳定性。较差的血浆稳定性特征可以

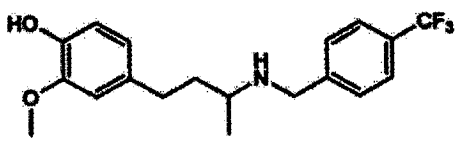
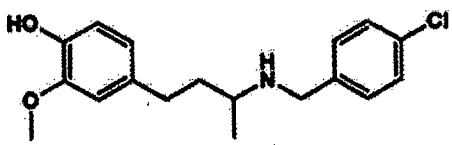
趋向于具有较低的生物利用度。较好的血浆稳定性可以定义为，在 30 分钟后剩余高于 50% 的分析物，在 45 分钟后剩余高于 50% 的分析物，且优选为在 60 分钟后剩余高于 50% 的分析物。

[0162] 本文使用的术语“代谢稳定性”是指化合物从首过代谢（口服药物的肠和肝降解或结合）中幸存的能力。这可以例如在体外通过将化合物暴露于小鼠或人肝脏微粒体而评测。在一些实施方式中，较好的代谢稳定性是指在将化合物暴露于小鼠或人肝脏微粒体时，$t_{1/2}$>5min、>10min、>15 分钟、>20 分钟、优选 >30 分钟。在一些实施方式中，较好的代谢稳定性是指内在清除率 (Cl_{int})<300uL/min/mg，优选≤ 200uL/min/mg，且更优选≤ 100uL/min/mg。

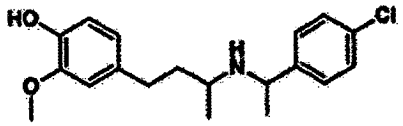
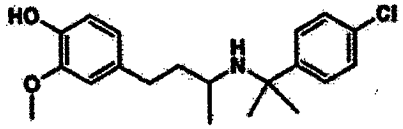
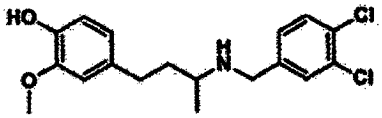
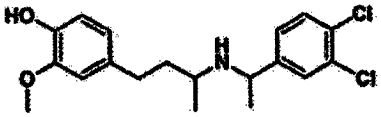
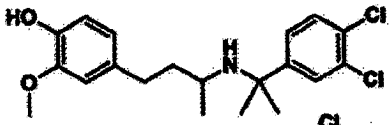
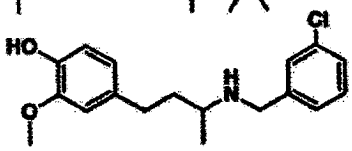
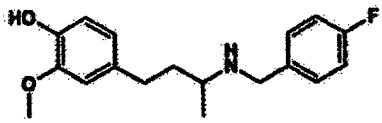
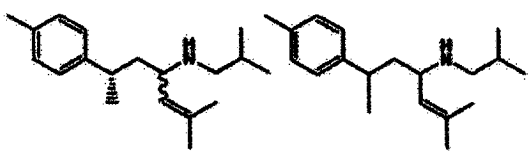
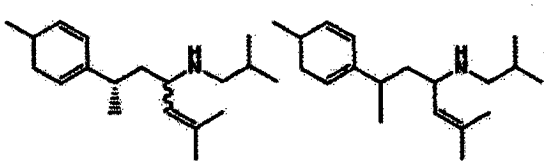
[0163] 在一些实施方式中，排除某些现有技术化合物，其已知为 σ-2 拮抗剂，并且要么 (i) 已知与 σ-2 受体结合并减少或消除 Abeta 诱导的病理，例如神经元细胞中膜运输的缺陷或突触减少，要么 (ii) 已知针对阿尔茨海默病症状具有活性而不涉及 σ-2 受体相互作用。在一些实施方式中，表 1A 中记载的化合物关于本公开内容的组合物或方法而不要求保护，关于表 1A，R、R_1 和 R_2 可以独立地为烷基、烷氧基、卤素、卤代烷基或卤代烷氧基，且 n = 0 ～ 8。

[0164] 表 1A. 不要求保护的化合物

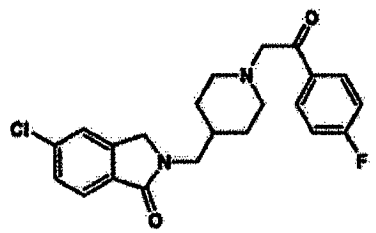
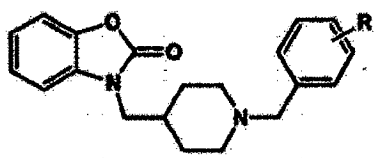
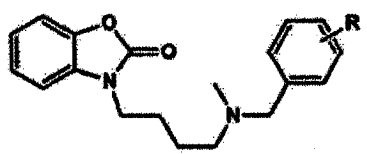
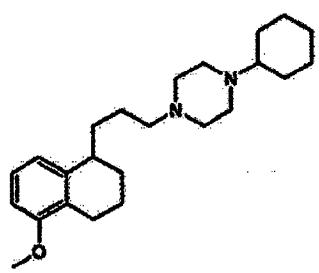
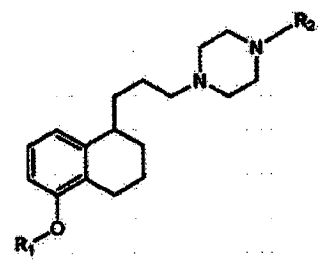
[0165]

不要求保护的化合物	参考文献
	CogRx Rishton, Catalano WO 2010/118055 A1 实施例 2., pp. 46-47
	CogRx Rishton, Catalano WO 2010/118055 A1 实施例 1., pp. 43-44

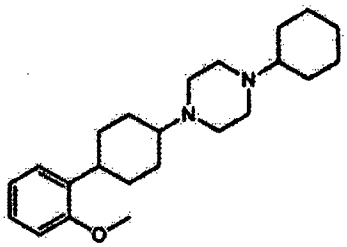
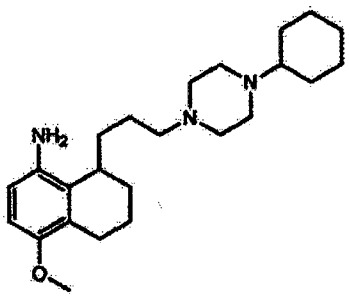
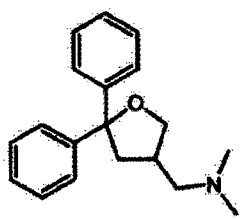
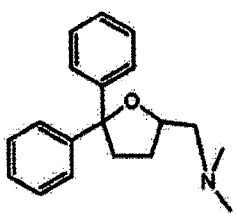
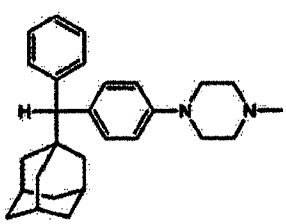
[0166]

不要求保护的化合物	参考文献
 	CogRx Rishton, Catalano WO 2010/118055 A1 p. 32
 	CogRx Rishton, Catalano WO 2010/118055 A1 p. 32
 	CogRx Rishton, Catalano WO 2010/118055 A1 p. 32
	CogRx Rishton, Catalano WO 2010/118055 A1 p. 32
	CogRx Rishton, Catalano WO 2011/014880 A1 实施例 1 , pp. 41-42
	CogRx Rishton, Catalano WO 2011/014880 A1

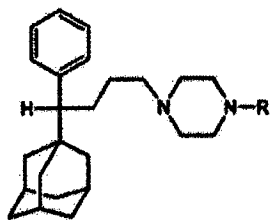
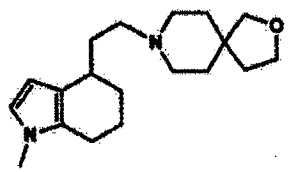
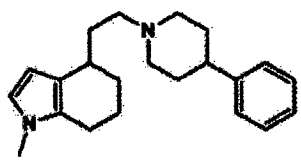
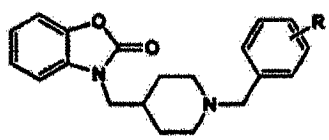
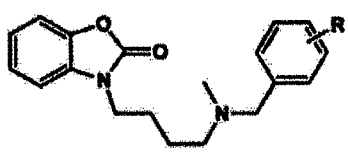
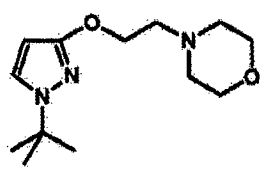
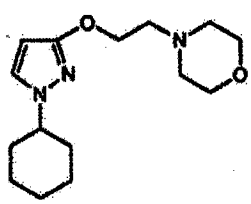
[0167]

不要求保护的化合物	参考文献
	实施例 1 , p. 42
	Rocher, Yamabe WO 2001/64670 A1 Laurini, *et al.* Bioorganic and Medicinal Chemistry Letters 20 (2010) 2954-2957
	Rocher, Yamabe WO 2001/64670 A1 Laurini, *et al.* Bioorganic and Medicinal Chemistry Letters 20 (2010) 2954-2957
	Rocher, Yamabe WO 2001/64670 A1 Laurini, *et al.* Bioorganic and Medicinal Chemistry Letters 20 (2010) 2954-2957
	Colabufo WO 2007/077543 A2
	Colabufo WO 2007/077543 A2

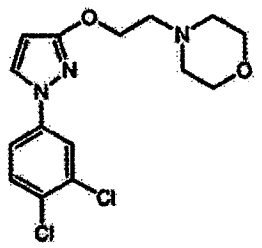
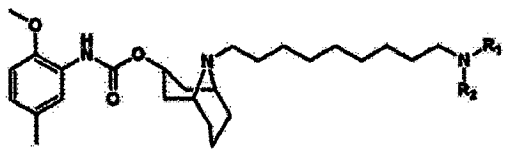
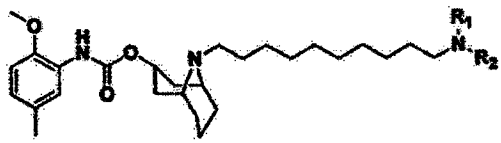
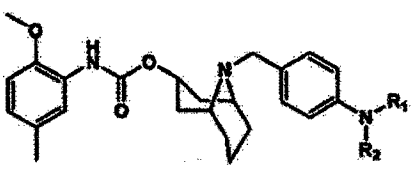
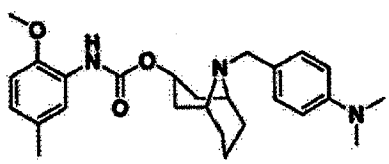
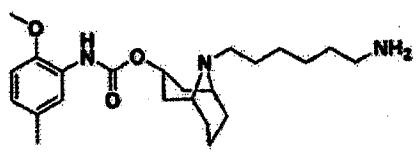
[0168]

不要求保护的化合物	参考文献
	Colabufo WO 2007/077543 A2
	Abate WO 2009/104058 A1
	Anavex US 2010/0069484 A1 Anavex WO 2008/087458 A2
	Anavex US 2010/0069484 A1 Anavex WO 2008/087458 A2
	Anavex US 2010/0069484 A1 Anavex WO 2008/087458 A2

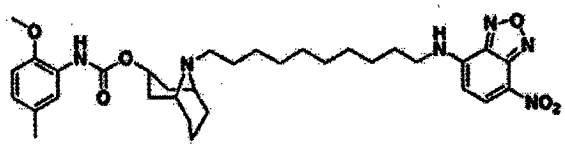
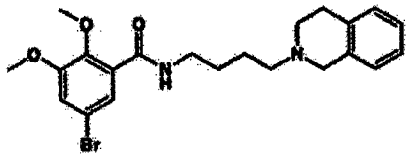
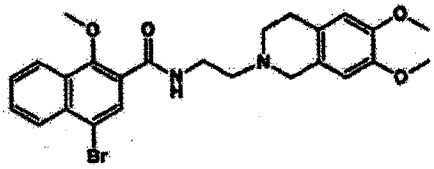
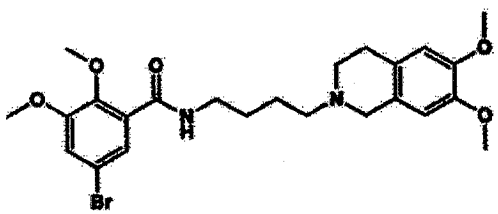
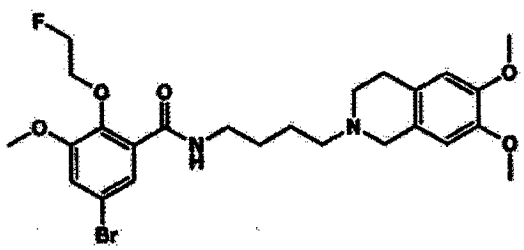
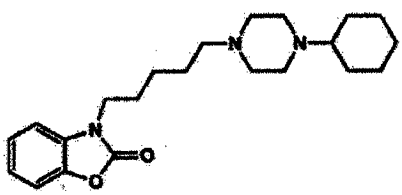
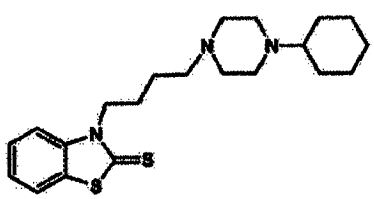
[0169]

不要求保护的化合物	参考文献
	Anavex US 2010/0069484 A1 Anavex WO 2008/087458 A2
	Corbrera EP 1829862 A1 2007
	Corbrera EP 1829862 A1 2007
	Rocher, Yamabe WO 0164670 A1
	Rocher, Yamabe WO 0164670 A1
	Cuberes EP 1829867 A1 2007
	Cuberes EP 1829867 A1 2007

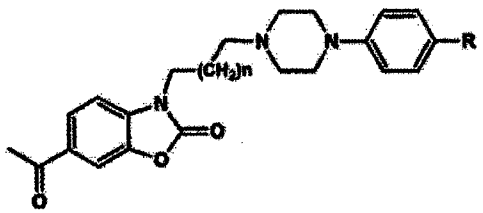
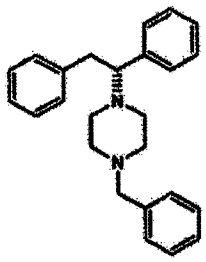
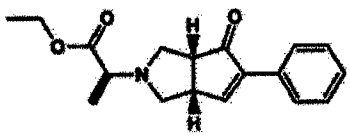
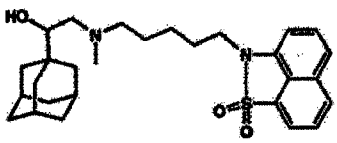
[0170]

不要求保护的化合物	参考文献
	Cuberes EP 1829867 A1 2007
	Hawkins, Mach US 2009/0176705 A1
	Hawkins, Mach US 2009/0176705 A1
	Hawkins, Mach US 2009/0176705 A1
	Hawkins, Mach US 2009/0176705 A1
	化合物 WC-26 Hawkins, Mach US 2009/0176705 A1
	化合物 SV-119 Hawkins, Mach US 2009/0176705 A1

[0171]

不要求保护的化合物	參考文献
	化合物 SW-120 Hawkins, Mach US 2009/0176705 A1
	Mach US 2005/0107398 A1
	Mach US 2005/0107398 A1
	Mach US 2005/0107398 A1
	Mach US 2005/0107398 A1
	McCurdy WO 2009/026227 A2
	McCurdy JPET 333:491–500, 2010 US 2011/0280804 A1

[0172]

不要求保护的化合物	参考文献
	McCurdy WO 2009/026227 A2
	Mita JP 2001/26584
	Pericas WO 2007/128458 A1
	Rocher, Yamabe WO 1996/05185

[0173] 在一些实施方式中，关于本公开内容的组合物或方法，已知为 RHM-1、RHM-4、PB28、SM-21、M-14、NE100、BD1008、BD1047、氟伏沙明、PPBP、戊唑星或氟哌啶醇的化合物不要求保护。在某些检定中，西拉美辛、SV-119 和 WC-26 是功能性 σ-2 受体激动剂。在一些实施方式中，本公开内容的组合物和方法不包括西拉美辛、SV-119 或 WC-26。

[0174] σ-2 激动剂引起细胞毒性

[0175] 不拘泥于理论，提出 Abeta 寡聚体是与 σ 蛋白复合物结合并能够引起多种有害的 Abeta 作用例如神经元毒性、异常运输和突触损失的 σ-2 受体激动剂。本文证实，已知的 σ-2 受体激动剂例如西拉美辛、SV-119 和 WC-26 对肿瘤细胞和神经元有细胞毒性，如通过杀死肿瘤细胞以及引起神经元中异常核形态（图 9A 和 9B）的能力所表现的。σ-2 激动剂（西拉美辛、SV-119），尽管能够以低浓度阻断寡聚体诱导的运输缺陷，但其高浓度引起细胞毒性和 caspase-3 活化（参见图 10A 中的激动剂西拉美辛和图 10B 中的 SV119）。σ-2 拮抗剂例如化合物 II 以及 IXa、IXb 能够阻断神经元细胞中由 σ-2 受体激动剂例如 SV-119 引起的 caspase-3 活化，如图 10D 中所示。σ-2 拮抗剂在所有的测试浓度阻断 Abeta 寡聚体诱导的运输缺陷而不引起细胞毒性，例如，参见图 11A 中的 II 和图 11B 中的 RHM-1。

[0176] 本文公开了具有治疗表型和较好药物样特性的高亲和性的选择性 σ-2 功能性拮抗剂可以用于治疗 Abeta 寡聚体诱导的突触功能障碍。

[0177] 在某些实施方式中，本发明的组合物包括对 σ 受体具有高结合亲和性的选择性 σ-2 功能性拮抗剂。σ 受体包括 σ-1 和 σ-2 亚型两者。参见 Hellewell, S. B. and Bowen, W. D., Brain Res. 527:224-253(1990)； 和 Wu, X.-Z. 等 人 , J. Pharmacol. Exp. Ther. 257:351-359(1991)。对两种 σ 位点的推定配体的结合亲和性进行定量的 σ 受体结合检定（针对 ^{3}H-DTG，其以约相等的亲和性标记两种位点）经 Weber 等人，Proc. Natl. Acad. Sci (USA) 83:8784-8788(1986) 公开。或者，[^{3}H] 戊唑辛可以用来在结合检定中选择性地标记 σ-1 结合位点。[^{3}H]DTG 和未标记的 (+) 戊唑辛的混合物用来在结合检定中选择性地标记 σ-2 位点。本发明还涉及包括某些对 σ-1 和 σ-2 受体具有选择性并用作为 σ-2 功能性拮抗剂的配体的组合物，以及这些组合物在治疗 Abeta 寡聚体诱导的突触功能障碍中的用途。这样的对两种 σ 受体亚型中的一种具有选择性的配体的发现可能是在识别可有效治疗中枢神经系统病症而副作用最小化的化合物中的重要因素。

[0178] 在一些实施方式中，σ-2 拮抗剂选自小分子或抗体、或其活性结合片段，对 σ-2 受体具有高亲和性，且具有阻断可溶 Abeta 寡聚体结合或 Abeta 寡聚体诱导的突触功能障碍的能力。

[0179] Abeta 抗体

[0180] 一些 Abeta 单克隆抗体处于用于治疗阿尔茨海默病的临床研发。在一些实施方式中，本公开内容提供包括 σ-2 受体拮抗剂与 Abeta 抗体的组合物。例如，巴匹珠单克隆抗体 (AAB-00 ; Janssen, Elan, Pfizer) 是静脉治疗轻度到重度阿尔茨海默病的 III 期临床研发中的 β-淀粉样蛋白人化 IgG_1 单克隆抗体。在 II 期临床研究中，某些接受 2mg/kg 高剂量的患者经历了可逆的血管性水肿。尽管在初级效力分析中没有发现显著差异，但元分析显示出非 APOE ε4 携带者中的潜在治疗差异。Salloway 等人，2009, A phase2ascending dose trial of bapineuzumab in mild to moderate Alzheimer's disease Neurol. 2009 ; 73(24):2061-70。现在进行的 III 期研究，巴匹珠单克隆抗体约每 13 周一次通过静脉输注以 0.5 或 1.0mg/kg 施用，同时施用允许的胆碱脂酶抑制剂或 memantidine。巴匹珠单克隆抗体识别 Abeta : $Abeta_{1-5}$ 的 N 端表位。其他 Aβ 人化单克隆抗体处于临床研发的各个阶段中，包括针对 $Abeta_{13-28}$ 提出的茄尼醇单克隆抗体 (LY2062430 ; Lilly)、靶向 $Abeta_{33-40}$ 的 PF-04360365(Pfizer) ; MABT5102A(Genentech) ; GSK933776(GlaxoSmithKline) 和更汀芦单克隆抗体 (gantenerumab) (R1450, RO4909832, Hoffman-LaRoche)。近来报道，茄尼醇单克隆抗体在 III 期临床试验结果的二次分析中在患有轻度 AD 的患者而非患有中度 AD 的患者中显示出统计显著的认知衰退的减缓。在一个实施方式中，将包括 σ-2 拮抗剂和茄尼醇单克隆抗体的组合物用于减缓患有轻度 AD 的患者中认知衰退的方法。

[0181] 公认的是外周施用的抗体可能不能到达目标组织，尽管被动免疫似乎在小鼠中可行。一个假设是，将抗体循环到 Aβ 改变 Aβ 肽从脑脊液到血浆的平衡，间接降低脑的 Aβ 负荷。Kerchner 等人，2010, Bapineuzumab, Expert Opin Biol Ther., 10(7):1121-1130。或者，提出可能静脉施用的抗体可以在脑中与 Aβ 直接结合。参见，例如 Yamada 等人，2009, Aβ immunotherapy: Intracerebral sequestration of Aβ by an anti-Ab monoclonal antibody266with high affinity to soluble Aβ. J Neurosci29(36):11393-11398。不幸的是，迄今，静脉 β 淀粉样蛋白特异性单克隆抗体还没有被证实为特别有效；例如，近来，静脉巴匹珠单克隆抗体的研发因在患有轻度到中度阿尔茨海默病的患者的两个后期试验中

缺乏效力而终结。

[0182] Abeta多克隆抗体在静脉免疫球蛋白(IVIg或IGIV)的混合制剂中天然出现,其已被FDA批准用于治疗其他神经病症。至少两个在AD中使用IVIg的临床试验正通过Baxter和Octpharma而在进行中。Kerchner等人,2010同上。在一些实施方式中,本公开内容提供用于治疗认知衰退或阿尔茨海默病的方法和组合物,其中组合物包括σ-2受体拮抗剂化合物和Abeta抗体以及药学上可接受的载体。

[0183] σ-2受体抗体

[0184] 在一些实施方式中,σ-2受体拮抗剂化合物是σ-2受体特异性抗体、或其活性结合片段,其具有阻断可溶Abeta寡聚体结合或Abeta寡聚体诱导的突触功能障碍的能力。在优选实施方式中,用在本文公开方法中的σ-2拮抗剂抗体或其免疫特异性片段不会在将要治疗的动物例如人中引发有害的免疫应答。在某些实施方式中,用在本文公开的治疗方法中的σ-2拮抗剂抗体或其活性结合片段可以使用领域认可的技术改性以降低其免疫原性。例如,抗体可以被人化、灵长类化(primatized)、去免疫化,可以制备合成或嵌合抗体。这些类型的抗体源自非人抗体,通常是鼠科或灵长类抗体,其保持有或基本保持有亲代抗体的抗原结合特性,但是其在人中具有较小免疫原性。这可以通过多种方法实现,例如但不限于,(a)将整个非人可变结构域植入人恒定区中,以产生嵌合抗体,(b)将一种或多种非人互补决定区(CDR)的至少一部分植入人类框架和恒定区中,保留或不保留关键框架残基,(c)移植整个非人可变结构域,但是通过表面残基的替换而用人样区域将其"掩盖",或(d)使用遗传改性的小鼠,其中小鼠工程设计为表达人的全部特征点,例如人免疫球蛋白重链和轻链可变结构域。这样的方法公开于Morrison等人,Proc. Natl. Acad. Sci. 81:6851-6855(1984);Morrison等人,Adv. Immunol. 44:65-92(1988);Verhoeyen等人,Science239:1534-1536(1988);Padlan,Molec. Immun. 28:489-498(1991);Padlan,Molec. Immun. 31:169-217(1994),Peterson,ILAR Journal46(3):314-319(2005),Lonberg,Nat. Biotechnol. 23(9):1119-1125(2005)以及美国专利第5,585,089号、第5,693,761号、第5,693,762号、第6,190,370号和US2012/0021409中公开,所有这些均全部并入本文以供参考。

[0185] 去免疫化也可以用于降低抗体的免疫原性。本文使用的术语"去免疫化"包括改变抗体以使T细胞表位改性(参见,例如WO9852976A1、WO0034317A2)。例如,分析来自起始抗体的V_H和V_L序列,且人T细胞表位从示出与互补决定区(CDR)相关的表位和序列内其他关键残基的位置的各个V区域进行"映射(map)"。分析来自T细胞表位映射的各个T细胞表位,以便识别具有较小的改变最终抗体活性风险的可选氨基酸取代。设计一系列可选的V_H和V_L序列,包括氨基酸取代的组合,并且这些序列随后引入到一系列结合多肽中,例如用在本文公开的方法中的σ-2拮抗剂抗体或其免疫特异性片段,之后测试其功能。通常而言,产生并测试12～24个变体抗体。之后,将包括改性的V和人C区的完整重链和轻链基因克隆到表达载体中,随后质粒被引入至细胞系中,以用于产生整个抗体。之后将抗体在合适的生物化学和生物检定中进行比较,并识别最佳变体。

[0186] 用在本发明方法中的σ-2拮抗剂抗体或其片段可以通过任何本领域已知的适当方法产生。多克隆抗体可以通过本领域熟知的多种程序来产生。例如,σ-2多肽片段可以施用到多种宿主动物,包括但不限于兔、小鼠、大鼠等,以诱导含有对抗原特异的多克

隆抗体的血清的制备。多种佐剂可以根据宿主物种用于增加免疫学应答，包括但不限于Freund's（完整和非完整），矿物凝胶例如氢氧化铝，表面活性物质例如溶血卵磷脂、复合(pluronic)多元醇、聚阴离子、肽、油乳剂、钥孔虫戚血蓝蛋白、二硝基酚和潜在可用的人佐剂例如BCG（卡介苗）和短棒状杆菌。这样的佐剂也在领域内熟知。

[0187] 单克隆抗体可以使用本领域已知的多种技术进行制备，包括杂交瘤、重组和噬菌体展示技术或其组合的使用。例如，单克隆抗体可以使用杂交瘤技术进行制备，包括领域内所知的和例如在Harlow等人，Antibodies:A Laboratory Manual,Cold Spring Harbor Laboratory Press,2nd ed.(1988)；Hammerling等人，Monoclonal Antibodies and T-Cell Hybridomas Elsevier,N.Y.,563-681(1981)中教导的那些（所述参考文献全部并入本文以供参考）。本文使用的术语“单克隆抗体”不限于经由杂交瘤技术制备的抗体。术语“单克隆抗体”是指源自单个克隆（包括任何真核、原核或噬菌体克隆）而非制备其的方法的抗体。因此，术语“单克隆抗体”不限于经由杂交瘤技术制备的抗体。单克隆抗体可以使用本领域已知的多种技术制备，包括杂交瘤和重组和噬菌体展示技术的使用。

[0188] 在一些实施方式中，对σ-2受体特异的σ-2拮抗剂，例如单克隆抗体或其活性结合片段，可以使用本领域已知的任何可得技术经工程设计为增强跨越血脑屏障的能力。重组蛋白疗法通常无法用于脑输送，因为它们无法跨越血脑屏障；然而，本领域已知用于生物学疗法的脑输送的技术。例如，参见Pardridge and Boado,Reengineering biopharmaceuticals for targeted delivery across the blood-brain barrier,Methods Enzymol.2012;503:269-292，其并入本文以供参考。Pardridge和Boado报道，重组蛋白可以再工程设计为BBB渗透的IgG融合蛋白，其中IgG部分是针对内源性BBB受体例如人胰岛素受体(HIR)或转铁蛋白受体(TfR)的遗传工程单克隆抗体(MAb)。在融合蛋白疗法中，IgG与内源性胰岛素受体或TfR结合以引发跨越BBB的转运，并用作为分子特洛伊木马(MTH)以运送到脑中。IgG融合蛋白的药代动力学(PK)特性不同于典型的MAb药物，并由于快速被外周组织以及脑摄取而类似于小分子的PK分布。IgG融合蛋白的脑摄取，2～3%的注射剂量/脑，与小分子的脑摄取可比。IgG融合蛋白已经在小鼠模型中慢性施用，且免疫应答为低效价(titer)，且对体内的从血或脑摄取中清除融合蛋白没有作用。例如，Zhou等人使用“特洛伊木马”来将Abeta淀粉样蛋白抗体(AAA)再工程设计为与血脑屏障(BBB)分子特洛伊木马的融合蛋白。AAA被工程设计为单链Fv(ScFv)抗体，且ScFv融合到针对小鼠转铁蛋白受体(TfR)的嵌合单克隆抗体(Mab)的重链中。cTfRMAb-ScFv蛋白从血液经BBB TfR上的转运而穿透小鼠脑，且脑摄取在静脉施用后为3.5%的注射剂量/克脑。Zhou等人，Receptor-mediated Abeta Amyloid Antibody Targeting to Alzheimer's Disease Mouse Brain.Mol.Pharm.2011,Feb7;8(1):280-285。BBB MTH技术使得能够再工程设计广谱重组蛋白疗法，以用于对脑的靶向药物输送。

[0189] 在一些实施方式中，抗体可以通过Yu等人，2011的方法工程设计为增强脑摄取。由构成BBB的内皮细胞高度表达的靶标转铁蛋白受体的抗体已经被报道为经由受体介导的胞转作用而跨越BBB。该方法的一个问题是靶向转铁蛋白受体的高亲和性抗体可以降低抗体从CNS脉管系统释放的可能性。Yu等人设计了对转铁蛋白具有低亲和性的抗体，以增加抗体从脑脉管内皮释放，并增强对脑的摄取和分布。Yu报道，较低亲和性TfR抗体显示出增加的脑摄取。在一些实施方式中，σ-2受体抗体是经Y.Joy Yu等人，Sci Transl

Med3, 84ra44(2011) 和 US2012/0171120(各自并入本文以供参考)的方法制备的双特异性抗体,其中一个臂包括低亲和性转铁蛋白受体抗体,而另一个臂包括高亲和性 σ-2 受体抗体或 PGRMC1 抗体。

[0190] 在一些实施方式中,σ-2 拮抗剂选自任何 PGRMC1 抗体,或选自对结合 σ-2 受体特异且还阻断 Abeta 寡聚体结合或 Abeta 寡聚体诱导的突触功能障碍或用作为功能性神经元拮抗剂或阻断 Abeta 寡聚体结合和 Abeta 作用的任何抗体或其片段。

[0191] 在一些实施方式中,σ-2 受体抗体或其结合片段可以再工程设计为 BBB 渗透的 IgG 融合蛋白或结合体,其中 IgG 部分是针对内源性 BBB 受体例如人胰岛素受体 (HIR) 或转铁蛋白受体 (TfR) 的遗传工程单克隆抗体 (MAb)。σ-2 受体抗体或其片段经由例如壳聚糖纳米颗粒与 HIR 或 TfR Mabs 的结合物,或聚苹果酸结合物。参见,例如 Yemisci 等人 ,Transport of a caspase inhibitor across the blood-brain barrier by chitosan nanoparticles, Methods Enzymol., 2012 ;508:243-269 ; 和 Ding 等 人 , Inhibition of brain tumor growth by intravenous poly(b-L-malic acid)nanobioconjugate with a pH-dependent drug release. PNAS, 2010107(42)18143-18148,其各自并入本文以供参考。在一些实施方式中,针对进行跨越血脑屏障的细胞摄入的受体提出的单个结构域抗体被采用于融合蛋白或结合物中。参见,例如 Abulrob 等人 , J Neurochem2005, 95(4):1201-121, 其并入本文以供参考。

[0192] 在一些实施方式中,σ-2 受体抗体被提出 (raised) 为人膜相关的孕酮受体成分 1(人 PGRMC1) 或其同工型、同源物、变体、胞外结构域或片段的表位。例如,人 PGRMC1 的一个蛋白序列为 195 氨基酸 (aa) 蛋白 ;GI:48146103 :

[0193] maaedvvatg adpsdlesgg llheiftspl nllllglcif llykivrgdq paasgdsddd eppplprlkr rdftpaelrr fdgvqdpril maingkvfdv tkgrkfygpe gpygvfagrd asrglatfcl dkealkdeyd dlsdltaaqq etlsdwesqf tfkyhhvgkl lkegeeptvy sdeeepkdes arknd SEQ ID NO:1。

[0194] 例如,孕酮受体膜成分 1,同工型 CRA_a[智人],195aa 蛋白 ;GI:119610285 :

[0195] maaedvvatg adpsdlesgg llheiftspl nllllglcif llykivrgdq paasgdsddd eppplprlkr rdftpaelrr fdgvqdpril maingkvfdv tkgrkfygpe gpygvfagrd asrglatfcl dkealkdeyd dlsdltaaqq etlsdwesqf tfkyhhvgkl lkegeeptvy sdeeepkdes arknd SEQ ID NO:2。

[0196] 例如,孕酮受体膜成分 1,同工型 CRA_b[智人],170aa 蛋白 ;GI:119610286 :

[0197] maaedvvatg adpsdlesgg llheiftspl nllllglcif llykivrgdq paasgdsddd eppplprlkr rdftpaelrr fdgvqdpril maingkvfdv tkgrkfygpe gpygvfagrd asrglatfcl dkemrknqkm rvpgkmikaf sgsisifvfc kiicnsplcl SEQ ID NO:3。

[0198] 例如,孕酮受体膜成分 1,同工型 CRA_c[智人],143aa 蛋白 ;GI:119610287 :

[0199] maaedvvatg adpsdlesgg llheiftspl nllllglcif llykivrgdq paasgdsddd eppplprlkr rdftpaelrr fdgvqdpril maingkvfdv tkgrkfygpv kyhhvgkllk egeeptvysd eeepkdesar knd SEQ ID NO:4。

[0200] 人 PGRMC1 的同源物包括例如大鼠 PGRMC1。例如大鼠 PGRMC1,243aa 蛋白 ; GI:11120720 :

[0201] maaedvvatg adpseleggg llqeiftspl nllllglcif llykivrgdq pgasgdnddd eppplprlkp rdftpaelrr ydgvqdpril maingkvfdv tkgrkfygpe gpygvfagrd asrglatfcl dkealkdeyd dlsdltpaqq etlndwdsqf sspsstitwg kllegaeepi vysddeeqkm rllgrvteav sgaylflyfa ksfvtfqsvf ttw SEQ ID NO:5。

[0202] 另一个同源物是大鼠 PGRMC1，195aa 蛋白；GI:38303845：

[0203] maaedvvatg adpseleggg llqeiftspl nllllglcif llykivrgdq pgasgdnddd eppplprlkp rdftpaelrr ydgvqdpril maingkvfdv tkgrkfygpe gpygvfagrd asrglatfcl dkealkdeyd dlsdltpaqq etlndwdsqf tfkyhhvgkl lkegeeptvy sddeepkdea arksd SEQ ID NO:6。

[0204] 在一些实施方式中，特异性 σ-2 受体拮抗剂化合物是阻断可溶 Abeta 寡聚体与 σ-2 受体之间的结合的 σ-2 受体抗体。在一些实施方式中，σ-2 受体抗体识别与 PGRMC1 氨基酸序列相应的表位。在一些实施方式中，σ-2 受体特异性抗体可以对于与如下氨基酸序列相应的表位具有特异性，该氨基酸序列源自与 PGRMC1 相应的 N 端序列、C 端序列、内部序列或全长蛋白。在实施方式中，σ-2 受体特异性抗体可以对于与 SEQ ID NOs:1、2、3、4、5、6、7、8、9 或 10 中的一个或多个的结合具有特异性。在一些实施方式中，特异性 σ-2 受体拮抗剂化合物为识别合成肽 EPKDESARKND(SEQ ID NO:7)（与人 PGRMC1 的 C 端氨基酸 185 ～ 195 相应）的 PGRMC1 抗体。在一些实施方式中，σ-2 受体拮抗剂化合物不是对人 PGRMC1 蛋白的 N 端残基 1 ～ 46(MAAEDVVATGADPSDLESGGLLHEIFTSPLNLLLLGLCIFLLYKI(SEQ ID NO:9)，#sc-98680, Santa Cruz) 具有特异性的抗体。

[0205] 在一些实施方式中，σ-2 受体抗体包括针对或在任何情况下识别任何已知的全长 PGRMC1 蛋白或其任何变体、片段、免疫原或表位的那些；包括 PGRMC1 或其同源物、免疫原或变体的 N 端、中央片段或 C 端区域。分离的、纯化的或合成的蛋白或肽可以用作免疫原。蛋白或片段任选地通过本领域已知的多种方法成为佐剂或结合物，以增强免疫原性。PGRMC1 的异名包括孕酮结合蛋白、HPR6.6；HGNC:16090、孕酮受体膜结合成分 1 和 MPR。在一个实施方式中，片段或表位是 EPKDESARKND SEQ ID NO:7，与人 PGRMC1 的 C 端氨基酸 185 ～ 195 相应。该片段用于引出市售可得的山羊抗人 PGRMC1 多克隆抗体（例如，Abcam ab48012；Sigma-Aldrich SAB2500782；和 Everest Biotech, Ltd. EB07207)。另一片段由人 PGRMC1 的残基 50 ～ 150 组成，taaqq etlsdwesqf tfkyhhvgkl lkegeeptvy sdeeepkdes arknd(SEQ ID NO:10)；该片段通过本领域已知的方法与 KLH 结合；生成兔抗 PGRMC1 多克隆抗体；市售可得为 Abcam ab88948。其他市售可得的抗体包括 Santa Cruz Biotechnology sc-98680（针对 N 端 aa1 ～ 46(MAAEDVVATG ADPSDLESGG LLHEIFTSPL NLLLLGLCIF LLYKIV(SEQ ID NO:9)）提出的山羊抗人 PGRMC1 多克隆抗体）；sc-82694（针对内部表位提出的山羊抗人 PGRMC1 多克隆抗体）；sc-133906（针对合成 PGRMC1 肽提出的兔抗人 PGRMC1 多克隆抗体）；sc-135720(PGRMC1(12B7) 小鼠 Mab)；sc-271275（针对 N 端 1 ～ 46 提出的 PGRMC1(c-3) 小鼠 Mab)；以及针对膜相关的孕酮受体成分 1 重组蛋白表位信号标签 (PrEST) 提出的 Sigma-Aldrich HPA002877PGRMC1 兔多克隆抗体。

[0206] 针对 σ-1 受体（阿片受体，σ-1；Oprs1) 蛋白、片段、表位或免疫原提出的其他抗体被用于本文提供的实施例中。这样的 σ-1 受体抗体包括 Thermo Scientific PAS-12326（针对与 KLH 结合的 OPRS1 的 N 端区域提出的兔 σ-1 受体多克隆抗体）；针对

σ-1 受体的内部区域提出的 Santa Cruz Biotechnology, Inc. σ 受体 (L-20) sd-16203，山羊抗人；针对兔抗人全长 σ 受体 aa1 ～ 223 提出的 Santa Cruz Biothechnology, Inc. σ 受体 (FL-223) sd-20935；针对人 σ 受体的内部区域提出的 Santa Cruz Biotechnology, Inc. σ 受体 (S-18) sc-22948 山羊抗人多克隆抗体；对人 σ-1 受体的内部区的氨基酸 136 ～ 169 之间的表位定位具有特异性的 Santa Cruz Biotechnology, Inc. σ 受体 (B-5) sc-137075，小鼠单克隆抗体 (Mab)；以及针对全长人 σ-1 受体的氨基酸 1 ～ 223 提出的 Santa Cruz Biotechnology, Inc. σ 受体 (F-5) 小鼠单克隆抗体。

[0207] 人 σ-1 受体是 223aa 蛋白；GI:74752153：

[0208] mqwavgrrwa waalllavaa vltqvvwlwl gtqsfvfqre eiaqlarqya gldhelafsr livelrrlhp ghvlpdeelq wvfvnaggwm gamcllhasl seyvllfgta lgsrghsgry waeisdtiis gtfhqwregt tksevfypge tvvhgpgeat avewgpntwm veygrgvips tlafaladtv fstqdfltlf ytlrsyargl rlelttylfg qdp SEQ ID NO:8。

[0209] 在一些实施方式中，可以采用任何 σ-1 受体全长蛋白、同源物变体或片段，包括 N 端、C 端、中央区域，来通过本领域已知的任何方法来引出作为 σ-1 受体拮抗剂的抗体。

[0210] 在一些实施方式中，σ-2 拮抗剂是对 σ-2 受体具有高亲和性的小分子化合物。

[0211] 选择为 σ-2 受体拮抗的 σ-2 受体配体

[0212] 在一些实施方式中，用于本发明的 σ-2 受体拮抗剂选自还满足附加选择标准的 σ-2 受体配体化合物。附加标准用来从 σ-2 受体配体中选择用于本发明的 σ-2 受体拮抗剂。附加选择标准包括：关于抑制可溶 Aβ 寡聚体诱导的突触损失以及抑制膜运输检定中可溶 Aβ 寡聚体诱导的的缺陷，用作为神经元细胞中的功能性拮抗剂；与任何其他非 σ 受体相比，对一种或多种 σ 受体具有高选择性；表现出 σ-2 受体处的高亲和性；以及表现出较好的药物样特性，包括较好的脑渗透性、较好的代谢稳定性和较好的血浆稳定性。在一些实施方式中，σ-2 受体拮抗剂基于表现出一种或多种以下附加特性而进行进一步选择：在不存在 Abeta 寡聚体时不影响运输或突触数目；不诱导神经元细胞中的 caspase-3 活性；抑制由 σ-2 受体激动剂对 caspase-3 活性的诱导；和 / 或降低或使免受神经元细胞中由 σ-2 受体激动剂引起的神经元毒性。

[0213] 在一些实施方式中，经受进一步的选择标准的某些 σ-2 受体配体化合物选自本文描述的化合物并可以根据本文描述的方法或在 WO2011/014880（申请第 PCT/US2010/044136 号）、WO2010/118055（申请第 PCT/US2010/030130 号）、申请第 PCT/US2011/026530 号和 WO2012/106426 中描述的方法进行合成，各个申请全部并入本文以供参考。其他用于制备这些化合物的选项在以下详细讨论。

[0214] 在一些实施方式中，σ-2 配体是任选取代的哌嗪、苯基四氢呋喃 -N,N- 二甲基甲胺、二苯基四氢呋喃 -N,N- 二甲基甲胺、4- 苯基戊基 - 哌嗪、苄基苯基 - 哌嗪、吲哚 - 氧杂 - 氮杂螺 - 癸烷、哌啶 - 吲哚、苯基哌啶 - 吲哚、吡唑 - 吗啉、吡唑 - 哌啶、吡唑 -N,N- 二乙基乙胺、吡唑 - 吡咯烷、苯基 - 吡唑 - 吗啉、苯甲酰胺 - 喹啉化合物或其衍生物。

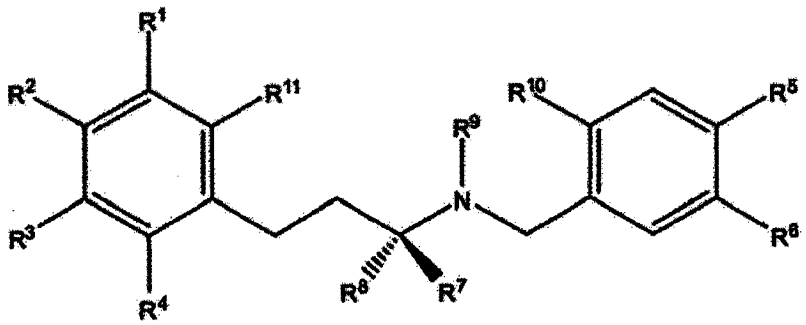
[0215] 在一些实施方式中，σ-2 配体为任选取代的 N-(4-(3,4- 二氢异喹啉 -2(1H)- 基）丁基）苯甲酰胺、1- 环己基 -4-(3-(1,2,3,4- 四氢萘 -1- 基）丙基）哌嗪、(5,5- 二苯基四氢呋喃 -3- 基）甲胺、1-(5,5- 二苯基四氢呋喃 -3- 基）-N,N- 二甲基甲胺、N,N- 二甲基 -1-(5- 苯基四氢呋喃 -3- 基）甲胺、1-(4- 苯基丁基）哌嗪、1-(4- 苄基苯基）-4- 甲

基哌嗪、环己基-4-(4-苯基环己基)哌嗪、1-环己基-4-(4-苯基环己基)哌嗪、环己基-4-(3-(1,2,3,4-四氢萘-1-基)丙基)哌嗪、1-环己基-4-(3-(1,2,3,4-四氢萘-1-基)丙基)哌嗪、8-(2-(4,5,6,7-四氢-1H-吲哚-4-基)乙基)-2-氧杂-8-氮杂螺[4.5]癸烷、4-(2-(4-苯基哌啶-1-基)乙基)-4,5,6,7-四氢-1H-吲哚、4-(2-(1H-吡唑-3-基氧)烷基)吗啉、4-(2-(1H-吡唑-3-基氧)乙基)吗啉、1-(2-(1H-吡唑-3-基氧)乙基)哌啶、1-(2-(1H-吡唑-3-基氧)烷基)哌啶、2-(1H-吡唑-3-基氧)-N,N-二乙基乙胺、2-(1H-吡唑-3-基氧)-N,N-二烷基-烷胺、3-(2-(吡咯烷-1-基)乙氧基)-1H-吡唑、3-(2-(吡咯烷-1-基)烷氧基)-1H-吡唑、1-(1H-吡唑-3-基氧)-3-(哌嗪-1-基)丙-2-醇、1-(1H-吡唑-3-基氧)-3-(哌嗪-1-基)烷-2-醇、1-(1-芳基-1H-吡唑-3-基氧)-3-(哌嗪-1-基)烷-2-醇、1-(1-苯基-1H-吡唑-3-基氧)-3-(哌嗪-1-基)烷-2-醇、1-(1-芳基-1H-吡唑-3-基氧)-3-(哌嗪-1-基)丙-2-醇、1-(1-杂芳基-1H-吡唑-3-基氧)-3-(哌嗪-1-基)烷-2-醇、1-(1-(二卤代芳基)-1H-吡唑-3-基氧)-3-(哌嗪-1-基)烷-2-醇、1-(1-二卤代杂芳基)-1H-吡唑-3-基氧)-3-(哌嗪-1-基)烷-2-醇、1-(1-(3,4-二卤代芳基)-1H-吡唑-3-基氧)-3-(哌啶-1-基)烷-2-醇、1-(1-(3,4-二卤代杂芳基)-1H-吡唑-3-基氧)-3-(哌啶-1-基)烷-2-醇、1-(1-(二卤代芳基)-1H-吡唑-3-基氧)-3-(二烷基氨基)烷-2-醇、1-(1-(二卤代杂芳基)-1H-吡唑-3-基氧)-3-(二烷基氨基)烷-2-醇、1-(1-(二氯杂芳基)-1H-吡唑-3-基氧)-3-(二烷基氨基)烷-2-醇、N-(4-(3,4-二氢异喹啉-2(1H)-基)丁基)苯甲酰胺、N-(4-(3,4-二氢异喹啉-2(1H)-基)烷基)苯甲酰胺、N-(4-(3,4-二氢异喹啉-2(1H)-基)烷基)-2-羟基苯甲酰胺、N-(4-(6,7-二甲氧基-3,4-二氢异喹啉-2(1H)-基)丁基)苯甲酰胺、N-(4-(6,7-二烷氧基-3,4-二氢异喹啉-2(1H)-基)烷基)苯甲酰胺、N-(4-(6,7-二甲氧基-3,4-二氢异喹啉-2(1H)-基)丁基)-2-萘甲酰胺、N-(4-(6,7-二烷氧基-3,4-二氢异喹啉-2(1H)-基)烷基)-2-萘甲酰胺、N-(4-(3,4-二氢异喹啉-2(1H)-基)丁基)-2-萘甲酰胺、N-(4-(3,4-二氢异喹啉-2(1H)-基)烷基)-2-萘甲酰胺、N-(2-(3,4-二氢异喹啉-2(1H)-基)乙基)-2,3-二甲氧基苯甲酰胺、N-(2-(3,4-二氢异喹啉-2(1H)-基)烷基)-2,3-二烷氧基苯甲酰胺、N-(2-(3,4-二氢异喹啉-2(1H)-基)乙基)苯甲酰胺、N-(2-(3,4-二氢异喹啉-2(1H)-基)烷基)苯甲酰胺、N-(2-(4-(2,3-二氯苯基)哌啶-1-基)乙基)-2-萘甲酰胺、N-(2-(4-(2,3-二卤代芳基)哌啶-1-基)烷基)-2-萘甲酰胺、2,3-二甲氧基-N-(4-(4-苯基哌啶-1-基)丁基)苯甲酰胺、2,3-二烷氧基-N-(4-(4-苯基哌啶-1-基)烷基)苯甲酰胺、N-(4-(4-(2,3-二卤代苯基)哌啶-1-基)烷基)-2,3-二烷氧基苯甲酰胺、5-卤代-N-(4-(4-(2,3-二卤代苯基)哌啶-1-基)烷基)-2,3-二烷氧基苯甲酰胺(其中2、3&5位卤素相同或独立地为F、Cl、Br或I)、N-(4-(6,7-二甲氧基-3,4-二氢异喹啉-2(1H)-基)丁基)-2-(2-氟乙氧基)-5-碘-3-甲氧基苯甲酰胺、N-(4-(6,7-二烷氧基-3,4-二氢异喹啉-2(1H)-基)烷基)-2-(2-卤代乙氧基)-5-卤代-3-甲氧基苯甲酰胺(其中卤素取代基相同或独立地为F、Cl、Br或I)、9-苄基-9-氮杂双环[3.3.1]壬-3-基苯基氨基甲酸酯、9-苄基-9-氮杂双环[3.3.1]壬-3-基2-烷氧基苯基氨基甲酸酯、9-(5-苯基烷基)-9-氮杂双环[3.3.1]壬-3-基2-甲氧基-5-甲基苯基氨基甲酸酯、9-烷基-9-氮杂双环[3.3.1]壬-3-基苯基氨基甲酸酯、3-(2-(4-环己基哌嗪-1-基)烷基)苯并[d]噁唑-2(3H)-酮、6-乙酰

基 -3-(4-(4- 环己基哌嗪 -1- 基) 烷基) 苯并 [d] 噁唑 -2(3H)- 酮、1- 苄基 -4-(1,2- 二苯基乙基) 哌嗪、1- 芳基 -4-(1,2- 二苯基乙基) 哌嗪、2-(6- 氧 -5- 苯基 -3,3a,6,6a- 四氢环戊 [c] 吡咯 -2(1H)- 基) 丙酸乙酯、2-(5- 烷基 -6- 氧 -3,3a,6,6a- 四氢环戊 [c] 吡咯 -2(1H)- 基) 丙酸乙酯、2-((3-(2H- 萘并 [1,8-cd] 异噻唑 -2- 基) 烷基)(甲基) 氨基) 烷醇、2-((1-(2-(1- 烷基 -1H- 吡咯 -2- 基)-2- 氧乙基) 哌啶 -4- 基) 烷基) 异吲哚啉 -1- 酮、2-((1-(2- 氧烷基) 哌啶 -4- 基) 烷基) 异吲哚啉 -1- 酮、1'-(4-(1-(4- 卤代芳基)-1H- 吲哚 -3- 基) 烷基)-3H- 螺 [异苯并呋喃 -1,4'- 哌啶]、1'-(4-(1-(4- 卤代杂芳基)-1H- 吲哚 -3- 基) 烷基)-3H- 螺 [异苯并呋喃 -1,4'- 哌啶]、3-(3- 烷基丁 -2- 炔基)-1,2,3,4,5,6- 六氢苯并 [d] 吖辛因 -8- 醇、戊唑辛化合物、或其类似物或衍生物。

[0216] 在一些实施方式中，σ -2 配体包括式 I 的化合物或其药学上可接受的盐：

[0217]



I

[0218] 其中：

[0219] R^1、R^2、R^3、R^4 和 R^{11} 各自独立地选自 H、OH、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基和 NH(C_{1-4} 烷基)；

[0220] R^5 和 R^6 各自独立地选自 H、C_{1-6} 卤代烷基、C_{1-6} 烷基和 C_{3-7} 环烷基和 NH(C_{1-4} 烷基)；

[0221] R^7 和 R^8 各自独立地选自 H、C_{1-6} 烷基、C_{1-6} 卤代烷基和 C_{3-7} 环烷基；

[0222] 或者 R^2 和 R^3 与它们连接的 C 原子一起形成任选地被 1、2、3、4 或 5 个独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代的 4 ～ 8 元环烷基、芳基、杂芳基、杂芳烷基、或杂环烷基，且 R^2 和 R^3 各自独立地选自键、C、N、S 和 O；

[0223] 或者 R^9 和 R^{10} 与它们连接的 N 和 C 原子一起形成任选地被 1、2、3、4 或 5 个独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代的 4 ～ 8 元杂环烷基或杂芳基，且 R^9 和 R^{10} 各自独立地选自键、C、N、S 和 O；

[0224] 或者 R^9 和 R^{11} 与它们连接的 N 和 C 原子一起形成任选地被 1、2、3、4 或 5 个独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代的 6 ～ 8 元杂环烷基或杂芳基，且 R^9 和 R^{11} 各自独立地选自键、C、N、S 和 O；

[0225] 或者 R^1 和 R^{11} 与它们连接的 C 原子一起形成任选地被 1、2、3、4 或 5 个独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂

芳烷基、环烷基和杂环烷基的取代基所取代的 4、5、6、7 或 8 元环烷基、杂环烷基、芳基或杂芳基，且 R^1 和 R^{11} 各自独立地选自键、C、N、S 和 O；

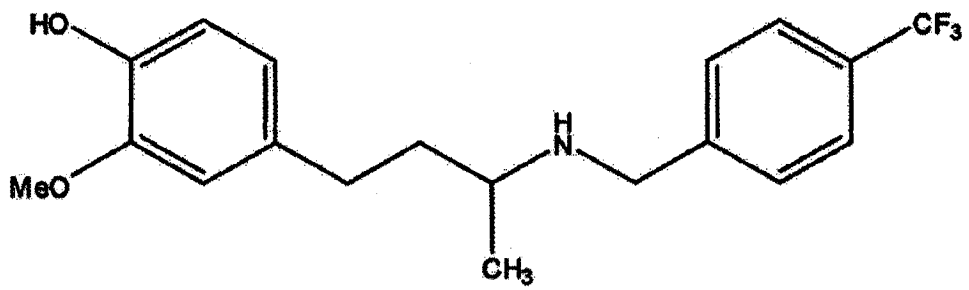
[0226] 或者 R^1 和 R^2 与它们连接的 C 原子一起形成任选地被 1、2、3、4 或 5 个独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代的 4、5、6、7 或 8 元环烷基、杂环烷基、芳基或杂芳基，且 R^1 和 R^2 各自独立地选自键、C、N、S 和 O；

[0227] 或者 R^3 和 R^4 与它们连接的 C 原子一起形成任选地被 1、2、3、4 或 5 个独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代的 4、5、6、7 或 8 元环烷基、杂环烷基、芳基或杂芳基，且 R^3 和 R^4 各自独立地选自键、C、N、S 和 O；

[0228] 其中 O、C_{1-6} 烷基、C_{1-6} 卤代烷基、杂芳基、芳基、杂芳基、杂环烷基和环烷基中的每一个任选且独立地被 1、2、3、4 或 5 个独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代。

[0229] 在一些实施方式中，σ -2 配体包括化合物 II 的外消旋混合物或对映异构体：

[0230]



II.

[0231] 在一些实施方式中，σ -2 配体是式 I 的化合物或药学上可接受的盐，其中 R^2 和 R^3 独立地选自 OH 和 C_{1-6} 烷氧基。

[0232] 在一些实施方式中，σ -2 配体是式 I 的化合物或药学上可接受的盐，其中 R^2 和 R^3 独立地选自 OH 和 NH(C_{1-4} 烷基)。

[0233] 在一些实施方式中，σ -2 配体是式 I 的化合物或药学上可接受的盐，其中 R^2 和 R^3 独立地选自 H、卤素和 C_{1-6} 卤代烷基。

[0234] 在一些实施方式中，σ -2 配体是式 I 的化合物或药学上可接受的盐，其中 R^2 和 R^3 各自独立地选自 OH 和 C_{1-6} 烷氧基，且 R^7 和 R^8 各自独立地为 C_{1-6} 烷基。在一些实施方式中，R^7 和 R^8 各自为甲基。

[0235] 在一些实施方式中，σ -2 配体是式 I 的化合物或药学上可接受的盐，其中 R^5 和 R^6 各自独立地选自 H 和 C_{1-6} 卤代烷基。

[0236] 在一些实施方式中，σ -2 配体是式 I 的化合物或药学上可接受的盐，其中 R^9 为 H。

[0237] 在一些实施方式中，σ -2 配体是式 I 的化合物或药学上可接受的盐，其中 R^2 和 R^3 或 R^3 和 R^4 与它们连接的 C 碳原子一起形成 6 元环烷基、环杂烷基、芳基或杂芳基环。在一些实施方式中，R^2 和 R^3 为 O。

[0238] 在一些实施方式中，σ-2 配体是式 I 的化合物或药学上可接受的盐，其中 R^7 为 C_{1-6} 烷基且 R^8 为 H。

[0239] 在一些实施方式中，σ-2 配体是式 I 的化合物或药学上可接受的盐，其中 R^7 为 H 且 R^8 为 C_{1-6} 烷基。

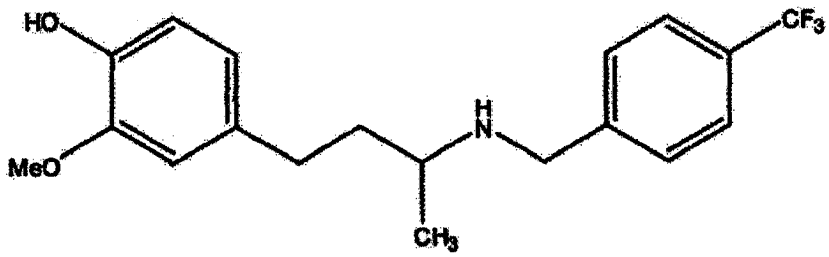
[0240] 在一些实施方式中，σ-2 配体是式 I 的化合物或药学上可接受的盐，其中 R^2 和 R^3 独立地选自 H、OH、卤素、C_{1-6} 烷氧基和 C_{1-6} 卤代烷基。

[0241] 在一些实施方式中，σ-2 配体是式 I 的化合物或药学上可接受的盐，其中 R^2 和 R^3 独立地选自 H、OH、Cl、F、-OMe 和 $-CF_3$。

[0242] 在一些实施方式中，σ-2 配体是式 I 的化合物或药学上可接受的盐，其中 R^2 和 R^3 独立地选自 H、OH、Cl、F、-OMe 和 $-CF_3$，其中 R^7 和 R^8 各自独立地选自 H 和 C_{1-6} 烷基，其中 R^9 为 H，且其中 R^5 和 R^6 各自独立地选自 H 和 C_{1-6} 卤代烷基。

[0243] 在一些实施方式中，式 I 的化合物是化合物 II 的基本纯的 (+) 或 (-) 对映异构体：

[0244]

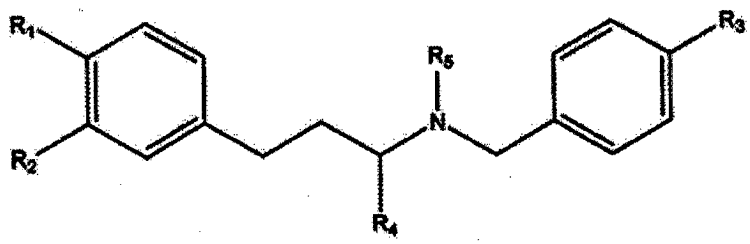


化合物 II

[0245] 其中，基本纯的对映异构体包括至少 90、91、92、93、94、95、96、97、98 或 99％的化合物 II 的一种对映异构体。在一些实施方式中，包括基本纯的化合物 II 对映异构体的组合物为至少 99.5％的一种对映异构体，且在其他实施方式中，组合物仅包括化合物 II 的一种对映异构体。

[0246] 在一个更具体的实施方式中，本发明的 σ-2 配体为由式 III 表示的新化合物：

[0247]



III

[0248] 其中，

[0249] R_1 和 R_2 独立地选自 H、OH、卤素、CN、NO_2、NH_2、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、C_{3-7} 环烷基、NH(C_{1-4} 烷基)、N(C_{1-4} 烷基)$_2$、NH(C_{3-7} 环烷基)、NHC(O)(C_{1-4} 烷基)、(C_{1-4} 烷基)$_2$N-C_{1-4} 亚甲基-O-、SH、S(C_{1-6} 烷基)、C(O)OH、C(O)O(C_{1-4} 烷基)、C(O)(C_{1-4} 烷基)、和 C(O)NH(C_{1-4} 烷基)，或者 R1 和 R2 连接在一起形成 -O-C_{1-4} 亚甲基-O- 基团，且其

中 R1 和 R2 中的至少一个不是 H；

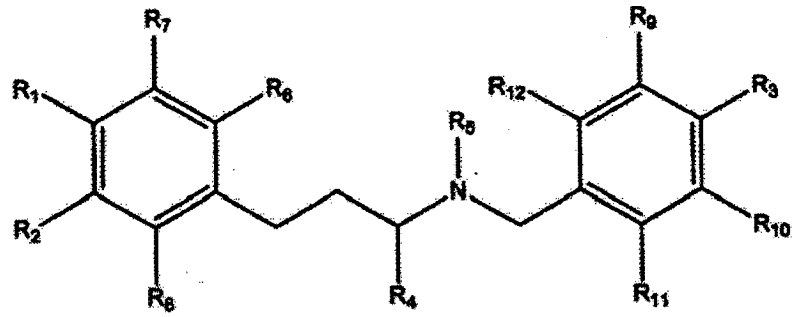
[0250] R_3 选自 H、OH、卤素、CN、NO_2、NH_2、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、C_{3-7} 环烷基、NH(C_{1-4} 烷基）、N(C_{1-4} 烷基）$_2$、NH(C_{3-7} 环烷基）、NHC(O)(C_{1-4} 烷基）、SH、S(C_{1-6} 烷基）、C(O)OH、C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）和 C(O)NH(C_{1-4} 烷基）；

[0251] R4 为 C_{1-6} 烷基；且

[0252] R_5 为 H、C_{1-6} 烷基和 C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）和 C(O)(C_{1-4} 卤代烷基）。

[0253] 在另一更加具体的实施方式中，本发明的 σ-2 配体为由式 IV 表示的新化合物：

[0254]



IV

[0255] 其中

[0256] R_1、R_2、R_6、R_7 和 R_8 独立地选自 H、OH、卤素、CN、NO_2、NH_2、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、C_{3-7} 环烷基、NH(C_{1-4} 烷基）、NH(C_{1-4} 烷基）$_2$、NH(C_{3-7} 环烷基）、NHC(O)(C_{1-4} 烷基）、SH、S(C_{1-6} 烷基）、C(O)OH、C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）和 C(O)NH(C_{1-4} 烷基），或者 R1 和 R2 连接在一起形成 -O-C_{1-4} 亚甲基 -O-，且其中 R_1、R_2、R_6、R_7 和 R_8 中的至少一个不是 H；

[0257] R_3 选自 H、卤素和 C_{1-6} 卤代烷基；

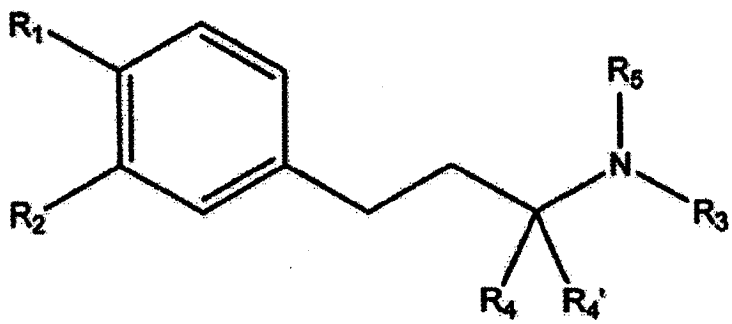
[0258] R_9、R_{10}、R_{11} 和 R_{12} 独立地选自 H、C_{1-6} 烷氧基和卤素。

[0259] R_4 为 C_{1-6} 烷基；且

[0260] R_5 为 H、C_{1-6} 烷基和 C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）、C(O)(C_{1-4} 卤代烷基）。

[0261] 在一些实施方式中，本发明的 σ-2 配体为式 Va 的那些或其药学上可接受的盐：

[0262]

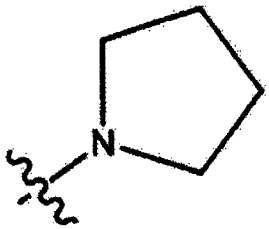


Va

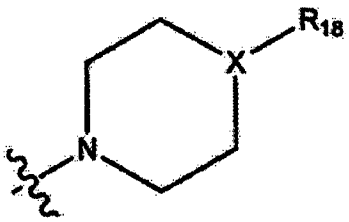
[0263] 其中，

[0264] R_1 和 R_2 独立地选自 H、OH、卤素、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、$(R_{16})(R_{17})N-C_{1-4}$ 亚烷基 -O-，或者 R1 和 R2 连接在一起形成 $-O-C_{1-2}$ 亚甲基 -O- 基团，其中

[0265] R_{16} 和 R_{17} 独立地为 C_{1-4} 烷基或苄基，或者 R_{16} 和 R_{17} 与氮一起形成选自



和



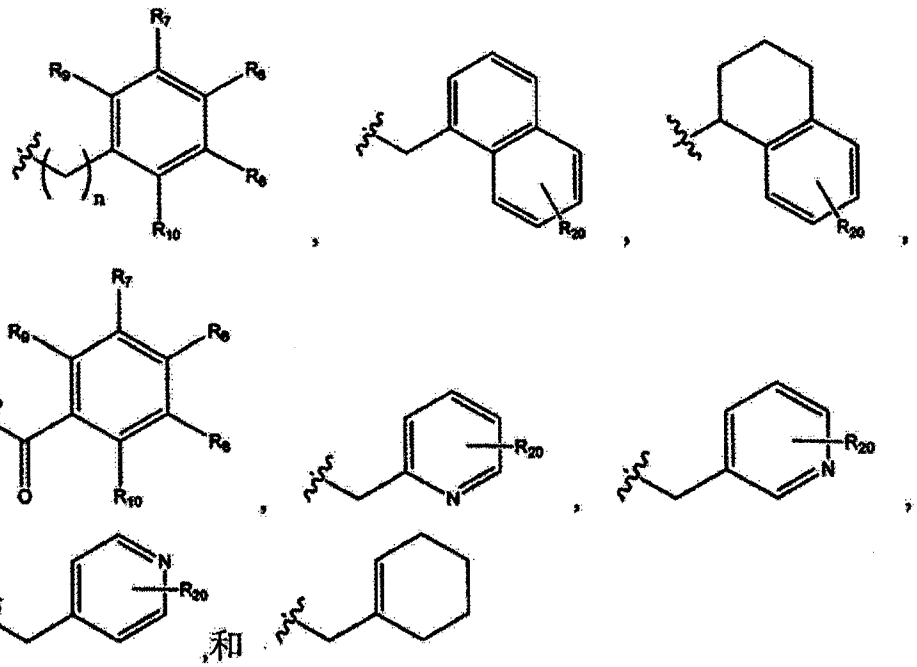
的环，其中

[0266] X 是 N 或 O，且 R_{18} 为 H 或未被取代的苯基；且

[0267] 其中 R_1 和 R_2 中的至少一个不是 H；

[0268] R_3 选自

[0269]



和

[0270] 其中

[0271] R_6、R_7、R_8、R_9 和 R_{10} 独立地选自 H、卤素、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基和 $S(O)_2-C_{1-6}$ 烷基；

[0272] R_{20} 为 H；且

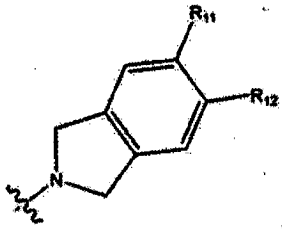
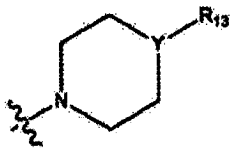
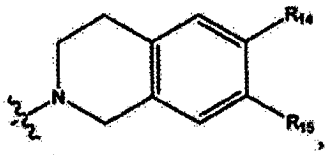
[0273] n 为 1 ～ 4，

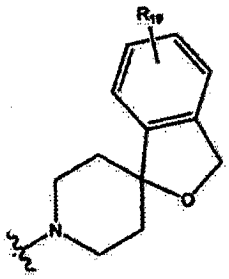
[0274] R_4 为 C_{1-6} 烷基；

[0275] $R_{4'}$ 为 H 或 C_{1-6} 烷基；且

[0276] R_5 为 H、C_{1-6} 烷基和 $C(O)O(C_{1-4}$ 烷基）、$C(O)(C_{1-4}$ 烷基）或 $C(O)(C_{1-4}$ 卤代烷基）；或

[0277] R_3 和 R_5 与氮一起形成选自

[0278]    和

 的环，其中

[0279] R_{11} 和 R_{12} 独立地选自 H、卤素和 C_{1-6} 卤代烷基，且

[0280] Y 为 CH 或 N；

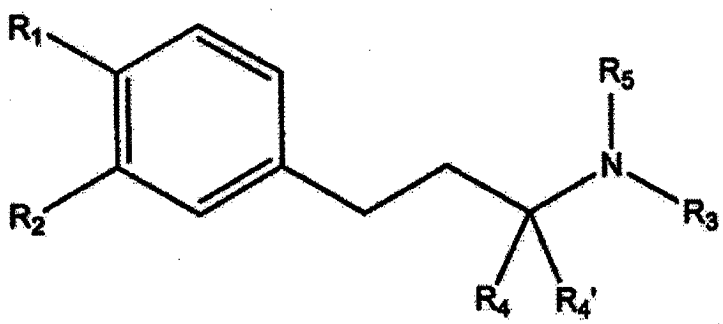
[0281] R_{13} 为 H、C_{1-6} 烷基、C_{3-6} 环烷基、未被取代的苯基或被 C_{1-6} 卤代烷基取代的苯基、或未被取代的苄基；

[0282] R_{14} 和 R_{15} 独立地选自 H 和卤素；

[0283] R_{19} 为 H。

[0284] 在一些实施方式中，本发明的 σ-2 配体为式 Va 的那些或其药学上可接受的盐：

[0285]

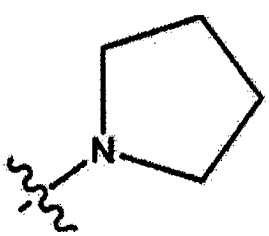
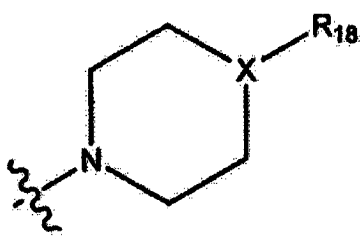


Va

[0286] 其中

[0287] R_1 和 R_2 独立地选自 H、OH、卤素、C_{1-6} 烷氧基、C_{1-6} 卤代烷基、C_{1-6} 卤代烷氧基、$(R_{16})(R_{17})N-C_{1-4}$ 亚烷基 -O-，或者 R1 和 R2 连接在一起形成 $-O-C_{1-2}$ 亚甲基 -O- 基团，其中

