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(54) **Title:** METHODS AND PHARMACEUTICAL COMPOSITIONS COMPRISING CARBOXYALKYLSULFONIC ACIDS

(57) **Abstract:** Pharmaceutical compositions and methods for using compounds of Formula (I), and pharmaceutically acceptable solvates thereof are described. The compounds of the invention are particularly useful as neuroprotective agents and for the prevention and/or treatment Aβ-amyloid related diseases, including Alzheimer's disease.

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METHODS AND PHARMACEUTICAL COMPOSITIONS COMPRISING CARBOXYALKYLSULFONIC ACIDS

Related Applications

This application claims priority to U.S. Provisional Application Serial No. 60/722,891, filed September 30, 2005, the entire contents of which are hereby incorporated herein by reference.

Background of the Invention

The Aβ peptide has been shown by several groups to be highly toxic to neurons. The amyloid plaques are directly associated with reactive gliosis, dystrophic neurites and apoptotic cells, suggesting that plaques induce neurodegenerative changes. *In vitro*, Aβ has been shown to be necrotic in rat PC-12 cells while it induces apoptosis in primary hippocampal cultures from fetal rat and in the predifferentiated human neurotype SH-SY5Y cell line (Li et al. (1996) *Brain Research* 738:196-204).

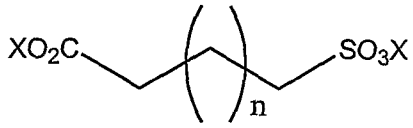
Numerous reports have shown that fibrillary Aβ can induce neurodegeneration, and non-fibrillar Aβ has also been shown to be cytotoxic to neurons. La Ferla et al. ((1997) *J. Clin. Invest.* 100(2):310-320) showed that neuronal cells exposed *in vitro* to soluble Aβ can become apoptotic. Once internalized, the Aβ peptide gets stabilized and induces DNA fragmentation, which is characteristic of apoptosis. In Alzheimer's disease, a progressive neuronal cell loss accompanies the deposition of Aβ amyloid fibrils in senile plaques.

Organisms eliminate unwanted cells by a process known as programmed cell death or apoptosis. Such cell death occurs as a normal aspect of animal development as well as in tissue homeostasis and aging (Glucksmann, A., *Biol. Rev. Cambridge Philos. Soc.* 26:59-86 (1951); Glucksmann, A., *Archives de Biologie* 76:419-437 (1965); Ellis et al., *Dev.* 112:591-603 (1991); Vaux et al., *Cell* 76:777-779 (1994)). Apoptosis regulates cell number, facilitates morphogenesis, removes harmful or otherwise abnormal cells and eliminates cells that have already performed their function. Additionally, apoptosis occurs in response to various physiological stresses, such as hypoxia or ischemia (PCT published application WO96/20721).

There are a number of morphological changes shared by cells experiencing programmed cell death, including plasma and nuclear membrane blebbing, cell shrinkage (condensation of nucleoplasm and cytoplasm), organelle relocalization and compaction, chromatin condensation and production of apoptotic bodies (membrane enclosed particles containing intracellular material) (Orrenius, S., *J. Internal Medicine* 237:529-536 (1995)).

Summary of the Invention

In one embodiment, the invention pertains, at least in part, to a pharmaceutical composition comprising a compound of Formula (I) or a pharmaceutically acceptable solvate thereof:



(I)

wherein

X is cationic group or ester-forming group independently chosen for each occurrence; and

n is 0, 1, 2 , 3, 4, 5, 6, 7, or 8.

In a further embodiment, the invention also pertains, at least in part, to a method for treating an Aβ-amyloid related disease in a subject, comprising administering to said subject, in need thereof, an effective amount of a pharmaceutical composition comprising a compound of Formula (I) or a pharmaceutically acceptable solvate thereof, such that the Aβ-amyloid related disease is treated in the subject.

In another embodiment, the invention also pertains, at least in part, to a method for treating a carboxyalkyl sulfonic acid responsive state in a subject. The method includes administering to the subject, in need thereof, an effective amount of a pharmaceutical composition comprising a compound of Formula (I) or a pharmaceutically acceptable solvate thereof, such that the carboxyalkyl sulfonic acid responsive state is treated in the subject.

Brief Descriptions of the Drawings

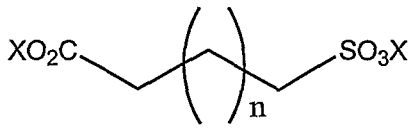
Figure 1 is a bar graph which shows the effect of the test compounds on Aβ1-42-induced apoptosis in primary rat neurons.

Figure 2 is a bar graph which shows the effect of the test compounds on Aβ1-42-induced apoptosis in neuroblastoma SH-SY5Y.

Detailed Description of the Invention

The present invention relates to 3-sulfo-1-propanoic acid, its salts and related carboxyalkylsulfonic acids and salts thereof. The present invention also relates to the use of those compounds as neuroprotective agents and for the prevention and/or treatment Aβ-amyloid related diseases.

The term "carboxyalkyl sulfonic acids" includes compounds comprising a carboxylate and a sulfonic acid group linked by a straight chain alkyl group and their salts. In a further embodiment, the carboxyalkyl sulfonic acids are of Formula (I):



(I)

wherein

X is a cationic group or ester-forming group independently chosen for each occurrence; and

n is 0, 1, 2 , 3, 4, 5, 6, 7, or 8.

The term "cationic group" includes groups with a positive charge and hydrogen atoms. Examples of cationic groups include inorganic and organic base addition salts, for example, ions of alkali or alkaline earth metals, such as lithium, sodium, potassium, calcium, magnesium, and aluminum and the like. Preferably, the salts are relatively non-toxic and are pharmaceutically acceptable. In a further embodiment, the cationic groups are H^+ or Na^+.

These compounds with cationic groups can be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary or tertiary amine. Representative organic amines useful as cationic groups include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

In another further embodiment, n is 0 or 1.

The term "ester-forming group" refers to alkyl or aryl groups which are capable forming esters with carboxylic acids or sulfonic acids. Examples of ester-forming group include methyl, ethyl, propyl, benzyl, or phenyl.

The invention pertains to both salt forms and acid forms of the compounds of Formula (I). For example, the invention pertains not only to the particular disodium salt form of 3-sulfo-1-propanoic acid shown herein, but it also includes other pharmaceutically acceptable salts, and the acid form of the compound. The invention also pertains to all salt forms of compounds shown herein.

In preferred embodiments, the compound is 3-sulfo-1-propanoic acid, disodium salt. In other embodiment, the compound is 3-sulfo-1-propanoic acid, dilithium salt; 3-sulfo-1-propanoic acid, dipotassium salt; 3-sulfo-1-propanoic acid, calcium salt; 3-sulfo-1-propanoic acid, magnesium salt; 3-sulfo-1-propanoic acid, aluminium salt; 3-sulfo-1-propanoic acid, ethylamine salt; 3-sulfo-1-propanoic acid, diethylamine salt; 3-sulfo-1-propanoic acid, ethylenediamine salt; 3-sulfo-1-propanoic acid, ethanolamine salt; 3-sulfo-1-propanoic acid, diethanolamine salt; and 3-sulfo-1-propanoic acid, piperazine salt. In another embodiment, the compound for use in the compositions and/or methods

according to the invention is a pharmaceutically acceptable salt or solvate of 3-sulfo-1-propanoic acid.

All acid, salt, and other ionic and non-ionic forms of the compounds described are included as compounds of the invention. For example, if a compound is shown as an acid herein, the salt forms of the compound are also included. Likewise, if a compound is shown as a salt, the acid form is also included.

The compounds may exist in several tautomeric forms including the enol form, the keto form, and mixtures thereof. Accordingly, the chemical structures depicted herein encompass all possible tautomeric forms of the illustrated compounds.

The disclosed compounds also include isotopically labeled compounds where one or more atoms have an atomic mass different from the atomic mass most abundantly found in nature. Examples of isotopes that may be incorporated into the compounds of the present invention include, but are not limited to, ^{2}H (D), ^{3}H (T), ^{11}C, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, etc.

