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| (54) Title: MERCAPTO DERIVATIVES AS INHIBITORS OF CYCLOOXYGENASES | | |
| (57) Abstract <p>This invention is directed to a pharmacologically acceptable composition for inhibiting cyclooxygenase in a mammal, which includes a mercapto derivative and a pharmaceutically acceptable carrier. The invention also concerns a method of inhibiting cyclooxygenase and treating various conditions where there is an advantage in inhibiting prostaglandins biosynthesis. The method includes the step of administering to a mammal a mercapto derivative in pure form or in a pharmaceutically acceptable carrier.</p> | | |

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**MERCAPTO DERIVATIVES AS
INHIBITORS OF CYCLOOXYGENASES****Related Application**

This application is a continuation in part of Application Serial No. 08/410,312 entitled "MERCAPTO DERIVATIVES AS INHIBITORS OF NITRIC OXIDE SYNTHASE" filed on March 24, 1995, which is incorporated herein in its entirety by reference.

Background of the Invention

The present invention relates to the use of mercapto derivatives as inhibitors of cyclooxygenases (COX).

5 Prostaglandins (PG's) are synthesized from arachidonic acid by a family of enzymes termed cyclooxygenases (COX). One isoform (COX-1) is constitutively present in a variety of tissues and releases PG's in low amounts. The continuous release of PG from COX-1 serves physiological purposes. For instance, prostacyclin, a vasodilatory and anti-aggregatory prostaglandin, reduces the adhesion
10 of platelets to the endothelial surface.

The inducible isoform of COX (COX-2) is expressed in response to immunological stimuli in multiple cell types including macrophages, vascular smooth muscle cells and epithelial cells, and produces large amounts of PG's, which can result in tissue injury.

5 There is substantial evidence that COX-2 plays an important role in the pathogenesis of a variety of inflammatory conditions. In animal models of endotoxic shock, endotoxin causes induction of COX-2. In addition, it is now thought that excess PG production may be involved in a number of other inflammatory
10 conditions, including arthritis and ulcerative colitis.

 Various inhibitors of COX have been proposed for therapeutic use. For example, non-steroidal anti-inflammatory drugs (acetylsalicylic acid, ibuprofen, etc.) are inhibitors of COX. Examples of United States patents directed to COX inhibition include 5,155,110;
15 5,360,925; 5,399,970; 5,409,944; 5,474,995; and 5,475,021. Although the COX inhibitors discussed above have therapeutic use, it is important to identify additional compounds which inhibit COX. It also is desirable to identify additional compounds which may have combined actions, i.e., inhibiting the activity of COX, as well as of
20 other pro-inflammatory enzymes.

Summary of the Invention

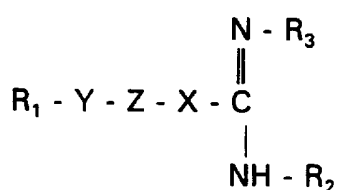
 This invention is directed to a pharmacologically acceptable composition for inhibiting COX in a mammal. The composition includes a mercapto derivative and a pharmaceutically

acceptable carrier, with the mercapto derivative present in the composition in an effective amount to inhibit COX in the mammal.

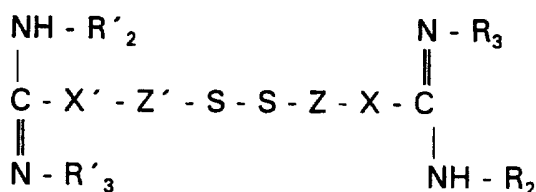
In this regard, the above-identified patent application relates to the use of the same mercapto compounds in the treatment of conditions associated with the overproduction of nitric oxide, a potent cytotoxic free radical. Thus, the mercapto compounds as COX inhibitors described in the present application have a distinct advantage over other classes of COX inhibitors, in that they also inhibit another class of inflammatory enzymes (nitric oxide synthases).

The invention also is directed to a method of inhibiting COX in a mammal, which includes the step of administering to the mammal a mercapto derivative in a pure form or in a pharmaceutically acceptable carrier.

The mercapto compound or derivative of the composition and method is defined by a formula selected from the group consisting of:



and



or a salt thereof, wherein

R_1 is H, alkyl, alkenyl, phenyl, alkylene, alkenylene, or phenylalkylene or a substituted derivative thereof;

When R_1 is alkylene or alkenylene, R_1 optionally may be joined to either of the amidino Ns, to Z or to X of the above formula
5 containing R_1 to form a 5-, 6- or 7- membered heterocyclic ring, with the proviso that, when R_1 is attached to Z, Z is alkylene or alkenylene or a substituted derivative thereof, and, when R_1 is attached to X, X is either CR_5 or N;

R_2 , R_3 , R'_2 and R'_3 are independently H, lower alkyl,
10 alkenyl, alkylene, alkenylene, amino, phenyl or phenylalkylene, or a substituted derivative thereof;

When R_2 or R'_2 is alkylene or alkenylene, R_2 or R'_2 optionally may be joined to the imino N residing on the adjacent amidino C to form a 5- or 6- membered heterocyclic ring;

15 Z and Z' are independently alkylene, alkenylene, cycloalkylene or cycloalkenylene, or a substituted derivative thereof;

When R_2 , R_3 , R'_2 or R'_3 is alkylene or alkenylene, R_2 , R_3 , R'_2 or R'_3 optionally may be joined to the adjacent Z or Z' to form a 5- or 6- membered heterocyclic ring including N, C and not more than
20 one atom of O or S, with the proviso that said Z or Z' so joined is an alkylene or alkenylene, said heterocyclic ring optionally being substituted with a lower alkyl, alkoxy, halo, hydroxy or amino;

X is N, NR_4 , O, CR_5 or CR_4R_5 ;

X' is N, NR'_4 , O, CR'_5 or $CR'_4R'_5$;

Y is S;

R_4 and R'_4 are independently H, alkyl, alkylene, alkenylene, thioalkylene or thioesteralkylene;

R_5 and R'_5 are independently H, alkyl, alkylene, alkenylene, thioalkylene, thioesteralkylene, amino or carboxyl; and

When R_4 or R'_4 is alkylene, alkenylene, thioalkylene, or thioesteralkylene, R_4 or R'_4 optionally may be joined to R_2 , R_3 , R'_2 or R'_3 to form a 5- or 6- membered heterocyclic ring including N, C and not more than one atom of O or S, with the proviso that said R_2 , R_3 , R'_2 or R'_3 so joined is alkylene, alkenylene, amino, phenyl, phenylalkylene, or a substituted derivative thereof wherein the substituted derivative is lower alkyl or halo.

Brief Description of the Drawings

Fig. 1 is a graph of the effect of mercaptoethylguanidine (MEG) on 6-keto prostaglandin F1 alpha production by (A) non-stimulated macrophages in the presence of arachidonic acid, and (B) immunostimulated J774 macrophages (stimulated with LPS and interferon-gamma for 6h).

Fig. 2 is a graph of the effect of mercaptoethylguanidine (MEG) on thromboxane B2 production by (A) non-stimulated macrophages in the presence of arachidonic acid, and (B) immunostimulated J774 macrophages (stimulated with LPS and interferon-gamma for 6h).

Fig. 3 is a graph of the effect of mercaptoethylguanidine (MEG), on 6-keto prostaglandin F1 alpha production by (A) purified COX-1, and (B) purified COX-2 in a cell-free system (N=3-6).

Detailed Description of the Invention

5 This invention is directed to a pharmacologically acceptable composition for inhibiting COX in a mammal. The composition includes a mercapto derivative and a pharmaceutically acceptable carrier, with the mercapto derivative present in the composition in an effective amount to inhibit COX in the mammal. The
10 invention also is directed to a method of inhibiting COX in a mammal, which includes the step of administering to the mammal a mercapto derivative in pure form or in a pharmaceutically acceptable carrier.

