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- (71) Applicant: **THE ROCKEFELLER UNIVERSITY**
[US/US]; 1230 York Avenue, New York, NY 10021-6299 (US).

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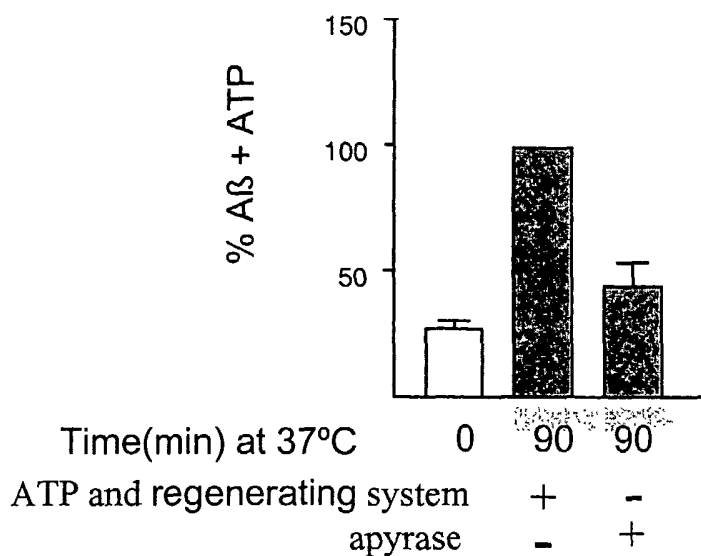
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- (72) Inventors: **NETZER, William, J.**; 450 East 63rd Street, New York, NY 10021 (US). **GREENGARD, Paul**; 362 East 69th Street, New York, NY 10021 (US). **XU, Huaxi**; 500 East 63rd Street, New York, NY 10021 (US).
- (74) Agents: **GEORGE, Nikolaos, C.** et al.; Pennie & Edmonds LLP, 1155 Avenue of the Americas, New York, NY 10036 (US).

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(54) Title: COMPOSITIONS AND METHODS FOR PREVENTION AND TREATMENT OF AMYLOID- β PEPTIDE-RELATED DISORDERS



(57) Abstract: The present invention provides methods and compositions for modulating levels of amyloid- β peptide ($A\beta$) exhibited by cells or tissues. The invention also provides pharmaceutical compositions and methods of screening for compounds that modulate $A\beta$ levels. The invention also provides modulation of $A\beta$ levels via selective modulation (*e.g.*, inhibition) of ATP-dependent- γ -secretase activity. The invention also provides methods of preventing, treating or ameliorating the symptoms of a disorder, including but not limited to an $A\beta$ -related disorder, by administering a modulator of γ -secretase, including, but not limited to, a selective inhibitor of ATP-dependent γ -secretase activity or an agent that decreases the formation of active (or optimally active) γ -secretase. The invention also provides the use of inhibitors of ATP-dependent γ -secretase activity to prevent, treat or

ameliorate the symptoms of Alzheimer's disease.



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**COMPOSITIONS AND METHODS FOR PREVENTION
AND TREATMENT OF AMYLOID- β PEPTIDE-RELATED DISORDERS**

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This invention was made with Government support under grant number 5P01AG009464-07 awarded by the National Institutes of Health. The United States Government may have certain rights in the invention.

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RELATED APPLICATIONS

This application claims benefit, under 35 U.S.C. § 119(e), of United States provisional application no. 60/345,009, filed on January 4, 2002, which is incorporated herein by reference in its entirety.

15

1. TECHNICAL FIELD

The present invention relates to methods and compositions for modulating levels of amyloid- β peptide ($A\beta$) exhibited by cells or tissues. The invention also relates to pharmaceutical compositions and methods of screening for compounds that modulate $A\beta$ levels. The invention also relates to modulation of $A\beta$ levels via selective modulation (*e.g.*, inhibition) of ATP-dependent γ -secretase activity. The invention also relates to methods of preventing, treating or ameliorating the symptoms of a disorder, including but not limited to an $A\beta$ -related disorder, by administering a modulator of γ -secretase, including, but not limited to, a selective inhibitor of ATP-dependent γ -secretase activity or an agent that decreases the formation of active (or optimally active) γ -secretase. The invention also relates to the use of inhibitors of ATP-dependent γ -secretase activity to prevent, treat or ameliorate the symptoms of Alzheimer's disease.

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2. BACKGROUND OF THE INVENTION

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Amyloid- β ($A\beta$) peptides are metabolites of the Alzheimer's disease-associated precursor protein, β -amyloid precursor protein (APP), and are believed to be the major pathological determinants of Alzheimer's disease (AD). These peptides consist mainly of 40 to 42 amino acids, $A\beta$ 1-40 (" $A\beta$ 40") and $A\beta$ 1-42 (" $A\beta$ 42"), respectively. $A\beta$ 40 and $A\beta$ 42 are generated by two enzymatic cleavages occurring close to the C-terminus of APP. The enzymes responsible for the cleavage, β -secretase and γ -secretase, generate the N- and C-termini of $A\beta$, respectively. The amino terminus of $A\beta$ is formed by β -secretase cleavage

between methionine residue 596 and aspartate residue 597 of APP (APP 695 isoform numbering) (see, *e.g.*, U.S. Patent No. 6,440,698; and U.S. Patent No. 5,744,346).

γ -secretase activity cleaves at varying positions 38-, 40- or 43-residues C-terminal of this β -secretase cleavage to release A β peptides (for a review of γ -secretase and γ -secretase activity, see, *e.g.*, U.S. Patent Application 20020025540). The complete molecular identity of γ -secretase enzyme is still unknown. Presenilin 1, or the closely related presenilin 2, is needed for γ -secretase activity. γ -secretase activity is reduced 80% in cultured cells derived from embryos genetically deleted for presenilin 1. All γ -secretase activity is lost in cells lacking both presenilin 1 and presenilin 2. Peptidomimetic inhibitors of γ -secretase activity can be crosslinked to presenilins 1 and 2, suggesting that these proteins are catalytic subunits for the cleavage. However, γ -secretase activity isolated from cells chromatographs as a large complex >1M daltons. Recent genetic studies have identified three more proteins required for γ -secretase activity; nicastrin, aph-1 and pen-1. (Francis *et al.*, 2002, Developmental Cell 3(1): 85-97; Steiner *et al.*, 2002, J. Biol. Chemistry: 277(42): 39062-39065; and Li *et al.*, 2002, J. Neurochem. 82(6): 1540-1548). Accumulation of presenilin into high molecular weight complexes is altered in cells lacking these proteins.

A third enzyme, α -secretase, cleaves the precursor protein between the β - and γ -cleavage sites, thus precluding A β production and releasing an approximately 3 kDa peptide known as P3, which is non-pathological. Both β - and α -secretase cleavage also result in soluble, secreted – terminal fragments of APP, known as sAPP β and sAPP α , respectively. The sAPP α fragment has been suggested to be neuroprotective.

In normal individuals, the A β peptide is found in two predominant forms, the majority A β -40 (also known as A β 1-40) form and the minority A β 42 (also known as A β 1-42) form, each having a distinct COOH-terminus. The major histological lesions of AD are neuritic plaques and neurofibrillary tangles occurring in affected brain regions. Neuritic plaques consist of A β peptides, primarily A β 40 and A β 42. Although healthy neurons produce at least ten times more A β 40 compared to A β 42, plaques contain a larger proportion of the less soluble A β 42. Patients with the most common form of familial Alzheimer's disease show an increase in the amount of the A β 42 form. The A β 40 form is not associated with early deposits of amyloid plaques. In contrast, the A β 42 form accumulates early and predominantly in the parenchymal plaques and there is strong evidence that A β 42 plays a major role in amyloid plaque deposits in familial Alzheimer's disease patients (Roher *et al.*, 1993, Proc. Natl. Acad. Sci. USA 90:10836; Iwatasubo, T., *et al.*, 1994 Neuron 13:45; Yamaguchi *et al.*, 1995, Amyloid Int. J. Clin. Invest. 2:7-16; and Mann *et al.*, 1996 Am. J. Pathol. 148:1257).

Neurofibrillary tangles consist of aggregated tau protein and their role in AD pathology is less clear. AD symptoms are most closely correlated with total brain A β rather

than plaques. About 10% of AD cases result from autosomal dominant inheritance of mutations in either the APP or the presenilin 1 and presenilin 2 genes. In both cases, increased production of total A β or A β 42 versus A β 40 results. There is strong evidence that A β peptides are critical in the pathogenesis of Alzheimer's disease. Therefore, compositions which modulate the activity and/or levels of these peptides are desired.

5 For example, U.S. Patent Application 20020128319 A1 states that certain non-steroidal anti-inflammatory drugs (NSAIDS) lower production and/or levels of A β 42 in cell cultures expressing A β 40 and A β 42 derived from the cleavage of APP. Since there is good evidence that high A β 42 levels are a major risk factor for AD, such drugs may be useful in preventing, delaying or reversing the progression of AD. The drawback of the use of such
10 drugs, however, is that large doses of NSAIDS are required for significant lowering of A β 42, and significant gastrointestinal side effects, including bleeding ulcers, are associated with prolonged use of NSAIDS at high doses (Langman *et al.*, 1994, Lancet 343: 1075-1078). In addition, there remains an unknown risk for Alzheimer's disease due to amyloid formation from A β 40 and other forms unaffected by A β 42 lowering agents.

15 There is, therefore, a need in the art to develop treatments for diseases or disorders related to the regulation of A β production. Furthermore, there is a need to develop methods of screening that can be used to identify compounds to treat such diseases or disorders.

20 Citation or identification of any reference in Section 2, or in any other section of this application, shall not be considered an admission that such reference is available as prior art to the present invention.

3. SUMMARY OF THE INVENTION

25 The present invention is based, in part, on Applicants' surprising discoveries that γ -secretase activity in cells requires adenosine triphosphate (ATP) in order to optimally produce amyloid- β peptide ("A β ") from APP and that selective competitors of ATP reduce A β levels, in particular, levels of secreted A β .

30 The present invention, therefore, relates, generally, to methods and compositions for preventing or treating an A β -related disorder such as Alzheimer's disease via administration of compounds that modulate, *e.g.*, inhibit, ATP-dependent enzymatic activity such as γ -secretase activity.

35 The invention provides a method for modulating amyloid- β peptide (A β) levels exhibited by a cell or tissue comprising contacting said cell or tissue with an amount of a compound sufficient to modulate said A β levels, wherein said compound modulates an ATP-dependent enzymatic activity.

In one embodiment, the A β levels are lowered. In another embodiment, the A β levels are raised. In another embodiment, the A β is A β 40. In another embodiment, the A β is A β 42.

In another embodiment, the modulating results in an increase in the ratio of A β 40 to A β 42. In another embodiment, the modulating results in an increase in C99.

5 In another embodiment, the compound binds an enzyme that exhibits an ATP-dependent enzymatic activity. In another embodiment, the compound binds a molecule that regulates the ATP-enzymatic activity.

In another embodiment, the compounds binds a molecule present in a complex with an enzyme that exhibits the ATP-dependent enzymatic activity, *e.g.*, γ -secretase activity.

10 In another embodiment, the molecule is a molecule that is allosterically regulated by ATP.

In another embodiment, the compound is an ATP modulator. In another embodiment, the the ATP modulator is a selective modulator.

15 In another embodiment, the ATP modulator competes with ATP for binding to an ATP-binding site. In another embodiment, the ATP-binding site is present on an enzyme that exhibits an ATP-dependent enzymatic activity. In another embodiment, the ATP-binding site is present on a molecule that regulates the ATP-dependent enzymatic activity.

20 In another embodiment, the compound does not affect total cellular levels of β -amyloid precursor product (APP). In another embodiment, the compound does not decrease levels of secreted APP (sAPP). In another embodiment, the compound increases levels of sAPP α . In another embodiment, levels of secreted A β are modulated. In another embodiment, the compound does not inhibit Notch-1 cleavage. In another embodiment, the compound does not inhibit tau phosphorylation.

In another embodiment, the compound crosses the blood-brain barrier.

25 In another embodiment, the enzymatic activity is a γ -secretase activity. In another embodiment, the compound inhibits γ -secretase activity. In another embodiment, the compound is an ATP modulator. In another embodiment, the ATP modulator is a selective modulator.

30 In another embodiment, the compound binds a γ -secretase enzyme exhibiting an ATP-dependent enzymatic activity. In another embodiment, the ATP modulator competes with ATP for binding to an ATP binding site.

35 In another embodiment, the compound is STI-571 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is a mesylate salt of STI-571. In another embodiment, the compound is WGB-BC-15 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is

Compound 1 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is Compound 2 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is Compound 3 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is Compound 4 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is Compound 5 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

In another embodiment, the enzymatic activity is a kinase activity. In another embodiment, the compound binds to a kinase enzyme exhibiting an ATP-dependent enzymatic activity. In another embodiment, the compound competes with ATP for binding to an ATP-binding site on the kinase. In another embodiment, the compound binds to a site other than an ATP-binding site on the kinase. In another embodiment, the kinase is a tyrosine kinase. In another embodiment, the tyrosine kinase is Abl kinase, BCR-Abl kinase, ARG kinase, src kinase, c-kit or platelet-derived growth factor receptor. In another embodiment, the kinase is a serine/threonine kinase, a carbohydrate kinase or a lipid kinase.

In another aspect, the invention provides a method for modulating A β levels exhibited by a cell or tissue comprising contacting said cell or tissue with an amount of an ATP modulator sufficient to modulate said A β levels.

In one embodiment, the A β levels are lowered. In another embodiment, the A β levels are raised. In another embodiment, the A β is A β 40. In another embodiment, the A β is A β 42.

In another embodiment, the modulating results in an increase in the ratio of A β 40 to A β 42. In another embodiment, the modulating results in an increase in C99. In another embodiment, the ATP modulator competes with ATP for binding to an ATP-binding site.

In another embodiment, the ATP-binding site is present on an enzyme that exhibits an ATP-dependent enzymatic activity. In another embodiment, the ATP-binding site is present on a molecule that regulates the ATP-dependent enzymatic activity. In another embodiment, the compound does not affect total cellular levels of β -amyloid precursor product (APP). In another embodiment, the compound does not decrease levels of secreted APP (sAPP). In another embodiment, the compound increases levels of sAPP α .

In another embodiment, levels of secreted A β are modulated.

In another embodiment, the compound does not inhibit Notch-1 cleavage. In another embodiment, the compound does not inhibit tau phosphorylation. In another embodiment, the compound crosses the blood-brain barrier. In another embodiment, the

enzymatic activity is a γ -secretase activity. In another embodiment, the compound inhibits γ -secretase activity.

In another embodiment, the the compound is an ATP modulator. In another embodiment, the ATP modulator is a selective modulator. In another embodiment, the compound binds a γ -secretase enzyme exhibiting an ATP-dependent enzymatic activity.

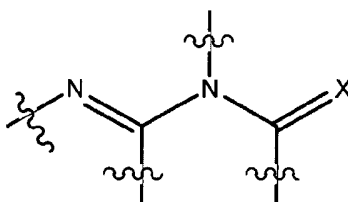
5 In another embodiment, the compound is STI-571 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is a mesylate salt of STI-571. In another embodiment, the compound is WGB-BC-15 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is any of
10 Compounds 1-5 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

In another embodiment, the enzymatic activity is a kinase activity. In another embodiment, the compound binds to a kinase enzyme exhibiting an ATP-dependent enzymatic activity. In another embodiment, the compound competes with ATP for binding
15 to an ATP-binding site on the kinase. In another embodiment, the compound binds to a site other than an ATP-binding site on the kinase. In another embodiment, the kinase is a tyrosine kinase. In another embodiment, the tyrosine kinase is Abl kinase, BCR-Abl kinase, ARG kinase, src kinase, c-kit or platelet-derived growth factor receptor. In another embodiment, the kinase is a serine/threonine kinase, a carbohydrate kinase or a lipid kinase.
20 In another embodiment, the kinase is a serine/threonine kinase, a carbohydrate kinase or a lipid kinase.

In another embodiment, the compound competes with ATP for binding to a target other than γ -secretase in the metabolic pathway that regulates the cleavage of APP to $A\beta$ by γ -secretase. In specific embodiments, the target is a kinase (or kinase domain), a protease
25 (*e.g.*, an AAA protease of the kind localized to the inner mitochondrial membrane), a phosphatase, or a molecular chaperone molecule (*e.g.*, hsp 60, hsp70, hsp90).

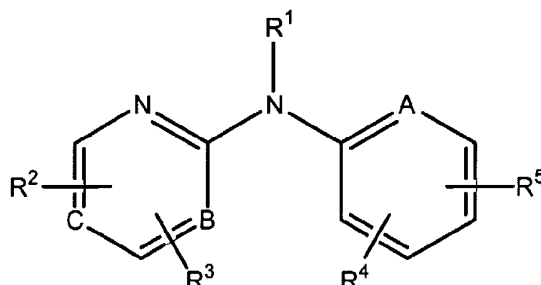
In one aspect in the invention provides a method for modulating $A\beta$ levels in a cell or tissue comprising contacting said cell or tissue with an amount of a compound sufficient to modulate said $A\beta$ levels, wherein said compound contains the following pharmacophore:
30

wherein X is CH-, O, NH or N-CO-.



In one embodiment, said pharmacophore containing compound comprises a compound of Figure I or a pharmaceutically acceptable salt thereof:

Figure I



5 A is CH or N;

B and C are independently CH, N or N⁺-O⁻;

R¹ is H, SO₂R^a, (C=O)_rO_sR^a;

R², R³, R⁴ and R⁵ are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl, (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl,
 10 (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl,
 (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^bR^c,

wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R⁶;

15 R⁶ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo, (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_s(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-NR^bR^c or (C₁-C₆)alkyl-heterocycl,

wherein said alkyl-heterocycl is optionally substituted with OH;

R^a is (C₁-C₆)alkyl, aryl or heterocycl; and

20 R^b and R^c independently are H, (C=O)_rO_s(C₁-C₁₀)alkyl, SO₂R^a, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl or CO₂R^a,

wherein r and s independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R⁶.

In another embodiment, A is CH, B is N and C is CH.

25 In another embodiment, R¹ is H.

In another embodiment, R² is H and R³ is heteroaryl.

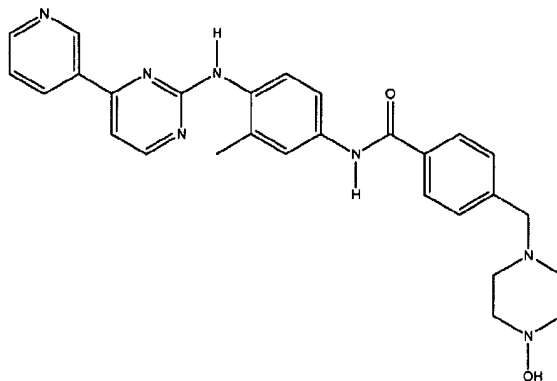
In another embodiment, R⁴ is (C=O)_rO_s(C₁-C₁₀)alkyl and R⁵ is (C₀-C₆)alkyl-NR^bR^c.

In another embodiment, R³ is 3-pyridinyl.

In another embodiment, r is 0, s is 0 and (C₁-C₁₀)alkyl is methyl.

In another embodiment, (C_0-C_6) alkyl is a direct bond (C_0), R^b is H and R^c is $(C=O)_rO_s$ heteroaryl or $(C=O)_rO_s$ heterocycl.

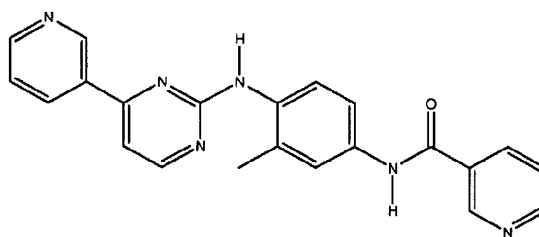
In another embodiment, $(C=O)_rO_s$ heterocycl is 4-hydroxy-1-piperazino, as illustrated below or a pharmaceutically acceptable salt thereof.



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In another embodiment, $(C=O)_rO_s$ heteroaryl is 3-pyridinyl, as illustrated below or a pharmaceutically acceptable salt thereof.

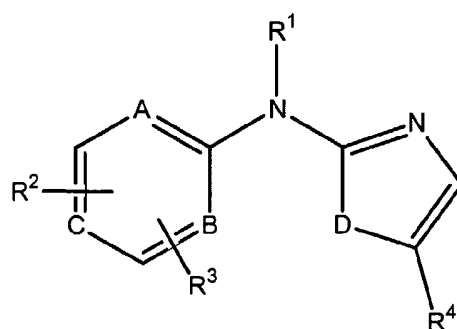
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In another embodiment, said pharmacophore containing compound, comprises a compound of Figure II or a pharmaceutically acceptable salt thereof:

Figure II



A, B and C are independently CH, N or N⁺-O⁻;

D is O, S or N-R⁵;

R¹ is H, SO₂R^a, (C=O)_rR^a or CO₂R^a;

R², R³ and R⁴ are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl,
 5 (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl,
 (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl,
 (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^bR^c,
 wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl,
 aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents
 10 selected from R⁶;

R⁵ is H, aryl or (C₁-C₆)alkyl;

R⁶ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo,
 (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_s(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-
 NR^bR^c or (C₁-C₆)alkyl-heterocycl;

15 R^a is (C₁-C₆)alkyl, aryl or heterocycl; and

R^b and R^c independently are H, (C=O)_rO_s(C₁-C₁₀)alkyl, SO₂R^a, (C=O)_rO_sheterocycl,
 (C=O)_rO_saryl, (C=O)_rO_sheteroaryl or CO₂R^a,

wherein r and s independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is
 optionally substituted with one or more substituents selected from R⁶.

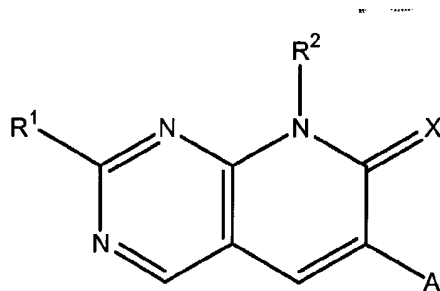
20 In another embodiment, R¹ is H.

In another embodiment, R² is H and R³ is (C₀-C₆)alkyl-NR^bR^c.

In another embodiment, R⁴ is heteroaryl.

In another embodiment, said pharmacophore containing compound, comprises a
 25 compound of Figure III or a pharmaceutically acceptable salt thereof:

Figure III



A is aryl or heteroaryl,

wherein said aryl or heteroaryl is optionally substituted with one or more substituents selected from R^3 ;

X is NH, N-acyl, O or S;

5 R^1 and R^2 are independently H, OH, CHO, CN, halogen, $(C=O)_rO_s(C_1-C_{10})$ alkyl, $(C=O)_rO_s(C_2-C_{10})$ alkenyl, $(C=O)_rO_s(C_2-C_{10})$ alkynyl, $(C=O)_rO_s$ cycloalkyl, $(C=O)_rO_s$ cycloalkenyl, $(C=O)_rO_s$ cycloalkynyl, $(C=O)_rO_s$ heterocycl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl, $(C=O)_rO_s$ perfluoroalkyl or (C_0-C_6) alkyl- NR^aR^b ,
 10 aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R^3 ;

R^3 is $(C=O)_rO_sNR^aR^b$, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heterocycl, halogen, OH, oxo, $(C=O)_rO_s(C_1-C_3)$ perfluoroalkyl, $(C=O)_rO_sS_t(C_1-C_6)$ alkyl, CHO, CO_2H , CN, (C_0-C_6) alkyl- NR^aR^b or (C_1-C_6) alkyl-heterocycl;

15 R^a and R^b independently are H, $(C=O)_rO_s(C_1-C_{10})$ alkyl, SO_2R^1 , $(C=O)_rO_s$ heterocycl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl or CO_2R^1 ,
 wherein r, s and t independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R^3 .

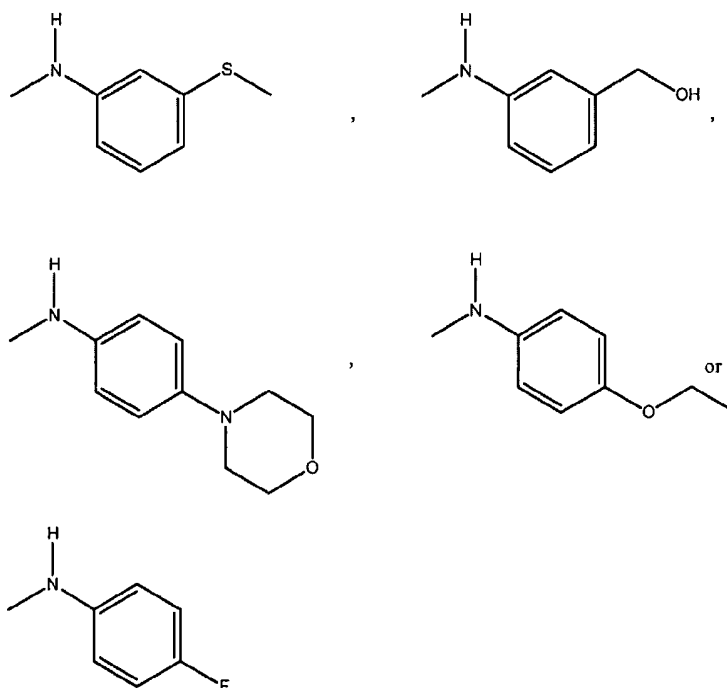
In another embodiment, A is aryl.

20 In another embodiment, the aryl is 2,5-dichlorophenyl.

In another embodiment, R^1 is (C_0-C_6) alkyl- NR^aR^b and R^2 is $(C=O)_rO_s(C_1-C_{10})$ alkyl.

In another embodiment, $(C=O)_rO_s(C_1-C_{10})$ alkyl is methyl.

In another embodiment, (C_0-C_6) alkyl- NR^aR^b is



or a pharmaceutically acceptable salt thereof.

In another aspect, the invention provides a pharmaceutical composition useful for modulating $A\beta$ level in a cell or tissue, comprising:

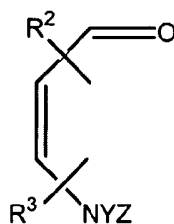
- 5 an amount of a compound described in any one of claims 1 to 21 sufficient to modulate said $A\beta$ level; and
a pharmaceutically acceptable excipient or carrier.

In one embodiment, the pharmaceutical composition is useful for modulating $A\beta$ levels exhibited by a cell or tissue.

- 10 In another embodiment, the compound is present in an amount sufficient to modulate said $A\beta$ levels.

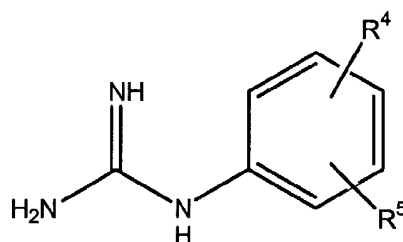
Compounds according to Figure I may be prepared by various processes known to one of skill in the art. In particular, compounds according to Figure I, wherein A is CH, B is N, C is CH and R^1 is H may be prepared by the following process:

- 15 a) a compound according to Figure IV
Figure IV



wherein Y and Z constitute (C₁-C₆)alkyl groups and R² and R³ are as above-defined is reacted with a compound according to Figure V

Figure V

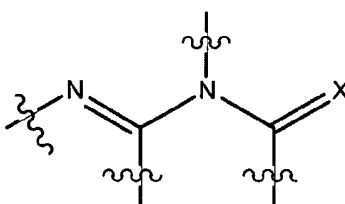


5 wherein R⁴ and R⁵ are as above-defined.

In one embodiment, a compound utilized by the methods of the invention contains the following pharmacophore:

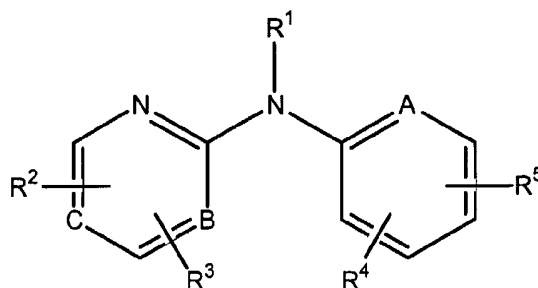
10

wherein X is CH-, O, NH or N-CO-.



In one embodiment, said pharmacophore containing compound comprises a compound of Figure I or a pharmaceutically acceptable salt thereof:

15 Figure I



A is CH or N;

B and C are independently CH, N or N⁺-O⁻;

R¹ is H, SO₂R^a, (C=O)_rO_sR^a;

R², R³, R⁴ and R⁵ are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl, (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl, (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^bR^c,
 5 wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R⁶;

R⁶ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo, (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_s(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-NR^bR^c or (C₁-C₆)alkyl-heterocycl,

wherein said alkyl-heterocycl is optionally substituted with OH;

15 R^a is (C₁-C₆)alkyl, aryl or heterocycl; and

R^b and R^c independently are H, (C=O)_rO_s(C₁-C₁₀)alkyl, SO₂R^a, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl or CO₂R^a,

wherein r and s independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R⁶.

20 In another embodiment, A is CH, B is N and C is CH.

In another embodiment, R¹ is H.

In another embodiment, R² is H and R³ is heteroaryl.

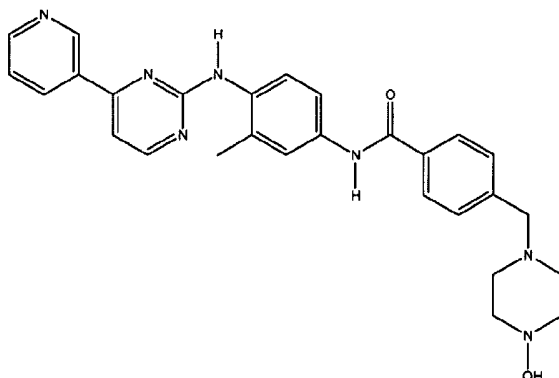
In another embodiment, R⁴ is (C=O)_rO_s(C₁-C₁₀)alkyl and R⁵ is (C₀-C₆)alkyl-NR^bR^c.

In another embodiment, R³ is 3-pyridinyl.

25 In another embodiment, r is 0, s is 0 and (C₁-C₁₀)alkyl is methyl.

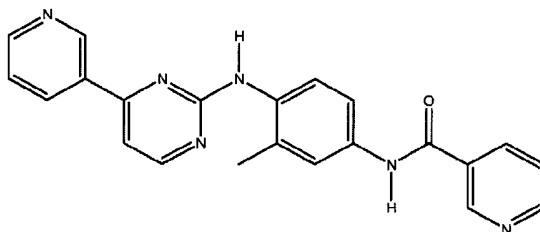
In another embodiment, (C₀-C₆)alkyl is a direct bond (C₀), R^b is H and R^c is (C=O)_rO_sheteroaryl or (C=O)_rO_sheterocycl.

In another embodiment, $(C=O)_rO_s$ heterocycl is 4-hydroxy-1-piperazino, as illustrated below or a pharmaceutically acceptable salt thereof.



5

In another embodiment, $(C=O)_rO_s$ heteroaryl is 3-pyridinyl, as illustrated below or a pharmaceutically acceptable salt thereof.

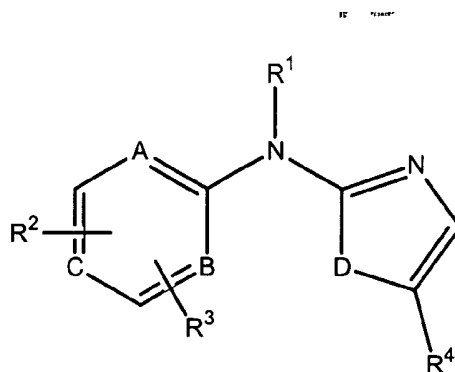


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15

In another embodiment, said pharmacophore containing compound, comprises a compound of Figure II or a pharmaceutically acceptable salt thereof:

Figure II



A, B and C are independently CH, N or N⁺-O⁻;

D is O, S or N-R⁵;

R¹ is H, SO₂R^a, (C=O)_rR^a or CO₂R^a;

R², R³ and R⁴ are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl,
 5 (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl,
 (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl,
 (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^bR^c,
 wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl,
 aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents
 10 selected from R⁶;

R⁵ is H, aryl or (C₁-C₆)alkyl;

R⁶ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo,
 (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_s(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-
 NR^bR^c or (C₁-C₆)alkyl-heterocycl;

15 R^a is (C₁-C₆)alkyl, aryl or heterocycl; and

R^b and R^c independently are H, (C=O)_rO_s(C₁-C₁₀)alkyl, SO₂R^a, (C=O)_rO_sheterocycl,
 (C=O)_rO_saryl, (C=O)_rO_sheteroaryl or CO₂R^a,

wherein r and s independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is
 optionally substituted with one or more substituents selected from R⁶.

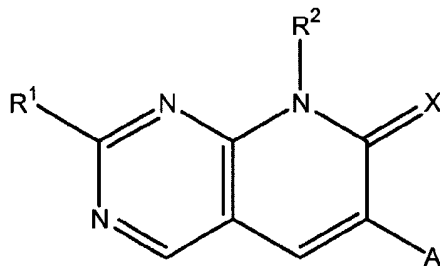
20 In another embodiment, R¹ is H.

In another embodiment, R² is H and R³ is (C₀-C₆)alkyl-NR^bR^c.

In another embodiment, R⁴ is heteroaryl.

In another embodiment, said pharmacophore containing compound, comprises a
 25 compound of Figure III or a pharmaceutically acceptable salt thereof:

Figure III



A is aryl or heteroaryl,

wherein said aryl or heteroaryl is optionally substituted with one or more substituents selected from R^3 ;

X is NH, N-acyl, O or S;

5 R^1 and R^2 are independently H, OH, CHO, CN, halogen, $(C=O)_rO_s(C_1-C_{10})$ alkyl, $(C=O)_rO_s(C_2-C_{10})$ alkenyl, $(C=O)_rO_s(C_2-C_{10})$ alkynyl, $(C=O)_rO_s$ cycloalkyl, $(C=O)_rO_s$ cycloalkenyl, $(C=O)_rO_s$ cycloalkynyl, $(C=O)_rO_s$ heterocycl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl, $(C=O)_rO_s$ perfluoroalkyl or (C_0-C_6) alkyl- NR^aR^b ,
 10 aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R^3 ;

R^3 is $(C=O)_rO_sNR^aR^b$, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heterocycl, halogen, OH, oxo, $(C=O)_rO_s(C_1-C_3)$ perfluoroalkyl, $(C=O)_rO_sS_t(C_1-C_6)$ alkyl, CHO, CO_2H , CN, (C_0-C_6) alkyl- NR^aR^b or (C_1-C_6) alkyl-heterocycl;

15 R^a and R^b independently are H, $(C=O)_rO_s(C_1-C_{10})$ alkyl, SO_2R^1 , $(C=O)_rO_s$ heterocycl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl or CO_2R^1 ,
 wherein r, s and t independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R^3 .

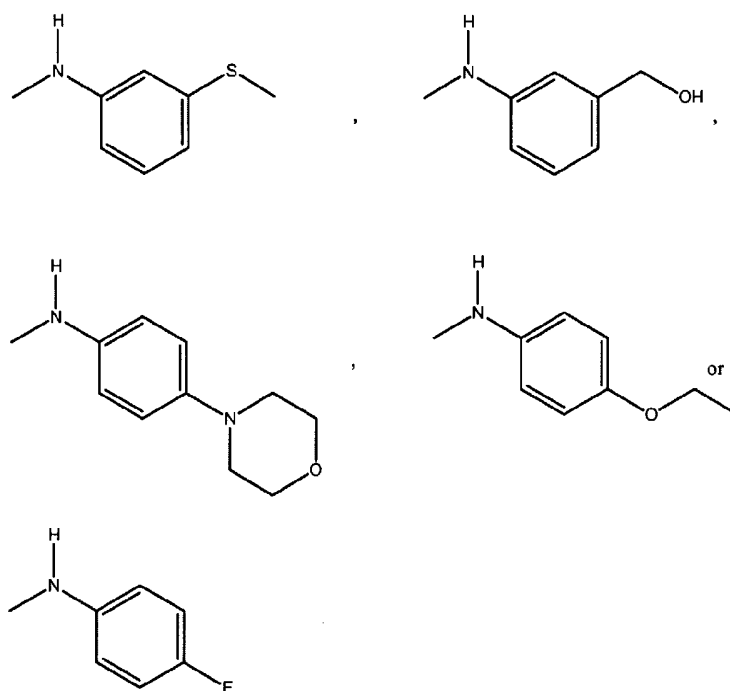
In another embodiment, A is aryl.