Certain compounds according to the invention may exist in multiple crystalline or amorphous forms. The compounds of the present invention may exhibit polymorphism. Polymorphs of compounds according to this invention may be prepared by crystallization under different conditions. For example, using different solvents or different solvent mixtures for recrystallization; crystallization at different temperatures; various modes of cooling ranging from very fast to very slow cooling during crystallization. The presence of polymorphs may be determined by solid probe NMR spectroscopy, IR spectroscopy, differential scanning calorimetry, powder X-ray diffraction or other such techniques. In general, all physical forms are equivalent for the uses contemplated herein and are intended to be within the scope of the present invention.

The compounds of the present invention may also exist in the form of solvates, for example, hydrates, ethanolate, n-proponalate, iso-propanolate, 1-butanolate, 2-butanolate and solvates of other physiologically acceptable solvents, such as the Class 3 solvents described in the *International Conference on Harmonization (ICH), Guidance for Industry, Q3C Impurities: Residual Solvents* (1997). The present invention includes each solvate and mixtures thereof.In an embodiment, the invention pertains to a method for treating an Aβ-amyloid related disease in a subject, by administering to the subject, in need thereof, an effective amount of a pharmaceutical composition comprising a compound of Formula (I), or a pharmaceutically acceptable solvate thereof, such that the Aβ-amyloid related disease is treated in the subject.

The term "amyloid" refers to amyloidogenic proteins, peptides, or fragments thereof which can be soluble (*e.g.*, monomeric or oligomeric) or insoluble (*e.g.*, having fibrillary structure or in amyloid plaque). *See, e.g.*, MP Lambert, *et al.*, *Proc. Nat'l*

Acad. Sci. USA 95, 6448-53 (1998). "Amyloidosis" or "amyloid disease" or "amyloid-related disease" refers to a pathological condition characterized by the presence of amyloid fibers. "Amyloid" is a generic term referring to a group of diverse but specific protein deposits (intracellular or extracellular) which are seen in a number of different diseases. Though diverse in their occurrence, all amyloid deposits have common morphologic properties, stain with specific dyes (*e.g.*, Congo red), and have a characteristic red-green birefringent appearance in polarized light after staining. They also share common ultrastructural features and common X-ray diffraction and infrared spectra.

The terms "Aβ-amyloid related diseases" or "amyloid–β diseases" refer to diseases or disorders which are associated with Aβ amyloidosis or are related to the undesirable formation and/or deposition of amyloid-β. Aβ-amyloid related diseases includes those diseases, disorders, conditions, pathologies, and other abnormalities of the structure or function of the brain, including components thereof, in which the causative agent is amyloid-β. Local deposition of amyloid-β is common in the brain, particularly in elderly individuals. The area of the brain affected in an amyloid–β disease may be the stroma including the vasculature or the parenchyma including functional or anatomical regions, or neurons themselves. The most frequent type of amyloid in the brain is composed primarily of Aβ peptide fibrils, resulting in dementia associated with e.g. Alzheimer's disease. A subject need not have received a definitive diagnosis of a specifically recognized amyloid–β disease.

Amyloid–β peptide (Aβ) is a 39-43 amino acid peptide derived by proteolysis from a large protein known as Beta Amyloid Precursor Protein ("βAPP"). Mutations in βAPP result in familial forms of Alzheimer's disease, Down's syndrome, cerebral amyloid angiopathy (e.g. hereditary cerebral hemorrhage) and senile dementia, characterized by cerebral deposition of plaques composed of Aβ fibrils and other components, which are described in further detail below. Known mutations in APP associated with Alzheimer's disease occur proximate to the cleavage sites of β or γ-secretase, or within Aβ. For example, position 717 is proximate to the site of gamma-secretase cleavage of APP in its processing to Aβ, and positions 670/671 are proximate to the site of β-secretase cleavage. Mutations at any of these residues may result in Alzheimer's disease, presumably by causing an increase in the amount of the 42/43 amino acid form of Aβ generated from APP. The familial form of Alzheimer's disease represents only 10% of the subject population. In fact, the incidence of sporadic Alzheimer's disease greatly exceeds forms shown to be hereditary. Nevertheless, fibril peptides forming plaques are very similar in both types.

The structure and sequence of Aβ peptides of various lengths are well known in the art. Such peptides can be made according to methods known in the art, or extracted from the brain according to known methods (*e.g.*, Glenner and Wong, *Biochem. Biophys. Res. Comm.* 129, 885-90 (1984); Glenner and Wong, *Biochem. Biophys. Res. Comm.* 122, 1131-35 (1984)). In addition, various forms of the peptides are commercially available.

The terms "β amyloid," "amyloid–β," and the like refer to amyloid β proteins or peptides, amyloid β precursor proteins or peptides, intermediates, and modifications and fragments thereof, unless otherwise specifically indicated. In particular, "Aβ" refers to any peptide produced by proteolytic processing of the APP gene product, especially peptides which are associated with amyloid pathologies, including Aβ1-39, Aβ1-40, Aβ1-41, Aβ1-42, Aβ1-43, Aβ3-40, Aβ3-42, Aβ3-43, Aβ3(pG)-40, Aβ3(pG)-42, and Aβ3(pG)-43. For convenience of nomenclature, "Aβ1-42" may be referred to herein as "Aβ(1-42)" or simply as "Aβ42" or "$A\beta_{42}$" (and likewise for any other amyloid peptides discussed herein). As used herein, the terms "β amyloid," "amyloid-β," and "Aβ" are synonymous. Unless otherwise specified, the term "amyloid" refers to amyloidogenic proteins, peptides, or fragments thereof which can be soluble (*e.g.*, monomeric or oligomeric) or insoluble (*e.g.*, having fibrillary structure or in amyloid plaque). *See, e.g.*, MP Lambert, *et al.*, *Proc. Nat'l Acad. Sci. USA* 95, 6448-53 (1998).

According to certain aspects of the invention, amyloid–β is a peptide having 39-43 amino-acids, or amyloid–β is an amyloidogenic peptide produced from βAPP. The Aβ-amyloid related diseases that are the subject of the present invention include, without limitation, age-related cognitive decline, early Alzheimer's disease as seen in Mild Cognitive Impairment ("MCI"), vascular dementia, or Alzheimer's disease ("AD"), which may be sporadic (non-hereditary) Alzheimer's disease or familial (hereditary) Alzheimer's disease. The Aβ-amyloid related disease may also be cerebral amyloid angiopathy ("CAA") or hereditary cerebral hemorrhage. The Aβ-amyloid related disease may be senile dementia, Down's syndrome, inclusion body myositis ("IBM"), or age-related macular degeneration ("ARMD").

Mild cognitive impairment ("MCI") is a condition characterized by a state of mild but measurable impairment in thinking skills, which is not necessarily associated with the presence of dementia. MCI frequently, but not necessarily, precedes Alzheimer's disease. It is a diagnosis that has most often been associated with mild memory problems, but it can also be characterized by mild impairments in other thinking skills, such as language or planning skills. However, in general, an individual with MCI will have more significant memory lapses than would be expected for someone of their age or educational background. As the condition progresses, a

physician may change the diagnosis to "Mild-to-Moderate Cognitive Impairment," as is well understood in this art.

Cerebral amyloid angiopathy ("CAA") refers to the specific deposition of amyloid fibrils in the walls of leptomingeal and cortical arteries, arterioles and in capillaries and veins. It is commonly associated with Alzheimer's disease, Down's syndrome and normal aging, as well as with a variety of familial conditions related to stroke or dementia (*see* Frangione, *et al.*, *Amyloid: J. Protein Folding Disord.* 8, Suppl. 1, 36-42 (2001)). CAA can occur sporadically or be hereditary. Multiple mutation sites in either Aβ or the APP gene have been identified and are clinically associated with either dementia or cerebral hemorrhage. Exemplary CAA disorders include, but are not limited to, hereditary cerebral hemorrhage with amyloidosis of Icelandic type (HCHWA-I); the Dutch variant of HCHWA (HCHWA-D; a mutation in Aβ); the Flemish mutation of Aβ; the Arctic mutation of Aβ; the Italian mutation of Aβ; the Iowa mutation of Aβ; familial British dementia; and familial Danish dementia. Cerebral amyloid angiopathy is known to be associated with cerebral hemorrhage (or hemorrhagic stroke).

