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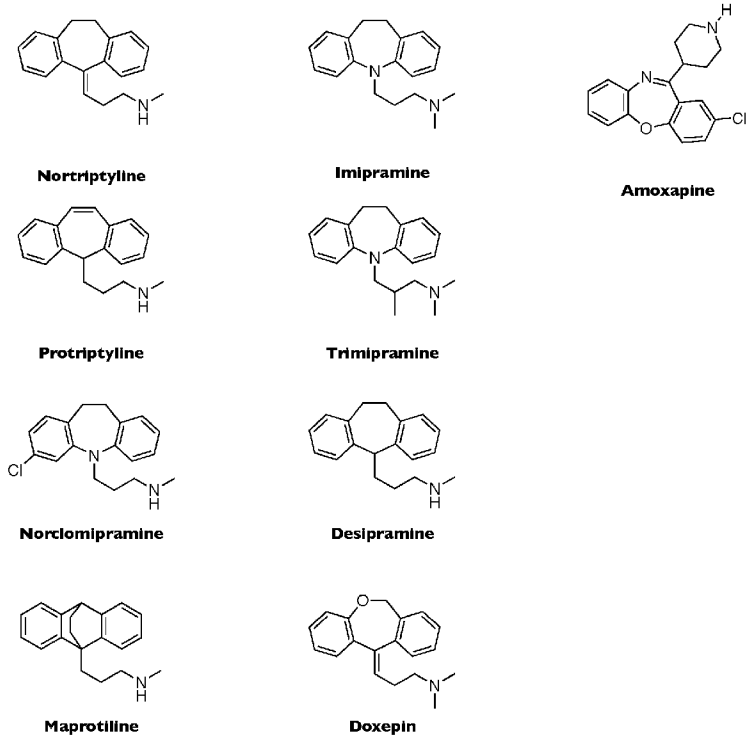
(54) Title: IMAGING OF ALPHA-SYNUCLEIN

(57) Abstract: Radiolabeled alpha-synuclein binding agents are provided that are useful in imaging alpha-synucleing deposits in the brain of subjects with a synucleinopathy or susceptible to a synucleinopathy. The agents are particularly useful in imaging via PET and SPECT nuclear medicine imaging. The imaging techniques may be used to diagnose a patient, follow the progression of disease, or follow treatment of the disease.

Figure 1A

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# IMAGING OF ALPHA-SYNUCLEIN

## Related Applications

**[0001]** The present invention claims priority under 35 U.S.C. § 119(e) to U.S. provisional patent application, USSN 60/953,379, filed August 1, 2007, which is incorporated herein by reference.

## Field of the Invention

**[0002]** The present invention relates to the treatment of neurodegenerative diseases, particularly synucleinopathies, such as Parkinson's disease (PD), diffuse Lewy body disease (DLBD), and multiple system atrophy (MSA).

## Background of the Invention

**[0003]** Synucleinopathies are a diverse group of neurodegenerative disorders that share a common pathologic lesion containing abnormal aggregates of insoluble α-synuclein protein in selectively vulnerable populations of neurons and glia. Certain evidence links the formation of filamentous aggregates to the onset and progression of clinical symptoms and the degeneration of affected brain regions in neurodegenerative disorders including Parkinson's disease (PD), diffuse Lewy body disease (DLBD), multiple system atrophy (MSA), and disorders of brain iron concentration including pantothenate kinase-associated neurodegeneration (*e.g.*, PANK1). The current treatment options for these diseases include symptomatic medications such as carbidopa-levodopa, anticholinergics, and monoamine oxidase inhibitors, with widely variable benefit. Even for the best responders, *i.e.*, patients with idiopathic Parkinson's Disease, an initial good response to levodopa is typically overshadowed by drug-induced complications such as motor fluctuations and debilitating dyskinesia, following the first five to seven years of therapy. For the rest of the disorders, the current medications offer marginal symptomatic benefit. Treatment designed to reduce α-synuclein deposits are of high interest, and a number of strategies (*e.g.*, RNAi, vaccine, small molecule) are already being explored in pre-clinical studies. The magnitude, extent, and rate of change in synuclein deposition effected by these therapeutic approaches will shortly become the critical question that needs to be answered as these strategies come into the clinic.

**[0004]** Given the importance of assessing α-synuclein deposits in the central nervous system in patients with synculeinopathies, there is a clear need in the art for novel approaches towards assessing the levels of α-synuclein deposits in a patient.

## Summary of the Invention

**[0005]** The present invention stems from recognizing the importance of assessing the levels of α-synuclein deposits in the central nervous system as an indicator of disease progression in synucleinopathic patients, such as patients with Parkinson's Disease (PD), Diffuse Lewy Body Disease (DLBD), Multiple System Atrophy (MSA), and disorders of brain iron concentration including pantothenate kinase-associated neurodegeneration (*e.g.*, PANK1). Assessing levels of α-synuclein deposits also allows one to follow treatment of a synucleinopathic subject. The present invention provides radiolabeled compounds that bind to, or otherwise interact with, α-synuclein. The radiolabeled compounds may be used *in vivo* or *in vitro*. The quantity and/or location of bound compound may be determined using an imaging technique known in the art. In certain embodiments, SPECT or PET imaging is used. Autoradiograms or scintillation counting may also be used to assess the levels of alpha-synuclein deposits in a biological sample or subject. The inventive technique may be useful in assessing various treatment modalities.

**[0006]** In one aspect, the invention provides compounds that bind to or interact with α-synuclein and are labeled with a radioisotope. In certain embodiments, the radioisotope is suitable for SPECT or PET imaging. In certain embodiments, the radioisotope is a radioisotope of a halogen such as fluorine (*e.g.*, F-18), chlorine, bromine (*e.g.*, Br-76), or iodine (*e.g.*, I-123). Any agent that binds α-synuclein may be used. In certain embodiments, the agent is a labeled derivative of a tricyclic antidepressant. For example, the agent may be a halogenated tricyclic antidepressant. Particular examples of agents that bind to α-synuclein include nortriptyline, protriptyline, norclomipramine, maprotiline, imipramine, trimipramine, desipramine, doxepin, amoxapine, amitriptyline, mirtazapine, clomipramine, cyproheptadine, cyclobenzaprine, and lofepramine (see *Figure 1*). These compounds may be fluorinated, chlorinated, brominated, or idodinated. In particular, the aromatic rings of the molecules may be used as sites for halogenation as norclomipramine has a chlorine atom on one of its aromatic rings.

**[0007]** In another aspect, the invention provides methods of using the inventive labeled compounds for imaging α-synuclein or assessing α-synuclein levels. In certain embodiments, a biological sample is contacted with an inventive radiolabeled compound at a sufficient

concentration to effect binding of the compound to any α-synuclein present in the sample. The sample is then analyzed to detect bound labeled compound. The detection of α-synuclein may be quantitative.

**[0008]** In another asepct, the invention provides methods of imaging using the labeled compounds of the invention. An amount of the labelled compound effective to bind to or interact with α-synuclein in the subject is administered to subject. The subject is then images using SPECT or PET imaging to detect α-synuclein deposits. In certain embodiments, the brain is imaged. In other embodiments, the nervous system is images. The inventive method may be used to diagnose a patient with a synucleinopathy such as Parkinson's Disease. In other embodiments, the inventive method is used to follow disease progression or treatment of a subject.

**[0009]** The present invention is a major advance in the diagnosis and treatment of synucleinopathies such as Parkinson's disease, diffuse Lewy Body disease, and multiple system atrophy. The invention provides a system useful for research as well as clinical purposes. The invention is particularly useful in assessing the treatment of a synucleinopathic patient using new treatment modalities such as agents that affect the synthesis, aggregation, deposition, and/or clearance of α-synuclein.

## Definitions

**[0010]** Definitions of specific functional groups and chemical terms are described in more detail below. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75$^{th}$ Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry," Thomas Sorrell, University Science Books, Sausalito: 1999, the entire contents of which are incorporated herein by reference.

**[0011]** Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including *cis*- and *trans*-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, including specific polymorphs, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

**[0012]** Isomeric mixtures containing any of a variety of isomer ratios may be utilized in accordance with the present invention. For example, where only two isomers are combined, mixtures containing 50:50, 60:40, 70:30, 80:20, 90:10, 95:5, 96:4, 97:3, 98:2, 99:1, or 100:0 isomer ratios are all contemplated by the present invention. Those of ordinary skill in the art will readily appreciate that analogous ratios are contemplated for more complex isomer mixtures.

**[0013]** If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

**[0014]** One of ordinary skill in the art will appreciate that the synthetic methods, as described herein, utilize a variety of protecting groups. By the term "protecting group", as used herein, it is meant that a particular functional moiety, *e.g.*, O, S, or N, is temporarily blocked so that a reaction can be carried out selectively at another reactive site in a multifunctional compound. In preferred embodiments, a protecting group reacts selectively in good yield to give a protected substrate that is stable to the projected reactions; the protecting group should be selectively removable in good yield by readily available, preferably non-toxic reagents that do not attack the other functional groups; the protecting group forms an easily separable derivative (more preferably without the generation of new stereogenic centers); and the protecting group has a minimum of additional functionality to avoid further sites of reaction. As detailed herein, oxygen, sulfur, nitrogen, and carbon protecting groups may be utilized. Hydroxyl protecting groups include methyl, methoxylmethyl (MOM), methylthiomethyl (MTM), *t*-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), *p*-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (*p*-AOM), guaiacolmethyl (GUM), *t*-butoxymethyl, 4-pentenyloxymethyl (POM), siloxymethyl, 2-methoxyethoxymethyl (MEM), 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 2-(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl (MTHP), 4-methoxytetrahydrothiopyranyl, 4-

methoxytetrahydrothiopyranyl S,S-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (CTMP), 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-(phenylselenyl)ethyl, *t*-butyl, allyl, *p*-chlorophenyl, *p*-methoxyphenyl, 2,4-dinitrophenyl, benzyl, *p*-methoxybenzyl, 3,4-dimethoxybenzyl, *o*-nitrobenzyl, *p*-nitrobenzyl, *p*-halobenzyl, 2,6-dichlorobenzyl, *p*-cyanobenzyl, *p*-phenylbenzyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl *N*-oxido, diphenylmethyl, *p,p'*-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, α-naphthyldiphenylmethyl, *p*-methoxyphenyldiphenylmethyl, di(*p*-methoxyphenyl)phenylmethyl, tri(*p*-methoxyphenyl)methyl, 4-(4'-bromophenacyloxyphenyl)diphenylmethyl, 4,4',4''-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4''-tris(levulinoyloxyphenyl)methyl, 4,4',4''-tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-yl)bis(4',4''-dimethoxyphenyl)methyl, 1,1-bis(4-methoxyphenyl)-1'-pyrenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodithiolan-2-yl, benzisothiazolyl S,S-dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylthexylsilyl, *t*-butyldimethylsilyl (TBDMS), *t*-butyldiphenylsilyl (TBDPS), tribenzylsilyl, tri-*p*-xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), *t*-butylmethoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, *p*-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantoate, crotonate, 4-methoxycrotonate, benzoate, *p*-phenylbenzoate, 2,4,6-trimethylbenzoate (mesitoate), alkyl methyl carbonate, 9-fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2-trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl carbonate (TMSEC), 2-(phenylsulfonyl) ethyl carbonate (Psec), 2-(triphenylphosphonio) ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate alkyl allyl carbonate, alkyl *p*-nitrophenyl carbonate, alkyl benzyl carbonate, alkyl *p*-methoxybenzyl carbonate, alkyl 3,4-dimethoxybenzyl carbonate, alkyl *o*-nitrobenzyl carbonate, alkyl *p*-nitrobenzyl carbonate, alkyl *S*-benzyl thiocarbonate, 4-ethoxy-1-napththyl carbonate, methyl dithiocarbonate, 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, *o*-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl, 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2,6-dichloro-4-

methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (*E*)-2-methyl-2-butenoate, *o*-(methoxycarbonyl)benzoate, α-naphthoate, nitrate, alkyl *N,N,N',N'*-tetramethylphosphorodiamidate, alkyl *N*-phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-dinitrophenylsulfenate, sulfate, methanesulfonate (mesylate), benzylsulfonate, and tosylate (Ts). For protecting 1,2- or 1,3-diols, the protecting groups include methylene acetal, ethylidene acetal, 1-*t*-butylethylidene ketal, 1-phenylethylidene ketal, (4-methoxyphenyl)ethylidene acetal, 2,2,2-trichloroethylidene acetal, acetonide, cyclopentylidene ketal, cyclohexylidene ketal, cycloheptylidene ketal, benzylidene acetal, *p*-methoxybenzylidene acetal, 2,4-dimethoxybenzylidene ketal, 3,4-dimethoxybenzylidene acetal, 2-nitrobenzylidene acetal, methoxymethylene acetal, ethoxymethylene acetal, dimethoxymethylene ortho ester, 1-methoxyethylidene ortho ester, 1-ethoxyethylidine ortho ester, 1,2-dimethoxyethylidene ortho ester, α-methoxybenzylidene ortho ester, 1-(*N,N*-dimethylamino)ethylidene derivative, α-(*N,N'*-dimethylamino)benzylidene derivative, 2-oxacyclopentylidene ortho ester, di-*t*-butylsilylene group (DTBS), 1,3-(1,1,3,3-tetraisopropyldisiloxanylidene) derivative (TIPDS), tetra-*t*-butoxydisiloxane-1,3-diylidene derivative (TBDS), cyclic carbonates, cyclic boronates, ethyl boronate, and phenyl boronate. Amino-protecting groups include methyl carbamate, ethyl carbamante, 9-fluorenylmethyl carbamate (Fmoc), 9-(2-sulfo)fluorenylmethyl carbamate, 9-(2,7-dibromo)fluoroenylmethyl carbamate, 2,7-di-*t*-butyl-[9-(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl carbamate (DBD-Tmoc), 4-methoxyphenacyl carbamate (Phenoc), 2,2,2-trichloroethyl carbamate (Troc), 2-trimethylsilylethyl carbamate (Teoc), 2-phenylethyl carbamate (hZ), 1-(1-adamantyl)-1-methylethyl carbamate (Adpoc), 1,1-dimethyl-2-haloethyl carbamate, 1,1-dimethyl-2,2-dibromoethyl carbamate (DB-*t*-BOC), 1,1-dimethyl-2,2,2-trichloroethyl carbamate (TCBOC), 1-methyl-1-(4-biphenylyl)ethyl carbamate (Bpoc), 1-(3,5-di-*t*-butylphenyl)-1-methylethyl carbamate (*t*-Bumeoc), 2-(2'- and 4'-pyridyl)ethyl carbamate (Pyoc), 2-(*N,N*-dicyclohexylcarboxamido)ethyl carbamate, *t*-butyl carbamate (BOC), 1-adamantyl carbamate (Adoc), vinyl carbamate (Voc), allyl carbamate (Alloc), 1-isopropylallyl carbamate (Ipaoc), cinnamyl carbamate (Coc), 4-nitrocinnamyl carbamate (Noc), 8-quinolyl carbamate, *N*-hydroxypiperidinyl carbamate, alkyldithio carbamate, benzyl carbamate (Cbz), *p*-methoxybenzyl carbamate (Moz), *p*-nitobenzyl carbamate, *p*-bromobenzyl carbamate, *p*-chlorobenzyl carbamate, 2,4-dichlorobenzyl carbamate, 4-methylsulfinylbenzyl carbamate (Msz), 9-anthrylmethyl carbamate, diphenylmethyl carbamate, 2-methylthioethyl carbamate, 2-methylsulfonylethyl carbamate, 2-(*p*-

toluenesulfonyl)ethyl carbamate, [2-(1,3-dithianyl)]methyl carbamate (Dmoc), 4-methylthiophenyl carbamate (Mtpc), 2,4-dimethylthiophenyl carbamate (Bmpc), 2-phosphonioethyl carbamate (Peoc), 2-triphenylphosphonioisopropyl carbamate (Ppoc), 1,1-dimethyl-2-cyanoethyl carbamate, *m*-chloro-*p*-acyloxybenzyl carbamate, *p*-(dihydroxyboryl)benzyl carbamate, 5-benzisoxazolylmethyl carbamate, 2-(trifluoromethyl)-6-chromonylmethyl carbamate (Tcroc), *m*-nitrophenyl carbamate, 3,5-dimethoxybenzyl carbamate, *o*-nitrobenzyl carbamate, 3,4-dimethoxy-6-nitrobenzyl carbamate, phenyl(*o*-nitrophenyl)methyl carbamate, phenothiazinyl-(10)-carbonyl derivative, *N'*-*p*-toluenesulfonylaminocarbonyl derivative, *N'*-phenylaminothiocarbonyl derivative, *t*-amyl carbamate, *S*-benzyl thiocarbamate, *p*-cyanobenzyl carbamate, cyclobutyl carbamate, cyclohexyl carbamate, cyclopentyl carbamate, cyclopropylmethyl carbamate, *p*-decyloxybenzyl carbamate, 2,2-dimethoxycarbonylvinyl carbamate, *o*-(*N,N*-dimethylcarboxamido)benzyl carbamate, 1,1-dimethyl-3-(*N,N*-dimethylcarboxamido)propyl carbamate, 1,1-dimethylpropynyl carbamate, di(2-pyridyl)methyl carbamate, 2-furanylmethyl carbamate, 2-iodoethyl carbamate, isoborynl carbamate, isobutyl carbamate, isonicotinyl carbamate, *p*-(*p'*-methoxyphenylazo)benzyl carbamate, 1-methylcyclobutyl carbamate, 1-methylcyclohexyl carbamate, 1-methyl-1-cyclopropylmethyl carbamate, 1-methyl-1-(3,5-dimethoxyphenyl)ethyl carbamate, 1-methyl-1-(*p*-phenylazophenyl)ethyl carbamate, 1-methyl-1-phenylethyl carbamate, 1-methyl-1-(4-pyridyl)ethyl carbamate, phenyl carbamate, *p*-(phenylazo)benzyl carbamate, 2,4,6-tri-*t*-butylphenyl carbamate, 4-(trimethylammonium)benzyl carbamate, 2,4,6-trimethylbenzyl carbamate, formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3-phenylpropanamide, picolinamide, 3-pyridylcarboxamide, *N*-benzoylphenylalanyl derivative, benzamide, *p*-phenylbenzamide, *o*-nitophenylacetamide, *o*-nitrophenoxyacetamide, acetoacetamide, (*N'*-dithiobenzyloxycarbonylamino)acetamide, 3-(*p*-hydroxyphenyl)propanamide, 3-(*o*-nitrophenyl)propanamide, 2-methyl-2-(*o*-nitrophenoxy)propanamide, 2-methyl-2-(*o*-phenylazophenoxy)propanamide, 4-chlorobutanamide, 3-methyl-3-nitrobutanamide, *o*-nitrocinnamide, *N*-acetylmethionine derivative, *o*-nitrobenzamide, *o*-(benzoyloxymethyl)benzamide, 4,5-diphenyl-3-oxazolin-2-one, *N*-phthalimide, *N*-dithiasuccinimide (Dts), *N*-2,3-diphenylmaleimide, *N*-2,5-dimethylpyrrole, *N*-1,1,4,4-tetramethyldisilylazacyclopentane adduct (STABASE), 5-substituted 1,3-dimethyl-1,3,5-triazacyclohexan-2-one, 5-substituted 1,3-dibenzyl-1,3,5-triazacyclohexan-2-one, 1-substituted 3,5-dinitro-4-pyridone, *N*-methylamine, *N*-allylamine, *N*-[2-(trimethylsilyl)ethoxy]methylamine (SEM), *N*-3-acetoxypropylamine, *N*-(1-isopropyl-4-

nitro-2-oxo-3-pyroolin-3-yl)amine, quaternary ammonium salts, *N*-benzylamine, *N*-di(4-methoxyphenyl)methylamine, *N*-5-dibenzosuberylamine, *N*-triphenylmethylamine (Tr), *N*-[(4-methoxyphenyl)diphenylmethyl]amine (MMTr), *N*-9-phenylfluorenylamine (PhF), *N*-2,7-dichloro-9-fluorenylmethyleneamine, *N*-ferrocenylmethylamino (Fcm), *N*-2-picolylamino *N'*-oxide, *N*-1,1-dimethylthiomethyleneamine, *N*-benzylideneamine, *N*-*p*-methoxybenzylideneamine, *N*-diphenylmethyleneamine, *N*-[(2-pyridyl)mesityl]methyleneamine, *N*-(*N',N'*-dimethylaminomethylene)amine, *N,N'*-isopropylidenediamine, *N*-*p*-nitrobenzylideneamine, *N*-salicylideneamine, *N*-5-chlorosalicylideneamine, *N*-(5-chloro-2-hydroxyphenyl)phenylmethyleneamine, *N*-cyclohexylideneamine, *N*-(5,5-dimethyl-3-oxo-1-cyclohexenyl)amine, *N*-borane derivative, *N*-diphenylborinic acid derivative, *N*-[phenyl(pentacarbonylchromium- or tungsten)carbonyl]amine, *N*-copper chelate, *N*-zinc chelate, *N*-nitroamine, *N*-nitrosoamine, amine *N*-oxide, diphenylphosphinamide (Dpp), dimethylthiophosphinamide (Mpt), diphenylthiophosphinamide (Ppt), dialkyl phosphoramidates, dibenzyl phosphoramidate, diphenyl phosphoramidate, benzenesulfenamide, *o*-nitrobenzenesulfenamide (Nps), 2,4-dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2-nitro-4-methoxybenzenesulfenamide, triphenylmethylsulfenamide, 3-nitropyridinesulfenamide (Npys), *p*-toluenesulfonamide (Ts), benzenesulfonamide, 2,3,6,-trimethyl-4-methoxybenzenesulfonamide (Mtr), 2,4,6-trimethoxybenzenesulfonamide (Mtb), 2,6-dimethyl-4-methoxybenzenesulfonamide (Pme), 2,3,5,6-tetramethyl-4-methoxybenzenesulfonamide (Mte), 4-methoxybenzenesulfonamide (Mbs), 2,4,6-trimethylbenzenesulfonamide (Mts), 2,6-dimethoxy-4-methylbenzenesulfonamide (iMds), 2,2,5,7,8-pentamethylchroman-6-sulfonamide (Pmc), methanesulfonamide (Ms), β-trimethylsilylethanesulfonamide (SES), 9-anthracenesulfonamide, 4-(4',8'-dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzylsulfonamide, trifluoromethylsulfonamide, and phenacylsulfonamide. Exemplary protecting groups are detailed herein. However, it will be appreciated that the present invention is not intended to be limited to these protecting groups; rather, a variety of additional equivalent protecting groups can be readily identified using the above criteria and utilized in the method of the present invention. Additionally, a variety of protecting groups are described in *Protective Groups in Organic Synthesis,* Third Ed. Greene, T.W. and Wuts, P.G., Eds., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference.

