

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
11 March 2004 (11.03.2004)

PCT

(10) International Publication Number
WO 2004/019986 A1

- (51) International Patent Classification⁷: **A61K 45/06**, 31/506, A61P 11/00
- (74) Agent: MIYAMOTO, Neil, G.; Berlex Biosciences, 2600 Hilltop Drive, P.O. Box 4099, Richmond, CA 94804 (US).
- (21) International Application Number: PCT/US2003/026462
- (22) International Filing Date: 22 August 2003 (22.08.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 60/406,739 29 August 2002 (29.08.2002) US
- (71) Applicant (for all designated States except US): **SCHERING AKTIENGESELLSCHAFT** [DE/DE]; Muellerstrasse 178, 13342 Berlin (DE).
- (72) Inventor; and
- (75) Inventor/Applicant (for US only): **PARKINSON, John, F.** [GB/US]; 6727 Waverly Road, Martinez, CA 94553 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS OF TREATING ACUTE RESPIRATORY DISTRESS SYNDROME

(57) Abstract: Methods of treating acute respiratory disease syndrome in a mammal, wherein the method comprises administering to the mammal in need thereof a therapeutically effective amount of a selective or partially selective iNOS inhibitor in combination with a therapeutically effective amount of a pulmonary vasodilator.

WO 2004/019986 A1

METHODS OF TREATING ACUTE RESPIRATORY DISTRESS SYNDROME

This application claims the benefit of U.S. Provisional Application Serial No. 60/406,739, filed August 29, 2002, which is incorporated herein in full by reference.

5

FIELD OF THE INVENTION

The invention relates to methods of using selective or partially selective iNOS inhibitors in combination with pulmonary vasodilators in the treatment of acute respiratory distress syndrome.

10

BACKGROUND OF THE INVENTION

Acute respiratory distress syndrome ("ARDS"), also referred to as adult respiratory distress syndrome, is a frequent complication in critically ill patients suffering from traumatic diseases of diverse etiology. These etiologies include, but are not limited to, overt sepsis and septic shock (Fein, A.M. *et al.* (2000) *Crit. Care Clin.* 16:289-317), hemorrhagic shock (Shoemaker, W.C. (1985) *Surg. Clin. North Am.* 65:931-963), multiple trauma, including head trauma (Nuytinck, H.K. *et al.* (1988) *Arch. Surg.* 123:1519-1524), localized bacterial pneumonia (Miesen, W.M. *et al.* (2001) *Neth. J. Med.* 59:57-61) or viral pneumonia (Byers, R.J. *et al.* (1996) *Eur. Respir. J.* 9:2313-2317), burn injury with and without smoke inhalation injury (Hollingsed, T.C. *et al.* (1993) *Am. J. Surg.* 166:592-597; Ruddy, R.M. (1994) *Pediatr. Clin. North Am.* 41:317-336), and surgery-induced or post-operative ARDS (Milot, J. *et al.* (2001) *Chest* 119:884-888). ARDS may also develop as a consequence of immunotherapy of cancer patients, such as interleukin-2 ("IL-2") treatment of malignant melanoma or renal cell carcinoma (Farrell, M.M. *et al.* (1992) *Oncol. Nurs. Forum* 19:475-480). ARDS is associated with the systemic inflammatory response syndrome ("SIRS"), and contributes to multiple organ failure, including acute renal failure (Croce, M.A. *et al.* (1999) *J. Trauma* 46:361-366; Karnik, A.M. *et al.* (1998) *Ren. Fail.* 20:103-116). ARDS can develop in pediatric patients as well as adults. The elderly are particularly at risk for ARDS and exhibit poorer recovery from available treatment (Ely, E.W. *et al.* (2002) *Ann. Intern. Med.* 136:25-36).

15

20

25

30

35

Cardiopulmonary measurements are frequently used as prognostic indicators for poor clinical outcome (e.g., survival and long-term recovery of pulmonary function) in ARDS. These include signs of ventilation/perfusion ("Va/Q") mismatch, such as low arterial oxygen tension ("PaO₂"), low arterial oxygen saturation ("CaO₂"), excessive right-to-left pulmonary shunt, and

low PaO₂/fractional inspired oxygen ("FiO₂") ratio ("P/F ratio <200"). High pulmonary artery pressure ("PAP") and low systemic artery pressure ("SAP") are also related to poor prognosis. These parameters and their relationship to ARDS outcomes are reviewed in Squara, P. *et al.* (1998) *Intensive Care Med.* 24:1018-1028.

5

Treating the symptoms of ARDS can be complicated by concurrent pathologies in other critical end organs. Sepsis can induce myocardial depression (Cain, B.S. *et al.* (1999) *Crit. Care Med.* 27:1309-1318) and severe hypotension requiring fluid resuscitation and vasoconstrictor (e.g., norepinephrine) support (Grover, R. *et al.* (1999) *Crit. Care Med.* 27:913-922). These pathologies also occur concurrently with capillary leak syndrome (Hauser, G.J. *et al.* (1988) *Crit. Care Clin.* 4:711-733) and ARDS in cancer patients treated with IL-2 (Glauser, F.L. *et al.* (1988) *Am. J. Med. Sci.* 296:406-412). Burn victims requiring fluid resuscitation to prevent dehydration and hypovolemia may also exhibit signs of myocardial depression (Reynolds, E.M. *et al.* (1995) *J. Pediatr. Surg.* 30:264-270). ARDS is also often associated with and may contribute to acute renal failure (Navarrete-Navarro, P. *et al.* (2001) *Intensive Care Med.* 27:1133-1140). Treatment of these concurrent complications may exacerbate ARDS. For example, excessive fluid resuscitation in sepsis or burn injury may contribute to the development of pulmonary edema and consequently adversely affect ARDS (Imm, A. *et al.* (1993) *Crit. Care Clin.* 9:313-333).

20

The pathology of ARDS is linked to excessive production of nitric oxide ("NO"). NO is an endogenous vasodilator synthesized from L-arginine through catalysis by nitric oxide synthase ("NOS"). NOS exists in at least three isoforms, which fall into two primary categories: constitutive and inducible. Two constitutive isoforms, which are calcium and calmodulin dependent, have been identified, and one inducible isoform has been identified.

25

The constitutive isoforms of NOS are NOS-1, a neuronal isoform, and NOS-3, an endothelial isoform. NOS-1, also referred to as nNOS or bNOS, is found in the brain and skeletal muscles. NOS-3, also referred to as eNOS, is expressed in the endothelium of blood vessels, as well as in the epithelium of the bronchial tree and in the brain. Under physiologic conditions, NO synthesis by eNOS in the vascular endothelium is generally believed to play a role in vascular regulation. In particular, it has been shown that eNOS has homeostatic and protective effects in many cardiopulmonary settings and that non-selective inhibition of eNOS can be deleterious and associated with "endothelial dysfunction". This is of particular relevance for the treatment of acute traumatic disease, such as ARDS or sepsis with ARDS, where non-selective NOS inhibitors such as N^g-monomethyl-L-arginine ("L-NMMA") and nitro-L-arginine

30
35

methyl ester ("L-NAME") can adversely affect cardiac output (Avontuur, J.A. *et al.* (1998) *Crit. Care Med.* 26:660-667; Petros, A. *et al.* (1994) *Cardiovasc. Res.* 28:34-39) or pulmonary function (Capellier, G. *et al.* (1996) *Eur. Respir. J.* 9:2531-2536; Rovira, I. *et al.* (1994) *J. Appl. Physiol.* 76:345-355).

5

The inducible isoform of NOS, NOS-2 or iNOS, is expressed in virtually all nucleated mammalian cells following exposure to inflammatory cytokines or lipopolysaccharide (Szabo, C. *et al.* (1995) *FEBS Lett.* 365:235-238). Its presence in macrophages and lung epithelial cells is particularly noteworthy. NO generated by iNOS has been implicated in the pathogenesis of several inflammatory diseases and has been demonstrated to be responsible for a series of complications and dysfunctions related to ARDS. Examples are impaired hypoxic pulmonary vasoconstriction ("HPV"); reduced arterial oxygenation (lowered PaO₂) (Ullrich, R. *et al.* (1999) *J. Clin. Invest.* 104:1421-1429); vascular hyperpermeability and edema formation (Kazutaka, S. *et al.* (2001) *Am. J. Respir. Crit. Care Med.* 163:745-752); and myocardial dysfunction (Kazutaka, S. *et al.* (2001) *Burn* 27:809-815). In all of these cases, iNOS inhibitors were found to have reduced the dysfunctions to various degrees.

10

15

NOS inhibitors with various selectivity towards iNOS versus nNOS or eNOS have been described (Babu, B.R. *et al.* (1998) *Curr. Opin. Chem. Biol.* 2:491-500; Parkinson, J.F. *et al.* (1998) in *The Pathophysiology & Clinical Applications of Nitric Oxide*, Harwood Academic, U.K., pp. 505-521). They can be direct inhibitors of NOS enzyme activity based on the substrate L-arginine (Parkinson, J.F. (1999) in *Handbook of Experimental Pharmacology - Nitric Oxide*, Springer-Verlag, Heidelberg, Vol. 143, pp. 111-135; Alderton, W.K. *et al.* (2001) *Biochem. J.* 357:593-615) or the enzyme cofactor tetrahydrobiopterin (Gorren, A.C. *et al.* (2001) *Nitric Oxide* 5:176-186). Alternatively, inhibitors that prevent the assembly of inactive NOS monomers into active NOS dimers, e.g., NOS dimerization inhibitors, have been described (McMillan, K. *et al.* (2000) *Proc. Natl. Acad. Sci. USA* 97:1506-1511; Blasko, E. *et al.* (2002) *J. Biol. Chem.* 277:295-302). An alternative strategy to directly inhibit NOS activity is to block or scavenge the formation of excess NO using NO binding species, such as modified hemoglobin (De Angelo, J. (1999) *Expert Opin. Pharmacother.* 1:19-29), or chemical scavenging agents (Roza, A.M. *et al.* (2000) *Transplantation* 69:227-231).

20

25

30

Pulmonary hypertension, which is frequently observed in patients with ARDS, is a result of pulmonary vasoconstriction which characterizes the early stages of ARDS where Va/Q mismatch is one of the negative diagnostic indicators. Vasodilation by selective pulmonary vasodilators (*i.e.*, vasodilators which selectively target the pulmonary system) in the ventilated

35

zones should decrease the Va/Q mismatch. Ideally, such selective pulmonary vasodilators would act on the pulmonary circulation while having little or no effect on systemic circulation.

Classes of selective pulmonary vasodilators include, but are not limited to: (1) inhaled NO gas (see, e.g., Kemming, G. *et al.* (2002) *Eur. Surg. Res.* 34:196-202; Zapol, W.M. *et al.* (1993)

- 5 *New Horiz.* 1:638-650) or a NO donating species, e.g., sodium nitroprusside, NONOATES, *etc.* (Jacobs, B.R. *et al.* (1998) *Am. J. Respir. Crit. Care Med.* 158:1536-1542; Schutte, H. *et al.* (1997) *J. Pharmacol. Exp. Ther.* 282:985-994); (2) inhaled or intravenously administered prostacylin, e.g., iloprost (Olschewski, H. *et al.* (2001) *J. Lab. Clin. Med.* 138:367-377; Hache, M. *et al.* (2001) *Can. J. Anaesth.* 48:924-929); (3) non-selective and selective cGMP or cAMP
- 10 phosphodiesterase ("PDE") inhibitors, e.g., pentoxifyline and theophylline (Schermuly, R.T. *et al.* (2001a) *Am. J. Respir. Crit. Care Med.* 164:1694-1700; Sheridan, B.C. *et al.* (1997) *J. Surg. Res.* 71:150-154), PDE3 inhibitors, such as milrinone or motapizone (Schermuly, R.T. *et al.* (2001a) *supra*; Schermuly, R.T. *et al.* (2001b) *Am. J. Physiol. Lung Cell Mol. Physiol.* 281:L1361-L1368), PDE3/4 inhibitors, such as tolafentrine (Schermuly, R.T. *et al.* (2000) *J.*
- 15 *Pharmacol. Exp. Ther.* 292:512-520) or zardaverine, PDE4 inhibitors, such as rolipram or mesopram (Dinter, H. *et al.* (2000) *J. Neuroimmunol.* 108:136-146; Genain, C.P. *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92:3601-3605; and Sommer, N. *et al.* (1995) *Nat. Med.* 1:244-248), PDE1/PDE5 inhibitors, such as zaprinast (Rabe, K.F. *et al.* (1994) *Am. J. Physiol.* 266:L536-L543; Holzmann, A. *et al.* (2001) *Intensive Care Med.* 27:251-257), PDE5 inhibitors, such as
- 20 dipyramidole (Schermuly, R.T. *et al.* (2001b) *supra*) or sildenafil (Ichinose, F. *et al.* (2001) *Crit. Care Med.* 29:1000-1005; Weimann, J. *et al.* (2000) *Anesthesiology* 92:1702-1712); (4) nonselective endothelin receptor antagonists, e.g., bostentan (Rubin, L.J. *et al.* (2002) *N. Engl. J. Med.* 346: 896-903); (5) selective endothelin receptor antagonists, e.g., PD 156707 (Hele, D.J. *et al.* (2000) *Br. J. Pharmacol.* 131:1129-1134), PD 155080 (Brunner, F. *et al.* (2002) *J.*
- 25 *Pharmacol. Exp. Ther.* 300:442-449), TBC 3711 (Perreault, T. *et al.* (2001) *Pediatr. Res.* 50:374-383), or sitaxsentan (Wu-Wong, J.R. (2001) *Curr. Opin. Investig. Drugs* 2:531-536); and (6) eNOS agonists.

Selective pulmonary vasodilators can be given systemically (intravenous, oral) or,

30 preferably, locally (intratracheal, inhalation/nebulization), to directly treat pulmonary dysfunction in ARDS. However, pulmonary vasodilators may or may not have desirable effects on extrapulmonary complications or may have undesirable systemic side effects depending on drug class and route of delivery.

35 The use of a non-selective iNOS inhibitor in combination with a pulmonary vasodilator, in particular, NO, has been evaluated in several ARDS models developed in sheep, the result of

which indicated that the non-selective NOS inhibitor adversely affected cardiac function (Weitzberg, E. *et al.* (1995) *Crit. Care Med.* 23:909-918; Suzuki, S. *et al.* (2001) *Pediatr. Int.* 43:343-349).

5 Accordingly, there exists a need for a therapy that will effectively treat the acute pulmonary dysfunction in patients having ARDS, while at the same time improving the survival outcome of such patients who are critically ill.

SUMMARY OF THE INVENTION

10

This invention is directed to methods of using selective or partially selective inhibitors of inducible nitric oxide synthase ("iNOS") in combination with pulmonary vasodilators for the treatment of cardiopulmonary distress and secondary multiple organ failure in humans having acute respiratory distress syndrome ("ARDS").

15

Accordingly, one aspect of this invention provides methods of treating ARDS in a mammal, wherein the methods comprise administering to the mammal in need thereof a therapeutically effective amount of a selective or partially selective iNOS inhibitor in combination with a therapeutically effective amount of a pulmonary vasodilator.

20

Another aspect of this invention provides pharmaceutical compositions comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of a selective or partially selective iNOS inhibitor in combination with a therapeutically effective amount of a pulmonary vasodilator.