Additionally, abnormal accumulation of APP and of amyloid–β protein in muscle fibers has been implicated in the pathology of sporadic inclusion body myositis ("IBM") (Askanas, *et al.*, *Proc. Natl. Acad. Sci. USA* 93, 1314-19 (1996); Askanas, *et al.*, *Current Opinion in Rheumatology* 7, 486-96 (1995)). Accordingly, the compounds of the invention can be used prophylactically or therapeutically in the treatment of disorders in which amyloid–β protein is abnormally deposited at non-neurological locations, such as treatment of IBM by delivery of the compounds to muscle fibers.

Additionally, it has been shown that Aβ is associated with abnormal extracellular deposits, known as drusen, that accumulate along the basal surface of the retinal pigmented epithelium in individuals with age-related macular degeneration (ARMD). ARMD is a cause of irreversible vision loss in older individuals. It is believed that Aβ deposition could be an important component of the local inflammatory events that contribute to atrophy of the retinal pigmented epithelium, drusen biogenesis, and the pathogenesis of ARMD (Johnson, *et al.*, *Proc. Natl. Acad. Sci. USA* 99(18), 11830-5 (2002)). Therefore, the invention also relates to the treatment of age-related macular degeneration.

APP is expressed and constitutively catabolized in most cells. The dominant catabolic pathway appears to be cleavage of APP within the Aβ sequence by the α-secretase enzyme, leading to release of a soluble ectodomain fragment known as APPsα. In contrast to this non-amyloidogenic pathway, APP can also be cleaved by enzymes known as β- and γ-secretase at the *N*- and *C*-termini of the Aβ, respectively, followed by

release of Aβ into the extracellular space. To date, BACE has been identified as β-secretase (Vasser, *et al.*, *Science* 286:735-741, 1999) and presenilins have been implicated in γ-secretase activity (De Strooper, *et al.*, *Nature* 391, 387-90 (1998)).

The 39-43 amino acid Aβ peptide is produced by sequential proteolytic cleavage of the amyloid precursor protein (APP) by the enzyme(s) β and γ secretases. Although Aβ40 is the predominant form produced, 5-7% of total Aβ exists as Aβ42 (Cappai *et al.*, *Int. J. Biochem. Cell Biol.* 31. 885-89 (1999)). The length of the Aβ peptide appears to dramatically alter its biochemical/biophysical properties. Specifically, the additional two amino acids at the *C*-terminus of Aβ42 are very hydrophobic, presumably increasing the propensity of Aβ42 to aggregate. For example, Jarrett, *et al.* demonstrated that Aβ42 aggregates very rapidly *in vitro* compared to Aβ40, suggesting that the longer forms of Aβ may be important pathological proteins that are involved in the initial seeding of the neuritic plaques in Alzheimer's disease (Jarrett, *et al.*, *Biochemistry* 32, 4693-97 (1993); Jarrett, *et al.*, *Ann. N.Y. Acad. Sci.* 695, 144-48 (1993)).

This hypothesis has been further substantiated by the recent analysis of the contributions of specific forms of Aβ in cases of genetic familial forms of Alzheimer's disease ("FAD"). For example, the "London" mutant form of APP (APPV717I) linked to FAD selectively increases the production of Aβ 42/43 forms versus Aβ 40 (Suzuki, *et al.*, *Science* 264, 1336-40 (1994)) while the "Swedish" mutant form of APP (APPK670N/M671L) increases levels of both Aβ40 and Aβ42/43 (Citron, *et al.*, *Nature* 360, 672-674 (1992); Cai, *et al.*, *Science* 259, 514-16, (1993)). Also, it has been observed that FAD-linked mutations in the Presenilin-1 ("PS1") or Presenilin-2 ("PS2") genes will lead to a selective increase in Aβ42/43 production but not Aβ40 (Borchelt, *et al.*, *Neuron* 17, 1005-13 (1996)). This finding was corroborated in transgenic mouse models expressing PS mutants that demonstrate a selective increase in brain Aβ42 (Borchelt, *op cit.*; Duff, *et al.*, *Neurodegeneration* 5(4), 293-98 (1996)). Thus the leading hypothesis regarding the etiology of Alzheimer's disease is that an increase in Aβ42 brain concentration due to an increased production and release of Aβ42 or a decrease in clearance (degradation or brain clearance) is a causative event in the disease pathology.

Multiple mutation sites in either Aβ or the APP gene have been identified and are clinically associated with either dementia or cerebral hemorrhage. In addition to the FAD mutations mentioned above, exemplary CAA disorders include, but are not limited to, hereditary cerebral hemorrhage with amyloidosis of Icelandic type (HCHWA-I); the Dutch variant of HCHWA (HCHWA-D; a mutation in Aβ); the Flemish mutation of Aβ; the Arctic mutation of Aβ; the Italian mutation of Aβ; the Iowa mutation of Aβ; familial British dementia; and familial Danish dementia. CAA may also be sporadic.

The term "treating" includes the application or administration of a composition of the invention to a subject, or application or administration of a composition of the invention to a cell or tissue from a subject, who has an Aβ-amyloid related disease or condition, has a symptom of such a disease or condition, or is at risk of (or susceptible to) such a disease or condition, with the purpose of curing, healing, alleviating, relieving, altering, remedying, ameliorating, preventing, improving, or affecting the disease or condition, the symptom of the disease or condition, or the risk of (or susceptibility to) the disease or condition. The term "treating" refers to any indicia of success in the treatment or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the subject; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a subject's physical or mental well-being; or, in some situations, preventing the onset of dementia. Treatment may be therapeutic or prophylactic. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, a psychiatric evaluation, or a cognition test such as CDR, MMSE, ADAS-Cog, or another test known in the art. For example, the methods of the invention successfully treat a subject's dementia by slowing the rate of or lessening the extent of cognitive decline.

The term "subject" includes living organisms in which Aβ-amyloidosis can occur, or which are susceptible to Aβ-amyloid diseases, *e.g.*, Alzheimer's disease, etc. Examples of subjects include humans, chickens, ducks, peking ducks, geese, monkeys, deer, cows, rabbits, sheep, goats, dogs, cats, mice, rats, and transgenic species thereof. The term "subject" preferably includes animals susceptible to states characterized by neuronal cell death, e.g. mammals, e.g. humans. The animal can be an animal model for a disorder, e.g., a transgenic mouse with an Alzheimer's-type neuropathology. In preferred embodiments, the subject is a human suffering from a neurodegenerative disease, such as Alzheimer's disease, Parkinson's disease, etc. Administration of the compositions of the present invention to a subject to be treated can be carried out using known procedures, at dosages and for periods of time effective to treat or prevent an Aβ-amyloid related disease, e.g. Alzheimer's disease, or to e.g. modulate amyloid aggregation or amyloid-induced toxicity or to stabilize cognitive decline in the subject as further described herein.

In certain embodiments of the invention, the subject is in need of treatment by the methods of the invention, and is selected for treatment based on this need. A subject in need of treatment is art-recognized, and includes subjects that have been identified as having a disease or disorder related to Aβ-amyloid-deposition or amyloidosis, has a symptom of such a disease or disorder, or is at risk of such a disease or disorder, and

would be expected, based on diagnosis, e.g., medical diagnosis, to benefit from treatment (e.g., curing, healing, preventing, alleviating, relieving, altering, remedying, ameliorating, improving, or affecting the disease or disorder, the symptom of the disease or disorder, or the risk of the disease or disorder).

In still a further embodiment, the subject is shown to be at risk by a cognitive test such as Clinical Dementia Rating ("CDR"), Alzheimer's Disease Assessment Scale-Cognition ("ADAS-Cog"), or Mini-Mental State Examination ("MMSE"). The subject may exhibit a worse average score on a cognitive test, as compared to a historical control of similar age and educational background. The subject may also exhibit a worse score as compared to previous scores of the subject on the same or similar cognition tests.