**[0015]** It will be appreciated that the compounds, as described herein, may be substituted with any number of substituents or functional moieties. In general, the term "substituted"

whether preceded by the term "optionally" or not, and substituents contained in formulas of this invention, refer to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms. Furthermore, this invention is not intended to be limited in any manner by the permissible substituents of organic compounds. Combinations of substituents and variables envisioned by this invention are preferably those that result in the formation of stable compounds useful in the treatment, for example, of infectious diseases or proliferative disorders. The term "stable", as used herein, preferably refers to compounds which possess stability sufficient to allow manufacture and which maintain the integrity of the compound for a sufficient period of time to be detected and preferably for a sufficient period of time to be useful for the purposes detailed herein.

**[0016]** The term "aliphatic", as used herein, includes both saturated and unsaturated, straight chain (*i.e.*, unbranched), branched, acyclic, cyclic, or polycyclic aliphatic hydrocarbons, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, "aliphatic" is intended herein to include, but is not limited to, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, and cycloalkynyl moieties. Thus, as used herein, the term "alkyl" includes straight, branched and cyclic alkyl groups. An analogous convention applies to other generic terms such as "alkenyl", "alkynyl", and the like. Furthermore, as used herein, the terms "alkyl", "alkenyl", "alkynyl", and the like encompass both substituted and unsubstituted groups. In certain embodiments, as used herein, "lower alkyl" is used to indicate those alkyl groups (cyclic, acyclic, substituted, unsubstituted, branched or unbranched) having 1-6 carbon atoms.

**[0017]** In certain embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl, alkenyl, and

alkynyl groups employed in the invention contain 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-4 carbon atoms. Illustrative aliphatic groups thus include, but are not limited to, for example, methyl, ethyl, n-propyl, isopropyl, cyclopropyl, -$CH_2$-cyclopropyl, vinyl, allyl, n-butyl, sec-butyl, isobutyl, tert-butyl, cyclobutyl, -$CH_2$-cyclobutyl, n-pentyl, sec-pentyl, isopentyl, tert-pentyl, cyclopentyl, -$CH_2$-cyclopentyl, n-hexyl, sec-hexyl, cyclohexyl, -$CH_2$-cyclohexyl moieties and the like, which again, may bear one or more substituents. Alkenyl groups include, but are not limited to, for example, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, and the like. Representative alkynyl groups include, but are not limited to, ethynyl, 2-propynyl (propargyl), 1-propynyl, and the like.

**[0018]** The term "alkoxy", or "thioalkyl" as used herein refers to an alkyl group, as previously defined, attached to the parent molecule through an oxygen atom or through a sulfur atom. In certain embodiments, the alkyl, alkenyl, and alkynyl groups contain 1-20 alipahtic carbon atoms. In certain other embodiments, the alkyl, alkenyl, and alkynyl groups contain 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl, alkenyl, and alkynyl groups contain 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups contain 1-4 aliphatic carbon atoms. Examples of alkoxy, include but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, tert-butoxy, neopentoxy, and n-hexoxy. Examples of thioalkyl include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, n-butylthio, and the like.

**[0019]** The term "alkylamino" refers to a group having the structure -NHR', wherein R' is aliphatic, as defined herein. In certain embodiments, the aliphatic group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the aliphatic group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the aliphatic group employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the aliphatic group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the aliphatic group contains 1-4 aliphatic carbon atoms. Examples of alkylamino groups include, but are not limited to, methylamino, ethylamino, n-propylamino, iso-propylamino, cyclopropylamino, n-butylamino, tert-butylamino, neopentylamino, n-pentylamino, hexylamino, cyclohexylamino, and the like.

**[0020]** The term "dialkylamino" refers to a group having the structure -NRR', wherein R and R' are each an aliphatic group, as defined herein. R and R' may be the same or different

in an dialkyamino moiety. In certain embodiments, the aliphatic groups contains 1-20 aliphatic carbon atoms. In certain other embodiments, the aliphatic groups contains 1-10 aliphatic carbon atoms. In yet other embodiments, the aliphatic groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the aliphatic groups contains 1-6 aliphatic carbon atoms. In yet other embodiments, the aliphatic groups contains 1-4 aliphatic carbon atoms. Examples of dialkylamino groups include, but are not limited to, dimethylamino, methyl ethylamino, diethylamino, methylpropylamino, di(n-propyl)amino, di(iso-propyl)amino, di(cyclopropyl)amino, di(n-butyl)amino, di(tert-butyl)amino, di(neopentyl)amino, di(n-pentyl)amino, di(hexyl)amino, di(cyclohexyl)amino, and the like. In certain embodiments, R and R' are linked to form a cyclic structure. The resulting cyclic structure may be aromatic or non-aromatic. Examples of cyclic diaminoalkyl groups include, but are not limted to, aziridinyl, pyrrolidinyl, piperidinyl, morpholinyl, pyrrolyl, imidazolyl, 1,3,4-trianolyl, and tetrazolyl.

**[0021]** Some examples of substituents of the above-described aliphatic (and other) moieties of compounds of the invention include, but are not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; arylalkyl; heteroarylalkyl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of $R_x$ independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein any of the aliphatic, heteroaliphatic, arylalkyl, or heteroarylalkyl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

**[0022]** In general, the terms "aryl" and "heteroaryl", as used herein, refer to stable mono- or polycyclic, heterocyclic, polycyclic, and polyheterocyclic unsaturated moieties having preferably 3-14 carbon atoms, each of which may be substituted or unsubstituted. Substituents include, but are not limited to, any of the previously mentioned substitutents, *i.e.*, the substituents recited for aliphatic moieties, or for other moieties as disclosed herein, resulting in the formation of a stable compound. In certain embodiments of the present invention, "aryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl,

indenyl, and the like. In certain embodiments of the present invention, the term "heteroaryl", as used herein, refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S, O, and N; zero, one, or two ring atoms are additional heteroatoms independently selected from S, O, and N; and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms, such as, for example, pyridyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl,oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

**[0023]** It will be appreciated that aryl and heteroaryl groups can be unsubstituted or substituted, wherein substitution includes replacement of one, two, three, or more of the hydrogen atoms thereon independently with any one or more of the following moieties including, but not limited to: aliphatic; heteroaliphatic; aryl; heteroaryl; arylalkyl; heteroarylalkyl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; -F; -Cl; -Br; -I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$, wherein each occurrence of $R_x$ independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein any of the aliphatic, heteroaliphatic, arylalkyl, or heteroarylalkyl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substitutents are illustrated by the specific embodiments shown in the Examples that are described herein.

**[0024]** The term "cycloalkyl", as used herein, refers specifically to groups having three to seven, preferably three to ten carbon atoms. Suitable cycloalkyls include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and the like, which, as in the case of other aliphatic, heteroaliphatic, or hetercyclic moieties, may optionally be substituted with substituents including, but not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; arylalkyl; heteroarylalkyl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; -F; -Cl; -Br; -I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$, wherein each occurrence of $R_x$ independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein any of the aliphatic, heteroaliphatic, arylalkyl, or

heteroarylalkyl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substitutents are illustrated by the specific embodiments shown in the Examples that are described herein.

**[0025]** The term "heteroaliphatic", as used herein, refers to aliphatic moieties that contain one or more oxygen, sulfur, nitrogen, phosphorus, or silicon atoms, *e.g.*, in place of carbon atoms. Heteroaliphatic moieties may be branched, unbranched, cyclic or acyclic and include saturated and unsaturated heterocycles such as morpholino, pyrrolidinyl, *etc.* In certain embodiments, heteroaliphatic moieties are substituted by independent replacement of one or more of the hydrogen atoms thereon with one or more moieties including, but not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; arylalkyl; heteroarylalkyl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; -F; -Cl; -Br; -I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$, wherein each occurrence of $R_x$ independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein any of the aliphatic, heteroaliphatic, arylalkyl, or heteroarylalkyl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substitutents are illustrated by the specific embodiments shown in the Examples that are described herein.

**[0026]** The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine, and iodine.

**[0027]** The term "heterocycloalkyl" or "heterocycle", as used herein, refers to a non-aromatic 5-, 6-, or 7- membered ring or a polycyclic group, including, but not limited to a bi- or tri-cyclic group comprising fused six-membered rings having between one and three heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein (i) each 5-membered ring has 0 to 1 double bonds and each 6-membered ring has 0 to 2 double bonds, (ii) the nitrogen and sulfur heteroatoms may be optionally be oxidized, (iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to a benzene ring. Representative heterocycles include, but are not limited to, pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, and

tetrahydrofuryl. In certain embodiments, a "substituted heterocycloalkyl or heterocycle" group is utilized and as used herein, refers to a heterocycloalkyl or heterocycle group, as defined above, substituted by the independent replacement of one, two or three of the hydrogen atoms thereon with but are not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; arylalkyl; heteroarylalkyl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; -F; -Cl; -Br; -I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$, wherein each occurrence of $R_x$ independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein any of the aliphatic, heteroaliphatic, arylalkyl, or heteroarylalkyl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substitutents are illustrated by the specific embodiments shown in the Examples which are described herein.

**[0028]** "Carbocycle": The term "carbocycle", as used herein, refers to an aromatic or non-aromatic ring in which each atom of the ring is a carbon atom.

**[0029]** "Independently selected": The term "independently selected" is used herein to indicate that the R groups can be identical or different.

**[0030]** "Labeled": As used herein, the term "labeled" is intended to mean that a compound has at least one element, isotope, or chemical compound attached to enable the detection of the compound. In general, labels typically fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes, including, but not limited to, $^{2}H$, $^{3}H$, $^{13}C$, $^{14}C$, $^{15}N$, $^{18}F$, $^{31}P$, $^{32}P$, $^{35}S$, $^{67}Ga$, $^{75}Br$, $^{76}Br$, $^{99m}Tc$ (Tc-99m), $^{111}In$, $^{123}I$, $^{125}I$, $^{131}I$, $^{169}Yb$, and $^{186}Re$; b) immune labels, which may be antibodies or antigens, which may be bound to enzymes (such as horseradish peroxidase) that produce detectable agents; and c) colored, luminescent, phosphorescent, or fluorescent dyes. It will be appreciated that the labels may be incorporated into the compound at any position that does not interfere with the biological activity or characteristic of the compound that is being detected.

**[0031]** "Tautomers": As used herein, the term "tautomers" are particular isomers of a compound in which a hydrogen and double bond have changed position with respect to the other atoms of the molecule. For a pair of tautomers to exist there must be a mechanism for interconversion. Examples of tautomers include keto-enol forms, imine-enamine forms, amide-imino alcohol forms, amidine-aminidine forms, nitroso-oxime forms, thio ketone-

enethiol forms, *N*-nitroso-hydroxyazo forms, nitro-*aci*-nitro forms, and pyridione-hydroxypyridine forms.

**[0032]** Definitions of non-chemical terms used throughout the specification include:

**[0033]** "Animal": The term animal, as used herein, refers to humans as well as non-human animals, including, for example, mammals, birds, reptiles, amphibians, and fish. Preferably, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a primate, a non-human primate, or a pig). The animal may be at any stage of development. A non-human animal may be a transgenic animal.

**[0034]** "Bind" or "binding": The term "bind" or "binding" refers to any interaction or association between the radiolabeled compound and α-synuclein. In certain embodiments, the binding is non-covalent. The binding preferably provides for quantitative measure of α-synuclein in a biological sample or subject.

**[0035]** "Biological sample": The term "biological sample" is broadly defined to include any cell, tissue, biological fluid, organ, multi-cellular organism, and the like. A biological sample may be derived, for example, from cells or tissue cultures *in vitro*. Alternatively, a biological sample may be derived from a living organism or from a population of single-cell organisms. A biological sample may be a live tissue such as live bone. The term "biological sample" is also intended to include samples such as cells, tissues or biological fluids isolated from a subject, as well as samples present within a subject.

**[0036]** "Effective amount": In general, the "effective amount" of an agent refers to an amount sufficient to bind alpha-synuclein to obtain a detectable signal upon imaging. As will be appreciated by those of ordinary skill in this art, the effective amount of a compound of the invention may vary depending on such factors as the affinity of the agent for alpha-synuclein, the pharmacokinetics of the compound, the disease being detected, the mode of administration, and the patient.

**[0037]** "Polynucleotide" or "oligonucleotide" refers to a polymer of nucleotides. The polymer may include natural nucleosides (*i.e.*, adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine), nucleoside analogs (*e.g.*, 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, 4-acetylcytidine, 5-(carboxyhydroxymethyl)uridine, dihydrouridine, methylpseudouridine, 1-methyl adenosine, 1-methyl guanosine, N6-methyl

adenosine, and 2-thiocytidine), chemically modified bases, biologically modified bases (*e.g.*, methylated bases), intercalated bases, modified sugars (*e.g.*, 2'-fluororibose, ribose, 2'-deoxyribose, 2′-O-methylcytidine, arabinose, and hexose), or modified phosphate groups (*e.g.*, phosphorothioates and 5' -N-phosphoramidite linkages).

**[0038]** A "protein" or "peptide" comprises a polymer of amino acid residues linked together by peptide bonds. The term, as used herein, refers to proteins, polypeptides, and peptide of any size, structure, or function. Typically, a protein will be at least three amino acids long. A protein may refer to an individual protein or a collection of proteins. Inventive proteins preferably contain only natural amino acids, although non-natural amino acids (*i.e.*, compounds that do not occur in nature but that can be incorporated into a polypeptide chain) and/or amino acid analogs as are known in the art may alternatively be employed. Also, one or more of the amino acids in an inventive protein may be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a hydroxyl group, a phosphate group, a farnesyl group, an isofarnesyl group, a fatty acid group, a linker for conjugation, functionalization, or other modification, *etc.* A protein may also be a single molecule or may be a multi-molecular complex. A protein may be just a fragment of a naturally occurring protein or peptide. A protein may be naturally occurring, recombinant, or synthetic, or any combination of these.

**[0039]** "Synucleinopathic subject": The term "synucleinopathic subject" refers to a subject that is affected by or at risk of developing a synucleinopathy (e.g. predisposed, for example genetically predisposed, to developing a synucleinopathy) and/or any neurodegenerative disorders characterized by pathological synuclein aggregations. Several neurodegenerative disorders including Parkinson's disease, Diffuse Lewy Body disease (DLBD) and Multiple System Atrophy (MSA) are collectively grouped as synucleinopathies.

**[0040]** "Treatment": According to the invention, the term "treatment" includes prophylaxis and therapy, and includes managing a synucleinopathic subject's symptoms and halting the progression of the synucleinopathy. Treatment includes preventing, slowing, stopping, or reversing (*e.g.*, curing) the development of a synucleinopathy, and/or the onset of certain symptoms associated with a synucleinopathy in a subject with, or at risk of developing, a synucleinopathy or a related disorder. Therapy includes preventing, slowing, stopping or reversing (*e.g.,* curing) the accumulation of α-synuclein in a subject with a synucleinopathy. Therapy also includes decreasing the amount of accumulated α-synuclein in a subject with a synucleinopathy.

## Brief Description of the Drawings

**[0041]** *Figure 1A* and *1B*. Chemical structures of various tricyclic antidepressants that may be labeled with radioisotopes and used in PET or SPECT imaging.

**[0042]** *Figure 2*. Nortriptyline binds to α-synuclein and affects the rate of structure formation in the presence of HFIP. A. Monitoring of Thioflavin T fluorescence. B. Monitoring of α-synuclein fluorescence polarization. Solvent alone (*solid line*), Nortriptyline at 50 μM (*open squares*), 100 μM (*open circles*), 200 μM (*open triangles*), or 400 μM (*open diamonds*).

**[0043]** *Figure 3*. Protriptyline and Maprotiline bind to α-synuclein and affect the rate of structure formation in the presence of HFIP. Monitoring of Thioflavin T fluorescence. A. Protriptyline. B. Maprotiline. Solvent alone (*solid line*), compound at 50 μM (*open squares*), 100 μM (*open circles*), 200 μM (*open triangles*), or 400 μM (*open diamonds*).

**[0044]** *Figure 4*. Norclomipramine and Nordoxepin bind to α-synuclein and affect the rate of structure formation in the presence of HFIP. Monitoring of Thioflavin T fluorescence. A. Norclomipramine. B. Nordoxepin. Solvent alone (*solid line*), compound at 50 μM (*open sqaures*), 100 μM (*open circles*), 200 μM (*open triangles*), or 400 μM (*open diamonds*).

**[0045]** *Figure 5*. Amoxapine and Doxepin bind to α-synuclein and affect the rate of structure formation in the presence of HFIP. Monitoring of Thioflavin T fluorescence. A. Amoxapine. B. Doxepin. Solvent alone (*solid line*), compound at 50 μM (*open squares*), 100 μM (*open circles*), 200 μM (*open triangles*), or 400 μM (*open diamonds*).

**[0046]** *Figure 6*. Desipramine and Trimipramine bind to α-synuclein and affect the rate of structure formation in the presence of HFIP. Monitoring of Thioflavin T fluorescence. A. Desipramine. B. Trimipramine. Solvent alone (*solid line*), compound at 20 μM (*closed sqaures*), or 200 μM (*open triangles*).

**[0047]** *Figure 7*. Clomipramine and Imipramine bind to α-synuclein and affect the rate of structure formation in the presence of HFIP. Monitoring of Thioflavin T fluorescence. A. Clomipramine. B. Imipramine. Solvent alone (*solid line*), compound at 20 μM (*closed sqaures*), or 200 μM (*open triangles*).

**[0048]** *Figure 8*. Nortriptyline delays aggregation of α-synuclein. A. Amount of α-synuclein monomer in solution. B. Monitoring of α -synuclein fluorescence polarization. Solvent alone (*black bars*), 100 μM Nortriptyline.

**[0049]** *Figure 9*. Nortriptyline delays aggregation of α-synuclein in a dose-dependent manner. Recombinant α-synuclein (70 μM) was incubated in the presence of solvent alone

(*solid line*), nortriptyline at 0.1 μM (*open squares*), 50 μM (*open cirlces*), or 200 μM (*open diamonds*).

**[0050]** *Figure 10.* Nortriptyline decreases α-synuclein neurotoxicity in dopaminergic neurons. Midbrain cultures from embryonic mice were infected with a recombinant lentivirus encoding A53T human α-synuclein (A53T) or a control virus (control), then treated for 3 days. The percent Tyrosine-Hydroxylase-positive neurons (TH+ cells) was determined as described.

**[0051]** *Figure 11.* Nortriptyline binding affects the accumulation of α-synuclein in transgenic mice. Quantification of α-synuclein by ELISA in the cytoplasmic (*black bars*) and membrane fraction (*white bars*) in three month old α-synuclein transgenic mice treated for 30 days. A. Cortex. B. Hippocampus. *, $p < 0.05$; **, $p < 0.01$, T-test.

**[0052]** *Figure 12.* Nortriptyline binding affects the accumulation of α-synuclein in transgenic mice. Number of cells positive for α-synuclein immunoreactivity in three month old α-synuclein transgenic mice treated for 30 days. A. Cortex. B. Hippocampus. ** $p < 0.01$, *** $P < 0.001$, T-test.

**[0053]** *Figure 13.* Hippocampus of three month old α-synuclein transgenic mice treated for 30 days with vehicle or Nortriptyline. Immunofluorescence analysis of brain sections immunostained for human α-synuclein *(green)* and NeuN *(red)*.

**[0054]** *Figure 14.* Nortriptyline binding affects the accumulation of α-synuclein in transgenic mice. Number of cells positive for human α-synuclein immunoreactivity six-month-old α-synuclein transgenic mice treated for 30 days. A. Cortex. B. Hippocampus. * $p < 0.05$, ** $p < 0.01$, *** $P < 0.001$, one-way ANOVA with Newman-Keuls comparison post-hoc test.

## Detailed Description of Certain Embodiments of the Invention

**[0055]** Deposition of alpha-synuclein is an invariant feature of synucleinopathies such as Parkinson's disease that is temporally and antomically linked to symptom progression. Treatment designed to reduce alpha-synuclein deposits are of high interest, and a number of such strategies using RNAi technology, vaccines, or small molecules are currently being explored pre-clinically. Assessing the success of these strategies will be aided by the efficient imaging of alpha-synuclein deposits in a biological sample, experimental animal, or human subject. The magnitude, extent, and rate of change in alpha-synuclein deposits effected by these new therapeutic approaches will soon become the critical question in

moving these approaches into the clinic. Based on this need, the present invention for imaging alpha-synuclein deposits or quantitating alpha-synuclein deposits in the nervous system of a subject was developed.

**[0056]** The invention provides a system for imaging and/or assessing the levels of α-synuclein in a biological sample or a subject. Compounds known to bind or interact with α-synuclein are labeled with a radioisotope to form scintigraphic biomarkers useful in imaging (*e.g.*, SPECT and PET imaging). The invention includes such labeled alpha-synuclein binding agents and pharmaceutical compositions thereof. The invention also includes methods of using the inventive compounds and compositions in diagnosing and following disease progression or treatment in a subject (*e.g.*, humans) including experimental animals. The inventive imaging methods may be combined with methods of treating a synucleinopathy.

*Synucleinopathies*

**[0057]** The inventive compound, compositions, kits, and methods may be used to diagnose or follow the progression of a synucleinopathy. In certain embodiments, the inventive system is used to assess a treatment of a synucleinopathy in a patient. Synucleinopathies are neurodegenerative disease that share a commong pathologic lesion containg α-synuclein deposits.

**[0058]** Synucleins are small proteins (123 to 143 amino acids) characterized by repetitive imperfect repeats SEQ ID NO: 8 (KTKEGV) distributed throughout most of the amino terminal half of the polypeptide in the acidic carboxy-terminal region. There are three human synuclein proteins termed α, β, and γ, and they are encoded by separate genes mapped to chromosomes 4221.3-q22, 5q23 and 10q23.2-q23.3, respectively. The most recently cloned synuclein protein synoretin, has a close homology to γ-synuclein and is predominantly expressed within the retina. α-synuclein, also referred to as non-amyloid component of senile plaques precursor protein (NACP), SYN1 or synelfin, is a heat-stable, "natively unfolded" protein of poorly defined function. It is predominantly expressed in the central nervous system (CNS) neurons where it is localized to presynaptic terminals. Electron microscopy studies have localized α-synuclein in close proximity to synaptic vesicles at axonal termini, suggesting a role for α-synuclein in neurotransmission or synaptic organization, and biochemical analysis has revealed that a small fraction of α-synuclein may be associated with vesicular membranes but most α-synuclein is cytosolic.

**[0059]** Genetic and histopathological evidence supports the idea that α-synuclein is the major component of several proteinaceous inclusions characteristic of specific neurodegenerative diseases. Pathological synuclein aggregations are restricted to the α-synuclein isoforms, as β and γ synucleins have not been detected in these inclusions. The presence of α-synuclein positive aggregates is disease specific. Lewy bodies, neuronal fibrous cytoplasmic inclusions that are histopathological hallmarks of Parkinson's Disease (PD) and Diffuse Lewy Body disease (DLBD) are strongly labeled with antibodies to α-synuclein. Dystrophic ubiquitin-positive neurites associated with PD pathology, termed Lewy neurites (LN) and CA2/CA3 ubiquitin neurites are also α-synuclein positive. Furthermore, pale bodies, putative precursors of LBs, thread-like structures in the perikarya of slightly swollen neurons and glial silver positive inclusions in the midbrains of patients with LB diseases are also immunoreactive for α-synuclein. α-synuclein is likely the major component of glial cell inclusions (GCIs) and neuronal cytoplasmic inclusions in MSA and Hallervorden-Spatz disease (brain iron accumulation type 1). α-synuclein immunoreactivity is also present in Alzheimer's disease, prion diseases (scrapie, mad cow disease, Gerstmann-Strassler Schenker, Creutzfeld Jakob disease, *etc.*), amyotrophic lateral sclerosis, Huntington's disease, and other neurodegenerative disorders.

