Title: NOVEL PHARMACEUTICAL FORMULATION SUITABLE FOR NEBULISATION

Abstract: The present invention relates to a pharmaceutical formulation suitable for nebulisation which comprises an aqueous suspension of (-)-3-[(14-aminocarbonyl)-1-piperidinyl]carbonyl]oxy[phenyl]-2-[2-(2-methylphenoxy)acetyl]amino]pentanoyl]amino] propanoic acid or a salt or solvate thereof. Methods and uses of the formulation in the treatment of respiratory disorders such as asthma are also described.
Novel Pharmaceutical Formulation Suitable for Nebulisation

The present invention relates to a pharmaceutical formulation for use in the administration of medicaments by inhalation. In particular, this invention relates to a pharmaceutical formulation for use in nebulisers. The invention also relates to methods for their preparation and to their use in therapy.

(2S)-3-[4-(((4-(Aminocarbonyl)-1-piperidinyl)carbonyl)oxy)phenyl]-2-(((2S)-4-methyl-2-[[2-(2-methylphenoxy)acetyl]amino]pentanoyl)amino] propanoic acid has recently been disclosed in International Patent Application (PCT/EP99/10000) as a novel antagonist of both α4β1 and α4β7 integrins which, as a consequence, results in effective anti-inflammatory properties.

Anti-inflammatory conditions, eg. asthma are typically treated by medicaments in the form of dry powders or aerosols containing small particles of the medicament, conventionally prepared by micronisation. Generally, these medicaments are administered by means of metered dose inhalers, which are designed to deliver a fixed unit dosage of medicament per actuation or "puff". However, some patients, in particular children and the elderly, have difficulty in co-ordinating actuation of a metered dose inhaler with inhalation, and are therefore unable to use this mode of administration effectively. Furthermore, a proportion of patients find inhalation of dry powders difficult or unpleasant. There is therefore a demand for a pharmaceutical formulation containing anti-inflammatory medicaments in a form suitable for nebulisation.

Thus, according to the present invention we provide a pharmaceutical formulation suitable for nebulisation which comprises:

an aqueous suspension of (2S)-3-[4-(((4-(Aminocarbonyl)-1-piperidinyl)carbonyl)oxy)phenyl]-2-(((2S)-4-methyl-2-[[2-(2-methylphenoxy)acetyl]amino]pentanoyl)amino] propanoic acid or a salt or solvate thereof.
Preferably, the formulation will contain one or more surfactants.

Preferably, the formulation will contain one or more isotonicity adjusting agents.

According to one particular aspect of the present invention we provide a pharmaceutical formulation which comprises:

(i) an aqueous suspension of \((2S)-3-[[4-(Aminocarbonyl)-1-piperidinyl]carbonyl]oxy)phenyl]-2-[[((2S)-4-methyl-2-[[2-(2-

methylphenoxy)acetyl]amino]pentanoyl]amino] propanoic acid or a salt or solvate thereof;

(ii) one or more surfactants; and

(iii) one or more isotonicity adjusting agents.

Examples of suitable salts include physiologically acceptable salts such as alkali metal salts, for example calcium, sodium and potassium salts and salts with (trishydroxymethyl)aminomethane.

Preferably, the \((2S)-3-[[4-(Aminocarbonyl)-1-piperidinyl]carbonyl]oxy)phenyl]-2-[[((2S)-4-methyl-2-[[2-(2-
methylphenoxy)acetyl]amino]pentanoyl]amino] propanoic acid is present as the free acid.

The aqueous component is preferably a high grade quality of water, most preferably purified water.