In determining the CDR, a subject is typically assessed and rated in each of six cognitive and behavioural categories: memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care. The assessment may include historical information provided by the subject, or preferably, a corroborator who knows the subject well. The subject is assessed and rated in each of these areas and the overall rating, (0, 0.5, 1.0, 2.0 or 3.0) determined. A rating of 0 is considered normal. A rating of 1.0 is considered to correspond to mild dementia. A subject with a CDR of 0.5 is characterized by mild consistent forgetfulness, partial recollection of events and "benign" forgetfulness. In one embodiment the subject is assessed with a rating on the CDR of above 0, of above about 0.5, of above about 1.0, of above about 1.5, of above about 2.0, of above about 2.5, or at about 3.0.

Another test is the Mini-Mental State Examination (MMSE), as described by Folstein "`Mini-mental state. A practical method for grading the cognitive state of patients for the clinician." J. Psychiatr. Res. 12:189-198, 1975. The MMSE evaluates the presence of global intellectual deterioration. See also Folstein "Differential diagnosis of dementia. The clinical process." Psychiatr Clin North Am. 20:45-57, 1997. The MMSE is a means to evaluate the onset of dementia and the presence of global intellectual deterioration, as seen in Alzheimer's disease and multi-infart dementia. The MMSE is scored from 1 to 30. The MMSE does not evaluate basic cognitive potential, as, for example, the so-called IQ test. Instead, it tests intellectual skills. A person of "normal" intellectual capabilities will score a "30" on the MMSE objective test (however, a person with a MMSE score of 30 could also score well below "normal" on an IQ test). See, e.g., Kaufer, J. Neuropsychiatry Clin. Neurosci. 10:55-63, 1998; Becke, Alzheimer Dis Assoc Disord. 12:54-57, 1998; Ellis, Arch. Neurol. 55:360-365, 1998; Magni, Int. Psychogeriatr. 8:127-134, 1996; Monsch, Acta Neurol. Scand. 92:145-150, 1995. In one embodiment, the subject scores below 30 at least once on the MMSE. In another embodiment, the subject scores below about 28, below about 26, below about 24, below

about 22, below about 20, below about 18, below about 16, below about 14, below about 12, below about 10, below about 8, below about 6, below about 4, below about 2, or below about 1.

Another means to evaluate cognition, particularly Alzheimer's disease, is the Alzheimer's Disease Assessment Scale (ADAS-Cog), or a variation termed the Standardized Alzheimer's Disease Assessment Scale (SADAS). It is commonly used as an efficacy measure in clinical drug trials of Alzheimer's disease and related disorders characterized by cognitive decline. SADAS and ADAS-Cog were not designed to diagnose Alzheimer's disease; they are useful in characterizing symptoms of dementia and are a relatively sensitive indicator of dementia progression. (See, e.g., Doraiswamy, Neurology 48:1511-1517, 1997; and Standish, J. Am. Geriatr. Soc. 44:712-716, 1996.) Annual deterioration in untreated Alzheimer's disease patients is approximately 8 points per year (See, eg., Raskind, M Prim. Care Companion J Clin Psychiatry 2000 Aug; 2(4):134-138).

The ADAS-cog is designed to measure, with the use of questionnaires, the progression and the severity of cognitive decline as seen in AD on a 70- point scale. The ADAS-cog scale quantifies the number of wrong answers. Consequently, a high score on the scale indicates a more severe case of cognitive decline. In one embodiment, a subject exhibits a score of greater than 0, greater than about 5, greater than about 10, greater than about 15, greater than about 20, greater than about 25, greater than about 30, greater than about 35, greater than about 40, greater than about 45, greater than about 50, greater than about 55, greater than about 60, greater than about 65, greater than about 68, or about 70.

In another embodiment, the subject exhibits no symptoms of Alzheimer's Disease. In another embodiment, the subject is a human who is at least 40 years of age and exhibits no symptoms of Alzheimer's Disease. In another embodiment, the subject is a human who is at least 40 years of age and exhibits one or more symptoms of Alzheimer's Disease.

In another embodiment, the subject has Mild Cognitive Impairment. In a further embodiment, the subject has a CDR rating of about 0.5. In another embodiment, the subject has early Alzheimer's disease. In another embodiment, the subject has cerebral amyloid angiopathy.

In another embodiment, the compounds of the invention are administered at a therapeutically effective dosage sufficient to reduce the levels of amyloid β peptides in a subject's plasma or cerebrospinal fluid (CSF) from levels prior to treatment from about 10 to about 100 percent, or even about 50 to about 100 percent.

The amount of amyloid β peptide in the brain, CSF, blood, or plasma of a subject can be evaluated by enzyme-linked immunosorbent assay ("ELISA") or quantitative immunoblotting test methods or by quantitative SELDI-TOF which are well known to those skilled in the art, such as is disclosed by Zhang, *et al.*, *J. Biol. Chem.* 274, 8966-72 (1999) and Zhang, *et al.*, *Biochemistry* 40, 5049-55 (2001). *See also*, A.K.Vehmas, *et al.*, *DNA Cell Biol.* 20(11), 713-21 (2001), P.Lewczuk, *et al.*, *Rapid Commun. Mass Spectrom.* 17(12), 1291-96 (2003); B.M.Austen, *et al.*, *J. Peptide Sci.* 6, 459-69 (2000); and H.Davies, *et al.*, *BioTechniques* 27, 1258-62 (1999). These tests are performed on samples of the brain or blood which have been prepared in a manner well known to one skilled in the art. Another example of a useful method for measuring levels of amyloid β peptides is by Europium immunoassay (EIA). *See, e.g.*, WO 99/38498 at p.11.

In another embodiment, the subject may have (or may be predisposed to developing or may be suspected of having or may be at risk of) e.g. Alzheimer's disease, dementia, vascular dementia, or senile dementia, Mild Cognitive Impairment, or early Alzheimer's disease. In addition to Alzheimer's disease, the subject may have e.g. another Aβ-amyloid related disease such as cerebral amyloid angiopathy, or the subject may have amyloid deposits, especially amyloid–β amyloid deposits in the brain. In still a further embodiment, the subject is shown to be at risk by a diagnostic brain imaging technique, for example, one that measures brain activity, plaque deposition, or brain atrophy.

In another embodiment, the invention pertains to a method for improving cognition in a subject suffering from an Aβ-amyloid related disease. The method includes administering an effective amount of a compound of the invention, such that the subject's cognition is stabilized or improved. The subject's cognition can be tested using methods known in the art such as the Clinical Dementia Rating ("CDR"), Mini-Mental State Examination ("MMSE"), and the Alzheimer's Disease Assessment Scale-Cognition ("ADAS-Cog").

In one embodiment, the compounds of the invention are administered at a therapeutically effective dosage sufficient to maintain a subject's CDR rating at its base line rating or at 0. In another embodiment, the compounds of the invention are administered at a therapeutically effective dosage sufficient to decrease (i.e. improve) a subject's CDR rating by about 0.25 or more, about 0.5 or more, about 1.0 or more, about 1.5 or more, about 2.0 or more, about 2.5 or more, or about 3.0 or more. In another embodiment, the compounds of the invention are administered at a therapeutically effective dosage sufficient to reduce the rate of the increase of a subject's CDR rating as compared to historical controls. In another embodiment, the compounds of the invention are administered at a therapeutically effective dosage sufficient to reduce the rate of increase of a subject's CDR rating by about 5% or more, about 10% or more,

about 20% or more, about 25% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 70% or more, about 80% or more, about 90% or more, or about 100%, of the increase of the historical or untreated controls.