**[0060]** Further evidence supports the notion that α-synuclein is the actual building block of the fibrillary components of LBs, LNs and GCIs. Immunoelectron microscopic studies have demonstrated that these fibrils are intensely labeled with α-synuclein antibodies *in situ*. Sarcosyl-insoluble α-synuclein filaments with straight and twisted morphologies can also be observed in extracts of DLBD and MSA brains. Moreover, α-synuclein can assemble *in vitro* into elongated homopolymers with similar widths as sarcosyl-insoluble fibrils or filaments visualized *in situ*. Polymerization is associated with a concomitant change in secondary structure from random coil to anti-parallel β-sheet structure consistent with the Thioflavine-S reactivity of these filaments. Furthermore, the PD-association with α-synuclein mutation, A53T, may accelerate this process, as recombinant A53T α-synuclein has a greater propensity to polymerize than wild-type α-synuclein. This mutation also affects the ultrastructure of the polymers; the filaments are slightly wider and are more twisted in appearance, as if assembled from two protofilaments. The A30P mutation may also modestly increase the propensity of α-synuclein to polymerize, but the pathological effects of this mutation also may be related to its reduced binding to vesicles. Interestingly, carboxyl-terminally truncated α-synuclein may be more prone to form filaments than the full-length protein.

**[0061]** In methods of the invention, the term "synucleionopathy" refers to neurological disorders that are characterized by a pathological accumulation of α-synuclein. This group of disorders includes PD, DLBD and MSA.

**[0062]** Parkinson's Disease (PD) is a neurological disorder characterized by bradykinesia, shuffling gait, postural instability, tremor, and a loss of automatic movement. It is due to the loss of dopamine-containing substantia nigra cells. It appears that about 50% of the cells need to be lost before symptoms appear. Associated symptoms often include rigidity, difficulty initiating movement (akinesia), small handwriting (micrographia), seborrhea, orthostatic hypertension, urinary difficulties, constipation, lymph pain, depression, dementia (up to a third of the patients), smelling disturbances (occurs early). Orthostatic hypertension might occur associated with the disease or as a complication of medication. Patients with Parkinsonism have greater mortality, about two times compared to general population without PD. This is attributed to greater frailty or reduced mobility.

**[0063]** The term "synucleinopathic subject" encompasses a subject that is affected by, or is at risk of developing PD. These subjects can be readily identified by persons of ordinary skill in the art by symptomatic diagnosis or by genetic screening, brain scans, SPEC, PET imaging, *etc.*

**[0064]** Diagnosis of PD is mainly clinical and is based on the clinical findings listed above. There are many conditions which may be mistaken for Parkinsonism. Among the most common are side effects of drugs, mainly the major tranquilizers, such as Haldol, strokes involving the basal ganglea, degenerative disorders, such as progressive supranuclear palsy (PSP), olivopontocerebellar degeneration (OPCD), MSA, and Huntington's Disease. The pathological hallmark of PD are Lewy bodies, which are intracytoplasmatic inclusion bodies in effected neurons of the substantion nigra. Recently, α-synuclein has been identified as the main component of Lewy bodies in sporadic Parkinsonism.

**[0065]** Although Parkinson's can be clearly traced to genetic factors, viruses, stroke, or toxins in few individuals for the most part the cause of Parkinson's in any particular case is unknown (this is referred to as sporadic PD). Environmental influences include drinking well water, farming and industrial exposure to heavy metals (iron, zinc, copper, mercury, magnesium and manganese), alkylated phosphates and orthonal chlorines. Paraquat (a herbicide) has been associated with increased prevalence of Parkinsonism, cigarette smoking is associated with the decrease incidence. The current consensus is that Parkinsonism may either be caused by an uncommon toxin combined with high genetic susceptibility or a common toxin combined with relatively low genetic susceptibility.

**[0066]** Subjects that are at risk of developing PD can be identified for example by genetic analysis. There is good evidence for genetic factors associated with PD. Large pedigrees of autosomal dominantly inherited PDs have been reported. A mutation in α-synuclein is responsible for one pedigree.

**[0067]** According to the invention, the term "synucleinopathic subject" also encompasses a subject that is affected by, or is at risk of developing DLBD. These subjects can be readily identified by persons of ordinary skill in the art by symptomatic diagnosis or by genetic screening, brain scans, SPEC, PET imaging, *etc.*

**[0068]** DLBD is the second commonest cause of neurodegenerative dementia in older people, it effects 7% of the general population older than 65 years and 30% of those aged over 80 years. It is part of a range of clinical presentations that share a neurotic pathology base of normal aggregation of the synaptic protein α-synuclein. DLBD has many of the clinical and pathological characteristics of the dementia that occurs during the course of Parkinson's Disease. An "one year rule" can been used to separate DLBD from PD. According to this rule, onset of dementia within 12 months of Parkinsonism qualifies as DLBD, whereas more than 12 months of Parkinsonism before onset of dementia qualifies as PD. The central features of DLBD include progressive cognitive decline of sufficient magnitude to interfere with normal social and occupational function. Prominent or persistent memory impairment does not necessarily occur in the early stages, but it is evident with progression in most cases. Deficits on tests of attention and of frontal cortical skills and visual spatial ability can be especially prominent.

**[0069]** Core diagnostic features, two of which are essential for diagnosis of probable and one for possible DLBD are fluctuating cognition with pronounced variations in attention and alertness, recurrent visual hallucinations that are typically well-formed and detailed, and spontaneous features of Parkinsonism. In addition, there can be some supportive features, such as repeated falls, syncope, transient loss of consciousness, neuroleptic sensitivity, systematized delusions, hallucinations and other modalities, REM sleep behavior disorder, and depression. Patients with DLBD do better than those with Alzheimer's Disease in tests of verbal memory, but worse on visual performance tests. This profile can be maintained across the range of severity of the disease, but can be harder to recognize in the later stages owing to global difficulties. DLBD typically presents with recurring episodes of confusion on a background of progressive deterioration. Patients with DLBD show a combination of cortical and subcortical neuropsychological impairments with substantial attention deficits

and prominent frontal subcortical and visual special dysfunction. These help differentiate this disorder from Alzheimer's Disease.

**[0070]** Rapid eye movement (REM), sleep behavior and disorder is a parasomnia manifested by vivid and frightening dreams associated with simple or complex motor behavior during REM sleep. This disorder is frequently associated with the synucleinopathies, DLBD, PD and MSA, but it rarely occurs in amyloidopathies and taupathies. The neuropsychological pattern of impairment in REM sleep behavior disorder/dementia is similar to that reported in DLBD and qualitatively different from that reported in Alzheimer's Disease. Neuropathological studies of REM sleep behavior disorder associated with neurodegenerative disorder have shown Lewy body disease or multiple system atrophy. REM sleep wakefulness disassociations (REM sleep behavior disorder, daytime hypersomnolence, hallucinations, cataplexy) characteristic of narcolepsy can explain several feature of DLBD, as well as PD. Sleep disorders could not contribute to the fluctuations typical of DLBD and their treatment can improve fluctuations and quality of life. Subjects at risk of developing DLBD can be identified. Repeated falls, syncope, transient loss of consciousness, and depression are common in older people with cognitive impairment and can serve as (a red flag) to a possible diagnosis of DLBD. By contrast, narcoleptic sensitivity in REM sleep behavior disorder can be highly predictive of DLBD. Their detection depends on the clinicians having a high index of suspicion and asking appropriate screening questions.

**[0071]** Clinical diagnosis of synucleinopathic subjects that are affected by or at risk of developing LBD can be supported by neuroimaging investigations. Changes associated with DLBD include preservation of hippocampal, and medialtemperalobe volume on MRI and sipital hyperprofusion on SPECT. Other features, such as generalized atrophy, white medichanges and rates of progression of whole brain atrophy are not helpful in differential diagnosis. Dopamine transported a loss in the caudate and putamen, a marker of nigrostriatal degeneration can be detected by dopomenergic SPECT and can prove helpful in clinical differential diagnosis. A sensitivity of 83% and specificity of 100% has been reported for an abnormal scan with an autopsy diagnosis of DLBD.

**[0072]** Consensus criteria for diagnosing DLBD include ubiquitin immunohistochemistry for Lewy body identification and staging into three categories; brain stem predominant, limbic, or neocortical, depending on the numbers and distribution of Lewy bodies. The recently-developed α-synuclein immunohistochemistry is a better marker that visualizes more Lewy bodies and also better source previously under recognized neurotic pathology, termed

Lewy neurites. Use of antibodies to α-synuclein moves the diagnostic rating for many DLBD cases from brain stem and limbic groups into the neocortical group.

**[0073]** In most patients with DLBD, there are no genetic mutations in the α-synuclein or other Parkinson's Disease genes. Pathological up-regulation of normal, wild-type α-synuclein due to increased mRNA expression is a possible mechanism, or Lewy bodies may form because α-synuclein becomes insoluble or more able to aggregate for some reason. Another possibility is that α-synuclein is abnormally processed, for example, by dysfunctional proteosome system and that toxic "proto fibrils" are therefore produced. Sequestering of these toxic fibrils into Lewy bodies could reflect an effort by the neurons to combat biological stress inside the cell, rather than their simply being neurodegenerative debris.

**[0074]** Target symptoms for the accurate of DLBD can include extrapyramidal motor features, cognitive impairment, neuropsychiatric features (including hallucinations, depression, sleep disorder, and associated behavioral disturbances) or autonomic dysfunction.

**[0075]** Methods of the invention can be used in combination with one or more alternative treatments DLBD. For example, lowest acceptable doses of levodopa can be used for treating DLBD. D2-receptor antagonists, particularly traditional neuroleptic agents can provoke severe sensitivity reactions in DLBD subjects with an increase in mortality of two to three times. Cholinsterase inhibitors dicussed above are also used in the treatment of DLBD.

**[0076]** According to the invention, the term "synucleinopathic subject" also encompasses a subject that is affected by, or is at risk of developing MSA. These subjects can be readily identified by persons of ordinary skill in the art by symptomatic diagnosis or by genetic screening, brain scans, SPEC, PET imaging, *etc.*

**[0077]** MSA is a neurodegenerative disease marked by a combination of symptoms; affecting movement, blood pressure, and other body functions, hence the label "multiple system atrophy". The cause of MSA is unknown. Symptoms of MSA vary in distribution of onset and severity from person to person. Because of this, three different diseases were initially described to accomplish this range of symptoms; Shy-Drager syndrome, striatonigral degeneration (SD), and olivopontocerebellar atrophy (OPCA).

**[0078]** In Shy-Drager syndrome, the most prominent symptoms are those involving the autonomic system; blood pressure, urinary function, and other functions not involving conscious control. Striatonigral degeneration causes Parkinsonism symptoms, such as slowed movements and rigidity, while OPCA principally effects balance, coordination and speech.

The symptoms for MSA can also include orthostatic hypertension, male impotence, urinary difficulties, constipation, speech and swallowing difficulties, and blurred vision.
The initial diagnosis of MSA is usually made by carefully interviewing the patient and performing a physical examination. Several types of brain imaging, including computer histomography, scans, magnetic resonance imaging (MRI), and positron emission tomography (PET), are used. Pharmacological challenge tests (administering certain drugs in the presence of various types of movement of the patient) may also be of help in those patients with typical Parkinsonism signs. An incomplete and relatively poor response to dopamine replacement therapy, such as Sinemet, may be a clue that MSA is present. A characteristic involvement of multiple brain systems is a defining feature of MSA and one that an autopsy confirms the diagnosis. Patients with MSA can have the presence of glial cytoplasmic inclusions in certain types of brain cells, as well. Lewy bodies are not present in MSA. In comparison to Parkinson's, in addition to the poor response to Sinemet, there are a few other observations that are suggested for MSA, such as low blood pressure on standing, difficulty with urination, use of a wheelchair, loud snoring or loud breathing, and frequent nighttime urination.

*α-Synuclein Binding Agent*

**[0079]** Any agent known to bind or interact with alpha-synuclein may be labeled and used in the inventive system. The compound may be a small molecule, organic compound, protein, peptide, or polynucleotide. In certain embodiments, the compound is a small molecule. In certain embodiments, the agent is an organic compound. In other embodiments, the compound is a protein or peptide. In certain embodiments, the unlabeled agent is a drug approved for use in humans by the U.S. Food and Drug Administration (FDA) or under consideration by the FDA (*e.g.*, nortriptyline).

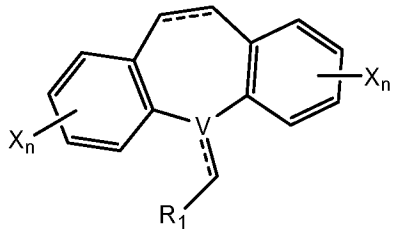
**[0080]** In certain embodiments, the agent has been discovered to prevent the aggregation of α-synuclein. Agents may be screened for their ability to bind aggregations of α-synuclein using any techniques known in the art. For example, agent may be identified by isothermal chemistry, solution NMR spectroscopy, capillary gel electrophoresis, high-throughput screening,or cell culture screening. Several assays for identifying compounds that prevent the aggregation of α-synuclein are described in U.S. provisional patent application, USSN 60/915,828, filed May 3, 2007, which is incorporated herein by reference. In certain embodiments, the assays involve testing for the aggregation of α-synuclein in

hexafluoroisopropanol. In other embodiments, the assay involves testing for the aggregation of α-synuclein in an aqueous solution.

**[0081]** In certain embodiments, the agent is an antidepressant. In certain embodiments, the agent is a tricyclic antidepressant. In certain embodiments, the agent is selected from the group consisting of nortriptyline, maprotiline, protriptyline, nordoxepin, trimipramine, imipramine, desipramine, doxepin, amoxapine, amitriptyline, clomipramine, cyclobenzaprine, lofepramine, mirtazapine, cyproheptadine, and norclomipramine. In certain particular embodiments, the agent is nortriptyline. In certain embodiments, the agent is norclomipramine. In certain embodiments, the agent is a monoamine reuptake inhibitor. The reuptake inhibitor may block the re-uptake of neurotransmitters such as norepinephrine, dopamine, serotonin, or combinations thereof. The reuptake inhibitor may be selective for a particular neurotransmitter, or it may be non-selective and block the reuptake of multiple neurotransmitters. In certain embodiments, the agent is selective serotonin reuptake inhibitor (SSRI). In certain embodiments, the agent is sertraline. In certain other embodiments, the agent is indatraline. In certain embodiments, the agent is fluoxetine. In certain embodiments, the agent is norfluoxetine.

**[0082]** The agent for use in the inventive system may be labeled with any radioisotope that emits detectable radiation. In certain embodiments, the radioisotope is detectable by positron emission tomography (PET) or single photon emission computed tomography (SPECT) imaging. In certain embodiments, the radioisotope is detectable by scintillation counting. In certain embodiments, the agent is labeled with a radioisotope of a halogen. In certain embodiments, the agent is labeled with a radioisotope of fluorine. In certain embodiments, the agent is labeled with F-18. In certain embodiments, the agent is labeled with a radioisotope of bromine. In certain embodiments, the agent is labeled with Br-75. In certain embodiments, the agent is labeled with Br-76. In certain embodiments, the agent is labeled with a radioisotope of chlorine. In certain embodiments, the agent is labeled with Cl-36. In certain embodiments, the agent is labeled with a radioisotope of iodine. In certain embodiments, the agent is labeled with I-123. In certain embodiments, the agent is labeled with I-131.

**[0083]** In certain embodiments, the inventive compound is of the formula:




wherein

each occurrence of a dashed line represent a bond or the absence of a bond;

each occurrence of X is independently a halogen, wherein the halogen is a radioisotope of fluorine, chlorine, bromine, or iodine;

each occurrence of n is independently an integer between 1 and 4;

V is N, C, or CH;

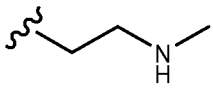
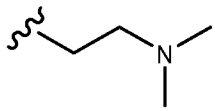
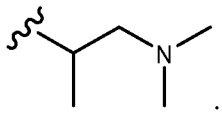
$R_1$ is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_A$; $-C(=O)R_A$; $-CO_2R_A$; -CN; -SCN; $-SR_A$; $-SOR_A$; $-SO_2R_A$; $-NO_2$; $-N_3$; $-N(R_A)_2$; $-NHC(=O)R_A$; $-NR_AC(=O)N(R_A)_2$; $-OC(=O)OR_A$; $-OC(=O)R_A$; $-OC(=O)N(R_A)_2$; $-NR_AC(=O)OR_A$; or $-C(R_A)_3$; wherein each occurrence of $R_A$ is independently a hydrogen, a protecting group, an aliphatic moiety, a heteroaliphatic moiety, an acyl moiety; an aryl moiety; a heteroaryl moiety; alkoxy; aryloxy; alkylthio; arylthio; amino, alkylamino, dialkylamino, heteroaryloxy; or heteroarylthio moiety; and pharmaceutically acceptable salts thereof.

**[0084]** In certain embodiments, X is F-18. In certain embodiments, X is Br-75. In certain embodiments, X is Br-76. In certain embodiments, X is I-123. In certain embodiments, X is I-131.

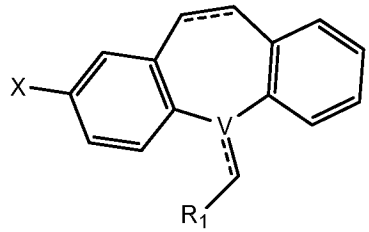
**[0085]** In certain embodiments, at least one occurrence of n is 1. In certain embodiments, one occurrence of n is 1, and the other occurrence of n is 0. In certain embodiments, both occurrences of n are 1. In certain embodiments, at least one occurrence of n is 2. In certain embodiments, at least one occurrence of n is 3.

**[0086]** In certain embodiments, V is CH. In certain embodiments, V is C. In certain embodiments, V is N.

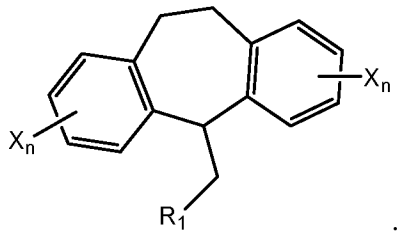
**[0087]** In certain embodiments, $R_1$ is cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic. In certain embodiments, $R_1$ is acyclic, unsubstituted, unbranched heteroaliphatic. In certain embodiments, $R_1$ is acyclic,

unsubstituted, branched heteroaliphatic. In certain embodiments, $R_1$ is acyclic, substituted, unbranched heteroaliphatic. In certain embodiments, $R_1$ is acyclic, substituted, branched heteroaliphatic. In certain embodiments, $R_1$ is aminoalkyl. In certain embodiments, $R_1$ is alkylaminoalkyl. In certain embodiments, $R_1$ is dialkylaminoalkyl. In certain embodiments, $R_1$ is  . In certain embodiments, $R_1$ is  . In certain embodiments, $R_1$ is  .

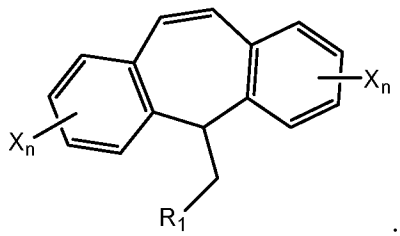
**[0088]** In certain embodiments, the inventive compound is of the formula:



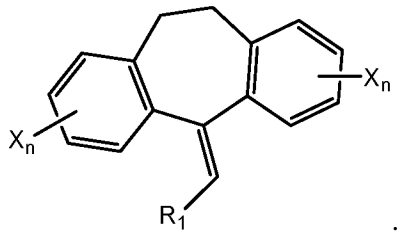
**[0089]** In certain embodiments, the inventive compound is of the formula:



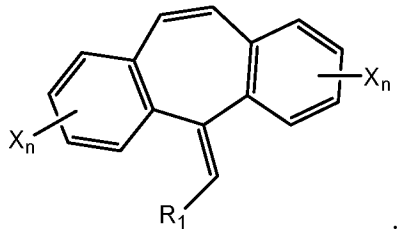
**[0090]** In certain embodiments, the inventive compound is of the formula:



**[0091]** In certain embodiments, the inventive compound is of the formula:



**[0092]** In certain embodiments, the inventive compound is of the formula:

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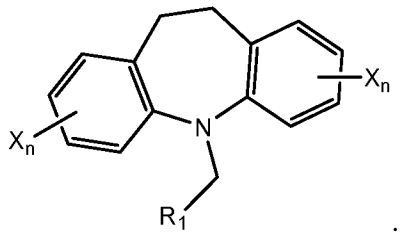
**[0093]** In certain embodiments, the inventive compound is of the formula:

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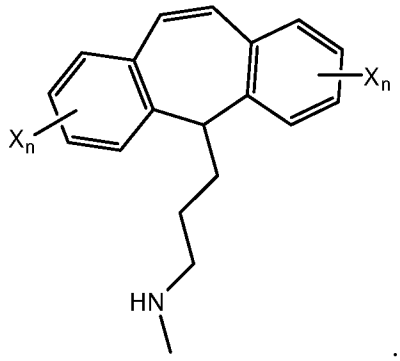
**[0094]** In certain embodiments, the inventive compound is of the formula:

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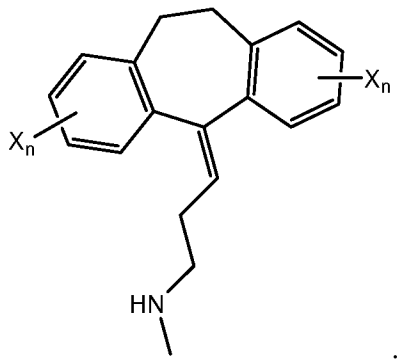
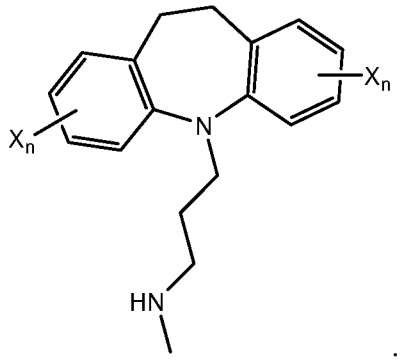
**[0095]** In certain embodiments, the inventive compound is of the formula:

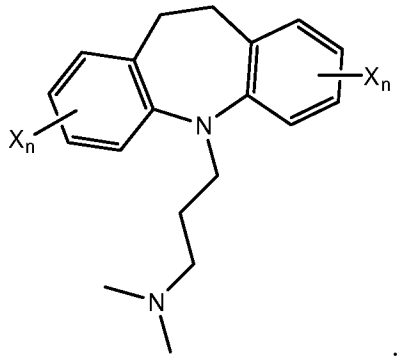



.

