METHODS AND COMPOUNDS TO BE USED IN THE TREATMENT OF NEURODEGENERATIVE DISEASES

The present invention relates to a compound having the general formula. These compounds specifically target early toxic protein aggregations and have a high affinity to proteins which are known to be involved in protein aggregation in neurodegenerative disorder such as amyloid β. Furthermore the compounds are stable in plasma and solution and are able to easily cross the blood brain barrier at high AUC brain / blood ratios.
DESCRIPTION

METHODS AND COMPOUNDS TO BE USED IN THE TREATMENT OF NEURODEGENERATIVE DISEASES

BACKGROUND

1. Field of the Invention

[0001] The present invention relates to means and methods for treating, ameliorating and/or preventing diseases associated with abnormal accumulation of proteins.

2. Description of Related Art

[0002] Protein misfolding and aggregation into toxic oligomers has been linked with the neurodegenerative process in several neurological disorders such as Alzheimer's Disease (AD), Parkinson's Disease (PD), fronto-temporal dementia (FTD), amyotrophic lateral sclerosis (ALS) and Huntington's Disease (FID) among others. Alzheimer's disease is the seventh most prevalent cause of death in the US and the leading cause of dementia, affecting more than 26 million worldwide. Without an effective therapy it is estimated that the number of patients with AD will double by the year 2050.

[0003] Diverse lines of evidence support the notion that the progressive accumulation of amyloid-β (Aβ) protein is causally involved in the pathogenesis of AD. Aβ protein is a 38-42 amino acid (aa) transmembrane peptide derived from the cleavage of the amyloid precursor protein (APP). However, in addition to Aβ a number of other proteins might be accumulating in the brains of patients with AD, including Tau, TDP43 and a-synuclein. The cognitive impairment in patients with AD is closely associated with synaptic loss in the neocortex and limbic system and increasing levels of Aβ might be involved in the mechanisms underlying this synaptic loss. The mechanisms through which accumulation of Aβ and other APP metabolites might contribute to synaptic damage and neurodegeneration are currently under investigation. Many studies support the hypothesis that formation of small Aβ aggregates also known as oligomers might play a major role in the mechanisms of neurotoxicity in AD. Oligomers of Aβ peptides can organize into dimers, trimers, tetramers, pentamers and other higher order arrays that can form annular structures. The levels of such oligomers in the brain are good predictors of the indices of dementia and synaptic loss in
patients with AD. In addition to the oligomers, Aβ can complex to form higher molecular weight aggregates with a fibrillar organization that includes probably hundreds or thousands of Aβ peptides. Such fibrillar aggregates are the main component of the amyloid plaques, which are considered useful indicators for the neuropathological diagnosis of AD. However, while the smaller circular oligomers are considered the toxic array, the fibrillar aggregates in the plaques are not directly toxic and might even represent an endogenous mechanism to isolate oligomers.

[0004] Accumulation of toxic Aβ oligomers in the brains of patients with AD is the result of an imbalance between rate of synthesis, aggregation and clearance. Most therapies currently under development for AD are designed at reducing Aβ synthesis by blocking the beta or gamma secretases or at increasing Aβ clearance with antibodies or molecular chaperones. However development of these therapies has been delayed because of problems crossing the blood brain barrier (BBB) or due to toxic effects. The need for alternatives to these strategies has been underscored by disappointing Phase II results of gamma secretase inhibitors including Tarenflurbil (Green RC, et al. JAMA 302(2009): 2557-2564) and Eli Lilly's Semagacestat (LY450139) (Portelius E, et al. J Alziieimers Dis 21(2010): 1005-12).

[0005] The existing state of the art includes inhibitors of Aβ aggregates (fibrils, proto-fibrils and large oligomers) utilizing for this purpose thiofiavme T or equivalent assays to screen large libraries of existing molecules. A number of relatively specific and non-specific Aβ inhibitors have been identified (Broersen K, et al. Alzheimers Res Ther 2(2010): 12). Most of these molecules such as the Aβ "breakers", small peptides, and phenolic and flavonoids including curcumin, tend to prevent amyloid formation and fibrillation and display antioxidant properties. However, most of the compounds described have less specific modes of action and target higher molecular weight aggregates and fibrils rather than the initial stages of the oligomerization process.

