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(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING ACRYLOYL DISTAMYCIN DERIVATIVES AND TOPOISOMERASE I AND II INHIBITORS

(57) **Abstract:** The present invention provides the combined use of acryloyl distamycin derivatives, in particular α -bromo- and α -chloro-acryloyl distamycin derivatives of formula (I), as set forth in the specification, and an antineoplastic topoisomerase I or II inhibitor, in the treatment of tumors. Also provided is the use of the said combinations in the treatment or prevention of metastasis or in the treatment of tumors by inhibition of angiogenesis.

COMBINED THERAPY AGAINST TUMORS COMPRISING SUBSTITUTED
ACRYLOYL DISTAMYcin DERIVATIVES AND TOPOISOMERASE I AND
II INHIBITORS

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The present invention relates to the field of cancer treatment and provides an antitumor composition comprising a substituted acryloyl distamycin derivative, more particularly an α -bromo- or α -chloro-acryloyl distamycin derivative, and a topoisomerase inhibitor of type I or II, having a synergistic antineoplastic effect.

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Distamycin A and analogues thereof, hereinafter referred to as distamycin and distamycin-like derivatives, are known in the art as cytotoxic agents useful in antitumor therapy.

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Distamycin A is an antibiotic substance with antiviral and antiprotozoal activity, having a polypyrrrole framework [*Nature* 203: 1064 (1964); *J. Med. Chem.* 32: 774-778 (1989)].

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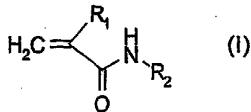
The international patent applications WO 90/11277, WO 98/04524, WO 98/21202, WO 99/50265, WO 99/50266 and WO 01/40181 (claiming priority from British patent application No. 9928703.9), all in the name of the applicant itself and herewith incorporated by reference, disclose acryloyl distamycin derivatives wherein the amidino moiety of distamycin is optionally replaced by nitrogen-containing ending groups such as, for instance, cyanamidino, N-methylamidino, guanidino, carbamoyl, amidoxime, cyano and the like, and/or wherein the polypyrrrole framework of distamycin, or part of it, is replaced by varying carbocyclic or heterocyclic moieties.

25

The present application generally relates to, in a first aspect, a pharmaceutical composition for use in antineoplastic therapy in mammals, including humans, comprising a pharmaceutically acceptable carrier or excipient;

30 an acryloyl distamycin derivative of formula (I):

35



wherein:

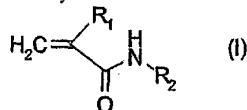
R₁ is a bromine or chlorine atom;

R₂ is a distamycin or distamycin-like framework; or a pharmaceutically acceptable salt thereof; and

5 an antineoplastic topoisomerase inhibitor of type I or II.

The present invention provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier or excipient and, as active ingredient,

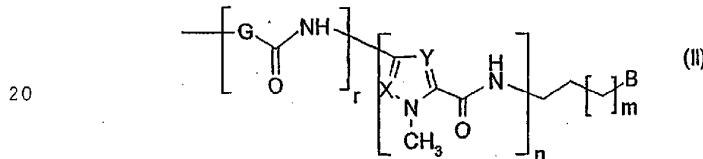
10 an acryloyl distamycin derivative of formula (I):



wherein:

15 R₁ is a bromine or chlorine atom;

R₂ is a group of formula (II):



wherein

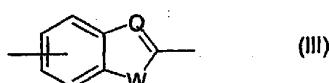
25 m is an integer from 0 to 2;

n is an integer from 2 to 5;

r is 0 or 1;

X and Y are, the same or different and independently for each heterocyclic ring, a nitrogen atom or a CH group;

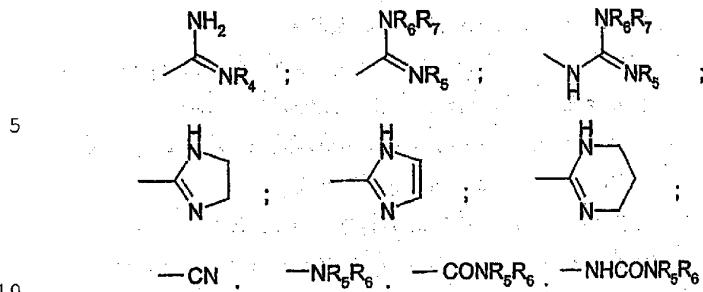
30 G is phenylene, a 5 or 6 membered saturated or unsaturated heterocyclic ring with from 1 to 3 heteroatoms selected among N, O or S, or it is a group of formula (III) below:



