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(54) Title: METHODS AND COMPOSITIONS FOR THE TREATMENT OF AMYLOID- AND EPILEPTOGENESIS-ASSOCI-  
ATED DISEASES

(57) Abstract: Methods of treating or preventing an amyloid-related disease in a subject by administering to a subject a therapeutic amount of a compound of the invention are described. Also included are methods for inhibiting epileptogenesis in a subject, by administering to a subject an effective amount of an anti-epileptogenic agent. Methods for treating a subject suffering from an epileptogenesis-associated condition, by administering to the subject an effective amount of an anti-epileptogenic agent are also included. Methods for treating convulsions in a subject by administering to the subject an effective amount of a therapeutic amount of a compound of the invention are also described.



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## METHODS AND COMPOSITIONS FOR THE TREATMENT OF AMYLOID- AND EPILEPTOGENESIS-ASSOCIATED DISEASES

### 5 Related Applications

This application claims priority to U.S. Patent Application No. 10/\_\_\_\_,\_\_\_\_, filed June 18, 2004 (identified by Attorney Docket No. NBI-163); U.S. Provisional Patent Application Serial No. 60/512,018, filed on October 17, 2003 and U.S. Provisional Patent Application Serial No. 60/480,928, filed on June 23, 2003, all entitled  
10 *Methods and Compositions for Treating Amyloid- and Epileptogenesis-Associated Diseases*.

This application is also related to U.S. Provisional Patent Application No. 60/512,017, filed October 17, 2003, U.S. Provisional Patent Application No. 60/480,918, filed June 23, 2003, and U.S. Patent Application No. 10/\_\_\_\_,\_\_\_\_, filed June 18, 2004  
15 (identified by Attorney Docket No. NBI-149), all entitled *Methods for Treating Protein Aggregation Disorders*.

This application is related to U.S. Provisional Patent Application No. 60/512,116, filed October 17, 2003, U.S. Provisional Patent Application No. 60/480,984, filed June 23, 2003, and U.S. application 10/\_\_\_\_,\_\_\_\_, filed June 18, 2004 (identified by  
20 Attorney Docket No. NBI-152), all entitled *Pharmaceutical Formulations of Amyloid-Inhibiting Compounds*.

This application is related to U.S. Provisional Patent Application No. 60/436,379, filed December 24, 2002, U.S. Provisional Patent Application No. 60/482,214, filed June 23 2003, entitled *Combination Therapy for the Treatment of*  
25 *Alzheimer's Disease*, U.S. Patent Application No. 10/746,138, filed December 24, 2003, International Patent Application No. PCT/CA2003/002011, and U.S. Patent Application No. 10/\_\_\_\_,\_\_\_\_, filed June 18, 2004 (identified by NBI-154CP), entitled *Therapeutic Formulations for the Treatment of Beta-Amyloid Related Diseases*.

This application is related to U.S. Provisional Patent Application No. 60/512,135, filed October 17, 2003, U.S. Provisional Patent Application No. 60/482,058, filed June 23, 2003, both entitled *Synthetic Process for Preparing Compounds for*  
30 *Treating Amyloidosis*, and U.S. Patent Application No. 10/\_\_\_\_,\_\_\_\_, filed June 18, 2004 (identified by Attorney Docket No. NBI-156), entitled *Improved Pharmaceutical Drug Candidates and Method for Preparation Thereof*.

This application is related to U.S. Provisional Patent Application No. 60/512,047, filed October 17, 2003, and U.S. Provisional Patent Application No. 60/480,906, filed June 23, 2003, U.S. Patent Application No. 10/\_\_\_\_,\_\_\_\_, filed June 18, 2004 (identified by Attorney Docket No. NBI-162A); and U.S. Patent Application No.

10/\_\_\_\_, filed June 18, 2004 (identified by Attorney Docket No. NBI-162B); all entitled *Methods and Compositions for Treating Amyloid-Related Diseases*.

This application is also related to *Method for Treating Amyloidosis*, U.S. Patent Application No. 08/463,548, now U.S. Pat. No. 5,972,328.

5 The entire contents of each of these patent applications and patents are hereby expressly incorporated herein by reference including without limitation the specification, claims, and abstract, as well as any figures, tables, or drawings thereof.

### **Background**

10 Amyloidosis refers to a pathological condition characterized by the presence of amyloid fibrils. 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  
15 characteristic red-green birefringent appearance in polarized light after staining. They also share common ultrastructural features and common X-ray diffraction and infrared spectra.

Amyloid-related diseases can either be restricted to one organ or spread to several organs. The first instance is referred to as "localized amyloidosis" while the  
20 second is referred to as "systemic amyloidosis."

Some amyloid diseases can be idiopathic, but most of these diseases appear as a complication of a previously existing disorder. For example, primary amyloidosis (AL amyloid) can appear without any other pathology or can follow plasma cell dyscrasia or multiple myeloma.

25 Secondary amyloidosis is usually seen associated with chronic infection (such as tuberculosis) or chronic inflammation (such as rheumatoid arthritis). A familial form of secondary amyloidosis is also seen in other types of familial amyloidosis, e.g., Familial Mediterranean Fever (FMF). This familial type of amyloidosis is genetically inherited and is found in specific population groups. In both primary and secondary amyloidosis,  
30 deposits are found in several organs and are thus considered systemic amyloid diseases.

"Localized amyloidoses" are those that tend to involve a single organ system. Different amyloids are also characterized by the type of protein present in the deposit. For example, neurodegenerative diseases such as scrapie, bovine spongiform encephalitis, Creutzfeldt-Jakob disease, and the like are characterized by the appearance  
35 and accumulation of a protease-resistant form of a prion protein (referred to as A<sub>Sc</sub>r or PrP-27) in the central nervous system. Similarly, Alzheimer's disease, another neurodegenerative disorder, is characterized by neuritic plaques and neurofibrillary tangles. In this case, the amyloid plaques found in the parenchyma and the blood vessel

is formed by the deposition of fibrillar A $\beta$  amyloid protein. Other diseases such as adult-onset diabetes (type II diabetes) are characterized by the localized accumulation of amyloid fibrils in the pancreas.

Once these amyloids have formed, there is no known, widely accepted therapy or treatment which significantly dissolves amyloid deposits *in situ*, prevents further amyloid deposition or prevents the initiation of amyloid deposition.

Each amyloidogenic protein has the ability to undergo a conformational change and to organize into  $\beta$ -sheets and form insoluble fibrils which may be deposited extracellularly or intracellularly. Each amyloidogenic protein, although different in amino acid sequence, has the same property of forming fibrils and binding to other elements such as proteoglycan, amyloid P and complement component. Moreover, each amyloidogenic protein has amino acid sequences which, although different, show similarities such as regions with the ability to bind to the glycosaminoglycan (GAG) portion of proteoglycan (referred to as the GAG binding site) as well as other regions which promote  $\beta$ -sheet formation. Proteoglycans are macromolecules of various sizes and structures that are distributed almost everywhere in the body. They can be found in the intracellular compartment, on the surface of cells, and as part of the extracellular matrix. The basic structure of all proteoglycans is comprised of a core protein and at least one, but frequently more, polysaccharide chains (GAGs) attached to the core protein. Many different GAGs have been discovered including chondroitin sulfate, dermatan sulfate, keratan sulfate, heparin, and hyaluronan.

In specific cases, amyloid fibrils, once deposited, can become toxic to the surrounding cells. For example, the A $\beta$  fibrils organized as senile plaques have been shown to be associated with dead neuronal cells, dystrophic neurites, astrogliosis, and microgliosis in patients with Alzheimer's disease. When tested *in vitro*, oligomeric (soluble) as well as fibrillar A $\beta$  peptide was shown to be capable of triggering an activation process of microglia (brain macrophages), which would explain the presence of microgliosis and brain inflammation found in the brain of patients with Alzheimer's disease. Both oligomeric and fibrillar A $\beta$  peptide can also induce neuronal cell death *in vitro*. See, e.g., MP Lambert, *et al.*, *Proc. Natl. Acad. Sci. USA* 95, 6448-53 (1998).

In another type of amyloidosis seen in patients with type II diabetes, the amyloidogenic protein IAPP, when organized in oligomeric forms or in fibrils, has been shown to induce  $\beta$ -islet cell toxicity *in vitro*. Hence, appearance of IAPP fibrils in the pancreas of type II diabetic patients contributes to the loss of the  $\beta$  islet cells (Langerhans) and organ dysfunction which can lead to insulinemia.

Another type of amyloidosis is related to  $\beta_2$  microglobulin and is found in long-term hemodialysis patients. Patients undergoing long term hemodialysis will develop

$\beta_2$ -microglobulin fibrils in the carpal tunnel and in the collagen rich tissues in several joints. This causes severe pains, joint stiffness and swelling.

Amyloidosis is also characteristic of Alzheimer's disease. Alzheimer's disease is a devastating disease of the brain that results in progressive memory loss leading to dementia, physical disability, and death over a relatively long period of time. With the aging populations in developed countries, the number of Alzheimer's patients is reaching epidemic proportions.

People suffering from Alzheimer's disease develop a progressive dementia in adulthood, accompanied by three main structural changes in the brain: diffuse loss of neurons in multiple parts of the brain; accumulation of intracellular protein deposits termed neurofibrillary tangles; and accumulation of extracellular protein deposits termed amyloid or senile plaques, surrounded by misshapen nerve terminals (dystrophic neurites) and activated microglia (microgliosis and astrocytosis). A main constituent of these amyloid plaques is the amyloid- $\beta$  peptide ( $A\beta$ ), a 39–43 amino-acid protein that is produced through cleavage of the  $\beta$ -amyloid precursor protein (APP). Extensive research has been conducted on the relevance of  $A\beta$  deposits in Alzheimer's disease, *see, e.g.,* Selkoe, *Trends in Cell Biology* 8, 447-453 (1998).  $A\beta$  naturally arises from the metabolic processing of the amyloid precursor protein ("APP") in the endoplasmic reticulum ("ER"), the Golgi apparatus, or the endosomal-lysosomal pathway, and most is normally secreted as a 40 (" $A\beta$ 1-40") or 42 (" $A\beta$ 1-42") amino acid peptide (Selkoe, *Annu. Rev. Cell Biol.* 10, 373-403 (1994)). A role for  $A\beta$  as a primary cause for Alzheimer's disease is supported by the presence of extracellular  $A\beta$  deposits in senile plaques of Alzheimer's disease, the increased production of  $A\beta$  in cells harboring mutant Alzheimer's disease associated genes, *e.g.,* amyloid precursor protein, presenilin I and presenilin II; and the toxicity of extracellular soluble (oligomeric) or fibrillar  $A\beta$  to cells in culture. *See, e.g.,* Gervais, *Eur. Biopharm. Review*, 40-42 (Autumn 2001); May, *DDT* 6, 459-62 (2001). Although symptomatic treatments exist for Alzheimer's disease, this disease cannot be prevented or cured at this time.

Alzheimer's disease is characterized by diffuse and neuritic plaques, cerebral angiopathy, and neurofibrillary tangles. Plaque and blood vessel amyloid is believed to be formed by the deposition of insoluble  $A\beta$  amyloid protein, which may be described as diffuse or fibrillary. Both soluble oligomeric  $A\beta$  and fibrillar  $A\beta$  are also believed to be neurotoxic and inflammatory.

Another type of amyloidosis is cerebral amyloid angiopathy (CAA). CAA is the specific deposition of amyloid- $\beta$  fibrils in the walls of leptomeningeal and cortical arteries, arterioles 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)).

Presently available therapies for treatment of  $\beta$ -amyloid diseases are almost entirely symptomatic, providing only temporary or partial clinical benefit. Although  
5 some pharmaceutical agents have been described that offer partial symptomatic relief, no comprehensive pharmacological therapy is currently available for the prevention or treatment of, for example, Alzheimer's disease.

Epilepsy is a serious neurological condition, associated with seizures, that affects hundreds of thousands of people worldwide. Clinically, a seizure results from a sudden  
10 electrical discharge from a collection of neurons in the brain. The resulting nerve cell activity is manifested by symptoms such as uncontrollable movements. Although epileptic seizures are rarely fatal, large numbers of patients require medication to avoid the disruptive, and potentially dangerous, consequences of seizures. In many cases, medication is required for extended periods of time, and in some cases, a patient must  
15 continue to take prescription drugs for life. Furthermore, drugs used for the management of epilepsy have side effects associated with prolonged usage, and the cost of the drugs can be considerable.

A variety of drugs are available for the management of epileptic seizures, including older anticonvulsant agents such as phenytoin, valproate and carbamazepine  
20 (ion channel blockers), as well as newer agents such as felbamate, gabapentin, and tiagabine.  $\beta$ -Alanine has been reported to have anticonvulsant activity, through NMDA inhibitory activity and GABAergic stimulatory activity, but has not been employed clinically. Currently available accepted drugs for epilepsy are anticonvulsant agents, where the term "anticonvulsant" is synonymous with "anti-seizure" or "anti-ictogenic;"  
25 these drugs can suppress seizures by blocking ictogenesis, but it is believed that they do not influence the development and/or progression of epilepsy because they do not block epileptogenesis. Thus, despite the numerous drugs available for the treatment of epilepsy (*i.e.*, through suppression of the convulsions associated with epileptic seizures), there are no generally accepted drugs for the treatment of the pathological changes  
30 which characterize epileptogenesis. There is no generally accepted method of inhibiting the epileptogenic process and there are no generally accepted drugs recognized as anti-epileptogenic.

A seizure is a single discrete clinical event caused by an excessive electrical discharge from a collection of neurons through a process termed "ictogenesis." As such,  
35 a seizure is merely the symptom of epilepsy. Epilepsy is a dynamic and often progressive process characterized by an underlying sequence of pathological transformations whereby normal brain is altered, becoming susceptible to recurrent seizures through a process termed "epileptogenesis." While it is believed that

ictogenesis and epileptogenesis have certain biochemical pathways in common, the two processes are not identical. Ictogenesis (the initiation and propagation of a seizure in time and space) is a rapid and definitive electrical/chemical event occurring over seconds or minutes. Epileptogenesis (the gradual process whereby normal brain is transformed into a state susceptible to spontaneous, episodic, time-limited, recurrent seizures, through the initiation and maturation of an "epileptogenic focus") is a slow biochemical or histological process which results in the appearance of seizures which generally occur months to years following the triggering event (e.g., brain trauma). Epileptogenesis is a two phase process. Phase 1 epileptogenesis is the initiation of the epileptogenic process prior to the first seizure, and is often the result of stroke, disease (e.g., meningitis), or trauma, such as an accidental blow to the head or a surgical procedure performed on the brain. Phase 2 epileptogenesis refers to the process during which a brain which is already susceptible to seizures, becomes still more susceptible to seizures of increasing frequency or severity. While the processes involved in epileptogenesis have not been definitively identified, some researchers believe that upregulation of excitatory coupling between neurons, mediated by *N*-methyl-D-aspartate (NMDA) receptors, is involved. Other researchers implicate downregulation of inhibitory coupling between neurons, mediated by gamma-amino-butyric acid (GABA) receptors.

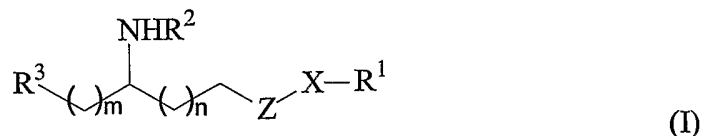
Patients with Alzheimer's disease can also experience seizures, which may be due to an imbalance in the activity of glutamate driven inhibitor neurons and downstream excitatory neurons.

### **Summary of The Invention**

The present invention relates to the use of certain compounds in the treatment of amyloid-related diseases, such as Alzheimer's disease. This invention also relates to methods and compounds useful for the treatment of epileptogenesis-associated conditions such as, for example, head trauma, neurosurgery, stroke, other events leading to neurotoxicity (or neurotoxic force) and neurological infections. This invention also relates to methods and compounds useful for the treatment of epilepsy, epileptogenesis, and/or convulsions. In some embodiments, the invention pertains to compounds and methods of using them in the simultaneous treatment of an amyloid-related disease and an epileptogenesis-associated condition in the same subject.

In particular, the invention relates to a method of treating or preventing an amyloid-related disease in a subject comprising administering to the subject a therapeutic amount of a compound of the invention. Among the compounds for use in the invention are those according to Formula I, such that, when administered, amyloid fibril formation, neurodegeneration, or cellular toxicity is reduced or inhibited.

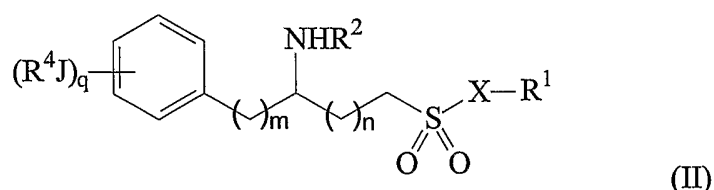
In one embodiment, the invention pertains at least in part to compounds of Formula (I):



wherein:

- 5 X is oxygen or nitrogen;  
 Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;  
 m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;  
 R<sup>1</sup> and R<sup>7</sup> are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to  
 10 form natural or unnatural amino acid residue, or  $-(\text{CH}_2)_p-\text{Y}$ ;  
 Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;  
 p is 0, 1, 2, 3, or 4;  
 15 R<sup>2</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxy carbonyl;  
 R<sup>3</sup> is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl, and  
 20 pharmaceutically acceptable salts, esters, and prodrugs thereof.

In another embodiment, the invention pertains to compounds of the Formula (II)



wherein:

- 25 X is oxygen or nitrogen;  
 m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;  
 q is 1, 2, 3, 4, or 5  
 R<sup>1</sup> is hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, or a moiety together with X to form a natural or unnatural amino  
 30 acid residue, or  $-(\text{CH}_2)_p-\text{Y}$ ;  
 Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

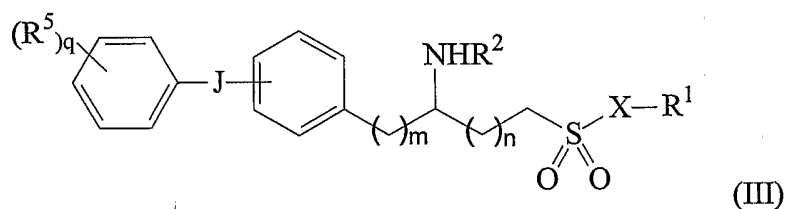
p is 0, 1, 2, 3, or 4;

$R^2$  is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxyalkyl;

each  $R^4$  is independently selected from the group consisting of hydrogen, halogen, hydroxyl, thiol, amino, cyano, nitro, alkyl, aryl, carbocyclic or heterocyclic;

J is absent, oxygen, nitrogen, sulfur, or a divalent link-moiety consisting of, without limiting to, lower alkylene, alkyleneoxy, alkyleneamino, alkyleneylthio, alkyleneoxyalkyl, alkyleneamoniakyl, alkyleneylthioalkyl, alkenyl, alkenyloxy, alkenylamino, or alkenylthio, and pharmaceutically acceptable salts, esters, amides and prodrugs thereof, provided that when J is a covalent bond, n is 1, m is 0 or 1, X is oxygen,  $R^4$  is hydrogen,  $R^2$  is not hydrogen or acyl; and provided that when q is 1, m is 0, n is 1, J is a covalent bond,  $R^4$  is methyl, ethyl, methoxy, ethoxy, chloro, bromo, hydroxy, or iodo in *para*-position, X is oxygen,  $R^2$  is not hydrogen; provided that when q is 2, m is 0, n is 1, J is a covalent bond,  $R^4$  is methyl in *ortho* and *para*-positions, X is oxygen,  $R^2$  is not hydrogen; provided that when provided that when q is 2, m is 0, n is 1, J is a covalent bond,  $R^4$  is chloro in 2 and 6 positions, X is oxygen,  $R^2$  is not acyl.

In a further embodiment, the invention pertains to compounds of the Formula (III):



wherein:

X is oxygen or nitrogen;

m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

q is 1, 2, 3, 4, or 5;

$R^1$  is hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, or a moiety together with X to form a natural or unnatural amino acid residue, or  $-(CH_2)_p-Y$ ;

Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

p is 0, 1, 2, 3, or 4;

$R^2$  is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxy carbonyl;

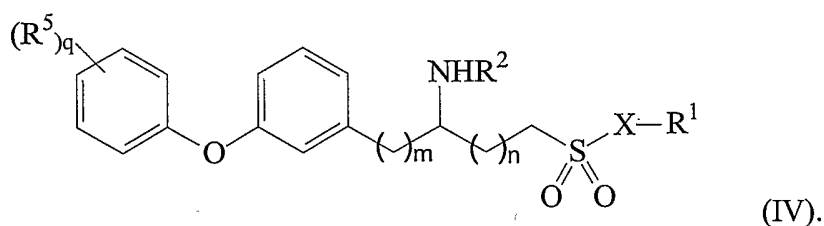
$R^5$  is selected from the group consisting of hydrogen, halogen, amino, nitro, hydroxy, carbonyl, thiol, carboxy, alkyl, alkoxy, alkoxy carbonyl, acyl, alkylamino, and acylamino;

q is an integer selected from 1 to 5;

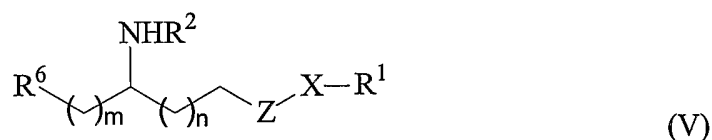
J is absent, oxygen, nitrogen, sulfur, or a divalent link-moiety consisting of, without limiting to, lower alkylene, alkyleneoxy, alkyleneamino, alkyleneylthio, alkyleneoxyalkyl, alkyleneamoniakyl, alkyleneylthioalkyl, alkenyl, alkenyloxy, alkenylamino, or alkenylthio; and

pharmaceutically acceptable salts, esters, and prodrugs thereof.

In yet another embodiment, the invention pertains to compounds of the Formula (IV):



In yet another embodiment, the invention pertains, at least in part, to compound of the Formula (V):



wherein:

X is oxygen or nitrogen;

Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;

m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

$R^1$  is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or  $-(\text{CH}_2)_p-\text{Y}$ ;

Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

p is 1, 2, 3, or 4;

$R^2$  is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxy carbonyl;

$R^6$  is a substituted or unsubstituted heterocyclic moiety,

$R^7$  is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, or aryl, and pharmaceutically acceptable salts, esters, and prodrugs thereof.

In one embodiment, the invention pertains to a method for inhibiting epileptogenesis in a subject. The method includes administering to the subject a therapeutic amount of a compound of the invention. In particular, the invention relates to a method of treating or preventing an epileptogenesis-associated condition comprising administering to the subject an effective amount of an anti-epileptogenic compound of the Formulae herein, e.g., Formula I. The invention also pertains to a method for treating convulsions in a subject comprising administering to the subject an effective amount of an anti-epileptogenic compound of the Formulae herein, e.g., Formula I. Likewise, the invention pertains to a method of treating or preventing epilepsy.

In one embodiment, the compounds disclosed herein prevent or inhibit amyloid protein assembly into insoluble fibrils which, *in vivo*, are deposited in various organs, or it favors clearance of deposits or slows deposition in patients already having deposits. In another embodiment, the compound may also prevent the amyloid protein, in its soluble, oligomeric form or in its fibrillar form, from binding or adhering to a cell surface and causing cell damage or toxicity. In yet another embodiment, the compound may block amyloid-induced cellular toxicity or microglial activation. In another embodiment, the compound may block amyloid-induced neurotoxicity or neurodegeneration. In another embodiment, the compounds may block astrogliosis. In another embodiment, the compounds may block mossy fiber formation. In another embodiment, the compounds may block the abnormal electroencephalographic changes associated with epileptogenesis and ictogenesis.

The compounds of the invention may be administered therapeutically or prophylactically to treat diseases associated with amyloid fibril formation, aggregation or deposition. The compounds of the invention may act to ameliorate the course of an amyloid-related diseases using any of the following mechanisms (this list is meant to be illustrative and not limiting): slowing the rate of amyloid fibril formation or deposition; lessening the degree of amyloid deposition; inhibiting, reducing, or preventing amyloid fibril formation; inhibiting neurodegeneration or cellular toxicity induced by amyloid; inhibiting amyloid induced inflammation; or enhancing the clearance of amyloid from amyloidotic organs (e.g., brain, pancreas).

In one embodiment, the compounds of the invention may be administered therapeutically or prophylactically to treat diseases associated with amyloid- $\beta$  fibril formation, aggregation or deposition. The compounds of the invention may act to

ameliorate the course of an amyloid- $\beta$  related disease using any of the following mechanisms (this list is meant to be illustrative and not limiting): slowing the rate of amyloid- $\beta$  fibril formation or deposition; lessening the degree of amyloid- $\beta$  deposition; inhibiting, reducing, or preventing amyloid- $\beta$  fibril formation; inhibiting  
5 neurodegeneration or cellular toxicity induced by amyloid- $\beta$ ; inhibiting amyloid- $\beta$  induced inflammation; or enhancing the clearance of amyloid- $\beta$  from the brain.

Therapeutic compounds of the invention may be effective in controlling amyloid- $\beta$  deposition either following their entry into the brain (following penetration of the blood brain barrier) or from the periphery. When acting from the periphery, a  
10 compound may alter the equilibrium of A $\beta$  between the brain and the plasma so as to favor the exit of A $\beta$  from the brain. An increase in the exit of A $\beta$  from the brain would result in a decrease in A $\beta$  brain concentration and therefore favor a decrease in A $\beta$  deposition. Alternatively, compounds that penetrate the brain could control deposition by acting directly on brain A $\beta$  *e.g.*, by maintaining it in a non-fibrillar form or favoring  
15 its clearance from the brain. These compounds could also prevent A $\beta$  in the brain from interacting with a cell surface and therefore prevent neurotoxicity or inflammation.

In an exemplary embodiment, the method is used to treat Alzheimer's disease (*e.g.*, sporadic or familial AD). The method can also be used prophylactically or therapeutically to treat other clinical occurrences of amyloid- $\beta$  deposition, such as in  
20 Down's syndrome individuals and in patients with cerebral amyloid angiopathy ("CAA") or hereditary cerebral hemorrhage.

Additionally, abnormal accumulation of APP and of amyloid- $\beta$  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, 1314-1319 (1996); Askanas, *et al.*,  
25 *Current Opinion in Rheumatology* 7, 486-496 (1995)). Accordingly, the compounds of the invention can be used prophylactically or therapeutically in the treatment of disorders in which amyloid-beta 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 A $\beta$  is associated with abnormal extracellular  
30 deposits, known as drusen, that accumulate along the basal surface of the retinal pigmented epithelium in individuals with age-related macular degeneration (AMD). AMD is a cause of irreversible vision loss in older individuals. It is believed that A $\beta$  deposition could be an important component of the local inflammatory events that contribute to atrophy of the retinal pigmented epithelium, drusen biogenesis, and the  
35 pathogenesis of AMD (Johnson, *et al.*, *Proc. Natl. Acad. Sci. USA* 99(18), 11830-5 (2002)).

The present invention therefore relates to the use of compounds of the invention in the prevention or treatment of amyloid-related diseases, including, *inter alia*,

Alzheimer's disease, cerebral amyloid angiopathy, inclusion body myositis, Down's syndrome, macular degeneration, other types of amyloidosis like Type II diabetes, primary (AL) amyloidosis, secondary (AA) amyloidosis and  $\beta_2$  microglobulin-related (dialysis-related) amyloidosis. .

- 5           The present invention also pertains to pharmaceutical compositions comprising a compound of any Formula herein and a pharmaceutically acceptable carrier, as well as methods of manufacturing such pharmaceutical compositions.

### **Detailed Description of The Invention**

- 10           The present invention relates to compounds, the use of compounds of the invention in the treatment of amyloid-related diseases, and the use of compounds of the invention in the treatment of epileptogenesis-associated conditions. For convenience, some definitions of terms referred to herein are set forth below.

15    *Amyloid-Related Diseases*  
    *AA (Reactive) Amyloidosis*

- Generally, AA amyloidosis is a manifestation of a number of diseases that provoke a sustained acute phase response. Such diseases include chronic inflammatory disorders, chronic local or systemic microbial infections, and malignant neoplasms. The most common form of reactive or secondary (AA) amyloidosis is seen as the result of long-standing inflammatory conditions. For example, patients with Rheumatoid Arthritis or Familial Mediterranean Fever (which is a genetic disease) can develop AA amyloidosis. The terms "AA amyloidosis" and "secondary (AA) amyloidosis" are used interchangeably.
- 20           AA fibrils are generally composed of 8,000 Dalton fragments (AA peptide or protein) formed by proteolytic cleavage of serum amyloid A protein (ApoSAA), a circulating apolipoprotein which is mainly synthesized in hepatocytes in response to such cytokines as IL-1, IL-6 and TNF. Once secreted, ApoSAA is complexed with HDL. Deposition of AA fibrils can be widespread in the body, with a preference for parenchymal organs.
- 25           The kidneys are usually a deposition site, and the liver and the spleen may also be affected. Deposition is also seen in the heart, gastrointestinal tract, and the skin.

- Underlying diseases which can lead to the development of AA amyloidosis include, but are not limited to inflammatory diseases, such as rheumatoid arthritis, juvenile chronic arthritis, ankylosing spondylitis, psoriasis, psoriatic arthropathy, Reiter's syndrome, Adult Still's disease, Behcet's syndrome, and Crohn's disease. AA deposits are also produced as a result of chronic microbial infections, such as leprosy, tuberculosis, bronchiectasis, decubitus ulcers, chronic pyelonephritis, osteomyelitis, and Whipple's disease. Certain malignant neoplasms can also result in AA fibril amyloid
- 35

deposits. These include such conditions as Hodgkin's lymphoma, renal carcinoma, carcinomas of gut, lung and urogenital tract, basal cell carcinoma, and hairy cell leukemia. Other underlying conditions that may be associated with AA amyloidosis are Castleman's disease and Schnitzler's syndrome.

5

#### *AL Amyloidosis*

AL amyloid deposition is generally associated with almost any dyscrasia of the B lymphocyte lineage, ranging from malignancy of plasma cells (multiple myeloma) to benign monoclonal gammopathy. At times, the presence of amyloid deposits may be a primary indicator of the underlying dyscrasia. AL amyloidosis is also described in detail in *Current Drug Targets*, 2004, 5 159-171.

Fibrils of AL amyloid deposits are composed of monoclonal immunoglobulin light chains or fragments thereof. More specifically, the fragments are derived from the N-terminal region of the light chain (kappa or lambda) and contain all or part of the variable ( $V_L$ ) domain thereof. Deposits generally occur in the mesenchymal tissues, causing peripheral and autonomic neuropathy, carpal tunnel syndrome, macroglossia, restrictive cardiomyopathy, arthropathy of large joints, immune dyscrasias, myelomas, as well as occult dyscrasias. However, it should be noted that almost any tissue, particularly visceral organs such as the kidney, liver, spleen and heart, may be involved.

20

There are many forms of hereditary systemic amyloidosis. Although they are relatively rare conditions, adult onset of symptoms and their inheritance patterns (usually autosomal dominant) lead to persistence of such disorders in the general population. Generally, the syndromes are attributable to point mutations in the precursor protein leading to production of variant amyloidogenic peptides or proteins. Table 1 summarizes the fibril composition of exemplary forms of these disorders.

25

**TABLE 1 - Fibril Composition of Exemplary Amyloid-Related Diseases**

| Fibril Peptide/Protein                        | Genetic Variant                     | Clinical Syndrome                                                                                                              |
|-----------------------------------------------|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| ATTR protein from Transthyretin and fragments | Met30, many others                  | Familial amyloid polyneuropathy (FAP), (Mainly peripheral nerves)                                                              |
| ATTR protein from Transthyretin and fragments | Thr45, Ala60, Ser84, Met111, Ile122 | Cardiac involvement predominant without neuropathy, familial amyloid polyneuropathy, senile systemic amyloidosis, Tenosynovium |

| Fibril Peptide/Protein                                                                                    | Genetic Variant                | Clinical Syndrome                                                                                                                    |
|-----------------------------------------------------------------------------------------------------------|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|
| N-terminal fragment of Apolipoprotein A1 (apoAI)                                                          | Arg26                          | Familial amyloid polyneuropathy (FAP), (mainly peripheral nerves)                                                                    |
| N-terminal fragment of Apolipoprotein A1 (ApoAI)                                                          | Arg26, Arg50, Arg 60, others   | Ostertag-type, non-neuropathic (predominantly visceral involvement)                                                                  |
| ApoAII from Apolipoprotein AII                                                                            |                                | Familial amyloidosis                                                                                                                 |
| Lysozyme (Alys)                                                                                           | Thr56, His67                   | Ostertag-type, non-neuropathic (predominantly visceral involvement)                                                                  |
| Fibrogen alpha chain fragment                                                                             | Leu554, Val 526                | Cranial neuropathy with lentic corneal dystrophy                                                                                     |
| Gelsolin fragment (Agel)                                                                                  | Asn187, Tyr187                 | Cranial neuropathy with lattice corneal dystrophy                                                                                    |
| Cystatin C fragment (ACys)                                                                                | Glu68                          | Hereditary cerebral hemorrhage (cerebral amyloid angiopathy) - Icelandic type                                                        |
| $\beta$ -amyloid protein ( $A\beta$ ) derived from Amyloid Precursor Protein (APP)                        | Gln693                         | Hereditary cerebral hemorrhage (cerebral amyloid angiopathy) - Dutch type                                                            |
| $\beta$ -amyloid protein ( $A\beta$ ) derived from Amyloid Precursor Protein (APP)                        | Ile717, Phe717, Gly717         | Familial Alzheimer's Disease                                                                                                         |
| $\beta$ -amyloid protein ( $A\beta$ ) derived from Amyloid Precursor Protein (APP), <i>e.g.</i> , bPP 695 | Gln 618                        | Alzheimer's disease, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis, Dutch type                                    |
| $\beta$ -amyloid protein ( $A\beta$ ) derived from Amyloid Precursor Protein (APP)                        | Asn670, Leu671                 | Familial Dementia – probably Alzheimer's Disease                                                                                     |
| Prion Protein (PrP, APrP <sup>Sc</sup> ) derived from Prp precursor protein (51-91 insert)                | Leu102, Val167, Asn178, Lys200 | Familial Creutzfeldt-Jakob disease; Gerstmann-Sträussler-Scheinker syndrome (hereditary spongiform encephalopathies, prion diseases) |
| AA derived from Serum amyloid A protein (ApoSAA)                                                          |                                | Familial Mediterranean fever, predominant renal involvement (autosomal recessive)                                                    |

| Fibril Peptide/Protein                                                | Genetic Variant  | Clinical Syndrome                                                   |
|-----------------------------------------------------------------------|------------------|---------------------------------------------------------------------|
| AA derived from Serum amyloid A protein (ApoSAA)                      |                  | Muckle-Well's syndrome, nephropathy, deafness, urticaria, limb pain |
| Unknown                                                               |                  | Cardiomyopathy with persistent atrial standstill                    |
| Unknown                                                               |                  | Cutaneous deposits (bullous, papular, pustulodermal)                |
| AH amyloid protein, derived from immunoglobulin heavy chain (gamma I) | A $\gamma$ I     | Myeloma associated amyloidosis                                      |
| ACal amyloid protein from (pro)calcitonin                             | (Pro) calcitonin | Medullary carcinomas of the thyroid                                 |
| AANF amyloid protein from atrial natriuretic factor                   |                  | Isolated atrial amyloid                                             |
| Apro from Prolactin                                                   |                  | Prolactinomas                                                       |
| Abri/ADan from ABri peptide                                           |                  | British and Danish familial Dementia                                |

Data derived from Tan SY, Pepys MB. Amyloidosis. *Histopathology*, 25(5), 403-414 (Nov 1994), WHO/IUIS Nomenclature Subcommittee, Nomenclature of Amyloid and Amyloidosis. Bulletin of the World Health Organisation 1993; 71: 10508; and Merlini *et al.*, Clin Chem Lab Med 2001; 39(11): 1065-75.

5

The data provided in Table 1 are exemplary and are not intended to limit the scope of the invention. For example, more than 40 separate point mutations in the transthyretin gene have been described, all of which give rise to clinically similar forms of familial amyloid polyneuropathy.

10 In general, any hereditary amyloid disorder can also occur sporadically, and both hereditary and sporadic forms of a disease present with the same characteristics with regard to amyloid. For example, the most prevalent form of secondary AA amyloidosis occurs sporadically, e.g. as a result of ongoing inflammation, and is not associated with Familial Mediterranean Fever. Thus general discussion relating to hereditary amyloid  
15 disorders below can also be applied to sporadic amyloidoses.

Transthyretin (TTR) is a 14 kiloDalton protein that is also sometimes referred to as prealbumin. It is produced by the liver and choroid plexus, and it functions in

transporting thyroid hormones and vitamin A. At least 50 variant forms of the protein, each characterized by a single amino acid change, are responsible for various forms of familial amyloid polyneuropathy. For example, substitution of proline for leucine at position 55 results in a particularly progressive form of neuropathy; substitution of methionine for leucine at position 111 resulted in a severe cardiopathy in Danish patients.

Amyloid deposits isolated from heart tissue of patients with systemic amyloidosis have revealed that the deposits are composed of a heterogeneous mixture of TTR and fragments thereof, collectively referred to as ATTR, the full length sequences of which have been characterized. ATTR fibril components can be extracted from such plaques and their structure and sequence determined according to the methods known in the art (*e.g.*, Gustavsson, A., *et al.*, Laboratory Invest. 73: 703-708, 1995; Kametani, F., *et al.*, Biochem. Biophys. Res. Commun. 125: 622-628, 1984; Pras, M., *et al.*, PNAS 80: 539-42, 1983).

Persons having point mutations in the molecule apolipoprotein AI (*e.g.*, Gly→Arg26; Trp→Arg50; Leu→Arg60) exhibit a form of amyloidosis ("Östertag type") characterized by deposits of the protein apolipoprotein AI or fragments thereof (AApoAI). These patients have low levels of high density lipoprotein (HDL) and present with a peripheral neuropathy or renal failure.

A mutation in the alpha chain of the enzyme lysozyme (*e.g.*, Ile→Thr56 or Asp→His57) is the basis of another form of Östertag-type non-neuropathic hereditary amyloid reported in English families. Here, fibrils of the mutant lysozyme protein (Alys) are deposited, and patients generally exhibit impaired renal function. This protein, unlike most of the fibril-forming proteins described herein, is usually present in whole (unfragmented) form (Benson, M.D., *et al.* CIBA Fdn. Symp. 199: 104-131, 1996).

Immunoglobulin light chains tend to form aggregates in various morphologies, including fibrillar (*e.g.*, AL amyloidosis and AH amyloidosis), granular (*e.g.*, light chain deposition disease (LCDD), heavy chain deposition disease (HCDD), and light-heavy chain deposition disease (LHCDD)), crystalline (*e.g.*, Acquired Farconi's Syndrome), and microtubular (*e.g.*, Cryoglobulinemia). AL and AH amyloidosis is indicated by the formation of insoluble fibrils of immunoglobulin light chains and heavy chain, respectively, and/or their fragments. In AL fibrils, lambda ( $\lambda$ ) chains such as  $\lambda$  VI chains ( $\lambda$ 6 chains), are found in greater concentrations than kappa ( $\kappa$ ) chains.  $\lambda$ III chains are also slightly elevated. Merlini *et al.*, CLIN CHEM LAB MED 39(11):1065-75 (2001). Heavy chain amyloidosis (AH) is generally characterized by aggregates of gamma chain amyloid proteins of the IgG1 subclass. Eulitz *et al.*, PROC NATL ACAD SCI USA 87:6542-46 (1990).

Comparison of amyloidogenic to non-amyloidogenic light chains has revealed that the former can include replacements or substitutions that appear to destabilize the folding of the protein and promote aggregation. AL and LCDD have been distinguished from other amyloid diseases due to their relatively small population monoclonal light chains, which are manufactured by neoplastic expansion of an antibody-producing B cell. AL aggregates typically are well-ordered fibrils of lambda chains. LCDD aggregates are relatively amorphous aggregations of both kappa and lambda chains, with a majority being kappa, in some cases  $\kappa$ IV. Bellotti *et al.*, JOURNAL OF STRUCTURAL BIOLOGY 13:280-89 (2000). Comparison of amyloidogenic and non-amyloidogenic heavy chains in patients having AH amyloidosis has revealed missing and/or altered components. Eulitz *et al.*, PROC NATL ACAD SCI USA 87:6542-46 (1990) (pathogenic heavy chain characterized by significantly lower molecular mass than non-amyloidogenic heavy chains); and Solomon *et al.* AM J HEMAT 45(2) 171-6 (1994) (amyloidogenic heavy chain characterized as consisting solely of the VH-D portion of the non-amyloidogenic heavy chain).

Accordingly, potential methods of detecting and monitoring treatment of subjects having or at risk of having AL, LCDD, AH, and the like, include but are not limited to immunoassaying plasma or urine for the presence or depressed deposition of amyloidogenic light or heavy chains, *e.g.*, amyloid  $\lambda$ , amyloid  $\kappa$ , amyloid  $\kappa$ IV, amyloid  $\gamma$ , or amyloid  $\gamma$ 1.

#### *Brain Amyloidosis*

The most frequent type of amyloid in the brain is composed primarily of A $\beta$  peptide fibrils, resulting in dementia associated with sporadic (non-hereditary) Alzheimer's disease. 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. Brain amyloidosis includes those diseases, conditions, pathologies, and other abnormalities of the structure or function of the brain, including components thereof, in which the causative agent is amyloid. The area of the brain affected in an amyloid-related disease may be the stroma including the vasculature or the parenchyma including functional or anatomical regions, or neurons themselves. A subject need not have received a definitive diagnosis of a specifically recognized amyloid-related disease. The term "amyloid related disease" includes brain amyloidosis.

Amyloid- $\beta$  peptide ("A $\beta$ ") is a 39-43 amino acid peptide derived by proteolysis from a large protein known as Beta Amyloid Precursor Protein (" $\beta$ APP"). Mutations in  $\beta$ APP result in familial forms of Alzheimer's disease, Down's syndrome, cerebral amyloid angiopathy, and senile dementia, characterized by cerebral deposition of plaques composed of A $\beta$  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  $\beta$  or  $\gamma$ -secretase, or within A $\beta$ . For example, position 717 is proximate to the site of gamma-secretase cleavage of APP in its processing to A $\beta$ , and positions 670/671 are proximate to the site of  $\beta$ -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 A $\beta$  generated from APP. The familial form of Alzheimer's disease represents only 10% of the subject population. Most occurrences of Alzheimer's disease are sporadic cases where APP and A $\beta$  do not possess any mutation. The structure and sequence of A $\beta$  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. APP is expressed and constitutively catabolized in most cells. The dominant catabolic pathway appears to be cleavage of APP within the A $\beta$  sequence by an enzyme provisionally termed  $\alpha$ -secretase, leading to release of a soluble ectodomain fragment known as APPs $\alpha$ . This cleavage precludes the formation of A $\beta$  peptide. In contrast to this non-amyloidogenic pathway, APP can also be cleaved by enzymes known as  $\beta$ - and  $\gamma$ -secretase at the N- and C-termini of the A $\beta$ , respectively, followed by release of A $\beta$  into the extracellular space. To date, BACE has been identified as  $\beta$ -secretase (Vasser, *et al.*, *Science* 286:735-741, 1999) and presenilins have been implicated in  $\gamma$ -secretase activity (De Strooper, *et al.*, *Nature* 391, 387-90 (1998)). The 39-43 amino acid A $\beta$  peptide is produced by sequential proteolytic cleavage of the amyloid precursor protein (APP) by the  $\beta$  and  $\gamma$  secretases enzyme. Although A $\beta$ 40 is the predominant form produced, 5-7% of total A $\beta$  exists as A $\beta$ 42 (Cappai *et al.*, *Int. J. Biochem. Cell Biol.* 31. 885-89 (1999)).

