



US007786162B2

(12) **United States Patent**
Greig et al.

(10) **Patent No.:** **US 7,786,162 B2**
(45) **Date of Patent:** ***Aug. 31, 2010**

(54) **AGENTS USEFUL FOR REDUCING
AMYLOID PRECURSOR PROTEIN AND
TREATING DEMENTIA AND METHODS OF
USE THEREOF**

2001/0007877 A1 7/2001 Burton et al.
2002/0004198 A1 1/2002 Hardwicke

(75) Inventors: **Nigel H. Greig**, Phoenix, MD (US);
Karen T. Y. Shaw, St. Laurent (CA);
Qiang-Sheng Yu, Lutherville, MD (US);
Harold W. Holloway, Middle River, MD
(US); **Tada Utsuki**, West Chester, PA
(US); **Timothy T. Soncrant**, Silver
Spring, MD (US); **Donald K. Ingram**,
Ellicott City, MD (US); **Arnold Brossi**,
Bethesda, MD (US); **Anthony**
Giordano, Phoenixville, PA (US);
Gordon Powers, Malvern, PA (US);
Diane M. Davidson, Collegeville, PA
(US); **Michael Sturgess**, Quakertown,
PA (US)

FOREIGN PATENT DOCUMENTS

EP	0506532	9/1992
JP	7-271066	10/1995
WO	WO 95/01334	1/1995
WO	WO 96/03377	2/1996
WO	WO 99/02154	1/1999
WO	WO 99/43654	9/1999
WO	WO 01/34146	5/2001
WO	WO 01/78721	10/2001

(73) Assignees: **Raptor Pharmaceutical Corp.**, Novato,
CA (US); **National Institutes of Health**
(**NIH**), Bethesda, MA (US)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

This patent is subject to a terminal dis-
claimer.

(21) Appl. No.: **12/357,115**

(22) Filed: **Jan. 21, 2009**

(65) **Prior Publication Data**

US 2009/0131501 A1 May 21, 2009

Related U.S. Application Data

(63) Continuation of application No. 11/455,959, filed on
Jun. 20, 2006, now abandoned.

(51) **Int. Cl.**
A61K 31/40 (2006.01)
C07D 487/00 (2006.01)

(52) **U.S. Cl.** **514/411**; 548/429

(58) **Field of Classification Search** 514/411;
548/429

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2,890,223	A	6/1959	Woley et al.
4,791,107	A	12/1988	Hamer et al.
4,900,748	A	2/1990	Brossi et al.
5,171,750	A	12/1992	Brossi et al.
5,378,723	A	1/1995	Brossi et al.
5,403,851	A	4/1995	D'Orlando et al.
5,726,323	A	3/1998	Glamkowski et al.
6,410,747	B1	6/2002	Greig et al.
6,683,105	B2	1/2004	Greig et al.
6,706,750	B1	3/2004	Bentley et al.
7,153,882	B2 *	12/2006	Greig et al. 514/411

OTHER PUBLICATIONS

Adem et al., "Muscarinic receptors in human SH-SY5Y neuroblastoma cell line: regulation by phorbol ester and retinoic acid-induced differentiation." *Dev. Brain Res.* 33:235-242 (1987).

Akiyama et al., "Inflammation and Alzheimer's disease." *Neurobiol. Aging* 21:383-421 (2000).

Atack et al., "Comparative Inhibitory Effects of Various Physostigmine Analogs Against Acetyl- and Butyrylcholinesterases." *J. Pharmacol. Exp. Ther.* 249:194 (1989).

Becker et al., "The second generation of cholinesterase inhibitors: clinical and pharmacological effects." *The Cholinergic Basis for Alzheimer Therapy*. Becker et al., eds. Birkhauser, Boston, 263-296 (1991).

Bhasker et al., "The Putative Iron-Responsive Element in the Human Erythroid 5-Aminolevulinatase Synthase mRNA Mediates Translational Control." *J. Biol. Chem.* 268(17):12699-12705 (Jun. 15, 1993).

Borchelt et al., "Accelerated Amyloid Deposition in the Brains of Transgenic Mice Coexpressing Mutant Presenilin 1 and Amyloid Precursor Proteins." *Neuron*. 19(4):939-945 (Oct. 1997).

Breitner, "Inflammatory Processes and Antiinflammatory Drugs in Alzheimer's Disease: A Current Appraisal." *Neurobiol. Aging* 17(5):789-794 (1996).

Bronfman et al., "Amyloid Precursor Protein Fragment and Acetylcholinesterase Increase with Cell Confluence and Differentiation in a Neuronal Cell Line." *Exp. Cell Res.* 229:93-99 (1996).

Brossi et al., "Phenserine, a Novel Anticholinesterase Related to Physostigmine: Total Synthesis, and Biological Properties." *Austr. J. Chem.* 49:171-181 (1996).

(Continued)

Primary Examiner—Rei-tsang Shiao

(74) *Attorney, Agent, or Firm*—Drinker Biddle & Reath LLP

(57) **ABSTRACT**

The present invention provides compounds and methods of administering compounds to a subject that can reduce β APP production and that is not toxic in a wide range of dosages. The present invention also provides non-carbamate compounds and methods of administering such compounds to a subject that can reduce β APP production and that is not toxic in a wide range of dosages. It has been discovered that either the racemic or enantiomerically pure non-carbamate compounds can be used to decrease β APP production.

21 Claims, 36 Drawing Sheets

OTHER PUBLICATIONS

- Buxbaum et al., "Processing of Alzheimer β /A4 amyloid precursor protein: Modulation by agents that regulate protein phosphorylation." *Proc. Natl. Acad. Sci. USA* 87(15):6003-6006 (Aug. 1990).
- Buxbaum et al., "Calcium regulates processing of the Alzheimer amyloid protein precursor in a protein kinase C-independent manner." *Proc. Natl. Acad. Sci. USA* 91:4489-4493 (May 1994).
- Buxbaum et al., "Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer β /A4 amyloid protein precursor." *Proc. Natl. Acad. Sci. USA* 89:10075-10078 (Nov. 1992).
- Caputi et al., Increase Secretion of the Amino-Terminal Fragment of Amyloid Precursor Protein in Brains of Rats with a Constitutive Up-Regulation of Protein Kinase C. *J. Neurochem.* 68(6):2523-2529 (1997).
- Checler, "Processing of β -Amyloid Precursor Protein and Its Regulation in Alzheimer's Disease." *J. Neurochem.* 65(4):1431-1444 (1995).
- Desdouts et al., "Amyloid β Peptide Formation in Cell-Free Preparations: Regulation by protein kinase C, calmodulin and calcineurin." *J. Biol. Chem.* 271(40):24670-24674 (Oct. 4, 1996).
- Desdouts-Magnen et al., "Regulation of Secretion of Alzheimer Amyloid Precursor Protein by the Mitogen-Activated Protein Kinase Cascade." *J. Neurochem.* 70(2):524-530 (1998).
- Dyrks et al., "Amyloid precursor protein secretion and β A4 amyloid generation are not mutually exclusive." *FEBS Lett.* 349:210-214 (1994).
- Eisenstein et al., "Iron-responsive Element-binding Protein: phosphorylation by protein kinase C." *J. Biol. Chem.* 268(36):27363-27370 (Dec. 25, 1993).
- Felder et al., "Muscarinic Acetylcholine Receptor Subtypes Associated with Release of Alzheimer Amyloid Precursor Derivatives Activate Multiple Signal Transduction Pathways." *Ann. NY Acad. Sci.* 695:15-18 (1993).
- Funato et al., "Astrocytes Containing Amyloid b-Protein (β)-Positive Granules Are Associated with $\text{A}\beta$ 40-Positive Diffuse Plaques in the Aged Human Brain." *Am. J. Path.* 152(4):983-992 (Apr. 1998).
- Giacobini et al., "The Effect of Cholinesterase Inhibitors on the Secretion of APPs from Rat Brain Cortex." *Ann. NY Acad. Sci.* 777:393-398 (1996).
- Greig et al., "Phenserine and Ring-C Hetero-Analogues: Drug Candidates for the Treatment of Alzheimer's Disease." *Med. Chem. Rev.* 15(1):3-31 (1995).
- Haroutunian et al., "Pharmacological modulation of Alzheimer's β -amyloid precursor protein levels in the CSF of rats with forebrain cholinergic system lesions." *Mol. Brain Res.* 46(1-2):161-168 (1997).
- He et al., Thiaphysovenine and Carbamate Analogues: A New Class of Potent Inhibitors of Cholinesterases. *Med. Chem. Res.* 2:229-237 (1992).
- Hentze et al., "Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron, nitric oxide, and oxidative stress." *Proc. Natl. Acad. Sci. USA.* 93:8175-8182 (Aug. 1996).
- Hung et al., "Selective ectodomain phosphorylation and regulated cleavage of β -amyloid precursor protein." *EMBO J.* 13(3):534-542 (1994).
- Hussaain et al., "Identification of a Novel Aspartic Protease (Asp 2) as β -Secretase." *Mol. Cell Neurosci.* 14:419-427 (1999).
- Jacobsen et al., The Release of Alzheimer's Disease β Amyloid Peptide Is Reduced by Phorbol Treatment: *J. Biol. Chem.* 269(11):8376-8382 (Mar. 18, 1994).
- Julian et al., "Studies in the Indole Series. IV. The Synthesis of d,1-Eserethole." *J. Am. Chem. Soc.* 57:563-566 (Mar. 1935).
- Kim et al., Identification of a Conserved and Functional Iron-responsive Element in the 5'-Untranslated Region of Mammalian Mitochondrial Aconitase. *J. Biol. Chem.* 271(39):24226-24230 (Sep. 27, 1996).
- Koike et al., "Thimet Oligopeptidase Cleaves the Full-Length Alzheimer Amyloid Precursor Protein at a β -Secretase Cleavage Site in COS Cell". *J. Biochem.* 126:235-242 (1999).
- Lahiri et al., "Effects of Cholinesterase Inhibitors on the Secretion of Beta-Amyloid Precursor Protein in Cell Cultures." *Ann. NY Acad. Sci.* 26(826):416-421 (1997).
- Lahiri et al., "Cholinesterase inhibitors, Beta-amyloid precursor protein and amyloid β -peptidases in Alzheimers disease." *Acta Neurol. Scand. Suppl.* 176:60-67 (2000).
- Lahiri et al., The secretion of amyloid β -peptides is inhibited in tacrine-treated human neuroblastoma cell. *Mol. Brain Res.* 62:131-140 (1998).
- LeBlanc et al., Protein Kinase C Activation Increases Release of Secreted Amyloid Precursor Protein without Decrease $\text{A}\beta$ Production in Human Primary Neuron Cultures. *J. Neurosci.* 18(8):2907-2913 (Apr. 15, 1998).
- Lee Wong, "High-performance liquid chromatographic enantioseparation of intermediates relating to the total synthesis of (-)-physostigmine." *J. Chromat.* 523:317-320 (1990).
- Lee et al., "Asymmetric Alkylation of Oxindoles: An Approach to the Total Synthesis of (-)-Physostigmine." *J. Org. Chem.* 56:872-875 (1991).
- Leli et al., "Distinct Mechanism of Differentiation of SH-SY5Y Neuroblastoma Cells by Protein Kinase C Activators and Inhibitors." *J. Neurochem.* 58(4):1191-1198 (1992).
- Matsuura et al., "Catalytic Asymmetric Synthesis of Either Enantiomer of the Calabar Alkaloids Physostigmine and Physovenine." *J. Am. Chem. Soc.* 120(26):6500-6503 (1998).
- Meleforts et, "Translational Control of 5-Aminolevulinic Synthase mRNA by Iron-responsive Elements in Erythroid Cells." *J. Biol. Chem.* 268(8):5974-5978 (Mar. 15, 1993).
- Nitsch et al., "Regulation of APP Processing by First Messengers" *Alzheimer Disease: Therapeutic Strategies.* Giacobini et al., eds. Birkhauser, Boston. pp. 54-61 (1994).
- Nitsch et al., "Release of Alzheimer Amyloid Precursor Derivatives Stimulated by Activation of Muscarinic Acetylcholine Receptors." *Science* 258:304-307 (Oct. 9, 1992).
- Patel et al., "Phenserine, a novel acetylcholinesterase inhibitor, attenuates impaired learning of rats in a 14-unit T-maze induced by blockade of the N-methyl-D-aspartate receptor." *Neuroreport.* 9(1):171-176 (1998).
- Pei et al., "125. Total Synthesis of Racemic and Optically Active Compounds Related to Physostigmine and Ring-C Heteroanalogues from 3-[2'-(Dimethylamino)ethyl]-1,2,3-dihydro-5-methoxy-1,3-dimethyl-1H-indol-2-ol." *Helv. Chem. Acta.* 77:1412-1422 (1994).
- Pei et al., "Facile Preparation of (3S)-1,3-Dimethyl-1,3-cyanomethyl-5-ethoxyoxindole from Julian's Nitrile Enriched in the (3S)-Enantiomer." *Heterocycles* 41(12):2823-2826 (1995).
- Pei et al., "Preparation and Selective Inhibition of Human Butyrylcholinesterase by N1-Phenethylnorphysostigmine Analogues." *Med. Chem. Res.* 5:455-461 (1995).
- Pei et al., "Inhibition of Human Acetylcholinesterase by Unnatural (+)-(3aR)-N1-Norphysostigmine and Arylcarbamate." *Med. Chem. Res.* 5:265-270 (1995).
- Pei et al., "Syntheses and Biological Evaluation of Ring-C Opened Analogues of the Cholinesterase Physostigmine, Phenserine and Cymserine." *Med. Chem. Res.* 9(1):50-60 (1999).
- Roberson et al., "Cholinergic activity and amyloid precursor protein metabolism." *Brain Res. Rev.* 25:50-69 (1997).
- Rogers et al., "Translation of the Alzheimer Amyloid Precursor Protein mRNA Is Up-regulated by Interleukin 1 through 5'-untranslated Region Sequences." *J. Biol. Chem.* 274(10):6421-6431 (Mar. 5, 1999).
- Savage et al., "Turnover of Amyloid β -Protein in Mouse Brain and Acute Reduction of Its Level by Phorbol Ester." *J. Neurosci.* 18(5):1743-1752 (Mar. 1, 1998).
- Schalinske et al., "Iron Differentially Stimulates Translation of Mitochondrial Aconitase and Ferritin mRNAs in Mammalian Cells: Implications for iron regulatory proteins as regulators of mitochondrial citrate utilization." *J. Biol. Chem.* 273(6):3740-3746 (Feb. 6, 1998).
- Selkoe, "Alzheimer's Disease: Genotypes, Phenotypes, and Treatments." *Science* 275:630-631 (Jan. 31, 1997).
- Sinha et al., "Purification and cloning of amyloid precursor protein β -secretase from human brain." *Nature* 402:537-540 (1999).

- Suzuki et al., "An Increased Percentage of Long Amyloid- β Protein Secreted by Familial Amyloid- β Protein-Precursor (13-APP717) Mutants." *Science* 264:1336-1340 (May 27, 1994).
- Vassar et al., " β -Secretase Cleavage of Alzheimer's Amyloid Precursor Protein by the Transmembrane Aspartic Protease BACE." *Science* 286:735-741 (Oct. 22, 1999).
- Waskiewicz et al., "Mitogen and stress response pathways: MAP kinase cascades and phosphates regulation in mammals and yeast." *Curr. Opin. Cell Biol.* 7:798-805 (1995).
- Wisniewski et al., "Review—David Oppenheimer Memorial Lecture 1995: Some neuropathological aspects of Alzheimer's disease and its relevance to other disciplines." *Neuropathol. Appl. Neurobiol.* 22(1):3-11 (1996).
- Xu et al., "Regulated Formation of Golgi Secretory Vesicles Containing Alzheimer β -Amyloid Precursor Protein." *J. Biol. Chem.* 270(40):23243-23245 (Oct. 6, 1995).
- Yan et al., "Membrane-anchored aspartyl protease with Alzheimer's disease β -secretase activity." *Nature* 402:533-537 (1999).
- Yu et al., "Synthesis and Anticholinesterase Activity of (-)-N1-Norphysostigmine, (-)-Eseramine and Other N(1)-Substituted Analogues of (-)-Physostigmine." *J. Med. Chem.* 31:2297-2300 (Dec. 1988).
- Yu et al., "Physovenines: Efficient Synthesis of (-)- and (+)-Physovenine and Synthesis of Carbamate Analogues of (-)-Physovenine. Anticholinesterase Activity and Analgesic Properties of Optically Active Physovenines." *Helv. Chimica. Acta.* 74:761-764 (1991).
- Yu et al., "Progress Towards a Practical Total Synthesis of Calabar Alkaloids: Total Synthesis of (-)-Esermethole and (-)-Physovenol Methyl Ether From (3s)-1,3 -Dimethyl-3-Carboxymethyl-5-Methoxyoxinbole." *Heterocycles* 36(6): 1279-1285 (1993).
- Yu et al., "Synthesis and Anticholinesterase Activities of (3aS)-N1,N8-Bisnorpheneserine, (3aS)-N1,N8-Bisnorphysostigmine, Their Antipodal Isomers, and Other Potential Metabolites of Phenenserine." *J. Med. Chem.* 41:2371-2379 (1998).
- Yu et al., "Synthesis of Novel Phenenserine-Based-Selective Inhibitors of Butyrylcholinesterase for Alzheimers Disease." *J. Med. Chem.* 42(10):1855-1861 (1999).
- Yu et al., "Total Synthesis of Racemic Physostigmine, Physovenine and Its Sulfur Analogue by the Oxindole-5-O-Tetrahydropyranyl Ether Route." *Heterocycles* 39(2):519-525 (1994).
- Yu et al., "Practical Synthesis of Unnatural (+)-Physostigmine and Carbamate Analogues." *Heterocycles* 27(3):745-751 (1988).
- Yu et al., "Total Synthesis and Anticholinesterase Activities of (3aS)-N(8)-Norphysostigmine, (3aS)-N(8)-Norpheneserine, Their Antipodal Isomers, and Other N(8)-Substituted Analogues." *J. Med. Chem.* 40:2895-2901 1997.
- Zhu et al., "A practical conversion of natural physostigmine into the potent butyrylcholinesterase inhibitor N1,N8-bisnorcymserine." *Tet. Lett.* 41:4861-4864 (2000).
- * cited by examiner

Total syntheses of analogues of (+)-physostigmine

1, Synthesis of intermediate (+)-N1-nor-esermethole

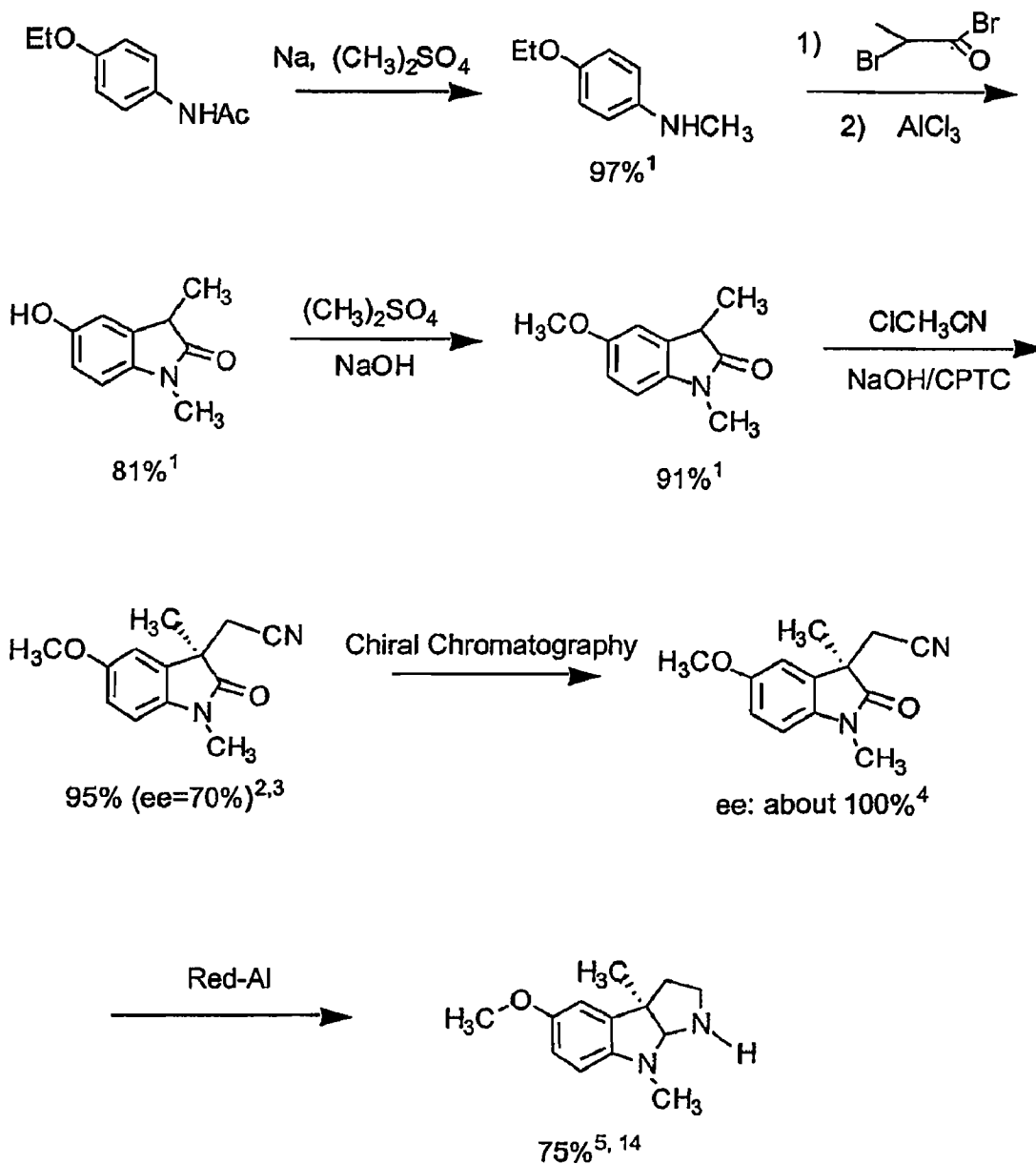
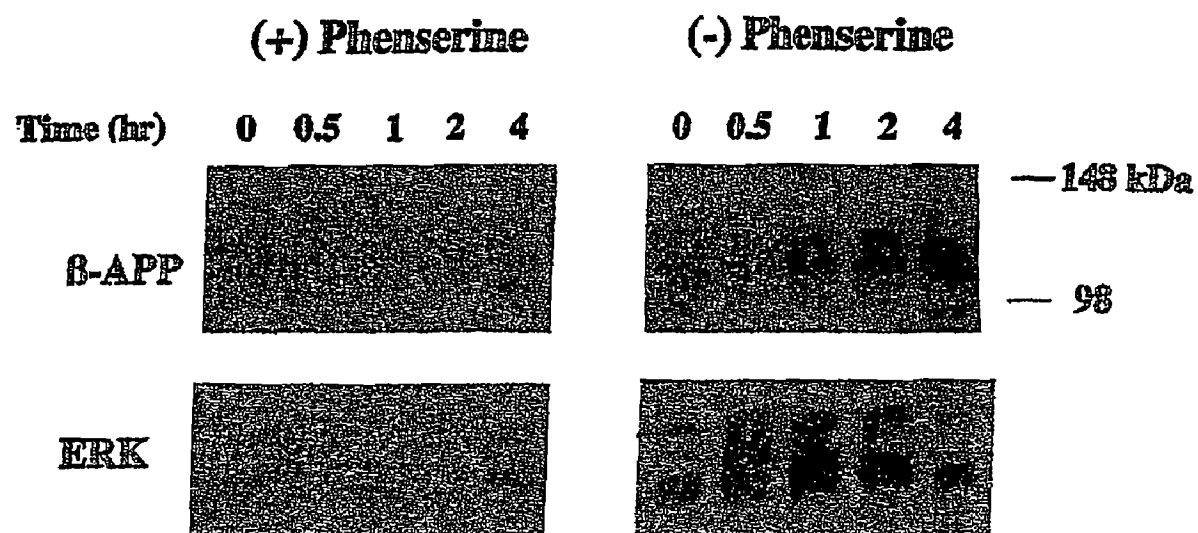


FIGURE 1

**FIGURE 3**

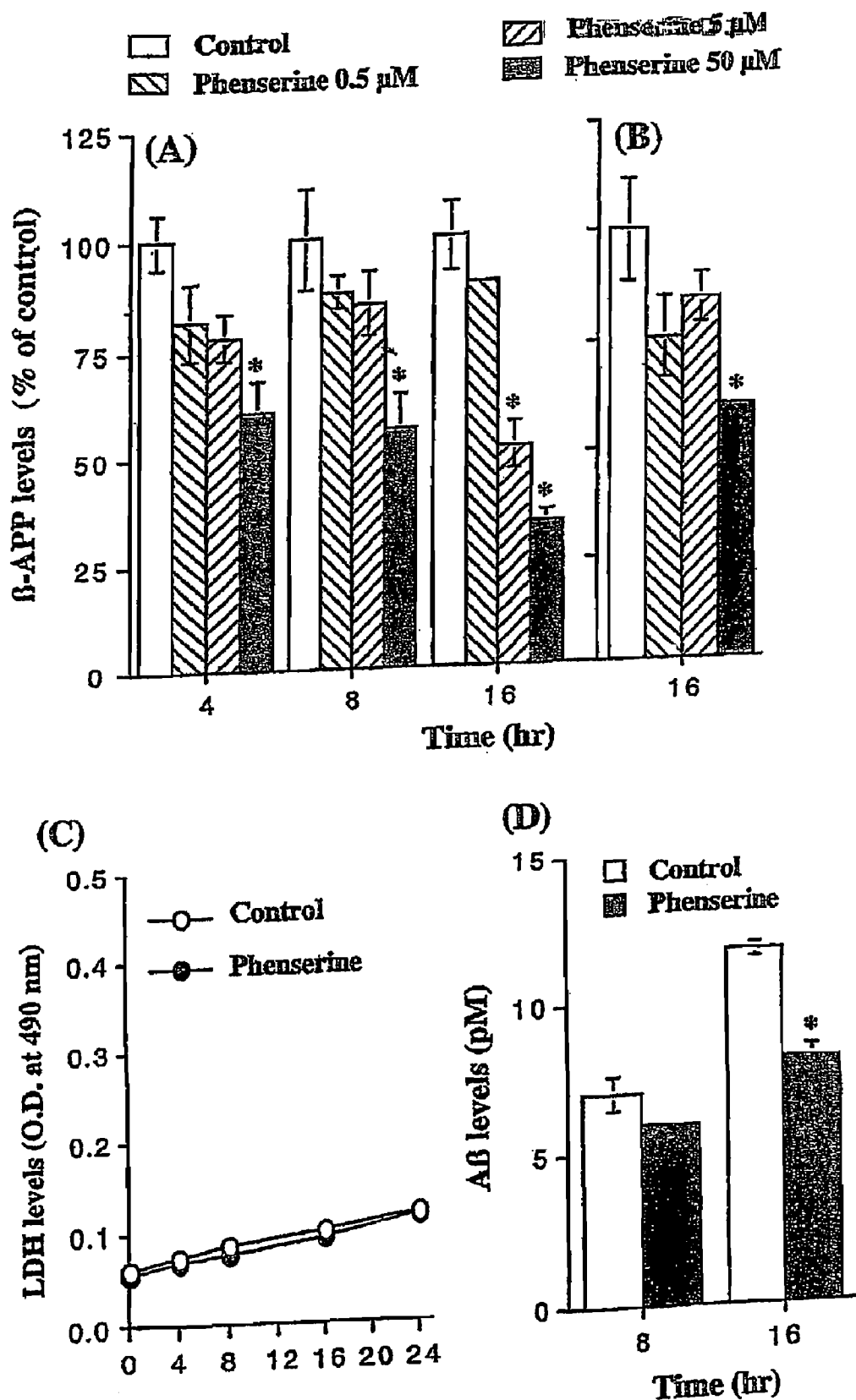


FIGURE 4

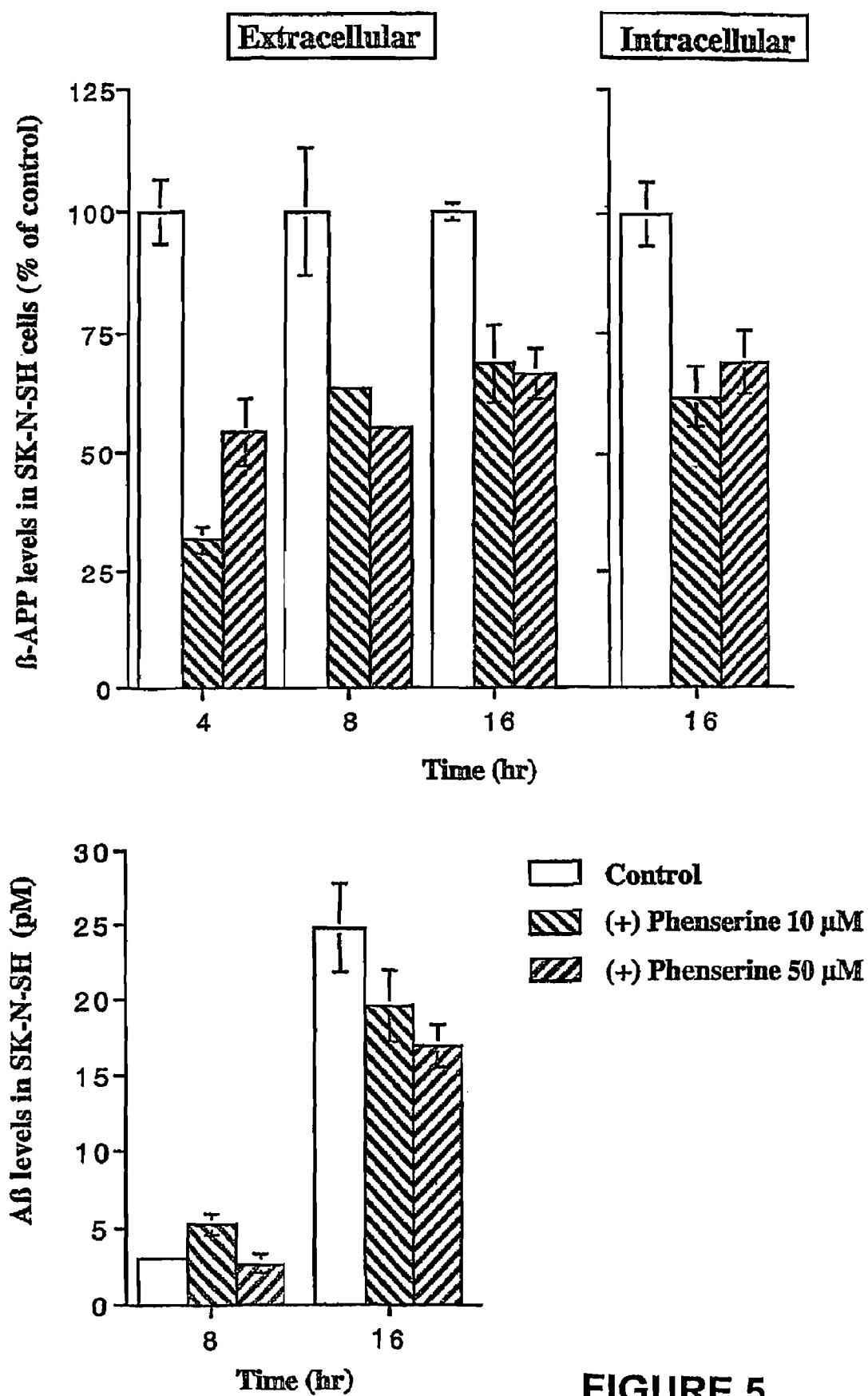


FIGURE 5

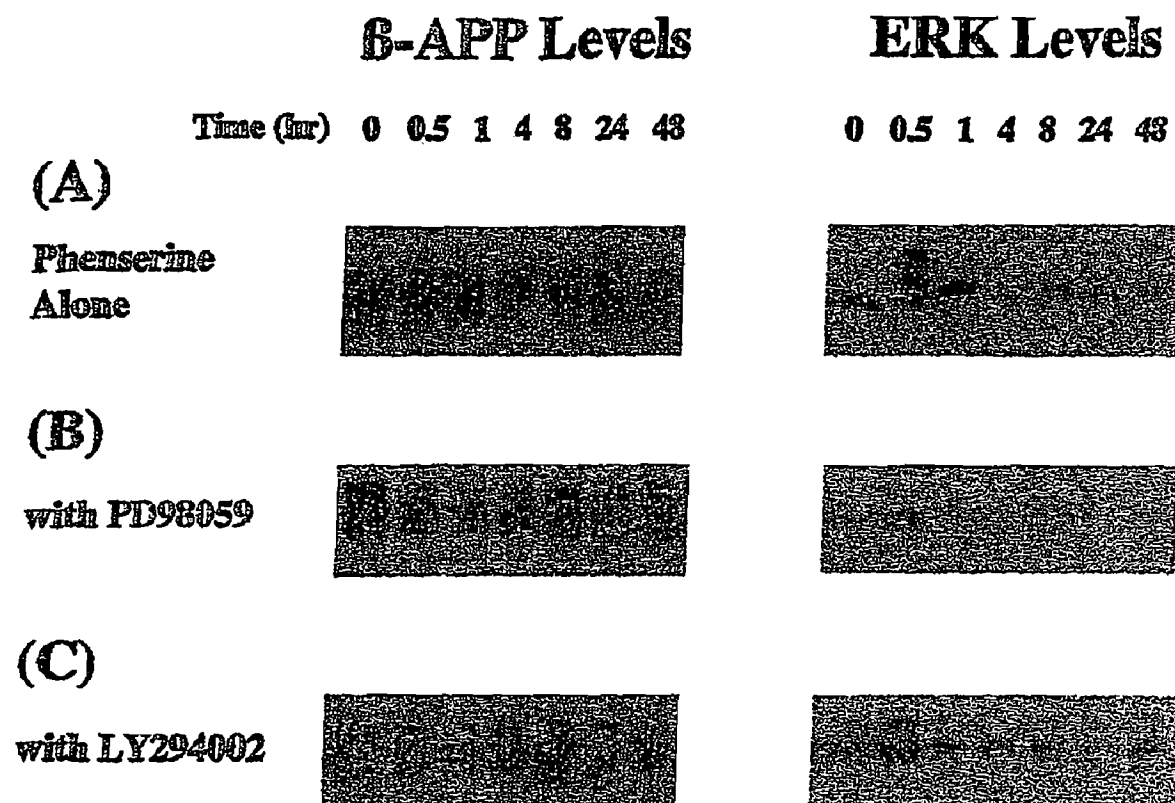


FIGURE 6

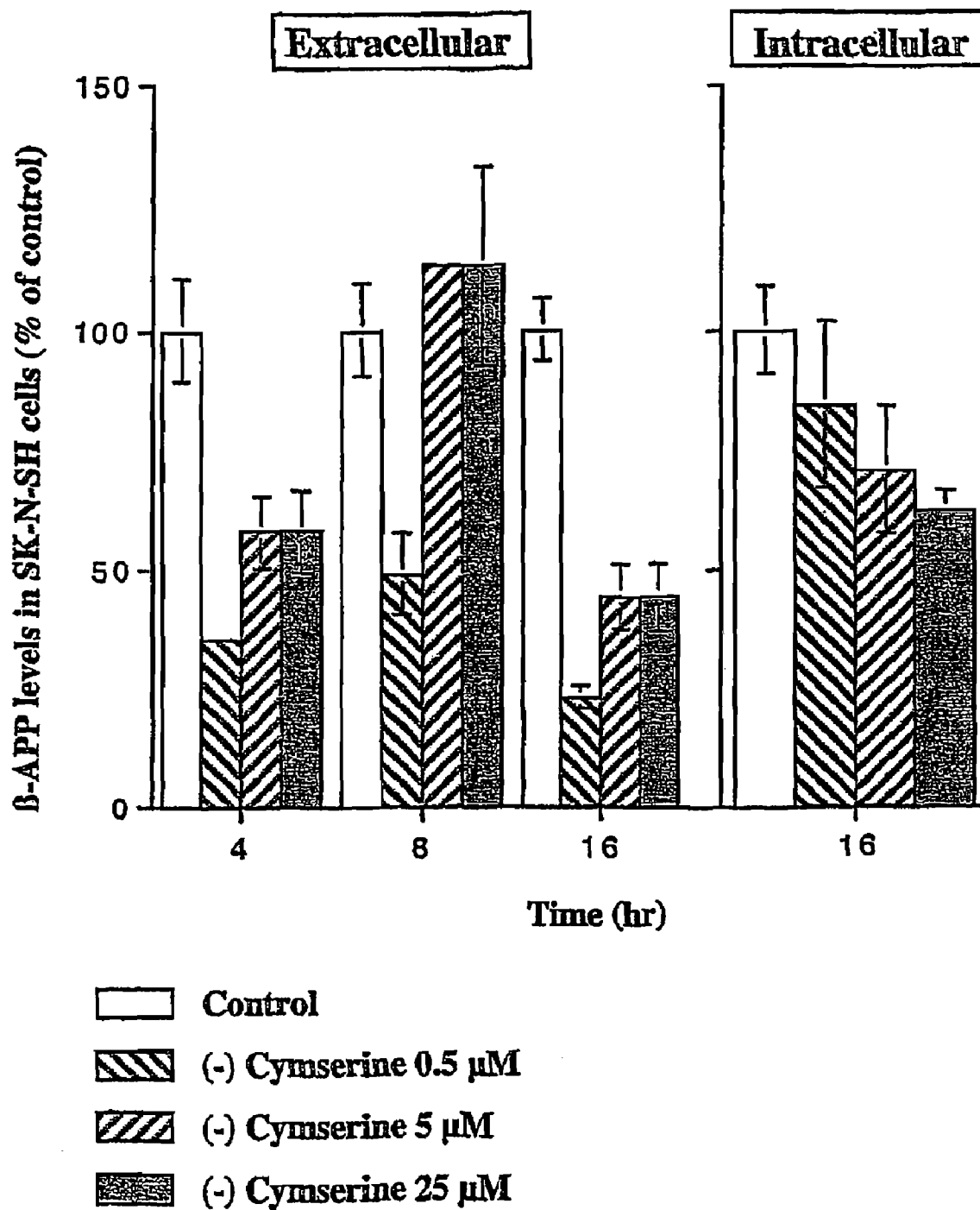
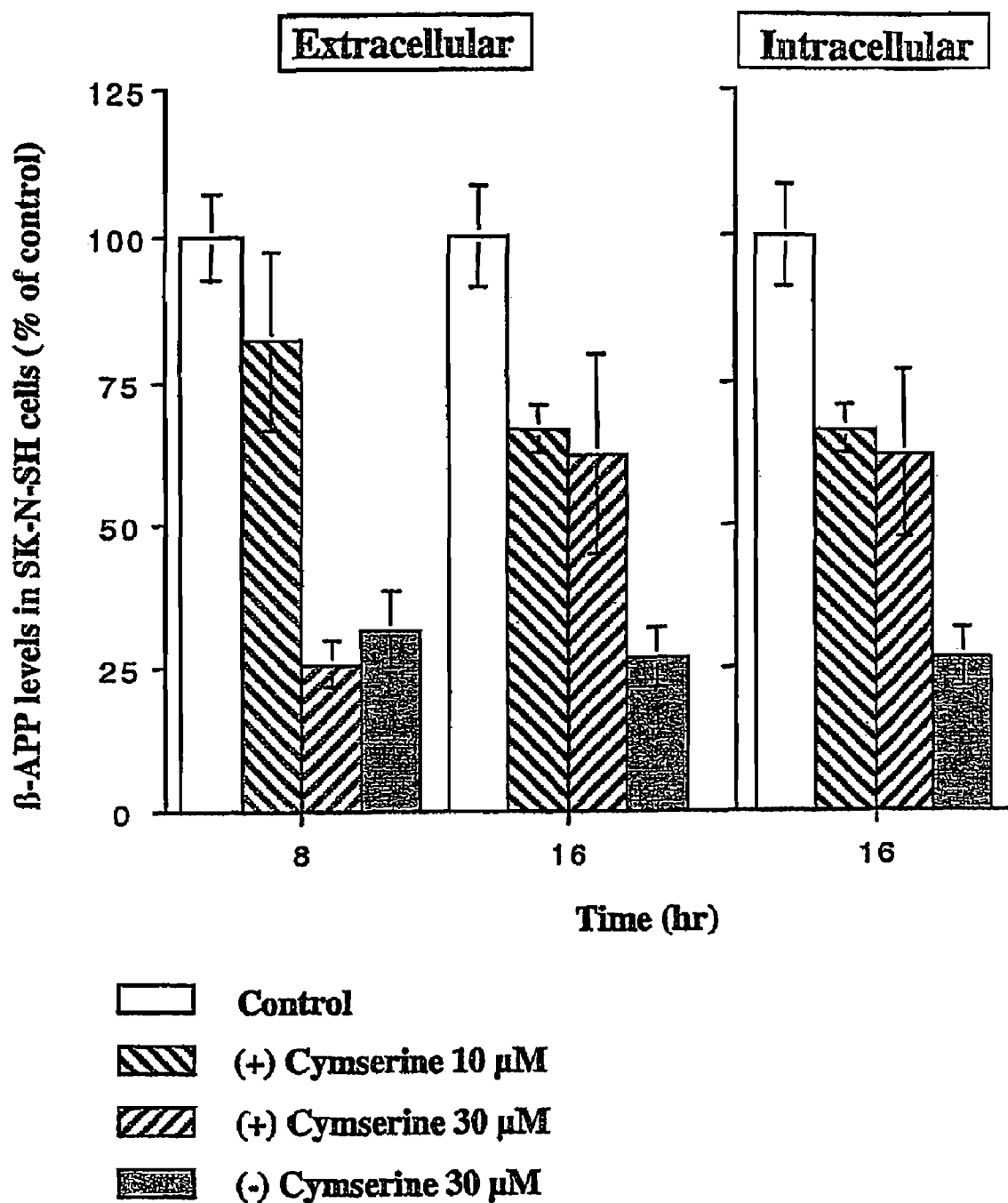


