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(71) Applicant (for all designated States except US): **ZAMBON GROUP S.P.A.** [IT/IT]; Via Della Chimica, 9, I-36100 Vicenza (IT).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **DEL TACCA, Mario** [IT/IT]; UNIVERSITA' DI PISA, Centro Interdip. Ric. Farmacol.Clinica e Terapia Speriment., Via Roma 55, I-56126 Pisa (IT). **BLANDIZZI, Corrado** [IT/IT]; UNIVERSITA' DI PISA, Centro Interdip.Ric.Farmacol. Clinica e Terapia Speriment., Via Roma 55, I-56126 Pisa (IT). **MORAZZONI, Gabriele** [IT/IT]; Via Labriola 12, I-20020 Lainate (MI) (IT).

(74) Agent: **LONGONI, Alessandra**; ZAMBON GROUP S.P.A., Intellectual Property Dept., Via Lillo Del Duca, 10, I-20091 Bresso (MI) (IT).

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(54) Title: USE OF MACROLIDES FOR TREATING INTESTINAL INFLAMMATION

(57) Abstract: The present invention relates to a compound of formula (IA) as defined in the specification or a pharmaceutically acceptable salt thereof, in the preparation of a medicament for both the treatment of inflammatory bowel diseases and the prevention of colon cancer.



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Use of macrolides for treating intestinal inflammation”

DESCRIPTION

The present invention relates to novel methods of treating an inflammatory response in
5 intestinal tissues associated with a disease such as inflammatory bowel disease involving
either or both the small and large bowel. In particular the present invention relates to a new
use of certain macrolides for the treatment of the inflammatory bowel disease.

Inflammatory bowel disease (IBD) is the generic term for a disease of unknown cause that
produces chronic inflammation or ulceration of the mucosa of the large and small intestine.

10 This inflammatory bowel disease includes such diseases as ulcerative colitis and Crohn's
disease.

Ulcerative colitis is a chronic, non-specific IBD which involves a diffuse inflammation in the
mucosa of the large intestine causing ulcerative lesions of the colon. Crohn's disease, also
known as regional enteritis or colitis granulomatosa, is most frequently located in the small
15 intestine (small bowel), especially in the ileum, but it can affect any part of the bowel,
including the rectum. In the latter case the differentiation of Crohn's disease from ulcerative
colitis may give rise to diagnostic problems. Generally, the inflammation differs from that of
ulcerative colitis by progressing to layers deeper than the mucosa and affecting the
epithelium to a lesser degree. Both diseases have become increasingly frequent especially in
20 the developed countries. Therefore, treatment of IBD has become an important problem of
modern medicine.

The presently available medical treatments for IBD generally involve drug therapy directed
towards the suppression of gastrointestinal inflammation. The most commonly used
medicaments to treat IBD are anti-inflammatory drugs such as the salicylates. The salicylate
25 preparations may be effective in treating mild to moderate disease. Examples of salicylates
include sulfasalazine, olsalazine, and mesalamine. All of these medications are given orally
in high doses for maximal therapeutic benefit. These medicines are not without side effects
including heartburn, nausea, vomiting, diarrhea, and headache.

