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(54) Title: NEW THIOSEMICARBAZONE COMPOUNDS AND ANALOGUES THEREOF

(57) Abstract: The present invention relates to new multi-functional compounds that are useful in the treatment of neurodegenerative diseases, such as Alzheimer's disease, to the preparation of the compounds, and to compositions including the compounds. The present invention also relates to the use of the compounds, as well as compositions including the compounds, in treating or preventing neurodegenerative diseases.



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New thiosemicarbazone compounds and analogues thereof

Field of the invention

The present invention relates to new multi-functional compounds that are useful in the treatment of neurodegenerative diseases, such as Alzheimer's disease, to the preparation of the compounds, and to compositions including the compounds. The present invention also relates to the use of the compounds, as well as compositions including the compounds, in treating or preventing neurodegenerative diseases.

Background of the invention

Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and Huntington's disease, remain an intractable health problem in the elderly. Dementia (including its most common form, Alzheimer's disease) is now the second leading cause of death in Australia, and no cure exists.

Notably, Alzheimer's disease is multi-faceted, with a number of coexisting and contributing pathological alterations including: (1) protein misfolding and β -amyloid aggregation with plaque formation; (2) oxidative stress and reactive oxygen species (ROS) generation; (3) dysregulation of autophagy, which limits the recycling of misfolded proteins; (4) dysregulation of metal metabolism; and (5) lowering of acetylcholine levels.

While the precise role of ROS in neurodegeneration is unclear, evidence does suggest that redox-active metals and ROS are involved. Notably, Alzheimer's disease is a disease of advancing age, and as iron levels also increase as a function of age, this may lead to increased ROS generation.

Senile plaques have remarkably high iron, copper and zinc levels, and copper and zinc ions are known to facilitate β -amyloid aggregation by modulating amyloid peptide conformation. The role of iron in Alzheimer's disease is further supported by the fact that expression of amyloid precursor protein (APP) is up-regulated by increased cellular iron (as APP plays an integral role in iron efflux from neuronal cells).

These data led to the assessment of various iron chelators for treatment of Alzheimer's disease. One such chelator (clioquinol; CQ) binds copper and zinc ions, is able to cross the blood brain barrier (BBB) and clinically attenuates cognitive loss. However, clioquinol is associated with myelinopathies and does not decrease brain β -amyloid.

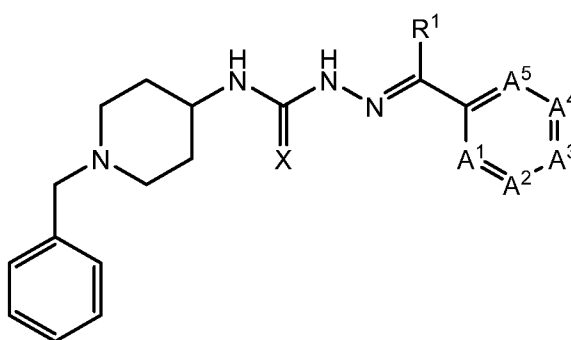
Therefore, there is still a need for agents that are effective in treating and/or preventing neurodegenerative diseases.

Reference to any prior art in the specification is not an acknowledgment or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be understood, regarded as relevant, and/or combined with other pieces of prior art by a skilled person in the art.

Summary of the invention

The present inventors have surprisingly found that the issues with current agents used in neurodegenerative therapy could be overcome by developing one multifunctional agent that is able to target the main hallmarks of neurodegenerative diseases.

In a first aspect, the present invention relates to a compound of formula (I):



or a pharmaceutically acceptable salt or prodrug thereof, wherein:

X is selected from O and S,

R¹ is selected from H, alkyl and heteroaryl,

A¹, A², A³, A⁴ and A⁵ are independently selected from CH and N, and

the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is optionally substituted with one or more substituents independently selected from halogen, OH, alkyl and heteroalkyl, and is optionally fused to an aryl group.

X may be S.

5 R¹ may be H. R¹ may be C₁ to C₃ alkyl (*i.e.*, methyl, ethyl or propyl). R¹ may be a heteroaryl group (*e.g.*, pyridine). Preferably, R¹ is H or C₁ to C₃ alkyl.

The aromatic ring containing A¹, A², A³, A⁴ and A⁵ may be substituted. At least one of the substituents may be OH (*e.g.*, one or more of A¹, A², A³, A⁴ and A⁵ may be C-OH). The aromatic ring containing A¹, A², A³, A⁴ and A⁵ may be substituted with one
10 or more additional substituents (*i.e.*, additional to OH). The aromatic ring containing A¹, A², A³, A⁴ and A⁵ may be substituted with one additional substituent, such as OH. The one additional substituent may be halogen (*e.g.*, bromine, chlorine or fluorine). The one additional substituent may be an alkyl group (*e.g.*, C₁ to C₃ alkyl). The one additional substituent may be a heteroalkyl group *e.g.*, O-alkyl (such as OCH₃, OCH₂CH₃) or
15 OCF₃. The aromatic ring containing A¹, A², A³, A⁴ and A⁵ may be substituted with two additional substituents, which may be the same or different (*e.g.*, two halogens, which may be the same or different, two OH groups, or one alkyl group and one heteroalkyl group).

One of A¹, A², A³, A⁴ and A⁵ may be N *i.e.*, the aromatic ring containing A¹, A²,
20 A³, A⁴ and A⁵ may be a heteroaryl group. The heteroaryl group may not be further substituted. Alternatively, the heteroaryl group may be substituted with one or more substituents. The one or more substituents may be independently selected from OH, an alkyl group (*e.g.*, methyl or ethyl) and a heteroalkyl group (*e.g.*, CH₂OH or CH₂CH₂OH).

The N and/or OH substituents may be situated on the aromatic ring containing
25 A¹, A², A³, A⁴ and A⁵ such that the compound of formula (I) is able to chelate metal ions (*e.g.*, iron, copper and zinc). Therefore, A¹ may be selected from N and C-OH. A¹ may be C-OH.

The aromatic ring containing A¹, A², A³, A⁴ and A⁵ may be fused (*e.g.*, at A² and A³ or A⁴ and A⁵) to an aryl group, to give a bicyclic aryl or heteroaryl group *e.g.*,
30 naphthyl or quinoline. The bicyclic aryl or heteroaryl group may be substituted with one or more substituents selected from OH, alkyl and heteroalkyl.

In a second aspect, the present invention relates to a pharmaceutical composition including a compound of formula (I) (according to the first aspect of the invention) together with a pharmaceutically acceptable carrier, diluent or excipient.

Compounds and pharmaceutical compositions according to the present invention
5 may be suitable for treating and/or preventing neurodegenerative diseases. Accordingly, in another aspect, the present invention relates to a method of treating and/or preventing a neurodegenerative disease in a subject, the method including administering to the subject an effective amount of a compound of formula (I) according to the first aspect of the invention or a pharmaceutical composition according to the
10 second aspect of the invention.

In a further aspect the present invention relates to the use of a compound of formula (I) according to the first aspect of the invention or a pharmaceutical composition according to the second aspect of the invention in the manufacture of a medicament for treating and/or preventing a neurodegenerative disease.

15 In a further aspect the present invention relates to the use of a compound of formula (I) according to the first aspect of the invention or a pharmaceutical composition according to the second aspect of the invention for the treatment and/or prevention of a neurodegenerative diseases in a subject.

In a further aspect the present invention relates to a compound of formula (I)
20 according to the first aspect of the invention or a pharmaceutical composition according to the second aspect of the invention for use in the treatment and/or prevention of a neurodegenerative diseases in a subject.

The neurodegenerative disease may be selected from dementia, Alzheimer's disease, Parkinson's disease, Huntington's disease and Friedreich's ataxia.

25 The compounds of formula (I) may be used in therapy alone or in combination with one or more other therapeutic agents, for example, as part of a combination therapy.

Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following
30 description, given by way of example and with reference to the accompanying drawings.

Brief description of the drawings

Figure 1. Effect of compounds 1-8 on ^{59}Fe mobilisation from prelabelled SK-N-MC cells. Results are presented as the mean $\pm$ SD of three independent experiments. *** $p < 0.001$ versus the control. ### $p < 0.001$ versus Dp44mT. ** $p < 0.01$, * $p < 0.001$ versus DFO.

Figure 2. Effect of compounds 1-8 on ^{59}Fe uptake from ^{59}Fe -transferrin ($^{59}\text{Fe}_2\text{-Tf}$) by SK-N-MC neuroepithelioma cells. Results are expressed as the mean $\pm$ SD of three independent experiments. ** $p < 0.01$, *** $p < 0.001$ versus the control. ### $p < 0.001$ versus Dp44mT. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus DFO.

Figure 3. The anti-proliferative activity of compounds 1-8 as determined by the MTT cell proliferation assay. Results are presented as IC_{50} values representing mean $\pm$ SD (three experiments).

Figure 4. Effect of iron complexes of compounds 1-8 on the oxidation of ascorbate. Results are mean $\pm$ SD (3 experiments). Compounds 6 and 7 were not determined (N.D.) due to their low solubility. ** $p < 0.01$, *** $p < 0.001$ versus the control. °°° $p < 0.001$ versus EDTA. ### $p < 0.001$ versus Dp44mT. *** $p < 0.001$ versus DFO.

Figure 5. Compounds 1-8 protect SK-N-MC neuroepithelioma cells against hydrogen peroxide-mediated cytotoxicity. Results are expressed as mean $\pm$ S.E.M. (3 experiments) as a percentage of the untreated control. * $p < 0.05$, *** $p < 0.001$ versus the control. ## $p < 0.01$, ### $p < 0.001$ versus control cells incubated with H_2O_2 .

Figure 6. The IC_{50} values of compounds 1-8 in terms of their inhibition of AChE. Results are of mean $\pm$ SD (one independent experiment) completed in quadruplicate. ** $p < 0.01$ and *** $p < 0.001$ versus donepezil. ## $p < 0.01$, ### $p < 0.001$ versus tacrine.

Figure 7. The effect of compounds 1-8 on inhibiting the Cu(II) -mediated aggregation of $\text{A}\beta_{1-40}$. Results are presented as mean $\pm$ S.E.M. (3 independent experiments) using quadruplicates in each experiment. * $p < 0.05$, *** $p < 0.001$ versus $\text{A}\beta + \text{Cu(II)}$; ### $p < 0.001$ versus $\text{A}\beta$.

Figure 8. The effect of compounds 1-8 on the levels of the autophagic marker p62 in SK-N-MC neuroepithelioma cells. (A, C, E, G) Western blots show the effect of compounds 1-8 (25 μ M) on the levels of p62 that is a marker of autophagy after a 24 h incubation at 37°C. **(B, D, F, H)** Densitometry of p62 protein levels relative to the corresponding loading control (β -actin). Results are presented as mean $\pm$ SD (3 independent experiments). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ *versus* control.

Figure 9. The compounds 1, 4, 6 and 8 stimulate autophagic flux in SK-N-MC neuroepithelioma cells as measured using the autophagy inhibitor, Bafilomycin A1 (Baf A1). **(A)** Schematic diagram demonstrating the mechanisms by which total LC3-II protein levels can be altered as a consequence of changes in autophagosomal flux. **(i)** Under basal conditions, the newly formed autophagosomes are degraded upon fusion with the lysosome, leaving LC3-II levels unaltered. **(ii)** Autophagosome degradation is inhibited in the presence of Baf A1, which prevents autophagosome-lysosome fusion and leads to the accumulation of LC3-II. **(iii)** In the presence Baf A1, as well as an agent that induces autophagy (*e.g.*, Dp44mT), autophagosome formation is increased relative to basal levels and leads to further accumulation of LC3-II relative to Baf A1 alone. **(B, D, F, H)** Western blots showing the effect of compounds 1-8 (25 μ M) on the levels of LC3-II (18 kDa) after a 24 h incubation at 37°C in the presence or absence of the late-stage autophagy inhibitor, Baf A1 (100 nM). **(C, E, G, I)** Densitometry of LC3-II protein levels relative to the corresponding loading control (β -actin). Results are presented as mean $\pm$ SD (3 experiments). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ *versus* control. # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ *versus* the Baf A1 control.

Detailed description of the embodiments

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

Compounds are generally described herein using standard nomenclature. For compounds having asymmetric centres, it will be understood that, unless otherwise specified, all of the optical isomers and mixtures thereof are encompassed. Compounds with two or more asymmetric elements can also be present as mixtures of

diastereomers. In addition, compounds with carbon-carbon double bonds may occur in *Z* and *E* forms, with all isomeric forms of the compounds being included in the present invention unless otherwise specified. Where a compound exists in various tautomeric forms, a recited compound is not limited to any one specific tautomer, but rather is intended to encompass all tautomeric forms. Recited compounds are further intended to encompass compounds in which one or more atoms are replaced with an isotope, *i.e.*, an atom having the same atomic number but a different mass number. By way of general example, and without limitation, isotopes of hydrogen include tritium and deuterium and isotopes of carbon include ^{11}C , ^{13}C , and ^{14}C .

Compounds according to the formula provided herein, which have one or more stereogenic centres, have an enantiomeric excess of at least 50%. For example, such compounds may have an enantiomeric excess of at least 60%, 70%, 80%, 85%, 90%, 95%, or 98%. Some embodiments of the compounds have an enantiomeric excess of at least 99%. It will be apparent that single enantiomers (optically active forms) can be obtained by asymmetric synthesis, synthesis from optically pure precursors, biosynthesis or by resolution of the racemates, for example, enzymatic resolution or resolution by conventional methods such as crystallization in the presence of a resolving agent, or chromatography, using, for example, a chiral HPLC column.

Certain compounds are described herein using a general formula that includes variables such as A^1 , A^2 , A^3 and R^1 . Unless otherwise specified, each variable within such a formula is defined independently of any other variable, and any variable that occurs more than one time in a formula is defined independently at each occurrence. Therefore, for example, if a group is shown to be substituted with 0, 1 or 2 R^* , the group may be unsubstituted or substituted with up to two R^* groups and R^* at each occurrence is selected independently from the definition of R^* . Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds, *i.e.*, compounds that can be isolated, characterized and tested for biological activity.

A "pharmaceutically acceptable salt" of a compound disclosed herein is an acid or base salt that is generally considered in the art to be suitable for use in contact with the tissues of human beings or animals without excessive toxicity or carcinogenicity,

and preferably without irritation, allergic response, or other problem or complication. Such salts include mineral and organic acid salts of basic residues such as amines, as well as alkali or organic salts of acidic residues such as carboxylic acids.

Suitable pharmaceutically acceptable salts include, but are not limited to, salts of acids such as hydrochloric, phosphoric, hydrobromic, malic, glycolic, fumaric, sulfuric, sulfamic, sulfanilic, formic, toluenesulfonic, methanesulfonic, benzenesulfonic, ethane disulfonic, 2-hydroxyethylsulfonic, nitric, benzoic, 2-acetoxybenzoic, citric, tartaric, lactic, stearic, salicylic, glutamic, ascorbic, pantoic, succinic, fumaric, maleic, propionic, hydroxymaleic, hydroiodic, phenylacetic, alkanolic (such as acetic, $\text{HOOC}-(\text{CH}_2)_n-\text{COOH}$ where n is any integer from 0 to 6, *i.e.*, 0, 1, 2, 3, 4, 5 or 6), and the like. Similarly, pharmaceutically acceptable cations include, but are not limited to sodium, potassium, calcium, aluminum, lithium and ammonium. A person skilled in the art will recognize further pharmaceutically acceptable salts for the compounds provided herein. In general, a pharmaceutically acceptable acid or base salt can be synthesized from a parent compound that contains a basic or acidic moiety by any conventional chemical method. Briefly, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two. Generally, the use of nonaqueous media, such as ether, ethyl acetate, ethanol, isopropanol or acetonitrile, is preferred.

It will be apparent that each compound of formula (I) may, but need not, be present as a hydrate, solvate or non-covalent complex. In addition, the various crystal forms and polymorphs are within the scope of the present invention, as are prodrugs of the compounds of formula (I) provided herein.

A "prodrug" is a compound that may not fully satisfy the structural requirements of the compounds provided herein, but is modified *in vivo*, following administration to a subject or patient, to produce a compound of formula (I) provided herein. For example, a prodrug may be an acylated derivative of a compound as provided herein. Prodrugs include compounds wherein hydroxy, carboxy, amine or sulfhydryl groups are bonded to any group that, when administered to a mammalian subject, cleaves to form a free hydroxy, carboxy, amino, or sulfhydryl group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate, phosphate and benzoate derivatives of

alcohol and amine functional groups within the compounds provided herein. Prodrugs of the compounds provided herein may be prepared by modifying functional groups present in the compounds in such a way that the modifications are cleaved *in vivo* to generate the parent compounds.

5 A "substituent" as used herein, refers to a molecular moiety that is covalently bonded to an atom within a molecule of interest. For example, a "ring substituent" may be a moiety such as a halogen, alkyl group, heteroalkyl group, haloalkyl group or other substituent described herein that is covalently bonded to an atom, preferably a carbon or nitrogen atom, that is a ring member. The term "substituted," as used herein, means
10 that any one or more hydrogens on the designated atom is replaced with a selection from the indicated substituents, provided that the designated atom's normal valence is not exceeded, and that the substitution results in a stable compound, *i.e.*, a compound that can be isolated, characterized and tested for biological activity. When a substituent is oxo, *i.e.*, =O, then two hydrogens on the atom are replaced. An oxo group that is a
15 substituent of an aromatic carbon atom results in a conversion of -CH- to -C(=O)- and a loss of aromaticity. For example a pyridyl group substituted by oxo is a pyridone. Examples of suitable substituents are alkyl (including haloalkyl *e.g.*, CF₃), heteroalkyl, halogen (for example, fluorine, chlorine, bromine or iodine atoms), C(O)OH, C(O)H, OH, =O, SH, SO₃H, NH₂, NH-alkyl, =NH, N₃ and NO₂ groups.

20 The term "alkyl" refers to a saturated, straight-chain or branched hydrocarbon group that contains from 1 to 20 carbon atoms, preferably from 1 to 10 carbon atoms, for example a *n*-octyl group, especially from 1 to 6, *i.e.*, 1, 2, 3, 4, 5, or 6, carbon atoms. Specific examples of alkyl groups are methyl, ethyl, propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, *iso*-pentyl, *n*-hexyl and 2,2-dimethylbutyl.

25 The term "heteroalkyl" refers to an alkyl group as defined above that contains one or more heteroatoms selected from oxygen, nitrogen and sulphur (especially oxygen and nitrogen). Specific examples of heteroalkyl groups are methoxy, trifluoromethoxy, ethoxy, *n*-propyloxy, *iso*-propyloxy, butoxy, *tert*-butyloxy, methoxymethyl, ethoxymethyl, -CH₂CH₂OH, -CH₂OH, methoxyethyl, 1-methoxyethyl, 1-ethoxyethyl, 2-methoxyethyl or
30 2-ethoxyethyl, methylamino, ethylamino, propylamino, *iso*-propylamino, dimethylamino, diethylamino, *iso*-propyl-ethylamino, methylamino methyl, ethylamino methyl, di-*iso*-

propylamino ethyl, methylthio, ethylthio, *iso*-propylthio, enol ether, dimethylamino methyl, dimethylamino ethyl, acetyl, propionyl, butyryloxy, acetyloxy, methoxycarbonyl, ethoxycarbonyl, propionyloxy, acetylamino, propionylamino, carboxymethyl, carboxyethyl, carboxypropyl, *N*-ethyl-*N*-methylcarbamoyl and *N*-methylcarbamoyl.

- 5 Further examples of heteroalkyl groups are nitrile, *iso*-nitrile, cyanate, thiocyanate, *iso*-cyanate, *iso*-thiocyanate and alkyl nitrile groups.

The term "aryl" refers to an aromatic group that contains one or more rings containing from 6 to 14 ring carbon atoms, preferably from 6 to 10 (especially 6) ring carbon atoms. Examples are phenyl, naphthyl and biphenyl groups.