35 wherein Q is a nitrogen atom or a CH group and W is an oxygen or sulfur atom or it is a group NR₃ wherein R₃ is hydrogen or C₁-C₄ alkyl;

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B is selected from the group consisting of



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wherein R₄ is cyano, amino, hydroxy or C₁-C₄ alkoxy; R₅, R₆ and R₇, the same or different, are hydrogen or C₁-C₄ alkyl.

or a pharmaceutically acceptable salt thereof; and

15 an antineoplastic topoisomerase inhibitor of type I or II.

The present invention includes, within its scope, the pharmaceutical compositions comprising any of the possible isomers covered by the compounds of formula (I), both considered separately or in admixture, as well as the metabolites and the pharmaceutically acceptable bio-precursors (otherwise known as pro-drugs) of the compounds of formula (I).

In the present description, unless otherwise specified, with the term distamycin or distamycin-like framework R₂ we intend any moiety structurally closely related to distamycin itself, for instance by optionally replacing the ending amidino moiety of distamycin and/or its polypyroline framework, or part of it.

Topoisomerase I and II inhibitors are known in the art as described in various scientific publications.

The main representatives for topoisomerase I inhibitors are camptothecin derivatives such as, for instance, CPT-11, Topotecan, 9-amino-camptothecin, 9-nitro-camptothecin and 10,11-methylenedioxy-camptothecin.

Among the topoisomerase II inhibitors are, in particular, the anthracycline derivatives such as doxorubicin, daunorubicin, epirubicin, nemorubicin and idarubicin; the podophyllotoxin compounds etoposide and teniposide; the anthraquinone derivative

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like mitoxantrone and losoxantrone; the acridine derivatives like amsacrine and actinomaycin D. See, for a reference, *Cancer, Principles and Practice of Oncology*, Lippincott-Raven Ed. (1997), 452-467.

5 According to a preferred embodiment of the invention, herewith provided are the above pharmaceutical compositions wherein the topoisomerase inhibitors are topoisomerase II inhibitors, in particular doxorubicin and etoposide.

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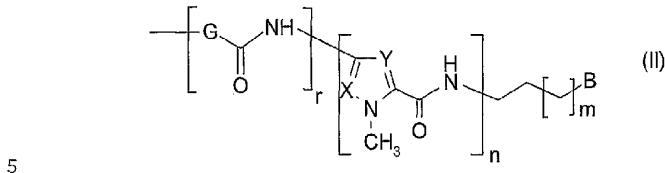
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According to another preferred embodiment of the invention, herewith provided are the above pharmaceutical compositions wherein, within the acryloyl distamycin derivative of formula (I), R_1 has the above reported meanings and R_2 is a group of formula (II) below:



wherein

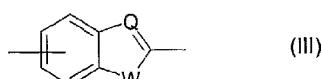
m is an integer from 0 to 2;

n is an integer from 2 to 5;

r is 0 or 1;

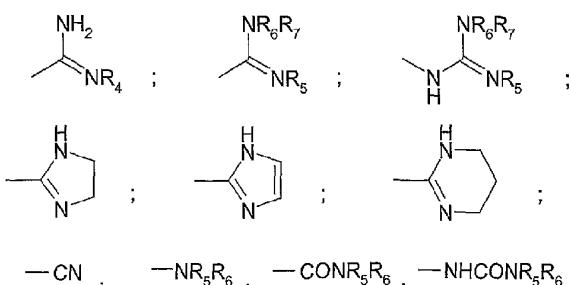
10 X and Y are, the same or different and independently for each heterocyclic ring, a nitrogen atom or a CH group;

G is phenylene, a 5 or 6 membered saturated or unsaturated heterocyclic ring with from 1 to 3 heteroatoms selected among N, O or S, or it is a group of formula (III) below:



15 wherein Q is a nitrogen atom or a CH group and W is an oxygen or sulfur atom or it is a group NR_3 wherein R_3 is hydrogen or C_1 - C_4 alkyl;

B is selected from the group consisting of



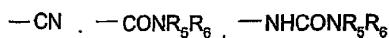
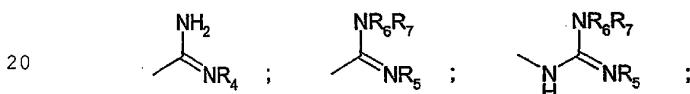
wherein R_4 is cyano, amino, hydroxy or C_1 - C_4 alkoxy; R_5 , R_6 and R_7 , the same or

different, are hydrogen or C₁-C₄ alkyl.

