

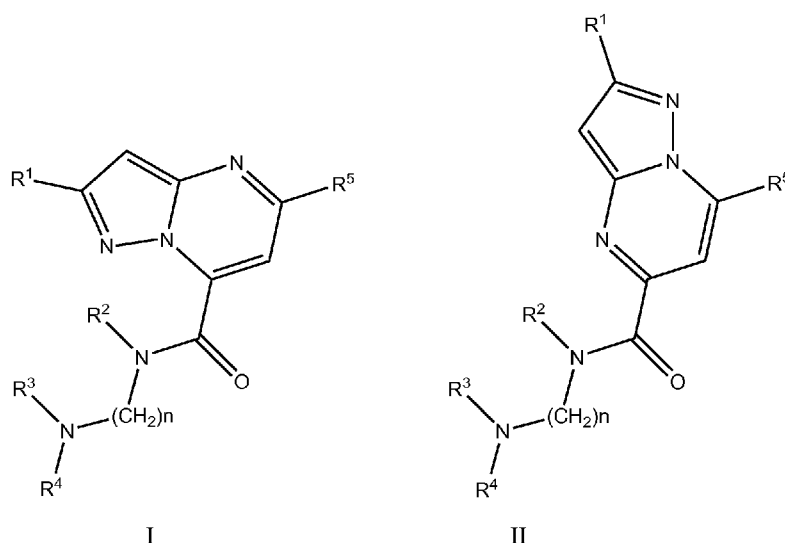
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(54) Title: PYRAZOLO[1,5-a]PYRIMIDINECARBOXAMIDE DERIVATIVES FOR TREATING COGNITIVE IMPAIRMENT

(57) Abstract: Compounds of formula I and II: Formula (I) or Formula (II) inhibit the interaction between fibrinogen and amyloid- β and are useful for treating cognitive disorders such as memory impairment and Alzheimer's disease.

PYRAZOLO[1,5-a]PYRIMIDINECARBOXAMIDE DERIVATIVES FOR TREATING COGNITIVE IMPAIRMENT

Federally Sponsored Research

[0001] The following invention was made with Government support under contract number NIH NS050537. The Government has certain rights in this invention.

Field of the Invention

[0002] The invention relates to N-substituted pyrazolo[1,5-a]pyrimidine-5-carboxamides and 7-carboxamides that inhibit the interaction between fibrinogen and amyloid- β . These compounds are useful to treat cognitive impairment and thrombotic disorders.

Background of the Invention

[0003] Accumulating evidence suggests that cerebrovascular risk factors play an important role in Alzheimer's disease (AD) pathophysiology. Many Alzheimer's disease patients suffer from altered cerebral blood flow, damaged cerebral vasculature, and increased cerebral microinfarcts, and a majority of patients with dementia present with both Alzheimer's disease and vascular pathologies. Furthermore, cerebral amyloid angiopathy (CAA), which is the deposition of the beta-amyloid (A β) peptide within cerebral blood vessels, results in degenerative vascular changes. Patients with both cerebral amyloid angiopathy and neurological pathology including neurofibrillary tangles and neuritic plaques have more severe cognitive impairment than patients with only Alzheimer's disease pathology or cerebral amyloid angiopathy alone, and reduction of cerebral amyloid angiopathy levels in Alzheimer's disease transgenic mice leads to memory improvement. Interestingly, a study by Mortimer [*Curr Alzheimer Res* 9, 621 (2012)] showed that one-third of the participants who had neurological Alzheimer's disease pathology were actually not demented at the time of death, but when Alzheimer's disease pathology was concomitant with brain infarcts, there was a high prevalence of dementia found in participants. Thus, the identification of a molecular association between these vascular and neurological pathologies could aid in more efficient diagnoses and effective treatments for Alzheimer's

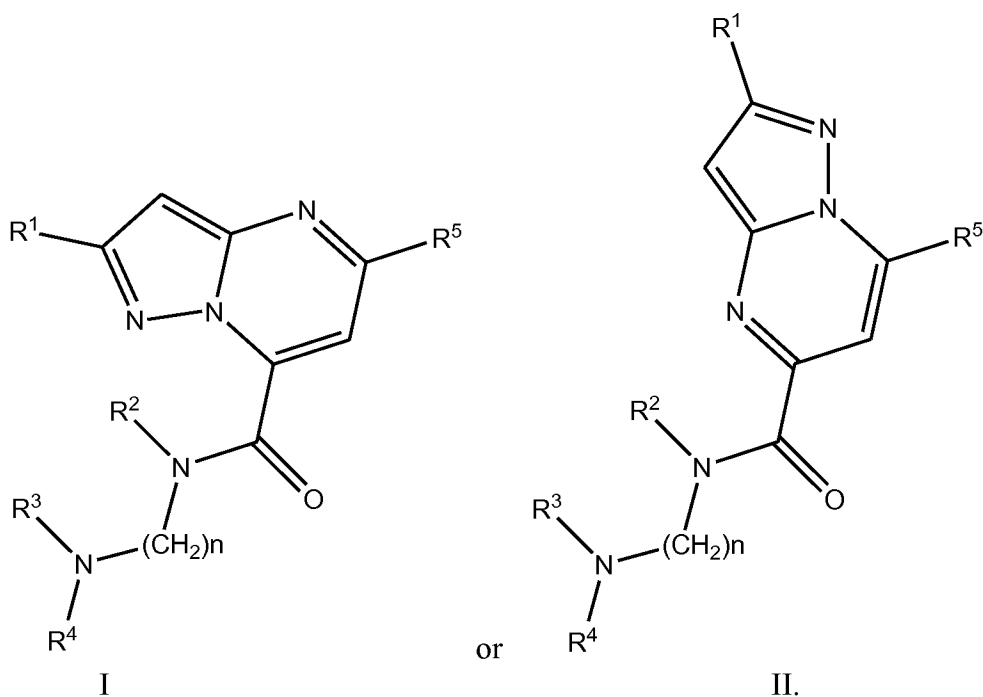
disease.

[0004] Recent studies have suggested that fibrinogen, a primary protein component of blood clots, serves as a molecular link between the vascular and neurological abnormalities observed in Alzheimer's disease patients. Normally, fibrin(ogen) is found in the blood and is excluded from the brain via the blood brain barrier. However, it has been shown that: 1) fibrin(ogen) is often localized to cerebral amyloid angiopathy in the brain's blood vessels and brain parenchyma in Alzheimer's disease patients and in mouse models of Alzheimer's disease; 2) fibrin deposition in the vasculature increases blood brain barrier dysfunction and neurovascular damage in AD mice; 3) A β binds specifically to fibrinogen; and 4) fibrin clots formed in the presence of A β have an abnormal structure, making them resistant to degradation by fibrinolytic enzymes.

Summary of the Invention

[0005] The foregoing observations indicate that in the presence of A β , any fibrin clots formed might be more persistent and may exacerbate neurovascular damage and cognitive impairment. Therefore, one might theorize that molecules that block the interaction of fibrinogen with amyloid- β , without affecting clotting in general, could restore altered thrombosis and fibrinolysis and protect against vascular damage in Alzheimer's disease patients, and could be used as therapeutic agents. In fact, this is confirmed in the experiments described below.

[0006] In one aspect, the invention relates to a method of inhibiting the interaction between fibrinogen and amyloid- β , comprising bringing amyloid- β into contact with a compound of formula I or II:



wherein

R^5 is (C₄-C₁₀) hydrocarbyl, aryl or heteroaryl, said hydrocarbyl, aryl or heteroaryl optionally substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆) alkylamino, and di(C₁-C₆)alkylamino;

R^1 is (C₃-C₁₀) heterocyclyl, (C₃-C₁₀) hydrocarbyl or (C₃-C₁₀) hydrocarbyl substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆) alkylamino, and di(C₁-C₆)alkylamino;

R^2 is (C₁-C₁₀) hydrocarbyl or (C₁-C₁₀) hydrocarbyl substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆) alkylamino, and di(C₁-C₆)alkylamino, wherein a benzene ring, if present, is not directly attached to the amide nitrogen and wherein halogen, if present, is only attached to a benzene ring;

R^3 is (C₁-C₁₀) hydrocarbyl;

R^4 is chosen from (C₁-C₈) acyl, (C₁-C₈) hydrocarbyl, and (C₁-C₈) hydrocarbyl substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆) alkylamino, and di(C₁-C₆)alkylamino, or,

taken together, any two of R^2 and R^3 or R^3 and R^4 , together with the nitrogens to which they are attached, may form a 4-7-membered, saturated nitrogen heterocycle; with the proviso that when R^2 and R^3 form a 6-membered, saturated nitrogen heterocycle and R^4 is chosen from (C_1-C_8) hydrocarbyl, and substituted (C_1-C_8) hydrocarbyl, then R^4 must be benzyl, substituted benzyl or phenyl substituted with (C_1-C_6) alkoxy; and n is 2, 3 or 4.

[0007] In another aspect, the invention relates to a method for treating cognitive disorders comprising administering to a mammal a therapeutically effective amount of a compound of formula I or II above.

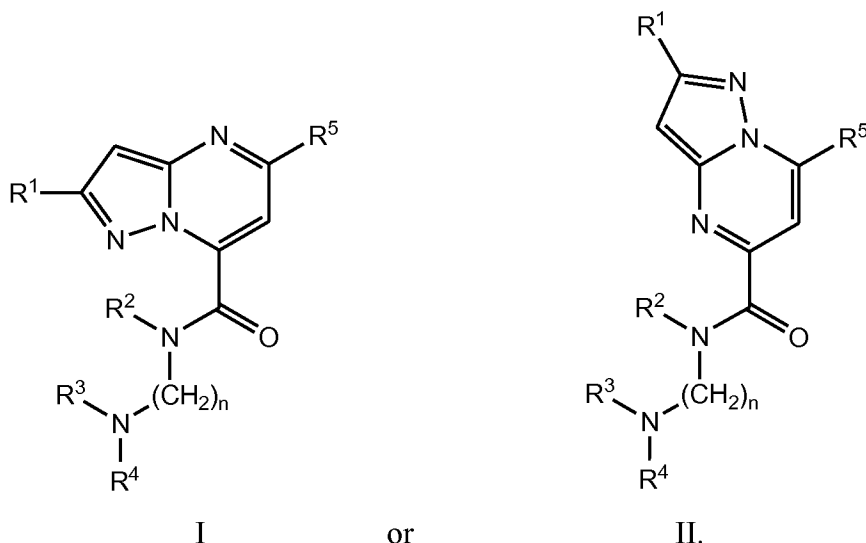
[0008] In another aspect, the invention relates to pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound of formula I or II.

[0009] In another aspect, the invention relates to compounds of formula I or II with the provisos that:

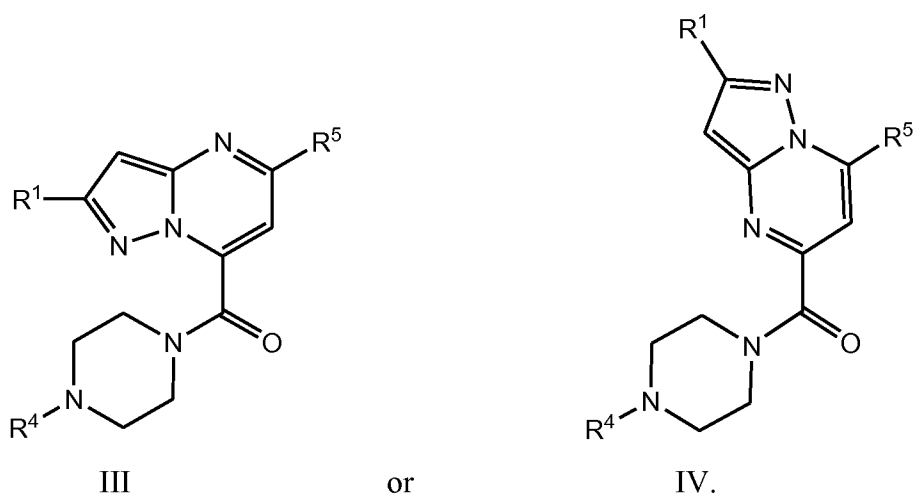
- (1) when n is 2 and R^2 is benzyl, then R^3 and R^4 are not methyl; and
- (2) when R^2 and R^3 form a 6-membered, saturated nitrogen heterocycle, then R^4 is (C_1-C_8) acyl, benzyl, substituted benzyl or phenyl substituted with (C_1-C_6) alkoxy; and R^5 is not phenyl, methoxyphenyl or fluorophenyl.

