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(54) Title: BIOLOGICALLY ACTIVE APHROCALLISTIN COMPOUNDS

(57) Abstract: The subject invention provides novel compositions of biologically active compounds which can advantageously be used in blocking cellular proliferation and/or treatment of cancer.

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DESCRIPTION

BIOLOGICALLY ACTIVE APHROCALLISTIN COMPOUNDS

FIELD OF THE INVENTION

This invention relates to organic compounds and compositions which have useful therapeutic properties. More particularly, the invention concerns novel compounds having anti-proliferative and antitumor activities, pharmaceutical compositions comprising such compounds, and methods of their use for therapeutic purposes.

BACKGROUND OF THE INVENTION

Of great importance to man is the control of pathological cellular proliferation such as that which occurs in the case of cancer. Considerable research and resources have been devoted to oncology and antitumor measures including chemotherapy. While certain methods and chemical compositions have been developed which aid in inhibiting, remitting, or controlling the growth of, for example, tumors, new methods and antitumor chemical compositions are needed. Anti-proliferative agents can also be useful in treating autoimmune diseases and inflammatory disease.

In searching for new biologically active compounds, it has been found that some natural products and organisms are potential sources for chemical molecules having useful biological activity of great diversity. For example, the diterpene commonly known as paclitaxel, isolated from several species of yew trees, is a mitotic spindle poison that stabilizes microtubules and inhibits their depolymerization to free tubulin (Fuchs, D.A., R.K. Johnson [1978] *Cancer Treat. Rep.* 62:1219-1222; Schiff, P.B., J. Fant, S.B. Horwitz [1979] *Nature* (London) 22:665-667). Paclitaxel is also known to have antitumor activity and has undergone a number of clinical trials which have shown it to be effective in the treatment of a wide range of cancers (Rowinski, E.K. R.C. Donehower [1995] *N. Engl. J. Med.* 332:1004-1014). See also, e.g., U.S. Patent Nos. 5,157,049; 4,960,790; and 4,206,221.

Marine sponges have also proven to be a source of biologically active chemical molecules. A number of publications disclose organic compounds derived from marine sponges including Scheuer, P.J. (ed.) *Marine Natural Products, Chemical and Biological Perspectives*, Academic Press, New York, 1978-1983, Vol. I-V; Uemura, D., K. Takahashi, T. Yamamoto, C.

Katayama, J. Tanaka, Y. Okumura, Y. Hirata (1985) *J. Am. Chem. Soc.* 107:4796-4798; Minale, L. *et al.* (1976) *Fortschr. Chem. org. Naturst.* 33:1-72 Faulkner, D.J., *Nat. Prod. Reports* 1984, 1, 251-551; *ibid.* 1987, 4, 539; *ibid* 1990, 7, 269; *ibid* 1993, 10, 497; *ibid* 1994, 11, 355; *ibid* 1995, 12, 22; *ibid* 1998, 15:113-58; *ibid* 2000 17:1-6; *ibid* 2000 17: 7-55; *ibid* 2001, 18: 1-49; 2002, 19: 1-48.; Gunasekera, S.P., M. Gunasekera, R.E. Longley and G.K. Schulte (1990) *J. Org. Chem.*, 55:4912-4915.; Horton, P.A., F.E. Koehn, R.E. Longley, and O.J. McConnell, (1994) *J. Am. Chem. Soc.* 116: 6015-6016.

The success of chemotherapy for the treatment of various cancers can be substantially negated though cellular mechanisms which have evolved to enable neoplastic cells to subvert the cytotoxic effects of the drug. Some cells have developed mechanism that confer resistance to a number of structurally unrelated drugs. This multi-drug resistance (or MDR) phenomenon may arise through a number of different mechanisms. One of these involves the ability of a cell to reduce intracellular concentrations of a given drug through efflux from cytoplasm through and out the cell membrane by a series of unique ATP-dependent transporter proteins called-P-glycoproteins (Pgp) (Casazza, A.M. and C.R. Fairchild [1996] "Paclitaxel (Taxol®): mechanisms of resistance" *Cancer Treat Res.* 87:149-171). The surface membrane, 170 kDa Pgp, is encoded by the *mdr-1* gene and appears to require substrate binding before transport begins. A wide range of compounds, including a number of structurally unrelated chemotherapeutic agents (adriamycin, vinblastine, colchicine, etoposide and Taxol), are capable of being transported by Pgp and render the cell resistant to the cytotoxic effects of these compounds.

While many normal cell types possess Pgp, in general, tumor cell lines, which possess high levels of mRNA specific for Pgp, also exhibit overexpression of membrane Pgp and demonstrate resistance to various drugs. This intrinsic resistance can be increased multifold by incubation of cells with stepwise increasing doses of a particular drug over a period of several months. This can be further facilitated by the addition of the MDR reversal agent, verapamil (Casazza, A.M. and C.R. Fairchild [1996] *supra*) in combination with the particular drug. Drug resistant cell lines produced in this fashion exhibit resistance to drug cytotoxicity from 20 to 500 fold, compared to parental cell lines.

An additional target for cancer drug discovery is a high molecular weight membrane protein associated with multi-drug resistance properties of certain tumor cells known as the multidrug resistance-associated protein (MRP). MRP is a 190 kD membrane-bound glycoprotein (Bellamy, W. T. [1996], *Annu. Rev. Pharmacol. Toxicol.*, 36: 161-183.) which belongs to the

