International Patent Classification: A61K 31/55

International Publication Number: WO 93/20822
International Publication Date: 28 October 1993 (28.10.93)

International Application Number: PCT/US93/03347
International Filing Date: 8 April 1993 (08.04.93)
Priority Data: 9207645.4 8 April 1992 (08.04.92) GB

Applicant (for all designated States except US): SMITHKLINE BEECHAM CORPORATION [US/US]; Corporate Patents - U.S., UW2220, 709 Smedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).

Inventor (for US only): FEUERSTEIN, Giora, Z. [IL/US]; 405 Ballyvore Road, Wynnewood, PA 19096 (US).

Agents: DUSTMAN, Wayne, J. et al.; SmithKline Beecham Corporation, Corporate Patents - U.S., UW2220, 709 Smedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).


Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

Title: COMPOSITIONS OF PLATELET ACTIVATING FACTOR ANTAGONISTS AND METHODS OF TREATING INTERLEUKIN-2 INDUCED LUNG INJURY THEREWITH

Abstract

Invented is a method of treating Interleukin-2 induced lung injury in a mammal, including human, which comprises administering to such mammal an effective amount of a platelet-activating factor antagonist.
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"Compositions of Platelet Activating Factor Antagonists and Methods of Treating Interleukin-2 Induced Lung Injury Therewith"

This invention relates to a method of treating interleukin-2 induced lung injury in a mammal, including a human, in need thereof which comprises administering to such mammal an effective amount of a platelet-activating factor antagonist.

Background of the Invention


The systemic use of IL-2 has, however, also been associated with disorders such as, microvascular injury and pulmonary edema (Clausner, F.L. et al. J. Appl. Physiol. 64:1030-1037 (1988) and Rosenstein, M. J. Immunol. 137:1735-1742 (1986)) (hereinafter "lung injury"). The acute pulmonary toxicity associated with IL-2 infusion has been proposed to result from Lymphocyte activation (Anderson, T.D. et al. Lab. Invest. 59:598-612 (1988), Damle, N.K. et al. J.

PAF (platelet activating factor) is phospholipid acetyl-glyceryl-ether-phosphoryl-choline (AGEPC) which is known as a potent lipid mediator released by animal and human proinflammatory cells. These cells include mainly basophilic and neutrophilic granulocytes, macrophages (from blood and tissue) and thrombocytes which are involved in inflammatory reactions.

Compounds which inhibit PAF are reported to be of potential value in the treatment of a variety of conditions including allergic, inflammatory and hypersecretory conditions such as asthma, arthritis, rhinitis, bronchitis and urticaria, the treatment of circulatory shock, gastric, ulceration, psoriasis and cardiovascular conditions, including angina, thrombosis and stroke, (WO 91/17162 published November 14, 1991).

Presently, PAF inhibitors are not known as having utility in treating IL-2-induced lung injuries.
Summary of the Invention

This invention relates to a method of treating interleukin-2 induced lung injury in a mammal, including a human, in need thereof which comprises administering to such mammal an effective amount of a platelet-activating factor antagonist.

Detailed Description of the Invention

Illustrative of compounds that have PAF activity are the following compounds

A)

\[
\begin{align*}
\text{H}_3\text{C} & \\
\text{N} & \\
\text{N} & \\
\text{O} & \\
\text{N} & \\
\text{N} & \\
\text{Cl} & \\
\end{align*}
\]

which is 5-[(2-Chlorophenyl)-3,4-dihydro-10-methyl-3-[(4-morpholinyl)carbonyl]-2H,7H-cyclopenta[4,5]thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine. This compound is disclosed and claimed in European Application No. 254245-A published January 27, 1988, as having platelet-activating factor antagonist activity.

B)

\[
\begin{align*}
\text{O} & \\
\text{N} & \\
\text{C(CH}_2\text{)}_2 & \\
\text{CH}_3 & \\
\text{N} & \\
\text{N} & \\
\text{N} & \\
\text{N} & \\
\text{Cl} & \\
\end{align*}
\]
which is 3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo-[4,3-a][1,4]-diazepin-2-yl]-1-(4-morpholiny)-1-propanon and which is disclosed in U.S. Patent No. 4968794 as having platelet-activating factor antagonist activity.