FIGURE 7A

**FIGURE 7B**

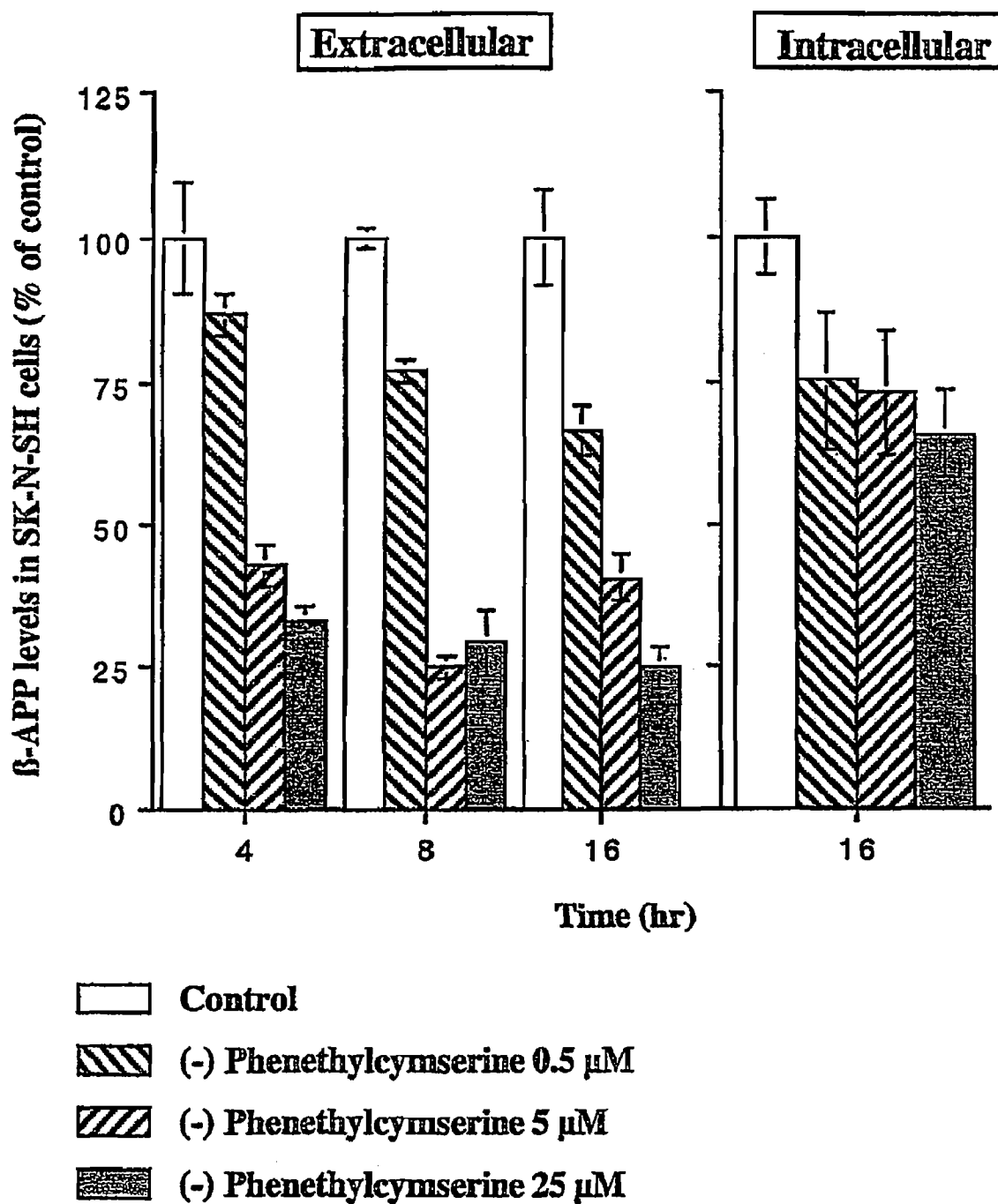
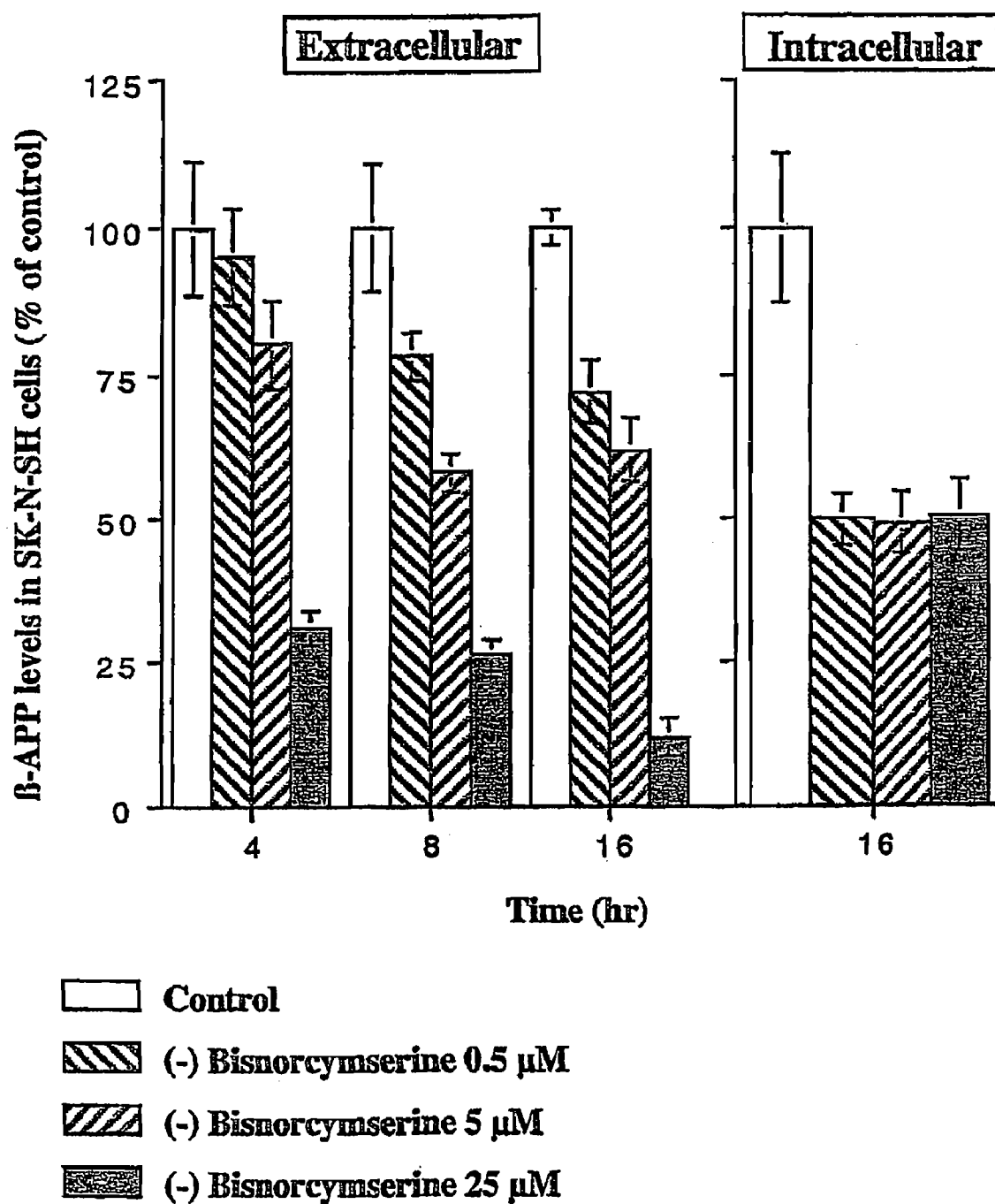


FIGURE 7C

**FIGURE 7D**

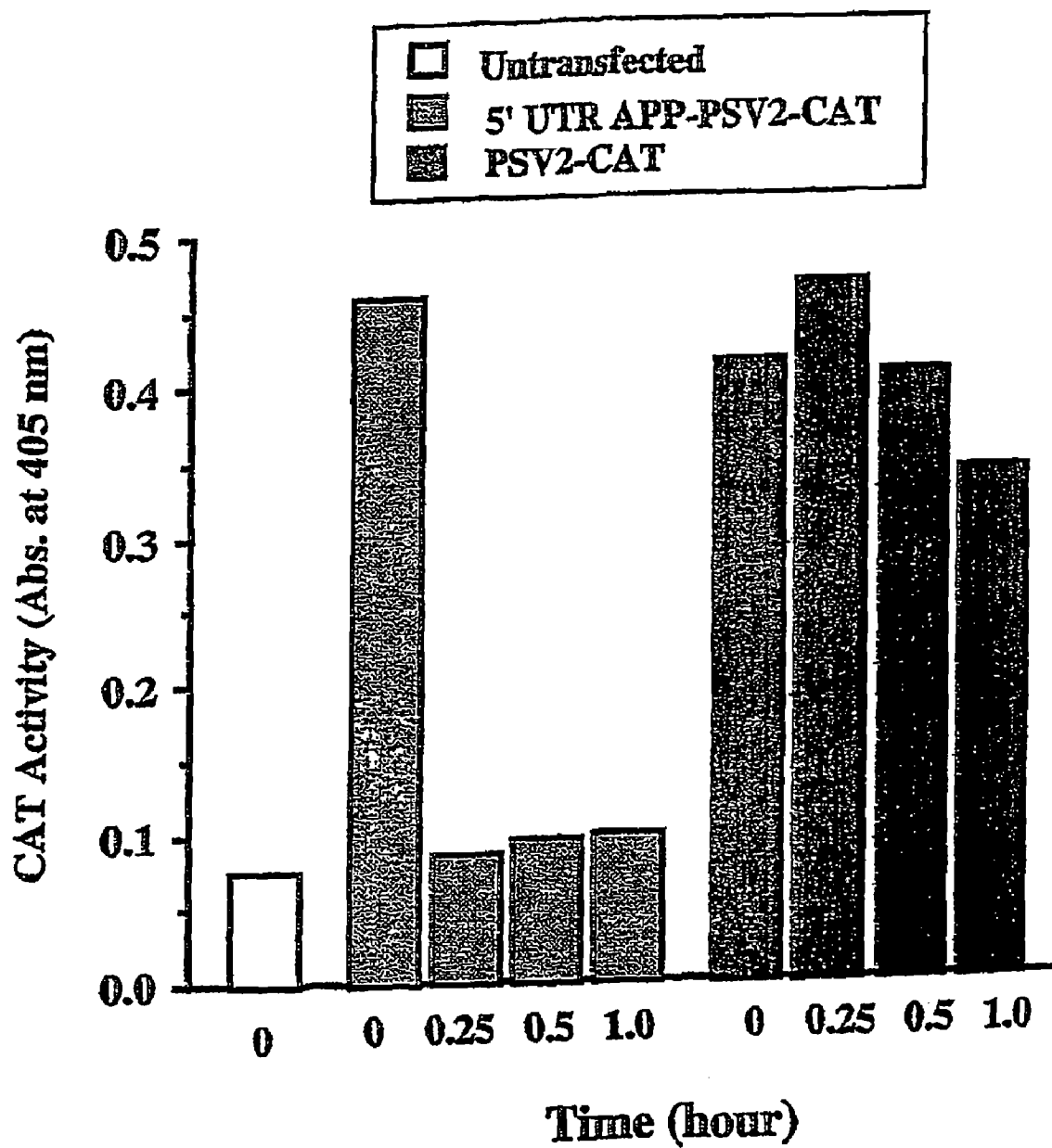


FIGURE 8A

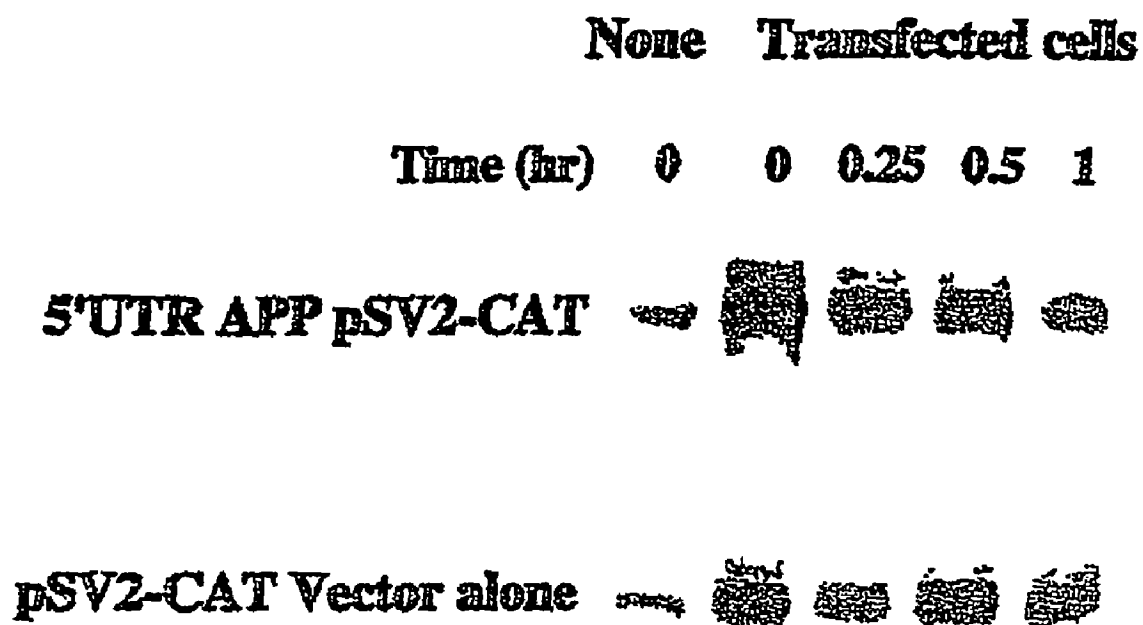
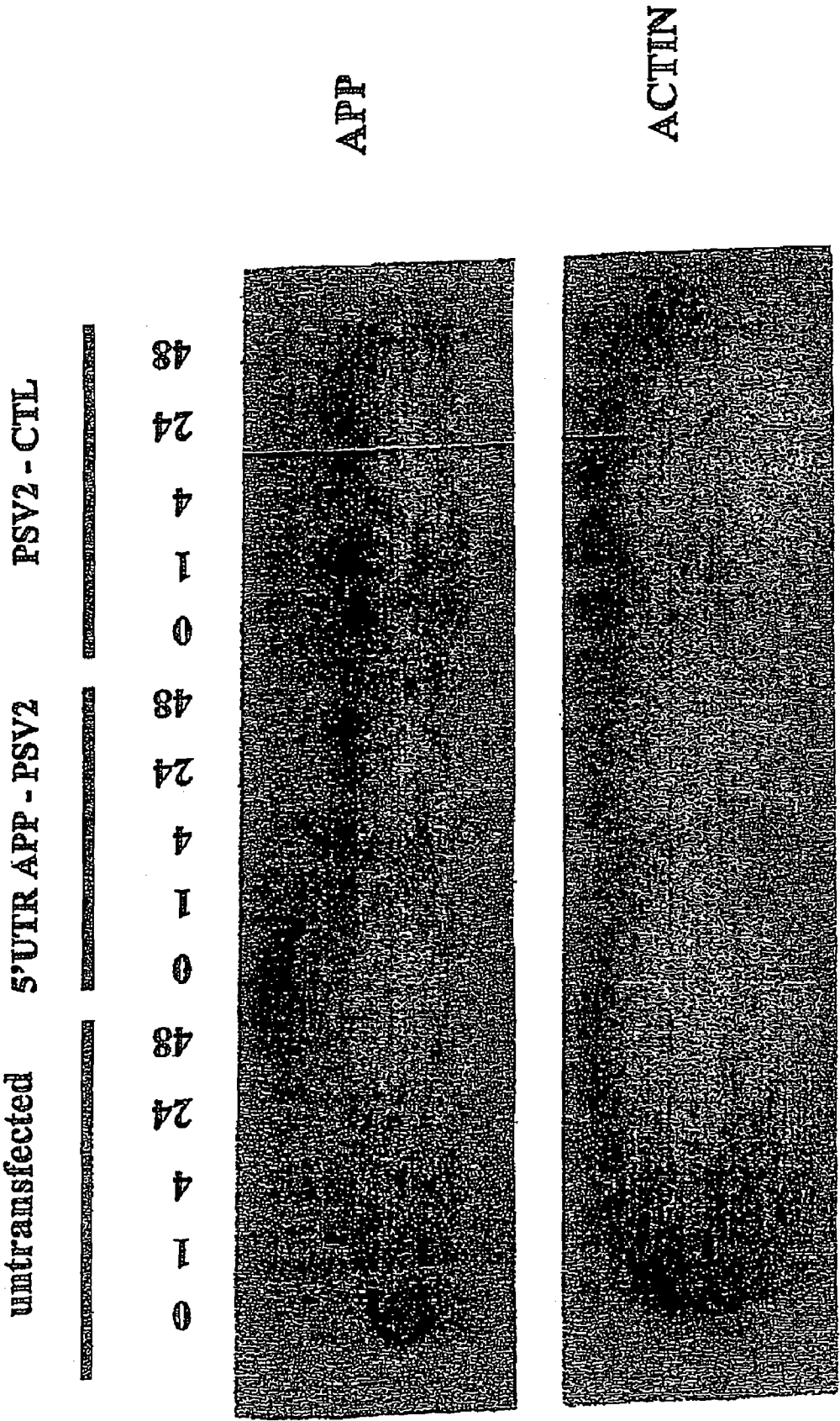
(B)**FIGURE 8B**

FIGURE 8C



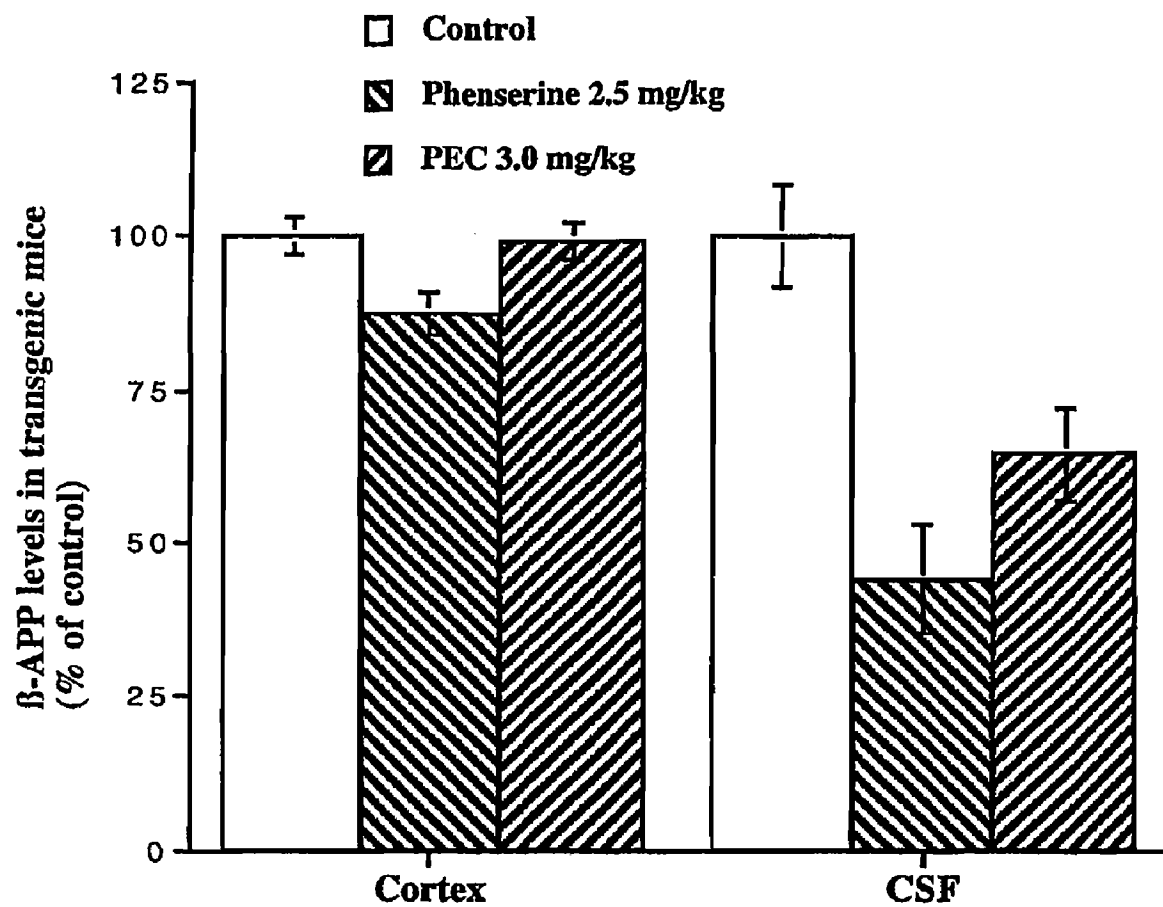


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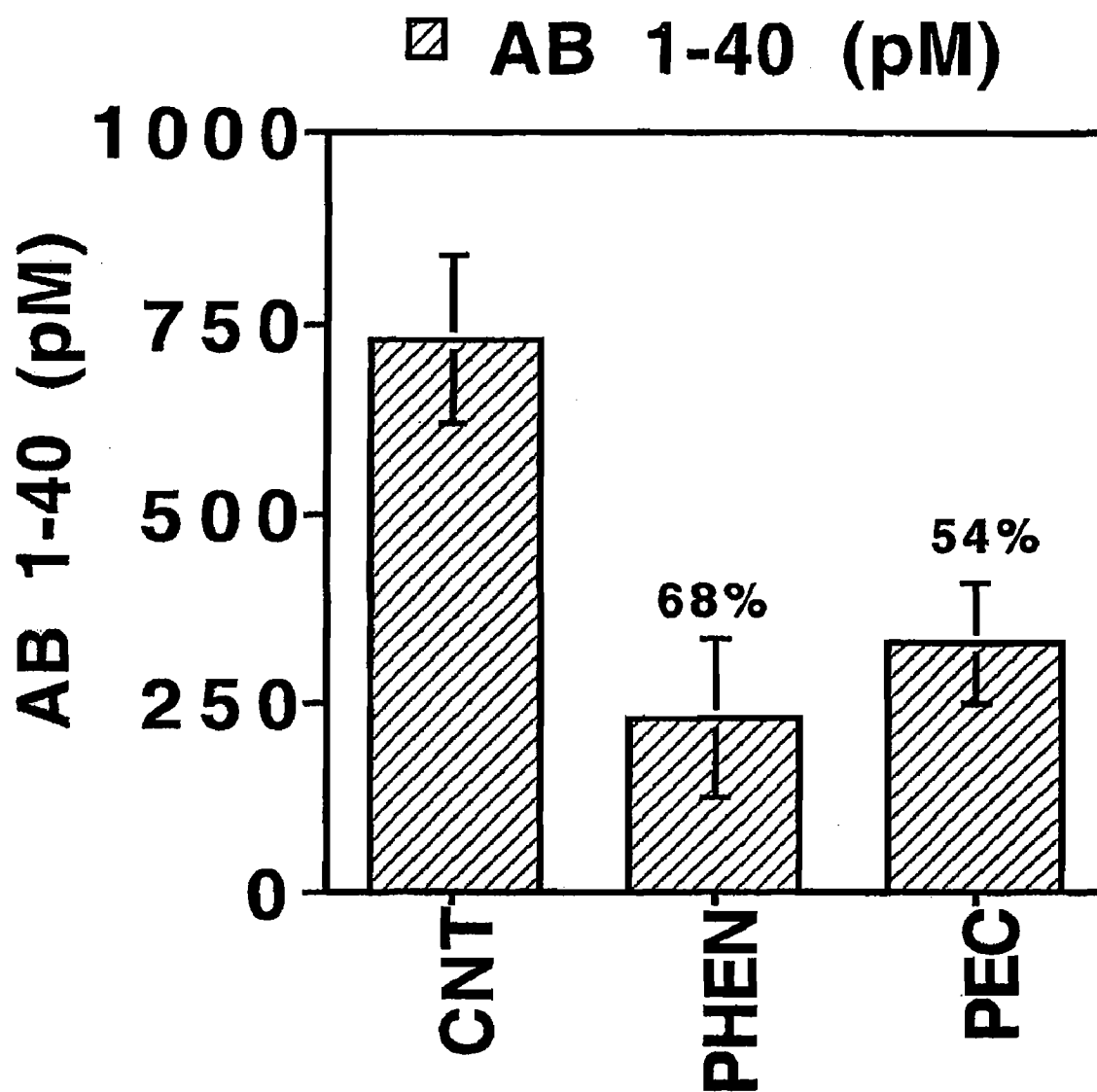


FIGURE 10A

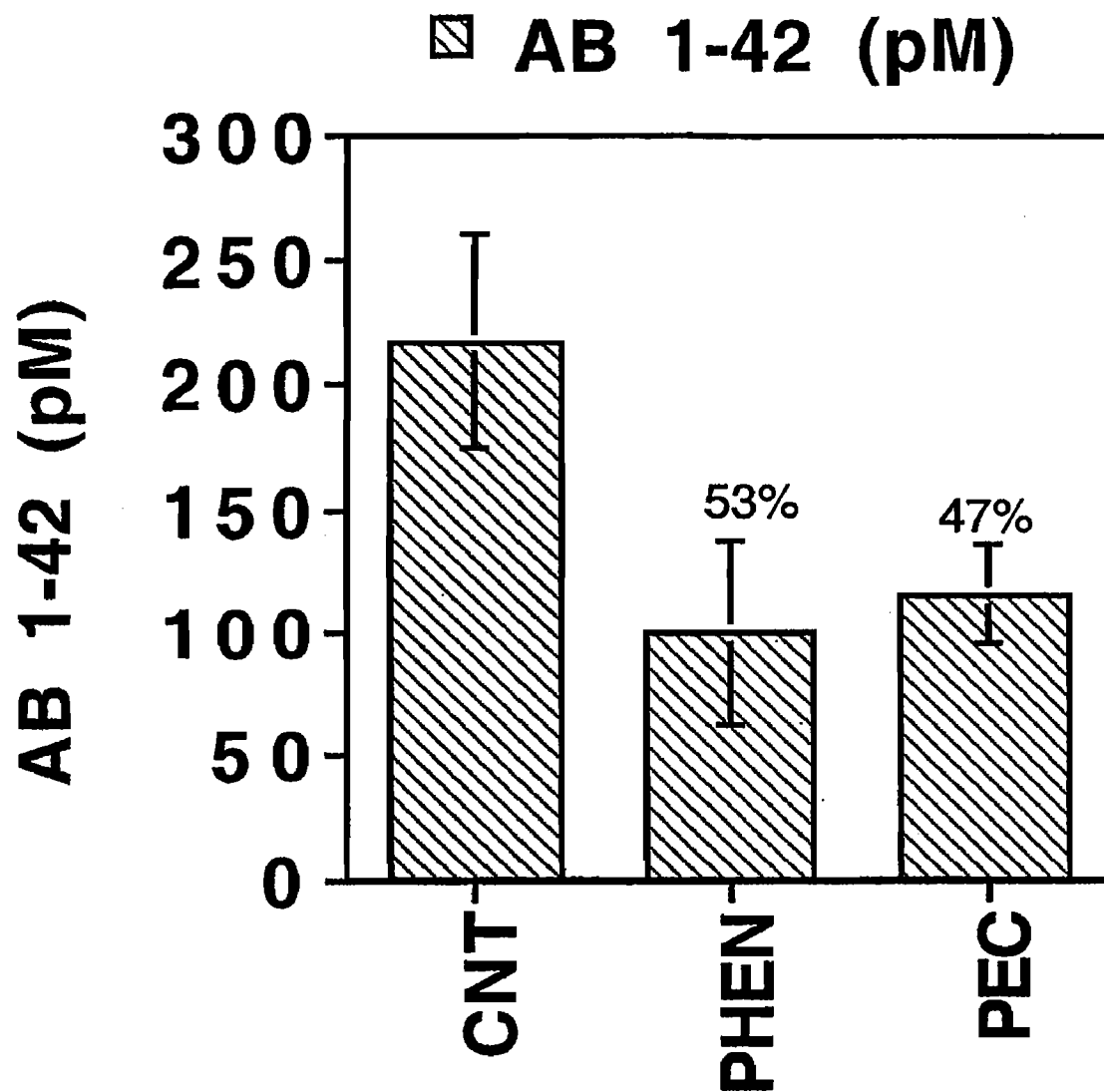


FIGURE 10B

FIGURE 11A

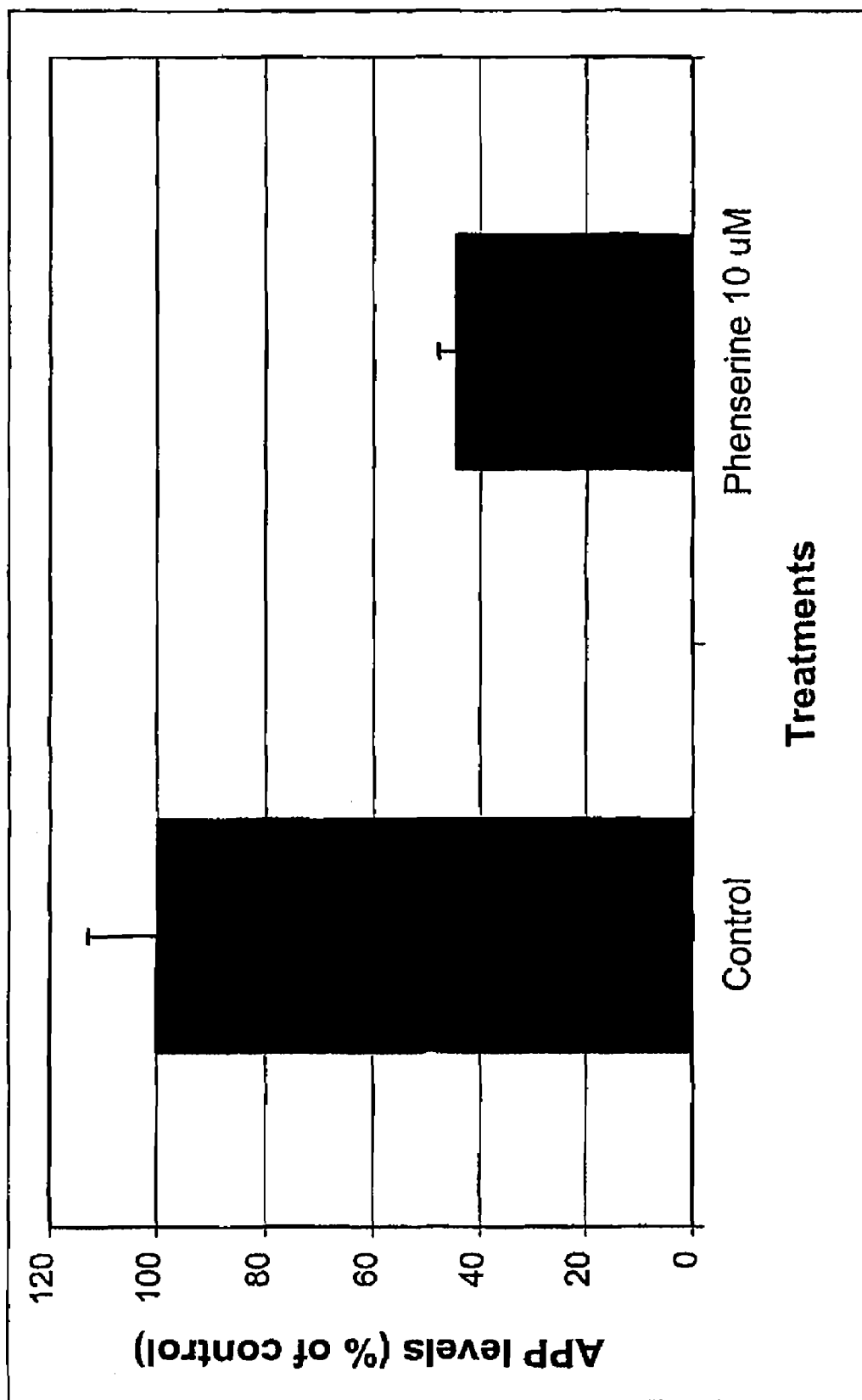


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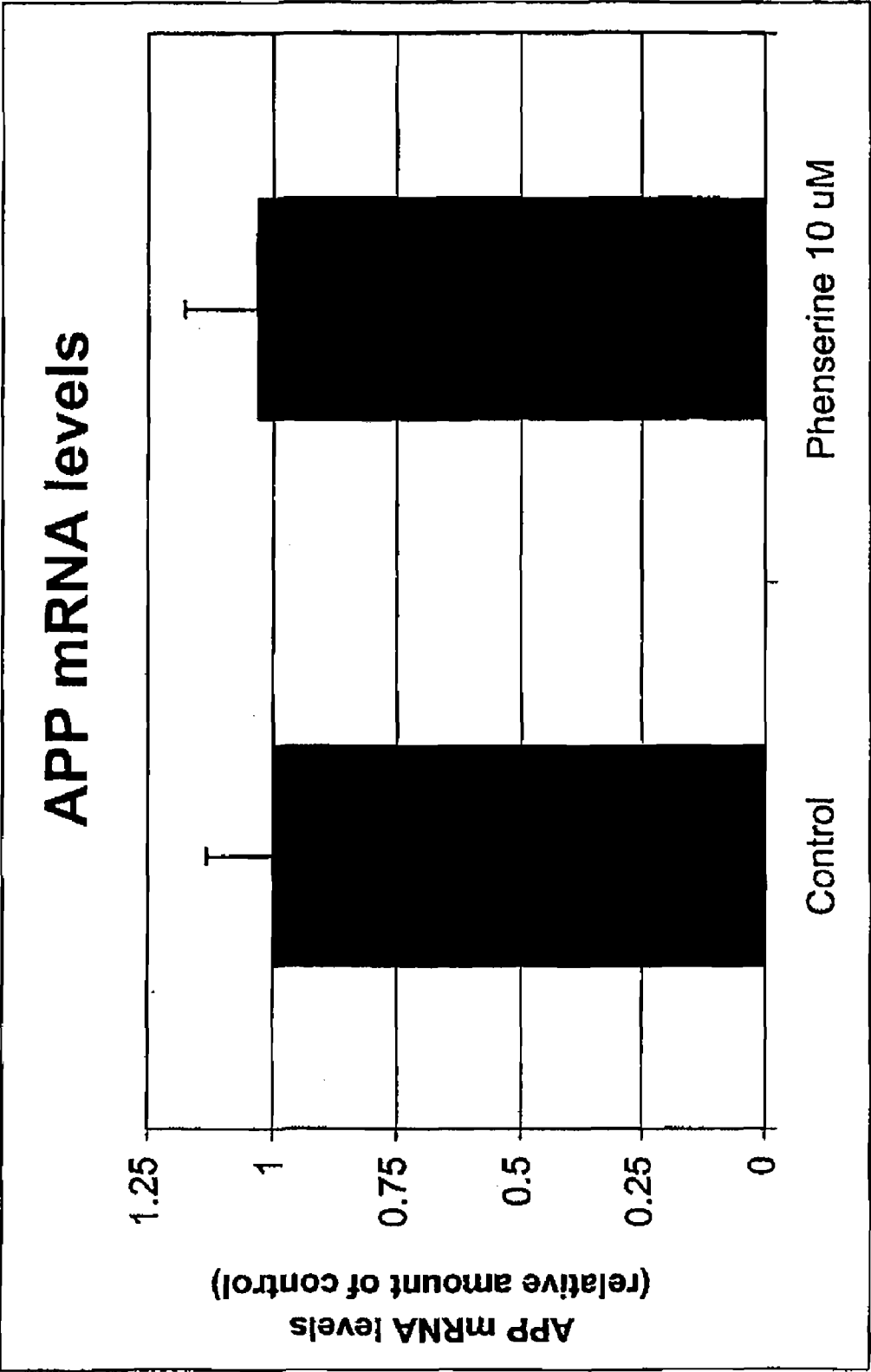