[0288] R_{16} 和 R_{17} 独立地为 C_{1-4} 烷基或苄基，或者 R_{16} 和 R_{17} 与氮一起形成选自

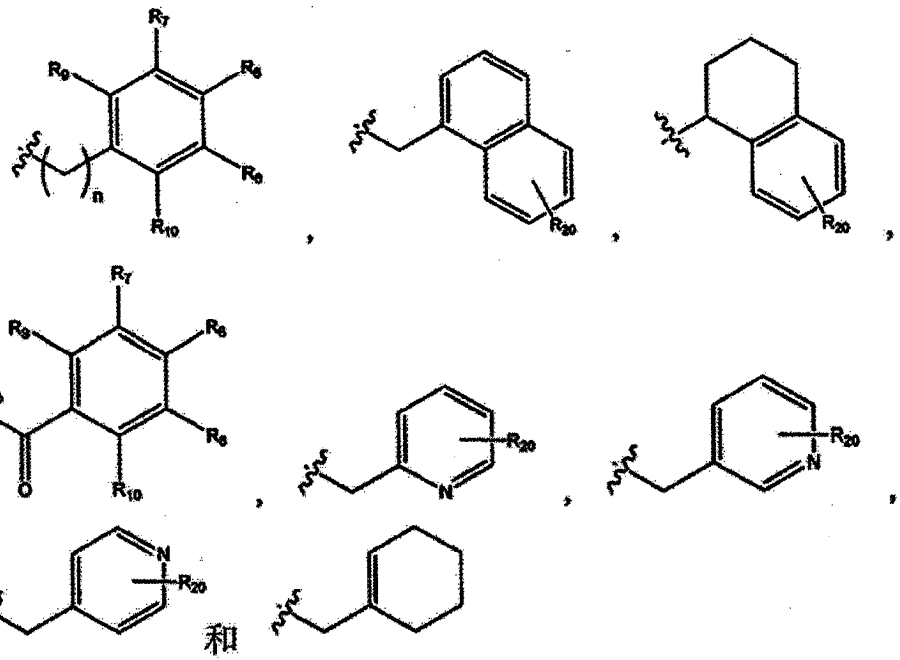
 和  的环，其中

[0289] X 为 N 或 O，且 R_{18} 不存在或为 H 或未被取代的苯基；且

[0290] 其中 R_1 和 R_2 中的至少一个不是 H；

[0291] R_3 选自

[0292]



和

[0293] 其中

[0294] R_6、R_7、R_8、R_9 和 R_{10} 独立地选自 H、卤素、C_{1-6} 烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷基和 $S(O)_2$-C_{1-6} 烷基；

[0295] R_{20} 为 H；且

[0296] n 为 1 ～ 4，

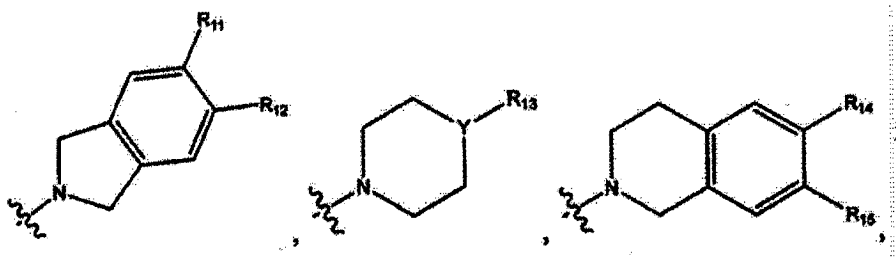
[0297] R_4 为 C_{1-6} 烷基；

[0298] $C_{4'}$ 为 H 或 C_{1-6} 烷基；且

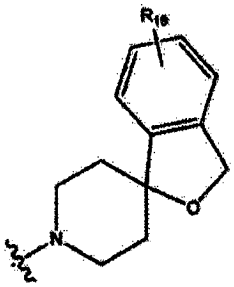
[0299] R_5 为 H、C_{1-6} 烷基和 C(O)O(C_{1-4} 烷基)、C(O)(C_{1-4} 烷基) 或 C(O)(C_{1-4} 卤代烷基)；或

[0300] R_3 和 R_5 与氮一起形成选自

[0301]



和



的环，其中

[0302] R_{11} 和 R_{12} 独立地选自 H、卤素和 C_{1-6} 卤代烷基，且

[0303] Y 是 CH 或 N；

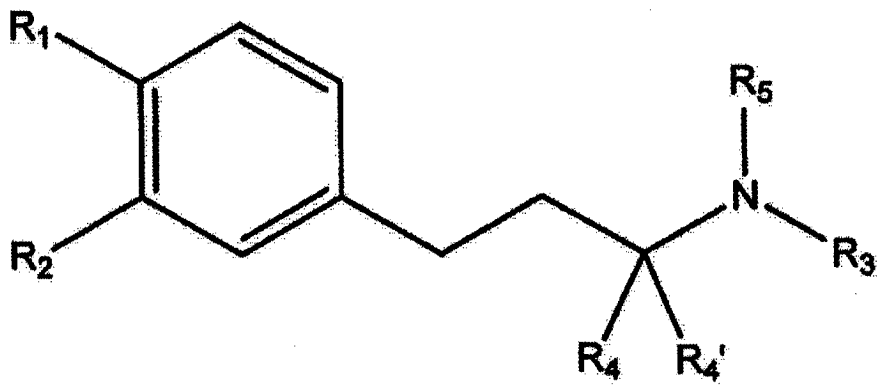
[0304] R_{13} 是 H、C_{1-6} 烷基、C_{3-6} 环烷基、未被取代的苯基或被 C_{1-6} 卤代烷基取代的苯基或未被取代的苄基；

[0305] R_{14} 和 R_{15} 独立地选自 H 和卤素；且

[0306] R_{19} 为 H。

[0307] 在一些实施方式中，本发明的 σ-2 配体为式 Va 的那些或其药学上可接受的盐：

[0308]

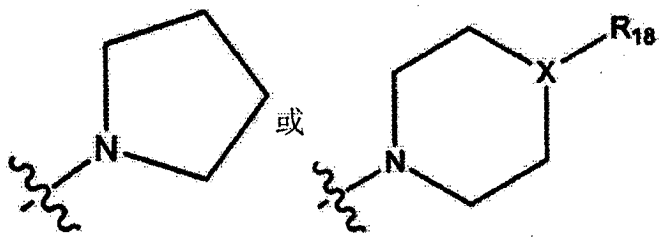


Va

[0309] 其中

[0310] R_1 选自 OH、OMe、F、Cl、CF_3、$(R_{16})(R_{17})N-$ 亚乙基 -O-，其中

[0311] R_{16} 和 R_{17} 各自为甲基、异丙基、正丁基或苄基，或者 R_{16} 和 R_{17} 与氮一起形成选自



或

的环，其中

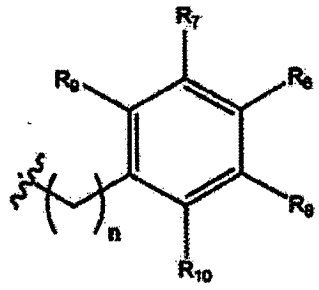
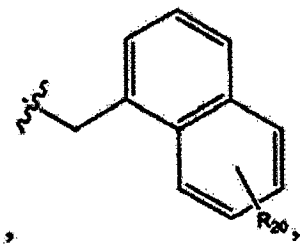
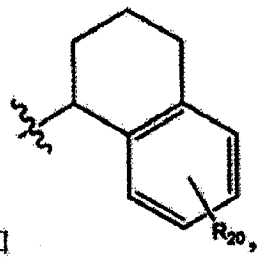
[0312] X 为 N 或 O，且 R_{18} 不存在或为未被取代的苯基；且

[0313] R_2 为 H、Cl、F、CF_3、OMe、OCF_3 或

[0314] R_1 和 R_2 连接在一起形成 $-O-C_{1-2}$ 亚甲基 -O- 基团，

[0315] R_3 选自

[0316]



和


[0317] 其中，

[0318] R_6 为 H、F、Cl、Me、异丙基、叔丁基、OMe、CF_3、或 $S(O)_2Me$，

[0319] R_7 和 R_8 独立地为 H、OMe、F、Cl 或 CF_3，

[0320] R_9 和 R_{10} 独立地选自 H、OMe、F 和 Cl，

[0321] R_{20} 为 H；且

[0322] n 为 1，

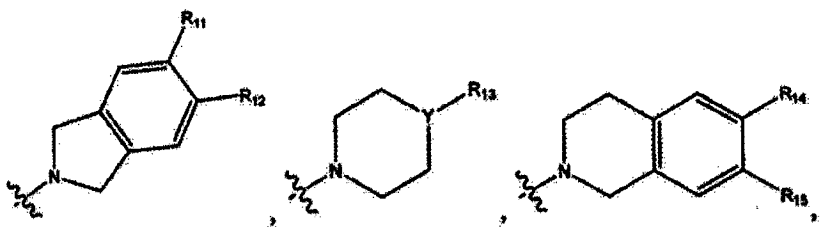
[0323] R_4 为 Me；

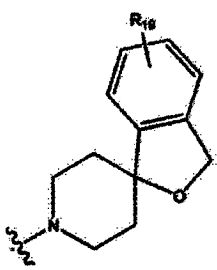
[0324] $R_{4'}$ 为 H 或 Me；且

[0325] R_5 为 H；或者

[0326] R_3 和 R_5 与氮一起形成选自

[0327]



[0328] 和  的环，其中

[0329] R_{11} 和 R_{12} 独立地选自 H、Cl 和 CF_3，且

[0330] Y 为 CH 或 N；

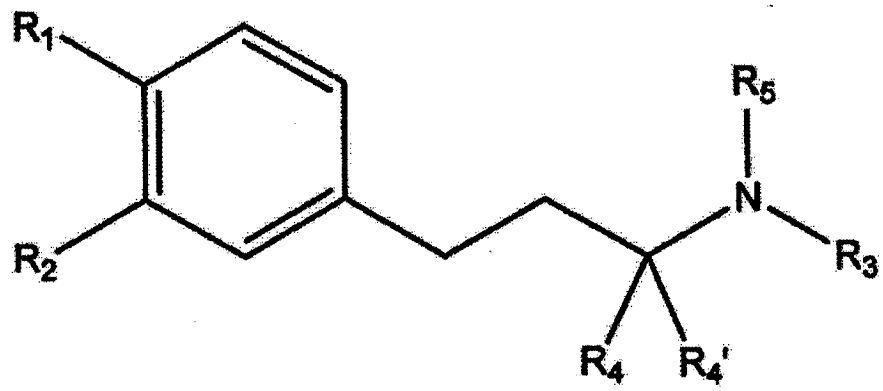
[0331] R_{13} 为 H、Me、环己基、未被取代的苯基或被 CF_3 取代的苯基或未被取代的苄基，

[0332] R_{14} 和 R_{15} 独立地选自 H 和 Cl；且

[0333] R_{19} 为 H。

[0334] 在一些实施方式中，本发明的 σ-2 配体为式 Va 的那些或其药学上可接受的盐：

[0335]

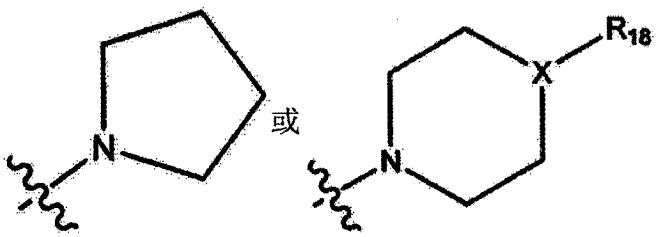


Va

[0336] 其中

[0337] R_1 选自 OH、OMe、F、Cl、CF_3、$(R_{16})(R_{17})N$- 亚乙基 -O-，其中

[0338] R_{16} 和 R_{17} 各自为甲基、异丙基、正丁基或苄基，或者 R_{16} 和 R_{17} 与氮一起形成选自



的环，其中

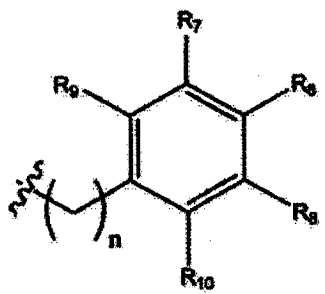
[0339] X 为 N 或 O，且 R_{18} 不存在或为未被取代的苯基；且

[0340] R_2 为 H、Cl、F、CF_3、OMe、OCF_3 或

[0341] R_1 和 R_2 连接在一起形成 -O-C_{1-2} 亚甲基 -O- 基团

[0342] R_3 选自

[0343]



[0344] 其中，

[0345] R_6 为 H、F、Cl、Me、异丙基、叔丁基、OMe、CF_3、或 $S(O)_2Me$，

[0346] R_7 和 R_8 独立地为 H、OMe、F、Cl 或 CF_3，

[0347] R_9 和 R_{10} 独立地选自 H、OMe、F 和 Cl，且

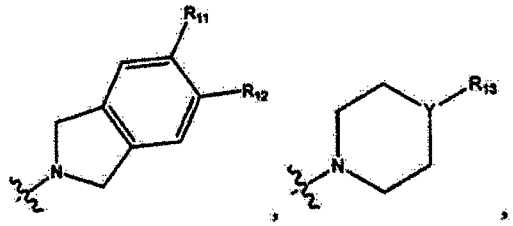
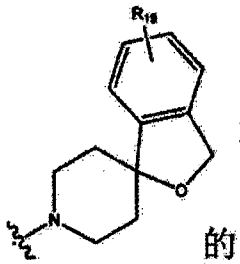
[0348] n 为 1

[0349] R_4 为 Me；

[0350] $R_{4'}$ 为 H；且

[0351] R_5 为 H；或者

[0352] R_3 和 R_5 与氮一起形成选自

[0353]  和  环，其中 的

[0354] R_{11} 和 R_{12} 独立地选自 H、Cl 和 CF_3，且

[0355] Y 为 CH 或 N；

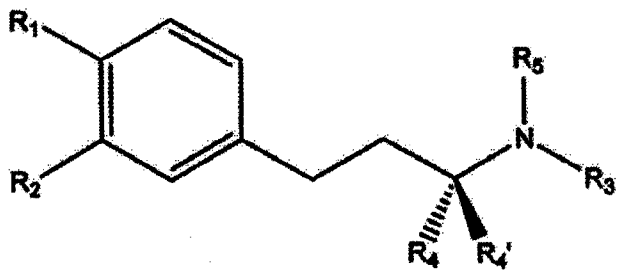
[0356] R_{13} 为 H、Me、环己基、未被取代的苯基或被 CF_3 取代的苯基或未被取代的苄基，

[0357] R_{14} 和 R_{15} 独立地选自 H 和 Cl；且

[0358] R_{19} 为 H。

[0359] 在一些更加具体的实施方式中，本发明的 σ-2 配体为式 Vb 的那些或其药学上可接受的盐：

[0360]

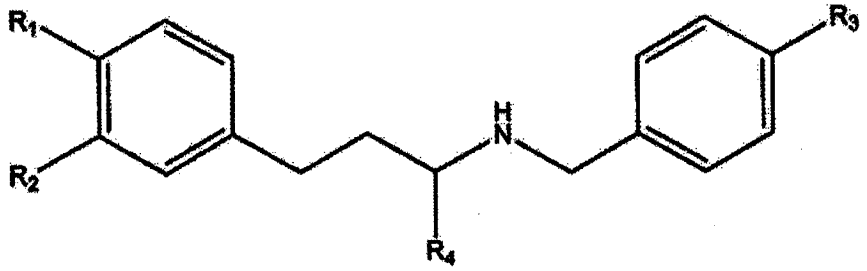


Vb

[0361] 其中 $R_{4'}$ 为 H，且剩余的基团如以上针对式 Va 的化合物所定义。

[0362] 在一些实施方式中，本发明的 σ-2 配体为式 IIIa 的那些或其药学上可接受的盐：

[0363]



IIIa

[0364] 其中

[0365] R_1 = 卤素、C_{1-6} 卤代烷基或 OH；

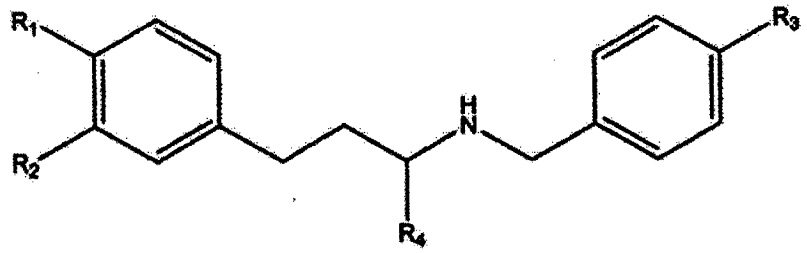
[0366] R_2 = H、卤素或 C_{1-6} 卤代烷基，或 R_1 和 R_2 连接在一起形成 -O- 亚甲基 -O- 基团；

[0367] R_3 = C_{1-6} 卤代烷基；且

[0368] R_4 = C_{1-6} 烷基。

[0369] 在一些更加具体的实施方式中，本发明的 σ-2 配体为式 IIIa 那些或其药学上可接受的盐：

[0370]



IIIa

[0371] 其中

[0372] R_1 = Cl、F、CF_3 或 OH；

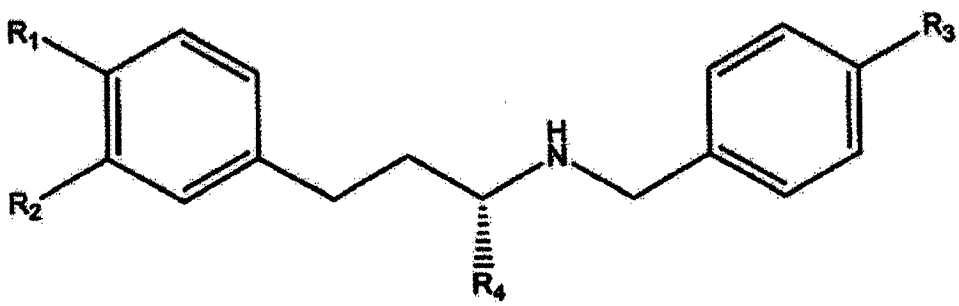
[0373] R_2 = H、Cl、F、CF_3，或者 R_1 和 R_2 连接在一起形成 -O- 亚乙基 -O- 基团；

[0374] R_3 = CF_3；且

[0375] R_4 = 甲基。

[0376] 在一些更加具体的实施方式中，本发明的 σ-2 配体为式 IIIb 那些或其药学上可接受的盐：

[0377]



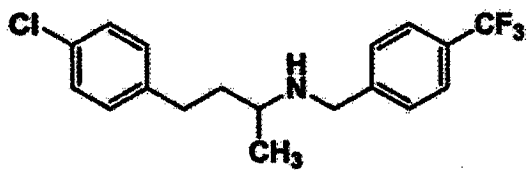
IIIc

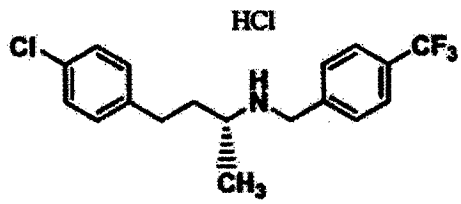
[0378] 其中 R_1 至 R_4 如以上针对式 IIIa 的化合物所定义。

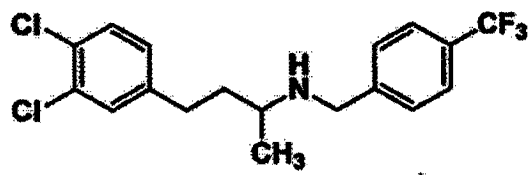
[0379] 式 III 和 IV 及 IIIa 和 IIIb 以及其他化合物的具体示例性化合物列在以下表 1B 中。

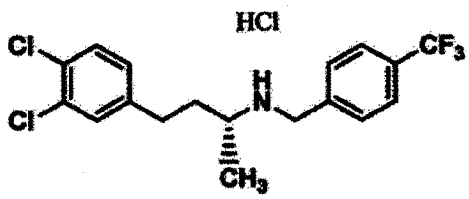
表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物

[0380]









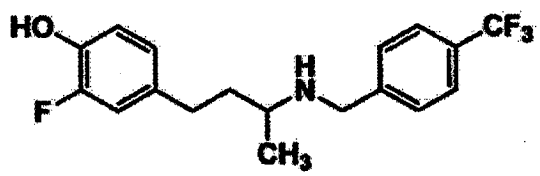
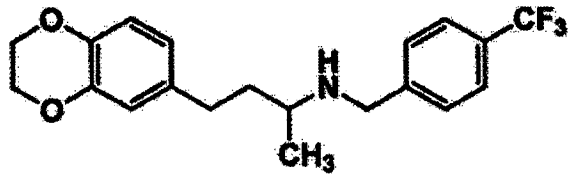
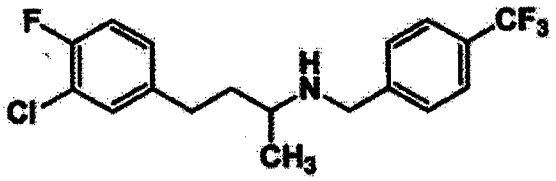
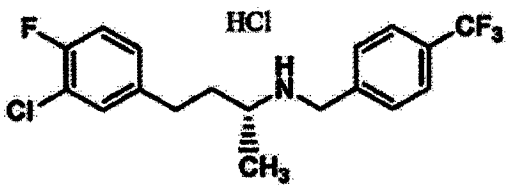


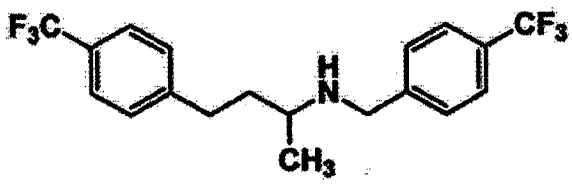
表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物

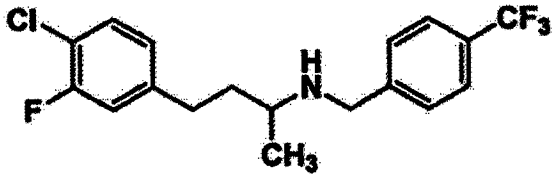


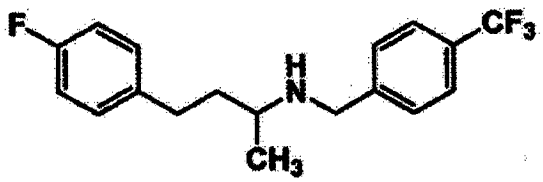


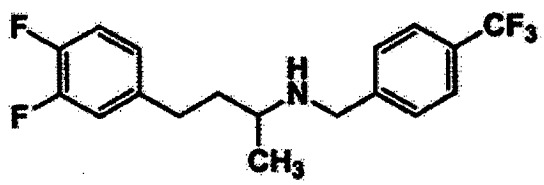


[0381]



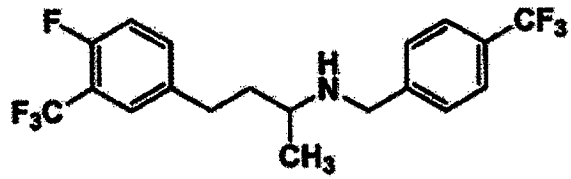
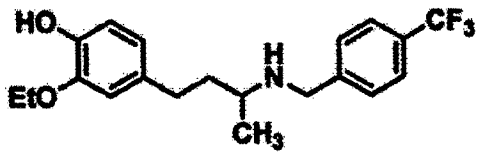
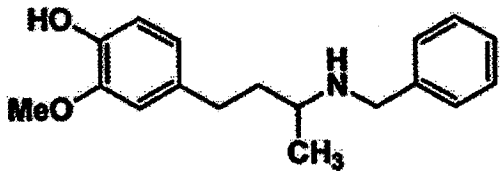
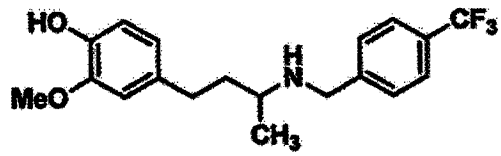
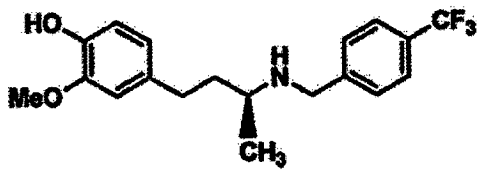
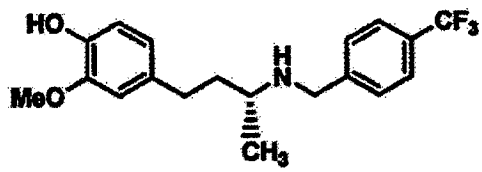
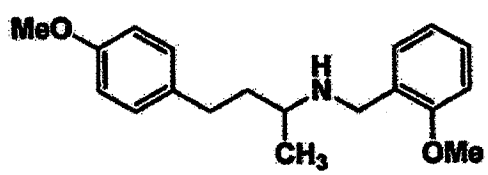






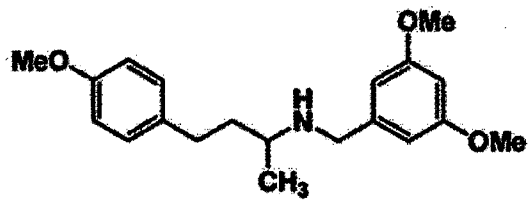
[0382]

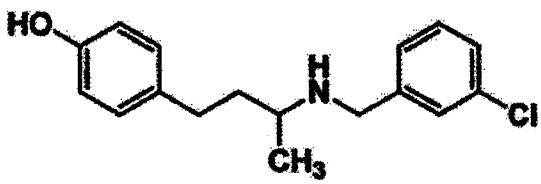
表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物

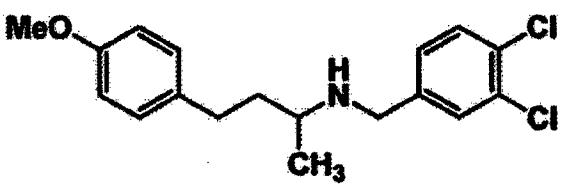








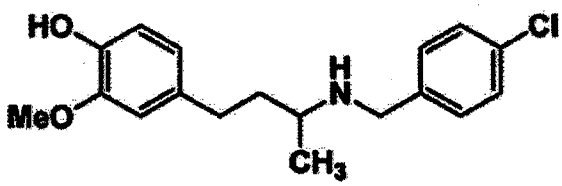
[0383]

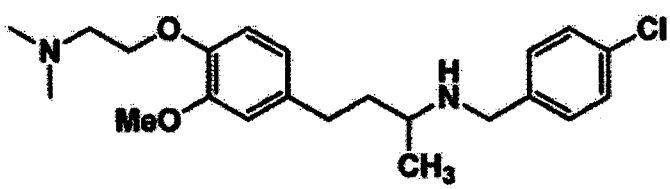
表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物





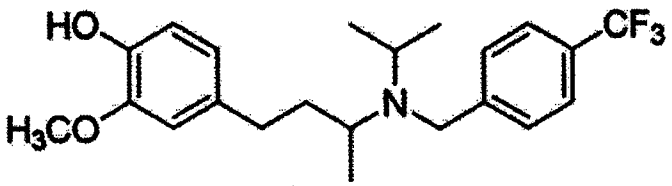


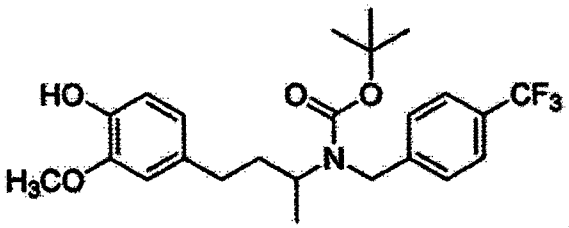




[0384]

表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物





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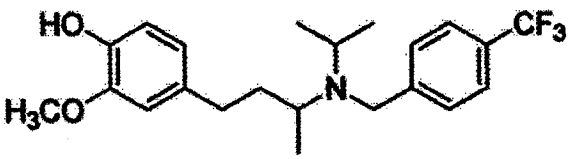

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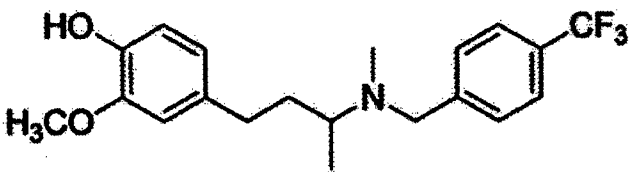

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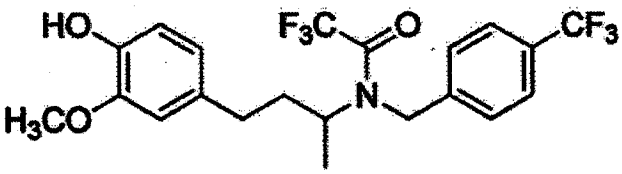

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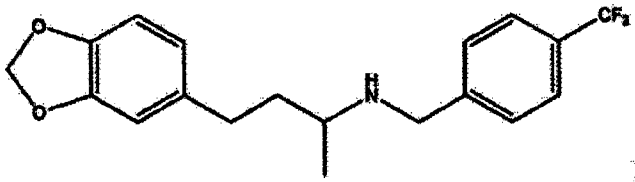

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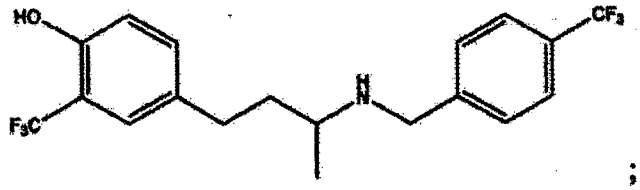
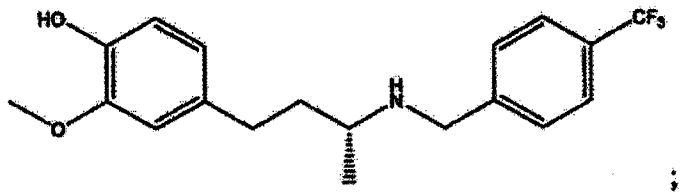
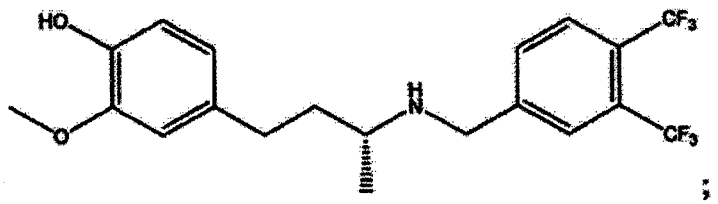
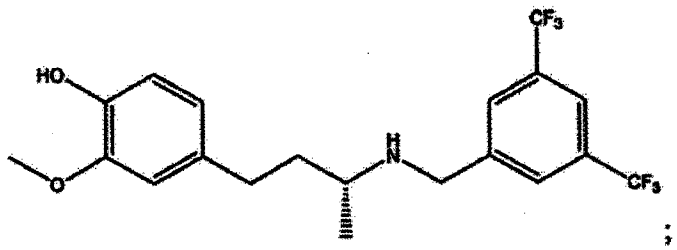
表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物

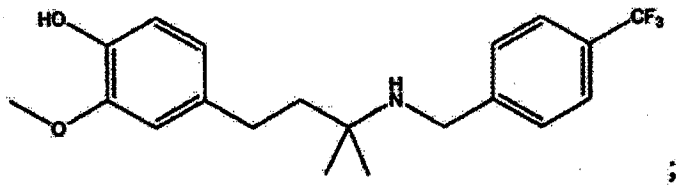
[0385]











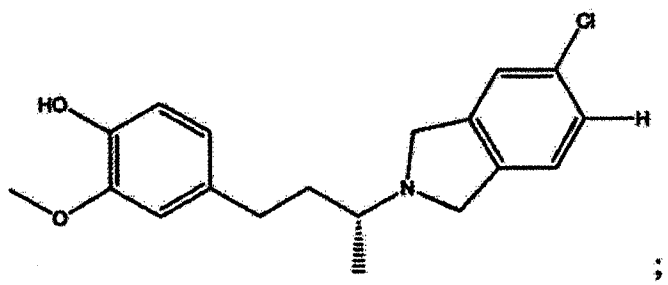
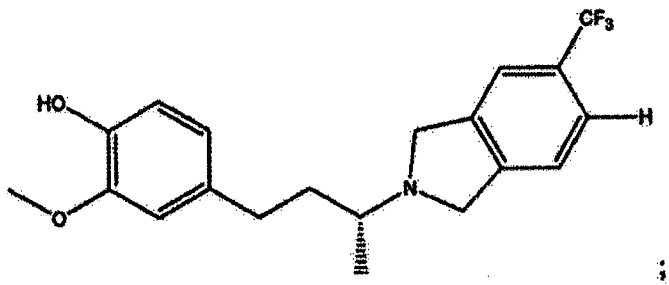


表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物

[0386]



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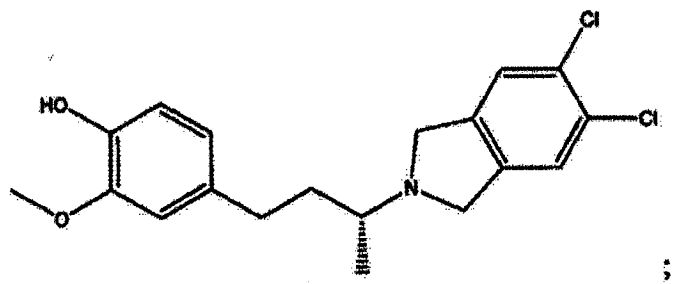

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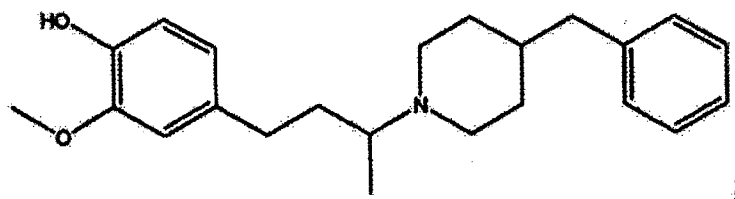

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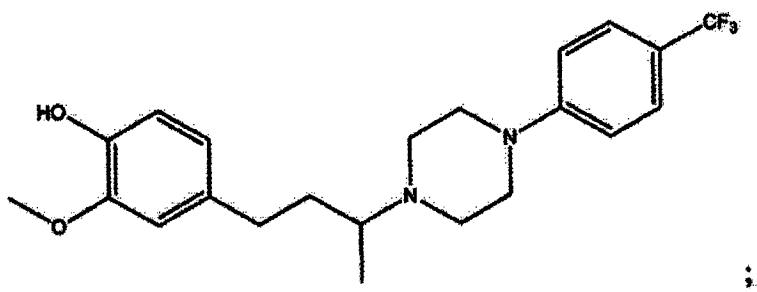

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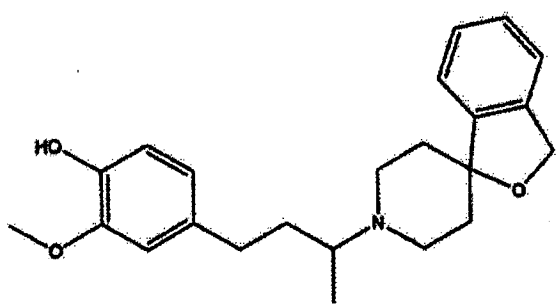

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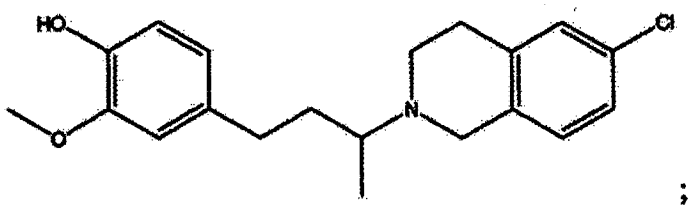
[0387]

表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物



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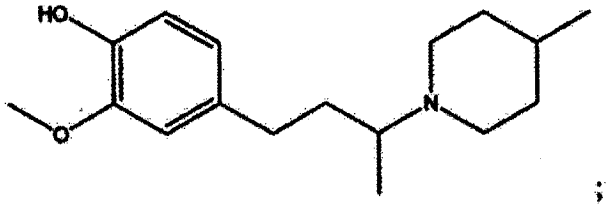

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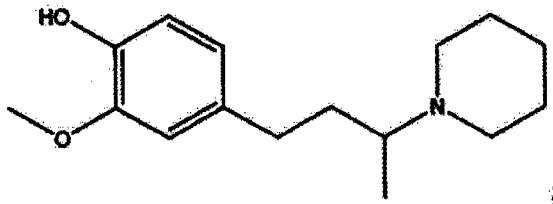

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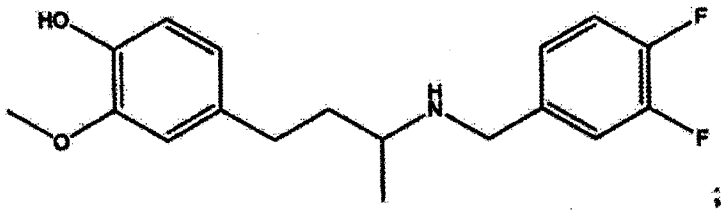

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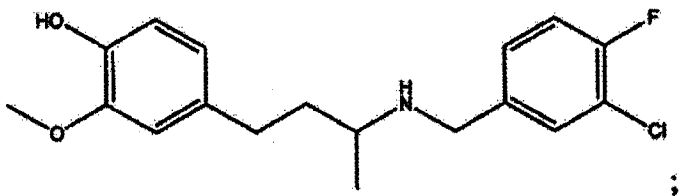

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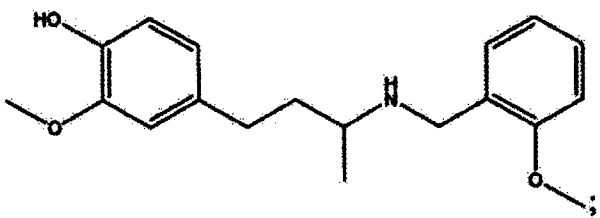

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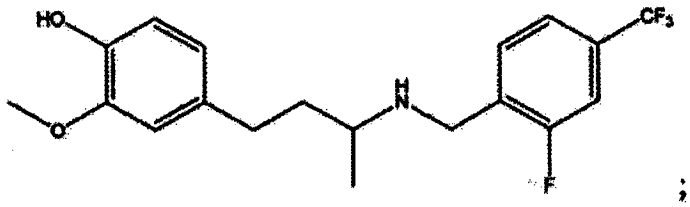
[0388]

表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物



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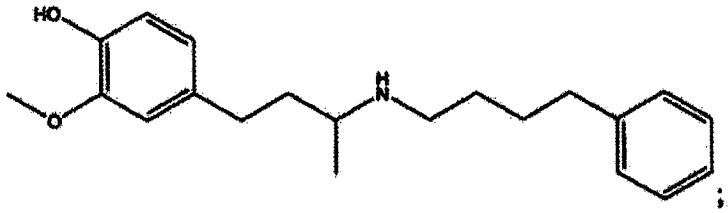

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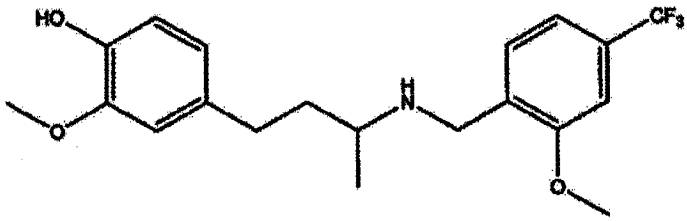

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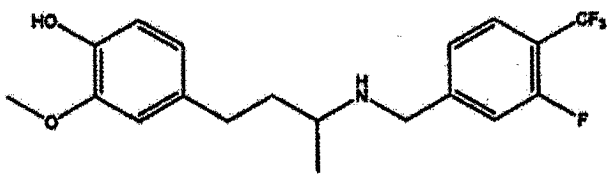

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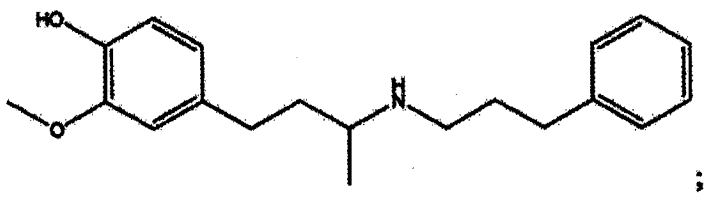

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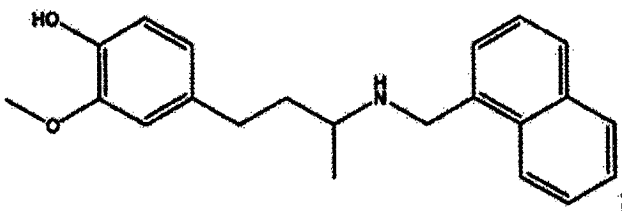

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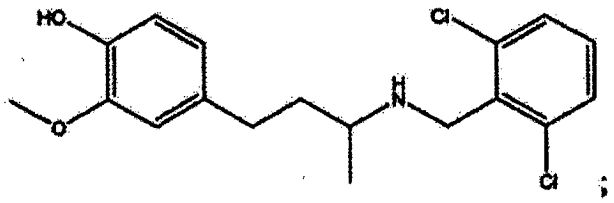
表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物

[0389]



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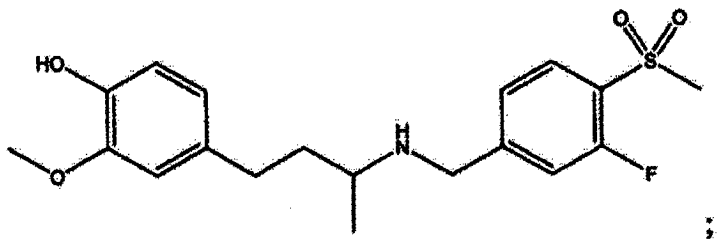

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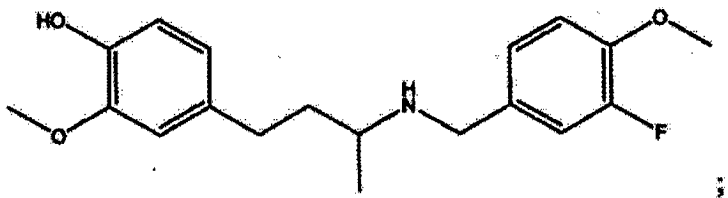

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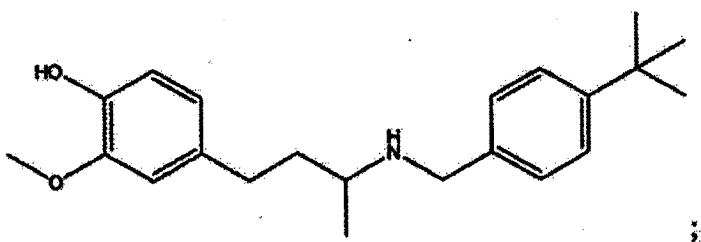

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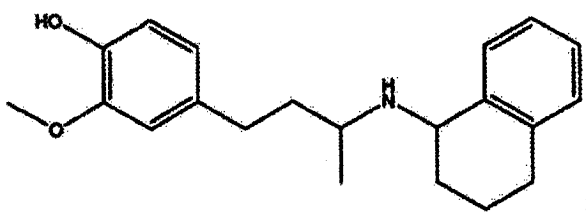

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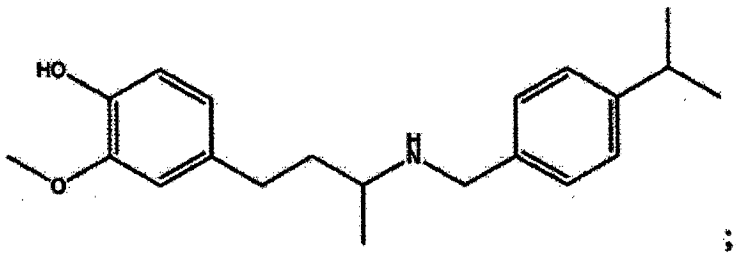
[0390]

表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物



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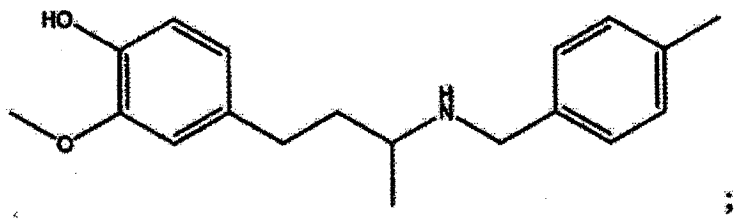

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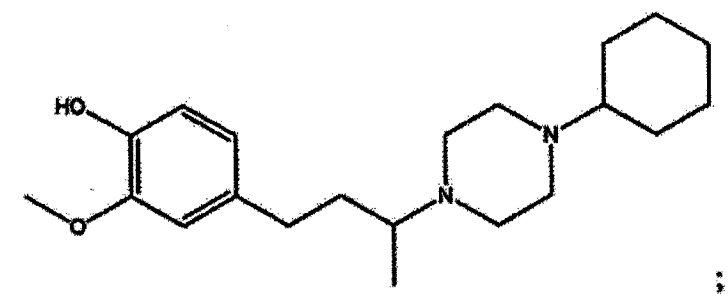

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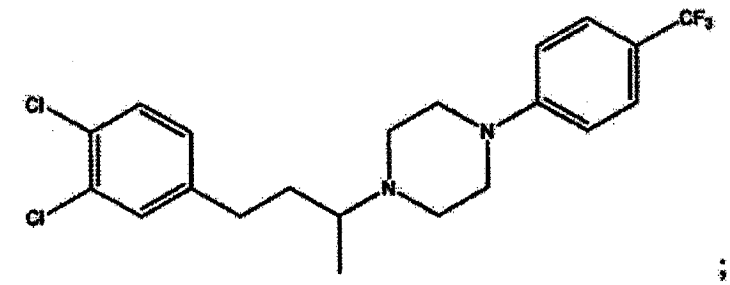

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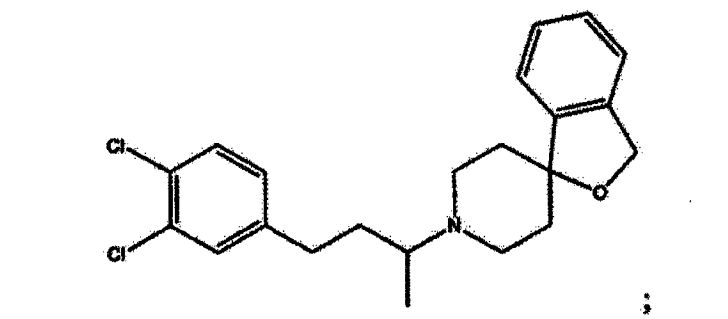

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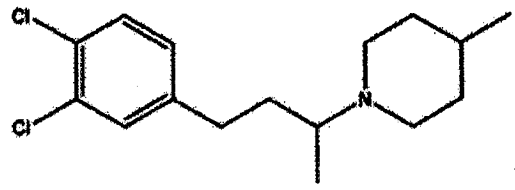
[0391]

表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物



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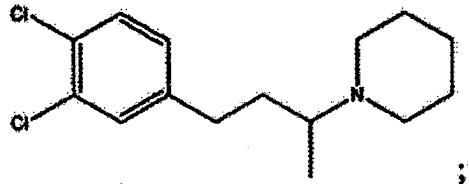

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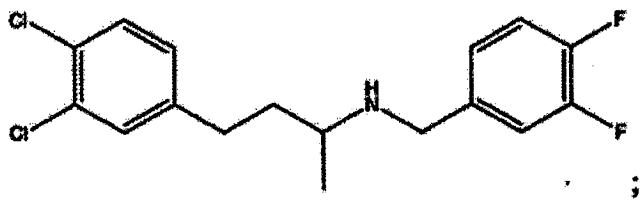

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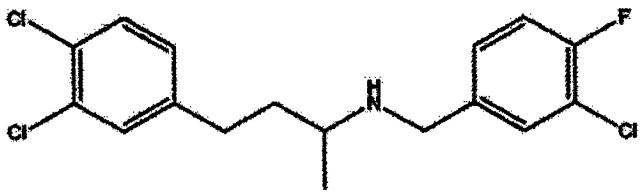

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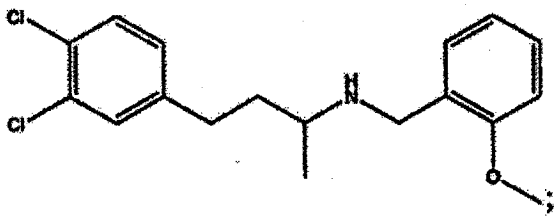

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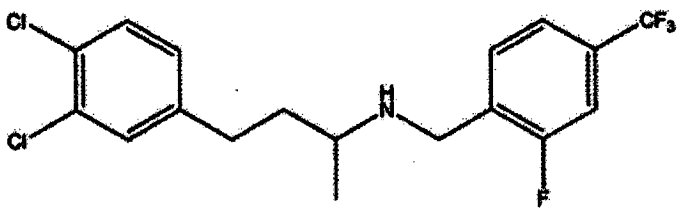

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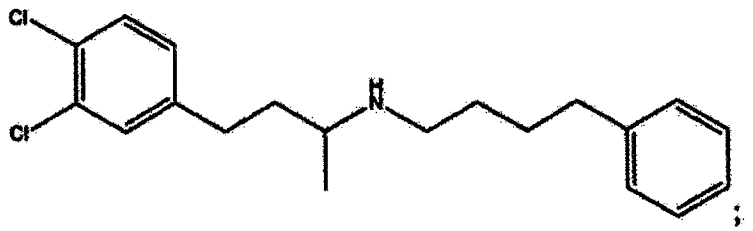
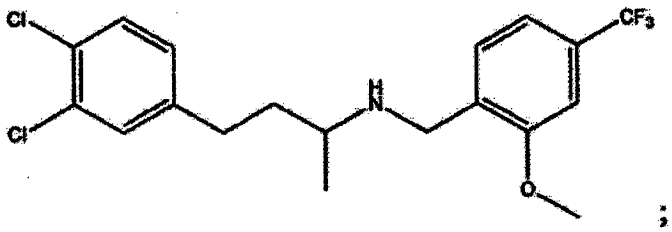
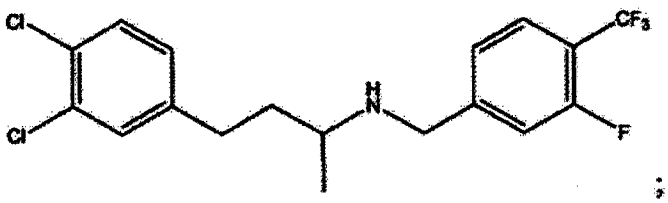
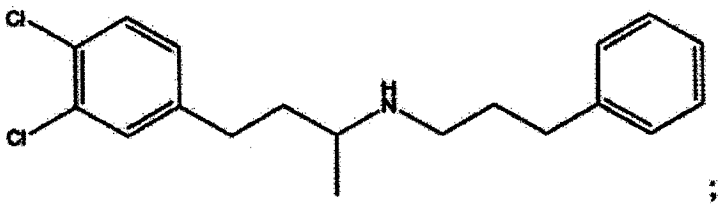
表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物

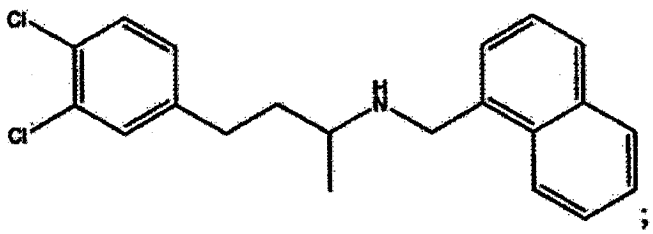
[0392]











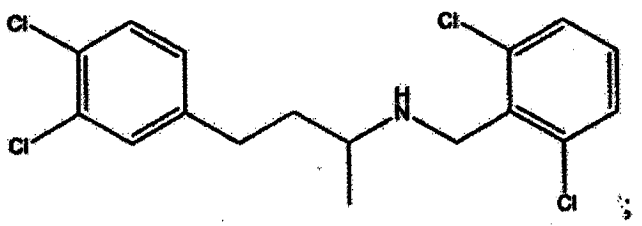
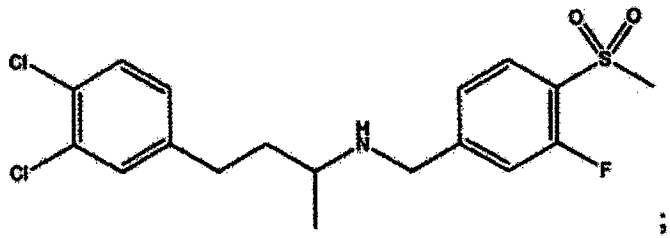


表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物



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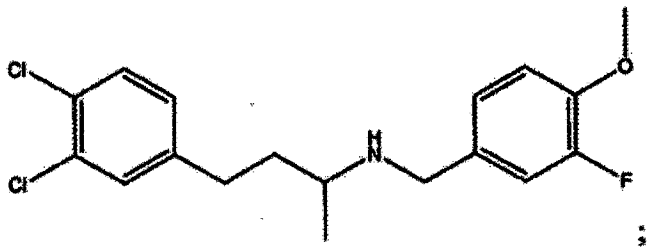

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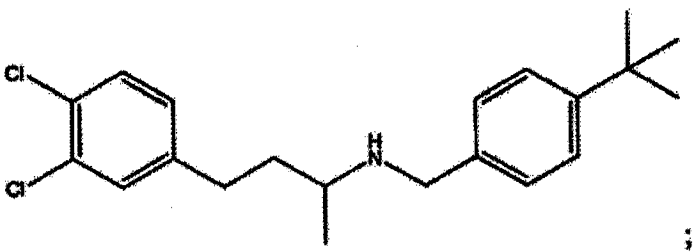
[0393]



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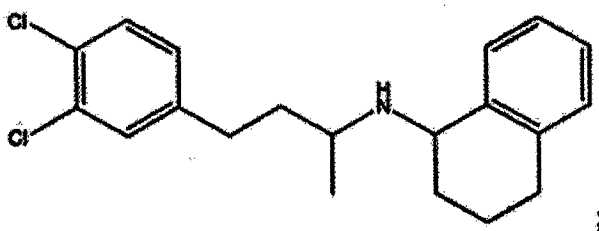

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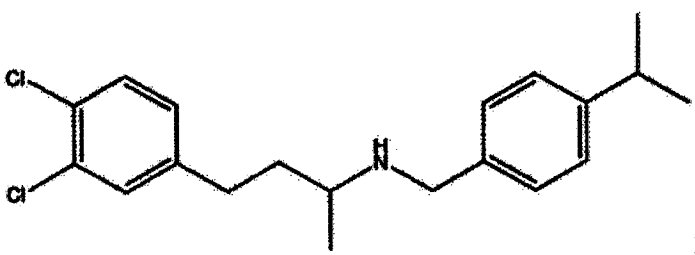

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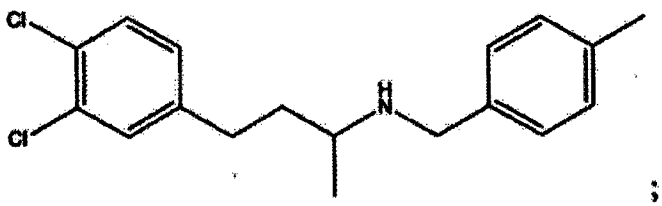
[0394]

表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物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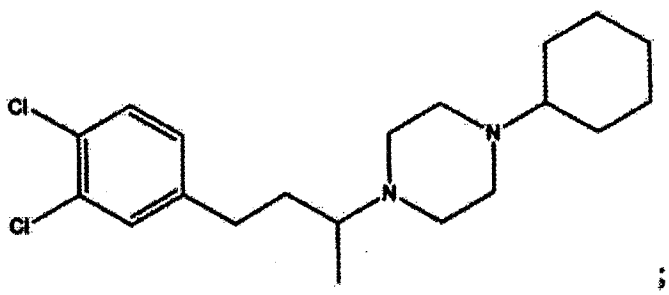
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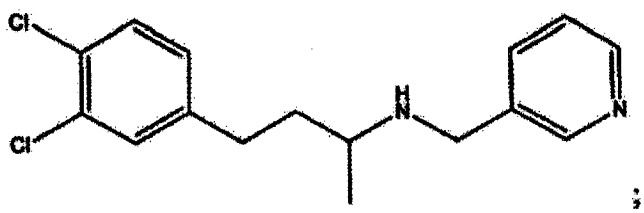
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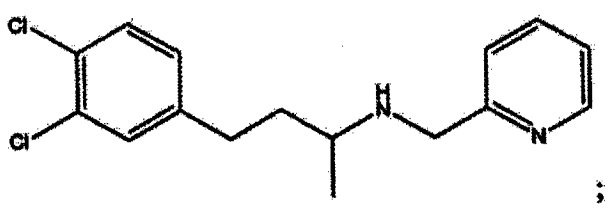
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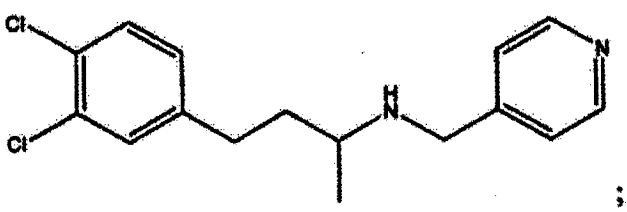
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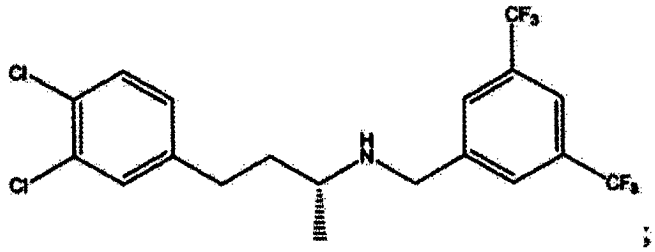
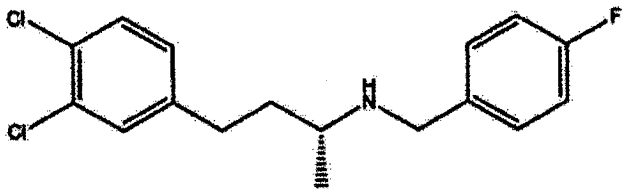
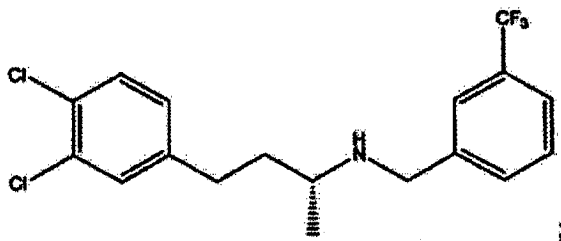
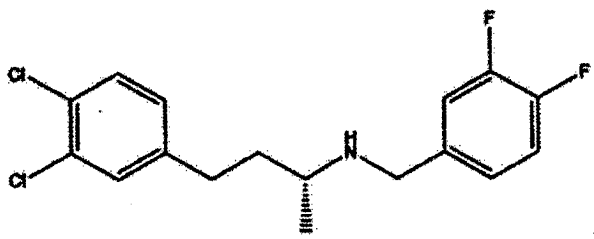
[0395]

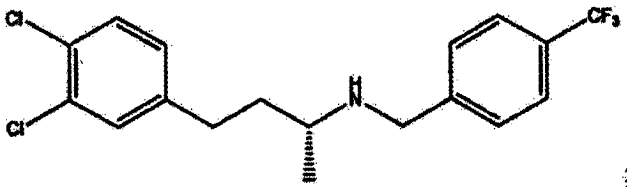
表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物

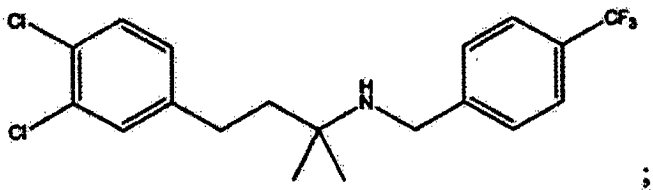






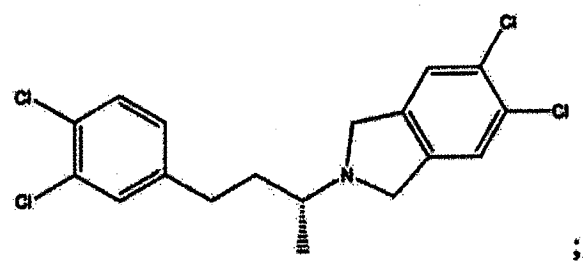






[0396]

表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物



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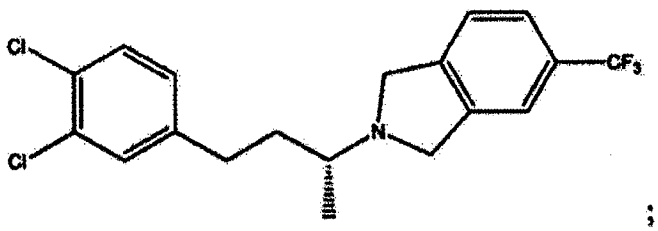

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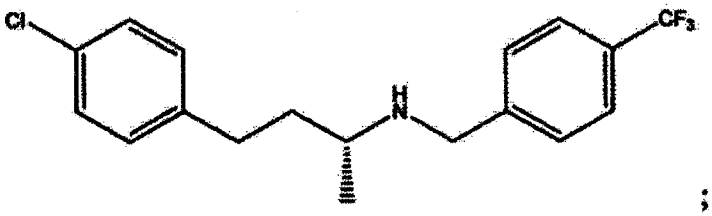

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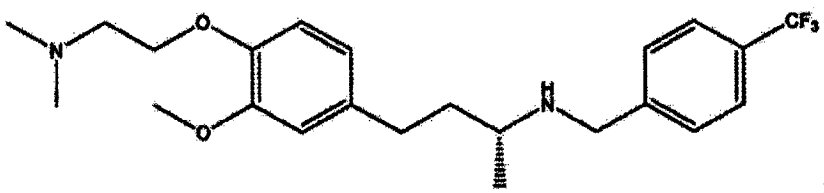

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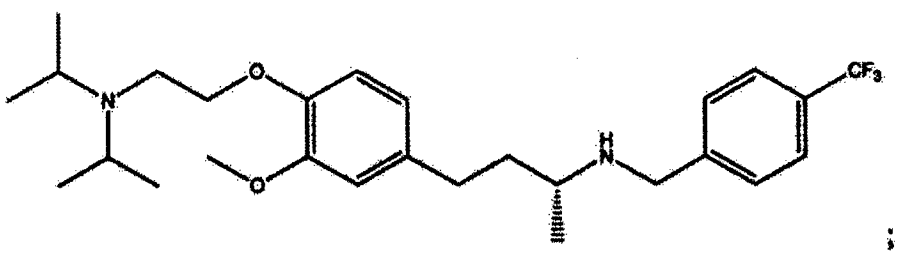

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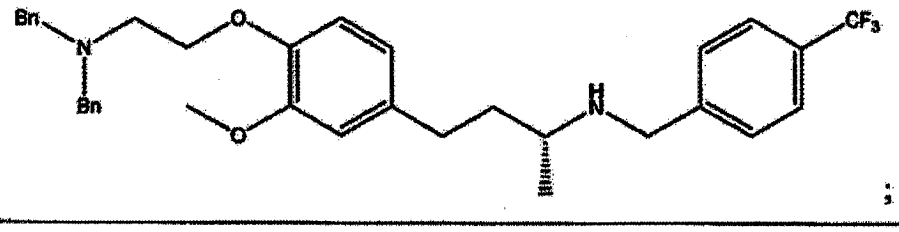
[0397]

表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物



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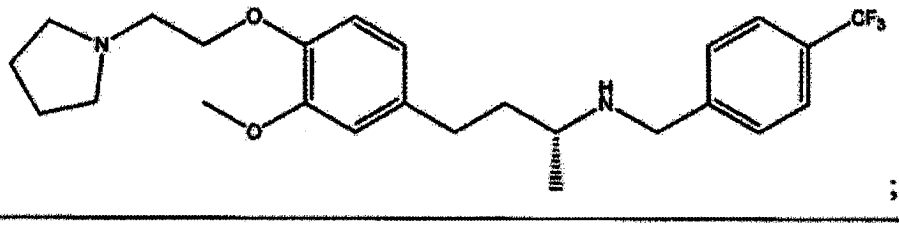

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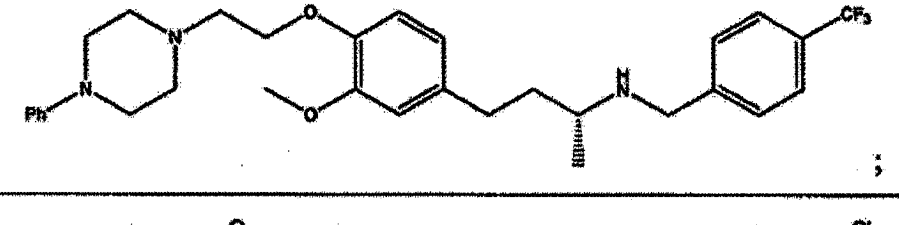

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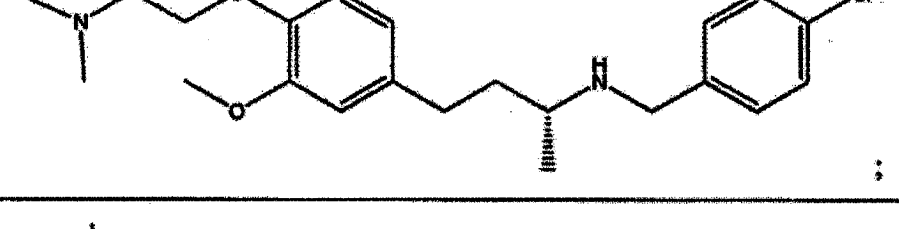

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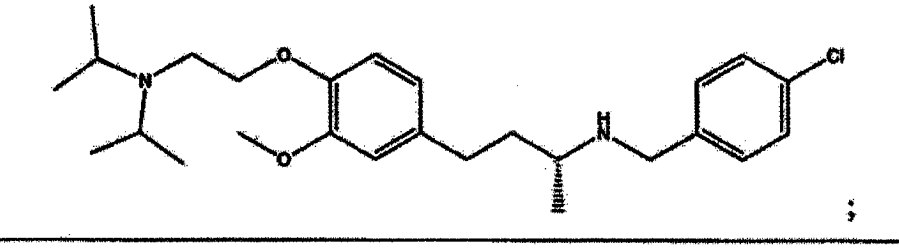

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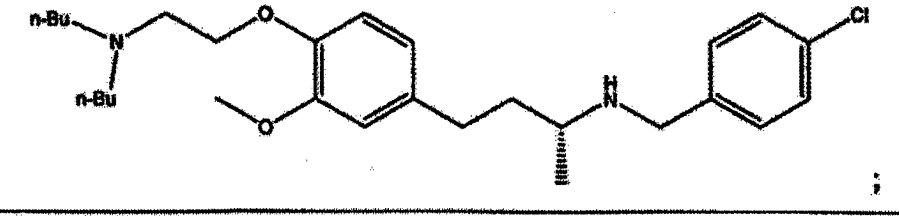

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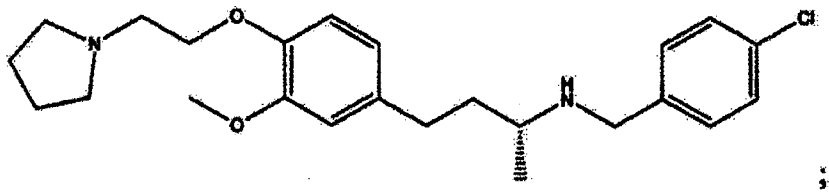
[0398]

表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物



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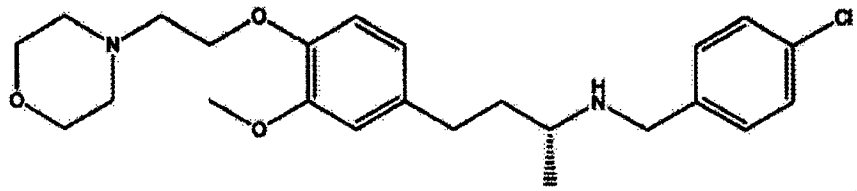

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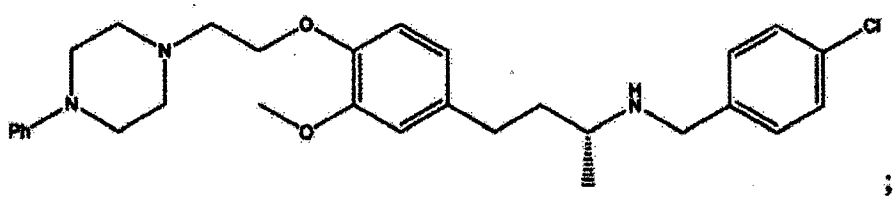

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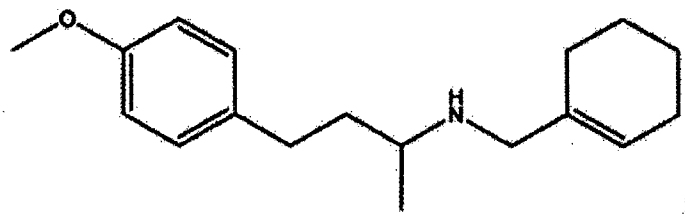

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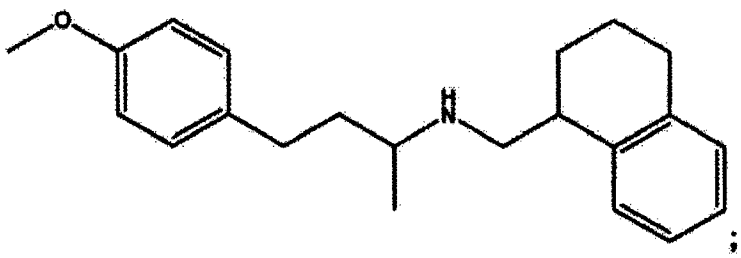

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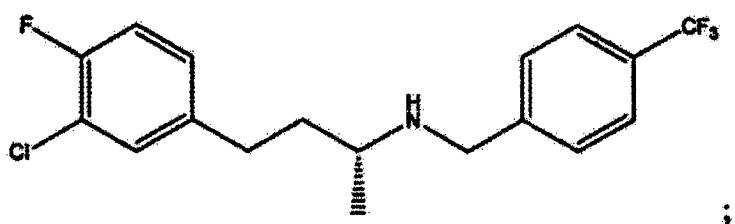

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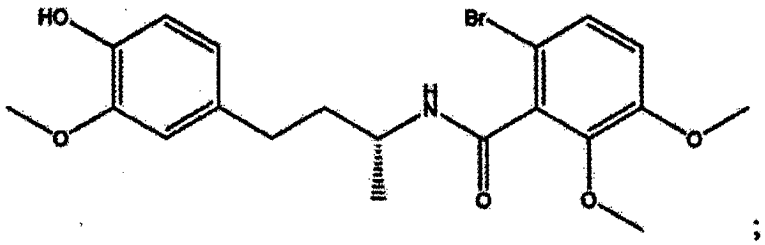
表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物



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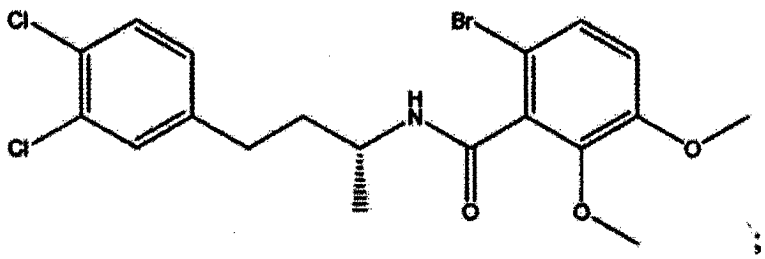

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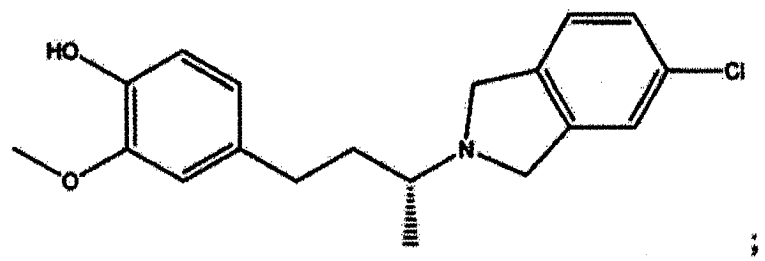
[0399]