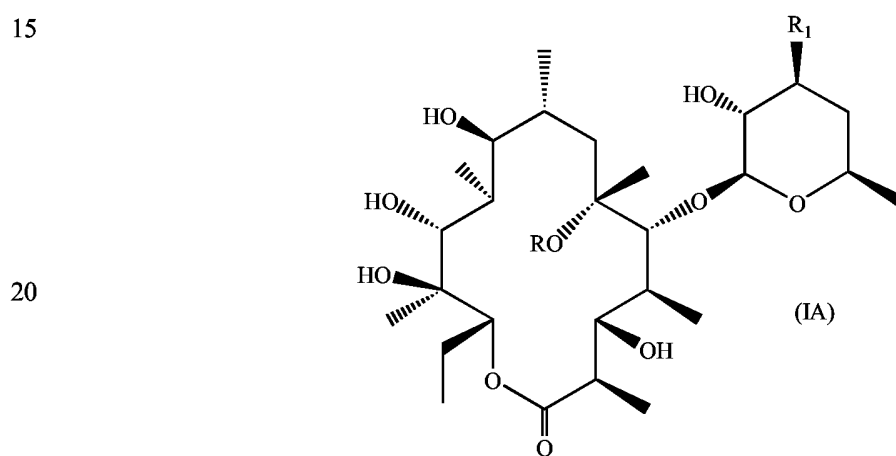
People with more severe IBD can be treated with corticosteroids, such as prednisone and
30 hydrocortisone, which are more potent and faster-acting than salicylates in the treatment of
IBD, but are endowed with potentially serious side effects.

In IBD patients that do not respond to salicylates or corticosteroids, medications that suppress the immune system are used. However, immunosuppressants cause increased risk of infection, renal failure, and may increase the need for hospitalization. In severe cases of disease, the patient may need surgery to remove the affected gut. Drugs like antidiarrheals, laxatives, and pain relievers can be also given to help relieve symptoms.

Since all the available medical treatments for IBD are rather unsatisfactory and often ineffective, there is currently a great need for novel drugs capable of treating IBD and preventing its relapse.

We have now found that some macrolides, encompassed by the general formula (I), as reported in the International patent application WO 2004/013153 and endowed with anti-inflammatory activity particularly in skin and lung inflammation, are also surprisingly effective in treating intestinal inflammation.

It is therefore a first object of the present invention the use of a compound of formula (IA)



wherein

25 R is hydrogen or methyl;

R₁ is an N-(C₁-C₄)acyl-N-(C₁-C₃)alkylamino group;

or a pharmaceutically acceptable salts thereof, in the preparation of a medicament for treating IBD.

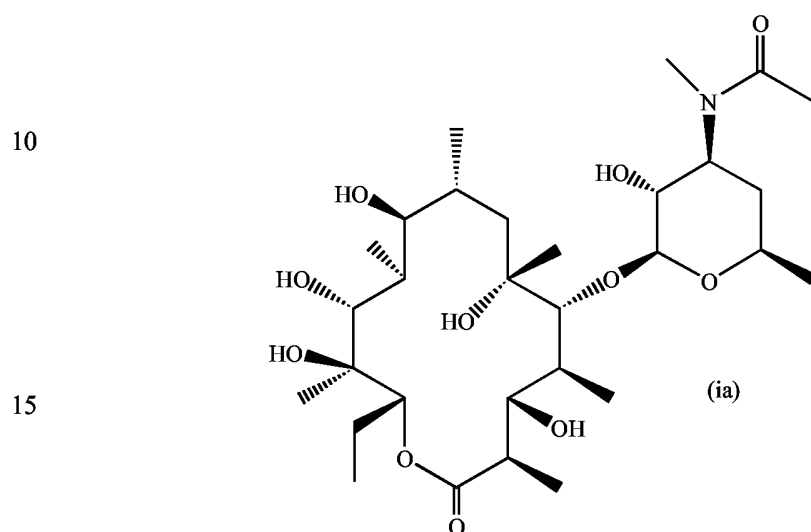
Examples of pharmaceutically acceptable salts of the compounds of formula (IA) are salts with organic or mineral acids such as hydrogen chloride, hydrogen bromide, hydrogen

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iodine, nitric acid, sulphuric acid, phosphoric acid, acetic acid, tartaric acid, citric acid, benzoic acid, succinic acid and glutaric acid.

In particular, the compounds of formula (IA) or a pharmaceutically acceptable salt thereof
5 can be used for treating ulcerative colitis or Crohn's disease.

Particularly preferred compound of formula (IA) according to the invention is the compound of formula (ia)



namely, (9S)-O5-[3-(1-oxoethyl)-methylamino-β-D-xylo-3,4,6-trideoxy-hexapyranosyl]-9-
20 hydroxy-9-deoxy-erythronolide-A, or a pharmaceutically acceptable salt thereof.