In another embodiment, the compounds of the invention are administered at a therapeutically effective dosage sufficient to maintain a subject's score on the MMSE. The compounds of the invention may be administered at a therapeutically effective dosage sufficient to increase a subject's MMSE score by about 1, about 2, about 3, about 4, about 5, about 7.5, about 10, about 12.5, about 15, about 17.5, about 20, or about 25 points. In another embodiment, the compounds of the invention are administered at a therapeutically effective dosage sufficient to reduce the rate of the decrease of a subject's MMSE score as compared to historical controls. In another embodiment, the compounds of the invention are administered at a therapeutically effective dosage sufficient to reduce the rate of decrease of a subject's MMSE score by about 5% or more, about 10% or more, about 20% or more, about 25% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 70% or more, about 80% or more, about 90% or more or about 100% or more, of the decrease of the historical or untreated controls.

In yet another embodiment, the compounds of the invention are administered at a therapeutically effective dosage sufficient to maintain a subject's score on the ADAS-Cog. In another embodiment, the compounds of the invention are administered at a therapeutically effective dosage sufficient to decrease a subject's ADAS-Cog score by about 1 point or greater, by about 2 points or greater, by about 3 points or greater, by about 4 points or greater, by about 5 points or greater, by about 7.5 points or greater, by about 10 points or greater, by about 12.5 points or greater, by about 15 points or greater, by about 17.5 points or greater, by about 20 points or greater, or by about 25 points or greater. The compounds of the invention may also be administered at a therapeutically effective dosage sufficient to reduce the rate of the increase of a subject's ADAS-Cog score as compared to historical controls. In another embodiment, the compounds of the invention are administered at a therapeutically effective dosage sufficient to reduce the rate of increase of a subject's ADAS-Cog score by about 5% or more, about 10% or more, about 20% or more, about 25% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 70% or more, about 80% or more, about 90% or more or about 100% of the increase of the historical or untreated controls. In a further embodiment, the compounds of the invention may be administered at a therapeutically effective dosage sufficient to treat, slow or stop an Aβ-amyloid related disease associated with cognition such that the subject's cognition as measured by ADAS-Cog remains constant over a year. "Constant" includes fluctuations of no more

than 2 points. Remaining constant includes fluctuations of two points or less in either direction.

In another embodiment, the invention also pertains, at least in part, to a method for treating a carboxyalkylsulfonic acid responsive state in a subject. The method includes administering to the subject, in need thereof, an effective amount of a pharmaceutical composition comprising a compound of Formula (I), such that the carboxyalkylsulfonic acid responsive state is treated in the subject.

The term "carboxyalkylsulfonic acid responsive states" include states which can be treated by administering an effective amount of a compound of the invention. Examples of carboxyalkylsulfonic acid responsive states include, for example, Aβ-amyloid related diseases, states associated with neurotoxicity, and states associated with neuronal cell death. Neurotoxicity and neuronal cell death may be caused, for example, by natural or manmade toxic substances which alter the normal activity of the nervous system. Neurotoxicity may eventually disrupt or even kill neurons. Neurotoxicity and neuronal cell death can result from exposure to substances used in chemotherapy, radiation treatment, drug therapies, and organ transplants, as well as exposure to heavy metals such as lead and mercury, certain foods and food additives, pesticides, industrial and/or cleaning solvents, cosmetics, and some naturally occurring substances, such as amyloids. Neuronal cell death can result from injury, for example ischemia, or from neurodegeneration, for example associated with a neurodegenerative disease.

Another aspect of the invention pertains to a method of providing neuroprotection to a subject, comprising administering a compound of Formula (I)to the subject, such that neuroprotection is provided.

In another aspect, methods for inhibiting neuronal cell death, e.g. apoptosis, necrosis, etc., are provided. In another aspect, methods for inhibiting Aβ-induced neuronal cell death are provided, e.g., by administering an effective amount of a compound of Formula (I).

In another aspect, a method of treating a disease state characterized by neuronal cell death in a subject is provided, e.g., by administering an effective amount of a compound of Formula (I). In yet another aspect, a method of treating a disease state characterized by Aβ-induced neuronal cell death is provided, e.g., by administering an effective amount of a compound of Formula (I).

The term "neuroprotection" includes protection of neuronal cells of a subject from cell death that may result in initiation of processes such as, but not limited to: the destabilization of the cytoskeleton; DNA fragmentation; the activation of hydrolytic enzymes, such as phospholipase A2; activation of caspases, calcium-activated proteases and/or calcium-activated endonucleases; inflammation mediated by macrophages; calcium influx into a cell; membrane potential changes in a cell; the disruption of cell

junctions leading to decreased or absent cell-cell communication; and the activation of expression of genes involved in cell death.

The term "neuronal cell death associated state" includes a disorder, disease or condition characterized by neuronal cell death e.g. Aβ-induced neuronal cell death. Examples of such disorders include Alzheimer's Disease, Aβ-amyloid related diseases, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, and spongiform encephalitis.

In a further embodiment, the invention pertains to a pharmaceutical composition comprising compounds of Formula (I), as described above. The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier. In a further embodiment, the compound of the invention may be provided in an effective amount to treat Aβ-amyloid related disease, such as, for example, Alzheimer's disease, CAA, etc. In another further embodiment, the effective amount may be effective to treat carboxyalkylsulfonic acid responsive states.

The invention further relates to a pharmaceutical composition comprising compounds of Formula (I) and/or solvates thereof. As useed herein, the term "solvate" refers to a physical association of a compound of this invention with one or more solvent molecules, whether organic or inorganic. This physical association includes hydrogen bonding. In certain instances, the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. "Solvate" encompasses both solution-phase and isolable solvates. Exemplary solvates include hydrates, ethanolates, methanolates, hemiethanolates, and the like.

Pharmaceutical compositions comprising the compounds of the invention can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound of the invention and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, wafers, and the like. The percentage of the compound in the compositions and preparations may, of course, be varied. The amount of the compound of the invention in such therapeutically useful compositions is such that a suitable dosage will be obtained.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of the compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The specification for the dosage unit forms of the invention are dictated by and

directly dependent on (a) the unique characteristics of the compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such a compound for the treatment of amyloid deposition in subjects.

The present invention therefore includes pharmaceutical formulations comprising the compound of the invention, in pharmaceutically acceptable vehicles for oral and parenteral administration. In accordance with the present invention, a compound of the invention may be administered orally or through inhalation as a solid.

Pharmaceutical compositions may also be coated by conventional methods, typically with pH or time-dependent coatings, such that the subject agent is released in the gastrointestinal tract in the vicinity of the desired topical application, or at various times to extend the desired action. Such dosage forms typically include, but are not limited to, one or more of cellulose acetate phthalate, polyvinylacetate phthalate, hydroxypropyl methyl cellulose phthalate, ethyl cellulose, waxes, and shellac.

Other compositions useful for attaining systemic delivery of the subject agents include sublingual, buccal and nasal dosage forms. Such compositions typically comprise one or more of soluble filler substances such as sucrose, sorbitol and mannitol; and binders such as acacia, microcrystalline cellulose, carboxymethyl cellulose and hydroxypropyl methyl cellulose. Glidants, lubricants, sweeteners, colorants, antioxidants and flavoring agents as are known in the art may also be included.

In one embodiment, the compounds of the invention are administered at a therapeutically effective dosage sufficient to inhibit Aβ-amyloid deposition in a subject and/or treat a Aβ-amyloid related disease in a subject. An "effective" dosage may inhibit Aβ-amyloid deposition by, for example, at least about 20%, or by at least about 40%, or even by at least about 60%, or by at least about 80% relative to untreated subjects. In another embodiment, a "therapeutically effective" dosage stabilizes cognitive function or prevents a further decrease in cognitive function (*i.e.*, preventing, slowing, or stopping disease progression) in a subject, e.g., a subject having Alzheimer's disease, CAA, etc.

Furthermore, the compounds may be administered at a therapeutically effective dosage sufficient to decrease deposition in a subject of amyloid protein, *e.g.*, Aβ40 or Aβ42. A therapeutically effective dosage decreases amyloid deposition by, for example, at least about 15%, or by at least about 40%, or even by at least 60%, or at least by about 80% relative to untreated subjects.