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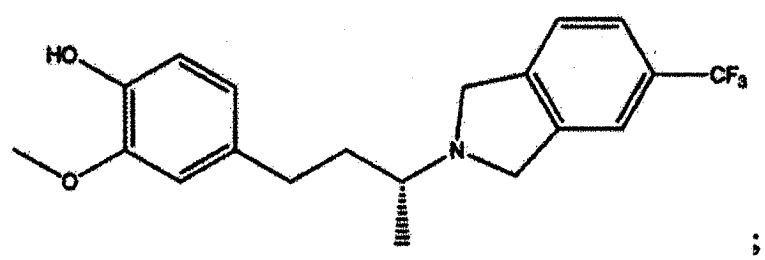

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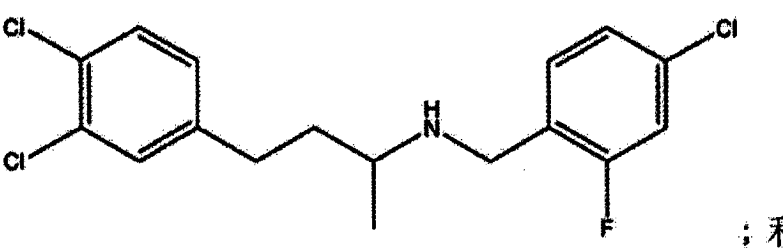
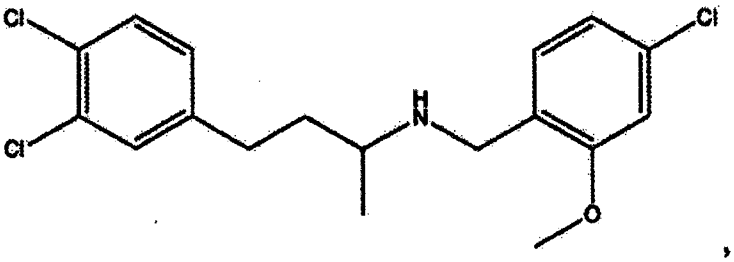


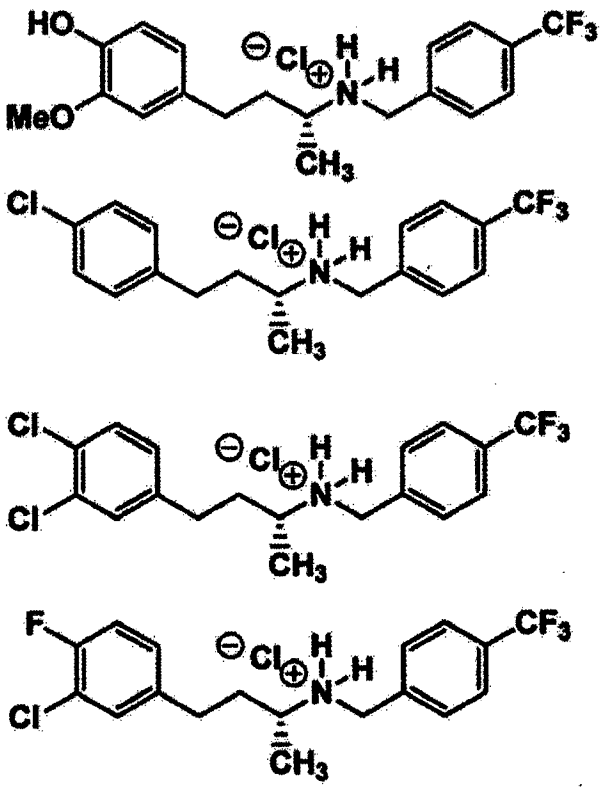
; 和

表 1B.式 III、IIIa、IIIb、IIIc、IV、Va 和 Vb 的示例性化合物

[0400] 

[0401] 或其药物学可接受的盐。

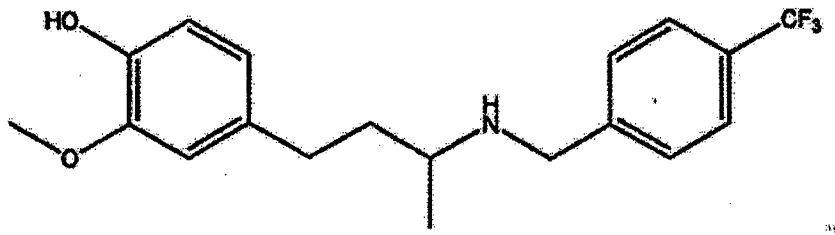
[0402] 用于本发明的优选盐包括以上化合物的盐酸盐，包括以下：

[0403] 

[0404] 这些已经根据本文提供的一般方法以及任何额外步骤均在本领域技术内的具体合成实例进行了合成。数种这些化合物已经在本文详述的各个检定中进行了检测并已经被发现有活性。通过参考 WO2010/110855 中公开的化合物，测试的化合物还表现出增加的生物利用度。

[0405] 化合物 II 具有式：

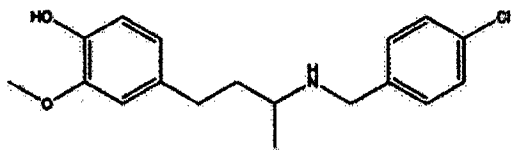
[0406]

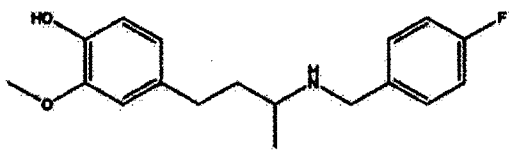


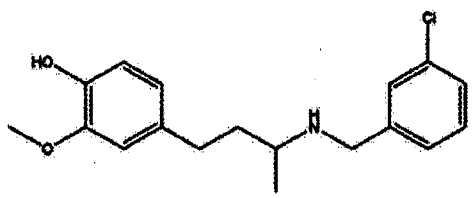
[0407] 在一些实施方式中，各个以上通式可以包含排除式 II 化合物的条件。

[0408] 在一些实施方式中，各个以上通式可以包含排除一种或多种以下化合物的条件：

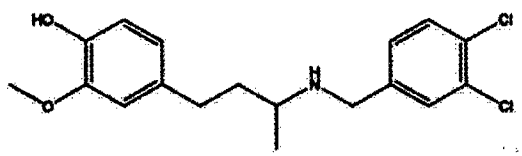
[0409]







和



[0410] 这些已经根据本文提供的一般方法以及任何额外步骤均在本领域技术内的具体合成实例进行了合成。数种这些化合物已经在本文详述的各个检定中进行了检测并已经被发现有活性。通过参考 WO2010/110855（其并入本文以供参考）中公开的化合物，测试的化合物还表现出增加的生物利用度。

[0411] 本文使用的术语“氢键受体基团”是指能够接受氢键的基团。氢键受体基团的实例为已知的且包括但不限于烷氧基、噁唑烷 -2- 酮基团、-O-C(O)-N- ;-C(O)-N- ;-O- ;环杂烷基中的杂原子（例如氧）;-N-SO_2- 等。基团可以在任一方向结合，并且可以与另一个碳或杂原子连接。氢键受体基团还可以存在于疏水脂族基团中或邻近疏水脂族基团。例如，四氢呋喃基包括氢键受体集团和疏水脂族基团两者。存在于四氢呋喃环中的氧用作为氢键受体且四氢呋喃环中的碳用作为疏水脂族基团。

[0412] 本文使用的术语“疏水脂族基团”是指碳链或碳环。碳链可以存在于环杂烷基中，但是疏水脂族基团不包括杂原子。以上提供的四氢呋喃实例是一个这样的例子，但是还有很多其他的。在一些实施方式中，疏水脂族基团是任选取代的 C1 ～ C6 烷基、环烷基或杂环烷基的 C1 ～ C6 碳。“疏水脂族基团”不是疏水芳族基团。

[0413] 本文使用的术语“可正离子化的基团”是指存在于结构中的原子或原子群，其可

以在某些条件例如存在于溶液或细胞中的生物学条件下荷正电。在一些实施方式中，可正离子化的基团是氮。在一些实施方式中，可正离子化的基团是存在于环杂烷基环中的氮。例如，在哌嗪基团中，两个氮被认为是两个可正离子化的基团。然而，在一些实施方式中，与可正离子化的基团连接的碳不被认为是疏水脂族基团。在一些实施方式中，可正离子化的基团是含氮环。含氮环的实例包括但不限于哌嗪、哌啶、三嗪（trizinane）、四嗪（tetrazinane）等。在一些关于可正离子化的基团的实施方式中，含氮环包括1、2、3或4个氮。在一些实施方式中，可正离子化的基团不是存在于 $-N-SO_2-$ 基团中的氮。

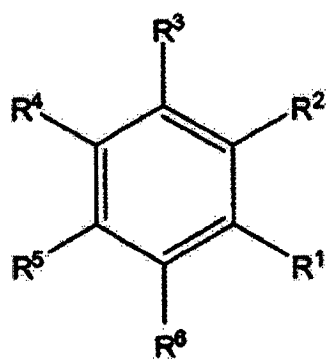
[0414] 在一些实施方式中，基团包括氢键受体和可正离子化的基团。例如，吗啉基团同时包括氧基中的氢键受体和氮中的可正离子化的基团。

[0415] 本文使用的术语“氢键供体”是指能够提供氢键的基团。氢键供体基团的实例包括但不限于 -OH 等。

[0416] 在一些实施方式中，σ-2 受体配体是任选取代的哌嗪、苯基四氢呋喃 -N,N- 二甲基甲胺、二苯基四氢呋喃 -N,N- 二甲基甲胺、4- 苯基戊基 - 哌嗪、苄基苯基 - 哌嗪、吲哚 - 氧杂 - 氮杂螺 - 癸烷、哌啶 - 吲哚、苯基哌啶 - 吲哚、吡唑 - 吗啉、吡唑 - 哌啶、吡唑 -N,N- 二乙基乙胺、吡唑 - 吡咯烷、苯基 - 吡唑 - 吗啉、苯甲酰胺 - 喹啉化合物或其衍生物。

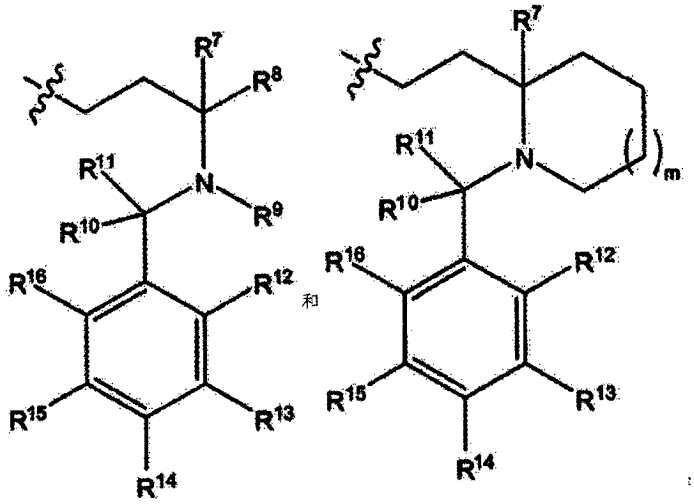
[0417] 此外，σ-2 受体配体可以是任何记载于 WO2011/014880（申请第 PCT/US2010/044136 号）、WO2010/118055（申请第 PCT/US2010/030130 号）和申请第 PCT/US2011/026530 号和 WO2012/106426 的化合物，各个申请全部并入本文以供参考。例如，在一些实施方式中，σ-2 配体是式 V 的化合物：

[0418]



V

[0419] 或其药物学上可接受的盐，其中：R^1 选自 (A1) 和 (A2)：

[0420] 

(A1) (A2) ;

[0421] 其中在式 V 的化合物中，R^2、R^3、R^4、R^5 和 R^6 各自独立地选自 H、OH、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、卤素、CN、NO_2、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基、NH_2、NH(C_{1-4} 烷基)、NH(C_{3-7} 环烷基)、N(C_{1-4} 烷基)$_2$、NHC(O)(C_{1-4} 烷基)、SH、S(C_{1-6} 烷基)、C(O)OR^a、C(O)R^b、C(O)NR^cR^d、OC(O)R^b、OC(O)NR^cR^d、NR^cR^d、$NR^cC(O)R^b$、$NR^cC(O)OR^a$、$NR^cS(O)_2R^b$、$NR^cS(O)_2NR^cR^d$、S(O)R^b、S(O)$_2R^b$ 和 S(O)$_2NR^cR^d$；

[0422] 其中在式 V 的化合物中，R^7 是 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、或 C_{3-7} 环烷基；

[0423] 其中在式 V 的化合物中，R^8 为 C_{1-6} 烷基、C_{1-6} 卤代烷基、或 C_{3-7} 环烷基；

[0424] 其中在式 V 的化合物中，R^9 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基或 C_{3-7} 环烷基；

[0425] 其中在式 V 的化合物中，R^{10} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、或 C_{3-7} 环烷基；

[0426] 其中在式 V 的化合物中，R^{11} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、或 C_{3-7} 环烷基；

[0427] 其中在式 V 的化合物中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 各自独立地选自 H、OH、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、卤素、CN、NO_2、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基、NH_2、NH(C_{1-4} 烷基)、NH(C_{3-7} 环烷基)、N(C_{1-4} 烷基)$_2$、NHC(O)(C_{1-4} 烷基)、SH、S(C_{1-6} 烷基)、C(O)OR^{a1}、C(O)R^{b1}、C(O)$NR^{c1}R^{d1}$、OC(O)R^{b1}、OC(O)$NR^{c1}R^{d1}$、$NR^{c1}R^{d1}$、$NR^{c1}C(O)R^{b1}$、$NR^{c1}C(O)OR^{a1}$、$NR^{c1}S(O)_2R^{b1}$、$NR^{c1}S(O)_2NR^{c1}R^{d1}$、S(O)$R^{b1}$、S(O)$_2R^{b1}$ 和 S(O)$_2NR^{c1}R^{d1}$；

[0428] 其中在式 V 的化合物中，各个 R^a 独立地选自 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、芳烷基、杂芳烷基、环烷基烷基、杂环烷基烷基、C_{2-6} 烯基、C_{2-6} 炔基、芳基、环烷基、杂芳基和杂环烷基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、芳烷基、杂芳烷基、环烷基烷基、杂环烷基烷基、C_{2-6} 烯基、C_{2-6} 炔基、芳基、环烷基、杂芳基和杂环烷基中的各个任选地被 1、2、3、4 或 5 个独立选自 OH、CN、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代；

[0429] 其中在式 V 的化合物中，各个 R^b 独立地选自 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、芳烷基、杂芳烷基、环烷基烷基、杂环烷基烷基、C_{2-6} 烯基、C_{2-6} 炔基、芳基、环烷基、杂芳基、杂环烷基、芳烷基、杂芳烷基、环烷基烷基和杂环烷基烷基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、芳烷基、杂芳烷基、环烷基烷基、杂环烷基烷基、C_{2-6} 烯基、C_{2-6} 炔基、芳基、环烷基、杂芳基、杂环烷基、芳烷基、杂芳烷基、环烷基烷基和杂环烷基烷基中的各个任选地被 1、2、3、4 或 5 个独立选自 OH、

氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代；

[0430] 其中在式 V 的化合物中，R^c 和 R^d 独立地选自 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、芳烷基、杂芳烷基、环烷基烷基、杂环烷基烷基、C_{2-6} 烯基、C_{2-6} 炔基、芳基、杂芳基、环烷基、杂环烷基、芳烷基、杂芳烷基、环烷基烷基和杂环烷基烷基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、芳烷基、杂芳烷基、环烷基烷基、杂环烷基烷基、C_{2-6} 烯基、C_{2-6} 炔基、芳基、杂芳基、环烷基、杂环烷基、芳烷基、杂芳烷基、环烷基烷基和杂环烷基烷基中的各个任选地被 1、2、3、4 或 5 个独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代；

[0431] 或者其中在式 V 的化合物中，R^c 和 R^d 与它们连接的 N 原子一起形成任选地被 1、2、3、4 或 5 个独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代的 4、5、6 或 7 元杂环烷基；

[0432] 其中在式 V 的化合物中，各个 R^{a1} 独立地选自 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、芳烷基、杂芳烷基、环烷基烷基、杂环烷基烷基、C_{2-6} 烯基、C_{2-6} 炔基、芳基、环烷基、杂芳基和杂环烷基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、芳烷基、杂芳烷基、环烷基烷基、杂环烷基烷基、C_{2-6} 烯基、C_{2-6} 炔基、芳基、环烷基、杂芳基和杂环烷基中的各个任选地被 1、2、3、4 或 5 个独立选自 OH、CN、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代；

[0433] 其中在式 V 的化合物中，各个 R^{b1} 独立地选自 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、芳烷基、杂芳烷基、环烷基烷基、杂环烷基烷基、C_{2-6} 烯基、C_{2-6} 炔基、芳基、环烷基、杂芳基、杂环烷基、芳烷基、杂芳烷基、环烷基烷基和杂环烷基烷基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、芳烷基、杂芳烷基、环烷基烷基、杂环烷基烷基、C_{2-6} 烯基、C_{2-6} 炔基、芳基、环烷基、杂芳基、杂环烷基、芳烷基、杂芳烷基、环烷基烷基和杂环烷基烷基中的各个任选地被 1、2、3、4 或 5 个独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代；

[0434] 其中在式 V 的化合物中，R^{c1} 和 R^{d2} 独立地选自 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、芳烷基、杂芳烷基、环烷基烷基、杂环烷基烷基、C_{2-6} 烯基、C_{2-6} 炔基、芳基、杂芳基、环烷基、杂环烷基、芳烷基、杂芳烷基、环烷基烷基和杂环烷基烷基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、芳烷基、杂芳烷基、环烷基烷基、杂环烷基烷基、C_{2-6} 烯基、C_{2-6} 炔基、芳基、杂芳基、环烷基、杂环烷基、芳烷基、杂芳烷基、环烷基烷基和杂环烷基烷基中的各个任选地被 1、2、3、4 或 5 个独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代；

[0435] 或者其中在式 V 的化合物中，R^{c1} 和 R^{d1} 与它们连接的 N 原子一起形成任选地被 1、2、3、4 或 5 个独立选自 OH、氨基、卤素 C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代的 4、5、6 或 7 元杂环烷基；且

[0436] m 为 0、1 或 2。

[0437] 在一些实施方式中，在式 V 的化合物中，当 R^1 为 (A1) 的组成部分时，则 R^2、R^3、R^4、

R^5 和 R^6 中的两个独立地选自 OH、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基。

[0438] 在一些实施方式中，在式 V 的化合物中，当 R^1 为 (A1) 的组成部分时，则 R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个不是 H。

[0439] 在一些实施方式中，在式 V 的化合物中，R^2、R^3、R^4、R^5 和 R^6 中的两个独立地选自 OH、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基。在一些其他实施方式中，R^2、R^3、R^4、R^5 和 R^6 中剩余的每一个为 H。

[0440] 在一些实施方式中，在式 V 的化合物中，R^2、R^3、R^4、R^5 和 R^6 各自独立地选自 H、OH、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、卤素、CN、NO_2、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基、NH_2、NH(C_{1-4} 烷基)、NH(C_{3-7} 环烷基)、N(C_{1-4} 烷基)$_2$、NHC(O)(C_{1-4} 烷基)、SH、S(C_{1-6} 烷基)、C(O)OH、C(O)O(C_{1-4} 烷基)、C(O)(C_{1-4} 烷基) 和 C(O)NH(C_{1-4} 烷基)。

[0441] 在一些实施方式中，在式 V 的化合物中，R^2、R^3、R^4、R^5 和 R^6 中的一个为 OH；且 R^2、R^3、R^4、R^5 和 R^6 中的一个为 OH、C_{1-6} 烷氧基、或 C_{1-6} 卤代烷氧基。在一些其他实施方式中，R^2、R^3、R^4、R^5 和 R^6 中剩余的每一个为 H。

[0442] 在一些实施方式中，在式 V 的化合物中，R^2、R^3、R^4、R^5 和 R^6 中的一个为 OH；且 R^2、R^3、R^4、R^5 和 R^6 中的一个为 C_{1-3} 烷氧基或 C_{1-3} 卤代烷氧基（在一些其他实施方式中，R^2、R^3、R^4、R^5 和 R^6 中剩余的每一个为 H)。在一些其他实施方式中，R^2、R^3、R^4、R^5 和 R^6 中的一个为 OH；且 R^2、R^3、R^4、R^5 和 R^6 中的一个为甲氧基或三卤代甲氧基（在一些其他实施方式中，R^2、R^3、R^4、R^5 和 R^6 中剩余的每一个为 H)。在其他实施方式中，R^2、R^3、R^4、R^5 和 R^6 中的一个为 OH；且 R^2、R^3、R^4、R^5 和 R^6 中的一个为甲氧基（在一些其他实施方式中，R^2、R^3、R^4、R^5 和 R^6 中剩余的每一个为 H)。

[0443] 在一些实施方式中，在式 V 的化合物中，R^4 为 OH；且 R^5 为甲氧基。在一些其他实施方式中，R^4 为 OH；R^5 为甲氧基；且 R^2、R^3 和 R^6 各自为 H。

[0444] 在一些实施方式中，在式 V 的化合物中，R^7 为 H 或 C_{1-6} 烷基。在一些其他实施方式中，R^7 为 H 或 C_{1-3} 烷基。

[0445] 在一些实施方式中，在式 V 的化合物中，R^7 为 C_{1-3} 烷基。在一些其他实施方式中，R^7 为甲基或乙基。在其他实施方式中，R^7 为甲基。

[0446] 在一些实施方式中，在式 V 的化合物中，R^7 为 H。

[0447] 在一些实施方式中，在式 V 的化合物中，R^8 为 C_{1-6} 烷基。在一些其他实施方式中，R^8 为 C_{1-3} 烷基。在其他实施方式中，R^8 为甲基。

[0448] 在一些实施方式中，在式 V 的化合物中，R^9 为 H 或 C_{1-6} 烷基。在一些其他实施方式中，R^9 为 H 或 C_{1-3} 烷基。

[0449] 在一些实施方式中，在式 V 的化合物中，R^9 为 H。

[0450] 在一些实施方式中，在式 V 的化合物中，R^9 为 C_{1-3} 烷基。

[0451] 在一些实施方式中，在式 V 的化合物中，R^{10} 为 H 或 C_{1-6} 烷基。在一些其他实施方式中，R^{10} 为 H 或 C_{1-3} 烷基。在其他实施方式中，R^{10} 为 H。在其他实施方式中，R^{10} 为 C_{1-3} 烷基。

[0452] 在一些实施方式中，在式 V 的化合物中，R^{11} 为 H 或 C_{1-6} 烷基。在一些其他实施方式中，R^{11} 为 H 或 C_{1-3} 烷基。在其他实施方式中，R^{11} 为 H。在其他实施方式中，R^{11} 为 C_{1-3} 烷基。

[0453] 在一些实施方式中，在式 V 的化合物中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个不是 H。

[0454] 在一些实施方式中，在式 V 的化合物中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 各自独立地选自 H、OH、

C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、卤素、CN、NO_2、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基、NH_2、NH(C_{1-4} 烷基）、NH(C_{3-7} 环烷基）、N(C_{1-4} 烷基）$_2$、NHC(O)(C_{1-4} 烷基）、SH、S(C_{1-6} 烷基）、C(O)OH、C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）、和 C(O)NH(C_{1-4} 烷基）。

[0455] 在一些实施方式中，在式 V 的化合物中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 各自独立地选自 H、卤素、CN、NO_2、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基、C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）和 C(O)NH(C_{1-4} 烷基）。

[0456] 在一些实施方式中，在式 V 的化合物中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个选自卤素、CN、NO_2、C_{1-6} 卤代烷基、C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）和 C(O)NH(C_{1-4} 烷基）。

[0457] 在一些实施方式中，在式 V 的化合物中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个选自卤素和 C_{1-6} 卤代烷基。在一些其他实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个选自卤素和 C_{1-6} 卤代烷基，且 R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中剩余的每一个为 H。在其他实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的一个或两个选自卤素和 C_{1-6} 卤代烷基，且 R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中剩余的每一个为 H。在其他实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的一个选自卤素和 C_{1-6} 卤代烷基，且 R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中剩余的每一个为 H。

[0458] 在一些实施方式中，在式 V 的化合物中，R^{14} 为卤素或 C_{1-6} 卤代烷基（在一些其他实施方式中，R^{12}、R^{13}、R^{15} 和 R^{16} 中的每一个为 H）。在一些其他实施方式中，R^{14} 为卤素或 C_{1-3} 卤代烷基（在一些其他实施方式中，R^{12}、R^{13}、R^{15} 和 R^{16} 中的每一个为 H）。在其他实施方式中，R^{14} 为卤素或 C_1 卤代烷基（在一些其他实施方式中，R^{12}、R^{13}、R^{15} 和 R^{16} 中的每一个为 H）。

[0459] 在一些实施方式中，在式 V 的化合物中，R^{14} 为卤素（在一些其他实施方式中，R^{12}、R^{13}、R^{15} 和 R^{16} 中的每一个为 H）。在一些实施方式中，R^{14} 为 Cl 或 F。在一些实施方式中，R^{14} 为 Cl。在一些实施方式中，R^{14} 为 F。

[0460] 在一些实施方式中，在式 V 的化合物中，R^{14} 为 C_{1-6} 卤代烷基（在一些其他实施方式中，R^{12}、R^{13}、R^{15} 和 R^{16} 中的每一个为 H）。在一些其他实施方式中，R^{14} 为 C_{1-3} 卤代烷基。在其他实施方式中，R^{14} 为 C_1 卤代烷基。在其他实施方式中，R^{14} 为 CF_3。

[0461] 在一些实施方式中，在式 V 的化合物中，R^{15} 为卤素或 C_{1-6} 卤代烷基（在一些其他实施方式中，R^{12}、R^{13}、R^{14} 和 R^{16} 中的每一个为 H）。在一些其他实施方式中，R^{15} 为卤素或 C_{1-3} 卤代烷基（在一些其他实施方式中，R^{12}、R^{13}、R^{14} 和 R^{16} 中的每一个为 H）。在其他实施方式中，R^{15} 为卤素或 C_1 卤代烷基（在一些其他实施方式中，R^{12}、R^{13}、R^{14} 和 R^{16} 中的每一个为 H）。

[0462] 在一些实施方式中，在式 V 的化合物中，R^{15} 为卤素。在一些实施方式中，R^{15} 为 Cl 或 F。在一些实施方式中，R^{15} 为 Cl。在一些实施方式中，R^{15} 为 F。

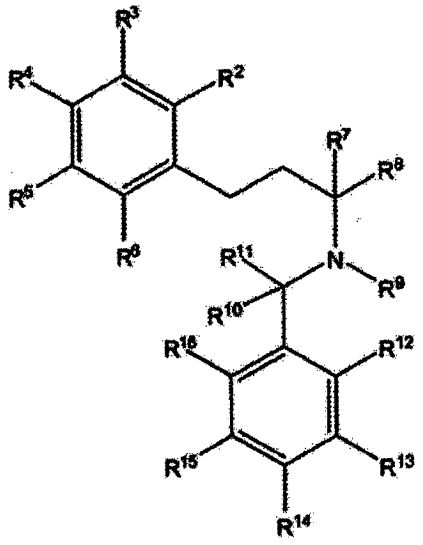
[0463] 在一些实施方式中，在式 V 的化合物中，R^{15} 为 C_{1-6} 卤代烷基。在一些其他实施方式中，R^{15} 为 C_{1-3} 卤代烷基。在其他实施方式中，R^{15} 为 C_1 卤代烷基。在其他实施方式中，R^{15} 为 CF_3。

[0464] 在一些实施方式中，在式 V 的化合物中，R^{14} 和 R^{15} 各自独立地为卤素或 C_{1-3} 卤代烷基（在一些其他实施方式中，R^{12}、R^{13} 和 R^{16} 中的每一个为 H）。在一些其他实施方式中，R^{14} 和 R^{15} 各自独立地为卤素或 C_1 卤代烷基。

[0465] 在一些实施方式中，在式 V 的化合物中，R^{14} 和 R^{15} 各自独立地为卤素。

[0466] 在一些实施方式中，式 V 的化合物为式 VI 的化合物：

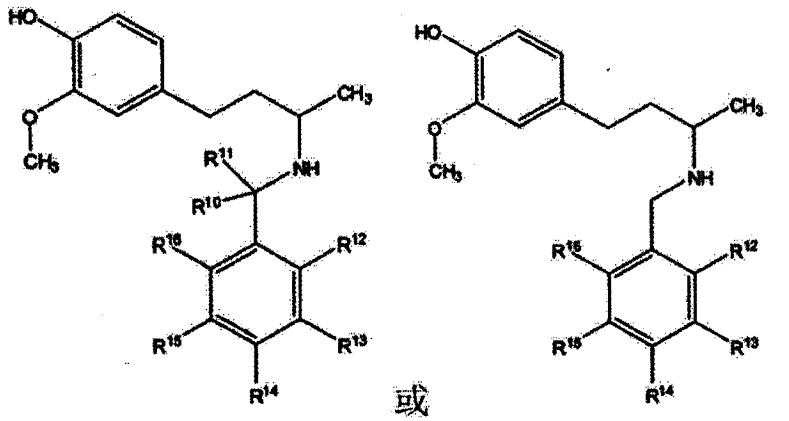
[0467]



VI.

[0468] 在一些实施方式中，式 VI 的化合物或其药学上可接受的盐为式 VIa 或 VIb 的化合物或其药学上可接受的盐：

[0469]



VIa 或 VIb

[0470] 在一些实施方式中，式 VI 的化合物为式 VIa 的化合物。在一些其他实施方式中，R^{10} 和 R^{11} 各自独立地选自 H 和 C_{1-3} 烷基。在其他实施方式中，R^{10} 和 R^{11} 各自独立地选自 H 和甲基。在其他实施方式中，R^{10} 和 R^{11} 各自为 H。

[0471] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{10} 和 R^{11} 中的一个选自 H 和 C_{1-3} 烷基且另一个为 H。在一些其他实施方式中，R^{10} 和 R^{11} 中的一个为 C_{1-3} 烷基。在其他实施方式中，R^{10} 和 R^{11} 中的一个为甲基。

[0472] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{10} 和 R^{11} 两者均选自 C_{1-3} 烷基。在一些其他实施方式中，R^{10} 和 R^{11} 两者均为甲基。

[0473] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 各自独立地选自 H、OH、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、卤素、CN、NO_2、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基、NH_2、NH(C_{1-4} 烷基)、NH(C_{3-7} 环烷基)、N(C_{1-4} 烷基)$_2$、NHC(O)(C_{1-4} 烷基)、SH、S(C_{1-6} 烷基)、C(O)OH、C(O)O(C_{1-4} 烷基)、C(O)(C_{1-4} 烷基) 和 C(O)NH(C_{1-4} 烷基)。

[0474] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 各自独立地选自 H、卤素、CN、NO_2、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基、C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）和 C(O)NH(C_{1-4} 烷基）。

[0475] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 各自独立地选自 H、卤素、CN、NO_2、C_{1-6} 烷基、C_{1-6} 卤代烷基和 C_{3-7} 环烷基。在一些其他实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 各自独立地选自 H、卤素、CN、C_{1-6} 烷基和 C_{1-6} 卤代烷基。在其他实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 各自独立地选自 H、卤素、C_{1-6} 烷基和 C_{1-6} 卤代烷基。

[0476] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个选自卤素和 C_{1-6} 卤代烷基，且 R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中剩余的每一个为 H。在一些其他实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的一个或两个选自卤素和 C_{1-6} 卤代烷基，且 R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中剩余的每一个为 H。在其他实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的一个选自卤素和 C_{1-6} 卤代烷基，且 R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中剩余的每一个为 H。

[0477] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个选自卤素、CN、NO_2、C_{1-6} 卤代烷基、C(O)O(C_{1-6} 烷基）、C(O)(C_{1-4} 烷基）和 C(O)NH(C_{1-4} 烷基）。

[0478] 在式 VIa 的化合物的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个选自卤素和 C_{1-6} 卤代烷基。

[0479] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个选自卤素和 C_{1-3} 卤代烷基。在一些其他实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个选自卤素和 C_1 卤代烷基。

[0480] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{14} 为卤素或 C_{1-6} 卤代烷基。在一些其他实施方式中，R^{14} 为卤素或 C_{1-3} 卤代烷基。在其他实施方式中，R^{14} 为卤素或 C_1 卤代烷基。

[0481] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{14} 为卤素（在一些其他实施方式中，R^{12}、R^{13}、R^{15} 和 R^{16} 中的每一个为 H)。在一些实施方式中，R^{14} 为 Cl 或 F。在一些实施方式中，R^{14} 为 Cl。在一些实施方式中，R^{14} 为 F。

[0482] 在式 IIa 的化合物的一些实施方式中，R^{14} 为 C_{1-6} 卤代烷基（在一些其他实施方式中，R^{12}、R^{13}、R^{15} 和 R^{16} 中的每一个为 H)。在一些其他实施方式中，R^{14} 为 C_{1-3} 卤代烷基。在其他实施方式中，R^{14} 为 C_1 卤代烷基。在其他实施方式中，R^{14} 为 CF_3。

[0483] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{14} 为卤素或 C_{1-6} 卤代烷基，且 R^{12}、R^{13}、R^{15} 和 R^{16} 中的每一个为 H。

[0484] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{15} 为卤素或 C_{1-6} 卤代烷基（在一些其他实施方式中，R^{12}、R^{13}、R^{14} 和 R^{16} 中的每一个为 H)。在一些其他实施方式中，R^{15} 为卤素或 C_{1-3} 卤代烷基。在其他实施方式中，R^{15} 为卤素或 C_1 卤代烷基。

[0485] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{15} 为卤素。在一些实施方式中，R^{15} 为 Cl 或 F。在一些实施方式中，R^{15} 为 Cl。在一些实施方式中，R^{15} 为 F。

[0486] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{15} 为 C_{1-6} 卤代烷基。在一些其他实施方式中，R^{15} 为 C_{1-3} 卤代烷基。在其他实施方式中，R^{15} 为 C_1 卤代烷基。在其他实施方式中，R^{15} 为 CF_3。

[0487] 在式 VIa 的化合物或其药学上可接受的盐的一些实施方式中，R^{14} 和 R^{15} 各自独立地为卤素或 C_{1-3} 卤代烷基（在一些其他实施方式中，R^{12}、R^{13} 和 R^{16} 中的每一个为 H）。在一些其他实施方式中，R^{14} 和 R^{15} 各自独立地为卤素或 C_1 卤代烷基。在其他实施方式中，R^{14} 和 R^{15} 各自独立地为卤素。

[0488] 在一些实施方式中，式 VI 的化合物或其药学上可接受的盐为式 VIb 的化合物或其药学上可接受的盐。

[0489] 在式 VIb 的化合物或其药学上可接受的盐的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 各自独立地选自 H、OH、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、卤素、CN、NO_2、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基、NH_2、NH(C_{1-4} 烷基）、N(C_{1-4} 烷基）$_2$、NHC(O)(C_{1-4} 烷基）、SH、S(C_{1-6} 烷基）、C(O)OH、C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）和 C(O)NH(C_{1-4} 烷基）。

[0490] 在式 VIb 的化合物或其药学上可接受的盐的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 各自独立地选自 H、卤素、CN、NO_2、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基、C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）和 C(O)NH(C_{1-4} 烷基）。

[0491] 在式 IIb 的化合物或其药学上可接受的盐的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 各自独立地选自 H、卤素、CN、NO_2、C_{1-6} 烷基、C_{1-6} 卤代烷基和 C_{3-7} 环烷基。在一些其他实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 各自独立地选自 H、卤素、CN、C_{1-6} 烷基和 C_{1-6} 卤代烷基。在其他实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 各自独立地选自 H、卤素、C_{1-6} 烷基和 C_{1-6} 卤代烷基。

[0492] 在式 VIb 的化合物或其药学上可接受的盐的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个选自卤素和 C_{1-6} 卤代烷基，且 R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中剩余的每一个为 H。在一些其他实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的一个或两个选自卤素和 C_{1-6} 卤代烷基，且 R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中剩余的每一个为 H。在其他实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的一个选自卤素和 C_{1-6} 卤代烷基，且 R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中剩余的每一个为 H。

[0493] 在式 VIb 的化合物或其药学上可接受的盐的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个选自卤素、CN、NO_2、C_{1-6} 卤代烷基、C(O)O(C_{1-4} 烷基）、C(O)(C_{1-4} 烷基）和 C(O)NH(C_{1-4} 烷基）。

[0494] 在式 VIb 的化合物的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个选自卤素和 C_{1-6} 卤代烷基。

[0495] 在式 VIb 的化合物或其药学上可接受的盐的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个选自卤素和 C_{1-3} 卤代烷基。在一些其他实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个选自卤素和 C_1 卤代烷基。

[0496] 在式 VIb 的化合物或其药学上可接受的盐的一些实施方式中，R^{14} 为卤素或 C_{1-6} 卤代烷基。在一些其他实施方式中，R^{14} 为卤素或 C_{1-3} 卤代烷基。在其他实施方式中，R^{14} 为卤素或 C_1 卤代烷基。

[0497] 在式 VIb 的化合物或其药学上可接受的盐的一些实施方式中，R^{14} 为卤素（在一些其他实施方式中，R^{12}、R^{13}、R^{15} 和 R^{16} 中的每一个为 H）。在一些实施方式中，R^{14} 为 Cl 或 F。在一些实施方式中，R^{14} 为 Cl。在一些实施方式中，R^{14} 为 F。

[0498] 在式 VIb 的化合物的一些实施方式中，R^{14} 为 C_{1-6} 卤代烷基（在一些其他实施方式中，R^{12}、R^{13}、R^{15} 和 R^{16} 中的每一个为 H）。在一些其他实施方式中，R^{14} 为 C_{1-3} 卤代烷基。在其他实施方式中，R^{14} 为 C_1 卤代烷基。在其他实施方式中，R^{14} 为 CF_3。

[0499] 在式 VIb 的化合物或其药学上可接受的盐的一些实施方式中，R^{14} 为卤素或 C_{1-6} 卤代烷基，且 R^{12}、R^{13}、R^{15} 和 R^{16} 中的每一个为 H。

[0500] 在式 VIb 的化合物或其药学上可接受的盐的一些实施方式中，R^{15} 为卤素或 C_{1-6} 卤代烷基（在一些其他实施方式中，R^{12}、R^{13}、R^{14} 和 R^{16} 中的每一个为 H）。在一些其他实施方式中，R^{15} 为卤素或 C_{1-3} 卤代烷基。在其他实施方式中，R^{15} 为卤素或 C_1 卤代烷基。

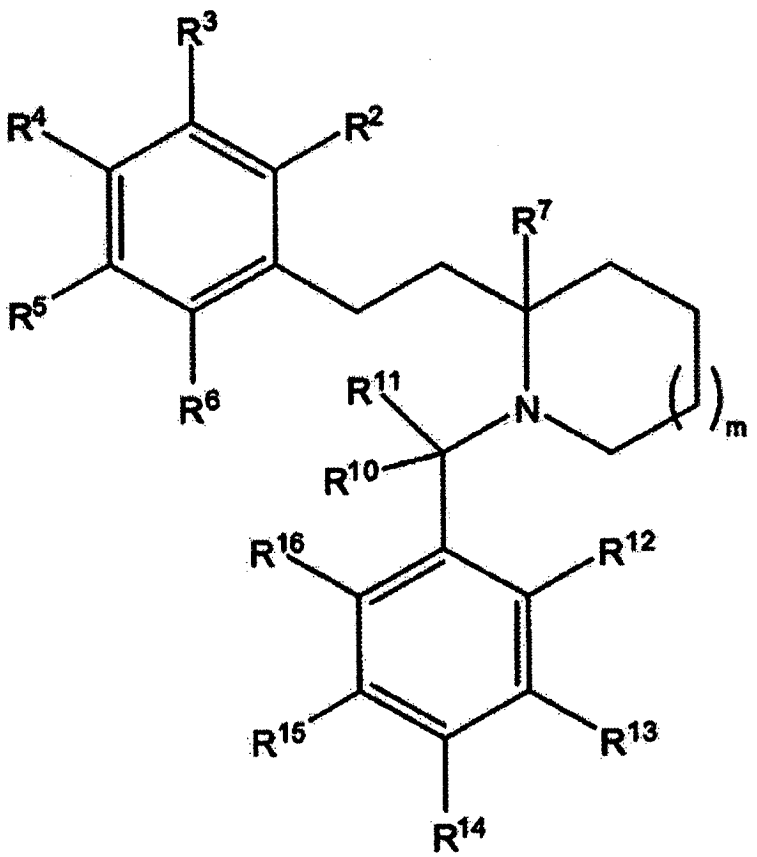
[0501] 在式 VIb 的化合物或其药学上可接受的盐的一些实施方式中，R^{15} 为卤素。在一些实施方式中，R^{15} 为 Cl 或 F。在一些实施方式中，R^{15} 为 Cl。在一些实施方式中，R^{15} 为 F。

[0502] 在式 VIb 的化合物或其药学上可接受的盐的一些实施方式中，R^{15} 为 C_{1-6} 卤代烷基。在一些其他实施方式中，R^{15} 为 C_{1-3} 卤代烷基。在其他实施方式中，R^{15} 为 C_1 卤代烷基。在其他实施方式中，R^{15} 为 CF_3。

[0503] 在式 VIb 的化合物或其药学上可接受的盐的一些实施方式中，R^{14} 和 R^{15} 各自独立地为卤素或 C_{1-3} 卤代烷基（在一些其他实施方式中，R^{12}、R^{13} 和 R^{16} 中的每一个为 H）。在一些其他实施方式中，R^{14} 和 R^{15} 各自独立地为卤素或 C_1 卤代烷基。在其他实施方式中，R^{14} 和 R^{15} 各自独立地为卤素。

[0504] 在一些实施方式中，式 V 的化合物为式 VII 的化合物：

[0505]



VII.

[0506] 在式 VII 的化合物或其药学上可接受的盐的一些实施方式中，m 为 1。

[0507] 在式 VII 的化合物或其药学上可接受的盐的一些实施方式中，m 为 0。

[0508] 在式 VII 的化合物或其药学上可接受的盐的一些实施方式中，R^2、R^3、R^4、R^5 和 R^6 中的至少一个选自 OH、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基。

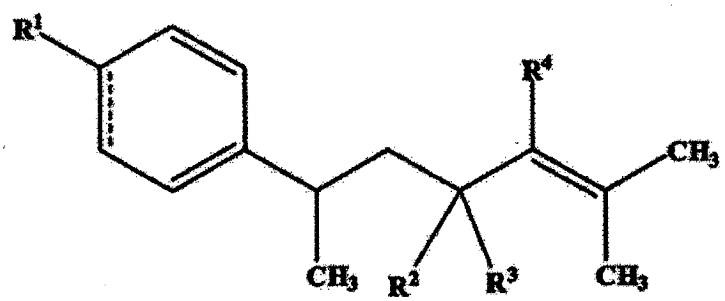
[0509] 在式 VII 的化合物或其药学上可接受的盐的一些实施方式中，R^2、R^3、R^4、R^5 和 R^6 中的至少两个独立地选自 OH、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基。

[0510] 在式 VII 的化合物或其药学上可接受的盐的一些实施方式中，R^{12}、R^{13}、R^{14}、R^{15} 和 R^{16} 中的至少一个不是 H。

[0511] 在式 VII 的化合物或其药学上可接受的盐的一些实施方式中，R^{14} 和 R^{15} 各自独立地为卤素或 C_{1-3} 卤代烷基。在一些其他实施方式中，R^{14} 和 R^{15} 各自独立地为卤素或 C_1 卤代烷基。

[0512] 在一些实施方式中，σ-2 配体为式 VIII 的化合物：

[0513]



VIII

[0514] 或其药学上可接受的盐，其中：

[0515] ---------- 是单键或双键；

[0516] R_1 为 H、CH_3、CF_3、F、Cl、Br 或 $-OCF_3$；

[0517] R_2 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基或 C_{6-10} 芳基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基或 C_{6-10} 芳基中的每一个被 0、1、2、3、4 或 5 个各自独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基的取代基所取代；

[0518] R^3 为 OH 或 $NR_{3a}NR_{3b}$；

[0519] R_{3a} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、环烷基烷基、C_{3-7} 环烷基、芳烷基或 C_{6-10} 芳基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基、芳烷基或 C_{6-10} 芳基中的每一个被 0、1、2、3、4 或 5 个各自独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基的取代基所取代；

[0520] R^{3b} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、环烷基烷基、C_{3-7} 环烷基、芳烷基、或 C_{6-10} 芳基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基或 C_{6-10} 芳基中的每一个被 0、1、2、3、4 或 5 个各自独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基的取代基所取代；

[0521] 或者 R^{3a} 和 R^{3b} 与它们连接的 N 原子一起形成被 0、1、2、3、4 或 5 个各自独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代的 4、5、6 或 7 元杂环烷基；且

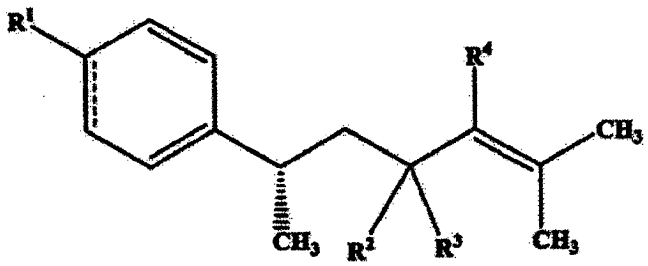
[0522] R^4 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基或 C_{6-10} 芳基，其中 C_{1-6} 烷基、C_{1-6} 卤代

烷基、C_{3-7} 环烷基或 C_{6-10} 芳基中的每一个被 0、1、2、3、4 或 5 个各自独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基的取代基所取代。

[0523] 在化合物 VIII 的一些实施方式中，当 ⋯⋯ 是双键且 R^3 为 OH 时，则 R^1、R^2 和 R^4 中的至少一个不是 H。在一些实施方式中，式 VIII 的化合物不是 2- 甲基 -6- 对甲苯基庚 -2- 烯 -4- 醇。

[0524] 在一些实施方式中，本发明的化合物或其药学上可接受的盐为式 VIIIa 的化合物：

[0525]

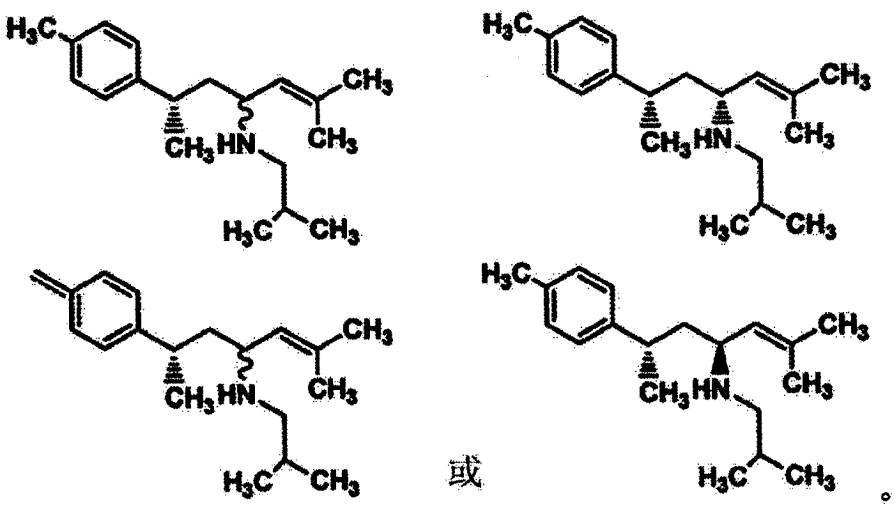


VIIIa

[0526] 或其药学上可接受的盐。

[0527] 在一些实施方式中，VIII 种类选自一种或多种以下化合物：

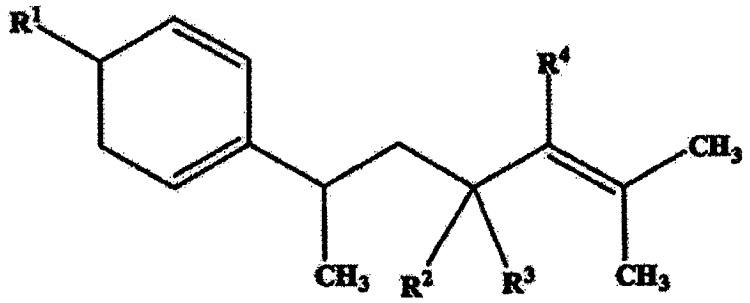
[0528]



[0529] 在一些实施方式中，式 VIIIa 的化合物不是 (6S)-2- 甲基 -6- 对甲苯基庚 -2- 烯 -4- 醇。

[0530] 在一些实施方式中，本发明的化合物或其药学上可接受的盐为式 VIIIb 的化合物：

[0531]

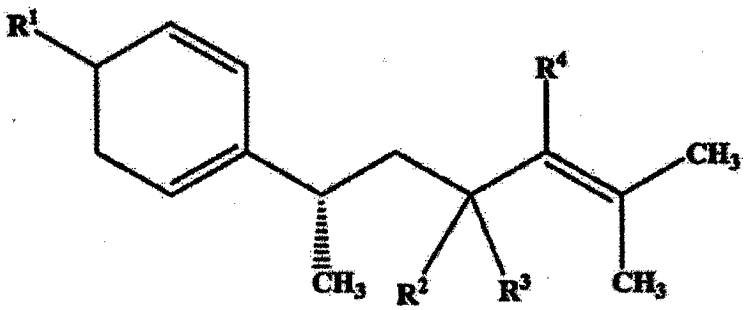


VIIIb

[0532] 或其药学上可接受的盐。

[0533] 在一些实施方式中，本发明的 σ-2 配体或其药学上可接受的盐为式 VIIIc 的化合物：

[0534]

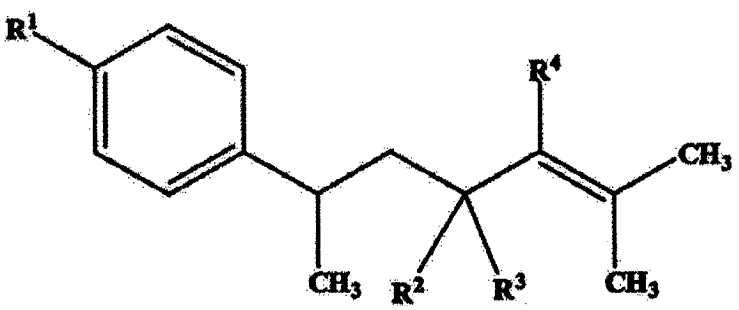


VIIIc

[0535] 或其药学上可接受的盐。

[0536] 在一些实施方式中，本发明的 σ-2 配体或其药学上可接受的盐为式 VIIId 的化合物：

[0537]

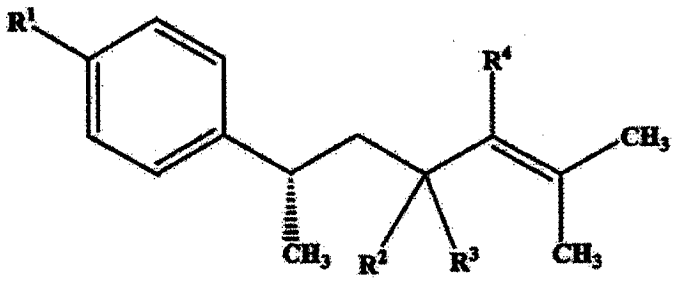


VIIId

[0538] 或其药学上可接受的盐。

[0539] 在一些实施方式中，本发明的化合物或其药学上可接受的盐为式 VIIIe 的化合物：

[0540]

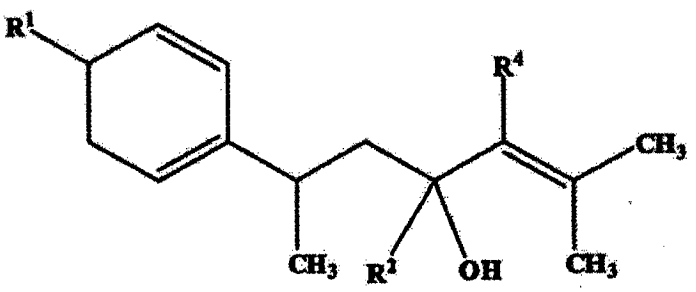


VIIIe

[0541] 或其药学上可接受的盐。

[0542] 在一些实施方式中，本发明考虑的 σ-2 配体或其药学上可接受的盐为式 VIIIf 的化合物：

[0543]

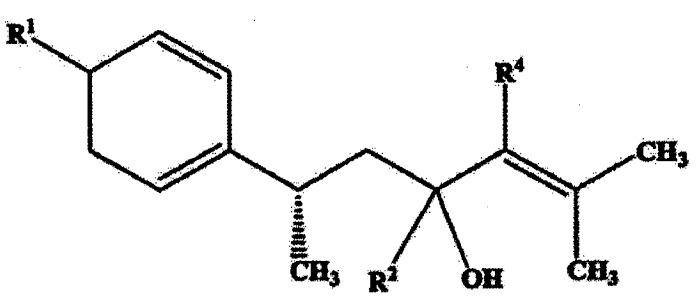


VIIIf

[0544] 或其药学上可接受的盐。

[0545] 在一些实施方式中，本发明的化合物或其药学上可接受的盐为式 VIIIg 的化合物：

[0546]

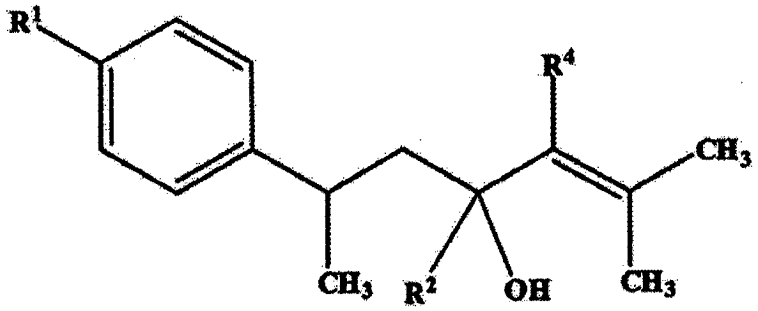


VIIIg

[0547] 或其药学上可接受的盐。

[0548] 在一些实施方式中，本发明考虑的 σ-2 配体或其药学上可接受的盐为式 VIIIh 的化合物：

[0549]

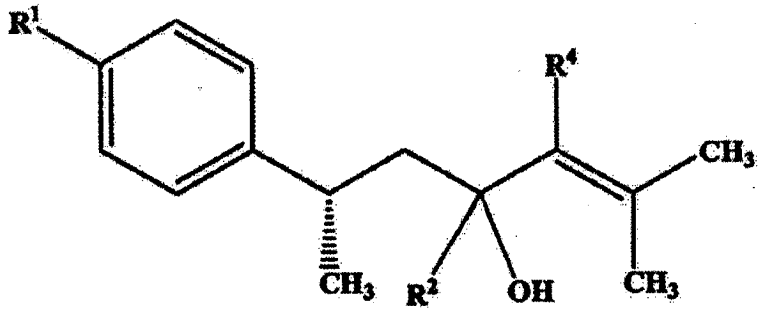


VIIIh

[0550] 或其药学上可接受的盐。

[0551] 在一些实施方式中，本发明的化合物或其药学上可接受的盐为式 VIIIi 的化合物：

[0552]

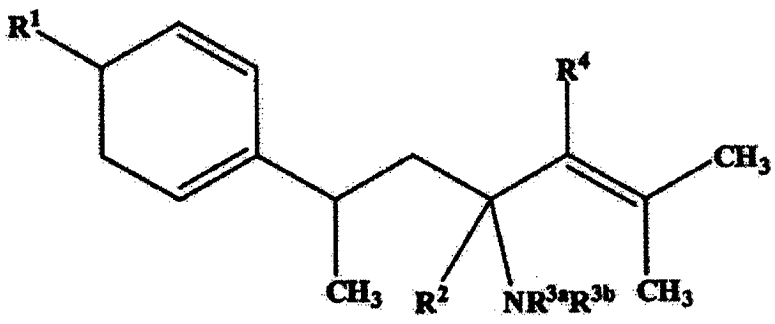


VIIIi

[0553] 或其药学上可接受的盐。

[0554] 在一些实施方式中，本发明的化合物或其药学上可接受的盐为式 VIIIj 的化合物：

[0555]

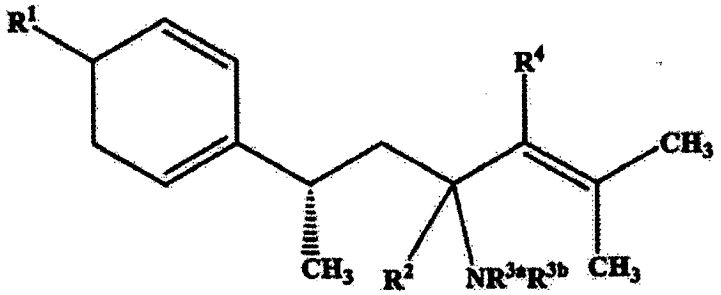


VIIIj

[0556] 或其药学上可接受的盐。

[0557] 在一些实施方式中，本发明考虑的 σ-2 配体或其药学上可接受的盐为式 VIIIk 的化合物：

[0558]

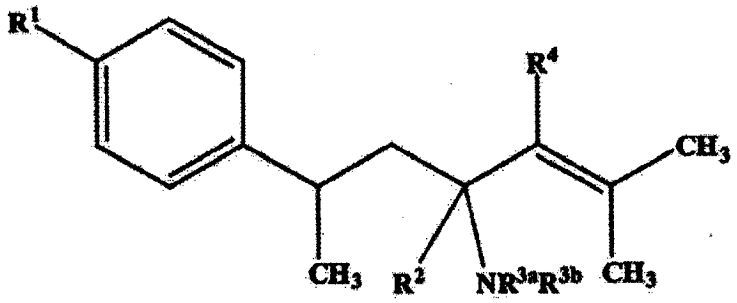


VIIIk

[0559] 或其药学上可接受的盐。

[0560] 在一些实施方式中，本发明的化合物或其药学上可接受的盐为式 VIIIm 的化合物：

[0561]

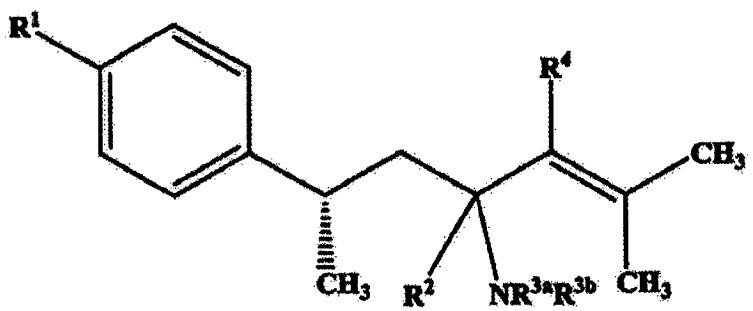


VIIIm

[0562] 或其药学上可接受的盐。

[0563] 在一些实施方式中，本发明的化合物或其药学上可接受的盐为式 VIIIn 的化合物：

[0564]



VIIIn

[0565] 或其药学上可接受的盐。

[0566] 在化合物 VIII 和其子集通式的一些实施方式中，R^1 为 H、CH_3 或 CF_3。在一些其他实施方式中，R^1 为 CH_3 或 CF_3。在其他实施方式中，R^1 为 CH_3。

[0567] 在化合物 VIII 和其子集通式的一些实施方式中，R^1 为 H 或 CH_3。

[0568] 在化合物 VIII 和其子集通式的一些实施方式中，R^1 为 F、Cl 或 Br。在其他实施方

式中，R^1 为 OCF_3。

[0569] 在化合物 VIII 和其子集通式的一些实施方式中，R^2 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基或 C_{6-10} 芳基。在一些其他实施方式中，R^2 为 H、C_{1-6} 烷基或 C_{3-7} 环烷基。

[0570] 在化合物 VIII 和其子集通式的一些实施方式中，R^2 为 H 或 C_{1-6} 烷基。在一些其他实施方式中，R^2 为 H 或甲基。在其他实施方式中，R 为 H。

[0571] 在化合物 VIII 和其子集通式的一些实施方式中，R^1 为 H、CH_3 或 CF_3；且 R^2 为 H 或 C_{1-6} 烷基。在一些其他实施方式中，R^1 为 CH_3 或 CF_3；且 R^2 为 H。在其他实施方式中，R^1 为 CH_3；且 R^2 为 H。

[0572] 在化合物 VIII 和其子集通式的一些实施方式中，R^{3a} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、环烷基烷基、C_{3-7} 环烷基、芳烷基或 C_{6-10} 芳基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、环烷基烷基、C_{3-7} 环烷基、芳烷基或 C_{6-10} 芳基中的每一个被 0、1、2、3、4 或 5 个各自独立选自卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基的取代基所取代。在一些其他实施方式中，R^{3a} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、环烷基烷基、C_{3-7} 环烷基、芳烷基或 C_{6-10} 芳基。

[0573] 在化合物 VIII 和其子集通式的一些实施方式中，R^{3b} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、环烷基烷基、C_{3-7} 环烷基、芳烷基或 C_{6-10} 芳基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、环烷基烷基、C_{3-7} 环烷基、芳烷基或 C_{6-10} 芳基中的每一个被 0、1、2、3、4 或 5 个各自独立选自卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基的取代基所取代。在一些其他实施方式中，R 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、环烷基烷基、C_{3-7} 环烷基、芳烷基或 C_{6-10} 芳基。

[0574] 在化合物 VIII 和其子集通式的一些实施方式中，R^{3a} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基或 C_{6-10} 芳基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基或或 C_{6-10} 芳基中的每一个被 0、1、2、3、4 或 5 个各自独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基的取代基所取代。

[0575] 在化合物 VIII 和其子集通式的一些实施方式中，R^{3b} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基或 C_{6-10} 芳基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基或 C_{6-10} 芳基中的每一个被 0、1、2、3、4 或 5 个各自独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基的取代基所取代。

[0576] 在化合物 VIII 和其子集通式的一些实施方式中，R^{3a} 为 H；且 R^{3b} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、环烷基烷基、C_{3-7} 环烷基、芳烷基或 C_{6-10} 芳基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、环烷基烷基、C_{3-7} 环烷基、芳烷基或 C_{6-10} 芳基中的每一个被 0、1、2、3、4 或 5 个各自独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基的取代基所取代。在一些其他实施方式中，R^{3a} 为 H；且 R^{3b} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、环烷基烷基、C_{3-7} 环烷基、芳烷基或 C_{6-10} 芳基，其中 C_{1-6} 烷基、C_{1-6} 卤代烷基、环烷基烷基、C_{3-7} 环烷基、芳烷基或 C_{6-10} 芳基中的每一个被 0、1、2、3、4 或 5 个各自独立选自卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基和 C_{1-6} 卤代烷氧基的取代基所取代。

[0577] 在化合物 VIII 和其子集通式的一些实施方式中，R^{3a} 为 H；且 R^{3b} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、环烷基烷基、C_{3-7} 环烷基、芳烷基或 C_{6-10} 芳基。

[0578] 在化合物 VIII 和其子集通式的一些实施方式中，R^{3a} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基或 C_{6-10} 芳基；且 R^{3b} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基或 C_{6-10} 芳基。

[0579] 在化合物 VIII 和其子集通式的一些实施方式中，R^{3a} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、

C_{3-7} 环烷基或 C_{6-10} 芳基；且 R^{3b} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基或 C_{6-10} 芳基；

[0580] 在化合物 VIII 和其子集通式的一些实施方式中，R^{3a} 为 H；且 R^{3b} 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基或 C_{3-7} 环烷基。

[0581] 在化合物 VIII 和其子集通式的一些实施方式中，R^{3a} 为 H；且 R^{3b} 为 C_{1-6} 烷基或 C_{1-6} 卤代烷基。

[0582] 在化合物 VIII 和其子集通式的一些实施方式中，R^{3a} 为 H；且 R^{3b} 为 C_{1-6} 烷基。

[0583] 在化合物 VIII 和其子集通式的一些实施方式中，R^{3a} 和 R^{3b} 与它们连接的 N 原子一起形成吡咯烷基、哌啶基、哌嗪基或吗啉基，其各自被 0、1、2、3、4 或 5 个各自独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、芳基、芳烷基、杂芳基、杂芳烷基、环烷基和杂环烷基的取代基所取代。

[0584] 在化合物 VIII 和其子集通式的一些实施方式中，R^{3a} 和 R^{3b} 与它们连接的 N 原子一起形成吡咯烷基、哌啶基、哌嗪基或吗啉基，其各自被 0、1、2、3、4 或 5 个各自独立选自 OH、氨基、卤素、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{1-6} 烷氧基、C_{1-6} 卤代烷氧基、苯基和苄基的取代基所取代。

[0585] 在化合物 VIII 和其子集通式的一些实施方式中，R^4 为 H、C_{1-6} 烷基、C_{1-6} 卤代烷基、C_{3-7} 环烷基或 C_{6-10} 芳基。在一些其他实施方式中，R^4 为 H、C_{1-6} 烷基或 C_{3-7} 环烷基。

[0586] 在一些实施方式中，R^4 为 H 或 C_{1-6} 烷基。在一些其他实施方式中，R^4 为 H 或甲基。在其他实施方式中，R^4 为 H。

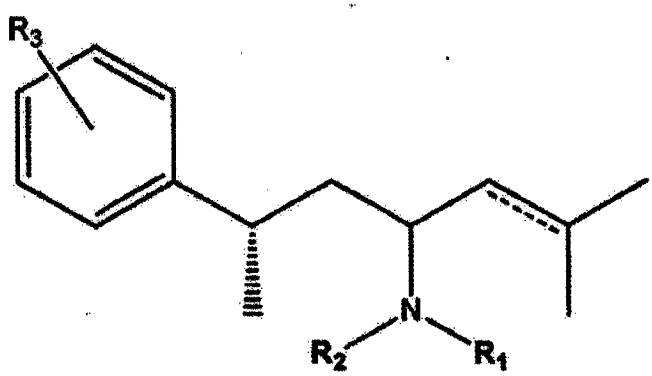
[0587] 在化合物 VIII 和其子集通式的一些实施方式中，R^2 为 H 或 C_{1-6} 烷基，且 R^4 为 H 或 C_{1-6} 烷基。

[0588] 在化合物 VIII 和其子集通式的一些实施方式中，R^2 为 H 或甲基且 R^4 为 H 或甲基。

[0589] 在化合物 VIII 和其子集通式的一些实施方式中，R^1 为 H、CH_3、或 CF_3；R^2 为 H 或 C_{1-6} 烷基，且 R^4 为 H 或 C_{1-6} 烷基。在一些其他实施方式中，R^1 为 CH_3 或 CF_3；R^2 为 H；且 R^4 为 H。在其他实施方式中，R^1 为 CH_3；R^2 为 H；且 R^4 为 H。

[0590] 在另一实施方式中，本发明的 σ-2 配体为式 VIIIo 的那些或其药学上可接受的盐：

[0591]



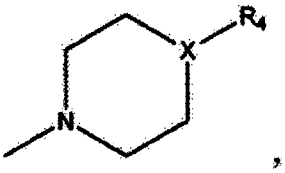
VIIIo

[0592] 其中：

[0593] ——为单键或双键；

[0594] R_1 为 C_{1-6} 烷基、C_{1-6} 卤代烷基、未被取代的苄基或被卤素、C_{1-6} 烷基或 C_{1-6} 卤代烷基取代的苄基；

[0595] R_2 为 H，或者

[0596] R_1 和 R_2 与氮一起形成环  其中，

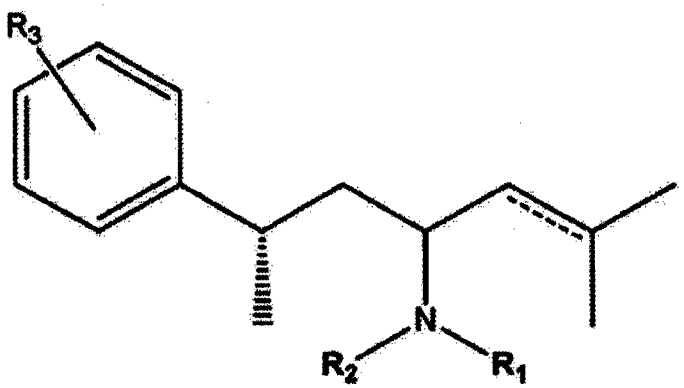
[0597] X 为 CH、N 或 O，且

[0598] R_4 不存在或为 H、C_{1-6} 烷基或未被取代的苯基或被卤素、C_{1-6} 烷基或 C_{1-6} 卤代烷基取代的苯基；且

[0599] R_3 为 C_{1-4} 烷基、卤素或 C_{1-6} 卤代烷氧基。

[0600] 在一些实施方式中，本发明的 σ-2 配体为式 VIIIo 的那些或其药学上可接受的盐：

[0601]



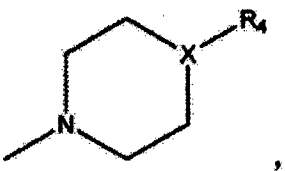
VIIIo

[0602] 其中：

[0603] ▬▬▬ 为单键或双键；

[0604] R_1 为异丁基、苄基或被氯、甲基或 CF_3 取代的苄基；

[0605] R_2 为 H，或者

[0606] R_1 和 R_2 与氮一起形成环  其中，

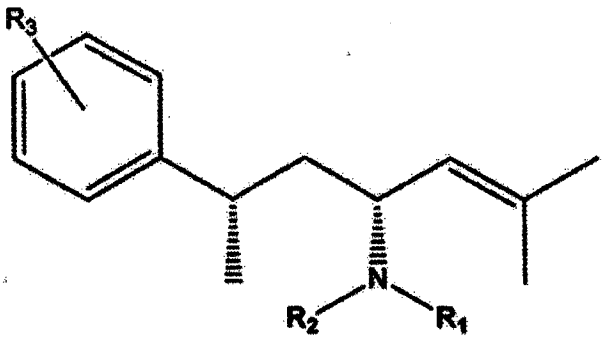
[0607] X 为 CH、N 或 O，且

[0608] R_4 不存在或为 H、异丙基或未被取代的苯基；且

[0609] R_3 为邻 -Me、间 -Me、对 -Me、对 -F 或对 $-OCF_3$。

[0610] 在一些更加具体的实施方式中，本发明的 σ-2 配体为式 VIIIp 的那些或其药学上可接受的盐：

[0611]

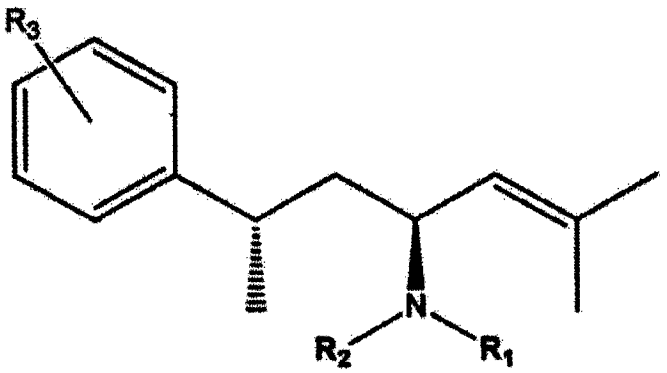


VIIIp

[0612] 其中 R_1 ～ R_3 如以上针对式 VIIIa 所定义。

[0613] 在一些更加具体的实施方式中，本发明的 σ-2 配体为式 VIIIq 的那些或其药学上可接受的盐：

[0614]

