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(54) **Title:** COMPOSITIONS AND METHODS FOR TREATING PARKINSON'S DISEASE

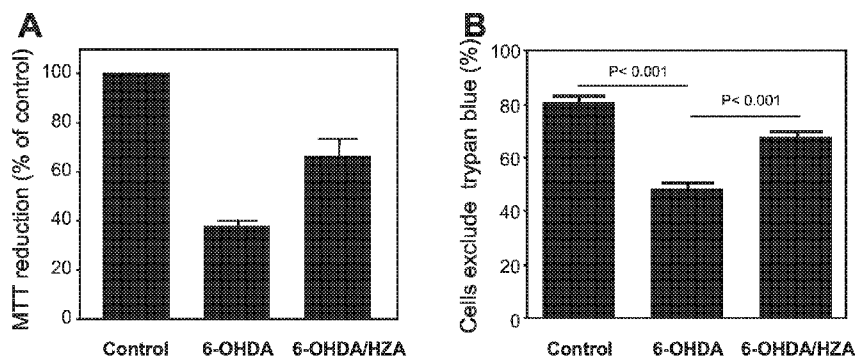


Figure 1

(57) **Abstract:** The invention generally relates to compositions and methods for treating Parkinson's disease. Aspects of the inven-
tion provide composition including an alpha/beta unsaturated aldehyde scavenger compound in a therapeutically effective amount
for treating a patient with Parkinson's disease.

COMPOSITIONS AND METHODS FOR TREATING PARKINSON'S DISEASE

Related Application

The present application claims the benefit of and priority to U.S. provisional application
5 serial number 62/136,007, filed March 20, 2015, the content of which is incorporated by
reference herein in its entirety.

Field of the Invention

The invention generally relates to compositions and methods for treating Parkinson's
10 disease.

Background

Parkinson's disease (PD) is a chronic and progressive movement disorder. Nearly one
million people in the United States are living with Parkinson's disease. Parkinson's disease
15 involves malfunction and death of vital nerve cells in the brain, called neurons. Parkinson's
disease affects neurons in an area of the brain known as the substantia nigra. Some of those
dying neurons produce dopamine, a chemical that sends messages to the part of the brain that
controls movement and coordination. As Parkinson's disease progresses, the amount of
dopamine produced in brain areas decreases, leaving a person unable to control movement
20 normally.

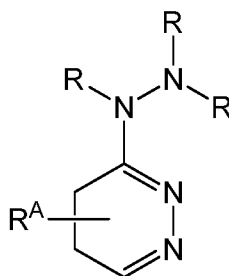
Summary

The invention recognizes that oxidative stress and the generation of free radicals are
implicated as important contributors to nigral cell death in Parkinson's disease. Aspects of the
25 invention are based on the fact that compounds that include alpha/beta unsaturated aldehydes
(e.g., acrolein, methylenedioxyamphetamine (MDA), or hydroxynonenal (HNE)), are capable of
directly damaging nerve cells and generating free radicals. Specifically, such compounds play a
particularly damaging role through the perpetuation of oxidative stress, enhancing cellular
degeneration and functional loss. In that manner, compounds that include alpha/beta unsaturated
30 aldehydes present a novel and effective target for therapeutic interventions aimed at suppressing

oxidative stress, reducing dopamine cell death, and reducing the pathological role of α -synuclein or alleviating the symptoms of Parkinson's disease.

Accordingly, aspects of the invention provide compositions that include an alpha/beta unsaturated aldehyde scavenger compound (or combinations of such compounds) in a therapeutically effective amount for treating a patient with Parkinson's disease. By scavenging these alpha/beta unsaturated aldehyde compounds, compositions of the invention suppress oxidative stress, reduce dopamine cell death, agglomeration of α -synuclein and reduce or alleviate the symptoms of Parkinson's disease.

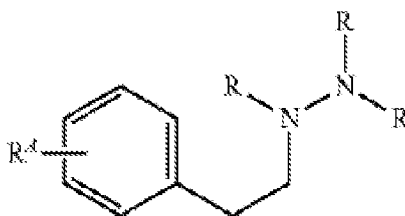
The alpha/beta unsaturated aldehyde scavenger compounds can include hydrazinopyridazines, fused hydrazinopyridazines, phenylethylhydrazines, or a combination thereof. In certain embodiments, the fused hydrazinopyridazine is a compound of the formula



or a pharmaceutically acceptable salt thereof. R is independently selected in each instance from hydrogen, acyl, or sulfonyl; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl comprising 3-10 ring members, heteroaryl comprising 3-10 ring members, arylalkyl comprising 3-10 ring members, or heteroarylalkyl comprising 3-10 ring members, each of which is optionally substituted. R^A represents three substituents, one of which is selected from the group consisting of hydrogen, halo, hydroxy and derivatives thereof, amino and derivatives thereof, thio and derivatives thereof, acyl, carboxylate or a derivative thereof, hydroxylamino and derivatives thereof, hydrazino and derivatives thereof, sulfonyl or a derivative thereof, or sulfonyl or a derivative thereof; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl comprising 3-10 ring members, heteroaryl comprising 3-10 ring members, arylalkyl comprising 3-10 ring members, or heteroarylalkyl comprising 3-10 ring members, each of which is optionally substituted; and two of R^A are taken together with the attached carbons to form an optionally substituted saturated, unsaturated, or aromatic carbocycle or heterocycle.

In certain embodiments, R^A represents a hydrogen; or R^A includes an optionally substituted benzo group; or R^A includes an optionally substituted fused piperidine; or R^A includes a hydrazino or derivative thereof; or R^A includes a hydrazino; or R^A includes amino or a derivative thereof; or R^A includes dialkylamino, where each alkyl is independently selected, and independently optionally substituted. In certain embodiments, each R is hydrogen; or at least one R is acyl; or at least one R is optionally substituted alkoxy carbonyl. In preferred embodiments, the fused hydrazinopyridazine is hydralazine, dihydralazine, and endralazine.

In other embodiments, the phenylethylhydrazine is a compound of the formula



or a pharmaceutically acceptable salt thereof. R is independently selected in each instance from hydrogen, acyl, or sulfonyl; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted, or two R are taken together with the attached nitrogen to form a hydrazine. R^A represents three substituents selected from the group consisting of hydrogen, halo, hydroxy and derivatives thereof, amino and derivatives thereof, thio and derivatives thereof, acyl, carboxylate or a derivative thereof, hydroxylamino and derivatives thereof, hydrazino and derivatives thereof, sulfonyl or a derivative thereof, or sulfonyl or a derivative thereof; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted; or two of R^A are taken together with the attached carbons to form an optionally substituted saturated, unsaturated, or aromatic carbocycle or heterocycle. In certain preferred embodiments, the phenylethylhydrazine is phenelzine.

In other embodiments, the alpha/beta unsaturated aldehyde compound scavenger is dimercaperol. In other embodiments, the alpha/beta unsaturated aldehyde compound scavenger is N-acetylcysteine, phloretin, carnosine and homocarnosine, sodium borohydride and sodium bisulfite. In other embodiments, the alpha/beta unsaturated aldehyde scavenger compound is an acrolein scavenger compound.

Other aspects of the invention provide methods for treating a patient with Parkinson's disease that involve providing to a patient a composition including an alpha/beta unsaturated aldehyde scavenger compound (or combinations of such compounds) in a therapeutically effective amount for treatment of Parkinson's disease. Any of the above described compounds may be used with methods of the invention.

Brief Description of the Drawings

FIG. 1 panels A-B show the effect of hydralazine on PC 12 cell viability by MTT assay and Dye Exclusion of Trypan blue assay. Cells were incubated with 400 uM acrolein for 2 hr, and some treated were with 500 uM hydralazine after a 15-min delay. Cell viability was determined by absorbance of the MTT reduction product at 550 nm. However for the Typan Blue assay the data are expressed as a percentage of Trypan blue-stained cells over the total number of cells. 6OHDA induced PC 12 cell death (based on MTT reduction and Trypan blue dye excursion assays) can be reduced by Hydralazine. Values are expressed as the percentage of control cells. Cell viability was significantly improved by hydralazine treatment in a compared with the group treated with 6 OHDA only (*P < 0.001) and (*P < 0.001) for the MTT and trypan blue assay respectively.

FIG. 2 panels A-B show the effect of hydralazine on MES 23.5 dopaminergic cell viability by MTT assay and Dye Exclusion of Trypan blue assay. Cells were incubated with 400 uM acrolein for 2 hr, and some treated were with 500 uM hydralazine after a 15-min delay. Cell viability was determined by absorbance of the MTT reduction product at 550nm. However for the Typan Blue assay the data are expressed as a percentage of trypan blue-stained cells over the total number of cells. 6OHDA induced dopaminergic cell death (based on MTT reduction and Trypan blue dye excursion assays) can be reduced by Hydralazine. Values are expressed as the percentage of control cells. Cell viability was significantly improved by hydralazine treatment in a compared with the group treated with 6 OHDA only (*P < 0.01) and (*P < 0.001) for the MTT and trypan blue assay respectively. 6 OHDA induced MES23.5 dopaminergic cell death (based on MTT reduction and Trypan blue dye excursion assays) can be reduced by Hydralazine.

FIG. 3 panel A show tissue samples from rat striatum analyzed by western blot with antibody against acrolein modified proteins. The band close to 75kD was chosen to provide a rough estimation of the level of acrolein-modified protein in the sample. Sham: Substantia nigra

treatment with saline solution. 6-OHDA: Substantia nigra treatment with 6-OHDA solution. Acrolein: Substantia nigra treatment with acrolein solution. FIG. 3 panel B is a graph showing quantification of the band highlighted in FIG. 3 panel A.

FIG. 4 shows three and two - dimensional representations of Exploratory Behavior as a Function of Groups: The activity levels and patterns of behavior in the exploratory box for Control, 6 OHDA injured and 6 OHDA injured- treated animals. The X and Y axis represent position in the in the box and the Z axes represent time (in seconds). Each graph shows typical behavior of one animal from each group. In A, the control animal displayed thigmotaxic (wall-following) behavior as evidenced by the square pattern of the activity. Further, in comparison to B and C (injured animals) the animal was more active and explored a greater area of the box repeatedly. While the 6 OHDA injured showed a less active, and explored lesser area at a much lower frequency. The animal also showed a circling behavior evidenced by the arrow. For the 6 OHDA injured, hydralazine treated showed much similar pattern of exploration to A by following the walls and also less circling behavior was evidenced.

FIG. 5 panel A is a graph showing the data for the mean Distance travelled and FIG. 5 panel B is a graph showing the mean Area covered in the Exploration boxes that were examined for control, sham, 6 OHDA injured and 6 OHDA injured, hydralazine treated as a function of time. Uninjured animals were significantly more active covering most of the area and traversed a greater distance than either injury groups. The injured, control (sham) animals did not differ from the uninjured, control, in mean area coverage and distance travelled. However, the 6 OHDA injured treated did differ significantly from the 6 OHDA injured animals on this behavioral measure.

FIG. 6 panel A is a graph showing the top speed and FIG. 6 panel B is a graph showing maximum time on the rotarod, examined for Control, sham, 6 OHDA and 6 OHDA injured, hydralazine treated groups. FIG. 6 panel A graphically depicts the top speed (averaged across trials) for each time the rat were assessed on the rotarod. For each measure, there is no significant difference in performance between the control and the sham groups. However, the 6 OHDA injured, hydralazine treated Displayed superior performance in comparison to the 6 OHDA injured groups. It shows that the performance of hydralazine treated rat improved to a greater degree when compared to the injured untreated groups. Thus, Hydralazine facilitated behavioral recovery for those motor deficit that are sensitive and measurable by the rotarod.

FIG. 7 panel A shows tissue samples from the rat substantia nigra that were analyzed by western blot with anti-alpha-synuclein antibody. The band chosen for quantification represents the dimer form of alpha-synuclein. Sham: Substantia nigra treatment with saline solution. Acrolein: Substantia nigra treatment with acrolein solution. Control: The anti-alpha-synuclein antibody was blocked with purified alpha-synuclein before perform the western blot, thus to identify any non specific band in the western blot. The tissue sample used is the same as that of acrolein group. FIG. 7 panel B is a graph showing quantification of the band highlighted in FIG. 7 panel A.

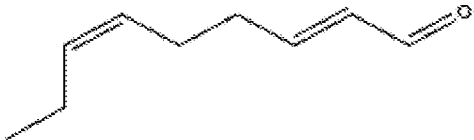
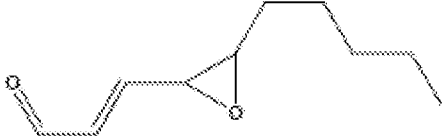
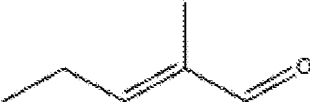
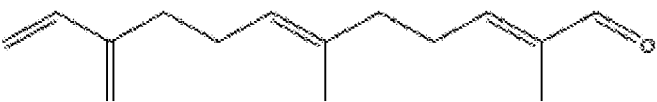
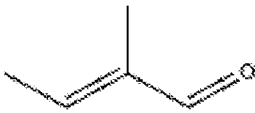
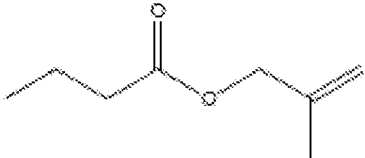
FIG. 8 panels A-C show histological analysis of Tyrosine hydroxylase (TH) immunohistochemistry of the rat striatum. Photomicrograph of 15 μ m coronal brain section showing TH immunolabeling of the striatum; (panel A) control, (panel B) 6-OHDA lesioned, A 6-OHDA concentration of 2ug/ μ L caused a 93.75% reduction of TH immunoreactivity, (panel C) 6-OHDA lesioned hydralazine treated. IV treatment of 6 OHDA lesioned rats with hydralazine at a dosage of 5mg/Kg improved significantly the TH immunoreactivity of 6 OHDA lesioned rats by 4 fold. FIG. 8 panel D shows an enlarged portion indicated in panel A. FIG. 8 panel E shows an enlarged portion indicated in panel B. FIG. 8 panel F shows an enlarged portion indicated in panel C. Rat was injected with 6-OHDA into the SNC and MFB. FIG. 8 panel G shows a one way ANOVA analysis indicates significance ($P < 0.001$). Multiple post hoc comparison with Neuman-kuel are significantly different, $P < 0.05$.

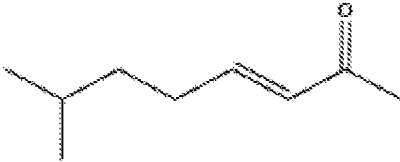
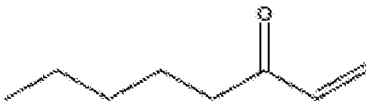
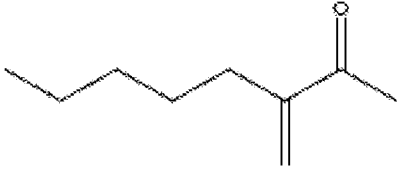
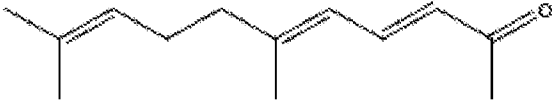
Detailed Description

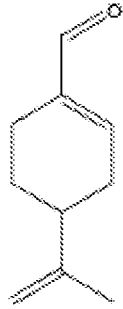
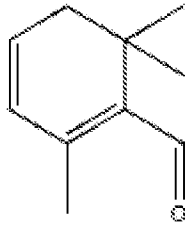
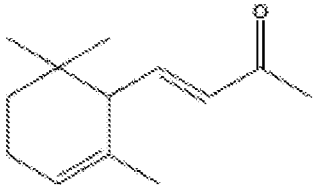
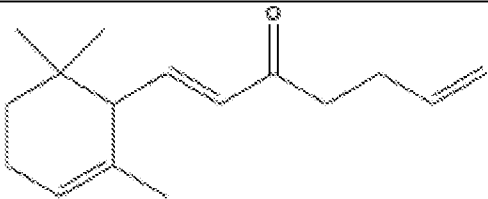
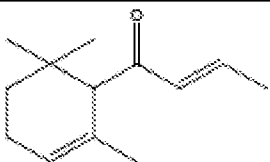
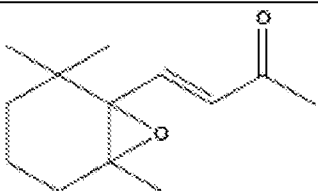
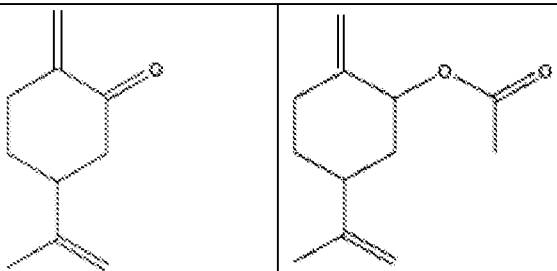
The invention generally relates to compositions and methods for treating Parkinson's disease. Aspects of the invention provide composition including an alpha/beta unsaturated aldehyde scavenger compound (or combinations of such compounds) in a therapeutically effective amount for treating a patient with Parkinson's disease. Generally, a scavenger is a chemical substance added to a mixture or sample or medium in order to remove or de-activate one or more molecules within the mixture or sample or medium. A scavenger can be administered to a patient to remove or de-activate one ore more molecules circulating within the patient. In the context of the invention, the compositions include compounds that can remove from circulation within a body of a patient or de-activate within a body of a patient compounds that include alpha/beta unsaturated aldehydes.