20 In another embodiment, the aryl is 2,5-dichlorophenyl.

In another embodiment, R^1 is (C_0-C_6) alkyl- NR^aR^b and R^2 is $(C=O)_rO_s(C_1-C_{10})$ alkyl.

In another embodiment, $(C=O)_rO_s(C_1-C_{10})$ alkyl is methyl.

In another embodiment, (C_0-C_6) alkyl- NR^aR^b is



or a pharmaceutically acceptable salt thereof.

The invention also provides an article of manufacture comprising packaging material and a pharmaceutical composition comprising a compound that lowers ATP-dependent A β levels, and a pharmaceutically acceptable carrier contained within the packaging material, said pharmaceutical composition in a form suitable for administration to a subject. In one embodiment, the article of manufacture further comprises printed instructions regarding the use or administration of the pharmaceutical composition. In one embodiment, the instructions suggest a dosing regimen for the prevention, treatment, or amelioration of a symptom of an A β -related disorder. In another embodiment, the instructions suggest a dosing regimen for the prevention, treatment, or amelioration of a symptom of Alzheimer's disease.

In another embodiment, the article of manufacture further comprises a label regarding the use or administration of the pharmaceutical composition. In another embodiment, the label suggests a dosing regimen for the prevention, treatment, or amelioration of a symptom of an A β -related disorder. In another embodiment, the label suggests a dosing regimen for the prevention, treatment, or amelioration of a symptom of Alzheimer's disease.

In another embodiment, the article of manufacture further comprises an antioxidant, a non-selective COX inhibitor or an acetylcholinesterase inhibitor. In another embodiment,

the pharmaceutical composition further comprises an antioxidant, a non-selective COX inhibitor or an acetylcholinesterase inhibitor.

The invention also provides method of identifying a compound that modulates A β levels exhibited by a cell or tissue comprising:

- (a) determining a first level of γ -secretase activity in said cell or tissue;
- 5 (b) contacting said cell or tissue with a test compound; and
- (c) determining a second level of γ -secretase activity in said cell or tissue,

wherein a difference in said first level and said second level of γ -secretase activity is indicative of the ability of said test compound to modulate A β levels.

10 In one embodiment, the difference in γ -secretase activity is indicative of the ability of said test compound to modulate the A β levels. In another embodiment, A β levels are modulated. In another embodiment, the γ -secretase activity is an ATP-dependent enzymatic activity. In another embodiment, the γ -secretase activity is production of A β .

The invention also provides a method of identifying a compound that modulates A β levels exhibited by a cell or tissue comprising:

- 15 (a) contacting said cell or tissue with a test compound; and
- (b) determining a level of activity of γ -secretase in said cell or tissue;

wherein a difference in said level and a control level of γ -secretase activity in a comparable cell or tissue not contacted with the test compound is indicative of the ability of said test compound to modulate A β levels.

20 In one embodiment, the difference in γ -secretase activity is indicative of the ability of said test compound to modulate the A β levels. In another embodiment, A β levels are modulated. In another embodiment, the γ -secretase activity is an ATP-dependent enzymatic activity. In another embodiment, the γ -secretase activity is production of A β .

The invention also provides a method for identifying an agent to be tested for an ability to treat an A β -related disorder in a patient in need of such treatment comprising:

- 25 (a) contacting in a cell or tissue γ -secretase with a potential agent; and
- (b) detecting the amount of γ -secretase activity

wherein the agent is identified if a decrease in γ -secretase activity is detected in the presence of the potential agent and wherein the agent modulates A β levels.

30 In one embodiment, the ability to treat the A β -related disorder is tested. In another embodiment, the γ -secretase activity is an ATP-dependent enzymatic activity. In another embodiment, the γ -secretase activity is production of A β .

In another aspect, the invention provides a method for identifying an agent to be tested for an ability to modulate A β levels exhibited by a cell or tissue comprising:

- 35 (a) determining a first level of γ -secretase activity in said cell or tissue;
- (b) contacting said cell or tissue with a potential agent; and

(c) determining a second level of γ -secretase activity in said cell or tissue, wherein a difference in said first level and said second level of γ -secretase activity is indicative of the ability of said potential agent to modulate $A\beta$ levels. In one embodiment, the method comprises the additional step of:

(d) determining whether the $A\beta$ levels are modulated.

5 In another embodiment, the γ -secretase activity is an ATP-dependent enzymatic activity. In another embodiment, the γ -secretase activity is production of $A\beta$.

The invention also provides a method for identifying an agent to be tested for an ability to modulate $A\beta$ levels exhibited by a cell or tissue comprising:

(a) contacting said cell or tissue with a potential agent; and

10 (b) determining a level of γ -secretase activity in said cell or tissue;

wherein a difference in said level and a control level of γ -secretase activity in a comparable cell or tissue not contacted with the test compound is indicative of the ability of said potential agent to modulate $A\beta$ levels.

In one embodiment, the method comprises the additional step of:

15 (c) determining whether the $A\beta$ levels are modulated.

In another embodiment, the γ -secretase activity is an ATP-dependent enzymatic activity.

In another embodiment, the γ -secretase activity is production of $A\beta$.

The invention also provides a method for selecting a potential therapeutic agent for use in the treatment of an $A\beta$ -related disorder comprising:

(a) administering a potential therapeutic agent to an animal;

(b) measuring the response of said animal to said potential therapeutic agent;

(c) comparing the response of said animal with that of a control animal to which the potential therapeutic agent has not been administered; and

25 (d) selecting a potential therapeutic agent based on the difference in responses observed between said animal and said control animal,

wherein the potential therapeutic agent modulates ATP-dependent γ -secretase activity.

In one embodiment, the animal is a guinea pig. In another embodiment, the disorder is Alzheimer's disease.

30 The invention also provides a method for selecting a potential therapeutic agent for use in the treatment of an $A\beta$ -related disorder comprising:

(a) administering a potential therapeutic agent to an animal;

(b) measuring the response of said animal to administration of an agent that modulates activity of γ -secretase;

35 (c) comparing the response of said animal with that of a control animal to which the potential therapeutic agent has not been administered; and

- (d) selecting a potential therapeutic agent based on the difference in responses observed between said animal and said control animal,

wherein the potential therapeutic agent modulates ATP-dependent γ -secretase activity.

In one embodiment, the animal is a guinea pig. In another embodiment, the disorder is Alzheimer's disease.

5 The invention also provides a method for selecting a potential therapeutic agent for use in the treatment of an $A\beta$ -related disorder comprising:

- (a) administering a potential therapeutic agent to an animal;
(b) measuring the response of said animal, wherein the response is selected from the group consisting of:

- 10 (i) exhibition of behavior in a Morris water maze; and
(i) exhibition of behavior in a Y-maze

- (c) comparing the response of said animal with that of a control animal to which the potential therapeutic agent has not been administered; and

- (d) selecting a potential therapeutic agent based on the difference in responses
15 observed between said animal and said control animal,

wherein the potential therapeutic agent modulates $A\beta$ levels.

In one embodiment, the animal is a guinea pig. In another embodiment, the disorder is Alzheimer's disease.

20 The invention also provides a method for preventing, delaying or reversing the progression of an $A\beta$ -related disorder comprising:

- (a) identifying a mammal in need of prevention, delay, or reversal of the progression of the disorder; and
(b) administering to said mammal an amount of an agent sufficient to modulate ATP-dependent γ -secretase activity,

25 wherein $A\beta$ levels are modulated.

In one embodiment, the agent inhibits or decreases ATP-dependent γ -secretase activity.

In another embodiment, the mammal is human. In another embodiment, the disorder is Alzheimer's disease.

30 In another embodiment, the agent promotes or increases ATP-dependent γ -secretase activity.

In another embodiment, the agent is administered orally.

In another embodiment, the agent is administered with a NSAID. In specific embodiment, the NSAID is sulindac sulfide, flufenamic acid, ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen, meclofenamic acid.
35

In another embodiment, the agent is administered with an

antioxidant. In another embodiment, the antioxidant is selected from the group consisting of vitamin E, vitamin C, curcumin, and Ginkgo biloba.

In another embodiment, the agent the agent is administered with an acetylcholinesterase inhibitor.

5 The invention also provides a method of treating, or ameliorating a symptom of, an A β -related disorder comprising administering to a subject in need of such treating or ameliorating an amount of a compound sufficient to reduce A β levels in the subject, wherein the compound modulates an ATP-dependent enzymatic activity, such that the A β -related disorder is treated or a symptom of the A β related disorder is ameliorated.

10 In one embodiment, the A β related disorder is Alzheimer's disease. In another embodiment, progression of the A β related disorder is slowed. In another embodiment, progression of the A β related disorder is reversed.

In another embodiment, the subject is a human subject. In a specific embodiment, the subject is a subject at risk for a familial form of Alzheimer's disease.

15 In another embodiment, the ATP-dependent enzymatic activity is lowered. In another embodiment, the compound crosses the blood-brain barrier. In another embodiment, the compound is administered orally.

In another embodiment, the compound is administered with a NSAID. In a specific embodiment, the NSAID is sulindac sulfide, flufenamic acid, ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen, meclofenamic acid.

20 In another embodiment, the compound is administered with an antioxidant. In a specific embodiment, the antioxidant is selected from the group consisting of vitamin E, vitamin C, curcumin, and Ginkgo biloba.

In another embodiment, the compound is administered with an acetylcholinesterase inhibitor.

25 In another embodiment, the compound is an ATP modulator. In another embodiment, the compound is ATP modulator is a selective modulator.

In another embodiment, the enzymatic activity is a γ -secretase activity. In another embodiment, the compound binds a γ -secretase enzyme exhibiting an ATP-dependent enzymatic activity.

30 In another embodiment, the ATP modulator competes with ATP for binding to an ATP binding site.

35 In one embodiment, the compound is STI-571 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is a mesylate salt of STI-571. In another embodiment, the compound is WGB-BC-15 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable

salt thereof. In another embodiment, the compound is any of Compounds 1-5 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

In one embodiment, the enzymatic activity is a kinase activity. In one embodiment, the compound binds to a kinase enzyme exhibiting an ATP-dependent enzymatic activity. In one embodiment, the compound competes with ATP for binding to an ATP-binding site
5 on the kinase. In another embodiment, the compound binds to a site other than an ATP-binding site on the kinase.

In a specific embodiment, the kinase is a tyrosine kinase. In another embodiment, the tyrosine kinase is Abl kinase, BCR-Abl kinase, ARG kinase, src kinase, c-kit, platelet-derived growth factor receptor.

10 In a specific embodiment, the kinase is a serine/threonine kinase, a carbohydrate kinase or a lipid kinase.

In another embodiment, the enzymatic activity is activity of a protease (*e.g.*, AAA protease of the kind localized to the inner mitochondrial membrane), phosphatase, or a molecular chaperone molecule (*e.g.*, hsp 60, hsp70, hsp90).

15 The invention also provides a method of treating, or ameliorating a symptom of, an A β -related disorder comprising administering to a subject in need of such treating or ameliorating an amount of an ATP modulator sufficient to reduce A β levels in the subject, such that the A β -related disorder is treated or a symptom of the A β related disorder is ameliorated.

20 In one embodiment, the A β related disorder is Alzheimer's disease. In one embodiment, the subject is a human subject. In another embodiment,

In one embodiment, the subject is a subject at risk for a familial form of Alzheimer's disease.

In another embodiment, the ATP-dependent enzymatic activity is lowered.

25 In another embodiment, the ATP modulator crosses the blood-brain barrier.

In another embodiment, the ATP modulator is administered orally. In another embodiment, the ATP modulator is administered with a NSAID. In another embodiment, the NSAID is sulindac sulfide, flufenamic acid, ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen, meclofenamic acid.

30 In another embodiment, the ATP modulator is administered with an antioxidant. In another embodiment, the antioxidant is selected from the group consisting of vitamin E, vitamin C, curcumin, and Ginkgo biloba.

In another embodiment, the ATP modulator is administered with an acetylcholinesterase inhibitor.

35 In another embodiment, the ATP modulator is a selective modulator.

In another embodiment, the enzymatic activity is a γ -secretase activity.

In one embodiment, the compound binds a γ -secretase enzyme exhibiting an ATP-dependent enzymatic activity. In another embodiment, the ATP modulator competes with ATP for binding to an ATP binding site.

In one embodiment, the compound is STI-571 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is a mesylate salt of STI-571. In another embodiment, the compound is WGB-BC-15 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is any of Compounds 1-5 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

In one embodiment, the enzymatic activity is a kinase activity. In one embodiment, the compound binds to a kinase enzyme exhibiting an ATP-dependent enzymatic activity. In one embodiment, the compound competes with ATP for binding to an ATP-binding site on the kinase. In another embodiment, the compound binds to a site other than an ATP-binding site on the kinase.

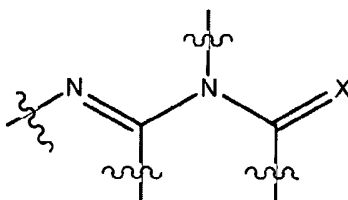
In a specific embodiment, the kinase is a tyrosine kinase. In another embodiment, the tyrosine kinase is Abl kinase, BCR-Abl kinase, ARG kinase, src kinase, c-kit, platelet-derived growth factor receptor.

In a specific embodiment, the kinase is a serine/threonine kinase, a carbohydrate kinase or a lipid kinase.

In another embodiment, the enzymatic activity is activity of a protease (*e.g.*, AAA protease of the kind localized to the inner mitochondrial membrane), phosphatase, or a molecular chaperone molecule (*e.g.*, hsp 60, hsp70, hsp90).

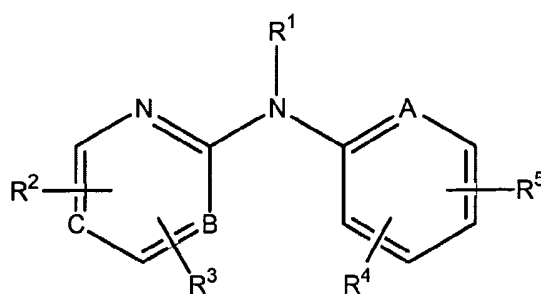
The invention also provides a method of treating, or ameliorating a symptom of, an A β -related disorder comprising administering to a subject in need of such treating or ameliorating an amount of a compound sufficient to reduce A β levels in the subject, such that the A β -related disorder is treated or a symptom of the A β -related disorder is ameliorated, wherein the compound contains the following pharmacophore:

wherein X is CH-, O, NH or N-CO-.



In one embodiment, said pharmacophore containing compound comprises a compound of Figure I or a pharmaceutically acceptable salt thereof:

Figure I



A is CH or N;

B and C are independently CH, N or N⁺-O⁻;

R¹ is H, SO₂R^a, (C=O)_rO_sR^a;

- 5 R², R³, R⁴ and R⁵ are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl, (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl, (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^bR^c,
 10 wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R⁶;

R⁶ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo, (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_s(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-NR^bR^c or (C₁-C₆)alkyl-heterocycl,

- 15 wherein said alkyl-heterocycl is optionally substituted with OH;

R^a is (C₁-C₆)alkyl, aryl or heterocycl; and

R^b and R^c independently are H, (C=O)_rO_s(C₁-C₁₀)alkyl, SO₂R^a, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl or CO₂R^a,

- 20 wherein r and s independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R⁶.

In another embodiment, A is CH, B is N and C is CH.

In another embodiment, R¹ is H.

In another embodiment, R² is H and R³ is heteroaryl.

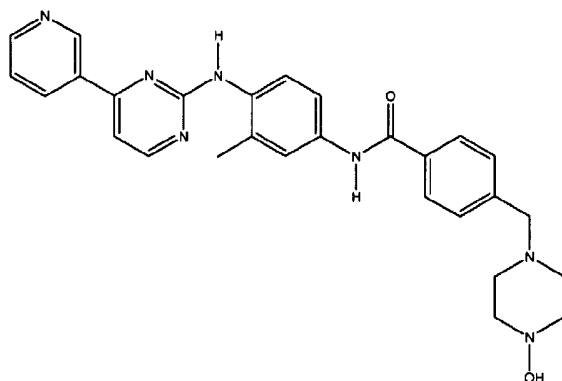
In another embodiment, R⁴ is (C=O)_rO_s(C₁-C₁₀)alkyl and R⁵ is (C₀-C₆)alkyl-NR^bR^c.

- 25 In another embodiment, R³ is 3-pyridinyl.

In another embodiment, r is 0, s is 0 and (C₁-C₁₀)alkyl is methyl.

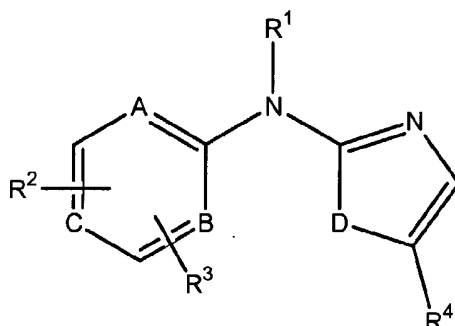
In another embodiment, (C₀-C₆)alkyl is a direct bond (C₀), R^b is H and R^c is (C=O)_rO_sheteroaryl or (C=O)_rO_sheterocycl.

In another embodiment, $(C=O)_rO_s$ heterocycyl is 4-hydroxy-1-piperazino, as illustrated below or a pharmaceutically acceptable salt thereof.



In another embodiment, the pharmacophore containing compound comprises a
5 compound of Figure II or a pharmaceutically acceptable salt thereof:

Figure II



A, B and C are independently CH, N or N^+-O^- ;

D is O, S or $N-R^5$;

10 R^1 is H, SO_2R^a , $(C=O)_rR^a$ or CO_2R^a ;

R^2 , R^3 and R^4 are independently H, OH, CHO, CN, halogen, $(C=O)_rO_s(C_1-C_{10})$ alkyl, $(C=O)_rO_s(C_2-C_{10})$ alkenyl, $(C=O)_rO_s(C_2-C_{10})$ alkynyl, $(C=O)_rO_s$ cycloalkyl, $(C=O)_rO_s$ cycloalkenyl, $(C=O)_rO_s$ cycloalkynyl, $(C=O)_rO_s$ heterocycyl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl, $(C=O)_rO_s$ perfluoroalkyl or (C_0-C_6) alkyl- NR^bR^c ,

15 wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycyl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R^6 ;

R^5 is H, aryl or (C_1-C_6) alkyl;

R^6 is $(C=O)_rO_sNR^aR^b$, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heterocycyl, halogen, OH, oxo, $(C=O)_rO_s(C_1-C_3)$ perfluoroalkyl, $(C=O)_rO_s(C_1-C_6)$ alkyl, CHO, CO_2H , CN, (C_0-C_6) alkyl-NR^bR^c or (C_1-C_6) alkyl-heterocycyl;

R^a is (C_1-C_6) alkyl, aryl or heterocycyl; and

R^b and R^c independently are H, $(C=O)_rO_s(C_1-C_{10})$ alkyl, SO_2R^a , $(C=O)_rO_s$ heterocycyl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl or CO_2R^a ,

wherein r and s independently are 0 or 1 and said alkyl, heterocycyl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R^6 .

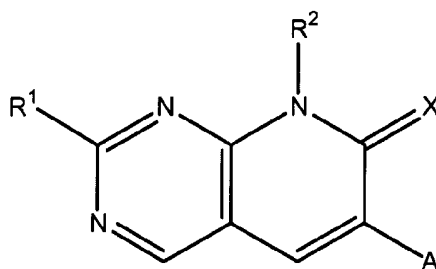
In another embodiment, R^1 is H.

In another embodiment, R^2 is H and R^3 is (C_0-C_6) alkyl-NR^bR^c.

In another embodiment, R^4 is heteroaryl.

In another embodiment, said pharmacophore containing compound, comprises a compound of Figure III or a pharmaceutically acceptable salt thereof:

Figure III



A is aryl or heteroaryl,

wherein said aryl or heteroaryl is optionally substituted with one or more substituents selected from R^3 ;

X is NH, N-acyl, O or S;

R^1 and R^2 are independently H, OH, CHO, CN, halogen, $(C=O)_rO_s(C_1-C_{10})$ alkyl, $(C=O)_rO_s(C_2-C_{10})$ alkenyl, $(C=O)_rO_s(C_2-C_{10})$ alkynyl, $(C=O)_rO_s$ cycloalkyl, $(C=O)_rO_s$ cycloalkenyl, $(C=O)_rO_s$ cycloalkynyl, $(C=O)_rO_s$ heterocycyl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl, $(C=O)_rO_s$ perfluoroalkyl or (C_0-C_6) alkyl-NR^aR^b,

wherein said alkykl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycyl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R^3 ;

R^3 is $(C=O)_rO_sNR^aR^b$, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heterocycyl, halogen, OH, oxo, $(C=O)_rO_s(C_1-C_3)$ perfluoroalkyl, $(C=O)_rO_s(C_1-C_6)$ alkyl, CHO, CO_2H , CN, (C_0-C_6) alkyl-NR^aR^b or (C_1-C_6) alkyl-heterocycyl;

R^a and R^b independently are H, $(C=O)_rO_s(C_1-C_{10})$ alkyl, SO_2R^1 , $(C=O)_rO_s$ heterocycl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl or CO_2R^1 ,
wherein r, s and t independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R^3 .

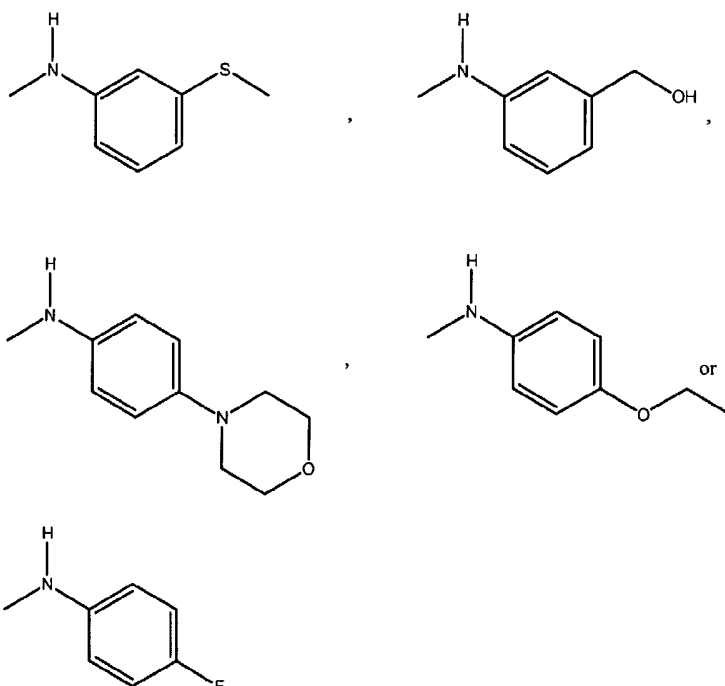
In another embodiment, A is aryl.

5 In another embodiment, aryl is 2,5-dichlorophenyl.

In another embodiment, R^1 is (C_0-C_6) alkyl- NR^aR^b and R^2 is $(C=O)_rO_s(C_1-C_{10})$ alkyl.

In another embodiment, $(C=O)_rO_s(C_1-C_{10})$ alkyl is methyl.

In another embodiment, (C_0-C_6) alkyl- NR^aR^b is



10 or a pharmaceutically acceptable salt thereof.

In another embodiment, the agent is administered orally.

In one embodiment, the compound is administered with a NSAID. In another embodiment, the NSAID is sulindac sulfide, flufenamic acid, ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen, meclofenamic acid.

15 In one embodiment, the compound is administered with an antioxidant. In a specific embodiment, the antioxidant is selected from the group consisting of vitamin E, vitamin C, curcumin, and Ginkgo biloba.

In another embodiment, the compound is administered with an acetylcholinesterase inhibitor.

20

3.1. TERMINOLOGY

As used herein, enzymatic activity of an enzyme, *e.g.*, γ -secretase, is "ATP-dependent" if (i) the activation state (level of activity) of the enzyme is modulated by the binding of ATP to the enzyme; (ii) the activity of the enzyme is modulated by the binding of ATP to a molecule in an enzyme complex that contains the enzyme; (iii) the activity of the enzyme is modulated by the binding of ATP to a molecule that effects the assembly, localization or stability of the enzyme complex; or (iv) the activity of the enzyme is modulated by the binding of ATP to a molecule that affects the activation state (level of activity) of the enzyme complex.

As used herein, the terms "modulate," "modulates," "modulated" or "modulation" shall have their usual meanings, and encompass the meanings of the words "enhance," "promote," "increase," "agonize," "inhibit," "decrease" or "antagonize."

As used herein, an "agonist" is any compound that acts directly or indirectly on a molecule to produce a pharmacological effect, while an "antagonist" is any compound that blocks the stimulation of a pharmacological effect.

As used herein, a "sufficient amount" of a compound, or "an amount of a compound sufficient to . . ." refers to an amount that contains at least the minimum amount necessary to achieve the intended result. Such an amount can routinely be determined by one of skill in the art based on data from studies using methods of analysis such as those disclosed herein. Such data may include, but not be limited to, results from IC₅₀ determinations, as discussed hereinbelow.

As used herein, the term "A β -related disorder" or an "A β disorder" is a disease (*e.g.*, Alzheimer's disease) or a condition (*e.g.*, senile dementia) that involves an aberration or dysregulation of A β levels. An A β -related disorder includes, but is not limited to Alzheimer's disease, Down's syndrome and inclusion body myositis.

As used herein, the terms "disorder" and "disease" are used interchangeably to refer to a condition in a subject.

As used herein, a "small organic molecule" is an organic compound (or organic compound complexed with an inorganic compound (*e.g.*, metal)) that generally has a molecular weight of less than about 3 kilodaltons, preferably less than about 1.5 kilodaltons. Preferably, the small organic molecule can be orally administered and/or cross the blood-brain barrier.

As used herein, the term "about" means within 10 to 15%, preferably within 5 to 10%.

As used herein, the term "ATP modulator," refers to a compound that modulates ATP function in a cell. For example, an ATP modulator can be a compound that binds to a molecule normally affected by ATP binding and, by binding to the molecule, modulates,

e.g., inhibits, ATP's usual effect on the molecule. Such an ATP modulator can bind the molecule at an ATP-binding site on the molecule or at some other site on the molecule. The ATP modulator can act by competing with ATP for binding to the molecule, *e.g.*, by competing for binding to an ATP-binding site. Preferably the ATP modulator is a selective ATP modulator.

5 As used herein, the terms "selective ATP modulator" or "selective ATP modulation" refers to a modulator (or modulation) that discriminates among the molecules that affect ATP function in the cell and only modulates a specific individual molecule, or class or subset of the molecules (a "modulatory profile"). For example, a compound may be assayed for activity in inhibiting a collection of various kinases, and is recognized as a
10 selective ATP modulator if it modulates ATP function in a related class of kinases. In one embodiment, a selective ATP modulator is one that exhibits a modulatory profile identical to or substantially similar to that exhibited by STI-571, a mesylate salt of STI-571, Compound 1 or Compound 2.

 As used herein, the terms "treat", "treatment" and "treating" refer to the
15 amelioration of a A β -related disorder that results from the administration of one or more compounds that lower A β levels exhibited by a cell or tissue. It also refers to the managing of the disorder, or the slowing and/or reversing of the progression of the disorder.

 Symptoms of A β -related disorders are well known to those of skill in the art. For example, symptoms of Alzheimer's disease are well known in the art and can include, *e.g.*,
20 memory loss, mild cognitive impairment, cognitive decline, severe cognitive impairment and personality changes that result in loss of functional ability, *e.g.*, over the course of a decade. In debilitated states, patients usually exhibit severe impairment, and retain only vegetative neurologic function. Symptoms of Alzheimer's disease can also include certain art-known neuropathological lesions, including intracellular neurofibrillary tangles and
25 extracellular parenchymal and cerebrovascular amyloid.

 As used herein, the terms "manage," "managing" and "management" refer to the beneficial effects that a subject derives from a compound, such as a compound that lowers A β levels exhibited by a cell or tissue, which does not result in a cure of the disease. In certain embodiments, a subject is administered one or more such agents to "manage" a
30 disorder so as to prevent or slow the progression or worsening of the disorder.

 As used herein, the terms "prevent", "preventing" and "prevention" refer to the impediment of the recurrence or onset of an A β -related disorder or one or more symptoms of a A β -related disorder in a subject.

 As used herein, a "protocol" includes dosing schedules and dosing regimens. The
35 protocols herein are methods of use and include prophylactic and therapeutic protocols.

As used herein, the terms "subject" and "patient" are used interchangeably. As used herein, the terms "subject" and "subjects" refer to an animal, preferably a mammal including a non-primate (*e.g.*, a cow, pig, horse, donkey, goat, camel, cat, dog, guinea pig, rat, mouse, sheep) and a primate (*e.g.*, a monkey, such as a cynomolgous monkey, gorilla, chimpanzee, and a human), preferably a human. In one embodiment, the subject is a
5 subject with Alzheimer's disease.

The term "pharmaceutically acceptable" as used herein means a composition, *e.g.*, a carrier, excipient, or salt, approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly, in humans.

10 4. DESCRIPTION OF THE FIGURES

FIG. 1 is a bar graph quantitating the effect of adding an ATP-regenerating system to a cell-free assay system for A β generation. The system is derived from mouse N2a cells
15 expressing both the Swedish variant of human APP and the presenilin-1 (PS1) mutation.

FIG. 2 is an autoradiogram showing that the β -secretase cleavage product, C99 (also known as β CTF), accumulates in an ATP-depleted cell free system.

FIG. 3 is a bar graph showing strong ATP-dependence of C99 cleavage to A β in isolated membranes.

20 FIG. 4 is a graph of the dose-response for reduction of A β levels in isolated membranes by Compound 1.

FIG. 5 is a mass spectrographic profile of peptides immunoprecipitated from the supernatant of N2a cells, expressing both the Swedish variant of human APP and the presenilin-1 (PS1) mutation, with anti-A β antibody 6E10 (Senetek, Napa, California),
25 showing decreased A β 40 ("A β 1-40") and A β 42 ("A β 1-42") in the presence of Compound 1. Y-axis, concentration of Compound 1 (M).

FIG. 6 is a graph of the dose-dependent inhibition by the mesylate salt of STI-571 (imatinib mesylate, GLEEVECTM, Novartis Pharmaceuticals; Calbiochem) of A β formation by intact N2a cells expressing the Swedish variant of human APP. The graph also
30 demonstrates that APP levels do not change in response to STI-571 administration.

FIG. 7 is a graph of the dose-dependent inhibition by Compound 1 of A β formation in intact N2a cells expressing the Swedish variant of human APP. The graph also demonstrates that APP levels do not change in response to STI-571 administration.

35 FIGS. 8A-B. FIG. 8A is a bar graph showing the reduction of A β produced from primary neuronal cells cultured from rat brain in response to Compound 1. FIG. 8B is an

autoradiogram showing the amount of APP produced (top panel) and the amount of A β produced at different concentrations of Compound 1 (bottom panel).

FIG. 9 is an autoradiogram illustrating consistent secretion of soluble APP- α ("APP α ") from primary neuronal cells cultured from rat brain in the presence of Compound 1.

5 FIG. 10 is a bar graph showing the reduction of A β produced from primary neuronal cells cultured from rat brain in response to the presence of Compound 2.

FIG. 11 is a bar graph showing the time course (in hours) of the effect of STI-571 (mesylate salt) on A β generation from primary neuronal cells cultured from rat brain.

10 FIG. 12 is a bar graph showing the time course of Compound 1 effect on A β generation from primary neuronal cells cultured from rat brain.

FIG. 13 is a bar graph showing the dose response to STI-571 on A β generation from primary neuronal cells cultured from rat brain.

15 FIG. 14 is a bar graph showing the dose response to Compound 1 on A β generation from primary neuronal cells cultured from rat brain. X-axis, DMSO control and Compound 1 concentration (M).

FIGS. 15A-D show the quantitation of STI-571 mesylate salt ("STI")-dependent decreases in A β 1-40(15A), A β 1-42 (15B) and increases in C99 (15C and 15D), *in vivo* in guinea pig. FIG. 15C also demonstrates that full-length β APP levels do not change in response to treatment. **, $p < 0.01$; *, $0.01 < p < 0.05$.

20 FIGS. 16A-D show the quantitation of Compound 1 ("CMPD1")-dependent decreases in A β 1-40(16A), A β 1-42 (16B) and increases in C99 (16C and 16D), *in vivo* in guinea pig. FIG. 16C also demonstrates that full-length β APP levels do not change in response to treatment. *, $p < 0.01$; *, $0.01 < p < 0.05$.

25 FIG. 17 is a graph and an autoradiogram showing stable levels of Notch cleavage in intact cells treated with STI-571 (mesylate salt). The figure presents results demonstrating that while STI-571 administration reduces A β levels in a dose-dependent manner, the level of Notch and Notch cleavage product (Notch intracellular domain, NICD) remains stable.

FIG. 18 is a graph showing stable levels of Notch cleavage in intact cells treated with Compound 1.

30 FIG. 19 is an autoradiogram illustrating that a standard γ -secretase inhibitor (" γ -secretase inhibitor"; Calbiochem) inhibits Notch cleavage in a cell-free system derived from m Δ e-Notch 1 transfected N2a cells, and that γ -secretase activity is therefore necessary for Notch cleavage in this system. The transfected cells were permeabilized and incubated either with or without a standard ATP energy regenerating system with GTP, and either in
35 the presence or absence of a standard γ -secretase inhibitor (" γ -secretase inhibitor,"

Calbiochem). γ -sec, γ -secretase. ERS, energy regenerating system. NICD, Notch intracellular domain.

FIG. 20 is a diagram of the chemical structures of ST-571, the mesylate salt GLEEVEC™, STI-571 variant ("WGB-BC-15"), Compound 1 (PD173955, Moasser *et al.*, 1999, Cancer Research 59: 6145–6152; Wisniewski *et al.*, 2002, 62(15):4244-55),
5 Compound 2 (PD166326; Wisniewski *et al.*, 2002, 62(15):4244-55), Compound 3 (PD173956; Bauer *et al.*, 2001, Thromb. Haemost. 85(2):331-40; Maschberger *et al.*, 2000, J. Biol. Chem. 275(25):19159-66), Compound 4 (PD173952; Dorsey *et al.*, 2002, Leukemia 16(9):1589-95), Compound 5 (PD-173958; Dorsey *et al.*, 2002, Leukemia 16(9):1589-95; Joseloff *et al.*, 2002, J. Biol. Chem. 277(14):12318-23).

10

5. DETAILED DESCRIPTION OF THE INVENTION

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections set forth below.

15

5.1. METHODS FOR MODULATING AMYLOID β PEPTIDE ($A\beta$) LEVELS

20

The invention provides a method for modulating amyloid- β peptide ($A\beta$) levels exhibited by a cell or tissue comprising contacting said cell or tissue with an amount of a compound sufficient to modulate said $A\beta$ levels, wherein said compound modulates an ATP-dependent enzymatic activity.