In the present description, unless otherwise specified, with the term C₁-C₄ alkyl or alkoxy group we intend a straight or branched group selected from methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy or tert-butoxy.

Even more preferred are the pharmaceutical compositions of the invention comprising the above acryloyl distamycin derivative of formula (I) wherein R₁ is bromine or chlorine; R₂ is the above group of formula (II) wherein r is 0, m is 0 or 1, n is 4 and B has the above reported meanings.

Still more preferred, within this class, are the pharmaceutical compositions comprising the compounds of formula (I) wherein R_1 is bromine or chlorine; R_2 is the above group of formula (II) wherein r is 0, m is 0 or 1, n is 4, X and Y are both CH groups and B is selected from:



wherein R₄ is cyano or hydroxy and R₅, R₆ and R₇, the same or different, are hydrogen or C₁-C₄ alkyl.

Pharmaceutically acceptable salts of the compounds of formula (I) are those with pharmaceutically acceptable inorganic or organic acids such as, for instance, hydrochloric, hydrobromic, sulfuric, nitric, acetic, propionic, succinic, malonic, citric, tartaric, methanesulfonic, p-toluenesulfonic acid and the like.

Examples of acryloyl distamycin derivatives of formula (I), optionally in the form of pharmaceutically acceptable salts, preferably with hydrochloric acid, are:

1. N-(5-{{[5-{{[5-{{[2-({[amino(imino)methyl]amino}ethyl]amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-

y]amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;

2. N-(5-{{(5-{{(2-{{[amino(imino)methyl]amino}propyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;

5 3. N-(5-{{(5-{{(5-{{(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;

10 4. N-(5-{{(5-{{(5-{{(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-imidazole-2-carboxamide hydrochloride;

15 5. N-(5-{{(5-{{(5-{{(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-3-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrazole-5-carboxamide hydrochloride;

20 6. N-(5-{{(5-{{(5-{{(3-amino-3-oxopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-3-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrazole-5-carboxamide;

7. N-(5-{{(5-{{(5-{{(2-{{[amino(imino)methyl]amino}ethyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;

25 8. N-(5-{{(5-{{(3-{{[amino(imino)methyl]amino}propyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;

9. N-(5-{{(5-{{(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride; and

30

10. N-[(5-[(5-[(3-[(aminocarbonyl)amino]propyl)amino]carbonyl]-1-methyl-1H-pyrrol-3-yl)amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide.

5

10 The above compounds of formula (I), either specifically identified as such or by means of the general formula, are known or easily prepared according to known methods as reported, for instance, in the aforementioned international patent applications WO 90/11277, WO 98/04524, WO 98/21202, WO 99/50265, WO 99/50266 and WO 01/40181.

15

The present invention further provides a product comprising an acryloyl distamycin derivative of formula (I), as defined above, and an antineoplastic topoisomerase I or II inhibitor, as a combined preparation for simultaneous, separate or sequential use in antitumor therapy.

20

A further aspect of the present invention is to provide a method of treating a mammal, including humans, suffering from a neoplastic disease state, which method comprises administering to said mammal the above acryloyl distamycin derivative of formula (I) and an antineoplastic topoisomerase I or II inhibitor, in amounts effective to produce a synergistic antineoplastic effect.

25

The present invention also provides a method for lowering the side effects caused by antineoplastic therapy with an antineoplastic agent in a mammal in need thereof, including humans, the method comprising administering to said mammal a combined preparation comprising an antineoplastic topoisomerase I or II inhibitor and an acryloyl distamycin derivative of formula (I), as defined above, in amounts effective to produce a synergistic antineoplastic effect.