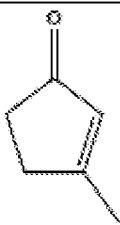
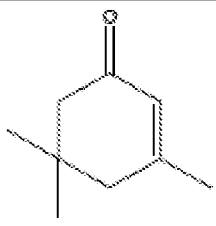
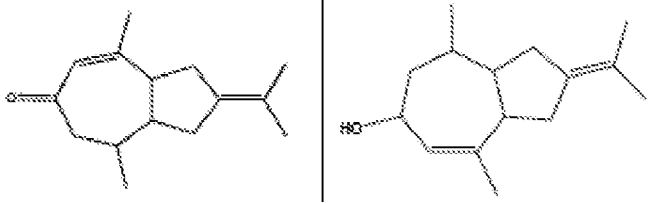
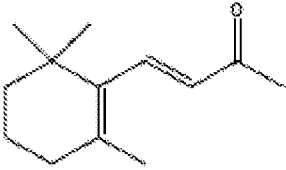
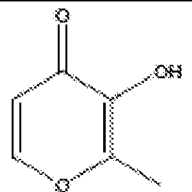
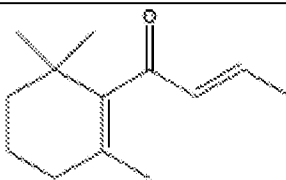
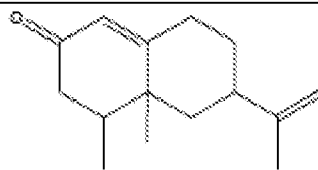
An alpha/beta unsaturated aldehyde is a functional group of a molecule, and may have the general formula of $(\text{O}=\text{CR})-\text{C}^{\alpha}=\text{C}^{\beta}-\text{R}$. In this functional group, the carbonyl group is conjugated with an alkene. Unlike the case for simple carbonyls, α,β -unsaturated aldehyde functional groups are often attacked by nucleophiles at the β carbon. Exemplary compounds that include alpha/beta unsaturated aldehydes are acrolein, methylenedioxyamphetamine (MDA), or hydroxynonenal (HNE). Other exemplary alpha/beta unsaturated aldehydes are shown in Table 1 below.

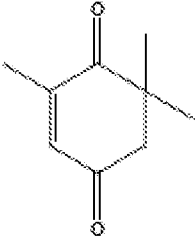
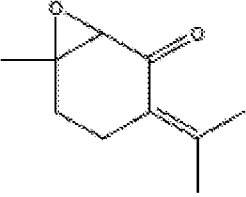
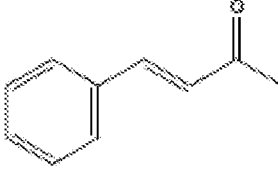
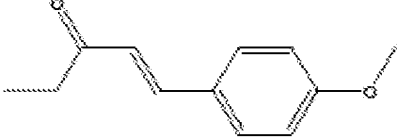
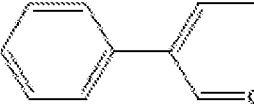
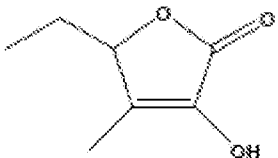
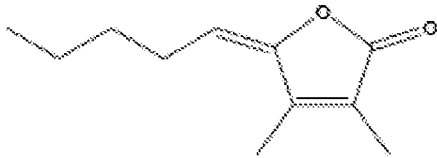
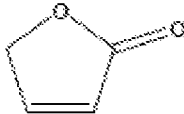
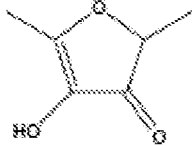
Table 1: Alpha/beta unsaturated aldehydes

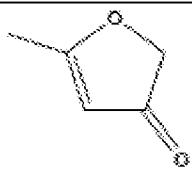
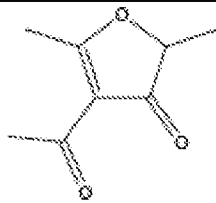
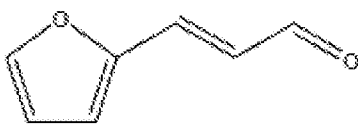
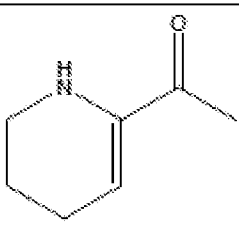
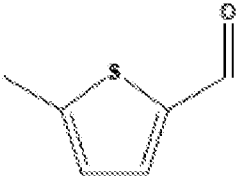
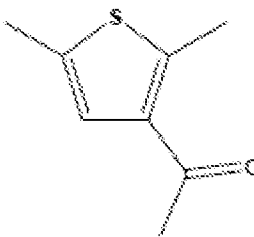
Compound Name	Compound Structure	
Hex-2(trans)-enal		
Nona-2(trans),6(cis)-dienal		
Oct-2-enal		
4,5-Epoxydec-2(trans)-enal		
2-Methylpent-2-enal		
beta-Sinensal		
2-Methylprop-2-enal or the precursor 2-Methylallyl butyrate		

Hexa-2(trans),4(trans)-dienal	
Deca-2(trans),4(trans)-dienal	
4-Methylpent-3-en-2-one	
7-Methyl-3-octenone-2	
Pent-1-en-3-one	
Oct-1-en-3-one	
2-Pentylbut-1-en-3-one	
6-Methylhepta-3,5-dien-2-one	
Pseudo-ionone	

p-Mentha-1,8-dien-7-al	
2,6,6-Trimethylcyclohexa-1,3-diene-1- carbaldehyde	
alpha-Ionone	
Allyl alpha-ionone	
alpha-Damascone	
beta-Ionone epoxide	
1(7),8-p-menthadien-2-one or the precursor 1(7),8-p-Menthadien-2-yl acetate (mixture of (E) and (Z) isomers)	

3-Methyl-2-cyclopenten-1-one	
3,5,5-Trimethylcyclohex-2-en-1-one	
2,6-Dimethyl-9-(1-methylethylidene)-bicyclo[5.3.0]dec-2-en-4-one or its precursor Vetiverol	
beta-Ionone	
Maltol	
beta-Damascone	
Nootkatone	

2,6,6-Trimethylcyclohex-2-en-1,4-dione	
Piperitenone oxide	
4-Phenylbut-3-en-2-one	
1-(4-Methoxyphenyl)-4pent-1-en-3-one	
2-Phenylcrotonaldehyde	
5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one	
3,4-Dimethyl-5-pentylidenefuran-2(5H)-one	
Furan-2(5H)-one	
4-Hydroxy-2,5-dimethylfuran-3(2H)-one	

5-Methylfuran-3(2H)-one	
4-Acetyl-2,5-dimethylfuran-3(2H)-one	
3-(2-Furyl)acrylaldehyde	
4-(2-Furyl)but-3-en-2-one	
2-Acetyl-1,4,5,6-tetrahydropyridine	
5-Methyl-2-thiophenecarbaldehyde	
3-Acetyl-2,5-dimethylthiophene	

With alpha/beta unsaturated aldehydes, the carbonyl group draws electrons away from the alkene, and the alkene group is, therefore, deactivated towards an electrophile, such as bromine or hydrochloric acid. As a general rule with asymmetric electrophiles, hydrogen

attaches itself at the α -position in an electrophilic addition. On the other hand, these compounds are activated towards nucleophiles in nucleophilic conjugate addition. Since α,β -unsaturated compounds are electrophiles, many α,β -unsaturated carbonyl compounds are toxic, mutagenic and carcinogenic. DNA can attack the β carbon and thus be alkylated.

5 In certain embodiments, compounds that include alpha/beta unsaturated aldehydes may be generated in the body via metabolism of polyamine compounds. The term "polyamine" herein represents a straight-chain aliphatic hydrocarbon having two or more primary amino groups. Known biogenic polyamines may include, but are not limited to, putrescine, cadaverine, spermidine, spermine, 1,3-diaminopropane, caldine, homospermidine, 3-aminopropylcadaverine,
10 norspermine, thermospermine, caldopentamine, and so on. Meanwhile, preferred polyamines in the present invention may be putrescine, spermidine and spermine.

 The above polyamines may be metabolized by oxidation, acetylation, transamination and carbamoylation, and polyamine oxidase is the enzyme that involves in the oxidation of polyamine. The term "polyamine oxidase" herein represents an enzyme that oxidizes diamine or
15 polyamine as a good substrate and generates hydrogen peroxide. Polyamine receives oxidative deamination by polyamine oxidase, thereby aldehyde compounds such as acrolein would be produced. The preferred aldehyde compound in the present invention may be acrolein, but is not so limited to it.

 As used herein, alpha/beta unsaturated aldehyde compounds may also include adducts
20 formed when an alpha/beta unsaturated aldehyde compound binds to another compound. For example, acrolein, an alpha/beta unsaturated aldehyde compound) is known to bind to proteins, such as alpha-synuclein. The complex formed when acrolein binds alpha-synuclein is considered an alpha/beta unsaturated aldehyde compound within the context of the invention because the alpha/beta unsaturated aldehyde function group of the acrolein molecule remains
25 exposed for scavenging by compositions of the invention.

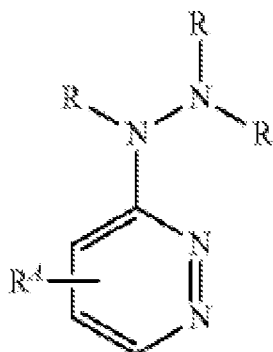
 Without being bound by any particular theory or mechanism of action, it is believed that alpha/beta unsaturated aldehyde compounds play a role in Parkinson's disease. For example, one of the toxicities of acrolein is its ability to damage proteins through adduct formation. Acrolein-bound proteins are likely to undergo profound structural changes causing both
30 functional alteration and toxicity. Alpha-synuclein is an abundant neuronal protein that is thought to play an important role in PD pathogenesis. Alpha-synuclein is a major component of

characteristic ‘Lewy body’ inclusions in the brains of PD patients, and mutations in the alpha-synuclein gene are involved in some forms of familial PD. From this neuropathological and genetic evidence, the current model indicates that alpha-synuclein aggregation plays an important role in DA cell death. Since alpha-synuclein possesses structural components that are known to be vulnerable to acrolein adduction, it is likely that acrolein-mediated structural alterations of alpha-synuclein lead to aggregation and further that these conformational changes may contribute to neurodegeneration in PD.

Accordingly, by scavenging acrolein or any other alpha/beta unsaturated aldehyde compound, structural alternations to proteins within the body can be avoided (such as structural alterations of alpha-synuclein) preventing Lewy body formation and aggregation and thereby ameliorating or eliminating the symptoms of Parkinson’s disease.

In certain embodiments, the alpha/beta unsaturated aldehyde scavenger compound is selected from the group consisting of a hydrazinopyridazine, a fused hydrazinopyridazine, and a phenylethylhydrazines.

In particular embodiment, the hydrazinopyridazine or fused hydrazinopyridazine is a compound of the formula

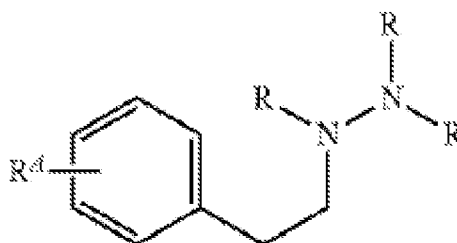


or a pharmaceutically acceptable salt thereof, wherein:

R is independently selected in each instance from hydrogen, acyl, or sulfonyl; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted; and

R^A represents three substituents selected from the group consisting of hydrogen, halo, hydroxy and derivatives thereof, amino and derivatives thereof, thio and derivatives thereof, acyl, carboxylate or a derivative thereof, hydroxylamino and derivatives thereof, hydrazino and derivatives thereof, sulfinyl or a derivative thereof, or sulfonyl or a derivative thereof; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted; or two of R^A are taken together with the attached carbons to form an optionally substituted saturated, unsaturated, or aromatic carbocycle or heterocycle.

In another embodiment, described herein is a method wherein the phenylethylhydrazine is a compound of the formula

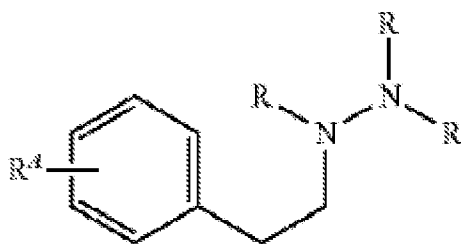


or a pharmaceutically acceptable salt thereof, wherein:

R is independently selected in each instance from hydrogen, acyl, or sulfonyl; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted; and

R^A represents three substituents selected from the group consisting of hydrogen, halo, hydroxy and derivatives thereof, amino and derivatives thereof, thio and derivatives thereof, acyl, carboxylate or a derivative thereof, hydroxylamino and derivatives thereof, hydrazino and derivatives thereof, sulfinyl or a derivative thereof, or sulfonyl or a derivative thereof; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted; or two of R^A are taken together with the attached carbons to form an optionally substituted saturated, unsaturated, or aromatic carbocycle or heterocycle.

In another embodiment, the phenylethylhydrazine is a compound of the formula

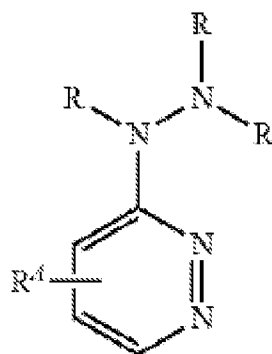


or a pharmaceutically acceptable salt thereof, wherein:

R is independently selected in each instance from hydrogen, acyl, or sulfonyl; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted; and

R^A represents three substituents selected from the group consisting of hydrogen, halo, hydroxy and derivatives thereof, amino and derivatives thereof, thio and derivatives thereof, acyl, carboxylate or a derivative thereof, hydroxylamino and derivatives thereof, hydrazino and derivatives thereof, sulfinyl or a derivative thereof, or sulfonyl or a derivative thereof; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted; or two of R^A are taken together with the attached carbons to form an optionally substituted saturated, unsaturated, or aromatic carbocycle or heterocycle.

In another embodiment, the hydrazinopyridazine or fused hydrazinopyridazine is a compound of the formula



or a pharmaceutically acceptable salt thereof, wherein:

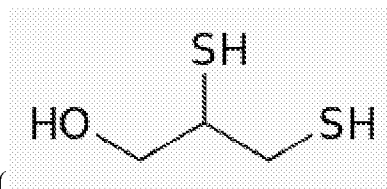
R is independently selected in each instance from hydrogen, acyl, or sulfonyl; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted; and

- 5 R^A represents three substituents selected from the group consisting of hydrogen, halo, hydroxy and derivatives thereof, amino and derivatives thereof, thio and derivatives thereof, acyl, carboxylate or a derivative thereof, hydroxylamino and derivatives thereof, hydrazino and derivatives thereof, sulfinyl or a derivative thereof, or sulfonyl or a derivative thereof; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted; or two of R^A are taken together with the attached carbons to form an optionally substituted saturated, unsaturated, or aromatic carbocycle or heterocycle.