C)

which is tetrahydro-4,7,8,10 methyl-(chloro-2phenyl)-6[(dimethoxy-3,4phenyl)thio)methylthiocarbonyl-9 pyrido[4',3'-4,5]thieno[3,2-f]triazolo-1,2,4[4,3- a]diazepine-1,4 (hereinafter compound C) and which is disclosed in U.S. Patent No. 5,049,559 as a specific platelet-activating factor antagonist.

D)
which is (+) N-(3-benzoylphenyl)3-(3 pyridyl)-1H, 3H pyrrolo [1,2-c] thiazole-7 carboxamide and which is disclosed and claimed in U.S. Patent 4,783,472 as having platelet-activating factor antagonist activity.

By the term "treating" as used herein is meant prophylactic or therapeutic therapy.

By the term "U" as used herein is meant a unit of IL-2 activity as specified by the manufacturer (i.e. Hoffman-La Roche, Nutley, NJ).

It has now been discovered that compounds which are antagonists of platelet-activating factor are useful in treating interleukin-2 (IL-2) induced lung injury.

Compound C was tested for its in vivo potency in treating IL-2 induced lung injury. To perform the experiments recombinant human IL-2 (obtained from Hoffman-La Roche, Nutley, NJ) was reconstituted before use with 1 ml of 0.9% NaCl per 10^6 U IL-2. A 10^5 U dose of IL-2 was prepared by diluting the above 10^6 U dose 10 times. Compound C was solubilized in 64% DMSO, in 0.9% NaCl to reach a concentration of 5 mg/ml. Male Sprague-Dawley Rats (obtained from Charles River, Wilmington, MA) were housed in groups of three in standard cages, and kept with food and water ad libitum in a temperature controlled room (22°C) on a 12 hour dark/light cycle, until surgery.

Following anesthesia with pentobarbital (30 mg/kg, i.p.) the animals were randomly assorted into three groups.

Group I. IL-2 at 10^5 U/kg (n=14) (as used herein the term "n" means the number of rats), 10^6 U/kg (n=18) or vehicle (n=12) was infused intravenously for one hour (syringe infusion pump 22, Harvard apparatus, South Natick, MA).

Group II. IL-2 at 10^6 U/kg was administered by bolus injection followed by IL-2 at 10^6
U/kg intravenous injection for one hour (n=6), vehicle treated animals served as controls (n=6).

Group III. IL-2 at 10^6 U/kg was administered by intravenous injection for 1 hour, 30 minutes after pretreatment with compound C (10 mg/kg, I.P.) (n=6) or compound C vehicle (n=6).

At the end of the experimental period the left lung was removed and immediately frozen on dry ice until assayed. When defrosted the lung was weighed to determine wet weight. Dry weight was determined after the lung was dried at 80°C for 36 hours, and the pulmonary water content was calculated by subtracting the lung dry weight from the wet lung weight.

The wet (485 ±4 mg), dry (92 ±4 mg) and wet-dry (pulmonary water content, 393 ±4 mg) lung weight did not differ among the control (vehicle treated) groups. IL-2 increased wet (P<0.05), dry and wet-dry (P<0.01) lung weight in a dose-dependent manner. The wet-dry/dry ratio however remained unchanged. Pretreatment with compound C prevented the responses (P<0.05).

The results of the above experiments clearly demonstrates the therapeutic utility of platelet-activating factor antagonist on treating IL-2 induced lung injury.

This invention discloses platelet-activating factor antagonist and pharmaceutically acceptable salts or hydrates or solvates thereof as being useful for treating IL-2 induced lung injury in mammals, including humans.

A platelet-activating factor antagonist or a pharmaceutically acceptable salt or hydrate or solvate thereof can be administered to a subject in a conventional dosage form prepared by combining a platelet-activating factor antagonist or a
pharmaceutically acceptable salt or hydrate or solvate thereof, with a conventional pharmaceutically acceptable carrier or diluent according to known techniques.

It will be recognized by one of skill in the art that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. A platelet-activating factor antagonist or a pharmaceutically acceptable salt or hydrate or solvate thereof is administered to a mammal, including a human, in an amount sufficient to prevent or alleviate IL-2 induced lung injury.

The route of administration of the platelet-activating factor antagonist is not critical but is usually oral or parenteral, preferably oral.