30

35 The present invention also provides a method for treating tumors, or a method for the prevention or treatment of metastasis or in the treatment of tumors by angiogenesis, the method comprising administering an acryloyl distamycin derivative of formula (I), as

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defined above, and an antineoplastic topoisomerase I or II inhibitor to a mammal in need thereof.

By the term "synergistic antineoplastic effect", as used herein, it is meant the inhibition
5 of the growth tumor, preferably the complete regression of the tumor, by administering

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an effective amount of the combination comprising an acryloyl distamycin derivative of formula (I) and a topoisomerase I or II inhibitor to mammals, including humans.

By the term "administered" or "administering", as used herein, it is meant parenteral and/or oral administration; the term "parenteral" means intravenous, subcutaneous and 5 intramuscular administration.

In the method of the present invention, the acryloyl distamycin derivative may be administered simultaneously with the compound having topoisomerase I or II inhibitory activity, for example with a compound of the camptothecin, anthracycline, 10 mitoxantrone, epipodophyllotoxin, or acridine class. Alternatively, both compounds may be administered sequentially in either order.

In this respect, it will be appreciated that the actual preferred method and order of administration will vary according to, inter alia, the particular formulation of the acryloyl distamycin of formula (I) being used, the particular formulation of the 15 topoisomerase I or II inhibitor being used, for instance the camptothecins such as CPT-11, topotecan, 9-AC; the anthracyclines such as doxorubicin, daunorubicin, epirubicin, idarubicin, nemorubicin; the anthraquinones such as mitoxantrone and losoxantrone; the epipodophyllotoxins such as etoposide, teniposide; the acridine derivatives such as 20 amsacrine and actinomycin D, the particular tumor model being treated as well as the particular host being treated.

To administer the acryloyl distamycin derivative of formula (I), according to the method of the invention, the course of therapy generally employed comprises doses varying from about 0.05 to about 100 mg/m² of body surface area and, more preferably, from about 0.1 to about 50 mg/m² of body surface area.

25 For the administration of the topoisomerase I or II inhibitor, according to the method of the invention, the course of therapy generally employed comprises
- when administering camptothecins: doses varying from about 1 to about 1000 mg/m² of body surface area and, more preferably, from about 10 to about 500 mg/m² of body surface area;
30 - when administering anthracyclines: doses varying from about 0.1 to about 1000 mg/m² of body surface area and, more preferably, from about 0.5 to about 500

mg/m² of body surface area;

- 5 - when administering epipodophyllotoxins: doses varying from about 1 to about 500 mg/m² of body surface area and, more preferably, from about 10 to about 400 mg/m² of body surface area;
- 10 - when administering anthraquinones: doses varying from about 1 to about 300 mg/m² of body surface area and, more preferably, from about 5 to about 100 mg/m² of body surface area.
- 15 - when administering acridine and actinomycin D derivatives: doses varying from about 1 to about 1000 mg/m² of body surface area and, more preferably, from about 10 to about 500 mg/m² of body surface area.

20 The antineoplastic therapy of the present invention is particularly suitable for treating breast, ovary, lung, colon, kidney, stomach, pancreas, liver, melanoma, leukemia and brain tumors in mammals, including humans.

25 In a further aspect, the present invention provides use of an acryloyl distamycin derivative of formula (I), as defined above, in the preparation of a medicament for use in combination therapy with an antineoplastic topoisomerase I or II inhibitor in the treatment of tumors or in the preparation of a medicament for use in the prevention or treatment of metastasis or in the treatment of tumors by inhibition of angiogenesis.

30 In a further aspect, the present invention provides use of an acryloyl distamycin derivative of formula (I), as defined above and an antineoplastic topoisomerase I or II inhibitor in amounts effective to produce a synergistic antineoplastic effect, in the preparation of an medicament for treating a mammal suffering from a neoplastic disease state.

35 In a further aspect, the present invention provides use of a combined preparation comprising an antineoplastic topoisomerase I or II inhibitor and an acryloyl distamycin derivative of formula (I), as defined above in amounts effective to produce a synergistic antineoplastic effect, in the preparation of a medicament for lowering side effects caused by antineoplastic therapy with an antineoplastic agent, in a

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mammal in need thereof.

