INSTRUCTIONS

(a) If Convention application insert "Convention"

601554

(a) Convention
AUSTRALIA

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APPLICATION FOR A (b) STANDARD/~~PETTY~~ PATENT

(b) Delete one

I/We (c) **MERRELL DOW PHARMACEUTICALS INC.**

(c) Insert FULL name(s) of applicant(s)

of (d) **2110 East Galbraith Road**
Cincinnati, Ohio 45215
United States of America

(d) Insert FULL address(es) of applicant(s)

hereby apply for the grant of a (e) Standard/~~Petty~~ Patent for an invention entitled (f)

(e) Delete one

METHOD OF INHIBITING INTERLEUKIN-1 RELEASE

(f) Insert TITLE of invention

which is described in the accompanying (g) **complete** specification.

(g) Insert "complete" or "provisional" or "petty patent"

(**Note:** *The following applies only to Convention applications*)

Details of basic application(s)

(h)

Application No.	Country	Filing Date
026,587	United States of America	March 17, 1987
151,521	United States of America	February 18, 1988

(h) Insert number, country and filing date for the/or each basic application

Address for Service: PHILLIPS ORMONDE AND FITZPATRICK
Patent and Trade Mark Attorneys
367 Collins Street
Melbourne, Australia 3000

APPLICATION ACCEPTED AND AMENDMENTS ALLOWED 11.7.90

(i) Insert date of signing

Dated (i) February 22, 1988

(j) Signature of applicant(s) (For body corporate see headnote*)

(j) MERRELL DOW PHARMACEUTICALS INC.

By Gary D. Street
Gary D. Street
Managing Patent Counsel

(k) Corporate seal if any

(k)

Note: No legalization or other witness required

1271A AU

PHILLIPS ORMONDE & FITZPATRICK
Patent and Trademark Attorneys
367 Collins Street
Melbourne, Australia

LODGED AT SUB-OFFICE
16 MAR 1988
Melbourne

AUSTRALIA

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DECLARATION FOR A PATENT APPLICATION

INSTRUCTIONS

(a) Insert "Convention" if applicable

(b) Insert FULL name(s) of applicant(s)

(c) Insert "of addition" if applicable

(d) Insert TITLE of invention

(e) Insert FULL name(s) AND address(es) of declarant(s) (See headnote*)

(f) Insert FULL name(s) AND address(es) of actual inventor(s)

(g) Recite how applicant(s) derive(s) title from actual inventor(s) (See headnote**)

(h) Insert country, filing date, and basic applicant(s) for the/or EACH basic application

(k) Insert PLACE of signing

(l) Insert DATE of signing

(m) Signature(s) of declarant(s)

Note: No legalization or other witness required

In support of the (a) **convention** application made by
(b)

MERRELL DOW PHARMACEUTICALS INC.

(hereinafter called "applicant(s)" for a patent (c) for an invention entitled (d)

METHOD OF INHIBITING INTERLEUKIN-1- RELEASE

I/~~We~~ (e) **Gary D. Street, Managing Patent Counsel**
MERRELL DOW PHARMACEUTICALS INC.
2110 East Galbraith Road
Cincinnati, Ohio 45215, United States of America

do solemnly and sincerely declare as follows:

~~1. I am/We are the applicant(s).~~
*(**or**, in the case of an application by a body corporate)*

1. I am/~~We~~ are authorized to make this declaration on behalf of the applicant(~~s~~).

~~2. I am/We are the actual inventor(s) of the invention.~~
*(**or**, where the applicant(s) is/are not the actual inventor(s))*

2. (f) **George Ku**
5700 Winton Road, Apt 312A
Cincinnati, Ohio 45232
United States of America

Niall Doherty
8024 Pepper Pike
West Chester, Ohio 45069
United States of America

~~is~~/are the actual inventor(s) of the invention and the facts upon which the applicant(~~s~~) is/~~are~~ entitled to make the application are as follows:

(g) **Applicant is the assignee of the above-entitled invention by virtue of a deeds of Assignment from the actual inventors dated March 17, 1987 and February 17, 1988.**

(**Note:** *Paragraphs 3 and 4 apply* **only** *to Convention applications)*

3. The basic application(~~s~~) for patent or similar protection on which the application is based is/~~are~~ identified by **country, filing date, and basic applicant(s)** as follows:

(h) **United States of America - March 17, 1987**
By: George Ku and Niall Doherty

United States of America - February 18, 1988
By: George Ku and Niall Doherty

4. The basic application(s) referred to in paragraph 3 hereof ~~was~~/were the first application(s) made in a Convention country in respect of the invention the subject of the application.

Declared at (k) **Cincinnati, Ohio, U.S.A.**

Date (l) **February 22, 1988**

(m) **MERRELL DOW PHARMACEUTICALS INC.**

By Gary D Street
Gary D. Street
Managing Patent Counsel

To: The Commissioner of Patents

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PHILLIPS ORMONDE & FITZPATRICK
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(12) PATENT ABRIDGMENT **(11)** Document No. **AU-B-13161/88**
(19) AUSTRALIAN PATENT OFFICE **(10)** Acceptance No. **601554**

(54) Title
METHOD OF INHIBITING INTERLEUKIN-1 RELEASE

International Patent Classification(s)
(51)[4] **A61K 031/145** **A61K 031/075** **A61K 031/135**

(21) Application No. : **13161/88** (22) Application Date : **16.03.88**

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151521	**18.02.88**	**US UNITED STATES OF AMERICA**

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MERRELL DOW PHARMACEUTICALS INC.

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PHILLIPS,ORMONDE & FITZPATRICK

(56) Prior Art Documents
US 1782111

(57) Claim

1. A method of inhibiting the release of interleukinl in animals which comprises administering to an animal in need thereof an effective amount of a compound selected from the group consisting of disulfiram, 2,4-di-isobutyl-6-(N,N-dimethylaminomethyl)-phenol, and tetrakis [3-(2,6-di-tert-butyl-4-hydroxyphenyl)propionyloxy methyl]methane.

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COMPLETE SPECIFICATION
(ORIGINAL)

Class Int. Class

Application Number:
Lodged:

Complete Specification Lodged:
Accepted:
Published:

Priority

Related Art:

APPLICANT'S REFERENCE: MO1271A AU

Name(s) of Applicant(s):

Merrell Dow Pharmaceuticals Inc

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UNITED STATES OF AMERICA.

Address for Service is:

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367 Collins Street
Melbourne 3000 AUSTRALIA

Complete Specification for the invention entitled:

METHOD OF INHIBITING INTERLEUKIN-1 RELEASE

Our Ref : 86177
POF Code: 1432/1432

The following statement is a full description of this invention, including the best method of performing it known to applicant(s):

METHOD OF INHIBITING INTERLEUKIN-1 RELEASE

Background of The Invention

Field of the Invention

Interleukin-1 (IL-1) is the name for a family of molecules which have multiple biological effects. The name interleukin-1 was proposed in 1979; and earlier literature reports refer to it by some other name. Murphy, *British Journal of Rheumatology*, 1985; **24(suppl 1)**: 6-9, and Oppenheim et al., *Immunology Today*, vol. 2, 45-55(1986). IL-1 is secreted by stimulated macrophages, and has several significant biological effects, such as mediation of T-lymphocyte proliferation and pyrogenic and proinflammatory effects.

IL-1 activities are summarized in the two above papers. IL-1 has been described to mediate the acute phase response in inflammation, and to have pyrogenic and proinflammatory effects. IL-1 induces connective tissue changes, and has been demonstrated to induce the release of degradative enzymes from mesenchymal cells that are present at the sites of bony erosion in inflammatory disease states, such as rheumatoid arthritis. Billingham,

Brit. J. Rheumatology, 1985:24(suppl 1):25-28. Dayer, *Brit. J. Rheumatology*, 1985:24(suppl 1):15-20. The production of acute phase proteins in the hepatocytes during the acute phase of inflammation is mediated by IL-1. Whicher, *Brit. J. Rheumatology*, 1985:24(suppl 1):21-24.

