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(71) Applicant(s)
Pharmacia Italia S.p.A.

(72) Inventor(s)
Beria, Italo; Geroni, Maria Cristina Rosa; Cozzi, Paolo

(74) Agent/Attorney
Griffith Hack, 509 St Kilda Road, Melbourne, VIC, 3004

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(71) Applicant *(for all designated States except US)*: **PHARMACIA ITALIA S.P.A.** [IT/IT]; Via Robert Koch, 1.2, I-20152 Milan (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GERONI, Maria, Cristina, Rosa** [IT/IT]; Via Correggio, 48, I-20149 Milano (IT). **COZZI, Paolo** [IT/IT]; Via Zanella 48/5, I-20133 Milano (IT). **BERIA, Italo** [IT/IT]; Via S. Anna, 16, I-20014 Nerviano (IT).

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(54) Title: COMBINED THERAPY AGAINST TUMORS COMPRISING SUBSTITUTED ACRYLOYL DISTAMYCIN DERIVATIVES AND ALKYLATING AGENTS

(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 alkylating agent, in the treatment of tumors. Also provided is the use of the said combination in the treatment or prevention of metastasis or in the treatment of tumors by inhibition of angiogenesis.

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COMBINED THERAPY AGAINST TUMORS COMPRISING SUBSTITUTED ACRYLOYL DISTAMYCIN DERIVATIVES AND ALKYLATING AGENTS

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 an alkylating agent, having a synergistic antineoplastic effect.

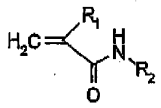
Distamycin A and analogues thereof, hereinafter referred to as distamycin and distamycin-like derivatives, are known in the art as cytotoxic agents useful in antitumor therapy.

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

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 polypyrrole framework of distamycin, or part of it, is replaced by varying carbocyclic or heterocyclic moieties.

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

- an acryloyl distamycin derivative of formula (I):



(I)

wherein:

R_1 is a bromine or chlorine atom;

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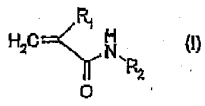
R_2 is a distamycin or distamycin-like framework; or a pharmaceutically acceptable salt thereof; and

- an alkylating agent.

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

- an acryloyl distamycin derivative of formula (I):

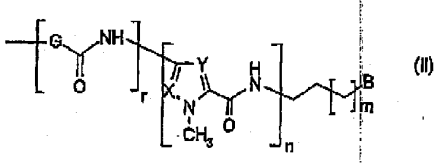


(I)

wherein:

R_1 is a bromine or chlorine atom;

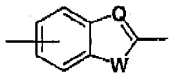
R_2 is a group of formula (II):



(II)

wherein

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



(III)

wherein Q is a nitrogen atom or a CH group and W is an O or S atom or is a group NR_3 wherein R_3 is H or C_1-C_4 alkyl;

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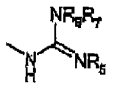
m is 0;
n is 4;
r is 0;
X and Y are both a CH group;
B is the group:



wherein R_5, R_6 and R_7, the same or different, are hydrogen or C_1-C_4 alkyl; or a pharmaceutically acceptable salt thereof; and

- an alkylating agent.

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 R_2 we intend any moiety structurally closely related to distamycin itself, for instance by optionally replacing the ending amidino moiety of distamycin and/or its polypyrrole framework, or part of it.

Alkylating agents are widely known in the art as described in various scientific publications.

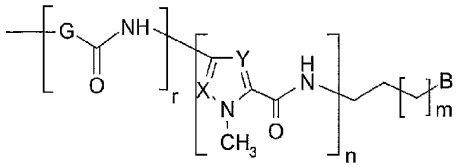
Representatives for this class of compounds are, for instance, mustards such as melphalan, chlorambucil, mechlorethamine, cyclophosphamide, ifosfamide and busulfan; nitrosoureas such as carmustine, lomustine, semustine and fotemustine; tetrazines such as dacarbazine and temozolomide; aziridines such as thiotepa and mitomycin C and platinum derivatives such as cisplatin, carboplatin, oxaliplatin, nedaplatin and lobaplatin and the like.