The length of the A $\beta$  peptide appears to dramatically alter its biochemical/biophysical properties. Specifically, the additional two amino acids at the C-terminus of A $\beta$ 42 are very hydrophobic, presumably increasing the propensity of A $\beta$ 42 to aggregate. For example, Jarrett, *et al.* demonstrated that A $\beta$ 42 aggregates very rapidly *in vitro* compared to A $\beta$ 40, suggesting that the longer forms of A $\beta$  may be the 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 A $\beta$  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 A $\beta$  42/43 forms versus A $\beta$  40 (Suzuki, *et al.*, *Science* 264, 1336-40 (1994)) while the

“Swedish” mutant form of APP (APPK670N/M671L) increases levels of both A $\beta$ 40 and A $\beta$ 42/43 (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 A $\beta$ 42/43 production but not A $\beta$ 40 (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 A $\beta$ 42 (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 A $\beta$ 42 brain concentration due to an increased production and release of A $\beta$ 42 or a decrease in clearance (degradation or brain clearance) is a causative event in the disease pathology.

Multiple mutation sites in either A $\beta$  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 A $\beta$ ); the Flemish mutation of A $\beta$ ; the Arctic mutation of A $\beta$ ; the Italian mutation of A $\beta$ ; the Iowa mutation of A $\beta$ ; familial British dementia; and familial Danish dementia. CAA may also be sporadic.

As used herein, the terms “ $\beta$  amyloid,” “amyloid- $\beta$ ,” and the like refer to amyloid  $\beta$  proteins or peptides, amyloid  $\beta$  precursor proteins or peptides, intermediates, and modifications and fragments thereof, unless otherwise specifically indicated. In particular, “A $\beta$ ” refers to any peptide produced by proteolytic processing of the APP gene product, especially peptides which are associated with amyloid pathologies, including A $\beta$ 1-39, A $\beta$ 1-40, A $\beta$ 1-41, A $\beta$ 1-42, and A $\beta$ 1-43. For convenience of nomenclature, “A $\beta$ 1-42” may be referred to herein as “A $\beta$  (1-42)” or simply as “A $\beta$ 42” or “A $\beta$ <sub>42</sub>” (and likewise for any other amyloid peptides discussed herein). As used herein, the terms “ $\beta$  amyloid,” “amyloid- $\beta$ ,” and “A $\beta$ ” 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). “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.

Gelsolin is a calcium binding protein that binds to fragments and actin filaments. Mutations at position 187 (*e.g.*, Asp→Asn; Asp→Tyr) of the protein result in a form of hereditary systemic amyloidosis, usually found in patients from Finland, as well as persons of Dutch or Japanese origin. In afflicted individuals, fibrils formed from gelsolin fragments (Agel), usually consist of amino acids 173-243 (68 kDa carboxyterminal fragment) and are deposited in blood vessels and basement membranes, resulting in corneal dystrophy and cranial neuropathy which progresses to peripheral neuropathy, dystrophic skin changes and deposition in other organs. (Kangas, H., *et al.* Human Mol. Genet. 5(9): 1237-1243, 1996).

Other mutated proteins, such as mutant alpha chain of fibrinogen (AfibA) and mutant cystatin C (Acys) also form fibrils and produce characteristic hereditary disorders. AfibA fibrils form deposits characteristic of a nonneuropathic hereditary amyloid with renal disease; Acys deposits are characteristic of a hereditary cerebral amyloid angiopathy reported in Iceland (Isselbacher, Harrison's Principles of Internal Medicine, McGraw-Hill, San Francisco, 1995; Benson, *et al.*). In at least some cases, patients with cerebral amyloid angiopathy (CAA) have been shown to have amyloid fibrils containing a non-mutant form of cystatin C in conjunction with amyloid beta protein (Nagai, A., *et al.* Molec. Chem. Neuropathol. 33: 63-78, 1998).

Certain forms of prion disease are now considered to be heritable, accounting for up to 15% of cases, which were previously thought to be predominantly infectious in nature. (Baldwin, *et al.*, in Research Advances in *Alzheimer's Disease and Related Disorders*, John Wiley and Sons, New York, 1995). In hereditary and sporadic prion disorders, patients develop plaques composed of abnormal isoforms of the normal prion protein (PrP<sup>Sc</sup>).

A predominant mutant isoform, PrP<sup>Sc</sup>, also referred to as AScr, differs from the normal cellular protein in its resistance to protease degradation, insolubility after detergent extraction, deposition in secondary lysosomes, post-translational synthesis, and high  $\beta$ -pleated sheet content. Genetic linkage has been established for at least five mutations resulting in Creutzfeldt-Jacob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI). (Baldwin, *supra*) Methods for extracting fibril peptides from scrapie fibrils, determining sequences and making such peptides are known in the art (*e.g.*, Beekes, M., *et al.* J. Gen. Virol. 76: 2567-76, 1995).

For example, one form of GSS has been linked to a PrP mutation at codon 102, while telencephalic GSS segregates with a mutation at codon 117. Mutations at codons 198 and 217 result in a form of GSS in which neuritic plaques characteristic of Alzheimer's disease contain PrP instead of A $\beta$  peptide. Certain forms of familial CJD

have been associated with mutations at codons 200 and 210; mutations at codons 129 and 178 have been found in both familial CJD and FFI. (Baldwin, *supra*).

#### *Cerebral Amyloidosis*

5           Local deposition of amyloid is common in the brain, particularly in elderly individuals. The most frequent type of amyloid in the brain is composed primarily of A $\beta$  peptide fibrils, resulting in dementia or sporadic (non-hereditary) Alzheimer's disease. The most common occurrences of cerebral amyloidosis are sporadic and not familial. For example, the incidence of sporadic Alzheimer's disease and sporadic CAA greatly  
10           exceeds the incidence of familial AD and CAA. Moreover, sporadic and familial forms of the disease cannot be distinguished from each other (they differ only in the presence or absence of an inherited genetic mutation); for example, the clinical symptoms and the amyloid plaques formed in both sporadic and familial AD are very similar, if not identical.

15           Cerebral amyloid angiopathy (CAA) refers to the specific deposition of amyloid fibrils in the walls of leptomeningeal and cortical arteries, arterioles 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  
20           sporadically or be hereditary.

#### *Senile Systemic Amyloidosis*

            Amyloid deposition, either systemic or focal, increases with age. For example, fibrils of wild type transthyretin (TTR) are commonly found in the heart tissue of elderly  
25           individuals. These may be asymptomatic, clinically silent, or may result in heart failure. Asymptomatic fibrillar focal deposits may also occur in the brain (A $\beta$ ), corpora amylacea of the prostate ( $\beta_2$  microglobulin), joints and seminal vesicles.

#### *Dialysis-related Amyloidosis (DRA)*

30           Plaques composed of  $\beta_2$  microglobulin ( $\beta_2$ M) fibrils commonly develop in patients receiving long term hemodialysis or peritoneal dialysis.  $\beta_2$  microglobulin is a 11.8 kiloDalton polypeptide and is the light chain of Class I MHC antigens, which are present on all nucleated cells. Under normal circumstances,  $\beta_2$ M is usually distributed in the extracellular space unless there is an impaired renal function, in which case  $\beta_2$ M is  
35           transported into tissues where it polymerizes to form amyloid fibrils. Failure of clearance such as in the case of impaired renal function, leads to deposition in the carpal tunnel and other sites (primarily in collagen-rich tissues of the joints). Unlike other fibril proteins,  $\beta_2$ M molecules are not produced by cleavage of a longer precursor

protein and are generally present in unfragmented form in the fibrils. (Benson, *supra*). Retention and accumulation of this amyloid precursor has been shown to be the main pathogenic process underlying DRA. DRA is characterized by peripheral joint osteoarthropathy (e.g., joint stiffness, pain, swelling, etc.). Isoforms of  $\beta_2$ M, glycosylated  $\beta_2$ M, or polymers of  $\beta_2$ M in tissue are the most amyloidogenic form (as opposed to native  $\beta_2$ M). Unlike other types of amyloidosis,  $\beta_2$ M is confined largely to osteoarticular sites. Visceral depositions are rare. Occasionally, these deposits may involve blood vessels and other important anatomic sites.

Despite improved dialysis methods for removal of  $\beta_2$ M, the majority of patients have plasmatic  $\beta_2$ M concentrations that remain dramatically higher than normal. These elevated  $\beta_2$ M concentrations generally lead to Diabetes-Related Amyloidosis (DRA) and comorbidities that contribute to mortality.

#### *Islet Amyloid Polypeptide and Diabetes*

Islet hyalinosis (amyloid deposition) was first described over a century ago as the presence of fibrous protein aggregates in the pancreas of patients with severe hyperglycemia (Opie, EL., *J Exp. Med.* 5: 397-428, 1901). Today, islet amyloid, composed predominantly of islet amyloid polypeptide (IAPP), or amylin, is a characteristic histopathological marker in over 90% of all cases of Type II diabetes (also known as Non-Insulin Dependent Diabetes, or NIDDM). These fibrillar accumulations result from the aggregation of the islet amyloid polypeptide (IAPP) or amylin, which is a 37 amino acid peptide, derived from a larger precursor peptide, called pro-IAPP.

IAPP is co-secreted with insulin in response to  $\beta$ -cell secretagogues. This pathological feature is not associated with insulin-dependent (Type I) diabetes and is a unifying characteristic for the heterogeneous clinical phenotypes diagnosed as NIDDM (Type II diabetes).

Longitudinal studies in cats and immunocytochemical investigations in monkeys have shown that a progressive increase in islet amyloid is associated with a dramatic decrease in the population of insulin-secreting  $\beta$ -cells and increased severity of the disease. More recently, transgenic studies have strengthened the relationship between IAPP plaque formation and  $\beta$ -cell apoptosis and dysfunction, indicating that amyloid deposition is a principal factor in increasing severity of Type II diabetes.

IAPP has also been shown to induce  $\beta$ -islet cell toxicity *in vitro*, indicating that appearance of IAPP fibrils in the pancreas of Type II or Type I diabetic patients (post-islet transplantation) could contribute to the loss of the  $\beta$ -cell islets (Langerhans) and organ dysfunction. In patients with Type II diabetes, the accumulation of pancreatic IAPP leads to formation of oligomeric IAPP, leading to a buildup of IAPP-amyloid as insoluble fibrous deposits which eventually destroys the

insulin-producing  $\beta$  cells of the islet, resulting in  $\beta$  cell depletion and failure (Westermarck, P., Grimelius, L., *Acta Path. Microbiol. Scand., sect. A.* 81: 291-300, 1973; de Koning, EJP., *et al.*, *Diabetologia* 36: 378-384, 1993; and Lorenzo, A., *et al.*, *Nature* 368: 756-760, 1994). Accumulation of IAPP as fibrous deposits can also have an impact on the ratio of pro-IAPP to IAPP normally found in plasma by increasing this ratio due to the trapping of IAPP in deposits. Reduction of  $\beta$  cell mass can be manifested by hyperglycemia and insulinemia. This  $\beta$ -cell mass loss can lead to a need for insulin therapy.

Diseases caused by the death or malfunctioning of a particular type or types of cells can be treated by transplanting into the patient healthy cells of the relevant type of cell. This approach has been used for Type I diabetes patients. Often pancreatic islet cells from a donor are cultured *in vitro* prior to transplantation, to allow them to recover after the isolation procedure or to reduce their immunogenicity. However, in many instances islet cell transplantation is unsuccessful, due to death of the transplanted cells. One reason for this poor success rate is IAPP, which organizes into toxic oligomers. Toxic effects may result from intracellular and extracellular accumulation of fibril oligomers. The IAPP oligomers can form fibrils and become toxic to the cells *in vitro*. In addition, IAPP fibrils are likely to continue to grow after the cells are transplanted and cause death or dysfunction of the cells. This may occur even when the cells are from a healthy donor and the patient receiving the transplant does not have a disease that is characterized by the presence of fibrils. For example, compounds of the present invention may also be used in preparing tissues or cells for transplantation according to the methods described in International Patent Application (PCT) number WO 01/003680.

The compounds of the invention may also stabilize the ratio of the concentrations of Pro-IAPP/IAPP, pro-Insulin/Insulin and C-peptide levels. In addition, as biological markers of efficacy, the results of the different tests, such as the arginine-insulin secretion test, the glucose tolerance test, insulin tolerance and sensitivity tests, could all be used as markers of reduced  $\beta$ -cell mass and/or accumulation of amyloid deposits. Such class of drugs could be used together with other drugs targeting insulin resistance, hepatic glucose production, and insulin secretion. Such compounds might prevent insulin therapy by preserving  $\beta$ -cell function and be applicable to preserving islet transplants.

### 35 *Hormone-derived Amyloidoses*

Endocrine organs may harbor amyloid deposits, particularly in aged individuals. Hormone-secreting tumors may also contain hormone-derived amyloid plaques, the fibrils of which are made up of polypeptide hormones such as calcitonin (medullary

carcinoma of the thyroid), and atrial natriuretic peptide (isolated atrial amyloidosis). Sequences and structures of these proteins are well known in the art.

#### *Miscellaneous Amyloidoses*

5           There are a variety of other forms of amyloid disease that are normally manifest as localized deposits of amyloid. In general, these diseases are probably the result of the localized production or lack of catabolism of specific fibril precursors or a predisposition of a particular tissue (such as the joint) for fibril deposition. Examples of such idiopathic deposition include nodular AL amyloid, cutaneous amyloid, endocrine  
10 amyloid, and tumor-related amyloid. Other amyloid-related diseases include those described in Table 1, such as familial amyloid polyneuropathy (FAP), senile systemic amyloidosis, Tenosynovium, familial amyloidosis, Ostertag-type non-neuropathic amyloidosis, cranial neuropathy, hereditary cerebral hemorrhage, familial dementia, chronic dialysis, familial Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker  
15 syndrome, hereditary spongiform encephalopathies, prion diseases, familial Mediterranean fever, Muckle-Well's syndrome, nephropathy, deafness, urticaria, limb pain, cardiomyopathy, cutaneous deposits, multiple myeloma, benign monoclonal gammopathy, macroglobulinaemia, myeloma associated amyloidosis, medullary carcinomas of the thyroid, isolated atrial amyloid, and diabetes.

20

          The compounds of the invention may be administered therapeutically or prophylactically to treat diseases associated with amyloid fibril formation, aggregation or deposition, regardless of the clinical setting. The compounds of the invention may act to ameliorate the course of an amyloid-related disease using any of the following  
25 mechanisms, such as, for example but not limited to: slowing the rate of amyloid fibril formation or deposition; lessening the degree of amyloid deposition; inhibiting, reducing, or preventing amyloid fibril formation; inhibiting amyloid induced inflammation; enhancing the clearance of amyloid from, for example, the brain; or protecting cells from amyloid induced (oligomers or fibrillar) toxicity.

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          In an embodiment, the compounds of the invention may be administered therapeutically or prophylactically to treat diseases associated with amyloid- $\beta$  fibril formation, aggregation or deposition. The compounds of the invention may act to ameliorate the course of an amyloid- $\beta$  related disease using any of the following mechanisms (this list is meant to be illustrative and not limiting): slowing the rate of  
35 amyloid- $\beta$  fibril formation or deposition; lessening the degree of amyloid- $\beta$  deposition; inhibiting, reducing, or preventing amyloid- $\beta$  fibril formation; inhibiting neurodegeneration or cellular toxicity induced by amyloid- $\beta$ ; inhibiting amyloid- $\beta$

induced inflammation; enhancing the clearance of amyloid- $\beta$  from the brain; or favoring greater catabolism of A $\beta$ .

Compounds of the invention may be effective in controlling amyloid- $\beta$  deposition either following their entry into the brain (following penetration of the blood brain barrier) or from the periphery. When acting from the periphery, a compound may  
5 alter the equilibrium of A $\beta$  between the brain and the plasma so as to favor the exit of A $\beta$  from the brain. An increase in the exit of A $\beta$  from the brain would result in a decrease in A $\beta$  brain concentration and therefore favor a decrease in A $\beta$  deposition. In addition, compounds that penetrate the brain may control deposition by acting directly  
10 on brain A $\beta$ , e.g., by maintaining it in a non-fibrillar form or favoring its clearance from the brain. The compounds may slow down APP processing; may increase degradation of A $\beta$  fibrils by macrophages or by neuronal cells; or may decrease A $\beta$  production by activated microglia. These compounds could also prevent A $\beta$  in the brain from  
15 interacting with the cell surface and therefore prevent neurotoxicity, neurodegeneration, or inflammation.

In a preferred embodiment, the method is used to treat Alzheimer's disease (e.g., sporadic or familial AD). The method can also be used prophylactically or therapeutically to treat other clinical occurrences of amyloid- $\beta$  deposition, such as in Down's syndrome individuals and in patients with cerebral amyloid angiopathy  
20 ("CAA"), hereditary cerebral hemorrhage, or early Alzheimer's disease.

In another embodiment, the method is used to treat mild cognitive impairment. 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  
25 disease.

Additionally, abnormal accumulation of APP and of amyloid- $\beta$  protein in muscle fibers has been implicated in the pathology of sporadic inclusion body myositis (IBM) (Askanas, V., *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93: 1314-1319; Askanas, V. *et al.* (1995) *Current Opinion in Rheumatology* 7: 486-496). Accordingly, the compounds of  
30 the invention can be used prophylactically or therapeutically in the treatment of disorders in which amyloid-beta 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 A $\beta$  is associated with abnormal extracellular deposits, known as drusen, that accumulate along the basal surface of the retinal  
35 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 A $\beta$  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)).

In another embodiment, the invention also relates to a method of treating or preventing an amyloid-related disease in a subject (preferably a human) comprising administering to the subject a therapeutic amount of a compound according to the following Formulae or otherwise described herein, such that amyloid fibril formation or deposition, neurodegeneration, or cellular toxicity is reduced or inhibited. In another embodiment, the invention relates to a method of treating or preventing an amyloid-related disease in a subject (preferably a human) comprising administering to the subject a therapeutic amount of a compound according to the following Formulae or otherwise described herein, such that cognitive function is improved or stabilized or further deterioration in cognitive function is prevented, slowed, or stopped in patients with brain amyloidosis, *e.g.*, Alzheimer's disease, Down's syndrome or cerebral amyloid angiopathy. These compounds can also improve quality of daily living in these subjects.

The therapeutic compounds of the invention may treat amyloidosis related to type II diabetes by, for example, stabilizing glycemia, preventing or reducing the loss of  $\beta$  cell mass, reducing or preventing hyperglycemia due to loss of  $\beta$  cell mass, and modulating (*e.g.*, increasing or stabilizing) insulin production. The compounds of the invention may also stabilize the ratio of the concentrations of pro-IAPP/IAPP.

The therapeutic compounds of the invention may treat AA (secondary) amyloidosis and/or AL (primary) amyloidosis, by stabilizing renal function, decreasing proteinuria, increasing creatinine clearance (*e.g.*, by at least 50% or greater or by at least 100% or greater), reducing serum creatinine, reducing amyloid content as determined, *e.g.*, by visceral SAP scintigraphy, or by leading to remission of chronic diarrhea or weight gain (*e.g.*, 10% or greater).

#### Compounds of the Invention

The present invention pertains to methods and compounds (and pharmaceutical formulations thereof) useful for the treatment of epilepsy and convulsive disorders, for inhibition of epileptogenesis, and for inhibition of ictogenesis; and to methods for preparing the anti-epileptogenic agents of the invention. The invention further pertains to pharmaceutical compositions for treatment of epileptogenesis or epileptogenic conditions, and to kits including the anti-epileptogenic agents of the invention.

The present invention also relates, at least in part, to the use of certain chemical compounds (and pharmaceutical formulations thereof) in the prevention or treatment of amyloid-related diseases, including, *inter alia*, Alzheimer's disease, cerebral amyloid angiopathy, inclusion body myositis, Down's syndrome, diabetes related amyloidosis, hemodialysis-related amyloidosis ( $\beta_2$ M), primary amyloidosis (e.g.,  $\lambda$  or  $\kappa$  chain-related), familial amyloid polyneuropathy (FAP), senile systemic amyloidosis, familial amyloidosis, Ostertag-type, non-neuropathic amyloidosis, cranial neuropathy, hereditary cerebral hemorrhage, familial dementia, chronic dialysis, familial Creutzfeldt-Jakob disease; Gerstmann-Sträussler-Scheinker syndrome, hereditary spongiform encephalopathies, prion diseases, familial Mediterranean fever, Muckle-Well's syndrome, nephropathy, deafness, urticaria, limb pain, cardiomyopathy, cutaneous deposits, multiple myeloma, benign monoclonal gammopathy, macroglobulinaemia, myeloma associated amyloidosis, medullary carcinomas of the thyroid, and isolated atrial amyloid.

The chemical structures herein are drawn according to the conventional standards known in the art. Thus, where an atom, such as a carbon atom, as drawn appears to have an unsatisfied valency, then that valency is assumed to be satisfied by a hydrogen atom even though that hydrogen atom is not necessarily explicitly drawn. The structures of some of the compounds of this invention include stereogenic carbon atoms. It is to be understood that isomers arising from such asymmetry (e.g., all enantiomers and diastereomers) are included within the scope of this invention unless indicated otherwise. That is, unless otherwise stipulated, any chiral carbon center may be of either (*R*)- or (*S*)-stereochemistry. Such isomers can be obtained in substantially pure form by classical separation techniques and by stereochemically-controlled synthesis. Furthermore, alkenes can include either the *E*- or *Z*- geometry, where appropriate. In addition, the compounds of the present invention may exist in unsolvated as well as solvated forms with acceptable solvents such as water, THF, ethanol, and the like. In general, the solvated forms are considered equivalent to the unsolvated forms for the purposes of the present invention.

A "small molecule" refers to a compound that is not itself the product of gene transcription or translation (e.g., protein, RNA, or DNA) and preferably has a low molecular weight, e.g., less than about 2500 amu.

In general, the term "nucleophile" is art-recognized to mean a chemical group having a reactive pair of electrons that reacts with a compound by displacing a leaving group (commonly another nucleophile), such as commonly occur in aliphatic chemistry as unimolecular (known as " $S_N1$ ") or bimolecular (" $S_N2$ ") reactions. Examples of nucleophiles include uncharged compounds such as amines, mercaptans, and alcohols, and charged groups such as alkoxides, thiolates, carbanions, and a variety of organic and

inorganic anions. Illustrative anionic nucleophiles include, *inter alia*, simple anions such as azide, cyanide, thiocyanate, acetate, formate, or chloroformate, and bisulfite. Organometallic reagents such as organocuprates, organozincs, organolithiums, Grignard reagents, enolates, and acetylides, will under appropriate reaction conditions, be suitable nucleophiles.

Similarly, an "electrophile" means an atom, molecule, or ion able to accept an electron pair, particularly a pair of electrons from a nucleophile, such as typically occurs during an electrophilic substitution reaction. In an electrophilic substitution reaction, an electrophile binds to a substrate with the expulsion of another electrophile, *e.g.*, the substitution of a proton by another electrophile such as a nitronium ion on an aromatic substrate (*e.g.*, benzene). Electrophiles include cyclic compounds such as epoxides, aziridines, episulfides, cyclic sulfates, carbonates, lactones, and lactams; and non-cyclic electrophiles include sulfates, sulfonates (*e.g.*, tosylates), chlorides, bromides, and iodides. Generally, an electrophile may be a saturated carbon atom (*e.g.*, a methylene group) bonded to a leaving group; however, an electrophile may also be an unsaturated group, such as an aldehyde, ketone, ester, or conjugated ( $\alpha,\beta$ -unsaturated) analog thereof, which upon reaction with a nucleophile forms an adduct.

The term "leaving group" generally refers to a group that is readily displaced and substituted by a nucleophile (*e.g.*, an amine, a thiol, an alcohol, or cyanide). Such leaving groups are well known and include carboxylates, *N*-hydroxysuccinimide ("NHS"), *N*-hydroxybenzotriazole, a halogen (fluorine, chlorine, bromine, or iodine), alkoxides, and thioalkoxides. A variety of sulfur-based leaving groups are routinely used in synthetic chemistry, including alkane sulfonyloxy groups (*e.g.*, C<sub>1</sub>-C<sub>4</sub> alkane such as methane sulfonyloxy, ethane sulfonyloxy, propane sulfonyloxy, and butane sulfonyloxy groups) and the halogenated analogs (*e.g.*, halogeno(C<sub>1</sub>-C<sub>4</sub> alkane) sulfonyloxy groups, such as trifluoromethane sulfonyloxy (*i.e.*, triflate), 2,2,2-trichloroethane sulfonyloxy, 3,3,3-tribromopropane sulfonyloxy, and 4,4,4-trifluorobutane sulfonyloxy groups), as well as arylsulfonyloxy groups (*e.g.*, C<sub>6</sub>-C<sub>10</sub> aryl optionally substituted with 1 to 3 C<sub>1</sub>-C<sub>4</sub> alkyl groups, such as benzene sulfonyloxy,  $\alpha$ -naphthylsulfonyloxy,  $\beta$ -naphthylsulfonyloxy, *p*-toluenesulfonyloxy (*i.e.*, tosylates), 4-*tert*-butylbenzene sulfonyloxy, mesitylene sulfonyloxy, and 6-ethyl- $\alpha$ -naphthylsulfonyloxy groups).

"Activated esters" may be represented by the formula -COL, where L is a leaving group, typical examples of which include *N*-hydroxysulfosuccinimidyl and *N*-hydroxysuccinimidyl groups; aryloxy groups substituted with electron-withdrawing groups (*e.g.*, *p*-nitro, pentafluoro, pentachloro, *p*-cyano, or *p*-trifluoromethyl); and carboxylic acids activated by a carbodiimide to form an anhydride or mixed anhydride, *e.g.*, -OCOR<sup>a</sup> or -OCNR<sup>a</sup>NHR<sup>b</sup>, where R<sup>a</sup> and R<sup>b</sup> are independently C<sub>1</sub>-C<sub>6</sub> alkyl,

C<sub>5</sub>-C<sub>8</sub> alkyl (e.g., cyclohexyl), C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, or C<sub>1</sub>-C<sub>6</sub> alkoxy groups. An activated ester may be formed *in situ* or may be an isolable reagent. Sulfosuccinimidyl esters, pentafluorothiophenol esters, and sulfotetrafluorophenol are preferred activated esters. However, the ester leaving group may be, for example, substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl (such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *sec*-butyl, *tert*-butyl, pentyl, or hexyl), or substituted or unsubstituted C<sub>6</sub>-C<sub>14</sub> aryl or heterocyclic groups, such as 2-fluoroethyl, 2-chloroethyl, 2-bromoethyl, 2,2-dibromoethyl, 2,2,2-trichloroethyl, 3-fluoropropyl, 4-chlorobutyl, methoxymethyl, 1,1-dimethyl-1-methoxymethyl, ethoxymethyl, *N*-propoxymethyl, isopropoxymethyl, *N*-butoxymethyl, *tert*-butoxymethyl, 1-ethoxyethyl, 1-methyl-1-methoxyethyl, 1-(isopropoxy)ethyl, 3-methoxypropyl-4-methoxybutyl, fluoromethoxymethyl, 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 3-fluoropropoxymethyl, 4-chlorobutoxyethyl, dibromomethoxyethyl, 2-chloroethoxypropyl, fluoromethoxybutyl, 2-methoxyethoxymethyl, ethoxymethoxyethyl, methoxyethoxypropyl, methoxyethoxybutyl, benzyl, phenethyl, 3-phenylpropyl, 4-phenylbutyl,  $\alpha$ -naphthylmethyl,  $\beta$ -naphthylmethyl, diphenylmethyl, triphenylmethyl,  $\alpha$ -naphthylmethyl, 9-anthrylmethyl, 4-methylbenzyl, 2,4,6-trimethylbenzyl, 3,4,5-trimethylbenzyl, 4-methoxybenzyl, 4-methoxyphenyldiphenylmethyl, 2-nitrobenzyl, 4-nitrobenzyl, 4-chlorobenzyl, 4-bromobenzyl, 4-cyanobenzyl, 4-cyanobenzylmethyl, or bis(2-nitrophenyl)methyl groups.

The term “electron-withdrawing group” is art-recognized and describes the ability of a substituent to attract valence electrons (*e.g.*, pi-electrons) from neighboring atoms, *e.g.*, the substituent is more electronegative than neighboring atoms, or it draws electrons to itself more than a hydrogen atom would at the same position. The Hammett sigma value ( $\sigma$ ) is an accepted measure of a group’s electron-donating and withdrawing ability, especially the sigma para value ( $\sigma_p$ ). *See, e.g.*, “Advanced Organic Chemistry” by J. March, 5<sup>th</sup> Ed., John Wiley & Sons, Inc., New York, pp.368-75 (2001). The Hammett constant values are generally negative for electron-donating groups ( $\sigma_p=-0.66$  for  $\text{NH}_2$ ) and positive for electron-withdrawing groups ( $\sigma_p=0.78$  for a nitro group),  $\sigma_p$  indicating para substitution. Exemplary electron-withdrawing groups include nitro, acyl (ketone), formyl (aldehyde), sulfonyl, trifluoromethyl, halogeno (*e.g.*, chloro and fluoro), and cyano groups, among others. Conversely, an “electron-donating group” designates a substituent that contributes electrons more than hydrogen would if it occupied the same position in the molecule. Examples include amino (including alkylamino and dialkylamino), aryl, alkoxy (including aralkoxy), aryloxy, mercapto and alkylthio, and hydroxyl groups, among others.

As used herein, “alkyl” groups include saturated hydrocarbons having one or more carbon atoms, including straight-chain alkyl groups (*e.g.*, methyl, ethyl, propyl,

- butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, etc.), cyclic alkyl groups (or “cycloalkyl” or “alicyclic” or “carbocyclic” groups) (*e.g.*, cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, etc.), branched-chain alkyl groups (isopropyl, *tert*-butyl, *sec*-butyl, isobutyl, etc.), and alkyl-substituted alkyl groups
- 5 (*e.g.*, alkyl-substituted cycloalkyl groups and cycloalkyl-substituted alkyl groups). The term “aliphatic group” includes organic moieties characterized by straight or branched-chains, typically having between 1 and 22 carbon atoms. In complex structures, the chains may be branched, bridged, or cross-linked. Aliphatic groups include alkyl groups, alkenyl groups, and alkynyl groups.
- 10 In certain embodiments, a straight-chain or branched-chain alkyl group may have 30 or fewer carbon atoms in its backbone, *e.g.*, C<sub>1</sub>-C<sub>30</sub> for straight-chain or C<sub>3</sub>-C<sub>30</sub> for branched-chain. In certain embodiments, a straight-chain or branched-chain alkyl group may have 20 or fewer carbon atoms in its backbone, *e.g.*, C<sub>1</sub>-C<sub>20</sub> for straight-chain or C<sub>3</sub>-C<sub>20</sub> for branched-chain, and more preferably 18 or fewer. Likewise, preferred
- 15 cycloalkyl groups have from 4-10 carbon atoms in their ring structure, and more preferably have 4-7 carbon atoms in the ring structure. The term “lower alkyl” refers to alkyl groups having from 1 to 6 carbons in the chain, and to cycloalkyl groups having from 3 to 6 carbons in the ring structure.
- Unless the number of carbons is otherwise specified, “lower” as in “lower
- 20 aliphatic,” “lower alkyl,” “lower alkenyl,” etc. as used herein means that the moiety has at least one and less than about 8 carbon atoms. In certain embodiments, a straight-chain or branched-chain lower alkyl group has 6 or fewer carbon atoms in its backbone (*e.g.*, C<sub>1</sub>-C<sub>6</sub> for straight-chain, C<sub>3</sub>-C<sub>6</sub> for branched-chain), and more preferably 4 or fewer. Likewise, preferred cycloalkyl groups have from 3-8 carbon atoms in their ring
- 25 structure, and more preferably have 5 or 6 carbons in the ring structure. The term “C<sub>1</sub>-C<sub>6</sub>” as in “C<sub>1</sub>-C<sub>6</sub> alkyl” means alkyl groups containing 1 to 6 carbon atoms.
- Moreover, unless otherwise specified the term alkyl includes both “unsubstituted alkyls” and “substituted alkyls,” the latter of which refers to alkyl groups having substituents replacing one or more hydrogens on one or more carbons of the
- 30 hydrocarbon backbone. Such substituents may include, for example, alkenyl, alkynyl, halogeno, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino,
- 35 dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl,

sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclic, alkylaryl, or aromatic (including heteroaromatic) groups.

An "arylalkyl" group is an alkyl group substituted with an aryl group (e.g., phenylmethyl (*i.e.*, benzyl)). An "alkylaryl" moiety is an aryl group substituted with an alkyl group (e.g., *p*-methylphenyl (*i.e.*, *p*-tolyl)). The term "*n*-alkyl" means a straight-chain (*i.e.*, unbranched) unsubstituted alkyl group. An "alkylene" group is a divalent analog of the corresponding alkyl group. The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous to alkyls, but which contain at least one double or triple carbon-carbon bond respectively. Suitable alkenyl and alkynyl groups include groups having 2 to about 12 carbon atoms, preferably from 2 to about 6 carbon atoms.

The term "aromatic group" or "aryl group" includes unsaturated and aromatic cyclic hydrocarbons as well as unsaturated and aromatic heterocycles containing one or more rings. Aryl groups may also be fused or bridged with alicyclic or heterocyclic rings that are not aromatic so as to form a polycycle (e.g., tetralin). An "arylene" group is a divalent analog of an aryl group. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (e.g., tetralin).

The term "heterocyclic group" includes closed ring structures analogous to carbocyclic groups in which one or more of the carbon atoms in the ring is an element other than carbon, for example, nitrogen, sulfur, or oxygen. Heterocyclic groups may be saturated or unsaturated. Additionally, heterocyclic groups (such as pyrrolyl, pyridyl, isoquinolyl, quinolyl, purinyl, and furyl) may have aromatic character, in which case they may be referred to as "heteroaryl" or "heteroaromatic" groups.

Unless otherwise stipulated, aryl and heterocyclic (including heteroaryl) groups may also be substituted at one or more constituent atoms. Examples of heteroaromatic and heteroalicyclic groups may have 1 to 3 separate or fused rings with 3 to about 8 members per ring and one or more N, O, or S heteroatoms. In general, the term "heteroatom" includes atoms of any element other than carbon or hydrogen, preferred examples of which include nitrogen, oxygen, sulfur, and phosphorus. Heterocyclic groups may be saturated or unsaturated or aromatic.

Examples of heterocycles include, but are not limited to, acridinyl; azocinyl; benzimidazolyl; benzofuranyl; benzothiofuranyl; benzothiophenyl; benzoxazolyl; benzthiazolyl; benztriazolyl; benztetrazolyl; benzisoxazolyl; benzisothiazolyl; benzimidazolinyl; carbazolyl; 4aH-carbazolyl; carbolinyl; chromanyl; chromenyl; cinnolinyl; decahydroquinolinyl; 2H,6H-1,5,2-dithiazinyl; dihydrofuro[2,3-b]tetrahydrofuran; furanyl; furazanyl; imidazolidinyl; imidazolyl; imidazolyl; 1H-indazolyl; indolenyl; indolinyl; indolizynyl; indolyl; 3H-indolyl;

isobenzofuranyl; isochromanyl; isoindazolyl; isoindolinyl; isoindolyl; isoquinolinyl; isothiazolyl; isoxazolyl; methylenedioxyphenyl; morpholinyl; naphthyridinyl; octahydroisoquinolinyl; oxadiazolyl; 1,2,3-oxadiazolyl; 1,2,4-oxadiazolyl; 1,2,5-oxadiazolyl; 1,3,4-oxadiazolyl; oxazolidinyl; oxazolyl; oxazolidinyl; pyrimidinyl; 5 phenanthridinyl; phenanthrolinyl; phenazinyl; phenothiazinyl; phenoxathiinyl; phenoxazinyl; phthalazinyl; piperazinyl; piperidinyl; piperidonyl; 4-piperidonyl; piperonyl; pteridinyl; purinyl; pyranyl; pyrazinyl; pyrazolidinyl; pyrazolinyl; pyrazolyl; pyridazinyl; pyridooxazole; pyridoimidazole; pyridothiazole; pyridinyl; pyridyl; pyrimidinyl; pyrrolidinyl; pyrrolinyl; 2H-pyrrolyl; pyrrolyl; quinazolinyl; quinolinyl; 10 4H-quinolizinyll; quinoxalinyll; quinuclidinyl; tetrahydrofuranyl; tetrahydroisoquinolinyl; tetrahydroquinolinyl; tetrazolyl; 6H-1,2,5-thiadiazinyl; 1,2,3-thiadiazolyl; 1,2,4-thiadiazolyl; 1,2,5-thiadiazolyl; 1,3,4-thiadiazolyl; thianthrenyl; thiazolyl; thienyl; thienothiazolyl; thienooxazolyl; thienoimidazolyl; thiophenyl; triazinyl; 1,2,3-triazolyl; 1,2,4-triazolyl; 1,2,5-triazolyl; 1,3,4-triazolyl; and xanthenyl. Preferred heterocycles 15 include, but are not limited to, pyridinyl; furanyl; thienyl; pyrrolyl; pyrazolyl; pyrrolidinyl; imidazolyl; indolyl; benzimidazolyl; 1H-indazolyl; oxazolidinyl; benzotriazolyl; benzisoxazolyl; oxindolyl; benzoxazolinyll; and isatinoyl groups. Also included are fused ring and spiro compounds containing, for example, the above heterocycles.

20 A common hydrocarbon aryl group is a phenyl group having one ring. Two-ring hydrocarbon aryl groups include naphthyl, indenyl, benzocyclooctenyl, benzocycloheptenyl, pentalenyl, and azulenyl groups, as well as the partially hydrogenated analogs thereof such as indanyl and tetrahydronaphthyl. Exemplary three-ring hydrocarbon aryl groups include acephthylene, fluorenyl, phenalenyl, 25 phenanthrenyl, and anthracenyl groups.

Aryl groups also include heteromonocyclic aryl groups, *i.e.*, single-ring heteroaryl groups, such as thienyl, furyl, pyranyl, pyrrolyl, imidazolyl, pyrazolyl, pyridinyl, pyrazinyl, pyrimidinyl, and pyridazinyl groups; and oxidized analogs thereof such as pyridonyl, oxazolonyl, pyrazolonyl, isoxazolonyl, and thiazolonyl groups. The 30 corresponding hydrogenated (*i.e.*, non-aromatic) heteromonocyclic groups include pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazolinyll, pyrazolidinyl, pyrazolinyl, piperidyl and piperidino, piperazinyl, and morpholino and morpholinyl groups.

Aryl groups also include fused two-ring heteroaryls such as indolyl, isoindolyl, indolizinyll, indazolyl, quinolinyl, isoquinolinyl, phthalazinyl, quinoxalinyll, 35 quinazolinyl, cinnolinyl, chromenyl, isochromenyl, benzothienyl, benzimidazolyl, benzothiazolyl, purinyl, quinolizinyll, isoquinolonyll, quinolonyll, naphthyridinyl, and pteridinyl groups, as well as the partially hydrogenated analogs such as chromanyl, isochromanyl, indolinyl, isoindolinyl, and tetrahydroindolyl groups. Aryl groups also

include fused three-ring groups such as phenoxathiinyl, carbazolyl, phenanthridinyl, acridinyl, perimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxazinyl, and dibenzofuranyl groups.

Some typical aryl groups include substituted or unsubstituted 5- and 6-membered single-ring groups. In another aspect, each Ar group may be selected from the group consisting of substituted or unsubstituted phenyl, pyrrolyl, furyl, thienyl, thiazolyl, isothiazolyl, imidazolyl, triazolyl, tetrazolyl, pyrazolyl, oxazolyl, isooxazolyl, pyridinyl, pyrazinyl, pyridazinyl, and pyrimidinyl groups. Further examples include substituted or unsubstituted phenyl, 1-naphthyl, 2-naphthyl, biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalyl, 5-quinoxalyl, 3-quinolyl, and 6-quinolyl groups.

The term "amine" or "amino," as used herein, refers to an unsubstituted or substituted moiety of the formula  $-NR^aR^b$ , in which  $R^a$  and  $R^b$  are each independently hydrogen, alkyl, aryl, or heterocyclyl, or  $R^a$  and  $R^b$ , taken together with the nitrogen atom to which they are attached, form a cyclic moiety having from 3 to 8 atoms in the ring. Thus, the term amino includes cyclic amino moieties such as piperidinyl or pyrrolidinyl groups, unless otherwise stated. Thus, the term "alkylamino" as used herein means an alkyl group having an amino group attached thereto. Suitable alkylamino groups include groups having 1 to about 12 carbon atoms, preferably from 1 to about 6 carbon atoms. The term amino includes compounds or moieties in which a nitrogen atom is covalently bonded to at least one carbon or heteroatom. The term "dialkylamino" includes groups wherein the nitrogen atom is bound to at least two alkyl groups. The term "arylamino" and "diarylamino" include groups wherein the nitrogen is bound to at least one or two aryl groups, respectively. The term "alkylarylamino" refers to an amino group which is bound to at least one alkyl group and at least one aryl group. The term "alkaminoalkyl" refers to an alkyl, alkenyl, or alkynyl group substituted with an alkylamino group. The term "amide" or "aminocarbonyl" includes compounds or moieties which contain a nitrogen atom which is bound to the carbon of a carbonyl or a thiocarbonyl group.

The term "alkylthio" refers to an alkyl group, having a sulfhydryl group attached thereto. Suitable alkylthio groups include groups having 1 to about 12 carbon atoms, preferably from 1 to about 6 carbon atoms.

The term "alkylcarboxyl" as used herein means an alkyl group having a carboxyl group attached thereto.

The term "alkoxy" as used herein means an alkyl group having an oxygen atom attached thereto. Representative alkoxy groups include groups having 1 to about 12 carbon atoms, preferably 1 to about 6 carbon atoms, *e.g.*, methoxy, ethoxy, propoxy, *tert*-butoxy and the like. Examples of alkoxy groups include methoxy, ethoxy, isopropoxy, propoxy, butoxy, and pentoxy groups. The alkoxy groups can be substituted with groups such as alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moieties. Examples of halogen substituted alkoxy groups include, but are not limited to, fluoromethoxy, difluoromethoxy, trifluoromethoxy, chloromethoxy, dichloromethoxy, trichloromethoxy, *etc.*, as well as perhalogenated alkoxy groups.

The term "acylamino" includes moieties wherein an amino moiety is bonded to an acyl group. For example, the acylamino group includes alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido groups.

The terms "alkoxyalkyl", "alkylaminoalkyl" and "thioalkoxyalkyl" include alkyl groups, as described above, which further include oxygen, nitrogen or sulfur atoms replacing one or more carbons of the hydrocarbon backbone.