Detailed Description of the Invention

[0010] In its most basic aspects, the invention relates to inhibiting the interaction between fibrinogen and amyloid- β by bringing amyloid- β into contact with a compound of formula I or II:



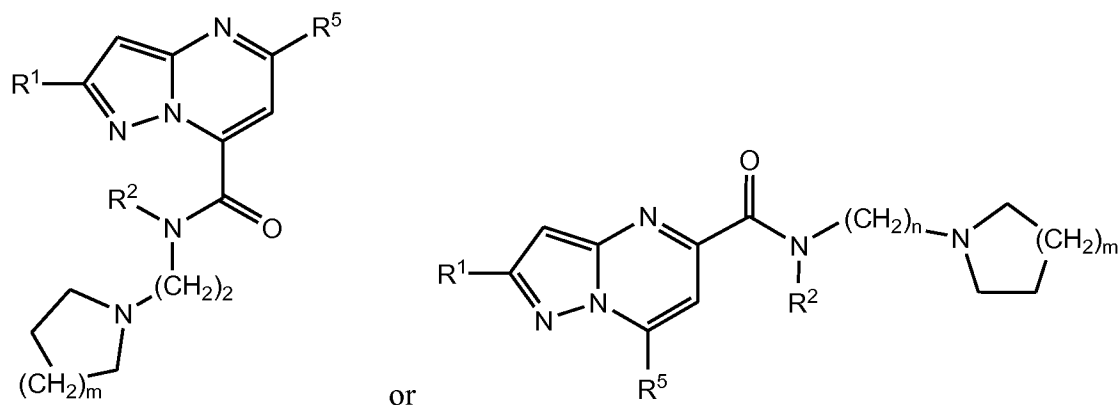
[0011] In some embodiments, R^5 is (C_4-C_{10}) hydrocarbyl, for example t-butyl. In some embodiments R^5 is aryl, wherein aryl is optionally substituted phenyl, particularly phenyl or phenyl substituted with halogen or (C_1-C_6) alkoxy. In some embodiments, R^4 is (C_1-C_8) acyl, particularly (C_1-C_6) acyl; in other embodiments R^4 is (C_1-C_8) hydrocarbyl. In other embodiments, R^4 is phenyl substituted with (C_1-C_6) alkoxy. In some embodiments, R^1 is (C_3-C_{10}) heterocyclyl, particularly (C_3-C_6) heterocyclyl; in other embodiments R^1 is (C_3-C_{10}) hydrocarbyl, particularly (C_3-C_{10}) alkyl and (C_3-C_{10}) cycloalkyl; in some other embodiments, R^1 is optionally substituted phenyl. In some embodiments, R^1 is (C_3-C_6) oxygen heterocyclyl; in some other embodiments R^1 is phenyl or t-butyl. In some embodiments, R^2 is (C_1-C_{10}) hydrocarbyl, for example benzyl. In other embodiments, R^2 and R^3 taken together form a 5 or 6-membered heterocycle, for example a piperazine. Such compounds may be represented by the formula III or IV:



In these compounds, R^4 is as previously defined. In particular, when R^4 is a phenyl

substituent (i.e. an aromatic (C₁-C₈) hydrocarbon or substituted aromatic (C₁-C₈) hydrocarbon in which the benzene ring is directly attached to the nitrogen as shown by the R⁴-N bond), R⁴ can only be alkoxy-substituted phenyl. In some embodiments R⁴ may be (C₁-C₈) hydrocarbyl or (C₁-C₆) acyl. In certain embodiments, when R² and R³ taken together form a 5 or 6-membered heterocycle, such as the compounds of formulae III and IV, R⁴ is phenyl substituted with (C₁-C₆)alkoxy. In some embodiments, R³ and R⁴ are independently chosen from (C₁-C₈) hydrocarbyl, for example (C₁-C₄)alkyl. In other embodiments, R³ and R⁴, together with the nitrogen to which they are attached, form a piperidine or pyrrolidine ring.

or



wherein m is 1 or 2.

[0012] In one particular embodiment, R¹ is chosen from (C₃-C₁₀) alkyl, (C₃-C₁₀) cycloalkyl and optionally substituted phenyl; R² and R³ taken together form a piperazine; and R⁴ is (C₁-C₁₀) hydrocarbyl. In another particular embodiment, R¹ is chosen from (C₃-C₁₀) alkyl, (C₃-C₁₀) cycloalkyl and optionally substituted phenyl; R² is benzyl; and R³ and R⁴ are independently chosen from (C₁-C₄)alkyl.

[0013] The invention also relates to a compound of formula I and II, as identified above. In some embodiments, R⁵ is (C₄-C₁₀) hydrocarbyl, for example t-butyl. In some embodiments R⁵ is aryl, wherein aryl is optionally substituted phenyl, particularly phenyl or phenyl substituted with halogen or (C₁-C₆)alkoxy. In some embodiments, R¹ is (C₃-C₁₀) heterocyclyl, particularly (C₃-C₆) heterocyclyl; in other embodiments R¹ is (C₃-C₁₀) hydrocarbyl, particularly (C₃-C₁₀) alkyl and (C₃-C₁₀) cycloalkyl; in some other embodiments, R¹ is optionally substituted phenyl. In some embodiments, R¹ is (C₃-C₆) oxygen heterocyclyl; in some other embodiments R¹ is phenyl or t-butyl. In some embodiments, R² is (C₁-C₁₀) hydrocarbyl, for example benzyl. In some embodiments, R⁴ is (C₁-C₈) acyl; in

other embodiments R^4 is (C_1-C_8) hydrocarbyl. In other embodiments, R^2 and R^3 taken together form a 5 or 6-membered heterocycle, for example a piperazine. Such compounds may be represented by the formula IV, as identified above. In these compounds, R^4 is as previously defined. In particular, when n is 2 and R^2 is benzyl, then R^3 and R^4 are not methyl, and when R^2 and R^3 form a 6-member heterocycle, then R^4 is (C_1-C_8) acyl, benzyl, substituted benzyl or phenyl substituted with (C_1-C_6) alkoxy; and R^5 is not phenyl, methoxyphenyl or fluorophenyl. In some embodiments R^4 may be (C_1-C_8) hydrocarbyl or (C_1-C_6) acyl. In some embodiments, R^3 and R^4 are independently chosen from (C_1-C_8) hydrocarbyl, for example (C_1-C_4) alkyl. In other embodiments, R^3 and R^4 , together with the nitrogens to which they are attached, form a piperidine or pyrrolidine ring

[0014] In one particular embodiment, R^1 is chosen from (C_3-C_6) heterocyclyl, (C_3-C_{10}) alkyl, (C_3-C_{10}) cycloalkyl and optionally substituted phenyl; R^2 and R^3 taken together form a piperazine and R^4 is (C_1-C_8) hydrocarbyl. In another particular embodiment, R^1 is chosen from (C_3-C_6) heterocyclyl, (C_3-C_{10}) alkyl, (C_3-C_{10}) cycloalkyl and optionally substituted phenyl; R^2 is benzyl; and R^2 and R^3 are independently chosen from (C_1-C_4) alkyl.

[0015] Throughout this specification the terms and substituents retain their definitions.

[0016] The term “hydrocarbon” (or “hydrocarbyl”, when used as a substituent) includes alkyl, cycloalkyl, polycycloalkyl, alkenyl, alkynyl, aryl and combinations thereof. Examples include benzyl, phenethyl, cyclohexylmethyl, adamantyl, camphoryl and naphthylethyl. Hydrocarbyl refers to any substituent or scaffold comprised of hydrogen and carbon as the only elemental constituents. Aliphatic hydrocarbons are hydrocarbons that are not aromatic; they may be saturated or unsaturated, cyclic, linear or branched. Examples of aliphatic hydrocarbons include isopropyl, 2-butenyl, 2-butylnyl, cyclopentyl, cyclohexyl, norbornyl, etc. Aromatic hydrocarbons include benzene (phenyl), naphthalene (naphthyl), anthracene, etc.

[0017] Unless otherwise specified, alkyl (or alkylene) is intended to include linear or branched saturated hydrocarbon structures and combinations thereof. Alkyl refers to alkyl groups from 1 to 20 carbon atoms, preferably 1 to 10 carbon atoms, more preferably 1 to 6 carbon atoms. Examples of alkyl groups include methyl, ethyl, propyl, isopropyl, n-butyl, s-butyl, t-butyl and the like.

[0018] Cycloalkyl is a subset of hydrocarbon and includes cyclic hydrocarbon groups of from 3 to 8 carbon atoms. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, norbornyl and the like.

[0019] Unless otherwise specified, the term “carbocycle” is intended to include ring systems in which the ring atoms are all carbon but of any oxidation state. Thus (C₃-C₁₀) carbocycle refers to both non-aromatic and aromatic systems, including such systems as cyclopropane, benzene and cyclohexene; (C₈-C₁₂) carbopolycycle refers to such systems as norbornane, decalin, indane and naphthalene. Carbocycle, if not otherwise limited, refers to monocycles, bicycles and polycycles.

[0020] Heterocycle means an aliphatic or aromatic carbocycle residue in which from one to four carbons is replaced by a heteroatom selected from the group consisting of N, O, and S. The nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. Unless otherwise specified, a heterocycle may be non-aromatic (heteroaliphatic) or aromatic (heteroaryl). Examples of heterocycles include pyrrolidine, pyrazole, pyrrole, indole, quinoline, isoquinoline, tetrahydroisoquinoline, benzofuran, benzodioxan, benzodioxole (commonly referred to as methylenedioxyphenyl, when occurring as a substituent), tetrazole, morpholine, thiazole, pyridine, pyridazine, pyrimidine, thiophene, furan, oxazole, oxazoline, isoxazole, dioxane, tetrahydrofuran and the like. Examples of heterocyclyl residues include piperaziny, piperidinyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyrazinyl, oxazolidinyl, isoxazolidinyl, thiazolidinyl, isothiazolyl, quinuclidinyl, isothiazolidinyl, benzimidazolyl, thiadiazolyl, benzopyranyl, benzothiazolyl, tetrahydrofuryl, tetrahydropyranyl, thienyl (also historically called thiophenyl), benzothienyl, thiamorpholinyl, oxadiazolyl, triazolyl and tetrahydroquinolinyl.

[0021] Alkoxy or alkoxyl refers to groups of from 1 to 20 carbon atoms, preferably 1 to 10 carbon atoms, more preferably 1 to 6 carbon atoms of a straight or branched configuration attached to the parent structure through an oxygen. Examples include methoxy, ethoxy, propoxy, isopropoxy and the like. Lower-alkoxy refers to groups containing one to four carbons. For the purpose of this application, alkoxy and lower alkoxy include methylenedioxy and ethylenedioxy.

[0022] The term “halogen” means fluorine, chlorine, bromine or iodine atoms. In one embodiment, halogen may be a fluorine or chlorine atom.