 Suitable mercapto derivatives for use in the composition or method may be made according to the methods of synthesis taught
15 in the following articles which are incorporated herein in their entirety by reference:

 (1) Joseph X. Khym et al., "Ion Exchange Studies of Transguanylation Reactions. I. Rearrangement of S,2-Aminoethylisothiurea to 2-Mercaptoethylguanidine and 2-Aminothiazoline", *Journal of the American Chemical Society*, Vol. 79,
20 pp. 5663-5666, November 5, 1957;

 (2) David G. Doherty, et al., "Synthesis of Aminoalkylisothiuronium Salts and their Conversion to Mercaptoalkylguanidines and Thiazolines", *Journal of the American Chemical Society*, Vol. 79, pp. 5667-5671, November 5, 1957;
25

 (3) Joseph X. Khym, et al., "Ion Exchange Studies of Transguanylation Reactions. II. Rearrangement of 3-Aminopropylisothiurea and N-Substituted Aminoethyl- and Aminopropylisothiureas to Mercaptoalkylguanidines and 2-

Aminothiazolines or Penthiazolines", *Journal of the American Chemical Society*, Vol. 80, pp. 3342-3349, July 5, 1958;

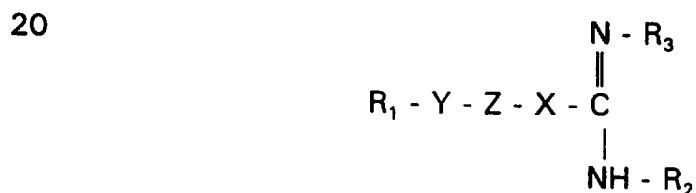
(4) David G. Doherty et al. "Synthesis of D- and L-2-Aminobutylisothiourethane Dihydrobromide Isomers and their Conversion to Guanidothiols, Disulfides, and Thiazolines", *Journal of Organic Chemistry*, Vol. 28, pp. 1339-1342, 1963.

(5) Shih-Hsi Chu et al., "Potential Antiradiation Agents. II. Selenium Analogs of 2-Aminoethylisothiuronium Hydrobromide and Related Compounds", *Journal of the American Chemical Society*, Vol. 27, pp. 2899-2901, August, 1962.

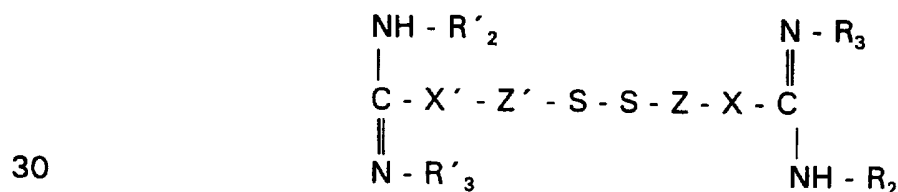
(6) Tohru Hino et al., "Radiation-protective Agents. I. Studies on N-Alkylated-2-(2-aminoethyl)thiopseudoureas and 1,1-(Dithioethylene)diguandines", *Chemical & Pharmaceutical Bulletin*, Vol. 14, No. 11, pp. 1193-1201, November, 1966.

Suitable mercapto derivatives also may be made according to the examples provided at the end of this detailed description of the invention.

The mercapto derivative of the composition and method is defined by a formula selected from the group consisting of:



and



or a salt thereof, wherein

R_1 is H, alkyl, alkenyl, phenyl, alkylene, alkenylene, or phenylalkylene or a substituted derivative thereof;

When R_1 is alkylene or alkenylene, R_1 optionally may be joined to either of the amidino Ns, to Z or to X of the above formula containing R_1 to form a 5-, 6- or 7- membered heterocyclic ring, with the proviso that, when R_1 is attached to Z, Z is alkylene or alkenylene or a substituted derivative thereof, and, when R_1 is attached to X, X is either CR_5 or N;

R_2 , R_3 , R'_2 and R'_3 are independently H, lower alkyl, alkenyl, alkylene, alkenylene, amino, phenyl or phenylalkylene, or a substituted derivative thereof;

When R_2 or R'_2 is alkylene or alkenylene, R_2 or R'_2 optionally may be joined to the imino N residing on the adjacent amidino C to form a 5- or 6- membered heterocyclic ring;

Z and Z' are independently alkylene, alkenylene, cycloalkylene or cycloalkenylene, or a substituted derivative thereof;

When R_2 , R_3 , R'_2 or R'_3 is alkylene or alkenylene, R_2 , R_3 , R'_2 or R'_3 optionally may be joined to the adjacent Z or Z' to form a 5- or 6- membered heterocyclic ring including N, C and not more than one atom of O or S, with the proviso that Z is an alkylene or alkenylene, said heterocyclic ring optionally being substituted with a lower alkyl, alkoxy, halo, hydroxy or amino;

X is N, NR_4 , O, CR_5 or CR_4R_5 ;

X' is N, NR'_4 , O, CR'_5 or $CR'_4R'_5$;

Y is S;

R_4 and R'_4 are independently H, alkyl, alkylene, alkenylene, thioalkylene or thioesteralkylene;

R_5 and R'_5 are independently H, alkyl, alkylene, alkenylene, thioalkylene, thioesteralkylene, amino or carboxyl; and

5 When R_4 or R'_4 is alkylene, alkenylene, thioalkylene, or thioesteralkylene, R_4 or R'_4 optionally may be joined to R_2 , R_3 , R'_2 or R'_3 to form a 5- or 6- membered heterocyclic ring including N, C and not more than one atom of O or S, with the proviso that R_2 , R_3 , R'_2 or R'_3 is alkylene, alkenylene, amino, phenyl, phenylalkylene, or a
10 substituted derivative thereof wherein the substituted derivative is lower alkyl or halo.

 As used herein, the term "salt" refers to any addition salt derived from any pharmaceutically acceptable organic or inorganic acid. Examples of suitable acids include hydrochloric, hydrobromic, sulfuric,
15 nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene p sulfonic, tartaric, acetic, citric, methanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic and benzenesulphonic acids. Additionally, as used herein, any alkyl or alkylene may be straight chain, branched or cyclic, and "halo" includes bromine,
20 chlorine, fluorine and iodine.

 As mentioned above, R_1 is H, alkyl, alkenyl, phenyl, alkylene, alkenylene or phenylalkylene, or a substituted derivative thereof. If desired, this R_1 derivative may be substituted with one or more alkoxy, halo, hydroxy, amino or nitro groups. Additionally, as

noted above, R_2 , R_3 , R'_2 and R'_3 are independently H, lower alkyl, alkenyl, alkylene, alkenylene, amino, phenyl or phenylalkylene, or a substituted derivative thereof. If desired, the R_2 , R_3 , R'_2 and R'_3 derivative may be substituted with a lower alkyl or halo.

5 If the R_4 , R_5 , R'_4 or R'_5 substituent is thioalkylene, the thioalkylene preferably has a formula $[-(CH_2)_n-SH]$ where n is independently 1 to 4. If R_4 , R_5 , R'_4 or R'_5 is thioesteralkylene, the thioesteralkylene preferably has the formula $[-(CH_2)_n-S-R_6]$ where R_6 is independently a lower alkyl and n is independently 1 to 4.

10 The Z and Z' substituents of the mercapto derivative are independently alkylene, alkenylene, cycloalkylene or cycloalkenylene, or a substituted derivative thereof. When such a substituted derivative is employed, the substituent may include an alkoxy, halo, hydroxy, amino or nitro group.