The term "carbonyl" or "carboxy" includes compounds and moieties which contain a carbon connected with a double bond to an oxygen atom. Examples of moieties which contain a carbonyl include aldehydes, ketones, carboxylic acids, amides, esters, anhydrides, *etc.*

The term "ether" or "ethereal" includes compounds or moieties which contain an oxygen bonded to two carbon atoms. For example, an ether or ethereal group includes "alkoxyalkyl" which refers to an alkyl, alkenyl, or alkynyl group substituted with an alkoxy group.

A "sulfonate" group is a  $-\text{SO}_3\text{H}$  or  $-\text{SO}_3^-\text{X}^+$  group bonded to a carbon atom, where  $\text{X}^+$  is a cationic counter ion group. Similarly, a "sulfonic acid" compound has a  $-\text{SO}_3\text{H}$  or  $-\text{SO}_3^-\text{X}^+$  group bonded to a carbon atom, where  $\text{X}^+$  is a cationic group. A "sulfate" as used herein is a  $-\text{OSO}_3\text{H}$  or  $-\text{OSO}_3^-\text{X}^+$  group bonded to a carbon atom, and a "sulfuric acid" compound has a  $-\text{SO}_3\text{H}$  or  $-\text{OSO}_3^-\text{X}^+$  group bonded to a carbon atom, where  $\text{X}^+$  is a cationic group. According to the invention, a suitable cationic group may be a hydrogen atom. In certain cases, the cationic group may actually be another group

on the therapeutic compound that is positively charged at physiological pH, for example an amino group.

A "counter ion" is required to maintain electroneutrality. Examples of anionic counter ions include halide, triflate, sulfate, nitrate, hydroxide, carbonate, bicarbonate, acetate, phosphate, oxalate, cyanide, alkylcarboxylate, *N*-hydroxysuccinimide, *N*-hydroxybenzotriazole, alkoxide, thioalkoxide, alkane sulfonyloxy, halogenated alkane sulfonyloxy, arylsulfonyloxy, bisulfate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, citrate, maleate, fumarate, succinate, tartrate, naphthylate mesylate, glucoheptonate, or lactobionate. Compounds containing a cationic group covalently bonded to an anionic group may be referred to as an "internal salt."

The term "nitro" means -NO<sub>2</sub>; the term "halogen" or "halogeno" or "halo" designates -F, -Cl, -Br or -I; the term "thiol," "thio," or "mercapto" means SH; and the term "hydroxyl" or "hydroxy" means -OH.

The term "acyl" refers to a carbonyl group that is attached through its carbon atom to a hydrogen (*i.e.*, a formyl), an aliphatic group (*e.g.*, acetyl), an aromatic group (*e.g.*, benzoyl), and the like. The term "substituted acyl" includes acyl groups where one or more of the hydrogen atoms on one or more carbon atoms are replaced by, for example, an alkyl group, alkynyl group, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylaryl amino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety.

Unless otherwise specified, the chemical moieties of the compounds of the invention, including those groups discussed above, may be "substituted or unsubstituted." In some embodiments, the term "substituted" means that the moiety has substituents placed on the moiety other than hydrogen (*i.e.*, in most cases, replacing a hydrogen), which allow the molecule to perform its intended function. Examples of substituents include moieties selected from straight or branched alkyl (preferably C<sub>1</sub>-C<sub>5</sub>), cycloalkyl (preferably C<sub>3</sub>-C<sub>8</sub>), alkoxy (preferably C<sub>1</sub>-C<sub>6</sub>), thioalkyl (preferably C<sub>1</sub>-C<sub>6</sub>), alkenyl (preferably C<sub>2</sub>-C<sub>6</sub>), alkynyl (preferably C<sub>2</sub>-C<sub>6</sub>), heterocyclic, carbocyclic, aryl (*e.g.*, phenyl), aryloxy (*e.g.*, phenoxy), aralkyl (*e.g.*, benzyl), aryloxyalkyl (*e.g.*, phenoxyalkyl), arylacetamidoyl, alkylaryl, heteroaralkyl, alkylcarbonyl and arylcarbonyl or other such acyl group, heteroarylcarbonyl, and heteroaryl groups, as well

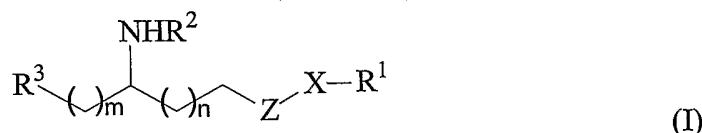
as  $(\text{CR}'\text{R}'')_{0-3}\text{NR}'\text{R}''$  (e.g.,  $-\text{NH}_2$ ),  $(\text{CR}'\text{R}'')_{0-3}\text{CN}$  (e.g.,  $-\text{CN}$ ),  $-\text{NO}_2$ , halogen (e.g.,  $-\text{F}$ ,  $-\text{Cl}$ ,  $-\text{Br}$ , or  $-\text{I}$ ),  $(\text{CR}'\text{R}'')_{0-3}\text{C}(\text{halogen})_3$  (e.g.,  $-\text{CF}_3$ ),  $(\text{CR}'\text{R}'')_{0-3}\text{CH}(\text{halogen})_2$ ,  $(\text{CR}'\text{R}'')_{0-3}\text{CH}_2(\text{halogen})$ ,  $(\text{CR}'\text{R}'')_{0-3}\text{CONR}'\text{R}''$ ,  $(\text{CR}'\text{R}'')_{0-3}(\text{CNH})\text{NR}'\text{R}''$ ,  $(\text{CR}'\text{R}'')_{0-3}\text{S}(\text{O})_{1-2}\text{NR}'\text{R}''$ ,  $(\text{CR}'\text{R}'')_{0-3}\text{CHO}$ ,  $(\text{CR}'\text{R}'')_{0-3}\text{O}(\text{CR}'\text{R}'')_{0-3}\text{H}$ ,  
 5  $(\text{CR}'\text{R}'')_{0-3}\text{S}(\text{O})_{0-3}\text{R}'$  (e.g.,  $-\text{SO}_3\text{H}$ ),  $(\text{CR}'\text{R}'')_{0-3}\text{O}(\text{CR}'\text{R}'')_{0-3}\text{H}$  (e.g.,  $-\text{CH}_2\text{OCH}_3$  and  $-\text{OCH}_3$ ),  $(\text{CR}'\text{R}'')_{0-3}\text{S}(\text{CR}'\text{R}'')_{0-3}\text{H}$  (e.g.,  $-\text{SH}$  and  $-\text{SCH}_3$ ),  $(\text{CR}'\text{R}'')_{0-3}\text{OH}$  (e.g.,  $-\text{OH}$ ),  $(\text{CR}'\text{R}'')_{0-3}\text{COR}'$ ,  $(\text{CR}'\text{R}'')_{0-3}$ (substituted or unsubstituted phenyl),  $(\text{CR}'\text{R}'')_{0-3}(\text{C}_3\text{-C}_8$  cycloalkyl),  $(\text{CR}'\text{R}'')_{0-3}\text{CO}_2\text{R}'$  (e.g.,  $-\text{CO}_2\text{H}$ ), and  $(\text{CR}'\text{R}'')_{0-3}\text{OR}'$  groups, wherein  $\text{R}'$  and  $\text{R}''$  are each independently hydrogen, a  $\text{C}_1\text{-C}_5$  alkyl,  $\text{C}_2\text{-C}_5$  alkenyl,  $\text{C}_2\text{-C}_5$  alkynyl, or aryl  
 10 group; or the side chain of any naturally occurring amino acid.

In another embodiment, a substituent may be selected from straight or branched alkyl (preferably  $\text{C}_1\text{-C}_5$ ), cycloalkyl (preferably  $\text{C}_3\text{-C}_8$ ), alkoxy (preferably  $\text{C}_1\text{-C}_6$ ), thioalkyl (preferably  $\text{C}_1\text{-C}_6$ ), alkenyl (preferably  $\text{C}_2\text{-C}_6$ ), alkynyl (preferably  $\text{C}_2\text{-C}_6$ ), heterocyclic, carbocyclic, aryl (e.g., phenyl), aryloxy (e.g., phenoxy), aralkyl (e.g.,  
 15 benzyl), aryloxyalkyl (e.g., phenyloxyalkyl), arylacetamidoyl, alkylaryl, heteroaralkyl, alkylcarbonyl and arylcarbonyl or other such acyl group, heteroarylcarbonyl, or heteroaryl group,  $(\text{CR}'\text{R}'')_{0-10}\text{NR}'\text{R}''$  (e.g.,  $-\text{NH}_2$ ),  $(\text{CR}'\text{R}'')_{0-10}\text{CN}$  (e.g.,  $-\text{CN}$ ),  $\text{NO}_2$ , halogen (e.g.,  $\text{F}$ ,  $\text{Cl}$ ,  $\text{Br}$ , or  $\text{I}$ ),  $(\text{CR}'\text{R}'')_{0-10}\text{C}(\text{halogen})_3$  (e.g.,  $-\text{CF}_3$ ),  $(\text{CR}'\text{R}'')_{0-10}\text{CH}(\text{halogen})_2$ ,  $(\text{CR}'\text{R}'')_{0-10}\text{CH}_2(\text{halogen})$ ,  $(\text{CR}'\text{R}'')_{0-10}\text{CONR}'\text{R}''$ ,  
 20  $(\text{CR}'\text{R}'')_{0-10}(\text{CNH})\text{NR}'\text{R}''$ ,  $(\text{CR}'\text{R}'')_{0-10}\text{S}(\text{O})_{1-2}\text{NR}'\text{R}''$ ,  $(\text{CR}'\text{R}'')_{0-10}\text{CHO}$ ,  $(\text{CR}'\text{R}'')_{0-10}\text{O}(\text{CR}'\text{R}'')_{0-10}\text{H}$ ,  $(\text{CR}'\text{R}'')_{0-10}\text{S}(\text{O})_{0-3}\text{R}'$  (e.g.,  $-\text{SO}_3\text{H}$ ),  $(\text{CR}'\text{R}'')_{0-10}\text{O}(\text{CR}'\text{R}'')_{0-10}\text{H}$  (e.g.,  $-\text{CH}_2\text{OCH}_3$  and  $-\text{OCH}_3$ ),  $(\text{CR}'\text{R}'')_{0-10}\text{S}(\text{CR}'\text{R}'')_{0-3}\text{H}$  (e.g.,  $-\text{SH}$  and  $-\text{SCH}_3$ ),  $(\text{CR}'\text{R}'')_{0-10}\text{OH}$  (e.g.,  $-\text{OH}$ ),  $(\text{CR}'\text{R}'')_{0-10}\text{COR}'$ ,  $(\text{CR}'\text{R}'')_{0-10}$ (substituted or unsubstituted phenyl),  $(\text{CR}'\text{R}'')_{0-10}(\text{C}_3\text{-C}_8$  cycloalkyl),  
 25  $(\text{CR}'\text{R}'')_{0-10}\text{CO}_2\text{R}'$  (e.g.,  $-\text{CO}_2\text{H}$ ), or  $(\text{CR}'\text{R}'')_{0-10}\text{OR}'$  group, or the side chain of any naturally occurring amino acid; wherein  $\text{R}'$  and  $\text{R}''$  are each independently hydrogen, a  $\text{C}_1\text{-C}_5$  alkyl,  $\text{C}_2\text{-C}_5$  alkenyl,  $\text{C}_2\text{-C}_5$  alkynyl, or aryl group, or  $\text{R}'$  and  $\text{R}''$  taken together are a benzylidene group or a  $-(\text{CH}_2)_2\text{O}(\text{CH}_2)_2-$  group.

It will be understood that "substitution" or "substituted with" includes the  
 30 implicit proviso that such substitution is in accordance with the permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term "substituted" is meant to include all permissible substituents of organic compounds. In a broad aspect,  
 35 the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. The permissible substituents can be one or more.

In some embodiments, a "substituent" may be selected from the group consisting of, for example, halogeno, trifluoromethyl, nitro, cyano, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> alkylcarbonyloxy, arylcarbonyloxy, C<sub>1</sub>-C<sub>6</sub> alkoxy carbonyloxy, aryloxy carbonyloxy, C<sub>1</sub>-C<sub>6</sub> alkylcarbonyl, C<sub>1</sub>-C<sub>6</sub> alkoxy carbonyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> alkylthio, arylthio, heterocyclyl, aralkyl, and aryl (including heteroaryl) groups.

Compounds of the invention include, for example, compounds of Formula (I):



wherein:

10 X is oxygen or nitrogen;

Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;

m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

R<sup>1</sup> and R<sup>7</sup> are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or -(CH<sub>2</sub>)<sub>p</sub>-Y;

Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

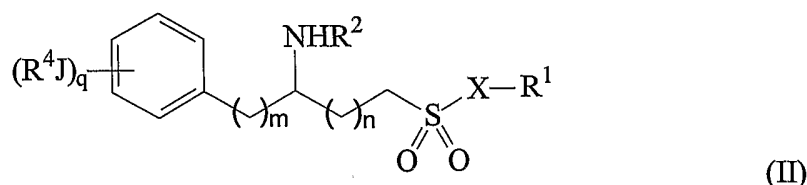
p is 0, 1, 2, 3, or 4;

20 R<sup>2</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxy carbonyl;

R<sup>3</sup> is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl, and pharmaceutically acceptable salts, esters, and prodrugs thereof.

In a further embodiment, m is 0, 1, or 2. In another further embodiment, n is 0, 1, or 2. In another further embodiment, R<sup>3</sup> is aryl, e.g., heteroaryl or phenyl. In yet another embodiment, Z is S(O)<sub>2</sub>.

In another embodiment, the compound of the invention is of the Formula (II)



wherein:

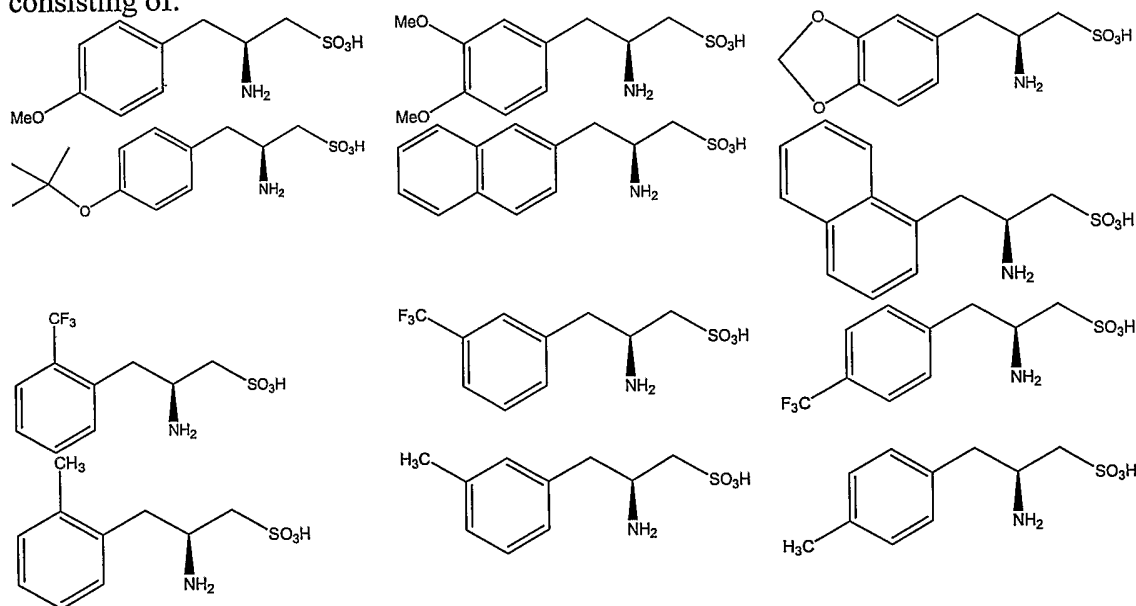
each  $R^4$  is independently selected from the group consisting of hydrogen, halogen, hydroxyl, thiol, amino, cyano, nitro, alkyl, aryl, carbocyclic or heterocyclic;

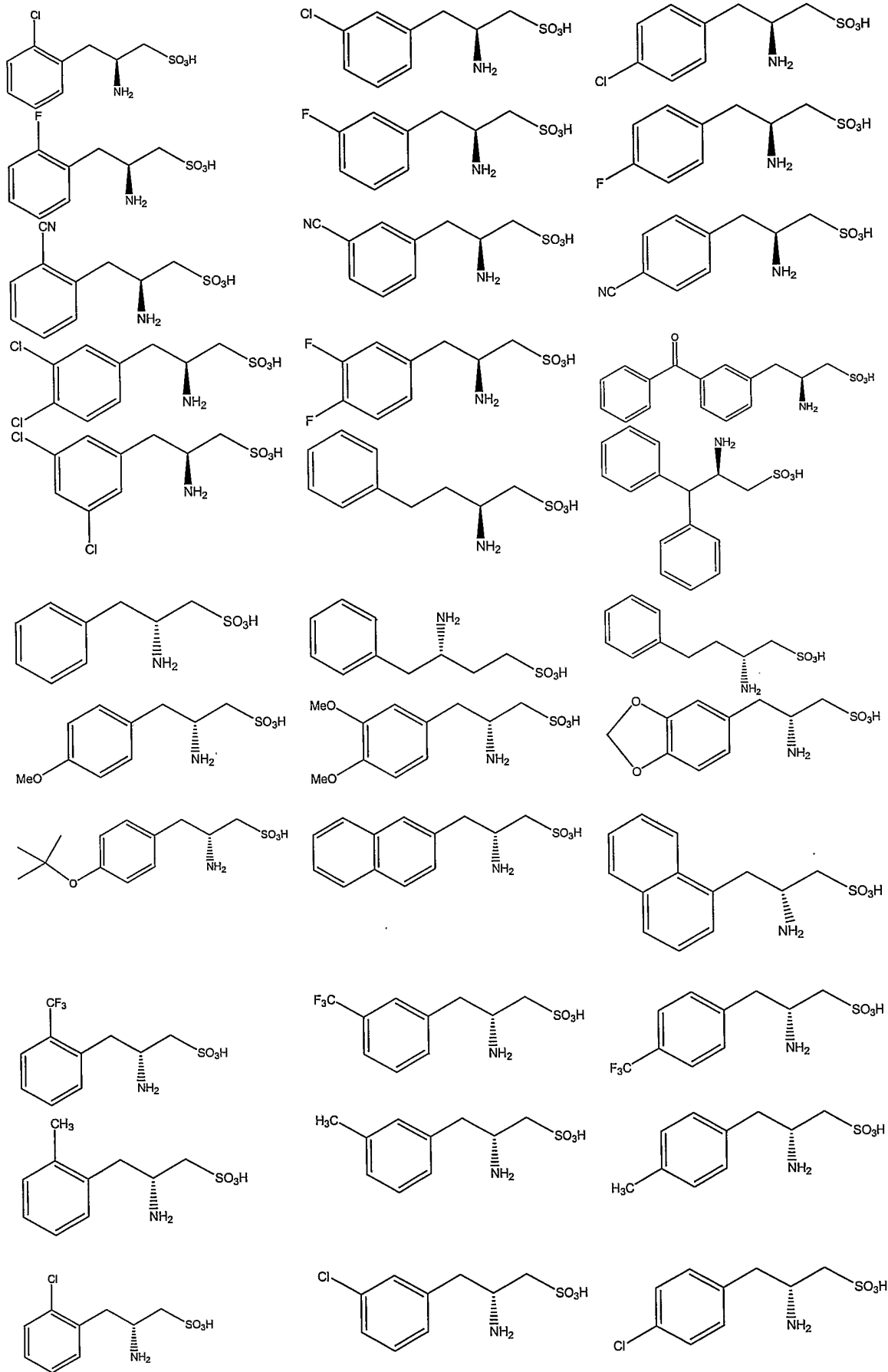
J is absent, oxygen, nitrogen, sulfur, or a divalent link-moiety comprising, without being limiting to, lower alkylene, alkyleneoxy, alkyleneamino, alkyleneylthio, alkyleneoxyalkyl, alkyleneylaminoalkyl, alkyleneylthioalkyl, alkenyl, alkenyloxy, alkenylamino, or alkenylthio; and

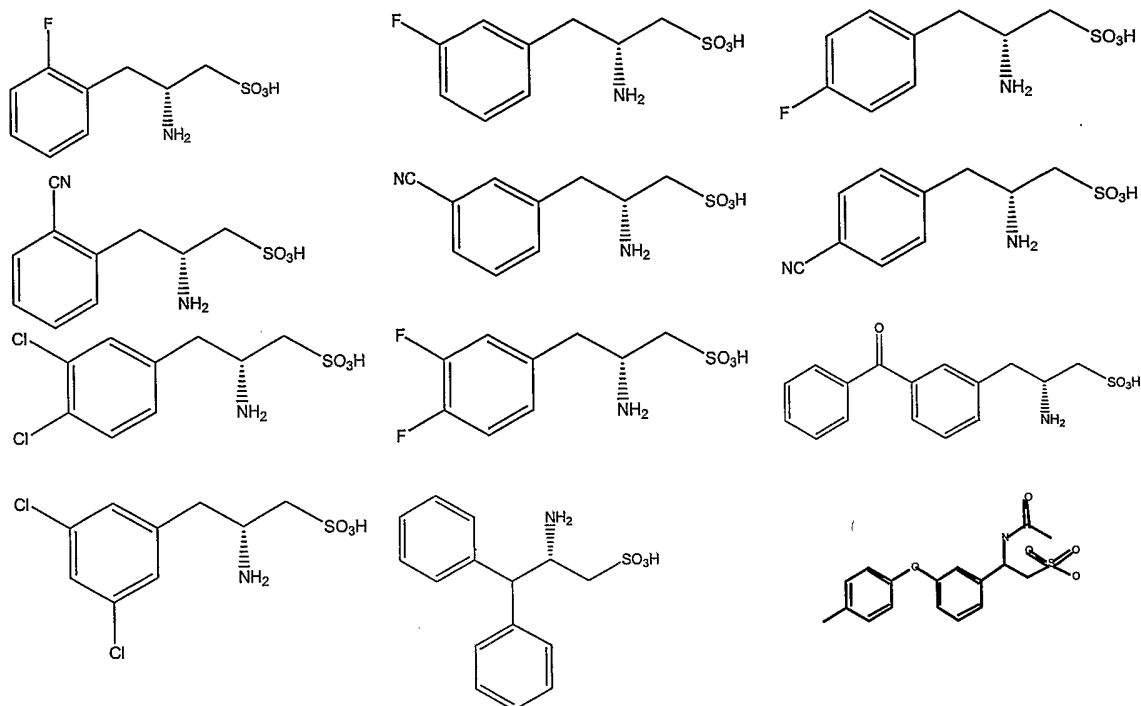
q is 1, 2, 3, 4, or 5, and pharmaceutically acceptable salts, esters and prodrugs thereof, provided that when J is a covalent bond, n is 1, m is 0 or 1, X is oxygen,  $R^4$  is hydrogen,  $R^2$  is not hydrogen or acyl; and provided that when q is 1, m is 0, n is 1, J is a covalent bond,  $R^4$  is methyl, ethyl, methoxy, ethoxy, chloro, bromo, hydroxy, or iodo in *para*-position, X is oxygen,  $R^2$  is not hydrogen; provided that when q is 2, m is 0, n is 1, J is a covalent bond,  $R^4$  is methyl in *ortho* and *para*-positions, X is oxygen,  $R^2$  is not hydrogen; provided that when q is 2, m is 0, n is 1, J is a covalent bond,  $R^4$  is chloro in 2 and 6 positions, X is oxygen,  $R^2$  is not acyl..

In a further embodiment,  $R^4$  is aryl, e.g., substituted or unsubstituted phenyl. In another embodiment,  $R^4$  is halogen (e.g., chlorine, fluorine, bromine, or iodine). In yet another embodiment,  $R^4$  is alkyl, e.g., methyl, ethyl, propyl, butyl, pentyl, trifluoromethyl, etc. In another embodiment, J is absent or oxygen. In a further embodiment, m is 1 or n is 1. In another further embodiment, the compound can be R- or S- isomer.

In a further embodiment, the compound is selected from the group consisting of:

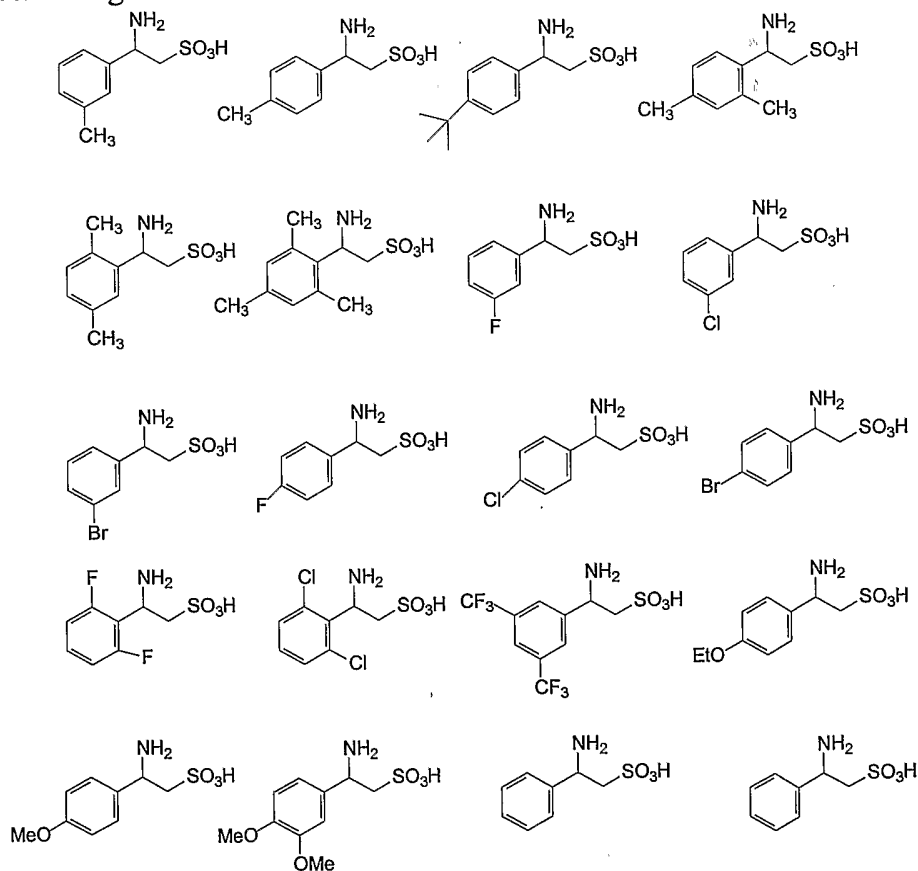


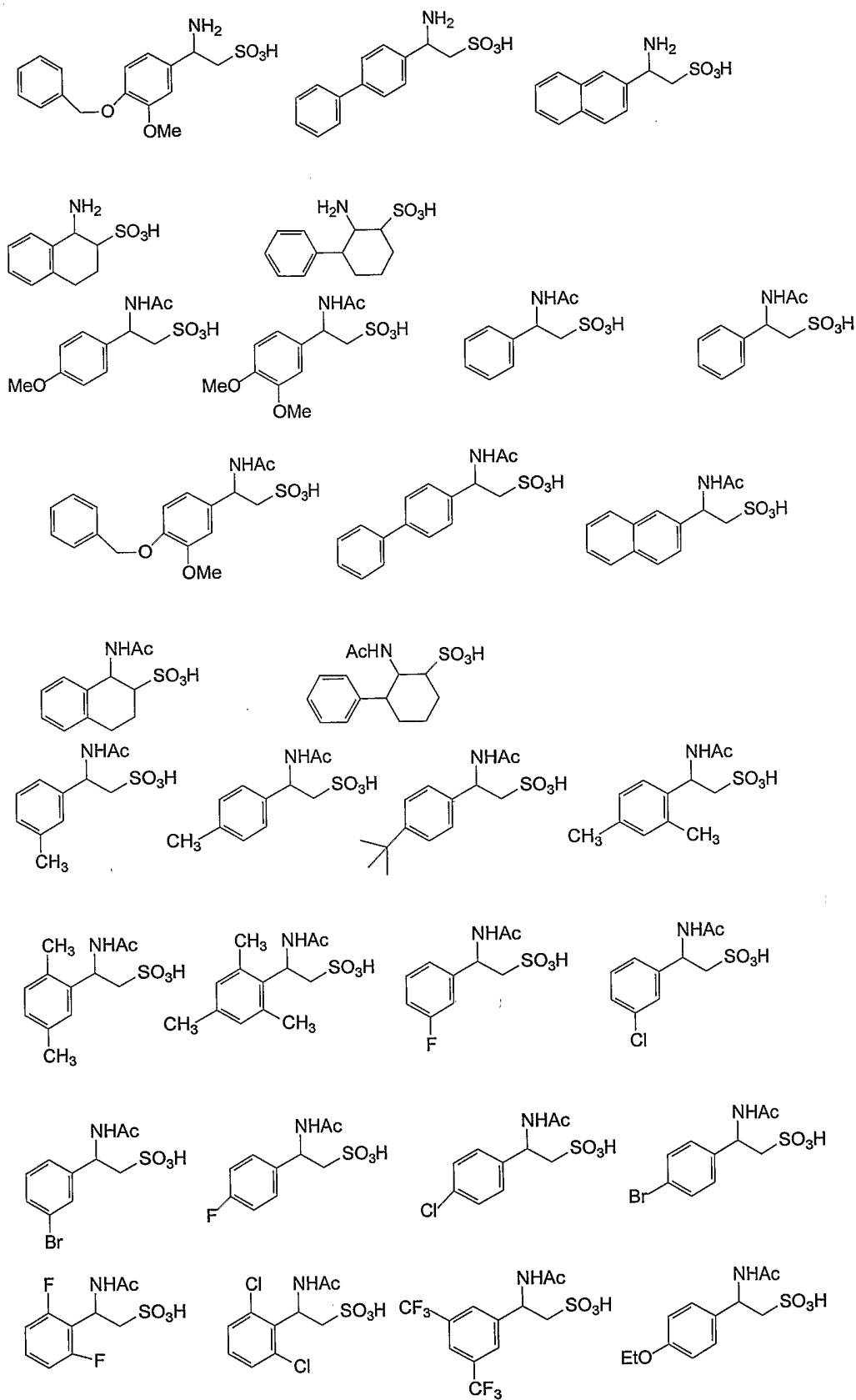




and pharmaceutically acceptable salts, prodrugs, and esters thereof.

In a further embodiment, the compound is selected from the group consisting of:

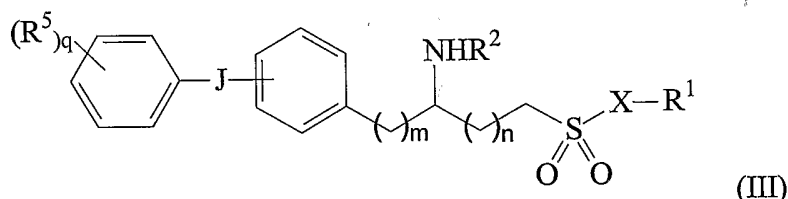




and pharmaceutically acceptable salts, prodrugs, and esters thereof.

In a further embodiment, the compound of the invention is not a compound described in Xu, *Tet. Assym.* (2002) 13(11), 1129-1134, Higashuira *et al. J. Chem. Soc. Perkins Trans.* 1989, (8), 1479-81; Gennari *et al. Chemistry, J. Europ.* (1998), 4(10), 1924-1931; Gennari *et al. J. Org. Chem.*, (1998), 63(16), 5312-5313; de Bont *et al. Biorg. & Med. Chem. Lett.* (1996), 6(24), 3035-3040; Gude, *Tet. Lett.*, (1996), 37(47), 8589-8592; Higashiura, *J. Org. Chem.* (1992), 57(2), 764-6; Higashuira *et al.* JP 02115147 A2; Enders *Synlett*, (2002) (2), 304-306; Abbenante, *et al. Aus. J. Chem.* (1997) 50(6), 523-527; Cordero *et al. Eur. J. Org. Chem.* (2002) (8), 1407-1411; Kerr *et al. Mol. Neuropharm.* (1992), 2(2), 159-61; Ong, J. *et al., J. Pharmacol.* (1991) 205(3), 319-22; Aboskalova, *et al. Z. Org. Khimii* (1991) 27(6), 1357-8; Meyle *et al. Liebigs Annalen der Chemie*, (1985) (4) 802-12; Potapov, *et al. Z. Obshchei Khimii* (1959), 29 953-4; Latif *et al. J. Ind. Chem. Soc.* (1959), 36, 212-14; Terent'ev *et al. Z. Obshchei Khimii* (1959), 29, 949-52; Terent'ev *Z. Obshchei Khimii* (1956), 26 2934-7; Palazzo *Gazz. Chim. Ital.* (1949) 79 13-24; Lambert *et al. J. Chem. Soc. Abs.* (1949) 46-9; and Sheehan, *J. Org. Chem.*, (1975) 40(8), 1179.

In a further embodiment, the compound of the invention is of the Formula (III):



wherein:

X is oxygen or nitrogen;

m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

q is 1, 2, 3, 4, or 5;

R<sup>1</sup> is hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, or a moiety together with X to form a natural or unnatural amino acid residue, or  $-(CH_2)_p-Y$ ;

Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

p is 0, 1, 2, 3, or 4;

R<sup>2</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxy carbonyl;

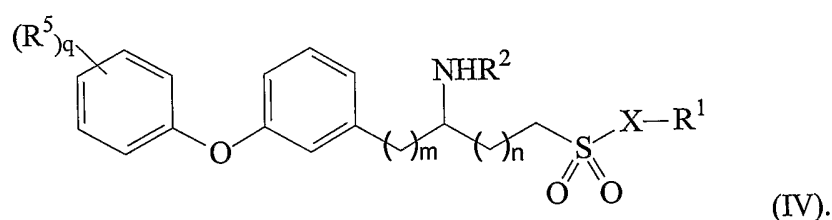
$R^5$  is selected from the group consisting of hydrogen, halogen, amino, nitro, hydroxy, carbonyl, thiol, carboxy, alkyl, alkoxy, alkoxycarbonyl, acyl, alkylamino, and acylamino;

$q$  is an integer selected from 1 to 5;

- 5 J is absent, oxygen, nitrogen, sulfur, or a divalent link-moiety comprising, with out being limited to, lower alkylene, alkyleneoxy, alkyleneamino, alkyleneylthio, alkyleneoxyalkyl, alkyleneylaminoalkyl, alkyleneylthioalkyl, alkenyl, alkenyloxy, alkenylamino, or alkenylthio; and

pharmaceutically acceptable salts, esters, and prodrugs thereof.

- 10 In yet another embodiment, the compound of the invention is:

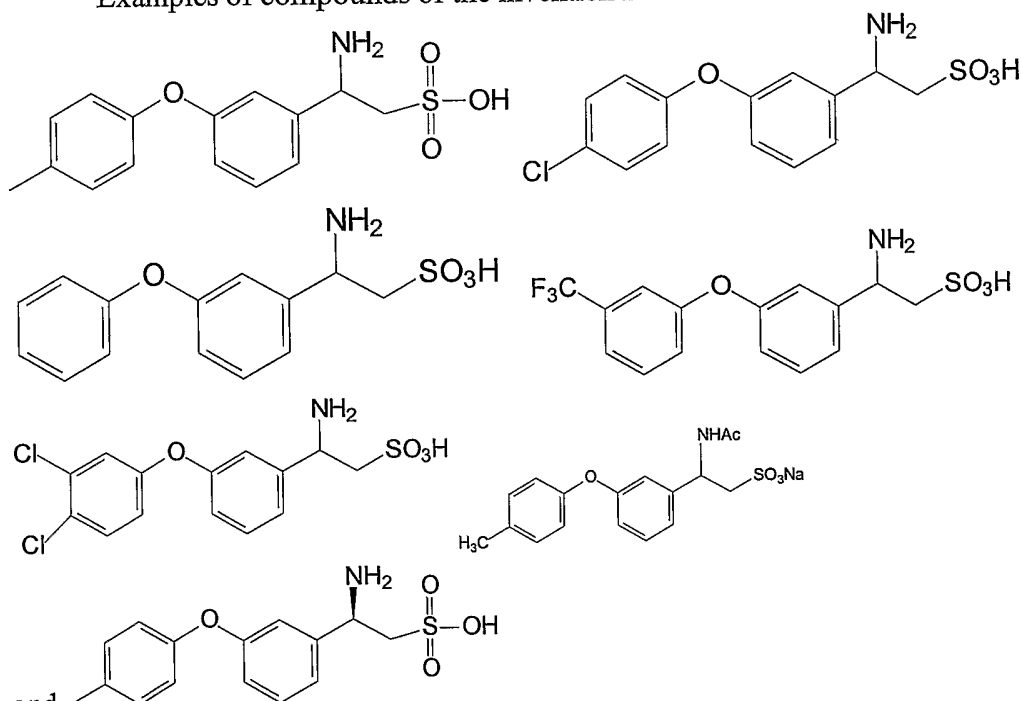


and pharmaceutically acceptable salts, esters and prodrugs thereof.

In a further embodiment,  $m$  is 0.

15

Examples of compounds of the invention include:

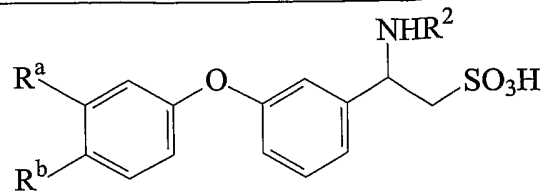


20

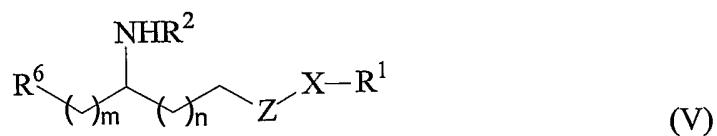
and pharmaceutically acceptable salts, esters and prodrugs thereof.

Further examples of compounds of the invention include compounds of Table 2:

Table 2

|  |               |                |                 |                 |
|------------------------------------------------------------------------------------|---------------|----------------|-----------------|-----------------|
| No. in Series                                                                      | Configuration | R <sup>2</sup> | R <sup>a</sup>  | R <sup>b</sup>  |
| 1.                                                                                 | D,L-          | H              | H               | H               |
| 2.                                                                                 | D,L-          | H              | H               | Cl              |
| 3.                                                                                 | D,L-          | H              | Cl              | Cl              |
| 4.                                                                                 | D,L-          | H              | H               | CH <sub>3</sub> |
| 5.                                                                                 | D,L-          | H              | CF <sub>3</sub> | H               |
| 6.                                                                                 | D-            | H              | H               | H               |
| 7.                                                                                 | D-            | H              | H               | Cl              |
| 8.                                                                                 | D-            | H              | Cl              | Cl              |
| 9.                                                                                 | D-            | H              | H               | CH <sub>3</sub> |
| 10.                                                                                | D-            | H              | CF <sub>3</sub> | H               |
| 11.                                                                                | L-            | H              | H               | H               |
| 12.                                                                                | L-            | H              | H               | Cl              |
| 13.                                                                                | L-            | H              | Cl              | Cl              |
| 14.                                                                                | L-            | H              | H               | CH <sub>3</sub> |
| 15.                                                                                | L-            | H              | CF <sub>3</sub> | H               |
| 16.                                                                                | D,L-          | Ac             | H               | H               |
| 17.                                                                                | D,L-          | Ac             | H               | Cl              |
| 18.                                                                                | D,L-          | Ac             | Cl              | Cl              |
| 19.                                                                                | D,L-          | Ac             | H               | CH <sub>3</sub> |
| 20.                                                                                | D,L-          | Ac             | CF <sub>3</sub> | H               |
| 21.                                                                                | D-            | Ac             | H               | H               |
| 22.                                                                                | D-            | Ac             | H               | Cl              |
| 23.                                                                                | D-            | Ac             | Cl              | Cl              |
| 24.                                                                                | D-            | Ac             | H               | CH <sub>3</sub> |
| 25.                                                                                | D-            | Ac             | CF <sub>3</sub> | H               |
| 26.                                                                                | L-            | Ac             | H               | H               |
| 27.                                                                                | L-            | Ac             | H               | Cl              |
| 28.                                                                                | L-            | Ac             | Cl              | Cl              |
| 29.                                                                                | L-            | Ac             | H               | CH <sub>3</sub> |
| 30.                                                                                | L-            | Ac             | CF <sub>3</sub> | H               |

In another embodiment, the invention pertains to compounds of Formula (V):



wherein:

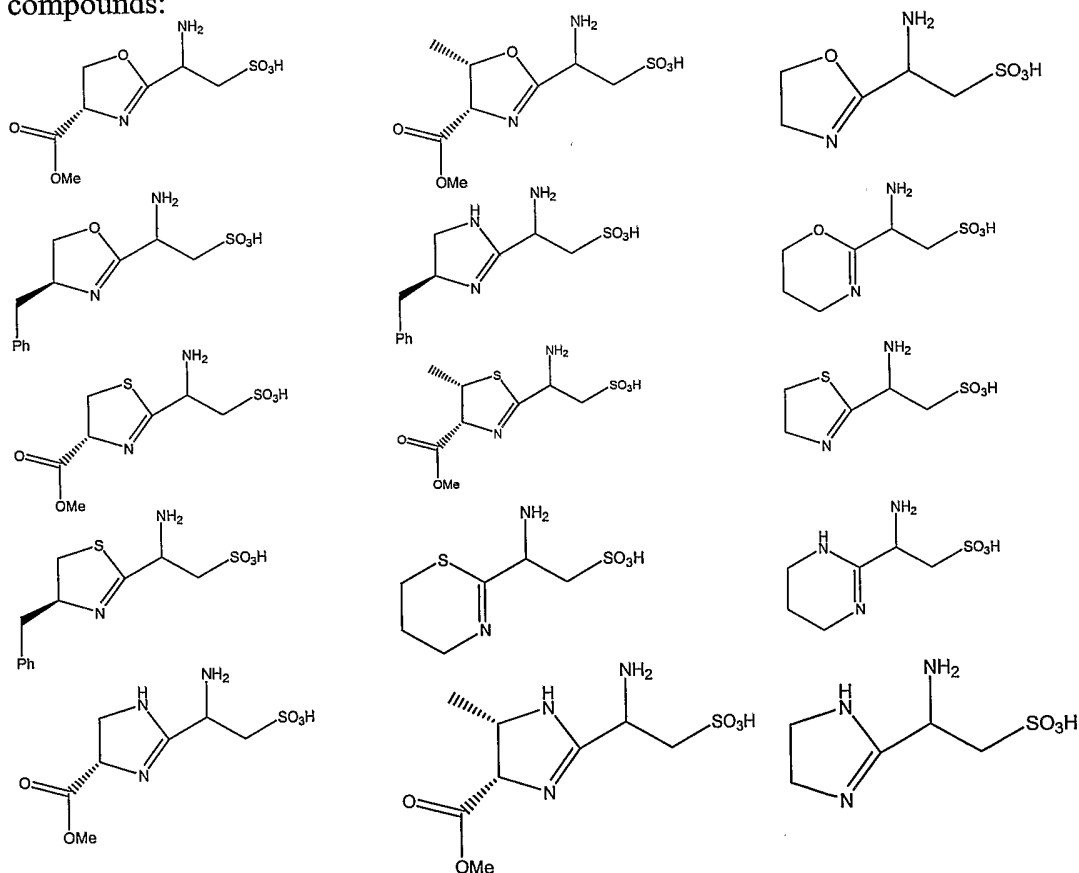
$\text{R}^6$  is a substituted or unsubstituted heterocyclic moiety, and pharmaceutically acceptable salts, esters, and prodrugs thereof.

