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# DESCRIPTION

## TECHNICAL FIELD

[0001] The present invention relates to compounds comprising a potassium channel opener with superoxide ion ( $O_2^-$ ) catalytic degradation properties and to pharmaceutical compositions comprising them. These compounds are useful for treatment, prevention and/or managing of diseases, disorders and conditions associated with oxidative stress or endothelial dysfunction.

## BACKGROUND ART

[0002] Openers of the mitochondrial ATP-regulated potassium channel have been shown to provide cytoprotection in experimental models of redox stress induced by ischemia-reperfusion or inflammation. However, the specificity for the mitochondrial rather than the sarcolemmal  $K^+$ -ATP channel is important to eliminate the risk of systemic hypotension.

[0003] Given the demonstrated benefit in these pathological settings of therapeutic administration of either antioxidants or  $K^+$ -ATP channel openers, a more substantial benefit may accrue from (i) the concomitant removal of reactive oxygen species (ROS) and (ii) stimulation of endogenous mechanisms (via opening  $K^+$ -ATP channels) to protect against redox stress. This effect would be maximized if both these actions co-localized in space and time. However, such co-localization would be unlikely to occur by the co-administration of two distinct drugs, such as a separate  $K^+$ -ATP channel opener and an anti-oxidant molecule, because of the unpredictability of their tissue distribution, metabolism, clearance, excretion, and intracellular localization.

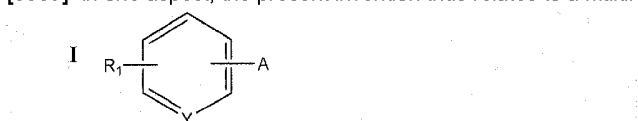
[0004] US2006/0025449 discloses carbamate nitroxide derivatives useful to treat disorder caused by the presence of reactive oxygen species.

[0005] WO2004/000331 describes the combination of a potassium channel opener such as Pinacidil and an antioxidant such as Tempol.

## SUMMARY OF INVENTION

[0006] It has been found in accordance with the present invention that administration of a conjugate of a pyridinocyanoguanidine moiety and the 3-amino-2,2,5,5-tetramethylpyrrolidinyloxy, free radical, more particularly, the oxy radical of 2-cyano-1-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)-3-(pyridin-3-yl) guanidine, is highly beneficial in treatment, prevention and/or managing of various diseases, disorders or conditions associated with oxidative stress or endothelial dysfunction such as myocardial ischemia-reperfusion injury and renal ischemia-reperfusion injury, as well as acute chlorine inhalation injury.

[0007] In one aspect, the present invention thus relates to a multifunctional nitroxide derivative of the general formula I:

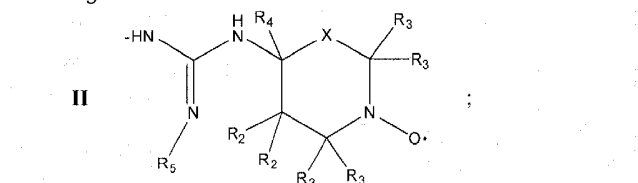


wherein Y is N, CH or  $N(\rightarrow O)$ ,

or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt or solvate thereof,

wherein

A is a moiety of the general formula II linked through its terminal -NH group to any carbon atom of the pyridine, phenyl, or pyridine oxide ring:



X is absent or  $-(CR_2R_2)_n$ ;

$R_1$  is absent or 1 to 5 substituents each independently selected from halogen,  $-CN$ ,  $-OH$ ,  $-NO_2$ ,  $-N(R_6)_2$ ,  $-OCF_3$ ,  $-CF_3$ ,  $-OR_6$ ,  $-COR_6$ ,  $-COOR_6$ ,  $-CON(R_6)_2$ ,  $-OCOOR_6$ ,  $-OCON(R_6)_2$ ,  $-(C_1-C_8)alkyl$ ,  $-(C_1-C_8)alkylene-COOR_6$ ,  $-SR_6$ ,  $-SO_2R_6$ ,  $-SO_2N(R_6)_2$ , or  $-S(=O)R_6$ , wherein said  $-(C_1-C_8)alkyl$  and  $-(C_1-C_8)alkylene-COOR_6$  may optionally be substituted with  $-OH$ ,  $-OR_3$ ,  $-OCF_3$ ,  $-CF_3$ ,  $-COR_3$ ,  $-COOR_3$ ,  $-OCOOR_3$ ,  $-OCON(R_3)_2$ ,  $-(C_1-C_8)alkylene-COOR_3$ ,  $-CN$ ,  $-NH_2$ ,  $-NO_2$ ,  $-SH$ ,  $-SR_3$ ,  $-(C_1-C_8)alkyl$ ,  $-O-(C_1-C_8)alkyl$ ,  $-N(R_3)_2$ ,  $-CON(R_3)_2$ ,  $-SO_2R_3$ , or  $-S(=O)R_3$ , or two adjacent  $R_1$  groups and the carbon atoms to which they are attached form a 5- or 6-membered carbocyclic or heterocyclic ring,  $(C_6-C_{10})aryl$ , or 6- to 10-membered heteroaryl;

$R_2$  each independently is selected from H, halogen,  $-OCF_3$ ,  $-CF_3$ ,  $-OR_7$ ,  $-COR_7$ ,  $-COOR_7$ ,  $-OCOOR_7$ ,  $-OCON(R_7)_2$ ,  $-(C_1-C_8)alkylene-COOR_7$ ,  $-CN$ ,  $-NO_2$ ,  $-SH$ ,  $-SR_7$ ,  $-(C_1-C_8)alkyl$ ,  $-N(R_7)_2$ ,  $-CON(R_7)_2$ ,  $-SO_2R_7$ ,  $-SO_2N(R_7)_2$ , or  $-S(=O)R_7$ ; or two  $R_2$  groups and the carbon atom to which they are attached form a 5- or 6-membered carbocyclic or heterocyclic ring;

$R_3$  each independently is selected from  $(C_1-C_8)alkyl$ ,  $(C_2-C_8)alkenyl$ , or  $(C_2-C_8)alkynyl$ ;

$R_4$  is selected from H,  $-COOR_7$ ,  $-(C_1-C_8)alkylene-COOR_7$ ,  $-CN$ ,  $-(C_1-C_8)alkyl$ , or  $-CON(R_7)_2$ ;

$R_5$  is selected from H,  $-OH$ ,  $-O-(C_1-C_8)alkyl$ ,  $-CO-(C_1-C_8)alkyl$ ,  $-COO-(C_1-C_8)alkyl$ ,  $-CN$ , or  $-NH_2$ ;

$R_6$  each independently is selected from H,  $(C_1-C_8)alkyl$ ,  $(C_3-C_{10})cycloalkyl$ , 4-12-membered heterocyclyl,  $(C_6-C_{14})aryl$ , or  $-(C_1-C_8)alkylene-NH_2$ ;

$R_7$  each independently is selected from H,  $(C_1-C_8)alkyl$ ,  $-(C_1-C_8)alkylene-NH_2$ ,  $(C_3-C_{10})cycloalkyl$ , 4-12-membered heterocyclyl, or  $(C_6-C_{14})aryl$ , each of which other than H may optionally be substituted with  $-OR_6$ ,  $-COR_6$ ,  $-COOR_6$ ,  $-OCOOR_6$ ,  $-OCON(R_6)_2$ ,  $-(C_1-C_8)alkylene-COOR_6$ ,  $-CN$ ,  $-NO_2$ ,  $-SR_6$ ,  $-(C_1-C_8)alkyl$ ,  $-N(R_6)_2$ ,  $-CON(R_6)_2$ ,  $-SO_2R_6$ , or  $-S(=O)R_6$ ; and

n is an integer of 1 or 2.

**[0008]** In another aspect, the present invention relates to a pharmaceutical composition comprising a multifunctional nitroxide derivative of the general formula I as defined above, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt or solvate thereof, and a pharmaceutically acceptable carrier.

**[0009]** The multifunctional nitroxide derivatives and pharmaceutical compositions of the invention are useful for treatment, prevention and/or managing of diseases, disorders and conditions associated with high levels of reactive oxygen species (ROS) and oxidative stress. Thus, in a further aspect, the present invention provides a multifunctional nitroxide derivative of the general formula I as defined above, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt, solvate or prodrug thereof, for use in treatment of a disease, disorder or condition associated with high levels of ROS, and oxidative stress.

**[0010]** Described herein is a method for treatment of a disease, disorder or condition associated with high levels of ROS, and oxidative stress, said method comprising administering to an individual in need a therapeutically effective amount of a multifunctional nitroxide derivative of the general formula I as defined above, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt or solvate thereof.

## BRIEF DESCRIPTION OF DRAWINGS

**[0011]**

**Fig. 1** shows the mean arterial pressure (mmHg) measured in rats subjected to a model of myocardial ischemia-reperfusion (I/R) injury (MIRI) as described in Example 11, indicating that compound **2**, when administered prior to reperfusion at 1 mg/kg IV bolus followed by a continuous IV infusion of 2 mg/kg/h, did not produce systemic hypotension.

**Figs. 2A-2B** demonstrate the histological protection afforded by compound **2** in a rat model of MIRI described in Example 11, indicating that compound **2**, when administered prior to reperfusion at 1 mg/kg IV bolus followed by a continuous IV infusion of 2 mg/kg/h, significantly reduced both myocardial myeloperoxidase (MPO) (**2A**) and tissue infarction (**2B**) ( $p < 0.01$  vs. I/R+vehicle;  $n=10$  rats per group). The results were analyzed by one-way ANOVA followed by a Bonferroni post-hoc test for multiple

comparisons. All values and text are expressed as mean $\pm$ standard error of the mean (SEM) of N number of animals.

**Figs. 3A-3C** illustrates the histological effects of MIRI, i.e., severe myocyte necrosis, edema and neutrophil infiltration, in rats subjected to coronary artery occlusion (20 min) followed by reperfusion (2 hours) as described in Example 11 (3A); sham controls, i.e., rats subjected to identical surgical procedures except for coronary artery occlusion (**3B**); and rats subjected to identical surgical procedures and treated with compound **2**, prior to reperfusion at 1 mg/kg IV bolus followed by a continuous IV infusion of 2 mg/kg/h (**3C**). As shown, compound **2** profoundly attenuated virtually all histologic features of injury induced by MIRI.

**Figs. 4A-4C** show that addition of compound **2** before reperfusion in a murine model of renal ischemia-reperfusion injury described in Example 12 profoundly attenuated elevations in plasma blood urea nitrogen (BUN) and creatinine (**4A** and **4B**, respectively), as well as in renal myeloperoxidase (MPO) (**4C**) ( $p < 0.01$  vs. vehicle control).

**Figs. 5A-5B** show that IP administration of compound **2** (30 mg/kg/dose in 0.5 ml D5W) following chlorine exposure, in a murine model of acute chlorine inhalation injury (CILI), reduced the elevation in MPO (**5A**), indicating polymorphonuclear leukocytes (PMN) infiltration, and histologic lung damage (**5B**) in male Balb/c mice by 52% ( $p < 0.0001$ ) and 43% ( $p < 0.001$ ), respectively, relatively to placebo (D5W).

**Fig. 6** shows histology scores demonstrating that compound **2**, formulated in saline solution and given in 0.25 ml volume, IP, at 2 h and 6 h after Cl<sub>2</sub> exposure, attenuated lung injury in a dose-dependent manner, ranging from 3 mg to 80 mg per dose (or 6 mg to 160 mg per day). Values are expressed as mean $\pm$ standard error of the mean (SEM) of N number of animals. Histology scores are: Sham/vehicle: mean=0 (n=2 mice, score 0,0); Cl<sub>2</sub>+vehicle: mean 3.2, SE 0.37 (n=5, scores 2,3,3,4,4); Cl<sub>2</sub>+80 mg/kg: 0.70, SE 0.45 (n=5, scores 0,1,1,1,1.5); Cl<sub>2</sub>+30 mg/kg: 1.4 SE 0.51 (n=5, scores 0,1,1,2,3); Cl<sub>2</sub>+10 mg/kg: 2.2, SE 0.37 (n=5, scores 1,2,2,3,3); Cl<sub>2</sub>+3 mg/kg: 3.0, SE 0.32 (n=5, scores 2,3,3,3,4).

**Fig. 7** shows that compound **2**, given by IP bolus to male Balb/c mice, did not exhibit any significant effect on blood glucose levels compared with saline vehicle group.

## DETAILED DESCRIPTION OF THE INVENTION

**[0012]** The present invention provides chemical compounds of the general formula I as defined above, herein also termed "multifunctional nitroxide derivatives", comprising a potassium channel opener as well as a reactive oxygen species (ROS) degradation catalyst that can act as an anti-oxidant. The term "reactive oxygen species (ROS) degradation catalyst", as used herein, refers to a group capable of acting as a scavenger of, or catalytically detoxifying, superoxide or other ROS including superoxide, hydroxyl radicals, peroxynitrite, peroxycarbonate, hypochlorous acid, and hydrogen peroxide. An anti-oxidant that preferentially scavenges, or catalytically detoxifies, superoxide is termed a "superoxide dismutase mimic" ("SOD-mimic") or "superoxide dismutase mimetic" ("SOD-mimetic"). The ROS superoxide, hydroxyl radicals, peroxynitrite, peroxycarbonate, hypochlorous acid and hydrogen peroxide are considered biologically undesirable. This ROS degradation catalyst is a nitroxide free radical (NO) group.

**[0013]** The multifunctional nitroxide derivatives of the present invention are useful for the prevention and treatment of inflammatory and reperfusion diseases, disorders or conditions. By virtue of the potassium channel opener and ROS degrading activities being covalently linked, the compounds of the invention ensure that adding potassium channel opener is accompanied contemporaneously and spatially by reduced levels of ROS, including peroxynitrite, peroxycarbonate, hydrogen peroxide, hydroxyl radicals, and other oxidant metabolites thereof.

**[0014]** The term "halogen" as used herein includes fluoro, chloro, bromo, and iodo, and is preferably fluoro, chloro or bromo.

**[0015]** The term "alkyl" as used herein typically means a straight or branched saturated hydrocarbon radical having 1-8 carbon atoms and includes, e.g., methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, 2,2-dimethylpropyl, n-hexyl, n-heptyl, n-octyl and the like. Preferred are (C<sub>1</sub>-C<sub>6</sub>)alkyl groups, more preferably (C<sub>1</sub>-C<sub>4</sub>)alkyl groups, most preferably methyl and ethyl. The terms "alkenyl" and "alkynyl" typically mean straight and branched hydrocarbon radicals having 2-8 carbon atoms and 1 double or triple bond, respectively, and include ethenyl, propenyl, 3-buten-1-yl, 2-ethenylbutyl, 3-octen-1-yl, and the like, and propynyl, 2-butyne-1-yl, 3-pentyn-1-yl, and the like. C<sub>2</sub>-C<sub>6</sub> alkenyl and alkynyl radicals are preferred, more preferably C<sub>2</sub>-C<sub>4</sub> alkenyl and alkynyl.

**[0016]** The term "alkylene" typically means a divalent straight or branched hydrocarbon radical having 1-8 carbon atoms and includes, e.g., methylene, ethylene, propylene, butylene, 2-methylpropylene, pentylene, 2-methylbutylene, hexylene, 2-methylpentylene, 3-methylpentylene, 2,3-dimethylbutylene, heptylene, octylene and the like. Preferred are (C<sub>1</sub>-C<sub>6</sub>)alkylene, more preferably (C<sub>1</sub>-C<sub>4</sub>)alkylene, most preferably (C<sub>1</sub>-C<sub>2</sub>)alkylene.

**[0017]** The term "cycloalkyl" as used herein means a mono- or bicyclic saturated hydrocarbonyl group having 3-10 carbon atoms such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, adamantyl, bicyclo[3.2.1]octyl, bicyclo[2.2.1]heptyl, and the like, that may be substituted, for example, by one or more alkyl groups.