25

DETAILED DESCRIPTION OF THE INVENTION

Definitions

"Alkyl" refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, containing no unsaturation, having from one to eight carbon atoms, and which is attached to the rest of the molecule by a single bond, e.g., methyl, ethyl, *n*-propyl, 1-methylethyl (*iso*-propyl), *n*-butyl, *n*-pentyl, 1,1-dimethylethyl (*t*-butyl), and the like. Alkyl radicals may also be indicated herein by the notation "[C_x-C_y, alkyl]" where x and y indicate the number of carbons present. Alkyl radicals may be optionally substituted by one or more substituents independently selected from the group consisting of halo, hydroxy, alkoxy, carboxy, cyano, carbonyl, alkoxycarbonyl, cyano, amino, monoalkylamino, dialkylamino, nitro, alkylthio, amidino,

30
35

aryl, heterocyclyl, aryloxy, aralkoxy, acylamino, aminocarbonyl, monoalkylaminocarbonyl, and dialkylaminocarbonyl.

"Alkenyl" refers to a straight or branched chain monovalent or divalent radical consisting solely of carbon and hydrogen, containing at least one double bond and having from two to eight carbon atoms, e.g., ethenyl, prop-1-enyl, but-1-enyl, pent-1-enyl, penta-1,4-dienyl, and the like. Alkenyl radicals may be indicated herein by the notation "[C_x-C_y alkenyl]" where x and y indicate the number of carbons present.

"Alkynyl" refers to a straight or branched chain monovalent or divalent radical consisting solely of carbon and hydrogen, containing at least one triple bond and having from two to eight carbon atoms, e.g., ethynyl, prop-1-ynyl, but-1-ynyl, pent-1-ynyl, pent-3-ynyl, and the like. Alkynyl radicals may be indicated herein by the notation "[C_x-C_y alkyl]" where x and y indicate the number of carbons present.

"Alkoxy" refers to a radical of the formula -OR_a where R_a is an alkyl radical as defined above, e.g., methoxy, ethoxy, propoxy, and the like.

"Alkoxy carbonyl" refers to a radical of the formula -C(O)OR_a where R_a is an alkyl radical as defined above, e.g., methoxycarbonyl, ethoxycarbonyl, *n*-propoxycarbonyl, and the like.

"Alkoxy carbonyl alkyl" refers to a radical of the formula -R_a-C(O)OR_a where each R_a is independently an alkyl radical as defined above, e.g., 2-(methoxycarbonyl)ethyl, 3-(ethoxycarbonyl)propyl, 4-(*n*-propoxycarbonyl)butyl, and the like.

"Alkylsulfonylamino" refers to a radical of the formula -N(H)S(O)₂-R_a where R_a is an alkyl radical as defined above, e.g., methylsulfonylamino, ethylsulfonylamino, and the like.

"Alkylsulfonyl" refers to a radical of the formula -S(O)₂-R_a where R_a is an alkyl radical as defined above, e.g., methylsulfonyl, ethylsulfonyl, and the like.

"Alkylthio" refers to a radical of the formula -S-R_a where R_a is an alkyl radical as defined above, e.g., methylthio, ethylthio, *n*-propylthio, and the like.

"Amidino" refers to a radical of the formula -C(NH)-NH₂.

"Amino" refers to a radical of the formula -NH_2 .

"Aminocarbonyl" refers to a radical of the formula -C(O)NH_2 .

5 "Aminosulfonyl" refers to a radical of the formula $\text{-S(O)}_2\text{NH}_2$.

"Aryl" refers to a phenyl or naphthyl radical. The aryl radical may be optionally substituted by one or more substituents selected from the group consisting of hydroxy, mercapto, halo, alkyl, alkenyl, alkynyl, phenyl, phenylalkyl, phenylalkenyl, alkoxy, phenoxy, 10 phenylalkoxy, haloalkyl, haloalkoxy, formyl, nitro, cyano, cycloalkyl, hydroxyalkyl, alkoxyalkyl, phenoxyalkyl, phenylalkoxyalkyl, amidino, ureido, alkoxycarbonylamino, amino, monoalkylamino, dialkylamino, monophenylamino, monophenylalkylamino, sulfonylamino, alkylsulfonylamino, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, monophenylaminoalkyl, monophenylalkylaminoalkyl, acyl, carboxyalkyl, alkoxycarbonylalkyl, aminocarbonyl, 15 monoalkylaminocarbonyl, dialkylaminocarbonyl, aminocarbonylalkyl, monoalkylaminocarbonylalkyl, and dialkylaminocarbonylalkyl, as defined herein.

"Aralkyl" refers to a radical of the formula $\text{-R}_a\text{R}_b$ where R_a is an alkyl radical as defined above and R_b is an aryl radical as defined above, e.g., benzyl, and the like. The aryl radical 20 may be optionally substituted as described above.

"Aryloxy" refers to a radical of the formula -OR_b where R_b is an aryl radical as defined above, e.g., phenoxy and naphthoxy, and the like. The aryl radical may be optionally substituted as described above.

25

"Aryloxycarbonyl" refers to a radical of the formula -C(O)OR_b where R_b is an aryl radical as defined above, e.g., phenoxycarbonyl.

"Aralkoxy" refers to a radical of the formula -OR_c where R_c is an aralkyl radical as defined above, e.g., benzyloxy, and the like. The aralkyl radical may be optionally substituted as described above.

30 "Aralkoxycarbonyl" refers to a radical of the formula -C(O)OR_c where R_c is an aralkyl radical as defined above, e.g., benzyloxycarbonyl, and the like. The aralkyl radical may be 35 optionally substituted as described above.

"Arylamincarbonyl" refers to a radical of the formula $-C(O)N(R_b)H$ where R_b is an aryl radical as defined above, e.g., phenylaminocarbonyl, and the like. The aryl radical may be optionally substituted as described above.

5 "Arylaminosulfonyl" refers to a radical of the formula $-S(O)_2N(R_b)H$ where R_b is an aryl radical as defined above, e.g., phenylaminosulfonyl, and the like. The aryl radical may be optionally substituted as described above.

10 "Arylsulfonyl" refers to a radical of the formula $-S(O)_2R_b$ where R_b is an aryl radical as defined above, e.g., phenylsulfonyl, and the like. The aryl radical may be optionally substituted as described above.

15 "Arylsulfonylaminocarbonyl" refers to a radical of the formula $-C(O)N(H)S(O)_2R_b$ where R_b is an aryl radical as defined above, e.g., phenylsulfonylaminocarbonyl, and the like. The aryl radical may be optionally substituted as described above.

20 "Acyl" refers to a radical of the formula $-C(O)R_a$ and $-C(O)R_b$ where R_a is an alkyl radical as defined above and R_b is an aryl radical as defined above, e.g., acetyl, propionyl, benzoyl, and the like.

"Acylamino" refers to a radical of the formula $-N(H)-C(O)R_a$ and $-N(H)-C(O)R_b$ where R_a is an alkyl radical as defined above and R_b is an aryl radical as defined above, e.g., acetylamino, benzoylamino and the like.

25 "Alkylene" refers to straight or branched chain divalent radical consisting solely of carbon and hydrogen, containing no unsaturation and having from one to eight carbon atoms, e.g., methylene, ethylene, propylene, *n*-butylene, and the like. The alkylene radical may be optionally substituted by one or more substituents selected from the group consisting of alkyl, hydroxy, $-N(R^{16})R^{21}$ or $-C(O)N(R^1)R^{16}$ where R^1 , R^{16} , and R^{21} are as defined below in the
30 Preferred Embodiments.

"Amino acid" refers to a divalent radical of the formula $-N(R^4)-R^{23}-C(O)-$ where R^4 is as described below in the Preferred Embodiments and R^{23} is an amino acid residue. "Amino acid residue" refers to the alkylene chain between the nitrogen atom and the carboxy group, which is
35 substituted by the various "side chains" of the known amino acids. For example, amino acid residues of α -amino acids include the α -carbon (to which the carboxy group and the nitrogen

atom is attached) and the side chain. For example, the amino acid residue of alanine is -C(CH₃)-, the amino acid residue of serine is -C(CH₂OH)-, and so forth. The term "amino acid" is therefore intended to include α -amino acids, β -amino acids, γ -amino acids, and so forth, and all optical isomers thereof. Examples of such amino acids include alanine, asparagine,

5 *N*- β -trityl-asparagine, aspartic acid, aspartic acid- β -*t*-butyl ester, arginine, *N*⁶-Mtr-arginine, cysteine, *S*-trityl-cysteine, glutamic acid, glutamic acid- γ -*t*-butyl ester, glutamine, *N*- γ -trityl-glutamine, glycine, histidine, *N*¹*m*-trityl-histidine, isoleucine, leucine, lysine, *N*⁶-Boc-lysine, methionine, phenylalanine, proline, serine, *O*-*t*-butyl-serine, threonine, tryptophan, *N*¹*n*-Boc-tryptophan, tyrosine, valine, sarcosine, L-alanine, chloro-L-alanine,

10 2-aminoisobutyric acid, 2-(methylamino)isobutyric acid, D,L-3-aminoisobutyric acid, (*R*)-(-)-2-aminoisobutyric acid, (*S*)-(+)-2-aminoisobutyric acid, D-leucine, L-leucine, D-norvaline, L-norvaline, L-2-amino-4-pentenoic acid, D-isoleucine, L-isoleucine, D-norleucine, 2,3-diaminopropionic acid, L-norleucine, D,L-2-aminocaprylic acid, β -alanine, D,L-3-aminobutyric acid, 4-aminobutyric acid, 4-(methylamino)butyric acid, 5-aminovaleric acid,

15 5-aminocaproic acid, 7-aminoheptanoic acid, 8-aminocaprylic acid, 11-aminodecanoic acid, 12-aminododecanoic acid, carboxymethoxylamine, D-serine, D-homoserine, L-homoserine, D-allothreonine, L-allothreonine, D-threonine, L-threonine, D,L-4-amino-3-hydroxybutyric acid, D-,L-3-hydroxynorvaline, (3*S*,4*S*)-(-)-statine, 5-hydroxy-D,L-lysine, 1-amino-1-cyclopropanecarboxylic acid, 1-amino-1-cyclopentanecarboxylic acid,

20 1-amino-1-cyclohexanecarboxylic acid, 5-amino-1,3-cyclohexadiene-1-carboxylic acid, 2-amino-2-norbornanecarboxylic acid, (*S*)-(-)-2-azetidinecarboxylic acid, *cis*-4-hydroxy-D-proline, *cis*-4-hydroxy-L-proline, *trans*-4-hydroxy-L-proline, 3,4-dehydro-D,L-proline, 3,4-dehydro-L-proline, D-pipecolinic acid, L-pipecolinic acid, nipecotic acid, isonipecotic acid, mimosine, 2,3-diaminopropionic acid, D,L-2,4-diaminobutyric acid,

25 (*S*)-(+)-diaminobutyric acid, D-ornithine, L-ornithine, 2-methylornithine, *N*⁶,*n*-methyl-L-lysine, *N*-methyl-D-aspartic acid, D,L-2-methylglutamic acid, D,L-2-aminoadipic acid, D-2-aminoadipic acid, L-2-aminoadipic acid, (+/-)-3-aminoadipic acid, D-cysteine, D-penicillamine, L-penicillamine, D,L-homocysteine, *S*-methyl-L-cysteine, L-methionine, D-ethionine, L-ethionine, *S*-carboxymethyl-L-cysteine, (*S*)-(+)-2-phenylglycine, (*R*)-(-)-2-phenylglycine,

30 *N*-phenylglycine, *N*-(4-hydroxyphenyl)glycine, D-phenylalanine, thienylalanine, (*S*)-(-)-indoline-2-carboxylic acid, α -methyl-D,L-phenylalanine, β -methyl-D,L-phenylalanine, D-homophenylalanine, L-homophenylalanine, D,L-2-fluorophenylglycine, D,L-2-fluorophenylalanine, D,L-3-fluorophenylalanine, D,L-4-fluorophenylalanine, D,L-4-chlorophenylalanine, L-4-chlorophenylalanine, 4-bromo-D,L-phenylalanine,

35 4-iodo-D-phenylalanine, 3,3',5-triiodo-L-thyronine, (+)-3,3',5-triiodo-L-thyronine, D-thyronine,

L-thyronine, D,L-m-tyrosine, D-4-hydroxyphenylglycine, D-tyrosine, L-tyrosine, O-methyl-L-tyrosine, 3-fluoro-D,L-tyrosine, 3-iodo-L-tyrosine, 3-nitro-L-tyrosine, 3,5-diiodo-L-tyrosine, D,L-dopa, L-dopa, 2,4,5-trihydroxyphenyl-D,L-alanine, 3-amino-L-tyrosine, 4-amino-D-phenylalanine, 4-amino-L-phenylalanine, 5 4-amino-D,L-phenylalanine, 4-nitro-L-phenylalanine, 4-nitro-D,L-phenylalanine, 3,5-dinitro-L-tyrosine, D,L- α -methyltyrosine, L- α -methyltyrosine, (-)-3-(3,4-dihydroxyphenyl)-2-methyl-L-alanine, D,L-threo-3-phenylserine, *trans*-4-(aminomethyl)cyclohexane carboxylic acid, 4-(aminomethyl)benzoic acid, D,L-3-aminobutyric acid, 3-aminocyclohexane carboxylic acid, *cis*-2-amino-1-cyclohexane 10 carboxylic acid, γ -amino- β -(p-chlorophenyl)butyric acid (Baclofen), D,L-3-aminophenylpropionic acid, 3-amino-3-(4-chlorophenyl)propionic acid, 3-amino-3-(2-nitrophenyl)propionic acid, and 3-amino-4,4,4-trifluorobutyric acid.

"ARDS" as used herein refers to acute respiratory distress syndrome, which occurs in 15 adults and children of all ages. The syndrome follows a direct pulmonary or systemic insult, such as trauma, burn, sepsis, aspiration, and hypoxia, resulting in injury to the alveolar-capillary unit. ARDS is characterized by progressive hypoxemia, decreased lung compliance, intrapulmonary shunting, and noncardiogenic pulmonary edema associated with diffuse 20 pulmonary infiltrates.

20

"Carbocyclyl" refers to a stable 3- to 15-membered ring radical consisting solely of carbon and hydrogen atoms. For purposes of this invention, the carbocyclyl radical may be a monocyclic, bicyclic or tricyclic ring system, and may include fused or bridged ring systems, and the ring system may be partially or fully saturated or aromatic, and the carbon atoms in the ring 25 system may be optionally oxidized. Examples of such carbocyclyl radicals include, but are not limited to, cycloalkyl radicals (as defined herein), norbornane, norbornene, adamantyl, bicyclo[2.2.2]octane, phenyl, naphthalenyl, indanyl, indenyl, azulenyl, fluorenyl, anthracenyl, and the like. The carbocyclyl ring may be substituted by R⁶ as described below in the Preferred Embodiments, or by one or more substituents selected from the group consisting of hydroxy, 30 mercapto, halo, alkyl, alkenyl, alkynyl, phenyl, phenylalkyl, phenylalkenyl, alkoxy, phenoxy, phenylalkoxy, haloalkyl, haloalkoxy, formyl, nitro, cyano, cycloalkyl, hydroxyalkyl, alkoxyalkyl, phenoxyalkyl, phenylalkoxyalkyl, amidino, ureido, alkoxycarbonylamino, amino, monoalkylamino, dialkylamino, monophenylamino, monophenylalkylamino, sulfonylamino, alkylsulfonylamino, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, monophenylaminoalkyl, 35 monophenylalkylaminoalkyl, acyl, carboxyalkyl, alkoxycarbonylalkyl, aminocarbonyl,

monoalkylaminocarbonyl, dialkylaminocarbonyl, aminocarbonylalkyl, monoalkylaminocarbonylalkyl, and dialkylaminocarbonylalkyl, as defined herein.

