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(54) ANTITUMORAL ANALOGS

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EP-A- 0 559 838 **WO-A-00/18233**
WO-A-00/69862

- **MARTINEZ E J ET AL: "PHTHALASCIDIN, A SYNTHETIC ANTITUMOR AGENT WITH POTENCY AND MODE OFACTION COMPARABLE TO ECTEINASCIDIN 743" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 96, no. 7, March 1999 (1999-03), pages 3496-3501, XP001026021 ISSN: 0027-8424**

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Description

[0001] The present invention relates to derivatives of the ecteinascidins, particularly ecteinascidin 736 (ET-736), pharmaceutical compositions containing them and their use as antitumoral compounds.

BACKGROUND OF THE INVENTION

[0002] The ecteinascidins are exceedingly potent antitumour agents isolated from the marine tunicate *Ecteinascidia turbinata*. Several ecteinascidins have been reported previously in the patent and scientific literature. See, for example:

U.S. Patent No 5.256.663, which describes pharmaceutical compositions comprising matter extracted from the tropical marine invertebrate, *Ecteinascidia turbinate*, and designated therein as ecteinascidins, and the use of such compositions as antibacterial, antiviral, and/ar antitumour agents in mammals.

U.S.Patent No 5.089.273, which describes novel compositions of matter extracted from the tropical marine invertebrate, *Ecteinascidia turbinata*, and designated therein as ecteinascidins 729, 743, 745, 759A, 759B and 770. These compounds are useful as antibacterial and/or antitumour agents in mammals.

U.S. Patent No 5.149.804 which describes Ecteinascidins 722 and 736 (Et's 722 and 736) isolated from the Caribbean tunicate *Ecteinascidia turbinata* and their structures. Et's 722 and 736 protect mice in vivo at very low concentrations against P388 lymphoma, B16 melanoma, and Lewis lung carcinoma.

U.S. Patent No 5.478.932, which describes ecteinascidins isolated from the Caribbean tunicate Ecteinascidia turbinata, which provide in vivo protection against P388 lymphoma, B16 melanoma, M5076 ovarian sarcoma, Lewis lung carcinoma, and the LX-1 human lung and MX-1 human mammary carcinoma xenografts.

U.S. Patent No 5.654.426, which describes several ecteinascidins isolated from the Caribbean tunicate *Ecteinascidia turbinata*, which provide in vivo protection against P388 lymphoma, B316 melanoma, M5076 ovarian sarcoma, Lewis lung carcinoma, and the LX-1 human lung and MX-1 human mammary carcinoma xenografts.

U.S. Patent No 5.721.362 which describes a synthetic process for the formation of ecteinascidin compounds and related structures.

U.S. Patent No 6.124.292 which describes a series of new ecteinascidin-like compounds.

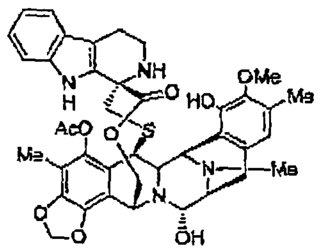
WO 0177115, WO 0187894 and WHO 0187895, which describe new synthetic compounds of the ecteinascidin series, their synthesis and biological properties.

[0003] See also: Corey, E.J., J. Am. Chem. Soc., 1996, 118 pp. 9202-9203; Rinehart, et al., Journal of Natural Products, 1990, "Bioactive Compounds from Aquatic and Terrestrial Sources", vol. 53, pp. 771-792; Rinehart et al., Pure and Appl. Chem., 1990, "Biologically active natural praducts", vol 62, pp. 1277-1280; Rinehart, et al., J. Org. Chem., 1990, "Ecteinascidins 729, 743, 745, 759A, 759B, and 770: potent Antitumour Agents from the Caribbean Tunicate Ecteinascidia turninata", vol. 55, pp. 4512-4515; Wright et al., J. Org. Chem., 1990, "Antitumour Tetrahydroisoquinoline Alkaloids from the Colonial ascidian Ecteinascidia turbinata", vol. 55, pp. 4508-4512; Sakai et al., Proc. Natl. Acad. Sci. USA 1992, "Additional anitumor ecteinascidins from a Caribbean tunicate: Crystal structures and activities in vivo", vol. 89, 11456-11460; Science 1994, "Chemical Prospectors Scour the Seas for Promising Drugs", vol. 266, pp.1324; Koenig, K.E., "Asymmetric Synthesis", ed. Morrison, Academic Press, Inc., Orlando, FL, vol. 5, 1985, p. 71; Barton, et al., J. Chem Soc. Perkin Trans., 1, 1982, "Synthesis and Properties of a Series of Sterically Hindered Guanidine bases", pp. 2085; Fukuyama et al., J. Am. Chem. Soc., 1982, "Stereocontrolled Total Synthesis of (+)-Saframycin B", vol. 104, pp. 4957; Fukuyama et al., J. Am. Chem. Soc., 1990, "Total Synthesis of (+) - Saframycin A", vol. 112, p. 3712; Saito, et al., J. Org. Chem., 1989, "Synthesis of Saframycins. Preparation of a Key tricyclic Lactam Intermediate to Saframycin A", vol. 54, 5391; Still, et al., J Org. Chem., 1978, "Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution", vol. 43, p. 2923; Kofron, W.G.; Baclawski, L.M., J. Org. Chem., 1976, vol. 41, 1879; Guan et al., J. Biomolec. Struc. & Dynam., vol. 10, pp. 793-817 (1993); Shamma et al., "Carbon-13 NMR Shift Assignments of Amines and Alkaloids", p. 206 (1979); Lown et al., Biochemistry, 21, 419-428 (1982); Zmijewski et al., Chem. Biol. Interactions, 52, 361-375 (1985); Ito, CRC Crit. Rev. Anal. Chem., 17, 65-143 (1986); Rinehart et al., "Topics in Pharmaceutical Sciences 1989", pp. 613-626, D. D. Breimer, D. J. A. Cromwelin, K. K. Midha, Ends., Amsterdam Medical Press B. V., Noordwijk, The Netherlands (1989); Rinehart et al., "Biological Mass Spectrometry", 233-258 eds. Burlingame et al., Elsevier Amsterdam (1990); Guan et al., Jour. Biomolec. Struct. & Dynam., vol. 10 pp. 793-817 (1993); Nakagawa et al., J. Amer. Chem. Soc., 111: 2721-2722 (1989);; Lichter et al., "Food and Drugs from the Sea Proceedings" (1972), Marine Technology Society, Washington, D.C. 1973, 117-127; Sakai et al., J. Amer. Chem. Soc., 1996, 118, 9017; Garcia-Rocha et al., Brit. J. Cancer, 1996, 73: 875-883; and pommier et al., Biochemistry, 1996, 35: 13303-13309;

[0004] In 2000, a hemisynthetic process for the formation of ecteinascidin compounds and related structures such as phthalascidin starting from natural bis(tetrahydroisoquinoline) alkaloid such as the saframycin and safracin antibiotics available from different culture broths was reported; See Manzanares et al.,Org. Lett, 2000, "Synthesis of Ecteinascidin ET-743 and Phthalascidin Pt-650 from Cyanosafracin B", Vol. 2, No 16, pp. 2545-2548; and International Patent Application WO 00 69862.

[0005] Proc. Natl. Acad. Sci. USA vol 96 pp 3496-3501 is concerned with phthalascilin, a synthetic antitumor agent with potency and node of action comparable to ecteinascidin 743.

[0006] Ecteinascidin 736 was first discovered by Rinehart and features a tetrahydro-β-carboline unit in place of the tetrahydroisoquinoline unit more usually found in the ecteinascidin compounds isolated from natural sources; See for example Sakai et al., Proc. Natl. Acad. Sci. USA 1992, "Additional antitumor ecteinascidins from a Caribbean tunicate: Crystal structures and activities in vivo", vol. 89, 11456-11460.



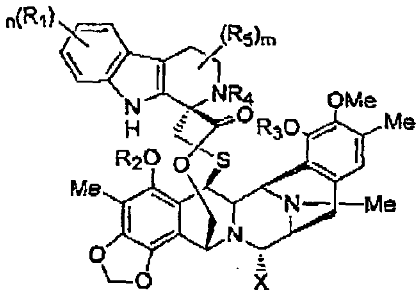
Et-736

[0007] WO 9209607 claims ecteinascidin 736, as well as ecteinascidin 722 with hydrogen in place of methyl on the nitrogen common to rings C and D of ecteinascidin 736 and O-methylecteinascidin 736 with methoxy in place of hydroxy on ring C of ecteinascidin 736.

[0008] Despite the positive results obtained in clinical applications in chemotherapy, the search in the field of ecteinascidin compounds is still open to the identification of new compounds with optimal features of cytotoxicity and selectivity toward the tumour and with a reduced systemic toxicity and improved pharmacokinetic properties.

SUMMARY OF THE INVENTION

[0009] The present invention is directed to the compounds of claim 1. These compounds are part of a class of compounds of general formula I:



I

wherein the substituent groups for R_1, R_2, R_3, R_4, and R_5 are each independently selected from the group consisting of H, OH, OR^-, SH, SR^-, SOR^-, SO_2R^-, C(=O)R', C(=O)OR', NO_2, NH_2, NHR^-, $N(R^-)_2$, $NHC(O)R^-$, CN, halogen, =O, substituted or unsubstituted C_1-C_{18} alkyl, substituted or unsubstituted C_2-C_{18} alkenyl, substituted or unsubstituted C_2-C_{18} alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heterocyclic;
wherein X is independently selected of OR', CN, (=O), or H.
wherein each of the R^- groups is independently selected from the group consisting of H, OH, NO_2 NH_2, SH, CN, halogen,

=O, C(=O)H, C(=O)CH_3, CO_2H, substituted or unsubstituted C_1-C_{18} alkyl, substituted or unsubstituted C_2-C_{18} alkenyl, substituted or unsubstituted C_2-C_{18} alkynyl, substituted or unsubstituted aryl;
wherein m is 0, 1 or 2; and
wherein n is 0, 1, 2, 3 or 4.

[0010] In another aspect, the invention relates to pharmaceutical compositions comprising a compound of claim 1.

[0011] In another aspect, the invention relates to the use of compounds of claim 1 in the treatment of cancer. We hereby provide information on the class of compounds of general formula I. Alkyl groups preferably have from 1 to 24 carbon atoms. One more preferred class of alkyl groups has 1 to about 12 carbon atoms, yet more preferably 1 to about 8 carbon atoms, still more preferably 1 to about 6 carbon atoms, and most preferably 1, 2, 3 or 4 carbon atoms. Another more preferred class of alkyl groups has 12 to about 24 carbon atoms, yet more preferably 12 to about 18 carbon atoms, and most preferably 13, 15 or 17 carbon atoms. Methyl, ethyl and propyl including isopropyl are particularly preferred alkyl groups. As used herein, the term alkyl, unless otherwise modified, refers to both cyclic and noncyclic groups, although cyclic groups will comprise at least three carbon ring members.

[0012] Preferred alkenyl and alkynyl groups have one or more unsaturated linkages and from 2 to about 12 carbon atoms, more preferably 2 to about 8 carbon atoms, still more preferably 2 to about 6 carbon atoms, even more preferably 1, 2, 3 or 4 carbon atoms. The terms alkenyl and alkynyl as used herein refer to both cyclic and noncyclic groups, although straight or branched noncyclic groups are generally more preferred.

[0013] Preferred alkoxy groups include groups having one or more oxygen linkages and from 1 to about 12 carbon atoms, more preferably from 1 to about 8 carbon atoms, and still more preferably 1 to about 6 carbon atoms, and most preferably 1, 2, 3 or 4 carbon atoms.

[0014] Preferred alkylthio groups have one or more thioether linkages and from 1 to about 12 carbon atoms, more preferably from 1 to about 8 carbon atoms, and still more preferably 1 to about 6 carbon atoms. Alkylthio groups having 1, 2, 3 or 4 carbon atoms are particularly preferred.

[0015] Preferred alkylsulphinyl groups include those groups having one or more sulphoxide (SO) groups and from 1 to about 12 carbon atoms, more preferably from 1 to about 8 carbon atoms, and still more preferebly 1 to about 6 carbon atoms. Alkylsulphinyl groups having 1, 2, 3 or 4 carbon atoms are particularly preferred.

[0016] Preferred alkylsulphonyl groups include those groups having one or more sulphonyl (SO_2) groups and from 1 to about 12 carbon atoms, more preferably from 1 to about 8 carbon atoms, and still more preferably 1 to about 6 carbon atoms. Alkylsulphonyl groups having 1, 2, 3 or 4 carbon atoms are particularly preferred.

[0017] Preferred aminoalkyl groups include those groups having one or more primary, secondary and/or tertiary amine groups, and from 1 to about 12 carbon atoms, more preferably 1 to about 8 carbon atoms, still more preferably 1 to about 6 carbon atoms, even more preferably 1, 2, 3 or 4 carbon atoms. Secondary and tertiary amine groups are generally more preferred than primary amine moieties.

[0018] Suitable heterocyclic groups include heteroaromatic and heteroalicyclic groups. Suitable heteroaromatic groups contain one, two or three heteroatoms selected from N, O or S atoms and include, e.g., coumarinyl including 8-coumarinyl, quinolinyl including 8-quinolinyl, pyridyl, pyrazinyl, pyrimidyl, furyl, pyrrolyl, thienyl, thiazolyl, oxazolyl, imidazolyl, indolyl, benzofuranyl and benzothiazol. Suitable heteroalicyclic groups contain one, two or three heteroatoms selected from N, O or S atoms and include, e.g., tetrahydrofuranyl, tetrahydropyranyl, piperidinyl, morpholino and pyrrolindinyl groups.

[0019] Suitable carbocyclic aryl groups include single and multiple ring compounds, including multiple ring compounds that contain separate and/or fused aryl groups. Typical carbocyclic aryl groups contain 1 to 3 separate or fused rings and from 6 to about 18 carbon ring atoms. Specifically preferred carbocyclic aryl groups include phenyl including substituted phenyl such as 2-substituted phenyl, 3-substituted phenyl, 2.3-substituted phenyl, 2.5-substituted phenyl, 2.3.5-substituted and 2.4.5-substituted phenyl, including where one or more of the phenyl substituents is an electron-withdrawing group such as halogen, cyano, nitro, alkanoyl, sulphinyl, sulphonyl and the like; naphthyl including 1-naphthyl and 2-naphthyl; biphenyl; phenanthryl; and anthracyl.