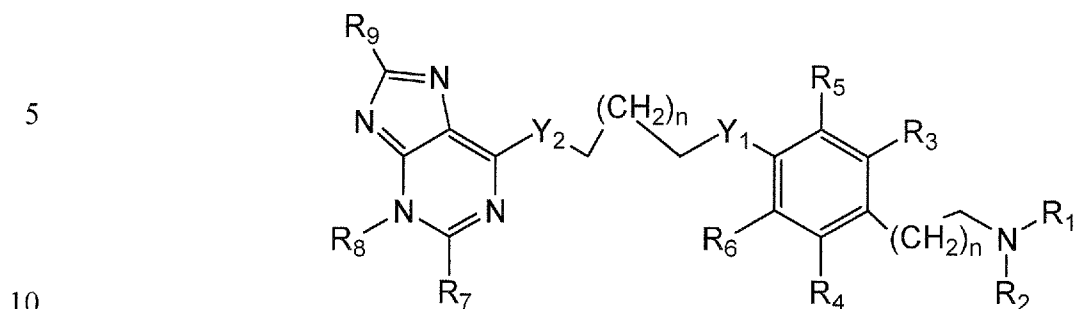
same family of proteins as the p-glycoprotein pump P-gp (Broxterman, H. J., Giaccone, G., and Lankelma, J. [1995], *Current Opinion in Oncology*, 7:532-540.) but shares less than 15% homology of amino acids with P-gp (Komorov, P. G., Shtil, A. A., Holian, O., Tee, L., Buckingham, L., Mechetner, E. B., Roninson, I. B., and Coon, J. S. [1998], *Oncology Research*, 10: 185-192.). MRP has been found to occur naturally in a number of normal tissues, including liver, adrenal, testis, and peripheral blood mononuclear cells (Krishan, A., Fitz, C. M., and Andritsch, I. [1997], *Cytometry*, 29: 279-285). MRP has also been identified in tissues of the lung, kidney, colon, thyroid, urinary bladder, stomach, spleen (Sugawara, I. [1998] *The Cancer Journal*, 8(2) and skeletal muscle (Kruh, G. D., Gaughan, K. T., Godwin, A., and Chan, A. [1995], *Journal of the National Cancer Institute*, 87(16): 1256-1258.). High levels of MRP have been implicated in multidrug resistance (MDR) in cancers of the lung and pancreas (Miller, D. W., Fontain, M., Kolar, C., and Lawson, T. [1996]. *Cancer Letters*, 107: 301-306.), and in neuroblastomas, leukemias and cancer of the thyroid (Kruh, G. D., Gaughan, K. T., Godwin, A., and Chan, A. [1995], *Journal of the National Cancer Institute*, 87(16): 1256-1258.), as well as bladder, ovarian and breast cancers (Barrand, M., Bagrij, T., and Neo, S. [1997]., *General Pharmacology*, 28(5): 639-645.).

MRP-mediated MDR involves some of the same classes of compounds as those which are mediated by P-gp, including vinca alkaloids, epipodophyllotoxins, anthracyclins and actinomycin D (Barrand, M., Bagrij, T., and Neo, S. [1997]., *General Pharmacology*, 28(5): 639-645). However, the substrate specificity has been demonstrated to differ from that of P-gp (Komorov, P. G., Shtil, A. A., Holian, O., Tee, L., Buckingham, L., Mechetner, E. B., Roninson, I. B., and Coon, J. S. [1998], *Oncology Research*, 10: 185-192.). Drugs which would inhibit or which are not substrates for the MDR pump would, therefore, be useful as chemotherapeutic agents.

BRIEF SUMMARY OF THE INVENTION

The subject invention is provides novel compositions of biologically active compounds that have utility in inhibiting cellular proliferation. In a specific embodiment, the compounds and compositions of the subject invention can be used in the treatment of cancer, including multi-drug resistant cancers.

The subject invention provides compounds of the following structure:



wherein R_{1-9} are the same or different and are selected from | H, | OH, halogen, | R, | OR, -COR, | OA, and NZZ (wherein the Zs can be the same or different);

Y_1 is NZ, O, or S;

Y_2 is NZ, O, or S;

Z is independently selected from | H, | R, | OH, and | COR;

R is C1-C8 alkyl or C1-C8 alkoxy, mesyl, or tosyl;

A is | R | phenyl; and

n is 1-6.

In a preferred embodiment:

R_1 is COR;

R_2 is CH_3 ;

n is 1;

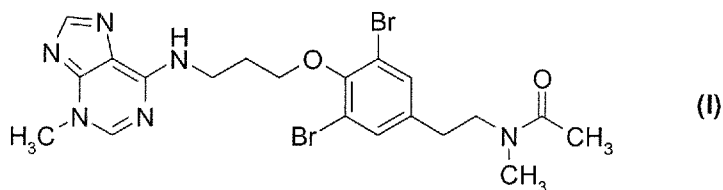
$R_3=R_4=H$;

$R_5=R_6=Br$;

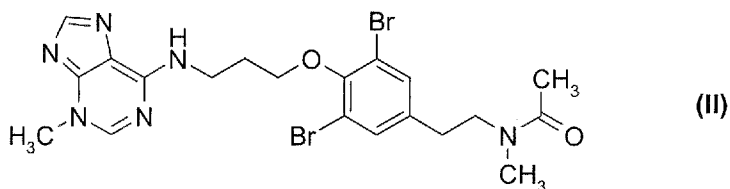
$R_7=R_9=H$; and

$R_8=CH_3$.

One aspect of the current invention concerns the novel compounds aphrocallistin A (I) and B (II). Advantageously, these compounds can inhibit unwanted cellular proliferation, including the proliferation of tumor cells.



Aphrocallistin A



Aphrocallistin B

In a specific embodiment, the novel compositions and methods of the subject invention can be used in the treatment of an animal hosting cancer cells including, for example, inhibiting the growth of tumor cells in a mammalian host. More particularly, the subject compounds can be used for inhibiting in a human the growth of tumor cells, including cells of breast, colon, CNS, ovarian, renal, prostate, liver, pancreatic, uterine, or lung tumors, as well as human leukemia or melanoma cells. The mechanisms for achieving anticancer activity exhibited by the subject compounds would lead a person of ordinary skill in the art to recognize the applicability of the subject compounds, compositions, and methods to additional types of cancer as described herein.

In specific embodiments, the subject invention provides new compounds, as exemplified by Aphrocallistin A (I) and Aphrocallistin B. Aphrocallistins A and B have not been isolated previously from a natural source nor have they been previously synthesized. One embodiment of the subject invention provides an approximately 3:2 mixture of these compounds with anti-cancer activity.

In accordance with the subject invention, methods for inhibiting cancer cells in a host include contacting tumor cells with an effective amount of the new pharmaceutical compositions of the invention. The cancer cells inhibited by the invention are those which are susceptible to the subject compounds described herein or compositions comprising those compounds.

Additional aspects of the invention include the provision of methods for producing the new compounds and compositions.

Other objects and further scope of applicability of the present invention will become apparent from the detailed descriptions given herein; it should be understood, however, that the detailed descriptions, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent from such descriptions.