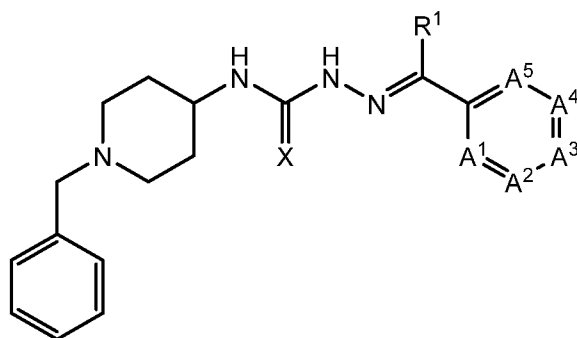
- 10 The term "heteroaryl" refers to an aromatic group that contains one or more rings containing from 5 to 14 ring atoms, preferably from 5 to 10 (especially 5 or 6) ring atoms, where one or more of the ring atoms are replaced with one or more (preferably 1, 2, 3 or 4) oxygen, nitrogen, phosphorus or sulfur ring atoms (preferably O, S or N). Examples are pyridine, imidazole, thiazole, *iso*-thiazole, 1,2,3-triazole, 1,2,4-triazole, 15 oxadiazole, thiadiazole, indole, indazole, tetrazole, pyrazine, pyrimidine, pyridazine, oxazole, isoxazole, triazole, tetrazole, isoxazole, indazole, benzimidazole, benzoxazole, benzisoxazole, benzthiazole, pyridazine, quinoline, isoquinoline, pyrrole, purine, carbazole, acridine, and *iso*-quinoline groups.

- The expression "halogen" or "halogen atom" as used herein means fluorine, 20 chlorine, bromine, or iodine.

- The term "optionally substituted" refers to a group in which one, two, three or more hydrogen atoms have been replaced independently of each other by, for example, halogen (for example, fluorine, chlorine, bromine or iodine atoms), C(O)OH, C(O)H, OH, =O, SH, =S, SO₃H, NH₂, NH-alkyl, =NH, N₃ or NO₂ groups. This expression also refers 25 to a group that is substituted by one, two, three or more alkyl or heteroalkyl groups. These groups may themselves be substituted. For example, an alkyl group substituent may be substituted by one or more halogen atoms (*i.e.*, may be a haloalkyl group). The term "haloalkyl" refers to an alkyl group (as defined above) that is substituted by one or more halogen atoms (as also defined above). Specific examples of haloalkyl groups are 30 trifluoromethyl, dichloroethyl, dichloromethyl and iodoethyl.

As used herein a wording defining the limits of a range of length such as, for example, "from 1 to 5" means any integer from 1 to 5, i. e. 1, 2, 3, 4 and 5. In other words, any range defined by two integers explicitly mentioned is meant to comprise and disclose any integer defining said limits and any integer comprised in said range.

5 As discussed above, the present invention relates to a compound of formula (I):



or a pharmaceutically acceptable salt or prodrug thereof, wherein:

X is selected from O and S,

R¹ is selected from H, alkyl and heteroaryl,

10 A¹, A², A³, A⁴ and A⁵ are independently selected from CH and N, and

the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is optionally substituted with one or more substituents independently selected from halogen, OH, alkyl and heteroalkyl, and is optionally fused to an aryl group.

X may be S.

15 R¹ may be H. R¹ may be C₁ to C₃ alkyl (*i.e.*, methyl, ethyl or propyl). R¹ may be a heteroaryl group (*e.g.*, pyridine). Preferably, R¹ is H or C₁ to C₃ alkyl.

The aromatic ring containing A¹, A², A³, A⁴ and A⁵ may be substituted. At least one of the substituents may be OH (*e.g.*, one or more of A¹, A², A³, A⁴ and A⁵ may be C-OH). The aromatic ring containing A¹, A², A³, A⁴ and A⁵ may be substituted with one or more additional substituents (*i.e.*, additional to OH). The aromatic ring containing A¹, A², A³, A⁴ and A⁵ may be substituted with one additional substituent, such as OH. The one additional substituent may be halogen (*e.g.*, bromine, chlorine or fluorine). The one additional substituent may be an alkyl group (*e.g.*, C₁ to C₃ alkyl). The one additional substituent may be a heteroalkyl group *e.g.*, O-alkyl (such as OCH₃, OCH₂CH₃) or

OCF₃. The aromatic ring containing A¹, A², A³, A⁴ and A⁵ may be substituted with two additional substituents, which may be the same or different (*e.g.*, two halogens, which may be the same or different, two OH groups, or one alkyl group and one heteroalkyl group).

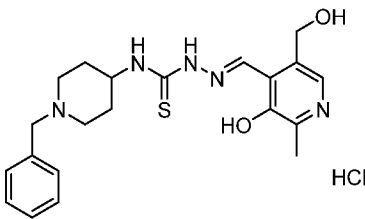
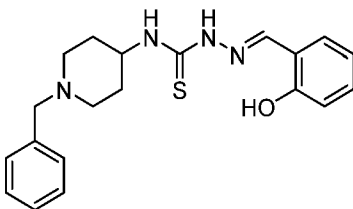
5 One of A¹, A², A³, A⁴ and A⁵ may be N *i.e.*, the aromatic ring containing A¹, A², A³, A⁴ and A⁵ may be a heteroaryl group. The heteroaryl group may not be further substituted. Alternatively, the heteroaryl group may be substituted with one or more substituents. The one or more substituents may be independently selected from OH, an alkyl group (*e.g.*, methyl or ethyl) and a heteroalkyl group (*e.g.*, CH₂OH or CH₂CH₂OH).

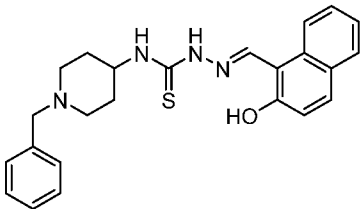
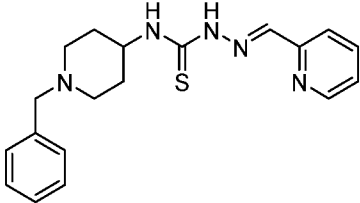
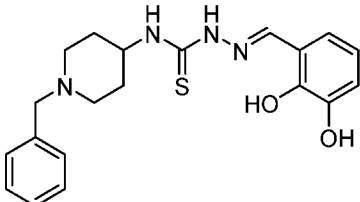
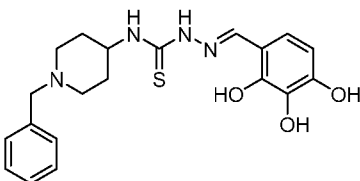
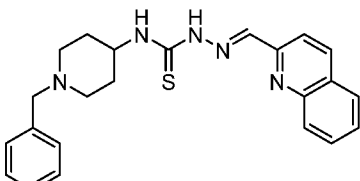
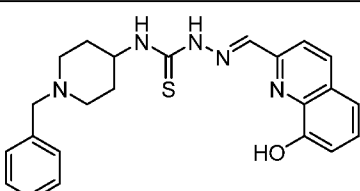
10 The N and/or OH substituents may be situated on the aromatic ring containing A¹, A², A³, A⁴ and A⁵ such that the compound of formula (I) is able to chelate metal ions (*e.g.*, iron, copper and zinc). Therefore, A¹ may be selected from N and C-OH. Preferably, A¹ is C-OH.

The aromatic ring containing A¹, A², A³, A⁴ and A⁵ may be fused (*e.g.*, at A² and A³ or A⁴ and A⁵) to an aryl group, to give a bicyclic aryl or heteroaryl group *e.g.*, naphthyl or quinoline. The bicyclic aryl or heteroaryl group may be substituted with one or more substituents selected from OH, alkyl and heteroalkyl.

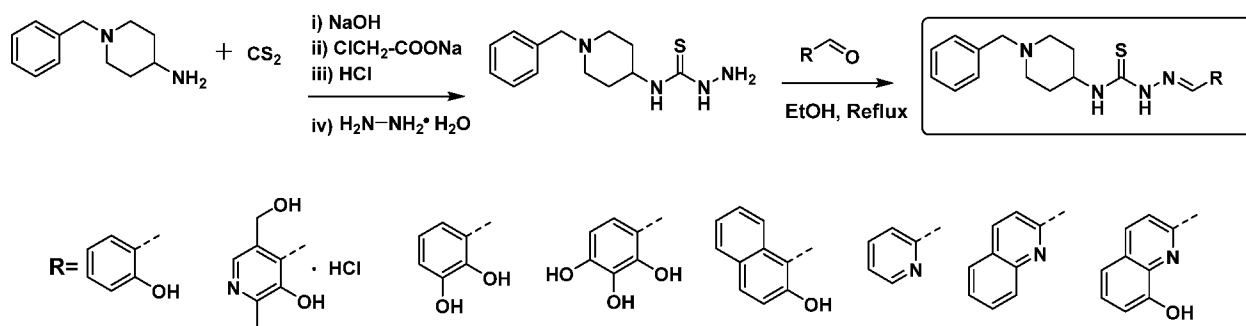
Specific examples of the compounds of the present invention are given in Table 1, below.

20 *Table 1. Examples of compounds of the present invention.*

Compound	Structure
1	
2	

Compound	Structure
3	
4	
5	
6	
7	
8	

The compounds of the present invention can be synthesised by any suitable method known to a person skilled in the art. One general synthesis is given below in Scheme 1.



Scheme 1

The compounds of the present invention are multifunctional agents that may be effective in treating and/or preventing a number of neurodegenerative disorders. The compounds of the present invention may achieve this by targeting the main hallmarks (such as protein misfolding and β -amyloid aggregation with plaque formation, oxidative stress and ROS generation, dysregulation of autophagy, dysregulation of metal metabolism, and lowering of acetylcholine levels) of neurodegenerative disorders.

As discussed above, increased iron, copper and/or zinc levels are thought to lead to β -amyloid aggregation with plaque formation, and ROS generation. Without wishing to be bound by theory, the present inventors postulate that the compounds of the present invention (in particular, the hydrazone/thiosemicarbazone moieties, in conjunction with the N or C-OH functionality on the aromatic ring) may be effective metal chelators. Therefore, the compounds of the present invention may be effective at preventing or ameliorating the aggregation of β -amyloid, and may also inhibit production of ROS, resulting in prevention or amelioration of ROS-induced cell death. Particularly preferred in this regard are compounds having a “hard” donor atom (such as O) at A¹ (e.g., in the form of a C-OH group), as such compounds strongly chelate Fe³⁺, thereby preventing reduction of Fe³⁺ to Fe²⁺ (Fe²⁺ is the iron species that is considered to be responsible for the aetiologies involved in neurodegenerative disorders).

The present inventors also postulate that the depletion of iron (*via* chelation of the metal) may induce pro-survival autophagy (specifically, beneficial clearing of protein aggregates).

In the brain, acetylcholine (a neurotransmitter) is produced in several locations, including the basal forebrain. Acetylcholine-producing cells in the basal forebrain are damaged in the early stages of Alzheimer's disease, which may contribute to the

memory impairments that are an early symptom of the disease. The present inventors postulate that, by including in the compounds of the present invention a moiety (such as the 1-benzylpiperidine group) that acts as a cholinesterase inhibitor, the decrease in acetylcholine levels that occurs *via* this pathway can be mitigated.

5 The compounds of the present invention are also highly lipophilic, which contributes to their blood-brain barrier (BBB) permeability.

 The present inventors have also found that the absence of substitution on the imine nitrogen results in the compounds of the present invention having lower cytotoxicity compared with compounds that are substituted at this position. Thus, while
10 compounds having substitution at the imine nitrogen may be useful in anti-cancer therapy, they would not be expected to be useful in other therapies (*e.g.*, for treatment of neurodegenerative disorders), due to their cytotoxicity.

 The therapeutic use of compounds of formula (I), their pharmaceutically acceptable salts, solvates or hydrates and also formulations and pharmaceutical
15 compositions (including mixtures of the compounds of formula (I)) are within the scope of the present invention. Accordingly, the present invention also relates to pharmaceutical compositions including a therapeutically effective amount of the compounds of formula (I), or its pharmaceutically acceptable salt, solvate or hydrate thereof, and one or more pharmaceutically acceptable excipients.

20 Pharmaceutical compositions may be formulated for any appropriate route of administration including, for example, topical (for example, transdermal or ocular), oral, buccal, nasal, vaginal, rectal or parenteral administration. The term parenteral as used herein includes subcutaneous, intradermal, intravascular (for example, intravenous), intramuscular, spinal, intracranial, intrathecal, intraocular, periocular, intraorbital,
25 intrasynovial and intraperitoneal injection, as well as any similar injection or infusion technique. In certain embodiments, compositions in a form suitable for oral use or parenteral use are preferred. Suitable oral forms include, for example, tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs.

30 Aqueous suspensions contain the active ingredient(s) in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include suspending agents such as sodium carboxymethylcellulose, methylcellulose,

hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as naturally-occurring phosphatides (for example, lecithin), condensation products of an alkylene oxide with fatty acids such as polyoxyethylene stearate, condensation products of ethylene oxide with long chain aliphatic alcohols such as heptadecaethyleneoxycetanol, condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol mono-oleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides such as polyethylene sorbitan monooleate. Aqueous suspensions may also comprise one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more colouring agents, one or more flavouring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and/or flavoring agents may be added to provide palatable oral preparations. Such suspensions may be preserved by the addition of an antioxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, such as sweetening, flavouring and colouring agents, may also be present.

Pharmaceutical compositions may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil such as olive oil or arachis oil, a mineral oil such as liquid paraffin, or a mixture thereof. Suitable emulsifying agents include naturally-occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soy bean lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides such as sorbitan monooleate, and condensation products of partial esters derived from fatty acids and hexitol with ethylene oxide such as

polyoxyethylene sorbitan monoleate. An emulsion may also comprise one or more sweetening and/or flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, such as glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also comprise one or more
5 demulcents, preservatives, flavouring agents and/or colouring agents.

A composition may further include one or more components adapted to improve the stability or effectiveness of the applied formulation, such as stabilizing agents, suspending agents, emulsifying agents, viscosity adjusters, gelling agents, preservatives, antioxidants, skin penetration enhancers, moisturizers and sustained
10 release materials. Examples of such components are described in Martindale – The Extra Pharmacopoeia (Pharmaceutical Press, London 1993) and Martin (ed.), Remington's Pharmaceutical Sciences. Formulations may comprise microcapsules, such as hydroxymethylcellulose or gelatin-microcapsules, liposomes, albumin microspheres, microemulsions, nanoparticles or nanocapsules.

15 A pharmaceutical composition may be formulated as inhaled formulations, including sprays, mists, or aerosols. For inhalation formulations, the compounds provided herein may be delivered *via* any inhalation methods known to a person skilled in the art. Such inhalation methods and devices include, but are not limited to, metered dose inhalers with propellants such as CFC or HFA or propellants that are
20 physiologically and environmentally acceptable. Other suitable devices are breath operated inhalers, multidose dry powder inhalers and aerosol nebulizers. Aerosol formulations for use in the subject method typically include propellants, surfactants and co-solvents and may be filled into conventional aerosol containers that are closed by a suitable metering valve.

25 Inhalant compositions may comprise liquid or powdered compositions containing the active ingredient that are suitable for nebulization and intrabronchial use, or aerosol compositions administered *via* an aerosol unit dispensing metered doses. Suitable liquid compositions comprise the active ingredient in an aqueous, pharmaceutically acceptable inhalant solvent such as isotonic saline or bacteriostatic water. The solutions
30 are administered by means of a pump or squeeze-actuated nebulized spray dispenser, or by any other conventional means for causing or enabling the requisite dosage amount of the liquid composition to be inhaled into the patient's lungs. Suitable

formulations, wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Pharmaceutical compositions may also be prepared in the form of suppositories such as for rectal administration. Such compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Suitable excipients include, for example, cocoa butter and polyethylene glycols.

Pharmaceutical compositions may be formulated as sustained release formulations such as a capsule that creates a slow release of modulator following administration. Such formulations may generally be prepared using well-known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Carriers for use within such formulations are biocompatible, and may also be biodegradable. Preferably, the formulation provides a relatively constant level of modulator release. The amount of modulator contained within a sustained release formulation depends upon, for example, the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

For the treatment of the conditions discussed herein, the dose of the biologically active compound according to the invention may vary within wide limits and may be adjusted to individual requirements. Active compounds according to the present invention are generally administered in a therapeutically effective amount. Preferred doses range from about 0.1 mg to about 140 mg per kilogram of body weight per day (*e.g.*, about 0.5 mg to about 7 g per patient per day). The daily dose may be administered as a single dose or in a plurality of doses. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. Dosage unit forms will generally contain between about 1 mg to about 500 mg of an active ingredient.

However, it will be understood, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination (*i.e.*,

other drugs being used to treat the patient), and the severity of the particular disorder undergoing therapy.

The terms “therapeutically effective amount” or “effective amount” refer to an amount of the compound of formula (I) that results in an improvement or remediation of the neurodegenerative disease.

Preferred compounds of the invention will have certain pharmacological properties. Such properties include, but are not limited to oral bioavailability and BBB permeability, such that the preferred oral dosage forms discussed above can provide therapeutically effective levels of the compound *in vivo*.

The compounds of the present invention are preferably administered to a patient (for example, a human) orally or parenterally, and are present within at least one body fluid or tissue of the patient. Accordingly, the present invention further provides methods for treating and/or preventing neurodegenerative diseases.

As used herein, the term “treatment” encompasses both disorder-modifying treatment and symptomatic treatment. It refers to therapeutic treatment, *i.e.*, after the onset of symptoms, in order to reduce the severity and/or duration of symptoms, and/or to cure the condition or disorder. As used herein, the term “prevention” encompasses prophylactic treatment, *i.e.*, before the onset of symptoms, in order to prevent, delay or reduce the severity of symptoms and/or the condition or disorder. Patients may include but are not limited to primates, especially humans, domesticated companion animals such as dogs, cats, horses, and livestock such as cattle, pigs, sheep, and poultry, with dosages as described herein.

Compounds of the present invention may be useful for the treatment and/or prevention of neurodegenerative diseases in a subject. Accordingly, the present invention also relates to a method of treating or preventing neurodegenerative diseases in a patient including administration to the patient of a therapeutically effective amount of a compound of formula (I), or a pharmaceutically-acceptable salt, solvate or hydrate thereof. The present invention also relates to the use of a therapeutically effective amount of a compound of formula (I), or a pharmaceutically-acceptable salt, solvate or hydrate thereof, for treating or preventing neurodegenerative diseases. The present invention also provides a pharmaceutical composition for use in treating or preventing neurodegenerative diseases, in any of the embodiments described in the specification.

The present invention also relates to the use of a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt, solvate or hydrate thereof, for the manufacture of a medicament for treating or preventing neurodegenerative diseases.

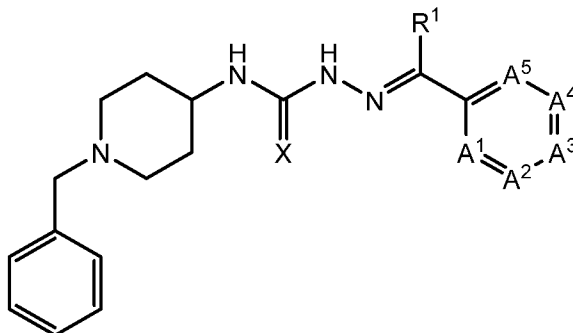
5 The present invention also relates to a compound of formula (I), or a pharmaceutically acceptable salt, solvate or hydrate thereof, when used in a method of treating or preventing neurodegenerative diseases. The present invention also relates to a composition having an active ingredient for use in treating or preventing neurodegenerative diseases, wherein the active ingredient is a compound of formula (I),
10 or a pharmaceutically acceptable salt, solvate or hydrate thereof. The present invention also relates to the use of a pharmaceutical composition containing a compound of the formula (I), or a pharmaceutically acceptable salt, solvate or hydrate thereof, in treating or preventing neurodegenerative diseases, such as described above. In one embodiment, the compound of formula (I) is essentially the only active ingredient of the
15 composition. In one embodiment, the neurodegenerative disease is selected from dementia, Alzheimer's disease, Parkinson's disease, Huntington's disease and Friedreich's ataxia.

It is also within the present invention that the compounds according to the invention are used as or for the manufacture of a diagnostic agent, whereby such
20 diagnostic agent is for the diagnosis of the disorders and conditions which can be addressed by the compounds of the present invention for therapeutic purposes as disclosed herein.

For various applications, the compounds of the invention can be labelled by isotopes, fluorescence or luminescence markers, antibodies or antibody fragments, any
25 other affinity label like nanobodies, aptamers, peptides etc., enzymes or enzyme substrates. These labelled compounds of this invention are useful for mapping the location of receptors *in vivo*, *ex vivo*, *in vitro* and *in situ* such as in tissue sections *via* autoradiography and as radiotracers for positron emission tomography (PET) imaging, single photon emission computerized tomography (SPECT) and the like, to characterize
30 those receptors in living subjects or other materials. The labelled compounds according to the present invention may be used in therapy, diagnosis and other applications such as research tools *in vivo* and *in vitro*, in particular the applications disclosed herein.