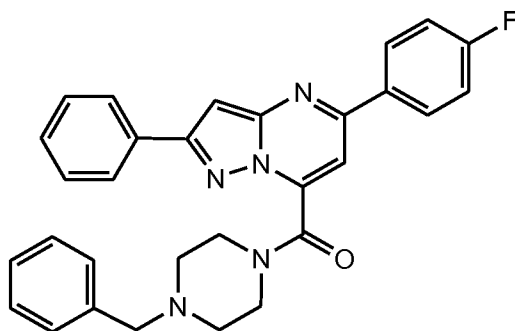
[0023] As used herein, the term “optionally substituted” may be used interchangeably with “unsubstituted or substituted”. The term “substituted” refers to the replacement of one or more hydrogen atoms in a specified group with a specified radical. For example, substituted alkyl, aryl, cycloalkyl, heterocyclyl etc. refer to alkyl, aryl, cycloalkyl, or heterocyclyl wherein one or more H atoms in each residue are replaced with halogen, haloalkyl, alkyl, acyl, alkoxyalkyl, hydroxy lower alkyl, carbonyl, phenyl, heteroaryl, benzenesulfonyl, hydroxy, lower alkoxy, haloalkoxy, oxaalkyl, carboxy, alkoxycarbonyl [-C(=O)O-alkyl], alkoxycarbonylamino [HNC(=O)O-alkyl], aminocarbonyl (also known as carboxamido) [-C(=O)NH₂], alkylaminocarbonyl [-C(=O)NH-alkyl], cyano, acetoxy, nitro, amino, alkylamino, dialkylamino, (alkyl)(aryl)aminoalkyl, alkylaminoalkyl (including cycloalkylaminoalkyl), dialkylaminoalkyl, dialkylaminoalkoxy, heterocyclylalkoxy, mercapto, alkylthio, sulfoxide, sulfone, sulfonylamino, alkylsulfinyl, alkylsulfonyl, acylaminoalkyl, acylaminoalkoxy, acylamino, amidino, aryl, benzyl, heterocyclyl, heterocyclylalkyl, phenoxy, benzyloxy, heteroaryloxy, hydroxyimino, alkoxyimino, oxaalkyl, aminosulfonyl, trityl, amidino, guanidino, ureido, benzyloxyphenyl, and benzyloxy. “Oxo” is also included among the substituents referred to in “optionally substituted”; it will be appreciated by persons of skill in the art that, because oxo is a divalent radical, there are circumstances in which it will not be appropriate as a substituent (e.g. on phenyl). In one embodiment, 1, 2, or 3 hydrogen atoms are replaced with a specified radical. In the case of alkyl and cycloalkyl, more than three hydrogen atoms can be replaced by fluorine; indeed, all available hydrogen atoms could be replaced by fluorine. In preferred embodiments, substituents are halogen, haloalkyl, alkyl, acyl, hydroxyalkyl, hydroxy, alkoxy, haloalkoxy, aminocarbonyl oxaalkyl, carboxy, cyano, acetoxy, nitro, amino, alkylamino, dialkylamino, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylsulfonylamino arylsulfonyl, arylsulfonylamino, and benzyloxy. Most preferred are halogen, (C₁-C₄)alkyl, halo(C₁-C₄)alkyl, (C₁-C₄)alkoxy, halo(C₁-C₄)alkoxy, and aminocarbonyl.

[0024] Substituents Rⁿ are generally defined when introduced and retain that definition throughout the specification and in all independent claims.

[0025] Unless otherwise specified, acyl refers to formyl and to groups of 1, 2, 3, 4, 5, 6, 7

and 8 carbon atoms of a straight, branched, cyclic configuration, saturated, unsaturated and aromatic and combinations thereof, attached to the parent structure through a carbonyl functionality. Examples include acetyl, benzoyl, propionyl, isobutyryl and the like. Lower-acyl refers to groups containing one to four carbons. The double bonded oxygen, when referred to as a substituent itself is called “oxo”.

[0026] As used herein, and as would be understood by the person of skill in the art, the recitation of “a compound” - unless expressly further limited - is intended to include salts of that compound. Thus, for example, the recitation “a compound of formula”



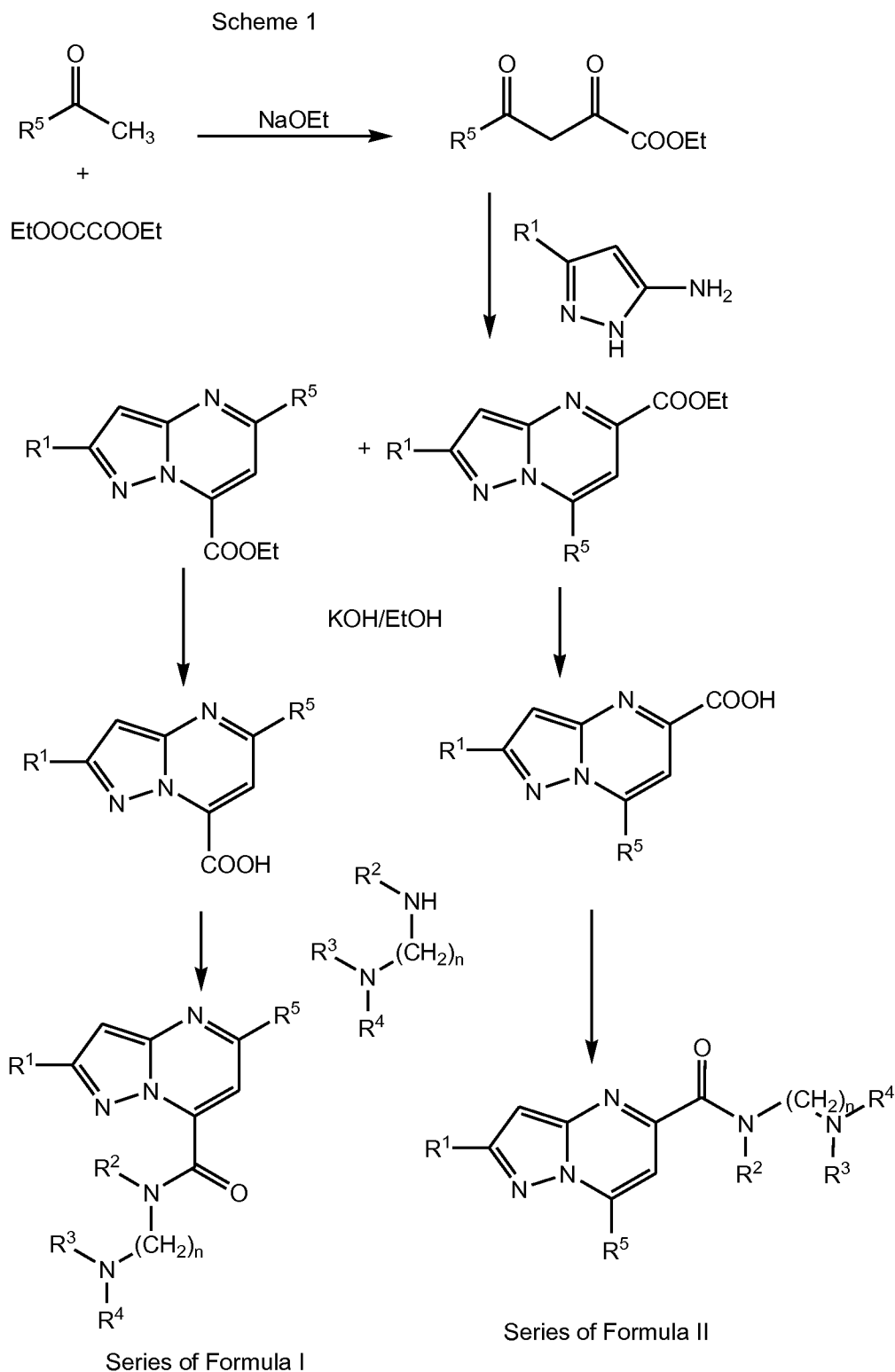
as depicted above, would include salts in which, for example, the piperazine nitrogen is protonated and is paired with any counterion. In a particular embodiment, the term “compound” refers to the compound or a pharmaceutically acceptable salt thereof. The term “pharmaceutically acceptable salt” refers to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. The compounds of the present invention are usually basic, and salts may be prepared from pharmaceutically acceptable non-toxic acids including inorganic and organic acids. Suitable pharmaceutically acceptable acid addition salts for the compounds of the present invention include acetic, adipic, alginic, ascorbic, aspartic, benzenesulfonic (besylate), benzoic, boric, butyric, camphoric, camphorsulfonic, carbonic, citric, ethanedisulfonic, ethanesulfonic, ethylenediaminetetraacetic, formic, fumaric, glucoheptonic, gluconic, glutamic, hydrobromic, hydrochloric, hydroiodic, hydroxynaphthoic, isethionic, lactic, lactobionic, laurylsulfonic, maleic, malic, mandelic, methanesulfonic, mucic, naphthylenesulfonic, nitric, oleic, pamoic, pantothenic, phosphoric, pivalic, polygalacturonic, salicylic, stearic, succinic, sulfuric, tannic, tartaric acid, teoclastic, p-toluenesulfonic, and the like. Further pharmaceutically acceptable salts include, when appropriate, sulfonate and phosphonate anions attached to alkyl having from 1 to 20 carbon atoms.

[0027] Unless otherwise stated or depicted, structures depicted herein are also meant to

include all stereoisomeric (e.g., enantiomeric, diastereomeric, and cis-trans isomeric) forms of the structure; for example, the R and S configurations for each asymmetric center, (Z) and (E) double bond isomers, and (Z) and (E) conformational isomers. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and cis-trans isomeric (or conformational) mixtures of the present compounds are within the scope of the invention. Unless otherwise stated, all tautomeric forms of the compounds of the invention are within the scope of the invention.

[0028] All of the compounds falling within the foregoing parent genera I and II and their subgenera inhibit the interaction between fibrinogen and amyloid- β , but not all the compounds are novel. In particular, certain known species fall within the genus I or II, although no utility in inhibiting the interaction between fibrinogen and amyloid- β has been suggested for these species. It may be found upon examination that compounds that have been excluded from the claims to compounds or compounds that have been excluded from the claims to methods are patentable to the inventors in this application; it may also be found that additional species and genera not presently excluded are not patentable to the inventors in this application. In either case, the exclusion of species and genera in applicants' claims are to be considered artifacts of patent prosecution and not reflective of the inventors' concept or description of their invention. The invention, in a composition aspect, encompasses all compounds of formula I and II except those that are in the public's possession.

[0029] Compounds of formula I and II may be synthesized by the general route shown in Scheme 1:



[0030] As a specific example, compound 10 was synthesized as follows:

Synthesis of ethyl 4-(4-fluorophenyl)-2,4-dioxobutanoate: In a 2L round bottom flask equipped with a reflux condenser and a CaCl₂ drying tube metallic sodium (49.9 g, 2.17 mol, 3.0 equiv.) was dissolved in ethanol (1.0 L) carefully. The resulting sodium ethylate solution was cooled to about 10 °C then the mixture of 1-(4-fluorophenyl)ethan-1-one

(100.0 g, 0.72 mol) and diethyl oxalate (2) (295 mL, 2.17 mol, 3.0 equiv.) was poured slowly into it. The reaction mixture was stirred for about 15 minutes, and then was allowed to stand in a refrigerator overnight. The reaction mixture was poured into a mixture of conc. HCl solution (300 mL) and ice (c.a. 1 kg). The resulting precipitate was filtered off and washed with plenty of water, finally dried in a vacuum desiccator over P₂O₅/KOH. Yield: 165.5 g (3) (96%) as a light yellow powder.