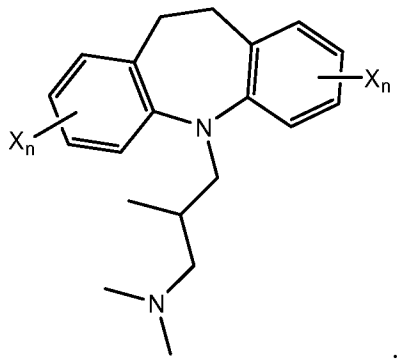
**[0096]** In certain embodiments, the inventive compound is of the formula:



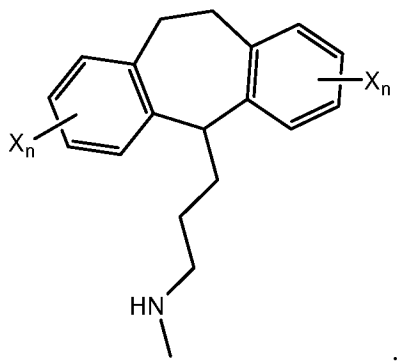

[0097] In certain embodiments, the inventive compound is of the formula:



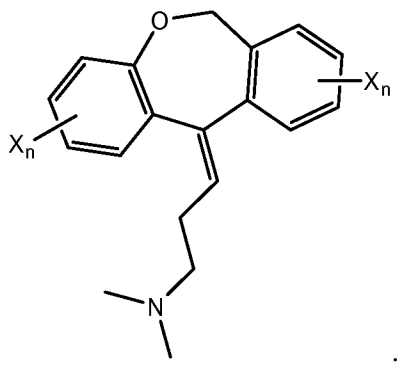

[0098] In certain embodiments, the inventive compound is of the formula:



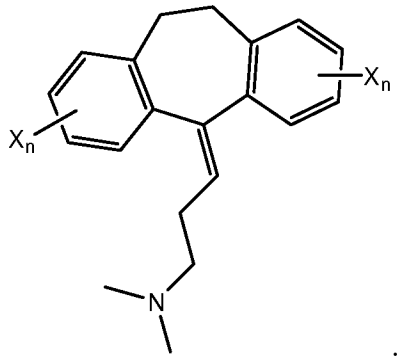

[0099] In certain embodiments, the inventive compound is of the formula:



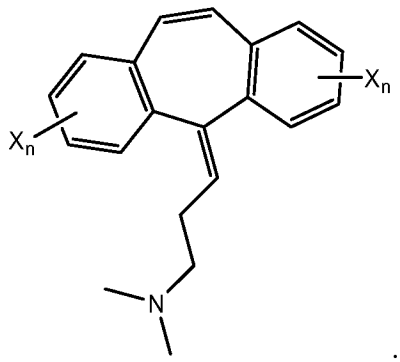

[00100] In certain embodiments, the inventive compound is of the formula:



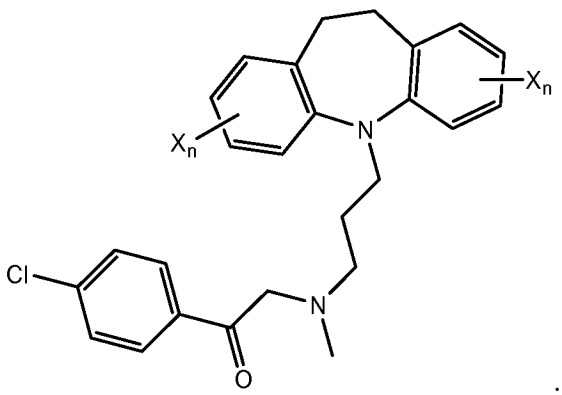

[00101] In certain embodiments, the inventive compound is of the formula:



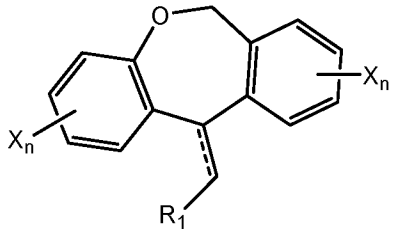

[00102] In certain embodiments, the inventive compound is of the formula:




[00103] In certain embodiments, the inventive compound is of the formula:




[00104] In certain embodiments, the inventive compound is of the formula:



wherein

each occurrence of a dashed line represent a bond or the absence of a bond;

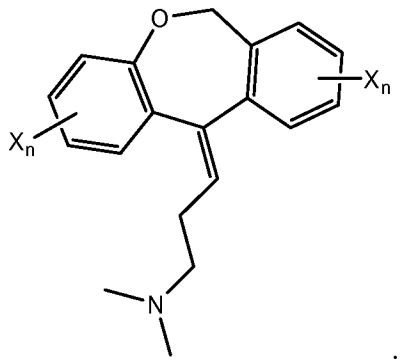
each occurrence of X is independently a halogen, wherein the halogen is a radioisotope of fluorine, chlorine, bromine, or iodine;

each occurrence of n is independently an integer between 1 and 4;

V is N, C, or CH;

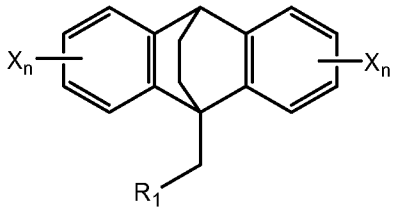
$R_1$ is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_A$; $-C(=O)R_A$; $-CO_2R_A$; -CN; -SCN; $-SR_A$; $-SOR_A$; $-SO_2R_A$; $-NO_2$; $-N_3$; $-N(R_A)_2$; $-NHC(=O)R_A$; $-NR_AC(=O)N(R_A)_2$; $-OC(=O)OR_A$; $-OC(=O)R_A$; $-OC(=O)N(R_A)_2$; $-NR_AC(=O)OR_A$; or $-C(R_A)_3$; wherein each occurrence of $R_A$ is independently a hydrogen, a protecting group, an aliphatic moiety, a heteroaliphatic moiety, an acyl moiety; an aryl moiety; a heteroaryl moiety; alkoxy; aryloxy; alkylthio; arylthio; amino, alkylamino, dialkylamino, heteroaryloxy; or heteroarylthio moiety; and pharmaceutically acceptable salts thereof.

**[00105]** In certain embodiments, the inventive compound is of the formula:



.

**[00106]** In certain embodiments, the inventive compound is of the formula:



wherein

each occurrence of a dashed line represent a bond or the absence of a bond;

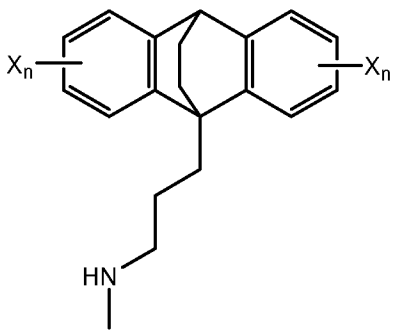
each occurrence of X is independently a halogen, wherein the halogen is a radioisotope of fluorine, chlorine, bromine, or iodine;

each occurrence of n is independently an integer between 1 and 4;

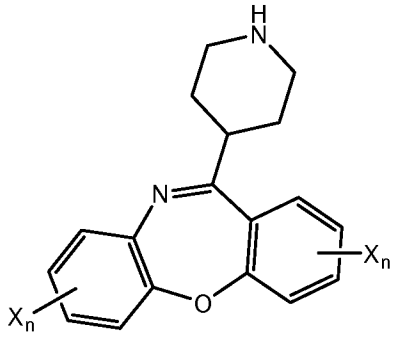
V is N, C, or CH;

$R_1$ is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_A$; $-C(=O)R_A$; $-CO_2R_A$; -CN; -SCN; $-SR_A$; $-SOR_A$; $-SO_2R_A$; $-NO_2$; $-N_3$; $-N(R_A)_2$; $-NHC(=O)R_A$; $-NR_AC(=O)N(R_A)_2$; $-OC(=O)OR_A$; $-OC(=O)R_A$; $-OC(=O)N(R_A)_2$; $-NR_AC(=O)OR_A$; or $-C(R_A)_3$; wherein each occurrence of $R_A$ is independently a hydrogen, a protecting group, an aliphatic moiety, a heteroaliphatic moiety, an acyl moiety; an aryl moiety; a heteroaryl moiety; alkoxy; aryloxy; alkylthio; arylthio; amino, alkylamino, dialkylamino, heteroaryloxy; or heteroarylthio moiety; and pharmaceutically acceptable salts thereof.

**[00107]** In certain embodiments, the inventive compound is of the formula:



**[00108]** In certain embodiments, the inventive compound is of the formula:



wherein

each occurrence of a dashed line represent a bond or the absence of a bond;

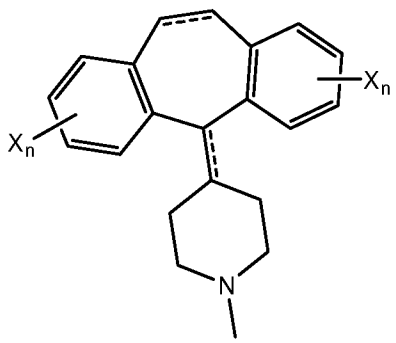
each occurrence of X is independently a halogen, wherein the halogen is a radioisotope of fluorine, chlorine, bromine, or iodine;

each occurrence of n is independently an integer between 1 and 4;

V is N, C, or CH;

$R_1$ is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_A$; $-C(=O)R_A$; $-CO_2R_A$; -CN; -SCN; $-SR_A$; $-SOR_A$; $-SO_2R_A$; $-NO_2$; $-N_3$; $-N(R_A)_2$; $-NHC(=O)R_A$; $-NR_AC(=O)N(R_A)_2$; $-OC(=O)OR_A$; $-OC(=O)R_A$; $-OC(=O)N(R_A)_2$; $-NR_AC(=O)OR_A$; or $-C(R_A)_3$; wherein each occurrence of $R_A$ is independently a hydrogen, a protecting group, an aliphatic moiety, a heteroaliphatic moiety, an acyl moiety; an aryl moiety; a heteroaryl moiety; alkoxy; aryloxy; alkylthio; arylthio; amino, alkylamino, dialkylamino, heteroaryloxy; or heteroarylthio moiety; and pharmaceutically acceptable salts thereof.

**[00109]** In certain embodiments, the inventive compound is of the formula:



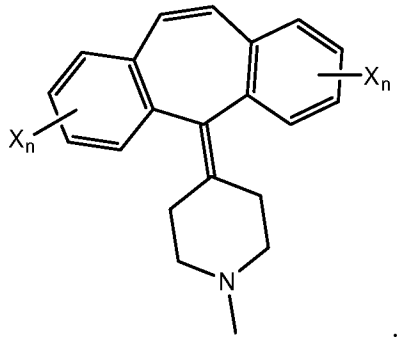
wherein

each occurrence of a dashed line represent a bond or the absence of a bond;

each occurrence of X is independently a halogen, wherein the halogen is a radioisotope of fluorine, chlorine, bromine, or iodine;

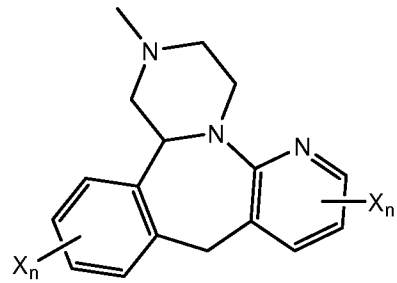
each occurrence of n is independently an integer between 1 and 4; and pharmaceutically acceptable salts thereof.

**[00110]** In certain embodiments, the inventive compound is of the formula:




.

**[00111]** In certain embodiments, the inventive compound is of the formula:




wherein

each occurrence of X is independently a halogen, wherein the halogen is a radioisotope of fluorine, chlorine, bromine, or iodine;

each occurrence of n is independently an integer between 1 and 4; and pharmaceutically acceptable salts thereof.

**[00112]** The present invention also includes methods of preparing the inventive radiolabeled α-synuclein binding agents. For example, a compound such as nortriptyline, protriptyline, imipramine, or desipramine may be radiolabeled with a radioisotope of fluorine, bromine, or iodine using methods known in the art. In certain embodiments, the compound is labeled with F-18, Br-76, and I-123. In certain embodiments, a radioisotope of bromine or iodine is added to the α-synuclein binding agent (*e.g.*, a tricyclic antidepressant) by halostannylation. In certain embodiments, a radioisotope of fluorine is added to the α-synuclein binding agent (*e.g.*, a tricyclic antidepressant) by nucleophilic substitution. In certain particular embodiments, the the substitution reaction involves substitution of a nitro group. In certain embodiments, the α-synuclein binding agent is iodinated using a ICl

reagent as described in Humphreys *et al. Mol. Pharm.* 36:620-26, 1989; Cassel *et al. Analytical Chem.* 170:63-67, 1988; Helmkamp *et al. Appl. Radiat. Isotopes* 18:747-754, 1967; Contreras *et al. Methods Enzymol.* 92:277-92, 1983; each of which is incorporated herein by reference. The ICl reagent may be labeled with I-123 or another radioisotope of iodine. In certain embodiments, the radioisotope has a short half-life requiring that the labeling of the α-synuclein binding agent be done near the facility were the agent will be eventually used.

**[00113]** It should be appreciated that in any of the aspects or embodiments described herein, the α-synuclein binding compounds may be provided in any suitable stereoisomeric form, and/or pharmaceutically acceptable acid or base addition salt form.

**[00114]** The "pharmaceutically acceptable acid or base addition salts" mentioned herein are meant to comprise the therapeutically active non-toxic acid and non-toxic base addition salt forms that the compounds are able to form. The compounds that have basic properties can be converted into their pharmaceutically acceptable acid addition salts by treating the base form with an appropriate acid. Appropriate acids include, for example, inorganic acids such as hydrohalic acids, *e.g.*, hydrochloric or hydrobromic acid; sulfuric; nitric; phosphoric and the like acids; or organic acids such as, for example, acetic, propanoic, hydroxyacetic, lactic, pyruvic, oxalic, malonic, succinic (*i.e.*, butanedioic acid), maleic, fumaric, malic, tartaric, citric, methanesulfonic, ethanesulfonic, benzenesulfonic, p-toluenesulfonic, cyclamic, salicylic, p-aminosalicylic, pamoic and the like acids.

**[00115]** The compounds that have acidic properties can be converted into their pharmaceutically acceptable base addition salts by treating the acid form with a suitable organic or inorganic base. Appropriate base salt forms include, for example, the ammonium salts, the alkali and earth alkaline metal salts, *e.g.*, the lithium, sodium, potassium, magnesium, calcium salts and the like, salts with organic bases, *e.g.*, the benzathine, N-methyl-D-glucamine, hydrabamine salts, and salts with amino acids such as, for example, arginine, lysine and the like.

**[00116]** The terms acid or base addition salt also comprise the hydrates and the solvent addition forms which the compounds are able to form. Examples of such forms are e.g. hydrates, alcoholates and the like.

**[00117]** The term stereochemically isomeric forms of compounds, as used herein, include all possible compounds made up of the same atoms bonded by the same sequence of bonds but having different three-dimensional structures which are not interchangeable, which the compounds may possess. Unless otherwise mentioned or indicated, the chemical designation

of a compound encompasses the mixture of all possible stereochemically isomeric forms that the compound can take. The mixture can contain all diastereomers and/or enantiomers of the basic molecular structure of the compound. All stereochemically isomeric forms of the compounds both in pure form or in admixture with each other are intended to be embraced within the scope of the present invention.

**[00118]** Some of the compounds may also exist in their tautomeric forms. Such forms although not explicitly indicated in the above formula are intended to be included within the scope of the present invention.

**[00119]** The methods and structures described herein relating to compounds and compositions of the invention also apply to the pharmaceutically acceptable acid or base addition salts and all stereoisomeric forms of these compounds and compositions.

*Uses*

**[00120]** The labeled compounds are useful in detecting alpha-synuclein. They may be used in assays, autoradiographic experiments, or imaging studies to determine the presence of alpha-synuclein. The binding of the labeled compound may be performed *in vivo* or *in vitro*. The compound bound to alpha-synuclein is then detected by the radiation that is emitted from the radioactive label (*e.g.*, I-123, I-131, F-18, Br-76, or Br-75).

**[00121]** In certain embodiments, the invention provides methods of detecting and/or quantifying alpha-synuclein deposits in a biological sample. The method includes contacting a biological sample with an effective amount of a labeled, alpha-synuclein binding agent and then assessing the quantity of alpha-synuclein binding agent bound to the biological sample based on detection of radiation emitted from the label. In certain embodiments, the radiation is detected by scintillation counting. In certain embodiments, the radiation is detected by autoradiogram. In certain embodiments, the biological sample is a biological fluid such as plasma, serum, blood, sweat, cerebral spinal fluid, lymph, *etc.* In certain embodiments, the biological sample is a tissue sample (*e.g.*, a brain section). In certain embodiments, the biological sample is a biopsy sample. In certain embodiments, the biological sample is derived from the nervous system of an animal. In certain embodiments, the biolgical sample is derived from a mammal. In certain embodiments, the biological sample is derived from a primate. In certain embodiments, the biological sample is derived from a human. In certain embodiments, the biological sample is derived from an experimental animal such as a pig, dog, rat, or mouse.

**[00122]** In certain embodiments, the invention provides methods of detecting and/or quantifying alpha-synuclein deposits in a subject. Such methods may be useful in diagnosing a patient suspected of having a synucleinopathy, in following the progression of disease in a subject, and/or in following treatment of a subject. The method includes administering an effective amount of a labeled, alpha-synuclein binding agent to a subject followed by imaging of the subject or a portion thereof. In certain embodiments, the binding agent is administered as part of a pharmaceutical composition. The binding agent may be administered using any technique known in the art. In certain embodiments, the agent is administered parenterally (*e.g.*, intravenously). In other embodiments, the agent is administered orally. In certain embodiments, the agent is administered intrathecally. The effective amount of the labeled agent may be from about 10ng/kg of body weight to about 1000mg/kg of body weight. A certain amount of time may be allowed to pass between the time when the labeled agent is administered and the time the imaging is performed. The delay between administration and imaging may range from minutes to days. In certain embodiments, the delay ranges from 5 minutes to 30 minutes. In certain embodiments, the delay ranges from 30 minutes to 6 hours. In certain embodiments, the delay ranges from 6 hours to 12 hours. In certain embodiments, the delay ranges from 12 hours to 24 hours. In certain embodiments, the delay ranges from 24 hours to 36 hours. In certain embodiments, the delay ranges from 36 hours to 48 hours. In certain embodiments, the delay is approximately 1 week. After the appropriate delay, the subject is imaged to determine the extent of labeled alpha-synuclein binding agent present in the subject. The subject may be imaged using any technique known in the art to detect radiation from a radioisotope. In certain embodiments, PET imaging is used. In other embodiments, SPECT imaging is used. The whole subject may be imaged or only a portion of the subject may be imaged. In certain embodiments, the head and neck of the subject are imaged. In certain embodiments, the brain of the subject is imaged. In certain embodiments, the central nervous system of the subject is imaged. The subject may be any animal. In certain embodiments, the subject is a mammal. In certain embodiments, the subject is a primate. In certain embodiments, the subject is a rodent. In certain embodiments, the subject is a mouse. In certain embodiments, the subject is a rat. In certain embodiments, the subject is a transgenic animal that expresses alpha-synuclein. In certain embodiments, the subject is a human.

**[00123]** The subject being imaged using the inventive method may be susceptible to developing a synucleinopathy such as Parkinson's disease. For example, the subject may be starting to exhibit some of the symptoms associated with Parkinson's disease such as rigidity,

tremor, or bradykinesia. Therefore, imaging the patient may assist the attending physician in diagnosing and treating the subject. In certain embodiments, the patient is already diagnosed with a synucleinopathy and is being treated for the disease. Periodic imaging of the subject using the inventive ystem may be used to assess the success of the treatment or to assess disease progression. The subject may be being treated with an RNAi, a vaccine, or a small molecule based therapy. The subject may be imaged every month, 6 months, 12 months, 24 months, or 36 months, for example.

*Kits*

**[00124]** The invention also provides kits for performing the inventive methods. Typically, a kit includes the necessary quantity of a labeled alpha-synuclein binding agent to detect alpha-synuclein in a biological sample or in a subject. The labeled agent may be provided in a convenient composition for use in the inventive methods. In certain embodiments, the labeled agent is provided in a pharmaceutical composition for administration to a subject for the imaging of synuclein. The kit may also include labeling and/or instructions for use of the labeled alpha-synuclein binding agent. The invention provides diagnostic kits for performing *in vivo* imaging. The invention also provides kits for research purposes.

**[00125]** In certain embodiments, the kit is designed for labeling an alpha-synuclein binding agent with a radioisotope. For example, an agent such as a tricyclic antidepressant known to bind alpha-synuclein may be provided in the kit with the necessary reagents for labeling the agent. In certain embodiments, the radioisotope has such a short half-life that it is preferable to label the agent soon before its use. The isotope for labeling may be provided by the user and used with the kit. For example, the radioisotope may be roduced by a cyclotron on site.

*Pharmaceutical Compositions*

**[00126]** In another aspect, the present invention provides "pharmaceutically acceptable" compositions, which comprise a therapeutically effective amount of one or more of the compounds described herein, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.*, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for

application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary and to other mucosal surfaces.

**[00127]** The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

**[00128]** The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in pharmaceutical formulations.

**[00129]** As set out herein, certain embodiments of the present compounds may 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 acid addition salts of compounds of the present invention. These salts can be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, or by

separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed during subsequent purification. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. See, for example, Berge *et al.* (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19.

**[00130]** The pharmaceutically acceptable salts of the subject compounds include the conventional nontoxic salts or quaternary ammonium salts of the compounds, e.g., from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts include those derived from inorganic acids such as hydrochloride, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicyclic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

**[00131]** In other cases, the compounds 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 compounds of the present invention. These salts can likewise be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, with ammonia, or with a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative 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. (See, for example, Berge *et al.*, *supra*).

**[00132]** Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

**[00133]** Examples of pharmaceutically-acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

**[00134]** Formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, and the particular mode of administration. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, this amount will range from about 1% to about 99% of active ingredient, preferably from about 5% to about 70%, most preferably from about 10% to about 30%.

**[00135]** In certain embodiments, a formulation of the present invention comprises an excipient selected from the group consisting of cyclodextrins, liposomes, micelle forming agents, *e.g.*, bile acids, and polymeric carriers, e.g., polyesters and polyanhydrides; and a compound of the present invention. In certain embodiments, an aforementioned formulation renders orally bioavailable a compound of the present invention.

**[00136]** Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

**[00137]** Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the

present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

**[00138]** In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol, glycerol monostearate, and non-ionic surfactants; absorbents, such as kaolin and bentonite clay; lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-shelled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

**[00139]** A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made in a suitable machine in which a mixture of the powdered compound is moistened with an inert liquid diluent.

**[00140]** The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be formulated for rapid release, e.g., freeze-dried. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions that can be dissolved in sterile water, or some other sterile injectable medium immediately before use.

