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(54) Title: SEMI-SYNTHETIC TAXANES WITH ANTITUMOR AND ANTIANGIOGENETIC ACTIVITIES

(57) Abstract: Novel derivatives obtained by oxidation, stereospecific reduction and esterification of baccatine III and the use thereof as antitumor, antiangiogenetic and anti-arthrosis agents. The compounds of the invention have cytotoxicity comparable to that of other taxanes but lower systemic toxicity and they can be administered intravenously or orally.

SEMI-SYNTHETIC TAXANES WITH ANTITUMOR AND ANTIANGIOGENETIC ACTIVITIES

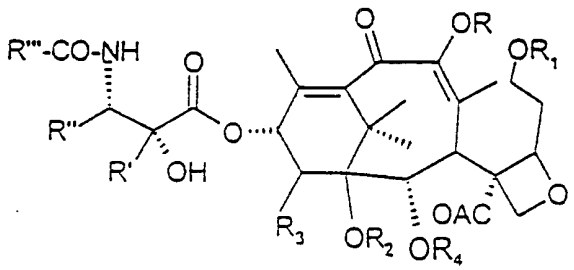
The present invention relates to seco-baccatine III derivatives.

Taxane-skeleton diterpenes, in particular Paclitaxel and Docetaxel, are at present used in medicine for the treatment of tumors of different origin.

However, the presently available taxane derivatives have remarkable side effects and also quickly induce resistance, analogously to other antitumor drugs.

The present invention relates to derivatives of seco-baccatine III, which is disclosed in US 5,756,776, characterized by bioavailability through the oral route, reduced toxicity and extremely high antiangiogenetic activity.

The compounds of the present invention have the following general formula (I):



wherein

- R and R_1, which can be the same or different, are hydrogen, a C_1-C_{18} acyl group, an optionally substituted aroyl group or a -$CONR_6R_7$ group wherein R_6 and R_7, which can be the same or different, are C_1-C_4 alkyl, benzyl or phenyl groups;
- R_2 is hydrogen or it forms with R_3 a carbonate or thiocarbonate residue;
- R_3 is hydrogen or a -OR_5 group wherein R_5 is hydrogen, or it forms with R_2 a carbonate or thiocarbonate residue;
- R_4 is a benzoyl group optionally substituted at the meta- position, or a hetaroyl group;
- R' is hydrogen or C_1-C_4 alkyl;

- R'' is C_1-C_4 alkyl, C_2-C_6 alkenyl, aryl or hetaryl;
- R''' is a C_1-C_4 alkyl, C_1-C_{18} acyl, aryl or tert-butoxy group,

with the proviso that R and R_1 cannot be both hydrogen.

A C_1-C_{18} acyl group is preferably a formyl, acetyl, n-propanoyl, n-hexanoyl group.

An optionally substituted aroyl group is preferably benzoyl, optionally substituted with one or three substituents selected from halogen atoms or C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, cyano, nitro groups.

A meta-substituted benzoyl group is preferably a 3- halo -benzyl or 3-methoxy-benzoyl group.

A hetaroyl group is preferably a 5- or 6- membered heteroaryl having one or two oxygen, nitrogen or sulfur atoms in the ring and substituted with a carbonyl group, for example 2- or 3-thenoyl, nicotinoyl, 2- or 3-furoyl.

Aryl is preferably phenyl and hetaryl is preferably 2- or 3- furyl, 2- or 3-thienyl, 2-, 3- or 4-pyridyl.

A preferred group of compounds of formula (I) is that in which:

- R and R_1, which are the same, are a C_1-C_{18} acyl group, an optionally substituted benzoyl group as defined above or a $CONR_6R_7$ group, more preferably R and R_1 are acetyl or 3,4,5-trimethoxy-benzoyl;
- R_2 is hydrogen;
- R_3 is hydrogen;
- R_4 is benzoyl;
- R' is hydrogen or methyl;
- R'' is C_1-C_4 alkyl or C_2-C_6 alkenyl, more preferably isobutyl or isobutenyl;
- R''' is a tert-butoxy group.

A further group of preferred compounds is the one in which R is hydrogen and R_1 is an acyl, aroyl or $CONR_6R_7$ group as defined above, R_2 and R_3 are hydrogen, R_4

is benzoyl, R_1 is hydrogen or methyl, R'' is C_1-C_4 alkyl or C_2-C_6 alkenyl and R''' is tert-butoxy.

