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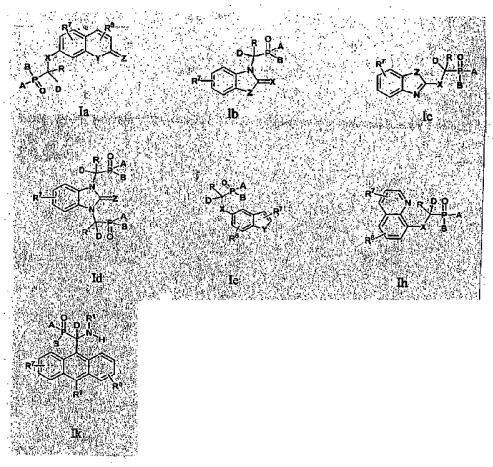
(54) **Title:** SUBSTITUTED PHOSPHONATES AND THEIR USE IN DECREASING AMYLOID AGGREGATES



(57) **Abstract:** The invention relates to novel and known substituted phosphonates for use in ameliorating amyloid aggregates, particularly for use in the treatment of Alzheimer's disease.

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# SUBSTITUTED PHOSPHONATES AND THEIR USE IN DECREASING AMYLOID AGGREGATES

The invention relates to novel and known substituted phosphonates for use in decreasing amyloid aggregates, particularly for use in the treatment of Alzheimer's disease (AD).

Diseases linked to amyloidosis include, for example, Alzheimer's disease, type 2 diabetes, Huntington's disease and Parkinson's disease. The diseases share a common characteristic whereby abnormal amyloid deposits are found in the affected organs. AD in particular displays abnormal amyloid deposits, typically found as of neuritic plaques in brain parenchyma and amyloidosis in cerebral blood vessels. The A[beta], 42 residue peptide, is the major constituent of these plaques. To date the cause of A[beta] deposits is unknown but a successful treatment of the disease may follow from the prevention of these deposits. Various compounds leading to modulation of the amyloid formation have been reported but so far no compound is available as a treatment. Examples of reports on such compounds deal with aminocarboxylates such as DP-109 J-Y Lee, JE Friedman, I Angel, A Kozak, J-Y Koh, Neurobiology of Aging, 2004, 25, 1315-1321 and WO9916741, desferrioxamine DFO (DR Crapper Maclachlan, AJ Dalton, TPA Kruck, MY Bell, WL Smith W Kalow, DF Andrews Lancet, 1991, 337, 1304.).Clioquinol, CC Curtain, KJ Barnham and AI Bush, Curr. Med. Chem.- Immun., Endoc. & Metab. Agents, 2003, 3, 309-315, US2006074104; N-thiazolyl amides, FR2865206; phosphonocarboxylate US2006135479; amine compounds :US2004077867, benzothiepine derivatives US2002128308; succinate esters US2006281692, benzoate an benzamide compounds US2006167108; bi- and tricyclic pyridine derivatives WO9825930; pyrazolylpyrimidines WO03080609.

One possible drug Clioquinol, has been shown to reverse the formation of plaques. Clioquinol chelates zinc and copper *in vitro*. Copper and zinc have particularly high concentration in the β-amyloid plaques in the brains of AD patients.

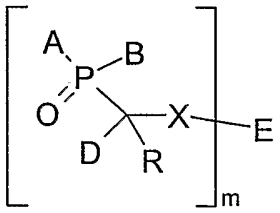
Clioquinol was approved by the FDA as an anti-biotic and anti-fungal agent, but was removed from the market over 30 years ago due to adverse side effects including the loss of vitamin B-12. A clinical trial using clioquinol together with vitamin B-12 was conducted to determine whether this drug is useful in the treatment of Alzheimer's without the previously seen side effects. Other side effects included eye damage with long term usage and nerve damage and loss of sensitivity. The first clinical trials of clioquinol (known as PTB1 in these trials) reached the final stages, when it was discovered that the PTB1 manufacturing process contained mutagenic impurities that could not be removed to acceptable levels. Thus, PTB1 trials were ceased. A follow on compound, known as PTB2 was developed, and has now finished phase 1 trials, although the results of this are unknown.

Thus, there remains a need for a way of reducing amyloid plaques in diseases, such as AD, without the adverse side effects seen in trials of the drugs mentioned above. In addition, there is still a clear need for improved therapies that not only improve on current treatment with regard to cognitive defects but also reduce side effects.

Accordingly, the present invention provides novel and known compounds of general formula I, described as fluorescent phosphonates in PCT publication number WO2004/101579, and the preparation and use of these novel and known compounds of general formula I for medical treatments of diseases linked to amyloidosis and compositions comprising such compounds.

The current invention is based on evidence generated showing that substituted phosphonates promote disaggregation of amyloidic aggregates, amyloidic load in cellular systems expressing amyloid, and amyloid plaques and reduce parenchymal zinc in the brain and therefore these compounds may provide treatments for disease states linked to amyloidosis and linked to zinc.

In a first aspect of the present invention, there is provided a compound of general formula I for use in medicine. Such compounds have been shown to be useful in the disaggregation of amyloidic deposits.




I

Examples of specific compounds for use in the invention are tabulated below in Table 1. Novel compounds 4, 5a, 6, 7a, 8, 8a, 14 and 10 are also provided.

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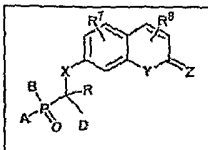
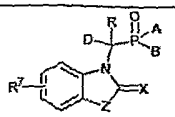
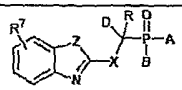
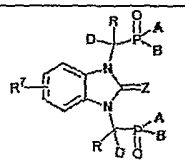
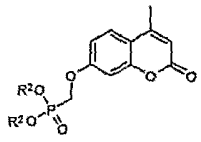
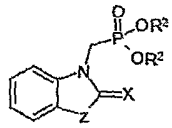
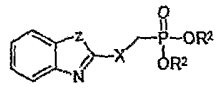
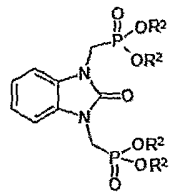
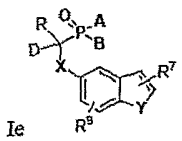
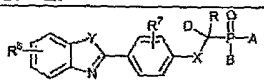
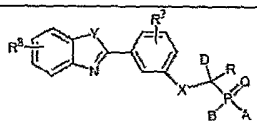
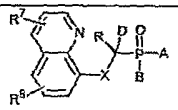
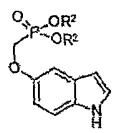
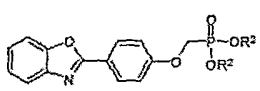
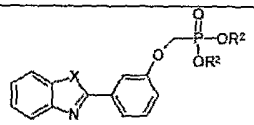
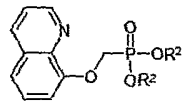
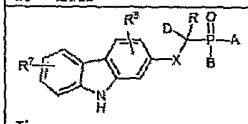
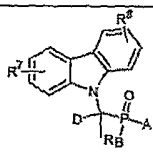
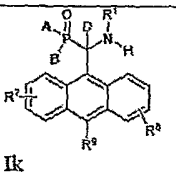
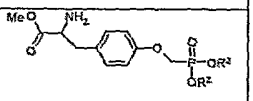
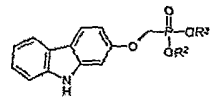
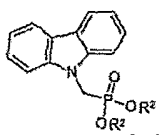
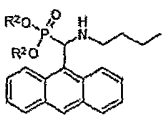
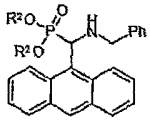
|  Ia |  Ib |  Ic |  Id |
|  Compound 1 $R^2/R^2$ = Et/Et<br>Compound 2 $R^2/R^2$ =Et/H<br>Compound 3 $R^2/R^2$ = H/H |  Compound 4 X = O, Z= O<br>Compound 5 X = S, Z = O<br>Compound 6 X = O, Z = S<br>Compound 7 X = S, Z = S<br>Compound 8 X = O, Z = NH<br>Compound 9 X = S, Z = NH<br>$R^2$ = Et |  Compound 4a X = O, Z= O<br>Compound 5a X = S, Z = O<br>Compound 6a X = O, Z = S<br>Compound 7a X = S, Z = S<br>Compound 8a X = O, Z = NH<br>Compound 9a X = S, Z = NH<br>$R^2$ = Et |  Compound 10<br>$R^2$ = Et |
|  Ie |  If |  Ig |  Ih |
|  Compound 11<br>$R^2$ = Et/H |  Compound 12<br>$R^2$ = Et/H |  Compound 13 X = O<br>Compound 14 X = S<br>$R^2$ = Et/H |  Compound 15 $R^2/R^2$ = Et<br>Compound 16 $R^2/R^2$ = Na |
|  Ii |  Ij |  Ik |  Compound 22 |
|  Compound 17<br>$R^2/R^2$ = Et<br>Compound 18<br>$R^2/R^2$ = H |  Compound 19 $R^2/R^2$ = Et<br>Compound 20 $R^2/R^2$ = H |  Compound 21 $R^2/R^2$ = Et |  Compound 23 |

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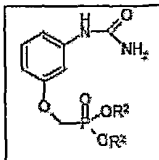
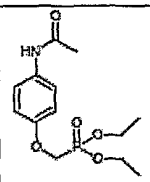
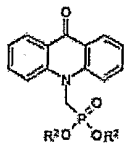
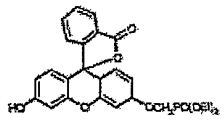
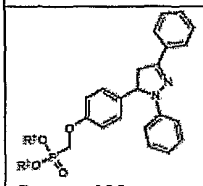
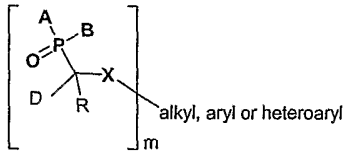
|  Compound 24 |  Compound 25 |  Compound 26 |  Compound 27 |
|  Compound 28 | | | |

wherein R is hydrogen or a substituted or unsubstituted linear or branched $C_{1-40}$ alkyl, aryl or heteroaryl, preferably $C_{1-20}$ alkyl, more preferably $C_{1-12}$ alkyl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkyl; E is a subsistuted or unsubstituted linear or branched $C_{1-40}$ alkyl, aryl, heteroaryl, or hydrogen where X is O, S, or $NR^1$ or when E is aryl or heteroaryl, X can be N integral to the aryl or heteroaryl ring; wherein $R^1$ is a hydrogen, a linear or branched $C_{1-40}$ alkyl, preferably $C_{1-20}$ alkyl, more preferably $C_{1-12}$ alkyl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkyl, $C_{2-40}$ alkenyl, preferably $C_{2-20}$ alkenyl, more preferably $C_{2-12}$ alkenyl, most preferably $C_{2-6}$ i.e. $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkenyl or $C_{2-40}$ alkynyl, preferably $C_{2-20}$ alkynyl, more preferably $C_{2-12}$ alkynyl, most preferably $C_{2-6}$ i.e. $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkynyl group, an aryl, a heteroaryl or $C_{1-40}$ alkylaryl, preferably $C_{1-20}$ alkylaryl, more preferably $C_{1-12}$ alkylaryl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkylaryl or alkylheteroaryl, preferably $C_{1-20}$ alkylaryl or alkylheteroaryl, more preferably $C_{1-12}$ alkylaryl or alkylheteroaryl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkylaryl or alkylheteroaryl group and where A and B are independently $OR^2$, $SR^2$, $NR^3R^4$ where $R^2$, $R^3$, and $R^4$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl, preferably $C_{1-20}$ alkyl, more preferably $C_{1-12}$ alkyl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkyl, $C_{2-40}$ alkenyl, preferably $C_{2-20}$ alkenyl, more preferably $C_{2-12}$ alkenyl, most preferably $C_{2-6}$ i.e. $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkenyl or $C_{2-40}$ alkynyl, preferably $C_{2-20}$ alkynyl, more preferably $C_{2-12}$ alkynyl, most preferably $C_{2-6}$ i.e. $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkynyl group, an aryl or $C_{1-40}$ alkylaryl, preferably $C_{1-20}$

alkylaryl, more preferably $C_{1-12}$ alkylaryl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkylarylgroup, or optionally a complex metal ion $M^{n+}/n$ wherein n is an integer from 1 to 8, or a linear or branched $C_{1-40}$ $NR^5R^6$ terminated alkyl chain, preferably $C_{1-20}$ $NR^5R^6$ terminated alkyl chain, more preferably $C_{1-12}$ $NR^5R^6$ terminated alkyl chain, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ $NR^5R^6$ terminated alkyl chain where $R^5$ and $R^6$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl, preferably $C_{1-20}$ alkyl, more preferably $C_{1-12}$ alkyl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkyl; and wherein m is an integer from 1 to 8; and wherein D is hydrogen or a linear or branched $C_{1-40}$ alkyl, preferably $C_{1-20}$ alkyl, more preferably $C_{1-12}$ alkyl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkyl, $C_{2-40}$ alkenyl, preferably $C_{2-20}$ alkenyl, more preferably $C_{2-12}$ alkenyl, most preferably $C_{2-6}$ i.e. $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkenyl or $C_{2-40}$ alkynyl, preferably $C_{2-20}$ alkynyl, more preferably $C_{2-12}$ alkynyl, most preferably $C_{2-6}$ i.e. $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkynyl group, an aryl, a heteroaryl or $C_{1-40}$ alkylaryl or alkylheteroaryl, preferably $C_{1-20}$ alkylaryl or alkylheteroaryl, more preferably $C_{1-12}$ alkylaryl or alkylheteroaryl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkylaryl or alkylheteroaryl group or a linear or branched $C_{1-40}$ alkyl $NR^5R^6$ chain, preferably $C_{1-20}$ alkyl $NR^5R^6$ chain, more preferably $C_{1-12}$ alkyl $NR^5R^6$ chain, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkyl $NR^5R^6$ chain , or a linear or branched $C_{1-40}$ mono or di alkyl ester, preferably $C_{1-20}$ mono or di alkyl ester, more preferably $C_{1-12}$ mono or di alkyl ester, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ mono or di alkyl ester $C_{1-40}$ alkylphosphonate, preferably $C_{1-20}$ alkylphosphonate, more preferably $C_{1-12}$ alkylphosphonate, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkylphosphonate or a linear or branched $C_{1-40}$ alkylphosphonic acid, preferably $C_{1-20}$ alkylphosphonic acid, more preferably $C_{1-12}$ alkylphosphonic acid, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkylphosphonic acid Y and Z are O, S, or $NR^1$. $R^7$ and $R^8$ represent one or more ring substituents which can be a hydrogen, a halide, a linear or branched $C_{1-40}$ alkyl, preferably $C_{1-20}$ alkyl, more preferably $C_{1-12}$ alkyl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkyl, $C_{2-40}$ alkenyl, preferably $C_{2-20}$ alkenyl, more preferably $C_{2-12}$ alkenyl, most preferably $C_{2-6}$ i.e. $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkenyl or $C_{2-40}$ alkynyl, preferably $C_{2-20}$ alkynyl, more preferably $C_{2-12}$ alkynyl, most preferably $C_{2-6}$ i.e. $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkynyl group, an aryl, a heteroaryl or $C_{1-40}$ alkylaryl or

alkylheteroaryl, preferably $C_{1-20}$ alkylaryl or alkylheteroaryl, more preferably $C_{1-12}$ alkylaryl or alkylheteroaryl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkylaryl or alkylheteroaryl group, a nitrile, a sulfonic acid or salt of sulfonic acid, a carboxy, an oxo, a carboxyalkyl, a carboxyalkoxy, a carboxylalkylamino, carboxyalkylthio, an amide, a sulfonamide, a $C_{1-6}$ alkylalkoxy, a $C_{1-6}$alkylamino group ,$OR^2$, $SR^2$, $NR^3R^4$ where $R^2$, $R^3$, and $R^4$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl, preferably $C_{1-20}$ alkyl, more preferably $C_{1-12}$ alkyl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkyl, $C_{2-40}$ alkenyl, preferably $C_{2-20}$ alkenyl, more preferably $C_{2-12}$ alkenyl, most preferably $C_{2-6}$ i.e. $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkenyl or $C_{2-40}$ alkynyl, preferably $C_{2-20}$ alkynyl, more preferably $C_{2-12}$ alkynyl, most preferably $C_{2-6}$ i.e. $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkynyl group, an aryl or $C_{1-40}$ alkylaryl, preferably $C_{1-20}$ alkylaryl, more preferably $C_{1-12}$ alkylaryl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkylaryl group.

In an alternative embodiment, formula I can be represented as:




wherein R is hydrogen or a linear or branched substituted or unsubstituted $C_{1-40}$ alkyl; X is O, S or N, which can be integral to the aryl or heteroaryl ring or NR ¦ where $R^1$ is a hydrogen, a linear or branched $C_{1-40}$ alkyl, $C_{2-40}$ alkenyl, or $C_{2-40}$ alkynyl, or aryl, a heteroaryl or $C_{1-40}$ alkylaryl or alkylheteroaryl; one or both of A and B is $OR^2$, $SR^2$, $NR^3$, $R^4$ where $R^2$, $R^3$ and $R^4$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl, $C_{2-40}$ alkenyl, $C_{2-40}$ alkynyl, $C_{1-40}$ alkylaryl or optionally a complex metal ion $M^{n+}/n$ wherein n is an integer from 1 to 8, or a linear or branched $C_{1-40}$ $NR^5R^6$ terminated alkyl chain, where $R^5$ and $R^6$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl; m is an integer from 1 to 8; and wherein D is hydrogen or a linear or branched $C_{1-40}$ alkyl, $C_{2-40}$ alkenyl, $C_{2-40}$ alkynyl, an aryl, a heteroaryl or $C_{1-40}$ alkylayl or alkylheteroaryl or a linear or branched $C_{1-40}$ alkyl $NR^5R^6$ chain or a linear or branched $C_{1-40}$ mono or di alkyl ester or di alkyl ester $C_{1-40}$ alkylphosphonate or a linear or branched $C_{1-40}$ alkylphosphonic acid.