[0006] Therefore there is a need for developing new compounds that will block the formation of Aβ oligomers by interfering with aggregation or promoting degradation. Early attempts at developing anti-aggregation compounds for in vivo use have not been successful. Probably in part because of their lack of specificity and inability to cross the blood brain barrier. Most of these compounds have been discovered by screening large libraries for molecules that block Aβ fibrillation rather than oligomerization. Moreover such compounds are generic blockers of amyloid formation not only for Aβ and might be acting through
general non-specific mechanisms such as anti-oxidant effects. While $\alpha\beta$ fibrils are contained in the amyloid plaques, the more toxic $\alpha\beta$ oligomers are soluble and diffuse around neurons and these soluble oligomers have been linked to deficits in learning and memory. Current evidence supports the view that it is the $\alpha\beta$ oligomers rather than the fibrils themselves that are toxic, therefore the development of anti-fibrillation compounds, as is the current state of the art, is only of limited use.

Therefore the identification of new and more effective molecules that will target the propagation of dimers and block the oligomerization of $\alpha\beta$ peptides might be of significant importance for developing new therapies for AD and related conditions.

**SUMMARY OF THE INVENTION**

The present invention relates to compounds having the general formula

![Chemical Structure](image)

wherein

- $X$ is an aliphatic group comprising 1 to 6 carbon atoms,
- $Y$ is an aliphatic group comprising 1 to 6 carbon atoms,
- $R_1$, $R_2$, $R_1'$, and $R_2'$ are independently nil, hydrogen or an aliphatic group comprising 1 to 6 carbon atoms,
- $G_1$, $G_2$, $G_3$, $G_4$, $G_5$, $G_6$, $G_7$, $G_8$, $G_9$ and $G_{10}$ are independently N or CH, wherein $G_1$, $G_4$, $G_7$ and $G_{10}$ are C when $R_1$, $R_2$, $R_1'$ and $R_2'$ is an aliphatic group comprising 1 to 6 carbon atoms or hydrogen,
- or a pharmaceutically acceptable ester or salt thereof,

wherein at least one of substituents $G_1$, $G_2$, $G_3$, $G_4$, $G_5$, $G_6$, $G_7$, $G_8$, $G_9$ and $G_{10}$ is an...
unsubstituted N.

[0009] The compounds of the present invention are unique in that they specifically block the early formation of toxic protein aggregation, e.g. the formation of Aβ oligomers, in a human or animal body. These compounds specifically target very early toxic protein aggregations and have a high affinity to proteins which are known to be involved in protein aggregation in neurodegenerative disorder such as amyloid β. Furthermore the compounds of the present invention are stable in plasma and solution and are able to easily cross the blood brain barrier at high AUC brain/ blood ratios. The compounds of the present invention can be synthesized as shown below and with methods known in the art.

[0100] The compounds of the present invention have alternating hydrophobic and polar regions in their chemical structures that specifically target the earliest forms of amyloid β (Aβ) oligomers, for instance, and prevent further accumulation of more toxic species. These anti-Aβ compounds work by preventing further docking of neighboring Aβ peptides that can result in the formation of toxic oligomers. Therefore these compounds work by interfering at "early" stages the process of Aβ oligomerization (e.g. dimer formation). They can also work by preventing already formed oligomers (e.g. trimers and tetramers) from growing further and forming ring like structures (e.g. pentamers, hexamers).

[0011] The heterocyclic organic compounds described in this invention that block, among others, Aβ oligomer formation, have a central moiety comprising cyclohexane which is linked through linker segments, which can be aliphatic groups comprising 1 to 6 carbon atoms, to an amino group to heteroaromatic rings with at least one nitrogen atom. The heteroaromatic ring may comprise more than one nitrogen atom. These further nitrogen atoms can either be free or connected to an alkyl group with 1-6 carbon atoms and being positively charged and thus forming a salt with negatively charged counter ions such as halogen, tosylate, citrate, or any counter ion used in the formulation of drugs. The heteroaromatic ring may either be unsubstituted or alternatively has attached to it in any position on the ring structure a hydrophobic group consisting of alkyl, alkoxy, fluorinated alkoxy, or halogen atoms including chlorine, bromine or iodide.