[0031] *Synthesis of ethyl 2-(tert-butyl)-5-(4-fluorophenyl)pyrazolo[1,5-a]pyrimidine-7-carboxylate and ethyl 2-(tert-butyl)-7-(4-fluorophenyl)pyrazolo[1,5-a]pyrimidine-5-carboxylate:* Ethyl 4-(4-fluorophenyl)-2,4-dioxobutanoate (17.85 g, 75 mmol) and 5-amino-3-tert-butylpyrazole (4) (10.44 g, 75 mmol) were dissolved in dry ethanol (250 mL). The reaction mixture was stirred at room temperature overnight, by which time the reaction was complete. The reaction mixture was evaporated to dryness, resulting in 27 g of crude regioisomer mixture, which was separated by column chromatography on silica gel with gradient elution from hexanes to 5% THF in hexanes. The first regioisomer (upper spot on TLC) was obtained as a yellow powder (3 g). It was recrystallized from ethanol-water mixture and resulted 2.4 g (9 %) of *ethyl 2-(tert-butyl)-5-(4-fluorophenyl)pyrazolo[1,5-a]pyrimidine-7-carboxylate* as yellow crystals. The second regioisomer (lower spot on TLC) was obtained as a yellow thick oil (12 g). It was crystallized with isopropyl ether, and 9 g (35 %) of *ethyl 2-(tert-butyl)-7-(4-fluorophenyl)pyrazolo[1,5-a]pyrimidine-5-carboxylate* was obtained as a yellow crystalline powder.

[0032] *Synthesis of 2-(tert-butyl)-5-(4-fluorophenyl)pyrazolo[1,5-a]pyrimidine-7-carboxylic acid:* Ethyl 2-(tert-butyl)-5-(4-fluorophenyl)pyrazolo[1,5-a]pyrimidine-7-carboxylate (2.05 g, 6.0 mmol) was dissolved in ethanol (40 mL) and 50 % aqueous potassium hydroxide solution (1.35 mL, 2.0 equiv.) was added to it. The reaction mixture was stirred at room temperature for three hours, by which time the reaction was complete. (The reaction was followed by TLC with an eluent of 1,2-dichloroethane- ethanol 4:1 mixture.). The solvent was evaporated in vacuum; the residue was dissolved in water (80 mL) and washed twice with diethyl ether (25 mL). The aqueous layer was acidified with 10% aq. HCl solution; the resulting precipitate was filtered off, washed with plenty of water and isopropyl ether, and finally dried in a vacuum desiccator over P₂O₅/KOH. Yield: 1.54 g of 2-(tert-butyl)-5-(4-fluorophenyl)pyrazolo[1,5-a]pyrimidine-7-carboxylic acid (81 %) as a yellow powder. ¹H NMR (500 MHz, Benzene-*d*₆) δppm 14.68 (s, 1H), 7.71 (dd,

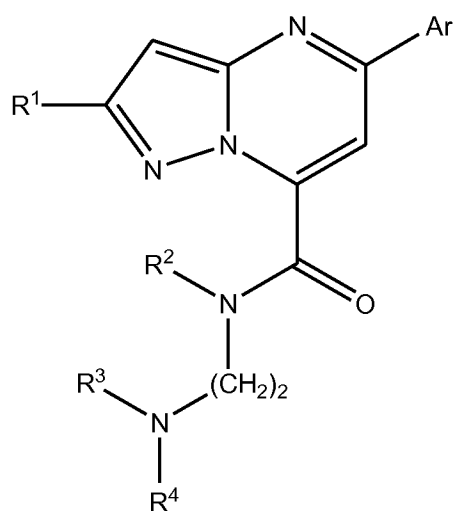
2H, $J_1 = 8.7$ Hz, $J_2 = 5.4$ Hz), 7.62 (s, 1H), 6.82 (dd, 2H, $J_1 = J_2 = 8.7$ Hz), 6.40 (s, 1H), 1.23 (s, 9H).

[0033] *Synthesis of N-benzyl-2-(tert-butyl)-5-(4-fluorophenyl)-N-(2-(pyrrolidin-1-yl)ethyl)pyrazolo[1,5-a] pyrimidine-7-carboxamide:* In a 25 mL screw capped vial the 2-(tert-butyl)-5-(4-fluorophenyl)pyrazolo[1,5-a]pyrimidine-7-carboxylic acid from the previous step (313 mg; 1.0 mmol) was dissolved in dry THF (10 mL) and 1,1'-carbonyldiimidazole (170 mg, 1.05 mmol, 1.05 equiv.) was added to it. The mixture was stirred at RT for 100 min., then N-benzyl-2-(pyrrolidin-1-yl)ethan-1-amine (215 mg, 1.05 mmol, 1.05 equiv.) was added to it. The reaction mixture was warmed at 40 °C for two hours and then allowed to stand overnight. Upon completion the reaction mixture was evaporated. The residue was dissolved in dichloromethane (40 mL), washed with water (20 mL), saturated aq. NaHCO₃ solution (20 mL) and brine (20 mL), dried over MgSO₄ and evaporated again. The crude product was recrystallized from ethanol and water mixture. The precipitated crystals were filtered off, washed with ethanol-water 1:1 mixture and dried in a vacuum desiccator over P₂O₅/KOH. Yield: 150 mg (30 %) of N-benzyl-2-(tert-butyl)-5-(4-fluorophenyl)-N-(2-(pyrrolidin-1-yl)ethyl)pyrazolo[1,5-a] pyrimidine-7-carboxamide **10** as a light yellow crystalline powder. From the mother liquor a second crop (58 mg) was obtained with the same purity; combined yield: 208 mg (42%). ¹H NMR (500 MHz, DMSO-*d*₆, T=296K) showed two rotamers in about a 1:3 ratio: Major component δ 8.33 (dd, $J_1 = 8.1$ Hz, $J_2 = 5.5$ Hz, 2H), 7.83 (s, 1H), 7.63 (d, $J = 7.3$ Hz, 2H), 7.39 (dd, $J_1 = J_2 = 8.1$ Hz, 2H), 7.45-7.20 (m, overlapping, 3H), 6.77 (s, 1H), 3.13 (m, 1H) and 3.01 (m, 1H), 2.57 (m, 1H) and 2.47 (m, overlapping, 1H), 2.07 (br, 4H), 1.45 (br, 4H), 1.41 (s, 9H) Minor component. δ 8.25 (2H, dd, $J_1 = 8.1$ Hz, $J_2 = 5.5$ Hz), 7.72 (s, 1H), 7.45-7.20 (m, overlapping, 7H), 6.71 (s, 1H), 3.51 (m, 2H), 2.71 (m, 2H), 2.46 (br, 4H), 1.65 (br, 4H), 1.39 (s, 9H). ¹³C NMR (DEPTq 125.8 MHz, DMSO-*d*₆, T=296K) also showed two rotamers in about a 1:3 ratio: Major component δ 168.4 (s), 163.7 (d, $J_{CF} = 249$ Hz), 161.8 (s), 153.8 (s), 148.3 (s), 141.1 (s), 136.5 (s), 132.9 (s), 129.5 (dd, $J_{CF} = 8$ Hz), 128.4* (d), 127.9* (d), 127.6* (d), 127.2 (d), 115.9 (dd, $J_{CF} = 22$ Hz), 102.3 (d), 93.1 (d), 53.6 (t) or 53.4 (t), 53.3 (t), 47.2 (t), 46.2 (t), 32.7 (s), 30.1 (q), 22.9 (t); Minor component δ 168.1 (s), 163.6 (d, $J_{CF} = 249$ Hz), 161.3 (s), 153.7 (s), 148.5 (s), 141.0 (s), 135.9 (s), 133.0 (s), 129.5 (dd, $J_{CF} = 8$ Hz), 128.4* (d), 127.9* (d), 127.6* (d), 127.1 (d), 115.8 (dd, $J_{CF} = 22$ Hz), 102.2 (d), 93.0 (d), 53.6 (t) or 53.7 (t), 52.4 (t), 52.1 (t), 42.8 (t), 32.6 (s), 30.1 (q), 23.2 (t) [asterisks denote unassigned signals].

[0034] The N-benzyl-2-(pyrrolidin-1-yl)ethan-1-amine used in the synthesis above was obtained by reductive amination of benzaldehyde: In a 500 mL round bottom flask equipped with a reflux condenser and a CaCl₂ drying tube 1-(2-aminoethylpyrrolidine (5.02g, 44 mmol) and benzaldehyde (4.7 mL, 46 mmol, 1.05 equiv.) were dissolved in ethanol (100 mL). The reaction mixture was stirred and refluxed for two hours and then allowed to stand at RT overnight. The resulting solution was diluted with methanol (50 mL), cooled down to about 10 °C and sodium borohydride (1.75 g, 46 mmol, 1.05 equiv.) was added in small portions with stirring, while the flask was kept in an ice-water cooling bath. (Intense foaming!) The reaction mixture was allowed to warm up to RT and stirred further for an hour. The mixture was evaporated in a rotary evaporator to almost dryness. The residue was diluted with water (200 mL), acidified by addition of 10% aq. HCl solution and washed with ether (3x30 mL). The solution was neutralized by addition of 10% aq. NaOH solution (pH 7-8) and washed with chloroform (3x30 mL). The aqueous phase was basified by addition of 20% aq. NaOH solution to pH 12-14 and it was extracted with dichloromethane (3x50 mL). The combined organic phases were washed with brine, dried over MgSO₄, and evaporated. Yield: 7.4 g (82%) of N-benzyl-2-(pyrrolidin-1-yl)ethan-1-amine as light yellow liquid. ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 7.30 (m, 4H), 7.21 (m, 1H), 3.69 (s, 2H), 2.57 (t, 2H), 2.48 (q, 2H), 2.38 (m, 4H), 2.2-1.8 (br, 1H), 1.64 (m, 4H)

[0035] The following compounds may be synthesized by processes analogous to those described above. All except compound 10 were purchased from commercial sources.

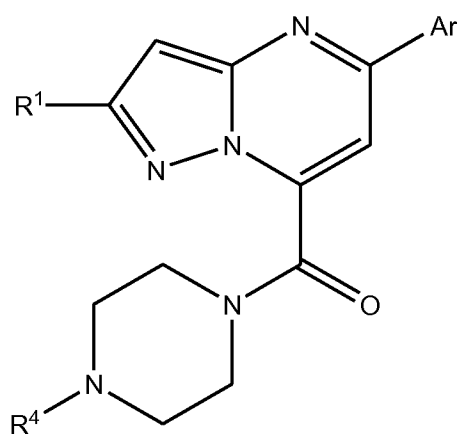
Table 1



Example #	Ar	R ¹	R ²	R ³	R ⁴
1	2-fluorophenyl	t-Bu	benzyl	methyl	methyl
2	2-chlorophenyl	t-Bu	benzyl	methyl	methyl
3	phenyl	t-Bu	benzyl	methyl	methyl
4	4-fluorophenyl	t-Bu	benzyl	methyl	methyl
30	4-fluorophenyl	4-methylphenyl	benzyl	methyl	methyl
31	4-fluorophenyl	2-methylphenyl	benzyl	methyl	methyl

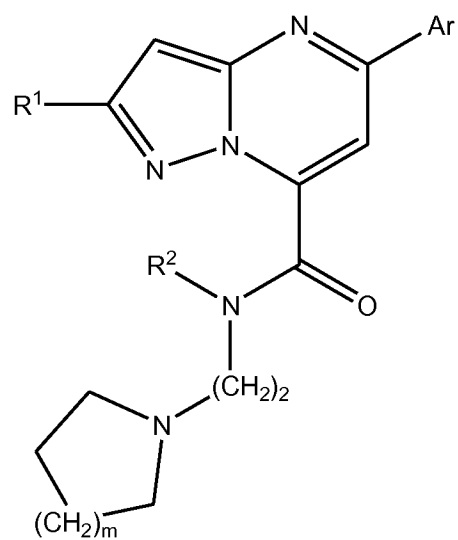
[0036]

