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NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____

NO__ _X_ _____

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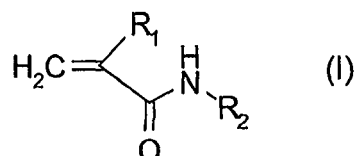
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(54) Title: COMBINED THERAPY AGAINST TUMORS COMPRISING SUBSTITUTED ACRYLOYL DISTAMYCIN DERIVATIVES AND TOPOISOMERASE I AND II INHIBITORS

(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.

CLAIMS

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



wherein:

R₁ is a bromine or chlorine atom;

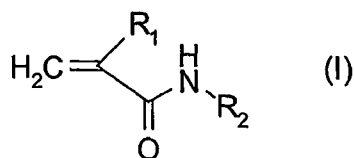
- 10 R₂ is a distamycin or distamycin-like framework; or a pharmaceutically acceptable salt thereof; and
- an antineoplastic topoisomerase inhibitor of type I or II.

2. A pharmaceutical composition according to claim 1 wherein the topoisomerase
15 inhibitor is a topoisomerase II inhibitor selected from anthracycline derivatives, including doxorubicin, daunorubicin, epirubicin, nemorubicin and idarubicin; epipodophyllotoxin compounds including etoposide and teniposide; anthraquinone derivatives including mitoxantrone and losoxantrone; acridine derivatives including amsacrine and dactinomycin.

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3. A pharmaceutical composition according to claim 2 wherein the topoisomerase II inhibitor is doxorubicin or etoposide.

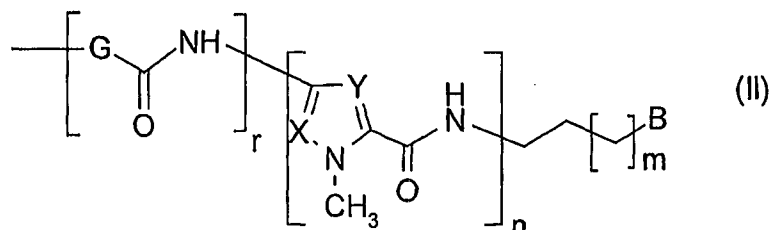
4. A pharmaceutical composition according to claim 1 comprising an acryloyl
25 distamycin derivative of formula (I)



wherein:

R₁ is a bromine or chlorine atom;

R₂ is a group of formula (II)



wherein

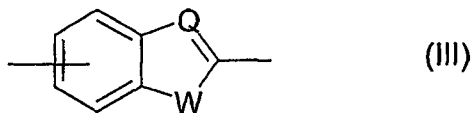
m is an integer from 0 to 2;

5 n is an integer from 2 to 5;

r is 0 or 1;

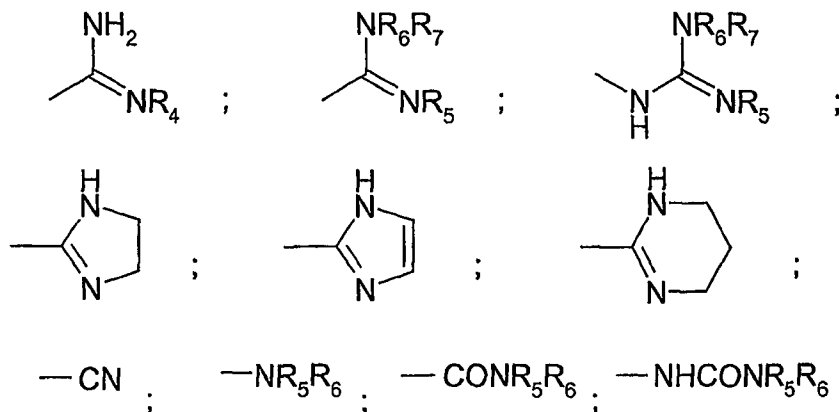
X and Y are, the same or different and independently for each heterocyclic ring, a nitrogen atom or a CH group;

G is phenylene, a 5 or 6 membered saturated or unsaturated heterocyclic ring with from 1
10 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;