In the context of the present invention, $C_{1-40}$ alkyl, preferably $C_{1-20}$ alkyl, more preferably $C_{1-12}$ alkyl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$, or $C_6$ alkyl refers to a straight, branched or cyclic hydrocarbon chain having from one to forty carbon atoms. The $C_{1-40}$ alkyl, preferably $C_{1-20}$ alkyl, more preferably $C_{1-12}$ alkyl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$, or $C_6$ alkyl, group may be substituted with one or more substituents selected from nitro, chloro, fluoro, bromo, nitrile, sulfonic acid or salt of sulfonic acid, carboxy, oxo, aryl, heteroaryl, carboxyalkyl, carboxyalkoxy, carboxylalkylamino, carboxyalkylthio, $C_{1-6}$ alkoxy, di $C_{1-40}$ alkyl phosphonate, $C_{1-40}$ alkyl phosphonate, phosphonic acid, amino, amino $C_{1-40}$ alkyl or amino di ($C_{1-40}$ alkyl). Examples include methyl, ethyl, isopropyl, *n*-propyl, butyl, *tert*-butyl, *n*-hexyl, *n*-decyl, *n*-dodecyl, cyclohexyl, octyl, *iso*-octyl, hexadecyl, octadecyl, *iso*-octadecyl and docosyl. A $C_{1-12}$-alkyl group has from one to twelve carbon atoms.

In the context of the present invention, $C_{2-40}$ alkenyl, preferably $C_{2-20}$ alkenyl, more preferably $C_{2-12}$ alkenyl, most preferably $C_{2-6}$ i.e. $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkenyl refers to a straight, branched or cyclic hydrocarbon chain having from one to forty carbon atoms and including at least one carbon-carbon double bond. The $C_{2-40}$ alkenyl, preferably $C_{2-20}$ alkenyl, more preferably $C_{2-12}$ alkenyl, most preferably $C_{2-6}$ i.e. $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkenyl, group may be substituted with one or more substituents selected from nitro, chloro, fluoro, bromo, nitrile, sulfonic acid or salt of sulfonic acid, carboxy, oxo, alkyl, carboxyalkyl, carboxyalkoxy, carboxylalkylamino, carboxyalkylthio, $C_{1-6}$-alkoxy, di $C_{1-40}$ alkyl phosphonate, $C_{1-40}$ alkyl phosphonate, phosphonic acid, amino, amino $C_{1-40}$-alkyl or amino di ($C_{1-40}$-alkyl). Examples include ethenyl, 2-propenyl, cyclohexenyl, octenyl, *iso*-octenyl, hexadecenyl, octadecenyl, *iso*-octadecenyl and docosenyl.

In the context of the present invention, $C_{2-40}$ alkynyl, preferably $C_{2-20}$ alkynyl, more preferably $C_{2-12}$ alkynyl, most preferably $C_{2-6}$ i.e. $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkynyl refers to a straight, branched or cyclic hydrocarbon chain having from one to forty carbon atoms and including at least one carbon-carbon triple bond. The $C_{2-40}$ alkynyl, preferably $C_{2-20}$ alkynyl, more preferably $C_{2-12}$ alkynyl, most preferably $C_{2-6}$ i.e. $C_2$,

$C_3$, $C_4$, $C_5$ or $C_6$ alkynyl, group may be substituted with one or more substituents selected from nitro, chloro, fluoro, bromo, nitrile, sulfonic acid or salt of sulfonic acid, carboxy, oxo, carboxyalkyl, carboxyalkoxy, carboxylalkylamino, carboxyalkylthio, $C_{1-6}$-alkoxy, di $C_{1-40}$ alkyl phosphonate, $C_{1-40}$ alkyl phosphonate, phosphonic acid, amino, amino $C_{1-40}$-alkyl or amino di ($C_{1-40}$-alkyl). Examples include ethynyl, 2-propynyl octynyl, *iso*-octynyl, hexadecynyl, octadecynyl, *iso*-octadecynyl and docosynyl.

$C_{1-6}$ alkoxy refers to a straight or branched hydrocarbon chain having from one to six carbon atoms and attached to an oxygen atom. Examples include methoxy, ethoxy, propoxy, *tert*-butoxy and *n*-butoxy.

The term aryl refers to a five or six membered cyclic, 8-10 membered bicyclic or 10-14 membered tricyclic group or up to a 10 fused ringed polyaromatic system with aromatic character and includes systems which contain one or more heteroatoms, for example, N, O or S. The aryl group may be substituted with one or more substituents selected from nitro, chloro, fluoro, bromo, nitrile, sulfonic acid or salt of sulfonic acid, carboxy, oxo, alkyl, alkoxy, carboxyalkyl, carboxyalkoxy, carboxylalkylamino, carboxyalkylthio, $C_{1-6}$-alkoxy, di $C_{1-40}$ alkyl phosphonate, $C_{1-40}$ alkyl phosphonate, phosphonic acid, amino, amino $C_{1-40}$-alkyl or amino di ($C_{1-40}$-alkyl).

Heteroaryl, as used herein, is an aromatic group that contains at least one heteroatom (a non-carbon atom forming the ring structure) and is optionally a single, two, three, four, five, six ringed structure or a fused 2-, 3-, 4-, 5-, 6-, 7- or 8-ring structure. Examples include pyrrolyl, pyridyl, thienyl, furanyl, oxazolyl, isoazolyl, oxadiazolyl, imidazolyl, benzoxazolyl, benzothiazolyl, benzimidazolyl, quinolyl, benzofuranyl, indolyl, carbazolyl, coumarins and benzocoumarins. The heteroaryl group may be substituted with one or more substituents selected from nitro, chloro, fluoro, bromo, nitrile, sulfonic acid or salt of sulfonic acid, carboxy, oxo, alkyl, alkoxy, carboxyalkyl, carboxyalkoxy, carboxylalkylamino, carboxyalkylthio, $C_{1-6}$-alkoxy, di $C_{1-40}$ alkyl phosphonate, $C_{1-40}$ alkyl phosphonate, phosphonic acid, amino, amino $C_{1-}$

$_{40}$-alkyl or amino di ($C_{1-40}$-alkyl). Such substituents are typically used to modify the spectral properties, affinity, selectivity, solubility or any combination of these factors.

The term $C_{1-40}$ alkylaryl, preferably $C_{1-20}$ alkylaryl, more preferably $C_{1-12}$ alkylaryl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkylaryl, group refers to a straight or branched hydrocarbon chain having from one to forty carbon atoms linked to an aryl group. The $C_{1-40}$ alkylaryl, preferably $C_{1-20}$ alkylaryl, more preferably $C_{1-12}$ alkylaryl, most preferably $C_{1-6}$, i.e. $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ or $C_6$ alkylaryl, group may be substituted with one or more substituents selected from nitro, chloro, fluoro, bromo, nitrile, sulfonic acid or salt of sulfonic acid, carboxy, oxo, carboxyalkyl, carboxyalkoxy, carboxylalkylamino, carboxyalkylthio, $C_{1-6}$-alkoxy, di $C_{1-40}$ alkyl phosphonate, $C_{1-40}$ alkyl phosphonate, phosphonic acid, amino, amino $C_{1-40}$-alkyl or amino di ($C_{1-40}$-alkyl). Examples include benzyl, phenylethyl and pyridylmethyl. In a $C_{1-8}$ alkylaryl group, the alkyl chain has from one to eight carbon atoms.

Compounds in which R and D are each independently hydrogen, X is either oxygen or a nitrogen with hydrogen attached or sulfur, and A and B are $OR^9$ where $R^9$ is a hydrogen, a $C_{1-6}$ alkyl or an optionally complex metal ion $M^{n+}/n$ wherein n is an integer from 1 to 8; and compounds in which R is hydrogen, X is either oxygen or nitrogen, A is a alklyaryl group, B is a known aryl or heteroaryl fluorophore and A and B are $OR^2$ where $R^2$ is hydrogen, a $C_{1-6}$ alkyl or an optionally complex metal ion $M^{n+}/n$ wherein n is an integer from 1 to 8; are especially preferred.

In a preferred embodiment of the first aspect, the compound is one or more selected from the group consisting of formula Ia, Ib, Ic, Id, Ie, Ih or Ik. More preferably, the compound is one or more selected from the group consisting of compound 1, 8, 8a, 10, 11, 15, 21, 23, 26 and 27.

The compound of the first aspect may be used in decreasing amyloid aggregates *in vitro* or *in vivo*. By this it is meant that aggregates of the amyloid peptide may be dispersed, or disaggregated, by the compound of the invention. Thus, the size, number and/or density of aggregates will be reduced.

A further embodiment of the first aspect provides the compounds of the invention for use in the treatment of a disease characterised by amyloid deposition. Such a disease may be Alzheimer's disease, type 2 diabetes, Huntington's disease, Parkinson's disease and others. Most particularly, the compounds of the invention may be used for treating Alzheimer's disease.

By amyloid deposition, it is meant a build up of aggregates of amyloid, also known as amyloid plaques. The compounds of the invention may treat such diseases by reducing the size, density and/or number of plaques in a subject with the disease.

A second aspect of the invention provides the use of a compound of general formula I in the manufacture of a medicament for use in the treatment of a disease characterised by amyloid deposition. Such a disease, preferably, is Alzheimer's disease.

In a preferred embodiment of the second aspect, the compound is selected from the group consisting of formula Ia, Ib, Ic, Id, Ie, and Ik. More preferably the compound is one or more selected from the group consisting of compounds 1 to 28 and 4a to 9a. Most preferably the compound is one or more selected from the group consisting of compounds 1, 8, 8a, 10, 11, 15, 21, 23, 26 and 27.

As a third aspect, the invention provides novel compounds of formula I. Specifically, such compounds are compounds 4, 5a, 6, 7a, 8, 8a, 14 and 10, as defined in table 1.

The present invention provides these novel compounds for use in medicine and in the manufacture of a medicament for use in the treatment of a disease characterised by amyloid deposition. Such a disease may be Alzheimer's disease. The compounds may be used in decreasing amyloid aggregates, *in vitro* or *in vivo*.

The invention also provides a composition comprising a compound of general formula I and the composition in the manufacture of a medicament for use in the treatment of a disease characterised by amyloid deposition.

Medicaments in accordance with the invention will usually be supplied as part of a sterile, pharmaceutical composition which will normally include a pharmaceutically acceptable carrier. This pharmaceutical composition may be in any suitable form, (depending upon the desired method of administering it to a subject).

It may be provided in unit dosage form, will generally be provided in a sealed container and may be provided as part of a kit. Such a kit would normally (although not necessarily) include instructions for use. It may include a plurality of said unit dosage forms.

The pharmaceutical composition may be adapted for administration by any appropriate route, for example by the oral (including buccal or sublingual), topical (including buccal, sublingual or transdermal), or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) route. Such compositions may be prepared by any method known in the art of pharmacy, for example by admixing the active ingredient with the carrier(s) or excipient(s) under sterile conditions.

Pharmaceutical compositions adapted for oral administration may be presented as discrete units such as capsules or tablets; as powders or granules; as solutions, syrups or suspensions (in aqueous or non-aqueous liquids; or as edible foams or whips; or as emulsions)

Suitable excipients for tablets or hard gelatine capsules include lactose, maize starch or derivatives thereof, stearic acid or salts thereof. Suitable excipients for use with soft gelatine capsules include for example vegetable oils, waxes, fats, semi-solid, or liquid polyols etc.

For the preparation of solutions and syrups, excipients which may be used include for example water, polyols and sugars. For the preparation of suspensions oils (e.g. vegetable oils) may be used to provide oil-in-water or water in oil suspensions.

Pharmaceutical compositions adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. For example, the active ingredient may be delivered from the patch by iontophoresis as generally described in *Pharmaceutical Research*, **3(6)**:318 (1986).

Pharmaceutical compositions adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils. For infections of the eye or other external tissues, for example mouth and skin, the compositions are preferably applied as a topical ointment or cream. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an oil-in-water cream base or a water-in-oil base.

Pharmaceutical compositions adapted for topical administration to the eye include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent.

Pharmaceutical compositions adapted for parenteral administration include aqueous and non-aqueous sterile injection solution which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation substantially isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. Excipients which may be used for injectable solutions include water, alcohols, polyols, glycerine and vegetable oils, for example. The compositions 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 the sterile liquid carried, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

The pharmaceutical compositions may contain preserving agents, solubilising agents, stabilising agents, wetting agents, emulsifiers, sweeteners, colourants, odourants, salts (substances of the present invention may themselves be provided in the form of a pharmaceutically acceptable salt), buffers, coating agents or antioxidants. They may also contain therapeutically active agents in addition to the substance of the present invention.

Dosages of the substances of the present invention can vary between wide limits, depending upon the disease or disorder to be treated, the condition of the individual to be treated, etc. and a veterinarian will ultimately determine appropriate dosages to be used. The dosage may be repeated as often as appropriate. If side effects develop the amount and/or frequency of the dosage can be reduced, in accordance with normal clinical practice.

All preferred features of each aspect apply to all other aspects *mutatis mutandis*.

The invention will now be described by way of the following non-limiting examples, with reference to the Figures, in which;

Fig. 1 Shows the disaggregation of β-amyloid peptide aggregates with Clioquinol;
Fig. 2 Shows the disaggregation of β-amyloid peptide aggregates with compound 15;
Fig. 3 Shows the disaggregation of β-amyloid peptide aggregates with compound 1;
Fig. 4 Shows the disaggregation of β-amyloid peptide aggregates with compound 11;
Fig. 5 Shows the disaggregation of β-amyloid peptide aggregates with compound mixture T;
Fig. 6 Shows L929 non-neural cell line viability with clioquinol to determine toxicity;
Fig. 7 Shows L929 non-neural cell line viability with compound 15 to determine toxicity. H4 cell survival and amyloid accumulation (expressed as the average value of 2-3 separate experiments) is shown for all the primary compounds at 1 μM;
Fig. 8 Shows human neuroblastoma cell line H4 cell viability with compound 11 using Neutral Red;

Fig. 9 Shows human neuroblastoma cell line H4 cell viability with compound 1 using Neutral Red;

Fig. 10 Shows human neuroblastoma cell line cell viability/survival and amyloid accumulation data for compound 1;

Fig. 11 Shows human neuroblastoma cell line cell viability/survival and amyloid accumulation data for compound 11;

Fig. 12 Shows human neuroblastoma cell line cell viability/survival and amyloid accumulation data for compound T;

Fig. 13 Shows human neuroblastoma cell line cell viability/survival and amyloid accumulation data for compound 21;

Fig. 14 Shows the intracellular distribution of amyloid in human neuroblastoma cell line H4 cells after incubation with compounds 11, 27, 21, T and clioquinol;

Fig. 15 Shows natural fluorescence of compound 27 and amyloid labelling, in the absence of compound 27;

Fig. 16 Shows effects of compounds 1, 10, 11, T, 21, 22 and clioquinol on primary sensory neurons;

Fig. 17 Shows images of primary sensory neurons after exposure to control, compound 1 and compound 11;

Fig. 18 Shows autometallographic images of brain parenchyma after exposure to compounds 11, T, 21, 1 and clioquinol;

Fig. 19 Images of brain tissue after post treatment with compounds 11, T, 21 and clioquinol.

**Examples:**

The compounds promote disaggregation of zinc promoted accumulations of amyloidic deposits *in vitro* and reduce amyloidic load in H4 cellular models. They show no overt cellular or *in vitro* toxicity and reduce the plaque load in APP/Tau transgenic mice. The parenchymal zinc load is reduced in treated CD1 albino male mice. The body of evidence supports the claims that these compounds may be effective therapeutic agents in neurodegenerative disease states with amyloid or zinc based pathologies.

**Compound Synthesis:**

The following compounds were synthesized and stored in 1 mM stock solutions in DMSO or ethanol, as previously described in WO2004/101579.

*Diethyl 7-oxomethylphosphonate-4-methylcoumarin* (compound 1)

*Ethyl 7-oxymethylphosphonic acid-4-methylcoumarin* (compound 2)

*4-Methyl-7-oxymethylphosphonic acid coumarin* (compound 3)

*Diethyl 5-indolyloxy methylphosphonate* (compound 11)
The corresponding phosphonic acid ($\delta_P$ (109.7 MHz, DMSO) 16.2) is obtained as described.

*Diethyl 4-(2-benzoxazolyl) phenoxymethylphosphonate* (compound 12)

*Diethyl 2-(2-benzoxazolyl) phenoxymethylphosphonate* (compound 13)
The corresponding phosphonic acid $\delta_P$ (109.3 MHz, DMSO) 15.06 is obtained as described.

*Diethyl 8-quinolyloxy methylphosphonate* (compound 15)

*Disodium 8-quinolyloxy methylphosphonate* (compound 16)

*Diethyl -2-carbazolyloxymethylphosphonate* (compound 17)

*2-carbazolyloxymethyl phosphonic acid* (compound 18)

*Diethyl N-carbazolylmethyl phosphonate* (compound 19)

*Disodium N-carbazolylmethylphosphonate* (compound 20)

*Diethyl (9-anthracyl)–N-butylaminemethylphosphonate* (compound 21)

*Diethyl N-(tert-butoxycarbonyl)-L-tyrosyl methyl ester methylphosphonate* (compound 22)

*Diethyl (9-anthracyl)–N-benzylaminemethylphosphonate* (compound 23)

*Diethyl (9-anthracyl)–N-butylaminemethylphosphonate* (compound 24)

*Diethyl 4-acetamidophenoxy methylphosphonate* (compound 25)

*Diethyl N-acridonyloxymethyl phosphonate* (compound 26)

Compounds **27** and **28** may be obtained in the manner described for 26 from fluorescein and hydroxy pyrazole reagents.

**Novel compounds**

**Example 1** (compound 4)

*Diethyl 2-oxo-benzoxazolyl-3-methylphosphonic acid diethyl ester*

A solution of 2-hydroxy-benzoxazole (2.7 g, 20 mmol) in dimethyl sulfoxide (15 ml) was added to sodium hydride (60%, 0.83 g, 21 mmol, washed with hexane) and left to stir for 1h under nitrogen after the initial reaction had subsided. Diethyl 4-chlorophenylsulfonyloxy methylphosphonate (7.2 g, 21 mmol) dissolved in dimethyl sulfoxide (30 ml) was added and the solution was left to stir for 96 h at room temperature. The reaction mixture was poured into water (50 ml), and then extracted with ethyl acetate (2 x 75 ml). The combined organic extract was washed with brine dried over magnesium sulfate and concentrated under reduced pressure. The resultant oil was passed down a silica gel column using pet.ether: ethyl acetate (7:3) followed by 5% methanol in ethyl acetate: pet ether (8:2) an oil (4.1 g);

High Resolution Mass Spec. Found: 286.0837, $C_{12}H_{16}NO_5P$ $(M+H)^+$ requires 286.0839, $\delta_H$ (270 MHz, $CDCl_3$) 7.15 (4H, m), 4.17 (2H, d, *J* 10 Hz), 4.15 (4H, dq, *J* 6.8 Hz), 1.3 (6H, t, *J* 6 Hz), $\delta_P$ (109.3 MHz, $CDCl_3$) 18.9

Infra red $\nu(cm^{-1})$ 1770 (s)

The corresponding phosphonic acid ($\delta_P$ 14.8) is obtained as described in WO2004/101579.