Unless otherwise specified, the term "treatment" as used herein relates to both treatment in order to cure or alleviate a disease or a condition, and to treatment in order to prevent the development or relapse of a disease or a condition.

The term "patient", as used herein, relates to any human or non-human mammal in need of
25 treatment according to the invention.

In IBD, the constant process of inflammatory injury and repair of the lining of the colon (colonic mucosa) is believed to make the individual more susceptible to the cancer. Colon cancer doesn't distinguish between active disease and remission. Patients whose disease has been quiet have the same risk as those who have more active disease.

30 For example, patients with prolonged ulcerative colitis are at increased risk for developing

colon cancer. The risk of cancer increases with the duration and the extent of involvement of colonic mucosa. For example, if only the lower colon and rectum are involved, the risk of cancer is no higher than normal. However, if the whole colon is involved, the risk of cancer may be higher.

It is therefore a further object of the present invention the use of a compound of formula (IA) as defined above or a pharmaceutically acceptable salt thereof for the preparation of a medicament for preventing colon cancer.

In a further aspect, the present invention relates to a method for treating IBD, which comprises administering to a patient in need thereof a therapeutically effective amount of a compound of formula (IA) or a pharmaceutically acceptable salt thereof.

In a still further aspect the present invention relates to a method for preventing colon cancer, which comprises administering to a patient in need thereof a therapeutically effective amount of a compound of formula (IA) or a pharmaceutically acceptable salt thereof.

The pharmacological activity of the compound of formula (ia), as a representative compound of the compounds of the present invention, was evaluated in *in vivo* models of intestinal inflammation, as illustrated in the following EXPERIMENTAL PART.

Brief description of the drawings

In the Experimental Part below reference is made to the appended drawings on which:

Fig. 1 shows the scheme of drug treatments. Oral drugs were suspended in 1% methocel and given in a volume of 0.5 ml.

Fig. 2 shows the macroscopic damage score criteria. The final score is obtained by the sum of all values.

Fig. 3 shows values of macroscopic damage score indicating the severity of DNBS-induced colitis in rats. Each column indicates the mean value obtained from 6-8 animals $\pm$ S.E.M. (vertical lines). Significant difference from control values * $P < 0.05$ (one way ANOVA following by Student-Newman-Keuls test).

EXPERIMENTAL PART

Methods

Induction of colitis

Albino male Sprague-Dawley rats, 200-250 g body weight, were treated with intrarectal DNBS (2,4-dinitrobenzensulfonic acid, 30 mg in 0.25 ml of 50% ethanol) under a light anaesthesia with diethyl ether. In control experiments, the animals received 0.25 ml of saline (NaCl 0.9%). Animals were subjected to subsequent experimental procedures 6 days after induction of colitis with DNBS (Blandizzi et al., Altered prejunctional modulation of intestinal cholinergic and noradrenergic pathways by alpha-2-adrenoceptors in the presence of experimental colitis. *Br J Pharmacol* 139, 309-320, 2003).

Experimental design

- 10 The compound of formula (ia), from now on the macrolide, was suspended in 1% methocel and administered to animals by intragastric gavage in a volume of 0.5 ml at the doses of 100 or 300 $\mu\text{mol/kg/day}$ for 7 consecutive days. Induction of colitis was performed on day 2 as reported above. Animals were subjected to subsequent experimental procedures on day 7, four hours later from administration of the last dose of the macrolide or its vehicle (Fig. 1).
- 15 Each experimental series included the following treatment groups:
- 1) controls (intragastric drug vehicle for 7 days plus intra-colonic saline on day 2);
 - 2) intragastric macrolide for 7 days plus intra-colonic saline on day 2;
 - 3) intragastric drug vehicle for 7 days plus intra-colonic DNBS on day 2;
 - 4) intragastric macrolide for 7 days plus intra-colonic DNBS on day 2.
- 20 Each data point represents the mean value of results obtained from at least 6-8 animals. The effect of the macrolide was compared with that of dexamethasone (1 mg/kg/day, by oral route), known to exert a good anti-inflammatory action in the rat model of TNBS-induced colitis (Bobin-Dubigeon et al., Effects of tumor necrosis factor- α synthesis inhibitors on rat trinitrobenzene sulfonic acid-induced chronic colitis. *Eur J Pharmacol* **431**, 103-110, 2001).
- 25 Dexamethasone was administered once daily following the same time schedule adopted for the macrolide.