B is selected from the group consisting of



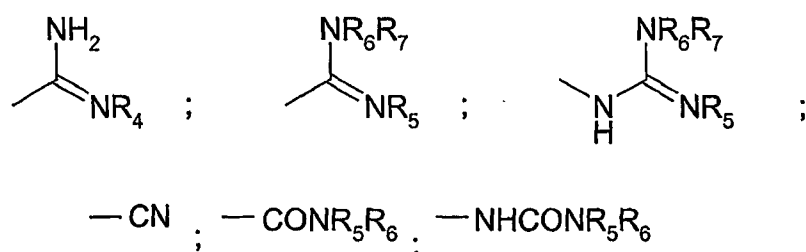
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wherein R₄ is cyano, amino, hydroxy or C₁-C₄ alkoxy; R₅, R₆ and R₇, the same or different, are hydrogen or C₁-C₄ alkyl.

5. A pharmaceutical composition according to claim 4 comprising an acryloyl

distamycin derivative of formula (I) wherein R_1 , R_2 and B are as defined in claim 4, r is 0, m is 0 or 1 and n is 4.

6. A pharmaceutical composition according to claim 5 comprising an acryloyl
5 distamycin derivative of formula (I) wherein R_1 and R_2 are as defined in claim 4, r is 0, m is 0 or 1, n is 4, X and Y are both CH groups and B is selected from:



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.

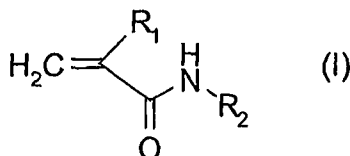
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7. A pharmaceutical composition according to claim 1 comprising an acryloyl
distamycin derivative, optionally in the form of a pharmaceutically acceptable salt,
selected from the group consisting of:

1. N-(5-([(5-([(5-([(2-[amino(imino)methyl]amino)ethyl]amino)carbonyl]-1-
15 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-
20 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
25 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-[[[(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-[[[(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-[[[(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-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide.

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



wherein:

R₁ is a bromine or chlorine atom;

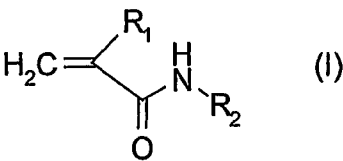
R₂ is a distamycin or distamycin-like framework; or a pharmaceutically acceptable salt thereof; and an antineoplastic topoisomerase inhibitor of type I or II, as a combined

preparation for simultaneous, separate or sequential use in the treatment of tumors.

9. Products according to claim 8 wherein the topoisomerase inhibitor is a topoisomerase II inhibitor selected from anthracycline derivatives, including doxorubicin, daunorubicin, epirubicin, nemorubicin and idarubicin; epipodophyllotoxin compounds including etoposide and teniposide; anthraquinone derivatives including mitoxantrone and losoxantrone; acridine derivatives including amsacrine and dactinomycin.

10. Products according to claim 9 wherein the topoisomerase II inhibitor is doxorubicin or etoposide.

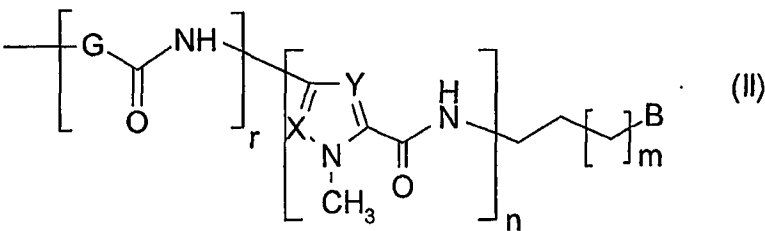
11. Products according to claim 8 comprising an acryloyl distamycin derivative of formula (I)



wherein:

R₁ is a bromine or chlorine atom;

R₂ is a group of formula (II)



wherein

m is an integer from 0 to 2;

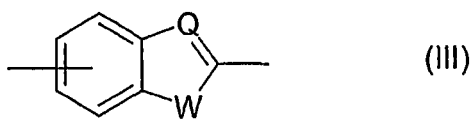
n is an integer from 2 to 5;

r is 0 or 1;