Table 2



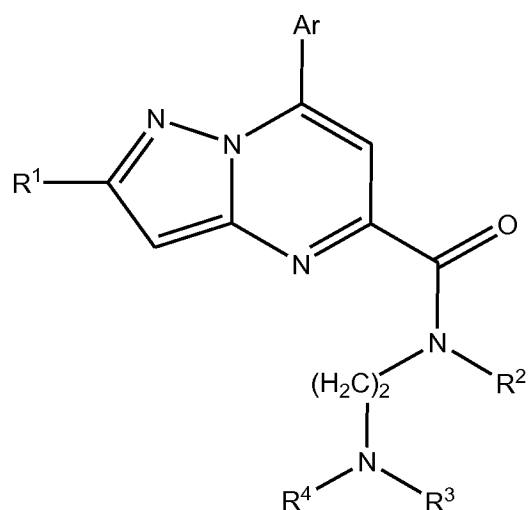
Example #	Ar	R ¹	R ⁴
6	4-fluorophenyl	phenyl	benzyl
7	2-fluorophenyl	t-butyl	benzyl
8	4-fluorophenyl	t-butyl	acetyl
9	phenyl	t-butyl	acetyl

Table 3



Example #	Ar	R ¹	R ²	m
10	4-fluorophenyl	t-butyl	benzyl	1

Table 4

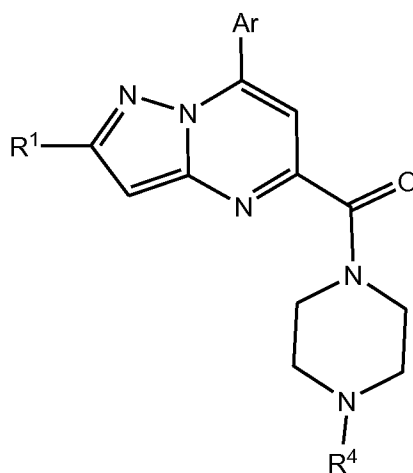


Example #	Ar	R ¹	R ²	R3	R4
11	4-fluorophenyl	t-butyl	benzyl	methyl	methyl
12	2-fluorophenyl	phenyl	benzyl	methyl	methyl
32	4-fluorophenyl	cyclopropyl	benzyl	methyl	methyl
33	4-fluorophenyl	phenyl	benzyl	methyl	methyl
34	4-methoxyphenyl	t-butyl	benzyl	methyl	methyl
35	4-cyanophenyl	t-butyl	benzyl	methyl	methyl
36	4-fluorophenyl	4-methylphenyl	benzyl	methyl	methyl

37	phenyl	t-butyl	benzyl	methyl	methyl
39	4-fluorophenyl	oxan-4-yl	benzyl	methyl	methyl

[0037]

Table 5

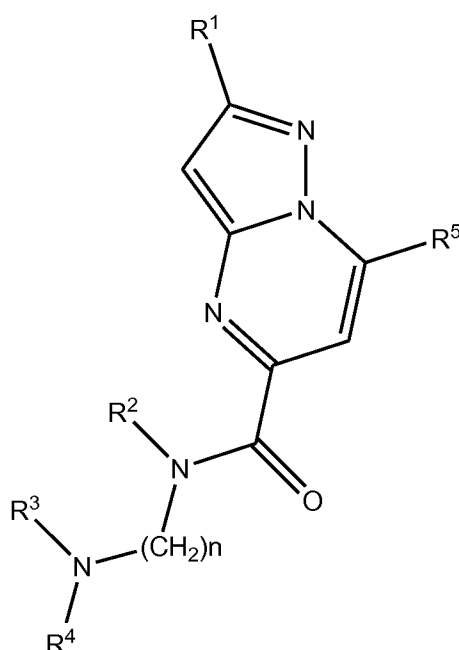


Example #	Ar	R ¹	R ⁴
13	3- methoxyphenyl	phenyl	benzyl
14	2- methoxyphenyl	t-butyl	2-ethoxyphenyl
15	3- methoxyphenyl	t-butyl	2-methoxyphenyl
16	2- fluorophenyl	t-butyl	2-ethoxyphenyl
17	3- methoxyphenyl	t-butyl	2-ethoxyphenyl
18	2- fluorophenyl	t-butyl	2-methoxyphenyl
19	2- methoxyphenyl	t-butyl	4-methoxyphenyl
20	3- methoxyphenyl	t-butyl	benzyl
21	2-fluorophenyl	phenyl	benzyl
22	3- fluorophenyl	t-butyl	2-ethoxyphenyl
23	2-fluorophenyl	t-butyl	3,4-methylenedioxybenzyl
24	2- methoxyphenyl	t-butyl	3,4-methylenedioxybenzyl
25	2- methoxyphenyl	phenyl	2-ethoxyphenyl

Example #	Ar	R ¹	R ⁴
26	2-methoxyphenyl	phenyl	benzyl
27	3- methoxyphenyl	t-butyl	3,4-methylenedioxybenzyl

[0038]

Table 6



Example #	R ⁵	R ¹	R ²	R ³	R ⁴
38	t-butyl	t-butyl	benzyl	methyl	methyl

[0039] Compounds of the genera I and II were screened using fluorescence polarization (FP) and AlphaLISA assays in a complementary fashion to cross check the activity of the compounds. Primarily, compounds were screened using FP, which measured the changes in the anisotropy induced by binding of a 5-carboxy-tetramethylrhodamine (TAMRA)-labeled A β peptide to fibrinogen. Then, hits from FP were screened using AlphaLISA to independently confirm the activity of the inhibitors identified in the FP assay. The compounds were screened using AlphaLISA at a concentration of 20 μ M for Examples 1-29 and at a concentration of 25 μ M for Examples 30-39. The combined experiments showed that the compounds disclosed herein are inhibitors of the A β -fibrinogen interaction. Table 7 presents the results of testing of the compounds of the formulae I and II in the AlphaLISA

screen:

Table 7

Example #	% inhibition
1	51
2	44
3	54
4	48
6	40
7	50
8	36
9	33
10	67
11	72
12	29
13	0
14	31
15	30
16	35
17	40
18	36
19	27
20	13
21	24
22	30
23	37
24	N/A
25	15
26	35
27	26
30	0
31	66
32	15

33	67
34	61
35	49
36	74
37	69
38	72
39	2

[0040] Since the interaction between A β 42 and fibrinogen induces a structurally abnormal fibrin clot and delays fibrin clot degradation during fibrinolysis, it is useful to determine whether a test compound restores A β -induced delayed fibrinolysis. When fibrinogen associates into a fibrin meshwork after cleavage by thrombin, the fine structure of this fibrin clot scatters light and the solution increases in turbidity. Thus, the kinetics of turbidity can be used as a read-out to analyze fibrin network formation and degradation. Compounds were therefore tested to see whether they restored A β -induced altered thrombosis and fibrinolysis *in vitro*. Test compound (20 μ M) or vehicle (0.4% DMSO) was incubated for 10 min with purified human fibrinogen and plasminogen in the presence or absence of A β 42. Fibrin clot formation and degradation were analyzed by measuring turbidity immediately after adding thrombin and tissue plasminogen activator (tPA) to the mixture. In the presence of A β 42, the maximum turbidity of the fibrin clot was decreased, since A β altered fibrin clot structure, and the dissolution of the fibrin clot was delayed. An example of a compound of genus I was tested. The compound of example 11 restored the A β -induced decrease in turbidity during fibrin clot formation and significantly reduced the delay in fibrin degradation in the presence of A β . Moreover, the compound of example 11 did not have any effect on fibrin clot formation and degradation in the absence of A β . This result confirms that a compound of the invention effectively restores A β -induced altered fibrin clot structure and delayed degradation without affecting normal clot formation and fibrinolysis.

[0041] To elucidate how compounds of the invention inhibit the A β -fibrinogen interaction, surface plasmon resonance (SPR) was used to analyze the binding characteristics of example 11. Hexafluoroisopropanol-treated monomerized A β 42 was immobilized to the sensor chip surface, and example 11 was injected for 2 min at 30 μ L/min. Sulindac sulfide was used as positive control and sulindac as negative control. A structural analog of example 11 that falls outside formula I and II, and that did not inhibit the A β -fibrinogen

interaction in AlphaLISA assay, was used as a negative control in these experiments. While the analog weakly binds to A β 42, example 11 showed a strong binding response to A β 42. Since it is known that sulindac sulfide binds A β , we tested whether sulindac sulfide could inhibit the A β -fibrinogen interaction by AlphaLISA and found that it had no effect. These results suggest that example 11 inhibits the A β -fibrinogen interaction through A β binding, but A β binding itself is not enough to inhibit the interaction between A β and fibrinogen. While not wishing to be held to a particular theory, it may be that in order to effectively inhibit the A β -fibrinogen interaction, a compound optimally possesses both an A β -specific binding moiety and a moiety responsible for inhibiting fibrinogen's binding to A β .

[0042] To assess whether compounds of the invention could restore A β -induced altered thrombosis and fibrinolysis *in vivo*, these parameters were examined in a transgenic mouse model of Alzheimer's disease. Cerebral blood flow and thrombosis were analyzed by a FeCl₃-induced thrombosis model combined with intravital microscopy. Example 11 or vehicle (35 mg/kg dose, every other day) were administered to 4-month-old Tg6799 mice and wild-type littermates (WT) for four months (analyzed at 8 months-of-age). Brains of 7-month-old Tg6799 or WT mice were exposed by craniotomy, and blood flow was observed using injected fluorescence-conjugated dextran. Three concentrations of FeCl₃ (5%, 10%, and 15%) were incrementally administered to the brain surface to induce thrombosis. Clot formation was revealed by the appearance of an enlarging shadow superimposed on normal blood flow. The length of all visible vessels whose diameters were larger than 20 μ m was measured before FeCl₃ treatment, and the length of occluded vessels was measured 5 min after the addition of each concentration of FeCl₃ for both Tg6799 and WT. There was no significant difference in the percentage of occluded vessels before FeCl₃ treatment or after 5% and 10% FeCl₃ treatment among groups. However, there was a significant difference between the percentage of occluded vessels after 15% FeCl₃ treatment in vehicle-treated WT and Tg6799 mice. Approximately half (52.7% $\pm$ 12.1) of the vessels were occluded in vehicle-treated WT mice, but 95.6% $\pm$ 3.5 of vessels were occluded in vehicle-treated Tg6799 mice. Example 11 treatment significantly lowered the vessel occlusion in Tg6799 mice to 60.7% $\pm$ 8.7, but did not change vessel occlusion in WT mice (54.2% $\pm$ 11.8). These results indicate that compounds such as example 11 can restore altered thrombosis and fibrinolysis in AD mice without affecting normal thrombosis and fibrinolysis in WT littermates.

[0043] Cerebral amyloid angiopathy has been implicated in vascular degeneration of Alzheimer's disease. Previous studies had shown that the A β -fibrinogen interaction increases A β fibrillization. Therefore, we investigated whether treatment of Tg6799 mice with compounds of the invention for four months could decrease A β deposition in blood vessels. A β deposits were stained using Congo red, and blood vessels were labeled using laminin. We quantified cerebral amyloid angiopathic area in the cortex by measuring Congo red deposits inside blood vessels, and A β plaque deposition was quantified by measuring Congo red outside blood vessels. The cerebral amyloid angiopathic area of Tg6799 mice treated with example 11 ($0.025\% \pm 0.006$, cortex) was significantly decreased from that of vehicle-treated Tg6799 mice ($0.046\% \pm 0.004$, cortex). However, there was no significant difference in A β plaque area in the cortex between vehicle-treated Tg6799 mice and mice treated with example 11. This result indicates that inhibition of the A β -fibrinogen interaction by a compound of the invention reduced cerebral amyloid angiopathy deposits in blood vessels of AD mice.

[0044] To determine whether long-term treatment could have behavioral effects on AD mice, seven-month-old Tg6799 mice were treated for three months with test compound in a protocol to assess contextual fear conditioning. Treatment with example 11 had no effect on baseline freezing behavior in WT and Tg6799 mice. Looking at contextual memory of Tg6799 and WT mice 24 hours after training, it is seen that vehicle-treated Tg6799 mice show a severe memory deficit compared to vehicle-treated WT mice. Tg6799 mice treated with compound 11 exhibited significantly improved memory compared to their vehicle-treated AD counterparts, while long-term treatment with example 11 in WT mice did not affect basal freezing behavior or contextual memory.