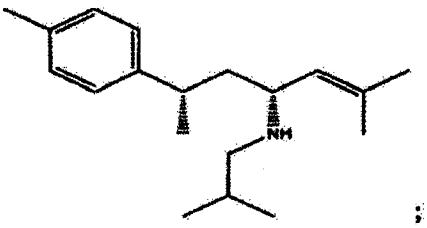


VIIIq

[0615] 其中 R_1 ～ R_3 如以上针对式 VIIIa 所定义。

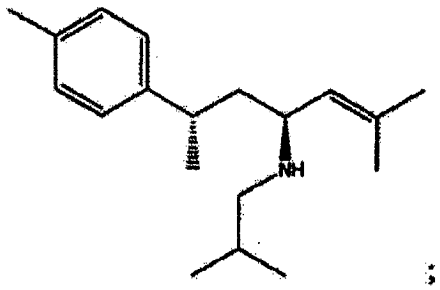
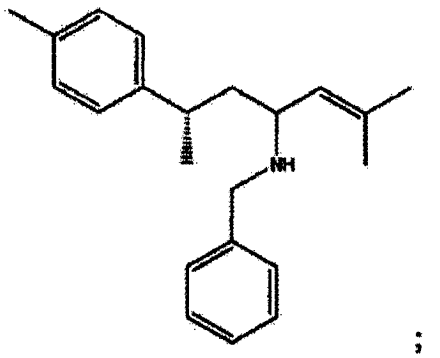
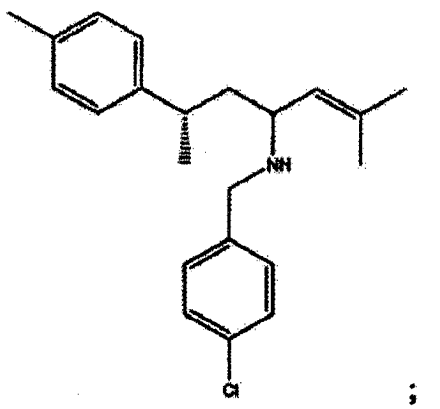
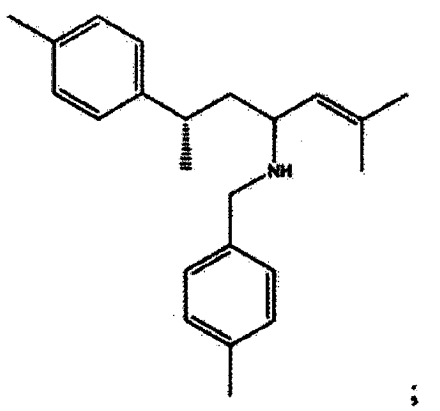
[0616] 表 1C：本发明的式 VIIIo ～ q 化合物的具体示例性化合物列于以下表格中：

[0617]

式 VIIIo~q 的化合物


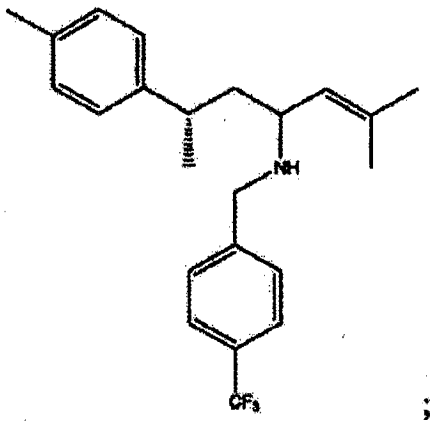
;

[0618]

式 VIIIo~q 的化合物
 ;
 ;
 ;
 ;

[0619]

式 VIIIo~q 的化合物



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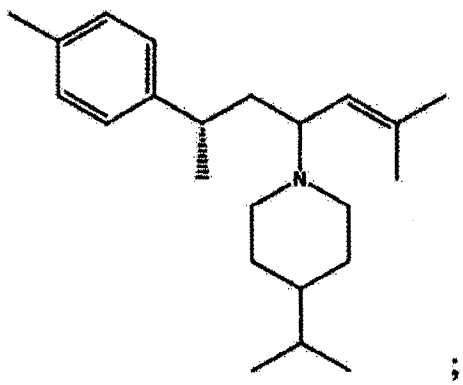

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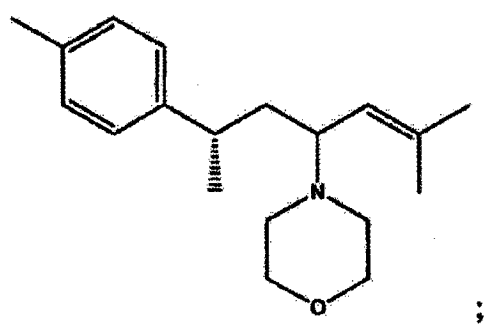

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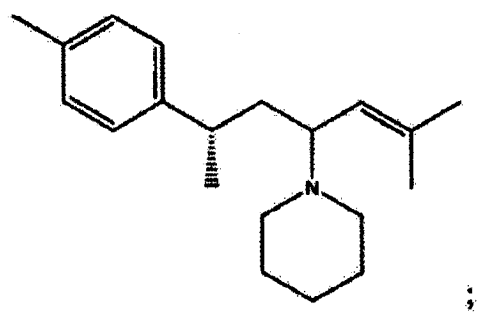
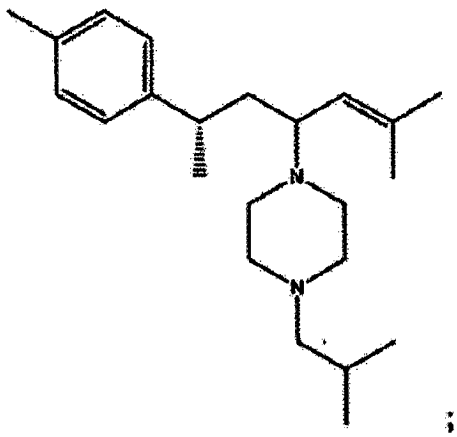
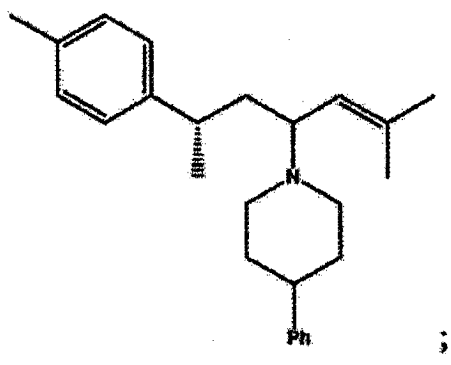
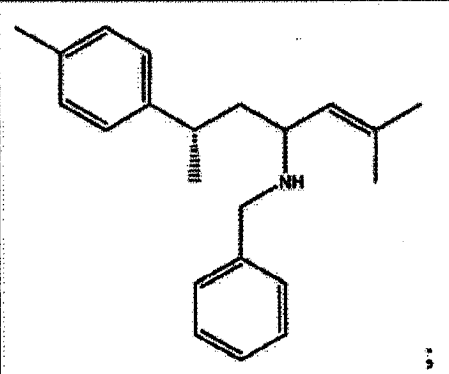
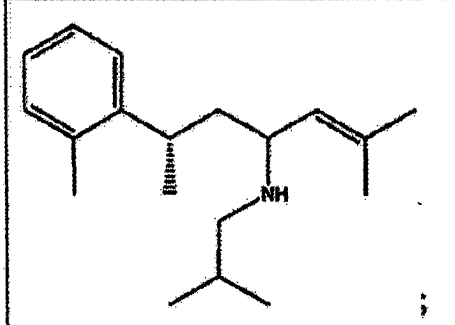


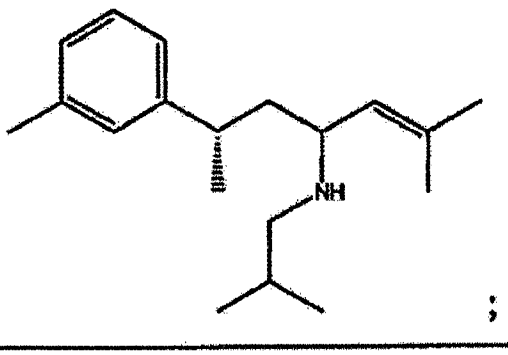
;

[0620]

式 VIIIo~q 的化合物
 ;
 ;
 ;
 ;

[0621]

式 VIIIo~q 的化合物



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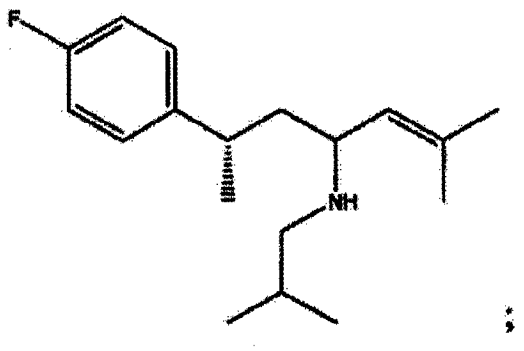

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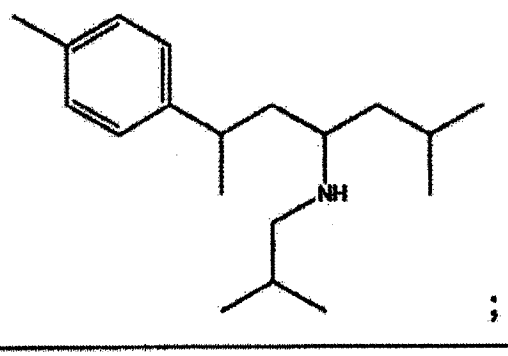
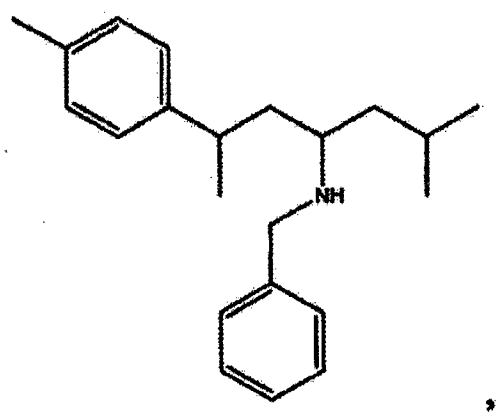


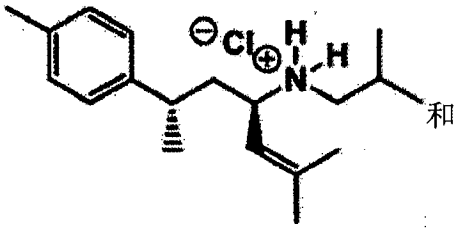
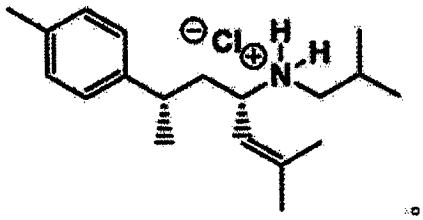
; 和



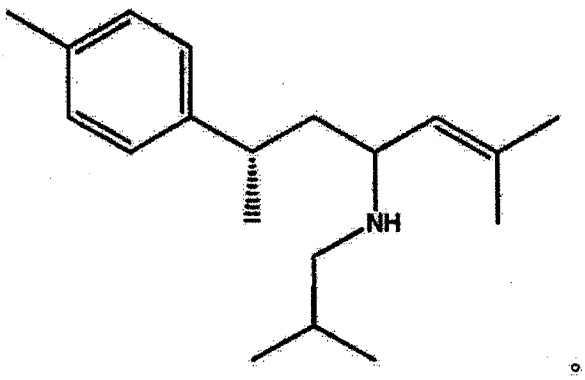
,

[0622] 或其药学上可接受的盐。

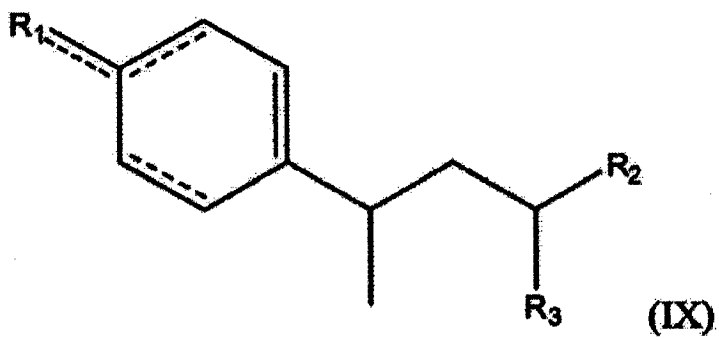
[0623] 用于本发明的优选盐包括上述化合物的盐酸盐，包括以下：

[0624]  和  。

[0625] 在一些实施方式中，以上通式中的每一个可以包含排除一种或多种以下化合物的条件：

[0626]  。

[0627] 在其他实施方式中，σ-2 配体为式 IX 的化合物：

[0628] 

[0629] 或其药学上可接受的盐，其中

[0630] ▬▬▬ 为单键或双键；

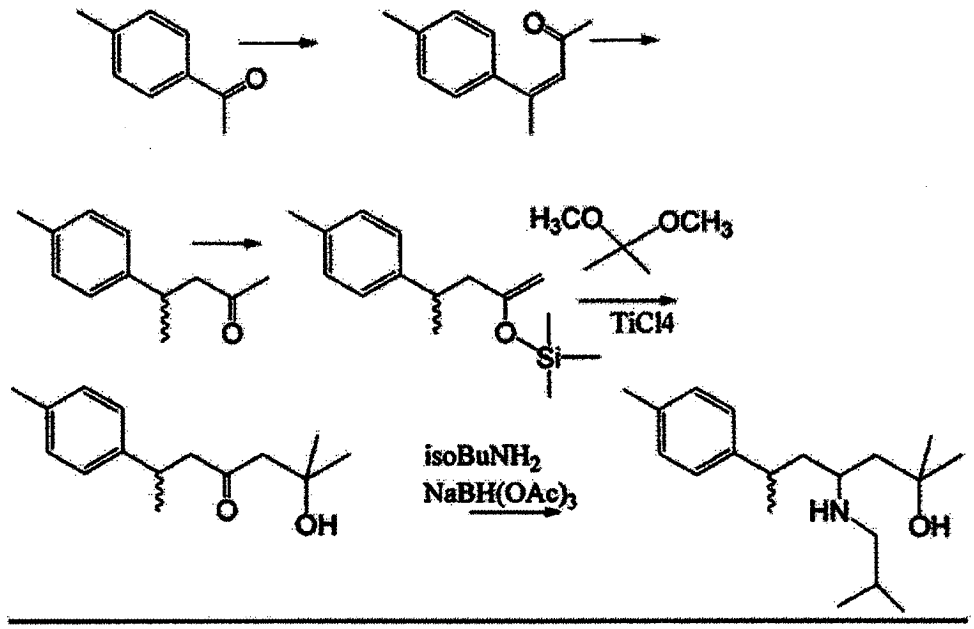
[0631] R_1 选自 CH_3、CH_2、F、Cl、Br、CF_3、O-烷基和 OCF_3；

[0632] R_2 选自 $CH_2C(CH_3)_2OH$ 和 $CH=C(CH_3)_2$；

[0633] R_3 选自 OH 或 $NHCH_2CH(CH_3)_2$ 或其混合物。

[0634] 在一些实施方式中，式 IX 的化合物可以通过还原胺化来制备，例如方案 8 中所示的路线。

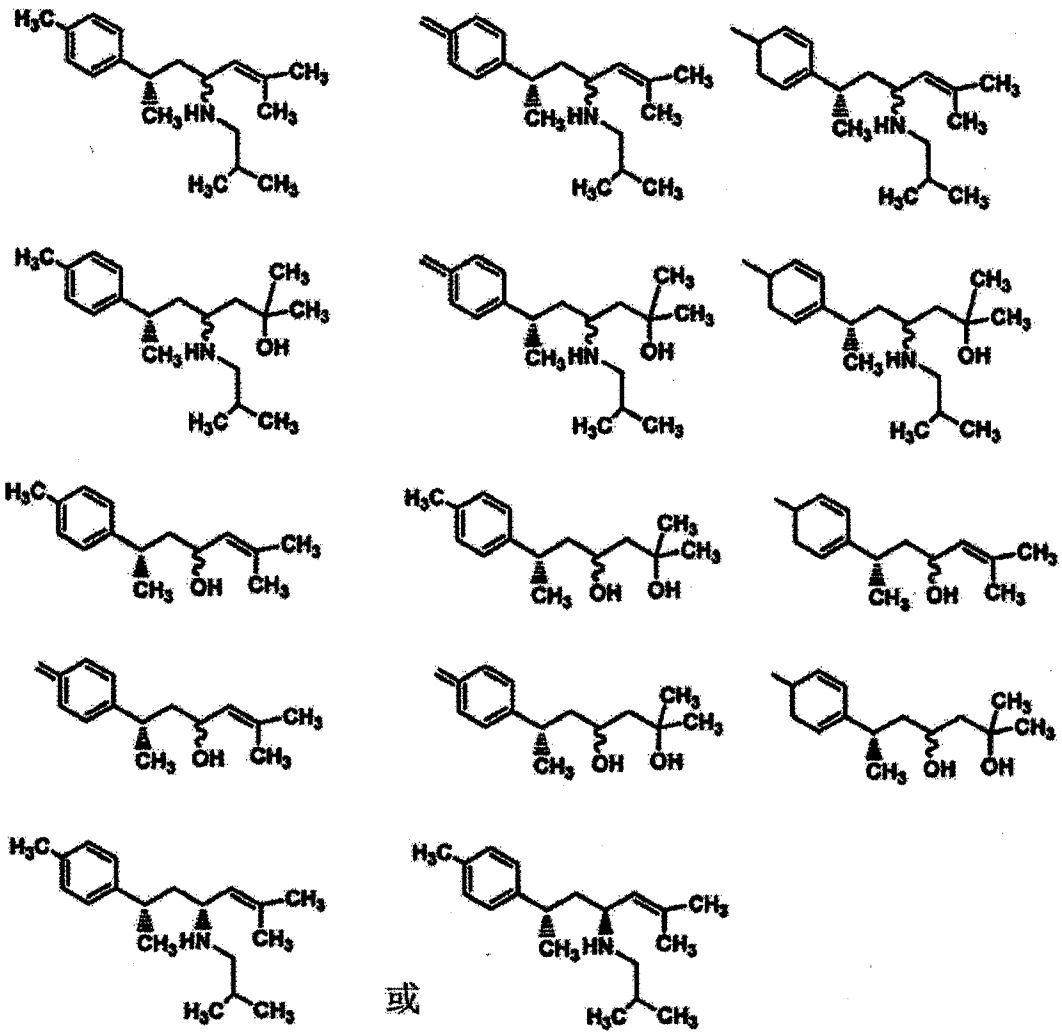
[0635]



方案 8

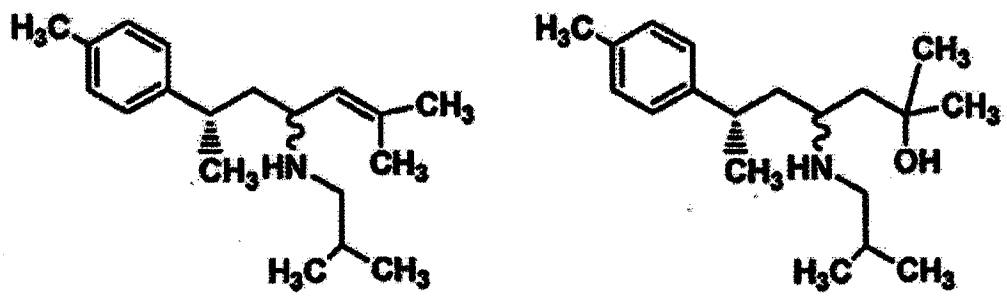
[0636] 在一些实施方式中，式 IX 的种类可以选自：

[0637]



[0638] 式 IX 的化合物的实例包括以下化合物，其为非对映异构体的混合物，并包括活性芳族胺烯烃和氨基醇成分 IXa 和 IXb

[0639]

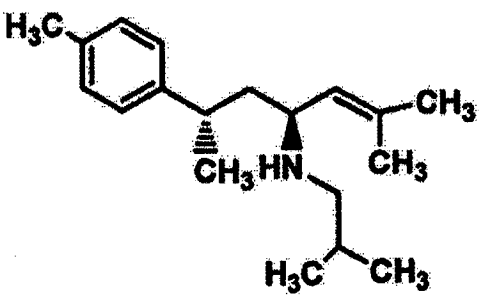


IXa IXb

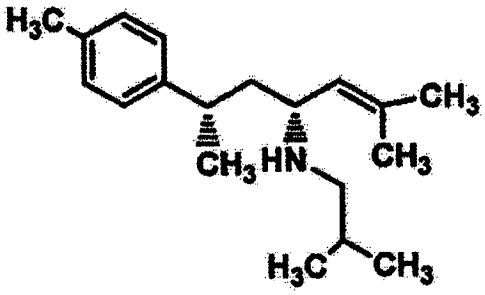
[0640] 在具体实施方式中，σ-2 受体拮抗剂选自化合物 IXa 和 IXb 以及对映异构体和药学上可接受的盐。

[0641] 在具体实施方式中，选择性的、高亲和性 σ-2 受体拮抗剂选自 IXa-1 和 IXa-2

[0642]



IXa-1



IXa-2

[0643] 本发明的新化合物的盐、溶剂化物、立体异构体、衍生物、前药和活性代谢物

[0644] 本发明还包括任意上式化合物的盐、溶剂化物、立体异构体、前药和活性代谢物。

[0645] 术语“盐”可以包括酸加成盐或游离碱的加成盐。优选地，盐为药学上可接受的。可以用于形成药学上可接受的酸加成盐的酸的实例包括但不限于得自无毒无机酸以及得自无毒有机酸的盐，无机酸为例如硝酸、磷酸、硫酸或氢溴酸、氢碘酸、氢氟酸、亚磷酸，有机酸为例如脂族单羧酸和二羧酸、苯基取代的烷酸、羟基烷酸、烷二酸、芳族酸、脂族和芳族磺酸、和醋酸、马来酸、琥珀酸或柠檬酸。这样的盐的非限制性实例包括萘二磺酸盐、苯磺酸盐、硫酸盐、焦硫酸盐、硫酸氢盐、亚硫酸盐、亚硫酸氢盐、硝酸盐、磷酸盐、磷酸一氢盐、磷酸二氢盐、偏磷酸盐、焦磷酸盐、氯化物、溴化物、碘化物、醋酸盐、三氟醋酸盐、丙酸盐、辛酸盐、异丁酸盐、草酸盐、丙二酸盐、琥珀酸盐、辛二酸盐、癸二酸盐、富马酸盐、马来酸盐、扁桃酸盐、苯甲酸盐、氯苯甲酸盐、甲基苯甲酸盐、二硝基苯甲酸盐、邻苯二甲酸盐、苯磺酸盐、甲苯磺酸盐、苯基醋酸盐、柠檬酸盐、乳酸盐、马来酸盐、酒石酸盐、甲磺酸盐等。还包括氨基酸的盐例如精氨酸盐等以及葡糖酸盐、半乳糖醛酸盐（参见，例如 Berge 等人“Pharmaceutical Salts,”J. Pharma. Sci. 1977 ;66:1)

[0646] 在一些实施方式中，σ-2 受体配体化合物选自以下表 1D 中的化合物。

[0647] 表 1D. σ-2 受体配体盐酸盐化合物。

[0648]

HCl 盐制剂	σ-1 (Ki,nM)	σ-2 (Ki,nM)	MTTX (EC50,uM)
W	270	120	1.2
CF	180	50	12
CB	19	48	6.5
CU	5.8	1.3	3.9
DC	330	3200	10
DB	530	4000	5

[0649] 任意上式的化合物的酸加成盐可以通过使游离碱形式与足量的所需酸接触而制备，从而以常规方式产生盐。游离碱形式可以通过使盐形式与碱接触并以常规方式分离游离碱而再生。游离碱形式在某些物理特性（例如极性溶剂中的溶解度）方面某种程度上不同于其各自的盐形式，但其他方面，出于本发明目的，盐等同于其各自的游离碱。

[0650] 还包括全部盐和部分盐，也就是说相对于每摩尔的例如式 I 化合物的酸具有 1、2 或 3 优选为 2 当量的碱的盐，或者相对于每摩尔的任意上式化合物的碱具有 1、2 或 3 当量优选为 1 当量的酸的盐。

[0651] 出于分离或纯化的目的，也可以使用药学上不可接受的盐。然而，仅药学上可接受的非毒性盐在治疗上使用，因此被优选。

[0652] 药学上可接受的碱加成盐与金属或胺形成，例如碱金属和碱土金属或有机胺。用作阳离子的金属的实例为钠、钾、镁、钙等。合适的胺的实例为 N,N'-二苄基乙二胺、氯普鲁卡因、胆碱、二乙醇胺、二环己胺、乙二胺、N-甲基葡糖胺和普鲁卡因。

[0653] 该酸性化合物的碱加成盐通过使游离酸形式与足量的所需碱接触而制备，从而以常规方式产生盐。游离酸形式可以通过使盐形式与酸接触并分离游离酸而再生。

[0654] 本发明的化合物可以同时具有碱性和酸性中心，因此可以为两性离子形式或内盐形式。

[0655] 通常而言，任意上式的化合物的药学上可接受的盐可以容易地通过合适地使用所需酸或碱而制备。盐可以从溶液中沉淀并可以通过过滤而收集或者可以通过蒸发溶剂而回收。例如，酸例如盐酸的水溶液可以加至任意上式的化合物的水性混悬液中，并将所得的混合物蒸发至干（冻干）以获得作为固体的酸加成盐。或者，任意上式的化合物可以溶解在

合适的溶剂中，例如醇如异丙醇中，且酸可以加到同一溶剂或另一合适溶剂中。所得的酸加成盐之后可以直接沉淀，或通过添加极性较小的溶剂例如二异丙基醚或己烷来沉淀，并通过过滤而分离。

[0656] 有机化学领域的技术人员应理解，很多有机化合物可以与它们在其中反应的或它们从其中沉淀或结晶的溶剂形成复合物。这些复合物被称为“溶剂化物”。例如，与水的复合物被称为“水合物”。本发明化合物的溶剂化物在本发明的范围内。任意上式的化合物的盐可以形成溶剂化物（例如，水合物），且本发明还包括所有这些溶剂化物。词语“溶剂化物”的含义被本领域技术人员熟知为通过溶剂和溶质的相互作用（即溶剂化）形成的化合物。用于制备溶剂化物的技术在领域内已较好地建立（参见，例如Brittain. Polymorphism in Pharmaceutical solids. Marcel Decker, New Youk, 1999）。

[0657] 本发明还包括式I化合物的N氧化物。术语“N氧化物”是指，对于含有另外未被取代的sp^2N原子的杂环，N原子可以带有共价结合的O原子，即-N→O。这样的N氧化物取代的杂环的实例包括吡啶基N氧化物、嘧啶基N氧化物、吡嗪基N氧化物和吡唑基N氧化物。

[0658] 任何上式的化合物可以具有一个或多个手性中心，且根据单个取代基的性质，也可以具有几何异构体。空间上原子排布不同的异构体被称为“立体异构体”。不互为镜像的立体异构体被称为“非对映异构体”，且互为非重合镜像的立体异构体被称为“对映异构体”。当化合物具有手性中心时，一对对映异构体是可能的。对映异构体的特征可以是其非对称中心的绝对构型，并且通过Cahn和Prelog的R--和S-排序规则而描述，或者通过分子旋转偏振光的平面的方式而描述，并命名为右旋或左旋（即，分别为(+)或(-)异构体）。手性化合物可以以单个对映异构体或对映异构体的混合物存在。含有等比例对映异构体的混合物称为“外消旋混合物”。含有非等比例对映异构体的混合物被描述为具有R或S化合物的“对映异构体过量”(ee)。混合物中一种对映异构体的过量经常用下式确定的%对映异构体过量（% ee）值来描述：

[0659] $\%\ ee = (R)-(S)/(R)+(S)$

[0660] 对映异构体的比率还可以通过“光学纯度”来定义，其中将对映异构体的混合物旋转平面偏振光的度数与单个光学纯的R和S化合物进行比较。光学纯度可以使用下式确定：

[0661] 光学纯度＝对映异构体$_{主要}$/（对映异构体$_{主要}$+对映异构体$_{次要}$）。

[0662] 化合物还可以是本文所述化合物的基本纯的(+)或(-)对映异构体。在一些实施方式中，包括基本纯的对映异构体的组合物包括至少90、91、92、93、94、95、96、97、98或99％的一种对映异构体。在一些实施方式中，包括基本纯的对映异构体的组合物为至少99.5％的一种对映异构体。在一些实施方式中，组合物仅包括本文所述化合物的一种对映异构体。

[0663] 本发明包括任意上式化合物的所有单个异构体。说明书和权利要求书中对特定化合物的描述或命名意在包括单个对映异构体或其混合物、外消旋物或其他。用于确定立体异构体的立体化学和拆分或立体定向合成的方法为本领域熟知。具体而言，在任意上式化合物中显示有手性中心，其引起一组对映异构体。其他手性中心可以根据取代基而存在。

[0664] 对于很多应用，优选执行立体选择性合成和/或使反应产物进行合适的纯化步

骤，从而制备基本光学纯的材料。用于制备光学纯的材料的合适的立体选择性合成程序为领域内熟知，用于将外消旋混合物纯化为光学纯的部分的程序也是。本领域技术人员还将认识到，本发明化合物可以以多晶型存在，其中化合物能够以不同形式结晶。用于识别和分离多晶型的适当方法为本领域已知。

[0665] 非对映异构体在物理特性和化学反应性两方面不同。非对映异构体的混合物可以基于溶解度、分级结晶或色谱特性例如薄层色谱、柱色谱或 HPLC 而分离成对映异构体对。

[0666] 将非对映结构体的复杂混合物纯化为对映异构体通常需要两个步骤。在第一步骤中，将非对映结构体的混合物拆分为对映异构体对，如上所述。在第二步骤中，还将对映异构体对纯化为富集一种或另一种对映异构体的组成，更优选拆分为含有纯对映异构体的组成。对映异构体的拆分通常需要与手性试剂例如溶剂或柱基质的反应或分子相互作用。可以例如通过与第二试剂（即拆分剂）的纯对映异构体的反应，将对映异构体的混合物例如外消旋混合物转化为非对映异构体的混合物而实现拆分。之后可以分离两种所得的非对映异构产物。分离的非对映异构体之后通过逆转初始的化学变换而再转化为纯对映异构体。

[0667] 对映异构体的拆分还可以通过它们与手性物质的非共价结合的差异而实现，例如，通过在纯手性吸附剂上的层析。对映异构体与层析吸附剂之间的非共价结合创建非对映异构复合物，引起层析体系中移动和结合状态的差异。因此，两种对映异构体经层析体系例如柱以不同速率移动，使得它们分离。

[0668] 手性拆分柱在领域内熟知，并市售可得（例如，从 MetaChem Technologies Inc.，ANSYS Technologies, Inc., Lake Forest, CA 的分部）。对映异构体可以使用例如 HPLC 用的手性固定相 (CSP) 而分析和纯化。手性 HPLC 柱通常含有固定至二氧化硅填充料的表面的一种形式的对映异构体化合物。

[0669] D- 苯基甘氨酸和 L- 亮氨酸是 I 型 CSP 的实例并使用 π - π 相互作用、氢键、偶极 - 偶极相互作用和空间相互作用的组合来实现手性识别。为在 I 型柱上拆分，分析物对映异构体必须含有与 CSP 的官能性互补的官能性，以使分析物进行与 CSP 的重要相互作用。样品应该优选包含一种以下的官能团：π - 酸或 π - 碱、氢键供体和 / 或受体、或酰胺偶极。有些时候使用衍生来向缺乏相互作用位点的化合物添加相互作用位点。最常见的衍生物涉及由胺和羧酸形成酰胺。

[0670] MetaChiral ODM™ 是 II 型 CSP 的实例。形成溶质 -CSP 复合物的主要机制是通过吸引相互作用，但是包合复合物也起重要作用。氢键、π - π 相互作用和偶极堆叠对于 MetaChiral™ ODM 上的手性拆分较为重要。当溶质分子不包含溶质 - 柱相互作用所需的基团时，衍生可能是必须的。通常向苄基酰胺的衍生对于一些强极性分子例如胺和羧酸是所需的，否则其会经由非特异性立体相互作用而与固定相强烈相互作用。

[0671] 在适用的地方，任意上式的化合物可以通过例如经由柱色谱或硅胶上 TLC 的分离而分离成非对映异构体对。这些非对映异构体对在本文中称为具有高 TLC Rf 的非对映异构体以及具有低 TLC Rf 的非对映异构体。非对映异构体还可以使用本领域熟知的方法例如本文描述的那些而富集特定对映异构体，或拆分为单个对映异构体。

[0672] 非对映异构体对的相对构型可以通过应用理论模型或规则（例如 Cram 规则、Felkin-Ahn 模型）或使用计算化学程序产生的更可靠的三维模型来进行推导。在很多情况中，这些方法能够预测哪个非对映异构体是化学变换的能量上有利的产物。作为可选的方

法，非对映异构体的相对构型可以通过发现非对映异构体对的一种（或两种）中的单个对映异构体的绝对构型而间接确定。

[0673] 立体中心的绝对构型可以通过本领域技术人员非常熟知的方法（例如，X 射线衍射、圆二色性）而确定。绝对构型的确定还可用于证实理论模型的可预测性，并且可有助于将这些模型的使用拓展到通过具有类似机制的反应（例如，经由氢化物的酮还原或酮的还原胺化）制备的相似分子。

[0674] 本发明还可以包括由于不直接与环连接的双键上的 R_2 ～ R_3 取代基而产生的 Z-E 型的立体异构体及其混合物。当 m 不是 1 且 m 和 n 不同时，会遇到其他 Z-E 立体异构体。应用 Cahn-Ingold-Prelog 优先级规则来确定由于双键连接的取代基的双键平面中的各个位置引起的立体异构体是否为 Z 或 E。如果最高优先级的 2 个基团位于经过 C ＝ C 键的参考平面的同一侧，则立体异构体被命名为 Z(zusammen ＝一起）。另一种立体异构体被命名为 E(entgegen ＝相反）。

[0675] E-Z 型的立体异构体的混合物可以使用基于这些化合物的不同化学物理特性的经典纯化方法而分离（和 / 或表现）为它们的组分。包括在这些方法中的是分级结晶，通过低、中或高压技术执行的色谱，分级蒸馏和本领域技术人员非常熟知的任何其他方法。

[0676] 本发明还包括任意上式化合物的前药，即，当施用至哺乳动物受试者时，在体内释放任意上式的活性药物的化合物。前药是药理上有活性的化合物，或者更通常地为无活性化合物，其通过代谢转化而转化成药理学活性剂。任意上式化合物的前药通过对任意上式化合物中存在的官能团进行修饰而制备，使得该修饰可以在体内断裂以释放母体化合物。在体内，前药容易在生理条件下进行化学变化（例如，水解或通过天然出现的酶而作用），引起药理学活性剂的释放。前药包括任意上式的化合物，其中羟基、氨基或羧基与可以在体内断裂的任意基团键合以分别再生游离羟基、氨基或羧基。前药的实例包括但不限于任意上式化合物的酯（例如乙酸酯、甲酸酯和苯甲酸酯衍生物）或任何其他在生理 pH 时或通过酶作用被转化为活性母体药物的衍生物。选择和制备适当前药衍生物的常规程序在本领域中有记载（参见，例如，Bundgaard. Design of Prodrugs. Elsevier, 1985）。

[0677] 前药可以以与它们所转化的活性成分相同的方式进行施用，或者它们可以以储库 (reservoir) 形式例如经皮贴剂或其他适用于（通过提供酶或其他合适试剂）将前药随时间缓慢转化为活性成分并将活性成分输送至患者的其他储库而进行输送。

[0678] 除非特别指明，术语“活性成分”被理解为指代本文定义的任意上式的化合物。

[0679] 本发明还包括代谢物。本文公开化合物的“代谢物”是在化合物被代谢时形成的化合物的衍生物。术语“活性代谢物”是指当化合物被代谢时形成的化合物的生物学活性衍生物。术语“代谢”是指特定物质在活体内变化的过程的总和。简单而言，身体内存在的所有化合物通过身体内的酶操控，以获取能量和 / 或从将其从身体中去除。特定的酶对化合物生成特定的结构变化。例如，细胞色素 P450 催化多种氧化和还原反应，而尿苷二磷酸葡糖醛酸基转移酶催化活化的葡糖醛酸分子转移到芳族醇、脂族醇、羧酸、胺和游离硫基。其他代谢信息可以从 The Pharmacological Basis of Therapeutics，第九版，McGraw-Hill (1996)，第 11 ～ 17 页中获得。本文公开的化合物的代谢物可以通过将化合物施用至宿主并分析宿主的组织样品、或通过将化合物与肝细胞在体外孵育并分析所得化合物而进行识别。两种方法均为本领域熟知。

[0680] σ-2 受体拮抗剂的使用

[0681] 在一些实施方式中，本发明提供通过施用 σ-2 受体拮抗剂来抑制与神经元细胞暴露于 Abeta 种类相关的突触数目减退或膜运输异常的方法。本发明还提供在患者中治疗认知衰退和 / 或神经退行性疾病例如阿尔茨海默病或轻度认知障碍 (MCI) 的方法，包括向患者施用本文记载的 σ-2 拮抗剂，例如任意本文所述通式包括的那些或其药学上可接受的盐。在一些实施方式中，抑制或治疗认知衰退和 / 或神经退行性疾病例如阿尔茨海默病的方法包括抑制或治疗一种或多种选自记忆丧失、混乱、判断受损、人格改变、定向障碍和语言技能丧失的认知衰退症状。在一些实施方式中，方法包括抑制或治疗由 Abeta 寡聚体介导的或与 Abeta 寡聚体相关的疾病或病症或障碍 (参见段落 002)。在一些实施方式中，抑制或治疗认知衰退和 / 或神经退行性疾病例如阿尔茨海默病的方法包括以下的一种或多种 :(i) 恢复经电生理测量可检测的长时程增强效应 (LTP)、长时程抑制效应 (LTD) 或突触可塑性或者任何在以上术语定义中提及的认知功能的负面变化 ;和 / 或 (ii) 抑制或治疗神经退行 ;和 / 或 (iii) 抑制或治疗一般的淀粉样变性 ;和 / 或 (iv) 抑制或治疗淀粉样蛋白产生、淀粉样蛋白组装、淀粉样蛋白聚集和淀粉样蛋白寡聚体结合和淀粉样蛋白沉积中的一种或多种 ;和 / 或 (v) 抑制、治疗和 / 或减轻一种或多种 Abeta 寡聚体对神经元细胞的作用、特别是非致死作用。在一些实施方式中，抑制、治疗和 / 或减轻认知衰退和 / 或神经退行性疾病例如阿尔茨海默病的方法包括抑制、治疗和 / 或减轻淀粉样蛋白产生、淀粉样蛋白组装、一种或多种 Abeta 寡聚体对神经元细胞的活性 / 作用、淀粉样蛋白聚集、淀粉样蛋白结合和淀粉样蛋白沉积中的一种或多种。在一些实施方式中，抑制、治疗和 / 或减轻认知衰退和 / 或神经退行性疾病例如阿尔茨海默病的方法包括抑制、治疗和 / 或减轻一种或多种 Abeta 寡聚体对神经元细胞的一种或多种的活性 / 作用。

[0682] 在一些实施方式中，一种或多种 Abeta 寡聚体对神经元细胞、淀粉样蛋白聚集和淀粉样蛋白结合的活性 / 作用是 Abeta 寡聚体对膜运输或突触数目的作用。在一些实施方式中，σ-2 拮抗剂抑制对膜运输或突触数目或 Abeta 寡聚体结合的 Abeta 寡聚体作用。

[0683] 在一些实施方式中，本发明提供治疗与 Abeta 寡聚体毒性 (具体为非致死 Abeta 寡聚体作用) 相关的蛋白病态 (proteopathic) 疾病的方法。在一些实施方式中，方法包括使具有这样的蛋白病态疾病的受试者与本发明的与 σ-2 受体结合的 σ-2 拮抗剂或含有该拮抗剂的组合物接触。

[0684] 在一些实施方式中，蛋白病态疾病是 CNS 蛋白病态，特征为 Abeta 蛋白增加，例如 MCI、唐氏综合症、黄斑变性或阿尔茨海默病等。

[0685] 在一些实施方式中，本发明提供通过施用本发明的 σ-2 拮抗剂来治疗一种或多种轻度认知障碍 (MCI) 或痴呆的方法。在一些实施方式中，本发明提供治疗 MCI 和痴呆的方法。

[0686] 在一些实施方式中，本发明提供用本发明的 σ-2 拮抗剂治疗个体，以在受到 Abeta 种类例如 Abeta 寡聚体不利影响的功能方面部分地或全部地将受试者的细胞恢复到正常表型的方法。实例是可以通过多种方法包括本文所述检定测量的突触数目减少和膜运输异常。正常的表型可以是例如正常的膜运输。在一些实施方式中，正常的表型是正常的认知能力。“正常”表型可以通过将受试者的结果与正常受试者的样品进行比较而确定。样品可以少至 1 个受试者或 1 个样品，或者可以多于 10 个样品或受试者，且标准是基于多个

受试者计算的均值。

[0687] 在一些实施方式中，方法包括向遭受认知衰退或神经退行性疾病的受试者施用与σ-2蛋白结合并抑制β淀粉样蛋白病理的化合物或组合物。在一些实施方式中，β淀粉样蛋白病理是膜运输缺陷、突触数目的减少、树突棘数目的减少、树突棘形态的变化、LTP的变化、LTD的变化、动物的记忆和学习测量中的缺陷或其任意组合等。以上使用得自发明者举出的以下证据：

[0688] 以上通式内的σ-2受体配体已经显示为选择性的、高亲和性σ-2受体配体。例如化合物II显示出在大鼠新皮质匀浆的σ-2受体处的[^{3}H]DTG/300nM(+)-戊唑辛替代的K_i9+/-4nM以及在人Jurkat细胞膜的σ-1受体处的[3H]-((+)-戊唑辛替代的K_i500+/-200nM。化合物IXa、IXb显示出在大鼠新皮质匀浆的σ-2受体处的[^{3}H]DTG/300nM(+)-戊唑辛替代的K_i54+/-22nM以及在人Jurkat细胞膜的σ-1受体处的[3H]-((+)-戊唑辛替代的K_i31+/12nM。相似地，化合物II显示出在大鼠肝脏匀浆的σ-2受体处的[^{3}H]DTG/500nM(+)-戊唑辛替代的K_i59.7+/-10.4nM以及在豚鼠脑膜的σ-1受体处的[3H]-((+)-戊唑辛替代的K_i108.1+/-19.9nM。化合物IXa、IXb显示出在大鼠肝脏匀浆的σ-2受体处的[^{3}H]DTG/500nM(+)-戊唑辛替代的K_i30.8+/-2.3nM以及在豚鼠脑膜的σ-1受体处的[3H]-((+)-戊唑辛替代的K_i6.37+/0.81nM。

[0689] 以上通式内的σ-2受体配体已经显示出用作为σ-2受体功能性神经元拮抗剂；例如，化合物II以及IXa和IXb已在本文显示为抑制神经元细胞中与可溶Abeta寡聚体相关的突触减少，以及当在引入Abeta寡聚体之前或之后添加时，抑制神经元细胞中的膜运输异常（例如，使用下述的MTT检定），其伴随于将这些细胞暴露于合成制剂或从阿尔茨海默病人脑中分离的制剂形式（后者在体外介导淀粉样病理中更有效力）的Abeta寡聚体。以上通式内的其他化合物也显示为抑制膜运输中的异常。化合物II以及化合物IXa和IXb在本文中还显示为抑制在如本文所述的阿尔茨海默病的转基因和诱导动物模型中展现的认知缺陷，认知缺陷与认知衰退和记忆丧失相关。化合物II以及以上通式内的其他化合物例如化合物B还已经在药代动力学研究中显示为全身性吸收并跨越血脑屏障并可生物利用。由于这些特性，并鉴于将强作用[看这个]归因于Abeta寡聚体和其他Abeta种类例如淀粉样蛋白病理例如阿尔茨海默病早期的发展中的组装的技术状态，预期化合物II和本文公开的其他化合物会在治疗和使免受轻度认知障碍以及治疗（如本文所述）阿尔茨海默病中有活性。此外，因为与化合物II结构相似以及因为已经证实化合物II的以上体外活性，以上具体公开的化合物中的式I、II、III、IV、V、VI和VII内的典型数目的其他化合物，即式I、II、III、IV、V、VI和VII内的所有化合物的药代动力学特性和σ-2配体状态预期在体内具有相似活性。类似地，因为与化合物IXa和IXb结构相似以及因为已经证实化合物IXa和IXb的上述体外活性，式VIII和IX特别是式IX内的所有化合物也预期在体内具有相似活性。

[0690] 化合物II行为效力：小鼠条件性恐惧中Abeta寡聚体诱导的记忆缺陷是在哥伦比亚大学Ottavio Arancio博士的实验室中建立的模型(Puzzo'08)。一些制药公司在他们的发现工作中使用该同一模型。情境条件性恐惧是与人认知功能特别是新记忆创建相关的联想记忆形成的已接受模型(Delgado' 06)。将Abeta寡聚体在条件性训练前即刻注射到野生型动物的海马中，且在24小时后经僵立行为(freezing behavior)评估记忆。参见，例如

图 4 和 8。详情提供在实施例 9 中。其中，化合物 II 能够完全消除小鼠中的记忆缺陷而不抑制记忆（当单独给药时）或引起任何行为或动作毒性。选择该模型系统是因为寡聚体的海马内施用使得可以快速地比较评估化合物活性和脱靶毒性。结果图示在图 4 中。

[0691] 化合物 II 还在两个转基因阿尔茨海默病模型中进行体内测试，以示出在化合物在逆转 Abeta 寡聚体相关的记忆丧失中的作用。具体而言，化合物 II 恢复两个不同突变小鼠模型的能力（该突变小鼠模型随着年龄增长而逐渐发展出以记忆丧失为特征的认知衰退），以记起记忆丧失发生前获取的机能。此外，在上述条件性恐惧检定中，化合物 II 以及化合物 IXa 和 IXb 显著抑制野生型小鼠的海马 Abeta 寡聚体暴露的作用，从而保护小鼠获取新记忆的能力。

[0692] 这些行为研究统一证实了，化合物 II 在两个不同行为任务中引起学习和记忆的改善，使用两个不同的阿尔茨海默病模型，以两种性别，并按照短期或长期给药，并且证实了体外检定与体内活性相关。尽管结构不同，化合物 IXa/IXb 具有相似的体外和体内活性，并且也是 σ-2 拮抗剂。最后，一些 σ-2 拮抗剂还显示出体外活性，尽管结构不同。因此，综合来说，这些结果表明化合物 II 可以用于治疗神经退行性疾病例如阿尔茨海默病。式 I、II、III、IV、V、VI 和 VII 内的其他化合物也被发现与 σ-2 受体结合并具有与化合物 II 相似的体外活性。基于它们与化合物 II 的相似性，预期它们具有与化合物 II 相似的体外活性和体内活性。确实，到这些化合物已经在体外进行测试的程度，它们具有与化合物 II 相同类型的活性，因此预期它们具有相似的体内活性，因此可用于相同的治疗适应症。式 I、II、III、IV、V、VI 和 VII 内的多种其他 σ-2 拮抗剂化合物已经或将在本文所述的突触减少和 / 或膜运输检定中进行测试，并预期在抑制 Abeta 寡聚体相关的突触损失中以及在抑制 Abeta 寡聚体相关的膜运输异常中具有活性，并且在抑制例如认知衰退和治疗阿尔茨海默病中具有相似活性。类似地，因为化合物 IXa 和 IXb 还显示出在体外和体内模型中有活性，因此结构相似的式 VIII 和 IX 特别是式 IX 的化合物也大体预期为同时在体外和体内具有活性，因此可用于本发明的治疗方法中。到被检测的程度，这些化合物为 σ-2 配体，且预期剩余者为 σ-2 配体。

[0693] 如本文所讨论的，证据表明，由膜运输介导的神经元表面受体表达中 Abeta 寡聚体介导的减少是突触可塑性 (LTP) 以及因此的学习和记忆的电生理测量的寡聚体抑制的基础（参见 Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, Sisodia S, Malinow R. APP processing and synaptic function. Neuron. 2003Mar27 ; 37(6):925-37 ; 和 Hsieh H, Boehm J, Sato C, Iwatsubo T, Tomita T, Sisodia S, Malinow R. AMPAR removal underlies Abeta oligomer-induced synaptic depressionand dendritic spine loss. Neuron. 2006Dec7 ;52(5):831-43)。经甲臜形态转变测量由寡聚体诱导的膜运输率变化已被用于细胞系中，以发现阻断 Abeta 寡聚体的药物 [Maezawa I, Hong HS, Wu HC, Battina SK, Rana S, Iwamoto T, Radke GA, Pettersson E, Martin GM, Hua DH, Jin LW. A novel tricyclic pyrone compound ameliorates cell death associated with intracellular amyloid-beta oligomeric complexes. J Neurochem. 2006Jul ; 98(1):57-67 ;Liu Y, Schubert D. Cytotoxic amyloid peptides inhibit cellular3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT)reduction by enhancing MTT formazan exocytosis. J Neurochem. 1997Dec ;69(6):2285-93 ;Liu

Y, Dargusch R, Banh C, Miller CA, Schubert D. Detecting bioactive amyloid beta peptide species in Alzheimer's disease. J Neurochem. 2004Nov ;91(3):648-56 ;Liu Y, Schubert D. Treating Alzheimer's disease by inactivating bioactive amyloid beta peptide. Curr Alzheimer Res. 2006Apr ;3(2):129-35 ;Rana S, Hong HS, Barrigan L, Jin LW, Hua DH. Syntheses of tricyclic pyrones and pyridinones and protection of Abeta-peptide induced MC65neuronal cell death. Bioorg Med Chem Lett. 2009Feb 1 ;19(3):670-4. Epub2008Dec24 ;和 Hong HS, Maezawa I, Budamagunta M, Rana S, Shi A, Vassar R, Liu R, Lam KS, Cheng RH, Hua DH, Voss JC, Jin LW. Candidate anti-Abeta fluorene compounds selected from analogs of amyloid imaging agents. Neurobiol Aging. 2008Nov18. (电子出版)], 其在啮齿动物体内降低 Abeta 脑水平 [Hong HS, Rana S, Barrigan L, Shi A, Zhang Y, Zhou F, Jin LW, Hua DH. Inhibition of Alzheimer's amyloid toxicity with a tricyclic pyrone molecule in vitro and in vivo. J Neurochem. 2009Feb ;108(4):1097-1108]。因此,上述测试已经建立了在识别化合物以治疗早期阿尔茨海默病和轻度认知障碍中的相关性。

[0694] 在一些实施方式中,关于抑制 Abeta 寡聚体对神经元(例如脑中的神经元)、淀粉样蛋白组装或其破裂和淀粉样蛋白(包括淀粉样蛋白寡聚体)结合和淀粉样蛋白沉积的一种或多种作用,任意上式的化合物具有小于 100μM、50μM、20μM、15μM、10μM、5μM、1μM、500nM、100nM、50nM 或 10nM 的 IC_{50} 值。在一些实施方式中,关于抑制 Abeta 种类例如寡聚体对神经元(例如中枢神经系统神经元)的活性/作用,化合物具有小于 100μM、50μM、20μM、15μM、10μM、5μM、1μM、500nM、100nM、50nM 或 10nM 的 IC_{50} 值。

[0695] 在一些实施方式中,本发明化合物对 Abeta 种类例如寡聚体对神经元(例如脑中神经元)的一种或多种作用(例如淀粉样蛋白(包括淀粉样蛋白寡聚体)与突触的结合以及由 Abeta 寡聚体介导的膜运输异常)的抑制百分比在 10nM 至 10μM 的浓度进行测量。在一些实施方式中,所测量的抑制百分比为约 1%至约 20%、约 20%至约 50%、约 1%至约 50%、或约 1%至约 80%。抑制可以例如通过在暴露于 β 淀粉样蛋白种类之前和之后对神经元的突触数目进行定量或者在同时存在 σ-2 拮抗剂和 Abeta 种类时对突触数目进行定量而评测,其中 σ-2 拮抗剂与 Abeta 种类暴露同时、或在之前或在之后。作为另一实例,抑制可以通过在存在和不存在 Abeta 种类时以及在存在和不存在本发明 σ-2 拮抗剂时确定膜运输并对测量胞吐速率和程度、胞吞速率和程度或其他细胞代谢指示物的一个或多个参数进行比较而进行评测。本发明的发明者已举出生化检定证据,即本发明的化合物还抑制淀粉样蛋白聚集(数据未示出)。

[0696] 在一些实施方式中,本文所述的化合物与 σ-2 受体特异性地结合。与特异受体特异性结合的化合物是指相对于另一受体偏好一种受体的化合物。例如,尽管化合物可能能够同时结合 σ-1 和 σ-2 受体,但当以高出 σ-1 受体至少 10%的亲和性结合时,化合物可以被认为对 σ-2 受体特异。在一些实施方式中,特异性对于一种结合配偶体(例如受体)相比于第二结合配偶体高出至少 10、20、30、40、50、60、70、80、90、100、200、300、400、500 或 1000%。

[0697] 在一些实施方式中,本发明提供使用标记的 σ-2 配体在动物中测量 β 淀粉样蛋白相关的认知衰退的方法。在一些实施方式中,方法包括使动物与本发明的标记 σ-2 配体接触并测量 σ-2 活性或表达。在一些实施方式中,方法包括将动物中的 σ-2 活性或表达

与已知具有 β 淀粉样蛋白诱导的认知衰退的动物进行比较。如果活性或表达与已知具有 β 淀粉样蛋白诱导的认知衰退的动物相同，认为该动物具有相同水平的认知衰退。动物可以根据与在 β 淀粉样蛋白诱导的认知衰退的各个阶段中的已知活性或表达的相似度来分级。本文所述的任意 σ-2 配体可以被标记，以使标记的 σ-2 配体可以在体内使用。

[0698] 在确定任意上式的化合物和描述为以上 σ-2 拮抗剂的其他化合物否可有效治疗本文所述的各种病症时，可以使用体外检定。已经使用化合物 II 将体外检定与体内作用进行了相关。例如，如果与化合物 II 结构相似的式 III-IV 化合物例如在本文所述体外检定中有活性，则其也可以在体内使用以治疗或改善本文所述的病症，包括抑制或恢复突触损失、调节神经元细胞中的膜运输变化、使免受或恢复记忆丧失，以及治疗认知衰退病症、疾病和障碍例如 MCI 和阿尔茨海默病。检定部分基于 β 淀粉样蛋白寡聚体和它们在突触处结合神经元的功能和 β 淀粉样蛋白寡聚体对神经元的体外作用。在一些实施方式中，本发明的发明者相信包括 σ-2 蛋白的神经元中的 Abeta 寡聚体受体与本文所述的 β 淀粉样蛋白组装接触，且与 σ-2 蛋白结合的式 I、III、IV、V、VI 和 VII 的化合物会抑制 β 淀粉样蛋白组装与受体的结合。在竞争性放射性配体结合检定中，本发明的发明者已经显示出本发明的化合物对 σ-2 受体有特异性。本发明的发明者还已经显示出，本发明的化合物抑制 Abeta 寡聚体与其在神经元表面上的在此之前未识别的受体进行结合。在一些实施方式中，提供方法来确定任意上式化合物在神经元信号传导中的 σ-2 配体效力。在一些实施方式中，方法包括使细胞例如但不限于初级神经元与 σ-2 配体接触并测量神经元功能。在一些实施方式中，细胞在体外接触。在一些实施方式中，细胞在体内接触。神经元活性可以是信号传导活性、电活性、突触蛋白的产生或释放等。增强或恢复信号传导的 σ-2 拮抗剂被识别为可有效调节神经元活性的化合物。在一些实施方式中，细胞得自病理样品。在一些实施方式中，细胞得自患有神经退行性疾病的受试者。在一些实施方式中，神经退行性疾病为 MCI 或阿尔茨海默病，特别是轻度阿尔茨海默病。

[0699] 受体结合检定和化合物筛选

[0700] 本发明还提供识别抑制认知衰退或治疗神经退行性疾病的另一化合物的方法。在一些实施方式中，方法包括使细胞与结合 σ-2 受体的化合物接触。在一些实施方式中，方法包括确定化合物是否抑制 β 淀粉样蛋白病理，其中抑制 β 淀粉样蛋白病理的化合物被识别为与 σ-2 受体结合并抑制认知衰退或治疗神经退行性疾病的化合物。在一些实施方式中，方法还包括识别与 σ-2 受体结合的其他化合物。在一些实施方式中，识别与 σ-2 受体结合的化合物的方法包括竞争性结合检定，其中在已知 σ-2 配体例如任意上式的化合物和描述为以上 σ-2 配体的其他化合物的存在下使测试化合物与 σ-2 受体接触，其中竞争性地抑制已知配体的结合的测试化合物被识别为 σ-2 受体配体。

[0701] 确定化合物是否能够与 σ-2 受体结合的方法是已知的，并且可以使用任何方法。例如，通过合同研究组织来进行测试，可以用来确定化合物是否与 σ-2 结合。可以进行多种检定以确定化合物是否与 σ-2 受体结合。在一些实施方式中，使用稳定表达人受体包括但不限于 σ-2 受体的同源群体的细胞，例如但不限于人胚胎肾细胞 (HEK293)、Jurkat 细胞或中国仓鼠卵巢 (CHO) 细胞。在其他情况下，使用 σ-2 受体的组织来源例如啮齿动物新皮质膜。该实例在本文的实施例部分中描述。

[0702] 在一些实施方式中，测试化合物与细胞或细胞膜接触，以确定测试化合物是否可

以与 σ-2 受体结合。在一些实施方式中，测试化合物溶解在载体或媒介物中，例如但不限于二甲基亚砜。在一些实施方式中，培养细胞直至铺满 (confluent)。在一些实施方式中，在铺满时，细胞可以通过温柔的刮擦来脱离。在一些实施方式中，细胞通过胰蛋白酶化或任何其他合适的脱离方式来脱离。

[0703] 在一些实施方式中，测试化合物与 σ-2 受体的结合可以通过例如竞争性放射性配体结合检定来确定。放射性配体结合检定可以在稳定表达人受体的完整细胞或组织来源上进行。脱离的细胞或组织可以例如被洗涤、离心和/或再混悬于缓冲液中。测试化合物可以根据包括但不限于本文所述方法的任何方法来进行放射标记。放射性配体可以在多种浓度（范围可以是例如 10^{10} ～ 10^{3}M 或 10^{11} ～ 10^{4}M）的竞争药物的不存在和存在下以 0.1 μCi 的固定浓度进行使用。这些药物可以添加到缓冲液中的组织或细胞（～例如 50,000 个细胞），并使其孵育。非特异性结合可以在各个受体亚型的广谱活化剂或抑制剂或功能性激动剂或拮抗剂的存在下（例如，对于 σ 受体，在例如 10 μM 的各个受体的合适配体的存在下）进行确定。反应可以通过快速过滤而终止，之后可以用冰冷缓冲液洗涤两次。干燥的过滤盘上的放射活性可以使用任何方法测量，包括但不限于液体闪烁分析仪。可以绘制出替代曲线并且对于受体亚型的测试配体的 Ki 值可以使用例如 GraphPad Prism(GraphPad Software Inc.，San Diego，CA) 进行确定。特异性结合的百分比可以通过将总结合（每分钟衰变）与非特异性结合（每分钟衰变）之间的差值除以总结合（每分钟衰变）而确定。

[0704] 在一些实施方式中，对于细胞系或组织来源中的结合研究，在各个实验内添加变化浓度的各个药物，一式两份，且各个 IC_{50} 值使用例如 GraphPad Prism 软件确定。各个配体的 Ki 值可以根据由 Cheng 和 Prusoff(1973) 描述的等式而确定，且最终数据可以呈现为 pKi±S.E.M.，其中在一些实施方式中，测试数目为约 1 ～ 6。

[0705] 在一些实施方式中，方法还包括确定与 σ-2 受体结合的化合物是否通过抑制可溶 Aβ 寡聚体诱导的神经毒性（有关于抑制可溶 Aβ 寡聚体诱导的突触损失）且抑制膜运输检定中 Aβ 寡聚体诱导的缺陷而用作为 σ-2 受体处的功能性拮抗剂。在一些实施方式中，方法还确定 σ-2 受体拮抗剂在不存在 Abeta 寡聚体时不影响运输或突触数目；不诱导神经元细胞中的 caspase-3 活性；抑制经由 σ-2 受体激动剂对 caspase-3 活性的诱导；且/或降低或使免受神经元细胞中由 σ-2 受体激动剂引起的神经元毒性。

[0706] 测试还可以包括确定测试化合物对结合配偶体（其可以是，但不限于 σ-2 受体）功能的作用的功能性检定。可以使用多种标准检定技术。例如，可以使用方法来测量化合物在活细胞或组织中的功能性激动剂样或拮抗剂样活性。方法包括但不限于，确定 cAMP 浓度和 IP1 水平的 TR-FRET，监测钙能量的实时荧光，测量阻抗调节、回肠收缩、肿瘤细胞凋亡的细胞介电谱。还可以通过例如确定化合物是否与 σ-1 受体、σ-2 受体、两者均不或两者均结合来确定测试化合物的特异性。用于确定测试化合物是否与 σ-1 受体结合的方法在 Ganapathy，M.E 等人 (1999) J. Pharmacol. Exp. Ther.，289:251-260 中记载，其全部并入本文以供参考。用于确定测试化合物是否与 σ-1 受体结合的方法在 Bowen，W.D 等人 (1993) Mol. Neuropharmacol.，3:117-126 中（其全部并入本文以供参考）以及 Xu，J. 等人，Nature Communications，2011，2:380DOI:10.1038/ncomms1386（其全部也并入本文以供参考）中记载。

[0707] 在多个实施方式中，本公开内容提供用于识别能够通过抑制可溶 Aβ 寡聚体诱导

的神经毒性（有关于抑制可溶 Aβ 寡聚体诱导的突触损失）而用作为 σ-2 受体处的功能性拮抗剂、抑制膜运输检定中可溶 Aβ 寡聚体诱导的的缺陷、在不存在 Abeta 寡聚体时不影响运输或突触数目、且如本文所述表现出较好的药物样特性的选择性、高亲和性 σ-2 受体配体的检定方案，使得由此识别的选择性、高亲和性 σ-2 受体拮抗剂化合物可以用于在体内治疗可溶 Aβ 寡聚体诱导的突触功能障碍。

[0708] 在一些实施方式中，提供用于识别在减轻或使免受非致死 Abeta 寡聚体毒性中有活性的化合物的筛选方法，其大大受益于将测试化合物与 σ-2 受体结合的能力引入为筛选标准，该能力例如通过其替代已知配体的能力或通过任何其它方法来评估。此外，测试化合物应进行至少一种可以评估化合物阻断或减轻 Abeta 寡聚体对神经元的非致死有害作用的能力的体外测试，例如本文所述的膜运输检定或突触数目或寡聚体结合检定，或者评估认知衰退的治疗的体内检定，例如本文所述的那些。

[0709] 在一些实施方式中，本发明提供确定受试者是否应该用 σ-2 拮抗剂治疗的方法，其中受试者被怀疑具有认知衰退或神经退行性疾病或本文所述的其他病症、疾病或障碍。在一些实施方式中，方法包括使得自患者的样品与 σ-2 拮抗剂接触以及确定 σ-2 调节化合物是否抑制或改善样品中的 β 淀粉样蛋白病理，其中显示出抑制或改善样品中存在的 β 淀粉样蛋白病理的样品表明受试者应该用 σ-2 拮抗剂治疗。

[0710] 此外，本发明包括识别抑制 Aβ 寡聚体诱导的突触数目减少等的 σ-2 拮抗剂的方法。在一些实施方式中，可以使用方法来识别用于治疗 β 淀粉样蛋白病理的 σ-2 拮抗剂。在一些实施方式中，使用方法来确定治疗 β 淀粉样蛋白病理的治疗效力。在一些实施方式中，β 淀粉样蛋白病理是膜运输缺陷、突触功能障碍、动物中的记忆和学习缺陷、突触数目的减少、树突棘长度或棘形态的变化、LTP 缺陷或 Tau 蛋白磷酸化的增加。

[0711] 用于本公开内容中的 β 淀粉样蛋白

[0712] 人 β 淀粉样蛋白是集中于神经元突触中的整合膜蛋白即淀粉样蛋白前体蛋白 (APP) 的剪切产物。β 淀粉样蛋白自缔合形成可代谢的寡聚组装。在更高的浓度下，Abeta 会聚合并组装成线形原纤维，由较低 pH 促进。目前并不清楚原纤维是否由寡聚体形成。β 淀粉样蛋白寡聚体已经被证实通过诱导神经元突触的阻断学习和记忆的变化而引起动物模型中的阿尔茨海默病，且 β 淀粉样蛋白原纤维长久以来与动物和人的晚期阿尔茨海默病相关。实际上，阿尔茨海默病的现代可行的且已得到大量支持的假设是，Abeta 组装尤其是 Abeta 寡聚体处于与阿尔茨海默病相关的早期病理的中心以及与较少严重痴呆相关的病理例如 MCI 和轻度 AD 的中心。Cleary, James P. 等人 "Natural oligomers of the amyloid-β protein specifically disrupt cognitive function."NatureNeuroscience Vol.8(2005):79-84;Klyubin, I. 等人 "Amyloid beta protein dimer-containing human CSF disrupts synaptic plasticity:prevention by systemic passive immunization."J Neurosci. Vol.28(2008):4231-4237。然而，几乎不知道寡聚体如何形成以及寡聚体的结构状态。例如，缔合形成寡聚体的 β 淀粉样蛋白亚基的数目在目前是未知的，如同寡聚体的结构形式或暴露哪些残基。有证据表明寡聚体的多于一种结构状态是有神经活性的。Reed, Jess D. 等人 "MALDI-TOF mass spectrometry of oligomeric food polyphenols."Phytochemistry66:18(2005 年 9 月):2248-2263;Cleary, James P. 等人 "Natural oligomers of the amyloid-β protein specifically disrupt cognitive function."Nature

Neuroscience Vol.8(2005):79 - 84。

[0713] β 淀粉样蛋白对脑中发现的很多蛋白具有亲和性，包括 ApoE 和 ApoJ。然而，不清楚分子伴侣或其他蛋白是否与能影响其最终结构状态和 / 或其神经活性的蛋白形成缔合。

[0714] 可溶的 Abeta 肽很可能通过扰乱突触功能障碍和认知过程而在 AD 早期中发挥重要作用。例如，Origlia 等人显示出可溶 Abeta(Abeta42) 通过神经元受体来破损害嗅皮质的长时程增强效应 (LTP)，该神经元受体用于 p38MAPK 的糖基化终末产物 (RAGE) 介导的活化。Origlia 等人 2008, Receptor for advanced glycation end product-dependnet activation of p38mitogen-activated protein kinase contributes to amyloid-beta-mediated cortical synaptic dysfunction. J. Neuroscience28(13):3521-3530，其并入本文以供参考。

[0715] 突触功能障碍涉及到阿尔茨海默病的早期中。β 淀粉样蛋白肽已经显示出改变突触功能。Puzzo 等人报道到，Abeta 的合成原纤维形式损害 LTP 的晚期蛋白合成依赖期而不影响早期蛋白合成期。该报道与 Abeta 寡聚体对细胞高度毒性并涉及到突触功能障碍中的早期报道一致。Puzzo 等人，2006, Curr Alzheimer's Res3(3):179-183，其并入本文以供参考。已经发现 Abeta 通过多种第二信使级联反应包括一氧化氮级联反应而显著地损害海马长时程增强效应 (LTP)。NO/cGMP/cGK/CREB。Puzzo 等人，J Neurosci. 2005。在一些实施方式中，公开内容提供包括 σ-2 受体拮抗剂的组合物和方法，以用于抑制神经元细胞的 β 淀粉样蛋白寡聚体诱导的突触功能障碍，以及抑制由神经元对 Abeta 寡聚体的暴露引起的海马长时程增强效应的压制。

[0716] 任何形式的 β 淀粉样蛋白可以用于实施本发明的筛选方法和检定，包括 β 淀粉样蛋白单体、寡聚体、原纤维以及与蛋白缔合的 β 淀粉样蛋白（“蛋白复合物”）且更一般地 β 淀粉样蛋白组装。例如，筛选方法可以采用多种形式的可溶 β 淀粉样蛋白寡聚体，例如在美国专利申请序列号 13/021,872；美国专利公开 2010/0240868；国际专利申请 WO/2004/067561；国际专利申请 WO/2010/011947；美国专利公开 20070098721；美国专利公开 20100209346；国际专利申请 WO/2007/005359；美国专利公开 20080044356；美国专利公开 20070218491；WO/2007/126473；美国专利公开 20050074763；国际专利申请 WO/2007/126473；国际专利申请 WO/2009/048631、和美国专利公开 20080044406、美国专利第 7,902,328 号和美国专利第 6,218,506 号中公开的，其各自并入本文以供参考。

[0717] β 淀粉样蛋白的形式，包括 β 淀粉样蛋白的单体或寡聚体，可以得自任何来源。例如，在一些实施方式中，市售可得的 β 淀粉样蛋白单体和 / 或 β 淀粉样蛋白寡聚体可以用于水溶液中，且在其他实施方式中，用于水性蛋白溶液中的 β 淀粉样蛋白单体和 / 或 β 淀粉样蛋白寡聚体可以通过技术人员使用任何数目的已知技术来分离并纯化。通常而言，用于制备各个实施方式的蛋白和 β 淀粉样蛋白的水溶液的 β 淀粉样蛋白单体和 / 或 β 淀粉样蛋白寡聚体可以在水溶液中可溶。因此，水溶液的蛋白和 β 淀粉样蛋白均可以是可溶的。

[0718] 添加的 β 淀粉样蛋白可以是任何同工型。例如，在一些实施方式中，β 淀粉样蛋白单体可以是 β 淀粉样蛋白 1 ～ 42，且在其他实施方式中，β 淀粉样蛋白单体可以是 β 淀粉样蛋白 1 ～ 40。在其他实施方式中，β 淀粉样蛋白可以是 β 淀粉样蛋白 1 ～ 39 或 β 淀粉样蛋白 1 ～ 41。因此，各个实施方式的 β 淀粉样蛋白可以包括 β 淀粉样蛋白的 C

端同工型。其他实施方式包括N端已磨损的 β 淀粉样蛋白，且在一些实施方式中，上述任何 β 淀粉样蛋白C端同工型的N端可以是氨基酸2、3、4、5或6。例如，β 淀粉样蛋白1～42可以包括 β 淀粉样蛋白2～42、β 淀粉样蛋白3～42、β 淀粉样蛋白4～42或 β 淀粉样蛋白5～42及其混合物，而且相似地，β 淀粉样蛋白1～40可以包括 β 淀粉样蛋白2～40、β 淀粉样蛋白3～40、β 淀粉样蛋白4～40或 β 淀粉样蛋白5～40。

[0719] 用在各个实施方式中的 β 淀粉样蛋白形式可以是野生型，即具有与大部分群体在体内合成的 β 淀粉样蛋白的氨基酸序列相同的氨基酸序列，或者在一些实施方式中，β 淀粉样蛋白可以是突变 β 淀粉样蛋白。实施方式不限于任何特定种类的突变 β 淀粉样蛋白。例如，在一些实施方式中，引入水溶液中的 β 淀粉样蛋白可以包括已知的突变，例如具有“道奇”(Dutch)突变(E22Q)或“Arctic”(E22G)突变的 β 淀粉样蛋白。这些突变的单体可以包括天然出现的突变，例如从易患例如阿尔茨海默病的个体群中分离的 β 淀粉样蛋白形式，β 淀粉样蛋白的家族形式。在其他实施方式中，突变 β 淀粉样蛋白单体可以通过使用分子技术而合成产生，从而生成具有特定突变的 β 淀粉样蛋白突变体。在其他实施方式中，突变 β 淀粉样蛋白单体可以包括先前未识别的突变，例如在随机生成的 β 淀粉样蛋白突变体中发现的那些突变体。本文使用的术语“β 淀粉样蛋白”意在包括 β 淀粉样蛋白的野生型形式以及 β 淀粉样蛋白的任何突变形式。

[0720] 在一些实施方式中，水性蛋白溶液中的 β 淀粉样蛋白可以是单个同工型。在其他实施方式中，β 淀粉样蛋白的多种C端同工型和/或 β 淀粉样蛋白的多种N端同工型可以组合形成能够在水性蛋白溶液中提供的 β 淀粉样蛋白混合物。在其他实施方式中，β 淀粉样蛋白可以得自添加至含蛋白水溶液并原位剪切的淀粉样蛋白前体蛋白(APP)，并且在这些实施方式中，β 淀粉样蛋白的多种同工型可以包含在溶液中。在添加 β 淀粉样蛋白后，可能水溶液中发生N端的磨损和/或C端氨基酸的移除。因此，即使在初始将单个同工型添加到溶液中时，如本文所述制备的水溶液也可以包括多种 β 淀粉样蛋白同工型。