X and Y are, the same or different and independently for each heterocyclic ring, a

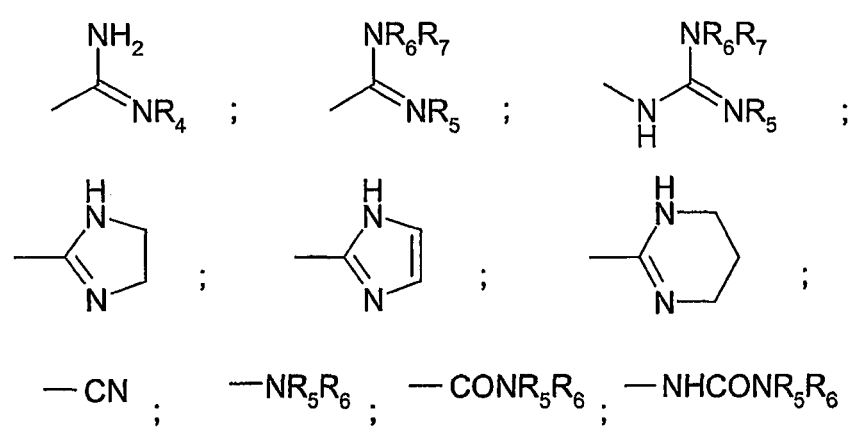
nitrogen atom or a CH group;

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:



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.

10

12. Products 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 from 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.

14. Use according to claim 13 wherein the medicament further comprises the said topoisomerase I or II inhibitor.

20

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.

17. Use according to any one of claims from 13 to 16 wherein the tumor is selected
5 from breast, ovary, lung, colon, kidney, stomach, pancreas, liver, melanoma, leukemia and brain tumors.

18. Use of an acryloyl distamycin derivative of formula (I), as defined in any one of
claims from 1 to 7 in the preparation of a medicament for use in combination therapy
10 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.

15

20. A method of treating a mammal, including humans, suffering from a neoplastic
disease state, which method comprises administering to said mammal the acryloyl
distamycin derivative of formula (I), as defined in any one of claims from 1 to 7, and
an antineoplastic topoisomerase I or II inhibitor, in amounts effective to produce a
20 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 including humans, the method
comprising administering to said mammal a combined preparation comprising an
25 antineoplastic topoisomerase I or II inhibitor and an acryloyl distamycin derivative of
formula (I), as defined in any one of claims from 1 to 7, in amounts effective to
produce a synergistic antineoplastic effect.

22. A pharmaceutical composition according to claim 1 wherein the distamycin
30 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)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 or doxorubicin.

5 February 1998 (05.02.98)

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

Acryloyl substituted distamycin derivatives of formula (I) wherein n is 2, 3, or 4; R₁ and R₂ are selected, each independently, from: hydrogen, halogen, and C₁-C₄ alkyl; R₃ is hydrogen or halogen; B is selected from: (1), (2), (3), (4), (5), (6) and (7); wherein R₄, R₅, R₆, R₇, and R₈ are, each independently, hydrogen or C₁-C₄ alkyl, with the proviso that at least one of R₄, R₅ and R₆ is C₁-C₄ alkyl; and pharmaceutically acceptable salts thereof. Such compounds are useful as antineoplastic and antiviral agents.

FAB-MS: m/z 724, (10, [M+H]⁺)

PMR (DMSO-d₆) δ :

10.27 (s, 1H), 9.94 (s, 1H), 9.91 (s, 1H), 9.88 (s, 1H),
9.5 (b.s., 2H), 8.5 (b.s., 1H), 7.98 (t, J=5.9 Hz, 1H),
5 7.3-6.8 (m, 8H), 6.66 (d, J=2.9 Hz, 1H), 6.21 (d, J=2.9 Hz,
1H), 3.84 (s, 6H), 3.83 (s, 3H), 3.79 (s, 3H), 3.38 (b.s.,
2H), 2.31 (b.s., 2H).