FIGURE 12A

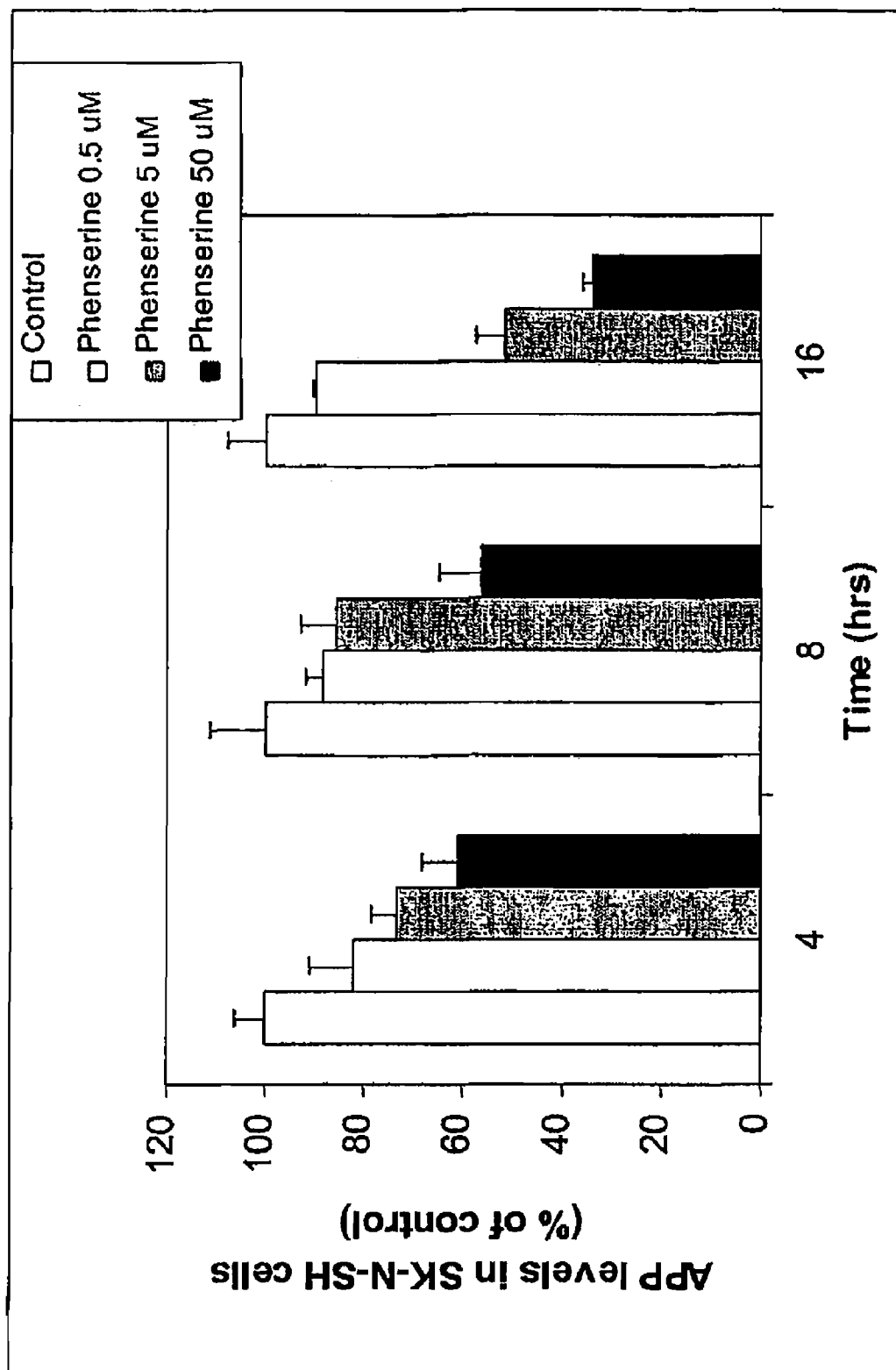


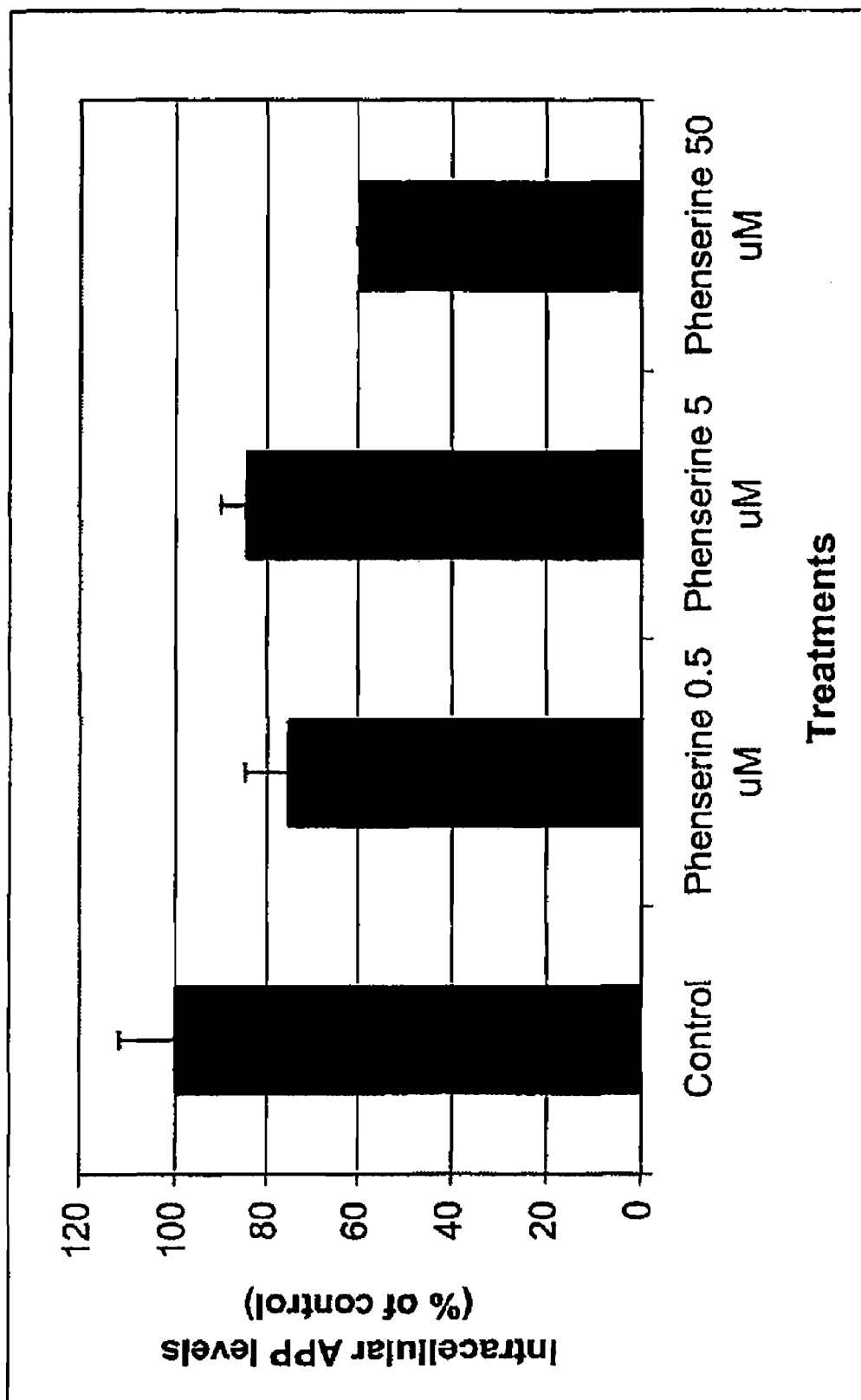
FIGURE 12B

FIGURE 13A

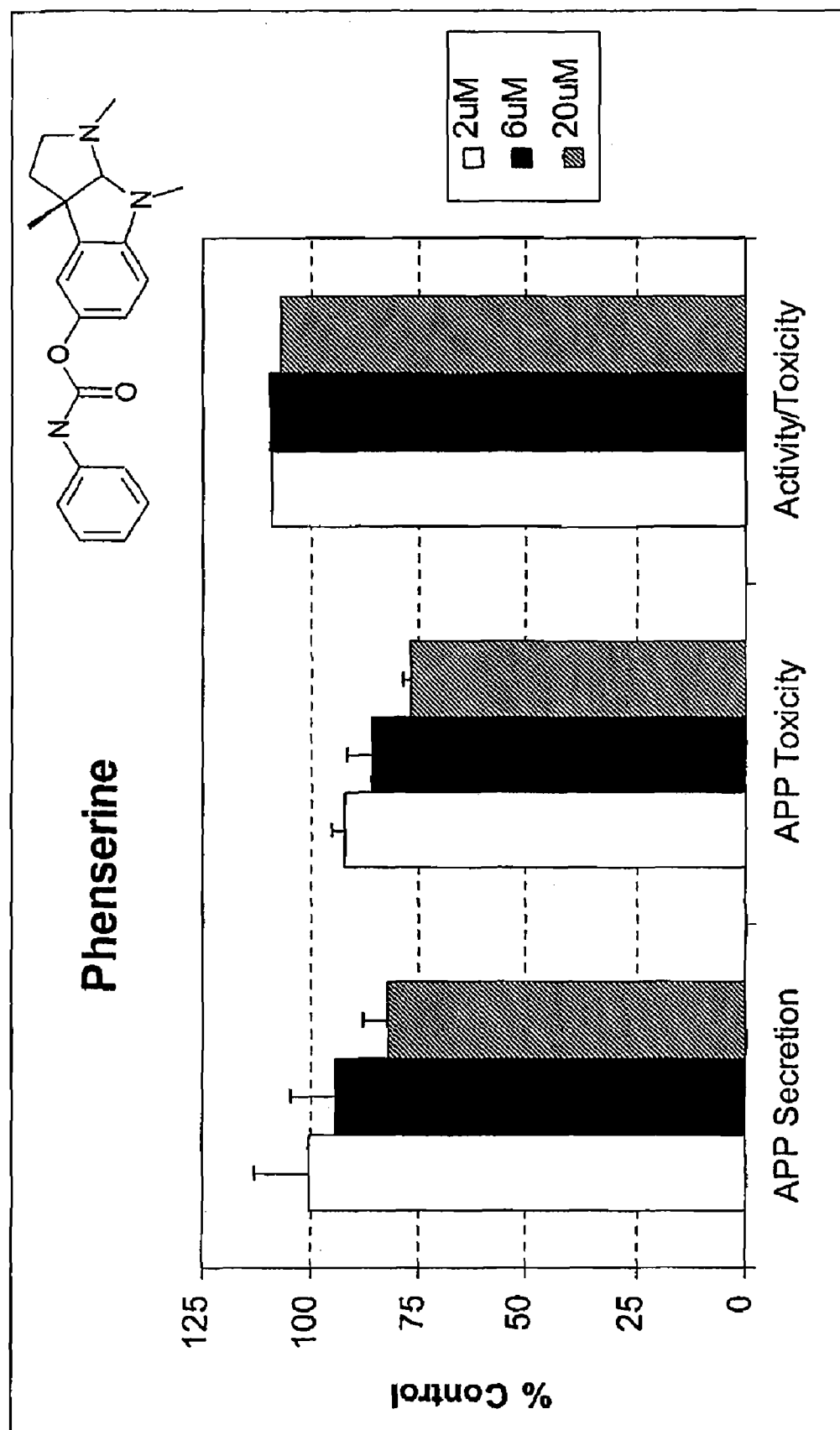


FIGURE 13B

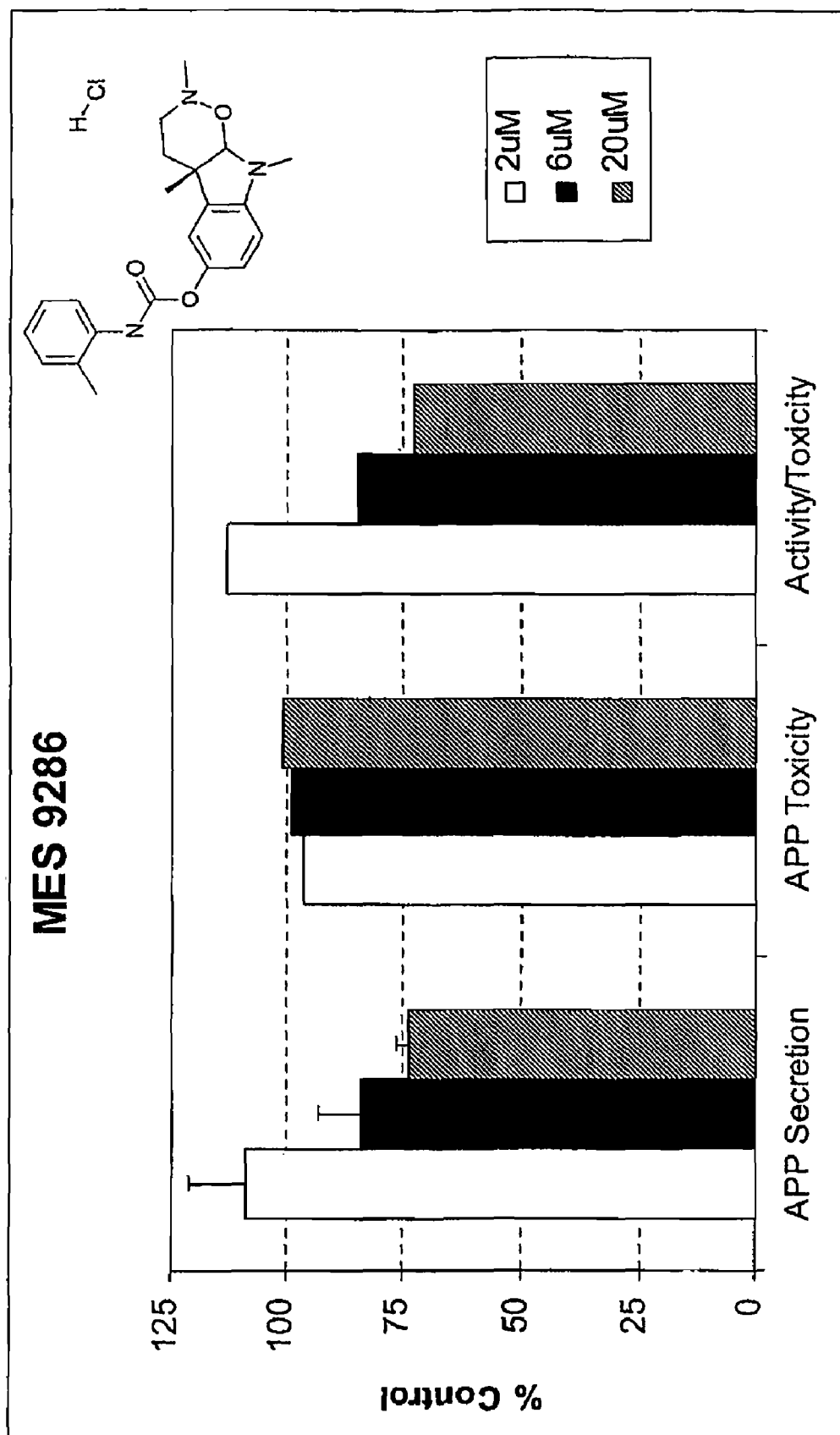


FIGURE 13C

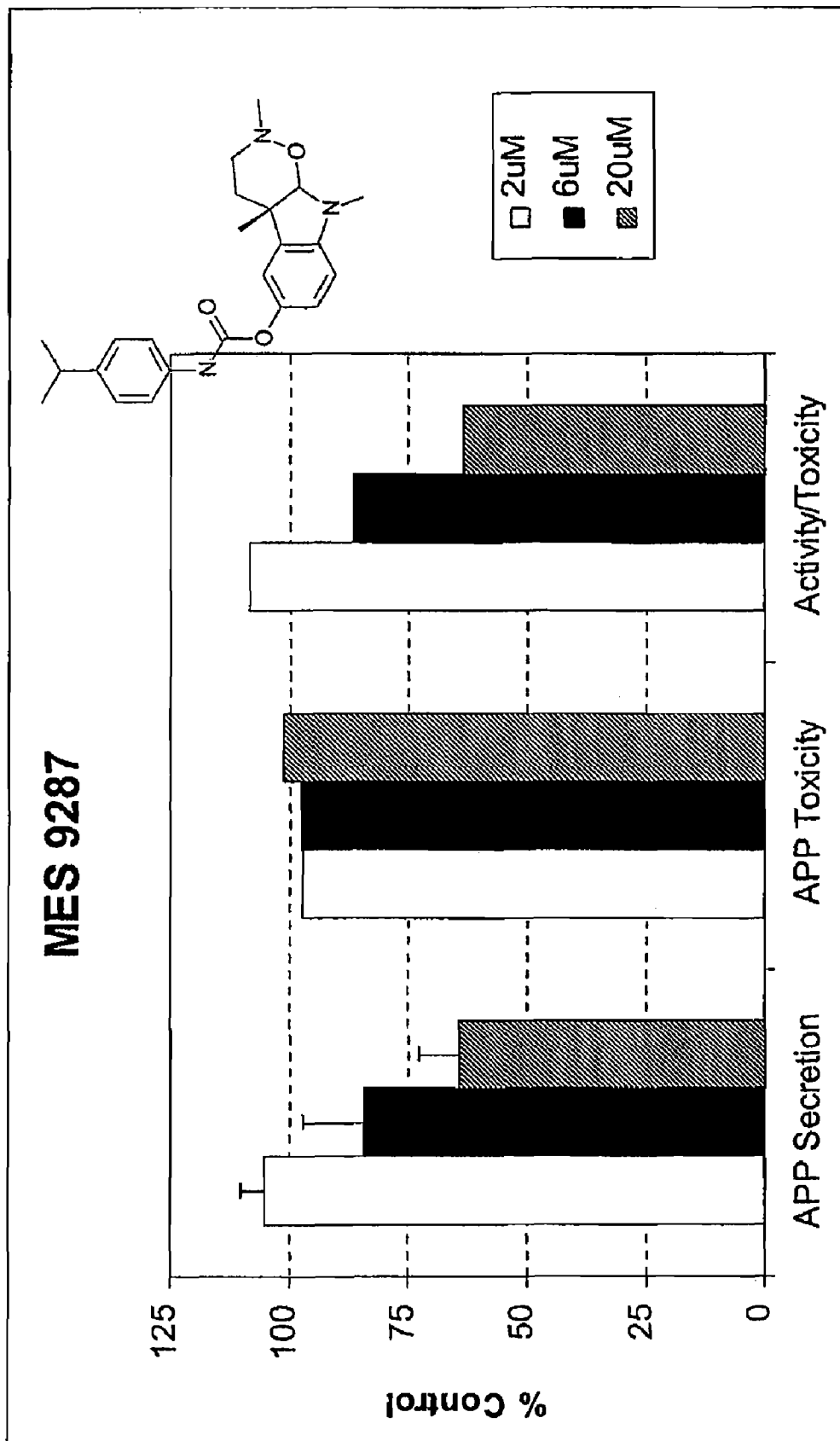


FIGURE 13D

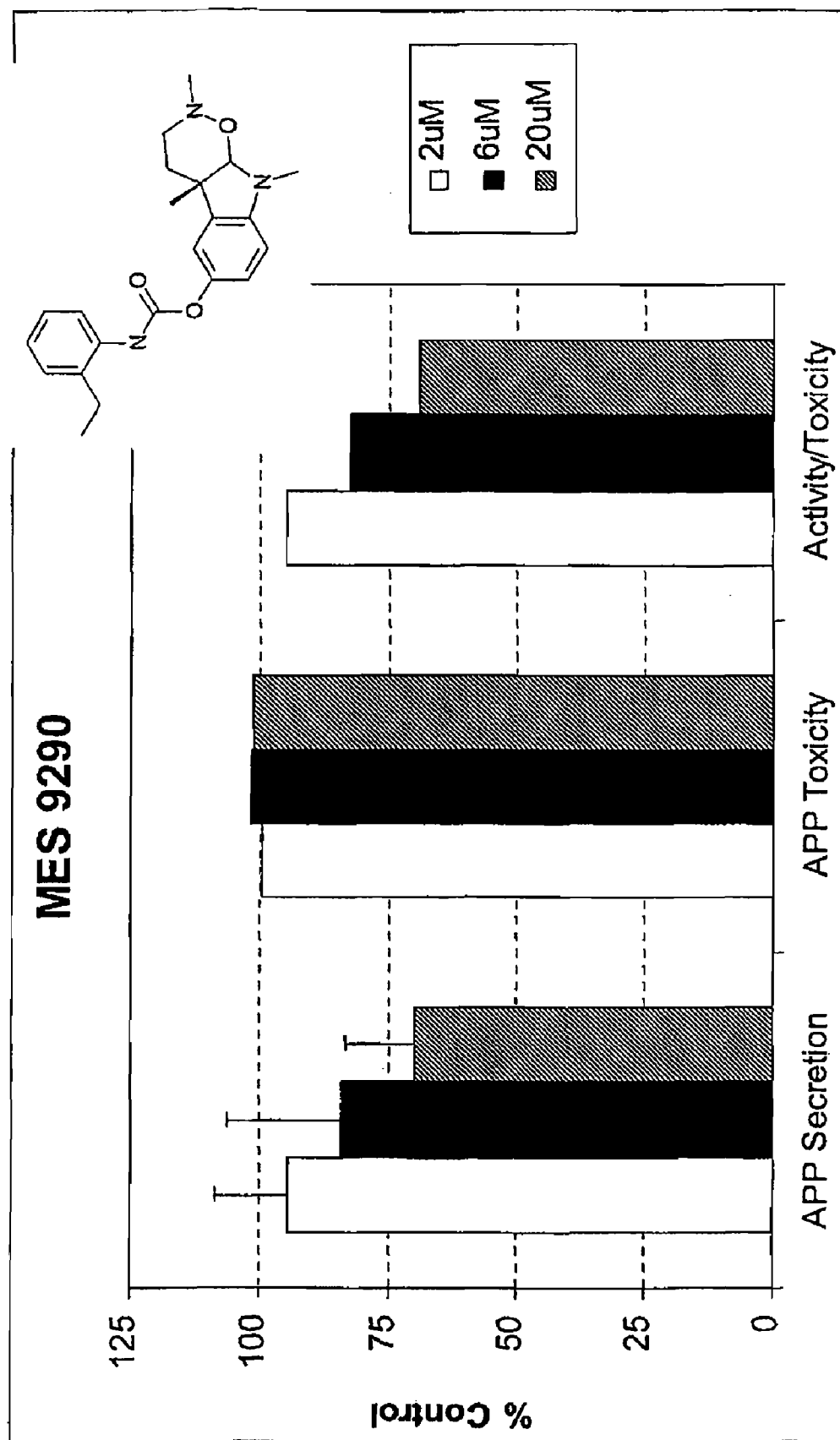


FIGURE 13E

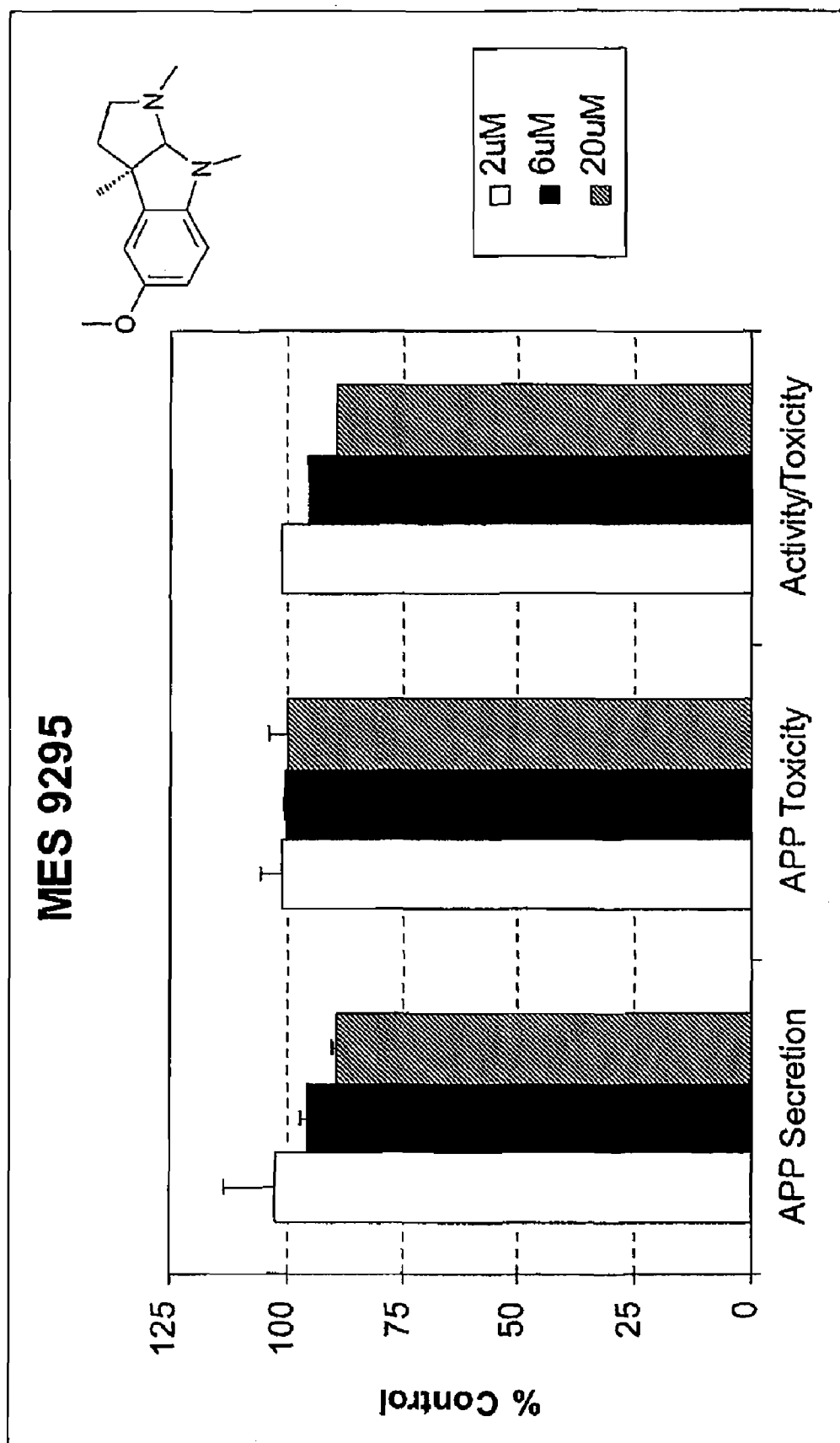


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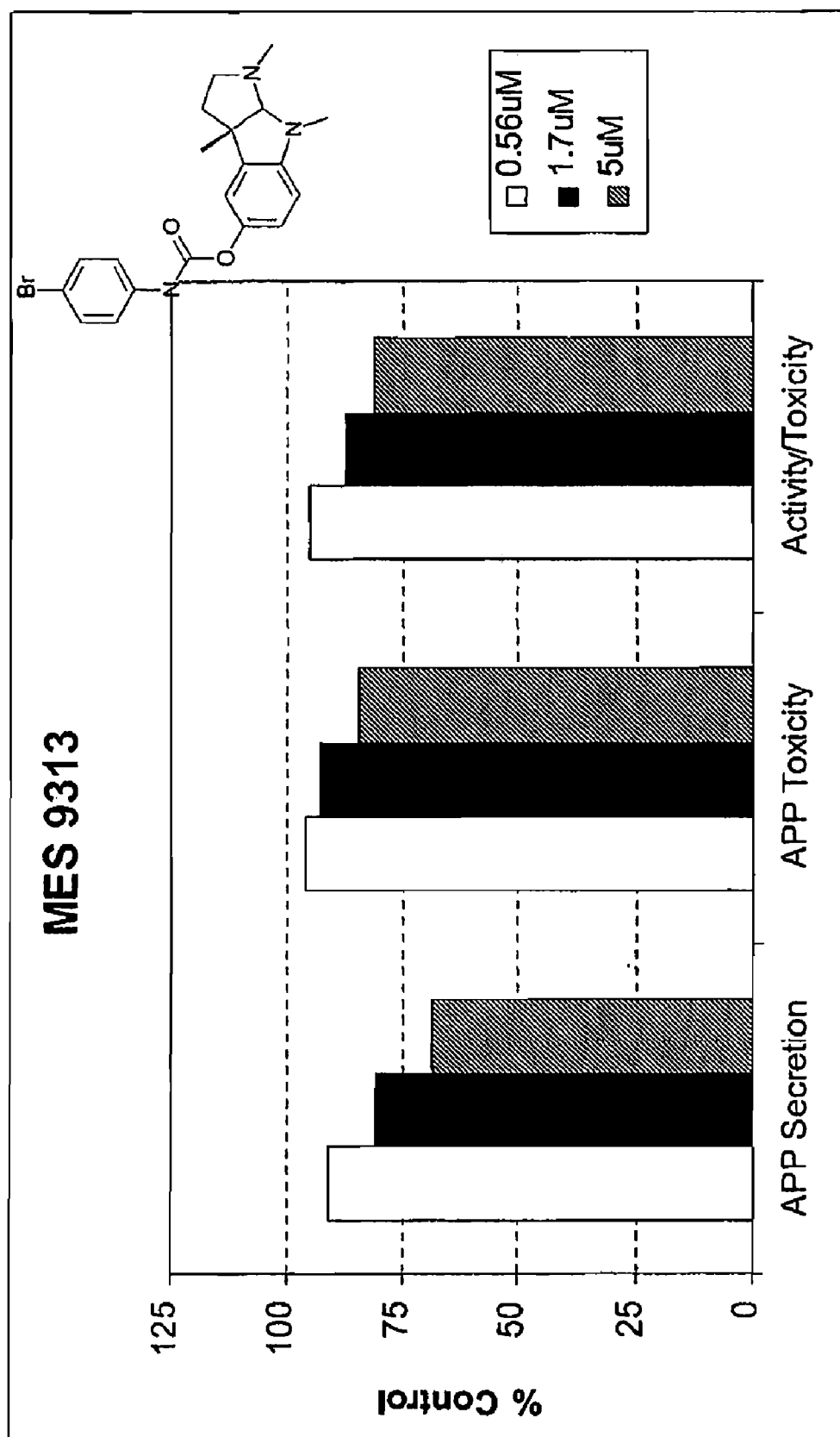