BRIEF DESCRIPTION OF THE FIGURE

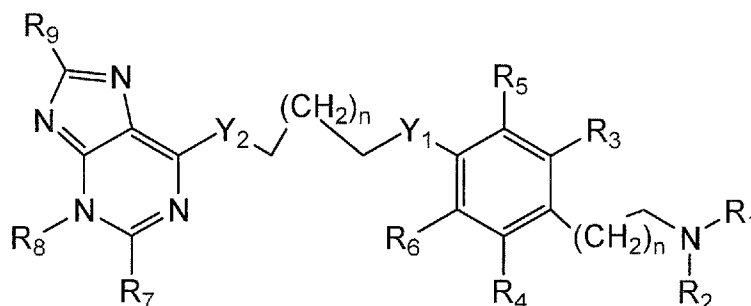
Figure 1 shows a NMR Spectrum of Aphrocallistins A and B as a 3:2 mixture A:B (600 MHZ, d4-methanol).

DETAILED DISCLOSURE OF THE INVENTION

The subject invention provides novel compositions of biologically active compounds that are useful for inhibiting pathological cellular proliferation. In a preferred embodiment, these compounds can be used for treating cancer.

More specifically, the novel compounds, compositions and methods of use can advantageously be used to inhibit the growth of tumor and other cancer cells in a mammalian host. More particularly, the subject compounds can be used for inhibiting in a human the growth of tumor cells, including cells of breast, prostate, colon, CNS, ovarian, renal, liver, pancreatic, uterine, or lung tumors, as well as human leukemia or melanoma cells. The compounds also have utility in the treatment of multi-drug resistant cancer cells.

The subject invention provides compounds of the following structure:



wherein R_{1-9} are the same or different and are selected from | H, | OH, halogen, | R, | OR, -COR, | OA, and NZZ (wherein the Zs can be the same or different);

Y_1 is NZ, O, or S;

5 Y_2 is NZ, O, or S;

Z is independently selected from | H, | R, | OH, and | COR;

R is C1-C8 alkyl or C1-C8 alkoxy, mesyl, or tosyl;

A is | R | phenyl; and

n is 1-6

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In a preferred embodiment:

R_1 is COR;

R_2 is CH₃;

n is 1;

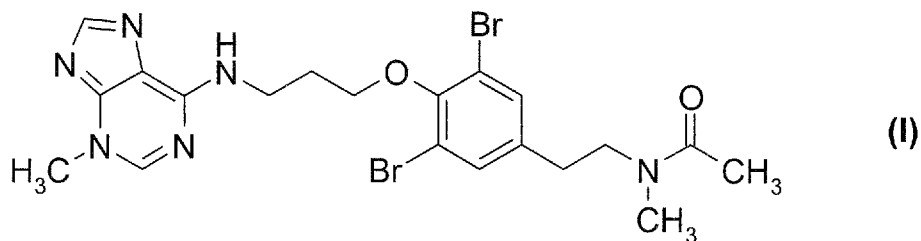
15 $R_3=R_4=H$;

$R_5=R_6=Br$;

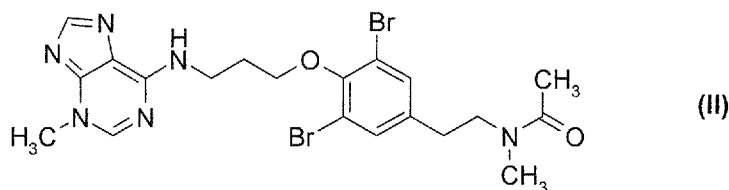
$R_7=R_9=H$; and

$R_8=CH_3$

20 In a preferred embodiment, the subject invention provides compounds having the following formula:



Aphrocallistin A



Aphrocallistin B

In accordance with the subject invention, methods for inhibiting cancer in a host include contacting cancer cells with an effective amount of the new pharmaceutical compositions of the invention. The tumor cells inhibited by the invention are those which are susceptible to the subject compounds described herein or compositions comprising those compounds.

The subject invention further provides methods of use of the new compounds and compositions of the invention, *e.g.*, methods of inhibiting tumors and other cancer cells in an animal, preferably a mammal. Most preferably, the invention comprises a method for the antitumor treatment of a human in need of such treatment, *i.e.*, a human hosting cancer cells, including breast, colon, liver, pancreatic, uterine, or lung tumor cells, or leukemia cells including multi-drug resistant cancer cells.

In preferred embodiments of the invention, the compounds are substantially pure, *i.e.*, contain at least 95% of the compound as determined by established analytical methods.

In further preferred methods of the invention, salts within the scope of the invention are made by adding mineral acids, *e.g.*, HCl, H₂SO₄, or strong organic acids, *e.g.*, formic, oxalic, in appropriate amounts to form the acid addition salt of the parent compound or its derivative. Also, synthesis type reactions may be used pursuant to known procedures to add or modify various groups in the preferred compounds to produce other compounds within the scope of the invention.

The scope of the invention is not limited by the specific examples and suggested procedures and uses related herein since modifications can be made within such scope from the information provided by this specification to those skilled in the art.

As used in this application, the terms "analogs," refers to compounds which are substantially the same as another compound but which may have been modified by, for example, adding or removing side groups.

A more complete understanding of the invention can be obtained by reference to the following specific examples of compounds, compositions, and methods of the invention. The following examples illustrate procedures for practicing the invention. These examples should not

be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted. It will be apparent to those skilled in the art that the examples involve use of materials and reagents that are commercially available from known sources, *e.g.*, chemical supply houses, so no details are given respecting them.

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Example 1 — Isolation and Structure Elucidation of Aphrocallistins A (I) and B (II).

A. Collection and Taxonomy of the Source Organism.

A sample of the sponge *Aphrocallistes beatrix* Gray, 1858 (Phylum: Porifera, Class Hexactinellida, Sub Class Hexasterophora Order Hexactinosida, Family Aphrocallistidae) was collected by manned submersible at a depth of 725.4 m approximately 40 nautical miles east of Fort Pierce on the Fort Pierce Pinnacles, Lophelia Pinnacle #TS-4 (latitude 27 39.4305'N, longitude 79 34.9679'W). The sponge is vasiform with the appearance of folded plates and fingers. It is crispy in texture and friable. It was collected growing in association with yellow sponge zooanthids. A reference sample preserved in ethanol has been deposited in the Harbor Branch Oceanographic Museum (catalog number 02:00022, DBMR number 20-V-04-1-005) and is available for taxonomic evaluation by those skilled in the art.