UV: c=4.16mg/l (MeOH) λ_{MAX}=307.8 ε=51155

- 10 By analogous procedure and by using the opportune starting materials the following products can be obtained:

3-[1-methyl-4[1-methyl-4[1-methyl-4(α-bromoacrylamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime; and

- 15 3-[1-methyl-4[1-methyl-4[1-methyl-4[1-methyl-4(α-chloroacrylamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime.

20 **EXAMPLE 5**

2-[1-methyl-4[1-methyl-4[1-methyl-4[1-methyl-4(α-bromoacrylamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine hydrochloride

25

Step I The intermediate 2-aminoethylguanidine dihydrochloride

- A solution of commercial N-BOC-ethylendiamine (1 g) in dry ethanol (100 ml) and 2-methyl-2-thiopseudourea hydroiodide
30 (1.5 g) was refluxed for 8 hours. The solvent was removed at reduced pressure and the crude residue purified by flash

animal body by therapy.

Furthermore, the present invention provides a method for treating tumors and viral infections in a patient in need of it, which comprises administering to said patient a
5 composition of the invention.

A further object of the present invention is a combined method for treating cancer or for ameliorating the conditions of mammals, including humans, suffering from cancer, said method comprising administering a compound of
10 formula (I), or a pharmaceutically acceptable salt thereof, and an additional antitumor agent, close enough in time and in amounts sufficient to produce a therapeutically useful effect.

15 The present invention also provides combined preparations for simultaneous, separate or sequential use in anti-cancer therapy, comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof, and an additional antitumour agent.

20 The term "antitumor agent" is meant to comprise both a single antitumor drug and "cocktails" i.e. a mixture of such drugs, according to the clinical practice. Examples of antitumor agents that can be formulated with a compound of formula (I), or alternatively, can be administered in a
25 combined method of treatment, include doxorubicin, daunomycin, epirubicin, idarubicin, etoposide, fluorouracil, melphalan, cyclo-phosphamide, 4-demethoxy daunorubicin, bleomycin, vinblastin, and mitomycin, or mixtures thereof.

30

The following examples are given to better illustrate the invention, but do not limit the scope of the invention


D₂
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/EP99/01823 (22) International Filing Date: 17 March 1999 (17.03.99) (30) Priority Data: 9806692.1 27 March 1998 (27.03.98) GB (71) Applicant (for all designated States except US): PHARMACIA & UPJOHN S.P.A. [IT/IT]; Via Robert Koch, 1.2, I-20152 Milan (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): COZZI, Paolo [IT/IT]; Via Zanella, 48/5, I-20133 Milan (IT). BARALDI, Pier, Giovanni [IT/IT]; Via Tulipani, 73, I-44100 Ferrara (IT). BERIA, Italo [IT/IT]; Via G. Matteotti, 39, I-45030 Villamarzana (IT). CALDARELLI, Marina [IT/IT]; Via Benzenanica, 9, I-20147 Milan (IT). CAPOLONGO, Laura [IT/IT]; Via P. Rembrandt, 11, I-20147 Milan (IT). ROMAGNOLI, Romeo [IT/IT]; Via Bologna, 291, I-44100 Ferrara (IT).		(81) Designated States: AE, AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BI, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.
(54) Title: BENZOHETEROCYCLIC DISTAMYCIN DERIVATIVES, PROCESS FOR PREPARING THEM, AND THEIR USE AS ANTITUMOR AGENTS		
(57) Abstract Compounds which are benzoheterocyclic distamycin derivatives of formula (I), wherein n is 2, 3 or 4; A is a heteroatom selected from O and S or is a group NR, wherein R is hydrogen or C₁-C₄ alkyl; B is CH or N; R₁ is hydrogen or C₁-C₄ alkyl; G is selected from the group consisting of (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), and -C≡N; wherein R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁ and R₁₂ are, independently from each other, hydrogen or C₁-C₄ alkyl; T is a group of formula (II) or (III) as defined above, wherein p is 0 or 1; R₂ and R₃ are, independently from each other, hydrogen, C₁-C₄ alkyl optionally substituted by one or more fluorine atoms, or C₁-C₄ alkoxy; R₄ is C₁-C₄ alkyl or C₁-C₃ haloalkyl; X₁ and X₂ are halogen atoms or pharmaceutically acceptable salts thereof; provided that at least one of R₅, R₆ and R₇ is alkyl; are useful as antitumor agents.		