Embodiments of the invention will now be discussed.

In a first embodiment, the present invention relates to a compound of formula (I):



5 or a pharmaceutically acceptable salt or prodrug thereof, wherein:

X is selected from O and S,

R¹ is selected from H, alkyl and heteroaryl,

A¹, A², A³, A⁴ and A⁵ are independently selected from CH and N, and

10 the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is optionally substituted with one or more substituents independently selected from halogen, OH, alkyl and heteroalkyl, and is optionally fused to an aryl group.

In a second embodiment, the present invention relates to a compound of formula (I) according to the first embodiment, wherein X is S.

15 In a third embodiment, the present invention relates to a compound of formula (I) according to the first or second embodiment, wherein R¹ is H.

In a fourth embodiment, the present invention relates to compound of formula (I) according to the first or second embodiment, wherein R¹ is C₁ to C₃ alkyl.

In a fifth embodiment, the present invention relates to compound of formula (I) according to the fourth embodiment, wherein R¹ is methyl, ethyl or propyl.

20 In a sixth embodiment, the present invention relates to compound of formula (I) according to the first or second embodiment, wherein R¹ is a heteroaryl group.

In a seventh embodiment, the present invention relates to compound of formula (I) according to the sixth embodiment, wherein R¹ is pyridine.

In an eighth embodiment, the present invention relates to compound of formula (I) according to any of the first to seventh embodiments, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted.

5 In a ninth embodiment, the present invention relates to compound of formula (I) according to the eighth embodiment, wherein at least one substituent is OH.

In a tenth embodiment, the present invention relates to compound of formula (I) according to the ninth embodiment, wherein one or more of A¹, A², A³, A⁴ and A⁵ is C-OH.

10 In an eleventh embodiment, the present invention relates to compound of formula (I) according to the ninth or tenth embodiments, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted with one or more additional substituents.

In a twelfth embodiment, the present invention relates to compound of formula (I) according to the eleventh embodiment, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted with one additional substituent.

15 In a thirteenth embodiment, the present invention relates to compound of formula (I) according to the twelfth embodiment, wherein the one additional substituent is OH.

In a fourteenth embodiment, the present invention relates to compound of formula (I) according to the eleventh embodiment, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted with two additional substituents.

20 In a fifteenth embodiment, the present invention relates to compound of formula (I) according to the fourteenth embodiment, wherein the two additional substituents are different to each other.

In a sixteenth embodiment, the present invention relates to compound of formula (I) according to the fourteenth embodiment, wherein the two additional substituents are
25 the same as each other.

In a seventeenth embodiment, the present invention relates to compound of formula (I) according to any of the fourteenth to sixteenth embodiments, wherein the two additional substituents are selected from OH, an alkyl group and a heteroalkyl group.

In an eighteenth embodiment, the present invention relates to compound of formula (I) according to any of the first to seventeenth embodiments, wherein one of A¹, A², A³, A⁴ and A⁵ is N to give a heteroaryl group.

5 In a nineteenth embodiment, the present invention relates to compound of formula (I) according to the eighteenth embodiment, wherein the heteroaryl group is substituted with one or more substituents.

In a twentieth embodiment, the present invention relates to compound of formula (I) according to the nineteenth embodiment, wherein the one or more substituents are independently selected from OH, an alkyl group and a heteroalkyl group.

10 In a twenty-first embodiment, the present invention relates to compound of formula (I) according to the twentieth embodiment, wherein the alkyl group is methyl or ethyl.

In a twenty-second embodiment, the present invention relates to compound of formula (I) according to the twentieth embodiment, wherein the heteroalkyl group is
15 CH₂OH or CH₂CH₂OH.

In a twenty-third embodiment, the present invention relates to compound of formula (I) according to any of the first to twenty-second embodiments, wherein A¹ is selected from N and C-OH.

20 In a twenty-fourth embodiment, the present invention relates to compound of formula (I) according to the twenty-third embodiment, wherein A¹ is C-OH.

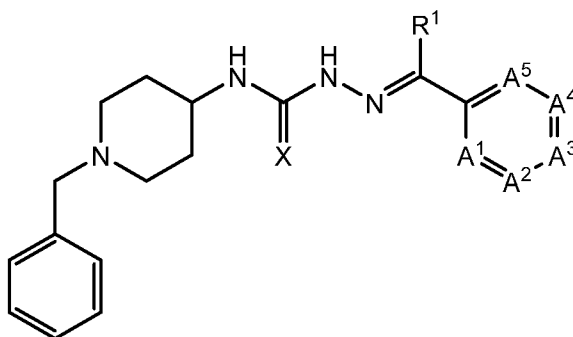
In a twenty-fifth embodiment, the present invention relates to compound of formula (I) according to any of the first to twenty-fourth embodiments, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is fused to an aryl group to give a bicyclic aryl or heteroaryl group.

25 In a twenty-sixth embodiment, the present invention relates to compound of formula (I) according to the twenty-fifth embodiment, wherein the bicyclic aryl or heteroaryl group is naphthyl or quinoline.

In a twenty-seventh embodiment, the present invention relates to compound of formula (I) according to the twenty-fifth or twenty-sixth embodiments, wherein the
30 bicyclic aryl or heteroaryl group is substituted by one or more substituents selected from OH, alkyl and heteroalkyl.

In a twenty-eighth embodiment, the present invention relates to compound of formula (I) according to the twenty-fifth or twenty-seventh embodiments, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is fused to the aryl group at A² and A³ or A⁴ and A⁵.

- 5 In a twenty-ninth embodiment, the present invention relates to a pharmaceutical composition including a compound of formula (I):



or a pharmaceutically acceptable salt or prodrug thereof, wherein:

- 10 X is selected from O and S,

R¹ is selected from H, alkyl and heteroaryl,

A¹, A², A³, A⁴ and A⁵ are independently selected from CH and N, and

- the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is optionally substituted with one or more substituents independently selected from halogen, OH, alkyl and
15 heteroalkyl, and is optionally fused to an aryl group,

together with a pharmaceutically acceptable carrier, diluent or excipient.

In a thirtieth embodiment, the present invention relates to a pharmaceutical composition according to the twenty-ninth embodiment, wherein the composition is suitable for parenteral or oral administration.

- 20 In a thirty-first embodiment, the present invention relates to a pharmaceutical composition according to the twenty-ninth or thirtieth embodiment, wherein X is S.

In a thirty-second embodiment, the present invention relates to a pharmaceutical composition according to any of the twenty-ninth to thirty-first embodiments, wherein R¹ is H.

In a thirty-third embodiment, the present invention relates to a pharmaceutical composition according to any of the twenty-ninth to thirty-second embodiments, wherein R^1 is C_1 to C_3 alkyl.

5 In a thirty-fourth embodiment, the present invention relates to a pharmaceutical composition according to the thirty-third embodiment, wherein R^1 is methyl, ethyl or propyl.

In a thirty-fifth embodiment, the present invention relates to a pharmaceutical composition according to any of the twenty-ninth to thirty-first embodiments, wherein R^1 is a heteroaryl group.

10 In a thirty-sixth embodiment, the present invention relates to a pharmaceutical composition according to the thirty-fifth embodiment, wherein R^1 is pyridine.

In a thirty-seventh embodiment, the present invention relates to a pharmaceutical composition according to any of the twenty-ninth to thirty-sixth embodiments, wherein the aromatic ring containing A^1 , A^2 , A^3 , A^4 and A^5 is substituted.

15 In a thirty-eighth embodiment, the present invention relates to a pharmaceutical composition according to the thirty-seventh embodiment, wherein at least one substituent is OH.

In a thirty-ninth embodiment, the present invention relates to a pharmaceutical composition according to the thirty-eighth embodiment, wherein one or more of A^1 , A^2 , A^3 , A^4 and A^5 is C-OH.

20 In a fortieth embodiment, the present invention relates to a pharmaceutical composition according to the thirty-eighth or thirty-ninth embodiments, wherein the aromatic ring containing A^1 , A^2 , A^3 , A^4 and A^5 is substituted with one or more additional substituents.

25 In a forty-first embodiment, the present invention relates to a pharmaceutical composition according to the fortieth embodiment, wherein the aromatic ring containing A^1 , A^2 , A^3 , A^4 and A^5 is substituted with one additional substituent.

In a forty-second embodiment, the present invention relates to a pharmaceutical composition according to the forty-first embodiment, wherein the one additional substituent is OH.

30

In a forty-third embodiment, the present invention relates to a pharmaceutical composition according to the fortieth embodiment, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted with two additional substituents.

5 In a forty-fourth embodiment, the present invention relates to a pharmaceutical composition according to the forty-third embodiment, wherein the two additional substituents are different to each other.

In a forty-fifth embodiment, the present invention relates to a pharmaceutical composition according to the forty-third embodiment, wherein the two additional substituents are the same as each other.

10 In a forty-sixth embodiment, the present invention relates to a pharmaceutical composition according to any of the forty-third to forty-fifth embodiments, wherein the two additional substituents are selected from OH, an alkyl group and a heteroalkyl group.

15 In a forty-seventh embodiment, the present invention relates to a pharmaceutical composition according to any of the twenty-ninth to forty-sixth embodiments, wherein one of A¹, A², A³, A⁴ and A⁵ is N to give a heteroaryl group.

In a forty-eighth embodiment, the present invention relates to a pharmaceutical composition according to the forty-seventh embodiment, wherein the heteroaryl group is substituted with one or more substituents.

20 In a forty-ninth embodiment, the present invention relates to a pharmaceutical composition according to the forty-eighth embodiment, wherein the one or more substituents are independently selected from OH, an alkyl group and a heteroalkyl group.

25 In a fiftieth embodiment, the present invention relates to a pharmaceutical composition according to the forty-ninth embodiment, wherein the alkyl group is methyl or ethyl.

In a fifty-first embodiment, the present invention relates to a pharmaceutical composition according to the forty-ninth embodiment, wherein the heteroalkyl group is CH₂OH or CH₂CH₂OH.

In a fifty-second embodiment, the present invention relates to a pharmaceutical composition according to any of the twenty-ninth to fifty-first embodiment, wherein A¹ is selected from N and C-OH.

In a fifty-third embodiment, the present invention relates to a pharmaceutical composition according to the fifty-second embodiment, wherein A¹ is C-OH.

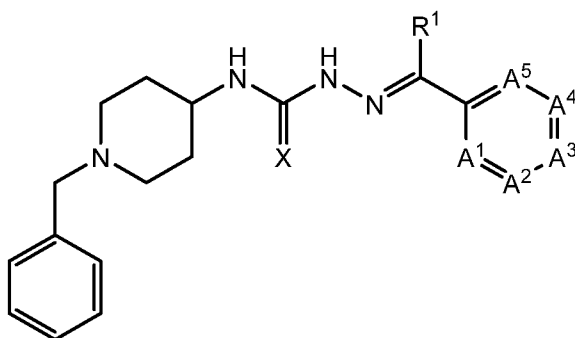
In a fifty-fourth embodiment, the present invention relates to a pharmaceutical composition according to any of the twenty-ninth to fifty-third embodiments, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is fused to an aryl group to give a bicyclic aryl or heteroaryl group.

In a fifty-fifth embodiment, the present invention relates to a pharmaceutical composition according to the fifty-fourth embodiment, wherein the bicyclic aryl or heteroaryl group is naphthyl or quinoline.

In a fifty-sixth embodiment, the present invention relates to a pharmaceutical composition according to the forty-ninth or fifty-fifth embodiment, wherein the bicyclic aryl or heteroaryl group is substituted by one or more substituents selected from OH, alkyl and heteroalkyl.

In a fifty-seventh embodiment, the present invention relates to a pharmaceutical composition according to any of the fifty-fourth to fifty-sixth embodiments, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is fused to the aryl group at A² and A³ or A⁴ and A⁵.

In a fifty-eighth embodiment, the present invention relates to a method of treating and/or preventing a neurodegenerative disease in a subject, the method including administering to the subject an effective amount of a compound of formula (I):



or a pharmaceutically acceptable salt or prodrug thereof, wherein:

X is selected from O and S,

R¹ is selected from H, alkyl and heteroaryl,

A¹, A², A³, A⁴ and A⁵ are independently selected from CH and N, and

5 the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is optionally substituted with one or more substituents independently selected from halogen, OH, alkyl and heteroalkyl, and is optionally fused to an aryl group.

In a fifty-ninth embodiment, the present invention relates to a method according to the fifty-eighth embodiment, wherein the neurodegenerative disease is selected from
10 dementia, Alzheimer's disease, Parkinson's disease, Huntington's disease and Friedreich's ataxia.

In a sixtieth embodiment, the present invention relates to a method according to the fifty-eighth or fifty-ninth embodiment, wherein X is S.

In a sixty-first embodiment, the present invention relates to a method according
15 to any of the fifty-eighth to sixtieth embodiments, wherein R¹ is H.

In a sixty-second embodiment, the present invention relates to a method according to any of the fifty-eighth to sixtieth embodiments, wherein R¹ is C₁ to C₃ alkyl.

In a sixty-third embodiment, the present invention relates to a method according to the sixty-second embodiment, wherein R¹ is methyl, ethyl or propyl.

20 In a sixty-fourth embodiment, the present invention relates to a method according to any of the fifty-eighth to sixtieth embodiments, wherein R¹ is a heteroaryl group.

In a sixty-fifth embodiment, the present invention relates to a method according to the sixty-fourth embodiment, wherein R¹ is pyridine.

In a sixty-sixth embodiment, the present invention relates to a method according
25 to any of the fifty-eighth to sixty-fifth embodiments, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted.

In a sixty-seventh embodiment, the present invention relates to a method according to the sixty-sixth embodiment, wherein at least one substituent is OH.

In a sixty-eighth embodiment, the present invention relates to a method according to sixty-seventh embodiment, wherein one or more of A¹, A², A³, A⁴ and A⁵ is C-OH.

5 In a sixty-ninth embodiment, the present invention relates to a method according to the sixty-seventh or sixty-eighth embodiment, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted with one or more additional substituents.

In a seventieth embodiment, the present invention relates to a method according to the sixty-ninth embodiment, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted with one additional substituent.

10 In a seventy-first embodiment, the present invention relates to a method according to the seventieth embodiment, wherein the one additional substituent is OH.

In a seventy-second embodiment, the present invention relates to a method according to the seventieth embodiment, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted with two additional substituents.

15 In a seventy-third embodiment, the present invention relates to a method according to the seventy-second embodiment, wherein the two additional substituents are different to each other.

In a seventy-fourth embodiment, the present invention relates to a method according to the seventy-second embodiment, wherein the two additional substituents
20 are the same as each other.

In a seventy-fifth embodiment, the present invention relates to a method according to any of the seventy-second to seventy-fourth embodiments, wherein the two additional substituents are selected from OH, an alkyl group and a heteroalkyl group.

25 In a seventy-sixth embodiment, the present invention relates to a method according to any of the fifty-eighth to seventy-fifth embodiments, wherein one of A¹, A², A³, A⁴ and A⁵ is N to give a heteroaryl group.

In a seventy-seventh embodiment, the present invention relates to a method according to the seventy-sixth embodiment, wherein the heteroaryl group is substituted with one or more substituents.

In a seventy-eighth embodiment, the present invention relates to a method according to the seventy-seventh embodiment, wherein the one or more substituents are independently selected from OH, an alkyl group and a heteroalkyl group.

In a seventy-ninth embodiment, the present invention relates to a method
5 according to the seventy-eighth embodiment, wherein the alkyl group is methyl or ethyl.

In an eightieth embodiment, the present invention relates to a method according to the seventy-eighth embodiment, wherein the heteroalkyl group is CH₂OH or CH₂CH₂OH.

In an eighty-first embodiment, the present invention relates to a method
10 according to any of the fifty-eighth to eightieth embodiments, wherein A¹ is selected from N and C-OH.

In an eighty-second embodiment, the present invention relates to a method according to the eighty-first embodiment, wherein A¹ is C-OH.

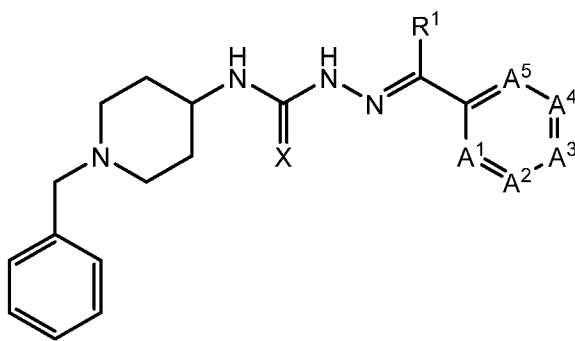
In an eighty-third embodiment, the present invention relates to a method
15 according to any of the fifty-eighth to eighty-second, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is fused to an aryl group to give a bicyclic aryl or heteroaryl group.

In an eighty-fourth embodiment, the present invention relates to a method according to the eighty-third embodiment, wherein the bicyclic aryl or heteroaryl group is
20 naphthyl or quinoline.

In an eighty-fifth embodiment, the present invention relates to a method according to the eighty-third or eighty-fourth embodiment, wherein the bicyclic aryl or heteroaryl group is substituted by one or more substituents selected from OH, alkyl and heteroalkyl.

25 In an eighty-sixth embodiment, the present invention relates to a method according to any of the eighty-third to eighty-fifth embodiments, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is fused to the aryl group at A² and A³ or A⁴ and A⁵.

In an eighty-seventh embodiment, the present invention relates to a method of treating and/or preventing a neurodegenerative disease in a subject, the method
30 including administering to the subject an effective amount of a pharmaceutical composition including a compound of formula (I):



or a pharmaceutically acceptable salt or prodrug thereof, wherein:

X is selected from O and S,

5 R^1 is selected from H, alkyl and heteroaryl,

A^1 , A^2 , A^3 , A^4 and A^5 are independently selected from CH and N, and

the aromatic ring containing A^1 , A^2 , A^3 , A^4 and A^5 is optionally substituted with one or more substituents independently selected from halogen, OH, alkyl and heteroalkyl, and is optionally fused to an aryl group,

10 together with a pharmaceutically acceptable carrier, diluent or excipient.

In an eighty-eighth embodiment, the present invention relates to a method according to the eighty-seventh embodiment, wherein the neurodegenerative disease is selected from dementia, Alzheimer's disease, Parkinson's disease, Huntington's disease and Friedreich's ataxia.

15 In an eighty-ninth embodiment, the present invention relates to a method according to the eighty-seventh or eighty-eighth embodiment, wherein X is S.

In a ninetieth embodiment, the present invention relates to a method according to any of the eighty-seventh to eighty-ninth embodiments, wherein R^1 is H.

20 In a ninety-first embodiment, the present invention relates to a method according to any of the eighty-seventh to eighty-ninth embodiments, wherein R^1 is C_1 to C_3 alkyl.

In a ninety-second embodiment, the present invention relates to a method according to the ninety-first embodiment, wherein R^1 is methyl, ethyl or propyl.

In a ninety-third embodiment, the present invention relates to a method according to any of the eighty-seventh to eighty-ninth embodiments, wherein R¹ is a heteroaryl group.

In a ninety-fourth embodiment, the present invention relates to a method
5 according to the ninety-third embodiment, wherein R¹ is pyridine.

In a ninety-fifth embodiment, the present invention relates to a method according to any of the eighty-seventh to ninety-fourth embodiments, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted.

In a ninety-sixth embodiment, the present invention relates to a method
10 according to the ninety-fifth embodiment, wherein at least one substituent is OH.

In a ninety-seventh embodiment, the present invention relates to a method according to the ninety-sixth embodiment, wherein one or more of A¹, A², A³, A⁴ and A⁵ is C-OH.

In a ninety-eighth embodiment, the present invention relates to a method
15 according to the ninety-sixth or ninety-seventh embodiment, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted with one or more additional substituents.

In a ninety-ninth embodiment, the present invention relates to a method according to the ninety-eighth embodiment, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted with one additional substituent.

20 In a one hundredth embodiment, the present invention relates to a method according to the ninety-ninth embodiment, wherein the one additional substituent is OH.

In a one hundred and first embodiment, the present invention relates to a method according to the ninety-eighth embodiment, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted with two additional substituents.