[0721] 添加到水溶液中的 β 淀粉样蛋白单体可以从天然来源例如活组织中分离，且在其它实施方式中，β 淀粉样蛋白可以得自合成来源例如转基因小鼠或经培养的细胞。在一些实施方式中，β 淀粉样蛋白形式，包括单体、寡聚体或其组合，从正常受试者和/或被诊断为具有认知衰退或与其相关的疾病例如但不限于阿尔茨海默病的患者中分离。在一些实施方式中，β 淀粉样蛋白单体、寡聚体或其组合是已从正常受试者或疾病患者中分离的Abeta组装。在一些实施方式中，Abeta组装具有高分子量，例如大于100KDa。在一些实施方式中，Abeta组装具有中等分子量，例如10～100KDa。在一些实施方式中，Abeta组装小于10kDa。

[0722] 一些实施方式的 β 淀粉样蛋白寡聚体可以由任何数目的 β 淀粉样蛋白单体组成，与“寡聚体”的常用定义一致。例如，在一些实施方式中，β 淀粉样蛋白寡聚体可以包括约2至约300、约2至约250、约2至约200个 β 淀粉样蛋白单体，且在其它实施方式中，β 淀粉样蛋白寡聚体可以由约2至约150、约2至约100、约2至约50、或约2至约25个 β 淀粉样蛋白单体组成。在一些实施方式中，β 淀粉样蛋白寡聚体可以包括2个或更多个单体。各个实施方式的 β 淀粉样蛋白寡聚体可以基于单体的确认而与 β 淀粉样蛋白原纤维和 β 淀粉样蛋白初原纤维区别开。具体地，β 淀粉样蛋白寡聚体的 β 淀粉样蛋白单体通常是由 β 折叠片组成的球状，而原纤维和初原纤维的 β 淀粉样蛋白单体的二级结构是平

行 β 片。

[0723] 具有阿尔茨海默病或存在具有阿尔茨海默病风险的受试者的识别

[0724] 通过大脑皮质中细胞外 β 淀粉样蛋白 (Aβ) 斑块和神经元内神经原纤维缠结的存在来从组织学上定义阿尔茨海默病 (AD)。各种诊断和预后生物标记物在本领域内已知，例如磁共振成像、单光子发射断层成像术、FDG PET、PiB PET、CSF tau 和 Abeta 分析，以及其诊断精确性的可得数据在 Alves 等人，2012, Alzheimer's disease:a clinical practice-oriented review, Frontiers in Neurology, April, 2012, vol3, Article63, 1-20 中讨论，其引入本文以供参考。

[0725] 痴呆的诊断以及对将患痴呆的人的预测受到磁共振成像和通过使用 [(18)F] 氟脱氧葡萄糖 (FDG) 的正电子发射断层成像术 (PET) 的帮助。这些技术不特定用于AD。参见例如 Vallabhajosula S. Positron emission tomography radiopharmaceuticals for imaging brain Beta-amyloid. Semin Nucl Med. 2011Jul ;41(4):283-99。最近 FDA 批准用于成像的对于具有认识障碍的患者中的频繁淀粉样蛋白神经斑温和的另一 PET 配体是 Florbetapir F18 注射剂，(4-((1E)-2-(6-{2-(2-(2-(18F) 氟乙氧基) 乙氧基) 乙氧基} 吡啶 -3- 基) 乙烯基)-N- 甲基苯胺，AMYVID®, Lilly)。Florbetapir 与原纤维 Abeta 特异结合，但不与神经原纤维缠结特异结合。参见例如，Choi SR 等人，Correlation of amyloid PET ligand florbetapir F18binding with A β aggregation and neuritic plaque deposition in postmortem brain tissue. Alzheimer Dis Assoc Disord. 2012Jan ;26(1):8-16。PET 配体 florbetapir 相对于 PET 扫描的定性视觉评估具有较低特异性。Camus 等人，2012, Eur J Nucl Med Mol Imaging39:621-631。然而，许多具有神经斑的人看似认知正常。

[0726] 对于阿尔茨海默病的 CSF 标记物包括总 tau、磷 -tau 和 Abeta42。参见例如，Andreasen, Sjogren and Blennow, World J Biol Psyciatry, 2003, 4(4):147-155，其引入本文以供参考。在许多研究中已经发现，在 AD 中 Abeta 的 42 氨基酸形式 (Abeta42) 的 CSF 水平降低且总 tau 的 CSF 水平增高。此外，对于可用于识别存在患 AD 风险的受试者的 APP 基因中的突变，存在已知的基因标记物。参见例如，Goate 等人，Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease, Nature, 349, 704-706, 1991，其引入本文以供参考。在实施方式中，可以采用任何已知的诊断或预后方法来识别具有阿尔茨海默病或存在具有阿尔茨海默病风险的受试者。

[0727] 包括 σ-2 受体拮抗剂的药物组合物

[0728] 通过本发明的方式识别的 σ-2 受体拮抗剂化合物、抗体或片段可以以药物组合物的形式施用。这些组合物可以以制药领域熟知的方式制备，且可以通过多种途径施用，取决于是否需要局部或全身治疗以及待治疗的区域。

[0729] 因此，本发明的另一实施方式包括药物组合物，该药物组合物包括药学上可接受的赋形剂或稀释剂以及治疗有效量的本发明的 σ-2 受体拮抗剂化合物 (包括其对映异构体、非对映异构体、N- 氧化物或药学上可接受的盐)。

[0730] 尽管化合物可以作为散装物质 (bulk substance) 施用，但优选以药物制剂提供活性成分，例如，其中活性剂与药学上可接受的载体混合，该药学上可接受的载体关于既定的施用途径和标准的制药实践进行选择。

[0731] 因此，一方面，本发明提供一种药物组合物，其包括任何上式以及描述为以上 σ-2

受体拮抗剂的其它化合物的至少一种化合物、抗体或片段，或其药学上可接受的衍生物（例如盐或溶剂化物），以及任选地，药学上可接受的载体。具体地，本发明提供一种药物组合物，其包括治疗有效量的任何上式的至少一种化合物或其药学上可接受的衍生物，以及任选地，药学上可接受的载体。

[0732] 组合

[0733] 对于本发明的组合物和方法，任何上式的化合物和描述为以上 σ-2 受体拮抗剂的其它化合物可以与其它治疗和 / 或活性剂组合使用。

[0734] 在一些实施方式中，σ-2 拮抗剂化合物可以与胆碱酯酶抑制剂、N- 甲基 -D- 天冬氨酸 (NMD A) 谷氨酸受体拮抗剂、β 淀粉样蛋白特异抗体、β 分泌酶 1(BACE1，β 位淀粉样蛋白前体蛋白分裂酶 1) 抑制剂、肿瘤坏死因子 α (TNFα) 调节剂、静脉内免疫球蛋白 (IVIG)、或朊蛋白拮抗剂中的一种或多种组合。在一些实施方式中，σ-2 受体拮抗剂与选自他克林（**COGNEX®**；Sciel)、多奈哌齐（**ARICEPT®**；Pfizer)，卡巴拉汀（**EXELON®**；Novartis)、或加兰他敏（**RAZADYNE®**；Ortho-McNeil-Janssen) 的胆碱酯酶抑制剂组合。在一些实施方式中，σ-2 受体拮抗剂与作为髓周依那西普（**ENBREL®**，Amgen/Pfizer) 的 TNFα 调节剂组合。在一些实施方式中，σ-2 受体拮抗剂与选自巴匹珠单克隆抗体 (Pfizer)、茄尼醇单克隆抗体 (Lilly)、PF-04360365(Pfizer)、GSK933776(GlaxoSmithKline)、Gammagard(Baxter) 或 Octagam(Octapharma) 的 β 淀粉样蛋白特异性抗体组合。在一些实施方式中，σ-2 受体拮抗剂与作为美金刚（**NAMENDA®**；Forest) 的 NMDA 受体拮抗剂组合。在一些实施方式中，BACE1 抑制剂是 MK-8931(Merck)。在一些实施方式中，σ-2 受体拮抗剂与 IVIG 组合，如描述于 Magga 等人，J Neuroinflam2010, 7:90, Human intravenous immunoglobulin provides protection against Ab toxicity by multiple mechanisms in a mouse model of Alzheimer's disease，以及 Whaley 等人，2011, Human Vaccines7:3, 349-356, Emerging antibody products and Nicotiana manufacturing；其各自引入本文以供参考。在一些实施方式中，σ-2 受体拮抗剂与阮蛋白拮抗剂组合，如公开于 Strittmatter 等人，US2010/0291090，其引入本文以供参考。

[0735] 因此，另一方面，本发明提供一种药物组合物，其包括任意上式的至少一种化合物或其药学上可接受的衍生物、第二活性剂、以及任选地药学上可接受的载体。

[0736] 当组合到同一制剂中时，应理解，两种化合物、抗体或片段须稳定且互相相容且与制剂的其它组分相容。当单独配制时，它们可以以任何便利的制剂提供，便利得对于这样的化合物领域内已知。

[0737] 可以在药物组合物中提供防腐剂、稳定剂、染料以及甚至调味剂。防腐剂的实例包括苯甲酸钠、抗坏血酸和对羟基苯甲酸酯。还可使用抗氧化剂和助悬剂。

[0738] 关于生物学例如单克隆抗体或片段，可以采用合适的赋形剂来防止抗体或片段在具有低内毒素的溶液中的聚集并使其稳定，通常用于肠道外例如静脉给药。例如，参见 Formulation and Delivery Issues for Monoclonal Antibody Therapeutics, Daugherty 等人，在 Current Trends in Monoclonal Antibody Development and Manufacturing 中，第 4 部分，2010, Springer, New York pp103-129。

[0739] 本发明的化合物可以使用已知的研磨程序例如湿磨进行研磨，以获得适用于片剂形成和其他制剂类型的粒径。本发明化合物的细分（纳米颗粒）制剂可以通过本领域已知的方法进行制备，例如参见 WO02/00196(SmithKline Beecham)。

[0740] 施用途径和单位剂型

[0741] 施用（输送）途径包括但不限于以下一种或多种：口服（例如作为片剂、胶囊或作为可吸收的溶液）、局部、粘膜（例如作为吸入用鼻腔喷雾剂或气雾剂）、肠胃外（例如通过可注射形式）、胃肠、脊柱内、腹膜内、肌内、静脉内、脑室内、或其它储库 (depot) 施用等。

[0742] 因此，本发明的组合物包括尤其配制用于施用模式的形式的组合物。在某些实施方式中，本发明的药物组合物配制成适用于口服输送的形式。例如，化合物 CB 和化合物 CF 是在动物模型中口服可生物利用且已每天口服一次在条件性恐惧模型中显示效力的 σ-2 受体拮抗剂化合物，参见例如图 12B。本文所述的口服可生物利用的化合物可以以口服制剂制备。在一些实施方式中，σ-2 拮抗剂化合物是口服可生物利用的化合物，适用于口服输送。在其它实施方式中，本发明的药物组合物配制成适用于肠胃外输送的形式。在一些实施方式中，σ-2 受体拮抗剂化合物是其抗体或片段，其中抗体或片段配制成肠胃外组合物。例如，阻断 Abeta 寡聚体与 σ-2 受体的结合的 σ-2 受体抗体例如 PGRMC1 抗体可以配制用于肠胃外输送。

[0743] 本发明的化合物可以配制成以用于人或兽药的任何便利方式施用，因此本发明的范围内包括含有适用于人或兽药的本发明化合物的药物组合物。这样的组合物可以在一种或多种合适载体的帮助下以常规方式进行使用。用于治疗用途的可接受的载体在制药领域内熟知，且描述于例如 Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985)。药物载体可以关于既定的施用途径和标准的制药实践进行选择。除载体之外，药物组合物还可以包括任何合适的粘合剂、润滑剂、助悬剂、涂覆剂和 / 或增溶剂作为载体。

[0744] 根据不同的输送体系可存在不同的组合物 / 制剂要求。应理解，并不是所有的化合物都需要通过相同的途径施用。同样地，如果组合物包括多于一种活性组分，则这些组分可以通过不同途径施用。通过实施例的方式，本发明的药物组合物可以配制成使用微型泵，或通过粘膜途径例如作为吸入用鼻腔喷雾剂或气雾剂或可吸收溶液，或肠胃外（其中，组合物配制成可注射形式，以便通过例如静脉内、肌内或皮下途径进行输送）进行输送。或者，制剂可以设计成通过多个途径进行输送。

[0745] 本发明的抗体或抗体片段分子可以通过多种途径中的任意种进行配制和施用，并以对适应症或出于所寻求的目的治疗有效的浓度进行施用。为完成该目标，抗体可以使用多种本领域已知的可接受的赋形剂进行配制。通常而言，抗体通过注射而施用，例如静脉内注射。实现该施用的方法为本领域普通技术人员已知。例如，Gokarn 等人，2008，J Pharm Sci97(8):3051-3066（其并入本文以供参考）描述了多种高浓度抗体自缓冲制剂。例如，自缓冲制剂中的单克隆抗体，以例如 5.25% 山梨糖醇，pH5.0 中的 50mg/mL mAb，或 5% 山梨糖醇、0.01% 聚山梨醇酯 20，pH5.2 中的 60mg/mL mAb；或常规缓冲制剂，例如 5.25% 山梨糖醇、25 或 50mM 乙酸盐、谷氨酸盐或琥珀酸盐，pH5.0 中的 50mg/mL mAb1；或 10mM 乙酸盐或谷氨酸盐、5.25% 山梨糖醇、0.01% 聚山梨醇酯 20，pH5.2 中的 60mg/mL；可以采用其他低浓度制剂，如本领域已知。

[0746] 因为本发明的优选 σ-2 受体拮抗剂化合物跨越血脑屏障，因此它们可以以多种方式进行施用，例如全身（例如通过 iv、SC、口服、粘膜、经皮途径）或局部方式（例如颅内）。在本发明的化合物经由胃肠粘膜进行粘膜输送时，其应该能够在经由胃肠道的运输过程中保持稳定；例如，其应该耐受蛋白质降解，在酸 pH 下稳定，且耐受胆汁的净化 (detergent) 作用。例如，选自 σ-2 配体并制备用于上述的口服的 σ-2 拮抗剂化合物可以由肠溶包衣层包衣。肠溶包衣层材料可以分散或溶解于水或合适有机溶剂中。作为肠溶包衣层聚合物，可以单独或组合使用以下的一种或多种：例如甲基丙烯酸共聚物、邻苯二甲酸乙酸纤维素、乙酸丁酸纤维素、羟丙甲基纤维素邻苯二甲酸酯、醋酸羟丙甲基纤维素琥珀酸酯、聚乙酸乙烯邻苯二甲酸酯、乙酸纤维素偏苯三酸酯、羧甲基乙基纤维素、虫胶、或其它合适的肠溶包衣层聚合物的溶液或分散液。由于环境原因，优选水性包衣工艺。在这样的水性工艺中，最优选甲基丙烯酸共聚物。

[0747] 合适时，药物组合物可通过吸入、通过使用皮肤贴剂施用，以含有赋形剂如淀粉或乳糖的片剂形式、或者以单独或与赋形剂混合的胶囊或胚珠 (ovule) 形式、或者以含有调味剂或着色剂的酏剂、溶液或混悬液形式口服施用，或者它们可以经肠胃外注射，例如静脉内、肌内或皮下。对于颊 (buccal) 或舌下施用，组合物可以以片剂或锭剂的形式施用，其可以以常规方式进行配制。

[0748] 在本发明的组合物经胃肠外施用时，这样的施用包括但不限于：静脉内、动脉内、鞘内、心室内、颅内、肌内或皮下施用本发明的化合物，和 / 或通过使用输注技术。抗体或片段通常经肠道外施用，例如静脉施用。

[0749] 适用于注射或输注的药物组合物可以是无菌水溶液、分散液或无菌粉末形式，其含有调整（若需要）用于这样的适用于输注或注射的无菌溶液或分散液制剂的活性成分。该制剂可任选地包入脂质体中。在所有情况下，最终制剂在制备和储存条件下须无菌、液态且稳定。为提高储存稳定性，这样的制剂还可含有防腐剂以防止微生物生长。对微生物作用的防止可通过添加各种抗细菌剂和抗真菌剂来实现，例如尼泊金类 (paraben)、氯丁醇或抗坏血酸。在很多情况下，推荐等张物质例如糖、缓冲液和氯化钠，以保证与体液特别是血液相似的渗透压。对这样的可注射混合物的延长吸收可通过引入吸收延迟剂例如单硬脂酸铝或明胶来实现。

[0750] 分散液可以在液体载体或媒介物 (intermediate) 例如甘油、液体聚乙二醇、三醋酸甘油酯油及其混合物中制备。液体载体或媒介物可以是溶剂或液体分散介质，其含有例如水、乙醇、多元醇（例如丙三醇、丙二醇等）、植物油、非毒性甘油酯及其合适混合物。合适的流动性可以通过生成脂质体、在分散液的情况下施用合适的粒径或通过添加表面活性剂来得到保持。

[0751] 对于肠胃外施用，最好以无菌水溶液的形式使用化合物，该无菌水溶液可含有其它物质，例如足够的盐或葡萄糖以使溶液与血液等张。若需要，水溶液应被适当地缓冲（优选到 3 ～ 9 的 pH）。在无菌条件下制备合适的肠胃外制剂可通过本领域技术人员熟知的标准制药技术来容易地完成。

[0752] 无菌注射液可以通过使式 I 的化合物与合适的溶剂以及一种或多种前述载体混合，随后无菌过滤来制备。在适用于制备无菌注射液的无菌粉末的情况下，优选的制备方法包括在真空下干燥以及冻干，这提供 σ-2 受体拮抗剂和所需赋形剂的粉状混合物以便随

后制备无菌溶液。

[0753] 根据本发明的化合物可以配制成通过注射（例如通过静脉内推注或输注或经肌内、皮下或鞘内途径）用于人或兽药且可以以单位剂型、以安瓿或其它单位剂量容器、或以多剂量容器提供，若需要添加防腐剂。注射用组合物可以是混悬液、溶液或乳液的形式，处于油性或水性媒介物中，且可以含有配制剂例如助悬剂、稳定剂、增溶剂和/或分散剂。或者，活性成分可以是在使用前与合适的媒介物例如无菌无热原水重构的无菌粉末形式。

[0754] 本发明的化合物可以以片剂、胶囊、胚珠 (ovules)、酏剂、溶液或混悬液的形式施用，用于即释、延释、调释、缓释、脉冲释放或控释应用。

[0755] 本发明的化合物还可以以适用于口服或颊服的形式提供用于人或兽用，例如以溶液、凝胶、糖浆或混悬液形式，或在使用前与水或其它合适的媒介物重构的干燥粉末形式。还可以使用固体组合物例如片剂、胶囊、锭剂、软锭剂、丸剂、大丸剂 (boluses)、粉末、糊剂、颗粒、药筒 (bullets) 或预混制剂。用于口服用途的固体和液体组合物可以根据领域内熟知的方法制备。这样的组合物还可以含有一种或多种可为固体或液体形式的药学上可接受的载体和赋形剂。

[0756] 片剂可含有赋形剂例如微晶纤维素、乳糖、柠檬酸钠、碳酸钙、二碱式磷酸钙和甘氨酸，崩解剂例如淀粉（优选玉米、马铃薯或木薯淀粉）、羟乙酸淀粉钠、交联羧甲基纤维素钠和某些复合硅酸盐，以及制粒粘合剂例如聚乙烯吡咯烷酮、羟丙甲基纤维素 (HPMC)、羟丙基纤维素 (HPC)、蔗糖、明胶和阿拉伯胶。

[0757] 另外，可以包括润滑剂例如硬脂酸镁、硬脂酸、山嵛酸甘油酯和滑石。

[0758] 组合物可以口服施用，以速释或控制的片剂、微粒、微型片剂、胶囊、囊剂 (sachets)、以及口服溶液或混悬液、或用于其制备的粉末的形式。口服制剂可以任选地包括各种标准的制药载体和赋形剂，例如粘合剂、填充剂、缓冲剂、润滑剂、助流剂、染料、崩解剂、香味剂、甜味剂、表面活性剂、脱模剂、防粘合剂和包衣。一些赋形剂在组合物中可以具有多种作用，例如同时起粘合剂和崩解剂的作用。

[0759] 可用于本发明的口服组合物的药学上可接受的崩解剂的实例包括但不限于淀粉、预胶化淀粉、羟乙酸淀粉钠、羧甲基纤维素钠、交联羧甲基纤维素钠、微晶纤维素、藻酸盐、树脂、表面活性剂、泡腾组合物、水性硅酸铝和交联聚乙烯吡咯烷酮。

[0760] 本文可用的口服组合物的药学上可接受的粘合剂的实例包括但不限于阿拉伯胶；纤维素衍生物，例如甲基纤维素、羧甲基纤维素、羟丙甲基纤维素、羟丙基纤维素、或羟乙基纤维素；明胶、葡萄糖、右旋糖、木糖醇、聚甲基丙烯酸酯、聚乙烯吡咯烷酮、山梨醇、淀粉、预胶化淀粉、黄蓍胶、黄嘌呤树脂、藻酸盐、硅酸镁铝、聚乙二醇或膨润土。

[0761] 口服组合物的药学上可接受的填充剂的实例包括但不限于乳糖、脱水乳糖、一水合乳糖、蔗糖、右旋糖、甘露醇、山梨醇、淀粉、纤维素（特别是微晶纤维素）、磷酸二氢钙或无水磷酸钙 (anhydro-calcium phosphate)、碳酸钙和硫酸钙。

[0762] 可用于本发明的组合物的药学上可接受的润滑剂的实例包括但不限于硬脂酸镁、滑石、聚乙二醇、氧化乙烯的聚合物、月桂基硫酸钠、月桂基硫酸镁、油酸钠、硬脂酰富马酸钠、和胶体二氧化硅。

[0763] 口服组合物的合适的药学上可接受的香味剂的实例包括但不限于合成香料和天然芳香油例如油、花、果（例如香蕉、苹果、酸樱桃、桃）以及其组合的提取物，以及类似的香

料。它们的使用取决于很多因素，最重要的是将要服用该药物组合物的群体的感官可接受性。

[0764] 口服组合物的合适的药学上可接受的染料的实例包括但不限于合成和天然染料例如二氧化钛、β-胡萝卜素和葡萄柚皮提取物。

[0765] 口服组合物的通常用于促进吞咽、改变释放性质、改善外观且/或掩盖组合物的味道的有用的药学上可接受的包衣的实例包括但不限于羟丙甲基纤维素、羟丙基纤维素和丙烯酸酯-甲基丙烯酸酯共聚物。

[0766] 口服组合物的药学上可接受的甜味剂的合适实例包括但不限于阿司帕坦、糖精、糖精钠、甜蜜素、木糖醇、甘露醇、山梨醇、乳糖和蔗糖。

[0767] 药学上可接受的缓冲剂的合适实例包括但不限于柠檬酸、柠檬酸钠、碳酸氢钠、二碱式磷酸钠、氧化镁、碳酸钙和氢氧化镁。

[0768] 药学上可接受的表面活性剂的合适实例包括但不限于月桂基硫酸钠和聚山梨醇酯。

[0769] 相似类型的固体组合物还可用作明胶胶囊中的填充剂。鉴于此，优选的赋形剂包括乳糖、淀粉、纤维素、奶糖或高分子量聚乙二醇。对于水性混悬液和/或酏剂，试剂可以与各种甜味剂或调味剂、着色物质或染料，与乳化剂和/或助悬剂，且与稀释剂例如水、乙醇、聚乙二醇和甘油，及其组合进行组合。

[0770] 如所示，本发明的化合物可以经鼻内或通过吸入进行施用，且以干粉吸入剂或气雾喷雾剂形式从加压容器、泵、喷雾或雾化器使用合适的抛射剂例如二氯二氟甲烷、三氯氟甲烷、二氯四氟乙烷、氢氟烷烃例如1,1,1,2-四氟乙烷(HFA134AT)或1,1,1,2,3,3,3-七氟丙烷(HFA227EA)、二氧化碳或其它合适的气体进行便利的输送。在加压气雾剂的情况下，剂量单位可以通过设置筏来确定以输送计量的量。加压容器、泵、喷雾或雾化器可以含有活性化合物的溶液或混悬液，例如使用乙醇和抛射剂的混合物作为溶剂，其可额外含有润滑剂例如脱水山梨糖醇三油酸酯。

[0771] 用于吸入剂或吹入剂的胶囊和药筒(例如，由明胶制成)可以配制为含有化合物和合适粉末基底例如乳糖或淀粉的粉末混合物。

[0772] 对于通过吸入进行的局部施用，可以经雾化器输送用于人或兽药的本发明化合物。

[0773] 本发明的药物组合物可以含有0.01～99% wt/v活性材料。对于局部施用，例如，组合物通常含有0.01-10%、更优选0.01-1%的活性材料。

[0774] 化合物还可以以脂质体输送体系的形式进行施用，例如小单层囊泡、大单层囊泡和多层囊泡。脂质体可以由多种磷脂例如胆固醇、硬脂酰胺或卵磷脂形成。

[0775] 本发明的药物组合物或单位剂型可以根据通过鉴于以上指导而进行的常规测试所限定的剂量和施用方案来进行施用，以便获得最佳活性，同时使对于具体患者的毒性或副作用最小化。然而，治疗方案的这样的微调鉴于本文所给出的指导是常规的。

[0776] 本发明的化合物剂量可以根据多种因素变化，例如潜在的疾病状态，个体的状态、体重、性别和年龄，以及施用模式。用于治疗病症的有效量可以通过本领域普通技术人员已知的经验方法来容易地确定，例如通过建立剂量和施用频率的矩阵并比较矩阵中各点处的一组实验单元或受试者。施用于患者的精确量会根据病症的状态和严重程度以及患者的身

体状态而变化。任何症状或参数的可测量的改善可以通过本领域技术人员确定或通过患者向医师报告。应理解，尿道病症的任何症状或参数的任何临床或统计学显著的减轻或改善都在本发明的范围内。临床显著的减轻或改善是指对于患者和/或医师可感知。

[0777] 待施用的化合物的量的范围可以为约 0.01 ～ 25mg/kg/ 天，通常约 0.1 ～ 10mg/kg/ 天，且最通常约 0.2 ～ 5mg/kg/ 天。应理解，本发明的药物制剂并不必然需要含有可有效治疗病症的全部量的化合物，因为这样的有效量可以通过施用多个分开剂量的这样的药物制剂来达到。

[0778] 在本发明的优选实施方式中，化合物 I 配制成胶囊或片剂，通常含有 10 ～ 200mg 本发明的化合物，且优选以 10 ～ 300mg、优选 20 ～ 150mg 且最优选约 50mg 的全天剂量施用于患者。

[0779] 用于肠胃外给药的药物组合物基于 100wt％的总药物组合物含有约 0.01wt％至约 100wt％的本发明的活性化合物。

[0780] 通常，经皮剂型相对于 100wt％的总剂型含有约 0.01wt％至约 100wt％的活性化合物。

[0781] 药物组合物或单位剂型可以以单天剂量施用，或者全天剂量可以以分开剂量施用。另外，共同施用或相继施用用于治疗病症的另一化合物可以是理想的。为此目的，组合的活性成分配制到单个剂量单位中。

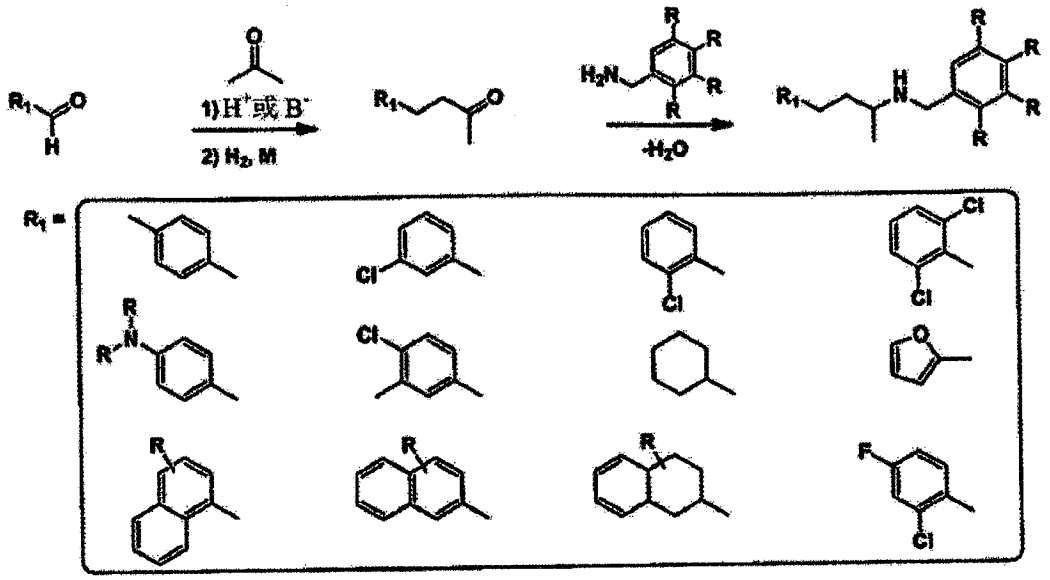
[0782] 本发明化合物的合成

[0783] 式 I 和 II 的化合物及其对映异构体、非对映异构体、N- 氧化物和药学上可接受的盐可以通过下文概述的一般方法来制备，该方法构成本发明的又一方面。在以下描述中，$R_{1\sim6}$ 基团具有针对任意上式的化合物所限定的含义，除非另外说明。

[0784] 本领域技术人员应理解，理想的是使用化合物 I 的制备中所使用的中间体的受保护衍生物。官能团的保护和去保护可以通过领域内已知的方法进行（参见例如，Green and Wuts Protective Groups in Organic Synthesis. John Wiley and Sons, New York, 1999）。羟基或氨基可以用任何羟基或氨基保护基团进行保护。氨基保护基团可以通过常规技术移除。例如，酰基，例如烷酰基、烷氧基羰基和芳酰基，可以通过溶剂分解例如通过酸性或碱性条件下的水解来移除。芳基甲氧基羰基（例如苄基氧基羰基）可以在催化剂例如铂/木炭的存在下通过氢解来裂解。

[0785] 目标化合物的合成通过使用本领域技术人员熟知的标准技术移除任何可存在于倒数第二个中间体的保护基来完成。然后使用标准技术例如硅胶层析、硅胶上的 HPLC 等，或通过重结晶来按需纯化去保护的终产物。上述化合物可以经任何合成路线来合成。例如，可以根据以下方案（方案 1）来制备化合物。

[0786]



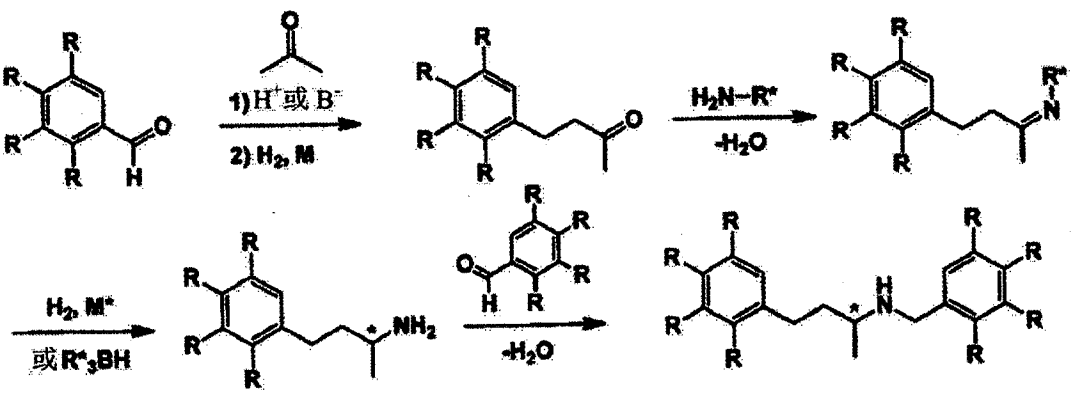
方案 1

[0787] 该方案可以产生本文描述的类似物的外消旋混合物。

[0788] 额外的 R1 基团还可以用于产生其它类似物。

[0789] 在一些实施方式中，不对称地进行合成，以便产生一个类似物的基本上纯的或纯的对映异构体。在一些实施方式中，本文所述化合物的不对称合成根据方案 2(* 表示手性中心）制备：

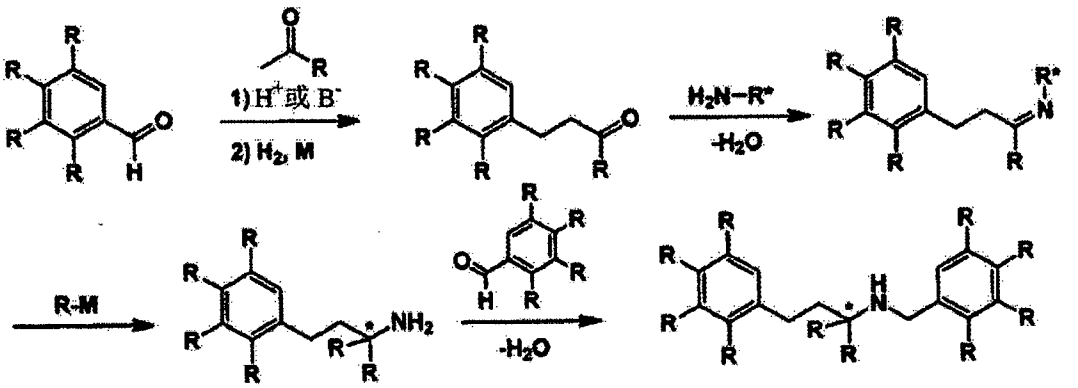
[0790]



方案 2

[0791] 在一些实施方式中，本文所述的化合物的不对称合成根据方案 3(* 表示手性中心）制备：

[0792]

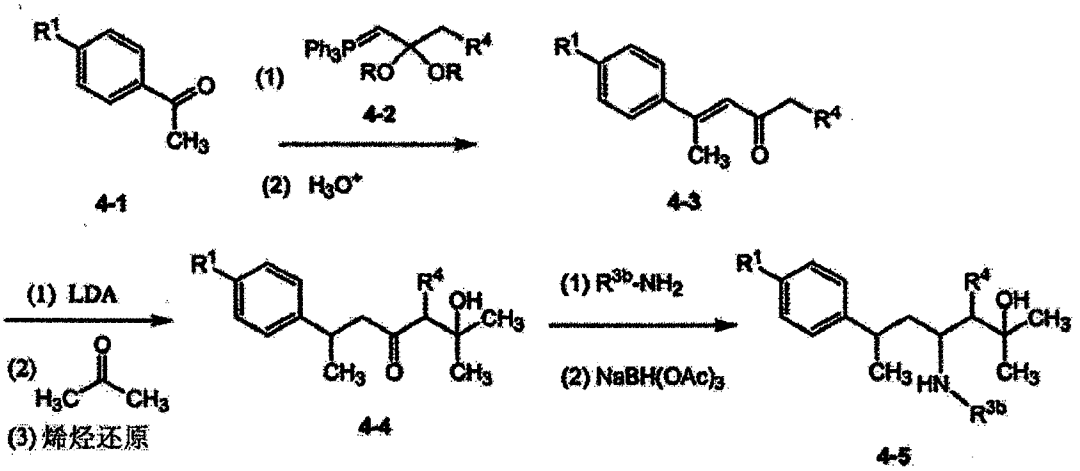


方案 3

[0793] 合成方案可以根据所需的终产物而改变。“R”基团是例示性的且可以被本文所述的任何取代基取代。

[0794] 以下是用于制备式 IX 的化合物的一般方法。如方案 4 所示，酮 4-1 可以与维蒂希试剂例如 4-2 反应，之后水解（例如在酸性条件下），得到酮 4-3。使用试剂例如 LDA，酮 4-3 的烯醇化物与丙酮缩合，后接烯烃的共轭还原，以形成酮 4-4。在合适的氢化物例如硼氢化钠的存在下，酮 4-5 与合适的胺 $R^{3b}NH_2$ 的还原胺化可以得到胺 4-6。胺 4-6 的不同非对映异构体可以通过本领域技术人员已知的方法例如柱层析而分离。

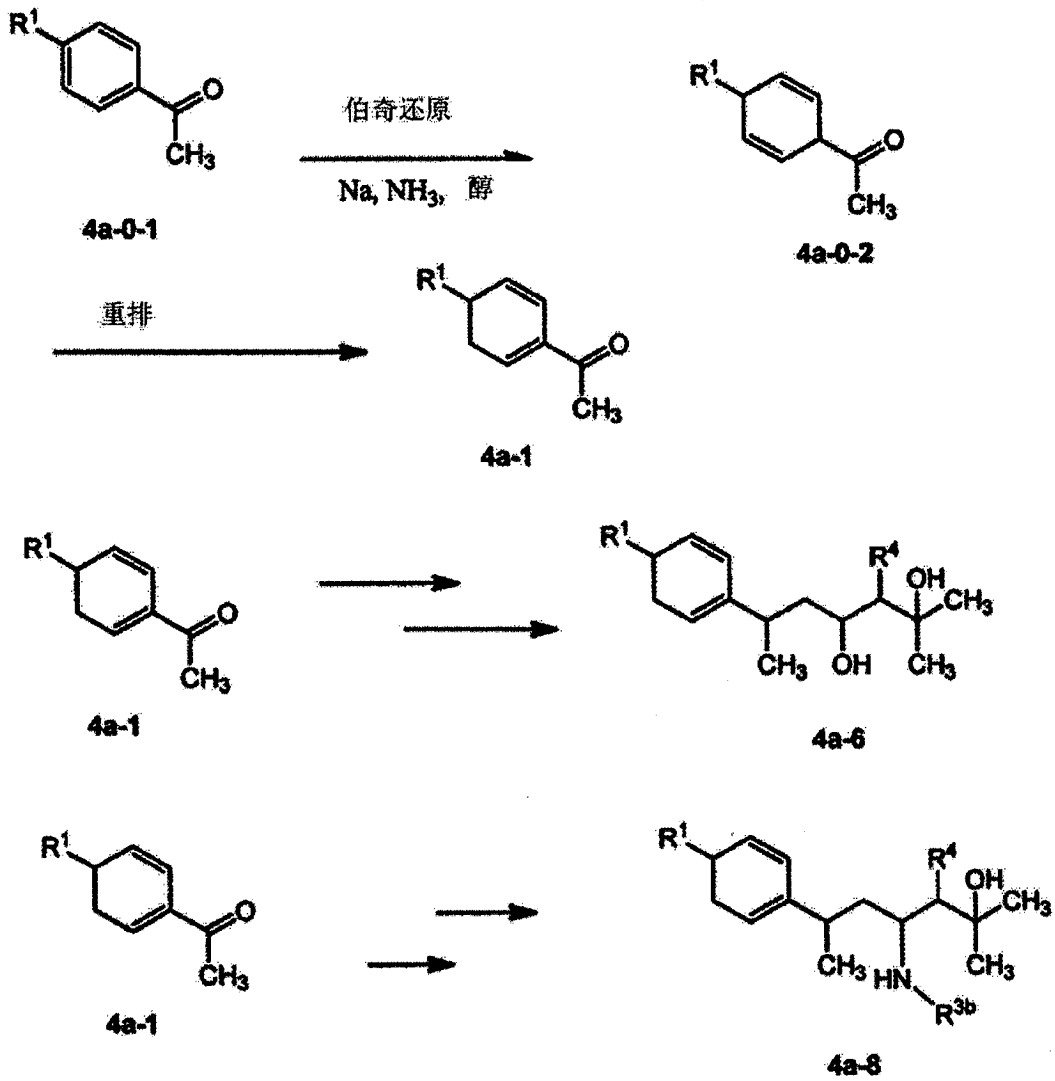
[0795]



方案 4

[0796] 如方案 4a 所示，芳族化合物 4a-0-1 可以在伯奇还原条件下还原为环己 -1,4- 二烯 4a-02。参见例如 Rabideau, P.W., "The metal-ammonia reduction of aromatic compounds", Tetrahedron, 第 45 卷，第 6 期，1989，第 1579-1603 页。在酸性条件下（例如在催化量的 HCl 或乙酸的存在下），环己 -1,4- 二烯 4a-02 可以重排为在热力学上更稳定的环己 -1,3- 二烯 4a-1。环己 -1,3- 二烯 4a-1 可以根据与方案 4 中所述相似的方法转化为醇 4a-6 或胺 4a-8。

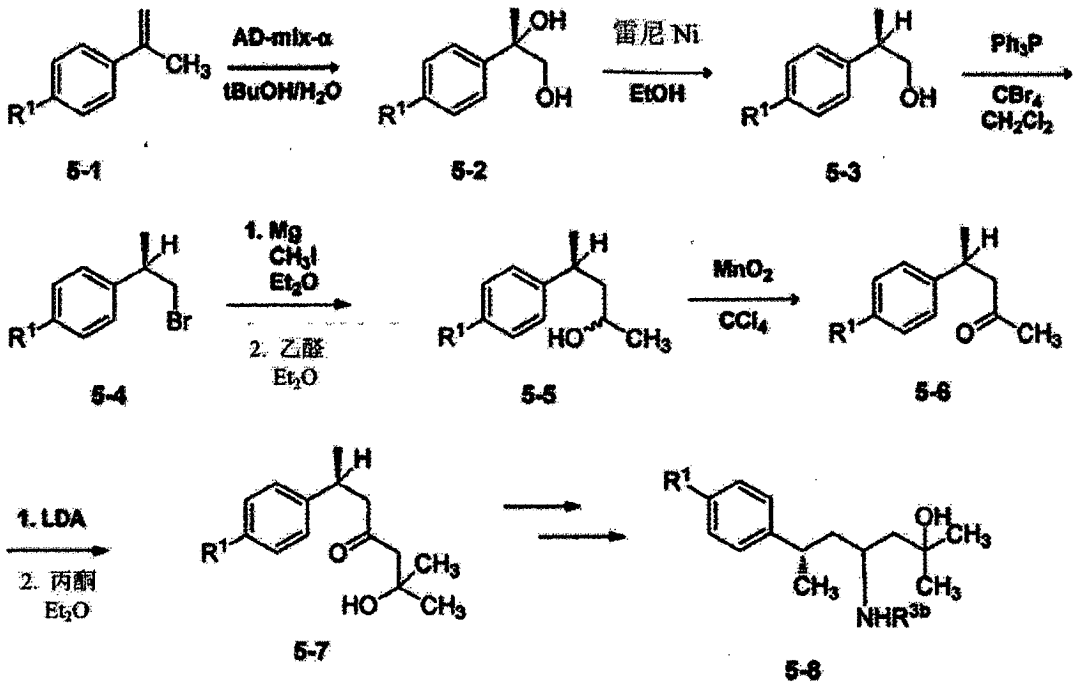
[0797]



方案 4a

[0798] 如方案 5 所示，用 AD-mix-α 处理苯乙烯衍生物 5-1（参见，例如 Sharpless, K. B. ;Amberg, W. ;Bennani, Y. L. ;Crispino, G. A. ;等人 J. Org. Chem. 1992, 57, 2771）得到二醇 5-2。参见 A. Li 等人 "Total asymmetricsynthesis of (7S, 9R)-(+)-bisacumol", Tetrahedron: Asymmetry (2003), 14(1), 75-78。用雷尼镍使二醇 5-2 的苄 OH 立体选择性还原，得到醇 5-3。参见，同上。5-2 的两种异构体均可以基于 Sharpless 催化剂的选择而获得。用合适溶剂例如二氯甲烷中的 PPh_3 和 CBr_4 处理醇 5-3，得到溴化物 5-4。在镁粉末和 CH_3I 的存在下将溴化物 5-4 转化为相应的格氏试剂（通过金属 - 卤素交换），后接与乙醛的反应，得到醇 5-5。醇 5-5 的不同非对应异构体可以通过本领域技术人员已知的方法例如柱层析而分离。参见，同上。醇 5-5 可以使用与方案 4 概述相似的方法而转化为其相应的氨化合物 5-6。胺化合物 5-8 的异构体可以通过立体选择性亚胺还原而得到。

[0799]

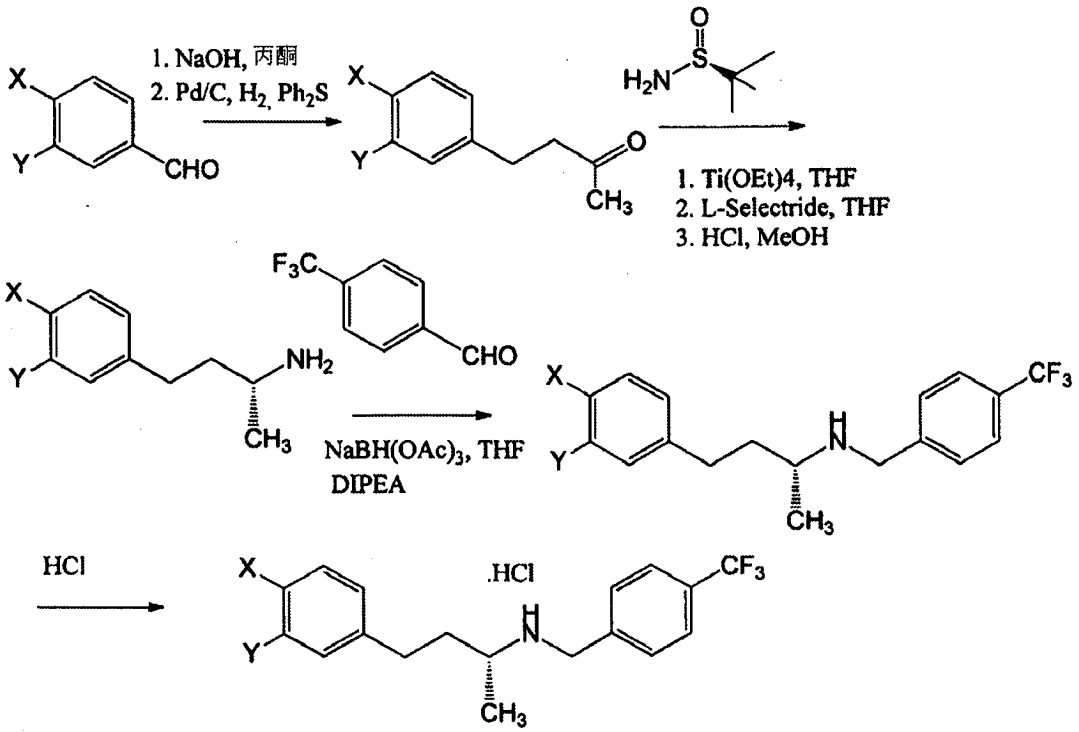


方案 5

[0800] 本领域技术人员可以识别出，在本文记载的所有方案中，如果在取代基例如 R^1、R^2、R^3 和 R^4 等上存在官能（反应）团，若合适和 / 或需要，可以做出进一步的修饰。例如，OH 基团可以转化为更好的离去基团例如甲磺酸根，其进而适用于亲核取代，例如通过 Br。因此，具有含官能团的取代基的式 I 化合物（例如方案 4 的化合物 4 ～ 8）可以转化为式 I 的具有不同取代基的另一化合物。

[0801] 在一些实施方式中，式 I ～ VI 的某些化合物例如通过方案 6 中所示的对映选择性路线进行制备。

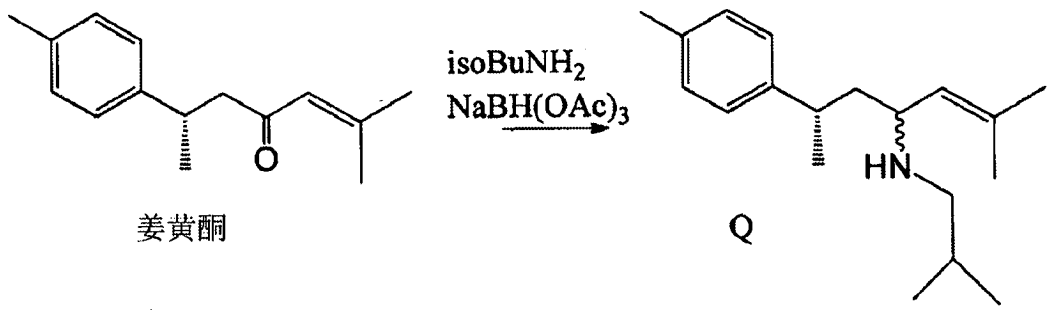
[0802]



方案 6. 式 I~VI 的化合物的对映选择性合成

[0803] 在一些实施方式中，σ-2 拮抗剂是式 VIIIa 的化合物。各个式 VIII 的某些化合物可以通过相应酮中间体的还原胺化来制备，例如通过方案 7 所示的代表性路线。

[0804]



方案 7

[0805] 工作例和合成例

[0806] 实施例 1 和 2 描述可以用于例如本文所述实验的 Abeta 寡聚体制剂。用在膜运输和寡聚体结合 / 突触减少检定中的特定制剂以及用在以下所述体内检定中的特定制剂各自在其所属的实施例中描述。

[0807] 实施例 1：制备 β 淀粉样蛋白寡聚体

[0808] 重新创建 β 淀粉样蛋白可以在神经组织中寡聚的条件，即其可以缔合的水性可溶蛋白的环境，从而识别 β 淀粉样蛋白寡聚体和原纤维的更多的疾病相关的结构状态。通过超速离心由大鼠脑制备水性可溶蛋白。具体而言，将每克脑组织 5 体积的 TBS 缓冲液 (20mM Tris-HCL, pH7.5, 34mM NaCl 和完整蛋白酶抑制剂混合物 (cocktail) (Santa Cruz)) 添加至冰上的大鼠脑组织。之后用紧定杵进行杜恩斯匀浆。之后将匀浆后的脑组织在 4℃以

150,000x g离心1小时(40,000rpmTy65)。之后除去次层清液(infranatant,在漂浮的髓磷脂与丸粒(pellet)之上半cm之间)并将等份在-75℃冰冻。之后将丸粒再悬于TBS中至原始体积,并以等份冷冻于-75℃。将合成的单体人β淀粉样蛋白1～42添加到该混合物以提供终浓度为1.5uM的β淀粉样蛋白,并将溶液在4℃孵育24小时。混合物以5,800g离心10分钟,以移除原纤维组装然后使用6E10结合的琼脂离心柱(Pierce Chemical Company)在4℃进行24小时免疫沉淀。之后将洗脱的β淀粉样蛋白寡聚体进行MALDI-Tof质谱分析,以识别样品的内容物,图1。

[0809] β淀粉样蛋白在含有蛋白的溶液中自缔合,以形成22,599Da的5亚基五聚体以及31,950Da的7亚基7聚体的亚基组装。49,291Da处的另一个峰可能表示12亚基的12聚体,尽管这不是β淀粉样蛋白12聚体的精确分子量。值得注意的是,在表示β淀粉样蛋白单体和二聚体的4518Da或9036Da处均没有观察到峰。然而,9,882Da和14,731Da处的峰可分别表示与786Da(或2x393Da)脂质或蛋白缔合的β淀粉样蛋白二聚体以及与3x393Da脂质或蛋白缔合的β淀粉样蛋白三聚体。此外,19,686Da处峰的存在表明可能涉及三聚体复合物和4954Da大鼠β淀粉样蛋白片段的组装状态。因此,这些数据可以反映出较小的脂质或蛋白与β淀粉样蛋白的二聚体和三聚体的缔合,这可以指引生理系统独特的构象状态的组装。

[0810] 实施例2:制备β淀粉样蛋白寡聚体

[0811] 将1.5uM单体人β淀粉样蛋白1-42于大鼠脑可溶蛋白的混合物中的溶液如实施例1所述在4℃孵育24小时。之后用三氟乙醇(TFE)在进行光谱前处理该溶液。在TFE中,组装的蛋白结构和非共价结合的蛋白复合物解离成变性蛋白,且预期与组装的寡聚体相关的峰消失。大多数在实施例1中观察到的蛋白峰消失,包括以上识别的9822Da、14,731Da、31,950Da和49,291Da峰。然而,在4518Da处观察到高丰度峰,其表示β淀粉样蛋白单体峰。4954.7处的峰很明显,其可表示与β淀粉样蛋白1-46相似的较长Abeta片段。在7086Da处观察到额外的峰,其在实施例1所述的制剂中不存在,可能表示与2550Da共价结合的蛋白缔合的β淀粉样蛋白单体。

[0812] 实施例3:从人AD脑组织中分离β淀粉样蛋白寡聚体

[0813] TBS可溶提取物:经组织病理学分析特征为Braak Stage V/VI阿尔茨海默病(AD)的人患者的死后脑组织的样品得自医院脑组织库。年龄和性别匹配的AD和正常组织样本在含有1mM EDTA和1mg/ml完整蛋白酶抑制剂混合物(Sigma P8340)的20mM Tris-HCL、137mM NaCl,pH7.6中稀释为0.15gm组织/ml并进行匀浆。在Beckman Optima XL-80K超速离心器中以105,000g对组织匀浆进行超速离心1小时。将所得的TBS可溶部分使用蛋白-A和蛋白-G琼脂糖柱(Pierce Chemical)免疫耗竭,然后用Amicon Ultra3、10&100kDa NMWCO过滤器(Millipore Corporation)进行尺寸分级。

[0814] 免疫沉淀:将尺寸分级且免疫耗竭的TBS可溶提取物在合适的NMWCO Amicon Ultra过滤器中浓缩到约200ul。将浓缩的TBS可溶提取物用TBS样品缓冲液(Pierce Chemical)稀释到400ul,并以5,800g离心10分钟以移除原纤维。然后将所得的上清液用6E10-结合的琼脂糖小球在4℃免疫沉淀过夜,随后使用高渗强度Gentle洗脱缓冲液(Pierce Chemical)进行抗原洗脱以分离含有Abeta的蛋白种类。

[0815] MALDI-质谱:使用Applied Biosystems(ABI)Voyager DE-Pro MALDI-Tof仪器对免

疫分离的 β 淀粉样蛋白进行质谱分析。根据分析的目标分子量范围，使用多种基质类型例如 α - 氰基 -4- 羟基肉桂酸 (CHCA)、芥子酸 (SA) 或 6- 氮杂 -2- 硫代胸腺嘧啶 (ATT) 来分析样品。仪器以线性正离子模式以及可变提取延迟来运行。非累积谱表示每次获取 100 次“热点”击中，而累积谱由每次获取 200 次激光击中的各个点的 12 个单独区域表示。

[0816] 数据分析：使用 Voyager's Data Explorer 软件包来进行数据获取和分析。质谱的标准处理除信噪比的变化之外还包括光谱的平滑和基线扣减功能。

[0817] 用于 Ab 定量的 ELISA：使用改良的夹心 ELISA 技术对免疫沉淀的 TBS 可溶部分分析“总”Abeta 和 Abeta 寡聚体浓度。简单来说，将 6E10 和 4G8 涂覆的 Nunc MaxiSorp96 孔板用含有 Abeta 的样品进行孵育，然后用生物素化的 4G8 检测抗体进行探测。与链霉亲和素 -HRP(Rockland) 的孵育以及之后的四甲基联苯胺 (TMB) 底物的显色使得可以在 BioTEk Synergy HT 酶标仪上对 Abeta 进行比色检测 (OD450)。使用单体 Abeta1-42 来产生标准曲线，并且连同 GEN5 软件一起使得可以对免疫沉淀的样品中的 Abeta 水平进行定量。

[0818] 实施例 4A：受体结合检定

[0819] 化合物 II 与数种受体通过阻断其激动剂或拮抗剂的结合或作用而相互作用。测试化合物 II 以观察其是否与已知的细胞受体或信号传导蛋白直接相互作用。测试化合物 II(10 μ M) 对给定人受体的已知激动剂或拮抗剂的结合的替代能力，该人受体在细胞系中过表达或从组织中分离。还测试其阻断由给定人受体的激动剂或拮抗剂所诱导的下游信号传导的能力。测试化合物 II 在 100 个已知受体处的作用，并且化合物 II 仅在 5 个这些受体处显示出活性 >50%（检定窗）(表 1E)。这表明化合物 II 仅在小子集的 CNS 相关的受体处具有高度特异性和活性。其以最高亲和性结合 σ -2 受体，因此是 σ -2 配体。

[0820] 表 1E. 化合物 II(10uM) 对已知受体结合的抑制

[0821]

化合物 II（10 uM）对已知受体结合的抑制	%对照抑制	SEM%对照
σ-2（激动剂放射性配体）	89	0.6
Mu（MOP）(h)（激动剂放射性配体）	60	1.4
Na+通道（位点 2）（拮抗剂放射性配体）	54	4.7
D3（h）（激动剂作用）	66	4.0
α1A（h）（拮抗剂作用）	56	1.1

[0822] 使用相同的方案，测试膜运输数据已经在表 5(以下) 中给出的化合物对 σ -2 受体的识别。结果证实，与化合物 II 结构相似的这些化合物为 σ -2 受体配体，即优选与 σ -2 受体结合。最后，与化合物 IXa 和 IXb 相似的化合物，例如式 VIII 和 IX 的化合物也是 σ -2 受体配体。

[0823] 竞争性放射性配体结合检定

[0824] 通过商业合同研究组织进行 σ -1 受体和 σ -2 受体的放射性配体结合检定。对于 σ -1 结合，使用从 100uM 至 1nM 的各个浓度的测试化合物，在 Jurkat 细胞膜上替代内源受

体的 8nM[^{3}H](+) 戊唑辛 (Ganapathy ME 等人 1991, J Pharmacol. Exp. Ther. 289:251-260)。使用 10μM 的氟哌啶醇来定义非特异性结合。对于 σ-2 受体，在存在 300nM(+) 戊唑辛掩盖 σ-1 受体的情况下，使用从 100μM 至 1nM 的各个浓度的测试化合物，在大鼠大脑皮质的膜上替代内源受体的 5nM[^{3}H]1,3-二-(2-甲苯基)胍。(Bowen WD 等人，1993, Mol. Neuropharmcol3:117-126)。使用 10μM 的氟哌啶醇来定义非特异性结合。使用 Brandel12R 细胞收集器经由 Whatman GF/C 过滤器通过快速过滤来终止反应，之后用冰冷缓冲液进行两次洗涤。干燥过滤盘上的放射活性使用液体闪烁分析仪 (Tri-Carb2900TR ;PerkinElmer Life and Analytical Sciences) 来测量。绘制替代曲线，并使用 GraphPad Prism(GraphPad Software Inc., San Diego, CA) 来确定测试配体对受体亚型的 Ki 值。特异性结合的百分比通过将总结合（每分钟衰变）与非特异性结合（每分钟衰变）之间的差值除以总结合（每分钟衰变）而确定。

[0825] 对于已知的现有技术化合物，对于 σ-1 和 σ-2 受体的亲和性得自已公开的研究，已公开的研究使用脑组织匀浆，用 [^{3}H](+) 戊唑辛测量来自 σ-1 受体的替代且在 300nM(+) 戊唑辛的存在下用 [^{3}H]1,3-二-(2-甲苯基)胍来测量来自 σ-2 受体的替代。结果显示在表 2 中。

[0826] 表 2. σ-2 和 σ-1 受体亲和性。

[0827]

化合物	σ-1 结合 Ki (nM)	σ-2 结合 Ki (nM)
II（三个不同批次：外消旋混合物、(+)异构体和（-）异构体）	500 100 46	9 52 63
化合物 A	47	16
化合物 B	47	16
化合物 E	1890(对 σ-1 受体基本上没有亲和性)	16
化合物 P	320	110
化合物 R'	26	27
化合物 S'	31	27
化合物 IXa	89	21
化合物 IXb	190	23
化合物 W	270	120
化合物 AC	23	240
化合物 AE	16	35
化合物 AF	8	110

[0828]

化合物	σ-1 结合 Ki (nM)	σ-2 结合 Ki (nM)
化合物 AH	23	50
化合物 AI	250	130
化合物 AL	3100	690
化合物 AX	620	440
化合物 AY	5	23
化合物 AZ	34	340
化合物 BB	0.72	5.2
化合物 BC	4.2	13
化合物 BD	2.1	19
化合物 BE	7.4	14
化合物 BH	4	7.4
化合物 BJ	6.2	25
化合物 BP	53	8.9
化合物 BT	1	4
化合物 CB	19	48
化合物 CC	12	3.9
化合物 CD	56	2.7
化合物 CE	33	2.2

[0829]

化合物	σ-1 结合 Ki (nM)	σ-2 结合 Ki (nM)
化合物 CF	180	50
化合物 CG	360	3200
化合物 CJ	44	810
化合物 CL	190	5,000
化合物 CO	130	7,200
化合物 CR	3.5	16
化合物 CS	78	85
化合物 DH	23	8.3
化合物 DR	330	3,200
其他化合物	σ-1 结合 Ki（nM）（活性类型）	σ-2 结合 Ki（nM）
BD1047	0.9（拮抗剂）	47
DTG	88（激动剂）	35
氟哌啶醇	5（拮抗剂）	110
艾芬地尔	26	4.9
Mach-14	12,900	8
NE 100	1.1（拮抗剂）	170

[0830]

化合物	σ-1 结合 Ki (nM)	σ-2 结合 Ki (nM)
PB 28	15	0.8
PRE-84	2.2（激动剂）	13,091
SM-21	1050（拮抗剂）	145
苏-艾芬地尔	59	0.9（激动剂）*
舍曲林	8.6（拮抗剂）	170
PPBP	0.8（激动剂）	1
BD1008	2.2（拮抗剂）	8
氟伏沙明	13（激动剂）	710
BD1063	8.8（拮抗剂）	625
SFK10047	597（激动剂）	39,740
西拉美新	19	0.19（激动剂）

[0831] *Monassier 等人，JPET，322(1)：341-350，2007。

[0832] 竞争性放射性配体结合检定 2

[0833] 候选 σ-2 配体化合物在 σ-1 和 σ-2 受体处的亲和性还通过对不同的已知的标记 σ-2 或 σ-1 配体的替代而确定。过滤检定根据之前公开的步骤（Xu 等人，2005）进行。测试化合物溶解在N，N-二甲基甲酰胺（DMF）、二甲基亚砜（DMSO）或乙醇中，之后在含有 150mM NaCl 和 100mM EDTA 的 50mM Tris-HCl pH7.4 缓冲液中稀释。由豚鼠脑制备用于 σ-1 结合检定的膜匀浆且由大鼠肝脏制备用于 σ-2 结合检定的膜匀浆。膜匀浆用 50mM Tris-HCl 缓冲液 pH8.0 稀释，并在具有浓度为 0.1nM ～ 10uM 的放射性配体和测试化合物的 96 孔板中以总体积 150uL 于 25℃孵育。在完成孵育后，使用 96 通道移液器（Fisher Scientific，Pittsburgh，PA）通过添加 150uL 的冰冷洗涤缓冲液（10mM Tris HCl，150mM NaCl，pH7.4）来终止反应，收集样品并通过经 100uL 的 50mM Tris-HCl 缓冲液预浸的 96 孔纤维玻璃滤板（Millipore，Billerica，MA）进行快速过滤。各个过滤器用 200uL 冰冷洗涤缓冲液（10mM Tris-HCl，150mM NaCl，pH7.4）洗涤 4 次。使用 Wallac1450MicroBeta 液体闪烁计数仪（Perkin Elmer，Boston，MA）对结合放射活性进行定量。

[0834] 使用豚鼠脑膜匀浆（～ 300ug 蛋白）和～ 5nM[^{3}H](+)-戊唑辛（34.9Ci/mmol，Perkin Elmer，Boston，MA）来进行 σ-1 受体结合检定，室温下孵育时间为 90min。由含有 10μM 的冷氟哌啶醇的样品确定非特异性结合。

[0835] 使用大鼠肝脏膜匀浆（～ 300ug 蛋白）和仅～ 2nM σ-2 高度选择性放射性配体[^{3}H]RHM-1（没有其他阻断物）（America Radiolabeled Chemicals Inc. St. Louis，MO）、～ 10nM[^{3}H]DTG（58.1Ci/mmol，Perkin Elmer，Boston，MA）或～ 10nM[^{3}H] 氟哌啶醇（America Radiolabeled Chemicals In.，St. Louis，MO）在 1uM 阻断 σ-1 位点的（+）-戊唑辛的存在下进行 σ-2 受体结合检定，室温下孵育时间对于[^{3}H]RHM-1 为 6 分钟，对于[^{3}H]DTG 和[^{3}H]

氟哌啶醇为 120 分钟。由含有 10uM 的冷氟哌啶醇的样品确定非特异性结合。

[0836] 使用非线性回归分析来对竞争性抑制实验中的数据进行建模，以确定抑制 50％放射性配体的特异性结合的抑制剂浓度（IC_{50} 值）。使用 Cheng 和 Prusoff 的方法来计算结合亲和性 Ki 值。豚鼠脑中用于 [^{3}H](+)- 戊唑辛的 Kd 值为 7.89nM，大鼠肝脏中用于 [^{3}H] RHM-1 和 [^{3}H]DTG 的 Kd 值分别为 0.66nM 和 30.73nM。标准化合物氟哌啶醇用于质量保证。化合物 IXa、IXb 和化合物 II 在 σ-1 和 σ-2 受体处的亲和性数据显示在表 3 中。因此，可以采用领域内已知的任何 σ-2 受体结合检定来确定候选化合物的 Ki 或 IC50。

[0837] 表 3. 竞争性放射性配体结合检定 2 中候选 σ-2 配体的 σ-2 和 σ-1 受体亲和性。

[0838]

编号	化合物	σ-1 （Ki，nM）±平均 SEM	σ-2 （Ki，nM）±平均 SEM
1	IXa、IXb	6.37±0.81	30.8±2.3
2	II	108.1±19.9	59.7±10.4

[0839] 实施例 4B. 受体抗体介导的寡聚体与受体结合的降低

[0840] 如本文所述，近来孕酮受体膜成分 1(PGRMC1) 被 Xu 等人 2011 识别为 σ-2 受体活性的关键 25kDa 成分。具体而言，通过使用光亲和性探针 WC-21 在大鼠肝脏中识别出 PGRMC1，该光亲和性探针 WC-21 可逆地标记大鼠肝脏中的 σ-2 受体。Xu 等人，Identification of the PGRMC1 protein complex as the putative sigma-2receptor binding site. Nature Communications2, article number380, July5, 2011，其并入本文以供参考。因此，在这些实验中采用对人 PGRMC1 的多种 C 端或 N 端氨基酸序列特异的单克隆抗体。

[0841] 使用以下一般检定程序来测试受体抗体影响 Abeta 寡聚体结合的能力。阳性对照：6E10 抗体 (Covance)（识别所有 Abeta 种类的 N 端，且将通过在溶液中在受体结合前与寡聚体的高亲和性结合而降低 Abeta 与神经元的结合）。准备培养液中的神经元以用于膜运输检定。阴性对照：非免疫 IgG。方法：

[0842] 1. 抽吸板至 10uL 体积

[0843] 2. 添加 30uL 指定浓度的受体单克隆抗体 (mAb)、6E10 或非免疫 IgG，37℃ 30 分钟

[0844] 3. 添加 10uL 指定浓度的 Abeta 寡聚体；37℃孵育 1 小时

[0845] 4. 固定，并用 PBS 洗涤板 3 次

[0846] 5. 使用封闭化合物 (1L PBS，50mL 正常山羊血清，50mL10％ TritonX) 封闭板 1 小时

[0847] 6. 抽吸板至 10uL

[0848] 7. 添加 25uL 检测一抗 4G8 和 MAP2；密封并冷藏过夜。

[0849] 8. 用封闭化合物洗涤板 3 次（抽吸至 10uL，用 60uL 封闭液洗涤）

[0850] 9. 添加 25uL 的二抗 (Alexa fluor647 山羊抗小鼠、Alexa flour488 山羊抗兔、Alexa fluor646 山羊抗鸡）；室温下静置 1 小时。

[0851] 10. 抽吸板至 10uL，用 60uL PBS 洗涤，抽吸板至 10uL，添加 60uL Dapi，抽吸板至 10uL，用 60uL PBS 洗涤。

[0852] 11. 用铝密封物覆盖

[0853] 12. 用专门的 Cellomics Arrayscan 方案来扫描并分析图像。

[0854] 特异性抗体阻断实验

[0855] 在这些实验中采用识别与人 PGRMC1(#EB07207,Everest Biosciences) 的 C 端氨基酸 185 ～ 195 相应的合成肽 C-EPKDESARKND(SEQ ID NO:7) 或人 PGRMC1 蛋白 N 端残基 1 ～ 46(MAAEDVVATGADPSDLESGGLLHEIFTSPLNLLLLGLCIFLLYKI(SEQ ID NO:9),#sc-98680,Santa Cruz) 或非免疫对照 IgG 的抗体。将各个抗体以 38nM(5x)、58nM(7.5x) 或 77nM(10x) 最终检定浓度应用于神经元 30 分钟。

[0856] 之后以 500nM 总 Abeta 浓度添加 Abeta1 ～ 42 寡聚体并使其与神经元再结合 15 分钟。之后固定培养物并使用 6E 抗体来对结合的 Abeta 种类进行免疫标记。以特征性点状模式与突触后膜结合的寡聚体通过自动图像处理进行定量。经 MAP2 免疫标记而识别出神经元。对于神经元健康状况的定量测量例如平均核面积通过图像处理进行定量,且结果显示在图 13A 至 13H 中。

[0857] 与对照 Abeta(图 13A) 相比,用 C 端抗体 (图 13C) 而非 N 端抗体 (图 13G) 或非免疫 IgG(图 13E) 孵育的神经元与 Abeta 寡聚体的结合的强度呈剂量依赖的降低。该强度降低看似由寡聚体阳性点的数目和面积降低组成。该寡聚体结合强度的降低是由于寡聚体与抗体之间对结合表位的竞争还是由于抗体介导的表面 PGRMC1 蛋白表达的降低是未知的。在一些实施方式中,阻断可溶 Abeta 寡聚体与 σ-2 受体之间的结合的 σ-2 受体抗体和 PGRMC1 抗体被认为是 σ-2 受体拮抗剂化合物。

[0858] 实施例 5 :转基因小鼠的记忆丧失 :Morris 游泳测试

[0859] 测试化合物 II 以确定其是否能够逆转较老的阿尔茨海默病转基因小鼠模型 (其中寡聚体随年龄增加) 中见到的记忆丧失。对于该研究,选择在鼠科 Thy-1 启动子控制下表达人 APP751 瑞典 (670/671) 和伦敦 (717) 突变的 hAPP 小鼠。这些小鼠的 Abeta 量表现出年龄依赖性增加,斑块在 3 ～ 6 个月开始形成,并在 8 个月年龄时表现出建立的认知缺陷。在该研究中,治疗已经建立的缺陷,而非防止缺陷发生。这些研究按照不知道实验条件的科学家的服务合同而进行。在 8 个月大的雌性小鼠中经皮下微型泵将化合物以 0.5 和 0.1mg/kg/ 天输注一个月,并且在 Morris 水迷宫 (一种基于海马的空间学习和记忆的测试) 中测试认知表现。该小鼠模型不表现神经元丧失,因此记忆的恢复不能归因于凋亡的避免。

[0860] 将游泳速度作为 Morris 测量的一部分进行分析,以确定是否存在任何的运动或动机缺陷。媒介物是 5% DMSO/5% Solutol、90% 盐水混合物。用低剂量 (0.1mg/kg/ 天) 和高剂量 (0.5mg/kg/ 天) 的化合物 II 处理转基因动物。确定连续四天中的每天三次试验的平均值。可检测没有显著的运动缺陷或任何种类的异常行为,且在研究的过程中仅损失了一只转基因媒介物组的动物,低于在该年龄的预期死亡数。另外,保留了周期性抽血的哨动物组,以监测化合物的血浆水平,且这些显示出初步 PK 研究中所见的血浆水平非常小的改变。

[0861] 进行 Morris 水迷宫测试中的逃跑潜伏期测量。在测试的第二天,观察到野生型和转基因动物之间的显著差异,野生型学得比转基因型更快。在这一天,还观察到转基因表现的较高化合物剂量相对于媒介物的显著改善。因此,结论为,以 0.5mg/kg/ 天施用的化合物 II 能够改善 AD 转基因模型中的认知表现。

