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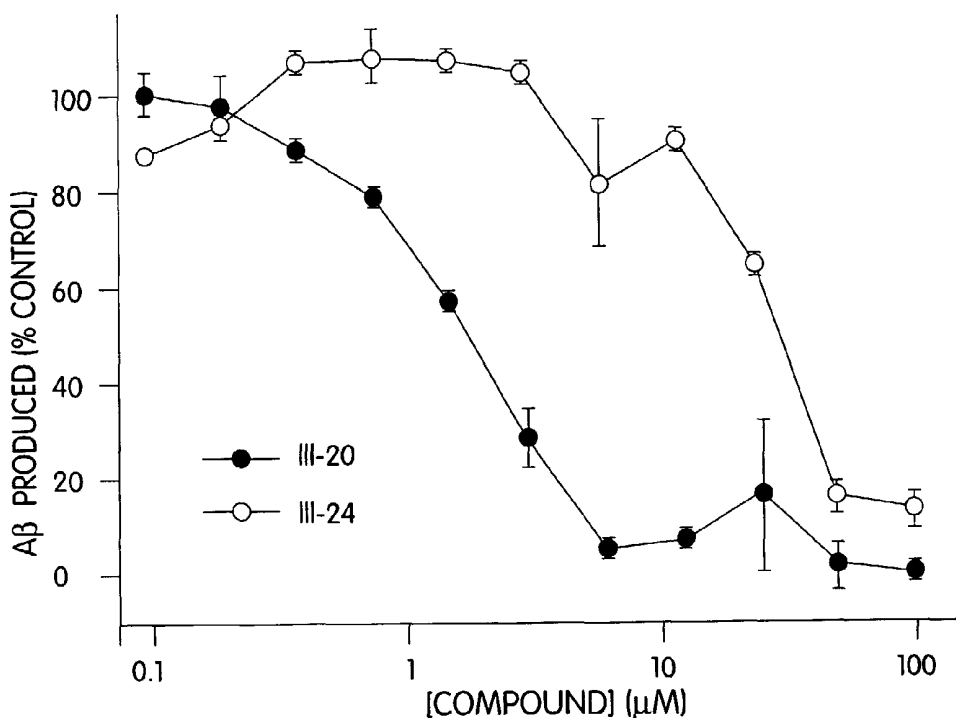
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(54) Title: (HYDROXYETHYL)UREAS AS INHIBITORS OF ALZHEIMER'S BETA-AMYLOID PRODUCTION



(57) Abstract: Novel (hydroxyethyl)ureas are described. These compounds are effective inhibitors of certain aspartyl proteases, notably secretases involved in the enzymatic cleavage of amyloid precursor protein (APP) to yield amyloid- β peptide. Methods are provided for administering the novel compounds to treat β -amyloid-associated diseases, notably Alzheimer's disease.

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**(HYDROXYETHYL)UREAS AS INHIBITORS OF ALZHEIMER'S
BETA-AMYLOID PRODUCTION**

5

RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application No. 60/225,043, filed August 11, 2000, the entire contents of which are hereby incorporated by reference.

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GOVERNMENT RIGHTS

This invention was funded in part under National Institute of Health Grant No. NS37537. The government may retain certain rights in these inventions.

FIELD OF THE INVENTION

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The invention relates to methods and compositions for inhibiting certain aspartyl proteases. More particularly it relates to methods and compounds for inhibiting the enzymatic activity of secretases involved in converting amyloid precursor protein to amyloid- β peptide. The methods and compounds of the invention can be used in the treatment of neurodegenerative disorders, notably Alzheimer's disease.

20

BACKGROUND OF THE INVENTION

Accumulating biochemical, histological, and genetic evidence supports the hypothesis that the 4 kDa β -amyloid protein ($A\beta$) is an essential component in the pathogenesis of Alzheimer's disease (AD). Selkoe DJ, *Science* 275:630-631 (1997). Hardy J, *Proc Natl Acad Sci USA* 94:2095-2097 (1997). Despite the intense interest in the role of $A\beta$ in the etiology of AD, the molecular mechanism of $A\beta$ biosynthesis is poorly understood. The 39-43-residue $A\beta$ is formed via the sequential cleavage of the integral membrane amyloid precursor protein (APP) by β - and γ -secretases. Selkoe DJ, *Annu Rev Cell Biol* 10:373-403 (1994). β -Secretase cleavage of APP occurs near the membrane, producing the soluble APP_s- β and a 12 kDa C-terminal membrane-associated fragment (CTF). The latter is processed by γ -secretase, which cleaves within the transmembrane domain of the substrate to generate $A\beta$. An alternative proteolytic event carried out by α -secretase occurs within the

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A β portion of APP, releasing APP $_{8-\alpha}$, and subsequent processing of the resulting membrane-bound 10 kDa CTF by γ -secretase leads to the formation of a 3 kDa N-terminally truncated version of A β called p3.

Heterogeneous proteolysis of the 12 kDa CTF by γ -secretase generates primarily two
5 C-terminal variants of A β , 40- and 42-amino acid versions (A β_{40} and A β_{42}), and parallel processing of the 10 kDa CTF generates the corresponding C-terminal variants of p3.

Although A β_{42} represents only about 10% of secreted A β , this longer and more hydrophobic variant is disproportionally present in the amyloid plaques observed post mortem in AD patients (Roher AE et al., *Proc Natl Acad Sci USA* 90:10836-40 (1993); Iwatsubo T et al.,
10 *Neuron* 13:45-53 (1994)), consistent with in vitro studies illustrating the kinetic insolubility of A β_{42} vis-à-vis A β_{40} . Jarrett JT et al., *Biochemistry* 32:4693-4697 (1993). Importantly, all genetic mutations associated with early-onset (<60 years) familial Alzheimer's disease (FAD) result in increased A β_{42} production. Selkoe DJ, *Science* 275:630-631 (1997); Hardy J, *Proc Natl Acad Sci USA* 94:2095-2097 (1997). An understanding of the production of A β in
15 general and that of A β_{42} in particular is essential for elucidating the molecular mechanism of AD pathogenesis and may also lead to the development of new chemotherapeutic agents which strike at the etiological heart of the disease.

Both γ -secretase and β -secretase are attractive targets for inhibitor design for the purpose of inhibiting production of A β . While γ -secretase is an attractive target for inhibitor
20 design, little is known about the structure, mechanism, or binding requirements of this unidentified protease.

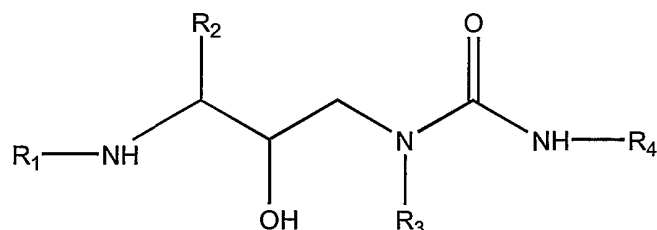
In view of the foregoing, a need still exists to develop compositions and methods for treating disorders characterized by the production and deposition of β -amyloid.

25 SUMMARY OF THE INVENTION

The present invention relates to novel compounds useful for inhibiting certain aspartyl proteases, particularly those involved in generating β -amyloid from APP. The compounds are useful for treating a subject having or at risk of having a β -amyloid-associated disease.

In a first aspect, the invention provides novel compounds of Formula I

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Formula I

wherein R_1 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, heteroaromatic, $R_6O(C=O)$, and $R_7R_8N(C=O)$, wherein R_6 , R_7 , and R_8 are independently
 5 selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and

heteroaromatic, provided R_1 is not bonded to the Formula I nitrogen via a group $Z\overset{\overset{X}{||}}{C}-$,
 wherein Z is C and X is O, S, or N; R_2 and R_3 are each independently selected from the group
 consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic; $NH-R_4$ is peptidyl or
 R_4 is independently selected from the group consisting of hydrogen, alkyl, cycloalkyl,
 10 aromatic, and heteroaromatic; and non-hydrogen R_1 , R_2 , R_3 , R_4 , R_6 , R_7 , and R_8 can
 independently be substituted with alkylamino, alkoxy, amino, halide, nitro, sulfate,
 sulfonamide, sulfoxide, or thiol ether.

In some preferred embodiments R_1 is t-butyloxycarbonyl (Boc).

In these and other preferred embodiments R_2 is bulky and is selected from the group
 15 consisting of cyclohexyl, benzyl and other amino acid side chains. In a more preferred
 embodiment R_2 is benzyl, i.e., the side chain of phenylalanine.

In these and other preferred embodiments R_3 is selected from the group consisting of
 methyl, isopropyl, isobutyl, benzyl and other amino acid side chains. In a more preferred
 embodiment R_3 is benzyl, i.e., the side chain of phenylalanine. Also in these and other
 20 preferred embodiments R_3 is selected from the group consisting of the side chains of alanine,
 leucine, and valine.

In these and other preferred embodiments $NH-R_4$ is selected from the group
 consisting of alanine-phenylalanine O-methyl ester, leucine-alanine O-methyl ester, leucine-
 leucine O-methyl ester, leucine-phenylalanine O-methyl ester, leucine-valine O-methyl ester,
 25 and valine-phenylalanine O-methyl ester. In some preferred embodiments $NH-R_4$ is selected
 from the group consisting of leucine-valine-alanine O-methyl ester, leucine-valine-leucine O-

methyl ester, leucine-valine-phenylalanine O-methyl ester, and leucine-valine-valine O-methyl ester.

In one particularly preferred embodiment R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is benzyl; and NH-R₄ is leucine-leucine O-methyl ester.

5 In another particularly preferred embodiment R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is isobutyl; and NH-R₄ is leucine-leucine O-methyl ester.

In another particularly preferred embodiment R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is benzyl; and NH-R₄ is alanine-phenylalanine O-methyl ester.

10 In another particularly preferred embodiment R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is benzyl; and NH-R₄ is leucine-valine O-methyl ester.

In another particularly preferred embodiment R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is benzyl; and NH-R₄ is valine-phenylalanine O-methyl ester.

In another particularly preferred embodiment R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is benzyl; and NH-R₄ is leucine-valine-phenylalanine O-methyl ester.

15 According to this aspect of the invention, certain embodiments embrace a stereoisomer of the compound of Formula I. Other related embodiments embrace a mixture of different stereoisomers of the compound of Formula I. In certain preferred embodiments all stereocenters are R.

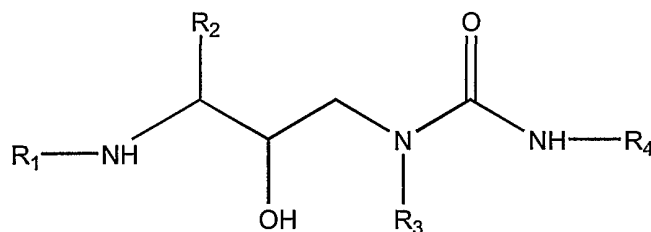
20 Yet another embodiment is a pharmaceutically acceptable salt of the compound of Formula I.

Also provided according to this aspect of the invention is a pharmaceutical composition comprising a compound of Formula I and further comprising a pharmaceutically acceptable carrier. Preferably the pharmaceutically acceptable carrier is adapted for oral administration of a compound of Formula I to a subject. More preferably the
25 pharmaceutically acceptable carrier is adapted for promoting delivery of a compound of Formula I to a brain of a subject.

The invention also provides a method for making a pharmaceutical composition. The method comprises placing a compound of Formula I according to this aspect of the invention in a pharmaceutically acceptable carrier. The method specifically embraces placing the
30 above-identified preferred embodiments of the compound of Formula I in a pharmaceutically acceptable carrier.

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In a second aspect the invention provides novel compounds of Formula I



Formula I

5 wherein R₁ is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, heteroaromatic, acyl (R₅C=O), R₆O(C=O), and R₇R₈N(C=O), wherein R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic; R₂ and R₃ are each independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic; NH-R₄ is peptidyl or R₄ is
 10 independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic; R₅ is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic; and non-hydrogen R₁, R₂, R₃, R₄, and R₅ can independently be substituted with alkylamino, alkoxy, amino, halide, nitro, sulfate, sulfonamide, sulfoxide, or thiol ether.

15 Preferred embodiments specifically exclude compounds previously disclosed in Getman DP et al., *J Med Chem* 36:288-291 (1993). Thus, preferred embodiments according to this aspect of the instant invention exclude compounds having Formula I, in which (using the convention of Formula I above) R₂ is benzyl and R₁ is -CO-CH(NHR)CH₂CONH₂, wherein:

20 R is carbobenzyloxy, R₃ is methyl, and R₄ is methyl;
 R is carbobenzyloxy, R₃ is methyl, and R₄ is n-butyl;
 R is carbobenzyloxy, R₃ is isobutyl, and R₄ is methyl;
 R is carbobenzyloxy, R₃ is isobutyl, and R₄ is n-butyl;
 R is quinolinyl-2-carboxamide, R₃ is isobutyl, and R₄ is n-butyl;
 25 R is carbobenzyloxy, R₃ is isobutyl, and R₄ is n-propyl;
 R is carbobenzyloxy, R₃ is isobutyl, and R₄ is ethyl;
 R is carbobenzyloxy, R₃ is isobutyl, and R₄ is isopropyl;

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R is carbobenzyloxy, R₃ is isobutyl, and R₄ is tert-butyl;

R is quinolinyl-2-carboxamide, R₃ is isobutyl, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is isopentyl, and R₄ is tert-butyl;

R is quinolinyl-2-carboxamide, R₃ is isopentyl, and R₄ is tert-butyl;

5 R is carbobenzyloxy, R₃ is CH₂C₆H₁₁, and R₄ is tert-butyl;

R is quinolinyl-2-carboxamide, R₃ is CH₂C₆H₁₁, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is benzyl, and R₄ is tert-butyl;

R is quinolinyl-2-carboxamide, R₃ is benzyl, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is (*R*)-CH(CH₃)-phenyl, and R₄ is tert-butyl;

10 R is carbobenzyloxy, R₃ is (*S*)-CH(CH₃)-phenyl, and R₄ is tert-butyl;

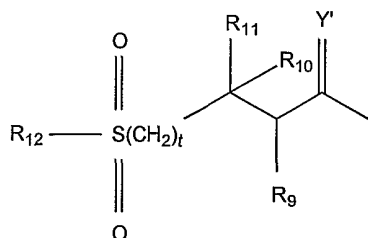
R is carbobenzyloxy, R₃ is CH₂(4-pyridyl), and R₄ is tert-butyl; or

R is quinolinyl-2-carboxamide, R₃ is CH₂(4-pyridyl), and R₄ is tert-butyl.

Preferred embodiments also specifically exclude compounds SC-52151 and SC-55389A previously disclosed in Bryant M et al., *Antimicrob Agents Chemother* 39:2229-2234 (1995) and Smidt ML et al., *Antimicrob Agents Chemother* 41:515-522 (1997). Thus, preferred embodiments according to this aspect of the instant invention exclude compounds having Formula I, in which (using the convention of Formula I above) R₁ is -CO-CH(C(CH₃)₃)NHR wherein R is COCH₂NHCH₃ HCl, R₂ is benzyl, R₃ is isopentyl, and R₄ is tert-butyl.