- In another embodiment of the above formulae, R^A represents three hydrogens. In another embodiment of the above formulae, R^A includes an optionally substituted benzo group. In another embodiment of the above formulae, R^A includes an optionally substituted fused piperidine. In another embodiment of the above formulae, R^A includes a hydrazino or derivative thereof. In another embodiment of the above formulae, R^A includes a hydrazino. In another embodiment of the above formulae, R^A includes amino or a derivative thereof. In another embodiment of the above formulae, R^A includes dialkylamino, where each alkyl is independently selected, and independently optionally substituted. In another of the above formulae, each R is hydrogen. In another embodiment of the above formulae, at least one R is acyl. In another of the above formulae, at least one R is optionally substituted alkoxy carbonyl.

- In another embodiment, the alpha/beta unsaturated aldehyde scavenger compound is hydralazine, cadralazine, dihydralazine, endralazine, phenelzine, or pharmaceutically acceptable salts of the foregoing, or combinations thereof.

In another embodiment, the alpha/beta unsaturated aldehyde scavenger compound is

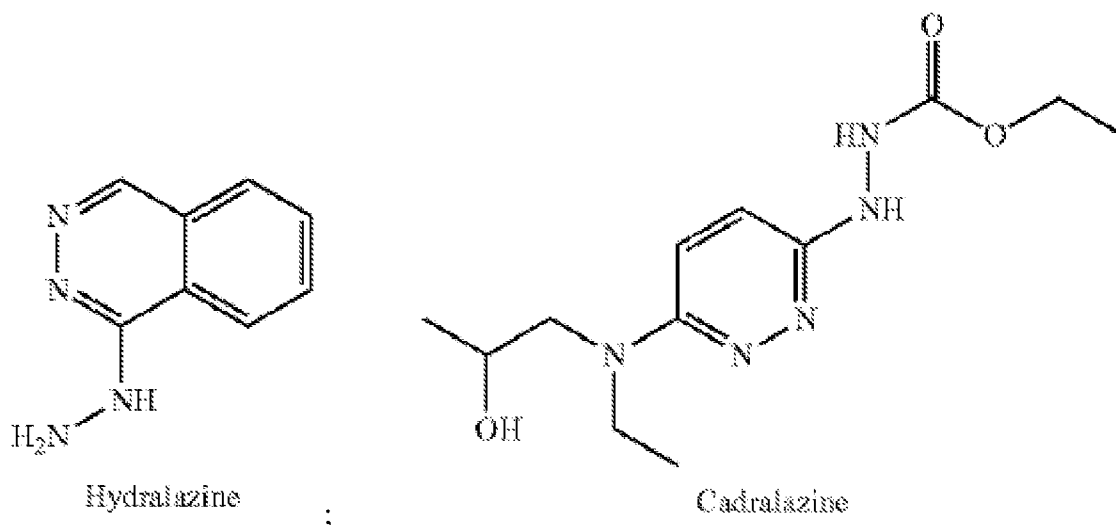


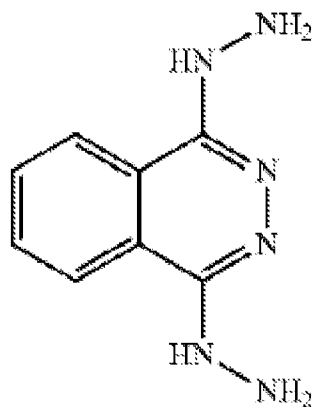
dimercaprol (). In other embodiments, the alpha/beta unsaturated aldehyde compound is N-acetylcysteine, phloretin, carnosine and homocarnosine, sodium

borohydride, sodium bisulfite, lipoic acid, (MESNA)--2-mercaptoethanesulfonate, or (D3T)--2-dithiole-3-thione.

Illustratively, hydralazine, a fused hydrazinopyridazine, is a compound that may be included in the compositions. It has been discovered herein that acrolein is significantly increased as a symptom of PD. It has also been discovered herein that the compounds and compositions described herein, such as hydralazine, phenelzine, dimercaprol and the like, are effective acrolein scavengers and may be used to trap acrolein in a patient with PD. It has been observed herein that acrolein is significantly increased when the behavioral deficits emerge in animal models. Illustratively, hydralazine, phenelzine, dimercaprol, and like treatments are efficacious in alleviating and/or reducing the symptoms of PD. Without being bound by theory, it is believed herein that the ability of the compounds like hydralazine and phenelzine and dimercaprol to treat PD is due at least in part to the capability of interacting with, blocking, or otherwise intervening in the pathology of acrolein (or other alpha/beta unsaturated aldehyde compounds) in vivo.

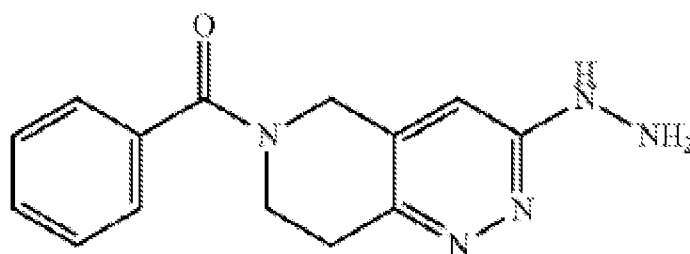
Illustrative hydrazinopyridazines and fused hydrazinopyridazines are of the formulae





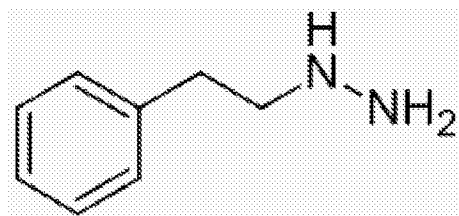
Dihydralazine

;



Endralazine

; or



;

Phenelzine

and pharmaceutically acceptable salts thereof, and analogs and derivatives of the foregoing.

The compositions of the invention may be formulated in unit dosage form. The compositions of the invention include a therapeutically effective amount of one or more compounds described herein that treats Parkinson's disease. In certain embodiments, the compositions of the invention include a therapeutically effective amount of one or more compounds described herein that treats Parkinson's disease, but is not therapeutically effective or clinically effective for treating hypertension. In another embodiment, the compositions of the invention includes a therapeutically effective amount of one or more compounds described herein that is at least about 2-fold, at least about 3-fold, at least about 4-fold, or at least about 5-fold lower than the therapeutically effective or clinically effective dose for treating hypertension.

In another embodiment, the compositions of the invention include a therapeutically effective amount of one or more compounds described herein that is at least about 10-fold, at least about 20-fold, at least about 30-fold, or at least about 50-fold lower than the therapeutically effective or clinically effective dose for treating hypertension. In another embodiment, the compositions of the invention include a therapeutically effective amount of one or more compounds described herein that is does not cause, or substantially cause, hypotension.

In another embodiment, the compositions of the invention include a therapeutically effective amount of one or more compounds described herein that is not therapeutically effective or clinically effective for treating depression or anxiety. In another embodiment, the compositions of the invention include a therapeutically effective amount of one or more compounds described herein that is at least about 2-fold, at least about 3-fold, at least about 4-fold, or at least about 5-fold lower than the therapeutically effective or clinically effective dose for treating depression or anxiety. In another embodiment, the compositions of the invention include a therapeutically effective amount of one or more compounds described herein that is at least about 10-fold, at least about 20-fold, at least about 30-fold, or at least about 50-fold lower than the therapeutically effective or clinically effective dose for treating depression or anxiety.

In another embodiment, the compositions of the invention include a therapeutically effective amount of one or more compounds described herein, such as the equivalent of about 0.01 mg/kg to about 2 mg/kg, about 0.01 mg/kg to about 1.5 mg/kg, about 0.01 mg/kg to about 1 mg/kg, or about 0.01 mg/kg to about 0.5 mg/kg, administered orally.

In another embodiment, the compositions of the invention include a therapeutically effective amount of one or more compounds described herein, such as the equivalent of about 0.05 mg/kg to about 2 mg/kg, about 0.05 mg/kg to about 1.5 mg/kg, about 0.05 mg/kg to about 1 mg/kg, or about 0.05 mg/kg to about 0.5 mg/kg, administered orally.

In another embodiment, the compositions of the invention include a therapeutically effective amount of one or more compounds described herein, such as the equivalent of about 0.1 mg/kg to about 2 mg/kg, about 0.1 mg/kg to about 1.5 mg/kg, about 0.1 mg/kg to about 1 mg/kg, or about 0.1 mg/kg to about 0.5 mg/kg, administered orally.

In another embodiment, the compositions of the invention include a therapeutically effective amount of one or more compounds described herein, such as the equivalent of about 0.5

mg/kg to about 5 mg/kg, about 0.5 mg/kg to about 3 mg/kg, about 0.5 mg/kg to about 2 mg/kg, about 0.5 mg/kg to about 1 mg/kg, administered orally.

In each of the foregoing embodiments, it is to be understood that the dose may be single or divided. In addition, it is to be understood that the therapeutically effective amount be administered following any of a wide variety of dosing schedules, including q.d., b.i.d., three times daily, four times daily, and the like.

Accordingly, an illustrative dosing schedule for an adult of average weight may be about 5 mg to 15 mg, p.o. twice, thrice, or four times daily, or about 5 mg to about 10 mg, p.o. twice, thrice, or four times daily.

In another embodiment, described herein are packages for daily administration of one or more compounds or compositions described herein according to the methods or uses described herein, including a unit dosage form as described above wherein the unit dosage form is a single or divided daily dose that sums to a daily amount of about 1 mg to about 50 mg of the compound, administered orally.

In another embodiment, described herein are packages for daily administration of one or more compounds or compositions described herein according to the methods or uses described herein, including a unit dosage form is a single or divided daily dose that sums to a daily amount of about 1 mg to about 40 mg of the compound, administered orally.

In another embodiment, described herein are packages for daily administration of one or more compounds or compositions described herein according to the methods or uses described herein, including a unit dosage form is a single or divided daily dose that sums to a daily amount of about 1 mg to about 30 mg of the compound, administered orally.

In another embodiment, described herein are packages for daily administration of one or more compounds or compositions described herein according to the methods or uses described herein, including a unit dosage form is a single or divided daily dose that sums to a daily amount of about 1 mg to about 25 mg of the compound, administered orally.

In another embodiment, described herein are packages for daily administration of one or more compounds or compositions described herein according to the methods or uses described herein, including a unit dosage form is a single or divided daily dose that sums to a daily amount of about 1 mg to about 20 mg of the compound, administered orally.

In another embodiment, described herein are packages for daily administration of one or

more compounds or compositions described herein according to the methods or uses described herein, including a unit dosage form is a single or divided daily dose that sums to a daily amount of about 1 mg to about 15 mg of the compound, administered orally.

5 In another embodiment, described herein are packages for daily administration of one or more compounds or compositions described herein according to the methods or uses described herein, including a unit dosage form is a single or divided daily dose that sums to a daily amount of about 1 mg to about 10 mg of the compound, administered orally.

10 It is to be understood that other routes of administration may be used, including buccal, sublingual, parenteral, and the like. It is appreciated herein that when other routes of administration that lead to higher bioavailability are used, the illustrative oral doses described herein will be reduced accordingly.

In addition to the foregoing illustrative dosages and dosing protocols, it is to be understood that an effective amount of any one or a mixture of the compounds described herein can be readily determined by the attending diagnostician or physician by the use of known
15 techniques and/or by observing results obtained under analogous circumstances. In determining the effective amount or dose, a number of factors are considered by the attending diagnostician or physician, including, but not limited to the species of mammal, including human, its size, age, and general health, the specific disease or disorder involved, the degree of or involvement or the severity of the disease or disorder, the response of the individual patient, the particular
20 compound administered, the mode of administration, the bioavailability characteristics of the preparation administered, the dose regimen selected, the use of concomitant medication, and other relevant circumstances.

In another embodiment, described herein are pharmaceutical compositions, unit doses, and unit dosage forms as described above further comprising one or more carriers, diluents, or
25 excipients, or a combination thereof.

In making the pharmaceutical compositions of the compounds described herein, a therapeutically effective amount of one or more compounds in any of the various forms described herein may be mixed with one or more excipients, diluted by one or more excipients, or enclosed within such a carrier which can be in the form of a capsule, sachet, paper, or other
30 container. Excipients may serve as a diluent, and can be solid, semi-solid, or liquid materials, which act as a vehicle, carrier or medium for the active ingredient. Thus, the formulation

compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders. The compositions may contain anywhere from about 0.1% to about 99.9% active ingredients, depending upon the selected dose and dosage form.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxybenzoates; sweetening agents; and flavoring agents. The compositions can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art. It is appreciated that the carriers, diluents, and excipients used to prepare the compositions described herein are advantageously GRAS (generally regarded as safe) compounds.

In another embodiment, described herein is a method for treating a patient Parkinson's disease, the method comprising the step of administering to the patient a therapeutically effective amount of one or more compounds, as described herein, capable of scavenging acrolein or other alpha/beta unsaturated aldehyde compounds.

In each of the foregoing and following embodiments, it is to be understood that the formulae include and represent not only all pharmaceutically acceptable salts of the compounds, but also include any and all hydrates and/or solvates of the compound formulae. It is appreciated that certain functional groups, such as the hydroxy, amino, and like groups form complexes and/or coordination compounds with water and/or various solvents, in the various physical forms of the compounds. Accordingly, the above formulae are to be understood to include and represent those various hydrates and/or solvates. In each of the foregoing and following embodiments, it is also to be understood that the formulae include and represent each possible isomer, such as stereoisomers and geometric isomers, both individually and in any and all possible mixtures. In each of the foregoing and following embodiments, it is also to be understood that the formulae include and represent any and all crystalline forms, partially crystalline forms, and non crystalline and/or amorphous forms of the compounds.

As used herein, the terms hydrazinopyridazines, fused hydrazinopyridazines, and phenylethylhydrazines generally refer to the compounds described herein and analogs and derivatives thereof, but are not limited to those compounds. Other compounds that are hydrazinopyridazines, fused hydrazinopyridazines, and phenylethylhydrazines are also useful in the methods, uses, pharmaceutical compositions, formulations, and unit dosage forms described herein. It is also to be understood that in each of the foregoing, any corresponding pharmaceutically acceptable salt is also included in the illustrative embodiments described herein.

In preferred embodiments, the alpha/beta unsaturated aldehyde compound scavenger is hydralazine. Hydralazine is an excellent scavenger of reactive lipid oxidation products, such as acrolein and HNE, and also prevents the lipid modification and crosslinking of proteins. In addition to reducing reactive oxygen species (ROS) and lipid peroxidation, hydralazine also prevents aldehyde mediated cell death, NADPH/monoamine and xanthine oxidases, NOS and COX 2 enzyme activities. Without being limited by any particular theory or mechanism of action, the scavenging efficacy of hydralazine is primarily due to its atoms in the ring and hydralazide group. Additionally, hydralazine effectively reduced AB production in primary neuronal cells from the brains of the transgenic mouse model.

Illustrative derivatives include, but are not limited to, both those compounds that may be synthetically prepared from the compounds described herein, as well as those compounds that may be prepared in a similar way as those described herein, but differing in the selection of starting materials. For example, it is to be understood that derivatives of those compounds also include the compounds having for example different functional groups on aromatic rings than those explicitly set forth in the compound genera described herein. In addition, it is to be understood that derivatives of those compounds also include the compounds having those same or different functional groups at different positions on the aromatic ring.

It is to be understood that such derivatives may include prodrugs of the compounds described herein, compounds described herein that include one or more protection or protecting groups, including compounds that are used in the preparation of other compounds described herein.