**Example 2** (compound 5a)

*Diethyl 2-benzoxazolyl thiomethylphosphonate*

A solution of 2-mercaptobenzoxazole (3.02 g, 20 mmol) in dimethyl sulfoxide (15 ml) was added to sodium hydride (60%, 0.83 g, 21 mmol, washed with hexane) and left to stir for 1h under nitrogen after the initial reaction had subsided. Diethyl 4-chlorophenylsulfonyloxy methylphosphonate (7.2 g, 21 mmol) dissolved in dimethyl sulfoxide (30 ml) was added and the solution was left to stir for 96 h at room temperature. The reaction mixture was poured into water (50 ml), and then extracted with ethyl acetate (2 x 75 ml). The combined organic extract was washed with brine dried over magnesium sulfate and concentrated under reduced pressure. The resultant oil was passed down a silica gel column using first pet.ether: ethyl acetate (7:3) and then with 5% methanol in ethyl acetate: pet ether (8:2). The combined fractions from the second elution were subjected to further column chromatography using pet.ether: ethyl acetate 8:2 to give diethyl (2-benzoxazolyl) thiomethylphosphonate (2.5 g, 40 %) as an oil. High Resolution Mass Spec. Found: 302.0608, $C_{12}H_{16}NO_4PS$ $M^+$+H requires 302.0610, $\delta_H$ (270 MHz, $CDCl_3$) 7.58 (1H, d, *J* 10Hz), 7.44 (1H, d, *J* 10 Hz), 7.3 (2H, m,), 4.12 (4H, dq, *J* 6.8 Hz), 3.65 (2H, d, *J* 13 Hz),1.3 (6H, dt, *J* 6 Hz), $\delta_P$ (109.3 MHz, $CDCl_3$) 21.44,

The corresponding phosphonic acid ($\delta_P$ 19.31) is obtained as described in WO2004/101579.

**Example 3** (compound 6)

*Diethyl 2-oxo-benzthiazolyl-3-methylphosphonic acid diethyl ester*

A solution of 2-hydroxybenzthiazole (3.0 g, 20 mmol) in dimethyl sulfoxide (15 ml) was added to sodium hydride (60%, 0.83 g, 21 mmol, washed with hexane) and left to stir for 1h under nitrogen after the initial reaction had subsided. Diethyl 4-chlorophenylsulfonyloxy methylphosphonate (7.2 g, 21 mmol) dissolved in dimethyl sulfoxide (30 ml) was added and the yellow solution was left to stir for 96 h at room temperature. The reaction mixture was poured into water (50 ml), and then extracted

with ethyl acetate (2 x 75 ml). The combined organic extract was washed with brine dried over magnesium sulfate and concentrated under reduced pressure. The resultant oil was passed down a silica gel column using first pet.ether: ethyl acetate (7:3) to elute traces of starting materials and then ethyl acetate:pet.ether (9:1) and finally 10% MeOH in ethyl acetate to give diethyl 2-benzthiazolyl oxymethylphosphonate (2.5 g, 42 %) as an oil.

High Resolution Mass Spec. Found: 302.0610, $C_{12}H_{16}NO_4PS$ $(M+H)^+$ requires 302.0607, $\delta_H$ (270 MHz, $CDCl_3$) 7.7 (1H, dd), 7.38 (1H, d), 7.25 (1H, m), 7.13 (1H, t), 4.34 (2H, d, *J* 11 Hz), 4.15 (4H, m), 1.25 (6H, t, *J* 7 Hz), $\delta_P$ (109.3 MHz, $CDCl_3$) 19.02

Infra red $\nu(cm^{-1})$ 1685 (s)

The corresponding phosphonic acid $\delta_P$ (109.3 MHz, DMSO)14.87 is obtained as described in WO2004/101579.

**Example 4** (compound 7a)

*Diethyl 2-benzthiazolyl thiomethylphosphonate*

A solution of 2-mercaptobenzthiazole (3.35 g, 20 mmol) in dimethyl sulfoxide (15 ml) was added to sodium hydride (60%, 0.83 g, 21 mmol, washed with hexane) and left to stir for 1h under nitrogen after the initial reaction had subsided. Diethyl 4-chlorophenylsulfonyloxy methylphosphonate (7.2 g, 21 mmol) dissolved in dimethyl sulfoxide (30 ml) was added and the solution was left to stir for 96 h at room temperature. The reaction mixture was poured into water (50 ml), and then extracted with ethyl acetate (2 x 75 ml). The combined organic extract was washed with brine dried over magnesium sulfate and concentrated under reduced pressure. The resultant oil was passed down a silica gel column using first pet.ether: ethyl acetate (6:4) to elute traces of starting materials and then with 10% methanol in ethyl acetate (8:2) to elute products to give diethyl 2-benzthiazolyl thiomethylphosphonate (4.3 g, 68 %) as an oil.

High Resolution Mass Spec. Found: 318.0381, $C_{12}H_{16}NO_3PS_2$ $(M+H)^+$ requires 318.0382, $\delta_H$ (270 MHz, $CDCl_3$) 7.85 (1H, d, *J* 7.5Hz), 7.75 (1H, d, *J* 7.5 Hz),7.4

(1H, t, *J* 7.5 Hz), 7.29 (1H, t, *J* 7.5 Hz), 4.15 (4H, dq, *J* 6.4 Hz,), 3.79 (2H, d, *J* 16 Hz),1.28 (6H, t, *J* 7.5 Hz), $\delta_P$ (109.3 MHz, $CDCl_3$) 22.13.

*2-benzthiazolyl thiomethylphosphonic acid*

To a stirred solution of diethyl 2-benzthiazolyl thiomethylphosphonate (0.2 g, 0.63 mmol) dissolved in dry dichloromethane (3.3 ml) under an atmosphere of nitrogen was added trimethylsilyl iodide (0.36 ml). The red solution was stirred for 2h then methanol (5.1 ml) was added. After 2h the solvent was removed under reduced pressure and then water (20 ml) was added to the residue. The mixture was concentrated under reduced pressure. Water (2 ml) was added and the mixture was concentrated under reduced pressure. This was repeated four times. The residue was washed finally with water then acetone to give 2-benzthiazolyl thiomethylphosphonic acid (0.06 g, 36%), , $\delta_P$(109.3 MHz, DMSO) 15.49; $\delta_H$ (270 MHz, DMSO) 8.05 (1H, d, *J* 7.5Hz), 7.75 (1H, d, *J* 7.5 Hz),7.5 (1H, t, *J* 7.5 Hz), 7.35 (1H, t, *J* 7.5 Hz), 3.6 (2H, d, *J* 16 Hz)

**Example 5**

*Phosphonomethylation of 2-hydroxybenzimidazole*

A solution of 2-hydroxybenzimidazole (2.68 g, 20 mmol) in dimethyl sulfoxide (15 ml) was added to sodium hydride (60%, 0.83 g, 21 mmol, washed with hexane) and left to stir for 1h under nitrogen after the initial reaction had subsided. Diethyl 4-chlorophenylsulfonyloxy methylphosphonate (7.2 g, 21 mmol) dissolved in dimethyl sulfoxide (30 ml) was added and the solution was left to stir for 96 h at room temperature. The reaction mixture was poured into water (50 ml), and then extracted with ethyl acetate (2 x 75 ml). The combined organic extract was washed with brine dried over magnesium sulfate and concentrated under reduced pressure. The resultant oil was passed down a silica gel column using first pet.ether: ethyl acetate (1:1), then: ethyl acetate:pet.ether (9:1) and finally ethyl acetate:pet.ether (9:1)with 5% methanol. The compounds 8, 8a and 10 below were obtained:

*2-oxo-benzimidazolyl-3-dimethylphosphonic acid diethyl ester* (compound 8)

Mass Spec (CI). Found: 284.9, $C_{12}H_{1H}O_4N_2P$ $(M+H)^+$ requires 285.2; $\delta_H$ (270 MHz, $CDCl_3$) 7.4 (4H, m, $J_1$ 12Hz, $J_2$ 6Hz, $J_3$ 4Hz,), 4.28 (2H, d, $J$ 10.8 Hz), 4.15 (4H, qq, $J$ 5 Hz,), 1.25(6H, t, $J$ 8Hz); $\delta_P$ (109.3 MHz, $CDCl_3$) 20.4. Structure confirmed by single crystal XRD.

*2-oxo-benzimidazolyl-3,3"-bis-methylphosphonic acid diethyl ester* (compound 10)

High Resolution Mass Spec. Found: 435.1446, $C_{17}H_{29}O_7N_2P_2$ $M^+$+H requires 435.1445; $\delta_H$ (270 MHz, $CDCl_3$) 7.4 (4H, m, $J_1$ 12Hz, $J_2$ 6Hz, $J_3$ 4Hz,), 4.26 (4H, d, $J$ 10.5 Hz), 4.15 (8H, qq, $J$ 5 Hz,), 1.25(12H, t, $J$ 8Hz); $\delta_P$ (109.3 MHz, $CDCl_3$) 20.2

*Diethyl 2-benzimidazolyloxymethylphosphonate* (compound 8a); $\delta_H$ (270 MHz, $CDCl_3$) 7.4 (4H, m, $J_1$ 12Hz, $J_2$ 6Hz, $J_3$ 4Hz,), 4.12 (2H, d, $J$ 8 Hz), 3.9 (4H, dq, $J$ 5 Hz,), 1.15(6H, t, $J$ 8Hz); $\delta_P$ (109.3 MHz, $CDCl_3$) 20.6; Isolated from a reaction similar to that above except that the 2-hydroxybenzimidazole was treated with potassium carbonate instead of sodium hydride.

**Example 6** (compound 14)

*Diethyl 2-(2-benzthiazolyl)phenoxymethylphosphonate*

To sodium hydride (60%, 0.83 g, 21 mmol, washed with dry hexane) was added dropwise, with stirring under nitrogen, 2-(2-benzthiazolyl) phenol (4.54 g, 20 mmol) in dimethyl sulfoxide (15 ml). After a further 1 h stirring at at 80 °C diethyl 4-chlorophenylsulfonyloxy methylphosphonate (7.2 g, 21 mmol) dissolved in dimethyl sulfoxide (30 ml) was added and the solution was stirred for a further 96 h at 80 °C. The reaction mixture was treated with water (50 ml) and then extracted with ethyl acetate (3 x 50 ml). The combined organic extracts were washed with brine and then dried. Evaporation of the solvent left an oily solid that on elution from silica, first with pet. ether–ethyl acetate (4:6) to remove traces of starting materials, then pet. ether–ethyl acetate (3:7) and finally ethyl acetate gave diethyl 2-(2-benzoxazolyl) phenoxymethylphosphonate as an oil $\delta_H$ (270 MHz, DMSO) 8.42 (1H, d, $J$ 7.7Hz), 8.11 (1H, d, $J$ 7.7Hz), 8.04 (1H, d, $J$ 7.7Hz), 7.53 (2H, m), 7.32 (2H, m), 7.42 (2H,

m), 7.21(1H, t, *J* 7.7Hz), 4.73 (2H, d, *J* 10.5 Hz), 4.18 (4H, dq, $J_1$ 6.7Hz) and 1.28 (6H, t, *J* 7Hz), $\delta_P$ (109.7 MHz, DMSO) 19.72

$\delta_H$ (270 MHz, $CDCl_3$) 8.52 (1H, d, *J* 7.7Hz), 8.1 (1H, d, *J* 7.7Hz), 8.04 (1H, d, *J* 7.7Hz), 7.48 (2H, q, *J* 7.7Hz), 7.36 (H, t, *J* 7.7Hz), 7.14 (1H, t, *J* 7.7Hz), 4.5 (2H, d, *J* 10 Hz), 4.25 (4H, dq, $J_1$ 7Hz) and 1.35 (6H, t, *J* 7Hz), $\delta_P$ (109.7 MHz, $CDCl_3$) 18.9

High Resolution Mass Spec. Found: 378.0925, $C_{18}H_{20}NO_4P$ $(M + H)^+$ requires 378.0923,

*2-(2-benzthiazolyl) phenoxymethylphosphonic acid*

To a stirred solution of diethyl 2-(2-benzthiazolyl) phenoxymethylphosphonate (0.2 g, 0.53 mmol) dissolved in dry dichloromethane (2.7 ml) under an atmosphere of nitrogen was added trimethylsilyl iodide (0.31 ml). The red solution was stirred for 2h then methanol (4.3 ml) was added. After 2h the solvent was removed under reduced pressure and then water (3.5 ml) was added to the residue. The mixture was concentrated under reduced pressure. Water (2 ml) was added and the mixture was concentrated under reduced pressure. This was repeated four times. The residue was washed finally with water then acetone to give 2-(2-benzthiazolyl) phenoxymethylphosphonic acid (0.13 g, 77%) $\delta_H$ (270 MHz, DMSO) 8.43 (1H, d, *J* 8 Hz), 8.04 (2H, m), 7.53 (2H, m), 7.44 (2H, m), 7.21(1H, t, *J* 8Hz), 4.4 (2H, d, *J* 12 Hz), $\delta_P$ (109.7 MHz, DMSO) 14.4

**Biological Activity of compounds according to the invention**

**Compound Testing Regimes**

**Example 7**

β-amyloid peptide was aggregated *in vitro* in the presence of Zn, and the formation of aggregates was assessed in the presence or absence of the substituted phosphonates. The method used to assess effects on aggregation was based on a turbidimetry assay, as previously described in literature (Klug et al., 2003; Qahwash et al., 2003). Aβ1-40 (25 μM) was incubated for 48 h in the absence or presence of zinc (100 μM). Substituted phosphonates (1 μM) were added at the beginning of the incubation. The

tests were conducted in multiwell culture plates, and the plates gently shaken during incubation. After 48 h, absorbance was measured at 405 nm using an ELISA plate reader. Clioquinol (1 μM) was used as a reference compound.

Compounds proposed for therapeutic treatment of amyloidopathy related disease states such as AD possess the ability the dissagregate zinc or copper aggregated amyloid in vitro as a first indicator of therapeutic potential. Using a turbidimetry-based approach (Figures 1-5), and clioquinol (1 μM) as the reference compound substantial disaggregation, presented as average % inhibition of aggregation in the presence of Zn, was seen for the substituted phosphonates shown in Figures 1-5 as well as several other phosphonates shown in the Table in the form of their di sodium phosphonate derivatives instead of ethyl ester derivatives.

**Example 8**

A first assessment of the potential toxicity of the substituted phosphonates was carried out in a non-neuronal cell line, using a tetrazolium salt (MTT) colorimetric assay, based on the method described by Mossman (1983), which gives a reflection of mitochondrial activity and cell integrity. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form dark blue formazan crystals which cannot cross cell membranes. The result is that healthy cells will accumulate more of the blue formazan crystals. Solubilisation of the cells by the addition of a detergent results in the liberation of the crystals. The number of surviving cells is directly proportional to the level of the formazan product created. Using the L929 immortalised fibroblast cell line, cells were seeded into a multiwell culture plate and were exposed to increasing concentrations of metal-chelating compounds (0.3 nM to 1 μM) for 30 minutes, 4 hours and 24 hours. They were then incubated for 2 h after addition of MTT (1 mg/ml) and the absorbance was read at 570 nm. Control wells were incubated either in the presence of culture medium, or in the presence of culture medium to which ethanol or DMSO was added, at the highest concentration used in the experiment (corresponding to the 1 μM concentration of substituted phosphonates). The toxicity

of the reference compound clioquinol (0.3 nM to 1 μM) was assessed under the same conditions, for comparison.

Compounds proposed for *in vivo* therapeutic treatments should not display overt toxicity.

A first assessment of toxicity of the substituted phosphonates was carried out in a non-neuronal cell line, using a tetrazolium salt (MTT) colorimetric assay, based on the method described by Mossman (1983), which gives a reflection of mitochondrial activity and cell integrity (Figs. 6 and 7). The toxicity of the reference compound clioquinol (0.3 nM to 1 μM) was assessed under the same conditions, for comparison.

The analysis of cell viability using the MTT assay, shows that under the conditions used, neither clioquinol nor any of the substituted phosphonates screened showed any toxicity, as reflected in cell viability after up to 24 h exposure to compounds over a wide range of concentrations.

**Example 9**

H4 cells (human neuroblastoma cell line) were used to test the ability of the substituted phosphonates to disrupt amyloid accumulation. Parallel 96 well plates were also set up with the H4 cells to assess the effect of the compounds on cell survival. Clioquinol was used as the reference compound. All tested compounds were made up in the growth media at the following concentrations: 1 μM, 2 μM and 10 μM. The compounds were initially tested over 48 hours either with a single dose of the compound or with 2 doses. This was extended to a 5 day period again, either with a single dose of the compound or with repeated daily doses of the compounds.

***Cell survival***

For the cell survival, two cell viability/toxicity assays were used: the assay based on MTT (described above), and an assay based on the use of neutral red (NR).

The MTT assay was carried out at the end of the culture periods using the standard protocol described above for the fibroblast cell line, and the absorbance read on a microplate reader.

The Neutral Red (NR) cytotoxicity assay procedure also measures cell survival/viability. It is based on the ability of cells to incorporate and bind NR, a supravital dye. Alterations of the cell surface or of the sensitive lysosomal membrane lead to lysosomal fragility, which results in the cells becoming unable to bind the NR dye. The NR assay was compared to the MTT cytotoxity assay. Both assays yielded comparable data but as the optical density values with the NR assay were about twice that obtained with the MTT assay, this provided us with a more sensitive test and required fewer cells for analysis. It also allowed to check that the compounds did not have a direct effect on the lysosomes or interfer with mitochondrial enzymes which would have resulted in greater toxicity in the NR *vs.* the MTT assays, respectively.

Cells were grown and once they reached the desired density, they were rinsed with phosphate-buffered saline and 200µl of NR-containing medium (40 µg/ml) was added to each well and cells were incubated with the dye for 3 hrs. The NR medium was removed and the cells washed with 200 µl phosphate-buffered saline (PBS). 200 µl of an acetic acid/ethanol mixture (1ml glacial acetic acid in 100 ml 50% ethanol) was used to desorb the NR for 15 min at room temperature. The plate was then placed on a shaker at 60 rpm for 30 min to form a homogenous solution. The absorbance was measured at 540 nm in a microplate reader, using the blank as a reference.

