

CONVENTION

AUSTRALIA

Patents Act 1990

NOTICE OF ENTITLEMENT

We, **MERRELL DOW PHARMACEUTICALS INC.** of 2110 East Galbraith Road, P. O. Box 156300, Cincinnati, Ohio 45215-6300, United States of America state the following in connection with Australian Application No. 38072/93:

1. We are the nominated person.
2. The nominated person is the assignee of the actual inventors.
3. The nominated person is the applicant of the basic application listed in the declaration under Article 8 of the PCT.
4. The basic application is the application first made in a Convention country in respect of the invention.

Dated: 9 September 1994

By **PHILLIPS ORMONDE & FITZPATRICK**  
Patent Attorneys for the Applicant  
By:

.....*David B. Fitzpatrick*.....

To: The Commissioner of Patents

**PHILLIPS ORMONDE & FITZPATRICK**  
367 Collins Street  
Melbourne, Australia, 3000

Our Ref: 382841

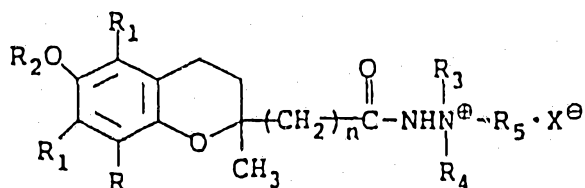
1925x



AU9338072

(12) PATENT ABRIDGMENT (11) Document No. AU-B-38072/93  
 (19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 675784

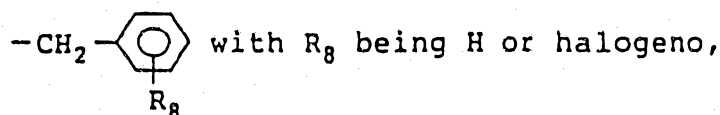
- (54) Title  
HYDRAZIDE DERIVATIVES OF 3,4-DIHYDRO-2H-1-BENZOPYRANS
- International Patent Classification(s)  
 (51)<sup>5</sup> C07D 311/66 A61K 031/35 C07D 311/58
- (21) Application No. : 38072/93 (22) Application Date : 12.03.93
- (87) PCT Publication Number : WO93/20059
- (30) Priority Data
- (31) Number (32) Date (33) Country  
 92400972 07.04.92 EP EUROPEAN PATENT OFFICE (EP)
- (43) Publication Date : 08.11.93
- (44) Publication Date of Accepted Application : 20.02.97
- (71) Applicant(s)  
MERRELL DOW PHARMACEUTICALS INC.
- (72) Inventor(s)  
J. MARTIN GRISAR; MARGARET A. PETTY; FRANK BOLKENIUS
- (74) Attorney or Agent  
PHILLIPS ORMONDE & FITZPATRICK, 367 Collins Street, MELBOURNE VIC 3000
- (56) Prior Art Documents  
 AU 69217/91 C07D 311/66  
 AU 77986/91 C07D 311/66  
 AU 60895/90 C07D 311/66
- (57) Claim 1. Compounds of the formula



(1)

the stereoisomers and mixtures thereof, their inner salts,  
~~and the pharmaceutically acceptable salts thereof wherein~~

R is H or C<sub>1-6</sub> alkyl,  
 R<sub>1</sub> is C<sub>1-6</sub> alkyl,  
 R<sub>2</sub> is H or -C(O)R, R<sub>3</sub> and R<sub>4</sub> are independently C<sub>1-6</sub> alkyl;  
 R<sub>5</sub> is C<sub>1-6</sub> alkyl, or



n is zero, 1, 2, or 3 and

(11) AU-B-38072/93  
(10) 675784

-2-

X is a halide,  $-S(O)_3R_6$ , or nothing when the inner salt is formed.

$R_6$  is H,  $C_{1-6}$  alkyl, phenyl or 4-methylphenyl.

12. A method of treating a patient for reperfusion damage by administering an effective amount of a compound of Claim 1.

13. A method of treating a patient for inflammatory bowel disease by administering an effective amount of a compound of Claim 1.

OPI DATE 08/11/93 APPLN. ID 38072/93  
 AOJP DATE 13/01/94 PCT NUMBER PCT/US93/02311



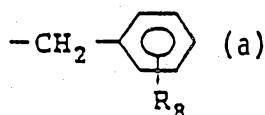
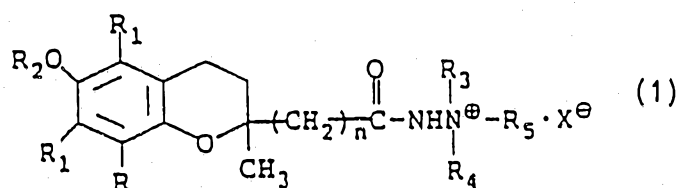
AU9338072

Y (PCT)

(51) International Patent Classification 5 : <b>C07D 311/66, 311/58</b>		<b>A1</b>	(11) International Publication Number: <b>WO 93/20059</b>
			(43) International Publication Date: 14 October 1993 (14.10.93)
(21) International Application Number: PCT/US93/02311		(74) Agent: MOON, Carolyn, D.; Merrell Dow Pharmaceuticals Inc., 2110 East Galbraith Road, P.O. Box 156300, Cincinnati, OH 45215-6300 (US).	
(22) International Filing Date: 12 March 1993 (12.03.93)			
(30) Priority data: 92400972.3 7 April 1992 (07.04.92) EP		(81) Designated States: AU, CA, FI, HU, JP, KR, NO, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(34) Countries for which the regional or international application was filed: FR et al.		Published With international search report.	
(71) Applicant (for all designated States except US): MERRELL DOW PHARMACEUTICALS INC. [US/US]; 2110 East Galbraith Road, P.O. Box 156300, Cincinnati, OH 45215-6300 (US).			
(72) Inventors; and			
(75) Inventors/Applicants (for US only) : GRISAR, J., Martin [US/FR]; 7, rue de Mulhouse, F-67160 Wissembourg (FR). PETTY, Margaret, A. [GB/FR]; 2, allée de la Robertsau, F-67000 Strasbourg (FR). BOLKENIUS, Frank [DE/DE]; Bierkellerstrasse 15B, D-7640 Kehl (DE).			