In a further aspect, the present invention provides use of an acryloyl distamycin derivative of formula (I), as defined above in combination therapy with an

5 antineoplastic topoisomerase I or II inhibitor in the treatment of tumors.

In a further aspect, the present invention provides use of an acryloyl distamycin derivative of formula (I), as defined above for a combination therapy with an

10 antineoplastic topoisomerase I or II inhibitor in the prevention or treatment of metastasis or in the treatment of tumors by inhibition of angiogenesis.

In further aspect, the present invention provides use of an acryloyl distamycin derivative of formula (I), as defined above and an antineoplastic topoisomerase I or II inhibitor, in amounts effective to produce a synergistic antineoplastic effect in a

15 mammal suffering from a neoplastic disease state.

In a further aspect, the present invention provides use of a combined preparation comprising an antineoplastic topoisomerase I or II inhibitor and an acryloyl distamycin derivative of formula (I), as defined above in amounts effective to produce a synergistic

20 antineoplastic effect for lowering side effects caused by antineoplastic therapy with an antineoplastic agent, in a mammal in need thereof.

As stated above, the effect of an acryloyl distamycin derivative of formula (I) and a

25 topoisomerase I or II inhibitor, such as an anthracycline or etoposide derivative, is significantly increased without a parallel increase of toxicity. In other words, the combined therapy of the present invention enhances the antitumoral effects of the acryloyl distamycin derivative and of the topoisomerase I or II inhibitor and, hence, 30 provides the most effective and least toxic treatment for tumors.

The superadditive effects of the combined preparations of the invention are shown, for instance, by the following *in vivo* tests, which are intended to illustrate the present

35 invention without posing any limitation to it.

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5 Table 1 shows the antileukemic activity on disseminated L1210 murine leukemia obtained by combining N-(5-{[(5-{[(2-
10 (amino(imino)methyl]amino)ethyl]amino]carbonyl}-1-methyl-1H-pyrrol-3-
15 yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-
yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride, as
15 a representative compound of formula (I) - internal code PNU 166196, with
doxorubicin. At the dose of 10 mg/kg of doxorubicin alone (day +1 after tumor
injection and 2 hours after PNU 166196 administration) and at the dose of 0.52 mg/kg
of PNU 166196 alone (days +1) were associated, without toxicity, ILS% values of 58
and 33, respectively. Combining doxorubicin and PNU 166196 at the same doses with
the same schedule, an increase of activity with ILS% value of 100 was observed, thus
indicating a synergistic effect.

20 Table 1: Antileukemic activity against disseminated L1210¹ murine leukemia of an
acryloyl distamycin derivative (I) in combination with doxorubicin

Compound	Treatment ² schedule	Dose (mg/kg/day)	ILS% ³	Tox ⁴
PNU 166196	iv +1	0.52	33	0/10
Doxorubicin	iv +1(*)	10	58	0/10
PNU 166196 +	iv +1	0.52 + 10	100	0/10
Doxorubicin	iv +1(*)			

25

1. L1210 leukemia cells (10^5 /mouse CD2F1) are injected IV on Day 0.
2. Treatment is given IV.
3. Increase in life span: [(median survival time of treated mice/median survival time of controls) x 100] -100.
4. Number of toxic deaths/number of mice.

30 (*) doxorubicin was administered 2 h after PNU 166196 administration.

35 For these experiments, PNU 166196 and doxorubicin were solubilized in water for injection.

In the claims which follow and in the preceding description of the invention, except
where the context requires otherwise due to express language or necessary implication,

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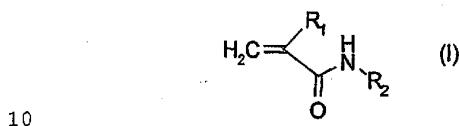
the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

- 5 It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

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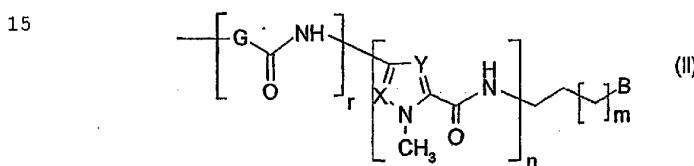
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or excipient and, as active ingredient,
 5 - an acryloyl distamycin derivative of formula (I):