Further object of the present invention are the compounds of formula (I) for use in a method for treating the human or animal body by therapy.

Furthermore, the present invention provides a method for
5 treating tumors in a patient in need of it, which comprises administering to said patient a composition of the invention.

A further object of the present invention is a combined method for treating cancer or for ameliorating the
10 conditions of mammals, including humans, suffering from cancer, said method comprising administering a compound of formula (I), or a pharmaceutically acceptable salt thereof, and an additional antitumor agent, close enough in time and in amounts sufficient to produce a therapeutically useful
15 effect.

The present invention also provides products containing a compound of formula (I), or a pharmaceutically acceptable salt thereof, and an additional antitumour agent as a combined preparation for simultaneous, separate or
20 sequential use in anti-cancer therapy.

The term "antitumor agent" is meant to comprise both a single antitumor drug and "cocktails" i.e. a mixture of such drugs, according to the clinical practice. Examples of antitumor agents that can be formulated with a compound of
25 formula (I), or alternatively, can be administered in a combined method of treatment, include doxorubicin, daunomycin, epirubicin, idarubicin, etoposide, fluoro-uracil, melphalan, cyclophosphamide, 4-demethoxy daunorubicin, bleomycin, vinblastin, and mitomycin, or
30 mixtures thereof.

The following examples are given to better illustrate the present invention, but do not limit the scope of the invention itself.

35 **EXAMPLE 1**

3-[1-methyl-4[1-methyl-4[1-methyl-4[5(α -
bromoacrylamido)benzofurane-2-carboxamido]pyrrole-2-

carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine hydrochloride (11)

Step I: The intermediate 5- α -bromoacrylamidobenzofurane-2-carboxylic acid

- 5 To a solution of 500 mg of commercial α -bromoacrylic acid in 5 ml of acetonitrile, a solution of 343 mg of N,N-dicyclohexylcarbodiimide in 15 ml of acetonitrile was slowly added. After one hour, the solution obtained after filtration of the precipitate was added to a solution of
- 10 294 mg of 5-amino-2-benzofuranic acid, prepared as reported in *Helv.Chim.Acta* 31, 75 (1948), and 229 mg of sodium bicarbonate in 20 ml of water. The reaction was stirred at room temperature for one hour, then 2N hydrochloric acid was added until pH=4. The solution was extracted with ethyl
- 15 acetate (3x10 ml), dried over sodium sulfate and evaporated to dryness in vacuo and the crude residue purified by flash chromatography with a methylene chloride/methanol mixture to yield 500 mg of the intermediate as a pale yellow solid.
- 20 By analogous procedure and by using the opportune starting materials the following products can be obtained:
- 5- α -bromoacrylamidobenzothiophene-2-carboxylic acid;
- 5- α -bromoacrylamidoindole-2-carboxylic acid;
- 1-methyl-5- α -bromoacrylamidoindole-2-carboxylic acid;
- 25 5- α -bromoacrylamidoindazole-2-carboxylic acid;
- 1-methyl-5- α -bromoacrylamidoindazole-2-carboxylic acid;
- 5- α -chloroacrylamidoindole-2-carboxylic acid;
- 1-methyl-5- α -chloroacrylamidoindole-2-carboxylic acid.

30 **Step II:** The title compound

- A solution of 250 mg of N-deformyl-distamycin A N,N'-dimethyl dihydrochloride, prepared as reported in WO 97/28123, in 5 ml of dry DMF was cooled to 5°C and added with 0.086 ml of N,N'-diisopropylethylamine. After 10 min,
- 35 180 mg of the intermediate obtained from step I, and 192 mg

3-[1-methyl-4[1-methyl-4[1-methyl-4[5-N,N-bis(2-chloroethyl)aminobenzofurane-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propioncyanamidine (24).