It is understood that appropriate doses depend upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the compound will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner

desires the compound to have upon the subject. Exemplary doses include milligram or microgram amounts of the compound per kilogram of subject or sample weight (*e.g.*, about 50 micrograms per kilogram to about 500 milligrams per kilogram, about 1 milligram per kilogram to about 100 milligrams per kilogram, about 1 milligram per kilogram to about 50 milligram per kilogram, about 1 milligram per kilogram to about 10 milligrams per kilogram, or about 3 milligrams per kilogram to about 5 milligrams per kilogram). Additional exemplary doses include doses of about 5 to about 500 mg, or about 25 to about 300 mg, or about 25 to about 200 mg, preferably about 50 to about 150 mg, more preferably about 50, about 100, about 150 mg, about 200 mg or about 250 mg, and, preferably, daily or twice daily, or lower or higher amounts.

It is furthermore understood that appropriate doses depend upon the potency. Such appropriate doses may be determined using the assays described herein. When one or more of these compounds is to be administered to an animal (*e.g.*, a human), a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific agent employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, and any drug combination.

Standard Methods for testing the compounds of the invention

The compounds according to the invention can be further analyzed, tested or validated using a variety of *in vitro* assays, or *in vivo* assays to confirm their ability of providing neuroprotection and/or preventing neuronal cell death. For example, a variety of different parameters can be monitored to assess toxicity. Examples of such parameters include, but are not limited to, cell proliferation, monitoring activation of cellular pathways for toxicological responses by gene or protein expression analysis, DNA fragmentation, changes in the composition of cellular membranes, membrane permeability, activation of components of death-receptors or downstream signaling pathways (e.g., caspases), generic stress responses, NF-kappaB activation and responses to mitogens. Related assays are used to assay for apoptosis (a programmed process of cell death) and necrosis, including cGMP formation and NO formation. The following are illustrative of the type of biological assays that can be conducted to assess whether a compound has a protective effect against neuronal injury or disease.

A. Morphological Changes

Apoptosis in many cell types is correlated with altered morphological appearances. Examples of such alterations include, but are not limited to, plasma membrane blebbing, cell shape change, loss of substrate adhesion properties. Such changes are readily detectable with a light microscope. Cells undergoing apoptosis can also be detected by fragmentation and disintegration of chromosomes. These changes can be detected using light microscopy and/or DNA or chromatin specific dyes.

B. Altered Membrane Permeability

Often the membranes of cells undergoing apoptosis become increasingly permeable. This change in membrane properties can be readily detected using vital dyes (e.g., propidium iodide and trypan blue). Dyes can be used to detect the presence of necrotic cells. For example, certain methods utilize a green-fluorescent LIVE/DEAD Cytotoxicity Kit #2, available from Molecular Probes. The dye specifically reacts with cellular amine groups. In necrotic cells, the entire free amine content is available to react with the dye, thus resulting in intense fluorescent staining. In contrast, only the cell-surface amines of viable cells are available to react with the dye. Hence, the fluorescence intensity for viable cells is reduced significantly relative to necrotic cells (see, e. g., Haugland, 1996 Handbook of Fluorescent Probes and Research Chemicals, 6th ed., Molecular Probes, OR).

C. Dysfunction of Mitochondrial Membrane Potential

Mitochondria provide direct and indirect biochemical regulation of diverse cellular processes as the main energy source in cells of higher organisms. These process include the electron transport chain activity, which drives oxidative phosphorylation to produce metabolic energy in the form of adenosine triphosphate (i.e., ATP). Altered or defective mitochondrial activity can result in mitochondrial collapse called the "permeability transition" or mitochondrial permeability transition. Proper mitochondrial functioning requires maintenance of the membrane potential established across the membrane. Dissipation of the membrane potential prevents ATP synthesis and thus halts or restricts the production of a vital biochemical energy source.

Consequently, a variety of assays designed to assess toxicity and cell death involve monitoring the effect of a test agent on mitochondrial membrane potentials or on the mitochondrial permeability transition. One approach is to utilize fluorescent indicators (see, e.g., Haugland, 1996 Handbook of Fluorescent Probes and Research Chemicals, 6th ed., Molecular Probes, OR, pp. 266-274 and 589- 594). Various non-fluorescent probes can also be utilized (see, e.g., Kamo et al. (1979) J. Membrane Biol. 49:105). Mitochondrial membrane potentials can also be determined indirectly from

mitochondrial membrane permeability (see, e.g., Quinn (1976) The Molecular Biology of Cell Membranes, University Park Press, Baltimore, Md., pp. 200-217). Further guidance on methods for conducting such assays is provided in PCT publication WO 00/19200 to Dykens et al.

D. Caspase Activation

Apoptosis is the process of programmed cell death and involves the activation of a genetic program when cells are no longer needed or have become seriously damaged. Apoptosis involves a cascade of biochemical events and is under the regulation of a number of different genes. One group of genes act as effectors of apoptosis and are referred to as the interleukin-1βconverting enzyme (ICE) family of genes. These genes encode a family of cysteine proteases whose activity is increased in apoptosis. The ICE family of proteases is generically referred to as caspase enzymes. The "C" in the name reflects the fact that the enzymes are cysteine proteases, while "Caspase" refers to the ability of these enzymes to cleave after aspartic acid residues.

Consequently, some assays for apoptosis are based upon the observation that caspases are induced during apoptosis. Induction of these enzymes can be detected by monitoring the cleavage of specifically-recognized substrates for these enzymes. A number of naturally occurring and synthetic protein substrates are known (see, e.g., Ellerby et al. (1997) J. Neurosci. 17:6165; Kluck, et al. (1997) Science 275:1132; Nicholson et al. (1995) Nature 376:37; and Rosen and Casciola- Rosen (1997) J. Cell Biochem. 64:50). Methods for preparing a number of different substrates that can be utilized in these assays are described in U.S. Pat. No. 5,976,822. This patent also describes assays that can be conducted using whole cells that are amendable to certain of the microfluidic devices described herein. Other methods using FRET techniques are discussed in Mahajan, et al. (1999) Chem. Biol. 6:401-9; and Xu, et al. (1998) Nucl. Acids. Res. 26:2034-5.

E. Cytochrome C Release

In healthy cells, the inner mitochondrial membrane is impermeable to macromolecules. Thus, one indicator of cell apoptosis is the release or leakage of cytochrome C from the mitochondria. Detection of cytochrome C can be performed using spectroscopic methods because of the inherent absorption properties of the protein. Thus, one detection option with the present devices is to place the cells within a holding space and monitor absorbance at a characteristic absorption wavelength for cytochrome C. Alternatively, the protein can be detected using standard immunological methods (e.g., ELISA assays) with an antibody that specifically binds to cytochrome C (see, e.g., Liu et al. (1996) Cell 86:147).

F. Assays for Cell Lysis

The final stage of cell death is typically lysis of the cell. When cells die they typically release a mixture of chemicals, including nucleotides, and a variety of other substances (e.g., proteins and carbohydrates) into their surroundings. Some of the substances released include ADP and ATP, as well as the enzyme adenylate cyclase, which catalyzes the conversion of ADP to ATP in the presence of excess ADP. Thus, certain assays involve providing sufficient ADP in the assay medium to drive the equilibrium towards the generation of ATP which can subsequently be detected via a number of different means. One such approach is to utilize a luciferin/luciferase system that is well known to those of ordinary skill in the art in which the enzyme luciferase utilizes ATP and the substrate luciferin to generate a photometrically detectable signal. Further details regarding certain cell lysis assays that can be performed are set forth in PCT publication WO 00/70082.