675784

(54) Title: HYDRAZIDE DERIVATIVES OF 3,4-DIHYDRO-2H-1-BENZOPYRANS



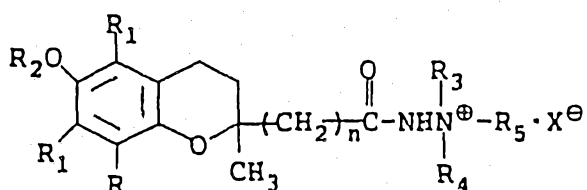
(57) Abstract

This invention relates to novel hydrazide acyl hydrazinium derivatives of certain 3,4-dihydro-2H-1-benzopyrans of formula (1), the stereoisomers and mixtures thereof, their inner salts, and the pharmaceutically acceptable salts thereof wherein R is H or C<sub>1-6</sub> alkyl, R<sub>1</sub> is C<sub>1-6</sub> alkyl, R<sub>2</sub> is H or -C(O)R, R<sub>3</sub> and R<sub>4</sub> are independently C<sub>1-6</sub> alkyl; R<sub>5</sub> is C<sub>1-6</sub> alkyl, or (a) with R<sub>8</sub> being H or halogeno, n is zero, 1, 2, or 3 and X is a halide, -S(O)<sub>3</sub>R<sub>6</sub>, or nothing when the inner salt is formed, R<sub>6</sub> is H, C<sub>1-6</sub> alkyl, phenyl or 4-methylphenyl, to the intermediates, processes and techniques for their preparation, to their ability to manifest the property of being free radical scavengers, and to their end-use application in the treatment of disease conditions capable of being ameliorated by free radical scavengers.

HYDRAZIDE DERIVATIVES OF 3,4-DIHYDRO-2H-1-BENZOPYRANS

This invention relates to novel hydrazide acyl  
 5 hydrazinium derivatives of certain 3,4-dihydro-2H-1-benzo-  
 pyrans, to the intermediates, processes and techniques for  
 their preparation, to their ability to manifest the  
 property of being free radical scavengers, and to their  
 end-use application in the treatment of disease conditions  
 capable of being ameliorated by free radical scavengers.

10 More specifically this invention relates to compounds of  
 the formula



(1)

20 the stereoisomers and mixtures thereof, their inner salts,  
~~and the pharmaceutically acceptable salts thereof~~ wherein

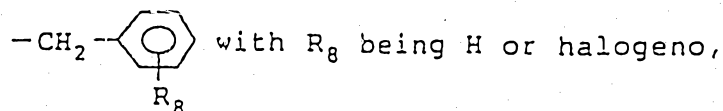
R is H or C<sub>1-6</sub> alkyl,

R<sub>1</sub> is C<sub>1-6</sub> alkyl,

25 R<sub>2</sub> is H or -C(O)R,

R<sub>3</sub> and R<sub>4</sub> are independently C<sub>1-6</sub> alkyl;

$R_5$  is  $C_{1-6}$  alkyl, or



5  $n$  is zero, 1, 2, or 3 and

$X$  is a halide,  $-\text{S}(\text{O})_3R_6$ , or nothing when the inner salt is formed

$R_6$  is H,  $C_{1-6}$  alkyl, phenyl or 4-methylphenyl.

The present invention also provides a pharmaceutical composition including a compound of the invention and a pharmaceutically acceptable carrier.

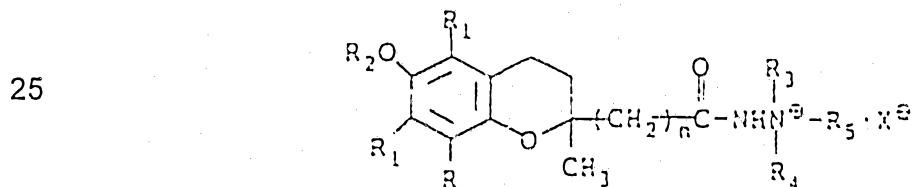
10 The present invention further provides a method of treating a patient for reperfusion damage by administering an effective amount of a compound of the invention.

The present invention still further provides a method of treating a patient for inflammatory bowel disease by administering an effective amount of a compound of the invention.

15 The present invention still further provides use of a compound of the invention in the treatment of inflammatory bowel disease or reperfusion damage.

Moreover, the present invention provides a use of a compound according to the invention, optionally in combination with a pharmaceutically acceptable carrier, for the preparation of a pharmaceutical composition according to the invention.

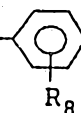
20 Still further, the present invention provides a process of making a compound of the formula



(1)

the stereoisomers and mixture thereof, their inner salts, wherein  $R$  is H or  $C$  alkyl,

$R_1$  is  $C_{1-6}$  alkyl,  
 $R_2$  is H or  $-C(O)R$ ,  
 $R_3$  and  $R_4$  are independently  $C_{1-6}$  alkyl;  
 $R_5$  is  $C_{1-6}$  alkyl, or

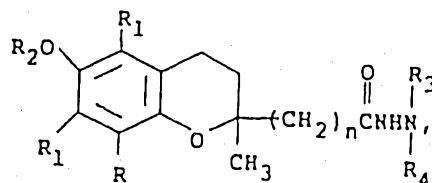
$-CH_2-$   with  $R_8$  being H or halogeno,

$n$  is zero, 1, 2, or 3 and

$X$  is a halide,  $-S(O)_3R_6$ , or nothing when the inner salt is formed,

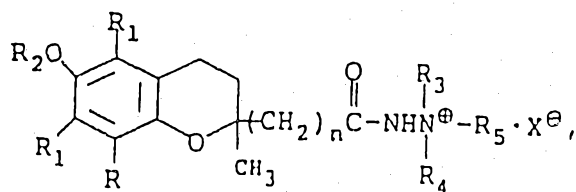
$R_6$  is H,  $C_{1-6}$  alkyl, phenyl or 4-methylphenyl,

including the steps of reacting the hydrazine



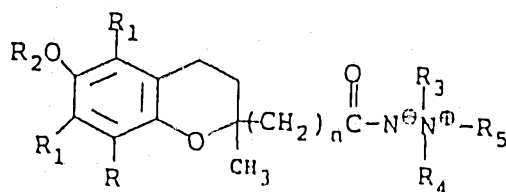
(4)

as previously defined, with  $R_5X'$  wherein  $X'$  is a halide or  $-S(O)_3R_6$  as previously defined and refluxing to produce the acyl hydrazinium salt



(5)

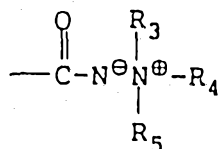
as previously defined, and optionally treating the foregoing acyl hydrazinium with a base in order to produce the inner salt



(6)

As used herein the term alkyl includes the straight or branched saturated aliphatic hydrocarbyl moieties having the designated number of carbon atoms, preferably methyl or ethyl, but including propyl, isopropyl, n-butyl, t-butyl, hexyl and the like. The term -C(O)r includes those acyl moieties where R includes H and C<sub>1-6</sub> alkyl, embracing formyl and methylcarbonyl as preferred species but including ethylcarbonyl and the like. In the acyl hydrazinium moiety (i.e., -C(O)NHN<sup>⊕</sup>(R<sub>3</sub>)(R<sub>4</sub>)(R<sub>5</sub>).X<sup>⊖</sup>) the R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> preferably are the same alkyl with methyl and ethyl being preferred and when R<sub>5</sub> is not alkyl the preferred radicals are benzyl or R<sub>8</sub>-substituted benzyl moieties with R<sub>8</sub> preferably being chloro or bromo. The X moiety includes a halogen, preferably chloro or bromo, or a -S(O)<sub>3</sub>R<sub>6</sub> moiety wherein R<sub>6</sub> is H, C<sub>1-6</sub> alkyl, phenyl, or, preferably, 4-methylphenyl.

