



(51) International Patent Classification:

C07D 211/94 (2006.01) A61K 31/4545 (2006.01)
C07D 401/12 (2006.01) A61P 43/00 (2006.01)
A61K 31/4436 (2006.01)

(21) International Application Number:

PCT/US2016/039877

(22) International Filing Date:

28 June 2016 (28.06.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/186,268	29 June 2015 (29.06.2015)	US
62/186,131	29 June 2015 (29.06.2015)	US
62/187,701	1 July 2015 (01.07.2015)	US

(71) Applicant: UNIVERSITY OF PITTSBURGH - OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION [US/US]; 200 Gardner Steel Conference Center, Thackeray and O'Hara Streets, Pittsburgh, PA 15260 (US).

(72) Inventors: WIPE, Peter; Department of Chemistry, Chevron Science Center (1301 CHVRN), 219 Parkman Avenue, Pittsburgh, PA 15260 (US). MICHAEL, Adrian; 200 Gardner Steel Conference Center, Thackeray and O'Hara Streets, Pittsburgh, PA 15260 (US).

(74) Agent: RUPERT, Wayne; Klarquist Sparkman, LLP, One World Trade Center, Suite 1600, 121 SW Salmon Street, Portland, OR 97204 (US).

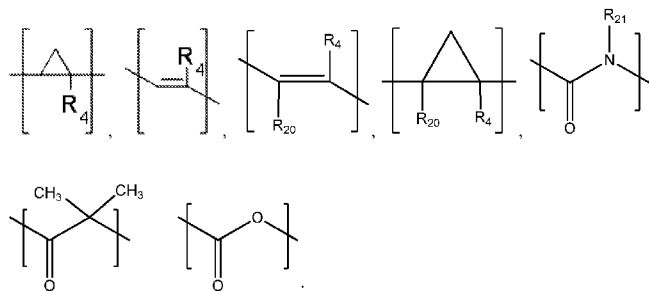
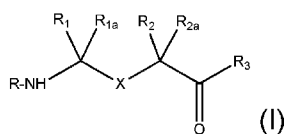
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LI, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: BISNITROXIDES



(57) Abstract: A compound, or a pharmaceutically acceptable salt thereof, having a structure of: Formula I wherein X is, or R₁, R_{1a}, R₂, R_{2a}, R₄, R₂₀ and R₂₁ are each independently hydrogen, halo, or an optionally substituted alkyl; R₃ is -N(R₅)(R₁₀), wherein R₅ and R₁₀ are each independently H or an -N-O•, -N-OH or -N=O containing group, provided at least one of R₅ is an -N-O•, -N-OH or -N=O containing group; and R is -C(O)-R₆, -C(O)O-R₆, or -P(O)-(R₆)₂, wherein R₆ is an optionally substituted alkyl or an -N-O•, -N-OH or -N=O containing group, provided that if only one of R₅ or R₁₀ is an -N-O•, -N-OH or -N=O containing group then R₆ is an optionally substituted alkyl or an -N-O•, -N-OH or -N=O containing group.

BISNITROXIDES

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of U.S. Provisional Application No. 62/186,131, filed June 29, 2015; U.S. Provisional Application No. 62/186,268, filed June 29, 2015; and U.S. Provisional Application No. 62/187,701, filed July 1, 2015, all of which are incorporated by reference in their entirety.

ACKNOWLEDGMENT OF GOVERNMENT SUPPORT

10 This invention was made with government support under grant number NS081744 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

15 Microdialysis is a powerful analytical technique used to recover analytes from the extracellular space of the brain (neurotransmitters, metabolites, amino acids, neuropeptides). Microdialysis probes consist of inlet tubing that leads to a semi-permeable membrane at which analytes diffuse across the membrane and are collected through outlet tubing. This simple technique can be coupled to various analytical methods (high-performance liquid
20 chromatography, mass spectrometry, capillary electrophoresis) for simultaneous detection of small molecules. Due to the simplicity and versatility of the method, brain microdialysis has significantly impacted our understanding of brain function, neurological diseases, drug addiction, and traumatic brain injury.

Commonly used probes are 200-300 μm in diameter. Implantation of probes into the
25 brain damages the surrounding tissue decreasing blood flow, neurons and increasing microglia and astrocytes. This penetration injury causes a progressive decline in dopamine (DA) an important neurotransmitter often studied using microdialysis, specifically, probe implantation significantly decreases evoked DA release in the surrounding tissue. Although, DA terminals survive probe implantation, they do not seem to function on a normal level in tissue surrounding
30 the probe.

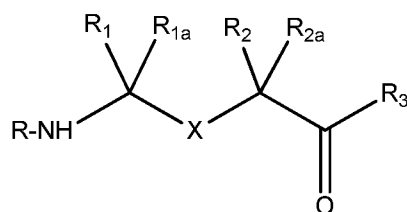
Retrodialysis of dexamethasone (DEX), an anti-inflammatory steroid increases blood flow to the area surrounding the probe and reduces gliosis for at least 5 days. By improving the health of the tissue DEX preserves neurons, DA terminals, and subsequently evoked DA release near probes at 4 and 24 hours. Pharmacological mitigation of probe induced tissue

damage through the use of an anti-inflammatory provides a platform for improving long-term microdialysis. A concern with DEX is that steroids are can affect neurotransmission, some steroids acting specifically on the central dopaminergic systems. Therefore it is of important to investigate other non-steroidal anti-inflammatory agents and their ability to prevent tissue damage and loss of evoked DA release near probes.

SUMMARY

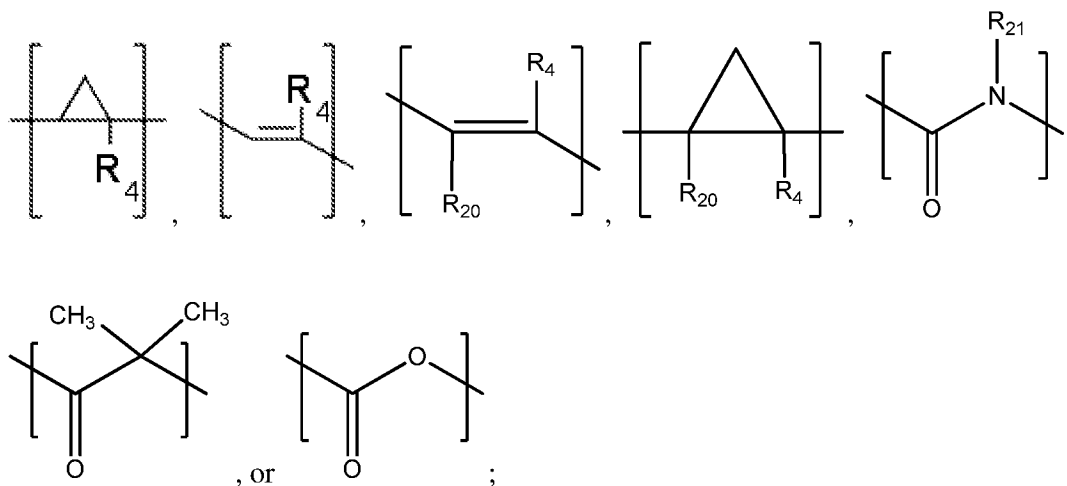
Disclosed herein are novel bisnitroxides or higher nitroxides.

In one embodiment, there is disclosed a compound, or a pharmaceutically acceptable salt thereof, having a structure of:



Formula 1

wherein X is



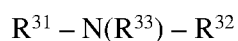
R_1 , R_{1a} , R_2 , R_{2a} , R_4 , R_{20} and R_{21} are each independently hydrogen, halo, or an optionally substituted alkyl;

R_3 is $-N(R_5)(R_{10})$, wherein R_5 and R_{10} are each independently H or an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group, provided at least one of R_5 is an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group; and

R is -C(O)-R₆, -C(O)O-R₆, or -P(O)-(R₆)₂, wherein R₆ is an optionally substituted alkyl or an -N-O•, -N-OH or -N=O containing group, provided that if only one of R₅ or R₁₀ is an -N-O•, -N-OH or -N=O containing group then R₆ is an optionally substituted alkyl or an -N-O•, -N-OH or -N=O containing group.

5

In another embodiment, there is disclosed a compound, or a pharmaceutically acceptable salt thereof, having a structure of:

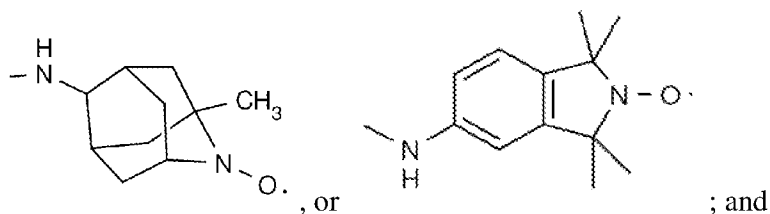
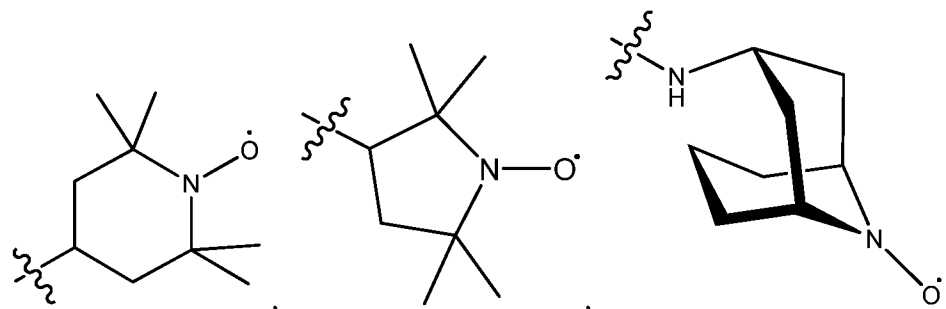


10

Formula 2

wherein R³¹ and R³² are each independently

15



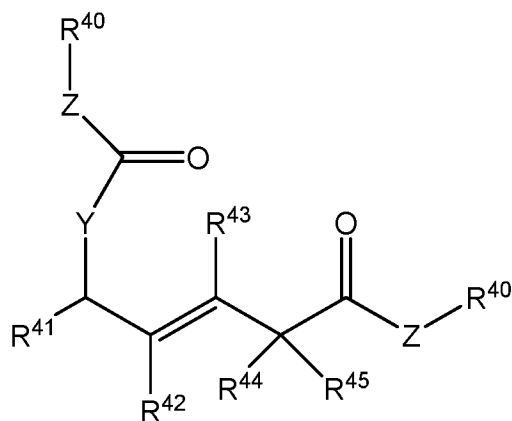
; and

20

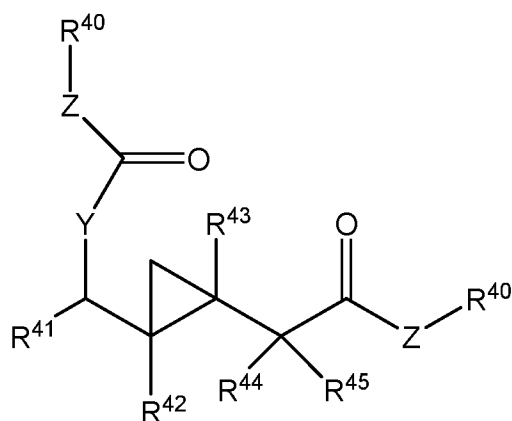
R³³ is H or an optionally substituted alkyl.

Further disclosed herein are compounds, or pharmaceutically acceptable salts thereof, having a structure of:

25



Formula 3A ; or



Formula 3B

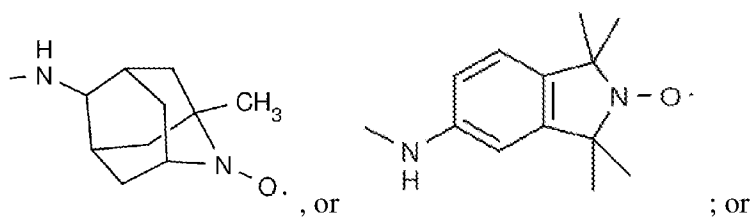
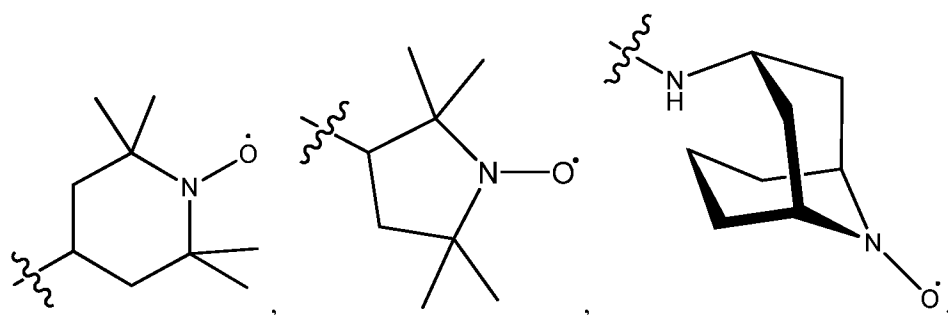
wherein Y is -NH-, -O-, -N(alkyl)-, -N(aryl)-, -N(cycloalkyl)-, -N(heteroaryl)-, or -CH(R⁴¹)-;

10 R⁴¹ is H, optionally substituted aliphatic, optionally substituted cycloalkyl, optionally substituted aryl, or optionally heteroaryl;

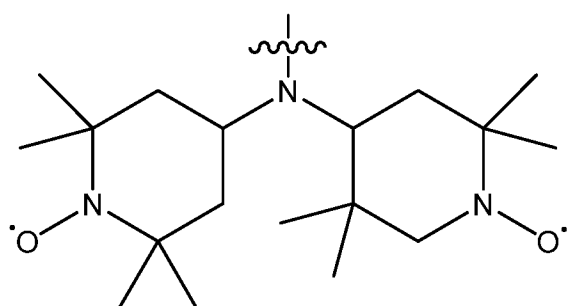
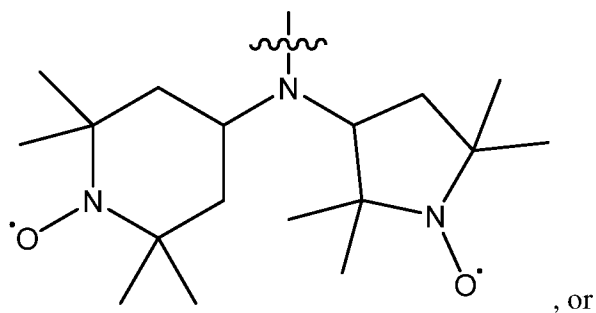
each of R⁴² – R⁴⁵ is H, optionally substituted aliphatic, optionally substituted cycloalkyl, optionally substituted aryl, optionally heteroaryl, or fluorine;

each Z is independently -O-, -NH- or -CH₂-; and

15 each R⁴⁰ is independently



5

each -Z^{R40} is independently

10

Further disclosed herein is a method for mitigating probe-induced tissue damage in a subject, comprising administering to a subject in need thereof, at least one compound disclosed herein.

15

The foregoing will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Top panel: Chemical structures of anti-inflammatory agents. Bottom panel: Chemical structures of reactive oxygen species scavengers.

FIGS. 2A-2C. Average ($\pm$ SEM) DA responses to a 25 s stimulation of the MFB recorded in the striatum premicrodialysis probe implantation (blue), post-probe implantation (red), and post-probe, post-nomifensine (green). Microdialysis probes were perfused with either DEX (Fig. 2A), IBU (Fig. 2B), or PPads (Fig. 2C) (n=6 rats per agent). Solid lines represent the mean response and dotted lines the SEM. Black diamond indicate the beginning and end of stimulation. DEX data previously published.

FIGS. 3A and 3B. Effects of anti-inflammatory retrodialysis of agents on maximum evoked DA (FIG. 3A) post-probe implantation and (FIG. 3B) post-nomifensine. (FIG. 3A) As DA was non-detectable in the case of aCSF a one-sample t-tests was performed to compare each post-probe response to zero. DEX, IBU and PPads significantly increased maximum evoked DA compared to aCSF: One-sample, one-tailed t-test: DEX $t(5)=8.21$, *** $p<0.0005$, IBU $t(5)=4.11$, ** $p<0.005$, PPads $t(5)=3.46$, * $p<0.05$. DEX is significantly different from IBU and PPads: one-way ANOVA $F(2,15)=5.20$, $p<0.05$, Post-hoc tukey DEX is significantly different from IBU $p<0.05$, PPads $p<0.05$. (FIG. 3B) Post-nomifensine, DEX, IBU, and Ppads increased evoked DA compared to aCSF: One-way ANOVA $F(3,20)=7.28$, $p<0.005$, Post-hoc tukey DEX: ** $p<0.005$, IBU: ** $p<0.005$, PPads: * $p<0.05$.

FIGS. 4A-4C. Average ($\pm$ SEM) DA responses to a 25 s stimulation of recorded in the striatum pre-microdialysis probe implantation (blue), post-probe implantation (red), and post-probe, post-nomifensine (green). Microdialysis probes were perfused with either XJB (Fig. 4A), JP4 (Fig. 4B), or JRS (Fig. 4C) (n=6 rats per agent). Solid lines represent the mean response and dotted lines the SEM. Black diamond indicate the beginning and end of stimulation. XJB data previously published.

FIGS. 5A and 5B. Effects ROS scavengers retrodialysis of maximum evoked DA (FIG. 5A) post-probe implantation and (FIG. 5B) postnomifensine. (FIG. 5A) As DA was non-detectable in the case of aCSF a one-sample t-tests was performed to compare each post-probe response to zero. XJB, and JRS significantly increased maximum evoked DA compared to aCSF: One sample, one-tailed t-test XJB $t(5)=2.02$, * $p<0.05$, JRS $t(5)=2.24$, * $p<0.05$. (FIG. 5B) Post-nomifensine, JRS

increased evoked DA compared to aCSF: One-way ANOVA $F(3,20)=3.98$, $p<0.05$, post-hoc tukey test: JRS: $*p<0.05$.