5

EXAMPLE 2

3-[1-methyl-4[1-methyl-4[1-methyl-4[1-methyl-5(α -bromoacrylamido)indole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile (23)

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Step I: The intermediate 3-[1-methyl-4[1-methyl-4[1-methyl-4[1-methyl-5(α -bromoacrylamido)indole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride.

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A solution of 260 mg of N-deformyldistamycin A dihydrochloride, prepared as reported in J. Med. Chem. 32, 774-778 (1989), in 5 ml of dry dimethylformamide (DMF) was cooled to 5°C and added with 0.086 ml of N,N'-diisopropylethylamine. After 10 min, 180 mg of 5- α -bromoacrylamidoindole-2-carboxylic acid and 190 mg of N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide (EDCI) were added. The reaction was stirred at room temperature for 10 hours, then 2N hydrochloric acid was added up to pH=4. The solvent was removed under reduced pressure and the crude residue purified by flash chromatography (methylene chloride/methanol:8/2) to give 240 mg of the title compound.

25

FAB-MS: m/z 760, (100, [M+H]⁺)

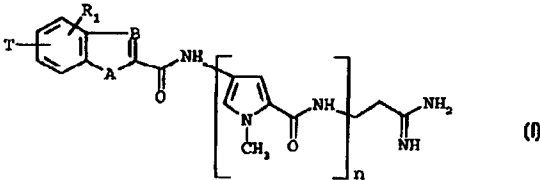
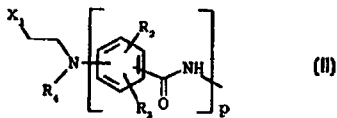
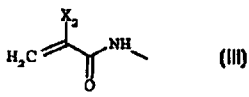
30 PMR (DMSO-d₆) δ :

10.44 (s, 1H), 10.23 (s, 1H), 10.00 (s, 1H), 9.93 (s, 1H), 8.97 (b.s., 2H), 8.60 (b.s., 2H), 8.22 (t, J=5.7 Hz, 1H), 8.05 (d, J=1.8 Hz, 1H), 7.54 (d, J=8.9 Hz, 1H), 7.47 (dd, J=8.9 Hz and J=1.9 Hz, 1H), 7.25 (d, J=1.8 Hz, 1H), 7.21 (s, 1H), 7.19 (d, J=1.8 Hz, 1H), 7.12 (d, J=1.8 Hz, 1H), 7.07 (d, J=1.8 Hz, 1H), 7.05 (d, J=1.8 Hz, 1H), 6.95 (d,

35


D₃
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/EP97/05986 (22) International Filing Date: 23 October 1997 (23.10.97) (30) Priority Data: 9623522.1 11 November 1996 (11.11.96) GB (71) Applicant (for all designated States except US): PHARMACIA & UPJOHN S.P.A. [IT/IT]; Via Robert Koch, 1.2, I-20152 Milan (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): COZZI, Paolo [IT/IT]; Via Zanella, 48/5, I-20133 Milan (IT). BARALDI, Pier, Giovanni [IT/IT]; Via Tulipani, 73, I-44100 Ferrara (IT). BERIA, Italo [IT/IT]; Via G. Matteotti, 39, I-45030 Villamarzana (IT). CALDARELLI, Marina [IT/IT]; Via Besenhanica, 9, I-20147 Milan (IT). CAPOLONGO, Laura [IT/IT]; Via P. Rembrandt, 11, I-20147 Milan (IT). SPALLUTO, Giampiero [IT/IT]; Via Pomposa, 20, I-44100 Ferrara (IT). ROMAGNOLI, Romeo [IT/IT]; Via Bologna, 291, I-44100 Ferrara (IT).		(81) Designated States: AU, BG, BR, CA, CN, CZ, EE, HU, IL, JP, KR, LT, LV, MX, NO, NZ, PL, SG, SI, TR, UA, US, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: BENZOHETEROCYCLIC DISTAMYCIN DERIVATIVES, PROCESS FOR PREPARING THEM, AND THEIR USE AS ANTITUMOR AND ANTIVIRAL AGENTS  (I)  (II)  (III)		
(57) Abstract Benzoheterocyclic distamycin derivatives of formula (I) wherein: n is 2, 3 or 4; A is O, S, or NR, wherein R is hydrogen or C₁-C₄ alkyl; B is CH or N; R₁ is hydrogen or C₁-C₄ alkyl; T is selected from: (i) formula (II) wherein: p is zero or 1; R₂ and R₃ are selected, each independently, from: hydrogen, C₁-C₄ alkyl optionally substituted by one or more fluorine atoms, and C₁-C₄ alkoxy; R₄ is C₁-C₄ alkyl or C₁-C₃ haloalkyl; X₁ is a halogen atom; and (ii) formula (III) wherein X₂ is a halogen atom; and pharmaceutically acceptable salts thereof, are described. Such compounds are useful as antineoplastic and antiviral agents.		