25

A cell or tissue may include, but not be limited to: an excitable cell, *e.g.*, a sensory neuron, motorneuron, or interneuron; a glial cell; a primary culture of cells, *e.g.*, a primary culture of neuronal or glial cells; cell(s) derived from a neuronal or glial cell line; dissociated cell(s); whole cell(s) or intact cell(s); permeabilized cell(s); a broken cell preparation; an isolated and/or purified cell preparation; a cellular extract or purified enzyme preparation; a tissue or organ, *e.g.*, brain, brain structure, brain slice, spinal cord, spinal cord slice, central nervous system, peripheral nervous system, or nerve; tissue slices, and a whole animal. In certain embodiments, the brain structure is cerebral cortex, the hippocampus, or their anatomical and/or functional counterparts in other mammalian species. In certain embodiments, the cell or tissue is an N2a cell, a primary neuronal culture or a hippocampal tissue explant.

30

In one embodiment, the $A\beta$ levels are lowered. In another embodiment, the $A\beta$ levels are raised. In another embodiment, the $A\beta$ is $A\beta$ 40. In another embodiment, the $A\beta$ is $A\beta$ 42.

35

In another embodiment, the modulating results in an increase in the ratio of $A\beta$ 40 to $A\beta$ 42. In another embodiment, the modulating results in an increase in C99.

In another embodiment, the compound binds an enzyme that exhibits an ATP-dependent enzymatic activity. In another embodiment, the compound binds a molecule that regulates the ATP-enzymatic activity.

In another embodiment, the molecule is a molecule that is allosterically regulated by ATP.

5 In another embodiment, the compound is an ATP modulator. In another embodiment, the ATP modulator is a selective modulator.

In another embodiment, the ATP modulator competes with ATP for binding to an ATP-binding site. In another embodiment, the ATP-binding site is present on an enzyme that exhibits an ATP-dependent enzymatic activity. In another embodiment, the ATP-
10 binding site is present on a molecule that regulates the ATP-dependent enzymatic activity.

Binding may be measured under any standard art-known physiological conditions, according to methods well known in the art.

In another embodiment, the compound does not affect total cellular levels of β -amyloid precursor product (APP). In another embodiment, the compound does not decrease
15 levels of secreted APP (sAPP). In another embodiment, the compound increases levels of sAPP α . In another embodiment, levels of secreted A β are modulated. In another embodiment, the compound does not inhibit Notch-1 cleavage. In another embodiment, the compound does not affect tau phosphorylation.

In another embodiment, the compound crosses the blood-brain barrier.

20 In another embodiment, the enzymatic activity is a γ -secretase activity. In another embodiment, the compound inhibits γ -secretase activity. In another embodiment, the compound is an ATP modulator. In another embodiment, the ATP modulator is a selective modulator.

In another embodiment, the compound binds a γ -secretase enzyme exhibiting an
25 ATP-dependent enzymatic activity. In another embodiment, the ATP modulator competes with ATP for binding to an ATP binding site.

In another embodiment, the compound is STI-571 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another
30 embodiment, the compound is a mesylate salt of STI-571. In another embodiment, the compound is WGB-BC-15 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is Compound 1 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is Compound 2 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In
35 another embodiment, the compound is Compound 3 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the

compound is Compound 4 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is Compound 5 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

5 In another embodiment, the enzymatic activity is a kinase activity. In another embodiment, the compound binds to a kinase enzyme exhibiting an ATP-dependent enzymatic activity. In another embodiment, the compound competes with ATP for binding to an ATP-binding site on the kinase. In another embodiment, the compound binds to a site other than an ATP-binding site on the kinase. In another embodiment, the kinase is a tyrosine kinase. In another embodiment, the tyrosine kinase is Abl kinase, BCR-Abl kinase, 10 ARG kinase, src kinase, c-kit or platelet-derived growth factor receptor. In another embodiment, the kinase is a serine/threonine kinase, a carbohydrate kinase or a lipid kinase.

In another aspect, the invention provides a method for modulating A β levels exhibited by a cell or tissue comprising contacting said cell or tissue with an amount of an ATP modulator sufficient to modulate said A β levels.

15 In one embodiment, the A β levels are lowered. In another embodiment, the A β levels are raised. In another embodiment, the A β is A β 40. In another embodiment, the A β is A β 42.

In another embodiment, the modulating results in an increase in the ratio of A β 40 to A β 42. In another embodiment, the modulating results in an increase in C99. In another 20 embodiment, the ATP modulator competes with ATP for binding to an ATP-binding site.

In another embodiment, the ATP-binding site is present on an enzyme that exhibits an ATP-dependent enzymatic activity. In another embodiment, the ATP-binding site is present on a molecule that regulates the ATP-dependent enzymatic activity. In another 25 embodiment, the compound does not affect total cellular levels of β -amyloid precursor product (APP). In another embodiment, the compound does not decrease levels of secreted APP (sAPP). In another embodiment, the compound increases levels of sAPP α .

In another embodiment, levels of secreted A β are modulated.

30 In another embodiment, the compound does not inhibit Notch-1 cleavage. In another embodiment, the compound does not affect tau phosphorylation. In another embodiment, the compound crosses the blood-brain barrier. In another embodiment, the enzymatic activity is a γ -secretase activity. In another embodiment, the compound inhibits γ -secretase activity.

35 In another embodiment, the compound is an ATP modulator. In another embodiment, the ATP modulator is a selective modulator. In another embodiment, the compound binds a γ -secretase enzyme exhibiting an ATP-dependent enzymatic activity.

In another embodiment, the compound is STI-571 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is a mesylate salt of STI-571. In another embodiment, the compound is WGB-BC-15 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is any of
5 Compounds 1-5 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

In another embodiment, the enzymatic activity is a kinase activity. In another embodiment, the compound binds to a kinase enzyme exhibiting an ATP-dependent enzymatic activity. In another embodiment, the compound competes with ATP for binding
10 to an ATP-binding site on the kinase. In another embodiment, the compound binds to a site other than an ATP-binding site on the kinase. In another embodiment, the kinase is a tyrosine kinase. In another embodiment, the tyrosine kinase is Abl kinase, BCR-Abl kinase, ARG kinase, src kinase, c-kit or platelet-derived growth factor receptor. In another embodiment, the kinase is a serine/threonine kinase, a carbohydrate kinase or a lipid kinase.
15 In another embodiment, the kinase is a serine/threonine kinase, a carbohydrate kinase or a lipid kinase.

In another embodiment, the compound competes with ATP for binding to a target other than γ -secretase in the metabolic pathway that regulates the cleavage of APP to A β by γ -secretase. In specific embodiments, the target is a kinase (or kinase domain), a protease
20 (*e.g.*, an AAA protease of the kind localized to the inner mitochondrial membrane), a phosphatase, or a molecular chaperone molecule (*e.g.*, hsp 60, hsp70, hsp90).

In certain embodiments, γ -secretase activity may reflect assembly, allosteric activation, or stabilization of a protein complex. In certain embodiments, the level of γ -secretase activity is a level of this protein complex, and the level of the protein complex can
25 be determined by measuring the levels of the component proteins in the complex by methods well known in the art, *e.g.*, by immunoblotting.

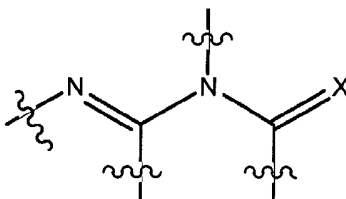
In another embodiment, the compound inhibits γ -secretase activity either by binding to a site on γ -secretase, or on a component protein of γ -secretase (as in a γ -secretase multimolecular complex that can comprise presenilin, nicastrin and APH1), that otherwise
30 can be occupied by ATP (where ATP-binding is necessary for kinase activity or for accelerating γ -secretase activity). According to the invention, such compounds antagonize ATP. Alternatively, in certain embodiments, these compounds may bind to a modulator of γ -secretase activity at an ATP binding site, wherein said modulator is a factor that is necessary for assembly, stability, trafficking or activation of γ -secretase activity and is
35 antagonized by binding of the compound.

In other embodiments, the level of A β is modulated via modulation of γ -secretase activity.

In one aspect, the invention provides a method for modulating A β levels in a cell or tissue comprising contacting said cell or tissue with an amount of a compound sufficient to modulate said A β levels, wherein said compound contains the following pharmacophore:

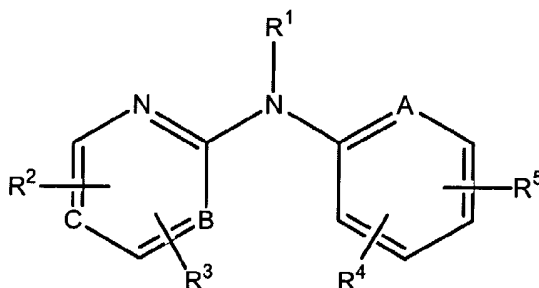
5

wherein X is CH-, O, NH or N-CO-.



In one embodiment, said pharmacophore containing compound comprises a compound of Figure I or a pharmaceutically acceptable salt thereof:

10 Figure I



A is CH or N;

B and C are independently CH, N or N⁺-O⁻;

R¹ is H, SO₂R^a, (C=O)_rO_sR^a;

15 R², R³, R⁴ and R⁵ are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl, (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl, (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^bR^c,

20 wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R⁶;

R⁶ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo, (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_s(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-NR^bR^c or (C₁-C₆)alkyl-heterocycl,

wherein said alkyl-heterocycl is optionally substituted with OH;

R^a is (C_1-C_6) alkyl, aryl or heterocycl; and

R^b and R^c independently are H, $(C=O)_rO_s(C_1-C_{10})$ alkyl, SO_2R^a , $(C=O)_rO_s$ heterocycl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl or CO_2R^a ,

wherein r and s independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is

5 optionally substituted with one or more substituents selected from R^6 .

In another embodiment, A is CH, B is N and C is CH.

In another embodiment, R^1 is H.

In another embodiment, R^2 is H and R^3 is heteroaryl.

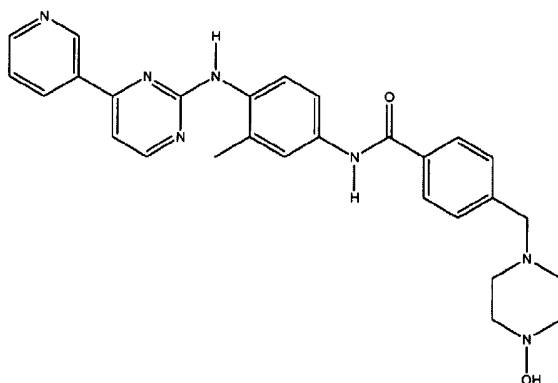
In another embodiment, R^4 is $(C=O)_rO_s(C_1-C_{10})$ alkyl and R^5 is (C_0-C_6) alkyl- NR^bR^c .

10 In another embodiment, R^3 is 3-pyridinyl.

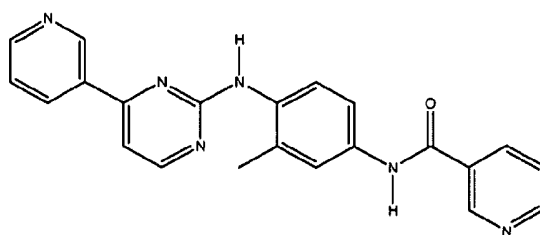
In another embodiment, r is 0, s is 0 and (C_1-C_{10}) alkyl is methyl.

In another embodiment, (C_0-C_6) alkyl is a direct bond (C_0), R^b is H and R^c is $(C=O)_rO_s$ heteroaryl or $(C=O)_rO_s$ heterocycl.

15 In another embodiment, $(C=O)_rO_s$ heterocycl is 4-hydroxy-1-piperazino, as illustrated below or a pharmaceutically acceptable salt thereof.

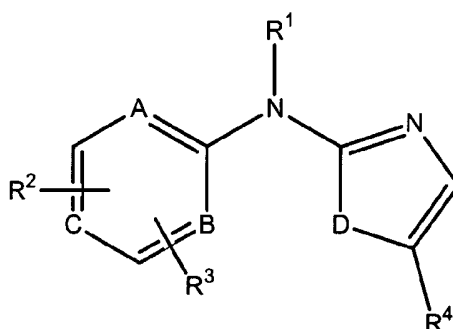


20 In another embodiment, $(C=O)_rO_s$ heteroaryl is 3-pyridinyl, as illustrated below or a pharmaceutically acceptable salt thereof.



- 5 In another embodiment, said pharmacophore containing compound, comprises a compound of Figure II or a pharmaceutically acceptable salt thereof:

Figure II



- A, B and C are independently CH, N or N⁺-O⁻;
 10 D is O, S or N-R⁵;
 R¹ is H, SO₂R^a, (C=O)_rR^a or CO₂R^a;
 R², R³ and R⁴ are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl,
 (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl,
 (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl,
 15 (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^bR^c,
 wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl,
 aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents
 selected from R⁶;
 R⁵ is H, aryl or (C₁-C₆)alkyl;

R^6 is $(C=O)_rO_sNR^aR^b$, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heterocycyl, halogen, OH, oxo, $(C=O)_rO_s(C_1-C_3)$ perfluoroalkyl, $(C=O)_rO_s(C_1-C_6)$ alkyl, CHO, CO_2H , CN, (C_0-C_6) alkyl- NR^bR^c or (C_1-C_6) alkyl-heterocycyl;

R^3 is (C_1-C_6) alkyl, aryl or heterocycyl; and

R^b and R^c independently are H, $(C=O)_rO_s(C_1-C_{10})$ alkyl, SO_2R^a , $(C=O)_rO_s$ heterocycyl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl or CO_2R^a ,

wherein r and s independently are 0 or 1 and said alkyl, heterocycyl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R^6 .

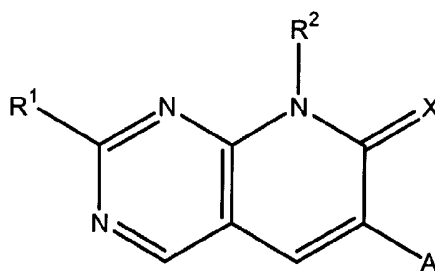
In another embodiment, R^1 is H.

In another embodiment, R^2 is H and R^3 is (C_0-C_6) alkyl- NR^bR^c .

In another embodiment, R^4 is heteroaryl.

In another embodiment, said pharmacophore containing compound, comprises a compound of Figure III or a pharmaceutically acceptable salt thereof:

Figure III



15

A is aryl or heteroaryl,

wherein said aryl or heteroaryl is optionally substituted with one or more substituents selected from R^3 ;

X is NH, N-acyl, O or S;

R^1 and R^2 are independently H, OH, CHO, CN, halogen, $(C=O)_rO_s(C_1-C_{10})$ alkyl, $(C=O)_rO_s(C_2-C_{10})$ alkenyl, $(C=O)_rO_s(C_2-C_{10})$ alkynyl, $(C=O)_rO_s$ cycloalkyl, $(C=O)_rO_s$ cycloalkenyl, $(C=O)_rO_s$ cycloalkynyl, $(C=O)_rO_s$ heterocycyl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl, $(C=O)_rO_s$ perfluoroalkyl or (C_0-C_6) alkyl- NR^aR^b ,

wherein said alkykl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycyl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R^3 ;

R^3 is $(C=O)_rO_sNR^aR^b$, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heterocycyl, halogen, OH, oxo, $(C=O)_rO_s(C_1-C_3)$ perfluoroalkyl, $(C=O)_rO_sS_t(C_1-C_6)$ alkyl, CHO, CO_2H , CN, (C_0-C_6) alkyl- NR^aR^b or (C_1-C_6) alkyl-heterocycyl;

R^a and R^b independently are H, $(C=O)_rO_s(C_1-C_{10})$ alkyl, SO_2R^1 , $(C=O)_rO_s$ heterocycl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl or CO_2R^1 ,
 wherein r, s and t independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R^3 .

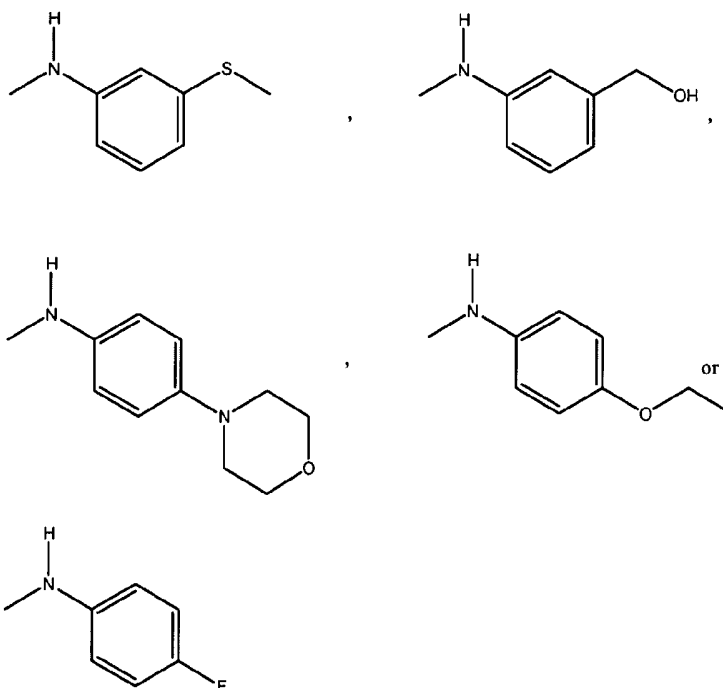
In another embodiment, A is aryl.

5 In another embodiment, the aryl is 2,5-dichlorophenyl.

In another embodiment, R^1 is (C_0-C_6) alkyl- NR^aR^b and R^2 is $(C=O)_rO_s(C_1-C_{10})$ alkyl.

In another embodiment, $(C=O)_rO_s(C_1-C_{10})$ alkyl is methyl.

In another embodiment, (C_0-C_6) alkyl- NR^aR^b is



10 or a pharmaceutically acceptable salt thereof.

In another aspect, the invention provides a pharmaceutical composition useful for modulating $A\beta$ level in a cell or tissue, comprising:

an amount of the compound described hereinabove sufficient to modulate said $A\beta$ level; and

15 a pharmaceutically acceptable excipient or carrier.

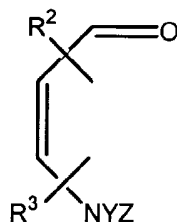
In one embodiment, the pharmaceutical composition is useful for modulating $A\beta$ levels exhibited by a cell or tissue.

In another embodiment, the compound is present in an amount sufficient to modulate said $A\beta$ levels.

Compounds according to Figure I may be prepared by various processes known to one of skill in the art. In particular, compounds according to Figure I, wherein A is CH, B is N, C is CH and R¹ is H may be prepared by the following process:

a) a compound according to Figure IV

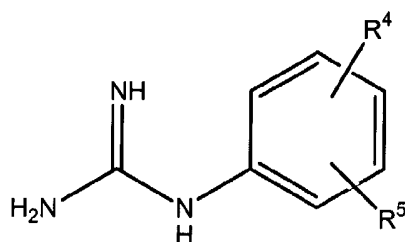
Figure IV



5

wherein Y and Z constitute (C₁-C₆)alkyl groups and R² and R³ are as above-defined is reacted with a compound according to Figure V

Figure V



10 wherein R⁴ and R⁵ are as above-defined.

In certain embodiments, the compound is a compound identified by the methods of the invention, wherein the compound modulates γ -secretase activity, *e.g.*, ATP-dependent γ -secretase activity, and wherein modulation of γ -secretase activity results in an alteration in the level of A β exhibited by a cell or tissue. γ -secretase activity is well known in the art, and includes cleavage of APP at the γ -secretase cleavage region.

15

In one embodiment, a method is provided for modulating γ -secretase activity in cells or tissues of interest *in vitro*.

In another embodiment, γ -secretase activity in cells or tissues of interest is modulated *in situ* or *in vivo*. The *in vitro*, *in situ* and *in vivo* applications may include, but are not limited to modulating activity in any of the cells or tissues disclosed hereinabove.

20

According to the invention, a substance "exhibited by a cell or tissue" encompasses a substance that it produced, sequestered, taken up or released by, the cell or tissue and that either remains within the cell (or tissue), *i.e.*, intracellularly, or is secreted or released by the cell (or tissue) (extracellularly).

5.1.1. THERAPEUTIC METHODS

The present invention provides methods for prevention, treatment, *e.g.*, management, of an A β -related disorder, or amelioration of a symptom of an A β -related disorder such as Alzheimer's disease. It is understood that the methods described herein in the context of treating and/or ameliorating a symptom can also routinely be utilized as part of a prevention protocol.

The invention also provides a method of treating, or ameliorating a symptom of, an A β -related disorder comprising administering to a subject in need of such treating or ameliorating an amount of a compound sufficient to reduce A β levels in the subject, wherein the compound modulates an ATP-dependent enzymatic activity, such that the A β -related disorder is treated or a symptom of the A β related disorder is ameliorated.

In one embodiment, the A β related disorder is Alzheimer's disease. In another embodiment, progression of the A β related disorder is slowed. In another embodiment, progression of the A β related disorder is reversed.

In another embodiment, the subject is a human subject. In a specific embodiment, the subject is a subject at risk for a familial form of Alzheimer's disease.

In another embodiment, the ATP-dependent enzymatic activity is lowered. In another embodiment, the compound crosses the blood-brain barrier. In another embodiment, the compound is administered orally. As used herein, this can mean either concurrent or sequential administration.

In another embodiment, the compound is administered with a NSAID. In a specific embodiment, the NSAID is sulindac sulfide, flufenamic acid, ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen, meclufenamic acid.

In another embodiment, the compound is administered with an antioxidant. In a specific embodiment, the antioxidant is selected from the group consisting of vitamin E, vitamin C, curcumin, and Gingko biloba.

In another embodiment, the compound is administered with an acetylcholinesterase inhibitor.

In another embodiment, the compound is an ATP modulator. In another embodiment, the compound is ATP modulator is a selective modulator.

In another embodiment, the enzymatic activity is a γ -secretase activity. In another embodiment, the compound binds a γ -secretase enzyme exhibiting an ATP-dependent enzymatic activity.

In another embodiment, the ATP modulator competes with ATP for binding to an ATP binding site.

In one embodiment, the compound is STI-571 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the

compound is a mesylate salt of STI-571. In another embodiment, the compound is WGB-BC-15 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is any of Compounds 1-5 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

5 In one embodiment, the enzymatic activity is a kinase activity. In one embodiment, the compound binds to a kinase enzyme exhibiting an ATP-dependent enzymatic activity. In one embodiment, the compound competes with ATP for binding to an ATP-binding site on the kinase. In another embodiment, the compound binds to a site other than an ATP-binding site on the kinase.

10 In a specific embodiment, the kinase is a tyrosine kinase. In another embodiment, the tyrosine kinase is Abl kinase, BCR-Abl kinase, ARG kinase, src kinase, c-kit, platelet-derived growth factor receptor.

In a specific embodiment, the kinase is a serine/threonine kinase, a carbohydrate kinase or a lipid kinase.

15 In another embodiment, the enzymatic activity is activity of a protease (*e.g.*, AAA protease of the kind localized to the inner mitochondrial membrane), phosphatase, or a molecular chaperone molecule (*e.g.*, hsp 60, hsp70, hsp90).

20 The invention also provides a method of treating, or ameliorating a symptom of, an A β -related disorder comprising administering to a subject in need of such treating or ameliorating an amount of an ATP modulator sufficient to reduce A β levels in the subject, such that the A β -related disorder is treated or a symptom of the A β related disorder is ameliorated.

In one embodiment, the A β related disorder is Alzheimer's disease. In one embodiment, the subject is a human subject.

25 In one embodiment, the subject is a subject at risk for a familial form of Alzheimer's disease.

In another embodiment, the ATP modulator crosses the blood-brain barrier.

30 In another embodiment, the ATP modulator is administered orally. In another embodiment, the ATP modulator is administered with a NSAID. In another embodiment, the NSAID is sulindac sulfide, flufenamic acid, ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen, meclofenamic acid.

In another embodiment, the ATP modulator is administered with an antioxidant. In another embodiment, the antioxidant is selected from the group consisting of vitamin E, vitamin C, curcumin, and Ginkgo biloba.

35 In another embodiment, the ATP modulator is administered with an acetylcholinesterase inhibitor.

In another embodiment, the ATP modulator is a selective modulator.

In another embodiment, the enzymatic activity is a γ -secretase activity.

In one embodiment, the compound binds a γ -secretase enzyme exhibiting an ATP-dependent enzymatic activity. In another embodiment, the ATP modulator competes with ATP for binding to an ATP binding site.

In one embodiment, the compound is STI-571 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is a mesylate salt of STI-571. In another embodiment, the compound is WGB-BC-15 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is any of Compounds 1-5 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

In one embodiment, the enzymatic activity is a kinase activity. In one embodiment, the compound binds to a kinase enzyme exhibiting an ATP-dependent enzymatic activity. In one embodiment, the compound competes with ATP for binding to an ATP-binding site on the kinase. In another embodiment, the compound binds to a site other than an ATP-binding site on the kinase.

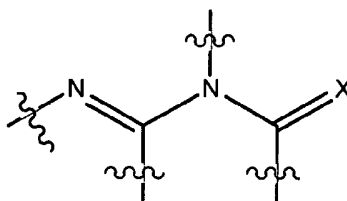
In a specific embodiment, the kinase is a tyrosine kinase. In another embodiment, the tyrosine kinase is Abl kinase, BCR-Abl kinase, ARG kinase, src kinase, c-kit, platelet-derived growth factor receptor.

In a specific embodiment, the kinase is a serine/threonine kinase, a carbohydrate kinase or a lipid kinase.

In another embodiment, the enzymatic activity is activity of a protease (*e.g.*, AAA protease of the kind localized to the inner mitochondrial membrane), phosphatase, or a molecular chaperone molecule (*e.g.*, hsp 60, hsp70, hsp90).

The invention also provides a method of treating, or ameliorating a symptom of, an A β -related disorder comprising administering to a subject in need of such treating or ameliorating an amount of a compound sufficient to reduce A β levels in the subject, such that the A β -related disorder is treated or a symptom of the A β -related disorder is ameliorated, wherein the compound contains the following pharmacophore:

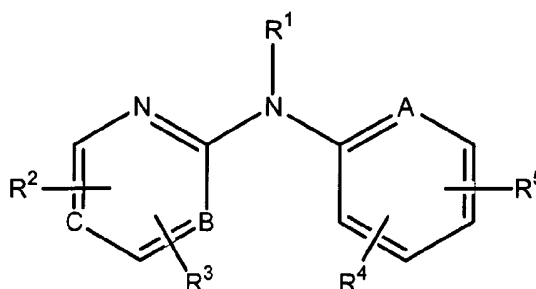
wherein X is CH-, O, NH or N-CO-.



30

In one embodiment, said pharmacophore containing compound comprises a compound of Figure I or a pharmaceutically acceptable salt thereof:

Figure I



A is CH or N;

5 B and C are independently CH, N or N⁺-O⁻;

R¹ is H, SO₂R^a, (C=O)_rO_sR^a;

R², R³, R⁴ and R⁵ are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl, (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl, (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^bR^c,
 10 wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R⁶;

R⁶ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo,
 15 (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_s(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-NR^bR^c or (C₁-C₆)alkyl-heterocycl,

wherein said alkyl-heterocycl is optionally substituted with OH;

R^a is (C₁-C₆)alkyl, aryl or heterocycl; and

R^b and R^c independently are H, (C=O)_rO_s(C₁-C₁₀)alkyl, SO₂R^a, (C=O)_rO_sheterocycl,
 20 (C=O)_rO_saryl, (C=O)_rO_sheteroaryl or CO₂R^a,

wherein r and s independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R⁶.

In another embodiment, A is CH, B is N and C is CH.

In another embodiment, R¹ is H.

25 In another embodiment, R² is H and R³ is heteroaryl.

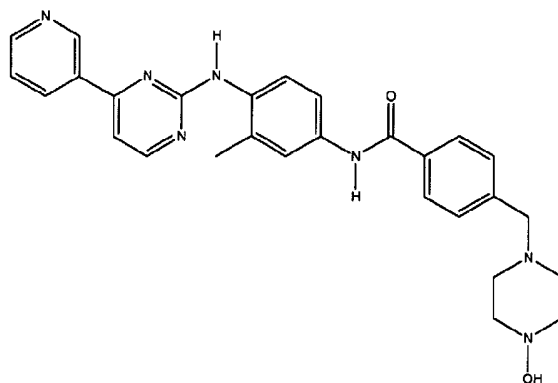
In another embodiment, R⁴ is (C=O)_rO_s(C₁-C₁₀)alkyl and R⁵ is (C₀-C₆)alkyl-NR^bR^c.

In another embodiment, R³ is 3-pyridinyl.

In another embodiment, r is 0, s is 0 and (C₁-C₁₀)alkyl is methyl.

In another embodiment, (C_0-C_6) alkyl is a direct bond (C_0), R^b is H and R^c is $(C=O)_rO_s$ heteroaryl or $(C=O)_rO_s$ heterocycl.

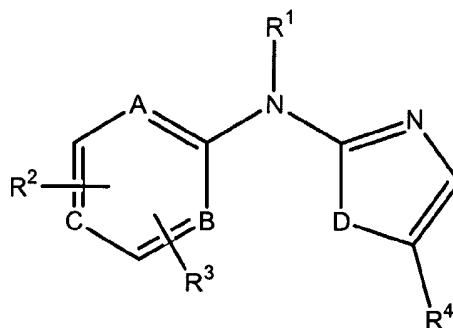
In another embodiment, $(C=O)_rO_s$ heterocycl is 4-hydroxy-1-piperazino, as illustrated below or a pharmaceutically acceptable salt thereof.



5

In another embodiment, the pharmacophore containing compound comprises a compound of Figure II or a pharmaceutically acceptable salt thereof:

Figure II



- 10 A, B and C are independently CH, N or N^+-O^- ;
 D is O, S or $N-R^5$;
 R^1 is H, SO_2R^a , $(C=O)_rR^a$ or CO_2R^a ;
 R^2 , R^3 and R^4 are independently H, OH, CHO, CN, halogen, $(C=O)_rO_s(C_1-C_{10})$ alkyl,
 $(C=O)_rO_s(C_2-C_{10})$ alkenyl, $(C=O)_rO_s(C_2-C_{10})$ alkynyl, $(C=O)_rO_s$ cycloalkyl,
 15 $(C=O)_rO_s$ cycloalkenyl, $(C=O)_rO_s$ cycloalkynyl, $(C=O)_rO_s$ heterocycl, $(C=O)_rO_s$ aryl,
 $(C=O)_rO_s$ heteroaryl, $(C=O)_rO_s$ perfluoroalkyl or (C_0-C_6) alkyl- NR^bR^c ,

wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R⁶;

R⁵ is H, aryl or (C₁-C₆)alkyl;

5 R⁶ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo, (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_s(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-NR^bR^c or (C₁-C₆)alkyl-heterocycl;

R^a is (C₁-C₆)alkyl, aryl or heterocycl; and

10 R^b and R^c independently are H, (C=O)_rO_s(C₁-C₁₀)alkyl, SO₂R^a, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl or CO₂R^a, wherein r and s independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R⁶.

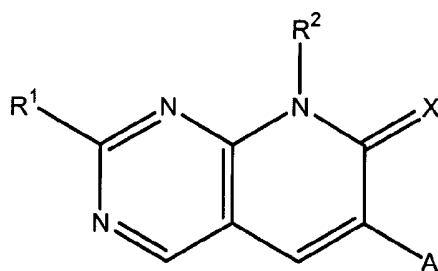
In another embodiment, R¹ is H.

In another embodiment, R² is H and R³ is (C₀-C₆)alkyl-NR^bR^c.

In another embodiment, R⁴ is heteroaryl.

15 In another embodiment, said pharmacophore containing compound, comprises a compound of Figure III or a pharmaceutically acceptable salt thereof:

Figure III



A is aryl or heteroaryl,

20 wherein said aryl or heteroaryl is optionally substituted with one or more substituents selected from R³;

X is NH, N-acyl, O or S;

25 R¹ and R² are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl, (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl, (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^aR^b, wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R³;

R^3 is $(C=O)_rO_sNR^aR^b$, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heterocycl, halogen, OH, oxo, $(C=O)_rO_s(C_1-C_3)$ perfluoroalkyl, $(C=O)_rO_sS_t(C_1-C_6)$ alkyl, CHO, CO_2H , CN, (C_0-C_6) alkyl- NR^aR^b or (C_1-C_6) alkyl-heterocycl;

R^a and R^b independently are H, $(C=O)_rO_s(C_1-C_{10})$ alkyl, SO_2R^1 , $(C=O)_rO_s$ heterocycl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl or CO_2R^1 ,

5 wherein r , s and t independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R^3 .

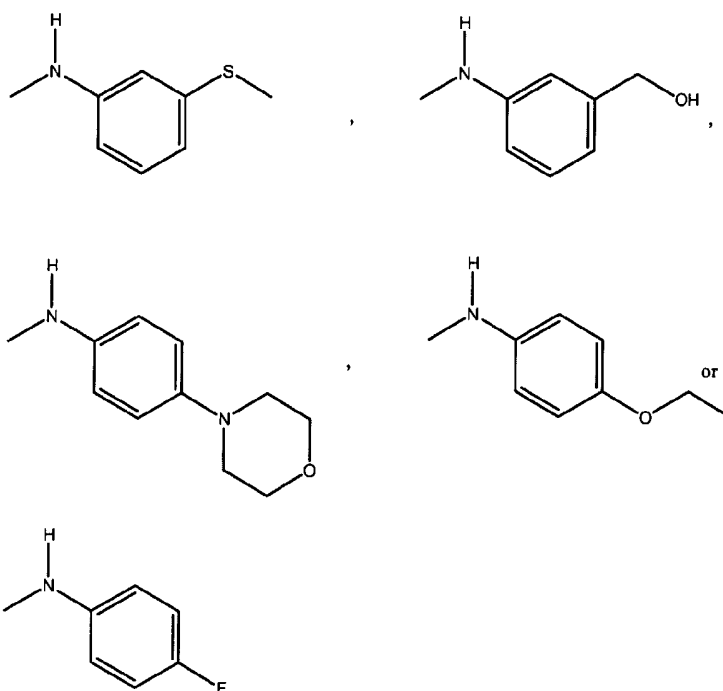
In another embodiment, A is aryl.

In another embodiment, aryl is 2,5-dichlorophenyl.

In another embodiment, R^1 is (C_0-C_6) alkyl- NR^aR^b and R^2 is $(C=O)_rO_s(C_1-C_{10})$ alkyl.

10 In another embodiment, $(C=O)_rO_s(C_1-C_{10})$ alkyl is methyl.

In another embodiment, (C_0-C_6) alkyl- NR^aR^b is



or a pharmaceutically acceptable salt thereof.

In another embodiment, the agent is administered orally.

15 In one embodiment, the compound is administered with a NSAID. In another embodiment, the NSAID is sulindac sulfide, flufenamic acid, ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen, meclofenamic acid.

In one embodiment, the compound is administered with an antioxidant. In a specific embodiment, the antioxidant is selected from the group consisting of vitamin E, vitamin C, curcumin, and Ginkgo biloba.

20

In another embodiment, the compound is administered with an acetylcholinesterase inhibitor.

5.1.2. MODULATING COMPOUNDS

5 5.1.2.1. COMPOUNDS THAT MODULATE A β LEVELS

The present invention also provides compositions for modulating A β levels exhibited by a cell or tissue, including, but not limited to the following compounds (or agents) disclosed hereinbelow. The invention also provides compositions for modulating the level A β via modulation of the activity of γ -secretase, including, but not limited to the following agents, drugs, compounds or small molecules disclosed hereinbelow.

