

constituents listed above and various concentrations of N<sup>6</sup>-(hydrazinoiminomethyl)-L-lysine (HAA), N<sup>G</sup>-methyl-L-arginine (NMMA), or N<sup>G</sup>-amino-L-arginine (NAA). NMMA and NAA are previously described, well-characterized inhibitors of the inducible isozyme of nitric oxide synthase (Gross, S. S. et al. (1990) Biochem. Biophys. Res. Commun. 170, 96-103). Nitric oxide formation was monitored by following the increase in the concentration of nitrite in the culture medium. Nitric oxide spontaneously oxidizes to nitrite under the incubator conditions. The experimental results are shown in FIG. 2. As shown in FIG. 2, HAA is a substantially better inhibitor of the enzyme than is NAA or NMMA, the best competitive inhibitors previously identified. As indicated in FIG. 2, the doses required for approximately 50% inhibition (ED 50% approx.) were 0.125 millimolar for NMMA, 0.0625 millimolar for NAA and 0.0156 millimolar for HAA, indicating that HAA is approximately 4 times more potent than NAA and approximately 8 times more potent than NMMA.

#### EXAMPLE VI

In vitro studies with rat aortic rings were carried out to test the effect of N<sup>6</sup>-(hydrazinoiminomethyl)-L-lysine relative to other known inhibitors of the constitutive isoform of nitric oxide synthase. Aortic rings (2-3 mm) were isolated, suspended under a resting tension of 2.00 gm in 5 ml glass chambers containing Krebs-Henseleit solution gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub> at 37° C. as described previously for vascular rings from guinea pigs (O. A. Fasehun et al, (1990) J. Pharmacol. Exp. Ther. 255, 1348-1353). The aortic rings were then contracted by addition of 0.03 μM phenylephrine to the bathing solution, and the tension of the rings was measured. Acetylcholine, an agonist that stimulates the formation of nitric oxide by constitutive nitric oxide synthase in the vascular endothelium, was next added to a final concentration of 1 μM. The nitric oxide formed in response to acetylcholine causes the vascular smooth muscle of the aortic rings to relax, and the decrease in tension is measured. Inhibitors of nitric oxide synthase decrease the extent to which acetylcholine causes relaxation of the phenylephrine-contracted aortic rings. In the study, rings were contracted with phenylephrine, relaxed with acetylcholine, and then increasing doses of inhibitor were added to the bathing solution to overcome the effect of acetylcholine. The results are shown in FIG. 3. The dose of inhibitor is denoted on the X-axis. On the Y-axis, which is labeled "Inhibition of Relaxation (%)", 0% indicates the state where there is no

inhibition of the relaxation caused by acetylcholine and 100% indicates the state of maximum constriction in response to inhibitor where the relaxant effect of acetylcholine has been completely overcome. Potency of inhibitors is typically compared on the basis of the concentration needed to overcome 50% of the relaxation caused by acetylcholine (ED<sub>50</sub>). As indicated in FIG. 3, the ED<sub>50</sub>'s for N<sup>G</sup>-nitro-L-arginine (NNA), N<sup>G</sup>-amino-L-arginine (NAA), N<sup>G</sup>-methyl-L-arginine (NMMA) and N<sup>6</sup>-(hydrazinoiminomethyl)-L-lysine (HAA) were determined to be about 1.5 μM, 4.2 μM, 10.2 μM, and 10.3 μM, respectively. This indicates that N<sup>6</sup>-(hydrazinoiminomethyl)-L-lysine (HAA) is a much less effective inhibitor of the constitutive isoform of nitric oxide synthase than either NNA or NAA, and is comparable to NMMA. As shown in Example V, N<sup>6</sup>-(hydrazinoiminomethyl)-L-lysine is much more effective than NMMA and NAA as an inhibitor of the inducible isoform of nitric oxide synthase. Of the compounds named, NNA is the least effective inhibitor of the inducible isoform (S. S. Gross et al (1990) Biochem. Biophys. Res. Commun. 170, 96-103).

When an equimolar amount of D,L-lysine is substituted for the L-lysine in Example I, pure N<sup>6</sup>-(hydrazinoiminomethyl)-D,L-lysine monohydrochloride is obtained.

When in Examples IV and V, N<sup>6</sup>-(hydrazinoiminomethyl)-D,L-lysine is substituted for N<sup>6</sup>-(hydrazinoiminomethyl)-L-lysine in twice the dosage or concentration, substantially equal results of blood pressure increase and inhibition of nitrite formation by induced endothelial cells are obtained.

Many variations of inventive embodiments will be obvious to those skilled in the art. Thus, the invention is defined by the claims.

What is claimed is:

1. N<sup>6</sup>-(hydrazinoiminomethyl)lysine containing L-enantiomer, or a pharmaceutically acceptable acid addition salt thereof.
2. The compound of claim 1 which is monohydrochloride acid addition salt of N<sup>6</sup>-(hydrazinoiminomethyl)lysine.
3. The compound of claim 1 containing from 50% to 100% L-enantiomer with any remainder being D-enantiomer.
4. The compound of claim 3 which is 100% L-enantiomer.
5. The compound of claim 3 which is 50% L-enantiomer and 50% D-enantiomer.

\* \* \* \* \*

55

60

65