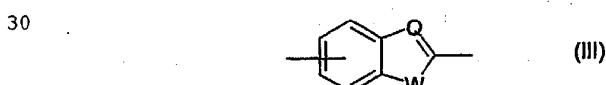
wherein:

R1 is a bromine or chlorine atom;
 R2 is a group of formula (II):



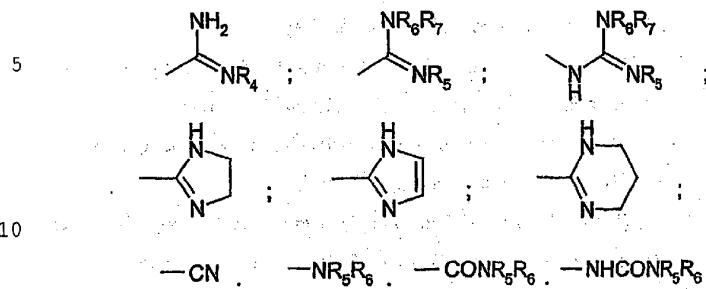
20 wherein

m is an integer from 0 to 2;
 n is an integer from 2 to 5;
 r is 0 or 1;
 25 X and Y are, the same or different and independently for each heterocyclic ring, a nitrogen atom or a CH group;
 G is phenylene, a 5 or 6 membered saturated or unsaturated heterocyclic ring with from 1 to 3 heteroatoms selected among N, O or S, or it is a group of formula (III) below:



35 wherein Q is a nitrogen atom or a CH group and W is an oxygen or sulfur atom or it is a group NR3 wherein R3 is hydrogen or C1-C4 alkyl;

B is selected from the group consisting of



wherein R₄ is cyano, amino, hydroxy or C₁-C₄ alkoxy; R₅, R₆ and R₇, the same or different, are hydrogen or C₁-C₄ alkyl.

15 or a pharmaceutically acceptable salt thereof; and
- an antineoplastic topoisomerase inhibitor of type I or II

2. A pharmaceutical composition according to claim 1 wherein the topoisomerase
20 inhibitor is a topoisomerase II inhibitor selected from the group consisting of
anthracycline derivatives, epipodophyllotoxin compounds, anthraquinone derivatives,
and acridine derivatives.

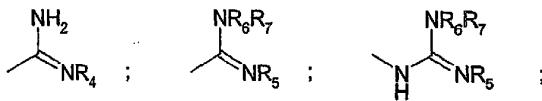
3. A pharmaceutical composition according to claim 2 wherein the anthracycline derivatives are selected from the group consisting of doxorubicin, daunorubicin, epirubicin, nemorubicin and idarubicin; the epipodophyllotoxin compounds are selected from etoposide or teniposide; the anthraquinone derivatives are selected from mitoxantrone or losoxantrone and the acridine derivatives are selected from amsacrine or dactinomycin.

30 4. A pharmaceutical composition according to claim 3 wherein the topoisomerase
II inhibitor is doxorubicin or etoposide.

5. A pharmaceutical composition according to any one of claims 1 to 4 comprising
35 an acryloyl distamycin derivative of formula (I) wherein R₁, R₂ and B are as defined in
claim 1, r is 0, m is 0 or 1 and n is 4.

6. A pharmaceutical composition according to any one of claims 1 to 5 comprising an acryloyl distamycin derivative of formula (I) wherein R₁ and R₂ are as defined in claim 1, r is 0, m is 0 or 1, n is 4, X and Y are both CH groups and B is selected from:

5



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wherein R₄ is cyano or hydroxy and R₅, R₆ and R₇, the same or different, are hydrogen or C₁-C₄ alkyl.

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7. A pharmaceutical composition according to claim 1 comprising an acryloyl distamycin derivative, optionally in the form of a pharmaceutically acceptable salt, selected from the group consisting of:

1. N-(5-{[(5-{[(2-{[amino(imino)methyl]amino}ethyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;
2. N-(5-{[(5-{[(2-{[amino(imino)methyl]amino}propyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;
3. N-(5-{[(5-{[(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;
4. N-(5-{[(5-{[(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-imidazole-2-carboxamide

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hydrochloride;

5. N-(5-{{(5-{{(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-3-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrazole-5-carboxamide hydrochloride;