15 A preferred subgroup of the mercapto derivative includes mercapto derivatives where: R_1 is H or lower alkyl; R_2 is H; R_3 is H; R'_2 is H; R'_3 is H; X is NR_4 ; X' is NR'_4 ; R_4 and R'_4 are independently H, methyl or ethyl; and Z and Z' are independently alkylene. A few nonlimiting examples include mercaptoethylguanidine, mercaptopropylguanidine, S-methyl-mercaptoethylguanidine, S-methyl-mercaptopropylguanidine, and guanidinoethyldisulfide. Another preferred subgroup of mercapto derivatives is formed wherein: R_1 is H; R_2 is H; R_3 is H; R'_2 is H; R'_3 is H; X is NR_4 ; X' is NR'_4 ; R_4 is H; R'_4 is H; and Z and Z' are independently a C_{1-6} alkylene. Nonlimiting

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examples include mercaptoethylguanidine, mercaptopropylguanidine, and guanidinoethyldisulfide.

5 The mercapto derivative, in pure form or in a pharmaceutically acceptable carrier, will find benefit in treating conditions and disorders where there is an advantage in inhibiting the cyclooxygenase enzymes. For example, the mercapto derivative may be used to treat a circulatory shock including its various aspects such as vascular and myocardial dysfunction, metabolic failure including the inhibition of mitochondrial enzymes and cytochrome P450-mediated
10 drug metabolism, and multiple organ dysfunction syndrome including adult respiratory distress syndrome. Circulatory shock may be a result of gram-negative and gram positive sepsis, trauma, hemorrhage, burn injury, anaphylaxis, cytokine immunotherapy, liver failure, kidney failure or systemic inflammatory response syndrome. Mercapto derivatives
15 also may be beneficial for patients receiving therapy with cytokines such as TNF, IL-1 and IL-2 or therapy with cytokine-inducing agents, or as an adjuvant to short term immunosuppression in transplant therapy. In addition, mercapto derivatives may be useful to inhibit PG synthesis in patients suffering from inflammatory conditions in which
20 COX-2 activity contributes to the pathophysiology of the condition, such as arthritis, inflammatory bowel disease, and myocarditis, for example.

 There is also evidence that COX-2 may be involved in the pathophysiology of autoimmune and/or inflammatory conditions such

as arthritis, rheumatoid arthritis and systemic lupus erythematosus (SLE) and in insulin-dependent diabetes mellitus, and, therefore, mercapto derivatives may prove helpful in treating these conditions.

Furthermore, it is now clear that there are a number of
5 additional inflammatory and noninflammatory diseases that are associated with COX-2 induction. Examples of such physiological disorders include: inflammatory bowel diseases such as ileitis and Crohn's disease; inflammatory lung disorders such as asthma and chronic obstructive airway disease; inflammatory disorders of the eye
10 including corneal dystrophy, trachoma, onchocerciasis, uveitis, sympathetic ophthalmitis and endophthalmitis; chronic inflammatory disorders of the gum including periodontitis; chronic inflammatory disorders of the joints including arthritis and osteoarthritis, tuberculosis, leprosy, glomerulonephritis sarcoid, and nephrosis; disorders of the skin
15 including sclerodermatitis, psoriasis and eczema; inflammatory diseases of the central nervous system, including chronic demyelinating diseases such as multiple sclerosis, dementia including AIDS-related neurodegeneration and Alzheimer's disease, encephalomyelitis and viral or autoimmune encephalitis; autoimmune diseases including immune-
20 complex vasculitis, systemic lupus and erythematodes; and disease of the heart including ischemic heart disease and cardiomyopathy. Additional diseases which may benefit from the use of mercapto derivatives include adrenal insufficiency; hypercholesterolemia; atherosclerosis; bone disease associated with increased bone

resorption, e.g., osteoporosis, pre-eclampsia, eclampsia, uremic complications; chronic liver failure, noninflammatory diseases of the central nervous system (CNS) including stroke and cerebral ischemia; and various forms of cancer.

5 Pharmaceutical formulations of the mercapto derivative may include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration, or for administration by inhalation or insufflation. The formulations may, where appropriate, be
10 conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All such pharmacy methods include the steps of bringing into association the active compound with liquid carriers or finely divided solid carriers or both as needed and then, if necessary, shaping the product into the
15 desired formulation.

 Pharmaceutical formulations suitable for oral administration may conveniently be presented: as discrete units, such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; or as a
20 solution, a suspension or as an emulsion. The active ingredient may also be presented as a bolus electuary or paste, and be in a pure form, i.e., without a carrier. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrant or wetting agents. A tablet may be made by

compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be coated according to methods well known in the art. Oral fluid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives. The tablets may optionally be formulated so as to provide slow or controlled release of the active ingredient therein.

Formulations for parenteral administration include: aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized)

condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Alternatively, the formulations may be presented for continuous infusion. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter or polyethylene glycol. Formulations for topical administration in the mouth, for example buccally or sublingually, include lozenges, comprising the active ingredient in a flavored base such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a base such as gelatin and glycerin or sucrose and acacia. For intranasal administration the compounds of the invention may be used as a liquid spray or dispersible powder or in the form of drops. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents. Liquid sprays are conveniently delivered from pressurized packs.

For administration by inhalation the compounds according to the invention are conveniently delivered from an insufflator, nebulizer pressurized packs or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as 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 a metered amount.

Alternatively, for administration by inhalation or
5 insufflation, the compounds according to the invention may take the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form, in for example, capsules, cartridges, gelatin or blister packs from which the
10 powder may be administered with the aid of an inhalator or insufflator.

When desired the above described formulations, adapted to give sustained release of the active ingredient, may be employed. The pharmaceutical compositions according to the invention may also contain other active ingredients such as antimicrobial agents,
15 immunosuppressants or preservatives.

The compounds of the invention may also be used in combination with other therapeutic agents, for example, anti-inflammatory agents, particularly nitric oxide synthase inhibitors, superoxide or peroxynitrite scavengers, vasodilator prostaglandins
20 including prostacyclin and prostaglandin E₁, cancer chemotherapeutic agents including cisplatin, NO donors or NO inhalation therapy, or PAF - receptor antagonists.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may

include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

5 Preferred unit dosage formulations are those containing an effective dose, as recited below, or an appropriate fraction thereof, of the active ingredient.

For each of the aforementioned conditions, the mercapto derivative may be administered orally or via injection at a dose of from 0.1 to 250 mg/kg per day. The dose range for adult humans is
10 generally from 5 mg to 17.5 g/day, preferably 5 mg to 10 g/day and most preferably 100 mg to 3 g/day. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount which is effective at such dosage or as a multiple of the same, for instance, units containing 5 mg to 500 mg, usually around 100 mg
15 to 500 mg.

The pharmaceutical composition preferably is administered orally or by injection (intravenous or subcutaneous), and the precise amount administered to a patient will be the responsibility of the attendant physician. However, the dose employed will depend upon
20 a number of factors, including the age and sex of the patient, the precise disorder being treated, and its severity. Also the route of administration may vary depending upon the condition and its severity.

The following Examples are provided by way of illustration, and are not intended to limit the scope of the invention.