FIGS. 6A-6D. Effects of nomifensine added to perfusion fluid of microdialysis probes on evoked DA release near probes (n=3 rats/group). (FIG. 6A) Average ($\pm$ SEM) current responses to a 25 s stimulation of the recorded in the striatum postprobe implantation with aCSF (red), and post-probe with aCSF plus nomifensine (green). (FIG. 6B) Average ($\pm$ SEM) DA responses to a 25 s stimulation of the MFB recorded in the striatum post-probe implantation with DEX (red), and post-probe with DEX plus nomifensine (green). (FIG. 6C) There was no difference in maximum current post-probe implantation with aCSF (red), and post-probe with aCSF plus nomifensine (green), paired t-test ($p>0.05$). (FIG. 6D) There was no difference in maximum DA release post-probe implantation with DEX (red), and post-probe with DEX plus nomifensine (green), paired t-test ($p>0.05$).

DETAILED DESCRIPTION

Terminology

The following explanations of terms and methods are provided to better describe the present compounds, compositions and methods, and to guide those of ordinary skill in the art in the practice of the present disclosure. It is also to be understood that the terminology used in the disclosure is for the purpose of describing particular embodiments and examples only and is not intended to be limiting.

“Acyl” refers to a group having the structure $-C(O)R$, where R may be, for example, optionally substituted alkyl, optionally substituted aryl, or optionally substituted heteroaryl. “Lower acyl” groups are those that contain one to six carbon atoms.

“Administration” as used herein is inclusive of administration by another person to the subject or self-administration by the subject.

The term “aliphatic” is defined as including alkyl, alkenyl, alkynyl, halogenated alkyl and cycloalkyl groups. A “lower aliphatic” group is a branched or unbranched aliphatic group having from 1 to 10 carbon atoms.

Alkanediyl,” “cycloalkanediyl,” “aryldiyl,” “alkanearyldiyl” refers to a divalent radical derived from aliphatic, cycloaliphatic, aryl, and alkanearyl hydrocarbons.

“Alkenyl” refers to a cyclic, branched or straight chain group containing only carbon and hydrogen, and contains one or more double bonds that may or may not be conjugated. Alkenyl groups may be unsubstituted or substituted. “Lower alkenyl” groups contain one to six carbon atoms.

The term “alkoxy” refers to a straight, branched or cyclic hydrocarbon configuration and combinations thereof, including from 1 to 20 carbon atoms, preferably from 1 to 8 carbon atoms (referred to as a “lower alkoxy”), more preferably from 1 to 4 carbon atoms, that include an oxygen atom at the point of attachment. An example of an “alkoxy group” is represented by the formula –
 5 OR, where R can be an alkyl group, optionally substituted with an alkenyl, alkynyl, aryl, aralkyl, cycloalkyl, halogenated alkyl, alkoxy or heterocycloalkyl group. Suitable alkoxy groups include methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, i-butoxy, sec-butoxy, tert-butoxy cyclopropoxy, cyclohexyloxy, and the like.

“Alkoxy carbonyl” refers to an alkoxy substituted carbonyl radical, $-C(O)OR$, wherein R
 10 represents an optionally substituted alkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl or similar moiety.

The term “alkyl” refers to a branched or unbranched saturated hydrocarbon group of 1 to 24 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *t*-butyl, pentyl, hexyl, heptyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl and the like. A “lower alkyl” group is
 15 a saturated branched or unbranched hydrocarbon having from 1 to 6 carbon atoms. Preferred alkyl groups have 1 to 4 carbon atoms. Alkyl groups may be “substituted alkyls” wherein one or more hydrogen atoms are substituted with a substituent such as halogen, cycloalkyl, alkoxy, amino, hydroxyl, aryl, alkenyl, or carboxyl. For example, a lower alkyl or (C₁-C₆)alkyl can be methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec-butyl, pentyl, 3-pentyl, or hexyl; (C₃-C₆)cycloalkyl
 20 can be cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl; (C₃-C₆)cycloalkyl(C₁-C₆)alkyl can be cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl, cyclohexylmethyl, 2-cyclopropylethyl, 2-cyclobutylethyl, 2-cyclopentylethyl, or 2-cyclohexylethyl; (C₁-C₆)alkoxy can be methoxy, ethoxy, propoxy, isopropoxy, butoxy, iso-butoxy, sec-butoxy, pentoxy, 3-pentoxy, or hexyloxy; (C₂-C₆)alkenyl can be vinyl, allyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1-,
 25 pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, or 5-hexenyl; (C₂-C₆)alkynyl can be ethynyl, 1-propynyl, 2-propynyl, 1-butyne, 2-butyne, 3-butyne, 1-pentyne, 2-pentyne, 3-pentyne, 4-pentyne, 1-hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, or 5-hexynyl; (C₁-C₆)alkanoyl can be acetyl, propanoyl or butanoyl; halo(C₁-C₆)alkyl can be iodomethyl, bromomethyl, chloromethyl, fluoromethyl, trifluoromethyl, 2-chloroethyl, 2-
 30 fluoroethyl, 2,2,2-trifluoroethyl, or pentafluoroethyl; hydroxy(C₁-C₆)alkyl can be hydroxymethyl, 1-hydroxyethyl, 2-hydroxyethyl, 1-hydroxypropyl, 2-hydroxypropyl, 3-hydroxypropyl, 1-hydroxybutyl, 4-hydroxybutyl, 1-hydroxypentyl, 5-hydroxypentyl, 1-hydroxyhexyl, or 6-hydroxyhexyl; (C₁-C₆)alkoxy carbonyl can be methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, pentoxycarbonyl, or hexyloxycarbonyl; (C₁-C₆)alkylthio can

be methylthio, ethylthio, propylthio, isopropylthio, butylthio, isobutylthio, pentylthio, or hexylthio; (C₂-C₆)alkanoyloxy can be acetoxy, propanoyloxy, butanoyloxy, isobutanoyloxy, pentanoyloxy, or hexanoyloxy.

The term “amine” or “amino” refers to a group of the formula –NRR', where R and R' can be, independently, hydrogen or an alkyl, alkenyl, alkynyl, acyl, aryl, aralkyl, cycloalkyl, halogenated alkyl, or heterocycloalkyl group. For example, an “alkylamino” or “alkylated amino” refers to –NRR', wherein at least one of R or R' is an alkyl. A suitable amine or amino group is acetamido.

The term “aminoalkyl” refers to alkyl groups as defined above where at least one hydrogen atom is replaced with an amino group (e.g., –CH₂–NH₂).

“Aminocarbonyl” alone or in combination, means an amino substituted carbonyl (carbamoyl) radical, wherein the amino radical may optionally be mono- or di-substituted, such as, for example, with alkyl, aryl, acyl, aralkyl, cycloalkyl, cycloalkylalkyl, alkanoyl, alkoxycarbonyl, aralkoxycarbonyl and the like.

The term “amide” or “amido” is represented by the formula –C(O)NRR', where R and R' independently can be, for example, a hydrogen, alkyl, alkenyl, alkynyl, acyl, aryl, aralkyl, cycloalkyl, halogenated alkyl, or heterocycloalkyl group.

An “animal” refers to living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals. Similarly, the term “subject” includes both human and non-human subjects, including birds and non-human mammals, such as non-human primates, companion animals (such as dogs and cats), livestock (such as pigs, sheep, cows), as well as non-domesticated animals, such as the big cats. The term subject applies regardless of the stage in the organism's life-cycle. Thus, the term subject applies to an organism *in utero* or *in ovo*, depending on the organism (that is, whether the organism is a mammal or a bird, such as a domesticated or wild fowl).

The term “aralkyl” refers to an alkyl group wherein an aryl group is substituted for a hydrogen of the alkyl group. An example of an aralkyl group is a benzyl group.

“Aryl” refers to a monovalent unsaturated aromatic carbocyclic group having a single ring (e.g., phenyl) or multiple condensed rings (e.g., naphthyl or anthryl), which can optionally be unsubstituted or substituted. A “heteroaryl group,” is defined as an aromatic group that has at least one heteroatom incorporated within the ring of the aromatic group. Examples of heteroatoms include, but are not limited to, nitrogen, oxygen, sulfur, and phosphorous. Heteroaryl includes, but is not limited to, pyridinyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl,

benzimidazolyl, benzooxazolyl, quinoxaliny, and the like. The aryl or heteroaryl group can be substituted with one or more groups including, but not limited to, alkyl, alkynyl, alkenyl, aryl, halide, nitro, amino, ester, ketone, aldehyde, hydroxy, carboxylic acid, or alkoxy, or the aryl or heteroaryl group can be unsubstituted.

5 “Aryloxy” or “heteroaryloxy” refers to a group of the formula –OAr, wherein Ar is an aryl group or a heteroaryl group, respectively.

 The term “cycloalkyl” refers to a non-aromatic carbon-based ring composed of at least three carbon atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like. The term “heterocycloalkyl group” is a
10 cycloalkyl group as defined above where at least one of the carbon atoms of the ring is substituted with a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorous.

 The term “ester” refers to a carboxyl group-containing moiety having the hydrogen replaced with, for example, a C₁₋₆alkyl group (“carboxylC₁₋₆alkyl” or “alkylester”), an aryl or aralkyl group (“arylester” or “aralkylester”) and so on. CO₂C₁₋₃alkyl groups are preferred, such as for example,
15 methylester (CO₂Me), ethylester (CO₂Et) and propylester (CO₂Pr) and includes reverse esters thereof (e.g. –OCOMe, –OCOEt and –OCOPr).

 The terms “halogenated alkyl” or “haloalkyl group” refer to an alkyl group with one or more hydrogen atoms present on these groups substituted with a halogen (F, Cl, Br, I).

 The term “hydroxyl” is represented by the formula –OH.

20 The term “hydroxyalkyl” refers to an alkyl group that has at least one hydrogen atom substituted with a hydroxyl group. The term “alkoxyalkyl group” is defined as an alkyl group that has at least one hydrogen atom substituted with an alkoxy group described above.

 “Inhibiting” refers to inhibiting the full development of a disease or condition. “Inhibiting” also refers to any quantitative or qualitative reduction in biological activity or binding, relative to a
25 control.

 The term “reactive oxygen species” (“ROS”) includes, but is not limited to, superoxide anion, hydroxyl, and hydroperoxide radicals.

 The term “subject” includes both human and non-human subjects, including birds and non-human mammals, such as non-human primates, companion animals (such as dogs and cats),
30 livestock (such as pigs, sheep, cows), as well as non-domesticated animals, such as the big cats. The term subject applies regardless of the stage in the organism’s life-cycle. Thus, the term subject applies to an organism *in utero* or *in ovo*, depending on the organism (that is, whether the organism is a mammal or a bird, such as a domesticated or wild fowl).

“Substituted” or “substitution” refers to replacement of a hydrogen atom of a molecule or an R-group with one or more additional R-groups. Unless otherwise defined, the term “optionally-substituted” or “optional substituent” as used herein refers to a group which may or may not be further substituted with 1, 2, 3, 4 or more groups, preferably 1, 2 or 3, more preferably 1 or 2 groups. The substituents may be selected, for example, from C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₃₋₆cycloalkyl, hydroxyl, oxo, C₁₋₆alkoxy, aryloxy, C₁₋₆alkoxyaryl, halo, C₁₋₆alkylhalo (such as CF₃ and CHF₂), C₁₋₆alkoxyhalo (such as OCF₃ and OCHF₂), carboxyl, esters, cyano, nitro, amino, substituted amino, disubstituted amino, acyl, ketones, amides, aminoacyl, substituted amides, disubstituted amides, thiol, alkylthio, thioxo, sulfates, sulfonates, sulfinyl, substituted sulfinyl, sulfonyl, substituted sulfonyl, sulfonylamides, substituted sulfonamides, disubstituted sulfonamides, aryl, arC₁₋₆alkyl, heterocyclyl and heteroaryl wherein each alkyl, alkenyl, alkynyl, cycloalkyl, aryl and heterocyclyl and groups containing them may be further optionally substituted. Optional substituents in the case N-heterocycles may also include but are not limited to C₁₋₆alkyl i.e. *N*-C₁₋₃alkyl, more preferably methyl particularly *N*-methyl.

A “therapeutically effective amount” refers to a quantity of a specified agent sufficient to achieve a desired effect in a subject being treated with that agent. Ideally, a therapeutically effective amount of an agent is an amount sufficient to inhibit or treat the disease or condition without causing a substantial cytotoxic effect in the subject. The therapeutically effective amount of an agent will be dependent on the subject being treated, the severity of the affliction, and the manner of administration of the therapeutic composition.

“Treatment” refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop, or administering a compound or composition to a subject who does not exhibit signs of a disease or exhibits only early signs for the purpose of decreasing the risk of developing a pathology or condition, or diminishing the severity of a pathology or condition. As used herein, the term “ameliorating,” with reference to a disease or pathological condition, refers to any observable beneficial effect of the treatment. The beneficial effect can be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject, a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease, an improvement in the overall health or well-being of the subject, or by other parameters well known in the art that are specific to the particular disease. The phrase “treating a disease” refers to inhibiting the full development of a disease, for example, in a subject who is at risk for a disease such as cancer. “Preventing” a disease or condition refers to prophylactic administering a composition to a subject who does not exhibit signs of a disease or

exhibits only early signs for the purpose of decreasing the risk of developing a pathology or condition, or diminishing the severity of a pathology or condition.

“Pharmaceutical compositions” are compositions that include an amount (for example, a unit dosage) of one or more of the disclosed compounds together with one or more non-toxic pharmaceutically acceptable additives, including carriers, diluents, and/or adjuvants, and optionally other biologically active ingredients. Such pharmaceutical compositions can be prepared by standard pharmaceutical formulation techniques such as those disclosed in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA (19th Edition).

The terms “pharmaceutically acceptable salt or ester” refers to salts or esters prepared by conventional means that include salts, e.g., of inorganic and organic acids, including but not limited to hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, malic acid, acetic acid, oxalic acid, tartaric acid, citric acid, lactic acid, fumaric acid, succinic acid, maleic acid, salicylic acid, benzoic acid, phenylacetic acid, mandelic acid and the like. “Pharmaceutically acceptable salts” of the presently disclosed compounds also include those formed from cations such as sodium, potassium, aluminum, calcium, lithium, magnesium, zinc, and from bases such as ammonia, ethylenediamine, N-methyl-glutamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chlorprocaine, diethanolamine, procaine, N-benzylphenethylamine, diethylamine, piperazine, tris(hydroxymethyl)aminomethane, and tetramethylammonium hydroxide. These salts may be prepared by standard procedures, for example by reacting the free acid with a suitable organic or inorganic base. Any chemical compound recited in this specification may alternatively be administered as a pharmaceutically acceptable salt thereof. “Pharmaceutically acceptable salts” are also inclusive of the free acid, base, and zwitterionic forms. Descriptions of suitable pharmaceutically acceptable salts can be found in *Handbook of Pharmaceutical Salts, Properties, Selection and Use*, Wiley VCH (2002). When compounds disclosed herein include an acidic function such as a carboxy group, then suitable pharmaceutically acceptable cation pairs for the carboxy group are well known to those skilled in the art and include alkaline, alkaline earth, ammonium, quaternary ammonium cations and the like. Such salts are known to those of skill in the art. For additional examples of “pharmacologically acceptable salts,” see Berge et al., *J. Pharm. Sci.* 66:1 (1977).

“Pharmaceutically acceptable esters” includes those derived from compounds described herein that are modified to include a carboxyl group. An *in vivo* hydrolysable ester is an ester, which is hydrolysed in the human or animal body to produce the parent acid or alcohol. Representative esters thus include carboxylic acid esters in which the non-carbonyl moiety of the carboxylic acid portion of the ester grouping is selected from straight or branched chain alkyl (for

example, methyl, n-propyl, t-butyl, or n-butyl), cycloalkyl, alkoxyalkyl (for example, methoxymethyl), aralkyl (for example benzyl), aryloxyalkyl (for example, phenoxymethyl), aryl (for example, phenyl, optionally substituted by, for example, halogen, C.sub.1-4 alkyl, or C.sub.1-4 alkoxy) or amino); sulphonate esters, such as alkyl- or aralkylsulphonyl (for example, methanesulphonyl); or amino acid esters (for example, L-valyl or L-isoleucyl). A “pharmaceutically acceptable ester” also includes inorganic esters such as mono-, di-, or tri-phosphate esters. In such esters, unless otherwise specified, any alkyl moiety present advantageously contains from 1 to 18 carbon atoms, particularly from 1 to 6 carbon atoms, more particularly from 1 to 4 carbon atoms. Any cycloalkyl moiety present in such esters advantageously contains from 3 to 6 carbon atoms. Any aryl moiety present in such esters advantageously comprises a phenyl group, optionally substituted as shown in the definition of carbocyclyl above. Pharmaceutically acceptable esters thus include C₁-C₂₂ fatty acid esters, such as acetyl, t-butyl or long chain straight or branched unsaturated or omega-6 monounsaturated fatty acids such as palmoyl, stearoyl and the like. Alternative aryl or heteroaryl esters include benzoyl, pyridylmethyloyl and the like any of which may be substituted, as defined in carbocyclyl above. Additional pharmaceutically acceptable esters include aliphatic L-amino acid esters such as leucyl, isoleucyl and especially valyl.

For therapeutic use, salts of the compounds are those wherein the counter-ion is pharmaceutically acceptable. However, salts of acids and bases which are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound.

The pharmaceutically acceptable acid and base addition salts as mentioned hereinabove are meant to comprise the therapeutically active non-toxic acid and base addition salt forms which the compounds are able to form. The pharmaceutically acceptable acid addition salts can conveniently be obtained by treating the base form with such appropriate acid. Appropriate acids comprise, 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 (i.e. ethanedioic), malonic, succinic (i.e. butanedioic acid), maleic, fumaric, malic (i.e. hydroxybutanedioic acid), tartaric, citric, methanesulfonic, ethanesulfonic, benzenesulfonic, p-toluenesulfonic, cyclamic, salicylic, p-aminosalicylic, pamoic and the like acids. Conversely said salt forms can be converted by treatment with an appropriate base into the free base form.

The compounds containing an acidic proton may also be converted into their non-toxic metal or amine addition salt forms by treatment with appropriate organic and inorganic bases.

Appropriate base salt forms comprise, 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.

5 The term “addition salt” as used hereinabove also comprises the solvates which the compounds described herein are able to form. Such solvates are for example hydrates, alcoholates and the like.