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B. Isolation and Structure Elucidation of Aphrocallistins A (I) and B (II).

Isolation of Aphrocallistins A and B.

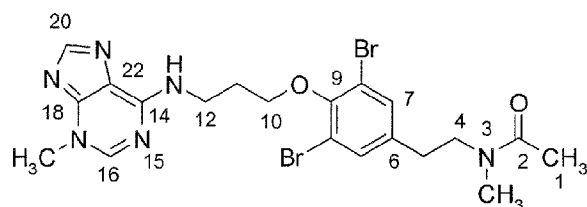
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The frozen sponge (106 g) was diced and extracted exhaustively with ethanol (Pharmco 100%). The combined ethanol extracts were concentrated to dryness and the residue partitioned between ethyl acetate and water. The ethyl acetate partition was concentrated to dryness to yield 0.493 g of an oil. The residue from the ethyl acetate partition was chromatographed by vacuum flash chromatography on a custom prepared RP-18 stationary phase using a step gradient of H₂O -CH₃CN-IPA as eluent. Column size was 150 mL. The eluent series is as follows: fraction 1, 100 mL of H₂O - CH₃CN (80:20 v/v); fraction 2, 100 mL of H₂O - CH₃CN (60:40 v/v); fraction 3, 100 mL of H₂O - CH₃CN (40:60 v/v); fraction 4, 100 mL of H₂O - CH₃CN (20:80 v/v); fraction 5, 100 mL of CH₃CN; Fraction 6 100 mL of H₂O - CH₃CN:TFA (20:80:0.1 v/v); Fraction 7: 100% CH₃CN; Fraction 8: 100% isopropanol. Fractions 6 and 7 (84.4 mg) were further purified by medium pressure liquid chromatography on a C-18 reversed-phase stationary phase using the Combiflash Companion with the following gradient program [Solvent A: H₂O/CH₃CN/TFA 95:5:0.1 in water v/v; Solvent B: CH₃CN/TFA 100:0.1; t= 0 minutes, A:B (100:0) hold for 1

minute; t=1 minutes A: B (100:0); t=11 minutes A: B (70:30); t=17 minutes, A:B (0:100); flow = 15 mL/min; Detected by uv absorption observed at 230 nm] to yield 40 mg of an inseparable mixture of Aphrocallistin A:B. in the proportions 3 parts Aphrocallistin A to 2 parts Aphrocallistin B.. Aphrocallistin A (I) Aphrocallistin B (mixture in proportion of 3:2, respectively): brown oil; MS: m/z observed 539.042786, calculated 539.040571 $\Delta=2.2$ mmu for formula $C_{20}H_{25}O_2N_6Br_2$ ($M+H^+$); See Table 1 for 1H and ^{13}C NMR data.



Aphrocallistin A

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Table 1. NMR data for Aphrocallistin A (d4-methanol, 600 MHz)

Position	^{13}C δ	Aphrocallistin A multiplicity	^{15}N δ'	1H δ	multiplicity	COSY ²	HMBC ³
1	20.0	CH ₃		2.02	s 3H		
2	173.4	C					H-1abc, N-3-CH ₃ , H-4a
3			111.5				H-1abc, H-5ab
4	50.1	CH ₂		3.50	2H dd J=7.6, 7.6	H-5ab	N3-CH ₃ abc, H-5ab
5	33.0	CH ₂		2.74	2H dd J=7.6, 7.6	H-4ab	H-4ab
6	139.9	C					H-4ab, H-5ab
7	134.4	CH, 2C		7.45	2H, s		H-5ab, H-7'
8	118.9	C, 2C					H-7
9	152.7	C					H-7, H-10ab
10	71.9	CH ₂		4.11	2H m	H-11ab	H-11ab, H-12ab
11	30.5	CH ₂		2.27	2H m	H-10ab, H-12ab	H-10ab, H-12ab
12	40.4	CH ₂		4.04	2H m	H-11ab	H-10ab, H-11ab
13			106.0				H-11ab
14	153.5	C					H-12ab, H-16
15			226.9				H-16
16	149.7	CH		8.6	s		N17-CH ₃ abc
17			148.7				H-16, N-17CH ₃

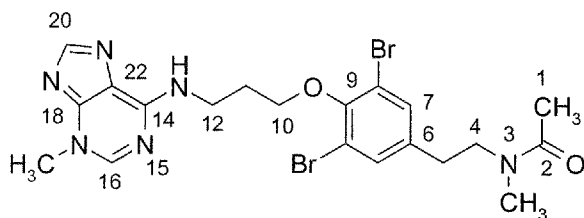
Table 1. NMR data for Aphrocallistin A (d4-methanol, 600 MHz)

Position	¹³ C δ	Aphrocallistin A		¹ H δ	multiplicity	COSY ²	HMBC ³
		multiplicity	¹⁵ N δ ¹				
18	149.0	C br					N-17CH ₃ , H-20
19			not observed				
20	145.6	CH br		8.4	s		
21			not observed				
22	112.6	C br					H-20
N3-CH ₃	36.9	CH ₃		2.98	3H, s		H-4ab
N-17 CH ₃	36.8	CH ₃		4.06	3H, s		H-18

¹ ¹⁵N chemical shifts detected indirectly through ¹⁵N-¹H HMBC

² From H # to

³ From atom at this position to H atoms listed



Aphrocallistin B

Table 2. NMR data for Aphrocallistin B (d4-methanol, 600 MHz)

position	Aphrocallistin B		¹ H	multiplicity
	¹³ C	¹⁵ N		
1	20.89		1.84	3H s
2	173.26			
3		111.5		
4	52.96		3.55	2H, dd J=6.9, 6.9
5	33.92		2.82	2H, dd J=6.9, 6.9
6	139.38			
7	134.61		7.47	2H, s
8	119.14			
9	153.06			
10	71.9		4.11	2H m
11	30.47		2.27	2H m
12	40.35		4.04	2H m
13		106.0		
14	153.5			

15		226.9		
16	149.7		8.6	s
17		148.7		
18	149.0			
19		not observed		
20	145.6		8.4	s
21		not observed		
22	112.6			
N3-CH ₃	33.76		2.88	3H s
N-17 CH ₃	36.85		4.06	s 3H

Example 2 — Antitumor Effects of the mixture of Aphrocallistin A (I) and B(II).