***Amyloid accumulation***

For the amyloid accumulation the cells were fixed with ice cold methanol for 3 minutes then washed with PBS 3 times 3 minutes. The 6E10 antibody (Abcam 1:5,000) was added and the cells incubated for 48 hours at 4°C. The primary antibody was removed and the cells washed with PBS 3 times 3 minutes, and the secondary antibody (anti-mouse HRP 1:4,000) was added for 2 hours. This was removed and the cells washed 3 times 3 minutes. They were then incubated with the OPD substrate (prepared according to the manufacturer's instructions) for 30 min, before reading the absorbance.

All 5 day experiments were repeated 3 times to confirm results, and all 48 hour studies were repeated twice.

In a further assessment of the compounds for toxicity and ability to modulate amyloidopathic pathways *in vitro* they were screened using H4 cells (human neuroblastoma cell line) (Figures 8-15 and Tables 1a and 2a). For the cell survival, two cell viability/toxicity assays were used: the assay based on MTT (described above), and an assay based on the use of NR. Clioquinol was used as the reference compound.

A summary of the H4 cell survival and amyloid accumulation (expressed as the average value of 2-3 separate experiments) is shown for all the primary compounds at 1 μM in Tables 1a and 2a, with the control cells grown in growth media (with the corresponding trace amounts of DMSO or ethanol added) representing the 100% value. The cell survival of the H4 cells assayed with either MTT or NR were comparable, and the graphs show the NR assay results only.

Clioquinol at 1 μM with a single dose or a repeated dose over the 5 day period has no toxicity, and decreases the accumulation of amyloid (Table 1a and Table 2a). In contrast, clioquinol leads to decreased cell survival at the 2 μM concentration, and there is extensive death of the H4 cells at the 10 μM concentration, either with a single dose or repeated doses of the drug.

As shown in Table 1a, the substituted phosphonates appear to be devoid of toxicity upon single exposure for 5 days to 1 μM. Repeated doses reduce in some cases cell viability (maximum 28% reduction seen with the compound 20). The analysis of cell viability using the NR assay gave comparable results to those obtained with the MTT assay, as exemplified for the compound 11. The exposure to 11 for 5 days (single exposure) did not affect significantly cell survival, whereas after 5 days and repeated dose, the compound reduced cell viability to approximately 60-70% of the control value. Similarly, 1 did not affect viability after single exposure, but reduced it to 70-80% after 5 days repeated dose.

As illustrated in Table 2a, the effect of the substituted phosphonates on amyloid accumulation is striking, with a majority of the compounds outperforming the reference compound clioquinol, in terms of effects on amyloid accumulation, at the lowest concentration tested (1 μM). For example, for a 5 day single dose, the compounds 1 and 21 reduce the amyloid accumulation to 37% of the value of controls, whereas the reference compound clioquinol reduced the accumulation to 64% of the value in controls.

Table 1a Summary of cell survival (%controls)

Table 2a Summary of amyloid reduction (% controls)

H4 cell survival and amyloid accumulation (expressed as the average value of 2-3 separate experiments) is shown for all the primary compounds at 1 μm

| Table 1a Summary of cell survival (% controls) | | |
|---|---|---|
| | 5 day single dose | 5 day repeated doses |
| **Control** | **100** | **100** |
| **clio 1 μM** | **99** | **96** |
| **1 1 μM** | **104** | **78** |
| **5** 1 μM | 100 | 113 |
| **7** 1 μM | 116 | 86 |
| **T 1 μM** | **87** | **97** |
| **11 1 μM** | **90** | **76** |
| 14 1 μM | 100 | 110 |
| 15 1 μM | 89 | 95 |
| 17 1 μM | 97 | 102 |
| 20 1 μM | 88 | 72 |
| 22 1 μM | 99 | 80 |
| **28** 1 μM | 107 | 85 |
| **24** 1 μM | 105 | 135 |
| **25** 1 μM | 98 | 97 |
| 27 1 μM | 114 | 113 |
| **21 1 μM** | **104** | **76** |
| 26 1 μM | 116 | 86 |

| Table 2a Summary of amyloid reduction (% controls) | | |
|---|---|---|
| | 5 day single dose | 5 day repeated doses |
| **control** | **100** | **100** |
| **clio 1 μM** | **64** | **76** |
| **1 1 μM** | **37** | **19** |
| **5** 1 μM | 38 | 74 |
| **7** 1 μM | 92 | 63 |
| **T 1 μM** | **47** | **31** |
| **11 1 μM** | **33** | **16** |
| 14 1 μM | 100 | 83 |
| 15 1 μM | 36 | 71 |
| 17 1 μM | 37 | 62 |
| 20 1 μM | 11 | 66 |
| 22 1 μM | 10 | 17 |
| **28** 1 μM | 58 | 82 |
| **24** 1 μM | 48 | 15 |
| **25** 1 μM | 24 | 62 |
| 27 1 μM | 30 | 61 |
| **21 1 μM** | **37** | **35** |
| **26** 1 μM | 57 | 52 |

**Example 10**

*Intracellular distribution of amyloid after incubation*

In separate experiments we also made observations with some of the compounds, on the intracellular distribution of amyloid in H4 cells. This was carried out by using the primary 6E10 antibody, followed by incubation of the cells with fluorescently-tagged secondary antibodies.

Under basal conditions, the intracellular labelling of amyloid in the H4 cells showed accumulations of peptides within the control cells throughout the cytoplasm of the cells. The addition of 1 μM clioquinol for 5 days reduced this intracellular accumulation in some of the cells, but brightly labelled areas could still be found. After addition of 1 μM of the compounds 11 and T, T = compounds 8 and 10 in 9:1 mole ratio, the number of cells with these brightly labelled amyloid accumulations was reduced (compared to cliquinol) but not entirely abolished. After incubation with 21, there were virtually no cells with these amyloid bright intracellular accumulations. The incubation with 27 (1 μM) also led to a reduction in the amyloid labelling in the H4 cells but there were still cells with bright labelling.

The figure shows H4 cells labelled for amyloid: control, clioquinol, 11, T, 27, and 21 treatment groups. Amyloid labelling was carried out after 5 days incubation of the cells with 1 μM compound (single dose).

A further indicator of potential therapeutic effect in amyloidiopathic pathways is determined by the effect on intracellular amyloid production.

In separate experiments we also made observations with some of the compounds, on the intracellular distribution of amyloid in H4 cells (Figure 15). This was carried out by using the primary 6E10 antibody, followed by incubation of the cells with fluorescently-tagged secondary antibodies. Under basal conditions, the intracellular

labelling of amyloid in the H4 cells showed accumulations of peptides within the control cells throughout the cytoplasm of the cells. The addition of 1 μM clioquinol for 5 days reduced this intracellular accumulation in some of the cells, but brightly labelled areas could still be found. After addition of 1 μM of the compounds 11 and T, the number of cells with these brightly labelled amyloid accumulations was reduced (compared to clioquinol). After incubation with 21, there were virtually no cells with these amyloid bright intracellular accumulations. The incubation with 27 (1 μM) also led to a reduction in the amyloid labelling in the H4 cells. The figure 15 shows H4 cells labelled for amyloid:control, clioquinol, 11, T, 27, and 21 treatment groups. Amyloid labelling was carried out after 5 days incubation of the cells with 1 μM compound (single dose). 11, T, 26, 21. The compound 27 is a fluorescein compound and we were able to detect it in cells using its natural fluorescence. These data show that substituted phosphonates reduced the accumulation of amyloid in the H4 cell line, without marked reduction in cell survival. Furthermore, there was also a reduction in the extracellular accumulation of amyloid after incubation with some substituted phosphonates which suggests that these compounds may act both extra- and intracellularly to reduce the amyloid accumulation. Many of the substituted phosphonates described herein contain a fluorogenic component and using 27 as example this compound was visualised within the cell located proximal to the labelled amyloid. These data show that the natural fluorescence of the substituted phosphonates provides a means of visualising the compounds as they target cellular and *in vitro* acellular amyloidic deposits.

**Example 11**

Cultures of dorsal root ganglia neurones from adult Wistar rats were incubated with clioquinol and with selected substituted phosphonates (compounds 11, T, 21, 1) at 1 μM for 24 hours and 4 days, to assess the toxicity of the compounds. Briefly, the method used was as follows:

Adult male Wistar rats where sacrificed by $CO_2$ inhalation and the dorsal root ganglia from all segmental levels were removed and dissociated into cells. The total number

of cells was counted using a haemocytometer and 500 cells plated onto pre-coated 8 well chambered slides. The cells were left to adhere for at least 6 hours before the addition of the test substituted phosphonates compounds or clioquinol to the media. Cells were grown for 1 day or 4 days and either had a single dose of the test compounds, or the media and compounds were changed every day over the 4 day period. Compounds were added at 1 μM and 10 μM concentrations, with duplicates of each concentration and each experiment repeated 4 times.

After 4 days the cells were fixed, then washed and incubated with primary antibody (anti-mouse beta tubulin III, Sigma, 1:1,000) for 24 hours at room temperature. The primary antibody was removed, the cells washed and then incubated with secondary antibody (donkey anti-mouse FITC labeled (Jackson 1:400)) for at least 2 hours at room temperature. The secondary antibody was removed, the cells washed and the slides were mounted with DABCO PBS:glycerol as an antifade. Analysis was done by counting all beta tubulin III positive cells in each well. Values were determined in triplicate and experiments were repeated 4 times (twice in case of 22).

Lack of toxicity to neuronal cells is a prerequisite for therapeutics targeting neurodegenerative disease states such as AD so the effects of the substituted phosphonates on primary sensory neuron cultures were assessed for toxicity in relation to the adult nervous tissue (Figures 16 and 17).

A single dose of clioquinol or the substituted phosphonate compounds (1 or 10 microMolar) was given at the start of the culture period and the neurons were incubated for 1 day, or the daily dose was repeated and the cells were maintained for 4 days. Viability was considerably better for the substituted phosphonate compounds than for the clioquinol reference compound.

**Example 12**

CD1 albino male mice (18-20 g, Charles River, UK) were treated for 4 days with the following compound: clioquinol 30 mg/kg p.o., clioquinol 10 mg/kg i.p., and compounds 11, T, 21, 1 at 10 mg/kg, i.p. (or the vehicle used to dissolve the drugs for

administration p.o.or i.p). On the 4th day of administration of the compounds or of the respective vehicle, mice received 1 hour before clioquinol or the substituted phosphonates an i.p. injection of sodium selenite 10 mg/kg. All animals were killed by cervical dislocation 2.5 hours after the administration of sodium selenite. The brain was frozen on dry ice, and then 15 μm sections were cut and stained to reveal the distribution of zinc using an autometallographic technique based of exposure of the tissue to a silver reagent enhancement kit (Aurion) (Wang et al, 2001). Some sections were counterstained with toluidine blue.

For therapeutics targeting neurodegenerative disease states such as AD prerequisites include lack of any overt toxicity, ability to access the CNS (central nervous system) and to provide neuro protection as evidenced for example in AD by modulating the formation of zinc containing amyloid deposits. In a first aspect, the assessment of the effect of the systemic administration of the substituted phosphonates on zinc distribution in the CNS (which is a reflection of the penetration of the blood-brain barrier, BBB, by the compounds) and a preliminary assessment of systemic toxicity after semi-chronic administration in mice was undertaken (Figure 18). All compounds tested were devoid of overt systemic toxicity using this administration paradigm, and no behavioural abnormalities were noted in animals receiving the new compounds. The autometallographic technique revealed the presence of an intense zinc signal in the brain of control animals, after administration of vehicle i.p. or vehicle p.o. (see images Figure 18). The administration of benchmark clioquinol or substituted phosphonates led to variable degrees of decrease in the zinc staining in the central nervous system. Marked decreases were seen in the zinc signal after administration of clioquinol 10 mg/kg, i.p. Decreases in the zinc signal were seen after clioquinol 30 mg/kg p.o. or 11, T, 21, 1 at 10 mg/kg, i.p. The decreased zinc staining signal is strong evidence for the passage of cliquinol (i.p, or p.o.) and of the substituted phosphonates compounds across the BBB, and their ability to chelate zinc in this environment. The systemic i.p. administration of substituted phosphonates leads to decrease in the signal that reflects central zincergic pathways. This supports the bioavailability of the compounds and their ability to reduce zinc in the brain parenchyma.

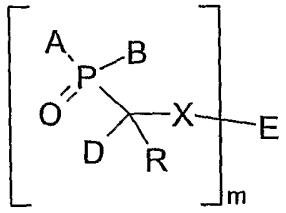
**Example 13**

Transgenic mice (APP and APP/PTau) and wild-type mice were treated for 4 weeks with the following compounds : clioquinol 30 mg/kg p.o., clioquinol 10 mg/kg i.p., and compounds 11, T, 21, at 10 mg/kg, i.p. (or the vehicle used to dissolve the drugs for administration p.o.or i.p). 24 hours after the last drug administration, animals were killed and the brain and heart, spleen, kidney and testis dissected out. One of the brain hemispheres was frozen on dry ice, and the other hemisphere was placed in 4% paraformaldehyde. Sections of the latter were cut and stained to reveal the distribution of amyloid plaques using the Campbell-Switzer silver staining method (analysis of the tissue courtesy of NSA Laboratories, USA).

For therapeutic targeting of amyloidic pathways in neurodegenerative disease states such as AD an effect on plaque load in treated mouse models may be an indicator of therapeutic effect. In this context chronic administration of the new substituted phosphonates on β-amyloid plaques in two mouse models of Alzheimer's disease, the APP and APP/Tau transgenic mice, was investigated (Figures 19). The chronic treatment did not reveal any overt signs of systemic toxicity or behavioural alterations in the treated animals, throughout the treatment, confirming thus the safety of the selected substituted phosphonates compounds at the dose chosen and using this regime of administration. In the APP transgenic mouse, there were significant amyloid plaques (large aggregates and also diffuse, punctate deposits) in the animals treated with vehicle only (p.o. or i.p.). Treatment with clioquinol led to apparent decreased plaque load, and especially a reduced load of large aggregates and a similar pattern was seen in the tissue from animals treated with the compound T while compounds 11 and 21 also reduced the plaque load.

Claims

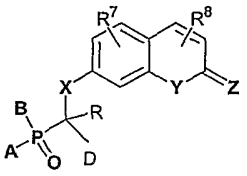
1. A compound of general formula I for use in medicine;



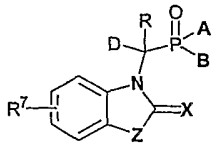

I

wherein R is hydrogen or a substituted or unsubstituted linear or branched $C_{1-40}$ alkyl, aryl or heteroaryl; E is a subsistuted or unsubstituted linear or branched $C_{1-40}$ alkyl, aryl, heteroaryl, or hydrogen; X is O, S or $NR^1$, or when E is aryl or heteroaryl, X can be N integral to the aryl or heteroaryl ring; wherein $R^1$ is a hydrogen, a linear or branched $C_{1-40}$ alkyl, $C_{2-40}$ alkenyl, or $C_{2-40}$ alkynyl, or aryl, a heteroaryl or $C_{1-40}$ alkylaryl or alkylheteroaryl; A and B are each independently $OR^2$, $SR^2$, $NR^3$, $R^4$ where $R^2$, $R^3$ and $R^4$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl, $C_{2-40}$ alkenyl, $C_{2-40}$ alkynyl, $C_{1-40}$ alkylaryl or optionally a complex metal ion $M^{n+}/n$ wherein n is an integer from 1 to 8, or a linear or branched $C_{1-40}$ $NR^5R^6$ terminated alkyl chain, where $R^5$ and $R^6$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl; m is an integer from 1 to 8; and wherein D is hydrogen or a linear or branched $C_{1-40}$ alkyl, $C_{2-40}$ alkenyl, $C_{2-40}$ alkynyl, an aryl, a heteroaryl or $C_{1-40}$ alkylayl or alkylheteroaryl or a linear or branched $C_{1-40}$ alkyl $NR^5R^6$ chain or a linear or branched $C_{1-40}$ mono or di alkyl ester or di alkyl ester $C_{1-40}$ alkylphosphonate or a linear or branched $C_{1-40}$ alkylphosphonic acid.

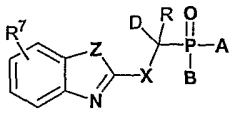
2. The compound as claimed in claim 1, wherein the compound is of formula Ia, Ib, Ic, Id, Ie. Ih or Ik



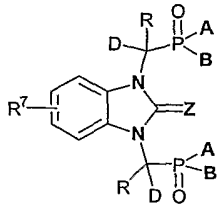
Ia



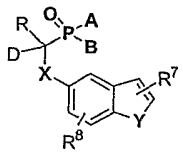
Ib



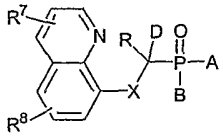
Ic



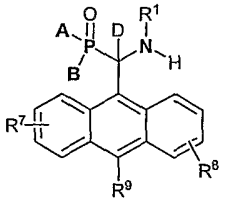
Id



Ie



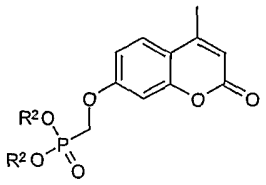
Ih



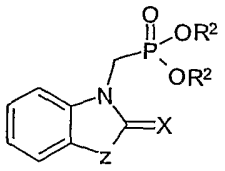
Ik

wherein R, A, B, X, D and M are as defined for claim 1, and Y and Z are O, S or $NR^1$ where $R^1$ is as defined in claim 1 and $R^7$ and $R^8$ and $R^9$ independently represent one or more ring substituents which can be a hydrogen, a halide, a linear or branched $C_{1-40}$ alkyl, a $C_{2-40}$ alkenyl, a $C_{2-40}$ alkynyl, an aryl, a heteroaryl or a $C_{1-40}$ alkylaryl or alkylheteroaryl, a nitrile, a sulphonic acid or salt of sulphonic acid, a carboxy, an oxo, a carboxyalkyl, a carboxyalkoxy, a carboxyalkylamino, a carboxyalkylthio, an amide, a sulphonamide, a $C_{1-6}$ alkylalkoxy, $C_{1-6}$ alkylamino, $OR^2$, $SR^2$, $NR^3$, $R^4$ where $R^2$, $R^3$ and $R^4$ are as defined in claim 1.