[0012] The heteroaromatic groups of the compound of the present invention can be identical in structure or can be distinct.
The term "pharmaceutically acceptable salt", as used herein, relates to salts which are toxicologically safe for human and animal administration. For example, suitable pharmaceutically acceptable salts include, but are not limited to, salts of pharmaceutically acceptable inorganic acids such as hydrochloric, sulphuric, phosphoric, nitric, carbonic, boric and sulfamic acids, or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, hydroxyxymalic, fumaric, maleic, citric, lactic, gluconic, benzoic, succinic, methanesulphonic, oxalic, phenylactic, toluenesulphonie, benzencesulphonic, salicyclic, sulphaniic, aspartic, glutamic, edelic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids.

According to a preferred embodiment of the present invention X is selected from the group consisting of a methylene, ethylene and propylene group, preferably a methylene group.

According to a further preferred embodiment of the present invention Y is selected from the group consisting of a methylene, ethylene and propylene group, preferably a methylene group.

The carbon and/or nitrogen atoms of the heteroaromatic rings of the compound of the present invention can be substituted either with hydrogen or an aliphatic group comprising 1 to 6 carbon atoms. A particularly preferred substituent of R₁ is selected from the group consisting of a methyl, ethyl and propyl group, preferably a methyl group. R₂ is preferably selected from the group consisting of a methyl, ethyl and propyl group, preferably a methyl group. According to a preferred embodiment of the present invention R₁ is selected from the group consisting of a methyl, ethyl and propyl group, preferably a methyl group. R₂ can be selected from the group consisting of a methyl, ethyl and propyl group, preferably a methyl group.

According to a particularly preferred embodiment of the present invention both heteroaromatic rings have the same structure and the same substituents.

According to a particularly preferred embodiment of the present invention the compounds of the present invention having general structure of formula (1) have the following substituents (Table A):
Table A:

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</table>

Of course the methyl groups of Table A can be exchanged, for instance, with ethyl or propyl groups.
According to a preferred embodiment of the present invention the compound is selected from the group consisting of N,N'-(1r,4r)-cyclohexane-1,4-diylibis(methylene))bis(pyridin-2-amine), N,N'-(1r,4r)-cyclohexane-1,4-diyibis(methylene))bis(pyrazin-2-amine), N,N'-(1r,4r)-cyclohexane-1,4-
diylibis(methylene))bis(pyridazin-3-amine), N,N'-(1r,4r)-cyclohexane-1,4-
diylibis(methylene))bis(pyridazin-3-amine), N,N'-(1r,4r)-cyclohexane-1,4-
diylibis(methylene))bis(pyridazine-3-amie), N,N'-(1r,4r)-cyclohexane-1,4-
diylibis(methylene))bis(3-methylpyridin-2-amine), N,N'-(1r,4r)-cyclohexane-1,4-
diylibis(methylene))bis(3,6-dimethylpyridin-2-amine), N,N'-(1r,4r)-cyclohexane-1,4-
diylibis(methylene))bis(3,6-dimethylpyrazine-2-amine), N,N'-(1r,4r)-cyclohexane-1,4-
diylibis(methylene))bis(3,6-dimethyl-1,2,4-triazin-5-amine), N,N'-(1r,4r)-cyclohexane-1,4-
diylibis(methylene))bis(4-methylpyridazin-3-amine) and N,N'-(1r,4r)-cyclohexane-1,4-
diylibis(methylene))bis(6-methyl-1,2,4,5-tetrazin-3-amine), whereby one of the most
preferred compounds is N,N'-(1r,4r)-cyclohexane-1,4-diylibis(methylene))bis(3-
methylpyridin-2-amine).

The compounds of the present invention can be synthesised using the following general synthesis scheme:

\[
\begin{align*}
\text{H}_2\text{N} & \text{-X} \quad \text{Y} \quad \text{NH}_2 \\
\text{K}_2\text{CO}_3 & \quad \text{DMF} \\
\text{G}_3 & \text{G}_4 \quad \text{G}_5 \\
\text{R}_1 & \quad \text{R}_2 \\
\text{G}_2 & \quad \text{G}_1 \\
\text{G}_6 & \text{G}_7 \\
\text{G}_8 & \quad \text{G}_9 \\
\text{R}_1 & \quad \text{R}_2 \\
\end{align*}
\]
[0021] The starting material used in this scheme can be obtained by methods well known in the art.

[0022] The compounds of the present invention are preferably used in a method for treating and/or preventing diseases associated with abnormal accumulation of proteins, preferably amyloid and/or alpha-synuclein protein and/or Lewy bodies, in organs.