 The term “quaternary amine” as used hereinbefore defines the quaternary ammonium salts which the compounds are able to form by reaction between a basic nitrogen of a compound and an
10 appropriate quaternizing agent, such as, for example, an optionally substituted alkylhalide, arylhalide or arylalkylhalide, e.g. methyl iodide or benzyl iodide. Other reactants with good leaving groups may also be used, such as alkyl trifluoromethanesulfonates, alkyl methanesulfonates, and alkyl p-toluenesulfonates. A quaternary amine has a positively charged nitrogen. Pharmaceutically acceptable counterions include chloro, bromo, iodo, trifluoroacetate and acetate. The counterion of
15 choice can be introduced using ion exchange resins.

 Prodrugs of the disclosed compounds also are contemplated herein. A prodrug is an active or inactive compound that is modified chemically through *in vivo* physiological action, such as hydrolysis, metabolism and the like, into an active compound following administration of the prodrug to a subject. The term “prodrug” as used throughout this text means the pharmacologically
20 acceptable derivatives such as esters, amides and phosphates, such that the resulting *in vivo* biotransformation product of the derivative is the active drug as defined in the compounds described herein. Prodrugs preferably have excellent aqueous solubility, increased bioavailability and are readily metabolized into the active inhibitors *in vivo*. Prodrugs of a compounds described herein may be prepared by modifying functional groups present in the compound in such a way that
25 the modifications are cleaved, either by routine manipulation or *in vivo*, to the parent compound. The suitability and techniques involved in making and using prodrugs are well known by those skilled in the art. For a general discussion of prodrugs involving esters see Svensson and Tunek, *Drug Metabolism Reviews* 165 (1988) and Bundgaard, *Design of Prodrugs*, Elsevier (1985).

 The term “prodrug” also is intended to include any covalently bonded carriers that release
30 an active parent drug of the present invention *in vivo* when the prodrug is administered to a subject. Since prodrugs often have enhanced properties relative to the active agent pharmaceutical, such as, solubility and bioavailability, the compounds disclosed herein can be delivered in prodrug form. Thus, also contemplated are prodrugs of the presently disclosed compounds, methods of delivering prodrugs and compositions containing such prodrugs. Prodrugs of the disclosed compounds

typically are prepared by modifying one or more functional groups present in the compound in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to yield the parent compound. Prodrugs include compounds having a phosphonate and/or amino group functionalized with any group that is cleaved *in vivo* to yield the corresponding amino and/or phosphonate group, respectively. Examples of prodrugs include, without limitation, compounds having an acylated amino group and/or a phosphonate ester or phosphonate amide group. In particular examples, a prodrug is a lower alkyl phosphonate ester, such as an isopropyl phosphonate ester.

Protected derivatives of the disclosed compounds also are contemplated. A variety of suitable protecting groups for use with the disclosed compounds are disclosed in Greene and Wuts, *Protective Groups in Organic Synthesis*; 3rd Ed.; John Wiley & Sons, New York, 1999.

In general, protecting groups are removed under conditions that will not affect the remaining portion of the molecule. These methods are well known in the art and include acid hydrolysis, hydrogenolysis and the like. One preferred method involves the removal of an ester, such as cleavage of a phosphonate ester using Lewis acidic conditions, such as in TMS-Br mediated ester cleavage to yield the free phosphonate. A second preferred method involves removal of a protecting group, such as removal of a benzyl group by hydrogenolysis utilizing palladium on carbon in a suitable solvent system such as an alcohol, acetic acid, and the like or mixtures thereof. A t-butoxy-based group, including t-butoxy carbonyl protecting groups can be removed utilizing an inorganic or organic acid, such as HCl or trifluoroacetic acid, in a suitable solvent system, such as water, dioxane and/or methylene chloride. Another exemplary protecting group, suitable for protecting amino and hydroxy functions amino is trityl. Other conventional protecting groups are known and suitable protecting groups can be selected by those of skill in the art in consultation with Greene and Wuts, *Protective Groups in Organic Synthesis*; 3rd Ed.; John Wiley & Sons, New York, 1999. When an amine is deprotected, the resulting salt can readily be neutralized to yield the free amine. Similarly, when an acid moiety, such as a phosphonic acid moiety is unveiled, the compound may be isolated as the acid compound or as a salt thereof.

Particular examples of the presently disclosed agents may include one or more asymmetric centers; thus these compounds can exist in different stereoisomeric forms. Accordingly, compounds and compositions may be provided as individual pure enantiomers or as stereoisomeric mixtures, including racemic mixtures. In certain embodiments the compounds disclosed herein are synthesized in or are purified to be in substantially enantiopure form, such as in a 90% enantiomeric excess, a 95% enantiomeric excess, a 97% enantiomeric excess or even in greater than a 99% enantiomeric excess, such as in enantiopure form.

The presently disclosed agents can have at least one asymmetric center or geometric center, cis-trans center(C=C, C=N). All chiral, diastomeric, racemic, meso, rotational and geometric isomers of the structures are intended unless otherwise specified. The compounds can be isolated as a single isomer or as mixture of isomers. All tautomers of the compounds are also considered part of the disclosure. The presently disclosed compounds also includes all isotopes of atoms present in the compounds, which can include, but are not limited to, deuterium, tritium, ¹⁸F, etc.

While the generation of ROS in small amounts is a typical byproduct of the cellular respiration pathway, certain conditions, including a disease or other medical condition, may occur in the patient when the amount of ROS is excessive to the point where natural enzyme mechanisms cannot scavenge the amount of ROS being produced. Therefore, compounds, compositions and methods that scavenge reactive oxygen species that are present within the mitochondrial membrane of the cell are useful and are provided herein. These compounds, compositions and methods have the utility of being able to scavenge an excess amount of ROS being produced that naturally occurring enzymes SOD and catalase, among others, cannot cope with.

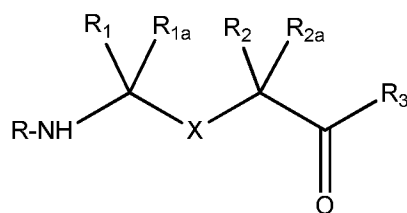
Compounds and compositions described herein have use in the prophylaxis and treatment of exposure to ionizing radiation, in anti-ageing therapies and, generally, in treating conditions that benefit from antioxidant treatment. Examples of these compounds are provided below and in the claims. For example, the effective mitochondrial concentration of mitochondria targeted conjugated nitroxides (-N-O, -N-OH or N=O containing compounds and groups) against γ -irradiation could be increased up to 1,000 times (and their required tissues concentrations can be reduced 1,000 times from 10 mM to 10 μ M) compared with parent non-conjugated nitroxides. Enrichment in mitochondria of mitochondria targeted nitroxides has been demonstrated by EPR spectroscopy as well as by MS analysis of their content in mitochondria obtained from cells incubated with mitochondria targeted nitroxides. Delivery of mitochondria targeted-nitroxides into mitochondria does not depend on the mitochondrial membrane potential. Therefore, mitochondria targeted nitroxides can accumulate not only in intact but also in de-energized or damaged mitochondria with low membrane potential. Moreover, mitochondria targeted nitroxide conjugates are delivered into mitochondria without affecting the mitochondrial membrane potential. Hence, they do not impair the major mitochondrial function, the energy production, in cells. In addition, the conjugated nitroxides provide a new important feature, post irradiation protection.

Like other nitroxides, conjugated mitochondria targeted nitroxides might potentially lower blood pressure and sympathetic nerve activity. However, the dramatically reduced dose of mitochondria targeted nitroxides (about 1,000-fold), compared to non-conjugated parental nitroxides, may be significantly below of those inducing side effects.

XJB-5-131 (XJB), a reactive oxygen species (ROS) scavenger also partially prevented evoked DA loss near probes for 4 hours by improving overall tissue health. This particular ROS scavenger is unique in that it targets the inner mitochondrial membrane and contains a potent nitroxide group responsible for electron and radical scavenging. The neuroprotective qualities of XJB make ROS's good candidates for pharmacologically enhanced microdialysis.

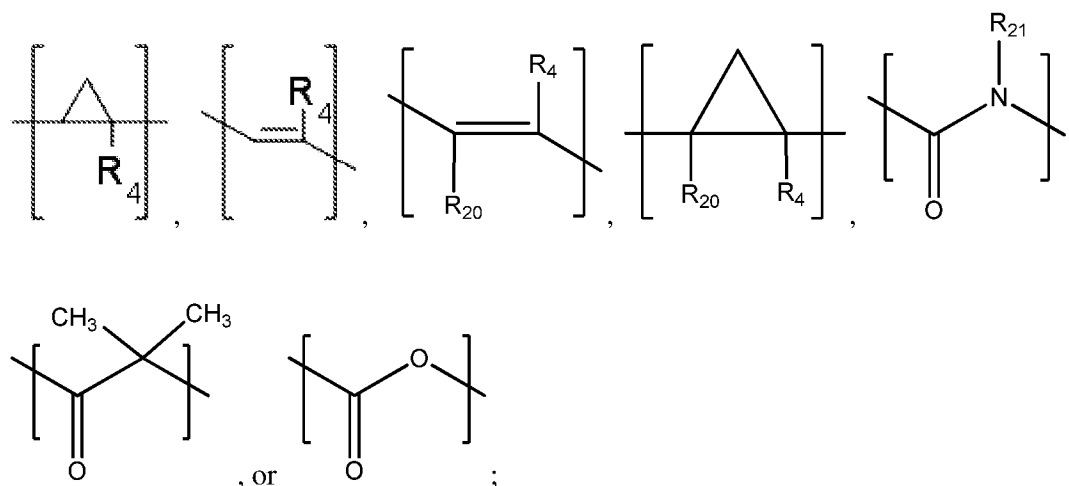
Disclosed herein are the effects of two non-steroidal anti-inflammatories, ibuprofen (IBU) and pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid tetrasodium salt (PPads) and two ROS scavengers, JP4-039 (JP4) and JRS527 (JRS) on the preservation of evoked DA responses near microdialysis probes. As the effects of both DEX and XJB were profound after only 4 hours, this timeframe was used for the present study. Carbon fiber microelectrodes were coupled with fast scan cyclic voltammetry (FSCV) for detection of evoked DA release in tissue near probes perfused separately with each pharmacological agent.

Disclosed herein are compounds, or pharmaceutically acceptable salts thereof, having a structure of:



Formula 1

wherein X is



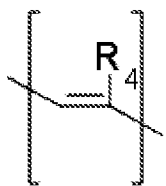
R_1 , R_{1a} , R_2 , R_{2a} , R_4 , R_{20} and R_{21} are each independently hydrogen, halo, or an optionally substituted alkyl;

R_3 is $-N(R_5)(R_{10})$, wherein R_5 and R_{10} are each independently H or an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group, provided at least one of R_5 is an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group; and

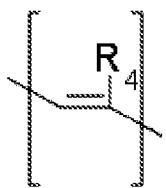
- 5 R is $-C(O)-R_6$, $-C(O)O-R_6$, or $-P(O)-(R_6)_2$, wherein R_6 is an optionally substituted alkyl or an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group, provided that if only one of R_5 or R_{10} is an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group then R_6 is an optionally substituted alkyl or an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group.

- 10 In certain embodiments, both R_5 and R_{10} are each an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group.

In certain embodiments, R_5 is an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group, R_{10} is H, and R_6 is an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group.



In certain embodiments, X is



In certain embodiments, X is , and R_4 is H.

- 15 In certain embodiments, R_{1a} , R_2 , and R_{2a} are each H.

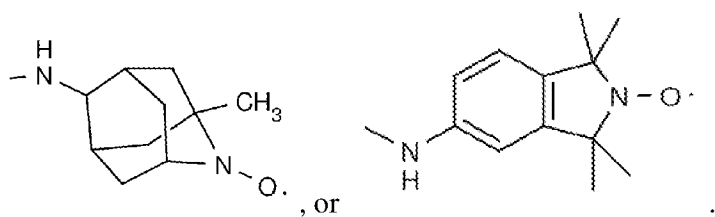
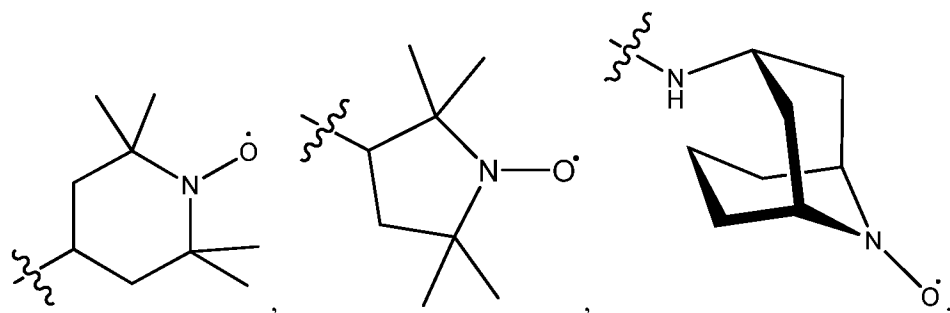
In certain embodiments, R_1 is alkyl, particularly C_1 - C_6 alkyl, most particularly propyl (e.g., isopropyl) or butyl (e.g., isobutyl).

In certain embodiments, R_{1a} and R_{2a} are H, and R_2 is aralkyl, particularly benzyl.

In certain embodiments, R_6 is alkyl or aralkyl, particularly isopropyl or benzyl.

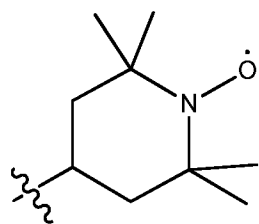
- 20 In certain embodiments, at least one of R_1 , R_{1a} , R_2 , R_{2a} , R_4 , R_{20} or R_{21} is C_1 - C_6 straight or branched-chain alkyl optionally substituted one or more phenyl ($-C_6H_5$) groups, and the phenyl group is optionally methyl-, ethyl-, hydroxyl- or fluoro-substituted.

In certain embodiments, the $-N-O\bullet$ containing group is selected from:

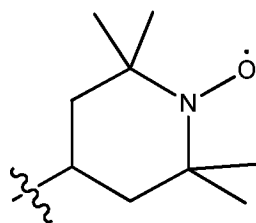


5

In certain embodiments, R_{10} is H and R_5 is

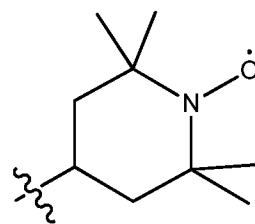


In certain embodiments, both R_5 and R_{10} are

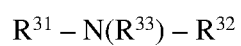


10

In certain embodiments, R_{10} is H, and R_5 and R_6 are both

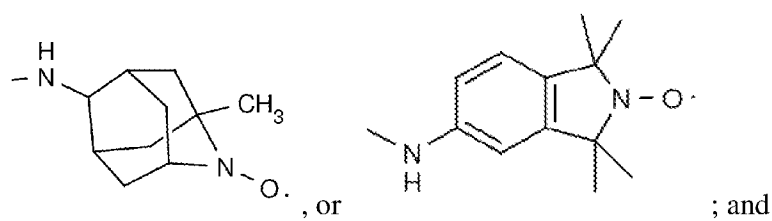
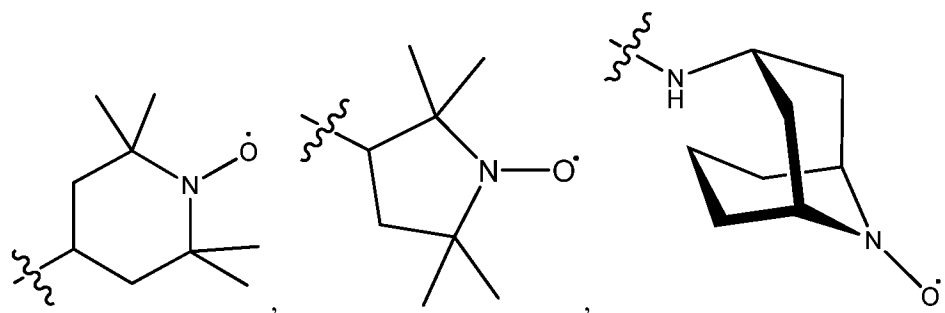


Also disclosed herein are compounds, or pharmaceutically acceptable salts thereof, having a structure of:



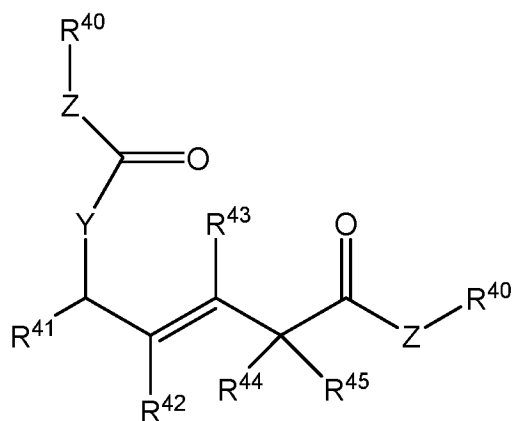
Formula 2

5 wherein R^{31} and R^{32} are each independently

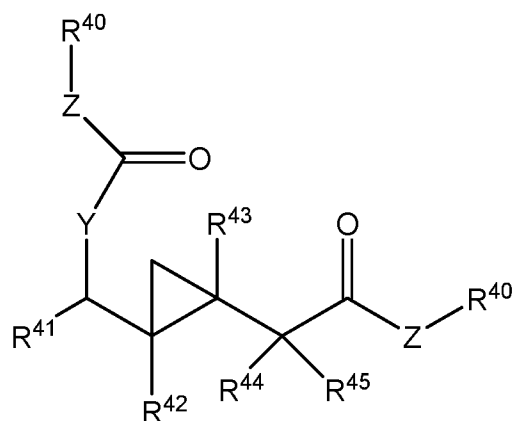


R^{33} is H or an optionally substituted alkyl.