The esterification of the hydroxyls at C-7 and C-9 induces, compared with known compounds, an increase in the cytotoxic activity on the resistant cell lines as well as improved absorption through the oral route. The compounds of the invention are less potent than Paclitaxel, taken as the reference drug, in binding with tubulin, while keeping comparable cytotoxicity on the sensitive cancer lines. These compounds mainly differ from those of the prior art in the antiangiogenetic activity. Table shows the in vivo activity of some C-seco-10-dehydro–10-deacetyl-7,9-bisacetyl-baccatine III and C-seco-10-dehydro-10-deacetyl-7,9-bisacetyl-1,14-carbonate-baccatine III derivatives having the same isoserine chain.

The antiangiogenic activity was evaluated by means of the Matrigel test, in which angiogenesis is induced by FGF-2 (150 mg/pellet) adsorbed on a Matrigel pellet (12.5 mg/ml, 0.5 mL) injected subcutaneously in C57BL6N mice.

The tested compound was administered through the oral route daily or through the intraperitoneal route on alternate days, at the shown concentration. After 7 days, the angiogenic response was evaluated by measuring the hemoglobin content in the pellets, according to the procedure by Drabkin.

Table - In vivo antiangiogenetic activity of the compound of example II.

Compound	Hemoglobin g/dl	%
Control	0.01 ± 0.001	-
FGF-2	0.03 ± 0.001	+ 300
Example II		
90 mg/kg i.p.	0.015 ± 0.001	- 50
150 mg/kg p.o.	0.009 ± 0.001	- 70
Example VII		
50 mg/kg i.p.	0.014	- 40
100 mg/kg p.o.	0.009	- 70

The compounds of the invention are prepared by reacting C-seco-10-dehydro-

10-deacetyl-7,9-hydroxy baccatine III described in US 5,756,776 with a carboxylic acid reactive derivative (chloride or anhydride), according to known acylation methods.

The C7 and C9 diesters can be prepared by using at least two equivalents of the reactive derivative. The carbamate groups can be introduced with conventional methods, for example by reaction with phosgene and an amine of formula R_6R_7NH.

The resulting compounds are then reacted, according to known procedures, with an isoserine derivative, usually an oxazolidine derivative, which, by acid treatment under mild conditions gives compounds (I).

The compounds of the invention are characterized by low systemic toxicity: at doses effective in inhibiting the tumor growth they induce neither weight loss nor evident neurotoxicity; in the nude mouse transplanted with human tumor cells, a dose of Paclitaxel, used as the reference drug, exerting the same antitumor activity, also induces tremors and weight loss up to 20%.

The compounds of the present invention, thanks to their high water solubility, can be easily formulated in injectable preparations.

Compounds (I) can also be formulated in the form of conventional oral compositions (capsules or tablets).

Thanks to their low toxicity, compounds (I) can be administered intravenously at dosages up to 600 mg/m^2 and orally at dosages up to 1000 mg/m^2. Dosages can be decreased to 50 mg/m^2 in the treatment of rheumatoid arthritis.

The following examples further illustrate the invention without limiting its scope.

Example I - preparation of C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl-baccatine III.

A solution of 300 mg of 10-dehydro-10-deacetylbaccatine III in 5 ml of methanol is added with 1 equiv. of $CeCl_3.3H_2O$ and the reaction mixture is stirred for 10 min. After complete dissolution, 80 mg of $NaBH_4$ are added in small portions.

After 10 min the solution is treated with an equal volume of a NH_4Cl aqueous solution and extracted with CH_2Cl_2. The chlorinated solvent is removed, the residue is taken up in 1 ml of pyridine, cooled to 0°C in 1 h, then added with 150 mg of acetic anhydride. The solution is left to stand for 2 h at 0°C, then diluted with 10 ml of water and back-extracted with CH_2Cl_2. The chlorinated solvent is distilled off under vacuum and the residue is chromatographed on silica gel eluting with a mixture of n-hexane/ethyl acetate to obtain 260 mg of C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl-baccatine III (m/z 630).

Example II - preparation of 13-[(2R, 3S)-3-iso-butyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl-baccatine III.

630 mg of C-seco-10-dehydro-10-deacetyl-7,9-bis-acetylbaccatine III are dissolved in 5 ml of toluene and added with 335 mg of dicyclohexylcarbodiimide (DCC), 500 mg of (4S,5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-5-oxazolidine-carboxylic acid and 20 mg of 4-dimethylaminopyridine. The solution is heated at 60°C for 24 h, then treated with ethyl acetate and a $NaHCO_3$ saturated solution. The organic phase is dried and filtered through silica gel to remove urea. The solvent is evaporated to dryness under vacuum and the residue is taken up in methanol/hydrochloric acid, keeping a temperature of 0°C for 1 h. The solution is neutralized to pH 5, then diluted with water and the desired compound is back-extracted with CH_2Cl_2. The solvent is evaporated off to obtain 700 mg of 13-[(2R, 3S)-3-iso-butyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl-baccatine III.