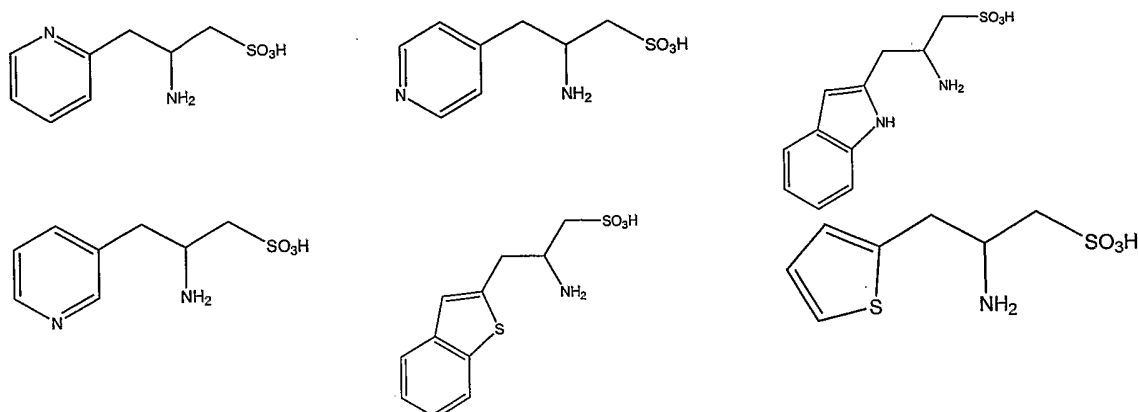
In a further embodiment,  $m$  is 0 or 1. In another embodiment,  $n$  is 0 or

1. In another further embodiment,  $\text{R}^6$  is thiazolyl, oxazolyl, pyrazolyl, indolyl, pyridinyl, thiazinyl, thiophenyl, benzothiophenyl, dihydroimidazolyl, dihydrothiazolyl, oxazolidinyl, thiazolidinyl, tetrahydropyrimidinyl, or oxazinyl. In yet another embodiment,  $\text{Z}$  is  $\text{S}(\text{O})_2$ .

In a further embodiment, the invention pertains to the following

compounds:





and pharmaceutically acceptable salts, esters and prodrugs thereof.

In one embodiment, the invention does not pertain to the compounds described in WO 00/64420 and W0 96/28187. In this embodiment, the invention does not pertain to methods of using the compounds described in WO 00/64420 and W0 96/28187 for the treatment of diseases or disorders described therein. In a further embodiment, the invention pertains to methods of using the compounds described in WO 00/64420 and W0 96/28187 for methods described in this application, which are not described in WO 00/64420 and W0 96/28187. Both of WO 00/64420 and W0 96/28187 are incorporated by reference herein in their entirety.

#### Subjects and Patient Populations

The term "subject" includes living organisms in which epilepsy, epileptogenesis, ictogenesis, convulsions, or amyloidosis can occur, or which are susceptible to epilepsy, epileptogenesis, ictogenesis, or amyloid-related diseases, *e.g.*, Alzheimer's disease, Down's syndrome, CAA, dialysis-related ( $\beta_2$ M) amyloidosis, secondary (AA) amyloidosis, primary (AL) amyloidosis, hereditary amyloidosis, diabetes, etc. Examples of subjects include humans, chickens, ducks, peking ducks, geese, monkeys, cows, rabbits, sheep, goats, dogs, cats, mice, rats, and transgenic species thereof.

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 modulate amyloid aggregation or amyloid-induced toxicity in the subject as further described herein. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the amount of amyloid already deposited at the clinical site in the subject, the age, sex, and weight of the subject, and the ability of the therapeutic compound to modulate amyloid aggregation in the subject. Dosage regimens can be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

In an exemplary aspect of the invention, the subject is a human. For example, the subject may be a human over 30 years old, human over 40 years old, a human over 50 years old, a human over 60 years old, a human over 70 years old, a human over 80 years old, a human over 85 years old, a human over 90 years old, or a human over 95 years old. The subject may be a female human, including a postmenopausal female human, who may be on hormone (estrogen) replacement therapy. The subject may also be a male human. In another embodiment, the subject is under 40 years old.

A subject may be a human at risk for Alzheimer's disease, *e.g.*, being over the age of 40 or having a predisposition for Alzheimer's disease. Alzheimer's disease predisposing factors identified or proposed in the scientific literature include, among others, a genotype predisposing a subject to Alzheimer's disease; environmental factors predisposing a subject to Alzheimer's disease; past history of infection by viral and bacterial agents predisposing a subject to Alzheimer's disease; and vascular factors predisposing a subject to Alzheimer's disease. A subject may also have one or more risk factors for cardiovascular disease (*e.g.*, atherosclerosis of the coronary arteries, angina pectoris, and myocardial infarction) or cerebrovascular disease (*e.g.*, atherosclerosis of the intracranial or extracranial arteries, stroke, syncope, and transient ischemic attacks), such as hypercholesterolemia, hypertension, diabetes, cigarette smoking, familial or previous history of coronary artery disease, cerebrovascular disease, and cardiovascular disease. Hypercholesterolemia typically is defined as a serum total cholesterol concentration of greater than about 5.2 mmol/L (about 200 mg/dL).

Several genotypes are believed to predispose a subject to Alzheimer's disease. These include the genotypes such as presenilin-1, presenilin-2, and amyloid precursor protein (APP) missense mutations associated with familial Alzheimer's disease, and  $\alpha$ -2-macroglobulin and LRP-1 genotypes, which are thought to increase the risk of acquiring sporadic (late-onset) Alzheimer's disease. E. van Uden, *et al.*, *J. Neurosci.* 22(21), 9298-304 (2002); J.J. Goto, *et al.*, *J. Mol. Neurosci.* 19(1-2), 37-41 (2002). Another genetic risk factor for the development of Alzheimer's disease are variants of ApoE, the gene that encodes apolipoprotein E (particularly the apoE4 genotype), a constituent of the low-density lipoprotein particle. WJ Strittmatter, *et al.*, *Annu. Rev. Neurosci.* 19, 53-77 (1996). The molecular mechanisms by which the various ApoE alleles alter the likelihood of developing Alzheimer's disease are unknown, however the role of ApoE in cholesterol metabolism is consistent with the growing body of evidence linking cholesterol metabolism to Alzheimer's disease. For example, chronic use of cholesterol-lowering drugs such as statins has recently been associated with a lower incidence of Alzheimer's disease, and cholesterol-lowering drugs have been shown to reduce pathology in APP transgenic mice. These and other studies suggest that cholesterol may affect APP processing. ApoE4 has been suggested to alter A $\beta$

trafficking (in and out of the brain), and favor retention of A $\beta$  in the brain. ApoE4 has also been suggested to favor APP processing toward A $\beta$  formation. Environmental factors have been proposed as predisposing a subject to Alzheimer's disease, including exposure to aluminum, although the epidemiological evidence is ambiguous. In addition, prior infection by certain viral or bacterial agents may predispose a subject to Alzheimer's disease, including the herpes simplex virus and chlamydia pneumoniae. Finally, other predisposing factors for Alzheimer's disease can include risk factors for cardiovascular or cerebrovascular disease, including cigarette smoking, hypertension and diabetes. "At risk for Alzheimer's disease" also encompasses any other predisposing factors not listed above or as yet identified and includes an increased risk for Alzheimer's disease caused by head injury, medications, diet, or lifestyle.

The methods of the present invention can be used for one or more of the following: to prevent Alzheimer's disease, to treat Alzheimer's disease, or ameliorate symptoms of Alzheimer's disease, or to regulate production of or levels of amyloid  $\beta$  (A $\beta$ ) peptides. In an embodiment, the human carries one or more mutations in the genes that encode  $\beta$ -amyloid precursor protein, presenilin-1 or presenilin-2. In another embodiment, the human carries the Apolipoprotein  $\epsilon$ 4 gene. In another embodiment, the human has a family history of Alzheimer's Disease or a dementia illness. In another embodiment, the human has trisomy 21 (Down's Syndrome). In another embodiment, the subject has a normal or low serum total blood cholesterol level. In another embodiment, the serum total blood cholesterol level is less than about 200 mg/dL, or less than about 180, and it can range from about 150 to about 200 mg/dL. In another embodiment, the total LDL cholesterol level is less than about 100 mg/dL, or less than about 90 mg/dL and can range from about 30 to about 100 mg/dL. Methods of measuring serum total blood cholesterol and total LDL cholesterol are well known to those skilled in the art and for example include those disclosed in WO 99/38498 at p.11, incorporated by reference herein. Methods of determining levels of other sterols in serum are disclosed in H. Gylling, *et al.*, "Serum Sterols During Stanol Ester Feeding in a Mildly Hypercholesterolemic Population", *J. Lipid Res.* 40: 593-600 (1999).

In another embodiment, the subject has an elevated serum total blood cholesterol level. In another embodiment, the serum total cholesterol level is at least about 200 mg/dL, or at least about 220 mg/dL and can range from about 200 to about 1000 mg/dL. In another embodiment, the subject has an elevated total LDL cholesterol level. In another embodiment, the total LDL cholesterol level is greater than about 100 mg/dL, or even greater than about 110 mg/dL and can range from about 100 to about 1000 mg/dL.

In another embodiment, the human is at least about 40 years of age. In another embodiment, the human is at least about 60 years of age. In another embodiment, the human is at least about 70 years of age. In another embodiment, the human is at least

about 80 years of age. In another embodiment, the human is at least about 85 years of age. In one embodiment, the human is between about 60 and about 100 years of age.

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  
5 deposition, or brain atrophy.

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  
10 may exhibit a below average score on a cognitive test, as compared to a historical control of similar age and educational background. The subject may also exhibit a reduction in 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  
15 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  
20 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  
25 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  
30 deterioration, as seen in Alzheimer's disease and multi-infert 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,  
35 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.

5           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  
10   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;  
15   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  
20   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.

25           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.

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

35           By using the methods of the present invention, the levels of amyloid  $\beta$  peptides in a subject's plasma or cerebrospinal fluid (CSF) can be reduced from levels prior to treatment from about 10 to about 100 percent, or even about 50 to about 100 percent.

          In an alternative embodiment, the subject can have an elevated level of amyloid  $A\beta_{40}$  and  $A\beta_{42}$  peptide in the blood and CSF prior to treatment, according to the present

methods, of greater than about 10 pg/mL, or greater than about 20 pg/mL, or greater than about 35 pg/mL, or even greater than about 40 pg/mL. In another embodiment, the elevated level of amyloid A $\beta$ <sub>42</sub> peptide can range from about 30 pg/mL to about 200 pg/mL, or even to about 500 pg/mL. One skilled in the art would understand that as  
5 Alzheimer's disease progresses, the measurable levels of amyloid  $\beta$  peptide in the CSF may decrease from elevated levels present before onset of the disease. This effect is attributed to increased deposition, *i.e.*, trapping of A $\beta$  peptide in the brain instead of normal clearance from the brain into the CSF.

In an alternative embodiment, the subject can have an elevated level of amyloid  
10 A $\beta$ <sub>40</sub> peptide in the blood and CSF prior to treatment, according to the present methods, of greater than about 5 pg A $\beta$ <sub>42</sub>/mL or greater than about 50 pg A $\beta$ <sub>40</sub>/mL, or greater than about 400 pg/mL. In another embodiment, the elevated level of amyloid A $\beta$ <sub>40</sub> peptide can range from about 200 pg/mL to about 800 pg/mL, to even about 1000 pg/mL.

In another embodiment, the subject can have an elevated level of amyloid A $\beta$ <sub>42</sub>  
15 peptide in the CSF prior to treatment, according to the present methods, of greater than about 5 pg/mL, or greater than about 10 pg/mL, or greater than about 200 pg/mL, or greater than about 500 pg/mL. In another embodiment, the level of amyloid  $\beta$  peptide can range from about 10 pg/mL to about 1,000 pg/mL, or even about 100 pg/mL to about 1,000 pg/mL.

In another embodiment, the subject can have an elevated level of amyloid A $\beta$ <sub>40</sub>  
20 peptide in the CSF prior to treatment according to the present methods of greater than about 10 pg/mL, or greater than about 50 pg/mL, or even greater than about 100 pg/mL. In another embodiment, the level of amyloid  $\beta$  peptide can range from about 10 pg/mL to about 1,000 pg/mL.

The amount of amyloid  $\beta$  peptide in the brain, CSF, blood, or plasma of a subject  
25 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  
30 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  $\beta$  peptides is by Europium immunoassay (EIA). *See, e.g.*, WO 99/38498 at p.11.

The methods of the invention may be applied as a therapy for a subject having Alzheimer's disease or a dementia, or the methods of the invention may be applied as a prophylaxis against Alzheimer's disease or dementia for subject with such a

predisposition, as in a subject, *e.g.*, with a genomic mutation in the APP gene, the ApoE gene, or a presenilin gene. The subject may have (or may be predisposed to developing or may be suspected of having) vascular dementia, or senile dementia, Mild Cognitive Impairment, or early Alzheimer's disease. In addition to Alzheimer's disease, the  
5 subject may have another amyloid-related disease such as cerebral amyloid angiopathy, or the subject may have amyloid deposits, especially amyloid- $\beta$  amyloid deposits in the subject's brain.

Methods for Treating Epileptogenesis, Epilepsy, and Other Methods of the Invention

10 In one embodiment, the invention provides a method for inhibiting epileptogenesis in a subject. The method includes the step of administering to a subject in need thereof an effective amount of a compound (*e.g.*, an anti-epileptogenic agent of the invention, *e.g.*, a compound of the Formulae herein) that modulates a process in a pathway associated with epileptogenesis, such that epileptogenesis is inhibited in the  
15 subject.

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 amyloid-deposition or amyloidosis, has a  
20 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).

25 The term "anti-epileptogenic agent" includes agents which are capable of, for example, inhibiting epileptogenesis, suppressing the uptake of synaptic GABA, blocking GABA transporters GAT-1, GAT-2 or GAT-3, depressing glutamatergic excitation, or interacting with an NMDA receptor (*e.g.*, at the strychnine insensitive glycine co-agonist site). Examples of anti-epileptogenic agents include compounds the Formulae herein,  
30 and pharmaceutically acceptable salts, esters, *N*-substituted analogs, and prodrugs thereof. In another embodiment, the compounds of the invention act as GABA receptor agonists to increase inhibition and counteract the excitotoxic action of neurotransmitters such as glutamate.

Up-regulation of excitatory coupling between neurons is in part mediated by  
35 *N*-methyl-D-aspartate (NMDA) receptors, and downregulation of inhibitory coupling between neurons is in part mediated by gamma-amino-butyric acid (GABA) receptors, and both have been implicated in epileptogenesis. Other processes in pathways associated with epileptogenesis include release of nitric oxide (NO), a neurotransmitter

implicated in epileptogenesis; release of calcium ( $\text{Ca}^{2+}$ ), which may mediate damage to neurons when released in excess; neurotoxicity due to excess zinc ( $\text{Zn}^{2+}$ ); neurotoxicity due to excess iron ( $\text{Fe}^{2+}$ ); and neurotoxicity due to oxidative cell damage. Accordingly, in certain embodiments, an agent to be administered to a subject to inhibit

5 epileptogenesis is capable of inhibiting one or more processes in at least one pathway associated with epileptogenesis. For example, an agent useful for inhibition of epileptogenesis can reduce the release of, or attenuate the epileptogenic effect of, NO in brain tissue; antagonize an NMDA receptor; augment endogenous GABA inhibition; block voltage-gated ion channels; reduce the release of, reduce the free concentration of

10 (e.g., by chelation), or otherwise reduce the epileptogenic effect of cations including  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$ , or  $\text{Fe}^{2+}$ ; inhibit oxidative cell damage; or the like. In certain embodiments, an agent to be administered to a subject to inhibit epileptogenesis is capable of inhibiting at least two processes in at least one pathway associated with epileptogenesis.

In one embodiment, the anti-epileptogenic agent antagonizes an NMDA receptor

15 and augments endogenous GABA inhibition. In certain embodiments, the anti-epileptogenic agent is administered intravenously or orally; for example, after the step of oral or intravenous administration, the anti-epileptogenic agent is transported to the nervous system of the subject by an active transport shuttle mechanism. A non-limiting example of an active transport shuttle is the large neutral amino acid transporter, which

20 is capable of transporting amino acids across the blood-brain barrier (BBB).

In an embodiment, an anti-epileptogenic agent of the invention may antantagonize NMDA receptors by interacting, e.g., binding to the glycine binding site of the NMDA receptors. In another embodiment, the agent augments GABA inhibition by decreasing glial GABA uptake. In certain other embodiments, the method further

25 includes administering the agent in a pharmaceutically acceptable vehicle, e.g., such that the anti-epileptogenic agent is suitable, e.g., for oral or intravenous administration. In another embodiment, the compound acts by binding directly to GABA receptors and acts as an agonist.

In still another embodiment, the invention provides a method of treating (e.g.,

30 preventing, alleviating, modulating, etc.) convulsions (e.g., seizures, e.g., associated with epilepsy, trauma, etc.). The method includes the step of administering to a subject (e.g., a subject suffering from, or at risk of suffering from convulsions or a disorder characterized by convulsions or seizures) an effective amount of an anti-epileptogenic compound of the invention such that the development of the convulsive prone state is

35 prevented. Examples of anti-epileptogenic agents of the invention include compounds of any Formula herein.

In another embodiment, the invention provides a method for inhibiting both a convulsive condition and epileptogenesis in a subject. The method includes the step of

administering to a subject in need thereof an effective amount of an agent that (a) blocks sodium or calcium ion channels, or opens potassium or chloride ion channels; and (b) has at least one activity selected from the group consisting of NMDA receptor antagonism; augmentation of endogenous GABA-mediated inhibition; binding to the glycine site of the NMDA receptor; calcium binding; iron binding; zinc binding; NO synthase inhibition; and antioxidant activity; such that epileptogenesis is inhibited in the subject.

Blockers of sodium or calcium ion channel activity are well known in the art and can be used as the A moiety in the compounds and methods of the present invention. Similarly, any compound that opens potassium or chloride ion channels can be used as the A moiety in the compounds and methods of the present invention. Antagonists of NMDA receptors and augmenters of endogenous GABA-mediated inhibition are also known to one of skill in the art and can be used in the methods and compounds of the invention. For example, 2,3-quinoxalinediones are reported to have NMDA receptor antagonistic activity (*see, e.g.*, U.S. Patent No. 5,721,234). Exemplary calcium and zinc chelators include moieties known in the art for chelation of divalent cations, including (in addition to those mentioned *supra*) ethylenediaminetetraacetic acid (EDTA), ethylene glycol bis(beta-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, and the like. Exemplary iron chelators include enterobactin, pyridoxal isonicotinyl hydrazones, *N,N'*-bis(2-hydroxybenzoyl)-ethylenediamine-*N,N'*-diacetic acid (HBED), 1-substituted-2-alkyl-3-hydroxy-4-pyridones, including 1-(2'-carboxyethyl)-2-methyl-3-hydroxy-4-pyridone, and other moieties known in the art to chelate iron. Compounds which inhibit NO synthase activity are known in the art and include, *e.g.*,  $N\gamma$ -substituted arginine analogs (especially of the L configuration), including L- $N\gamma$ -nitro-arginine (a specific inhibitor of cerebral NO synthase), L- $N\gamma$ -amino-arginine, and L- $N\gamma$ -alkyl-arginines; or an ester (for example, the methyl ester) thereof. Exemplary antioxidants include ascorbic acid, tocopherols including alpha-tocopherol, and the like.

The efficacy of anti-epileptogenic agents of the invention may be determined through screening assays. For example, the animal model of Phase 1 epileptogenesis described in the Examples, *infra*, can be employed to determine whether a particular compound has anti-epileptogenic activity against Phase 1 epileptogenesis. Chronic epileptogenesis can be modeled in rats (and candidate compounds screened) with the kindling assay described by Silver *et al.* (*Ann. Neurol.* (1991) 29:356). Similarly, compounds useful as anti-convulsants can be screened in conventional animal models, such as the mouse model described in Hotrod, R.W. *et al.*, *Eur. J. Pharmacol.* (1979) 59:75-83. Compounds or pharmacophores useful for, *e.g.*, binding to or inhibition of receptors or enzymes can be screened according to conventional methods known to the ordinarily skilled artisan. For example, binding to the GABA uptake transporter can be

quantified by the method of Ramsey *et al.* as modified by Schlewer (Schlewer, J., *et al.*, *J. Med. Chem.* (1991) 34:2547). Binding to the glycine site on an NMDA receptor can be quantified, *e.g.*, according to the method described in Kemp, A., *et al.*, *Proc. Natl. Acad. Sci. USA* (1988) 85:6547. Effect on the voltage-gated Na<sup>+</sup> channel can be  
5 evaluated *in vitro* by voltage clamp assay in rat hippocampal slices.

Epilepsy is believed to be associated with excessive activation of neuronal circuits either through the increase of excitatory (glutamatergic) neurotransmission or through a decreased inhibitory (GABA) input. Both presynaptic changes in the synthesis, release or catabolism of GABA and postsynaptic changes in the GABA signal  
10 transduction can decrease the inhibitory input.

GABA<sub>A</sub> receptor is involved in the development of epilepsy, status epilepticus and other forms of epileptic syndromes. Postsynaptic alterations in subunit composition, subunit structure, and ligand binding as well as genetic modifications of the receptor have been shown to impact susceptibility to various seizures both in humans and animal  
15 models.

The GABA<sub>A</sub> receptor is formed by the assembly of multiple subunit subtypes into a pentamer. The composition of this multimeric receptor varies considerably developmentally and spatially (in different brain regions). The various pharmacological responses to drugs such as benzodiazepines (diazepam, lorazepam or midazolam) can  
20 depend on the prevalence of various subunits that form these receptors. The composition of these receptors also responds to various stimuli. For instance, the induction of acute seizures (status epilepticus) by pilocarpine or kainic acid can change (decrease) the expression of specific subunits and the GABA<sub>A</sub> receptor subunit composition. Such changes are proposed to be responsible for some of the  
25 pharmacoresistance that is sometimes observed in epilepsy. Both in humans with TLE and in animal models, a decrease in the Alpha 1 subtype appear to be associated with the loss in the CA1 and CA3 interneurons.

GABA<sub>A</sub> receptor agonists can suppress seizures in animal models while antagonists which block this receptor (bicuculline and picrotoxin) induce seizures *in*  
30 *vivo*. Compounds which inhibit GABA reuptake (Tiagabine) or inhibit GABA breakdown (vigabatrin) are effective anticonvulsants.

Various mutations in GABA<sub>A</sub> subunits lead to familial epileptic forms such as febrile seizures plus (Gamma2 mutation which reduces the receptor mediated current amplitudes) childhood absence epilepsy (Gamma 2 L mutation which affects the  
35 benzodiazepine binding pocket and possibly the interaction with other endogenous unidentified ligands) or juvenile myoclonic epilepsy (alpha1 mutation leading to decreased receptor expression at cell surface). Finally, beta3 subunit knock-out mice display seizures and deficits in functional GABA<sub>A</sub> receptors.

It is believed that GABA dysfunction plays a role in the development of epilepsy. Therefore, compounds of the invention which act as GABA<sub>A</sub> receptor agonists may provide effective treatment against various types of epilepsies and prevent epileptogenesis. GABA<sub>A</sub> receptor agonists can be identified using methods described in  
5 the Examples.

The term "inhibiting epileptogenesis" includes both partial and complete prevention of epileptogenesis as well as reversal of the process. It also includes prevention of epileptogenesis or a decrease or slowing in the rate of epileptogenesis  
10 (e.g., a partial or complete stop in the rate of epileptogenic transformation of the brain or central nervous system tissue). It also includes any inhibition or slowing of the rate of the biochemical processes or events which take place during Phase 1 or Phase 2 epileptogenesis and lead to epileptogenic changes in tissue, i.e., in tissues of the central nervous system (CNS), e.g., the brain. Examples of processes in pathways associated  
15 with epileptogenesis, which may be inhibited by the compounds of the invention, are discussed in more detail, *infra*. It also includes the prevention, slowing, halting, or reversing of the process of epileptogenesis, i.e., the changes in brain tissue which result in epileptic seizures.

The term "convulsive disorder" or "convulsive condition" according to the  
20 invention includes conditions wherein a subject suffers from convulsions. Convulsive disorders include, but are not limited to, epilepsy, ictogenesis, and non-epileptic convulsions, and convulsions due to administration of a convulsive agent or trauma to the subject. The term "epileptogenesis-associated disorders" includes disorders of the central and peripheral nervous system which may advantageously be treated by the  
25 compounds of the invention. In an advantageous embodiment, the nervous system disorders are disorders associated or related to the process or the results of epileptogenic transformation of the brain or other nervous tissue. Examples of epileptogenesis-associated disorders include, but are not limited to, epilepsy, head trauma, neurosurgery, pain, stroke, anxiety, schizophrenia, multiple sclerosis, amyotrophic lateral sclerosis,  
30 psychoses, cerebral ischemia, Huntington's chorea, Parkinson's disease, motor neuron disease, Alzheimer's disease, dementia and other disorders (in humans or animals) in which excessive activity of NMDA receptors is a cause, at least in part, of the disorder (see, e.g., Schoepp *et al.*, *Eur. J. Pharmacol.* 203:237-243 (1991); Leeson *et al.*, *J. Med. Chem.* 34:1243-1252 (1991); Kulagowski *et al.*, *J. Med. Chem.* 37:1402-1405 (1994);  
35 Mallamo *et al.*, *J. Med. Chem.* 37:4438-4448 (1994); and references cited therein).

In another embodiment, the invention also includes a method for treating convulsions (e.g., seizures) in a subject. The method includes administering to a subject

an effective amount of an anti-epileptogenic agent (*e.g.*, compounds of the Formulae herein).

In one embodiment, the invention pertains to a method for inhibiting epileptogenesis in a subject. The method includes administering to the subject an effective amount of an anti-epileptogenic agent, such as, for example compounds of the Formulae herein (*e.g.*, Formula I). In one embodiment, the invention provides a method for treating epileptogenesis-associated conditions, epilepsy or ictogenesis.

The invention also pertains to methods for treating a subject suffering from an epileptogenesis-associated condition. The method includes administering to the subject an effective amount of an anti-epileptogenic agent (*e.g.*, compounds of the Formulae herein).

Other anti-epileptogenic agents which may be formulated into therapeutic compositions of the invention, include, but are not limited to, compounds of the Formulae herein and pharmaceutically acceptable salts, esters, *N*-substituted analogs, and prodrugs thereof.

#### Treatment of Amyloid-Related Diseases

The present invention also pertains to methods of using the compounds and pharmaceutical compositions thereof in the treatment and prevention of amyloid-related diseases. The pharmaceutical compositions of the invention may be administered therapeutically or prophylactically to treat diseases associated with amyloid (*e.g.*, AL amyloid protein ( $\lambda$  or  $\kappa$ -chain related, *e.g.*, amyloid  $\lambda$ , amyloid  $\kappa$ , amyloid  $\kappa$ IV, amyloid  $\lambda$ VI, amyloid  $\gamma$ , amyloid  $\gamma$ 1), A $\beta$ , IAPP,  $\beta$ <sub>2</sub>M, AA, or AH amyloid protein) fibril formation, aggregation or deposition.

The pharmaceutical compositions of the invention may act to ameliorate the course of an amyloid-related disease using any of the following mechanisms (this list is meant to be illustrative and not limiting): slowing the rate of amyloid fibril formation or deposition; lessening the degree of amyloid deposition; inhibiting, reducing, or preventing amyloid fibril formation; inhibiting neurodegeneration or cellular toxicity induced by amyloid; inhibiting amyloid induced inflammation; enhancing the clearance of amyloid from the brain, *e.g.*, -enhancing degradation of A $\beta$  in the brain, or favoring clearance of amyloid protein prior to its organization in fibrils.

"Inhibition" of amyloid deposition includes preventing or stopping of amyloid formation, *e.g.*, fibrillogenesis, clearance of amyloid, *e.g.*, soluble A $\beta$  from brain, inhibiting or slowing down of further amyloid deposition in a subject with amyloidosis, *e.g.*, already having amyloid deposits, and reducing or reversing amyloid fibrillogenesis or deposits in a subject with ongoing amyloidosis. Inhibition of amyloid deposition is determined relative to an untreated subject, or relative to the treated subject prior to

treatment, or, *e.g.*, determined by clinically measurable improvement, *e.g.*, or in the case of a subject with brain amyloidosis, *e.g.*, an Alzheimer's or cerebral amyloid angiopathy subject, stabilization of cognitive function or prevention of a further decrease in cognitive function (*i.e.*, preventing, slowing, or stopping disease progression), or  
5 improvement of parameters such as the concentration of A $\beta$  or tau in the CSF.

"Modulation" of amyloid deposition includes both inhibition, as defined above, and enhancement of amyloid deposition or fibril formation. The term "modulating" is intended, therefore, to encompass prevention or stopping of amyloid formation or accumulation, inhibition or slowing down of further amyloid aggregation in a subject  
10 with ongoing amyloidosis, *e.g.*, already having amyloid aggregates, and reducing or reversing of amyloid aggregates in a subject with ongoing amyloidosis; and enhancing amyloid deposition, *e.g.*, increasing the rate or amount of amyloid deposition *in vivo* or *in vitro*. Amyloid-enhancing compounds may be useful in animal models of amyloidosis, for example, to make possible the development of amyloid deposits in  
15 animals in a shorter period of time or to increase amyloid deposits over a selected period of time. Amyloid-enhancing compounds may be useful in screening assays for compounds which inhibit amyloidosis *in vivo*, for example, in animal models, cellular assays and *in vitro* assays for amyloidosis. Such compounds may be used, for example, to provide faster or more sensitive assays for compounds. Modulation of amyloid  
20 aggregation is determined relative to an untreated subject or relative to the treated subject prior to treatment.

As used herein, "treatment" of a subject 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  
25 has a 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, 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  
30 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,  
35 in some situations, preventing the onset of dementia. 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.

In one embodiment, the term "treating" includes maintaining a subject's CDR rating at its base line rating or at 0. In another embodiment, the term treating includes  
5 decreasing 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 term "treating" also includes reducing the rate of the increase of a subject's CDR rating as compared to historical controls. In another  
10 embodiment, the term includes reducing 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 term "treating" also includes maintaining a subject's  
15 score on the MMSE. The term "treating" includes increasing 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. The term also includes reducing the rate of the decrease of a subject's MMSE score as compared to historical controls. In another  
20 embodiment, the term includes reducing the rate of decrease of a subject's MMSE score by about 5% or less, about 10% or less, about 20% or less, about 25% or less, about 30% or less, about 40% or less, about 50% or less, about 60% or less, about 70% or less, about 80% or less, about 90% or less or about 100% or less, of the decrease of the historical or untreated controls.

In yet another embodiment, the term "treating" includes maintaining a subject's  
25 score on the ADAS-Cog. The term "treating" includes decreasing 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  
30 points or greater. The term also includes reducing the rate of the increase of a subject's ADAS-Cog score as compared to historical controls. In another embodiment, the term includes reducing 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  
35 80% or more, about 90% or more or about 100% of the increase of the historical or untreated controls.

In another embodiment, the term "treating" e.g., for AA or AL amyloidosis, includes an increase in serum creatinine, e.g., an increase of creatinine clearance of 10%

or greater, 20% or greater, 50% or greater, 80% or greater, 90% or greater, 100% or greater, 150% or greater, 200% or greater. The term "treating" also may include remission of nephrotic syndrome (NS). It may also include remission of chronic diarrhea and/or a gain in body weight, e.g., by 10% or greater, 15% or greater, or 20% or greater.

Without wishing to be bound by theory, in some aspects the pharmaceutical compositions of the invention contain a compound that prevents or inhibits amyloid fibril formation, either in the brain or other organ of interest (acting locally) or throughout the entire body (acting systemically). Pharmaceutical compositions of the invention may be effective in controlling amyloid deposition either following their entry into the brain (following penetration of the blood brain barrier) or from the periphery. When acting from the periphery, a compound of a pharmaceutical composition may alter the equilibrium of amyloidogenic peptide between the brain and the plasma so as to favor the exit of amyloidogenic peptide from the brain. It may also favor clearance (or catabolism) of the amyloid protein (soluble), and then prevent amyloid fibril formation and deposition due to a reduction of the amyloid protein pool in a specific organ, e.g., liver, spleen, pancreas, kidney, joints, brain, etc. An increase in the exit of amyloidogenic peptide from the brain would result in a decrease in amyloidogenic peptide brain concentration and therefore favor a decrease in amyloidogenic peptide deposition. In particular, an agent may lower the levels of amyloid  $\beta$  peptides, e.g., both A $\beta$ 40 and A $\beta$ 42 in the CSF and the plasma, or the agent may lower the levels of amyloid  $\beta$  peptides, e.g., A $\beta$ 40 and A $\beta$ 42 in the CSF and increase it in the plasma. Alternatively, compounds that penetrate the brain could control deposition by acting directly on brain amyloidogenic peptide e.g., by maintaining it in a non-fibrillar form or favoring its clearance from the brain, by increasing its degradation in the brain, or protecting brain cells from the detrimental effect of amyloidogenic peptide. An agent can also cause a decrease of the concentration of the amyloid protein (i.e., in a specific organ so that the critical concentration needed to trigger amyloid fibril formation or deposition is not reached). Furthermore, the compounds described herein may inhibit or reduce an interaction between amyloid and a cell surface constituent, for example, a glycosaminoglycan or proteoglycan constituent of a basement membrane, whereby inhibiting or reducing this interaction produces the observed neuroprotective and cell-protective effects. For example, the compound may also prevent an amyloid peptide from binding or adhering to a cell surface, a process which is known to cause cell damage or toxicity. Similarly, the compound may block amyloid-induced cellular toxicity or microglial activation or amyloid-induced neurotoxicity, or inhibit amyloid induced inflammation. The compound may also reduce the rate or amount of amyloid aggregation, fibril formation, or deposition, or the compound lessens the degree of

amyloid deposition. The foregoing mechanisms of action should not be construed as limiting the scope of the invention inasmuch as the invention may be practiced without such information.

5 The term "amyloid- $\beta$  disease" (or "amyloid- $\beta$  related disease," which terms as used herein are synonymous) may be used for mild cognitive impairment; vascular dementia; early Alzheimer's disease; Alzheimer's disease, including sporadic (non-hereditary) Alzheimer's disease and familial (hereditary) Alzheimer's disease; age-related cognitive decline; cerebral amyloid angiopathy ("CAA"); hereditary cerebral hemorrhage; senile dementia; Down's syndrome; inclusion body myositis ("IBM"); or  
10 age-related macular degeneration ("ARMD"). According to certain aspects of the invention, amyloid- $\beta$  is a peptide having 39-43 amino-acids, or amyloid- $\beta$  is an amyloidogenic peptide produced from  $\beta$ APP.

Mild cognitive impairment ("MCI") is a condition characterized by a state of mild but measurable impairment in thinking skills, which is not necessarily associated  
15 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  
20 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 leptomeningeal and cortical arteries, arterioles and in  
25 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 A $\beta$  or the APP gene have been identified and are clinically associated with  
30 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 A $\beta$ ); the Flemish mutation of A $\beta$ ; the Arctic mutation of A $\beta$ ; the Italian mutation of A $\beta$ ; the Iowa mutation of A $\beta$ ; familial British dementia; and familial Danish dementia. Cerebral  
35 amyloid angiopathy is known to be associated with cerebral hemorrhage (or hemorrhagic stroke).

Additionally, abnormal accumulation of APP and of amyloid- $\beta$  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- $\beta$  protein is abnormally deposited at non-  
5 neurological locations, such as treatment of IBM by delivery of the compounds to muscle fibers.

Additionally, it has been shown that A $\beta$  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).  
10 ARMD is a cause of irreversible vision loss in older individuals. It is believed that A $\beta$  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 or prevention of age-  
15 related macular degeneration.

Also, the invention relates to a method for preventing or inhibiting amyloid deposition in a subject. For example, such a method comprises administering to a subject a therapeutically effective amount of a compound capable of reducing the concentration of amyloid (*e.g.*, AL amyloid protein ( $\lambda$  or  $\kappa$ -chain related, *e.g.*, amyloid  $\lambda$ ,  
20 amyloid  $\kappa$ , amyloid  $\kappa$ IV, amyloid  $\lambda$ VI, amyloid  $\gamma$ , amyloid  $\gamma$ 1), A $\beta$ , IAPP,  $\beta$ <sub>2</sub>M, AA, AH amyloid protein, or other amyloids), such that amyloid accumulation or deposition is prevented or inhibited.

In another aspect, the invention relates to a method for preventing, reducing, or inhibiting amyloid deposition in a subject. For example, such a method comprises  
25 administering to a subject a therapeutically effective amount of a compound capable of inhibiting amyloid (*e.g.*, AL amyloid protein ( $\lambda$  or  $\kappa$ -chain related, *e.g.*, amyloid  $\lambda$ , amyloid  $\kappa$ , amyloid  $\kappa$ IV, amyloid  $\lambda$ VI, amyloid  $\gamma$ , amyloid  $\gamma$ 1), A $\beta$ , IAPP,  $\beta$ <sub>2</sub>M, AA, AH amyloid protein, or other amyloids), such that amyloid deposition is prevented, reduced, or inhibited.

30 The invention also relates to a method for modulating, *e.g.*, minimizing, amyloid-associated damage to cells, comprising the step of administering a compound capable of reducing the concentration of amyloid (*e.g.*, AL amyloid protein ( $\lambda$  or  $\kappa$ -chain related, *e.g.*, amyloid  $\lambda$ , amyloid  $\kappa$ , amyloid  $\kappa$ IV, amyloid  $\lambda$ VI, amyloid  $\gamma$ , amyloid  $\gamma$ 1), A $\beta$ , IAPP,  $\beta$ <sub>2</sub>M, AA, AH amyloid protein, or another amyloid), such that said amyloid-  
35 associated damage to cells is modulated. In certain aspects of the invention, the methods for modulating amyloid-associated damage to cells comprise a step of administering a compound capable of reducing the concentration of amyloid or reducing the interaction of an amyloid with a cell surface.

The invention also includes a method for directly or indirectly preventing cell death in a subject, the method comprising administering to a subject a therapeutically effective amount of a compound capable of preventing amyloid (*e.g.*, AL amyloid protein ( $\lambda$  or  $\kappa$ -chain related, *e.g.*, amyloid  $\lambda$ , amyloid  $\kappa$ , amyloid  $\kappa$ IV, amyloid  $\lambda$ VI, amyloid  $\gamma$ , amyloid  $\gamma$ 1), A $\beta$ , IAPP,  $\beta$ <sub>2</sub>M, AA, AH amyloid protein, or other amyloid) mediated events that lead, directly or indirectly, to cell death.

In an embodiment, the method is used to treat Alzheimer's disease (*e.g.* sporadic or familial AD). The method can also be used prophylactically or therapeutically to treat other clinical occurrences of amyloid- $\beta$  deposition, such as in Down's syndrome individuals and in patients with cerebral amyloid angiopathy ("CAA") or hereditary cerebral hemorrhage.

The compounds of the invention may be used prophylactically or therapeutically in the treatment of disorders in which amyloid-beta peptide is abnormally deposited at non-neurological locations, such as treatment of IBM by delivery of the compounds to muscle fibers, or treatment of macular degeneration by delivery of the compound(s) of the invention to the basal surface of the retinal pigmented epithelium.

The present invention also provides a method for modulating amyloid-associated damage to cells, comprising the step of administering a compound capable of reducing the concentration of A $\beta$ , or capable of minimizing the interaction of A $\beta$  (soluble oligomeric or fibrillary) with the cell surface, such that said amyloid-associated damage to cells is modulated. In certain aspects of the invention, the methods for modulating amyloid-associated damage to cells comprise a step of administering a compound capable of reducing the concentration of A $\beta$  or reducing the interaction of A $\beta$  with a cell surface.

In accordance with the present invention, there is further provided a method for preventing cell death in a subject, said method comprising administering to a subject a therapeutically effective amount of a compound capable of preventing A $\beta$ -mediated events that lead, directly or indirectly, to cell death.

The present invention also provides a method for modulating amyloid-associated damage to cells, comprising the step of administering a compound capable of reducing the concentration of IAPP, or capable of minimizing the interaction of IAPP (soluble oligomeric or fibrillary) with the cell surface, such that said amyloid-associated damage to cells is modulated. In certain aspects of the invention, the methods for modulating amyloid-associated damage to cells comprise a step of administering a compound capable of reducing the concentration of IAPP or reducing the interaction of IAPP with a cell surface.

In accordance with the present invention, there is further provided a method for preventing cell death in a subject, said method comprising administering to a subject a

therapeutically effective amount of a compound capable of preventing IAPP-mediated events that lead, directly or indirectly, to cell death.

This invention also provides methods and compositions which are useful in the treatment of amyloidosis. The methods of the invention involve administering to a  
5 subject a therapeutic compound which inhibits amyloid deposition. Accordingly, the compositions and methods of the invention are useful for inhibiting amyloidosis in disorders in which amyloid deposition occurs. The methods of the invention can be used therapeutically to treat amyloidosis or can be used prophylactically in a subject susceptible to (hereditary) amyloidosis or identified as being at risk to develop  
10 amyloidosis, e.g., hereditary, or identified as being at risk to develop amyloidosis. In certain embodiments, the invention includes a method of inhibiting an interaction between an amyloidogenic protein and a constituent of basement membrane to inhibit amyloid deposition. The constituent of basement membrane is a glycoprotein or proteoglycan, preferably heparan sulfate proteoglycan. A therapeutic compound used in  
15 this method may interfere with binding of a basement membrane constituent to a target binding site on an amyloidogenic protein, thereby inhibiting amyloid deposition.

In some aspects, the methods of the invention involve administering to a subject a therapeutic compound which inhibits amyloid deposition. "Inhibition of amyloid deposition," includes the prevention of amyloid formation, inhibition of further amyloid  
20 deposition in a subject with ongoing amyloidosis and reduction of amyloid deposits in a subject with ongoing amyloidosis. Inhibition of amyloid deposition is determined relative to an untreated subject or relative to the treated subject prior to treatment. In an embodiment, amyloid deposition is inhibited by inhibiting an interaction between an amyloidogenic protein and a constituent of basement membrane. "Basement  
25 membrane" refers to an extracellular matrix comprising glycoproteins and proteoglycans, including laminin, collagen type IV, fibronectin, perlecan, agrin, dermatan sulfate, and heparan sulfate proteoglycan (HSPG). In one embodiment, amyloid deposition is inhibited by interfering with an interaction between an amyloidogenic protein and a sulfated glycosaminoglycan such as HSPG, dermatan  
30 sulfate, perlecan or agrin sulfate. Sulfated glycosaminoglycans are known to be present in all types of amyloids (*see* Snow, *et al.* Lab. Invest. 56, 120-23 (1987)) and amyloid deposition and HSPG deposition occur coincidentally in animal models of amyloidosis (*see* Snow, *et al.* Lab. Invest. 56, 665-75 (1987) and Gervais, F. *et al.* Curr. Med. Chem., 3, 361-370 (2003)). Consensus binding site motifs for HSPG in amyloidogenic proteins  
35 have been described (*see, e.g.,* Cardin and Weintraub Arteriosclerosis 9, 21-32 (1989)). The ability of a compound to prevent or block the formation or deposition of amyloid may reside in its ability to bind to non-fibrillar, soluble amyloid protein and to maintain its solubility.