**[0018]** The term "carbocyclic ring" as used herein refers to a saturated or unsaturated, i.e., containing at least one unsaturated bond, ring consisting of 3-10 carbon atoms. Preferred are 5- or 6-membered carbocyclic rings such as cyclopentane, cyclopentene, cyclohexane, cyclohexene and the like.

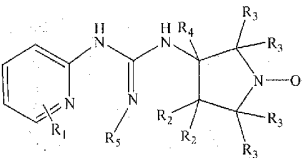
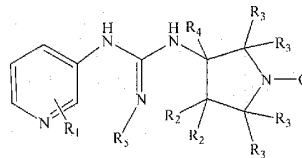
**[0019]** The term "heterocyclic ring" denotes a mono- or poly-cyclic non-aromatic ring of 4-12 atoms containing at least one carbon atom and one to three, preferably 1-2 heteroatoms selected from sulfur, oxygen or nitrogen, which may be saturated or unsaturated, i.e., containing at least one unsaturated bond. Preferred are 5- or 6-membered heterocyclic rings. The term "heterocyclyl" as used herein refers to any univalent radical derived from a heterocyclic ring as defined herein by removal of hydrogen from any ring atom. Examples of such radicals include, without limitation, piperidino, 4-morpholinyl, or pyrrolidinyl.

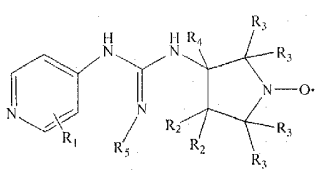
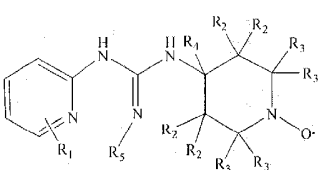
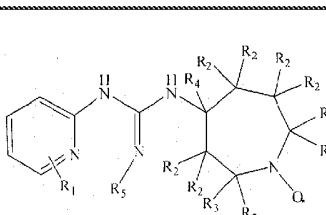
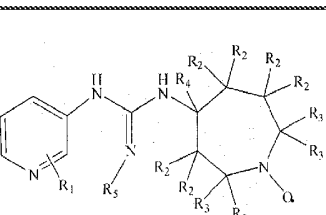
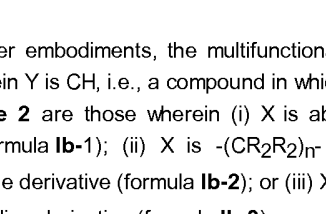
**[0020]** The term "aryl" denotes an aromatic carbocyclic group having 6-14 carbon atoms consisting of a single ring or multiple rings either condensed or linked by a covalent bond such as, but not limited to, phenyl, naphthyl, phenanthryl, and biphenyl. The aryl radical may optionally be substituted by one or more groups each independently selected from halogen, e.g., F, Cl or Br, (C<sub>1</sub>-C<sub>8</sub>)alkyl, -O-(C<sub>1</sub>-C<sub>8</sub>)alkyl, -COO(C<sub>1</sub>-C<sub>8</sub>)alkyl, -CN, or NO<sub>2</sub>.

**[0021]** The term "heteroaryl" refers to a radical derived from a mono- or poly-cyclic heteroaromatic ring containing one to three, preferably 1-2, heteroatoms selected from the group consisting of N, O and S. When the heteroaryl is a monocyclic ring, it is preferably a radical of a 5-6-membered ring such as, but not limited to, pyrrolyl, furyl, thienyl, thiazinyl, pyrazolyl, pyrazinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyridyl, pyrimidinyl, 1,2,3-triazinyl, 1,3,4-triazinyl, and 1,3,5-triazinyl. Polycyclic heteroaryl radicals are preferably composed of two rings such as, but not limited to, benzofuryl, isobenzofuryl, benzothienyl, indolyl, quinolinyl, isoquinolinyl, imidazo[1,2-a]pyridyl, benzimidazolyl, benzthiazolyl, benzoxazolyl, pyrido[1,2-a]pyrimidinyl and 1,3-benzodioxinyl. The heteroaryl may be substituted. It is to be understood that when a polycyclic heteroaryl is substituted, the substitution may be in any of the carbocyclic and/or heterocyclic rings.

**[0022]** In certain embodiments, the multifunctional nitroxide derivative of the present invention is a compound of the general formula I wherein Y is N, i.e., a compound in which group A is linked to position 2, 3, 4, 5 or 6 of a pyridine ring. Particular such compounds shown in **Table 1** are those wherein A is linked to position 2, 3 or 4 of the pyridine ring, and (i) X is absent, i.e., the oxy radical of a 1-(1-hydroxypyrrolidin-3-yl)-3-(pyridin-2-yl)guanidine derivative, 1-(1-hydroxy pyrrolidin-3-yl)-3-(pyridin-3-yl)guanidine derivative, or 1-(1-hydroxypyrrolidin-3-yl)-3-(pyridin-4-yl)guanidine derivative (formula **la-2**, **la-2** or **la-3**, respectively); (ii) X is -(CR<sub>2</sub>R<sub>2</sub>)<sub>n</sub> wherein n is 1, i.e., the oxy radical of a 1-(1-hydroxypiperidin-4-yl)-3-(pyridin-2-yl)guanidine derivative, 1-(1-hydroxypiperidin-4-yl)-3-(pyridin-3-yl)guanidine derivative, or 1-(1-hydroxy piperidin-4-yl)-3-(pyridin-4-yl)guanidine derivative (formula **la-4**, **la-5** or **la-6**, respectively); or (iii) X is -(CR<sub>2</sub>R<sub>2</sub>)<sub>n</sub> wherein n is 2, i.e., the oxy radical of a 1-(1-hydroxyazepan-4-yl)-3-(pyridin-2-yl)guanidine derivative, 1-(1-hydroxy azepan-4-yl)-3-(pyridin-3-yl)guanidine derivative, or 1-(1-hydroxyazepan-4-yl)-3-(pyridin-4-yl)guanidine derivative (formula **la-7**, **la-8**, or **la-9**, respectively). More particular such compounds are those wherein A is linked to position 3 or 4 of the pyridine ring.

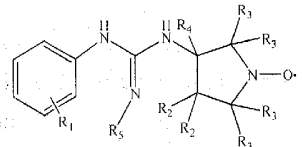
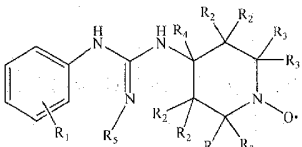
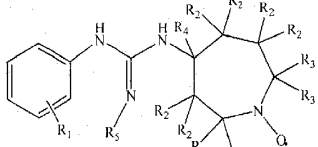
**Table 1:** Multifunctional nitroxide derivatives of the formulas **la-1** to **la-9**

<b>la-1</b>	<b>la-2</b>
	
<b>la-3</b>	<b>la-4</b>

	
<p style="text-align: center;"><b>Ia-5</b></p>	<p style="text-align: center;"><b>Ia-6</b></p>
	
<p style="text-align: center;"><b>Ia-7</b></p>	<p style="text-align: center;"><b>Ia-8</b></p>
	
<p style="text-align: center;"><b>Ia-9</b></p>	

**[0023]** In other embodiments, the multifunctional nitroxide derivative of the present invention is a compound of the general formula I wherein Y is CH, i.e., a compound in which group A is linked to any position of a phenyl ring. Particular such compounds shown in **Table 2** are those wherein (i) X is absent, i.e., the oxy radical of a 1-(1-hydroxypyrrolidin-3-yl)-3-phenylguanidine derivative (formula **Ib-1**); (ii) X is  $-(CR_2R_2)_n$  wherein n is 1, i.e., the oxy radical of a 1-(1-hydroxypiperidine-4-yl)-3-phenylguanidine derivative (formula **Ib-2**); or (iii) X is  $-(CR_2R_2)_n$  wherein n is 2, i.e., the oxy radical of a 1-(1-hydroxyazepan-4-yl)-3-phenylguanidine derivative (formula **Ib-3**).

**Table 2:** Multifunctional nitroxide derivatives of the formulas **Ib-1** to **Ib-3**

<p style="text-align: center;"><b>Ib-1</b></p> 	<p style="text-align: center;"><b>Ib-2</b></p> 
<p style="text-align: center;"><b>Ib-3</b></p> 	

**[0024]** In further embodiments, the multifunctional nitroxide derivative of the present invention is a compound of the general formula I wherein Y is N( $\rightarrow$ O), i.e., a compound in which group A is linked to position 2, 3, 4, 5 or 6 of a pyridine oxide ring. Particular such compounds shown in **Table 3** are those wherein A is linked to position 2, 3 or 4 of the pyridine oxide ring, and (i) X is absent, i.e., the oxy radical of a 1-(1-hydroxypyrrolidin-3-yl)-3-(1-oxypyridin-2-yl)guanidine derivative, 1-(1-hydroxypyrrolidin-3-yl)-3-(1-oxypyridin-3-yl)guanidine derivative, or 1-(1-hydroxypyrrolidin-3-yl)-3-(1-oxypyridin-4-yl)guanidine derivative (formula **1c-1**, **1c-2** or **1c-3**, respectively); (ii) X is  $-(\text{CR}_2\text{R}_2)_n$  wherein n is 1, i.e., the oxy radical of a 1-(1-hydroxypiperidin-4-yl)-3-(1-oxypyridin-2-yl)guanidine derivative, 1-(1-hydroxypiperidin-4-yl)-3-(1-oxypyridin-3-yl)guanidine derivative, or 1-(1-hydroxypiperidin-4-yl)-3-(1-oxypyridin-4-yl)guanidine derivative (formula **1c-4**, **1c-5** or **1c-6**, respectively); or (iii) X is  $-(\text{CR}_2\text{R}_2)_n$  wherein n is 2, i.e., the oxy radical of a 1-(1-hydroxyazepan-4-yl)-3-(1-oxypyridin-2-yl)guanidine derivative, 1-(1-hydroxy azepan-4-yl)-3-(1-oxypyridin-3-yl)guanidine derivative, or 1-(1-hydroxy azepan-4-yl)-3-(1-oxypyridin-4-yl)guanidine derivative (formula **1c-7**, **1c-8** or **1c-9**, respectively). More particular such compounds are those wherein A is linked to position 3 or 4 of the pyridine ring.

**Table 3:** Multifunctional nitroxide derivatives of the formulas **1c-1** to **1c-9**

<p><b>1c-1</b></p>	<p><b>1c-2</b></p>
<p><b>1c-3</b></p>	<p><b>1c-4</b></p>
<p><b>1c-5</b></p>	<p><b>1c-6</b></p>
<p><b>1c-7</b></p>	<p><b>1c-8</b></p>
<p><b>1c-9</b></p>	<p><b>1c-10</b></p>

**[0025]** According to the present invention, R<sub>1</sub> is absent or represents 1 to 5 substituents as defined above. Nevertheless, it should be understood that in cases wherein Y is N or N(→O), the maximal number of R<sub>1</sub> groups is limited to 4 only.



[0026] In certain embodiments, the multifunctional nitroxide derivative of the present invention is a compound of the general formulas **1a-1** to **1a-9**, a compound of the general formula **1b-1** to **1b-3**, or a compound of the general formula **1c-1** to **1c-9**, wherein R<sub>1</sub> is absent.

[0027] In other embodiments, the multifunctional nitroxide derivative of the present invention is a compound of the general formulas **1a-1** to **1a-9**, a compound of the general formula **1b-1** to **1b-3**, or a compound of the general formula **1c-1** to **1c-9**, wherein R<sub>1</sub> is 1, 2, 3, 4 or 5, preferably 1 or 2, more preferably 1, substituents each independently selected from halogen, -OH, -CN, -NO<sub>2</sub>, -N(R<sub>6</sub>)<sub>2</sub>, -OR<sub>6</sub>, -OCF<sub>3</sub>, -CF<sub>3</sub>, -COR<sub>6</sub>, -COOR<sub>6</sub>, -CON(R<sub>6</sub>)<sub>2</sub>, -OCOOR<sub>6</sub>, -OCON(R<sub>6</sub>)<sub>2</sub>, -(C<sub>1</sub>-C<sub>8</sub>)alkyl, -(C<sub>1</sub>-C<sub>8</sub>)alkylene-COOR<sub>6</sub>, -SR<sub>6</sub>, -SO<sub>2</sub>R<sub>6</sub>, -SO<sub>2</sub>N(R<sub>6</sub>)<sub>2</sub>, or -S(=O)R<sub>6</sub>, wherein R<sub>6</sub> each independently is H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, or -(C<sub>1</sub>-C<sub>8</sub>)alkylene-NH<sub>2</sub>, preferably H.

**[0028]** In further embodiments, the multifunctional nitroxide derivative of the present invention is a compound of the general formula **Ia-1** to **Ia-9**, a compound of the general formula **Ib-1** to **Ib-3**, or a compound of the general formula **Ic-1** to **Ic-9**, wherein two adjacent R<sub>1</sub> groups and the carbon atoms of the pyridine ring to which they are attached form a 5- or 6-membered carbocyclic or heterocyclic ring, (C<sub>6</sub>-C<sub>10</sub>)aryl, or 6- to 10-membered heteroaryl. In such embodiments, group A in the general formula I is linked, in fact, to a pyridine-, phenyl-, or pyridine oxide-moiety condensed to a monocyclic or bicyclic group selected from a 5- or 6-membered carbocyclic or heterocyclic ring, (C<sub>6</sub>-C<sub>10</sub>)aryl, or 6- to 10-membered heteroaryl. Non-limiting examples of such multifunctional nitroxide derivatives include (i) compounds of the general formula I wherein Y is N, in which group A is linked to a moiety of 6,7-dihydro-5H-cyclopenta[b]pyridine, 6,7-dihydro-5H-cyclopenta[c]pyridine, 5,6,7,8-tetrahydroquinoline, 5,6,7,8-tetrahydroisoquinoline, 2,3-dihydrofuro[3,2-b]pyridine, 3,4-dihydro-2H-pyrano[3,2-b]pyridine, quinoline, isoquinoline, benzo[g]quinoline, benzo[g]isoquinoline, 1,5-naphthyridine, 1,8-naphthyridine, pyrido[2,3-b]pyrazine, or pyrido[3,2-g]quinoline; (ii) compounds of the general formula I wherein Y is CH, in which group A is linked to a moiety of 2,3-dihydro-1H-indene, 1,2,3,4-tetrahydronaphthalene, 2,3-dihydrobenzofuran, chroman, naphthalene, anthracene, quinoline, quinoxaline, or benzo[g]quinoline; and (iii) compounds of the general formula I wherein Y is N(→O), in which group A is linked to a moiety of 1-oxy-6,7-dihydro-5H-cyclopenta[b]pyridine, 2-oxy-6,7-dihydro-5H-cyclopenta[c]pyridine, 1-oxy-5,6,7,8-tetrahydroquinoline, 2-oxy-5,6,7,8-tetrahydroisoquinoline, 4-oxy-2,3-dihydrofuro[3,2-b]pyridine, 5-oxy-3,4-dihydro-2H-pyrano[3,2-b]pyridine, 1-oxyquinoline, 2-oxyisoquinoline, 1-oxybenzo[g]quinoline, 2-oxybenzo[g]isoquinoline, 1-oxy-1,5-naphthyridine, 1-oxy-1,8-naphthyridine, 5-oxy-1-oxy-2,3-b]pyrazine, or 1-oxy-2,3-g]quinoline.

**[0029]** In certain embodiments, the multifunctional nitroxide derivative of the present invention is a compound of the general formulas **1a-1** to **1a-9**, a compound of the general formula **1b-1** to **1b-3**, or a compound of the general formula **1c-1** to **1c-9**, wherein R<sub>2</sub> each is H.