The inner salts include those compounds wherein the acyl hydrazinium moiety would have the structure configuration



as its terminal moiety.



The compounds of the present invention include stereo-isomers; the term "stereoisomer" is a general term for all isomers of individual molecules that differ only in the orientation of their atoms in space. It includes mirror image isomers (enantiomers), geometric isomers (cis/trans), and isomers of compounds with more than one chiral center that are not mirror images of one another (diastereoisomers).

10

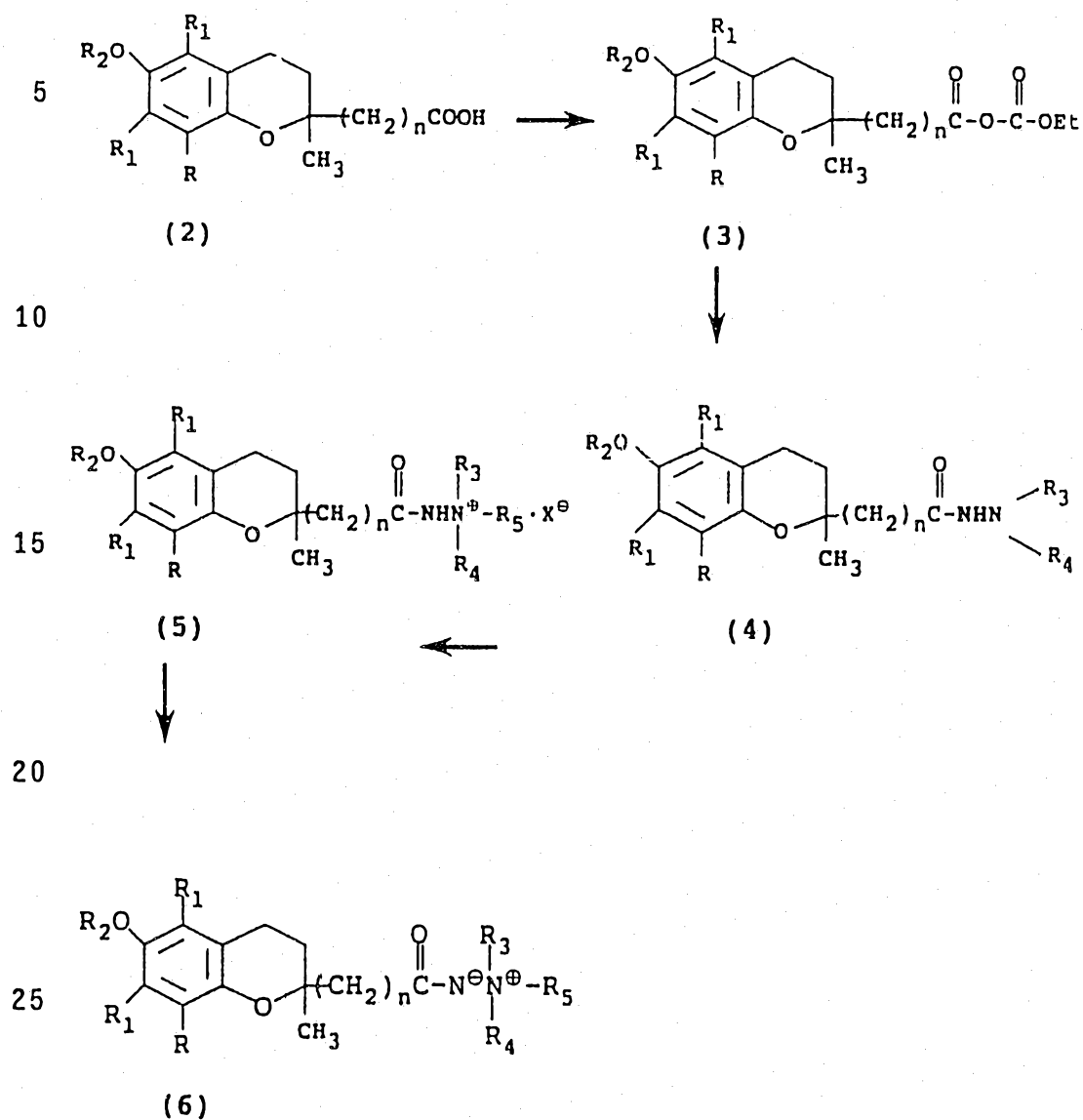
In general, the compounds of this invention may be prepared by standard chemical processes and techniques analogously known in the art from materials which are either known per se or which may be prepared in an analogous manner. Preferably the starting materials are in the desired enantiomeric form. The overall process for the preparation of the compounds of this invention may be depicted by the following reaction scheme with the chemical process and techniques being taught by the specific examples herein described.

20

25

30

35

REACTION SCHEME A

30 wherein R, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, n and X are as previously defined.

35

In essence, the reaction scheme entails the conversion of the acids (2) to a mixed anhydride (3) by reaction with ethylchloroformate at low temperatures (below 0°C) under anhydrous conditions. The resulting mixed anhydrides are  
5 treated with an unsymmetrical dialkylhydrazine, preferably at about room temperature to produce the hydrazides of formula (4). Upon reaction with the appropriate alkylhalide or alkylsulfonates (e.g.  $R_5X$  wherein X is a halide or  $SO_3R_5$ ) the hydrazide is converted to its corresponding acyl hydrazinium salt (5) and, by treatment with base, preferably  
10 sodium hydroxide, to the inner salt (6). Of course acylation of a hydroxy function at the 6-position of the 3,4-dihydro-benzopyran may be effected by reaction with an acid anhydride or acid halide to produce the desired formyl  
15 or alkylcarbonyloxy moieties. Conversion back to the 6-OH moiety may be effected by hydrolysis, said acylation and hydrolysis reaction being well known in the art.

The following examples illustrate the processes by  
20 which the compounds of this invention may be prepared.

25

30

35

EXAMPLE 1

2-[(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-yl)carbonyl]-1,1,1-trimethylhydrazinium 4-methylbenzenesulfonate

## STEP A:

A solution of 10.0 g (0.04 m) of 3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-carboxylic acid and 4.05 g (0.04 m) of triethylamine in 100 ml of dry tetrahydrofuran is cooled to -10°C under conditions that exclude moisture (drying tube) and a solution of 4.34 g (0.04 m) of ethyl chloroformate in 50 ml of tetrahydrofuran is added at such a rate that the internal temperature does not rise above 0°C. After completed addition (about 15 minutes) the mixture is stirred at 0°C for 35 minutes. Then, a solution of 2.40 g (0.04 m) of unsymmetrical dimethylhydrazine in 30 ml of tetrahydrofuran is added, the mixture is allowed to warm to room temperature and is stirred overnight. The solvent is evaporated under reduced pressure, water is added and the product is extracted with ethyl acetate. The extract is washed with water and sodium bicarbonate solution, dried over sodium sulfate, filtered and evaporated to give 9.24 g of an oil. It is crystallized and recrystallized from ethyl acetate/heptane to give 6.13 g of 3,4-dihydro-6-hydroxy-N',N',2,5,7,8-hexamethyl-2H-1-benzopyran-2-carboxhydrazide, m.p. 145-146°C.