25 In a one hundred and second embodiment, the present invention relates to a method according to the one hundredth embodiment, wherein the two additional substituents are different to each other.

In a one hundred and third embodiment, the present invention relates to a method according to the one hundred and first embodiment, wherein the two additional
30 substituents are the same as each other.

In a one hundred and fourth embodiment, the present invention relates to a method according to any of the one hundred and first to one hundred and third embodiments, wherein the two additional substituents are selected from OH, an alkyl group and a heteroalkyl group.

- 5 In a one hundred and fifth embodiment, the present invention relates to a method according to any of the eighty-seventh to one hundred and fourth embodiments, wherein one of A^1 , A^2 , A^3 , A^4 and A^5 is N to give a heteroaryl group.

In a one hundred and sixth embodiment, the present invention relates to a method according to the one hundred and fifth embodiment, wherein the heteroaryl
10 group is substituted with one or more substituents.

In a one hundred and seventh embodiment, the present invention relates to a method according to the one hundred and sixth embodiment, wherein the one or more substituents are independently selected from OH, an alkyl group and a heteroalkyl group.

- 15 In a one hundred and eighth embodiment, the present invention relates to a method according to the one hundred and seventh embodiment, wherein the alkyl group is methyl or ethyl.

In a one hundred and ninth embodiment, the present invention relates to a method according to the one hundred and seventh embodiment, wherein the heteroalkyl
20 group is CH_2OH or $\text{CH}_2\text{CH}_2\text{OH}$.

In a one hundred and tenth embodiment, the present invention relates to a method according to any of the eighty-seventh to one hundred and ninth embodiments, wherein A^1 is selected from N and C-OH.

In a one hundred and eleventh embodiment, the present invention relates to a
25 method according to the one hundred and tenth embodiment, wherein A^1 is C-OH.

In a one hundred and twelfth embodiment, the present invention relates to a method according to any of the eighty-seventh to one hundred and eleventh embodiment, wherein the aromatic ring containing A^1 , A^2 , A^3 , A^4 and A^5 is fused to an aryl group to give a bicyclic aryl or heteroaryl group.

In a one hundred and thirteenth embodiment, the present invention relates to a method according to the one hundred and twelfth embodiment, wherein the bicyclic aryl or heteroaryl group is naphthyl or quinoline.

In a one hundred and fourteenth embodiment, the present invention relates to a method according to the one hundred and twelfth or one hundred and thirteenth embodiment, wherein the bicyclic aryl or heteroaryl group is substituted by one or more substituents selected from OH, alkyl and heteroalkyl.

In a one hundred and fifteenth embodiment, the present invention relates to a method according to any of the one hundred and twelfth to one hundred and fourteenth embodiment, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is fused to the aryl group at A² and A³ or A⁴ and A⁵.

The nature of the present invention shall now be illustrated by the following non-limiting Examples.

Examples

15 Experimental Details

Materials and methods

All chemicals for syntheses were purchased from Sigma-Aldrich and used as received without further purification. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded on a Bruker Advance 400 NMR spectrometer using DMSO-*d*₆ as a solvent. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and other biochemicals were purchased from Sigma-Aldrich. Elemental analysis was performed on a Thermo Scientific Flash 2000 CHNS/O analyser. Electrospray ionization mass spectrometry (ESI-MS) measurements were performed on a Bruker amaZon SL mass spectrometer in enhanced resolution mode.

25

Synthesis of 1-benzylpiperidine-4-thiosemicarbazide

To an aqueous solution (100 mL) containing sodium hydroxide (1.0 g, 25.0 mmol), carbon disulfide (2.0 g, 26.3 mmol) followed by 4-amino-1-benzylpiperidine (4.65 g, 24.4 mmol) was added and stirred at room temperature. The solution turned to a yellow/orange colour and the organic layer slowly disappeared. After 4 h, sodium

30

chloroacetate (3.05 g, 26.2 mmol) was added and stirred for another 15 h at room temperature. The addition of conc. HCl (3 mL) to the reaction mixture resulted in the precipitation of the dithiocarbamate intermediate. Subsequently, hydrazine hydrate (2.50 g, 50 mmol) was added and the reaction mixture was heated to 90°C for 2 h and then cooled to room temperature. The white precipitate formed was collected by filtration, washed with water and dried. This afforded 3.9 g of 1-benzylpiperidine-4-thiosemicarbazide as a white solid. Overall yield: 60%. ¹H NMR: δ ppm (DMSO-*d*₆) 1.51 (2H, m, CH₂), 1.81 (2H, m, CH₂), 2.04 (2H, m, CH₂), 2.73 (2H, broad doublet, CH₂), 3.45 (2H, s, CH₂-Ph), 4.09 (1H, m, CH-NH), 4.46 (2H, s, NH₂), 7.27 (5H, m, 5×CH_{ph}), 7.56 (1H, d, *J* = 8.2 Hz, NH-CH), 8.61 (1H, s, NH-N). ¹³C NMR: δ ppm (DMSO-*d*₆) 32.0, 50.5, 52.5, 62.6, 127.3, 128.6, 129.2, 139.0, 180.7.

General procedure for preparation of compounds 1-8:

A solution of 1-benzylpiperidine-4-thiosemicarbazide (0.53 g, 2.0 mmol) in ethanol (15 mL) was refluxed with an equimolar amount of the desired aldehyde for 2 h in the presence of glacial acetic acid (5 drops). After cooling to room temperature, the precipitate formed was collected by filtration and was washed with adequate amounts of ethanol and dried under vacuum in a desiccator.

1

Yellow solid (0.82 g). Yield: 91%. ¹H NMR: δ ppm (DMSO-*d*₆) 1.98 (2H, m, CH₂), 2.11 (2H, m, CH₂), 2.42 (3H, s, CH₃), 3.02 (2H, bs, CH₂), 3.30 (2H, bs, CH₂), 4.23 (2H, bs, CH₂-Ph), 4.37 (1H, bs, CH-NH), 4.57 (2H, s, CH₂-OH), 5.34 (1H, bs, CH₂-OH), 7.45 (3H, m, 3×CH), 7.61 (2H, d, *J* = 3.3 Hz, 2×CH), 7.97 (1H, s, CH), 8.56 (1H, s, CH=N), 8.72 (1H, bs, NH-CH), 10.89 (1H, bs, OH), 11.82 (1H, s, NH-N). ¹³C NMR: δ ppm (DMSO-*d*₆) 19.6, 28.7, 50.0, 50.9, 59.5, 129.2, 129.7, 131.7, 133.0, 139.6, 141.7, 147.6, 149.6, 152.9, 178.0. ESI-MS in CH₃CN: found mass: 414.21 (100%), Calc. mass for C₂₁H₂₈N₅O₂S: 414.21 [M-Cl]⁺. Anal. Calc. for C₂₁H₂₇N₅O₂S·(HCl)(H₂O)₂(CH₃COOH)_{0.5} (%): C 51.20, H 6.64, N 13.57, S 6.21. Found (%): C 51.31, H 6.74, N 13.76, S 6.16.

2

White solid (0.69 g). Yield: 94%. ¹H NMR: δ ppm (DMSO-*d*₆) 1.74 (2H, m, CH₂), 1.84 (2H, m, CH₂), 2.03 (2H, t, *J* = 10.6 Hz, CH₂), 2.82 (2H, broad doublet, CH₂), 3.49

(2H, s, $\text{CH}_2\text{-Ph}$), 4.24 (1H, m, CH-NH), 6.85 (2H, m, $2\times\text{CH}$), 7.24 (2H, m, $2\times\text{CH}$), 7.32 (4H, m, $4\times\text{CH}$), 7.94 (1H, d, $J = 7.8$ Hz, CH), 8.04 (1H, d, $J = 8.6$ Hz, NH-CH), 8.38 (1H, s, CH=N), 9.96 (1H, bs, OH), 11.38 (1H, s, NH-N). ^{13}C NMR: δ ppm ($\text{DMSO-}d_6$) 31.5, 51.5, 52.7, 62.5, 116.5, 119.7, 120.8, 127.2, 127.3, 128.6, 129.2, 131.5, 139.1, 139.9, 156.9, 176.3. ESI-MS in CH_3CN : found mass: 369.17 (100%), Calc. mass for $\text{C}_{20}\text{H}_{25}\text{N}_4\text{OS}$: 369.18 $[\text{M}+\text{H}]^+$. Anal. Calc. for $\text{C}_{20}\text{H}_{24}\text{N}_4\text{OS}\cdot\text{CH}_3\text{COOH}$ (%): C 61.66, H 6.59, N 13.07, S 7.48. Found (%): C 61.82, H 6.48, N 13.28, S 7.36.

3

Yellow solid (0.78 g). Yield: 93%. ^1H NMR: δ ppm ($\text{DMSO-}d_6$) 1.68 (2H, m, CH_2), 1.89 (2H, m, CH_2), 2.10 (2H, m, CH_2), 2.76 (2H, broad doublet, CH_2), 3.48 (2H, s, $\text{CH}_2\text{-Ph}$), 4.25 (1H, m, CH-NH), 7.22 (1H, d, $J = 8.9$ Hz, CH), 7.26 (1H, m, CH), 7.32 (4H, m, $4\times\text{CH}$), 7.39 (1H, t, $J = 7.5$ Hz, CH), 7.59 (1H, m, CH), 7.89 (2H, m, $2\times\text{CH}$), 8.03 (1H, d, $J = 8.1$ Hz, CH), 8.46 (1H, d, $J = 8.6$ Hz, NH-CH), 9.03 (1H, s, CH=N), 10.64 (1H, bs, OH), 11.43 (1H, s, NH-N). ^{13}C NMR: δ ppm ($\text{DMSO-}d_6$) 31.4, 51.4, 52.4, 62.6, 110.4, 118.9, 123.0, 123.9, 127.3, 128.2, 128.6, 128.7, 129.2, 129.3, 132.0, 132.8, 139.0, 142.9, 156.9, 176.3. ESI-MS in CH_3CN : found mass: 419.18 (100%), Calc. mass for $\text{C}_{24}\text{H}_{27}\text{N}_4\text{OS}$: 419.19 $[\text{M}+\text{H}]^+$. Anal. Calc. for $\text{C}_{24}\text{H}_{26}\text{N}_4\text{OS}$ (%): C 68.87, H 6.26, N 13.39, S 7.66. Found (%): C 68.83, H 6.28, N 13.60, S 7.56.

4

White solid (0.75 g). Yield: 50%. ^1H NMR: δ ppm ($\text{DMSO-}d_6$) 1.77 (4H, m, $2\times\text{CH}_2$), 2.02 (2H, m, CH_2), 2.84 (2H, broad doublet, CH_2), 3.18 (2H, s, $\text{CH}_2\text{-Ph}$), 4.27 (1H, m, CH-NH), 7.25 (1H, m, CH), 7.33 (4H, m, $4\times\text{CH}$), 7.39 (1H, m, CH), 7.84 (1H, td, $J = 7.8, 1.6$ Hz, CH), 8.11 (1H, s, CH=N), 8.25 (1H, d, $J = 8.5$ Hz, CH), 8.30 (1H, d, $J = 8.1$ Hz, NH-CH), 8.57 (1H, d, $J = 4.4$ Hz, CH), 11.66 (1H, s, NH-N). ^{13}C NMR: δ ppm ($\text{DMSO-}d_6$) 31.3, 51.8, 52.7, 62.5, 120.9, 124.6, 127.3, 128.6, 129.1, 136.9, 139.2, 143.0, 149.8, 153.7, 176.7. ESI-MS in CH_3CN : found mass: 354.17 (100%), Calc. mass for $\text{C}_{19}\text{H}_{24}\text{N}_5\text{S}$: 354.17 $[\text{M}+\text{H}]^+$. Anal. Calc. for $\text{C}_{19}\text{H}_{23}\text{N}_5\text{S}\cdot(\text{H}_2\text{O})_{0.25}$ (%): C 63.74, H 6.62, N 19.56, S 8.96. Found (%): C 63.55, H 6.48, N 19.78, S 8.84.

5

White crystals (0.68 g). Yield: 88%. ^1H NMR: δ ppm ($\text{DMSO-}d_6$) 1.71 (2H, m, CH_2), 1.82 (2H, m, CH_2), 2.03 (2H, t, $J = 10.8$ Hz, CH_2), 2.80 (2H, broad doublet, CH_2),

3.48 (2H, s, $\text{CH}_2\text{-Ph}$), 4.23 (1H, m, CH-NH), 6.66 (1H, t, $J = 7.8$ Hz, CH), 6.81 (1H, dd, $J = 7.8, 1.4$ Hz, CH), 7.25 (1H, m, CH), 7.31 (4H, m, $4 \times \text{CH}$), 7.36 (1H, d, $J = 8.3$ Hz, CH), 8.02 (1H, d, $J = 8.4$ Hz, NH-CH), 8.38 (1H, s, CH=N), 9.26 (2H, bs, $2 \times \text{OH}$), 11.38 (1H, s, NH-N). ^{13}C NMR: δ ppm ($\text{DMSO-}d_6$) 31.4, 51.5, 52.7, 62.5, 116.9, 117.7, 119.5, 121.3, 127.3, 128.6, 129.2, 139.1, 140.6, 145.7, 146.0, 176.2. ESI-MS in CH_3CN : found mass: 385.18 (100%), Calc. mass for $\text{C}_{20}\text{H}_{25}\text{N}_4\text{O}_2\text{S}$: 385.17 $[\text{M}+\text{H}]^+$. Anal. Calc. for $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_2\text{S} \cdot \text{H}_2\text{O}$ (%): C 59.68, H 6.51, N 13.92, S 7.97. Found (%): C 59.65, H 6.54, N 13.99, S 7.82.

6

Yellowish brown solid (0.65 g). Yield: 81%. ^1H NMR: δ ppm ($\text{DMSO-}d_6$) 1.75 (2H, m, CH_2), 1.90 (2H, m, CH_2), 2.32 (2H, broad doublet, CH_2), 3.71 (2H, s, $\text{CH}_2\text{-Ph}$), 4.27 (1H, m, CH-NH), 6.37 (1H, d, $J = 8.6$ Hz, CH), 7.13 (1H, d, $J = 8.3$ Hz, CH), 7.33 (5H, m, $5 \times \text{CH}$), 7.98 (1H, d, $J = 7.7$ Hz, NH-CH), 8.26 (1H, s, CH=N), 9.13 (1H, bs, OH), 11.28 (1H, s, NH-N). ^{13}C NMR: δ ppm ($\text{DMSO-}d_6$) 30.6, 50.8, 52.1, 61.5, 108.2, 113.0, 118.7, 128.1, 129.9, 133.2, 142.4, 147.1, 148.8. ESI-MS in CH_3CN : found mass: 385.18 (100%), Calc. mass for $\text{C}_{20}\text{H}_{25}\text{N}_4\text{O}_2\text{S}$: 385.17 $[\text{M}+\text{H}]^+$. ESI-MS in CH_3CN : found mass: 401.16 (100%), Calc. mass for $\text{C}_{20}\text{H}_{25}\text{N}_4\text{O}_3\text{S}$: 401.17 $[\text{M}+\text{H}]^+$. Anal. Calc. for $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_3\text{S} \cdot (\text{CH}_3\text{COOH})_{0.5}(\text{H}_2\text{O})_{2.5}$ (%): C 53.05, H 6.57, N 11.78, S 6.74. Found (%): C 53.17, H 6.08, N 12.03, S 6.75.

7

Yellow solid (1.1 g). Yield: 63%. ^1H NMR: δ ppm ($\text{DMSO-}d_6$) 1.83 (4H, m, $2 \times \text{CH}_2$), 2.03 (2H, m, CH_2), 2.86 (2H, broad doublet, CH_2), 3.49 (2H, s, $\text{CH}_2\text{-Ph}$), 4.31 (1H, m, CH-NH), 7.27 (1H, m, CH), 7.32 (4H, m, $4 \times \text{CH}$), 7.63 (1H, m, CH), 7.78 (1H, td, $J = 7.2, 1.2$ Hz, CH), 8.01 (2H, m, $2 \times \text{CH}$), 8.26 (1H, s, CH=N), 8.39 (1H, m, CH), 8.48 (1H, d, $J = 8.8$ Hz, NH-CH), 11.83 (1H, s, NH-N). ^{13}C NMR: δ ppm ($\text{DMSO-}d_6$) 31.6, 52.0, 52.8, 62.5, 118.7, 127.3, 127.6, 128.3, 128.4, 128.6, 129.2, 129.3, 130.4, 136.7, 139.2, 143.1, 147.8, 154.3, 176.7. ESI-MS in CH_3CN : found mass: 404.19 (100%), Calc. mass for $\text{C}_{23}\text{H}_{26}\text{N}_5\text{S}$: 404.19 $[\text{M}+\text{H}]^+$. Anal. Calc. for $\text{C}_{23}\text{H}_{25}\text{N}_5\text{S}$ (%): C 68.46, H 6.24, N 17.35, S 7.94. Found (%): C 68.34, H 6.26, N 17.53, S 7.82.

8

Yellow solid (1.37 g). Yield: 69%. ^1H NMR: δ ppm (DMSO- d_6) 1.82 (4H, m, $2\times\text{CH}_2$), 2.03 (2H, td, $J = 11.2, 3.2$ Hz, CH_2), 2.86 (2H, broad doublet, CH_2), 3.49 (2H, s, $\text{CH}_2\text{-Ph}$), 4.31 (1H, m, CH-NH), 7.11 (1H, dd, $J = 7.2, 1.6$ Hz, CH), 7.26 (1H, m, CH),
5 7.33 (4H, m, $4\times\text{CH}$), 7.42 (2H, m, $2\times\text{CH}$), 8.29 (1H, s, CH=N), 8.31 (1H, s, CH), 8.38 (1H, d, $J = 8.5$ Hz, CH), 8.46 (1H, d, $J = 8.5$ Hz, NH-CH), 9.83 (1H, s, OH), 11.88 (1H, s, NH-N). ^{13}C NMR: δ ppm (DMSO- d_6) 31.3, 51.9, 52.8, 62.5, 112.6, 118.3, 119.0, 127.3, 128.5, 128.6, 129.2, 129.3, 136.5, 138.7, 139.2, 142.9, 152.2, 153.9, 176.7. ESI-MS in CH_3CN : found mass: 420.18 (100%), Calc. mass for $\text{C}_{23}\text{H}_{26}\text{N}_5\text{OS}$: 420.19 $[\text{M}+\text{H}]^+$. Anal.
10 Calc. for $\text{C}_{23}\text{H}_{25}\text{N}_5\text{OS}$ (%): C 65.85, H 6.01, N 16.69, S 7.64. Found (%): C 65.80, H 6.00, N 16.79, S 7.49.

Effect of the compounds 1-8 on Mobilising Cellular ^{59}Fe

The effect of compounds 1-8 on ^{59}Fe mobilisation in SK-N-MC cells was
15 determined by ^{59}Fe efflux experiments using a standard protocol.¹ The human SK-N-MC neuroepithelioma cell line was chosen for these initial studies as it is a neural cell line that has been used as a preliminary model for AD.^{2,3} Furthermore, it is a well characterised cell line for examining the effect of iron chelating agents.⁴ Briefly, SK-N-MC cells were seeded in 6-well plates and incubated overnight. The cell growth medium
20 was aspirated and then the cells prelabelled with $^{59}\text{Fe}_2\text{-Tf}$ (0.75 μM) in MEM media (1 mL) for 3 h at 37°C. Cells were washed four times with ice-cold PBS to remove extracellular $^{59}\text{Fe}_2\text{-Tf}$ and then incubated with medium alone (control) or medium containing compounds 1-8 (25 μM) and incubated for 3 h at 37°C. The well characterised chelators, di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone (Dp44mT;
25 25 μM) and desferrioxamine (DFO; 25 μM), and the AD therapeutic, donepezil (25 μM), were included in this study as controls. After incubation, cells were placed on ice and the media containing released ^{59}Fe was separated without dislodging cells. To the cells, PBS (1 mL) was added and then scraped using a plastic spatula. Radioactivity was measured in both the cell suspension and supernatant using a γ -scintillation counter
30 (Wallac Wizard 3, Turku, Finland).