[0030] In certain embodiments, the multifunctional nitroxide derivative of the present invention is a compound of the general formulas **1a-1** to **1a-9**, a compound of the general formula **1b-1** to **1b-3**, or a compound of the general formula **1c-1** to **1c-9**, wherein R<sub>3</sub> each independently is (C<sub>1</sub>-C<sub>4</sub>)alkyl, preferably (C<sub>1</sub>-C<sub>2</sub>)alkyl, more preferably methyl. In particular such embodiments, the multifunctional nitroxide derivative of the invention is such a compound, wherein R<sub>3</sub> are identical.

[0031] In certain embodiments, the multifunctional nitroxide derivative of the present invention is a compound of the general formulas **1a-1** to **1a-9**, a compound of the general formula **1b-1** to **1b-3**, or a compound of the general formula **1c-1** to **1c-9**, wherein R<sub>4</sub> is H.

[0032] In certain embodiments, the multifunctional nitroxide derivative of the present invention is a compound of the general formulas **1a-1** to **1a-9**, a compound of the general formula **1b-1** to **1b-3**, or a compound of the general formula **1c-1** to **1c-9**, wherein R<sub>5</sub> is -CN.

**[0033]** In certain particular embodiments, the multifunctional nitroxide derivative of the present invention is a compound of the general formula I, wherein Y is N; A is linked to position 2, 3, 4, 5 or 6 of the pyridine ring; R<sub>1</sub> is absent or 1 to 4 substituents each independently is halogen; X is absent or -(CR<sub>2</sub>R<sub>2</sub>)<sub>n</sub>- wherein n is 1 or 2; R<sub>2</sub> each is H; R<sub>3</sub> each independently is (C<sub>1</sub>-C<sub>4</sub>)alkyl, preferably (C<sub>1</sub>-C<sub>2</sub>)alkyl, more preferably methyl; R<sub>4</sub> is H; and R<sub>5</sub> is -CN. In certain more particular embodiments, R<sub>1</sub> is a sole substituent linked to any of the available carbon atoms of the pyridine ring, i.e., a halogen linked to the pyridine ring at position ortho, meta or para with respect to group A. In other more particular embodiments, R<sub>1</sub> represents 2 substituents each independently linked to any of the available carbon atoms of the pyridine ring, i.e., two halogens linked to the pyridine ring at position ortho, meta or para with respect to each other. Specific such compounds described in the specification are herein identified by the Arabic numbers **1-21** in bold (each one of the compounds **4-7**, **11-14** and **18-21** has three configurations

identified a-c), wherein their full chemical structures are depicted in **Table 4** hereinafter.

**[0034]** In certain specific embodiments, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is N; X is absent; R<sub>1</sub> is absent; R<sub>2</sub> is H; R<sub>3</sub> is methyl; and A is linked to position 2, 3 or 4 of the pyridine ring, i.e., the oxy radical of 2-cyano-1-(1-hydroxy-2,2,5,5-tetramethyl pyrrolidin-3-yl)-3-(pyridin-2-yl)guanidine (compound **1**); 2-cyano-1-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)-3-(pyridin-3-yl)guanidine (compound **2**); or 2-cyano-1-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)-3-(pyridin-4-yl)guanidine (compound **3**).

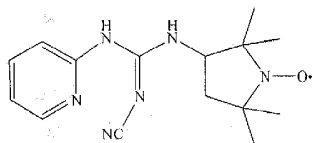
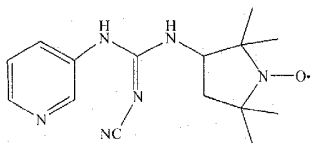
**[0035]** In other specific embodiments, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is N; X is absent; R<sub>1</sub> is F, Cl or Br, linked to position 6 of the pyridine ring; R<sub>2</sub> is H; R<sub>3</sub> is methyl; and A is linked to position 2, 3, 4 or 5 of the pyridine ring, i.e., the oxy radical of 2-cyano-1-(6-fluoropyridin-2-yl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl) guanidine, 2-cyano-1-(6-chloropyridin-2-yl)-3-(1-hydroxy-2,2,5,5-tetramethyl pyrrolidin-3-yl)guanidine, or 2-cyano-1-(6-bromopyridin-2-yl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine (compounds **4a-4c**, respectively); 2-cyano-1-(6-fluoropyridin-3-yl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl) guanidine, 2-cyano-1-(6-chloropyridin-3-yl)-3-(1-hydroxy-2,2,5,5-tetramethyl pyrrolidin-3-yl)guanidine, or 2-cyano-1-(6-bromopyridin-3-yl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine (compounds **5a-5c**, respectively); 2-cyano-1-(6-fluoropyridin-4-yl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl) guanidine, 2-cyano-1-(6-chloropyridin-4-yl)-3-(1-hydroxy-2,2,5,5-tetramethyl pyrrolidin-3-yl)guanidine, or 2-cyano-1-(6-bromopyridin-4-yl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine (compounds **6a-6c**, respectively); or 2-cyano-1-(6-fluoropyridin-5-yl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl) guanidine, 2-cyano-1-(6-chloropyridin-5-yl)-3-(1-hydroxy-2,2,5,5-tetramethyl pyrrolidin-3-yl)guanidine, or 2-cyano-1-(6-bromopyridin-5-yl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine (compounds **7a-7c**, respectively).

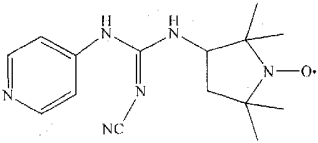
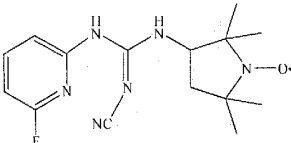
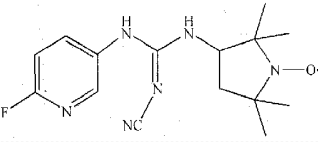
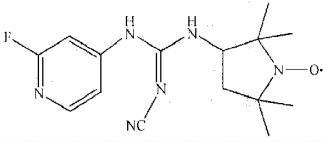
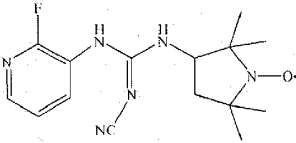
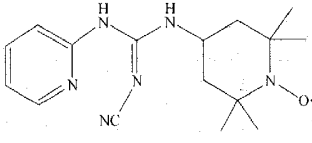
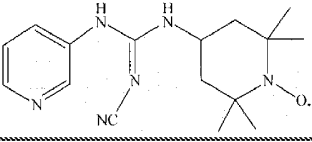
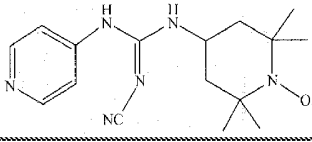
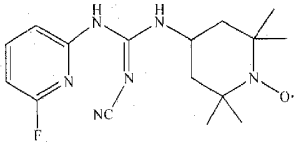
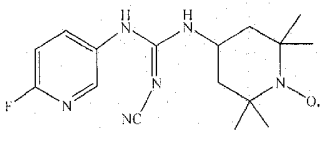
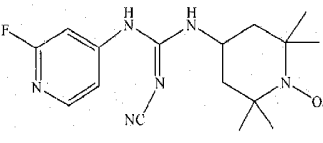
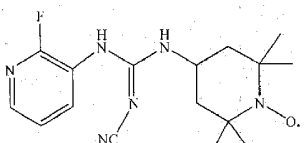
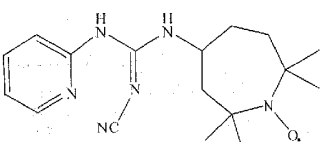
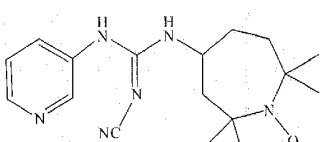
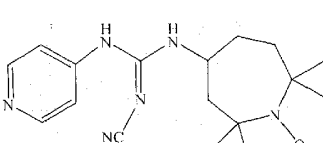
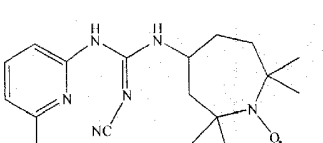
**[0036]** In further specific embodiments, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is N; X is  $-(CR_2R_2)_n-$  wherein n is 1; R<sub>1</sub> is absent; R<sub>2</sub> is H; R<sub>3</sub> is methyl; and A is linked to position 2, 3 or 4 of the pyridine ring, i.e., the oxy radical of 2-cyano-1-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)-3-(pyridin-2-yl)guanidine (compound **8**); 2-cyano-1-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)-3-(pyridin-3-yl)guanidine (compound **9**); or 2-cyano-1-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)-3-(pyridin-4-yl)guanidine (compound **10**).

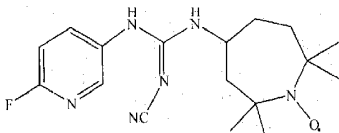
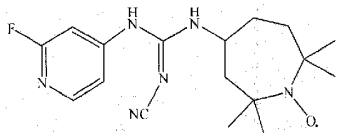
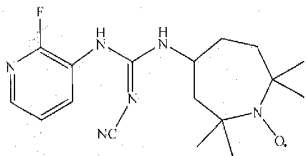
**[0037]** In still other specific embodiments, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is N; X is  $-(CR_2R_2)_n-$  wherein n is 1; R<sub>1</sub> is F, Cl or Br, linked to position 6 of the pyridine ring; R<sub>2</sub> is H; R<sub>3</sub> is methyl; and A is linked to position 2, 3, 4 or 5 of the pyridine ring, i.e., the oxy radical of 2-cyano-1-(6-fluoropyridin-2-yl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, 2-cyano-1-(6-chloropyridin-2-yl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, or 2-cyano-1-(6-bromo pyridin-2-yl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine (compounds **11a-11c**, respectively); 2-cyano-1-(6-fluoropyridin-3-yl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, 2-cyano-1-(6-chloropyridin-3-yl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, or 2-cyano-1-(6-bromo pyridin-3-yl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine (compounds **12a-12c**, respectively); 2-cyano-1-(6-fluoropyridin-4-yl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, 2-cyano-1-(6-chloropyridin-4-yl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, or 2-cyano-1-(6-bromo pyridin-4-yl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine (compounds **13a-13c**, respectively); or 2-cyano-1-(6-fluoropyridin-5-yl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, 2-cyano-1-(6-chloropyridin-5-yl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, or 2-cyano-1-(6-bromopyridin-5-yl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine (compounds **14a-14c**, respectively).

**[0038]** In yet other specific embodiments, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is N; X is  $-(CR_2R_2)_n-$  wherein n is 2; R<sub>1</sub> is absent; R<sub>2</sub> is H; R<sub>3</sub> is methyl; and A is linked to position 2, 3 or 4 of the pyridine ring, i.e., the oxy radical of 2-cyano-1-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)-3-(pyridin-2-yl)guanidine (compound **15**); 2-cyano-1-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)-3-(pyridin-3-yl)guanidine (compound **16**); or 2-cyano-1-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)-3-(pyridin-4-yl) guanidine (compound **17**).

**Table 4:** Multifunctional nitroxide derivatives herein identified compounds **1-21**

1	2
	

<b>3</b>	<b>4<sub>a</sub>*</b>
	
<b>5<sub>a</sub>*</b>	<b>6<sub>a</sub>*</b>
	
<b>7<sub>a</sub>*</b>	<b>8</b>
	
<b>9</b>	<b>10</b>
	
<b>11<sub>a</sub>*</b>	<b>12<sub>a</sub>*</b>
	
<b>13<sub>a</sub>*</b>	<b>14<sub>a</sub>*</b>
	
<b>15</b>	<b>16</b>
	
<b>17</b>	<b>18<sub>a</sub>*</b>
	

<b>19<sub>a</sub>*</b>	<b>20<sub>a</sub>*</b>
	
<b>21<sub>a</sub>*</b>	
	
<p>* The compound in which the F atom is replaced by Cl or Br has the same identification number with configuration "b" or "c", respectively</p>	

[0039] In yet further specific embodiments, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is N; X is  $(\text{CR}_2\text{R}_2)_n$  wherein n is 2; R<sub>1</sub> is F, Cl or Br, linked to position 6 of the pyridine ring; R<sub>2</sub> is H; R<sub>3</sub> is methyl; and A is linked to position 2, 3, 4 or 5 of the pyridine ring, i.e., the oxy radical of 2-cyano-1-(6-fluoropyridin-2-yl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine, 2-cyano-1-(6-chloropyridin-2-yl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine, or 2-cyano-1-(6-bromopyridin-2-yl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine (compounds **18<sub>a</sub>**-**18<sub>c</sub>**, respectively); 2-cyano-1-(6-fluoropyridin-3-yl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine, 2-cyano-1-(6-chloropyridin-3-yl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine, or 2-cyano-1-(6-bromopyridin-3-yl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine (compounds **19<sub>a</sub>**-**19<sub>c</sub>**, respectively); 2-cyano-1-(6-fluoropyridin-4-yl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine, 2-cyano-1-(6-chloropyridin-4-yl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine, or 2-cyano-1-(6-bromopyridin-4-yl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine (compounds **20<sub>a</sub>**-**20<sub>c</sub>**, respectively); or 2-cyano-1-(6-fluoropyridin-5-yl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine, 2-cyano-1-(6-chloropyridin-5-yl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine, or 2-cyano-1-(6-bromopyridin-5-yl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine (compounds **21<sub>a</sub>**-**21<sub>c</sub>**, respectively).

[0040] In certain particular embodiments, the multifunctional nitroxide derivative of the present invention is a compound of the general formula I, wherein Y is CH; A is linked to any position of the phenyl ring; R<sub>1</sub> is absent or 1 to 5 substituents each independently is halogen; X is absent or  $(\text{CR}_2\text{R}_2)_n$  wherein n is 1 or 2; R<sub>2</sub> each is H; R<sub>3</sub> each independently is (C<sub>1</sub>-C<sub>4</sub>)alkyl, preferably (C<sub>1</sub>-C<sub>2</sub>)alkyl, more preferably methyl; R<sub>4</sub> is H; and R<sub>5</sub> is -CN. In certain more particular embodiments, R<sub>1</sub> is a sole substituent linked to any of the available carbon atoms of the phenyl ring, i.e., a halogen linked to the phenyl ring at position ortho, meta or para with respect to group A. In other more particular embodiments, R<sub>1</sub> represents 2 substituents each independently linked to any of the available carbon atoms of the phenyl ring, i.e., two halogens linked to the phenyl ring at position ortho, meta or para with respect to each other. Specific such compounds described in the specification are herein identified by the Arabic numbers **22-39** in bold (each one of the compounds **23-25**, **33-35** and **37-39** has three configurations identified a-c), wherein their full chemical structures are depicted in **Table 5** hereinafter.

[0041] In a specific embodiment, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is CH; X is absent; R<sub>1</sub> is absent; R<sub>2</sub> is H; and R<sub>3</sub> is methyl, i.e., the oxy radical of 2-cyano-1-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)-3-phenylguanidine (compound **22**).

[0042] In other specific embodiments, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is CH; X is absent; R<sub>2</sub> is H; R<sub>3</sub> is methyl; and R<sub>1</sub> is F, Cl or Br, linked to the phenyl ring at position ortho, meta or para with respect to A, i.e., the oxy radical of 2-cyano-1-(2-fluorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine, 2-cyano-1-(2-chlorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine, or 2-cyano-1-(2-bromophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine (compounds **23<sub>a</sub>**-**23<sub>c</sub>**, respectively); 2-cyano-1-(3-fluorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine, 2-cyano-1-(3-chlorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine, or 2-cyano-1-(3-bromophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine (compounds **24<sub>a</sub>**-**24<sub>c</sub>**, respectively); or 2-cyano-1-(4-fluorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine, 2-

cyano-1-(4-chlorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine, or 2-cyano-1-(4-bromophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine (compounds **25a-25c**, respectively).