In one embodiment, the invention provides a composition for modulating A β level exhibited by a cell or tissue comprising a modulator of ATP-dependent γ -secretase activity and a pharmaceutically acceptable vehicle. In one aspect of this embodiment, the A β level is lowered. In another aspect, the A β level is raised. In another aspect, the A β is A β 40. In another aspect, the A β is A β 42. In another aspect, the modulator binds to a kinase. In another aspect, the modulator competes with ATP for binding to the ATP-binding site on a kinase. In another aspect, the compound binds to a site other than an ATP-binding site on the kinase. In another aspect, the compound is an ATP modulator. In another aspect, the kinase may be, *e.g.*, Abl kinase, ARG kinase, BCR-Abl kinase, src kinase, c-kit or platelet-derived growth factor receptor. In another aspect, the modulator does not modulate Abl kinase activity. In another aspect, the modulator is an inhibitor of ATP-dependent enzymatic activity. In another aspect, the modulator does not inhibit Notch-1 cleavage. In another aspect, the modulator can cross the blood-brain barrier. In another aspect, the inhibitor is a selective inhibitor. In another aspect, the selective inhibitor is STI-571, WGB-BC-15, Compound 1, Compound 2, Compound 3, Compound 4, Compound 5 or a blood-brain barrier permeable variant thereof.

Among the preferred compounds are those that are ATP modulators.

In certain embodiments, the ATP modulator, such as an ATP inhibitor, may include, but not be limited to, a phenylaminopyrimidine tyrosine kinase inhibitor, *e.g.*, 2-phenylaminopyrimidine, a pyrimidinyl pyridione tyrosine kinase inhibitor, 2-(Purin-9-yl)-tetrahydrofuran-3,4-diol derivatives; pyridoxine and pyridoxal analogues; N-6 heterocyclic 8-modified adenosine derivatives; N-6 heterocyclic 5'-modified adenosine derivatives; allosteric inhibitors of pyruvate kinase; 8-phenylxanthines, 8-cycloalkylxantines or 8-substituted xanthine derivatives; N-6 substituted adenosine-5'-uronamides; purine, pyrrolo[2,3,d]pyrimidine and pyrazolo[3,4,d]pyrimidine nucleoside analogs.

In another embodiment, the compound, *e.g.*, ATP modulator, may include, but not be limited to, the following compounds:

Heterocyclic-hydroxyimino-fluorene nuclei compounds; 3-anilinomethylene oxindoles; 3-(4'-bromobenzylidenyl)-2-indolinone analogues; indeno[1,2,c]-naphthol[1,2,c] and benzo[6,7]cyclohepta[1,2,c]pyrazole derivatives; 3'-epimeric k-252a derivatives; quinazolines; 3-cyano-[1,7], [1,5] and [1,8]-naphthyridine analogues; N-[4-(3-chloro-4-fluoro-phenylamino)-7-(3-morpholin-4-yl-propoxy)-quinazolin-6-yl]-acrylamide; pyrimidine derivatives; benzimidazoles; bicyclic heteroaromatic compounds; pyrrolopyrimidines; quinoline and quinoxaline derivatives; indolinones; 2-pyrimidineamine derivatives; substituted pyrido[3,2,d]pyrimidines; fused polycyclic 2-aminopyrimidine derivatives; bicyclic 4-alkylaminopyrimidine derivatives; N-7-heterocycl pyrrolo[2,3,d]pyrimidines; 3-cyano quinoline derivatives; pyrazole derivatives; pyrimido[5,4,d]pyrimidines; 4-anilinoquinazoline derivatives; 6-aryl naphthyridines; N-oxides of amino containing pyrido[2,3,d]pyrimidines; 5-aminopyrazoles; 5,10-dihydropyrimido[4,5,b]quinolin-4(1H)-one; quinolymethylen-oxindole analogues; acrylonitrile-sulfonamide derivatives; 3-(4'-dimethylaminobenzylidenyl)-2-indolines; 3-(2'-alkoxybenzylidenyl)-2-indolines; 3-(4'-bromobenzylidenyl)-2-indolines; benzylidene-Z-indoline compounds; 4,6-dianilino-pyrimidine derivatives; substituted indolylmethylene-oxindole analogues; hydrosoluble 3-arylidene-2-oxindole analogues; 3-(2'-halobenzylidenyl)-2-indolinone compounds; 3-heteroaryl-2-indolinone compounds; benzoylethylene derivatives; urea and thiourea-type compounds; benzopyran derivatives; pyrido[2,3,d]pyrimidines; 6-aryl-pyrido[2,3,d]pyrimidines and naphthyridines; substituted 3-arylidene-7-azaoxindole compounds; thienyl compounds; aryl and heteroaryl quinazoline compounds; arylidene and heteroarylidene oxindole derivatives; N-substituted-beta-aryl- and beta-heteroaryl-alpha-cyanoacrylamide derivatives; 2-iminochromene derivatives; 4-aminopyrrolo[2,3,d]pyrimidines; 4-aminopyrazolo(3,4,d)pyrimidine derivatives; 4-aminopyrazolo(3,4,d)pyridine derivatives; 3-(cycloalkanoheteroarylidenyl)-2-indolinones; isoxazole-4-carboxamide compounds; 3-(cycloalkanoheteroarylidenyl)-2-indolinones; substituted phenylacrylonitrile compounds; benzylidene-Z-indoline compounds; 3-(2'-halobenzylidenyl)-2-indolinone compounds; benzopyran compounds; or 4-aminopyrimidines; isoxazole compounds. Such compounds can, for example, be utilized as kinase modulators, *e.g.*, inhibitors.

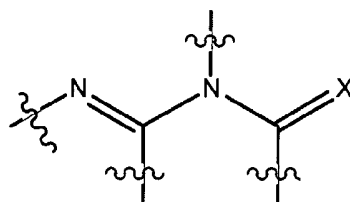
Appropriate compounds can also include, for example, STI-571, mesylate salt of STI-571 (imatinib mesylate, GLEEVEC™, Novartis Pharmaceuticals), and congeners thereof, members of the 2-phenylaminopyrimidine class of compounds, such as Compounds 1-5, as well as compounds that compete for the ATP binding sites of Bcr-Abl, Abl kinase, or c-kit, platelet-derived growth factor receptors (PDGFR), and on any other kinases known

or later shown to be inhibited by STI-571, or a related compound, such as the pyridone tyrosine kinase inhibitor class, would be candidates for use in the present invention.

In another embodiment, agents of the invention inhibit γ -secretase activity either by binding to a site on γ -secretase, or on a component protein of γ -secretase.

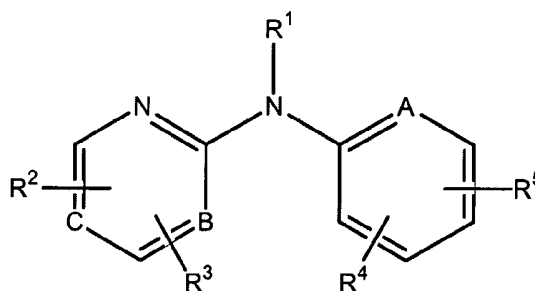
5 Still further, compounds that can be used as part of the methods of the invention include, but are not limited to, compounds that contain the following pharmacophore:

wherein X is CH-, O, NH or N-CO-.



10 In one embodiment, said pharmacophore containing compound comprises a compound of Figure I or a pharmaceutically acceptable salt thereof:

Figure I



A is CH or N;

B and C are independently CH, N or N⁺-O⁻;

15 R¹ is H, SO₂R^a, (C=O)_rO_sR^a;

R², R³, R⁴ and R⁵ are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl, (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl, (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycyl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^bR^c,

20 wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycyl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R⁶;

R^6 is $(C=O)_rO_sNR^aR^b$, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heterocycl, halogen, OH, oxo, $(C=O)_rO_s(C_1-C_3)$ perfluoroalkyl, $(C=O)_rO_s(C_1-C_6)$ alkyl, CHO, CO₂H, CN, (C_0-C_6) alkyl-NR^bR^c or (C_1-C_6) alkyl-heterocycl,

wherein said alkyl-heterocycl is optionally substituted with OH;

R^a is (C_1-C_6) alkyl, aryl or heterocycl; and

5 R^b and R^c independently are H, $(C=O)_rO_s(C_1-C_{10})$ alkyl, SO₂R^a, $(C=O)_rO_s$ heterocycl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl or CO₂R^a,

wherein r and s independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R⁶.

In another embodiment, A is CH, B is N and C is CH.

10 In another embodiment, R¹ is H.

In another embodiment, R² is H and R³ is heteroaryl.

In another embodiment, R⁴ is $(C=O)_rO_s(C_1-C_{10})$ alkyl and R⁵ is (C_0-C_6) alkyl-NR^bR^c.

In another embodiment, R³ is 3-pyridinyl.

In another embodiment, r is 0, s is 0 and (C_1-C_{10}) alkyl is methyl.

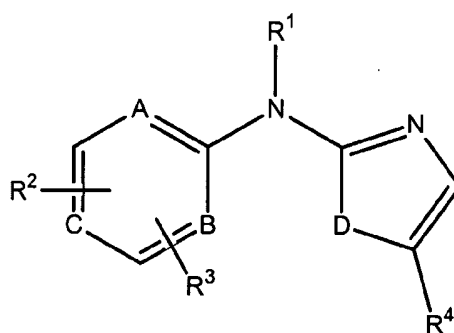
15 In another embodiment, (C_0-C_6) alkyl is a direct bond (C₀), R^b is H and R^c is $(C=O)_rO_s$ heteroaryl or $(C=O)_rO_s$ heterocycl.

In another embodiment, $(C=O)_rO_s$ heterocycl is 4-hydroxy-1-piperazino, as illustrated below or a pharmaceutically acceptable salt thereof.

20 In another embodiment, $(C=O)_rO_s$ heteroaryl is 3-pyridinyl, as illustrated below or a pharmaceutically acceptable salt thereof.

In another embodiment, said pharmacophore containing compound, comprises a compound of Figure II or a pharmaceutically acceptable salt thereof:

Figure II



25 A, B and C are independently CH, N or N⁺-O⁻;

D is O, S or N-R⁵;

R¹ is H, SO₂R^a, $(C=O)_rR^a$ or CO₂R^a;

R^2 , R^3 and R^4 are independently H, OH, CHO, $\overset{..}{\text{CN}}$, halogen, $(\text{C}=\text{O})_r\text{O}_s(\text{C}_1-\text{C}_{10})$ alkyl, $(\text{C}=\text{O})_r\text{O}_s(\text{C}_2-\text{C}_{10})$ alkenyl, $(\text{C}=\text{O})_r\text{O}_s(\text{C}_2-\text{C}_{10})$ alkynyl, $(\text{C}=\text{O})_r\text{O}_s$ cycloalkyl, $(\text{C}=\text{O})_r\text{O}_s$ cycloalkenyl, $(\text{C}=\text{O})_r\text{O}_s$ cycloalkynyl, $(\text{C}=\text{O})_r\text{O}_s$ heterocycl, $(\text{C}=\text{O})_r\text{O}_s$ aryl, $(\text{C}=\text{O})_r\text{O}_s$ heteroaryl, $(\text{C}=\text{O})_r\text{O}_s$ perfluoroalkyl or (C_0-C_6) alkyl- NR^bR^c ,

wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R^6 ;

R^3 is H, aryl or (C_1-C_6) alkyl;

R^6 is $(\text{C}=\text{O})_r\text{O}_s\text{NR}^a\text{R}^b$, $(\text{C}=\text{O})_r\text{O}_s$ aryl, $(\text{C}=\text{O})_r\text{O}_s$ heterocycl, halogen, OH, oxo, $(\text{C}=\text{O})_r\text{O}_s(\text{C}_1-\text{C}_3)$ perfluoroalkyl, $(\text{C}=\text{O})_r\text{O}_s(\text{C}_1-\text{C}_6)$ alkyl, CHO, CO_2H , CN, (C_0-C_6) alkyl- NR^bR^c or (C_1-C_6) alkyl-heterocycl;

R^a is (C_1-C_6) alkyl, aryl or heterocycl; and

R^b and R^c independently are H, $(\text{C}=\text{O})_r\text{O}_s(\text{C}_1-\text{C}_{10})$ alkyl, SO_2R^a , $(\text{C}=\text{O})_r\text{O}_s$ heterocycl, $(\text{C}=\text{O})_r\text{O}_s$ aryl, $(\text{C}=\text{O})_r\text{O}_s$ heteroaryl or CO_2R^a ,

wherein r and s independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R^6 .

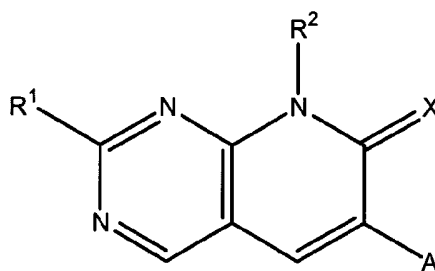
In another embodiment, R^1 is H.

In another embodiment, R^2 is H and R^3 is (C_0-C_6) alkyl- NR^bR^c .

In another embodiment, R^4 is heteroaryl.

In another embodiment, said pharmacophore containing compound, comprises a compound of Figure III or a pharmaceutically acceptable salt thereof:

Figure III



A is aryl or heteroaryl,

wherein said aryl or heteroaryl is optionally substituted with one or more substituents selected from R^3 ;

X is NH, N-acyl, O or S;

R^1 and R^2 are independently H, OH, CHO, CN, halogen, $(\text{C}=\text{O})_r\text{O}_s(\text{C}_1-\text{C}_{10})$ alkyl, $(\text{C}=\text{O})_r\text{O}_s(\text{C}_2-\text{C}_{10})$ alkenyl, $(\text{C}=\text{O})_r\text{O}_s(\text{C}_2-\text{C}_{10})$ alkynyl, $(\text{C}=\text{O})_r\text{O}_s$ cycloalkyl,

(C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl,
 (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^aR^b,

wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl,
 aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents
 selected from R³;

5 R³ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo,
 (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_sS_t(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-
 NR^aR^b or (C₁-C₆)alkyl-heterocycl;

R^a and R^b independently are H, (C=O)_rO_s(C₁-C₁₀)alkyl, SO₂R¹, (C=O)_rO_sheterocycl,
 (C=O)_rO_saryl, (C=O)_rO_sheteroaryl or CO₂R¹,

10 wherein r, s and t independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is
 optionally substituted with one or more substituents selected from R³.

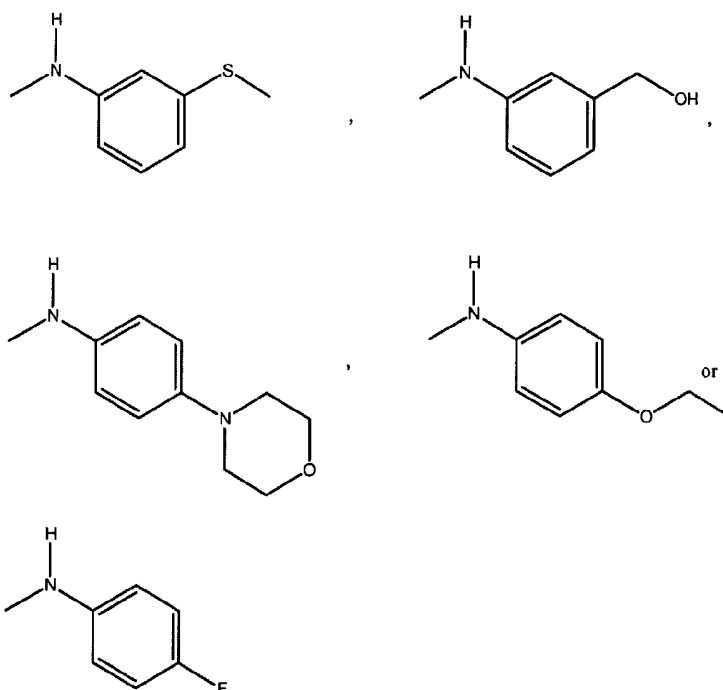
In another embodiment, A is aryl.

In another embodiment, the aryl is 2,5-dichlorophenyl.

In another embodiment, R¹ is (C₀-C₆)alkyl-NR^aR^b and R² is (C=O)_rO_s(C₁-C₁₀)alkyl.

15 In another embodiment, (C=O)_rO_s(C₁-C₁₀)alkyl is methyl.

In another embodiment, (C₀-C₆)alkyl-NR^aR^b is

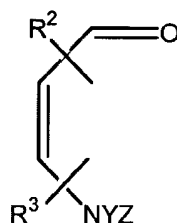


or a pharmaceutically acceptable salt thereof.

Compounds according to Figure I may be prepared by various processes known to one of skill in the art. In particular, compounds according to Figure I, wherein A is CH, B is N, C is CH and R¹ is H may be prepared by the following process:

a) a compound according to Figure IV

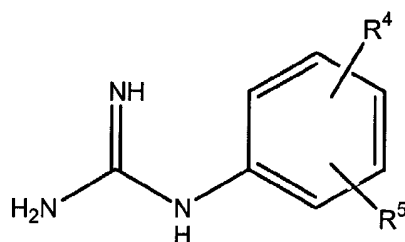
Figure IV



5

wherein Y and Z constitute (C₁-C₆)alkyl groups and R² and R³ are as above-defined is reacted with a compound according to Figure V

Figure V

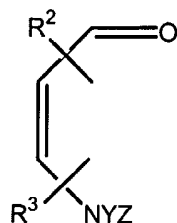


10 wherein R⁴ and R⁵ are as above-defined.

Compounds according to Figure II may be prepared by various processes known to one of skill in the art. In particular, compounds according to Figure II, wherein A is N, B is N, C is CH and R¹ is H may be prepared by the following process:

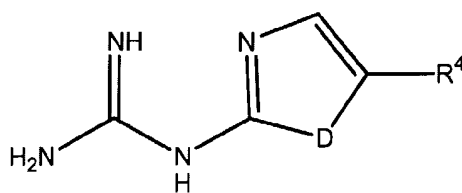
15 a) a compound according to Figure VI

Figure VI



wherein Y and Z constitute (C₁-C₆)alkyl groups and R² and R³ are as above-defined is reacted with a compound according to Figure VII

Figure VII



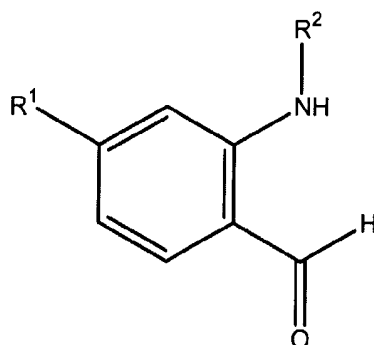
wherein D and R⁴ are as above-defined.

5

Compounds according to Figure III may be prepared by various processes known to one of skill in the art. In particular, compounds according to Figure III, wherein X is O may be prepared by the following process:

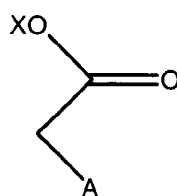
a) a compound according to Figure VIII

10 Figure VIII



wherein R¹ and R² are as above-defined is reacted under basic conditions with a compound according to Figure IX

Figure IX



15

wherein X is a (C₁-C₆)alkyl group and A is as above-defined.

In one embodiment, the compounds utilized by the methods of the invention are none of those disclosed in U.S. Pat. No. 5,385,915 (incorporated herein by reference in its entirety), particularly at column 11, lines 13-20.

Candidate compounds may be routinely assayed to determine which are appropriate ATP modulators using standard assays, *e.g.*, Xu *et al.*, 1997, Proc. Natl. Acad. Sci. USA 94: 3748–3752 or Zhang *et al.*, 2001, Biochemistry 40: 5049-5055.

Preferably the compound (or agent) administered in the method of treatment can cross through the blood brain barrier in sufficient quantities and at a sufficient rate so as to allow the treatment of an A β -related disorder. In one such embodiment, the compound is administered intravenously. In another embodiment, the compound is administered orally. More preferably the compound can cross the blood brain barrier without a carrier (for methods and routes of administration, see Section 5.1.2.5).

A β , however, exists not only in brain tissues and in the central nervous system (CNS), but also outside the brain in peripheral tissues, such as blood and in most organs where A β is also produced by the processing of APP. A balance exists between brain A β and A β outside the brain (DeMattos *et al.*, 2001, Proc. Natl. Acad. Sci. USA 98(15):8850–8855). Reduction of A β in the periphery results in a reduction of brain A β , presumably by re-partitioning of soluble A β . Thus, even compounds that do not cross the blood-brain barrier can be utilized in modulating A β levels and preventing or treating an A β -related disorder, or a symptom thereof.

5.1.2.2. PHARMACEUTICAL COMPOSITIONS AND FORMULATIONS

The present invention provides pharmaceutical compositions of the agents, drugs or compounds of the invention disclosed hereinabove.

In one embodiment, the invention provides a pharmaceutical composition comprising an amount of a compound described hereinabove sufficient to modulate A β levels in a cell or tissue, and a pharmaceutically acceptable excipient or carrier.

In another embodiment, the pharmaceutical composition comprising a compound that lowers ATP-dependent A β levels in a cell or tissue, a compound selected from the group consisting of: an NSAID, *e.g.* sulindac sulfide, flufenamic acid, ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen, meclofenamic acid, an antioxidant, *e.g.*, vitamin E, vitamin C, curcumin, and Ginkgo biloba, a non-selective COX inhibitor and an acetylcholinesterase inhibitor, and a pharmaceutically acceptable excipient or carrier.

The agent, drug or compound, or their physiologically acceptable salts or solvates, may be formulated for administration for injection, or for oral, topical, nasal, inhalation, insufflation (either through the mouth or the nose) buccal, parenteral, rectal administration or other forms of administration. In certain embodiments, administration is accomplished by intrathecal administration via osmotic pumps, by oral gavage, or by intraperitoneal injection.

The invention provides pharmaceutical compositions comprising sufficient amounts of an agent(s) of the invention together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants, excipients and/or carriers. Such compositions include diluents of various buffer content (*e.g.*, Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (*e.g.*, Tween 80, Polysorbate 80), anti-oxidants (*e.g.*, ascorbic acid, sodium metabisulfite), preservatives (*e.g.*, Thimerosal, benzyl alcohol) and bulking substances (*e.g.*, lactose, mannitol).

The compositions may also be incorporated into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or liposomes.

Hyaluronic acid may also be used. Biocompatible absorbable polymers may be selected from the group consisting of aliphatic polyesters, copolymers and blends, which include, but are not limited to, homopolymers and copolymers of lactide (which include D-, L-, lactic acid and D-, L- and meso lactide), glycolide (including glycolic acid), epsilon-caprolactone, p-dioxanone (1,4-dioxan-2-one, which is disclosed in U.S. Pat. No. 4,052,988), alkyl substituted derivatives of p-dioxanone (*i.e.*, 6,6-dimethyl-1,4-dioxan-2-one which is disclosed in U.S. Pat. No. 5,703,200), triethylene carbonate (1,3-dioxan-2-one), alkyl substituted derivatives of 1,3-dioxanone (which are disclosed in U.S. Pat. No. 5,412,068), delta-valerolactone, beta- butyrolactone, gamma-butyrolactone, epsilon-decalactone, hydroxybutyrate, hydroxyvalerate, 1,4-dioxepan-2-one (disclosed in U.S. Pat. No. 4,052,988 and its dimer 1,5,8,12-tetraoxacyclotetradecane-7,14 dione), 1,5-dioxepan-2-one, and polymer blends thereof.

Such compositions may influence physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the present proteins and derivatives. See, *e.g.*, Remington's Pharmaceutical Sciences, 18th ed., (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712). The compositions may be prepared in liquid form, or be in dried powder, such as lyophilized form.

Contemplated for use herein are oral solid dosage forms, which are disclosed generally in Remington's Pharmaceutical Sciences, 18th Ed. 1990 (Mack Publishing Co. Easton PA 18042) at Chapter 89. Solid dosage forms include tablets, capsules, pills, troches or lozenges, cachets or pellets. Also, liposomal or proteinoid encapsulation may be used to formulate the present compositions (as, for example, proteinoid microspheres

reported U.S. Patent No. 4,925,673). Liposomal encapsulation may be used and the liposomes may be derivatized with various polymers (e.g., U.S. Patent No. 5,013,556). A description of possible solid dosage forms for the therapeutic is given by Marshall, K. In: Modern Pharmaceutics Edited by G.S. Banker and C.T. Rhodes Chapter 10, 1979). In general, the formulation will include the agent and inert ingredients (which allow for protection against the stomach environment and release of the biologically active material in the intestine).

To ensure full gastric resistance a coating impermeable to at least pH 5.0 is useful. Examples of the more common inert ingredients that are used as enteric coatings are cellulose acetate trimellitate (CAT), hydroxypropylmethylcellulose phthalate (HPMCP), HPMCP 50, HPMCP 55, polyvinyl acetate phthalate (PVAP), Eudragit L3OD, Aquateric, cellulose acetate phthalate (CAP), Eudragit L, Eudragit S, and Shellac. These coatings may be used as mixed films.

A coating or mixture of coatings can also be used on tablets, which are not intended for protection against the stomach. This can include sugar coatings, or coatings that make the tablet easier to swallow. Capsules may consist of a hard shell (such as gelatin) for delivery of dry therapeutic, *i.e.*, powder; for liquid forms, a soft gelatin shell may be used. The shell material of cachets may be thick starch or other edible paper. For pills, lozenges, molded tablets or tablet triturates, moist massing techniques can be used.

The therapeutic can be included in the formulation as fine multi-particulates in the form of granules or pellets. The formulation of the material for capsule administration can also be as a powder, lightly compressed plugs or even as tablets. The therapeutic can also be prepared by compression.

Colorants and flavoring agents may all be included. For example, the protein (or derivative) may be formulated (such as by liposome or microsphere encapsulation) and then further contained within an edible product, such as a refrigerated beverage containing colorants and flavoring agents.

One may dilute or increase the volume of the therapeutic with an inert material or filler. These diluents or fillers can include carbohydrates, especially mannitol, α -lactose, anhydrous lactose, cellulose (e.g., microcrystalline cellulose), sucrose, calcium hydrogen phosphate modified dextrans and starch. Certain inorganic salts may be also be used as fillers including calcium triphosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and Avicell.

Disintegrants may be included in the formulation of the therapeutic into a solid dosage form. Materials used as disintegrates include, but are not limited to, starch (e.g., potato starch or the commercial disintegrant based on starch, Explotab). Sodium starch

glycolate, Amberlite, sodium carboxymethylcellulose, ultramylopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. Another form of the disintegrants are the insoluble cationic exchange resins. Powdered gums may be used as disintegrants and as binders and these can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also
5 useful as disintegrants.

Binders may be used to hold the therapeutic agent together to form a hard tablet and include materials from natural products such as acacia, tragacanth, starch (*e.g.*, pregelatinised maize starch) and gelatin. Others include methyl cellulose (MC), ethyl cellulose (EC) and carboxymethyl cellulose (CMC). Polyvinyl pyrrolidone (PVP) and
10 hydroxypropylmethyl cellulose (HPMC) can both be used in alcoholic solutions to granulate the therapeutic.

An anti-frictional agent may be included in the formulation of the therapeutic to prevent sticking during the formulation process. Lubricants may be used as a layer between the therapeutic and the die wall, and these can include but are not limited to; stearic acid
15 including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes, talc and silica. Soluble lubricants may also be used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, Carbowax 4000 and 6000.

Glidants that can improve the flow properties of the drug during formulation and to aid rearrangement during compression can be added. The glidants can include starch, talc,
20 pyrogenic silica and hydrated silicoaluminate.

To aid dissolution of the therapeutic into the aqueous environment a surfactant can be added as a wetting agent. Surfactants may include anionic detergents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic
25 detergents can be used and can include benzalkonium chloride or benzethonium chloride. The list of potential non-ionic detergents that can be included in the formulation as surfactants are lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. These surfactants can be present
30 in the formulation of the protein or derivative either alone or as a mixture in different ratios.

Additives that potentially enhance uptake of the agent are, for example, the fatty acids oleic acid, linoleic acid and linolenic acid.

Controlled release oral formulation may be desirable. The agent can be incorporated into an inert matrix that permits release by either diffusion or leaching mechanisms, *e.g.*,
35 gums. Slowly degenerating matrices may also be incorporated into the formulation. Some enteric coatings also have a delayed release effect.

Another form of a controlled release of this therapeutic is by a method based on the Oros therapeutic system (Alza Corp.), *i.e.* the drug is enclosed in a semipermeable membrane, which allows water to enter and to push the drug out through a single small opening due to osmotic effects.

Other coatings may be used for the formulation. These include a variety of sugars that can be applied in a coating pan. The therapeutic agent can also be given in a film coated tablet and the materials used in this instance are divided into two groups. The first are the nonenteric materials and include methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, methylhydroxy-ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl-methyl cellulose, sodium carboxy-methyl cellulose, providone and the polyethylene glycols. The second group consists of the enteric materials that are commonly esters of phthalic acid.

A mix of materials can be used to provide the optimum film coating. Film coating may be carried out in a pan-coater or in a fluidized bed or by compression coating.

Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-*p*-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Nasal delivery of the agent is also contemplated. Nasal delivery allows the passage of the protein to the blood stream directly after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with dextran or cyclodextran.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative.

The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

5 The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations disclosed previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. 10 Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may 15 for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

Other pharmaceutical compositions can be co-administered (sequentially or concurrently) with the pharmaceutical composition of the invention. Co-administration can be accomplished by administration of such other pharmaceutical compositions in the same 20 or in a different pharmaceutical composition as those described above. In one embodiment, an NSAID is co-administered, *e.g.*, sulindac sulfide, flufenamic acid, ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen or meclofenamic acid. In another embodiment, a non-selective COX inhibitor is co-administered. In another embodiment, a non-specific secretase inhibitor is co-administered. In another embodiment, 25 an antioxidant is co-administered. In specific embodiments, the antioxidant is selected from the group consisting of vitamin E, vitamin C, curcumin, and *Gingko biloba*. In another embodiment, an acetylcholinesterase inhibitor is co-administered.

5.1.2.3. ARTICLES OF MANUFACTURE

30 The present invention also provides an article of manufacture comprising packaging material and a pharmaceutical composition of the invention contained within the packaging material, said pharmaceutical composition in a form suitable for administration to a subject, preferably a human, or in a format that can be diluted or reconstituted for administration to the subject. In one embodiment, the article of manufacture further comprises printed 35 instructions and/or a label directing the use or administration of the pharmaceutical composition.

In particular, the article of manufacture can comprise packaging material and a pharmaceutical composition comprising a compound that lowers ATP-dependent A β levels, and a pharmaceutically acceptable carrier contained within the packaging material, said pharmaceutical composition in a form suitable for administration to a subject. Such an article of manufacture can further comprise printed instructions regarding the use or
5 administration of the pharmaceutical composition. For example, the instructions suggest a dosing regimen for the prevention, treatment, or amelioration of a symptom of an A β -related disorder such as for the prevention, treatment, or amelioration of a symptom of Alzheimer's disease. An article of manufacture of the invention can also further comprise a label regarding the use or administration of the pharmaceutical composition. For example,
10 the label can suggest a dosing regimen for the prevention, treatment, or amelioration of a symptom of an A β -related disorder such as Alzheimer's disease. In one embodiment, the article of manufacture can further comprises an antioxidant, a non-selective COX inhibitor or an acetylcholinesterase inhibitor.

As with any pharmaceutical product, the packaging material and container of the
15 articles of manufacture of the invention are designed to protect the stability of the product during storage and shipment. More specifically, the invention provides an article of manufacture comprising packaging material, such as a box, bottle, tube, vial, container, sprayer, insufflator, intravenous (i.v.) bag, envelope and the like; and at least one unit dosage form of a pharmaceutical composition of the invention contained within said
20 packaging material.

5.1.2.4. DOSAGES

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the
25 LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such
30 compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or
35 no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of

the invention, the therapeutically sufficient dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

In certain embodiments, treatment can begin at the first signs of mild cognitive impairment in a subject or patient, using standard methods for assessing mild cognitive impairment known in the art. In other embodiments, for example when a subject is at risk for familial Alzheimer's disease, treatment can begin before onset of mild cognitive impairment.

In certain embodiments, a treatment regimen can be used as a prophylactic or preventative measure. For example, in certain embodiments, a treatment regimen is administered to an elderly person, *e.g.*, age 65 or older. In another embodiment, demographic data available in the art may be consulted to determine the average age of onset of Alzheimer's disease in a population of interest.

In a specific embodiment, a pharmaceutical composition of the invention is administered chronically, *e.g.*, daily. In one embodiment, a pharmaceutical composition, *e.g.*, a blood-brain permeable version of the mesylate salt of STI-571 (or its mesylate salt) is administered daily via oral administration, with a minimum of 10 µg/day and a maximum of 800 µg/day administered.

5.1.2.5. ROUTES OF ADMINISTRATION

The component or components of a therapeutic composition of the invention may be introduced parenterally, topically, or transmucosally, *e.g.*, orally, nasally, or rectally, or transdermally. In certain embodiments, administration is parenteral, *e.g.*, via intravenous injection, and also including, but not limited to, intra-arteriole, intramuscular, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial administration. In certain embodiments, the component or components of a therapeutic composition of the invention is introduced by intrathecal administration via osmotic pumps (*e.g.*, a refillable osmotic pump), by oral gavage, or by intraperitoneal injection.

In preferred embodiments of the invention, an agent (or drug or compound) can cross and more preferably, readily pass through the blood-brain barrier, which permits, *e.g.*, oral, parenteral or intravenous administration. Alternatively, the agent can be modified or otherwise altered so that it can cross or be transported across the blood brain barrier. Many strategies known in the art are available for molecules crossing the blood-brain barrier, including but not limited to, increasing the hydrophobic nature of a molecule; introducing

the molecule as a conjugate to a carrier, such as transferring, targeted to a receptor in the blood-brain barrier, or to docosahexaenoic acid etc.

In a specific embodiment, the agent is delivered orally and can readily pass through the blood-brain barrier.

In another embodiment, an agent of the present invention is administered via the standard procedure of drilling a small hole in the skull to administer the agent.

In other embodiments, the molecule can be administered intracranially or intraventricularly. In another embodiment, osmotic disruption of the blood-brain barrier can be used to effect delivery of agent to the brain (Nilaver *et al.*, 1995, Proc. Natl. Acad. Sci. USA 92:9829-9833). In yet another embodiment, an agent can be administered in a liposome targeted to the blood-brain barrier. Administration of pharmaceutical agents in liposomes is known (see Langer, 1990, Science 249:1527-1533; Treat *et al.*, 1989, in Liposomes in the Therapy of infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317-327 and 353-365). All of such methods are envisioned in the present invention.

The rate and extent of entry of a compound into the brain are generally considered to be determined by partition coefficient, ionization constant(s), and molecular size. The octanol water system, for example, has received particular attention, and Hansch and coworkers have suggested that a partition coefficient in this system of about 100 is optimal for entry into the central nervous system (CNS) (Glave and Hansch, 1972, J. Pharm. Sci. 61:589; Hansch *et al.*, 1987, J. Pharm. Sci. 76:663). The octanol-water partition system provides a qualitative indication of the capability of a compound to cross the blood-brain barrier. Other factors, besides the octanol-water partition can be manipulated to influence the propensity to cross the blood-brain barrier. For example, decreasing over-all hydrogen binding ability of a compound can be used to facilitate a compound's crossing of the blood-brain barrier. Further, methodology as used by Begley *et al.* can be used and includes: (1) measuring the brain uptake index (BUI) with the equation for a tritiated agent compound:

$$\text{BUI} = [(\text{brain } ^3\text{H}/\text{brain } ^{14}\text{C}) / (\text{injectate } ^3\text{H}/\text{injectate } ^{14}\text{C})] \times 100$$
 where the ^{14}C reference compound is ^{14}C butanol or an analogous solvent; (2) Brain perfusion studies; (3) Intravenous bolus injection studies; and (4) Studies with cultured cerebral capillary endothelium.