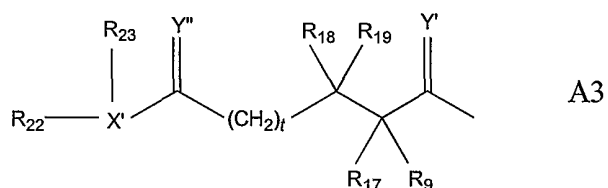
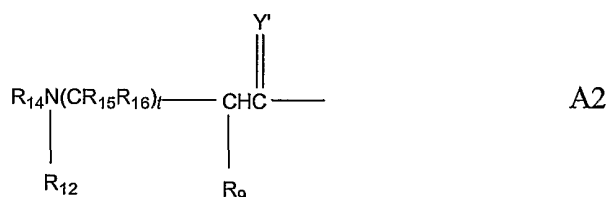
20 Preferred embodiments also specifically exclude compounds disclosed in U.S. Patent No. 5,457,013 (issued to Talley et al.). Thus, preferred embodiments according to this aspect of the invention exclude compounds having Formula I in which R₁ is a radical represented by any of the formulas A1, A2, A3 below:

25



A1

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5

wherein:

R_{14} represents hydrogen and alkoxy carbonyl, aralkoxy carbonyl, alkyl carbonyl, cycloalkyl carbonyl, cycloalkyl alkoxy carbonyl, cycloalkyl alkanoyl, alkanoyl, aralkanoyl, aroyl, aryloxy carbonyl, aryloxy alkanoyl, heterocyclyl carbonyl, heterocyclyloxy carbonyl, heterocyclyl alkanoyl, heterocyclyl alkoxy carbonyl, heteroalkoxy carbonyl, heteroaryloxy carbonyl, heteroalkanoyl, heteroaryl, alkyl, aryl, aralkyl, aryloxy alkyl, heteroaryloxy alkyl, hydroxy alkyl, aminocarbonyl, amino alkanoyl, and mono- and disubstituted aminocarbonyl and mono- and disubstituted amino alkanoyl radicals wherein the substituents are selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroalkyl, heteroalkyl and heterocycloalkylalkyl radicals or in the case of a disubstituted amino alkanoyl radical, said substituents along with the nitrogen atom to which they are attached form a heterocycloalkyl or heteroaryl radical;

R_{12} represents hydrogen and radicals as defined for R_{13} or R_{14} and R_{12} together with the nitrogen to which they are attached form a heterocycloalkyl or heteroaryl radical or when R_1 is A1, R_{12} represents hydrogen, radicals as defined for R_{13} and aralkoxy carbonylalkyl and aminocarbonylalkyl and aminoalkyl radicals wherein said amino group may be mono- or disubstituted with substituents selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroalkyl, heteroalkyl, and heterocycloalkylalkyl radicals;

t represents either 0 or 1;

R_9 represents hydrogen, $-\text{CH}_2\text{SO}_2\text{NH}_2$, $-\text{CO}_2\text{CH}_3$, $-\text{CH}_2\text{CO}_2\text{CH}_3$, $-\text{CONH}_2$, $-\text{CH}_2\text{C}(\text{O})\text{NHCH}_3$, $-\text{CH}_2\text{C}(\text{O})\text{N}(\text{CH}_3)_2$, $-\text{CONHCH}_3$, $-\text{CONH}(\text{CH}_3)_2$, $-\text{C}(\text{CH}_3)_2(\text{SCH}_3)$,

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-C(CH₃)₂(S[O]CH₃), -C(CH₃)₂(S[O]₂CH₃), alkyl, haloalkyl, alkenyl, alkynyl and cycloalkyl radicals and amino acid side chains selected from asparagine, S-methyl cysteine and the corresponding sulfoxide and sulfone derivatives thereof, glycine, leucine, isoleucine, allo-isoleucine, tert-leucine, phenylalanine, ornithine, alanine, histidine, norleucine, glutamine, valine, threonine, serine, aspartic acid, beta-cyano alanine, and allo-threonine side chains;

R₁₅ and R₁₆ independently represent hydrogen and radicals as defined for R₉, or one of R₁₅ and R₁₆, together with R₉ and the carbon atoms to which they are attached, represent a cycloalkyl radical;

R₁₃ represents alkyl, alkenyl, alkynyl, hydroxyalkyl, alkoxyalkyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, heterocycloalkylalkyl, aryl, aralkyl, heteroaralkyl, aminoalkyl and mono- and disubstituted aminoalkyl radicals where said substituents are selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroalkyl, heterocycloalkyl, and heterocycloalkylalkyl radicals or, in the case of a disubstituted aminoalkanoyl radical, said substituents along with the nitrogen atom to which they are attached, form a heterocycloalkyl or a heteroaryl radical;

X' represents O, C(R₂₁) where R₂₁ represents hydrogen and alkyl radicals and N;

Y' and Y'' independently represent O, S and NR₂₀ wherein R₂₀ represents hydrogen and radicals as defined for R₁₃;

R₁₀, R₁₁, R₁₇, R₁₈ and R₁₉ represent radicals as defined for R₉, or one of R₉ and R₁₇ together with one of R₁₈ and R₁₉ and the carbon atoms to which they are attached form a cycloalkyl radical; and

R₂₂ and R₂₃ independently represent hydrogen and radicals as defined for R₁₃, or R₂₂ and R₂₃ together with X' represent cycloalkyl, aryl, heterocyclyl and heteroaryl radicals, provided that when X' is O, R₂₃ is absent.

In these and other preferred embodiments R₂ is bulky and is selected from the group consisting of cyclohexyl, benzyl and other amino acid side chains. In a more preferred embodiment R₂ is benzyl, i.e., the side chain of phenylalanine.

In these and other preferred embodiments R₃ is selected from the group consisting of methyl, isopropyl, isobutyl, benzyl and other amino acid side chains. In a more preferred embodiment R₃ is benzyl, i.e., the side chain of phenylalanine. Also in these and other preferred embodiments R₃ is selected from the group consisting of the side chains of alanine, leucine, and valine.

In these and other preferred embodiments NH-R₄ is selected from the group consisting of alanine-phenylalanine O-methyl ester, leucine-alanine O-methyl ester, leucine-leucine O-methyl ester, leucine-phenylalanine O-methyl ester, leucine-valine O-methyl ester, and valine-phenylalanine O-methyl ester. In some preferred embodiments NH-R₄ is selected
5 from the group consisting of leucine-valine-alanine O-methyl ester, leucine-valine-leucine O-methyl ester, leucine-valine-phenylalanine O-methyl ester, and leucine-valine-valine O-methyl ester.

In one particularly preferred embodiment R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is benzyl; and NH-R₄ is leucine-leucine O-methyl ester.

10 In another particularly preferred embodiment R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is isobutyl; and NH-R₄ is leucine-leucine O-methyl ester.

In another particularly preferred embodiment R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is benzyl; and NH-R₄ is alanine-phenylalanine O-methyl ester.

15 In another particularly preferred embodiment R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is benzyl; and NH-R₄ is leucine-valine O-methyl ester.

In another particularly preferred embodiment R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is benzyl; and NH-R₄ is valine-phenylalanine O-methyl ester.

In another particularly preferred embodiment R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is benzyl; and NH-R₄ is leucine-valine-phenylalanine O-methyl ester.

20 According to this aspect of the invention, certain embodiments embrace a stereoisomer of the compound of Formula I. Other related embodiments embrace a mixture of different stereoisomers of the compound of Formula I. In certain preferred embodiments all stereocenters are R.

25 Yet another embodiment is a pharmaceutically acceptable salt of the compound of Formula I.

Also provided is a pharmaceutical composition comprising a compound of Formula I and further comprising a pharmaceutically acceptable carrier. Preferably the pharmaceutically acceptable carrier is adapted for oral administration of a compound of Formula I to a subject. More preferably the pharmaceutically acceptable carrier is adapted
30 for promoting delivery of a compound of Formula I to a brain of a subject.

The invention according to this aspect also provides a method for making a pharmaceutical composition. The method comprises placing a compound of Formula I

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according to this aspect of the invention in a pharmaceutically acceptable carrier. The method specifically embraces placing the above-identified preferred embodiments of the compound of Formula I in a pharmaceutically acceptable carrier.

5 In a third aspect the invention provides a method for treating a subject having or at risk of having a β -amyloid-associated disease. The method according to this aspect of the invention involves administering to a subject having or at risk of having a β -amyloid-associated disease a therapeutically effective amount of a compound of Formula I according to the first aspect or second aspect of the invention as described above.

10 A compound of Formula I as used throughout this application shall refer to a compound of Formula I according to the first aspect of the invention as described above or to a compound of Formula I according to the second aspect of the invention as described above. These two aspects differ primarily from one another in the nature of the R_1 group. Compounds of a first aspect of the invention have an R_1 group which is not bonded to the

15 Formula I nitrogen via a group $\begin{array}{c} X \\ || \\ ZC - \end{array}$, wherein Z is C and X is O, S, or N. Compounds of a second aspect of the invention have an R_1 group that can be bonded to the Formula I nitrogen

via a group $\begin{array}{c} X \\ || \\ ZC - \end{array}$, wherein Z is C and X is O, S, or N.

20 In a preferred embodiment the β -amyloid-associated disease is a neurodegenerative disease. In a more preferred embodiment the β -amyloid-associated disease is Alzheimer's disease.

In certain embodiments the subject is free of symptoms otherwise calling for treatment with a compound of Formula I. Preferably the subject is free of symptoms of retrovirus infection. More preferably the subject is free of symptoms of human immunodeficiency virus (HIV) infection.

25 According to this aspect of the invention the compound of Formula I is as described above, including preferred embodiments. The compound of Formula I can be packaged in unit dose form for convenience in dosing.

Preferably the compound of Formula I is administered orally.

In some preferred embodiments R_1 is t-butyloxycarbonyl.

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In these and other preferred embodiments R_2 is bulky and is selected from the group consisting of cyclohexyl, benzyl and other amino acid side chains. In a more preferred embodiment R_2 is benzyl, i.e., the side chain of phenylalanine.

In these and other preferred embodiments R_3 is selected from the group consisting of methyl, isopropyl, isobutyl, benzyl and other amino acid side chains. In a more preferred embodiment R_3 is benzyl, i.e., the side chain of phenylalanine. Also in these and other preferred embodiments R_3 is selected from the group consisting of the side chains of alanine, leucine, and valine.

In these and other preferred embodiments $NH-R_4$ is selected from the group consisting of alanine-phenylalanine O-methyl ester, leucine-alanine O-methyl ester, leucine-leucine O-methyl ester, leucine-phenylalanine O-methyl ester, leucine-valine O-methyl ester, and valine-phenylalanine O-methyl ester. In some preferred embodiments $NH-R_4$ is selected from the group consisting of leucine-valine-alanine O-methyl ester, leucine-valine-leucine O-methyl ester, leucine-valine-phenylalanine O-methyl ester, and leucine-valine-valine O-methyl ester.

In one particularly preferred embodiment R_1 is t-butyloxycarbonyl; R_2 is benzyl; R_3 is benzyl; and $NH-R_4$ is leucine-leucine O-methyl ester.

In another particularly preferred embodiment R_1 is t-butyloxycarbonyl; R_2 is benzyl; R_3 is isobutyl; and $NH-R_4$ is leucine-leucine O-methyl ester.

In another particularly preferred embodiment R_1 is t-butyloxycarbonyl; R_2 is benzyl; R_3 is benzyl; and $NH-R_4$ is alanine-phenylalanine O-methyl ester.

In another particularly preferred embodiment R_1 is t-butyloxycarbonyl; R_2 is benzyl; R_3 is benzyl; and $NH-R_4$ is leucine-valine O-methyl ester.

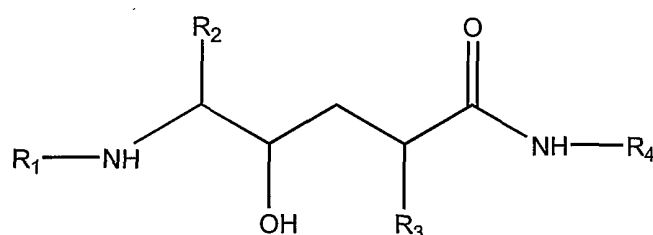
In another particularly preferred embodiment R_1 is t-butyloxycarbonyl; R_2 is benzyl; R_3 is benzyl; and $NH-R_4$ is valine-phenylalanine O-methyl ester.

In another particularly preferred embodiment R_1 is t-butyloxycarbonyl; R_2 is benzyl; R_3 is benzyl; and $NH-R_4$ is leucine-valine-phenylalanine O-methyl ester.

According to this aspect of the invention, certain embodiments embrace a stereoisomer of the compound of Formula I. Other related embodiments embrace a mixture of different stereoisomers of the compound of Formula I. In certain preferred embodiments all stereocenters are R.

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According to one embodiment of this aspect of the invention, a compound of Formula I is administered to the subject in combination with an effective amount of another agent useful in the treatment of β -amyloid-associated disease. The method thus embraces the administration of a compound of Formula I in combination with an acetylcholinesterase inhibitor. In other embodiments a compound of Formula I is administered in combination with a compound of Formula II



Formula II

wherein R_1 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, heteroaromatic, acyl ($R_5C=O$), $R_6O(C=O)$, and $R_7R_8N(C=O)$, wherein R_5 , R_6 , R_7 , and R_8 are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic; R_2 and R_3 are each independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic; $NH-R_4$ is peptidyl or R_4 is independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic; and non-hydrogen R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 can independently be substituted with alkylamino, alkoxy, amino, halide, nitro, sulfate, sulfonamide, sulfoxide, or thiol ether.

In a fourth aspect the invention provides a method for inhibiting activity of an aspartyl protease. The method involves contacting an aspartyl protease, under conditions in which aspartyl protease is enzymatically active, with a compound of Formula I according to the first aspect or the second aspect of the invention as described above.

In certain preferred embodiments the aspartyl protease is not a retroviral protease. Preferably the aspartyl protease is not HIV protease.

In certain preferred embodiments the aspartyl protease is not renin.

In a preferred embodiment the aspartyl protease is a γ -secretase.

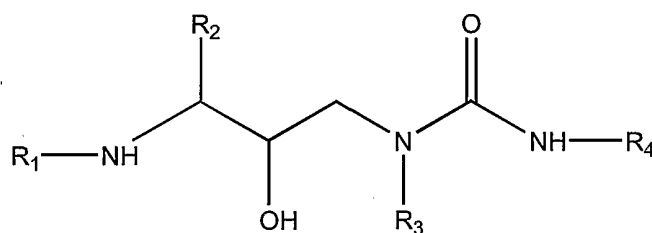
In another preferred embodiment the aspartyl protease is a β -secretase.

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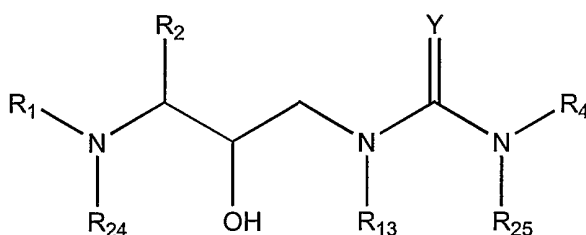
In a more preferred embodiment the contacting results in a decrease in the generation of amyloid- β peptide.

In certain embodiments the method according to this aspect of the invention is performed in vitro. In certain embodiments the method according to this aspect of the invention is performed in vivo.

In a fifth aspect the invention provides a method for treating a subject having or at risk of having a β -amyloid-associated disease. The method involves administering to a subject having or at risk of having a β -amyloid-associated disease and free of symptoms otherwise calling for treatment with a compound of Formula IA or Formula IB, a therapeutically effective amount of a compound of Formula IA or Formula IB



Formula IA



Formula IB

wherein, with respect to Formula IA:

(i) R_2 is benzyl and R_1 is $-\text{CO}-\text{CH}(\text{NHR})\text{CH}_2\text{CONH}_2$, wherein:

R is carbobenzyloxy, R_3 is methyl, and R_4 is methyl;

R is carbobenzyloxy, R_3 is methyl, and R_4 is n-butyl;

R is carbobenzyloxy, R_3 is isobutyl, and R_4 is methyl;

R is carbobenzyloxy, R_3 is isobutyl, and R_4 is n-butyl;

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R is quinolinyl-2-carboxamide, R₃ is isobutyl, and R₄ is n-butyl;

R is carbobenzyloxy, R₃ is isobutyl, and R₄ is n-propyl;

R is carbobenzyloxy, R₃ is isobutyl, and R₄ is ethyl;

R is carbobenzyloxy, R₃ is isobutyl, and R₄ is isopropyl;

5 R is carbobenzyloxy, R₃ is isobutyl, and R₄ is tert-butyl;

R is quinolinyl-2-carboxamide, R₃ is isobutyl, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is isopentyl, and R₄ is tert-butyl;

R is quinolinyl-2-carboxamide, R₃ is isopentyl, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is CH₂C₆H₁₁, and R₄ is tert-butyl;

10 R is quinolinyl-2-carboxamide, R₃ is CH₂C₆H₁₁, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is benzyl, and R₄ is tert-butyl;

R is quinolinyl-2-carboxamide, R₃ is benzyl, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is (*R*)-CH(CH₃)-phenyl, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is (*S*)-CH(CH₃)-phenyl, and R₄ is tert-butyl;

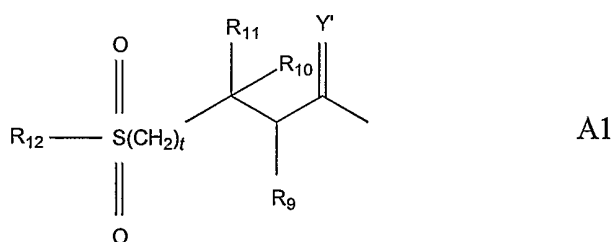
15 R is carbobenzyloxy, R₃ is CH₂(4-pyridyl), and R₄ is tert-butyl; or

R is quinolinyl-2-carboxamide, R₃ is CH₂(4-pyridyl), and R₄ is tert-butyl; or

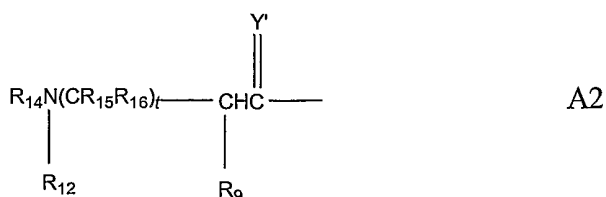
(ii) R₁ is -CO-CH(C(CH₃)₃)NHR, wherein: R is COCH₂NHCH₃ HCl, R₂ is benzyl, R₃ is isopentyl, and R₄ is tert-butyl;

and, with respect to Formula IB,

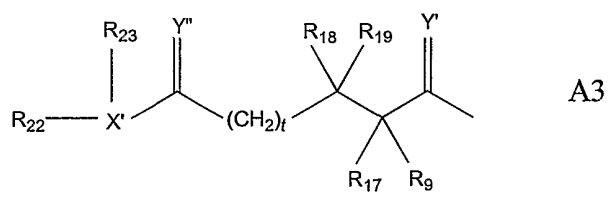
20 R₁ is a radical represented by any of the formulas A1, A2, A3 below:



25

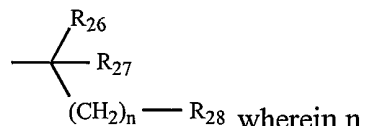


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5 R_2 represents alkyl, aryl, cycloalkyl, cycloalkylalkyl and aralkyl radicals, which radicals are optionally substituted with a group selected from $-\text{NO}_2$, $-\text{OR}_{30}$, $-\text{SR}_{30}$, and halogen radicals, wherein R_{30} represents hydrogen and alkyl radicals;

R_4 represents radicals represented by the formula:



10 represents an integer of from 0 to 6, R_{26} and R_{27} independently represent radicals as defined for R_{13} and amino acid side chains selected from the group consisting of valine, isoleucine, glycine, alanine, allo-isoleucine, asparagine, leucine, glutamine, and t-butylglycine or R_{26} and R_{27} together with the carbon atom to which they are attached form a cycloalkyl radical; and R_{28} represents cyano, hydroxyl, alkyl, alkoxy, cycloalkyl, aryl, aralkyl, heterocycloalkyl and heteroaryl radicals and radicals represented by the formulas $\text{C}(\text{O})\text{R}_{29}$, CO_2R_{29} , SO_2R_{29} , SR_{29} , $\text{CONR}_{29}\text{R}_{21}$, OR_{29} , CF_3 and $\text{NR}_{29}\text{R}_{21}$ wherein R_{29} and R_{21} independently represent hydrogen and radicals as defined for R_{13} or R_{29} and R_{21} together with a nitrogen to which they are attached in the formula $-\text{NR}_{29}\text{R}_{21}$ represent heterocycloalkyl and heteroaryl radicals;

R_{24} represents hydrogen and alkyl radicals;

R_{25} independently represents hydrogen and radicals as defined by R_{13} ; and

20 R_{13} represents alkyl, alkenyl, alkynyl, hydroxyalkyl, alkoxyalkyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, heterocycloalkylalkyl, aryl, aralkyl, heteroaralkyl, aminoalkyl and mono- and disubstituted aminoalkyl radicals where said substituents are selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroalkyl, heterocycloalkyl, and heterocycloalkylalkyl radicals or, in the case of a

25 disubstituted aminoalkanoyl radical, said substituents along with the nitrogen atom to which they are attached, form a heterocycloalkyl or a heteroaryl radical;

wherein:

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R₁₄ represents hydrogen and alkoxy carbonyl, aralkoxy carbonyl, alkyl carbonyl, cycloalkyl carbonyl, cycloalkyl alkoxy carbonyl, cycloalkyl alkanoyl, alkanoyl, aralkanoyl, aroyl, aryloxy carbonyl, aryloxy alkanoyl, heterocyclyl carbonyl, heterocyclyloxy carbonyl, heterocyclyl alkanoyl, heterocyclyl alkoxy carbonyl, heteroaralkoxy carbonyl, heteroaryloxy carbonyl, heteroaralkanoyl, heteroaroyl, alkyl, aryl, aralkyl, aryloxy alkyl, heteroaryloxy alkyl, hydroxy alkyl, aminocarbonyl, amino alkanoyl, and mono- and disubstituted aminocarbonyl and mono- and disubstituted amino alkanoyl radicals wherein the substituents are selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkyl alkyl, heteroaryl, heteroaralkyl, heteroalkyl and heterocycloalkyl alkyl radicals or in the case of a disubstituted amino alkanoyl radical, said substituents along with the nitrogen atom to which they are attached form a heterocycloalkyl or heteroaryl radical;

R₁₂ represents hydrogen and radicals as defined for R₁₃ or R₁₄ and R₁₂ together with the nitrogen to which they are attached form a heterocycloalkyl or heteroaryl radical or when R₁ is A1, R₁₂ represents hydrogen, radicals as defined for R₁₃ and aralkoxy carbonyl alkyl and aminocarbonyl alkyl and amino alkyl radicals wherein said amino group may be mono- or disubstituted with substituents selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkyl alkyl, heteroaryl, heteroaralkyl, heteroalkyl, and heterocycloalkyl alkyl radicals;

t represents either 0 or 1;

R₉ represents hydrogen, -CH₂SO₂NH₂, -CO₂CH₃, -CH₂CO₂CH₃, -CONH₂, -CH₂C(O)NHCH₃, -CH₂C(O)N(CH₃)₂, -CONHCH₃, -CONH(CH₃)₂, -C(CH₃)₂(SCH₃), -C(CH₃)₂(S[O]CH₃), -C(CH₃)₂(S[O]₂CH₃), alkyl, haloalkyl, alkenyl, alkynyl and cycloalkyl radicals and amino acid side chains selected from asparagine, S-methyl cysteine and the corresponding sulfoxide and sulfone derivatives thereof, glycine, leucine, isoleucine, allo-isoleucine, tert-leucine, phenylalanine, ornithine, alanine, histidine, norleucine, glutamine, valine, threonine, serine, aspartic acid, beta-cyano alanine, and allo-threonine side chains;

R₁₅ and R₁₆ independently represent hydrogen and radicals as defined for R₉, or one of R₁₅ and R₁₆, together with R₉ and the carbon atoms to which they are attached, represent a cycloalkyl radical;

X' represents O, C(R₂₁) where R₂₁ represents hydrogen and alkyl radicals and N;

Y, Y' and Y'' independently represent O, S and NR₂₀ wherein R₂₀ represents hydrogen and radicals as defined for R₁₃;

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R₁₀, R₁₁, R₁₇, R₁₈ and R₁₉ represent radicals as defined for R₉, or one of R₉ and R₁₇ together with one of R₁₈ and R₁₉ and the carbon atoms to which they are attached form a cycloalkyl radical; and

R₂₂ and R₂₃ independently represent hydrogen and radicals as defined for R₁₃, or R₂₂ and R₂₃ together with X' represent cycloalkyl, aryl, heterocyclyl and heteroaryl radicals, provided that when X' is O, R₂₃ is absent, whereby the β -amyloid disease is treated.

BRIEF DESCRIPTION OF THE DRAWINGS

The following figures are provided for illustrative purposes only and are not required for understanding or practicing the invention.

Figure 1 is a graph that depicts the inhibitory effect of compounds III-20 (filled circles) and III-24 (open circles) on the production of A β peptide as measured by enzyme-linked immunosorbent assay (ELISA).

Figure 2 is a graph that depicts the inhibitory effect of compound III-20 on the production of total A β peptide (filled circles) and A β ₁₋₄₂ (open circles) as measured by ELISA.

Figure 3 is a graph that depicts the inhibitory effect of four (hydroxyethyl)urea compounds on the production of total A β peptide as measured by ELISA. Open circles, F Ψ FFF; filled circles, F Ψ FLL; open triangles, F Ψ FLF; and filled circles, F Ψ FLV (see Example 3).

DETAILED DESCRIPTION

I. Definitions

Alkyl groups can be linear or branched, saturated or unsaturated, and have up to about ten carbon atoms. One or more hydrogen atoms can also be replaced by a substituent group selected from acyl, amino, carboalkoxy, carboxy, carboxyamido, cyano, halo, hydroxy, nitro, thio, alkyl, cycloalkyl, alkoxy, aryloxy, sulfoxy, and guanido groups. Preferred alkyl groups are "lower alkyl" groups having one to about four carbon atoms. Equally preferred alkyl groups are unsubstituted or include amino, carboxy, carboxyamido, hydroxy, thio and guanido groups. More preferred alkyl groups are methyl, isopropyl, isobutyl, 1-methylpropyl, thiomethylethyl, hydroxymethyl, 1-hydroxyethyl, thiomethyl,

carboxyamidomethyl, carboxyamidoethyl, carboxymethyl, carboxyethyl, aminobutyl and guanido.

Cycloalkyl groups have, preferably, saturated or partially unsaturated ring systems, each containing zero to four hetero atoms selected from oxygen, nitrogen and sulfur in a single or fused carbocyclic or heterocyclic ring system having from three to fifteen ring members. Cycloalkyl groups include multicyclic groups having two, three, or more saturated or partially unsaturated rings that can be single, fused, or a combination of single and fused rings. One or more hydrogen atoms may also be replaced by a substituent group selected from acyl, amino, carboalkoxy, carboxy, carboxyamido, cyano, halo, hydroxy, nitro, oxo, thio, alkyl, aryl, cycloalkyl, alkoxy, aryloxy, and guanido groups or two substituents together may form a fused cycloalkyl ring. Examples of a cycloalkyl group include cyclopropyl, cyclobutyl, cyclohexyl, cycloheptyl, morpholinyl, piperidinyl, and pyrrolidinyl. An example of a multicyclic cycloalkyl group is adamantyl. An alkoxy group denotes an oxygen atom substituted with an acyl, alkyl or cycloalkyl group. Examples include methoxy, tert-butyl, 10 butyloxy, benzyloxy, and cyclohexyloxy. An aryloxy group denotes an oxygen atom substituted with an aryl group. Examples of aryloxy groups are phenoxy, 4-carbobenzyloxyphenoxy, 4-phenoxyphenoxy. Sulfoxy groups comprise a hexavalent sulfur atom bound to two or three substituents selected from the group consisting of oxo, alkyl, aryl and cycloalkyl groups, wherein at least one of said substituents is oxo.

Aromatic groups can contain a single or fused carbocyclic ring system, having from five to fifteen ring members. One or more hydrogen atoms may also be replaced by a substituent group selected from acyl, amino, carboalkoxy, carboxy, carboxyamido, cyano, halo, hydroxy, nitro, thio, alkyl, aryl, cycloalkyl, alkoxy, aryloxy, sulfoxy, and guanido groups. Arylalkyl groups embrace aryl-substituted alkyl groups. Preferred arylalkyl groups include benzyl, 3-indolylmethyl, 4-hydroxybenzyl, 5-imidazolylmethyl.

Heteroaromatic groups can contain one to four hetero atoms selected from oxygen, nitrogen and sulfur in a single or fused heterocyclic ring system, having from five to fifteen ring members. One or more hydrogen atoms may also be replaced by a substituent group selected from acyl, amino, carboalkoxy, carboxy, carboxyamido, cyano, halo, hydroxy, nitro, thio, alkyl, aryl, cycloalkyl, alkoxy, aryloxy, sulfoxy, and guanido groups. Arylalkyl groups embrace aryl-substituted alkyl groups. Preferred arylalkyl groups include benzyl, 3-indolylmethyl, 4-hydroxybenzyl, 5-imidazolylmethyl.

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Peptidyl groups can contain one to ten amino acid residues, amino acid side chains, or amino acid analog residues, usually, but not always, joined in a linear or cyclic fashion by peptide linkages. Amino acid residues can include naturally-occurring and non-naturally-occurring amino acids, examples of which are well known in the art. In certain embodiments amino acid residues or peptidyl groups can be terminated by O-methyl ester linkages. In other embodiments adjacent amino acid residues can be joined together by peptide linkages. In certain preferred embodiments, the peptidyl group includes one to four amino acid residues. In certain more preferred embodiments, a peptidyl group includes two amino acid residues. In certain more preferred embodiments, a peptidyl group includes three amino acid residues. In certain more preferred embodiments, a peptidyl group is selected from the group alanine, leucine, phenylalanine, valine, alanine-phenylalanine, leucine-alanine, leucine-leucine, leucine-phenylalanine, leucine-valine, valine-phenylalanine, leucine-valine-alanine, leucine-valine-leucine, leucine-valine-phenylalanine, and leucine-valine-valine.

Notwithstanding the definitions above, with respect to preferred embodiments of the novel compounds of Formula I of the instant invention, compounds previously disclosed in Getman DP et al., *J Med Chem* 36:288-291 (1993), related U.S. Patent Nos. 5,457,013 and 5,698,569 (issued to Talley et al.), and Smidt ML et al., *Antimicrob Agents Chemother* 41:515-522 (1997) are specifically excluded. Thus, preferred embodiments according to the first and second aspects of the invention exclude compounds having Formula I in which (using the convention of Formula I above) R_2 is benzyl and R_1 is $-\text{CO}-\text{CH}(\text{NHR})\text{CH}_2\text{CONH}_2$, wherein:

- R is carbobenzyloxy, R_3 is methyl, and R_4 is methyl;
- R is carbobenzyloxy, R_3 is methyl, and R_4 is n-butyl;
- R is carbobenzyloxy, R_3 is isobutyl, and R_4 is methyl;
- R is carbobenzyloxy, R_3 is isobutyl, and R_4 is n-butyl;
- R is quinolinyl-2-carboxamide, R_3 is isobutyl, and R_4 is n-butyl;
- R is carbobenzyloxy, R_3 is isobutyl, and R_4 is n-propyl;
- R is carbobenzyloxy, R_3 is isobutyl, and R_4 is ethyl;
- R is carbobenzyloxy, R_3 is isobutyl, and R_4 is isopropyl;
- R is carbobenzyloxy, R_3 is isobutyl, and R_4 is tert-butyl;
- R is quinolinyl-2-carboxamide, R_3 is isobutyl, and R_4 is tert-butyl (compound SC-52151);
- R is carbobenzyloxy, R_3 is isopentyl, and R_4 is tert-butyl;

- 20 -

R is quinolinyl-2-carboxamide, R₃ is isopentyl, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is CH₂C₆H₁₁, and R₄ is tert-butyl;

R is quinolinyl-2-carboxamide, R₃ is CH₂C₆H₁₁, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is benzyl, and R₄ is tert-butyl;

5 R is quinolinyl-2-carboxamide, R₃ is benzyl, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is (*R*)-CH(CH₃)-phenyl, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is (*S*)-CH(CH₃)-phenyl, and R₄ is tert-butyl;

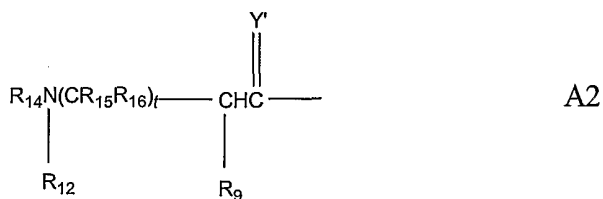
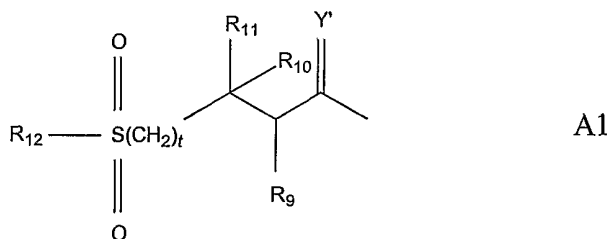
R is carbobenzyloxy, R₃ is CH₂(4-pyridyl), and R₄ is tert-butyl; or

R is quinolinyl-2-carboxamide, R₃ is CH₂(4-pyridyl), and R₄ is tert-butyl.

10 Preferred embodiments according to the first and second aspects of the invention also specifically exclude compound SC-55389A previously disclosed Smidt ML et al., *Antimicrob Agents Chemother* 41:515-522 (1997), i.e., compounds having Formula I, in which (using the convention of Formula I above) R₂ is benzyl and R₁ is -CO-CH(C(CH₃)₃)NHR, wherein R is COCH₂NHCH₃ HCl, R₃ is isopentyl, and R₄ is tert-butyl.