[0043] In further specific embodiments, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is CH; X is absent; R<sub>2</sub> is H; R<sub>3</sub> is methyl; and R<sub>1</sub> represents 2 substituents each independently is F, Cl or Br, linked to the phenyl ring at position ortho, meta or para with respect to A. In particular such embodiments, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein R<sub>1</sub> represents 2 substituents each is Cl, linked to the phenyl ring at positions ortho and ortho, i.e., the oxy radical of 2-cyano-1-(2,6-dichlorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethyl pyrrolidin-3-yl)guanidine (compound **26**); ortho and meta, i.e., the oxy radical of 2-cyano-1-(2,5-dichlorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl) guanidine or 2-cyano-1-(2,3-dichlorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethyl pyrrolidin-3-yl)guanidine (compounds **27** and **28**, respectively); ortho and para, i.e., the oxy radical of 2-cyano-1-(2,4-dichlorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethyl pyrrolidin-3-yl)guanidine (compound **29**); meta and meta, i.e., the oxy radical of 2-cyano-1-(3,5-dichlorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl) guanidine (compound **30**); or meta and para, i.e., the oxy radical of 2-cyano-1-(3,4-dichlorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine (compound **31**), with respect to A.

[0044] In another specific embodiment, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is CH; X is - (CR<sub>2</sub>R<sub>2</sub>)<sub>n</sub>- wherein n is 1; R<sub>1</sub> is absent; R<sub>2</sub> is H; and R<sub>3</sub> is methyl, i.e., the oxy radical of 2-cyano-1-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)-3-phenyl guanidine (compound **32**).

[0045] In still other specific embodiments, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is CH; X is -(CR<sub>2</sub>R<sub>2</sub>)<sub>n</sub>- wherein n is 1; R<sub>2</sub> is H; R<sub>3</sub> is methyl; and R<sub>1</sub> is F, Cl or Br, linked to the phenyl ring at position ortho, meta or para with respect to A, i.e., the oxy radical of 2-cyano-1-(2-fluorophenyl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl) guanidine, 2-cyano-1-(2-chlorophenyl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, or 2-cyano-1-(2-bromophenyl)-3-(1-hydroxy-2,2,6,6-tetramethyl piperidin-4-yl)guanidine (compounds **33a-c**, respectively); 2-cyano-1-(3-fluoro phenyl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, 2-cyano-1-(3-chlorophenyl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, or 2-cyano-1-(3-bromophenyl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl) guanidine (compounds **34a-c**, respectively); or 2-cyano-1-(4-fluorophenyl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, 2-cyano-1-(4-chlorophenyl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, or 2-cyano-1-(4-bromo phenyl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine (compounds **35a-c**, respectively).

[0046] In yet other specific embodiments, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is CH; X is - (CR<sub>2</sub>R<sub>2</sub>)<sub>n</sub>- wherein n is 1; R<sub>2</sub> is H; R<sub>3</sub> is methyl; and R<sub>1</sub> represents 2 substituents each independently is F, Cl or Br, linked to the phenyl ring at position ortho, meta or para with respect to A.

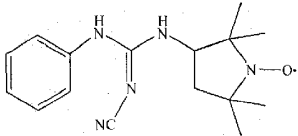
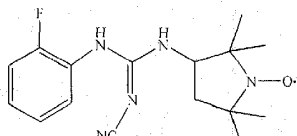
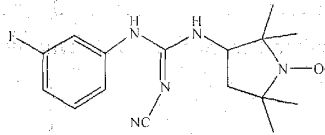
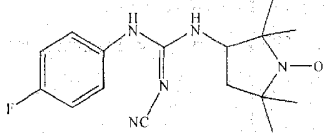
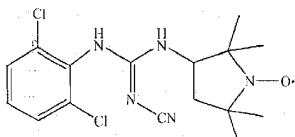
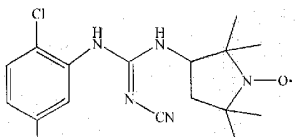
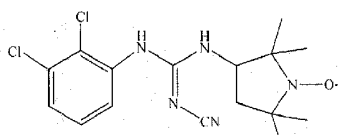
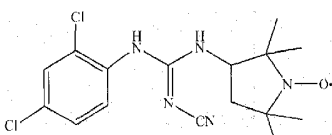
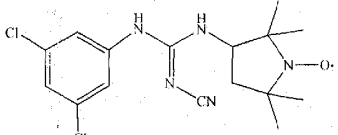
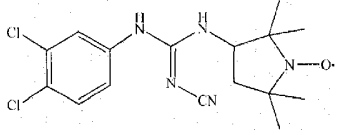
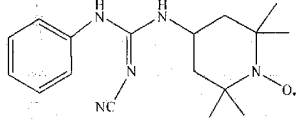
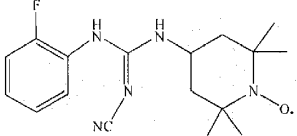
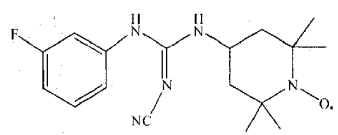
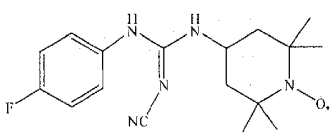
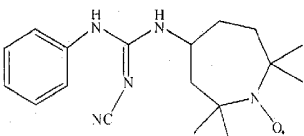
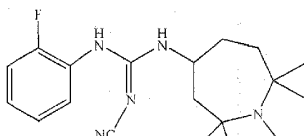
[0047] In a further specific embodiment, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is CH; X is - (CR<sub>2</sub>R<sub>2</sub>)<sub>n</sub>- wherein n is 2; R<sub>1</sub> is absent; R<sub>2</sub> is H; and R<sub>3</sub> is methyl, i.e., the oxy radical of 2-cyano-1-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)-3-phenylguanidine (compound **36**).

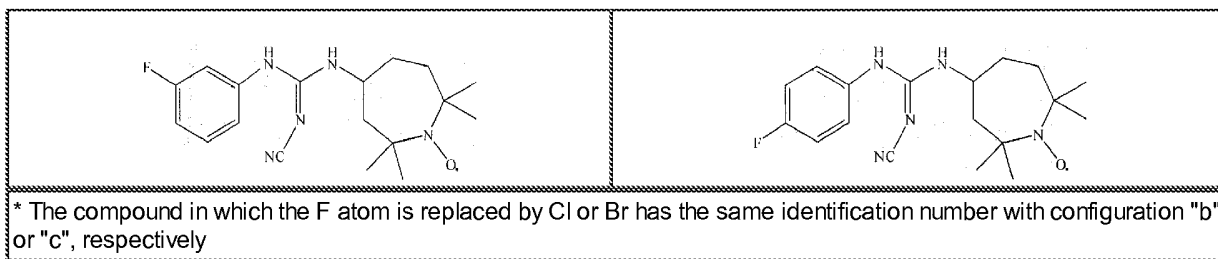
[0048] In still further specific embodiments, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is CH; X is -(CR<sub>2</sub>R<sub>2</sub>)<sub>n</sub>- wherein n is 2; R<sub>2</sub> is H; R<sub>3</sub> is methyl; and R<sub>1</sub> is F, Cl or Br, linked to the phenyl ring at position ortho, meta or para with respect to A, i.e., the oxy radical of 2-cyano-1-(2-fluorophenyl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine, 2-cyano-1-(2-chlorophenyl)-3-(1-hydroxy-2,2,7,7-tetramethyl azepan-4-yl)guanidine, or 2-cyano-1-(2-bromophenyl)-3-(1-hydroxy-2,2,7,7-tetra methylazepan-4-yl)guanidine (compounds **37a-c**, respectively); 2-cyano-1-(3-fluoro phenyl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine, 2-cyano-1-(3-chlorophenyl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine, or 2-cyano-1-(3-bromophenyl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine (compounds **38a-c**, respectively); or 2-cyano-1-(4-fluorophenyl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine, 2-cyano-1-(4-chlorophenyl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine, or 2-cyano-1-(4-bromophenyl)-3-(1-hydroxy-2,2,7,7-tetramethylazepan-4-yl)guanidine (compounds **39a-c**, respectively).

[0049] In yet further specific embodiments, the multifunctional nitroxide derivative of the invention is a compound of the general formula I, wherein Y is CH; X is -(CR<sub>2</sub>R<sub>2</sub>)<sub>n</sub>- wherein n is 1; R<sub>2</sub> is H; R<sub>3</sub> is methyl; and R<sub>1</sub> represents 2 substituents each independently is F, Cl or Br, linked to the phenyl ring at position ortho, meta or para with respect to A.

**Table 5:** Multifunctional nitroxide derivatives herein identified compounds **22-39** &

<b>22</b>	<b>23<sub>a</sub>*</b>
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<b>24<sub>a</sub>*</b>	<b>25<sub>a</sub>*</b>
	
<b>26</b>	<b>27</b>
	
<b>28</b>	<b>29</b>
	
<b>30</b>	<b>31</b>
	
<b>32</b>	<b>33<sub>a</sub>*</b>
	
<b>34<sub>a</sub>*</b>	<b>35<sub>a</sub>*</b>
	
<b>36</b>	<b>37<sub>a</sub>*</b>
	
<b>38<sub>a</sub>*</b>	<b>39<sub>a</sub>*</b>



**[0050]** In certain particular embodiments, the multifunctional nitroxide derivative of the present invention is a compound of the general formula I, wherein Y is N( $\rightarrow$ O); A is linked to position 2, 3, 4, 5 or 6 of the pyridine ring; R<sub>1</sub> is absent or 1 to 4 substituents each independently is halogen; X is absent or -(CR<sub>2</sub>R<sub>2</sub>)<sub>n</sub> wherein n is 1 or 2; R<sub>2</sub> each is H; R<sub>3</sub> each independently is (C<sub>1</sub>-C<sub>4</sub>)alkyl, preferably (C<sub>1</sub>-C<sub>2</sub>)alkyl, more preferably methyl; R<sub>4</sub> is H; and R<sub>5</sub> is -CN. In certain more particular embodiments, R<sub>1</sub> is a sole substituent linked to any of the available carbon atoms of the pyridine oxide ring, i.e., a halogen linked to the pyridine oxide ring at position ortho, meta or para with respect to group A. In other more particular embodiments, R<sub>1</sub> represents 2 substituents each independently linked to any of the available carbon atoms of the pyridine oxide ring, i.e., two halogens linked to the pyridine oxide ring at position ortho, meta or para with respect to each other.

**[0051]** The various multifunctional nitroxide derivatives of the present invention may be synthesized according to any technology or procedure known in the art, e.g., as described in the Examples section hereinafter.

**[0052]** The multifunctional nitroxide derivatives of the present invention may have one or more asymmetric centers, and may accordingly exist both as enantiomers, i.e., optical isomers (R, S, or racemate, wherein a certain enantiomer may have an optical purity of 90%, 95%, 99% or more) and as diastereoisomers. Specifically, those chiral centers may be, e.g., in each one of the carbon atoms of the oxy radical of 1-hydroxypyrrolidin-3-yl, 1-hydroxypiperidin-4-yl, or 1-hydroxyazepan-4-yl moiety; as well as in either or both the -NH groups of the guanidino moiety. It should be understood that the present invention encompasses all such enantiomers, optical isomers and mixtures thereof, as well as pharmaceutically acceptable salts or solvates thereof.

**[0053]** Optically active forms of the multifunctional nitroxide derivatives of the invention may be prepared using any method known in the art, e.g., by resolution of the racemic form by recrystallization techniques; by chiral synthesis; by extraction with chiral solvents; or by chromatographic separation using a chiral stationary phase. A non-limiting example of a method for obtaining optically active materials is transport across chiral membranes, i.e., a technique whereby a racemate is placed in contact with a thin membrane barrier, the concentration or pressure differential causes preferential transport across the membrane barrier, and separation occurs as a result of the non-racemic chiral nature of the membrane that allows only one enantiomer of the racemate to pass through. Chiral chromatography, including simulated moving bed chromatography, can also be used. A wide variety of chiral stationary phases are commercially available.

**[0054]** As stated above, administration of compound 2 was found to be highly beneficial in treatment, prevention and/or managing of various diseases, disorders or conditions associated with oxidative stress or endothelial dysfunction.

**[0055]** As shown in Example 11, compound 2 significantly reduced both myocardial myeloperoxidase (MPO) and tissue infarction in a rat model of myocardial ischemia-reperfusion injury (MIRI). In particular, whereas MIRI induced severe myocyte necrosis, edema and neutrophil infiltration (center) compared to a sham control, administration of compound 2 prior to reperfusion profoundly attenuated virtually all histologic features of injury. Furthermore, in an *ex vivo* rat vascular ring system precontracted with norepinephrine, compound 2 was found to be a less potent *ex vivo* vasodilator compared to pinacidil, having ED<sub>50</sub> that is one log greater than that of pinacidil, indicative of a loss in sarcolemmal K<sup>+</sup>-ATP channel activation.

**[0056]** Example 12 shows that compound 2 was further protective in a murine model of renal ischemia-reperfusion injury. More particularly, addition of compound 2 to CD mice underwent bilateral clamping of the renal pedicles, before reperfusion, profoundly attenuated elevations in plasma blood urea nitrogen and creatinine, as well as in renal myeloperoxidase, a marker of neutrophil infiltration; and as revealed by histologic studies, provided near total protection against severe renal tubular necrosis.

**[0057]** In addition to its therapeutic properties in ischemia-reperfusion injury model, compound 2 has further been found to be effective as a rescue therapy in a murine model of acute chlorine inhalation injury as demonstrated in Example 13. As particularly shown, compound 2 administered to Balb/c mice 15 min after a continuous exposure to chlorine remarkably reduced both the elevation in polymorphonuclear leukocytes (PMN) infiltration as reflected in the level of myocardial myeloperoxidase (MPO) and

histologic lung damage. As further shown, compound 2 attenuated lung injury in a dose-dependent manner, as exemplified by improved histology scores.

**[0058]** Example 14 shows that compound 2 did not exhibit any significant effect on blood glucose levels compared with saline vehicle group.

**[0059]** In another aspect, the present invention thus provides a pharmaceutical composition comprising a multifunctional nitroxide derivative of the general formula I as defined above, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt or solvate thereof, and a pharmaceutically acceptable carrier. In particular embodiments, the pharmaceutical composition of the invention comprises a multifunctional nitroxide derivative selected from compounds 1-39, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt or solvate thereof.

**[0060]** The multifunctional nitroxide derivatives and compositions of the present invention can be provided in a variety of formulations, e.g., in a pharmaceutically acceptable form and/or in a salt or solvate, e.g., hydrate, form, as well as in a variety of dosages.

**[0061]** In one embodiment, the pharmaceutical composition of the present invention comprises a non-toxic pharmaceutically acceptable salt of a multifunctional nitroxide derivative of the general formula I. Suitable pharmaceutically acceptable salts include acid addition salts such as, without being limited to, the mesylate salt; the maleate salt, the fumarate salt, the tartrate salt, the hydrochloride salt, the hydrobromide salt, the esylate salt; the p-toluenesulfonate salt, the benzoate salt, the acetate salt, the phosphate salt, the sulfate salt, the citrate salt, the carbonate salt, and the succinate salt. Additional pharmaceutically acceptable salts include salts of ammonium ( $\text{NH}_4^+$ ) or an organic cation derived from an amine of the formula  $\text{R}_4\text{N}^+$ , wherein each one of the Rs independently is selected from H, C<sub>1</sub>-C<sub>22</sub>, preferably C<sub>1</sub>-C<sub>6</sub> alkyl, such as methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, 2,2-dimethylpropyl, n-hexyl, and the like, phenyl, or heteroaryl such as pyridyl, imidazolyl, pyrimidinyl, and the like, or two of the Rs together with the nitrogen atom to which they are attached form a 3-7 membered ring optionally containing a further heteroatom selected from N, S and O, such as pyrrolidine, piperidine and morpholine. Furthermore, where the multifunctional nitroxide derivatives of the invention carry an acidic moiety, suitable pharmaceutically acceptable salts thereof may include metal salts such as alkali metal salts, e.g., lithium, sodium or potassium salts, and alkaline earth metal salts, e.g., calcium or magnesium salts.