See, for a general reference, Cancer Principles and Practice of Oncology, Lippincott-Raven Ed. (1997), 405-432.

According to a preferred embodiment of the invention, herewith provided are the above pharmaceutical compositions wherein the alkylating agent is selected from mustards and platinum derivatives such as cisplatin, carboplatin and oxaliplatin.

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:



(II)

wherein

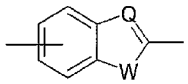
m is an integer from 0 to 2;

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;

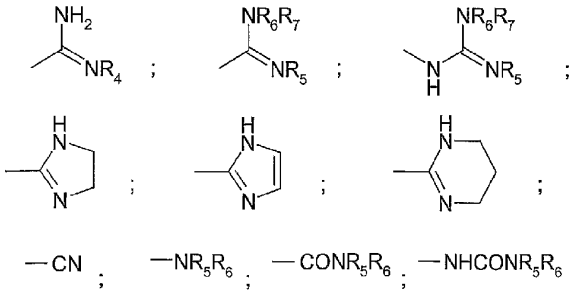
G is phenylene, a 5 or 6 membered saturated or unsaturated heterocyclic ring with from 1 to 3 heteroatoms selected among N, O or S, or it is a group of formula (III) below:



(III)

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 C_1-C_4 alkyl.

In the present description, unless otherwise specified, with the term C_1-C_4 alkyl or

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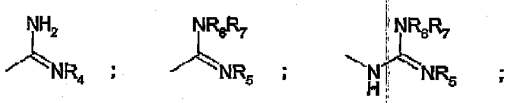
alkoxy group we intend a straight or branched group selected from methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy or tert-butoxy.

Even more preferred are the pharmaceutical compositions of the invention comprising the above acryloyl distamycin derivative of formula (I) wherein 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 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:



$$-CN \; ; \; -CONR_5R_6 \; ; \; -NHCONR_6R_6$$

wherein R_4 is cyano or hydroxy and R_5, R_6 and R_7, the same or different, are hydrogen or C_1-C_4 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-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;

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

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methyl-1H-pyrrol-3-yl}-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide.

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 and WO 99/50266 as well as in WO 01/40181.

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

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 alkylating agent, in amounts effective to produce a synergistic antineoplastic effect.

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 alkylating agent and an acryloyl distamycin derivative of formula (I), as defined above, in amounts effective to produce a synergistic antineoplastic effect.

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 inhibition of angiogenesis, comprising administering an acryloyl distamycin derivative of formula (I), as defined above, and an alkylating agent to a mammal in need thereof.

By the term "synergistic antineoplastic effect", as used herein, it is meant the inhibition of the growth tumor, preferably the complete regression of the tumor, by administering an effective amount of the combination comprising an acryloyl distamycin derivative of formula (I) and an alkylating agent 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 intramuscular administration.

In the method of the present invention, the acryloyl distamycin derivative may be administered simultaneously with the alkylating agent or, 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 alkylating agent being used, 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/m^2 of body surface area and, more preferably, from about 0.1 to about 50 mg/m^2 of body surface area.

For the administration of the alkylating agent, according to the method of the invention, the course of therapy generally employed comprises:

for the administration of mustard compounds doses varying from about 1 mg/m^2 to about 5000 mg/m^2 of body surface area and, more preferably, from about 10 to about 1000 mg/m^2 of body surface area.

for the administration of nitrosourea derivatives doses varying from about 1 mg/m^2 to about 1000 mg/m^2 of body surface area and, more preferably, from about 10 to about 1000 mg/m^2 of body surface area.

for the administration of tetrazine and aziridine compounds doses varying from about 1 mg/m^2 to about 1000 mg/m^2 of body surface area and, more preferably, from about 10 to about 1000 mg/m^2 of body surface area.

for the administration of platinum derivatives doses varying from about 1 mg/m^2 to about 1000 mg/m^2 of body surface area and, more preferably, from about 10 to about 500 mg/m^2 of body surface area.

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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.

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 alkylating agent in the treatment of tumors or in the prevention or treatment of metastasis or in the treatment of tumors by inhibition of angiogenesis.