FIGURE 13G

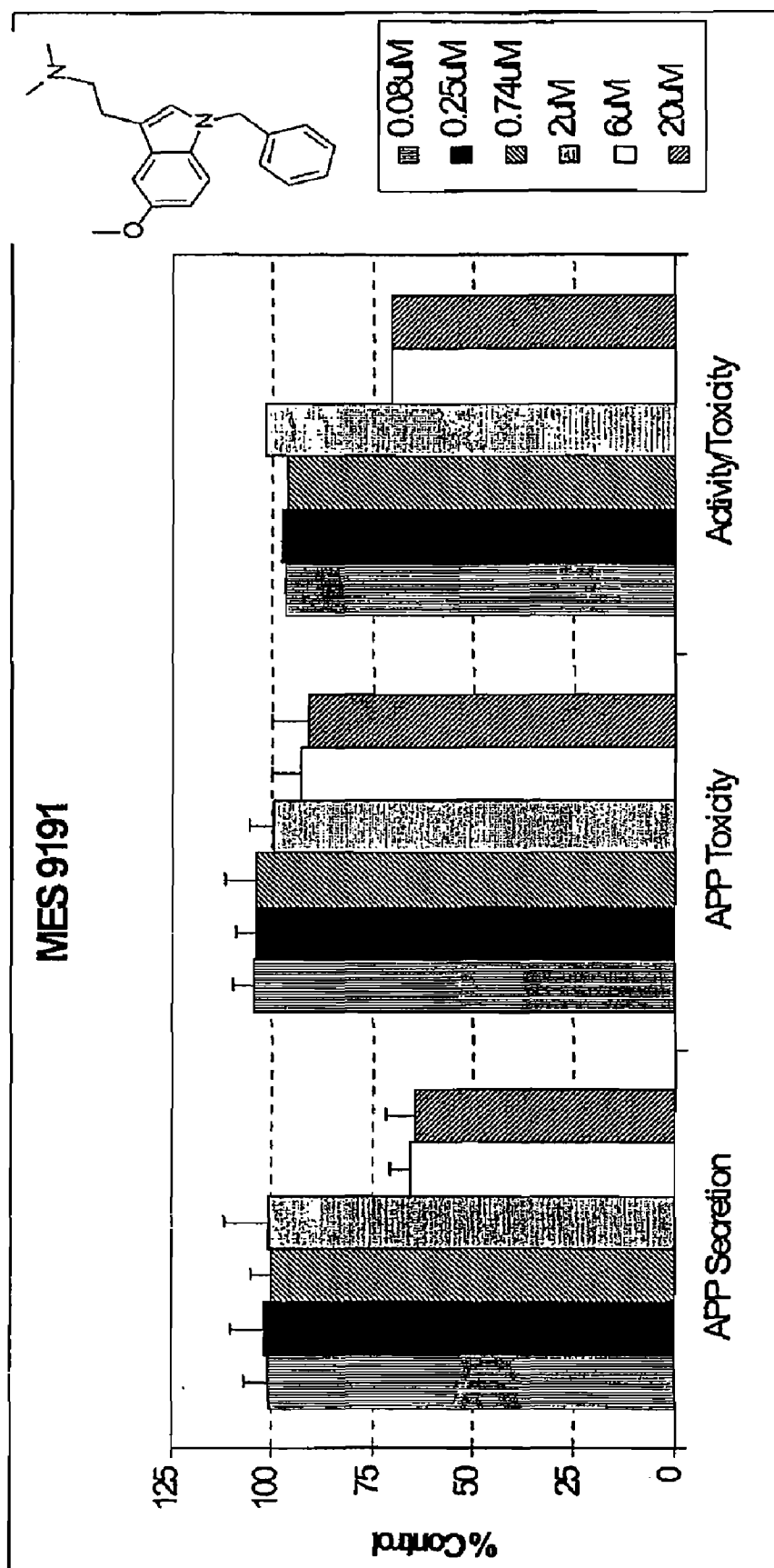


FIGURE 13H

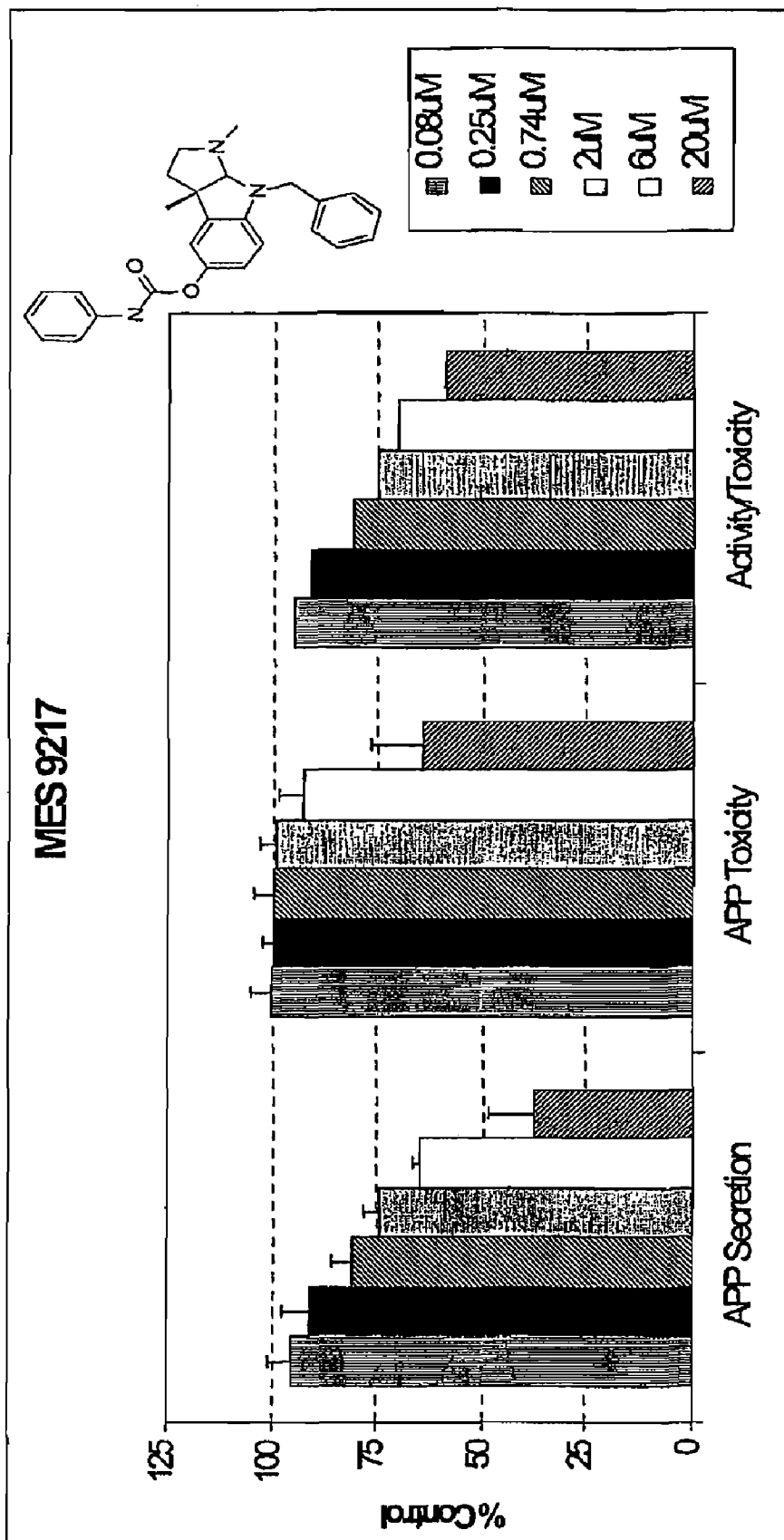


FIGURE 13I

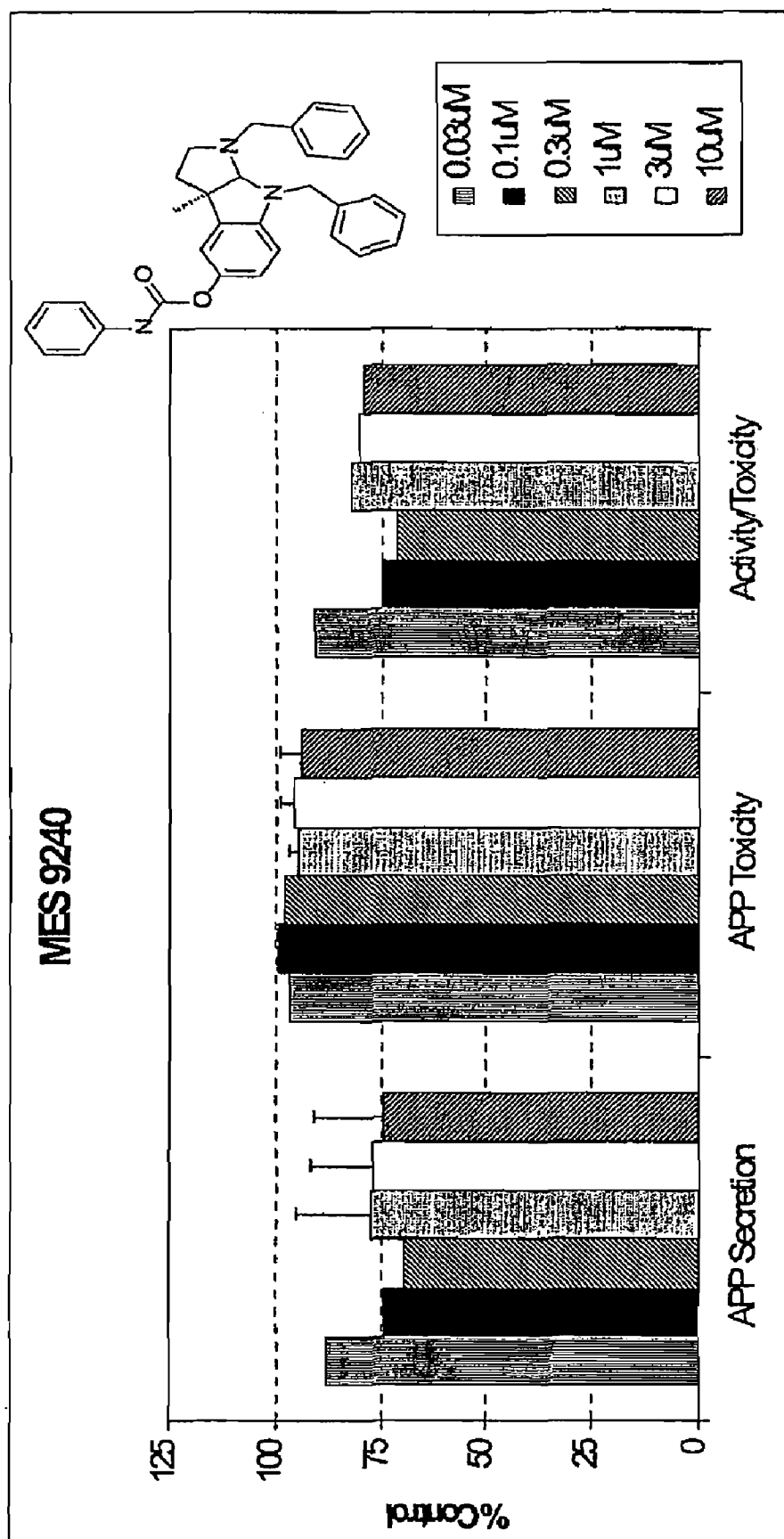


FIGURE 13J

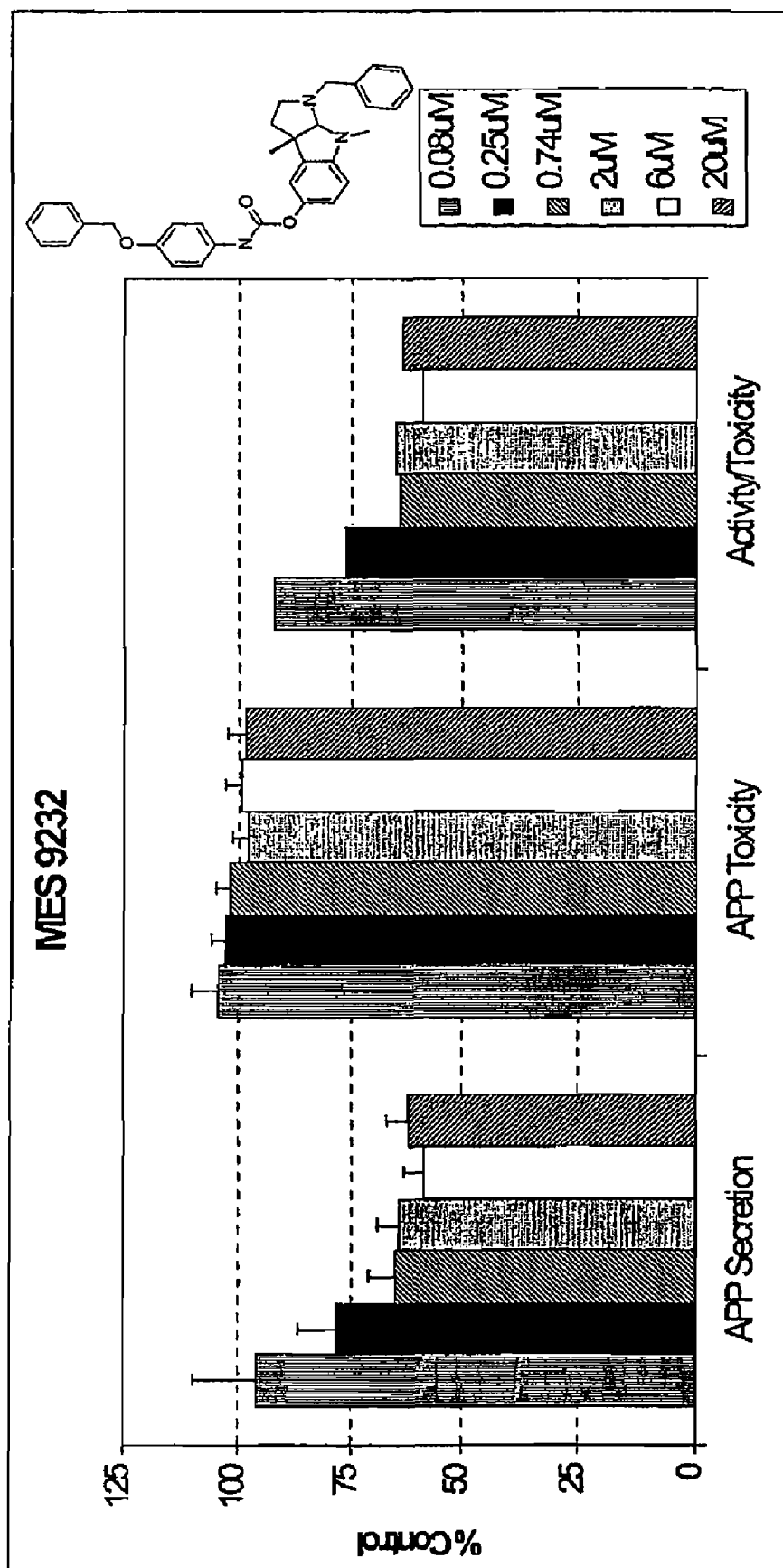


FIGURE 13K

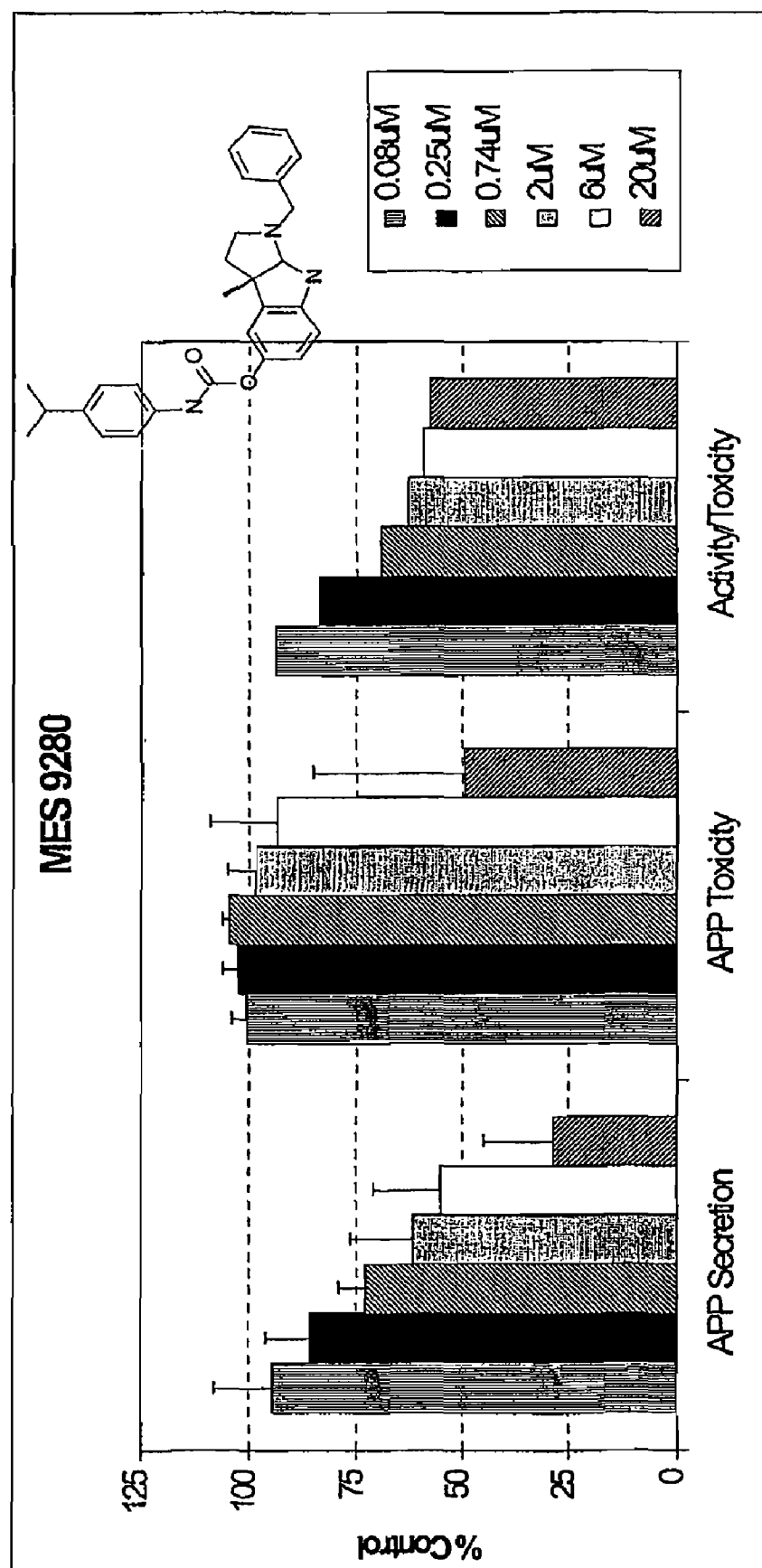


FIGURE 13L

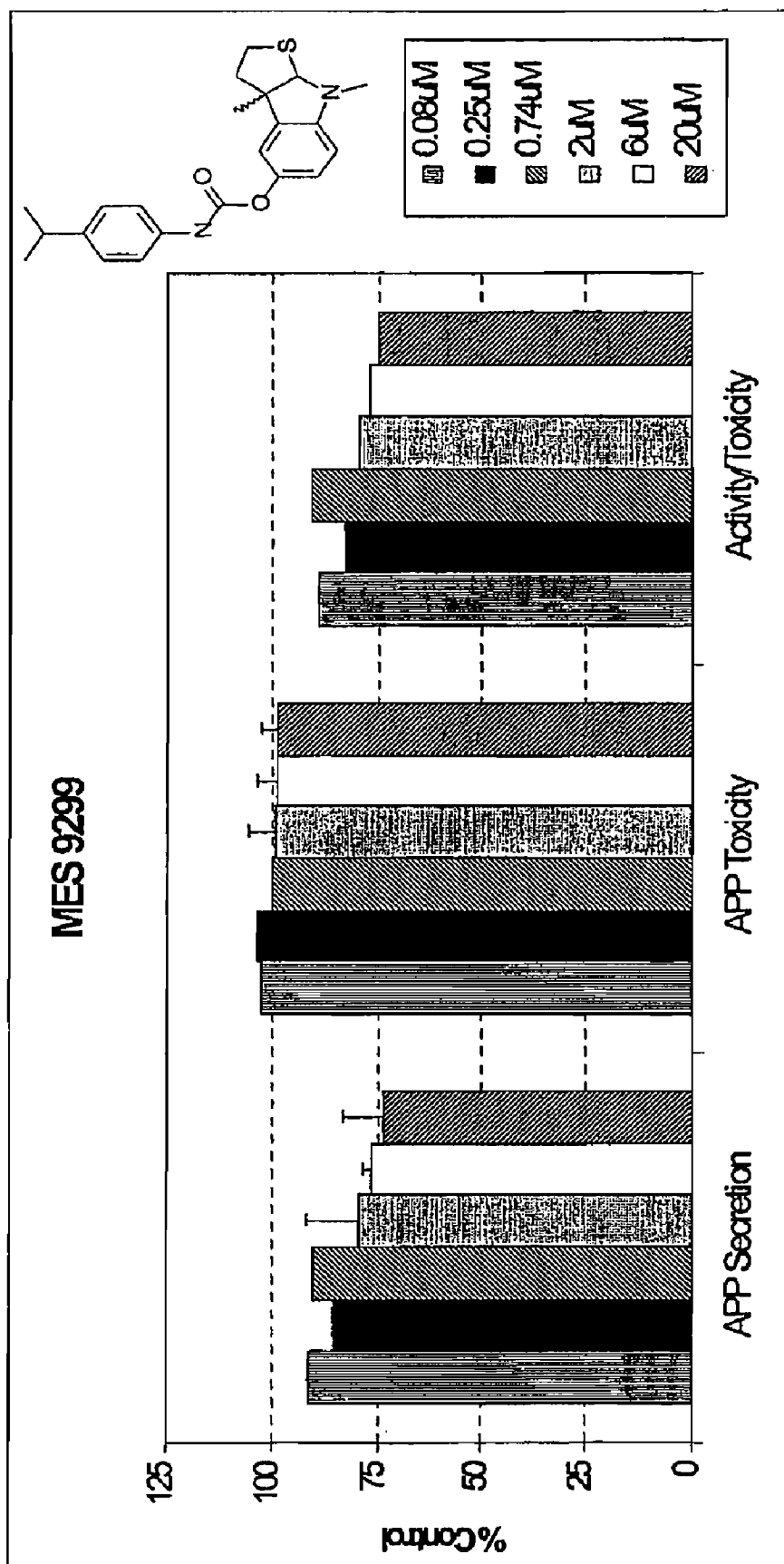


FIGURE 13M

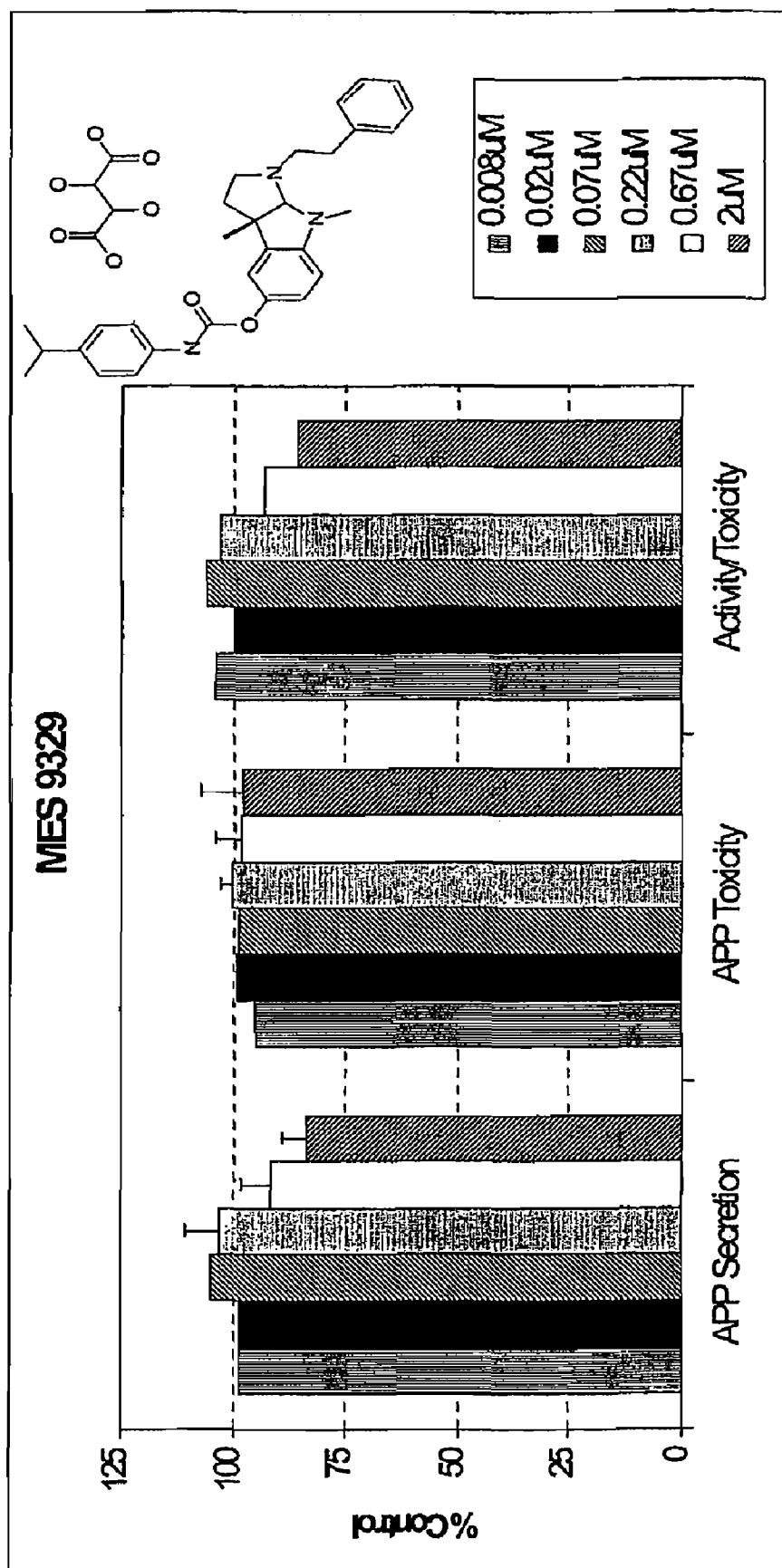


FIGURE 14

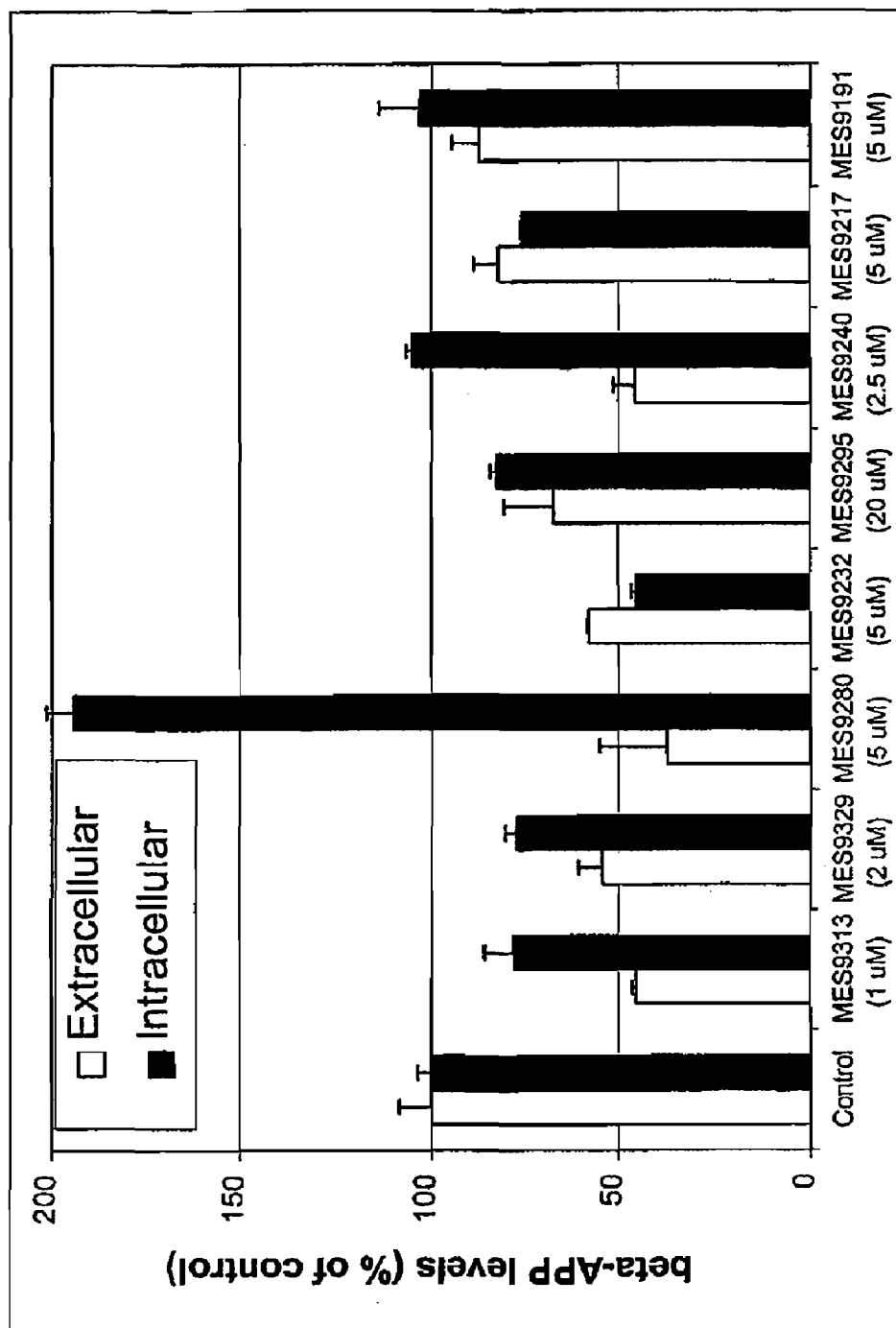
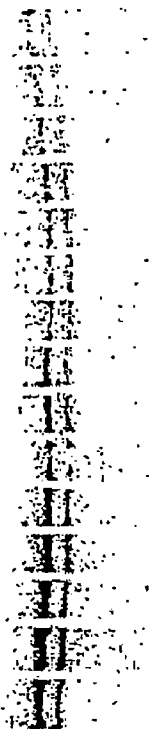


FIGURE 15A

Control	MES9295 (20 μ M)	MES9191 (5 μ M)	MES9217 (5 μ M)	MES9240 (2.5 μ M)
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Control	MES9313 (1 μ M)	MES9329 (2 μ M)	MES9280 (1 μ M)	MES9232 (10 μ M)
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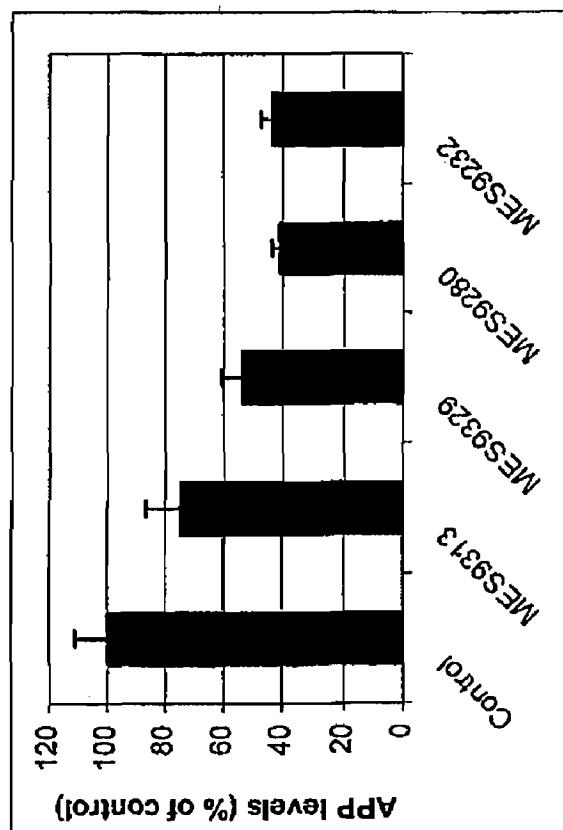
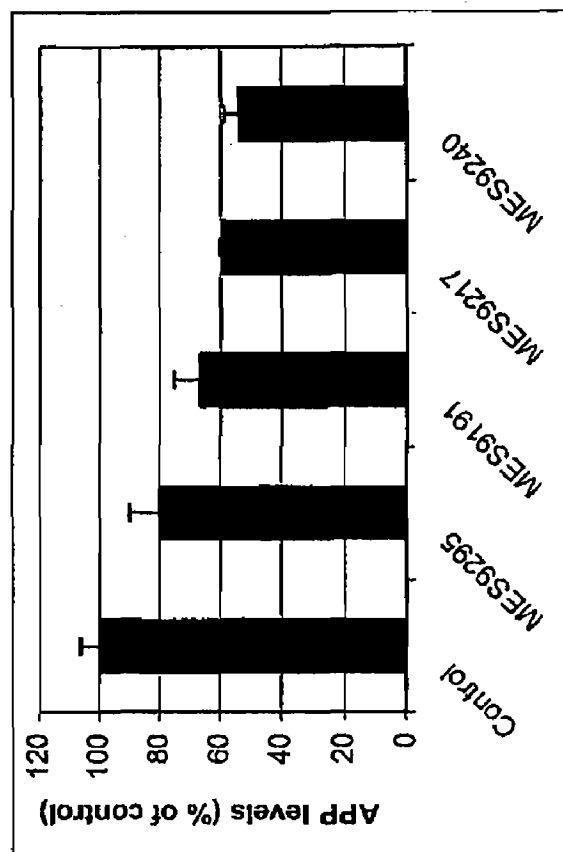
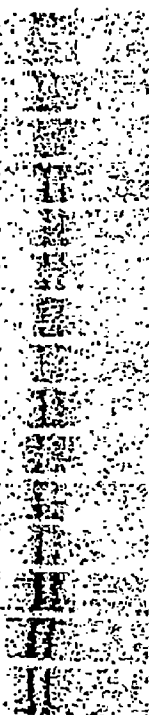
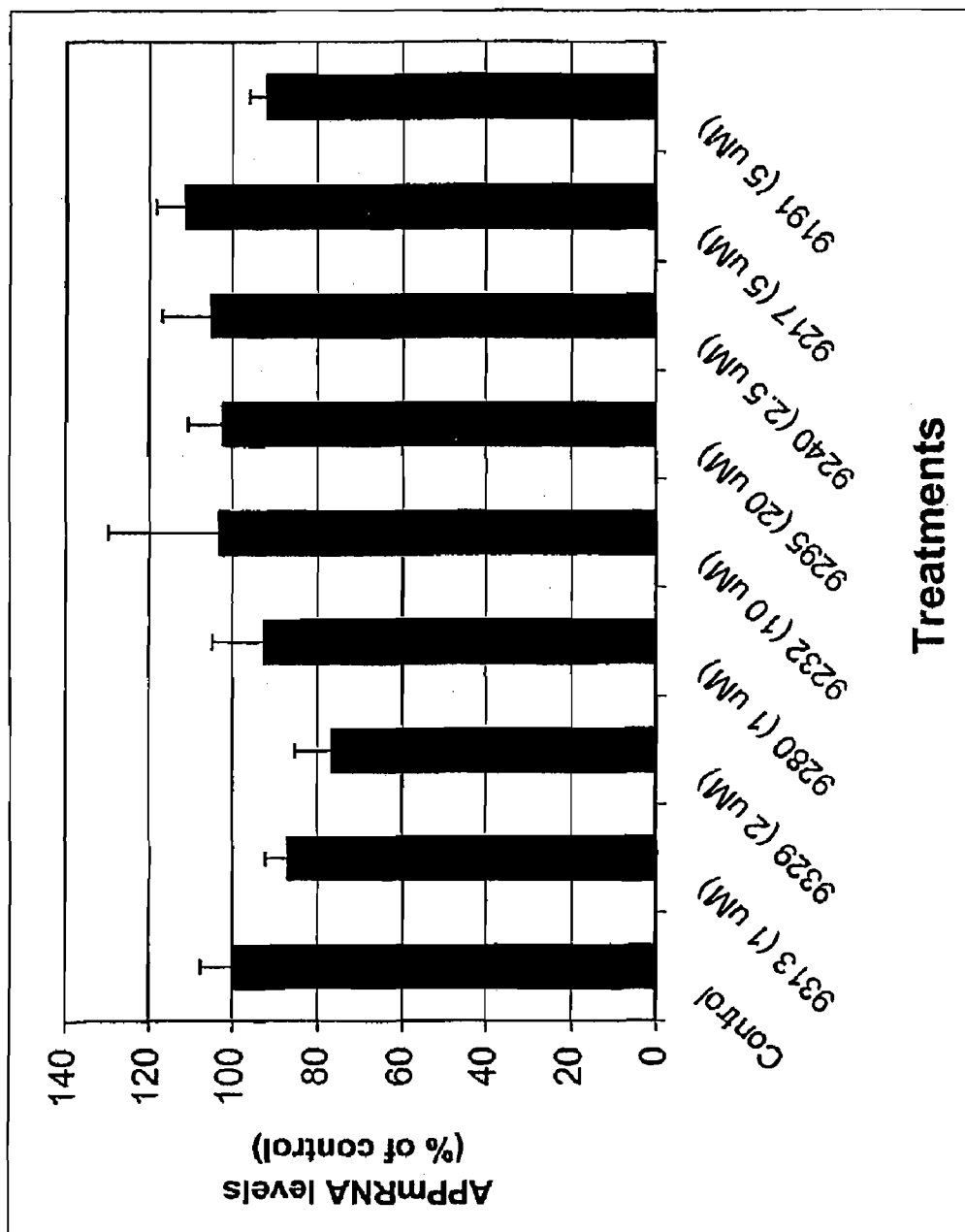


FIGURE 15B



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AGENTS USEFUL FOR REDUCING AMYLOID PRECURSOR PROTEIN AND TREATING DEMENTIA AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATION

The present application is a continuation of and claims the benefit of U.S. application Ser. No. 11/455,959, filed Jun. 20, 2006 now abandoned, which claims priority to U.S. application Ser. No. 10/415,765, filed Feb. 6, 2004, now issued as U.S. Pat. No. 7,153,882, which is a 35 U.S.C. §371 national phase application from, and claims priority to, International Application PCT/US01/48175, filed Nov. 2, 2001 and published under PCT Article 21(2) in English, which claims priority to U.S. Provisional Application No. 60/245,329, filed Nov. 2, 2000, which applications are incorporated herein in their entireties by this reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to agents useful for reducing amyloid precursor protein and methods of use thereof.

2. Background Art

The major pathological hallmarks of Alzheimer's disease (AD), a progressive neurodegenerative condition leading to loss of memory, are characterized by the appearance of senile plaques which are primarily composed of A β and neurofibrillary tangle aggregates (Selkoe, 1997; Roberson and Harrell, 1997). A β , a 40-42 residue peptide, is derived from a larger protein, β APP (695-770 amino acids), whose biological functions remain to be fully determined but whose pathological role may be separated on the basis of its final proteolyzed form (Checker, 1995; Selkoe, 1997). β APP derivatives are generated by three enzymatic activities termed α -, β - and γ -secretases, to produce different protein fragments that are either neuroprotective or amyloidogenic. An aspartyl protease with β -secretase like properties has been identified (Hussein et al., 1999; Sinha et al., 1999; Vassar et al., 1999; Yan et al., 1999), that may serve as a therapeutic marker. However, its value as a target for drug development is complicated by its location within two membranes (plasma and Golgi apparatus). Furthermore, the role of alternative compensatory activities remains unclear. Indeed, a second enzyme, Thimet oligopeptidase, was found capable of β -secretase activity in transfected COS cells (Koike et al., 1999). A major pharmaceutical industry focus has been to look for agents that reduce amyloidogenic processing using compounds that can manipulate β APP to produce non-amyloidogenic by-products. However, it is important to note that the role of alternative β APP fragments in AD is unclear.

Regarding regulatory mechanisms involved in β APP processing, environmental agents have been demonstrated to accelerate β APP turnover into its pathological A β form (Selkoe, 1997). Furthermore, the cellular surrounding of neurons, particularly astrocytes and microglia, are additional and non-neuronal sources of β APP (Funato et al., 1998; Akiyama et al., 2000). Thus, amyloid plaque occurrence is often associated with enlarged microglia which produce interleukin-1 (IL-1), a potent mediator of astroglial proliferation and β APP production (Akiyama et al., 2000). The fact that IL-1 can influence this process suggests that signaling pathways induced by cytokines are interconnected with β APP metabolism. Another example of receptor-signaling association and β APP homeostasis is demonstrated through the activation of muscarinic m1 and m3 receptors which modify β APP synthesis and processing through MAP kinase dependent and independent pathways (Felder et al., 1993; Nitsch et al., 1992

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and 1994). Reductions in muscarinic receptors, as in AD, may alter β APP metabolism and result in subsequent A β deposition. Cholinergic system impairment has been reversed with moderate success by the use of anticholinesterases (Greig et al., 1995; Brossi et al., 1996), the only approved drugs for AD treatment.

A family of novel anticholinesterases, phenserine and analogues, has been synthesized. Phenserine dramatically improves cognitive performance in rodents and is in clinical trials (Greig et al., 1995; Patel et al., 1998). Studies of rats with forebrain cholinergic lesions that are known to dramatically increase β APP in cholinergic projection areas have shown that phenserine can protect against this and additionally, reduce β APP production in naive animals (Haroutunian et al., 1997). As both β APP processing and cholinesterase activity are affected in the AD brain (Bronfman et al., 1996) and as the anticholinesterase, tacrine, has been shown to decrease β APP and A β in neuronal cells in vitro (Lahiri et al., 1998), current studies have focused on the molecular changes induced by phenserine. In these studies, naturally-occurring phenserine (the (-)-enantiomer) was used.

It is the cholinergic action of anticholinesterases such as (-)-phenserine, rivastigmine (Exellon®, Novartis®), donepezil (Aricept®, Pfizer®), galanthamine (Jansen®), tacrine (Cognex®, Warner Lambert®), (-)-physostigmine (Synapton®, Forest®), that provides the compounds their ability to improve cognitive performance in both animal models and humans. Likewise, it is the cholinergic action that is also dose limiting for these same compounds (nausea, sweating, GI effects) (Becker et al., 1991). Conversely, the (+)-enantiomers are unable to inhibit either acetylcholinesterase (AChE., EC 3.1.1.7.) or butyrylcholinesterase (BChE., EC 3.1.1.8.), and hence have no cholinergic action. The (+)-enantiomers are also unnatural isomers and thus, need to be synthesized. Synthetic procedures provide a mixture of (+)- and (-)-forms that require early separation into optically pure forms to eventually obtain the final products.

Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

SUMMARY OF THE INVENTION

The present invention provides compounds and methods of administering compounds to a subject that can reduce β APP production and that is not toxic in a wide range of dosages. The present invention also provides non-carbamate compounds and methods of administering such compounds to a subject that can reduce β APP production and that is not toxic in a wide range of dosages. It has been discovered that either the racemic or enantiomerically pure non-carbamate compounds can be used to decrease β APP production.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the synthesis of analogues of (+)-physostigmine.

FIG. 2 shows the transformation of (+)-oxindole to analogues of (+)-physostigmine. Compound numbers refer to compounds in Table 1.

FIG. 3 shows effects of (+) and (-)-phenserine treatment of SH-SY5Y neuroblastoma cells on β APP protein levels and ERK transcription factor levels.

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FIG. 4 shows effects of (-)-phenserine treatment of SK-N-SH neuroblastoma cells on extracellular β APP protein levels (4A), intracellular β APP protein levels (4B), toxicity (4C) and A β levels (4D).

FIG. 5 shows A β and β APP levels in SK-N-SH cells after administration of (+)-phenserine.

FIG. 6 shows (-)-phenserine treatment of U373 MG astrocytoma alone or in combination with ERK and PI 3 kinase inhibitors.

FIG. 7 shows β APP protein levels in SK-N-SH cells after administration of cymserine and its analogs.

FIG. 8 shows effects of (-)-phenserine treatment on reporter gene expression in the presence and absence of the β APP-mRNA 5' UTR (8A), intracellular β APP protein levels (8B), and on β APP RNA levels (8C) in transfected U373 MG astrocytoma cells.

FIG. 9 shows KNAPP levels in transgenic mice after administration of (-)-phenserine and (-)-phenethylcymserine.

FIGS. 10A-10B show A β ₁₋₄₀ and A β ₁₋₄₂ levels in transgenic mice after administration of (-)-phenserine and (-)-phenethylcymserine derived from animals described in FIG. 9.

FIG. 11A shows the translational regulation of phenserine (Rate of APP Synthesis) in SH-SY5Y cells. FIG. 11B shows the effects of (-)-phenserine on steady state APP mRNA levels in SH-SY5Y cells.

FIGS. 12A and B show the effects of (-)-phenserine on extracellular and intracellular APP levels in SK-N-SH, respectively.

FIG. 13A shows the effects of (-)-phenserine on secreted APP levels and cell-viability in SH-SY5Y cells. FIGS. 13B-M show the effects of several compounds of the invention on secreted APP levels and cell-viability in SH-SY5Y cells.

FIG. 14 shows the effects of several compounds of the invention on extra- and intracellular APP levels in SH-SY5Y cells.

FIG. 15A shows the translational regulation by compounds (rate of APP Synthesis) in SH-SY5Y cells using several compounds of the invention. FIG. 15B shows the effects of several compounds of the invention on the steady state APP mRNA levels in SH-SY5Y cells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention may be understood more readily by reference to the following detailed description of desired embodiments of the invention and the Examples included therein.

Before the present compounds, compositions and methods are disclosed and described, it is to be understood that this invention is not limited to specific synthetic methods, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

Throughout this application, where publications and patents are referenced, the disclosures of these publications and patents in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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Variables, such as R₁-R₁₅, n, A, D, E, G, X, Y, and Z throughout the application are the same variables as previously defined unless stated to the contrary.

In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meaning.

The term "alkyl" as used herein refers to a branched or unbranched saturated hydrocarbon group of 1 to 4, 1 to 8, or 1 to 20 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, pentyl, hexyl, heptyl, octyl, and the like. Examples of cycloalkyl groups include cyclopentyl and cyclohexyl.

The term "alkenyl" as used herein refers to a hydrocarbon group of 2 to 4, 2 to 8, or 2 to 20 carbon atoms and structural formula containing a carbon-carbon double bond.

The term "alkynyl" as used herein refers to a hydrocarbon group of 2 to 4, 2 to 8, or 2 to 20 carbon atoms and a structural formula containing a carbon-carbon triple bond.

The term "aryl" is defined as any carbon-based aromatic group including, but not limited to, phenyl, benzene, naphthalene, anthracene, phenanthrene, pyrene, and benzo[a]pyrene, etc.

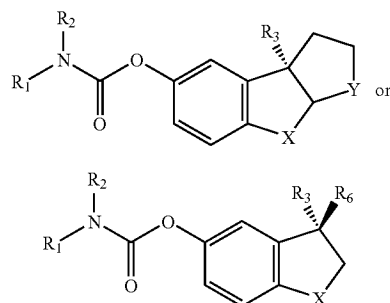
The term "substituted aryl" is defined as an aryl group having at least one group attached to the aryl group that is not hydrogen. Examples of groups that can be attached to the aryl group include, but are not limited to, alkyl, alkynyl, alkenyl, aryl, heterocyclic, halide, nitro, amino, ester, ketone, aldehyde, hydroxy, carboxylic acid, alkoxy, cyano, alkoxy, thioalkyl, haloalkyl, hydroxyalkyl, alkylamino, dialkylamino, or acyl. In various embodiments, a substituent is bound to carbon 2, 3, 4, 5, or 6 of one of these moieties. Examples of alkoxy substituents include, but are not limited to, methoxy, ethoxy, and isopropoxy groups. Examples of acyl substituents include acetyl and benzoyl groups.

The term "aralkyl" is defined as an aryl group having an alkyl, alkynyl, or alkenyl group attached to the aryl group. An example of an aralkyl group is a benzyl group.

The term "heteroaryl" is defined as an aryl group that has at least one heteroatom such as nitrogen, sulfur, or oxygen incorporated within the ring of the aryl group.

The term "heteroalkyl" is defined as an alkyl group that has at least one heteroatom, such as nitrogen, sulfur, oxygen, or phosphate, incorporated within the alkyl group or attached to the alkyl group.

The invention, in one aspect, relates to a compound having the formula I or II



wherein R₁ and R₂ are, independently, hydrogen, branched or straight chain C₁-C₈ alkyl, substituted or unsubstituted aryl, or aralkyl;

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R₃ is branched or straight chain C₁-C₄ alkyl or heteroalkyl or C₄-C₈ alkyl or heteroalkyl, or substituted or unsubstituted aryl;

X and Y are, independently, O, S, alkyl, hydrocarbon moiety, C(H)R₄, or NR₅, wherein R₄ and R₅ are, independently, hydrogen, oxygen, branched or straight chain C₁-C₈ alkyl, C₂-C₈ alkenyl or C₂-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl; and

R₆ is hydrogen; C₁-C₈ alkyl, C₁-C₈ alkenyl, C₂-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl, or (CH₂)_nR₇, where R₇ is hydroxy, alkoxy, cyano, ester, carboxylic acid, substituted or unsubstituted amino, and n is from 1 to 4,

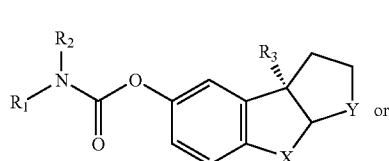
wherein the compound having the formula I or II is the substantially pure (+)-enantiomer,

with the proviso that the compound is not (+)-physostigmine, (+)-octylcarbamoylseroline, (+)-benzylcarbamoylseroline, (+)-physovenine, (+)-N-methylphysostigmine, (+)-phenserine, (+)-3-(1-methylamino)-ethyl-2H-indol-5-yl-phenylcarbamate, (+)-N¹-

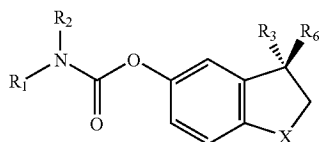
benzylmorphysostigmine, (+)-N¹-benzylmorphenserine, (+)-N¹-benzylmorphoserine, (+)-N¹-benzylmorphoserine, (+)-N¹-norphenserine, (+)-N¹-nortolserine, (+)-N¹-norcymserine, (+)-N⁸-benzylmorphysostigmine, (+)-N⁸-benzylmorphenserine, (+)-N⁸-morphysostigmine, (+)-N⁸-morphenserine, (+)-N¹,N⁸-bisbenzylmorphysostigmine, (+)-N¹,N⁸-bisbenzylmorphenserine, (+)-N¹,N⁸-bisbenzylmorphoserine, or (+)-N¹,N⁸-bisbenzylmorphoserine,

with the proviso that when the compound is formula I, X is NCH₃, and Y is NR₅, where R₅ is hydrogen, loweralkyl, arylloweralkyl, heteroarylloweralkyl, cycloalkylmethyl, or loweralkenyl methyl, R₃ is not methyl or at least one of R₁ or R₂ is not H or alkyl.