Macroscopic and histological assessment of intestinal inflammation

- At the end of treatments, animals were sacrificed and the severity of colonic inflammation was macroscopically evaluated in accordance with the criteria previously reported by
- 30 Wallace and Keenan (Wallace JL and Keenan CM, An orally active inhibitor of leukotriene

synthesis accelerates healing in a rat model of colitis. *Am J Physiol*, **258**, G527-G534, 1990) with minor modifications (Blandizzi et al., 2003). Briefly, the macroscopic evaluation is based on the following criteria: presence of adhesions between the colon and other intra-abdominal organs; consistency of colonic faecal material (as an indirect marker of diarrhea); thickening of the colonic wall; presence and extension of hyperemia and macroscopic mucosal damage (assessed with the aid of a ruler) (Fig. 2). All parameters of macroscopic damage were recorded and scored for each rat by two independent observers blinded to the treatment.

10 Results

The obtained results are illustrated in Fig. 3. Control rats exhibited a negligible macroscopic damage score, accounting for 1.0 ± 0.2 . In DNBS-treated animals, the extent of inflammatory lesions was significantly higher than that observed in control rats (10.7 ± 0.4), indicating the occurrence of colitis. The macrolide exerted significant anti-inflammatory effects both at 100 and 300 $\mu\text{mol/kg}$ (-24% and -51%, respectively). Dexamethasone induced anti-inflammatory effect similar to that observed with the macrolide 100 $\mu\text{mol/kg}$.

Conclusions

Data obtained from the present study indicate a good pattern of anti-inflammatory activity of the macrolide against experimental colitis. In this setting, the efficacy of the macrolide seems to be comparable with that of dexamethasone, a well known anti-inflammatory drug.

It is thus clear from the experimental evidence reported above that a compound of formula (IA), especially the compound of formula (ia) or a pharmaceutically acceptable salt thereof, may be effectively used in the treatment of IBD and in the prevention of colon cancer.

The therapeutical effective amounts of a compound of formula (IA) or a pharmaceutically acceptable salt thereof will depend on the age and the general physiological state of the patient, the route of administration and the pharmaceutical formulation used; the therapeutic doses will generally be between about 10 and 2000 mg/day and preferably between about 30 and 1500 mg/day.

The compounds of the present invention for use in the treatment and/or prophylaxis of the above mentioned diseases will preferably be used in a pharmaceutical form that is suitable

for oral, rectal, sublingual, parenteral, topical, transdermal and inhalational administration.

It is therefore a further object of the present invention to provide pharmaceutical formulations containing a therapeutical effective amount of a compound of formula (IA) or a
5 pharmaceutically acceptable salt thereof together with a pharmaceutical acceptable vehicle.