[0023] The amyloid protein is preferably amyloid beta.

[0024] The disease is preferably selected from the group consisting of Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and Huntington's Disease.

[0025] Another aspect of the present invention relates to a pharmaceutical composition comprising an effective amount of at least one compound according to the present invention or a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable solvate of said compound or salt, and one or more pharmaceutically acceptable excipients.

[0026] The compounds of the present invention or a pharmaceutically acceptable salt thereof may be formulated by following any number of techniques known in the art of drug delivery. The compounds or pharmaceutically acceptable salts thereof may of course be administered by a number of means keeping in mind that not all formulations not suitable for every route of administration. They can be administered in solid or liquid form. The application may be oral, rectal, nasal, topical (including buccal and sublingual) or by inhalation. The compounds of the invention or a pharmaceutically acceptable salt thereof may be administered together with conventional pharmaceutically acceptable adjuvants, carriers and/or diluents. The solid dosage forms comprise tablets, capsules, powders, pills, pastilles, suppositories, gels and granular forms of administration. They may also include carriers or additives, such as flavors, dyes, diluents, softeners, binders, preservatives, lasting agents and/or enclosing materials. Liquid forms of administration include solutions, suspensions and emulsions. These may also be offered together with the above-mentioned additives.

[0027] Solutions and suspensions of the compounds of the invention or pharmaceutically acceptable salts thereof (provided of course that these solutions and suspensions have a suitable viscosity) may be injected. If the suspension is too viscous for
injection the pharmaceutical preparation may be implanted using devices designed for such purposes. Sustained release forms are generally administered via parenteral or enteric means. Parenteral administration is another route of administration of the compounds of the present invention or pharmaceutically acceptable salts thereof.

[0028] The administration of the compounds of the present invention may involve an oral dosage form. Oral dose formulations are preferably administered once or twice daily, three times daily in the form of a capsule or tablet, for instance, or alternatively as an aqueous based solution. If the compounds of the present invention are administered intravenously, the administration may occur either daily, continuously, once a week or three times a week.

[0029] It is also possible to provide pharmaceutical compositions which in addition to the compounds of the present invention comprise other substances which are suited for treating, preventing or relieving the symptoms of synucleopathies and Parkinson's-like disorders. These combinations may be administered in solid or liquid form in a single formulation or composition or in separate formulations or compositions.

[0030] According to a preferred embodiment of the present invention the pharmaceutical compositions contain from about 0.01 mg to about 5.0 g, preferably from about 0.05 mg to 2 g, more preferably from about 0.5 mg to 1 g, even more preferably from about 1 mg to 500 mg, of the compound of the present invention. The compounds of the present invention can be administered to a patient in an amount of about 0.01 mg to about 5 g, preferably of about 0.05 mg to 2 g, more preferably from about 0.5 mg to 1 g, even more preferably from about 1 mg to about 500 mg per kg body weight.

[0031] The compounds of the present invention may also be provided as sustained release oral formulations. These formulations generally comprise the compounds of the invention having decreased solubility in order to delay absorption into the bloodstream. In addition, these formulations may include other components, agents, carriers, etc., which may also serve to delay absorption of the compounds. Microencapsulation, polymeric entrapment systems, and osmotic pumps, which may or may not be bioerodible, may also be used to allow delayed or controlled diffusion of the compounds from a capsule or matrix.

[0032] As used herein, the term "effective amount" in the context of treating or preventing alpha-synucleopathies or Parkinson's-like disorders, especially PD, relates to the
administration or addition of an amount of the compound of the present invention that is effective for the prevention and/or treatment of existing synucleopathies or Parkinson's-like disorder. The effective amount will vary depending on the health and physical condition of the individual to be treated, the taxonomic group of the individual to be treated, the formulation of the composition, the assessment of the medical situations and other relevant factors.

[0033] A further aspect of the present invention relates to the use of a compound according to the present invention for the manufacture of a medicament for treating and/or preventing diseases associated with abnormal accumulation of proteins, preferably amyloid and/or alpha-synuclein protein and/or Lewy bodies, in organs.