G. Ischemic Model Systems

Methods for assaying whether a compound can confer protective neurological effects against ischemia and stroke are discussed by Aarts, et al. (Science 298:846-850, 2002). In general, this assay involves subjecting rats to a middle cerebral artery occlusion (MCAO) for a relatively short period of time (e.g., about 90 minutes). MCAO can be induced using various methods, including an intraluminal suture method (see, e.g., Longa, E. Z. et al. (1989) Stroke 20:84; and Belayev, L., et al. (1996) Stroke 27:1616). A composition containing the putative inhibitor is introduced into the rat using conventional methods (e.g., via intravenous injection). To evaluate the compositions prophylactic effect, the composition is administered before performing MCAO. If the compound is to be evaluated for its ability to mitigate against an ischemic event that has already occurred, the composition with the compound is introduced after MCAO has been initiated. T he extent of cerebral infarction is then evaluated using various measures of neurological function. Examples of such measures include the postural reflex test (Bederson, J. B. et al. (1986) Stroke 17:472) and the forelimb placing test (De Ryck, M. et al. (1989) Stroke 20:1383). Methods are also described in Aarts et al assessing the effects of NMDA-induced excitotoxicity using in vitro assays.

H. MTT Cytotoxicity Assay

The MTT assay is another assay which has been widely used to assess cytotoxicity in neuronal cells. The cellular toxicity can assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Trevigen, Gaithersburg, Md.) following the recommendations of the manufacturer.

I. Trypan Blue Cell Viability Measurement

Cell viability can be measured using the trypan blue exclusion method as we previously described. Yao et al., The Gingko biloba extract EGb 761 rescues PC12 neuronal cells from β-amyloid-induced cell death by inhibiting the formation of β-amyloid-derived diffusible neurotoxic ligands, *Brain Res.*, 889, 181-190 (2001).

J. Determination of Cellular ATP Levels

Cellular ATP Levels can be indicative of cell viability. Cellular ATP concentrations can be measured using the ATPLite-M® luminescence assay (Packard BioSciences Co.). For example, in this assay, cells typically are cultured on black 96-well ViewPlate® and the ATP concentrations are measured on a TopCount NXT® counter (Packard BioSciences Co.) following the recommendations of the manufacturer.

K. Animal Models

Various animal models can be used to the efficacy and/or potency of the compound according to the invention. For example, certain transgenic animal models have been described, for example, in U.S. Pat. Nos. 5,877,399; 5,612,486; 5,387,742; 5,720,936; 5,850,003; 5,877,015, and 5,811,633, and in Ganes et al., 1995, Nature 373:523. Preferred are animals that exhibit characteristics associated with the pathophysiology of AD. Administration of the compound inhibitors of the invention to the transgenic mice described herein provides an alternative method for demonstrating the inhibitory activity of the compounds. Administration of the compounds in a pharmaceutically effective carrier and via an administrative route that reaches the target tissue in an appropriate therapeutic amount is also preferred.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents are considered to be within the scope of this invention and covered by the claims appended hereto. The contents of all references, issued patents, and published patent applications cited throughout this application are hereby incorporated by reference. The invention is further illustrated by the following examples, which should not be construed as further limiting.

Examples

Example 1: Hoechst Staining on Primary Rat Neurons and Neuroblastoma SH-SY5Y

Preparation of primary rat neurons

Primary rat neurons were isolated and cultured according to standard literature techniques.

Maintenance of human undifferentiated neuroblastoma SH-SY5Y

Materials

Item	Company	Catalogue #
SH-SY5Y, human neuroblastoma cell line, established from a subline of SK-N-SH	American Type Culture Collection (ATCC)	CRL-2266
Fetal Bovine Serum (FBS)	Gibco	10099-141
Eagle's Minimum Essential Medium (EMEM)	Sigma	4655
Ham's F12 Nutrient mixture with L-Glutamine	Gibco	11765-054
MEM non-essential amino acids	Gibco	1140-050
Trypsin/EDTA (2.5 g Trypsin and 0.38 g EDTA-4Na/L in HBSS without Ca^{++} and Mg^{++})	Gibco	2500-056

Procedure

SH-SY5Y cells were cultured and sub-cultured according to ATCC's recommendations. Briefly, cells were grown in a culture medium containing 10 % fetal bovine serum (FBS), 1x non-essential amino acids in a 1:1 mixture of Eagle's minimum essential medium and Ham's F12 medium.

For passage, cells were trypsinized with 0.25% (w/vol) Trypsin/ Ethylenediaminetetraacetic (EDTA) for 5 minutes at 37°C, and then centrifuged for 5 minutes at 300 x g (GS-6R™ Beckman Centrifuge). The pellet was resuspended in the culture medium and the cell density was adjusted.

3- Preparation of Aβ and Test Compounds

Preparation of $A\beta_{1-42}$

Synthetic $A\beta_{1-42}$ was purchased from American Peptide Company, Sunnyvale, CA. $A\beta_{1-42}$ stock solution was prepared according to the standard literature procedure BCM-6012-02.

The soluble $A\beta_{1-42}$ solution was evaporated to remove the HFIP and resuspended in a buffer containing 0.04 M Tris-HCl , 0.3 M NaCl, pH 7.4, at a final concentration of 120 μM. This solution was stored frozen for later use.

Preparation of the Test Compounds

Code	Test Compounds	MW	Diluents	Stock Conc.
A	**3-Amino-1-propanesulfonic acid**	139.17	PBS 1% DMSO	10 mM
B	**3-Sulfo-1-propanoic acid, disodium salt**	198.10	PBS 1% DMSO	10 mM

The test compounds were dissolved in phosphate buffered saline (PBS) (without calcium and magnesium), 1% dimethyl sulfoxide (DMSO), pH 7.4, filtered through a 0.22 µm syringe filter, aliquoted and stored at -80 °C until use.

4- Cell treatment

Primary rat neurons

Following isolation, primary rat neurons were seeded on glass coverslips (Fisher, cat. # 12-545-82) coated with Poly-L-Lysine (Sigma, cat. # P-7890) in a 24-well plate at a density of 10^5 cells/well. Treatments were performed on 4-day old cultures. Cells were incubated for 72 hours with 5 µM $A\beta_{1-42}$, diluted (in the Neurobasal medium) from the 120 µM stock in the presence or absence of 100 µM of the test compounds (1:20 Aβ:drug ratio).

SH-SY5Y

SH-SY5Y cells were seeded on glass coverslips in a 24-well plate at a density of 3×10^5 cells/well. Treatments were performed the next day. Cells were incubated for 24 hours with 10 µM $A\beta_{1-42}$, diluted (in the culture medium) from the 120 µM stock in the presence or absence of 200 µM the test compounds (1:20 Aβ:drug ratio).

5- Hoechst Staining

Materials

ITEM	Company	Catalogue #
Paraformaldehyde (PFA)	Electron Microscopy Science (EMB)	15714
Methanol	Fisher	A452-4
Phosphate Buffered Saline (PBS)	Gibco	14040-133
Hoechst Dye 33342	Molecular Probes	H-3570 (10 mg/mL in water)
Water	Sigma	W-3500
Prolong Gold Anti-fade Reagent	Molecular Probes	P36930

Procedure

The stock solution of Hoechst 33342 was diluted to 100 µg/ml in water and stored at 2-8 °C. Primary rat neurons or neuroblastoma SH-SY5Y cells were incubated for 10 to 60 minutes with 500 µl of Hoechst solution at a final concentration of 2 µg/ml in the culture medium. Cells were washed 3 times with PBS. Primary rat neurons were fixed in ice cold methanol for 5 minutes at room temperature and SH-SY5Y cells were fixed in 4% PFA for 30 minutes at room temperature. After 3 washes in PBS, the coverslips were mounted onto glass slides using prolong anti-fade reagent.

Counting Method and Data Analysis

Nuclear morphology was observed using an Olympus™ fluorescent microscope IX50™ equipped with an Olympus™ Camera (20x objectif and a bandpass filter (Ex /Em: 355 nm/465 nm). Live cells and cells considered morphologically apoptotic were counted. Apoptotic nuclei of primary rat neurons appear both fragmented and condensed (visualized as brighter blue) whereas apoptotic undifferentiated SH-SY5Y appear more often condensed and only occasionally fragmented.

Five random fields were captured for each condition in a blinded fashion. Apoptotic and normal nuclei in each field were quantified by manual examination.