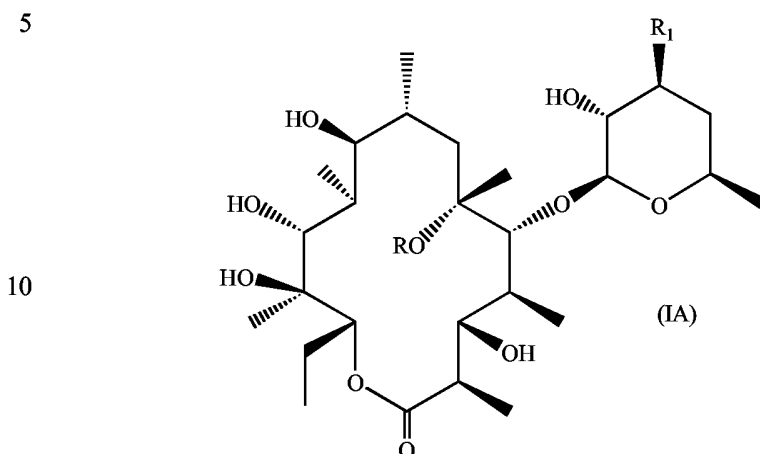
The pharmaceutical formulations of the present invention may be liquid, suitable for oral and/or parenteral administration, for instance drops, syrups, solutions, injectable solutions ready to use or prepared via dilution of a lyophilizate, but preferably solid, for instance tablets, capsules, granules, powders, pellets, pessaries, suppositories, creams, pomades, gels
10 or ointments; or alternatively solutions, suspensions, emulsions or other forms suitable for inhalation and transdermal administration.

Depending on the type of formulation, these formulations will contain, besides a therapeutical effective amount of a compound of formula (IA) or a pharmaceutically acceptable salt thereof, solid or liquid excipients or diluents for pharmaceutical use and
15 optionally other additives normally used in the preparation of pharmaceutical formulations, for instance thickeners, aggregating agents, lubricants, disintegrants, flavourings and colourings.

The pharmaceutical formulations according to the invention, may be produced according to conventional techniques. As an example, hard gelatine capsules for oral administration
20 comprising from 100 mg to 300 mg of the macrolide and a pharmaceutically acceptable vehicle may be prepared to be administered to a patient in need thereof.

CLAIMS

1. The use of a compound of formula (IA)

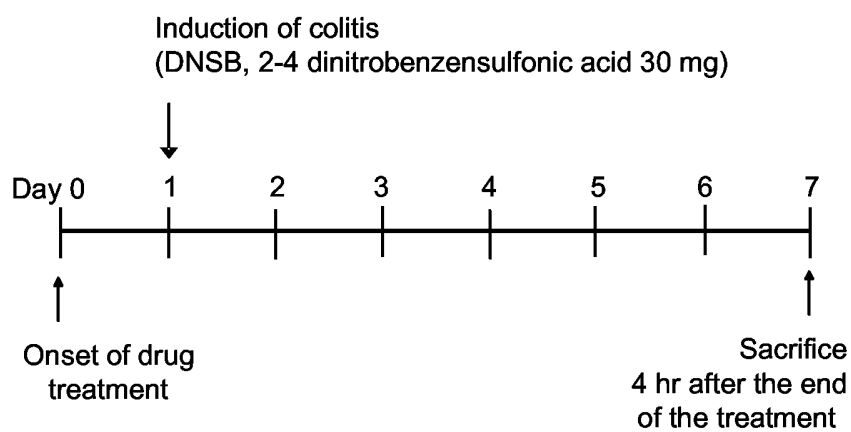


wherein

- 15 R is hydrogen or methyl;
R₁ is an N-(C1-C4)acyl-N-(C1-C3)alkylamino group;
or a pharmaceutically acceptable salts thereof, in the preparation of a medicament for the
treatment of inflammatory bowel diseases.
2. The use according to claim 1, wherein the compound of formula (IA) is (9S)-O5-[3-(1-
20 oxoethyl)-methylamino-β-D-xylo-3,4,6-trideoxy-hexapyranosyl]-9-hydroxy-9-deoxo-
erythronolide-A, or a pharmaceutically acceptable salt thereof.
3. The use according to claim 1 or 2, wherein the inflammatory bowel disease is ulcerative
colitis.
4. The use according to claim 1 or 2, wherein the inflammatory bowel disease is Crohn's
25 disease.
5. The use of a compound of formula (IA) as defined in claim 1, for the prevention of colon
cancer.
6. The use according to claim 5, wherein the compound of formula (IA) is (9S)-O5-[3-(1-
30 oxoethyl)-methylamino-β-D-xylo-3,4,6-trideoxy-hexapyranosyl]-9-hydroxy-9-deoxo-
erythronolide-A, or a pharmaceutically acceptable salt thereof.