The invention also relates to a compound having the formula III or IV



(III)



(IV)

wherein R₁ and R₂ are, independently, hydrogen, branched or straight chain C₁-C₈ alkyl, substituted or unsubstituted aryl, or aralkyl;

R₃ is branched or straight chain C₁-C₄ alkyl, or substituted or unsubstituted aryl;

X and Y are, independently, O, S, alkyl, hydrocarbon moiety, C(H)R₄, or NR₅, wherein R₄ and R₅ are, independently, hydrogen, oxygen, branched or straight chain C₁-C₈ alkyl, q-C₈ alkenyl, or C₂-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl; and

R₆ is hydrogen; C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl, or (CH₂)_nR₇, where R₇ is hydroxy, alkoxy, cyano, ester, carboxylic acid, substituted or unsubstituted amino, and n is from 1 to 4,

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The chiral center of compounds I-IV is the carbon atom that has R₃ bonded to it. Here, the (+)-enantiomer has R₃ pointing behind the plane of the page. In various embodiments, the compounds having the structure I or II have an enantiomeric purity for the (+)-enantiomer of from 55 to 100%, desirably from 75 to 100%, more desirably from 85 to 100%, more desirably from 95 to 100%, and even more desirably 100%.

In one embodiment, when the compound is formula I, R₃ is methyl and X is NCH₃.

In one embodiment, when the compound is formula I or II, R₃ is not methyl. In particular embodiments, R₃ is a branched or straight chain alkyl or heteroalkyl group of 2, 3, 4, 5, 6, 7, or 8 carbons or substituted or unsubstituted aryl.

In another embodiment, when the compound has the structure I or II, Y is C(H)R₄ or X is O, S, or C(H)R₄.

In another embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, and Y is NCH₃. In one embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, Y is NCH₃, and R₁ is C₁-C₈ straight chain alkyl or benzyl and R₂ is hydrogen. In one embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, Y is NCH₃, and R₁ is substituted or unsubstituted phenyl and R₂ is hydrogen. In one embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, Y is NCH₃, and R₁ and R₂ are, independently, methyl or ethyl.

In another embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, and Y is O. In one embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, Y is O, R₁ is C₁-C₈ straight chain alkyl or benzyl, and R₂ is hydrogen. In one embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, Y is O, and R₁ and R₂ are, independently, methyl or ethyl. In one embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, Y is O, and R₁ is substituted or unsubstituted phenyl and R₂ is hydrogen.

In another embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, and Y is S. In one embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, Y is S, R₁ is C₁-C₈ straight chain alkyl or benzyl, and R₂ is hydrogen. In one embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, Y is S, and R₁ and R₂ are, independently, methyl or ethyl. In one embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, Y is S, R₁ is substituted or unsubstituted phenyl, and R₂ is hydrogen.

In another embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, and Y is NR₅. In one embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, and Y is NR₅, wherein R₅ is -CH₂CH=CH₂, -CH₂CH₂Ph, benzyl, or hydrogen.

In another embodiment, when the compound has the formula I, R₃ is methyl, Y is NCH₃, and X is NCH₃, wherein R₄ is benzyl or hydrogen.

In another embodiment, when the compound is formula I, R₃ is methyl, X is NCH₃, Y is NR₅, wherein each R₄ and R₅ is, independently, hydrogen or benzyl.

In another embodiment, when the compound is formula I, R₃ is phenyl, X is NCH₃, and Y is NCH₃.

In another embodiment, when the compound is formula I, R₃ is methyl, and X is NCH₃, and Y is not NH or NHCH₂Ph.

In another embodiment, when the compound is formula II, R₃ is methyl, X is C(H)CH₃, and R₆ is (CH₂)₂R₇, where R₇ is a substituted or unsubstituted amino group.

In another embodiment, the compound having the formula I or II can be found in Table 1. Although only the (+)-isomer is illustrated to save space, it is the intent of the invention to claim the (+)-isomer, (-)-isomer, and mixtures of both isomers (e.g., racemic 1:1 mixtures) of all of the compounds of the invention unless such compounds are specifically excluded.

TABLE 1

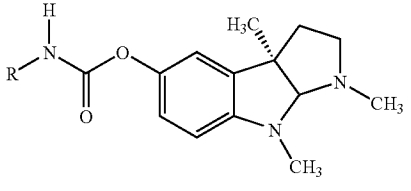
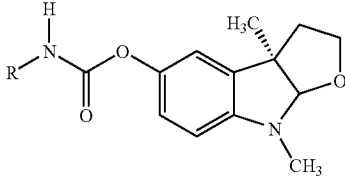
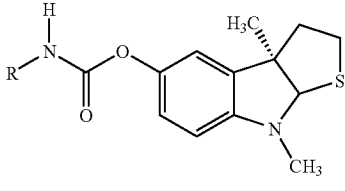
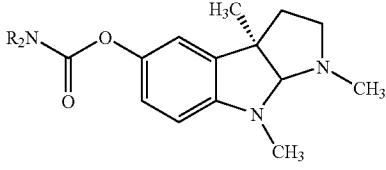
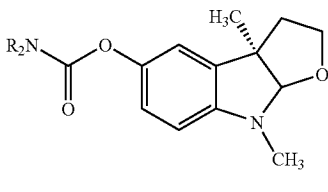
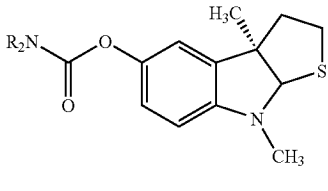
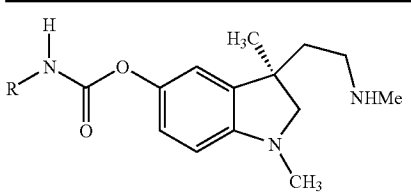
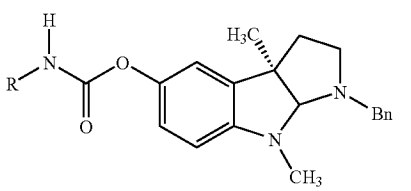
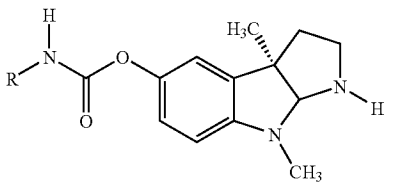
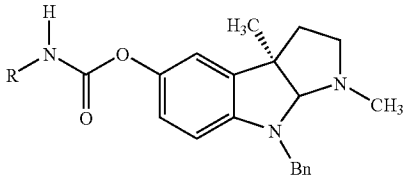
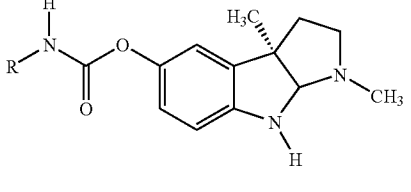
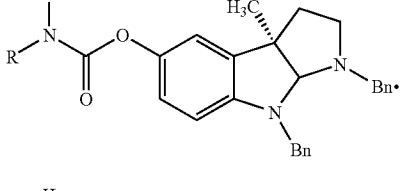
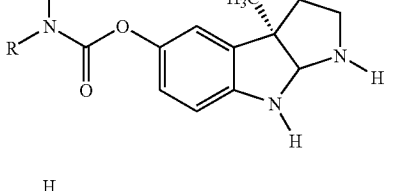
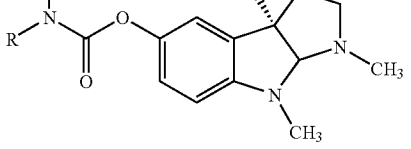
Structure	No.	R	Compounds
	1	CH ₃	(+)-Physostigmine
	2	C ₂ H ₅	(+)-Ethylcarbamoyleseroline
	3	(CH ₂) ₂ CH ₃	(+)-Propylcarbamoyleseroline
	4	CH ₂ (CH ₃) ₂	(+)-Isopropylcarbamoyleseroline
	5	(CH ₂) ₃ CH ₃	(+)-Butylcarbamoyleseroline
	6	(CH ₂) ₄ CH ₃	(+)-Pentylcarbamoyleseroline
	7	(CH ₂) ₅ CH ₃	(+)-Hexylcarbamoyleseroline
	8	(CH ₂) ₆ CH ₃	(+)-Heptylcarbamoyleseroline
	9	(CH ₂) ₇ CH ₃	(+)-Octylcarbamoyleseroline
	10	CH ₂ C ₆ H ₅	(+)-Benzylcarbamoyleseroline
	11	CH ₃	(+)-Physovenine
	12	C ₂ H ₅	(+)-Ethylcarbamoylphysovenol
	13	(CH ₂) ₂ CH ₃	(+)-Propylcarbamoylphysovenol
	14	CH ₂ (CH ₃) ₂	(+)-Isopropylcarbamoylphysovenol
	15	(CH ₂) ₃ CH ₃	(+)-Butylcarbamoylphysovenol
	16	(CH ₂) ₄ CH ₃	(+)-Pentylcarbamoylphysovenol
	17	(CH ₂) ₅ CH ₃	(+)-Hexylcarbamoylphysovenol
	18	(CH ₂) ₆ CH ₃	(+)-Heptylcarbamoylphysovenol
	19	(CH ₂) ₇ CH ₃	(+)-Octylcarbamoylphysovenol
	20	CH ₂ C ₆ H ₅	(+)-Benzylcarbamoylphysovenol
	21	CH ₃	(+)-Thiaphysovenine
	22	C ₂ H ₅	(+)-Ethylcarbamoylthiaphysovenol
	23	(CH ₂) ₂ CH ₃	(+)-Propylcarbamoylthiaphysovenol
	24	CH ₂ (CH ₃) ₂	(+)-Isopropylcarbamoylthiaphysovenol
	25	(CH ₂) ₃ CH ₃	(+)-Butylcarbamoylthiaphysovenol
	26	(CH ₂) ₄ CH ₃	(+)-Pentylcarbamoylthiaphysovenol
	27	(CH ₂) ₅ CH ₃	(+)-Hexylcarbamoylthiaphysovenol
	28	(CH ₂) ₆ CH ₃	(+)-Heptylcarbamoylthiaphysovenol
	29	(CH ₂) ₇ CH ₃	(+)-Octylcarbamoylthiaphysovenol
	30	CH ₂ C ₆ H ₅	(+)-Benzylcarbamoylthiaphysovenol
	31	CH ₃	(+)-N-Methylphysostigmine
	32	C ₂ H ₅	(+)-Diethylcarbamoyleseroline
	33	CH ₃	(+)-N-Methylphysovenine
	34	C ₂ H ₅	(+)-Diethylcarbamoylphysovenol
	35	CH ₃	(+)-N-Methylthiaphysovenine
	36	C ₂ H ₅	(+)-Diethylcarbamoylthiaphysovenol

TABLE 1-continued

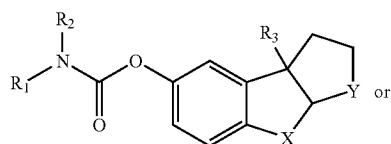
Structure	No.	R	Compounds
	37	H	(+)-Phenserine
	38	2'-CH ₃	(+)-Tolserine
	39	3'-CH ₃	(+)-3'-Methylphenserine
	40	4'-CH ₃	(+)-4'-Methylphenserine
	41	2'-CH ₂ CH ₃	(+)-2'-Ethylphenserine
	42	3'-CH ₂ CH ₃	(+)-3'-Eethylphenserine
	43	4'-CH ₂ CH ₃	(+)-4'-Eethylphenserine
	44	2'-CH ₂ (CH ₃) ₂	(+)-2'-Isopropylphenserine
	45	3'-CH ₂ (CH ₃) ₂	(+)-3'-Isopropylphenserine
	46	4'-CH ₂ (CH ₃) ₂	(+)-Cymserine
	47	2',3'-CH ₃	(+)-2',3'-Dimethylphenserine
	48	2',4'-CH ₃	(+)-2',4'-Dimethylphenserine
	49	2',5'-CH ₃	(+)-2',5'-Dimethylphenserine
	50	2',6'-CH ₃	(+)-2',6'-Dimethylphenserine
	51	3',4'-CH ₃	(+)-3',4'-Dimethylphenserine
	52	3',5'-CH ₃	(+)-3',5'-Dimethylphenserine
	53	3',6'-CH ₃	(+)-3',6'-Dimethylphenserine
	54	2',4',6'-CH ₃	(+)-2',4',6'-Trimethylphenserin
	55	H	(+)-Phenylcarbamoyl-physovenol
	56	2'-CH ₃	(+)-2'-Methylphenylcarbamoyl-physovenol
	57	3'-CH ₃	(+)-3'-Methylphenylcarbamoyl-physovenol
	58	4'-CH ₃	(+)-4'-Methyl phenylcarbamoyl-physovenol
	59	2'-CH ₂ CH ₃	(+)-2'-Ethylphenylvarbamoyl-physovenol
	60	3'-CH ₂ CH ₃	(+)-3'-Eethylphenylcarbamoyl-physovenol
	61	4'-CH ₂ CH ₃	(+)-4'-Eethylphenylcarbamoyl-physovenol
	62	2'-CH ₂ (CH ₃) ₂	(+)-2'-Isopropylphenylcarbamoyl-physovenol
	63	3'-CH ₂ (CH ₃) ₂	(+)-3'-Isopropylphenylcarbamoyl-physovenol
	64	4'-CH ₂ (CH ₃) ₂	(+)-4'-Isopropylphenylcarbamoyl-physovenol
	65	2',3'-CH ₃	(+)-2',3'-Dimethylphenylcarbamoyl-physovenol
	66	2',4'-CH ₃	(+)-2',4'-Dimethylphenylcarbamoyl-physovenol
	67	2',5'-CH ₃	(+)-2',5'-Dimethylphenylcarbamoyl-physovenol
	68	2',6'-CH ₃	(+)-2',6'-Dimethylphenylcarbamoyl-physovenol
	69	3',4'-CH ₃	(+)-3',4'-Dimethylphenylcarbamoyl-physovenol
	70	3',5'-CH ₃	(+)-3',5'-Dimethylphenylcarbamoyl-physovenol
	71	3',6'-CH ₃	(+)-3',6'-Dimethylphenylcarbamoyl-physovenol
	72	2',4',6'-CH ₃	(+)-2',4',6'-Trimethylphenylcarbamoyl-physovenol
	55	H	(+)-Phenylcarbamoyl-thiaphysovenol
	56	2'-CH ₃	(+)-2'-Methylphenylcarbamoyl-thiaphysovenol
	57	3'-CH ₃	(+)-3'-Methylphenylcarbamoyl-thiaphysovenol
	58	4'-CH ₃	(+)-4'-Methyl phenylcarbamoyl-thiaphysovenol
	59	2'-CH ₂ CH ₃	(+)-2'-Ethylphenylvarbamoyl-thiaphysovenol
	60	3'-CH ₂ CH ₃	(+)-3'-Eethylphenylcarbamoyl-thiaphysovenol
	61	4'-CH ₂ CH ₃	(+)-4'-Eethylphenylcarbamoyl-thiaphysovenol
	62	2'-CH ₂ (CH ₃) ₂	(+)-2'-Isopropylphenylcarbamoyl-thiaphysovenol
	63	3'-CH ₂ (CH ₃) ₂	(+)-3'-Isopropylphenylcarbamoyl-thiaphysovenol
	64	4'-CH ₂ (CH ₃) ₂	(+)-4'-Isopropylphenylcarbamoyl-thiaphysovenol
	65	2',3'-CH ₃	(+)-2',3'-Dimethylphenylcarbamoyl-thiaphysovenol
	66	2',4'-CH ₃	(+)-2',4'-Dimethylphenylcarbamoyl-thiaphysovenol
	67	2',5'-CH ₃	(+)-2',5'-Dimethylphenylcarbamoyl-thiaphysovenol
	68	2',6'-CH ₃	(+)-2',6'-Dimethylphenylcarbamoyl-thiaphysovenol
	69	3',4'-CH ₃	(+)-3',4'-Dimethylphenylcarbamoyl-thiaphysovenol
	70	3',5'-CH ₃	(+)-3',5'-Dimethylphenylcarbamoyl-thiaphysovenol
	71	3',6'-CH ₃	(+)-3',6'-Dimethylphenylcarbamoyl-thiaphysovenol
	72	2',4',6'-CH ₃	(+)-2',4',6'-Trimethylphenylcarbamoyl-thiaphysovenol
	73	CH ₃	(+)-N ¹ -Allylnorphysostigmine
	74	phenyl	(+)-N ¹ -Allylnorphenserine
	75	2'-tolyl	(+)-N ¹ -Allylnortolserine
	76	4'-cymyl	(+)-N ¹ -Allylnorcymserine
	77	CH ₃	(+)-N ¹ -Phenethylnorphysostigmine
	78	phenyl	(+)-N ¹ -Phenethylnorphenserine
	79	2'-tolyl	(+)-N ¹ -Phenethylnortolserine
	80	4'-cymyl	(+)-N ¹ -Phenethylnorcymserine

TABLE 1-continued

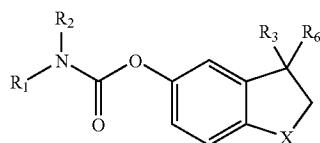
Structure	No.	R	Compounds
	81	CH ₃	(+)-3-(1-Methylamino)-ethyl-2H-indol-5yl-methylcarbamate
	82	phenyl	(+)-3-(1-Methylamino)-ethyl-2H-indol-5yl-phenylcarbamate
	83	2'-tolyl	(+)-3-(1-Methylamino)-ethyl-2H-indol-5yl-tolylcarbamate
	84	4'-cymyl	(+)-3-(1-Methylamino)-ethyl-2H-indol-5yl-cymylcarbamate
	85	CH ₃	(+)-N ¹ -Benzylmorphysostigmine
	86	phenyl	(+)-N ¹ -Benzylmorphenserine
	87	2'-tolyl	(+)-N ¹ -Benzylmortolserine
	88	4'-cymyl	(+)-N ¹ -Benzylmorcymserine
	89	CH ₃	(+)-N ¹ -Norphysostigmine
	90	phenyl	(+)-N ¹ -Norphenserine
	91	2'-tolyl	(+)-N ¹ -Nortolserine
	92	4'-cymyl	(+)-N ¹ -Norocymserine
	93	CH ₃	(+)-N ⁸ -Benzylmorphysostigmine
	94	phenyl	(+)-N ⁸ -Benzylmorphenserine
	95	2'-tolyl	(+)-N ⁸ -Benzylmortolserine
	96	4'-cymyl	(+)-N ⁸ -Benzylmorcymserine
	97	CH ₃	(+)-N ⁸ -Norphysostigmine
	98	phenyl	(+)-N ⁸ -Norphenserine
	99	2'-tolyl	(+)-N ⁸ -Nortolserine
	100	4'-cymyl	(+)-N ⁸ -Norcymserine
	101	CH ₃	(+)-N ¹ ,N ⁸ -Bisbenzylmorphysostigmine
	102	phenyl	(+)-N ¹ ,N ⁸ -Bisbenzylmorphenserine
	103	2'-tolyl	(+)-N ¹ ,N ⁸ -Bisbenzylmortolserine
	104	4'-cymyl	(+)-N ¹ ,N ⁸ -Bisbenzylmorcymserine
	105	CH ₃	(+)-N ¹ ,N ⁸ -Bisnorphysostigmine
	106	phenyl	(+)-N ¹ ,N ⁸ -Bisnorphenserine
	107	2'-tolyl	(+)-N ¹ ,N ⁸ -Bisnortolserine
	108	4'-cymyl	(+)-N ¹ ,N ⁸ -Bisnorcymserine
	109	CH ₃	(+)-3a-Phenylphysostigmine
	110	phenyl	(+)-3a-Phenylphenserine
	111	2'-tolyl	(+)-3a-Phenyltolserine
	112	4'-cymyl	(+)-3a-Phenylcymserine

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The invention, in one aspect, a compound having the formula XIV or XV



(XIV) 5



(XV) 10

wherein R₁ and R₂ are, independently, hydrogen, branched or straight chain C₁-C₈ alkyl, substituted or unsubstituted aryl, or aralkyl;

R₃ is branched or straight chain C₁-C₄ alkyl or heteroalkyl or C₄-C₈ alkyl or heteroalkyl, or substituted or unsubstituted aryl;

X and Y are, independently, O, S, alkyl, hydrocarbon moiety, C(H)R₄, or NR₅, wherein R₄ and R₅ are, independently, hydrogen, oxygen, branched or straight chain C₁-C₈ alkyl, C₂-C₈ alkenyl, or C₂-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl; and

R₆ is hydrogen; C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl, or (CH₂)_n R₇, where R₇ is hydroxy, alkoxy, cyano, ester, carboxylic acid, substituted or unsubstituted amino, and n is from 1 to 4,

wherein the compound having the formula XIV or XV is a racemic mixture or the substantially pure (-)-enantiomer.

In one embodiment, the compounds having the structure XV and XV have an enantiomeric purity for the (-)-enantiomer of from 55 to 100%, desirably from 75 to 100%, more desirably from 85 to 100%, more desirably from 95 to 100%, and even more desirably 100%.

In another embodiment, when the compound has the structure XIV or XV, Y is C(H)R₄ or X is O, S, or C(H)R₄.

In yet another embodiment, the compound of formula XIV is MES9280 (FIG. 13K), EES9232 (FIG. 13J), MES9313 (FIG. 13F), or a (+)-isomer or a racemic mixture thereof.

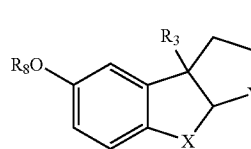
In another embodiment, when the compound has the formula XIV, where X and Y are nitrogen, and the compound is the substantially pure (-)-enantiomer, then R₃ is not methyl. In another embodiment, when the compound has the formula XIV, where X is nitrogen, Y is nitrogen or oxygen, and the compound is the substantially pure (-)-enantiomer, then R₅ is not hydrogen or C₁-C₁₀ alkyl. In another embodiment, when the compound has the formula XIV, where X is nitrogen and Y is sulfur, and the compound is the substantially pure (-)-enantiomer, then R₃ is not methyl. In another embodiment, the compound is not (-)-phenserine, (-)-physostigmine, (-)-heptyl-physostigmine, (-)-physovenine, (-)-N(1)-norphysostigmine, MES9217 (FIG. 13H), MES9299 (FIG. 13L), and MES9329 (FIG. 13M). In one embodiment, when the compound is formula XIV or XV, R₃ is not methyl. In particular embodiments, R₃ is a branched or straight chain alkyl or heteroalkyl group of 2, 3, 4, 5, 6, 7, or 8 carbons or substituted or unsubstituted aryl.

FIGS. 1 and 2 show one approach to the synthesis of compounds having the structure I and II. Using techniques

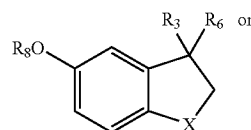
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known in the art (see Julian et al., *J. Am. Chem. Soc.*, 1935, 57, 563; Lee et al., *J. Org. Chem.*, 1991, 56, 872; Pei et al., *Heterocycles*, 1995, 41, 2823; Lee et al., *J. Chromatography*, 1990, 523, 317; and Pei et al., *Heterocycles*, 1994, 39, 557, which are incorporated by reference in their entirety), the physostigmine compound A (FIG. 1) can be prepared in high yield and enantiopurity. Compound A is an important intermediate, and can be used to produce a variety of compounds having the structure I and II. FIG. 2 shows that by using techniques known in the art (see Yu et al., *Heterocycles*, 1988, 27, 745; Yu et al., *Helv. Chem. Res.*, 1991, 74, 761; He et al., *Med. Chem. Res.*, 1992, 2, 229; Pei et al., *Med. Chem. Res.*, 1995, 5, 455; Yu et al., *J. Med. Chem.*, 1998, 31, 2297; Zhu, *Tet. Lett.*, 2000, 41, 4861; Pei et al., *Med. Chem. Res.*, 1995, 5, 455; and Yu et al., *J. Med. Chem.*, 1997, 40, 2895, which are incorporated by reference in their entirety) compound A can be converted to a number of different compounds having the structure I and II. The numbers below each compound in FIG. 2 correspond to the compound numbers in Table 1. The racemic mixture of I and II as well as compounds XIV and XV can be prepared using the synthetic procedure outlined in FIGS. 1 and 2, where a chiral chromatography step is not performed to produce the racemic mixture or the (-)-enantiomer is isolated instead of the (+)-enantiomer.

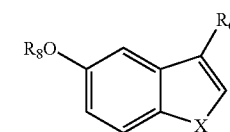
The invention also relates to a compound having the formula V, VI, or VII



(V)



(VI)



(VII)

wherein R₈ is hydrogen, branched or straight chain C₁-C₈ alkyl, substituted or unsubstituted aryl, aralkyl, or CR₉R₁₀OR₁₁, where R₉ and R₁₀ are, independently, hydrogen or alkyl, and R₁₁ is alkyl;

R₃ is branched or straight chain C₁-C₄ alkyl, or substituted or unsubstituted aryl;

X and Y are, independently, O, S, C(H)R₄, or NR₅, wherein R₄ and R₅ are, independently, hydrogen, oxygen, branched or straight chain C₁-C₈ alkyl, C₂-C₈ alkenyl, or C₂-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl; and

R₆ is hydrogen; C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl, or (CH₂)_n R₇, where R₇ is hydroxy, alkoxy, cyano, ester, carboxylic acid, substituted or unsubstituted amino, and n is from 1 to 4,

with the proviso that when the compound is formula V, X is NCH₃, and Y is NR₅, where R₅ is hydrogen, loweralkyl,

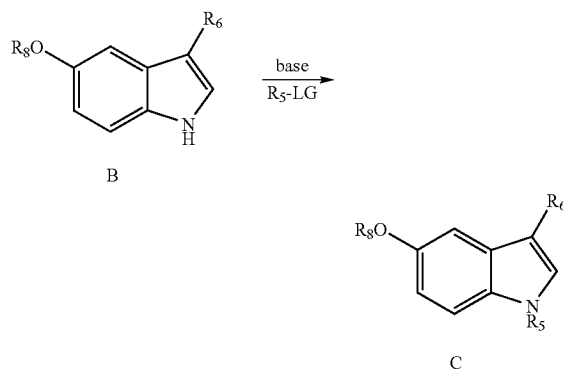
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arylloweralkyl, heteroarylloweralkyl, cycloalkylmethyl, or loweralkenyl methyl, R_3 is not methyl or R_8 is not H or, loweralkyl.

The compounds having the formula V-VII as well as compounds VIII-XIII described below are referred to herein as "non-carbamate compounds" because they do not possess the carbamate group present in compounds I-IV, XIV-XVI. Compounds V-XIII are generally the synthetic precursors to compounds I-IV. FIG. 1 provides a general synthesis to intermediate A, which is a species of compound V. Intermediate A is shown as the (+)-enantiomer; however, the (-)-enantiomer can also be isolated using chiral chromatography. Similarly, the (+)- and (-)-enantiomers of compound VI can also be produced using similar methodology. Furthermore, in the absence of the chiral chromatography step in FIG. 1, the racemic compounds V and VI can be produced.

In another embodiment, when the non-carbamate compound has the formula VII, where X is NR_5 , the reaction depicted in Scheme 1 can be used to produce the compounds. For example, in Scheme I, compound B (compound VII where X is NH) can be treated with a base, such as $NaNH_2$, then treated with an alkyl or aralkyl halide compound to produce compound C.

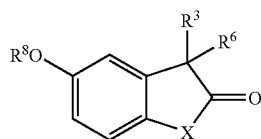
SCHEME 1



In one embodiment, when the compound is formula V, X and Y are NR_5 , wherein R_5 is branched or straight chain C_1 - C_8 alkyl, desirably methyl. In another embodiment, when the compound is formula V, R_3 is methyl, X and Y are NCH_3 , and R_8 is C_1 - C_8 straight chain alkyl, desirably methyl.

In another embodiment, when the compound is formula VII, X is NR_5 , wherein R_5 is branched or straight chain C_1 - C_8 alkyl or aralkyl, desirably benzyl. In another embodiment, when the compound is formula VII, R_6 is $(CH_2)_nR_7$, where R_7 is a substituted or unsubstituted amino group. In another embodiment, when the compound is formula VII, X is NR_5 , where R_5 is benzyl, R_6 is $(CH_2)_2N(CH_3)_2$, and R_8 is methyl.

The invention also relates to a compound having the formula VIII



(VIII)

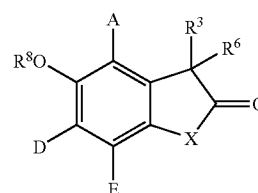
wherein R^8 is hydrogen, branched or straight chain C_1 - C_8 alkyl, substituted or unsubstituted aryl, aralkyl, or $CR_9R_{10}OR_{11}$, where R_9 and R_{10} are, independently, hydrogen or alkyl, and R_{11} is alkyl;

R^3 is branched or straight chain C_1 - C_4 alkyl, or substituted or unsubstituted aryl;

X is O, S, $C(H)R_4$, or NR_5 , wherein R_4 and R_5 are, independently, hydrogen, oxygen, branched or straight chain C_1 - C_8 alkyl, C_1 - C_8 alkenyl, or C_2 - C_8 alkynyl, aralkyl, or substituted or unsubstituted aryl; and

R^6 is hydrogen; C_1 - C_8 alkyl, C_2 - C_8 alkenyl, C_2 - C_8 alkynyl, aralkyl, or substituted or unsubstituted aryl, or $(CH_2)_nR_7$, where R_7 is hydroxy, alkoxy, cyano, ester, carboxylic acid, substituted or unsubstituted amino, and n is from 1 to 4.

The invention further relates to a compound having the formula IX



(IX)

wherein R^8 is hydrogen, branched or straight chain C_1 - C_8 alkyl, substituted or unsubstituted aryl, aralkyl, or $CR_9R_{10}OR_{11}$, where R_9 and R_{10} are, independently, hydrogen or alkyl, and R_{11} is alkyl;

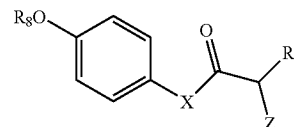
R^3 is branched or straight chain C_1 - C_4 alkyl, or substituted or unsubstituted aryl;

R^6 is hydrogen; C_1 - C_8 alkyl, C_2 - C_8 alkenyl, C_2 - C_8 alkynyl, aralkyl, or substituted or unsubstituted aryl, or $(CH_2)_nR_7$, where R_7 is hydroxy, alkoxy, cyano, ester, carboxylic acid, substituted or unsubstituted amino, and n is from 1 to 4;

X is O, S, $C(H)R_4$, or NR_5 , wherein R_4 and R_5 are, independently, hydrogen, oxygen, branched or straight chain C_1 - C_8 alkyl, C_2 - C_8 alkenyl, or C_2 - C_8 alkynyl, aralkyl, or substituted or unsubstituted aryl; and

A, D, and E are, independently, hydrogen, hydroxy, alkoxy, halide, alkyl, aralkyl, or amino.

The invention also relates to a compound having the formula X



(X)

wherein R^8 is hydrogen, branched or straight chain C_1 - C_8 alkyl, substituted or unsubstituted aryl, aralkyl, or $CR_9R_{10}OR_{11}$, where R_9 and R_{10} are, independently, hydrogen or alkyl, and R_{11} is alkyl;

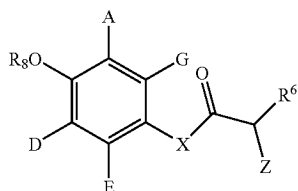
R^6 is hydrogen; C_1 - C_8 alkyl, C_2 - C_8 alkenyl, C_2 - C_8 alkynyl, aralkyl, or substituted or unsubstituted aryl, or $(CH_2)_nR_7$, where R_7 is hydroxy, alkoxy, cyano, ester, carboxylic acid, substituted or unsubstituted amino, and n is from 1 to 4;

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X is O, S, C(H)R₄, or NR₅, wherein R₄ and R₅ are, independently, hydrogen, oxygen, branched or straight chain C₁-C₈ alkyl, C₂-C₈ alkenyl, or C₂-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl; and

Z is halide, hydroxy, or alkoxy.

The invention also relates to a compound having the formula XI



wherein R⁸ is hydrogen, branched or straight chain C₁-C₈ alkyl, substituted or unsubstituted aryl, aralkyl, or CR₉R₁₀OR₁₁, where R₉ and R₁₀ are, independently, hydrogen or alkyl, and R₁₁ is alkyl;

R⁶ is hydrogen; C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl, or (CH₂)_nR₇, where R₇ is hydroxy, alkoxy, cyano, ester, carboxylic acid, substituted or unsubstituted amino, and n is from 1 to 4;

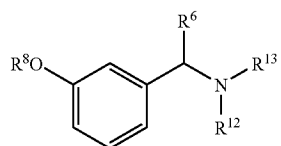
X is O, S, C(H)R₄, or NR₅, wherein R₄ and R₅ are, independently, hydrogen, oxygen, branched or straight chain C₁-C₈ alkyl, C₂-C₈ alkenyl, or q-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl;

A, D, E, and G are, independently, hydrogen, hydroxy, alkoxy, halide, alkyl, aralkyl, or amino; and

Z is halide, hydroxy, or alkoxy.

In one embodiment, steps 3-5 in FIG. 1 can be used to prepare compounds having the formula VII-XI.

The invention further relates to a compound having the formula XII



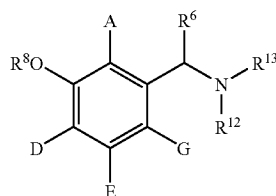
wherein R⁸ is hydrogen, branched or straight chain C₁-C₈ alkyl, substituted or unsubstituted aryl, aralkyl, or CR₉R₁₀OR₁₁, where R₉ and R₁₀ are, independently, hydrogen or alkyl, and R₁₁ is alkyl;

R⁶ is hydrogen; C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl, or (CH₂)_nR₇, where R₇ is hydroxy, alkoxy, cyano, ester, carboxylic acid, substituted or unsubstituted amino, and n is from 1 to 4; and

R¹² and R¹³ are, independently, hydrogen; C₁-C₈ alkyl; aryl or substituted aryl; aralkyl; or (CH₂)_nR¹⁴, wherein R¹⁴ is hydroxy, alkoxy, ester, carboxylic acid, substituted or unsubstituted amino, and n is from 1 to 4.

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The invention also relates to compound having the formula XIII



wherein R⁸ is hydrogen, branched or straight chain C₁-C₈ alkyl, substituted or unsubstituted aryl, aralkyl, or CR₉R₁₀OR₁₁, where R₉ and R₁₀ are, independently, hydrogen or alkyl, and R₁₁ is alkyl;

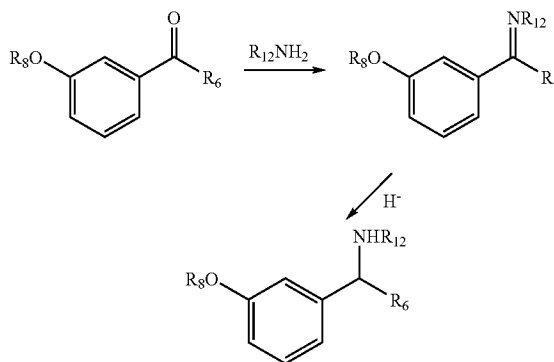
R⁶ is hydrogen; C₁-C₈ alkyl, C₂-C₈ alkenyl, q-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl, or (CH₂)_nR₇, where R₇ is hydroxy, alkoxy, cyano, ester, carboxylic acid, substituted or unsubstituted amino, and n is from 1 to 4; and

R¹² and R¹³ are, independently, hydrogen; C₁-C₈ alkyl; aryl or substituted aryl; aralkyl; or (CH₂)_nR¹⁴, wherein R¹⁴ is hydroxy, alkoxy, ester, carboxylic acid, substituted or unsubstituted amino, and n is from 1 to 4; and

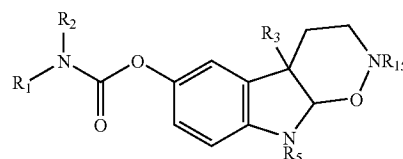
A, D, E, and G are, independently, hydrogen, hydroxy, alkoxy, halide, alkyl, aralkyl, or amino.

In one embodiment, compounds having the formula XII and XIII can be prepared by the general reaction shown in Scheme 2.

SCHEME 2



The invention also relates to a compound having the formula XVI



wherein R₁ and R₂ are, independently, hydrogen, branched or straight chain C₁-C₈ alkyl, substituted or unsubstituted aryl, or aralkyl;

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R₃ is branched or straight chain C₁-C₄ alkyl, or substituted or unsubstituted aryl;

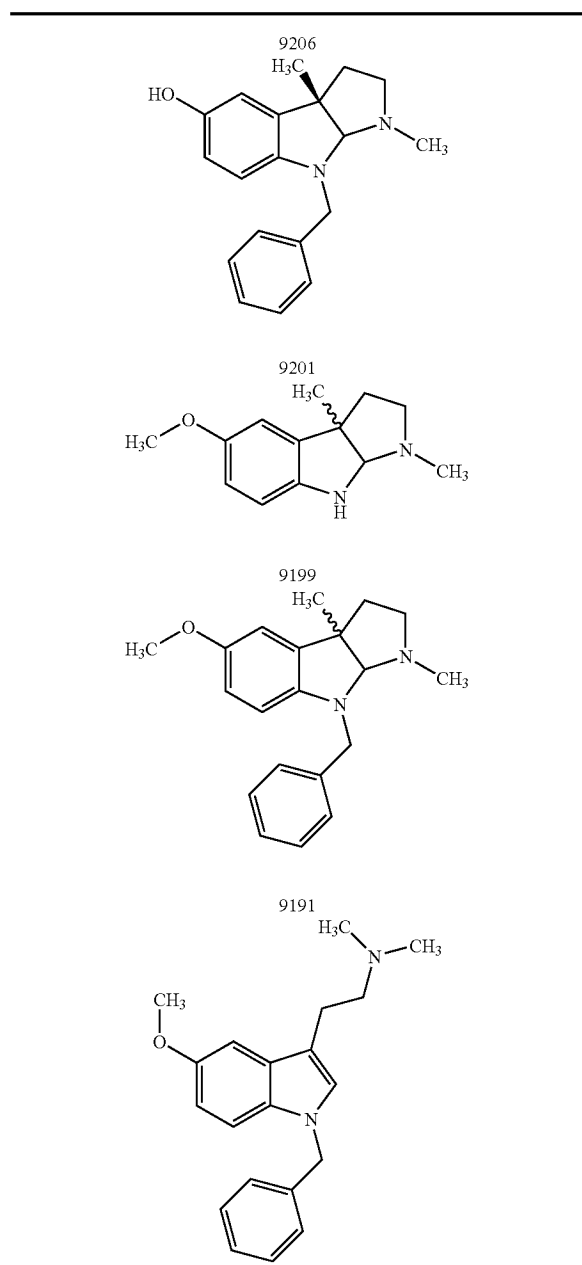
R₅ and R₁₅ are, independently, hydrogen, oxygen, branched or straight chain C₁-C₈ alkyl, C₂-C₈ alkenyl, or C₂-C₈ alkynyl, aralkyl, or substituted or unsubstituted aryl; and

wherein the compound is a racemic mixture, the substantially pure (-)-enantiomer, or the substantially pure (+)-enantiomer.

In a related aspect, the invention features the compound disclosed in FIG. 13C (MES9287) and MES9286 (Table 2).