[0862] Abeta42 寡聚体引起突触数目降低 18% ;100% 的该类损失由化合物 II 及其对映

异构体消除。与化合物 II 相似，一些其他 σ-2 受体拮抗剂也阻断突触损失。已知的现有技术 σ-2 受体配体 NE-100 和氟哌啶醇完全消除突触损失，而选择性 σ-1 配体 SM-21 在消除突触损失中仅有较弱活性（20％恢复）。

[0863] 还使用相似的检定测试化合物 IXa 和 IXb 的混合物。化合物 IXa 和 IXb 的混合物（1mg/kg/ 天，N = 8 或 10mg/kg/ 天，N = 8）或媒介物（5％ DMSO/5％ Solutol/90％盐水，N = 15）经皮下给药（Alzet 微型泵）向 9 个月大的雄性 hAPPSL 转基因小鼠（N = 8）或非转基因同窝仔（N = 6）全身性施用 20 天并在 Morris 水迷宫中评价这些小鼠的空间学习和记忆。在处理的最后 4 天中，以 3 次试验 / 天测试小鼠以寻找隐藏的平台。计算机化的追踪系统自动定量逃跑潜伏期或游泳长度。

[0864] 在测试的任何一天，转基因动物相对于非转基因动物的表现均没有显著差异（分析限于这两组；重复测量的双因素（基因型和时间）ANOVA，后接 Bonferroni 事后比较测试）。限于转基因组（处理和时间）的相似分析显示出，用 10mg/kg/ 天的化合物 IXa 和 IXb 的混合物处理的转基因动物在测试的第二天和第四天表现得显著好于媒介物处理的转基因动物（$p<0.05$，经学生 t 检验分析）。媒介物处理的非转基因动物在测试的第一天和第二天表现得显著好于媒介物处理的转基因动物。用化合物 IXa 和 IXb 的混合物进行的处理，与媒介物处理相比，在测试的第一天（两种剂量）、第二天（10mg/kg/ 天剂量）和第四天（10mg/kg/ 天剂量）显著改善转基因动物的表现（$p<0.05$；游泳长度）。

[0865] 这表明，化合物 IXa 和 IXb 的混合物能够以剂量依赖的方式在老龄转基因动物中逆转学习和记忆中已建立的行为缺陷。

[0866] 实施例 6：在膜运输检定中抑制 Abeta 寡聚体对神经元细胞的作用

[0867] 对选自以上表 2 的 σ-2 配体测试其抑制 β 淀粉样蛋白对细胞的作用的能力。σ-2 配体能够抑制经膜运输 / 胞吐检定（MTT 检定）测量的 β 淀粉样蛋白作用。结果显示在以下表 5 中。该检定的基本原理如下：

[0868] 因为突触和记忆缺陷而非广泛的细胞死亡在阿尔茨海默病的最早期中占主导地位，测量这些变化的检定特别适合于发现寡聚体活性的小分子抑制剂。通常使用 MTT 检定作为培养中毒性的测量。黄色的四唑盐被细胞胞吞并在胞内体途径中被还原为不溶的紫色甲臜。紫色甲臜的水平反映出培养中活性代谢细胞的数目，且甲臜的量的减少被认为是培养中的细胞死亡或代谢毒性的测量。当经由显微镜观察时，紫色甲臜首先在填充细胞的胞内囊泡中可见。随着时间推移，囊泡被胞吐，且在不溶甲臜暴露于水性介质环境时，甲臜在细胞质膜的外表面上沉淀为针状结晶。Liu 和 Schubert（'97）发现，细胞通过选择性地加速还原甲臜的胞吐速率来应答 Abeta 寡聚体的亚致死水平，而不影响胞吞速率。本发明的发明者已经在培养的成熟初级神经元中重复了这些发现，并经由自动显微镜术和图像处理来对这些形态变化进行定量。在这些情况下，还原甲臜的总量没有总体改变，仅仅是反映其形成和 / 或从细胞中排出的速度变化的形态学具有变化。本发明的发明者已经证实了先前的发现，即该检定对不引起细胞死亡的低水平寡聚体敏感（Liu 和 Schubert'04，Hong 等人'07）。确实，引起 LTP 抑制的低量寡聚体不引起细胞死亡（Tong 等人'04）并且预期不会改变培养（或脑切片）中甲臜的总量。

[0869] 由其他研究者举出的证据表明，由膜运输介导的神经元表面受体表达中 Abeta 寡聚体介导的降低是突触可塑性 (LTP) 以及因此的学习和记忆的的电生理测量的寡聚体抑制的基础 (Kamenetz 等人' 03, Hseih 等人' 06)。经由甲臜形态变化测量由寡聚体诱导的膜运输速率改变已经被用在细胞系中以发现降低啮齿动物体内 Abeta 脑水平 (Hong 等人' 09) 的阻断 Abeta 寡聚体的药物 (Maezawa 等人 '06, Liu 和 Schubert'97、'04、'06, Rana 等人 '09, Hong 等人'08)。用于胞吐检定 /MTT 检定的相似程序可以在文献中找到。参见，例如 Liu Y 等人，Detecting bioactive amyloid beta peptide species in Alzheimer's disease. J Neurochem. 2004Nov ;91(3):648-56 ;Liu Y 和 Schubert D. "Cytotoxic amyloid peptides inhibit cellular3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT)reduction by enhancing MTT formazan exocytosis. "J Neurochem. 1997Dec ; 69(6):2285-93 ; 以 及 Liu Y 和 Schubert D. "Treating Alzheimer's disease by inactivating bioactive amyloid beta peptide"Curr. Alzheimer Res. 2006Apr ; 3(2):129-35。因此该方法是有效的。

[0870] 本发明的胞吐检定适用于体外生长 3 周的成熟初级神经元培养物。参见 WO/2011/106785，其全部并入本文以供参考。Abeta 寡聚体对填充有还原的紫色甲臜的胞内囊泡 (点) 的量引起剂量依赖性降低，这使用 Cellomics VTI 自动显微系统经图像处理而测量。例如比较图 1A(仅暴露于媒介物的培养神经元细胞的显微照片，示出填充有甲臜的囊泡) 与图 1B(暴露于媒介物 +Abeta 寡聚体的神经元细胞的显微图片，示出相当少的填充有甲臜的囊泡，相反，胞吐的甲臜在遇到胞外环境时沉淀为结晶)。增加 Abeta 寡聚体的量最终引起明显的毒性。因此，用在检定中的神经活性 Abeta 寡聚体的浓度远低于引起细胞死亡的浓度。本发明的发明者通过显示 Abeta 寡聚体的作用在添加 Abeta 抗体时被阻断但仅抗体自身无作用 (数据未示出)，证实检定是有效的。当以该方式配置时，检定能够检测抑制 Abeta 寡聚体的非致死作用的化合物是否经由破坏寡聚体、抑制寡聚体与神经元的结合或对抗由寡聚体结合起始的作用的信号传导机制而起作用。

[0871] 用于产生结果的方法如下示于膜运输 / 胞吐 (MTT) 检定。

[0872] 将 E18Sprague-Dawley 大鼠胚胎的初级海马神经元以优化浓度在 NB 培养基 (Invitrogen) 的 384 孔板中进行铺板。将神经元保持培养 3 周，每周两次提供 N_2 补充的 NB 培养基 (Invitrogen)。这些神经元表达成熟脑中神经元特有的突触蛋白的全补体 (full complement)，并表现出活性依赖的电信号传导的复杂网络。这样的培养中的神经元和胶质细胞具有表现出优异的登记 (registration) 且脑回路完整的分子信号传导网络，且出于该原因，已被用作学习和记忆的模型系统多于 20 年 (参见例如 Kaech S, Banker G. Culturing hippocampal neurons. NatProtoc. 2006 ;1(5):2406-15. Epub2007Jan11 ; 同时参见 Craig AM, Graf ER, Linhoff MW. How to build a central synapse:clues from cell culture. Trends Neurosci. 2006Jan ;29(1):8-20. Epub2005Dec7. 综述)。

[0873] 以 100uM 至 0.001nM 的浓度向细胞中添加测试化合物，之后添加媒介物或 Abeta 寡聚体制剂 (3μM 部 Abeta 蛋白浓度)，并在 5% CO_2 中于 37℃孵育 1 ～ 24 小时。将 MTT 试剂 (3-(4,5- 二甲基噻唑 -2- 基)-2,5- 二苯基溴化四唑) (Roche Molecular Biochemicals) 在磷酸盐缓冲盐水中重构为 5mg/mL。将 10μM 的 MTT 标记试剂添加到各个孔中，并在 37℃

孵育 1 小时，然后拍照。通过自动显微术和图像处理来评价胞吐，以对胞吞和胞吐的甲臆的量进行定量。

[0874] 使各个检定板格式化，使得在各个板上在具有和不具有 Abeta 寡聚体的情况下测试化合物。该设计在筛选级联的早期消除毒性或代谢活性的化合物（在初级筛选的水平）。还原的甲臆首先在胞内囊泡中可见。最终的甲臆胞吐经 Abeta 寡聚体加速。图 1A 和 1B 是神经元显微图的实例，第一个是首先看到甲臆的胞内囊泡，第二个是覆盖有经胞吐排出的不溶紫色染料的神经元。染料沉淀在培养的水性环境中并在神经元的表面上形成针状结晶。

[0875] 在 15 微摩尔化合物 II 的存在下，图 1B 中采集的膜运输变化被阻断（参见 1C）且图 1C 中的细胞无法与媒介物处理的神经元区分开。此外，化合物 II 的该作用看似与化合物 II 是在细胞暴露于 Abeta 寡聚体之前还是之后添加无关，这表明治疗以及预防效果。参见图 1D，膜运输变化的图（剂量响应曲线）表达为，在添加各种量的化合物 II 或 IXa 与 IXb 的混合物之前（图 1D）或之后（图 1E）添加的各种量的 Abeta 寡聚体的存在下，在图像处理中见到的囊泡百分比相对于化合物 II 对数浓度。单独的 Abeta 寡聚体由图 1D 和 1E 左下角的圆圈表示。单独的囊泡由实心方块表示。当在寡聚体之前添加时（预防模式），化合物 II 以 EC_{50} = 2.2uM 阻断寡聚体作用，且化合物 IXa、IXb 以 EC_{50} = 4.9uM 阻断寡聚体作用。当在寡聚体之后添加时（治疗模式），化合物 II 以 EC_{50} = 4.1uM 阻断寡聚体作用，且化合物 IXa、IXb 以 EC_{50} = 2.0uM 阻断寡聚体作用。在任一情况中，化合物 II 或 IXa 与 IXb 的混合物各自阻断 Abeta 寡聚体的膜运输作用，如该检定中所见。递增剂量的来自两个结构不同系列（II 和 IXa、IXb）的选择性、高亲和性 σ-2 受体拮抗剂化合物阻止寡聚体作用并使得培养物看上去更像媒介物处理的培养物。

[0876] 基于这些结果，如本文公开的可有效用于抑制 Abeta 寡聚体毒性的选择性、高亲和性 σ-2 受体拮抗剂化合物，作为 β 淀粉样蛋白寡聚体毒性相关的认知衰退（例如在阿尔茨海默病中见到的）的治疗（非常早的阶段）和预防方式是有前景的。与 Abeta 寡聚体的可饱和的竞争性结合不能在这些实验中表明，因为毒性限制更高浓度。

[0877] 在膜运输检定中进行合成的 Abeta 寡聚体的给药，如图 1F 和 1G 所示，其中其表现出 820nM 的 EC50。针对数个浓度的各个选择性、高亲和性 σ-2 受体拮抗剂化合物药物候选物 II 和 IXa、IX（其各自引起 EC_{50} 右移几乎两个数量级），测试各个浓度的 Abeta。当将数据拟合到经典的线性和非线性模型时，数据对于 Schild 分析为线性（斜率 nH 为 1），其表明 σ-2 受体化合物在寡聚体与化合物之间对介导膜运输的靶标表现出真实药理学竞争。得自阿尔茨海默病患者脑的 Abeta 寡聚体针对这些化合物进行给药，如图 1J 和 1K 所示，也通过化合物暴露而显示出右移。具体而言，以有效剂量，化合物 II 和 IXa、IXb 表现出与合成的（图 1F、G，Schild 斜率 = -0.75、-0.51）和得自人阿尔茨海默病患者（图 1J、1K）的寡聚体的药理学竞争。其净效果为这两种选择性、高亲和性 σ-2 受体拮抗剂化合物候选药物有效地使 Abeta 寡聚体具有更少的突触毒性，且这些是目前为止我们所知的已证实该特性的仅有的疗法。不拘泥于理论，作用的最简单可能的机制是 σ-2 受体化合物起到竞争性受体拮抗剂的作用。

[0878] 在相关实验中，基于 0 或 20 μM 的化合物 II 对映异构体的效果观察到剂量响应曲线中的右移（%囊泡针对 Abeta 寡聚体浓度）：参见以下表 4。(+) 对映异构体被示出为在

较高浓度 Abeta 寡聚体下更有效。

[0879] 表 4

[0880]

化合物	针对 0 uM 浓度 Abeta 的 EC50	针对 20 μM 浓度 Abeta 的 EC50	曲线移动（倍）	使用单一浓度 Abeta 寡聚体的筛选检定中的 EC50
Cpd II 的(+)对映异构体	1.19	1.46	2.86	5.6 uM
Cpd II 的(-)对映异构体	1.05	2.82	1.22	10.9 uM

[0881] 如以上表 4 所示，20 μ M 对映异构体相对于 0 μ M 对映异构体（即单独 Abeta 寡聚体）在％囊泡针对 Abeta 寡聚体浓度的剂量响应曲线中的右移，在较高浓度的 Abeta 寡聚体下对于 (+) 对映异构体显著地更明显。

[0882] 实验对照：

[0883] 根据公开的方法制备的 Abeta1-42 寡聚体用作阳性对照。[参见，例如 Dahlgren 等 人，"Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability"J Biol Chem. 2002Aug30 ;277(35):32046-53. Epub2002Jun10. ;LeVine H3rd. "Alzheimer's beta-peptide oligomer formation at physiologic concentrations"Anal Biochem. 2004Dec 1 ;335(1):81-90 ;Shrestha 等 人，"Amyloid beta peptide adversely affects spine number and motility in hippocampal neurons"Mol Cell Neurosci. 2006Nov ;33(3):274-82. Epub2006Sep8 ; Puzzo 等 人，"Amyloid-beta peptide inhibits activation of the nitric oxide/cGMP/cAMP-responsive element-binding protein pathway during hippocampal synaptic plasticity"J Neurosci. 2005Jul20 ;25(29):6887-97 ;Barghorn 等人，"Globular amyloid beta-peptide oligomer-a homogenous and stable neuropathological protein in Alzheimer's disease"J Neurochem. 2005Nov ;95(3):834-47. Epub2005Aug31 ;Johansson 等 人，Physiochemical characterization of the Alzheimer's disease-related peptides A beta1-42Arctic and A beta 1-42wt. FEBS J. 2006Jun ;273(12):2618-30] 以及得自脑的 Abeta 寡聚体（参见，例如 Walsh 等人，Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature(2002). 416, 535-539 ;Lesne 等 人，A specific amyloid-beta protein assembly in the brain impairs memory. Nature. 2006Mar 16 ;440(7082):352-7 ;Shankar 等人，Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med. 2008Aug ;14(8):837-42. Epub2008Jun22）。应该注意到，任何 Abeta 寡聚体制剂可以用在该检定中或作为对照，包括以上引用并全部并入

以作参考的专利文献中记载的制剂。

[0884] 多种不同的 Abeta 寡聚体制剂被证实引起膜运输检定中的 Abeta 作用，包括尤其是从阿尔茨海默病患者的脑中分离的寡聚体制剂。

[0885] 寡聚体从死后人海马或前额皮质中分离而使用用清洁剂，并以剂量依赖的方式以 6 皮摩尔的 Kd 抑制膜运输。与媒介物（第一条，图 1J）相比，得自人阿尔茨海默病患者的 Abeta 寡聚体（137pM，图 1J 中的第二条）产生统计学上显著的膜运输抑制。化合物 II（第三条）消除由得自 AD 脑的 Abeta 寡聚体诱导的膜运输缺陷，但是当不存在 Abeta 而给药时不影响运输（第四阴影 (hatched) 条）。对 3 次实验 (n = 3) 的数据取平均值。

[0886] 尽管各种 Abeta 寡聚体制剂的效力不同（例如，天然阿尔茨海默病分离物比任何所测试的合成制剂更有效力 - 数据未示出），但结果在性质上相同：由寡聚体介导的病理被本发明包括 σ -2 受体拮抗剂化合物的组合物所对抗。

[0887] 在黑条所示的过量化合物 II 的存在下 (15uM，第三条，图 1J)，完全消除寡聚体诱导的膜运输缺陷。当其自身进行给药时，化合物 II 对膜运输没有显著作用（黑色斜条，图 1J）。

[0888] 相反，从取自认知正常的年龄匹配的个体的同一死后脑区分离的寡聚体通常相对于每克组织重量以较低的浓度存在，相对于 137pM 的 90pM（图 1K，第二条），并且相对于媒介物（图 1K，第一条）不产生显著的膜运输缺陷。在这些条件下，化合物 II 在与寡聚体一起给药或单独给药时没有作用（图 1K，分别为第三和第四条）。对数据也取平均值 (n = 3，除了其中 n = 5 的第二条）。

[0889] 阴性对照包括媒介物处理的神经元以及用超生理的 28 μ M 浓度的美金刚处理的神经元。美金刚在该剂量产生 50％的寡聚体作用抑制。各给板上的这些对照用作为标准化工具，以校准逐个板的检定表现。

[0890] 初级神经元培养物

[0891] 基于对 Abeta 寡聚体的细胞应答（使用胞吐检定作为读出）、以及培养物中胶质细胞与神经元的相对比例的免疫组化分析来确定最佳细胞密度。用免疫组化和基于图像处理的定量来每周监测培养物，以监测神经元相对于胶质细胞的培养物百分比。排除在 21 天的体外筛选年龄 (21DIV) 含有多余 20％的胶质细胞（对于 GFAP 为阳性）相对于神经元（针对 MAP2 用 1:5000（浓度可变）（鸡多克隆）抗体 (Millipore) 阳性染色）的培养物。

[0892] Abeta 寡聚体制剂

[0893] 从大量商业供应商例如 California Pepride 获得人淀粉样蛋白肽 1 ～ 42，视质量控制分析而定具有大量选择。寡聚体制剂的质量控制由确定寡聚体尺寸范围和相对浓度的蛋白印迹以及证实胞吐加速而无毒性的 MTT 检定构成。在各个基于图像的检定中经由使用 DNA 结合蓝色染料 DAPI (Invitrogen) 可视化的核形态的定量而监测毒性。片段化的细胞核被认为处于晚期细胞凋亡 (Majno and Joris '95)，且测试可以被排除。产生不寻常的肽尺寸范围或在标准 1.5 μ M 浓度对神经元产生显著毒性的肽群 (lots) 也将被排除。

[0894] 基于板的对照 - 当重新格式化的板在常规基础上在媒介物与 Abeta 寡聚体处理的神经元之间实现统计学显著的两倍分离的最小值 ($p<0.01$，学生 t 检验，不等方差），且各板间不多于 10％ CV 时，检定优化被视为完成。

[0895] 统计软件和分析：

[0896] 通过 Cellomics VTI 图像分析软件和 STORE 自动化数据库软件完成数据处理和分析。因为在培养三周后较低的动态范围和神经元孔间的可变性，经成对 Tukey-Kramer 分析进行统计表学比较，以确定化合物 +Abeta 寡聚体与单独的 Abeta 之间以及单独的化合物与媒介物之间的分离的显著性。成熟初级神经元更密切地接近于电生理介导的成人脑信号传导网络的能力证实该筛选策略合理。对于使假阴性最小化的大量重复筛选孔（例如，N = 4）设定检定力分析 (power analysis)。本发明的测试化合物显著逆转 Abeta 寡聚体对膜运输的作用且不影响神经元代谢本身。

[0897] 所选择的本发明通式内的化合物，包括以下表 5 中示出的式 III ～ IV、VIIIq 和 VIIIo，在本文所述的 MTT 检定中在 Abeta 寡聚体添加前给药，并显示出以所指示的 EC_{50} 阻断 Abeta 寡聚体诱导的膜运输缺陷。具体而言，这些结果表明，化合物以微摩尔浓度阻断 / 减轻 Abeta 寡聚体对神经元细胞的膜运输的活性 / 作用。

[0898] 表 5. σ -2 受体配体和抑制淀粉样蛋白寡聚体对膜运输的作用的能力

[0899]

σ-2 受体配体	通过膜运输检定测量的抑制细胞中β淀粉样蛋白作用的 EC_{50}	Abeta 的最大抑制（%）
化合物 II（三个不同批次：外消旋混合物、(+)异构体和(-)异构体）	2.2 uM 5.6 uM 10.9 uM	68 （78 （64）
化合物 A	3.4 uM	78
化合物 B	5.5 uM	84
化合物 C	5.4 uM	93
化合物 D	8.9 uM	58
化合物 E	8.2 uM	68
化合物 F	2.6 uM	69
化合物 G	5.8 uM	92
化合物 H	2.2 uM	-
化合物 I	3.4 uM	100
化合物 J	3.9 uM	97
化合物 K	14 uM	13
化合物 L	2.4 uM	34
化合物 M	0.6 uM	60
化合物 N	5.2 uM	46
化合物 O	2.7 uM	27
化合物 P	20.0 uM （19.5 uM）	43 （73）
化合物 Q	0.5 uM	82
化合物 R	6.7 uM	55
化合物 R'	39 uM（无活性）	38
化合物 S	5.4 uM	100

[0900]

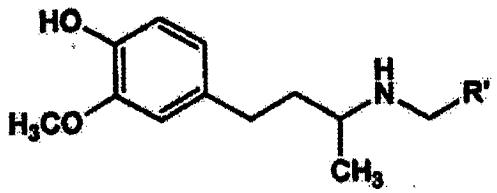
化合物 S'	>30 uM（无活性）	0
化合物 T	7.7 uM	45
化合物 IXa	4.9 uM	76
化合物 IXb	6.9 uM	97
化合物 AC	2.4 uM	-
化合物 AD	0.7 uM	-
化合物 AG	6.1 uM	-
化合物 BA	<1.0 uM	-
化合物 BT	0.4 uM	-
化合物 BY	0.8 uM	-
化合物 CA	1.9 uM	-
化合物 CB	18.2 uM	-
化合物 CR	1 uM	-
化合物 CS	6.9 uM	-
化合物 CT	3 uM	-
化合物 CW	2.5 uM	-
化合物 CX	1.3 uM	-
化合物 CY	14 uM	-
化合物 DE	>20.0 uM	-
化合物	通过膜运输检定测量的抑制细胞中β淀粉样蛋白作用的 EC_{50}	Abeta 的最大抑制（%）
BD1047	11	100
DTG	N/A	23
氟哌啶醇	6.2	100
艾芬地尔	1.3	38
Mach-14	1	80
NE 100	9.1	98
PB 28	2.2	84
PRE-84	N/A	38
SM-21	11	65
苏-艾芬地尔	N/A	39

[0901] 表 5 中的化合物显示为以所示的 EC_{50} 阻断 Abeta 寡聚体诱导的胞吐加速。因此，表 5 中的化合物显著地阻断 Abeta 寡聚体介导的膜运输变化。这些结果表明，化合物阻断 / 减轻 Abeta 寡聚体对神经元细胞的活性 / 作用，且 σ-2 配体可以用来阻断 Abeta 寡聚体诱导的膜运输异常。

[0902] 表 6A 显示出对于某些其他化合物的膜运输 EC_{50}。

[0903] 表 6A :其他膜运输数据

[0904]



[0905]

条目	R'	膜运输 EC_{50}(μM)
1	4-CF3-Ph-	2.2
2	4-Cl-Ph-	12
3	Ph-	20
4	isoBu-	>30
5	H-	30

[0906] 运输检定与 σ 受体结合数据之间的相关性

[0907] 使用 Spotfire 软件来分析化合物与 σ-1 或 σ-2 受体的结合亲和性(表 2)以及它们在膜运输检定中的 EC_{50} 和最大作用(表 5),以发现受体结合与检定活性之间的相关性。计算 MTTX 中 EC_{50} 的对数相对于对数 σ-1 和 σ-2 结合 Ki、Abeta 的最大抑制相对于对数 σ-1 和 σ-2 结合 Ki、以及运输检定中对数 EC_{50} 相对于 σ-1 结合 Ki 与 σ-2 结合 Ki 的比率的拟合优度。对于 4 组化合物进行所有计算 :A) 表 5 中所列的粗体化合物 + 已知的现有技术化合物,B) 仅表 5 中的粗体化合物,C) 仅表 5 中的参照化合物,以及 D) 除 σ-1 拮抗剂(NE-100、氟哌啶醇、BD1047、SM21)以外的所有 A 组化合物。

[0908] 表 6B :σ 结合亲和性与运输检定活性之间的相关性

[0909]

Y 轴	X 轴	R^2(P 值)所有 19 种化合物	R^2(P 值)表 5 粗体化合物	R^2(P 值)10 种参照化合物	R^2(P 值)W/O σ-1 拮抗剂

[0910]

MTTX log(EC 50)	Vs Log S1 Ki	0.06 (ns)	0.01 (ns)	0.02 (ns)	0.02 (ns)
	Vs Log S2 Ki	0.15 (ns)	0.70 (< 0.001)	0.27 (ns)	0.78 (< 0.001)
Abeta 的最大抑制	Vs Log S1 Ki	0.00 (ns)	0.14 (ns)	0.06 (ns)	0.00 (ns)
	Vs Log S2 Ki	0.00 (ns)	0.00 (ns)	0.00 (ns)	0.00 (ns)
MTTX log(EC 50)	S1/S2	0.01 (ns)	0.11 (ns)	0.04 (ns)	0.01 (ns)

[0911] 从表 6B 中可以看出，单独粗体化合物的最高相关性在其 MTTX 中的 Log(EC_{50}) 与 σ-2 结合的 Log(Ki) 之间 (R^2 = 0.70, P<0.001)。其他比较均为统计学不显著。当将参照化合物添加到分析中时，该相同的相关性统计学不显著 (R^2 = 0.15, P>0.05)，且单独的参照化合物在这些参数之间不显示统计学显著的相关性 (R^2 = 0.27, P>0.05)。

[0912] 该相关性的不同表现的图还显示在图 5A ～ 5D 中。

[0913] 在已知的现有技术化合物中，Mach-14 对于 σ-2 受体具有高选择性 (σ-1Ki = 12,900nM, σ-2Ki = 9nM)，且其抑制运输检定中 80％的 Abeta 作用。相反，PRE-84 对于 σ-1 受体具有高特异性 (σ-1Ki = 2.2nM, σ-2Ki = 13,091nM)，且在运输检定中为较差的抑制剂 (最大抑制 38％)。该结果如下理论一致，即 σ-2 受体结合而非 σ-1 受体结合与逆转 Abeta 在运输检定中的作用相关。该检定的结果还对治疗表型的研发提供支持。

[0914] 当对表 5 中的粗体化合物以及已知的现有技术化合物 PB28、氟哌啶醇和 Mach14 进行作图时 (图 5A)，在 σ-2 结合亲和性与运输检定中的效力之间具有强相关性 (R^2 = 0.79, P<0.001)。相比之下，在 σ-1 受体结合与 MTTX 检定中的效力之间不存在显著相关性 (R^2 = 0.02, P = 0.18) (图 5C)。该结果显示出结合 σ-2 受体与抑制运输中 Abeta 作用之间的较强关系，且看似表明结合 σ-1 受体与抑制 Abeta 之间的较弱关系。

[0915] 三种其他已知的现有技术化合物 (BD1047、NE100 和 SM-21) 在膜运输 MTTX 检定中相比于可归因于它们单独的 σ-2 结合亲和性的效力更有效力。氟哌啶醇、PB28 和 Mach-14 表明了 σ-2 结合与运输检定中的效力之间的紧密相关性。

[0916] PRE-084 在运输检定中无活性，且这与其是有效的 σ-1 激动剂而在 σ-2 受体处没有效力这一观察一致。

[0917] 两种粗体化合物 R' 和 S' 在运输检定中无活性，尽管它们对 σ-2 受体具有较大的亲和性。在一些实施方式中，化合物 R' 和 S' 不满足治疗分布。

[0918] 此外，化合物 IXa 和 IXb 的混合物以 5.2μM+/-1.1 的可重现 EC_{50} 协同地抑制 100% 的寡聚体对膜运输的作用（图 7）。相似地，其他化合物将在该实施例报道的检定中进行测试。这些也将选自式 I、II、III ～ VII 和 IX 以及由其他通式（例如式 XIII）包括的化合物和描述为以上 σ-2 配体的其他化合物。

[0919] 表 2 中所选的化合物在膜运输检定中进行给药，并显示为以所示的 EC_{50} 阻断 Abeta 寡聚体诱导的膜运输异常。因此，表 2 中的化合物显著地阻断 Abeta 寡聚体介导的膜运输变化。这些结果表明，化合物阻断 / 减轻 Abeta 寡聚体对神经元细胞的活性 / 作用，且 σ-2 受体配体可以用作阻断 Abeta 寡聚体诱导的膜运输异常的候选化合物。因为由以上通式包括的化合物也预期为 σ-2 配体，因此也可用于阻断 Abeta 寡聚体诱导的胞吐加速。

[0920] 实施例 7. 药代动力学和代谢稳定性研究

[0921] 通过商业合同研究组织在小鼠的微粒体中进行第一个药代动力学研究。该研究根据 Obach, R. S 等人 (1997) J. Pharmacol. Exp. Ther., 283:46-58 来进行，其引入本文以供参考。表 7A 中所测试的化合物的半衰期为 2 ～ 72 分钟，且剩余化合物的半衰期预期在大约相同的范围内。

[0922] 微粒体中半衰期的结果显示在表 7A 和 7B 中。

[0923] 表 7A：化合物小鼠微粒体稳定性

[0924]

化合物	小鼠微粒体中 t1/2
II	16
A	33
B	55
C	10
D	2
E	46
F	72
G	42
H	24
I	33
J	47

[0925] 结果表明，一些测试化合物在小鼠肝脏微粒体中比化合物 II 具有实质上更长的

半衰期。该结果预示这些化合物后在口服后更大的生物利用度。相同的化合物已通过上述的膜运输检定进行测试，以及其本文所指的活性。

[0926] 化合物 II 的内在清除的速度较为快速，表明大量的首过代谢。为改善药代动力学性质，设计其它化合物以提高代谢稳定性并改善药物样特性。进行微粒体稳定性实验和血浆稳定性实验以确定候选化合物的代谢和肝脏稳定性。

[0927] 第二个 PK 研究在体内进行并涉及测量经多种途径以急性或慢性方式施用的测试化合物的血浆水平和脑水平，具体如下：

[0928] HPLC-MS 优化

[0929] 制备各个测试化合物的溶液并经注射泵以恒定速率输入到 TSQ Quantum 光谱仪 (Fisher Thermo Scientific) 源中。进行全扫描 MS（质谱）分析，并对于各个测试化合物以正离子化和负电离子化模式产生总离子流色谱图和相应的质谱。MS/MS 的前体离子选自正质谱和负质谱，作为各离子丰度的函数。此外，进行产物离子 MS/MS 分析，以确定用于定量分析的合适选择的裂解反应。选择最终反应监测参数，以使对存在于复杂的组分混合物中的测试化合物进行定量的能力最大化。在识别出待用于各个测试化合物的特定 SRM 跃迁之后，使用 TSQ Quantum 化合物优化工作区中的自动操作指南来优化检测参数。最后，通过在合适的 LC 柱中注射并分离分析物并按需调整梯度条件而识别用于 LC-MS 分析的色谱条件。

[0930] 用于 IV 给药的配方

[0931] 首先通过视觉检查来评价测试化合物在磷酸盐缓冲盐水 pH7.4(PBS) 中的溶解度。如果化合物在目标浓度可溶，则使用 PBS 作为媒介物。（如果化合物在 PBS 中不完全溶解，则可以评价与 IV 给药相容的其他媒介物。这样的媒介物除其它之外包括 DMSO、聚乙二醇 (PEG400)、Solutol HS15 和 Cremophor EL。）在此处报道的实验中，IV 施用单次推注的 10mg/kg 化合物 II。

[0932] 用于 PO 给药的配方：首先评价测试化合物在 PBS 中的溶解度。如果化合物在目标浓度可溶，则使用 PBS 作为媒介物。（如果测试化合物在各个浓度在 PBS 中不完全溶解，则可以使用 DMSO/Solutol HS15/PBS(5/5/90，v/v/v) 或 DMSO/1％甲基纤维素 (5/95，v/v)。）

[0933] 血浆中的线性

[0934] 在等份的血浆中掺入 (spiked) 指定浓度的测试化合物。使用乙腈沉淀处理所掺的样品，并通过 HPLC-MS 或 HPLC-MS/MS 分析。构建峰面积相对于浓度的校准曲线。确定该检定的可报告的线性范围，以及定量下限 (LLQ)。

[0935] 血浆样品的定量生物分析

[0936] 使用乙腈沉淀处理血浆样品，并通过 HPLC-MS 或 HPLC-MS/MS 分析。生成血浆校准曲线。在等份的无药血浆中掺入指定浓度水平的测试化合物。将所掺的血浆样品与未知的血浆样品一起使用相同的步骤进行处理。处理的血浆样品（干燥提取物）在 HPLC-MS 或 HPLC-MS/MS 分析之前通常冷冻储存 (-20℃)。将干燥提取物重构到合适的溶剂中且在离心后通过 HPLC-MS 或 HPLC-MS/MS 分析。记录峰面积，且使用各校准曲线确定未知血浆样品中测试化合物的浓度。确定该检定的可报告的线性范围，以及定量下限 (LLQ)。

[0937] 用于研究的动物是各自称重为 20-30g 的雄性 C57BL/6 小鼠或称重为 180-250g 的雄性 Sprague-Dawley 大鼠。针对各个施用条件和各个时间点处理三只动物，使得各个动物仅抽血一次。通过腹膜内注射完成皮下化合物施用。通过灌胃完成口服。经颈静脉导管完

成静脉内施用。

[0938] 在施用各种浓度的化合物后，在 10、30、60、120、240、360、480 和 1440min 收集血浆样品。

[0939] 从小鼠和大鼠收集血浆样品

[0940] 在一般吸入麻醉（3%异氟烷）下使动物镇静，以通过心脏穿刺（小鼠）或颈静脉导管（大鼠）收集血液。将血液等份（300 ～ 400μL）收集在涂布有肝素锂的管中，温和混合，之后保持在冰上并在 4℃于 2,500x g 离心 15 分钟，在 1 小时内收集。之后收获血浆并在 -20℃保持冷冻直至进一步处理。

[0941] 动物给药设计 - 体内 PK- 非插管、非禁食动物

[0942] 组 1 :SC，每个时间点 n ＝ 3 只动物（共 24 只动物）或

[0943] IV，每个时间点 n ＝ 3 只动物（共 24 只动物）

[0944] 组 2 :PO，每个时间点 n ＝ 3 只动物（共 24 只动物）

[0945] 组 3 :对照动物（用于无药血液），n ＝ 5 只小鼠

[0946] 各个动物进行一次抽血和一次脑收集。

[0947] 从动物收集脑样品

[0948] 紧接着血液取样，将动物断头，并快速取出整个脑，用冷盐水（0.9% NaCl，g/mL）清洗，使表面脉管系统破裂，用纱布擦干，称重，保持于冰上直到进一步处理，在一小时内收集。使各个脑在冰上使用 Power Gen125 在 1.5mL 冷磷酸盐缓冲盐水 pH7.4（小鼠＝ 1.5mL，大鼠＝）中匀浆 10 秒钟。然后将来自各个脑的脑匀浆储存在 -20℃直到进一步处理。

[0949] 脑样品中的线性

[0950] 在等份的脑匀浆中掺入指定浓度的测试化合物。向各个脑等份中，添加等体积的冷 26%（g/mL）中性右旋糖（来自 Sigma，平均分子量 65,000-85,000，目录号 D-1390）溶液以得到 13%的最终右旋糖浓度。将匀浆在 4℃以 54000x g 离心 15 分钟。随后使用乙腈沉淀处理上清液并通过 HPLC-MS/MS 分析。构建峰面积相对于浓度的校准曲线。确定该检定的可报告的线性范围，以及定量下限（LLQ）。

[0951] 脑样品的定量分析

[0952] 向各个脑匀浆等份中，添加等体积的冷 26%（g/mL）中性右旋糖（来自 Sigma，平均分子量 65,000-85,000，目录号 D-1390）溶液以得到 13%的最终右旋糖浓度。将匀浆在 4℃以 54000x g 离心 15 分钟。随后使用乙腈沉淀处理上清液并通过 HPLC-MS/MS 分析。生成脑校准曲线。在等份无药脑匀浆中掺入指定浓度水平的测试化合物。将所掺的脑匀浆样品与未知的脑匀浆样品一起使用相同的步骤进行处理。将处理的脑样品在 -20℃储存直到 LC-MS/MS 分析，分析时记录峰面积，并使用各校准曲线确定未知脑样品中的测试化合物的浓度。确定该检定的可报告的线性范围，以及定量下限（LLQ）。

[0953] 脑渗透性

[0954] 通过 LC-MS/MS 确定测试化合物在脑中（ng/g 组织）和血浆中（ng/mL）的浓度以及各个时间点脑浓度与血浆浓度的比率，并如上所述进行报告。

[0955] 药代动力学

[0956] 构建化合物的血浆浓度相对于时间的图。口腔和 SC 给药后化合物的基本药代动力学参数（AUClast、AUCINF、Tl/2、Tmax 和 Cmax）得自使用 WinNonlin(Pharsight) 的

血浆数据的非房室分析 (NCA)。非房室分析不需要假定用于药物或代谢物的特定的房室模型。NCA 允许应用梯形法则来测量血浆浓度 - 时间曲线下的面积 (Gabrielsson, J. and Weiner, D. Pharmacokinetic and Pharmacodynamic Data Analysis:Concepts and Applications. Swedish Pharmaceutical Press. 1997)。

[0957] 报告项的定义

[0958] 曲线下面积 (AUC)- 达到全身循环的未变化的药物的总量的量度。曲线下面积是几何测量，其通过绘制浓度相对于时间的图并对各个梯形的增量面积求和来计算。

[0959] WinNonlin 具有两种用于计算该面积的方法 :线性梯形法和线性对数梯形法。因为线性梯形法对浓度 - 时间曲线的下降部分可能提供有偏结果并高估 AUC，因此 WinNonlin 对 AUC 的计算提供线性对数选项。默认使用对数线性梯形法来测量 Tmax 后剩余血浆浓度 - 时间曲线的面积。

[0960] AUC_{last} :大于定量限的从给药时间至最后观察时间的曲线下面积。

[0961] AUC_{INF} :从给药时间外推到无穷大的曲线下面积。

[0962] C_{max}- 在给药时间与最后观察时间点之间口服或非 IV 施用药物后的最大血浆药物浓度。

[0963] T_{max}- 在施用药物后观察的最大血浆浓度 (Cmax) 处以分钟计的时间。

[0964] $T_{1/2}$-IV 及非 IV 给药的末端消除半衰期。

[0965] 其中 λ Z(z) 是与血浆浓度 - 时间曲线的末端 (对数线性) 部分相关的一级速率常数。Z 通过时间相对于对数浓度的线性回归而估计。

[0966] 结果显示，当以范围为 0.1 ～ 0.5mg/kg 的剂量急性或慢性 (在 5 天内每天) 施用时，所测试的化合物 II 和 B 生物利用度高且脑渗透性高。急性施用化合物 II 的结果显示于图 2A 中。图 2A 是其中化合物的血浆水平以 ng/mL 为单位显示于左边 y 轴的图。脑水平以绿色以 ng/g 为单位显示于右边 y 轴。X 轴显示在零时推注 IV 或 SC 施用之后的时间。在以 10mg/kg i. v. 急性 IV 施用之后，化合物 II 达到高脑浓度且在给药后 180 分钟仍具有 171ng/g 的浓度 (57x 体内有效脑剂量，由空心菱形显示)。在急性 SC 施用之后为相似的模式。化合物 B 在肠胃外施用时显示相同水平的生物利用度，但通过口腔途径生物利用度显著更大。发现两种所测试的化合物均具有明显较高的 BBB 渗透性，且化合物 II 在 3 小时具有 8 的脑 / 血浆比率。

[0967] 化合物 B 的结果和化合物 II 急性施用的结果显示在表 8 中。

[0968] 表 8. 化合物 B 和 II 在小鼠中的药代动力学

[0969]

参数	化合物 B-10 mg/kg		化合物 II-10 mg/kg	
	PO 血浆	SC 血浆	PO 血浆	SC 血浆
T_{max}（min）	30	120	NC	30
C_{max}（ng/mL）	599.3	607.7	NC	279
$t_{1/2}$（min）	210	218	NC	100
AUC_{all} ((ng*hr)/mL)	2,851.1	5,242.3	NC	384.7
F_{rel}（%）	54.4		NC	

[0970] 如该表中所示，化合物 B 的口服生物利用度及它的其它 PK 参数相对于化合物 II 有改善。

[0971] 在 5 天的时程内慢性施用，0.1、0.35 和 0.5mg/kg 范围内的给药提供化合物 II 相对稳定的血浆水平，脑暴露较好且脑 / 血浆比率与急性设置相似。结果显示于图 2B 中。图 2B 是在每天一次皮下施用不同量的化合物 II（0.5mg/kg/ 天：实心下三角；0.35mg/kg/ 天：实心上三角；以及 0.1mg/ 天：实心方块）时在血浆（左纵坐标）中以及在 SC 施用相同量（各自是空心下三角、空心上三角和空心方块）的化合物 II 时在脑中获得的药代动力学数据的图。

[0972] 化合物 B 的脑水平测量比化合物 II 显示出更低的峰浓度，但处于更高的持续水平。在三小时点处，脑中化合物 II 的量相比于针对化合物 II 确定的 50ng/mL 有效剂量，对 PO 几乎高 40x 且对 SC 高 60x。然而，化合物 II 的三小时点相比于该化合物的有效剂量对于 SC 仍高 >3x（数据未显示）。

[0973] 当在小鼠中以 1mg/kg 静脉给药时，化合物 IXa 和 IXb 的混合物显示出 2.7 小时的半衰期。然而，当化合物以 5mg/kg 口服给药时，其在血浆中显示出可忽略量的药物。对化合物 IXa 和 IXb 的混合物 + 药物标准物在小鼠肝脏微粒体中随后的研究（表 6）测量混合物的半衰期为 8.7 分钟且内在清除率为 267microL/min/mg，表明混合物易受首过代谢影响。通常，CNS 活性化合物倾向于具有低至中度的内在清除率（CL_{int} ≤ 100 微升 /min/mg）(Wager 等人'10)。13 个 CNS 活性药物的人肝脏微粒体稳定性数据提供 51±29 分钟的平均半衰期 (Orbach'99)。因此，化合物 II 首过代谢的改善的合理目标是 $T_{1/2}$>30 分钟且 CL_{int} ≤ 100 微升 /min/mg。化合物 IXa、IXb 相比于某些已知的药物表现出可比的肝脏稳定性，如表 9 中所示。

[0974] 表 9. 对于化合物 IXa 和 IXb 的小鼠肝脏微粒体数据并选择 CNS 药物标准物。CL' int = 内在清除率。通过合同研究组织进行实验，且以化合物 II 作为标准物进行。

[0975]

药物	作用模式	微粒体浓度（mg/mL）	T1/2（min）	CL' INT（uL/min/mg）
化合物 IXa 和 IXb 的混合物	候选化合物	0.3	8.7+/-0.1	267
丙咪嗪	抗抑郁	0.3	11.5	200

[0976]

普萘洛尔	β阻断剂	0.3	16.4+/-0.5	141
特非那定	抗组胺	14.6	8.7+/-1.1	159
维拉帕米	Ca++通道阻断剂	0.3	11.4+/-0.5	204

[0977] 实施例 8 :Abeta1 ～ 42 寡聚体结合和突触损失检定

[0978] 在该检定中，使 Abeta 寡聚体与培养中的成熟初级神经元接触，且通过免疫组化（Abeta 抗体）确定其结合并通过图像处理进行定量。神经元树突中的 Abeta 的量通过对神经炎上标记点的数目进行计数来评价。已知 Abeta 寡聚体与初代培养中以海马神经元显著百分数（30 ～ 50％）存在的突触后神经元的子集饱和（Kd 约 400nM ;Laurén2009）且高亲和性地结合（Lacor 等人，2004 ;Lambert 等人，2007），且这与阿尔茨海默病患者的脑中的 Abeta 结合的观察很好地相关（Lambert 等人，2007）。该标记与突触相关联，与突触后支架蛋白 PSD-95 共定位（Lacor 等人 '04）。还已知 Abeta 寡聚体介导突触损失（在脑切片的人海马神经元中报道为 18％（Schef 等人，2007））且抑制长时程增强效应（LTP）。突触数目还可以在该检定中通过免疫荧光化学进行定量。用于结合检定的相似步骤可在文献中找到。参见例如，Look GC 等人 Discovery of ADDL-targeting small molecule drugs for Alzheimer's disease. Curr Alzheimer Res. 2007Dec ;4(5) :562-7. Review。

[0979] 与神经元表面结合的 Abeta 量的测量可以用作识别经一种或多种以下机制起作用的化合物的第二筛选 :通过干扰 Abeta 寡聚体与神经元表面的结合或通过影响寡聚体自身的变化（反向激动或寡聚体解离）或寡聚体结合的表面受体的变化（变构调节或经典受体拮抗）来阻断 Abeta 作用。其还可以将这些化合物与在下游信号传导事件中起作用的化合物区分开。因此，该检定与特征为神经元上的 Abeta 寡聚体非致死作用的疾病状态相关，且形成本发明的发明人所采用的筛选级联的一部分，从而识别临床相关的化合物。重要地，本文公开的化合物之一即化合物 II 在膜运输检定且在该结合 / 突触损失检定中具有活性，且已被证明还在两种不同的阿尔茨海默病转基因模型中且在诱导的模型中也具有活性。因此，这以及膜运输检定可用于识别临床相关的化合物且似乎具有用于体内结果的预测值。该检定的预测有效性通过使用本发明范围外的化合物证实其预测化合物性质的能力来确认。

[0980] 初级海马神经元培养物如在以上膜运输检定中确立。添加化合物 II（以 10^{-8} ～ 30 微摩尔的浓度），且将任何其它待在将来测试的化合物（以 10^{-8} ～ 30 微摩尔的浓度）添加到板中，随后以达到饱和结合的浓度添加含有 Abeta1-42 寡聚体的制剂。用待测试的化合

物进行的预处理持续 1 小时，且以 70ul 的终浓度添加 Abeta 寡聚体或无寡聚体（单独的媒介物），之后孵育另外 23 小时。

[0981] 用 3.7％的多聚甲醛于磷酸盐缓冲盐水中的溶液将板固定 15min。然后用 PBS 洗涤板 3x，每次 5min。在室温在 PBS 中的 5％山羊血清和 0.5％ Triton X-100 中将板封闭 1 小时。将一抗 (MAP2 多克隆，Millipore#AB5622 和 β 淀粉样蛋白 6E10 单克隆，Covance#SIG-39300，以 1 微克 /ml，以及兔多克隆突触素抗体 Anaspe，以 0.2 微克 /ml) 在 PBS 中的 5％山羊血清中 1:1000 稀释。将一抗在 4℃孵育过夜。然后用 PBS 洗涤板 3x，每次 5min。将二抗 (Alex Flor488 多克隆，Invitrogen#A11008 和 Alexa Flor647 单克隆，Invitrogen#A21235) 在 PBS 中的 5％山羊血清中 1:1000 稀释。将二抗在室温孵育 1 小时。用 PBS 将板洗涤一次。然后以 0.03ug/ul 施用 DAPI(4',6- 二脒基 -2- 苯基吲哚，Invitrogen) 且将其在室温孵育 5min，然后用 PBS 洗涤。结果显示，如预期，如下详述制备且根据所使用的制剂以 3 或 1μM 给药的 Abeta 寡聚体与突触处的神经元结合，如由红色染料所显示。在具有早期阿尔茨海默病的人中，与年龄匹配的认知正常的个体相比，海马中的突触数目显示为减少 18％ (Scheff 等人 '07)，且该结果还可以在该检定中通过荧光点以及因此的突触数目的 20％消退而可视化。然而，在化合物 II(15uM) 的共存下，Abeta 结合基本上减少到对照水平，且绿色荧光未受影响，表明突触数目未减少。参见图 3A、3B、3C 和 3D。在图 3A- 板块 A 中，Abeta42 寡聚体与突触后棘结合，图 3A- 板块 B 显示突触前棘在初级神经元中经突触素标记 (21DIV)。图 3A- 板块 C 和 D 分别显示当已将 IXa、IXb 添加到培养物时处于基本上对照水平的突触后棘和突触。如在图 3C 的条形图中所定量显示，相比于单独的媒介物（第一条），单独添加的 Abeta42 寡聚体在 24 小时后（第四条）引起突触素点密度降低 20％（如计算）。该损失被化合物 II 或 IXa、IXb（第五或第六条）逆转，且该结果统计学显著。在不存在 Abeta 寡聚体的情况下，化合物 IXa、IXb 或化合物 II 均不影响突触数目（阴影条）且其保持于与对照（单独的媒介物）可比的水平。比例尺＝ 20um。$p<0.001$ANOVA。图 3D($p<0.001$ANOVA) 也是条形图且显示通过 Abeta 点计算的 Abeta 结合强度在化合物 II 或 IXa、IXb 的存在下减少 18％，而该降低足以允许突触计数在该化合物的存在下达到对照水平。

[0982] 此外，在以浓度依赖方式存在化合物 IXa 和 IXb 的混合物时，点状突触 Abeta 寡聚体结合减少 38％，IC_{50} 为 1.2μM（数据未示出）。点强度的条形图显示，正常双峰结合群（具有亮点的神经元和具有较不亮点的群）在药物存在下左移（数据未示出）。已经报道对 Abeta 寡聚体结合的部分抑制恢复 100％的 LTP 功能 (Strittmatter SM 等人，Cellular Prion Protein Mediates Impairment of Synaptic Plasticity by Amyloid-Beta Oligomers Nature(2009)457(7233:1128-32))。此外，如图 3C 所示，与媒介物处理（第一条）相比，Abeta 寡聚体（第四条）在 24 小时后引起突触素点密度降低 20％，其被 5μM 的化合物 IXa 和 IXb 的混合物逆转（第五条）。不存在 Abeta 时（第二条），化合物 IXa 和 IXb 的混合物不影响突触数目。Abeta 寡聚体引起突触数目降低 18.2％；该损失的 100％被 5μM 的化合物 IXa、IXb 或 II 消除（图 3C）。化合物 IXa 和 IXb 的混合物以 1.21uM 的 IC_{50} 引起 Abeta 标记点密度降低 17.7％（图 3D）。

[0983] 用 DAPI 可视化的细胞核表现出正常的形态，表明不存在神经退行。使用选自被式 I ～ IX 包括的那些、以及描述为以上 σ-2 配体的其他化合物的其他测试化合物重复该步

骤。

[0984] Abeta 寡聚体制剂：

[0985] 人淀粉样蛋白肽 1 ～ 42 得自 California Peptide，视质量控制分析而定具有大量选择。根据上述的公开方法来制备 Abetal ～ 42 寡聚体。[参见，例如 Dahlgren 等 人，"Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability"J Biol Chem. 2002Aug30 ;277(35):32046-53. Epub2002Jun10. ;LeVine H3rd. "Alzheimer's beta-peptide oligomer formation at physiologic concentrations"Anal Biochem. 2004Dec 1 ;335(1):81-90 ;Shrestha 等 人，"Amyloid beta peptide adversely affects spine number and motility in hippocampal neurons"Mol Cell Neurosci. 2006Nov ;33(3):274-82. Epub2006Sep8 ; Puzzo 等 人，"Amyloid-beta peptide inhibits activation of the nitric oxide/cGMP/cAMP-responsive element-binding protein pathway during hippocampal synaptic plasticity"J Neurosci. 2005Jul20 ;25(29):6887-97 ;Barghorn 等人，"Globular amyloid beta-peptide oligomer-a homogenous and stable neuropathological protein in Alzheimer's disease"J Neurochem. 2005Nov ;95(3):834-47. Epub2005Aug31 ;Johansson 等 人，Physiochemical characterization of the Alzheimer's disease-related peptides A betal-42Arctic and A beta 1-42wt. FEBS J. 2006Jun ;273(12):2618-30] 以及得自脑的 Abeta 寡聚体（参见，例如 Walsh 等人，Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature(2002). 416, 535-539 ;Lesne 等 人，A specific amyloid-beta protein assembly in the brain impairs memory. Nature. 2006Mar16 ;440(7082):352-7 ;Shankar 等人，Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med. 2008Aug ;14(8):837-42. Epub2008Jun22）。寡聚体制剂的质量控制由确定寡聚体尺寸范围和相对浓度的蛋白印迹以及证实胞吐加速而没有毒性的 MTT 检定构成。在各个基于图像的检定中经由使用 DNA 结合染料 DAPI(Invitrogen) 可视化的核形态的定量而监测毒性。片段化的细胞核被认为处于晚期细胞凋亡，且测试被排除 (Majno and Joris Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol1995 ;146:3-16)。产生不寻常的肽尺寸范围或在标准浓度对神经元产生显著毒性的肽群也将被排除。

[0986] 对照

[0987] 用寡聚体制剂对 Abeta 抗体 6E10 的预吸附以剂量依赖的方式 ($7.84x10^{-6}$) 抑制突触结合，并用作阳性对照。抗体以 1:1000(1 微克 /ml) 使用。对于突触损失检定，NMDA 拮抗剂地佐环平 (MK-801) 以 80uM 用作阳性对照。

[0988] 图像处理

[0989] 拍摄图像，并用 Cellomics VTI 自动显微平台使用神经元分布 (profiling) 算法进行分析。对于统计分析，使用不等方差的 Turkey-Kramer 成对比较。

[0990] 蛋白免疫印迹

[0991] 将含有 Abetal ～ 42 的样品在非还原示踪样品缓冲液 (Pierce#1859594) 中稀释 (1:5)。将 30 微升 (μL) 样品装载到十八个孔的预制 4 ～ 15% Tris-HCl 凝胶

(BIORAD#345-0028) 中。在BIO-RAD标准预制凝胶系统中使用Tris-甘氨酸缓冲液在125伏特 (V) 进行90分钟的电泳。在Tris-甘氨酸/10%甲醇缓冲液中,在30V,经120分钟将凝胶转印到0.2μM硝酸纤维素膜上。将膜在PBS溶液中煮5分钟并用TBS/5%牛奶溶液在4℃封闭过夜。将膜在TBS/1%牛奶溶液中用稀释成10μg/mL的6E10-HRP(Covance#SIG-39345)在室温杂交 (probed)1小时。将膜用TBS/0.05%吐温-20的溶液洗涤三次,每次40分钟,并用ECL试剂 (BIO-RAD#162-0112) 显色5分钟。在Alpha InnotechFluoChem Q定量成像系统中进行图像获取,并用Alpha View Q软件分析。

[0992] 活性

[0993] 示出化合物II,且预期选自本文具体公开的那些的化合物会显示出根据结合检定(使用成像处理算法)部分地阻断约25%的Abeta寡聚体配体与神经元的结合。

[0994] 实施例9:条件性恐惧检定

[0995] 在已知为条件性恐惧的记忆依赖的行为任务的动物模型中测试化合物II。基于所公开的方案来设计研究方案(参见,例如Puzzo D,Privitera L,Leznik E,Fa M,Staniszewski A,Palmeri A,Arancio O.Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus.J Neurosci.2008Dec31;28(53):14537-45)。情境记忆的形成取决于内侧颞叶结构例如海马的完整性。在该检定中,将小鼠训练成记住,特定的突出情境(条件性刺激,CS)与厌恶事件相关(在该情况下为轻度足部电击)(非条件性刺激,US)。示出较好学习的动物在放回到相同情境时会增加僵立行为。该僵立不存在于新情境中。情境中增加的僵立表明动物中较强的海马依赖的记忆形成。条件性恐惧中测试的记忆对可溶Aβ的升高较为敏感。化合物II可有效阻止Abeta寡聚体介导的对膜运输的作用。当在施用Abeta寡聚体前施用于动物时,化合物II以剂量依赖的方式阻断寡聚体对记忆的作用。化合物以2pmol的剂量完全阻断寡聚体介导的记忆缺陷。

[0996] 确实,如图4所示,化合物II完全消除Abeta寡聚体诱导的记忆缺陷(黑条)而当单独给药时不影响记忆(阴影条)。单独Abeta寡聚体的作用由红条示出。此外,如图6所示,化合物IXa和IXb的混合物提供相似的结果。该行为效力表明,膜运输检定能够预测哪些化合物可有效治疗由寡聚体引起的行为记忆丧失。如本文所述进行对于记忆的恐惧条件模型。在任何剂量均没有观察到不利的行为变化。因此,在该化合物在膜运输检定中的表现与其在条件性恐惧检定中的表现之间具有相关性,后者是记忆丧失的标识。预测表2中所列的化合物会在条件性恐惧检定中有活性,因此会显示出可有效治疗记忆丧失。化合物在恐惧条件模型中的表现与其在治疗记忆丧失中的有用性之间的相关性已经在文献中确定。(Delgado MR,Olsson A,Phelps EA."Extending animal models of fear conditioning to humans"Biol.Psychol.2006Jul;73(1):39-48)。

[0997] 实施例10.对大鼠、猕猴和人死后脑样品的放射自显影研究

[0998] 对于σ-2和σ-1受体配体的神经学和药理学分布的放射自显影成像研究通过改良先前由Xu等人,2010报道的方案来进行Xu,J.,Hassanzadeh B,Chu W,Tu Z,Vangveravong S,.Tones LA,Leudtke RR,Perlmutter JS,Mintun MA,Mach RH.[^{3}H]4-(Dimethylamino)-N-[4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl]benzamide,a selective radioligand for dopamine D(3)receptors.II.Quantitative analysis of dopamine D3and D2receptor

density ratio in the caudate-putamen. Synapse64:449-459(2010)，其并入本文以供参考。标记的 RHM-1 通过 Xu J, Tu Z, Jones LA, Wheeler KT, Mach RH. [^{3}H]N-[4-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1 H)-yl)butyl]-2-methoxy-5-methylbenzamide:a Novel Sigma-2Receptor Probe. Eur. J. Pharmacol. 525:8-17(2005) 的方法得到，其并入本文以供参考。

[0999] 对来自大鼠、猕猴和死后人脑的 20 μ M 厚的脑切片使用 Microm 冷冻切片机进行切片，并贴附在 superfrost plus 载玻片 (Fisher Scientific, Pittsburgh, PA) 上，并在该研究中使用经过大脑皮质和海马的脑区的连续切片。在 σ -1 受体阻断剂 (+)- 戊唑辛的存在下，将脑切片与 5nM[^{3}H](+)- 戊唑辛 (用于 σ -1 受体分布)、4nM[^{3}H]RHM-1(仅用于 σ -2 受体表征)、10nM[^{3}H]DTG 和 [^{3}H] 氟哌啶醇孵育，以对 σ -2 受体分布进行成像 ；在与放射性配体孵育 30 分钟后，将含有脑切片的载玻片用冰冷缓冲液清洗 5 次，每次一分钟。

[1000] 将载玻片干燥，并通过在自由侧涂覆铜箔带而使其导电，并将其放置在气体检测器 Beta Imager2000Z Digital Beta 成像系统 (Biospace, France) 的气体室 [氩和三乙胺 (Sigma-Aldrich, USA) 的混合物] 中。在气体较好混合并达到均质状态时，再暴露 24 小时至 48 小时直至观察到高质量图像。同时对 [^{3}H]Microscale([^{3}H] 微尺度)(American Radiolabeled Chemicals, Inc., St. Louis, MO) 计数，作为对总放射性定量分析的参考，即将 cpm/mm2 转化为 nCi/mg 组织。对感兴趣的解剖区域 (ROI) 用 Beta-Image Plus 程序 (BioSpace, France) 进行定量分析，即，在皮质和海马区域获得定量放射性摄取 (cpm/nlln2)。基于相应放射性配体的特定活性以及标准 [^{3}H]Microscale 的校准曲线，将结合密度标准化为 fmol/mg 组织。使用定量放射自显影，对于四种放射性配体 [^{3}H](+)- 戊唑辛、[^{3}H]RHM-1、[^{3}H]DTG 和 [^{3}H] 氟哌啶醇，测试一系列稀释的候选化合物 (10nM、100nM、1,000nM 和 10,000nM) 对结合位点的竞争，之后分析特异性结合 (%对照) 以得到皮质和海马区域 (齿状回、海马 CA I 和 CA3) 的结合亲和性。

[1001] σ -1 和 σ -2 受体处的放射自显影分别示于图 8A 和 8B 中。图 8C 示出 (A) 正常患者、路易体痴呆 (DLB) 患者或阿尔茨海默病 (AD) 患者的人额皮质切片中的 [^{3}H]-(+)- 戊唑辛 (σ -1 受体配体) 放射自显影，以及 (B) 与对照相比的特异性结合的图。如图 8A 所示，与正常对照相比，σ -1 受体在阿尔茨海默病和可能的 DLB 中在统计学上下调。该发现证实了 Mishina 等人的发现，Mishina 等人报道早期阿尔茨海默病中的低密度 σ -1 受体。Mishina 等人，2008, Low density of sigmal receptors in early Alzheimer's disease. Ann. Nucl Med22:151-156。图 8B 示出 (A) 正常患者、路易体痴呆 (DLB) 患者或阿尔茨海默病 (AD) 患者的人额皮质切片中的 [^{125}I]-RHM-4(σ -2 受体配体) 放射自显影，以及 (B) 与对照相比的特异性结合的图。σ -2 受体在 AD 中在统计学上没有下调。图 8C 示出 (A) 在猴额皮质、猴海马或人颞皮质中 σ -2 配体对 18.4nM[^{3}H]-RHM-1 的替代，以及 (B) 在具有和不具有 1 μ M 西拉美新和化合物 IXA、IXB 中每一个的情况下 [^{3}H]-RHM-1 的结合密度图，且西拉美新以及化合物 IXA、IXB 和 II 部分替代目标组织中的 [^{3}H]-RHM-1。

[1002] 实施例 11. MTS 检定 ：确定多种 σ -2 配体的激动剂或拮抗剂活性

[1003] 以下示出的化合物的细胞毒性使用 CellTiter96Aqueous One Solution 检定 (Promega, Madison, WI) 来确定。简单而言，将 MDA-MB-435 或 MDA-MB231 或 SKOV-3 细胞以 2000 细胞 / 孔的密度在用 σ -2 受体选择性配体处理前的一天接种在 96 孔板中。在 24 小

时处理后，向各个孔加入 CellTiter96Aqueous One Solution 试剂，且将板在 37℃孵育 2 小时。将板在 Victor3 酶标仪 (PerkinElmer Life and Analytical Sciences, Shelton, CT) 中于 490nm 读取。EC^{50} 值，定义为相对于未处理细胞抑制 50％细胞活力所需的 σ 配体浓度，从各个细胞系的剂量响应曲线中确定。西拉美新被接收为激动剂。σ-2 配体的激动剂和拮抗剂被定义如下：如果 σ-2 配体的 EC50 小于西拉美新 EC50 的 2 倍，该 σ-2 配体被认为是激动剂；如果 σ-2 配体的 EC50 为西拉美新 EC50 的 2 ～ 10 倍，该 σ-2 配体被认为是部分激动剂；如果 σ-2 配体的 EC50 大于西拉美新 EC50 的 10 倍，该 σ-2 配体被认为是拮抗剂。用于研究的 σ-2 配体是：激动剂（西拉美新和 SV119）、部分激动剂 (WC26)、拮抗剂 (RHM-1) 和候选化合物 (II 和 IXa、IXb)。结果显示在图 9A 中。图 9A 的数据显示在表 10 中。

[1004] 表 10. 肿瘤细胞活力检定的 IC_{50} 值

[1005]

化合物	IC_{50}，48 小时 (uM)	作用
RHM-1	203±13	拮抗剂
西拉美新	11.8±2.7	完全激动剂
SV-119	21.7±2.9	完全激动剂
WC-26	65.6±6.3	部分激动剂
IXa、IXb	169±9	拮抗剂
II	150±12	拮抗剂

[1006] 将神经元培养物用多种浓度的 σ 化合物处理 24 小时，并测量相对于媒介物的细胞核强度。与在测试浓度不降低细胞核强度的 σ-2 拮抗剂 (RHM-1、IXa、IXb 和 II) 相反，σ-2 激动剂（西拉美新、SV-119、WC-26）在神经元中引起显著异常的细胞核形态，如图 9B 所示。因此，σ-2 受体激动剂对神经元和癌症细胞有细胞毒性；然而，σ-2 受体拮抗剂无毒性且进一步阻断由 σ-2 受体激动剂引起的细胞毒性。

[1007] 实施例 12. caspase-3 检定．确定 σ-2 配体的激动剂或拮抗剂活性

[1008] 如本文所述，Xu 等人将 PGRMC1 蛋白复合物识别为推定的 σ-2 受体结合位点。Xu 等人，2011. Nature Commun. 2, 文章数 380，其并入本文以供参考。σ-2 受体激动剂可以诱导 caspase-3 依赖的细胞死亡。Xu 等人 2011 公开了检测 PGRMC1 通过 σ-2 受体激动剂 WC-26 来调节 caspase-3 活化的能力的功能性检定。

[1009] Abeta 寡聚体引起低水平的 caspase-3 活化，并引起 LTD。高水平的 Abeta 寡聚体和 caspase-3 活化引起细胞死亡。Li 等人，2010；Olsen 和 Sheng，2012。本文已表明，σ-2 受体激动剂 (SV-119、西拉美新）使肿瘤细胞和神经元中的 caspase-3 活化；参见，例如图 10A 和 10B。σ-2 受体拮抗剂 RHM-1 抑制肿瘤细胞中的活化（图 10A)，但是在该实验中不能阻断神经元中由激动剂 SV-119 引起的活化（图 10B)。测试化合物 II 和 IXa、IXb（所有这些为 σ-2 受体拮抗剂，如下所示）能够抑制肿瘤细胞中的 caspase-3 活化，并阻断神经元中 σ-2 受体激动剂 SV-119 对 caspase-3 的活化。因此，测试化合物 II 和 IXa、IXb 用作

为肿瘤细胞和神经元的 caspase-3 检定中的 σ-2 受体拮抗剂，如该实施例所证实。

[1010] 根据制造商的操作指南，使用 caspase-3 比色活性检定试剂盒 (Milipore, Billerica, MA) 来测量内源 caspase-3 经由 σ-2 受体配体的活化。简单而言，将 MDA-MB435 或 MDA-MB231 细胞以 $0.5x10^6$ 细胞 /100mm Ⅲ进行铺板。在铺板后 24 小时，将 σ-2 配体添加到培养皿中，以诱导 caspase-3 的活化。σ-2 配体的终浓度为其 EC50。在处理后 24 小时，收集细胞，在 300uL 的细胞裂解缓冲液中裂解，并以 10,000x g 离心 5 分钟。收集上清液，并与 caspase-3 底物 DEVD-pNA 在 37℃孵育 2 小时。使用 Dc 蛋白检定试剂盒 (Bio-Rad, Hercules, CA1) 确定蛋白浓度。使用 $Victor^3$ 酶标仪 (PerkinEliner Life and Analytical Sciences, Shelton, CT) 在 405nm 测量所得的游离 pNA。所测试的配体包括：σ-2 激动剂 (西拉美新、SV119、WC26) 和 σ-2 拮抗剂 RHMWU-I-102(RHM-1) 以及候选化合物 (II 和 IXa、IXb)。使 caspase3 活化的配体被认为是激动剂，而不使 caspase3 活化的配体被认为是拮抗剂。如图 10A 所示，σ-2 激动剂西拉美新诱导 caspase-3 的活性，而 σ-2 拮抗剂 RHM-1 以及候选化合物 II 和 IXa、IXb 不诱导 caspase-3 的活性。图 10B 显示出由 σ-2 激动剂 SV-119 引起的 caspase-3 活化，其被化合物 IXa、IXb 和 II 阻断。化合物 IXa、IXb 和 II 在癌细胞和神经元中表现得类似于 σ-2 拮抗剂。

[1011] 实施例 13. 治疗表型

[1012] 在一些实施方式中，公开内容提供可预测行为效力的体外检定平台。(1) 以高亲和性与 σ-2 受体选择性结合；且 (2) 用作为神经元中的功能性拮抗剂的化合物，如果阻断 Aβ 诱导的膜运输缺陷、阻断 Aβ 诱导的突触损失且在不存在 Abeta 寡聚体时不影响运输或突触数目，则被预测为具有行为效力。该体外检定中的该活性模式被称为“治疗表型”。σ-2 受体拮抗剂阻断成熟神经元中的 Abeta 寡聚体作用且在不存在 Abeta 寡聚体时不影响正常功能的能力是治疗表型的一个标准。在不存在寡聚体时影响运输或突触数目的化合物并非在行为上有效。仅有选择性地阻断寡聚体而不影响正常运输或改变突触数目的那些化合物，在预防和治疗 Abeta 寡聚体诱导的记忆丧失中是行为上有效的。在一个实施方式中，体外检定平台可以预测行为效力。因此，该平台检定中的该活性模式是治疗表型。

[1013] 例如，参见表 11A。

[1014] 表 11A. 治疗表型

[1015]

化合物	阻断 Aβ 诱导的膜运输缺陷 EC50 (uM)	阻断 Aβ 诱导的突触损失	不存在 Aβ 时的检定效果	行为效力
II	2.2	++	否	是
Z	6.1	+++	是	否
Z'	4.3	+++	是	否
IXa+IXb	4.9	+++	否	是

[1016] 概括起来，在 σ-2 受体处具有高亲和性 (优选 Ki 小于约 600nM、500nM、400nM、300nM、200nM、150nM、100nM 或 70nM) 且相比于其他非 σ CNS 或靶标受体对 σ 受体具有高于约 20 倍、30 倍、50 倍、70 倍或优选高于 100 倍选择性的 σ-2 拮抗剂具有较好的药物样特性

（包括脑渗透性和较好的代谢和 / 或血浆稳定性），且具有治疗表型的 σ-2 拮抗剂被预测为具有行为效力且可以用于在需要的患者中治疗 Abeta 寡聚体诱导的突触功能障碍。

[1017] 预测为具有口服生物利用度的一些化合物 II 类似物的功能性神经元表型示于表 11B 中。

[1018] 表 11B. 功能性神经元表型

[1019]

选择性	化合物	抑制 Abeta 寡聚体诱导的膜运输 EC50（uM）	S1 结合 Ki（nM）	S2 结合 Ki（nM）	阻断突触损失	功能性神经元表型
在 σ-2 处较高的亲和性	II	2.2	500	9	100%	拮抗剂
	II(+) 异构体	5.6	100	80	47%	拮抗剂
	W	8.7	110	36	43%	拮抗剂
	S'	>20	25	8	0%	无活性
	P	>20	320	110	0%	无活性
在 σ-1 处较高的亲和性	A	3.4	3	13	100%	拮抗剂
	B	5.5	1.3	3.9	100%	拮抗剂
	X	6.1	3.5	16	100%	拮抗剂
	E	8.2	2	3.6	34%	拮抗剂
	II(-) 异构体	10.9	46	63	0%	拮抗剂
在 σ-2 和 σ-1 处的相当的亲和性	Y	4.3	78	85	100%	拮抗剂
	R'	>20	11	16	33%	无活性

[1020] 治疗表型

[1021] 表 11C 示出对 σ-2 或 σ-1 受体具有高亲和性的已知现有技术化合物。一些 σ-2 配体落入三种功能神经元表型：拮抗剂（阻断 Abeta 信号传导）；激动剂（以 U 形剂量响应曲线以及高剂量下的毒性阻断 Abeta 信号传导）；以及无活性（在神经元培养物中没有作用）。已知的现有技术 σ-1 受体配体落入两个类别：拮抗剂（阻断 Abeta 信号传导）和无活性（在神经元培养物中没有作用）。大多数现有技术化合物具有低选择性，即它们对其他非 σ 受体具有显著亲和性。一些现有技术化合物可能无法穿透血脑屏障 (BBB) 且很可能是氧化代谢的底物，因此不符合治疗分布。