[0020] References herein to substituted R' groups refer to the specified moiety, typically alkyl or alkenyl, that may be substituted at one or more available positions by one or more suitable groups, e.g., halogen such as fluoro, chloro, bromo and iodo; cyano; hydroxyl; nitro; azido; alkanoyl such as a C1-6 alkanoyl group such as acyl and the like; carboxamido; alkyl groups including those groups having 1 to about 12 carbon atoms or from 1 to about 6 carbon atoms and more preferably 1-3 carbon atoms; alkenyl and alkynyl groups including groups having one or more unsaturated linkages and from 2 to about 12 carbon or from 2 to about 6 carbon atoms; alkoxy groups having those having one or more oxygen linkages and from 1 to about 12 carbon atoms or 1 to about 6 carbon atoms; aryloxy such as phenoxy; alkylthio groups including those moieties having one or more thioether linkages and from 1 to about 12 carbon atoms or from 1 to about 6 carbon atoms; alkylsulphinyl groups including those moieties having one or more sulphinyl linkages and from 1 to about 12 carbon atoms or from 1 to about 6 carbon atoms; alkylsulphonyl groups including those moieties having one or more sulphonyl linkages and from 1 to about 12 carbon atoms or from 1 to about 6 carbon atoms; aminoalkyl groups such as groups having one or more N atoms and from 1 to about 12 carbon atoms or from 1 to about 6 carbon atoms; carbocylic aryl having 6 or more carbons, particularly phenyl (e.g., R being a substituted or unsubstituted biphenyl moiety); and aralkyl such as benzyl; heterocyclic groups including heteroalicyclic and heteroaromatic groups, especially with 5 to 10 ring atoms of which 1 to 4 are heteroatoms, more preferably heterocyclic groups with 5 or 6 ring atoms and 1 or 2 heteratoms or with 10 ring atoms and 1 to 3 heteroatoms.

[0021] Preferred R' groups are present in groups of formula R', COR' or OCOR' and include alkyl or alkenyl, that may be substituted at one or more available positions by one or more suitable groups, e.g.; halogen such as fluoro, chloro, bromo and iodo, especially ω-chloro or perfluoro; aminoalkyl groups such as groups having one or more N atoms and from 1 to about 12 carbon atoms or from 1 to about 6 carbon atoms, and especially including amino acid, notably glycine, alanine, arginine, asparagine, asparaginic acid, cystein, glutamine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine or valine, especially protected forms of such amino acids; carbocylic aryl having 6 or more carbons, particularly phenyl; and aralkyl such as benzyl; heterocyclic groups including heteroalicyclic and heteroaromatic groups, especially with 5 to 10 ring atoms of which 1 to 4 are heteroatoms, more preferably heterocyclic groups with 5 or 6 ring atoms and 1 or 2 heteratoms or with 10 ring atoms and 1 to 3 heteroatoms, the heterocyclic groups optionally being substituted with one or more of the subsitituents permitted for R' and especially amino such as dimethylamino or with keto.

[0022] Compounds where R1 is not hydrogen have higher activity, a wider therapeutic window and improved pharmacokinetic properties.

[0023] Compounds where R_5 is not hydrogen are another class of preferred compounds. See for example compounds 37, 38 and 42. These compounds tend to be less active (cytotoxic) but have lower toxicity and improved pharmacokinetic properties. When R_5 is not hydrogen a chiral center is generated, and we have found that there is difference in activity between the diastereoisomers.

[0024] Compounds wherein R_2 is an ester, in general, have improved toxicology properties and thus give a wider therapeutic window.

[0025] There are compounds that have good ADME properties (absorption-distribution-metabolism-excretion) which are good indicative of pharmacokinetics.

[0026] As mentioned above, compounds of the present invention, preferably those with bulky substituted groups, have a good therapeutic window and the estherification of the phenols with different acids and carbonates, results in a general enhancement of the pharmaceutical properties: there is a significant decrease in hepatocyte toxicity, and also a good profile on drug-drug interactions since these derivatives do not show cytochrome inhibition having moreover higher metabolic stability.

[0027] Several active antitumor compounds have been prepared and it is believed that many more compounds may be formed in accordance with the teachings of the present disclosure.

[0028] Antitumoural activities of these compounds include leukaemias, lung cancer, colon cancer, kidney cancer, prostate cancer, ovarian cancer, breast cancer, sarcomas and melanomas.

[0029] Another especially preferred embodiment of the present invention is pharmaceutical compositions useful as antitumour agents which contain as active ingredient a compound or compounds of the invention, as well as the processes for their preparation.

[0030] Examples of pharmaceutical compositions include any solid (tablets, pills, capsules, granules etc) or liquid (solutions, suspensions or emulsions) with suitable compositions or oral, topical or parenteral administration.

[0031] Administration of the compounds or compositions of the present invention may be any suitable method, such as intravenous infusion, oral preparation, intraperitoneal and intravenous preparation.

[0032] The compounds and compositions of this invention may be used with other drugs to provide a combination therapy. The other drugs may form part of the same composition, or be provided as a separate composition for administration at the same time or a different time. The identity of the other drug is not particularly limited, and suitable candidates include:

a) drugs with antimitotic effects, especially those which target cytoskeletal elements, including microtubule modulators such as taxane drugs (such as taxol, paclitaxel, taxotere, dacetaxel), podophylotoxins or vinca alkaloids (vincristine, vinblastine);

b) antimetabolite drugs such as 5-fluorouracil, cytarabine, gemcitabine, purine analogues such as pentostatin, methotrexate);

c) alkylating agents such as nitrogen mustards (such as cyclophosphamide or ifosphamide);

d) drugs which target DNA such as the antracycline drugs adriamycin, doxorubicin, pharmorubicin or epirubicin;

e) drugs which target topoisomerases such as etoposide;

f) hormones and hormone agonists or antagonists such as estrogens, antiestrogens (tamoxifen and related compounds) and androgens, flutamide, leuprorelin, goserelin, cyprotrone or octreotide;

g) drugs which target signal transduction in tumour cells including antibody derivatives such as herceptin;

h) alkylating drugs such as platinum drugs (cis-platin, carbonplatin, oxaliplatin, paraplatin) or nitrosoureas;

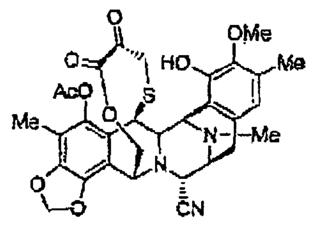
i) drugs potentially affecting metastasis of tumors such as matrix metalloproteinase inhibitors;

j) gene therapy and antisense agents;
k) antibody therapeutics;
l) other bioactive compounds of marine origin, notably the didemnins such as aplidine;
m) steroid analogues, in particular dexamethasone;
n) anti-inflammatory drugs, in particular dexamethasone; and
o) anti-emetic drugs, in particular dexamethasone.

[0033] Yet another especially preferred embodiment of the present invention is the synthetic intermediates of the compounds of the present invention as described in detail below.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0034] Disclosed in international patent application WO 0069862 is compound 36 (an intermediate in the conversion of cyanosafracin B to Ecteinascidin 743).



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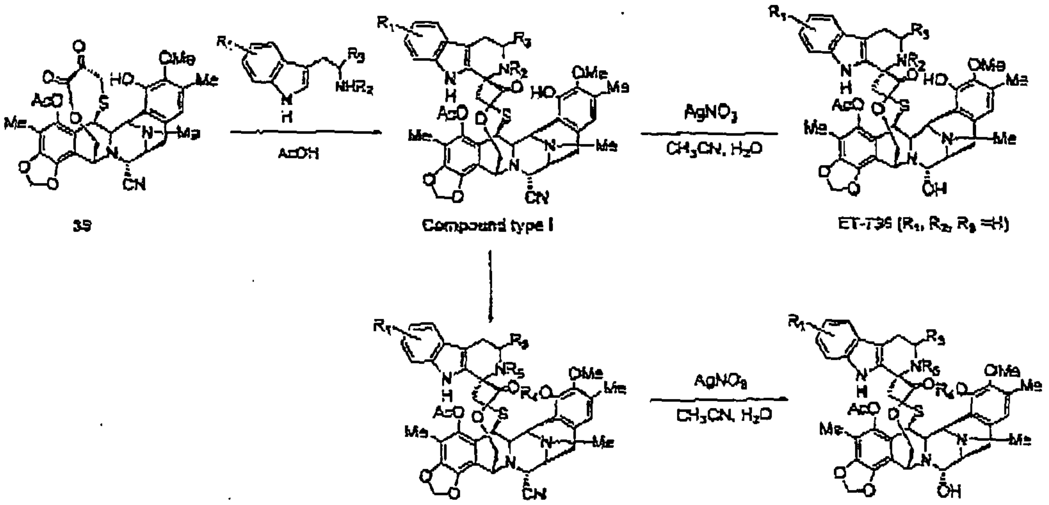
[0035] This hemi-synthetic intermediate has served as the starting material for the synthesis of ecteinascidin 736, a further member of the naturally occurring ecteinascidin family with potential antitumor therapeutic activity.
[0036] The preferred method of producing compounds related to ecteinascidin 736 with different substituents in the tetrahydro-β-carboline unit and in the position 18 (-OR$_4$) are described below in the following reaction scheme.

Scheme 1



[0037] As illustrated in Scheme 1, intermediate 36 can be converted to ET-736 (or a substituted derivative) in two steps.
[0038] The first step for producing the preferred compounds type I of the present invention from compound 36 is the introduction of the tetrahydro-β-carboline unit by reaction with the corresponding primary or secondary amine.

[0039] The second step is the transformation of the CN group into an OH group by reaction with silver nitrate in ACN/H_2O.

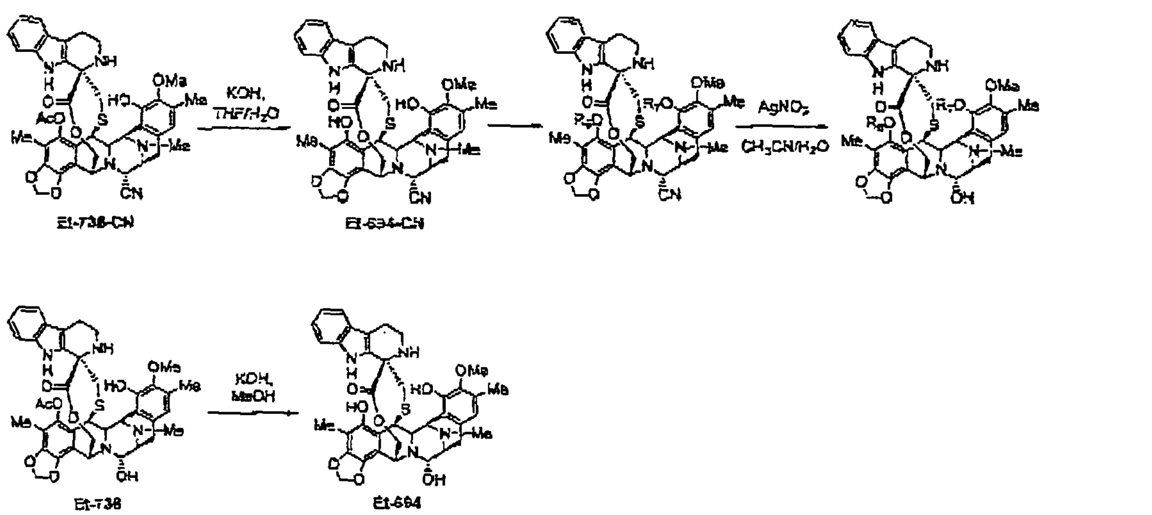
[0040] Also is possible to obtain new derivatives with different substituent groups (-OR_4, position 18 and =NR_5) through a acylation or alkylation reaction from the preferred compounds 1. In all theses cases R_1 and R_2 in the starting material is an hydrogen atom. From the same intermediate and through an alkylation reaction with allyl bromide or an acylation reaction with vinylchloroformiate it can be obtained N and O allylated and N and O vinyl derivatives. All these compounds by reaction with silver nitrate lead to the final products wherein the CN group is transformed into an OH group.

[0041] As the skilled artisan will readily appreciate, the reaction scheme described herein may be modified by use of a wide range of substituted primary amines to produce a series of substituted ecteinascidin 736 derivatives to generate compounds that are part of this invention.

[0042] In particular the reaction conditions can be varied to suit other combinations of the substituent groups in the tetrahydro-β-carboline unit,

[0043] The preferred method of producing ecteinascidin 694 and related compounds with different substituents in the position 5 and 18 (-OR_6 and -OR_7) are described below in the following reaction scheme.

Scheme 2



[0044] In Scheme 2 the hydrolisis of the acetyl group in C-5 in basic conditions allows to prepare the intermediate with the hydroxyl group in this position. From this compound and by an acylation reaction with anhydrides, acid chlorides or carboxylic acids are prepared new derivatives mono-O sustitued and mono and di-O sustitued (in C-5 and C-18). The reaction to tranform the CN group into the OH is performed in the classic conditions (silver nitrate in CH_3CN/H_2O). On the other hand Et-694 can be obtained from Et-736 through the hydrolisis of the acetyl group in C-5 with KOH/MeOH.

[0045] As the skilled artisan will readily appreciate, the reaction scheme described herein may be modified by use of a wide range of substituted primary amines to produce a series of substituted ecteinascidin 736-CN derivatives to generate compounds that are part of this invention.

[0046] In particular the reaction conditions can be varied to suit other combinations of the substituent groups in the tetrahydro-β-carboline unit and in positions C-5 and C-18.

[0047] The present invention will be further illustrated with reference to the following examples which aid in the understanding, but which are not to be construed as limitations thereof. The numbering of the compounds is not fully sequential, because of deletions.

EXPERIMENTAL PART

Scheme 1

[0048] **Method 1.-** To a solution of 1 equiv. of starting material in acetic acid (5.33 E-5M) under argon at room temperature was added 3.5 equiv. of tryptamine. The reaction mixture was stirred during 24 h and then the acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2 and the organic layers were dried over Na_2SO_4. Flash chromatography gives pure compounds.

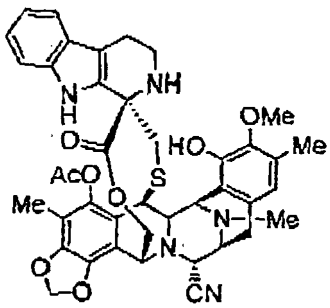
[0049] Method 2.- To a solution of 1 equiv. of compound **1** in CH_2Cl_2 (0.032M) under Argon at room temperature were added 2 equiv, of Et_3N and 2 equiv. of the butyryl chloride or Boc anhydride (3 equiv.) or vinylchloroformiate. The reaction was followed by TLC and quenched with an aqueous saturated solution of $NaHCO_3$, extracted with CH_2Cl_2 and the organic layers dried over Na_2SO_4. Flash chromatography gives pure compound.