Effect of Compounds 1-8 at Preventing Cellular ^{59}Fe Uptake

In order to estimate the ability of compounds 1-8 to prevent the cellular uptake of ^{59}Fe from the Fe transport protein, $^{59}\text{Fe}_2\text{-Tf}$, ^{59}Fe uptake experiments were performed using standard procedures.¹ Briefly, SK-N-MC cells were incubated with $^{59}\text{Fe}_2\text{-Tf}$ (0.75 μM) and the compounds (25 μM) in MEM media for 3 h at 37°C. The media was then removed and cells washed four times with ice-cold PBS to eliminate an excess of extracellular $^{59}\text{Fe}_2\text{-Tf}$ and the chelator. Subsequently, cells were incubated with Pronase (1 mg/mL; Sigma-Aldrich), a general protease, for 30 min at 4°C. The monolayer of cells was then scraped using a plastic spatula and centrifuged at 14,000 rpm for 3 min at 4°C. The supernatant media that contain membrane-bound ^{59}Fe was removed and the settled pellet containing internalised ^{59}Fe was resuspended in 1 mL of PBS and the ^{59}Fe levels in both supernatant and cell suspension were measured on a γ -scintillation counter. Internalised ^{59}Fe uptake was calculated as a percentage of the control (medium alone). As utilised in the ^{59}Fe efflux experiments, Dp44mT (25 μM), DFO (25 μM) and donepezil (25 μM) were included as controls.

Effect of Compounds 1-8 on Cellular Proliferation

The cytotoxic potential of compounds 1-8 was determined by the [1-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium] (MTT) assay against SK-N-MC cells using standard techniques.⁵ The cells were seeded in 96-well microtiter plates at the density of 1.5×10^4 cells/well. The compounds were dissolved in DMSO (20 mM) and diluted further using MEM media to result in a final concentration of DMSO $\leq 0.5\%$ (v/v) at which DMSO has no effect on cellular proliferation.⁵ After 24 h, the cells were exposed to various concentrations of chelators (0–100 μM) and incubated for 72 h at 37°C. After this incubation, 10 μL of MTT (5 mg/mL in PBS) was added to each well and incubated further for 2 h at 37°C. Culture medium was aspirated carefully and 100 μL of DMSO was added to dissolve the formazan crystals. The plates were shaken for 5 min and the absorbance was measured at 570 nm using a scanning multi-well spectrophotometer. The half maximal inhibitory concentration (IC_{50}) was defined as the chelator concentration necessary to reduce the absorbance to 50% of the untreated control.

Ascorbate Oxidation Assay

The ability of the iron complexes of compounds 1-8 to catalyse the oxidation of ascorbate was examined as previously described.^{4,6,7} The assay was carried out in PBS buffer (10 mM, pH = 7.4) containing 5% (v/v) acetonitrile and sodium citrate (500 μ M).
5 Freshly prepared sodium ascorbate (50 μ M) was incubated with FeCl₃ alone (10 μ M, control) or in the presence of chelators at an iron-binding equivalent (IBE) of 1. This IBE results in a fully occupied coordination sphere of Fe(III) upon complexation (Fe(III)–EDTA (1:1 ratio), Fe(III)–DFO (1:1 ratio), Fe(III)–Dp44mT (1:2 ratio), and Fe(III)–compounds 1-8 (1:2 ratio)). The standard iron chelators, ethylenediaminetetraacetic
10 acid (EDTA), Dp44mT, and DFO were included as controls. The absorbance at 265 nm was measured using UV-visible spectrophotometer after 10 and 40 min of incubation at RT and the decrease in absorbance between the two time points was calculated and expressed as a percentage of ascorbate oxidation relative to the control (100%).

15 Protection Against Hydrogen Peroxide-Mediated Cytotoxicity

The ability of compounds 1-8 to protect SK-N-MC neuroepithelioma cells against H₂O₂-mediated cytotoxicity was assessed using the MTT assay.⁸ SK-N-MC cells were seeded in 96-well microtiter plates at a density of 1.5×10^4 cells/well and incubated at 37°C/24 h. The cells were then pre-incubated for 2 h/37°C with serum-free medium
20 alone (control), compounds 1-8, or the relevant controls, including DFO and Donepezil, at 10 μ M (final DMSO concentration $\leq 0.2\%$ v/v) in serum-free medium. The medium was then removed and the cells incubated with fresh serum-free medium alone, or serum-free medium containing H₂O₂ (150 μ M), for a further 24 h/37°C. The cellular viability was then measured using the MTT assay.

25

Acetylcholinesterase (AChE) Inhibition

Since acetylcholinesterase (AChE) is considered as a viable therapeutic target for the symptomatic improvement in AD with several agents already being utilised in the clinics including donepezil,^{9,10} the *in vitro* inhibitory activities of compounds 1-8 against
30 isolated AChE were evaluated. In these studies, the activities of compounds 1-8 were

compared with the well-known AChE inhibitors donepezil and tacrine, which were used as positive controls.¹¹

The ability of compounds 1-8 to inhibit AChE was measured using standard procedures.^{12,13} Stock solutions of the compounds were prepared in DMSO (10 mM).

- 5 The working solutions were prepared by dissolving 12.5 μ L of compounds or DMSO (control) in 997.5 μ L of freshly prepared HEPES buffer (50 mM, 150 mM, NaCl; pH 8). The compound (206 μ L) was added to a cuvette containing 238 μ L of DTNB (3 mM in HEPES buffer) and 37.5 μ L of AChE at a concentration of 0.26 U/mL and incubated for 15 min. After the addition of 12.5 μ L of AChI (15 mM), the enzymatic reaction was
- 10 followed by measuring the absorbance at 405 nm for the first 5 min using UV-vis spectrophotometer in kinetic mode to calculate initial rate of the reaction. A slope (m) was calculated from each kinetic graph. The percentage of enzymatic activity was calculated using formula given below.

$$\% \text{ activity} = 100 - [m_{\text{sample}}/m_{\text{control}}]$$

- 15 Where m_{sample} is the slope of obtained from the sample and m_{control} is the slope obtained from the control (DMSO) reaction. Each experiment was carried out in quadruplicate.

Effect of Compounds 1-8 on Inhibition of Copper-Mediated A β ₁₋₄₀ Aggregation

- 20 An established turbidity assay was used to evaluate the ability of compounds 1-8 to inhibit A β ₁₋₄₀ aggregation induced by Cu(II).¹⁴ Synthetic human A β ₁₋₄₀ (1 mg; purchased from ChinaPeptides, Shanghai, China) was dissolved in 50 μ L of DMSO and then 910 μ L of Milli-Q water was added just prior the experiment to provide a 250 μ M solution. Stock solutions of agents, including DTPA, Dp44mT, Donepezil, Tacrine and
- 25 compounds 1-8, were prepared in DMSO (10 mM) and diluted further with HEPES buffer (20 mM HEPES, 150 mM NaCl, pH 6.6). This solution was passed through Chelex resin to remove trace metal ions and filtered using a 0.2 μ m filter to achieve a working stock of 250 μ M. A stock solution (250 μ M) of CuCl₂ (analytical grade, Sigma-Aldrich) was also prepared in HEPES buffer. Solutions of Cu(II) (5 μ L) and A β ₁₋₄₀ (5 μ L)
- 30 were added to HEPES buffer (35 μ L) in the 384-well plate, which was then incubated with the ligands (5 μ L) in a final volume of 50 μ L in quadruplicates. The final

concentrations of the ligands, Cu(II) and A β ₁₋₄₀ were 25 μ M. The solutions were incubated for 2 h/37 °C and absorbance measured at 405 nm using a FluoStar Omega Plate Reader (BMG Labtech, Ortenberg, Germany). The absorbance from Cu(II), ligand and buffer were used as blank and subtracted from corresponding wells. Both Cu(II) and peptide were used alone as positive controls to demonstrate the effect on peptide aggregation in the absence of the ligand.

Western blotting

Western blotting was performed by standard methodology, as previously described.^{15,16} Briefly, SK-N-MC cells were incubated with compounds 1-8 (25 μ M) or Dp44mT (25 μ M), which is known to activate the autophagy pathway,^{15,16} for 24 h at 37°C in the presence or absence of the late-stage autophagy inhibitor, Baf A1 (100 nM). Proteins were extracted from the cell lysates and were separated by SDS-PAGE using the MiniProtein Tetra Cell System (Bio-Rad; CA, USA). The gel was electroblotted overnight at 30 V/4°C onto a polyvinylidene difluoride (PVDF) membrane (0.45 μ m pore size) in 1x transfer buffer. PVDF was activated by 100% methanol and soaked in 1x transfer buffer prior to transfer. The primary antibodies used for immunoblotting were LC3 (Cat. #: MBPM036; 1:2,000 dilution; Abacus), p62 (Cat. #: ab56416; 1:5,000 dilution; Abcam) and β -actin (Cat. #: A1978; 1:10,000; Sigma). The secondary antibodies used included horseradish peroxidase (HRP)–conjugated anti-rabbit (Cat. #: A6154) and anti-mouse (Cat. #: A4416) antibodies from Sigma-Aldrich. Both antibodies were used at a 1:10,000 dilution. PVDF membranes were incubated with Luminata Forte Western HRP substrate (Millipore, Billerica, MA, USA). Signals were detected using a ChemiDoc MP Imaging System (Bio-Rad) and densitometry performed using Quantity One software (Bio-Rad) and normalized to the relative β -actin-loading control.

Results

Effect of Compounds 1-8 on ⁵⁹Fe Release from Prelabelled Cells

Iron loading is a common feature of Alzheimer's disease,¹⁷ which leads to oxidative stress through the generation of excess ROS and β -amyloid aggregation. The effect of compounds 1-8 on iron efflux from cells was examined with the aim of

evaluating their efficacy to chelate intracellular iron and release it from cells. These results were compared with four controls, namely: (1) Dp44mT, a well characterised thiosemicarbazone with high iron mobilisation efficacy;¹⁸ (2) DFO, a bacterial siderophore used for the treatment of iron overload diseases;¹⁹ and (3) donepezil, which is an AD therapeutic with AChE inhibitory activity.¹⁰ The results are shown in Figure 1.

As previously observed,⁴ the incubation of SK-N-MC cells with control medium alone resulted in minimal cellular ⁵⁹Fe release ($6 \pm 1\%$ of total cellular iron) from prelabelled SK-N-MC cells. The positive control, Dp44mT (25 μ M), increased ⁵⁹Fe mobilisation to $49 \pm 5\%$ and was significantly ($p < 0.001$) increased relative to that of the control medium. In contrast, the iron chelator, DFO, showed limited ability to induce ⁵⁹Fe release from cells, leading to the mobilisation of $15 \pm 4\%$ of cellular ⁵⁹Fe at 25 μ M. Notably, although DFO showed poor iron chelation efficacy, it was significantly ($p < 0.001$) increased relative to the control medium, but was significantly ($p < 0.001$) less active than Dp44mT. The control compound donepezil demonstrated similar levels of ⁵⁹Fe mobilisation to that of the control medium alone. In fact, donepezil mediated a slight, but significant ($p < 0.05$), decrease in ⁵⁹Fe release from cells ($4.5 \pm 1.5\%$) than the control medium.

Compounds 1-8 showed the ability to significantly ($p < 0.001$) increase ⁵⁹Fe mobilisation compared to control. However, compounds 1-8 were significantly ($p < 0.001$) less effective than Dp44mT at mediating ⁵⁹Fe mobilisation. Notably, compounds 1 ($31.4 \pm 3.8\%$), 2 ($27.7 \pm 2.4\%$) and 5 ($29.4 \pm 1.9\%$) showed significantly ($p < 0.001$) greater levels of cellular ⁵⁹Fe release than DFO. Compounds 3 ($15.7 \pm 2.5\%$), 4 ($17.7 \pm 3.4\%$), 6 ($13.6 \pm 1.2\%$) and 8 ($16.5 \pm 2.3\%$) showed comparable ⁵⁹Fe mobilisation efficacy relative to DFO. Finally, compound 7 ($10.4 \pm 1.6\%$) showed the poorest ability to mediate the release of cellular ⁵⁹Fe of this series of analogues and was significantly ($p < 0.01$) less active than DFO.

No correlation was observed between the ability of the piperidine-thiosemicarbazones to mediate cellular ⁵⁹Fe release and either their Log P_{calc} values ($R^2 = 0.3274$) or IC₅₀ values ($R^2 = 0.02379$). This suggested that their ability to mediate ⁵⁹Fe release was not dependent on their Log P_{calc} values and their anti-proliferative activity was not dependent on their ability to mediate ⁵⁹Fe release. Collectively, amongst this

series of analogues, compounds 1, 2 and 5 showed the most promising ability to mobilise cellular ^{59}Fe .

Effect of Compounds 1-8 on Inhibiting ^{59}Fe Uptake from ^{59}Fe -Transferrin

Considering the ability of compounds 1-8 to induce cellular iron release, experiments were then performed to examine the activity of these agents to inhibit the uptake of iron by SK-N-MC cells from $^{59}\text{Fe}_2\text{-Tf}$. As utilised in the iron efflux studies above, Dp44mT, DFO and donepezil were used as controls. The results are shown in Figure 2.

As previously observed,¹⁸ Dp44mT could markedly and significantly ($p < 0.001$) inhibit cellular ^{59}Fe uptake to $5 \pm 1\%$ of the control. Additionally, DFO could significantly ($p < 0.001$) decrease cellular ^{59}Fe uptake to $85 \pm 4\%$ of the control. However, was significantly ($p < 0.001$) less effective at inhibiting ^{59}Fe uptake relative to Dp44mT. Interestingly, donepezil slightly but significantly ($p < 0.001$) decreased cellular ^{59}Fe uptake to $96 \pm 2\%$ of the control.

Overall, the compounds from these analogues that demonstrated marked ability to mobilise cellular ^{59}Fe (Figure 1) also markedly prevented cellular ^{59}Fe uptake (Figure 2). Although compounds 1-8 were significantly ($p < 0.001$ - 0.01) more effective at inhibiting ^{59}Fe uptake relative to the control medium, all analogues were significantly ($p < 0.001$) less effective than Dp44mT (Figure 2). Of these analogues, compound 1 was the most effective, inhibiting cellular ^{59}Fe uptake to $41 \pm 3\%$ of the control, which was significantly ($p < 0.001$) less than that of DFO (Figure 2). Compounds 2 ($57 \pm 5\%$), 3 ($68 \pm 7\%$), 4 ($63 \pm 7\%$) and 5 ($63 \pm 5\%$) showed significantly ($p < 0.001$) decreased ^{59}Fe uptake in comparison to DFO (Figure 2). The compounds 6 ($93 \pm 6\%$), 7 ($89 \pm 8\%$) and 8 ($78 \pm 8\%$) were the least effective at inhibiting ^{59}Fe uptake from $^{59}\text{Fe}_2\text{-Tf}$. While both compounds 7 and 8 showed comparable efficacy to DFO, compound 6 showed significantly ($p < 0.05$) poorer ^{59}Fe uptake efficacy relative to DFO (Figure 2).

As observed with the ^{59}Fe mobilisation experiments, no correlation was observed between the ability of compounds 1-8 to inhibit ^{59}Fe uptake with either their Log P_{calc} values ($R^2 = 0.2064$) or their IC_{50} values ($R^2 = 0.308$). This observation suggested that

the ability of these compounds to inhibit ^{59}Fe uptake was not dependent on their $\text{Log}P_{\text{calc}}$ values and their anti-proliferative activity was not dependent on their ability to inhibit cellular ^{59}Fe uptake. In conclusion, compounds 1, 2, 3, 4 and 5 showed the most promising ability to inhibit the internalisation of ^{59}Fe from $^{59}\text{Fe}_2\text{-Tf}$.

5 Cytotoxicity of Compounds 1-8 in SK-N-MC Neuroepithelioma Cells

Having demonstrated that compounds 1-8 exhibited iron chelation activity, studies then assessed their cytotoxicity in SK-N-MC cells. The results are shown in Figure 3. Three controls were utilised, including the well-known iron chelators, Dp44mT and DFO, as their anti-proliferative activity is well described in this cell-type.²⁰⁻²²

Moreover, the cytotoxicity of the therapeutic activity of donepezil was also assessed.

In these studies, Dp44mT and DFO displayed IC_{50} values of $0.03 \pm 0.04 \mu\text{M}$ and $16.0 \pm 4.4 \mu\text{M}$, respectively. Additionally, the AD therapeutic, donepezil, showed no appreciable cytotoxic activity with an IC_{50} value of $> 100 \mu\text{M}$. Importantly, compounds 1-8 showed poor anti-proliferative activity and were significantly ($p < 0.001$) less cytotoxic than the anti-cancer agent, Dp44mT (Fig. 3). Notably, compound 1 demonstrated an IC_{50} value of $>100 \mu\text{M}$ and was the least cytotoxic of all of novel compounds examined (Fig. 3). Similarly, 2 (IC_{50} : $34 \pm 4 \mu\text{M}$), 6 (IC_{50} : $77 \pm 3 \mu\text{M}$) and 8 (IC_{50} : $33 \pm 4 \mu\text{M}$) showed poor anti-proliferative activity and were significantly ($p < 0.001$) less cytotoxic than both DFO and Dp44mT (Fig. 3). Both compounds 5 (IC_{50} : $16 \pm 4 \mu\text{M}$) and 7 (IC_{50} : $18 \pm 1 \mu\text{M}$) showed IC_{50} values that were significantly ($p < 0.001$) greater than Dp44mT, but were comparable ($p > 0.05$) to DFO (Fig. 3). Compounds 3 (IC_{50} : $7 \pm 2 \mu\text{M}$) and 4 (IC_{50} : $3 \pm 1 \mu\text{M}$) were the most cytotoxic, with IC_{50} values that were significantly ($p < 0.001$) less than DFO, but were significantly ($p < 0.001$) greater than Dp44mT (Fig. 3).

No relationship ($R^2 = 0.1898$) was observed between the $\text{Log} P_{\text{calc}}$ and the anti-proliferative activity of compounds 1-8, suggesting that other factors were involved in the cytotoxicity observed. In summary, these results demonstrate the low cytotoxic effects of compounds 1-8 in SK-N-MC neuroepithelioma cells and showed properties suitable for the treatment of AD.

Effect of the Iron Complexes of Compounds 1-8 on Ascorbate Oxidation

An important property of chelators for the treatment of iron-loading conditions is that they should form iron complexes that are not redox active. This is critical in diseases such as Alzheimer's disease where Fe accumulation occurs within plaques and has been reported to play a role in oxidative stress.¹⁷ Hence, the removal of the iron accumulation without the generation of redox stress is an important property of compounds with therapeutic potential.

To examine this property, the Fe(III) complexes of compounds 1-8 were assessed in terms of their activity to catalyse ascorbate oxidation *via* iron-mediated Fenton chemistry.^{4,23} In these studies, ascorbate was used as a substrate because of its abundance in neurons and its strong anti-oxidant potential to protect these cells from oxidative stress.²⁴⁻²⁶ For comparison, the well-known, redox-active Fe(III) complexes of EDTA and Dp44mT were included as positive controls.²⁷ On the other hand, DFO was also used as a redox-inactive Fe(III) complex.²⁷ Moreover, we also assessed the ability of donepezil to mediate ascorbate oxidation, as this AD therapeutic was used as a scaffold for the design of compounds 1-8. The ascorbate oxidation activity of the Fe complexes of 6 and 7 could not be determined (N.D.) due to their low solubility under the conditions used in this assay. The results of this experiment are shown in Figure 4.

In accordance with previously published results,²⁷ the EDTA- and Dp44mT-Fe(III) complexes markedly and significantly ($p < 0.001$) accelerated the oxidation of ascorbate to 436% and 285% of the control, respectively. In contrast, the redox inactive Fe(III)-DFO complex significantly ($p < 0.001$) reduced ascorbate oxidation, decreasing it to 29% of the control, confirming its anti-oxidative behaviour. Donepezil in the presence of Fe(III) showed comparable ($p > 0.05$) levels of ascorbate oxidation (98%) relative to control.

Generally, all but one of the Fe (III) complexes of compounds 1-8 did not accelerate ascorbate oxidation. In fact, the Fe (III) complexes of these agents significantly ($p < 0.001$) decreased ascorbate oxidation when compared to the Fe(III) complexes of EDTA or Dp44mT. However, all iron complexes of compounds 1-8 showed significantly ($p < 0.001$) increased levels of ascorbate oxidation relative to DFO. The Fe(III) complexes of compounds 1, 3, 5 and 8 significantly ($p < 0.001$) reduced ascorbate oxidation to 61%, 52%, 79% and 69% of the control, respectively, suggesting

their anti-oxidant activity. In contrast, the Fe(III) complex of 4 mediated significantly ($p < 0.001$) increased levels of ascorbate oxidation to 212% of the control, suggesting its pro-oxidative activity. The Fe(III) complex of 2 demonstrated a slightly, but significantly ($p < 0.01$) lower level of ascorbate oxidation (93%) relative to the control. Collectively, the Fe(III) complexes of compounds 1, 3, 5 and 8 inhibited ascorbate oxidation relative to the control. These observations suggest that these ligands have the potential to alleviate the Fe-mediated oxidative stress observed in AD.