Further disclosed herein are compounds, or pharmaceutically acceptable salts thereof,
 15 having a structure of:



Formula 3A ; or



Formula 3B

5

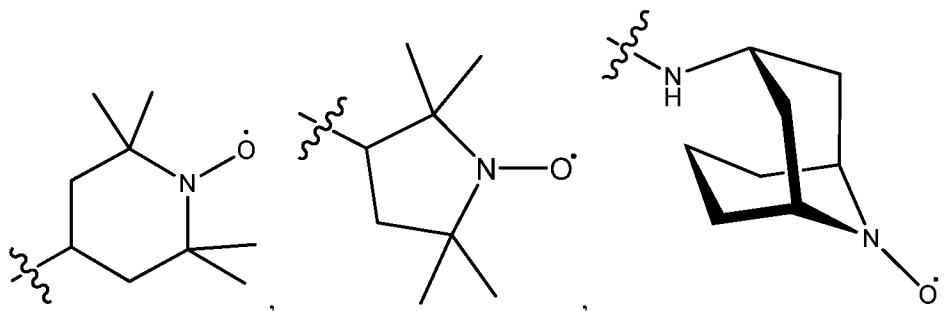
wherein Y is -NH-, -O-, -N(alkyl)-, -N(aryl)-, -N(cycloalkyl)-, -N(heteroaryl)-, or -CH(R⁴¹)-;

R⁴¹ is H, optionally substituted aliphatic, optionally substituted cycloalkyl, optionally substituted aryl, or optionally heteroaryl;

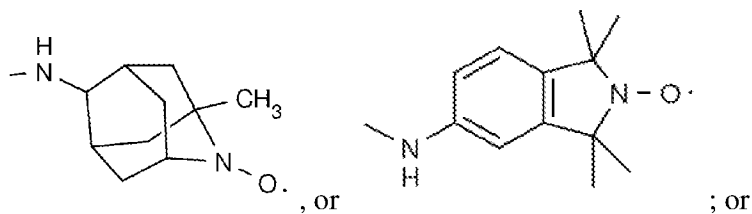
10 each of R⁴² - R⁴⁵ is H, optionally substituted aliphatic, optionally substituted cycloalkyl, optionally substituted aryl, optionally heteroaryl, or fluorine;

each Z is independently -O-, -NH- or -CH₂-; and

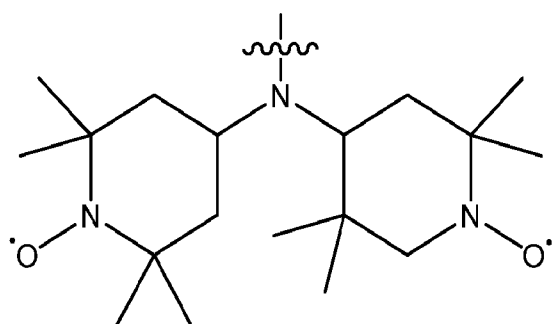
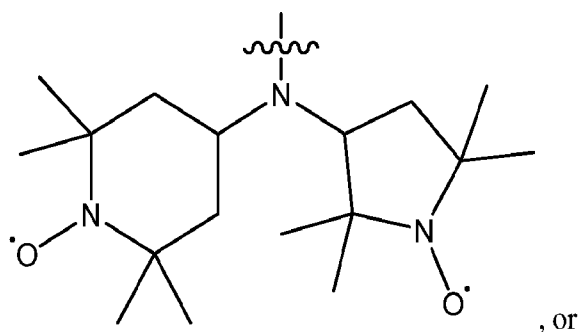
each R⁴⁰ is independently



15



each -ZR⁴⁰ is independently



5

In certain embodiments, each of R⁴¹-R⁴⁵ is H or (C₁-C₆)alkyl.

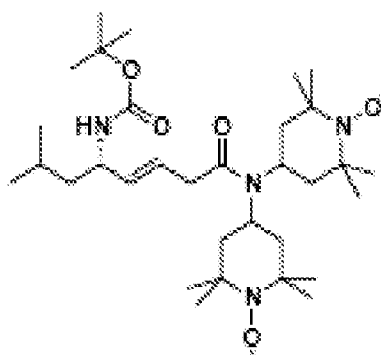
In certain embodiments, R⁴¹ is propyl or butyl, particularly isobutyl.

In certain embodiments, Y is -NH-.

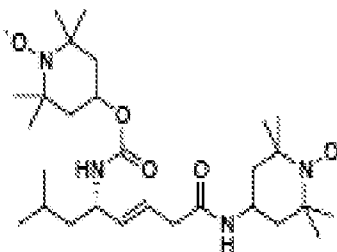
In certain embodiments, R⁴²-R⁴⁵ are each H.

10

Illustrative compounds are shown below:



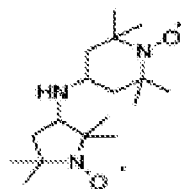
JR3527-44



JR5527-084



JR5527-33



JR5527-38

5

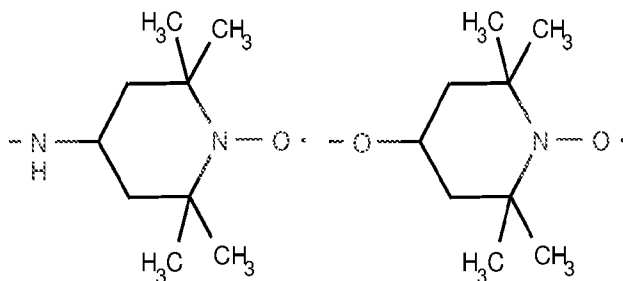
As indicated above, R₅ is an -N-O•, -N-OH or -N=O containing group (not -N-O•, -N-OH or -N=O, but groups containing those moieties, such as TEMPO, *etc.*, as described herein). It should be recognized that the nitroxides (-N-O•) disclosed herein may undergo reduction *in vivo* to their corresponding hydroxylamines (-N-OH) and/or may undergo oxidation *in vivo* to their corresponding nitrosonium ions. All three forms may be in equilibrium and their ratio depends on the redox environment. Any one of the three forms, or a mixture thereof, could be administered to a subject.

10

As is known to one ordinarily skilled in the art, nitroxide and nitroxide derivatives, including TEMPOL and associated TEMPO derivatives are stable radicals that can withstand biological environments. Therefore, the presence of the 4-amino-TEMPO, TEMPOL or another nitroxide "payload" within the mitochondria membrane can serve as an effective and efficient

15

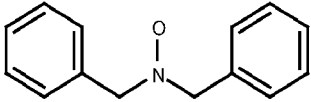
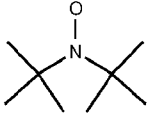
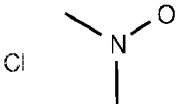
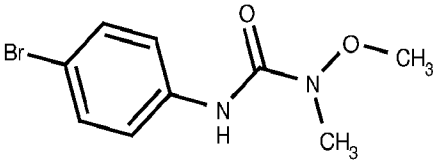
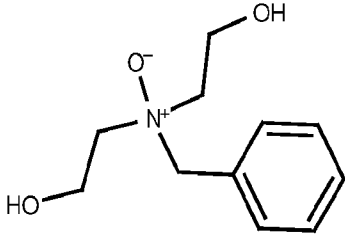
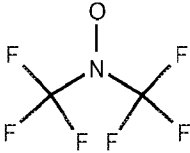
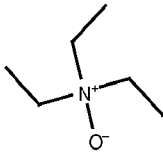
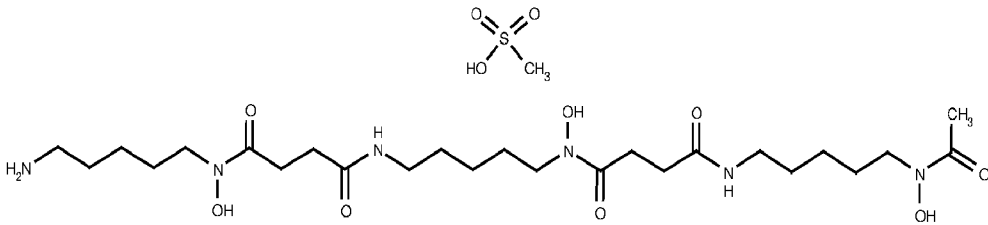
electron scavenger of the ROS being produced within the membrane. Non-limiting examples of this include TEMPO (2,2,6,6-Tetramethyl-4-piperidine 1-oxyl) and TEMPOL (4-Hydroxy-TEMPO), in which, when incorporated into the compound described herein, form, for example, when R₃ is -NH-R₅, -O-R₅:

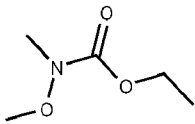
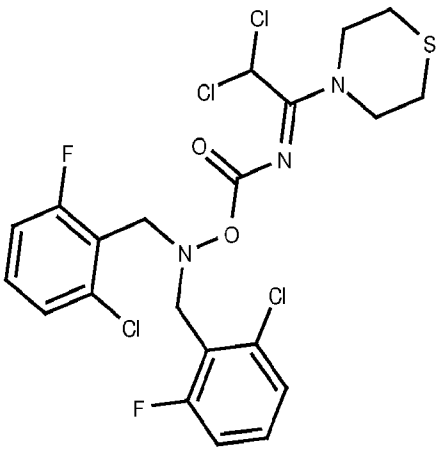
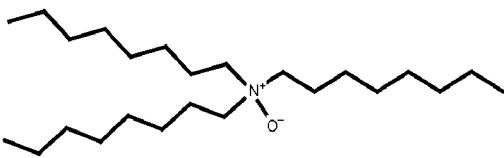
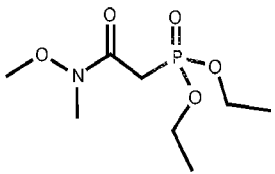
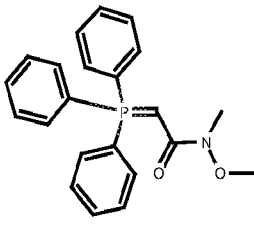


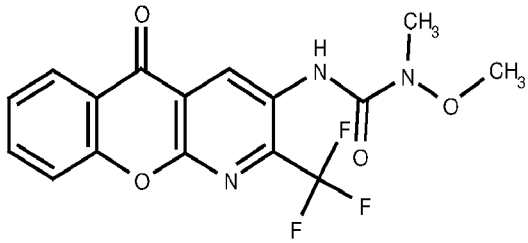
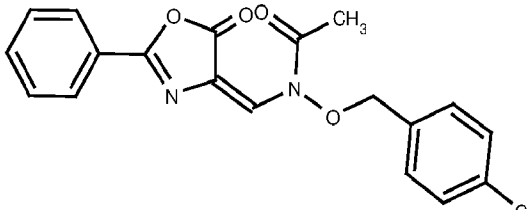
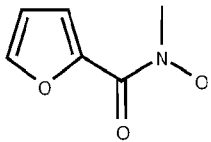
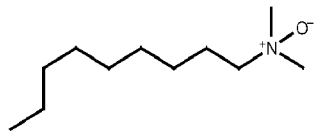
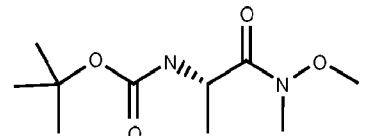
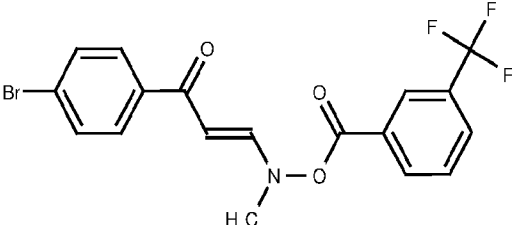
Additional non-limiting examples of -N-O•, -N-OH or N=O containing group are provided in Table 1 (from Jiang, J., *et al.* "Structural Requirements for Optimized Delivery, Inhibition of Oxidative Stress, and Antiapoptotic Activity of Targeted Nitroxides", J Pharmacol Exp Therap. 2007, 320(3):1050-60). A person of ordinary skill in the art would be able to conjugate (covalently attach) any of these compounds to the rest of the compound using common linkers and/or conjugation chemistries, such as the chemistries described herein. Table 1 provides a non-limiting excerpt from a list of over 300 identified commercially-available -N-O•, -N-OH or N=O containing compounds that may be useful in preparation of the compounds or compositions described herein.

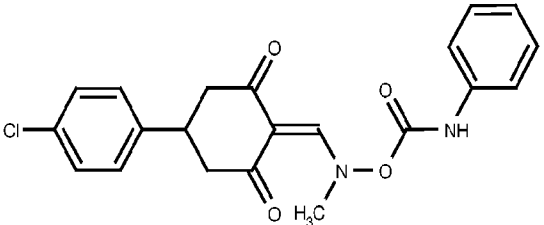
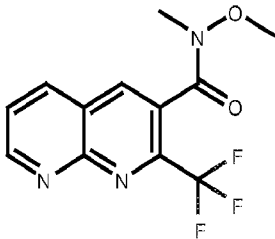
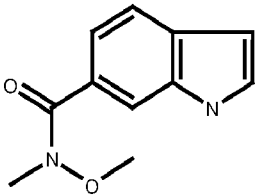
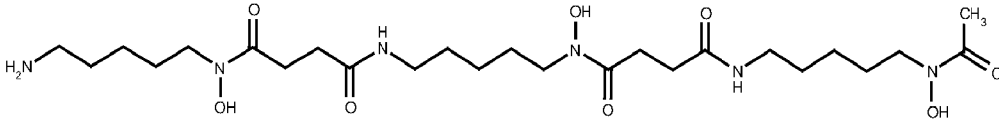
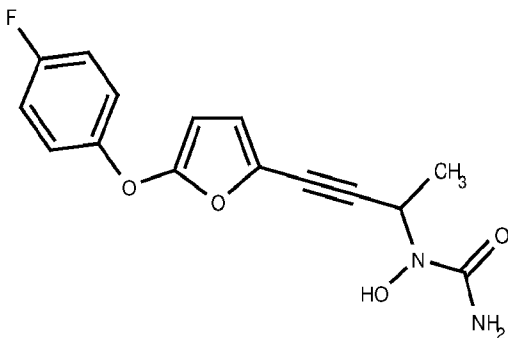
Table 1 - Commercially-available -N-O•, -N-OH or N=O containing groups

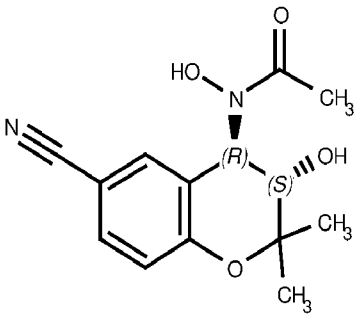
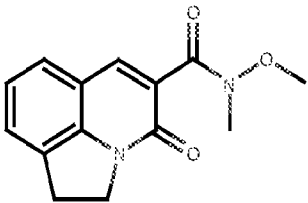
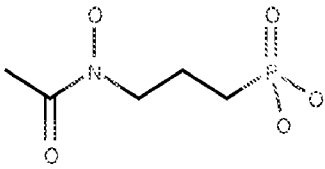
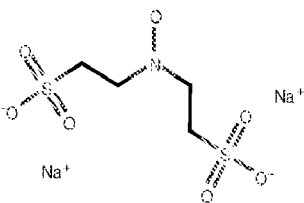
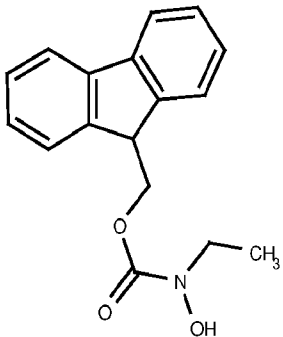
Structure	Name	CAS No.
	Trimethylamine N-Oxide	1184-78-7
	N,N-Dimethyldodecylamine N-Oxide	1643-20-5 70592-80-2
	N-Benzoyl-N-Phenylhydroxylamine	304-88-1
	N,N-Diethylhydroxylamine	3710-84-7

	N,N-Dibenzylhydroxylamine	14165-27-6 621-07-8
	Di-Tert-Butyl Nitroxide	2406-25-9
	N,N-Dimethylhydroxylamine Hydrochloride	16645-06-0
	Metobromuron	3060-89-7
	Benzyl-Di-Beta-Hydroxy Ethylamine-N-Oxide	
	Bis(Trifluoromethyl)Nitroxide	2154-71-4
	Triethylamine N-Oxide	2687-45-8
		

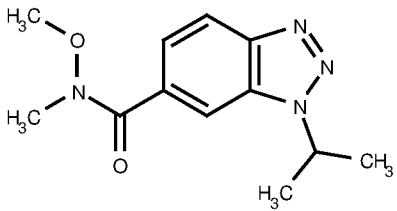
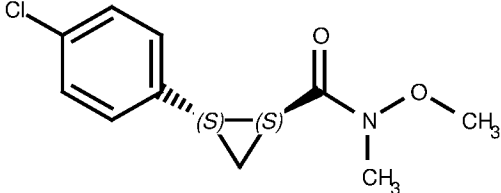
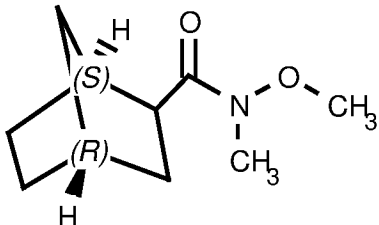
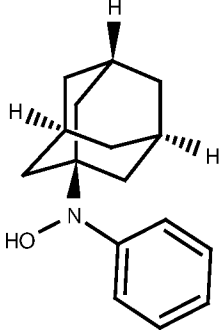
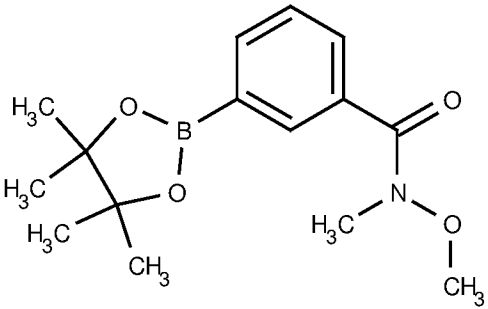
	N-Methoxy-N-Methylcarbamate	6919-62-6
	N,N-BIS(2-CHLORO-6-FLUOROBENZYL)-N-([2,2-DICHLORO-1-(1,4-THIAZINAN-4-YL+)ETHYLIDENE]AMINO)CARBONYLOXY]AMINE	
	Tri-N-Octylamine N-Oxide	13103-04-3
	DIETHYL (N-METHOXY-N-METHYLCARBAMOYLMETHYL)PHOSPHONATE	124931-12-0
	N-Methoxy-N-Methyl-2-(Triphenylphosphorylidene)Acetamide	129986-67-0