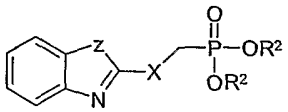
3. The compound as claimed in claim 1 or claim 2, wherein the compound is one of compounds 1, 8, 8a, 10, 11, 15, 21, 22, 23, 26 or 27;



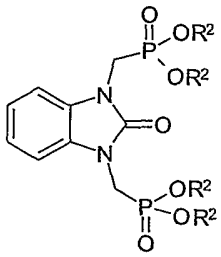
Compound 1, wherein $R^2/R^2$ = Et/Et



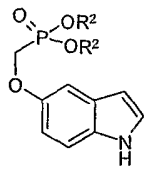
Compound 8, wherein X = O, Z = NH



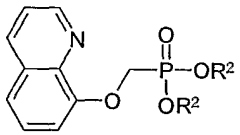
Compound 8a, wherein X = O, Z = NH



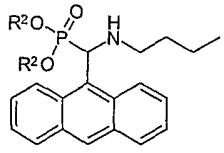
Compound 10 wherein $R^2$ = Et



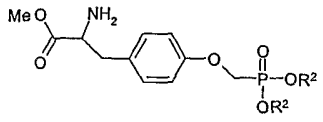
Compound 11, wherein 11 $R^2$ = Et/H



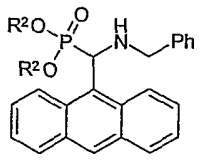
Compound 15, wherein $R^2/R^2$ = Et



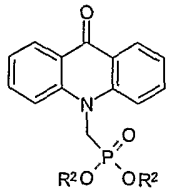
Compound 21, wherein $R^2/R^2$ = Et



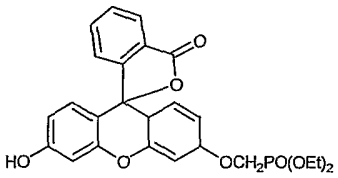
Compound 22



Compound 23



Compound 26



Compound 27

wherein $R^2$ is hydrogen, a linear or branched $C_{1-40}$ alkyl, $C_{2-40}$ alkenyl, $C_{2-40}$ alkynyl, $C_{1-40}$ alkylaryl or optionally a complex metal ion $M^{n+}/n$ wherein n is an integer from 1 to 8, or a linear or branched $C_{1-40}$ $NR^5R^6$ terminated alkyl chain, where $R^5$ and $R^6$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl.

4. The compound as claimed in claims 1 to 3 for use in decreasing amyloid aggregates.

5. The compound as claimed in claims 1 to 3 for use in the treatment of a disease characterised by amyloid deposition.

6. The compound as claimed in claims 1 to 3 or claim 5, wherein the disease is Alzheimer's disease.

7. Use of a compound of general formula I as defined in claim 1 in the manufacture of a medicament for use in the treatment of a disease characterised by amyloid deposition.

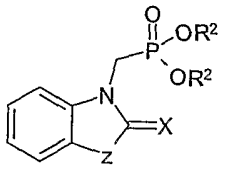
8. Use as claimed in claim 7, wherein the disease is Alzheimer's disease.

9 Use as claimed in claims 4 to 8, wherein the compound is one or more selected from the group consisting of formulae Ia, Ib, Ic, Id, Ie, Ih and Ik, as defined in claim 2.

10. Use as claimed in claims 4 to 9, wherein the compound is one or more selected from the group consisting of compounds 1 to 28 and 4a to 9a, as defined in table 1.

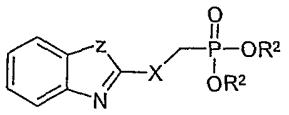
11. Use as claimed in claims 4 to 10, wherein the compound is selected from the group consisting of compounds 1, 8, 8a, 10, 11, 15, 21, 22, 23, 26 and 27.

12. Compound of formula 4;



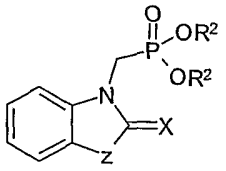
wherein X = O and Z = O and $R^2$ is hydrogen, a linear or branched $C_{1-40}$ alkyl, $C_{2-40}$ alkenyl, $C_{2-40}$ alkynyl, $C_{1-40}$ alkylaryl or optionally a complex metal ion $M^{n+}/n$ wherein n is an integer from 1 to 8, or a linear or branched $C_{1-40}$ $NR^5R^6$ terminated alkyl chain, where $R^5$ and $R^6$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl.

13. Compound of formula 5a;



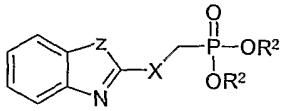
wherein X = S and Z = O and $R^2$ is hydrogen, a linear or branched $C_{1-40}$ alkyl, $C_{2-40}$ alkenyl, $C_{2-40}$ alkynyl, $C_{1-40}$ alkylaryl or optionally a complex metal ion $M^{n+}/n$ wherein n is an integer from 1 to 8, or a linear or branched $C_{1-40}$ $NR^5R^6$ terminated alkyl chain, where $R^5$ and $R^6$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl.

14. Compound of formula 6;



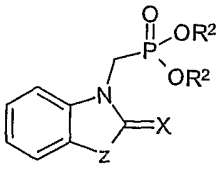
wherein X = O and Z = S and $R^2$ is hydrogen, a linear or branched $C_{1-40}$ alkyl, $C_{2-40}$ alkenyl, $C_{2-40}$ alkynyl, $C_{1-40}$ alkylaryl or optionally a complex metal ion $M^{n+}/n$ wherein n is an integer from 1 to 8, or a linear or branched $C_{1-40}$ $NR^5R^6$ terminated alkyl chain, where $R^5$ and $R^6$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl.

15. Compound of formula 7a;



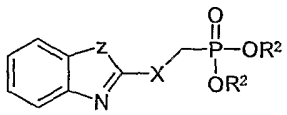
wherein X = S and Z = S and $R^2$ is hydrogen, a linear or branched $C_{1-40}$ alkyl, $C_{2-40}$ alkenyl, $C_{2-40}$ alkynyl, $C_{1-40}$ alkylaryl or optionally a complex metal ion $M^{n+}/n$ wherein n is an integer from 1 to 8, or a linear or branched $C_{1-40}$ $NR^5R^6$ terminated alkyl chain, where $R^5$ and $R^6$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl.

16. Compound of formula 8;



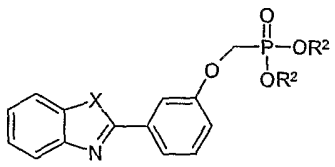
wherein X = O and Z = NH and $R^2$ is hydrogen, a linear or branched $C_{1-40}$ alkyl, $C_{2-40}$ alkenyl, $C_{2-40}$ alkynyl, $C_{1-40}$ alkylaryl or optionally a complex metal ion $M^{n+}/n$ wherein n is an integer from 1 to 8, or a linear or branched $C_{1-40}$ $NR^5R^6$ terminated alkyl chain, where $R^5$ and $R^6$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl.

17. Compound of formula 8a;



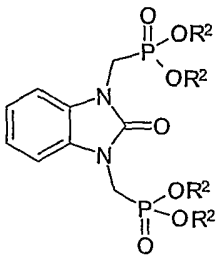

wherein X = O and Z = NH and $R^2$ is hydrogen, a linear or branched $C_{1-40}$ alkyl, $C_{2-40}$ alkenyl, $C_{2-40}$ alkynyl, $C_{1-40}$ alkylaryl or optionally a complex metal ion $M^{n+}/n$ wherein n is an integer from 1 to 8, or a linear or branched $C_{1-40}$ $NR^5R^6$ terminated alkyl chain, where $R^5$ and $R^6$ are each independently hydrogen, a linear or branched $C_{1-40}$ alkyl.

18. Compound of formula 14;




wherein X = S and $R^2$ = Et/H.

19. Compound of formula 10;



wherein $R^2 = Et$ .

20. A compound as claimed in any one of claims 12 to 19 for use in medicine.

21. Compounds as claimed in claims 12 to 19 in the manufacture of a medicament for use in the treatment of a disease characterised by amyloid deposition.

22. A composition comprising a compound of general formula I as defined in claim 1 and a pharmaceutically acceptable carrier or diluent.

23. The composition as claimed in claim 22, wherein the compound is one or more selected from the group consisting of formulae Ia, Ib, Ic, Id, Ie, Ih and Ik, as defined in claim 2.

24. The composition as claimed in claims 22 or 23, wherein the compound is one or more selected from the group consisting of 1, 8, 8a, 10, 11, 15, 21, 23, 26 and 27.

25. The use of the composition of any one of claims 22 to 24 for use in treatment of a disease characterised by amyloid deposition.

FIG. 1

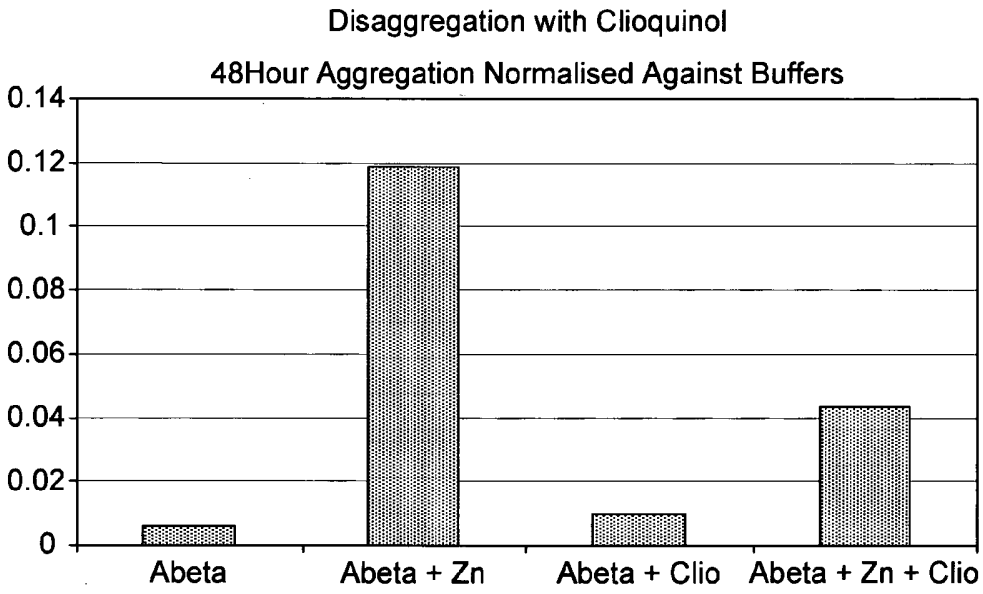

Disaggregation with Clioquinol
48Hour Aggregation Normalised Against Buffers
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Abeta
Abeta + Zn
Abeta + Clio
Abeta + Zn + Clio

FIG. 2

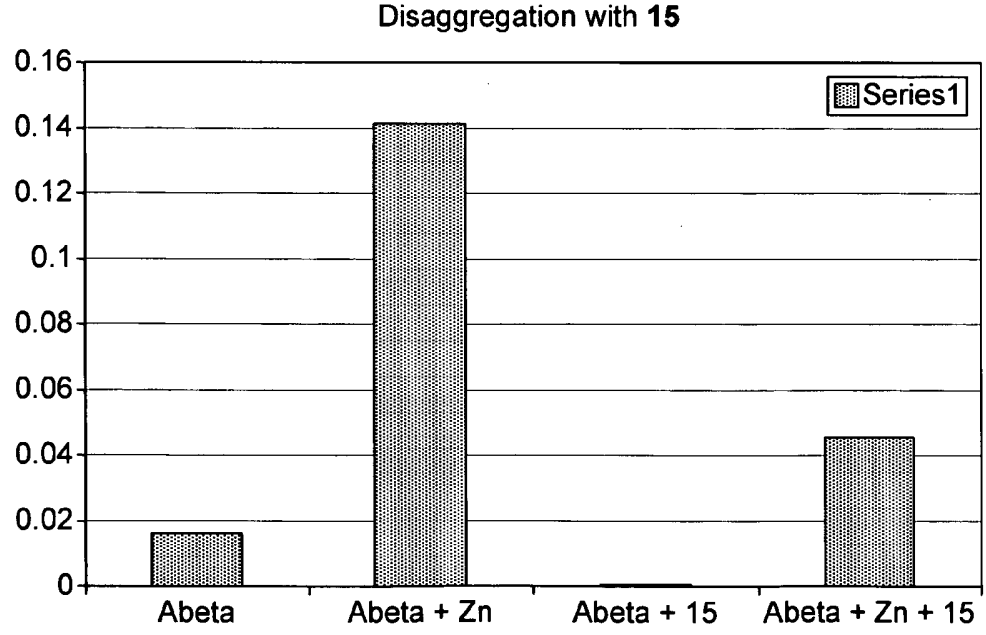

Disaggregation with **15**
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Series1
Abeta
Abeta + Zn
Abeta + 15
Abeta + Zn + 15

FIG. 3

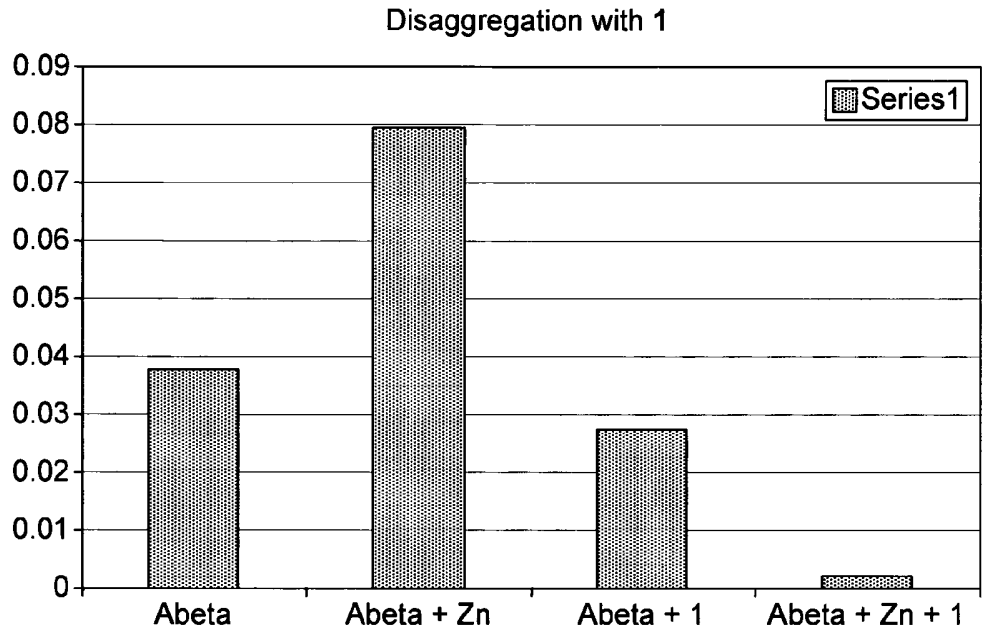

Disaggregation with 1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Series1
Abeta
Abeta + Zn
Abeta + 1
Abeta + Zn + 1

FIG. 4

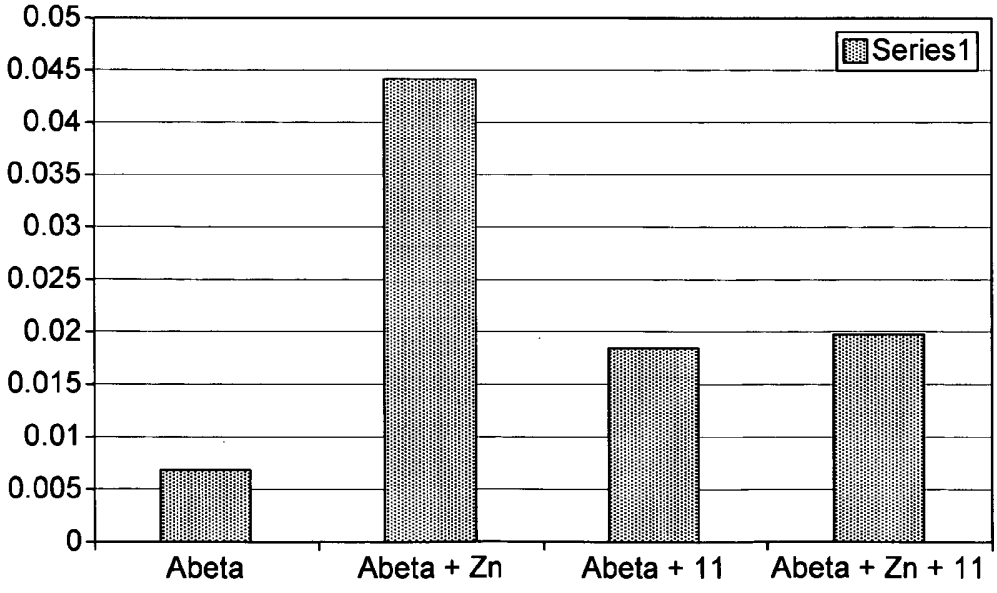

Disaggregation with compound 11
0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
Series1
Abeta
Abeta + Zn
Abeta + 11
Abeta + Zn + 11