In another embodiment used to reduce systemic side effects, the therapeutic compound is delivered in a vesicle, in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat *et al.*, 1989, in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss: New York, pp. 317-327 and 353-365).

In another embodiment, the therapeutic compound can be delivered in a controlled release system. For example, the agent may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used (see Langer, 1990, *Science* 249:1527-1533; ; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:201; Buchwald *et al.*, 1980, *Surgery* 88:507; Saudek *et al.*, 1989, *N. Engl. J. Med.* 321:574). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), 1974, CRC Press: Boca Raton, Florida; *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), 1984, Wiley: New York; Ranger and Peppas, 1983, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61; see also Levy *et al.*, 1985, *Science* 228:190; During *et al.*, 1989, *Ann. Neurol.* 25:351; Howard *et al.*, 1989, *J. Neurosurg.* 71:105). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, *i.e.*, the brain, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer (1990, *Science* 249:1527-1533).

5.2. METHODS FOR SCREENING FOR COMPOUNDS THAT MODULATE A β LEVELS

The present invention provides, *in vivo*, *in situ*, and *in vitro*, methods of identifying an agent, drug or compound for modulating A β levels exhibited by a cell or tissue. In certain embodiments, the agent has the ability to either stimulate or inhibit A β levels through modulation of the activity of γ -secretase. In one embodiment, the γ -secretase activity is ATP-dependent γ -secretase activity.

In one aspect, the method comprises identifying an agent to be tested for an ability to treat a disorder, including, but not limited to, an A β -related disorder. Such methods can be used alone or in conjunction with each other.

In certain embodiments, the invention provides methods of identifying an agent, drug or compound that modulate ATP-dependent A β peptide production and/or levels of A β in cells and tissues.

In certain embodiments, the invention provides methods of identifying an agent, drug or compound for modulating γ -secretase activity based on the determination of γ -secretase activity both before and after treatment of an ATP-dependent A β generating system with the compound to be tested. Such a system can be established from either broken cells (Xu *et al.*, 1997, *Proc. Natl. Acad. Sci. USA* 94: 3748-3752) or isolated membranes (Zhang *et al.*, 2001, *Biochemistry* 40: 5049-5055).

The invention also provides method of identifying a compound that modulates A β levels exhibited by a cell or tissue comprising:

- (a) determining a first level of γ -secretase activity in said cell or tissue;
- (b) contacting said cell or tissue with a test compound; and
- (c) determining a second level of γ -secretase activity in said cell or tissue,

5 wherein a difference in said first level and said second level of γ -secretase activity is indicative of the ability of said test compound to modulate A β levels.

In one embodiment, the difference in γ -secretase activity is indicative of the ability of said test compound to modulate the A β levels. In another embodiment, A β levels are modulated. In another embodiment, the γ -secretase activity is an ATP-dependent enzymatic activity. In another embodiment, the γ -secretase activity is production of A β .

The invention also provides a method of identifying a compound that modulates A β levels exhibited by a cell or tissue comprising:

- (a) contacting said cell or tissue with a test compound; and
- (b) determining a level of activity of γ -secretase in said cell or tissue;

15 wherein a difference in said level and a control level of γ -secretase activity in a comparable cell or tissue not contacted with the test compound is indicative of the ability of said test compound to modulate A β levels.

In one embodiment, the difference in γ -secretase activity is indicative of the ability of said test compound to modulate the A β levels. In another embodiment, A β levels are modulated. In another embodiment, the γ -secretase activity is an ATP-dependent enzymatic activity. In another embodiment, the γ -secretase activity is production of A β .

The invention also provides a method for identifying an agent to be tested for an ability to treat an A β -related disorder in a patient in need of such treatment comprising:

- (a) contacting in a cell or tissue γ -secretase with a potential agent; and
- (b) detecting the amount of γ -secretase activity

25 wherein the agent is identified if a decrease in γ -secretase activity is detected in the presence of the potential agent and wherein the agent modulates A β levels.

In one embodiment, the ability to treat the A β -related disorder is tested. In another embodiment, the γ -secretase activity is an ATP-dependent enzymatic activity. In another embodiment, the γ -secretase activity is production of A β .

In another aspect, the invention provides a method for identifying an agent to be tested for an ability to modulate A β levels exhibited by a cell or tissue comprising:

- (a) determining a first level of γ -secretase activity in said cell or tissue;
- (b) contacting said cell or tissue with a potential agent; and
- (c) determining a second level of γ -secretase activity in said cell or tissue,

35

wherein a difference in said first level and said second level of γ -secretase activity is indicative of the ability of said potential agent to modulate $A\beta$ levels. In one embodiment, the method comprises the additional step of:

- (d) determining whether the $A\beta$ levels are modulated.

In another embodiment, the γ -secretase activity is an ATP-dependent enzymatic activity. In another embodiment, the γ -secretase activity is production of $A\beta$.

The invention also provides a method for identifying an agent to be tested for an ability to modulate $A\beta$ levels exhibited by a cell or tissue comprising:

- (a) contacting said cell or tissue with a potential agent; and
- (b) determining a level of γ -secretase activity in said cell or tissue;

wherein a difference in said level and a control level of γ -secretase activity in a comparable cell or tissue not contacted with the test compound is indicative of the ability of said potential agent to modulate $A\beta$ levels.

In one embodiment, the method comprises the additional step of:

- (c) determining whether the $A\beta$ levels are modulated.

In another embodiment, the γ -secretase activity is an ATP-dependent enzymatic activity.

In another embodiment, the γ -secretase activity is production of $A\beta$.

The invention also provides a method for selecting a potential therapeutic agent for use in the treatment of an $A\beta$ -related disorder comprising:

- (a) administering a potential therapeutic agent to an animal;
- (b) measuring the response of said animal to said potential therapeutic agent;
- (c) comparing the response of said animal with that of a control animal to which the potential therapeutic agent has not been administered; and
- (d) selecting a potential therapeutic agent based on the difference in responses observed between said animal and said control animal,

wherein the potential therapeutic agent modulates ATP-dependent γ -secretase activity.

In one embodiment, the animal is a guinea pig. In another embodiment, the disorder is Alzheimer's disease.

The invention also provides a method for selecting a potential therapeutic agent for use in the treatment of an $A\beta$ -related disorder comprising:

- (a) administering a potential therapeutic agent to an animal;
- (b) measuring the response of said animal to administration of an agent that modulates activity of γ -secretase;
- (c) comparing the response of said animal with that of a control animal to which the potential therapeutic agent has not been administered; and

- (d) selecting a potential therapeutic agent based on the difference in responses observed between said animal and said control animal,

wherein the potential therapeutic agent modulates ATP-dependent γ -secretase activity.

In one embodiment, the animal is a guinea pig. In another embodiment, the disorder is Alzheimer's disease.

5 The invention also provides a method for selecting a potential therapeutic agent for use in the treatment of an $A\beta$ -related disorder comprising:

- (a) administering a potential therapeutic agent to an animal;
(b) measuring the response of said animal, wherein the response is selected from the group consisting of:

- 10 (i) exhibition of behavior in a Morris water maze; and
(i) exhibition of behavior in a Y-maze

- (c) comparing the response of said animal with that of a control animal to which the potential therapeutic agent has not been administered; and

- (d) selecting a potential therapeutic agent based on the difference in responses
15 observed between said animal and said control animal,

wherein the potential therapeutic agent modulates $A\beta$ levels.

In one embodiment, the animal is a guinea pig. In another embodiment, the disorder is Alzheimer's disease.

20 The invention also provides a method for preventing, delaying or reversing the progression of an $A\beta$ -related disorder comprising:

- (a) identifying a mammal in need of prevention, delay, or reversal of the progression of the disorder; and
(b) administering to said mammal an amount of an agent sufficient to modulate ATP-dependent γ -secretase activity,

25 wherein $A\beta$ levels are modulated.

In one embodiment, the agent inhibits or decreases ATP-dependent γ -secretase activity.

In another embodiment, the mammal is human. In another embodiment, the disorder is Alzheimer's disease.

30 In another embodiment, the agent promotes or increases ATP-dependent γ -secretase activity.

In another embodiment, the agent is administered orally.

In another embodiment, the agent is administered with a NSAID. In specific embodiment, the NSAID is sulindac sulfide, flufenamic acid, ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen, meclofenamic acid.
35

In another embodiment, the agent is administered with an

antioxidant. In another embodiment, the antioxidant is selected from the group consisting of vitamin E, vitamin C, curcumin, and Gingko biloba.

In another embodiment, the agent the agent is administered with an acetylcholinesterase inhibitor.

5 According to the invention, a "control" level means a separate baseline level exhibited by a comparable cell or tissue not contacted with a test compound or a level that is exhibited by a cell or tissue prior to contacting it with a test compound. A control level can return to a known standard reference level.

10 As would be clearly understood by a person of ordinary skill in the art, any and/or all of the embodiments disclosed herein for identifying an agent, drug or compound that can modulate A β levels, including such procedures that incorporate rational drug design, as disclosed herein, can be combined to form additional drug screens and assays, all of which are contemplated by the present invention.

15 According to the methods of the invention, γ -secretase activity and/or A β level may be determined both before and after treatment of cells or tissues with a test compound.

20 One of skill would understand that according to the invention, once a compound is identified as capable of producing, *e.g.*, γ -secretase activity and/or A β level similar to those produced by known ameliorative compounds, the compound may be used to treat an A β -related disorder. Such conditions would include, but not be limited to, Alzheimer's disease, Down's syndrome and inclusion body myositis. In the context of the present invention, the compounds identified would be administered as a sufficient dose or amount which can be determined by one of skill in the art based on data from studies such as presented in this specification. Such data would include, but not be limited to, results from IC50 determinations.

25 To determine whether a modulator is a selective ATP modulator, the skilled artisan would determine, using methods well known in the art, whether a candidate modulator discriminates among the molecules that affect ATP function in a cell of interest, and whether it only modulates a specific individual molecule, or class or subset of the molecules. For example, a compound may be assayed for activity in inhibiting a collection of various kinases, and would be recognized as a selective ATP modulator if it modulates ATP function in a related class of kinases. In certain embodiments, a battery of standard kinase assays is run to determine whether a modulator only modulates ATP function of a specific subset or class of kinases. In a specific embodiment, a standard luciferase (ATP-dependency) assay can be used. In certain embodiments, a non-specific ATP modulator will modulate luciferase production in the assay, whereas a specific inhibitor will not.

35 In other embodiments, a selective ATP modulator does not affect tau phosphorylation (see, *e.g.*, U.S. Patent No. 5,955,444) or Notch cleavage (see Section 10).

In one embodiment, the selectivity profile of the ATP modulator is similar or identical to that of STI-571 (or its mesylate salt, GLEEVECT[™]), STI-571 variant WGB-BC-15 or any of Compounds 1-5.

In certain embodiments, the modulator modulates an ATP-dependent cellular process, as disclosed herein. In other embodiments, however, it is contemplated that the modulator may modulate other energy-dependent (*e.g.*, GTP-dependent) processes that utilize sources of energy other than ATP (*e.g.*, GTP).

The present invention also provides *in vivo* methods of identifying agents that can modulate the level of A β via modulation of γ -secretase activity. Such methods can be employed alone or in conjunction with *in vitro* and *in situ* methods as disclosed herein.

According to the invention, in one embodiment of the invention, the skilled artisan would administer a modulator of A β levels, *e.g.*, an ATP modulator or an inhibitor of γ -secretase activity, based on results of screening for levels of A β peptides produced in the presence and absence of the inhibitor. Such screening of compounds is routine to one of skill in the art (Durkin *et al.*, 1999, *J. Biol. Chem.* 274 (29): 20499–20504; Petanceska and Gandy, 1999, *J. Neurochem.* 73: 2316–2320; De Strooper *et al.*, 1999, *Nature* 398: 518–522; Vandermeerena *et al.*, 2001, *Neuroscience Letters* 315: 145–148). Any compound identified as having selective γ -secretase inhibitory activity or to selectively inhibit A β production would be administered, *e.g.*, in a pharmaceutically acceptable vehicle, to an animal, *e.g.*, a human.

One such *in vivo* method comprises administering the agent to a non-human mammal. The amount (and/or rate) of activation of γ -secretase is then determined. An agent is identified as capable of modulating the level of A β , via modulation of γ -secretase activity, when the amount (and/or rate) of γ -secretase activation is increased or decreased in the presence of the agent relative to in the absence of the agent. In a specific embodiment, the γ -secretase activity is ATP-dependent γ -secretase activity. In one embodiment, the non-human mammal is a rodent, *e.g.*, a wild-type guinea pig. In other embodiments, the non-human mammal is an animal model for a disease or disorder. Such animal models are disclosed herein.

In another embodiment, an experimental animal is used to ascertain the effect of a potential agent on a disorder, including but not limited to an A β -related disorder. A potential modulator that ameliorates the disorder can then be selected.

For example, in certain embodiments, a learning and/or memory behavioral response of an animal can be determined in the presence and absence of the agent. In specific embodiment, learning and/or memory behavioral response of the animal, *e.g.*, a transgenic mouse such as a Tg2576 mouse. The Tg2576 transgenic mouse expresses human APP 695, develops the neuropathological signs of Alzheimer's disease, including amyloid plaques,

and exhibits learning and memory deficits (Hsiao *et al.*, 1996, Science 274, 99-102; U.S. Patent No. 5,877,399). Another useful model is a transgenic mouse expressing human APP and a familial Alzheimer's disease gene ("human APP-Swedish") (Borchelt *et al.*, 1996, Neuron 17:1005-1013). The learning and/or memory behavioral response of such an animal model can be measured using standard methods, *e.g.*, a Y-maze or a Morris water maze (see, *e.g.*, U.S. Patent No. 5,877,399).

Methods of testing a potential therapeutic agent (*e.g.*, a candidate drug, potential modulator, etc.) in animals or animal models are well known in the art. Thus potential therapeutic agents can be used to treat whole animals. The potential modulators can be administered by a variety of ways including topically, orally, subcutaneously, or intraperitoneally (such as by intraperitoneal injection) depending on the proposed use. Optimal dose will be empirically defined.

The potential efficacy of these compounds in relieving pathological symptoms of a disorder, including but not limited to, an A β -related disorder, can be assessed in animal models for disease. For example, in one embodiment, the animal model is a Tg2576, a transgenic mouse that expresses human APP 695. The Tg2576 mouse develops the neuropathological signs of Alzheimer's disease, including amyloid plaques, and exhibits learning and memory deficits (Hsiao *et al.*, 1996, Science 274, 99-102; U.S. Patent No. 5,877,399). Another useful model is a transgenic mouse expressing human APP and a familial Alzheimer's disease gene ("human APP-Swedish") (Borchelt *et al.*, 1996, Neuron 17:1005-1013).

In certain embodiments of the invention, modulation of A β 1-40 levels is screened for. A β 1-40 is the major product of catabolism of APP by β -secretase and γ -secretase enzyme activities. The amino acid sequence of A β 1-40 corresponds to residues 597-636 of the 695 amino acid isotype of human APP:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr

1

Glu Val His His Gln Lys Leu Val Phe Phe

21

Ala Glu Asp Val Gly Ser Asn Lys Gly Ala

31

Ile Ile Gly Leu Met Val Gly Gly Val Val [SEQ ID NO: 1].

In other embodiments, modulation of A β 1-42 level is screened for. A β 1-42 is a minor product of APP catabolism, typically produced at 10% the level of A β 1-40, that

aggregates into fibrillar forms very rapidly. The amino acid sequence of A β 1-42 corresponds to residues 597-638 of human APP:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr
 11
 5 Glu Val His His Gln Lys Leu Val Phe Phe
 21
 Ala Glu Asp Val Gly Ser Asn Lys Gly Ala
 31
 10 Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala [SEQ ID NO: 2].

Models such as these can be used to assess the efficacy of any potential therapeutic agents as disclosed hereinbelow. Generally, at least two groups of animals are used in the assay, with at least one group being a control group in which the administration vehicle is administered without the potential therapeutic agent.

Another aspect of the invention is a method for selecting a therapeutic agent for potential use in the treatment of disorder, including but not limited to an A β -related disorder, which comprises administering a suspected therapeutic agent to an animal model, and measuring and/or determining the putative therapeutic agent's effect on any of the phenotypic characteristics of the animal model, which may be believed to be related to said disorder.

Potential therapeutic agents are selected on the basis of whether there is a statistical significance between a test response and a normal (*i.e.*, naive or control) response. Potential therapeutic agents are selected that show a statistically significant change in the characteristic measured/determined. In a preferred embodiment, the response of a control (or vehicle-treated) animal in the presence of a therapeutic agent is characteristically different from the response of a control (or vehicle-treated) animal to which the agent has not been administered.

A still further aspect of the invention is a method for selecting a therapeutic agent for possible use in the treatment of a disorder, including but not limited to an A β -related disorder, which comprises administering a suspected therapeutic agent to an animal model for a disorder and measuring and/or determining the putative therapeutic agent's effect on any of the phenotypic characteristics outlined above which may be believed to be related to said disorder.

In other embodiments, the agent is administered along with an antagonist. The amount (and/or rate) of modulation of γ -secretase activity is then determined. Since the

administration of *e.g.*, an antagonist, in the absence of the agent, should result in an increase in γ -secretase activity, an agent is identified as capable of modulating the activity of γ -secretase when the amount (and/or rate) of activation is significantly increased or decreased in the presence of the agent relative to in the absence of the agent.

5 In other embodiments, the agent is administered along with a nucleoside triphosphate. The amount (and/or rate) of modulation of γ -secretase activity is then determined. Since the administration of a nucleoside triphosphate, in the absence of the agent should result in a increase in γ -secretase activity, an agent is identified as capable of modulating the activity of γ -secretase when the amount (and/or rate) of activation is significantly increased or decreased in the presence of the agent relative to in the absence of
10 the agent.

In certain embodiments, combinatorial libraries of chemical compounds, based on different structural skeletons (*e.g.*, purines), as well as unrelated naturally occurring compounds, can be tested as drug candidates. In a preferred embodiment of this type, the assay is performed using high throughput technology with automated robotic technology as
15 disclosed herein. Positive results ("hits") represent either the reduced or increased activity of γ -secretase, as compared to the control reactions (in which the drug candidate is not included in the assay).

Once a drug candidate is selected, structural variants of the drug candidate can be tested. These compounds can also be scrutinized and modified with parameters such as
20 membrane permeability, specificity of effects, and toxicity. The selected (*e.g.*, the most potent) compounds of this secondary screening can then be evaluated *in situ* and in animal models (see Section 9) to determine whether the selected compounds alter the activity of γ -secretase, and/or induce predicted behavioral alterations with minimal to no side-effects. Such behavioral abnormalities may include, but not be limited to, testing locomotor activity
25 or learning and memory, as disclosed herein (see also, *e.g.*, U.S. Patent No. 5,877,399; and Kosten *et al.*, J. Pharmacol., Exp. Ther. 269:137-144 (1994). In specific embodiments, methods for testing for learning and memory commonly known in the art, *e.g.*, a Y-maze or a Morris water test can be used (see, *e.g.*, U.S. Patent No. 5,877,399).

These tests can be then be followed by human trials in clinical studies. Alternatively,
30 in certain embodiments, human trials in clinical studies can be performed without animal testing. Compounds affecting targets other than γ -secretase can also be similarly screened, using alternative targets exemplified below.

Alternatively, modulators (*e.g.*, activators or inhibitors) of γ -secretase activity can be obtained by screening, *e.g.*, a random peptide library produced by recombinant
35 bacteriophage (see, *e.g.*, Scott and Smith, Science 249:386-390 (1990); Cwirla *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:6378-6382; and Devlin *et al.*, 1990, Science 249:404-406) or

a chemical library. Using the "phage method" very large libraries can be constructed (106-108 chemical entities). A second approach may be to use chemical methods, of which the Geysen method (Geysen *et al.*, 1986, *Molecular Immunology* 23:709-715; Geysen *et al.*, 1987, *J. Immunologic Method* 102:259-274) and the method of Fodor *et al.* (1991, *Science* 251:767-773) are examples. Furka *et al.* (1988, 14th international Congress of Biochemistry, Volume 5, Abstract FR:013; Furka, 1991, *Int. J. Peptide Protein Res.* 37:487-493), Houghton (U.S. Patent No. 4,631,211) and Rutter *et al.* (U.S. Patent No. 5,010,175) disclose methods to produce a mixture of peptides. Such peptides can be tested as potential modulators of γ -secretase activity.

In another aspect, synthetic libraries (Needels *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* 90:10700-4; Ohlmeyer *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* 90:10922-10926; Lam *et al.*, International Patent Publication No. WO 92/00252; Kocis *et al.*, International Patent Publication No. WO 94/28028), and the like can be used to screen for modulators of γ -secretase activation, according to the present invention. Once a potential modulator is identified, chemical analogues can be either selected from a library of chemicals as are commercially available (*e.g.*, from Chembridge Corporation, San Diego, CA or Evotec OAI, Abingdon, UK), or alternatively synthesized *de novo*. The prospective agent (drug) can be placed into any standard assay to test its effect on the activity of γ -secretase activation. A drug is then selected that modulates the activity of γ -secretase activation.

The present invention also contemplates screens for small molecules, analogs thereof, as well as screens for natural modulators of γ -secretase, such as those molecules that bind to and inhibit or activate, *e.g.*, γ -secretase *in vivo*. Alternatively, natural products libraries can be screened using assays of the invention for molecules that modulate *e.g.*, γ -secretase activity.

In another aspect of the present invention, a potential modulator can be assayed for its ability to modulate the level of A β , either independently, or subsequent to, a binding assay as disclosed herein. Such assays are known in the art. Thus, according to the methods of the invention, a modulator of γ -secretase activity that modulates the level of A β is then selected

In another embodiment, a potential modulator can be added to a cultured cell line or tissue explant. Samples of the cells or tissues can be treated with various concentrations of a potential modulator and the samples can then be analyzed for A β levels. Potential modulators of A β levels can also be tested for example, on intact neurons *in situ*. The effects of these compounds can be tested by empirically defining the optimal concentration and time of incubation.

The present invention also includes compositions identified by the methods described herein. One of skill in the art would understand that once identified as capable of

modulating A β levels in the method of the present invention, the compound of the invention could be used therapeutically to modulate A β levels in neuronal cells in order to treat conditions in which A β levels may be involved.

One of skill will understand that once identified as capable of modulating γ -secretase activity in the methods of the present invention, the compound may be used therapeutically to modulate γ -secretase activity in cells, *e.g.*, neurons, in order to treat conditions in which γ -secretase activity may be involved. Such conditions include, but are not limited to, an A β -related disorder.

The present invention further provides methods for performing rational drug design to develop drugs that can modulate activity of γ -secretase and thereby ameliorate a disorder, including but not limited to an A β -related disorder. Such rational drug design can be performed using compounds that have been identified as inhibitors (or activators) of γ -secretase as a starting point. Thus, the present invention provides screens and assays to allow more specific inhibitors (or activators) to be identified. Such methods of rational drug design are well-known in the art. In a specific embodiment, the rational drug design methods disclosed in U.S. Patent Application Nos. 09/419,379, by Bibb *et al.*, entitled "Methods of Identifying Agents That Regulate Phosphorylation/Dephosphorylation in Dopamine Signaling," filed October 15, 1999, and 09/687,959, by Bibb *et al.*, entitled "Methods of Identifying Agents That Regulate Phosphorylation/Dephosphorylation in Dopamine Signaling," filed October 13, 2000, incorporated herein by reference in their entireties, are used.

Indeed, potential modulators can be examined through the use of computer modeling using a docking program such as GRAM, DOCK, or AUTODOCK (Dunbrack *et al.*, 1997, *Folding & Design* 2:27-42), to identify compounds as potential modulators of an ATP-dependent enzymatic activity, *e.g.*, ATP modulators such as those that modulate γ -secretase. These modulators can then be tested for their effect. In one embodiment, this procedure can include computer fitting of potential modulators to the γ -secretase complex to ascertain how well the shape and the chemical structure of the potential modulator will bind to γ -secretase (*see, e.g.*, Bugg *et al.*, 1993, *Scientific American* 269(6):92-98; West *et al.*, 1995, *TIPS*, 16:67-74). Computer programs can also be employed to estimate the attraction, repulsion, and steric hindrance of the subunits with a modulator/inhibitor. Generally the tighter the fit, the lower the steric hindrances, and the greater the attractive forces, the more potent the potential modulator since these properties are consistent with a tighter binding constant. Furthermore, the more specificity in the design of a potential drug the more likely that the drug will not interact as well with other proteins. This will minimize potential side-effects due to unwanted interactions with other proteins.

Initially, in one embodiment, compounds known to bind to γ -secretase on any molecule that regulates γ -secretase activity or affects an enzyme complex that contain γ -secretase can be systematically modified by computer modeling programs until one or more promising potential analogs are identified. In addition systematic modification of selected analogs can then be systematically modified by computer modeling programs until one or more potential analogs are identified. Such analyses are well known to those of skill in the art and have been shown to be effective in the development of, *e.g.*, HIV protease inhibitors (see, *e.g.*, Lam *et al.*, 1994, Science 263:380-384; Wlodawer *et al.*, 1993, Ann. Rev. Biochem. 62:543-585; Appelt, 1993, Perspectives in Drug Discovery and Design 1:23-48; Erickson, 1993, Perspectives in Drug Discovery and Design 1:109-128).

Any of the potential agents or targets for the potential agents (*e.g.*, γ -secretase, A β , kinases, ATP) can be labeled. Suitable labels include enzymes (*e.g.*, alkaline phosphatase or horseradish peroxidase), fluorophores (*e.g.*, fluorescein isothiocyanate (FITC), phycoerythrin (PE), Texas red (TR), rhodamine, free or chelated lanthanide series salts, especially Eu³⁺, to name a few fluorophores), chromophores, radioisotopes, chelating agents, dyes, colloidal gold, latex particles, ligands (*e.g.*, biotin), chemiluminescent agents, magnetic beads or magnetic resonance imaging labels. When a control marker is employed, the same or different labels may be used for the receptor and control marker.

In embodiments wherein a radioactive label, such as the isotopes ³H, ¹⁴C, ³²P, ³⁵S, ³⁶Cl, ⁵¹Cr, ⁵⁷Co, ⁵⁸Co, ⁵⁹Fe, ⁹⁰Y, ¹²⁵I, ¹³¹I, and ¹⁸⁶Re is used, standard counting procedures known in the art may be utilized.

In embodiments wherein the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques known in the art.

A direct label is an example of a label that can be used according to the methods of the present invention. A direct label is an entity that, in its natural state, is readily visible, either to the naked eye (for example, by visual inspection through a compound or dissecting light microscope), or with the aid of an optical filter and/or applied stimulation, *e.g.*, U.V. light to promote fluorescence. Examples of colored labels that can be used according to the methods of the present invention, include metallic sol particles, for example, gold sol particles such as those disclosed by Leuving (U.S. Patent 4,313,734); dye sol particles such as disclosed by Gribnau *et al.* (U.S. Patent 4,373,932) and May *et al.* (WO 88/08534); dyed latex such as disclosed by May *et al.* (WO 88/08534), Snyder (EP-A 0280 559 and 0 281 327); or dyes encapsulated in liposomes as disclosed by Campbell *et al.* (U.S. Patent 4,703,017).

Other direct labels include a radionucleotide, a luminescent moiety, or a fluorescent moiety including, but not limited, to, *e.g.*, a modified/fusion chimera of green fluorescent

protein (as disclosed in U.S. Patent No. 5,625,048, issued April 29, 1997, and WO 97/26333, published July 24, 1997, each of which is incorporated herein by reference in its entirety).

In addition to these direct labeling devices, indirect labels comprising enzymes can also be used according to the present invention. Various types of enzyme-linked immunoassays are well known in the art, for example, enzyme-linked immunoassays using alkaline phosphatase, horseradish peroxidase, lysozyme, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, or urease. These and other similar assays are well known in the art and are disclosed, *e.g.*, in Engvall (1980, "Enzyme Immunoassay ELISA and EMIT," in *Methods in Enzymology*, 70: 419-439) and in U.S. Patent 4,857,453.

In certain embodiments, proteins can be labeled by metabolic labeling. Metabolic labeling occurs during *in vitro* incubation of the cells that express the protein in the presence of culture medium supplemented with a metabolic label, such as [³⁵S]-methionine or [³²P]-orthophosphate. In addition to metabolic (or biosynthetic) labeling with [¹⁴C]-methionine, the invention further contemplates labeling with [¹⁴C]-amino acids and [³H]-amino acids (with the tritium substituted at non-labile positions) (see, *e.g.*, U.S. Patent Application Nos. 09/419,379, by Bibb *et al.*, entitled "Methods of Identifying Agents That Regulate Phosphorylation/Dephosphorylation in Dopamine Signaling," filed October 15, 1999, and 09/687,959, by Bibb *et al.*, entitled "Methods of Identifying Agents That Regulate Phosphorylation/Dephosphorylation in Dopamine Signaling," filed October 13, 2000, incorporated herein by reference in their entireties).

5.2.1. ASSAYS FOR γ -SECRETASE ACTIVITY

According to the methods of the invention, modulators (*e.g.*, inhibitors or activators) of γ -secretase, can be detected and isolated using methods commonly known in the art, and/or identified by direct assay of isolated enzyme. Such assays are well known to those of skill in the art.

In one embodiment, the methods of Roberts *et al.* (U.S. Patent Application 20020025540, published February 28, 2002, entitled "Isolation of functionally active gamma-secretase protein complex and methods for detection of activity and inhibitors thereof") are used.

In another embodiment, the methods of Zhang *et al.* (2001, *Biochemistry* 40: 5049-5055) are used.

In another embodiment, γ -secretase activity may be assayed according to the methods known in the art, *e.g.*, the methods of De Strooper *et al.*, 1999, *Nature* 398: 518-522.; Hsiao *et al.*, 1996, *Science* 274: 99-102.; Li *et al.*, 2000, *Proc. Natl. Acad. Sci. USA* 97: 6138-6143.; Lichtenthaler *et al.*, 1999, *FEBS Lett.* 453: 288-292; McLendon *et al.*,

2000; FASEB J. 14: 2383-2386; or Seiffert *et al.*, 2000, *Brain. Res. Mol. Brain Res.* 84: 115-126.

In another embodiment, γ -secretase activity may be assayed according to the methods that assay for cleavage of proteins other than APP or APP-like proteins. For example, proteolytic cleavage of the Notch protein, which is a single transmembrane domain cell surface receptor that mediates many cell fate decisions in vertebrates and invertebrates (Artavanis-Tsakonas *et al.*, 1996, *Science* 268:225-232; Kopan and Turner, 1996, *Curr. Opin. Neurobiol.* 6:594-601; Weinmaster, 1997, *Mol. Cell. Neurosci.* 9:91-102) may be assayed using the methods of Ray *et al.* (1999, *J. Biol. Chem.* 274:36801-36807) or Schroeter *et al.* (1998, *Nature* 393:382-386), which are incorporated herein by reference in their entireties.

γ -secretase may be immunoprecipitated from brain homogenate using standard methods, using, *e.g.*, a commercial antibody directed against γ -secretase.

In yet another embodiment, γ -secretase activity is assayed according to the methods disclosed hereinbelow, *e.g.*, in Example 4.

5.2.2. ASSAYS FOR A β LEVELS

A β levels may be determined according to any method known in the art. In one embodiment, A β levels are determined according to the methods of Xu *et al.* (1997, *Proc. Natl. Acad. Sci. USA* 94: 3748-3752).

In other embodiments, A β levels are determined according to the methods disclosed hereinbelow, *e.g.*, in Examples 1, 2 or 3.

In another embodiment, A β peptide may be assayed for, *e.g.*, in a brain homogenate, using a sandwich ELISA (*e.g.*, Biosource International, Camarillo, CA) according to the manufacturer's recommendations.

5.2.3. ANIMAL MODELS

In vivo animal models of APP processing into A β and of A β -related disorders such as Alzheimer's disease are well known to those of skill in the art. Such animal models can, for example, be used to further screen compounds identified via the methods presented herein. Alternatively, such models can be used to routinely validate compounds for efficacy in use in the modulation, prevention, treatment, and symptom amelioration methods of the present invention.

In one embodiment, the wild-type guinea pig is used as an animal model. For example, wild-type guinea pigs may be used as models for APP processing into A β , for A β production and/or accumulation of A β in brain. They are known to produce A β peptides that are immunologically identical to human A β . Therefore, the guinea pigs provide, for

example, a model in which to examine perturbations in APP metabolism with and without drug treatment.

In other embodiments, a mouse model of Alzheimer's disease is used, *e.g.*, a transgenic mouse expressing human APP-Swedish (see, *e.g.*, Borchelt *et al.*, 1996, Neuron 17:1005–1013), which exhibits neuropathological symptoms similar to those of Alzheimer's disease patients. Moreover, a rat model of Alzheimer's disease can be used. In another embodiment, a transgenic mouse model of Alzheimer's disease can be used, *e.g.*, the Tg2576 transgenic mouse (U.S. Patent No. 5,877,399). Such an animal model may be used to screen for compounds useful in the treatment of Alzheimer's disease due to its exhibition of, *e.g.*, an altered level of human A β .

Models such as these can also be used to assess the efficacy of any potential therapeutic agents as disclosed hereinbelow. Generally, at least two groups of animals are used in the assay, with at least one group being a control group in which the administration vehicle is administered without the potential therapeutic agent.

According to the invention, animal models may be employed that have, in certain embodiments, an altered physiological regulation of the nervous system such that the animal or tissues derived from it can be utilized for screening of potential therapeutic agents and/or therapeutic regimens that act at the level of γ -secretase activity, especially at the level of competing (directly or indirectly) with ATP for activation of γ -secretase. Drugs that can reverse any of the defects exhibited by such an animal model act at some point in the catabolic pathway underlying the cleavage of APP to A β , and are thus of potential use therapeutically. Additionally, since some defects occur at the behavioral level, the alteration or modulation of these behavioral defects can have a high predictive value for therapeutic use in modification of such behaviors.

According to the methods of the invention, animal models thus can be used, as screening tools to elucidate the mechanisms of APP cleavage to A β involved in both normal and diseased patient populations. For example, an animal model can thus be utilized to assess the response to a variety of potential therapeutic strategies and therapeutic agents that can thus be used in the treatment of patients suffering from a variety of neurological diseases and disturbances.

Using animal models, various small molecule drugs can be screened for potentially advantageous effects, including enhanced potency as well as minimization of side effects. Typical candidates for such screening may be obtained from any of several commercially available drug libraries.

Specific disorders for which an animal model can be utilized include, but are not limited to, an A β -related disorders, as disclosed herein. By measuring various characteristics in an animal model in response to administration of endogenous or

exogenous agents, and comparing these characteristics to those in an animal treated with a potential therapeutic agent, an assessment of the utility of the potential therapeutic agent in the prevention and/or treatment of a particular disorder or disease state can be made. For instance, the potential therapeutic agent can be administered to an animal model for a particular A β -related disorder, and the A β level in a control animal (*e.g.*, an animal administered vehicle) can then provide an indication of the value of the potential therapeutic agent.

Another aspect of the invention is a method for selecting a therapeutic agent for possible use in the treatment of a disorder, including but not limited to, an A β -related disorder, which comprises administering a suspected therapeutic agent to an animal model, and measuring and/or determining the putative therapeutic agent's effect on any phenotypic characteristics which may be believed to be related to said disorder.

In one embodiment of this aspect of the invention, a suspected therapeutic agent is administered to an animal model, *e.g.*, an animal model for an A β -related disorder, and the A β level is measured for the animal model, wherein the normal A β level of the animal model in the absence of a therapeutic agent is characteristically different than that of control (*e.g.*, wild-type) animals. The potential therapeutic agents are selected on the basis of whether there is a statistical significance between a test characteristic, *e.g.*, a test A β level, and a normal characteristic, *e.g.*, a normal A β level. Potential therapeutic agents are selected that show a statistically significant change in the characteristic that is measured or determined. In a preferred embodiment, the test characteristic of the animal model in the absence of a therapeutic agent is different than that of control (*e.g.*, wild-type) animals to which the potential therapeutic has not been administered.