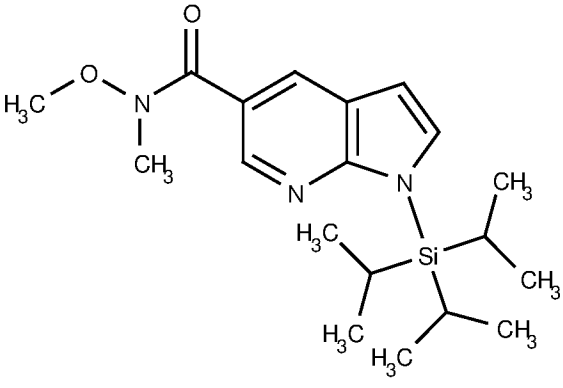
	N-Methoxy-N-Methyl-N'-[5-Oxo-2-(Trifluoromethyl)-5h-Chromeno[2,3-B]Pyridin-3-yl]Urea	
	N-[(4-Chlorobenzyl)Oxy]-N-([5-Oxo-2-Phenyl-1,3-Oxazol-4(5h)-Yliden]Methyl)Acetamide	
	N-Methylfurohydroxamic Acid	109531-96-6
	N,N-Dimethylnonylamine N-Oxide	2536-13-2
	N-(Tert-Butoxycarbonyl)-L-Alanine N'-Methoxy-N'-Methylamide	87694-49-3
	1-(4-Bromophenyl)-3-(Methyl([3-(Trifluoromethyl)Benzoyl]Oxy)Amino)-2-Propen-1-One	

	2- (((Anilinocarbonyl)Oxy)(Methyl)Amino]Methylene)-5-(4-Chlorophenyl)-1,3+ - Cyclohexanedione	
	N-Methoxy-N-Methyl-2-(Trifluoromethyl)-1,8-Naphthyridine-3-Carboxamide	
	N-Methoxy-N-Methyl-Indole-6-Carboxamide	
 Desferrioxamin		
	AKOS 91254	127408-31-5

	N-[(3s,4r)-6-Cyano-3,4-Dihydro-3-Hydroxy-2,2-Dimethyl-2h-1-Benzopyran-4-Y+L]-N-Hydroxyacetamide	127408-31-5
	N-Methoxy-N-Methyl-1,2-Dihydro-4-Oxo-Pyrrolo[3,2,1-Ij]Quinoline-5-Carboxamide	
	Fr-900098	
	2,2'-(Hydroxyimino)Bis(s-Ethanesulfonic Acid Disodium Salt	133986-51-3
	Fmoc-N-Ethyl-Hydroxylamine	

	Bis(N,N-Dimethylhydroxamido)Hydroxooxovanadate	
	Pyraclostrobin	175013-18-0
	1-Boc-5-Chloro-3-(Methoxy-Methyl-Carbamoyl)Indazole	
	N-Methoxy-N-Methyl-Thiazole-2-Carboxamide	
	4,4-Difluoro-N-Methyl-N-Methoxy-L-Prolinamide Hcl	
	3-Fluoro-4-(Methoxy(Methyl)Carbamoyl)Phenylboronic Acid	913835-59-3

	1-Isopropyl-N-Methoxy-N-Methyl-1h-Benzo[D][1,2,3]Triazole-6-Carboxamide	467235-06-9
	(Trans)-2-(4-Chlorophenyl)-N-Methoxy-N-Methylcyclopropanecarboxamide	
	Bicyclo[2.2.1]Heptane-2-Carboxylic Acid Methoxy-Methyl-Amide	
	Akos Bc-0582	
	3-(N,O-Dimethylhydroxylaminocarbonyl)Phenylboronic Acid, Pinacol Ester	

	1- Triisopropylsilanyl -1h-Pyrrolo[2,3- B]Pyridine-5- Carboxylic Acid Methoxy+ -Methyl-Amide	
---	--	--

The compounds described above can be synthesized by any useful method.

Methods of Use and Pharmaceutical Compositions

- 5 In a therapeutic embodiment, a method of scavenging free-radicals in a subject (e.g., a patient in need of treatment with a free-radical scavenger) is provided, comprising administering to the subject an amount of one or more compound described herein and having a free-radical scavenging group, such as a nitroxide-containing group effective to scavenge free radicals. As described above, a number of diseases, conditions or injuries can be ameliorated or otherwise
- 10 treated or prevented by administration of free radical scavenging compounds, such as those described herein.

Any agent or agents used for prevention, mitigation or treatment in a subject of injury caused by radiation exposure may be administered in an amount effective to prevent, mitigate or treat such injury, namely in an amount and in a dosage regimen effective to prevent injury or to

15 reduce the duration and/or severity of the injury resulting from radiation exposure. According to one non-limiting embodiment, an effective dose ranges from 0.1 or 1 mg/kg to 100 mg/kg, including any increment or range therebetween, including 1 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, 25 mg/kg, 50 mg/kg, and 75 mg/kg. However, for each compound described herein, an effective dose or dose range is expected to vary from that of other compounds described herein for any

20 number of reasons, including the molecular weight of the compound, bioavailability, specific activity, etc. The therapeutic window between the minimally-effective dose, and maximum tolerable dose in a subject can be determined empirically by a person of skill in the art, with end points being determinable by in vitro and in vivo assays, such as those described herein and/or are acceptable in the pharmaceutical and medical arts for obtaining such information regarding

25 radioprotective agents. Different concentrations of the agents described herein are expected to achieve similar results, with the drug product administered, for example and without limitation,

once prior to an expected radiation dose, such as prior to radiation therapy or diagnostic exposure to ionizing radiation, during exposure to radiation, or after exposure in any effective dosage regimen. The compounds can be administered continuously, such as intravenously, one or more times daily, once every two, three, four, five or more days, weekly, monthly, etc., including increments
5 therebetween. A person of ordinary skill in the pharmaceutical and medical arts will appreciate that it will be a matter of simple design choice and optimization to identify a suitable dosage regimen for prevention, mitigation or treatment of injury due to exposure to radiation.

The compounds described herein also are useful in preventing, mitigating (to make less severe) and/or treating injury caused by radiation exposure. By radiation, in the context of this
10 disclosure, it is meant types of radiation that result in the generation of free radicals, e.g., reactive oxygen species (ROS), as described herein. The free radicals are produced, for example and without limitation, by direct action of the radiation, as a physiological response to the radiation and/or as a consequence of damage/injury caused by the radiation. In one embodiment, the radiation is ionizing radiation. Ionizing radiation consists of highly-energetic particles or waves that
15 can detach (ionize) at least one electron from an atom or molecule. Examples of ionizing radiation are energetic beta particles, neutrons, and alpha particles. The ability of light waves (photons) to ionize an atom or molecule varies across the electromagnetic spectrum. X-rays and gamma rays can ionize almost any molecule or atom; far ultraviolet light can ionize many atoms and molecules; near ultraviolet and visible light are ionizing to very few molecules. Microwaves and radio waves
20 typically are considered to be non-ionizing radiation, though damage caused by, e.g., microwaves, may result in the production of free-radicals as part of the injury and/or physiological response to the injury.

The compounds typically are administered in an amount and dosage regimen to prevent, mitigate or treat the effects of exposure of a subject to radiation. The compounds may be
25 administered in any manner that is effective to treat, mitigate or prevent damage caused by the radiation. Examples of delivery routes include, without limitation: topical, for example, epicutaneous, inhalational, enema, ocular, otic and intranasal delivery; enteral, for example, orally, by gastric feeding tube and rectally; and parenteral, such as, intravenous, intraarterial, intramuscular, intracardiac, subcutaneous, intraosseous, intradermal, intrathecal, intraperitoneal,
30 transdermal, iontophoretic, transmucosal, epidural and intravitreal, with oral, intravenous, intramuscular and transdermal approaches being preferred in many instances.

In certain embodiments, the compounds disclosed herein may be useful for treating neurodegenerative disorders such as, for example, Alzheimer's disease, ataxia telangiectasia, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease.

In some embodiments, one or more of the disclosed compounds are mixed or combined with a suitable pharmaceutically acceptable carrier to prepare a pharmaceutical composition. Pharmaceutical carriers or vehicles suitable for administration of the compounds provided herein include any such carriers known to be suitable for the particular mode of administration.

5 *Remington: The Science and Practice of Pharmacy*, The University of the Sciences in Philadelphia, Editor, Lippincott, Williams, & Wilkins, Philadelphia, PA, 21st Edition (2005), describes exemplary compositions and formulations suitable for pharmaceutical delivery of the compounds disclosed herein. In addition, the compounds may be formulated as the sole pharmaceutically active ingredient in the composition or may be combined with other active ingredients.

10 Upon mixing or addition of the compound(s) to a pharmaceutically acceptable carrier, the resulting mixture may be a solution, suspension, emulsion, or the like. Liposomal suspensions may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the
15 compound in the selected carrier or vehicle. Where the compounds exhibit insufficient solubility, methods for solubilizing may be used. Such methods are known and include, but are not limited to, using cosolvents such as dimethylsulfoxide (DMSO), using surfactants such as Tween®, and dissolution in aqueous sodium bicarbonate. Derivatives of the compounds, such as salts or prodrugs may also be used in formulating effective pharmaceutical compositions. The disclosed
20 compounds may also be prepared with carriers that protect them against rapid elimination from the body, such as time-release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, microencapsulated delivery systems.

The disclosed compounds and/or compositions can be enclosed in multiple or single dose containers. The compounds and/or compositions can also be provided in kits, for example,
25 including component parts that can be assembled for use. For example, one or more of the disclosed compounds may be provided in a lyophilized form and a suitable diluent may be provided as separated components for combination prior to use. In some examples, a kit may include a disclosed compound and a second therapeutic agent for co-administration. The compound and second therapeutic agent may be provided as separate component parts. A kit may include a
30 plurality of containers, each container holding one or more unit dose of the compound. The containers are preferably adapted for the desired mode of administration, including, but not limited to tablets, gel capsules, sustained-release capsules, and the like for oral administration; depot products, pre-filled syringes, ampoules, vials, and the like for parenteral administration; and patches, medipads, creams, and the like for topical administration.

The active compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the subject treated. A therapeutically effective concentration may be determined empirically by testing the compounds in known *in vitro* and *in vivo* model systems for the treated disorder. In some examples, a therapeutically effective amount of the compound is an amount that lessens or ameliorates at least one symptom of the disorder for which the compound is administered. Typically, the compositions are formulated for single dosage administration. The concentration of active compound in the drug composition will depend on absorption, inactivation, and excretion rates of the active compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art.

In some examples, about 0.1 mg to 1000 mg of a disclosed compound, a mixture of such compounds, or a physiologically acceptable salt or ester thereof, is compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in a unit dosage form. The amount of active substance in those compositions or preparations is such that a suitable dosage in the range indicated is obtained. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. In some examples, the compositions are formulated in a unit dosage form, each dosage containing from about 1 mg to about 1000 mg (for example, about 2 mg to about 500 mg, about 5 mg to 50 mg, about 10 mg to 100 mg, or about 25 mg to 75 mg) of the one or more compounds. In other examples, the unit dosage form includes about 0.1 mg, about 1 mg, about 5 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, or more of the disclosed compound(s).

The disclosed compounds or compositions may be administered as a single dose, or may be divided into a number of smaller doses to be administered at intervals of time. The therapeutic compositions can be administered in a single dose delivery, by continuous delivery over an extended time period, in a repeated administration protocol (for example, by a multi-daily, daily, weekly, or monthly repeated administration protocol). It is understood that the precise dosage, timing, and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test data. It is to be noted that concentrations and dosage values may also vary with the severity of the condition

to be alleviated. In addition, it is understood that for a specific subject, dosage regimens may be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only.

5 When administered orally as a suspension, these compositions are prepared according to techniques well known in the art of pharmaceutical formulation and may contain microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners/flavoring agents. As immediate release tablets, these compositions may contain microcrystalline cellulose, dicalcium phosphate, starch, magnesium
10 stearate and lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants. If oral administration is desired, the compound is typically provided in a composition that protects it from the acidic environment of the stomach. For example, the composition can be formulated in an enteric coating that maintains its integrity in the stomach and releases the active compound in the intestine. The composition may also be formulated in combination with an
15 antacid or other such ingredient.

 Oral compositions will generally include an inert diluent or an edible carrier and may be compressed into tablets or enclosed in gelatin capsules. For the purpose of oral therapeutic administration, the active compound or compounds can be incorporated with excipients and used in the form of tablets, capsules, or troches. Pharmaceutically compatible binding agents and adjuvant
20 materials can be included as part of the composition. The tablets, pills, capsules, troches, and the like can contain any of the following ingredients or compounds of a similar nature: a binder such as, but not limited to, gum tragacanth, acacia, corn starch, or gelatin; an excipient such as microcrystalline cellulose, starch, or lactose; a disintegrating agent such as, but not limited to, alginic acid and corn starch; a lubricant such as, but not limited to, magnesium stearate; a gildant,
25 such as, but not limited to, colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; and a flavoring agent such as peppermint, methyl salicylate, or fruit flavoring.

 When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials, which modify the physical form of the dosage unit, for example, coatings of sugar and
30 other enteric agents. The compounds can also be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings, and flavors.

When administered orally, the compounds can be administered in usual dosage forms for oral administration. These dosage forms include the usual solid unit dosage forms of tablets and capsules as well as liquid dosage forms such as solutions, suspensions, and elixirs. When the solid dosage forms are used, it is preferred that they be of the sustained release type so that the compounds need to be administered only once or twice daily. In some examples, an oral dosage form is administered to the subject 1, 2, 3, 4, or more times daily. In certain examples, the oral dosage is from about 1 mg/day to about 500 mg/day, about 2 mg/day to about 200 mg/day, or about 5 mg/day to about 50 mg/day. It is understood that while a subject may be started at one dose, that dose may be varied over time as the subject's condition changes.

In additional examples, the compounds can be administered orally to humans in a dosage range of 1 to 1000 mg/kg body weight in single or divided doses. One illustrative dosage range is 0.1 to 200 mg/kg body weight orally (such as 0.5 to 100 mg/kg body weight orally) in single or divided doses. For oral administration, the compositions may be provided in the form of tablets containing about 1 to 1000 milligrams of the active ingredient, particularly 1, 5, 10, 15, 20, 25, 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 750, 800, 900, or 1000 milligrams of the active ingredient. It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

Injectable solutions or suspensions may also be formulated, using suitable non-toxic, parenterally-acceptable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution or isotonic sodium chloride solution, or suitable dispersing or wetting and suspending agents, such as sterile, bland, fixed oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid. Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include any of the following components: a sterile diluent such as water for injection, saline solution, fixed oil, a naturally occurring vegetable oil such as sesame oil, coconut oil, peanut oil, cottonseed oil, and the like, or a synthetic fatty vehicle such as ethyl oleate, and the like, polyethylene glycol, glycerine, propylene glycol, or other synthetic solvent; antimicrobial agents such as benzyl alcohol and methyl parabens; antioxidants such as ascorbic acid and sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates, and phosphates; and agents for the adjustment of tonicity such as sodium chloride and dextrose. Parenteral preparations can be enclosed in ampoules, disposable syringes, or multiple

dose vials made of glass, plastic, or other suitable material. Buffers, preservatives, antioxidants, and the like can be incorporated as required.

Where administered intravenously, suitable carriers include physiological saline, phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents such as glucose, polyethylene glycol, polypropyleneglycol, and mixtures thereof. Liposomal suspensions including tissue-targeted liposomes may also be suitable as pharmaceutically acceptable carriers.

The compounds can be administered parenterally, for example, by IV, IM, depo-IM, SC, or depo-SC. When administered parenterally, a therapeutically effective amount of about 0.1 to about 500 mg/day (such as about 1 mg/day to about 100 mg/day, or about 5 mg/day to about 50 mg/day) may be delivered. When a depot formulation is used for injection once a month or once every two weeks, the dose may be about 0.1 mg/day to about 100 mg/day, or a monthly dose of from about 3 mg to about 3000 mg.

The compounds can also be administered sublingually. When given sublingually, the compounds should be given one to four times daily in the amounts described above for IM administration.

The compounds can also be administered intranasally. When given by this route, the appropriate dosage forms are a nasal spray or dry powder. The dosage of the compounds for intranasal administration is the amount described above for IM administration. When administered by nasal aerosol or inhalation, these compositions may be prepared according to techniques well known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents.

The compounds can be administered intrathecally. When given by this route, the appropriate dosage form can be a parenteral dosage form. The dosage of the compounds for intrathecal administration is the amount described above for IM administration.

The compounds can be administered topically. When given by this route, the appropriate dosage form is a cream, ointment, or patch. When administered topically, an illustrative dosage is from about 0.5 mg/day to about 200 mg/day. Because the amount that can be delivered by a patch is limited, two or more patches may be used.

The compounds can be administered rectally by suppository. When administered by suppository, an illustrative therapeutically effective amount may range from about 0.5 mg to about 500 mg. When rectally administered in the form of suppositories, these compositions may be prepared by mixing the drug with a suitable non-irritating excipient, such as cocoa butter, synthetic

glyceride esters of polyethylene glycols, which are solid at ordinary temperatures, but liquefy and/or dissolve in the rectal cavity to release the drug.

Examples

5 **Control Experiments.** In all of the following experiments evoked DA release was measured in the striatum during a 25 s stimulation of the MFB. Responses measured pre-probe implantation showed robust maximum evoked DA release ranging from 12-20 μ M (blue responses in Figure 2 and 4). As previously reported, microdialysis probes perfused with aCSF (no pharmacological agent) eliminated evoked DA release nearby (70-100 μ m away). To determine if any DA
10 terminals survived probe implantation, a competitive DA transporter inhibitor, nomifensine was administered by i.p. injection. Nomifensine revived evoked DA release near probes. The immediate response to stimulation proves that DA terminals survive directly next to the electrode, no further than 70 μ m from the probe.

15 **Anti-Inflammatory and ROS Scavenger Agents.** This study compares the effects of three anti-inflammatory agents and three ROS scavengers on preserving evoked DA release in tissue surrounding microdialysis probes. DEX and XJB have previously been reported on (Nesbitt et al. (2013) Pharmacological mitigation of tissue damage during brain microdialysis, *Anal Chem.* 85, 8173-8179), and are represented here for comparison purposes. The chemical structures of all pharmacological agents are represented in Figure 1. The top panel illustrates the
20 inflammatory agents and the bottom panel the ROS scavengers.

