(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 August 2009 (27.08.2009)

PCT

(10) International Publication Number
WO 2009/104150 A1

(51) **International Patent Classification**:
A61K 31/513 (2006.01) ***A61P 35/00*** (2006.01)
A61K 31/337 (2006.01)

(21) **International Application Number**:
PCT/IB2009/050679

(22) **International Filing Date**:
19 February 2009 (19.02.2009)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
PCT/IB2008/050608
20 February 2008 (20.02.2008) IB

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2009/104150 A1

(54) **Title**: COMBINATION COMPRISING BOSENTAN FOR TREATING OVARIAN CANCER

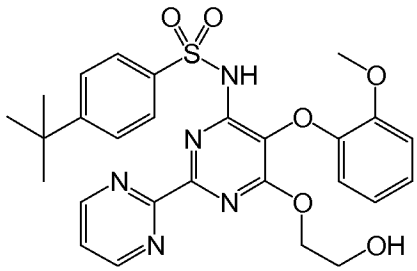
(57) **Abstract**: The invention relates to the combination of bosentan with paclitaxel, and in particular to this combination for therapeutic use, simultaneously, separately or over a period of time, in the treatment of ovarian cancer.

COMBINATION COMPRISING BOSENTAN FOR TREATING OVARIAN CANCER

The present invention concerns the combination of bosentan with paclitaxel for therapeutic use, simultaneously, separately or over a period of time, in the treatment of ovarian cancer.

Ovarian cancer is one of the most common cancers in women. A common complication of ovarian cancer is ascite formation. Today, there is no satisfactory treatment for ovarian cancer or for its complications such as ascite formation.

Bosentan is the active principle of Tracleer®. It is a dual endothelin receptor antagonist compound (i.e. a compound with affinity for both endothelin ET_A and ET_B receptors) which has the following formula



Bosentan and its preparation have been described notably in EP 0 526 708 or US 5,292,740.

Paclitaxel (the active principle of a medicament sold under the trademark Taxol® in the United States) is an anti-microtubule agent extracted from the needles and bark of the Pacific yew tree, *Taxus brevifolia*. This compound is currently approved in the European Union and the United States for, among others, the treatment of advanced cancer of the ovary.

The combination of endothelin receptor A (ET_AR) antagonists with paclitaxel in the treatment of ovarian cancer has already been suggested in literature.

For example, L. Rosano et al (*Cancer Res.* (2003), **63**, 2447-2453) teach that the selective ET_AR antagonist ABT-627 (atrasentan) combined with paclitaxel produced additive antitumor, apoptotic and antiangiogenic effects.

Besides, L. Rosano et al (*Mol. Cancer Ther.* (2007), **6**(7), 2003-2011) disclosed that ZD4054, a specific ET_AR antagonist, inhibits tumor growth and enhances paclitaxel activity in human ovarian carcinoma *in vitro* and *in vivo*.

On the other hand, L. Rosano et al (*Mol. Cancer Ther.* (2006), **5**(4), 833-842) also showed that BQ 788, a selective endothelin receptor B (ET_BR) antagonist, contrarily to ET_AR antagonists, was ineffective in inhibiting cell adhesiveness of ovarian tumor cells *in vitro*.

The applicant has now found that bosentan produces surprisingly high effects in an *in vivo* model of ovarian cancer when combined with paclitaxel. Besides, in the same *in vivo* model, the applicant found that the use of the combination of bosentan with paclitaxel prevents the formation of ascites. As a result, bosentan in combination with paclitaxel may be used for the preparation of a medicament, and is suitable, for the treatment of ovarian cancer and/or the prevention or treatment of ascite formation associated with ovarian cancer.

The invention thus firstly relates to a product containing bosentan or a pharmaceutically acceptable salt or hydrate of this compound, in combination with paclitaxel, or a pharmaceutically acceptable salt thereof, and to said product for therapeutic use, simultaneously, separately or over a period of time, in the treatment of ovarian cancer.

The following paragraphs provide definitions of the various terms used in the present patent application and are intended to apply uniformly throughout the specification and claims, unless an otherwise expressly set out definition provides a broader or narrower definition.