conditions of mammals, including humans, suffering from cancer, said method comprising administering a compound of formula (I), or a pharmaceutically acceptable salt thereof, and an additional antitumor agent, close enough in time and in amounts sufficient to produce a therapeutically useful effect.

The present invention also provides products containing a compound of formula (I), or a pharmaceutically acceptable salt thereof, and an additional antitumour agent as a combined preparation for simultaneous, separate or sequential use in anti-cancer therapy.

The term "antitumor agent" is meant to comprise both a single antitumor drug and "cocktails" i.e. a mixture of such drugs, according to the clinical practice. Examples of antitumor agents that can be formulated with a compound of formula (I), or alternatively, can be administered in a combined method of treatment, include doxorubicin, daunomycin, epirubicin, idarubicin, etoposide, fluorouracil, melphalan, cyclophosphamide, 4-demethoxy daunorubicin, bleomycin, vinblastin, and mitomycin, or mixtures thereof.

The following examples are given to better illustrate the invention, but do not limit the scope of the invention itself.

Example 1

3- [1-methyl-4 [1-methyl-4 [1-methyl-4 [5-N,N-bis (2-chloroethyl) aminoindole-2-carboxamido] pyrrole-2-carboxamido] pyrrole-2-carboxamido] pyrrole-2-

- 3- [1-methyl-4 [1-methyl-4 [1-methyl-4 [1-methyl-5- [4-N,N-bis(2-chloroethyl) aminobenzene-1-carboxamido] indole-2-carboxamido] pyrrole-2-carboxamido] pyrrole-2-carboxamido] propionamidine hydrochloride;
- 3- [1-methyl-4 [1-methyl-4 [5- [4-N,N-bis(2-bromoethyl) aminobenzene-1-carboxamido] indole-2-carboxamido] pyrrole-2-carboxamido] pyrrole-2-carboxamido] propionamidine hydrochloride;
- 3- [1-methyl-4 [1-methyl-4 [1-methyl-4 [5- [4-N,N-bis(2-bromoethyl) aminobenzene-1-carboxamido] indole-2-carboxamido] pyrrole-2-carboxamido] pyrrole-2-carboxamido] propionamidine hydrochloride;
- 3- [1-methyl-4 [1-methyl-4 [1-methyl-4 [5- [4-N-ethyl-N(2-chloroethyl) aminobenzene-1-carboxamido] indole-2-carboxamido] pyrrole-2-carboxamido] pyrrole-2-carboxamido] propionamidine hydrochloride;
- 3- [1-methyl-4 [1-methyl-4 [1-methyl-4 [5(α-bromoacrylamido) benzoimidazole-2-carboxamido] pyrrole-2-carboxamido] pyrrole-2-carboxamido] propionamidine hydrochloride.
- Example 2**
- 3- [1-methyl-4 [1-methyl-4 [1-methyl-4 [5(α-bromoacrylamido) benzofurane-2-carboxamido] pyrrole-2-carboxamido] pyrrole-2-carboxamido] propionamidine hydrochloride