25 **IBU and Ppads Preserve Evoked DA Release Near Probes.** During an inflammatory event such as microdialysis probe implantation, cells up-regulate production of pro-inflammatory proteins causing activation of phospholipase A2. Through cyclooxygenase (COX) modification a cascade of inflammatory mediators are produced. Both DEX and IBU work to intervene at
30 different stages of the inflammatory cellular mechanism. DEX acts by down-regulating pro-inflammatory proteins in cells and up-regulating anti-inflammatory proteins produced by the cell. IBU is considered a COX-1 and COX-2 inhibitor thereby inhibiting COX modification and preventing inflammatory mediator signaling. The non-steroidal anti-inflammatory IBU, when perfused through microdialysis probes prevented complete loss of evoked DA release near
30 probes (Figure 2-middle panel, red). Administration of nomifensine greatly impacted maximum evoked DA release near probes, increasing it to nearly pre-probe implantation amplitude (Figure 2-middle panel, green). IBU had similar effects as DEX suggesting that DEX's effects on evoked DA release are due to both its anti-inflammatory actions, not steroidal actions on DA terminals.

PPads is a non-selective P2 purinergic antagonist that blocks P2Y receptors. PPads reduces inflammation in the brain by blocking purine receptors on microglia limiting their response. Microglia are immune cells that are the first to respond to injury in the central nervous system. In a study investigating the migration of microglia, PPads significantly decreased the number and motility of the microglia responding to the site of an injury. PPads perfused through microdialysis also preserved evoked DA release near probes (Figure 2-right panel, red). Nomifensine further increased evoked DA release but not as dramatically as DEX and IBU (Figure 2-right panel, green).

DEX, IBU, and PPads all significantly increased evoked DA release near probes (Figure 3).

DEX's effect was more significant than IBU and PPads, and this is most likely due to DEX's dual action to both decrease pro-inflammatory proteins and increase anti-inflammatory proteins. Post-nomifensine maximum evoked DA responses were subject to a one-way ANOVA. DEX, IBU, and PPads all significantly increased maximum evoked DA release, DEX and IBU to above 80% of the pre-probe response. These findings demonstrate the ability of anti-inflammatories to preserve DA release and uptake activity in tissue near microdialysis probes.

JP4 and JRS. Inflammation is known to induce mitochondrial dysfunction and dopaminergic neurodegeneration in the nigrostriatal system. XJB, a first generation ROS scavenger, infiltrates mitochondria and has been shown to be effective in neuroprotection. XJB prevents neuronal loss and preserves evoked DA near probes. A second generation isostere of XJB, the small molecule JP4, was developed offering an improved pharmacokinetic profile while maintaining its impressive ROS scavenging abilities. JP4 specifically targets mitochondria, similar to XJB and catalyzes the dismutation of superoxide radical anions and other reactive oxygen species. JP4 protective qualities mitigate radiation damage in blood cells in vitro and in vivo. JRS, a modified analog of JP4, represents the first bis-nitroxide in this series and is expected to increase potency (Figure 1).

JRS Preserve Evoked DA Release Near Probes. Similar to anti-inflammatory drugs, probes perfused with JP4 and JRS decreased but did not abolish evoked DA release near probes (Figure 4). JP4 and JRS all substantially increased the post-nomifensine response. Histograms representing ROS effect on evoked DA release are represented in Figure 5. XJB and JRS significantly increased evoked DA release near probes (Figure 5). All responses post-probe with JP4 gave a measurable DA signal however, this increase was not significantly greater than zero. JRS significantly increased evoked DA release compared to aCSF (Figure 5). One-way ANOVA comparing post-nomifensine responses with ROS scavengers and aCSF). Nomifensine increase

all ROS DA responses, but it is clear that JRS had the greatest effect on protective evoked DA near probes, which was expected.

Nomifensine Perfusion does not Impact DA Terminals 70-100 μ m Away. In a final study, nomifensine was administrated by perfusion through microdialysis probes instead of an i.p.

5 injection. Voltammetry near probes perfused with nomifensine was performed in two groups of rats (n=3 rats/group), one group with perfusion of aCSF plus 1 μ M nomifensine, and the other with perfusion of DEX plus 1 μ M nomifensine.

Post-probe implantation responses were recorded before nomifensine was added to the perfusion fluid and gave similar results to experiments performed previously. At probes perfused
10 with only aCSF DA was not detected (Figure 6a). At probes perfused with DEX, evoked DA was measured (Figure 6b). Note that the aCSF results are reported as current (Figure 6a and 6c) as DA was undetected (aka. results did not produce a corresponding DA cyclic voltammograms). Nomifensine was added to each perfusion fluid and evoked DA responses were monitored near probes every 20 minutes for at least 2 hours. Nomifensine had no effect on evoked DA responses
15 near probes whether DEX was present or not in the perfusion fluid.

Nomifensine, a competitive DA transporter inhibitor was unable to act on DA terminals 70-100 μ m away from the probe. However nomifensine perfusion through microdialysis is known to increase extracellular levels of DA within 10 minutes of administration. When measured by microdialysis probes, Nomifensine is a smaller molecule than DEX; therefore it would expect
20 to diffuse through brain tissue more efficiently than DEX. We have previously shown that DEX's effects on evoked responses are attributable to its anti-inflammatory actions, not direct actions on DA terminals. This evidence further supports our conclusion that DEX is acting on the tissue to improve cellular health during inflammation as opposed to DEX acting on DA terminals, specifically the DA transporter 70 μ m away.

It has previously shown that microdialysis probe implantation decreased evoked DA
25 release in tissue near probes. Through mitigation of the injury site with an anti-inflammatory steroid, DEX, and a novel ROS scavenger, XJB, DA activity is preserved. Herein it is shown that other non-steroidal anti-inflammatory agents and ROS scavengers that protect DA release and uptake near microdialysis probes. IBU, PPads, XJB and JRS significantly attenuate the loss of
30 DA activity in tissue near microdialysis probes. As we have previously shown long-term protection with DEX preserves evoked DA release measured in tissue near the probe and at the outlet of probes, these drugs present other options to improve chronic microdialysis.

The protective effects were only partial, not completely restoring evoked DA release to its pre-probe response amplitude. Combination (e.g., via co-administration) of these

pharmacologically agents may also further promote evoked DA release in probe induced tissue damage. Overall, pharmacological enhanced microdialysis provides new insight into acute mitigation of microdialysis probe penetration injury and has the potential to successfully mitigate chronic implantation for long-term in vivo monitoring of neurochemicals.

5

METHODS

Reagents and Solutions. Dexamethasone sodium phosphate (DEX: AAP

Pharmaceuticals LLC, Schaumburg, IL), ibuprofen (IBU: Sigma Aldrich, St. Louis, MO) pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid tetrasodium salt (PPads: Sigma Aldrich, 10 St. Louis, MO), were used as received from their suppliers. XJB-5-131 (XJB), and JP4-039 (JP4), were prepared as described by Wipf and co-workers. JRS527 (JRS) was prepared as described below. All solutions were prepared with ultrapure water (Nanopure, Barnstead, Dubuque, IA). Artificial cerebrospinal fluid (aCSF: 142 mM NaCl, 1.2 mM CaCl₂, 2.7 mM KCl, 1.0 mM MgCl₂, 2.0 mM NaH₂PO₄, pH 7.4) was used as the perfusion fluid for 15 microdialysis.

DEX, and PPads were diluted to 10 µM in aCSF. IBU, XJB, JP4, and JRS were dissolved in aCSF containing 1% DMSO (Sigma Aldrich, St. Louis, MO). Nomifensine (20 mg/kg, i.p., Sigma Aldrich, St. Louis, MO) was used as received. Nomifensine was dissolved in phosphate-buffered saline (PBS: 155 mM NaCl, 100 mM NaH₂PO₄, pH 7.40) for i.p. injections and 20 diluted to 1 µM in aCSF for microdialysis perfusion. Electrodes were pre-treated in isopropyl alcohol (Sigma Aldrich, St. Louis, MO) and decolorizing carbon (Fisher, Pittsburgh, PA). Post- calibration of electrodes was performed with DA (Sigma Aldrich, St. Louis, MO) standards prepared in N₂-purged aCSF.

Synthetic Procedure of JRS527. A solution of 4-hydroxy-TEMPO (67 mg, 0.38 mmol) in DMF 25 (700 µL) was treated with *N,N'*-disuccinimidyl carbonate (115 mg, 0.45 mmol) and pyridine (62 µL, 0.075 mmol) and the resulting mixture was heated at 40 °C for 14 h. The solution was cooled to room temperature and directly used for the next step.

To a solution of JP4-039 (160 mg, 0.377 mmol) in CH₂Cl₂ (3.5 mL) was added trifluoroacetic acid (565 µL, 7.53 mmol) at 0 °C. The reaction was stirred and warmed to room temperature for 3 30 h and the solvent was removed under reduced pressure. The crude material was dissolved in EtOAc, washed with 10% aq. Na₂CO₃ and the aqueous layers was extracted with further portions of EtOAc. The combined organic layer was dried (Na₂CO₃), filtered and the solvent was removed under reduced pressure. To the crude product dissolved in DMF (2.1 mL) and H₂O (700

μL) was then added K_3PO_4 (83 mg, 0.38 mmol) and freshly prepared TEMPO-succinimidyl carbonate. After stirring at rt for 16 h the mixture was diluted with water (20 mL) and EtOAc (10 mL). The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organics were washed with 5% aqueous LiCl, dried over MgSO_4 , and concentrated to dryness.

The residue was purified by flash column chromatography (SiO_2 , 40–80% EtOAc/hexanes) to provide JRS527-84 (110 mg, 56%) as a pale orange solid. HRMS (ESI) m/z calcd for $\text{C}_{28}\text{H}_{51}\text{N}_4\text{O}_5$ $[\text{M}+\text{H}]^+$ 523.3846, found 523.3854; IR (neat) 3310, 2972, 2934, 2871, 1713, 1649, 1528, 1465, 1363, 1316, 1239, 1178, 1117, 1085, 1049, 971, 776, 742, 685; m.p. 89–92 °C;

$[\alpha]_{\text{D}}^{20} +0.032$ ($c=0.5$)

Voltammetry and Microdialysis. Carbon fiber microelectrodes (7 μm in diameter, and 400 μm in length) were coupled with fast scan cyclic voltammetry for in vivo measurement of evoked DA. Concentric microdialysis probes (300 μm diameter, 4 mm length) were constructed in house as previously described with hollow fiber 13 kDa MWCO membranes (Spectra/Por RC, Spectrum Laboratories Inc., Ranco Dominguez, CA). The probe inlet tubing (PE-20, Becton Dickinson, Franklin Lakes, NJ) was connected to a syringe pump (Harvard Apparatus, Holliston, MA) at a rate of 0.610 $\mu\text{L}/\text{min}$. The outlet made of fused silica capillary was led to waste as the dialysate fluid was not analyzed in this experiment.

Voltammetry Next to Microdialysis Probes. All procedures involving animals were approved by the University of Pittsburgh's Animal Care and Use Committee. Male Sprague-Dawley rats (250–350g; Charles Rivers, Raleigh, NC) were anesthetized with isoflurane (2.5 % by volume) and placed in a stereotaxic frame. Voltammetry next to microdialysis probes was performed in six groups of rats (6 rats per perfusion fluid unless otherwise noted).

A carbon fiber electrode was implanted at a 5° angle into the striatum of each rat. A stimulating electrode was lowered towards the medial forebrain bundle (MFB) until maximum evoked DA was observed at the electrode (4.3 mm below bregma, 1.2 mm lateral to bregma, and 7.2–8.5 mm below dura). Electrical stimulation of the MFB was performed for 25 s at 45 Hz (waveform: biphasic, square, constant current, 300 μA) and evoked DA release was measured in the striatum in twenty minute intervals.

Once consistent evoked DA responses were observed, a microdialysis probe was slowly (over 30 minutes) implanted next to the electrode (0.7 mm above bregma, 2.5 mm lateral from bregma, and 7 mm below dura). In its final position, the microdialysis probe was 70 μm from the tip of the carbon fiber, and 100 μm from the base of the electrode. The probe was left to

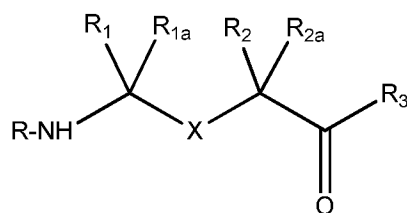
perfuse in the brain for 2 hours. Following this, three more stimulus responses were recorded. Finally, nomifensine was administered (20 mg/kg i.p.) and one more stimulus response was collected.

Data Analysis. For comparison, evoked DA responses near probes perfused with only aCSF (no pharmacological agent) were used as the control. These results have previously been reported in Nesbitt et al 2013. For statistical analysis, maximum DA release was normalized with respect to the maximum amplitude of the responses recorded before each probe was implanted and presented in histograms (Figure 3 and 4). As in the case of probe perfusion with aCSF, DA was undetected after probe implantation but before nomifensine, a one-sample t-test was performed comparing DEX, IBU, PPads, XJB, JP4, and JRS to zero. Statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) 22 software. Details of statistical test of in the figure captions.

In view of the many possible embodiments to which the principles of the disclosed compounds, compositions, and methods may be applied, it should be recognized that the illustrated embodiments are only preferred examples of the invention and should not be taken as limiting the scope of the invention.

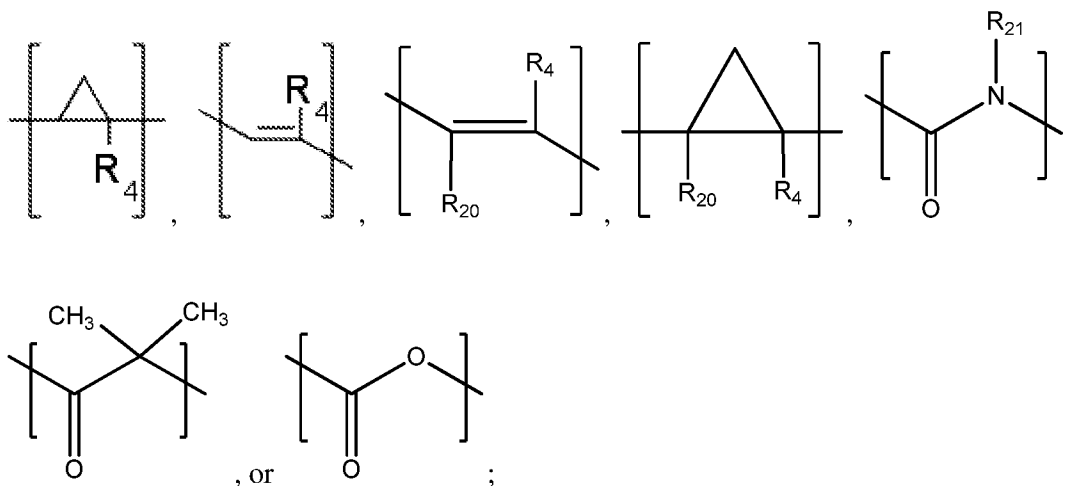
What is claimed is:

1. A compound, or a pharmaceutically acceptable salt thereof, having a structure of:



Formula 1

wherein X is

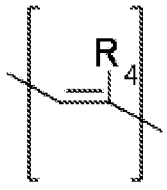


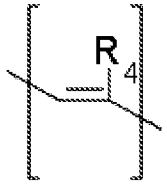
R_1 , R_{1a} , R_2 , R_{2a} , R_4 , R_{20} and R_{21} are each independently hydrogen, halo, or an optionally substituted alkyl;

R_3 is $-N(R_5)(R_{10})$, wherein R_5 and R_{10} are each independently H or an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group, provided at least one of R_5 is an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group; and R is $-C(O)-R_6$, $-C(O)O-R_6$, or $-P(O)-(R_6)_2$, wherein R_6 is an optionally substituted alkyl or an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group, provided that if only one of R_5 or R_{10} is an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group then R_6 is an optionally substituted alkyl or an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group.

2. The compound of claim 1, wherein both R_5 and R_{10} are each an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group.

3. The compound of claim 1, wherein R_5 is an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group, R_{10} is H, and R_6 is an $-N-O\bullet$, $-N-OH$ or $-N=O$ containing group.

5 4. The compound of any one of claims 1 to 3, wherein X is .

5. The compound of any one of claims 1 to 3, wherein X is , and R_4 is H.

6. The compound of any one of claims 1 to 5, wherein R_{1a} , R_2 , and R_{2a} are each H.

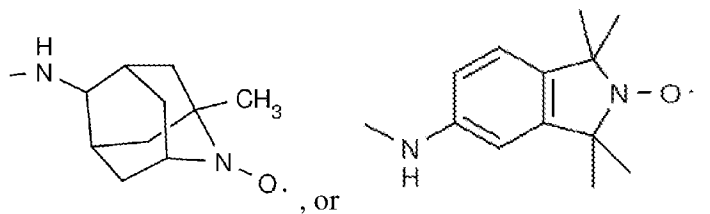
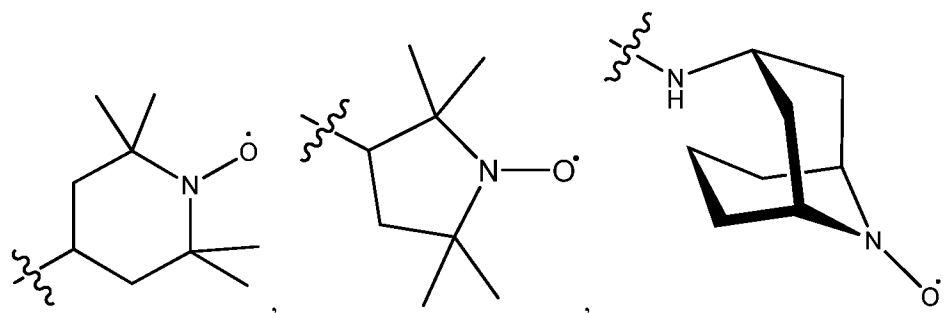
10 7. The compound of any one of claims 1 to 6, wherein R_1 is alkyl.

8. The compound of any one of claims 1 to 5 or 7, wherein R_{1a} and R_{2a} are H, and R_2 is aralkyl.

15 9. The compound of any one of claims 1 to 8, wherein R_6 is alkyl or aralkyl.

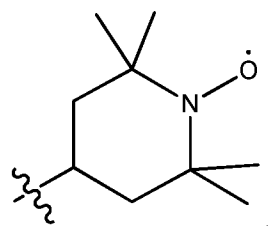
10. The compound of claim 1, wherein at least one of R_1 , R_{1a} , R_2 , R_{2a} , R_4 , R_{20} or R_{21} is C_1 - C_6 straight or branched-chain alkyl optionally substituted one or more phenyl ($-C_6H_5$) groups, and the phenyl group is optionally methyl-, ethyl-, hydroxyl- or fluoro-substituted.