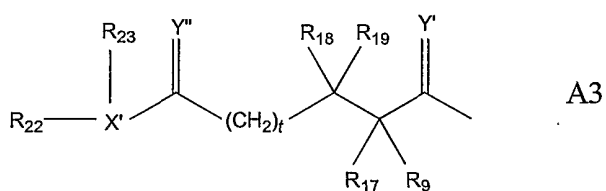
15 Preferred embodiments also specifically exclude compounds disclosed in U.S. Patent No. 5,457,013 (issued to Talley et al.). Thus, preferred embodiments according to the first and second aspects of the invention exclude compounds having Formula I in which R₁ is a radical represented by any of the formulas A1, A2, A3 below:

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wherein:

- 5 R₁₄ represents hydrogen and alkoxy carbonyl, aralkoxy carbonyl, alkyl carbonyl, cycloalkyl carbonyl, cycloalkyl alkoxy carbonyl, cycloalkyl alkanoyl, alkanoyl, aralkanoyl, aroyl, aryloxy carbonyl, aryloxy alkanoyl, heterocyclyl carbonyl, heterocyclyloxy carbonyl, heterocyclyl alkanoyl, heterocyclyl alkoxy carbonyl, heteroaralkoxy carbonyl, heteroaryloxy carbonyl, heteroaralkanoyl, heteroaroyl, alkyl, aryl, aralkyl, aryloxy alkyl, heteroaryloxy alkyl, hydroxy alkyl, aminocarbonyl, amino alkanoyl, and mono- and
- 10 disubstituted aminocarbonyl and mono- and disubstituted amino alkanoyl radicals wherein the substituents are selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkyl alkyl, heteroaryl, heteroaralkyl, heteroalkyl and heterocycloalkyl alkyl radicals or in the case of a disubstituted amino alkanoyl radical, said substituents along with the nitrogen atom to which they are
- 15 attached form a heterocycloalkyl or heteroaryl radical;

- R₁₂ represents hydrogen and radicals as defined for R₁₃ or R₁₄ and R₁₂ together with the nitrogen to which they are attached form a heterocycloalkyl or heteroaryl radical or when R₁ is A1, R₁₂ represents hydrogen, radicals as defined for R₁₃ and aralkoxy carbonyl alkyl and aminocarbonyl alkyl and amino alkyl radicals wherein said amino group may be mono- or
- 20 disubstituted with substituents selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkyl alkyl, heteroaryl, heteroaralkyl, heteroalkyl, and heterocycloalkyl alkyl radicals;

t represents either 0 or 1;

- R₉ represents hydrogen, -CH₂SO₂NH₂, -CO₂CH₃, -CH₂CO₂CH₃, -CONH₂, -CH₂C(O)NHCH₃, -CH₂C(O)N(CH₃)₂, -CONHCH₃, -CONH(CH₃)₂, -C(CH₃)₂(SCH₃),
- 25 -C(CH₃)₂(S[O]CH₃), -C(CH₃)₂(S[O]₂CH₃), alkyl, haloalkyl, alkenyl, alkynyl and cycloalkyl radicals and amino acid side chains selected from asparagine, S-methyl cysteine and the corresponding sulfoxide and sulfone derivatives thereof, glycine, leucine, isoleucine, allo-isoleucine, tert-leucine, phenylalanine, ornithine, alanine, histidine, norleucine, glutamine, valine, threonine, serine, aspartic acid, beta-cyano alanine, and allo-threonine side chains;

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R₁₅ and R₁₆ independently represent hydrogen and radicals as defined for R₉, or one of R₁₅ and R₁₆, together with R₉ and the carbon atoms to which they are attached, represent a cycloalkyl radical;

R₁₃ represents alkyl, alkenyl, alkynyl, hydroxyalkyl, alkoxyalkyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, heterocycloalkylalkyl, aryl, aralkyl, heteroaralkyl, aminoalkyl and mono- and disubstituted aminoalkyl radicals where said substituents are selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroalkyl, heterocycloalkyl, and heterocycloalkylalkyl radicals or, in the case of a disubstituted aminoalkanoyl radical, said substituents along with the nitrogen atom to which they are attached, form a heterocycloalkyl or a heteroaryl radical;

X' represents O, C(R₂₁) where R₂₁ represents hydrogen and alkyl radicals and N;

Y' and Y'' independently represent O, S and NR₂₀ wherein R₂₀ represents hydrogen and radicals as defined for R₁₃;

R₁₀, R₁₁, R₁₇, R₁₈ and R₁₉ represent radicals as defined for R₉, or one of R₉ and R₁₇ together with one of R₁₈ and R₁₉ and the carbon atoms to which they are attached form a cycloalkyl radical; and

R₂₂ and R₂₃ independently represent hydrogen and radicals as defined for R₁₃, or R₂₂ and R₂₃ together with X' represent cycloalkyl, aryl, heterocyclyl and heteroaryl radicals, provided that when X' is O, R₂₃ is absent.

Notwithstanding the foregoing, the invention also embraces the second medical use of the above-noted known compounds in a method for treating a subject having or susceptible to having a β -amyloid-associated disease wherein the subject is free of symptoms otherwise calling for treatment with such compounds, e.g., symptoms of infection with a retrovirus such as HIV.

The pharmaceutically acceptable salts of the compounds of Formula I include acid addition salts and base addition salts. The term "pharmaceutically acceptable salts" embraces salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. The nature of the salt is not critical, provided that it is pharmaceutically acceptable. Suitable pharmaceutically acceptable acid addition salts of the compounds of Formula I may be prepared from an inorganic acid or an organic acid. Examples of such inorganic acids are hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic,

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arylaliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which are formic, acetic, propionic, succinic, glycolic, gluconic, maleic, embonic (pamoic), methanesulfonic, ethanesulfonic, 2-hydroxyethanesulfonic, pantothenic, benzenesulfonic, toluenesulfonic, sulfanilic, mesylic, cyclohexylaminosulfonic, stearic, algenic, β -

5 hydroxybutyric, malonic, galactic, and galacturonic acid. Suitable pharmaceutically acceptable base addition salts of compounds of Formula I include, but are not limited to, metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from N,N'-dibenzylethylene-diamine, chlorprocaine, choline, diethanolamine, ethylenediamine, N-methylglucamine and procaine. All of these salts may

10 be prepared by conventional means from the corresponding compound of Formula I by treating, for example, the compound of Formula I with the appropriate acid or base.

The compounds of Formula I have centers of asymmetry, i.e., chiral centers, including one at the alcohol. The absolute configuration of these centers can be assigned by the stereochemical descriptors R and S, this R and S notation corresponding to the rules

15 described in *Pure Appl Chem* 45:11-30 (1976). Unless otherwise indicated, the chemical designation of compounds as used herein includes all possible stereochemical isomeric forms.

The compounds of Formula I are thus capable of existing in the form of optical isomers as well as in the form of racemic or non-racemic mixtures thereof. The compounds of Formula I can be utilized in the present invention as a single diastereomer or as a mixture

20 of stereochemical isomeric forms. Diastereoisomers can be separated by conventional means such as chromatography, distillation, crystallization or sublimation. The optical isomers can be obtained by resolution of the racemic mixtures according to conventional processes, for example by formation of diastereoisomeric salts by treatment with an optically active acid or base. Examples of appropriate acids are tartaric, diacetyltartaric, dibenzoyltartaric,

25 ditoluoyltartaric and camphorsulfonic acid. The mixture of diastereomers can be separated by crystallization followed by liberation of the optically active bases from these salts. An alternative process for separation of optical isomers includes the use of a chiral chromatography column optimally chosen to maximize the separation of the enantiomers. Still another available method involves synthesis of covalent diastereoisomeric molecules by

30 reacting compounds of Formula I with an optically pure acid in an activated form or an optically pure isocyanate. The synthesized diastereoisomers can be separated by conventional means such as chromatography, distillation, crystallization or sublimation, and

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then hydrolyzed to obtain the enantiomerically pure compound. The optically active compounds of Formula I can likewise be obtained by utilizing optically active starting materials. These isomers may be in the form of a free acid, a free base, an ester or a salt.

The invention also embraces isolated compounds. An isolated compound refers to a compound which represents at least 10 percent of the compound present in the mixture and exhibits a detectable (i.e., statistically significant) biological activity when tested in conventional biological assays such as those described herein. Preferably the isolated compound represents at least 50 percent of the mixture; more preferably at least 80 percent of the mixture; and most preferably at least 90 percent or at least 95 percent of the mixture.

The invention embraces compounds and methods useful for inhibiting the enzymatic activity of certain aspartyl proteases. An aspartyl protease refers to an enzyme with an active site characterized by two aspartate residues which are adjacent to one another in three-dimensional space and coordinated by a water molecule, that catalyzes the hydrolysis of its substrate. Aspartyl proteases include cathepsin D, renin, HIV protease, pepsin, and β - and γ -secretase. Vassar R et al., *Science* 286:735-741 (1999); Leung D et al., *J Med Chem* 43:305-341 (2000); Wolfe MS et al., *Biochemistry* 38:4720-4727 (1999).

As mentioned elsewhere herein, in certain embodiments a subject is free of symptoms of retrovirus infection, including in particular infection by human immunodeficiency virus (HIV). Retroviruses are RNA viruses that belong to the family Retroviridae. These viruses characteristically contain an RNA-dependent DNA polymerase (reverse transcriptase) that directs the synthesis of a DNA form of the viral genome after infection of a host cell. The Retroviridae family includes the subfamilies Oncovirinae (oncogenic viruses), for example human T-cell lymphotropic virus (HTLV), Rous sarcoma virus, Abelson leukemia virus, murine mammary tumor virus, and Mason-Pfizer monkey virus; Lentivirinae (slow viruses), which includes HIV-1, HIV-2, Visna virus, and feline immunodeficiency virus; and Spumavirinae (foamy viruses), for example Simian foamy virus and human foamy virus. As their names suggest, many of these viruses cause symptoms related to malignant transformation of infected cells and induction of immunodeficiency that leads to opportunistic infections. Symptoms of infection by specific retroviruses are well known in the art and are described, for example, in Harrison's Principles of Internal Medicine, 14th Ed., Fauci AS et al., eds, New York: McGraw-Hill, 1998, Chapters 192 and 308. As used herein, symptoms of HIV infection include both clinical symptoms and levels of viremia associated

with any stage of HIV disease, including acute HIV syndrome, asymptomatic stage, early symptomatic disease, neurologic disease, secondary infections, neoplasms, and organ-specific syndromes.

5 II. Description

According to one aspect of the invention, compounds of Formula I are provided. The compounds are useful for inhibiting the enzymatic activity of certain aspartyl proteases. The compounds are particularly potent inhibitors of γ -secretase, the enzyme that catalyzes the final step in the generation of amyloid- β peptide from the amyloid- β precursor protein (APP).

10 The compounds are also believed by the applicants to be particularly potent inhibitors of β -secretase, another enzyme that catalyzes a late step in the generation of amyloid- β peptide from APP.

γ -Secretase catalyzes the final step in the generation of amyloid- β peptide ($A\beta$) from the amyloid- β precursor protein (APP) and plays a central role in the pathogenesis of
15 Alzheimer's disease (AD). Inhibition of this enzyme by transition-state analogues is consistent with an aspartyl protease mechanism, while substrate modeling and mutagenesis suggest an intramembranous proteolysis. The polytopic presenilins are intimately associated with γ -secretase activity. The requirement for two transmembrane aspartates for both γ -secretase activity and presenilin endoproteolysis into two subunits suggests that presenilin is
20 the catalytic unit of γ -secretase, an intramembrane-cleaving protease that undergoes autoactivation from a holoprotein zymogen.

This aspect of the invention is based, in part, on the discovery that certain transition-state analogue inhibitors, designed to interact with the γ -secretase active site, bind directly to presenilin subunits. Although not wishing to be bound to any particular theory or
25 mechanism, it is believed that the compounds of Formula I function as small peptidomimetics containing a (hydroxyethyl)urea moiety, an aspartyl protease transition-state mimetic that allows incorporation of a P1' substituent. Compounds of this type can be readily synthesized without undue experimentation in a few simple steps from commercially available materials. Significantly, compounds of Formula I can inhibit γ -secretase activity in whole cells at sub-
30 micromolar concentrations. Production of total $A\beta$ and the more fibrillogenic $A\beta_{42}$ is effectively blocked by these compounds, and membrane-associated APP C-terminal fragments (γ -secretase substrates), are elevated in a dose-dependent manner.

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A preferred class of compounds of Formula I includes compounds in which R₁ is t-butylloxycarbonyl.

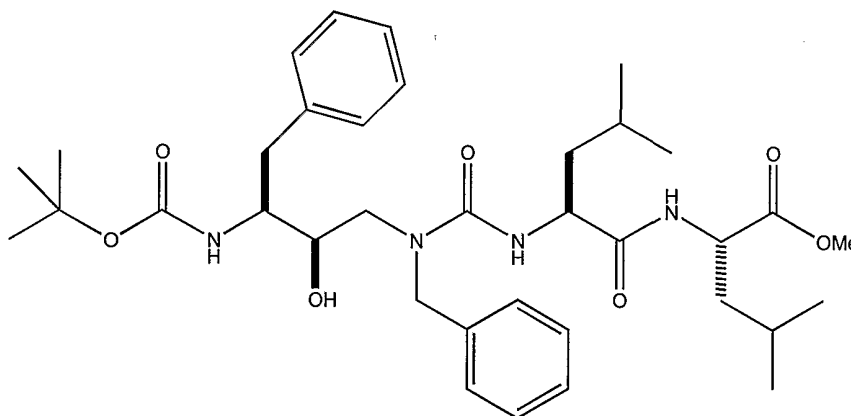
In these and other preferred classes of compounds of Formula I, R₂ is bulky and is selected from cyclohexyl, benzyl or other amino acid side chains.

5 In these and yet other preferred classes of compounds of Formula I, R₃ is methyl, isopropyl, isobutyl, benzyl or other amino acid side chains.

Any of the compounds shown in Table 1 below are non-limiting, representative examples of this preferred class of compounds. These are characterized by R₁ that is t-butylloxycarbonyl, R₂ and R₃ that are independently selected amino acid side chains, and
10 NH-R₄ that is peptidyl O-methyl ester.

In these and other preferred classes of compounds of Formula I includes compounds in which NH-R₄ is leucine-leucine O-methyl ester or leucine-phenylalanine O-methyl ester.

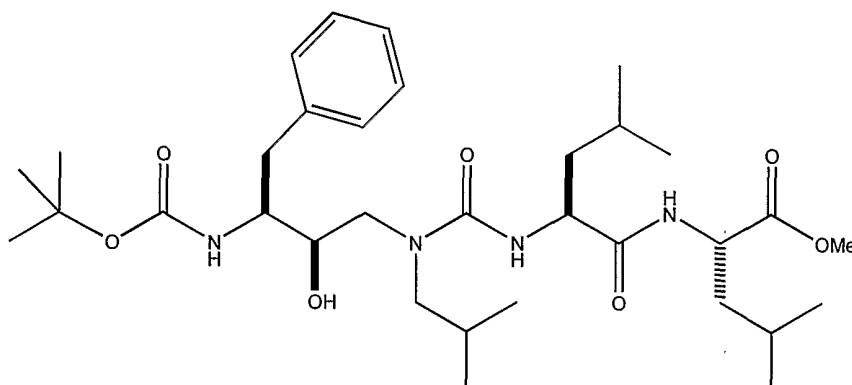
In a more preferred class of compounds of Formula I, R₁ is t-butylloxycarbonyl; R₂ is benzyl; R₃ is benzyl; and NH-R₄ is leucine-leucine O-methyl ester. This compound has the
15 structural formula



This particular compound is referred to as III-20 in the Examples section below.

20 In a second more preferred class of compounds of Formula I, R₁ is t-butylloxycarbonyl; R₂ is benzyl; R₃ is isobutyl; and NH-R₄ is leucine-leucine O-methyl ester. This compound has the structural formula

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In another preferred class of compounds of Formula I, R₁ is t-butyloxycarbonyl; R₂ is
 5 benzyl, R₃ is benzyl, and NH-R₄ is alanine-phenylalanine O-methyl ester.

In another preferred class of compounds of Formula I, R₁ is t-butyloxycarbonyl; R₂ is
 benzyl, R₃ is benzyl, and NH-R₄ is leucine-valine O-methyl ester.

In another preferred class of compounds of Formula I, R₁ is t-butyloxycarbonyl; R₂ is
 benzyl, R₃ is benzyl, and NH-R₄ is valine-phenylalanine O-methyl ester.

10 In yet another preferred class of compounds of Formula I, R₁ is t-butyloxycarbonyl;
 R₂ is benzyl, R₃ is benzyl, and NH-R₄ is leucine-valine-phenylalanine O-methyl ester.

In certain embodiments the compound of Formula I can be a pure R stereoisomer. In
 alternative embodiments, the compound of Formula I can be a pure S stereoisomer. In yet
 other alternative embodiments, a compound of Formula I can include a mixture of R and S
 15 stereoisomers, wherein the ratio of the contribution of one stereoisomer to the other can range
 from about 1:99 to about 99:1. In certain preferred embodiments the compound of Formula I
 is a pure R stereoisomer.

Certain embodiments embrace a salt of a compound of Formula I. In a preferred
 embodiment, the salt of the compound is a pharmaceutically acceptable salt as defined above.

20 In yet other embodiments, the composition of the invention is a pharmaceutical
 composition that includes a compound of Formula I prepared in a pharmaceutically
 acceptable carrier. The pharmaceutically acceptable carrier can be selected on the basis of
 the desired route of administration of the compound. For example, in a preferred
 embodiment the carrier is suitable for oral administration. In a more preferred embodiment,
 25 the carrier is suitable for promoting delivery of the compound to the brain. Carriers that can

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promote delivery of the compound to the brain can include any carrier that promotes translocation across the blood-brain barrier and any carrier that promotes uptake of the compound by neural cells. Examples of such carriers include those disclosed in U.S. Patents 5,604,198 (issued to Poduslo et al.), 5,827,819 (issued to Yatvin et al.), 5,919,815 (issued to
5 Bradley et al.), 5,955,459 (issued to Bradley et al.), and 5,977,174 (issued to Bradley et al.).

The compounds of the present invention are active against a variety of β -amyloid-associated diseases including, for example, Alzheimer's disease and the dementia of Down's syndrome. These neurodegenerative disorders occur in association with, and are believed to be caused by deposition of, amyloid- β peptide in neural tissue, i.e., β -amyloid plaques.

10 In addition to APP, secretases also have as their substrates members of the Notch family of receptors. De Strooper B et al., *Nature* 398:518-522 (1999). Notch proteins are ligand-activated transmembrane receptors involved in cell-fate selection throughout development. Notch activation results in transcriptional changes in the nucleus through an association with members of the CSL family of DNA-binding proteins (where CSL stands for
15 CBF1, Su(H), Lag-1). It is believed that Notch is cleaved by a protease, enabling the cleaved fragment to enter the nucleus. Signaling by a constitutively active membrane-bound Notch-1 protein requires the proteolytic release of the Notch intracellular domain (NICD), which interacts preferentially with CSL. Schroeter EH et al., *Nature* 393:382-386 (1998).