EXAMPLE 1

This example (Fig. 1) illustrates the effect of mercaptoethyguanine on arachidonic acid or immunostimulation-induced 6-keto prostaglandin F1 alpha formation in J774.2 macrophages. J774 macrophage cell lines were obtained from the American Type Culture Collection (ATCC) and were grown using standard methods in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, glutamine, penicillin (10,000 U/l) and streptomycin (10,000 U/l). Cells were grown in 96-well plates for measure of the production of prostaglandin metabolites and cell viability. All the experiments were carried out without fetal calf serum in order to avoid interference with radioimmunoassay. Concentration of 6-keto prostaglandin 1 alpha, the stable metabolite product of prostacyclin in the culture medium, was determined by radioimmunoassay. Supernatant or reaction samples were diluted 1:5 in a buffer containing 0.1% polyvinylpyrrolidone, 0.9% NaCl, 50 mM Tris base, 1.7 mM MgSO₄ and 0.16 mM CaCl₂ (pH 7.4) before radioimmunoassay. The stable metabolite of prostacyclin, 6-keto-PGF1 alpha, was determined by radioimmunoassay as described (Wise WC, Cook JA, Haluskha PV. "Arachidonic Acid Metabolism in Endotoxin Tolerance", *Adv. Shock*, Vol. 10, pp. 131-142, 1983)

Measurement of cyclooxygenase 1 activity in J774 cells

Cells were plated in 96-well plates at 90-100% confluence (200 μ M-3mM). Inhibitors were given as a 30-minute pretreatment.

Cells were then stimulated with arachidonic acid (16 μ M) in order to activate the constitutive cyclooxygenase (COX-1). Cells were then incubated for a further period of 30 minutes and the supernatant was collected for the measurement of arachidonic acid metabolism evaluation by radioimmunoassay.

Measurement of cyclooxygenase 2 activity in J774 cells

Cells were placed in 96-well plates at 90-100% confluence (200 μ L final volume) and pretreated for 30 minutes with MEG (1 μ M-3mM). Inhibitors were given as a 30-minute pretreatment. Cells were then stimulated with endotoxin of *E.coli* (LPS, 10 μ g/mL) and interferon γ (IFN, 150 U/ml) in order to induce the expression of the inducible cyclooxygenase (COX-2). Cells were then incubated for a further period of 6 hours and the supernatant was collected for the measurement of arachidonic acid metabolism evaluation by radioimmunoassay.

Mitochondrial respiration, an indicator of cell viability, was assessed by the mitochondrial-dependent reduction of MTT [3- (4,5 - dimethylthiazol-2-yl) - 2,5 - diphenyltetrazolium bromide] to formazan. Cells in 96-well plates were incubated (37 °C) with MTT (0.2 mg/ml for 60 minutes). Culture medium was removed by aspiration and the cells solubilized in dimethylsulfoxide (DMSO) (100 μ l). The extent of reduction of MTT to formazan within cells was quantitated by measurement of OD₅₅₀ using a microplate reader. The calibration curve for the reduction of MTT to formazan was prepared in DMSO.

Formazan production by cells was expressed as a percentage of the values obtained from untreated cells.

Cells stimulated with arachidonic acid (panel a) or endotoxin (panel b) released 6-keto prostaglandin 1 alpha into the culture medium. This was dose-dependently inhibited by the mercapto derivatives, mercaptoethylguanidine (MEG). The inhibition of PG production was not due to cell killing, as these agents in their effective doses did not decrease cellular viability (not shown). Similar to MEG, other related mercapto derivatives exhibited inhibition of COX-2 activity to a variable degree (Table 1).

TABLE 1

| Half-maximal inhibitory potencies of selected mercapto derivatives on COX-2 activity in immunostimulated J744 macrophages | |
|--|---------------------------------|
| Compound | EC50 (μM) |
| MEG | 55 |
| S-methyl-MEG | 40 |
| N-methyl-MEG | 55 |
| MPG | 55 |
| GED | 75 |

EXAMPLE 2

This example (Fig. 2) illustrates the effect of mercaptoethylguanidine on arachidonic acid or immunostimulation-induced thromboxane B2 formation in J774.2 macrophages. J774 macrophage cell lines were cultured and treated as described in

example 1. Concentration of thromboxane B2, the stable metabolite of thromboxane A2 was determined by radioimmunoassay. Supernatant or reaction samples were diluted 1:5 in a buffer containing 0.1% polyvinylpyrrolidone, 0.9% NaCl, 50mM Tris base, 1.7 mM MgSO₄ and 0.16 mM CaCl₂ (pH 7.4) before radioimmunoassay. Thromboxane B2, the stable metabolite of thromboxane A2, was determined by radioimmunoassay as described (Wise WC, Cook JA, Haluskha PV, "Arachidonic Acid Metabolism in Endotoxin Tolerance", *Adv. Shock*, Vol. 10, pp. 131-142, 1983).

Cells stimulated with arachidonic acid (A) or endotoxin (B) released thromboxane A2 into the culture medium. This was dose-dependently inhibited by the mercapto derivatives, mercaptoethylguanidine (MEG). The inhibition of PG production was not due to cell killing, as these agents in their effective doses did not decrease cellular viability (not shown).

EXAMPLE 3

This example (Fig. 3) illustrates the effect of mercaptoethylguanidine on 6-keto prostaglandin F1 alpha formation by purified COX-1 (A) and COX-2 (B) isoenzymes. In a test tube containing 0.1 M Tris-HCL buffer (pH 8.0, final volume 2 mL) with 1 mM EDTA, 0.2 mM phenol and 1 μ M hemin, 10 units of COX-1 or COX-2 were allowed to react with 100 μ M arachidonic acid for 2 minutes at 37° C in the presence or absence of MEG (1 μ M-3mM). The reaction was quenched by addition of 50 μ L stannous chloride

solution (100 mg/ml in 1 M HCl). the reaction was allowed to proceed for an additional 10 minutes and it was stopped thereafter by addition of 5 mL of a buffer containing 0.1% polyvinylpyrrolidine, 0.9% NaCl, 50 mM Tris base, 1.7 mM MgSO₄ and 0.16 mM CaCl₂ (pH 7.4).

5 Concentration of 6-keto prostaglandin 1 alpha, the stable metabolite product of prostacyclin in the culture medium, was determined by radioimmunoassay. Supernatant or reaction samples were diluted 1:5 in a buffer containing 0.1% polyvinylpyrrolidine, 0.9% NaCl, 50 mM Tris base, 1.7 mM MgSO₄ and 0.16 mM CaCl₂ (pH 7.4) before radioimmunoassay. The stable metabolite of prostacyclin, 10 6-keto-PGF1a, was determined by radioimmunoassay as described (Wise WC, Cook JA, Haluskha PV, "Arachidonic Acid Metabolism in Endotoxin Tolerance", *Adv. Shock*, Vol. 10, pp. 131-142, 1983).

 There was a significant production of 6-keto prostaglandin 15 1 alpha into the reaction mixture. This was dose-dependently inhibited by the mercapto derivatives, mercaptoethylguanidine (MEG).

EXAMPLE 4

 This Example illustrates a method for synthesizing mercaptoethylguanidine sulphate. Mercaptoethylamine hydrochloride 20 (2g) was dissolved in methanol (5 ml) and cooled in a salt/ice bath. A cold solution of potassium hydroxide (0.99 g) in methanol (10 ml) was added and the mixture stirred. After 1 hour, the solution was filtered and S-methylisothiurea (2g) was added to 12 ml of the filtrate. The solution was stirred at room temperature (18°C) for 16 hours under

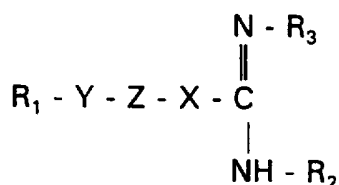
nitrogen. The solution then was filtered and ether was added to precipitate the crude product which was then recrystallized from an ether/ethanol mixture.

5 The detailed description of the invention presented above is provided by way of illustration, and it is not intended to limit the scope of the invention which is to be determined by the following claims.