20 11. The compound of any one of claims 1 to 10, wherein the $-N-O\bullet$ containing group is selected from:

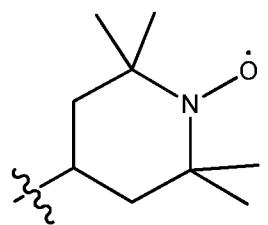


5

12. The compound of any one of claims 1 to 11, wherein R_{10} is H and R_5 is

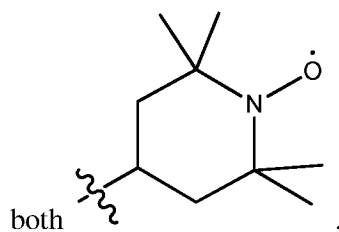


13. The compound of any one of claims 1 to 11, wherein both R_5 and R_{10} are

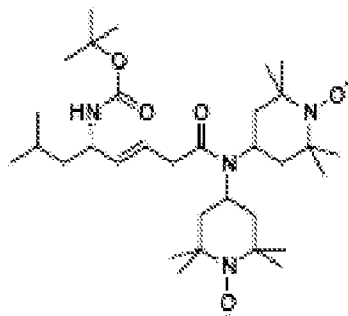


10

14. The compound of any one of claims 1 to 11, wherein R_{10} is H, and R_5 and R_6 are

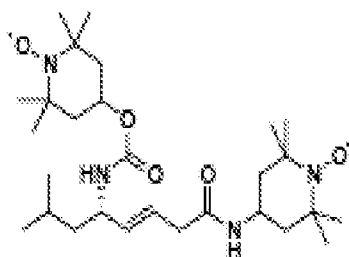


15. The compound of claim 1, wherein the compound is



JR5527-44

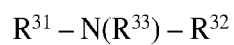
or



JR5527-064

5

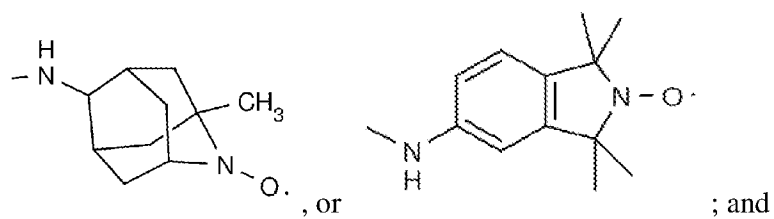
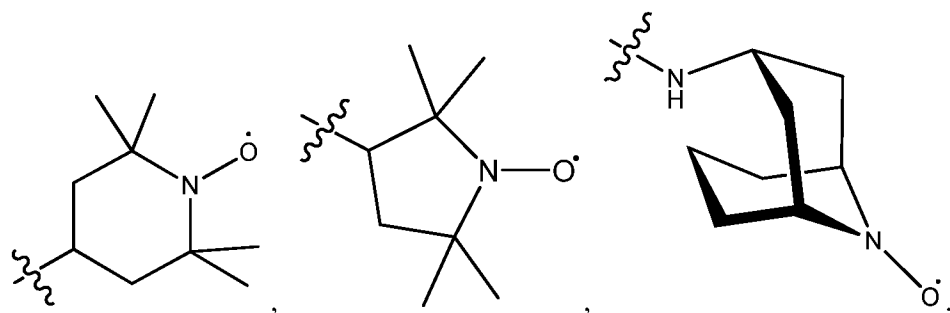
16. A compound, or a pharmaceutically acceptable salt thereof, having a structure of:



10

Formula 2

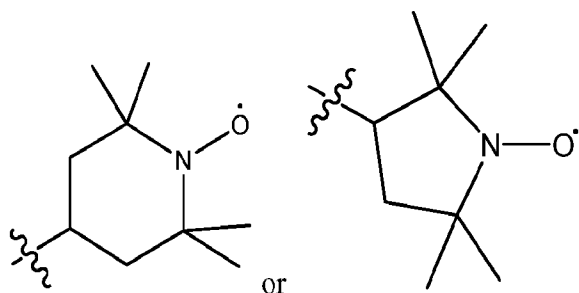
wherein R^{31} and R^{32} are each independently



5

R^{33} is H or an optionally substituted alkyl.

17. The compound of claim 16, wherein R^{31} and R^{32} are each independently

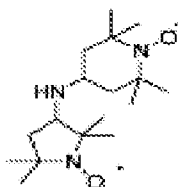


- 5 18. The compound of claim 16, wherein the compound is



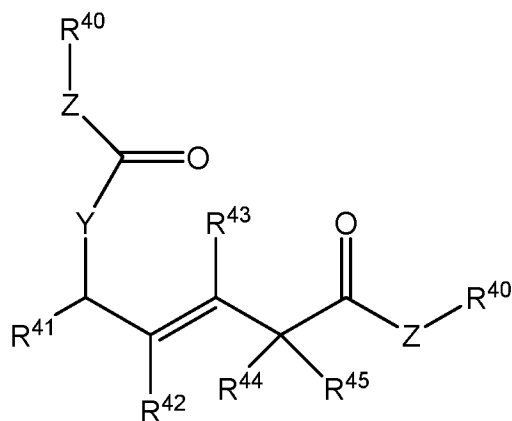
JRS527-33

or

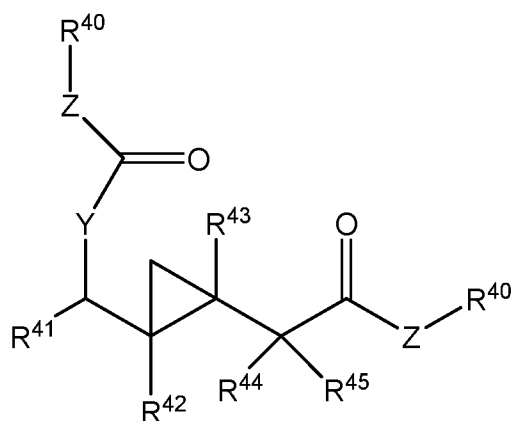


JRS527-36

- 10 19. A compound, or a pharmaceutically acceptable salt thereof, having a structure of:



Formula 3A ; or



Formula 3B

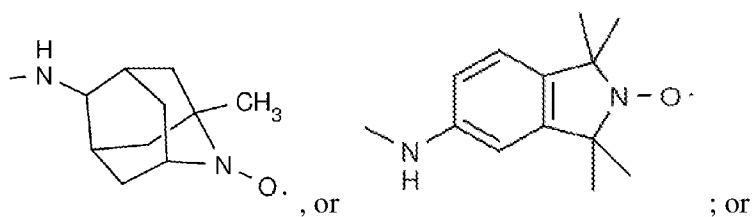
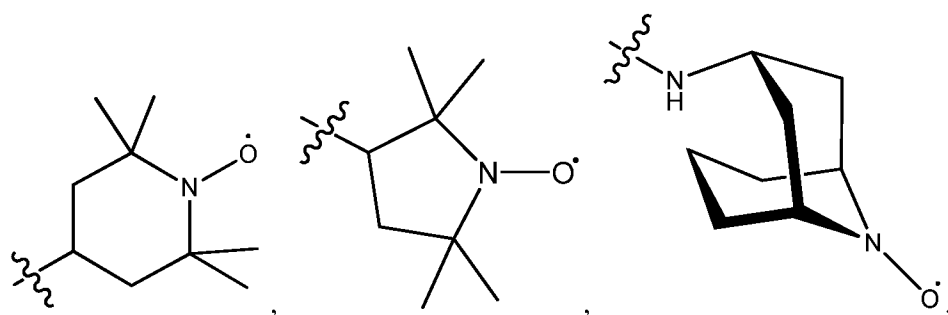
wherein Y is -NH-, -O-, -N(alkyl)-, -N(aryl)-, -N(cycloalkyl)-, -N(heteroaryl)-, or -CH(R⁴¹)-;

10 R⁴¹ is H, optionally substituted aliphatic, optionally substituted cycloalkyl, optionally substituted aryl, or optionally heteroaryl;

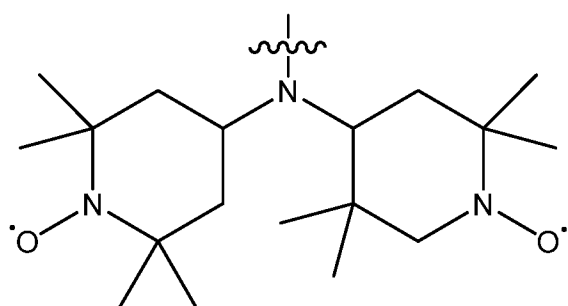
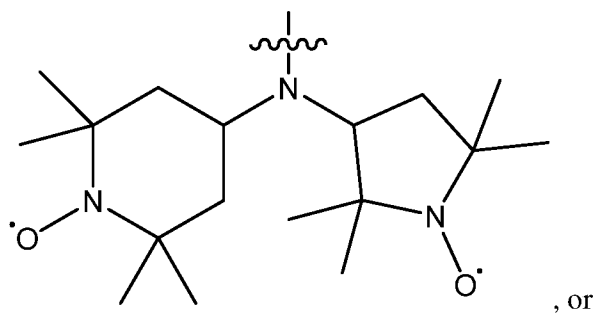
each of R⁴² – R⁴⁵ is H, optionally substituted aliphatic, optionally substituted cycloalkyl, optionally substituted aryl, optionally heteroaryl, or fluorine;

each Z is independently -O-, -NH- or -CH₂-; and

15 each R⁴⁰ is independently



5

each -Z^{R40} is independently

10

20. A pharmaceutical composition comprising at least one compound of any one of claims 1 to 19, and at least one pharmaceutically acceptable additive.

21. A method for mitigating probe-induced tissue damage in a subject, comprising administering to a subject in need thereof, at least one compound of any one of claims 1 to 19.