"Cycloalkyl" refers to a stable 3- to 10-membered monocyclic or bicyclic radical which is saturated, and which consist solely of carbon and hydrogen atoms, *e.g.*, cyclopropyl, cyclobutyl, cyclobutyl, cyclohexyl, decalanyl, and the like. Unless otherwise stated specifically in the specification, the term "cycloalkyl" is meant to include cycloalkyl radicals which are optionally substituted by one or more substituents independently selected from the group consisting of alkyl, halo, hydroxy, amino, cyano, nitro, alkoxy, carboxy, and alkoxycarbonyl.

"Carboxy" refers to the radical of the formula $-C(O)OH$.

"Carboxyalkyl" refers to a radical of the formula $-R_a-C(O)OH$ where R_a is an alkyl radical as defined above, *e.g.*, carboxymethyl, 2-carboxyethyl, 3-carboxypropyl, and the like.

"Di(alkoxy)alkyl" refers to a radical of the formula $-R_a(-OR_a)_2$ where each R_a is independently an alkyl radical as defined above and where the $-OR_a$ groups may be attached to any carbon in the R_a group, *e.g.*, 3,3-dimethoxypropyl, 2,3-dimethoxypropyl, and the like.

"Dialkylamino" refers to a radical of the formula $-N(R_a)R_a$ where each R_a is independently an alkyl radical as defined above, *e.g.*, dimethylamino, diethylamino, (methyl)(ethyl)amino, and the like.

"Dialkylaminocarbonyl" refers to a radical of the formula $-C(O)N(R_a)R_a$ where each R_a is independently an alkyl radical as defined above, *e.g.*, dimethylaminocarbonyl, methylethylaminocarbonyl, diethylaminocarbonyl, dipropylaminocarbonyl, ethylpropylaminocarbonyl, and the like.

"Dialkylaminosulfonyl" refers to a radical of the formula $-S(O)_2N(R_a)R_a$ where each R_a is independently an alkyl radical as defined above, *e.g.*, dimethylaminosulfonyl, methylethylaminosulfonyl, diethylaminosulfonyl, dipropylaminosulfonyl, ethylpropylaminosulfonyl, and the like.

" FiO_2 " as used herein refers to fractional inspired oxygen, which is the amount of oxygen delivered to a patient through mechanical means, *i.e.*, a ventilator. The amount of oxygen can range between 21% (room air) to 100%.

"Halo" refers to bromo, chloro, iodo or fluoro.

5 "Haloalkyl" refers to an alkyl radical, as defined above, that is substituted by one or more halo radicals, as defined above, *e.g.*, trifluoromethyl, difluoromethyl, trichloromethyl, 2,2,2-trifluoroethyl, 1-fluoromethyl-2-fluoroethyl, 3-bromo-2-fluoropropyl, 1-bromomethyl-2-bromoethyl, and the like.

10 "Haloalkoxy" refers to a radical of the formula $-OR_d$ where R_d is an haloalkyl radical as defined above, *e.g.*, trifluoromethoxy, difluoromethoxy, trichloromethoxy, 2,2,2-trifluoroethoxy, 1-fluoromethyl-2-fluoroethoxy, 3-bromo-2-fluoropropoxy, 1-bromomethyl-2-bromoethoxy, and the like.

15 "Heterocyclyl" refers to a stable 3- to 15-membered ring radical, which consists of carbon atoms and from one to five heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur. For purposes of this invention, the heterocyclyl radical may be a monocyclic, bicyclic or tricyclic ring system, which may include fused or bridged ring systems, and the nitrogen, carbon or sulfur atoms in the heterocyclyl radical may be optionally oxidized, the nitrogen atom may be optionally quaternized, and the heterocyclyl radical may be partially
20 or fully saturated or aromatic. The heterocyclyl radical may be attached to the main structure at any heteroatom or carbon atom that results in the creation of a stable compound. Examples of such heterocyclyl radicals include, but are not limited to, azepinyl, azetidiny, acridinyl, benzimidazolyl, benzodioxolyl, benzodioxanyl, benzothiazolyl, benzoxazolyl, benzopyranly, benzofuranyl, benzothienyl, carbazolyl, cinnolinyl, decahydroisoquinolyl, dioxolanyl, furyl,
25 isothiazolyl, quinuclidinyl, imidazolyl, imidazoliny, imidazolidinyl, isothiazolidinyl, indolyl, isoindolyl, indoliny, isoindoliny, indoliziny, isoxazolyl, isoxazolidinyl, morpholiny, naphthyridinyl, oxadiazolyl, octahydroindolyl, octahydroisoindolyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, 2-oxoazepinyl, oxazolyl, oxazolidinyl, perhydroazepinyl, piperidinyl, piperazinyl, 4-piperidonyl, phenazinyl, phenothiazinyl, phenoxazinyl, phthalazinyl, pteridinyl,
30 purinyl, pyrrolyl, pyrrolidinyl, pyrazolyl, pyrazolidinyl, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinazolinyl, quinoxalinyl, quinolinyl, quinuclidinyl, isoquinolinyl, thiazolyl, thiazolidinyl, thiadiazolyl, triazolyl, tetrazolyl, tetrahydrofuryl, tetrahydropyranly, tetrahydroisoquinolyl, thienyl, thiomorpholinyl, thiomorpholinyl sulfoxide, and thiomorpholinyl sulfone. The heterocyclyl radical may be optionally substituted by R^6 as defined below in the Preferred Embodiments or may be
35 optionally substituted by one or more substituents selected from the group consisting of hydroxy, mercapto, halo, alkyl, alkenyl, alkynyl, phenyl, phenylalkyl, phenylalkenyl, alkoxy,

phenoxy, phenylalkoxy, haloalkyl, haloalkoxy, formyl, nitro, cyano, amidino, cycloalkyl, hydroxyalkyl, alkoxyalkyl, phenoxyalkyl, phenylalkoxyalkyl, amidino, ureido, alkoxycarbonylamino, amino, monoalkylamino, dialkylamino, monophenylamino, monophenylalkylamino, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, monophenylaminoalkyl, monophenylalkylaminoalkyl, alkylcarbonyl, carboxyalkyl, alkoxycarbonylalkyl, aminocarbonyl, monoalkylaminocarbonyl, dialkylaminocarbonyl, aminocarbonylalkyl, monoalkylaminocarbonylalkyl, dialkylaminocarbonylalkyl, and imidazolyl, as defined herein.

10 "Heterocyclylalkyl" refers to a radical of the formula $-R_a-R_e$ where R_a is an alkyl radical as defined above and R_e is a heterocyclyl radical as defined above, e.g., 2-(1,3-benzodioxol-5-yl)ethyl, and 3-(1,4-benzodioxan-6-yl)propyl, and the like.

"Hypoxia" as used herein refers to a condition in which there is a decrease of oxygen to the tissue in spite of adequate blood flow to the tissue.

"Hypoxemia" as used herein refers to a severe impairment of gas exchange in the arterial blood. Hypoxemia can be clinically defined as an arterial oxygen tension/fractional concentration of inspired oxygen ratio ("P/F ratio") of less than 150 without positive end-expiratory pressure ("PEEP") and less than 200 with PEEP.

"Monoalkylamino" refers to a radical of the formula $-N(H)R_a$ where R_a is an alkyl radical as defined above, e.g., methylamino, ethylamino, propylamino, and the like.

25 "Monoalkylaminocarbonyl" refers to a radical of the formula $-C(O)N(H)R_a$ where R_a is an alkyl radical as defined above, e.g., methylaminocarbonyl, ethylaminocarbonyl, propylaminocarbonyl, and the like.

30 "Monoalkylaminosulfonyl" refers to a radical of the formula $-S(O)_2N(H)R_a$ where R_a is an alkyl radical as defined above, e.g., methylaminosulfonyl, ethylaminosulfonyl, propylaminosulfonyl, and the like.

"N-heterocyclyl" refers to a heterocyclyl radical as defined above which contains at least one nitrogen atom and which is attached to the main structure through the nitrogen atom. The N-heterocyclyl radical may contain up to three additional heteroatoms. Examples include piperidinyl, piperazinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, azetidiny, indolyl, pyrrolyl,

imidazolyl, tetrahydroisoquinolyl, perhydroazepinyl, tetrazolyl, triazolyl, oxazinyl, and the like, and may be optionally substituted as described above for heterocyclyl radicals. In addition to being optionally substituted by the substituents listed above for a heterocyclyl radical, the *N*-heterocyclyl radical may also be optionally substituted by R^6 as defined below in the

5 Preferred Embodiments.

"Phenylalkyl" refers to an alkyl radical as defined above substituted by a phenyl radical, e.g., benzyl, and the like.

10 "Optional" or "optionally" means that the subsequently described event of circumstances may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted aryl" means that the aryl radical may or may not be substituted and that the description includes both substituted aryl radicals and aryl radicals having no substitution. The term " $-[C_2-C_8 \text{ alkyl}]-R^{10}$ "
15 (optionally substituted by hydroxy)" means that the alkyl has the optional substitution. The same goes for the term " $-[C_1-C_8 \text{ alkyl}]-R^{11}$ (optionally substituted by hydroxy)". The term "optionally substituted $-S(O)_tR^{22n}$ " means that the R^{22} substituents all have the optional substitution.

20 "Phenylalkenyl" refers to an alkenyl radical as defined above substituted by a phenyl radical.

The term "pharmaceutically acceptable salt" refers to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. When the compounds of the present invention are basic, salts may
25 be prepared from pharmaceutically acceptable non-toxic acids including inorganic and organic acids. Suitable pharmaceutically acceptable acid addition salts for the compounds of the present invention include acetic, benzenesulfonic (besylate), benzoic, camphorsulfonic, citric, ethenesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic,
30 sulfuric, tartaric acid, *p*-toluenesulfonic, and the like. When the compounds contain an acidic side chain, suitable pharmaceutically acceptable base addition salts for the compounds of the present invention include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from lysine, *N,N'*-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (*N*-methylglucamine),
35 and procaine.

“Pulmonary hypertension” as used herein is defined as a mean pulmonary artery pressure greater than 20 mm Hg. In ARDS, various vasoconstrictors (e.g., thromboxanes and endothelins), are released into the circulation, which leads to a narrowing of the arterial lumen and reduced compliance of the vessel wall which contributes to the maintenance of the hypertension.

“Pulmonary edema” as used herein refers to signs or symptoms representing the permeation of fluids from intravascular compartments to interstitium and consequently to the alveolus. Pulmonary edema of ARDS leads to small airway closure and microatelectasis, with a resulting reduction in lung volume, especially functional residual capacity and a stiff, noncompliant lung.

“PaO₂” as used herein refers to arterial partial pressure of oxygen, which is an indication of the level of oxygenation in the arterial blood. PaO₂ typically reflects the adequacy of lung function, the matching of ventilation and perfusion (“Va/Q”), diffusion, alveolar ventilation, and the degree of right to left shunting.

“P/F ratio” as used herein refers to PaO₂ to FiO₂ ratio. A ratio of <200 torr is an indication of severe oxygenation abnormality and a primary clinical characteristics of ARDS.

“Right to left shunting” or “intrapulmonary shunting” as used herein is defined as a substantial fraction of mixed venous blood traversing airspaces that are non-ventilated as a result of alveolar flooding or collapse. Because the mixed venous blood that goes to the alveolar capillary exchange unit will not have an increase in oxygenation and blood will be shunted across that segment, supplemental oxygen administration (i.e., increasing the inspired oxygen) will not improve PaO₂.

“Partially selective iNOS inhibitors” as used herein refer to those iNOS inhibitors that substantially inhibit iNOS (or NOS-2), but also inhibit eNOS (or NOS-3), nNOS (or NOS-1), or both eNOS and nNOS. A partially selective iNOS inhibitor has a selectivity ratio ~50 or lower (IC₅₀ or K_i in enzyme assays) when comparing iNOS versus eNOS or nNOS (see Parkinson, J.F. (2003) in *Lung Biology in Health and Disease: Therapeutic Targets in Airway Inflammation*, Marcell Dekker, N.Y., Vol. 177, pp. 199-221). Examples of partially selective iNOS inhibitors that exhibit high iNOS versus eNOS selectivity, but only moderate iNOS versus nNOS selectivity, include N⁶-iminoethyl-L-lysine (“L-NIL”, see, for example, Alderton, W.K. *et al.* (2001) *supra*) and its prodrug derivative L-N⁶-(1-iminoethyl)lysine-5-tetrazole-amide (Hallinan,

E.A. *et al.* (2002) *J. Med. Chem.* 45:1686-1689), and heterocyclic amidines (Moormann, A.E. *et al.* (2001) *Bioorg. Med. Chem. Lett.* 11:2651-2653). See Table 1 in Parkinson, J.F. (2003) *supra* for IC₅₀ or K_i in enzyme assays and selectivity ratios for a variety of selective and partially selective iNOS inhibitors.

5

"Selective iNOS inhibitors" as used herein refer to those iNOS inhibitors that substantially inhibit iNOS (or NOS-2) without inhibiting eNOS (or NOS-3). Varying levels of iNOS versus nNOS (or NOS-1) selectivity may be tolerated. A selective iNOS inhibitor has a selectivity ratio ~50 or higher when comparing iNOS versus eNOS or nNOS. An example of selective iNOS inhibitors that exhibit high iNOS versus eNOS selectivity, but only moderate iNOS versus nNOS selectivity, includes dihydro-1-isoquinolinamines (Beaton, H. *et al.* (2001) *Bioorg. Med. Chem. Lett.* 11:1023-1026). Examples of selective iNOS inhibitors that exhibit high selectivity for iNOS versus both nNOS and eNOS include the selective and potent iNOS dimerization inhibitors described in McMillan, K. *et al.* (2000) *supra*, and Blasko, E. *et al.* (2002) *supra*. These inhibitors are disclosed and claimed in PCT Published Patent Application, WO 01/14371 and PCT Published Patent Application, WO 98/37019. Further examples of highly selective iNOS inhibitors are 1400W (Alderton, W.K. *et al.* (2001) *supra*) and recently described sulfur-containing acetamidines, such as GW273629 and GW274150 (Alderton, W.K. *et al.* (2001) *supra*; Young, R.J. *et al.* (2000) *Bioorg. Med. Chem. Lett.* 10:597-600).

20

The beneficial effects of a highly selective iNOS dimerization inhibitor are manifold. Pulmonary benefits include improved gas exchange (PaO₂/FiO₂ ratio), decreased intrapulmonary shunt fraction, decreased edema, decreased permeability index, decreased lung lymphatic flow, decreased bronchiolar congestion and obstruction, as well as decreased pulmonary inflammation. Additionally, there is strong evidence of a multitude of extrapulmonary benefits, such as improved cardiac performance including cardiac index, cardiac output, left arterial pressure and left ventricle stroke work index, together indicating prevention of injury-induced myocardial depression. Prevention of systemic vascular leak, improved renal function (urinary output, creatinine clearance and glomerular filtration rate), and other positive effects on net fluid balance have also been observed.

30

It is further notable that selective iNOS inhibitors of the invention do not significantly increase mean arterial pressure, systemic vascular resistance index, or decrease cardiac output, as have been observed with non-selective NOS inhibitors such as L-NAME and L-NMMA.