[0050] Method 3.- To a solution of 1 equiv. of compound **1** in DMF (0.032M) under Argon at room temperature were added 3 equiv. of Cs_2CO_3 and 3 equiv. of the allyl bromide. The reaction was followed by TLC and quenched with an aqueous saturated solution of $NaHCO_3$, extracted with CH_2Cl_2 and the organic layers dried over Na_2SO_4. Flash chromatography gives a mixture of two pure compounds: compound **24** (ET-736-CN-All) and compound **25** (ET-736-CN-diAll).

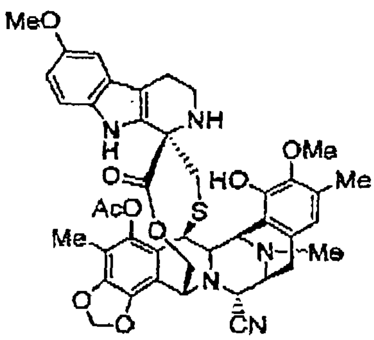
[0051] Method 4.- To a solution of 1 equiv. of starting material in CH_3CN/H_2O 3:2 (0.009M) were added 30 equiv. of $AgNO_3$. After 24 h the reaction was quenched with a mixture 1:1 of saturated solutions of brine and $NaHCO_3$, stirred for 10 min and diluted and extracted with CH_2Cl_2. The organic layer was dried with Na_2SO_4. Chromatography gives pure compounds.

Method 1:

[0052]

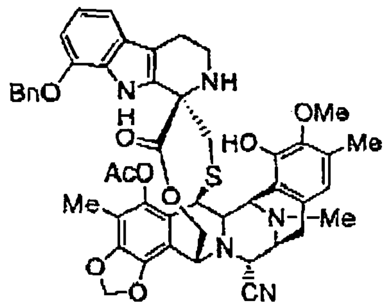


Compound 1 (reference compound): ^1H-NMR (300 MHz, $CDCl_3$): δ 7.74 (s, 1H); 7.38 (d, 1H); 7.25 (d, 1H); 7.08 (t, 1H); 7.00 (t, 1H); 6.66 (s, 1H); 6.22 (d, 1H); 6.02 (d, 1H); 5.79 (s, 1H); 5.08(d, 1H); 4.55(s, 1H); 4.32(s, 1H); 4.27(d, 1H); 4.21 (s, 1H); 4.19(d, 1H); 3.81(s, 3H); 3.44-3.40(m, 2H); 3.18-2.78(m, 4H); 2.71-2.51(m, 3H); 2.37(s, 3H); 2.26(s, 3H); 2.21 (s, 3H); 2.06 (s, 3H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.7, 168.9, 148.2, 145.9, 143.2, 141.3, 140.5, 135.7, 130.8, 130.6, 129.5, 127.0, 122.2, 120.9, 120.8, 119.5, 118.6, 118.4, 113.8, 111.1, 110.5, 102.2, 62.5, 61.5, 60.8, 60.5, 59.7, 55.9, 54.8, 42.1, 41.7, 40.0, 39.5, 29.9, 24.0, 21.7, 20.8, 16.1, 9.9.
ESI-MS m/z; Calcd. for $C_{41}H_{41}N_5O_8S$: 763.3 Found ($M+H^+$): 764.2.

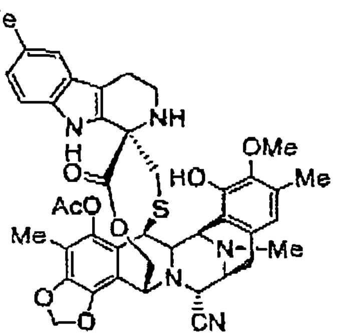


Compound 2: ^1H-NMR (300 MHz, $CDCl_3$): δ 7.64(s, 1H); 7.12 (d, 1H); 6.81 (d, 1H); 6.73 (dd, 1H); 6.65 (s, 1H); 6.19 (s, 1H); 6.00 (s, 1H); 5.79 (s, 1H); 5.0 (d, 1H); 4.54 (s, 1H); 4.30 (s, 1H); 4.27 (d, 1H); 4.20 (s, 1H); 4.18 (d, 1H); 3.80 (s, 3H); 3.78 (s, 3H); 3.43-3.40 (m, 2H); 3.18-2.77 (m, 4H); 2.66-2.49 (m, 3H); 2.37 (s, 3H); 2.34-2.20 (m, 1H); 2.26 (s, 3H); 2.21 (s, 3H); 2.05 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.4, 168.6, 153.7, 148.0, 145.6, 142.9, 141.0, 140.2,

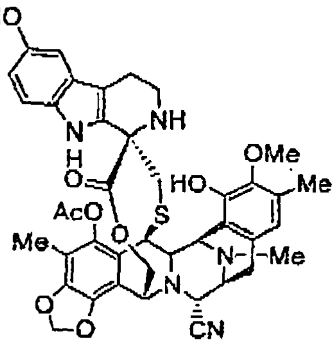
131.1, 130.6, 130.5, 129.2, 127.0, 120.6, 120.5, 118.2, 113.6, 111.9, 111.6, 110.0, 102.0, 100.3, 62.3, 61.2, 60.5, 60.2, 59.4, 55.7, 54.6, 54.5, 41.8, 41.4, 39.7, 39.2, 31.5, 29.6, 23.8, 22.6, 21.5, 20.5, 15.8, 14.4, 9.7. ESI-MS m/z: Calcd. for $C_{42}H_{43}N_5O_9S$: 793.3 Found ($M+H^+$): 794.7.



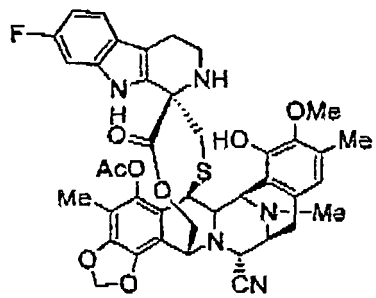
Compound 3: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.85(s, 1H); 7.45-7.36(m, 5H); 7.01(t, 1H); 6.91(t, 1H); 6.65-6.63(m, 2H); 5.87(s, 1H); 5.77(s, 1H); 5.63(s, 1H); 5.13(s, 2H); 5.05(d, 1H); 4.53(s, 1H); 4.27-4.19(m, 4H); 3.80(s, 3H); 3.46-3.39(m 2H); 3.06-2.79(m, 4H); 2.68-2.50(m 2H); 2.42-2.20(m, 1H); 2.36(s, 3H); 2.27(s, 3H); 2.20(s, 3H); 2.03(s, 3H).
ESI-MS m/z: Calcd. for $C_{48}H_{47}N_5O_9S$: 869.3 Found ($M+H^+$): 870.3.



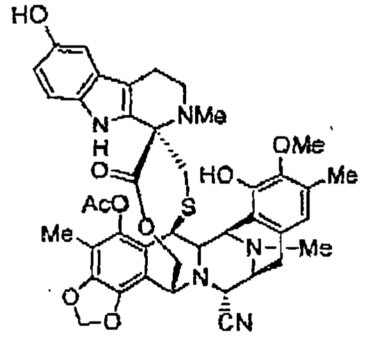
Compound 5: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.63(s, 1H); 7.15-7.11(m, 2H); 6.91(dd, 1H); 6.65(s, 1H); 6.21(d, 1H); 6.01 (s, 1H); 5.78(s, 1H); 5.07(d, 1H); 4.54(s, 1H); 4.31(s, 1H); 4.27(d, 1H); 4.21-4.16(m, 2H); 3.81(s, 3H); 3.44-3.40(m, 2H); 3.17-2.77(m, 4H); 2.66-2.46(m, 3H); 2.31(s, 6H); 2.26(s, 3H); 2.21(s, 3H); 2.06(s, 3H).
ESI-MS m/z: Calcd. for $C_{42}H_{43}N_5O_8S$: 777.3 Found ($M+Na^+$): 800.7.



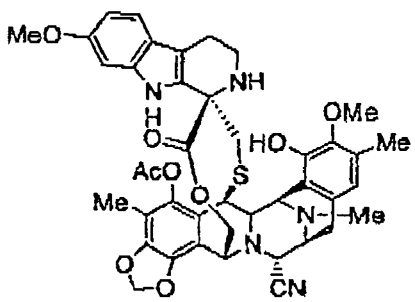
Compound 6: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.66(s, 1H); 6.95(d, 1H); 6.64(s, 2H); 6.56(dd, 1H); 6.15(s, 1H); 5.97(s, 1H); 5.81(s, 1H); 5.06(d, 1H); 4.53(s, 1H); 4.29(s, 1H); 4.26(d, 1H); 4.19(s, 1H); 4.17(d, 1H); 3.80(s, 3H); 4.41-3.39(m, 2H); 3.12-2.73(m, 4H); 2.55-2.27(m, 3H); 2.36(s, 3H); 2.25(s, 3H); 2.20(s, 3H); 2.04(s, 3H).
ESI-MS m/z: Calcd. for $C_{41}H_{41}N_5O_9S$: 779.3 Found ($M+H^+$): 780.3.



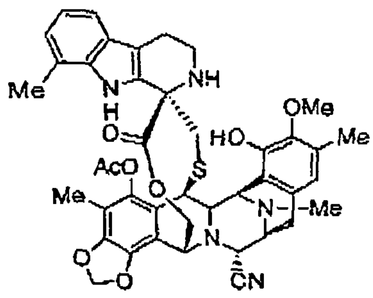
Compound 7: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.75(s, 1H); 7.26(dd, 1H); 6.93(dd, 1H); 6.76(ddd, 1H); 6.65(s, 1H); 6.22 (d, 1H); 6.01(d, 1H);5.79(s, 1H); 5.08(d, 1H); 4.55(s, 1H); 4.31(s, 1H); 4.25(d, 1H); 4.20(s, 1H); 4.18(dd, 1H); 3.80(s, 3H); 3.43-3.40(m, 2H); 3.18-2.77(m, 4H); 2.64-2.50(m, 3H); 2.36(s, 3H); 2.26(s, 3H); 2.21(s, 3H); 2.06(s, 3H), ESI-MS m/z: Calcd. for $C_{41}H_{40}FN_5O_8S$: 781.3 Found ($M+H^+$): 782.3.



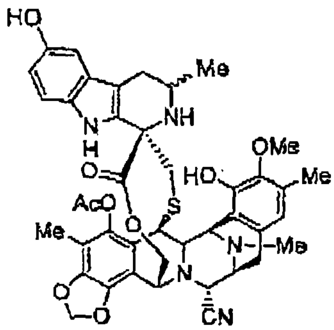
Compound 8: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.93(d, 1H); 6.80(s, 1H); 6.73(s, 1H); 6.67(dd, 1H); 6.46(s, 1H); 6.20(s, 1H); 6.06(s, 1H); 5.72(s, 1H); 4.96(d, 1H); 4.45(s, 1H); 4.37(d, 1H); 4.25(d, 1H); 4.05-4.01(m, 2H); 3.79(s, 3H); 3.63(d, 1H); 3.39(d, 1H); 3.03-3.91(m, 2H); 2.76-2.34(m, 5H); 3.30(s, 3H); 2.28(s, 3H); 2.21(s, 3H); 2.18(s, 3H); 2.04(s, 3H). ESI-MS m/z: Calcd. for $C_{42}H_{43}N_5O_9S$: 793.3 Found ($M+H^+$): 794.3.



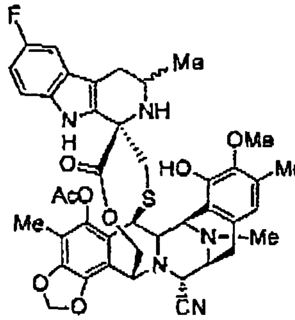
Compound 9: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.63(s, 1H); 7.24(d, 1H); 6.75(d, 1H); 6.66(dd, 1H); 6.65(s, 1H); 6.20(s, 1H); 6.00(s, 1H); 5.79(s, 1H); 5.07(d, 1H); 4.54(s, 1H); 4.31(s, 1H); 4.27(d, 1H); 4.20(d, 1H); 4.17(dd, 1H); 3.80(s, 3H); 3.71(s, 3H); 3.43-3.40(m, 2H); 3.16-2.78(m, 4H); 2.64-2.49(m, 3H), 2.36(s, 3H); 2.25(s, 3H); 2.21(s, 3H); 2.06(s, 3H). ESI-MS m/z: Calcd. for $C_{42}H_{43}N_5O_9S$: 793.3 Found ($M+H^+$): 794.3.



Compound 11: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.48 (s, 1H); 7.22 (d, 1H); 6.96-6.88 (m, , 2H); 6.65 (s, 1H); 6.15 (d, 1H); 6.04 (d, 1H); 5.78 (s, 1H); 5.09 (d, 1H); 4.55 (s, 1H); 4,34 (s, 1H); 4.28-4.20 (m, 3H); 3.81 (s, 3H); 3.48 (d, 1H); 3.42 (d, 1H); 3.12-2.78 (m, 4H); 2.69-2.43 (m, 3H); 2.37 (s, 3H); 2.36 (s, 3H); 2.28 (s, 3H); 2.21 (s, 3H); 2.06 (s, 3H).
ESI-MS m/z: Calcd. for $C_{42}H_{43}N_5O_8S$: 777.3 Found ($M+H^+$): 778.3



Compound 12 (first isomer): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.68 (s, 1H); 7.05 (d, 1H); 6.63-6.57 (m, , 3H); 6.22 (d, 1H); 6.02 (d, 1H); 5.73 (s, 1H); 5.12 (d, 1H); 4.58 (s, 1H); 4.36 (s, 1H); 4.34-4.22 (m, 3H); 3.80 (s, 3H); 3.47-3.42 (m, 2H); 3.05-2.86 (m, 2H); 2.67-2.35 (m, 2H); 2.32-2.05 (m, 3H); 2.31 (s, 3H); 2.28 (s, 3H); 2.15 (s, 3H); 2.03 (s, 3H); 1.07 (d, 3H).
ESI-MS m/z: Calcd. for $C_{42}H_{43}N_5O_9S$: 793.2 Found ($M+H^+$): 794.2.
Compound 13 (second isomer): ^{1}H-NMR (300 MHz, $CDCl_3$); δ 7.54 (s, 1H); 7.08 (d, 1H); 6.73 (d, 1H); 6.63 (dd, 1H); 6.57 (s, 1H); 6.20 (d, 1H); 6.00 (d, 1H); 5.74 (s, 1H); 5.02 (d, 1H); 4.60 (s, 1H); 4.33 (s, 1H); 4.27 (d, 1H); 4.22 (d, 1H); 4.12 (dd, 1H); 3.80 (s, 3H); 3.44-3.32 (m, 3H); 3.05-2.89 (m, 2H); 2.49-2.03 (m, 4H); 2.32 (s, 3H); 2.24 (s, 3H); 2.18 (s, 3H); 2.07 (s, 3H); 1.21 (d, 3H).
ESI-MS m/z: Calcd. for $C_{42}H_{43}N_5O_9S$: 793.2 Found ($M+H^+$): 794.2.