The active \((2S)-3-[[4-(Aminocarbonyl)-1-piperidinyl]carbonyl]oxy) phenyl]-2-[[((2S)-4-methyl-2-[[2-(2-
methylphenoxy)acetyl]amino]pentanoyl]amino] propanoic acid medicament (or a salt or solvate thereof) will suitably have a mass mean diameter (MMD) of less than 20\(\mu\)m, preferably between 0.5-10\(\mu\)m, especially around 3-5\(\mu\)m, eg. 2\(\mu\)m. Particle size reduction, if necessary, may be achieved eg. by micronisation. Preferably, the particles will be crystalline, prepared for

For introduction of the (2S)-3-[4-(((4-(Aminocarbonyl)-1-piperidiny1)[carbony1]oxy)phenyl]-2-[(((2S)-4-methyl-2-[[2-(2-methylphenoxy)acetyl]amino]pentanoyl]amino] propanoic acid (or a salt or solvate thereof) into the lungs, the droplet size of the nebulised formulation is an important parameter. Droplet size depends to some extent on the type of nebuliser used, whether a facemask or a mouthpiece is used and, for jet nebulisers, the pressure or flow rate of the compressed gas, as well as on the physical properties of the formulation for nebulisation. The nebulised formulation will be heterodisperse, i.e. droplets will cover a range of sizes. Typically, mean droplet size will be in the range of 1 to 15 microns, preferably 1 to 10 microns, more preferably less than 7 microns.

The formulation according to the invention desirably contains 1 to 50mg of (2S)-3-[4-(((4-(Aminocarbonyl)-1-piperidiny1)[carbony1]oxy)phenyl]-2-[(((2S)-4-methyl-2-[[2-(2-methylphenoxy)acetyl]amino]pentanoyl]amino] propanoic acid (or a salt or solvate thereof) per 2ml dose.

The surfactants which may be used in the formulations of the present invention must be physiologically acceptable upon administration by inhalation. Within this category are included surfactants such as oleic acid, sorbitan trioleate, sorbitan monooleate, sorbitan monolaurate, polyoxyethylene (20) sorbitan monolaurate, polyoxyethylene (20) sorbitan monooleate, natural lecithin, oleyl polyoxyethylene (2) ether, stearyl polyoxyethylene (2) ether, lauryl polyoxyethylene (4) ether, block copolymers of ethylene oxide and of propylene oxide, synthetic lecithin, diethylene glycol dioleate, tetrahydrofurfuryl oleate, ethyl oleate, isopropyl
myristate, glyceryl monooleate, glyceryl monostearate, glyceryl monoricinoleate, cetyl alcohol, stearyl alcohol, polyethylene glycol 400 or glyceryl monolaurate, or cationic surfactants, such as cetylpyridinium chloride or benzalkonium chloride. Other examples of surfactants include synthetic phosphatides eg. distearoylphosphatidylcholine. Preferably, the surfactant will be present within the formulation at an amount between 0.01 and 20% (w/w).

Particularly preferred surfactants of use in the formulations of the present invention are sorbitan monolaurate and polyoxyethylene (20) sorbitan monolaurate (also known as polysorbate 20).

The formulations according to the invention will desirably be isotonic with the fluids of the lung. The formulations may be adjusted to isotonicity by addition of a suitable salt, for example, sodium chloride, dextrose or calcium chloride.

Thus, in a preferred embodiment, the formulations according to the invention additionally comprise sufficient sodium chloride, or another suitable pharmaceutically acceptable salt, to provide an isotonic composition.

In a particularly preferred embodiment, the invention provides a formulation suitable for administration by nebulisation, which formulation consists of:

(a) (2S)-3-[4-([(4-(Aminocarbonyl)-1-piperidiny]carbonyl)oxy]phenyl]-2-[((2S)-4-methyl-2-[[2-(2-methylphenoxy)acetyl]amino]pentanoyl)amino] propanoic acid (or a salt or solvate thereof) 0.1 - 20mg;
(b) poloxyethylene (20) sorbitan monolaurate 0.1 - 0.2mg;
(c) sorbitan monolaurate 0.01 - 0.03mg
(d) sodium chloride 10 – 40 mg; and
(e) water for injection to 2.0ml.
The chemical and physical stability and the pharmaceutical acceptability of the formulations according to the invention may be determined by techniques well known to those skilled in the art. Thus, for example, the chemical stability of the components may be determined by HPLC assay, for example, after prolonged storage of the product.