35

"Pulmonary vasodilators" or "selective pulmonary vasodilators" are used herein interchangeably to refer to vasodilators that selectively target the pulmonary system. The ideal pulmonary vasodilator should decrease the pulmonary artery pressure ("PAP") and the resistance across the pulmonary bed, while at the same time increase the cardiac output without worsening the ventilation-perfusion matching. It must also minimize the risk of systemic hypotension. In general, selective pulmonary vasodilators should have localized (pulmonary) beneficial effects in increasing the $\text{PaO}_2/\text{FiO}_2$ ratio, restoring mismatched V_a/Q , and reducing vascular hyperpermeability in ARDS models.

Examples of selective pulmonary vasodilators include the compound classes described above in the Background of the Invention. Those skilled in the art will recognize that some agents that have been shown to provide pulmonary vasodilatory effects may or may not also provide an anti-inflammatory effect. These distinct profiles can be exploited therapeutically in combinations with iNOS inhibitors for the treatment of ARDS and related critical care indications. Endothelin receptor antagonists, such as bosentan, have pulmonary vasodilatory effects (Yamamoto, S. *et al.* (1997) *Am. J. Physiol.* 272:H1239-H1249) as well as anti-inflammatory effects (Finsnes, F. *et al.* (2001) *Am. J. Physiol. Lung Cell Mol. Physiol.* 280:L659-L665) *in vivo*. NO donating agents and inhaled NO gas are selective pulmonary vasodilators (Adrie, C. *et al.* (1998) *Anesthesiology* 88:190-195; Frostell, C. *et al.* (1991) *Circulation* 83:2038-2047). In addition, inhaled NO gas and NO-donating agents have anti-inflammatory effects (Razavi, H.M. *et al.* (2002) *Crit. Care Med.* 30:868-873; Cicala, C. *et al.* (2000) *Br. J. Pharmacol.* 130:1399-1405). A broad class of PDE inhibitors are found to promote vasodilatation with varying levels of anti-inflammatory effects. For example, selective PDE 5 inhibitors, such as sildenafil, are pulmonary vasodilators (Ichinose, F. *et al.* (2001) *supra*, Abrams, D. *et al.* (2000) *Heart* 84:E4), but are not recognized to have potent anti-inflammatory effects *in vivo*. In contrast, PDE 4 inhibitors, such as rolipram, mesopram, and ariflo (SB-207499), have pulmonary vasodilator effects in addition to potent anti-inflammatory effects *in vivo* (Schermuly, R.T. *et al.* (1999) *Am. J. Respir. Crit. Care Med.* 160:1500-1506, Dinter, H. *et al.* (2000) *supra*, Torphy, T.J. *et al.* (1999) *Pulm. Pharmacol. Ther.* 12:131-135). PDE inhibitors with mixed profiles, such as the PDE 3/4, PDE 4/5, and PDE 1/4/5 (e.g., ZK-803616) inhibitors, are expected to also have pulmonary vasodilatory and anti-inflammatory effects. Since there are now at least 12 known PDE isoforms, it can be anticipated that new pharmacological agents with defined pulmonary vasodilating and anti-inflammatory profiles will continue to be developed. Iloprost and other prostacyclin derivatives also have pulmonary vasodilator and anti-inflammatory effects *in vivo* (Olschewski, H. *et al.* (2001) *supra*; see Jung, S. *et al.* (1997) *J. Autoimmun.* 10:519-529 for review).

Since each class of vasodilator has a distinct pharmacological profile (e.g., endothelin antagonists, NO gas or NO donors, non-selective PDE inhibitors, selective PDE 5 and PDE 4 inhibitors, and mixed PDE 4 and 5 inhibitors), different combined therapeutic outcomes can be expected depending on profile, dose, and route of delivery.

"Stable compound" and "stable structure" are meant to indicate a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent.

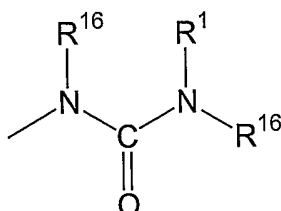
"Therapeutically effective amount" refers to that amount of a selective iNOS inhibitor and that amount of a pulmonary vasodilator of the invention which, when administered to a human in need thereof, is sufficient to effect treatment, as defined below, for conditions resulting from ARDS. The amount of the selective iNOS inhibitor or pulmonary vasodilator will vary depending on the compound, the condition and its severity, and the age of the human to be treated, but can be determined routinely by one of ordinary skill in the art having regard to his own knowledge and to this disclosure.

"Treating" or "treatment" as used herein covers the treatment of a condition in a human, which condition results from ARDS, and includes:

- (i) preventing the condition from occurring in a human, in particular, when such human is predisposed to the condition but has not yet been diagnosed as having it;
- (ii) inhibiting the condition, *i.e.*, arresting its development; or
- (iii) relieving the condition, *i.e.*, causing regression of the condition.

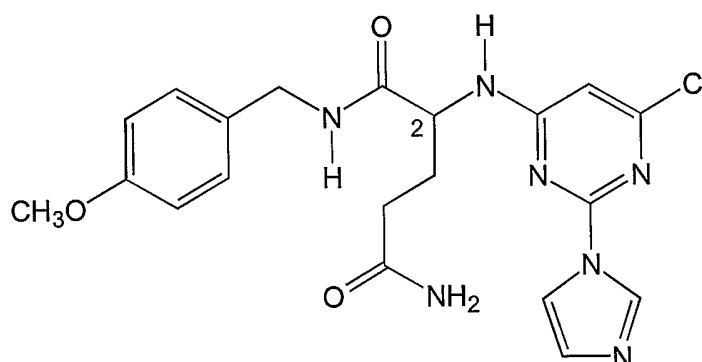
"Ventilation and perfusion (Va/Q) mismatch" as used herein refers to an imbalance between alveolar ventilation and pulmonary capillary blood flow, which contributes to hypoxemia. Va/Q mismatch is the result of impaired hypoxic pulmonary vasoconstriction ("HPV"), a regulatory reflex which tends to direct the pulmonary capillary blood flow to well ventilated space in the lung, *i.e.*, oxygenated alveolar spaces, in order to allow the perfusion of oxygen into blood. Mismatched ventilation and perfusion occurs when the pulmonary capillary blood flow is unable to be directed to a well-ventilated area in the lung, resulting in arterial hypoxemia.

The use of parentheses in a formula is used to conserve space. Accordingly, the use of parenthesis in a formula indicates that the group enclosed within the parentheses is attached directly to the atom preceding the parenthesis. For example, the term $-N(R^{16})C(O)N(R^1)R^{16}$ can be drawn as follows:



5

The nomenclature used herein is a modified form of the I.U.P.A.C. nomenclature system wherein the compounds of the invention are named herein as amide derivatives. For example, the following compound of the invention:



- 10 would be named herein as 2-[[6-chloro-2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]amino]-*N*-[(4-methoxyphenyl)methyl]pentanediamide. Unless otherwise indicated, compound names are intended to include any single stereoisomer, enantiomer, racemate, or mixtures thereof.

Most of the selective iNOS inhibitors described herein contain one or more asymmetric
 15 centers and may thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (*R*)- or (*S*)- or as (*D*)- or (*L*)- for amino acids. The present invention is meant to include all such possible isomers, as well as, their racemic and optically pure forms. Optically active (*R*)- and (*S*)- or (*D*)- and (*L*)- isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional
 20 techniques. When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both *E* and *Z* geometric isomers. Likewise, all tautomeric forms are also intended to be included.

Utility of the Invention

One of the goals of the present invention is to overcome a critical phase of respiratory failure associated with ARDS. This can be achieved by increasing the patient's $\text{PaO}_2/\text{FiO}_2$ ratio as fast and as high as possible (P/F ratio lower than 200 is considered clinically as ARDS). The present invention envisions a broad range of treatments for ARDS and related diseases using a selective or partially selective iNOS inhibitor in combination with various pulmonary vasodilators. The combination treatment is expected to enhance the therapeutic effects of an iNOS inhibitor on both pulmonary and extrapulmonary function in ARDS. Additive or synergistic effects could be evaluated readily by comparison of therapeutic and sub-therapeutic doses of the combined compounds in relevant animal models.

Methods for Testing the Invention

The primary concept behind the invention is that the combination of a selective or partially selective iNOS inhibitor with a pulmonary vasodilator will enhance pulmonary functional benefits (additive or synergistic effects) in patients having ARDS. In addition, depending on the pharmacological profile and route of delivery of the pulmonary vasodilator, the systemic (extrapulmonary) benefits of the iNOS inhibitor, such as anti-inflammatory effects, may be enhanced. There are many animal models in which acute lung injury ("ALI") can be induced with pulmonary dysfunction that mimics certain aspects of ARDS. Some of these models are associated with a robust systemic inflammatory response, others promote a more direct and focal inflammatory response in the lung.

ALI with pulmonary edema can be induced by endotoxin in rabbits and is attenuated by the non-selective iNOS inhibitor aminoguanidine (Mikawa, K. *et al.* (1998) *Crit. Care Med.* 26:905-911). Intestinal ischemia-reperfusion injury in rats causes enhanced pulmonary microvascular leak that is attenuated by L-NIL (Turnage, R.H. *et al.* (1998) *Surgery* 124:457-463). A systemic capillary leak syndrome evidenced by pulmonary edema and pleural effusions is induced by IL-2 in mice and these pathological endpoints are attenuated by NOS inhibitors (Orucevic, A. *et al.* (1997) *Lab Invest.* 76:53-65). Endotoxin-treated mice also exhibit both cardiac and pulmonary dysfunction that is attenuated by iNOS inhibitors or in iNOS -/- mice (Ullrich, R. *et al.* (2000) *Circulation* 102:1440-1446, Ullrich, R. *et al.* (1999) *supra*). These models can serve as test systems for the effects of the compounds of the invention on iNOS-dependent pulmonary dysfunction. The endotoxin and IL-2 models also provide a test system for extrapulmonary effects. Additional models where the extrapulmonary effects of an iNOS inhibitor can be tested are endotoxin-induced shock, where evidence for iNOS-dependent vascular leak in several tissues and iNOS-dependent systemic hypotension are well-established

(Garvey, E.P. *et al.* (1997) *J. Biol. Chem.* 272:4959-4963, Wray, G.M. *et al.* (1998) *Shock* 5:329-335).

Similarly, there are many models for evaluating the *in vivo* effects of pulmonary vasodilators. The effects of endothelin antagonists on pulmonary vascular leak and pulmonary hypertension are described in Hele, D.J. *et al.* (2000) *supra* and Chen, S.J. *et al.* (1995) *J. Appl. Physiol.* 79:2122-2131. The PDE 5 inhibitor sildenafil has shown positive effects on hypoxia-induced pulmonary hypertension in mice (Zhao, L. *et al.* (2001) *Circulation* 104:424-428). Thus, rodent and rabbit models exist where the effects of combining a NOS inhibitor, preferably a selective iNOS inhibitor, with a pulmonary vasodilator can be evaluated for efficacy on pulmonary or extrapulmonary (heart, liver, kidney, gut) functional endpoints. The compounds could be tested systematically *via* oral, parenteral (intravenous, subcutaneous, intraperitoneal) or local (intratracheal, aerosol or nebulization) delivery.

Those skilled in the art will recognize that the complex pathophysiology of ARDS, particularly in the setting of concomitant pulmonary and extrapulmonary organ dysfunction, is difficult to study in rodents. This is because measuring the integrated cardiopulmonary and systemic hemodynamic physiological responses requires sophisticated and extensive instrumentation not amenable to small species. For this reason, ARDS, sepsis, and sepsis with ARDS models have been developed in higher order species that permit integrated physiological monitoring. These include endotoxemia and sepsis models in pigs (Weitzberg, E. *et al.* (1995) *supra*), piglets (Suzuki, S. *et al.* (2001) *supra*), dogs (Kaszaki, J. *et al.* (1996) *Shock* 6:279-285), and sheep (Fischer, S.R. *et al.* (1997) *Am. J. Respir. Crit. Care Med.* 156:833-839), as well as combined smoke inhalation and burn injury in sheep (Alpard, S.K. *et al.* (2000) *Crit. Care Med.* 28:1469-1476). Some of these models have been used to evaluate a combined effect of non-selective NOS inhibitors with NO inhalation therapy, which confirmed the adverse effects of L-NAME on cardiac function (Weitzberg, E. *et al.* (1995) *supra*, Suzuki, S. *et al.* (2001) *supra*). In the sheep smoke inhalation and burn injury model, the effects of a non-selective guanidine-based NOS inhibitor (with peroxynitrite scavenging properties) and NO inhalation therapy have been tested individually, but not in combination (Soejima, K. *et al.* (2001) *Burns* 27:809-815, Soejima, K. *et al.* (2001) *Am. J. Respir. Crit. Care Med.* 163:745-752, Soejima, K. *et al.* (2000) *Shock* 13:261-266, Booke, M. *et al.* (1997) *J. Burn Care Rehabil.* 18:27-36). In addition, endothelins are upregulated in this model (Cox, R.A. *et al.* (2001) *J. Burn Care Rehabil.* 22:375-383).

The sheep smoke and burn injury model thus affords a highly relevant system in which the current invention can be tested since both pulmonary and extrapulmonary organ functions can be measured simultaneously. In addition, the drug classes of the current invention (iNOS inhibitors, various pulmonary vasodilators) are active in the model. The sheep are maintained in laboratory ICU setting with mechanical ventilation support, allowing for administration of drugs systemically (including bolus or constant intravenous infusion) or directly to the pulmonary compartment (aerosol, nebulization, intratracheal injection). A full hemodynamic, pulmonary, gas exchange and fluid balance profile can be evaluated. Endpoints include, but not limited to: cardiac index, cardiac output, left and right ventricle stroke work indices, mean arterial pressure, PAP, PCW, PaO₂, FiO₂, PaO₂/FiO₂ ratio, pulmonary edema, lung lymphatic flow, hematocrit, urinary output, glomerular filtration rate, and so on. Tissues, fluids, blood, and plasma samples can be isolated during and at the end of the model for assessment of drug treatments on biological mediators. For example, measurement of iNOS enzyme activity, cAMP or cGMP levels in lung or cardiac tissue extracts, nitric oxide metabolite levels in plasma or lymphatic fluids, *etc.*

Pharmaceutical Compositions

The selective iNOS inhibitors and pulmonary vasodilators of the invention, or their respective pharmaceutically acceptable salts, in pure forms or in appropriate pharmaceutical compositions as described herein, can be administered to a human to treat the symptoms of ARDS *via* any of the accepted modes of administration of agents for serving similar utilities. For purposes of the following description, the term "active ingredient" refers to either a selective iNOS inhibitor or a pulmonary vasodilator as described herein, and the term "active ingredients" refers to both a selective iNOS inhibitor and a pulmonary vasodilator as described herein.

In general, pharmaceutical compositions containing the active ingredients, either alone or in combination, can be prepared by combining the selective iNOS inhibitor and the pulmonary vasodilator with an appropriate pharmaceutically acceptable carrier, diluent or excipient, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols.

Typical methods of administering the selective iNOS inhibitor and pulmonary vasodilator in pure forms or the pharmaceutical compositions containing the active ingredients, either alone or in combination, include, without limitation, oral, buccal, topical, transdermal, inhalation, parenteral, sublingual, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. Pharmaceutical compositions of the invention are formulated so as to allow the active ingredient(s) contained therein to be bioavailable upon administration of the pharmaceutical composition to a patient for treatment of ARDS in accordance with the teachings of this invention. Pharmaceutical compositions that will be administered to a subject or patient take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of a compound of the invention in aerosol form may hold a plurality of dosage units. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington's Pharmaceutical Sciences*, 18th Ed., Mack Publishing Company, Easton, Pennsylvania, 1990.