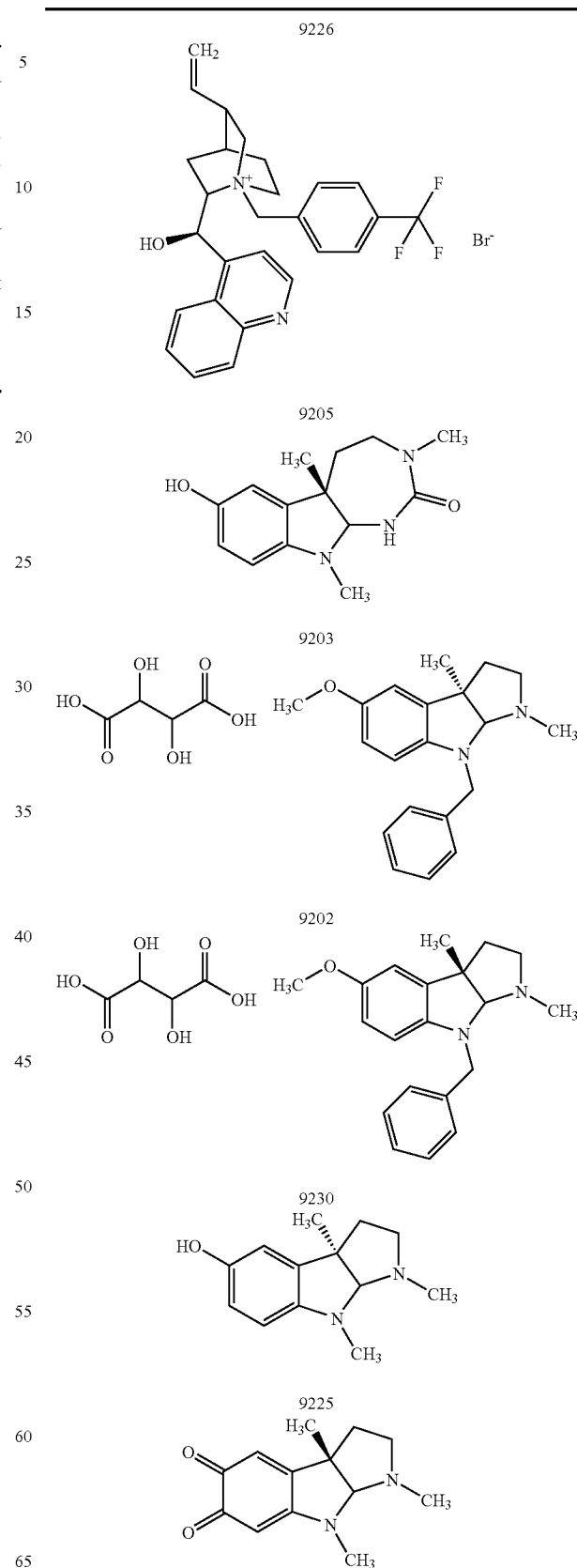
Additional non-carbamate compounds of the present invention are shown in Table 2.

TABLE 2



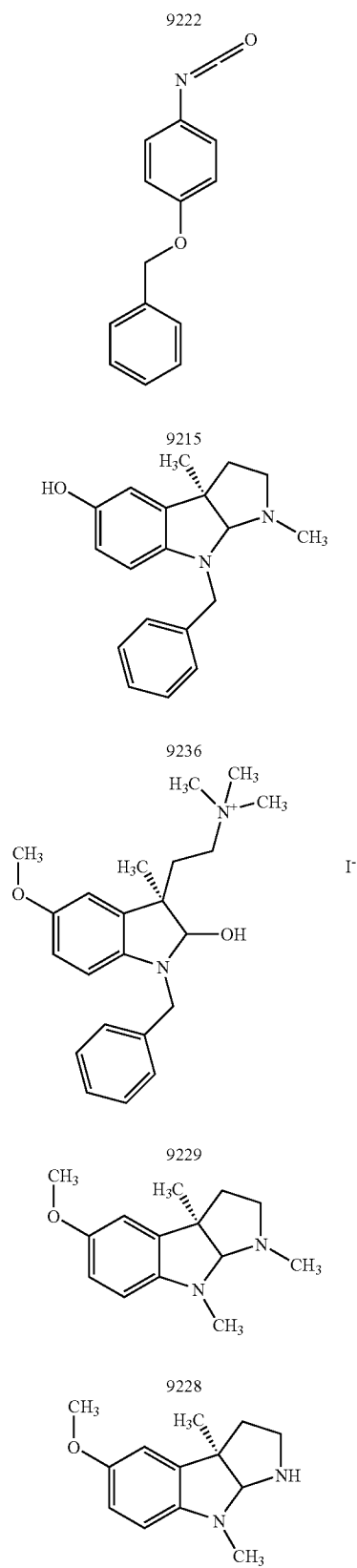
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TABLE 2-continued



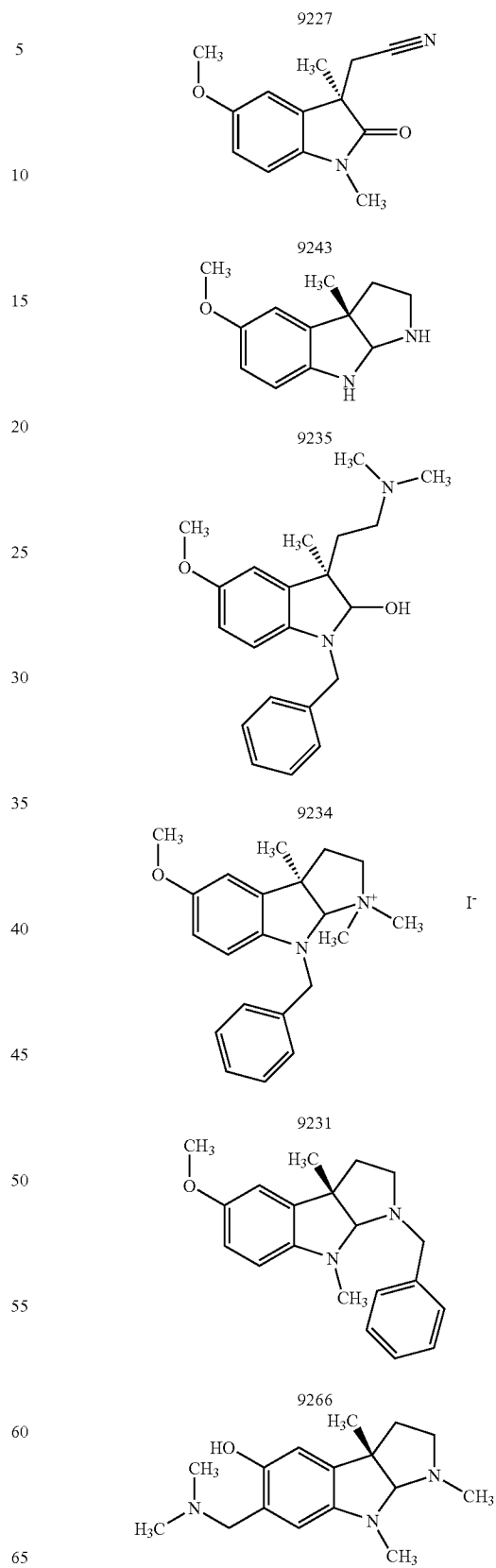
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TABLE 2-continued



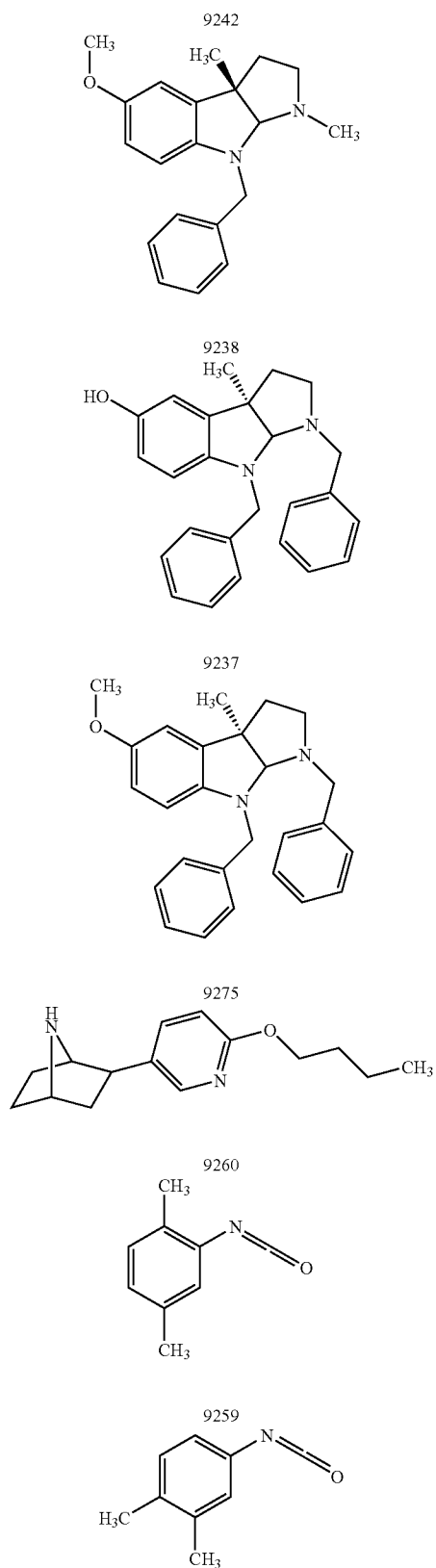
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TABLE 2-continued



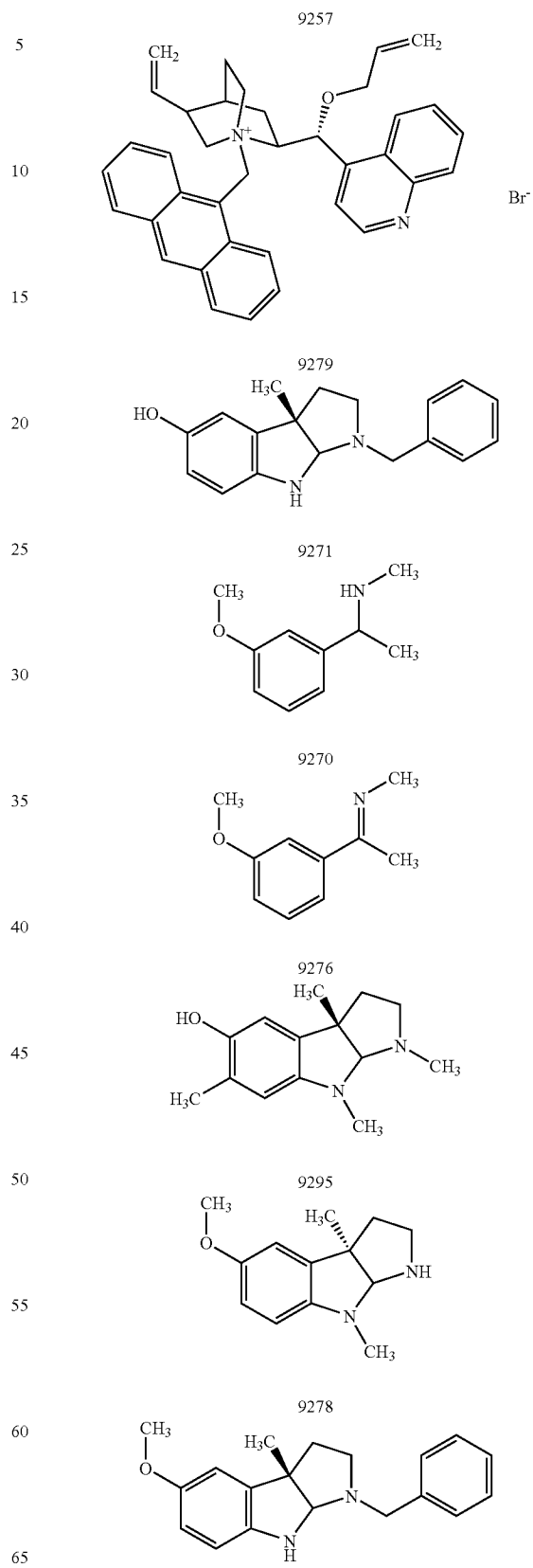
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TABLE 2-continued



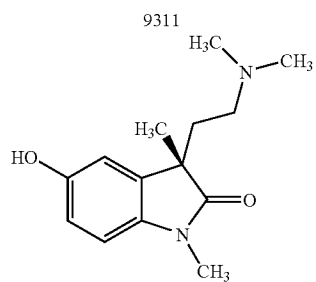
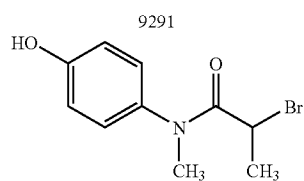
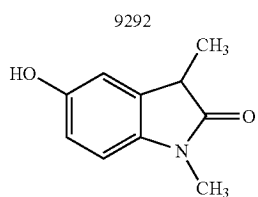
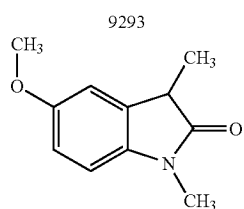
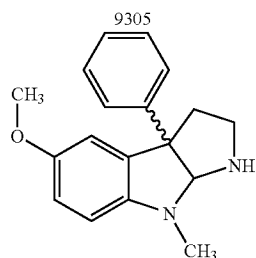
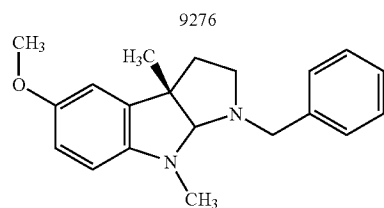
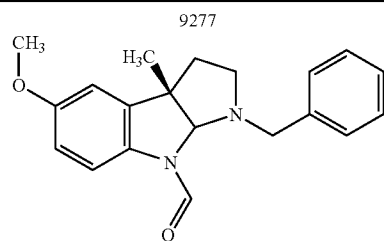
24

TABLE 2-continued



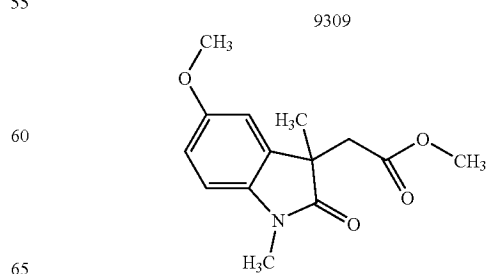
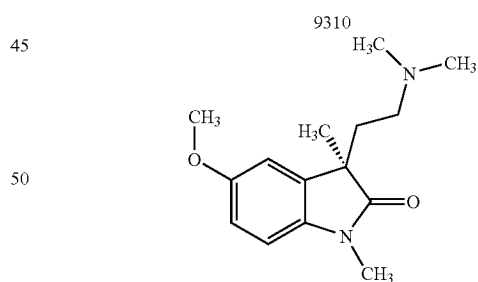
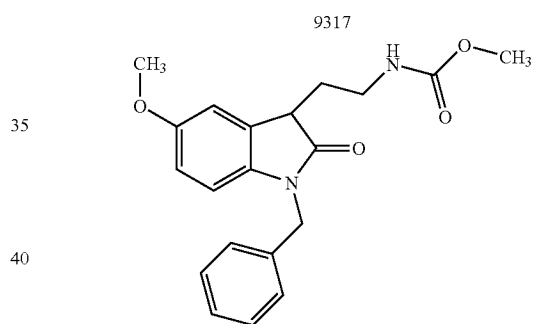
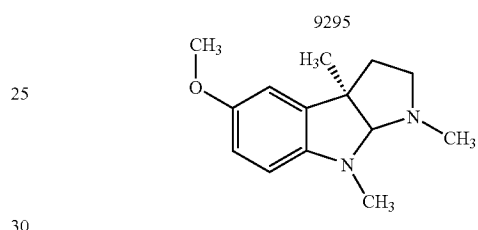
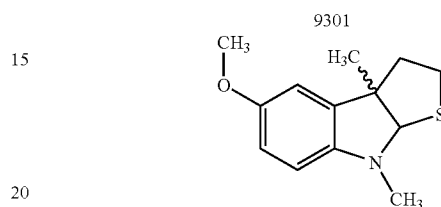
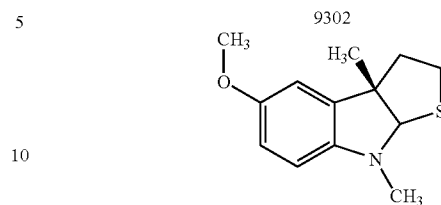
25

TABLE 2-continued



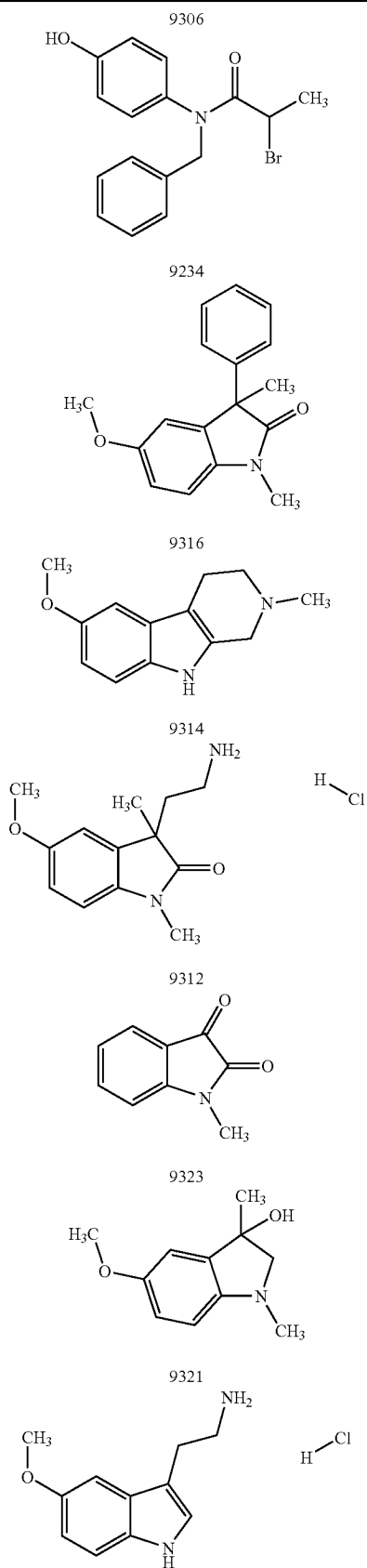
26

TABLE 2-continued



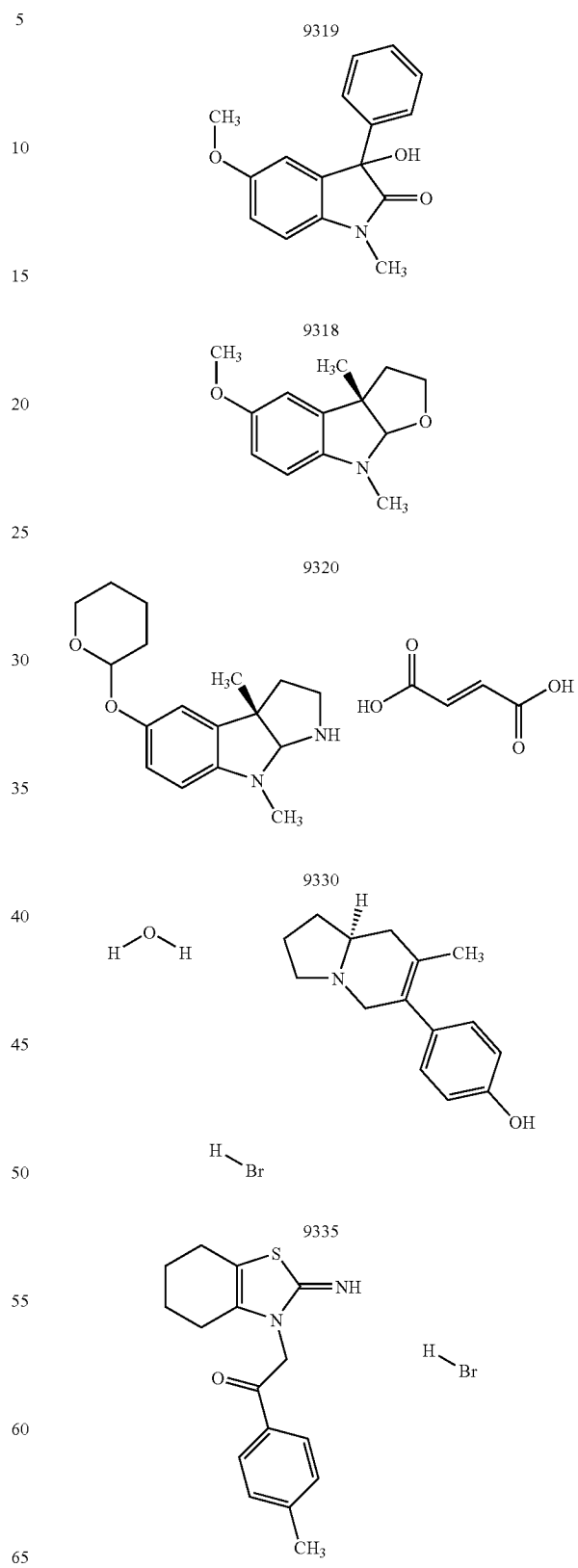
27

TABLE 2-continued



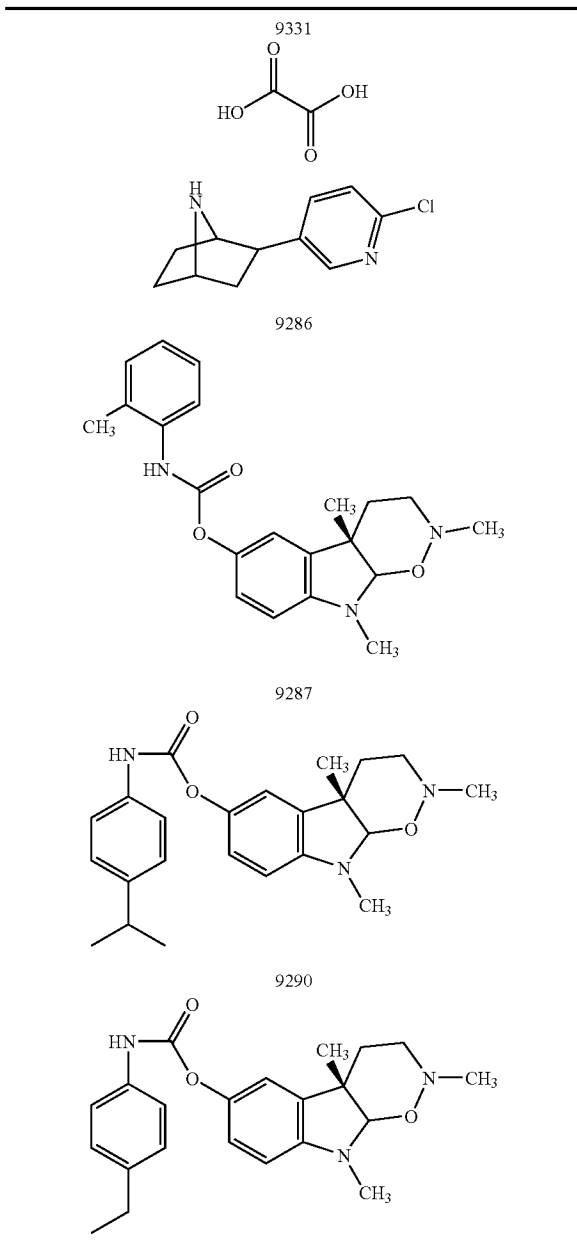
28

TABLE 2-continued



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TABLE 2-continued



In one embodiment, the present invention provides a method of inhibiting production of amyloid precursor protein in a cell, comprising contacting the cell with a compound having the formula I-XVI and any combination thereof. As used herein, "inhibiting" means decreasing the amount or concentration of amyloid precursor protein. "Inhibition" also refers to halting or reducing the production of amyloid precursor protein, wherein the concentration of amyloid precursor protein is reduced. Thus, the inhibition of production of amyloid precursor protein can be measured, for example, by comparing the amount of amyloid precursor protein produced by cells after contacting the cells with the compound having the formula I-XVI and any combination thereof, with the amount of amyloid precursor protein produced by control cells that have not been contacted with a compound having the formula I-XVI and any combination thereof. In one embodiment, the cell that is contacted with the compound is

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in vivo, ex vivo, or in vitro. The cell of this invention can be a mammalian cell, desirably a human cell.

In a desirable embodiment, the compounds inhibit production of amyloid precursor protein, $A\beta_{1-40}$, and/or $A\beta_{1-42}$ in a cell or a mammal by at least 30, 50, 60, 70, 80, 90, 95, or 100% compared to a buffer control, as measured using standard assays such as those described herein. In another desirable embodiment, the compound inhibits production of amyloid precursor protein, $A\beta_{1-40}$, and/or $A\beta_{1-42}$ in a cell or a mammal by at least 2, 5, 10, 20, or 50-fold compared to a buffer control, as measured using standard assays such as those described herein.

As used herein, "contacting" means exposure of at least one cell to a compound of the present invention. The cell of this invention can be, but is not limited to, a neural cell or supporting cell (e.g., glial or astrocyte). The term "neural cell" is defined as any cell that can be located in the central or peripheral nervous system or is a precursor or derivative thereof, including, for example, but not limited to, neuronal cells, glial cells, neural stem cells, neuronal stem cells and neuroblasts. The cell can be contacted in vitro with the compound, for example, by adding the compound to the culture medium (by continuous infusion, by bolus delivery, or by changing the medium to a medium that contains the compound), or the cell can be contacted with the compound in vivo (e.g., by local delivery, systemic delivery, intravenous injection, bolus delivery, or continuous infusion). In vitro contact may be preferred, for example, for measuring the effect of the compound on a population of cells. In vivo contact would be employed for inhibiting production of amyloid precursor protein in a subject in need of such inhibition, (e.g., a subject) with a neurodegenerative disease, for example, Alzheimer's Disease.

The subject of this invention can be any mammal that produces amyloid precursor protein, such as a primate and more desirably, a human. The subject of this invention can also be domesticated animals, such as cats, dogs, etc., livestock (e.g., cattle, horses, pigs, sheep, goats, etc.), and laboratory animals (e.g., mouse, rabbit, rat, guinea pig, etc.).

The duration of contact with a cell or population of cells is determined by the time the compound is present at physiologically effective levels or at presumed physiologically effective levels in the medium or extracellular fluid bathing the cell or cells. Desirably, the duration of contact is 1-48 hours and, more desirably, for 24 hours, but such time would vary based on the half-life of the compound and could be optimized by one skilled in the art using routine experimentation.

Examples of compounds used in the methods of this invention for inhibiting amyloid precursor protein include, but are not limited to, (+)-phenserine, (+)-cymserine, (+)-N¹-phenethylnorcymserine, or (+)-N¹,N⁸-bisnorcymserine. In a desired embodiment, the compound is (+)-phenserine, compound V, wherein X and Y are NCH₃, R₃ and R₈ are methyl, and the compound is the substantially pure (+)-enantiomer, or compound VII, wherein X is NR₅, where R₅ is benzyl, R₆ is (CH₂)₂N(CH₃)₂, and R₈ is methyl.

In another embodiment, the present invention also provides a method of inhibiting production of amyloid precursor protein in a subject, comprising administering to the subject an effective amount of a compound having the structure I-XVI and any combination thereof in a pharmaceutically acceptable carrier, whereby the compound inhibits production of amyloid precursor protein in the subject.

In a desirable embodiment, the compounds of the invention inhibit production of amyloid precursor protein, $A\beta_{1-40}$, and/or $A\beta_{1-42}$ in the subject or in a sample from the subject by at

least 30, 50, 60, 70, 80, 90, 95, or 100% compared to a buffer control, as measured using standard assays such as those described herein. In another desirable embodiment, the compound inhibits production of amyloid precursor protein, $A\beta_{1-40}$, and/or $A\beta_{1-42}$ in the subject or in a sample from the subject by at least 2, 5, 10, 20, or 50-fold compared to a buffer control, as measured using standard assays such as those described herein.

The compounds of the present invention can be administered in vivo to a subject in need thereof by commonly employed methods for administering compounds in such a way to bring the compound in contact with cells. The compounds of the present invention can be administered orally, parenterally, transdermally, extracorporeally, topically or the like, although oral or parenteral administration is typically desired. Parenteral administration of the compounds of the present invention, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. As used herein, "parenteral administration" includes intradermal, subcutaneous, intramuscular, intraperitoneal, intravenous, intra-articular and intratracheal routes. Additionally, the compound can be administered via a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Pat. No. 3,610,795, which is incorporated by reference herein in its entirety. The compounds can also be administered using polymer based delivery systems, including, for example, microencapsulation, which techniques are well known in the art.

The dosage of the compound varies depending on the weight, age, sex and condition of the subject as well as the method and route of administration. As an example, the dosage of the compound is from about 0.1 mg/kg to about 100 mg/kg of body weight. The lower limit for the dosage can be about 0.1, 0.5, 1, 2, 5, 10, 15, 20, 25, 30, or 40 mg/kg and the upper limit can be about 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100 mg/kg. Any lower limit can be used with any upper limit. More desirably, the compound is administered in vivo in an amount of about 1 to about 20 mg/kg. Thus, an administration regimen could include long-term, daily treatment. By "long-term" is meant at least two weeks and, desirably, several weeks, months, or years of duration. Necessary modifications in this dosage range may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. See *Remington's Pharmaceutical Sciences* (Martin, E. W., ed., latest edition), Mack Publishing Co., Easton, Pa. The dosage can also be adjusted by the individual physician in the event of any complication.

The compounds can be administered conventionally as compositions containing the active compound as a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent (i.e., carrier or vehicle). Depending on the intended mode of administration, the compound can be in pharmaceutical compositions in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, suspensions, lotions, creams, gels, or the like, desirably in unit dosage form suitable for single administration of a precise dosage. The compositions will include, as noted above, an effective amount of the selected compound in combination with a pharmaceutically acceptable carrier and, in addition, may include other medicinal compounds, pharmaceutical compounds, carriers, adjuvants, diluents, etc. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual

along with the selected compound without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

For solid compositions, conventional nontoxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talc, cellulose, glucose, sucrose, magnesium carbonate, and the like. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc. an active compound as described herein and optional pharmaceutical adjuvants in an excipient, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying compounds, pH buffering compounds and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, etc. Thus, the compositions are administered in a manner compatible with the dosage formulation and in a therapeutically effective amount. As discussed above, precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual.

For oral administration, fine powders or granules may contain diluting, dispersing, and/or surface active compounds, and may be presented in water or in a syrup, in capsules or sachets in the dry state, or in a nonaqueous solution or suspension wherein suspending compounds may be included, in tablets wherein binders and lubricants may be included, or in a suspension in water or a syrup. Where desirable or necessary, flavoring, preserving, suspending, thickening, or emulsifying compounds may be included. Tablets and granules are desired oral administration forms, and these may be coated.

In another embodiment, the present invention provides a method of treating a disorder associated with abnormal production of amyloid precursor protein, such as, for example, dementia in a subject, comprising administering to the subject an effective amount of the compound having the formula I-XVI and any combination thereof in a pharmaceutically acceptable carrier, whereby the compound treats the disorder in the subject. As used herein, the term "dementia" describes a neurodegenerative disorder that results from an organic brain disease in which a subject experiences usually irreversible deterioration of intellectual faculties with accompanying emotional disturbances. An example of dementia includes, but is not limited to, Alzheimer's disease. An example of another disorder that can be treated by the methods of this invention includes, but is not limited to, cerebral amyloidosis. In a desirable embodiment, a compound used for the treatment of dementia improves a symptom associated with dementia or Alzheimer's, stabilizes a symptom, or delays the worsening of a symptom. In other desirable embodiments, the compound increases the life-span of a subject compared to the average life-span of corresponding subjects not administered the compound. In yet other desirable embodiments, the compound is used to prevent or delay the onset of dementia or Alzheimer's.

In general, an "effective amount" of a compound is that amount needed to achieve the desired result or results. Thus, for example, administering to a subject (e.g., a human) with Alzheimer's disease an effective amount of a compound of the present invention can result in slowing, stopping, or even possibly reversing the deterioration of the subject's intellectual faculties and other accompanying neurological signs and symptoms. Therefore, the inhibition of the production of

amyloid precursor protein, by the methods of the present invention, treats the subject with Alzheimer's disease. The effective amount of the compound needed to treat dementia is from about 0.5 mg to about 200 mg. The lower limit for the effective amount of the compound can be about 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 100, or 150 mg, and the upper limit can be about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg. Any lower limit can be used with any upper limit. In one embodiment, when the subject is a human, the effective amount of compound to treat dementia is from about 0.5 to about 100 mg. In another embodiment, (+)-phenserine, (+)-cymserine, (+)-N¹-phenethylnorcymserine, (+)-N¹,N⁸-bisnorcymserine, compound V, wherein X and Y are NCH₃, R₃ and R₈ are methyl, and the compound is the substantially pure (+)-enantiomer, or compound VII, wherein X is NR₅, where R₅ is benzyl, R₆ is (CH₂)₂N(CH₃)₂, and R₈ is methyl can be used in these amounts to treat dementia. In a desired embodiment, (+)-phenserine can be used in these amounts to treat dementia in a subject.

In a further embodiment, the present invention relates to a method of binding an amyloid precursor protein messenger RNA 5' untranslated region (5'UTR) in a cell, comprising contacting the cell with a compound having the formula I-XVI and any combination thereof, whereby the compound binds the amyloid precursor protein messenger RNA 5' untranslated region in the cell, thereby inhibiting amyloid protein production. The amyloid precursor protein messenger RNA 5'UTR confers translational control of β APP protein synthesis. In one embodiment, (+)-phenserine, (+)-cymserine, (+)-N¹-phenethylnorcymserine, (+)-N¹,N⁸-bisnorcymserine, compound V, wherein X and Y are NCH₃, R₃ and R₈ are methyl, and the compound is the substantially pure (+)-enantiomer, or compound VII, wherein X is NR₅, where R₅ is benzyl, R₆ is (CH₂)₂N(CH₃)₂, and R₈ is methyl can be used to bind the amyloid precursor protein with messenger RNA 5' UTR. In a desired embodiment, (+)-phenserine can be used to bind to an amyloid precursor protein messenger RNA 5' untranslated region in a cell. In a desirable embodiment, at least 30, 50, 60, 70, 80, 90, 95, or 100% of the amyloid precursor protein mRNA in a cell is bound by the compound.

In a further embodiment, the present invention relates to a method of inhibiting translation of an amyloid precursor protein messenger RNA in a cell, comprising contacting the cell with a compound having the formula I-XVI and any combination thereof, whereby the compound binds the amyloid precursor protein messenger RNA 5' and/or 3' untranslated region in the cell, or binds a protein that interacts with the amyloid precursor protein messenger RNA 5' and/or 3' untranslated region in the cell, or alters a process, either indirectly or directly, such as glycosylation or phosphorylation that then changes the binding of a specific regulatory protein to amyloid precursor protein messenger RNA 5' and/or 3' untranslated region in the cell, thereby inhibiting amyloid protein production. In one embodiment, (+)-phenserine, (+)-cymserine, (+)-N¹-phenethylnorcymserine, (+)-N¹,N⁸-bisnorcymserine, compound V, wherein X and Y are NCH₃, R₃ and R₈ are methyl, and the compound is the substantially pure (+)-enantiomer, or compound VII, wherein X is NR₅, where R₅ is benzyl, R₆ is (CH₂)₂N(CH₃)₂, and R₈ is methyl can be used to inhibit the translation of the amyloid precursor protein with messenger RNA. In a desired embodiment, (+)-phenserine can be used to inhibit the translation of the amyloid precursor protein messenger RNA by interfering with the post-transcriptional regulation of the amyloid precursor protein RNA in a cell. In a desirable embodiment, the compound inhibits production of amyloid precursor protein, A β ₁₋₄₀, and/or A β ₁₋₄₂ by at least 30, 50, 60, 70, 80, 90, 95, or 100%

compared to a buffer control, as measured using standard assays such as those described herein. In another desirable embodiment, the compound inhibits production of amyloid precursor protein, A β ₁₋₄₀, and/or A β ₁₋₄₂ by at least 2, 5, 10, 20, or 50-fold compared to a buffer control, as measured using standard assays such as those described herein.

The present invention also provides a method of screening a compound for the ability to inhibit production of amyloid precursor protein, A β ₁₋₄₀, and/or A β ₁₋₄₂, comprising (a) contacting the cell with the compound having the formula I-XVI and any combination thereof, and (b) detecting a decrease in amyloid precursor protein, A β ₁₋₄₀, and/or A β ₁₋₄₂ production in a cell contacted with the compound as compared to the amount of amyloid precursor protein, A β ₁₋₄₀, and/or A β ₁₋₄₂ production in a control cell not contacted with the compound, whereby decreased production of amyloid precursor protein, A β ₁₋₄₀, and/or A β ₁₋₄₂ in the cell identifies the compound as having the ability to inhibit the production of amyloid precursor protein, A β ₁₋₄₀, and/or A β ₁₋₄₂ in a cell. As shown in the Examples section below, a person of skill in the art can measure the amount of β APP, A β ₁₋₄₀, and/or A β ₁₋₄₂ production in a control population of cells and compare the production of β APP, A β ₁₋₄₀, and/or A β ₁₋₄₂ in a population of cells contacted with a compound to be screened by the methods of the present invention. A decrease in the production of β APP, A β ₁₋₄₀, and/or A β ₁₋₄₂ in a population of cells contacted with a compound as compared to the production of β APP, A β ₁₋₄₀, and/or A β ₁₋₄₂ in a population of control cells identifies the compound as having the ability to inhibit production of amyloid precursor protein, A β ₁₋₄₀, and/or A β ₁₋₄₂. In a desirable embodiment, the compound inhibits production of amyloid precursor protein, A β ₁₋₄₀, and/or A β ₁₋₄₂ by at least 30, 50, 60, 70, 80, 90, 95, or 100% compared to a buffer control, as measured using standard assays such as those described herein. In another desirable embodiment, the compound inhibits production of amyloid precursor protein, A β ₁₋₄₀, and/or A β ₁₋₄₂ by at least 2, 5, 10, 20, or 50-fold compared to a buffer control, as measured using standard assays such as those described herein.

The present invention further provides a method of screening a compound for the ability to inhibit amyloid precursor protein production by binding an amyloid precursor protein messenger RNA 5' untranslated region, comprising (a) contacting the messenger RNA with the compound; (b) detecting the binding of the compound to the amyloid precursor protein-messenger RNA 5' untranslated region; and (c) detecting the inhibition of amyloid precursor protein production from an amyloid precursor protein-messenger RNA 5' untranslated region, thereby identifying a compound having the ability to inhibit amyloid precursor protein messenger RNA 5' untranslated region. The binding of the compound to the amyloid precursor protein messenger RNA 5' untranslated region inhibits β amyloid precursor protein (β APP) from the messenger RNA by directly preventing the binding of the ribosomal translational subunit with the mRNA through steric hindrance. The detection of binding of a compound to the 5' UTR of the amyloid precursor protein messenger RNA can be carried out by methods standard in the art for detecting the binding of substances to nucleic acids such as RNA. The detection of inhibition of amyloid precursor protein production upon contact with the compound can be carried out by the methods provided in the Examples herein, as well as protocols well known in the art. The messenger RNA can be in a cell or in a cell-free environment (e.g., a cell-free translation system). In a desirable embodiment, at least 30, 50, 60, 70, 80, 90, 95, or 100% of the amyloid precursor protein mRNA in a cell is bound by the compound.