## STEP B:

A solution of 5.58 g (0.01922 m) of the above dimethylhydrazide and 3.94 g (0.02114 m) of methyl p-toluenesulfonate in 60 ml of acetonitrile is refluxed for 6 hours. The mixture is allowed to cool overnight and crystalline material that formed is collected and recrystallized from acetonitrile/water to give 6.52 g of the title compound,

m.p. 244°C (decomposition). Elemental analysis, UV, IR and <sup>1</sup>H-NMR spectra confirm the structure.

5

EXAMPLE 2

2-[(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-yl)carbonyl]-1,1,1-trimethylhydrazinium, inner salt

10

To a solution of 3.0 g (0.00627 m) of the compound described in Example 1 in 10 ml of water and 10 ml of ethanol is added 3.2 ml of 2N NaOH. The solution is evaporated to dryness and the residue is slurried in 15 ml of water. The solid is collected and is recrystallized twice from water to give the title compound, m.p. 187°C (decomposition).

20

EXAMPLE 3

2-(3,4-Dihydro-6-hydroxy-2,5,8-tetramethyl-2H-1-benzopyran-2-acetyl)-1,1,1-trimethylhydrazinium 4-methylbenzenesulfonate

25

Using the procedure described in Example 1 but starting with 3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-acetic acid gives the dimethylhydrazide derivative of that acid, m.p. 151-152°C.

30

Treatment with 10% excess methyl p-toluenesulfonate in refluxing acetonitrile, as described in Example 1, gives the title compound, m.p. 182-183°C.

35

EXAMPLE 4

2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-acetyl)-1,1,1-trimethylhydrazinium, inner salt

5

Treatment of the tosylate described in the preceding example with an equivalent of base, as described in Example 2, gives the internal salt, m.p. 219°C (decomposition). Elemental analysis, UV, IR and <sup>1</sup>H-NMR spectra confirm the structure.

10

EXAMPLE 5

Resolution of 2S(-)- and 2R(+)-2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-acetyl)-1,1,1-trimethylhydrazinium 4-methylbenzenesulfonate and inner salt

15

Step A:

Resolution of 3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-acetic Acid

20

To a hot solution of 132.16 g of the title compound in 700 ml of isopropanol is added 60.59 g of S(-)-α-methylbenzylamine and 100 ml of ethyl acetate. Slow crystallization overnight in a refrigerator gives somewhat more than half the theoretical amount of crystalline material (checked by evaporating the filtrate to dryness). This material is recrystallized in a like manner three times and the resulting pure diastereomeric salt is converted to free acid by shaking in 200 ml of 2N hydrochloric acid and 400 ml of ethyl acetate. The aqueous phase is separated and extracted with ethyl acetate. The combined organic phase is washed with 2N hydrochloric acid, water, and a saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The resulting

25

30

35

solid is recrystallized from ethyl acetate/heptane to give 40.85 g (62%) of the S-(-)-enantiomer of the title compound,  $[\alpha]_D^{25} = -9.61^\circ$  (0.95% in MeOH). The enantiomeric purity, as determined by HPLC is ee = 99.9%. Elemental analysis was within 0.3% of theory.

The combined filtrates of the above diastereomeric salt crystallizations are evaporated and converted to free acid as described to give 92.02 g of material. It is dissolved in 600 ml of isopropanol and 42.19 g of R-(+)- $\alpha$ -methylbenzylamine is added as well as 200 ml of ethyl acetate. Slow crystallization and two recrystallizations give, after conversion to free acid and one final recrystallization, 41.50 g (63%) of the R-(+)-enantiomer of the title compound,  $[\alpha]_D^{25} = +9.35^\circ$  (0.96% in MeOH) ee = 99.9%. Anal. C, H.

It is possible to recover the unresolved balance of material from the filtrates as well as the enantiomeric amines for use in a subsequent resolution.

## 20 STEP B:

Using the procedure described in Examples 1 and 3, but starting with the 2S-(-)- and 2R-(+)- enantiomers of the acid described in Example 5, Step A, gives the 4-methylbenzenesulfonates of the two enantiomers of the title compound.

These 4-methylbenzenesulfonate salts are converted to the inner salts by the procedure described in Examples 2 and 4 to give the two enantiomers of the title compound,  $[\alpha]_D^{25} = + 2.30^\circ$  and  $-3.07^\circ$ , respectively (1% in MeOH).

The compounds of this invention are free radical scavengers. Free radical reactions have been implicated in the pathology of more than 50 human diseases. Radicals and other reactive oxygen species are formed constantly in the human body both by deliberate synthesis (e.g. by activated phagocytes) and by chemical side-reactions. They are removed by enzymic and non-enzymic antioxidant defence systems. Oxidative stress, occurring when antioxidant defences are inadequate, can damage lipids, proteins, carbohydrates and DNA. A few clinical conditions are caused by oxidative stress, but more often the stress results from the disease and can make a significant contribution to the disease pathology. For a more detailed review see B. Halliwell in *Drugs*, 1991, 42, 569-605.

When the blood supply to parts of the heart muscle is blocked, a myocardial infarct (heart attack) results and the deprived muscle tissue dies with the result of permanent heart damage. If the blood supply can be re-established within hours after ischemia, the heart muscle tissue remains viable and permanent damage can be reduced. This can be accomplished by surgical as well as pharmacologic (thrombolysis) procedures and these processes are known as reperfusion.

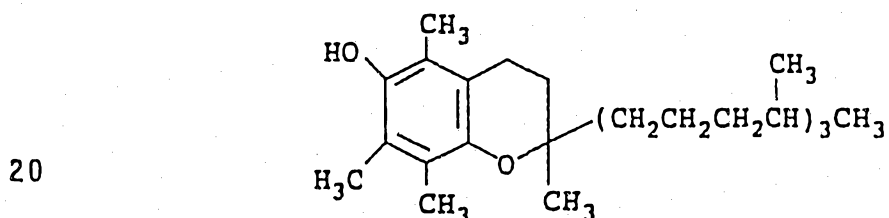
Ischemia followed by reperfusion causes formation of oxygen-derived free radicals and increased lipid peroxidation and results in tissue injury. Administration of free radical scavengers to animals subjected to coronary infarction/reperfusion or ischemia/reperfusion or surgical interventions/reperfusion reduces these effects in heart, lung, kidney, pancreas, brain and other tissues.