As noted above, a pharmaceutical composition of the invention may be in the form of a solid or liquid. In one aspect, the carrier(s) are particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) may be liquid, with the compositions being, for example, an oral syrup, injectable liquid, or an aerosol, which is useful in, e.g., inhalatory administration.

When intended for oral administration, the pharmaceutical composition is preferably in either solid or liquid form, where semi-solid, semi-liquid, suspension, and gel forms are included within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the pharmaceutical composition may be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer, or the like form. Such a solid composition will typically contain one or more inert diluents or edible carriers. In addition, one or more of the following may be present: binders, such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, gum tragacanth, or gelatin; excipients, such as starch, lactose or dextrins; disintegrating agents, such as alginic acid,

sodium alginate, Primogel, corn starch, and the like; lubricants, such as magnesium stearate or Sterotex; glidants, such as colloidal silicon dioxide; sweetening agents, such as sucrose or saccharin; a flavoring agent, such as peppermint, methyl salicylate, or orange flavoring; and a coloring agent.

5

When the pharmaceutical composition is in the form of a capsule, *e.g.*, a gelatin capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as polyethylene glycol or oil.

10

The pharmaceutical composition may be in the form of a liquid, *e.g.*, an elixir, syrup, solution, emulsion, or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred compositions contain, in addition to the present compounds, one or more of a sweetening agent, preservatives, dye/colorant, and flavor enhancer. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer, and isotonic agent may be included.

15

The liquid pharmaceutical compositions of the invention, whether they be solutions, suspensions, or other like form, may include one or more of the following adjuvants: sterile diluents, such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils, such as synthetic mono or diglycerides, which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol, or other solvents; antibacterial agents, such as benzyl alcohol or methyl paraben; antioxidants, such as ascorbic acid or sodium bisulfite; chelating agents, such as ethylenediaminetetraacetic acid; buffers, such as acetates, citrates, or phosphates; and agents for the adjustment of tonicity, such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes, or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile.

20

25

30

A liquid pharmaceutical composition of the invention intended for either parenteral or oral administration should contain an amount of either a selective iNOS inhibitor or a pulmonary

vasodilator or an amount of a selective iNOS inhibitor together with an amount of a pulmonary vasodilator such that a suitable dosage will be obtained. Typically, this amount is at least 0.01% of the active ingredient(s) in the composition. When intended for oral administration, this amount may be varied to be between 0.1 and about 70% of the weight of the composition.

5 Preferred oral pharmaceutical compositions contain between about 4% and about 50% of an active ingredient. Preferred pharmaceutical compositions and preparations according to the present invention are prepared so that a parenteral dosage unit contains between 0.01 to 1% by weight of the active ingredient(s).

10 The pharmaceutical composition of the invention may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment, or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, bee wax, mineral oil, diluents, such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition
15 for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device. Topical formulations may contain a concentration of the active ingredient from about 0.1 to about 10% w/v (weight per unit volume).

The pharmaceutical composition of the invention may be intended for rectal
20 administration, in the form, e.g., of a suppository, which will melt in the rectum and release the active ingredient(s). The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter, and polyethylene glycol.

25 The pharmaceutical composition of the invention may include various materials, which modify the physical form of a solid or liquid dosage unit. For example, the composition may include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and may be selected from, for example, sugar, shellac, and other enteric coating agents. Alternatively, the active ingredients may be encased in a
30 gelatin capsule.

The pharmaceutical composition of the invention in solid or liquid form may include an agent that binds to the compound of the invention and thereby assists in the delivery of the compound. Suitable agents that may act in this capacity include a monoclonal or polyclonal antibody, a protein, or a liposome.

5

The pharmaceutical composition of the invention may consist of dosage units that can be administered as an aerosol. The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery may be by a liquefied or compressed gas or by a suitable pump system that dispenses the active ingredients. Aerosols of compounds of the invention may be delivered in single phase, bi-phasic, or tri-phasic systems in order to deliver the active ingredient(s). Delivery of the aerosol includes the necessary container, activators, valves, subcontainers, and the like, which together may form a kit. One skilled in the art, without undue experimentation may determine preferred aerosols.

15

Whether in solid, liquid or gaseous form, the pharmaceutical compositions of the present invention may contain one or more known pharmacological agents used in the treatment of ARDS in a mammal.

20 **Methods of Administration**

The selective iNOS inhibitor and the pulmonary vasodilator, either alone or in combination, are administered in a therapeutically effective amount, which will vary depending upon a variety of factors, including the activity of the specific active ingredient employed; the metabolic stability and length of action of the active ingredient; the age, body weight, general health, sex, and diet of the patient undergoing treatment; the mode and time of administration; the rate of excretion; the specific combination of the active ingredients; and the severity of the ARDS. Generally, a therapeutically effective daily dose of either active ingredient is from about 0.1 mg to about 20 mg/kg of body weight per day of an active ingredient; preferably, from about 0.1 mg to about 10 mg/kg of body weight per day; and most preferably, from about 0.1 mg to about 7.5 mg/kg of body weight per day.

30

A preferred route of administration of the active ingredients is intranasal administration or administration by inhalation wherein the active ingredients are delivered in the form of a solution or suspension from a suitable container. For example, the active ingredients may be delivered intranasally or by inhalation from a pump spray container that can be squeezed or pumped, either mechanically or manually, to deliver the desired dosage. Alternatively, the active ingredients may be delivered intranasally or by inhalation in pure form or in the form of a solution or suspension from a pressurized container or nebulizer, preferably with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver the desired therapeutically effective amount of the active ingredient(s).

Preferably both active ingredients are administered intranasally or by inhalation. This route of administration targets primarily the pulmonary tissue (*i.e.*, lung tissue), and would be advantageous in those cases of ARDS where there is a profound inflammation of the pulmonary tissue coupled with pulmonary hypertension, with no signs of systemic infection or extrapulmonary organ dysfunction. For example, ARDS due to smoke inhalation alone without cutaneous burn injury or ARDS secondary to a localized pneumonia with no signs of systemic infection or extrapulmonary organ dysfunction.

20

Alternatively, the selective iNOS inhibitor can be administered by a route of administration that will result in a systemic effect and the pulmonary vasodilator can be administered by a route of administration that will target primarily the pulmonary tissue. Routes of administration that result in systemic effects include, but are not limited to, oral, buccal, topical, transdermal, parenteral, sublingual, rectal, or vaginal administration, while routes targeting primarily the pulmonary tissue include, but are not limited to, intranasal administration or administration by inhalation. Administering the selective iNOS inhibitor and the pulmonary vasodilator separately by these routes has an advantage when the pulmonary and extrapulmonary effects of a selective iNOS inhibitor are desired and the deleterious systemic side effects of the pulmonary vasodilator are not. For example, sildenafil, a known pulmonary vasodilator, can lower systolic and diastolic blood pressure through cardiac side effects which

30

may be undesirable in the setting of ARDS-associated myocardial depression, hypovolemia or hypotension. Alternatively, a PDE 4 inhibitor, such as rolipram, may not have an adequate safety profile when delivered systemically, but may exert potent anti-inflammatory and pulmonary vasodilatory effects when delivered directly to the pulmonary tissue (*i.e.*, lung tissue) through intranasal or inhalation administration.

Alternatively, the selective iNOS inhibitor and the pulmonary vasodilator can both be delivered by a route of administration that will result in a systemic effect of both active ingredients. Such routes of administration include, but are not limited to, oral, buccal, topical, transdermal, parenteral, sublingual, rectal, or vaginal administration. This route of administration has the advantage when the pulmonary and extrapulmonary (*i.e.*, systemic) therapeutic effects of both the selective iNOS inhibitor and the pulmonary vasodilator are desirable. For example, endothelin antagonists can prevent both pulmonary and portal (*i.e.*, extrapulmonary) hypertension. Thus, a endothelin antagonist can be used as the pulmonary vasodilator in combination with the selective iNOS inhibitor to treat a patient with ARDS complicated by sepsis when hepatic perfusion preservation is desired.

Use of Co-medications and Other Treatment

It is well recognized that the management of ARDS in various clinical settings is associated with frequent co-medications and respiratory support (mechanical ventilation with oxygen support, *etc*). Fluid resuscitation is used in burn patients, patients with sepsis, and hemorrhagic shock. The use of colloids to prevent hypovolemia and dehydration is used in burn patients with ARDS. Vasoconstrictors are also used clinically to maintain blood pressure in sepsis patients with hypotension. Sepsis patients and patients at high risk for sepsis are treated or prophylactically administered antibiotics.

Those skilled in the art will recognize that the present invention may need to be used in combination with these treatments clinically. The impact of the current invention on the need for these additional supporting treatment modalities can be assessed using variants of the animal models described and also clinically. For example, it is well known that iNOS is a major mediator of hypotension in sepsis and that iNOS inhibitors can reduce the need for vasopressor

support in this clinical setting (Grover, R. *et al.* (1999) *supra*). NOS inhibitors can lead to an “overshoot” phenomenon whereby a co-administered vasopressor causes systemic and pulmonary vascular resistance levels higher than baseline. It is also known that appropriate fluid and antibiotic support (Hollenberg, S.M. *et al.* (2000) *Circ. Res.* 86:774-778) can enhance the effect of iNOS inhibition on promoting survival in rodents with bacterial septic shock. Variants of these models in rodents and higher species can be adapted to assess these questions. Combination treatments of the present invention may promote survival (with and without antibiotics), promote superior net fluid balance (reduced need for fluid or colloidal support), weaning from vasopressor support to prevent hypotension, *etc.*

Preferred Embodiments

A preferred method of using the invention is to administer a therapeutically effective amount of a selective iNOS inhibitor in combination with a therapeutically effective amount of a pulmonary vasodilator wherein the combination is delivered locally to the lungs, *i.e.*, both active ingredients are delivered *via* inhalation, aerosol, or nebulization. This method of use would target pulmonary effects, but limit extrapulmonary effects. Such a combination would have an advantage where there is profound local lung inflammation and pulmonary hypertension without an underlying systemic inflammatory response in other end organs.

Another preferred method is to administer concurrently a therapeutically effective amount of a selective iNOS inhibitor and a therapeutically effective amount of a pulmonary vasodilator locally to the lung, wherein the selective iNOS inhibitor is delivered systemically (*e.g.*, intravenous bolus, intravenous infusion, oral, subcutaneous, intraperitoneal, transdermal patch, or other means of systemic drug delivery) and the pulmonary vasodilator is delivered locally to the lung (*e.g.*, inhalation, aerosol, or nebulization). This combination would have an advantage where the pulmonary and extrapulmonary therapeutic effects of an iNOS inhibitor are desired and the pulmonary vasodilator being used to suppress pulmonary hypertension may have unwanted side effects if delivered systemically.

Another preferred method is to administer sequentially a therapeutically effective amount of a selective iNOS inhibitor and a therapeutically effective amount of a pulmonary

vasodilator locally to the lung, wherein the selective iNOS inhibitor is delivered systemically (e.g., intravenous bolus, intravenous infusion, oral, subcutaneous, intraperitoneal, transdermal patch, or other means of systemic drug delivery) and the pulmonary vasodilator is delivered locally to the lung (e.g., inhalation, aerosol, or nebulization).

5

Yet another preferred method is to systemically administer (e.g., intravenous bolus, intravenous infusion, oral, subcutaneous, intraperitoneal, transdermal patch, or other means of systemic drug delivery) a therapeutically effective amount of a selective iNOS inhibitor in combination with a therapeutically effective amount of a pulmonary vasodilator. This combination would have an advantage where the pulmonary and extrapulmonary therapeutic effects of both the iNOS inhibitor and the pulmonary vasodilator are desirable.

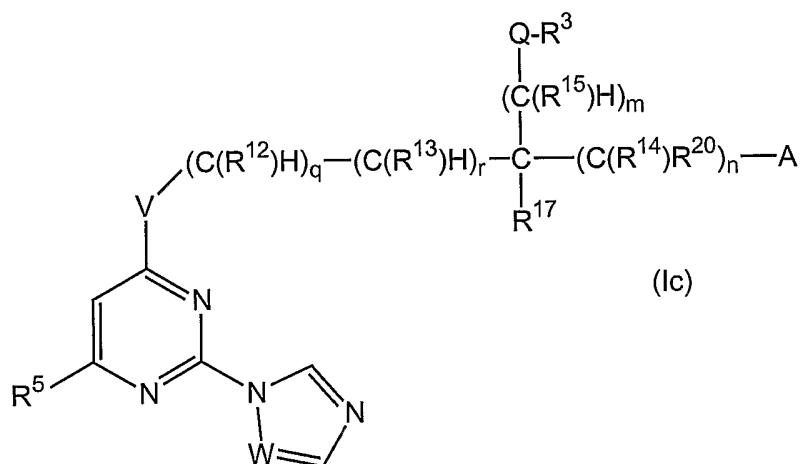
10

Yet another preferred method is to administer a therapeutically effective amount of a selective iNOS inhibitor locally to the lung in combination with the systemic administration of a therapeutically effective amount of a pulmonary vasodilator. Such a combination would have an advantage where the iNOS inhibitor has undesirable systemic side effects (e.g., L-NAME or L-NMMA) or a high potential for drug-drug interactions due to inhibition of hepatic cytochromes P450.