IL-1 is also involved as a mediator in the infalmmatory skin disease, psoriasis. Camp et al., J. Immunology 1986: 137: 3469-3474, and Ristow, Proc. Natl. Acad. Sci. USA 1987: 84: 1940-1944. It is cytotoxic for insulin producing beta cells in the pancreas, and is thus a causative factor in the development of diabetes mellitus. Bendtzen et al., Science 1986: 232: 1545-1547 and Marx, Science 1988: 239: 257-258, . IL-1 also appears to be involved in the development of atherosclerotic lesions or atherosclerotic plaque. Marx, Science 1988: 239: 257-258. In the absence or suppression of endogenous prostaglandins, IL-1 stimulates growth and proliferation of vascular smooth muscle cells, which could lead to thickening of vascular walls, such as occurs in atherogenesis. Libby et al., Fed. Proc. March 1, 1987: Vol. 46, no. 3: 975, Abstract 3837.

It would be advantageous to control the release of IL-1, and to be able to treat IL-1-mediated effects. It would also be advantageous to control or treat IL-1 mediated inflammations, without production of concomitant side effects known to accompany the use of antiinflammatory steroids and non-steroidal antiinflammatory agents.

Summary of the Invention

It has now been found that certain pharmaceutically-acceptable compounds can be used to inhibit the the release of IL-1, and thus to control or treat IL-1

mediated conditions. Compounds useful in practicing the method of the invention include disulfiram, tetrakis [3-(2,6-di-tert-butyl-4-hydroxyphenyl) propionyloxy methyl] methane and 2,4-di-isobutyl-6-(N, N-dimethylaminomethyl)-phenol. Although the compounds have diverse structures, the compounds all have antioxidant activity. Tetrakis [3-(2,6-di-tert-butyl-4-hydroxyphenyl) propionyloxy methyl] methane (also named as Irganox 1010 or as benzenepropanoic acid: 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, tetraester with 2,2-bis(hydroxymethyl)1,3-propanediol) is commercially used as an anantioxidant. The causal mechanism of any relationship between antioxidant activity of the compounds and their ability to inhibit IL-1 release is not known, and the invention is not limited to any particular theoretical mechanism.

Such compounds can be administered to animals to inhibit secretion of IL-1; to inhibit or treat IL-1-mediated effects; and to inhibit or treat IL-1-mediated inflammation.

In the method of the invention, one or more compound is administered to an animal, typically to a mammal in need of inhibition of IL-1 secretion, inhibition of IL-1-mediated effects, or inhibition of IL-1-mediated inflammation, in an amount effective to produce such inhibition. The compounds can be administered to inhibit or treat IL-1-mediated effects in conditions such as inflammation, psoriasis, atherosclerosis, and diabetes.

The compounds can be administered by conventional routes, oral administration being preferred. The dosage to be employed will vary according to factors such as the species, age, weight and condition of the particular animal being treated, and the particular compound

employed. Optimum dosages in particular situations can be determined by conventional dose range finding techniques.

In general, dosage levels for the use of the compounds to inhibit IL-1 release can be ascertained by conventional range finding studies. The compounds are preferably administered orally at daily dosages from about one to about 300 milligrams of active ingredient per kilogram of animal body weight. Useful results in inhibition of IL-1 release have been obtained with daily oral dosages of 100 milligrams per kilogram of animal body weight (mg/kg).

Although some of the compounds of the method of the invention, such as disulfiram, are known to produce pharmacologic effects unrelated to IL-1 release, they can be usefully employed in the method of the invention with animals which are not in need of such other pharmacologic action. In certain cases, the other pharmacologic action may be regarded as an undesirable side effect, when the desired result is inhibition of IL-1 release. Thus, with disulfiram, for example, concomitant administration of ethanol should be avoided, to avoid the well known reaction to alcohol.