Reperfusion is now widely and successfully applied and it has been claimed that fatalities due to myocardial infarction can be reduced by 20-30%. However, reperfusion



also poses problems. Oxygen-deprived (ischemic) tissue finds itself in an abnormal state and is vulnerable when suddenly exposed to oxygen-rich blood. This has been termed the "oxygen paradox" and leads to reperfusion damage in the form of cell death. It has been postulated that this damage is due to oxygen-derived free radicals. Evidence for this hypothesis has been obtained in animal experiments. B.R. Lucchesi and coworkers showed that the enzyme superoxide dismutase, as well as the free radical scavenger N-(mercaptopropionyl)-glycine reduce canine myocardial reperfusion injury (Cir. Res., 1984, 54, 277-285; J. Cardiovasc. Pharmacol., 1986, 8, 978-88; Fed. Proc., 1987, 46, 2413-21).

15 Vitamin E, i.e.,  $\alpha$ -tocopherol, a well known compound of  
the formula



is a natural anti-oxidant that reacts with oxygen-derived free radicals as well as hydrogen peroxide. It has been shown that it is intercalated in lipid membranes and that its biological function is to protect biomembranes against oxidative attack. The anti-oxidant 3,4-dihydro-2,5,7,8-tetramethyl-2H-2-benzopyran-6-ol moiety of  $\alpha$ -tocopherol is constantly regenerated by the ubiquitous redox systems.

30 The compounds of the present invention are also useful  
in treating the process of inflammation which is known to  
involve the release of superoxide radicals from phagocytic  
cells which cause some of the symptoms of rheumatoid  
arthritis and other inflammatory diseases such as  
35 ulcerative colitis and inflammatory dermatological

disorders such as psoriasis. Of particular use of this anti-inflammatory effect of the compounds of this invention is the treatment of inflammatory lower bowel disease. .

5 Inhalation injury of the lungs is typically caused by heat and chemical irritation, and chemical injury is the leading lethal cause of smoke inhalation injury. Smoke inhalation leads to lung injury due to an increase in pulmonary microvasculature and pulmonary edema. This  
10 process is accompanied by increased lipid peroxidation in lung tissue. An inhibitor of lipid peroxidation was shown to reduce these symptoms in animals subjected to hot sawdust smoke by Z. Min et al., (*J. Med. Cell. PLA*, 1990, 5, 176-180). They suggest the use of antioxidants in treatment  
15 of smoke inhalation-lung injury, adult respiratory distress syndrome and emphysema.

Reactive oxygen species also play a role in the formation of foam cells in atherosclerotic plaques  
20 (reviewed by D. Steinberg et al., *New Engl. J. Med.*, 1989, 320, 915-924) and the free radical scavenger probucol has a marked antiatherosclerotic effect in hyperlipidemic rabbits (Carew et al., *Proc. Nat. Acad. Sci. USA*, 1987, 84, 7725-7729. Degenerative retinal damage and diabetogenic retinopathy  
25 have also been listed as target for treatment with free radical scavengers (cf. J.W. Baynes, *Diabetes*, 1991, 40, 405-412; S.P. Wolff et al., *Free Rad. Biol. Med.*, 1991, 10, 339-352).

30 The compounds may also be useful in the treatment of cancers, and degenerative diseases related to aging, stroke, and head trauma, since oxygen-derived free radicals have been identified among causative factors. For reviews, see B. Halliwell and C. Gutteridge, *Biochem. J.*, 1984, 219,  
35 1-14; *TINS* 1985, 22-6. Antioxidants have also been shown to

be useful in the treatment of cataracts, *Free Rad. Biol. Med.*, 12:251-261 (1992).

*In vitro* and *in vivo* activity for the compounds of this  
5 invention may be determined by the use of standard assays which demonstrate the free radical scavenging property, affinity for cardiac tissue and cardioprotective properties, as well as by comparison with agents known to be effective for these purposes.

10

Exemplary of the assay useful for determining the free-radical scavenging property of the compounds of this invention is by the *in vitro* inhibition of lipid peroxidation in rat brain homogenates.

15

The free radical scavenging properties of the compounds may readily be evaluated wherein superoxide radicals are generated by 4 ml of xanthine oxidase in the presence of 0.1 mM xanthine and detected by reduction of 40  $\mu$ M nitro  
20 blue tetrazolium (NBT) to the diformazan dye in a spectrophotometric assay as described by C. Beauchamp and I. Fridovick, (*Analyt. Biochem.* 1971, 44, 276-287). 30 U of superoxide dismutase inhibited this reduction by 90% which is due to superoxide radicals. In the presence of a  
25 superoxide scavenger (test compound) there is a competition for the superoxide radical and thus a reduction in the color formation of NBT demonstrates the superoxide radical scavenging property of the test compound.

30 Inhibiting the process of lipid peroxidation may be assayed using tissue homogenates for measuring the antioxidant activity of biological fluids by the methodology of J. Stocks et al., (*Clin. Sci. Mol. Med.*, 1974, 47, 215-222), wherein a brain tissue homogenate of treated  
35 adult Sprague Dawley rats is utilized. Samples of total

volume 1 ml of diluted brain homogenate and with the scavenger at an appropriate dilution are incubated. Non-incubated samples are taken as background. Controls are run without scavenger and a sample containing only buffer is taken as blank. After incubation at 37°C for 30 minutes, 200 µl of 35% perchloric acid is added, the samples centrifuged and 800 µl of the supernatants mixed with 200 µl of 1% thiobarbituric acid. The pink condensation product of thiobarbituric acid reactive material is developed at 100°C in a boiling water bath for 15 minutes, and absorbance read at 532 nm.

For *ex vivo* inhibition of tissue including heart tissue, lipid peroxidation in mice may be utilized to demonstrate the ability of the compounds to penetrate and act as free radical scavengers in these tissues. This assay involves pretreatment of male CD1 mice by subcutaneous administration of the test compound. One hour later the tissues are excised, homogenized 1+9 (w/v) in 20 mM potassium phosphate buffer at pH 7.3 (0.14 M KCl) and incubated at 1/100 concentration in 1 ml of buffer at 37°C for 30-120 minutes. At the end of the incubation 200 µl of 35% perchloric acid is added and proteins removed by centrifugation. To 800 µl of the supernatant are added 200 µl of 1% TBA and the samples are treated to 100°C for 15 minutes. The TBA-adduct is extracted into 2 times 1 ml of n-butanol. The fluorescence is measured at an excitation wavelength of 515 nm and an emission wavelength of 553 nm against a standard prepared from malondialdehyde dimethylacetal.

30

Stimulated human leukocytes release radicals and other oxygen metabolites, which, during inflammation, act as microbial agents. At the same time, they release proteolytic enzymes, such as elastase, which are also microbicidal but potentially threaten the connective tissue

35

of the host. An endogenous  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ Pi) normally protects the host tissue from proteolytic digestion.  $\alpha_1$ Pi is however, inactivated by the leukocyte-derived oxidants. Antagonism of the inactivation of  $\alpha_1$ Pi is an indication of the disclosed radical scavengers. The concentration needed to protect 50% of the elastase inhibitory capacity of  $\alpha_1$ Pi ( $PC_{50}$ ) depends on the amount of stimulated leukocytes present.