15

20

Of the selective iNOS inhibitors described herein, preferred selective iNOS inhibitors are those disclosed in PCT Published Patent Application WO 01/14371 and PCT Published Patent Application WO 98/37019, and which have the following formula (Ic):



wherein :

A is R^1 , $-OR^1$, $-C(O)N(R^1)R^2$, $-N(R^{16})C(O)N(R^1)R^{16}$, $-N(R^1)C(O)R^2$ or $-N(R^1)R^{21}$;

V is $N(R^4)$;

W is CH; and

5 Q is chosen from the group consisting of a direct bond, $-C(O)-$, $-O-$, $-C(=N-R^1)-$, $-S(O)_t$, and $-N(R^6)-$;

m is zero or an integer from 1 to 4;

n is zero or an integer from 1 to 3;

q is zero or one;

10 r is zero or one, provided that when Q is a heteroatom, m, q, and r cannot all be zero; when A is $-OR^1$, $-N(R^1)C(O)R^2$, $-N(R^1)R^{21}$, $-N(R^{16})C(O)N(R^1)R^{16}$, n, q, and r cannot all be zero; and when Q is a heteroatom and A is $-OR^1$, $-N(R^1)C(O)R^2$, $-N(R^1)R^{21}$, $-N(R^{16})C(O)N(R^1)R^{16}$, m and n cannot both be zero;

t is zero, one or two;

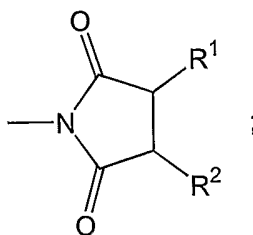
15 each R^1 and R^2 are independently chosen from the group consisting of hydrogen, optionally substituted C_1 - C_{20} alkyl, optionally substituted cycloalkyl, $-[C_0$ - C_8 alkyl]- R^9 , $-[C_2$ - C_8 alkenyl]- R^9 , $-[C_2$ - C_8 alkynyl]- R^9 , $-[C_2$ - C_8 alkyl]- R^{10} (optionally substituted by hydroxy), $-[C_1$ - C_8]- R^{11} (optionally substituted by hydroxy), and optionally substituted heterocyclyl; or R^1 and R^2 together with the nitrogen atom to which they are attached is an optionally substituted *N*-heterocyclyl;

R^3 is chosen from the group consisting of hydrogen, alkyl, cycloalkyl, optionally substituted aryl, haloalkyl, $-[C_1$ - C_8 alkyl]- $C(O)N(R^1)R^2$, $-[C_1$ - C_8 alkyl]- $N(R^1)R^2$, $-[C_1$ - C_8 alkyl]- R^8 , $-[C_2$ - C_8 alkyl]- R^{10} , $-[C_1$ - C_8 alkyl]- R^{11} , and heterocyclyl (optionally substituted by one or more substituents selected from the group consisting of halo, alkyl, alkoxy and imidazolyl);

25 or when Q is $-N(R^6)-$ or a direct bond to R^3 , R^3 may additionally be aminocarbonyl, alkoxycarbonyl, alkylsulfonyl, monoalkylaminocarbonyl, dialkylaminocarbonyl and $-C(=NR^{18})-NH_2$;

or $-Q-R^3$ taken together represents $-C(O)OH$, $-C(O)N(R^1)R^2$, $-C(=NH)-N(R^1)R^2$ or

- 32 -



R⁴ is chosen from the group consisting of hydrogen, alkyl, aryl, aralkyl and cycloalkyl, provided that when A is -R¹ or -OR¹, R⁴ cannot be hydrogen, and when V is CH, R⁴ may additionally be hydroxy;

5 R⁵ is chosen from the group consisting of hydrogen, halo, alkyl, haloalkyl, optionally substituted aralkyl, optionally substituted aryl, -OR¹⁶, -S(O)_t-R¹⁶, -N(R¹⁶)R²¹, -N(R¹⁶)C(O)N(R¹)R¹⁶, -N(R¹⁶)C(O)OR¹⁶, -N(R¹⁶)C(O)R¹⁶, -[C₀-C₈ alkyl]-C(O)OR¹⁶, -[C₀-C₈ alkyl]-C(H)[C(O)OR¹⁶]₂, and -[C₀-C₈ alkyl]-C(O)N(R¹)R¹⁶;

10 R⁶ is chosen from the group consisting of hydrogen, alkyl, cycloalkyl, -[C₁-C₈ alkyl]-R⁸, -[C₂-C₈ alkyl]-R¹⁰, -[C₁-C₈ alkyl]-R¹¹, acyl, -C(O)R⁸, -C(O)-[C₁-C₈ alkyl]-R⁸, alkoxycarbonyl, optionally substituted aryloxycarbonyl, optionally substituted aralkoxycarbonyl, alkylsulfonyl, optionally substituted aryl, optionally substituted heterocyclyl, alkoxycarbonylalkyl, carboxyalkyl, optionally substituted arylsulfonyl, aminocarbonyl, monoalkylaminocarbonyl, dialkylaminocarbonyl, optionally substituted arylaminocarbonyl, aminosulfonyl, 15 monoalkylaminosulfonyl, dialkylaminosulfonyl, arylaminosulfonyl, arylsulfonylaminocarbonyl, optionally substituted *N*-heterocyclyl, -C(=NH)-N(CN)R¹, -C(O)-R²³-N(R¹)R², -C(O)-R²³-N(R¹)C(O)-R²³-N(R¹)R², and -C(O)-N(R¹)-R²³-C(O)OR¹;

20 each R⁸ and R⁹ are independently chosen from the group consisting of haloalkyl, cycloalkyl (optionally substituted with halo, cyano, alkyl or alkoxy), carbocyclyl (optionally substituted with one or more substituents selected from the group consisting of halo, alkyl and alkoxy), and heterocyclyl (optionally substituted with alkyl, aralkyl or alkoxy);

25 each R¹⁰ is independently chosen from the group consisting of halo, alkoxy, optionally substituted aryloxy, optionally substituted aralkoxy, optionally substituted -S(O)_t-R²², acylamino, amino, monoalkylamino, dialkylamino, (triphenylmethyl)amino, hydroxy, mercapto, and alkylsulfonamido;

each R¹¹ is independently chosen from the group consisting of cyano, di(alkoxy)alkyl, carboxy, alkoxycarbonyl, aminocarbonyl, monoalkylaminocarbonyl and dialkylaminocarbonyl;

each R¹², R¹³, R¹⁴, R¹⁵, R¹⁷ and R²⁰ is independently hydrogen or alkyl;

- 33 -

each R^{16} is independently hydrogen, alkyl, optionally substituted aryl, optionally substituted aralkyl or cycloalkyl;

R^{18} is hydrogen, NO_2 , or toluenesulfonyl;

each R^{21} is independently hydrogen, alkyl, cycloalkyl, optionally substituted aryl, optionally substituted aralkyl, $-\text{C}(\text{O})\text{R}^{22}$ or $-\text{SO}_2\text{R}^{22}$;

or R^{21} taken together with R^1 and the nitrogen to which they are attached is an optionally substituted *N*-heterocyclyl;

or R^{21} taken together with R^{16} and the nitrogen to which they are attached is an optionally substituted *N*-heterocyclyl;

each R^{22} is independently alkyl, cycloalkyl, optionally substituted aryl or optionally substituted aralkyl; and

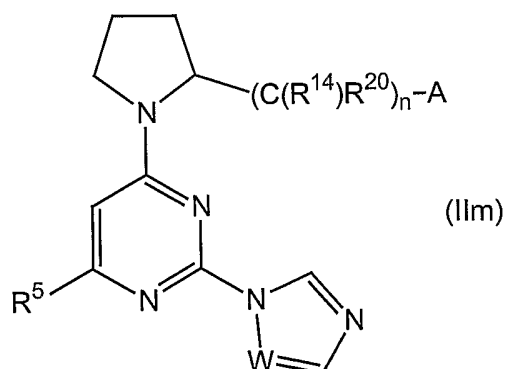
R^{23} is an amino acid residue;

as single stereoisomers or mixtures thereof, or pharmaceutically acceptable salts thereof.

Of this group of compounds, a preferred subgroup of compounds is that subgroup of compounds of formula (Ic) wherein A is R^1 , $-\text{N}(\text{R}^1)\text{C}(\text{O})\text{R}^2$ or $-\text{N}(\text{R}^1)\text{R}^2$.

Of this subgroup of compounds of formula (Ic), a preferred compound is 2-(1*H*-imidazol-1-yl)-6-methyl-4-[[3-[(1,3-benzodioxol-5-yl)methyl]aminopropyl]-(methyl)amino]pyrimidine.

Of the compounds disclosed in the above mentioned patent applications, another preferred group of compounds is that group of compounds which are selective iNOS inhibitors and which have the following formula (IIIm):



wherein:

A is $-OR^1$, $-C(O)N(R^1)R^2$, $-N(R^{16})C(O)N(R^1)R^{16}$, $-N(R^1)C(O)R^2$ or $-N(R^1)R^2$;

W is CH;

n is zero or an integer from 1 to 3;

5 t is zero, one or two;

each R^1 and R^2 are independently chosen from the group consisting of hydrogen, optionally substituted C_1 - C_{20} alkyl, optionally substituted cycloalkyl, $-[C_0$ - C_8 alkyl]- R^9 , $-[C_2$ - C_8 alkenyl]- R^9 , $-[C_2$ - C_8 alkynyl]- R^9 , $-[C_2$ - C_8 alkyl]- R^{10} (optionally substituted by hydroxy), $-[C_1$ - $C_8]$ - R^{11} (optionally substituted by hydroxy), optionally substituted heterocyclyl;

10 or R^1 and R^2 together with the nitrogen atom to which they are attached is an optionally substituted *N*-heterocyclyl;

R^5 is chosen from the group consisting of hydrogen, halo, alkyl, haloalkyl, optionally substituted aralkyl, optionally substituted aryl, $-OR^{16}$, $-S(O)_tR^{16}$, $-N(R^{16})R^{21}$, $-N(R^{16})C(O)N(R^1)R^{16}$, $-N(R^{16})C(O)OR^{16}$, $-N(R^{16})C(O)R^{16}$, $-[C_0$ - C_8 alkyl]- $C(O)OR^{16}$, $-[C_0$ - C_8 alkyl]- $C(H)[C(O)OR^{16}]_2$, and $-[C_0$ - C_8 alkyl]- $C(O)N(R^1)R^{16}$;

15 each R^9 is independently chosen from the group consisting of haloalkyl, cycloalkyl (optionally substituted with halo, cyano, alkyl or alkoxy), carbocyclyl (optionally substituted with one or more substituents selected from the group consisting of halo, alkyl and alkoxy), and heterocyclyl (optionally substituted with alkyl, aralkyl or alkoxy);

20 each R^{10} is independently chosen from the group consisting of halo, alkoxy, optionally substituted aryloxy, optionally substituted aralkoxy, optionally substituted $-S(O)_tR^{22}$, acylamino, amino, monoalkylamino, dialkylamino, (triphenylmethyl)amino, hydroxy, mercapto, alkylsulfonamido;

25 each R^{11} is independently chosen from the group consisting of cyano, di(alkoxy)alkyl, carboxy, alkoxycarbonyl, aminocarbonyl, monoalkylaminocarbonyl and dialkylaminocarbonyl;

each R^{14} and R^{20} are independently hydrogen or alkyl;

each R^{16} is independently hydrogen, alkyl, optionally substituted aryl, optionally substituted aralkyl or cycloalkyl;

30 each R^{21} is independently hydrogen, alkyl, cycloalkyl, optionally substituted aryl, optionally substituted aralkyl, $-C(O)R^{22}$ or $-SO_2R^{22}$;

or R²¹ taken together with R¹ and the nitrogen to which they are attached is an optionally substituted *N*-heterocyclyl;

or R²¹ taken together with R¹⁶ and the nitrogen to which they are attached is an optionally substituted *N*-heterocyclyl; and

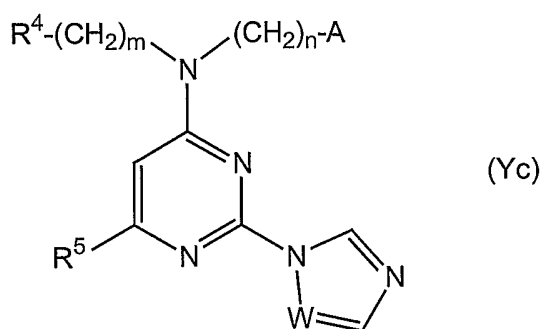
5 each R²² is independently alkyl, cycloalkyl, optionally substituted aryl or optionally substituted aralkyl; as single stereoisomers or mixtures thereof, or pharmaceutically acceptable salts thereof.

10 Of this group of compounds, a preferred subgroup of compounds is that subgroup of compounds of formula (IIIm) where A is -C(O)N(R¹); R¹ is hydrogen; R² is lower alkyl or -[C₁-C₈ alkyl]-R⁹; R⁵ is hydrogen, halo, alkyl or alkoxy; and n is zero or one.

15 Of this subgroup of compounds, a preferred class of compounds is that class of compounds of formula (IIIm) where the -(C(R¹⁴)R²⁰)_n-A substituent is in the *R* configuration with respect to the pyrrolidine carbon to which it is attached.

Of this class of compounds, a preferred compound is
N-[(1,3-benzodioxol-5-yl)ethyl]-1-[2-(1*H*-imidazol-1-yl)-6-methylpyrimidin-4-yl]
 pyrrolidine-2-carboxamide.

20 Of the compounds disclosed in the above mentioned patent applications, another preferred group of compounds is that group of compounds which are selective iNOS inhibitors and which have the following formula (Yc):



25 wherein:

n and m are independently an integer from 1 to 4;

t is zero, one or two;

A is $-\text{C}(\text{O})\text{OR}^1$ or $-\text{C}(\text{O})\text{N}(\text{R}^1)\text{R}^2$;

each W is N or CH;

each R^1 is independently hydrogen, alkyl, aryl or aralkyl;

5 each R^2 is independently hydrogen, $\text{C}_1\text{-C}_{20}$ alkyl, $-(\text{CH}_2)_n\text{-N}(\text{R}^1)_2$, heterocyclalkyl (optionally substituted by alkyl, halo, haloalkyl or alkoxy), aralkyl (optionally substituted by halo, alkyl, alkoxy, or $-\text{N}(\text{R}^1)_2$);

when m is an integer from 2 to 4, R^4 can be hydroxy, $-\text{N}(\text{R}^1)\text{R}^2$, $-\text{N}(\text{R}^1)\text{-C}(\text{O})\text{-R}^1$, $-\text{N}(\text{R}^1)\text{-C}(\text{O})\text{OR}^1$, $-\text{N}(\text{R}^1)\text{-S}(\text{O})_t\text{-R}^1$, or $-\text{N}(\text{R}^1)\text{-C}(\text{O})\text{-N}(\text{R}^1)_2$;

10 when m is an integer from 1 to 4, R^4 can also be cyano or heterocycl;

R^5 is hydrogen, halo, alkyl, aryl, aralkyl, or haloalkyl;

as a single stereoisomer or mixture thereof, or a pharmaceutically acceptable salt thereof.

15 Of this group of compounds, a preferred subgroup of compounds is that subgroup of compounds of formula (Yc) wherein n is 1; m is 2 or 3; A is $-\text{C}(\text{O})\text{OR}^1$ or $-\text{C}(\text{O})\text{N}(\text{R}^1)\text{R}^2$; W is CH; R^1 is hydrogen or alkyl; and R^2 is hydrogen, alkyl, $-(\text{CH})_n\text{-N}(\text{R}^1)_2$, optionally substituted heterocyclalkyl or optionally substituted aralkyl.

20 Of this subgroup of compounds, a preferred class of compounds is that class of compounds of formula (Yc) wherein R^4 is $-\text{N}(\text{R}^1)\text{R}^2$ where R^1 is hydrogen or alkyl and R^2 is heterocyclalkyl selected from the group consisting of (1,3-benzodioxol-5-yl)methyl or (1,4-benzodioxan-6-yl)methyl.

25 Of this class of compounds, a preferred compound is 2-[[3-[[[(1,3-benzodioxol-5-yl)methyl](methyl)amino]propyl][2-(1H-imidazol-1-yl)-6-methylpyrimidin-4-yl]amino]acetamide.

The following specific example is provided as a guide to assist in the practice of the invention, and are not intended as a limitation on the scope of the invention.

30

EXAMPLE

Surgical Preparation

Twenty Four female sheep can be surgically prepared for chronic study under halothane anesthesia. The right femoral artery and vein are cannulated with Silastic catheters (Intracath[®], 16GA, 24IN, Becton Dickinson Vascular Access, Sandy, UT). A thermodilution catheter (Swan Ganz[®], model 131F7, Baxter, Edwards Critical-Care Division, Irvine, CA) is introduced through the right external jugular vein into the pulmonary artery. Through the fifth intercostal space, a catheter (Durastic Silicone Tubing[®] DT08, 0.062 in. ID, 0.125 in. OD; Allied Biomedical, Paso Robles, CA) is positioned in the left atrium. The animals are given 5–7 days to recover from the surgical procedure with free access to food and water.