Illustrative analogs include, but are not limited to, those compounds that share functional and in some cases structural similarity to those compounds described herein. For example,

described herein are compounds that include a benzopyridazine ring system. Illustrative analogs include, but are not limited to, the corresponding ring expanded or ring contracted compounds, and the like. Other illustrative analogs include, but are not limited to, the corresponding ring systems that include additional heteroatoms, or where the ring fusion is made at a different pair of atoms, and the like.

In addition, as used herein the terms hydrazinopyridazines, fused hydrazinopyridazines, and phenylethylhydrazines also refer to prodrug derivatives of the compounds described herein, and including prodrugs of the various analogs and derivatives thereof. In addition, as used herein, the terms hydrazinopyridazines, fused hydrazinopyridazines, and phenylethylhydrazines also refer to both the amorphous as well as any and all morphological forms of each of the compounds described herein. In addition, as used herein, the terms hydrazinopyridazines, fused hydrazinopyridazines, and phenylethylhydrazines also refer to any and all hydrates, or other solvates, of the compounds described herein.

It is to be understood that each of the foregoing embodiments may be combined in chemically relevant ways to generate subsets of the embodiments described herein. Accordingly, it is to be further understood that all such subsets are also illustrative embodiments of the invention described herein. For example, in another embodiment, when each R is hydrogen, R^A includes an optionally substituted benzo group; or when at least one R is acyl, R^A includes a hydrazine; or when at least one R is acyl, R^A includes an optionally substituted benzo group; and the like.

The compounds described herein may contain one or more chiral centers, or may otherwise be capable of existing as multiple stereoisomers. It is to be understood that in one embodiment, the invention described herein is not limited to any particular stereochemical requirement, and that the compounds, and compositions, methods, uses, and medicaments that include them may be optically pure, or may be any of a variety of stereoisomeric mixtures, including racemic and other mixtures of enantiomers, other mixtures of diastereomers, and the like. It is also to be understood that such mixtures of stereoisomers may include a single stereochemical configuration at one or more chiral centers, while including mixtures of stereochemical configuration at one or more other chiral centers.

Similarly, the compounds described herein may include geometric centers, such as cis, trans, E, and Z double bonds. It is to be understood that in another embodiment, the invention

described herein is not limited to any particular geometric isomer requirement, and that the compounds, and compositions, methods, uses, and medicaments that include them may be pure, or may be any of a variety of geometric isomer mixtures. It is also to be understood that such mixtures of geometric isomers may include a single configuration at one or more double bonds, while including mixtures of geometry at one or more other double bonds.

In certain embodiments, additional compounds are combined with the composition that includes an alpha/beta unsaturated aldehyde scavenger compound. Such additional compounds can off-set or counter certain side-effects of the alpha/beta unsaturated aldehyde scavenger compound. For example, hydralazine, an alpha/beta unsaturated aldehyde scavenger compound, has vasoactive effects. The anti-hypertensive effect can result in tachyphylaxis which can be reduced by co administration of Beta Blockers and a diuretic. Accordingly, certain embodiments of the invention provide a composition that includes a combination of an alpha/beta unsaturated aldehyde scavenger compound (such as hydralazine), with beta blocker, a diuretic, or a combination thereof. Exemplary beta blockers include Propranolol, Bucindolol, Carteolol, Carvedilol, Labetalol, Nadolol, Oxprenolol, Penbutolol, Pindolol, Sotalol, Timolol, Eucommia bark, Acebutolol, Atenolol, Betaxolol, Bisoprolol, Celiprolol, Esmolol, Metoprolol, Butaxamine, and Nebivolol. Diuretics include loop diuretics (such as furosemide), Thiazide-type diuretics such as hydrochlorothiazide, Carbonic anhydrase inhibitors, diuretics which do not promote the secretion of potassium into the urine (Aldosterone antagonists or Epithelial sodium channel blockers), calcium-sparing diuretic, osmotic diuretics (e.g. mannitol or glucose).

In other embodiments, the alpha/beta unsaturated aldehyde scavenger compound is combined with a vasoconstrictor. Exemplary vasoconstrictors include 25I-NBOMe, Amphetamines, AMT, Antihistamines, Caffeine, DOM, LSA, Methylphenidate, Mephedrone, Oxymetazoline, Phenylephrine, Propylhexedrine, Pseudoephedrine, Stimulants, and Tetrahydrozoline hydrochloride.

As used herein, the term "alkyl" includes a chain of carbon atoms, which is optionally branched. As used herein, the term "alkenyl" and "alkynyl" includes a chain of carbon atoms, which is optionally branched, and includes at least one double bond or triple bond, respectively. It is to be understood that alkynyl may also include one or more double bonds. It is to be further understood that in certain embodiments, alkyl is advantageously of limited length, including C₁-C₂₄, C₁-C₁₂, C₁-C₈, C₁-C₆, and C₁-C₄. It is to be further understood that in certain embodiments

alkenyl and/or alkynyl may each be advantageously of limited length, including C₂-C₂₄, C₂-C₁₂, C₂-C₈, C₂-C₆, and C₂-C₄. It is appreciated herein that shorter alkyl, alkenyl, and/or alkynyl groups may add less lipophilicity to the compound and accordingly will have different pharmacokinetic behavior. Illustrative alkyl groups are, but not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, 2-pentyl, 3-pentyl, neopentyl, hexyl, heptyl, octyl and the like.

As used herein, the term "cycloalkyl" includes a chain of carbon atoms, which is optionally branched, where at least a portion of the chain is cyclic. It is to be understood that cycloalkylalkyl is a subset of cycloalkyl. It is to be understood that cycloalkyl may be polycyclic.

Illustrative cycloalkyl include, but are not limited to, cyclopropyl, cyclopentyl, cyclohexyl, 2-methylcyclopropyl, cyclopentyleth-2-yl, adamantyl, and the like. As used herein, the term "cycloalkenyl" includes a chain of carbon atoms, which is optionally branched, and includes at least one double bond, where at least a portion of the chain is cyclic. It is to be understood that the one or more double bonds may be in the cyclic portion of cycloalkenyl and/or the non-cyclic portion of cycloalkenyl. It is to be understood that cycloalkenylalkyl and cycloalkylalkenyl are each subsets of cycloalkenyl. It is to be understood that cycloalkyl may be polycyclic. Illustrative cycloalkenyl include, but are not limited to, cyclopentenyl, cyclohexylethen-2-yl, cycloheptenylpropenyl, and the like. It is to be further understood that chain forming cycloalkyl and/or cycloalkenyl is advantageously of limited length, including C₃-C₂₄, C₃-C₁₂, C₃-C₈, C₃-C₆, and C₅-C₆. It is appreciated herein that shorter alkyl and/or alkenyl chains forming cycloalkyl and/or cycloalkenyl, respectively, may add less lipophilicity to the compound and accordingly will have different pharmacokinetic behavior.

As used herein, the term "heteroalkyl" includes a chain of atoms that includes both carbon and at least one heteroatom, and is optionally branched. Illustrative heteroatoms include nitrogen, oxygen, and sulfur. In certain variations, illustrative heteroatoms also include phosphorus, and selenium. As used herein, the term "cycloheteroalkyl" including heterocyclyl and heterocycle, includes a chain of atoms that includes both carbon and at least one heteroatom, such as heteroalkyl, and is optionally branched, where at least a portion of the chain is cyclic. Illustrative heteroatoms include nitrogen, oxygen, and sulfur. In certain variations, illustrative heteroatoms also include phosphorus, and selenium. Illustrative cycloheteroalkyl include, but are

not limited to, tetrahydrofuryl, pyrrolidinyl, tetrahydropyranyl, piperidinyl, morpholinyl, piperazinyl, homopiperazinyl, quinuclidinyl, and the like.

As used herein, the term "aryl" includes monocyclic and polycyclic aromatic carbocyclic groups, each of which may be optionally substituted. Illustrative aromatic carbocyclic groups described herein include, but are not limited to, phenyl, naphthyl, and the like. As used herein, the term "heteroaryl" includes aromatic heterocyclic groups, each of which may be optionally substituted. Illustrative aromatic heterocyclic groups include, but are not limited to, pyridinyl, pyrimidinyl, pyrazinyl, triazinyl, tetrazinyl, quinolinyl, quinazolinyl, quinoxalinyl, thienyl, pyrazolyl, imidazolyl, oxazolyl, thiazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, thiadiazolyl, triazolyl, benzimidazolyl, benzoxazolyl, benzthiazolyl, benzisoxazolyl, benzisothiazolyl, and the like.

As used herein, the term "amino" includes the group NH_2 , alkylamino, and dialkylamino, where the two alkyl groups in dialkylamino may be the same or different, i.e. alkylalkylamino. Illustratively, amino includes methylamino, ethylamino, dimethylamino, methylethylamino, and the like. In addition, it is to be understood that when amino modifies or is modified by another term, such as aminoalkyl, or acylamino, the above variations of the term amino are included therein. Illustratively, aminoalkyl includes H_2N -alkyl, methylaminoalkyl, ethylaminoalkyl, dimethylaminoalkyl, methylethylaminoalkyl, and the like. Illustratively, acylamino includes acylmethylamino, acylethylamino, and the like.

As used herein, the term "amino and derivatives thereof" includes amino as described herein, and alkylamino, alkenylamino, alkynylamino, heteroalkylamino, heteroalkenylamino, heteroalkynylamino, cycloalkylamino, cycloalkenylamino, cycloheteroalkylamino, cycloheteroalkenylamino, arylamino, arylalkylamino, arylalkenylamino, arylalkynylamino, heteroarylamino, heteroarylalkylamino, heteroarylalkenylamino, heteroarylalkynylamino, acylamino, and the like, each of which is optionally substituted. The term "amino derivative" also includes urea, carbamate, and the like.

As used herein, the term "hydroxy and derivatives thereof" includes OH, and alkyloxy, alkenyloxy, alkynyloxy, heteroalkyloxy, heteroalkenyloxy, heteroalkynyloxy, cycloalkyloxy, cycloalkenyloxy, cycloheteroalkyloxy, cycloheteroalkenyloxy, aryloxy, arylalkyloxy, arylalkenyloxy, arylalkynyloxy, heteroaryloxy, heteroarylalkyloxy, heteroarylalkenyloxy,

heteroarylalkynyloxy, acyloxy, and the like, each of which is optionally substituted. The term "hydroxy derivative" also includes carbamate, and the like.

As used herein, the term "thio and derivatives thereof" includes SH, and alkylthio, alkenylthio, alkynylthio, heteroalkylthio, heteroalkenylthio, heteroalkynylthio, cycloalkylthio, cycloalkenylthio, cycloheteroalkylthio, cycloheteroalkenylthio, arylthio, arylalkylthio, arylalkenylthio, arylalkynylthio, heteroarylthio, heteroarylalkylthio, heteroarylalkenylthio, heteroarylalkynylthio, acylthio, and the like, each of which is optionally substituted. The term "thio derivative" also includes thiocarbamate, and the like.

As used herein, the term "acyl" includes formyl, and alkylcarbonyl, alkenylcarbonyl, alkynylcarbonyl, heteroalkylcarbonyl, heteroalkenylcarbonyl, heteroalkynylcarbonyl, cycloalkylcarbonyl, cycloalkenylcarbonyl, cycloheteroalkylcarbonyl, cycloheteroalkenylcarbonyl, arylcarbonyl, arylalkylcarbonyl, arylalkenylcarbonyl, arylalkynylcarbonyl, heteroarylcarbonyl, heteroarylalkylcarbonyl, heteroarylalkenylcarbonyl, heteroarylalkynylcarbonyl, acylcarbonyl, and the like, each of which is optionally substituted.

As used herein, the term "carbonyl and derivatives thereof" includes the group C(O), C(S), C(NH) and substituted amino derivatives thereof.

As used herein, the term "carboxylate and derivatives thereof" includes the group CO₂H and salts thereof, and esters and amides thereof, and CN.

As used herein, the term "sulfinyl or a derivative thereof" includes SO₂H and salts thereof, and esters and amides thereof.

As used herein, the term "sulfonyl or a derivative thereof" includes SO₃H and salts thereof, and esters and amides thereof.

As used herein, the term "hydroxylamino and derivatives thereof" includes NHOH, and alkyloxylnH alkenyloxylnH alkynyloxylnH heteroalkyloxylnH heteroalkenyloxylnH heteroalkynyloxylnH cycloalkyloxylnH cycloalkenyloxylnH cycloheteroalkyloxylnH cycloheteroalkenyloxylnH aryloxylnH arylalkyloxylnH arylalkenyloxylnH arylalkynyloxylnH heteroaryloxylnH heteroarylalkyloxylnH heteroarylalkenyloxylnH heteroarylalkynyloxylnH acyloxy, and the like, each of which is optionally substituted.

As used herein, the term "hydrazino and derivatives thereof" includes alkylNHNH, alkenylNHNH, alkynylNHNH, heteroalkylNHNH, heteroalkenylNHNH, heteroalkynylNHNH, cycloalkylNHNH, cycloalkenylNHNH, cycloheteroalkylNHNH, cycloheteroalkenylNHNH,

arylNHNH, arylalkylNHNH, arylalkenylNHNH, arylalkynylNHNH, heteroarylNHNH, heteroarylalkylNHNH, heteroarylalkenylNHNH, heteroarylalkynylNHNH, acylNHNH, and the like, each of which is optionally substituted.

The term "optionally substituted" as used herein includes the replacement of hydrogen atoms with other functional groups on the radical that is optionally substituted. Such other functional groups illustratively include, but are not limited to, amino, hydroxyl, halo, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, heteroaryl, heteroarylalkyl, heteroarylheteroalkyl, nitro, sulfonic acids and derivatives thereof, carboxylic acids and derivatives thereof, and the like. Illustratively, any of amino, hydroxyl, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, heteroaryl, heteroarylalkyl, heteroarylheteroalkyl, and/or sulfonic acid is optionally substituted.

As used herein, the terms "optionally substituted aryl" and "optionally substituted heteroaryl" include the replacement of hydrogen atoms with other functional groups on the aryl or heteroaryl that is optionally substituted. Such other functional groups illustratively include, but are not limited to, amino, hydroxyl, halo, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, heteroaryl, heteroarylalkyl, heteroarylheteroalkyl, nitro, sulfonic acids and derivatives thereof, carboxylic acids and derivatives thereof, and the like. Illustratively, any of amino, hydroxyl, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, heteroaryl, heteroarylalkyl, heteroarylheteroalkyl, and/or sulfonic acid is optionally substituted.

Illustrative substituents include, but are not limited to, a radical $-(CH_2)_xZ^X$, where x is an integer from 0-6 and Z^X is selected from halogen, hydroxy, alkanoyloxy, including C_1 - C_6 alkanoyloxy, optionally substituted aroyloxy, alkyl, including C_1 - C_6 alkyl, alkoxy, including C_1 - C_6 alkoxy, cycloalkyl, including C_3 - C_8 cycloalkyl, cycloalkoxy, including C_3 - C_8 cycloalkoxy, alkenyl, including C_2 - C_6 alkenyl, alkynyl, including C_2 - C_6 alkynyl, haloalkyl, including C_1 - C_6 haloalkyl, haloalkoxy, including C_1 - C_6 haloalkoxy, halocycloalkyl, including C_3 - C_8 halocycloalkyl, halocycloalkoxy, including C_3 - C_8 halocycloalkoxy, amino, C_1 - C_6 alkylamino, $(C_1$ - C_6 alkyl) $(C_1$ - C_6 alkyl)amino, alkylcarbonylamino, N -- $(C_1$ - C_6 alkyl)alkylcarbonylamino, aminoalkyl, C_1 - C_6 alkylaminoalkyl, $(C_1$ - C_6 alkyl) $(C_1$ - C_6 alkyl)aminoalkyl, alkylcarbonylaminoalkyl, N -- $(C_1$ - C_6 alkyl)alkylcarbonylaminoalkyl, cyano, and nitro; or Z^X is selected from $--CO_2R^4$ and $--CONR^5R^6$, where R^4 , R^5 , and R^6 are each independently selected in each occurrence from hydrogen, C_1 - C_6 alkyl, aryl- C_1 - C_6 alkyl, and heteroaryl- C_1 - C_6 alkyl.