The present invention further provides use of an acryloyl distamycin derivative of formula (I), as defined above, and an alkylating agent in amounts effective to produce a synergistic antineoplastic effect, for the manufacture of a medicament for treating a mammal suffering from a neoplastic disease state.

The present invention also provides use of a combined preparation comprising an alkylating agent and an acryloyl distamycin derivative of formula (I), as defined above, in amounts effective to produce a synergistic antineoplastic effect, for the manufacture of a medicament for lowering side effects caused by antineoplastic therapy with an antineoplastic agent, to a mammal in need thereof.

The present invention also provides use of an acryloyl distamycin derivative of formula (I) as defined above for combination therapy with an alkylating agent in the treatment of tumors or in the prevention or treatment of metastasis or in the treatment of tumors by inhibition of angiogenesis.

The present invention also provides use of an acryloyl distamycin derivative of formula (I) as defined above and an alkylating agent, in amounts effective to produce a synergistic antineoplastic effect in a mammal suffering from a neoplastic disease state.

The present invention still also provides use of a combined preparation comprising an alkylating agent and an acryloyl distamycin derivative of formula (I) as defined above, in amounts effective to produce a synergistic antineoplastic effect for lowering the side effects caused by antineoplastic therapy with an antineoplastic agent, in a mammal in need thereof.

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As stated above, the effect of an acryloyl distamycin derivative of formula (I) and an alkylating agent, for instance cisplatin and carboplatin, 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 alkylating agent and, hence, provides the most effective and least toxic treatment for tumors.

The synergistic or superadditive effect of the combined preparations of the invention is shown, for instance, by the following *in vivo* tests which are intended to illustrate the present invention without posing any limitation to it.

Table 1 shows the antileukemic activity on disseminated L1210 murine leukemia obtained by combining the representative compound of formula (I) of the invention 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)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride - internal code PNU 166196, with cisplatin.
At the dose of 5.9 mg/kg of cisplatin alone (day +3) and at the dose of 0.26 mg/kg of PNU 166196 alone (days +1,2) were associated, without toxicity, with ILS% values of 67 and 33, respectively.

Combining cisplatin and PNU 166196 at the same doses with the same schedule, an increase of activity with ILS% values of 125 were observed, thus indicating a synergistic antitumor effect.

Table 2 shows the antileukemic activity on disseminated L1210 murine leukemia obtained by combining the above PNU 166196 derivative with carboplatin.
At the dose of 135 mg/kg of carboplatin alone (day +3) and at the dose of 0.26 mg/kg of PNU 166196 alone (days +1,2) were associated, without toxicity, with ILS% values of 50 and 33, respectively.
By combining carboplatin and PNU 166196 at the same doses and with the same schedule, an increase of activity with ILS% values of 92 were observed, again indicating a more than additive effect.

Table 3 shows the antitumor effect on subcutaneous implanted HCT-116 human colon carcinoma obtained by combining PNU 166196 with cisplatin.
At the dose of 2 mg/kg of cisplatin alone (q7dx3) and at the dose of 0.4 mg/kg of PNU 166196 alone (q7dx3) were associated, without toxicity, T/C% values of 92 and 61, respectively.
By combining cisplatin and PNU 166196, instead, a significant increase in tumor growth delay was observed, hence indicating a therapeutic advantage of the combination (synergism) in comparison to the administration of the drugs alone.

For these experiments PNU 166196 was solubilized in water for injection, while standard pharmaceutical preparations were used for cisplatin and carboplatin.

Table 1: Antileukemic activity against disseminated L1210[1] murine leukemia of an acryloyl distamycin derivative (I) in combination with cisplatin.

Compound	Treatment[2] schedule	Dose (mg/kg/day)	ILS%[3]	Tox[4]
PNU 166196	iv +1,2	0.26	33	0/10
Cisplatin	iv +3	5.9	67	0/10
PNU 166196 + Cisplatin	iv +1,2 iv +3	0.26 + 5.9	120	0/10

[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.