Burn and Smoke Inhalation Injury

Before the injury, a tracheostomy can be performed under ketamine anesthesia (Ketaset[®], Fort Dodge Animal Health, Fort Dodge, IA), and a cuffed tracheostomy tube (10 mm diameter, Shiley, Irvine, CA) is inserted, and then anesthesia is continued with halothane. All animals then receive a combined injury with a 40% TBSA third-degree burn and 48 breaths of cotton smoke inhalation. After shaving the wool, a 20% TBSA third-degree flame burn is made on one side of the flank using a Bunsen burner. Thereafter, inhalation injury is induced with a modified bee smoker. The bee smoker is filled with 40 g of burning cotton toweling and attached to the tracheostomy tube *via* a modified endotracheal tube containing an indwelling thermistor from the Swan Ganz[®] catheter. Four sets of 12 breaths of smoke (total 48) are delivered and the carboxyhemoglobin level is determined immediately after smoke inhalation. The temperature of the smoke was not allowed to exceed 40°C during the smoking procedure. After smoke insufflation, another 20% TBSA third-degree burn was made on the remainder of the flank.

Measured Variables

Vascular pressures, mean arterial pressure (MAP, mm Hg), pulmonary arterial pressure (PAP, mm Hg), left arterial pressure (LAP, mm Hg), and central venous pressure (CVP, mm Hg) were measured using pressure transducers (Model PX-1800, Baxter, Edwards Critical-Care Division, Irvine, CA) that are adapted with a continuous flushing device. The transducers are connected to a hemodynamic monitor (Model 78304A, Hewlett Packard, Santa Clara, CA). All hemodynamic measurements are made in the standing position on animals that are awake. Zero calibrations are taken at the level of the olecranon joints on the front leg of the animals. Cardiac output is measured by the thermodilution technique using a cardiac output computer

(COM-1™, Baxter, Edward Critical-Care Division, Irvine, CA). A 5% dextrose solution is used as the indicator. For evaluation of cardiac function, cardiac index (CI , $\text{l min}^{-1} \text{m}^{-2}$), stroke volume index (SVI , $\text{ml beat}^{-1} \text{m}^{-2}$), left ventricular stroke work index (LVSWI , g m m^{-2}), and systemic vascular resistance index (SVRI , $\text{dyn s cm}^{-5} \text{m}^{-2}$) can be calculated using standard equations. Blood gases and acid base balance are measured using a blood gas analyzer (Model IL 1600, Instrumentation Laboratory, Lexington, MA). The blood gas results are corrected for the body temperature of the sheep. Oxyhemoglobin saturation and carboxyhemoglobin concentration are analyzed with a co-oximeter (Model IL 482, Instrumentation Laboratory, Lexington, MA). Hematocrit (Ht) was measured in heparinized micro-hematocrit capillary tubes (Fisherbrand®, Pittsburgh, PA).

Experimental Protocols

Twenty-four hours prior to the experiment, vascular catheters are connected to the monitoring devices and maintenance fluid administration (Ringer's lactate, 2 ml kg^{-1}) via the femoral vein was started. After baseline measurements and sample collections are completed, all animals receive burn and smoke inhalation combined injury, as described above. A silicone Foley catheter (Dover®, 14Fr., 5 ml, Sherwood Medical, St. Louis, MO) was placed in the urinary bladder to determine urine output. Immediately after injury, anesthesia is discontinued and the animals are allowed to awaken but are maintained on mechanical ventilation (Servo Ventilator® 900C, Seimens–Elema, Sweden) throughout the 48 hr experimental period. Ventilation is performed with a positive end-expiratory pressure ("PEEP") of $5 \text{ cm H}_2\text{O}$ and a tidal volume of 15 ml kg^{-1} . During the first 3 hr following injury, the inspiratory O_2 concentration is maintained at 100% and the respiratory rate is kept at 30 per minute to induce rapid clearance of carboxyhemoglobin after smoke inhalation. Then ventilation is adjusted according to blood gas analysis to maintain the arterial O_2 saturation above 90% and the PCO_2 between 25 and 30 mm Hg. Fluid resuscitation during the experiment is performed with Ringer's lactate solution following the Parkland formula ($4 \text{ ml \% burned surface area/kg body weight}$ for the first 24 hr and $2 \text{ ml \% burned surface area/kg body weight}$ for the next 24 hr). One-half of the volume for the first day is infused in the initial 8 hr, and the remainder is infused in the next 16 hr. Fluid balance is determined by urine output every 3 hr subtracted from total fluid volume infused. Net fluid balance accumulation is calculated and represented as $\text{ml kg}^{-1} \text{hr}^{-1}$. During this experiment, the animals are allowed free access to food, but not to water, so as to accurately measure fluid intake.

In the treatment methods wherein the pulmonary vasodilators are to be inhaled, such as NO gas, the animals are connected to a different ventilator, which is equipped with a

chemoluminescence NO sensor (Microgas, Micro Medical Ltd., Rochester, United Kingdom). Hemodynamic readings are made and blood gases are taken from the awake sheep. Thereafter, NO gas or other pulmonary vasodilators in nebulized form can be added to the inspired air in ascending concentrations of 10, 20, 50, and 100 ppm. Each concentration is
5 given for 10 minutes, and then hemodynamic reading are taken, and samples obtained for blood gas analysis.

The animals are randomized into four groups: a combined treatment group, an iNOS inhibitor treatment group, a vasodilator treatment group, and a control group. In the combined
10 treatment group, the animals are given 100 µg/kg/hr of a selective iNOS inhibitor 1 hr after injury and then every 8 hr for 41 hr, n= 6, either contemporaneously or sequentially, in combination with a selective pulmonary vasodilator. In the control group, 0.9% NaCl is given in the same manner as is the iNOS inhibitor. The infusion rate was 150 ml hr⁻¹. The lymph and blood samples for determination of total protein concentration, and colloid osmotic pressure are
15 collected at 3, 6, 12, 18, 24, 36, and 48 hr following injury in all four groups. Hemodynamic variables and blood gases were obtained at 3, 6, 12, 18, 24, 30, 36, 42, and 48 hr post-injury in all groups.

Selective iNOS inhibitors in combination with pulmonary vasodilators when tested in this
20 animal model for ARDS demonstrated the ability to effectively treat ARDS in a mammal.

* * * * *

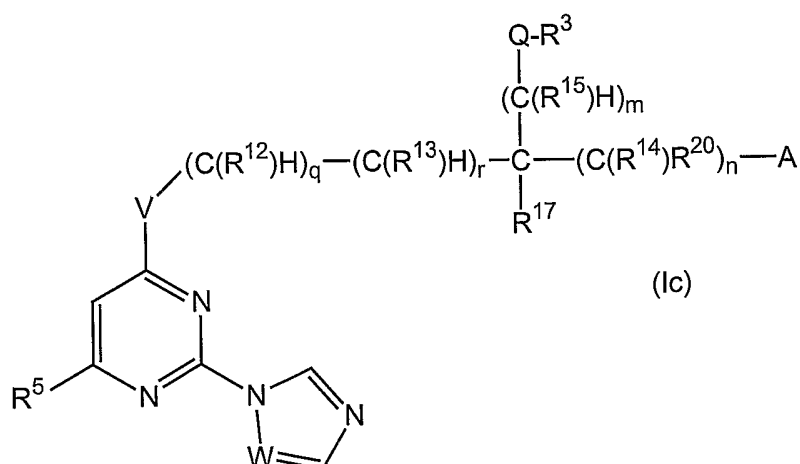
All of the U.S. patents, U.S. patent application publications, U.S. patent applications,
25 foreign patents, foreign patent applications, and non-patent publications referred to in this specification, are incorporated herein by reference, in their entirety.

While the present invention has been described with reference to the specific
embodiments thereof, it should be understood by those skilled in the art that various changes
30 may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

What is claimed is:

1. A method of treating acute respiratory distress syndrome in a mammal, wherein the method comprises administering to the mammal in need thereof a therapeutically effective amount of a selective or partially selective iNOS inhibitor in combination with a therapeutically effective amount of a pulmonary vasodilator.
2. The method of Claim 1 wherein the selective iNOS inhibitor and the pulmonary vasodilator are administered concurrently.
3. The method of Claim 1 wherein the selective iNOS inhibitor and the pulmonary vasodilator are administered sequentially.
4. The method of Claim 2 or 3 wherein the selective iNOS inhibitor and the pulmonary vasodilator are both administered topically (locally) to the lung.
5. The method of Claim 2 or 3 wherein the selective iNOS inhibitor is administered topically (locally) to the lung and the pulmonary vasodilator is administered systemically.
6. The method of Claim 2 or 3 wherein the selective iNOS inhibitor is administered systemically and the pulmonary vasodilator is administered topically (locally) to the lung.
7. The method of Claim 2 or 3 wherein the selective iNOS inhibitor and the pulmonary vasodilator are both administered systemically.
8. The method of Claims 4-6 wherein the route of administration to the lung is by inhalation.
9. The method of Claims 1-8 wherein the selective iNOS inhibitor is a compound of formula (Ic):

- 41 -



wherein:

A is R^1 , $-OR^1$, $-C(O)N(R^1)R^2$, $-N(R^{16})C(O)N(R^1)R^{16}$, $-N(R^1)C(O)R^2$ or $-N(R^1)R^{21}$;

V is $N(R^4)$;

W is CH; and

Q is chosen from the group consisting of a direct bond, $-C(O)-$, $-O-$, $-C(=N-R^1)-$, $-S(O)_t$, and $-N(R^6)-$;

m is zero or an integer from 1 to 4;

n is zero or an integer from 1 to 3;

q is zero or one;

r is zero or one, provided that when Q is a heteroatom, m, q, and r cannot all be zero; when A is $-OR^1$, $-N(R^1)C(O)R^2$, $-N(R^1)R^{21}$, $-N(R^{16})C(O)N(R^1)R^{16}$, n, q, and r cannot all be zero; and when Q is a heteroatom and A is $-OR^1$, $-N(R^1)C(O)R^2$, $-N(R^1)R^{21}$, $-N(R^{16})C(O)N(R^1)R^{16}$, m and n cannot both be zero;

t is zero, one or two;

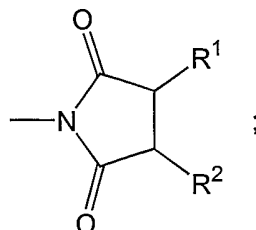
each R^1 and R^2 are independently chosen from the group consisting of hydrogen, optionally substituted C_1 - C_{20} alkyl, optionally substituted cycloalkyl, $-[C_0$ - C_8 alkyl]- R^9 , $-[C_2$ - C_8 alkenyl]- R^9 , $-[C_2$ - C_8 alkynyl]- R^9 , $-[C_2$ - C_8 alkyl]- R^{10} (optionally substituted by hydroxy), $-[C_1$ - $C_8]$ - R^{11} (optionally substituted by hydroxy), and optionally substituted heterocyclyl;

or R^1 and R^2 together with the nitrogen atom to which they are attached is an optionally substituted *N*-heterocyclyl;

R^3 is chosen from the group consisting of hydrogen, alkyl, cycloalkyl, optionally substituted aryl, haloalkyl, $-[C_1$ - C_8 alkyl]- $C(O)N(R^1)R^2$, $-[C_1$ - C_8 alkyl]- $N(R^1)R^2$, $-[C_1$ - C_8 alkyl]- R^8 , $-[C_2$ - C_8 alkyl]- R^{10} , $-[C_1$ - C_8 alkyl]- R^{11} , and heterocyclyl (optionally substituted by one or more substituents selected from the group consisting of halo, alkyl, alkoxy and imidazolyl);

or when Q is $-N(R^6)-$ or a direct bond to R^3 , R^3 may additionally be aminocarbonyl, alkoxycarbonyl, alkylsulfonyl, monoalkylaminocarbonyl, dialkylaminocarbonyl and $-C(=NR^{18})-NH_2$;

or $-Q-R^3$ taken together represents $-C(O)OH$, $-C(O)N(R^1)R^2$, $-C(=NH)-N(R^1)R^2$ or



R^4 is chosen from the group consisting of hydrogen, alkyl, aryl, aralkyl and cycloalkyl, provided that when A is $-R^1$ or $-OR^1$, R^4 cannot be hydrogen, and when V is CH, R^4 may additionally be hydroxy;

R^5 is chosen from the group consisting of hydrogen, halo, alkyl, haloalkyl, optionally substituted aralkyl, optionally substituted aryl, $-OR^{16}$, $-S(O)_t-R^{16}$, $-N(R^{16})R^{21}$, $-N(R^{16})C(O)N(R^1)R^{16}$, $-N(R^{16})C(O)OR^{16}$, $-N(R^{16})C(O)R^{16}$, $-[C_0-C_8 \text{ alkyl}]-C(O)OR^{16}$, $-[C_0-C_8 \text{ alkyl}]-C(H)[C(O)OR^{16}]_2$, and $-[C_0-C_8 \text{ alkyl}]-C(O)N(R^1)R^{16}$;

R^6 is chosen from the group consisting of hydrogen, alkyl, cycloalkyl, $-[C_1-C_8 \text{ alkyl}]-R^8$, $-[C_2-C_8 \text{ alkyl}]-R^{10}$, $-[C_1-C_8 \text{ alkyl}]-R^{11}$, acyl, $-C(O)R^8$, $-C(O)-[C_1-C_8 \text{ alkyl}]-R^8$, alkoxycarbonyl, optionally substituted aryloxycarbonyl, optionally substituted aralkoxycarbonyl, alkylsulfonyl, optionally substituted aryl, optionally substituted heterocyclyl, alkoxycarbonylalkyl, carboxyalkyl, optionally substituted arylsulfonyl, aminocarbonyl, monoalkylaminocarbonyl, dialkylaminocarbonyl, optionally substituted arylaminocarbonyl, aminosulfonyl, monoalkylaminosulfonyl, dialkylaminosulfonyl, arylaminosulfonyl, arylsulfonylaminocarbonyl, optionally substituted *N*-heterocyclyl, $-C(=NH)-N(CN)R^1$, $-C(O)-R^{23}-N(R^1)R^2$, $-C(O)-R^{23}-N(R^1)C(O)-R^{23}-N(R^1)R^2$, and $-C(O)-N(R^1)-R^{23}-C(O)OR^1$;

each R^8 and R^9 are independently chosen from the group consisting of haloalkyl, cycloalkyl (optionally substituted with halo, cyano, alkyl or alkoxy), carbocyclyl (optionally substituted with one or more substituents selected from the group consisting of halo, alkyl and alkoxy), and heterocyclyl (optionally substituted with alkyl, aralkyl or alkoxy);

each R^{10} is independently chosen from the group consisting of halo, alkoxy, optionally substituted aryloxy, optionally substituted aralkoxy, optionally substituted $-S(O)_t-R^{22}$, acylamino, amino, monoalkylamino, dialkylamino, (triphenylmethyl)amino, hydroxy, mercapto, and alkylsulfonamido;

each R^{11} is independently chosen from the group consisting of cyano, di(alkoxy)alkyl, carboxy, alkoxycarbonyl, aminocarbonyl, monoalkylaminocarbonyl and dialkylaminocarbonyl;

each R^{12} , R^{13} , R^{14} , R^{15} , R^{17} and R^{20} is independently hydrogen or alkyl;

each R^{16} is independently hydrogen, alkyl, optionally substituted aryl, optionally substituted aralkyl or cycloalkyl;

R^{18} is hydrogen, NO_2 , or toluenesulfonyl;

each R^{21} is independently hydrogen, alkyl, cycloalkyl, optionally substituted aryl, optionally substituted aralkyl, $-\text{C}(\text{O})\text{R}^{22}$ or $-\text{SO}_2\text{R}^{22}$;
or R^{21} taken together with R^1 and the nitrogen to which they are attached is an optionally substituted *N*-heterocyclyl;

or R^{21} taken together with R^{16} and the nitrogen to which they are attached is an optionally substituted *N*-heterocyclyl;

each R^{22} is independently alkyl, cycloalkyl, optionally substituted aryl or optionally substituted aralkyl; and

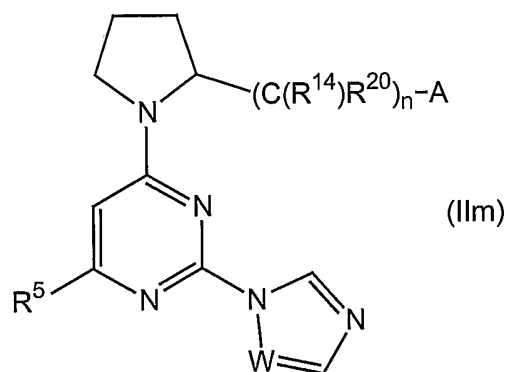
R^{23} is an amino acid residue;

as single stereoisomers or mixtures thereof, or pharmaceutically acceptable salts thereof.