These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

**[00141]** Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

**[00142]** Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming, and preservative agents.

**[00143]** Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

**[00144]** Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

**[00145]** The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

**[00146]** Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain

customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

**[00147]** Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Dissolving or dispersing the compound in the proper medium can make such dosage forms. Absorption enhancers can also be used to increase the flux of the compound across the skin. Either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel can control the rate of such flux.

**[00148]** Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

**[00149]** Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more compounds of the invention in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain sugars, alcohols, antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

**[00150]** Examples of suitable aqueous and nonaqueous carriers, which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

**[00151]** These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms upon the subject compounds may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

**[00152]** In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be

accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution, which in turn, may depend upon crystal size and crystalline form.

Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

**[00153]** Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions, which are compatible with body tissue.

**[00154]** In certain embodiments, a compound or pharmaceutical preparation is administered orally. In other embodiments, the compound or pharmaceutical preparation is administered intravenously. Alternative routs of administration include sublingual, intramuscular, and transdermal administrations.

**[00155]** When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1% to 99.5% (more preferably, 0.5% to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

**[00156]** The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given in forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administrations are preferred.

**[00157]** The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticulare, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

**[00158]** The phrases “systemic administration, “administered systemically,” “peripheral administration” and “administered peripherally” as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such

that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

**[00159]** These compounds may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

**[00160]** Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

**[00161]** Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired imaging for a particular patient, composition, and mode of administration, without being toxic to the patient.

**[00162]** The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion or metabolism of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

**[00163]** A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required to achieve the desired therapeutic effect and then gradually increasing the dosage until the desired effect is achieved.

**[00164]** Generally doses of the compounds of this invention for a patient, when used for the indicated effects, will range from about 0.0001 mg to about 100 mg per kg of body weight. Preferably the daily dosage will range from 0.001 mg to 50 mg of compound per kg of body weight, and even more preferably from 0.01 mg to 10 mg of compound per kg of body weight. However, lower or higher doses can be used. In some embodiments, the dose administered to a subject for imaging may be modified as the physiology of the subject changes due to age, disease progression, weight, or other factors.

**[00165]** While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition) as described above.

**[00166]** The compounds according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other pharmaceuticals.

**[00167]** According to the invention, compounds for treating neurological conditions or diseases can be formulated or administered using methods that help the compounds cross the blood-brain barrier (BBB). The vertebrate brain (and CNS) has a unique capillary system unlike that in any other organ in the body. The unique capillary system has morphologic characteristics which make up the blood-brain barrier (BBB). The blood-brain barrier acts as a system-wide cellular membrane that separates the brain interstitial space from the blood.

**[00168]** The unique morphologic characteristics of the brain capillaries that make up the BBB are: (a) epithelial-like high resistance tight junctions which literally cement all endothelia of brain capillaries together, and (b) scanty pinocytosis or transendothelial channels, which are abundant in endothelia of peripheral organs. Due to the unique characteristics of the blood-brain barrier, hydrophilic drugs and peptides that readily gain access to other tissues in the body are barred from entry into the brain or their rates of entry and/or accumulation in the brain are very low.

**[00169]** In one aspect of the invention, alpha-synuclein binding compounds that cross the BBB are particularly useful for imaging in the present invention. In one embodiment, it is expected that alpha-synuclein binding compounds that are non-charged (*e.g.*, not positively charged) and/or non-lipophilic may cross the BBB with higher efficiency than charged (*e.g.*, positively charged) and/or lipophilic compounds. Therefore, it will be appreciated by a person of ordinary skill in the art that some of the compounds of the invention might readily cross the BBB. Alternatively, the compounds of the invention can be modified, for example, by the addition of various substitutuents that would make them less hydrophilic and allow them to more readily cross the BBB.

**[00170]** Other approaches to circumventing the blood-brain barrier utilize pharmacologic-based procedures involving drug latentiation or the conversion of hydrophilic drugs into lipid-soluble drugs. The majority of the latentiation approaches involve blocking the hydroxyl, carboxyl and primary amine groups on the drug to make it more lipid-soluble and therefore more easily able to cross the blood-brain barrier.

**[00171]** Another approach to increasing the permeability of the BBB to drugs involves the intra-arterial infusion of hypertonic substances which transiently open the blood-brain barrier to allow passage of hydrophilic drugs. However, hypertonic substances are potentially toxic and may damage the blood-brain barrier.

**[00172]** The permeability of the blood brain barrier can be increased by administering a blood brain barrier agonist, for example bradykinin (US 5,112,596 incorporated herein in its entirety by reference), or polypeptides called receptor mediated permeabilizers (RMP) (US 5,268,164 incorporated herein in its entirety by reference). Exogenous molecules can be administered to the host's bloodstream parenterally by subcutaneous, intravenous or intramuscular injection or by absorption through a bodily tissue, such as the digestive tract, the respiratory system or the skin. The form in which the molecule is administered (e.g., capsule, tablet, solution, emulsion) depends, at least in part, on the route by which it is administered. The administration of the exogenous molecule to the host's bloodstream and the intravenous injection of the agonist of blood-brain barrier permeability can occur simultaneously or sequentially in time. For example, a therapeutic drug can be administered orally in tablet form while the intravenous administration of an agonist of blood-brain barrier permeability is given later (e.g. between 30 minutes later and several hours later). This allows time for the drug to be absorbed in the gastrointestinal tract and taken up by the bloodstream before the agonist is given to increase the permeability of the blood-brain barrier to the drug. On the other hand, an agonist of blood-brain barrier permeability (e.g. bradykinin) can be administered before or at the same time as an intravenous injection of a drug. Thus, the term "co administration" is used herein to mean that the agonist of blood-brain barrier and the exogenous molecule will be administered at times that will achieve significant concentrations in the blood for producing the simultaneous effects of increasing the permeability of the blood-brain barrier and allowing the maximum passage of the exogenous molecule from the blood to the cells of the central nervous system.

**[00173]** In other embodiments, compounds of the invention can be formulated as a prodrug with a fatty acid carrier (and optionally with another neuroactive drug). The prodrug is stable in the environment of both the stomach and the bloodstream and may be delivered by ingestion. The prodrug passes readily through the blood brain barrier. The prodrug preferably has a brain penetration index of at least two times the brain penetration index of the drug alone. Once in the central nervous system, the prodrug is hydrolyzed into the fatty acid carrier and the alpha-synuclein binding agent. The carrier preferably is a normal component of the central nervous system and is inactive and harmless.

The compound and/or drug, once released from the fatty acid carrier, is capable of binding alpha-synuclein. Preferably, the fatty acid carrier is a partially-saturated straight chain molecule having between about 16 and 26 carbon atoms, and more preferably 20 and 24 carbon atoms. Examples of fatty acid carriers are provided in U.S. Patents 4,939,174; 4,933,324; 5,994,932; 6,107,499; 6,258,836; and 6,407,137; the disclosures of which are incorporated herein by reference in their entirety.

**[00174]** The function and advantage of these and other embodiments of the present invention will be more fully understood from the examples described below. The following examples are intended to illustrate the benefits of the present invention, but do not exemplify the full scope of the invention.

## Examples

**[00175]** Nortriptyline, protriptyline, maprotiline, norclomipramine, nordoxepin, amoxapine, doxepin, desipramine, trimipramine, clomipramine, and imipramine (*Figure 1*) were found to bind to α-synuclein and affect the rate of protein aggregation in the presence of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). HFIP induces rapid structure formation and aggregation of α-synuclein (Munishkina *et al.* (2003). "Conformational behavior and aggregation of alphα-synuclein in organic solvents: modeling the effects of membranes" *Biochemistry* 42(9):2720-30; Maiti *et al.* (2004). "Raman spectroscopic characterization of secondary structure in natively unfolded proteins: α-synuclein" *J. Am. Chem. Soc.* 126(8):2399-408; each of which is incorporated herein by reference). Compounds were incubated with purified recombinant human α-synuclein (20 μM) in a buffer containing HFIP (25 mM Tris, pH 8.0, 3.1% HFIP) at room temperature. Structure formation was monitored by Thioflavin T flourescence and fluorescence polarization. Thioflavin T fluorescence can be used to measures aggregation of amyloidogenic proteins, including α-synuclein (exctiation, 440 nm; emission, 495 nm) (Naiki *et al.* (1989). "Fluorometric determination of amyloid fibrils in vitro using the fluorescent dye, thioflavin T1" *Anal. Biochem.* 177(2):244-9; Conway *et al.* (2000). "Fibrils formed in vitro from alphα-synuclein and two mutant forms linked to Parkinson's disease are typical amyloid" *Biochemistry* 39(10):2552-63; incorporated herein by reference). Average molecular size was monitored by fluorescence polarization using human α-synuclein covalently conjugated to Alexa Fluor 594 (exctiation, 546 nm; emission, 620 nm). Assays were performed in a 384-well plate and readings taken directly from each well over time. Nortriptyline (*Figure 2*) causes a dose-dependent increase in the rate of structure formation of α-synuclein in the presence of HFIP as determined by Thioflavin T fluorescence and fluorescence polarization. Dose-dependent effects on α-synuclein aggregation were also observed by Thioflavin T fluorescence measurements for protriptyline, maprotiline (*Figure 3*), norclomipramine, nordoxepin (*Figure 4*) , amoxapine, doxepin (*Figure 5*), desipramine, trimipramine (*Figure 6*) clomipramine, and imipramine (*Figure 7*).

**[00176]** Nortriptyline also binds to α-synuclein in a buffer system relevant to physiological conditions as monitored by effects on aggregation. The rate of the α-synuclein aggregation was determined by monitoring the amount of α-synuclein monomer in solution and by fluorescence polarization. Recombinant α-synuclein (70 μM, 20 mM Bis-tris propane, 100

mM LiCl, pH 7.4, 700 µl total volume) was incubated at 37 °C with gentle agitation. Samples (40 µl) were separated on a 2 ml Shodex KW-G gel filtration column (20 mM Bis-tris propane, 100 mM LiCl, pH 7.4, 0.5 ml/min). Absorbance at 280 nm was recorded and the monomer peak height was quantified. At the same time points, aliquots (5 µl) were diluted ten-fold and fluorescence polarization measured in a 384-well plate. At a single concentration (100 µM), nortriptyline delayed loss of α-synuclein monomer from solution (*Figure 8A*) and also the formation of larger structures (*Figure 8B*). This effect was dose-dependent (*Figure 9*).

**[00177]** Nortriptyline decreased α-synuclein neurotoxicity toward dopaminergic neurons. Midbrain cultures will be prepared from E17 rat ventral mesencephalon as described in a published protocol (Xu *et al.* (2002). "Dopamine-dependent neurotoxicity of alphα-synuclein: a mechanism for selective neurodegeneration in Parkinson disease" *Nat. Med.* 8(6):600-6; incorporated herein by reference). Cultured cells were infected with a recombinant lentivirus encoding human A53T α-synuclein (A53T) or a control virus (none). Cells were treated with various concentrations of nortriptyline (white bars) for 3 days. Cells were then fixed and immunostained for Microtubule-associated protein 2, which stains all neurons, and Tyrosine Hydroxylase, a marker for dopaminergic neurons. Toxicity of A53T α-synuclein toward dopaminergic neurons was determined by calculating the percentage neurons positive for tyrosine hydroxylase ($TH^+$ cells). Nortriptyline diminished toxicity of A53T α-synuclein toward dopaminergic neurons in a dose-dependent manner (*Figure 10*).

*Animal Data*

**[00178]** Nortriptyline was administered to mice of the α-synuclein transgenic line described in Masliah *et al.* ((2000). "Dopaminergic loss and inclusion body formation in alphα-synuclein mice: implications for neurodegenerative disorders" *Science* 287(5456):1265-9; which is incorporated herein by reference). Animals from this line have α-synuclein neuronal inclusions in the cortex, hippocampus, and the olfactory bulb (Masliah *et al.* (2000). "Dopaminergic loss and inclusion body formation in α-synuclein mice: implications for neurodegenerative disorders" *Science* 287(5456):1265-9). Transgenic mice were administered nortriptyline in saline (0.9%) or the same volume of vehicle alone once a day intraperitoneally for 30 days. At the end of treatment, mice were sacrificed and the brains removed and hemisected. One hemisphere of each was fixed in 4% paraformaldehyde/PBS (pH 7.4), cryoperserved, then sectioned for histology. From the other

hemisphere, the cortex and hippocampus were dissected, homogenized, and processed into cytoplasmic and membrane fractions.

**[00179]** Administration of nortriptyline to transgenic animals decreases α-synuclein protein accumulation. Three month old animals were treated with 25 mg/kg nortriptyline once a day for 30 days. Total α-synuclein levels were analyzed by a sandwich ELISA assay similar to one previously described (El-Agnaf *et al.* (2006). "Detection of oligomeric forms of α-synuclein protein in human plasma as a potential biomarker for Parkinson's disease" *Faseb J.* 20(3):419-25; which is incorporated herein by reference). Three month old transgenic mice that received nortripytline for 30 days had lower total α-synuclein protein levels than vehicle-treated transgenic mice in the cytoplasmic and membrane fractions of both the hippocampus and cortex (*Figure 11*). Formation of α-synuclein inclusions in the cortex and hippocampus was probed by immunostaining with an antibody specific for human α-synuclein. Cells positive for human α-synuclein were quantified. In both regions, three month old transgenic mice that received nortriptyline for 30 days had significantly fewer α-synuclein-positive cells per $mm^2$ than those treated with vehicle (*Figure 12*). Representative images from the hippocampus are shown in *Figure 13*. Six month old transgenic animals that received 0.5, 5, or 25 mg/kg nortriptyline for 30 days also had significantly fewer α-synuclein-positive cells per $mm^2$ than vehicle-treated animals in both the cortex and hippocampus (*Figure 14*).

**[00180]** Having now described some illustrative embodiments of the invention, it should be apparent to those skilled in the art that the foregoing is merely illustrative and not limiting, having been presented by way of example only. Numerous modifications and other illustrative embodiments are within the scope of one of ordinary skill in the art and are contemplated as falling within the scope of the invention. In particular, although many of the examples presented herein involve specific combinations of method acts or system elements, it should be understood that those acts and those elements may be combined in other ways to accomplish the same objectives. Acts, elements and features discussed only in connection with one embodiment are not intended to be excluded from a similar role in other embodiments. Further, for the one or more means-plus-function limitations recited in the following claims, the means are not intended to be limited to the means disclosed herein for performing the recited function, but are intended to cover in scope any means, known now or

later developed, for performing the recited function. Use of ordinal terms such as "first", "second", "third", *etc.*, in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements. Similarly, use of a), b), *etc.*, or i), ii), *etc.* does not by itself connote any priority, precedence, or order of steps in the claims. Similarly, the use of these terms in the specification does not by itself connote any required priority, precedence, or order.

**[00181]** The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by examples provided, since the examples are intended as a single illustration of one aspect of the invention and other functionally equivalent embodiments are within the scope of the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. The advantages and objects of the invention are not necessarily encompassed by each embodiment of the invention.

## Claims

What is claimed is:

1. A method of determining levels of alpha-synuclein deposits in a biological sample, the method comprising steps of:

contacting an alpha-synuclein binding agent with a biological samples, wherein the alpha-synuclein binding agent comprises a radiolabel; and

determing the quantity of alpha-synuclein binding agent bound to the biological sample based on detection of the radiolabel.

2. The method of claim 1, wherein the radiolabel is a radiolabel of fluorine, bromine, chlorine, or iodine.

3. The method of claim 1, wherein the radiolabel is I-123.

4. The method of claim 1, wherein the radiolabel is I-131.

5. The method of claim 1, wherein the radiolabel is F-18.

6. The method of claim 1, wherein the radiolabel is Br-76.

7. The method of claim 1, wherein the alpha-synuclein binding agent is a small molecule.

8. The method of claim 1, wherein the alpha-synuclein binding agent is a protein or peptide.

9. The method of claim 1, wherein the alpha-synuclein binding agent is radiolabeled antidepressant.

10. The method of claim 1, wherein the alpha-synuclein binding agent is a radiolabeled tricyclic antidepressant.

11. The method of claim 1, wherein the alpha-synuclein binding agent is a radiolabeled version of nortriptyline, maprotiline, nordoxepin, trimipramine, imipramine, desipramine, doxepin, amoxapine, amitriptyline, clomipramine, cyclobenzaprine, lofepramine, mirtazapine, cyproheptadine, or norclomipramine.

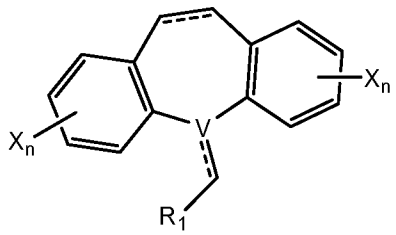
12. The method of claim 1, wherein the alpha-synuclein binding agent is a radiolabeled version of nortriptyline.

13. The method of claim 1, wherein the alpha-synuclein binding agent is a radiolabeled version of protriptyline.

14. The method of claim 1, wherein the alpha-synuclein binding agent is a radiolabeled version of imipramine.

15. The method of claim 1, wherein the alpha-synuclein binding agent is a radiolabeled version of desipramine.

16. The method of claim 1, wherein the alpha-synuclein binding agent is of formula:




wherein

each occurrence of a dashed line represent a bond or the absence of a bond;

each occurrence of X is independently a halogen, wherein the halogen is a radioisotope of fluorine, chlorine, bromine, or iodine;

each occurrence of n is independently an integer between 1 and 4;

V is N, C, or CH;

$R_1$ is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_A$; $-C(=O)R_A$; $-CO_2R_A$; -CN; -SCN; $-SR_A$; $-SOR_A$; $-SO_2R_A$; $-NO_2$;

$-N_3$; $-N(R_A)_2$; $-NHC(=O)R_A$; $-NR_AC(=O)N(R_A)_2$; $-OC(=O)OR_A$; $-OC(=O)R_A$; $-OC(=O)N(R_A)_2$; $-NR_AC(=O)OR_A$; or $-C(R_A)_3$; wherein each occurrence of $R_A$ is independently a hydrogen, a protecting group, an aliphatic moiety, a heteroaliphatic moiety, an acyl moiety; an aryl moiety; a heteroaryl moiety; alkoxy; aryloxy; alkylthio; arylthio; amino, alkylamino, dialkylamino, heteroaryloxy; or heteroarylthio moiety; and pharmaceutically acceptable salts thereof.

17. The method of claim 16, wherein X is F-18.

18. The method of claim 16, wherein X is Br-76.

19. The method of claim 16, wherein X is I-123.

20. The method of claim 16, wherein X is I-131.

21. The method of claim 16, wherein at least one occurrrence of n is 1.

22. The method of claim 16, wherein V is C or CH.

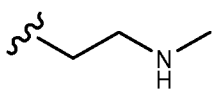
23. The method of claim 16, wherein V is N.

24. The method of claim 16, wherein $R_1$ is acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic.

25. The method of claim 16, wherein $R_1$ is aminoalkyl.

26. The method of claim 16, wherein $R_1$ is alkylaminoalkyl.

27. The method of claim 16, wherein $R_1$ is dialkylaminoalkyl.

28. The method of claim 16, wherein $R_1$ is 

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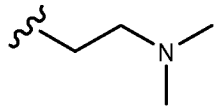
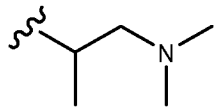
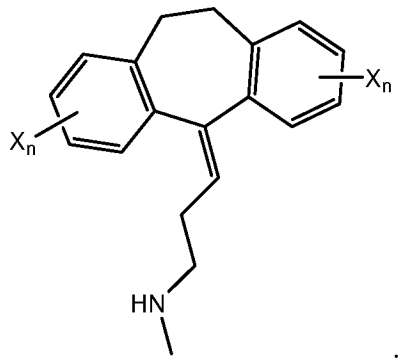
29. The method of claim 16, wherein $R_1$ is  .

30. The method of claim 16, wherein $R_1$ is  .

31. The method of claim 16, wherein the alpha-synuclein binding agent is of formula:



32. The method of claim 1, wherein the biological sample is a mammalian biological sample.

33. The method of claim 1, wherein the biological sample is a human biological sample.

34. The method of claim 1, wherein the biological sample is nervous system tissue.

35. The method of claim 1, wherein the biological sample is central nervous system tissue.

36. The method of claim 1, wherein the biological sample is brain tissue.

37. The method of claim 1, wherein the biological sample is cerebral spinal fluid.

38. The method of claim 1, wherein the biological sample is derived from a transgenic animal that overexpresses alpha-synuclein.

39. The method of claim 1, wherein the biological sample is derived from a subject with a synucleinopathy.

40. The method of claim 1, wherein the step of determining comprises detecting the radiolabel by scintillation counting.

41. The method of claim 1, wherein the step of determing comprises detecting the radiolabel by autoradiogram.

42. The method of claim 1, wherein the step of determing comprises detecting the radiolabel by positron emission tomography (PET) or single photon emission tomography (SPECT).

43. A method of imaging alpha-synuclein deposits in a subject, the method comprising steps of:

administering an alpha-synuclein binding agent to a subject, wherein the alpha-synuclein binding agent comprises a radiolabel; and

imaging alpha-synuclein deposits in a subject based on detection of the radiolabel.

44. The method of claim 43, wherein the subject is a mammal.

45. The method of claim 43, wherein the subject is a human.

46. The method of claim 43, wherein the subject has been diagnosed with a synucleinopathy.

47. The method of claim 43, wherein the subject is thought to have a synucleinopathy.

48. The method of claim 43, wherein the subject have been diagnosed with Parkinson's disease or is susceptible to having Parkinson's disease.

49. The method of claim 43, wherein the step of imaging comprises imaging by PET.

50. The method of claim 43, wherein the step of imaging comprises imaging by SPECT.

51. A method of diagnosing a subject with a synucleinopathy, the method comprising steps of:

administering to a subject an alpha-synuclein binding agent, wherein the alpha-synuclein binding agent comprises a radiolabel;

imaging the subject using SPECT or PET imaging; and

diagnosing the subject based on quantity of radiolabel seen by SPECT or PET imaging.

52. The method of claim 51, wherein the synculeinopathy is selected from the group consisting of Parkinson's disease, diffuse Lewy body disease, multiple system atrophy, and pantothenate kinase-associated neurodegeneration.

53. A method of monitoring disease progression in a subject with a synucleinopathy, the method comprising steps of:

(a) administering to a subject an alpha-synuclein binding agent, wherein the alpha-binding agent comprises a radiolabel;

(b) imaging the subject using SPECT or PET imaging;

(c) repeating steps (a) and (b) after a length of time.

54. The method of claim 53, wherein the length of time ranges from 1 month to 10 years.

55. The method of claim 53, wherein the length of time ranges from 6 months to 5 years.

56. The method of claim 53, wherein the length of time ranges from 3 months to 3 years.

57. The method of claim 53, wherein the length of time ranges from 1 years to 5 years.

58. A method of assessing a treatment for a synuclienopathy, the method comprising steps of:

administering to a subject an alpha-synculein binding agent, wherein the alpha-binding agent comprises a radiolabel;

imaging the subject using SPECT or PET imaging;

administering a treatment for a synucleinopathy;

administering to a subject an alpha-synculein binding agent, wherein the alpha-binding agent comprises a radiotracer, after a length a time; and

imaging the subject using SPECT or PET imaging.