The ability of a therapeutic compound of the invention to inhibit an interaction between an amyloidogenic protein and a glycoprotein or proteoglycan constituent of a basement membrane can be assessed by an in vitro binding assay, such as that described in US 5,164,295, the contents of which are hereby incorporated by reference.

5 Alternatively, the ability of a compound to bind to an amyloidogenic protein or to inhibit the binding of a basement membrane constituent (e.g. HSPG) to an amyloidogenic protein (e.g. A $\beta$ ) can be measured using a mass spectrometry assay where soluble protein, e.g. A $\beta$ , IAPP,  $\beta_2$ M is incubated with the compound. A compound which binds to, e.g. A $\beta$ , will induce a change in the mass spectrum of the protein. The protocol can  
10 readily be modified to adjust the sensitivity of the data, e.g., by adjusting the amount of protein and/or compound employed. Thus, e.g., binding might be detected for test compounds noted as not having detectable binding employing less sensitive test protocols.

Alternative methods for screening compounds exist and can readily be employed  
15 by a skilled practitioner to provide an indication of the ability of test compounds to bind to, e.g., fibrillar A $\beta$ . One such screening assay is an ultraviolet absorption assay. In an exemplary protocol, a test compound (20  $\mu$ M) is incubated with 50  $\mu$ M A $\beta$ (1-40) fibers for 1 hour at 37°C in Tris buffered saline (20 mM Tris, 150 mM NaCl, pH 7.4 containing 0.01 sodium azide). Following incubation, the solution is centrifuged for 20  
20 minutes at 21,000 g to sediment the A $\beta$ (1-40) fibers along with any bound test compound. The amount of test compound remaining in the supernatant can then be determined by reading the absorbance. The fraction of test compound bound can then be calculated by comparing the amount remaining in the supernatants of incubations with A $\beta$  to the amount remaining in control incubations which do not contain A $\beta$  fibers.  
25 Thioflavin T and Congo Red, both of which are known to bind to A $\beta$  fibers, may be included in each assay run as positive controls. Before assaying, test compounds can be diluted to 40  $\mu$ M, which would be twice the concentration in the final test, and then scanned using the Hewlett Packard 8453 UV/VIS spectrophotometer to determine if the absorbance is sufficient for detection.

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

In another embodiment, the invention pertains to a method for treating a subject for an amyloid-related disease. The method includes administering a cognitive test to a subject prior to administration of a compound of the invention, administering an effective amount of a compound of the invention to the subject, and administering a cognitive test to the subject subsequent to administration of the compound, such that the subject is treated for the amyloid-related disease, wherein the subject's score on said cognitive test is improved.

"Improvement," "improved" or "improving" in cognition is present within the context of the present invention if there is a statistically significant difference in the direction of normality between the performance of subjects treated using the methods of the invention as compared to members of a placebo group, historical control, or between subsequent tests given to the same subject.

In one embodiment, a subject's CDR is maintained at 0. In another embodiment, a subject's CDR is decreased (e.g., improved) 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 rate of increase of a subject's CDR rating is reduced 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 increase of the historical or untreated controls.

In one embodiment, a subject's score on the MMSE is maintained. Alternatively, the subject's score on the MMSE may be increased 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 alternative, the rate of the decrease of a subject's MMSE score as compared to historical controls is reduced. For example, the rate of the decrease of a subject's MMSE score may be reduced 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 one embodiment, the invention pertains to a method for treating, slowing or stopping an amyloid-related disease associated with cognitive impairment, by administering to a subject an effective amount of a therapeutic compound of the invention, wherein the annual deterioration of the subject's cognition as measured by ADAS-Cog is less than 8 points per year, less than 6 points per year, less than 5 points per year, less than 4 points per year, or less than 3 points per year. In a further embodiment, the invention pertains to a method for treating, slowing or stopping an amyloid-related disease associated with cognition by administering an effective amount

of a therapeutic compound of the invention 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 a further embodiment, the subject's cognition improves by 2 points or greater per year, 3 points or greater per year, 4 point or greater per year, 5 points or greater per year, 6 points or greater per year, 7 points or greater per year, 8 points or greater per year, etc. as measured by the ADAS-Cog. In another alternative, the rate of the increase of a subject's ADAS-Cog score as compared to historical controls is reduced. For example, the rate of the increase of a subject's ADAS-Cog score may be reduced 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 ratio of A $\beta$ 42:A $\beta$ 40 in the CSF or plasma of a subject decreases by about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 45% or more, or about 50% or more. In another embodiment, the levels of A $\beta$  in the subject's cerebrospinal fluid decrease by about 15% or more, about 25% or more, about 35% or more, about 45% or more, about 55% or more, about 75% or more, or about 90% or more.

#### Pharmaceutical Preparations

In one embodiment, the invention pertains to pharmaceutical compositions, which include an effective amount of an anti-epileptogenic agent and a pharmaceutical acceptable carrier. The anti-epileptogenic agent may be a compound of any Formula herein and pharmaceutically acceptable salts, esters, *N*-substituted analogs, and prodrugs thereof.

In another embodiment, the present invention relates to pharmaceutical compositions comprising agents according to any of the Formulae herein for the treatment of an amyloid-related disease, as well as methods of manufacturing such pharmaceutical compositions.

In general, the agents of the present invention may be prepared by the methods illustrated in the general reaction schemes as, for example, in the patents and patent applications referred to herein, or by modifications thereof, using readily available starting materials, reagents and conventional synthesis procedures. In these reactions, it is also possible to make use of variants which are in themselves known, but are not mentioned here. Functional and structural equivalents of the agents described herein and which have the same general properties, wherein one or more simple variations of

substituents are made which do not adversely affect the essential nature or the utility of the agent are also included.

The agents of the invention may be supplied in a solution with an appropriate solvent or in a solvent-free form (*e.g.*, lyophilized). In another aspect of the invention, the agents and buffers necessary for carrying out the methods of the invention may be packaged as a kit, optionally including a container. The kit may be commercially used according to the methods described herein and may include instructions for use in a method of the invention. Additional kit components may include acids, bases, buffering agents, inorganic salts, solvents, antioxidants, preservatives, or metal chelators. The additional kit components are present as pure compositions, or as aqueous or organic solutions that incorporate one or more additional kit components. Any or all of the kit components optionally further comprise buffers.

The term "container" includes any receptacle for holding the therapeutic compound. For example, in one embodiment, the container is the packaging that contains the compound. In other embodiments, the container is not the packaging that contains the compound, *i.e.*, the container is a receptacle, such as a box or vial that contains the packaged compound or unpackaged compound and the instructions for use of the compound. Moreover, packaging techniques are well known in the art. It should be understood that the instructions for use of the therapeutic compound may be contained on the packaging containing the therapeutic compound, and as such the instructions form an increased functional relationship to the packaged product.

The therapeutic agent may also be administered parenterally, intraperitoneally, intraspinally, or intracerebrally. Dispersions can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

To administer the therapeutic agent by other than parenteral administration, it may be necessary to coat the agent with, or co-administer the agent with, a material to prevent its inactivation. For example, the therapeutic agent may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan *et al.*, *J. Neuroimmunol.* 7, 27 (1984)).

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists.

It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi.

Suitable pharmaceutically acceptable vehicles include, without limitation, any non-immunogenic pharmaceutical adjuvants suitable for oral, parenteral, nasal, mucosal, 5 transdermal, intravascular (IV), intraarterial (IA), intramuscular (IM), and subcutaneous (SC) administration routes, such as phosphate buffer saline (PBS).

The vehicle can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity 10 can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, isotonic agents are included, for 15 example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

Sterile injectable solutions can be prepared by incorporating the therapeutic 20 agent in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the therapeutic agent into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable 25 solutions, the methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient (*i.e.*, the therapeutic agent) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The therapeutic agent can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The therapeutic agent and other ingredients may 30 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 therapeutic agent may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of the therapeutic agent in the compositions and preparations may, 35 of course, be varied. The amount of the therapeutic agent 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 therapeutic agent 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  
5 directly dependent on (a) the unique characteristics of the therapeutic agent and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such a therapeutic agent for the treatment of amyloid deposition in subjects.

The present invention therefore includes pharmaceutical formulations comprising  
10 the agents of the Formulae described herein, including pharmaceutically acceptable salts thereof, in pharmaceutically acceptable vehicles for aerosol, oral and parenteral administration. Also, the present invention includes such agents, or salts thereof, which have been lyophilized and which may be reconstituted to form pharmaceutically acceptable formulations for administration, as by intravenous, intramuscular, or  
15 subcutaneous injection. Administration may also be intradermal or transdermal.

In accordance with the present invention, an agent of the Formulae described herein, and pharmaceutically acceptable salts thereof, may be administered orally or through inhalation as a solid, or may be administered intramuscularly or intravenously as a solution, suspension or emulsion. Alternatively, the agents or salts may also be  
20 administered by inhalation, intravenously or intramuscularly as a liposomal suspension.

Pharmaceutical formulations are also provided which are suitable for administration as an aerosol, by inhalation. These formulations comprise a solution or suspension of the desired agent of any Formula herein, or a salt thereof, or a plurality of solid particles of the agent or salt. The desired formulation may be placed in a small  
25 chamber and nebulized. Nebulization may be accomplished by compressed air or by ultrasonic energy to form a plurality of liquid droplets or solid particles comprising the agents or salts. The liquid droplets or solid particles should have a particle size in the range of about 0.5 to about 5 microns. The solid particles can be obtained by processing the solid agent of any Formula described herein, or a salt thereof, in any appropriate  
30 manner known in the art, such as by micronization. The size of the solid particles or droplets will be, for example, from about 1 to about 2 microns. In this respect, commercial nebulizers are available to achieve this purpose.

A pharmaceutical formulation suitable for administration as an aerosol may be in the form of a liquid, the formulation will comprise a water-soluble agent of any Formula  
35 described herein, or a salt thereof, in a carrier which comprises water. A surfactant may be present which lowers the surface tension of the formulation sufficiently to result in the formation of droplets within the desired size range when subjected to nebulization.

Peroral compositions also include liquid solutions, emulsions, suspensions, and the like. The pharmaceutically acceptable vehicles suitable for preparation of such compositions are well known in the art. Typical components of carriers for syrups, elixirs, emulsions and suspensions include ethanol, glycerol, propylene glycol, polyethylene glycol, liquid sucrose, sorbitol and water. For a suspension, typical suspending agents include methyl cellulose, sodium carboxymethyl cellulose, tragacanth, and sodium alginate; typical wetting agents include lecithin and polysorbate 80; and typical preservatives include methyl paraben and sodium benzoate. Peroral liquid compositions may also contain one or more components such as sweeteners, flavoring agents and colorants disclosed above.

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 disclosed above may also be included.

The compositions of this invention can also be administered topically to a subject, *e.g.*, by the direct laying on or spreading of the composition on the epidermal or epithelial tissue of the subject, or transdermally *via* a "patch". Such compositions include, for example, lotions, creams, solutions, gels and solids. These topical compositions may comprise an effective amount, usually at least about 0.1%, or even from about 1% to about 5%, of an agent of the invention. Suitable carriers for topical administration typically remain in place on the skin as a continuous film, and resist being removed by perspiration or immersion in water. Generally, the carrier is organic in nature and capable of having dispersed or dissolved therein the therapeutic agent. The carrier may include pharmaceutically acceptable emollients, emulsifiers, thickening agents, solvents and the like.

The language "effective amount" of an anti-epileptogenic compound includes that amount necessary or sufficient to treat or prevent an epileptogenesis-associated state, *e.g.*, to prevent the various symptoms of an epileptogenesis-associated state. The effective amount can vary depending on such factors as the size and weight of the subject, the type of illness, or the particular anti-epileptogenic agent. For example, the

choice of the anti-epileptogenic agent can affect what constitutes an “effective amount.” One of ordinary skill in the art would be able to study the aforementioned factors and make the determination regarding the effective amount of the anti-epileptogenic agent without undue experimentation. In a further embodiment, the effective amount is  
5 effective to treat an epileptogenesis-associated state in a subject. Examples of such states, include, but are not limited to, epilepsy, head trauma, pain, stroke, anxiety, schizophrenia, other psychoses, cerebral ischemia, Huntington’s chorea, motor neuron disease, Alzheimer’s disease, Parkinson’s disease, neurosurgery, cancer, fibrile seizures, and dementia.

10 In another embodiment, active agents are administered at a therapeutically effective dosage sufficient to inhibit amyloid deposition in a subject. A “therapeutically effective” dosage inhibits 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 the case of an Alzheimer’s subject, a “therapeutically effective”  
15 dosage stabilizes cognitive function or prevents a further decrease in cognitive function (*i.e.*, preventing, slowing, or stopping disease progression). The present invention accordingly provides therapeutic drugs. By “therapeutic” or “drug” is meant an agent having a beneficial ameliorative or prophylactic effect on a specific disease or condition in a living human or non-human animal.

20 In the case of AA or AL amyloidosis, the agent may improve or stabilize specific organ function. As an example, renal function may be stabilized or improved by 10% or greater, 20% or greater, 30% or greater, 40% or greater, 50% or greater, 60% or greater, 70% or greater, 80% or greater, or by greater than 90%.

In the case of IAPP, the agent may maintain or increase  $\beta$ -islet cell function, as  
25 determined by insulin concentration or the Pro-IAPP/IAPP ratio. In a further embodiment, the Pro-IAPP/IAPP ratio is increased by about 10% or greater, about 20% or greater, about 30% or greater, about 40% or greater, or by about 50%. In a further embodiment, the ratio is increased up to 50%. In addition, a therapeutically effective amount of the agent may be effective to improve glycemia or insulin levels.

30 In another embodiment, the active agents are administered at a therapeutically effective dosage sufficient to treat AA (secondary) amyloidosis and/or AL (primary) amyloidosis, by stabilizing renal function, decreasing proteinuria, increasing creatinine clearance (*e.g.*, by at least 50% or greater or by at least 100% or greater), remission of chronic diarrhea, or by weight gain (*e.g.*, 10% or greater). In addition, the agents may  
35 be administered at a therapeutically effective dosage sufficient to improve nephrotic syndrome.

Furthermore, active agents may be administered at a therapeutically effective dosage sufficient to decrease deposition in a subject of amyloid protein, *e.g.*, A $\beta$ 40 or

A $\beta$ 42. 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.

5 In another embodiment, active agents are administered at a therapeutically effective dosage sufficient to increase or enhance amyloid protein, *e.g.*, A $\beta$ 40 or A $\beta$ 42, in the blood, CSF, or plasma of a subject. A therapeutically effective dosage increases the concentration 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.

10 In yet another embodiment, active agents 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 active agents are administered at a therapeutically effective dosage sufficient to decrease 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 active agents are administered  
15 at a therapeutically effective dosage sufficient to reduce the rate of the increase of a subject's CDR rating as compared to historical or untreated controls. In another embodiment, the therapeutically effective dosage is sufficient to reduce the rate of increase of a subject's CDR rating (relative to untreated subjects) by about 5% or greater, about 10% or greater, about 20% or greater, about 25% or greater, about 30% or  
20 greater, about 40% or greater, about 50% or greater, about 60% or greater, about 70% or greater, about 80% or greater, about 90% or greater or about 100% or greater.

In yet another embodiment, active agents are administered at a therapeutically effective dosage sufficient to maintain a subject's score on the MMSE. In another embodiment, the active agents are administered at a therapeutically effective dosage  
25 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 active agents 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 therapeutically effective  
30 dosage is sufficient to reduce the rate of decrease of a subject's MMSE score may be about 5% or less, about 10% or less, about 20% or less, about 25% or less, about 30% or less, about 40% or less, about 50% or less, about 60% or less, about 70% or less, about 80% or less, about 90% or less or about 100% or less, of the decrease of the historical or untreated controls.

35 In yet another embodiment, active agents are administered at a therapeutically effective dosage sufficient to maintain a subject's score on the ADAS-Cog. In another embodiment, the active agents are administered at a therapeutically effective dosage sufficient to decrease a subject's ADAS-Cog score 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. In another embodiment, the active agents are administered at a therapeutically effective dosage sufficient to reduce the rate of the increase of a subject's ADAS-Cog scores as compared to historical or untreated controls. In another embodiment, the therapeutically effective dosage is sufficient to reduce the rate of increase of a subject's ADAS-Cog scores (relative to untreated subjects) by about 5% or greater, about 10% or greater, about 20% or greater, about 25% or greater, about 30% or greater, about 40% or greater, about 50% or greater, about 60% or greater, about 70% or greater, about 80% or greater, about 90% or greater or about 100% or greater.

In another embodiment, active agents are administered at a therapeutically effective dosage sufficient to decrease the ratio of A $\beta$ 42:A $\beta$ 40 in the CSF or plasma of a subject by about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 45% or more, or about 50% or more.

In another embodiment, active agents are administered at a therapeutically effective dosage sufficient to lower levels of A $\beta$  in the CSF or plasma of a subject by about 15% or more, about 25% or more, about 35% or more, about 45% or more, about 55% or more, about 75% or more, or about 95% or more.

Toxicity and therapeutic efficacy of such agents can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD50/ED50, and usually a larger therapeutic index is more efficacious. While agents that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to unaffected cells and, thereby, reduce side effects.

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 small molecule 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 small molecule to have upon the subject. Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per

kilogram to about 50 micrograms per kilogram). 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.

The ability of an agent to inhibit amyloid deposition can be evaluated in an animal model system that may be predictive of efficacy in inhibiting amyloid deposition in human diseases, such as a transgenic mouse expressing human APP or other relevant animal models where A $\beta$  deposition is seen or for example in an animal model of AA amyloidosis. Likewise, the ability of an agent to prevent or reduce cognitive impairment in a model system may be indicative of efficacy in humans. Alternatively, the ability of an agent can be evaluated by examining the ability of the agent to inhibit amyloid fibril formation *in vitro*, e.g., using a fibrillogenesis assay such as that described herein, including a ThT, CD, or EM assay. Also the binding of an agent to amyloid fibrils may be measured using a MS assay as described herein. The ability of the agent to protect cells from amyloid induced toxicity is determined *in vitro* using biochemical assays to determine percent cell death induced by amyloid protein. The ability of an agent to modulate renal function may also be evaluated in an appropriate animal model system.

The therapeutic agent of the invention may be also be administered *ex vivo* to inhibit amyloid deposition or treat certain amyloid-related diseases, such as  $\beta_2$ M amyloidosis and other amyloidoses related to dialysis. *Ex vivo* administration of the therapeutic agents of the invention can be accomplished by contacting a body fluid (e.g., blood, plasma, etc.) with a therapeutic compound of the invention such that the therapeutic compound is capable of performing its intended function and administering the body fluid to the subject. The therapeutic compound of the invention may perform its function *ex vivo* (e.g., dialysis filter), *in vivo* (e.g., administered with the body fluid), or both. For example, a therapeutic compound of the invention may be used to reduce plasma  $\beta_2$ M levels and/or maintain  $\beta_2$ M in its soluble form *ex vivo*, *in vivo*, or both.

### Blood-Brain Barrier

The compound may reverse or favor deposition of amyloid or the compound may favor plaque clearance or slow deposition. For example, the compound may decrease the amyloid concentration in the brain of a subject versus an untreated subject. The

compound may penetrate into the brain by crossing the blood-brain barrier ("BBB") to exert its biological effect. The compound may maintain soluble amyloid in a non-fibrillar form, or alternatively, the compound may increase the rate of clearance of soluble amyloid from the brain of a subject versus an untreated subject. The compound  
5 may also increase the rate of degradation of A $\beta$  in the brain prior to organization into fibrils. A compound may also act in the periphery, causing a change in the equilibrium of the amyloid protein concentration in the two compartments (*i.e.*, systemic *vs.* central), in which case a compound may not be required to penetrate the brain to decrease the concentration of A $\beta$  in the brain (a "sink" effect).

10 Agents of the invention that exert their physiological effect *in vivo* in the brain may be more useful if they gain access to target cells in the brain. Non-limiting examples of brain cells are neurons, glial cells (astrocytes, oligodendrocytes, microglia), cerebrovascular cells (muscle cells, endothelial cells), and cells that comprise the meninges. The blood brain barrier ("BBB") typically restricts access to brain cells by  
15 acting as a physical and functional blockade that separates the brain parenchyma from the systemic circulation (*see, e.g.*, Pardridge, *et al.*, *J. Neurovirol.* 5(6), 556-69 (1999); Rubin, *et al.*, *Rev. Neurosci.* 22, 11-28 (1999)). Circulating molecules are normally able to gain access to brain cells *via* one of two processes: lipid-mediated transport through the BBB by free diffusion, or active (or catalyzed) transport.

20 The agents of the invention may be formulated to improve distribution *in vivo*, for example as powdered or liquid tablet or solution for oral administration or as a nasal spray, nose drops, a gel or ointment, through a tube or catheter, by syringe, by packtail, by pledget, or by submucosal infusion. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic agents. To ensure that the more hydrophilic  
25 therapeutic agents of the invention cross the BBB, they may be formulated, for example, in liposomes. For methods of manufacturing liposomes, *see, e.g.*, U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs ("targeting moieties" or "targeting groups" or "transporting vectors"), thus providing targeted drug  
30 delivery (*see, e.g.*, V.V. Ranade *J. Clin. Pharmacol.* 29, 685 (1989)). Likewise, the agents may be linked to targeting groups that facilitate penetration of the blood brain barrier. In one embodiment, the method of the present invention employs a naturally occurring polyamine linked to an agent that is a small molecule and is useful for inhibiting *e.g.*, A $\beta$  deposition.

35 To facilitate transport of agents of the invention across the BBB, they may be coupled to a BBB transport vector (for review of BBB transport vectors and mechanisms, *see*, Bickel, *et al.*, *Adv. Drug Delivery Reviews* 46, 247-79 (2001)). Exemplary transport vectors include cationized albumin or the OX26 monoclonal

antibody to the transferrin receptor; these proteins undergo absorptive-mediated and receptor-mediated transcytosis through the BBB, respectively. Natural cell metabolites that may be used as targeting groups, include, *inter alia*, putrescine, spermidine, spermine, or DHA. Other exemplary targeting moieties include folate or biotin (*see, e.g.*,  
5 U.S. Pat. No. 5,416,016); mannosides (Umezawa, *et al.*, *Biochem. Biophys. Res. Commun.* 153, 1038 (1988)); antibodies (P.G. Bloeman, *et al.*, *FEBS Lett.* 357, 140 (1995); M. Owais, *et al.*, *Antimicrob. Agents Chemother.* 39, 180 (1995)); surfactant protein A receptor (Briscoe, *et al.*, *Am. J. Physiol.* 1233, 134 (1995)); gp120 (Schreier, *et al.*, *J. Biol. Chem.* 269, 9090 (1994)); *see also*, K. Keinänen and M.L. Laukkanen,  
10 *FEBS Lett.* 346, 123 (1994); J.J. Killian and I.J. Fidler, *Immunomethods* 4, 273 (1994).

Examples of other BBB transport vectors that target receptor-mediated transport systems into the brain include factors such as insulin, insulin-like growth factors ("IGF-I," and "IGF-II"), angiotensin II, atrial and brain natriuretic peptide ("ANP," and "BNP"), interleukin I ("IL-1") and transferrin. Monoclonal antibodies to the receptors  
15 that bind these factors may also be used as BBB transport vectors. BBB transport vectors targeting mechanisms for absorptive-mediated transcytosis include cationic moieties such as cationized LDL, albumin or horseradish peroxidase coupled with polylysine, cationized albumin or cationized immunoglobulins. Small basic oligopeptides such as the dynorphin analogue E-2078 and the ACTH analogue ebitatide may also cross the  
20 brain *via* absorptive-mediated transcytosis and are potential transport vectors.

Other BBB transport vectors target systems for transporting nutrients into the brain. Examples of such BBB transport vectors include hexose moieties, *e.g.*, glucose and monocarboxylic acids, *e.g.*, lactic acid and neutral amino acids, *e.g.*, phenylalanine and amines, *e.g.*, choline and basic amino acids, *e.g.*, arginine, nucleosides, *e.g.*,  
25 adenosine and purine bases, *e.g.*, adenine, and thyroid hormone, *e.g.*, triiodothyridine. Antibodies to the extracellular domain of nutrient transporters may also be used as transport vectors. Other possible vectors include angiotensin II and ANP, which may be involved in regulating BBB permeability.

In some cases, the bond linking the therapeutic agent to the transport vector may  
30 be cleaved following transport into the brain in order to liberate the biologically active agent. Exemplary linkers include disulfide bonds, ester-based linkages, thioether linkages, amide bonds, acid-labile linkages, and Schiff base linkages. Avidin/biotin linkers, in which avidin is covalently coupled to the BBB drug transport vector, may also be used. Avidin itself may be a drug transport vector.

35 Transcytosis, including receptor-mediated transport of compositions across the blood brain barrier, may also be suitable for the agents of the invention. Transferrin receptor-mediated delivery is disclosed in U.S. Pat. Nos. 5,672,683; 5,383,988; 5,527,527; 5,977,307; and 6,015,555. Transferrin-mediated transport is also known.

P.M. Friden, *et al.*, *Pharmacol. Exp. Ther.* 278, 1491-98 (1996); H.J. Lee, *J. Pharmacol. Exp. Ther.* 292, 1048-52 (2000). EGF receptor-mediated delivery is disclosed in Y. Deguchi, *et al.*, *Bioconjug. Chem.* 10, 32-37 (1999), and transcytosis is described in A. Cerletti, *et al.*, *J. Drug Target.* 8, 435-46 (2000). Insulin fragments have also been  
5 used as carriers for delivery across the blood brain barrier. M. Fukuta, *et al.*, *Pharm. Res.* 11, 1681-88 (1994). Delivery of agents *via* a conjugate of neutral avidin and cationized human albumin has also been described. Y.S. Kang, *et al.*, *Pharm. Res.* 1, 1257-64 (1994).

Other modifications in order to enhance penetration of the agents of the  
10 invention across the blood brain barrier may be accomplished using methods and derivatives known in the art. For example, U.S. Pat. No. 6,024,977 discloses covalent polar lipid conjugates for targeting to brain and central nervous system. U.S. Pat. No. 5,017,566 discloses cyclodextrin derivatives comprising inclusion complexes of lipoidal forms of dihydropyridine redox targeting moieties. U.S. Pat. No. 5,023,252 discloses the  
15 use of pharmaceutical compositions comprising a neurologically active drug and a compound for facilitating transport of the drug across the blood-brain barrier including a macrocyclic ester, diester, amide, diamide, amidine, diamidine, thioester, dithioester, thioamide, ketone or lactone. U.S. Pat. No. 5,024,998 discloses parenteral solutions of aqueous-insoluble drugs with cyclodextrin derivatives. U.S. Pat. No. 5,039,794 discloses  
20 the use of a metastatic tumor-derived egress factor for facilitating the transport of compounds across the blood-brain barrier. U.S. Pat. No. 5,112,863 discloses the use of *N*-acyl amino acid derivatives as antipsychotic drugs for delivery across the blood-brain barrier. U.S. Pat. No. 5,124,146 discloses a method for delivery of therapeutic agents across the blood-brain barrier at sites of increase permeability associated with brain  
25 lesions. U.S. Pat. No. 5,153,179 discloses acylated glycerol and derivatives for use in a medicament for improved penetration of cell membranes. U.S. Pat. No. 5,177,064 discloses the use of lipoidal phosphonate derivatives of nucleoside antiviral agents for delivery across the blood-brain barrier. U.S. Pat. No. 5,254,342 discloses receptor-mediated transcytosis of the blood-brain barrier using the transferrin receptor in  
30 combination with pharmaceutical compounds that enhance or accelerate this process. U.S. Pat. No. 5,258,402 discloses treatment of epilepsy with imidate derivatives of anticonvulsive sulfamate. U.S. Pat. No. 5,270,312 discloses substituted piperazines as central nervous system agents. U.S. Pat. No. 5,284,876 discloses fatty acid conjugates of dopamine drugs. U.S. Pat. No. 5,389,623 discloses the use of lipid dihydropyridine  
35 derivatives of anti-inflammatory steroids or steroid sex hormones for delivery across the blood-brain barrier. U.S. Pat. No. 5,405,834 discloses prodrug derivatives of thyrotropin releasing hormone. U.S. Pat. No. 5,413,996 discloses acyloxyalkyl phosphonate conjugates of neurologically-active drugs for anionic sequestration of such drugs in

brain tissue. U.S. Pat. No. 5,434,137 discloses methods for the selective opening of abnormal brain tissue capillaries using bradykinin infused into the carotid artery. U.S. Pat. No. 5,442,043 discloses a peptide conjugate between a peptide having a biological activity and incapable of crossing the blood-brain barrier and a peptide which exhibits  
5 no biological activity and is capable of passing the blood-brain barrier by receptor-mediated endocytosis. U.S. Pat. No. 5,466,683 discloses water soluble analogues of an anticonvulsant for the treatment of epilepsy. U.S. Pat. No. 5,525,727 discloses compositions for differential uptake and retention in brain tissue comprising a conjugate of a narcotic analgesic and agonists and antagonists thereof with a lipid form of  
10 dihydropyridine that forms a redox salt upon uptake across the blood-brain barrier that prevents partitioning back to the systemic circulation.

Nitric oxide is a vasodilator of the peripheral vasculature in normal tissue of the body. Increasing generation of nitric oxide by nitric oxide synthase causes vasodilation without loss of blood pressure. The blood-pressure-independent increase in blood flow  
15 through brain tissue increases cerebral bioavailability of blood-born compositions. This increase in nitric oxide may be stimulated by administering L-arginine. As nitric oxide is increased, cerebral blood flow is consequently increased, and drugs in the blood stream are carried along with the increased flow into brain tissue. Therefore, L-arginine may be used in the pharmaceutical compositions of the invention to enhance delivery of agents  
20 to brain tissue after introducing a pharmaceutical composition into the blood stream of the subject substantially contemporaneously with a blood flow enhancing amount of L-arginine, as described in WO 00/56328.

Still further examples of modifications that enhance penetration of the blood brain barrier are described in International (PCT) Application Publication Number  
25 WO 85/02342, which discloses a drug composition comprising a glycerolipid or derivative thereof. PCT Publication Number WO 089/11299 discloses a chemical conjugate of an antibody with an enzyme which is delivered specifically to a brain lesion site for activating a separately-administered neurologically-active prodrug. PCT Publication Number WO 91/04014 discloses methods for delivering therapeutic and  
30 diagnostic agents across the blood-brain barrier by encapsulating the drugs in liposomes targeted to brain tissue using transport-specific receptor ligands or antibodies. PCT Publication Number WO 91/04745 discloses transport across the blood-brain barrier using cell adhesion molecules and fragments thereof to increase the permeability of tight junctions in vascular endothelium. PCT Publication Number WO 91/14438 discloses the  
35 use of a modified, chimeric monoclonal antibody for facilitating transport of substances across the blood-brain barrier. PCT Publication Number WO 94/01131 discloses lipidized proteins, including antibodies. PCT Publication Number WO 94/03424 discloses the use of amino acid derivatives as drug conjugates for facilitating transport

across the blood-brain barrier. PCT Publication Number WO 94/06450 discloses conjugates of neurologically-active drugs with a dihydropyridine-type redox targeting moiety and comprising an amino acid linkage and an aliphatic residue. PCT Publication Number WO 94/02178 discloses antibody-targeted liposomes for delivery across the blood-brain barrier. PCT Publication Number WO 95/07092 discloses the use of drug-growth factor conjugates for delivering drugs across the blood-brain barrier. PCT Publication Number WO 96/00537 discloses polymeric microspheres as injectable drug-delivery vehicles for delivering bioactive agents to sites within the central nervous system. PCT Publication Number WO 96/04001 discloses omega-3-fatty acid conjugates of neurologically-active drugs for brain tissue delivery. PCT WO 96/22303 discloses fatty acid and glycerolipid conjugates of neurologically-active drugs for brain tissue delivery.

In general, it is well within the ordinary skill in the art to prepare an ester, amide or hydrazide derivative of an agent of the invention, for example, from the corresponding carboxylic acid and a suitable reagent. For instance, a carboxylic acid-containing compound, or a reactive equivalent thereof, may be reacted with a hydroxyl-containing compound, or a reactive equivalent thereof, so as to provide the corresponding ester. *See, e.g.*, "Comprehensive Organic Transformations," 2<sup>nd</sup> Ed., by R.C. Larock, VCH Publishers John Wiley & Sons, Ltd. (199989); "March's Advanced Organic Chemistry," 5<sup>th</sup> Ed., by M.B. Smith and J. March, John Wiley & Sons, Ltd. (2000).

### Prodrugs

The present invention is also related to prodrugs of the agents of the Formulae disclosed herein. Prodrugs are agents which are converted *in vivo* to active forms (*see, e.g.*, R.B. Silverman, 1992, "The Organic Chemistry of Drug Design and Drug Action," Academic Press, Chp. 8). Prodrugs can be used to alter the biodistribution (*e.g.*, to allow agents which would not typically enter the reactive site of the protease) or the pharmacokinetics for a particular agent. For example, a carboxylic acid group, can be esterified, *e.g.*, with a methyl group or an ethyl group to yield an ester. When the ester is administered to a subject, the ester is cleaved, enzymatically or non-enzymatically, reductively, oxidatively, or hydrolytically, to reveal the anionic group. An anionic group can be esterified with moieties (*e.g.*, acyloxymethyl esters) which are cleaved to reveal an intermediate agent which subsequently decomposes to yield the active agent. The prodrug moieties may be metabolized *in vivo* by esterases or by other mechanisms to carboxylic acids.

Examples of prodrugs and their uses are well known in the art (*see, e.g.*, Berge, *et al.*, "Pharmaceutical Salts", *J. Pharm. Sci.* 66, 1-19 (1977)). The prodrugs can be

prepared *in situ* during the final isolation and purification of the agents, or by separately reacting the purified agent in its free acid form with a suitable derivatizing agent. Carboxylic acids can be converted into esters *via* treatment with an alcohol in the presence of a catalyst.

- 5           Examples of cleavable carboxylic acid prodrug moieties include substituted and unsubstituted, branched or unbranched lower alkyl ester moieties, (*e.g.*, ethyl esters, propyl esters, butyl esters, pentyl esters, cyclopentyl esters, hexyl esters, cyclohexyl esters), lower alkenyl esters, dilower alkyl-amino lower-alkyl esters (*e.g.*, dimethylaminoethyl ester), acylamino lower alkyl esters, acyloxy lower alkyl esters  
10 (*e.g.*, pivaloyloxymethyl ester), aryl esters (phenyl ester), aryl-lower alkyl esters (*e.g.*, benzyl ester), substituted (*e.g.*, with methyl, halo, or methoxy substituents) aryl and aryl-lower alkyl esters, amides, lower-alkyl amides, dilower alkyl amides, and hydroxy amides.

15           Pharmaceutically Acceptable Salts

- Certain embodiments of the present agents can contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable acids. The term "pharmaceutically acceptable salts" in this respect, refers to the relatively non-toxic, inorganic and organic  
20 acid addition salts of agents of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the agents of the invention, or by separately reacting a purified agent of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed.

- Representative salts include the hydrohalide (including hydrobromide and  
25 hydrochloride), sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, 2-hydroxyethanesulfonate, and laurylsulphonate salts and the like. *See, e.g.*, Berge *et al.*, "Pharmaceutical Salts", *J. Pharm. Sci.* 66, 1-19 (1977).

- 30           In other cases, the agents of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of agents of the present invention.

- 35           These salts can likewise be prepared *in situ* during the final isolation and purification of the agents, or by separately reacting the purified agent 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 alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

“Pharmaceutically acceptable salts” also includes, for example, derivatives of agents modified by making acid or base salts thereof, as described further below and elsewhere in the present application. Examples of pharmaceutically acceptable salts include mineral or organic acid salts of basic residues such as amines; and alkali or organic salts of acidic residues such as carboxylic acids. Pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent agent formed, for example, from non-toxic inorganic or organic acids. Such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, and nitric acid; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, and isethionic acid. Pharmaceutically acceptable salts may be synthesized from the parent agent which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts may be prepared by reacting the free acid or base forms of these agents with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two.

All acid, salt, base, 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 and/or basic forms are also included.

#### Synthesis of Compounds of the Invention

In general, the compounds of the present invention may be prepared by the methods illustrated in the general reaction schemes as, for example, described below, or by modifications thereof, using readily available starting materials, reagents and conventional synthesis procedures. In these reactions, it is also possible to make use of variants which are in themselves known, but are not mentioned here. Functional and structural equivalents of the compounds described herein and which have the same general properties, wherein one or more simple variations of substituents are made which do not adversely affect the essential nature or the utility of the compound are also included.

The compounds of the present invention may be readily prepared in accordance with the synthesis schemes and protocols described herein, as illustrated in the specific procedures provided. However, those skilled in the art will recognize that other synthetic pathways for forming the compounds of this invention may be used, and that the following is provided merely by way of example, and is not limiting to the present invention. *See, e.g.*, "Comprehensive Organic Transformations" by R. Larock, VCH Publishers (1989). It will be further recognized that various protecting and deprotecting strategies will be employed that are standard in the art (*See, e.g.*, "Protective Groups in Organic Synthesis" by Greene and Wuts). Those skilled in the relevant arts will recognize that the selection of any particular protecting group (*e.g.*, amine and carboxyl protecting groups) will depend on the stability of the protected moiety with regards to the subsequent reaction conditions and will understand the appropriate selections.

Further illustrating the knowledge of those skilled in the art is the following sampling of the extensive chemical literature: "Chemistry of the Amino Acids" by J.P. Greenstein and M. Winitz, John Wiley & Sons, Inc., New York (1961); "Comprehensive Organic Transformations" by R. Larock, VCH Publishers (1989); T.D. Ocain, *et al.*, J. Med. Chem. 31, 2193-99 (1988); E.M. Gordon, *et al.*, J. Med. Chem. 31, 2199-10 (1988); "Practice of Peptide Synthesis" by M. Bodansky and A. Bodanszky, Springer-Verlag, New York (1984); "Protective Groups in Organic Synthesis" by T. Greene and P. Wuts (1991); "Asymmetric Synthesis: Construction of Chiral Molecules Using Amino Acids" by G.M. Coppola and H.F. Schuster, John Wiley & Sons, Inc., New York (1987); "The Chemical Synthesis of Peptides" by J. Jones, Oxford University Press, New York (1991); and "Introduction of Peptide Chemistry" by P.D. Bailey, John Wiley & Sons, Inc., New York (1992).

The synthesis of compounds of the invention is carried out in a solvent. Suitable solvents are liquids at ambient room temperature and pressure or remain in the liquid state under the temperature and pressure conditions used in the reaction. Useful solvents are not particularly restricted provided that they do not interfere with the reaction itself (that is, they preferably are inert solvents), and they dissolve a certain amount of the reactants. Depending on the circumstances, solvents may be distilled or degassed. Solvents may be, for example, aliphatic hydrocarbons (*e.g.*, hexanes, heptanes, ligroin, petroleum ether, cyclohexane, or methylcyclohexane) and halogenated hydrocarbons (*e.g.*, methylenechloride, chloroform, carbontetrachloride, dichloroethane, chlorobenzene, or dichlorobenzene); aromatic hydrocarbons (*e.g.*, benzene, toluene, tetrahydronaphthalene, ethylbenzene, or xylene); ethers (*e.g.*, diglyme, methyl-*tert*-butyl ether, methyl-*tert*-amyl ether, ethyl-*tert*-butyl ether, diethylether, diisopropylether, tetrahydrofuran or methyltetrahydrofurans, dioxane, dimethoxyethane, or

diethyleneglycol dimethylether); nitriles (*e.g.*, acetonitrile); ketones (*e.g.*, acetone); esters (*e.g.*, methyl acetate or ethyl acetate); and mixtures thereof.

In general, after completion of the reaction, the product is isolated from the reaction mixture according to standard techniques. For example, the solvent is removed  
5 by evaporation or filtration if the product is solid, optionally under reduced pressure. After the completion of the reaction, water may be added to the residue to make the aqueous layer acidic or basic and the precipitated compound filtered, although care should be exercised when handling water-sensitive compounds. Similarly, water may be added to the reaction mixture with a hydrophobic solvent to extract the target compound.  
10 The organic layer may be washed with water, dried over anhydrous magnesium sulphate or sodium sulphate, and the solvent is evaporated to obtain the target compound. The target compound thus obtained may be purified, if necessary, *e.g.*, by recrystallization, reprecipitation, chromatography, or by converting it to a salt by addition of an acid or base.

15 The compounds of the invention may be supplied in a solution with an appropriate solvent or in a solvent-free form (*e.g.*, lyophilized). In another aspect of the invention, the compounds and buffers necessary for carrying out the methods of the invention may be packaged as a kit. The kit may be commercially used for treating or preventing amyloid-related disease according to the methods described herein and may  
20 include instructions for use in a method of the invention. Additional kit components may include acids, bases, buffering agents, inorganic salts, solvents, antioxidants, preservatives, or metal chelators. The additional kit components are present as pure compositions, or as aqueous or organic solutions that incorporate one or more additional kit components. Any or all of the kit components optionally further comprise buffers.

25 It is to be understood that wherever values and ranges are provided herein, *e.g.*, in ages of subject populations, dosages, and blood levels, all values and ranges encompassed by these values and ranges, are meant to be encompassed within the scope of the present invention. Moreover, all values in these values and ranges may also be  
30 the upper or lower limits of a range.

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,  
35 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

The pilocarpine, MES and PTZ assays are performed by the Anticonvulsant Drug Development (ADD) Program in the Epilepsy Branch of the NIH (*see, e.g., Stables and Kupferberg (1997) The NIH anticonvulsant Drug Development (ADD) Program: Preclinical Anticonvulsant Screening Project, Libby & Sons*). All compounds are tested with either male Carworth Farms #1 mice or male Sprague-Dawley rats. Each test compound is administered *via* an i.p. injection at 300, 100, and 30 mg/kg.

#### 10 Pilocarpine Assay

A seizure model is performed using adult male Sprague-Dawley rats in accordance with the guidelines of the Canada Council on Animal Care and under the supervision of the Queen's University Animal Ethics Committee. This test procedure was adopted from previous work by Turski *et al.* (1984) *Brain Res.* 321:237. The test compounds are administered at 100mg/kg by intraperitoneal (i.p.) injection. Seizures are induced 20 minutes afterwards by i.p. administration of pilocarpine hydrochloride (350 mg/kg). Protection is defined as the absence of clonic spasms over a 30 minute observation period after pilocarpine administration.

#### 20 MES Induced Seizure Model

For the maximal electroshock seizure test (MES), corneal electrodes primed with a drop of electrolyte solution (0.9% NaCl) are applied to the eyes of the animal and an electrical stimulus (50 mA for mice, 150 mA for rats; 60 Hz) is delivered for 0.2 second at the time of the peak effect of the test compound. The animals are restrained by hand and are released at the moment of stimulation in order to permit observation of the seizure. Abolition of hind-leg tonic-extensor component (hind-leg tonic extension does not exceed a 90° angle to the plane of the body) indicates that the compound prevents MES-induced seizure spread.