10. The method of Claim 9 wherein the selective iNOS inhibitor is a compound of formula (Ic) wherein A is R^1 , $-\text{N}(\text{R}^1)\text{C}(\text{O})\text{R}^2$ or $-\text{N}(\text{R}^1)\text{R}^2$.

11. The method of Claim 10 wherein the selective iNOS inhibitor is 2-(1*H*-imidazol-1-yl)-6-methyl-4-[[3-[(1,3-benzodioxol-5-yl)methyl]aminopropyl]-(methyl)amino] pyrimidine.

12. The method of Claims 1-8 wherein the selective iNOS inhibitor is a compound of formula (IIIm):



wherein:

A is $-\text{OR}^1$, $-\text{C}(\text{O})\text{N}(\text{R}^1)\text{R}^2$, $-\text{N}(\text{R}^{16})\text{C}(\text{O})\text{N}(\text{R}^1)\text{R}^{16}$, $-\text{N}(\text{R}^1)\text{C}(\text{O})\text{R}^2$ or $-\text{N}(\text{R}^1)\text{R}^2$;

W is CH;

n is zero or an integer from 1 to 3;

t is zero, one or two;

each R^1 and R^2 are independently chosen from the group consisting of hydrogen, optionally substituted C_1 - C_{20} alkyl, optionally substituted cycloalkyl, $-[C_0-C_8 \text{ alkyl}]-R^9$, $-[C_2-C_8 \text{ alkenyl}]-R^9$, $-[C_2-C_8 \text{ alkynyl}]-R^9$, $-[C_2-C_8 \text{ alkyl}]-R^{10}$ (optionally substituted by hydroxy), $-[C_1-C_8]-R^{11}$ (optionally substituted by hydroxy), optionally substituted heterocyclyl;

or R^1 and R^2 together with the nitrogen atom to which they are attached is an optionally substituted *N*-heterocyclyl;

R^5 is chosen from the group consisting of hydrogen, halo, alkyl, haloalkyl, optionally substituted aralkyl, optionally substituted aryl, $-OR^{16}$, $-S(O)_t-R^{16}$, $-N(R^{16})R^{21}$, $-N(R^{16})C(O)N(R^1)R^{16}$, $-N(R^{16})C(O)OR^{16}$, $-N(R^{16})C(O)R^{16}$, $-[C_0-C_8 \text{ alkyl}]-C(O)OR^{16}$, $-[C_0-C_8 \text{ alkyl}]-C(H)[C(O)OR^{16}]_2$, and $-[C_0-C_8 \text{ alkyl}]-C(O)N(R^1)R^{16}$;

each R^9 is independently chosen from the group consisting of haloalkyl, cycloalkyl (optionally substituted with halo, cyano, alkyl or alkoxy), carbocyclyl (optionally substituted with one or more substituents selected from the group consisting of halo, alkyl and alkoxy), and heterocyclyl (optionally substituted with alkyl, aralkyl or alkoxy);

each R^{10} is independently chosen from the group consisting of halo, alkoxy, optionally substituted aryloxy, optionally substituted aralkoxy, optionally substituted $-S(O)_t-R^{22}$, acylamino, amino, monoalkylamino, dialkylamino, (triphenylmethyl)amino, hydroxy, mercapto, alkylsulfonamido;

each R^{11} is independently chosen from the group consisting of cyano, di(alkoxy)alkyl, carboxy, alkoxycarbonyl, aminocarbonyl, monoalkylaminocarbonyl and dialkylaminocarbonyl;

each R^{14} and R^{20} are independently hydrogen or alkyl;

each R^{16} is independently hydrogen, alkyl, optionally substituted aryl, optionally substituted aralkyl or cycloalkyl;

each R^{21} is independently hydrogen, alkyl, cycloalkyl, optionally substituted aryl, optionally substituted aralkyl, $-C(O)R^{22}$ or $-SO_2R^{22}$;

or R^{21} taken together with R^1 and the nitrogen to which they are attached is an optionally substituted *N*-heterocyclyl;

or R^{21} taken together with R^{16} and the nitrogen to which they are attached is an optionally substituted *N*-heterocyclyl; and

each R^{22} is independently alkyl, cycloalkyl, optionally substituted aryl or optionally substituted aralkyl;

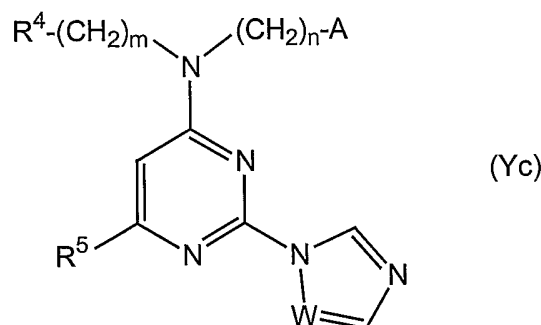
as single stereoisomers or mixtures thereof, or pharmaceutically acceptable salts thereof.

13. The method of Claim 12 wherein the selective iNOS inhibitor is a compound of formula (IIIm) wherein A is $-\text{C}(\text{O})\text{N}(\text{R}^1)$; R^1 is hydrogen; R^2 is lower alkyl or $-\text{[C1-C8 alkyl]}-\text{R}^9$; R^5 is hydrogen, halo, alkyl, or alkoxy; and n is zero or one.

14. The method of Claim 13 wherein the selective iNOS inhibitor is a compound of formula (IIIm) wherein the $-(\text{C}(\text{R}^{14})\text{R}^{20})_n\text{-A}$ substituent is in the R configuration with respect to the pyrrolidine carbon to which it is attached.

15. The method of Claim 14 wherein the selective iNOS inhibitor is $\text{N}-[(1,3\text{-benzodioxol-5-yl})\text{ethyl}]-1-[2-(1\text{H-imidazolyl-1-yl})-6\text{-methylpyrimidin-4-yl}]$ pyrrolidine-2-carboxamide.

16. The method of Claims 1-8 wherein the selective iNOS inhibitor is a compound of formula (Yc):



wherein:

n and m are independently an integer from 1 to 4;

t is zero, one or two;

A is $-\text{C}(\text{O})\text{OR}^1$ or $-\text{C}(\text{O})\text{N}(\text{R}^1)\text{R}^2$;

each W is N or CH;

each R^1 is independently hydrogen, alkyl, aryl or aralkyl;

each R^2 is independently hydrogen, $\text{C}_1\text{-C}_{20}$ alkyl, $-(\text{CH}_2)_n\text{-N}(\text{R}^1)_2$, heterocyclalkyl (optionally substituted by alkyl, halo, haloalkyl or alkoxy), aralkyl (optionally substituted by halo, alkyl, alkoxy, or $-\text{N}(\text{R}^1)_2$);

when m is an integer from 2 to 4, R^4 can be hydroxy, $-\text{N}(\text{R}^1)\text{R}^2$, $-\text{N}(\text{R}^1)\text{-C}(\text{O})\text{-R}^1$, $-\text{N}(\text{R}^1)\text{-C}(\text{O})\text{OR}^1$, $-\text{N}(\text{R}^1)\text{-S}(\text{O})_t\text{-R}^1$, or $-\text{N}(\text{R}^1)\text{-C}(\text{O})\text{-N}(\text{R}^1)_2$;

when m is an integer from 1 to 4, R^4 can also be cyano or heterocyclyl;

R^5 is hydrogen, halo, alkyl, aryl, aralkyl, or haloalkyl;

as a single stereoisomer or mixture thereto, or a pharmaceutically acceptable salt thereof.

17. The method of Claim 16 wherein the selective iNOS inhibitor is a compound of formula (Yc) wherein n is 1; m is 2 or 3; A is $-\text{C}(\text{O})\text{OR}^1$ or $-\text{C}(\text{O})\text{N}(\text{R}^1)\text{R}^2$; W is CH; R^1 is hydrogen or alkyl; and R^2 is hydrogen, alkyl, $-(\text{CH})_n-\text{N}(\text{R}^1)_2$, optionally substituted heterocyclalkyl or optionally substituted aralkyl.
18. The method of Claim 16 wherein the selective iNOS inhibitor is a compound of formula (Yc) wherein R^4 is $-\text{N}(\text{R}^1)\text{R}^2$ where R^1 is hydrogen or alkyl and R^2 is heterocyclalkyl selected from the group consisting of (1,3-benzodioxol-5-yl)methyl and (1,4-benzodioxan-6-yl)methyl.
19. The method of Claim 17 wherein the selective iNOS inhibitor is
2-[[3-[[[(1,3-benzodioxol-5-yl)methyl](methyl)amino]propyl][2-(1*H*-imidazol-1-yl)-6-methylpyrimidin-4-yl]amino]acetamide.
20. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of a selective or partially selective iNOS inhibitor in combination with a therapeutically effective amount of a pulmonary vasodilator.

INTERNATIONAL SEARCH REPORT

Internatic application No
PCT/US 03/26462

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K45/06 A61K31/506 A61P11/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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

EPO-Internal, PAJ, WPI Data, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 45294 A (ASTRA PHARMACEUTICALS) 15 October 1998 (1998-10-15) claims 1,10-15,18,19 page 16, line 1-6 ---	1-3,20
X	WO 00 00200 A (ASTRA PHARMACEUTICALS) 6 January 2000 (2000-01-06) claims 1,19 page 1, line 3-6 page 4, line 12-25 page 11, line 14-16 page 12, line 11-16 --- -/--	1-3,20

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

* & * document member of the same patent family

Date of the actual completion of the international search

21 November 2003

Date of mailing of the international search report

02/12/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Peeters, J

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 03/26462

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M.BARDOU E.A.: "Hypoxic vasoconstriction of rat main pulmonary artery: role of endogenous nitric oxide, potassium channels, and phosphodiesterase inhibition" JOURNAL OF CARDIOVASCULAR PHARMACOLOGY, vol. 38, no. 2, 2001, pages 325-334, XP009018830 page 325	1,20
P,X	WO 03 048108 A (CALYX THERAPEUTICS) 12 June 2003 (2003-06-12) claims 1,19,20,23,26,31 page 31, paragraph 3 page 34, paragraph 3 -page 36, paragraph 1	1-3,20
A	WO 98 37079 A (BERLEX LABORATORIES) 27 August 1998 (1998-08-27) cited in the application claims 1-64	9-15
A	WO 01 14371 A (BERLEX LABORATORIES) 1 March 2001 (2001-03-01) cited in the application claims 1-15	16-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 03/26462

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: —
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.: —
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

Continuation of Box I.2

Present claims 1-8,20 relate to a product/compound/method defined by reference to a desirable characteristic or property, namely:

- 1) "iNOS inhibitor
- 2) "pulmonary vasodilator"

The claims cover all products/compounds/methods having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products/compounds/methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product/compound/method by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely the compounds specified in claims 9-19 and on page 4 of the present application with due regard to the general idea underlying the present application

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US 03/26462

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9845294	A	15-10-1998	AU 7091098 A	30-10-1998
			BR 9807950 A	08-03-2000
			CN 1259131 T	05-07-2000
			EE 9900448 A	17-04-2000
			EP 0973772 A1	26-01-2000
			HU 0001865 A2	29-01-2001
			JP 2001519805 T	23-10-2001
			NO 994900 A	19-11-1999
			NZ 338006 A	27-04-2001
			PL 336141 A1	05-06-2000
			WO 9845294 A1	15-10-1998
			SK 128999 A3	16-05-2000
			TR 9902495 T2	21-07-2000
			US 6303613 B1	16-10-2001
WO 0000200	A	06-01-2000	AU 4944799 A	17-01-2000
			BR 9911666 A	20-03-2001
			CA 2334515 A1	06-01-2000
			CN 1313769 T	19-09-2001
			EE 200000766 A	17-06-2002
			EP 1100505 A1	23-05-2001
			JP 2002519324 T	02-07-2002
			NO 20006559 A	28-02-2001
			PL 345028 A1	19-11-2001
			WO 0000200 A1	06-01-2000
			SK 18482000 A3	10-07-2001
			TR 200003808 T2	21-06-2001
			US 6248745 B1	19-06-2001
			ZA 200007076 A	28-02-2002
WO 03048108	A	12-06-2003	WO 03048108 A2	12-06-2003
WO 9837079	A	27-08-1998	AU 732969 B2	03-05-2001
			AU 6174998 A	09-09-1998
			CN 1100777 B	05-02-2003
			EP 0968206 A1	05-01-2000
			GB 2338957 A ,B	12-01-2000
			HK 1025952 A1	12-04-2002
			HU 0204228 A2	28-03-2003
			JP 2002515058 T	21-05-2002
			NO 993996 A	18-10-1999
			NZ 337861 A	23-02-2001
			PL 335235 A1	10-04-2000
			SK 113599 A3	11-07-2000
			WO 9837079 A1	27-08-1998
			US 2003060452 A1	27-03-2003
			US 2003004137 A1	02-01-2003
			US 2002183323 A1	05-12-2002
			US 2003073669 A1	17-04-2003
			US 2003027794 A1	06-02-2003
			US 2003078265 A1	24-04-2003
			US 2002165203 A1	07-11-2002
			US 2003092678 A1	15-05-2003
			US 2003083332 A1	01-05-2003
			US 2003069210 A1	10-04-2003
			US 6432947 B1	13-08-2002
WO 0114371	A	01-03-2001	US 6432947 B1	13-08-2002

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internatic Application No
PCT/US 03/26462

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0114371 A		AU 7067100 A	19-03-2001
		BG 106440 A	29-11-2002
		BR 0014144 A	21-05-2002
		CA 2376355 A1	01-03-2001
		CN 1382136 T	27-11-2002
		CZ 20020642 A3	14-08-2002
		EE 200200091 A	15-04-2003
		EP 1206467 A1	22-05-2002
		HU 0202450 A2	28-11-2002
		JP 2003507476 T	25-02-2003
		LT 2002028 A , B	25-09-2002
		NO 20020925 A	16-04-2002
		NZ 517411 A	26-09-2003
		SI 20818 A	31-08-2002
		SK 2622002 A3	10-09-2002
		WO 0114371 A1	01-03-2001
		US 2003060452 A1	27-03-2003
		US 2003004137 A1	02-01-2003
		US 2002183323 A1	05-12-2002
		US 2003073669 A1	17-04-2003
		US 2003027794 A1	06-02-2003
		US 2003078265 A1	24-04-2003
		US 2002165203 A1	07-11-2002
		US 2003092678 A1	15-05-2003
		US 2003083332 A1	01-05-2003
		US 2003069210 A1	10-04-2003