The particle size distribution of the formulations according to the invention on nebulisation may be measured by conventional techniques, for example by cascade impaction or by the "Twin Impinger" analytical process. As used herein reference to the "Twin Impinger" assay means "Determination of the deposition of the emitted dose in pressurised inhalations using apparatus A" as defined in British Pharmacopoeia 1988, pages A204-207, Appendix XVII C. Such techniques enable the "respirable fraction" of the formulations to be calculated. As used herein reference to "respirable fraction" means the amount of active ingredient collected in the lower impingement chamber per actuation expressed as a percentage of the total amount of active ingredient delivered per actuation using the twin impinger method described above.

The formulations according to the invention may be prepared by conventional methods for the preparation of nebuliser formulations. Typically the (2S)-3-[4-([(4-(Aminocarbonyl)-1-piperidinyl)carbonyl]oxy) phenyl]-2-[(2S)-4-methyl-2-[[2-(2-methylphenoxy)acetyl]amino]pentanoyl]amino] propanoic acid (or a salt or solvate thereof) is contacted with a small amount of surfactant solution so as to "wet" it before addition to the bulk liquid containing the remaining excipients. As the formulation is a suspension formulation, constant mixing is essential to maintain a homogeneous suspension. The formulation is sterilised, conveniently by means of thermal sterilisation using steam. Aliquots of the formulation are conveniently filled into sterile containers, for example unit dose containers such as vials or ampoules which are suitably moulded from thermoplastics.
Optionally a further particulate active ingredient suitable for inhalation therapy may be incorporated into the formulation such as a corticosteroid (e.g. fluticasone propionate) or a bronchodilator (e.g. salmeterol or albuterol or a salt thereof).

Examples of disease states in which the formulation of the present invention has potentially beneficial anti-inflammatory effects include respiratory disorders, more particularly asthma.

Thus, according to a further aspect of the invention we provide a pharmaceutical formulation of the present invention for use in the treatment or prophylaxis of respiratory disorders such as asthma by inhalation.

We also provide a use of a pharmaceutical formulation of the present invention in the manufacture of a medicament for the treatment or prophylaxis of respiratory disorders such as asthma by inhalation.

We also provide a method of treatment of respiratory disorders such as asthma which comprises administering to a patient by inhalation a pharmaceutically acceptable amount of the formulation of the present invention.

The invention is further illustrated by the following non-limiting examples:

**Example A:** (2S)-3-[4-(((4-(Aminocarbonyl)-1-piperidinyl)carbonyl)oxy)phenyl]-2-(((2S)-4-methyl-2-[[2-(2-methylphenoxy)acetyl]amino]pentanoyl]amino] propanoic acid

To Wang resin (50g) was added a solution of (2S)-3-[4-(allyloxy)phenyl]-2-[[tert-butoxycarbonyl]amino]propanoic acid (115.8g) and 1-hydroxybenzotriazole (48.6g) in DMF (475ml). After 15 minutes 1,3-diisopropylcarbodiimide (56.5ml) was added and the mixture was stirred for 24h at 45°C. The resin was filtered and washed with DMF (3 x 360ml), methanol (3 x 360ml) and dichloromethane (3 x 700ml). To a slurry of the resin in dichloromethane (644ml) was added
pyridine (14.7ml). Acetic anhydride (26.9ml) was added and the mixture was
stirred for 12h at 20°C. The resin was filtered and washed with dichloromethane
(3 x 550ml), methanol (3 x 370ml) and dichloromethane (3 x 550ml).

A slurry of 20g of the resin in dichloromethane (100ml) was cooled to 2-5°C and
treated with a solution of phenol (20g) in dichloromethane (80ml).

Chlorotrimethylsilane (20ml) was added dropwise and the mixture was stirred for
6h at 2-5°C. The resin was filtered and washed with dichloromethane (3 x
200ml), methanol (3 x 200ml), 10% water in DMF (2 x 200ml), 10%
diisopropylethylamine in DMF (3 x 200ml), DMF (200ml), methanol (3 x 200ml)
and dichloromethane (3 x 200ml).

A slurry of the resin in DMF (55ml) was treated with a solution of Fmoc-leucine
(32.7g) and 1-hydroxybenzotriazole (12.5g) in DMF (85ml). After 5 minutes 1,3-
diisopropylcarbodiimide (19.3ml) was added and the mixture was stirred for 15h
at 20°C. The resin was filtered and washed with DMF (3 x 150ml), methanol (3 x
150ml) and dichloromethane (3 x 150ml).