59. The method of claim 58, wherein the synucleinopathy is selected from the group consisting of Parkinson's disease, diffuse Lewy body disease, multiple system atrophy, and pantothenate kinase-associated neurodegeneration.

60. The method of claim 58, wherein the step of administering a treatment comprises administering a farnesyl transferase inhibitor to the subject.

61. The method of claim 58, wherein the step of administering a treatment comprises administering a dopamine agonist, a DOPA decarboxylase inhibitor, a dopamine precursor, a monoamine oxidase inhibitor, a cathechol O-methyl transferase inhibitor, an anticholinergic, a NMDA antagonist, carbidopa-levodopa, an anticholinergic, or a combination thereof to the subject.

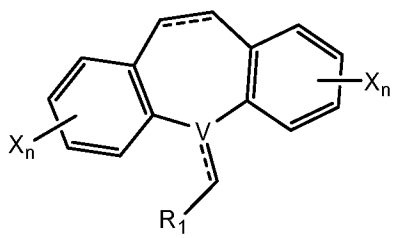
62. The method of claim 58, wherein the length of time ranges from 1 month to 5 years.

63. The method of claim 58, wherein the length of time ranges from 6 months to 3 years.

64. The method of claim 58, wherein the length of time ranges from 1 year to 5 years.

65. The method of claim 58, wherein the length of time ranges from 1 year to 3 years.

66. A radiolabeled compoud of formula:




wherein

each occurrence of a dashed line represent a bond or the absence of a bond;

each occurrence of X is independently a halogen, wherein the halogen is a radioisotope of fluorine, chlorine, bromine, or iodine;

each occurrence of n is independently an integer between 1 and 4;

V is N, C, or CH;

$R_1$ is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR_A$; $-C(=O)R_A$; $-CO_2R_A$; -CN; -SCN; $-SR_A$; $-SOR_A$; $-SO_2R_A$; $-NO_2$; $-N_3$; $-N(R_A)_2$; $-NHC(=O)R_A$; $-NR_AC(=O)N(R_A)_2$; $-OC(=O)OR_A$; $-OC(=O)R_A$; $-OC(=O)N(R_A)_2$; $-NR_AC(=O)OR_A$; or $-C(R_A)_3$; wherein each occurrence of $R_A$ is independently a hydrogen, a protecting group, an aliphatic moiety, a heteroaliphatic moiety, an acyl moiety; an aryl moiety; a heteroaryl moiety; alkoxy; aryloxy; alkylthio; arylthio; amino, alkylamino, dialkylamino, heteroaryloxy; or heteroarylthio moiety; and pharmaceutically acceptable salts thereof.

67. The compound of claim 66, wherein X is F-18.

68. The compound of claim 66, wherein X is Br-76.

69. The compound of claim 66, wherein X is I-123.

70. The compound of claim 66, wherein X is I-131.

71. The compound of claim 66, wherein at least one occurrrence of n is 1.

72. The compound of claim 66, wherein V is C or CH.

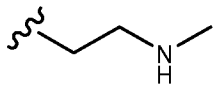
73. The compound of claim 66, wherein V is N.

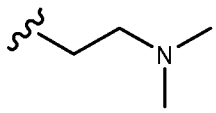
74. The compound of claim 66, wherein $R_1$ is acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic.

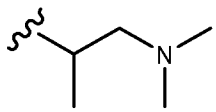
75. The compound of claim 66, wherein $R_1$ is aminoalkyl.

76. The compound of claim 66, wherein $R_1$ is alkylaminoalkyl.

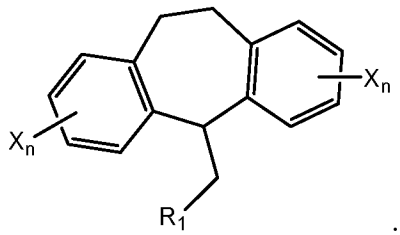
77. The compoung of claim 66, wherein $R_1$ is dialkylaminoalkyl.

78. The compound of claim 66, wherein $R_1$ is  .

79. The compound of claim 66, wherein $R_1$ is  .

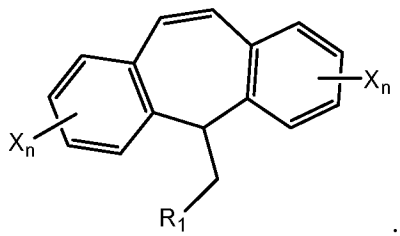
80. The compound of claim 66, wherein $R_1$ is  .

81. The compound of claim 66 of formula:



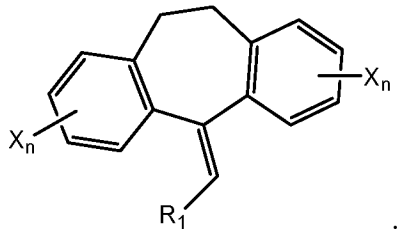

.

82. The compound of claim 66 of formula:

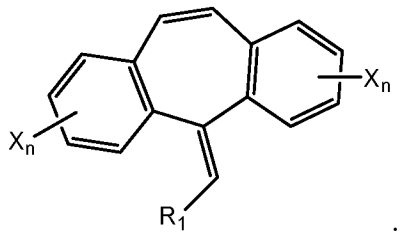



.

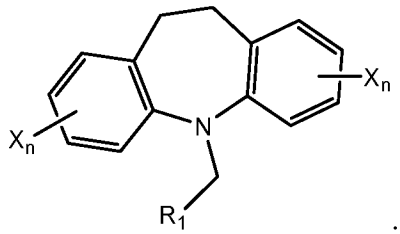
83. The compound of claim 66 of formula:



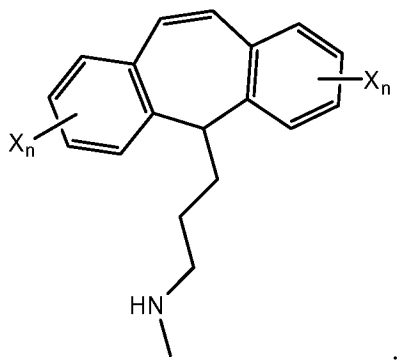

84. The compound of claim 66 of formula:



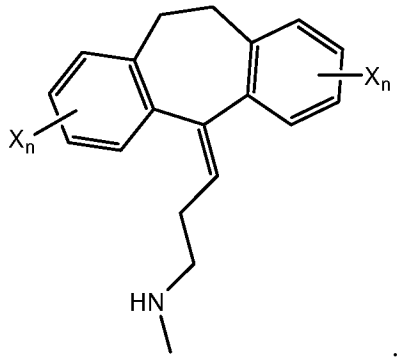

85. The compound of claim 66 of formula:



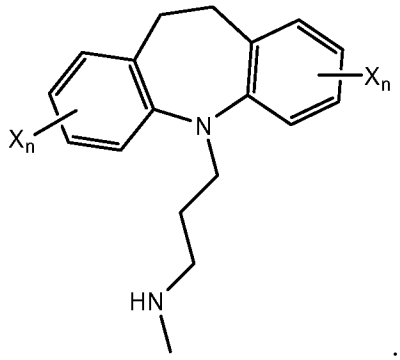

86. The compound of claim 66 of formula:



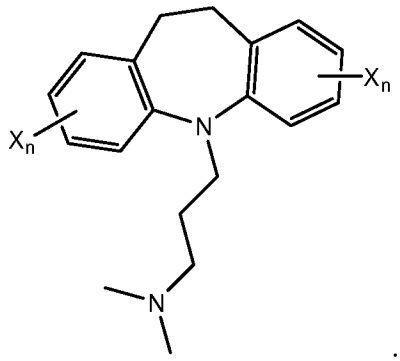

87. The compound of claim 66 of formula:



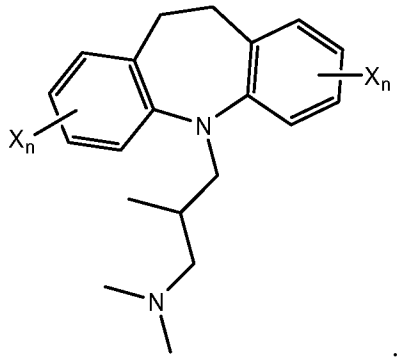

88. The compound of claim 66 of formula:



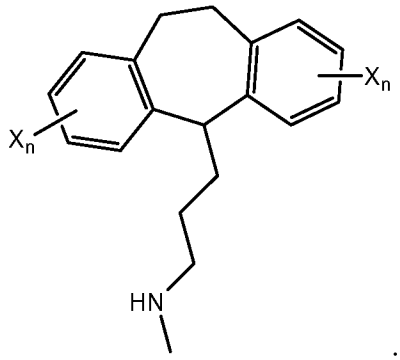

89. The compound of claim 66 of formula:



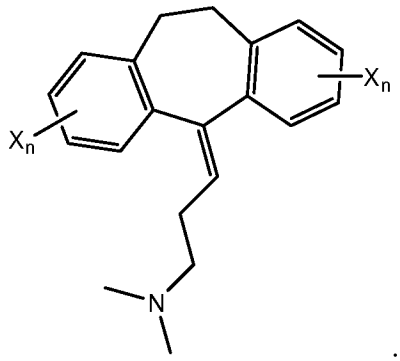

90. The compound of claim 66 of formula:



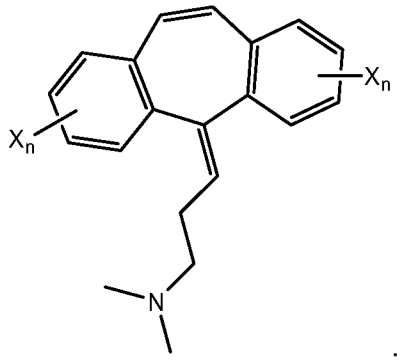

91. The compound of claim 66 of formula:



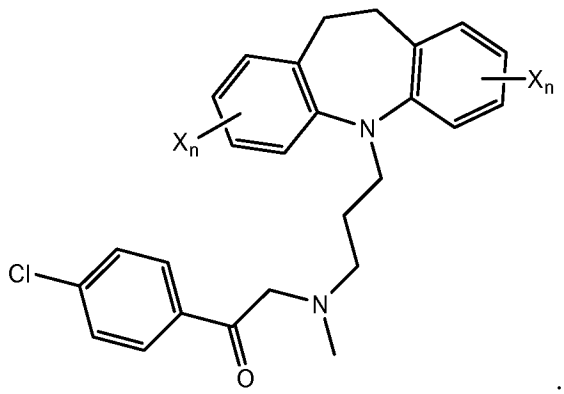

92. The compound of claim 66 of formula:



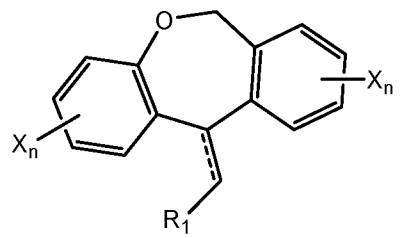

93. The compound of claim 66 of formula:




94. The compound of claim 66 of formula:




95. A radiolabled compound of formula:




wherein

each occurrence of a dashed line represent a bond or the absence of a bond;

each occurrence of X is independently a halogen, wherein the halogen is a radioisotope of fluorine, chlorine, bromine, or iodine;

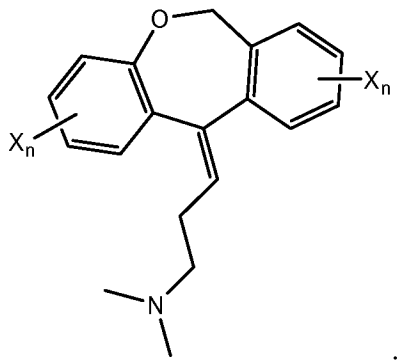
each occurrence of n is independently an integer between 1 and 4;

V is N, C, or CH;

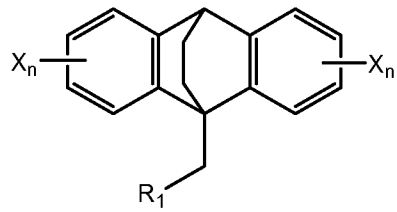
$R_1$ is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or

unbranched heteroaryl; -$OR_A$; -$C(=O)R_A$; -$CO_2R_A$; -CN; -SCN; -$SR_A$; -$SOR_A$; -$SO_2R_A$; -$NO_2$; -$N_3$; -$N(R_A)_2$; -$NHC(=O)R_A$; -$NR_AC(=O)N(R_A)_2$; -$OC(=O)OR_A$; -$OC(=O)R_A$; -$OC(=O)N(R_A)_2$; -$NR_AC(=O)OR_A$; or -$C(R_A)_3$; wherein each occurrence of $R_A$ is independently a hydrogen, a protecting group, an aliphatic moiety, a heteroaliphatic moiety, an acyl moiety; an aryl moiety; a heteroaryl moiety; alkoxy; aryloxy; alkylthio; arylthio; amino, alkylamino, dialkylamino, heteroaryloxy; or heteroarylthio moiety; and pharmaceutically acceptable salts thereof.

96. The compound of claim 95 of formula:




97. A radiolabeled compound of formula:




wherein

each occurrence of a dashed line represent a bond or the absence of a bond;

each occurrence of X is independently a halogen, wherein the halogen is a radioisotope of fluorine, chlorine, bromine, or iodine;

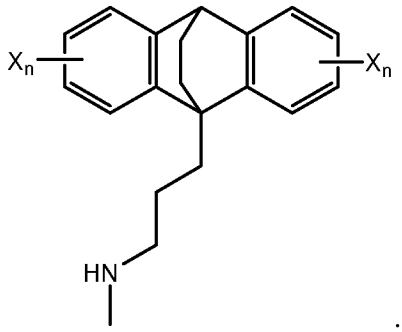
each occurrence of n is independently an integer between 1 and 4;

V is N, C, or CH;

$R_1$ is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; -$OR_A$; -$C(=O)R_A$; -$CO_2R_A$; -CN; -SCN; -$SR_A$; -$SOR_A$; -$SO_2R_A$; -$NO_2$;

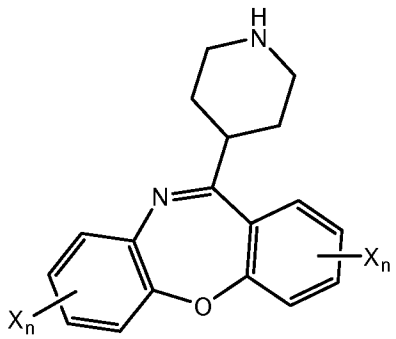
$-N_3$; $-N(R_A)_2$; $-NHC(=O)R_A$; $-NR_AC(=O)N(R_A)_2$; $-OC(=O)OR_A$; $-OC(=O)R_A$; $-OC(=O)N(R_A)_2$; $-NR_AC(=O)OR_A$; or $-C(R_A)_3$; wherein each occurrence of $R_A$ is independently a hydrogen, a protecting group, an aliphatic moiety, a heteroaliphatic moiety, an acyl moiety; an aryl moiety; a heteroaryl moiety; alkoxy; aryloxy; alkylthio; arylthio; amino, alkylamino, dialkylamino, heteroaryloxy; or heteroarylthio moiety; and pharmaceutically acceptable salts thereof.

98. The compound of claim 97 of formula:




.

99. A radiolabeled compound of formula:




wherein

each occurrence of a dashed line represent a bond or the absence of a bond;

each occurrence of X is independently a halogen, wherein the halogen is a radioisotope of fluorine, chlorine, bromine, or iodine;

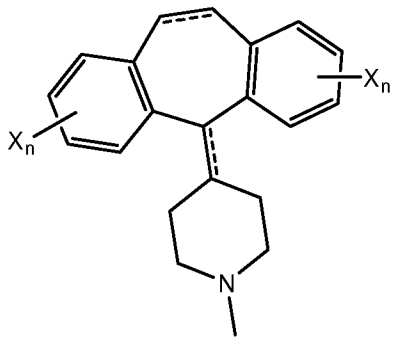
each occurrence of n is independently an integer between 1 and 4;

V is N, C, or CH;

$R_1$ is hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or

unbranched heteroaryl; -$OR_A$; -$C(=O)R_A$; -$CO_2R_A$; -CN; -SCN; -$SR_A$; -$SOR_A$; -$SO_2R_A$; -$NO_2$; -$N_3$; -$N(R_A)_2$; -$NHC(=O)R_A$; -$NR_AC(=O)N(R_A)_2$; -$OC(=O)OR_A$; -$OC(=O)R_A$; -$OC(=O)N(R_A)_2$; -$NR_AC(=O)OR_A$; or -$C(R_A)_3$; wherein each occurrence of $R_A$ is independently a hydrogen, a protecting group, an aliphatic moiety, a heteroaliphatic moiety, an acyl moiety; an aryl moiety; a heteroaryl moiety; alkoxy; aryloxy; alkylthio; arylthio; amino, alkylamino, dialkylamino, heteroaryloxy; or heteroarylthio moiety; and pharmaceutically acceptable salts thereof.

100. A radiolabled compound of formula:



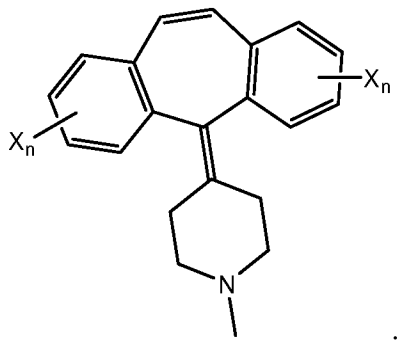

wherein

each occurrence of a dashed line represent a bond or the absence of a bond;

each occurrence of X is independently a halogen, wherein the halogen is a radioisotope of fluorine, chlorine, bromine, or iodine;

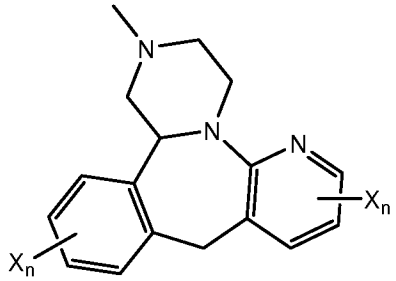
each occurrence of n is independently an integer between 1 and 4; and pharmaceutically acceptable salts thereof.

101. The compound of claim 100 of formula:




.

102. A radiolabeled compound of formula:



wherein

each occurrence of X is independently a halogen, wherein the halogen is a radioisotope of fluorine, chlorine, bromine, or iodine;

each occurrence of n is independently an integer between 1 and 4; and pharmaceutically acceptable salts thereof.

103. A pharmaceutical composition comprising a radiolabeled compound of any one of claims 66-102 and a pharmaceutically acceptable excipient.

104. A kit comprising a radiolabled compound of any one of claims 66-102 and instructions for adminsitering the compound.

105. A kit comprising a compounds selected from the group consisting of nortriptyline, maprotiline, nordoxepin, trimipramine, imipramine, desipramine, doxepin, amoxapine, amitriptyline, clomipramine, cyclobenzaprine, lofepramine, mirtazapine, cyproheptadine, and norclomipramine; and reagents suitable for radiolabeling the compound.

106. The kit of claim 102 wherein the reagents suitable for radiolabeling the compound comprise I-123, I-131, F-18, or Br-76.

Figure 1A

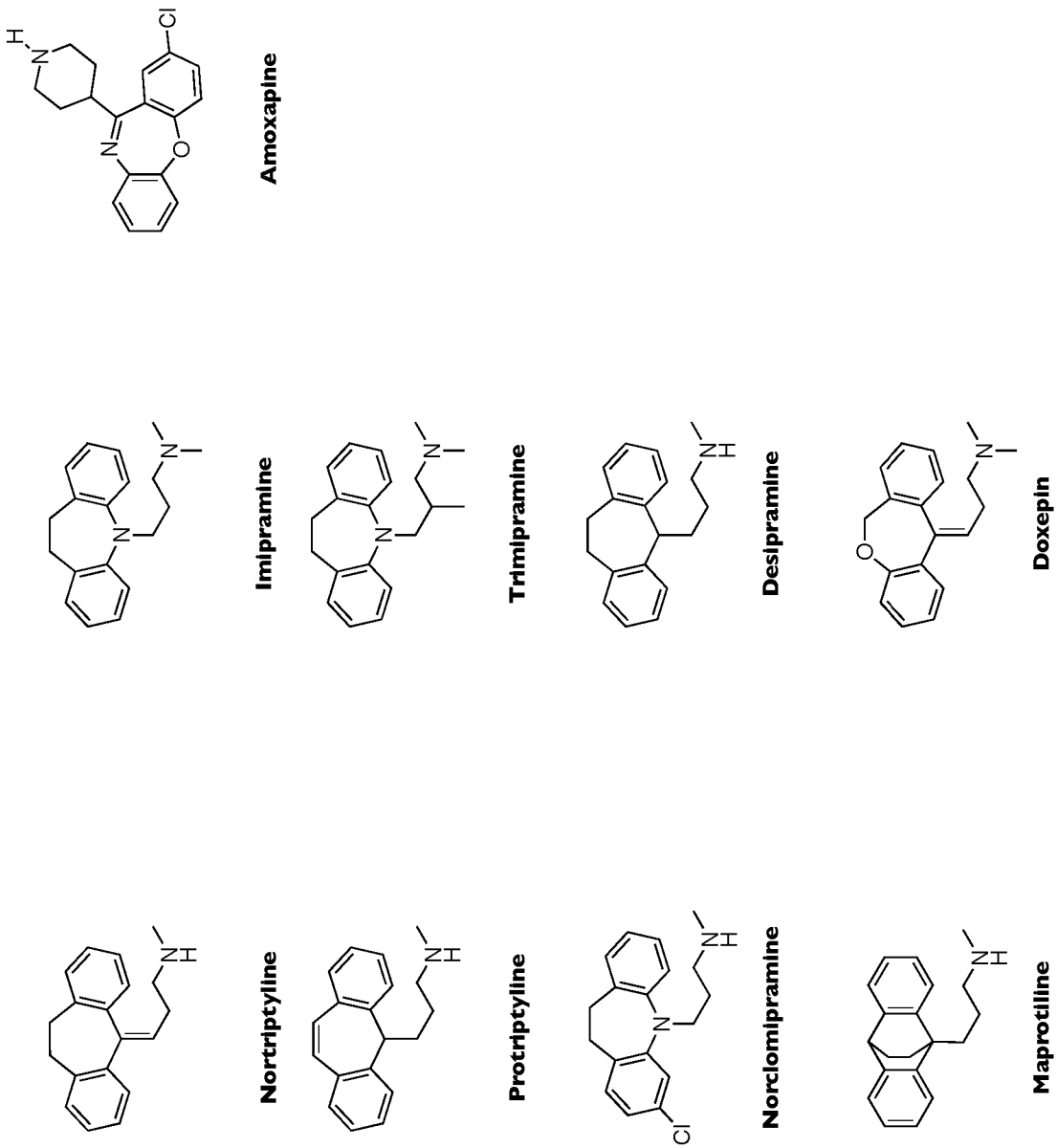
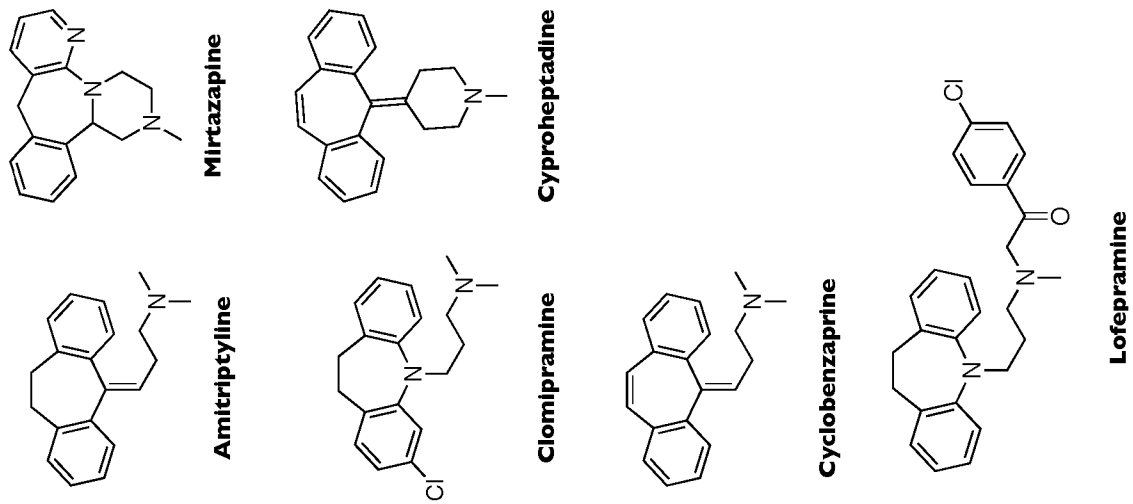
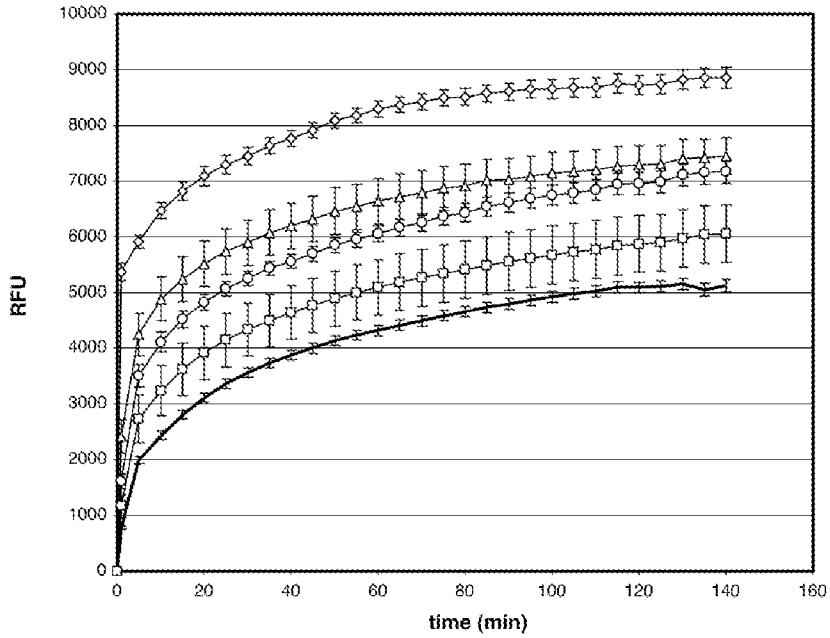