[0034] Another aspect of the present invention relates to a method for treating, ameliorating and/or preventing diseases associated with abnormal accumulation of proteins, preferably amyloid and/or alpha-synuclein protein and/or Lewy bodies, in organs by administering to an individual suffering or being at risk to suffer from said diseases an effective amount of a compound or a pharmaceutical preparation according to the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] The present invention is further illustrated in the following figures and examples, however, without being restricted thereto.

[0036] Fig. 1 shows a general reaction scheme to obtain the compounds of the present invention.

[0037] Fig. 2 shows that compound 7 reverses oligomeric-mediated increases in calcium mobilization.

[0038] Fig. 3 shows that compound 7 reverses oligomeric-mediated decreases in calcein fluorescence.

[0039] Fig. 4 shows that compound 7 reduces Abeta-protein dimers in a cell free preparation.

[0040] Figs. 5A and 5B show the most preferred compounds of the present invention.
Example 1: Synthesis of Compound 1 (N,N'-(lr,4r)-cyclohexane-1,4-diylnbis(methylene)bis(pyridin-2-amine))

A mixture of 2-fluoropyridine (3eq) and 1 eq. 1,4-cyclohexane-methylamine was heated at 100°C for 2 hours with stirring followed by heating at 150°C for 8 hours. After cooling to room temperature the reaction mixture was poured into water and the product was extracted with methylene chloride. The organic phase was washed with water, dried over anhydrous sodium sulfates, filtered and the clear colorless solution was evaporated under reduced pressure to dryness. The crude product was purified by flash column chromatography using chloroform/hexane at a ratio of 1:4 as an eluent to yield 96% pure compound in 30% yield. H NMR (400 MHz, MeOD), d 0.82 (m), 1.49 (m), 1.81 (m), 3.12 (m), 3.48 (M), 6.50 (S), 7.38 (M), 7.95 (M).

Example 2: Synthesis of Compound 2 (N,N'-(lr,4r)-cyclohexane-1,4-diylnbis(methylene)bis(pyrazin-2-amine))

2-chloropyrazine (3 eq.) and 1 eq. 1,4-cyclohexane-methylamine were suspended in dimethylformamide in a round bottom flask with stirring and excess sodium carbonate was added. The mixture was heated to 140°C for 8 hours and cooled to ambient temperature. The flask content was poured into water and the product extracted with chloroform. After evaporation of the chloroform the crude product was resuspended in hot ethanol. Upon cooling at 4°C over night a near white solid was obtained in 45% yield.

Example 3: Synthesis of Compound 3 (N,N'-(lr,4r)-cyclohexane-1,4-diylnbis(methylene)bis(pyridazin-3-amine))

A mixture of 2-cloropyridazine (3 eq.) and 1 eq. 1,4-cyclohexane methylamine (1 eq.) and DABC (5-Chloro-1,3-benzenediamine) were dissolved in N-methylpyrrolidone and heated over night at 120°C to obtain compound 3 in 85% yield. H NMR (400, MeOD), d = 4.52 (8), 5.09 (V), 7.38 (S), 7.82 (m), 7.88 (m), 7.91 (m), 8.12 (m).

Example 4: Synthesis of Compound 4 (N,N'-(lr,4r)-cyclohexane-1,4-diylnbis(methylene)bis(pyrimidin-4-amine))
[0044] 2-chloro-pyrimidine (3 eq.) and 1 eq 1,4 cyclohexanemethylamine and 5 eq of
pottasium carbonate all in dimethylformamide were stirred for 8 hours at 130°C.

[0045] Upon cooling the mixture and then adding water a precipitate formed and the
product was extracted from methylene chloride and purified by crystallization from a
mixture of ethanol/hexane to obtain the product in 60% yield.

Example 5: Synthesis of Compound 5 (N,N'-(lr,4r)-cyclohexane-1,4-
diylbis(methylene))bis(1,2,4-triazin-5-amine))

[0046] A mixture of 6-chloro(1,2,4 triazme) and 1,4 cyclohexane methylamine and
triethylamine all in a 3:1 molar ratio were dissolved in dry dimethylformamide and heated to
reflux for 4 hours. The crude reaction product was purified as compound 1 in example 1. The
yield of product was 57%.