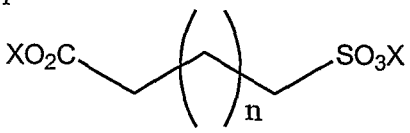
The data are expressed as a percentage of toxicity, corresponding to the number of apoptotic cells divided by total cell number (apoptotic + non apoptotic cells). The total number of cells counted in each condition ranged from 30 to 180 cells for primary rat neurons (**Figure 1**) and from 60 to 550 for SH-SY5Y (**Figure 2**).

The data was analyzed with SigmaPlot™ software. Student t-test (Excel software) was used to compare the % toxicity in Aβ treatment in presence of compound to the Aβ treatment alone, using the average obtained from all experiments. A significance level of $P < 0.05$ was considered for the t-test.

Under these conditions, the two test compounds, 3-Amino-1-Propanesulfonic Acid (Compound A) and 3-sulfo-1-propanoic acid, disodium salt (Compound B) were observed to provide neuroprotection in both assays.

CLAIMS:

1. A pharmaceutical composition comprising a compound of Formula (I) or a pharmaceutically acceptable solvate thereof:



(I)

wherein

X is cationic group or ester-forming group independently chosen for each occurrence; and

n is 0, 1, 2 , 3, 4, 5, 6, 7, or 8, and a pharmaceutically acceptable carrier.

2. The pharmaceutical composition of claim 1, wherein X is hydrogen for each occurrence.

3. The pharmaceutical composition of claim 1, wherein X is Na^+ for each occurrence.

4. The pharmaceutical composition of any one of claims 1-3, wherein n is 0.

5. The pharmaceutical composition of claim 1, wherein said compound is 3-sulfo-1-propanoic acid, disodium salt.

6. The pharmaceutical composition of any one of claims 1-5, wherein said pharmaceutical composition comprises an effective amount of said compound to treat an Aβ-amyloid related disease.

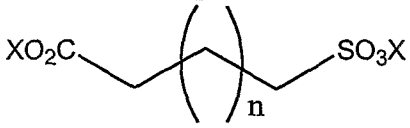
7. The pharmaceutical composition of claim 6, wherein said Aβ-amyloid related disease is Alzheimer's disease.

8. The pharmaceutical composition of claim 7, wherein said Aβ-amyloid related disease is CAA.

9. The pharmaceutical composition of any one of claims 1-5, wherein said pharmaceutical composition comprises an effective amount of said compound to treat a carboxyalkylsulfonicacid responsive state.

10. The pharmaceutical composition of claim 9, wherein said carboxyalkylsulfonic acid responsive state is associated with neurotoxicity.

11. A method for treating an Aβ-amyloid related disease in a subject, comprising administering to a subject in need thereof an effective amount of a a compound of Formula I or a pharmaceutically acceptable solvate thereof:



(I)

wherein

X is cationic group or ester-forming group independently chosen for each occurrence; and

n is 0, 1, 2 , 3, 4, 5, 6, 7, or 8.

12. The method of claim 11, wherein X is hydrogen for each occurrence.

13. The method of claim 11, wherein X is Na^+ for each occurrence.

14. The method of any one of claims 11-13, wherein n is 0.

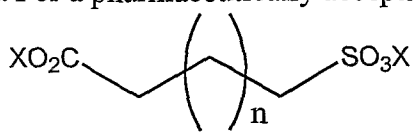
15. The method of any one of claims 11-14, wherein said compound is 3-sulfo-1-propanoic acid, disodium salt.

16. The method of any one of claims 11-15, wherein said subject is a human.

17. The method of any one of claims 11-16, wherein said Aβ-amyloid related disease is Alzheimer's disease.

18. The method of any one of claims 11-16, wherein said Aβ-amyloid related disease is CAA.

19. A method for treating a carboxyalkylsulfonicacid responsive state in a subject, comprising administering to a subject in need thereof an effective amount of a compound of Formula I or a pharmaceutically acceptable or solvate thereof:



(I)

wherein

X is cationic group or ester-forming group independently chosen for each occurrence; and

n is 0, 1, 2 , 3, 4, 5, 6, 7, or 8.

20. The method of claim 19, wherein said carboxyalkyl sulfonic acid responsive state is associated with neurotoxicity.

21. The method of any one of claim 19 or 20, wherein said compound is 3-sulfo-1-propanoic acid, disodium salt.

FIGURE 1 (Primary rat neurons)

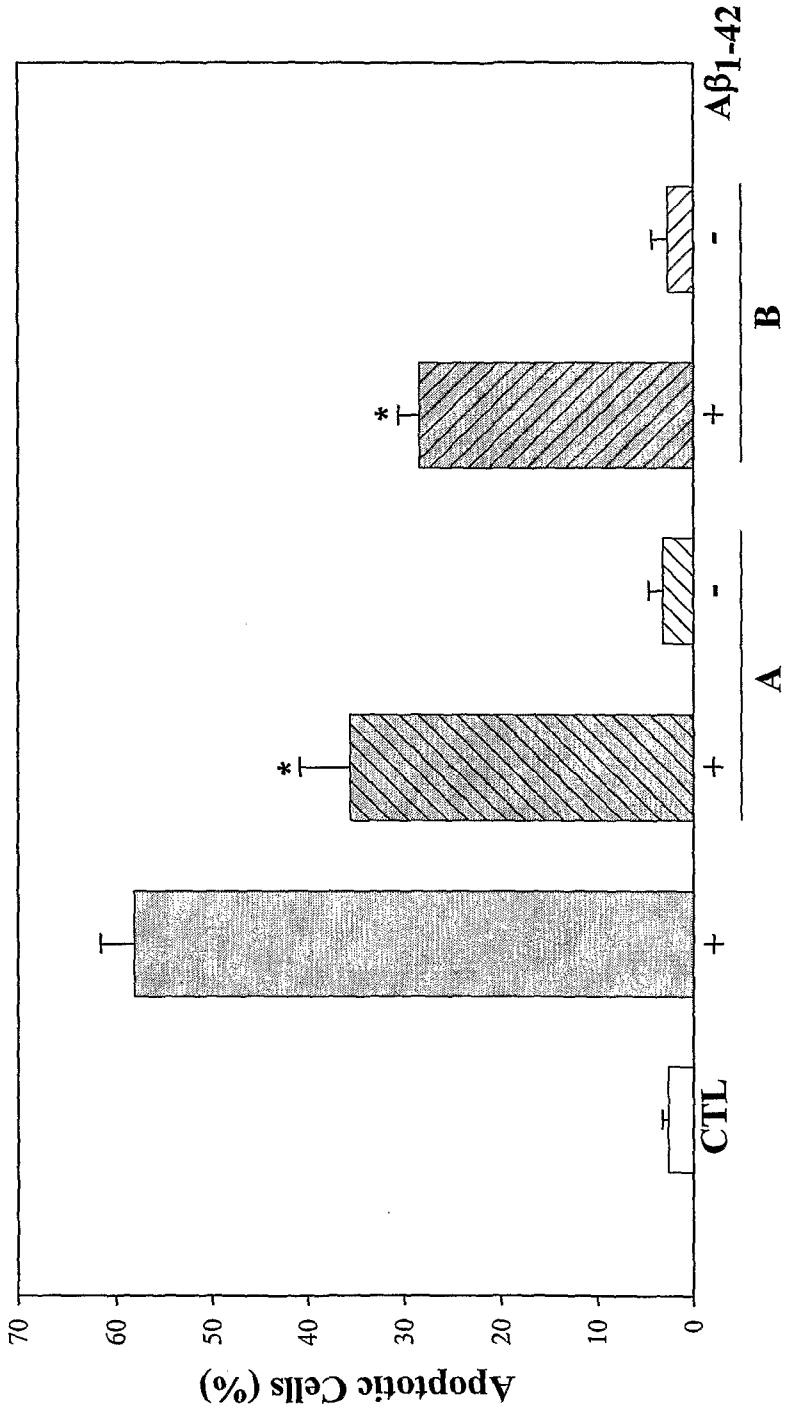


FIGURE 2 (Neuroblastoma SH-SY5Y)