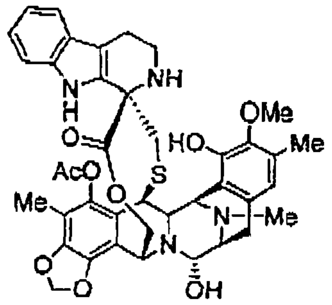


[0053] Compound 17 (second isomer): ^{1}H-NMR (300 MHz, $CDCl_3$); δ 7,65 (s, 1H); 7.18 (dd, 1H); 6.99 (dd, 1H); 6.83 (ddd, 1H); 6.58 (s, 1H); 6.22 (d, 1H); 6.01 (d, 1H); 5.74 (s, 1H); 5.03 (d, 1H); 4.61 (s, 1H); 4.34 (s, 1H); 4.27 (d,

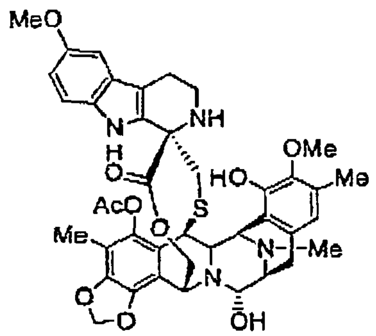
1H); 4.22 (d, 1H); 4.14-4.10 (m, 1H); 3.80(s, 3H); 3.44 (d, 2H); 3.38-3.30 (m, 1H); 3.06-2.99 (m, 2H); 2.50 (dd, 1H); 2.43 (d, 1H); 2.32 (s, 3H); 2.24 (s, 3H); 2.18 (s, 3H); 2.16-2.11 (m, 2H); 2.08 (s, 3H); 1.20 (d, 3H).
ESI-MS m/z: Calcd. for $C_{42}H_{42}FN_5O_8S$: 795.3 Found ($M+H^+$): 796.2.

Method 4:

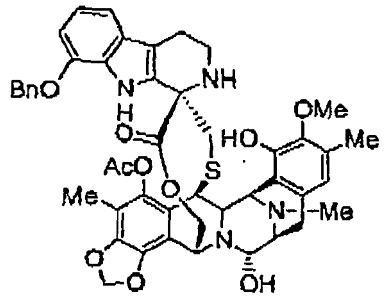
[0054]



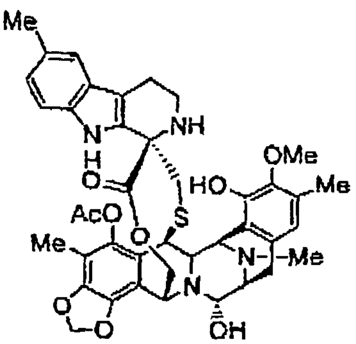
Compound 26 (Reference Compound): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.70 (s, 1H); 7.38 (d, 1H); 7.24 (d, 1H); 7.08 (t, 1H); 7.00 (t, 1H); 6.67 (s, 1H); 6.20 (d, 1H); 5.99 (d, 1H); 5.74 (s, 1H); 5.20 (d, 1H); 4.82 (s, 1H); 4.347-4.38 (m, 3H); 4.16-4.10 (m, 2H); 3.81 (s, 3H); 3.49 (d, 1H); 3.22-3.13 (m, 2H); 3.00 (d, 1H); 2.88-2.79 (m, 2H); 2.71-2.52 (m, 3H); 2.37 (s, 3H); 2.28-2.24 (m, 1H); 2.25 (s, 3H); 2.19 (s, 3H); 2.05 (s, 3H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.4, 168.7, 147.8, 1.45.4, 142.8, 141.0, 140.6, 135.4, 131.2, 130.9, 1,29.0, 126.8, 121.8, 121.3, 120.9, 119.1, 118.3, 118.1, 115.5, 112.8, 110.8, 110.1, 101.7, 81.9, 62.3, 61.8, 60.2.57.6, 57.4, 55.8, 54.9, 42.1, 41.2, 39.7, 39.2, 31.5, 23.5, 22.6, 21.5, 20.5, 15.8, 14.0, 9.6.
ESI-MS m/z: Calcd. for $C_{40}H_{42}N_4O_9S$: 754.3 Found ($M-H_2O+H^+$): 737.2.



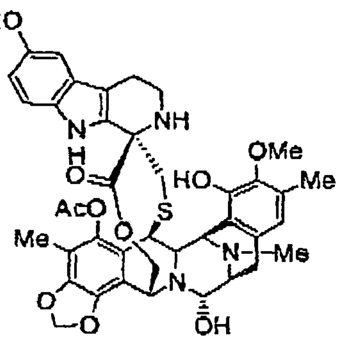
Compound 27: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.59 (s, 1H); 7.13 (d, 1H); 6.81 (s, 1H); 6.73 (dd, 1H); 6.67 (s, 1H); 6.19 (d, 1H); 5.99 (d, 1H); 5.74 (s, 1H); 5.19 (d, 1H); 4.82 (s, 1H); 4.49-4.47 (m, 2H); 4.16-4.09 (m, 2H); 3.81 (s, 3H); 3.79 (s, 3H); 3.50-3.45 (m, 2H); 3.24-3.13 (m, 2H); 3.02 (d, 1H); 2.88-2.79 (m, 2H); 2.67-2.48 (m, 3H); 2.37 (s, 3H); 2.30-2.24 (m, 1H); 2,25 (s, 3H); 2.19 (s, 3H); 2.04 (s, 3H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.6, 154.0, 148.1, 145.6, 143.1, 141.3, 140.9, 131.9, 131.4, 130.8, 129.3, 127.4, 121.5, 121.2, 115.7, 113.1, 112.1, 111.8, 110.1, 102.0, 100.6, 82.1, 62.6, 62.0, 60.5, 57.9, 57.6, 56.1, 56.0, 55.2, 42.4, 41.5, 40.0, 39.4, 31.8, 29.9, 23.8, 22.8, 21.8, 20.8, 16.0, 14.6, 9.9.
ESI-MS m/z: Calcd. for $C_{40}H_{42}N_4O_9S$: 784.4 Found ($M-H_2O+H^+$): 767.2.



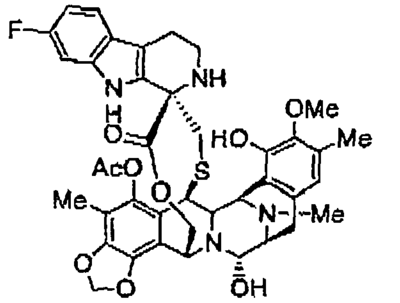
Compound 28: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.81 (s, 1H); 7.43-7.36 (m, 5H); 7.01 (d, 1H); 6.91 (t, 1H); 6.66 (s, 1H); 6.63 (d, 1H); 5.84 (s, 1H); 5.75 (s, 1H); 5.60 (s, 1H); 5.20-5.09 (m, 3H); 4.78 (s, 1H); 4.49 (d, 1H); 4.44 (s, 1H); 4.16 (s, 1H); 4.14-4.12 (m, 1H); 3.81 (s, 3H); 3.52 (d, 1H); 3.47 (s, 2H); 3.22-2.80 (m, 5H); 2.68-2.51 (m, 2H); 2.36 (s, 3H); 2.39-2.21 (m, 1H); 2.27 (s, 3H); 2.18 (s, 3H); 2.02 (s, 3H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.4, 148.0, 145.6, 145.2, 143.1, 141.1, 140.8, 137.3, 131.6, 130.7, 129.3, 128.8, 128.5, 128.2, 128.0, 126.2, 121.4, 121.3, 119.8, 118.1, 115.5, 113.0, 111.8, 111.0, 103.8, 101.8, 82.1, 70.6, 62.9, 61.9, 60.5, 58.0, 57.7, 56.1, 55.1, 42.3, 41.5, 40.0, 39.5, 29.9, 23.9, 22.0, 20.7, 16.0, 9.8.
ESI-MS m/z: Calcd. for $C_{47}H_{48}N_4O_{10}S$: 860.3 Found ($M-H_2O+H^+$): 843.3



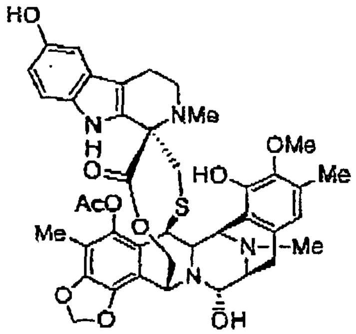
Compound 30: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.61 (s, 1H); 7.16-7.11 (m, 2H); 6.91 (d, 1H); 6.67 (s, 1H); 6.20 (d, 1H); 5.99 (d, 1H); 5.75 (s, 1H); 5.20 (d, 1H); 4.82 (s, 1H); 4.49 (d, 1H); 4.35 (s, 1H); 4.16 (d, 2H); 4.11 (dd, 1H); 3.81 (s, 3H); 3.48 (s, 1H); 3.23-2.79 (m, 5H); 2.67-2.47 (m, 3H); 2.37 (s, 6H); 2.25 (s, 3H); 2.19 (s, 3H); 2.05 (s, 3H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 174.5, 171.6, 168.9, 148.1, 145.6, 143.1, 141.3, 140.9, 134.0, 131.2, 128.6, 127.3, 123.6, 121.5, 121.2, 118.3, 115.7, 109.9, 102.0, 82.1, 62.6, 62.0, 60.5, 57.9, 57.7, 56.1, 56.2, 42.4, 41.5, 40.0, 39.4, 29.9, 23.8, 21.7, 21.6, 20.8, 16.0, 9.9.
ESI-MS m/z: Calcd. for $C_{41}H_{44}N_4O_9S$: 768.3 Found ($M-H_2O+H^+$): 751.3.



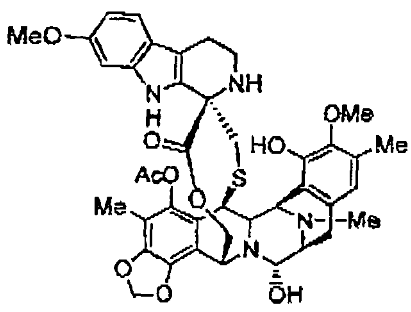
Compound 31: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.60 (s, 1H); 7.00 (d, 1H); 6.69 (d, 1H); 6.66 (s, 1H); 6.69 (dd, 1H); 6.16 (s, 1H); 5.96 (s, 1H); 5.78 (s, 1H); 5.19 (d, 1H); 4.82 (s, 1H); 4.49 (d, 1H); 4.46 (s, 1H); 4.17 (d, 1H); 4.10 (d, 1H); 3.81 (s, 3H); 3.72-3.59 (m, 2H); 3.64 (d, 2H); 3.50 (d, 1H); 3.23-2.76 (m, 4H); 2.55-2.29 (m, 3H); 2.37 (s, 3H); 2.25 (s, 3H); 2.19 (s, 3H); 2.03 (s. 3H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.3, 166.9, 149.2, 147.9, 145.4, 142.9, 141.0, 140.7, 131.2, 130.7, 127.5, 121.2, 120.9, 115.5, 111.5, 103.1, 101.8, 81.9, 62.3, 61.8, 60.3, 57.7, 57.4, 55.8, 54.9, 42.1.41.2, 39.6, 39.1, 29.6, 23.5, 22.6, 21.4, 20.5, 15.8, 14.1, 9.6.
ESI-MS m/z: Calcd. for $C_{40}H_{42}N_4O_{10}S$: 770.3 Found ($M-H_2O+H^+$): 753.3.



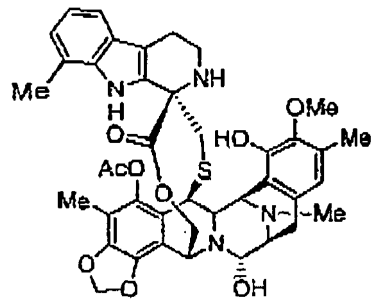
Compound 32: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.72 (s, 1H); 7.27 (dd, 1H); 6.94 (dd, 1H); 6.76 (ddd, 1H); 6.66 (s, 1H); 6.20 (s, 1H); 5.99 (s, 1H); 5.75 (s, 1H); 5.19 (d, 1H); 4.83 (s, 1H); 4.49 (d, 1H); 4.16-4.09 (m, 2H); 3.81 (s, 3H); 3.50-3.48 (m, 1H); 3.48 (s, 1H); 3.22-2.79 (m, 5H); 2.65-2.51 (m, 3H); 2.37 (s, 3H); 2.25 (s, 3H); 2.19 (s, 3H); 2.05 (s, 3H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.5, 169.0, 148.1, 145.7, 143.1, 141.3, 140.9, 131.5, 129.3, 123.7, 121.5, 121.1, 119.3, 119.1, 118.3, 115.7, 110.4, 108.2, 107.8, 102.0, 97.8, 97.5, 82.1, 62.4, 62.1, 60.5, 57.9, 57.6, 56.1, 55.1, 42.4, 41.5, 39.9, 39.4, 29.9, 23.8, 21.7, 20.8, 16.0, 9.9.
ESI-MS m/z: Calcd. for $C_{40}H_{41}FN_4O_9S$: 772.3 Found ($M-H_2O+H^+$): 755.3.



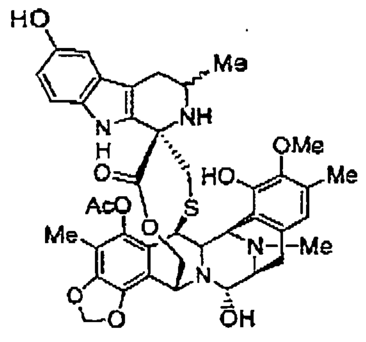
Compound 33: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.85 (d, 1H); 6.80 (s, 1H); 6.71-6.64 (m, 2H); 6.48 (s, 1H); 6.18 (s, 1H); 6.02 (s, 1H); 5.74 (s, 1H); 5.03 (d, 1H); 4.88 (d, 1H); 4.39 (d, 1H); 4.36 (s, 1H); 4.16 (d, 1H); 3.98 (ddm 1H); 3.80 (s, 3H); 3.71 (d, 1H); 3.48 (s, 1H); 3.22 (d, 1H); 2.93-2.83 (m, 2H); 2.73-2.39 (m, 4H); 2.29 (s, 3H); 2.28-2.05 (m, 1H); 2.26 (s, 3H); 2.22 (s, 3H); 2.18 (s, 3H); 2.03 (s, 3H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 168.9, 149.3, 147.6, 145.5, 142.7, 141.6, 140.7, 131.3, 131.1, 129.3, 126.9, 121.2, 115.7, 111.9, 111.0, 110.7, 103.1, 102.0, 83.4, 69.8, 63.7, 60.2, 58.5, 57.7, 55.1, 54.7, 59.5, 43.1, 41.4, 40.6, 35.0, 29.6, 24.6, 22.6, 21.1, 20.2, 15.5, 9.7.
ESI-MS m/z: Calcd. for $C_{41}H_{44}N_4O_{10}S$: 784.3 Found ($M-H_2O+H^+$) 767.3.