The resin was treated with 20% piperidine in DMF (180ml) and stirred for 1h at
20°C. The resin was filtered and washed with DMF (3 x 150ml),
dichloromethane (3 x 150ml), DMF (3 x 150ml) and dichloromethane (3 x
150ml). To a slurry of this in DMF (50ml) was added a solution of (2-
methylphenoxy)acetic acid (17.9g) and 1-hydroxybenzotriazole (14.6g) in DMF
(100ml). After 5 minutes 1,3-diisopropylcarbodiimide (16.9ml) was added and
the mixture was stirred for 65h at 20°C. The resin was filtered and washed with
DMF (2 x 150ml), methanol (3 x 150ml) and dichloromethane (3 x 150ml).

A slurry of the resin in dichloromethane (60ml) was treated with a solution of
tetrakis(triphenylphosphine)palladium(0) (5.21g) in dichloromethane (140ml)
followed by morpholine (13ml). The mixture was stirred for 2h at 20°C then the
resin was filtered and washed with dichloromethane (7 x 200ml).

A slurry of the resin in dichloromethane (160ml) was treated with
diisopropylethylamine (12.4ml) followed by 4-nitrophenyl chloroformate (24.8g)
in 3 portions at 5 minute intervals. The mixture was stirred for 1h at 20°C. The
resin was filtered and washed with dichloromethane (3 x 200ml). The resin was treated with a solution of isonipecotamide (15.8g) in DMF (180ml) and the mixture was stirred for 1.5h at 20°C. The resin was filtered and washed with DMF (4 x 200ml) and dichloromethane (2 x 200ml).

The resin was treated with 50% TFA in dichloromethane (200ml). After stirring for 1h at 20°C the resin was filtered and washed with dichloromethane (5 x 200 ml). The combined filtrate and washings were evaporated in vacuo. The residue was azeotroped with toluene (2 x 100ml) then triturated with ether (50ml) and the resulting white solid filtered. To this was added acetonitrile (150ml) and the mixture was heated to reflux. The resulting suspension was allowed to cool to 20°C and stirred for 18h.. The mixture was filtered to give the title compound as a white solid (4.9g).

**Example 1**

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<tr>
<td>Polyoxyethylene (20) sorbitan monolaurate</td>
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<td>Sodium chloride</td>
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<tr>
<td>Water for injection (Stilmas) to</td>
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Example 2

(2S)-3-[4-(((4-(Aminocarbonyl)-1-piperidinyl)carbonyloxy)phenyl]-2-(((2S)-4-methyl-2-[[2-(2-methylphenoxy)acetylamino]pentanoyl]amino)propanoic acid potassium salt (micronised) (prepared according to Example A)

Polyoxyethylene (20) sorbitan monolaurate 0.16
Sorbitan monolaurate 0.02
Sodium chloride 15
Water for injection (Stilmas) to 2.00ml

Throughout the specification and the claims which follow, unless the context requires otherwise, the word ‘comprise’, and variations such as ‘comprises’ and ‘comprising’, will be understood to imply the inclusion of a stated integer or step or group of integers but not to the exclusion of any other integer or step or group of integers or steps.

Above mentioned patent applications are hereinbefore incorporated by reference.
Claims

1. A pharmaceutical formulation suitable for nebulisation which comprises:

2. A formulation according to claim 1 which comprises one or more surfactants.

3. A formulation according to claim 2 wherein the surfactant is selected from: oleic acid, sorbitan trioleate, sorbitan monooleate, sorbitan monolaurate, polyoxyethylene (20) sorbitan monolaurate, polyoxyethylene (20) sorbitan monooleate, natural lecithin, oleyl polyoxyethylene (2) ether, stearyl polyoxyethylene (2) ether, lauryl polyoxyethylene (4) ether, block copolymers of ethylene oxide and of propylene oxide, synthetic lecithin, diethylene glycol dioleate, tetrahydrofurfuryl oleate, ethyl oleate, isopropyl myristate, glyceryl monooleate, glyceryl monostearate, glyceryl monoricinoleate, cetyl alcohol, stearyl alcohol, polyethylene glycol 400 or glycerol monolaurate, or cationic surfactants, such as cetylpyridinium chloride or benzalkonium chloride. Other examples of surfactants include synthetic phosphatides eg. distearoylphosphatidylcholine.