The term "prodrug" as used herein generally refers to any compound that when administered to a biological system generates a biologically active compound as a result of one or more spontaneous chemical reaction(s), enzyme-catalyzed chemical reaction(s), and/or metabolic chemical reaction(s), or a combination thereof. In vivo, the prodrug is typically acted upon by an enzyme (such as esterases, amidases, phosphatases, and the like), simple biological chemistry, or other process in vivo to liberate or regenerate the more pharmacologically active drug. This activation may occur through the action of an endogenous host enzyme or a non-endogenous enzyme that is administered to the host preceding, following, or during administration of the prodrug. Additional details of prodrug use are described in U.S. Pat. No. 5,627,165; and Pathalk et al., *Enzymic protecting group techniques in organic synthesis*, Stereosel. Biocatal. 775-797 (2000). It is appreciated that the prodrug is advantageously converted to the original drug as soon as the goal, such as targeted delivery, safety, stability, and the like is achieved, followed by the subsequent rapid elimination of the released remains of the group forming the prodrug.

Prodrugs may be prepared from the compounds described herein by attaching groups that ultimately cleave in vivo to one or more functional groups present on the compound, such as --OH-, --SH, --CO₂H, --NR₂. Illustrative prodrugs include but are not limited to carboxylate esters where the group is alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, acyloxyalkyl, alkoxycarbonyloxyalkyl as well as esters of hydroxyl, thiol and amines where the group attached is an acyl group, an alkoxycarbonyl, aminocarbonyl, phosphate or sulfate. Illustrative esters, also referred to as active esters, include but are not limited to 1-indanyl, N-oxysuccinimide; acyloxyalkyl groups such as acetoxymethyl, pivaloyloxymethyl, .beta.-acetoxylethyl, .beta.-pivaloyloxyethyl, 1-(cyclohexylcarbonyloxy)prop-1-yl, (1-aminoethyl)carbonyloxymethyl, and the like; alkoxycarbonyloxyalkyl groups, such as ethoxycarbonyloxymethyl, .alpha.-ethoxycarbonyloxyethyl, .beta.-ethoxycarbonyloxyethyl, and the like; dialkylaminoalkyl groups, including di-lower alkylamino alkyl groups, such as dimethylaminomethyl, dimethylaminoethyl, diethylaminomethyl, diethylaminoethyl, and the like; 2-(alkoxycarbonyl)-2-alkenyl groups such as 2-(isobutoxycarbonyl) pent-2-enyl, 2-(ethoxycarbonyl)but-2-enyl, and the like; and lactone groups such as phthalidyl, dimethoxyphthalidyl, and the like.

Further illustrative prodrugs contain a chemical moiety, such as an amide or phosphorus group functioning to increase solubility and/or stability of the compounds described herein.

Further illustrative prodrugs for amino groups include, but are not limited to, (C₃-C₂₀)alkanoyl; halo-(C₃-C₂₀)alkanoyl; (C₃-C₂₀)alkenoyl; (C₄-C₇)cycloalkanoyl; (C₃-C₆)-cycloalkyl(C₂-C₁₆)alkanoyl; optionally substituted aroyl, such as unsubstituted aroyl or aroyl substituted by 1 to 3 substituents selected from the group consisting of halogen, cyano,

5 trifluoromethanesulphonyloxy, (C₁-C₃)alkyl and (C₁-C₃)alkoxy, each of which is optionally further substituted with one or more of 1 to 3 halogen atoms; optionally substituted aryl(C₂-C₁₆)alkanoyl and optionally substituted heteroaryl(C₂-C₁₆)alkanoyl, such as the aryl or heteroaryl radical being unsubstituted or substituted by 1 to 3 substituents selected from the group consisting of halogen, (C₁-C₃)alkyl and (C₁-C₃)alkoxy, each of which is optionally further
10 substituted with 1 to 3 halogen atoms; and optionally substituted heteroarylalkanoyl having one to three heteroatoms selected from O, S and N in the heteroaryl moiety and 2 to 10 carbon atoms in the alkanoyl moiety, such as the heteroaryl radical being unsubstituted or substituted by 1 to 3 substituents selected from the group consisting of halogen, cyano, trifluoromethanesulphonyloxy, (C₁-C₃)alkyl, and (C₁-C₃)alkoxy, each of which is optionally
15 further substituted with 1 to 3 halogen atoms. The groups illustrated are exemplary, not exhaustive, and may be prepared by conventional processes.

It is understood that the prodrugs themselves may not possess significant biological activity, but instead undergo one or more spontaneous chemical reaction(s), enzyme-catalyzed chemical reaction(s), and/or metabolic chemical reaction(s), or a combination thereof after
20 administration in vivo to produce the compound described herein that is biologically active or is a precursor of the biologically active compound. However, it is appreciated that in some cases, the prodrug is biologically active. It is also appreciated that prodrugs may often serve to improve drug efficacy or safety through improved oral bioavailability, pharmacodynamic half-life, and the like. Prodrugs also refer to derivatives of the compounds described herein that
25 include groups that simply mask undesirable drug properties or improve drug delivery. For example, one or more compounds described herein may exhibit an undesirable property that is advantageously blocked or minimized may become pharmacological, pharmaceutical, or pharmacokinetic barriers in clinical drug application, such as low oral drug absorption, lack of site specificity, chemical instability, toxicity, and poor patient acceptance (bad taste, odor, pain at
30 injection site, and the like), and others. It is appreciated herein that a prodrug, or other strategy

using reversible derivatives, can be useful in the optimization of the clinical application of a drug.

The term "therapeutically effective amount" as used herein, refers to that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated. In one aspect, the therapeutically effective amount is that which may treat or alleviate the disease or symptoms of the disease at a reasonable benefit/risk ratio applicable to any medical treatment. However, it is to be understood that the total daily usage of the compounds and compositions described herein may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors, including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, gender and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidentally with the specific compound employed; and like factors well known to the researcher, veterinarian, medical doctor or other clinician of ordinary skill

It is also appreciated that the therapeutically effective amount, whether referring to monotherapy or combination therapy, is advantageously selected with reference to any toxicity, or other undesirable side effect, that might occur during administration of one or more of the compounds described herein. Further, it is appreciated that the co-therapies described herein may allow for the administration of lower doses of compounds that show such toxicity, or other undesirable side effect, where those lower doses are below thresholds of toxicity or lower in the therapeutic window than would otherwise be administered in the absence of a cotherapy.

As used herein, the term "composition" generally refers to any product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combinations of the specified ingredients in the specified amounts. It is to be understood that the compositions described herein may be prepared from isolated compounds described herein or from salts, solutions, hydrates, solvates, and other forms of the compounds described herein. It is also to be understood that the compositions may be prepared from various

amorphous, non-amorphous, partially crystalline, crystalline, and/or other morphological forms of the compounds described herein. It is also to be understood that the compositions may be prepared from various hydrates and/or solvates of the compounds described herein. Accordingly, such pharmaceutical compositions that recite compounds described herein are to be understood to include each of, or any combination of, the various morphological forms and/or solvate or hydrate forms of the compounds described herein. Illustratively, compositions may include one or more carriers, diluents, and/or excipients. The compounds described herein, or compositions containing them, may be formulated in a therapeutically effective amount in any conventional dosage forms appropriate for the methods described herein. The compounds described herein, or compositions containing them, including such formulations, may be administered by a wide variety of conventional routes for the methods described herein, and in a wide variety of dosage formats, utilizing known procedures (see generally, Remington: The Science and Practice of Pharmacy, (21st ed., 2005)).

The term “providing” as used herein includes administering, but can be more than administering, such as a manufacturer providing the composition to a supplier or a doctor for administering. The term “administering” as used herein includes all means of introducing the compounds and compositions described herein to the patient, including, but are not limited to, oral (po), intravenous (iv), intramuscular (im), subcutaneous (sc), transdermal, inhalation, buccal, ocular, sublingual, vaginal, rectal, and the like. The compounds and compositions described herein may be administered in unit dosage forms and/or formulations containing conventional nontoxic pharmaceutically-acceptable carriers, adjuvants, and vehicles.

It is to be understood that in the methods described herein, the individual components of a co-administration, or combination can be administered by any suitable means, contemporaneously, simultaneously, sequentially, separately or in a single pharmaceutical formulation. Where the co-administered compounds or compositions are administered in separate dosage forms, the number of dosages administered per day for each compound may be the same or different. The compounds or compositions may be administered via the same or different routes of administration. The compounds or compositions may be administered according to simultaneous or alternating regimens, at the same or different times during the course of the therapy, concurrently in divided or single forms.

Illustrative routes of oral administration include tablets, capsules, elixirs, syrups, and the like.

Illustrative routes for parenteral administration include intravenous, intraarterial, intraperitoneal, epidural, intraurethral, intrasternal, intramuscular and subcutaneous, as well as any other art recognized route of parenteral administration. Illustrative means of parenteral administration include needle (including microneedle) injectors, needle-free injectors and infusion techniques, as well as any other means of parenteral administration recognized in the art. Parenteral formulations are typically aqueous solutions which may contain excipients such as salts, carbohydrates and buffering agents (preferably at a pH in the range from about 3 to about 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water. The preparation of parenteral formulations under sterile conditions, for example, by lyophilization, may readily be accomplished using standard pharmaceutical techniques well known to those skilled in the art. Parenteral administration of a compound is illustratively performed in the form of saline solutions or with the compound incorporated into liposomes. In cases where the compound in itself is not sufficiently soluble to be dissolved, a solubilizer such as ethanol can be applied.

The dosage of each compound of the claimed combinations depends on several factors, including: the administration method, the condition to be treated, the severity of the condition, whether the condition is to be treated or prevented, and the age, weight, and health of the person to be treated. Additionally, pharmacogenomic (the effect of genotype on the pharmacokinetic, pharmacodynamic or efficacy profile of a therapeutic) information about a particular patient may affect the dosage used.

The effective use of the compounds, compositions, and methods described herein for treating or ameliorating one or more effects of PD using one or more compounds described herein may be based upon animal models, such as murine, canine, porcine, and non-human primate animal models of disease. For example, it is understood that PD in humans may be characterized by a loss of function, and/or the development of symptoms, each of which may be elicited in animals, such as mice, and other surrogate test animals. In particular the animal PD model may be used to evaluate the methods of treatment and the pharmaceutical compositions described herein to determine the therapeutically effective amounts described herein.

Incorporation by Reference

References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made throughout this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes.

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Equivalents

Various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including references to the scientific and patent literature cited herein. The subject matter herein contains important information, exemplification and guidance that can be adapted to the practice of this invention in its various embodiments and equivalents thereof.

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EXAMPLES

In Examples herein show using a combination of both in vitro and in vivo testing, coupled with anatomical, functional and behavioral examination, in order to present evidence that acrolein plays a critical role in the pathogenesis in the 6-OHDA rat, a typical animal model of Parkinsons' diseases (PD). This is based on the findings that acrolein is elevated in 6-OHDA-injected rats, and behavioral deficits associated with 6-OHDA can be mimicked by injection of acrolein in a similar manner. The Examples also provide data that the pathogenic of acrolein in 6-OHDA rats is through the promotion of the oligomerization of alpha-synulien, the basis of the Lewy body formation, and a signature pathology in human PD. Hydralazine, an acrolein scavenger, when applied systemically, can significantly alleviate PD-like motor deficits and doparminergic cell death in 6-OHDA rats. Such neuroprotection offered by hydralazine in 6-OHDA rats can also be repeated in in vitro experimentation using cell cultures of Doparmingeic and PC-12 cells. These data are not only indicating a role of acrolein in the pathogenic of PD, but provide evidence of anti-acrolein therapy as an effective intervention to alleviate tissue damage and motor deficits associated with PD.

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Example 1: Material and methods

PC12 cells were grown in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, La

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Jolla, CA) supplemented with 12.5% horse serum, 2.5% fetal bovine serum, 50 U/ml penicillin, and 5 mg/ml streptomycin. The incubator was set at 5% CO₂ at 37°C. Culture media were changed every other day, and cells were split every week. Cells were switched from DMEM-supplemented culture medium to Hank's balanced salt solution (HBSS) before they were treated with 6 OHDA. 6 OHDA (Sigma, St. Louis, MO), was prepared fresh in phosphate-buffered saline (PBS) as stock solutions and diluted to the specific concentrations upon use. Hydralazine was dissolved at 30 mM, 45 mM, and 100 mM in double-distilled water as stock solutions. Hydralazine application was typically delayed for about 15 min after the application of 6 OHDA.

The MES23.5 is a mouse–rat hybrid dopaminergic cell line. The cells were routinely propagated in Sato's N1 medium (87.5% (v/v) DMEM, glutamine (4 mM), Newborn Calf Serum (NCS) (2%, v/v) Fetal Bovine Serum (FBS) (5%, v/v), penicillin/streptomycin (1%, v/v), 15 mM Hepes (pH 7.4), and 1X SATO (50X SATO: insulin, 0.25 mg/mL; human transferrin, 0.25 mg/mL; pyruvic acid, 2.43 mg/mL; putrescine, 0.2 mg/mL; sodium selenite, 0.25 µg/mL; progesterone, 0.315 µg/mL) as described were selected in media supplemented with G418 (600 µg/mL).

Trypan blue is a vital dye that is imbibed by cells after their membranes are damaged. Normally, undamaged cells exclude trypan blue, because the chromophore is negatively charged and cannot enter the cell in the absence of breaches to the membrane. All the cells excluding the dye were considered viable, whereas labeled cells were considered otherwise dying or dead. A cell suspension (0.5 ml, 1 x 10⁶ cells/ml in HBSS) was mixed thoroughly with 0.5 ml of 0.4% trypan blue for 2 min at room temperature. With a micropipette, 10 µl of the mixture was withdrawn to fill a hemocytometer on each side. The total number of cells and viable cells were counted under the light microscope. Percentage viability was calculated as:

$$\% \text{ Viability} = \frac{\text{Viable cells}}{\text{Total cells}} \times 100$$

The percentage viability was averaged by duplicate readings from both sides of the hemocytometer. Each experiment was repeated four times.

Cells were seeded in 12-well plates at 1 x 10⁶ cells/ml in HBSS. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) was reconstituted in PBS and added to each well

1 hr before the termination of the experiment. After incubation, an equal volume of MTT solubilization solution was added to each well to dissolve the remaining formazan crystals. The resulting absorbance was measured spectrophotometrically (SLT, Spectra) at 550 nm, and the background absorbance at 660 nm was subtracted from these values. For each experiment, the final MTT measurement for each sample was expressed as the percentage of control sample (no treatment).

Adult male Sprague-Dawley rats (weighing 250 – 300g) at the start of the experiment were obtained from Hilltop (Indianapolis, IN). Animals were housed under conditions of controlled temperature (25 OC) an illumination (12 h light; 12 h darkness) and free access to standard diet and water. Experiments were performed in compliance with the Purdue Animal Care Unit Center (PACUC) concerning the experimental use of animals.

Animal were anesthetized with a combination of ketamine (100 mg/kg) and xylazine (10 mg/Kg) and placed in a kofp sterotaxic instrument. With the head held firmly in place by sterotaxic frame, careful measurements were made of made the midline sagital suture bregma (the convergence of coronal suture with the sagital suture). A 2 cm midsagittal skin incision is made on the scalp to expose the skull. A dermal drill (size) was used to drill a hole in the skull to expose the dural matter.

A unilateral injection of 8 ug/2μL of 6-hydroxydopamine to the right side of the brain into the region of the MFB and substantia Nigra at a coordinate of AP: -1.5, ML: 4.0 lateral and 8.5 DV from bregma using a 10μL Hamilton syringes at a rate of 1 μl/min for a 2 minute duration into the nigrostriatal pathway. Sham operated animal received the vehicle (saline with ascorbic acid 0.01%) and used as injured control. In addition to this no injured animal control were used. (Aguiar, 2006). Following the 6-OHDA infusion, the infusion needle is allowed to sit in place for 5 minutes, then slowly withdrawn and the skin incision closed with stainless steel wound clips.