Amoxapine
Imipramine
Trimipramine
Desipramine
Doxepin
Nortriptyline
Protriptyline
Norclomipramine
Maprotiline

Figure 1B

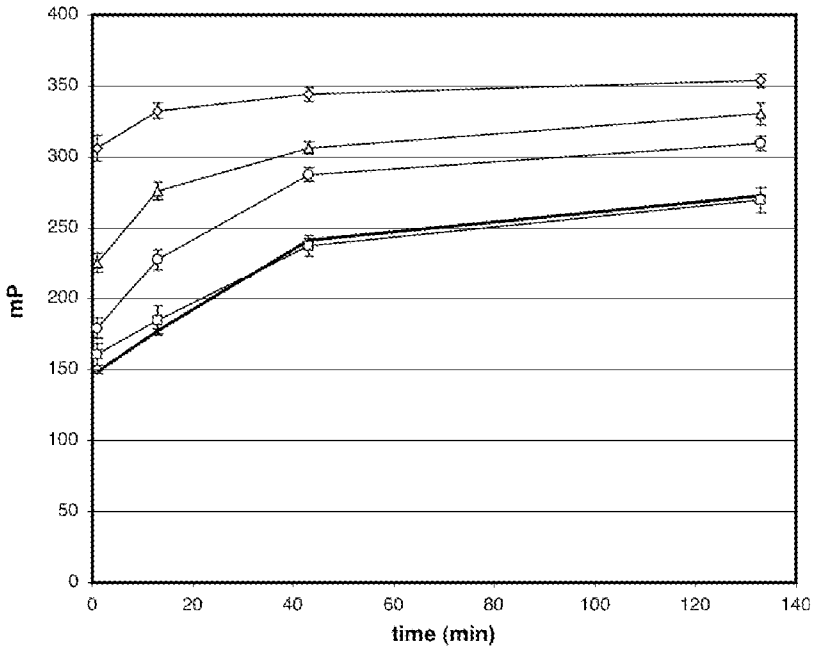

Mirtazapine
Cyproheptadine
Amitriptyline
Clomipramine
Cyclobenzaprine
Lofepramine

# Figure 2

A.

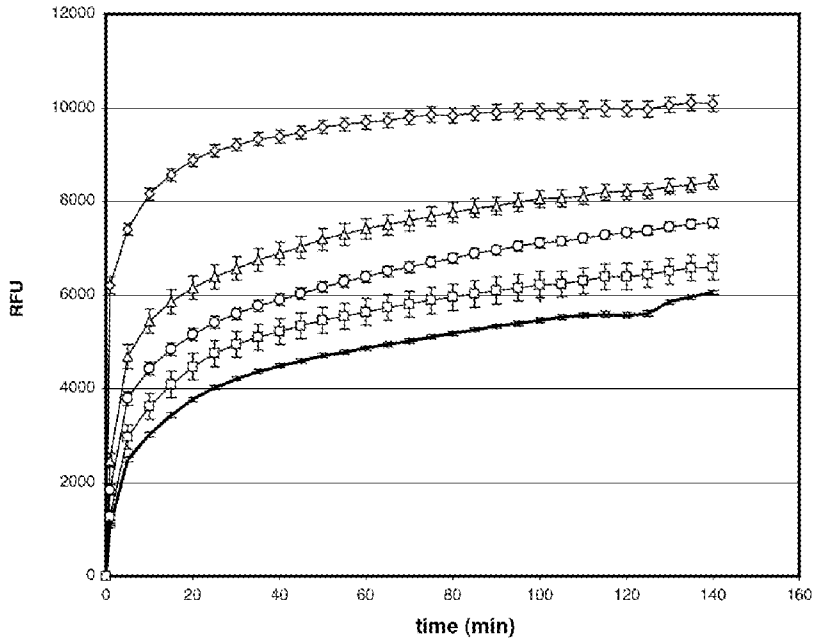

RFU
time (min)
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
0
20
40
60
80
100
120
140
160

B.

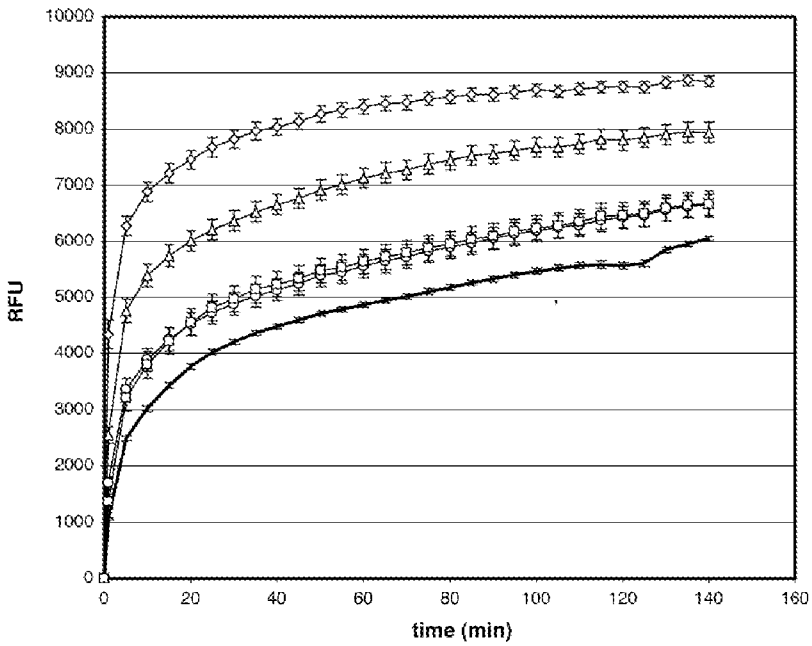

mP
time (min)
0
50
100
150
200
250
300
350
400
0
20
40
60
80
100
120
140

# Figure 3

A.

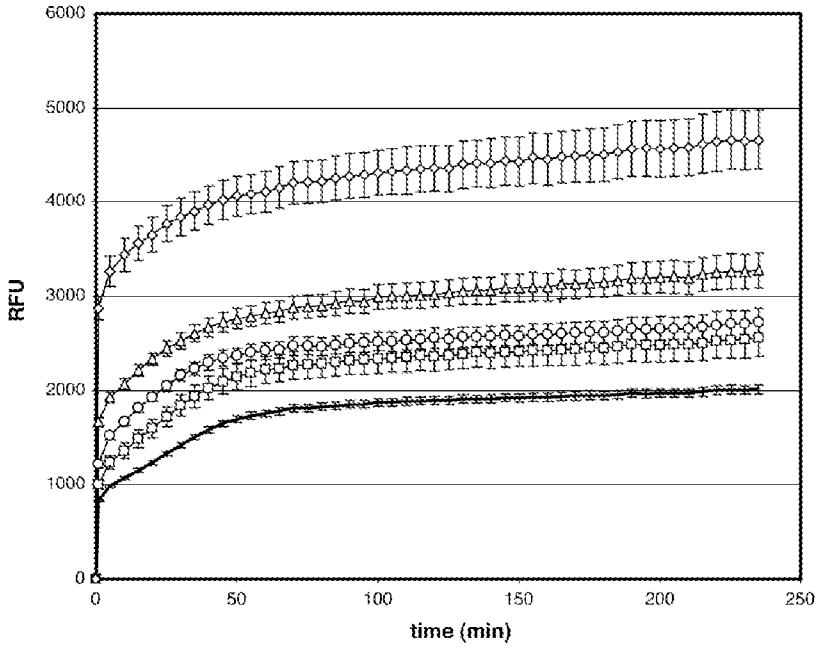

12000
10000
8000
6000
4000
2000
0
RFU
0
20
40
60
80
100
120
140
160
time (min)

B.

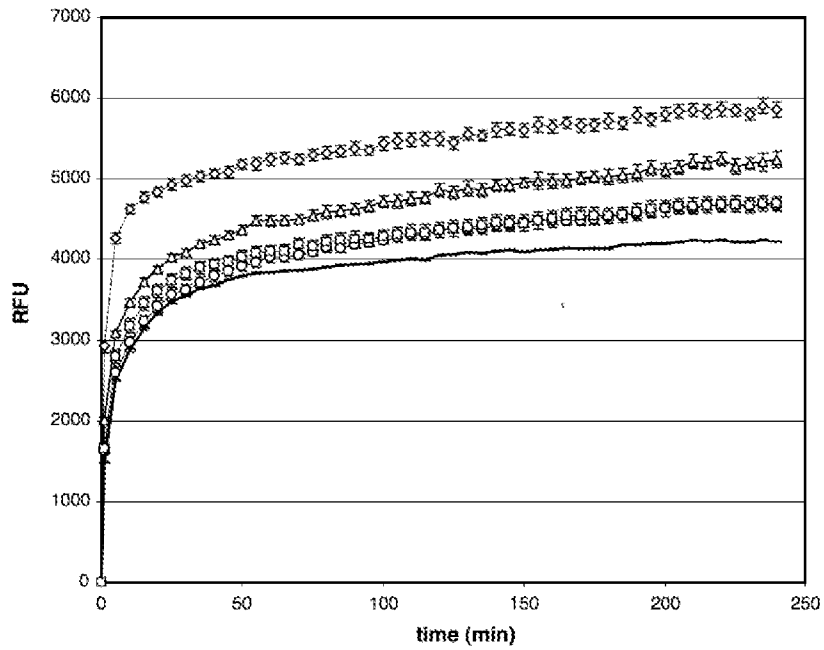

10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
RFU
0
20
40
60
80
100
120
140
160
time (min)

# Figure 4

A.

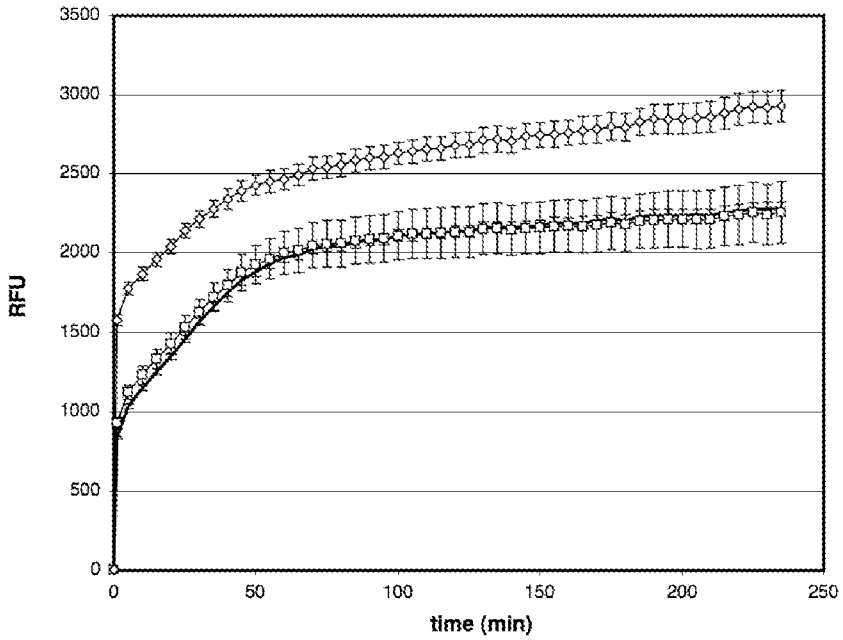

6000
5000
4000
3000
2000
1000
0
RFU
0
50
100
150
200
250
time (min)

B.

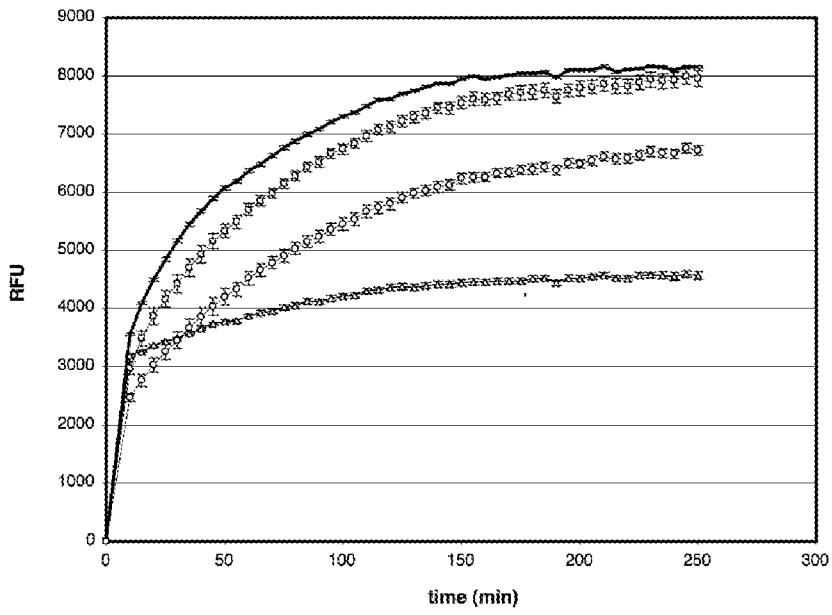

7000
6000
5000
4000
3000
2000
1000
0
RFU
0
50
100
150
200
250
time (min)

# Figure 5

A.

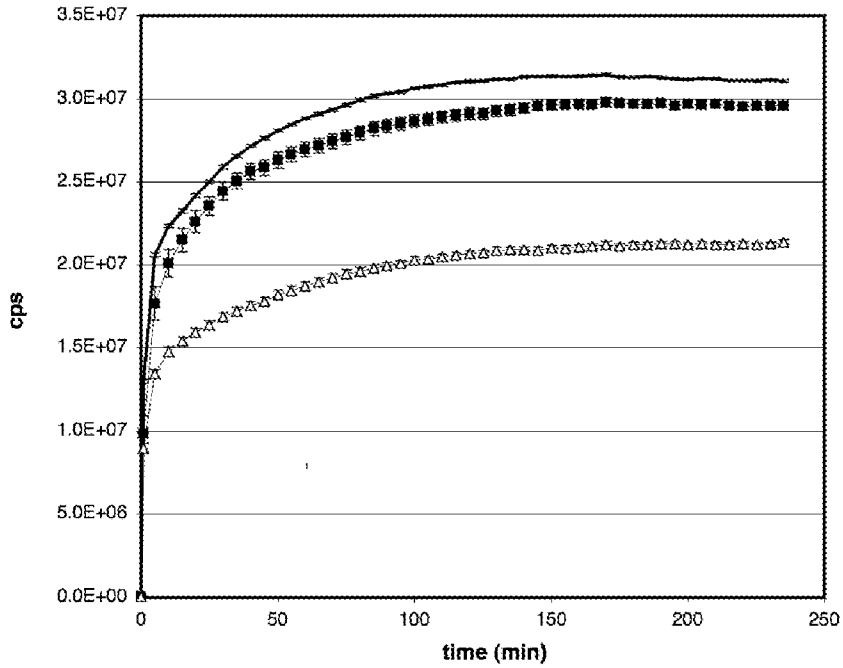

3500
3000
2500
2000
1500
1000
500
0
RFU
0
50
100
150
200
250
time (min)

B.

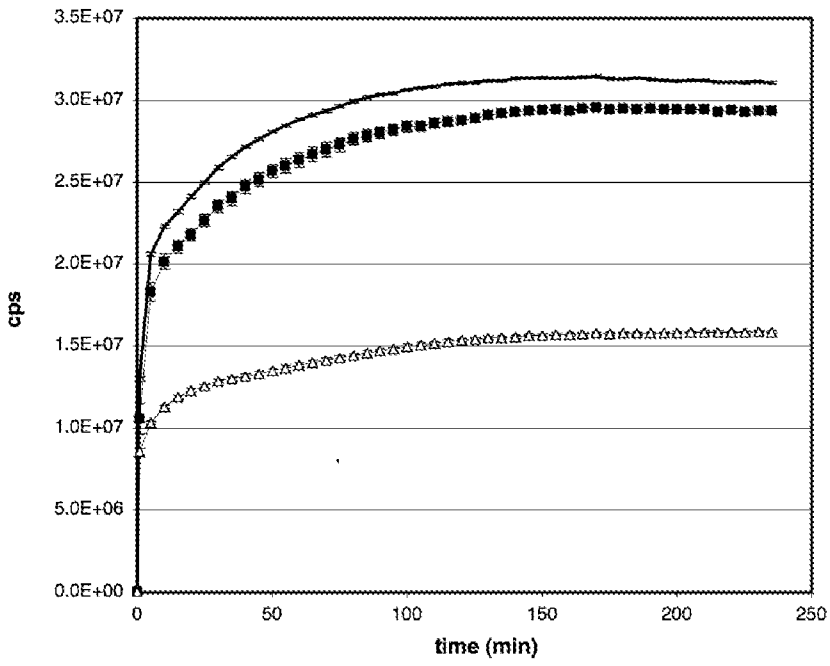

9000
8000
7000
6000
5000
4000
3000
2000
1000
0
RFU
0
50
100
150
200
250
300
time (min)

# Figure 6

A.

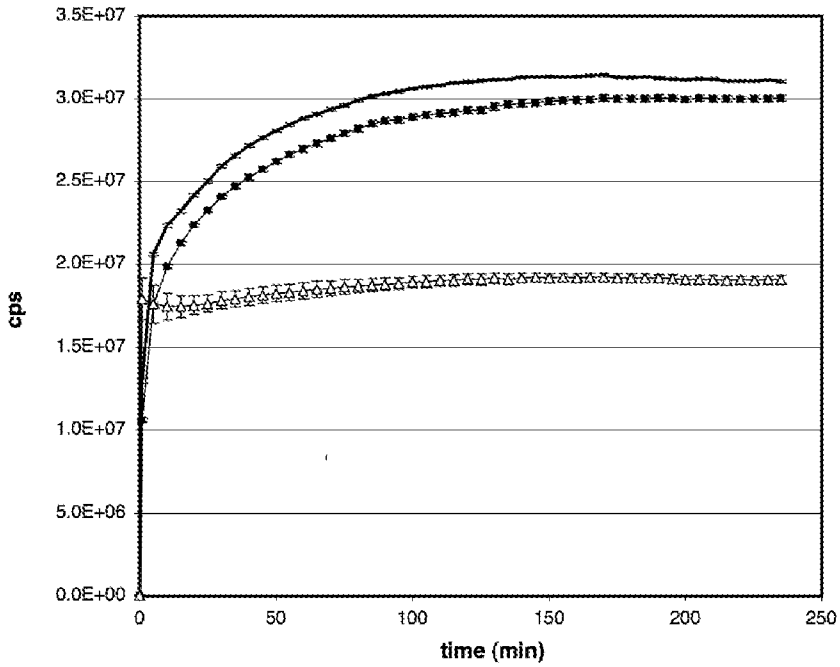

3.5E+07
3.0E+07
2.5E+07
2.0E+07
1.5E+07
1.0E+07
5.0E+06
0.0E+00
cps
0
50
100
150
200
250
time (min)

B.