The Ability of Compounds 1-8 to Inhibit Hydrogen Peroxide-Mediated Cytotoxicity

The ability of compounds 1-8 to protect SK-N-MC cells from hydrogen peroxide (H_2O_2)-mediated cytotoxicity was examined as oxidative stress is a hallmark of AD.¹⁷ The results of this assay are shown in Figure 5. The incubation of control cells with medium containing H_2O_2 led to a significant ($p < 0.001$) decrease in cellular proliferation to 56% of the control. Importantly, over the short incubation utilized (24 h), the control agents, DFO and donepezil alone, did not exhibit any significant ($p > 0.05$) anti-proliferative activity relative to the control. Additionally, these agents also protected cells from H_2O_2 -mediated cytotoxicity, with DFO and donepezil significantly ($p < 0.001$) increasing cellular proliferation to 89% and 74%, respectively, relative to control cells incubated with H_2O_2 .

Pre-incubation of cells with 1 or 2 alone did not demonstrate any significant ($p > 0.05$) effect on proliferation relative to control cells. In contrast, 3, 4, 5, 6, 7 or 8 alone mediated a significant ($p < 0.001$ -0.05) decrease in cellular proliferation (58-94%) relative to control cells. All of compounds 1-8, except 3, could alleviate the H_2O_2 -mediated inhibition of proliferation and significantly ($p < 0.001$ -0.01) increased proliferation to 66-87% of the control relative to control cells incubated with H_2O_2 alone.

Collectively, these studies demonstrate that compounds 1-8 (except 3) could alleviate H_2O_2 -mediated anti-proliferative activity. Both 1 and 2 were able to alleviate H_2O_2 -mediated inhibition of proliferation without inducing cytotoxic effects on their own, suggesting their potential to prevent oxidative stress in AD.

Acetylcholinesterase (AChE) Inhibition

We evaluated the *in vitro* inhibitory activities of compounds 1-8 against isolated AChE. In these studies, the activities of the compounds 1-8 were compared with the well-known AChE inhibitors, donepezil and tacrine, which were used as positive controls.¹¹ The inhibition of AChE was expressed as IC₅₀ values, which are presented in Figure 6. As expected, the positive controls, donepezil and tacrine, showed potent inhibition of AChE with IC₅₀ values of 0.07 μ M and 0.1 μ M, respectively.

Compounds 1-8 demonstrated the ability to inhibit AChE, with IC₅₀ values observed between 1-23 μ M. However, all compounds showed significantly ($p < 0.001$ -0.01) decreased AChE inhibitory activity relative to both donepezil and tacrine. Compounds 5 and 3 showed the greatest ability to inhibit AChE, with IC₅₀ values of 1.0 μ M and 1.2 μ M, respectively. Compounds 1, 2, 7 and 8 demonstrated IC₅₀ values in a similar range, namely from 2.5 to 4.9 μ M. In contrast, compounds 4 and 6 showed the least potent AChE inhibitory activity and demonstrated IC₅₀ values of 7.2 μ M and 22.6 μ M, respectively. Collectively, compounds 3 and 5 showed the greatest promise as AChE inhibitors.

The Effect of Compounds 1-8 on Inhibiting Copper-Mediated A β ₁₋₄₀ Aggregation

It is well known that elevated levels of trace metal ions, including Cu, Zn and Fe, are detected in A β plaques.²⁸ Furthermore, Cu(II) and Zn(II) facilitate the self-aggregation of A β ₁₋₄₀ and A β ₁₋₄₂ peptides,²⁹ of which the A β ₁₋₄₀ isoform is abundant in cerebrospinal fluid.³⁰ Therefore, we examined the ability of compounds 1-8 to inhibit Cu(II)-mediated aggregation of A β ₁₋₄₀ using an established turbidity assay.¹⁴ Several controls were also implemented, including: **(1)** the chelator, diethylenetriamine pentaacetic acid (DTPA), which is a known inhibitor of Cu(II)-induced A β aggregation;¹⁴ **(2)** the well-characterized copper chelating thiosemicarbazone, Dp44mT; and **(3)** the clinically used AChE inhibitors, Donepezil and Tacrine.

As shown in Figure 7, the co-incubation of Cu(II) with A β ₁₋₄₀ significantly ($p < 0.001$) enhanced A β aggregation relative to A β ₁₋₄₀ alone. Upon addition of the positive control, DTPA, a significant ($p < 0.001$) reduction in Cu(II)-mediated A β ₁₋₄₀ aggregation was observed, which is likely to be due to its ability to chelate Cu(II).¹⁴ Similarly,

Dp44mT, markedly and significantly ($p < 0.001$) inhibited Cu(II)-mediated A β_{1-40} aggregation. In contrast, the AChE inhibitors, Donepezil and Tacrine, did not show any significant ($p > 0.05$) effect on Cu(II)-mediated A β_{1-40} aggregation, which is likely due to their inability to chelate Cu(II).

5 Of all of the novel compounds 1-8, compounds 4, 8 and especially 5, exhibited the greatest ability to inhibit Cu(II)-mediated A β_{1-40} aggregation, followed by 6, 1, 2 and 7. All of these aforementioned ligands significantly ($p < 0.001-0.05$) inhibited Cu(II)-mediated A β_{1-40} aggregation. In fact, the ability of 5 and 8 to inhibit Cu(II)-mediated A β_{1-40} aggregation was comparable to that observed with DTPA. The ability of 3 to inhibit the
10 Cu(II)-mediated aggregation of A β_{1-40} could not be determined (N.D.) due to its low solubility in this assay.

Overall, these studies demonstrated that compounds 4, 8 and especially 5 displayed the ability to inhibit Cu(II)-mediated A β_{1-40} aggregation, demonstrating their therapeutic value for AD treatment.

15

The Effect of Compounds 1-8 on the Expression of the Autophagic Marker, p62

Studies were performed to examine the effect of compounds 1-8 on autophagy, as it is a catabolic pathway involved in the clearance of damaged cellular proteins and organelles.³¹ Dysfunctional autophagy has been observed in AD and linked to the
20 accumulation of aggregated proteins, such as A β and tau.^{32,33} The effect of compounds 1-8 on the protein, p62, was examined as a useful marker of autophagy.³⁴ This protein is involved in the delivery of damaged cargo to autophagosomes and its levels are known to be inversely related to autophagic flux in the cell.³⁴ The compound, Dp44mT, which is known to activate the autophagic pathway^{15,16} was used as a control.

25 As shown in Figure 8, the levels of p62 were shown to be significantly ($p < 0.001$) decreased after incubation with Dp44mT compared to control cells, suggesting increased autophagic activity (Fig. 8A-H).

Incubation with compounds 3 (Fig. 8A, B) or 2 (Fig. 8E, F) led to a slight, but significant ($p < 0.01-0.05$) increase in p62 levels compared to control cells. However,
30 there was no significant ($p > 0.05$) change in p62 levels after incubation with 1, 6, 4, 7, 8, or 6 compared to control cells (Fig. 8A-H). The lack of change observed in p62 levels

after incubation with the latter compounds may be due to an increase in overall autophagic flux, *i.e.*, both increased formation and degradation of autophagosomes. Thus, studies next assessed the effect of these agents on the autophagic initiation and degradation pathway to assess the autophagic flux.

5 The Effect of Compounds 1-8 on Autophagic Initiation

As autophagy is a dynamic process (Fig. 9Ai), it was crucial to determine the ability of compounds 1-8 to affect autophagic flux. The late-stage autophagy inhibitor, Bafilomycin A1 (Baf A1) was implemented to assess this using standard procedures.³⁴ Cells were pre-incubated for 30 min with the late-stage autophagy inhibitor, Baf A1 (100
10 nM), followed by co-incubation of Baf A1 and Dp44mT (a known inducer of autophagy; 25 μ M)^{15,16} or compounds 1-8 (25 μ M) for 24 h/37°C (Fig. 9B-I). Incubation of cells with Baf A1 inhibits the late-stage autophagic degradation process *via*: **(1)** prevention of lysosomal acidification; and **(2)** inhibition of autophagosome-lysosome fusion (Fig. 9Aii).³⁴

15 The lysates obtained from SK-N-MC cells treated with compounds 1-8 with or without Baf A1 were assessed for LC3-II levels, which is a classical marker for autophagosomes.³⁴ Importantly, LC3-II is present on the autophagosome throughout its lifetime and is a reliable indicator of the quantity of autophagosomes present in the cell (Fig. 9Ai).³⁴

20 Autophagy is a catabolic process that constantly turns over cellular constituents. Thus, the synthesis and degradation of LC3-II is in constant flux under basal conditions (Fig. 9Ai). In the presence of Baf A1, the degradation of the autophagosome, and thus, LC3-II, is inhibited and results in an accumulation of this protein (Fig. 9Aii).³⁴ Upon the addition of an autophagic inducer (*e.g.*, Dp44mT),^{15,16} the synthesis of
25 autophagosomes, and thus, LC3-II, is enhanced in comparison to basal levels and leads to a further increase in LC3-II accumulation when autophagosome turnover is inhibited by Baf A1 (Fig. 9Aiii). In contrast, an agent that inhibits autophagosomal degradation would not mediate a further increase in LC3-II levels upon the addition of Baf A1 relative to the basal LC3-II levels in the presence of Baf A1. Hence, *via* the
30 utilization of Baf A1, the effect of agents on autophagosome turnover can be established.^{31,34}

As shown previously,¹⁶ a marked and significant ($p < 0.001$) increase in LC3-II (18 kDa) levels were observed after incubation with Baf A1 alone (*i.e.*, Baf A1-treated control cells) compared to the control cells (Fig. 9B-I). The levels of LC3-II observed after incubation of control cells with Baf A1 represents basal autophagic initiation and the basal number of autophagosomes generated (see Fig. 9Aii).³⁴ Notably, as shown previously,^{15,16} incubation of cells with Baf A1 and the positive control, Dp44mT, resulted in a significant ($p < 0.001$) increase in LC3-II levels compared to cells incubated with Baf A1 alone (Fig. 9B-I). This observation demonstrates that Dp44mT can initiate the autophagy pathway in SK-N-MC cells to increase autophagosome number (see Fig. 9Aiii), as demonstrated previously in other cell-types.^{15,16}

In cells incubated with 1, 4, 6, or 8 along with Baf A1, a significant ($p < 0.01-0.05$) increase in LC3-II levels was observed compared to control cells incubated with Baf A1 alone (Fig. 9B-E,H,I) and demonstrates that these agents can stimulate the autophagic initiation pathway in SK-N-MC cells. In contrast, there was no significant ($p > 0.05$) increase of LC3-II levels after incubation of cells with 2, 3, 6, or 7 along with Baf A1, when compared to control cells incubated with Baf A1 alone (Fig. 9B-G).

Moreover, there was a slight, but significant ($p < 0.001-0.05$) increase in LC3-II levels upon incubation with 3, 6 or 8 alone (in the absence of Baf A1) relative to the control (Fig. 9B-E,H,I). However, as shown above through BafA1 inhibitor studies, 3 and 6 were unable to induce autophagic initiation (Fig. 9B-E). Hence, the observed increase in LC3-II mediated by 3 and 6 can be attributed to inhibition of the autophagosomal degradation pathway by these compounds.

Taken together, these studies demonstrate that 1, 4, 6 and 8 increase autophagic initiation, while 2, 3, and 5 inhibit the autophagic degradation pathway in SK-N-MC cells. The ability of some of these agents (*e.g.*, compound 1) to increase autophagic initiation may be useful in order to clear protein aggregates (*e.g.*, A β), which is a major problem in AD.³²

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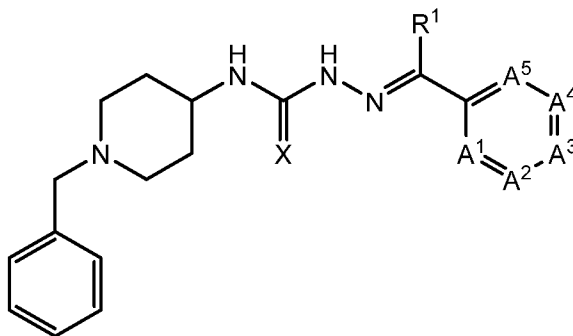
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CLAIMS

1. A compound of formula (I):



- 5 or a pharmaceutically acceptable salt or prodrug thereof, wherein:
- X is selected from O and S,
- R¹ is selected from H, alkyl and heteroaryl,
- A¹, A², A³, A⁴ and A⁵ are independently selected from CH and N, and
- the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is optionally substituted with
- 10 one or more substituents independently selected from halogen, OH, alkyl and heteroalkyl, and is optionally fused to an aryl group.
2. The compound of claim 1, wherein X is S.
3. The compound of claim 1 or claim 2, wherein R¹ is H.
4. The compound of claim 1 or claim 2, wherein R¹ is C₁ to C₃ alkyl.
- 15 5. The compound of claim 4, wherein R¹ is methyl, ethyl or propyl.
6. The compound of claim 1 or claim 2, wherein R¹ is a heteroaryl group.
7. The compound of claim 6, wherein R¹ is pyridine.
8. The compound of any one of the preceding claims, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted.
- 20 9. The compound of claim 8, wherein at least one substituent is OH.
10. The compound of claim 9, wherein one or more of A¹, A², A³, A⁴ and A⁵ is C-OH.

11. The compound of claim 9 or claim 10, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted with one or more additional substituents.

12. The compound of claim 11, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted with one additional substituent.

5 13. The compound of claim 12, wherein the one additional substituent is OH.

14. The compound of claim 11, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is substituted with two additional substituents.

15. The compound of claim 14, wherein the two additional substituents are different to each other.

10 16. The compound of claim 14, wherein the two additional substituents are the same as each other.

17. The compound of any one of claims 14 to 16, wherein the two additional substituents are selected from OH, an alkyl group and a heteroalkyl group.

15 18. The compound of any one of the preceding claims, wherein one of A¹, A², A³, A⁴ and A⁵ is N to give a heteroaryl group.

19. The compound of claim 18, wherein the heteroaryl group is substituted with one or more substituents.

20. The compound of claim 19, wherein the one or more substituents are independently selected from OH, an alkyl group and a heteroalkyl group.

20 21. The compound of claim 20, wherein the alkyl group is methyl or ethyl.

22. The compound of claim 20, wherein the heteroalkyl group is CH₂OH or CH₂CH₂OH.

23. The compound of any one of the preceding claims, wherein A¹ is selected from N and C-OH.

25 24. The compound of claim 23, wherein A¹ is C-OH.

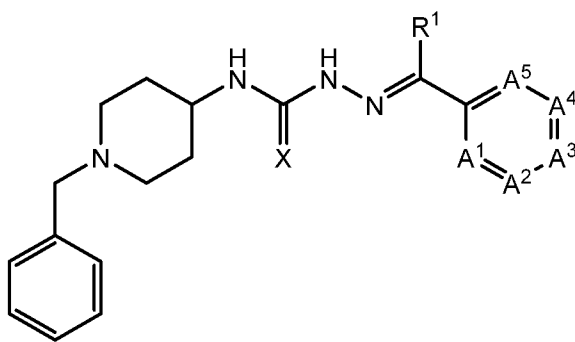
25. The compound of any one of the preceding claims, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is fused to an aryl group to give a bicyclic aryl or heteroaryl group.

26. The compound of claim 25, wherein the bicyclic aryl or heteroaryl group is naphthyl or quinoline.

27. The compound of claim 25 or 26, wherein the bicyclic aryl or heteroaryl group is substituted by one or more substituents selected from OH, alkyl and
5 heteroalkyl.

28. The compound of any one of claims 25 to 27, wherein the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is fused to the aryl group at A² and A³ or A⁴ and A⁵.

29. A pharmaceutical composition including a compound of formula (I):



10

or a pharmaceutically acceptable salt or prodrug thereof, wherein:

X is selected from O and S,

R¹ is selected from H, alkyl and heteroaryl,

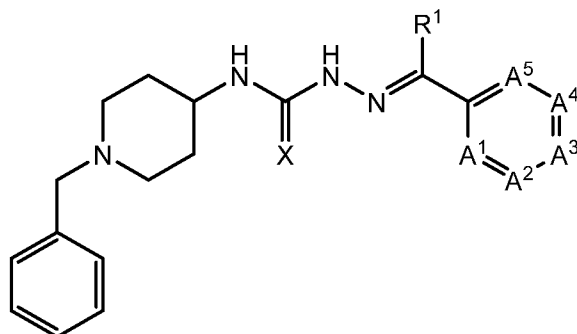
A¹, A², A³, A⁴ and A⁵ are independently selected from CH and N, and

15 the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is optionally substituted with one or more substituents independently selected from halogen, OH, alkyl and heteroalkyl, and is optionally fused to an aryl group,

together with a pharmaceutically acceptable carrier, diluent or excipient.

30. The pharmaceutical composition of claim 29, wherein the composition is
20 suitable for parenteral or oral administration.

31. A method of treating and/or preventing a neurodegenerative disease in a subject, the method including administering to the subject an effective amount of a compound of formula (I):



or a pharmaceutically acceptable salt or prodrug thereof, wherein:

X is selected from O and S,

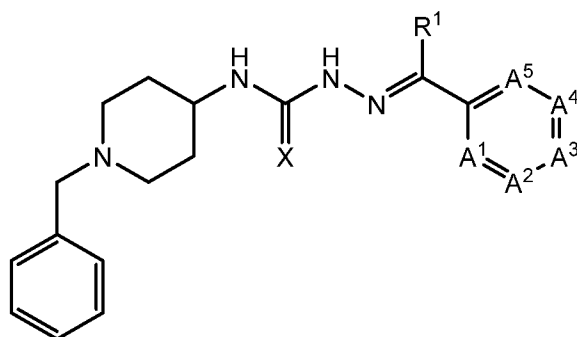
5 R^1 is selected from H, alkyl and heteroaryl,

A^1 , A^2 , A^3 , A^4 and A^5 are independently selected from CH and N, and

the aromatic ring containing A^1 , A^2 , A^3 , A^4 and A^5 is optionally substituted with one or more substituents independently selected from halogen, OH, alkyl and heteroalkyl, and is optionally fused to an aryl group.

10 32. The method of claim 31, wherein the administration is selected from parenteral and oral administration.

33. Use of an effective amount of a compound of formula (I):



or a pharmaceutically acceptable salt or prodrug thereof, wherein:

15 X is selected from O and S,

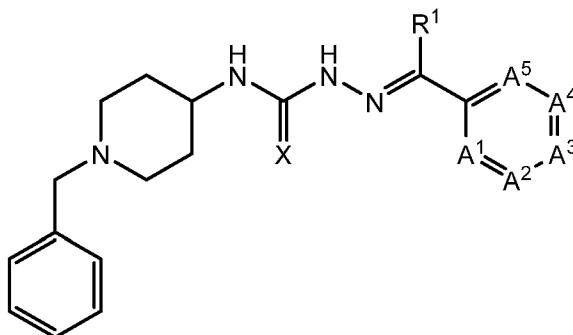
R^1 is selected from H, alkyl and heteroaryl,

A^1 , A^2 , A^3 , A^4 and A^5 are independently selected from CH and N, and

the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is optionally substituted with one or more substituents independently selected from halogen, OH, alkyl and heteroalkyl, and is optionally fused to an aryl group,

in the preparation of a medicament for the treatment of a neurodegenerative
5 disease.

34. Use of an effective amount of a compound of formula (I):



or a pharmaceutically acceptable salt or prodrug thereof, wherein:

X is selected from O and S,

10 R¹ is selected from H, alkyl and heteroaryl,

A¹, A², A³, A⁴ and A⁵ are independently selected from CH and N, and

the aromatic ring containing A¹, A², A³, A⁴ and A⁵ is optionally substituted with one or more substituents independently selected from halogen, OH, alkyl and heteroalkyl, and is optionally fused to an aryl group,

15 for the treatment of a neurodegenerative disease.

35. The method of claim 558, wherein the neurodegenerative disease is selected from dementia, Alzheimer's disease, Parkinson's disease, Huntington's disease and Friedreich's ataxia.