Figure 1

Scheme of drug treatment

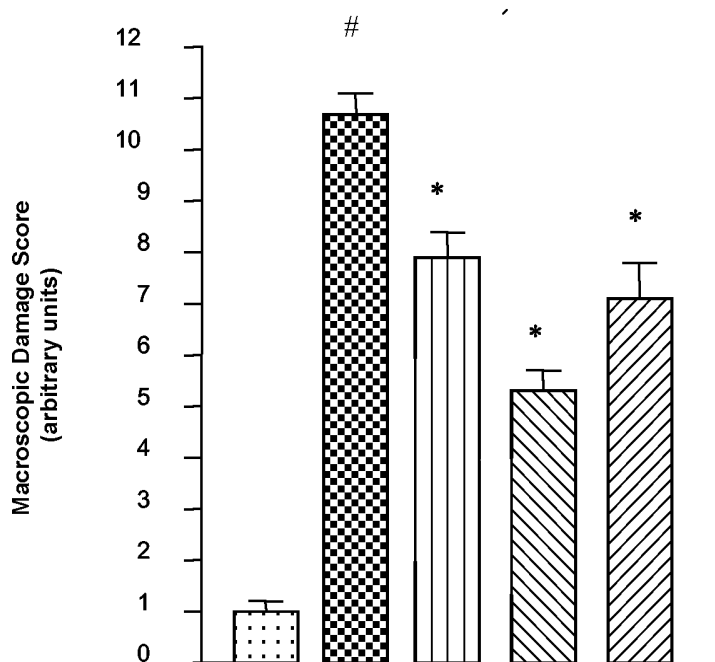
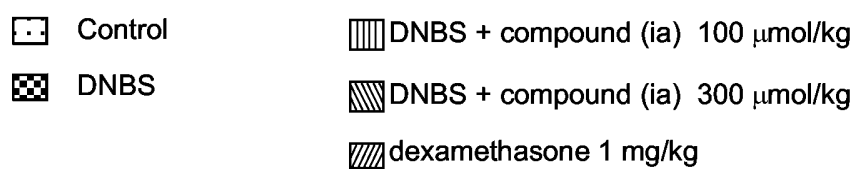


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[illegible]

Figure 3

Macroscopic damage score



* $p < 0.05$ vs DNBS

$p < 0.05$ vs control

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/060709

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/7048 A61P1/04 A61P35/00

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)

A61K

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

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

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 2005/075494 A (ZAMBON GROUP S.P.A; MEREU, ANDREA; NAPOLETANO, MAURO; ORNAGHI, FERNAND) 18 August 2005 (2005-08-18) page 15, line 18 claims; examples	1-6
X	WO 2004/013153 A (ZAMBON GROUP S.P.A; NAPOLETANO, MAURO; MORIGGI, ERMANNO; MEREU, ANDREA) 12 February 2004 (2004-02-12) cited in the application Compound RN: 654076-73-0 (compound (ia)) page 28, lines 3-13 examples claim 31	1-6

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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- "&" document member of the same patent family

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Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Veronese, A

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2006/060709

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	"The Merck Manual" 1999, MERCK RESEARCH LABORATORIES, USA, XP002391025 pages 302-312 -----	1-6
Y	US 5 712 253 A (LARTEY ET AL) 27 January 1998 (1998-01-27) column 2, lines 31-45 column 2, lines 60-67 claims -----	1-6
A	US 2002/035075 A1 (BORODY THOMAS JULIUS) 21 March 2002 (2002-03-21) See claim 3: clarithromycin paragraphs [0001], [0002]; examples 1,4-8 -----	1-6
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2006/060709

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