The term "pharmaceutically acceptable salt" refers to non-toxic, inorganic or organic acid and/or base addition salts. Reference can be made to "Salt selection for basic drugs", *Int. J. Pharm.* (1986), **33**, 201-217.

The term "hydrate" refers herein to products obtained through incorporation of water molecules into their crystalline structure. In the case of bosentan hydrates, bosentan monohydrate will be preferred.

"Simultaneously" or "simultaneous", when referring to a therapeutic use, means in the present application that the therapeutic use concerned consists in the administration of two or more active ingredients by the same route and at the same time.

"Separately" or "separate", when referring to a therapeutic use, means in the present application that the therapeutic use concerned consists in the administration of two or more active ingredients at approximately the same time by at least two different routes.

By therapeutic administration "over a period of time" is meant in the present application the administration of two or more ingredients at different times, and in particular an administration method according to which the entire administration of one of the active ingredients is completed before the administration of the other or others begins. In this way it is possible to administer one of the active ingredients for several months before administering the other active ingredient or ingredients. In this case, no simultaneous administration occurs. Therapeutic administration "over a period of time" also encompasses situations wherein the ingredients are not given with the same periodicity (e.g. wherein one ingredient is given once a day and another is given once a week).

By "prevention of ascite formation" or "prevent(ing) ascite formation" is meant in the present application that, following the administration of the appropriate preventive treatment according to this invention, the formation of ascites is either avoided or that this formation is reduced, or, alternatively, that the ascites nevertheless formed are eliminated or reduced.

By "treatment of ascite formation" or "treat(ing) ascite formation" is meant in the present application that, following the administration of the appropriate treatment according to this invention, the ascites present in the patient are eliminated or reduced.

In a preferred embodiment of this invention, the product containing bosentan or a pharmaceutically acceptable salt or hydrate of this compound, in combination with paclitaxel, or a pharmaceutically acceptable salt thereof, will be for therapeutic use, simultaneously, separately or over a period of time, in the prevention or treatment of ascite formation in patients having ovarian cancer.

According to one variant of this invention, bosentan or its pharmaceutically acceptable salt or hydrate will be intended to be administered by intravenous or intraperitoneal route.

According to another variant of this invention, bosentan or its pharmaceutically acceptable salt or hydrate will be intended to be administered by oral route.

Paclitaxel or its pharmaceutically acceptable salt will preferably be administered by intravenous or intraperitoneal route.

Though the exact administration doses of a product according to this invention will have to be determined by the treating physician, it is expected that a dose of 0.05 to 30 mg (and preferably 0.1 to 10 mg and more preferably 0.5 to 5 mg) of bosentan per kg of patient body weight per day combined with a dose of 0.1 to 10 mg (and preferably 1 to 3 mg) of paclitaxel per kg of patient body weight per day, will be appropriate.

The invention also relates to a pharmaceutical composition containing, as active principles, bosentan or a pharmaceutically acceptable salt or hydrate of this compound, in combination with paclitaxel, or a pharmaceutically acceptable salt thereof, as well as at least one non-toxic excipient.

Preferably, such a pharmaceutical composition will be in a liquid form suitable for intravenous or intraperitoneal administration. In particular, said pharmaceutical composition may contain bosentan or a pharmaceutically acceptable salt or hydrate of this compound and paclitaxel or a pharmaceutically acceptable salt thereof, in solution in a mixture of polyoxyethylated castor oil (e.g. Cremophor® EL) and ethanol (said mixture containing for example from 40 to 60% in volume of polyoxyethylated castor oil in ethanol).

Alternatively, bosentan or its pharmaceutically acceptable salt or hydrate of this compound may be formulated as a tablet as for commercial Tracleer®, whereas paclitaxel may be formulated as a solution in a mixture of polyoxyethylated castor oil (e.g. Cremophor® EL) and ethanol.