Acrolein was injected in a similar setting to the Medial Forebain Bundle (MFB) and Substantia nigra region(SN) a concentration 500uM/2uL. Acrolein was prepared from a net acrolein by preparing 1 Molar and diluting it to 500uM using saline and 0.01% ascorbic acid to prevent oxidation.

Hydralzine was injected to the lesioned animal through intraperitoneal injection (IP) every day for 15 days and every other day for the rest of the experiment.

Acrolein injection was made to the right side of the rat brain and the experiment was terminated in two weeks. After 15 days the animals were perfused with kreb's solutions and the brain sample was collected. Briefly, brain samples were homogenized by a glass homogenizer (Duell 21, Kontes Glass Co) with a bathing solution consisting of 3% Trion and anti-proteases: 2 mmol/L pefabloc, 15 μ mol/L pepstatin A, 20 μ g/mL aprotinin, and 25 μ g/mL leupeptin. The solution was centrifuged to pellet any large pieces of tissue for 30 minutes at 14000g after incubating on ice for at least 1 hr. Supernatants then were collected to store in -80°C and were kept for up to 2 weeks. An additional centrifugation was required before applying the sample with appropriate sample loading dye to the resolving gel system (7.5%). After heating the dye-sample mixture at 96°C for 3min, it was applied to the resolving gel. The sample was resolved under 200V usually for around 50 mins. Then the samples were further transferred from the resolving gel to the nitrocellulose membrane under 100 V for around 3 hr (Western Blot system, Bio-Rad). The membrane was blocked for 1 h in blocking buffer (0.2% casein and 0.1% Tween 20 in PBS). The membrane was then transferred to 1 : 1,000 polyclonal rabbit anti-acrolein (in blocking buffer with 2% goat serum and 0.025% sodium azide) (Novus Biologicals) for 18 h at 4 OC. The membrane was washed in blocking buffer and then transferred to 1: 10,000 alkaline phosphatase conjugated goat anti-rabbit IgG. The membrane was washed in blocking buffer followed by 0.1% Tween 20 in Tris-buffered saline. The membrane was then exposed to Bio-Rad Immuno-Star Substrate (Bio-Rad) and visualized by chemiluminescence. Density of bands was evaluated using Image J (NIH, Bethesda, MD, USA) and is expressed as % control values $\pm$ Standard Deviations (SD).

The rotarod test, developed in 1957 by Dunham and Miya, in which animals must balance on a rotating drum, is widely used to assess motor deficit in neurodegenerative disease models in rodents (Hammn et al.). Performance is measured by the duration that an animal stays on the rod as a function of drum speed.

The test of Rotarod was conducted based on that described by Jones & Roberts (1968). The rotarod was controlled by gradually increasing the speed of rotation led to greater sensitivity of the test. Animals are allowed first to remain stationary for 10s. The speed then gradually increased by 3 rpm per 10s until 30 rpm is reached (rotational speed at which naïve, uninjured rat will not fall of during 2 min test interval). The animal must remain on the apparatus for the remainder of the 2 min test interval at this 30 rpm speed. The trial will end if the rat completely

falls off the rungs, or grips the device and spins around twice without actually walking on the rungs.

The purpose of the Rotarod test is to assess the rat's sensor motor coordination and motor deficit, in which animals must balance on a rotating drum, in neurodegenerative disease models in rodents. The test is sensitive to damage in the basal ganglia and cerebellum and to drugs that affect motor function.

6-OHDA is a neurotransmitter analogue that depletes noradrenergic stores in nerve endings and induces a reduction of dopamine levels in the brain. Its mechanism of action is related to the production of free radicals and production of acrolein.

Four groups of rats were used, controls (no surgery), shame injury (surgery and saline), injured (surgery and 6-OHDA), and injured treated (surgery, 6 OHDA and hydralazine). Rats were tested at different time points after the lesion. We report that gradually increasing the speed of rotation is more sensitive to detect the presence of a lesion, the accelerating protocol provides a more discriminative test to correlate motor deficits against lesion.

Twelve hours after injury or sham, animals were placed in a Plexiglas activity box (100 cm×100 cm×20 cm) in a darkened room. Food was placed over the center of the box and the box was thoroughly cleaned with water between experiments to encourage the rat not to engage in thigmotaxic behavior. 4.5 cm off the ground, eight infrared beams in an X-Y matrix, 20 cm apart, were counted when broken by a Veeder-Root Series 7999 Mite Totalizer (ID# 79998D-110, Gurnee, IL). The lag time between counts was 14 ms. At 1/2 h, the amount of total beam breaks was tabulated.

Separate from the totalizer, custom-designed in house software recorded the state of the infrared beams at 200 ms intervals. With this data, the experimenter could determine the rat's position in space and time over the course of the experiment. The intent is to detect the animal's movement in real time.

After the last rotarod and behavioral tests, animals were deeply anaesthetized with ketamine and xylazine perfused transcardially with Krebs's solution containing ((115 mM NaCl, 5.9 mM KCl, 1.2 mM MgCl₂, 1.2 mM NaH₂PO₄, 1.2 mM Na₂SO₄, 2.5 mM CaCl₂, 25 mM NaHCO₃, 10 mM glucose, pH 7.4)) followed by a fixative solution containing 4% paraformaldehyde. Brains were removed and postfixed for 3 days. Cryoprotected with 15-30% sucrose and freezed with OCT compound and stored until sectioned. The brains were cut in 15

um sections on a cryostat and mounted on gelatin-coated slides. Serial coronal sections are made from rostral to caudal. One section for striatum and one section for substantia nigra are processed for tyrosine hydroxylase (TH) immunohistochemistry of dopamine-producing cells. These sections were washed in 1 M phosphate buffer and then incubated overnight at room temperature with anti-TH monoclonal antiserum (1: 10 000, Sigma). The sections then were processed by the ABC method (Vector, Vectastain, Burlingame, CA, USA) with anti-mouse antiserum IgG and horse serum and reacted with 3, 3'-diaminobenzidine tetrahydrochloride (0.6%), hydrogen peroxide (0.3%) and nickel solution. Some sections were processed to control for either monoclonal antiserum or antibody stain.

Example 2: 6-OHDA-mediated PC 12 cell death and its alleviation by hydralazine

Using the MTT test, we have found that 6-OHDA-mediated PC-12 cell death can be partially prevented by Hydralazine (FIG. 1 panel A). Specifically, in the presence of 6-OHDA cell viability was 38±3% of control value. However, cell viability increased to 66±7% when hydralazine was applied, a significant increase in cell viability compared to 6-OHDA only ($p < 0.001$ ANOVA, $n=6$ in all conditions). Similar results were obtained when cell viability was assessed using a trypan blue exclusion test (FIG. 1 panel B). In the control, about 80% of the cells excluded trypan blue. In the presence of 6-OHDA, there were only 48±2% of the PC 12 cells that were impermeable to trypan blue, a significant decrease was observed compared to the control group ($p < 0.01$). However, the addition of hydralazine led to an increase in the portion of the cells impermeable to trypan blue to 67.6±2, a significant increase ($p < 0.001$ ANOVA, $n=6$ in all conditions).

Example 3: 6-OHDA-mediated dopaminergic cell death and its alleviation by hydralazine

Using a dopaminergic cell line, we have found that 6-OHDA reduced cell survival (47.5±6%) which was significant compared to control (100%, $P < 0.001$) (FIG. 2 panel A). The addition of hydralazine (15 minutes after the incubation of 6-OHDA) can enhance cell viability to 71±1% ($P < 0.01$, ANOVA, $n = 9$ in all conditions). In another experiment, we applied hydralazine first for 30 min and then washed it off before the application of 6-OHDA. Therefore, there was little hydralazine in the extracellular space. This manipulation was meant to eliminate

the possibility, if any, that extracellular hydralazine will interfere with 6-OHDA before it entering cells. In such a manipulation, the cell viability was 75+5% ($P < 0.001$ compared to control). Similar results were also obtained using trypan blue exclusion test (FIG. 2 panel B). 6-OHDA reduced cell viability from a control level of 83+3% to 51.7+2%. This reduction can be partially and significantly reversed with the addition of hydralazine (71+1%, $P < 0.001$, $n=14$ in all conditions).

Example 4: Acrolein level is increased in 6-OHDA-injected rats and/or acrolein injected rats

It has been found that injection of either 6-OHDA or acrolein to the substantia nigra significantly increased the level of protein-bound acrolein detected 12 days after injection. Specifically, in 6-OHDA injected animals, proteins containing cross-linked acrolein could be detected at increased levels, particularly in a band near MW of 75 (FIG. 3 panel A). Such an increase could be mimicked to a greater extent when acrolein was injected in the same manner as 6-OHDA. Mean density of this band was analyzed and difference between various conditions was plotted in FIG. 3 panel B. A significant difference was revealed between 6-OHDA (0.602+0.17) and sham (0.226+0.12) ($P < 0.05$). Furthermore, the acrolein group was also significantly higher than 6-OHDA ($P < 0.05$) or sham group ($P < 0.001$).

Example 5: Motor deficits of 6-OHDA lesioned animals were alleviated by hydralazine examined using an activity box

6-OHDA lesioned rats showed a typical reduction of activity assessed by a well-established activity box (FIG. 4). There is a more-than-half reduction of distance travelled recorded for an hour induced by 6-OHDA (Fig. 5A). Specifically, uninjured rats (no surgery) traveled an average of 223+67 m within an hour. Rats in Sham group (surgery but no 6-OHDA) travelled about the similar distance, 245.5 + 71.2m ($P > 0.05$ when compared to control). In 6-OHDA injected rats, the travelling distance reduced to 97+32 m, a significant decrease compared to control or sham group ($P < 0.001$). Interestingly, acrolein injected in the same manner as that of 6-OHDA also produced reduction of the distance travelled similar to 6-OHDA (118+24, $P > 0.05$ when compared to 6-OHDA). However, 6-OHDA-induced reduction of distance travelled can be significantly alleviated by the application of daily injection of hydralazine. Hydralazine

application partially restored the distance travelled to 193.2 ± 19.5 , a significant increase compared to 6-OHDA group ($P < 0.01$) (FIG. 5 panel A).

When assessed by the area the rat covered within one hour, basically similar results as that assessed by the distance were obtained (FIG. 5 panel B). Specifically, 6-OHDA produced a significant decrease of area traveled compare to control and sham (43.3 ± 18.5 vs. 97.8 ± 2.1 or 94 ± 10.1 , $P < 0.01$). Again, acrolein injection in a similar manner as that of 6-OHDA can also cause reduction of area of covered by rat (64.5 ± 6.9 , $P < 0.05$ when compared to control, 97.8 ± 2.1 ; or shame group, 94 ± 10.1). However, 6-OHDA-mediated reduction of area travelled can be significantly reversed when hydralazine was applied daily for two weeks ($P < 0.01$ comparison between 6-OHDA, and 6-OHDA plus hydralazine, 43.3 ± 18.5 vs 80 ± 5.6).

Example 6: 6-OHDA-induced motor deficits and their alleviation by hydralazine based on Rotarod activity

It has been found that 6-OHDA produced a reduction of maximal speed that rat can sustain in a rotarod, an established behavioral test examining the motor capability (Fig 6). Specifically, the maximal speed for both control (no surgery) and shame (surgery and saline injected) are 29.7 and 28.9 rpm respectively. 6-OHDA or acrolein significantly reduced this value to 13.6 ± 3 and 17.2 ± 1.4 respectively ($P < 0.05$). Hydralazine injection in 6-OHDA treated rats has resulted a maximal speed of 20.1 ± 2.1 , which is significantly higher that 6-OHDA alone (13.6 ± 3.0 , $P < 0.05$) (FIG. 6 panel A).

When the maximal time that a rat can hang on to a moving rotarod at a speed of 30 rpm was used an indicator, similar results were obtained. Specifically, the maximal time for both control (no surgery) and shame (surgery and saline) are 174.9 ± 8.3 and 165 ± 13.4 sec. respectively. 6-OHDA or acrolein will reduce this value to 70.9 ± 10.7 and 85.4 ± 12.5 sec respectively. Hydralazine injection in 6-OHDA-treated rats has resulted a maximal time of 93.1 ± 17.9 sec, which is significantly higher that 6-OHDA alone (70.9 ± 10.7 sec, $P < 0.05$) (FIG. 6 panel B).

Example 7: Acrolein increases Alpha synuclein aggregation in rats

It has been found that injection of acrolein can significantly enhance alpha-synuclein aggregation detected 12 days after injection (FIG. 7 panel A). Specifically, in acrolein injected

animals, there is a significant increase of higher molecular alpha-synuclein detected by antibody that specifically targeting alpha-synuclein. Such increase is most obvious at a band near MW of approximately 37. Mean density of this band was analyzed and difference between various conditions were plotted in FIG. 7 panel B. It is clear that acrolein produced a band with a value of 3.51 ± 0.99 unit that significantly greater than shame (1 ± 0.43 unit) or antibody control (0.34 ± 0.25 unit) ($P < 0.001$).

Example 8: The reduction of DA neuron labeling in 6-OHDA rats and its attenuation by hydralazine

It has been found that 6-OHDA injection resulted in a 93.75% reduction of TH immunoreactivity examined at the striatum in a brain coronal section (FIG. 8 panels A and D) compared to control rats (FIG. 8 panels B and E). However, Systemic treatment of hydralazine at a dosage of 5mg/Kg improved TH immunoreactivity significantly by 4 fold in 6-OHDA-treated rats (FIG. 8 panel C).

Example 9: Alpha/beta unsaturated aldehyde scavenger compounds reduce or eliminate the symptoms of Parkinson's disease

Using a combination of both in vitro and in vivo testing, coupled with anatomical, functional and behavioral examination, evidence has been gathered indicating that acrolein plays a critical role in the pathogenesis in the 6-OHDA rat, a typical animal- model of PD. First, it was found that level of acrolein-lysine adducts is significantly elevated in the rat mid-brain 3 weeks following the injection of 6-OHDA. Secondly, injection of acrolein to the midbrain can produce PD-like motor deficits that are similar to those injected with 6-OHDA. Thirdly, application of hydralazine, an effective acrolein scavenger, can significantly alleviate PD-like motor deficits in 6-OHDA rats. Additionally, it has been found that hydralazine therapy has the capability to mitigate the death of dopaminergic cells, labeled as tyrosine positive cells, in the 6-OHDA rat. Finally, in cell culture experiments using two types of cells, it was noted that 6-OHDA-induced cell death can be effectively reduced with treatment using hydralazine. These data indicate that acrolein can cause significant neuronal cell death. The data shows that acrolein plays a critical role in mediating the 6-OHDA-induced neuropathology in both an animal and in vitro model of PD.

The increase in acrolein-lys adducts in 6-OHDA and acrolein-injected rats from this study is most consistently associated with the 75 Kd region (FIG. 3 panel A). In a post spinal cord injury model, we have found a wide spread and consistent increase of acrolein-lys adducts across many molecular weights . The reason for this discrepancy may lie in the nature of the injury. In trauma, the increase of acrolein is likely sudden and severe, potentially affecting a wide variety of proteins while in 6-OHDA rats the level of acrolein is significantly lower suggesting that it may take a significantly longer time period to develop a level of tissue damage able to produce pathological symptoms. In addition, instead of targeting a wide range of proteins when elevated acrolein levels are observed in trauma, the significantly lower level of acrolein measured in neurodegenerative disease models may result in the damage of a much more focused, yet critical group of proteins such as alpha-synuclein.

The western blot used in this study to estimate the level of acrolein is a semi-quantitative method. Therefore, the exact concentration of acrolein-lysine adducts is not currently known in the 6-OHDA rat model of PD. However it appears that acrolein-adducts are increased by nearly three times in the mid brain of 6-OHDA-treated rats (FIG. 3 panels A-B). Lovell and his colleagues reported that the acrolein levels were increased almost three times in amygdala region in human AD brain compared to healthy controls. Therefore, the ratio of increase of acrolein in amygdala region in AD patients appears to be consistent with our results observed the mid brain of PD-model animals.