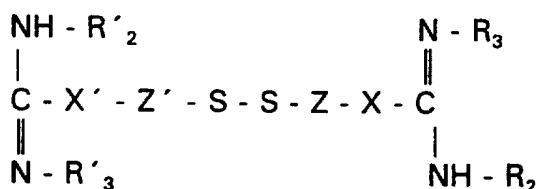
What is claimed is:

1. A pharmacologically acceptable composition for inhibiting cyclooxygenase in a mammal, comprising:

a compound having a formula selected from the group consisting of:



and



or a salt thereof, wherein

R_1 is H, alkyl, alkenyl, phenyl, alkylene, alkenylene, or phenylalkylene or a substituted derivative thereof;

When R_1 is alkylene or alkenylene, R_1 optionally may be joined to either of the amidino Ns, to Z or to X of the above formula containing R_1 to form a 5-, 6- or 7- membered heterocyclic ring, with the proviso that, when R_1 is attached to Z, Z is alkylene or alkenylene or a substituted derivative thereof, and, when R_1 is attached to X, X is either CR_5 or N;

R_2 , R_3 , R'_2 and R'_3 are independently H, lower alkyl, alkenyl, alkylene, alkenylene, amino, phenyl or phenylalkylene, or a substituted derivative thereof;

When R_2 or R'_2 is alkylene or alkenylene, R_2 or R'_2 optionally may be joined to the imino N residing on the adjacent amidino C to form a 5- or 6- membered heterocyclic ring;

Z and Z' are independently alkylene, alkenylene, cycloalkylene or cycloalkenylene, or a substituted derivative thereof;

When R_2 , R_3 , R'_2 or R'_3 is alkylene or alkenylene, R_2 , R_3 , R'_2 or R'_3 optionally may be joined to the adjacent Z or Z' to form a 5- or 6- membered heterocyclic ring including N, C and not more than one atom of O or S, with the proviso that Z is an alkylene or alkenylene, said heterocyclic ring optionally being substituted with a lower alkyl, alkoxy, halo, hydroxy or amino;

X is N, NR_4 , O, CR_5 or CR_4R_5 ;

X' is N, NR'_4 , O, CR'_5 or $CR'_4R'_5$;

Y is S;

R_4 and R'_4 are independently H, alkyl, alkylene, alkenylene, thioalkylene or thioesteralkylene;

R_5 and R'_5 are independently H, alkyl, alkylene, alkenylene, thioalkylene, thioesteralkylene, amino or carboxyl;

When R_4 or R'_4 is alkylene, alkenylene, thioalkylene, or thioesteralkylene, R_4 or R'_4 optionally may be joined to R_2 , R_3 , R'_2 or R'_3 to form a 5- or 6- membered heterocyclic ring including N, C and not more than one atom of O or S, with the proviso that R_2 , R_3 , R'_2 or R'_3 is independently alkylene, alkenylene, amino, phenyl,

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phenylalkylene, or a substituted derivative thereof wherein the substituted derivative is lower alkyl or halo; and

a pharmaceutically acceptable carrier, said mercapto derivative present in said composition in an effective amount to inhibit
55 cyclooxygenase in said mammal.

2. The composition of claim 1 wherein said substituted derivative of R_1 is selected from the group consisting of one or more of alkoxy, halo, hydroxy, amino and nitro.
3. The composition of claim 1 wherein said substituted derivative of R_2 , R_3 , R'_2 or R'_3 is independently selected from the group consisting of lower alkyl and halo.
4. The composition of claim 1 wherein said R_4 , R_5 , R'_4 or R'_5 thioalkylene has a formula $[-(CH_2)_n-SH]$ where n is independently 1 to 4.
5. The composition of claim 1 wherein said R_4 , R_5 , R'_4 or R'_5 thioesteralkylene has a formula $[-(CH_2)_n-S-R_6]$ where R_6 is independently a lower alkyl and n is independently 1 to 4.
6. The composition of claim 1 wherein said substituted derivative of Z or Z' is independently selected from the group consisting of alkoxy, halo, hydroxy, amino and nitro.
7. The composition of claim 1 wherein R_1 is selected from the group consisting of H and lower alkyl, R_2 is H, R_3 is H, R'_2 is H, R'_3 is H, X is NR_4 , X' is NR'_4 , R_4 and R'_4 are independently selected from the group consisting of H, methyl and ethyl, and Z and Z' are independently alkylene.

8. The composition of claim 1 wherein R_1 is H, R_2 is H, R_3 is H, R'_2 is H, R'_3 is H, X is NR_4 , X' is NR'_4 , R_4 is H, R'_4 is H and Z and Z' are independently a C_{1-6} alkylene.

9. The composition of claim 1 wherein said compound is selected from the group consisting of mercaptoethylguanidine, mercaptopropylguanidine, S-methyl-mercaptoethylguanidine, S-methyl-mercaptopropylguanidine, and guanidinoethyldisulfide.

10. The composition of claim 1 wherein said compound is present in an amount sufficient to treat a condition where there is an advantage in inhibiting cyclooxygenases.

11. The composition of claim 10 wherein said condition is selected from the group consisting of circulatory shock, systemic inflammatory response syndrome, therapy with cytokines, therapy with cytokine-inducing agents, transplantation, transplant rejection, local inflammatory responses, systemic inflammation, autoimmune diseases, adult respiratory distress syndrome, arthritis, rheumatoid arthritis, diabetes mellitus, ileitis, ulcerative colitis, Crohn's disease, asthma, periodontitis, nephrosis, chronic demyelinating diseases of the nervous system, multiple sclerosis, AIDS-related complications, Alzheimer's disease, ischemic heart disease, cardiomyopathy, adrenal insufficiency, hypercholesterolemia, atherosclerosis, bone diseases associated with increased bone resorption, pre-eclampsia, eclampsia, uremic complications, chronic liver failure, stroke, cerebral ischemia, and cancer.

12. The composition of claim 10 wherein said condition is selected from the group consisting of systemic inflammatory response syndrome and circulatory shock.

13. The composition of claim 1 formulated for oral, rectal, nasal, topical, buccal, sub-lingual, vaginal, parenteral, intramuscular, sub-cutaneous, intravenous, inhalation or insufflation administration.

14. The composition of claim 1 formulated for oral administration, said carrier including an ingredient selected from the group consisting of a binding agent, filler, lubricant, disintegrant, wetting agent, inert diluent, surface active agent, dispersing agent, suspending agent, emulsifying agent, edible oil, flavoring agent and mixtures thereof.

15. The composition of claim 1 formulated for topical administration in the mouth, said carrier including an ingredient selected from the group consisting of a flavor, sucrose, acacia, tragacanth, gelatin, glycerin and mixtures thereof.

16. The composition of claim 1 formulated for nasal administration, said carrier including an ingredient selected from the group consisting of a dispersing agent, solubilizing agent, suspending agent and mixtures thereof.

17. The composition of claim 1 formulated for administration by inhalation, said carrier including a propellant.

18. The composition of claim 17 wherein said propellant is selected from the group consisting of dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide and mixtures thereof.

19. The composition of claim 1 formulated for administration by inhalation or insufflation, said carrier including an ingredient selected from the group consisting of lactose, starch and mixtures thereof.

20. The composition of claim 1 formulated for parenteral administration, said carrier including an ingredient selected from the group consisting of an anti-oxidant, buffer, bacteriostat, suspending agent, thickening agent, saline, water and mixtures thereof.

21. The composition of claim 1 formulated for rectal administration, said carrier including an ingredient selected from the group consisting of cocoa butter, polyethylene glycol and mixtures thereof.

22. The composition of claim 1 formulated to include an ingredient selected from the group consisting of an antimicrobial agent, an immunosuppressant, a preservative and mixtures thereof.

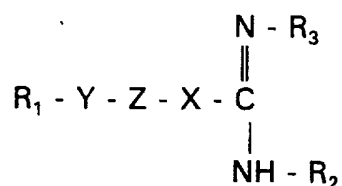
23. The composition of claim 1 formulated for administration at a dose of from about 5 mg to about 17.5 g/day of said compound.

24. The composition of claim 23 formulated for administration at a dose of from about 5 mg to about 10 g/day of said compound.