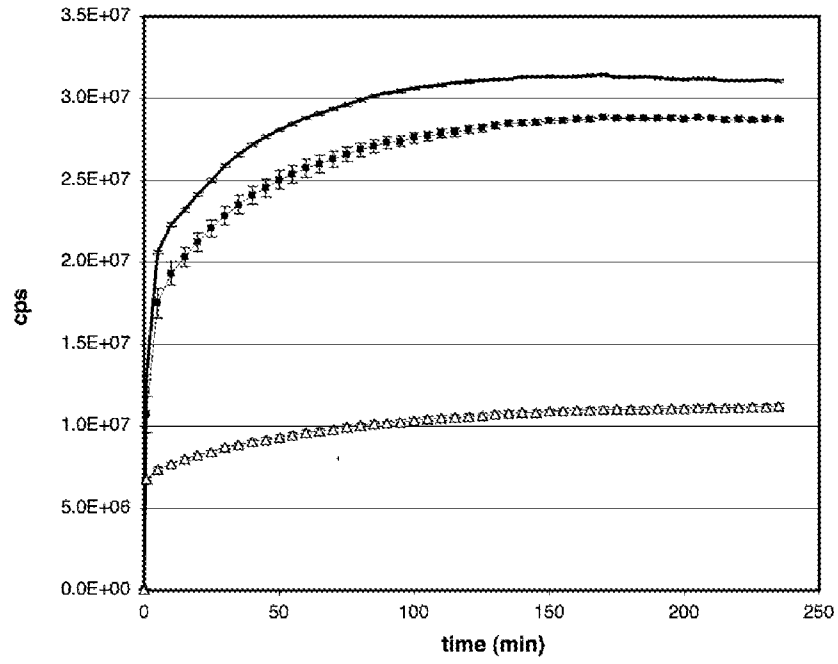

3.5E+07
3.0E+07
2.5E+07
2.0E+07
1.5E+07
1.0E+07
5.0E+06
0.0E+00
cps
0
50
100
150
200
250
time (min)

# Figure 7

A.

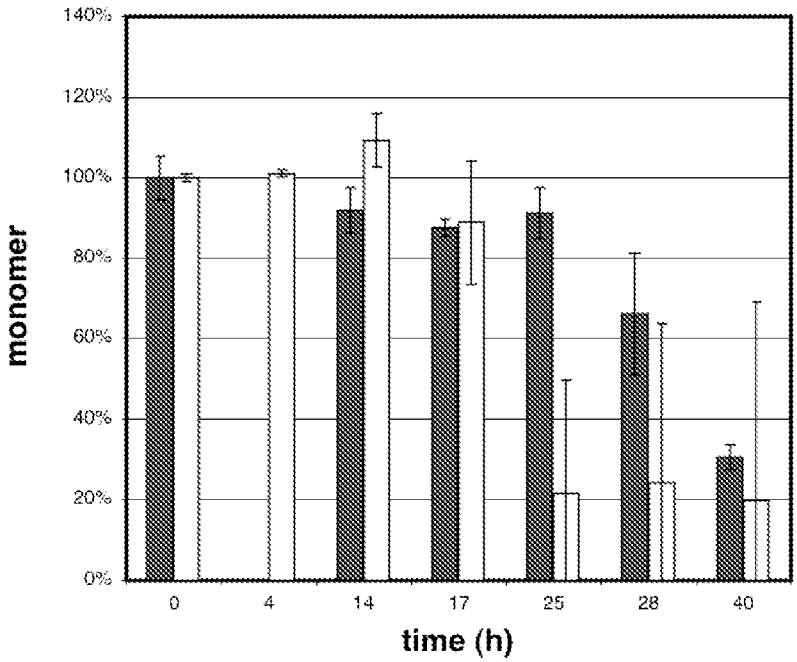

3.5E+07
3.0E+07
2.5E+07
2.0E+07
1.5E+07
1.0E+07
5.0E+06
0.0E+00
cps
0
50
100
150
200
250
time (min)

B.

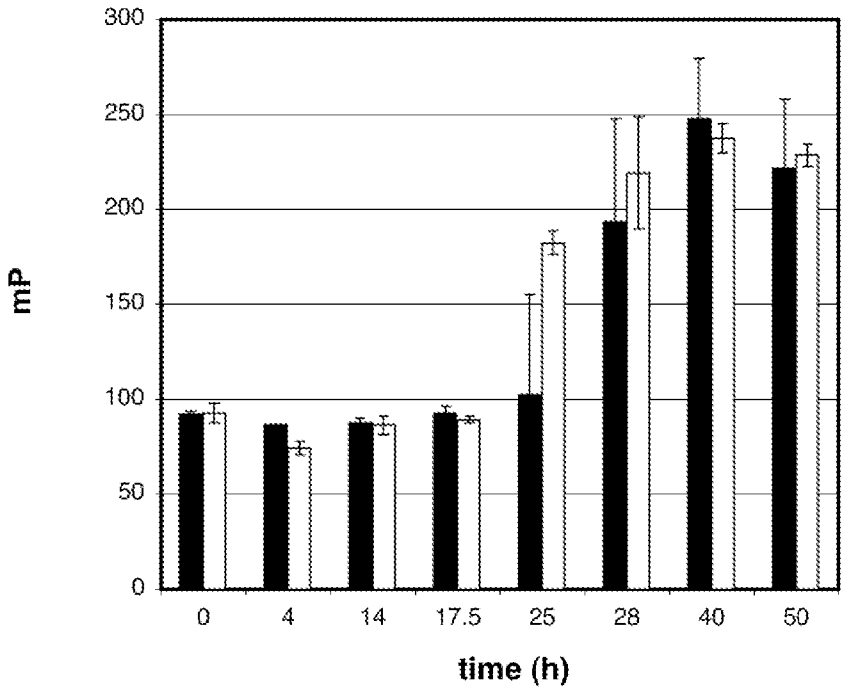

3.5E+07
3.0E+07
2.5E+07
2.0E+07
1.5E+07
1.0E+07
5.0E+06
0.0E+00
cps
0
50
100
150
200
250
time (min)

# Figure 8

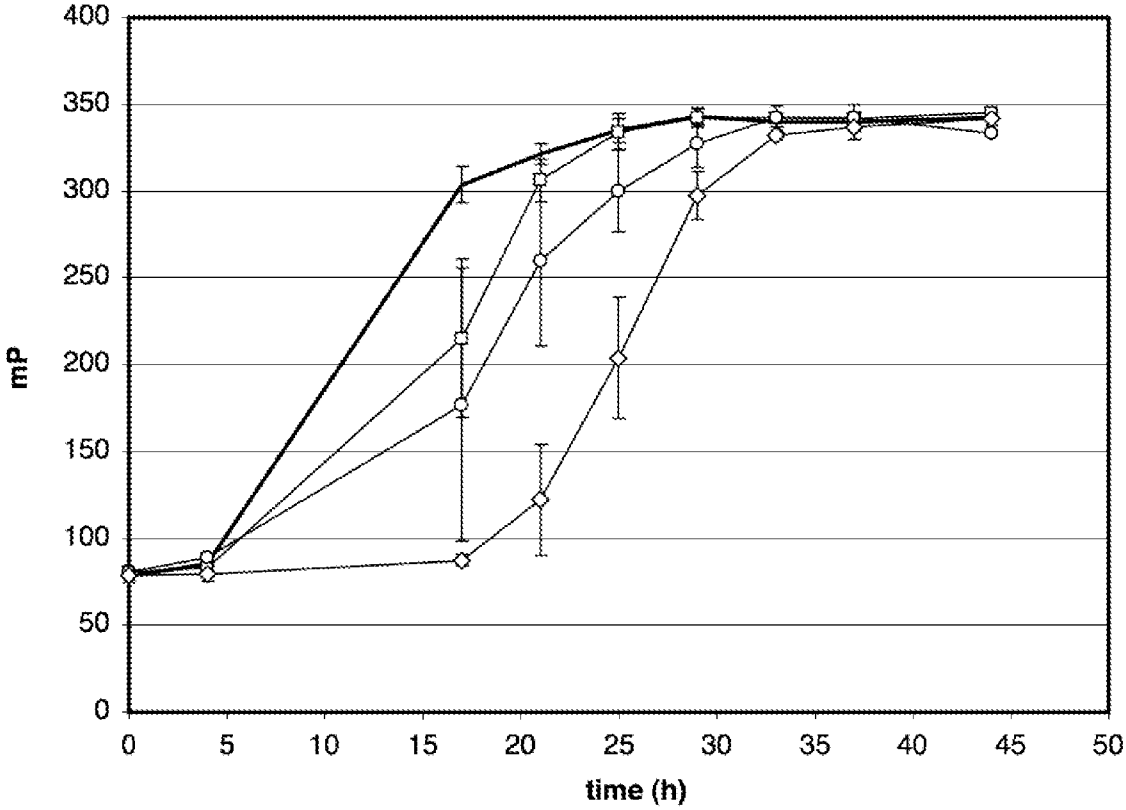
A.


140%
120%
100%
80%
60%
40%
20%
0%
monomer
0
4
14
17
25
28
40
time (h)

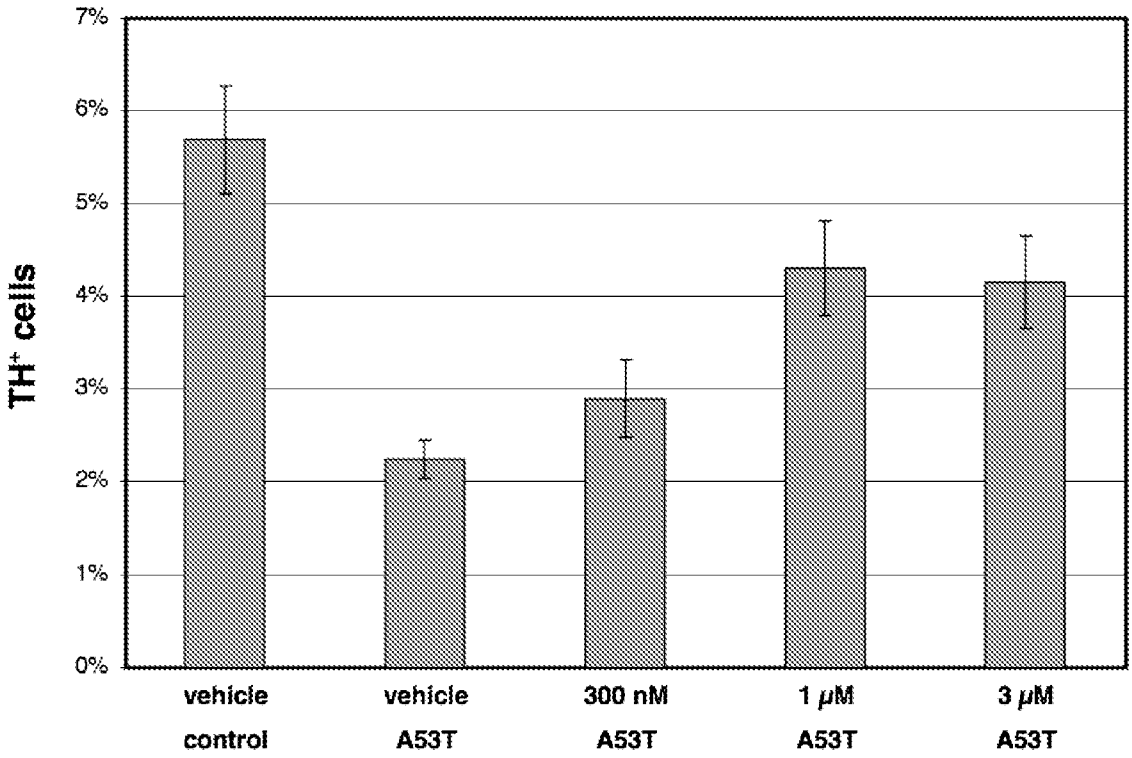
B.


300
250
200
150
100
50
0
mP
0
4
14
17.5
25
28
40
50
time (h)

# Figure 9

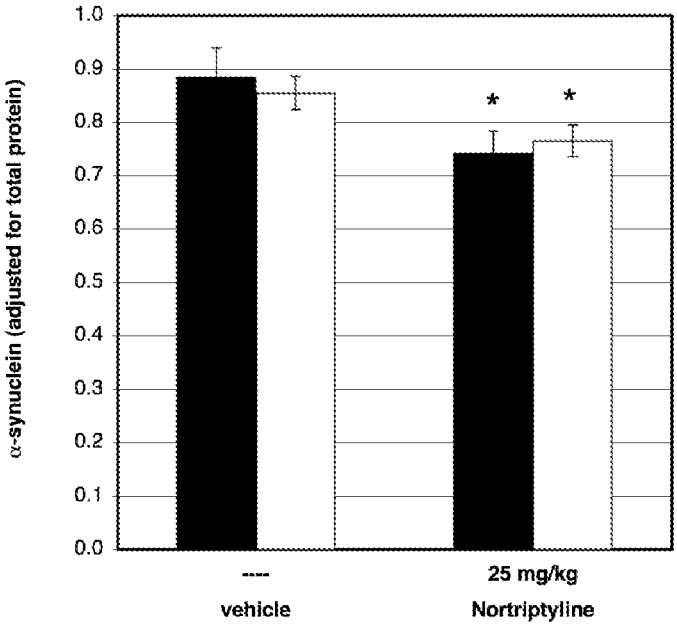

400
350
300
250
200
150
100
50
0
mP
0
5
10
15
20
25
30
35
40
45
50
time (h)

# Figure 10

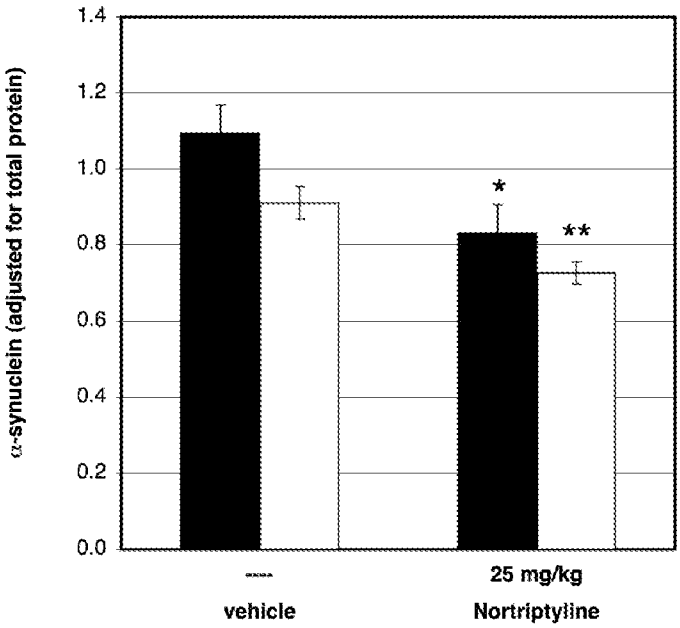

7%
6%
5%
4%
3%
2%
1%
0%
TH+ cells
vehicle control
vehicle A53T
300 nM A53T
1 μM A53T
3 μM A53T

# Figure 11

A.

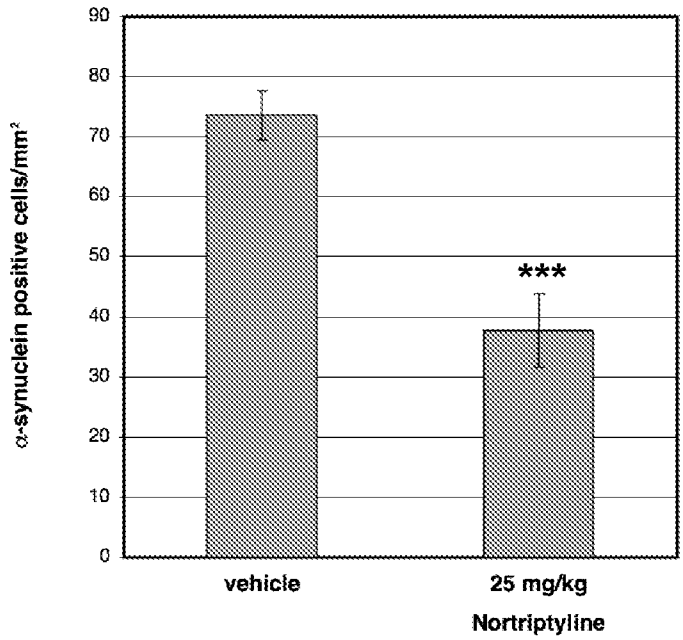

α-synuclein (adjusted for total protein)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
*
----
vehicle
25 mg/kg
Nortriptyline

B.

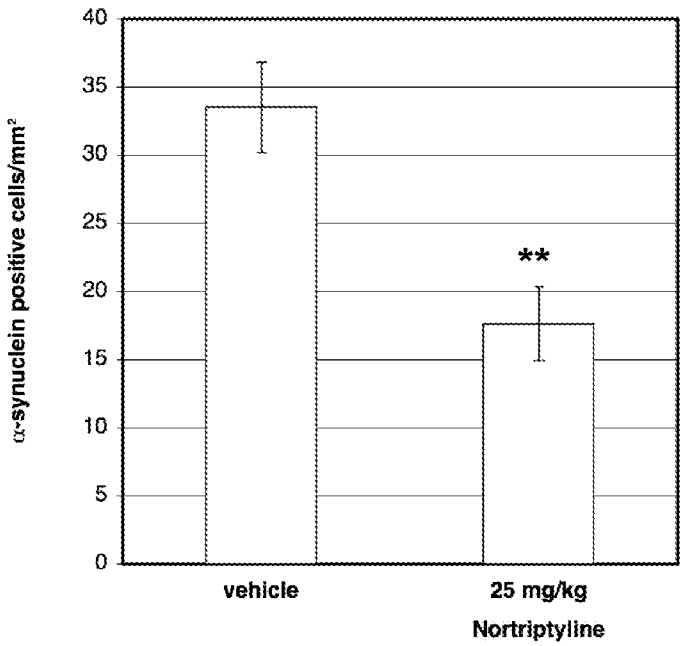

α-synuclein (adjusted for total protein)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
**
----
vehicle
25 mg/kg
Nortriptyline

# Figure 12

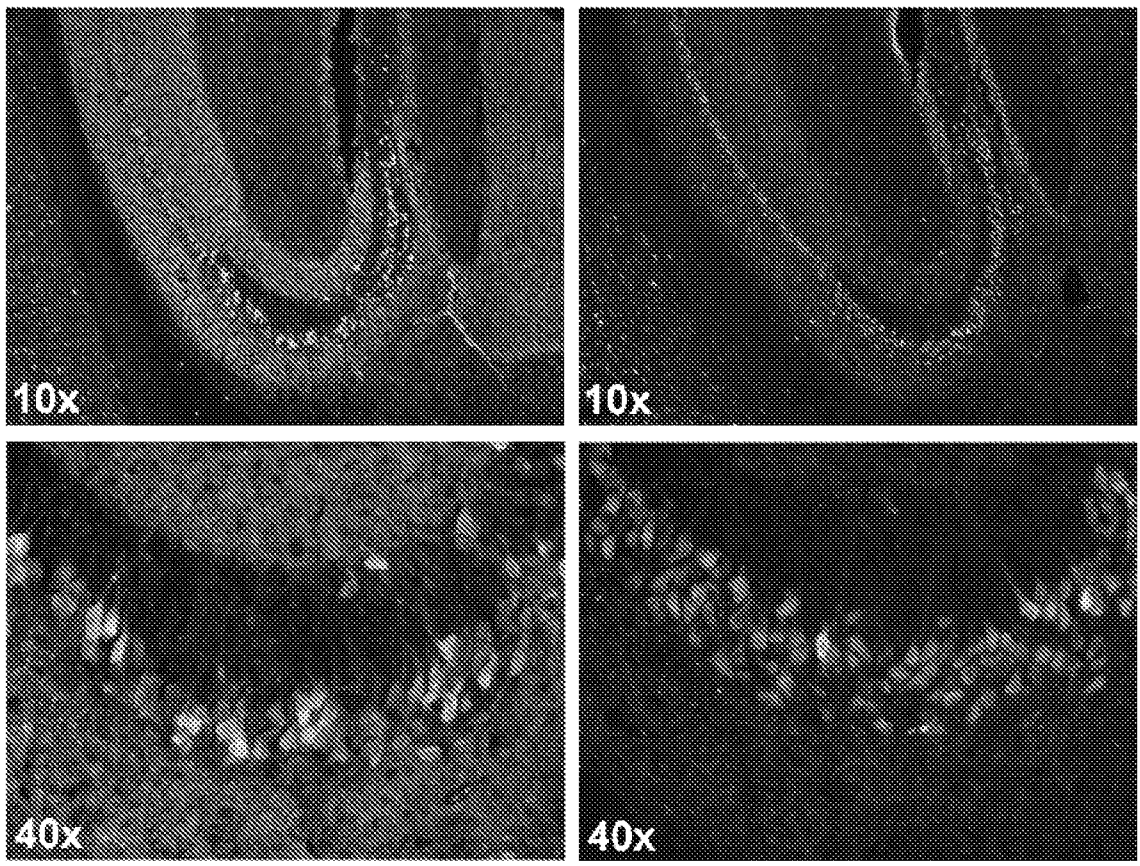
A.


90
80
70
60
50
40
30
20
10
0
α-synuclein positive cells/mm²
***
vehicle
25 mg/kg
Nortriptyline

B.

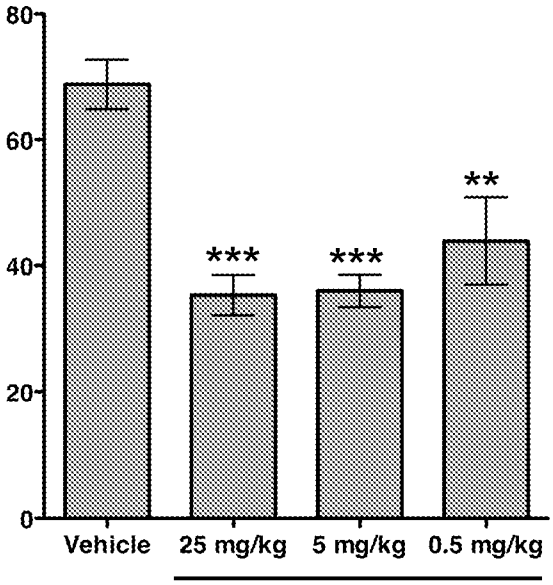

40
35
30
25
20
15
10
5
0
α-synuclein positive cells/mm²
**
vehicle
25 mg/kg
Nortriptyline

# Figure 13

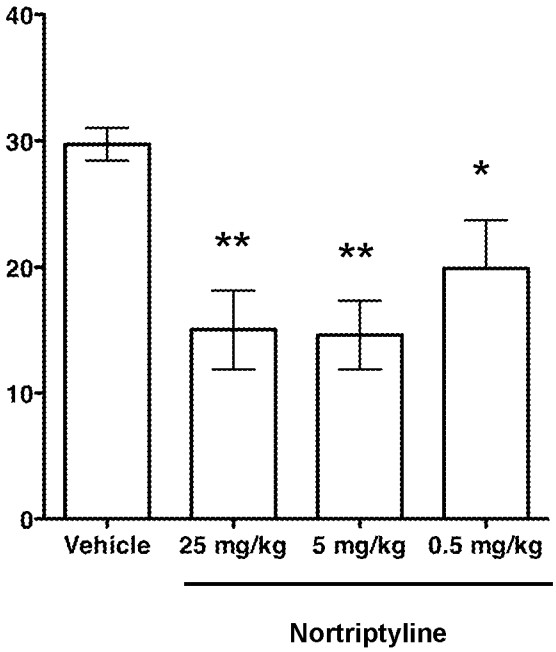


# Figure 14

A.


80
60
40
20
0
***
***
**
Vehicle
25 mg/kg
5 mg/kg
0.5 mg/kg
Nortriptyline

B.


40
30
20
10
0
**
**
*
Vehicle
25 mg/kg
5 mg/kg
0.5 mg/kg
Nortriptyline