Example 6: Synthesis of Compound 6 (N,N'-(lr,4r)-cyclohexane-1,4-
diylbis(methylene))bis(1,2,4,5-tetrazin-3-amine))

[0047] Following the procedure given for compound 5 (example 5), a mixture of 6-
chloro(1,2,4,5 tetrazine) and 1,4 cyclohexane methylamine at 3:1 molar ratio and DABCO
were reacted with stirring for 24 hours at 110°C. Purification was done as in example 1 for
compound 1. The yield of product was 70%.

Example 7: Synthesis of Compound 7 (N,N'-(lr,4r)-cyclohexane-1,4-
diylbis(methylene))bis(3-methylpyridin-2-amine))

[0048] A mixture of 2 chloro-3-methylpyridine (30 mmoles) and 1,4
cyclohexymethyiamme (12 mmoles) dissolved in dimethylactamide was stirred at room
temperature and 30 mmoles of DABCO (1,1-diazabicyclo)ocatane was added to the clear
solution. The reaction vessel was heated to 90°C for 18 hours. After cooling down to ambient
temperature the reaction mixture was diluted with water, forming a precipitate which as
filtered off and recrystallized from a mixture of isopropanol/hexane. The yield of purified
compound was 45%.

Example 8: Synthesis of Compound 9 (N,N'-(ir,4r)-cyclohexane-1,4-
diylbis(methylene))bis(3,6-dimethylpyrazin-2-amine))

[0049] A mixture of dimethylpyrazine chloride (4 eq), cesium carbonate (5 eq),
lithium fluoride (2.5 eq) and 1,4-chyclodenaxe-methylamine all dissolved in
dimethylfonnamide was heated to 135°C for 16 hours with stirring. After cooling to room
temperature the reaction mixture was poured into water, extracted with methylene chloride and the solvent evaporated under reduced pressure. The nearly colorless solid was dried over anhydrous sodium sulfate. The crude product was purified by flash chromatography using chloroform/hexane at a volume ratio of 1:4 and eluted to yield 98% pure compound in 35% yield. 

$\text{H}^1\text{NMR} \ (400 \ \text{MHz} \ \text{MeOD}) \ \delta = 1.09 \ \text{(m)}, \ 1.66 \ \text{(m)}, \ 1.93 \ \text{(m)}, \ 2.48 \ \text{(s)}, \ 3.38 \ \text{(m)} 3.52\text{(m)}, \ 4.37 \ \text{(m)}$  

Example 9: Beta-amyloid (Abeta)-protein oligomer formation  

[0050] Recombinant Abeta-protein (American Peptide Company) was prepared at a concentration of 100 nM and incubated for either 16 hours at 37°C (monomer control) or 37°C+56°C for 16 + 6 hours (oligomer control). Compound 7 (N,N'-((1r,4r)-cyclohexane-1,4-diylbis(methylene))bis(3-methylypyriditi-2-amine); NPT 420-7) was co-incubated at concentrations from 0 to 12 µM, and resulting control and compound oligomeric solutions were used in subsequent cell based assays (calcium and calcein mobilization assays) or cell free western blots.  

Calcium mobilization assay  

[0051] Assessment of calcium influx was carried out using a modified protocol of the commercially available Fluo-4 NW Calcium imaging assay kit (Life Technologies). Briefly, plated B103 cells were treated with prepared vehicle or Abeta-protein monomer and oligomeric solutions (as described above) for 24 hours at 37°C. After treatment, media was replaced by 100 ml of HBSS (Hank's Buffered Salt Solution) buffer and 100 ml of calcium dye was added to each well. As a positive control of calcium influx, 0.6 ng of ionomycin (Sigma) was added to additional wells. Cells were kept in the incubator at 37°C for 1 hr before measuring fluorescence with excitation/emission filter at 470-495/515-575 nm on a DTX 880 Multimode Detector (Beckman Coulter). The data in Fig. 2 are presented as (A) mean relative fluorescent unit (RFU) values ± standard error of the mean (SEM), and (B) as % reversal of oligomeric mediated increases in calcium fluorescence. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc Tukey-Kramer tests (Prism Graph Pad Software, San Diego, CA, USA). The significance level was set at p <0.05, and denoted as ***p<0.001 versus vehicle control (No Abeta) group or ###p<0.0001 vs. compound 7 Control (No Abeta).  