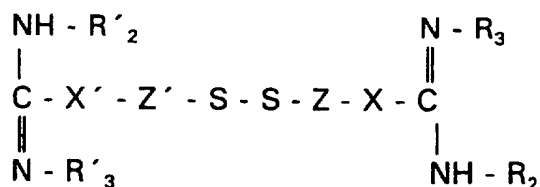
25. The composition of claim 24 formulated for administration at a dose of from about 100 mg to about 3 g/day of said compound.

26. A method for inhibiting cyclooxygenase in a mammal comprising:

administering to the mammal an effective amount of a compound to inhibit cyclooxygenase in the mammal, said compound having a formula selected from the group consisting of:



and



or a salt thereof, wherein

R_1 is H, alkyl, alkenyl, phenyl, alkylene, alkenylene, or phenylalkylene or a substituted derivative thereof;

When R_1 is alkylene or alkenylene, R_1 optionally may be joined to either of the amidino Ns, to Z or to X of the above formula containing R_1 to form a 5-, 6- or 7- membered heterocyclic ring, with the proviso that, when R_1 is attached to Z, Z is alkylene or alkenylene or a substituted derivative thereof, and, when R_1 is attached to X, X is either CR_5 or N;

R_2 , R_3 , R'_2 and R'_3 are independently H, lower alkyl, alkenyl, alkylene, alkenylene, amino, phenyl or phenylalkylene, or a substituted derivative thereof;

When R_2 or R'_2 is alkylene or alkenylene, R_2 or R'_2
30 optionally may be joined to the imino N residing on the adjacent
amidino C to form a 5- or 6- membered heterocyclic ring;

Z and Z' are independently alkylene, alkenylene,
cycloalkylene or cycloalkenylene, or a substituted derivative thereof;

When R_2 , R_3 , R'_2 or R'_3 is alkylene or alkenylene, R_2 , R_3 ,
35 R'_2 or R'_3 optionally may be joined to the adjacent Z or Z' to form a
5- or 6- membered heterocyclic ring including N, C and not more than
one atom of O or S, with the proviso that Z is an alkylene or
alkenylene, said heterocyclic ring optionally being substituted with a
lower alkyl, alkoxy, halo, hydroxy or amino;

40 X is N, NR_4 , O, CR_5 or CR_4R_5 ;

X' is N, NR'_4 , O, CR'_5 or $CR'_4R'_5$;

Y is S;

R_4 and R'_4 are independently H, alkyl, alkylene,
alkenylene, thioalkylene or thioesteralkylene;

45 R_5 and R'_5 are independently H, alkyl, alkylene,
alkenylene, thioalkylene, thioesteralkylene, amino or carboxyl; and

When R_4 or R'_4 is alkylene, alkenylene, thioalkylene, or
thioesteralkylene, R_4 or R'_4 optionally may be joined to R_2 , R_3 , R'_2 or
 R'_3 to form a 5- or 6- membered heterocyclic ring including N, C and
50 not more than one atom of O or S, with the proviso that R_2 , R_3 , R'_2 or
 R'_3 is alkylene, alkenylene, amino, phenyl, phenylalkylene, or a

substituted derivative thereof wherein the substituted derivative is lower alkyl or halo.

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27. The method of claim 26 wherein said substituted derivative of R_1 is selected from the group consisting of one or more of alkoxy, halo, hydroxy, amino and nitro.

28. The method of claim 26 wherein said substituted derivative of R_2 , R_3 , R'_2 or R'_3 is independently selected from the group consisting of lower alkyl and halo.

29. The method of claim 26 wherein said R_4 , R_5 , R'_4 or R'_5 thioalkylene has a formula $[-(CH_2)_n-SH]$ where n is independently 1 to 4.

30. The method of claim 26 wherein said R_4 , R_5 , R'_4 or R'_5 thioesteralkylene has a formula $[-(CH_2)_n-S-R_6]$ where R_6 is independently a lower alkyl and n is independently 1 to 4.

31. The method of claim 26 wherein said substituted derivative of Z or Z' is independently selected from the group consisting of alkoxy, halo, hydroxy, amino and nitro.

32. The method of claim 26 wherein R_1 is selected from the group consisting of H and lower alkyl, R_2 is H, R_3 is H, R'_2 is H, R'_3 is H, X is NR_4 , X' is NR'_4 , R_4 and R'_4 are independently selected from the group consisting of H, methyl and ethyl, and Z and Z' are independently alkylene.

33. The method of claim 26 wherein R_1 is H, R_2 is H, R_3 is H, R'_2 is H, R'_3 is H, X is NR_4 , X' is NR'_4 , R_4 is H, R'_4 is H and Z and Z' are independently a C_{1-6} alkylene.

34. The method of claim 26 wherein said compound is selected from the group consisting of mercaptoethylguanidine, mercaptopropylguanidine, S-methyl-mercaptoethylguanidine, S-methyl-mercaptopropylguanidine, and guanidinoethyldisulphide.

35. The method of claim 26 conducted for treating a condition where there is an advantage in inhibiting cyclooxygenase.

36. The method of claim 35 wherein said condition is selected from the group consisting of circulatory shock, systemic inflammatory response syndrome, therapy with cytokines, therapy with cytokine-inducing agents, transplantation, transplant rejection, local inflammatory responses, systemic inflammation, autoimmune diseases, adult respiratory distress syndrome, arthritis, rheumatoid arthritis, diabetes mellitus, ileitis, ulcerative colitis, Crohn's disease, asthma, periodontitis, nephrosis, chronic demyelinating diseases of the nervous system, multiple sclerosis, AIDS-related complications, Alzheimer's disease, ischemic heart disease, cardiomyopathy, adrenal insufficiency, hypercholesterolemia, atherosclerosis, bone diseases associated with increased bone resorption, pre-eclampsia, eclampsia, uremic complications, chronic liver failure, stroke, cerebral ischemia, and cancer.

37. The method of claim 35 wherein said condition is selected from the group consisting of arthritis and gastrointestinal inflammatory disorders.

38. The method of claim 26 by administering said compound by a method selected from the group consisting of oral, rectal, nasal, topical, buccal, sub-lingual, vaginal, parenteral, intramuscular, sub-cutaneous, intravenous, inhalation and insufflation administration.

39. The method of claim 26 by orally administering said compound in a pharmacologically acceptable carrier, said carrier including an ingredient selected from the group consisting of a binding agent, filler, lubricant, disintegrant, wetting agent, inert diluent, surface active agent, dispersing agent, suspending agent, emulsifying agent, edible oil, flavoring agent and mixtures thereof.

40. The method of claim 26 by topically administering said compound in a pharmacologically acceptable carrier in the mouth, said carrier including an ingredient selected from the group consisting of a flavor, sucrose, acacia, tragacanth, gelatin, glycerin and mixtures thereof.

41. The method of claim 26 by nasally administering said compound in a pharmacologically acceptable carrier, said carrier including an ingredient selected from the group consisting of a dispersing agent, solubilizing agent, suspending agent and mixtures thereof.

42. The method of claim 26 by administering said compound in a pharmacologically acceptable carrier by inhalation, said carrier including a propellant.

43. The method of claim 42 wherein said propellant is selected from the group consisting of dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide and mixtures thereof.

44. The method of claim 26 by administering said compound in a pharmacologically acceptable carrier by inhalation or insufflation, said carrier including an ingredient selected from the group consisting of lactose, starch and mixtures thereof.

45. The method of claim 26 by administering said compound in a pharmacologically acceptable carrier parenterally, said carrier including an ingredient selected from the group consisting of an antioxidant, buffer, bacteriostat, suspending agent, thickening agent, saline, water and mixtures thereof.