**[0062]** Further pharmaceutically acceptable salts include salts of a cationic lipid or a mixture of cationic lipids. Cationic lipids are often mixed with neutral lipids prior to use as delivery agents. Neutral lipids include, but are not limited to, lecithins; phosphatidylethanolamine; diacyl phosphatidylethanolamines such as dioleoyl phosphatidylethanolamine, dipalmitoyl phosphatidylethanolamine, palmitoyloleoyl phosphatidylethanolamine and distearoyl phosphatidylethanolamine; phosphatidylcholine; diacyl phosphatidylcholines such as dioleoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, palmitoyloleoyl phosphatidylcholine and distearoyl phosphatidylcholine; phosphatidylglycerol; diacyl phosphatidylglycerols such as dioleoyl phosphatidylglycerol, dipalmitoyl phosphatidylglycerol and distearoyl phosphatidylglycerol; phosphatidylserine; diacyl phosphatidylserines such as dioleoyl- or dipalmitoyl phosphatidylserine; and diphosphatidylglycerols; fatty acid esters; glycerol esters; sphingolipids; cardiolipin; cerebroside; ceramides; and mixtures thereof. Neutral lipids also include cholesterol and other 3 $\beta$  hydroxy-sterols.

**[0063]** Examples of cationic lipid compounds include, without being limited to, Lipofectin<sup>®</sup> (Life Technologies, Burlington, Ontario) (1:1 (w/w) formulation of the cationic lipid N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride and dioleoylphosphatidylethanolamine); Lipofectamine<sup>™</sup> (Life Technologies, Burlington, Ontario) (3:1 (w/w) formulation of polycationic lipid 2,3-dioleoyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate and dioleoylphosphatidylethanolamine), Lipofectamine Plus (Life Technologies, Burlington, Ontario) (Lipofectamine and Plus reagent), Lipofectamine 2000 (Life Technologies, Burlington, Ontario) (Cationic lipid), Effectene (Qiagen, Mississauga, Ontario) (Non liposomal lipid formulation), Metafectene (Biontex, Munich, Germany) (Polycationic lipid), Eu-fectins (Promega Biosciences, San Luis Obispo, Calif.) (ethanolic cationic lipids numbers 1 through 12: C<sub>52</sub>H<sub>106</sub>N<sub>6</sub>O<sub>4</sub>·4CF<sub>3</sub>CO<sub>2</sub>H, C<sub>88</sub>H<sub>178</sub>N<sub>8</sub>O<sub>4</sub>S<sub>2</sub>·4CF<sub>3</sub>CO<sub>2</sub>H, C<sub>40</sub>H<sub>84</sub>N<sub>3</sub>O<sub>3</sub>P·CF<sub>3</sub>CO<sub>2</sub>H, C<sub>50</sub>H<sub>103</sub>N<sub>7</sub>O<sub>3</sub>·4CF<sub>3</sub>CO<sub>2</sub>H, C<sub>55</sub>H<sub>116</sub>N<sub>8</sub>O<sub>2</sub>·6CF<sub>3</sub>CO<sub>2</sub>H, C<sub>49</sub>H<sub>102</sub>N<sub>6</sub>O<sub>3</sub>·4CF<sub>3</sub>CO<sub>2</sub>H, C<sub>44</sub>H<sub>89</sub>N<sub>5</sub>O<sub>3</sub>·2CF<sub>3</sub>CO<sub>2</sub>H, C<sub>100</sub>H<sub>206</sub>N<sub>12</sub>O<sub>4</sub>S<sub>2</sub>·8CF<sub>3</sub>CO<sub>2</sub>H, C<sub>162</sub>H<sub>330</sub>N<sub>22</sub>O<sub>9</sub>·13CF<sub>3</sub>CO<sub>2</sub>H, C<sub>43</sub>H<sub>88</sub>N<sub>4</sub>O<sub>2</sub>·2CF<sub>3</sub>CO<sub>2</sub>H, C<sub>43</sub>H<sub>88</sub>N<sub>4</sub>O<sub>3</sub>·2CF<sub>3</sub>CO<sub>2</sub>H, C<sub>41</sub>H<sub>78</sub>N<sub>3</sub>O<sub>3</sub>P); Cytofectene (Bio-Rad, Hercules, Calif.) (mixture of a cationic lipid and a neutral lipid), GenePORTER<sup>®</sup> (Gene Therapy Systems, San Diego, Calif.) (formulation of a neutral lipid (Dope) and a cationic lipid) and FuGENE 6 (Roche Molecular Biochemicals, Indianapolis, Ind.) (Multi-component lipid based non-liposomal reagent).

**[0064]** The pharmaceutically acceptable salts of the present invention may be formed by conventional means, e.g., by reacting



the free base form of the active agent, i.e., the multifunctional nitroxide derivative of the invention, with one or more equivalents of the appropriate acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is removed *in vacuo* or by freeze drying, or by exchanging the anion/cation on a suitable ion exchange resin.

**[0065]** In one embodiment, the pharmaceutical composition of the present invention is formulated as nanoparticles.

**[0066]** The pharmaceutical compositions provided by the present invention may be prepared by conventional techniques, e.g., as described in Remington: The Science and Practice of Pharmacy, 19th Ed., 1995. The compositions can be prepared, e.g., by uniformly and intimately bringing the active agent, e.g., the multifunctional nitroxide derivative of the invention, into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into the desired formulation. The compositions may be in solid, semisolid or liquid form and may further include pharmaceutically acceptable fillers, carriers, diluents or adjuvants, and other inert ingredients and excipients. The compositions can be formulated for any suitable route of administration, e.g., oral, nasogastric, nasointestinal, orogastric, parenteral (e.g., intramuscular, subcutaneous, intraperitoneal, intravenous, intraarterial or subcutaneous injection, or implant), gavage, buccal, nasal, sublingual or topical administration, as well as for inhalation. The dosage will depend on the state of the patient, and will be determined as deemed appropriate by the practitioner.

**[0067]** The pharmaceutical composition of the present invention may be in a form suitable for oral use, e.g., as tablets, troches, lozenges, aqueous, or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and may further comprise one or more agents selected from sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients, which are suitable for the manufacture of tablets. These excipients may be, e.g., inert diluents such as calcium carbonate, sodium carbonate, lactose, calcium phosphate, or sodium phosphate; granulating and disintegrating agents, e.g., corn starch or alginic acid; binding agents, e.g., starch, gelatin or acacia; and lubricating agents, e.g., magnesium stearate, stearic acid, or talc. The tablets may be either uncoated or coated utilizing known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated using the techniques described in the US Patent Nos. 4,256,108, 4,166,452 and 4,265,874 to form osmotic therapeutic tablets for control release. The pharmaceutical composition of the invention may also be in the form of oil-in-water emulsion.

**[0068]** The pharmaceutical composition of the present invention may be in the form of a sterile injectable aqueous or oleaginous suspension, which may be formulated according to the known art using suitable dispersing, wetting or suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent. Acceptable vehicles and solvents that may be employed include, without limiting, water, Ringer's solution and isotonic sodium chloride solution.

**[0069]** The pharmaceutical compositions of the invention may be in any suitable form, e.g., tablets such as matrix tablets, in which the release of a soluble active agent is controlled by having the active diffuse through a gel formed after the swelling of a hydrophilic polymer brought into contact with dissolving liquid (*in vitro*) or gastro-intestinal fluid (*in vivo*). Many polymers have been described as capable of forming such gel, e.g., derivatives of cellulose, in particular the cellulose ethers such as hydroxypropyl cellulose, hydroxymethyl cellulose, methylcellulose or methyl hydroxypropyl cellulose, and among the different commercial grades of these ethers are those showing fairly high viscosity.

**[0070]** The pharmaceutical compositions of the present invention may comprise the active agent formulated for controlled release in microencapsulated dosage form, in which small droplets of the active agent are surrounded by a coating or a membrane to form particles in the range of a few micrometers to a few millimeters, or in controlled-release matrix.

**[0071]** Another contemplated formulation is depot systems, based on biodegradable polymers, wherein as the polymer degrades, the active agent is slowly released. The most common class of biodegradable polymers is the hydrolytically labile polyesters prepared from lactic acid, glycolic acid, or combinations of these two molecules. Polymers prepared from these individual monomers include poly (D,L-lactide) (PLA), poly (glycolide) (PGA), and the copolymer poly (D,L-lactide-co-glycolide) (PLG).

**[0072]** Pharmaceutical compositions according to the present invention, when formulated for inhalation, may be administered utilizing any suitable device known in the art, such as metered dose inhalers, liquid nebulizers, dry powder inhalers, sprayers, thermal vaporizers, electrohydrodynamic aerosolizers, and the like.

[0073] The multifunctional nitroxide derivatives and pharmaceutical compositions of the invention are useful for treating, preventing and/or managing diseases, disorders or conditions associated with high levels of reactive oxygen species (ROS), i.e., oxidative stress, or endothelial dysfunction.

[0074] In one embodiment, said disease, disorder or condition associated with oxidative stress or endothelial dysfunction is a disease, disorder or condition associated with ischemia-reperfusion injury. Non-limiting examples of such diseases include sepsis, septic shock, stroke, cataract formation, glaucoma, geographic atrophy, macular degeneration, angina, hemorrhagic shock, superantigen-induced circulatory shock, renal reperfusion injury, contrast agent-induced nephropathy, retinopathy of prematurity, necrotizing enterocolitis, neonatal respiratory distress syndrome, lung ischemia reperfusion injury, e.g., following transplantation, complications of IL-2 biotherapy, myocardial infarction, complications of cardiopulmonary bypass surgery, limb reperfusion injury, post-prostatectomy related erectile dysfunction, reperfusion complications related to vascular surgery including carotid endarterectomy, aortic aneurysm repair, peripheral arterial embolectomy and thrombectomy, crush injury, compartment syndrome, organ preservation, head trauma, and spinal cord injury.

[0075] In another embodiment, said disease, disorder or condition associated with oxidative stress or endothelial dysfunction is a neurodegenerative disease such as, without being limited to, Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis.

[0076] In a further embodiment, said disease, disorder or condition associated with oxidative stress or endothelial dysfunction is an inflammatory or immune disease. In certain particular embodiments, said inflammatory or immune diseases is selected from sepsis, uveitis, rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, inflamed joints, eczema, inflammatory skin conditions, inflammatory eye conditions, conjunctivitis, tissue necrosis resulting from inflammation, tissue rejection following transplant surgery, graft vs. host disease, Crohn's disease and ulcerative colitis, airway inflammation, asthma, bronchitis, systemic lupus erythematosus, multiple sclerosis, glaucoma, smoking-induced lung injury, pulmonary fibrosis, pancreatitis, cardiomyopathy including chemotherapy-induced cardiomyopathy, complications of IL-2 biotherapy, diabetes, diabetic complications including diabetic retinopathy, peripheral neuropathy, acute macular degeneration, skin ulcers, renal disease, pneumonia, mucositis, adult respiratory distress syndrome, smoke inhalation, or cutaneous burn injury. In other particular embodiments, said inflammatory disease is an inflammatory disease of the lung caused by inhalation of toxic agents or irritants such as chlorine, phosgene, and smoke.

[0077] In still another embodiment, said disease, disorder or condition associated with oxidative stress or endothelial dysfunction is cancer or a condition associated with chemotherapy or radiation treatment of cancer.

[0078] In a further aspect, the present invention thus provides a multifunctional nitroxide derivative of the general formula I as defined above, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt, solvate or prodrug thereof, for use in treatment of a disease, disorder or condition associated with oxidative stress or endothelial dysfunction.

[0079] Described herein is a method for treatment of a disease, disorder or condition associated with oxidative stress or endothelial dysfunction, said method comprising administering to an individual in need a therapeutically effective amount of a multifunctional nitroxide derivative of the general formula I as defined above, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt or solvate thereof.

[0080] The invention will now be illustrated by the following non-limiting Examples.

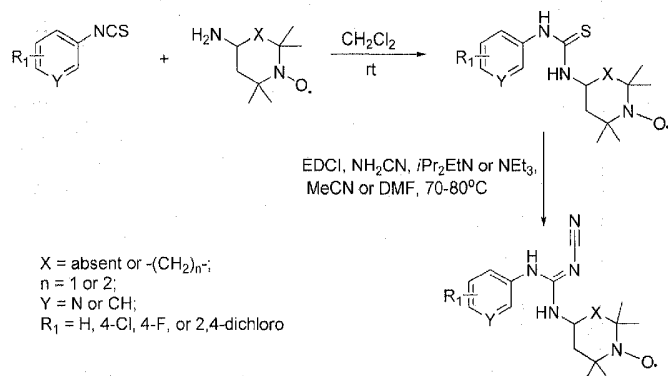
## EXAMPLES

### Example 1. Synthesis of compound 2

[0081] As generally depicted in **Scheme 1**, a mixture of 3-isothiocyanatopyridine (1.735 g) and 3-amino-2,2,5,5-tetramethylpyrrolidin-nitroxide (2.0 g) in methylene chloride (30 ml) was stirred at room temperature for 8 hr. Thin layer chromatography (TLC) showed some unreacted starting material. The reaction mixture was stirred overnight at room temperature, and it was then concentrated on rotavap. The residue, 1-(pyridin-3-yl)-3-(2,2,5,5-tetramethylpyrrolidin-nitroxide-3-yl)thiourea (3.730 g) obtained was used for further reaction.

**[0082]** A mixture of 1-(pyridin-3-yl)-3-(2,2,5,5-tetramethylpyrrolidin-nitroxide-3-yl)thiourea (3.6 g), cyanamide (5.125 g), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (2.805 g) and triethylamine (4.928 g) in acetonitrile (50 ml) was stirred at room temperature for 24 hr, and the mixture was then refluxed at 80°C for 4 hr. The reaction mixture was concentrated on rotavap and purified on silica gel column using methanol-methylene chloride (5-10%) to provide the oxy radical of 2-cyano-1-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)-3-(pyridin-3-yl)guanidine, **2** (3.050 g). MS (CI<sup>+</sup>) *m/z* 301.17 (M+1, 302.18).

**Scheme 1:** General procedure for the synthesis of nitroxide derivatives such as compounds **2**, **5<sub>b</sub>**, **25<sub>a-c</sub>**, **29** and **35<sub>a-c</sub>**



### Example 2. Synthesis of compound **3**

**[0083]** The synthesis of the oxy radical of 2-cyano-1-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)-3-(pyridin-4-yl)guanidine, **3**, commences with the preparation of thiourea from the reaction of 4-isothiocyanatopyridine and 3-amino-2,2,5,5-tetramethylpyrrolidin-1-nitroxide. The 1-(pyridin-4-yl)-3-(2,2,5,5-tetramethylpyrrolidin-nitroxide-3-yl)thiourea obtained is then reacted with cyanamide, EDCI and diisopropylethylamine in DMF, under the same conditions described in Example 1, to yield the desired cyanoguanidine product.

### Example 3. Synthesis of compound **5<sub>b</sub>**

**[0084]** As generally depicted in **Scheme 1**, a mixture of (3-amino-2,2,5,5-tetramethylpyrrolidin-1-yl)oxidanyl (1.580 g, 0.01 mol) and 6-chloro-3-pyridyl isothiocyanate (1.706 g, 0.01 mol) in methylene chloride (20 ml) was stirred at room temperature for 24 hr. The reaction mixture was concentrated on rotary evaporator. The residue obtained after concentration was purified on silica gel column using 5% methanol-methylene chloride to give the (3-[(6-chloropyridin-3-yl)carbamothioyl]amino)-2,2,5,5-tetramethylpyrrolidin-1-yl)oxidanyl as a yellow colored oil (3.130 g).