The term parenteral as used herein includes intravenous, intramuscular, subcutaneous, intranasal, intrarectal, transdermal, intravaginal or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred. The daily parenteral dosage regimen will preferably be from about 0.01 mg/kg to about 20 mg/kg of total body weight, most preferably, from about 0.1 mg/kg to about 5 mg/kg. Preferably, each parenteral dosage unit will contain the active ingredient in an amount of from about 2 mg to about 150 mg.

The platelet-activating factor antagonist which are active when given orally can be formulated as liquids, for example, syrups, suspensions or emulsions, tablets, capsules and lozenges.

A liquid formulation will generally consist of a suspension or solution of the compound or pharmaceutically acceptable salt in a suitable liquid carrier(s) for example, ethanol, glycerine, non-aqueous
solvent, for example polyethylene glycol, oils, or water with a suspending agent, preservative, flavoring or coloring agent.

A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier(s) routinely used for preparing solid formulations. Examples of such carriers include magnesium stearate, starch, lactose, sucrose and cellulose.

A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, pellets containing the active ingredient can be prepared using standard carriers and then filled into a hard gelatin capsule; alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier(s), for example aqueous gums, celluloses, silicates or oils and the dispersion or suspension then filled into a soft gelatin capsule.

The daily oral dosage regimen will preferably be from about 0.01 mg/kg to about 20 mg/kg of total body weight. Preferably each oral dosage unit will contain the active ingredient in an amount of from about 2 mg to about 150 mg.

While it is possible for an activate ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation.

It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a platelet-activating factor antagonist or a pharmaceutically acceptable salt or hydrate or solvate thereof will be determined by the nature and extent of the exact condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of a platelet-
activating factor antagonist or a pharmaceutically acceptable salt or hydrate or solvate thereof given per day and duration of therapy, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following examples are, therefore, to be construed as merely illustrative and not a limitation of the scope of the present invention in any way.

**Example 1 - Capsule Composition**

A oral dosage form for administering a platelet-activating factor antagonist is produced by filing a standard two piece hard gelatin capsule with the ingredients in the proportions shown in Table I, below.

**Table I**

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>AMOUNTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound C</td>
<td>25 mg</td>
</tr>
<tr>
<td>Lactose</td>
<td>55 mg</td>
</tr>
<tr>
<td>Talc</td>
<td>16 mg</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>4 mg</td>
</tr>
</tbody>
</table>

**Example 2 - Injectable Parenteral Composition**

An injectable form for administering a platelet-activating factor antagonist is produced by stirring 1.5% by weight of Compound C in 10% by volume propylene glycol in water.

**Example 3 - Tablet Composition**

The sucrose, calcium sulfate dihydrate and a platelet-activating factor antagonist shown in Table II below, are mixed and granulated in the proportions shown
with a 10% gelatin solution. The wet granules are screened, dried, mixed with the starch, talc and stearic acid, screened and compressed into a tablet.

Table II

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>AMOUNTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound C</td>
<td>20 mg</td>
</tr>
<tr>
<td>calcium sulfate dihydrate</td>
<td>30 mg</td>
</tr>
<tr>
<td>sucrose</td>
<td>4 mg</td>
</tr>
<tr>
<td>starch</td>
<td>2 mg</td>
</tr>
<tr>
<td>talc</td>
<td>1 mg</td>
</tr>
<tr>
<td>stearic acid</td>
<td>0.5 mg</td>
</tr>
</tbody>
</table>

While the above descriptions and examples fully describe the invention and the preferred embodiments thereof, it is understood that the invention is not limited to the particular disclosed embodiments coming within the scope of the following claims.
What is claimed is:


2. A use according to claim 1 wherein the mammal is a human.

3. A use according to claim 2 wherein the platelet-activating factor antagonist is 5-[(2-Chlorophenyl)-3,4-dihydro-10-methyl-3-[(4-morpholiny)carbonyl]-2H,7H-cyclopenta[4,5]thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine.

4. A use according to claim 2 wherein the platelet-activating factor antagonist is 3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]-diazepin-2-yl]-1-(4-morpholiny)-1-propanon.