A. Effects of Aphrocallistin A (I) and B(II) mixture on In Vitro Proliferation of Tumor Cell Lines.

5 Aphrocallistin A (I) and B(II) 3:2 mixture was analyzed as to its effects on the proliferation of a panel of tumor cell lines including both cell lines. The cell lines tested include: the NCI-ADR-RES (Formerly MCF-7/ADR) human ovarian carcinoma, DLD-1 human colorectal carcinoma, A549 human lung adenocarcinoma and PANC-1 human pancreatic carcinoma cell lines. A549, DLD-1, PANC-1, and NCI-ADR-RES cells were obtained from American Type

10 Culture Collection, Rockville, MD. The A549, DLD-1, NCI-ADR-RES and PANC-1 cell lines are maintained in Roswell Park Memorial Institute (RPMI) medium 1640 supplemented with 100 U/mL penicillin 100 µg/ml streptomycin, 60 µg/ml L-glutamine, 18 mM HEPES, 0.05 mg/mL gentamycin and 10% fetal bovine serum (for the PANC-1 and DLD-1 cell lines, the media is also supplemented with 100 µg/ml sodium pyruvate and 2.5 mg/ml glucose). Cell lines are cultured

15 in plastic tissue culture flasks and kept in an incubator at 37°C in humidified air containing 5% CO₂. To assess the antiproliferative effects of agents against the various cell lines, 200 µl cultures (96-well tissue culture plates, Nunc, Denmark) are first established at 3×10^4 cells/ml for adherent lines (NCI ADR-RES, PANC-1) in tissue culture medium and incubated for 24 hr at 37°C in 10% CO₂ in air in order to allow cells to attach. A volume of 100 µl of medium is

20 removed from each test well and 100 µl of medium containing serial, two-fold dilutions of the test agent is added to each well containing tumor cells. Medium without drug is also added to wells containing tumor cells which serve as no drug controls. Positive drug controls are included to monitor drug sensitivity of each of the cell lines. These include varying dilutions of 5-fluorouracil and doxorubicin. After 72-h exposures tumor cells are enumerated using 3-[4,5-

Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (M.C. Alley, et al., Cancer Res. 48:589, 1988) as follows:

A volume of 75 μ l of warm growth media containing 5 mg/ml MTT is added to each well, cultures returned to the incubator, and left undisturbed for 3 hours. To spectrophotometrically quantitate formation of reduced formazan, plates are centrifuged (900 x g, 5 minutes), culture fluids removed by aspiration, and 200 μ l of acidified isopropanol (2 ml concentrated HCl/liter isopropanol) added per well. The absorbance of the resulting solutions is measured at 570 nm with a plate reader (BMG Labtech NOVOSTar). The absorbance of tests wells is divided by the absorbance of drug-free wells, and the concentration of agent that results in 50% of the absorbance of untreated cultures (IC₅₀) is determined by linear regression of logit-transformed data (D. J. Finney, Statistical Method in Biological Assay, third ed., pp.316-348, Charles Griffin Co., London, 1978). A linear relationship between tumor cell number and formazan production has been routinely observed over the range of cell densities observed in these experiments. The two standard drug controls (indicated above) are included in each assay to monitor the drug sensitivity of each of the cell lines and IC₅₀ values are determined for each drug-cell combination.

A summary of results in these assays for compound I can be found in Table 2.

Table 2. Cytotoxicity Results for Aphrocallistin A (I) and B(II) mixture				
	A549 IC ₅₀ μ g/mL	PANC-1 IC ₅₀ μ g/mL	NCI/ADR-RES IC ₅₀ μ g/mL	DLD-1 IC ₅₀ μ g/mL
Aphrocallistin A (I)/B(II) 3:2 mixture	12.7 $\pm$ 3.7	13.1 $\pm$ 4.6	18.1 $\pm$ 0.7	3.7 $\pm$ 1.7

Example 3 — Formulation and Administration

The compounds of the invention are useful for various non-therapeutic and therapeutic purposes. It is apparent from the testing that the compounds of the invention are effective for inhibiting cell growth. Because of the antiproliferative properties of the compounds, they are useful to prevent unwanted cell growth in a wide variety of settings including *in vitro* uses. They are also useful as standards and for teaching demonstrations. As disclosed herein, they are also useful prophylactically and therapeutically for treating cancer cells in animals and humans.

Therapeutic application of the new compounds and compositions containing them can be accomplished by any suitable therapeutic method and technique presently or prospectively known to those skilled in the art. Further, the compounds of the invention have use as starting materials or intermediates for the preparation of other useful compounds and compositions.

5 The dosage administration to a host in the above indications will be dependent upon the identity of the cancer cells, the type of host involved, its age, weight, health, kind of concurrent treatment, if any, frequency of treatment, and therapeutic ratio.

The compounds of the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in detail in a
10 number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* by E.W. Martin describes formulations which can be used in connection with the subject invention. In general, the compositions of the subject invention will be formulated such that an effective amount of the bioactive compound(s) is combined with a suitable carrier in order to facilitate effective administration of the composition.

15 In accordance with the invention, pharmaceutical compositions comprising, as an active ingredient, an effective amount of one or more of the new compounds and one or more non-toxic, pharmaceutically acceptable carrier or diluent. Examples of such carriers for use in the invention include ethanol, dimethyl sulfoxide, glycerol, silica, alumina, starch, and equivalent carriers and diluents.