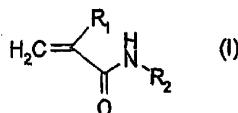
10. N-(5-{{(5-{{(3-amino-3-oxopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-3-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrazole-5-carboxamide;

15. N-(5-{{(5-{{(2-{{[amino(imino)methyl]amino}ethyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-chloroacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;

20. N-(5-{{(5-{{(3-{{[amino(imino)methyl]amino}propyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride; and

25. N-(5-{{(5-{{(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide.

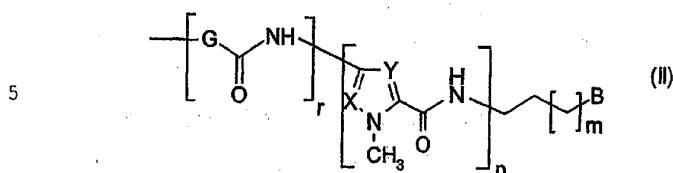
8. Product comprising an acryloyl distamycin derivative of formula (I):



35 wherein:

R_1 is a bromine or chlorine atom;

R₂ is a group of formula (II):



wherein

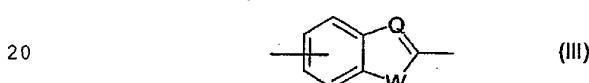
10 m is an integer from 0 to 2;

n is an integer from 2 to 5;

r is 0 or 1;

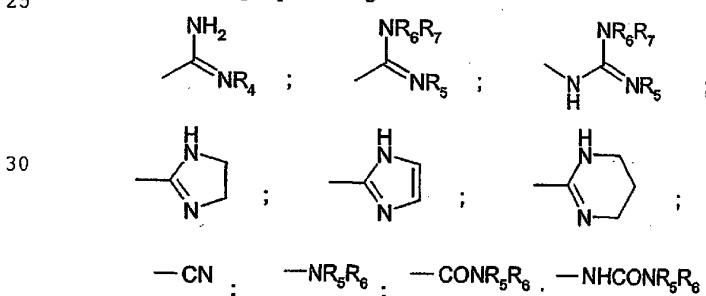
15 X and Y are, the same or different and independently for each heterocyclic ring, a nitrogen atom or a CH group;

G is phenylene, a 5 or 6 membered saturated or unsaturated heterocyclic ring with from 1 to 3 heteroatoms selected among N, O or S, or it is a group of formula (III) below:



wherein Q is a nitrogen atom or a CH group and W is an oxygen or sulfur atom or it is a group NR₃, wherein R₃ is hydrogen or C₁-C₄ alkyl;

25 B is selected from the group consisting of



35 wherein R₄ is cyano, amino, hydroxy or C₁-C₄ alkoxy; R₅, R₆ and R₇, the same or different, are hydrogen or C₁-C₄ alkyl.

or a pharmaceutically acceptable salt thereof; and an antineoplastic topoisomerase inhibitor of type I or II, as a combined preparation for simultaneous, separate or sequential use when used in the treatment of tumors.

- 5 9. Product according to claim 8 wherein the topoisomerase inhibitor is a topoisomerase II inhibitor selected from the group consisting of anthracycline derivatives, epipodophyllotoxin compounds, anthraquinone derivatives and acridine derivatives.
- 10 10. Product according to claim 9 wherein the anthracycline derivatives are selected from the group consisting of doxorubicin, daunorubicin, epirubicin, nemorubicin and idarubicin; the epipodophyllotoxin compounds are selected from etoposide or teniposide; the anthraquinone derivatives are selected from mitoxantrone or losoxantrone and the acridine derivatives are selected from amsacrine or dactinomycin.
- 15 11. Product according to claim 10 wherein the topoisomerase II inhibitor is doxorubicin or etoposide.
- 20 12. Product according to claim 8 wherein the acryloyl distamycin derivative is selected from the group as defined in claim 7.
13. Use of an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 7 in the preparation of a medicament for use in combination therapy with an antineoplastic topoisomerase I or II inhibitor in the treatment of tumors.
- 25 14. Use according to claim 13 wherein the medicament further comprises the said topoisomerase I or II inhibitor.
- 30 15. Use according to claim 13 or 14 wherein the topoisomerase inhibitor is a topoisomerase II inhibitor selected from etoposide or doxorubicin.
16. Use according to claim 13 or 14 wherein the acryloyl distamycin derivative is selected from the group as defined in claim 7.
- 35 17. Use according to any one of claims from 13 to 16 wherein the tumor is selected from the group consisting of breast, ovary, lung, colon, kidney, stomach, pancreas, liver, melanoma, leukemia and brain tumors.