[1022] 表 11C. 化合物 - 选择性 σ-2 和 σ-1 受体配体

[1023]

选择性	化合物	抑制 Abeta 寡聚体诱导的膜运输 EC50（uM）	S1 结合 Ki（nM）	S2 结合 Ki（nM）	功能性神经元表型
在 σ-2 处较高的亲和性	PB 28	1	15	0.8	拮抗剂
	西拉美新	1.3	19	0.19	激动剂
	WC-26	1.6	1,400	3	激动剂
	SM-21	2.2	1050	145	拮抗剂
	SV119	2.6	1,400	8	激动剂
	M-14	6.2	12,900	8	拮抗剂
	艾芬地尔	>20	26	4.9	无活性
	苏 - 艾芬地尔	>20	59	0.9	无活性
	DTG	>20	88	35	无活性
在 σ-1 处较高的亲和性	NE 100	1.3	1.1	170	拮抗剂
	BD1008	1.5	2.2	8	拮抗剂
	BD1047	2.4	0.9	47	拮抗剂
	氟伏沙明	2.5	13	710	拮抗剂
	戊唑辛	7.6	3	>5,000	拮抗剂
	PPBP	10.9	0.8	1	拮抗剂
	氟哌啶醇	11	5	110	拮抗剂
	PRE-84	>30	2.2	13,091	无活性
	BD1063	>30	8.8	625	无活性
	SKF 10,047	>30	149	>10,000	无活性

[1024] 尽管一些临床化合物具有期望的功能性表型，但它们不满足期望的治疗分布。具有期望的拮抗剂功能性神经元表型但是不满足治疗分布的标准（非选择性或无法跨越 BBB 或被预测为氧化底物并具有代谢不稳定性）的已知现有技术化合物示于表 11D。

[1025] 表 11D. 某些已知化合物的表征

[1026]

Cpd	膜运输	功能性神	σ-1 受体	σ-2 受体	选择性其	药物样特

[1027]

	IC50（uM）	经元表型	Ki（nM）	Ki（nM）	他活性	性
PB 28	I	拮抗剂	15	0.8	未报道	氧化底物
SM-21	2.2	拮抗剂	1050	145	毒蕈碱拮抗剂	氧化底物
M-14	6.2	拮抗剂	12,900	8	D3	不 BBB，氧化底物
NE 100	1.3	拮抗剂	1.1	170	未报道	氧化底物
BD1008	1.5	拮抗剂	2.2	8	未报道	氧化底物
BD1047	2.4	拮抗剂	0.9	47	D2，β_3-AR	氧化底物
氟伏沙明	2.5	拮抗剂	13	710	5HT、DA、AR 转运体	是
(+)戊唑辛	7.6	拮抗剂	3	>5,000	NMDAR、PCP	是
PPBP	10.9	拮抗剂	0.8	1	未报道	氧化底物
氟哌啶醇	11	拮抗剂	5	110	D2、D3、D4、5HT2A、a1B、a2A、a2B、a2C、NMDAR、Ach、GluR、Ca2+	是

[1028] 此外，与 σ-2 和 / 或 σ-1 受体结合的很多临床化合物不具有高选择性，如表 11E 所示。实际上，相比于其他非 σ 受体，对于 σ 受体为 10 倍、20 倍以及特别是 100 倍的选择性是很罕见的。

[1029] 表 11E. 临床化合物选择性

[1030]

药物	治疗作用	σ 亲和性（S1/S2）nM	其他靶标
氟西汀	SSRI 抗抑郁	75/260	5-HT2C（33 nM）、2A（141 nM）、毒蕈碱 M1~M4（500 nM）、五羟色胺转运体（1~270 nM）

[1031]

氟伏沙明	SSRI 抗抑郁	13/710	多巴胺转运体（1.5 nM）、五羟色胺（12 nM）、肾上腺素能转运体（299 nM）
戊唑辛	阿片类镇痛	1.2/5,000	NMDAR2B（2.5 uM）、2A（2.7 uM）、PCP（2.9 uM）
奥匹哌醇	抗抑郁，抗焦虑	7/56	5-HT2A、B、C（120 nM），α1A、1B（200 nM）
西拉美新	抗抑郁，抗焦虑	19/0.19	D2（800 nM），α1A、1B（300 nM）
库他美新	抗抑郁，中风	17/>1800	VAChT（50 nM），毒蕈碱 M1、2，D2（2 uM），α1 AR，5-HT1a/2，H1（2 uM）
Anavex 1-41	阿尔茨海默病	7/>10,000	毒蕈碱 M1~M4（18~114 nM）、Na+ch2（>10 uM）
Anavex 2-73	阿尔茨海默病	860/>20,000	毒蕈碱 M1~M4（3~5 uM）、NMDAR2B（8 uM）、Na+ch2（5 uM）
II	阿尔茨海默病	500/9	D3（4 uM）、Mu 阿片样物质（4 uM）、Na+ch2（2 uM）
IXa、IXb	阿尔茨海默病	31/54	DA 转运体（1.5 uM）

[1032] 实施例 14：体外毒性

[1033] 代表性 σ-2 拮抗剂 II 和 IXa、IXb 在急性或慢性体外给药时不引起神经元或胶质细胞毒性。σ-2 受体拮抗剂消除或降低 Abeta 寡聚体诱导的膜运输变化。当在不存在寡聚体的情况下给药时，没有出现化合物对膜运输的显著作用。当以 EC50 浓度（高达 50 μM II 或 IXa、IXb）在三天内测试达 10 次时，相对于神经元数目、胶质细胞数目、细胞核尺寸、细胞

核形态、轴突长度、细胞骨架形态没有毒性。参见表 12。表 12

[1034]

化合物	IXa、IXb	II
EC50(uM)	4.9	2.2
Abeta 的最大抑制（浓度）	100%（14）	85%（10）
计算的 Ki*	0.58	0.26
Ki 处的单独 Cpd	+9%	+1%

[1035] *Abeta 的 Km = 0.4uM；检定浓度 3μM 总 Abeta。

[1036] 在大量标准检定中测试化合物 II 的体外毒性。体外毒性研究中的测试显示，在 10μM 没有基因毒性（AMES，微核，细菌细胞毒性）。在 10μM 的 66%的 HepG2 毒性（在受体 x 处为 100 倍以上亲和性）可能是由于化合物亲脂性或 HepG2 肿瘤细胞系中的受体过表达。CYP450 酶 2D6、3A4 和 2C19 的部分抑制（46 ～ 73%）发生在 10uM。在 100nM 处观察到中度 hERG 抑制（24%）。化合物 II 在 PGP 处表现出非常弱（IC50>30uM）的活性。

[1037] 实施例 15：化合物对映异构体在膜运输检定中的分离和活性

[1038] 将化合物 II 分离成其（+）和（-）对映异构体。将外消旋混合物施用至手性柱 CHIRALPAK AD-H（直链淀粉三（3,5-二甲基苯基氨基甲酸酯）涂覆在硅胶上；4.6X250mm）。将 15 微升体积的样品注射到柱中。洗脱液为己烷 /EtOH/TEA(95/5/0.1)，25 摄氏度，流速为 1ml/min。两种对映异构体分离为不同的峰。（+）对映异构体在约 16 分钟洗脱到第一个峰，且（-）对映异构体在约 20 分钟洗脱到第二个峰。对映异构体为至少 98%纯。（+）对映异构体具有 +10.1 的比旋度（c1.80 于 MeOH 中），且（-）对映异构体具有 -7.2 的比旋度（c1.80 于 MeOH 中）。在实施例 6 所述的膜运输检定中，（+）对映异构体比（-）对映异构体更有效力。在一个样品中，在抑制膜运输检定中的 β 淀粉样蛋白诱导的缺陷中，（+）对映异构体具有 5.6 的 EC50，且（-）对映异构体具有 10.9μM 的 EC50。

[1039] 实施例 16. 口服可利用的化合物的行为效力-改善转基因阿尔茨海默病小鼠模型中的记忆缺陷

[1040] 将雄性 hAPP Swe/Ldn 转基因（Tg）小鼠用作 AD 的 TG 模型。用媒介物、10 或 30mg/kg/ 天的 CB 或 CF 经 p.o. 处理 5.5 个月的转基因小鼠，以及媒介物处理的非转基因同窝仔进行标准条件性恐惧范例。在情境条件性恐惧中，相对于媒介物处理的非转基因同窝仔，用媒介物 p.o. 处理 5.5 个月的媒介物处理 9 个月大雄性 hAPP Swe/Ldn 转基因（Tg）小鼠表现出显著的记忆缺陷。

[1041] 当在训练 24 小时后，测试动物的联想记忆时，使用反复测量的双因素（基因型和时间）ANOVA 没有检测出转基因和非转基因媒介物处理小鼠之间的总僵立时间的显著差异。然而，在各个定时的时间间隔内，僵立行为的更加敏感的分析表明，相比于媒介物处理的非转基因动物，转基因小鼠在 1 ～ 3 分钟时间间隔内表现得更差（Mann-Whitney U 测试，$p<0.05$）。在该时间间隔内，与媒介物相比，用 10 和 30mg/kg/ 天的 CB（$p<0.05$）和 30mg/kg/ 天的 CF（$p<0.005$）处理的转基因动物显著改善表现（Mann-Whitney U 测试）。结果显示在图

12 中，两种剂量的 CB 显著地逆转 AD 小鼠中的记忆缺陷；且较高剂量的 CF 逆转 AD 小鼠中的记忆缺陷。用 10 和 30mg/kg/ 天的 CB 或 30mg/kg/ 天的 CF 处理 Tg 动物，分别在 394±287、793±325 或 331±373nM(AVG±S.D.) 的测量脑浓度改善缺陷。对于口服可利用的化合物的脑 / 谷血浆和脑 / 峰血浆比率示于表 13 中。

[1042] 表 13. 对于口服可利用的化合物的脑 / 谷血浆和脑 / 峰血浆比率

[1043]

化合物	剂量 (p.o.)	脑 / 谷血浆比率	脑 / 峰血浆比率
CF	30mg/kg	13	0.5
CF	10mg/kg	11	0.2
CB	30mg/kg	14	0.4
CB	10mg/kg	17	0.7

[1044] 因此，化合物 CB 和 CF 均为口服可利用的，能够实现显著的脑渗透并在老年转基因阿尔茨海默病小鼠模型动物中在慢性长期给药后逆转已建立的记忆缺陷。没有观察到不利的行为效应。

[1045] CB 和 CF 均为选择性、高亲和性 σ-2 受体拮抗剂化合物。CB 和 CF 均以高亲和性与 σ-2 以及 σ-1 受体结合，如表 14 中所示。针对一组 40 种脑受体进行反筛选，且结果显示 CB 和 CF 对 σ 受体具有高选择性，如表 14 中所示。

[1046] 表 14. 对于口服生物可利用的化合物的受体亲和性

[1047]

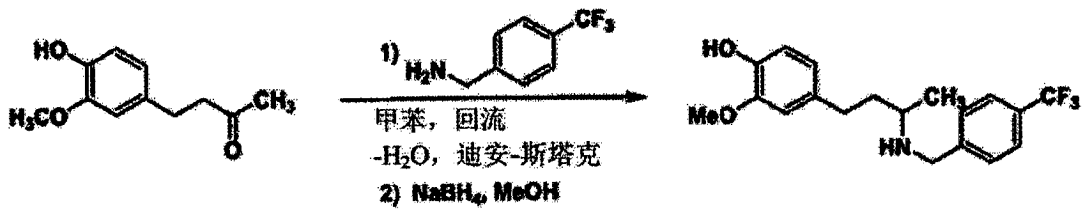
药物	治疗作用	σ 受体亲和性（σ-1/σ-2）（Ki，nM）	其他受体亲和性（Ki，nM）
CB	阿尔茨海默病	19/48	毒蕈碱 M1（1.5 uM）、M2（1.5 uM）、M3（1.8 uM）
			κ 阿片样物质（1.5 uM）
			Ca++ch-L-型（860 nM）
			转运体：NE（1.4 uM）、DA（220 nM）、5-HT（970 nM）
CF	阿尔茨海默病	180/50	毒蕈碱 M1（1.1 uM）、M2（2.5 uM）、M3（3.7 uM）
			κ 阿片样物质（6.1 uM）
			Ca++ch-L-型（2.5 uM）
			转运体：NE（1.9 uM）、DA（940 nM）、5-HT（3.2 uM）

[1048] 10mg/kg/ 天剂量的 CB 引起化合物脑水平处于或高于对 σ 和多巴胺转运体的 Ki，30mg/kg/ 天剂量则击中这些加上 Ca++ch 和 5-HT 转运体。随后的研究可以用来确定该化合物的最小有效剂量。30mg/kg/ 天剂量的 CF 引起化合物脑水平仅对 σ 受体具有选择性，因此其在 σ 受体处的亲和性解释了其在这些脑浓度的行为效力。

[1049] 合成例

[1050] 合成例 1 ：通过还原胺化合成化合物

[1051]



[1052] 将姜酮 (5.00g,25.7mmol) 溶于甲苯 (250mL) 并且添加 4- 三氟甲基苄基胺 (4.73g,27.0mmol)。将混合物保持在氮氛下并在回流下加热 16 小时，通过迪安 - 斯塔克蒸馏去除水。这时，移除迪安 - 斯塔克阱并将反应混合物在冰浴上冷却到 0℃。在大力搅拌下在 30 内分钟分批添加硼氢化钠 (5g) 于甲醇 (100mL) 中的溶液。当完成添加时，将混合物在回流下加热 16 小时。这时，将反应混合物冷却到室温并倒入饱和碳酸氢钠水溶液 (300mL)。将所得混合物通过旋转蒸发浓缩并且水性残余物在水与氯仿之间分隔开。使氯仿层在无水硫酸钠上干燥，然后过滤和浓缩。然后使用硅胶柱层析采用 5% 氨 - 甲醇于氯仿中的溶液的流动相纯化产物。将含有产物的馏分合并和浓缩，然后在高真空下干燥过夜，以提供浅棕色油 (6.72g,74%)。^{1}H NMR(500MHz, $CDCl_3$) δ :7.57(d, J = 7.8Hz, 2H), 7.43(d, J = 7.9Hz, 2H), 6.82(d, J = 7.3Hz, 1H), 6.65(m, 2H), 5.16-4.42(br s, 2H), 3.90(d, J = 13.7Hz, 1H), 3.84(s, 3H), 3.80(d, J = 13.7Hz, 1H), 2.76-2.70(m, 1H), 2.67-2.55(m, 2H), 1.84-1.77(m, 1H), 1.69-1.63(m, 1H), 1.17(d, J-6.3Hz, 3H). ^{13}C NMR(125MHz, $CDCl_3$) δ :146.7, 144.6, 143.9, 134.0, 129.1, 128.4, 127.5, 125.4, 125.3, 123.2, 120.8, 114.6, 111.0, 55.7, 52.1, 50.6, 38.8, 32.0, 20.1. MS(CI)m/z353(M^+).

[1053] 经 ^{1}H NMR 的化学位移测量可能变化例如高达 0.2ppm。经 ^{13}HNMR 的化学位移测量可能变化例如高达 0.5ppm。分析质谱可以具有 +/-0.3 的实验误差。

[1054] 纯度确定

[1055] 通过 HPLC 测量产物的纯度。保留时间为 2.22 分钟的主峰表明纯度大于约 80%、85%、90 或 95%。所使用的 HPLC 条件如下。

[1056] HPLC 条件 :

[1057] 流动相 A :13.3mM 甲酸铵 /6.7mM 甲酸于水中的溶液

[1058] 流动相 B :6mM 甲酸铵 /3mM 甲酸于水中的溶液 /CH_3CN(1/9, v/v)

[1059] 柱 :Synergi Fusion-RP100A Mercury, 2x20mm, 2.5 微米

[1060] (Phenomenex Part No00M-4423-B0_CE)

[1061] 梯度程序 :RT = 2.22 分钟

[1062]

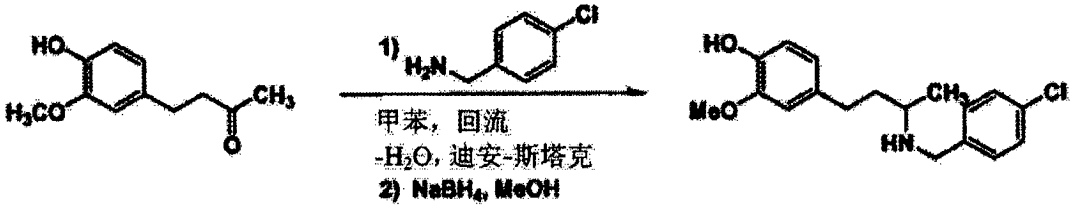
时间，分钟	%相 B	流速，ml/min
0	100	0.5
1	100	0.5
2.5	40	0.5
3.4	40	0.5

3.5	100	0.5
4.5	100	0.5

[1063] 还通过 ^{1}H NMR 测量产物的纯度，表明其是纯度大于 90％或 95％的单一化合物。本文所述的合成可以根据待合成的终产物进行修改。

[1064] 合成例 2：通过还原胺化合成化合物

[1065]



[1066] 将姜酮 (5.00g，25.7mmol) 溶于甲苯 (250mL) 并添加 4- 氯苄基胺 (4.73g，27.0mmol)。将混合物保持在氮氛下并在回流下加热 16 小时，通过迪安 - 斯塔克蒸馏去除水。这时，移除迪安 - 斯塔克阱并将反应混合物在冰浴上冷却到 0℃。在大力搅拌下在 30 分钟内分批添加硼氢化钠 (5g) 于甲醇 (100mL) 中的溶液。当完成添加时，将混合物在回流下加热 16 小时。这时，将反应混合物冷却到室温并倒入饱和碳酸氢钠水溶液 (300mL)。将所得混合物通过旋转蒸发浓缩并且水性残余物在水与氯仿之间分隔开。使氯仿层在无水硫酸钠上干燥，然后过滤和浓缩。然后使用硅胶柱层析采用 5％氨 - 甲醇于氯仿中的溶液的流动相纯化产物。将含有产物的馏分合并和浓缩，然后在高真空下干燥过夜，以提供浅棕色油 (6.16g，75％)。^{1}H NMR(500MHz, $CDCl_3$) δ：7.30-7.24(m, 4H), 6.81(d, J = 7.8Hz, 1H), 6.66-6.62(m, 2H), 4.25(br s, 2H), 3.82(s, 3H), 3.82(d, J = 13.2Hz, 1H), 3.72(d, J = 13.2Hz, 1H), 2.73(m, 1H), 2.66-2.51(m, 1H), 1.86-1.78(m, 1H), 1.72-1.63(m, 1H), 1.62-1.51(m, 1H), 1.17(d, J = 6.3Hz, 3H). ^{13}C NMR(125MHz, $CDCl_3$) δ：146.6, 143.8, 133.9132.8, 129.9, 129.7, 128.6, 120.8, 114.5, 110.9, 55.8, 51.9, 50.2, 38.5, 31.9, 31.6, 29.7, 26.9, 22.6, 19.9. MS(MH^+)：m/z320.

[1067] 经 ^{1}H NMR 的化学位移测量可能变化例如高达 0.2ppm。经 ^{13}HNMR 的化学位移测量可能变化例如高达 0.5ppm。分析质谱可以具有 +/-0.3 的实验误差。

[1068] 纯度确定

[1069] 通过 HPLC 测量产物的纯度。保留时间为 2.22 分钟的主峰表明纯度大于约 80％、85％、90 或 95％。所使用的 HPLC 条件如下。

[1070] HPLC 条件：

[1071] 流动相 A：13.3mM 甲酸铵 /6.7mM 甲酸于水中的溶液

[1072] 流动相 B：6mM 甲酸铵 /3mM 甲酸于水中的溶液 /CH_3CN(1/9, v/v)

[1073] 柱：Synergi Fusion-RP100A Mercury，2x20mm，2.5 微米

[1074] (Phenomenex Part No00M-4423-B0_CE)

[1075] 梯度程序：RT = 2.22 分钟

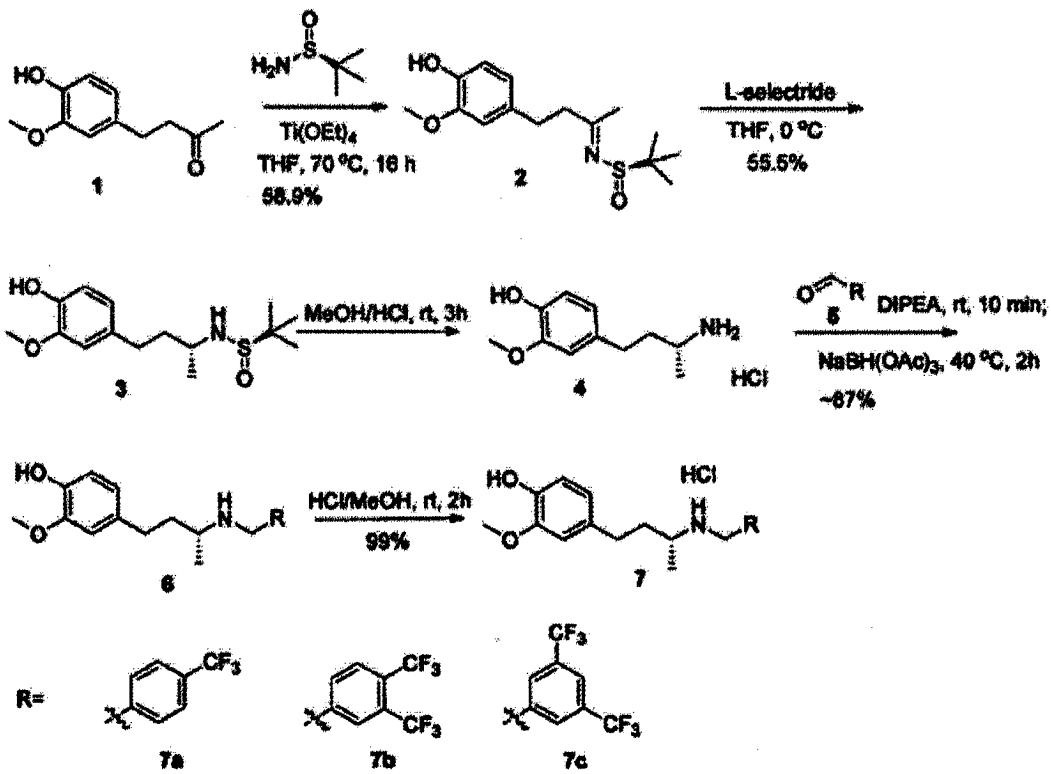
[1076]

时间,分钟	%相 B	流速,ml/min
0	100	0.5
1	100	0.5
2.5	40	0.5
3.4	40	0.5
3.5	100	0.5
4.5	100	0.5

[1077] 还通过 ^{1}H NMR 测量产物的纯度,表明其是纯度大于 90%或 95%的单一化合物。本文所述的合成可以根据待合成的终产物进行修改。

[1078] 合成例 3

[1079]



方案 9

[1080] 步骤 1:向 4-(4-羟基-3-甲氧基-苯基)-丁-2-酮 (38.8g,200mmol) 于 THF(600mL) 中的溶液中添加 Ti(OEt)$_4$(136.9g,600mmol) 和 (S)-(-)-叔丁基亚磺酰胺 (29g,240mmol)。将混合物在 70℃搅拌 16h,通过冰水猝灭,用 EA(3x300mL) 萃取,在 Na_2SO_4 上干燥,浓缩以获得粗产物,其通过柱层析 (PE/EA :3/1) 纯化以得到标题化合物 2(35g,59%)。

[1081] 步骤 2：在 0 ℃ 向化合物 2(18g,60mmol) 于 THF(180mL) 中的溶液中添加 L-selectride(三仲丁基硼氢化锂)(180mL,THF 中 1.0M,180mmol)。使反应在 3h 时间内温热至室温。经 TLC 对反应混合物的分析显示起始亚胺 2 的完全消耗。然后通过添加水使溶液猝灭，并通过 EA(3x200mL) 萃取。将合并的有机层用盐水洗涤，在 Na_2SO_4 上干燥，并在真空下浓缩，以得到残余物，其通过柱层析 (PE/EA :2/1) 纯化以提供产物。继续用 PE/EA(1:1) 通过重结晶纯化产物以得到产物 3(9.9g,55％)。通过 HPLC 确定 ee 值。

[1082] 步骤 3：向 3(7.0g,23.4mmol) 于 MeOH(20mL) 中的溶液中添加 HCl(MeOH 中 2M,20mL)，并将所得溶液在室温下搅拌 3h 时间。反应混合物的 TLC 分析显示化合物 3 的完全消耗。然后在真空下去除溶剂，并将所得残余物 4 直接用于下一步骤。

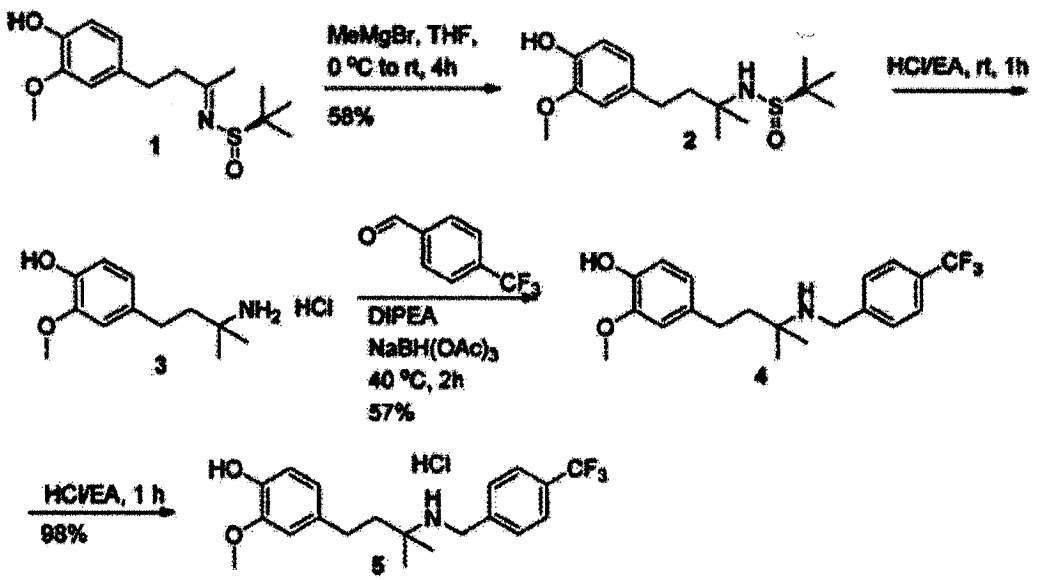
[1083] 步骤 4：向粗化合物 4(5.4g,23.4mmol) 于 THF(100mL) 中的溶液中添加 DIPEA(4.53g,35.1mmol) 和 4- 三氟甲基苯甲醛 5(4.28g,24.6mmol)。将所得溶液在室温搅拌 10min。然后，添加 $NaBH(OAc)_3$(14.9g,70.2mmol)，并将混合物在 40℃搅拌 2h。使混合物经 0℃水猝灭，过滤并通过 EtOAc 萃取。将有机层通过盐水洗涤，在硫酸钠上干燥，过滤，并在减压下浓缩滤液以提供残余物。将残余物通过柱层析 (PE/EA ＝ 1:2) 纯化以得到产物 6(7.0g,87％)。

[1084] 步骤 5：向 6(1.0g,2.8mmol) 于 MeOH(5mL) 中的溶液中添加 HCl(MeOH 中 2M,20mL)，并将所得溶液在室温搅拌 30min。去除溶剂以得到产物 7a(1.1g,99％)，其为白色固体。化合物 7b 和 7c 通过用合适的苯甲醛替换化合物 5 来相似地制备。

[1085] m/z(ESI+)(M+H)+：7a[354.2]；7b[422.2]；7c[422.2]。

[1086] 合成例 4

[1087]



[1088] 步骤 1：在 0 ℃ 向溴化甲镁于 THF(5mL) 中的溶液中添加 1(1.0g,3.3mmol) 于 THF(5mL) 中的溶液。将混合物在室温搅拌 4h，通过添加冰水猝灭，用乙酸乙酯 (3x30mL) 萃取，通过真空干燥，以提供粗产物，其通过柱层析 (PE/EA :3/1) 纯化以得到化合物 2(0.6g,58％)。

[1089] 步骤 2：在 0℃向 2 于 EA(10mL) 中的溶液中加入 HCl(EA 中 2M,3mL)，并将所得溶

液在室温搅拌 1h。经 TLC 对反应混合物的分析显示 2 的完全消耗。在真空下浓缩，将粗产物直接用于下一步骤。

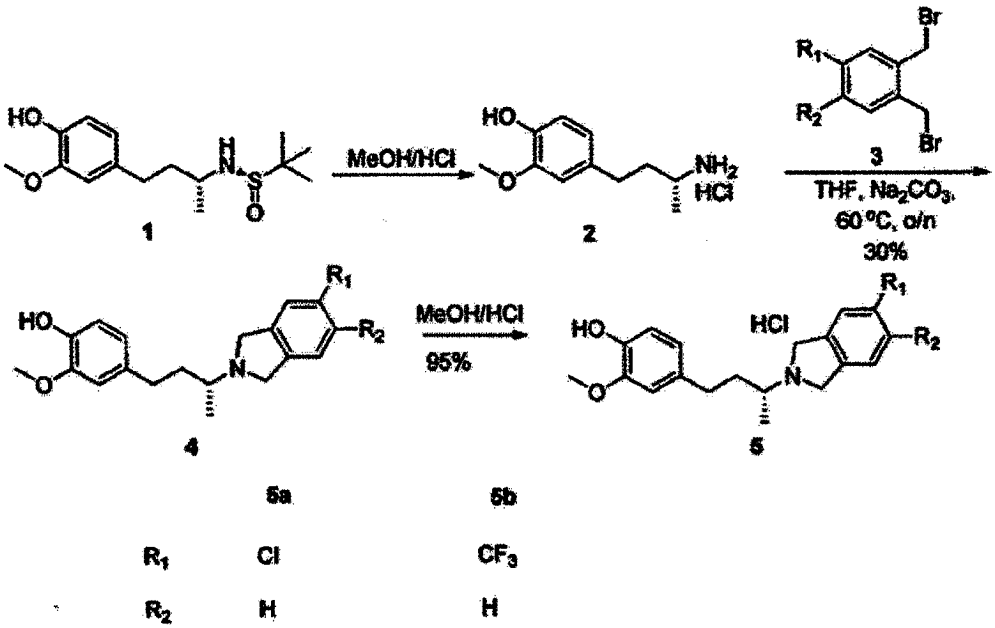
[1090] 步骤 3：随后，向 3(0.4g，1.9mmol) 于 THF(20mL) 中的溶液中加入 DIPEA(0.6g，4.6mmol) 和三氟甲基苯甲醛 (0.4g，2.3mmol)。将所得溶液在室温搅拌 10min。添加三乙酰氧基硼氢化钠 (1.63g，7.7mmol)，并将混合物在 40℃搅拌 2h。使混合物经 0℃水猝灭，过滤，并通过乙酸乙酯 (3x40mL) 萃取。将有机层用盐水洗涤，在硫酸钠上干燥，过滤，并在减压下浓缩滤液以提供残余物。将残余物通过柱层析 (PE/EA = 1:1) 纯化以得到 4(0.4g，57％)。

[1091] 步骤 4：向 4 于 EA(10mL) 中的溶液中添加 HCL(MeOH 中 2M，2mL)，并将所得溶液在室温下搅拌 1h。在真空浓缩之后，将残余物用乙酸乙酯洗涤以提供 5(0.4g，98％)。

[1092] m/z(ESI+) (M+H)+：5[368.2]。

[1093] 合成例 5

[1094]



方案 11

[1095] 步骤 1：向化合物 1(2g，6mmol) 于 MeOH(30mL) 中的溶液中添加 HCl(MeOH 中 2M，30mL)，并将所得溶液在室温搅拌 3h。反应混合物的 TLC 分析显示化合物 1 的完全消耗。然后在真空下去除溶剂，并将其直接用于下一步骤。

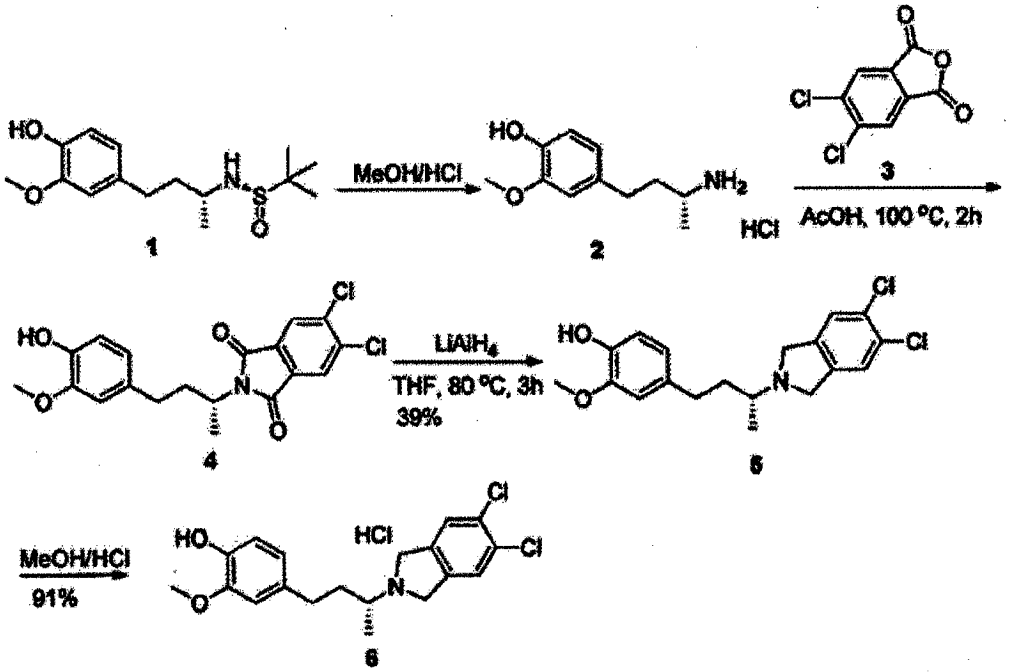
[1096] 步骤 2：向 2(0.4g，2mmol) 于 THF(10mL) 中的溶液中添加化合物 3a(0.54g，2mmol) 于 THF(5mL) 中的溶液。添加 Na_2CO_3(0.6g，6mmol)，并将所得溶液在 60℃搅拌过夜。在浓缩之后，将残余物通过 FCC 纯化以得到化合物 4(0.2g，30％)。

[1097] 步骤 3：向 4 于 EA(5mL) 中的溶液中添加 HCl(MeOH 中 2M，3mL)，并将所得溶液在室温搅拌 1h。在真空内浓缩之后，将残余物通过乙酸乙酯洗涤以得到化合物 5(0.2g，95％)。化合物 5b 通过用合适的二苄基溴替换化合物 3 来相似地制备。

[1098] m/z(ESI+) (M+H)+：5a[332.1]；5b[366.1]。

[1099] 合成例 6

[1100]



方案 12

[1101] 步骤 1 :向化合物 1(0.4g,1.3mmol) 于 MeOH(10mL) 中的溶液中添加 HCl(MeOH 中 2M,10mL),并将所得溶液在室温搅拌 3h。反应混合物的 TLC 分析显示化合物 1 的完全消耗。在真空中去除溶剂,并将其直接用于下一步骤。

[1102] 步骤 2 :将化合物 2(0.2g,1mmol) 和 3(0.2g,1mmol) 溶于乙酸 (10mL),在 100℃搅拌 2h。将混合物冷却到室温,经水 (10mL) 猝灭,通过 EtOAc(3x20mL) 萃取,干燥,浓缩,以得到标题化合物 4(0.3g,76%)。

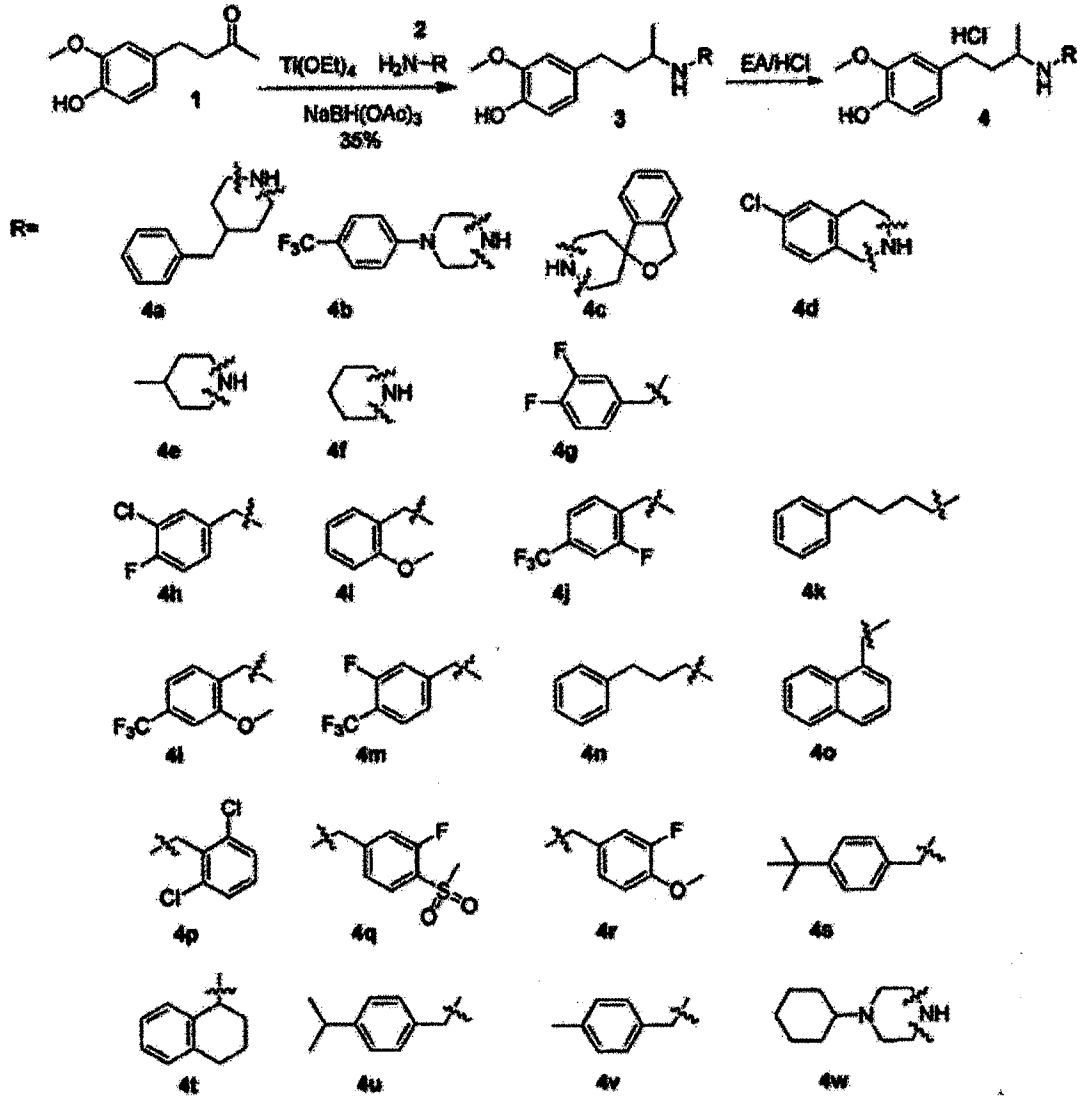
[1103] 步骤 3 :向 4(0.3g,0.7mmol) 于 THF(10mL) 中的溶液中添加 LAH(0.1g,3.5mmol)。将混合物在 80℃搅拌 3h。使混合物经水 (0.1mL)、15% NaOH(0.1mL) 和水 (0.3mL) 猝灭,过滤,浓缩。将粗产物通过柱层析 (PE/EA = 5:1) 纯化以得到化合物 5(0.1g,39%)。

[1104] 步骤 4 :向 5 于乙酸乙酯 (5mL) 中的溶液中添加 HCl(MeOH 中 2M,3mL) 并将所得溶液在室温搅拌 1h。将反应通过真空浓缩以提供标题化合物 6(0.16g,91%)。

[1105] m/z(ESI+) (M+H)+ :6[366.2]。

[1106] 合成例 7

[1107]



方案 13

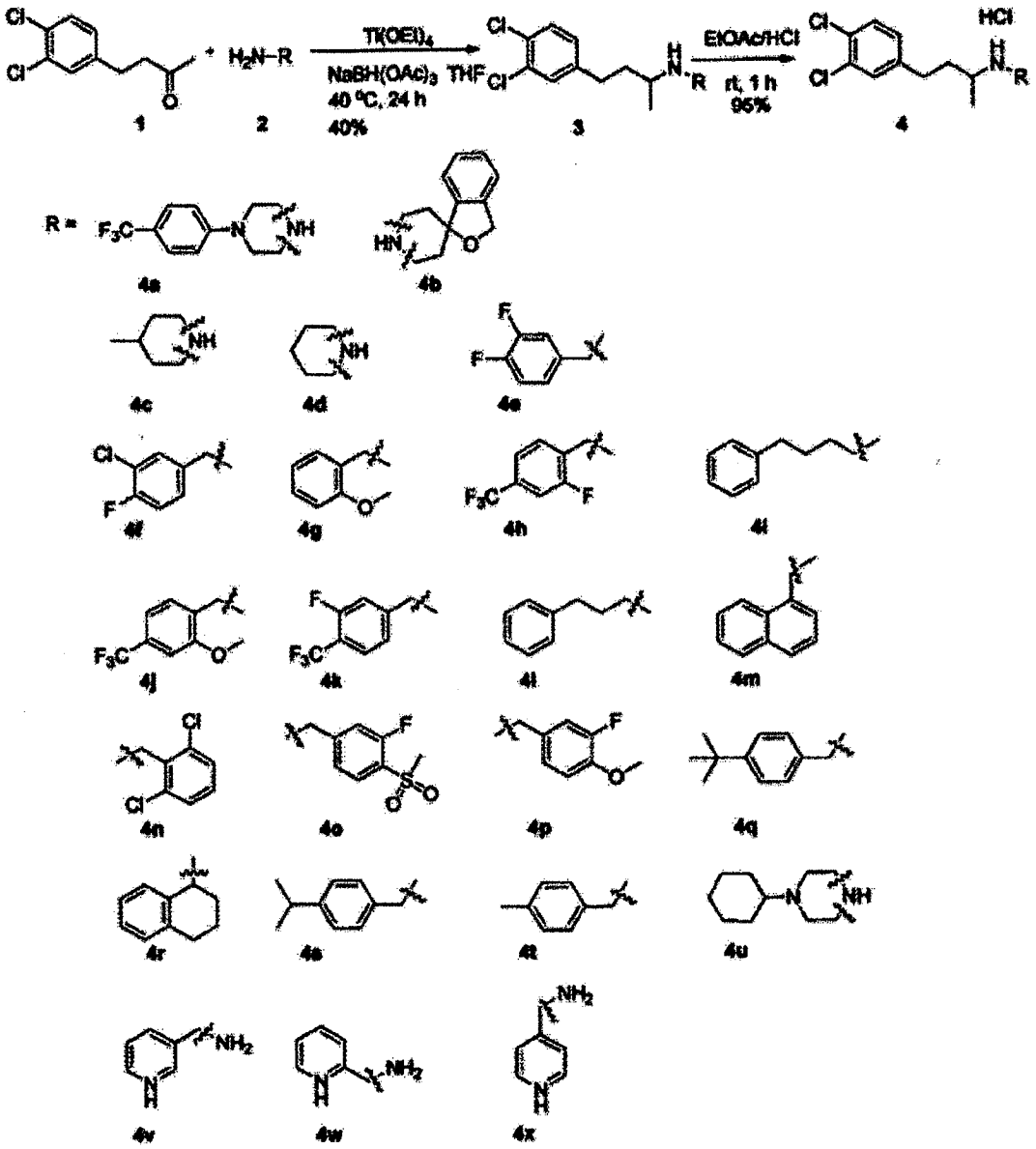
[1108] 步骤 1：向 1(0.278g,1.43mmol) 于 THF(20mL) 中的溶液中添加 $Ti(OEt)_4$(2.1g,9.2mmol) 和 4- 苄基哌啶 (0.34g,1.3mmol)。将混合物在 40℃搅拌一天，经冰水猝灭，用乙酸乙酯 (3x20mL) 萃取。在真空内浓缩之后，将粗产物通过柱层析 (PE/EA :1/1) 纯化以得到 3(205mg,35％)。

[1109] 步骤 2：向 3(0.2g,0.47mmol) 于乙酸乙酯 (5mL) 中的溶液中添加 HCl(MeOH 中 2M,3mL) 并将所得溶液在室温搅拌 1h。将反应通过真空浓缩以得到 4a(0.2g,95％)。化合物 4b ～ 4w 通过用合适的胺替换胺化合物 2 来相似地制备。

[1110] 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]。

[1111] 合成例 8

[1112]



方案 14

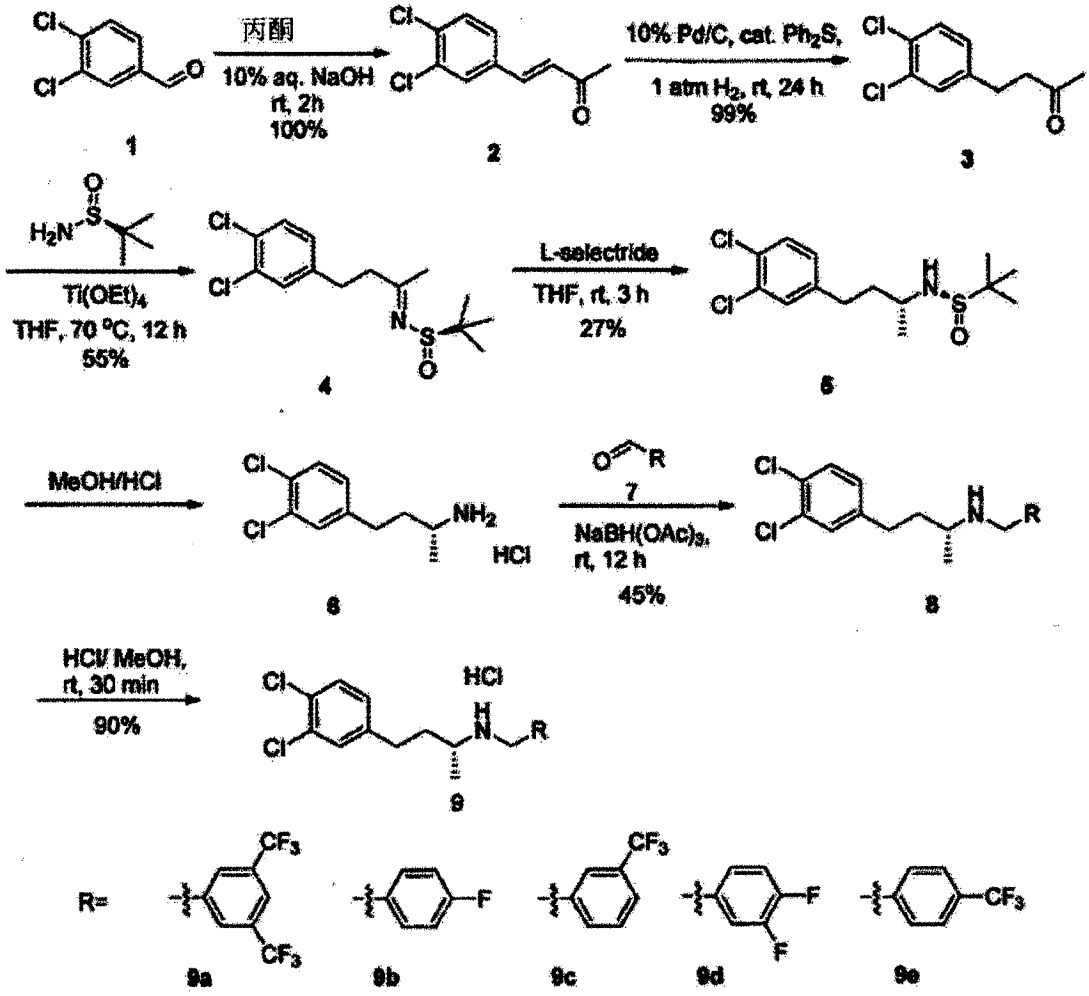
[1113] 步骤 1：向 1(0.31g,1.43mmol) 于 THF(20mL) 中的溶液中添加 $Ti(OEt)_4$(0.595g,2.58mmol) 和 N-(4- 三氟甲基苯基)- 哌嗪 2(0.3g,1.3mmol)。将混合物在 40℃搅拌 24h，通过添加冰水猝灭，用乙酸乙酯 (3x20mL) 萃取，干燥。通过柱层析 (PE/EA :1/1) 纯化得到产物 3(0.25g,41%)。

[1114] 步骤 2：向化合物 3(0.25g,0.58mmol) 于乙酸乙酯 (5mL) 中的溶液中添加 MeOH-HCl(2N,4mL)。将混合物在室温搅拌 1 h。真空内的浓缩得到化合物 4(0.25g,95%)。化合物 4b ～ 4x 通过用合适的胺替换胺化合物 2 来相似地制备。

[1115] 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]。

[1116] 合成例 9

[1117]



方案 15

[1118] 步骤 1：向 1(3.5g,20mmol) 于丙酮 (20mL) 和乙醇 (2mL) 中的溶液中添加 NaOH 水溶液 (10%,15mL) 和水 (80mL)。将混合物在室温搅拌 2h,用 EA(3x50mL) 萃取。将有机层干燥并浓缩以得到 2(4.3g,100%)。

[1119] 步骤 2：向 2(4.3g,20mmol) 于 MeOH(50mL) 中的溶液中添加二苯基硫 (0.15mL) 和 Pd/C(10%,0.43g)。将混合物在 25℃在 1atm 氢下大力搅拌 24h。将反应混合物经硅藻土 (Celite) 垫过滤,用甲醇洗涤,并将滤液浓缩以提供 3(4.3g,99%)。

[1120] 步骤 3：向 3(10g,46mmol) 于 THF(100mL) 中的溶液中添加 $Ti(OEt)_4$(21g,92mmol) 和 (S)-(-)-叔丁基亚磺酰胺 (6.1g,50mmol)。将混合物在 70℃搅拌 12h,经冰水猝灭,用乙酸乙酯 (3x250mL) 萃取。在真空浓缩之后,将粗产物通过柱层析 (PE/EA :10/1) 纯化以提供化合物 4(8.1g,55%)。

[1121] 步骤 4：在 0 ℃向化合物 4(3.3g,10mmol) 于 THF(30mL) 中的溶液中添加 L-Selectride(33mL,THF 中 1.0M,33mmol)。使反应在 3h 时间内温热至室温。经 TLC 对反应混合物的分析显示起始亚胺 4 的完全消耗。将溶液通过水猝灭,并通过乙酸乙酯 (3x30mL) 萃取。将合并的有机层用盐水洗涤,在 Na_2SO_4 上干燥,并在真空下浓缩以得到残余物,其通

过柱层析 (PE/EA :2/1) 纯化以提供产物 5(0.9g,27%)。

[1122] 步骤 5 :向化合物 5(5g,15.5mmol) 于 MeOH(10mL) 中的溶液中添加 HCl(MeOH 中 2M,10mL) 并将所得溶液在室温搅拌 3h。反应混合物的 TLC 分析显示化合物 5 的完全消耗。在真空中去除溶剂,并将粗 6(3.95g,100%) 直接用于下一步骤而无需进一步纯化。

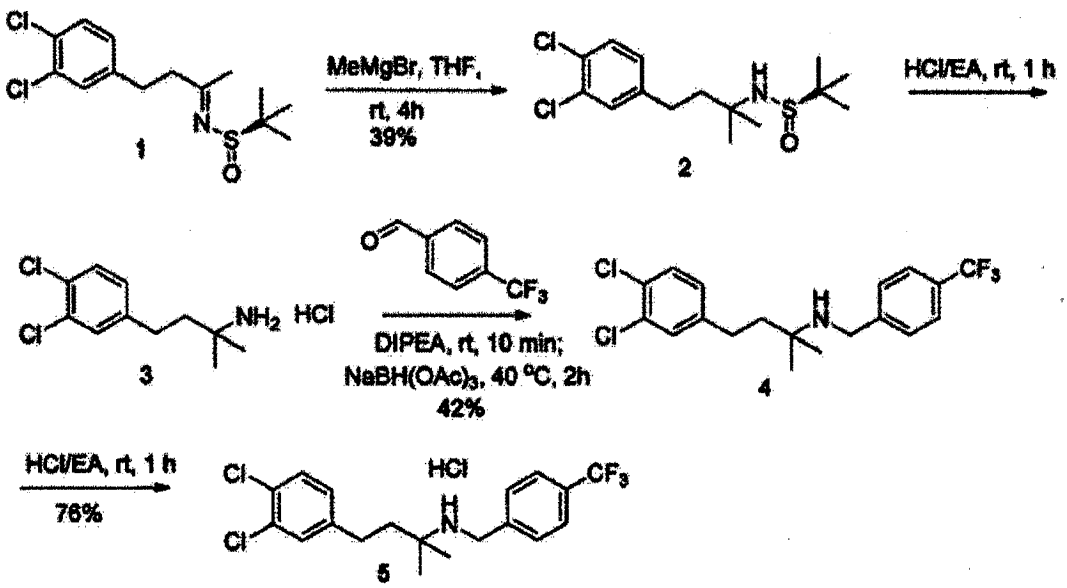
[1123] 步骤 6 :随后向 6(0.6g,2.4mmol) 于 THF(10mL) 中的溶液中添加 DIPEA(0.4g,3.1mmol) 和 3- 三氟甲基苯甲醛 (0.41g,2.4mmol)。将所得溶液在室温搅拌 10min。添加 $NaBH(OAc)_3$(1.0g,4.7mmol) 并将混合物搅拌 12h。将混合物经 0℃水猝灭,过滤并通过 EtOAc(3x30mL) 萃取。将有机层用盐水洗涤,在硫酸钠上干燥,过滤,并在减压下浓缩滤液以提供残余物。将残余物通过柱层析 (PE/EA = 1:1) 纯化以得到化合物 8(0.4g,45%)。

[1124] 步骤 7 :向 8(0.4g,1.08mmol) 于 MeOH(5mL) 中的溶液中添加 HCl(MeOH 中 2M,4mL) 并将所得溶液在室温搅拌 0.5h。将反应浓缩以得到胺 9a(0.4g,90%)。化合物 9b ～ 9e 通过用合适的苯甲醛替换化合物 7 来相似地制备。

[1125] m/z(ESI+) (M+H)+ :9a[444.2] ;9b[326.25] ;9c[376.2] ;9d[344.2] ;9e[376.1]。

[1126] 合成例 10

[1127]



方案 16

[1128] 步骤 1 :在 0℃向溴化甲镁于 THF(3M,15mL) 中的溶液中添加 1(1.5g,4.6mmol) 于 THF(20mL) 中的溶液。将混合物在室温搅拌 4h,经冰水猝灭,用乙酸乙酯 (3x30mL) 萃取。在浓缩之后,将粗产物通过柱层析 (PE/EA :3/1) 纯化以提供化合物 2(0.6g,39%)。

[1129] 步骤 2 :向化合物 2(0.6g,1.8mmol) 于乙酸乙酯 (10mL) 中的溶液中添加 HCl(MeOH 中 2M,3mL) 并将所得溶液在室温搅拌 1h。反应混合物的 TLC 分析显示出化合物 2 的完全消耗。然后在真空中去除溶剂,并将粗化合物 3 直接用于下一步骤。

[1130] 步骤 3 :向 3(0.43g,1.8mmol) 于 THF(20mL) 中的溶液中相继添加 DIPEA(0.54g,4.0mmol) 和 4- 三氟甲基苯甲醛 (0.36g,2.0mmol)。将所得溶液在室温搅拌 10min。添加 $NaBH(OAc)_3$(1.57g,7.4mmol) 并将混合物在 40℃搅拌 2h。将混合物经 0℃水猝灭,过滤,并通过 EtOAc(3x30mL) 萃取。将有机层通过盐水洗涤,在硫酸钠上干燥,过滤,并将滤液在减

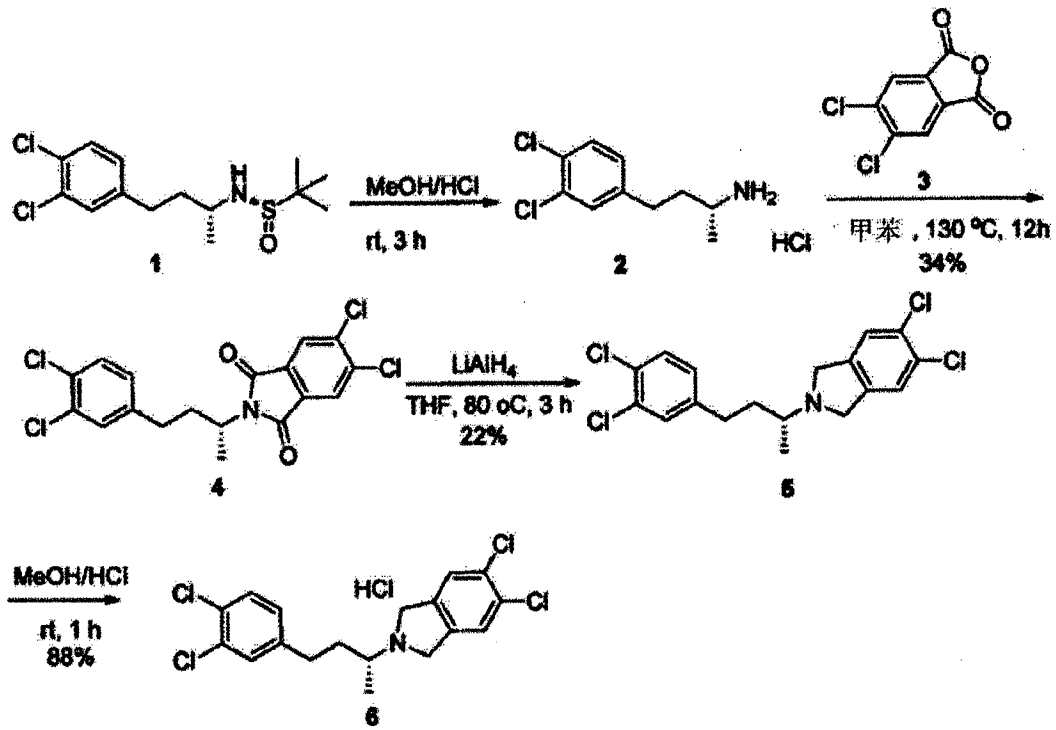
压下浓缩以提供残余物，其通过柱层析 (PE/EA = 3:1) 纯化以得到化合物 4(0.3g，43%)。

[1131] 步骤 4：向 4(0.3g，0.8mmol) 于乙酸乙酯 (10mL) 中的溶液中添加 HCl(MeOH 中 2M，2mL) 并将所得溶液在室温搅拌 1h。将沉淀过滤，得到化合物 5(0.25g，76%)。

[1132] m/z(ESI+) (M+H)+：5[390.14]。

[1133] 合成例 11

[1134]



方案 17

[1135] 步骤 1：将化合物 1(1.75g，5.43mmol) 于 MeOH-HCl(2M，10mL) 中的混合物在室温搅拌 3h。将反应混合物在真空内浓缩，得到粗 2，其用于下一步骤而无需进一步纯化。

[1136] 步骤 2：将化合物胺 2(1.4g，5.43mmol) 和酐 3(1.18g，5.43mmol) 于甲苯 (12mL) 中的溶液在 130℃加热 12h。将混合物冷却到室温并添加水 (10mL)，通过 EtOAc(3x20mL) 萃取，干燥，浓缩。将粗产物通过柱层析 (PE/EA = 10:1) 纯化得到产物 4(0.78g，34%)。

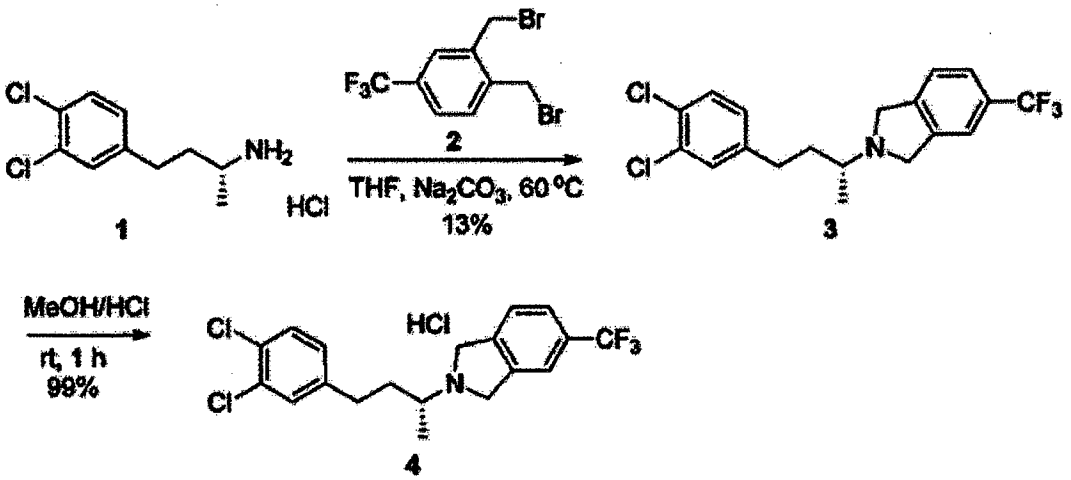
[1137] 步骤 3：向化合物 4(0.78g，1.87mmol) 于 THF(20mL) 中的溶液中添加 LAH(0.36g，9.1mmol)。将混合物在 80℃搅拌 3h。将冷却的混合物通过水 (3.46mL)、15% NaOH(3.46mL) 和水 (13.5mL) 猝灭。将反应混合物过滤，浓缩。将粗产物通过柱层析 (PE/EA = 5:1) 纯化得到产物 5(0.16g，23%)。

[1138] 步骤 4：将化合物 5(0.3g，0.8mmol) 溶于乙酸乙酯 (5mL)，添加 MeOH-HCl(2N，3mL)。将混合物在室温搅拌 1 h，浓缩以得到化合物 6(0.16g，89%)。

[1139] m/z(ESI+) (M+H)+：6[390.0]。

[1140] 合成例 12

[1141]



方案 18

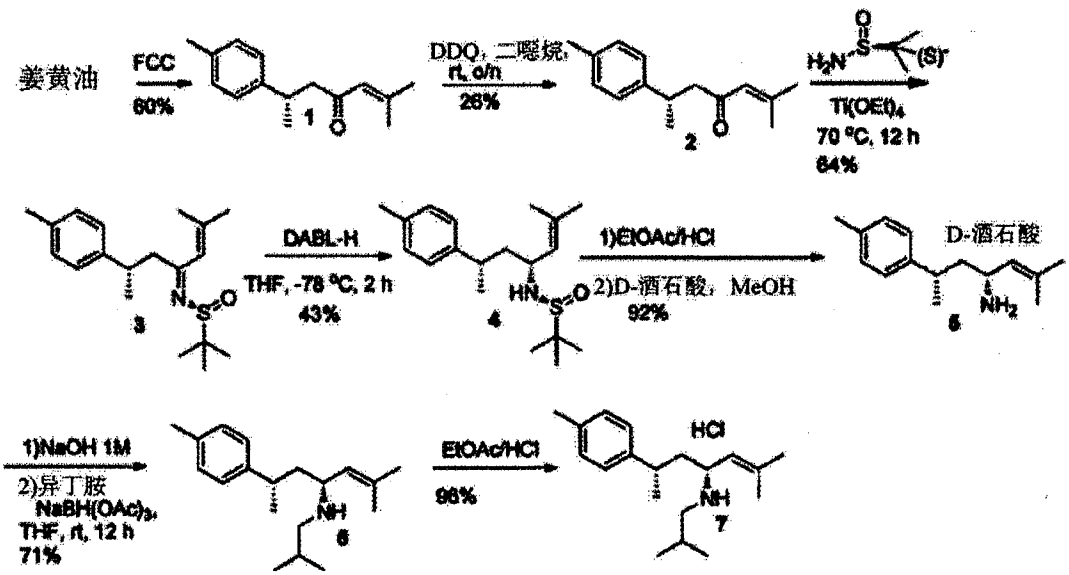
[1142] 步骤 1：向化合物 1(0.4g，2mmol) 于 DMF(6mL) 中的溶液中添加二溴化物 2(0.6g，2mmol)。将所得溶液在 80℃搅拌过夜，浓缩，通过制备 HPLC 纯化，得到化合物 3(0.1g，13％)。

[1143] 步骤 2：将化合物 3(0.1g，0.2mmol) 溶于乙酸乙酯 (5mL)，添加 MeOH-HCl(2N，3mL)。将混合物在室温搅拌 1 h。将混合物过滤以得到化合物 4(0.11g，99％)。

[1144] m/z(ESI+) (M+H)+：4[388.1]。

[1145] 合成例 13

[1146]



方案 19

[1147] 步骤 1：将姜黄油 (100g) 通过柱层析 (PE:EA/100:1) 纯化以提供粗产物 1(60g)。

[1148] 步骤 2：将粗产物 1(60g) 溶于二噁烷 (200mL)，添加 DDQ(81.7g，360mmol)。将混合物在室温搅拌过夜，然后经水 (500mL) 猝灭，通过硅藻土垫过滤。将滤液通过乙酸乙酯 (3x200mL) 萃取。将有机层干燥，浓缩并通过柱层析 (PE:EA/30:1) 纯化以提供化合物 2(15.6g，26％)。

[1149] 步骤 3：向 2(7.4g,34.2mmol) 于 $Ti(OEt)_4$(23.4g,102.6mmol) 中的溶液中添加 (S)-(-)-2- 甲基 -2- 丙烷亚磺酰胺。将混合物在 70℃搅拌 12h，经冰水猝灭，用乙酸乙酯 (3x100mL) 萃取，并干燥，以得到残余物，其通过柱层析 (PE/EA :5/1) 纯化得到产物 3(7.1g，64％)。

[1150] 步骤 4：在 -78℃向化合物 3(7.0g,21.9mmol) 于 THF(70mL) 中的溶液中添加 DIBAL-H(22mL，THF 中 1.5M,33mmol)。将所得溶液在 -78℃搅拌 2h。经 TLC 对反应混合物的分析显示出起始亚胺的完全消耗，得到亚磺酰胺化合物 4。将溶液经水猝灭，并通过乙酸乙酯 (3x200mL) 萃取。将合并的有机层用盐水洗涤，在 Na_2SO_4 上干燥，并浓缩以提供橙色油。使粗产物进行柱层析 (PE:EA/3:1) 以提供产物 4(3.1g,43％)。

[1151] 步骤 5：向化合物 4(1.6g,5.0mmol) 于乙酸乙酯 (10mL) 中的溶液中添加 HCl- 乙酸乙酯 (2N,10mL)，并将所得溶液在室温搅拌 3h。反应混合物的 TLC 分析显示出化合物 3 的完全消耗。在真空中去除溶剂。将残余物溶于水 (10mL)，并将 pH 通过 K_2CO_3 的饱和水溶液调整为 9 ～ 10，通过乙酸乙酯 (3x20mL) 萃取，干燥，并浓缩以得到游离胺。将游离胺 (1.1g，5.0mmol) 溶于甲醇 (15mL)。将 D- 酒石酸 (0.75g,5.0mmol) 添加到溶液。将混合物在回流下搅拌 1 h。将溶液缓慢冷却到室温。将所形成的结晶过滤，得到产物 5(1.7g,93％)。

[1152] Mp. 172 ～ 174℃。通过 X- 射线结晶学确定化合物 5 的绝对立体化学。

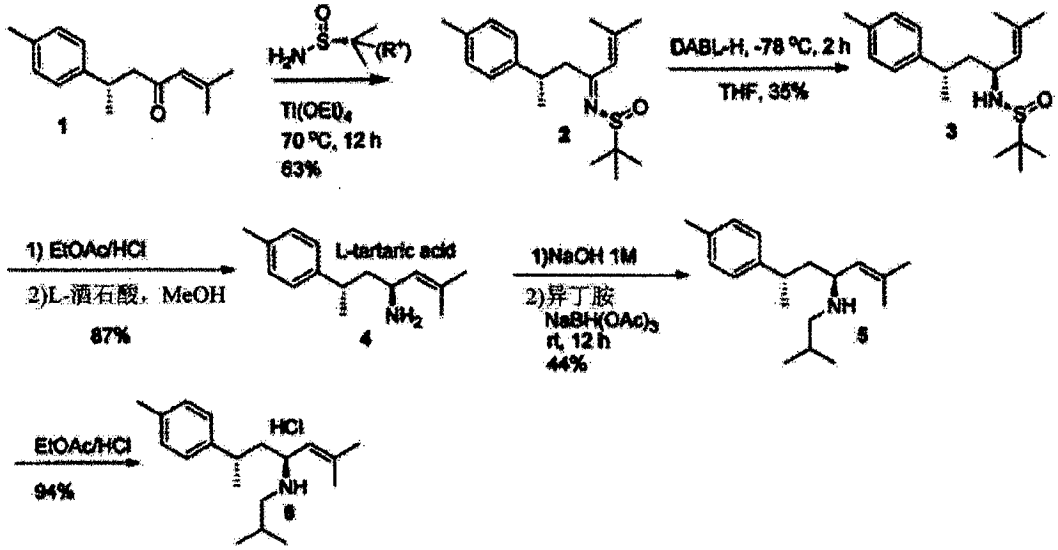
[1153] 步骤 6：将化合物 5(1.7g,4.6mmol) 于水 (20mL) 中的溶液通过 1M NaOH 调整为 pH9 ～ 10。将产物用乙酸乙酯 (3x20mL) 萃取。将有机层干燥，浓缩，以得到游离胺。将游离胺溶于 THF(10mL)，添加异丁基胺 (0.40g,5.5mmol) 和 $NaBH(OAc)_3$(3.90g,18.4mmol)。将混合物在室温搅拌 12h，用水猝灭，通过乙酸乙酯 (3x30mL) 萃取。将有机层干燥，浓缩，并通过柱层析 (PE:EA/3:1) 纯化以提供产物 6(0.9g,72％)。

[1154] 步骤 7：向化合物 6(0.9g,3.2mmol) 于乙酸乙酯 (10mL) 中的溶液中添加乙酸乙酯 -HCl(2N,5mL)。将混合物在室温搅拌 1 h。在真空中去除乙酸乙酯，得到化合物 7(0.95g，96％)。

[1155] m/z(ESI+) (M+H)+ :7[274.20]。

[1156] 合成例 14

[1157]



方案 20

[1158] 步骤 1：向 1(7.4g,34.2mmol) 于 $Ti(OEt)_4$(23.4g,102.6mmol) 中的溶液中添加 (R)-(+)-2- 甲基 -2- 丙烷亚磺酰胺。将混合物在 70℃搅拌 12h，经冰水猝灭，用乙酸乙酯 (3x100mL) 萃取，干燥。通过柱层析 (PE/EA :5/1) 纯化，得到产物 2(6.9g,63%)。

[1159] 步骤 2：将化合物 2(7.0g,21.9mmol) 溶于 THF(70mL) 并冷却到 -78℃。然后向容器中添加 DIBAL-H(22mL, THF 中 1.5M,33mmol)，并将所得溶液在 -78℃搅拌 2h。经 TLC 对反应混合物的分析显示出起始亚胺的完全消耗，得到亚磺酰胺化合物 3。然后将溶液经水猝灭并通过乙酸乙酯 (3x200mL) 萃取。将合并的有机层用盐水洗涤，在 Na_2SO_4 上干燥，并在真空下浓缩以提供橙色油。使粗产物进行柱层析 (PE:EA/3:1) 以提供产物 3(2.5g,36%)。