The production of the pharmaceutical compositions can be effected in a manner which will be familiar to any person skilled in the art (see for example Remington, *The Science and Practice of Pharmacy*, 21st Edition (2005), Part 5, "Pharmaceutical Manufacturing"

[published by Lippincott Williams & Wilkins]) by bringing the described compounds or their pharmaceutically acceptable salts or hydrates, optionally in combination with other therapeutically valuable substances, into a galenical administration form together with suitable, non-toxic, inert, therapeutically compatible solid or liquid carrier materials and, if desired, usual pharmaceutical adjuvants.

The invention further relates to the use of bosentan or a pharmaceutically acceptable salt or hydrate of this compound, in combination with paclitaxel, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament intended to treat ovarian cancer. It also relates to the use of bosentan or a pharmaceutically acceptable salt or hydrate of this compound, in combination with paclitaxel, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament intended to prevent or treat ascite formation in patients having ovarian cancer.

The invention further relates to a method of treating a patient having an ovarian cancer by administering to said patient a combination of bosentan or a pharmaceutically acceptable salt or hydrate of this compound, with paclitaxel or a pharmaceutically acceptable salt thereof. It also relates to a method of preventing or treating the formation of ascites in a patient having an ovarian cancer by administering to said patient a combination of bosentan or a pharmaceutically acceptable salt or hydrate of this compound, with paclitaxel or a pharmaceutically acceptable salt thereof.

Besides, preferences indicated for the product according to this invention of course apply *mutatis mutandis* to the pharmaceutical compositions and uses of this invention.

Particular embodiments of the invention are described in the following section, which serves to illustrate the invention in more detail without limiting its scope in any way.

Pharmacological properties of the invention product

Human SKOV3ip1 tumor growth inhibition assay in mice

Experimental methods:

Vehicle solution

An aqueous 0.5% (by weight) solution of methylcellulose is prepared by stirring the appropriate quantity of methylcellulose in the appropriate quantity of water for 4 hours. This solution can be prepared up to 3 days in advance. On the day of the experiment, 0.05% (by volume) of Tween 80 is dissolved in the methylcellulose solution previously obtained to yield the vehicle solution.

Experimental procedure

43 mice are injected i.p. with 10^6 SKOV3ip1 cells. Ten days later, the tumor weight is evaluated in three of the mice and treatment with a suspension of bosentan in the vehicle solution (10 mice), paclitaxel diluted 1:6 in phosphate buffered saline (PBS) for i.p. injections (10 mice), a suspension of bosentan in the vehicle solution as well as paclitaxel diluted 1:6 in PBS for i.p. injections (10 mice), or the vehicle solution only (10 mice), is administered to the mice using the following doses, frequencies and routes:

- paclitaxel: 5 mg/kg (125 µg paclitaxel in 200 µL PBS per mouse), once a week, i.p. route;
- bosentan: 300 mg/kg (as suspension in the vehicle solution at a concentration of up to 25 mg/mL), once a day, oral route.

After one month of treatment, the tumor incidence and weight are determined in each of the mice. At the same time, the ascite incidence and volume are also determined.

Results:

The following results were obtained with respect to tumor incidence and weight:

Treatment group	Body weight (g) Mean ± S.D.	Tumor incidence	Tumor weight (g) Median (range)	p
Control	23.1 ± 2.5	8/10	1.1 (0-1.8)	
Paclitaxel	23.6 ± 1.9	9/9	0.4 (0.1-0.5)	0.01
Bos	23.0 ± 2.1	9/10	1.1 (0-1.9)	0.9
Paclitaxel + Bos	23.4 ± 3.2	4/10	0 (0-1.1)	0.004

S.D. = standard deviation Bos = bosentan

The following results were obtained with respect to ascite incidence and volume:

Treatment group	Body weight (g) Mean ± S.D.	Ascite incidence	Ascites (mL) Median (range)	p
Control	23.1 ± 2.5	8/10	0.4 (0-0.9)	
Paclitaxel	23.6 ± 1.9	4/9	0.1 (0-0.2)	0.005
Bos	23.0 ± 2.1	5/10	0.1 (0-1.9)	0.9
Paclitaxel + Bos	23.4 ± 3.2	0/10	0	0.002