#### 30 PTZ Induced Seizure Model

In the subcutaneous pentylenetetrazole (PTZ)-induced seizure model, seizures are induced 0.5 and 4 hrs after test compound administration by i.p. injection of PTZ (85mg/kg in mice and 70 mg/kg in rats). Protection is defined as the inhibition of clonic spasms over a 30 min observation period.

35

#### Measurement of GABA<sub>A</sub> Receptor Binding In Rat Brain Membranes Using [<sup>3</sup>H]GABA

In this assay, [<sup>3</sup>H]GABA is used to determine binding (Enna SJ et al. Current protocols in pharmacology. New York: John Wiley & Sons, 1998). Specificity is

increased by treating the membranes with detergent. Incubation with Triton X-100 and multiple resuspensions and centrifugations destroys neuronal GABA uptake sites that may bind [ $^3\text{H}$ ]GABA and rids the tissue of endogenous GABA, which competes for binding sites with the radioligand. Detergent treatment is less important when [ $^3\text{H}$ ]muscimol is used to label GABA<sub>A</sub> receptors, since muscimol has a low affinity for the other subtypes of GABA transporters. [ $^3\text{H}$ ]muscimol may also be used for more specificity to measure binding to the GABA<sub>A</sub> receptor.

#### Preparation of Membranes

Crude synaptosomal membrane fractions are prepared from fresh brain as follows.

#### Materials

Fresh brain sample  
0.32 M sucrose, ice cold  
Potter-Elvehjem glass homogenizer with Teflon pestle  
Refrigerated centrifuge (Sorvall RC-5 with SS-34 and SM-24 rotors or equivalent)  
Tissue homogenizer (Polytron, Brinkmann; Tissumizer, Tekmar)  
10% Triton X-100 in Tris citrate buffer (see Basic Protocol 1 for buffer)  
[ $^3\text{H}$ ]GABA<sub>A</sub> (NEN Life Sciences)  
Tissue solubilizer (e.g., BTS-450, Beckman)  
Scintillation cocktail compatible with organic solvents  
15-ml polypropylene centrifuge tubes

#### Preparation of the membranes

Fresh brain tissue is placed into a 15 vol ice-cold 0.32 M sucrose solution in a Potter-Elvehjem glass homogenizer fitted with a Teflon pestle, and homogenized. The homogenate is then centrifuged for 10 minutes at 1000 x g, 4°C. The resultant pellet is discarded and the supernatant is centrifuged for 20 minutes at 20,000 x g, 4°C. The resultant pellet is resuspended in 20 ml ice-cold distilled water using a tissue homogenizer (midpoint setting) for 30 seconds. The suspension is then centrifuged at 20 minutes at 8000 x g, 4°C. The tube is then gently agitated by hand to suspend the soft buffy coat surrounding the pellet into the supernatant without disturbing the pellet itself. The pellet is then discarded and the suspension is centrifuged for 20 minutes at 48,000 x g, 4°C. The resultant supernatant is then discarded and the pellet (crude synaptic membrane pellet) is stored for at least 18 hr at -20°C prior to use for a GABA receptor binding assay.

### Preparation of GABA<sub>A</sub> receptors

In 50-ml polypropylene centrifuge tubes, previously frozen tissue or brain membranes are resuspended in 50 vol ice-cold Tris citrate buffer using the tissue homogenizer. The homogenate is centrifuged for 10 minutes at 50,000 x g at 4°C. The resultant pellet is resuspended in sufficient Tris citrate buffer to yield a concentration of 1 mg protein/ml. Then, sufficient 10% Triton X-100 in Tris citrate buffer is added to yield a 0.05% (v/v) concentration of detergent. The suspension is then incubated 20 minutes in a 37°C water bath and centrifuged for 10 minutes at 50,000 x g at 4°C. The resultant pellet is resuspended and centrifuged twice as before. Afterwards, the pellet is resuspended in sufficient Tris acetate buffer to yield a final concentration of 0.5 mg protein/ml.

### Measure [<sup>3</sup>H]GABA binding to GABA<sub>A</sub> receptors

For the competition assay, the following components are assembled in 1-ml volumes, diluted with Tris citrate buffer and placed in separate 15-ml polypropylene tubes on ice:

- 8.0 nM [<sup>3</sup>H]GABA (to determine total binding).
- 8.0 nM [<sup>3</sup>H]GABA + [200 μM (-)-bicuculline methiodine or 20 μM muscimol] (to determine nondisplace binding);
- 8.0 nM [<sup>3</sup>H]GABA + various concentrations of unlabeled compound.

All assays are performed in duplicate or triplicate. The final concentrations of [<sup>3</sup>H]GABA, bicuculline methiodine, and muscimol in the final 2-ml incubation volume are 4 nM, 100 μM, and 10 μM, respectively. The unlabeled bicuculline or unlabeled muscimol is used to define nonspecific binding (blank) which when subtracted from total binding (tissue in tubes containing [<sup>3</sup>H]GABA alone) reveals the amount of radioligand bound to the GABA<sub>A</sub> receptor.

To generate binding site saturation data by displacement, 1-ml solutions are prepared in tubes as described above but containing:

- 8.0 nM [<sup>3</sup>H]GABA;
- 8.0 nM [<sup>3</sup>H]GABA + various concentrations (2.0 to 1000 nM) of unlabeled GABA.

To begin the assays, 1 ml of the tissue suspension is added to each of the chilled tubes and gently vortexed to mix the contents of each tube. The final tissue concentration in the 2-ml incubation volume is about 0.25 mg protein/ml, which is

within the tissue linearity range for [ $^3\text{H}$ ]GABA binding to GABA<sub>A</sub> receptors. The tubes are incubated 5 minutes in an ice-water bath (4°C) to achieve binding equilibrium. The binding reaction is terminated by centrifuging the mixture 10 minutes at 50,000 x g, 4°C. The radioactive supernatant is discarded, and the tissue pellets are washed rapidly and superficially three times with 5 ml ice-cold Tris citrate buffer.

After 1 ml of tissue solubilizer is placed into each tube, the tissue is allowed to dissolve at room temperature. Once the tissue is dissolved, 4 ml of an organic solvent-compatible scintillation cocktail is added. The contents of each tube are placed into individual scintillation vials, and radioactivity is quantified using liquid scintillation spectrometry.

The data may be presented as the fraction of bound [ $^3\text{H}$ ]GABA (y-axis) over the log of the concentration of the competitor molecule.

#### SRS Model of Epilepsy

The "spontaneous recurrent seizures" (SRS) model of epilepsy is used to evaluate candidate compounds in a model for Phase 1 epileptogenesis (see, e.g., Mello, E. *et al.*, *Epilepsia* (1993) 34:985; Cavaleiro, J. *et al.*, *Epilepsia* (1991) 32:778). In the SRS model, an adult male Sprague-Dawley rat (c. 260 g) is given kainic acid by injection (380 mg/kg i.p.). Within 25 minutes, the animal enters *status epilepticus*, which typically lasts for 15-20 hours (although about 10% of animals die at this stage). The rat is allowed to spontaneously recover and is given food and water *ad lib.* and maintained on a 12 hour light/dusk cycle. Beginning on about day 13-15, the rats develop spontaneous recurrent seizures, which occur at the rate of about 4-5 per week. The rats are videotaped 8 hours per day, and the videotapes are reviewed for behavioral seizures (including head nodding, forelimb clonus, and rearing), which are counted. The animals are watched for a few weeks to a few months (2 weeks to three months), permitting evaluation of a sufficient number of seizures. An experimental compound for evaluation can be administered at either of two times: Time 1, on Day 1, after the cessation of *status epilepticus* but before the onset of SRS; or Time 2, on Day 30, when the rats have been experiencing SRS for about two weeks. Administration of the candidate compound at Time 1 permits evaluation for anti-epileptogenic properties (ability to prevent the onset of seizures); administration of compounds at Time 2 permits evaluation of drugs as anti-ictogenics with the ability to suppress established seizures.

As a reference, the standard anticonvulsant phenytoin was administered (20 mg/kg/day i.v. for 10 day) at either Time 1 or Time 2. As expected, phenytoin was ineffective in preventing the onset of seizures when administered at Time 1, but was 75% effective at decreasing seizure frequency by 50% or more when administered at Time 2.

*Binding and Antifibrillogenic Assays*

Test compounds were purchased from commercial sources or synthesized and screened by mass spectroscopy ("MS") assay. The MS assay gives data on the ability of compounds to bind to an amyloid.

- 5 In the MS assay for A $\beta$ 40, samples were prepared as aqueous solutions (adding 20% ethanol if necessary to solubilize in water), 200  $\mu$ M of a test compound and 20  $\mu$ M of solubilized A $\beta$ 40, or 400  $\mu$ M of a test compound and 40  $\mu$ M of solubilized A $\beta$ 40. The pH value of each sample was adjusted to 7.4 ( $\pm$ 0.2) by addition of 0.1% aqueous sodium hydroxide. The solutions were then analyzed by electrospray ionization mass
- 10 spectrometry using a Waters ZQ 4000 mass spectrometer. Samples were introduced by direct infusion at a flow-rate of 25  $\mu$ L/min within 2 hr. after sample preparation. The source temperature was kept at 70 °C and the cone voltage was 20 V for all the analysis. Data were processed using Masslynx 3.5 software. The MS assay gives data on the ability of compounds to bind to soluble A $\beta$ , whereas the ThT, EM and CD assays give
- 15 data on inhibition of fibrillogenesis. The results from the assay for binding to A $\beta$  are summarized in Table 3.

- The assay for IAPP was conducted under the same conditions except that 200  $\mu$ M of test compound and 20  $\mu$ M of solubilized IAPP were employed. The key below describes the codes used in Table 3 to quantify the binding based on the intensity of the
- 20 absorption.

Key to Table 3

| A $\beta$ 1-40               |      |                           |                           |
|------------------------------|------|---------------------------|---------------------------|
|                              | Code | 400 $\mu$ M               | 200 $\mu$ M               |
| Strong Binding               | +++  | 90-100%                   | 60-100%                   |
| Moderate Binding             | ++   | 70-89%                    | 30-69%                    |
| Weak Binding                 | +    | 45-59%                    | 20-44%                    |
| Little/no detectable binding | -    | 20-39%                    | 20-39%                    |
| IAPP                         |      |                           |                           |
|                              |      | 200 $\mu$ M<br>(20% EtOH) | 100 $\mu$ M<br>(20% EtOH) |
| Strong Binding               | +++  | > 75%                     | > 50%                     |
| Moderate Binding             | ++   | 40-75%                    | 30-50%                    |
| Weak Binding                 | +    | 20-40%                    | 15-30%                    |
| Little/no detectable binding | -    | 0-20%                     | 0-15%                     |

An ultraviolet absorption assay is also available, and this assay gives an indication of the ability of test compounds to bind to (fibrillar) A $\beta$ . The experiments are carried out in a blinded fashion. Test compound at 20  $\mu$ M is incubated with 50  $\mu$ M A $\beta$ (1-40) fibers for 1 hour at 37°C in Tris buffered saline (20 mM Tris, 150 mM NaCl, pH 7.4 containing 0.01 % sodium azide). Following incubation, the solution is centrifuged for 20 min at 21,000 g to sediment the A $\beta$ (1-40) fibers along with any bound test compound. The amount of test compound remaining in the supernatant is determined by reading the absorbance. The fraction of test compound bound is then calculated by comparing the amount remaining in the supernatants of incubations with A $\beta$  to the amount remaining in control incubations which do not contain A $\beta$  fibers. Thioflavin T and Congo Red, both of which are known to bind to A $\beta$  fibers, are included in each assay run as positive controls. Before assaying, test compounds are diluted to 40  $\mu$ M, which is twice the concentration in the final test, and then they are scanned using the Hewlett Packard 8453 UV/VIS spectrophotometer to determine if the absorbance is sufficient for detection.

Table 3

| ID # | M.W.   | Structure | MS Results<br>20% Ethanol |                |
|------|--------|-----------|---------------------------|----------------|
|      |        |           | IAPP                      | A $\beta$ 1-40 |
| A    | 307.36 |           | +                         | +++            |
| B    | 327.78 |           | +                         | +++            |
| C    | 293.34 |           | +                         | +++            |
| D    | 361.34 |           | +                         | +++            |
| E    | 362.23 |           | +                         | +++            |
| F    | 485.44 |           | +++                       | +              |

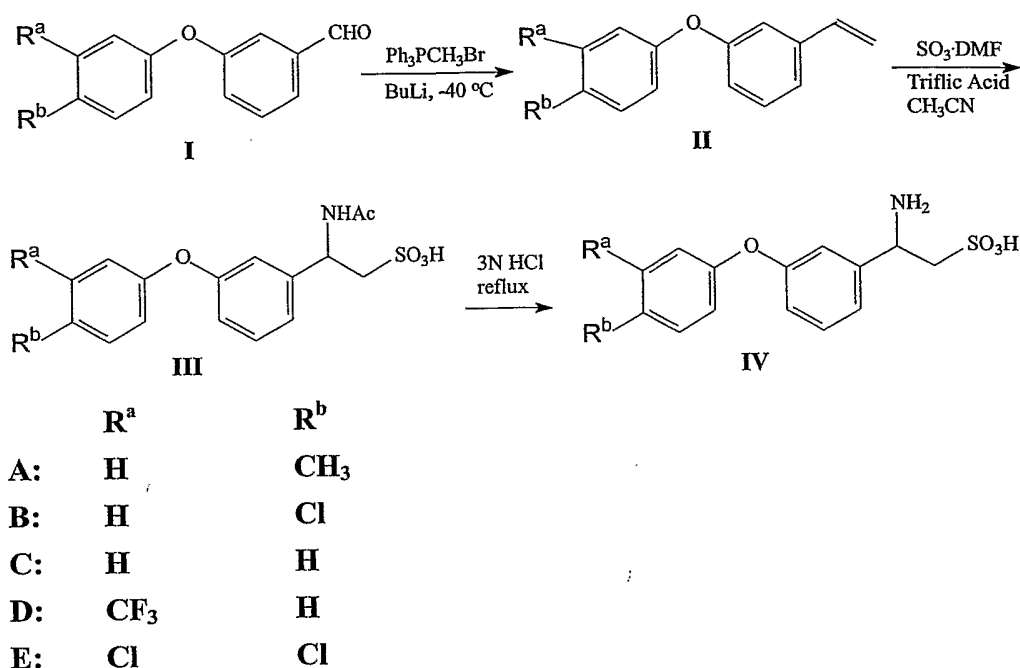
|   |        |  |  |     |
|---|--------|--|--|-----|
| G | 307.36 |  |  | +++ |
| H | 235.69 |  |  | ++  |
| I | 219.23 |  |  | +   |
| J | 215.27 |  |  | ++  |
| K | 235.69 |  |  | -   |
| L | 201.24 |  |  | -   |
| M | 280.14 |  |  | -   |
| N | 215.27 |  |  | +   |
| O | 251.3  |  |  | +   |
| P | 280.14 |  |  | -   |
| Q | 215.27 |  |  | +   |

The present invention also relates to novel compounds and the synthesis thereof. Accordingly, the following examples are presented to illustrate how some of those compounds may be prepared.

5

#### Synthesis of Compounds of the Invention

Racemic compounds and N-acetylated derivatives of compounds of the invention may be synthesized according to Scheme 1.



Scheme 1

*Wittig Reaction*

The starting aldehydes (**I**) are commercially available from Sigma-Aldrich. To a suspension of methyltriphenylphosphonium bromide (21.43 g, 60 mmol, 2 eq.) in THF (150 mL, anhydrous) at  $-40\text{ }^\circ\text{C}$ , was added *n*-BuLi (2.5 M in hexanes, 24 mL, 2 eq.). The mixture was stirred at  $-20\text{ }^\circ\text{C}$  for 45 min. Then the mixture was cooled to  $-30\text{ }^\circ\text{C}$ , and a solution of the aldehyde (30 mmol, 1 eq.) in THF (30 mL, anhydrous) was added to the mixture via a syringe. The reaction mixture was then warmed to rt and stirred for 3 h. Upon TLC showed the total disappearance of the starting aldehyde, water (100 mL) was added to quench the reaction. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with water and brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated *in vacuo*. Purification was performed using flash column chromatography (5% ethyl acetate in hexanes) to provide styrene **II** as a colorless oil (~90% yield).

**4-Methylphenoxy styrene (IIa)** colorless oil,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  ppm 7.29-7.26 (m, 1H), 7.16-7.12 (m, 3H), 7.05 (s, 1H), 6.94-6.87 (m, 3H), 6.67 (dd, 1H,  $J = 17.5, 11\text{ Hz}$ ), 5.72 (d, 1H,  $J = 17.5\text{ Hz}$ ), 5.25 (d, 1H,  $J = 11\text{ Hz}$ ), 2.35 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  ppm 158.26, 154.89, 139.61, 136.58, 133.16, 130.48, 129.92, 121.16, 119.29, 118.02, 116.21, 114.76, 20.94.

*Amino sulfonation of alkene*

SO<sub>3</sub>·DMF (30 mmol, 4.6 g, 1 eq.) was dissolved in acetonitrile (30 mL, anhydrous), then trifluoromethanesulfonic acid (triflic acid) (30 mmol, 2.64 mL) was added to the solution. The solution was stirred under nitrogen. The styrene derivative **II** (30 mmol) obtained from the Wittig reaction was dissolved in acetonitrile (10 mL) and was added to the solution via syringe. The reaction mixture was stirred overnight. Upon the total disappearance of the starting material monitored by TLC, the reaction mixture was concentrated to give a yellow gel as the crude product. MS showed as the desired product. No purification was conducted on this step.

*Deacetylation*

The crude product obtained from the previous step was dissolved in 3N HCl (20 mL) and refluxed overnight. Solid was formed during the reflux. The reaction was cooled to room temperature and concentrated to half of the volume. The solid was collected by vacuum filtration and purified by recrystallization from H<sub>2</sub>O/ acetonitrile.

**2-Amino-2-[3-(4-methylphenoxy)phenyl]ethanesulfonic acid (Compound A):** 40.1 % overall yield from the aldehyde, white solid, <sup>1</sup>H NMR (DMSO, 500 MHz) δ ppm 8.27 (s, 3H), 7.41-7.31 (m, 1H), 7.22-7.19 (m, 4H), 6.94-6.92 (m, 3H), 4.51 (d, *J* = 9 Hz, 1H), 3.10 (dd, *J* = 14.5, 11.5 Hz, 1H), 2.84 (dd, *J* = 14, 2.5 Hz, 1H), 2.30 (s, 3H). <sup>13</sup>C NMR (DMSO, 125 MHz) δ ppm 157.43, 153.74, 139.10, 132.94, 130.47, 130.42, 121.86, 119.04, 118.09, 116.97, 53.82, 51.78, 20.28.

**2-Amino-2-[3-(4-chlorophenoxy)phenyl]ethanesulfonic acid (Compound B):** white solid, 44.1 % overall yield from the aldehyde, <sup>1</sup>H NMR (DMSO, 500 MHz) δ ppm 8.28 (br, 3H), 7.47-7.42 (m, 3H), 7.26-7.21 (m, 2H), 7.07-7.02 (m, 3H), 4.54 (br, 1H), 3.10 (dd, 1H, *J* = 14, 11.5 Hz), 2.86 (dd, 1H, *J* = 14, 2 Hz); <sup>13</sup>C NMR (DMSO, 125 MHz) δ ppm 155.41, 155.28, 139.31, 130.61, 129.91, 127.41, 122.76, 120.40, 118.92, 117.69, 53.78, 51.68.

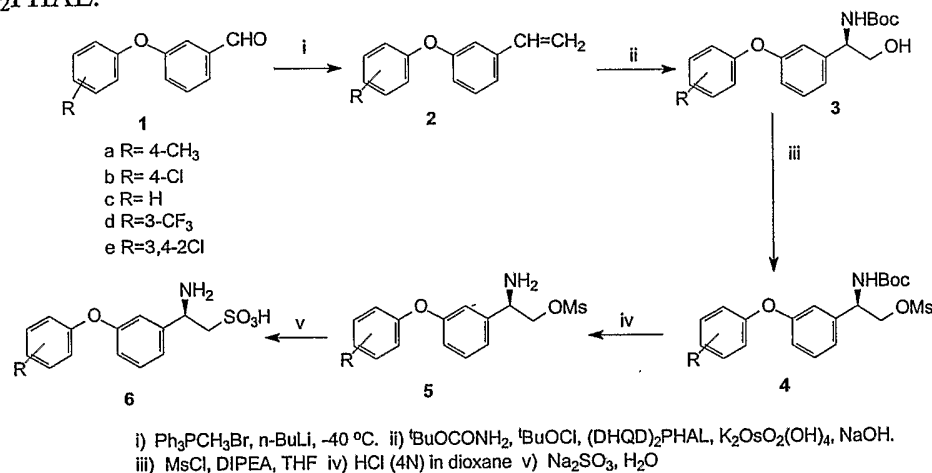
**2-Amino-2-(3-phenoxy)phenylethanesulfonic acid (Compound C),** white solid, 30.7 % overall yield from the aldehyde, <sup>1</sup>H NMR (DMSO, 500 MHz) δ ppm 8.27 (br, 3H), 7.42-7.38 (m, 3H), 7.22-7.13 (m, 3H), 7.02-6.96 (m, 3H), 4.51 (dd, 1H, *J* = 11.5, 2 Hz), 3.08 (dd, *J* = 14, 11 Hz, 1H), 2.85 (dd, *J* = 14.5, 2.5 Hz, 1H), <sup>13</sup>C NMR (DMSO, 125 MHz) δ ppm 156.78, 156.28, 139.20, 130.52, 130.10, 123.67, 122.33, 118.71, 118.67, 117.63, 53.78, 51.74. ES-MS 294 (M+1).

**2-Amino-2-[3-(3-trifluoromethoxyphenoxy)phenyl]ethanesulfonic acid (Compound D)**, 36.8 % overall yield from aldehyde, white solid,  $^1\text{H}$  NMR (DMSO, 500 MHz)  $\delta$  ppm 8.30 (s, 3H), 7.65-7.62 (m, 1H), 7.52-7.49 (m, 2H), 7.32-7.29 (m, 4H), 7.10-7.08 (m, 1H), 4.57 (dd, 1H,  $J = 11, 2$  Hz), 3.12 (dd, 1H,  $J = 14, 11$  Hz), 2.89 (dd, 1H,  $J = 14.5, 2.5$  Hz).  $^{13}\text{C}$  NMR (DMSO, 125 MHz)  $\delta$  ppm 157.84, 156.43, 140.19, 132.12, 131.48, 124.08, 122.90, 120.71, 120.68, 120.20, 119.08, 115.55, 115.52, 54.46, 52.34. ES-MS 362 (M+1).

**2-Amino-2-[3-(3,4-dichlorophenoxy)phenyl]ethanesulfonic acid (Compound E)**, 45.7 % overall yield from the aldehyde, white solid,  $^1\text{H}$  NMR (DMSO, 500 MHz)  $\delta$  ppm 8.28 (s, 3H), 7.65-7.64 (m, 1H), 7.48-7.45 (m, 1H), 7.30-7.26 (m, 3H), 7.10-7.09 (m, 1H), 7.05-7.02 (m, 1H), 4.56 (d, 1H,  $J = 11.5$  Hz), 3.10 (dd, 1H,  $J = 14, 11.5$  Hz), 2.88 (dd, 1H,  $J = 14.5, 1.5$  Hz).  $^{13}\text{C}$  NMR (DMSO, 125 MHz)  $\delta$  ppm 156.12, 155.77, 139.47, 132.04, 131.61, 130.75, 125.54, 123.41, 120.41, 119.46, 118.88, 118.17, 3.76, 51.63. ES-MS 363 (M+1).

### Synthesis of chiral (D, or L) compounds

Scheme 2 describes the synthesis of D-isomers. The corresponding L-isomers are synthesized in the same way by using the mirror-image of the chiral-reagent – (DHQD)<sub>2</sub>PHAL.



**Scheme 2**

### Wittig reaction

The starting aldehydes (1) are commercially available from Sigma-Aldrich. To a suspension of methyltriphenylphosphonium bromide (21.43 g, 60 mmol, 2 eq.) in THF (150 mL, anhydrous) at  $-40^\circ\text{C}$ , was added  $n\text{-BuLi}$  (2.5 M in hexanes, 24 mL, 2 eq.). The mixture was stirred at  $-20^\circ\text{C}$  for 45 min. Then the mixture was cooled to  $-30^\circ\text{C}$ , and a solution of the aldehyde (1) (30 mmol, 1 eq.) in THF (30 mL, anhydrous) was added to the mixture via a syringe. The reaction mixture was then warmed to rt and

stirred for 3 h. Upon TLC showed the total disappearance of the starting aldehyde (1), water (100 mL) was added to quench the reaction. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with water and brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrate *in vacuo*.

- 5 Purification was performed using flash column chromatography (5% ethyl acetate in hexanes) to provide styrene **2** as a colorless oil (~90% yield).

**4-Methylphenoxystyrene (2a)** colorless oil, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ ppm 7.29-7.26 (m, 1H), 7.16-7.12 (m, 3H), 7.05 (s, 1H), 6.94-6.87 (m, 3H), 6.67 (dd, 1H, *J* = 17.5, 11 Hz), 5.72 (d, 1H, *J* = 17.5 Hz), 5.25 (d, 1H, *J* = 11 Hz), 2.35 (s, 3H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ ppm 158.26, 154.89, 139.61, 136.58, 133.16, 130.48, 129.92, 121.16, 119.29, 118.02, 116.21, 114.76, 20.94.

#### *Sharpless Aminohydroxylation*

- 15 To a solution of *t*-butyl carbamate (3.16 g, 27 mmol, 3.1 eq.) in *n*-propanol (35 mL) was treated with aqueous NaOH (50 mL, 0.4 N) and *t*-BuOCl (27 mmol, 3.1 mL, 3.1 eq.). Then the solution was cooled to 0 °C. (DHQD)<sub>2</sub>PHAL (0.435 mmol, 340 mg, 0.05 eq.) in *n*-propanol (35 mL) was added. The solution was stirred for 10 min. 4-Chlorophenoxystyrene (**2b**) (8.7 mmol, 2 g, 1 eq.) in *n*-propanol (35 mL) was added, then followed by a solution of potassium osmate (160 mg, 0.35 mmol, 4% eq.) in aqueous NaOH (15 mL, 0.4 N). The solution was light green at the starting of the reaction, then gradually turned into light yellow. After 1 h, the starting material was totally disappeared, aqueous saturated sodium sulfite solution (30 mL) was added to quench the reaction. The mixture was stirred for 30 min. Then the mixture was concentrated under vacuum, and extracted with ethyl acetate (3 x 40 mL). The combined organic phases were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The product was purified by silica gel flash column chromatography (40% ethyl acetate in hexanes) to give **3b**, as white solid, 1.35 g, 42% yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ ppm 7.29-7.24 (m, 3H), 7.04-7.03 (m, 1H), 6.95-6.85 (m, 4H), 5.52 (br., 1H), 4.71 (br, 1H), 3.76-3.68 (br., 2H), 3.18 (br., 1H), 1.43 (s, 9H).

In the same manner, **3a**, **3c** to **3e** were prepared.

#### *Mesylation*

- 35 To a solution of compound **3b** (960 mg, 2.6 mmol) in THF (20 mL, anhydrous) was added DIPEA (diisopropylethylamine, 10 mmol, 1.74 mL, 3.8 eq.) via syringe. The solution was then cooled to 0 °C. Methanesulfonyl chloride (4 mmol, 0.31 mL, 1.5 eq.) was added dropwise via syringe. The reaction was stirred at rt overnight. The reaction was quenched by adding saturated aqueous NH<sub>4</sub>Cl solution (10 mL). The mixture was

extracted with DCM (3 x 30 mL). The combined organic layers were washed with brine and dried over Na<sub>2</sub>SO<sub>4</sub>. Compound **4b** (950 mg, 82%) was obtained as white solid via silica gel flash column chromatography (30% ethyl acetate in hexanes).

- 5 **4b** White solid, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ ppm 7.36-7.26 (m, 4H), 7.08-7.06 (m, 1H), 6.96-6.92 (m, 3H), 5.20-5.10 (br, 1H), 4.90-5.10 (br, 1H), 4.50-4.40 (br, 1H), 4.40-4.35 (br, 1H), 2.94 (s, 3H), 1.43 (s, 9H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ ppm 157.66, 155.68, 155.26, 140.22, 130.62, 130.09, 128.85, 121.97, 120.49, 118.60, 117.31, 80.62, 71.23, 53.63, 37.75, 28.51

10 *Deprotection of Boc*

Compound **4b** (950 mg, 2.15 mmol) obtained from the previous step was dissolved in HCl (10 mL, 4N in dioxane). The solution was stirred at rt overnight. Then the solvent was removed by reduced pressure. The light yellow solid obtained was recrystallised with H<sub>2</sub>O/acetonitrile to give **5b** (360 mg, 50%) as white solid.

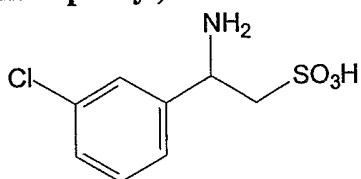
- 15 **5b** white solid, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ ppm 9.13 (s, 3H), 7.32-7.27 (m, 4H), 7.19 (s, 1H), 6.98-6.93 (m, 3H), 4.68-4.62 (m, 2H), 4.56-4.53 (m, 1H), 3.13 (s, 3H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ ppm 157.95, 155.09, 133.84, 131.30, 130.23, 129.30, 122.58, 120.87, 119.91, 118.41, 67.41, 54.62, 38.25.

20 *SN<sub>2</sub> replacement*

To a solution of compound **5a** (400 mg) in water (10 mL), was added sodium sulfite (1 g). The milky mixture was stirred overnight. The mixture was then extracted with ethyl acetate (3 x 10 mL). The combined organic layers were washed with brine and dried over Na<sub>2</sub>SO<sub>4</sub>. The final product (**6a**, **Compound G**) was obtained via

- 25 preparative HPLC. Yield 60 mg, white solid. [α]<sub>D</sub><sup>20</sup> +6.67 (2.7, MeOH). <sup>1</sup>H NMR (DMSO, 500 MHz) δ ppm 8.27 (s, 3H), 7.41-7.31 (m, 1H), 7.22-7.19 (m, 4H), 6.94-6.92 (m, 3H), 4.51 (d, *J* = 9 Hz, 1H), 3.10 (dd, *J* = 14.5, 11.5 Hz, 1H), 2.84 (dd, *J* = 14, 2.5 Hz, 1H), 2.30 (s, 3H). <sup>13</sup>C NMR (DMSO, 125 MHz) δ ppm 157.43, 153.74, 139.10, 132.94, 130.47, 130.42, 121.86, 119.04, 118.09, 116.97, 53.82, 51.78, 20.28. ES-MS  
30 307 (M+1).

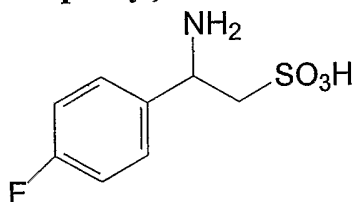
**Synthesis of 2-Amino-2-(3-chlorophenyl)ethanesulfonic acid (Compound H)**



To a solution of SO<sub>3</sub>DMF complex (1.22 g, 8.0 mmol) and triflic acid (0.7 mL, 8 mmol) in acetonitrile (20 mL, anhydrous) was added dropwise 3-chlorostyrene (1.0 g, 7.22 mmol) in acetonitrile (5 mL, anhydrous) at room temperature. The reaction mixture was stirred at room temperature overnight. H<sub>2</sub>O was added, the mixture was continued to stir for 3 hours. Concentration *in vacuo* afforded the crude acetylaminosulfonic acid, which could be directly used for subsequent hydrolysis. The crude product was dissolved in 3N HCl solution and then heated at a vigorous reflux overnight. The solution was then partially concentrated in vacuo and diluted with acetonitrile to precipitate the aminosulfonic acid, which was filtered, washed with acetonitrile.

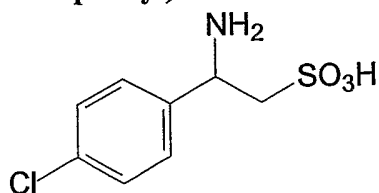
The product 2-Amino-2-(3-chlorophenyl)ethanesulfonic acid, white solid was obtained in 880 mg, yield 52 %. <sup>1</sup>H NMR (DMSO, 500 MHz) δ 8.31 (s, 3H), 7.59 (s, 1H), 7.44 (s, 3H), 4.57 (d, 1H, J=8.5 Hz), 3.14 (dd, 1H, J=14.5, 11.5 Hz), 2.90 (dd, 1H, J=14, 3 Hz). <sup>13</sup>C NMR (DMSO, 125 MHz) δ 139.37, 133.27, 130.68, 128.69, 127.50, 126.27, 53.52, 51.46.

#### Synthesis of 2-Amino-2-(4-fluorophenyl)ethanesulfonic acid (Compound I)



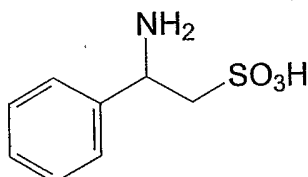
To a solution of SO<sub>3</sub>DMF complex (1.53 g, 10 mmol) and triflic acid (0.88 mL, 10 mmol) in acetonitrile (20 mL, anhydrous) was added dropwise 4-fluorostyrene (1.0 g, 8.2 mmol) in acetonitrile (5 mL, anhydrous) at room temperature. The reaction mixture was stirred at room temperature overnight. H<sub>2</sub>O was added, the mixture was continued to stir for 3 hours. Concentration in vacuo afforded the crude acetylaminosulfonic acid, which could be directly used for subsequent hydrolysis. The crude product was dissolved in 3N HCl solution and then heated at a vigorous reflux overnight. The solution was then partially concentrated in vacuo and diluted with acetonitrile to precipitate the aminosulfonic acid, which was filtered, washed with acetonitrile.

2-Amino-2-(4-fluorophenyl)ethanesulfonic acid was obtained as a white solid (1.43 g), yield 79.5 %. <sup>1</sup>H NMR (DMSO, 500 MHz) δ 8.27 (s, 3H), 7.54-7.51 (m, 2H), 7.27-7.24 (m, 2H), 4.56 (d, 1H, J=9.5 Hz), 3.15 (dd, 1H, J=14, 11.5 Hz), 2.85 (dd, 1H, J=14, 2.5 Hz). <sup>13</sup>C NMR (DMSO, 125 MHz) δ 162.13 (d, J=242.5 Hz), 133.36 (d, J=2.88 Hz), 129.75 (d, J=8.5 Hz), 115.60 (d, J=17.6 Hz), 53.74, 51.31.

**Synthesis of 2-Amino-2-(4-chlorophenyl)ethanesulfonic Acid (Compound K)**

To a solution of  $\text{SO}_3\cdot\text{DMF}$  complex (1.8 g, 12 mmol) and triflic acid (1.05 mL, 12 mmol) in acetonitrile (20 mL, anhydrous) was added dropwise 4-chlorostyrene (1.38 g, 10 mmol) in acetonitrile (5 mL, anhydrous) at room temperature. The reaction mixture was stirred at room temperature overnight.  $\text{H}_2\text{O}$  was added, the mixture was continued to stir for 3 hours. Concentration *in vacuo* afforded the crude acetylaminosulfonic acid, which could be directly used for subsequent hydrolysis. The crude product was dissolved in 3N HCl solution and then heated at a vigorous reflux overnight. The solution was then partially concentrated in vacuo and diluted with acetonitrile to precipitate the animosulfonic acid, which was filtered, washed with acetonitrile.

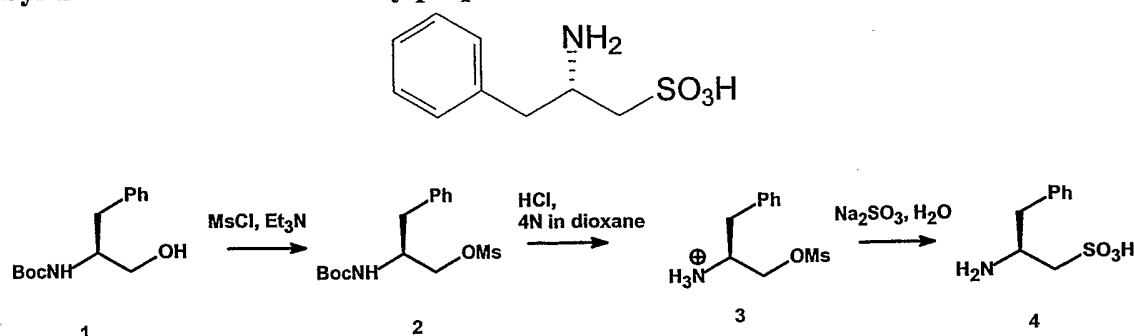
2-Amino-2-(4-chlorophenyl)ethanesulfonic acid was obtained as a white solid (1.0 g).  $^1\text{H}$  NMR was consistent with the structure.

**Synthesis of 2-Amino-2-Phenylethanesulfonic Acid (Compound L)**

To a solution of  $\text{SO}_3\cdot\text{DMF}$  complex (1.8 g, 12 mmol) and triflic acid (1.05 mL, 12 mmol) in acetonitrile (20 mL, anhydrous) was added dropwise styrene (1.04 g, 10 mmol) in acetonitrile (5 mL, anhydrous) at room temperature. The reaction mixture was stirred at room temperature overnight.  $\text{H}_2\text{O}$  was added, the mixture was continued to stir for 3 h. Concentration in vacuo afforded the crude acetylaminosulfonic acid, which could be directly used for subsequent hydrolysis. The crude product was dissolved in 3N HCl solution and then heated at a vigorous reflux overnight. The solution was then partially concentrated in vacuo and diluted with acetonitrile to precipitate the animosulfonic acid, which was filtered, washed with acetonitrile.

2-Amino-2-phenylethanesulfonic acid was obtained as a white solid (1.1 g).  $^1\text{H}$  NMR was consistent with the structure.

### Synthesis of 2-Amino-3-Phenylpropanesulfonic Acid (Compound J)



#### Step 1:

To a solution of compound 1 (2.51 g, 10 mmol) in dichloromethane (50 mL) was added triethylamine (10 mL, 75 mmol) via syringe. The solution was then cooled to 0°C. Methanesulfonyl chloride (0.94 mL, 12 mmol) was added dropwise via syringe. The reaction was stirred at room temperature overnight. The reaction was quenched by adding saturated aqueous NH<sub>4</sub>Cl solution (50 mL). The mixture was extracted with dichloromethane (3 x 30 mL). The combined organic layers were washed with brine and dried over Na<sub>2</sub>SO<sub>4</sub>. Pure product 2 (2.57 g, 80%) was obtained as white solid via silica gel flash column with 30% ethyl acetate in hexane.

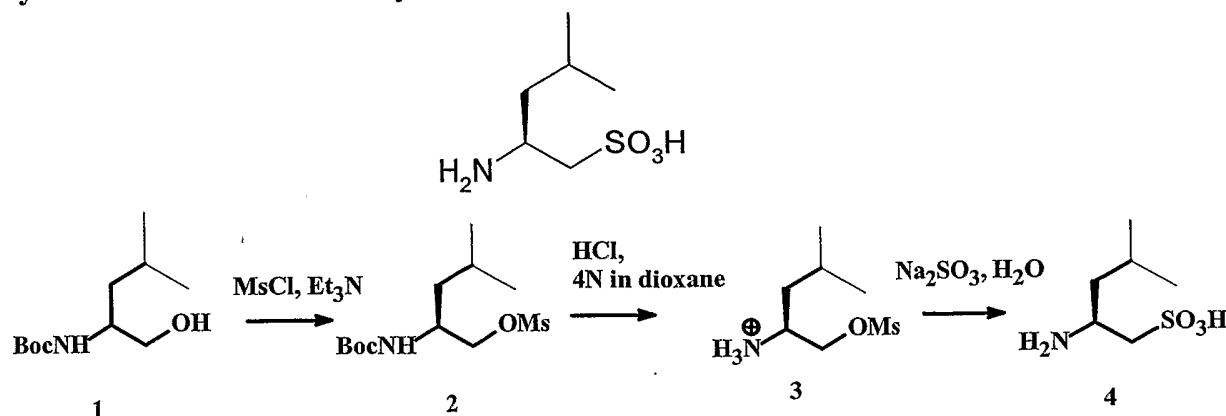
#### Step 2:

Compound 2 (2.57 g) obtained from the previous step was dissolved in HCl (20 mL, 4N in dioxane). The solution was stirred at room temperature overnight. Then the volume was reduced to half by reduced pressure. Acetonitrile (20 mL) was added to the solution. The mixture was cooled in an ice bath. The white crystal 3 formed was collected and dried under vacuum. Yield 1.4 g.

#### Step 3:

To a solution of compound 3 (1.4 g) in water (10 mL), was added sodium sulfite (1.5 g). The clear solution stirred overnight was gradually turned into milky mixture. The mixture was then diluted with water (100 mL) and passed through a acid resin column, rinsed with water. The eluent was concentrated and white solid was formed. The white solid was collected and washed with acetonitrile. 2-Amino-3-phenylpropanesulfonic acid was obtained as a white solid, yield 0.75 g. <sup>1</sup>H NMR (DMSO, 500 MHz) δ 7.92 (s, 3H), 7.37- 7.24 (m, 5H), 3.52-3.60 (m, 1H), 3.00 (dd, 1H, J=13, 5 Hz), 2.76 (dd, 1H, J=13, 9 Hz), 2.63-2.60 (m, 2H). <sup>13</sup>C NMR (DMSO, 125 MHz) δ 136.08, 129.39, 128.70, 127.0, 50.88, 50.03, 37.96.

### Synthesis of 2-Amino-4-Methyl-Pentane-1-Sulfonic Acid



5

#### Step 1:

To a solution of compound 1 (3.25 g, 15 mmol) in dichloromethane (50 mL) was added triethylamine (10 mL, 75 mmol) via syringe. The solution was then cooled to 0°C. Methanesulfonyl chloride (1.56 mL, 20 mmol) was added dropwise via syringe. The reaction was stirred at room temperature overnight. The reaction was quenched by adding saturated aqueous  $\text{NH}_4\text{Cl}$  solution (50 mL). The mixture was extracted with dichloromethane (3 x 30 mL). The combined organic layers were washed with brine and dried over  $\text{Na}_2\text{SO}_4$ . Pure product 2 (2.50 g) was obtained as white solid via silica gel flash column with 30% ethyl acetate in hexane.

15

#### Step 2:

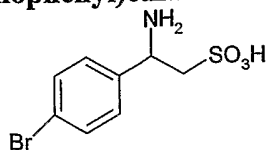
Compound 2 (2.5 g) obtained from the previous step was dissolved in HCl (20 mL, 4N in dioxane). The solution was stirred at room temperature overnight. Then the volume was reduced to half by reduced pressure. Acetonitrile (20 mL) was added to the solution. The mixture was cooled in an ice bath. The white crystal 3 formed was collected and dried under vacuum. Yield 1.8 g.