46. The method of claim 26 by administering said compound in a pharmacologically acceptable carrier rectally, said carrier including an ingredient selected from the group consisting of cocoa butter, polyethylene glycol and mixtures thereof.

47. The method of claim 26 wherein said compound includes an ingredient selected from the group consisting of an antimicrobial agent, an immunosuppressant, a preservative and mixtures thereof.

48. The method of claim 26 wherein said compound is administered at a dose of from about 5 mg to about 17.5 g/day.

49. The method of claim 48 wherein said compound is administered at a dose of from about 5 mg to about 10 g/day.

50. The method of claim 49 wherein said compound is administered at a dose of from about 100 mg to about 3 g/day.

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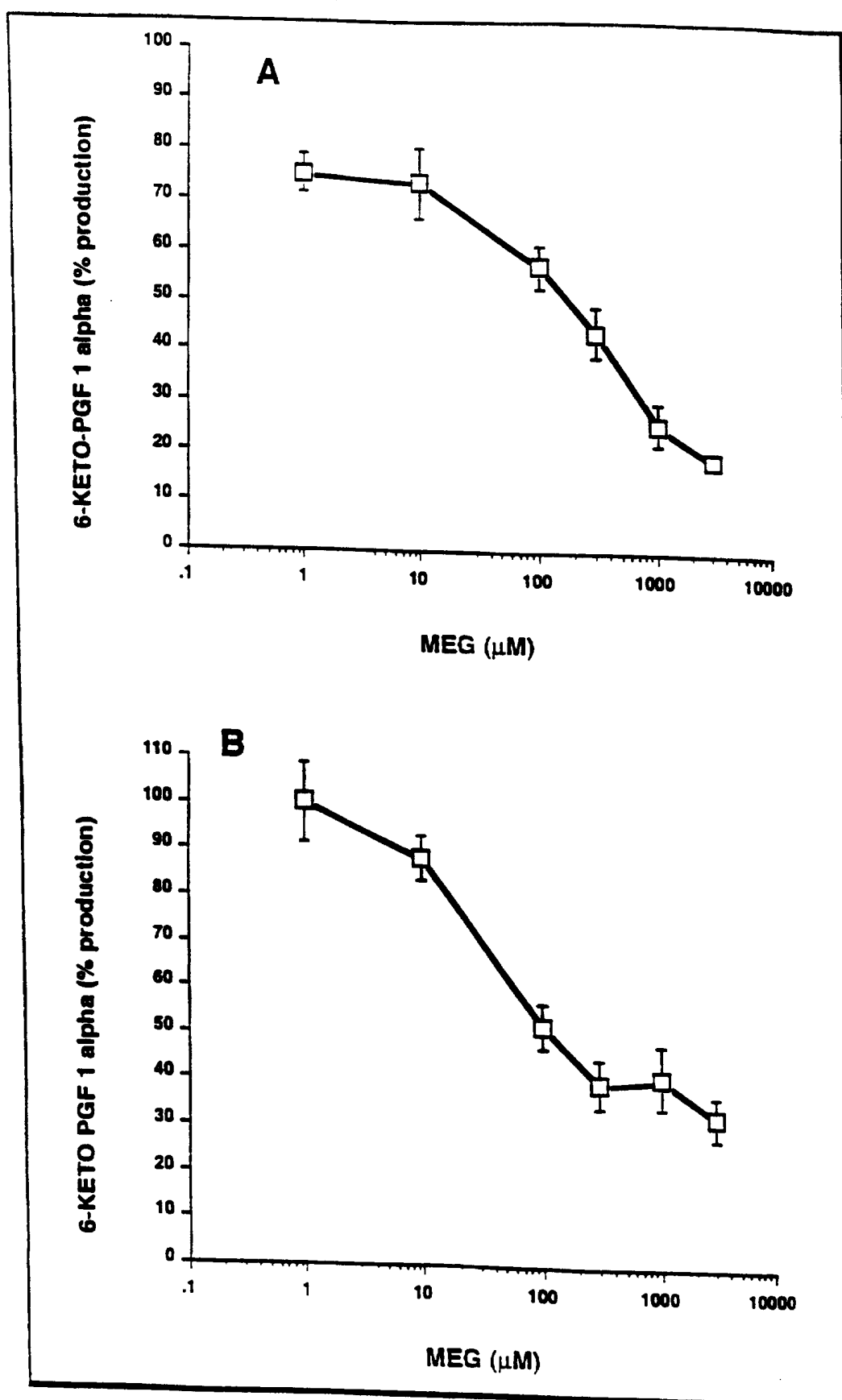


FIG. 1

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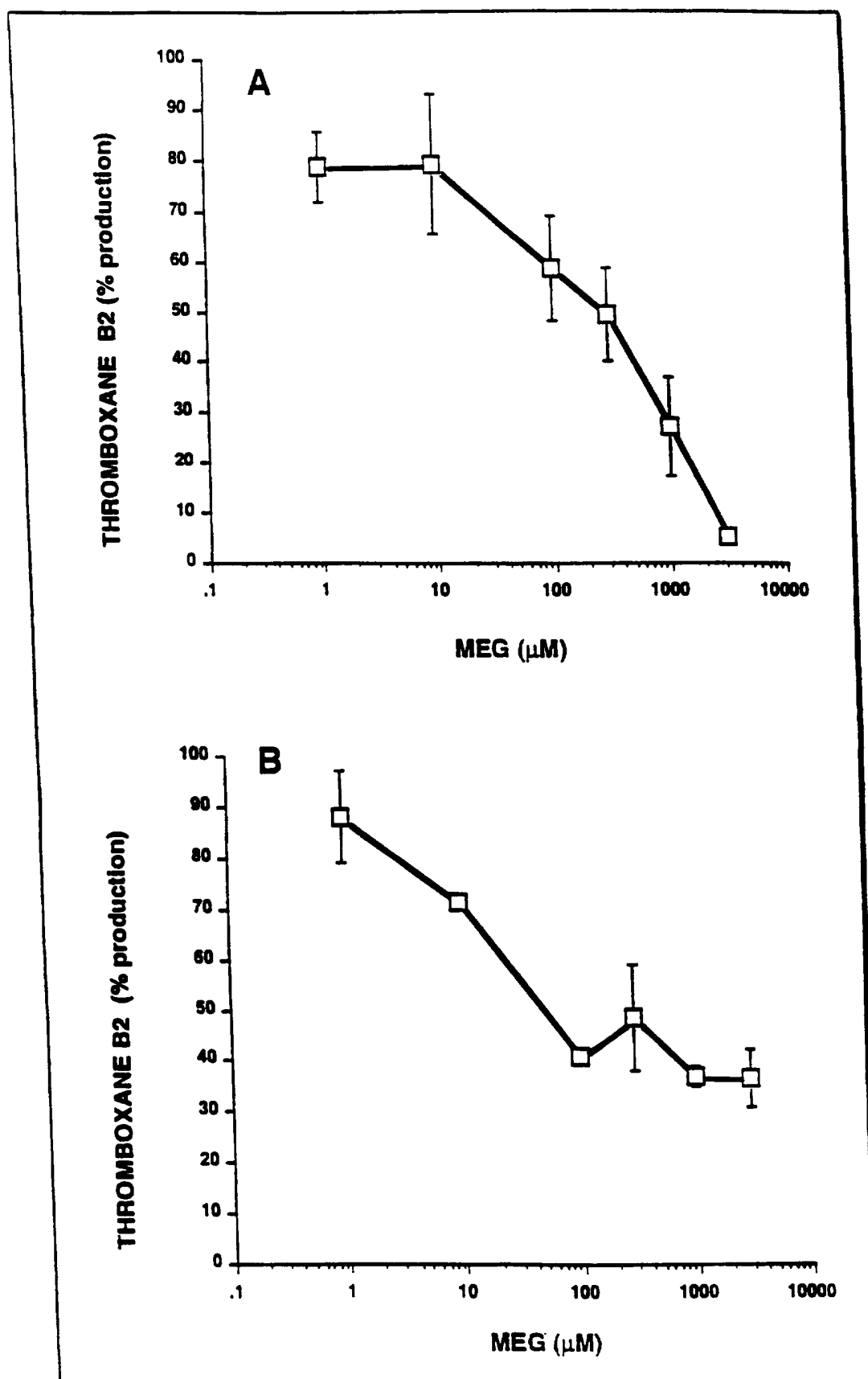