[1160] 步骤 3：向 3(2.5g,7.8mmol) 于乙酸乙酯 (10mL) 中的溶液中添加 2M HCl 于乙酸乙酯 (10mL) 中的溶液，并将所得溶液在室温搅拌 3h。反应混合物的 TLC 分析显示出化合物 3 的完全消耗。以真空去除溶剂。将残余物溶于水 (10mL)，通过添加饱和 K_2CO_3 将其 pH 调整为 9 ～ 10。将混合物通过乙酸乙酯 (3x20mL) 萃取，干燥，浓缩，以得到游离胺 4。将游离胺 4 溶于甲醇 (15mL)，添加 L- 酒石酸 (1.17g,7.8mmol)。将混合物在回流下搅拌 1h，冷却到室温，过滤以得到结晶盐 4(2.5g,87%)。

[1161] 步骤 4：将 L- 酒石酸盐 4(2.5g,6.8mmol) 溶于水 (20mL)，通过添加 1M NaOH 将其 pH 调整为 9 ～ 10。然后将混合物通过乙酸乙酯 (3x50mL) 萃取，干燥，浓缩以得到游离胺 4。将游离胺 4 再溶解于 THF，添加异丁基胺 (0.60g,8.2mmol) 和 $NaBH(OAc)_3$(5.85g,27.6mmol)。将混合物在室温搅拌 12h，经水猝灭，通过乙酸乙酯 (3x30mL) 萃取。将有机层干燥，浓缩，并通过柱层析 (PE:EA/3:1) 纯化以提供产物 5(0.83g,45%)。

[1162] 步骤 5：向 5(0.83g,3.0mmol) 于乙酸乙酯 (10mL) 中的溶液中添加 HCl(乙酸乙酯中 2M,5mL)，并将所得溶液在室温搅拌 1h。去除溶剂以得到产物 6(0.88g,94%)。

[1163] m/z(ESI+) (M+H)+ :6[274.20]

[1164] 所有在说明书 (包括摘要和附图) 中公开的特征、以及所有在任意所公开方法或工序中的步骤可以以任意组合进行组合，除其中至少一些这样的特征和 / 或步骤相互排斥的组合之外。说明书 (包括摘要和附图) 中公开的各个特征可以用提供相同、等同或相似

目的的可选特征进行替换，除非明确指出相反情况。因此，除非明确指出相反情况，所公开的各个特征仅是通用系列的等同或相似特征的一个实例。根据上述说明，除本文记载的那些外，本发明的多种修改对于本领域技术人员也是明显的。这些修改也意在落入所附权利要求的范围内。

[1165] 所有本文提及的公开物全部并入本文以供参考。本文中没有事物被认为是承认本发明由于在先的发明而没有早于这些公开的资格。

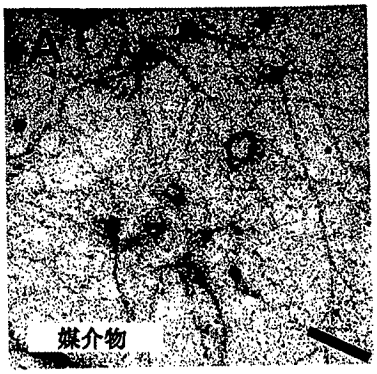


图 1A

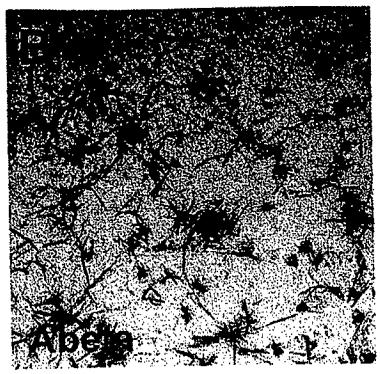


图 1B

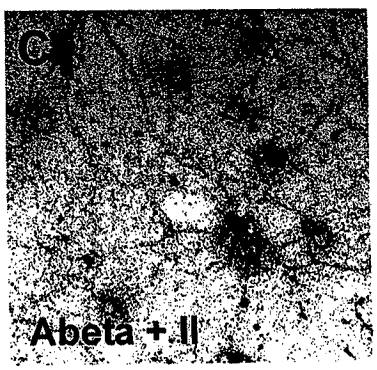


图 1C

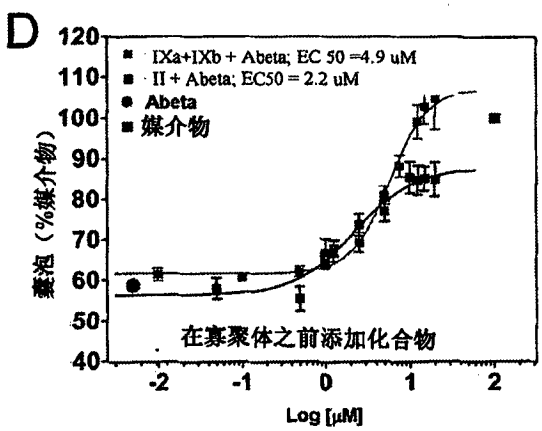


图 1D

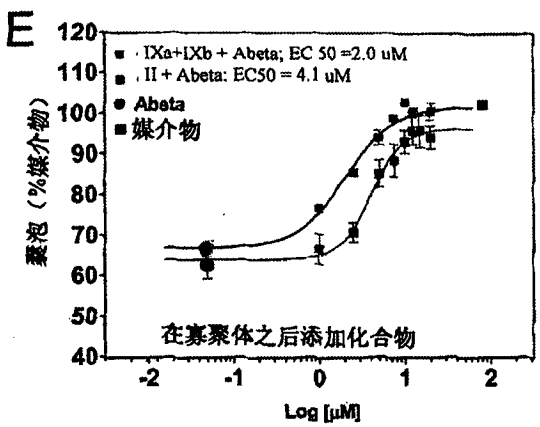


图 1E

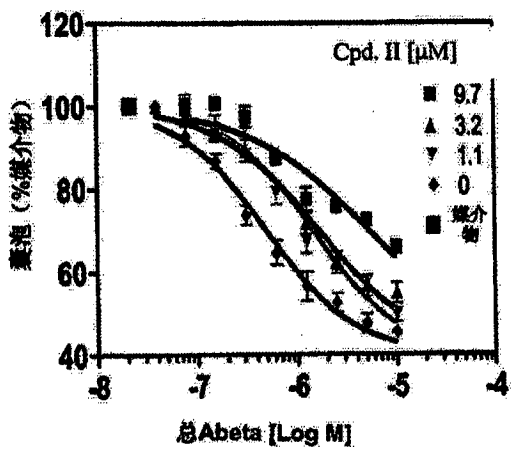


图 1F

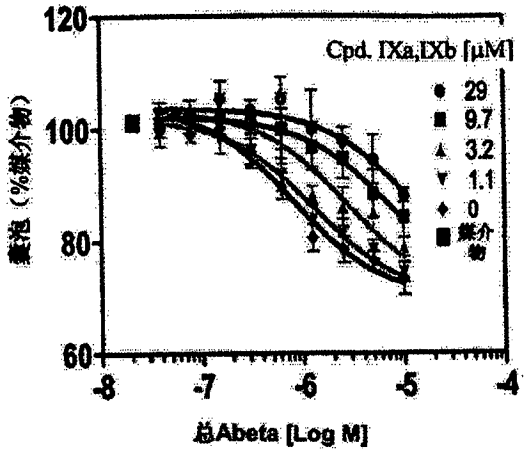
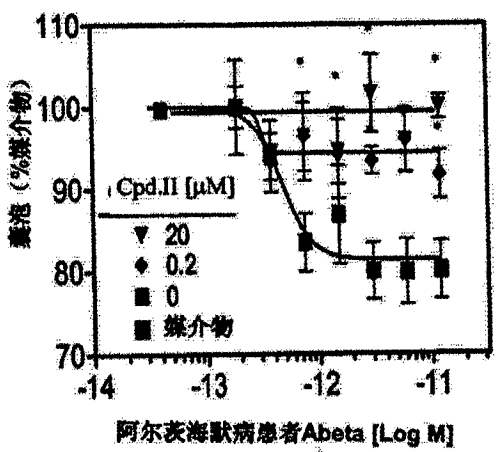
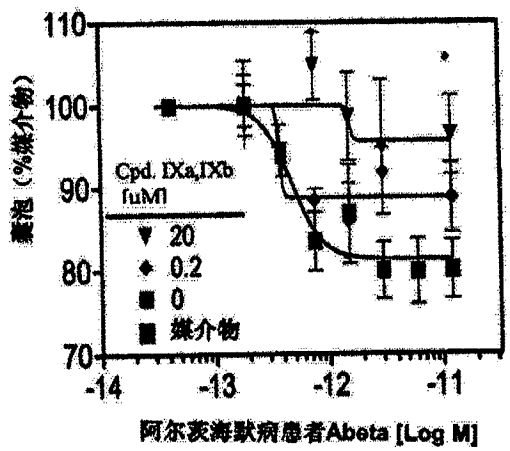


图 1G





* p<0.05 vs 对照 (ANOVA)

图1H 图1I

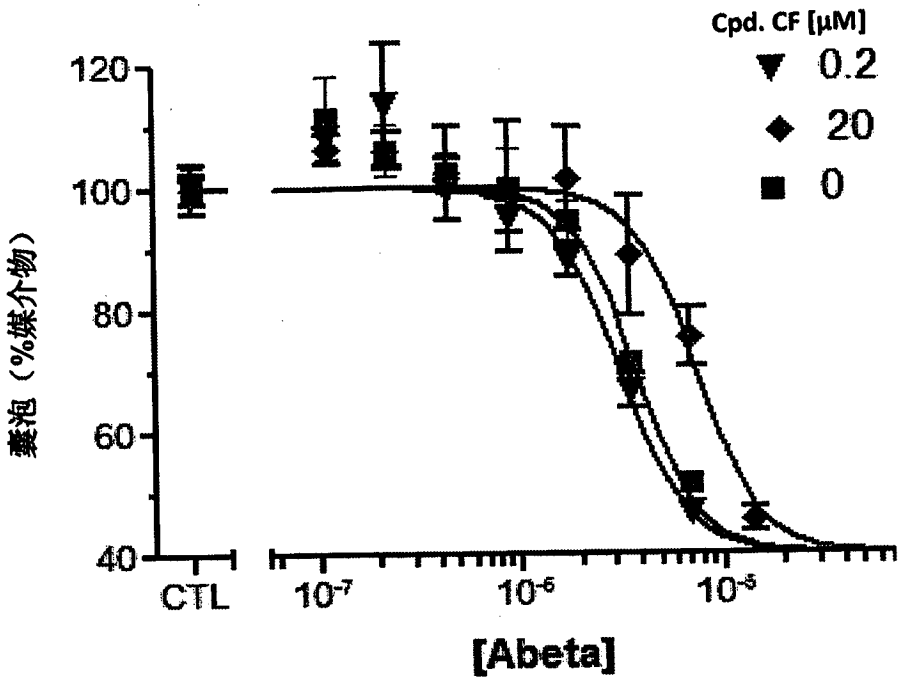

Cpd. CF [μM]
0.2
20
0
120
100
80
60
40
囊泡（%媒介物）
CTL
10-7
10-6
10-5
[Abeta]

图 1J

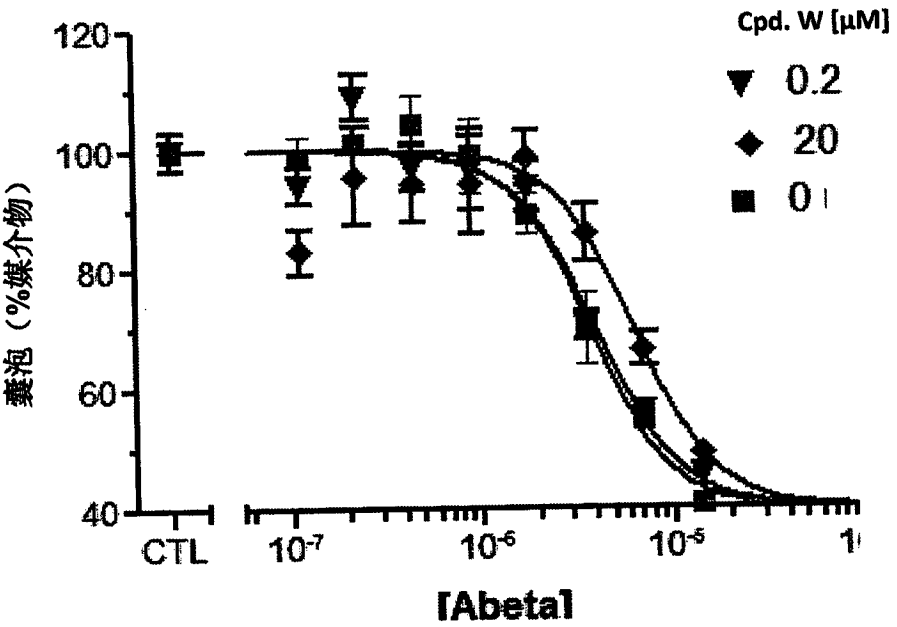

Cpd. W [μM]
0.2
20
0
120
100
80
60
40
囊泡（%媒介物）
CTL
10-7
10-6
10-5
[Abeta]

图 1K

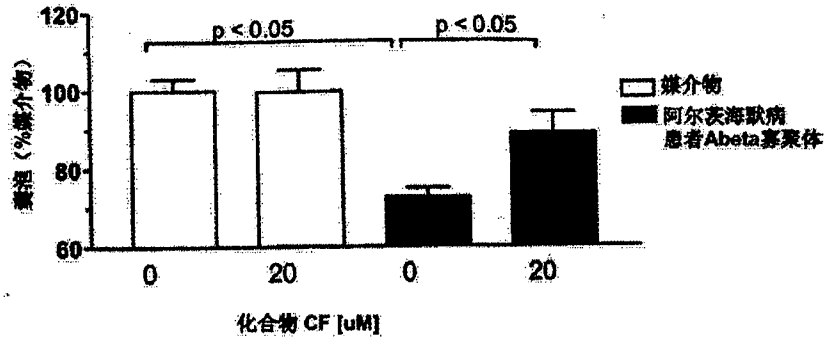

120
100
80
60
p < 0.05
p < 0.05
0
20
0
20
化合物 CF [uM]
媒介物
阿尔茨海默病患者Abeta寡聚体

图 1L

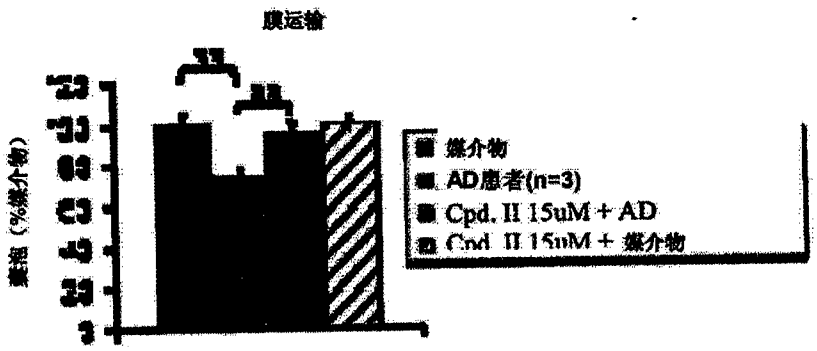

膜运输
媒介物
AD患者(n=3)
Cpd. II 15uM + AD
Cpd. II 15uM + 媒介物

图 1M

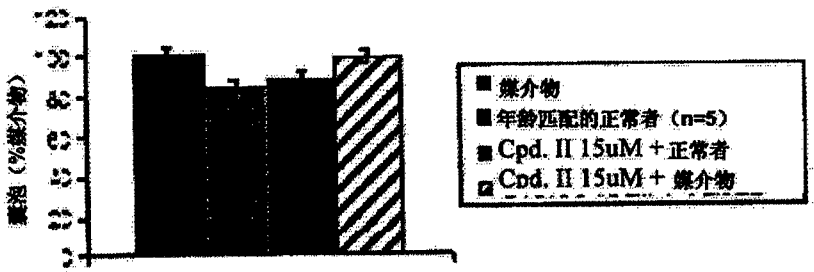

媒介物
年龄匹配的正常者 (n=5)
Cpd. II 15uM + 正常者
Cpd. II 15uM + 媒介物

图 1N

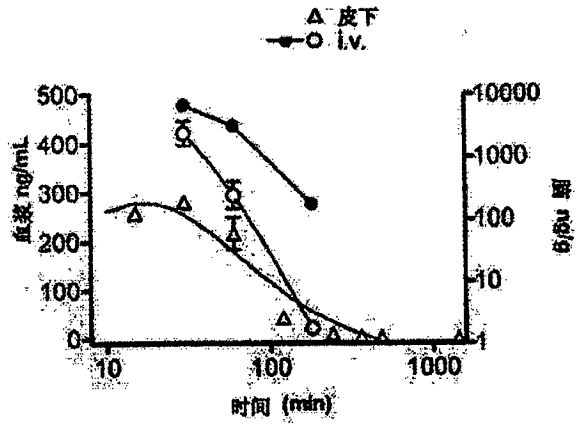

皮下
i.v.
500
400
300
200
100
0
血浆 ng/mL
10000
1000
100
10
1
10
100
1000
时间 (min)

图 2A

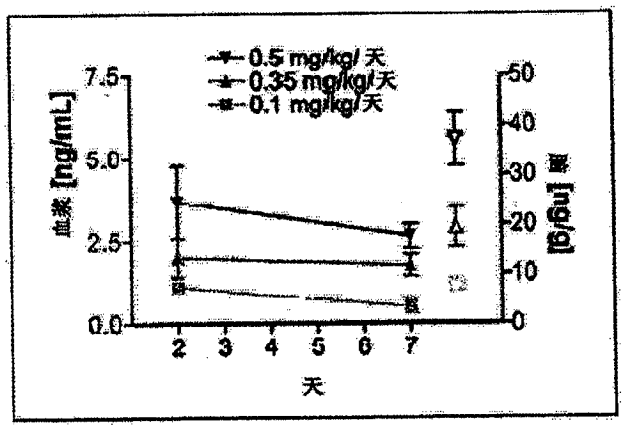

0.5 mg/kg/天
0.35 mg/kg/天
0.1 mg/kg/天
7.5
5.0
2.5
0.0
血浆 [ng/mL]
50
40
30
20
10
0
[ng/g]
2
3
4
5
6
7
天

图 2B

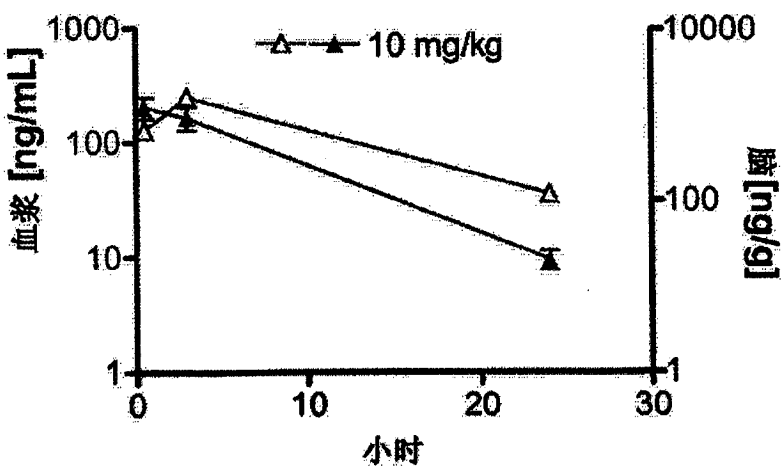

10 mg/kg
1000
100
10
1
血浆 [ng/mL]
10000
100
1
[ng/g]
0
10
20
30
小时

图 2C

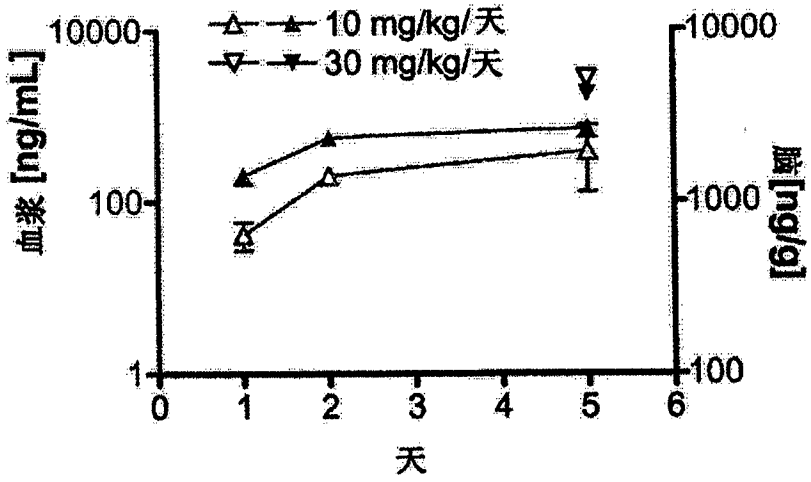

10 mg/kg/天
30 mg/kg/天
血浆 [ng/mL]
脑 [ng/g]
10000
100
1
10000
1000
100
0
1
2
3
4
5
6
天

图 2D

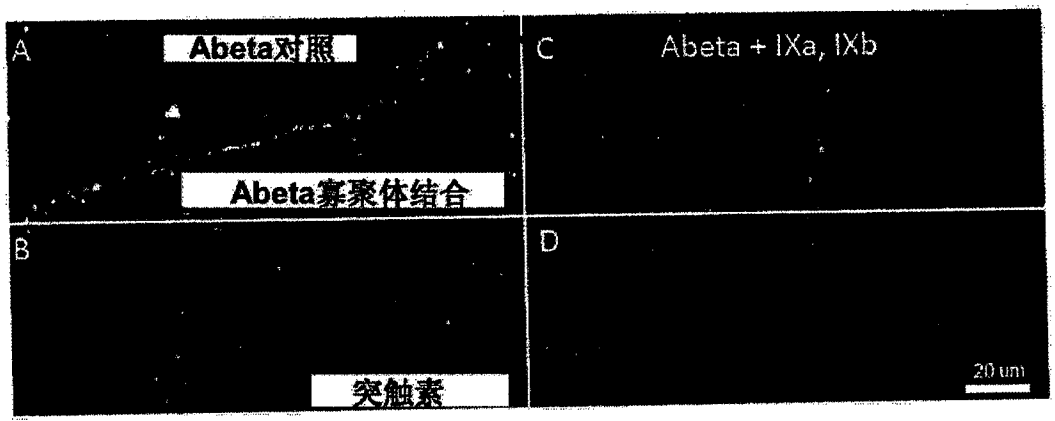

A
Abeta对照
Abeta寡聚体结合
C
Abeta + IXa, IXb
B
突触素
D
20 um

图 3A

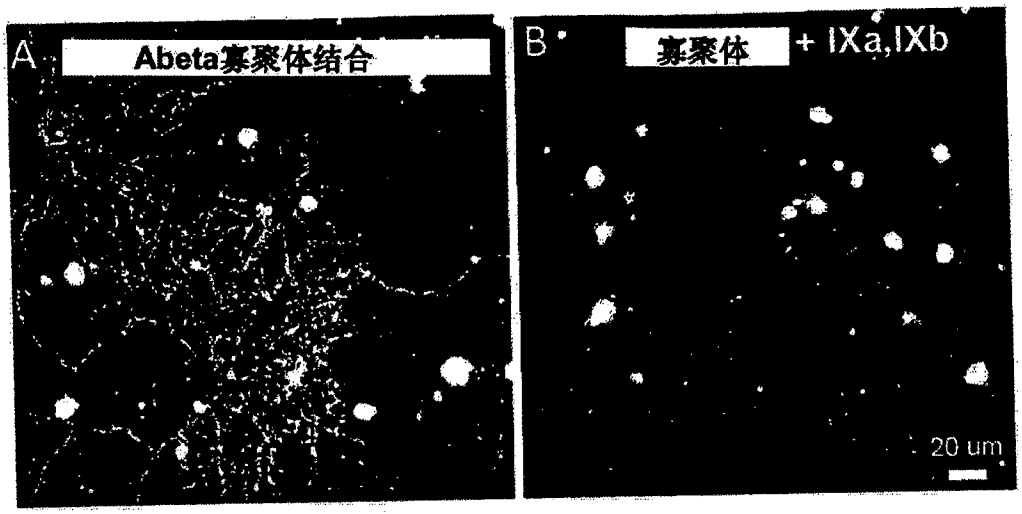

A
Abeta寡聚体结合
B
寡聚体
+ IXa,IXb
20 um

图 3B

**p<0.001, 单因素ANOVA
1.2
1.0
0.8
0.6
0.4
***
***
媒介物
IXa,IXb + 媒介物
II + 媒介物
Abeta
IXa,IXb + Abeta
II + Abeta

图 3C

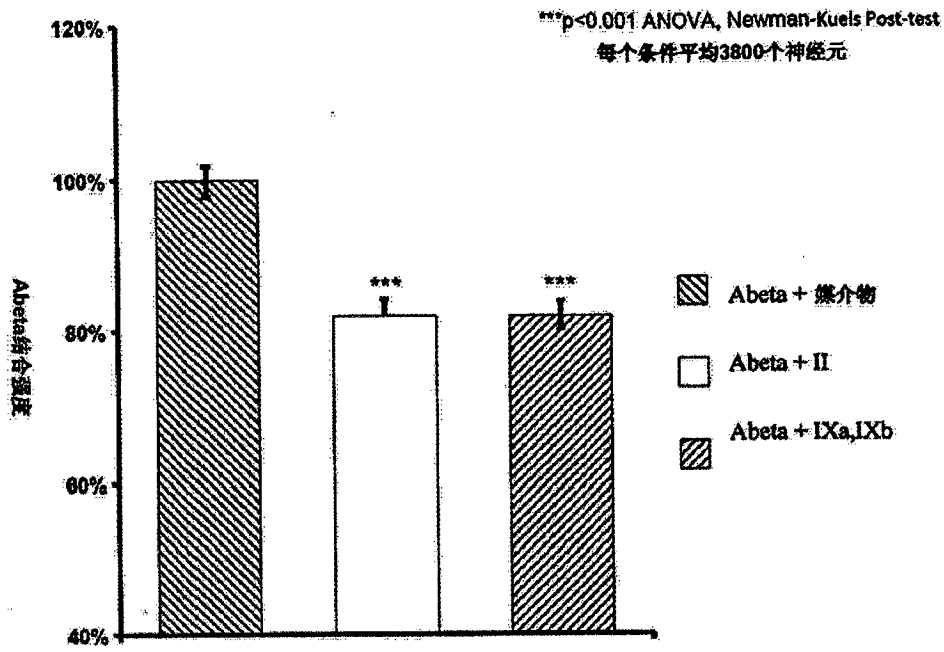

***p<0.001 ANOVA, Newman-Kuels Post-test
每个条件平均3800个神经元
120%
100%
80%
60%
40%
Abeta结合强度
***
***
Abeta + 媒介物
Abeta + II
Abeta + IXa,IXb

图 3D

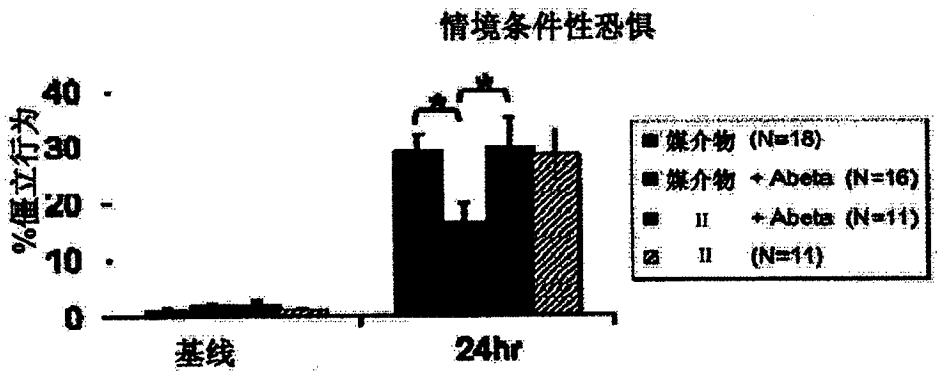

情境条件性恐惧
%僵立行为
40
30
20
10
0
基线
24hr
媒介物 (N=16)
媒介物 + Abeta (N=16)
II + Abeta (N=11)
II (N=11)

图 4

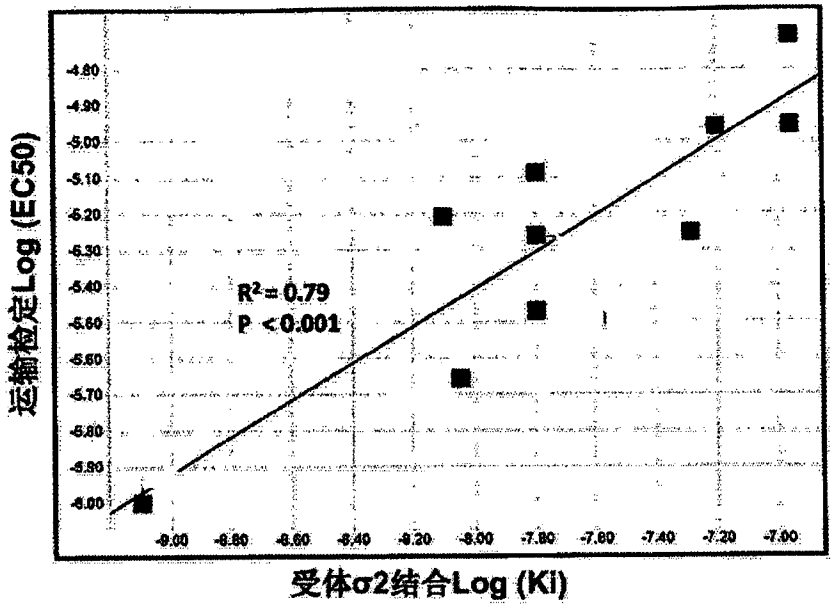

运输检定Log (EC50)
受体σ2结合Log (Ki)
R² = 0.79
P < 0.001

图 5A

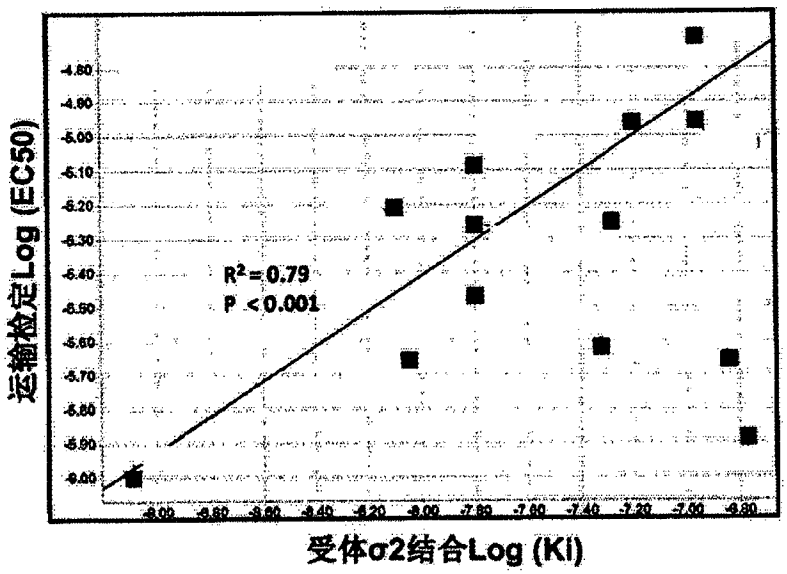

运输检定Log (EC50)
受体σ2结合Log (Ki)
R² = 0.79
P < 0.001

图 5B

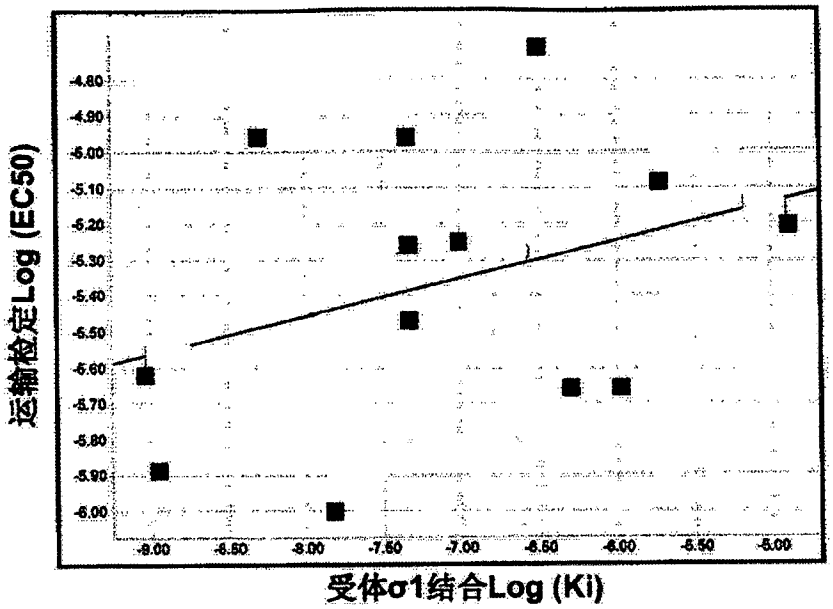

运输检定Log (EC50)
-4.80
-4.90
-5.00
-5.10
-5.20
-5.30
-5.40
-5.50
-5.60
-5.70
-5.80
-5.90
-6.00
-9.00
-8.50
-8.00
-7.50
-7.00
-6.50
-6.00
-5.50
-5.00
受体σ1结合Log (Ki)

图 5C

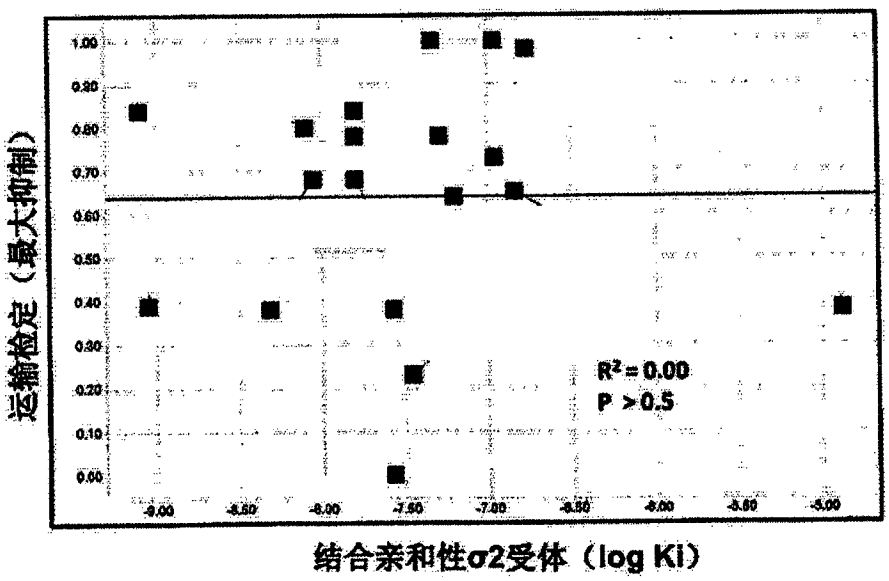

运输检定（最大抑制）
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
$R^2 = 0.00$
P > 0.5
-9.00
-8.50
-8.00
-7.50
-7.00
-6.50
-6.00
-5.50
-5.00
结合亲和性σ2受体（log Ki）

图 5D

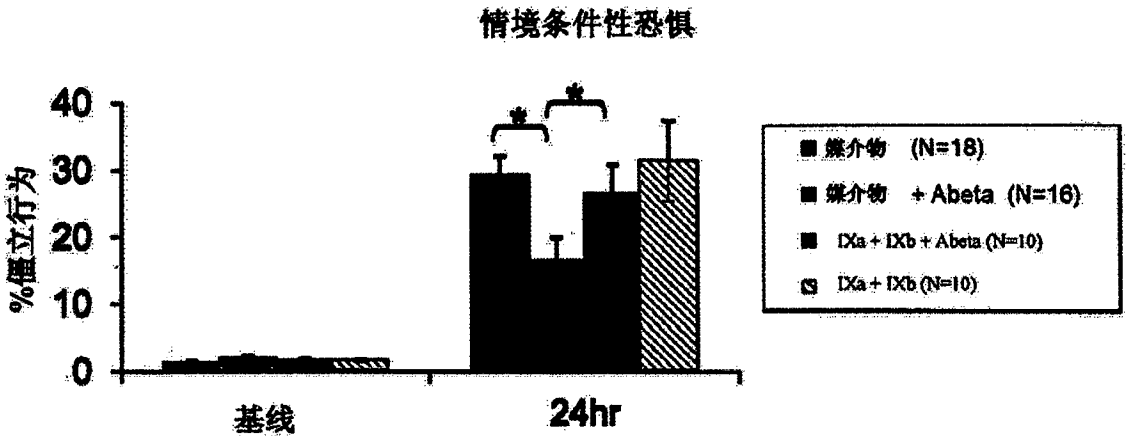

情境条件性恐惧
%僵立行为
40
30
20
10
0
基线
24hr
媒介物 (N=18)
媒介物 + Abeta (N=16)
IXa + IXb + Abeta (N=10)
IXa + IXb (N=10)

图 6

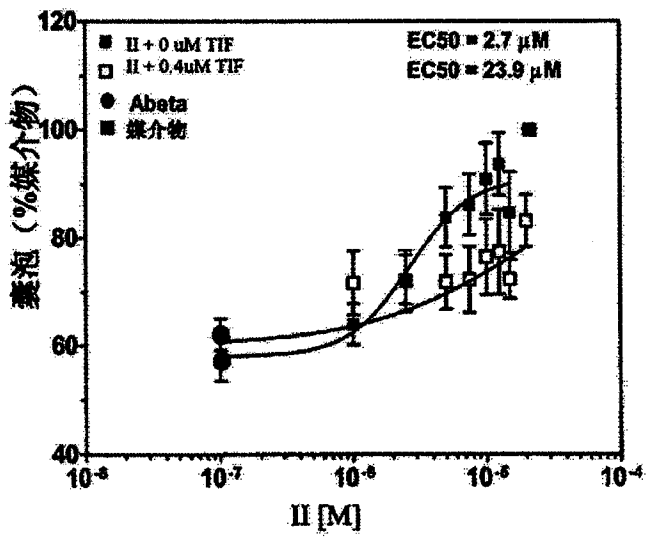

II + 0 uM TIF
II + 0.4uM TIF
Abeta
媒介物
EC50 = 2.7 μM
EC50 = 23.9 μM
囊泡（%媒介物）
120
100
80
60
40
II [M]

图 7A

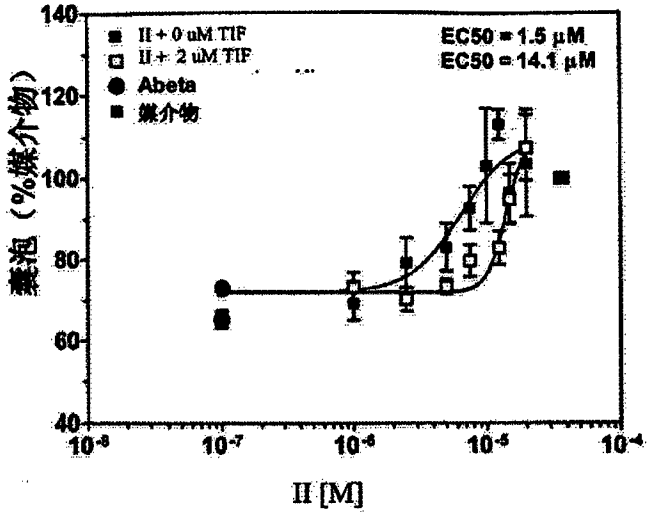

II + 0 uM TIF
II + 2 uM TIF
Abeta
媒介物
EC50 = 1.5 μM
EC50 = 14.1 μM
氚泡（%媒介物）
II [M]

图 7B

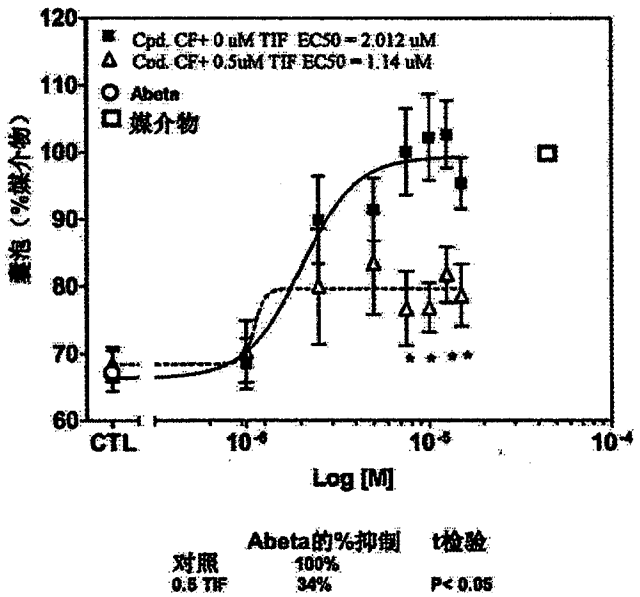

Cpd. CF+ 0 uM TIF EC50 = 2.012 uM
Cpd. CF+ 0.5uM TIF EC50 = 1.14 uM
Abeta
媒介物
氚泡（%媒介物）
CTL
Log [M]
Abeta的%抑制 t检验
对照 100%
0.5 TIF 34% P< 0.05

图 7C

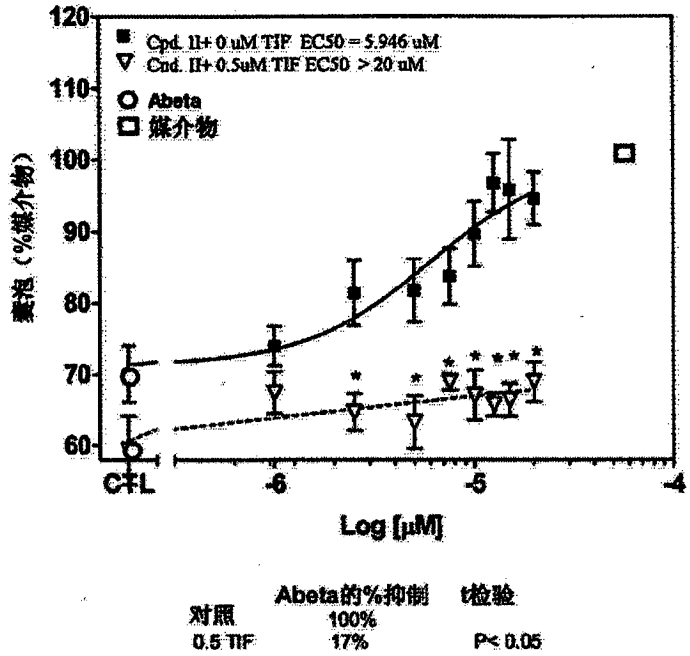

Cpd. II+ 0 uM TIF EC50 = 5.946 uM
Cpd. II+ 0.5uM TIF EC50 > 20 uM
Abeta
媒介物
氨泡（%媒介物）
120
110
100
90
80
70
60
C+L
-6
-5
-4
Log [μM]
Abeta的%抑制
t检验
对照
100%
0.5 TIF
17%
P< 0.05

图 7D

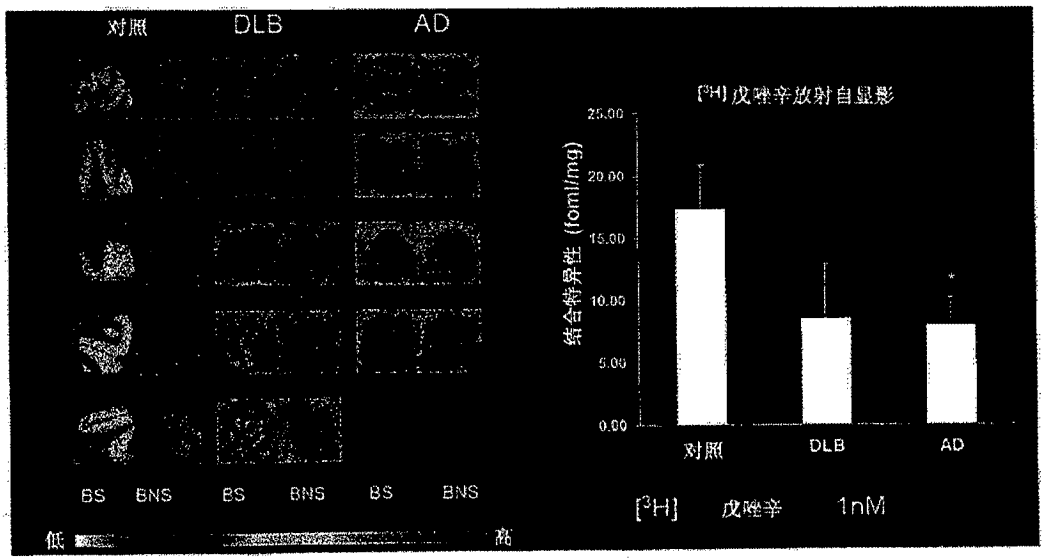

对照
DLB
AD
BS
BNS
BS
BNS
BS
BNS
低
高
[³H]戊唑辛放射自显影
结合特异性 (fomli/mg)
25.00
20.00
15.00
10.00
5.00
0.00
对照
DLB
AD
[³H] 戊唑辛 1nM

图 8A

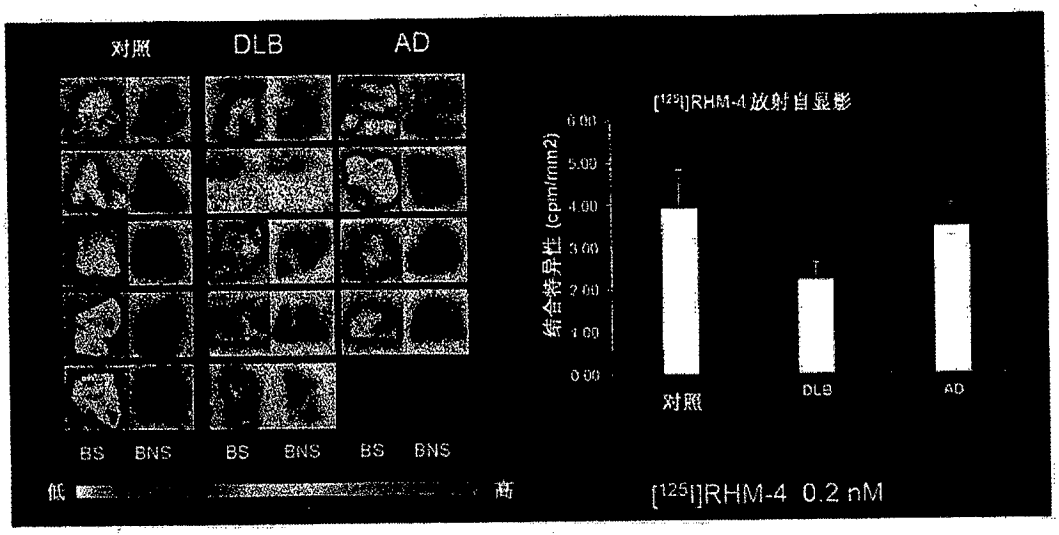

对照
DLB
AD
BS BNS BS BNS BS BNS
低
高
[125I]RHM-4 放射自显影
结合特异性 (cpm/mm2)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
对照
DLB
AD
[125I]RHM-4 0.2 nM

图 8B

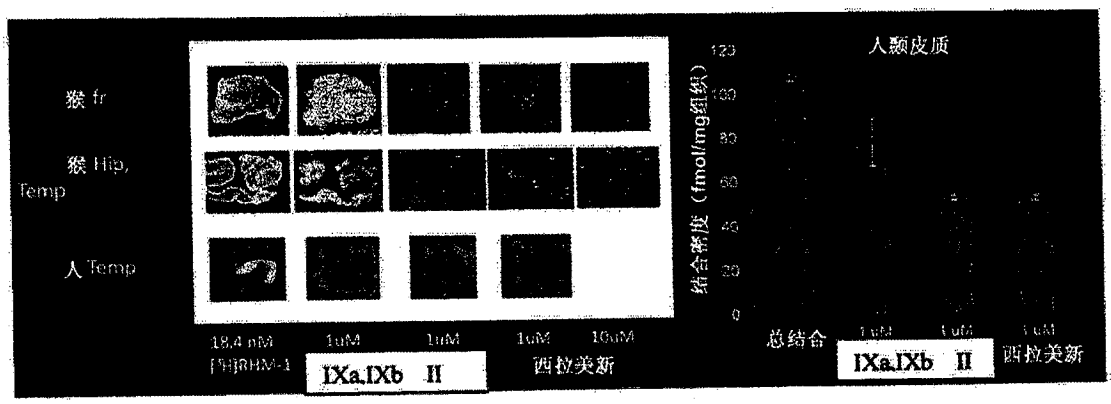

猴 fr
猴 Hip, Temp
人 Temp
18.4 nM [3H]RHM-1
1uM
1uM
1uM
10uM
IXa.IXb II
西拉美新
人额皮质
结合密度 (fmol/mg组织)
120
100
80
60
40
20
0
总结合
1uM
1uM
1uM
IXa.IXb II
西拉美新

图 8C

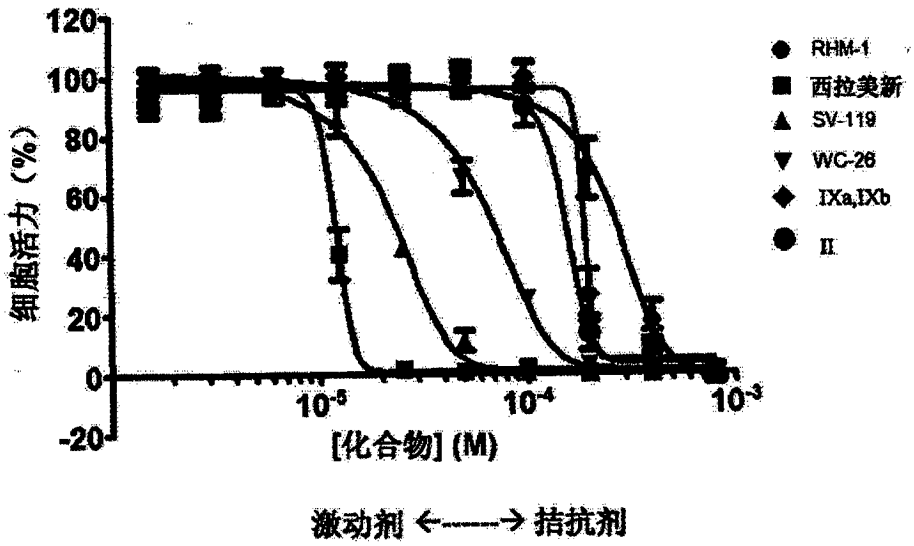

120
100
80
60
40
20
0
-20
细胞活力（%）
10^{-5}
10^{-4}
10^{-3}
[化合物] (M)
RHM-1
西拉美新
SV-119
WC-26
IXa,IXb
II
激动剂 ←——→ 拮抗剂

图 9A

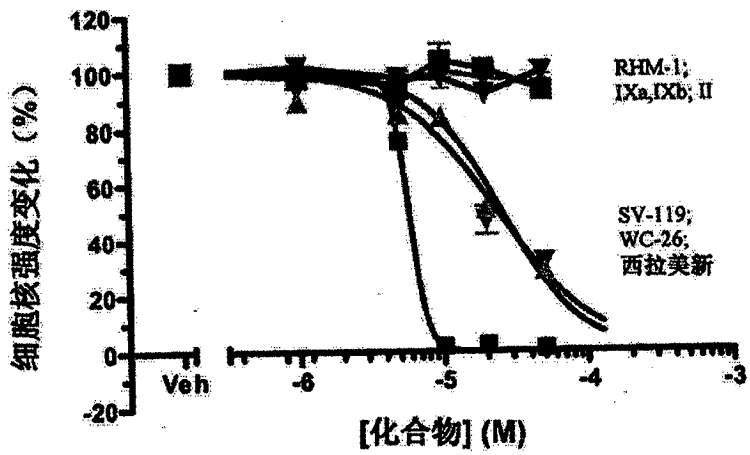

120
100
80
60
40
20
0
-20
细胞核强度变化（%）
Veh
-6
-5
-4
-3
[化合物] (M)
RHM-1;
IXa,IXb; II
SV-119;
WC-26;
西拉美新

图 9B

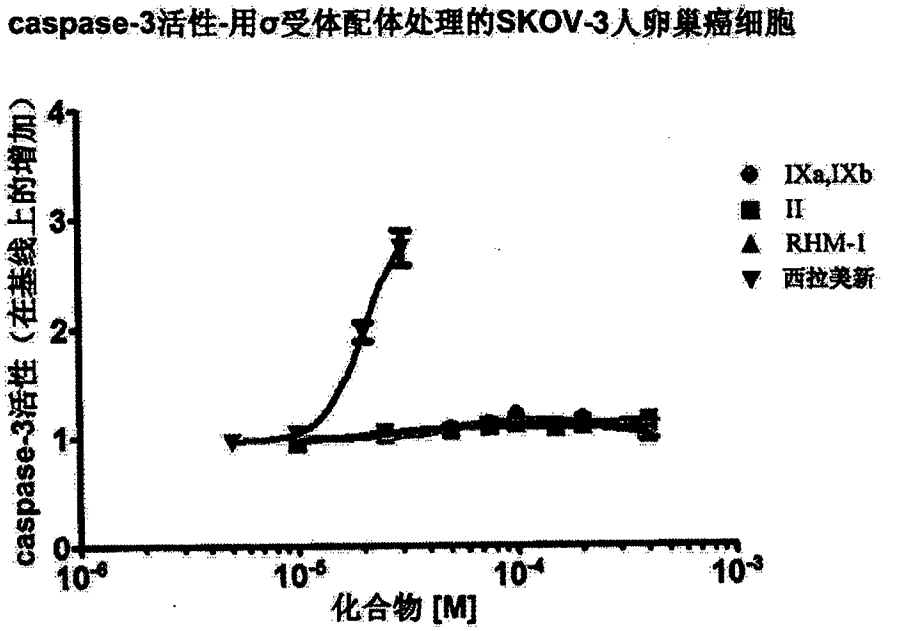

caspase-3活性-用σ受体配体处理的SKOV-3人卵巢癌细胞
caspase-3活性（在基线上的增加）
4
3
2
1
0
10-6
10-5
10-4
10-3
化合物 [M]
IXa,IXb
II
RHM-1
西拉美新

图 10A

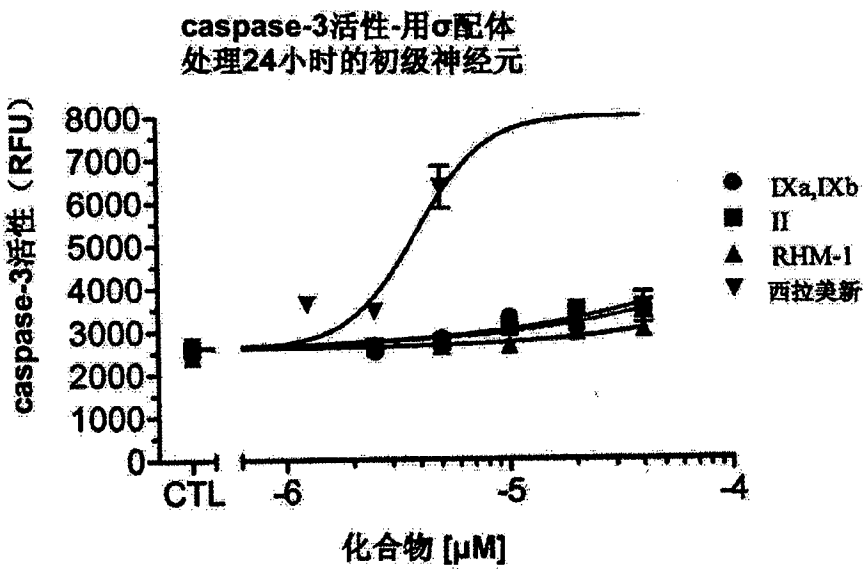

caspase-3活性-用σ配体
处理24小时的初级神经元
caspase-3活性（RFU）
8000
7000
6000
5000
4000
3000
2000
1000
0
CTL
-6
-5
-4
化合物 [μM]
IXa,IXb
II
RHM-1
西拉美新

图 10B

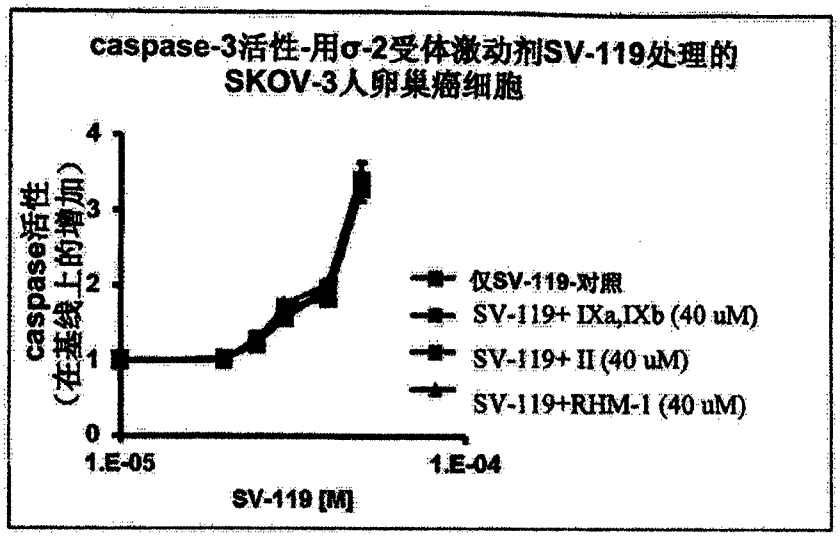

caspase-3活性-用σ-2受体激动剂SV-119处理的SKOV-3人卵巢癌细胞
caspase活性（在基线上的增加）
4
3
2
1
0
1.E-05
1.E-04
SV-119 [M]
仅SV-119-对照
SV-119+ IXa,IXb (40 uM)
SV-119+ II (40 uM)
SV-119+RHM-1 (40 uM)

图 10C

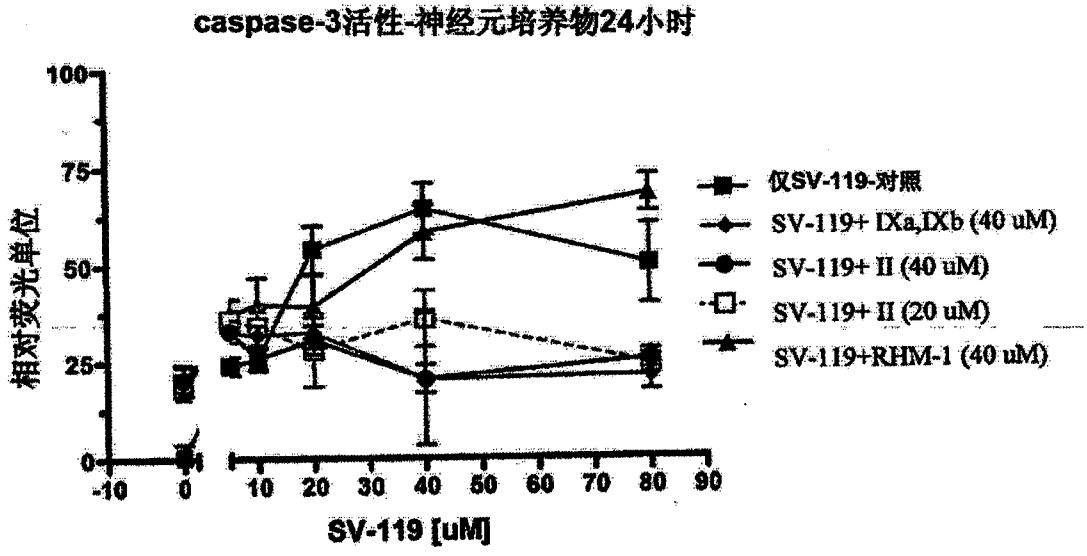

caspase-3活性-神经元培养物24小时
相对荧光单位
100
75
50
25
0
-10
0
10
20
30
40
50
60
70
80
90
SV-119 [uM]
仅SV-119-对照
SV-119+ IXa,IXb (40 uM)
SV-119+ II (40 uM)
SV-119+ II (20 uM)
SV-119+RHM-1 (40 uM)

图 10D

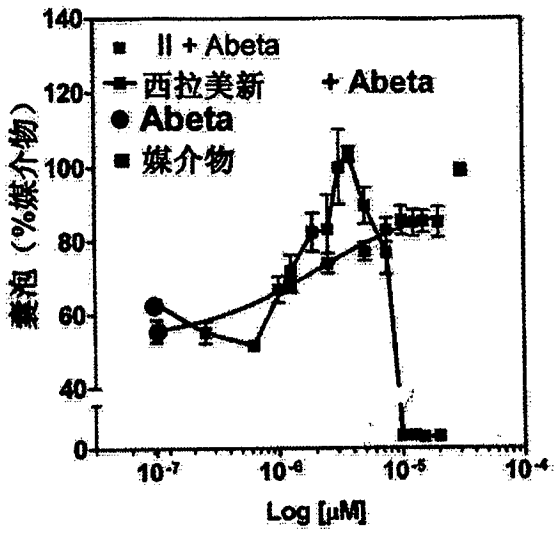


图 11A

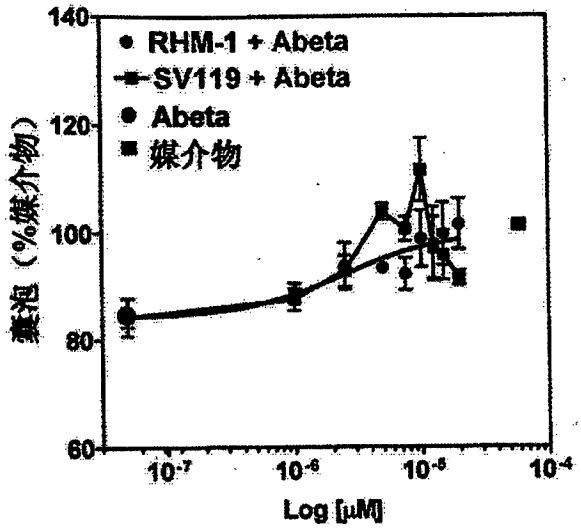


图 11B

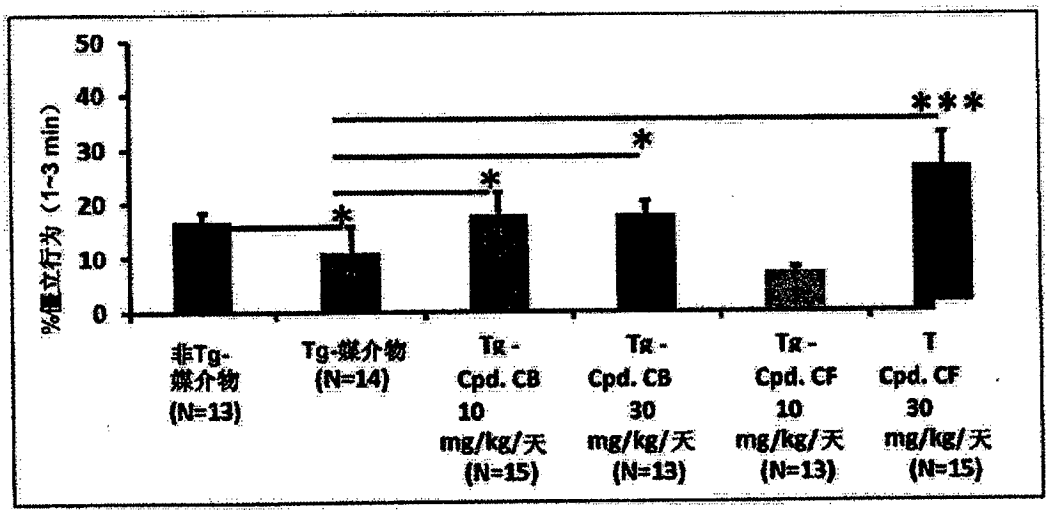


图 12A

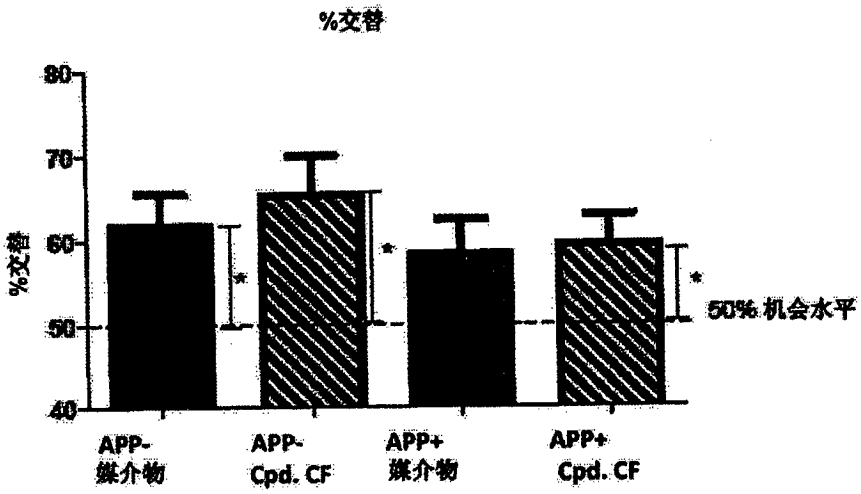

%交替
80
70
60
50
40
%交替
*
*
*
50% 机会水平
APP-
媒介物
APP-
Cpd. CF
APP+
媒介物
APP+
Cpd. CF

图 12B

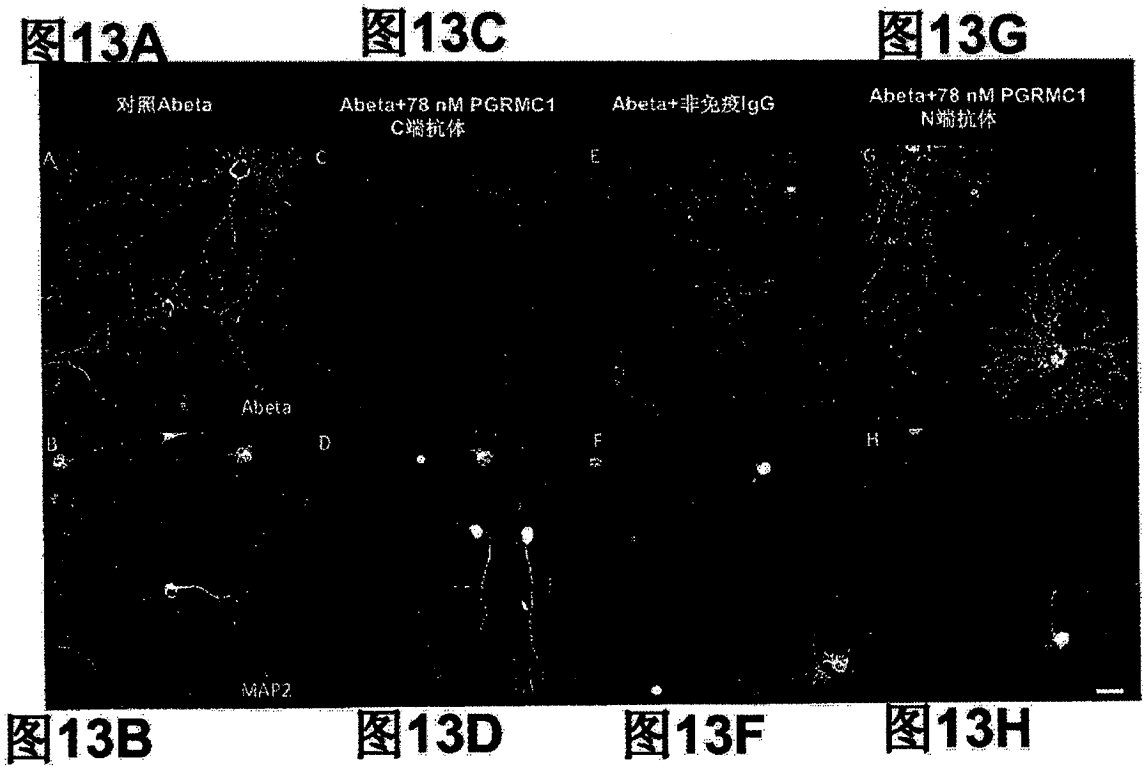


图13A
图13C
图13G
对照Abeta
Abeta+78 nM PGRMC1
C端抗体
Abeta+非免疫IgG
Abeta+78 nM PGRMC1
N端抗体
A
C
E
G
Abeta
B
D
F
H
MAP2
图13B
图13D
图13F
图13H

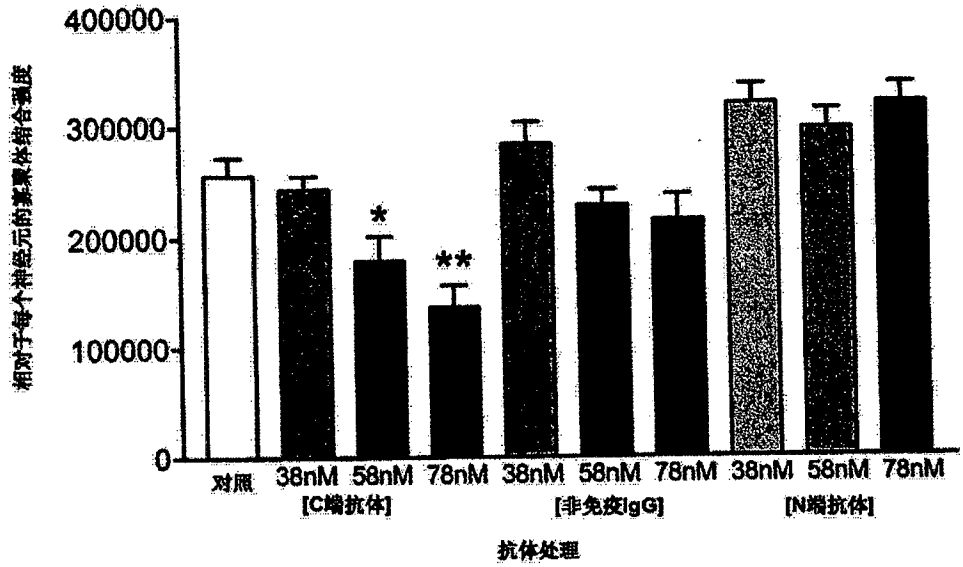

400000
300000
200000
100000
0
相对于每个神经元的聚集体结合强度
*
**
对照
38nM
58nM
78nM
38nM
58nM
78nM
38nM
58nM
78nM
[C端抗体]
[非免疫IgG]
[N端抗体]
抗体处理

图 14A

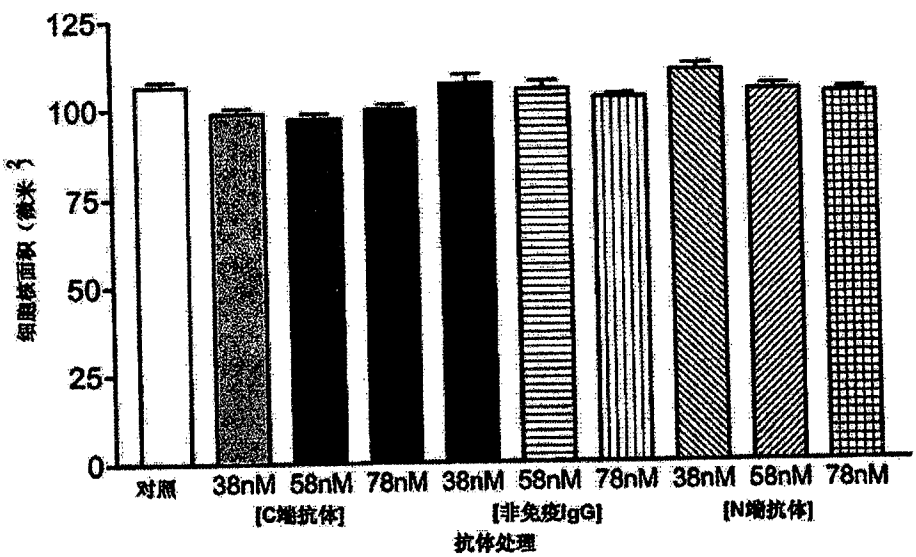

125
100
75
50
25
0
细胞核面积（微米²）
对照
38nM
58nM
78nM
38nM
58nM
78nM
38nM
58nM
78nM
[C端抗体]
[非免疫IgG]
[N端抗体]
抗体处理

图 14B