S.D. = standard deviation Bos = bosentan

As can be seen, the combination of bosentan with paclitaxel markedly increased the response to the paclitaxel treatment alone:

- six out of ten mice were tumor-free after the combination treatment while all mice still had tumors in the paclitaxel-treated group;
- the average tumor weight in the combination treatment group was close to zero, although in the mice treated with bosentan alone, the tumor weight was on average the same as in the control group and in the mice treated with paclitaxel alone the average tumor weight was still about a third of that in the mice of the control group; and

- no mouse treated with the combination developed ascites even though 4 out of 10 still had tumors, whereas ascites were present in 4 out of 9 mice treated with paclitaxel alone and in 5 out of 10 mice treated with bosentan alone.

Claims

1. A product containing bosentan or a pharmaceutically acceptable salt or hydrate of this compound, in combination with paclitaxel, or a pharmaceutically acceptable salt thereof.

2. The product of claim 1 for therapeutic use, simultaneously, separately or over a period of time, in the treatment of ovarian cancer.

3. The product of claim 1 for therapeutic use, simultaneously, separately or over a period of time, in the prevention or treatment of ascite formation in patients having ovarian cancer.

4. The product of claim 2 or 3, wherein bosentan or its pharmaceutically acceptable salt or hydrate is intended to be administered by intravenous or intraperitoneal route.

5. The product of claim 2 or 3, wherein bosentan or its pharmaceutically acceptable salt or hydrate is intended to be administered by oral route.

6. The product of one of claims 2 to 5, wherein paclitaxel or its pharmaceutically acceptable salt is intended to be administered by intravenous or intraperitoneal route.

7. A pharmaceutical composition containing, as active principles, bosentan or a pharmaceutically acceptable salt or hydrate of this compound, in combination with paclitaxel, or a pharmaceutically acceptable salt thereof, as well as at least one non-toxic excipient.

8. A pharmaceutical composition according to claim 7, which is in a liquid form suitable for intravenous or intraperitoneal administration.

9. A pharmaceutical composition according to claim 8, which contains bosentan or a pharmaceutically acceptable salt or hydrate of this compound, and paclitaxel, or a pharmaceutically acceptable salt thereof, in solution in a mixture of polyoxyethylated castor oil and ethanol.

10. A pharmaceutical composition according to claim 9, wherein the mixture of polyoxyethylated castor oil and ethanol is such that it contains from 40 to 60% in volume of polyoxyethylated castor oil in ethanol.

11. Use of bosentan or a pharmaceutically acceptable salt or hydrate of this compound, in combination with paclitaxel, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament intended to treat ovarian cancer.

12. Use of bosentan or a pharmaceutically acceptable salt or hydrate of this compound, in combination with paclitaxel, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament intended to prevent or treat ascite formation in patients having ovarian cancer.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2009/050679

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/513 A61K31/337 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 A61P

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, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MIKI ADACHI ET AL: "Identification of a region of the human endothelin ETA receptor required for interaction with bosentan" EUROPEAN JOURNAL OF PHARMACOLOGY. MOLECULAR PHARMACOLOGY SECTION, ELSEVIER SCIENCE BV, AMSTERDAM, NL, vol. 269, no. 2, 14 October 1994 (1994-10-14), pages 225-234, XP023817030 ISSN: 0922-4106 [retrieved on 1994-10-14] the whole document ----- -/--	1-12

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

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
12 May 2009	07/07/2009

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2009/050679

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROSANO LAURA ET AL: "ZD4054, a potent endothelin receptor A antagonist, inhibits ovarian carcinoma cell proliferation" EXPERIMENTAL BIOLOGY AND MEDICINE (MAYWOOD), vol. 231, no. 6, June 2006 (2006-06), pages 1132-1135, XP002527397 ISSN: 1535-3702 the whole document	1-12
Y	GODARA GEETA ET AL: "Role of endothelin axis in progression to aggressive phenotype of prostate adenocarcinoma" PROSTATE, vol. 65, no. 1, September 2005 (2005-09), pages 27-34, XP002527398 ISSN: 0270-4137 the whole document	1-12