20

#### Step 3:

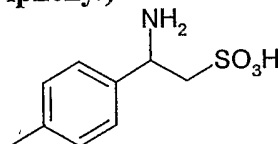
To a solution of compound 3 (1.8 g) in water (15 mL), was added sodium sulfite (4 g). The clear solution stirred overnight was gradually turned into milky mixture. The mixture was then diluted with water (100 mL) and passed through a acid resin column, rinsed with water. The eluent was concentrated and white solid was formed. The white solid was collected and washed with acetonitrile. Yield 1.2 g.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR were consistent with the structure with about 5 % impurities.

25

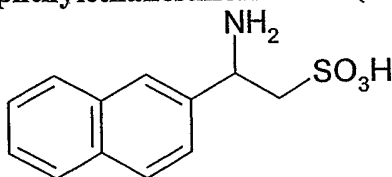
**Synthesis of 2-Amino-2-(4-bromophenyl)ethanesulfonic acid (Compound M)**

To a solution of  $\text{SO}_3 \cdot \text{DMF}$  complex (0.9 g, 6 mmol) and triflic acid (0.55 mL, 6 mmol) in acetonitrile (20 mL, anhydrous) was added dropwise 4-bromostyrene (1.0g, 5.46 mmol) in acetonitrile (5 mL, anhydrous) at room temperature. The reaction mixture was stirred at room temperature overnight.  $\text{H}_2\text{O}$  was added, the mixture was continued to stir for 3 hours. Concentration in vacuo afforded the crude acetaminosulfonic acid, which could be directly used for subsequent hydrolysis. The crude product was dissolved in 3N HCl solution and then heated at a vigorous reflux overnight. The solution was then partially concentrated in vacuo and diluted with acetonitrile to precipitate the animosulfonic acid, which was filtered, washed with acetonitrile.

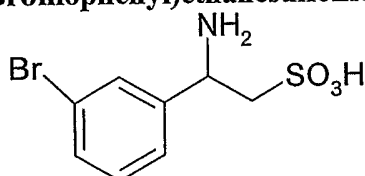
2-Amino-2-(4-bromophenyl)ethanesulfonic acid (0.9 g) was obtained as a white solid,  $^1\text{H}$  NMR (DMSO, 500 MHz) 8.30(s, 3H), 7.62 (d, 2H,  $J=8.5$  Hz), 7.42 (d, 2H,  $J=8.5$  Hz), 4.53 (br, 1H), 3.12 (dd, 1H,  $J=14, 11$  Hz), 2.85 (dd, 1H,  $J=14, 3\text{Hz}$ ).  $^{13}\text{C}$  NMR (DMSO, 125 MHz)  $\delta$  136.55, 131.69, 129.74, 122.03, 53.65, 51.46.

**Synthesis of -Amino-2-(4-methylphenyl)ethanesulfonic Acid (Compound N)**

To a solution of  $\text{SO}_3 \cdot \text{DMF}$  complex (1.8 g, 10 mmol) and triflic acid (0.88 mL, 10 mmol) in acetonitrile (20 mL, anhydrous) was added dropwise 4-methylstyrene (1.18, 10 mmol) in acetonitrile (5 mL, anhydrous) at room temperature. The reaction mixture was stirred at room temperature overnight.  $\text{H}_2\text{O}$  was added, the mixture was continued to stir for 3 h. Concentration in vacuo afforded the crude acetaminosulfonic acid, which could be directly used for subsequent hydrolysis. The crude product was dissolved in 3N HCl solution and then heated at a vigorous reflux overnight. The solution was then partially concentrated in vacuo and diluted with acetonitrile to precipitate the animosulfonic acid, which was filtered, washed with acetonitrile. 2-Amino-2-(4-methylphenyl)ethanesulfonic acid was obtained as a white solid (0.65 g).  $^1\text{H}$  NMR (DMSO, 500 MHz) 8.23 (s, 3H), 7.33 (d, 2H,  $J=8$  Hz), 7.22 (d, 2H,  $J=8$  Hz), 4.48 (d, 1H,  $J=4$  Hz), 3.13 (dd, 1H,  $J=14, 11$  Hz), 2.79 (dd, 1H,  $J=14, 1.5$  Hz), 2.30 (s, 3H).  $^{13}\text{C}$  NMR (DMSO, 125 MHz)  $\delta$  138.12, 134.12, 129.31, 127.19, 53.88, 51.78, 20.70.

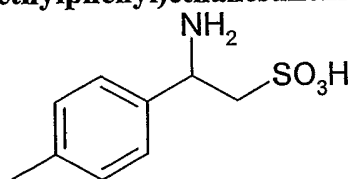
**Synthesis of 2-Amino-2-Naphthylethanesulfonic Acid (Compound O)**

To a solution of SO<sub>3</sub>DMF complex (1.8 g, 12 mmol) and triflic acid (1.05 mL) in acetonitrile (20 mL, anhydrous) was added dropwise 2-naphthaleneethylene (1.54 g, 10 mmol) in acetonitrile (5 mL, anhydrous) at room temperature. The reaction mixture was stirred at room temperature overnight. H<sub>2</sub>O was added, the mixture was continued to stir for 3 h. Concentration in vacuo afforded the crude acetylaminosulfonic acid, which could be directly used for subsequent hydrolysis. The crude product was dissolved in 3N HCl solution and then heated at a vigorous reflux overnight. The solution was then partially concentrated in vacuo and diluted with acetonitrile to precipitate the animosulfonic acid, which was filtered, washed with acetonitrile. 2-Amino-2-naphthylethanesulfonic acid was obtained as a white solid (0.73 g), <sup>1</sup>H NMR (DMSO, 500 MHz) 8.38 (s, 3H), 8.05-7.91 (m, 4H), 7.62-7.56 (m, 3H), 4.72-4.68 (br, 1H), 3.26 (dd, 1H, J=14.5, 11.5 Hz), 2.97 (dd, 1H, J=14.5, 3Hz). <sup>13</sup>C NMR (DMSO, 125 MHz) δ134.47, 132.76, 132.60, 128.52, 127.98, 127.59, 126.70, 126.65, 126.63, 124.74, 53.78, 52.21.

**20 Synthesis of 2-Amino-2-(3-Bromophenyl)ethanesulfonic Acid (Compound P)**

To a solution of SO<sub>3</sub>DMF complex (0.9 g, 6 mmol) and triflic acid (0.55 mL, 6 mmol) in acetonitrile (20 mL, anhydrous) was added dropwise 3-bromostyrene (1.0g, 5.46 mmol) in acetonitrile (5 mL, anhydrous) at room temperature. The reaction mixture was stirred at room temperature overnight. H<sub>2</sub>O was added, the mixture was continued to stir for 3 h. Concentration in vacuo afforded the crude acetylaminosulfonic acid, which could be directly used for subsequent hydrolysis. The crude product was dissolved in 3N HCl solution and then heated at a vigorous reflux overnight. The solution was then partially concentrated in vacuo and diluted with acetonitrile to precipitate the animosulfonic acid, which was filtered, washed with acetonitrile.

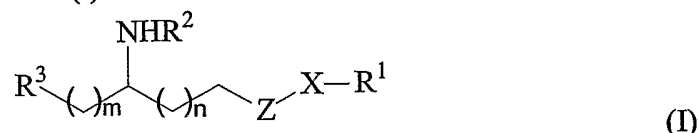
2-Amino-2-(3-bromophenyl)ethanesulfonic acid(1.16 g) was obtained as a white solid, <sup>1</sup>H NMR (DMSO, 500 MHz) and <sup>13</sup>C NMR was consistent with the structure.

**Synthesis of 2-Amino-2-(4-methylphenyl)ethanesulfonic Acid (Compound Q)**

- 5 To a solution of SO<sub>3</sub>DMF complex (1.8 g, 10 mmol) and triflic acid (0.88 mL, 10 mmol) in acetonitrile (20 mL, anhydrous) was added dropwise 3-methylstyrene (1.18, 10 mmol) in acetonitrile (5 mL, anhydrous) at room temperature. The reaction mixture was stirred at room temperature overnight. H<sub>2</sub>O was added, the mixture was continued to stir for 3 h. Concentration in vacuo afforded the crude acetylaminosulfonic acid, which
- 10 could be directly used for subsequent hydrolysis. The crude product was dissolved in 3N HCl solution and then heated at a vigorous reflux overnight. The solution was then partially concentrated in vacuo and diluted with acetonitrile to precipitate the animosulfonic acid, which was filtered, washed with acetonitrile. 2-Amino-2-(4-
- 15 methylphenyl)ethanesulfonic acid was obtained as a white solid (0.98 g). <sup>1</sup>H NMR and <sup>13</sup>C NMR was consistent with the structure.

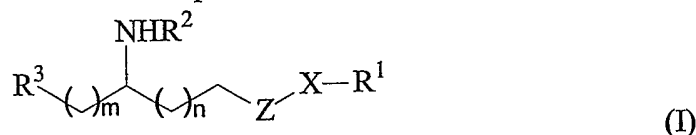
## CLAIMS

1. A method of treating or preventing an amyloid-related disease in a subject, comprising administering to a subject a therapeutic amount of a compound of Formula (I), such that said amyloid-related disease in a subject is treated or prevented, wherein said compound of Formula (I) is:



wherein:

- X is oxygen or nitrogen;  
 10 Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;  
 m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;  
 R<sup>1</sup> and R<sup>7</sup> are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or —(CH<sub>2</sub>)<sub>p</sub>—Y;  
 15 Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;  
 p is 0, 1, 2, 3, or 4;  
 R<sup>2</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxy carbonyl;  
 20 R<sup>3</sup> is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl; and pharmaceutically acceptable salts, esters, and prodrugs thereof.  
 25
2. A method for inhibiting epileptogenesis in a subject, comprising administering to a subject an effective amount of a compound, such that said epileptogenesis in said subject is inhibited, wherein said compound is of Formula I:



30 wherein:

- X is oxygen or nitrogen;  
 Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;  
 m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

$R^1$  and  $R^7$  are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form a natural or unnatural amino acid residue, or  $-(CH_2)_p-Y$ ;

Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

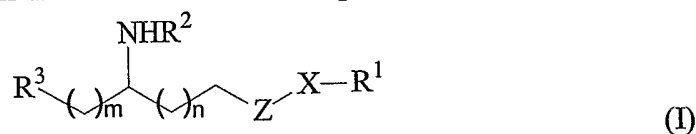
p is 0, 1, 2, 3, or 4;

$R^2$  is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxycarbonyl;

$R^3$  is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl; and pharmaceutically acceptable salts, esters, *N*-substituted analogs, and prodrugs thereof, provided that when Z is C=O, n is 1, 2, 3, or 4.

15

3. A method for treating a subject suffering from an epileptogenesis-associated condition, comprising administering to said subject an effective amount of a compound, such that said subject is treated wherein said compound is of Formula I:



20 wherein:

X is oxygen or nitrogen;

Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;

m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

$R^1$  and  $R^7$  are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form a natural or unnatural amino acid residue, or  $-(CH_2)_p-Y$ ;

Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

p is 0, 1, 2, 3, or 4;

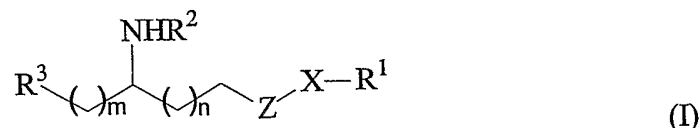
$R^2$  is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxycarbonyl;

$R^3$  is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl;

35

and pharmaceutically acceptable salts, esters, *N*-substituted analogs, and prodrugs thereof, provided that when Z is C=O, n is 1, 2, 3, or 4.

4. A method for treating convulsions in a subject comprising administering to said  
 5 subject an effective amount of a compound, such that said subject is treated, wherein said compound is of Formula I:

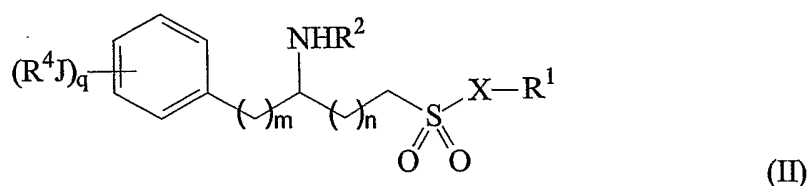


wherein:

- X is oxygen or nitrogen;  
 10 Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;  
 m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;  
 R<sup>1</sup> and R<sup>7</sup> are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or  $-(\text{CH}_2)_p-\text{Y}$ ;  
 15 Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;  
 p is 0, 1, 2, 3, or 4;  
 R<sup>2</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl,  
 20 alkylcarbonyl, arylcarbonyl, or alkoxycarbonyl;  
 R<sup>3</sup> is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl, and pharmaceutically acceptable salts, esters, *N*-substituted analogs, and prodrugs  
 25 thereof, provided that when Z is C=O, n is 1, 2, 3, or 4.

5. The method of any one of claims 1-4, wherein m is 0, 1, or 2.  
 6. The method of any one of claims 1-5, wherein n is 0, 1, or 2.  
 30 7. The method of any one of claims 1-6, wherein R<sup>3</sup> is aryl.  
 8. The method of claim 7, wherein R<sup>3</sup> is phenyl.  
 35 9. The method of any one of claims 1-8, wherein Z is S(O)<sub>2</sub>.

10. The method of any one of claims 1-4, wherein said compound is of the Formula (II)



wherein:

5 each  $R^4$  is independently selected from the group consisting of hydrogen, halogen, hydroxyl, thiol, amino, cyano, nitro, alkyl, aryl, carbocyclic or heterocyclic;

J is absent, oxygen, nitrogen, sulfur, or a divalent link-moiety comprising lower alkylene, alkyleneoxy, alkyleneamino, alkyleneylthio, alkyleneoxyalkyl, 10 alkyleneylammonialkyl, alkyleneylthioalkyl, alkenyl, alkenyloxy, alkenylamino, or alkenylthio; and

q is 1, 2, 3, 4, or 5, or pharmaceutically acceptable salts, esters, or prodrugs thereof.

15 11. The method of claim 10, wherein  $R^4$  is aryl.

12. The method of claim 11, wherein  $R^4$  is phenyl.

13. The method of claim 10, wherein  $R^4$  is halogen.

20

14. The method of claim 10, wherein  $R^4$  is alkyl.

15. The method of claim 14, wherein  $R^4$  is methyl or trifluoromethyl.

25 16. The method of any one of claims 10-15, wherein J is absent,

17. The method of any one of claims 10-16, wherein m is 0, 1, or 2.

18. The method of any one of claims 10-17, wherein n is 0 or 1.

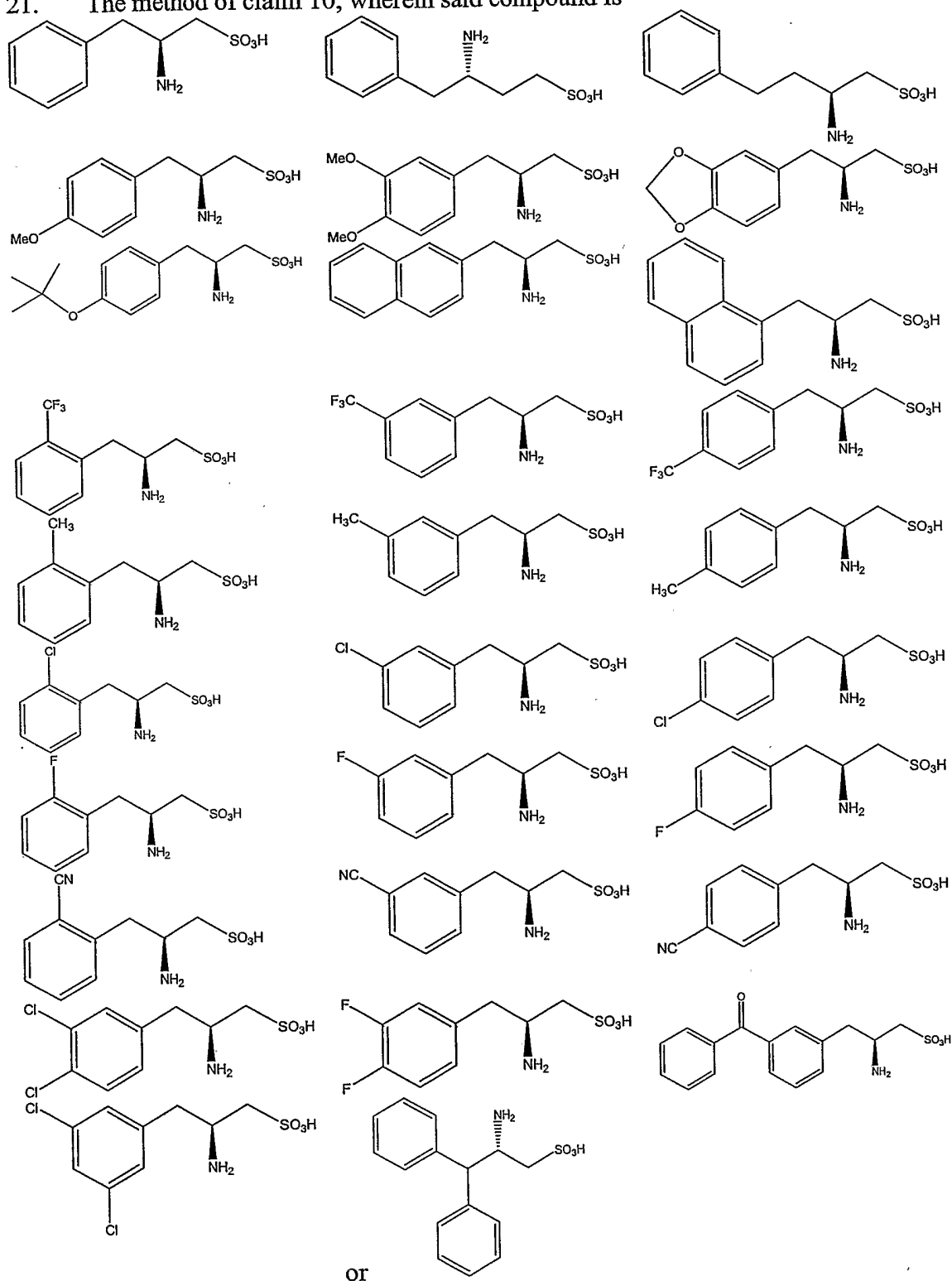
30

19. The method of claim 10-18, wherein a chiral carbon has the R-configuration.

20. The method of claim 10-18, wherein a chiral carbon has the S-configuration.

35

21. The method of claim 10, wherein said compound is



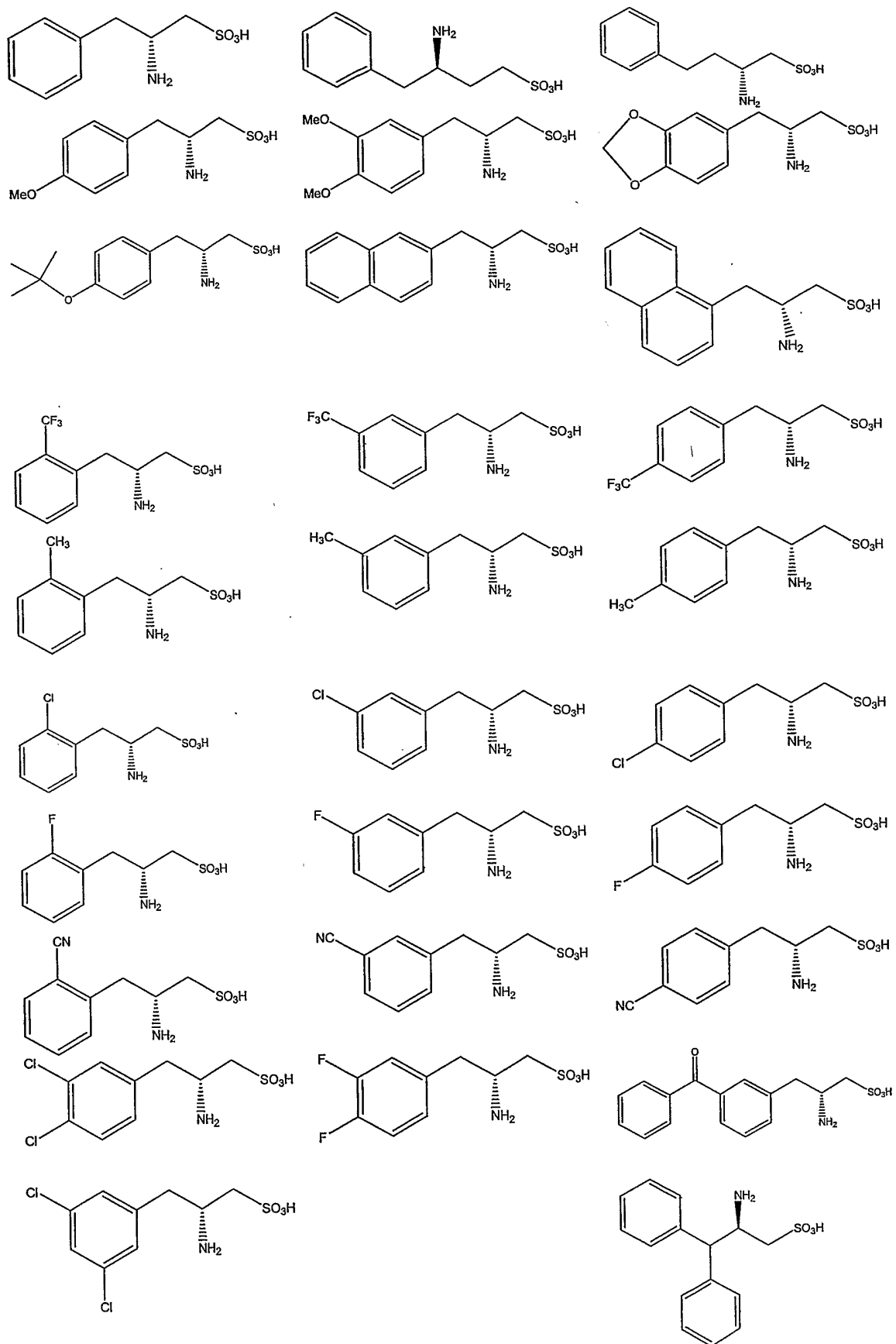
or

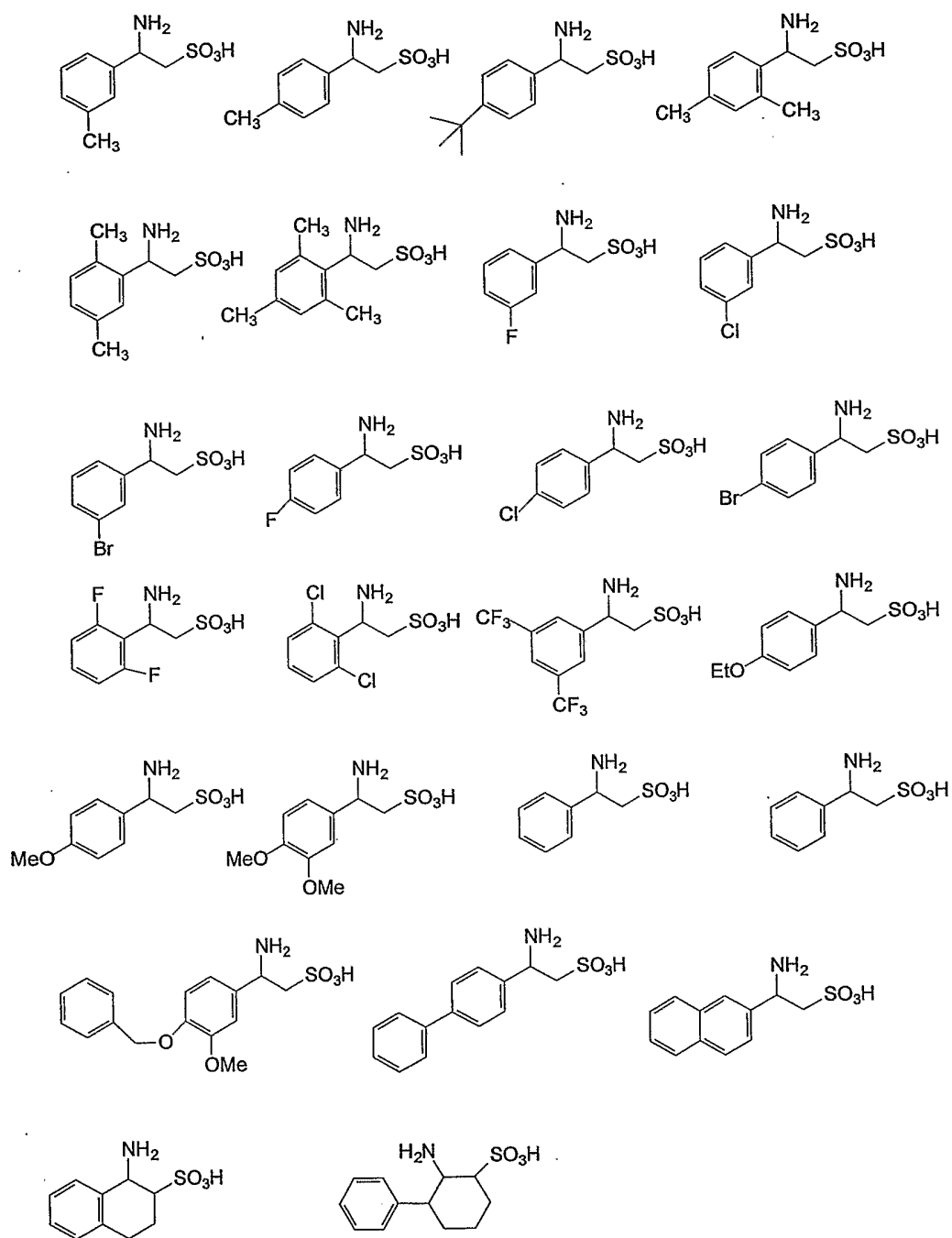
or pharmaceutically acceptable salts, esters, or prodrugs thereof.

5

22.

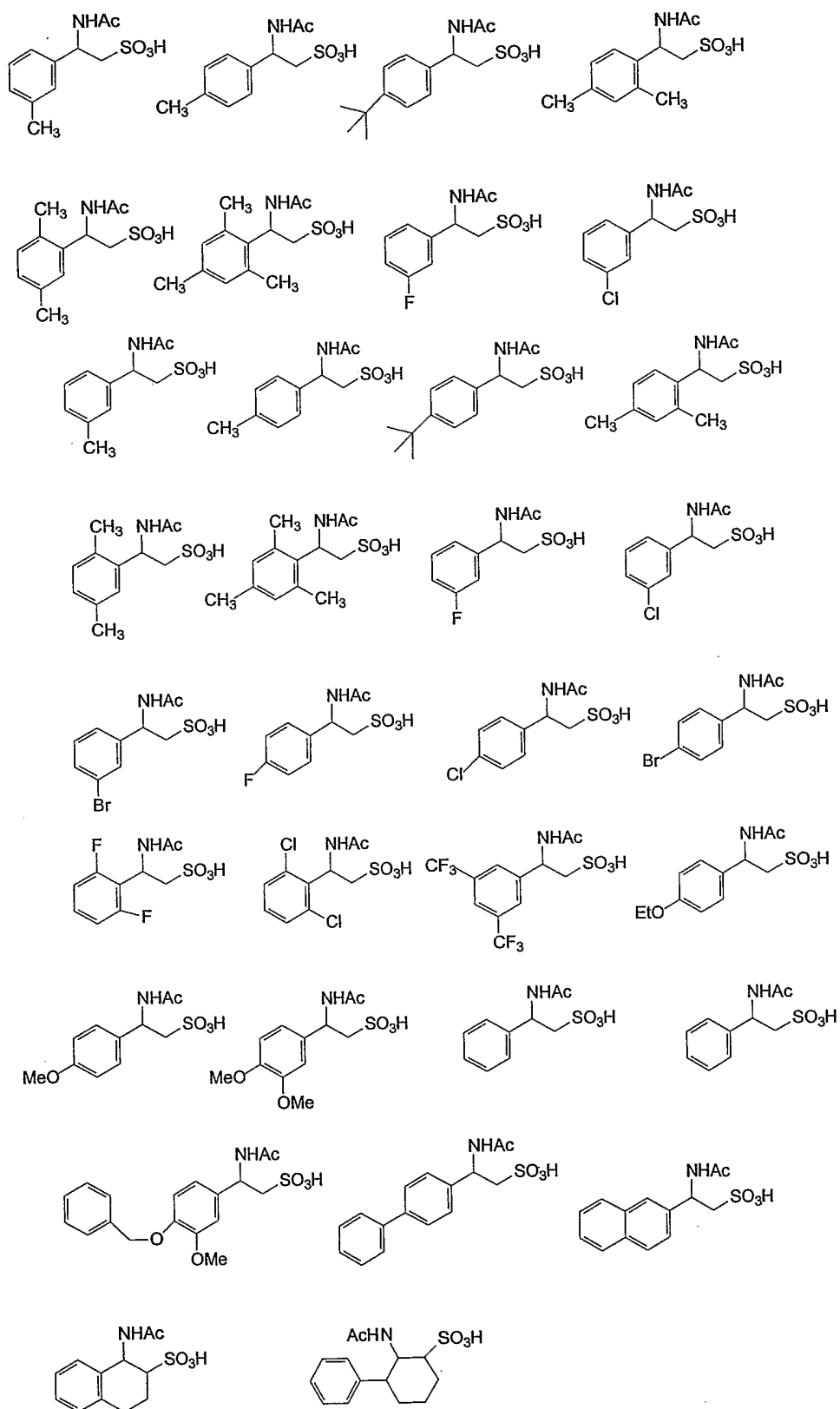
The method of claim 10, wherein said compound is:





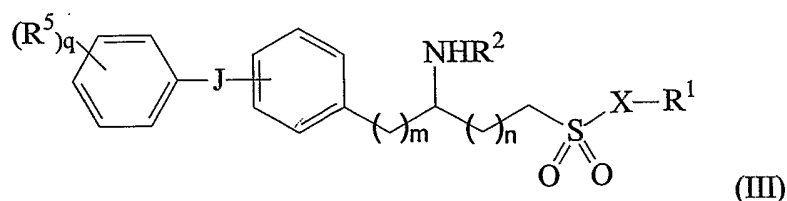
or pharmaceutically acceptable salts, esters, or prodrugs thereof.

- 5 23. The method of claim 10, wherein said compound is:



or pharmaceutically acceptable salts, esters, or prodrugs thereof.

- 5 24. The method of claim 10, wherein said compound is of the Formula:



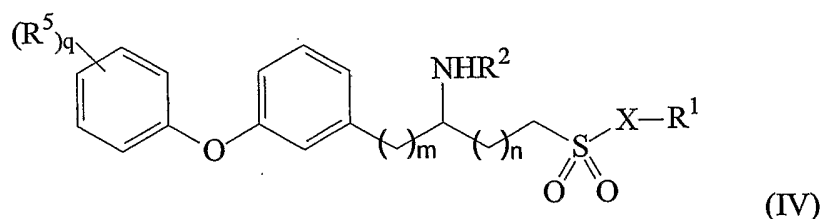
wherein:

$R^5$  is selected from the group consisting of hydrogen, halogen, amino, cyano, nitro, hydroxy, carbonyl, thiol, carboxy, alkyl, alkoxy, alkoxy carbonyl, acyl, alkylamino, and acylamino; and

$q$  is 1, 2, 3, 4, or 5;

or pharmaceutically acceptable salts, esters, or prodrugs thereof.

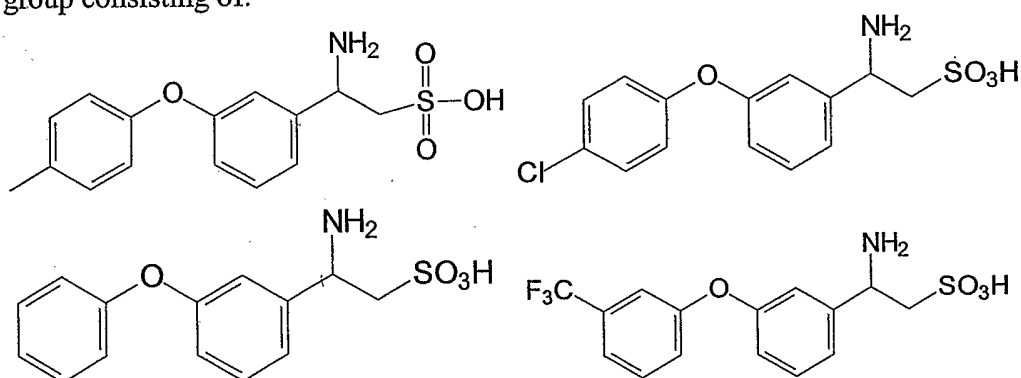
25. The method of claim 24, wherein said compound is of the Formula (IV):

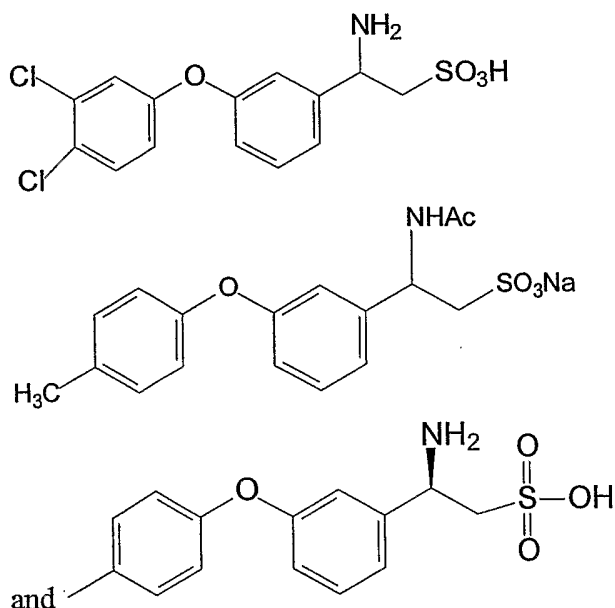


or pharmaceutically acceptable salts, esters, or prodrugs thereof.

26. The method of claim 25, wherein  $m$  is 0.

27. The method of claim 25, wherein said compound is selected from the group consisting of:



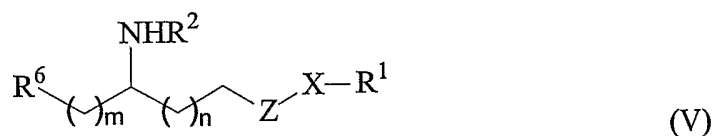


and pharmaceutically acceptable salts, esters, and prodrugs thereof.

5

28. The method of claim 25, wherein said compound is selected from the group consisting of the compounds in Table 2, or a pharmaceutically acceptable salt, ester, or prodrug thereof.

10 29. The method of any one of claims 1-4, wherein said compound is of Formula (V):



wherein:

15  $\text{R}^6$  is a substituted or unsubstituted heterocyclic moiety, or a pharmaceutically acceptable salt, ester, or prodrug thereof.

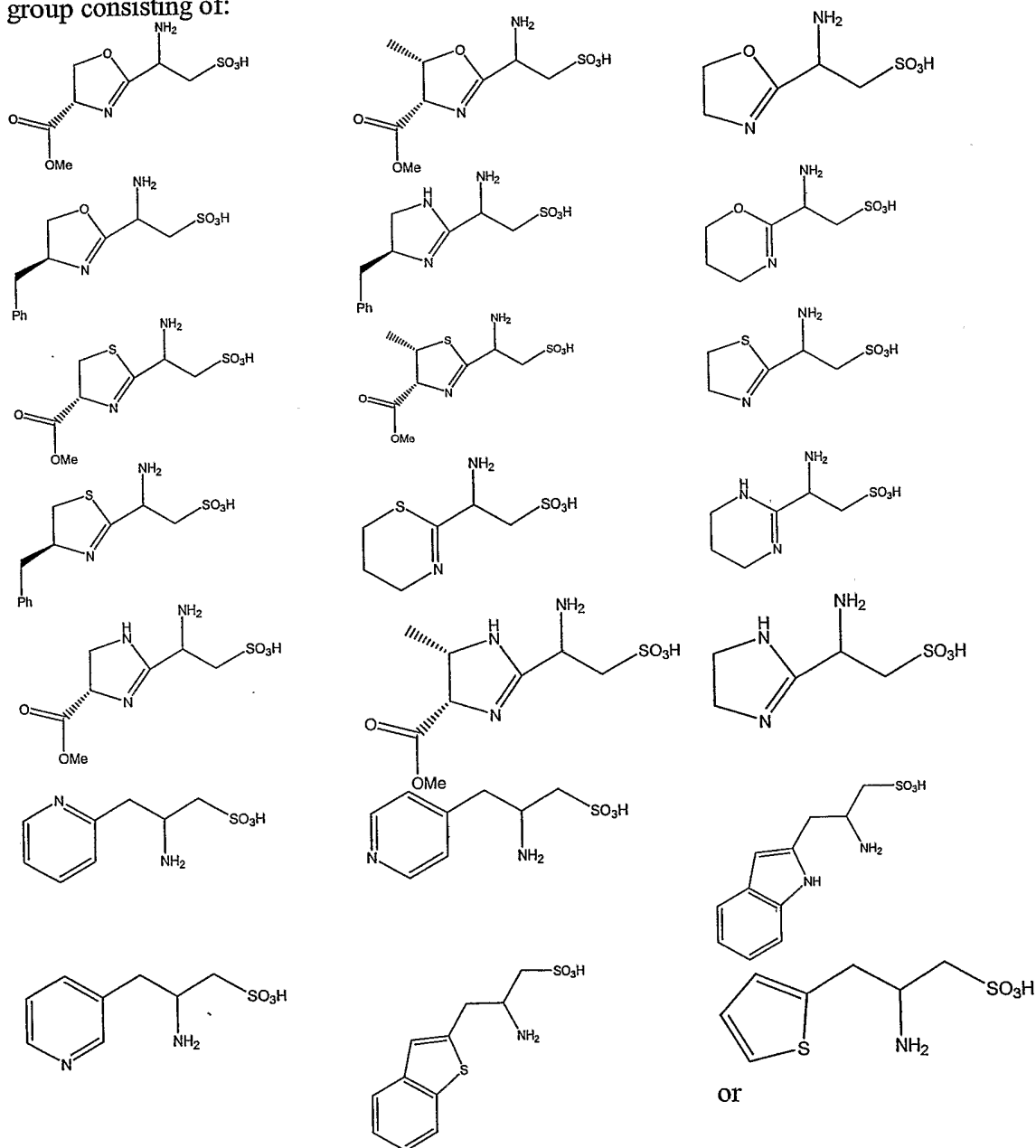
30. The method of claim 29, wherein m is 0 or 1.

20 31. The method of claim 29 or 30, wherein n is 0.

32. The method of anyone of claims 29-31, wherein  $\text{R}^6$  is thiazolyl, oxazolyl, pyrazolyl, indolyl, pyridinyl, thiazinyl, thiophenyl, benzothiophenyl, dihydroimidazolyl, dihydrothiazolyl, oxazolidinyl, thiazolidinyl, 25 tetrahydropyrimidinyl, or oxazinyl.

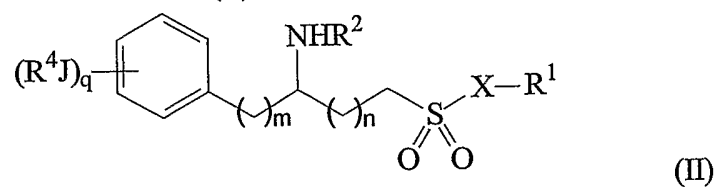
33. The method of any one of claims 29-32, wherein Z is S(O)<sub>2</sub>.

34. The method of claim 29, wherein said compound is selected from the group consisting of:



5 and pharmaceutically acceptable salts, esters, and prodrugs thereof.

35. A compound of the Formula (II)



wherein:

X is oxygen or nitrogen;

m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

q is 1, 2, 3, 4, or 5

- 5         $R^1$  is hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, or a moiety together with X to form a natural or unnatural amino acid residue, or  $-(CH_2)_p-Y$ ;

10        Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

p is 0, 1, 2, 3, or 4;

$R^2$  is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxy carbonyl;

- 15        each  $R^4$  is independently selected from the group consisting of hydrogen, halogen, hydroxyl, thiol, amino, cyano, nitro, alkyl, aryl, carbocyclic or heterocyclic;

J is absent, a covalent bond, oxygen, nitrogen, sulfur, or a divalent link-moiety consisting of, without limiting to, lower alkylene, alkyleneoxy, alkyleneamino, alkyleneylthio, alkyleneoxyalkyl, alkyleneylaminoalkyl, 20        alkyleneylthioalkyl, alkenyl, alkenyloxy, alkenylamino, or alkenylthio; and pharmaceutically acceptable salts, esters and prodrugs thereof, provided that when J is a covalent bond, n is 1, m is 0 or 1, X is oxygen, and  $R^4$  is hydrogen,  $R^2$  is not hydrogen or acyl; and provided that when q is 1, m is 0, n is 1, J is a covalent bond,  $R^4$  is methyl, ethyl, methoxy, ethoxy, chloro, bromo, 25        hydroxy, or iodo in *para*-position, and X is oxygen,  $R^2$  is not hydrogen; provided that when q is 2, m is 0, n is 1, J is a covalent bond,  $R^4$  is methyl in *ortho* and *para*-positions, and X is oxygen,  $R^2$  is not hydrogen; provided that when provided that when q is 2, m is 0, n is 1, J is a covalent bond,  $R^4$  is chloro in 2 and 6 positions, and X is oxygen,  $R^2$  is not acyl.

30

36.    The compound of claim 35, wherein  $R^4$  is aryl.

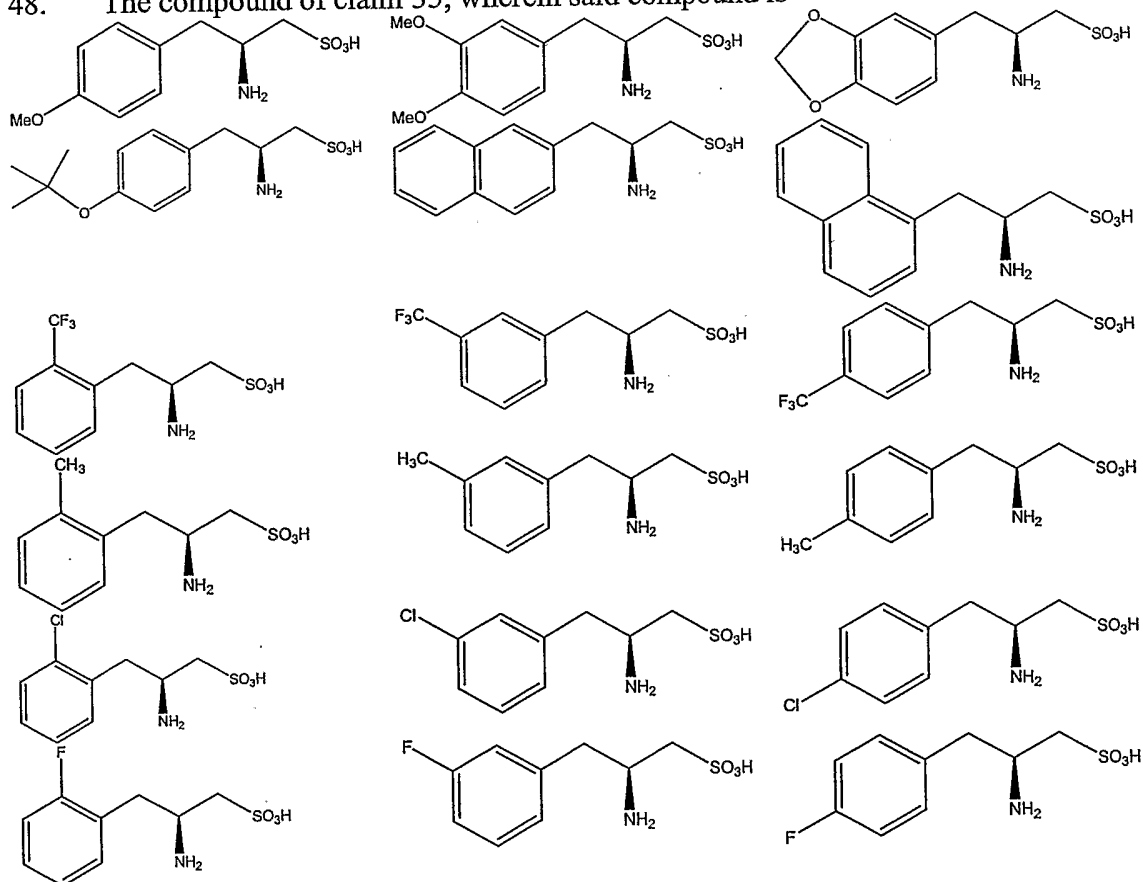
37.    The compound of claim 36, wherein  $R^4$  is phenyl.