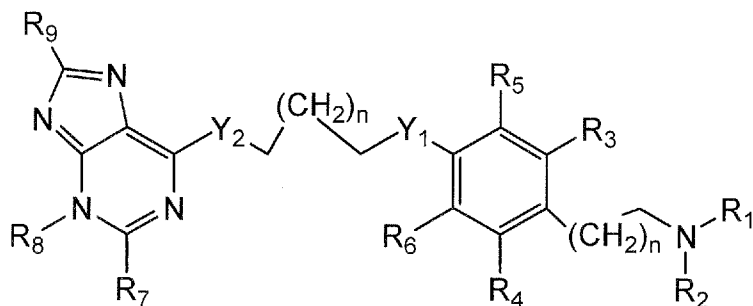
20 To provide for the administration of such dosages for the desired therapeutic treatment, new pharmaceutical compositions of the invention will advantageously comprise between about 0.1% and 45%, and especially, 1 and 15%, by weight of the total of one or more of the new compounds based on the weight of the total composition including carrier or diluent. Illustratively, dosage levels of the administered active ingredients can be: intravenous, 0.01 to
25 about 20 mg/kg; intraperitoneal, 0.01 to about 100 mg/kg; subcutaneous, 0.01 to about 100 mg/kg; intramuscular, 0.01 to about 100 mg/kg; orally 0.01 to about 200 mg/kg, and preferably about 1 to 100 mg/kg; intranasal instillation, 0.01 to about 20 mg/kg; and aerosol, 0.01 to about 20 mg/kg of animal (body) weight.

It should be understood that the examples and embodiments described herein are for
30 illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

CLAIMS

We claim:

1. A compound having the following structural formula:



wherein R_{1-9} are the same or different and are selected from | H, | OH, halogen, | R, | OR, -COR, | OA, and NZZ (wherein the Zs can be the same or different);

Y_1 is NZ, O, or S;

Y_2 is NZ, O, or S;

Z is independently selected from | H, | R, | OH, and | COR;

R is C1-C8 alkyl or C1-C8 alkoxy, mesyl, or tosyl;

A is | R | phenyl; and

n is 1-6,

or a salt thereof.

2. The compound, according to claim 1, wherein:

R_1 is COR;

R_2 is CH_3 ;

n is 1;

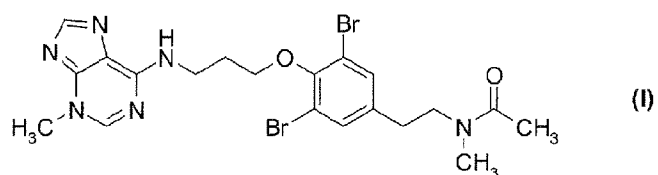
$R_3=R_4=H$;

$R_5=R_6=Br$;

$R_7=R_9=H$; and

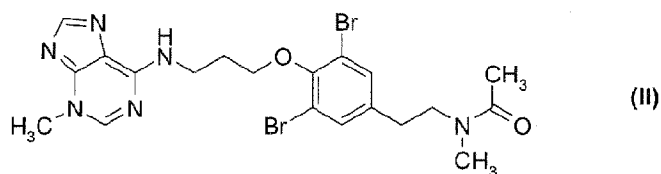
$R_8=CH_3$.

3. The compound, according to claim 1, having the following structure:



Aphrocallistin A

4. The compound, according to claim 1, having the following structure:



Aphrocallistin B

5. The compound, according to claim 1, having the following spectral data:

Table 1. NMR data for Aphrocallistin A (d4-methanol, 600 MHz)

Position	¹³ C δ	Aphrocallistin A		¹ H δ	multiplicity	COSY ²	HMBC ³
		multiplicity	¹⁵ N δ ¹				
1	20.0	CH ₃	111.5	2.02	s 3H		
2	173.4	C					H-1abc, N-3-CH ₃ , H-4a
3							H-1abc, H-5ab
4	50.1	CH ₂		3.50	2H dd J=7.6, 7.6	H-5ab	N3-CH ₃ abc, H-5ab
5	33.0	CH ₂		2.74	2H dd J=7.6, 7.6	H-4ab	H-4ab
6	139.9	C					H-4ab, H-5ab
7	134.4	CH, 2C		7.45	2H, s		H-5ab, H-7'

Table 1. NMR data for Aphrocallistin A (d4-methanol, 600 MHz)

Position	¹³ C δ	Aphrocallistin A		¹ H δ	multiplicity	COSY ²	HMBC ³
		multiplicity	¹⁵ N δ ¹				
8	118.9	C, 2C					H-7
9	152.7	C					H-7, H-10ab
10	71.9	CH ₂		4.11	2H m	H-11ab	H-11ab, H-12ab
11	30.5	CH ₂		2.27	2H m	H-10ab, H-12ab	H-10ab, H-12ab
12	40.4	CH ₂		4.04	2H m	H-11ab	H-10ab, H-11ab
13			106.0				H-11ab
14	153.5	C					H-12ab, H-16
15			226.9				H-16
16	149.7	CH		8.6	s		N17-CH ₃ abc
17			148.7				H-16, N-17CH ₃
18	149.0	C br					N-17CH ₃ , H-20
19			not observed				
20	145.6	CH br		8.4	s		
21			not observed				
22	112.6	C br					H-20
N3-CH ₃	36.9	CH ₃		2.98	3H, s		H-4ab
N-17 CH ₃	36.8	CH ₃		4.06	3H, s		H-18

¹ ¹⁵N chemical shifts detected indirectly through ¹⁵N-¹H HMBC

² From H # to

³ From atom at this position to H atoms listed

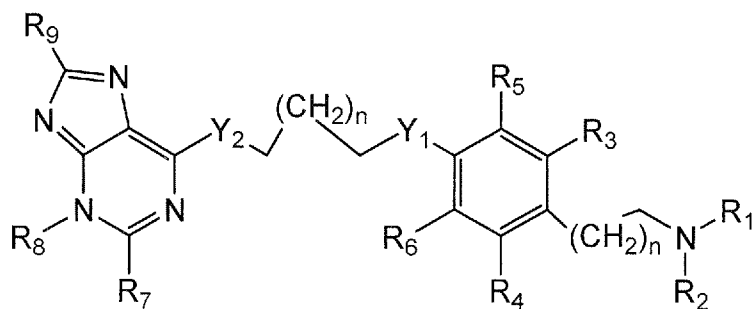
6. The compound, according to claim 1, having the following spectra data:

Table 2. NMR data for Aphrocallistin B (d4-methanol, 600 MHz)

position	Aphrocallistin B		¹ H	multiplicity
	¹³ C	¹⁵ N		
1	20.89		1.84	3H s
2	173.26			
3		111.5		
4	52.96		3.55	2H, dd J=6.9, 6.9
5	33.92		2.82	2H, dd J=6.9, 6.9
6	139.38			
7	134.61		7.47	2H, s
8	119.14			