**[0085]** A mixture of (3-[(6-chloropyridin-3-yl)carbamothioyl]amino)-2,2,5,5-tetramethylpyrrolidin-1-yl)oxidanyl (2.825 g, 0.0086 mol), cyanamide (3.612 g, 0.086 mol), EDCI (2.465 g, 0.0129 mol) and triethylamine (4.81 ml, 0.034 mol) in acetonitrile (40 ml) was refluxed at 80°C for 2 days. The reaction mixture was concentrated and the residue was purified on silica gel column using 5% methanol-methylene chloride to provide the oxy radical of 2-cyano-1-(6-chloropyridin-3-yl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl) guanidine, **5<sub>b</sub>**, as a pale yellow colored solid (1.8 g). MS (ES<sup>+</sup>) *m/z* 335.14 (M+1, 336.2).

### Example 4. Synthesis of compound **25<sub>a</sub>**

**[0086]** As generally depicted in **Scheme 1**, a mixture of (3-amino-2,2,5,5-tetramethylpyrrolidin-1-yl)oxidanyl (0.532 g, 0.0033 mol) and 4-fluorophenyl isothiocyanate (0.408 ml, 0.0033 mol) in methylene chloride (20 ml) was stirred at room temperature for 3 hr. The reaction mixture was concentrated on rotary evaporator, and the residue obtained after concentration was suspended in hexane. The liquid was removed and the solid was dried under vacuum to give the {2,2,5,5-tetramethyl-3-[(4-fluorophenyl-1-yl)carbamothioyl]amino}pyrrolidin-1-yl)oxidanyl as a yellow colored solid.

**[0087]** The {2,2,5,5-tetramethyl-3-[(4-fluorophenyl-1-yl)carbamothioyl]amino} pyrrolidin-1-yl)oxidanyl obtained was combined with cyanamide (1.385 g, 0.033 mol), EDCI (0.945 gm, 0.0049 mol) and triethylamine (1.850 ml, 0.012 mol) in acetonitrile (25 ml), and

was stirred at 80°C for 24 hr. The reaction mixture was concentrated and the residue was diluted with ethyl acetate and water (25 ml each). The organic layer was collected and concentrated on rotary evaporator. The crude product was then purified on silica gel column using 40-50% ethyl acetate-hexane to provide the oxy radical of 2-cyano-1-(4-fluorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine, **25<sub>a</sub>** (0.355 gm). MS (ES<sup>+</sup>) *m/z* 318.17 (M+1, 319.34).

#### Example 5. Synthesis of compound 25<sub>b</sub>

[0088] As generally depicted in **Scheme 1**, a mixture of (3-amino-2,2,5,5-tetramethylpyrrolidin-1-yl)oxidanyl (0.500 g, 0.0031 mol) and 4-chlorophenyl isothiocyanate (0.538 g, 0.0031 mol) in methylene chloride (25 ml) was stirred at room temperature for 6 hr. The reaction mixture was concentrated on rotary evaporator, and the residue obtained after concentration was suspended in hexane. The liquid was removed and the solid was dried under vacuum to give the {2,2,5,5-tetramethyl-3-[(4-chlorophenyl-1-ylcarbamothioyl)amino]pyrrolidin-1-yl}oxidanyl as a yellow colored solid.

[0089] The {2,2,5,5-tetramethyl-3-[(4-chlorophenyl-1-ylcarbamothioyl)amino] pyrrolidin-1-yl}oxidanyl obtained was combined with cyanamide (1.3 g, 0.031 mol), EDCI (0.710 g, 0.0037 mol) and triethylamine (1.250 g, 0.012 mol) in acetonitrile (30 ml), and was stirred at 80°C for 24 hr. The reaction mixture was concentrated and the residue was diluted with ethyl acetate and water (25 ml each). The organic layer was collected and concentrated on rotary evaporator. The crude product was then purified on silica gel column using 40% ethyl acetate-hexane to provide the oxy radical of 2-cyano-1-(4-chlorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl)guanidine, **25<sub>b</sub>** (0.175 g). MS (ES<sup>+</sup>) *m/z* 334.13 (M+1, 335.25).

#### Example 6. Synthesis of compound 25<sub>c</sub>

[0090] As generally depicted in **Scheme 1**, a mixture of (3-amino-2,2,5,5-tetramethylpyrrolidin-1-yl)oxidanyl (0.450 mg, 0.0028 mol) and 4-bromophenyl isothiocyanate (0.615 g, 0.0028 mol) in methylene chloride (30 ml) was stirred at room temperature for 4 hr. The reaction mixture was concentrated on rotary evaporator, and the residue obtained after concentration was suspended in hexane. The liquid was removed and the solid was dried under vacuum to give the {2,2,5,5-tetramethyl-3-[(4-bromophenyl-1-ylcarbamothioyl)amino]pyrrolidin-1-yl}oxidanyl as a yellow colored solid.

[0091] The {2,2,5,5-tetramethyl-3-[(4-bromophenyl-1-ylcarbamothioyl)amino] pyrrolidin-1-yl}oxidanyl obtained was combined with cyanamide (0.028 mol), EDCI (0.0042 mol) and triethylamine (0.011 mol) in acetonitrile (25 ml), and was stirred at 70°C for 24 hr. The reaction mixture was concentrated and the residue was diluted with ethyl acetate and water (25 ml each). The organic layer was collected and concentrated on rotary evaporator. The crude product was then purified on silica gel column using 40-100% ethyl acetate-hexane to provide the oxy radical of 2-cyano-1-(4-bromophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl) guanidine, **25<sub>c</sub>** (0.280 gm). MS (ES<sup>+</sup>) *m/z* 378.10 (M+1, 379.22).

#### Examples 7. Synthesis of compound 29

[0092] As generally depicted in **Scheme 1**, a mixture of (3-amino-2,2,5,5-tetramethylpyrrolidin-1-yl)oxidanyl (0.500 g, 0.0031 mol) and 2,4-dichlorophenyl isothiocyanate (0.0034 mol) in methylene chloride (25 ml) was stirred at room temperature for 6 hr. The reaction mixture was concentrated on rotary evaporator, and the residue obtained after concentration was suspended in hexane. The liquid was removed and the solid was dried under vacuum to give the {2,2,5,5-tetramethyl-3-[(2,4-dichlorophenyl-1-ylcarbamothioyl) amino]pyrrolidin-1-yl} oxidanyl as a yellow colored solid.

[0093] The {2,2,5,5-tetramethyl-3-[(2,4-dichlorophenyl-1-ylcarbamothioyl)amino] pyrrolidin-1-yl}oxidanyl obtained was combined with cyanamide (1.3 g, 0.031 mol), EDCI (0.710 g, 0.0037 mol) and triethylamine (1.250 g, 0.012 mol) in acetonitrile (30 ml) and was stirred at 80°C for 24 hr. The reaction mixture was concentrated and the residue was diluted with ethyl acetate and water (25 ml each). The organic layer was collected and concentrated on rotary evaporator. The crude product was then purified on silica gel column using 5% methanol-methylene chloride to provide the oxy radical of 2-cyano-1-(2,4-dichlorophenyl)-3-(1-hydroxy-2,2,5,5-tetramethylpyrrolidin-3-yl) guanidine, **29** (0.705 gm). MS (ES<sup>+</sup>) *m/z* 368.06 (M+1, 379.23).

#### Example 8. Synthesis of compound 35<sub>a</sub>

[0094] As generally depicted in **Scheme 1**, a mixture of (4-amino-2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl (0.500 g, 0.0029 mol) and 4-fluorophenyl isothiocyanate (0.0029 mol) in methylene chloride (20 ml) was stirred at room temperature for overnight. The reaction mixture was concentrated on rotary evaporator. The residue obtained after concentration was dried under vacuum to give the {2,2,6,6-tetramethyl-3-[(4-fluorophenyl-1-ylcarbamothioyl)amino] piperidin-1-yl}oxidanyl as a pale yellow colored solid.

[0095] The {2,2,6,6-tetramethyl-3-[(4-fluorophenyl-1-ylcarbamothioyl)amino] piperidin-1-yl}oxidanyl obtained was combined with cyanamide (1.220 g, 0.029 mol), EDCI (0.840 g, 0.0043 mol) and triethylamine (1.620 ml, 0.011 mol) in acetonitrile (20 ml) and was stirred at 70°C for 4 hr. The reaction mixture was concentrated and the residue was diluted with ethyl acetate and water (25 ml each). The organic later was collected and concentrated on rotary evaporator. The crude product was then purified on silica gel column using 10% methanol-methylene chloride to provide the oxy radical of 2-cyano-1-(4-fluorophenyl)-3-(1-hydroxy-2,2,6,6-tetramethyl piperidin-4-yl) guanidine, **35<sub>a</sub>**, (0.248 g). MS (ES<sup>+</sup>) *m/z* 332.19 (M+1, 333.4).

#### Example 9. Synthesis of compound **35<sub>b</sub>**

[0096] As generally depicted in **Scheme 1**, a mixture of (4-amino-2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl (0.500 g, 0.0029 mol) and 4-chlorophenyl isothiocyanate (0.490 g, 0.0029 mol) in methylene chloride (20 ml) was stirred at room temperature for overnight. The reaction mixture was concentrated on rotary evaporator. The residue obtained after concentration was dried under vacuum to give the {2,2,6,6-tetramethyl-3-[(4-chlorophenyl-1-ylcarbamothioyl)amino] piperidin-1-yl}oxidanyl as a pale yellow colored solid.

[0097] The {2,2,6,6-tetramethyl-3-[(4-chlorophenyl-1-ylcarbamothioyl)amino] piperidin-1-yl}oxidanyl obtained was combined with cyanamide (1.220 g, 0.029 mol), EDCI (0.840 g, 0.0043 mol) and triethylamine (1.620 ml, 0.011 mol) in acetonitrile (20 ml) and was stirred at 80°C for 24 hr. The reaction mixture was concentrated and the residue was diluted with ethyl acetate and water (25 ml each). The organic later was collected and concentrated on rotary evaporator. The crude product was then purified on silica gel column using 10% methanol-methylene chloride to provide the oxy radical of 2-cyano-1-(4-chlorophenyl)-3-(1-hydroxy-2,2,6,6-tetra methylpiperidin-4-yl) guanidine, **35<sub>b</sub>** (0.415 g). MS (ES<sup>+</sup>) *m/z* 348.1 (M+1, 339.30).

#### Example 10. Synthesis of compound **35<sub>c</sub>**

[0098] As generally depicted in **Scheme 3**, a mixture of (4-amino-2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl (0.600 g, 0.0035 mol) and 4-bromophenyl isothiocyanate (0.0035 mol) in methylene chloride (30 ml) was stirred at room temperature for 3 hr. The reaction mixture was concentrated on rotary evaporator and suspended in ethyl acetate and hexane (10 ml each). The solid was filtered and dried under vacuum to give the {2,2,6,6-tetramethyl-3-[(4-bromophenyl-1-ylcarbamothioyl)amino]piperidin-1-yl}oxidanyl as a pale yellow colored solid.

[0099] The {2,2,6,6-tetramethyl-3-[(4-bromophenyl-1-ylcarbamothioyl)amino] piperidin-1-yl}oxidanyl obtained was combined with cyanamide (1.470 g, 0.035 mol), EDCI (1.0 g, 0.0052 mol) and triethylamine (2.00 ml, 0.014 mol) in acetonitrile (20 ml) and was stirred at 75°C for 7 hr. The reaction mixture was concentrated and the residue was diluted with ethyl acetate and water (25 ml each). The organic later was collected and concentrated on rotary evaporator. The crude product was then purified on silica gel column using 50-100% ethyl acetate-hexane to provide the oxy radical of 2-cyano-1-(4-bromophenyl)-3-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)guanidine, **35<sub>c</sub>** (0.240 g). MS (ES<sup>+</sup>) *m/z* 392.24 (M+1, 393.26).

#### Example 11. Compound **2** reduces infarct size and myocardial damage in a rat model of myocardial ischemia-reperfusion injury (MIRI)

[0100] Male adult Wistar rats (250-300 g), anesthetized with thiopentone sodium and mechanically ventilated [fraction of inspired oxygen (FiO<sub>2</sub>)=30%; intermittent mandatory ventilation (IMV)=70 bpm, tidal volume (TV)=8-10 ml/kg], underwent placement of a ligature around the left anterior descending (LAD) coronary artery approximately 1-2 mm below its origin. Ischemia was induced by tightening the threads of the coronary suture and was maintained for 20 min. Reperfusion for 2 hours was obtained by reopening the chest and cutting the ligature around the coronary artery. Rats were randomly allocated to the following groups

(n=10 per experimental arm): (i) MIRI + vehicle group: rats were subjected to coronary artery occlusion (20 min) followed by reperfusion (2 hours); (ii) MIRI+compound **2** group: rats were subjected to the surgical procedures described above and were treated with compound **2** at 1 mg/kg IV bolus followed by a continuous IV infusion of 2 mg/kg/h; and (iii) Sham+vehicle group: rats were subjected to identical surgical procedures, except for coronary artery occlusion, and were kept under anaesthesia for the duration of the experiment. At the end of the 2 hours reperfusion period, the LAD was re-occluded, and 1 ml of Evans blue dye (2% wt/vol) was injected to the animal via the jugular vein. The area at risk (AAR), i.e., the non-perfused and thus non-stained myocardium, was separated from the non-ischemic (blue) tissue and expressed as a percentage of the left ventricle. The tissue from the AAR staining with p-Nitroblue tetrazolium was separated from the infarcted tissue and weighed, and the infarct size was expressed as a percentage of the AAR. Compound **2** did not produce systemic hypotension in rats subjected to MIRI, as shown in **Fig. 1**. This was significant because systemic hypotension would severely compromise and exacerbate regional coronary ischemia.

**[0101]** As shown in **Figs. 2A-2B**, compound **2** significantly reduced both myocardial myeloperoxidase (MPO) (**2A**) and tissue infarction (**2B**).

**[0102]** As further shown in **Figs. 3A-3C**, while MIRI induced severe myocyte necrosis, edema and neutrophil infiltration (**3A**) compared to a sham control (**3B**), administration of compound **2** prior to reperfusion profoundly attenuated virtually all histologic features of injury (**3C**).

**[0103]** In an *ex vivo* rat vascular ring system precontracted with norepinephrine (1  $\mu$ M), compound **2** (100  $\mu$ M) was found to be a less potent *ex vivo* vasodilator compared to pinacidil, having ED<sub>50</sub> that is one log greater than that of pinacidil, indicative of a loss in sarcolemmal K<sup>+</sup>-ATP channel activation.

#### **Example 12. Compound 2 is protective in a murine model of renal ischemia-reperfusion injury**

**[0104]** Anesthetized male CD mice underwent bilateral clamping of the renal pedicles for 30 minutes, followed by 6 hours of reperfusion. As shown in **Figs. 4A-4C**, addition of compound **2** before reperfusion profoundly attenuated elevations in plasma blood urea nitrogen (BUN) and creatinine (**4A** and **4B**, respectively), and in renal myeloperoxidase (MPO), a marker of neutrophil infiltration (**4C**) ( $p < 0.01$  vs. vehicle control). Histologic studies (not shown) revealed that compound **2** provided near total protection against severe renal tubular necrosis.