[0045] Another method of cognitive assessment is the Barnes maze, a behavioral test that assesses spatial learning and memory in rodents. Vehicle-treated Tg6799 mice spent a significantly longer time to find the target hole compared to vehicle-treated WT and Tg6799 mice treated with example 11. During the memory retention test in the probe trials, vehicle-treated Tg6799 mice also had significantly longer latency to reach the closed target hole and significantly fewer visits to the target hole compared to vehicle-treated WT and Tg6799 mice treated with compound 11. These results suggest that vehicle-treated Tg6799 mice have impaired spatial learning and memory, and that a compound of the invention can restore the cognitive impairment of Tg6799 mice.

[0046] When one measures total distance traveled during probe trials, Tg6799 mice moved significantly less than WT mice. However, there was no significant difference in distance traveled between untreated Tg6799 mice and mice treated with the compound of Example 11. This result suggests that the better performance of Example 11-treated Tg6799 mice compared to untreated Tg6799 mice is likely due to memory improvement and not effects on locomotion.

[0047] To further address any possible issue of hypoactivity in the Tg6799 mice and to test whether treatment had a similar effect on a different strain of AD transgenic mice, example 11 was administered to 4-month-old TgCRND8 mice for three months (analyzed at 7 months-of-age). During training, treatment with test compound did not lead to improvement in spatial learning in TgCRND8 mice. However, this treatment significantly reduced the latency to reach the target hole during the probe trial compared to vehicle-treated TgCRND8 mice. Furthermore, the number of visits to the target hole during the probe trial was significantly higher in treated TgCRND8 mice compared to control (vehicle-treated) TgCRND8 mice. In addition, vehicle-treated TgCRND8 mice showed similar locomotor activity during probe trials, indicating that the impaired performance of vehicle-treated TgCRND8 mice in Barnes maze test is more likely due to deficits in spatial memory. These results suggest that treatment with a compound of the invention substantially improved the deficits in spatial memory of TgCRND8 mice.

[0048] Materials and Methods

[0049] Tg6799 mice (Jackson Laboratory) are double transgenic mice for APP/Presenilin 1 that co-express five early onset familial AD mutations. TgCRND8 mice (provided by A. Chishti and D. Westaway, University of Toronto, Canada) have three APP mutations (K670N, M671L, and V717F) driven by the human prion protein promoter. Test compound was prepared in 2.5% EtOH, 4.5% Cremophor RH40 (Sigma-Aldrich), and 14% D5W (5% dextrose in water) in saline. A 35 mg/kg dose of test compound or vehicle was administered to Tg6799 mice and a 25 mg/kg dose or vehicle to TgCRND8 mice subcutaneously every other day for three months. Non-transgenic (wild-type; WT) littermates were used in all experiments. The assigned genotype of all the mice used in the experiments was double-checked by taking tail tissue the day of sacrifice. Only male mice were used in experiments, and all animals were maintained in The Rockefeller University's Comparative Biosciences Center and treated in accordance with IACUC-approved protocols.

[0050] Compounds were commercially available and were purchased from several vendors. The primary assay utilized fluorescence polarization (FP) to measure the changes in the anisotropy induced by binding of 5-carboxytetramethylrhodamine (TAMRA)-labeled A β 42 (Anaspec) to fibrinogen. TAMRA-A β 42 (2 nM) was mixed with 300 nM fibrinogen (Calbiochem) and 20 μ M compounds (dissolved in 1% DMSO (final)) in 50 mM PBS, pH 7.4, 0.001% Tween 20, and 0.001% BSA as 50 μ L final volume in black 384-well plates (Greiner) at room temperature. After binding reached equilibrium, polarization measurements were recorded with a Perkin-Elmer EnVision plate reader with excitation at 490 nm and emission at 535 nm. The FP response was monitored and plotted as milli-Polarization (mP) units.

[0051] Compounds that showed over 75% inhibition of the A β -fibrinogen interaction in the FP assay were screened by AlphaLISA as a secondary assay. Compounds (12.5 μ M) were plated in white 384-well plates (Greiner) and were incubated with 10 nM biotinylated A β 42 (Anaspec) and 1 nM fibrinogen for 30 min at RT in final volume of 10 μ L assay buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% Tween-20, 0.1% BSA). The mixture was incubated with anti-fibrinogen antibody (Dako), 20 μ g/mL streptavidin-conjugated donor, and protein A-conjugated acceptor beads (Perkin-Elmer) for 90 min at RT. Samples were read by a PerkinElmer EnVision plate reader.

[0052] The AlphaScreen TruHits kit (PerkinElmer) was used to detect those compounds that react with singlet oxygen and thus unspecifically quench the assay. The AlphaScreen TruHits kit also allows for the identification of color quenchers, light scatterers (insoluble compounds), and biotin mimetics interfering with the AlphaLISA signal. If inhibition by quenching was more than 30% at 10 μ M compound, the compound was considered inactive for the purpose of the invention. Compounds were then tested in a dose-response experiment at various concentrations (0.01 - 20 μ M) using FP and AlphaLISA. The data were fitted to sigmoidal dose-response equation ($Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{(\log \text{IC}_{50} - X) \cdot \text{Hill coefficient}})$) using GraphPad Prism 4 to calculate half-maximal inhibition (IC_{50}) of each compound. Compounds with $\text{IC}_{50} < 50$ μ M in both FP and AlphaLISA were retested in dose-response experiments using both assays.

[0053] To test whether compounds have an effect on fibrin clot formation and lysis, 20 μ M of test compound (dissolved in 0.4% DMSO (final)) or DMSO control was incubated with fibrinogen (1.5 μ M) in the presence or absence of A β 42 (3 μ M) for 10 min and then

mixed with plasminogen (0.25 μ M) in 20 mM HEPES buffer (pH 7.4) with 137 mM NaCl. Fibrin clot formation and degradation was analyzed measuring turbidity right after adding thrombin (0.5 U/mL), tPA (0.15 nM), and CaCl_2 (5 mM) in a final volume of 150 μ l. Assays were performed at RT in High Binding 96-well plates (Fisher Scientific) in triplicate and were measured at 450 nm using a Molecular Devices Spectramax Plus384 reader.

[0054] Surface Plasmon Resonance (SPR) experiments were performed to test whether compounds bind to A β 42 as described by Richter *et al.* [*Proc Natl Acad Sci U S A* **107**, 14597 (2010)]. Biacore 3000 instrument and CM5 sensor chips (GE Healthcare) were used for this assay. Hexafluoroisopropanol-treated monomerized A β 42 was immobilized to the sensor chip surface by amine coupling. Compounds were diluted to 40 μ M from DMSO stock solutions in PBS as running buffer (final 2% DMSO) and injected for 2 min at a flow rate of 30 μ L/min using the KINJECT command. After the dissociation phase the chip was rinsed with 20 mM HCl. Corresponding DMSO dilutions were used as a buffer blank, and a solvent correction assay was performed to correct the difference of DMSO response between empty reference surface and protein-immobilized surface. Sulindac sulfide and sulindac were used as positive control and negative controls, respectively.

[0055] In vivo toxicity study - Maximum tolerated dose studies were carried out to determine toxicity and to identify the optimal dose for *in vivo* assays. Single injection toxicity was performed at Absorption Systems LP (Exton, PA), and four different doses (200, 100, 50, and 20 mg/kg mouse) of test compound, along with saline and vehicle, were injected into male and female CD-1 mice intravenously. Mortality and overt clinical signs of toxicity were monitored for two days. All animals dosed with 200 mg/kg were found dead after single intravenous injection of the compound of example 11, and no clinical signs of toxicity were observed after single dose of 20, 50, or 100 mg/kg for two days after injection. Therefore, the maximum tolerated dose of example 11 after single intravenous dose in mice was established as 100 mg/kg.

[0056] Since Alzheimer's disease treatment would be long-term and toxicity of long-term treatment can be different from toxicity after a single injection, Tg6799 mice and WT littermates were treated with two doses (100 mg/kg and 50 mg/kg) of example 11 every other day for 3 months, and overt clinical signs of toxicity were monitored. After three months, mice were sent to the Laboratory of Comparative Pathology at Memorial Sloan-Kettering Cancer Center for complete necropsy and hematology reports to determine the effects after

long-term treatment. It was found that 100 mg/kg for long-term treatment was toxic to the AD mice, but 50 mg/kg showed no clinical signs of toxicity except local chronic inflammation at the injection site. To address the issue of local inflammation, doses were adjusted to 35 mg/kg for Tg6799 or 25mg/kg for TgCRND8 over 3 months.

[0057] To observe blood circulation and to induce thrombosis, a cranial window was prepared as described by Cortes-Canteli *et al.* [*Neuron* **66**, 695 (2010)]. Briefly, a cranial window was prepared over the parietal cortex of 7-month-old Tg6799 mice and WT littermates which were treated with test compound or vehicle for three months (n=5 per group). Mice were anesthetized by IP injection of 500 mg/kg tribromethanol and 0.04 mg/kg atropine and placed in a custom built restraint system. A 2.5-mm circular craniotomy was prepared using 5-10 circular brush strokes with a fine dental drill bit, and a 4 mm plastic ring surrounding the window was attached with dental acrylic and cyanoacrylate adhesive. Sterile saline was applied periodically to protect the brain surface and prevent drying. For imaging of blood flow, 100 μ l of 5 mg/ml 2 MDa FITC-conjugated dextran (Sigma) dissolved in PBS was administered by retro-orbital injection. During the entire imaging session, the body temperature of mice was kept at 37.5°C using a TC-1000 Mouse complete temperature control system (CWE Inc). Increasing concentrations of FeCl₃ (5%, 10%, and 15%) were added directly to the brain surface with an interval of 5 min, and thrombosis was recorded using real-time video acquisition with an upright Zeiss Axiovert 200 epi-fluorescence microscope (Metavue software). The total length of vessels with a diameter greater than 20 μ m before FeCl₃ treatment and the total length of occluded vessels at 5 min after each FeCl₃ treatment (5%, 10%, and 15%) were measured. All analysis was performed using NIH ImageJ software with the analyzer blinded to genotype and treatment of mice.

[0058] Immunohistochemistry - Mice were saline/heparin-perfused, and 20 mm coronal brain cryostat sections were fixed with 4% paraformaldehyde. Brain sections were incubated with Rabbit anti-Laminin antibody (Sigma) for overnight and then stained for 30 min with 0.2% Congo Red (Sigma) in 70% isopropanol. Images of all the areas with CAA and A β plaque were acquired and thresholded using Image J. The total area of CAA and A β plaques was analyzed as percentage of total cortex area with the analyzer blinded to treatment of mice. The average of 7–10 different sections from each mouse was determined (n=5 mice per group).

[0059] Behavioral Analysis - All behavioral experiments were performed and analyzed

with a researcher blinded to genotype and treatment. Forty mg/kg of test compound or vehicle was administered to 4-month-old Tg6799 mice and WT littermates and 25 mg/kg or vehicle to 4-month-old TgCRND8 mice and WT littermates subcutaneously every other day for three months (analyzed at 7 months-of-age). Mice were handled and allowed to acclimate to the testing room for 10 min per day for at least 5 days.

[0060] Contextual Fear Conditioning - During training, Tg6799 mice and WT littermates (n=8-10 per group) were allowed to explore the training chamber (Med Associates, Inc.) for the first 2 min and then received three mild footshocks (2 sec, 0.7 mA) spaced 1 min apart. Mice were removed from the training chamber 30 sec after the last foot shock. Contextual learning was assessed 24 h after training by re-exposing mice to the same training chamber for 3 min. Mouse behavior during training and testing was recorded, and freezing behavior was measured by observing mice every 5 sec.