9	153.06			
10	71.9	4.11	2H	m
11	30.47	2.27	2H	m
12	40.35	4.04	2H	m
13		106.0		
14	153.5			
15		226.9		
16	149.7	8.6	s	
17		148.7		
18	149.0			
19		not observed		
20	145.6	8.4	s	
21		not observed		
22	112.6			
N3-CH ₃	33.76	2.88	3H	s
N-17 CH ₃	36.85	4.06	s	3H

7. A method for inhibiting cellular proliferation, said method comprising administering to a patient in need of such treatment an effective amount of a compound having the following structure:



wherein R₁₋₉ are the same or different and are selected from | H, | OH, halogen, | R, | OR, -COR, | OA, and NZZ (wherein the Zs can be the same or different);

Y₁ is NZ, O, or S;

Y₂ is NZ, O, or S;

Z is independently selected from | H, | R, | OH, and | COR;

R is C1-C8 alkyl or C1-C8 alkoxy, mesyl, or tosyl;

A is | R | phenyl; and

n is 1-6

or a salt thereof.

8. The method, according to claim 7, wherein:

R_1 is COR;

R_2 is CH_3 ;

n is 1;

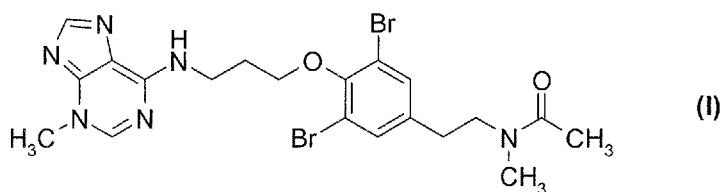
$R_3=R_4=H$;

$R_5=R_6=Br$;

$R_7=R_9=H$; and

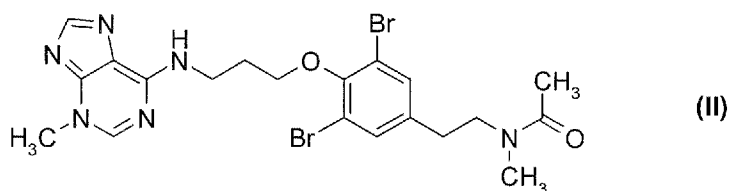
$R_8=CH_3$.

9. The method, according to claim 7, wherein the compound has the following structure:



Aphrocallistin A

10. The method, according to claim 7, wherein the compound has the following structure:



Aphrocallistin B

11. The method, according to claim 7, wherein said compound has the following spectral data:

Table 1. NMR data for Aphrocallistin A (d4-methanol, 600 MHz)

Position	¹³ C δ	Aphrocallistin A		¹ H δ	multiplicity	COSY ²	HMBC ³
		multiplicity	¹⁵ N δ ¹				
1	20.0	CH ₃		2.02	s 3H		
2	173.4	C					H-1abc, N-3-CH ₃ , H-4a
3			111.5				H-1abc, H-5ab
4	50.1	CH ₂		3.50	2H dd J=7.6, 7.6	H-5ab	N3-CH ₃ abc, H-5ab
5	33.0	CH ₂		2.74	2H dd J=7.6, 7.6	H-4ab	H-4ab
6	139.9	C					H-4ab, H-5ab
7	134.4	CH, 2C		7.45	2H, s		H-5ab, H-7'
8	118.9	C, 2C					H-7
9	152.7	C					H-7, H-10ab
10	71.9	CH ₂		4.11	2H m	H-11ab	H-11ab, H-12ab
11	30.5	CH ₂		2.27	2H m	H-10ab, H-12ab	H-10ab, H-12ab
12	40.4	CH ₂		4.04	2H m	H-11ab	H-10ab, H-11ab
13			106.0				H-11ab
14	153.5	C					H-12ab, H-16
15			226.9				H-16
16	149.7	CH		8.6	s		N17-CH ₃ abc
17			148.7				H-16, N-17CH ₃
18	149.0	C br					N-17CH ₃ , H-20
19			not observed				
20	145.6	CH br		8.4	s		
21			not observed				
22	112.6	C br					H-20
N3-CH ₃	36.9	CH ₃		2.98	3H, s		H-4ab
N-17 CH ₃	36.8	CH ₃		4.06	3H, s		H-18

¹ ¹⁵N chemical shifts detected indirectly through ¹⁵N-¹H HMBC² From H # to³ From atom at this position to H atoms listed

12. The method, according to claim 7, wherein said compound has the following spectra data:

Table 2. NMR data for Aphrocallistin B (d4-methanol, 600 MHz)

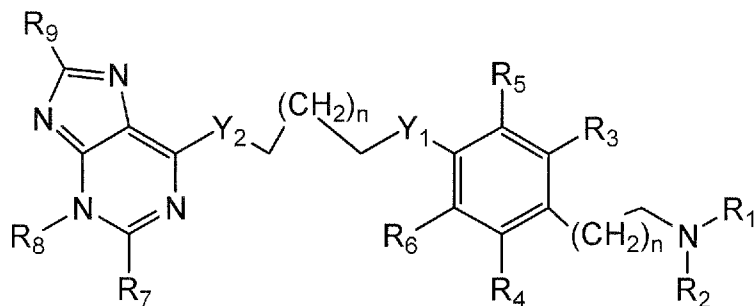
position	¹³ C	¹⁵ N	¹ H	multiplicity
1	20.89		1.84	3H s
2	173.26			
3		111.5		
4	52.96		3.55	2H, dd J=6.9, 6.9
5	33.92		2.82	2H, dd J=6.9, 6.9
6	139.38			
7	134.61		7.47	2H, s
8	119.14			
9	153.06			
10	71.9		4.11	2H m
11	30.47		2.27	2H m
12	40.35		4.04	2H m
13		106.0		
14	153.5			
15		226.9		
16	149.7		8.6	s
17		148.7		
18	149.0			
19		not observed		
20	145.6		8.4	s
21		not observed		
22	112.6			
N3-CH ₃	33.76		2.88	3H s
N-17 CH ₃	36.85		4.06	s 3H

13. The method, according to claim 7, wherein said cellular proliferation is cancer cell proliferation.

14. The method, according to claim 13, wherein said cancer is selected from the group consisting of breast cancer, colon cancer, CNS cancer, liver cancer, lung cancer, leukemia, melanoma, ovarian cancer, uterine cancer, renal cancer, pancreatic cancer and prostate cancer.