5. A use according to claim 2 wherein the platelet-activating factor antagonist is tetrahydro-4,7,8,10 methyl-(chloro-2phenyl)-6[(dimethoxy-3,4phenyl)thio)methylthiocarbonyl-9 pyrido[4',3'-4,5]thieno[3,2-f]triazolo-1,2,4[4,3-a]diazepine-1,4.

6. A use according to claim 2 wherein the platelet-activating factor antagonist is (+) N-(3-benzoylephenyl)3-(3 pyridyl)-1H, 3H pyrrolo[1,2-c] thiazole-7 carboxamide.

7. A use according to claim 2 wherein the platelet-activating factor antagonist is administered orally.

8. A use according to claim 7 wherein from about 0.01 mg/kg to about 20 mg/kg of the platelet-activating factor antagonist is administered per day.

9. A use according to claim 2 wherein the platelet-activating factor antagonist is administered parenterally.
10. A use according to claim 9 wherein from about 0.01 mg to about 20 mg/kg of the platelet-activating factor antagonist is administered per day.


12. A composition according to claim 11 wherein the mammal is a human.

13. A composition according to claim 12 wherein the platelet-activating factor antagonist is 5-[(2-Chlorophenyl)-3,4-dihydro-10-methyl-3-[(4-morpholinyl)carbonyl]-2H,7H-cyclopenta[4,5]thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine.

14. A composition according to claim 12 wherein the platelet-activating factor antagonist is 3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo-[4,3-a][1,4]-diazepin-2-yl]-1-(4-morpholinyl)-1-propanone.

15. A composition according to claim 12 wherein the platelet-activating factor antagonist is tetrahydro-4,7,8,10 methyl-(chloro-2phenyl)-6([(dimethoxy-3,4phenyl)thio)methylthiocarbonyl-9 pyrido[4',3'-4,5]thieno[3,2-f]triazolo-1,2,4[4,3-a]diazepine-1,4.

16. A composition according to claim 12 wherein the platelet-activating factor antagonist is (+) N-(3-benzoylephenyl)-3-(3 pyridyl)-1H, 3H pyrrolo[1,2-c] thiazole-7 carboxamide.

17. A composition according to claim 12 wherein the platelet-activating factor antagonist is administered orally.

18. A composition according to claim 17 wherein from about 0.01 mg/kg to about 20 mg/kg of the platelet-activating factor antagonist is administered per day.
19. A composition according to claim 12 wherein the platelet-activating factor antagonist is administered parenterally.

20. A composition according to claim 19 wherein from about 0.01 mg to about 20 mg/kg of the platelet-activating factor antagonist is administered per day.
**INTERNATIONAL SEARCH REPORT**

### A. CLASSIFICATION OF SUBJECT MATTER

- **IPC(5):** A61K 31/55
- **US Cl.:** 514/219

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)

- **U.S.:** 514/220, 338

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

Please See Extra Sheet.

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

- **STN:** Registry (structures) and Chemical Abstracts, APS lung, pulmonary, PAF, platelet activating factor, interleukin, injury, disease, toxicity, edema.

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<td>US, A, 4,783, 472 (Fabre et al) 08 November 1988</td>
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| X        | US, A, 5,049,559 (Braquet et al) 17 September 1991                            | 11,12,15              |
| Y        |                                                                                  | 1-20                  |

| X        | US, A 5,082,839 (Weber et al) 21 January 1992                                 | 11,12,14              |
| Y        |                                                                                  | 1-20                  |

| Y        |                                                                                  | 1-20                  |

[X] Further documents are listed in the continuation of Box C.  [ ] See patent family annex.

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<td>document published prior to the international filing date but later than the priority date claimed</td>
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**Date of the actual completion of the international search**  **15 JULY 1993**

**Date of mailing of the international search report**  **16 AUG 1993**

**Name and mailing address of the ISA/US Commissioner of Patents and Trademarks**

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**Authorized officer**

[Signature]

WILLIAM JARVIS

**Telephone No.** (703) 308-1235

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<td>Y</td>
<td>Chemical Abstracts Vol. 116(21), No. 212769w, 1992, Tavares de Lima et al.</td>
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B. FIELDS SEARCHED

Documentation other than minimum documentation that are included in the fields searched:

Form PCT/ISA/210 (extra sheet) (July 1992)