Example III - preparation of 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl-baccatine III.

630 mg of C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl-baccatine III are dissolved in 5 ml of toluene and added with 335 mg of DCC, 525 mg of (4S,5R)-N-

Boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-5-oxazolidine-carboxylic acid and 20 mg of 4-dimethylaminopyridine. The solution is heated at 60°C for 24 h, then treated with ethyl acetate and a $NaHCO_3$ saturated solution. The organic phase is dried and filtered through silica gel to remove urea. The solvent is evaporated to dryness under vacuum and the residue is taken up in methanol/hydrochloric acid, keeping a temperature of 0°C for 1 h. The solution is neutralized to pH 5, then diluted with water and the desired compound is back-extracted with CH_2Cl_2. The solvent is evaporated off to obtain 700 mg of 13-[(2R, 3S)-3-iso-butyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl-baccatine III, which is crystallized from ethyl acetate to yield 645 mg of pure compound.

Example IV - preparation of C-seco-10-dehydro-10-deacetyl-7,9-bis-trimethoxybenzoyl-baccatine III.

A solution of 546 mg of C-seco-10-dehydro-10-deacetyl-baccatine III in 3 ml of pyridine is added with 575 mg of trimethoxybenzoyl chloride in small portions. After 3 h the solution is poured into 30 ml of water and extracted with CH_2Cl_2; the organic phase is washed with acids until pyridine is completely removed. The solvent is evaporated off to obtain 905 mg of C-seco-10-dehydro-10-deacetyl-7,9-bis-trimethoxybenzoyl-baccatine III. (m/z 936).

Example V - preparation of 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-dehydro-10-deacetyl-7,9-bis-trimethoxybenzoyl-baccatine III.

930 mg of 13-[(2R, 3S)]-C-seco-10-dehydro-10-deacetyl-7,9-bis-trimethoxy-benzoyl-baccatine III are dissolved in 15 ml of toluene and added with 335 mg of DCC, 525 mg of (4S, 5R)-N-boc-2-(2,4-dimethoxyphenyl)-4-isobutyl-5-oxazolidinecarboxylic acid and 20 mg of 4-dimethylaminopyridine. The solution is heated at 60°C for 24 h, then treated with ethyl acetate and a $NaHCO_3$ saturated solution. The organic phase is dried and filtered through silica gel to remove urea. The solvent is evaporated to dryness under vacuum and the residue is taken up in

methanol/hydrochloric acid, keeping a temperature of 0°C for 1 h. The solution is neutralized to pH 5, then diluted with water and the desired compound is back-extracted with CH_2Cl_2. The solvent is evaporated off to obtain 940 mg of 13-(2R, 3S)-3-isobutyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-dehydro-10-deacetyl-7,9-bis-trimethoxybenzoyl-baccatine III, which is crystallized from ethyl acetate to yield 878 mg of pure compound.

Example VI - preparation of C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl-baccatine III 1,14-carbonate.

1 g of 10-deacetyl-14β-hydroxybaccatine III, prepared as disclosed in US 5,698,712, are dissolved in methanol and treated with 6 g of Cu(OAc)2 and the reaction mixture is stirred for 120 hrs. The salt is filtered off, the solvent is removed and the residue is chromatographed on silica gel column, eluting with a 6:4 mixture of hexane/ethyl acetate, to obtain 0.9 g of 10-dehydro-10-deacetyl-14β-hydroxy-baccatine III 1,14-carbonate (M+ 568). 300 mg of this compound are dissolved in methanol and treated with 1 equiv. of $CeCl_3.3H_2O$ and the reaction mixture is stirred for 10 min. After complete dissolution, 80 mg of $NaBH_4$ are added in small portions. After 10 min the solution is treated with an equal volume of a NH_4Cl aqueous solution and extracted with CH_2Cl_2. The chlorinated solvent is removed, the residue is taken up in 1 ml of pyridine, cooled to 0°C in 1 h, then added with 150 mg of acetic anhydride under stirring. The solution is left to stand for 2 h at 0°C, then diluted with 10 ml of water and back-extracted with CH_2Cl_2. The chlorinated solvent is distilled off under vacuum and the residue is chromatographed on silica gel eluting with a mixture of n-hexane/ethyl acetate to obtain 250 mg of C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl-baccatine III 1,14-carbonate (m/z 658).

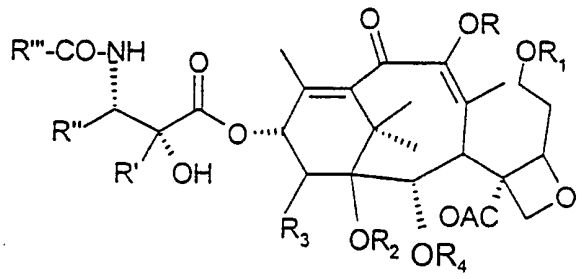
Example VII – preparation of 13-[(2R, 3S)-3-isobutyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl - baccatine III 1,14-carbonate.