10 *Method:* The procedure described by Skosey and Chow was followed (see J.L. Skosey and D.C. Chow in *Handbook of Methods for Oxygen Radical Research* (Greenwald, R.A., ed.) 1985, pp.413-416, CRC Press, Boca Raton). In short, human  $\alpha_1$ Pi was incubated with zymosan-stimulated human peripheral-blood  
15 leukocytes in the absence or presence of the scavengers. The amount of  $\alpha_1$ Pi protected from oxidative inactivation was determined by its residual elastase inhibitory capacity.

The relevance to inflammation matter has been reviewed  
20 by Weiss (see S.J. Weiss, *N. England J. Med.*, 1989, 320, 365-376). Lung emphysema is associated with a genetic defect in  $\alpha_1$ Pi; the disease is further enhanced by oxidants inhaled during cigarette smoking, which leads to oxidative inactivation of  $\alpha_1$ Pi in the lung tissue (see J. Travis and  
25 G.S. Salvesen, *Annu. Rev. Biochem.*, 1983, 52, 655-709). Oxidized  $\alpha_1$ Pi has also been isolated from rheumatoid synovial fluid (see P.S. Wong and J. Travis, *Biochem. Biophys. Roc. Commun.*, 1980, 06, 1440-1454). The degradation of hyaluronic acid, a macromolecule accounting for the  
30 viscosity of synovial fluid, is triggered by superoxyl radicals released from human leukocytes *in vitro* (see R.A. Greenwald and S.A. Moak, *Inflammation*, 1986, 10, 15-30). Furthermore, nonsteroidal anti-inflammatory drugs were shown to inhibit the release of superoxyl radicals from  
35 leukocytes (see H. Strom and I. Ahnfelt-Ronne, *Agents and*

Actions, 1989, 26, 235-237 and M. Roch-Arveiller, V. Revelant, D. Pharm Huy, L. Maman, J. Fontagne, J.R.J. Sorenson and J.P. Giroud, *Agents and Actions*, 1990, 31, 65-71), and 5-aminosalicylic acid may exert its therapeutic  
5 activity in inflammatory bowel disease by a radical scavenger mechanism (see I. Ahnfelt-Ronne, O.H. Nielsen, A. Christensen, E. Langholz, V. Binder and P. Riis, *Gastroenterology*, 1990, 98, 1162-1169). Therefore, it is believed that the compounds of this invention may be useful in the  
10 mentioned pathologic situations and that inflammatory bowel disease may be a special target. An immune stimulatory effect of antioxidants has also been reported in that they enhanced lymphocyte activity (R. Anderson and P.T. Lukey, *Ann. N.Y. Acad. Sci.*, 1987, 498, 229-247) *in vitro* in the presence  
15 of triggered leukocytes, and *ex vivo* after pretreatment of human volunteers.

Thus, using standard and well known methodology, as well as by comparison with known compounds found useful, it  
20 is to be found that the compounds are free radical scavengers useful in the prevention and treatment of such disease states related to neurotoxicity due to excessive glutamic acid release, to Huntington's disease, Alzheimer's disease and other cognitive dysfunctions, (e.g. memory,  
25 learning and attention deficits), amnesia, and Parkinson's disease, as well as the treatment and prevention of tissue damage in heart, lung, kidney, pancreas and brain tissues induced by ischemia/reperfusion, surgical intervention and infarction/reperfusion and to allay acute blood loss due to  
30 hemorrhagic shock.

The compounds of this invention are related to  $\alpha$ -tocopherol and also possess a 3,4-dihydro-6-hydroxy-2,5,7,8-tetraalkyl-2H-1-benzopyran-2-yl moiety, but the  
35 2-position lipophylic moiety of the  $\alpha$ -tocopherol molecule,

which is thought to be responsible for its ubiquitous incorporation into biomembranes, is replaced with a hydrophilic moiety to impart a greater affinity for cardiac tissue. Thus, the compounds of this invention are also  
5 useful as pharmacologic antioxidants and free radical scavengers and, in particular, as scavengers of superoxide anion radical  $O_2^-$ . They can be therapeutically employed where reperfusion damage due to oxygen-derived free radicals and hydrogen peroxide causes cell death in  
10 tissues. This situation arises when total or partial blockade of blood supply to tissues is removed, either spontaneously (transient ischemia) or by pharmacologic or surgical intervention (thrombolysis, angioplasty, by-pass, organ transplant and the like). Tissues subjected to  
15 transient ischemia or reperfusion in various disease states, or by their medical treatment, are those of heart, lung, kidney, pancreas and brain. In particular, the now rapidly increasing practice of pharmacologic thrombolysis, also known as reperfusion, after coronary infarct and  
20 stroke, will benefit by prior or concomitant administration of a free radical scavenger such as the compounds of this invention. Similarly, surgical interventions, such as percutaneous transluminal coronary angioplasty, where a dilating balloon is used to increase the luminal diameter  
25 in severely occluded atherosclerotic vessels, coronary by-pass operations, and organ transplant surgery create conditions where reperfusion damage due to oxygen-derived radicals takes place and can be reduced by scavengers. Transient ischemia is one of the causative factors that  
30 lead to angina pectoris, and thus the compounds of this invention are also useful as antianginal agents.

The compounds of this invention may also be evaluated in ligation-induced infarcted and reperfused rats as  
35 follows. One of two groups of rats is infused intravenously

with a solution of a test compound in saline at a rate of 2.3 ml/h (10 mg/kg/h). The control group is infused with saline at the same rate. After 10 minutes of drug infusion, coronary arteries are ligated surgically for 60 minutes, ligation is loosened to allow reperfusion for 30 minutes. The ligation is retied and a dye (Evans Blue) is injected. The animals are sacrificed and the heart ventricles are removed and weighed. The unstained tissue is dissected and weighed; this represents the "area at risk", i.e. the area that is deprived of blood supply by ligation. To determine the infarcted area, the tissue is incubated with 2,3,5-triphenyltetrazolium chloride. Infarcted tissue becomes light colored and can be dissected and weighed. Thus, for each rat that survives the ligation a measurement of "area at risk" (RA) and of "infarcted area" (IA) is obtained and the ratio IA/RA is calculated.

The compounds of this invention can be utilized both prophylactically and therapeutically. The amount of active ingredient for therapeutic administration can vary over a wide range and is dependent upon such factors as the species of patient to be treated, its age, health, sex, weight, nature and the severity of the condition being treated. The term "patient" refers to a warm-blooded animal such as, for example, rats, mice, dogs, cats, guinea pigs, primates, and humans. Generally, a therapeutically effective amount of the active ingredient to be administered will range from about 0.1 mg/kg to 30 mg/kg of body weight per day. For prophylactic administration, corresponding lower doses can be utilized. Preferably, the compounds of the present invention will be administered to the patient in combination with a pharmaceutically acceptable carrier which is any substance which aids in the administration of the compound without substantially affecting its therapeutic properties.



Most preferably, the compounds are administered intravenously particularly under crisis situations wherein it is essential that the therapeutic agent be gotten to its site of action as quickly as possible, such as in those emergency conditions caused by coronary infraction, stroke and surgical interventions, conditions which can cause severe reperfusion damage.