Figure 1

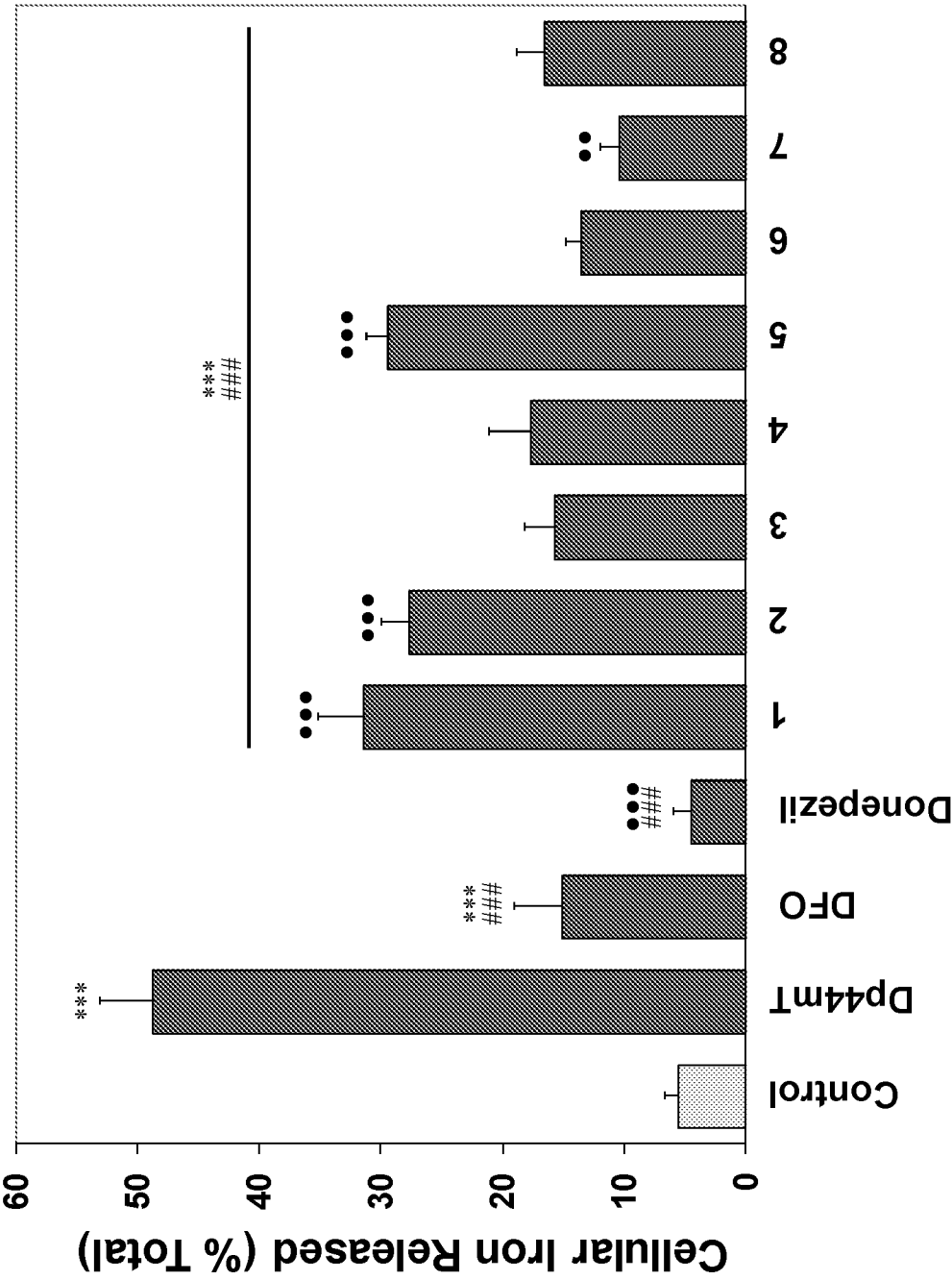


Figure 2

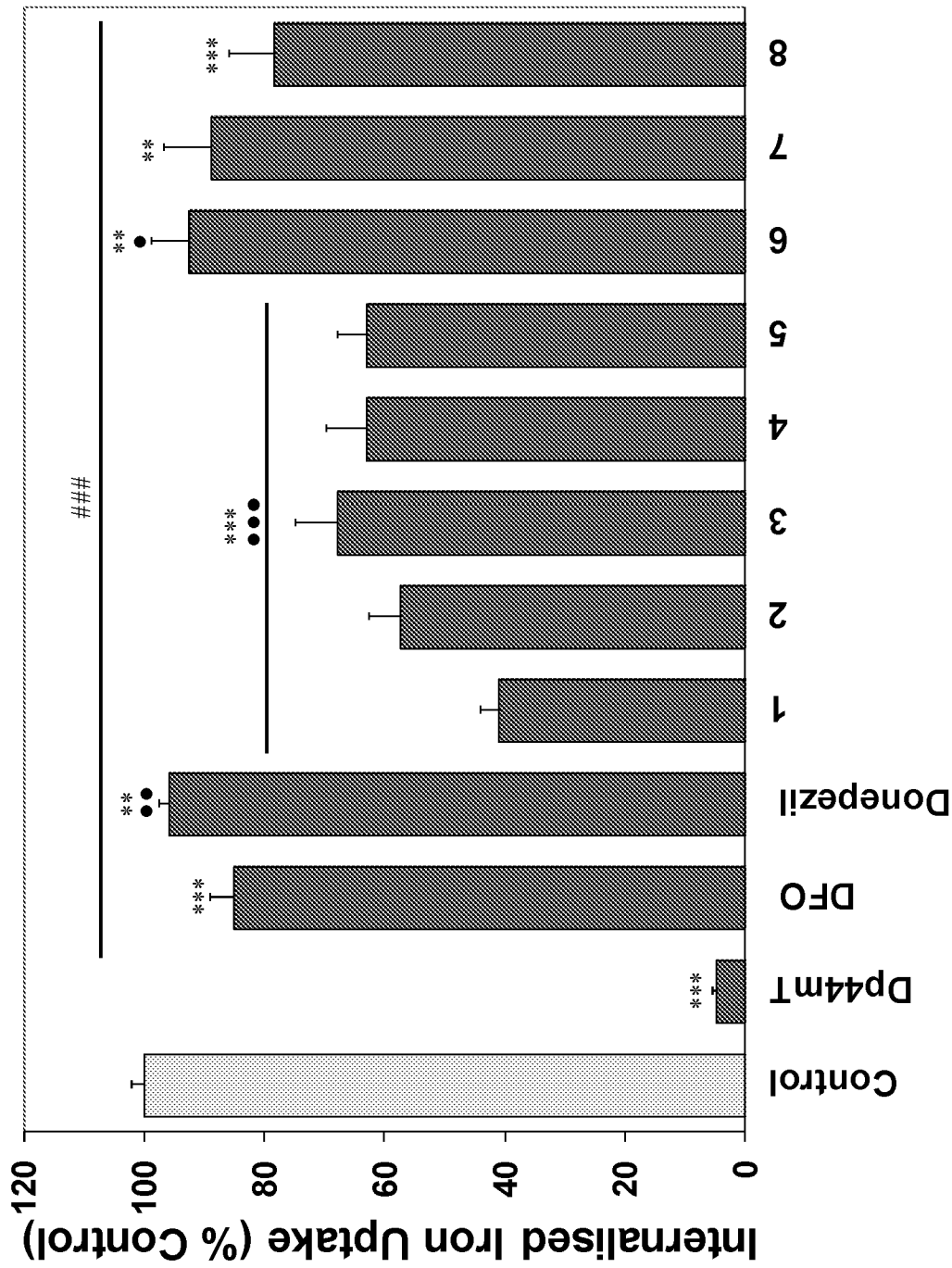


Figure 3

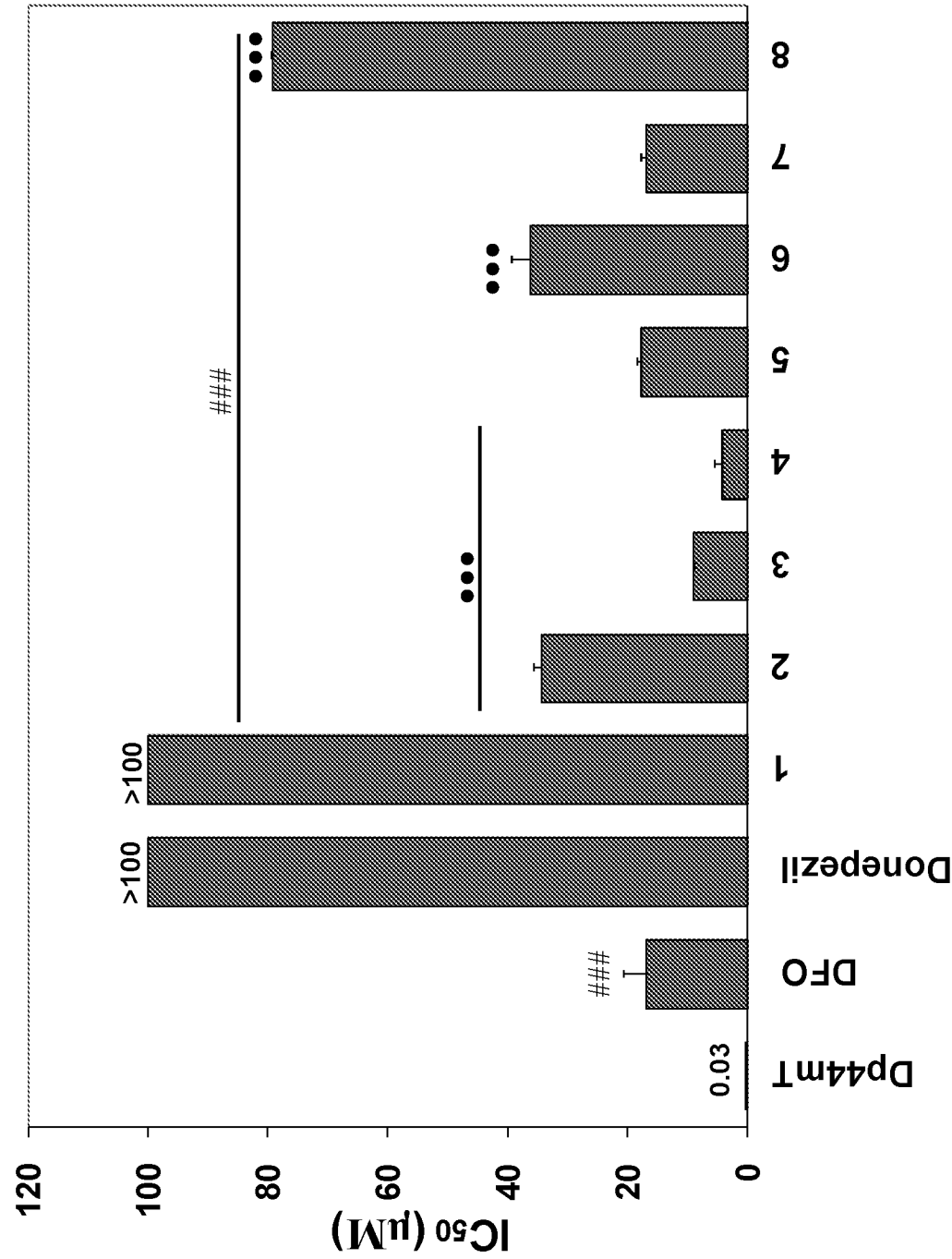


Figure 4

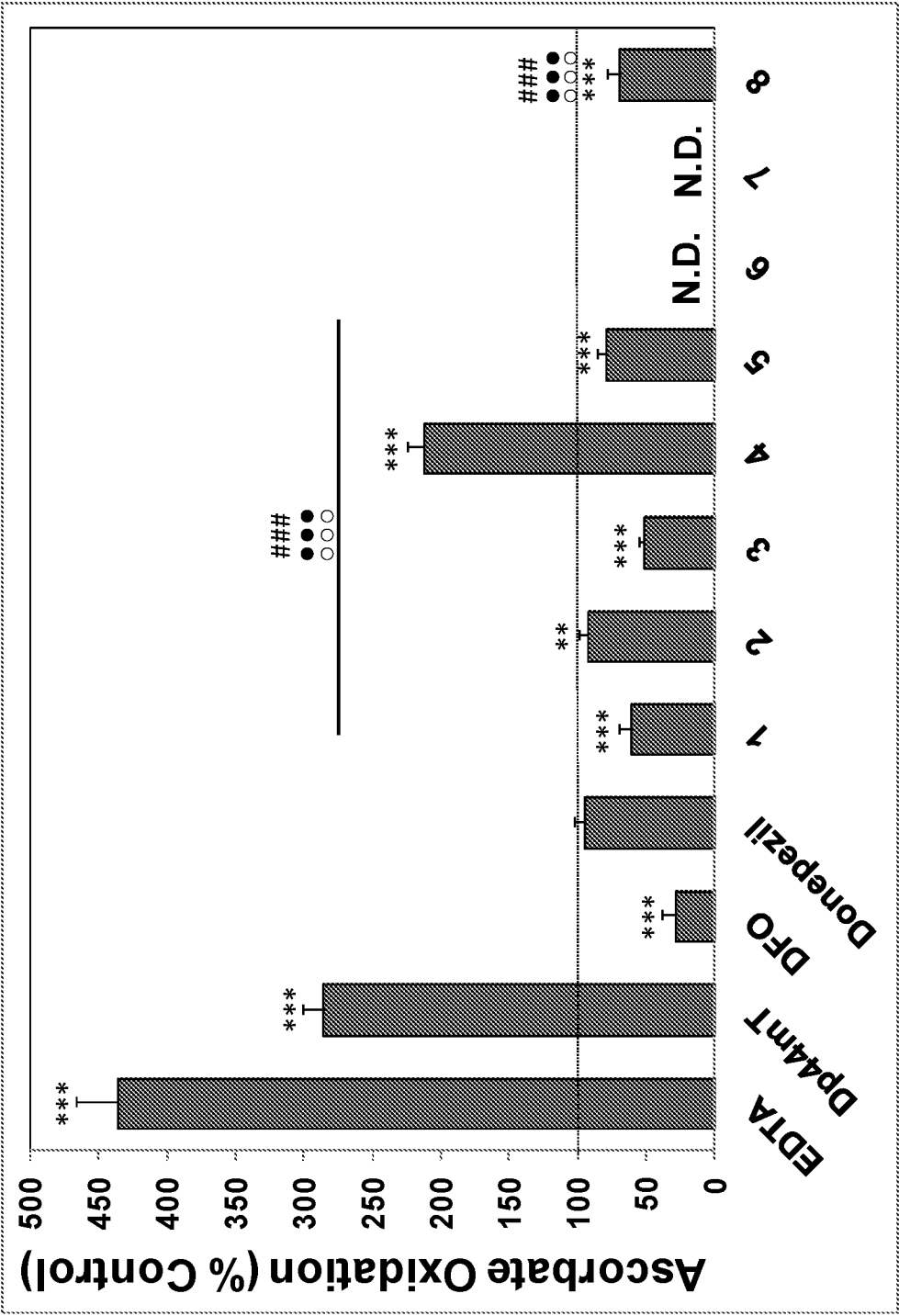


Figure 5

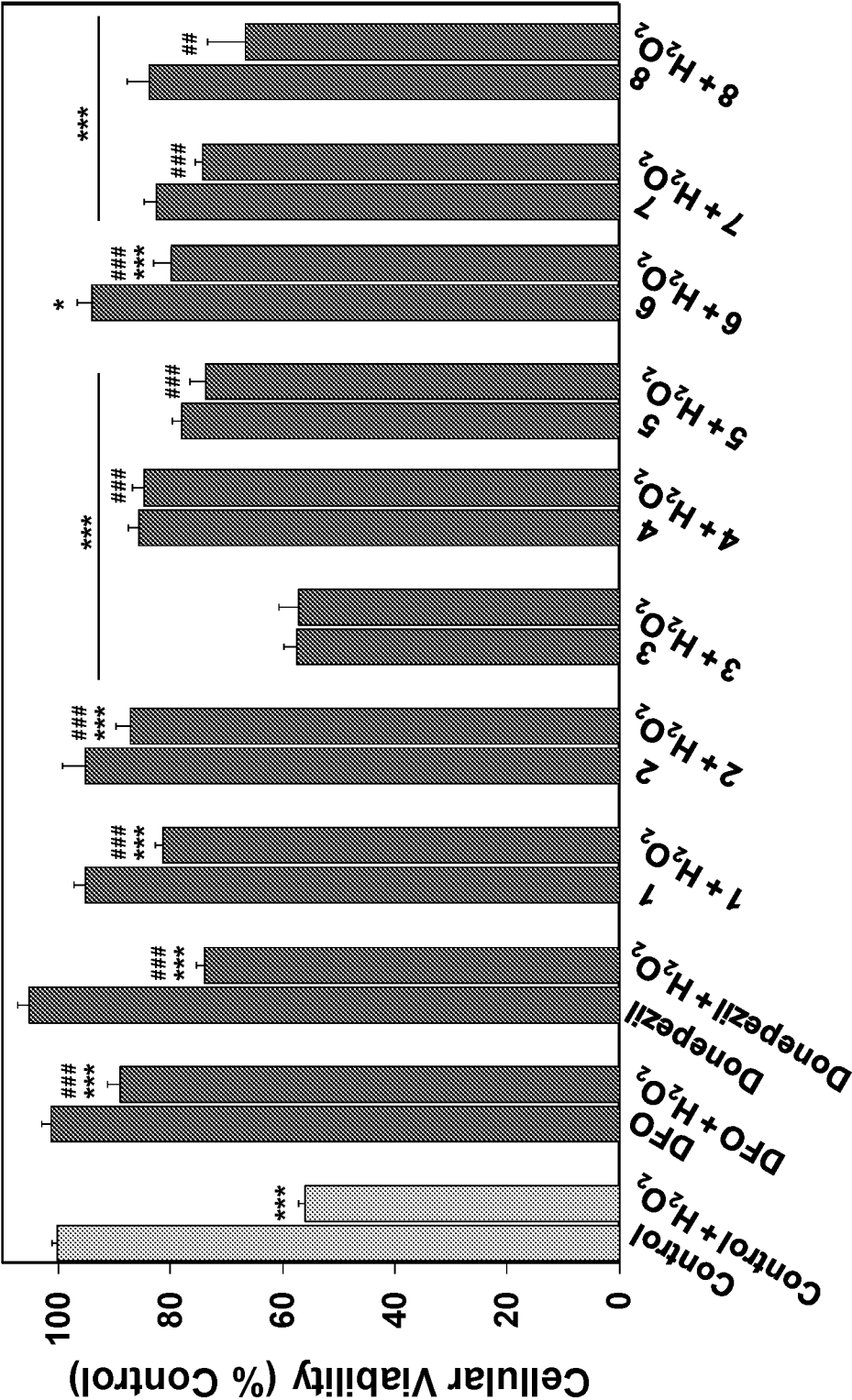


Figure 6

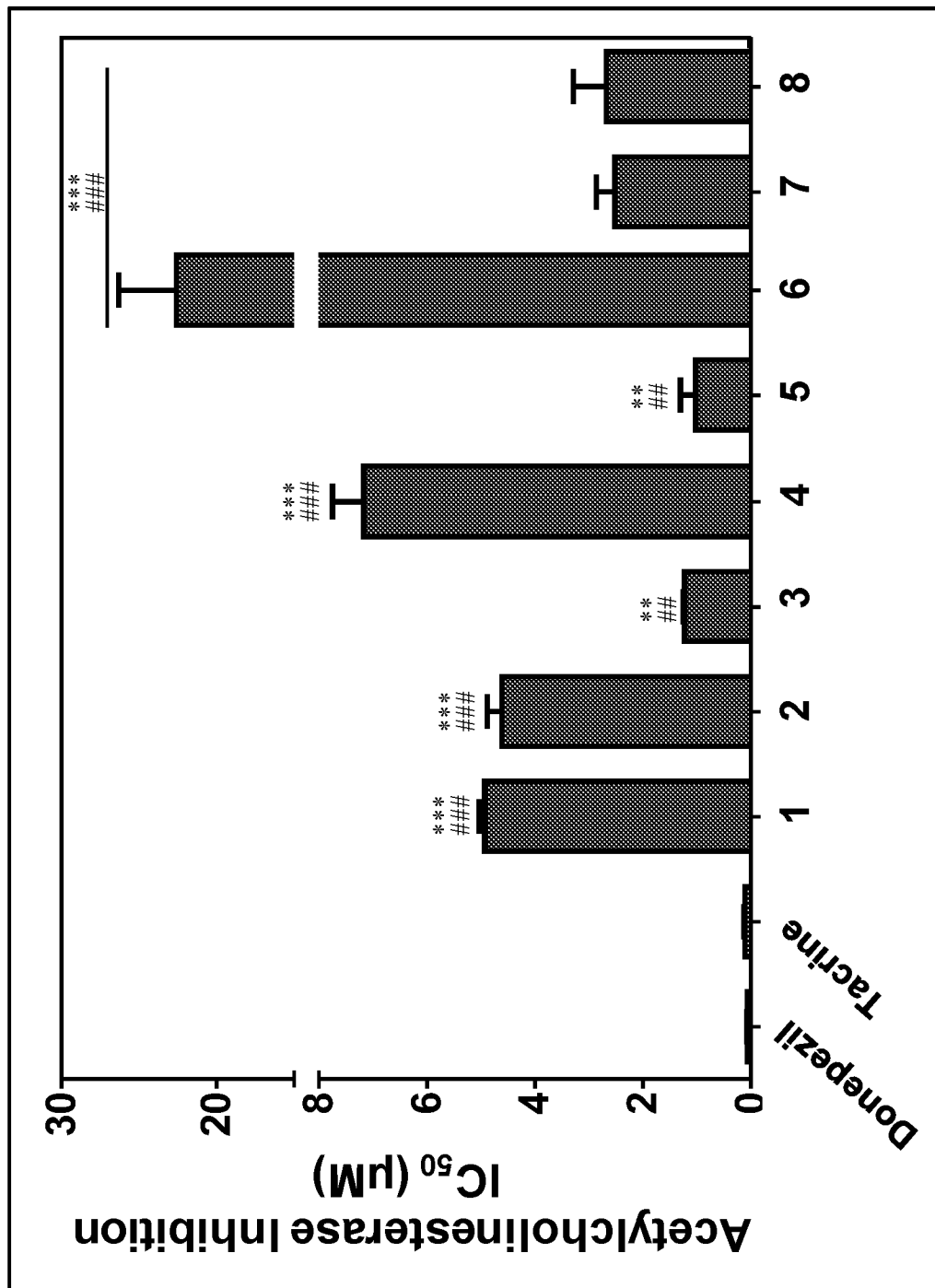


Figure 7

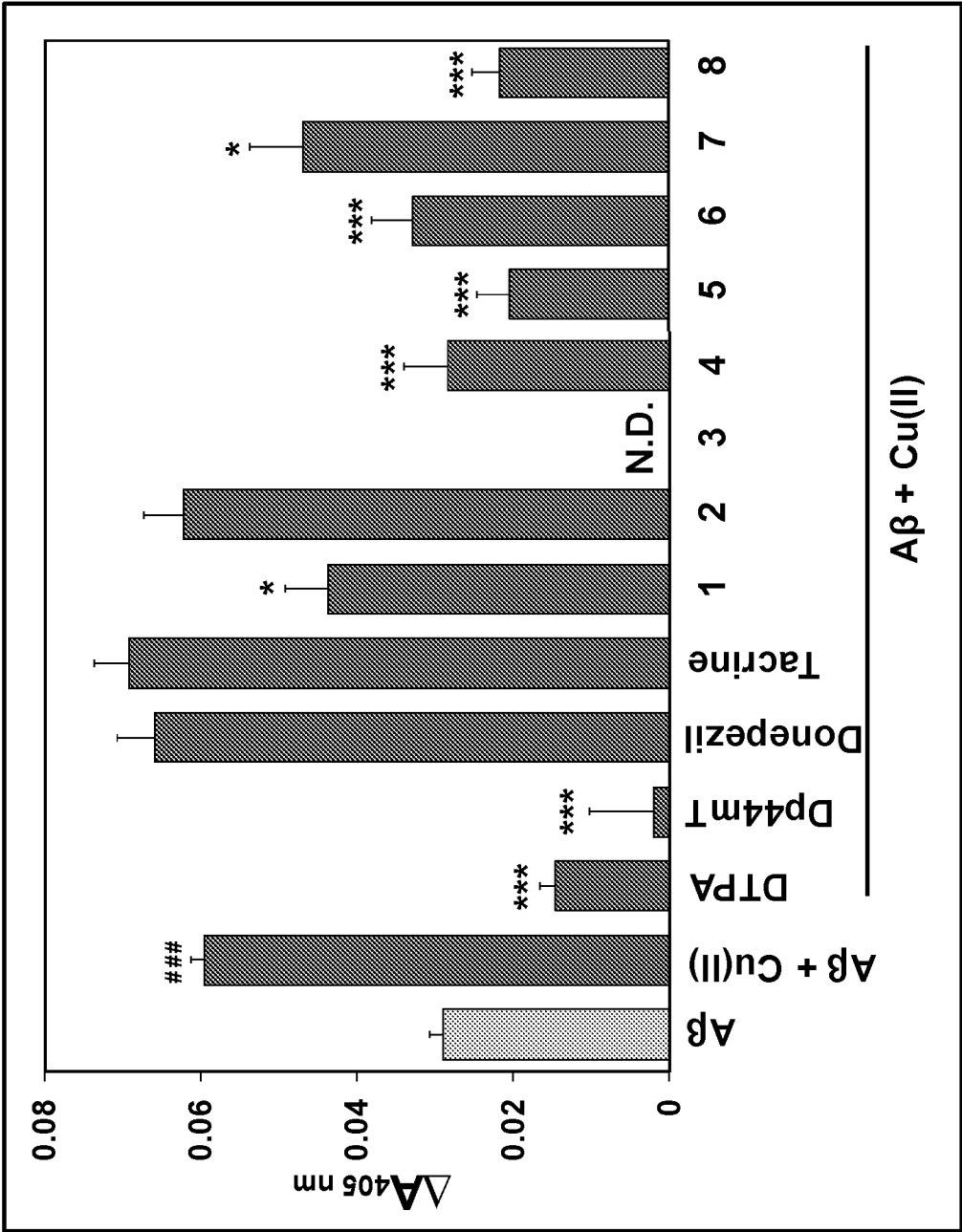


Figure 8

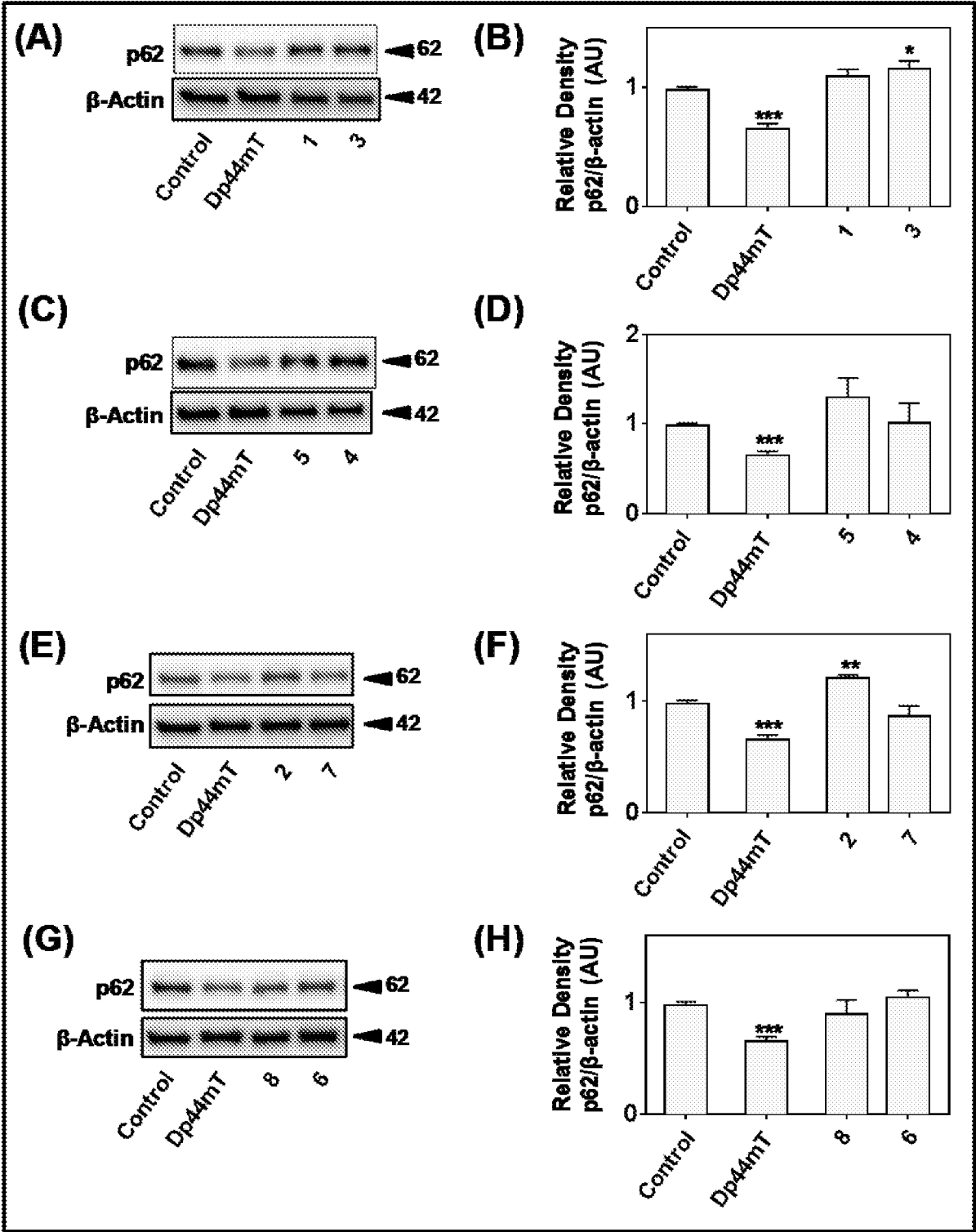


Figure 9

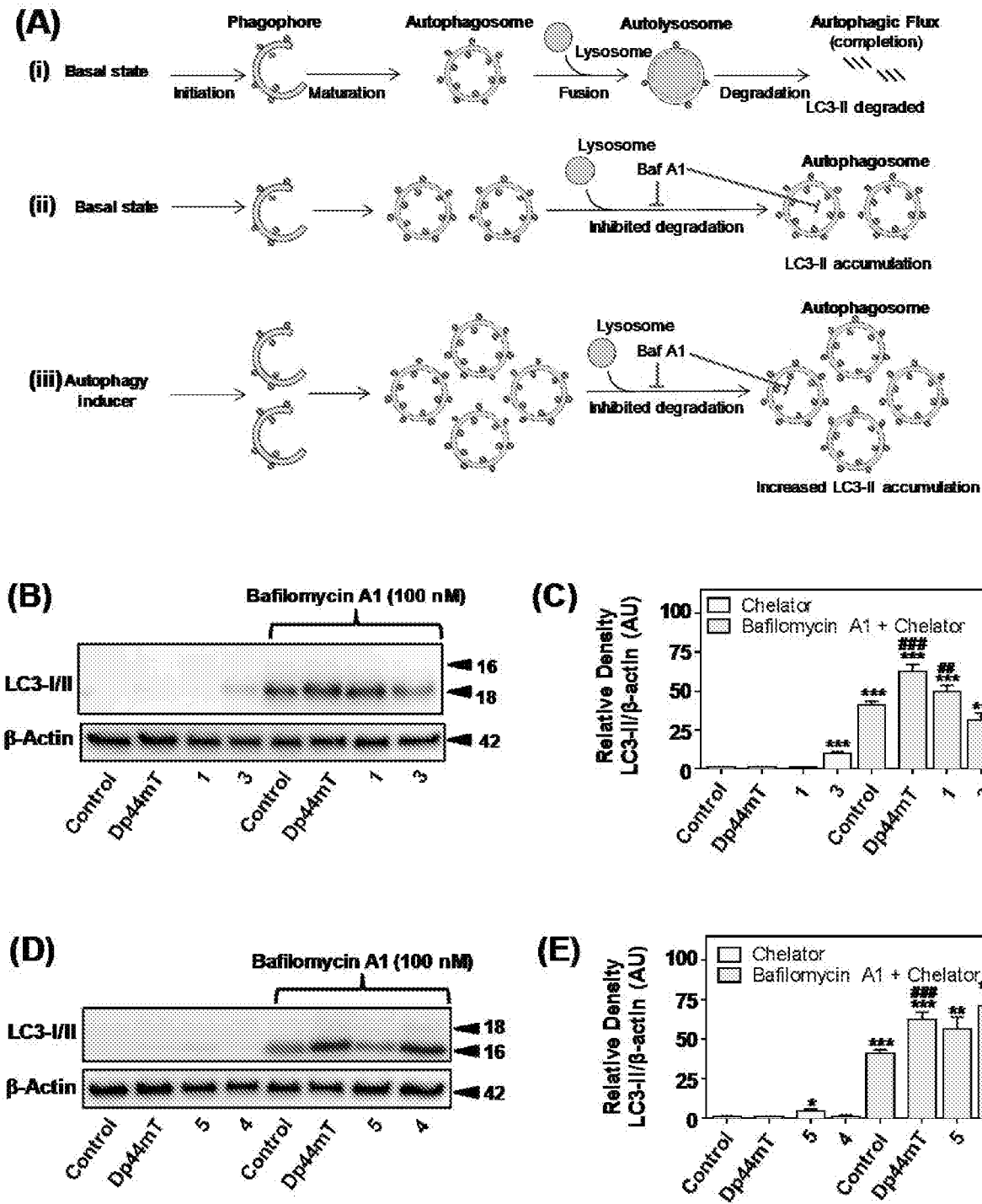
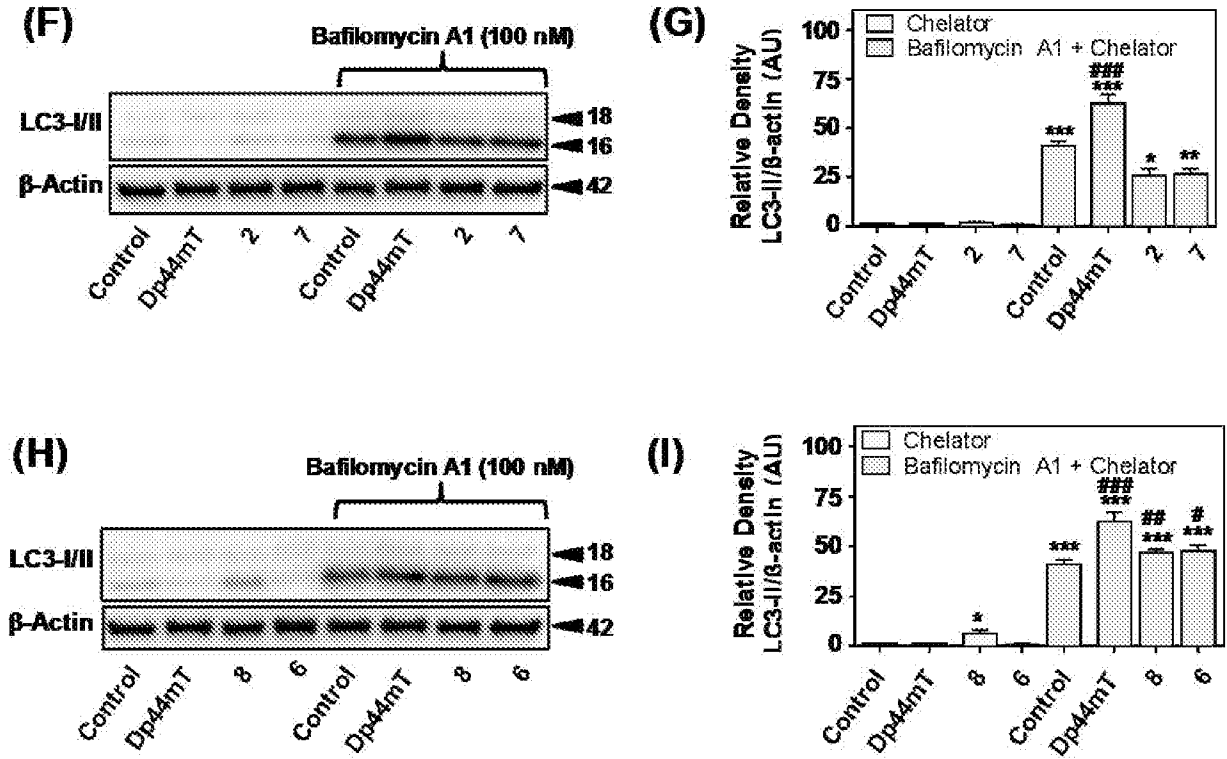


Figure 9 (continued)



INTERNATIONAL SEARCH REPORT

 International application No.
PCT/AU2017/050635

A. CLASSIFICATION OF SUBJECT MATTER

C07D 401/12 (2006.01) **C07D 211/58 (2006.01)** **A61K 31/4545 (2006.01)** **A61K 31/4468 (2006.01)**
A61K 31/4523 (2006.01) **A61P 25/28 (2006.01)** **A61P 25/16 (2006.01)** **A61P 25/14 (2006.01)** **A61P 25/02 (2006.01)**

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)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Invention Search: Database- STN Registry and CAPlus: Based upon substructure searches on compounds of Formula I.

Applicant/Inventor Search: Database- Patenscope: Keywords : 'THE UNIVERSITY OF SYDNEY' OR 'KALINOWSKI Danuta Sandra' OR 'PALANIMUTHU Duraippandi' OR 'RICHARDSON Des'

Applicant(s)/Inventor(s) name searched in internal databases provided by IP Australia

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier application or patent but published on or after the international filing date	"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
"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)	"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
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 29 September 2017	Date of mailing of the international search report 29 September 2017
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au	Authorised officer Will Findlay AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262832018