The compounds used in the invention can be formulated in conventional pharmaceutically-acceptable carriers to provide unit dosage forms convenient for administration. In general, known dosage forms and carrier materials can be used.

The invention is further illustrated in the following Example.

Example

Peritoneal macrophages obtained from CD-1 mice were collected and washed once with RPMI-1640 medium (GIBCO, Grand Island, New York) containing 100 Units/ml penicillin, 100 µg/ml streptomycin and 25 µg/ml fungizone (GIBCO, Grand Island, NY). Cells were suspended at 6 x 10^6 cells per ml, and one ml aliquots were plated into 6-well flat-bottom plates. After one hour incubation at 37° C in a humidified air chamber containing 5% CO_2, non-adherent cells were removed and 1 ml RPMI medium (with or without lipopolysaccharide (LPS) - 100 µg/well) was added to each well; LPS stimulates macrophages to release IL-1. Incubation was continued for 6 hours, after which the culture supernatant was collected and filtered through 0.22 micrometer Acrodisc filters (Gelman, Ann Arbor, MI). The fluid was stored at a temperature of -70°C until assayed for IL-1 activity.

IL-1 activity was determined by the C3H/HeJ thymocyte proliferation assay of Mizel et al., J. Immunol. 120:1497 (1978). In this procedure, thymocytes of C3H/HeJ mice are incubated with the macrophage culture supernatant in the presence of phytohemagglutinin, and pulsed by incubation with ^{3}H-thymidine. The cells are then harvested and radioactivity is determined by liquid scintillation counting. IL-1 activity was expressed as units defined according to Mizel et al, J. Immunol. 120:1497 (1978).

Compounds were tested in this procedure by oral administration to CD-1 mice 40, 24 and 16 hours prior to collection of peritoneal macrophages. The dosage used was 100 mg/kg. The compounds and results obtained are set out in the following table.

Compound	Per Cent Reduction of IL-1 Secretion
Disulfiram	79.0%
Tetrakis [3-(2,6-di-tert-butyl-4-hydroxyphenyl)propionyloxy methyl] methane	66.8%
2,4-Di-isobutyl-6-(N, N-dimethylaminomethyl)-phenol	92.5%

The above results indicate significant inhibition of IL-1 release was obtained with the test compounds.

The claims defining the invention are as follows:

1. A method of inhibiting the release of interleukinl in animals which comprises administering to an animal in need thereof an effective amount of a compound selected from the group consisting of disulfiram, 2,4-di-isobutyl-6-(N,N-dimethylaminomethyl)-phenol, and tetrakis [3-(2,6-di-tert-butyl-4-hydroxyphenyl)propionyloxy methyl]methane.

2. A method according to Claim 1 wherein the compound is 2,4-di-isobutyl-6-(N,N-dimethylaminomethyl)-phenol.

3. A method according to Claim 1 wherein the compound is d ,ulfiram.

4. A method according to Claim 1 wherein the compound is tetrakis [3-(2-6-di-tert-butyl-4-hydroxyphenyl)propionyloxy methyl]methane.

5. A method according to Claim 1 wherein the animal is suffering from inflammation and the compound is administered in an amount sufficient to alleviate the inflammation.

6. A method according to Claim 1 wherein the animal is suffering from psoriasis and the compound is administered in an amount sufficient to alleviate the psoriasis.

7. Method of Claim 1 wherein the animal is suffering from diabetes and the compound is administered in an amount sufficient to alleviate the diabetes.

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8. Method of Claim 1 wherein the animal is suffering from antherosclerosis and the compound is administered in an amount sufficient to alleviate the atherosclerosis.

9. A method according to Claim 1 substantially as hereinbefore described with reference to the accompanying example.

DATED: 15 March, 1988.

PHILLIPS ORMONDE AND FITZPATRICK
Attorneys for:
MERRELL DOW PHARMACEUTICALS INC.

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