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The present invention further provides a method of screening a compound for the ability to inhibit amyloid precursor protein, $A\beta_{1-40}$, and/or $A\beta_{1-42}$ production by inhibiting translation of the amyloid precursor protein messenger RNA, comprising (a) contacting the cell with the compound; and (b) detecting the inhibition of amyloid precursor protein, $A\beta_{1-40}$, and/or $A\beta_{1-42}$ production from an amyloid precursor protein RNA, thereby identifying a compound having the ability to inhibit amyloid precursor protein messenger RNA translation. In another embodiment, the screening method further comprises after step (a) detecting the amount of the amyloid precursor protein-messenger RNA. In one embodiment, the amount of amyloid precursor protein mRNA is not inhibited by the compound or is inhibited by less than 80, 80, 50, 40, 30, 20, or 10%. In this case, the compound primarily or only inhibits the translation of amyloid precursor protein. It is also contemplated that in other embodiments a compound may inhibit both transcription and translation of amyloid precursor protein or inhibit only transcription of amyloid precursor protein. While not meant to limit the invention to any particular mechanism of action, the inhibition of translation by the compound can result from the binding of the compound to the amyloid precursor protein messenger RNA 5' and/or 3' untranslated region(s) inhibiting β amyloid precursor protein (β APP) from the messenger RNA by directly preventing the binding of the ribosomal translational subunit with the mRNA through steric hindrance or by inhibiting the binding of an important regulatory protein, or the binding of the compound to the regulatory protein or by the compound modifying an important regulatory protein such that it no longer can interact with the amyloid precursor protein RNA. The direct detection of binding of a compound to the 5' and/or 3' UTR(s) of the amyloid precursor protein messenger RNA can be carried out by methods standard in the art for detecting the binding of substances to nucleic acids such as RNA, such as NMR or mass spectroscopy. The detection of inhibition of amyloid precursor protein production upon contact with the compound can be carried out by the methods provided in the Examples herein, as well as protocols well known in the art. The messenger RNA can be in a cell or in a cell-free environment (e.g., a cell-free translation system). In a desirable embodiment, the compound inhibits production of amyloid precursor protein, $A\beta_{1-40}$, and/or $A\beta_{1-42}$ by at least 30, 50, 60, 70, 80, 90, 95, or 100% compared to a buffer control, as measured using standard assays such as those described herein. In another desirable embodiment the compound inhibits production of amyloid precursor protein, $A\beta_{1-40}$, and/or $A\beta_{1-42}$ by at least 2, 5, 10, 20, or 50-fold compared to a buffer control, as measured using standard assays such as those described herein. In yet another desirable embodiment, at least 30, 50, 60, 70, 80, 90, 95, or 100% of the amyloid precursor protein mRNA in a cell is bound by the compound. In still another embodiment, at least 30, 50, 60, 70, 80, 90, 95, or 100% of the compound is directly or indirectly bound to an amyloid precursor protein messenger RNA 5' or 3' untranslated region or to an RNA binding protein that interacts with the amyloid precursor protein messenger RNA 5' or 3' untranslated region.

In another embodiment, the invention relates to a method of screening a compound for the ability to inhibit amyloid protein production by eliciting a change in reporter gene expression, comprising:

(a) contacting a cell transfected with a reporter gene containing the 5' and/or 3' UTR(s) of the amyloid precursor protein messenger RNA with the compound, and

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(b) detecting a decrease in reporter gene expression or activity; thereby identifying a compound having the ability to inhibit amyloid precursor protein messenger RNA translation.

In a desirable embodiment, the compound inhibits reporter gene expression or activity by at least 30, 50, 60, 70, 80, 90, 95, or 100% compared to a buffer control, as measured using standard assays such as those described herein. In another desirable embodiment, the compound inhibits reporter gene expression or activity by at least 2, 5, 10, 20, or 50-fold compared to a buffer control, as measured using standard assays such as those described herein.

The compounds used in the screening methods of this invention can be, but is not limited to, a compound having the structure I-XVI and any combination thereof. In various embodiments, the compound is a (+)-isomer, (-)-isomer, or a racemic mixture. In various embodiments of any of the methods of the invention, the compound is a (+)-isomer, (-)-isomer, or a racemic mixture of MES 9295 (FIG. 13E). In other embodiments of any of the methods of the invention, the compound is not a (+)-isomer, (-)-isomer, or a racemic mixture of MES 9295.

In desirable embodiment of any of the aspects of the invention, the compound inhibits cholinesterase activity, such as acetylcholinesterase or butyrylcholinesterase activity, by less than 80, 70, 60, 50, 40, 30, 20, 10, or 5% (in order of increasing preference) relative to a buffer only control. In other desirable embodiments, inhibition of cholinesterase activity, such as acetylcholinesterase or butyrylcholinesterase activity, by the compound is at least 2, 5, 10, 20, 50, or 100-fold less than the inhibition of cholinesterase activity by the corresponding amount of (-)-pheneserine. In other desirable embodiments, inhibition of cholinesterase activity, such as acetylcholinesterase or butyrylcholinesterase activity, by a (+) isomer or a racemic mixture is at least 2, 5, 10, 20, 50, or 100-fold less than the inhibition of cholinesterase activity by the corresponding amount of (-)-isomer. In yet other desirable embodiments, the compound is substantially free of cholinesterase inhibitory activity. Inhibition of cholinesterase activity can be measured using any standard assay. For example, the assay and the in vivo mouse model described in U.S. Pat. No. 4,791,107, which is incorporated by reference in its entirety, or the in vivo mouse model described herein can be used.

In other desirable embodiments of any of the aspects of the invention, the compound results in a less than 20, 10, 5, or 2-fold increase in the amount of released lactate dehydrogenase (a marker of cell viability and integrity) relative to the amount of released lactate dehydrogenase in the absence of the compound or in the presence of a buffer control. In still other desirable embodiments, the amount of compound that is administered to a subject per kg body weight of the subject does not cause tremors or death when administered in the in vivo mouse model described herein. In yet other desirable embodiments, less than 80, 70, 60, 50, 40, 30, 20, 10, or 5% of the neuronal cells contacted with the compound are killed by the compound. In another desirable embodiment, the dose of the compound is equal to or greater than 1 mg/kg of body weight, 5 mg/kg, or 10 mg/kg.

In other desirable embodiments of any of the aspects of the invention, the compound inhibits production of amyloid precursor protein, $A\beta_{1-40}$, and/or $A\beta_{1-42}$ by at least 30, 50, 60, 70, 80, 90, 95, or 100% compared to a buffer control. In another embodiment, the compound inhibits production of amyloid precursor protein, $A\beta_{1-40}$, and/or $A\beta_{1-42}$ by at least 30, 50, 60, 70, 80, 90, 95, or 100% compared to a buffer control, and inhibits cholinesterase activity by less than 80, 70, 60, 50, 40,

30, 20, 10, or 5% relative to a buffer only control. In other desirable embodiments, the compound inhibits intracellular and/or extracellular APP or A β production. In yet other desirable embodiments, the compound inhibits production of amyloid precursor protein in a cell or mammal by at least 2, 5, 10, 20, or 50-fold more than it inhibits cholinesterase activity in the cell or mammal. In still other desirable embodiments, the amount of compound required to inhibit production of amyloid precursor protein in a cell or mammal by 50% (IC₅₀ value) is at least 2, 5, 10, 20, 50, or 100-fold less than the amount of compound required to inhibit cholinesterase activity by 50% (IC₅₀ value) in the cell or mammal, as measured using standard assays.

In other desirable embodiments of any of the methods of the invention, the compound is (+)-3,3a,8,8a-Tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol methyl ether; (+)-3,3a,8,8a-Tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol; (+)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno-[2,3-b]indole-5-ol butyl carbamate; (+), -3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol heptylcarbamate; (+)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol phenylcarbamate; (+)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol 2'-methylphenylcarbamate; (+)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol 2'-ethylphenylcarbamate; (+)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol 2'-isopropylphenylcarbamate; (+)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol 4'-isopropylphenylcarbamate; (+)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol 2',4'-dimethylphenylcarbamate; (+)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol N,N-dimethylcarbamate; (+)-O-methyl-N(1)-noreseroline; (+)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol methyl ether; (+)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol ((-)-thiaphysovenol); (+)-3,3a,8,8a-Tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol 2',4'-dimethylphenylcarbamate; (+)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol 2'-methylphenylcarbamate; (+)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol 4'-isopropylphenylcarbamate, or a mixture of the (+)- and (-)-enantiomers thereof (e.g., a racemic mixture). In other embodiments, the compound is the (+)-enantiomer or a mixture of the (+)- and (-)-enantiomers thereof (e.g., a racemic mixture) of a compound disclosed in column 2, line 16 through column 3, line 40 of U.S. Pat. No. 5,378,723 (Brossi et al., issued Jan. 3, 1995).

In other desirable embodiments of any of the methods of the invention, the compound is a compound that falls with the general formula (e.g., column 1, lines 11-52) and or a specifically disclosed compound (e.g., a compound listed in column 21, line 59 through column 38, line 42) in U.S. Pat. No. 4,791,107 (Hamer et al., issued Dec. 13, 1988), and the compound has negligible cholinesterase inhibitory activity (e.g., causes no detectable cholinesterase inhibition or causes less than 50, 40, 30, 20, 10, or 5% inhibition). In other desirable embodiments, administration of the compound produces no adverse side-effects due to inhibition of cholinesterase activity. These compounds can be the (+)- or (-)-isomers or a mixture thereof (e.g., a racemic mixture). In other embodiments, the compound is a compound that falls with the general formula and or a specifically disclosed compound in U.S. Pat. No. 4,791,107, and the compound is administered in a dose of at least 1 mg/kg, 5 mg/kg, or 10 mg/kg.

In other desirable embodiments of any of the methods of the invention, the compound is a compound that falls with a general formula disclosed in U.S. Pat. No. 5,378,723 (Brossi et al., issued Jan. 3, 1995), U.S. Pat. No. 5,171,750 (Brossi et al., issued Dec. 15, 1992), or U.S. Pat. No. 5,998,460 (Brossi et al., issued Dec. 7, 1999), and the compound has negligible cholinesterase inhibitory activity (e.g., causes no detectable cholinesterase inhibition or causes less than 50, 40, 30, 20, 10, or 5% inhibition) or is administered in a dose of at least 1 mg/kg, 5 mg/kg, or 10 mg/kg. [As an alternative, these structures could be cut and pasted into the application.] In other desirable embodiments of any of the methods of the invention, the compound is the (+)-isomer or a racemic mixture of a compound that falls with a general formula or is specifically disclosed in U.S. Pat. No. 5,378,723, U.S. Pat. No. 5,171,750, or U.S. Pat. No. 5,998,460.

In other embodiments of any of the methods of the invention, the compound is not (-)-phenserine, (-)-physostigmine, (-)-heptyl-physostigmine, (-)-physovenine, (-)-N(1)-nor-physostigmine, MES9217 (FIG. 13H), MES9299 (FIG. 13L), or MES9329 (FIG. 13M). In other embodiments of any of the methods of the invention, the compound is not a compound disclosed in U.S. Pat. No. 5,378,723, U.S. Pat. No. 5,171,750, U.S. Pat. No. 5,998,460, or U.S. Pat. No. 4,791,107.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds claimed herein are made and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in ° C. and is at room temperature, and pressure is at or near atmospheric.

General Considerations

Phenserine: Phenserine is a member of a family of compounds that are phenylcarbamates of hexahydropyrrol indoles with specific side groups that provide it selectivity against either acetyl- or butyryl-cholinesterase, a high brain uptake and a long duration of pharmacological action (Greig et al., 1995; Brossi et al., 1996). The compound was synthesized in its optically (>99.9%) and chemically (>99.9%) pure (-)- and (+)-enantiomeric forms as a tartrate salt, as described (Yu and Brossi, 1988; Greig et al., 1995). The concentration of compound required to inhibit 50% AChE activity was 22 nM for (-)-phenserine, whereas >25,000 nM was inactive for optically pure (+)-phenserine.

Drug treatment: SK-N-SH neuroblastoma cells were cultured on 60 mm dishes at a concentration of 3×10^6 cells, and SH-SY-5Y neuroblastoma and U373 astrocytoma cell lines were plated in 100 mm dishes at a concentration of 3×10^5 cells. The cells were allowed to grow in complete medium (10% FBS, 2 mM glutamine in DMEM) for 3 to 4 days until they reached 70% confluence. To start the experiment, spent medium was removed and replaced with fresh medium (SKNSH—4 ml of DMEM+0.5% FBS; U373—5 ml of DMEM+2.5% FBS) containing 0, 5, or 50 μ M phenserine. The cells were incubated at 37° C., 5% CO₂ for the specific times indicated. Different media and sera were purchased from Life Technologies (Gaithersburg, Md.).

Inhibitor treatment: One day prior to drug treatment, confluent cultures of U373 cells were pretreated with 25 nM of ERK specific inhibitor, PD98059 (Calbiochem-Novabiochem, La Jolla, Calif.), in 4.5 ml of 2.5% FBS, 2 mM glutamine and DMEM for 16 hours. Phenserine was added to each assay plate and a final volume of 5 min was reached. To examine for PI 3 kinase involvement, an active 2 μ M concentration of the PI 3 kinase inhibitor, LY294002 (Calbiochem-Novabiochem, La Jolla, Calif.), in 4.5 ml of 2.5% FBS, 2 mM glutamine and DMEM was added to each assay plate and incubated for 30 minutes prior to the addition of phenserine. Appropriate vehicle controls were run alongside treated samples.

Lysate Preparation: At each time point, the spent medium was collected and stored at -70° C. for later analysis of secretory β APP levels. The cells were washed twice with PBS, pH 7.4 and incubated on ice for 15 min for lysis with 100 μ L of lysis buffer (20 mM HEPES, 2 mM EGTA, 50 mM β -glycophosphate, 1 mM sodium orthovanadate, 1% Triton X-100, 10% glycerol) containing appropriate protease inhibitors (2 mM PMSF, 100 μ g/mL aprotinin, 25 μ M leupeptin and 20 μ g/mL soybean trypsin inhibitor). Each lysate was microcentrifuged for 15 min at 14 000 rpm. Protein levels of the supernatant were analyzed by the Bradford protein assay (BioRad, Melville, N.Y.).

Western Blot: Fifteen μ g of protein from each sample was mixed with the appropriate volume of 5 \times Laemmli buffer and boiled for 5 min at 100° C. The samples were loaded onto a 10% NuPAGE Bis-Tris gel in 1 \times NuPAGE MOPS SDS running buffer (NOVEX, San Diego, Calif.) and the proteins separated at 200 V for 45 min. The gels then were transferred onto nitrocellulose at 25 V for 1.5 h. The blots were blocked with 5% non-fat dry milk in 10 mM Tris, pH 8.0 containing 150 mM NaCl for 1 h and washed twice for 15 min in large volumes of TBST (10 mM Tris, pH 8.0, 150 mM NaCl and 0.05% Tween 20). Each blot was probed for 2 h with either 22C11 anti- β APP-terminal antibody (Boehringer Mannheim, Indianapolis, Ind.), diluted to a concentration of 2.5 μ g/mL or anti-activated ERK antibody (Promega, Madison, Wis.), diluted to a concentration of 25 ng/mL. The blots were washed twice for 15 min in TBST and placed in secondary antibody, anti-mouse IgG- or anti-rabbit IgG conjugated to horse radish peroxidase (Sigma, St. Louis, Mo.), for 30 min. Three final TBST washes of 20 min duration each were performed before the samples were detected by chemiluminescence and exposed to film, as per the manufacturer's instructions Amersham Life Science Inc., Arlington Heights, Ill.). Additionally, all blots also were stained with Ponceau S (Sigma, St. Louis, Mo.) to determine equivalent loading of samples. Densitometric quantification of blots was undertaken by using a CD camera and NIH-IMAGE (version 4.1).

Lactate Dehydrogenase Assay: Measurement of released lactate dehydrogenase (LDH) in the conditioned medium was undertaken as a marker of cell viability and integrity, as described previously (Lahiri et al., 1997 and 1998).

Total A β Assay: Total A β peptide levels in SH-SY-5Y and SK-N-SH cultured samples were assayed by a sensitive ELISA (Suzuki et al., 1994). For total A β measurements in conditioned medium, the rabbit polyclonal antibody #3160 (1-40 residues of A β) was used as a capture antibody for all species of A β peptide (A β 1-40 and A β 1-42) while monoclonal antibody 4G8 (17-25 residues of A β) was used to detect A β peptide levels, and the values were expressed as the mean of six independent assays.

Transfection: One day prior to transfection, U373 cells were plated onto 100 mm dishes at a density of 3×10^5 cells. On the day of transfection, the cells were given 5 ml of fresh media containing 10% FBS, 2 mM glutamine in DMEM. The cells were transfected using a calcium phosphate precipitation method, as per the manufacturer's protocol and described previously (Rogers et al., 1999). Briefly, for each plate, three μ g of DNA (5'UTR APP-PSV2-CAT or PSV2-CAT vector) were placed in a final volume of 500 μ L of 250 mM CaCl_2 . The chloramphenicol acetyl transferase (CAT) gene was used as a reporter gene. The DNA solution was slowly pipetted into an aerated, equivalent volume of 2 \times HeBS, pH 7.05. The resulting precipitate was allowed to stand 10-20 min at RT before its addition to the cells. After 18 h, the medium was changed and the transfected cells were left for two days before drug treatment.

CAT Assay: The cell lysates from transfected U373 cells treated with phenserine were analyzed for their CAT activity using a calorimetric enzyme immunoassay (Boehringer Mannheim, Indianapolis, Ind.). Briefly, 50 μ g of protein (an amount previously found to lie within the linear range of the assay) were placed onto anti-CAT coated microtiter plate modules and allowed to bind for 1 h at 37° C. The plates were washed thoroughly after each step. Next, a digoxigenin-labeled anti-CAT antibody was added to the samples and incubated for 1 h at 37° C. A subsequent antibody, anti-digoxigenin conjugated to peroxidase, was placed in the wells for another hour under similar conditions. Finally peroxidase substrate, ABTS, was added to each well and the absorbance of each sample was measured at a wavelength of 405 nm.

Northern Blotting: Total RNA (10 μ g) was extracted and prepared from treated astrocytoma cells using an RNA-STAT kit (Tel-test, Friendswood, Tex.). The samples were denatured in formamide, MOPS buffer, formaldehyde, dye mix and ethidium bromide at 65° C. for 10 min, placed on ice for 5 min and electrophoresed on a 1.0% agarose-formaldehyde gel. The gel was blotted onto Hybond Nitrocellulose filters and immobilized by UV crosslinking and heating filters for 2 hours. Each filter was prehybridized in hybridization buffer (1% BSA, 7% SDS, 0.5 M phosphate buffer, pH 7, 1 mM EDTA) for at least 2 hours. The filter was hybridized overnight with probe. Following hybridization, the filters were washed twice with wash solution containing 0.5% BSA, 5% SDS, 40 mM phosphate buffer, pH 7, 1 mM EDTA for 30 min at 65° C. The β APP cDNA probe corresponded to a unique internal BglII/SpeI fragment generated from human KNAPP cDNA (provided by John Kusiak, Gerontology Research Center, IRP, NIA, NIH). Equal loading of samples was verified by rehybridizing the filter with a human actin gene using an actin β -cDNA probe (Clontech Laboratories, Palo Alto, Calif.).

Plasmid Constructs: The plasmid PSV2-APP-CatD was provided by Dr. Rogers (1999). Briefly, the pSV₂(APP)CAT construct was generated by inserting a 90 bp fragment of the β APP gene 5'-UTR immediately upstream of the CAT gene into the pSV₂ vector.

Statistics: A two-tailed Student's t-test was carried out to compare two means. When more than two means were compared, one-way analysis of variance, together with a Bartlett's test for homogeneity of variances and a Dunnett's multiple comparison test were used. The level of significance was defined as $P < 0.05$.

Phenserine Decreases β APP and AD Levels in Neuroblastoma Cells

β APP protein levels were measured after treatment of the SH-SY-5Y cells with 5 μ M (+)-phenserine and (-)-phenserine for 0.5, 1, 2, and 4 hours (FIG. 3). SH-SY5Y cells were incubated with 5 μ M (+)-phenserine or (-) phenserine for 0, 0.5, 1, 2 and 4 hours to determine the effect of the drug on β APP protein levels. Western blots of cell lysates containing 15 μ g of total protein per lane were analyzed. The blot was sectioned into two halves and the top portion of the blot was probed with an N-terminal directed anti- β APP antibody whereas the remaining blot was probed with an antibody directed to phosphorylated ERK. In accord with previous reports (Lahiri et al., 1997, 1998; Waskiewicz and Cooper, 1995), two high molecular weight bands corresponded to alternate forms of β APP (100-125 kDa) and ERK $\frac{1}{2}$ (42-46 kDa). The stereoisomeric forms of the drugs have opposite effects on cholinesterase activity: (+)-phenserine exhibits no anti-cholinesterase activity whereas (-)-phenserine has potent enzymatic activity (Greig et al., 1995; Brossi et al., 1996). In both experiments, the β APP levels in the cell lysates slowly decreased at each time point with the most dramatic decline observed after 4 hours. During this period, the cells were also examined for their ability to induce signal transduction pathways. Mitogen stimulated kinase, ERK1/2, was detected in the treated samples at all times and peaked at the 30 minute to 1 hour period. Stress activated transcription kinases, p38 and JNK, were not detected in the samples. Furthermore, media samples were analyzed for levels of A β at 4 hours and, additionally, at 8 and 16 hours to assess whether or not decline in β APP translated into a decline in total A β peptide levels. Levels of A β were below detectable levels in both control and (-)-phenserine treated cells. As a consequence, studies were repeated with SK-N-SH cells with (-)-phenserine and (+)-phenserine, which was used in all subsequent studies unless otherwise indicated.

SK-N-SH cells were incubated with either (-)-phenserine (FIG. 4) or with (+)-phenserine (FIG. 5) for up to 16 hours. FIG. 4 illustrates the effect of (-)-phenserine on β APP protein levels (FIGS. 4A and 4B), LDH levels (FIG. 4C) and total A β levels (FIG. 4D). β APP levels are shown as a percent of controls after pretreatment with 0.5, 5 and 50 μ M (-)-phenserine for 4, 8 and 16 hours (*significantly different from control, $p < 0.05$). Western blots of conditioned media (FIG. 4A) and cell lysates (FIG. 4B) were probed with a N-terminal directed anti- β APP antibody. Following phenserine treatment of SK-N-SH cells for 16 hours, β APP levels were reduced in a time- and concentration-dependent manner in both conditioned media (FIG. 4A) and cell lysates (FIG. 4B).

LDH levels were measured in media from cells treated with and without 50 μ M (-)-phenserine for up to 16 hours. There was no significant difference between treated and untreated levels up to 16 hours ($p > 0.05$). This was not associated with cellular dysfunction, as determined by measurement of LDH levels versus untreated controls (FIG. 4C).

Quantification of levels of total A β was undertaken at 8 and 16 hours and results shown in FIG. 4D demonstrate a (-)-phenserine induced reduction of 14% and 31% ($p < 0.002$), respectively, versus untreated controls. (+)-Phenserine possessed a similar concentration- and time-dependent action on β APP levels. The concentration of total A β peptide was measured in media from SK-N-SH cells that were incubated with 50 μ M (-)-phenserine for up to 16 hours. Levels fell from 6.95 to 5.95 μ M (14%) at 8 hours, and from 11.75 to 8.1 μ M (31%, $p < 0.02$) at 16 hours in control and (-)-phenserine treated cell, respectively (FIG. 4D). Likewise, (+)-phenserine reduced β APP protein levels and total A β peptide levels at 16 hours

(FIG. 5). This was not associated with toxicity, as assessed by measurement of cell number and viability (LDH levels).

Phenserine Associated Decrease of β APP Levels in Astrocytoma Cell Line U373 is Not Dependent on ERK Activation

Following an extended period of (-)-phenserine treatment, U373 cells exhibited a similar pattern of decreased β APP protein synthesis. FIG. 6 is a representative of 4 experiments that showed that β APP levels gradually decreased between 1 and 8 hours of treatment. U373 MG astrocytoma cells were treated with (-)-phenserine to determine its effect on β APP protein levels. The addition of ERK inhibitor, PD98059, and PI 3 kinase inhibitor, LY294002, was carried out to ascertain whether or not (-)-phenserine action on β APP was directed through these signaling pathways. Western blots of lysates (15 μ g per lane) of U373 cells incubated with 50 μ M of (-)-phenserine for 0, 0.5, 1, 4, 8, 24 and 48 hours were analyzed. The blot was divided into two sections. On the left panel, the blot was probed with anti- β APP antibody and on the right panel, the blot was probed with anti-phosphorylated ERK antibody. After 8 hours, a slow recovery of β APP was detectable (FIG. 6A) but its level was still lower than in untreated cells. The activation of ERK1/2 peaked at the 30 minute time point and remained elevated at a low level for the remainder of the assay.

In order to determine whether or not ERK involvement was directly related to phenserine treatment, the cells were pretreated with PD98059, a specific inhibitor of MAP kinase (FIG. 6B). U373 MG cells were pretreated with 25 nM PD98059 for 16 hours prior to (-)-phenserine treatment. Lysates were analyzed by western blots as described above. Although ERK levels decreased significantly, the pattern of β APP levels induced by phenserine remained largely similar to U373 cells treated with drug without PD98059. In all cases, β APP levels were decreased by in excess of 25%, as determined by densitometric quantification.

Phenserine action on β APP through ERK independent, phosphoinositol 3 kinase (PI 3 kinase) stimulation was also assessed. Treatment of astrocytoma cells with phenserine and LY294002, a specific inhibitor of PI 3 kinase, showed a similar pattern of β APP levels when compared to (-)-phenserine alone treated cells (FIG. 6C). U373 MG cells were pretreated with 200 μ M LY294002 for 1.5 hours prior to the addition of (-)-phenserine. The cell lysate of each sample was analyzed as described above. β APP protein levels were reduced by in excess of 25% ($p < 0.05$) with (-)-phenserine treatment in FIGS. 6A-C, as determined by densitometric quantification.

In summary, these studies demonstrate that the action of (-)-phenserine to reduce β APP protein and total A β peptide levels did not occur via classical cholinergic or neurotransmitter mediated mechanism, as has been suggested by Buxbaum et al., (1990, 1992, 1994) and Nitsch et al. (1992, 1994). This was supported by two previously unreported lines of evidence. First, studies with the (+)-enantiomer, (+)-phenserine, that is devoid of anticholinesterase activity and hence cholinergic action, still reduced β APP protein and total A β peptide levels (FIG. 5). Second, the actions of (-)-phenserine β APP protein and total A β peptide persisted after the classical pathways involved in cholinergic modulation were blocked (FIG. 6). In addition, in separate studies, when 50 μ M concentrations of the classical anticholinesterase, (-)-physostigmine, were applied to SK-N-SH cells, no reduction or change in β APP protein and total A β peptide levels was found.

To demonstrate that the actions on β APP protein and A β peptide were not restricted to enantiomers of phenserine, identical studies were undertaken with both enantiomers of

cymserine (compound 46 in Table 1 for (+)-enantiomer) and with (–)-N¹,N⁸-bisnorcymserine and (–)-N¹-phenethylcymserine. Similar to (–)-phenserine, the (–)-enantiomer of cymserine possessed anticholinesterase action and the (+)-enantiomer was devoid of it. As shown in FIGS. 7A and 7B, both enantiomers reduce β APP protein levels. Similarly, N¹,N⁸-bisnorcymserine and N¹-phenethylcymserine reduced β APP protein levels (FIGS. 7C and 7D). In each case, A β peptide levels were also reduced, and there was no toxicity (as assessed by cell number and viability, as assessed by LDH measurement). However, at higher concentrations of N¹,N⁸-bisnorcymserine and N¹-phenethylcymserine, the compounds are toxic.

Phenserine Decreases β APP Protein Levels Through the Action of a Translational Enhancer in the APP-mRNA 5' Untranslated Region

A recent report identified a 90 nt element from the 146 nt 5' untranslated region (5'UTR) of the β APP mRNA that is able to confer a 3 fold IL-1 responsive gene expression to CAT reporter mRNAs in astrocytoma cells (Rogers et al., 1999). Interleukin-1 was able to induce β APP protein levels in the absence of increased β APP mRNA synthesis. Parallel experiments with (–)-phenserine were examined for its ability to regulate β APP protein levels in an identical manner. U373 MG astrocytoma cells were transfected with 3 μ g of pSV₂ (APP) CAT plasmid or the parental vector pSV₂ CAT. Each set of transfection plates was left unstimulated or treated with 50 μ M of phenserine for the experimental times listed below.

FIG. 8A is a representative CAT assay that shows that (–)-phenserine is able to decrease the level of APP-mRNA 5'UTR enhancement to a CAT reporter mRNA in pSV₂ (APP) CAT transfected astrocytoma cells. CAT activity was assessed from lysates of transfected cells treated with (–)-phenserine for 0, 0.25, 0.5 and 1 hour and 50 mg from each sample was measured in duplicate for each assay. Quantitation of the fold stimulus of CAT gene activity conferred by the 5' UTR β APP-mRNA was measured. ELISA readings of CAT expression were measured at 405 nm. A 4-fold decrease after phenserine treatment was sustained after 1 hour. In control samples, pSV₂ CAT transfected cells exhibited no inhibition by (–)-phenserine at all time points, indicating that the parental vector was unresponsive to drug treatment. In other assays where the time of treatment was extended to 48 hours, only a 2-fold decrease in CAT reporter mRNA was detected with phenserine treatment. However, even in these extended assays, CAT activity in control cells remained undisturbed, indicating that the drug's effects were specific for the 5' UTR of β APP mRNA. The expression level of CAT in the control vector vs. the 5'UTR containing vector prior to (–)-phenserine treatment was similar.

(–)-Phenserine decreases the level of β APP levels through the influence of the β APP-mRNA 5'UTR region. Western blot analysis of β APP protein levels was performed on lysates of transfected cells treated with (–)-phenserine for 0, 0.25, 0.5 and 1 hour (FIG. 8B). The blot was probed with anti- β APP antibody. β APP protein levels in transfected U373 astrocytoma cells treated with phenserine showed a similar pattern of results as with the CAT assay (FIG. 8A). The introduction of the CAT reporter constructs increased the level of β APP over the endogenous untransfected cells, in accord with that reported by Rogers et al. (1999). However, after (–)-phenserine treatment, β APP levels in pSV₂ (APP) CAT transfected astrocytomas gradually approached levels seen in untransfected and untreated cells. In contrast to these results, phenserine treatment of U373 cells did not affect β APP mRNA levels.

FIG. 8C is a representative northern blot of U373 cells treated with phenserine for time points up to 48 hours. Phenserine does not affect the steady state levels of β APP-mRNA levels. Ten μ g of RNA isolated from untransfected and transfected cells treated with (–)-phenserine for 0, 1, 4, 24 and 48 hours were analyzed by northern blot. Phosphorimager analysis revealed steady-state expression of β APP-mRNA in each sample. The same filter was stripped and rehybridized with a labeled human actin probe to standardize the loading differences in individual samples. Standardization of each sample to actin mRNA expression showed consistent levels of β APP mRNA without any major fluctuations in densitometry readings. Clearly, phenserine's action of β APP protein is at the level of translation, as northern blot analysis of untransfected and transfected cells show little differences in levels of mRNA transcription.

In Vivo Studies-Toxicity of (–)-Phenserine vs. (+)-Phenserine

On administration of (–)-phenserine to rodents by the i.p. route (1 ml/kg in 0.9% saline) a fine tremor is observed at a dose of 5 mg/kg. This persisted for an hour and is related to central cholinergic overdrive. Tremor together with symptoms of peripheral cholinergic overdrive (specifically, salivation and lacrimation) were seen at a dose of 7.5 mg/kg for some 3 hours. Animals were incapacitated at 20 mg/kg (N=3 per dose group), and 2 were killed when moribund. Animals (N=2) administered 20 mg/kg (+)-phenserine were without clinical symptoms and appeared normal.

Illustrated in FIG. 9 is the action of (–)-phenserine (2.5 mg/kg i.p., once daily for 3 weeks) on brain cortex and CSF β APP levels in transgenic mice (N=12) that significantly overexpress β APP as a consequence of the human Swedish β APP mutation and mutant presenilin 1 (Borchelt et al., 1997). (–)-Phenserine significantly reduced β APP levels by 55% in CSF and 10% in cerebral cortex. Greater reductions could be achieved, but, as previously reported, doses of >5 mg/kg produce cholinergic side effects for the (–)-enantiomer but not for the (+)-enantiomer.

Samples of cerebral cortex from the transgenic mice then were analyzed for β -amyloid peptide (A β) levels. Specifically, A β ₁₋₄₀ and A β ₁₋₄₂ levels, following formic acid extraction, were determined by ELISA assay (Suzuki et al., 1994). As shown in FIG. 10A, (–)-phenserine treatment reduced A β ₁₋₄₀ levels by 68% (p<0.05), whereas (–)-N¹-phenethylcymserine reduced levels by 54% (p<0.05). In contrast, (–)-phenserine reduced A β ₁₋₄₂ levels by 53% (p<0.05), compared to a 47% reduction (p<0.05) induced by (–)-N¹-phenethylcymserine, as shown in FIG. 10B.

Hence, even over as short a duration as three weeks in the life span of transgenic mice (generally 18 to 24 months) that overexpress β APP and overproduce A β , daily phenserine and N¹-phenethylcymserine administration, in well tolerated doses, reduce both β APP and A β levels, thereby indicating that in vitro efficacy-translates to in vivo activity.

In summary, the (+)-enantiomers of this invention are the focus of the present application. They are unnatural and totally synthetic compounds. The described studies demonstrated that both (+)- and (–)-enantiomers possessed potent activity to reduce β APP protein and total A β peptide levels. However, the (+)-enantiomers are devoid of anticholinesterase activity, and hence lack cholinergic action. It is the cholinergic action that is dose limiting with regard to the use of the (–)-enantiomers in vivo. The reductions in levels of β APP protein demonstrated in tissue culture studies occurred in two different types of human neuroblastoma cell (SK-N-

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SH and SH-SY-5Y lines), as well as in astrocytoma cells (U 373 line). These in vitro effects translate into in vivo activity as demonstrated herein.

Translational Effect of Phenserine on APP

1. Rate of APP Synthesis

8×10⁶ SHSY-5Y cells were plated on 100 mm dishes with DMEM containing 10% FBS. After 36 hours, the culture medium was replaced with DMEM containing 0.5% FBS. The cells were incubated with low serum medium for 1 hour. Thereafter, the medium was replaced with fresh low serum medium with and without 10 μM of phenserine for 16 hours.

After treatment (16 hrs) with and without (–)-phenserine (10 μM), the cells were incubated with methionine and cystine free DMEM containing 4 mM of glutamine for 1 hour. After treatment with methionine and cystine free medium, 2 ml of ³⁵S-labeled DMEM (100 μMCl/ml) with and without phenserine (10 μM) were added and incubated for 10 minutes. Thereafter, the labeled medium was carefully removed and the cells were suspended in lysis buffer containing with protease inhibitors and frozen at –80 C for use in the immunoprecipitation assay.

APP protein was immunoprecipitated from 300 μg of total protein in each sample using the polyclonal antibody O443, which recognizes a 20 amino acid sequence in the carboxy terminal of APP, and protein A/G resin overnight at 4° C. Immunoprecipitated APP was eluted from the protein A/G resin with 30 μl of elution buffer (10% beta-mercaptoethanol). The samples were loaded onto 10% trys-glycine gels, and the proteins were separated at 150 V for 90 min. The gels were fixed and dried at 80 C for 60 min. The dried gels were exposed to Phosphor Screen (PACKARD Instrument Company, Inc., Meriden, Conn.) overnight and the βAPP signals were quantitated on a phosphor imager. Phenserine significantly decreased βAPP synthesis (52% reduction) without changing TCA precipitable counts (FIG. 11A). This change in APP protein was not reflected in a change in mRNA levels as phenserine did not alter levels of APP RNA (FIG. 11B).

2. Effect of Phenserine on Steady State APP Levels

3×10⁶ SK-N-SH cells were plated on 60 mm dishes with DMEM containing 10% FBS. After 36 hours, cells were incubated with low serum medium for 1 hour. Thereafter, the medium was replaced with fresh low serum medium (0.5% FBS). To start the experiments, spent medium was removed and replaced with fresh medium containing 0, 0.5, 5 or 50 μM of (+) or (–)-phenserine. The cells were incubated with and without phenserine for 16 hours. At the stated time (4, 8 and 16 hrs), 200 μl of medium was transferred from each dish for assessment of extracellular APP levels. At the end of experiments (16 hrs), the cells were lysed and collected for assessment of intracellular APP levels.

15 μl of medium samples and 15 μg of total protein from each lysed samples were loaded onto 10% trys-glycine gels, and the protein was separated at 150 V for 90 min. The gels were transferred onto polyvinylidene difluoride paper and probed with an affinity-purified anti-APP antibody (22C11), which recognizes the ectodomain of APP (residues 66-81). The APP signals were detected by chemiluminescence and exposed to film. The quantitation of the signals was determined by using a CD camera and NIH-IMAGE (version 4.0).

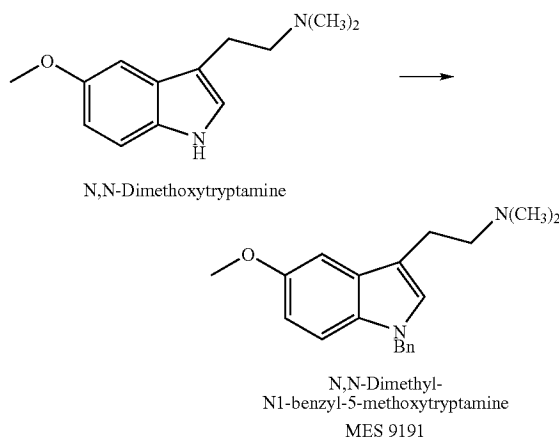
Phenserine significantly decreased steady state levels of extra- and intracellular βAPP in dose and time dependent manner (FIGS. 12A and B). Under these conditions, there was no significant toxicity as assessed by an LDH assay.

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Screening for Inhibitors of APP

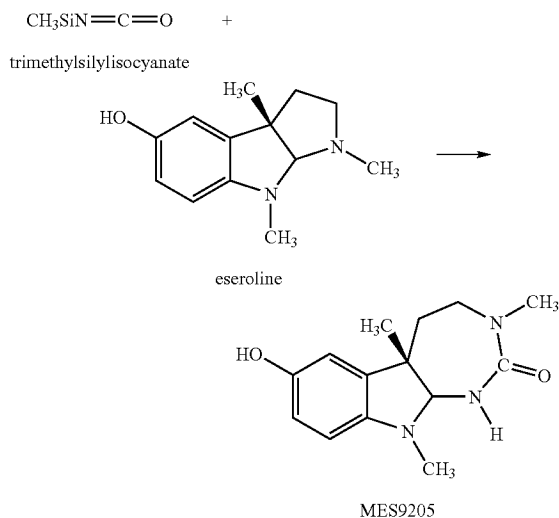
1. Synthesis of Compounds of Carbamate Non-Carbamate Compounds

N,N-Dimethyl-N1-benzyl-5-methoxytryptamine (MES 9191)



N,N-Dimethyl-5-methoxytryptamine (654 mg, 3.0 mmol) and NaNH₂ (234 mg, 60 mmol) were added into THF (10 ml), then benzyl bromide (513 mg, 3.0 mmol) was added. The mixture was refluxed under nitrogen with stirring for 2 days. Workup gave MES 9191 281 mg (30%).