15. The method, according to claim 7, used to treat multidrug resistant cancer.

16. A pharmaceutical composition comprising a compound having the following structure:



wherein R_{1-9} are the same or different and are selected from | H, | OH, halogen, | R, | OR, -COR, | OA, and NZZ (wherein the Zs can be the same or different);

Y_1 is NZ, O, or S;

Y_2 is NZ, O, or S;

Z is independently selected from | H, | R, | OH, and | COR;

R is C1-C8 alkyl or C1-C8 alkoxy, mesyl, or tosyl;

A is | R | phenyl; and

n is 1-6

or a salt thereof, together with a pharmaceutically-acceptable carrier.

17. The pharmaceutical composition, according to claim 16, wherein:

R_1 is COR;

R_2 is CH_3 ;

n is 1;

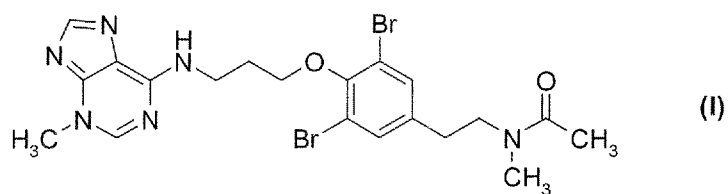
$R_3=R_4=H$;

$R_5=R_6=Br$;

$R_7=R_9=H$; and

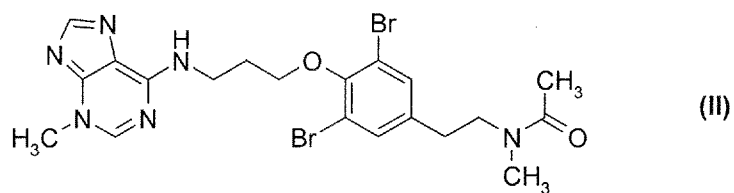
$R_8=CH_3$.

18. The pharmaceutical composition, according to claim 16, wherein the compound has the following formula:



Aphrocallistin A

19. The pharmaceutical composition, according to claim 16, wherein the compound has the following formula:



Aphrocallistin B

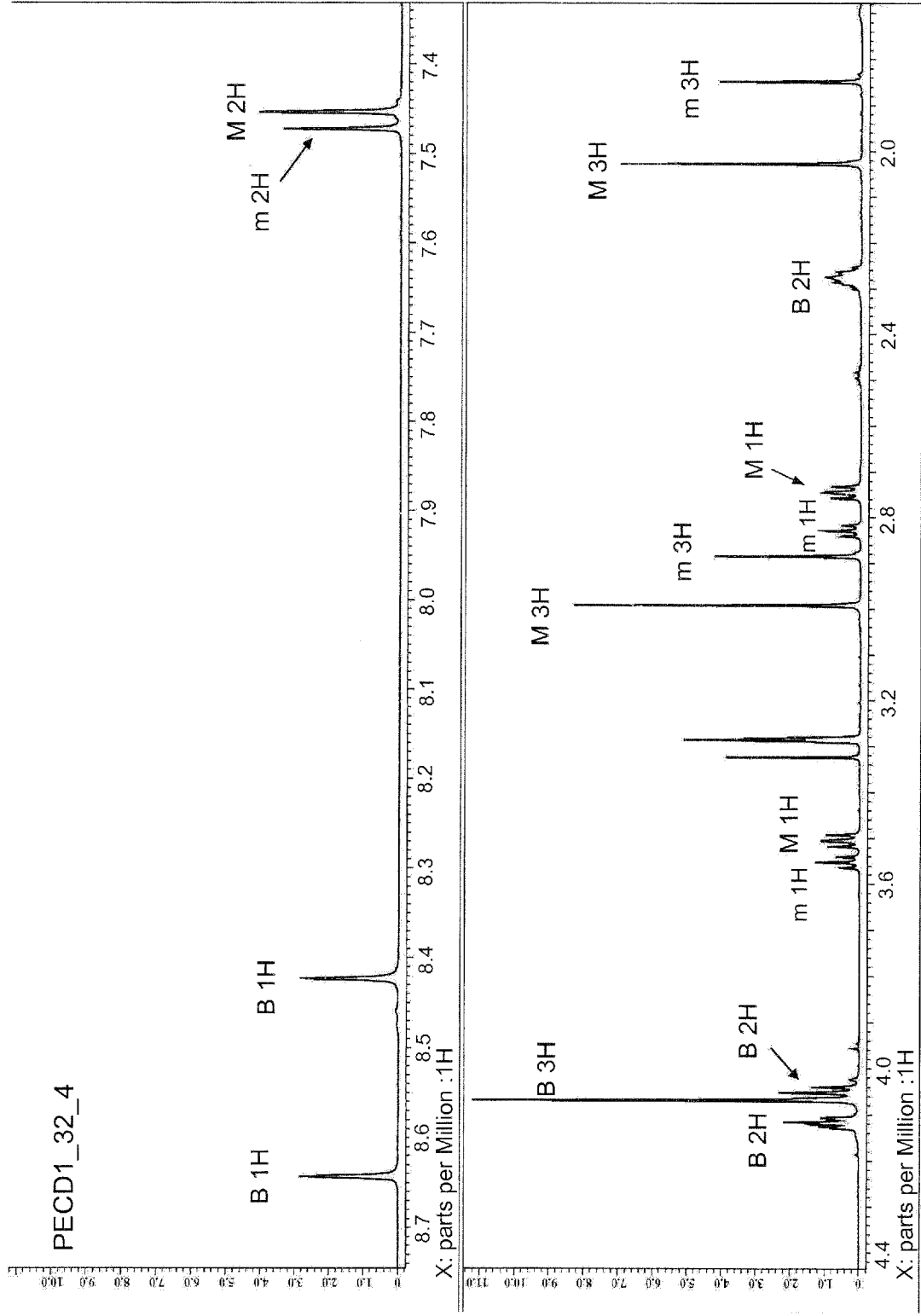


FIG. 1