FIG. 5

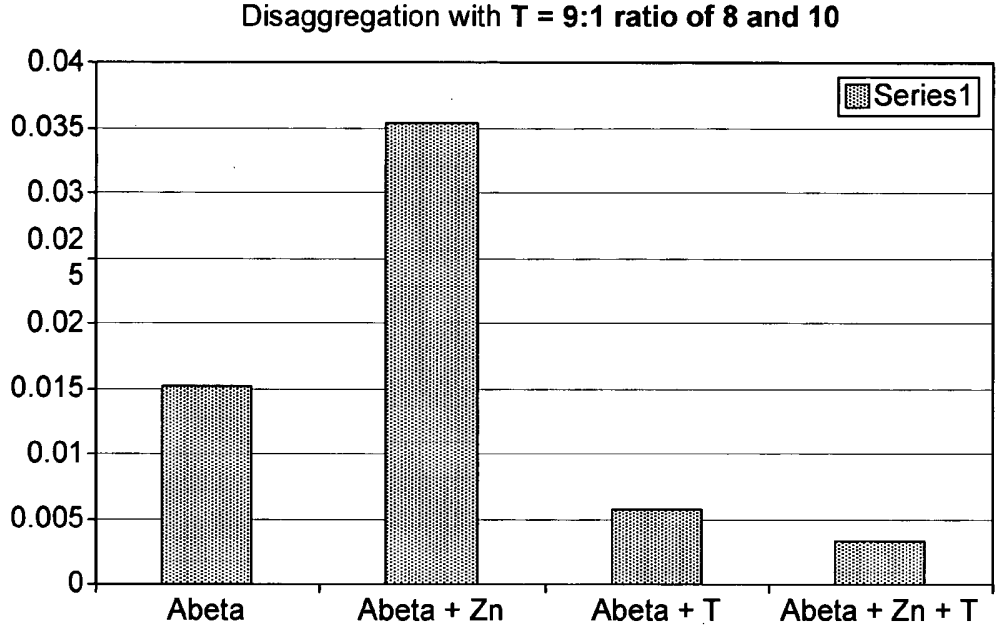
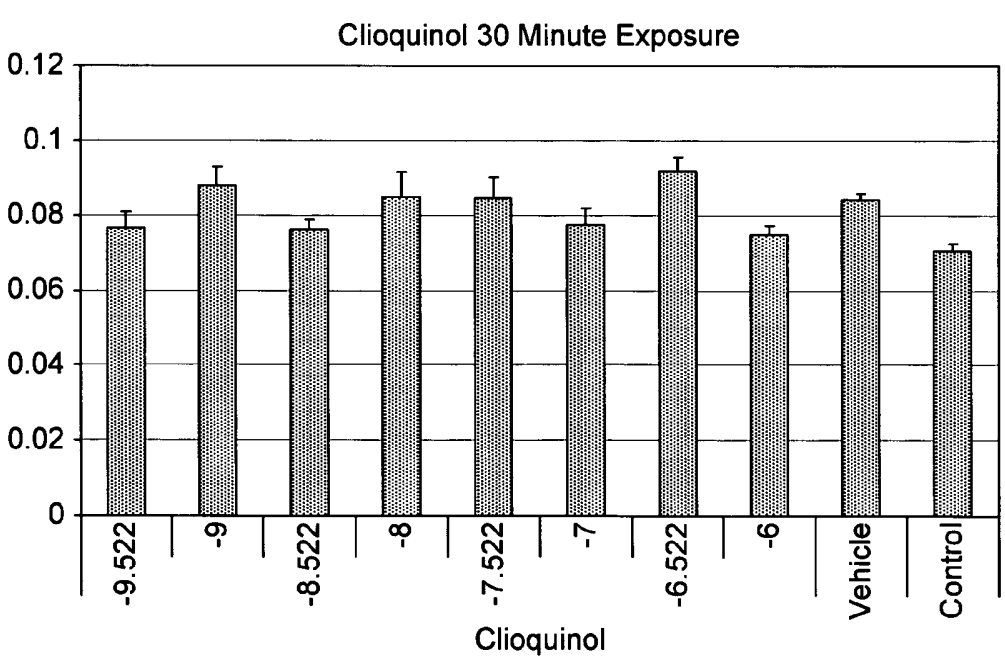
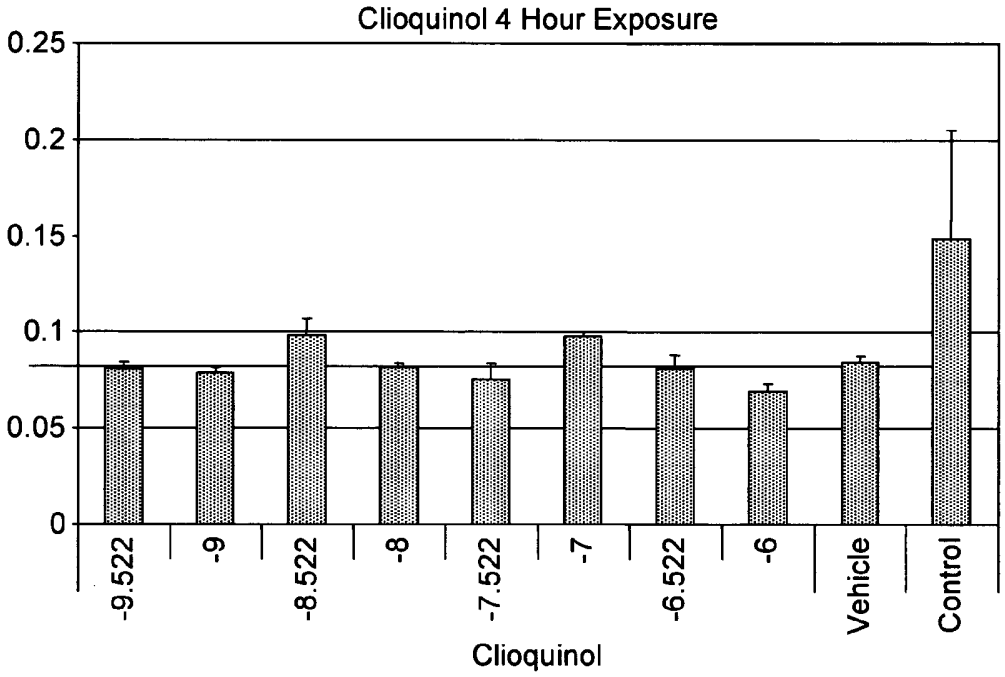
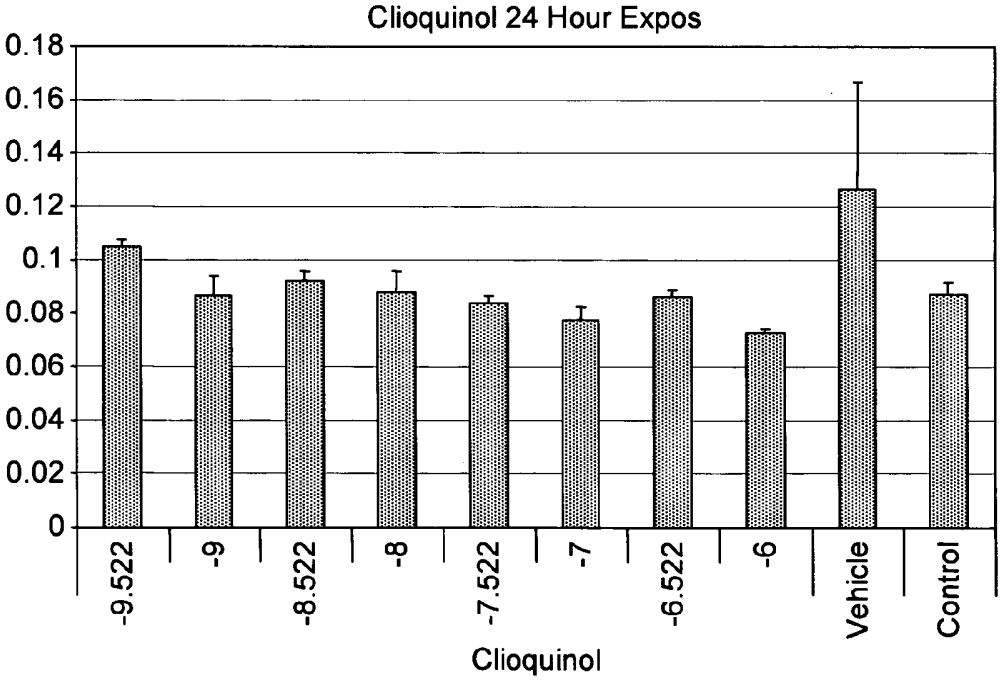

Disaggregation with T = 9:1 ratio of 8 and 10
0.04
0.035
0.03
0.02
5
0.02
0.015
0.01
0.005
0
Series1
Abeta
Abeta + Zn
Abeta + T
Abeta + Zn + T

FIG. 6


L929 viability with clioquinol
Clioquinol 30 Minute Exposure
0.12
0.1
0.08
0.06
0.04
0.02
0
-9.522
-9
-8.522
-8
-7.522
-7
-6.522
-6
Vehicle
Control
Clioquinol

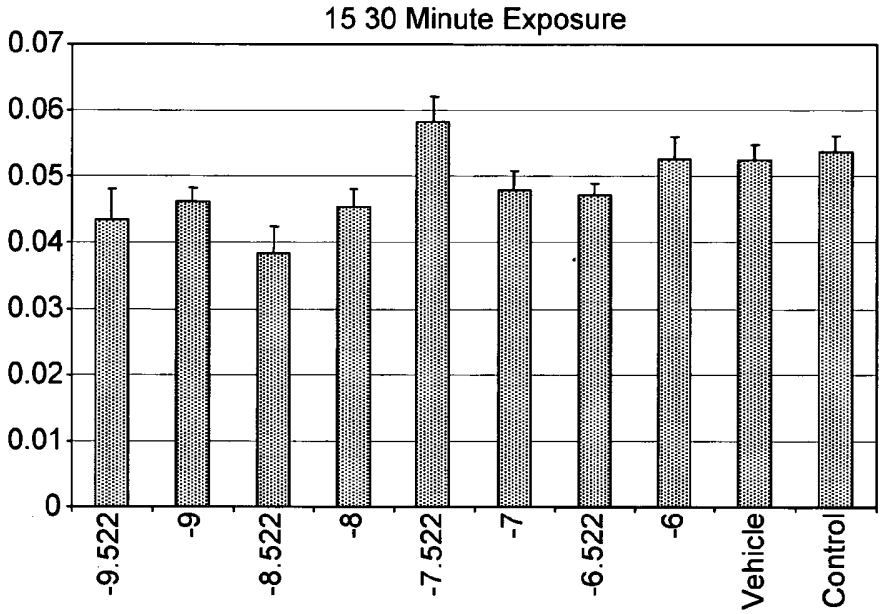
# FIG. 6 Cont'd


Clioquinol 4 Hour Exposure
0.25
0.2
0.15
0.1
0.05
0
-9.522
-9
-8.522
-8
-7.522
-7
-6.522
-6
Vehicle
Control
Clioquinol


Clioquinol 24 Hour Expos
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-9.522
-9
-8.522
-8
-7.522
-7
-6.522
-6
Vehicle
Control
Clioquinol

# FIG. 7

L929 viability with **15**



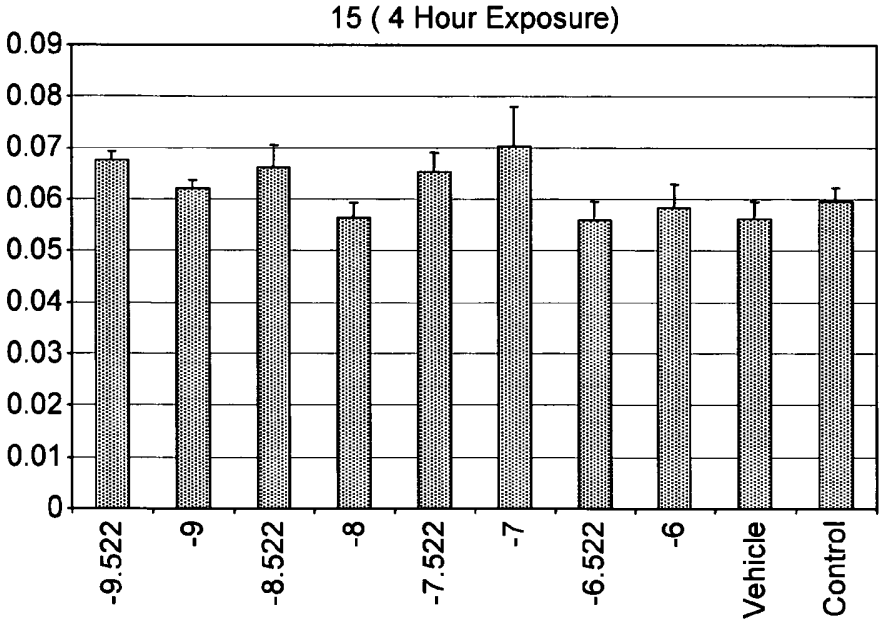



FIG. 7 Cont'd

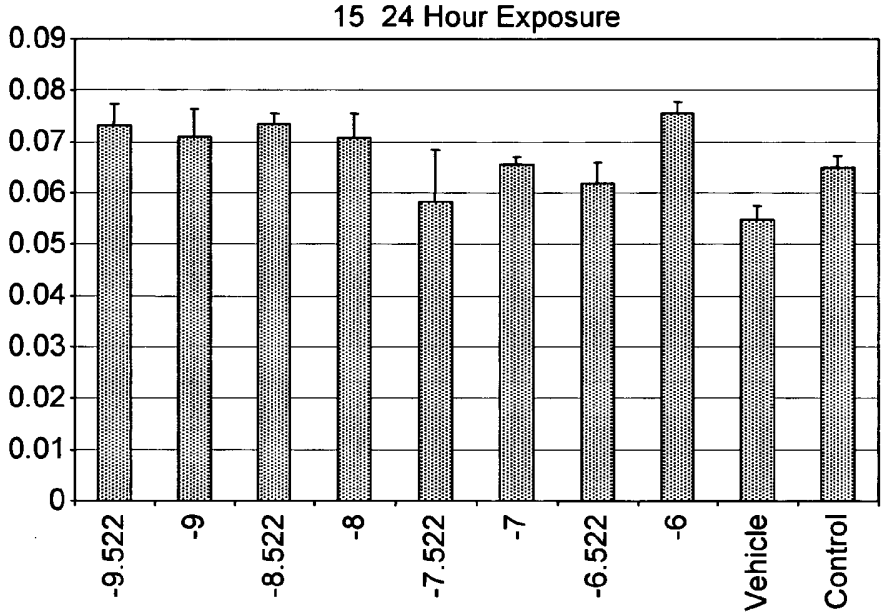



FIG. 8

H4 cell viability with **11** using Neutral Red
(Each group starts with control on left hand side)

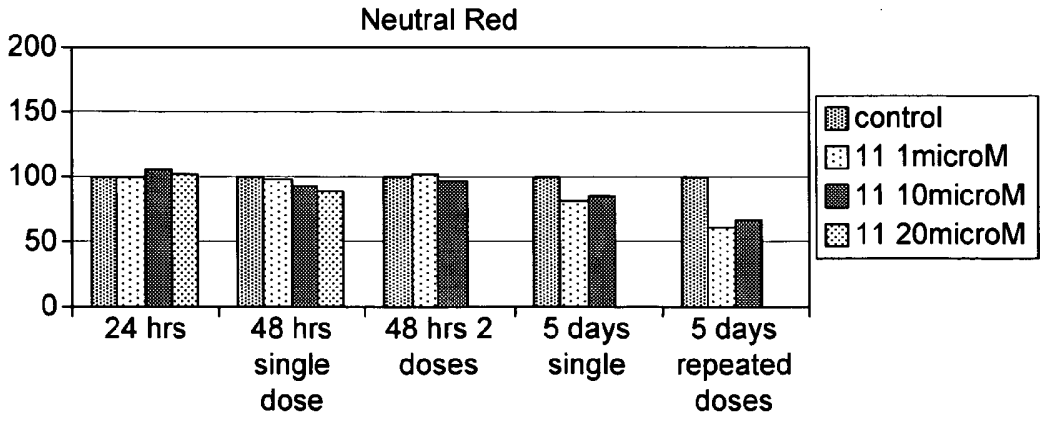


FIG. 9

H4 cell viability with 1 using Neutral Red

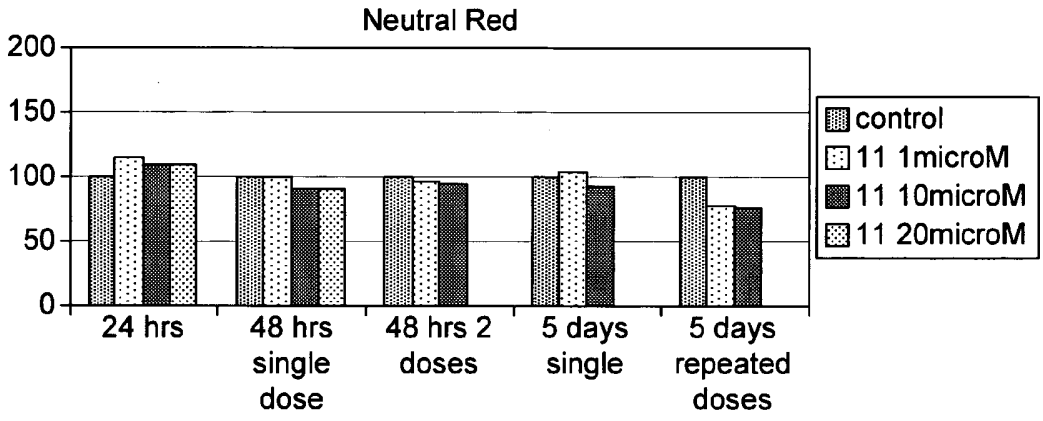
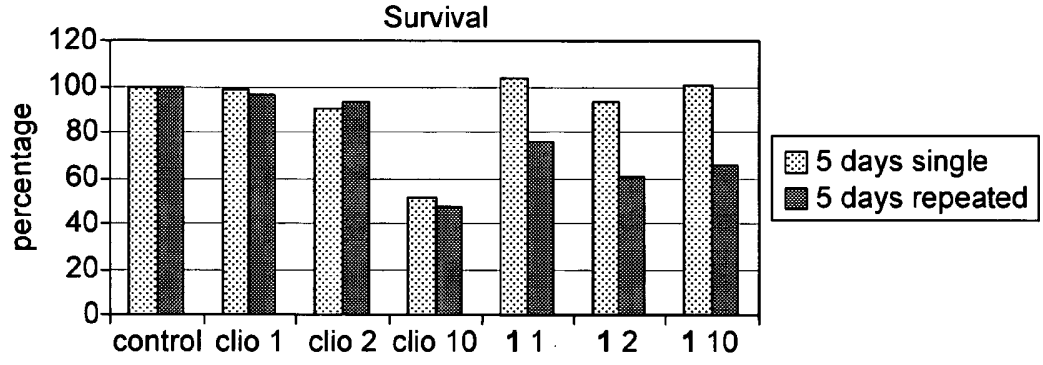
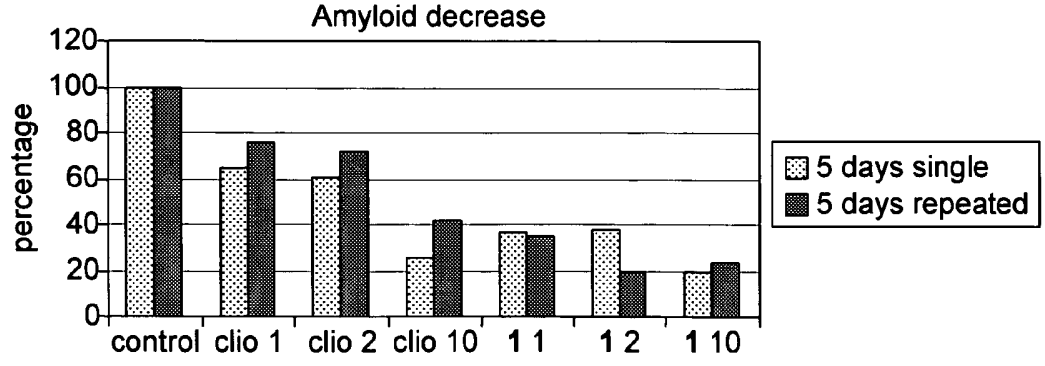