[0061] Barnes maze - The Barnes maze apparatus (TAP Plastics) consisted of a white circular platform (92 cm in diameter) with 20 equally spaced holes (5 cm in diameter; 7.5 cm between holes). Among these holes, one hole (target hole) was connected to a hidden black escape chamber. Bright lights (600 lx) were used to motivate the mice to find the target hole and enter into the escape chamber. Visual clues surrounded the maze. To remove any lingering scent on the maze from the previous animal, the platform and escape box were cleaned using 50% ethanol between mice. The entire experiment was recorded and analyzed using the Ethovision video tracking system (Noldus). For Tg6799 mice training consisted of two training trials per day over a period of 7 days (n=11-13 per group). During each trial, mice were placed in the center of the maze in a black starting box for 30 s. After 30 s, the box was removed, and mice were allowed to freely explore and find the target hole within 2 min. Latency to poke the target hole was recorded. If mice did not enter into the escape chamber within 2 min, they were gently guided into the escape chamber and placed in the chamber for 30 sec. To assess memory retention, a probe trial was conducted 24 h and 3 days after the last training. The target hole was closed like the other 19 holes, and the escape chamber was removed. Holes were kept in the same position as during the training. Mice were placed in the center of the maze in a black starting box for 30 s. After 30 s, the box was removed, and mice were allowed to freely explore for 90 s. The number of visits into each hole and the latency to reach the target hole were recorded. For analysis, scores of each mouse from both probe trials were combined and averaged. For TgCRND8 mice training

consisted of a trial per day over a period of 12 days (n=7-11 per group). During training, mice were placed in the center of the maze in a black starting box for 30 s. After 30 s, the box was removed, and mice were allowed to freely explore and find the target hole for 5 min. To assess memory retention, probe trials were conducted 24 h and 6 days after the last training. Mice were allowed to freely explore for 2 min during probe trials. The number of visits into each hole and the latency to reach the target hole were recorded. For analysis, scores of each mouse from both probe trials were combined and averaged.

[0062] All numerical values were calculated with a mean $\pm$ SEM. Statistical significance of most experiments was determined using two-tailed t-test analysis comparing control and experimental groups. The AlphaScreen TruHits assay was analyzed using one-way ANOVA and Bonferroni *post hoc* test. Comparison of training curves from the Barnes maze were analyzed using two-way ANOVA and Bonferroni *post hoc* test with repeated measure.

[0063] While it may be possible for the compounds described herein to be administered to patients as the raw chemical, it is usually desirable to present them as a pharmaceutical composition. According to a further aspect, the present invention provides a pharmaceutical composition comprising a compound together with one or more pharmaceutical carriers and optionally one or more other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[0064] The formulations for administration to patients include those suitable for oral, parenteral (including subcutaneous, intradermal, intramuscular, intravenous and intraarticular), rectal and topical (including dermal, buccal, sublingual and intraocular) administration. In most cases, parenteral administration will be preferred. The most suitable route may depend upon the condition and disorder of the recipient. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.

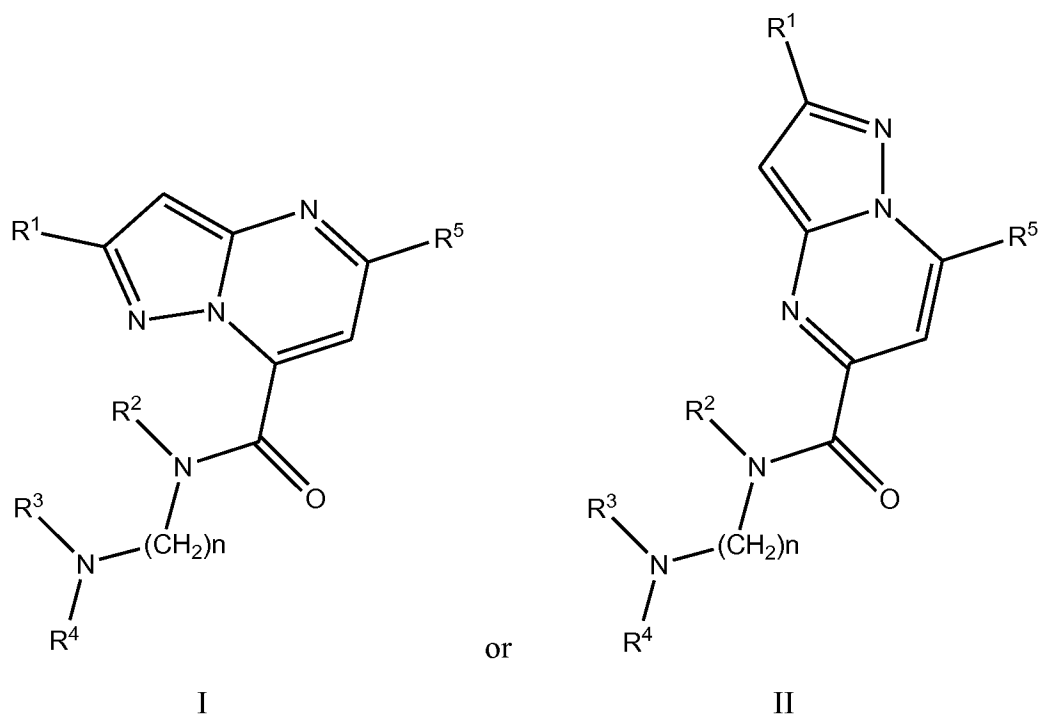
[0065] Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion

or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste. A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide sustained, delayed or controlled release of the active ingredient therein.

[0066] Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient. Formulations for parenteral administration also include aqueous and non-aqueous sterile suspensions, which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of a sterile liquid carrier, for example saline, phosphate-buffered saline (PBS) or the like, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

CLAIMS

1. A method of inhibiting the interaction between fibrinogen and amyloid- β , comprising bringing amyloid- β into contact with a compound of formula I or II:



wherein

R⁵ is (C₄-C₁₀) hydrocarbyl, aryl, or heteroaryl, said hydrocarbyl, aryl, or heteroaryl optionally substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆) alkylamino, and di(C₁-C₆)alkylamino;

R¹ is (C₃-C₁₀) heterocyclcyl, (C₃-C₁₀) hydrocarbyl or (C₃-C₁₀) hydrocarbyl substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆) alkylamino, and di(C₁-C₆)alkylamino;

R² is (C₁-C₁₀) hydrocarbyl or (C₁-C₁₀) hydrocarbyl substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆)alkylamino, and di(C₁-C₆)alkylamino, wherein a benzene ring, if present, is not directly attached to the amide nitrogen and wherein halogen, if present, is only attached to a benzene ring;

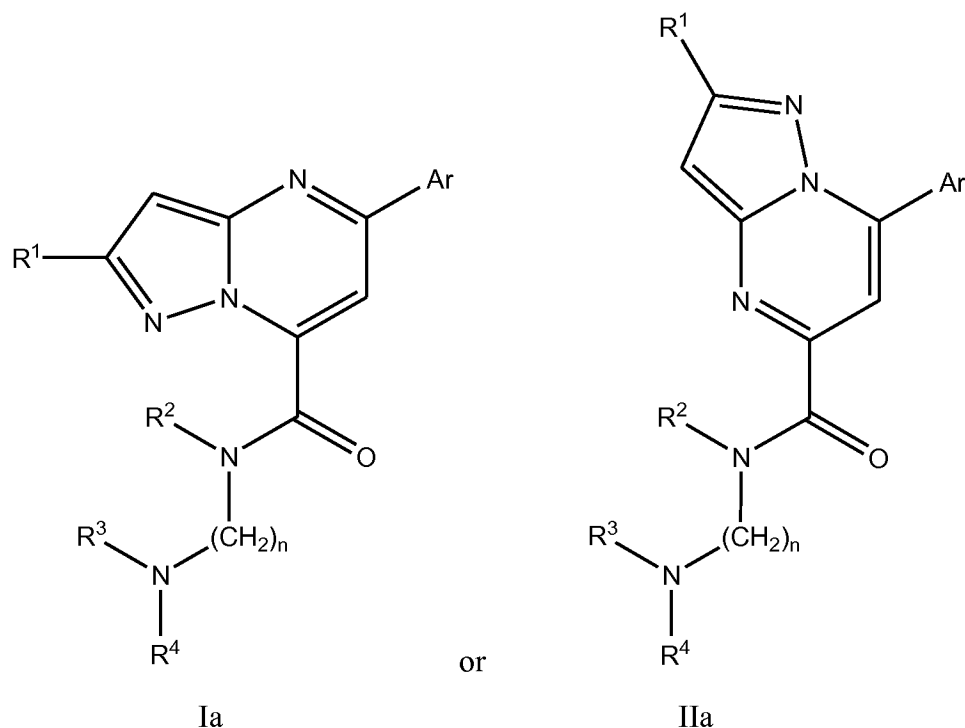
R³ is (C₁-C₁₀) hydrocarbyl;

R⁴ is chosen from (C₁-C₈) acyl, (C₁-C₈) hydrocarbonyl, and (C₁-C₈) hydrocarbonyl substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆) alkylamino, and di(C₁-C₆)alkylamino, or,

taken together, any two of R² and R³ or R³ and R⁴, together with the nitrogens to which they are attached, may form a 4-7-membered, saturated nitrogen heterocycle;

with the proviso that when R² and R³ form a 6-membered, saturated nitrogen heterocycle and R⁴ is chosen from (C₁-C₈) hydrocarbonyl, and substituted (C₁-C₈) hydrocarbonyl, then R⁴ must be benzyl, substituted benzyl or phenyl substituted with (C₁-C₆)alkoxy; and n is 2, 3 or 4.

2. A method according to claim 1, wherein R⁵ is aryl or heteroaryl, and the compounds are of formula Ia or IIa:



wherein

Ar is aryl or heteroaryl, said aryl or heteroaryl optionally substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆)alkylamino, and di(C₁-C₆)alkylamino;

R¹ is (C₃-C₁₀) heterocyclyl, (C₃-C₁₀) hydrocarbly or (C₃-C₁₀) hydrocarbly substituted with from one to three substituents chosen independently from the group

consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆) alkylamino, and di(C₁-C₆)alkylamino;

R² is (C₁-C₁₀) hydrocarbyl or (C₁-C₁₀) hydrocarbyl substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆) alkylamino, and di(C₁-C₆)alkylamino, wherein a benzene ring, if present, is not directly attached to the amide nitrogen and wherein halogen, if present, is only attached to a benzene ring;

R³ is (C₁-C₁₀) hydrocarbyl;