FIG.2

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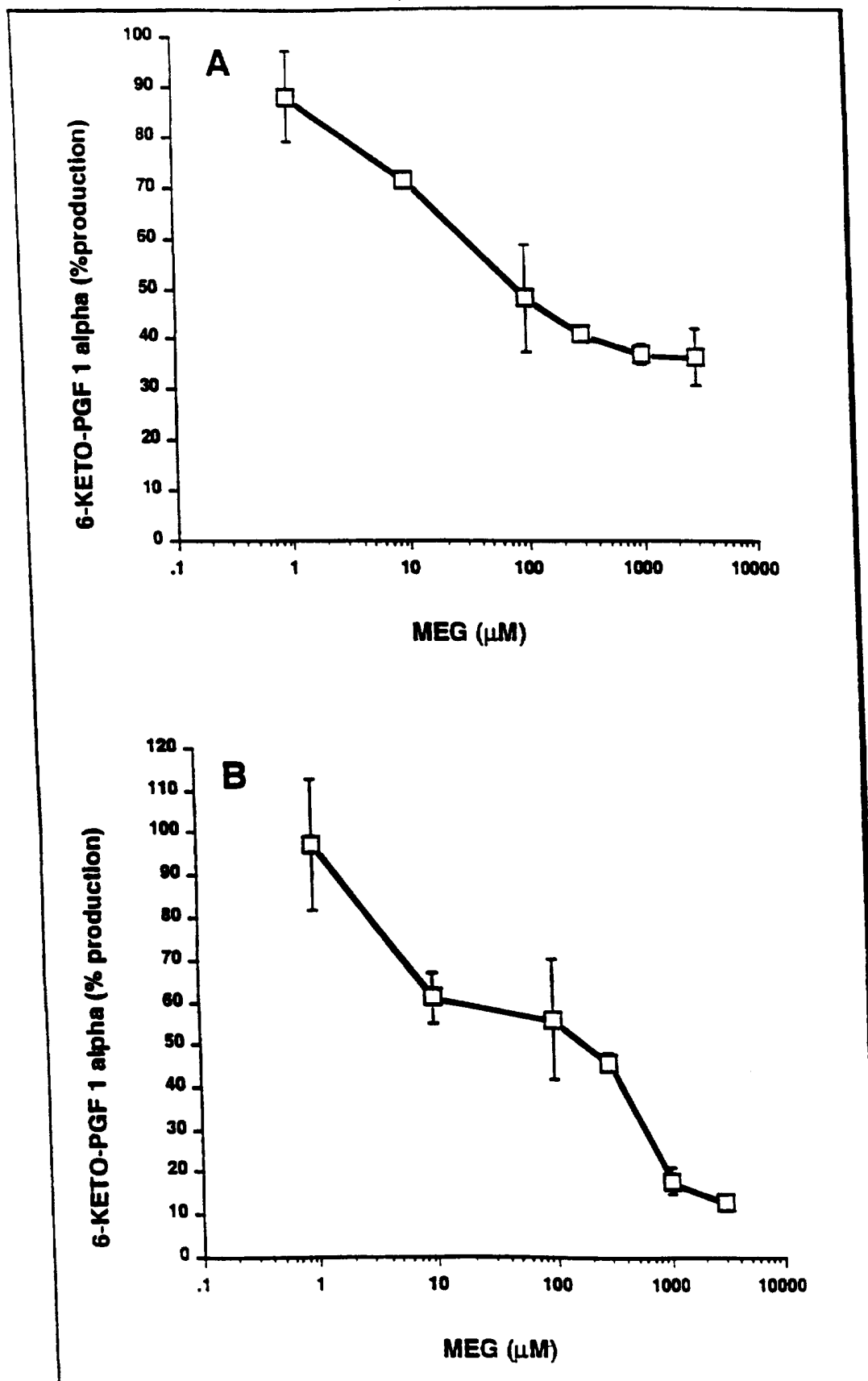


FIG. 3

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/03279

| | | |
|--|--|--|
| A. CLASSIFICATION OF SUBJECT MATTER IPC 6 A61K31/155 A61K31/54 A61K31/425 | | |
| 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 6 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) | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | |
| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | BRITISH JOURNAL OF PHARMACOLOGY, vol. 117, February 1996, pages 619-632, XP000575067 G. J. SOUTHAN ET AL: "Spontaneous rearrangement of aminoalkylisothioureas into mercaptoalkylguanidines, a novel class of nitric oxide synthase inhibitors with selectivity towards the inducible isoform" *cf. abstract, p. 621, right col., 1st para.* | 1-25 |
| X | --- EP 0 558 468 A (UNIV WASHINGTON) 1 September 1993 *cf. abstract, p.3, lines 35-41, claims 1-9* | 1-25 |
| A | *cf. p., lines 23-25* --- -/-- | 1-50 |
| <div style="display: flex; justify-content: space-between;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex. </div> | | |
| <div style="display: flex;"> <div style="flex: 1;"> <p>* Special categories of cited documents :</p> <p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*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)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>*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</p> <p>*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</p> <p>*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.</p> <p>*&* document member of the same patent family</p> </div> </div> | | |
| Date of the actual completion of the international search <div style="text-align: center; font-weight: bold;">20 June 1997</div> | | Date of mailing of the international search report <div style="text-align: center; font-weight: bold;">28.07.97</div> |
| 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 <div style="text-align: center; font-weight: bold;">Stoltner, A</div> |

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/03279

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| P,X | WO 96 30007 A (CHILDRENS HOSP MEDICAL CENTER) 3 October 1996 *cf. abstract, claims 1-26* --- | 1-25 |
| A | US 5 360 925 A (CHABRIER DE LASSAUNIERE PIERRE ET AL) 1 November 1994 *cf. cols. 1 bridging with col. 2, lines 7-55* ----- | 1-50 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/03279

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|---|--|
| EP 0558468 A | 01-09-93 | US 5246971 A AT 141790 T CA 2090574 A DE 69304225 D DE 69304225 T JP 6009380 A US 5358969 A | 21-09-93 15-09-96 29-08-93 02-10-96 30-01-97 18-01-94 25-10-94 |
| WO 9630007 A | 03-10-96 | AU 5319196 A | 16-10-96 |
| US 5360925 A | 01-11-94 | AT 401054 B AT 256092 A AU 664399 B AU 3049892 A BE 1006227 A CA 2085555 A CH 685629 A DE 4244539 A ES 2052452 A FR 2685869 A FR 2685916 A GB 2263111 A,B GR 1001443 B HK 22296 A IE 71675 B IT 1256761 B JP 5286916 A LU 88208 A NL 9300001 A NZ 245499 A PL 169432 B PT 101165 A SE 9203825 A US 5480999 A ZA 9210080 A | 25-06-96 15-10-95 16-11-95 08-07-93 14-06-94 05-07-93 31-08-95 08-07-93 01-07-94 09-07-93 09-07-93 14-07-93 30-12-93 16-02-96 26-02-97 15-12-95 02-11-93 15-04-93 02-08-93 26-07-95 31-07-96 28-02-94 05-07-93 02-01-96 02-08-93 |