Inhibiting γ -secretase may also be useful in the treatment of Notch-related diseases,
20 including cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). A Notch-related disease refers to a disease caused by abnormal Notch-related proteolysis or signaling. For example, a Notch-related disease can arise from a mutation in a Notch receptor causing inappropriate, constitutive Notch activity. Schroeter EH et al., *Nature* 393:382-386 (1998). CADASIL, the most common form of
25 familial vascular dementia, appears to be essentially a disorder of the arteries that is linked to single missense mutations in the Notch 3 gene locus on chromosome 19. Ruchoux MM and Mauraage CA, *J Neuropathol Exp Neurol* 56:947-964 (1997); Thomas NJ et al., *Ann N Y Acad Sci* 903:293-8 (2000). Other Notch-related diseases include certain neoplasms including, for example, acute lymphoblastic T-cell leukemia. Selkoe DJ, *Curr Opin Neurobiol* 10:50-7
30 (2000); Deftos ML and Bevan MJ, *Curr Opin Immunol* 12:166-72 (2000); Jeffries S and Capobianco AJ, *Mol Cell Biol* 20:3928-3941 (2000); Capobianco AJ et al., *Mol Cell Biol* 17:6265-6273 (1997); Zagouras P et al., *Proc Natl Acad Sci USA* 92:6414-6418 (1995).

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Thus the compounds of the present invention are also believed to be useful in the treatment of Notch-related diseases, including CADASIL and certain types of neoplasia, e.g., certain leukemias. According to this aspect, the method of treating a subject having or at risk of having a Notch-related disease comprises involves administering to a subject having or at risk of having a Notch-related disease a therapeutically effective amount of a compound of Formula I.

In accordance with another aspect of the invention, a method is provided wherein the compositions disclosed herein are used for treating a subject afflicted by or susceptible to a β -amyloid-associated disease. The method involves administering to a subject having or at risk of having a β -amyloid-associated disease a therapeutically effective amount of a compound of Formula I according to the first aspect of the invention or to the second aspect of the invention. Preferred subjects of the present invention have only one type of β -amyloid-associated disease. More preferably, subjects of the present invention have Alzheimer's disease and do not have any other β -amyloid-associated disease.

A subject having a β -amyloid-associated disease is a subject with at least one identifiable sign, symptom, or laboratory finding sufficient to make a diagnosis of a β -amyloid-associated disease in accordance with clinical standards known in the art for identifying such disorder. In some instances, the absence of identifiable signs, symptoms, or laboratory findings may be necessary to make a diagnosis. For example, the diagnosis of Alzheimer's disease is most often made as a diagnosis of exclusion based on positive findings in cognitive testing in conjunction with exclusion of other causes. See, for example, Bird TD, *In: Harrison's Principles of Internal Medicine*, 14th Ed., Fauci AS et al., eds, New York: McGraw-Hill, 1998, Chapters 26 and 367. In some instances it may be possible to make a tissue diagnosis.

A subject at risk of having a β -amyloid-associated disease is a subject with an identifiable risk factor for developing a β -amyloid-associated disease. For example, a subject at risk of having a β -amyloid-associated disease can include a member in a family with familial Alzheimer's disease. Another example of a subject at risk of having a β -amyloid-associated disease is a subject over the age of 40 with Down's syndrome.

According to this aspect of the invention, the novel compositions disclosed herein are placed in a pharmaceutically acceptable carrier and are delivered to a recipient subject

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(preferably a human) in accordance with known methods of drug delivery. The compounds of the present invention may be administered alone or in combination with at least one other agent known or believed by the applicants to be useful for treating a β -amyloid-associated disease. In general, the methods of the invention for delivering the compositions of Formula I in vivo utilize art-recognized protocols for delivering the agent with the only substantial procedural modification being the substitution of the compounds of Formula I for the drugs in the art-recognized protocols.

Other agents which are known to be useful in the treatment of a β -amyloid-associated disease include acetylcholinesterase inhibitors, particularly tetrahydroaminoacridine (tacrine hydrochloride, COGNEX® (Parke-Davis)).

Other agents which the applicants believe to be useful in the treatment of a β -amyloid-associated disease include structurally related hydroxyethylene isosteres of general structure of Formula II as described above.

Compounds of Formula II have been reported as inhibitors of aspartyl proteases cathepsin D (Argarwal NS and Rich DH, *J Med Chem* 29:2519-24 (1986)), renin (Kati WM et al., *Biochemistry* 26:7621-6 (1987)), and HIV protease (Huff JR, *J Med Chem* 34:2305-14 (1991)) and have more recently been reported as inhibitors of beta-amyloid production (U.S. Patent 5,703,129; Li YM et al., *Proc Natl Acad Sci USA* 97:6138-43 (2000)).

Surprisingly, the effect of replacing nitrogen in (hydroxyethyl)ureas of Formula I with carbon in hydroxyethylene compounds of Formula II is significant. This replacement removes a key amide bond which applicants believe renders compounds of Formula II more susceptible to metabolic degradation. The synthetic methods for making (hydroxyethyl)ureas of Formula I are relatively simple, rapid, versatile and readily capable of scaling up compared with the structurally related hydroxyethylenes of Formula II. Getman DP et al., *J Med Chem* 36:288-291 (1993). While compounds of Formula II have been reported as inhibitors of β -amyloid production, and compounds related to Formula I have been reported as inhibitors of other aspartyl proteases, compounds of Formula I have not previously been recognized as inhibitors of Alzheimer's aspartyl proteases.

The phrase "therapeutically effective amount" means that amount of a compound which prevents the onset of, alleviates the symptoms of, or stops the progression of a disorder or disease being treated. The phrase "therapeutically effective amount" means, with respect to a β -amyloid-associated disease, that amount of a compound of Formula I which prevents

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the onset of, alleviates the symptoms of, or stops the progression of a β -amyloid-related disorder or disease. In general such symptoms are, at least in part, the result of the accumulation of increased amounts of amyloid- β peptide in vivo. Thus, a " β -amyloid-associated disease" is a condition that is characterized by certain clinical features and which, it is generally believed, is associated with excessive amounts of amyloid- β peptide.

"Excessive," with respect to amounts of amyloid- β peptide, refers to an amount amyloid- β peptide which is (1) greater than the amount of amyloid- β peptide that occurs in a normal, healthy subject, and (2) results in an adverse medical condition. The term "treating" is defined as administering, to a subject, a therapeutically effective amount of a compound (e.g., of Formula I) that is sufficient to prevent the onset of, alleviate the symptoms of, or stop the progression of a disorder or disease being treated. The term "subject," as described herein, is defined as a mammal. In a preferred embodiment, a subject is a human.

The pharmaceutical preparations disclosed herein are prepared in accordance with standard procedures and are administered at dosages that are selected to reduce, prevent, or eliminate, or to slow or halt the progression of, the condition being treated (See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA, and Goodman and Gilman's The Pharmaceutical Basis of Therapeutics, Pergamon Press, New York, N.Y., the contents of which are incorporated herein by reference, for a general description of the methods for administering various agents for human therapy). The compositions of Formula I can be delivered using controlled or sustained-release delivery systems (e.g., capsules, bioerodable matrices). Exemplary delayed-release delivery systems for drug delivery that would be suitable for administration of the compositions of Formula I are described in U.S. Patent Nos. US 5,990,092 (issued to Walsh); 5,039,660 (issued to Leonard); 4,452,775 (issued to Kent); and 3,854,480 (issued to Zaffaroni).

The pharmaceutically acceptable compositions of the present invention comprise one or more compounds of Formula I in association with one or more nontoxic, pharmaceutically acceptable carriers and/or diluents and/or adjuvants and/or excipients, collectively referred to herein as "carrier" materials, and if desired other active ingredients.

The compounds of the present invention may be administered by any route, preferably in the form of a pharmaceutical composition adapted to such a route, and would be dependent on the condition being treated. The compounds and compositions may, for example, be administered orally, intravascularly, intramuscularly, subcutaneously, intraperitoneally, or

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topically. The preferred method of administration is oral administration. In one embodiment the method of administration involves direct administration to brain.

For oral administration, the pharmaceutical compositions may be in the form of, for example, a tablet, capsule, suspension or liquid. The pharmaceutical composition is preferably made in the form of a dosage unit containing a therapeutically effective amount of the active ingredient. Examples of such dosage units are tablets and capsules. For therapeutic purposes, the tablets and capsules can contain, in addition to the active ingredient, conventional carriers such as binding agents, for example, acacia gum, gelatin, polyvinylpyrrolidone, sorbitol, or tragacanth; fillers, for example, calcium phosphate, glycine, lactose, maize-starch, sorbitol, or sucrose; lubricants, for example, magnesium stearate, polyethylene glycol, silica, or talc; disintegrants, for example potato starch, flavoring or coloring agents, or acceptable wetting agents. Oral liquid preparations generally in the form of aqueous or oily solutions, suspensions, emulsions, syrups or elixirs may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous agents, preservatives, coloring agents and flavoring agents. Examples of additives for liquid preparations include acacia, almond oil, ethyl alcohol, fractionated coconut oil, gelatin, glucose syrup, glycerin, hydrogenated edible fats, lecithin, methyl cellulose, methyl or propyl para-hydroxybenzoate, propylene glycol, sorbitol, or sorbic acid.

The pharmaceutical compositions may also be administered parenterally via injection. Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions or suspensions may be prepared from sterile powders or granules having one or more of the carriers mentioned for use in the formulations for oral administration. The compounds may be dissolved in polyethylene glycol, propylene glycol, ethanol, corn oil, benzyl alcohol, sodium chloride, and/or various buffers.

For topical use the compounds of the present invention may also be prepared in suitable forms to be applied to the skin, or mucus membranes of the nose and throat, and may take the form of creams, ointments, liquid sprays or inhalants, lozenges, or throat paints. Such topical formulations further can include chemical compounds such as dimethylsulfoxide (DMSO) to facilitate surface penetration of the active ingredient. Suitable carriers for topical administration include oil-in-water or water-in-oil emulsions using mineral oils, petrolatum

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and the like, as well as gels such as hydrogel. Alternative topical formulations include shampoo preparations, oral pastes and mouthwash.

For application to the eyes or ears, the compounds of the present invention may be presented in liquid or semi-liquid form formulated in hydrophobic or hydrophilic bases as ointments, creams, lotions, paints or powders.

For rectal administration the compounds of the present invention may be administered in the form of suppositories admixed with conventional carriers such as cocoa butter, wax or other glyceride.

Alternatively, the compounds of the present invention may be in powder form for reconstitution at the time of delivery.

The dosage regimen for treating a β -amyloid-associated disease with the compound and/or compositions of this invention is selected in accordance with a variety of factors, including the type, age, weight, sex and medical condition of the patient, the severity of the β -amyloid-associated disease, the route and frequency of administration, and the particular compound employed. In general, dosages are determined in accordance with standard practice for optimizing the correct dosage for treating a β -amyloid-associated disease.

The dosage regimen can be determined, for example, by following the response to the treatment in terms of cognitive studies. Examples of such cognitive studies are well known in the art, and they include the mini-mental status examination. See, for example, Bird TD, *In: Harrison's Principles of Internal Medicine*, 14th Ed., Fauci AS et al., eds, New York: McGraw-Hill, 1998, Chapter 26. In addition, because the compounds of the invention are believed to inhibit the synthesis of β -amyloid in vivo, the dosage regimen can also be determined by measurement of β -amyloid. It should be noted that β -amyloid is released into the blood and the cerebrospinal fluid (CSF), and is not confined to neural tissue. Therefore, the dosage regimen can also be determined by correlating serial measurement of β -amyloid present in blood or in CSF to the dose of the compositions of this invention. Methods of measuring β -amyloid present in blood or in CSF can include, for example, methods based on A β -specific ELISA.

The compositions may contain from 0.01% to 99% by weight of the active ingredient, depending on the method of administration.

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In a further aspect of the invention, a method is provided for inhibiting activity of an aspartyl protease. The method involves contacting a compound of Formula I of the first aspect of the invention or of the second aspect of the invention with an aspartyl protease under conditions in which the aspartyl protease is enzymatically active upon its substrate when the compound is not present, in an amount effective to result in a detectable inhibition of the activity of the aspartyl protease. Also included in this method is contacting an aspartyl protease with a combination of two or more compounds of Formula I to inhibit the aspartyl protease. In a preferred embodiment of this aspect of the invention, the aspartyl protease is γ -secretase. In another preferred embodiment, the aspartyl protease is β -secretase. The compounds of Formula I can be used alone or in combination with other compounds that inhibit aspartyl protease activity. In certain embodiments a compound of the invention is contacted with an aspartyl protease in vitro. The aspartyl protease can be isolated or cellular for in vitro assays. In certain other embodiments a compound of the invention is contacted with an aspartyl protease in vivo.

In a preferred embodiment the class of compounds of Formula I useful for this aspect of the invention includes compounds in which R_1 is t-butyloxycarbonyl.

In another preferred embodiment the class of compounds of Formula I useful for this aspect of the invention includes compounds in which R_2 is cyclohexyl, benzyl, or other amino acid side chain.

In yet another preferred embodiment the class of compounds of Formula I useful for this aspect of the invention includes compounds in which R_3 is methyl, isopropyl, isobutyl, benzyl or other amino acid side chain.

In a further preferred embodiment the class of compounds of Formula I useful for this aspect of the invention includes compounds in which $NH-R_4$ is leucine-leucine O-methyl ester or leucine-phenylalanine O-methyl ester.

In a more preferred embodiment the class of compounds of Formula I useful for this aspect of the invention includes compounds in which R_1 is t-butyloxycarbonyl; R_2 is benzyl; R_3 is benzyl; and $NH-R_4$ is leucine-leucine O-methyl ester.

In a second more preferred embodiment the class of compounds of Formula I useful for this aspect of the invention includes compounds in which R_1 is t-butyloxycarbonyl; R_2 is benzyl; R_3 is isobutyl; and $NH-R_4$ is leucine-leucine O-methyl ester.

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In another preferred embodiment the class of compounds of Formula I useful for this aspect of the invention includes compounds in which R₁ is t-butyloxycarbonyl; R₂ is benzyl, R₃ is benzyl, and NH-R₄ is alanine-phenylalanine O-methyl ester.

5 In yet another preferred embodiment the class of compounds of Formula I useful for this aspect of the invention includes compounds in which R₁ is t-butyloxycarbonyl; R₂ is benzyl, R₃ is benzyl, and NH-R₄ is leucine-valine O-methyl ester.

In yet another preferred embodiment the class of compounds of Formula I useful for this aspect of the invention includes compounds in which R₁ is t-butyloxycarbonyl; R₂ is benzyl, R₃ is benzyl, and NH-R₄ is valine-phenylalanine O-methyl ester.

10 In yet another preferred embodiment the class of compounds of Formula I useful for this aspect of the invention includes compounds in which R₁ is t-butyloxycarbonyl; R₂ is benzyl, R₃ is benzyl, and NH-R₄ is leucine-valine-phenylalanine O-methyl ester.

The inhibitory effect of a compound of unknown inhibitory activity can be assessed by monitoring aspartyl protease activity according to standard techniques. For example, a γ -secretase enzyme is maintained under conditions suitable for β -amyloid formation, the enzyme is contacted with the compound to be tested, and formation of the β -amyloid is monitored by standard assay, such as by ELISA. More specifically for γ -secretase, since reduced γ -secretase activity leads to an increase in γ -secretase substrate, a γ -secretase enzyme is maintained under conditions suitable for β -amyloid formation, the enzyme is contacted with the compound to be tested, and accumulation or concentration of the γ -secretase substrate is monitored by standard assay, such as by Western blotting. A reduction in the enzyme activity measured in the presence of the compound, as compared with the activity in the absence of compound, is indicative of inhibition of γ -secretase activity by the compound. In an effort to insure the integrity of the assay, a parallel assay is conducted in which the inhibitory activity of a compound of Formula I with known inhibitory activity is assessed. Since the compounds of Formula I are known inhibitors of γ -secretase, they serve as positive controls for the assay of compounds of unknown inhibitory activity. The absence of inhibition in an assay using a compound of Formula I is indicative of a problem in the assay itself.

30

III. Examples

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The following examples are illustrative only and are not intended to limit the scope of the invention in any way.

Example 1. Synthesis of Compounds of Formula I.

5 The method of synthesis is similar to methods beginning with epoxides previously described by Getman, Talley, and Vasquez, the entire contents of which are incorporated herein by reference. Getman DP et al., *J Med Chem* 36:288-291 (1993); U.S. Patent No. 5,475,013 (issued to Talley et al.); U.S. Patent No. 5,830,897 (issued to Vasquez et al.). The epoxides can be readily obtained from commercial sources, such as Sigma-Aldrich. Epoxides
10 not commercially available can be prepared according to published methods, for example the method described by Luly, the entire contents of which are incorporated herein by reference. Luly JR et al., *J Org Chem* 52:1487-1492 (1987).

The following describes the synthetic scheme depicted below. Selection of particular starting compounds in order to arrive at the desired end product will be evident to those
15 skilled in the art. To 1 equivalent of commercially available epoxide A (100 mM in 2-propanol) is added 20 equivalents of amine RNH₂, and the reaction is refluxed for 18 hours. Solvent is then removed under reduced pressure providing the adduct B directly in the case of volatile amines (e.g., Me-NH₂, I-Pr-NH₂, I-Bu-NH₂). With nonvolatile amines (e.g., PhCH₂NH₂), the concentrated mixture is taken up into ethyl acetate and washed with water,
20 1N HCl, saturated NaHCO₃ solution, and brine. The organic layer is then dried over Na₂SO₄, filtered, and concentrated *in vacuo* to provide adduct B. Yields range from 85 to 100%.