10 The compounds of this invention also can be orally administered, preferably using more active ingredient per day than when parenterally administered, preferably taking divided doses 3 to 4 times per day. Preferably, enteral administration in post "crisis" situations, particularly after release from hospitalized conditions. The compounds can be used in standard dosage unit forms such as tablets, capsules, dragees, lozenges, elixirs, emulsions, suspensions, and in cases wherein topical application is preferred by suppository or sub-lingual administration.

20 Tablets and capsules containing from 100 to 400 mg of active ingredient are preferred modes of enteral administration. Of course, in the treatment of inflammation the preferred method of administration is by depot injection directly to the situs of the inflammation area with follow-up enteral means of administration.

In preparing solid dose forms such as tablets, the active ingredient is generally blended with conventional pharmaceutical carriers or excipients such as gelatin, various starches, lactose, calcium phosphate or powdered sugar. The term pharmaceutical carrier as used herein also includes lubricants employed to improve the flow of tablet granulations and which prevent adhesion of tablet material to the surfaces of tablet dies and punches. Suitable lubricants include, for example, talc stearic acid, calcium

stearate, magnesium stearate and zinc stearate. Also included within the definition of a pharmaceutical carrier as used herein, are disintegrating agents added to assist the breakup and dissolution of tablets following administration, as well as coloring and/or flavoring agents to enhance the aesthetic qualities of the tablets and make them more acceptable to the patient. For its own effects as an anti-oxidant, ascorbic acid also is advantageously utilized in formulations with the active ingredients of formula (1).

Suitable liquid excipients for the preparation of liquid dosage unit forms include water and alcohols such as ethanol, benzyl alcohol and the polyethylene glycols, either with or without the addition of a surfactant. In general, the preferred liquid excipients, particularly for injectable preparations, include water, physiological and saline solutions, dextrose and glycol solutions such as an aqueous propylene glycol or polyethylene glycol solutions. In order to minimize or eliminate irritation at the site of injection, such compositions may contain a non-ionic surfactant having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations ranges from about 5 to 15% by weight. The surfactant can be a single component having the above-identified HLB, or a mixture of two or more components having the desired HLB. Illustrative of surfactants useful in parenteral formulations are the class of polyoxyethylene sorbitan fatty acid esters as, for example, sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. In certain topical and parenteral preparations, various oils can be utilized as carriers or excipients. Illustrative of such oils are mineral oils, glyceride oils such as lard

oil, cod liver oil, peanut oil, sesame oil, corn oil and soybean oil. For insoluble compounds, suspending agents may be added as well as agents to control the viscosity, as for example, magnesium aluminum silicate or carboxymethyl-cellulose. In addition to these excipients, buffers, preservatives and emulsifying agents may also be added. Typical enema preparation of the retention type enema utilize small volumes, generally much less than about 150 mL for an adult, typically volumes of only a few milliliters are preferred. Excipients and solvents for use in retention enemas should, of course, be selected so as to avoid colonic irritation and should also be selected so as to minimize absorption of the various agents.

The compounds of this invention can also be administered topically. This can be accomplished by simply preparing a solution of the compound to be administered, preferably using a solvent known to promote transdermal absorption such as ethanol or dimethyl sulfoxide (DMSO) with or without other excipients. Preferably topical administration will be accomplished using a patch either of the reservoir and porous membrane type or of a solid matrix variety.

Some suitable transdermal devices are described in U.S. Pat. Nos. 3,742,951, 3,797,494, 3,996,934, and 4,031,894. These devices generally contain a backing member which defines one of its face surfaces, an active agent permeable adhesive layer defining the other face surface and at least one reservoir containing the active agent interposed between the face surfaces. Alternatively, the active agent may be contained in a plurality of microcapsules distributed throughout the permeable adhesive layer. In either case, the active agent is delivered continuously from the reservoir or microcapsules through a membrane into the active agent permeable adhesive, which is in contact

with the skin or mucosa of the recipient. If the active agent is absorbed through the skin, a controlled and predetermined flow of the active agent is administered to the recipient. In the case of microcapsules, the  
5 encapsulating agent may also function as the membrane.

In another device for transdermally administering the compounds in accordance with the present invention, the pharmaceutically active compound is contained in a matrix  
10 from which it is delivered in the desired gradual, constant and controlled rate. The matrix is permeable to the release of the compound through diffusion or microporous flow. The release is rate controlling. Such a system, which requires no membrane is described in U.S. Pat. No. 3,921,636. At  
15 least two types of release are possible in these systems. Release by diffusion occurs when the matrix is non-porous. The pharmaceutically effective compound dissolves in and diffuses through the matrix itself. Release by microporous flow occurs when the pharmaceutically effective compound is  
20 transported through a liquid phase in the pores of the matrix.

The compounds of the present invention may be incorporated into an aerosol preparation by means commonly  
25 known to those skilled in the art. The aerosol preparation may be prepared for use as a topical aerosol or may be prepared for inhalation. The aerosol preparation may be in the form of a solution or suspension and may contain other ingredients such as solvents, propellants and/or dispersing  
30 agents. Typical examples of aerosol preparations are shown in *Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Company, Easton Pennsylvania, pp. 1694-1712 (1990)* incorporated herein by reference.

Of course, as is true in most instances wherein certain classes of chemical compounds have been found to have beneficial therapeutic end-use applications, certain sub-generic groups and certain specific compounds are preferred. In this instance the preferred compounds of Formula I are those wherein  $R_3$ ,  $R_4$  and  $R_5$  are each methyl;  $R$  is methyl; each  $R_1$  is methyl,  $R_2$  is hydrogen;  $n$  is zero or one; and/or  $X$  is 4-methylbenzenesulfonate or nothing when the inner salt is formed.

10

15

20

25

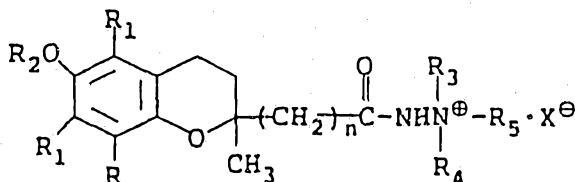
30

35

## WHAT IS CLAIMED IS:

## 1. Compounds of the formula

5

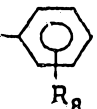


10

(1)

the stereoisomers and mixtures thereof, their inner salts,  
~~or~~  
~~and the pharmaceutically acceptable salts thereof~~ wherein

- 15 R is H or C<sub>1-6</sub> alkyl,  
 R<sub>1</sub> is C<sub>1-6</sub> alkyl,  
 R<sub>2</sub> is H or -C(O)R, R<sub>3</sub> and R<sub>4</sub> are independently C<sub>1-6</sub> alkyl;  
 R<sub>5</sub> is C<sub>1-6</sub> alkyl, or

- 20 -CH<sub>2</sub>  with R<sub>8</sub> being H or halogeno,  
 R<sub>8</sub>

n is zero, 1, 2, or 3 and

X is a halide, -S(O)<sub>3</sub>R<sub>6</sub>, or nothing when the inner salt  
 is formed.