4. A formulation according to claim 3 wherein the surfactants are sorbitan monolaurate and polyoxyethylene (20) sorbitan monolaurate.

5. A formulation according to any one of claims 2 to 4 wherein the surfactant is present within the formulation at an amount between 0.01 and 20% (w/w).
6. A formulation according to any one of claims 1 to 5 which comprises one or more isotonicity adjusting agents.

7. A formulation according to claim 6 wherein the isotonicity adjusting agent is selected from sodium chloride, dextrose or calcium chloride.

8. A formulation according to claim 7 wherein the isotonicity adjusting agent is sodium chloride.

9. A formulation according to any one of claims 1 to 10 which comprises:
   (i) an aqueous suspension of (2S)-3-[4-(((4-(Aminocarbonyl)-1-piperidinyl)carbonyl)oxy)phenyl]-2-[[2-(2-methylphenoxy)acetyl]amino]pentanoyl]amino] propanoic acid or a salt or solvate thereof;
   (ii) one or more surfactants; and
   (iii) one or more isotonicity adjusting agents.

10. A formulation according to any one of claims 1 to 9 wherein the (2S)-3-[[4-(Aminocarbonyl)-1-piperidinyl]carbonyl]oxy)phenyl]2-[[2-(2-methylphenoxy)acetyl]amino]pentanoyl]amino] propanoic acid is present as the free acid.

11. A formulation according to any one of claims 1 to 10 which contains 1 to 50mg of (2S)-3-[4-(((4-(Aminocarbonyl)-1-piperidinyl)carbonyl)oxy) phenyl]-2-[[2-(2-methylphenoxy)acetyl]amino]pentanoyl]amino] propanoic acid (or a salt or solvate thereof) per 2ml dose.

12. A formulation suitable for administration by nebulisation, which formulation consists of:
(a) (2S)-3-[4-(((4-(Aminocarbonyl)-1-piperidinyl)carbonyl)oxy) phenyl]-2-([(2S)-4-methyl-2-[(2-(2-methylphenoxy)acetyl]amino]pentanoyl]amino) propanoic acid (or a salt or solvate thereof) 0.1 - 20mg;
(b) polyoxyethylene (20) sorbitan monolaurate 0.1 - 0.2mg;
(c) sorbitan monolaurate 0.01 - 0.03mg
(d) sodium chloride 9 - 10 mg; and
(e) water for injection to 2.0ml

13. A pharmaceutical formulation according to any one of claims 1 to 12 for use in the treatment or prophylaxis of respiratory disorders by inhalation.

14. Use of a pharmaceutical formulation according to any one of claims 1 to 12 in the manufacture of a medicament for the treatment or prophylaxis of respiratory disorders by inhalation.

15. A method of treatment of respiratory disorders which comprises administering to a patient by inhalation a pharmaceutically acceptable amount of the formulation according to any one of claims 1 to 12.
### INTERNATIONAL SEARCH REPORT

#### A. CLASSIFICATION OF SUBJECT MATTER

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<th>A61P11/00</th>
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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):

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<th>A61K</th>
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

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

- EPO-Internal, WPI Data, PAJ, CHEM ABS Data, BIOSIS

#### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>P,A</td>
<td>WO 00 37444 A (JACK TORQUIL IAIN MACLEAN ; KEELING STEVEN PHILIP (GB); RAMSDEN NIG) 29 June 2000 (2000-06-29) cited in the application page 21, line 19 - page 23, line 31 page 57 - page 60; example 27 claims 1,14,15</td>
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<td>P,A</td>
<td>WO 01 28616 A (ANDERSON GREGOR JOHN MCLENNAN; GLAXO GROUP LTD (GB); ROBERTSON DUN) 26 April 2001 (2001-04-26) page 11, line 33 - page 12, line 23</td>
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Relevant to claim No. 1-15

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Further documents are listed in the continuation box C. Patent family members are listed in annex.

* Special categories of cited documents:
  *A* document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search: 3 September 2001

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