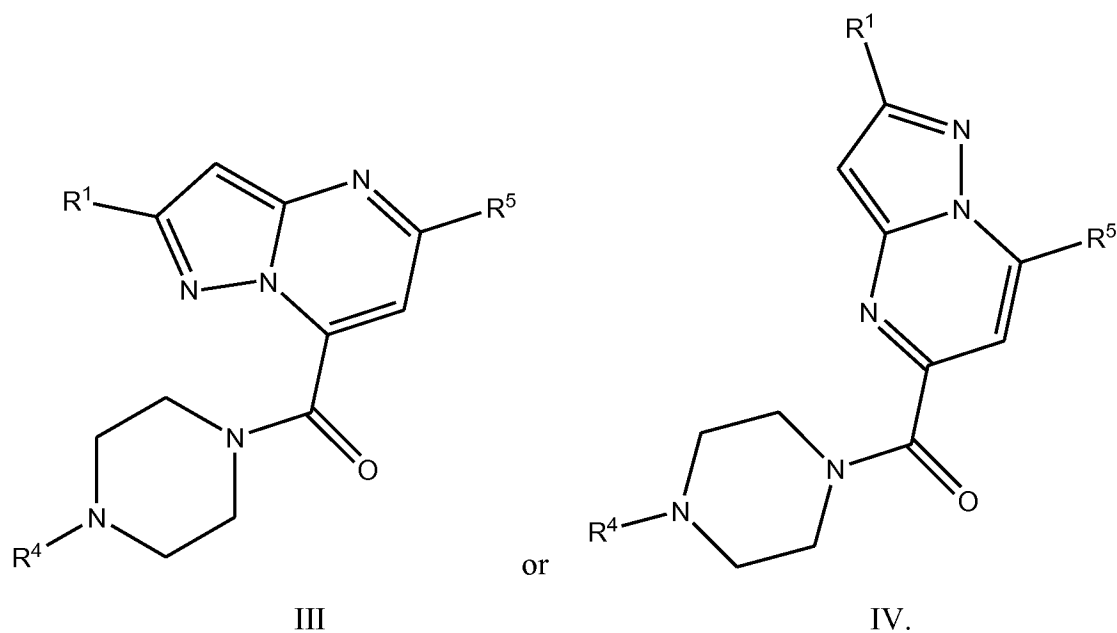
R⁴ is chosen from (C₁-C₈) acyl, (C₁-C₈) hydrocarbyl, and (C₁-C₈) hydrocarbyl substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆) alkylamino, and di(C₁-C₆)alkylamino, or,

taken together, any two of R² and R³ or R³ and R⁴, together with the nitrogens to which they are attached, may form a 4-7-membered, saturated nitrogen heterocycle; with the proviso that when R² and R³ form a 6-membered, saturated nitrogen heterocycle and R⁴ is chosen from (C₁-C₈) hydrocarbyl, and substituted (C₁-C₈) hydrocarbyl, then R⁴ must be benzyl, substituted benzyl or phenyl substituted with (C₁-C₆)alkoxy; and

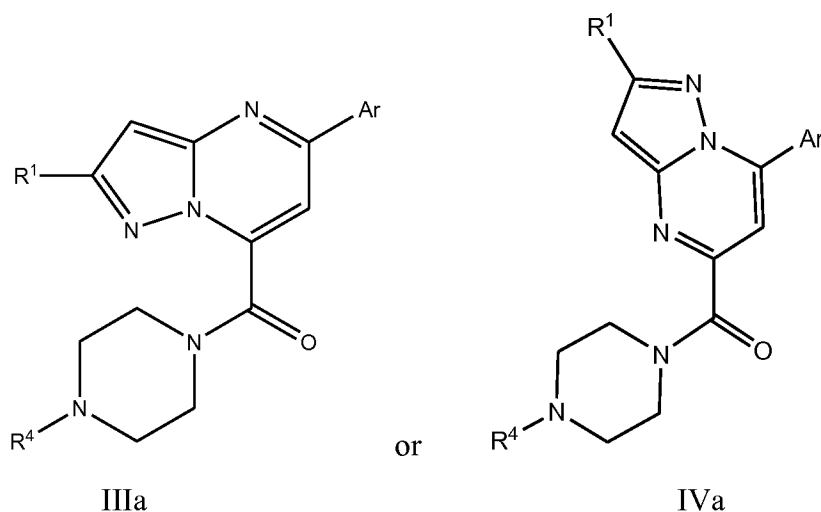
n is 2, 3 or 4.

3. A method according to claim 2 wherein Ar is optionally substituted phenyl.
4. A method according to claim 2 wherein Ar is phenyl or phenyl substituted with halogen or (C₁-C₆)alkoxy.
5. A method according to claim 1 wherein R⁵ is (C₄-C₁₀) hydrocarbyl.
6. A method according to claim 5 wherein R⁵ is t-butyl.
7. A method according to claim 2 wherein R¹ is (C₃-C₁₀) hydrocarbyl.
8. A method according to claim 2 wherein R¹ is chosen from (C₃-C₆) heterocyclyl, (C₃-C₁₀) alkyl, (C₃-C₁₀) cycloalkyl and optionally substituted phenyl.

9. A method according to claim 8 wherein R¹ is phenyl or t-butyl.
10. A method according to claim 8, wherein R¹ is (C₃-C₆) oxygen heterocyclyl.
11. A method according to claim 2 wherein R² is (C₁-C₁₀) hydrocarbyl.
12. A method according to claim 11 wherein R² is benzyl.
13. A method according to claim 2 wherein R² and R³ taken together form a 5 or 6-membered heterocycle.
14. A method according to claim 13 wherein R² and R³ taken together form a piperazine, and the compounds are of formula III or IV:



15. A method according to claim 14 wherein R⁵ is Ar and R² and R³ taken together form a piperazine, and the compounds are of formula IIIa or IVa:



16. A method according to claim 14 wherein R⁴ is chosen from (C₁-C₈) hydrocarbyl, (C₁-C₆) acyl and phenyl substituted with (C₁-C₆)alkoxy.

17. A method according to claim 2 wherein R^3 and R^4 are independently chosen from (C_1-C_8) hydrocarbyl.

18. A method according to claim 17 wherein R³ and R⁴ are independently chosen from (C₁-C₄)alkyl.

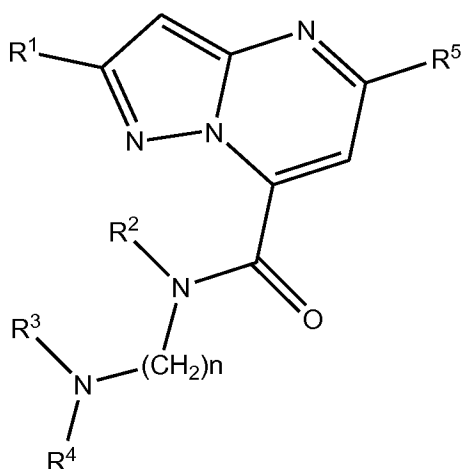
19. A method according to claim 2 wherein R³ and R⁴, together with the nitrogens to which they are attached, form a piperidine or pyrrolidine ring.

20. A method according to claim 4 wherein
R¹ is chosen from (C₃-C₁₀) alkyl, (C₃-C₁₀) cycloalkyl and optionally substituted
phenyl;

R² and R³ taken together form a piperazine and R⁴ is (C₁-C₈) hydrocarbonyl or phenyl substituted with (C₁-C₂)alkoxy; or

R² is benzyl and R³ and R⁴ are independently chosen from (C₁-C₄)alkyl.

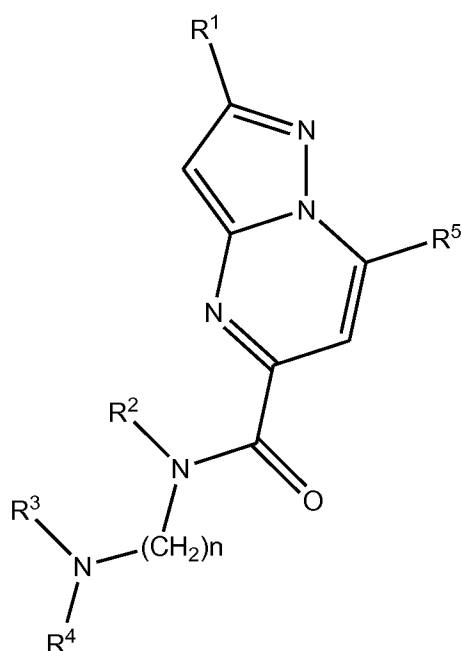
21. A method according to any of claims 1-20 wherein the compound has formula I:



I.

22. A method according to any of claims 1-20 wherein the compound has formula

II:



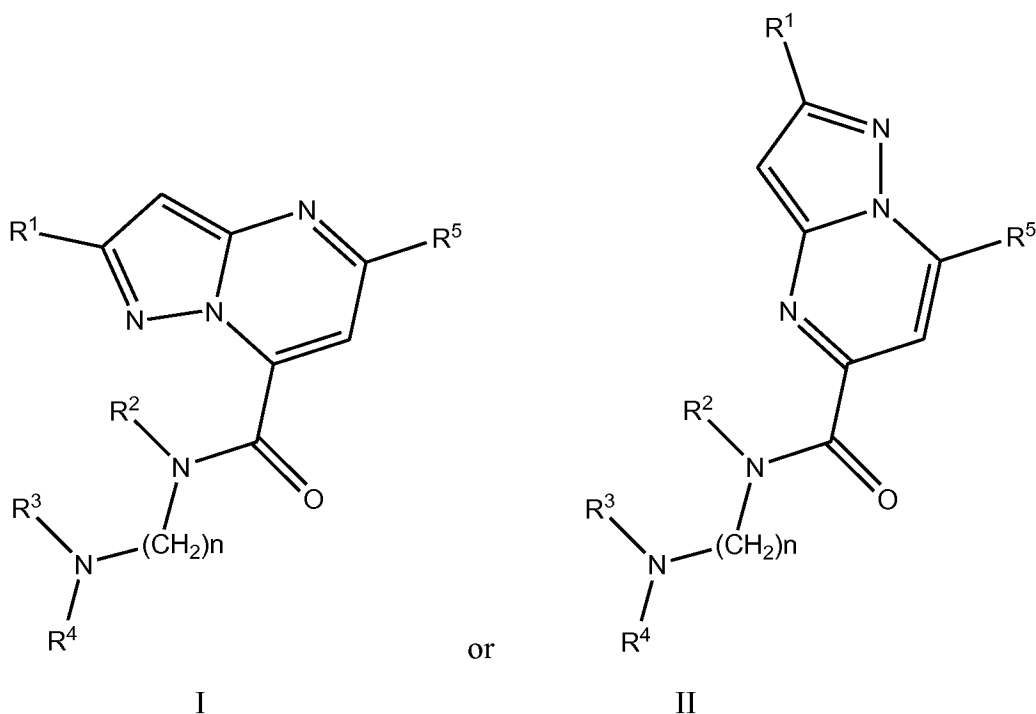
II.

23. A method for treating cognitive disorders comprising administering to a mammal a therapeutically effective amount of a compound of formula I or II, as defined in claim 1.

24. A method according to claim 23 wherein said cognitive disorder is memory impairment or Alzheimer's disease.

25. A method for reducing vascular amyloid deposits comprising administering to a mammal a therapeutically effective amount of a compound of formula I or II, as defined in claim 1.

26. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of formula I or II:



wherein

R⁵ is (C₄-C₁₀) hydrocarbyl, aryl or heteroaryl, said hydrocarbyl, aryl or heteroaryl optionally substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆) alkylamino, and di(C₁-C₆)alkylamino;

R¹ is (C₃-C₁₀) heterocyclyl, (C₃-C₁₀) hydrocarbyl or (C₃-C₁₀) hydrocarbyl substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆) alkylamino, and di(C₁-C₆)alkylamino;

R² is (C₁-C₁₀) hydrocarbonyl or (C₁-C₁₀) hydrocarbonyl substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆)alkylamino, and di(C₁-C₆)alkylamino, wherein a benzene ring, if present, is not directly attached to the amide nitrogen and wherein halogen, if present, is only attached to a benzene ring;

R^3 is (C₁-C₁₀) hydrocarbyl;

R^4 is chosen from (C₁-C₈) acyl, (C₁-C₈) hydrocarbyl, and (C₁-C₈) hydrocarbyl substituted with from one to three substituents chosen independently from the group consisting of halogen, acyl, hydroxy, (C₁-C₆)alkoxy, carboxy, (C₁-C₆)alkoxycarbonyl, cyano, acetoxy, nitro, amino, (C₁-C₆) alkylamino, and di(C₁-C₆)alkylamino, or,

taken together, any two of R^2 and R^3 or R^3 and R^4 , together with the nitrogens to which they are attached, may form a 4-7-membered, saturated nitrogen heterocycle; with the proviso that when R^2 and R^3 form a 6-membered, saturated nitrogen heterocycle and R^4 is chosen from (C₁-C₈) hydrocarbyl, and substituted (C₁-C₈) hydrocarbyl, then R^4 must be benzyl, substituted benzyl or phenyl substituted with (C₁-C₆)alkoxy;

and

n is 2, 3 or 4.

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