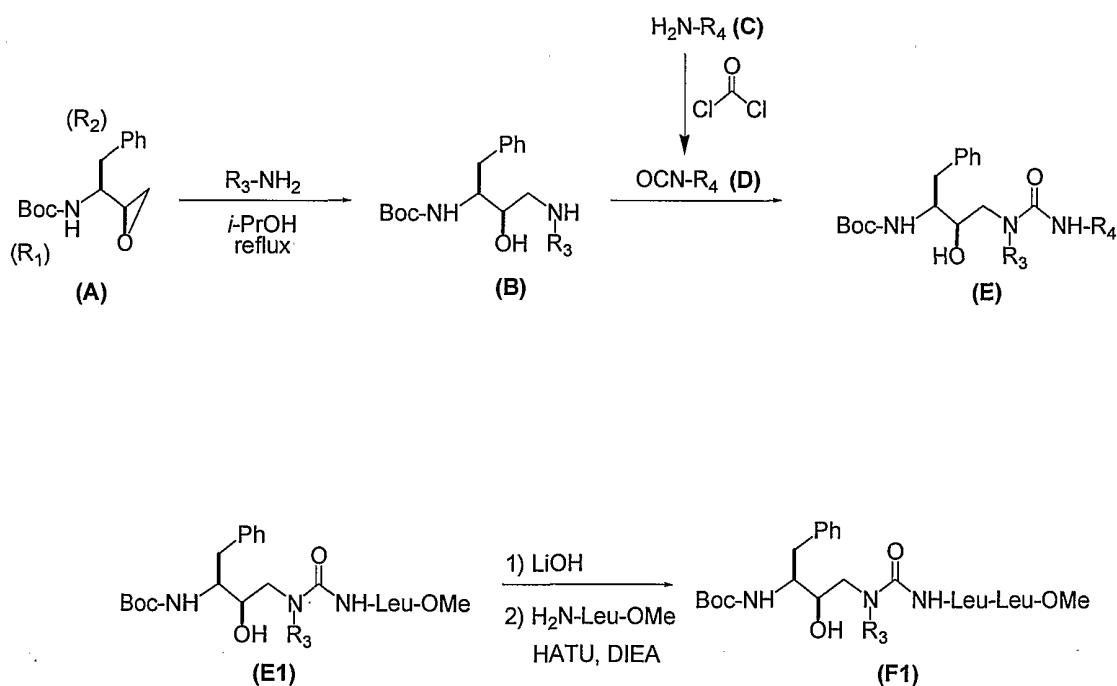
To a 100 mM solution of amine C in methylene chloride is added an equal volume of saturated NaHCO₃ solution. The mixture is stirred at 0°C for 10 minutes, whereupon stirring is ceased. 2 equivalents of phosgene (2 M solution in toluene) is quickly added via syringe to
25 the lower (organic) phase, and the mixture is vigorously stirred for 10 minutes. The mixture is poured into a separatory funnel and extracted three times with methylene chloride. The combined organic layers are dried over Na₂SO₄, filtered, and concentrated *in vacuo* to provide isocyanate D in quantitative yield.

1.2 equivalents of isocyanate D is added to a 70 mM solution of B in methylene
30 chloride, and the solution is stirred at ambient temperature for 18 hours. The solvent is then removed *in vacuo*, and the residue is passed through silica gel, eluting with methylene chloride:methanol (96:4), to provide urea E in 88-100% yield.

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To obtain compounds where R_4 is a dipeptide ester, a 0.13 M solution compound E1 is treated with 1.1 equivalent of lithium hydroxide (0.5 M in water) and stirred at 0°C for two hours. The mixture is then transferred to a separatory funnel, and the aqueous phase is brought to pH 1-2 with 1 N HCl and extracted three times with methylene chloride. The combined organic layers are dried over Na_2SO_4 , filtered, and concentrated. The residue is then taken up in dimethylformamide (100 mM) and treated with 1.1 equivalents of uronium coupling agent HATU (PerSeptive Biosystems, Cambridge, MA), 3 equivalents of N,N -diisopropylethylamine (DIEA), and 1.25 equivalents of amino ester. After stirring 18 hours, the mixture is taken up in ethyl acetate and washed three times with 1 N HCl, three times with saturated NaHCO_3 solution, and three times with brine. The organic layer is dried over Na_2SO_4 , filtered, and concentrated. The residue is then passed through silica gel, eluting with methylene chloride:methanol (96:4), to provide urea F1 in 88-97% yield.

Synthetic Scheme to (Hydroxyethyl)urea Inhibitors



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Example 2. Inhibition of $\text{A}\beta$ Peptide Production in vitro.

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Cell Lines, Compound Treatments, and ELISAs. Cell lines were Chinese hamster ovary (CHO) and SK-N-SH human neuroblastoma cells stably transfected with the 751- or 695-amino acid splicing variants of APP, respectively, and the *neo* gene, and human embryonic kidney (HEK) 293 cells carrying the same genes (APP695 plus *neo*) but with the K595N/M596L (“Swedish”) double mutation of APP. Xia W et al., *J Biol Chem* 272:7977-7982 (1997); Citron M et al., *Proc Natl Acad Sci USA* 93:13170-13175 (1996). Cells were grown to confluence in Dulbecco’s modified Eagle’s medium containing 200 µg/ml G418 (Gibco BRL). Stock concentrations of the (hydroxyethyl)urea compounds of the invention in DMSO were added to media to reach the final concentrations with 1% DMSO. Positive controls contained 1% DMSO alone. After 4 hours, the medium was removed and centrifuged at 3000 x g for 5 minutes, and the supernatant was stored at -80°C until the assays were carried out. Sandwich ELISAs for Aβ₄₀ and Aβ₄₂ were performed as described previously. Johnson-Wood K et al., *Proc Natl Acad Sci USA* 94:1550-1555 (1997); Seubert P et al., *Nature* 359:325-327 (1992). The capture antibodies were 2G3 (to Aβ₄₀ residues 33-40) for the Aβ₄₀ species and 21F12 (to Aβ₄₂ residues 33-42) for the Aβ₄₂ species. The reporter antibody was detected using 3,3',5,5'-tetramethylbenzidine (Pierce), measuring at 455 nm for calculating Aβ levels and at 595 nm for normalization.

Compound III-20 (shown above) or compound III-24 (identical to III-20 except R₄ is L-phenylalanine O-methyl ester) was added over a range of concentrations (0.1 - 100 µM) to APP-transfected cells in culture as described. In negative control cultures no compound was added. After 4 hours in culture, supernatants from test and control cultures were assayed by ELISA specific for Aβ peptides, and results were analyzed as percent total or specific Aβ produced compared to control.

As shown in Figure 1, compound III-20 caused a 50 percent inhibition of total Aβ peptide production at a concentration of about 1 µM. Compound III-24 also inhibited total Aβ peptide production in this assay, however it required a higher concentration, about 50 µM, for 50 percent inhibition.

As shown in Figure 2, the inhibitory effect of compound III-20 on the production of Aβ₄₂ closely followed the inhibition by compound III-20 of total Aβ production.

Example 3. Inhibition of Aβ Peptide Production in vitro.

A series of related (hydroxyethyl)urea compounds according to Formula I were synthesized and compared in terms of their ability to inhibit A β production in APP751- plus *neo*-transfected CHO cells in vitro. The (hydroxyethyl)urea compounds in this example all had t-butyloxycarbonyl (Boc) as R₁, a single amino acid side chain as R₂ and as R₃, and a dipeptide- or tripeptide- O-methyl ester as R₄. The particular compounds can be summarized as shown in Table 1, where amino acids or their side chains are designated using standard one-letter code (A = alanine, F = phenylalanine, L = leucine, V = valine); Boc represents t-butyloxycarbonyl, Ψ represents the core (hydroxyethyl)urea structure, and OMe represents O-methyl ester.

10

Table 1. (Hydroxyethyl)urea compounds for Example 3

Designation	R1	R2	R3	R4	IC ₅₀ (μ M)
F Ψ ALF	Boc	F	A	LF-OMe	3
F Ψ FAF	Boc	F	F	AF-OMe	0.2
F Ψ FFF	Boc	F	F	FF-OMe	25
F Ψ FLA	Boc	F	F	LA-OMe	2
F Ψ FLF	Boc	F	F	LF-OMe	0.5
F Ψ FLL	Boc	F	F	LL-OMe	0.5
F Ψ FLV	Boc	F	F	LV-OMe	0.2
F Ψ FVF	Boc	F	F	VF-OMe	0.2
F Ψ LLF	Boc	F	L	LF-OMe	0.5
F Ψ VLF	Boc	F	V	LF-OMe	5
F Ψ FLVA	Boc	F	F	LVA-OMe	0.8
F Ψ FLVF	Boc	F	F	LVF-OMe	0.2
F Ψ FLVL	Boc	F	F	LVL-OMe	0.4
F Ψ FLVV	Boc	F	F	LVV-OMe	0.8

Transfected CHO cells were prepared and maintained as described in Example 2. Stock concentrations of the (hydroxyethyl)urea compounds in Table 1 in DMSO were added to media to attain final concentrations of 0.005 to 100 μ M with 1% DMSO. Negative controls contained 1% DMSO alone. After 4 hours, the medium was removed and centrifuged at 3000 x g for 5 minutes, and the supernatant was stored at -80°C until the assays were carried out by ELISA as described for Example 2 above. Results were plotted as percent control A β produced versus (hydroxyethyl)urea compound concentration (μ M), and

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(hydroxyethyl)urea compound concentrations at which production was inhibited by 50 percent (IC₅₀) were determined. See Figure 3 and Table 1.

The results demonstrate that for the particular (hydroxyethyl)urea compounds shown in Table 1, IC₅₀ values as low as 0.2 μM were attained (FΨFAF, FΨFVF, FΨFLV, and FΨFLVF). All else being equal in this series, it was found that leucine-leucine O-methyl ester, leucine-phenylalanine O-methyl ester, and leucine-valine O-methyl ester were roughly equivalent as R₄. Of the (hydroxyethyl)urea compounds tested in this example, the least effective was FΨFFF, in which R₁ is Boc, R₂ is benzyl (i.e., the side group of phenylalanine), R₃ is benzyl (i.e., the side group of phenylalanine), and NH-R₄ is phenylalanine-phenylalanine O-methyl ester.

The results also demonstrate that the IC₅₀ values were relatively insensitive to the particular amino acid side chains selected for R₂ and R₃ and for the particular dipeptide and tripeptide O-methyl ester selected for NH-R₄. The compositions listed in Table 1 are thus exemplary and are not meant to be limiting with respect to selection of the particular amino acid side chains for R₂ and R₃ and for the particular dipeptide and tripeptide O-methyl ester for NH-R₄.

All of the references, patents and patent publications identified or cited herein are incorporated, in their entirety, by reference.

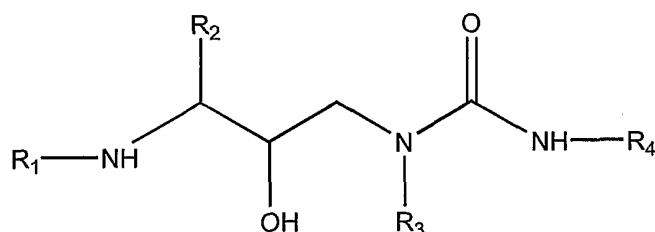
Although this invention has been described with respect to specific embodiments, the details of these embodiments are not to be construed as limitations. Various equivalents, changes and modifications may be made without departing from the spirit and scope of this invention, and it is understood that such equivalent embodiments are part of this invention.

We claim:

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CLAIMS

1. A composition comprising a compound of Formula I



5

Formula I

wherein:

- R₁ is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, heteroaromatic, R₆O(C=O), and R₇R₈N(C=O), wherein R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and

10

heteroaromatic, provided R₁ is not bonded to the Formula I nitrogen via a group $\begin{matrix} X \\ || \\ ZC- \end{matrix}$, wherein Z is C and X is O, S, or N;

R₂ and R₃ are each independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic;

15

NH-R₄ is peptidyl or R₄ is independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic; and

non-hydrogen R₁, R₂, R₃, R₄, R₆, R₇, and R₈ can independently be substituted with alkylamino, alkoxy, amino, halide, nitro, sulfate, sulfonamide, sulfoxide, or thiol ether.

20

2. The composition of claim 1 wherein the compound is a stereoisomer of the compound of Formula I.

3. The composition of claim 1 wherein the compound is a pharmaceutically acceptable salt of the compound of Formula I.

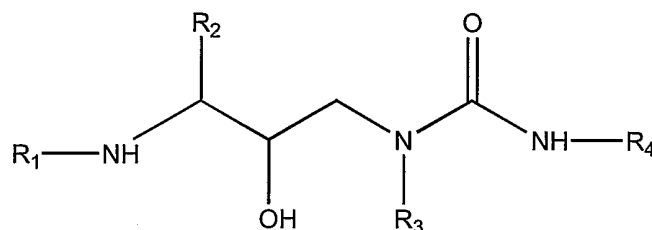
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4. The composition of claim 1 wherein R₁ is t-butyloxycarbonyl.

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5. The composition of claim 1 wherein R_2 is selected from the group consisting of cyclohexyl, benzyl and other amino acid side chains.
6. The composition of claim 1 wherein R_3 is selected from the group consisting of methyl, isopropyl, isobutyl, benzyl and other amino acid side chains.
7. The composition of claim 1 wherein $NH-R_4$ is selected from the group consisting of alanine-phenylalanine O-methyl ester, leucine-alanine O-methyl ester, leucine-leucine O-methyl ester, leucine-phenylalanine O-methyl ester, leucine-valine O-methyl ester, and valine-phenylalanine O-methyl ester.
8. The composition of claim 1 wherein $NH-R_4$ is selected from the group consisting of leucine-leucine O-methyl ester and leucine-phenylalanine O-methyl ester.
9. The composition of claim 1 wherein $NH-R_4$ is selected from the group consisting of leucine-valine-alanine O-methyl ester, leucine-valine-leucine O-methyl ester, leucine-valine-phenylalanine O-methyl ester, and leucine-valine-valine O-methyl ester.
10. The composition of claim 1 wherein R_1 is t-butyloxycarbonyl; R_2 is benzyl; R_3 is benzyl; and $NH-R_4$ is leucine-leucine O-methyl ester.
11. The composition of claim 1 wherein R_1 is t-butyloxycarbonyl; R_2 is benzyl; R_3 is isobutyl; and $NH-R_4$ is leucine-leucine O-methyl ester.
12. The composition of claim 1 wherein R_1 is t-butyloxycarbonyl; R_2 is benzyl, R_3 is benzyl, and $NH-R_4$ is alanine-phenylalanine O-methyl ester.
13. The composition of claim 1 wherein R_1 is t-butyloxycarbonyl; R_2 is benzyl, R_3 is benzyl, and $NH-R_4$ is leucine-valine O-methyl ester.
14. The composition of claim 1 wherein R_1 is t-butyloxycarbonyl; R_2 is benzyl, R_3 is benzyl, and $NH-R_4$ is valine-phenylalanine O-methyl ester.