22. The method of claim 21, wherein the probe is a microdialysis probe.

23. The method of claim 21 or 22, wherein the method mitigates loss of evoked
5 dopamine release.

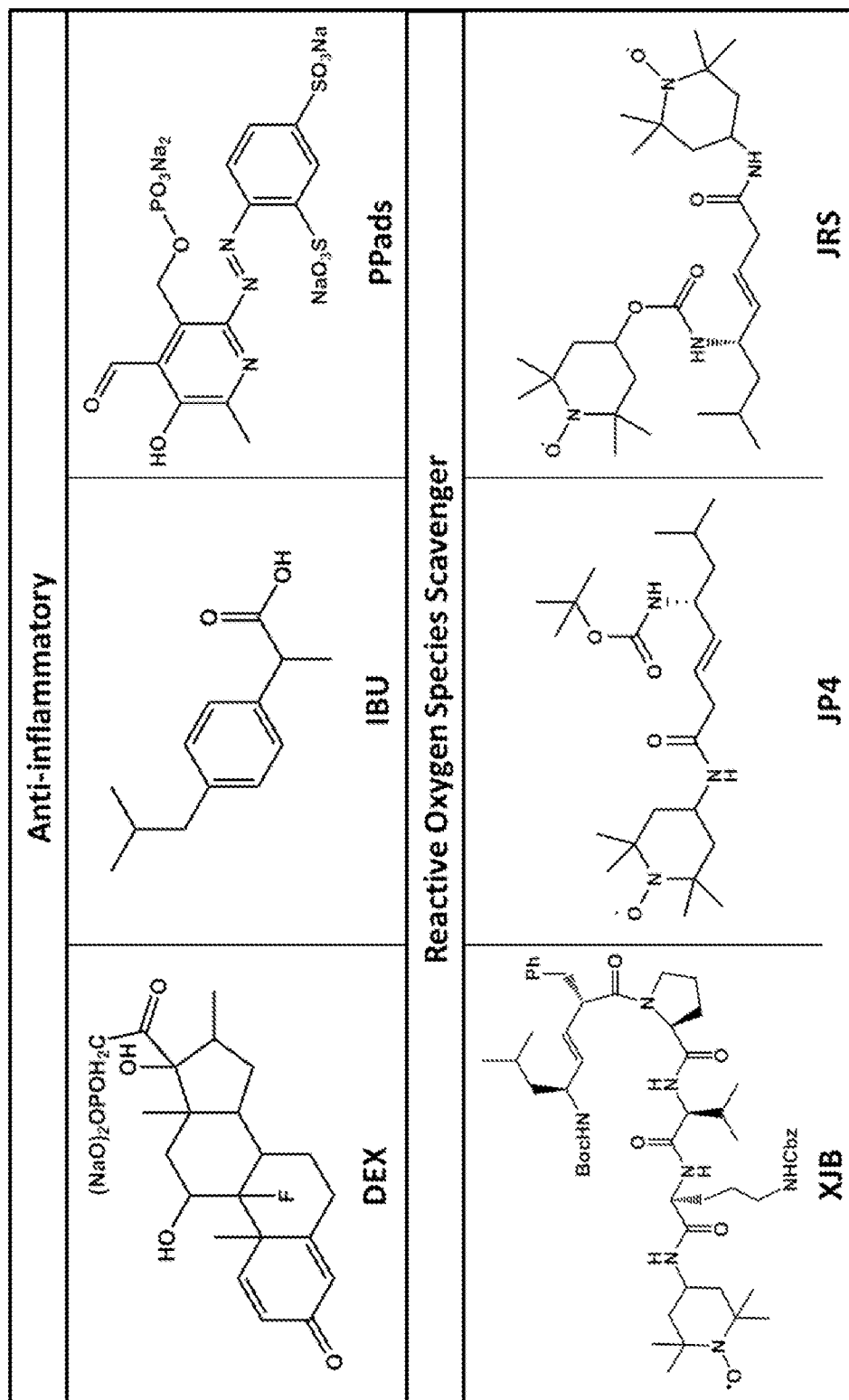


FIG. 1

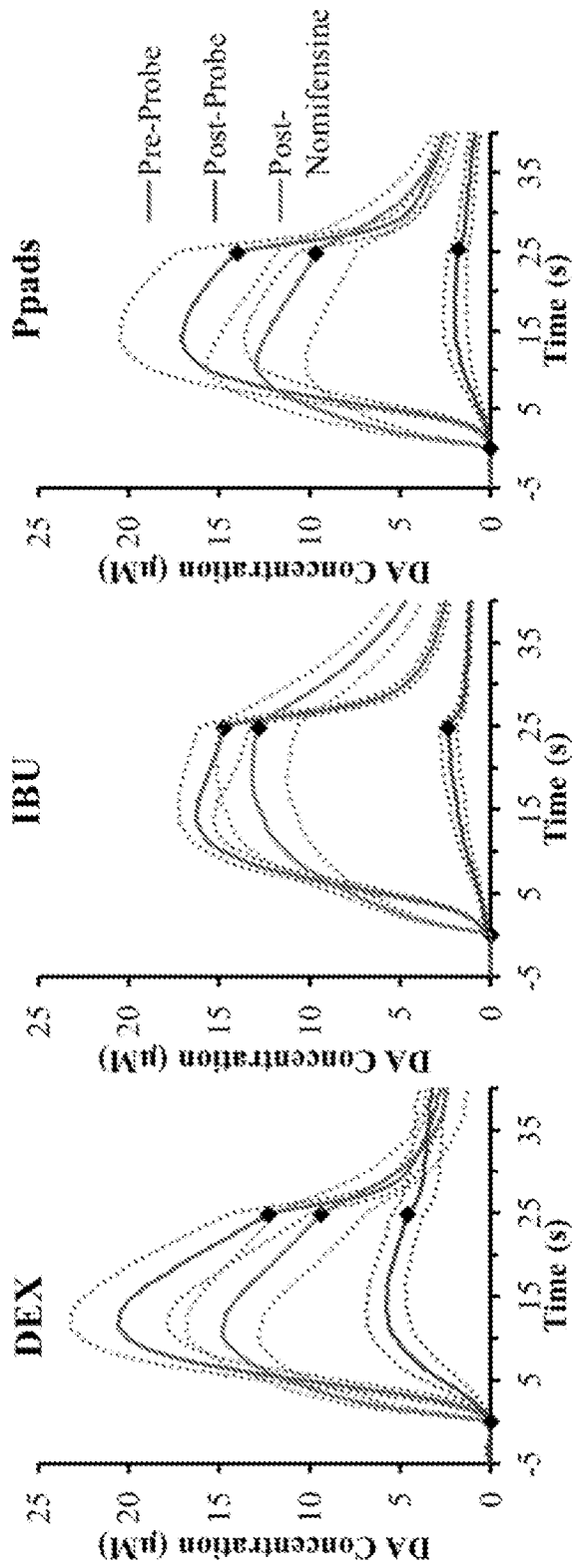


FIG. 2A

FIG. 2B

FIG. 2C

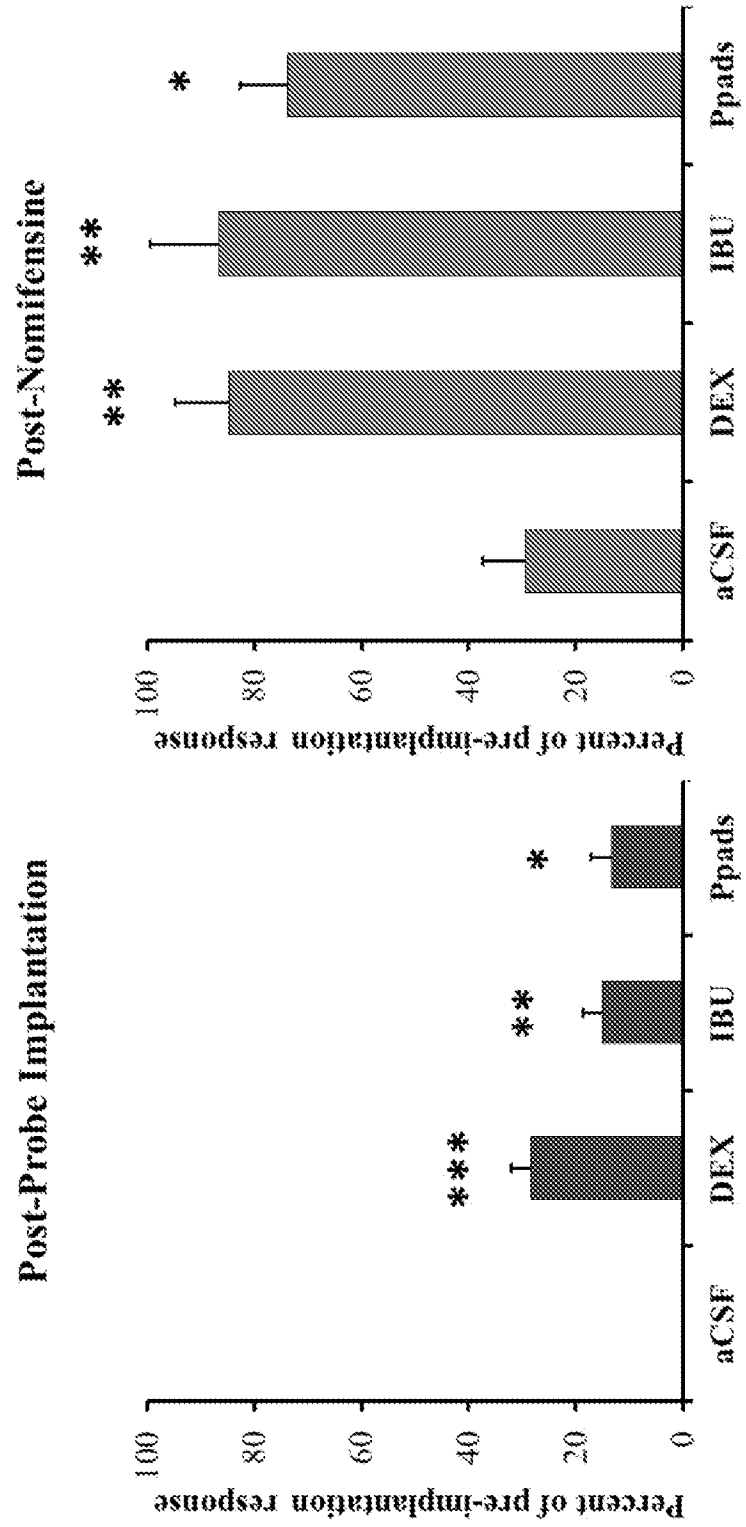


FIG. 3A

FIG. 3B

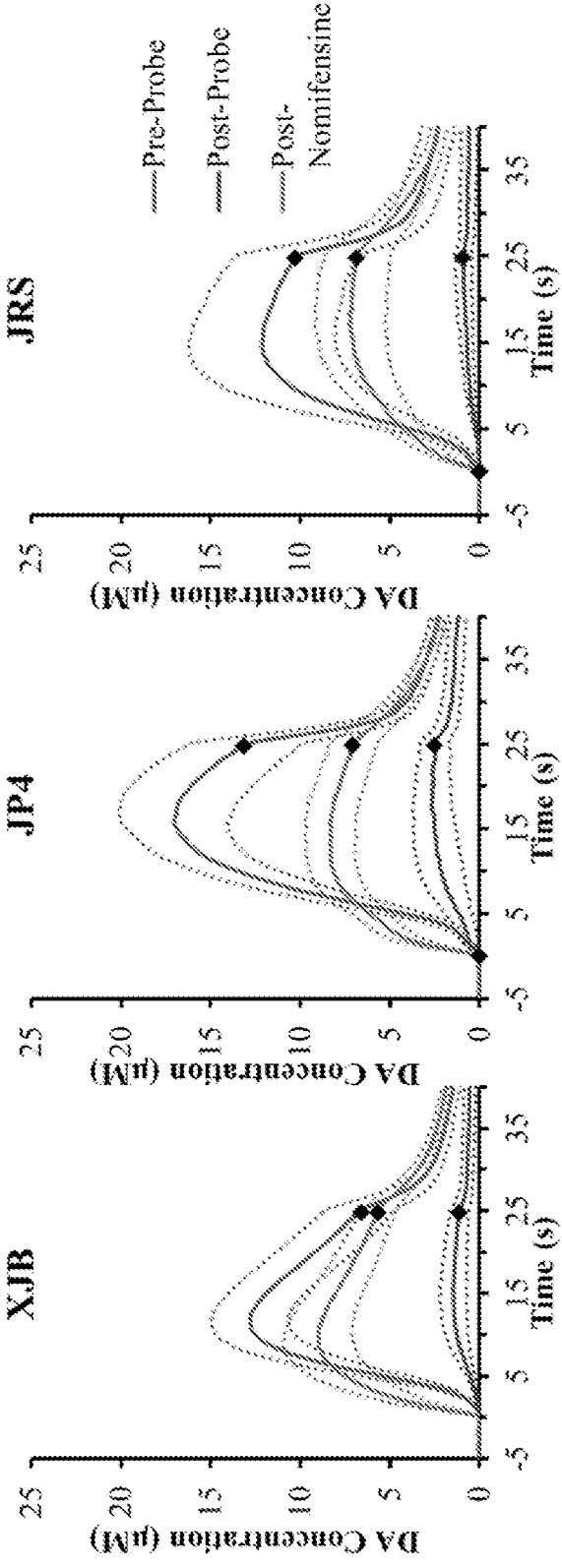


FIG. 4A

FIG. 4B

FIG. 4C

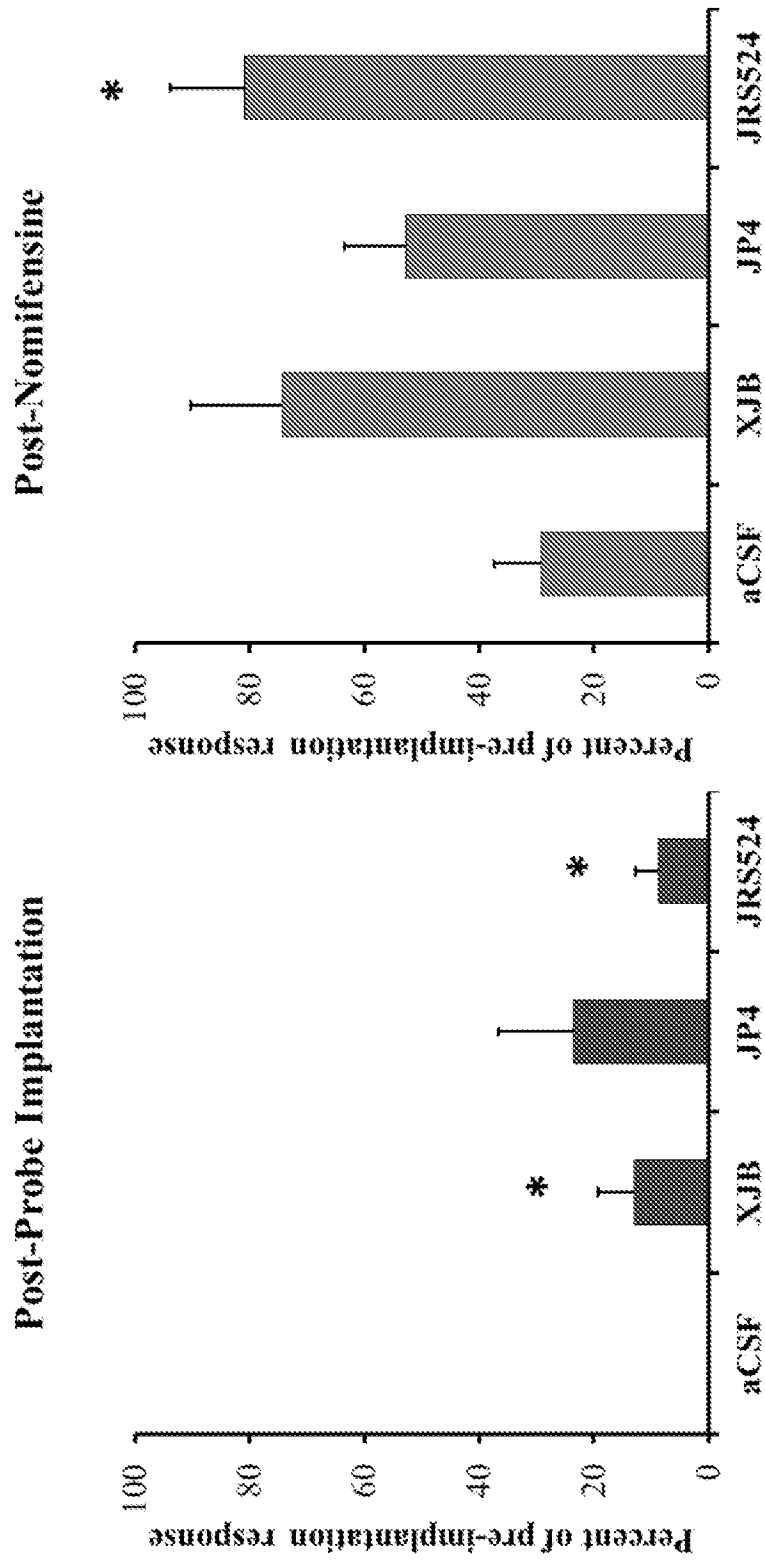


FIG. 5B

FIG. 5A

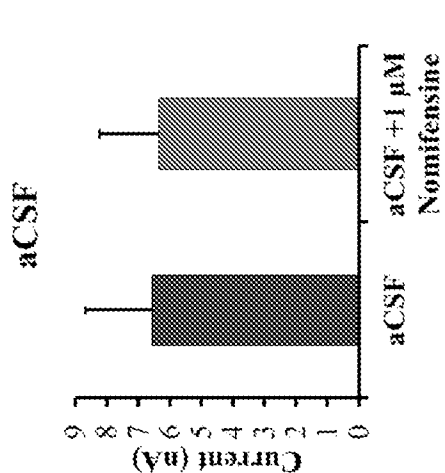


FIG. 6C

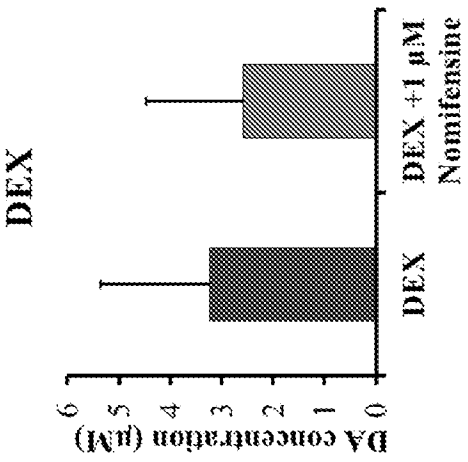


FIG. 6D

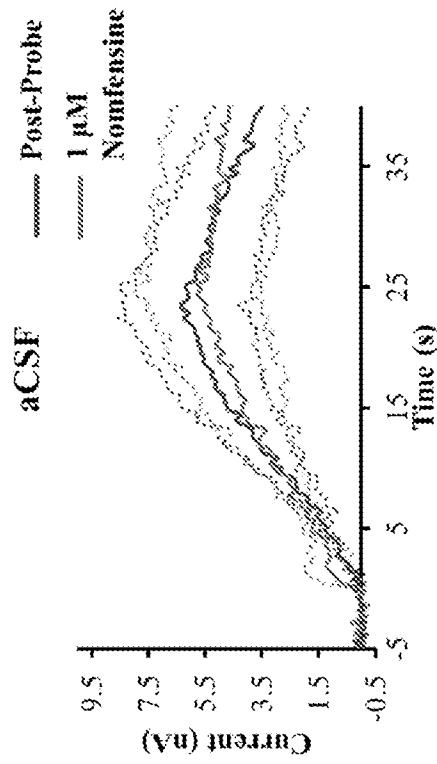


FIG. 6A

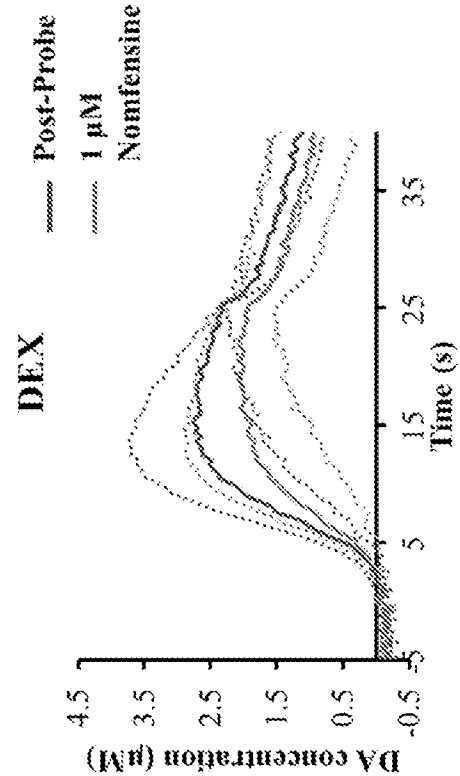


FIG. 6B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/039877

A. CLASSIFICATION OF SUBJECT MATTER IPC (2016.01) C07D 211/94, C07D 401/12, A61K 31/446800, A61K 31/454500, A61P 43/00 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC (2016.01) C07D 211/94, C07D 401/12, A61K 31/446800, A61K 31/454500 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Databases consulted: THOMSON INNOVATION, Google Patents, CAPLUS, REGISTRY, Google Scholar Search terms used: nitroxide, probe, induced, tissue, damage, microdialysis, dopamine, ROS (Reactive Oxygen Species) scavenger.		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012112851 A2 (UNIV PITTSBURGH [US]) 23 Aug 2012 (2012/08/23) The whole document, especially compound 26 (pages 55, 59), compound 35 (page 62), compounds 33, 20, JP4-039 (page 69), compounds 1, 2, 3, 4, 5, and 6 (page 68) and claim 17 (page 79).	1-15,20
Y	The whole document, especially claim 18	21-23
X	WO 2010009327 A1 (UNIV PITTSBURGH [US]) 21 Jan 2010 (2010/01/21) The whole document, especially : compounds A1, A2, and A3 (claims 5 and 13), compounds D1, D2, and D3 (claims 7 and 15) and page 10 (lines 8-11).	1-7,9-12,20
X	Frantz Marie-Celine et al, Synthesis of analogs of the radiation mitigator JP4-039 and Visualization of BODIPY derivatives in mitochondria, Org Biomol Chem. 2013 July 7; 11 (25): pages 4147-4153. Retrieved from the internet: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3729477/ > 07 Jul 2013 (2013/07/07) The whole document, especially compound 33 (page 19), JP4-039 (page 9)	1-15,20
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 13 Oct 2016		Date of mailing of the international search report 13 Oct 2016
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616		Authorized officer BARASH SHIFTAN Noga Telephone No. 972-2-5651672

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/039877

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	The whole document, especially page 5 lines 3-5	21-23
X	WO 2010009405 A2 (UNIV PITTSBURGH [US]) 21 Jan 2010 (2010/01/21) The whole document, especially JP4-049 (claim 21 page 96) and page 15 lines 17-18.	1-15,20
Y	Abstract	21-23
X	Kavala Miroslav, Preparation and Spectroscopic, Magnetic, and Electrochemical Studies of Mono-/Biradical TEMPO Derivatives, the Journal of Organic. Chemistry. 3 June 2013; Vol. 78, pages 6558-6569. 03 Jun 2013 (2013/06/03) compounds 6 and 7 (Figure 2 page 6558)	16-18
X	Wen-Guang Li et al. The relationship between structure and antioxidative activity of piperidine nitroxides, Journal of Pharmacy and Pharmacology (01 July 2006), Vol. 58, No. 7, pages 941-949. . Retrieved from the internet: < https://www.researchgate.net/publication/6978637_The_relationship_between_structure_and_antioxidative_activity_of_piperidine_nitroxides > 01 Jul 2006 (2006/07/01) Abstract and Introduction (page 941) and compound 4-Bis-Tempo (Figure 1 page 942)	16-18
Y	Nesbitt, Kathryn M. et al, Pharmacological Mitigation of Tissue Damage During Brain Microdialysis, Anal. Chem. (Published online: 20 August 2013), Vol. 85, No. 17, pages 8173-8179. Retrieved from the internet: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3799822/ > 20 Aug 2013 (2013/08/20) The whole document, especially Abstract and Conclusion	1-15,19-23
X	Nesbitt, Kathryn M. Retrodialysis of Pharmacological Agents Mitigates Tissue Damage during Brain Microdialysis and Preserves Dopamine Activity in Surrounding Tissue. Doctoral Dissertation, University of Pittsburgh. Pages 1-149. Retrieved from the internet: < http://d-scholarship.pitt.edu/24621/ >. Publication Date 22 June 2015. 22 Jun 2015 (2015/06/22) The whole document, especially Figure 4.2. (page 68),page 62 last paragraph, page 72, last line.	1-15,19-23

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2016/039877

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
WO 2012112851 A2	23 Aug 2012	WO 2012112851 A2	23 Aug 2012
		WO 2012112851 A3	15 Nov 2012
		US 2014018317 A1	16 Jan 2014
		US 9217000 B2	22 Dec 2015
		US 2016068556 A1	10 Mar 2016
WO 2010009327 A1	21 Jan 2010	WO 2010009327 A1	21 Jan 2010
		US 2012004263 A1	05 Jan 2012
		US 8937086 B2	20 Jan 2015
		US 2015018346 A1	15 Jan 2015
		US 9220711 B2	29 Dec 2015
		US 2016151269 A1	02 Jun 2016
WO 2010009405 A2	21 Jan 2010	WO 2010009405 A2	21 Jan 2010
		WO 2010009405 A9	25 Mar 2010
		WO 2010009405 A3	14 May 2010
		AU 2009270740 A1	21 Jan 2010
		AU 2009270740 B2	22 Jan 2015
		AU 2009270740 B8	09 Jul 2015
		CA 2768183 A1	21 Jan 2010
		CN 102159553 A	17 Aug 2011
		CN 102159553 B	17 Feb 2016
		DK 2328875 T3	02 Feb 2015
		EP 2328875 A2	08 Jun 2011
		EP 2328875 A4	21 Mar 2012
		EP 2328875 B1	15 Oct 2014
		ES 2527958 T3	02 Feb 2015
		JP 2011528378 A	17 Nov 2011
		JP 5719293 B2	13 May 2015
		PT 2328875 E	04 Feb 2015
		US 2010035869 A1	11 Feb 2010

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2016/039877

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
		US 8288551 B2	16 Oct 2012
		US 2013102626 A1	25 Apr 2013
		US 8609850 B2	17 Dec 2013
		US 2011172214 A1	14 Jul 2011
		US 8822541 B2	02 Sep 2014
		US 2014378444 A1	25 Dec 2014
		US 9216976 B2	22 Dec 2015
		US 2016137692 A1	19 May 2016
		WO 2010009389 A1	21 Jan 2010