Compound 34: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.58 (s, 1H); 7.23 (d, 1H); 6.76 (d, 1H); 6.67 (dd, 1H); 6.66 (s, 1H); 6.19 (d, 1H); 5.98 (d, 1H); 5.74 (s, 1H); 5.20 (d, 1H); 4.82 (s, 1H); 4.49 (d, 1H); 4.47 (s, 1H); 4.16 (d, 1H); 4.1.0 (dd, 1H); 3.81 (s, 3H); 3.78 (s, 3H); 3.51-3.47 (m, 1H); 3.22-2.80 (m, 5H); 2.65.2.50 (m, 3H); 2.36 (s, 3H); 2.25 (s, 3H); 2.19 (s, 3H); 2.05 (s, 3H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.7, 168.9, 156.5, 148.1, 145.6, 143.1, 141.3, 140.9, 136.5, 131.5, 129.8, 129.3, 121.6, 121.5, 121.2, 119.2, 115.8, 113.1, 110.3, 109.3, 102.2, 94.9, 82.2, 62.5, 62.0, 60.5, 57.9, 57.7, 56.1, 55.8, 55.2, 42.3, 41.5, 40.0, 39.5, 29.9, 23.8, 21.8, 20.8, 16.0, 9.9.
ESI-MS m/z: Calcd. for $C_{41}H_{44}N_4O_{10}S$: 784.3 Found ($M-H_2O+H^+$); 767.3.

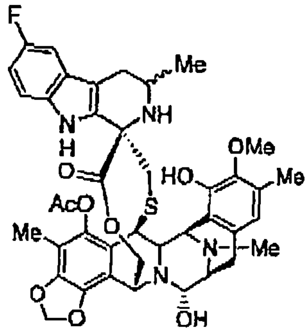


Compound 36: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.47 (s, 1H); 7.22 (d, 1H); 6.95-6.87 (m, 2H); 6.66 (s, 1H); 6.13 (d, 1H); 6.01 (d, 1H); 5.76 (s, 1H); 5.20 (d, 1H); 4.84 (s, 1H); 4.49 (d, 1H); 4.46 (s, 1H); 4.18-4.14 (m, 2H); 3.81 (s, 3H); 3.54 (d, 1H); 3.48(s, 1H); 3.22 (d, 1H); 3.20-2.80 (m, 4H); 2.70-2.42 (m, 3H); 2.36(s, 6H); 2.27 (s, 3H); 2.18 (s, 3H); 2.05 (s, 3H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.3, 169.0, 148.0, 145.6, 143.1, 141.4, 140.9, 135.3, 131.5, 131.1, 130.7, 129.4, 126.6, 122.8, 121.8, 121.3, 119.9, 119.6, 118.1, 116.3, 115.8, 102.0, 82.1, 62.1, 60.5, 58.0, 57.8, 56.1, 55.1, 42.4, 41.5, 40.1, 39.6, 29.9, 23.9, 21.9, 20.7, 16.6, 16.0, 9.9.
ESI-MS m/z: Calcd. for $C_{41}H_{44}N_4O_9S$: 768.2 Found ($M-H_2O+H^+$): 751.2.



Compound 37 (first isomer): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.70 (s, 1H); 7.06 (d, 1H); 6.67-6.61 (m, 3H); 6.20 (d, 1H); 5.98 (d, 1H); 5.70 (s, 1H); 5.20 (d, 1H); 4.86 (s, 1H); 4.53 (d, 1H); 4.48 (s, 1H); 4.18(s, 1H); 3.80(s, 3H); 3.72-3.54 (m,

4H); 3.24-3.22 (m, 1H); 3.01-2.56 (m, 5H); 2.31 (s, 3H); 2.27 (s, 3H); 2.15 (s, 3H); 2.02 (s 3H); 1.10 (d, 3H).
ESI-MS m/z: Calcd. for $C_{41}H_{44}N_4O_{10}S$: 784.3 Found ($M-H_2O+H^+$): 767.3.
Compound 38 (second isomer): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.51 (s, 1H); 7.10 (d, 1H); 6.75 (d, 1H); 6.64 (dd, 1H); 6,59 (s, 1H); 6.19 (d, 1H); 5.97 (d, 1H); 5.71 (s, 1H); 5.15 (d, 1H); 4.84 (s, 1H); 4.53-4.50 (m, 2H); 4.16 (s, 1H); 4.04 (dd, 1H); 3.80(s, 3H); 3.65-3,63 (m, 1H); 3.51-3.49 (m, 1H); 3.40-2.36 (m, 1H); 3.24-3.21 (m, 1H); 3.03-2.84 (m, 2H); 2.50-2.41 (m, 2H); 2.32 (s, 3H); 2.23 (s, 3H); 2.16 (s, 3H); 2.06 (s. 3H); 1.20 (d, 3H).
ESI-MS m/z: Calcd. for $C_{41}H_{44}N_4O_{10}S$: 784.3 Found ($M-H_2O+H^+$): 767.3.



Compound 42 (second isomer): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.62 (s, 1H); 7.18 (dd, 1H); 6.99 (dd, 1H); 6.82 (ddd, 1H); 6.59 (s, 1H); 6.20 (d, 1H); 5.98 (d, 1H); 5.71 (s, 1H); 5.15 (d, 1H); 4.85 (s, 1H); 4.52(s, 1H); 4.50 (d, 1H); 4.16 (d, 1H); 4.05 (dd, 1H); 3.80 (s, 3H); 3.50-3.48 (m, 1H); 3.42-3.36 (m, 1H); 3.23 (d, 1H); 3.00-2.81 (m, 2H); 2.50 (dd, 1H); 2.44 (d, 1H); 2.32 (s, 3H); 2.24 (s, 3H); 2.16 (s, 3H); 2.07 (s, 3H); 1.23 (d, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.9, 168.7, 156.2, 147.8, 145.6, 143.3, 141.8, 140.9, 132.7, 131.4, 131.1, 129.4, 129.0, 121.8, 121.4, 115.8, 113.1, 111.9, 111.8, 110.4, 110.0, 103.7, 103.4, 102.0, 81.9, 63.4, 61.8, 60.6, 58.0, 56.2, 55,2, 46.6, 42.3, 41.5, 41.0, 32.1, 29.5, 23.9, 22.9, 21.9, 20.7, 16.1, 14.3, 9.9.
ESI-MS m/z: Calcd. for $C_{41}H_{43}FN_4O_9S$: 786.2 Found ($M-H_2O+H^+$): 769.3.

BIOASSAYS FOR ANTITUMOR SCREENING

[0055] The finality of these assays is to interrupt the growth of a "in vitro" tumor cell culture by means of a continued exhibition of the cells to the sample to be testing.

CELL LINES

Name	N° ATCC	Species	Tissue	Characteristics
P-388	CCL-46	mouse	ascites fluid	lymphoid neoplasm
K-562	CCL-243	human	leukemia	erythroleukemia (pleural effusion)
A-549	CCL-185	human	lung	lung carcinoma "NSCL"
SK-MEL-28	HTB-72	human	melanoma	malignant melanoma
HT-29	HTB-38	human	colon	colon adenocarcinoma
LoVo	CCL-229	human	colon	colon adenocarcinoma
LoVo-Dox		human	colon	colon adenocarcinoma (MDR)
SW620	CCL-228	human	colon	colon adenocarcinoma (lymph node metastasis)
DU-145	HTB-81	human	prostate	prostate carcinoma, not androgen receptors
LNCaP	CRL-1740	human	prostate	prostate adenocarcinoma, with androgen receptors
SK-BR-3	HTB-30	human	breast	breast adenocarcinoma, Her2/neu$^+$, (pleural effusion)
MCF-7	HTB-22	human	breast	breast adenocarcinoma, (pleural effusion)
MDA-MB-231	HTB-26	human	breast	breast adenocarcinoma, Her2/neu+, (pleural effusion)
IGROV-1		human	ovary	ovary adenocarcinoma
IGROV-ET		human	ovary	ovary adenocarcinoma, characterized as ET-743 resistant cells
SK-OV-3	HTB-77	human	ovary	ovary adenocarcinoma (malignant ascites)
OVCAR-3	HTB-161	human	ovary	ovary adenocarcinoma

(continued)

CELL LINES				
HeLa	CCL-2	human	cervix	cervix epitheloid carcinoma
HeLa-APL	CCL-3	human	cervix	cervix epitheloid carcinoma, characterized as aplidine resistant cells
A-498	HTB-44	human	kidney	kidney carcinoma
PANC-1	CRL-1469	human	pancreas	pancreatic epitheloid carcinoma
HMEC1		human	endothelium	

Inhibition of cells growth by colorimetric assay

[0056] A colorimetric type of assay, using sulphorhodamine B (SRB) reaction has been adapted for a quantitative measurement of cell growth and viability following the technique described by Philip Skehan, et al. (1990), New colorimetric cytotoxicity assay for anticancer drug screening, J. Natl. Cancer Inst., 82:1107-1112]

[0057] This form of the assay employs 96 well cell culture microplates of 9 mm diameter (Faircloth, 1988; Mosmann, 1983). Most of the cell lines are obtained from American Type Culture Collection (ATCC) derived from different human cancer types.

[0058] Cells are maintained in RPMI 1640 10% FBS, supplemented with 0.1 g/l penicillin and 0.1 g/l streptomycin sulphate and then incubated at 37°C, 5% CO_2 and 98% humidity. For the experiments, cells were harvested from subconfluent cultures using trypsin and resuspended in fresh medium before plating.

[0059] Cells are seeded in 96 well microtiter plates, at 5 x 10^3 cells per well in aliquots of 195 μl medium, and they are allowed to attach to the plate surface by growing in drug free medium for 18 hours. Afterward, samples are added in aliquots of 5 μl in a ranging from 10 to 10^{-8} μg/ml, dissolved in DMSO/EtOH/PBS (0.5:0.5:99). After 48 hours exposure, the antitumor effect are measured by the SRB methodology: cells are fixed by adding 50 μl of cold 50% (wt/vol) trichloroacetic acid (TCA) and incubating for 60 minutes at 4°C. Plates are washed with deionized water and dried. One hundred μl of SRB solution (0.4% wt/vol in 1% acetic acid) is added to each microtiter well and incubated for 10 minutes at room temperature. Unbound SRB is removed by washing with 1% acetic acid. Plates are air dried and bound stain is solubilized with Tris buffer. Optical densities are read on a automated spectrophotometric plate reader at a single wavelength of 490 nm.

[0060] The values for mean +/- SD of data from triplicate wells are calculated. Some parameters for cellular responses can be calculated: GI = growth inhibition, TGI = total growth inhibition (cytostatic effect) and LC = cell killing (cytotoxic effect).

[0061] Obtained results may predict the usefulness of a certain drug as a potential cancer treatment. For this technique, compounds which show GI_{50} values smaller than 10 μg/ml are selected to continue with further studies. GI_{50} data allow to predict that not only could a drug be cystostatic, but also it could have a potential in terms of tumour reduction.

Activity Data (Molar).

		Compound 1 (Reference Compound)
A549	IC_{50}	1.31E-09
HT29	IC_{50}	1.31E-09

		Compound 2	Compound 3	Compound 5	Compound 6	Compound 7
A549	GI_{50}	6.30E-10	3.45E-07	1.29E-08	5.13E-09	7.67E-07
	TGI	6.30E-09	3.45E-06	1.29E-07	5.13E-08	1.28E-07
	LC_{50}	6.30E-05	5.75E-05	1.29E-05	1.28E-05	1.28E-06
HT29	GI_{50}	1.26E-09	2.30E-07	1.29E-08	5.13E-09	6.39E-08
	TGI	1.26E-09	2.30E-07	1.29E-08	5.13E-09	6.39E-08
	LC_{50}	6.30E-05	5.75E-05	5.14E-06	5.13E-06	1.28E-06
H-MEC-1	GI_{50}					
	TGI					
	LC_{50}					

		Compound 8	Compound 9	Compound 11	Compound 12	Compound 13
A549	GI_{50}	2.52E-09	2.52E-08	1.89E-08	7.28E-09	6.31E-09
	TGI	1.01E-08	1.01E-07	5.00E-08	8.35E-08	5.79E-08
	LC_{50}	1.26E-05	8.82E-06	1.29E-07	1.26E-05	1.26E-05
HT29	GI_{50}	2.52E-09	3.78E-07	3.09E-08	1.37E-08	3.33E-07
	TGI	2.52E-09	3.78E-07	1.29E-07	1.26E-07	1.26E-06
	LC_{50}	5.04E-06	1.26E-05	1.29E-05	1.26E-05	1.26E-05
H-MEC-1	GI_{50}					
	TGI					
	LC_{50}					

		Compound 17
A549	GI_{50}	5.39E-09
	TGI	4.41E-08
	LC_{50}	6.67E-06
HT29	GI_{50}	2.22E-08
	TGI	1.28E-08