Calcein Assay
Assessment of calcein fluorescence was carried out following the calcium mobilization assay procedure described above with modifications including use of calcein AM fluorescent indicator dye (Life Technologies, Carlsbad, CA; #C3100MP and a different cell plating density (30K vs. 25K cells per 96 well plate). Briefly, plated B103 cells were treated with prepared vehicle or Abeta-protein monomer and oligomeric solutions (as described above) for 16 hours at 37°. Calcein solution was prepared in phenol red free media with DMSO. 200uL of the resultant solution was added to each well and the plates were incubated at 37° for 2 hour. The fluorescence was read with excitation/emission filter at 470-495/515–575 nm on a DTX 880 Multimode Detector (Beckman Coulter, Fullerton, CA). The data are presented in Figure 3 as (A) mean relative fluorescent unit (RFU) values ± standard error of the mean (SEM), and (B) as % reversal of oligomeric mediated increases in calcein fluorescence. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc Tukey-Kramer tests (Prism Graph Pad Software, USA). The significance level was set at p <0.05, and denoted as #p<0.05 vs. NPT-420-7 Control (No Abeta).

Cell Free Western Blot Protocol

Samples were loaded onto a 4-12% SDS-polyacrylamide gel (Life Technologies), and blotted onto a nitrocellulose membrane. Blots were blocked with 5% non-fat milk in PBST and labeled with a primary monoclonal antibody against Abeta-protein (6E10, Signet Laboratories, Dedham, MA) followed by an anti-mouse secondary antibody (1:5000) (American Qualex, San Clemente, CA). Blots were then imaged with Western Lightening Plus-ECL (Perkin Elmer) on a VersaDoc MP 4000 series (Bio-Rad). Blot Quantity One analysis software (Bio-Rad) was utilized for analysis of blot images. The data are presented in Figure 4 as (A) the labeled blot image and (B) the quantified mean integrated pixel values for that blot.
All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.
1. Compound having the general formula

wherein

X is an aliphatic group comprising 1 to 6 carbon atoms,

Y is an aliphatic group comprising 1 to 6 carbon atoms,

R₁, R₂, R₃ and R₄ are independently nil, hydrogen or an aliphatic group comprising 1 to 6 carbon atoms, alicyclic groups with 3 to 8 membered rings or aromatic groups,

G₁, G₆, G₇, G₈, G₉ and G₁₀ are independently N or CH₃,

or a pharmaceutical(!) acceptable ester or salt thereof,

wherein at least one of substituents G₁, G₂, G₃, G₄, G₅, G₆, G₇, G₈, G₉ and G₁₀ is an unsubstituted N.

2. Compound according to claim 1, wherein X is selected from the group consisting of a methylene, ethylene and propylene group, preferably a methylene group.

3. Compound according to claim 1 or 2, wherein Y is selected from the group consisting of a methylene, ethylene and propylene group, preferably a methylene group.
4. Compound according to any one of claims 1 to 3, wherein R is selected from the group consisting of a methyl, ethyl and propyl group, preferably a methyl group.

5. Compound according to any one of claims 1 to 4, wherein R₂ is selected from the group consisting of a methyl, ethyl and propyl group, preferably a methyl group.

6. Compound according to any one of claims 1 to 5, wherein R₃ is selected from the group consisting of a methyl, ethyl and propyl group, preferably a methyl group.

7. Compound according to any one of claims 1 to 6, wherein R₄ is selected from the group consisting of a methyl, ethyl and propyl group, preferably a methyl group.

8. Compound according to any one of claims 1 to 7, wherein the compound is selected from the group consisting of N,N'-(lr,4r)-cyclohexane-1,4-diylbis(methylene)bis(pyridin-2-amine), N,N'-(lr,4r)-cyclohexane-1,4-diylbis(methylene)bis(pyridazin-2-amine), N,N'-(lr,4r)-cyclohexane-1,4-diylbis(methylene)bis(pyrimidin-4-amine), N,N'-(lr,4r)-cyclohexane-1,4-diylbis(methylene)bis(1,2,4-triazin-5-amine), N,N'-(lr,4r)-cyclohexane-1,4-diylbis(methylene)bis(3-methylpyridin-2-amine), N,N'-(lr,4r)-cyclohexane-1,4-diylbis(methylene)bis(3,6-dimethylpyridin-2-amine), N,N'-(lr,4r)-cyclohexane-1,4-diylbis(methylene)bis(3,6-dimethylpyrazin-2-amine), N,N'-(lr,4r)-cyclohexane-1,4-diylbis(methylene)bis(4-methylpyridazin-3-amine) and N,N'-(lr,4r)-cyclohexane-1,4-diylbis(methylene)bis(6-methyl-1,2,4,5-tetrazin-3-amine).