FIG. 10

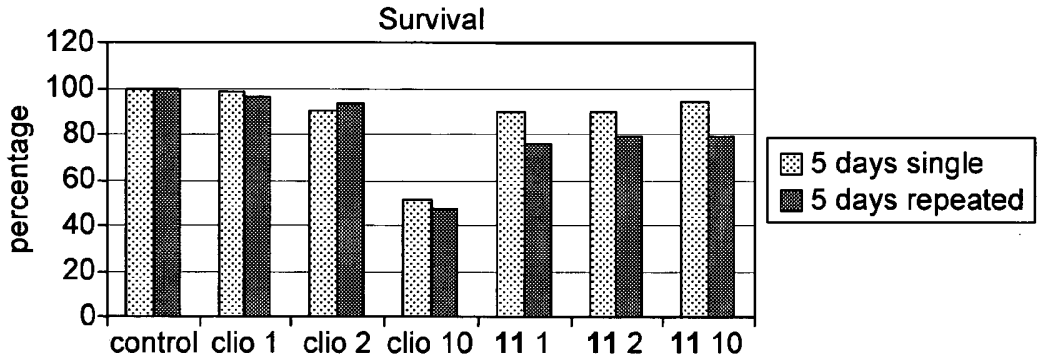
**Cell viability/survival and amyloid accumulation data for 1**

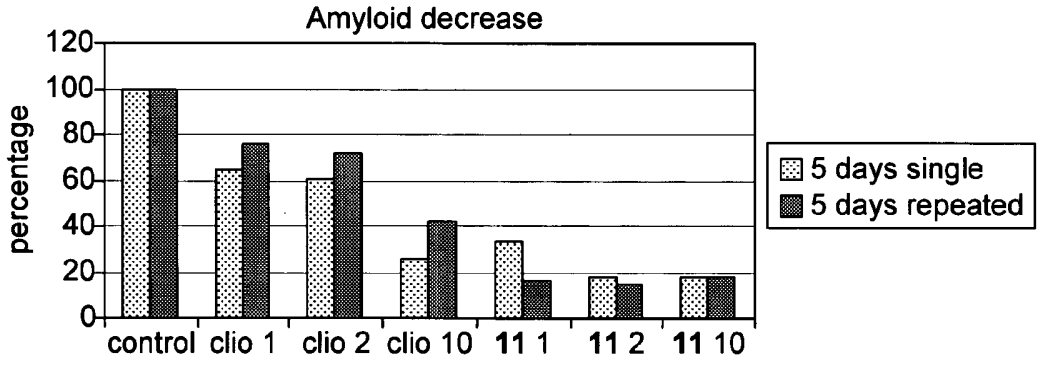


# FIG. 11

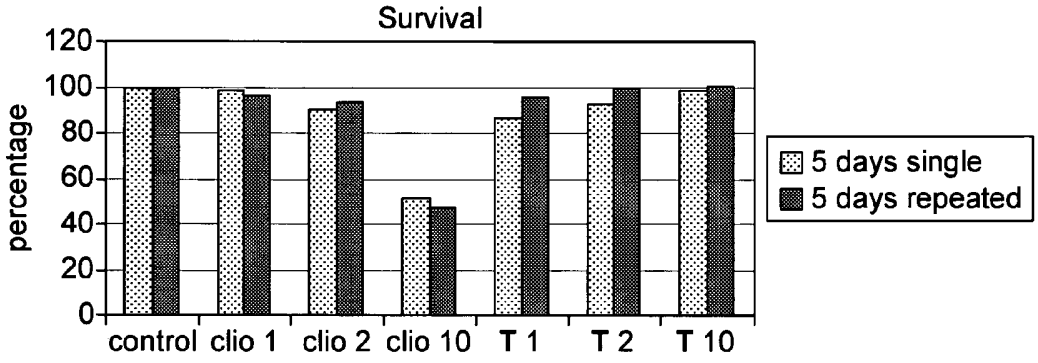
**Cell viability/survival and amyloid accumulation data for 11**



# FIG. 12

**Cell viability/survival and amyloid accumulation data for T**



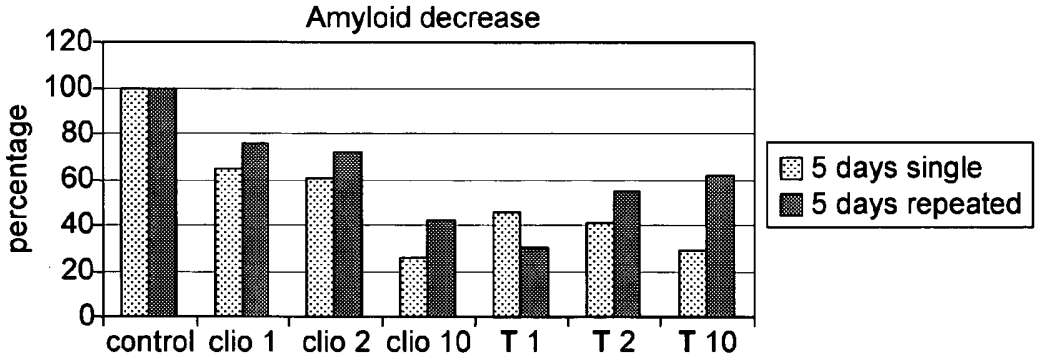
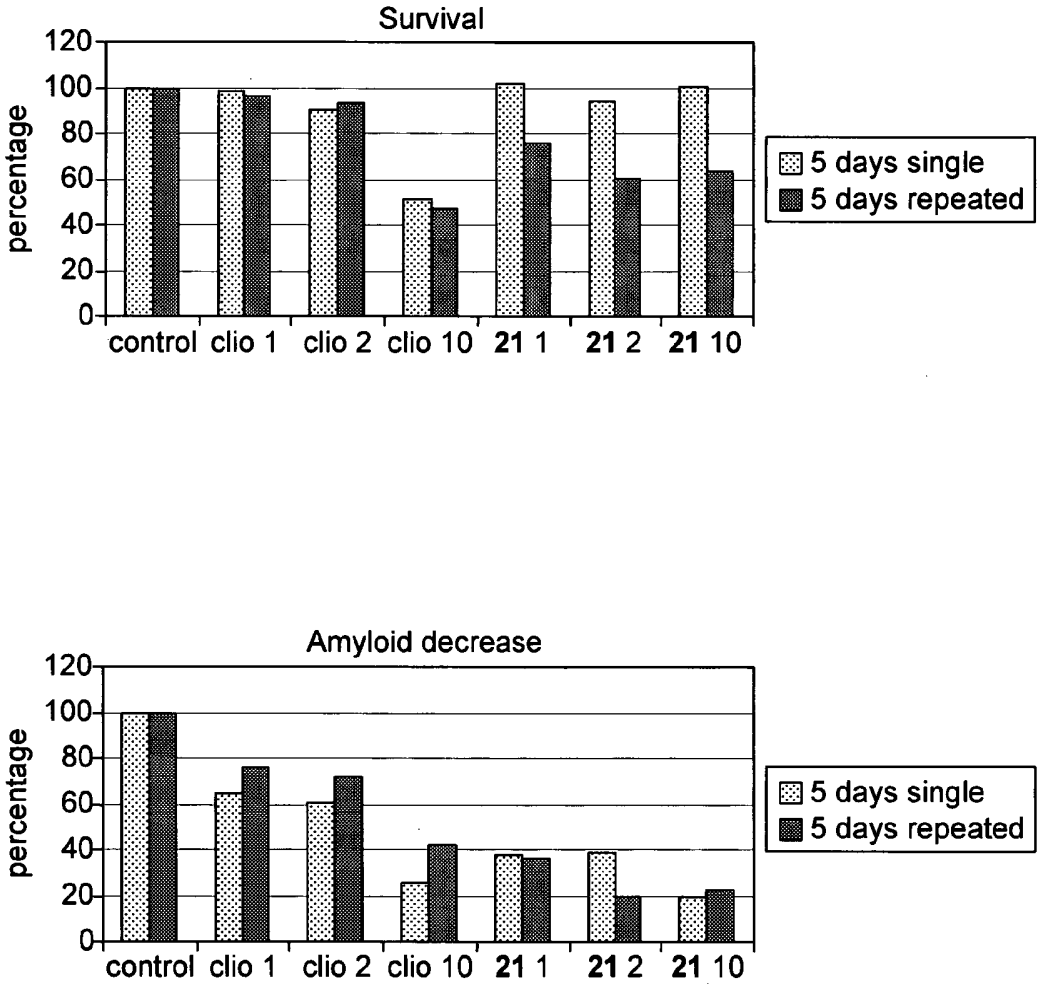



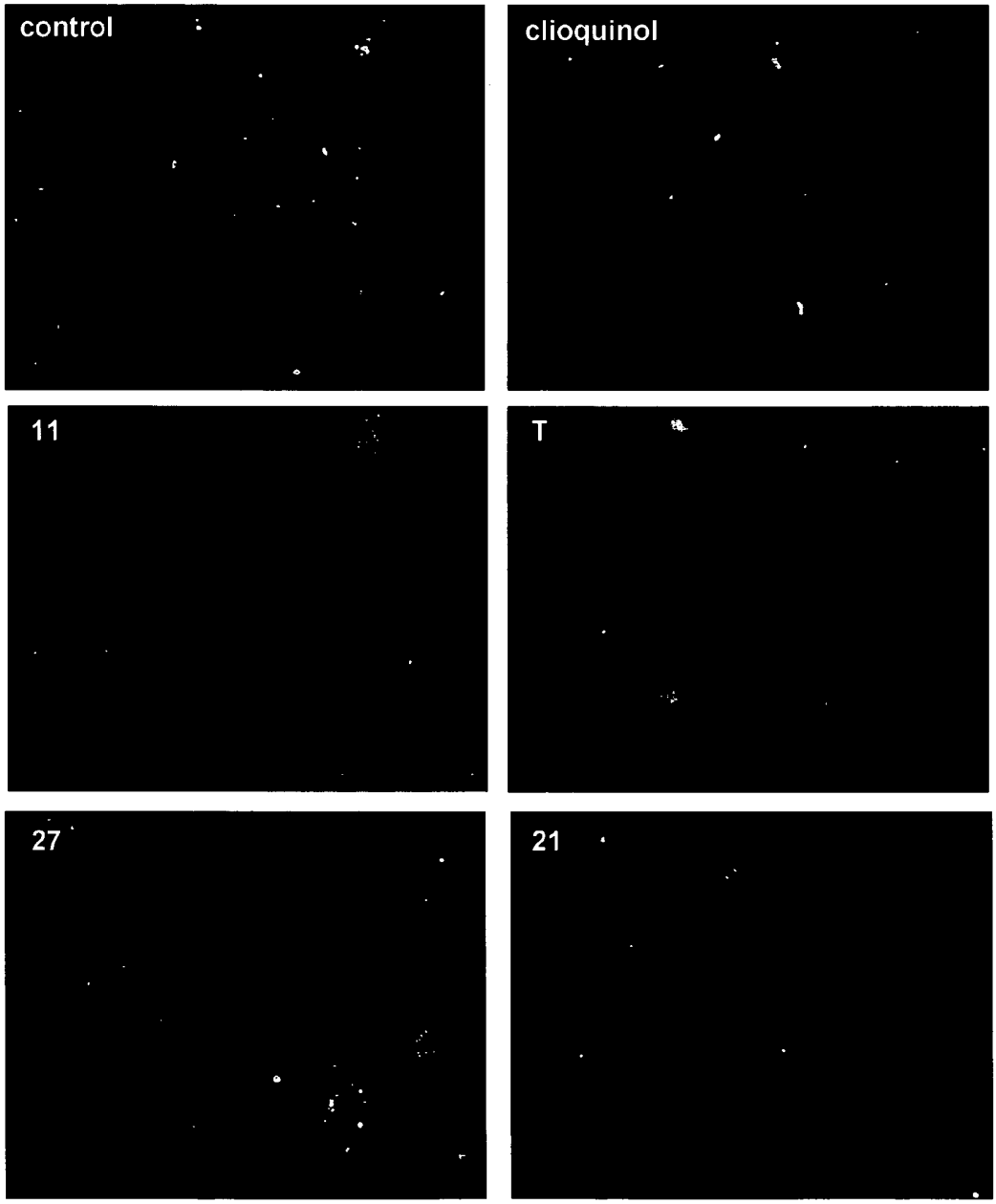
FIG. 13

**Cell viability/survival and amyloid accumulation data for 21**



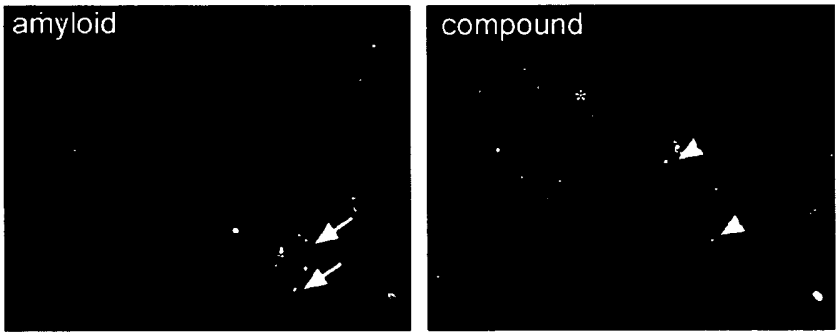
# FIG. 14

Observations on the intracellular distribution of amyloid after incubation with substituted phosphonates



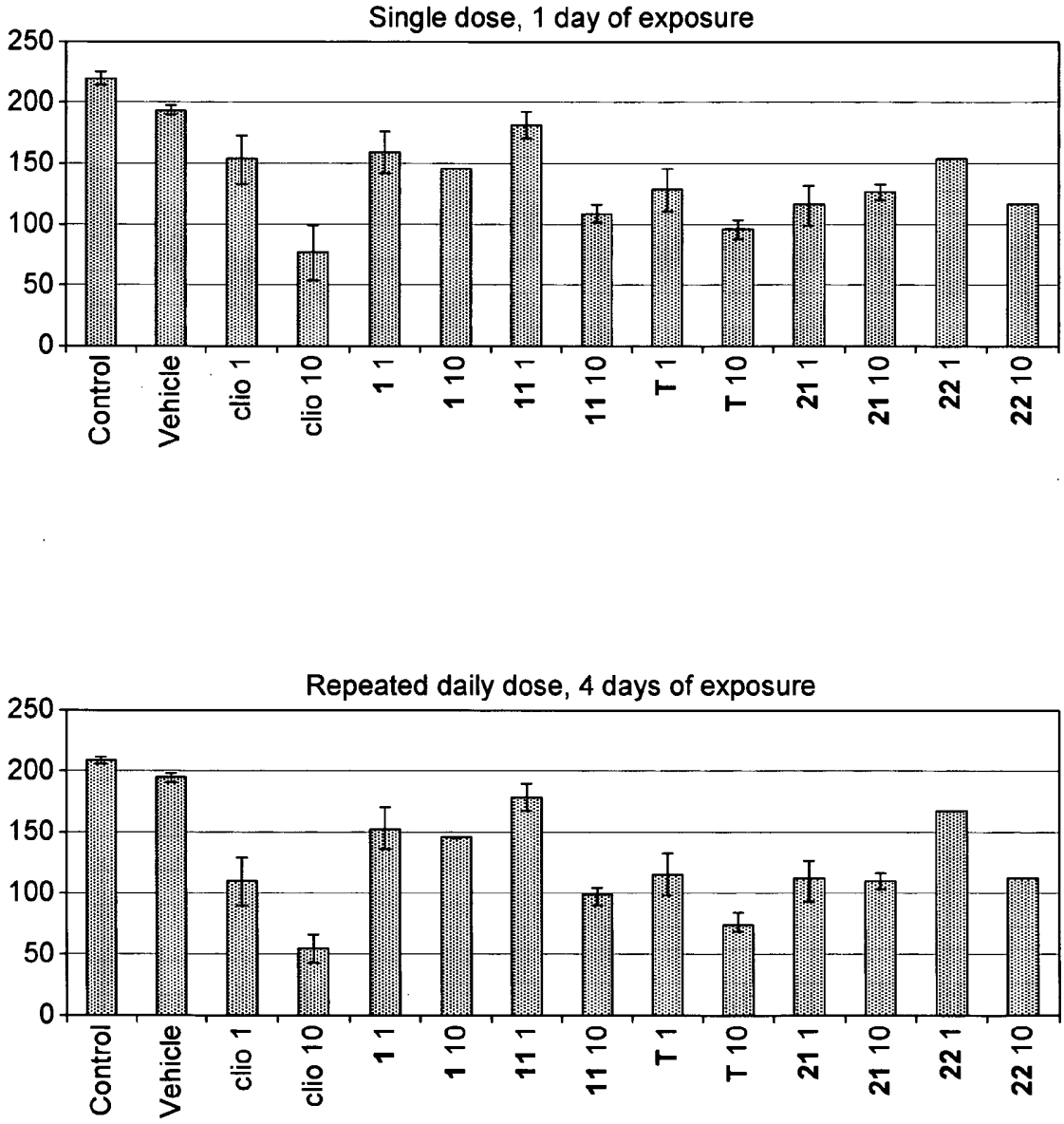
# FIG. 15

The figure below shows natural fluorescence of 27 and amyloid labelling in the right and left panels, respectively, after 1μM for 5 days, single dose. Arrows indicate H4 cells with strong amyloid and no 27 signal, arrowheads little amyloid and evidence of 27 in the cytoplasm and nucleus of the H4 cells. There is also overlap in some H4 cells between the **27** and the amyloid lebelling shown by the *.