It is noted that the toxic concentration (or threshold) of acrolein in vivo is probably significantly lower than the concentration used in in vitro studies (1-100 μM) due to longer exposures; hours in vitro vs. days in injury, or months to years in chronic neurodegenerative diseases. For example, it has been found that acrolein increased for 7 days following injury and 14 days after injection of 6-OHDA in rats. Thus, acrolein concentrations significantly less than 1 μM are likely to be toxic following prolonged exposure in vivo in the case of chronic neurodegenerative diseases.

This notion has two significant implications. First, it indicates that the expected decreased acrolein threshold in chronic exposures makes it easier for acrolein to inflict cellular damage in various pathological conditions. This is based on our previous study showing that acrolein cytotoxicity is dependent on incubation time, as in addition to the concentration of acrolein (). Indeed, chronic illnesses such as cancer, smoke-related illnesses, kidney diseases, and diabetes

have all been linked to acrolein exposure. Assuming the level of acrolein in the mid brain is similar to that in amygdala region, we can estimate a threefold-increase of acrolein in 6-OHDA rats reaching a level of 270 $\mu\text{mol/L}$ from baseline level. Considering it may take years to reach this level of acrolein in humans while it only takes less than three weeks to achieve this in rat following 6-OHDA injection, we conclude that the accelerated increase in acrolein may contribute to the swift induction of PD-like pathology in the rat model compared to humans, days vs years. This is supported by the current study that hydralazine can reduce cellular damage and motor effects associated with PD. Therefore, a correlation of acrolein concentration and the development of PD would offer a powerful clue as to the acrolein-mediated mechanism of PD as well as serve as a diagnostic and prognostic tool for PD.

The second significance of lower toxic level of acrolein in chronic diseases is that the therapeutic concentration of scavengers needed to attenuate toxicity of endogenously produced acrolein could be substantially lower than the concentrations used in in vitro studies, or in an accelerated animal model, such as the 6-OHDA PD model (100-500 μM). Therefore, this would imply a significantly increased likelihood of hydralazine and other acrolein scavengers can be used as effective treatments for neurodegenerative diseases in humans.

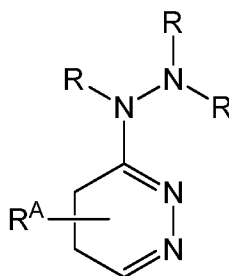
In the current study, we also noticed that when acrolein was directly injected to the mid brain, the rats not only exhibited typical PD-like motor deficits similar to that in 6-OHDA rats, but also resulted in alpha-synuclein aggregation (FIG. 7). Since alpha-synuclein is well known for its role in PD, we postulate that acrolein can induce PD symptoms by stimulating alpha-synuclein aggregation. It is well established that alpha-synuclein aggregation is the basis of the Lewy body formation, a signature pathology in human PD. Unfortunately, 6-OHDA rats usually do not develop Lewy bodies. Consistent with the literature, we did not detect significant increase of alpha-synuclein aggregation in 6-OHDA rats (data not shown). This could because acrolein level, although increased, did not reach critical levels capable of effectively causing significant alpha-synuclein aggregation. Consistent with this hypothesis, rats developed significant alpha-synuclein aggregation when directly injected with acrolein, an observation confirmed by western blot. Considering that the Lewy body is a signature pathology in human PD, the data indicate that an acrolein induced rat PD model is perhaps closer to the human pathology than a model induced by 6-OHDA. This is particularly valid considering the lack of Lewy body is considered a weakness of 6-OHDA PD rat.

In the current study, hydralazine was used in the 6-OHDA rats IP at a dosage of 5 mg/kg body weight. It has been reported that hydrazine has the capability to cross the BBB barrier. The fact that it can preserve nervous system cells provides additional supportive evidence of this finding. Also, we have noted that hydralazine can achieve an effective therapeutic concentration
5 two hours after a dosage of 5 mg/kg body weight is administered IP and that this dose did not cause any serious hypotension (data not shown). Therefore, based on the above results, we suggest that dosage of hydralazine used in the current study is likely achievable in Parkinson's patients. The data demonstrate the utility of acrolein scavengers as a new treatment modality.

In conclusion, we have presented in vitro and in vivo evidence that acrolein is a critical
10 mediator in the 6-OHDA rat model of PD. We also noted a significant increase of alpha-synuclein aggregation in an acrolein injected rat model which also produced PD-like motor deficits similar to 6-OHDA rat pathology. This demonstrated the ability of acrolein to enhance the aggregation of alpha-synuclein which may mediate its ability to cause PD like symptoms and pathological changes in the rat brain. Furthermore, anti-acrolein therapy using hydralazine
15 served as an effective intervention to alleviate PD motor deficits.

What is claimed is:

1. A composition comprising an alpha/beta unsaturated aldehyde scavenger compound in a therapeutically effective amount for treating a patient with Parkinson's disease.
2. The composition according to claim 1, wherein the alpha/beta unsaturated aldehyde scavenger compound is selected from the group consisting of a hydrazinopyridazine, a fused hydrazinopyridazine, and a phenylethylhydrazine.
3. The composition according to claim 2, wherein the fused hydrazinopyridazine is a compound of the formula



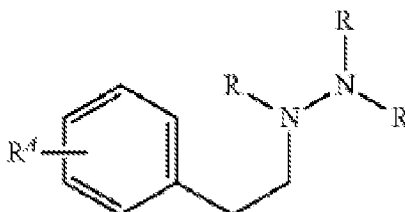
or a pharmaceutically acceptable salt thereof, wherein:

R is independently selected in each instance from hydrogen, acyl, or sulfonyl; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl comprising 3-10 ring members, heteroaryl comprising 3-10 ring members, arylalkyl comprising 3-10 ring members, or heteroarylalkyl comprising 3-10 ring members, each of which is optionally substituted; and

R^A represents three substituents, one of which is selected from the group consisting of hydrogen, halo, hydroxy and derivatives thereof, amino and derivatives thereof, thio and derivatives thereof, acyl, carboxylate or a derivative thereof, hydroxylamino and derivatives thereof, hydrazino and derivatives thereof, sulfonyl or a derivative thereof, or sulfonyl or a derivative thereof; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl comprising 3-10 ring members, heteroaryl comprising 3-10 ring members, arylalkyl comprising 3-10 ring members, or heteroarylalkyl comprising 3-10 ring members, each of which is optionally substituted; and two of R^A are taken together with the attached carbons to form an optionally substituted saturated,

unsaturated, or aromatic carbocycle or heterocycle.

4. The composition according to claim 3, wherein R^A represents a hydrogen; or
 R^A includes an optionally substituted benzo group; or
 R^A includes an optionally substituted fused piperidine; or
 R^A includes a hydrazino or derivative thereof; or
 R^A includes a hydrazino; or
 R^A includes amino or a derivative thereof; or
 R^A includes dialkylamino, where each alkyl is independently selected, and independently optionally substituted.
5. The composition according to claim 4, wherein each R is hydrogen; or at least one R is acyl; or at least one R is optionally substituted alkoxy carbonyl.
6. The composition according to claim 3, wherein the fused hydrazinopyridazine, is selected from the group consisting of hydralazine, dihydralazine, and endralazine.
7. The composition according to claim 1, wherein the alpha/beta unsaturated aldehyde compound scavenger is selected from the group consisting of: dimercaperol, N-acetylcysteine, phloretin, carnosine and homocarnosine, sodium borohydride, sodium bisulfite, lipoic acid, (MESNA)--2-mercaptoethanesulfonate, and (D3T)--2-dithiole-3-thione.
8. The composition according to claim 2, wherein the phenylethylhydrazine is a compound of the formula



or a pharmaceutically acceptable salt thereof, wherein:

R is independently selected in each instance from hydrogen, acyl, or sulfonyl; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted, or two R are taken together with the attached nitrogen to form a hydrazone; and

R^A represents three substituents selected from the group consisting of hydrogen, halo, hydroxy and derivatives thereof, amino and derivatives thereof, thio and derivatives thereof, acyl, carboxylate or a derivative thereof, hydroxylamino and derivatives thereof, hydrazino and derivatives thereof, sulfonyl or a derivative thereof, or sulfonyl or a derivative thereof; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted; or two of R^A are taken together with the attached carbons to form an optionally substituted saturated, unsaturated, or aromatic carbocycle or heterocycle.

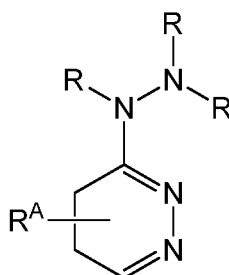
9. The composition according to claim 8, wherein the phenylethylhydrazines is phenelzine.

10. The composition according to claim 1, wherein the alpha/beta unsaturated aldehyde scavenger compound is an acrolein scavenger compound.

11. A method for treating a patient with Parkinson's disease, the method comprising providing to a patient a composition comprising an alpha/beta unsaturated aldehyde scavenger compound in a therapeutically effective amount for treatment of Parkinson's disease.

12. The method according to claim 11, wherein the alpha/beta unsaturated aldehyde scavenger compound is selected from the group consisting of a hydrazinopyridazine, a fused hydrazinopyridazine, and a phenylethylhydrazine.

13. The method according to claim 12, wherein the fused hydrazinopyridazine is a compound of the formula



or a pharmaceutically acceptable salt thereof, wherein:

R is independently selected in each instance from hydrogen, acyl, or sulfonyl; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl comprising 3-10 ring members, heteroaryl comprising 3-10 ring members, arylalkyl comprising 3-10 ring members, or heteroarylalkyl comprising 3-10 ring members, each of which is optionally substituted; and

R^A represents three substituents, one of which is selected from the group consisting of hydrogen, halo, hydroxy and derivatives thereof, amino and derivatives thereof, thio and derivatives thereof, acyl, carboxylate or a derivative thereof, hydroxylamino and derivatives thereof, hydrazino and derivatives thereof, sulfonyl or a derivative thereof, or sulfonyl or a derivative thereof; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl comprising 3-10 ring members, heteroaryl comprising 3-10 ring members, arylalkyl comprising 3-10 ring members, or heteroarylalkyl comprising 3-10 ring members, each of which is optionally substituted; and two of R^A are taken together with the attached carbons to form an optionally substituted saturated, unsaturated, or aromatic carbocycle or heterocycle.

14. The method according to claim 13, wherein R^A represents a hydrogen; or

R^A includes an optionally substituted benzo group; or

R^A includes an optionally substituted fused piperidine; or

R^A includes a hydrazino or derivative thereof; or

R^A includes a hydrazino; or

R^A includes amino or a derivative thereof; or

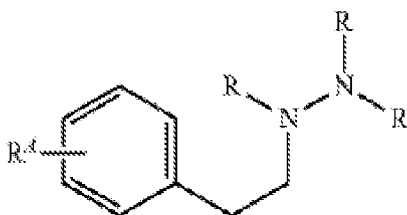
R^A includes dialkylamino, where each alkyl is independently selected, and independently optionally substituted.

15. The method according to claim 14, wherein each R is hydrogen; or at least one R is acyl; or at least one R is optionally substituted alkoxy carbonyl.

16. The method according to claim 13, wherein the fused hydrazinopyridazine, is selected from the group consisting of hydralazine, dihydralazine, and endralazine.

17. The method according to claim 11, wherein the alpha/beta unsaturated aldehyde compound scavenger is selected from the group consisting of: dimercaperol, N-acetylcysteine, phloretin, carnosine and homocarnosine, sodium borohydride, sodium bisulfite, lipoic acid, (MESNA)--2-mercaptoethanesulfonate, and (D3T)--2-dithiole-3-thione.

18. The method according to claim 12, wherein the phenylethylhydrazine is a compound of the formula



or a pharmaceutically acceptable salt thereof, wherein:

R is independently selected in each instance from hydrogen, acyl, or sulfonyl; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted, or two R are taken together with the attached nitrogen to form a hydrazone; and

R^A represents three substituents selected from the group consisting of hydrogen, halo, hydroxy and derivatives thereof, amino and derivatives thereof, thio and derivatives thereof, acyl, carboxylate or a derivative thereof, hydroxylamino and derivatives thereof, hydrazino and derivatives thereof, sulfonyl or a derivative thereof, or sulfonyl or a derivative thereof; or alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heteroalkyl, heteroalkenyl, cycloheteroalkyl, cycloheteroalkenyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, each of which is optionally substituted; or two of R^A are taken together with the attached carbons to form an optionally substituted saturated, unsaturated, or aromatic carbocycle or heterocycle.

19. The method according to claim 18, wherein the phenylethylhydrazines is phenelzine.
20. The method according to claim 11, wherein the alpha/beta unsaturated aldehyde scavenger compound is an acrolein scavenger compound.