(–)-(5aS)-3,5a,10-Trimethyl-1,3,4,5,5a,10a,10-heptahydro-1,3-diaza-2-one-7-hydroxycyclohept[2,3-b]indole (MES 9205)

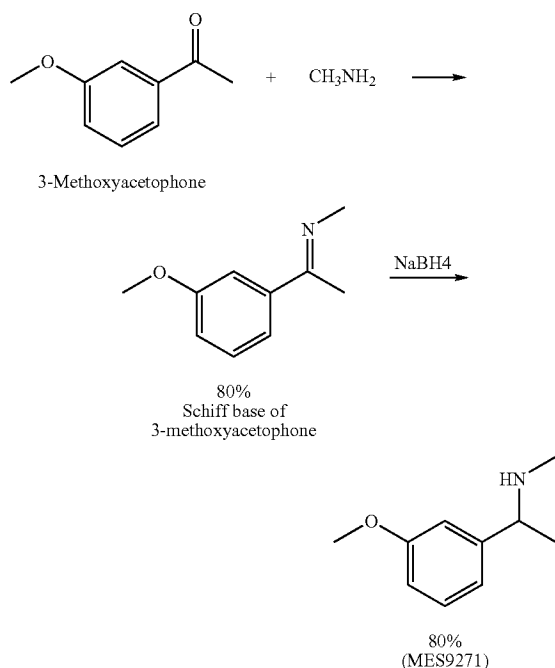


Method 1:¹⁵ Eseroline (180 mg, 0.82 mmol) and trimethylsilyl isocyanate (48 mg, 0.42 mmol) were dissolved in toluene (2 ml) in a sealed tube. The reaction mixture was stirred by a small magnetic bar and heated in an oil bath for 6 hours. The temperature of the oil was maintained between 100 and 110° C. After cooling to room temperature, the precipitated crys-

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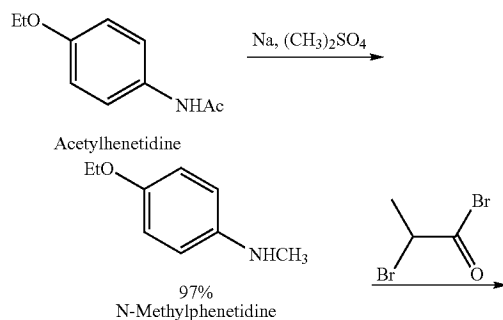
tals were filtered and recrystallized from MeOH to give carbamate MES 9205 (64 mg, 30%): m.p. 174-176° C.; $[\alpha]_D^{20} = -154^\circ$ (c=0.5, EtOH); CI-MS (NH₃) m/z, 262 (MH⁺); ¹HNMR (CD₃OD): 6.59 (d, J=3.0 Hz, 1H, C6-H), 6.53 (m, 1H, C8-H), 6.43 (d, J=8.0 Hz, 1H, C9-H), 3.88 (s, 1H, C10a-H), 3.20 (m, 1H, C4-H'), 2.95 (m, 1H, C4-H''), 2.84 (s, 3H, N10-CH₃), 2.62 (s, 3H, N3-CH₃), 2.19 (m, 1H, C5-H'), 1.81 (m, 1H, C5-H''), 1.30 (s, 3H, C5a-CH₃). Anal (C₁₄H₁₉N₃O₂) C, H, N.

3-Methoxy-(1'-N-methylamino)-ethyl-benzene
(MES 9271)



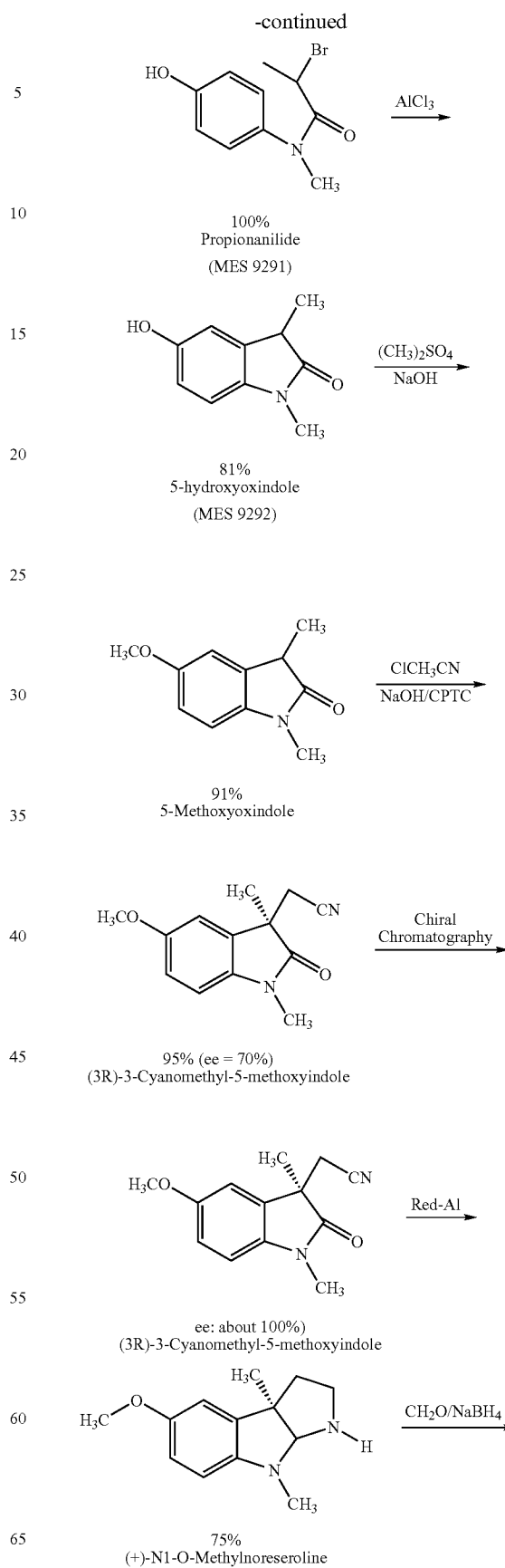
3-Methoxyacetophenone (2 g, 0.013 mol) with methylamine (6 g) was refluxed in ethanol for 4 h. After evaporation of solvent chromatography gave schiff base (1.7 g, 80%). The schiff base of 3-methoxyacetophenone (1.25 g, 0.008 mol) was dissolved in methanol (25 ml) and reduced by sodiumborohydride (0.24 g, 0.008 mol). Workup gave MES 9271 1 g (80%).

Propionanilide (MES 9291)



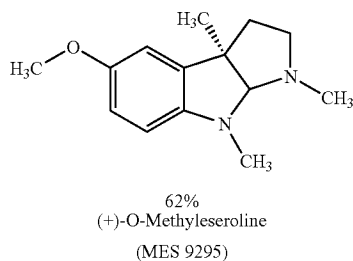
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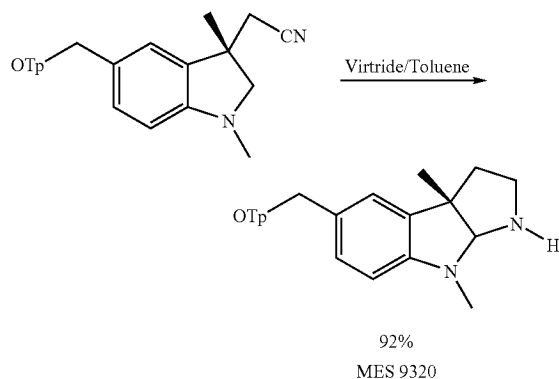


N-Methylphenetidine (340 g, 1.85 mol) was dissolved into 750 ml of benzene and cooled to 10° C., then α -bromopropionylbromide (200 g, 0.926 mol) was rapidly added. The mixture was stirred for 1.5 h at 40° C. then washed by water and 1.5% HCl. Evaporation gave product MES 9291 477 g (100%).

1,3-Dimethyl-5-hydroxyoxindole (MES 9292). The propionanilide (MES 9291) (477 g, 1.85 mol) was mixed with 450 g AlCl_3 then heated by an oil bath to 190° C. for 1 h. After reaction the mixture was poured into ice water, the precipitate was filtered and crystallized to give product MES 9292 148 g (91%).

(+)-O-Methyleseroline (MES 9295). The 1,3-dimethyl-5-hydroxyoxindole (MES 9292) was chiral alkylated by chloroacetonitrile in using the chiral catalyst CPTC to afford optical rich product, (3R)-3-cyanomethyl-5-methoxyoxindole, which then purified by chiral chromatography to give optical pure (3R)-3-cyanomethyl-5-methoxyoxindole. Reductive cyclization of above cyano-oxindole by reducing agent red-A1 gave (+)-N1-O-methylnoreseroline which then was methylated by formaldehyde and sodiumborohydride to give MES 9295 in the yields as described in the scheme.

(-)-O-Tetrahydropyranyl-N-1-noreseroline (MES 9320)

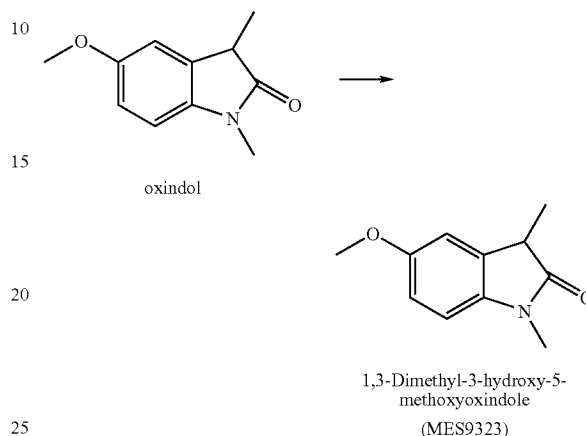


Starting material Nitrile (120 mg, 0.4 mmol) and virtride (0.15 ml, 0.4 mmol) were dissolved in toluene. The mixture was stirred under nitrogen at room temperature for 3 h, then 5 ml of 5% NaOH was added. The toluene layer was separated out and the aqueous layer was extracted with ether (2x5 ml).

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The combined organic layers were washed with brine, dried over sodium sulfate, evaporated in vacuum to give MES 9320 119 mg (92.5%).

1,3-Dimethyl-3-hydroxy-5-methoxyoxindole (MES 9323)



To a 25 ml flask was added benzene (12 ml) and oxindole (95.5 ml, 0.5 mmol), then 2 ml of 50% NaOH solution were added. The mixture after standing for 12 h was extracted with ether (3x10 ml). The combined extracts were washed with brine, and dried over MgSO_4 . After evaporation of solvent and chromatography (silica gel, petroleum ether: EtOH=3:1) gave MES 9323 62 mg (50%).

2. ELISA Assay for Identification of APP Inhibitors

An enzyme-linked immunosorbent assay was developed to detect secreted APP in SHSY5Y cells, a human neuroblastoma cell line. The purpose of the screen was to discover small molecules that inhibit APP protein synthesis in SHSY5Y cells. SHSY5Y cells were plated at 1×10^5 cells/well in 100 mL/well of DMEM containing 0.5% heat inactivated FBS in 96 well tissue culture treated plates (Falcon no. 35 3072). 144 MES compounds were tested at final concentrations of 20 mM, 6.7 mM, and 2.2 mM in 0.1% DMSO. Compounds were added and the plates incubated for 16 hours at 37° C./5% CO_2 . Maxisorp plates (Nunc no. 437958) were coated overnight with 2 mg/mL capture antibody (Biosource 44-100) diluted in Ca^{++} and Mg^{++} free PBS. Biosource 44-100 is a mouse mAb that recognizes aa 1-100 of human APP. Plates were blocked for 30 minutes with 1 mg/mL BSA in Ca^{++} and Mg^{++} free PBS. Plates were washed 3x with wash buffer (Ca^{++} and Mg^{++} free PBS+0.01% Tween20). 50 mL of supernatants and non-cultured medium controls were transferred from culture plates to ELISA plates. Culture plates were reserved for toxicity analysis. Supernatants were incubated for 4 hours at RT on a plate shaker. After supernatant incubation, plates were washed 3x. Primary antibody (Signet Clone 6E-10 biotin) was added at 0.3125 mg/mL and incubated overnight at 4° C. The recognition site of 6E-10 is aa 1-17 of Ab. For detection, a 1:3000 dilution of horseradish peroxidase conjugated streptavidin (Endogen no. N-100) was incubated for 30 minutes. Plates were washed 3x. Enzyme activity was assessed by incubation with 100 mL/well TMB substrate solution (Moss no. TMB-US) for 20 minutes. The reaction was stopped by the addition of 100 mL/well 0.18M sulfuric acid. Optical density was read at 450 nm on Wallac Victor2 plate reader. During the first incubation, toxicity was determined

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using MTS assay, Cell Titer96 AQ reagent (Promega no. G5430). The background values from the non-cultured medium control were subtracted from the sample values and secreted APP levels and toxicity were expressed as % vehicle control. Of the 144 MES compounds screened, 12 inhibited 5 secreted APP as determined by ELISA. The results are depicted in FIGS. 13A-M.

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Using the experimental procedure outlined above, APP secretion and toxicity studies were performed on several other non-carbamate compounds, and the results are shown in Tables 3 and 4. The compound structures are provided in Table 2 in the application, which are identified by their MES number.

TABLE 3

Number	NIH #	MES	APP Secretion by SH-SY-5Y Cells					
			Dose (uM) 0.08	Dose (uM) 0.25	Dose (uM) 0.74	Dose (uM) 2	Dose (uM) 6	Dose (uM) 20
6	594xp2	9191	100.7666667	101.6333333	99.86666667	100.74	65.3	64.36
15	y-1-15	9199				92.2	93.9	102.95
17	y-1-46	9201				97.25	110.75	124.65
18	y-1-48-1 (-)	9202				94.4	107.55	110.05
19	y-1-48-2 (+)	9203				103.1	103.866667	108.36667
21	y-1-59	9205	115.1	107.4	114.7	96.73333333	96.9	22.166667
22	y-1-60	9206				88.2	97.1	96.4
27	y-1-66	9215				94.55	108.1	6.2
34	y-2-1	9222				99.85	105.55	120.4
37	y-2-13	9225				110.45	109.2	59.75
38	y-2-15	9226				116.55	101.95	104.65
39	y-2-16	9227				101.15	99.45	120.5
40	y-2-18	9228				97.75	110.45	130.05
41	y-2-19	9229				111.6	111.4	125.1
42	y-2-20	9230				101.25	88.8	4.4
43	y-2-24	9231				104.9	133.5	154.2
46	y-2-31	9234				106.4	96.5	98.8
48	y-2-33	9236				105.8	103.2	101.65
49	y-2-34	9237				149.95	158.1	173.3
50	y-2-35	9238	117.6	107.7	109.7	48.5666667	39.23333333	23.566667
54	y-2-43-2	9242				116.75	116.3	116.1
55	y-2-49	9243				88.95	95.25	100.55
69	y-2-95	9257				111.2	98.9	99.033333
71	y-2-105	9259				90.7	101.1	111.1
72	y-2-106	9260				93.95	103.2	101.1
78	y-2-113	9266				94.4	95.2	94.85
79	y-2-114	9267	97.8	97.43333333	97.43333333	93.68	68.64	22.22
82	y-2-130	9270				95.8	95.9	98.15
83	y-2-131	9271	96.6	87.95	92.2	96.1	89.9	63.825
87	y-3-5	9276				96.15	116.95	133.4
89	y-3-15	9277				98.25	109	113.6
90	y-3-17	9278				118.4	116.766667	120.63333
91	y-3-19	9279	97.8	98.4	97	43.2666667	42.3666667	18.4
104	y-3-58	9291	98.2	99.5	99.7	100.133333	96.9	67.8
105	y-3-59	9292				106.15	99.55	100.15
106	y-3-60	9293				97.8	102.75	94.45
107	y-3-69	9294				105.25	90	111
108	y-3-71	9295				102.6	95.7	89.35
114	SH-281	9301				108.45	106.1	111.15
115	140xp2	9302				100.35	105.4	105.8
118	614xp1	9305				106.2	102.4	102.2
119	552xp3	9306				110.75	114.55	124.8
122	piy-1-79-2	9309				100	106.4	100.9
123	129xp3	9310				104.8	97.85	98.95
124	637xp4	9311				102.45	103.05	102.7
125	piy-1-79-3	9312				105.4	101.7	96.65
127	piy-1-79-4	9314				111.25	99.15	109.4
129	piy-1-79-5	9316				104.15	98.45	102
130	597xp4	9317				99.8666667	112.266667	122.03333
131	197xF3	9318				104.05	103.4	108.75
132	504xp2	9319				108.95	114.1	112.8
133	338xp2	9320				103.15	91.8	15.2
134	574xp1	9321				97.95	98.1	100.5
136	111xp1	9323				98.4	102.3	98.3
137	484xp2	9324				110.3	95.9	99.95
143	y-1-78-1	9330				99.6	101.5	110.65
144	y-1-78-2	9331				95.45	102.1	106.7

TABLE 4

			APP Toxicity SH-SY-5Y Cells					
Number	NIH #	MES	Dose (uM) 0.08	Dose (uM) 0.25	Dose (uM) 0.74	Dose (uM) 2	Dose (uM) 6	Dose (uM) 20
6	594xp2	9191	104.4666667	103.9	103.7	99.18	92.84	91.1
15	y-1-15	9199				100.5	101.65	101.9
17	y-1-46	9201				98.9	98.65	98.25
18	y-1-48-1 (-)	9202				99.15	97.85	90.25
19	y-1-48-2 (+)	9203				98.93333333	99.16666667	98.83333333
21	y-1-59	9205	108.7	105.8	105.6	97.9	97.73333333	33.33333333
22	y-1-60	9206				96	91.85	76.3
27	y-1-66	9215				92.3	78.6	12.9
34	y-2-1	9222				99.25	100.5	98.9
37	y-2-13	9225				104.6333333	100.1	66.5
38	y-2-15	9226				106.3666667	99.7	97.9
39	y-2-16	9227				103.6666667	99.26666667	96.06666667
40	y-2-18	9228				102.7666667	97.6	95.4
41	y-2-19	9229				99.96666667	96.06666667	91.46666667
42	y-2-20	9230				100.8333333	86.13333333	13.83333333
43	y-2-24	9231				95.7	90.73333333	85.86666667
46	y-2-31	9234				102.55	99.75	99.3
48	y-2-33	9236				97.6	101	100.05
49	y-2-34	9237				96.55	98.35	99.4
50	y-2-35	9238	101.8	103.8	106.4	41.8	40.46666667	31.63333333
54	y-2-43-2	9242				94.2	89.8	80.95
55	y-2-49	9243				99.1	98.4	99
69	y-2-95	9257				100.5333333	98.76666667	100
71	y-2-105	9259				102.65	102.9	98.75
72	y-2-106	9260				99.8	97.2	98.95
78	y-2-113	9266				96.55	97.75	94.15
79	y-2-114	9267	101.5333333	102	101.9333333	97.98	96.72	15.52
82	y-2-130	9270				99.4	101.1	100.4
83	y-2-131	9271	103.55	103.45	103.1	99	99.475	94.1
87	y-3-5	9276				92.85	93.5	90.65
89	y-3-15	9277				98.25	98.3	91.1
90	y-3-17	9278				97.63333333	98.4	96.33333333
91	y-3-19	9279	102.9	101.1	100.1	68.63333333	46.66666667	38.63333333
104	y-3-58	9291	99.1	100.5	97.1	102.3666667	99.86666667	99
105	y-3-59	9292				102.85	103.75	104.9
106	y-3-60	9293				100.8	99.85	101.95
107	y-3-69	9294				99.9	98.3	100.2
108	y-3-71	9295				101.15	100.4	99.85
114	SH-281	9301				99.15	97.35	95.9
115	140xp2	9302				100.7	101.2	99.05
118	614xp1	9305				95.8	96.73333333	94.83333333
119	552xp3	9306				102.3	101.45	106
122	piy-1-79-2	9309				100.85	98.6	101.1
123	129xp3	9310				101.85	99.9	97.7
124	637xp4	9311				101.1	101.25	99.55
125	piy-1-79-3	9312				99.95	103.85	106.95
127	piy-1-79-4	9314				100.15	100.85	100.6
129	piy-1-79-5	9316				99.15	98.05	98.75
130	597xp4	9317				97.26666667	97.23333333	93.5
131	197xF3	9318				96.2	96.45	96.05
132	504xp2	9319				99.8	101.05	102.7
133	338xp2	9320				92	84	28
134	574xp1	9321				99.9	101.15	99.45
136	111xp1	9323				97.2	100.1	101.15
137	484xp2	9324				102.15	100.4	98.65
143	y-1-78-1	9330				99.05	98.45	97.95
144	y-1-78-2	9331				100.45	99.9	98.95

2. Effect of Phenserine Analogues on Steady State APP Levels

3×10⁶ SK-N-SH cells were plated on 60 mm dishes with DMEM containing 10% FBS. After 36 hours, cells were incubated with low serum medium for 1 hour. Thereafter, the medium was replaced with fresh low serum medium (0.5% FBS). To start the experiments, spent medium was removed and replaced with fresh medium with and without phenserine analogues. The cells were incubated with and without compounds for 16 hours. At the end of experiments (16 hrs), 200 ml of medium and the cells were lysed and collected for assessment of intracellular APP levels.

20 ml of medium samples and 15 mg of total protein from each lysed samples were loaded onto 10% trys-glycine gels, and the protein was separated at 150 V for 90 min. The gels were transferred onto polyvinylidene difluoride paper and probed with an affinity-purified anti-APP antibody (22C11), which recognizes the ectodomain of APP (residues 66-81). The APP signals were detected by chemiluminescence. The quantitation of blots was undertaken by using a CD camera and NIH-IMAGE (version 4.0).

Several of the compounds of the invention decreased the extra- and intracellular APP levels. A few compounds, which have no carbamate group within the molecule, did not show

significant reductions of intracellular APP levels, but did not reduce extracellular APP levels. The treatment with 5 mM of MES9280 showed some toxicity (increase of LDH levels and morphological change). The results are shown in FIG. 14.

3. Effect of Phenserine Analogues on APP Translation

3×10⁶ SHSY-5Y cells were plated on 60 mm dishes with DMEM containing 10% FBS. After 36 hours, the culture medium was replaced with DMEM containing 0.5% FBS. The cells were incubated with low serum medium for 1 hour, thereafter, the medium was replaced with fresh low serum medium with and without phenserine analogues for 16 hours.

After treatments (16 hrs) with and without the compounds of the invention, the cells were incubated with methionine and cystine free DMEM containing 4 mM of glutamine for 1 hr. After treatment with methionine and cystine free medium, 1 ml of ³⁵S-labeled DMEM (100 uCi/ml) with and without the compounds were added and incubated for 10 minutes. Thereafter, the labeled medium were carefully removed and the cells were suspended within lysis buffer containing with protease inhibitors and frozen at -80° C. until immunoprecipitation assay.

APP were immunoprecipitated from 200 ug of total protein in each samples with polyclonal antibody O443, which recognizes 20 aminoacids sequences of APP carboxy terminal, and protein A/G resin for overnight at 4 C. Immunoprecipitated APP were eluted from protein A/G resin with 30 ul of elution buffer (10% beta-mercaptoethanol). The samples were loaded onto 10% trys-glycine gels, and the protein were separated at 150 V for 90 min. The gels were fixed with fixing buffer and dried at 80 C for 60 min. The dried gels were exposed onto Phosphor Screen (PACKARD, Instrument Company, Inc., Meriden, Conn.) for overnight and the APP signals were quantitated on phosphor image.

The levels of newly synthesized βAPP were normalized by TCA precipitable counts. Several phenserine analogues significantly decreased βAPP synthesis without changing TCA precipitable counts (FIG. 15A). In addition, there was no change in APP mRNA levels (FIG. 15B).

REFERENCES

Adem A, Mattsson M E K, Nordberg A, Pahlman S (1987) Muscarinic receptors in human SH-SY5Y neuroblastoma cell line: regulation by phorbol ester and retinoic acid-induced differentiation. *Develop Brain Res* 33:235-242.

Akijama H, Barger S, Barnum S, et al., (2000) Inflammation and Alzheimer's disease. *Neurobiol. Aging* 21:383-421.

Becker R E., Moriearty P., Unni L. The second generation of cholinesterase inhibitors: clinical and pharmacological effects. In, *The Cholinergic Basis for Alzheimer Therapy* (ed., Becker R E., Giacobini, E) Birkhauser, Boston, 263-296, 1991.

Bhasker C R, Burgiel G, Neupert B, Emery-Goodman A, Kuhn L C, May B K (1993) The putative iron-responsive element in the human erythroid 5-aminolevulinate synthase mRNA mediates translational control. *J Biol Chem* 268(17): 12699-1270.

Borchelt D R, Ratovitski T, van Lare J, Lee M K, Gonzales V, Jenkins N A, Copeland N G, Price D L, Sisodia S S. Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. *Neuron* 1997 October; 19(4):939-45.

Breitner J C S (1997) Inflammatory processes and anti-inflammatory drugs in Alzheimer's disease: a current appraisal. *Neurobiol Aging* 17(5): 789-794.

Bronfman F C, Fernandez H L, Inestrosa N C (1996) Amyloid precursor protein fragment and acetylcholinesterase increase with cell confluence and differentiation in a neuronal cell line. *Exp Cell Res* 229:93-99.

Brossi A, Pei X-F, Greig N H (1996) Phenserine, a novel anticholinesterase related to physostigmine: total synthesis, and biological properties. *Austr J Chem* 49:171-190.

Buxbaum J D, Gandy S E, Cicchetti P, Ehrlich M E, Czernik A J, Fracasso R P, Ramabhadran T V, Unterbeck A J, Greengard P (1990) Processing of Alzheimer beta/A4 amyloid precursor protein: modulation by agents that regulate protein phosphorylation. *Proc Natl Acad Sci USA* 1990 87(15):6003-6006.

Buxbaum J D, Oishi M, Chen H I, Pinkas-Kramarski R, Jaffe E A, Gandy S E, Greengard P (1992) Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer beta/A4 amyloid protein precursor. *Proc Natl Acad Sci USA* 89(21): 10075-10078.

Buxbaum J D, Ruefli A A, Parker C A, Cypess A M, Greengard P (1994) Calcium regulates processing of the Alzheimer amyloid protein precursor in a protein kinase C-independent manner. *Proc Natl Acad Sci USA* 91:4489-4493.

Caputi A, Barindelli S, Pastorino L, Cimino M, Buxbaum J D, Cattabeni F, Di Luca M (1997) Increased secretion of the amino-terminal fragment of amyloid precursor protein in brains of rats with a constitutive up-regulation of protein kinase C. *J Neurochem* 68(6):2523-2529.

Checler, F (1995) Processing of β-amyloid precursor protein and its regulation in Alzheimer's disease. *J Neurochem* 65:1431-1444.

Desdouits F, Buxbaum J D, Desdouits-Magnen, Nairn A C, Greengard P (1996) Amyloid b peptide formation in cell-free preparations: regulation by protein kinase C, calmodulin and calcineurin. *J Biol Chem* 271(40):24670-24674.

Desdouits-Magnen J, Desdouits F, Takeda S, Syu L, Saltiel A R, Buxbaum J D, Czernik A J, Nairn A C, Greengard P (1998) Regulation of secretion of Alzheimer amyloid precursor protein by the mitogen-activated protein kinase cascade. *J Neurochem* 70(2):524-530.

Dyrks T, Monning U, Beyreuther K, Turner J (1994) Amyloid precursor protein secretion and bA4 amyloid generation are not mutually exclusive. *FEBS Lett* 349:210-214.

Eisenstein R S, Tuazon P T, Schalinske K L, Anderson S A, Traugh J A (1993) Iron-responsive element-binding protein. Phosphorylation by protein kinase C. *J Biol Chem* 268(36):27363-27370.

Felder C C, Ma A L, Briley E M, Axelrod J (1993) Muscarinic acetylcholine receptor subtypes associated with release of Alzheimer amyloid precursor derivatives activate multiple signal transduction pathways. *Ann NY Acad Sci* 695:15-18.

Funato H, Yoshimura M, Yamazaki T, Sato T C, Ito Y, Yokofujita J, Okeda R, Ihara Y (1998) Astrocytes containing amyloid-protein (A)-positive granules are associated with A40-positive diffuse plaques in the aged human brain. *Am J Path* 152:983-992.

Greig N H, Pei X-F, Soncrant T, Ingram D, Brossi A (1995) Phenserine and ring-C hetero-analogues: drug candidates for the treatment of Alzheimer's disease. *Med Chem Rev* 15:3-31.

Haroutunian V, Greig N H, Pei X F, Utsuki T, Gluck R, Acevedo L D, Davis K L, Wallace W C (1997) Pharmacological modulation of Alzheimer's beta-amyloid precursor protein levels in the CSF of rats with forebrain cholinergic system lesions. *Brain Res Mol Brain Res* 46(1-2): 161-168.

Hentze M W, Kuhn L C (1996) Molecular control of vertebrate iron metabolism. mRNA-based regulatory circuits

operated by iron, nitric oxide, and oxidative stress. *Proc Natl Acad Sci USA* 93:8175-8182.

Hung A Y, Selkoe D J (1994) Selective ectodomain phosphorylation and regulated cleavage of beta-amyloid precursor protein. *EMBO J* 13(3):534-542.

Husaaain, I, Powell D, Howlett D R, Tew D G, Week T D, Chapman C, Golger I S, Murphy K E, Southan C D, Ryan D M, Smith T S, Simmons D L, Walsh F S, Dingwall C, Christie, G (1999) Identification of a novel aspartic protease (Asp 2) as beta-secretase. *Mol Cell Neurosci* 14: 419-427.

Jacobsen J S, Spruyt M A, Brown A M, Sahasrabudhe S R, Blume A J, Vitek M P, Muenkel H A, Sonnenberg-Reines J (1994) The release of Alzheimer's disease beta amyloid peptide is reduced by phorbol treatment. *J Biol Chem* 269(11):8376-8382.

Kim H-Y, LaVaute T, Iwai K, Klausner R D, Rounault T A (1996) Identification of a conserved and functional iron-responsive element in the 5'-untranslated region of mammalian mitochondrial aconitase. *J Biol Chem* 271(39): 24226-24230.

Koike H, Seki H, Kouchi Z, Ito M, Kinouchi T, Tomioka S, Sorimachi H, Saido T C, Maruyama K, Suzuki K, Ishiura S (1999) Thimet oligopeptidase cleaves the full-length Alzheimer amyloid precursor protein at a beta-secretase cleavage site in COS cells. *J Biochem* 126: 235-242.

Lahiri D K, Farlow M R, Nurnberger J I Jr, Greig N H (1997) Effects of cholinesterase inhibitors on the secretion of beta-amyloid precursor protein in cell cultures. *Ann N Y Acad Sci* 26; 826:416-421.

Lahiri D K, Farlow M R, Sambamurti K (1998) The secretion of amyloid beta-peptide is inhibited in tacrine-treated human neuroblastoma cells. *Mol. Brain. Res.* 62: 131-140.

Leblanc A C, Koutroumanis M, Goodyer C G (1998) Protein kinase C activation increases release of secreted amyloid precursor protein without decreasing Ab production in human primary neuron cultures. *J Neurosci* 18(8):2907-2913.

Leli U, Cataldo A, Shea T B, Nixon R A, Hauser G (1992) Distinct mechanism of differentiation of SH-SY5Y neuroblastoma cells by protein kinase C activators and inhibitors. *J Neurochem* 58(4): 1191-1198.

Melefors O, Goossen B, Johansson H E, Stripecke R, Gray N K, Hentze M W (1993) Translational control of 5-aminolevulinic synthase mRNA by iron-responsive elements in erythroid cells. *J Biol Chem* 268(8):5974-5978.

Nitsch R M, Growdon J H, Farber, S A, Deng M, Wurtman R J (1994) Regulation of APP processing by first messengers. In: *Alzheimer Disease: Therapeutic Strategies* (Giacobini E, Becker R, eds), pp 54-61. Boston:Birkhauser.

Nitsch R M, Slack B E, Wurtman R J, Growdon J H. (1992) Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. *Science* 258:304-307.

Patel N, Spangler E L, Greig N H, Yu Q S, Ingram D K, Meyer R C (1998) Phenserine, a novel acetylcholinesterase inhibitor, attenuates impaired learning of rats in a 14-unit T-maze induced by blockade of the N-methyl-D-aspartate receptor. *Neuroreport* 9(1):171-176.

Rogers J T, Leiter L, Mcphee J, Cahill C M, Zhan S S, Potter H, Nilsson L N (1999) Translation of the Alzheimer amyloid precursor protein mRNA is upregulated by interleukin 1 through 5'-untranslated region sequences. *J Biol Chem* 274: 6421-6431.

Roberson, M R, Harrell L E (1997) Cholinergic activity and amyloid precursor protein metabolism *Brain Res Rev* 25:50-69.

Savage M J, Trusko S P, Howland D S, Pinsker L R, Mistretta S, Reaume A G, Greenberg B D, Siman R, Scott R W (1998) Turnover of amyloid b-protein in mouse brain and acute reduction of its level by phorbol ester. *J Neurosci* 18(5):1743-1752.

Schalinske K L, Chen O S, Eisenstein R S (1998) Iron differentially stimulates translation of mitochondrial aconitase and ferritin mRNAs in mammalian cells. Implications for iron regulatory proteins as regulators of mitochondrial citrate utilization. *J Biol Chem* 273(6):3740-3746.

Selkoe D J (1997) Alzheimer's disease: genotypes, phenotypes, and treatments. *Science* 1997 275(5300):630-631.

Sinha S, Anderson J P, Barbour R, Basi G S, Caccavello R, Davis D, Doan M, Dovey H F, Frigon N, Hong J, Jacobson-Croak K, Jewett N, Keim P, Knops J, Lieberburg I, Power M, Tan H, Tatsuno G, Tung J, Schenk D, Seubert P, Suomensaaari S M, Wang S W, Walker D, Zhao J, McConlogue L, John V (1999) Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature* 402: 537-540.

Suzuki N, Cheung T, Cai X., Odaka A., Eckman C., Golde T, Younkin, S G (1994) An increased percentage of long amyloid-beta protein secreted by familial amyloid-beta protein-precursor (beta-APP (717)) mutants. *Science* 264: 1336-1340.

Vassar R, Bennett B D, Babu-Khan S, Kahn S, Mendiaz E A, Denis P, Teplow D B, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller L, Edenson S, Lile J, Jarosinski M A, Biere A L, Curran E, Burgess T, Louis J C, Collins F, Treanor J, Rogers G, Citron M (1999) beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 286: 735-741.

Waskiewicz A J, Cooper J A (1995) Mitogen and stress response pathways: MAP kinase cascades and phosphatase regulation in mammals and yeast. *Curr Opin Cell Biol* 7:798-805.

Wisniewski H M, Wegiel J, Kotula L (1996) Review. David Oppenheimer Memorial Lecture 1995: Some neuropathological aspects of Alzheimer's disease and its relevance to other disciplines. *Neuropathol Appl Neurobiol* 22(1):3-11.

Yan R Q, Bienkowski M J, Shuck M E, Miao H Y, Tory M C, Pauley A M, Brashler J R, Stratman N C, Mathews W R, Buhl A E, Carter D B, Tomasselli A G, Parodi L A, Henrikson R L, Gurney M E (1999) Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. *Nature* 402: 533-537.

Yu, Q S, Brossi A (1988) Practical Synthesis of Unnatural (+)-Physostigmine and Carbamate Analogues. *Heterocycles* 27: 745-751.

Xu H, Greengard P, Gandy S (1995) Regulated formation of Golgi secretory vesicles containing Alzheimer beta-amyloid precursor protein. *J Biol Chem* 270(40):23243-23245.

REFERENCES FOR FIG. 1

1. Qian-sheng Yu, Arnold Brossi. Practical Synthesis of Unnatural (+)-Physostigmine and Carbamate Analogues. *Heterocycles*, 1988, 27, 745-750.
2. Xue-Feng Pei, Nigel H. Greig, Sheng Bi, Arnold Brossi and V. Toom. Inhibition of Human Acetylcholinesterase by Unnatural (+)-(3aR)—N¹-Norphysostigmine and Arylcarbamate. *Medicinal Chemistry Research*, 1995, 5, 265-270.
3. Qian-sheng Yu, Xue-Feng Pei, Harold W. Holloway, Nigel H. Greig, Arnold Brossi. Total Synthesis and Anticholinesterase Activities of (3aS)—N(8)-Norphysostigmine,

- (3aS)—N(8)-Norphenserine, Their Antipodal Isomers, and Other N(8)-Substituted Analogues. *J. Med. Chem.*, 1997, 40, 2895-2901.
4. Qian-sheng Yu, Nigel H. Greig, Harold W. Holloway, Arnold Brossi. Syntheses and Anticholinesterase Activities of (3aS)—N¹,N⁸-Bisnorphenserine, (3aS)—N¹,N⁸-Bisnorphysostigmine, Their Antipodal Isomers and Other Potential Metabolites of Phenserine. *J. Med. Chem.*, 1998, 41, 2371-2379.
5. Xue-Feng Pei, Qian-sheng Yu, Harold W. Holloway, Arnold Brossi, Nigel H. Greig, Syntheses and Biological Evaluation of Ring-C Opened Analogues of The Cholinesterase Physostigmine, Phenserine and Cymserine. *Med. Chem. Res.*, 1999, 9, 50-60.

REFERENCES FOR FIG. 2

1. Julian, P. L.; Pikle, J. J., *J. Am. Chem. Soc.* 1935, 57, 563.
2. Lee, T. B. K and Wong, G. S. K., *J. Org. Chem.*, 1991, 56, 872.
3. Pei, X. F. and Brossi, A., *Heterocycles*, 1995, 41, 2823.
4. Lee, T. B. K. and Wong, G. S. K., *J. Chromatography*, 1990, 523, 317.
5. Pei, X. F. and Sheng, B., *Heterocycles*, 1994, 39, 557.
6. Yu, Q. S. and Brossi, A., *Heterocycles*, 1988, 27, 745.
7. Yu, Q. S. et al., *Helv. Chem. Res.* 1991, 74, 761.
8. He, X. S. et al., *Med. Chem. Res.* 1992, 2, 229.
9. Pei, X. F. et al., *Med. Chem. Res.* 1995, 5, 455.
10. Yu, Q. S. et al., *J. Med. Chem.* 1988, 31, 2297.
11. Zhu, X. X., *TL*, 2000, 41, 4861.
12. Pei, X. F. et al., *Med. Chem. Res.* 1995, 5, 455.
13. Yu, Q. S. et al., *J. Med. Chem.* 1997, 40, 2895.
14. Yu Q. S. et al., *Heterocycles*, 1993, 36, 1279.
15. Pei, X. F., *Helv. Chem. Acta*, 1994, 77, 1421.

What is claimed is:

1. A method of inhibiting production of amyloid precursor protein in a mammalian subject in need thereof, comprising administering orally to the mammalian subject an effective amount of a pharmaceutical composition comprising (+)-phenserine.
2. The method of claim 1, wherein the dosage of (+)-phenserine is from about 0.1 mg/kg to about 100 mg/kg of body weight.
3. The method of claim 2, wherein the dosage of (+)-phenserine is from about 1 mg/kg to about 20 mg/kg of body weight.

4. The method of claim 1, wherein the pharmaceutical composition is administered daily to the mammalian subject.
5. The method of claim 4, wherein the pharmaceutical composition is administered once daily to the mammalian subject.
6. The method of claim 1, wherein the pharmaceutical composition comprises a slow release system.
7. The method of claim 1, wherein the pharmaceutical composition comprises a sustained release system.
8. The method of claim 1, wherein the pharmaceutical composition is administered by a polymer based delivery system.
9. The method of claim 1, wherein the pharmaceutical composition comprises (+)-phenserine tartrate.
10. The method of claim 1, wherein the mammalian subject is human.
11. A method of treating dementia in a mammalian subject in need thereof, comprising administering orally to the mammalian subject an effective amount of a pharmaceutical composition comprising (+)-phenserine.
12. The method of claim 11, wherein the dementia is Alzheimer's Disease.
13. The method of claim 11, wherein the dosage of (+)-phenserine is from about 0.1 mg/kg to about 100 mg/kg of body weight.
14. The method of claim 13, wherein the dosage of (+)-phenserine is from about 1 mg/kg to about 20 mg/kg of body weight.
15. The method of claim 11, wherein the pharmaceutical composition is administered daily to the mammalian subject.
16. The method of claim 15, wherein the pharmaceutical composition is administered once daily to the mammalian subject.
17. The method of claim 11, wherein the pharmaceutical composition comprises a slow release system.
18. The method of claim 11, wherein the pharmaceutical composition comprises a sustained release system.
19. The method of claim 11, wherein the pharmaceutical composition is administered by a polymer based delivery system.
20. The method of claim 11, wherein the pharmaceutical composition comprises (+)-phenserine tartrate.
21. The method of claim 11, wherein the mammalian subject is human.

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