Yet another aspect of the present invention is a method for selecting a therapeutic agent for possible use in the treatment of Alzheimer's disease which comprises administering a suspected therapeutic agent to an animal model, *e.g.*, an animal model for Alzheimer's disease, and measuring and/or determining the putative therapeutic agent's effect on any of the phenotypic characteristics which may be believed to be related to Alzheimer's disease.

The following experimental examples are offered by way of illustration and not by way of limitation.

6. EXAMPLE 1: PRODUCTION OF A β IS ATP-DEPENDENT

The working example presented herein demonstrates that production of A β is ATP-dependent, and presents data indicating that β -secretase activity is at least partially ATP-independent, while optimal γ -secretase activity requires ATP.

5

6.1. MATERIALS AND METHODS

Whole N2a cells doubly transfected with the human Swedish APP mutation and the presenilin-1 (PS1) mutant, Δ E9, were ³⁵S-pulse-labeled before permeabilization and chased at 20°C to localize newly synthesized, ³⁵S-labeled APP to the Golgi apparatus, a major location for APP and the predominant site of A β production. Cells were then permeabilized according to the methods of Xu *et al.* (1997, Proc. Natl. Acad. Sci. USA 94: 3748–3752), depleted of cytosol and incubated at 37°C either with an ATP regenerating system or with apyrase to hydrolyze residual ATP and other nucleotide triphosphates, and A β production was assayed. The effect of adding an ATP-regenerating system to a cell-free assay system for A β generation was quantitated according to the methods of Xu *et al.* (1997, Proc. Natl. Acad. Sci. USA 94: 3748–3752).

For the assay of isolated γ -secretase activity, membranes were isolated from N2a cells expressing a recombinant γ -secretase substrate, C99, according to the methods of Zhang *et al.* (2001, Biochemistry 40: 5049-5055). Accumulation of human C99 (also known as β C-terminal fragment or β CTF) in this system results from expression of the transgene and not from endogenous BACE (β -secretase) cleavage of mouse APP; furthermore, the use of human specific anti-A β antibody does not effectively resolve the small amount of endogenous mouse A β produced under the experimental conditions, thus, allowing measurement of only A β produced from the action of γ -secretase on the human C99. Also, to determine whether ATP was primarily responsible for stimulating γ -secretase activity, the previous energy regenerating system was substituted with one designed to sustain concentrations of ATP alone but not GTP. A β release from isolated membranes was determined by ELISA (Biosource International, Camarillo, CA).

6.2. RESULTS

Studies were performed to examine possible energy requirements of A β production. A β production was reconstituted in a cell-free system consisting of permeabilized cells, the cytosol of which had been washed away, leaving intracellular membranes and organelles intact.

To identify steps in the APP processing pathway that might require ATP, accumulation of APP metabolites was studied using ³⁵S pulse labeling in a cell-free system

consisting of N2a cells doubly transfected with the human Swedish APP mutation and the presenilin-1 (PS1) mutant, $\Delta E9$. Both mutations result in familial forms of Alzheimer's disease characterized by a higher $A\beta_{42}/A\beta_{40}$ ratio (Borchelt *et al.*, 1996, Neuron 17:1005–1013). This cell line has been shown to produce large amounts of $A\beta$ peptides and of C99, a product of β -secretase cleavage of APP and an immediate precursor of $A\beta$. The responses of both β -secretase and γ -secretase activities to ATP depletion were measured and compared.

ATP stimulated $A\beta$ production above the initial level of $A\beta$ that had accumulated during the 20° C chase. The results of these experiments, showing radiolabeled $A\beta$ production from precursor accumulated in the Golgi apparatus, are shown in FIG. 1. In particular, the energy regenerating system resulted in a three-fold increase in $A\beta$ production compared to apyrase treated samples (FIG. 1) and concomitantly, a two-thirds decrease in C99 (also known as β C-terminal fragment or β CTF) (FIG. 2).

A much smaller quantity of $A\beta$ was produced in the absence of ATP, demonstrating that production of $A\beta$ is ATP-dependent.

By contrast, C99 production from APP was increased in the absence of ATP, indicating that the activity of β -secretase is at least partially ATP-independent. This result also indicates that the increase in C99 likely results from a decrease in γ -secretase activity, that allows for accumulation of the $A\beta$ precursor. This is evident in the autoradiogram shown in FIG. 2.

Virtually, identical results were achieved when apyrase was left out and samples were incubated in the absence of the energy regenerating system (data not shown).

These results indicate that, in the context of $A\beta$ production, optimal γ -secretase activity requires nucleotide triphosphates and that substantial β site APP cleaving enzyme (also known as β -secretase or "BACE") activity still occurs in their absence.

In order to test whether isolated γ -secretase activity is ATP-dependent, an assay of isolated γ -secretase activity was performed. Generation of $A\beta$ in the isolated γ -secretase assay was strongly stimulated by addition of ATP and a regenerating system (Xu *et al.*, 1997, Proc. Natl. Acad. Sci. USA 94: 3748–3752) (FIG. 3). A dose-dependent decrease in $A\beta$ generation was observed upon addition of Compound 1 (see below) to these membranes (FIG. 4).

In summary, the results presented herein demonstrate that production of $A\beta$ is ATP-dependent. The results further indicate that β -secretase activity is at least partially ATP-independent, and optimal γ -secretase activity requires ATP.

7. EXAMPLE 2: MODULATION OF $A\beta$ LEVELS

The working example presented herein demonstrates the successful use of compounds to modulate, in particular lower, A β levels.

7.1. MATERIALS AND METHODS

5 N2a cells transfected with the human Swedish APP mutation, and with either normal human presenilin 1 or Δ E9 mutant presenilin 1 (PS1), were used for assays of compound effect on A β generation.

Media was added with compound concentration of 0, 10^{-9} , 10^{-8} , 10^{-7} and 10^{-6} M; cells were incubated overnight at 37°C. The next day, cells were incubated with 35 S-methionine and 35 S-cysteine for four hours in the presence of the compound as above.

10 Media and cells were separately harvested and subjected to immunoprecipitation with antibody 4G8 (Senetek, Napa, California), which recognizes an epitope corresponding to residues 17-28 of A β . Antibody 4G8 immunoprecipitates A β from media, and A β plus full-length APP from cells. After removal of antibody 4G8 and bound A β from media, the supernatant was reacted with 22C11 (Chemicon International), an antibody that
15 immunoprecipitates sAPP β and sAPP α . Immunoprecipitates were subjected to PAGE and resolved by autoradiography according to standard methods. APP metabolites were compared by densitometry according to standard methods.

In other experiments, immunoprecipitation followed by Western blot analysis was carried out on unlabeled (no radioactive label) N2a cells, using standard methods and using
20 6E10 (Senetek, Napa, California) or 22C11 antibodies for detection.

Mass spectrometry was carried out on media from N2a cells to detect A β 40 and A β 42 peptides using the CiphergenProteinChip System (Ciphergen Biosystems Inc., Fremont, CA), which is based on Seldi and time-of-flight mass spectrometry, using standard
25 protocols as set out by the manufacturer. The experimental procedures were the same as those disclosed above for immunoprecipitation/Western blotting, without metabolic labeling. Unlike immunoprecipitation/Western blotting, media did not contain sodium lauryl sulfate (SDS) but did contain Triton X-100 detergent. Aliquots of detergent treated media were contacted to a metal chip that was previously contacted with an antibody, such as 6E10. The antibody binds human APP metabolites, including A β , in the applied sample.
30 Detection of bound antigen followed a laser pulse that dissociated antigen from antibody.

A β peptides were resolved graphically as peaks of various heights, situated on an x-axis denoting molecular mass. Thus, A β 40 and A β 42 were resolved separately at approximately 4333 and 4519 daltons, respectively. The relative heights or areas under the peaks in a given spectrum denoted the relative concentrations of the detected species. Peaks
35 corresponding to the same species, as conveyed by molecular mass, were compared among

different spectra, reflecting different samples, to measure relative quantities of A β among a set of samples.

These immunoprecipitation experiments were repeated using a minimal treatment time, 1 hour instead of overnight.

5 7.2. RESULTS

Compounds were tested for an ability to modulate A β levels. In particular, STI-571, a member of the phenyl aminopyrimidine class of pharmacophores was tested. Specifically, the mesylate salt of STI-571 (also known as GLEEVEC™, Novartis; see FIG. 20) was tested. STI-571 is an ATP competitor known to target the ATP-binding site on BCR-Abl tyrosine kinase, and also to inhibit Abl, c-kit and platelet-derived growth factor receptor (PDGFR). An STI-571 variant, WGB-BC-15 (see FIG. 20), was also tested. In addition, Compounds 1 and 2 (see FIG. 20), members of the pyrimidinyl pyridone tyrosine kinase inhibitor class of molecules, were tested.

At concentrations in the range of 1×10^{-6} M, STI-571 exhibited an IC₅₀ (that is, 15 inhibited total levels of A β by 50%) in N2a cells expressing APP and either normal human presenilin 1 or Δ E9 mutant presenilin 1. WGB-BC-15 also inhibited levels of secreted A β . Compound 1 inhibited total A β levels as well, with an IC₅₀ of less than 10^{-6} M.

Intracellular levels of A β also decreased in the presence of the compounds. These results were derived from immunoprecipitation/Western blot analysis and corroborated by 20 results of studies on APP incorporation of ³⁵S-methionine.

Levels of sAPP α and sAPP β , two of the major secreted metabolites of APP, were not inhibited by these compounds. Levels of sAPP α increased at higher concentrations of the compounds. These results demonstrate that the reduction observed in secreted A β levels was not due to some non-specific deleterious affect on the cells' protein secretion ability.

Mass spectrometry was carried out according to standard methods, by which A β ₄₀ and A β ₄₂ were clearly resolved, revealing that levels of both species were dramatically 25 reduced in the presence of Compound 1 (FIG. 5).

FIGS. 6 and 7 illustrate the dose-dependent decrease in A β observed after just one hour of treatment of ³⁵S labeled N2a cells expressing the human APP-Swedish transgene 30 with STI-571 or Compound 1, respectively.

8. EXAMPLE 3: MODULATION OF A β LEVELS IN PRIMARY NEURONAL CELL CULTURES

This example demonstrates that STI-571, Compound 1 and Compound 2 inhibit 35 production of A β peptides in normal rat primary neuronal cell cultures. These result correlate well with the *in vivo* results described below (see Section 9).

8.1. MATERIALS AND METHODS

Cultures of rat embryonic cortical neurons were established from day 18 pregnant SD rats using standard techniques (Gasparini *et al.*, 2001, *J. Neurosci.* 21(8):2561–2570). After several days of culture, new media was added along with STI-571, Compound 1 or Compound 2 at concentrations of 0, 10^{-9} , 10^{-8} , 10^{-7} , 2×10^{-6} and 10^{-6} M; cells were incubated overnight at 37°C.

The next day, cells were labeled with ^{35}S -methionine and cysteine for four hours in the presence of compounds as above. Media and cells were then harvested and subjected to immunoprecipitation with antibody 4G8, to detect A β and APP. The media was serially immunoprecipitated first with antibody 4G8 and then antibody 22C11, for detection of secreted A β , sAPP β and sAPP α . Immunoprecipitates were subjected to PAGE and resolved by autoradiography; APP metabolites were compared by densitometry.

8.2. RESULTS

Results similar to those obtained in Example 2 were obtained in these experiments with rat cortical neurons exposed to Compound 1. Compound 1 reduced levels of A β in both media (FIG. 8A) and cells. The predominant A β peptide produced by the neurons was A β 11-40, which is characteristic of rat primary neurons (FIG. 8B). Total sAPP was not reduced significantly nor was total cellular APP (FIG. 9), indicating that the compound was not cytotoxic nor did it non-specifically inhibit cellular secretion. Similar results were obtained with related Compound 2 (FIG. 10).

The time course and dose response of primary cortical neuronal cultures to STI-571 and Compound 1 were studied in more detail in a second set of experiments similar to those described above. Significant reductions in A β were observed in as little as 6 hours (FIGS. 11 and 12) and 5 μM STI-571 (FIG. 13) or 0.5 μM Compound 1 (FIG. 14).

9. EXAMPLE 4: MODULATION OF A β LEVELS IN AN ANIMAL MODEL OF A β PRODUCTION

The working example presented herein demonstrates the successful reduction of levels of brain A β 40 and A β 42 in wild-type guinea pigs, an animal model of A β production. Wild-type guinea pigs are an art-accepted model for normal A β production and are known to produce A β peptides that are immunologically identical to human A β . Therefore, wild-type guinea pigs provide an animal model for examining perturbations in APP metabolism with and without drug treatment, and, as such also represent an animal model for a physiological hallmark of Alzheimer's disease.

9.1. MATERIALS AND METHODS

Osmotic minipumps containing 200 μ l compound STI-571 (50 mM in saline buffer, final concentration of 15.4 mg/kg) were implanted intrathecally in 300-350g guinea pigs. Two hundred microliters of Compound 1 (10 mM, final 2.8 mg/kg) in 100% DMSO was placed in a small catheter attached to the minipump.

Compounds were tested for an ability to modulate A β levels. In particular, STI-571, a member of the phenyl aminopyrimidine class of pharmacophores was tested. Specifically, the mesylate salt of STI-571 (also known as GLEEVECTM, Novartis) was tested. STI-571 is an ATP competitor known to target the ATP binding site on BCR-Abl tyrosine kinase, and also to inhibit Abl, c-kit and platelet-derived growth factor receptor (PDGFR). An STI-571 variant, WGB-BC-15 (see FIG. 20), was also tested. In addition, Compounds 1 and 2 (see FIG. 20), members of the pyrimidinyl pyridone tyrosine kinase inhibitor class of molecules, were tested.

After 7 days of treatment, animals were sacrificed, and their brains homogenized in 25 mM Tris 7.5, 50 mM NaCl, 1 mM DTT, 5 mM EDTA, 1 mM EGTA, and 1X protease inhibitor cocktail (CompleteTM, Boehringer Biochemicals). Aliquots of brain homogenate were assayed for A β peptide by using a sandwich ELISA according the manufacturer's recommendations (Biosource International, Camarillo, CA) and for C99 by immunoprecipitation and Western blot analysis using standard methods.

9.2. RESULTS

Levels of brain A β 40 and A β 42 were reduced (FIGS. 15A and 15B), and C99 increased (FIGS. 15C and 15D), in animals treated with 15.4 mg/kg STI-571. Similar changes were seen with 2.8 mg/kg Compound 1 (FIGS. 16A-D). Animals appeared healthy and active at these doses of compound.

These data demonstrate that *in vivo*, STI-571 and Compound 1 inhibit production of A β peptides. Further, because the amounts of C99 rose significantly, the compounds represent putative γ -secretase inhibitors, as the reduction in A β levels is likely due to the specific cleavage of C99.

10. EXAMPLE 5: ADMINISTRATION OF STI-571 OR COMPOUND 1 DOES NOT INHIBIT THE CLEAVAGE OF NOTCH

A major concern related to the use of γ -secretase inhibitors is their potential for inhibition of cell signaling through the Notch cell surface receptor. Notch receptors function in a variety of cell fate decisions in the developing nervous system and function in adult life in the immune system. Thus, it is generally preferred that the A β inhibition not significantly inhibit cleavage of Notch. This working example demonstrates that administration of STI-571 or Compound 1 to cultured cells at levels that strongly inhibit A β

formation does not affect Notch cleavage. As such, these compounds represent selective inhibitors of γ -secretase activity.

10.1. MATERIALS AND METHODS

5 N2a cells were transfected with a constitutively active truncated form of Notch, mouse "deleted-extracellular" Notch (m Δ e-Notch), which supplies a direct substrate for γ -secretase (De Strooper *et al.*, 1999, Nature 398: 518-522).

10.2. RESULTS

10 At levels of STI-571 (FIG.17) or Compound 1 (FIG. 18) that strongly inhibit A β formation, cleavage of m Δ e-Notch to form Notch intracellular domain (NICD) was unaffected.

11. EXAMPLE 6: STI-571 AND COMPOUND 1 INHIBIT PRODUCTION OF A β BY INHIBITION OF ARG KINASE BUT NOT ABL KINASE

15 Abl tyrosine kinases are known targets of STI-571 (Okuda *et al.*, 2001, Blood 97(8): 2440-2448). Abl kinase is a known target of Compound 1. To determine whether Abl kinase is required for A β production and whether the effects of the inhibitors STI-571 and Compound 1 on A β production result from inhibition of Abl kinase, the experiments described herein were conducted. The results presented in this working example
20 demonstrate that STI-571 and Compound 1 inhibit production of A β whether or not Abl kinase is present.

11.1. MATERIALS AND METHODS

25 Abl knockout (Abl^{-/-}) 3T3 mouse fibroblasts and wild-type 3T3 mouse fibroblasts (see Liu *et al.*, 1996, Nature 384(6606):273-6) were grown separately and to confluence in 100mm Corning tissue culture plates. These fibroblast cell lines are permanent cell lines. Several plates were exposed to either 5 μ M or 10 μ M STI-571 dissolved in water, or to no STI-571. Several other plates were exposed to either 0.5 μ M or 1.0 μ M Compound 1 (dissolved in DMSO) or to DMSO without Compound 1.

30 For the above-described cell cultures, each of the drugs or control solvents was added to a standard culture medium (DMEM + 10% FBS + pen/strep) at a dilution of 1:1000 and cultures were then incubated at 37°C for 2 hours. The contacting of cell cultures and incubation with inhibitors and solvent, as described above, constituted "pretreatment" of cells.

35 The first culture medium was then removed and the cell cultures were rinsed with a second culture medium lacking methionine, cysteine and glutamate. The cell cultures were

incubated with this culture medium for 30 minutes (to stimulate subsequent uptake of amino acids). Then the second culture medium was exchanged for fresh first culture medium containing ³⁵S-labeled methionine and cysteine (NEN Express label 50μl/ml) and unlabeled glutamine (final concentration 2mM) and with inhibitors as described above, and incubated for 4 hours at 37°C.

5 Media was collected and immunoprecipitated with 4G8 mouse monoclonal antibody (Senetek, Napa, California) to detect Aβ peptides and resolved by PAGE and autoradiography, according to standard methods (as described hereinabove). Cells were dissolved in 3%SDS, diluted in immunoprecipitation buffer containing 1% Triton X-100 and immunoprecipitated with 369 rabbit polyclonal antiserum (produced according to
10 standard methods) to detect APP and resolved by PAGE and autoradiography.

11.2. RESULTS

Both wild-type 3T3 fibroblasts and Abl -/- 3T3 fibroblasts produced similar quantities of Aβ peptide and p3 peptide (both products of γ-secretase activity) compared to
15 the quantities of APP produced by each cell line (quantities of APP were also similar in both cell lines). This demonstrates that Abl kinase is not necessary for Aβ production or for γ-secretase activity.

Furthermore, both STI-571 and Compound 1 inhibited Aβ production in both the 3T3 fibroblast and Abl -/- 3T3 fibroblast cells lines to similar degrees, and this inhibition
20 was also similar to the degree of inhibition of Aβ production achieved by the inhibitors in N2a cells. This indicates that the target of the inhibitors, responsible for Aβ inhibition, was intact, whether or not Abl kinase was present.

12. EXAMPLE 7: MECHANISM OF ACTION OF γ-SECRETASE ACTIVITY

25 It was previously noted (in FIGS.17 and 18) that Notch-I cleavage is not inhibited by the inhibitors STI-571 and Compound 1. Significantly, Notch cleavage is known to be catalyzed by γ-secretase activity. Thus γ-secretase inhibitors that inhibit Aβ production but not Notch cleavage (*i.e.*, selective γ-secretase inhibitors) may be used as therapeutic agents because they potentially produce fewer side effects that could injure the immune system, as
30 well as other Notch dependent systems.

This working example demonstrates that Notch-1 cleavage is not energy or ATP-dependent, or differentially affected by the presence (versus the absence) of ATP. Thus, without wishing to be bound by any particular theory, ATP modulators, such as inhibitors that compete with ATP for binding to protein targets, do not inhibit Notch cleavage and will
35 not be cytotoxic or affect cellular or developmental processes regulated by the Notch protein.

12.1. MATERIALS AND METHODS

A cell-free system derived from mΔe-Notch 1 transfected N2a cells (Leem *et al.*, 2002, *Neurobiol Dis.* 11(1):64-82) was created according to the methods described in Example 1 (see also, Xu *et al.*, 1997, *Proc. Natl. Acad. Sci. USA* 94: 3748–3752). The cells were pulse-labeled with radioactivity and chased at 20°C for 2 hours to accumulate labeled
5 Notch in the trans Golgi network (TGN). The cells were then incubated at 37°C for 20 to 25 minutes to synchronize localization of mΔe-Notch 1 to the cell surface (considered the primary site for Notch 1 cleavage). The cells were then permeabilized and incubated either with or without a standard ATP energy regenerating system with GTP (Xu *et al.*, 1997, *Proc. Natl. Acad. Sci. USA* 94: 3748–3752). Samples were incubated, under the same
10 conditions, either with or without a standard γ -secretase inhibitor (Calbiochem).

To measure mΔe Notch-1 and Notch intracellular domain (NICD, the product of Notch cleavage), permeabilized cells were sedimented and both sedimented cells and supernatant were immunoprecipitated with anti-myc antibody according to standard methods to detect Notch and NICD (both were labeled with myc tags).
15

12.2. RESULTS

NICD was found in the supernatant because the cells are permeabilized and hence NICD, which would otherwise be in the cytosol, could be retrieved by sedimenting the permeabilized cells, thus separating them from the supernatant. Furthermore, the standard
20 γ -secretase inhibitor inhibited Notch cleavage as expected (FIG.19), showing that γ -secretase activity is necessary for Notch cleavage in this system.

It was previously shown, in Section 10, that at levels of STI-571 (FIG. 17) or Compound 1 (FIG. 18) that strongly inhibit A β formation, cleavage of mΔe-Notch to form Notch intracellular domain (NICD) was unaffected. In the present example, it was
25 demonstrated that Notch is cleaved equally effectively in the presence or absence of a standard γ -secretase inhibitor, and in the presence or absence of ATP. Thus Notch cleavage is not ATP-dependent.

The present invention is not to be limited in scope by the specific embodiments
30 described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

All references cited herein are incorporated herein by reference in their entireties and
35 for all purposes to the same extent as if each individual publication, patent or patent

application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

WHAT IS CLAIMED IS:

1. A method for modulating amyloid- β peptide ($A\beta$) levels exhibited by a cell or tissue comprising contacting said cell or tissue with an amount of a compound sufficient to modulate said $A\beta$ levels, wherein said compound modulates an ATP-dependent enzymatic activity.
2. The method of claim 1 wherein the $A\beta$ levels are lowered.
3. The method of claim 1 wherein the $A\beta$ levels are raised.
4. The method of claim 1 wherein the $A\beta$ is $A\beta_{40}$.
5. The method of claim 1 wherein the $A\beta$ is $A\beta_{42}$.
6. The method of claim 1 wherein the modulating results in an increase in the ratio of $A\beta_{40}$ to $A\beta_{42}$.
7. The method of claim 1 wherein the modulating results in an increase in C99.
8. The method of claim 1 wherein the compound binds an enzyme that exhibits an ATP-dependent enzymatic activity.
9. The method of claim 1 wherein compound binds a molecule that regulates the ATP-enzymatic activity.
10. The method of claim 9 wherein the molecule is a molecule that is allosterically regulated by ATP.
11. The method of claim 1 wherein the compound is an ATP modulator.
12. The method of claim 11 wherein the ATP modulator is a selective modulator.
13. The method of claim 11 wherein the ATP modulator competes with ATP for binding to an ATP-binding site.

14. The method of claim 13 wherein the ATP-binding site is present on an enzyme that exhibits an ATP-dependent enzymatic activity.
15. The method of claim 13 wherein the ATP-binding site is present on a molecule that regulates the ATP-dependent enzymatic activity.
- 5 16. The method of claim 1 wherein the compound does not affect total cellular levels of β -amyloid precursor product (APP).
- 10 17. The method of claim 1 wherein the compound does not decrease levels of secreted APP (sAPP).
18. The method of claim 1 wherein the compound increases levels of sAPP α .
19. The method of claim 1 wherein levels of secreted A β are modulated.
- 15 20. The method of claim 1 wherein the compound does not inhibit Notch-1 cleavage.
21. The method of claim 1 wherein the compound crosses the blood-brain barrier.
- 20 22. The method of claim 1 wherein the enzymatic activity is a γ -secretase activity.
- 25 23. The method of claim 22 wherein the compound inhibits γ -secretase activity.
24. The method of claim 22 wherein the compound is an ATP modulator.
25. The method of claim 24 wherein the ATP modulator is a selective modulator.
- 30 26. The method of claim 22 wherein the compound binds a γ -secretase enzyme exhibiting an ATP-dependent enzymatic activity.
- 35 27. The method of claim 24 wherein the ATP modulator competes with ATP for binding to an ATP binding site.

- 28. The method of claim 24 wherein the compound is STI-571 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.
29. The method of claim 28 wherein the compound is a mesylate salt of STI-571.
- 5 30. The method of claim 24 wherein the compound is WGB-BC-15 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.
31. The method of claim 24 wherein the compound is Compound 1 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.
- 10 32. The method of claim 24 wherein the compound is Compound 2 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.
33. The method of claim 1 wherein the enzymatic activity is a kinase activity.
- 15 34. The method of claim 33 wherein the compound binds to a kinase enzyme exhibiting an ATP-dependent enzymatic activity.
35. The method of claim 34 wherein the compound competes with ATP for
- 20 binding to an ATP-binding site on the kinase.
36. The method of claim 34 wherein the compound binds to a site other than an ATP-binding site on the kinase.
- 25 37. The method of claim 33 wherein the kinase is a tyrosine kinase.
38. The method of claim 37 wherein the tyrosine kinase is Abl kinase, BCR-Abl kinase, ARG kinase, src kinase, c-kit or platelet-derived growth factor receptor.
- 30 39. The method of claim 37 wherein the kinase is a serine/threonine kinase, a carbohydrate kinase or a lipid kinase.
40. The method of claim 1 wherein the compound is STI-571 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.
- 35 41. The method of claim 40 wherein the compound is a mesylate salt of STI-571

42. The method of claim 1 wherein the compound is WGB-BC-15 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

43. The method of claim 1 wherein the compound is Compound 1 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

5

44. The method of claim 1 wherein the compound is Compound 2 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

10

45. A method for modulating A β levels exhibited by a cell or tissue comprising contacting said cell or tissue with an amount of an ATP modulator sufficient to modulate said A β levels.

46. The method of claim 45 wherein the A β levels are lowered.

15

47. The method of claim 45 wherein the A β levels are raised.

48. The method of claim 45 wherein the A β is A β 40.

49. The method of claim 45 wherein the A β is A β 42.

20

50. The method of claim 45 wherein the modulating results in an increase in the ratio of A β 40 to A β 42.

25

51. The method of claim 45 wherein the modulating results in an increase in C99.

52. The method of claim 45 wherein the ATP modulator competes with ATP for binding to an ATP-binding site.

30

53. The method of claim 45 wherein the ATP-binding site is present on an enzyme that exhibits an ATP-dependent enzymatic activity.

54. The method of claim 45 wherein the ATP-binding site is present on a molecule that regulates the ATP-dependent enzymatic activity.

35

55. The method of claim 45 wherein the compound does not affect total cellular levels of β -amyloid precursor product (APP).
56. The method of claim 45 wherein the compound does not decrease levels of secreted APP (sAPP).
57. The method of claim 45 wherein the compound increases levels of sAPP α .
58. The method of claim 45 wherein levels of secreted A β are modulated.
59. The method of claim 45 wherein the compound does not inhibit Notch-1 cleavage.
60. The method of claim 45 wherein the compound crosses the blood-brain barrier.
61. The method of claim 45 wherein the enzymatic activity is a γ -secretase activity.
62. The method of claim 61 wherein the compound inhibits γ -secretase activity.
63. The method of claim 61 wherein the compound is an ATP modulator.
64. The method of claim 63 wherein the ATP modulator is a selective modulator.
65. The method of claim 61 wherein the compound binds a γ -secretase enzyme exhibiting an ATP-dependent enzymatic activity.
66. The method of claim 45 wherein the compound is STI-571 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.
67. The method of claim 45 wherein the compound is a mesylate salt of STI-571.
68. The method of claim 45 wherein the compound is WGB-BC-15 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

69. The method of claim 45 wherein the compound is Compound 1 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

70. The method of claim 45 wherein the compound is Compound 2 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

5

71. The method of claim 45 wherein the enzymatic activity is a kinase activity.

72. The method of claim 45 wherein the compound binds to a kinase enzyme exhibiting an ATP-dependent enzymatic activity.

10

73. The method of claim 72 wherein the compound competes with ATP for binding to an ATP-binding site on the kinase.

74. The method of claim 72 wherein the compound binds to a site other than an ATP-binding site on the kinase.

15

75. The method of claim 71 wherein the kinase is a tyrosine kinase.

76. The method of claim 75 wherein the tyrosine kinase is Abl kinase, BCR-Abl kinase, ARG kinase, src kinase, c-kit or platelet-derived growth factor receptor.

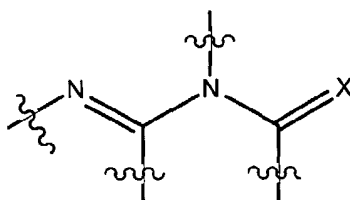
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77. The method of claim 71 wherein the kinase is a serine/threonine kinase, a carbohydrate kinase or a lipid kinase.

78. A method for modulating A β levels in a cell or tissue comprising contacting said cell or tissue with an amount of a compound sufficient to modulate said A β levels, wherein said compound contains the following pharmacophore:

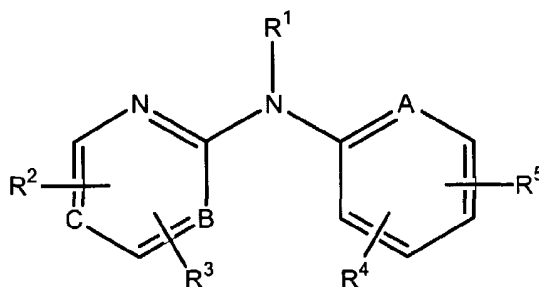
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wherein X is CH-, O, NH or N-CO-.



30

79. The method according to claim 78, wherein said pharmacophore containing compound comprises a compound of Figure I or a pharmaceutically acceptable salt thereof:
Figure I



A is CH or N;

5 B and C are independently CH, N or N⁺-O⁻;

R¹ is H, SO₂R^a, (C=O)_rO_sR^a;

R², R³, R⁴ and R⁵ are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl, (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl, (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^bR^c,

10 wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R⁶;

R⁶ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo, (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_s(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-NR^bR^c or (C₁-C₆)alkyl-heterocycl,

15 wherein said alkyl-heterocycl is optionally substituted with OH;

R^a is (C₁-C₆)alkyl, aryl or heterocycl; and

R^b and R^c independently are H, (C=O)_rO_s(C₁-C₁₀)alkyl, SO₂R^a, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl or CO₂R^a,

20 wherein r and s independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R⁶.

80. The method according to claim 79, wherein A is CH, B is N and C is CH.
25

81. The method according to claim 80, wherein R¹ is H.

82. The method according to claim 81, wherein R² is H and R³ is heteroaryl.

83. The method according to claim 82, wherein R^4 is $(C=O)_rO_s(C_1-C_{10})$ alkyl and R^5 is (C_0-C_6) alkyl- NR^bR^c .

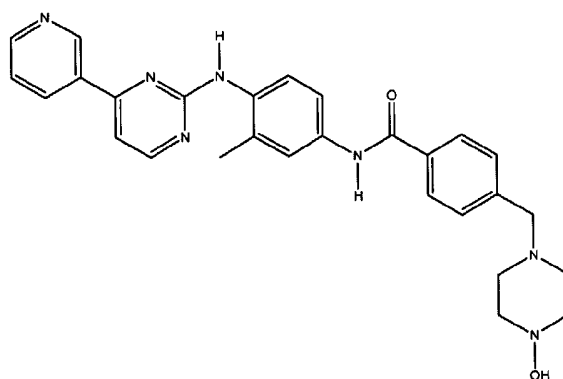
84. The method according to claim 82, wherein R^3 is 3-pyridinyl.

5 85. The method according to claim 84, wherein r is 0, s is 0 and (C_1-C_{10}) alkyl is methyl.

86. The method according to claim 85, wherein (C_0-C_6) alkyl is a direct bond (C_0) , R^b is H and R^c is $(C=O)_rO_s$ heteroaryl or $(C=O)_rO_s$ heterocycl.

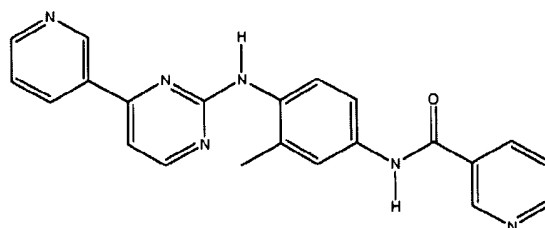
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87. The method according to claim 86, wherein $(C=O)_rO_s$ heterocycl is 4-hydroxy-1-piperazino, as illustrated below



or a pharmaceutically acceptable salt thereof.

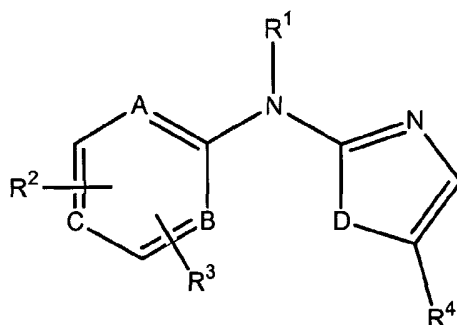
15 88. The method according to claim 86, wherein $(C=O)_rO_s$ heteroaryl is 3-pyridinyl, as illustrated below



or a pharmaceutically acceptable salt thereof.

89. The method according to claim 78, wherein said pharmacophore containing compound, comprises a compound of Figure II or a pharmaceutically acceptable salt thereof:

Figure II

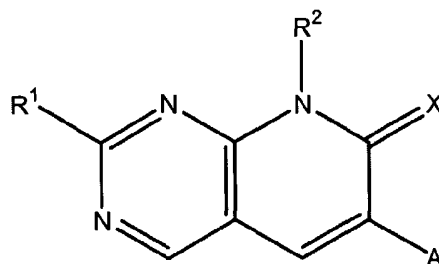


- 5 A, B and C are independently CH, N or N⁺-O⁻;
 D is O, S or N-R⁵;
 R¹ is H, SO₂R^a, (C=O)_rR^a or CO₂R^a;
 R², R³ and R⁴ are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl,
 (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl,
 10 (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl,
 (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^bR^c,
 wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl,
 aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents
 selected from R⁶;
- 15 R⁵ is H, aryl or (C₁-C₆)alkyl;
 R⁶ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo,
 (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_s(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-
 NR^bR^c or (C₁-C₆)alkyl-heterocycl;
 R^a is (C₁-C₆)alkyl, aryl or heterocycl; and
- 20 R^b and R^c independently are H, (C=O)_rO_s(C₁-C₁₀)alkyl, SO₂R^a, (C=O)_rO_sheterocycl,
 (C=O)_rO_saryl, (C=O)_rO_sheteroaryl or CO₂R^a,
 wherein r and s independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is
 optionally substituted with one or more substituents selected from R⁶.
- 25 90. The method according to claim 89, wherein R¹ is H.
91. The method according to claim 90, wherein R² is H and R³ is (C₀-C₆)alkyl-
 NR^bR^c.