15. The composition of claim 1 wherein R₁ is t-butyloxycarbonyl; R₂ is benzyl, R₃ is benzyl, and NH-R₄ is leucine-valine-phenylalanine O-methyl ester.
- 5 16. The composition of claim 1, further comprising a pharmaceutically acceptable carrier.
17. The composition of claim 1, further comprising a carrier to promote delivery of a compound of Formula I to a brain.
- 10 18. A composition comprising a compound of Formula I



Formula I

wherein:

- 15 R₁ is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, heteroaromatic, acyl (R₅C=O), R₆O(C=O), and R₇R₈N(C=O), wherein R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic;
- R₂ and R₃ are each independently selected from the group consisting of hydrogen, 20 alkyl, cycloalkyl, aromatic, and heteroaromatic;
- NH-R₄ is peptidyl or R₄ is independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic;
- R₅ is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and 25 heteroaromatic; and
- non-hydrogen R₁, R₂, R₃, R₄, and R₅ can independently be substituted with alkylamino, alkoxy, amino, halide, nitro, sulfate, sulfonamide, sulfoxide, or thiol ether, excluding compounds in which:
- (i) R₂ is benzyl and R₁ is -CO-CH(NHR)CH₂CONH₂, wherein:

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R is carbobenzyloxy, R₃ is methyl, and R₄ is methyl;

R is carbobenzyloxy, R₃ is methyl, and R₄ is n-butyl;

R is carbobenzyloxy, R₃ is isobutyl, and R₄ is methyl;

R is carbobenzyloxy, R₃ is isobutyl, and R₄ is n-butyl;

5 R is quinolinyl-2-carboxamide, R₃ is isobutyl, and R₄ is n-butyl;

R is carbobenzyloxy, R₃ is isobutyl, and R₄ is n-propyl;

R is carbobenzyloxy, R₃ is isobutyl, and R₄ is ethyl;

R is carbobenzyloxy, R₃ is isobutyl, and R₄ is isopropyl;

R is carbobenzyloxy, R₃ is isobutyl, and R₄ is tert-butyl;

10 R is quinolinyl-2-carboxamide, R₃ is isobutyl, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is isopentyl, and R₄ is tert-butyl;

R is quinolinyl-2-carboxamide, R₃ is isopentyl, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is CH₂C₆H₁₁, and R₄ is tert-butyl;

R is quinolinyl-2-carboxamide, R₃ is CH₂C₆H₁₁, and R₄ is tert-butyl;

15 R is carbobenzyloxy, R₃ is benzyl, and R₄ is tert-butyl;

R is quinolinyl-2-carboxamide, R₃ is benzyl, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is (*R*)-CH(CH₃)-phenyl, and R₄ is tert-butyl;

R is carbobenzyloxy, R₃ is (*S*)-CH(CH₃)-phenyl, and R₄ is tert-butyl;

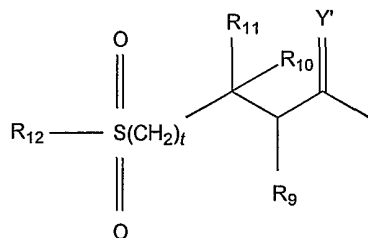
R is carbobenzyloxy, R₃ is CH₂(4-pyridyl), and R₄ is tert-butyl; or

20 R is quinolinyl-2-carboxamide, R₃ is CH₂(4-pyridyl), and R₄ is tert-butyl;

(ii) R₁ is -CO-CH(C(CH₃)₃)NHR, wherein: R is COCH₂NHCH₃ HCl, R₂ is benzyl, R₃ is isopentyl, and R₄ is tert-butyl; and

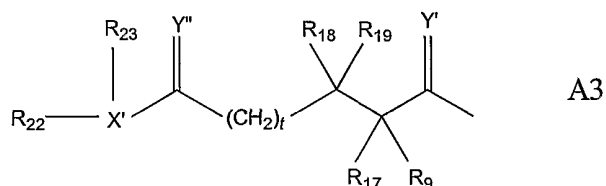
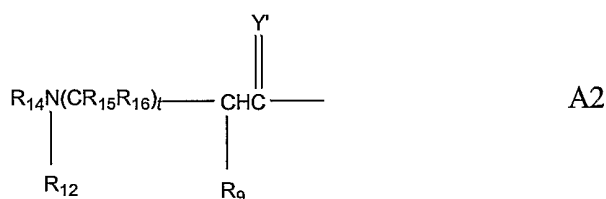
(iii) R₁ is a radical represented by any of the formulas A1, A2, A3 below:

25



A1

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5

wherein:

R_{14} represents hydrogen and alkoxy carbonyl, aralkoxy carbonyl, alkyl carbonyl, cycloalkyl carbonyl, cycloalkyl alkoxy carbonyl, cycloalkyl alkanoyl, alkanoyl, aralkanoyl, aroyl, aryloxy carbonyl, aryloxy alkanoyl, heterocyclyl carbonyl, heterocyclyloxy carbonyl, heterocyclyl alkanoyl, heterocyclyl alkoxy carbonyl, heteroaralkoxy carbonyl, heteroaryloxy carbonyl, heteroaralkanoyl, heteroaroyl, alkyl, aryl, aralkyl, aryloxy alkyl, heteroaryloxy alkyl, hydroxy alkyl, aminocarbonyl, amino alkanoyl, and mono- and disubstituted aminocarbonyl and mono- and disubstituted amino alkanoyl radicals wherein the substituents are selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkyl alkyl, heteroaryl, heteroaralkyl, heteroalkyl and heterocycloalkyl alkyl radicals or in the case of a disubstituted amino alkanoyl radical, said substituents along with the nitrogen atom to which they are attached form a heterocycloalkyl or heteroaryl radical;

R_{12} represents hydrogen and radicals as defined for R_{13} or R_{14} and R_{12} together with the nitrogen to which they are attached form a heterocycloalkyl or heteroaryl radical or when R_1 is A1, R_{12} represents hydrogen, radicals as defined for R_{13} and aralkoxy carbonyl alkyl and aminocarbonyl alkyl and amino alkyl radicals wherein said amino group may be mono- or disubstituted with substituents selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkyl alkyl, heteroaryl, heteroaralkyl, heteroalkyl, and heterocycloalkyl alkyl radicals;

t represents either 0 or 1;

25

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R₉ represents hydrogen, -CH₂SO₂NH₂, -CO₂CH₃, -CH₂CO₂CH₃, -CONH₂,
-CH₂C(O)NHCH₃, -CH₂C(O)N(CH₃)₂, -CONHCH₃, -CONH(CH₃)₂, -C(CH₃)₂(SCH₃),
-C(CH₃)₂(S[O]CH₃), -C(CH₃)₂(S[O]₂CH₃), alkyl, haloalkyl, alkenyl, alkynyl and
cycloalkyl radicals and amino acid side chains selected from asparagine, S-methyl
5 cysteine and the corresponding sulfoxide and sulfone derivatives thereof, glycine, leucine,
isoleucine, allo-isoleucine, tert-leucine, phenylalanine, ornithine, alanine, histidine,
norleucine, glutamine, valine, threonine, serine, aspartic acid, beta-cyano alanine, and
allo-threonine side chains;

R₁₅ and R₁₆ independently represent hydrogen and radicals as defined for R₉, or one
10 of R₁₅ and R₁₆, together with R₉ and the carbon atoms to which they are attached,
represent a cycloalkyl radical;

R₁₃ represents alkyl, alkenyl, alkynyl, hydroxyalkyl, alkoxyalkyl, cycloalkyl,
cycloalkylalkyl, heterocycloalkyl, heteroaryl, heterocycloalkylalkyl, aryl, aralkyl,
heteroaralkyl, aminoalkyl and mono- and disubstituted aminoalkyl radicals where said
15 substituents are selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl, heteroaryl,
heteroalkyl, heterocycloalkyl, and heterocycloalkylalkyl radicals or, in the case of a
disubstituted aminoalkanoyl radical, said substituents along with the nitrogen atom to
which they are attached, form a heterocycloalkyl or a heteroaryl radical;

X' represents O, C(R₂₁) where R₂₁ represents hydrogen and alkyl radicals and N;

20 Y' and Y'' independently represent O, S and NR₂₀ wherein R₂₀ represents hydrogen
and radicals as defined for R₁₃;

R₁₀, R₁₁, R₁₇, R₁₈ and R₁₉ represent radicals as defined for R₉, or one of R₉ and R₁₇
together with one of R₁₈ and R₁₉ and the carbon atoms to which they are attached form a
cycloalkyl radical; and

25 R₂₂ and R₂₃ independently represent hydrogen and radicals as defined for R₁₃, or R₂₂
and R₂₃ together with X' represent cycloalkyl, aryl, heterocyclyl and heteroaryl radicals,
provided that when X' is O, R₂₃ is absent.

19. The composition of claim 18 wherein the compound is a stereoisomer of the
30 compound of Formula I.

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20. The composition of claim 18 wherein the compound is a pharmaceutically acceptable salt of the compound of Formula I.
21. The composition of claim 18 wherein R₂ is selected from the group consisting of cyclohexyl, benzyl and other amino acid side chains.
22. The composition of claim 18 wherein R₃ is selected from the group consisting of methyl, isopropyl, isobutyl, benzyl and other amino acid side chains.
23. The composition of claim 18 wherein NH-R₄ is selected from the group consisting of alanine-phenylalanine O-methyl ester, leucine-alanine O-methyl ester, leucine-leucine O-methyl ester, leucine-phenylalanine O-methyl ester, leucine-valine O-methyl ester, and valine-phenylalanine O-methyl ester.
24. The composition of claim 18 wherein NH-R₄ is selected from the group consisting of leucine-leucine O-methyl ester and leucine-phenylalanine O-methyl ester.
25. The composition of claim 18 wherein NH-R₄ is selected from the group consisting of leucine-valine-alanine O-methyl ester, leucine-valine-leucine O-methyl ester, leucine-valine-phenylalanine O-methyl ester, and leucine-valine-valine O-methyl ester.
26. The composition of claim 18 further comprising a pharmaceutically acceptable carrier.
27. The composition of claim 18 further comprising a carrier to promote delivery of a compound of Formula I to a brain.
28. A method of treating a subject having or at risk of having a β -amyloid-associated disease, comprising:
- administering to a subject having or at risk of having a β -amyloid-associated disease a therapeutically effective amount of a compound of Formula I according to claim 1 or claim 18, whereby the β -amyloid-associated disease is treated.

29. The method of claim 28 wherein the β -amyloid-associated disease is a neurodegenerative disease.
- 5 30. The method of claim 28 wherein the β -amyloid-associated disease is Alzheimer's disease.
31. The method of any of claims 28-30, wherein R_1 is t-butyloxycarbonyl.
- 10 32. The method of any of claims 28-31, wherein R_2 is selected from the group consisting of cyclohexyl, benzyl and other amino acid side chains.
33. The method of any of claims 28-32, wherein R_3 is selected from the group consisting of methyl, isopropyl, isobutyl, benzyl and other amino acid side chains.
- 15 34. The method of any of claims 28-33, wherein $NH-R_4$ is selected from the group consisting of alanine-phenylalanine O-methyl ester, leucine-alanine O-methyl ester, leucine-leucine O-methyl ester, leucine-phenylalanine O-methyl ester, leucine-valine O-methyl ester, and valine-phenylalanine O-methyl ester.
- 20 35. The method of any of claims 28-34, wherein $NH-R_4$ is selected from the group consisting of leucine-leucine O-methyl ester and leucine-phenylalanine O-methyl ester.
- 25 36. The method of any of claims 28-35, wherein $NH-R_4$ is selected from the group consisting of leucine-valine-alanine O-methyl ester, leucine-valine-leucine O-methyl ester, leucine-valine-phenylalanine O-methyl ester, and leucine-valine-valine O-methyl ester.
- 30 37. The method of any of claims 28-36, wherein R_1 is t-butyloxycarbonyl; R_2 is benzyl; R_3 is benzyl; and $NH-R_4$ is leucine-leucine O-methyl ester.

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38. The method of claim 28 wherein R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is benzyl; and NH-R₄ is leucine-leucine O-methyl ester.

39. The method of claim 28 wherein R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is isobutyl; and NH-R₄ is leucine-leucine O-methyl ester.

40. The method of claim 28 wherein R₁ is t-butyloxycarbonyl; R₂ is benzyl, R₃ is benzyl, and NH-R₄ is alanine-phenylalanine O-methyl ester.

41. The method of claim 28 wherein R₁ is t-butyloxycarbonyl; R₂ is benzyl, R₃ is benzyl, and NH-R₄ is leucine-valine O-methyl ester.

42. The method of claim 28 wherein R₁ is t-butyloxycarbonyl; R₂ is benzyl, R₃ is benzyl, and NH-R₄ is valine-phenylalanine O-methyl ester.

43. The method of claim 28 wherein R₁ is t-butyloxycarbonyl; R₂ is benzyl, R₃ is benzyl, and NH-R₄ is leucine-valine-phenylalanine O-methyl ester.

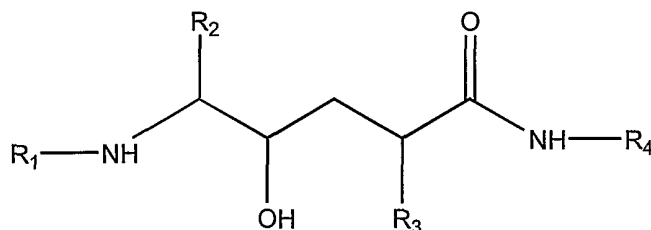
44. The method of claim 28 wherein the subject is free of symptoms of retrovirus infection.

45. The method of claim 28 wherein the compound is administered to the subject in combination with an effective amount of a second agent useful in the treatment of β -amyloid-associated disease.

46. The method of claim 45 wherein the second agent is an acetylcholinesterase inhibitor.

47. The method of claim 45 wherein the second agent is a hydroxyethylene compound of Formula II

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Formula II

5 wherein R₁ is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, heteroaromatic, acyl (R₅C=O), R₆O(C=O), and R₇R₈N(C=O), wherein R₅, R₆, R₇, and R₈ are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic;

R₂ and R₃ are each independently selected from the group consisting of hydrogen, alkyl, 10 cycloalkyl, aromatic, and heteroaromatic;

NH-R₄ is peptidyl or R₄ is independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aromatic, and heteroaromatic; and

non-hydrogen R₁, R₂, R₃, R₄, R₅, R₆, R₇, and R₈ can independently be substituted with 15 alkylamino, alkoxy, amino, halide, nitro, sulfate, sulfonamide, sulfoxide, or thiol ether.

48. The method of claim 28 wherein the compound is administered orally.

49. A method of inhibiting activity of an aspartyl protease, comprising:

contacting an aspartyl protease under conditions in which the aspartyl protease is 20 enzymatically active with an effective amount of a compound of Formula I according to claims 1 or 18, whereby activity of the aspartyl protease is decreased in the presence of the compound.

50. The method of claim 49 wherein R₁ is *t*-butyloxycarbonyl.

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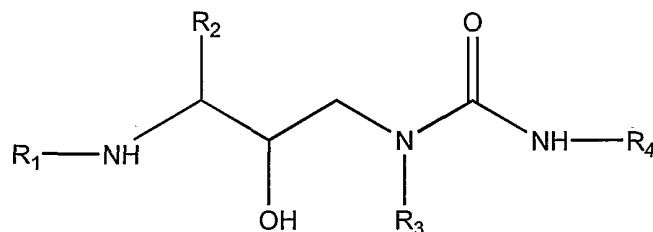
51. The method of claim 49 wherein R₂ is selected from the group consisting of cyclohexyl, benzyl and other amino acid side chains.

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52. The method of claim 49 wherein R₃ is selected from the group consisting of methyl, isopropyl, isobutyl, benzyl and other amino acid side chains.
53. The method of claim 49 wherein NH-R₄ is selected from the group consisting of
5 alanine-phenylalanine O-methyl ester, leucine-alanine O-methyl ester, leucine-leucine O-methyl ester, leucine-phenylalanine O-methyl ester, leucine-valine O-methyl ester, and valine-phenylalanine O-methyl ester.
54. The method of claim 49 wherein NH-R₄ is selected from the group consisting of
10 leucine-leucine O-methyl ester and leucine-phenylalanine O-methyl ester.
55. The method of claim 49 wherein NH-R₄ is selected from the group consisting of leucine-valine-alanine O-methyl ester, leucine-valine-leucine O-methyl ester, leucine-valine-phenylalanine O-methyl ester, and leucine-valine-valine O-methyl ester.
15
56. The method of claim 49 wherein R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is benzyl; and NH-R₄ is leucine-leucine O-methyl ester.
57. The method of claim 49 wherein R₁ is t-butyloxycarbonyl; R₂ is benzyl; R₃ is
20 isobutyl; and NH-R₄ is leucine-leucine O-methyl ester.
58. The method of claim 49 wherein R₁ is t-butyloxycarbonyl; R₂ is benzyl, R₃ is benzyl, and NH-R₄ is alanine-phenylalanine O-methyl ester.
- 25 59. The method of claim 49 wherein R₁ is t-butyloxycarbonyl; R₂ is benzyl, R₃ is benzyl, and NH-R₄ is leucine-valine O-methyl ester.
60. The method of claim 49 wherein R₁ is t-butyloxycarbonyl; R₂ is benzyl, R₃ is benzyl, and NH-R₄ is valine-phenylalanine O-methyl ester.
30
61. The method of claim 49 wherein R₁ is t-butyloxycarbonyl; R₂ is benzyl, R₃ is benzyl, and NH-R₄ is leucine-valine-phenylalanine O-methyl ester.