#### **Example 13. Compound 2 is effective as a rescue therapy in a murine model of acute chlorine inhalation injury**

**[0105]** Male Balb/c mice (25 g; n=6 per experimental group) were exposed in a closed environmental chamber to 400 ppm Cl<sub>2</sub> in air for 30 min. 15 min after the conclusion of Cl<sub>2</sub> exposure, mice were initiated on a q12h regimen of compound **2** (30 mg/kg/dose IP in 0.5 ml dextrose 5% in water [D5W]). At 24 h, mice were euthanized and lung tissue taken for examination of polymorphonuclear leukocytes (PMN) infiltration (as reflected in the level of MPO) and histology, by a pathologist blinded to the experimental arm assignment. As shown in **Figs. 5A-5B**, compound **2** therapy reduced the elevation in MPO (**5A**) and histologic lung damage (**5B**) by 52% ( $p < 0.0001$ ) and 43% ( $p < 0.001$ ), respectively, relative to placebo (D5W).

**[0106]** Dose-response evaluations were performed as described above with little modifications. Compound **2** was formulated in saline solution and given in 0.25 ml volume at 2 h and 6 h after Cl<sub>2</sub> exposure by IP. As found, compound **2** attenuated lung injury in a dose-dependent manner, ranging from 3 mg to 80 mg per dose (or 6 mg to 160 mg per day), as exemplified by the improved histology scores shown in **Fig. 6**.

#### **Example 14. Compound 2 does not affect blood glucose level**

**[0107]** In order to test the effect of compound **2** on blood glucose levels, 0.25 ml of drug formulated in saline solution was given by IP bolus to male Balb/c mice weighing approximately 25 g (n=5). Mice were fasted 4 hr before experiment and remained fast during study period. At indicated time points, 20  $\mu$ l of blood samples were collected from tail veins under ether anesthesia and glucose levels were measured by using glucometer. As shown in **Fig. 7**, compound **2** did not exhibit any significant effect on blood glucose levels compared with saline vehicle group.

## REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

### Patent documents cited in the description

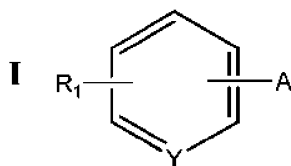
- US20060025449A [0004]
- WO2004000331A [0005]
- US4256108A [0067]
- US4166452A [0067]
- US4265874A [0067]

### Non-patent literature cited in the description

- **REMINGTON**The Science and Practice of Pharmacy19950000 [0066]

## PATENTKRAV

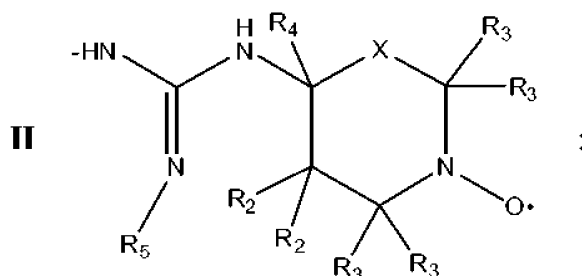
1. Forbindelse med den almene formel I:



hvor Y er N, CH eller N( $\rightarrow$ O),

5 eller en enantiomer, diastereomer, racemat, eller et farmaceutisk acceptabelt salt eller solvat deraf, hvor

A er en del af den almene formel II forbundet gennem dets terminal -NH-gruppe til et hvilket som helst carbonatom af pyridin-, phenyl- eller pyridinoxidringen:



10 X er fraværende eller  $-(CR_2R_2)_n$ ;

R<sub>1</sub> er fraværende eller 1 til 5 substituenten hver uafhængigt udvalgt fra halogen, -CN, -OH, -NO<sub>2</sub>, -N(R<sub>6</sub>)<sub>2</sub>, -OCF<sub>3</sub>, -CF<sub>3</sub>, -OR<sub>6</sub>, -COR<sub>6</sub>, -COOR<sub>6</sub>, -CON(R<sub>6</sub>)<sub>2</sub>, -OCOOR<sub>6</sub>, -OCON(R<sub>6</sub>)<sub>2</sub>, -  
 15 (C<sub>1</sub>-C<sub>8</sub>)-alkyl, -(C<sub>1</sub>-C<sub>8</sub>)-alkylen-COOR<sub>6</sub>, -SR<sub>6</sub>, -SO<sub>2</sub>R<sub>6</sub>, -SO<sub>2</sub>N(R<sub>6</sub>)<sub>2</sub>, eller -S(=O)R<sub>6</sub>, hvor -(C<sub>1</sub>-C<sub>8</sub>)-alkyl og -(C<sub>1</sub>-C<sub>8</sub>)-alkylen-COOR<sub>6</sub> eventuelt kan være substitueret med -OH, -OR<sub>3</sub>, -OCF<sub>3</sub>,  
 -CF<sub>3</sub>, -COR<sub>3</sub>, -COOR<sub>3</sub>, -OCOOR<sub>3</sub>, -OCON(R<sub>3</sub>)<sub>2</sub>, -(C<sub>1</sub>-C<sub>8</sub>)-alkylen-COOR<sub>3</sub>, -CN, -NH<sub>2</sub>, -NO<sub>2</sub>,  
 -SH, -SR<sub>3</sub>, -(C<sub>1</sub>-C<sub>8</sub>)-alkyl, -O-(C<sub>1</sub>-C<sub>8</sub>)-alkyl, -N(R<sub>3</sub>)<sub>2</sub>, -CON(R<sub>3</sub>)<sub>2</sub>, -SO<sub>2</sub>R<sub>3</sub>, eller -S(=O)R<sub>3</sub>, eller  
 to tilstødende R<sub>1</sub>-grupper og de carbonatomer, hvortil de er bundet, danner en 5- eller 6-leddet  
 carbocyklisk eller heterocyklisk ring, (C<sub>6</sub>-C<sub>10</sub>)-aryl, eller 6- til 10-leddet heteroaryl, under  
 forudsætning af at når Y er N eller N( $\rightarrow$ O), så er det maksimale antal af R<sub>1</sub>-grupper 4;

20 R<sub>2</sub> hver uafhængigt er udvalgt fra H, halogen, -OCF<sub>3</sub>, -CF<sub>3</sub>, -OR<sub>7</sub>, -COR<sub>7</sub>, -COOR<sub>7</sub>, -OCOOR<sub>7</sub>,  
 -OCON(R<sub>7</sub>)<sub>2</sub>, -(C<sub>1</sub>-C<sub>8</sub>)-alkylen-COOR<sub>7</sub>, -CN, -NO<sub>2</sub>, -SH, -SR<sub>7</sub>, -(C<sub>1</sub>-C<sub>8</sub>)-alkyl, -N(R<sub>7</sub>)<sub>2</sub>, -  
 CON(R<sub>7</sub>)<sub>2</sub>, -SO<sub>2</sub>R<sub>7</sub>, SO<sub>2</sub>N(R<sub>7</sub>)<sub>2</sub>, eller -S(=O)R<sub>7</sub>; eller to R<sub>2</sub>-grupper og det carbonatom, hvortil  
 de er bundet, danner en 5- eller 6-leddet carbocyklisk eller heterocyklisk ring;

R<sub>3</sub> hver uafhængigt er udvalgt fra (C<sub>1</sub>-C<sub>8</sub>)-alkyl, (C<sub>2</sub>-C<sub>8</sub>)-alkenyl, eller (C<sub>2</sub>-C<sub>8</sub>)-alkynyl;

25 R<sub>4</sub> er udvalgt fra H, -COOR<sub>7</sub>, -(C<sub>1</sub>-C<sub>8</sub>)-alkylen-COOR<sub>7</sub>, -CN, -(C<sub>1</sub>-C<sub>8</sub>)-alkyl, eller -CON(R<sub>7</sub>)<sub>2</sub>;  
 R<sub>5</sub> er udvalgt fra H, -OH, -O-(C<sub>1</sub>-C<sub>8</sub>)-alkyl, -CO-(C<sub>1</sub>-C<sub>8</sub>)-alkyl, -COO-(C<sub>1</sub>-C<sub>8</sub>)-alkyl, -CN, eller  
 -NH<sub>2</sub>;



R<sub>6</sub> hver uafhængigt er udvalgt fra H, (C<sub>1</sub>-C<sub>8</sub>)-alkyl, (C<sub>3</sub>-C<sub>10</sub>)-cycloalkyl, 4-12-leddet heterocyclyl, (C<sub>6</sub>-C<sub>14</sub>)-aryl, eller -(C<sub>1</sub>-C<sub>8</sub>)-alkylen-NH<sub>2</sub>;

R<sub>7</sub> hver uafhængigt er udvalgt fra H, (C<sub>1</sub>-C<sub>8</sub>)-alkyl, -(C<sub>1</sub>-C<sub>8</sub>)-alkylen-NH<sub>2</sub>, (C<sub>3</sub>-C<sub>10</sub>)-cycloalkyl, 4-12-leddet heterocyclyl, eller (C<sub>6</sub>-C<sub>14</sub>)-aryl, der hver især, på nær H, eventuelt kan være  
 5 substitueret med -OR<sub>6</sub>, -COR<sub>6</sub>, -COOR<sub>6</sub>, -OCOOR<sub>6</sub>, -OCON(R<sub>6</sub>)<sub>2</sub>, -(C<sub>1</sub>-C<sub>8</sub>)-alkylen-COOR<sub>6</sub>, -CN, -NO<sub>2</sub>, -SR<sub>6</sub>, -(C<sub>1</sub>-C<sub>8</sub>)-alkyl, -N(R<sub>6</sub>)<sub>2</sub>, -CON(R<sub>6</sub>)<sub>2</sub>, -SO<sub>2</sub>R<sub>6</sub>, eller -S(=O)R<sub>6</sub>; og n er et heltal på 1 eller 2.

2. Forbindelse ifølge krav 1, hvor (i) Y er N, og A er bundet til position 2, 3, 4, 5 eller 6  
 10 af pyridinringen; (ii) Y er CH, og A er bundet til en hvilken som helst position af phenylringen; eller (iii) Y er N(→O), og A er bundet til position 2, 3, 4, 5 eller 6 af 1-oxypyridinringen.

3. Forbindelse ifølge krav 1 eller 2, hvor  
 (i) R<sub>1</sub> er fraværende, eller 1, 2, 3, 4 eller 5 substituenten hver uafhængigt udvalgt fra halogen, -  
 15 OH, -CN, -NO<sub>2</sub>, -N(R<sub>6</sub>)<sub>2</sub>, -OR<sub>6</sub>, -OCF<sub>3</sub>, -CF<sub>3</sub>, -COR<sub>6</sub>, -COOR<sub>6</sub>, -CON(R<sub>6</sub>)<sub>2</sub>, -OCOOR<sub>6</sub>, -OCON(R<sub>6</sub>)<sub>2</sub>, -(C<sub>1</sub>-C<sub>8</sub>)-alkyl, -(C<sub>1</sub>-C<sub>8</sub>)-alkylen-COOR<sub>6</sub>, -SR<sub>6</sub>, -SO<sub>2</sub>R<sub>6</sub>, -SO<sub>2</sub>N(R<sub>6</sub>)<sub>2</sub>, eller -S(=O)R<sub>6</sub>, hvor R<sub>6</sub> hver uafhængigt er H, (C<sub>1</sub>-C<sub>8</sub>)-alkyl, eller -(C<sub>1</sub>-C<sub>8</sub>)-alkylen-NH<sub>2</sub>; eller  
 (ii) to tilstødende R<sub>1</sub>-grupper og de carbonatomer, hvortil de er bundet, danner en 5- eller 6-leddet carbocyklisk eller heterocyklisk ring, (C<sub>6</sub>-C<sub>10</sub>)-aryl, eller 6- til 10-leddet heteroaryl.

20

4. Forbindelse ifølge krav 1 eller 2, hvor (i) R<sub>2</sub> er H; eller (ii) R<sub>3</sub> hver uafhængigt er (C<sub>1</sub>-C<sub>4</sub>)-alkyl; eller (iii) R<sub>4</sub> er H; eller (iv) R<sub>5</sub> er -CN.

5. Forbindelse ifølge krav 4, hvor R<sub>3</sub> er identiske.

25

6. Forbindelse ifølge krav 1, hvor Y er N; A er bundet til position 2, 3, 4, 5 eller 6 af pyridinringen; R<sub>1</sub> er fraværende eller 1 til 4 substituenten, der uafhængigt er halogen; X er fraværende eller -(CR<sub>2</sub>R<sub>2</sub>)<sub>n</sub>-, hvor n er 1 eller 2; R<sub>2</sub> er H; R<sub>3</sub> hver uafhængigt er (C<sub>1</sub>-C<sub>4</sub>)-alkyl; R<sub>4</sub> er H; og R<sub>5</sub> er -CN.

30

7. Forbindelse ifølge krav 6, hvor:

(i) X er fraværende; R<sub>1</sub> er fraværende; R<sub>3</sub> er methyl; og A er bundet til position 2, 3 eller 4 af pyridinringen (herefter identificeret som henholdsvis forbindelserne **1-3**);  
 (ii) X er fraværende; R<sub>1</sub> er F, Cl eller Br, bundet til position 6 af pyridinringen; R<sub>3</sub> er  
 35 methyl; og A er bundet til position 2, 3, 4 eller 5 af pyridinringen (herefter identificeret som henholdsvis forbindelserne **4<sub>a-c</sub>-7<sub>a-c</sub>**);  
 (iii) X er -(CR<sub>2</sub>R<sub>2</sub>)<sub>n</sub>-, hvor n er 1; R<sub>1</sub> er fraværende; R<sub>3</sub> er methyl; og A er bundet til

position 2, 3 eller 4 af pyridinringen (herefter identificeret som henholdsvis forbindelserne **8-10**);

(iv) X er  $-(CR_2R_2)_n-$ , hvor n er 1;  $R_1$  er F, Cl eller Br, bundet til position 6 af pyridinringen;  $R_3$  er methyl; og A er bundet til position 2, 3, 4 eller 5 af pyridinringen (herefter identificeret som henholdsvis forbindelserne **11<sub>a-c</sub>-14<sub>a-c</sub>**);

(v) X er  $-(CR_2R_2)_n-$  hvor n er 2;  $R_1$  er fraværende;  $R_3$  er methyl; og A er bundet til position 2, 3 eller 4 af pyridinringen (herefter identificeret som henholdsvis forbindelserne **15-17**); eller

(vi) X er  $-(CR_2R_2)_n-$ , hvor n er 2;  $R_1$  er F, Cl eller Br, bundet til position 6 af pyridinringen;  $R_3$  er methyl; og A er bundet til position 2, 3, 4 eller 5 af pyridinringen (herefter identificeret som henholdsvis forbindelserne **18<sub>a-c</sub>-21<sub>a-c</sub>**).

8. Forbindelse ifølge krav 1, hvor Y er CH; A er bundet til en hvilken som helst position af phenylringen;  $R_1$  er fraværende eller 1 til 5 substituent, der uafhængigt er halogen; X er fraværende eller  $-(CR_2R_2)_n-$ , hvor n er 1 eller 2;  $R_2$  er H;  $R_3$  hver uafhængigt er (C<sub>1</sub>-C<sub>4</sub>)-alkyl;  $R_4$  er H; og  $R_5$  er -CN.