92. The method according to claim 91, wherein R⁴ is heteroaryl.

93. The method according to claim 78, wherein said pharmacophore containing compound, comprises a compound of Figure III or a pharmaceutically acceptable salt thereof:

5 Figure III



A is aryl or heteroaryl,

wherein said aryl or heteroaryl is optionally substituted with one or more substituents selected from R³;

10 X is NH, N-acyl, O or S;

R¹ and R² are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl, (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl, (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^aR^b,

15 wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R³;

R³ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo, (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_sS_t(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-NR^aR^b or (C₁-C₆)alkyl-heterocycl;

20 R^a and R^b independently are H, (C=O)_rO_s(C₁-C₁₀)alkyl, SO₂R¹, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl or CO₂R¹,

wherein r, s and t independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R³.

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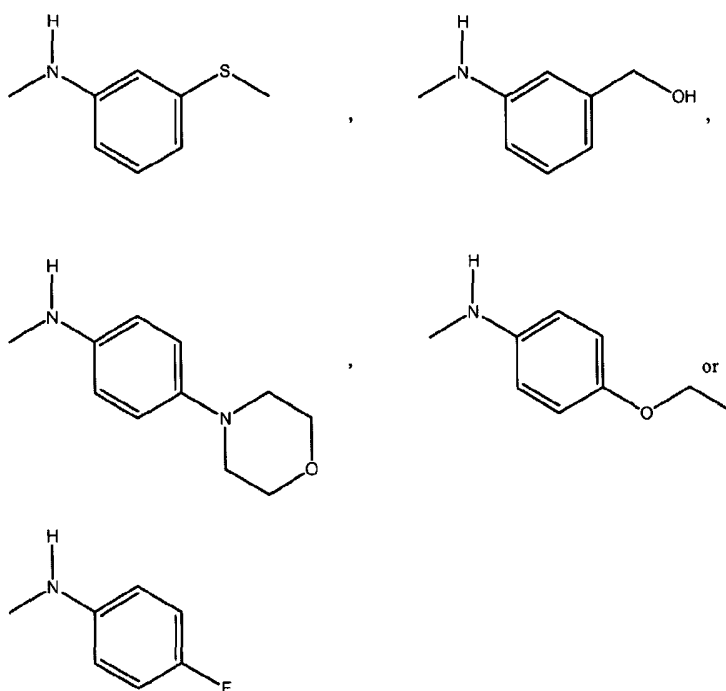
94. The method according to claim 93, wherein A is aryl.

95. The method according to claim 94, wherein the aryl is 2,5-dichlorophenyl.

96. The method according to claim 95, wherein R^1 is (C_0-C_6) alkyl- NR^aR^b and R^2 is $(C=O)_rO_s(C_1-C_{10})$ alkyl.

97. The method according to claim 96, wherein $(C=O)_rO_s(C_1-C_{10})$ alkyl is methyl.

5 98. The method according to claim 97, wherein (C_0-C_6) alkyl- NR^aR^b is



or a pharmaceutically acceptable salt thereof.

99. A pharmaceutical composition useful for modulating $A\beta$ level in a cell or
10 tissue, comprising:

an amount of a compound described in any one of claims 78 to 98 sufficient to
modulate said $A\beta$ level; and
a pharmaceutically acceptable excipient or carrier.

15 100. The pharmaceutical composition according to claim 99, wherein the
pharmaceutical composition is useful for modulating $A\beta$ levels exhibited by a cell or tissue.

101. The pharmaceutical composition according to claim 100, wherein the
compound is present in an amount sufficient to modulate said $A\beta$ levels.

20

102. A method of treating, or ameliorating a symptom of, an A β -related disorder comprising administering to a subject in need of such treating or ameliorating an amount of a compound sufficient to reduce A β levels in the subject, wherein the compound modulates an ATP-dependent enzymatic activity, such that the A β -related disorder is treated or a symptom of the A β related disorder is ameliorated.
- 5
103. The method of claim 102 wherein the A β related disorder is Alzheimer's disease.
104. The method of claim 102 wherein progression of the A β related disorder is
10 slowed.
105. The method of claim 102 wherein progression of the A β related disorder is reversed.
- 15
106. The method of claim 102 wherein the subject is a human.
107. The method of claim 106 wherein the subject is an subject at risk for a familial form of Alzheimer's disease.
- 20
108. The method of claim 102 wherein the ATP-dependent enzymatic activity is lowered.
109. The method of claim 102 wherein the compound crosses the blood-brain
25 barrier.
110. The method of claim 102 wherein the compound is administered orally.
111. The method of claim 102 wherein the compound is administered with a
30 NSAID.
112. The method of claim 111 wherein the NSAID is sulindac sulfide, flufenamic acid , ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen, meclofenamic acid.
- 35
113. The method of claim 102 wherein the compound is administered with an antioxidant.

114. The method of claim 113 wherein said antioxidant is selected from the group consisting of vitamin E, vitamin C, curcumin, and Gingko biloba.
115. The method of claim 102 wherein the compound is administered with an acetylcholinesterase inhibitor.
- 5 116. The method of claim 102 wherein the compound is an ATP modulator.
117. The method of claim 116 wherein the ATP modulator is a selective modulator.
- 10 118. The method of claim 102 wherein the enzymatic activity is a γ -secretase activity.
119. The method of claim 102 wherein the compound binds a γ -secretase enzyme exhibiting an ATP-dependent enzymatic activity.
- 15 120. The method of claim 116 wherein the ATP modulator competes with ATP for binding to an ATP binding site.
121. The method of claim 102 wherein the compound is STI-571 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.
- 20 122. The method of claim 121 wherein the compound is a mesylate salt of STI-571.
- 25 123. The method of claim 102 wherein the compound is WGB-BC-15 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.
124. The method of claim 102 wherein the compound is Compound 1 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.
- 30 125. The method of claim 102 wherein the compound is Compound 2 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.
- 35 126. The method of claim 102 wherein the enzymatic activity is a kinase activity.

127. The method of claim 102 wherein the compound binds to a kinase enzyme exhibiting an ATP-dependent enzymatic activity.

128. The method of claim 102 wherein the compound competes with ATP for binding to an ATP-binding site on the kinase.

5

129. The method of claim 102 wherein the compound binds to a site other than an ATP-binding site on the kinase.

130. The method of claim 126 wherein the kinase is a tyrosine kinase.

10

131. The method of claim 130 wherein the tyrosine kinase is Abl kinase, BCR-Abl kinase, ARG kinase, src kinase, c-kit, platelet-derived growth factor receptor.

132. The method of claim 126 wherein the kinase is a serine/threonine kinase.

15

133. The method of claim 126 wherein the kinase is a carbohydrate kinase or a lipid kinase.

134. The method of claim 102 wherein the compound modulates the activity of a protease, a phosphatase or a molecular chaperone molecule.

20

135. A method of treating, or ameliorating a symptom of, an A β -related disorder comprising administering to a subject in need of such treating or ameliorating an amount of an ATP modulator sufficient to reduce A β levels in the subject, such that the A β -related disorder is treated or a symptom of the A β related disorder is ameliorated.

25

136. The method of claim 135 wherein the A β related disorder is Alzheimer's disease.

30

137. The method of claim 135 wherein the subject is a human.

138. The method of claim 135 wherein the subject is an subject at risk for a familial form of Alzheimer's disease.

35

139. The method of claim 135 wherein the ATP modulator crosses the blood-brain barrier.

140. The method of claim 135 wherein the ATP modulator is administered orally.
141. The method of claim 135 wherein the ATP modulator is administered with a NSAID.
- 5 142. The method of claim 141 wherein the NSAID is sulindac sulfide, flufenamic acid, ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen, meclofenamic acid.
- 10 143. The method of claim 135 wherein the ATP modulator is administered with an antioxidant.
144. The method of claim 143 wherein said antioxidant is selected from the group consisting of vitamin E, vitamin C, curcumin, and Gingko biloba.
- 15 145. The method of claim 135 wherein the ATP modulator is administered with an acetylcholinesterase inhibitor.
- 20 146. The method of claim 135 wherein the ATP modulator is a selective modulator.
147. The method of claim 135 wherein the ATP modulator modulates a γ -secretase activity.
- 25 148. The method of claim 147 wherein the compound binds a γ -secretase enzyme exhibiting an ATP-dependent enzymatic activity.
149. The method of claim 135 wherein the ATP modulator competes with ATP for binding to an ATP binding site.
- 30 150. The method of claim 135 wherein the compound is STI-571 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.
- 35 151. The method of claim 150 wherein the compound is a mesylate salt of STI-571.

152. The method of claim 135 wherein the compound is WGB-BC-15 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

153. The method of claim 135 wherein the compound is Compound 1 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

5

154. The method of claim 135 wherein the compound is Compound 2 or a blood-brain barrier permeable variant thereof, or a pharmaceutically acceptable salt thereof.

155. The method of claim 135 wherein the ATP modulator modulates a kinase activity.

10

156. The method of claim 155 wherein the compound binds to a kinase enzyme exhibiting an ATP-dependent enzymatic activity.

157. The method of claim 156 wherein the compound competes with ATP for binding to an ATP-binding site on the kinase.

15

158. The method of claim 156 wherein the compound binds to a site other than an ATP-binding site on the kinase.

20

159. The method of claim 156 wherein the kinase is a tyrosine kinase.

160. The method of claim 159 wherein the tyrosine kinase is Abl kinase, BCR-Abl kinase, ARG kinase, src kinase, c-kit, platelet-derived growth factor receptor.

25

161. The method of claim 155 wherein the kinase is a serine/threonine kinase, a carbohydrate kinase or a lipid kinase.

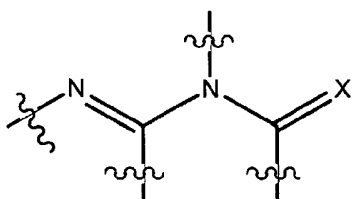
162. The method of claim 135 wherein the compound modulates the activity of a protease, a phosphatase or a molecular chaperone molecule.

30

163. A method of treating, or ameliorating a symptom of, an A β -related disorder comprising administering to a subject in need of such treating or ameliorating an amount of a compound sufficient to reduce A β levels in the subject, such that the A β -related disorder is treated or a symptom of the A β -related disorder is ameliorated, wherein the compound contains the following pharmacophore:

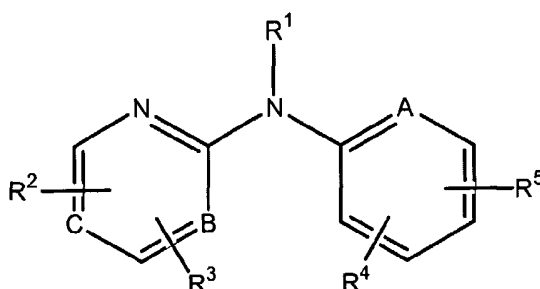
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wherein X is CH-, O, NH or N-CO-.



164. The method according to claim 163, wherein said pharmacophore containing compound comprises a compound of Figure I or a pharmaceutically acceptable salt thereof:

5 Figure I



A is CH or N;

B and C are independently CH, N or N⁺-O⁻;

R¹ is H, SO₂R^a, (C=O)_rO_sR^a;

10 R², R³, R⁴ and R⁵ are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl, (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl, (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^bR^c,

15 wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R⁶;

R⁶ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo, (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_s(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-NR^bR^c or (C₁-C₆)alkyl-heterocycl,

20 wherein said alkyl-heterocycl is optionally substituted with OH;

R^a is (C₁-C₆)alkyl, aryl or heterocycl; and

R^b and R^c independently are H, (C=O)_rO_s(C₁-C₁₀)alkyl, SO₂R^a, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl or CO₂R^a,

wherein r and s independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R⁶.

165. The method according to claim 164, wherein A is CH, B is N and C is CH.

5 166. The method according to claim 165, wherein R¹ is H.

167. The method according to claim 166, wherein R² is H and R³ is heteroaryl.

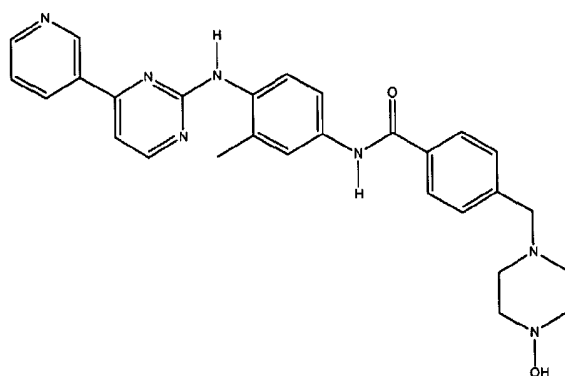
10 168. The method according to claim 157, wherein R⁴ is (C=O)_rO_s(C₁-C₁₀)alkyl and R⁵ is (C₀-C₆)alkyl-NR^bR^c.

169. The method according to claim 166, wherein R³ is 3-pyridinyl.

15 170. The method according to claim 168, wherein r is 0, s is 0 and (C₁-C₁₀)alkyl is methyl.

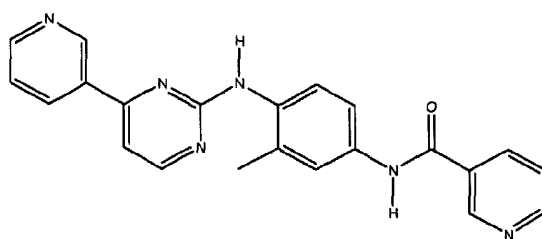
171. The method according to claim 168, wherein (C₀-C₆)alkyl is a direct bond (C₀), R^b is H and R^c is (C=O)_rO_sheteroaryl or (C=O)_rO_sheterocycl.

20 172. The method according to claim 171, wherein (C=O)_rO_sheterocycl is 4-hydroxy-1-piperazino, as illustrated below



or a pharmaceutically acceptable salt thereof.

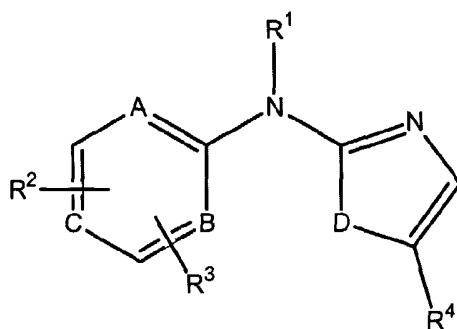
25 173. The method according to claim 9, wherein (C=O)_rO_sheteroaryl is 3-pyridinyl, as illustrated below



or a pharmaceutically acceptable salt thereof.

174. The method according to claim 163, wherein said pharmacophore containing compound, comprises a compound of Figure II or a pharmaceutically acceptable salt thereof:

5 Figure II



A, B and C are independently CH, N or N⁺-O⁻;

D is O, S or N-R⁵;

R¹ is H, SO₂R^a, (C=O)_rR^a or CO₂R^a;

10 R², R³ and R⁴ are independently H, OH, CHO, CN, halogen, (C=O)_rO_s(C₁-C₁₀)alkyl, (C=O)_rO_s(C₂-C₁₀)alkenyl, (C=O)_rO_s(C₂-C₁₀)alkynyl, (C=O)_rO_scycloalkyl, (C=O)_rO_scycloalkenyl, (C=O)_rO_scycloalkynyl, (C=O)_rO_sheterocycl, (C=O)_rO_saryl, (C=O)_rO_sheteroaryl, (C=O)_rO_sperfluoroalkyl or (C₀-C₆)alkyl-NR^bR^c,
 15 aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R⁶;

R⁵ is H, aryl or (C₁-C₆)alkyl;

20 R⁶ is (C=O)_rO_sNR^aR^b, (C=O)_rO_saryl, (C=O)_rO_sheterocycl, halogen, OH, oxo, (C=O)_rO_s(C₁-C₃)perfluoroalkyl, (C=O)_rO_s(C₁-C₆)alkyl, CHO, CO₂H, CN, (C₀-C₆)alkyl-NR^bR^c or (C₁-C₆)alkyl-heterocycl;

R^a is (C_1-C_6) alkyl, aryl or heterocycyl; and

R^b and R^c independently are H, $(C=O)_rO_s(C_1-C_{10})$ alkyl, SO_2R^a , $(C=O)_rO_s$ heterocycyl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl or CO_2R^a ,

wherein r and s independently are 0 or 1 and said alkyl, heterocycyl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R^6 .

5

175. The method according to claim 174, wherein R^1 is H.

176. The method according to claim 175, wherein R^2 is H and R^3 is (C_0-C_6) alkyl- NR^bR^c .

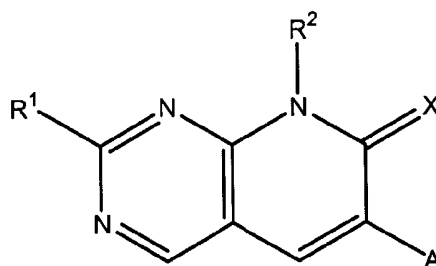
10

177. The method according to claim 176, wherein R^4 is heteroaryl.

178. The method according to claim 163, wherein said pharmacophore containing compound, comprises a compound of Figure III or a pharmaceutically acceptable salt thereof:

15

Figure III



A is aryl or heteroaryl,

wherein said aryl or heteroaryl is optionally substituted with one or more substituents selected from R^3 ;

20

X is NH, N-acyl, O or S;

R^1 and R^2 are independently H, OH, CHO, CN, halogen, $(C=O)_rO_s(C_1-C_{10})$ alkyl, $(C=O)_rO_s(C_2-C_{10})$ alkenyl, $(C=O)_rO_s(C_2-C_{10})$ alkynyl, $(C=O)_rO_s$ cycloalkyl, $(C=O)_rO_s$ cycloalkenyl, $(C=O)_rO_s$ cycloalkynyl, $(C=O)_rO_s$ heterocycyl, $(C=O)_rO_s$ aryl,

25

$(C=O)_rO_s$ heteroaryl, $(C=O)_rO_s$ perfluoroalkyl or (C_0-C_6) alkyl- NR^aR^b ,

wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycyl, aryl, heteroaryl and perfluoroalkyl is optionally substituted with one or more substituents selected from R^3 ;

R^3 is $(C=O)_rO_sNR^aR^b$, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heterocycl, halogen, OH, oxo, $(C=O)_rO_s(C_1-C_3)$ perfluoroalkyl, $(C=O)_rO_sS_1(C_1-C_6)$ alkyl, CHO, CO_2H , CN, (C_0-C_6) alkyl-NR^aR^b or (C_1-C_6) alkyl-heterocycl;

R^a and R^b independently are H, $(C=O)_rO_s(C_1-C_{10})$ alkyl, SO_2R^1 , $(C=O)_rO_s$ heterocycl, $(C=O)_rO_s$ aryl, $(C=O)_rO_s$ heteroaryl or CO_2R^1 ,

5 wherein r, s and t independently are 0 or 1 and said alkyl, heterocycl, aryl or heteroaryl is optionally substituted with one or more substituents selected from R^3 .

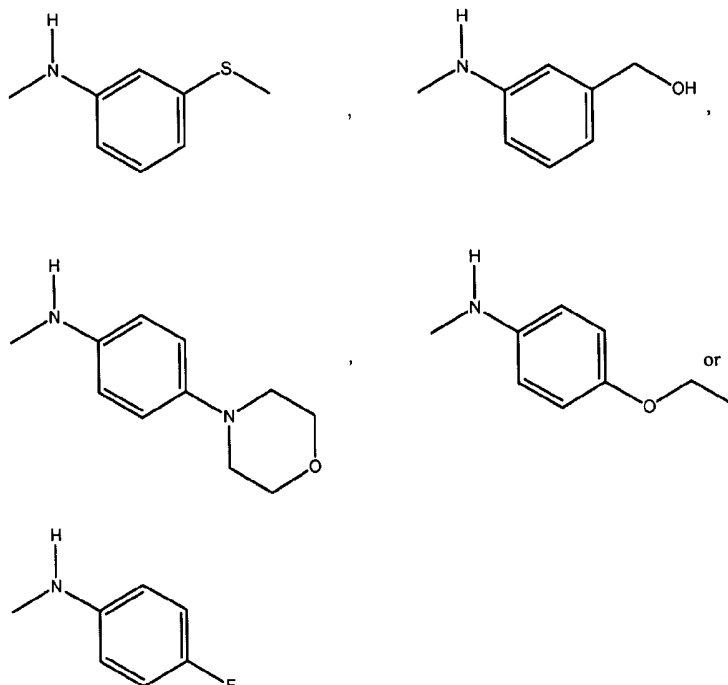
179. The method according to claim 178 wherein A is aryl.

10 180. The method according to claim 179 wherein the aryl is 2,5-dichlorophenyl.

181. The method according to claim 180, wherein R^1 is (C_0-C_6) alkyl-NR^aR^b and R^2 is $(C=O)_rO_s(C_1-C_{10})$ alkyl.

15 182. The method according to claim 181, wherein $(C=O)_rO_s(C_1-C_{10})$ alkyl is methyl.

183. The method according to claim 182, wherein (C_0-C_6) alkyl-NR^aR^b is



20 or a pharmaceutically acceptable salt thereof.

184. The method of claim 163 wherein the agent is administered orally.

185. The method of claim 163 wherein the compound is administered with a NSAID.

5 186. The method of claim 185 wherein the NSAID is sulindac sulfide, flufenamic acid, ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen, meclofenamic acid.

10 187. The method of claim 163 wherein the compound is administered with an antioxidant.

188. The method of claim 187 wherein said antioxidant is selected from the group consisting of vitamin E, vitamin C, curcumin, and Ginkgo biloba.

15 189. The method of claim 163 wherein the compound is administered with an acetylcholinesterase inhibitor.

20 190. A pharmaceutical composition comprising an amount of a compound described in any one of claims 163 to 183 sufficient to modulate A β levels in a cell or tissue, and a pharmaceutically acceptable excipient or carrier.

25 191. A pharmaceutical composition comprising a compound that lowers ATP-dependent A β levels in a cell or tissue, a compound selected from the group consisting of: an antioxidant, a non-selective COX inhibitor and an acetylcholinesterase inhibitor, and a pharmaceutically acceptable excipient or carrier.

30 192. An article of manufacture comprising packaging material and a pharmaceutical composition comprising a compound that lowers ATP-dependent A β levels, and a pharmaceutically acceptable carrier contained within the packaging material, said pharmaceutical composition in a form suitable for administration to a subject.

193. The article of manufacture of Claim 192, further comprising printed instructions regarding the use or administration of the pharmaceutical composition.

194. The article of manufacture of Claim 193, wherein the instructions suggest a dosing regimen for the prevention, treatment, or amelioration of a symptom of an A β -related disorder.

5 195. The article of manufacture of Claim 194, wherein the instructions suggest a dosing regimen for the prevention, treatment, or amelioration of a symptom of Alzheimer's disease.

10 196. The article of manufacture of Claim 192, further comprising a label regarding the use or administration of the pharmaceutical composition.

197. The article of manufacture of Claim 196, wherein the label suggests a dosing regimen for the prevention, treatment, or amelioration of a symptom of an A β -related disorder.

15 198. The article of manufacture of Claim 197, wherein the label suggests a dosing regimen for the prevention, treatment, or amelioration of a symptom of Alzheimer's disease.

20 199. The article of manufacture of claim 195 wherein said article of manufacture further comprises an antioxidant, a non-selective COX inhibitor or an acetylcholinesterase inhibitor.

25 200. The article of manufacture of claim 198 wherein said pharmaceutical composition further comprises an antioxidant, a non-selective COX inhibitor or an acetylcholinesterase inhibitor.

201. A method of identifying a compound that modulates A β levels exhibited by a cell or tissue comprising:

- 30 (a) determining a first level of γ -secretase activity in said cell or tissue;
(b) contacting said cell or tissue with a test compound; and
(c) determining a second level of γ -secretase activity in said cell or tissue,
wherein a difference in said first level and said second level of γ -secretase activity is indicative of the ability of said test compound to modulate A β levels.

35 202. The method of claim 201 wherein the difference in γ -secretase activity is indicative of the ability of said test compound to modulate the A β levels.

203. The method of claim 202 wherein A β levels are modulated.

204. The method of claim 203 wherein the γ -secretase activity is an ATP-dependent enzymatic activity.

5 205. The method of claim 204 wherein the γ -secretase activity is production of A β .

206. A method of identifying a compound that modulates A β levels exhibited by a cell or tissue comprising:

10 (a) contacting said cell or tissue with a test compound; and

(b) determining a level of activity of γ -secretase in said cell or tissue;

wherein a difference in said level and a control level of γ -secretase activity in a comparable cell or tissue not contacted with the test compound is indicative of the ability of said test compound to modulate A β levels.

15

207. The method of claim 206 wherein A β levels are modulated.

208. The method of claim 207 wherein the γ -secretase activity is an ATP-dependent enzymatic activity.

20

209. The method of claim 208 wherein the γ -secretase activity is production of A β .

25

210. A method for identifying an agent to be tested for an ability to treat an A β -related disorder in a patient in need of such treatment comprising:

(a) contacting in a cell or tissue γ -secretase with a potential agent; and

(b) detecting the amount of γ -secretase activity

wherein the agent is identified if a decrease in γ -secretase activity is detected in the presence of the potential agent and wherein the agent modulates A β levels.

30

211. The method of claim 210 wherein the ability to treat the A β -related disorder is tested.

35

212. The method of claim 211 wherein the γ -secretase activity is an ATP-dependent enzymatic activity.

213. The method of claim 212 wherein the γ -secretase activity is production of $A\beta$.

214. A method for identifying an agent to be tested for an ability to modulate $A\beta$ levels exhibited by a cell or tissue comprising:

- 5 (a) determining a first level of γ -secretase activity in said cell or tissue;
(b) contacting said cell or tissue with a potential agent; and
(c) determining a second level of γ -secretase activity in said cell or tissue,
wherein a difference in said first level and said second level of γ -secretase activity is
indicative of the ability of said potential agent to modulate $A\beta$ levels.

10 215. The method of claim 214 comprising the additional step of:

- (d) determining whether the $A\beta$ levels are modulated.

216. The method of claim 215 wherein the γ -secretase activity is an ATP-
dependent enzymatic activity.

15

217. The method of claim 216 wherein the γ -secretase activity is production of
 $A\beta$.

20

218. A method for identifying an agent to be tested for an ability to modulate $A\beta$ levels exhibited by a cell or tissue comprising:

- (a) contacting said cell or tissue with a potential agent; and
(b) determining a level of γ -secretase activity in said cell or tissue;
wherein a difference in said level and a control level of γ -secretase activity in a comparable
cell or tissue not contacted with the test compound is indicative of the ability of said
25 potential agent to modulate $A\beta$ levels.

219. The method of claim 218 comprising the additional step of:

- (c) determining whether the $A\beta$ levels are modulated.

30

220. The method of claim 219 wherein the γ -secretase activity is an ATP-
dependent enzymatic activity.

35

221. The method of claim 220 wherein the γ -secretase activity is production of
 $A\beta$.

222. A method for selecting a potential therapeutic agent for use in the treatment of an A β -related disorder comprising:
- (a) administering a potential therapeutic agent to an animal;
 - (b) measuring the response of said animal to said potential therapeutic agent;
 - (c) comparing the response of said animal with that of a control animal to which the potential therapeutic agent has not been administered; and
 - (d) selecting a potential therapeutic agent based on the difference in responses observed between said animal and said control animal,
- wherein the potential therapeutic agent modulates ATP-dependent γ -secretase activity.
223. The method of claim 222 wherein the animal is a guinea pig.
224. The method of claim 223 wherein the disorder is Alzheimer's disease.
225. A method for selecting a potential therapeutic agent for use in the treatment of an A β -related disorder comprising:
- (a) administering a potential therapeutic agent to an animal;
 - (b) measuring the response of said animal to administration of an agent that modulates activity of γ -secretase;
 - (c) comparing the response of said animal with that of a control animal to which the potential therapeutic agent has not been administered; and
 - (d) selecting a potential therapeutic agent based on the difference in responses observed between said animal and said control animal,
- wherein the potential therapeutic agent modulates ATP-dependent γ -secretase activity.
226. The method of claim 225 wherein the animal is a guinea pig.
227. The method of claim 226 wherein the disorder is Alzheimer's disease.
228. A method for selecting a potential therapeutic agent for use in the treatment of an A β -related disorder comprising:
- (a) administering a potential therapeutic agent to an animal;
 - (b) measuring the response of said animal, wherein the response is selected from the group consisting of:
 - (i) exhibition of behavior in a Morris water maze; and
 - (i) exhibition of behavior in a Y-maze

- (c) comparing the response of said animal with that of a control animal to which the potential therapeutic agent has not been administered; and
- (d) selecting a potential therapeutic agent based on the difference in responses observed between said animal and said control animal,

wherein the potential therapeutic agent modulates A β levels.

5

229. The method of claim 228 wherein the animal is a guinea pig.

230. The method of claim 229 wherein the disorder is Alzheimer's disease.

10

231. A method for preventing, delaying or reversing the progression of an A β -related disorder comprising:

- (a) identifying a mammal in need of prevention, delay, or reversal of the progression of the disorder; and
- (b) administering to said mammal an amount of an agent sufficient to modulate

15

ATP-dependent γ -secretase activity,
wherein A β levels are modulated.

232. The method of claim 231 wherein the agent inhibits or decreases ATP-dependent γ -secretase activity.

20

233. The method of claim 232 wherein the mammal is human.

234. The method of claim 233 wherein the disorder is Alzheimer's disease.

25

235. The method of claim 231 wherein the agent promotes or increases ATP-dependent γ -secretase activity.

236. The method of claim 231 wherein the agent is administered orally.

30

237. The method of claim 231 wherein the agent is administered with a NSAID.

238. The method of claim 237 wherein the NSAID is sulindac sulfide, flufenamic acid, ibuprofen, flurbiprofen, fenoprofen, mefenamic acid, indomethacin, carprofen, meclufenamic acid.

35

239. The method of claim 231 wherein the agent is administered with an

antioxidant.

240. The method of claim 240 wherein said antioxidant is selected from the group consisting of vitamin E, vitamin C, curcumin, and Ginkgo biloba.

5 241. The method of claim 231 wherein the agent is administered with an acetylcholinesterase inhibitor.

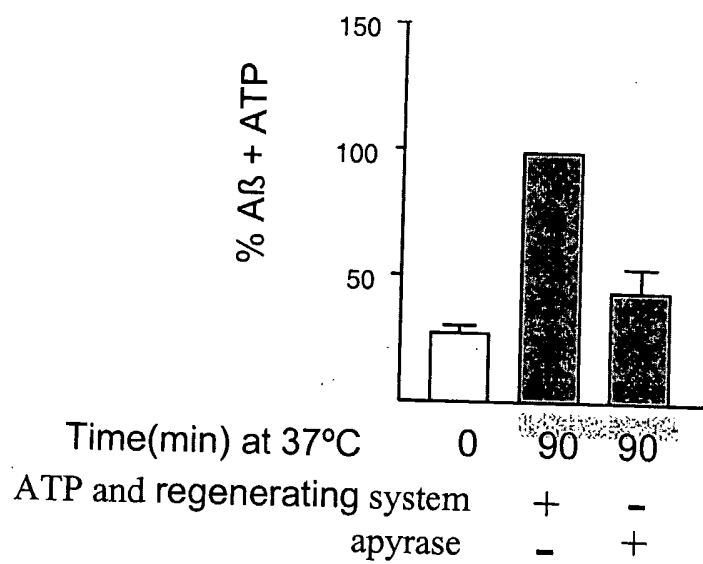


FIG. 1

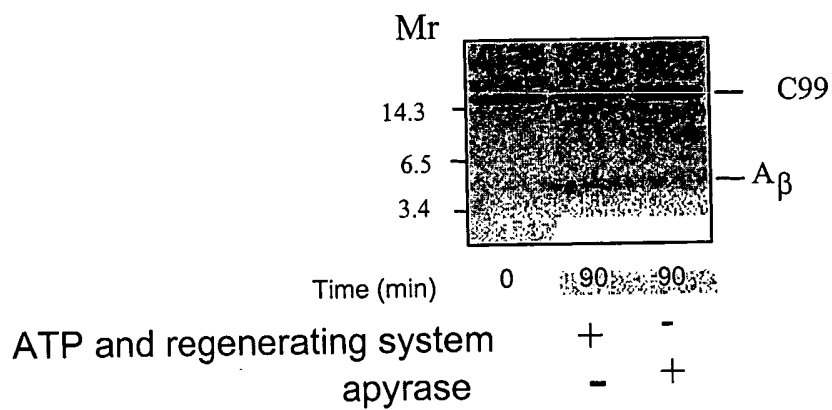


FIG. 2

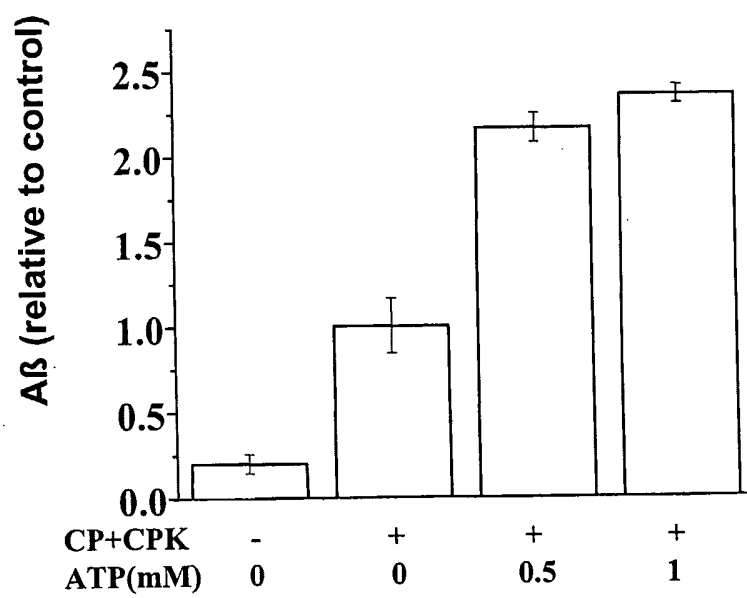


FIG. 3

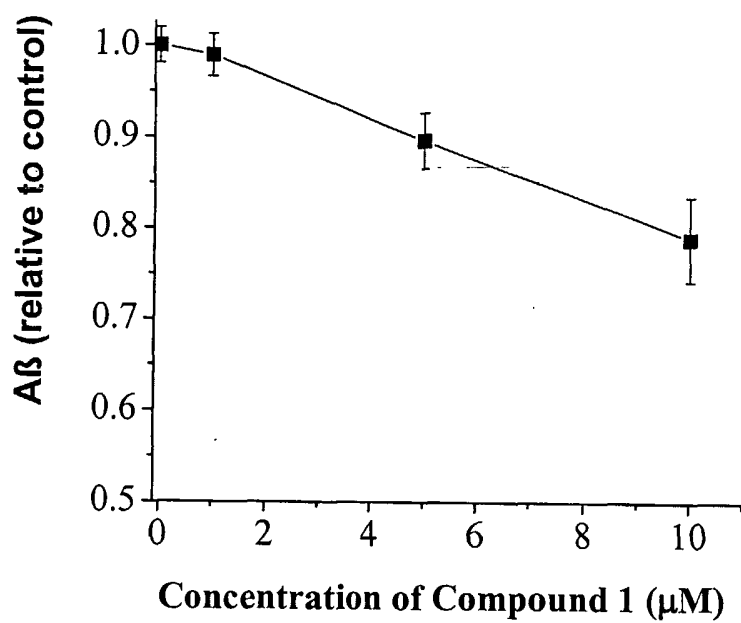


FIG. 4

COMPOUND 1 (M)