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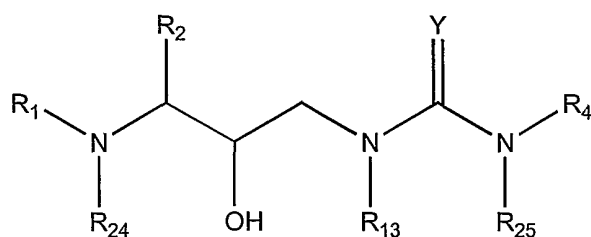
62. The method of claim 49 wherein the aspartyl protease is not a retroviral protease.
63. The method of claim 49 wherein the aspartyl protease is not HIV protease.
- 5
64. The method of claim 49 wherein the aspartyl protease is not renin.
65. The method of claim 49 wherein the aspartyl protease is a γ -secretase.
- 10 66. The method of claim 49 wherein the aspartyl protease is a β -secretase.
67. The method of claim 49 wherein the contacting results in a decrease in generation of amyloid- β peptide.
- 15 68. The method of claim 49 wherein the contacting occurs in vitro.
69. The method of claim 49 wherein the contacting occurs in vivo.
70. A method of treating a subject having or at risk of having a β -amyloid-associated
20 disease, comprising:
administering to a subject having or at risk of having a β -amyloid-associated disease and free of symptoms otherwise calling for treatment with a compound of Formula IA or Formula IB, a therapeutically effective amount of a compound of Formula IA or Formula IB



25

Formula IA

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Formula IB

5 wherein, with respect to Formula IA:

(i) R_2 is benzyl and R_1 is $-\text{CO}-\text{CH}(\text{NHR})\text{CH}_2\text{CONH}_2$, wherein:

R is carbobenzyloxy, R_3 is methyl, and R_4 is methyl;

R is carbobenzyloxy, R_3 is methyl, and R_4 is n-butyl;

R is carbobenzyloxy, R_3 is isobutyl, and R_4 is methyl;

10 R is carbobenzyloxy, R_3 is isobutyl, and R_4 is n-butyl;

R is quinolinyl-2-carboxamide, R_3 is isobutyl, and R_4 is n-butyl;

R is carbobenzyloxy, R_3 is isobutyl, and R_4 is n-propyl;

R is carbobenzyloxy, R_3 is isobutyl, and R_4 is ethyl;

R is carbobenzyloxy, R_3 is isobutyl, and R_4 is isopropyl;

15 R is carbobenzyloxy, R_3 is isobutyl, and R_4 is tert-butyl;

R is quinolinyl-2-carboxamide, R_3 is isobutyl, and R_4 is tert-butyl;

R is carbobenzyloxy, R_3 is isopentyl, and R_4 is tert-butyl;

R is quinolinyl-2-carboxamide, R_3 is isopentyl, and R_4 is tert-butyl;

R is carbobenzyloxy, R_3 is $\text{CH}_2\text{C}_6\text{H}_{11}$, and R_4 is tert-butyl;

20 R is quinolinyl-2-carboxamide, R_3 is $\text{CH}_2\text{C}_6\text{H}_{11}$, and R_4 is tert-butyl;

R is carbobenzyloxy, R_3 is benzyl, and R_4 is tert-butyl;

R is quinolinyl-2-carboxamide, R_3 is benzyl, and R_4 is tert-butyl;

R is carbobenzyloxy, R_3 is (*R*)- $\text{CH}(\text{CH}_3)$ -phenyl, and R_4 is tert-butyl;

R is carbobenzyloxy, R_3 is (*S*)- $\text{CH}(\text{CH}_3)$ -phenyl, and R_4 is tert-butyl;

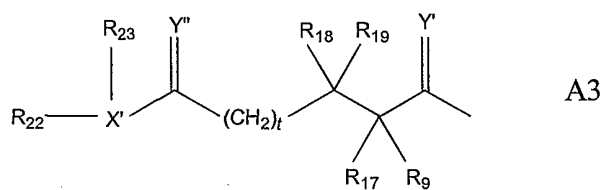
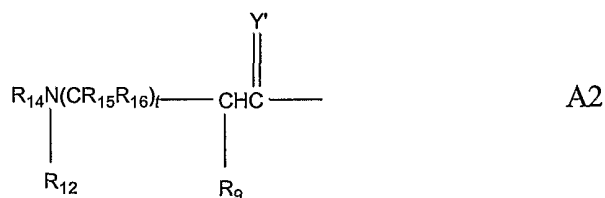
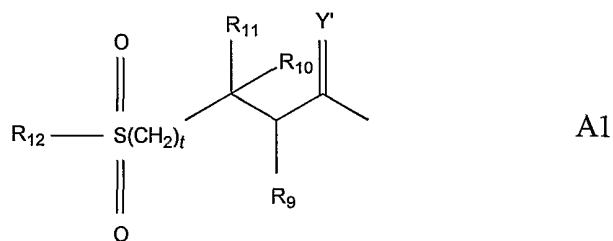
25 R is carbobenzyloxy, R_3 is $\text{CH}_2(4\text{-pyridyl})$, and R_4 is tert-butyl; or

R is quinolinyl-2-carboxamide, R_3 is $\text{CH}_2(4\text{-pyridyl})$, and R_4 is tert-butyl; or

(ii) R_1 is $-\text{CO}-\text{CH}(\text{C}(\text{CH}_3)_3)\text{NHR}$, wherein: R is $\text{COCH}_2\text{NHCH}_3$ HCl, R_2 is benzyl, R_3 is isopentyl, and R_4 is tert-butyl;

and, with respect to Formula IB,

R₁ is a radical represented by any of the formulas A1, A2, A3 below:



R₂ represents alkyl, aryl, cycloalkyl, cycloalkylalkyl and aralkyl radicals, which radicals are optionally substituted with a group selected from -NO₂, -OR₃₀, -SR₃₀, and halogen radicals, wherein R₃₀ represents hydrogen and alkyl radicals;

15 R₄ represents radicals represented by the formula: wherein n represents an integer of from 0 to 6, R₂₆ and R₂₇ independently represent radicals as defined for R₁₃ and amino acid side chains selected from the group consisting of valine, isoleucine, glycine, alanine, allo-isoleucine, asparagine, leucine, glutamine, and t-butylglycine or R₂₆ and R₂₇ together with the carbon atom to which they are attached

20 form a cycloalkyl radical; and R₂₈ represents cyano, hydroxyl, alkyl, alkoxy, cycloalkyl, aryl, aralkyl, heterocycloalkyl and heteroaryl radicals and radicals represented by the formulas C(O)R₂₉, CO₂R₂₉, SO₂R₂₉, SR₂₉, CONR₂₉R₂₁, OR₂₉, CF₃ and NR₂₉R₂₁ wherein

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R₂₉ and R₂₁ independently represent hydrogen and radicals as defined for R₁₃ or R₂₉ and R₂₁ together with a nitrogen to which they are attached in the formula -NR₂₉R₂₁ represent heterocycloalkyl and heteroaryl radicals;

R₂₄ represents hydrogen and alkyl radicals;

5 R₂₅ independently represents hydrogen and radicals as defined by R₁₃; and

R₁₃ represents alkyl, alkenyl, alkynyl, hydroxyalkyl, alkoxyalkyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, heterocycloalkylalkyl, aryl, aralkyl, heteroaralkyl, aminoalkyl and mono- and disubstituted aminoalkyl radicals where said substituents are selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroalkyl, heterocycloalkyl, and heterocycloalkylalkyl radicals or, in the case of a disubstituted aminoalkanoyl radical, said substituents along with the nitrogen atom to which they are attached, form a heterocycloalkyl or a heteroaryl radical;

10
15
20

wherein:

R₁₄ represents hydrogen and alkoxy carbonyl, aralkoxy carbonyl, alkyl carbonyl, cycloalkyl carbonyl, cycloalkylalkoxy carbonyl, cycloalkylalkanoyl, alkanoyl, aralkanoyl, aroyl, aryloxy carbonyl, aryloxyalkanoyl, heterocyclyl carbonyl, heterocyclyloxy carbonyl, heterocyclylalkanoyl, heterocyclylalkoxy carbonyl, heteroaralkoxy carbonyl, heteroaryloxy carbonyl, heteroaralkanoyl, heteroaroyl, alkyl, aryl, aralkyl, aryloxyalkyl, heteroaryloxyalkyl, hydroxyalkyl, aminocarbonyl, aminoalkanoyl, and mono- and disubstituted aminocarbonyl and mono- and disubstituted aminoalkanoyl radicals wherein the substituents are selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroaralkyl, heteroalkyl and heterocycloalkylalkyl radicals or in the case of a disubstituted aminoalkanoyl radical, said substituents along with the nitrogen atom to which they are attached form a heterocycloalkyl or heteroaryl radical;

25 R₁₂ represents hydrogen and radicals as defined for R₁₃ or R₁₄ and R₁₂ together with the nitrogen to which they are attached form a heterocycloalkyl or heteroaryl radical or when R₁ is A1, R₁₂ represents hydrogen, radicals as defined for R₁₃ and aralkoxy carbonylalkyl and aminocarbonylalkyl and aminoalkyl radicals wherein said amino group may be mono- or disubstituted with substituents selected from alkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroaralkyl, heteroalkyl, and heterocycloalkylalkyl radicals;

30

t represents either 0 or 1;

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R₉ represents hydrogen, -CH₂SO₂NH₂, -CO₂CH₃, -CH₂CO₂CH₃, -CONH₂,
-CH₂C(O)NHCH₃, -CH₂C(O)N(CH₃)₂, -CONHCH₃, -CONH(CH₃)₂, -C(CH₃)₂(SCH₃),
-C(CH₃)₂(S[O]CH₃), -C(CH₃)₂(S[O]₂CH₃), alkyl, haloalkyl, alkenyl, alkynyl and
cycloalkyl radicals and amino acid side chains selected from asparagine, S-methyl
5 cysteine and the corresponding sulfoxide and sulfone derivatives thereof, glycine, leucine,
isoleucine, allo-isoleucine, tert-leucine, phenylalanine, ornithine, alanine, histidine,
norleucine, glutamine, valine, threonine, serine, aspartic acid, beta-cyano alanine, and
allo-threonine side chains;

R₁₅ and R₁₆ independently represent hydrogen and radicals as defined for R₉, or one
10 of R₁₅ and R₁₆, together with R₉ and the carbon atoms to which they are attached,
represent a cycloalkyl radical;

X' represents O, C(R₂₁) where R₂₁ represents hydrogen and alkyl radicals and N;

Y, Y' and Y'' independently represent O, S and NR₂₀ wherein R₂₀ represents hydrogen
and radicals as defined for R₁₃;

15 R₁₀, R₁₁, R₁₇, R₁₈ and R₁₉ represent radicals as defined for R₉, or one of R₉ and R₁₇
together with one of R₁₈ and R₁₉ and the carbon atoms to which they are attached form a
cycloalkyl radical; and

R₂₂ and R₂₃ independently represent hydrogen and radicals as defined for R₁₃, or R₂₂
and R₂₃ together with X' represent cycloalkyl, aryl, heterocyclyl and heteroaryl radicals,
20 provided that when X' is O, R₂₃ is absent, whereby the β-amyloid disease is treated.

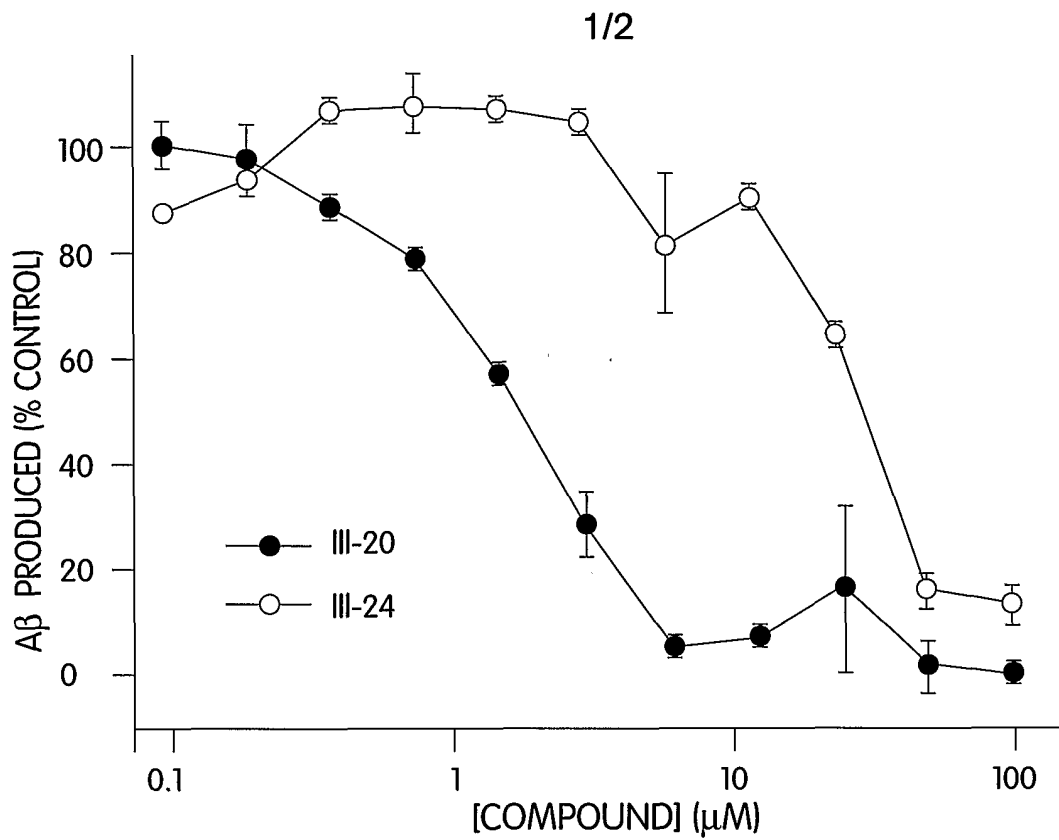


Fig. 1

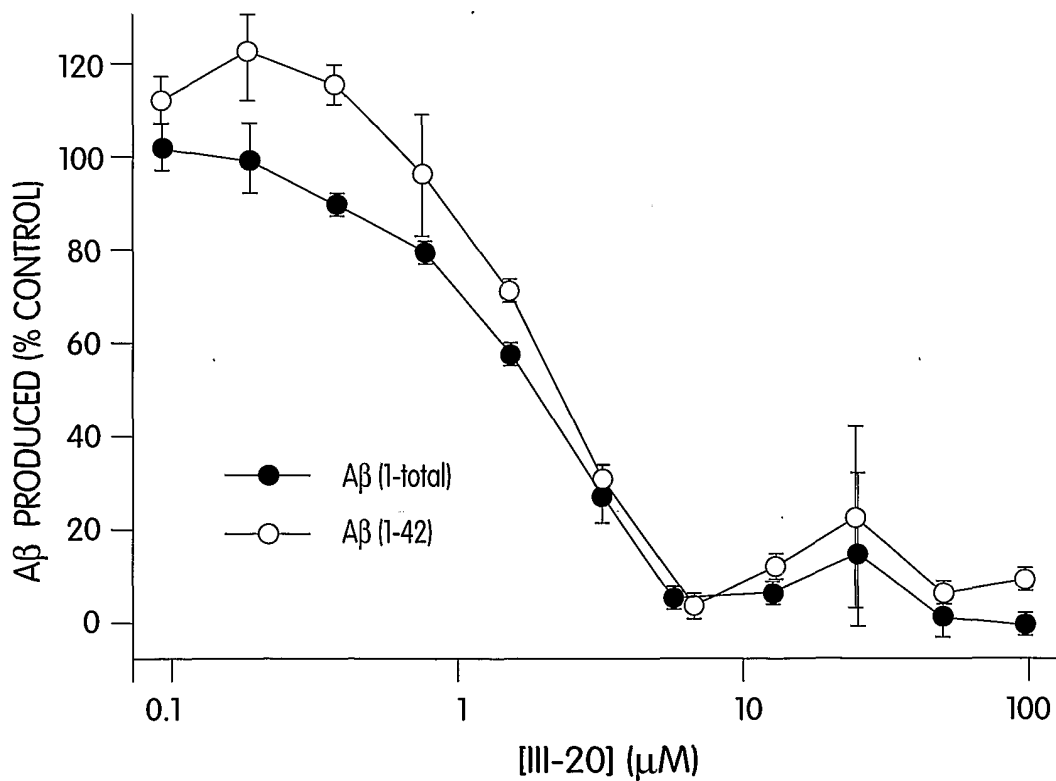


Fig. 2

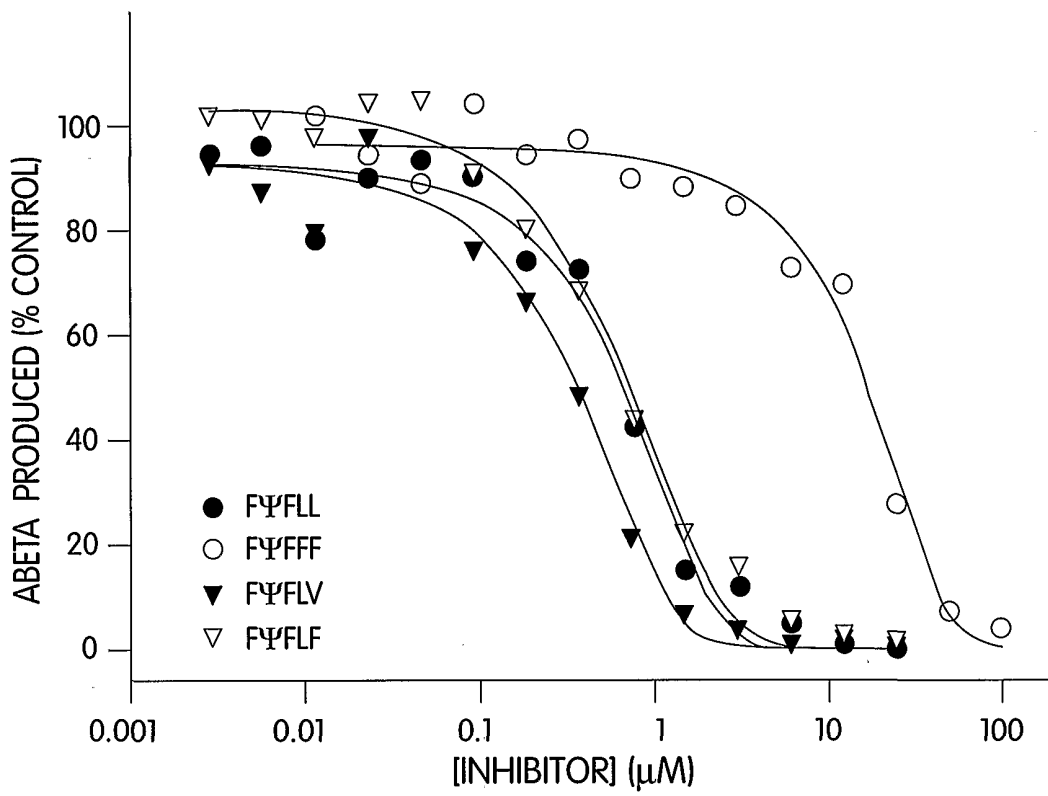


Fig. 3