Table 2: Antileukemic activity against disseminated L1210[1] murine leukemia of an acryloyl distamycin derivative in combination with carboplatin.

Compound	Treatment schedule	Dose[2] (mg/kg/day)	ILS%[3]	Tox [4]
PNU 166196	iv +1,2	0.26	33	0/10
Carboplatin	iv +3	135	50	0/10
PNU 166196 + Carboplatin	iv +1,2 iv +3	0.26 + 135	92	0/10

[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.

Table 3: Antitumor activity against human colon carcinoma HCT-116 (low/medium GST and MMR deficiency) of an acryloyl distamycin derivative in combination with cisplatin.

Compound	Dose[a] (mg/kg)	T/C %[b]	Log cell Kill total	Tumor free/total mice[c]	WL% (day of nadir)[d]
PNU-166196	0.4	61	0.15	0/8	12 (29)
Cisplatin	2	92	0	0/7	12 (24)
PNU-166196 + Cisplatin	0.4 + 2	36 (synergic)[e]	0.7	1/7	13 (27)

[a] Treatment IV started on day 7 after tumor implant; schedule q7dx3 of PNU 166196 administered 48 hours after cisplatin;

[b] Tumor regression (T/C %) on day 20 after treatment (according to NCI standards: T/C ≤ 42 active);

[c] On day 40 after tumor implant;

[d] 27 days after tumor implant;

[e] Fisher's test vs. both cisplatin and PNU 166196

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, 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.

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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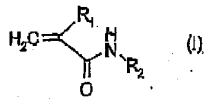
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or excipient and, as active ingredient,

- an acryloyl distamycin derivative of formula (I):

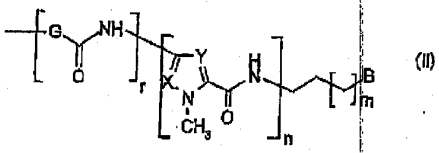


(I)

wherein:

R_1 is a bromine or chlorine atom;

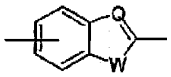
R_2 is a group of formula (II):



(II)

wherein

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



(III)

wherein Q is a nitrogen atom or a CH group and W is an O or S atom or is a group NR_3 wherein R_3 is H or C_1-C_4 alkyl;

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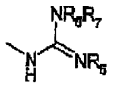
m is 0;
n is 4;
r is 0;
X and Y are both a CH group;
B is the group:



wherein R_5, R_6 and R_7, the same or different, are hydrogen or C_1-C_4 alkyl; or a pharmaceutically acceptable salt thereof; and

- an alkylating agent.

2. A pharmaceutical composition according to claim 1 wherein the alkylating agent is selected from the group consisting of mustards, nitrosoureas, tetrazines, aziridines and platinum derivatives.

3. A pharmaceutical composition according to claim 2 wherein the mustard is selected from the group consisting of melphalan, chlorambucil, mechlorethamine, cyclophosphamide, ifosfamide and busulfan; the nitrosourea is selected from the group consisting of carmustine, lormustine, semustine and fotemustine; the tetrazine is selected from dacarbazine or temozolomide; the azaridine is selected from thiotepa or mitomycin C; and the platinum derivative is selected from the group consisting of cisplatin, carboplatin, oxaliplatin, nedaplatin and lobaplatin.

4. 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; and
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.

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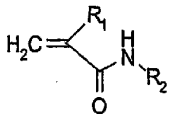
5. Product comprising an acryloyl distamycin derivative of formula (I):



(I)

as defined in claim 1 or a pharmaceutically acceptable salt thereof; and an alkylating agent, as a combined preparation for simultaneous, separate or sequential use when used in the treatment of tumors.

6. Product according to claim 5 wherein the alkylating agent is selected from mustards, nitrosoureas, tetrazines, aziridines and platinum derivatives.

7. Product according to claim 6 wherein the mustard is selected from the group consisting of melphalan, chlorambucil, mechlorethamine, cyclophosphamide, ifosfamide and busulfan; the nitrosourea is selected from the group consisting of carmustine, lormustine, semustine and fotemustine; the tetrazine is selected from dacarbazine or temozolomide; the azaridine is selected from thiotepa or mitomycin C; and the platinum derivative is selected from the group consisting of cisplatin, carboplatin, oxaliplatin, nedaplatin and lobaplatin.