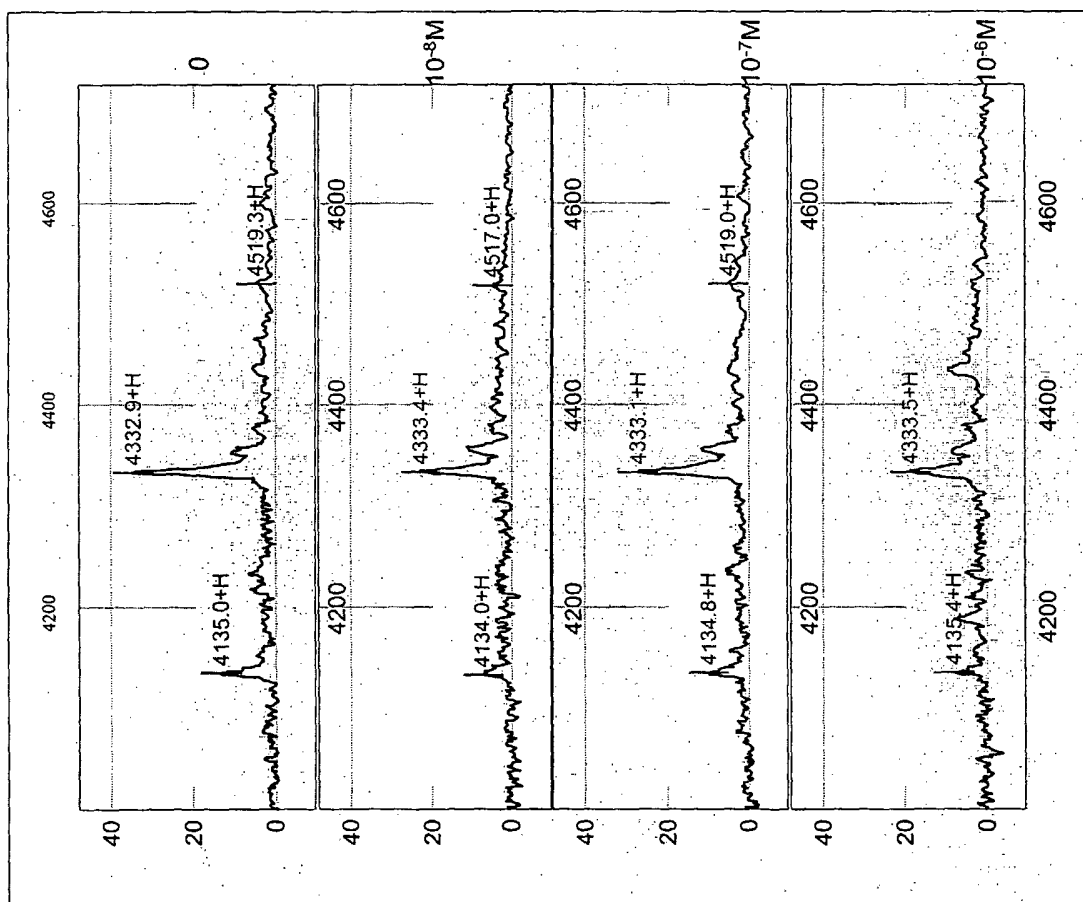


FIG. 5

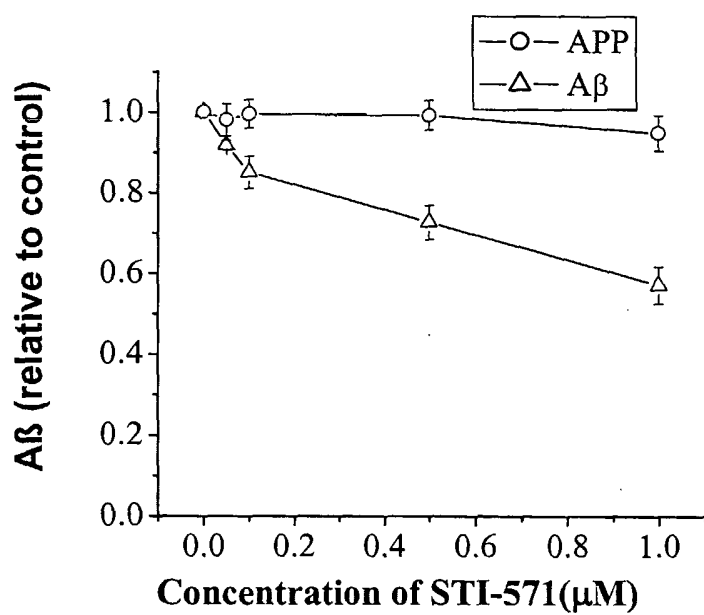


FIG. 6

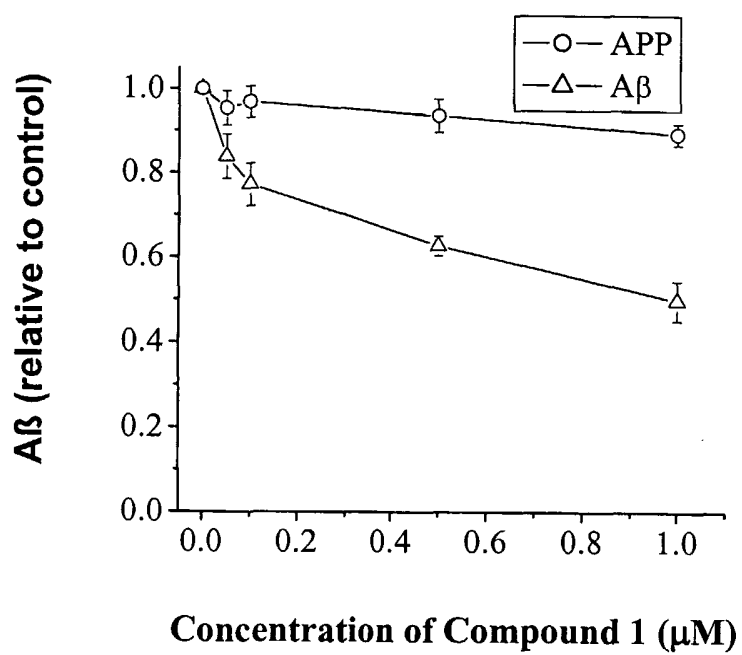


FIG. 7

FIG. 8A

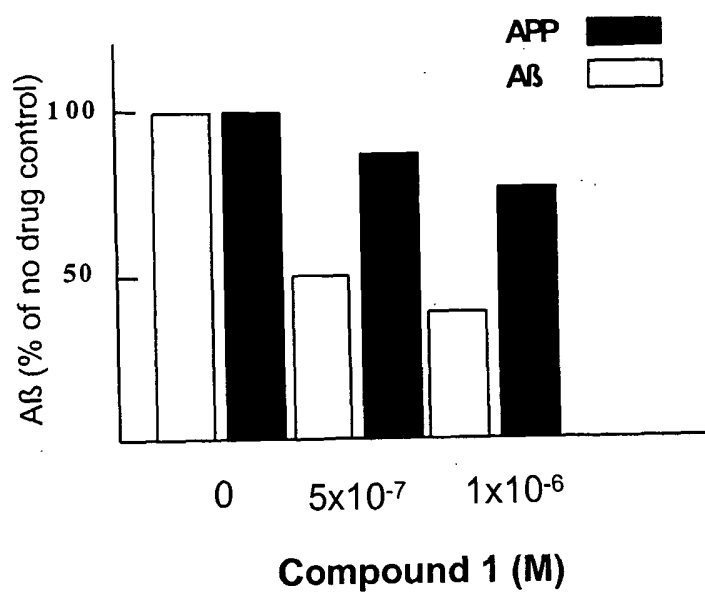
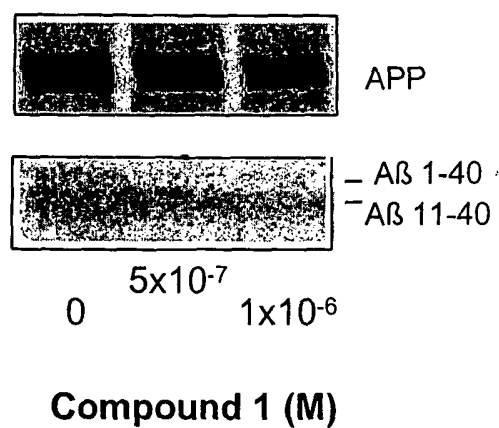


FIG. 8B



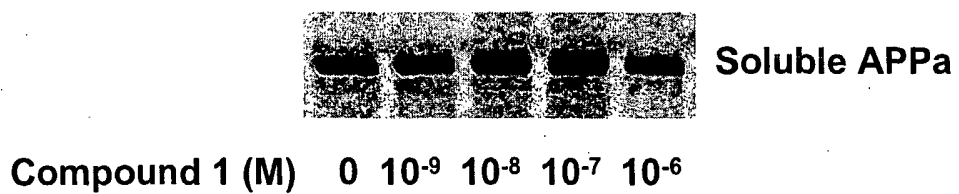


FIG. 9

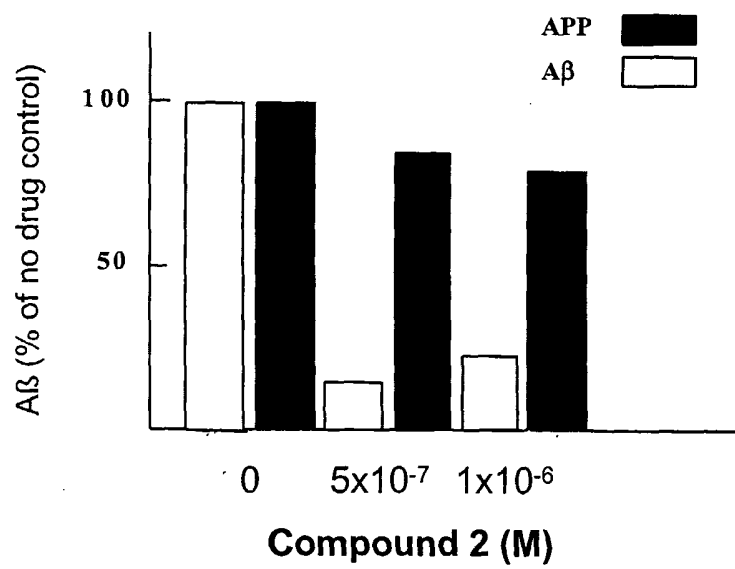


FIG. 10

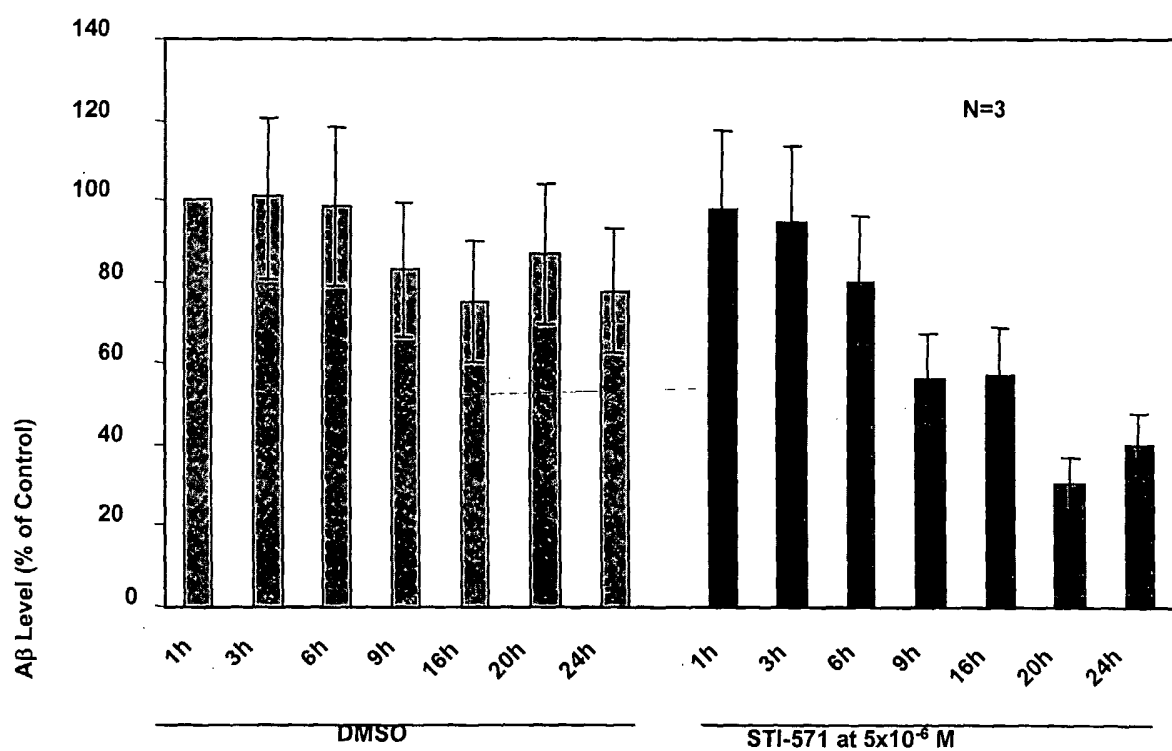


FIG. 11

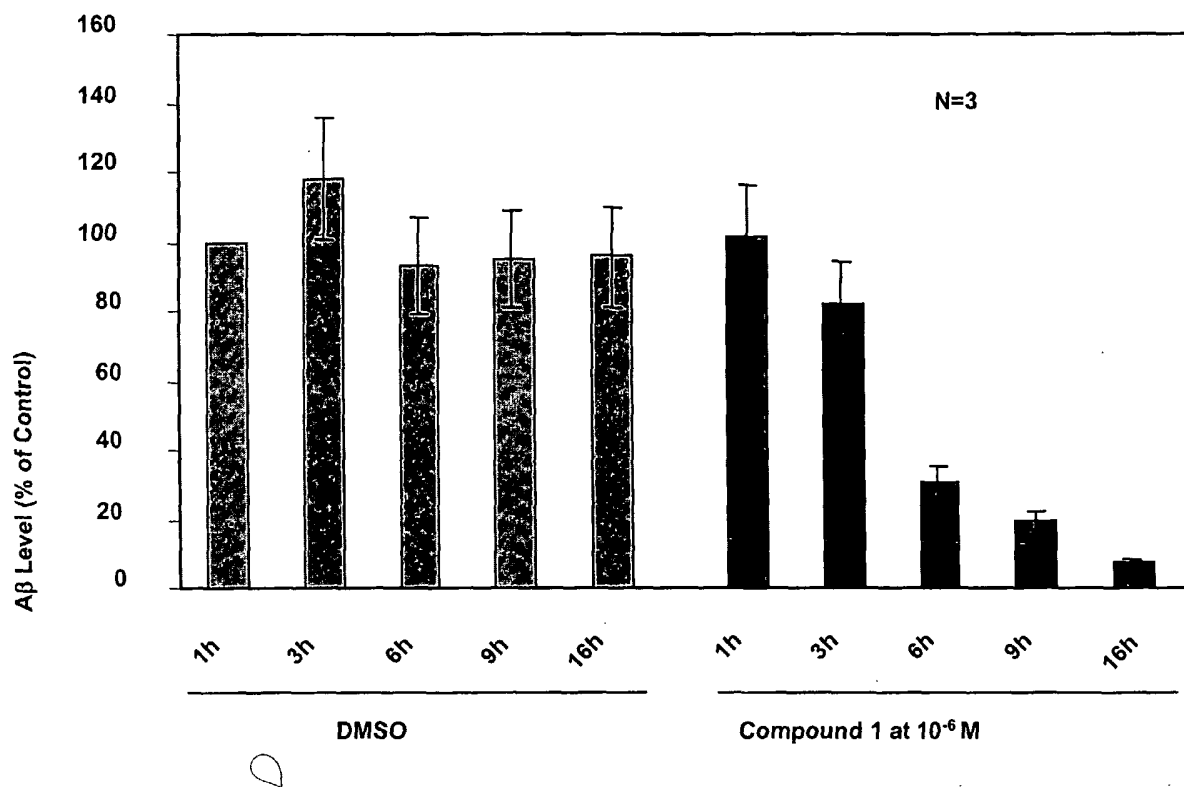


FIG. 12

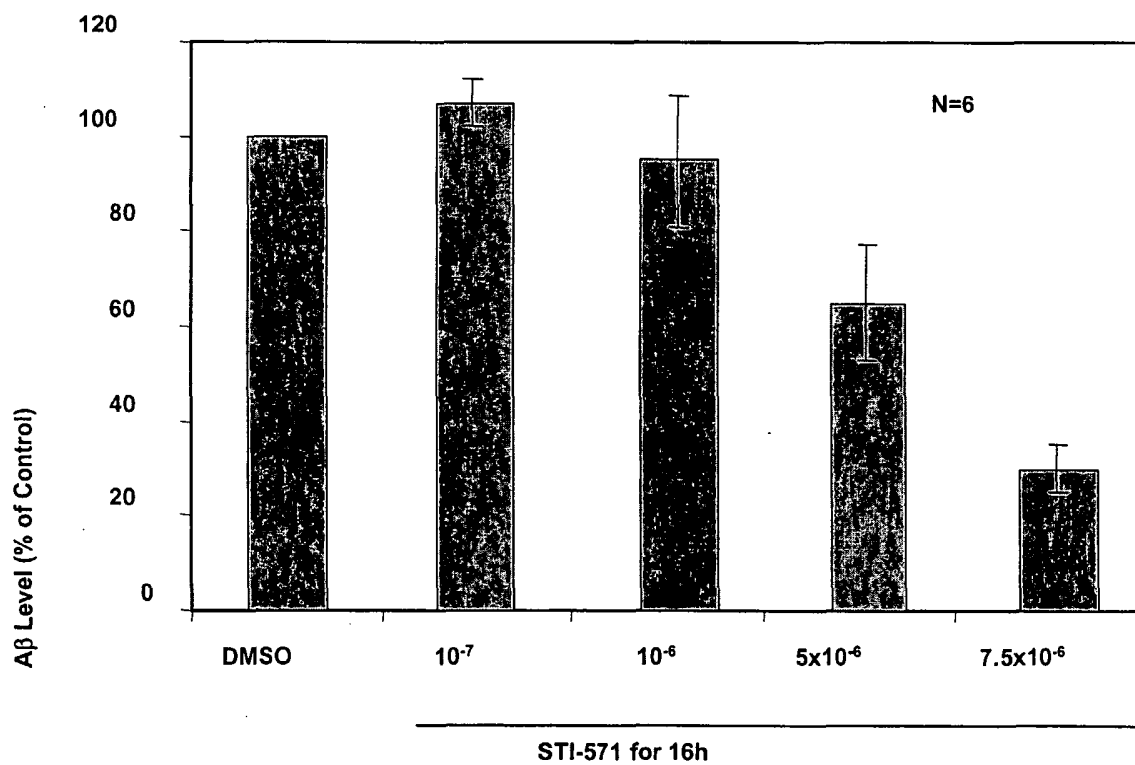


FIG. 13

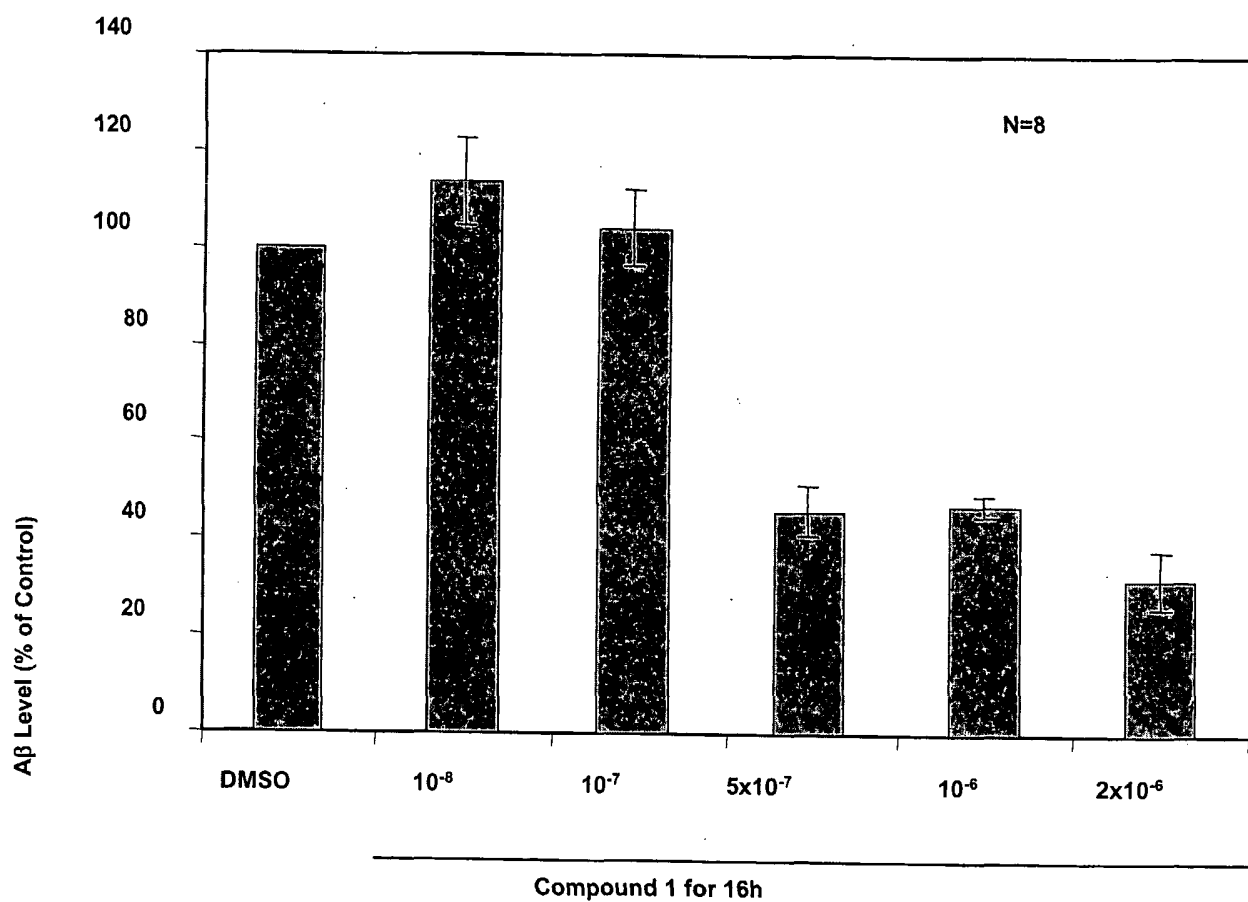


FIG. 14

FIG. 15A

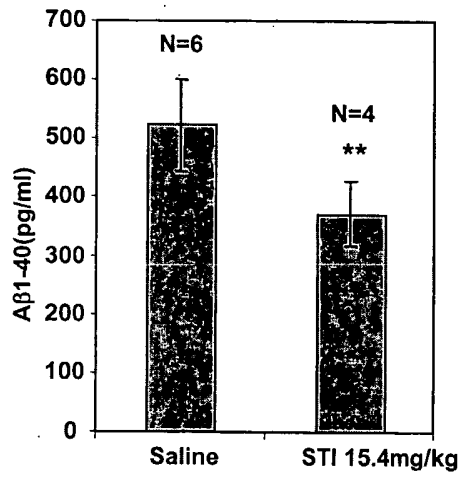


FIG. 15B

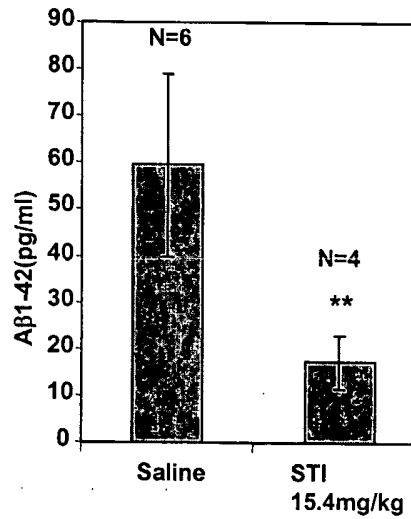


FIG. 15C

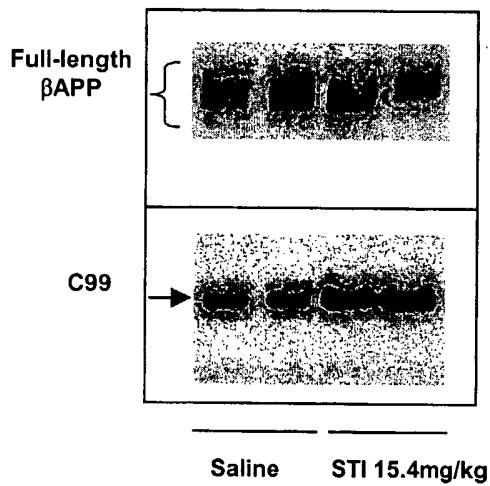


FIG. 15D

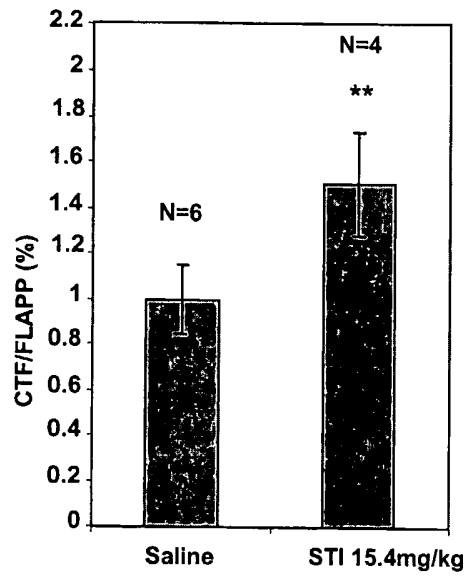


FIG. 16A

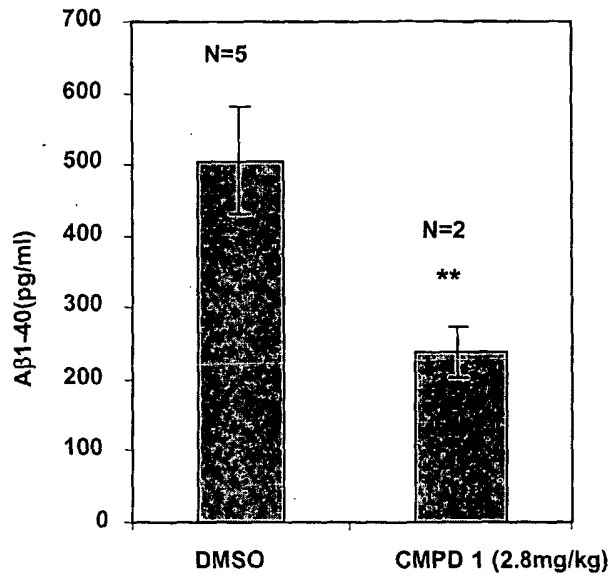


FIG. 16B

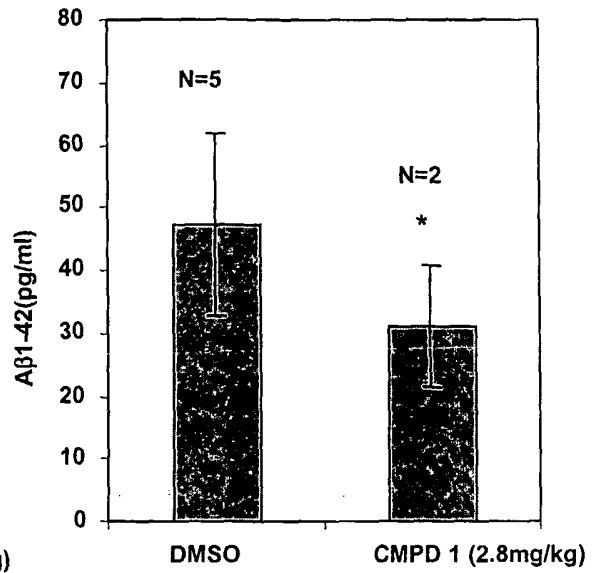


FIG. 16C

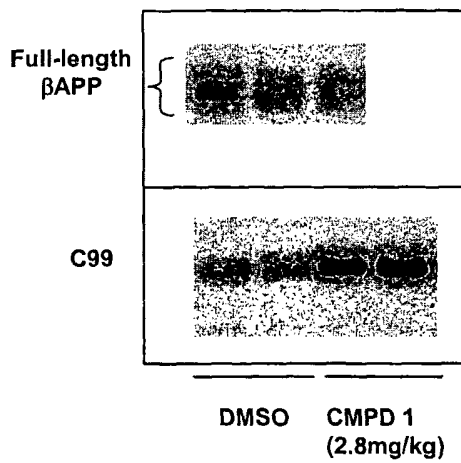
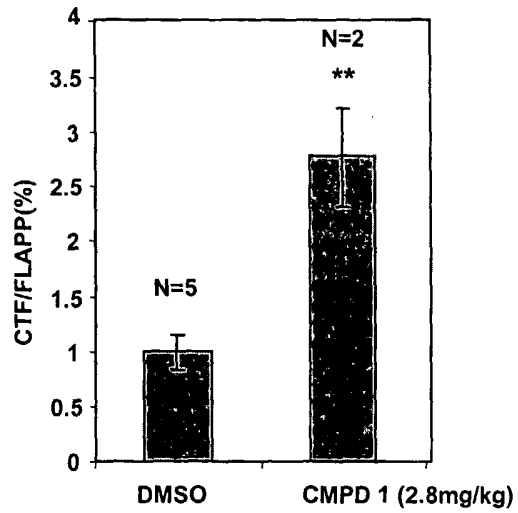


FIG. 16D



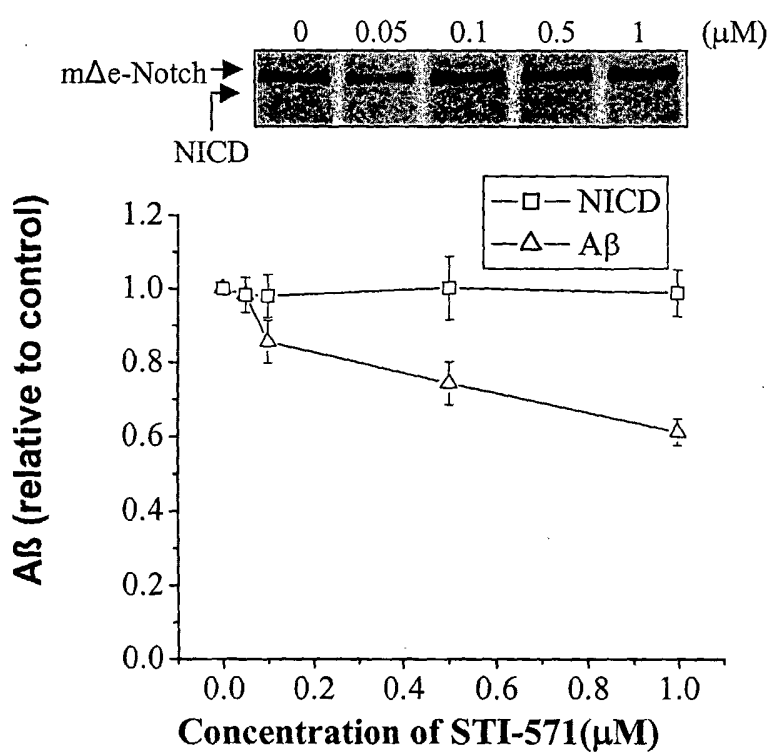


FIG. 17

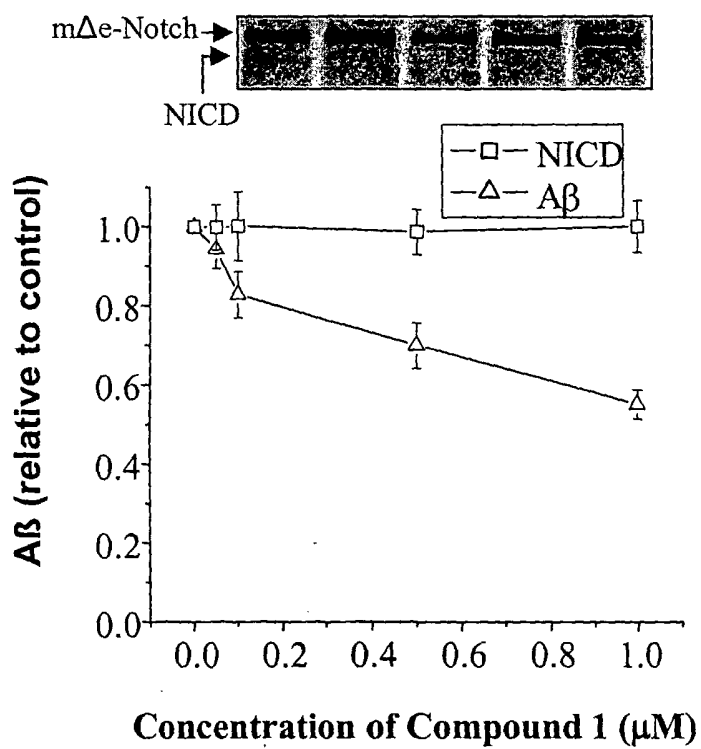


FIG. 18

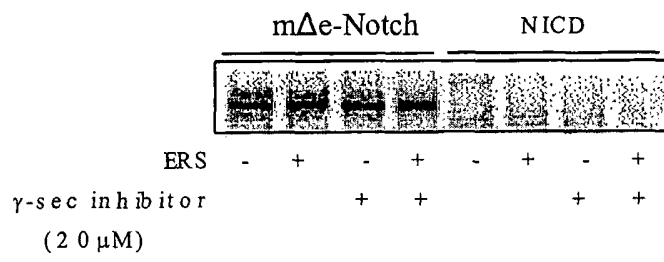
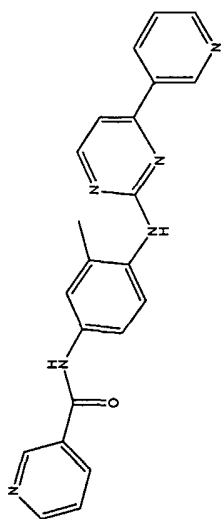
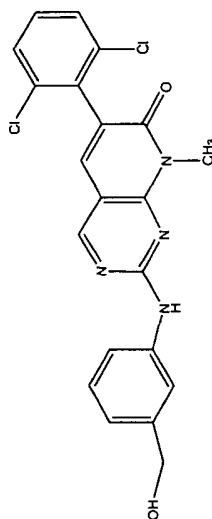


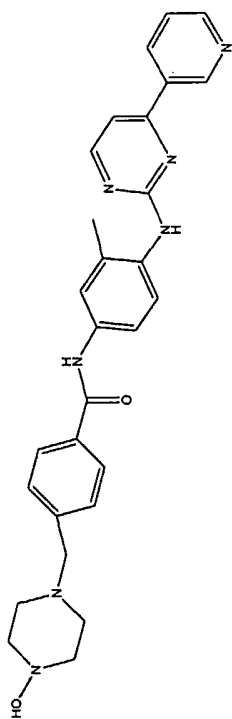
FIG. 19



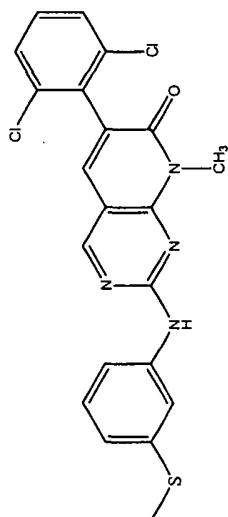
**STI-571 Variant
("WGB-BC-15")**



Compound 2



STI-571 ("Gleevec™")



Compound 1

FIG. 20

SEQUENCE LISTING

<110> The Rockefeller University

<120> COMPOSITIONS AND METHODS FOR PREVENTION AND TREATMENT OF AMYLOID- β
PEPTIDE-RELATED DISORDERS

<130> 11181-014-228

<140> TBA

<141> 2003-01-03

<150> 60/345,009

<151> 2002-01-04

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		35					40								

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