- 25 R<sub>6</sub> is H, C<sub>1-6</sub> alkyl, phenyl or 4-methylphenyl.

2. The compounds of Claim 1 wherein each of R<sub>3</sub>, R<sub>4</sub> and  
 R<sub>5</sub> are methyl.

- 30 3. The compounds of Claim 1, <sup>or Claim 2</sup> wherein each of R<sub>1</sub> are  
 methyl.

4. The compounds of Claims 1 <sup>any one</sup> <sup>to 3</sup> wherein X is -S(O)<sub>3</sub>R<sub>6</sub>.

35

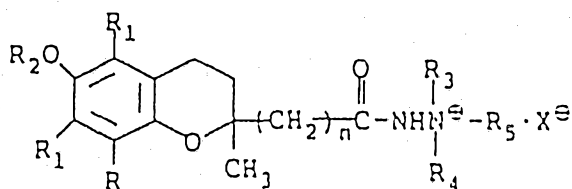
5. The compounds <sup>any one</sup> of Claims <sup>to 3</sup> 1 wherein X is nothing.
6. The compounds <sup>any one</sup> of Claims <sup>to 5</sup> 1 wherein R<sub>2</sub> is hydrogen.
7. The compounds of Claim 1 wherein the compound is 2-[(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-yl)carbonyl]-1,1,1-trimethylhydrazinium 4-methylbenzenesulfonate and enantiomers thereof.
8. The compounds of Claim 1 wherein the compound is 2-[(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-yl)carbonyl]-1,1,1-trimethylhydrazinium, inner salt and enantiomers thereof.
9. The compounds of Claim 1 wherein the compound is 2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-acetyl)-1,1,1-trimethylhydrazinium 4-methylbenzenesulfonate and enantiomers thereof.
10. The compounds of Claim 1 wherein the compound is 2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-acetyl)-1,1,1-trimethylhydrazinium, inner salt and enantiomers thereof.
11. A pharmaceutical composition <sup>including</sup> ~~comprising~~ a compound of Claim 1 and a pharmaceutically acceptable carrier.
12. A method of treating a patient for reperfusion damage by administering an effective amount of a compound of Claim 1.
13. A method of treating a patient for inflammatory bowel disease by administering an effective amount of a compound of Claim 1.

14. Use of a compound according to any one of Claims 1 to 10 in the treatment of inflammatory bowel disease or reperfusion damage.

15. Use of a compound according to any one of Claims 1 to 10, in combination with a pharmaceutically acceptable carrier, for the preparation of a pharmaceutical composition according to Claim 11.

16. A process of making a compound of the formula

10



(1)

15 the stereoisomers and mixtures thereof, their inner salts, wherein

R is H or C<sub>1-6</sub> alkyl,

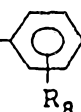
R<sub>1</sub> is C<sub>1-6</sub> alkyl,

R<sub>2</sub> is H or -C(O)R,

R<sub>3</sub> and R<sub>4</sub> are independently C<sub>1-6</sub> alkyl;

20 R<sub>5</sub> is C<sub>1-6</sub> alkyl, or



$-\text{CH}_2-$   with  $R_8$  being H or halogeno,

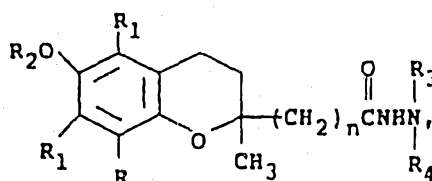
$n$  is zero, 1, 2, or 3 and

$X$  is a halide,  $-\text{S}(\text{O})_3R_6$ , or nothing when the inner salt  
5 is formed,

$R_6$  is H,  $C_{1-6}$  alkyl, phenyl or 4-methylphenyl,

~~including~~ ~~comprising~~ the steps of reacting the hydrazine

10

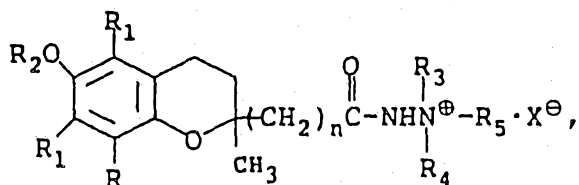


(4)

15

as previously defined, with  $R_5X'$  wherein  $X'$  is a halide or  
- $\text{S}(\text{O})_3R_6$  as previously defined and refluxing to produce the  
acyl hydrazinium salt

20

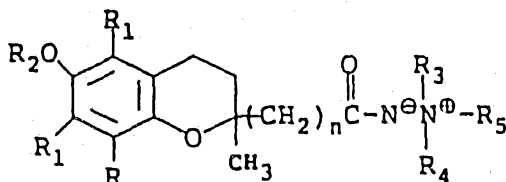


(5)

25

as previously defined, and optionally treating the fore-  
going acyl hydrazinium with a base in order to produce the  
inner salt

30



(6)

35

17. A compound according to Claim 1, substantially as herein described with reference to any one of the Examples.

18. A process of making a compound according to Claim 1, substantially as  
5 herein described with reference to any one of the Examples.

10 DATED: 13 December, 1996  
PHILLIPS ORMONDE & FITZPATRICK  
Attorneys for:  
**MERRELL DOW PHARMACEUTICALS INC.**

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/02311

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 C07D311/66; C07D311/58		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.Cl. 5	C07D	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
A	EP,A,0 369 083 (MERREL DOW PHARMACEUTICALS) 23 May 1990 see page 2 ---	1, 15
A	EP,A,0 036 169 (BASF) 23 September 1981 see page 1 ---	1
A	EP,A,0 293 078 (UPJOHN) 30 November 1988 see page 9, line 3 - page 11, line 37; example 23 -----	1, 15
<p><sup>10</sup> 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> <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</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>"A" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
14 JUNE 1993	21. 06. 93	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	RUSSELL F. ENGLISH	

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/02311

**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:  
Although claims 12 and 13 are directed to a method of treatment of the human or animal body, the search has been carried out and based on the alleged effects of the compound.
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:
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.

ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.

US 9302311  
SA 71584

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

14/06/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0369083	23-05-90	AU-B- 620660	20-02-92
		AU-A- 4468289	17-05-90
		EP-A- 0369874	23-05-90
		JP-A- 2178278	11-07-90
-----			
EP-A-0036169	23-09-81	DE-A- 3010505	01-10-81
		JP-C- 1518434	07-09-89
		JP-A- 56145283	11-11-81
		JP-B- 63066316	20-12-88
		US-A- 4511685	16-04-85
-----			
EP-A-0293078	30-11-88	AU-B- 624788	25-06-92
		AU-A- 1709888	02-12-88
		EP-A- 0358676	21-03-90
		EP-A- 0487510	27-05-92
		JP-T- 2503198	04-10-90
		WO-A- 8808424	03-11-88
US-A- 5120843	09-06-92		
-----			