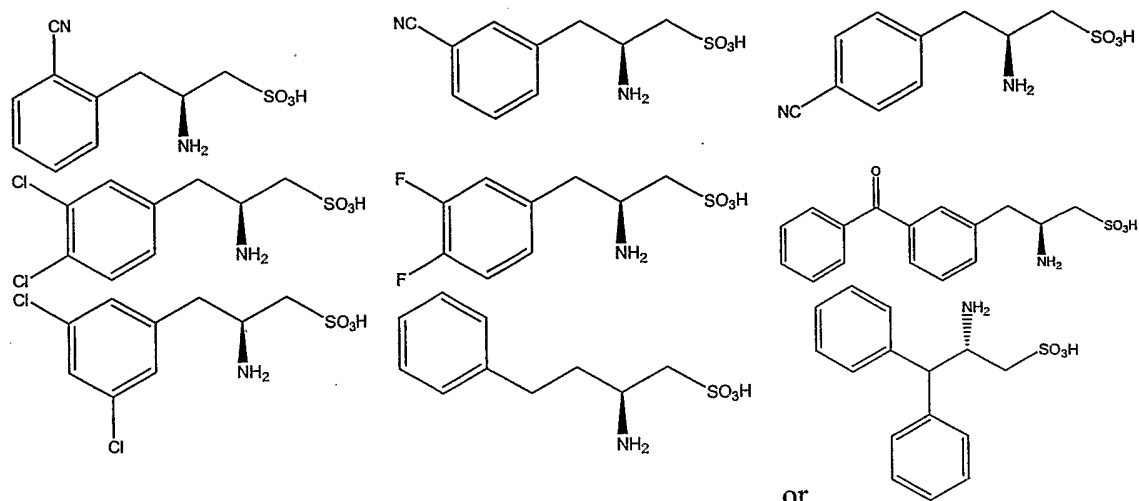
35    38.    The compound of any one of claims 35-37, wherein m is 0 or 1.

39.    The compound of any one of claims 35-38, wherein n is 0 or 1.

40. The compound of any one of claims 35-39, wherein X is oxygen.
41. The compound of any one of claims 35-40, wherein R<sup>1</sup> is hydrogen.
42. The compound of any one of claims 35-41, wherein R<sup>2</sup> is hydrogen.
43. The compound of any one of claims 35-42, wherein R<sup>4</sup> is alkyl.
44. The compound of claim 43, wherein R<sup>4</sup> is trifluoroalkyl or methyl.
45. The compound of any one of claims 35-44, wherein J is oxygen.
46. The compound of any one of claims 35-42, wherein R<sup>4</sup> is halogen.
47. The compound of any one of claims 35-42, wherein each R<sup>4</sup> is hydrogen.

48. The compound of claim 35, wherein said compound is

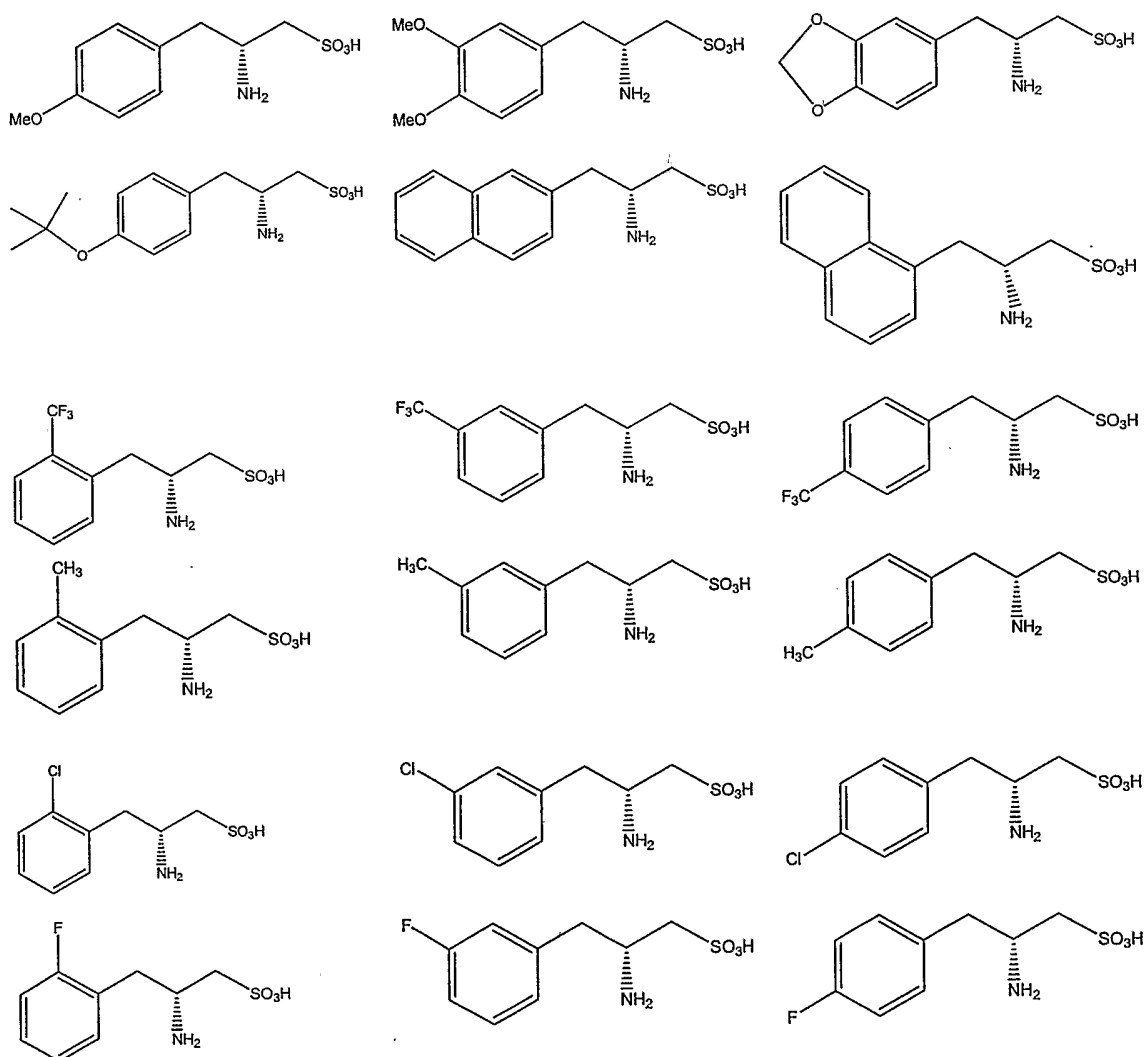


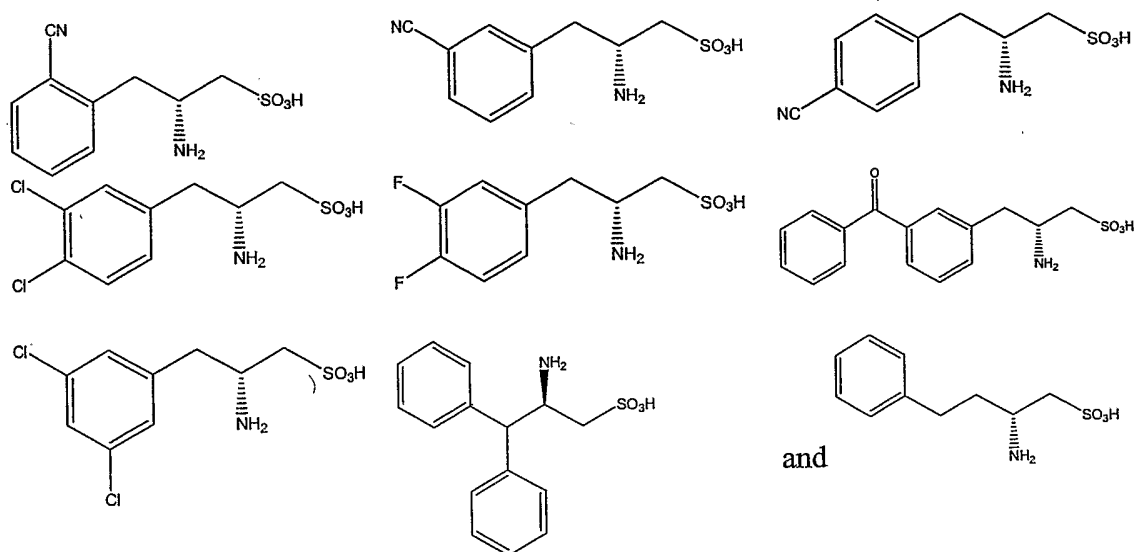


or

and pharmaceutically acceptable salts, esters, and prodrugs thereof.

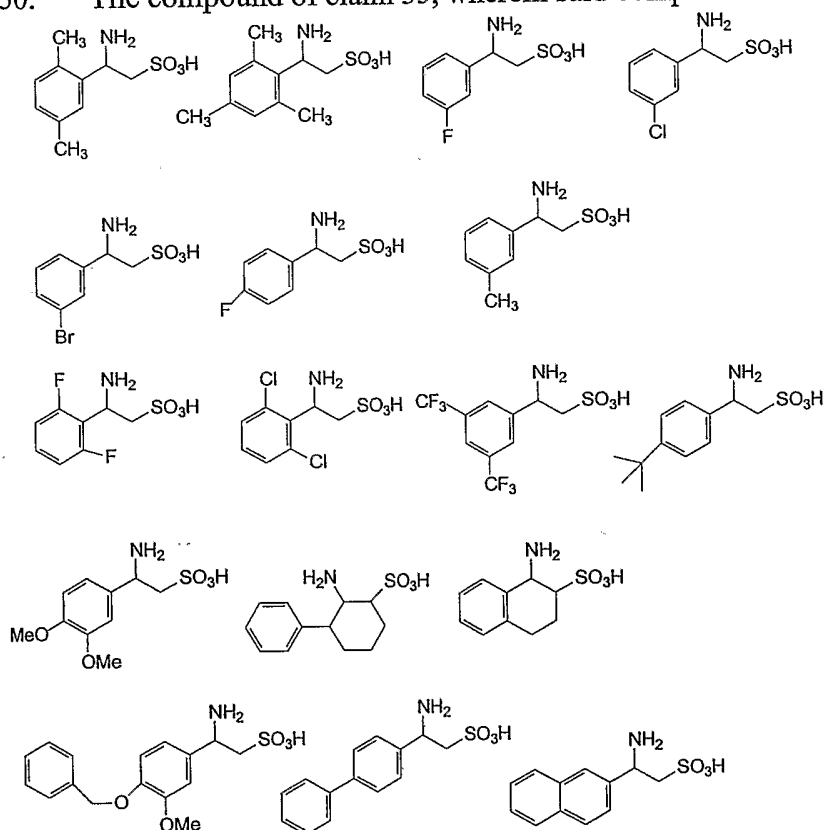
49. The compound of claim 35, wherein said compound is:





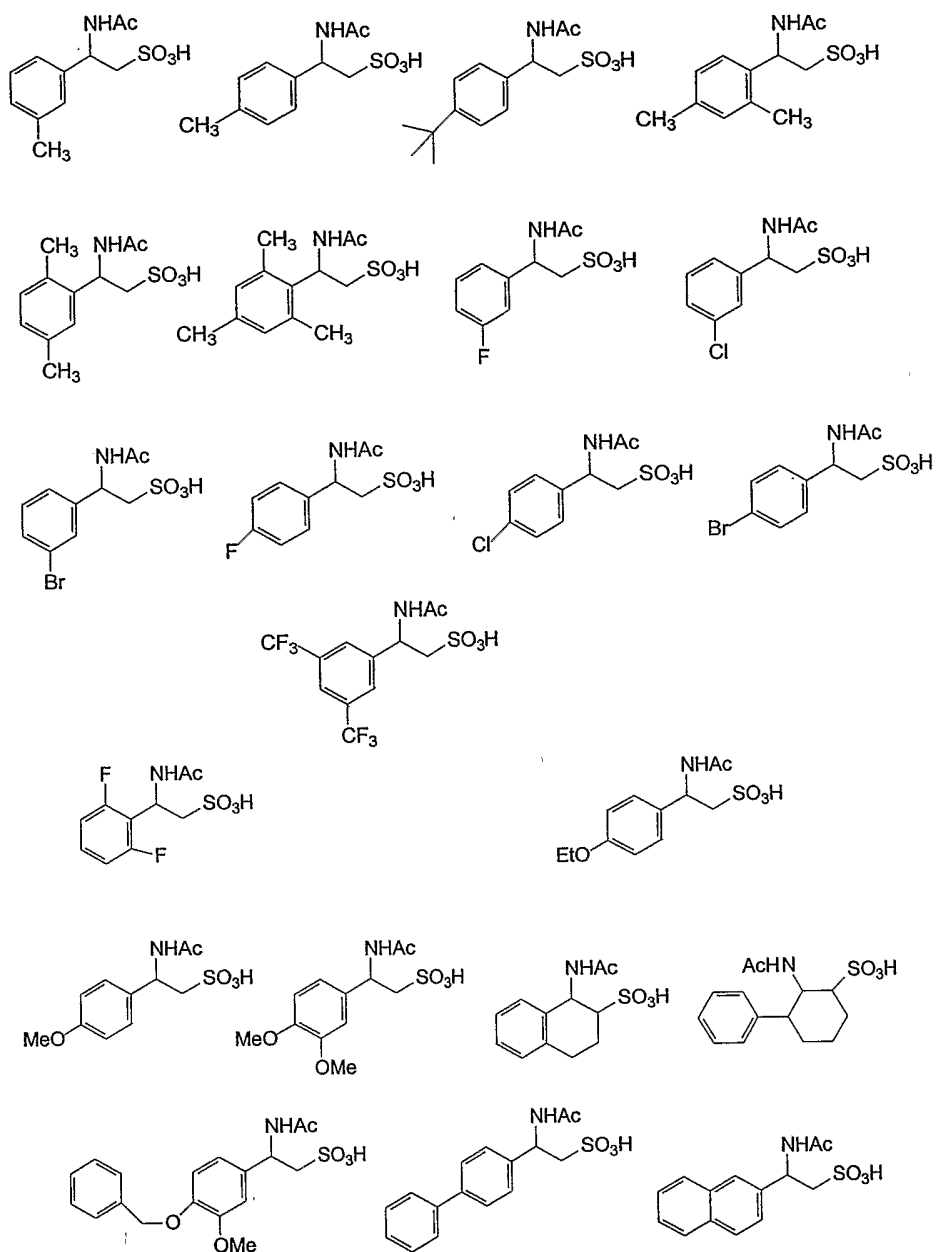
and pharmaceutically acceptable salts, esters, and prodrugs thereof.

50. The compound of claim 35, wherein said compound is:



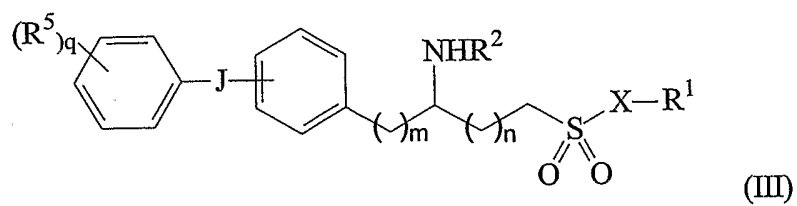
5 and pharmaceutically acceptable salts, esters, and prodrugs thereof.

51. The compound of claim 35, wherein said compound is:



and pharmaceutically acceptable salts, esters and prodrugs thereof.

- 5 52. The compound of claim 35, wherein said compound is of the Formula:

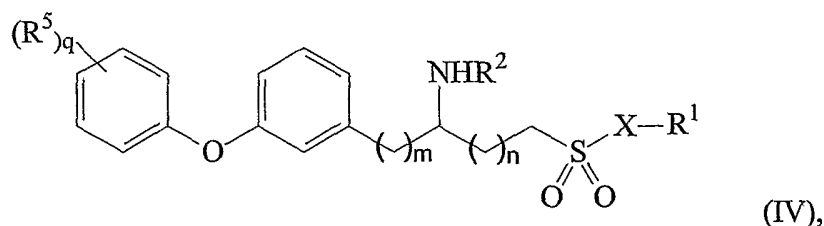


wherein:

$R^5$  is selected from the group consisting of hydrogen, halogen, amino, cyano, nitro, hydroxy, carbonyl, thiol, carboxy, alkyl, alkoxy, alkoxycarbonyl, acyl, alkylamino, and acylamino; and

$q$  is an integer selected from 1 to 5, and pharmaceutically acceptable salts, esters, and prodrugs thereof.

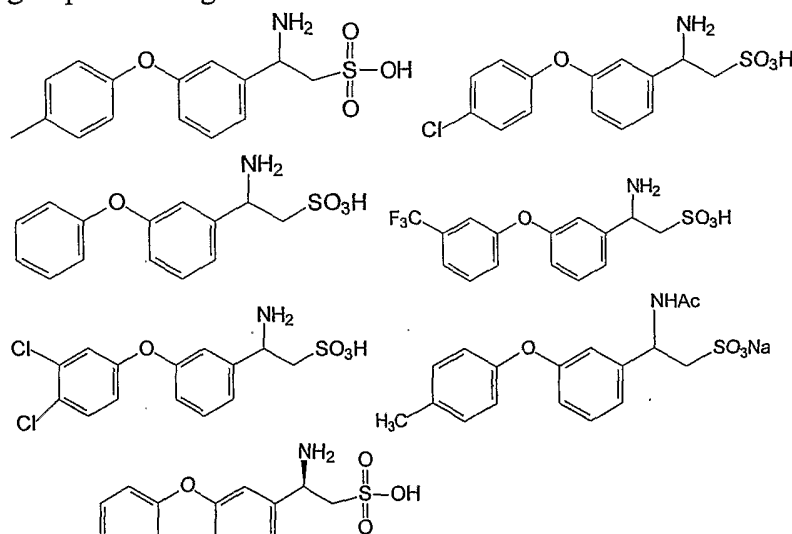
53. The compound of claim 52, wherein said compound is of the Formula (IV):



and pharmaceutically acceptable salts, esters and prodrugs thereof.

54. The compound of claim 53, wherein  $m$  is 0.

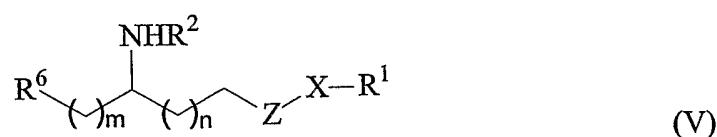
55. The compound of claim 53, wherein said compound is selected from the group consisting of:



and , and pharmaceutically acceptable salts, esters and prodrugs thereof.

56. The compound of claim 53, wherein said compound is selected from the group consisting of the compounds in Table 2, and pharmaceutically acceptable salts, esters and prodrugs thereof.

57. A compound of the Formula (V):



wherein:

- 5 X is oxygen or nitrogen;  
 Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;  
 m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;  
 R<sup>1</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl,  
 a moiety together with X to form natural or unnatural amino acid residue, or  
 —(CH<sub>2</sub>)<sub>p</sub>—Y;  
 10 Y is hydrogen or a heterocyclic moiety selected from the group  
 consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and  
 benzoimidazolyl;  
 p is 0, 1, 2, 3, or 4;  
 R<sup>2</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl,  
 15 alkylcarbonyl, arylcarbonyl, or alkoxycarbonyl;  
 R<sup>6</sup> is a substituted or unsubstituted heterocyclic moiety;  
 R<sup>7</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, or  
 aryl; and pharmaceutically acceptable salts, esters, and prodrugs thereof.

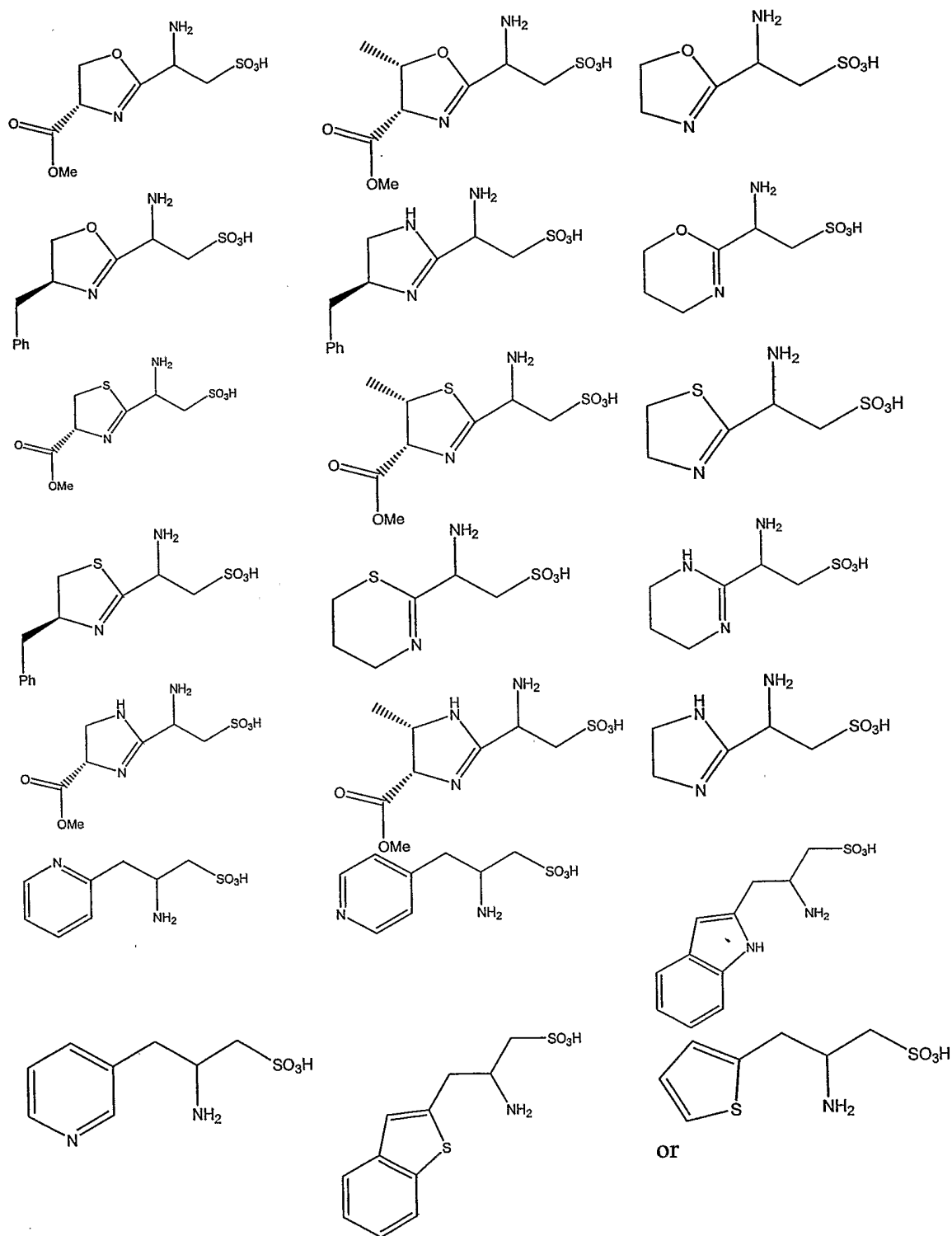
20 58. The compound of claim 57, wherein m is 0 or 1.

59. The compound of claim 57 or 58, wherein n is 0.

60. The compound of any one of claims 57-59, wherein R<sup>6</sup> is  
 25 thiazolyl, oxazolyl, pyrazolyl, indolyl, pyridinyl, thiazinyl, thiophenyl,  
 benzothiophenyl, dihydroimidazolyl, dihydrothiazolyl, oxazolidinyl,  
 thiazolidinyl, tetrahydropyrimidinyl, or oxazinyl.

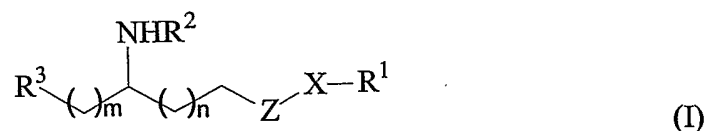
61. The compound of claim 57-60, wherein Z is S(O)<sub>2</sub>.

30 62. The compound of claim 57, wherein said compound is selected  
 from the group consisting of:



and pharmaceutically acceptable salts, esters and prodrugs thereof.

63. A kit for use in treating amyloid-related disease, comprising a compound of Formula (I), and instructions for use in the method of the instant invention, wherein said compound of Formula I is:



wherein:

X is oxygen or nitrogen;

Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;

5 m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

R<sup>1</sup> and R<sup>7</sup> are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or -(CH<sub>2</sub>)<sub>p</sub>-Y;

10 Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

p is 0, 1, 2, 3, or 4;

R<sup>2</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxy carbonyl;

15 R<sup>3</sup> is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl; and pharmaceutically acceptable salts, esters, and prodrugs thereof.

20 64. The method according to any one of the foregoing claims, wherein said disease is Alzheimer's disease, cerebral amyloid angiopathy, inclusion body myositis, macular degeneration, or Down's syndrome.

25 65. The method according to any one of the foregoing claims, wherein amyloid fibril formation or deposition, neurodegeneration, or cellular toxicity is reduced or inhibited.

66. The method according to any one of claims 1-4, wherein said compound is selected from those depicted in Tables and Figures.

30 67. The method according to any one of the foregoing claims, wherein said amyloid-related disease is Alzheimer's disease, cerebral amyloid angiopathy, Down's syndrome, inclusion body myositis, or macular degeneration.

35 68. The method according to any one of the foregoing claims, wherein said amyloid-related disease is type II diabetes.

69. The method according to any one of the foregoing claims, wherein said subject is a human.
- 5 70. The method of any claim herein, wherein said compound causes in an Alzheimer's patient a stabilization of cognitive function, prevention of a further decline in cognitive function, or prevention, slowing, or stopping of disease progression.
- 10 71. The method of any claim herein, wherein said subject is suffering from head trauma, pain, stroke, anxiety, schizophrenia, multiple sclerosis, amyotrophic lateral sclerosis, psychoses, cerebral ischemia, Huntington's chorea, meningitis, motor neuron disease, Alzheimer's disease, Parkinson's disease, cancer, fibrile seizures, or dementia.
- 15 72. The method of any of the foregoing claims, wherein said subject is suffering from an amyloid-related disease and an epileptogenesis-associated condition.
73. The method of claim 72, wherein said amyloid-related disease is Alzheimer's disease.
- 20 74. The method of claim 72, wherein said epileptogenesis-associated condition is epilepsy.
- 25 75. A method for inhibiting epileptogenesis in a subject, comprising administering to a subject an effective amount of a compound such that said epileptogenesis is inhibited, wherein said compound is of Formula II, III, IV, or V or defined in the foregoing claims, and pharmaceutically acceptable salts, esters, *N*-substituted analogs, and prodrugs thereof.
- 30 76. A method for treating a epileptogenesis-associated condition in a subject, comprising administering to said subject an effective amount of a compound such that said subject is treated for said epileptogenesis-associated condition, wherein said compound is of Formula II, III, IV, or V or defined in the foregoing claims, and pharmaceutically acceptable salts, esters, *N*-substituted analogs, and prodrugs thereof.

77. A method for treating convulsions in a subject, comprising administering to said subject an effective amount of a compound such that said subject is treated for said convulsions, wherein said compound is of Formula II, III, IV, or V or defined in the foregoing claims, and pharmaceutically acceptable salts, esters, *N*-substituted analogs, and prodrugs thereof.
78. The method of any one of claims 2-4 and 75-77, wherein said compound modulates GAT-1, GAT-2., or GAT-3.
79. The method of any one of claims 2-4 and 75-77, wherein said compound inhibits the uptake of synaptic GABA.
80. The method of any one of claims 2-4 and 75-77, wherein said compound is a GABA receptor agonist.
81. The method of any one of claims 2-4 and 75-77, wherein said compound is a glutamatergic excitation modulator.
82. The method of any one of claims 2-4 and 75-77, wherein said compound is a glutamatergic excitation inhibitor.
83. The method of any one of claims 2-4 and 75-77, wherein said compound interacts with the NMDA receptor.
84. The method of any one of claims 2-4 and 75-77, wherein said compound is capable of crossing the blood brain barrier.
85. The method of any one of claims 2-4 and 75-77, wherein said compound is not toxic.
86. The method of any of the foregoing claims, wherein said epileptogenesis-associated condition is head trauma, pain, stroke, anxiety, schizophrenia, other psychoses, cerebral ischemia, Huntington's chorea, motor neuron disease, meningitis, Alzheimer's disease, Parkinson's disease, neurosurgery, cancer, fibrile seizures, or dementia.
87. The method of claim 86, wherein said epileptogenesis-associated condition is epilepsy.

88. A pharmaceutical composition, comprising a therapeutically effective amount of a compound and a pharmaceutical acceptable carrier, wherein said compound is of claims 48-51, 55, 56, and 62, and pharmaceutically acceptable salts or esters, *N*-substituted analogs, and prodrugs thereof.

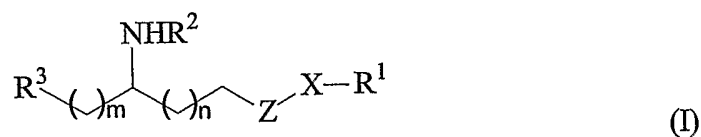
89. The pharmaceutical composition for treating an epileptogenesis-associated state comprising an effective amount of a compound of the Formula of any one of the claims 2-4 and 75-77, wherein said effective amount is effective to treat an epileptogenesis-associated state.

90. The pharmaceutical composition of claim 89, wherein said epileptogenesis-associated state is head trauma, pain, stroke, anxiety, schizophrenia, other psychoses, cerebral ischemia, Huntington's chorea, motor neuron disease, Alzheimer's disease, Parkinson's disease, neurosurgery, meningitis, cancer, fibrile seizures, or dementia.

91. The pharmaceutical composition of claim 90, wherein said epileptogenesis-associated state is epilepsy.

93. The method of any of the foregoing claims, wherein said compound inhibits or modulates GABA transaminase.

94. A method for inhibiting amyloid deposition in a subject comprising administering to the subject an effective amount of a therapeutic compound of Formula (I):



wherein:

X is oxygen or nitrogen;

Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;

m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

R<sup>1</sup> and R<sup>7</sup> are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or —(CH<sub>2</sub>)<sub>p</sub>—Y;

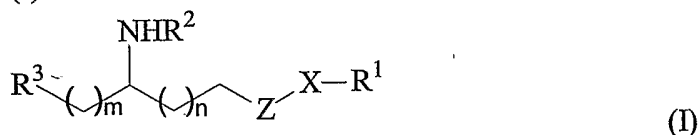
Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

p is 0, 1, 2, 3, or 4;

R<sup>2</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxycarbonyl;

R<sup>3</sup> is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl; and pharmaceutically acceptable salts, esters, and prodrugs thereof, such that said therapeutic compound inhibits an interaction between an amyloidogenic protein and a glycoprotein or proteoglycan constituent of a basement membrane to inhibit amyloid deposition.

95. A method for reducing amyloid deposits in a subject having amyloid deposits, the method comprising administering to said subject an effective amount of a therapeutic compound of Formula (I):



wherein:

X is oxygen or nitrogen;

Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;

m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

R<sup>1</sup> and R<sup>7</sup> are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or -(CH<sub>2</sub>)<sub>p</sub>-Y;

Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

p is 0, 1, 2, 3, or 4;

R<sup>2</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxycarbonyl;

R<sup>3</sup> is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl; and pharmaceutically acceptable salts, esters, and prodrugs thereof, such that amyloid deposits are reduced.

96. The method according to any one of claims 94-95, wherein the therapeutic compound is administered orally.

97. The method according to any one of claims 94-96, wherein said therapeutic compound is administered in a pharmaceutically acceptable vehicle.

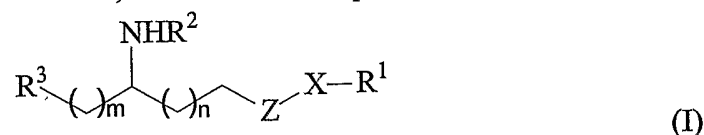
5 98. The method of claim 97, wherein the therapeutic compound is administered orally.

99. The method of claim 97 or 98, wherein said therapeutic compound is administered in a pharmaceutically acceptable vehicle.

10

100. A method for inhibiting amyloid deposition in a subject comprising administering to the subject an effective amount of a therapeutic compound of Formula (I), such that the biodistribution of the therapeutic compound for an intended target site is not prevented while maintaining activity of the therapeutic compound, and such that

15 amyloid deposition is inhibited, wherein said compound of Formula I is:



wherein:

X is oxygen or nitrogen;

Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;

20 m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

R<sup>1</sup> and R<sup>7</sup> are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or  $-(\text{CH}_2)_p-\text{Y}$ ;

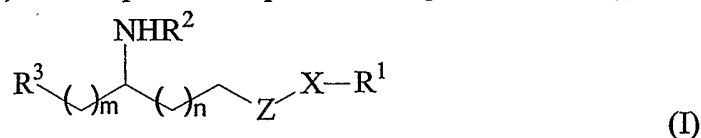
25 Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

p is 0, 1, 2, 3, or 4;

R<sup>2</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxycarbonyl;

30 R<sup>3</sup> is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl; and pharmaceutically acceptable salts, esters, and prodrugs thereof.

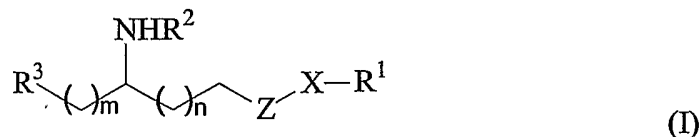
101. A method for inhibiting amyloid deposition in a subject comprising administering to the subject an effective amount of a therapeutic compound of Formula (I), such that the therapeutic compound inhibits an interaction between an amyloidogenic protein and a constituent of a basement membrane and amyloid deposition is inhibited, the therapeutic compound having the Formula (I):



wherein:

- X is oxygen or nitrogen;  
 Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;  
 10 m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;  
 R<sup>1</sup> and R<sup>7</sup> are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or  $-(\text{CH}_2)_p-\text{Y}$ ;  
 Y is hydrogen or a heterocyclic moiety selected from the group  
 15 consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;  
 p is 0, 1, 2, 3, or 4;  
 R<sup>2</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxy carbonyl;  
 20 R<sup>3</sup> is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl; and pharmaceutically acceptable salts, esters, and prodrugs thereof.

- 25 102. A method for inhibiting amyloid deposition in a subject comprising administering to the subject an effective amount of a compound of Formula (I), such that an interaction between an amyloidogenic protein and a constituent of a basement membrane is inhibited and amyloid deposition is inhibited, the therapeutic compound having the Formula



wherein:

- X is oxygen or nitrogen;  
 Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;  
 m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

$R^1$  and  $R^7$  are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or  $-(CH_2)_p-Y$ ;

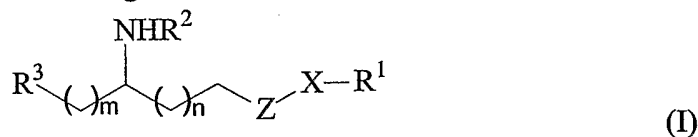
Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

p is 0, 1, 2, 3, or 4;

$R^2$  is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxycarbonyl;

$R^3$  is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl; and pharmaceutically acceptable salts, esters, and prodrugs thereof.

103. A method for reducing amyloid deposits in a subject having amyloid deposits, the method comprising administering to the subject an effective amount of a therapeutic compound of Formula (I), such that amyloid deposits are reduced in the subject, wherein the therapeutic compound having the Formula I is:



wherein:

X is oxygen or nitrogen;

Z is  $C=O$ ,  $S(O)_2$ , or  $P(O)OR^7$ ;

m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

$R^1$  and  $R^7$  are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or  $-(CH_2)_p-Y$ ;

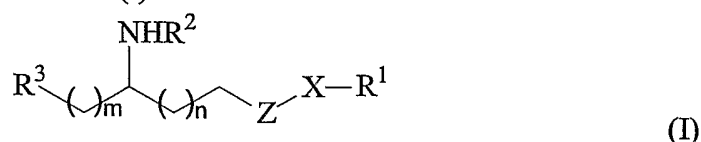
Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

p is 0, 1, 2, 3, or 4;

$R^2$  is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxycarbonyl;

$R^3$  is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl; and pharmaceutically acceptable salts, esters, and prodrugs thereof.

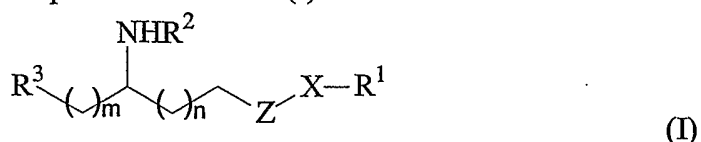
104. A method for inhibiting amyloid deposition in a subject comprising administering to the subject an effective amount of a therapeutic compound of Formula (I), such that the therapeutic compound inhibits an interaction between an amyloidogenic protein and a glycoprotein or proteoglycan constituent of a basement membrane, such that amyloid deposition is inhibited, wherein said therapeutic compound having the Formula (I):



wherein:

- 10 X is oxygen or nitrogen;  
 Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;  
 m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;  
 R<sup>1</sup> and R<sup>7</sup> are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or  $-(\text{CH}_2)_p-\text{Y}$ ;  
 Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;  
 p is 0, 1, 2, 3, or 4;  
 20 R<sup>2</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxycarbonyl;  
 R<sup>3</sup> is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl' and  
 25 pharmaceutically acceptable salts, esters, and prodrugs thereof.

105. The use of a compound according to any one of claims 35-53 of Formula (I) in the preparation of a medicament for the treatment or prevention of an amyloid-related disease, wherein said compound of Formula (I) is:



wherein:

- X is oxygen or nitrogen;  
 Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;  
 m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

$R^1$  and  $R^7$  are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or  $-(CH_2)_p-Y$ ;

Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

p is 0, 1, 2, 3, or 4;

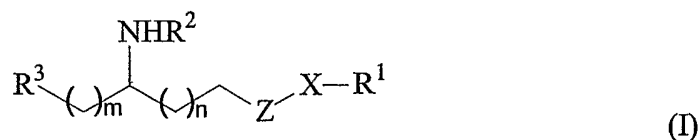
$R^2$  is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxy carbonyl;

$R^3$  is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl; and pharmaceutically acceptable salts, esters, and prodrugs thereof.

106. A pharmaceutical composition for the treatment of an amyloid-related disease comprising a compound according to any one of claims 35-62 or Formula (I).

107. A pharmaceutical composition comprising a compound according to any one of claims 35-62.

108. A pharmaceutical composition comprising a compound according to Formula (I), wherein said compound of Formula I is:



wherein:

X is oxygen or nitrogen;

Z is  $C=O$ ,  $S(O)_2$ , or  $P(O)OR^7$ ;

m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

$R^1$  and  $R^7$  are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or  $-(CH_2)_p-Y$ ;

Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

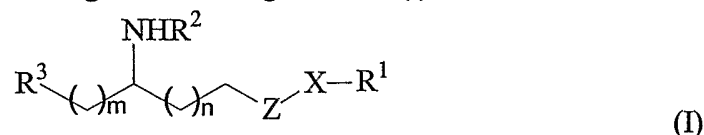
p is 0, 1, 2, 3, or 4;

$R^2$  is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxy carbonyl;

$R^3$  is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl; and pharmaceutically acceptable salts, esters, and prodrugs thereof.

5

109. A pharmaceutical composition for treating amyloidosis comprising a therapeutic compound in an amount sufficient to inhibit amyloid deposition in a subject, the therapeutic compound having the following Formula (I):



10 wherein:

X is oxygen or nitrogen;

Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;

m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

15  $R^1$  and  $R^7$  are each independently hydrogen, metal ion, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to form natural or unnatural amino acid residue, or  $-(\text{CH}_2)_p-\text{Y}$ ;

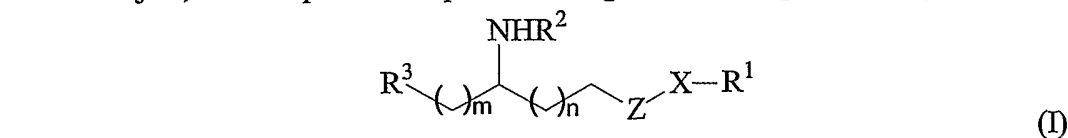
Y is hydrogen or a heterocyclic moiety selected from the group consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and benzoimidazolyl;

20 p is 0, 1, 2, 3, or 4;

$R^2$  is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, or alkoxycarbonyl;

25  $R^3$  is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl; and pharmaceutically acceptable salts, esters, and prodrugs thereof.

110. A pharmaceutical composition for treating amyloidosis comprising a therapeutic compound in an amount sufficient to inhibit an interaction between an amyloidogenic protein and a constituent of a basement membrane and to inhibit amyloid deposition in a subject, the therapeutic compound having the following Formula (I):



wherein:

X is oxygen or nitrogen;

Z is C=O, S(O)<sub>2</sub>, or P(O)OR<sup>7</sup>;

m and n are each independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10;

R<sup>1</sup> and R<sup>7</sup> are each independently hydrogen, metal ion, alkyl,  
mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl, a moiety together with X to  
5 form natural or unnatural amino acid residue, or  $-(CH_2)_p-Y$ ;

Y is hydrogen or a heterocyclic moiety selected from the group  
consisting of thiazolyl, triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, and  
benzoimidazolyl;

p is 0, 1, 2, 3, or 4;

10 R<sup>2</sup> is hydrogen, alkyl, mercaptoalkyl, alkenyl, alkynyl, cycloalkyl, aryl,  
alkylcarbonyl, arylcarbonyl, or alkoxycarbonyl;

R<sup>3</sup> is hydrogen, amino, cyano, alkyl, mercaptoalkyl, alkenyl, alkynyl,  
cycloalkyl, heterocyclic, substituted or unsubstituted aryl, heteroaryl, thiazolyl,  
triazolyl, tetrazolyl, imidazolyl, benzothiazolyl, or benzoimidazolyl; and  
15 pharmaceutically acceptable salts, esters, and prodrugs thereof.