# FIG. 16

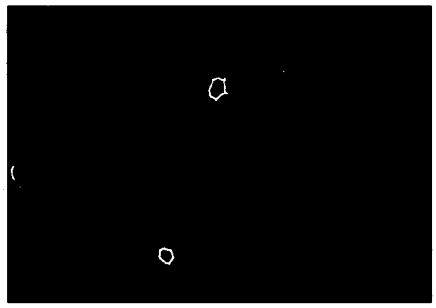
Effects on primary sensory neurons



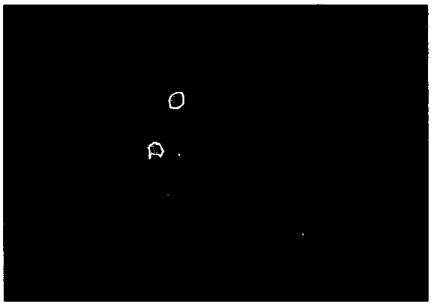
# FIG. 17

Images of primary sendory neurons after exposure to control, 1 and 11

Control 24 hours



1 24 hours



11 24 hours

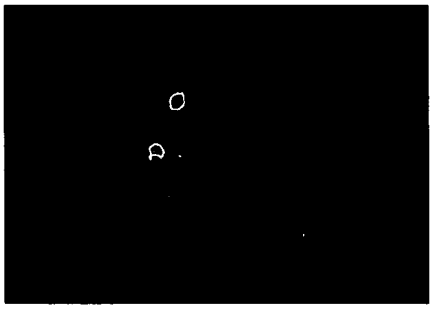


FIG. 18

Autometallographic images of brain parenchyma after exposure to compounds

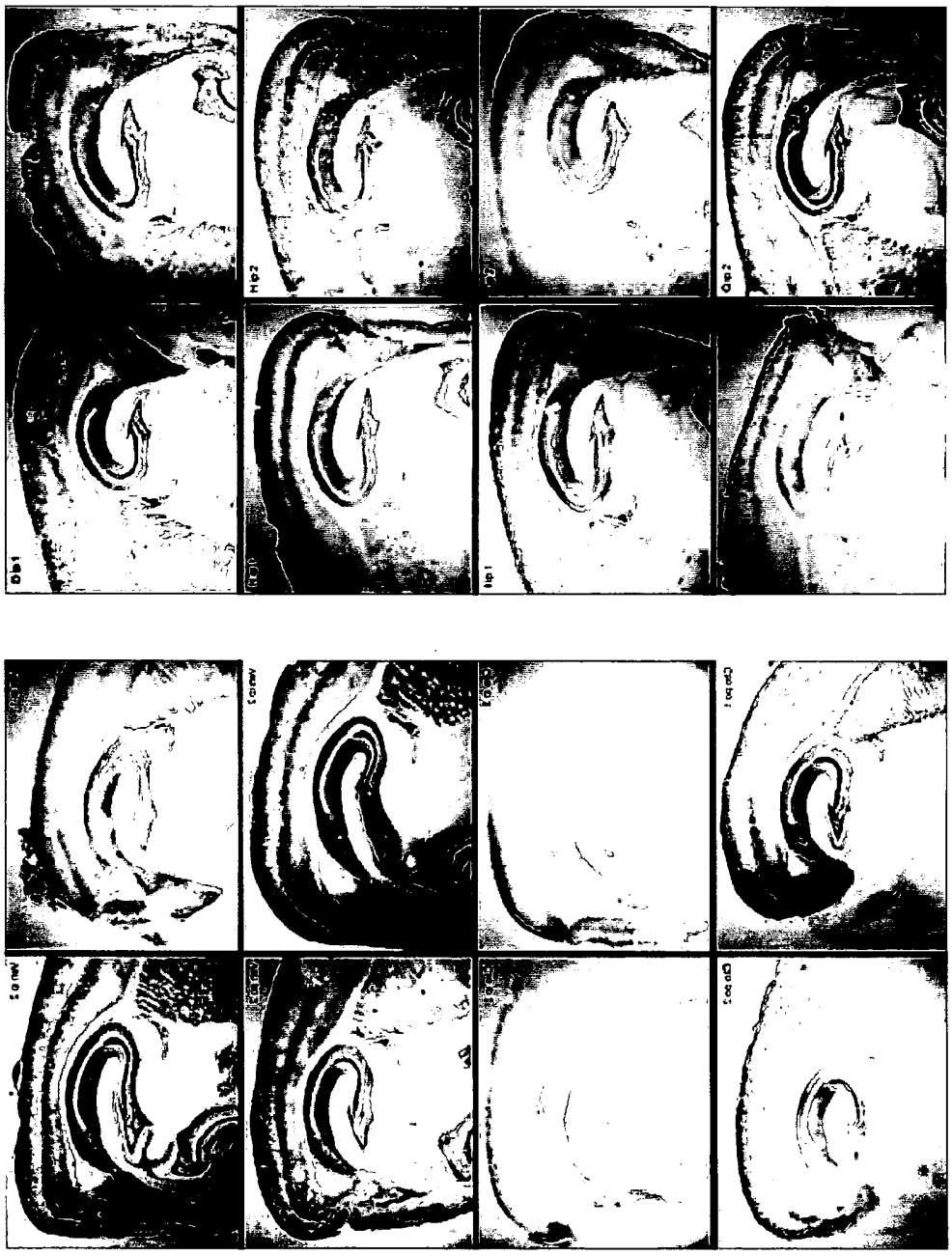


FIG. 19

Images of brain tissue after post treatment with compounds

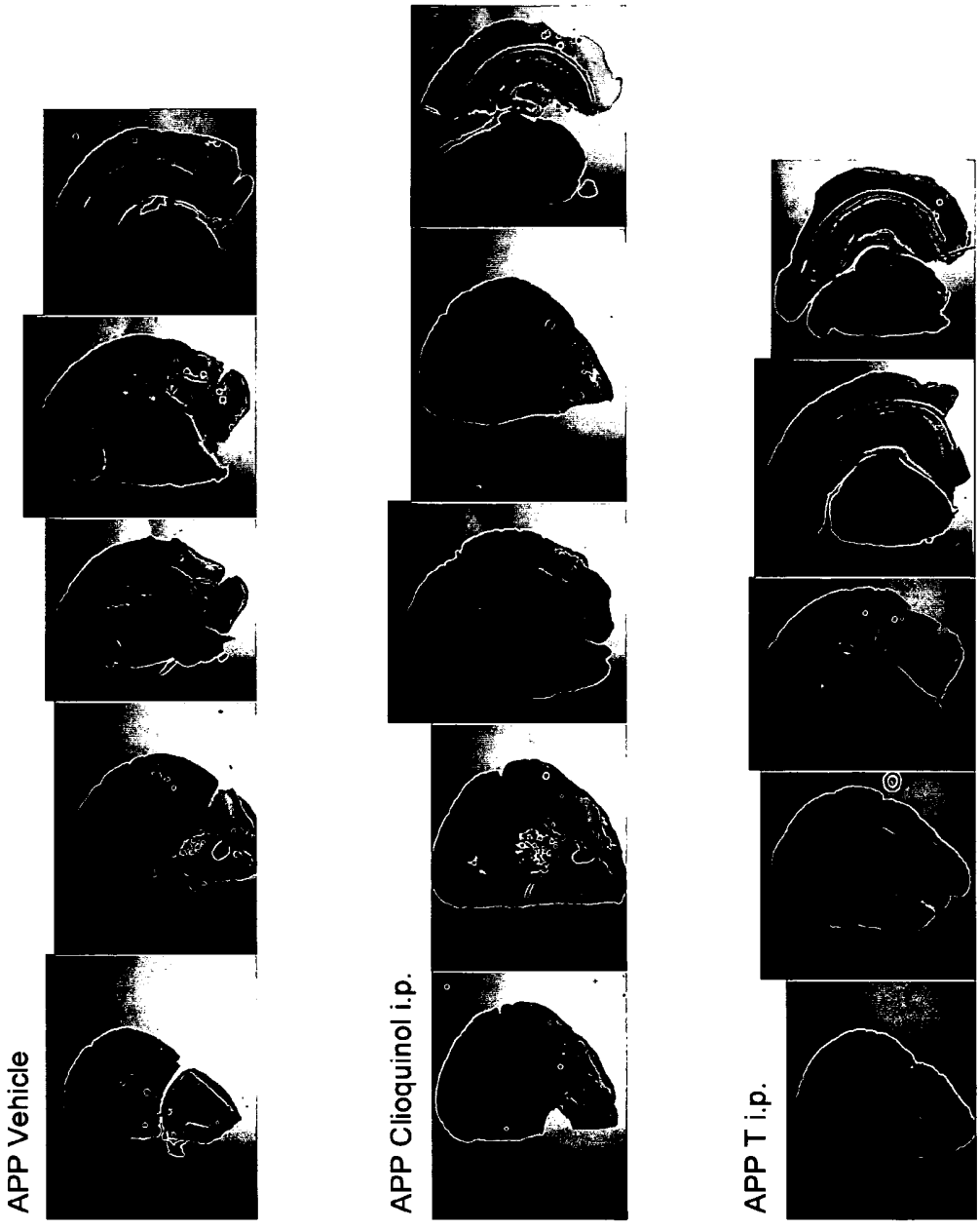
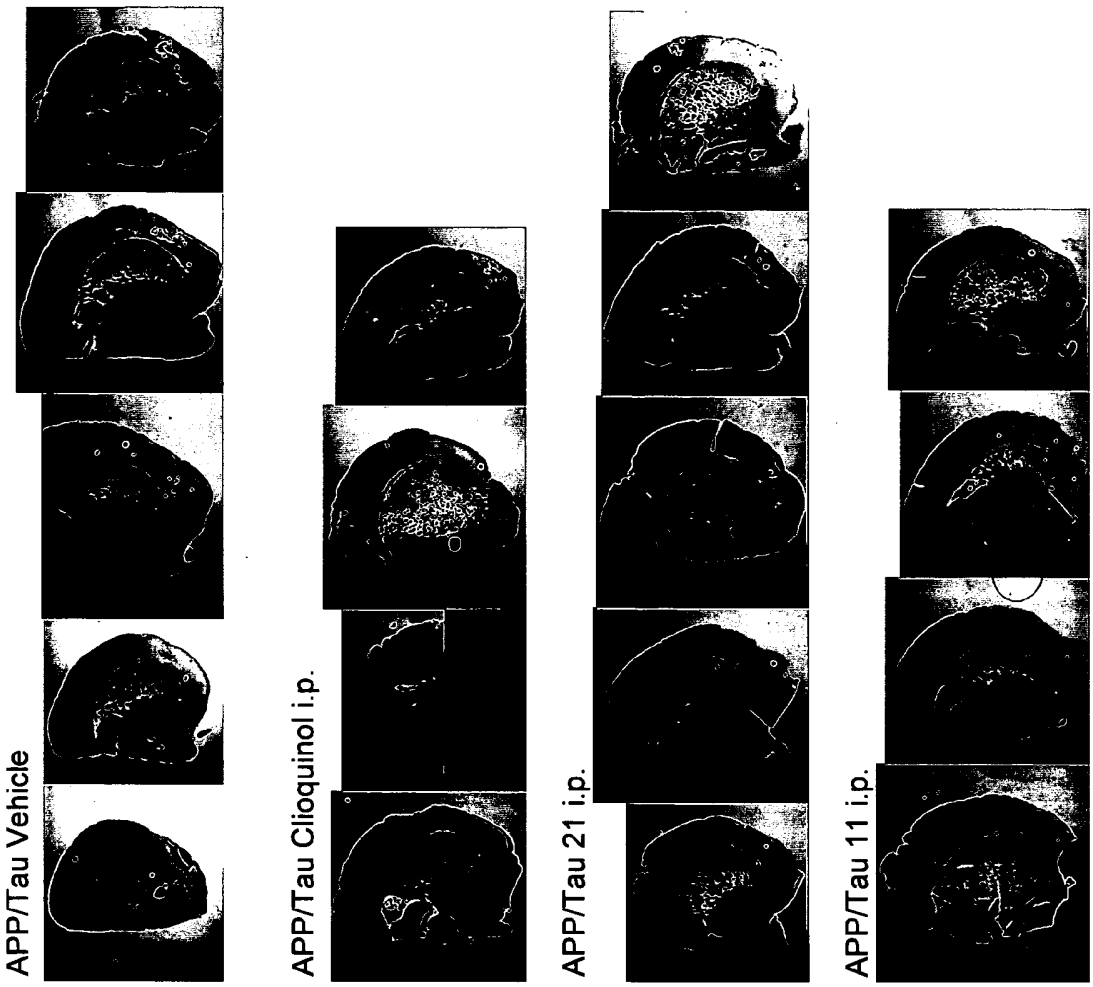


FIG. 19 Cont'd


APP/Tau Vehicle
APP/Tau Clioquinol i.p.
APP/Tau 21 i.p.
APP/Tau 11 i.p.

# INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2008/001540

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P25/28 C07F9/40 C07F9/572 C07F9/60 C07F9/64
C07F9/6506 C07F9/653 C07F9/655 C07F9/6561 A61K31/662
A61K31/665 A61K31/675

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)
C07F A61P A61K

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 practical, search terms used)
EPO-Internal, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 6 288 089 B1 (ZAWADA MICHAEL [US] ET AL) 11 September 2001 (2001-09-11)<br>claims 1,2,11,12<br>column 25, line 11 - line 12<br>column 4, line 24 - column 5, line 28 | 1,4-8, 22,25 |
| X | WO 01/85093 A (NEUROCHEM INC [CA])<br>15 November 2001 (2001-11-15)<br>* N-Phosphonomethylglycine; N-Phosphonomethylglycine, trisodium salt *<br>page 48; table III<br>abstract<br>page 32, line 13 - line 17 | 1,4-8, 22,25 |

-/--

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"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
"T" 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
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"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.
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 22 July 2008 | 29/09/2008 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Eberhard, Michael |

Form PCT/ISA/210 (second sheet) (April 2005)

# INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2008/001540

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 00/43394 A (USTAV EX BOTAN AKADEMIE VED CE [CZ]; LACHEMA AS [CZ]; HANUS JAN [CZ];) 27 July 2000 (2000-07-27)<br>page 27, line 1<br>page 25, line 27<br>table 8 | 1,4-8, 22,25 |
| X | WO 2005/080406 A (JERINI AG [DE]; KNOLLE JOCHEN [DE]; SCHUTKOWSKI MIKE [DE]; HUMMEL GERD) 1 September 2005 (2005-09-01)<br>compound 64<br>page 10, paragraph 7<br>claim 31 | 1,4-8, 22,25 |
| X | WO 2007/015122 A (GENEXEL INC [KR]; KIM JAESEOB [KR]; BAE EUNKYUNG [KR]; KIM JUNGMO [KR]) 8 February 2007 (2007-02-08)<br>the whole document | 1,4-8, 22,25 |
| X | CN 1 548 444 A (GUILIN JIQI PHARMACEUTICAL IND [CN]) 24 November 2004 (2004-11-24)<br>claim 1<br>abstract | 1,4-8, 22,25 |
| X | WO 99/03878 A (POLIFARMA SPA [IT]; CONSIGLIO NAZIONALE RICERCHE [IT]; POLITI VINCENZO) 28 January 1999 (1999-01-28)<br>abstract<br>claim 1 | 1,4-8, 22,25 |
| X | WO 2007/022059 A (UNIV CALIFORNIA [US]; HAMMOCK BRUCE D [US]; MORISSEAU CHRISTOPHE [US];) 22 February 2007 (2007-02-22)<br>page 38; compound 31 | 1,22 |
| X | WO 98/17672 A (CIBA GEIGY AG [CH]; ACKLIN PIERRE [CH]; ALLGEIER HANS [DE]; AUBERSON Y) 30 April 1998 (1998-04-30)<br>page 52, lines 6,7,11,12 | 1,22 |
| X | US 6 284 748 B1 (DANG QUN [US] ET AL)<br>4 September 2001 (2001-09-04)<br>example 5j; compounds (5.5),A | 1,22 |
| X | WO 2006/058411 A (MEDICURE INT INC [CA]; FRIESEN ALBERT [CA])<br>8 June 2006 (2006-06-08)<br>examples 9,11; compound XIII<br>abstract | 1,22 |
| P,X | WO 2007/134449 A (WARATAH PHARMACEUTICALS INC [CA]; SLON-USAKIEWICZ JACEK [CA])<br>29 November 2007 (2007-11-29)<br>abstract<br>* p. 126 2nd compound "C3H8NO5P" *<br>* p. 138 6th compound "C4H11NO8P2" * | 1,4-8, 22,25 |

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2008/001540

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see annex

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 4-8, 22,25 (in part)

A compound of formula I for use in medicine wherein E is a substituted or unsubstituted linear or branched C1-40 alkyl; use of such a compound in the manufacture of a medicament; a composition thereof with a pharmaceutically acceptable carrier or diluent; and the use of such a composition for use in the treatment of a disease characterised by amyloid deposition.

---

2. claims: 1-11, 22-25 (in part), 18 (fully)

A compound of formula I for use in medicine wherein E is aryl not including heteroaryls; use of such a compound in the manufacture of a medicament; a composition thereof with a pharmaceutically acceptable carrier or diluent; and the use of such a composition for use in the treatment of a disease characterised by amyloid deposition.

---

3. claims: 1-11, 22-25 (in part), 12-17, 19-21 (fully)

A compound of formula I for use in medicine wherein E is heteroaryl; use of such a compound in the manufacture of a medicament; a composition thereof with a pharmaceutically acceptable carrier or diluent; and the use of such a composition for use in the treatment of a disease characterised by amyloid deposition.

---

4. claims: 1,4-8,22,25 (in part)

A compound of formula I for use in medicine wherein E is hydrogen; use of such a compound in the manufacture of a medicament; a composition thereof with a pharmaceutically acceptable carrier or diluent; and the use of such a composition for use in the treatment of a disease characterised by amyloid deposition.

---

# INTERNATIONAL SEARCH REPORT

*Information on patent family members*

International application No
PCT/GB2008/001540

| Patent document cited in search report | | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|---|
| US 6288089 | B1 | 11-09-2001 | NONE | | |
| WO 0185093 | A | 15-11-2001 | AU | 8431301 A | 20-11-2001 |
| | | | BR | 0016652 A | 19-11-2002 |
| | | | CA | 2395314 A1 | 15-11-2001 |
| | | | CN | 1434706 A | 06-08-2003 |
| | | | EP | 1251837 A2 | 30-10-2002 |
| | | | JP | 2003532656 T | 05-11-2003 |
| | | | KR | 20080003945 A | 08-01-2008 |
| | | | MX | PA02006196 A | 09-12-2002 |
| WO 0043394 | A | 27-07-2000 | AT | 247115 T | 15-08-2003 |
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