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18. Use of an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 7 in the preparation of a medicament for use in combination therapy with an antineoplastic topoisomerase I or II inhibitor in the prevention or treatment of metastasis or in the treatment of tumors by inhibition of angiogenesis.
19. Use according to claim 18 wherein the medicament further comprises the said topoisomerase I or II inhibitor.
20. A method of treating a mammal suffering from neoplastic disease state, which method comprises administering to said mammal the acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 7, and an antineoplastic topoisomerase I or II inhibitor, in amounts effective to produce a synergistic antineoplastic effect.
21. A method for lowering the side effects caused by antineoplastic therapy with an antineoplastic agent, in a mammal in need thereof the method comprising administering to said mammal a combined preparation comprising an antineoplastic topoisomerase I or II inhibitor and an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 7, in amounts effective to produce a synergistic antineoplastic effect.
22. A pharmaceutical composition according to claim 1 wherein the distamycin derivative, optionally in the form of a pharmaceutically acceptable salt, is N-(5-{{(5-{{[(2-{{[amino(imino)methyl]amino}ethyl]amino}carbonyl}-1-methyl-1H-pyrrol-3-yl)amino}carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide, and wherein the topoisomerase inhibitor is a topoisomerase II inhibitor selected from the group consisting of etoposide and doxorubicin.
23. Use of an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 7 in combination therapy with an antineoplastic topoisomerase I or II inhibitor in the treatment of tumors.
24. A method of treating tumors, comprising administering an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 7 and an antineoplastic topoisomerase I or II inhibitor to a mammal in need thereof.
25. Use according to claim 23 or method according to claim 24, wherein the

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topoisomerase inhibitor is a topoisomerase II inhibitor selected from etoposide or doxorubicin.

26. Use according to claim 23 or method according to claim 24, wherein the acryloyl distamycin derivative is selected from the group as defined in claim 7.
27. Use according to claim 23 or method according to claim 24, wherein the tumor is selected from the group consisting of breast, ovary, lung, colon, kidney, stomach, pancreas, liver, melanoma, leukemia and brain tumors.
28. Use of an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 7 for a combination therapy with an antineoplastic topoisomerase I or II inhibitor in the prevention or treatment of metastasis or in the treatment of tumors by inhibition of angiogenesis.
29. A method for the prevention or treatment of metastasis or in the treatment of tumors by inhibition of angiogenesis, comprising administering an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 7 with an antineoplastic topoisomerase I or II inhibitor to a mammal in need thereof.
30. Use of an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 7 and an antineoplastic topoisomerase I or II inhibitor in amounts effective to produce a synergistic antineoplastic effect, in the preparation of a medicament for treating a mammal suffering from a neoplastic disease state.
31. Use of an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 7 and an antineoplastic topoisomerase I or II inhibitor, in amounts effective to produce a synergistic antineoplastic effect in a mammal suffering from a neoplastic disease state.
32. Use of a combined preparation comprising an antineoplastic topoisomerase I or II inhibitor and an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 7 in amounts effective to produce a synergistic antineoplastic effect, in the preparation of a medicament for lowering side effects caused by antineoplastic therapy with an antineoplastic agent, in a mammal in need thereof.
33. Use of a combined preparation comprising an antineoplastic topoisomerase I or

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II inhibitor and an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 7 in amounts effective to produce a synergistic antineoplastic effect for lowering side effects caused by antineoplastic therapy with an antineoplastic agent, in a mammal in need thereof.

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34. Method according to claim 20, 21, 24 or 29 or use according to any one of claims 30 to 32, wherein the mammal is a human.

10 35. Pharmaceutical compositions, products, uses or methods, substantially as herein described with reference to the accompanying examples.

Dated this 25th day of November 2005

PHARMACIA ITALIA S.p.A.

15 By their Patent Attorneys

GRIFFITH HACK

Fellows Institute of Patent and

Trade Mark Attorneys of Australia

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