	LC_{50}	1.28E-05
H-MEC-1	GI_{50}	
	TGI	
	LC_{50}	

		Compound 26 (Reference Compound)	Compound 27	Compound 28	Compound 30
A549	GI_{50}	2.65E-09	2.55E-10	3.48E-09	3.90E-11
	TGI	3.97E-09	8.92E-10	4.65E-08	2.60E-10
	LC_{50}	1.32E-08	6.37E-09	3.48E-08	1.04E-09
HT29	GI_{50}	3.97E-09	2.55E-10	1.16E-08	1.04E-10
	TGI	7.95E-09	7.64E-10	6.97E-08	3.90E-10
	LC_{50}	1.32E-08	1.15E-09	1.05E-07	1.04E-09
SW-620	GI_{50}	2.65E-09	2.55E-10	5.97E-09	2.60E-11
	TGI	7.95E-09	6.37E-10	2.32E-08	3.90E-10
	LC_{50}	7.95E-08	1.15E-09	9.29E-08	1.30E-09
MEL-28	GI_{50}	2.65E-09	2.55E-10	2.32E-08	2.60E-11
	TGI	5.30E-09	6.37E-10	3.48E-08	1.30E-10
	LC_{50}	1.06E-08	1.27E-09	8.13E-08	6.50E-10
OVCAR	GI_{50}				
	TGI				
	LC_{50}				
A498	GI_{50}	2.65E-09	2.55E-10	3.48E-09	1.30E-10
	TGI	5.62E-09	5.10E-10	1.16E-08	5.20E-10
	LC_{50}	2.65E-08	1.27E-09	5.81E-08	2.60E-09
DU145	GI_{50}	2.65E-09	2.55E-11	2.32E-09	1.30E-11
	TGI	3.97E-09	8.92E-11	3.48E-09	3.90E-11
	LC_{50}	1.06E-08	3.82E-09	9.29E-09	1.30E-10
MCF	GI_{50}	2.65E-09	2.55E-10	5.81E-09	2.60E-10
	TGI	5.30E-09	1.27E-09	2.32E-08	3.90E-10
	LC_{50}	1.19E-08	1.15E-08	1.16E-07	2.60E-09
MB231	GI_{50}	2.65E-09	2.55E-10	3.48E-09	2.60E-12
	TGI	5.30E-09	6.37E-09	9.29E-09	1.30E-10

	LC_{50}	1.32E-08	1.27E-08	1.16E-07	3.90E-09
H-MEC-1	GI_{50}				
	TGI				
	LC_{50}				
LNCAP	GI_{50}				
	TGI				
	LC_{50}				
SK-OV3	GI_{50}				
	TGI				
	LC_{50}				
IGROV	GI_{50}				
	TGI				
	LC_{50}				
IGROV-ET	GI_{50}				
	TGI				
	LC_{50}				
SK-BR3	GI_{50}				
	TGI				
	LC_{50}				
K562	GI_{50}				
	TGI				
	LC_{50}				
PANC-1	GI_{50}				
	TGI				
	LC_{50}				
LOVO	GI_{50}				
	TGI				
	LC_{50}				
LOVO-DOX	GI_{50}				
	TGI				
	LC_{50}				
HELA	GI_{50}				
	TGI				
	LC_{50}				
HELA-APL	GI_{50}				
	TGI				
	LC_{50}				

		Compound 31	Compound 32	Compound 33	Compound 34	Compound 36
A549	GI_{50}	2.59E-09	3.88E-09	3.82E-10	5.10E-09	2.84E-08
	TGI	5.19E-09	2.59E-08	1.27E-09	2.55E-08	5.07E-08

	LC_{50}	3.89E-08	9.06E-08	5.10E-09	8.92E-08	9.09E-08
HT29	GI_{50}	3.89E-09	7.76E-09	2.55E-10	7.64E-09	6.18E-08
	TGI	7.78E-09	2.59E-08	7.64E-10	2.54E-09	1.87E-07
	LC_{50}	1.30E-08	1.03E-07	1.15E-09	1.15E-08	1.30E-05
SW-620	GI_{50}	2.59E-09	5.17E-09	2.55E-10	1.02E-08	
	TGI	3.89E-09	2.59E-08	1.15E-09	3.82E-08	
	LC_{50}	1.17E-08	1.29E-07	1.02E-08	1.02E-07	
MEL-28	GI_{50}	2.59E-09	2.59E-09	2.55E-10	3.82E-09	2.16E-09
	TGI	3.89E-09	1.03E-08	6.37E-10	1.27E-08	5.23E-09
	LC_{50}	1.04E-08	2.59E-08	1.15E-09	6.37E-08	1.26E-08
OVCAR	GI_{50}					
	TGI					
	LC_{50}					
A498	GI_{50}	2.59E-09	2.59E-09	2.55E-10	3.82E-09	
	TGI	5.49E-09	9.06E-09	5.10E-10	1.27E-08	
	LC_{50}	1.30E-08	5.17E-08	1.27E-09	6.37E-08	
DU145	GI_{50}	1.30E-09	1.29E-09	3.82E-10	2.55E-09	5.41E-09
	TGI	3.89E-09	2.59E-09	8.92E-10	3.82E-09	9.39E-09
	LC_{50}	9.08E-09	3.88E-09	3.82E-09	1.02E-08	1.30E-05
MCF	GI_{50}	2.59E-09	9.06E-09	1.02E-09	5.10E-09	
	TGI	5.19E-09	2.59E-08	2.55E-09	2.55E-08	
	LC_{50}	1.17E-08	1.29E-07	1.15E-08	1.27E-07	
MB231	GI_{50}	1.30E-09	2.59E-09	2.55E-10	3.82E-09	
	TGI	5.19E-09	9.06E-09	1.27E-09	1.02E-08	
	LC_{50}	1.30E-08	9.06E-08	1.27E-08	1.27E-07	
H-MEC-1	GI_{50}					8.43E-08
	TGI					7.83E-07
	LC_{50}					1.30E-05
LNCAP	GI_{50}					6.14E-09
	TGI					9.48E-09
	LC_{50}					2.51E-08
SK-OV3	GI_{50}					5.07E-08
	TGI					2.05E-07
	LC_{50}					1.30E-05
IGROV	GI_{50}					3.72E-09
	TGI					7.21E-09
	LC_{50}					2.71E-08
IGROV-ET	GI_{50}					5.03E-08
	TGI					1.03E-07
	LC_{50}					1.30E-07
SK-BR3	GI_{50}					1.18E-08

	TGI					3.20E-08
	LC_{50}					8.64E-08
K562	GI_{50}					6.15E-09
	TGI					9.92E-09
	LC_{50}					1.09E-07
PANC-1	GI_{50}					4.21E-08
	TGI					1.04E-07
	LC_{50}					1.30E-05
LOVO	GI_{50}					2.73E-08
	TGI					5.46E-08
	LC_{50}					1.09E-07
LOVO-DOX	GI_{50}					7.04E-08
	TGI					9.77E-07
	LC_{50}					1.30E-05
HELA	GI_{50}					
	TGI					
	LC_{50}					
HELA-APL	GI_{50}					
	TGI					
	LC_{50}					

		Compound 37	Compound 38	Compound 42
A549	GI_{50}	4.23E-09	2.38E-09	2.20E-08
	TGI	2.17E-08	5.24E-09	4.09E-08
	LC_{50}	1.38E-07	1.15E-08	7.59E-08
HT29	GI_{50}	3.71E-09	4.20E-09	3.41E-08
	TGI	1.40E-08	1.69E-08	1.42E-07
	LC_{50}	1.27E-05	1.27E-05	1.27E-05
SW-620	GI_{50}			
	TGI			
	LC_{50}			
MEL-28	GI_{50}	2.40E-09	2.73E-09	4.04E-10
	TGI	6.82E-09	5.33E-09	9.77E-10
	LC_{50}	3.35E-08	1.04E-08	6.19E-09
OVCAR	GI_{50}			
	TGI			
	LC_{50}			
A498	GI_{50}			
	TGI			
	LC_{50}			
DU145	GI_{50}	4.60E-09	2.04E-09	3.51E-09

	TGI	1.06E-08	6.80E-09	7.97E-09
	LC_{50}	1.27E-05	1.27E-05	1.27E-08
MCF	GI_{50}			
	TGI			
	LC_{50}			
MB231	GI_{50}			
	TGI			
	LC_{50}			
H-MEC-1	GI_{50}	2.22E-09	3.06E-09	4.04E-08
	TGI	3.58E-08	8.93E-09	2.47E-07
	LC_{50}	1.27E-05	1.27E-05	1.27E-05
LNCAP	GI_{50}	2.60E-10	2.00E-10	3.74E-09
	TGI	8.56E-10	7.21E-10	6.61E-09
	LC_{50}	4.75E-09	3.07E-09	1.17E-08
SK-OV3	GI_{50}	3.57E-09	2.55E-09	7.37E-09
	TGI	1.07E-08	7.13E-09	7.92E-07
	LC_{50}	1.27E-05	1.27E-05	1.27E-05
IGROV	GI_{50}	2.82E-09	7.57E-10	2.44E-09
	TGI	7.06E-09	2.89E-09	5.12E-09
	LC_{50}	6.13E-07	8.40E-09	1.07E-08
IGROV-ET	GI_{50}	3.76E-08	1.57E-08	4.32E-08
	TGI	1.19E-07	6.59E-08	9.42E-08
	LC_{50}	1.27E-05	6.51E-06	1.27E-05
SK-BR3	GI_{50}	2.96E-09	1.07E-09	5.63E-09
	TGI	6.80E-09	3.38E-09	2.44E-08
	LC_{50}	1.41E-07	9.54E-09	8.73E-08
K562	GI_{50}	4.92E-10	4.22E-10	4.77E-09
	TGI	1.36E-09	8.18E-10	9.15E-09
	LC_{50}	1.27E-08	3.15E-09	2.81E-06
PANC-1	GI_{50}	3.12E-09	3.22E-09	1.69E-08
	TGI	1.18E-08	8.37E-09	8.25E-08
	LC_{50}	3.02E-06	4.28E-07	1.27E-05
LOVO	GI_{50}	2.92E-09	3.55E-09	1.35E-08
	TGI	8.97E-09	1.03E-08	4.37E-08
	LC_{50}	1.27E-05	1.27E-05	1.27E-07
LOVO-DOX	GI_{50}	6.17E-08	5.53E-08	4.59E-08
	TGI	4.10E-07	8.42E-07	2.05E-07
	LC_{50}	1.27E-05	1.27E-05	1.27E-05
HELA	GI_{50}			
	TGI			
	LC_{50}			

HELA-APL	GI_{50}						
	TGI						
	LC_{50}						

TOXICITY DATA

[0062] Toxicity was asssessed by the methods reported in Toxicology in Vitro, 15 (2001) 571-577, J. Luber Narod et al.:"Evaluation of the use of in vitro methodologies as tools for screening new compounds for potential in vivo toxicity".

Methods

[0063] In order to assess the cytotoxicity of the drugs to normal cells, we used 96 well plates plated at a density of 5000 cells per well (except for the FDC-P1 which were plated at 12,000 cells per well) with normal cell lines (ATCC, Table 1) maintained as per the directions of the ATCC: AML-12, normal mouse liver cells; NRK-52E, normal rat kidney cells; L8, normal rat skeletal muscle cells; FDC-P1, normal mouse myelogeous stem cells; and H9c2 (2-1), normal rat cardiac muscle cells. The cells in each plate were permitted to settle overnight before adding the test drug. In addition, primary neuronal cultures were prepared from embryonic (day e-17) whole brain (forebrain and brainstem) and spinal cord using established methods (Federoff and Richardson, 1997).

[0064] To each well (100µl medium) 10µl of drug in media was added at varying concentrations (1x10-10-0.01mg/ml final concentration) and further incubated overnight at 37°C with 5% CO_2. After 24h the following assays were performed. All experiments were repeated at least 3 times and were assayed in duplicate.

[0065] 1.MTS assay (CellTiter 96 aqueous) was performed according to the manufacturer's (Promega) directions (for all cell types). Cell viability (mitochondrial activity) is determined via enzymatic conversion of the formazan substrate.

Compound n°	Liver	Heart	Myelo	Skeletal	Kidney
26*	1.06E-06	6.43E-07	1.03E-07	3.71E-08	4.50E-08
27	1.48E-08	9.93E-08	1.75E-08	1.54E-08	1.01E-08
28	1.42E-07	1.84E-07	2.00E-07	1.45E-07	8.37E-08
30	2.72E-07	5.06E-07	7.58E-09	2.51E-08	4.19E-09
31	1.89E-08	6.65E-08	3.18E-08	1.35E-08	4.27E-08
32	6.00E-07	2.42E-07	5.25E-07	1.51E-08	1.45E-07
33	1.05E-08	1.27E-06	1.92E-08	1.41E-08	7.78E-09
34	2.55E-06	4.96E-07	1.15E-05	1.48E-08	2.74E-07
36	3.19E-07	8.86E-07	2.05E-07	2.71E-08	3.56E-07
37	5.46E-09	1.74E-08	4.59E-09	2.22E-08	2.92E-08
38	1.39E-10	2.96E-09	9.66E-11	1.29E-08	9.85E-08
42	1.86E-07	1.42E-07	6.41E-08	3.37E-08	1.12E-09
* - reference compound					

In vitro evaluation of the compounds for ADME-TOX profile

Partition Coefficient (log D)

[0066] The partition coefficient of a chemical compound provides a thermodynamic measure of its hydrophilicity-lipophylicity balance. Lipophilicity is a major structural factor that influences the pharmacokinetic and pharmacodynamic behavior of compounds. The partition coefficient between water or buffer and 1-octanol is the most widely used measure of chemical compound lipophilicity.

[0067] The measurement of partition coefficient was evaluated based on a miniaturized shake-flask procedure. Buffer

(Dulbecco's PBS, pH 7.40) was used as the aqueous phase, The tested compound was dissolved in DMSO, at the concentration of 100 μM. The final DMSO concentration (1%.) during the octanol-buffer partitioning are very low to avoid bias on the partitioning. The amount of compound in the buffer phase was determined by HPLC with photodiode array detection after an equilibration phase of 60 min. The amount of compound in the octanol phase is calculated by subtraction of the amount of compound in buffer from the total amount of compound, which is determined from a calibration sample.

[0068] Log D is calculated as the Log_{10} of the amount of compound in the octanol phase divided by the amount of compound in the buffer phase, The effective range of the log D microassay is approximately -0.5 to +4.5.

In vitro Intestinal Absorption Assays

[0069] The intestinal epithelium permeability is a critical characteristic that determines the rate and extent of human absorption and ultimately the bioavailability of a drug candidate. Caco-2 permeability assay allows a rapid assessment of membrane permeability and thus helps to rank-order compounds in terms of their absorption potential.

[0070] The Caco-2 cell line is a human colon adenocarcinoma cell line that differentiates in culture and resembles the epithelial lining of the human small intestine. It has been widely used as an *in vitro* intestinal epithelial model for drug transport and permeability screening of discovery compounds.

[0071] The apparent permeability coefficients (P_{app}) was determined in the apical-to-basolateral (A-to-B) direction across the cell monolayers (TC-7 sub-clone of the Caco-2) cultured on polycarbonate membrane filters, Compounds were tested at 50 μM with at a final DMSO concentration of 1%. Samples were analyzed by HPLC-MS or HPLC-MS/MS.