9. Forbindelse ifølge krav 8, hvor:

(i) X er fraværende;  $R_1$  er fraværende;  $R_2$  er H; og  $R_3$  er methyl (herefter identificeret som forbindelse 22);

(ii) X er fraværende;  $R_3$  er methyl; og  $R_1$  er F, Cl eller Br, bundet til phenylringen ved position ortho, meta eller para i forhold til A (herefter identificeret som henholdsvis forbindelserne **23<sub>a-c</sub>-25<sub>a-c</sub>**);

(iii) X er fraværende;  $R_3$  er methyl; og  $R_1$  repræsenterer 2 substituent, der uafhængigt er F, Cl eller Br, bundet til phenylringen ved position ortho, meta eller para i forhold til A;

(iv) X er  $-(CR_2R_2)_n-$  hvor n er 1;  $R_1$  er fraværende;  $R_2$  er H; og  $R_3$  er methyl (herefter identificeret som forbindelse **32**);

(v) X er  $-(CR_2R_2)_n-$  hvor n er 1;  $R_3$  er methyl; og  $R_1$  er F, Cl eller Br, bundet til phenylringen ved position ortho, meta eller para i forhold til A (herefter identificeret som henholdsvis forbindelserne **33<sub>a-c</sub>-35<sub>a-c</sub>**);

(vi) X er  $-(CR_2R_2)_n-$  hvor n er 1;  $R_3$  er methyl; og  $R_1$  repræsenterer 2 substituent, der uafhængigt er F, Cl eller Br, bundet til phenylringen ved position ortho, meta eller para i forhold til A; eller

(vii) X er  $-(CR_2R_2)_n-$  hvor n er 2;  $R_1$  er fraværende;  $R_2$  er H; og  $R_3$  er methyl (herefter identificeret som forbindelse **36**);

(viii) X er  $-(CR_2R_2)_n-$ , hvor n er 2;  $R_3$  er methyl; og  $R_1$  er F, Cl eller Br, bundet til phenylringen ved position ortho, meta eller para i forhold til A (herefter identificeret som

henholdsvis forbindelserne **37<sub>a-c</sub>**-**39<sub>a-c</sub>**);

(ix) X er  $-(CR_2R_2)_n$ - hvor n er 2; R<sub>3</sub> er methyl; og R<sub>1</sub> repræsenterer 2 substituent, der uafhængigt er F, Cl eller Br, bundet til phenylringen ved position ortho, meta eller para i forhold til A.

5

10. Forbindelse ifølge krav 9, hvor X er fraværende; R<sub>3</sub> er methyl; og R<sub>1</sub> repræsenterer 2 substituent, der hver er Cl, bundet til phenylringen ved positionerne ortho og ortho, ortho og meta, ortho og para, meta og meta, eller meta og para i forhold til A (herefter identificeret som henholdsvis forbindelserne **26-31**).

10

11. Forbindelse ifølge krav 1, hvor Y er N( $\rightarrow$ O); A er bundet til position 2, 3, 4, 5 eller 6 af pyridinringen; R<sub>1</sub> er fraværende eller 1 til 4 substituent, der uafhængigt er halogen; X er fraværende eller  $-(CR_2R_2)_n$ -, hvor n er 1 eller 2; R<sub>2</sub> er H; R<sub>3</sub> hver uafhængigt er (C<sub>1</sub>-C<sub>4</sub>)-alkyl; R<sub>4</sub> er H; og R<sub>5</sub> er -CN.

15

12. Farmaceutisk sammensætning, der omfatter en forbindelse ifølge et hvilket som helst af kravene 1 til 11, eller en enantiomer, diastereomer, racemat, eller et farmaceutisk acceptabelt salt eller solvat deraf, og en farmaceutisk acceptabel bærer.

20

13. Farmaceutisk sammensætning ifølge krav 12 til intravenøs, intramuskulær, subkutan, transdermal, oral, nasal, parenteral eller topisk administration, eller til administration ved inhalation.

25

14. Farmaceutisk sammensætning ifølge krav 13, hvor sammensætningen er til oral administration og formuleret som en tablet, kapsel, vandig eller olieholdig opløsning, suspension eller emulsion; eller sammensætningen er til topisk administration og formuleret som en creme, salve, gel, vandig eller olieholdig opløsning eller suspension, salve, patch, plaster, smøremiddel eller stikpiller.

30

15. Farmaceutisk sammensætning ifølge krav 12, hvor bæreren omfatter en biologisk nedbrydelig polymer.

35

16. Farmaceutisk sammensætning ifølge et hvilket som helst af kravene 12 til 15 til anvendelse til behandling af en sygdom, lidelse eller tilstand forbundet med oxidativ stress eller endotel dysfunktion, hvor sygdommen, lidelsen eller tilstanden forbundet med oxidativ stress eller endotel dysfunktion er (i) en sygdom, lidelse eller tilstand forbundet med iskæmisk reperfusionsskade; (ii) en neurodegenerativ sygdom; (iii) en inflammatorisk eller

17. Farmaceutisk sammensætning til anvendelse ifølge krav 16, hvor

- (i) sygdommen, lidelsen eller tilstanden forbundet med iskæmisk reperfusionsskade er udvalgt fra sepsis, septisk shock, apopleksi, dannelse af grå stær, glaukom, geografisk atrofi, makulær degeneration, angina, hæmoragisk shock, superantigen-induceret cirkulationsshock, renal reperfusionsskade, kontrastmiddelinduceret nefropati, retinopati hos præmature, nekrotiserende enterokolit, neonatal respiratorisk distress-syndrom, iskæmisk lungereperfusionsskade, komplikationer ved IL-2-bioterapi, myokardieinfarkt, komplikationer ved kardiopulmonal bypasskirurgi, reperfusionsskade i lem, post-prostatektomirelateret erektil dysfunktion, reperfusionskomplikationer relateret til vaskulær kirurgi, herunder carotid endarterektomi, reparation af aortisk aneurisme, perifer arteriel embolektomi og thrombektomi, knusningslæsioner, kompartmentsyndrom, organpræservasjon, hovedtraume eller rygmarvsskade;

(ii) den neurodegenerative sygdom er Parkinsons sygdom, Alzheimers sygdom eller amyotrofisk lateral sklerose;

(iii) den inflammatoriske sygdom eller immunsygdommen er udvalgt fra sepsis, uveitis, rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, betændte led, eksem, inflammatoriske hudtilstande, inflammatoriske øjentalstande, conjunctivitis, vævsnekrose som et resultat af inflammation, vævsafstødning efter transplantation, graft-versus-host-sygdom, Crohns sygdom og ulcerativ colitis, luftvejsinflammation, astma, bronkitis, systemisk lupus erythematoses, multipel sklerose, glaukom, rygningsforårsaget lungeskade, pulmonal fibrose, pankreatitis, kardiomyopati, herunder kemoterapi-induceret kardiomyopati, komplikationer ved IL-2-bioterapi, diabetes, diabetiske komplikationer, herunder diabetisk retinopati, perifer neuropati, akut makulær degeneration, hudsår, nyresygdom, lungebetændelse, mucositis, akut lungesvigt hos voksne (ARDS), røginhalation eller kutan brandskade; eller

(iv) den inflammatoriske sygdom er en inflammatorisk sygdom i lungerne forårsaget af inhalation af giftige stoffer eller irriterende stoffer udvalgt fra chlor, phosgen eller røg.

18. Forbindelse ifølge et hvilket som helst af kravene 1 til 11, eller en enantiomer, diastereomer, racemat, eller et farmaceutisk acceptabelt salt eller solvat deraf, til anvendelse til behandling af en sygdom, lidelse eller tilstand forbundet med oxidativ stress eller endotel dysfunktion, hvor sygdommen, lidelsen eller tilstanden forbundet med oxidativ stress eller endotel dysfunktion er (i) en sygdom, lidelse eller tilstand forbundet med iskæmisk reperfusionsskade; (ii) en neurodegenerativ sygdom; (iii) en inflammatorisk eller immunsygdom; eller (iv) en tilstand forbundet med kemoterapi eller

strålingsbehandling af cancer.

## DRAWINGS

Fig. 1

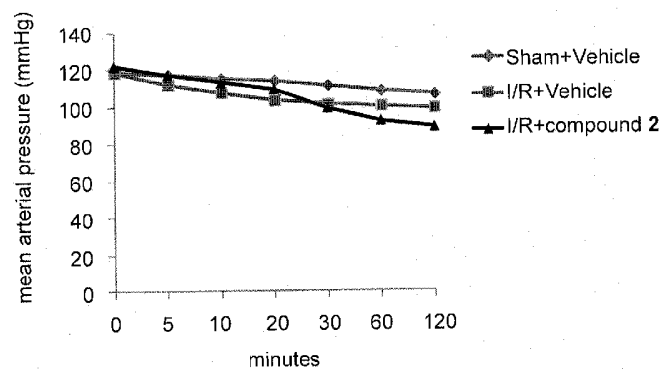


Fig. 2A

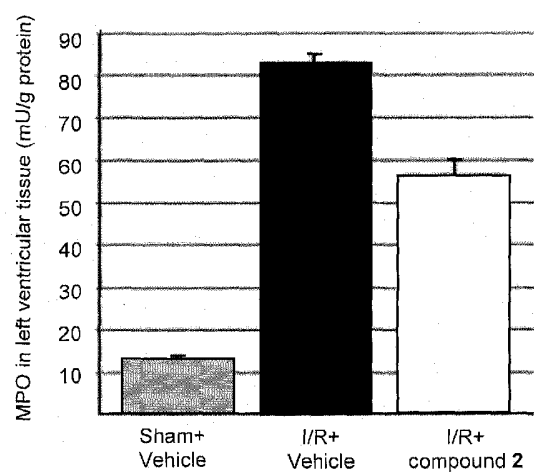
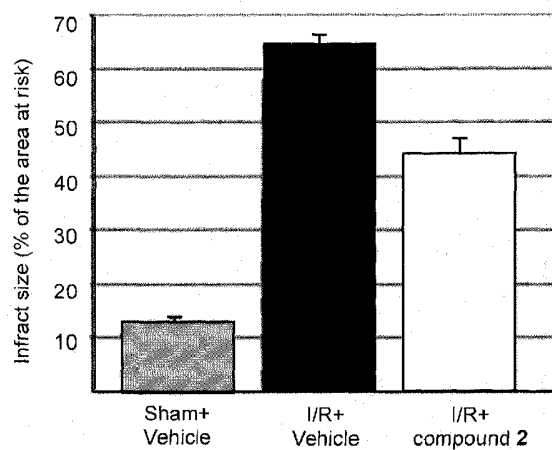
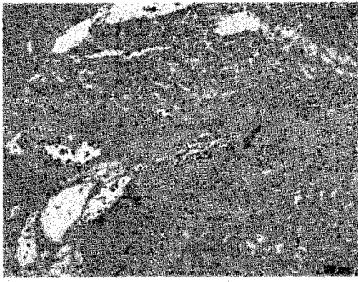


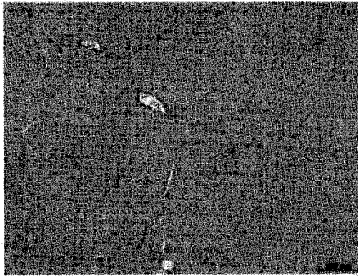
Fig. 2B



**Fig. 3A**



**Fig. 3B**



**Fig. 3C**

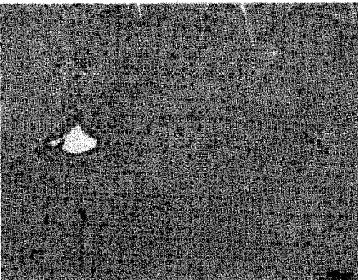




Fig. 4A

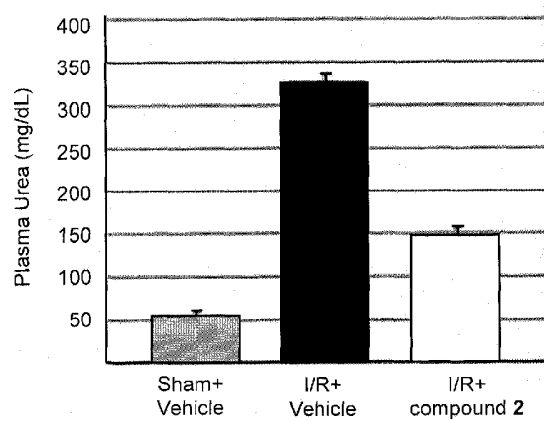


Fig. 4B

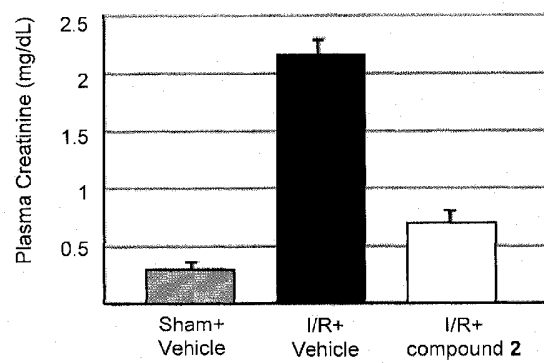


Fig. 4C

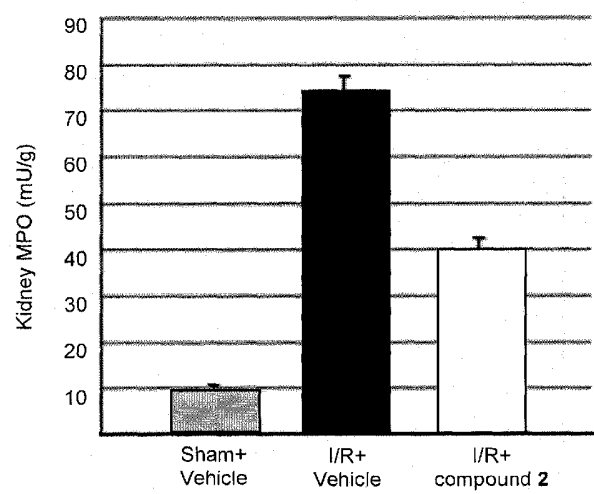


Fig. 5A

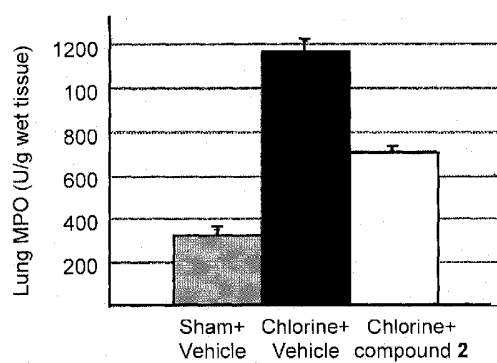


Fig. 5B

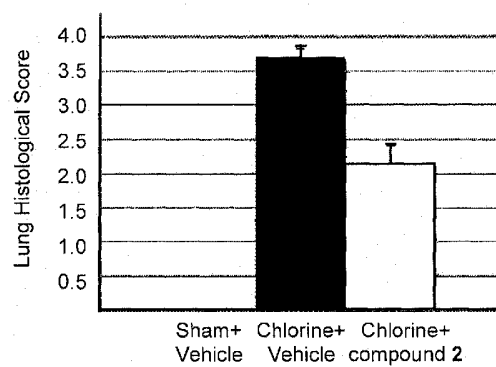


Fig. 6

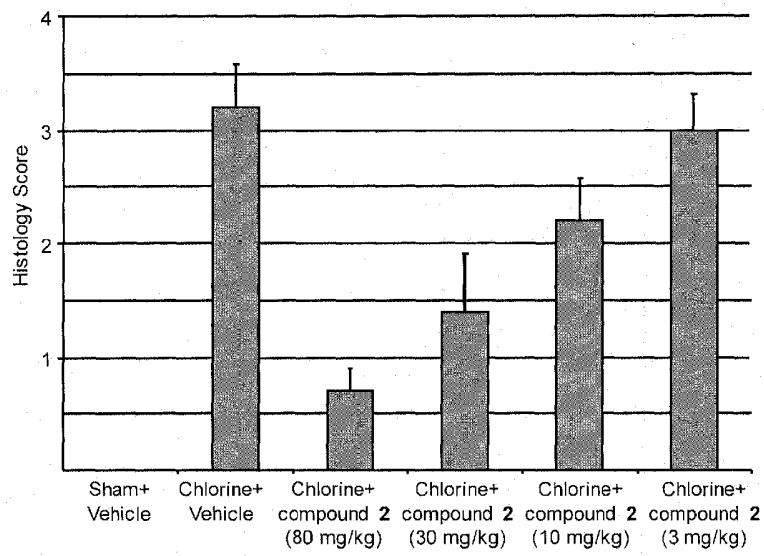


Fig. 7