9. Compound according to any one of claims 1 to 8 for use in a method for treating and/or preventing diseases associated with abnormal accumulation of proteins, preferably amyloid and/or alpha-synuclein protein and/or lewy bodies, in organs.

10. Compound according to claim 9, characterised in that the amyloid protein is amyloid beta.

11. Compound according to claim 9 or 10, characterised in that the disease is selected from the group consisting of Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and Huntington's Disease.
12. Pharmaceutical composition comprising an effective amount of at least one compound according to any one of claims 1 to 8 or a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable solvate of said compound or salt, and one or more pharmaceutically acceptable excipients.

13. Pharmaceutical composition according to claim 12, wherein the composition is adapted for oral administration.

14. Use of a compound according to any one of claims 1 to 8 for the manufacture of medicament for treating and/or preventing diseases associated with abnormal accumulation of proteins, preferably amyloid and/or alpha-synuclein protein and/or Lewy bodies, in organs.

15. Use according to claim 14, characterised in that the amyloid protein is amyloid beta.

16. Use according to claim 14 or 15, characterised in that the disease is selected from the group consisting of Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and Huntington's Disease.

17. Method for treating, ameliorating and/or preventing diseases associated with abnormal accumulation of proteins, preferably amyloid and/or alpha-synuclein protein and/or Lewy bodies, in organs by administering to an individual suffering or being at risk to suffer from said diseases an effective amount of a compound according to any one of claims 1 to 8 or a pharmaceutical preparation according to claim 12 or 13.
Fig. 1
B. In presence of

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1 μM NPT-420-7

A. Fig. 2

**RPU**

Vehicle control

**No Ap M**

50,000

100,000

150,000
A. 

![Graph showing RFU](image)

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B. 

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% reversal of impairment:

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compound No

1  \(N,N'-(1r,4r\text{-cyclohexane-1,4-diylbis(methylene)})\text{bis(pyridin-2-amine)}\)

2  \(N,N'-(1r,4r\text{-cyclohexane-1,4-diylbis(methylene)})\text{bis(pyrazin-2-amine)}\)

3  \(N,N'-(1r,4r\text{-cyclohexane-1,4-diylbis(methylene)})\text{bis(pyridazin-3-amine)}\)

4  \(N,N'-(1r,4r\text{-cyclohexane-1,4-diylbis(methylene)})\text{bis(pyrimidin-4-amine)}\)

5  \(N,N'-(1r,4r\text{-cyclohexane-1,4-diylbis(methylene)})\text{bis(1,2,4-triazin-5-amine)}\)

6  \(N,N'-(1r,4r\text{-cyclohexane-1,4-diylbis(methylene)})\text{bis(1,2,4,5-tetrazin-3-amine)}\)

Fig. 5A
$N,N'-(1\text{r},4\text{r})$-cyclohexane-1,4-diylbis(methylene))bis(3-methylpyridin-2-amine)

$N,N'-(1\text{r},4\text{r})$-cyclohexane-1,4-diylbis(methylene))bis(3,6-dimethylpyridin-2-amine)

$N,N'-(1\text{r},4\text{r})$-cyclohexane-1,4-diylbis(methylene))bis(3,6-dimethylpyrazin-2-amine)

$N,N'-(1\text{r},4\text{r})$-cyclohexane-1,4-diylbis(methylene))bis(3,6-dimethyl-1,2,4-triazin-5-amine)

$N,N'-(1\text{r},4\text{r})$-cyclohexane-1,4-diylbis(methylene))bis(4-methylpyridazin-3-amine)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
   INV. C07D403/12 C07D401/12 A61K31/4427 A61P25/00

ADD.
   According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
   Minimum documentation searched (classification system followed by classification symbols)
   C07D A61K A61P

   Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

   Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
   EPO-Internal, CHEMABS Data, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
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  "L" document which may throw doubts on priority claim(s) on which the international publication is based and, where shown, the earliest date for which such prior published document exists
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed

"I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"S" document member of the same patent family

Date of the actual completion of the international search
4 April 2013

Date of mailing of the international search report
12/04/2013

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Authorized officer
Menchaca, Roberto
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