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2017/050635
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CAS Registry number #1055125-26-2, STN entry date: 30 th September 2008, Compound name: 2-[[2-[[[1-(phenylmethyl)-4-piperidinyl]amino]thioxomethyl]hydrazinylidene]methyl]-benzoic acid Whole document	1-28
A	CAS Registry number #1054506-74 - 9, STN entry date: 29 th September 2008, Compound name: 2-[4-[[2-[[[1-(phenylmethyl)-4-piperidinyl]amino]thioxomethyl]hydrazinylidene]methyl]phenoxy]-acetic acid Whole document	1-28
A	CAS Registry number #1054423-57-2, STN entry date: 29 th September 2008, Compound name: 4-[[2-[[[1-(phenylmethyl)-4-piperidinyl]amino]thioxomethyl]hydrazinylidene]methyl]-benzoic acid Whole document	1-28
A	CAS Registry number #1054391-13-7, STN entry date: 29 th September 2008, Compound name: 2-[2-[[2-[[[1-(phenylmethyl)-4-piperidinyl]amino]thioxomethyl]hydrazinylidene]methyl]phenoxy]-acetic acid Whole document	1-28
A	OPLETALOVA V. et al., "Thiosemicarbazones and their antimycobacterial effects." Ceska a Slovenska Farmacie, 2013, Vol. 62, pages 78-83 Abstract; Compounds 6a-6s (Page 80, Figure 3); Experimental part (Page 81)	1-35
A	CN 104230845 A (Shenyang Pharmaceutical University) 24 December 2014 Abstract; Compounds of formula I (claim 1), Compounds 2-[(2,4-dichlorophenyl)methylene]-N-[6-[(4-methyl-1-piperidinyl)methyl]-2-benzothiazolyl]-hydrazinecarboxamide & 2-[[3,5-bis(1,1-dimethylethyl)-2-hydroxyphenyl]methylene]-N-[6-[(4-methyl-1-piperidinyl)methyl]-2-benzothiazolyl]-hydrazinecarboxamide	1-35
A	RAJAK H. et al., "Synthesis and anticonvulsant evaluation of some novel 2,5-disubstituted 1,3,4-thiadiazoles: pharmacophore model studies", Acta Poloniae Pharmaceutica-Drug Research, 2010, Vol. 67, No. 5, pages 503-510 Abstract; Pharmacophore model (figure 1); Compounds 1, 3, 4, 6,7 and 10 (scheme 1).	1-35
Form PCT/ISA/210 (fifth sheet) (July 2009)		