600 mg of C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl-baccatine III 1,14-

carbonate are treated as described in Example II, to obtain 680 mg of the title compound.

CLAIMS

1. Compounds of formula (I)



wherein

- R and R_1, which can be the same or different, are hydrogen, a C_1-C_{18} acyl group, an optionally substituted aroyl group or a -$CONR_6R_7$ group wherein R_6 and R_7, which can be the same or different, are C_1-C_4 alkyl, benzyl or phenyl groups;
- R_2 is hydrogen or it forms with R_3 a carbonate or thiocarbonate residue;
- R_3 is hydrogen or a -OR_5 group wherein R_5 is hydrogen, or it forms with R_2 a carbonate or thiocarbonate residue;
- R_4 is a benzoyl group optionally substituted at the meta- position, or a hetaroyl group;
- R' is hydrogen or C_1-C_4 alkyl;
- R'' is C_1-C_4 alkyl, C_2-C_6 alkenyl, aryl or hetaryl;
- R''' is a C_1-C_4 alkyl, C_1-C_{18} acyl, aryl or tert-butoxy group,

with the proviso that R and R_1 cannot be both hydrogen.

2. Compounds as claimed in claim 1 wherein:

- R and R_1, which are the same, are a C_1-C_{18} acyl group, an optionally substituted group benzoyl as defined in claim 1 or a group $CONR_6R_7$, more preferably R and R_1 are acetyl or 3,4,5-trimethoxybenzoyl;
- R_2 is hydrogen;
- R_3 is hydrogen;

- R_4 is benzoyl;
- R' is hydrogen or methyl;
- R" is C_1-C_4 alkyl or C_2-C_6 alkenyl, more preferably isobutyl or isobutenyl;
- R"' is a tert-butoxy group.

3. Compounds as claimed in claim 1 wherein R is hydrogen and R_1 is an acyl, aroyl or $CONR_6R_7$ group as defined in claim 1, R_2 and R_3 are hydrogen, R_4 is benzoyl, R' is hydrogen or methyl, R" is C_1-C_4 alkyl or C_2-C_6 alkenyl and R"' is tert-butoxy.

4. A compound as claimed in claim 1 selected from:

13-[(2R, 3S)-3-iso-butyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl-baccatine III,

13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl-baccatine III,

13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-dehydro-10-deacetyl-7,9-bis-trimethoxybenzoyl-baccatine III,

13-[(2R, 3S)-3-isobutyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-dehydro-10-deacetyl-7,9-bis-acetyl -baccatine III 1,14-carbonate.

5. Pharmaceutical compositions containing as active ingredient a compound of claims 1 - 4 in mixture with a suitable carrier.

6. The use of the compounds of claims 1 - 4 for the preparation of antitumor, antiangiogenetic and anti-arthrosis medicaments.

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/EP 01/00386

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D305/08 C07D305/14 A61K31/335

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)
IPC 7 C07D 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, PAJ, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 03394 A (INDENA SPA ;BOMBARDELLI EZIO (IT); BELLIS PAOLO DE (IT); GABETTA B) 8 February 1996 (1996-02-08) cited in the application page 7; claims 7-9,11-14	1-6

☐ Further documents are listed in the continuation of 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	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

Inter ›nal Application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9603394 A	08-02-1996	IT 1274356 B	17-07-1997
		IT RM950413 A	19-12-1996
		AT 176464 T	15-02-1999
		AU 684218 B	04-12-1997
		AU 3164195 A	22-02-1996
		BG 62851 B	29-09-2000
		BG 101165 A	29-08-1997
		CA 2195844 A	08-02-1996
		CZ 9700199 A	16-07-1997
		DE 69507702 D	18-03-1999
		DE 69507702 T	26-08-1999
		DK 773938 T	20-09-1999
		EP 0773938 A	21-05-1997
		ES 2126919 T	01-04-1999
		FI 970271 A	19-03-1997
		GR 3029309 T	28-05-1999
		HK 1011687 A	31-03-2000
		HU 76829 A	28-11-1997
		JP 2986550 B	06-12-1999
		JP 9512827 T	22-12-1997
		KR 220490 B	15-09-1999
		NO 970296 A	12-03-1997
		PL 318289 A	09-06-1997
		RO 115875 B	28-07-2000
		RU 2134688 C	20-08-1999
		SI 9520078 A,B	30-06-1997
		SK 10097 A	06-08-1997
		US 5756776 A	26-05-1998