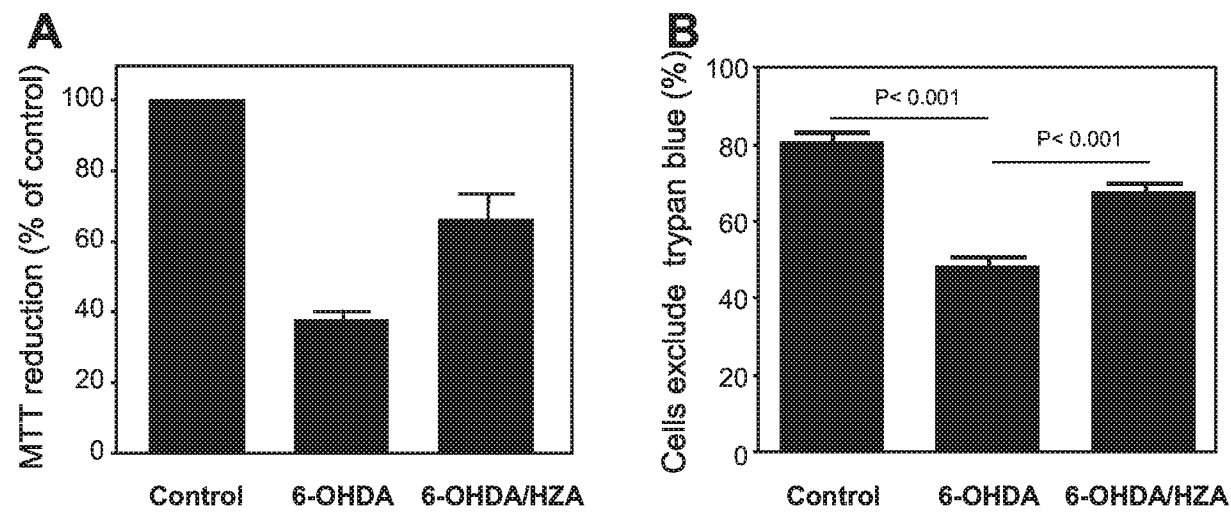


Figure 1

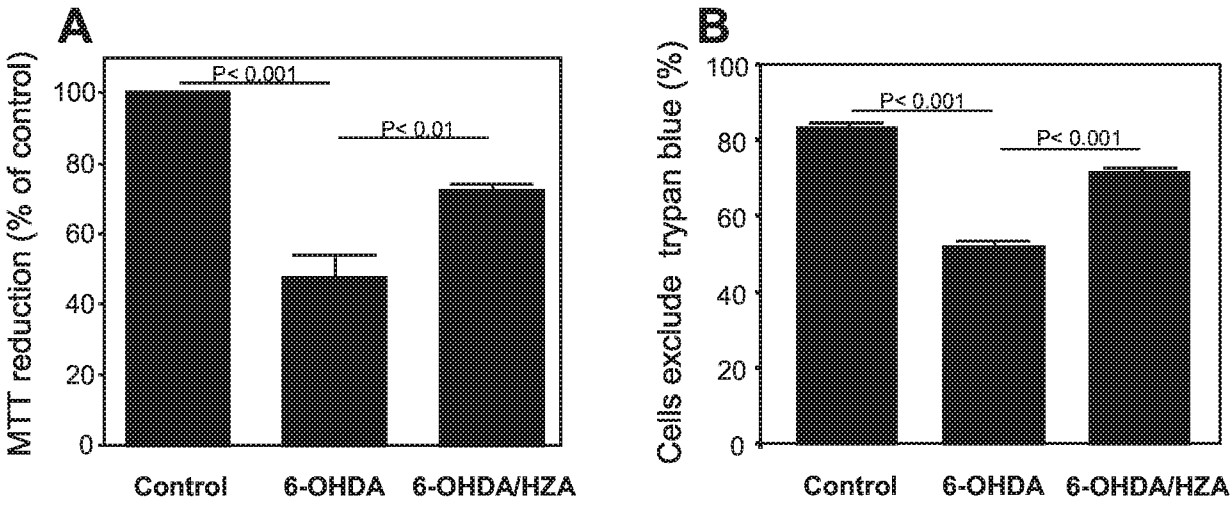


Figure 2

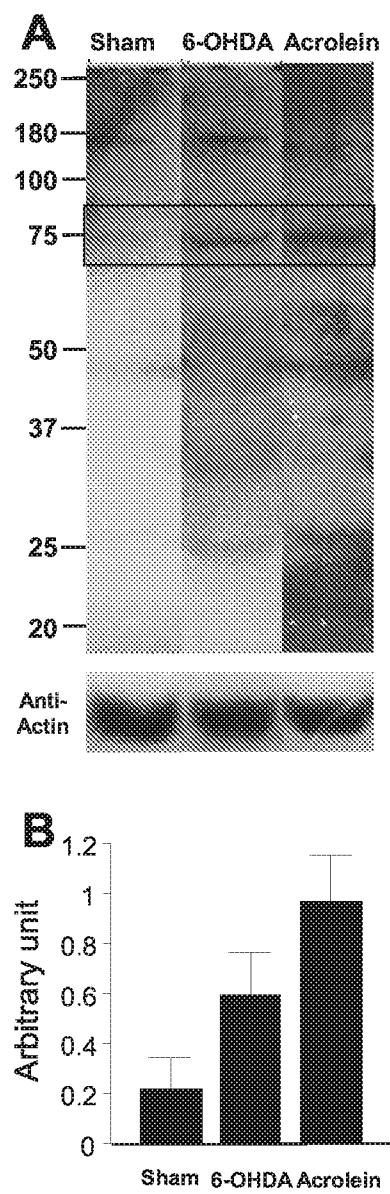


Figure 3

Open field test

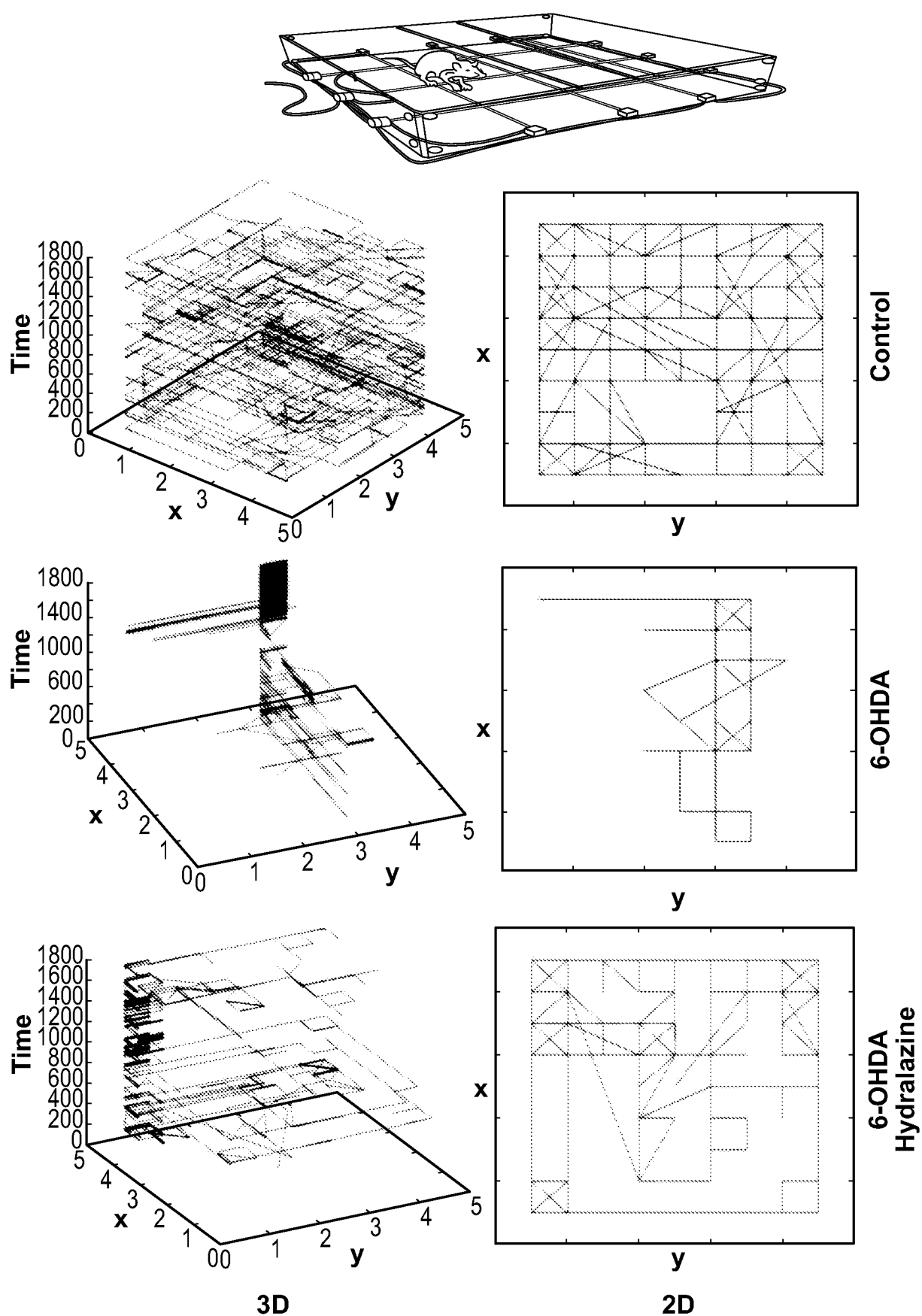


Figure 4

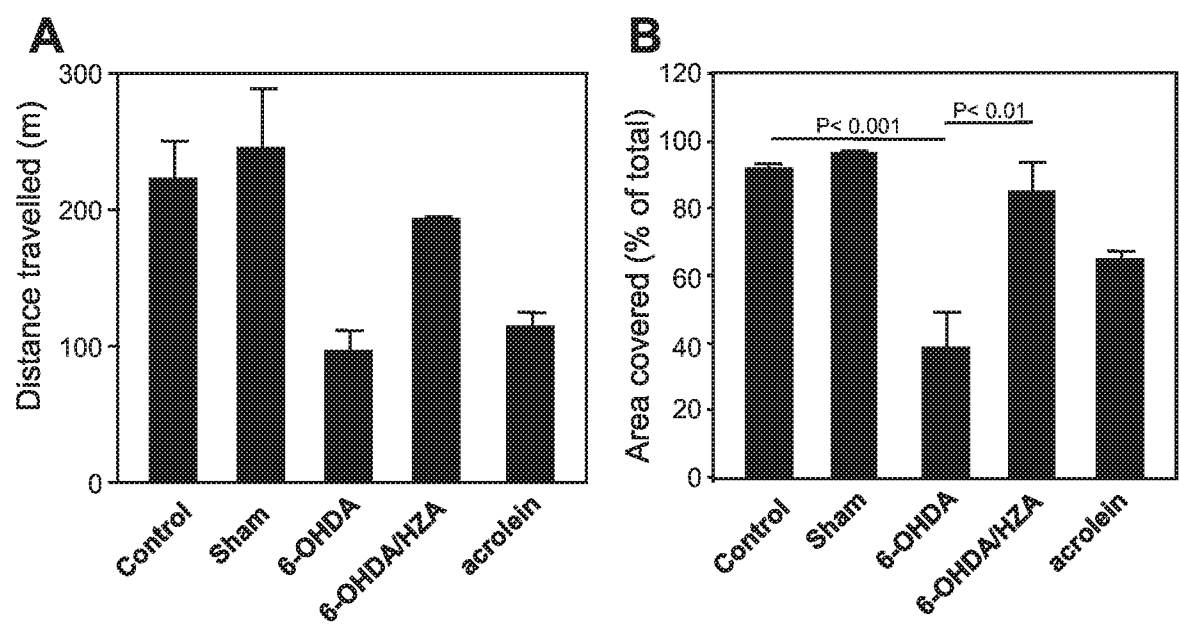


Figure 5

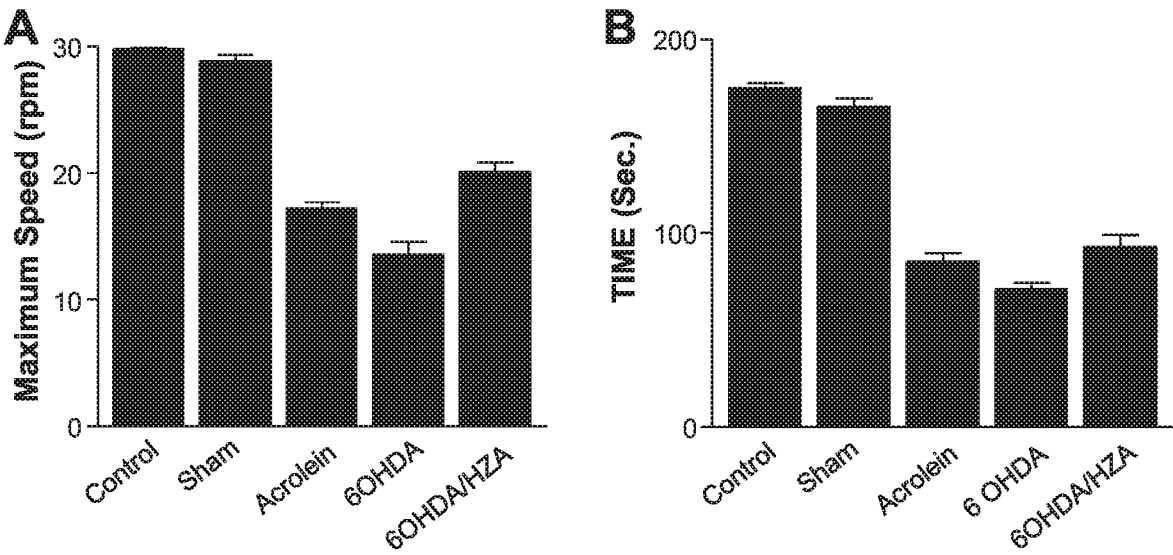


Figure 6

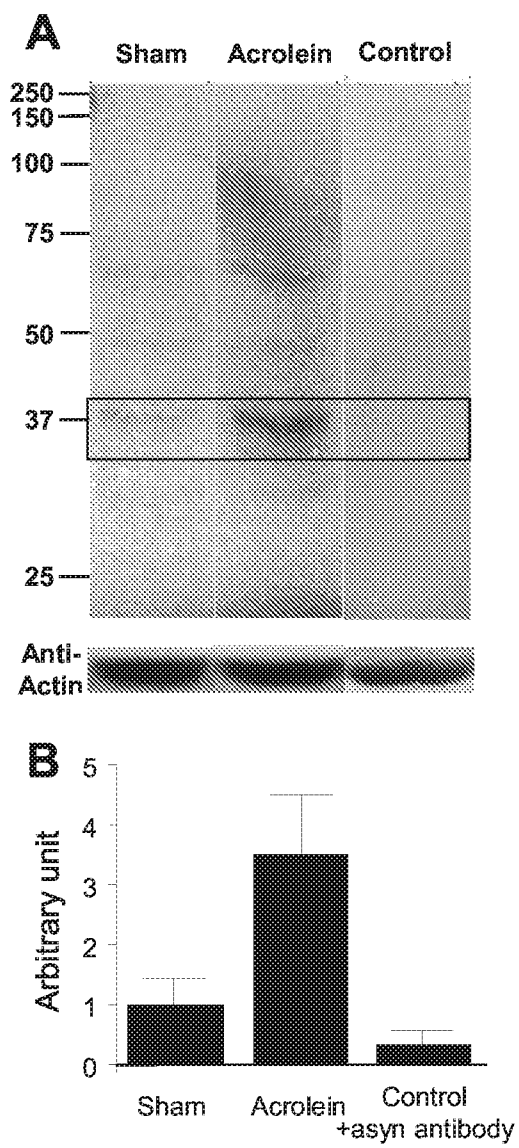


Figure 7

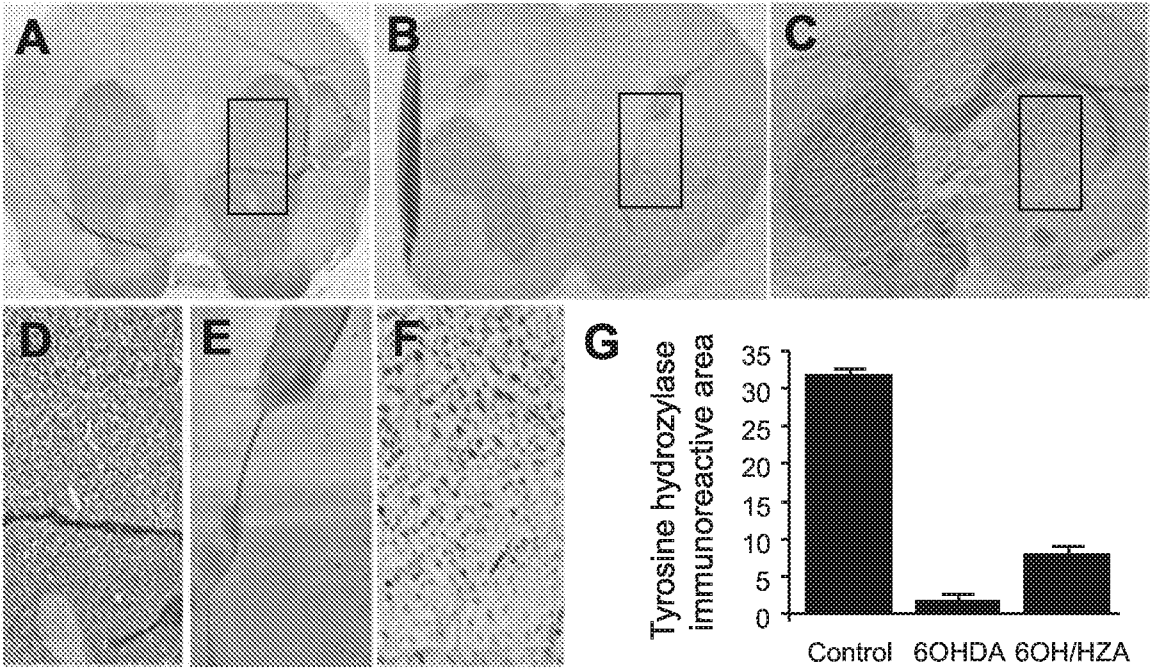


Figure 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/22622

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/50; C07D 237/04 (2016.01)

CPC - A61K31/50; C07D237/04

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(8): A61K 31/50; C07D 237/04 (2016.01)

CPC: A61K31/50; C07D237/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/247; 544/224

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

PatBase, Google Scholar, PubWEST

hydrazinopyridazine, phenelzine, aldehyde scavenger, Parkinson's, acrolein, methylenedioxyamphetamine, hydroxynonenal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2013/0143967 A1 (OTT) 06 June 2013 (06.06.2013) para [0002], [0108]-[0110]	1, 10, 11, 20 ----- 2-9, 12-19
Y	US 2013/0345424 A1 (SHI) 26 December 2013 (26.12.2013) para [0014], [0021]-[0023], [0027], [0034]	2-6, 8-9, 12-16, 18-19
Y	CN103315988 A (ZHENGZHOU TOBACCO RES CNTC) 25 September 2013 (25.09.2013) para [0029], [0043]	7, 17
A	US 2013/0302268 A1 (BOWDEN et al.) 14 November 2013 (14.11.2013) Entire Document	1-20

☐ Further documents are listed in the continuation of Box C.

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Date of the actual completion of the international search

01 May 2016 (01.05.2016)

Date of mailing of the international search report

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