8. Product according to any one of claims 5 to 7 wherein the acryloyl distamycin derivative is selected from the group as defined in claim 4.

9. Use of an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 4, in the preparation of a medicament for use in combination therapy with an alkylating agent in the treatment of tumors.

10. Use according to claim 9 wherein the medicament further comprises the said alkylating agent.

11. Use according to claim 9 or 10 wherein the alkylating agent is as defined in claim 2.

12. Use according to claim 9 or 10 wherein the acryloyl distamycin derivative is selected from the group as defined in claim 4.

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13. Use according to any one of claims 7 to 10 wherein the tumor is selected from the group consisting of breast, ovary, lung, colon, kidney, stomach, pancreas, liver, melanoma, leukemia and brain tumors.

14. Use of an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 4 in the preparation of a medicament for use in combination therapy with an alkylating agent in the prevention or treatment of metastasis or in the treatment of tumors by inhibition of angiogenesis.

15. Use of an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 4, and an alkylating agent, in amounts effective to produce a synergistic antineoplastic effect, for the manufacture of a medicament for treating a mammal suffering from a neoplastic disease state.

16. Use of a combined preparation comprising an alkylating agent and an acryloyl distamycin derivative of formula (I), as defined in any one of claims from 1 to 4, in amounts effective to produce a synergistic antineoplastic effect, for the manufacture of a medicament for lowering side effects caused by antineoplastic therapy with an antineoplastic agent, in a mammal in need thereof.

17. A pharmaceutical composition according to claim 1 wherein the acryloyl distamycin derivative, optionally in the form of a pharmaceutically acceptable salt, is 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)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]- 1-methyl-1H-pyrrole-2-carboxamide, and the alkylating agent is a platinum derivative selected from the group consisting of cisplatin, carboplatin, oxaliplatin, nedaplatin and lobaplatin.

18. Use of an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 4 for combination therapy with an alkylating agent in the treatment of tumors.

19. Use of an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 4 for combination therapy with an alkylating agent in the prevention or treatment of metastasis or in the treatment of tumors by inhibition of angiogenesis.

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20. Use of an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 4 and an alkylating agent, in amounts effective to produce a synergistic antineoplastic effect in a mammal suffering from a neoplastic disease state.

21. Use of a combined preparation comprising an alkylating agent and an acryloyl distamycin derivative of formula (I), as defined in any one of claims from 1 to 4, 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.

22. A method of treating tumors, comprising administering an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 4 and an alkylating agent to a mammal in need thereof.

23. A method according to claim 22 or use according to claim 18, wherein the alkylating agent is as defined in claim 2.

24. A method according to claim 22 or use according to claim 18, wherein the acryloyl distamycin derivative is selected from the group as defined in claim 4.

25. A method according to claim 22 or use according to claim 18, wherein the tumor is selected from the group consisting of breast, ovary, lung, colon, kidney, stomach, pancreas, liver, melanoma, leukemia and brain tumors.

26. 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 4 with an alkylating agent to a mammal in need thereof.

27. A method of treating a mammal suffering from a neoplastic disease state, comprising administering an acryloyl distamycin derivative of formula (I), as defined in any one of claims 1 to 4 and an alkylating agent, in amounts effective to produce a synergistic antineoplastic effect.

28. A method for lowering side effects caused by antineoplastic therapy with an antineoplastic agent in a mammal in need thereof, comprising administering a combined preparation comprising an alkylating agent and an acryloyl distamycin derivative of

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formula (I), as defined in any one of claims 1 to 4 in amounts effective to produce a synergistic antineoplastic effect.

29. Use according to any one of claims 15, 16, 23 or 24, or a method according to any one of claims 25 to 31, wherein the mammal is a human.

30. 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.
By their Patent Attorneys
GRIFFITH HACK
Fellows Institute of Patent and
Trade Mark Attorneys of Australia

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