[0072] The test compound was added to the apical side and the P_{app} was determined based on the rate of appearance of the test compound in the basolateral side after 2h-incubation. Two reference compounds, propranolol (highly permeable) and ranitidine (poorly permeable), are tested in each assay as controls. Results from this assay can be used to rank-order compounds in terms of their absorption potential. Compounds with P_{app} equals to or greater than 20 x 10-6 cm/s could be considered highly permeable and are likely to be "not permeability-limited". Compounds with P_{app} less than 5 x 10-6 cm/s are considered poorly permeable and are likely to be "permeability-limited". Compounds with P_{app} greater than 5 x 10-6 but less than 20 x 10-6 cm/s" are considered to have medium permeability.

Metabolism

[0073] Hepatic metabolism is a primary determinant of pharmacokinetic behavior, and rapid first-pass metabolism is a major cause of low bioavailability. Pooled liver microsomes and recombinant cytochrome P450's are used for the metabolic assessment of hits, leads, and new pharmaceutical compounds. The results of the metabolic screening studies are useful in:

- Determining the initial rate at which compounds are metabolized
- Investigating the major pathways of drug metabolism
- Predicting in vivo pharmacokinetic behavior
- Investigating the potential for drug-drug interactions

[0074] The *metabolic stability* was determined used human liver S9 homogenate that including both microsomal and cytosolic enzyme activities. The test compound was diluted in methanol (0.625%) and acetonitrile (0.625%) at the concentration 1 μM and incubated in the human liver pool (protein = 1mg/mL) during 60 min at 37°C. Peak areas corresponding to all analytes (metabolic products) were determined by HPLC-MS/MS. Areas were recorded and ratios of peak areas of analytes to that of internal standard for each analyte were determined. The ratio of precursor compound remaining after 60 minutes and the amount remaining at time zero, expressed as per cent, are reported as metabolic stability. Higher values mean higher metabolic stability.

Inhibition of cytochromes P450 (CYP450)

[0075] The cytochromes P450 are a group of related enzymes primarily located in the liver and responsible of the metabolism of drugs. The inhibition of these CYP by drugs are related with drug-drug interactions and toxicities.

[0076] CYP3A4 is the most common form of the CYP3A enzymes found in adults and is the form implicated in most drug interactions. CYP2D6 metabolize more than 25% of the clinically useful medications.

[0077] For CYP2D6 inhibition assay, the compound are tested at 10 μM in duplicate with a 0.25% final concentration of both methanol and acetonitrile in presence of the fluorescent substrate AMMC (3-[2-*N,N*-diethyl-*N*-methylammonium) ethyl]-7-methoxy-4-methylcoumarin) at the concentration of 1.5 μM). The conversion of the AMMC in AHMC (3-[2-*N*, *N*-diethyl-*N*-methylammonium)ethyl]-7-hydroxy-4-methylcoumarin) is determined spectrofluorimetry after incubation with the enzyme during 450 min at 37°C.

[0078] For CYP3A4 inhibition assay, the compound were tested at 10 μM in duplicate with a 0.25% final concentration of both methanol and acetonitrile in presence of the fluorescent substrate BFC (50 μM). The conversion of the BFC (7-benzyloxy-4-trifluoromethylcoumarin) in HFC (7-hydroxy-4-trifluoromethylcoumarin) was determined spectrofluormetry after incubation with the enzyme during 30 min at 37°C.

[0079] For both assays, the fluorescent intensity measured at t = 0 is subtracted from that measured after the appropriate incubation time. The ratio of signal-to-noise is calculated by comparing the fluorescence in incubations containing the test compound to the control samples containing the same solvent vehicle. The percent of control activity is calculated and reported as percent inhibition.

In vitro Safety Assessment. Cell Viability

[0080] The toxic potential of compounds was investigated *in vitro* using primary human hepatocytes (HEPG2). The compounds were tested at 30 μM in duplicate with a final DMSO concentration of 1%. After incubation during 24 h at 37°C the cell viability was determined by the conversion of oxided alarmarBlue (resazurin) to reduced alarmarBlue (resorufin). Chlorpromazine was used as reference compound. Results are expressed as a percent of inhibition of control values.

Results of the studies ADME -TOX

[0081]

Compound	Relative Peak Area of Principal Peak (Chromatographic Purity)	Solubility Dulbecco's PBS pH 7.4	log D n-Octanol-Dulbecco's PBS pH 7.4	Permeability A to B TC 7 Cells
	(%)	(μM)		P_{app} (10^{-6} cm/s)
31	97.4	18.28	3.61	< 0.32
Reference Compound 26 (ET-736)	62.9	< 1 est	>5.0	PD
27	84.9	2.99	>5.0	< 1.18
34	98.6	17.65	>5.0	5.00
33	82.6	68.94	3.50	7.77
28	88.8	< 1 est	>4.6	ND
30	100.0	2.24	>4.9	ND
32	92.8	14.28	>4.3	PD
36	98.4	26.03	>4.4	PD
42	96.8	10.37	>4.8	PD

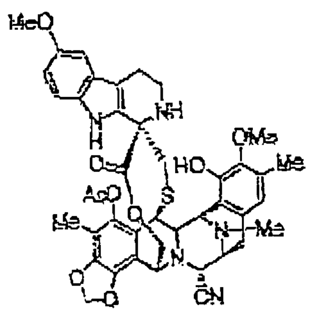
	Human Liver S9			

Compound	Metabolic Stability (% remaining)	CYP2D6 (% inhibition)	CYP3A4 (% inhibition)	Cell Viability (% inhibition)
31	17	-	84	22
Reference Compound 26 (ET-736)	3	12	45	-
27	7	13	53	-
34	24	28	80	45
33	10	28	84	32
28	33	34	79	17
30	6	-	25	-
32	9	12	83	39
36	2	15	78	33
42	5	19	78	67

Claims

1. A compound of formula:

Compound 2:



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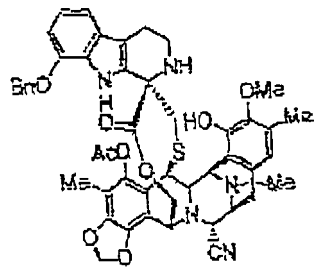
Compound 3:



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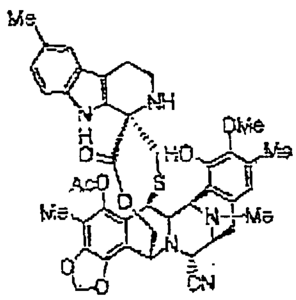
Compound 5:



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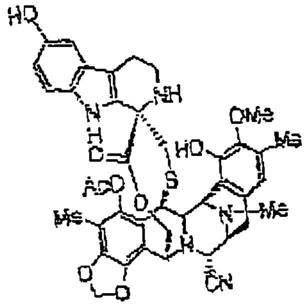
Compound 6:



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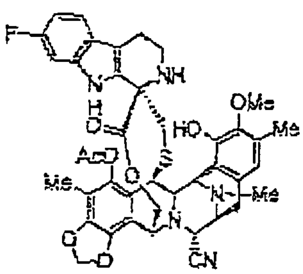
Compound 7:



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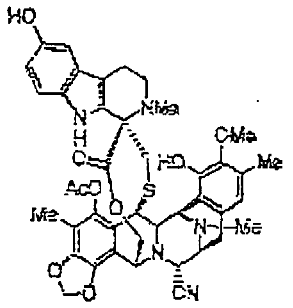

Compound 8:

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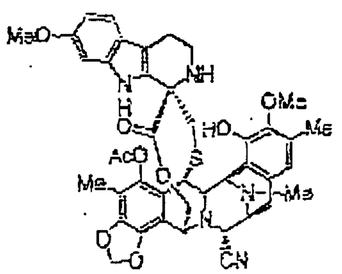

Compound 9:

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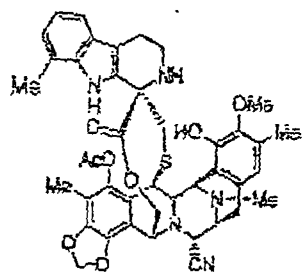

Compound 11:

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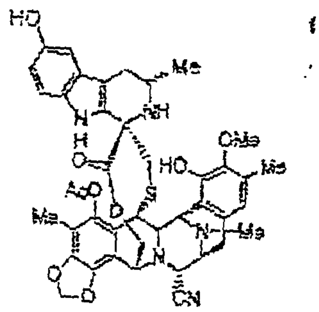
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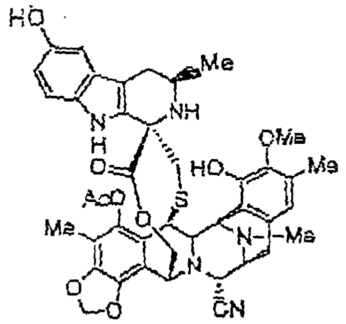
NH
N
H
OMe
HO
Me
AcO
S
Me
N
Me
N
CN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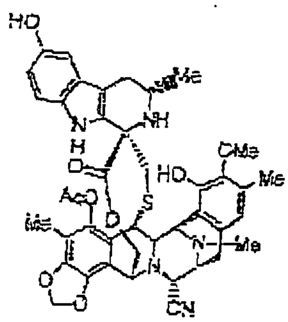
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N
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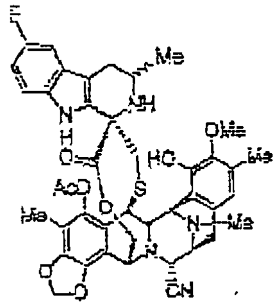

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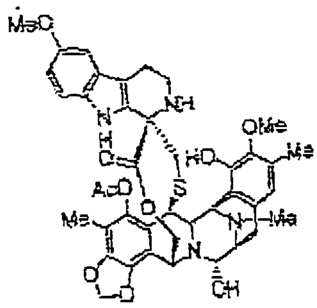
Compound 27:



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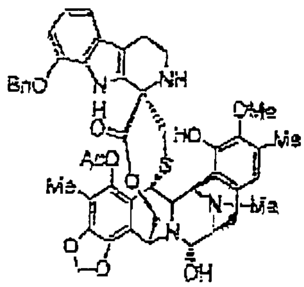
Compound 28:



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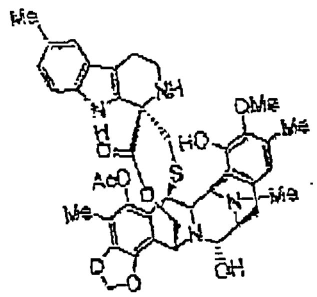
Compound 30:



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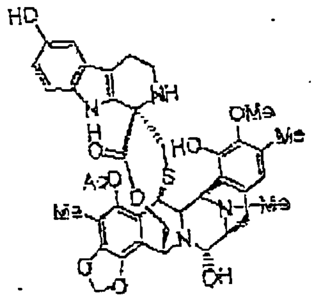
Compound 31:



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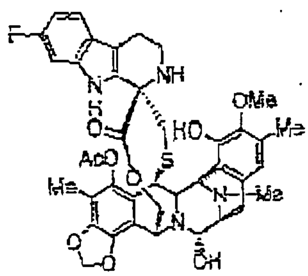
Compound 32:



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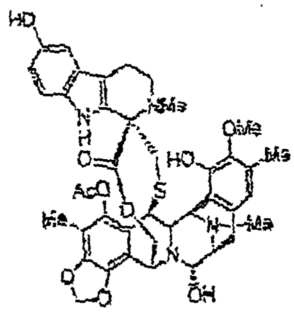
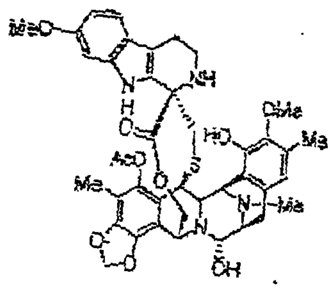
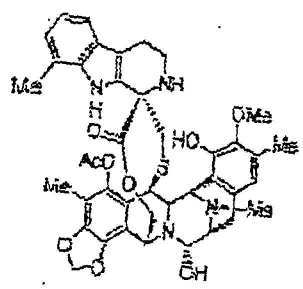


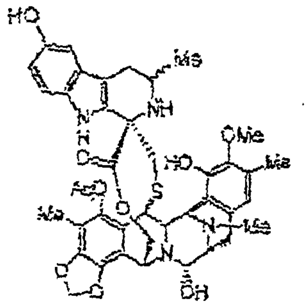
Compound 33:

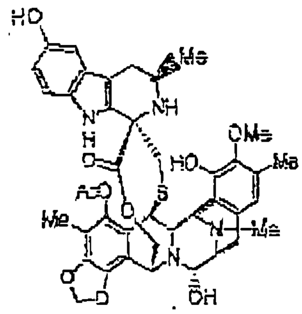


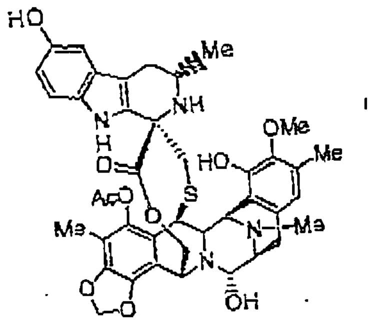
Compound 34:



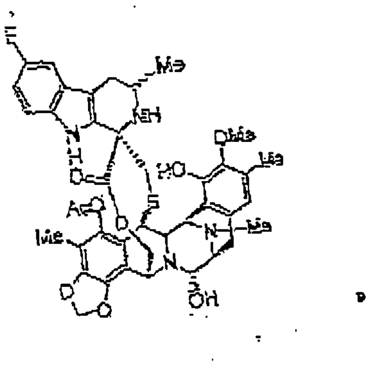
Compound 36:



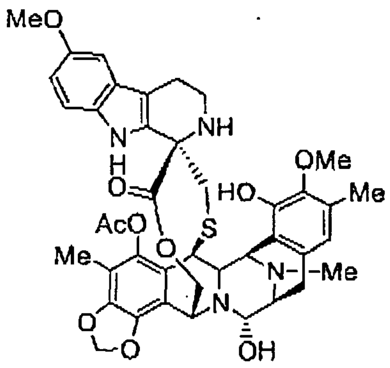




or

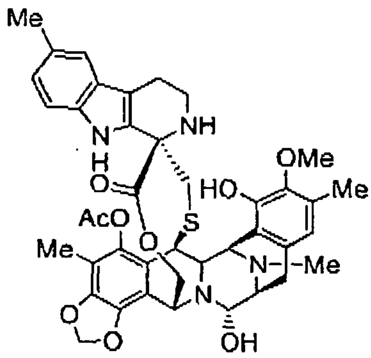


2. A compound according to claim 1 of formula (27):



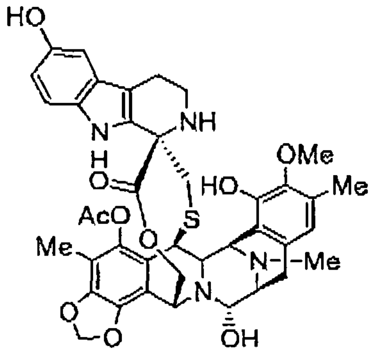
27

3. A compound according to claim 1 of formula (30):



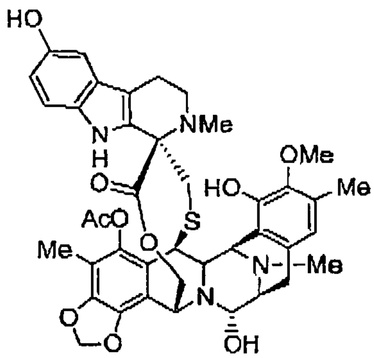
30

4. A compound according to claim 1 of formula (31):



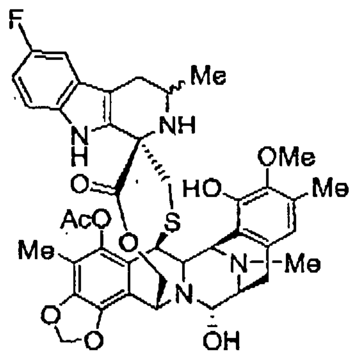
31

5. A compound according to claim 1 of formula (33):

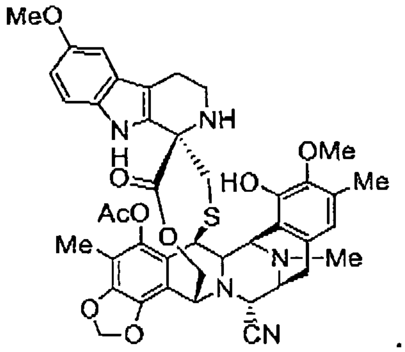


33

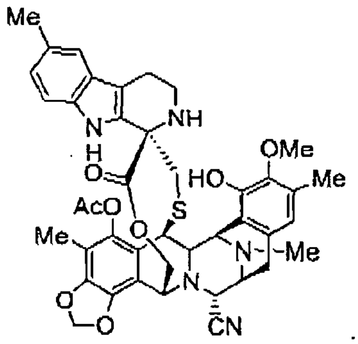
6. A compound according to claim 1 of formula:



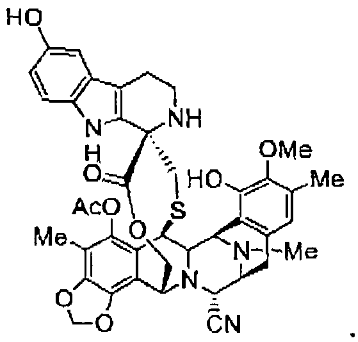
7. A compound according to claim 1 of formula (2):



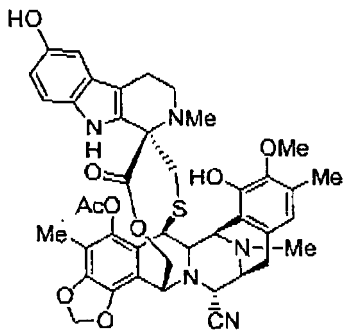
8. A compound according to claim 1 of formula (5):



9. A compound according to claim 1 of formula (6):



10. A compound according to claim 1 of formula (8):



.

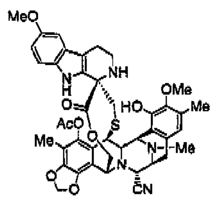
11. A pharmaceutical composition which contains a compound according to any preceding claim together with a pharmaceutically acceptable diluent.

12. The use of a compound according to any of claims 1 to 10 in the preparation of a medicament for treatment of cancer.

Patentansprüche

1. Verbindung der Formel:

Verbindung 2 :



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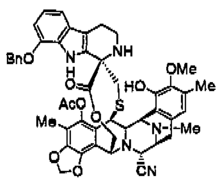
Verbindung 3:



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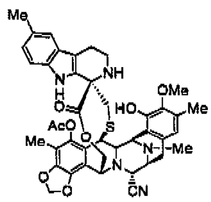
Verbindung 5:



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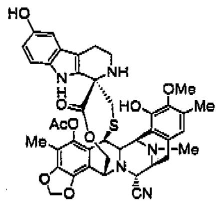
Verbindung 6:



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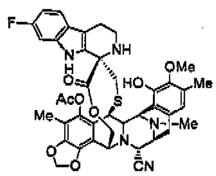
Verbindung 7:



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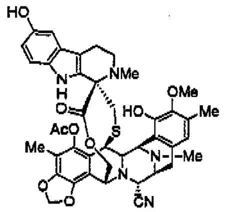
Verbindung 8:



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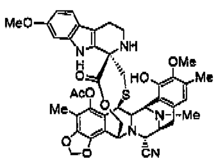

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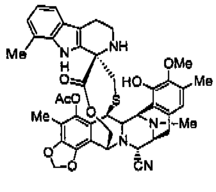

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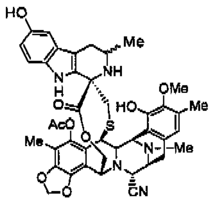

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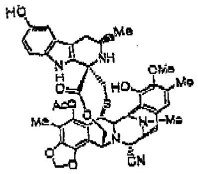

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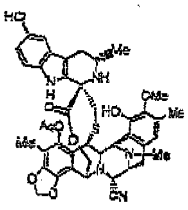

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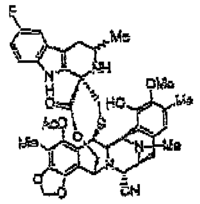

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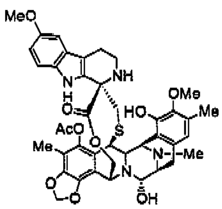
Verbindung 27:



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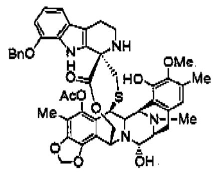
Verbindung 28:



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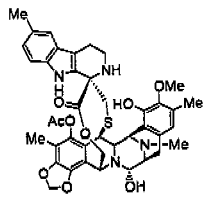
Verbindung 30:



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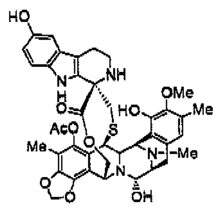
Verbindung 31:



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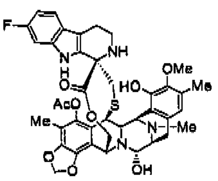

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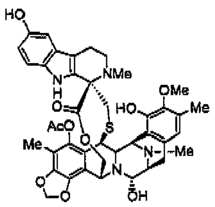

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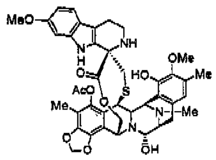
Verbindung 34:



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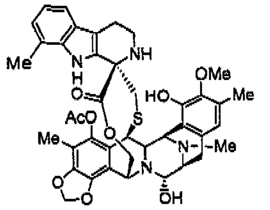

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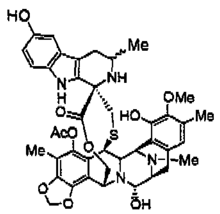

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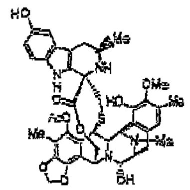

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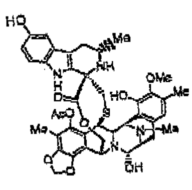

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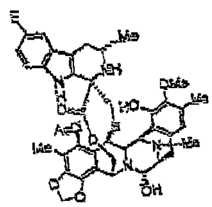
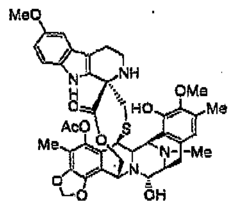
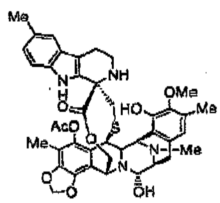


2. Verbindung nach Anspruch 1 mit der Formel (27):



27

3. Verbindung nach Anspruch 1 mit der Formel (30):



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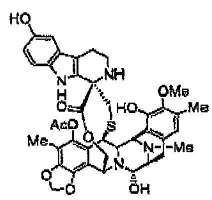
4. Verbindung nach Anspruch 1 mit der Formel (31):



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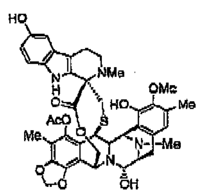
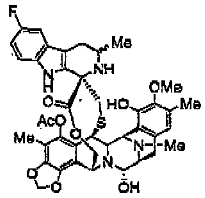
5. Verbindung nach Ansprach 1 mit der Formel (33):

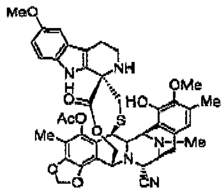


33

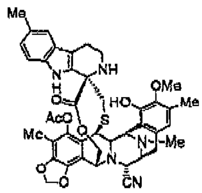
6. Verbindung nach Anspruch 1 mit der Formel :



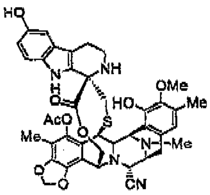
7. Verbindung nach Anspruch 1 mit der Formel (2) :



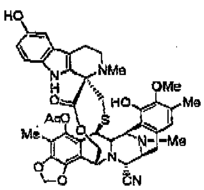
8. Verbindung nach Anspruch 1 mit der Formel (5) ;



9. Verbindung nach Anspruch 1 mit der Formel (6) :



10. Verbindung nach Anspruch 1 mit der Formel (8) ;



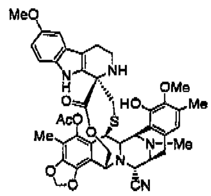
11. Eine pharmazeutische Zusammensetzung welche eine Verbindung nach einem der vorhergenden Ansprüche enthält, zusammen mit einem pharmazeutisch annehmbaren Verdünnungsmittel.

12. Verwendung einer Verbindung nach einem der vorhergehenden Ansprüche 1 bis 10 zur Herstellung eines Medikaments zur Krebsbehandlung.

Revendications

1. Un composé de formule:

Composé 2:



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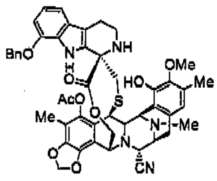
Composé 3:



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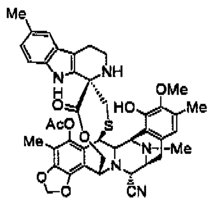
Composé 5:



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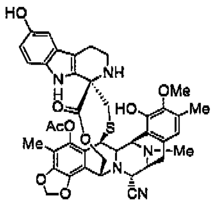
Composé 6:



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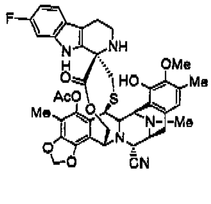
Composé 7:



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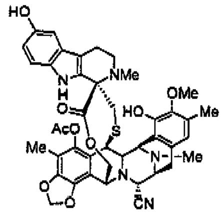
Composé 8:



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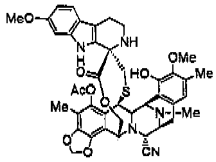
Composé 9:



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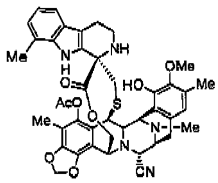
Composé 11:



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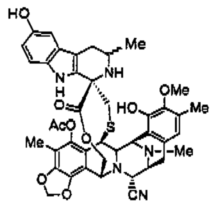

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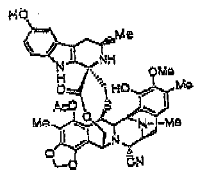

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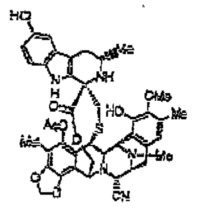

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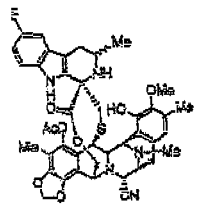

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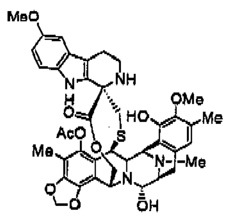
Composé 27 :



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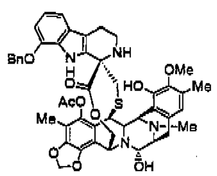
Composé 28:



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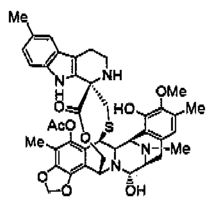

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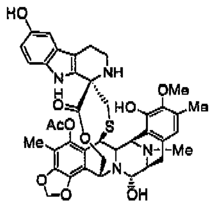
Composé 31:



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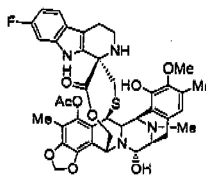

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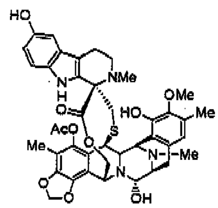

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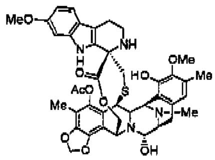

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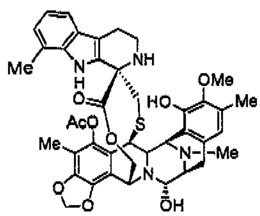

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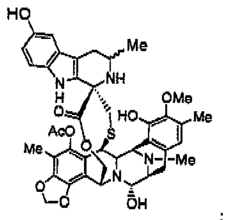

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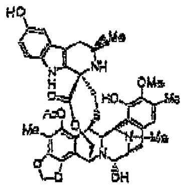

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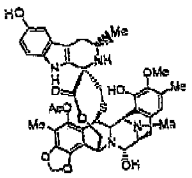

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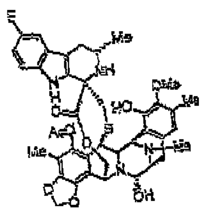

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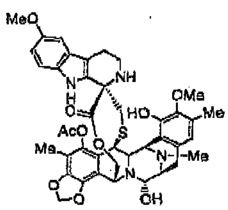
2. Composé selon la revendication 1 de formule (27) :



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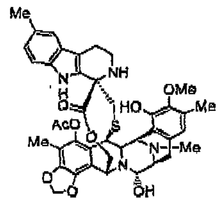
3. Composé selon la revendication 1 de formule (30) :



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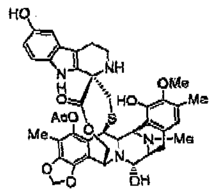
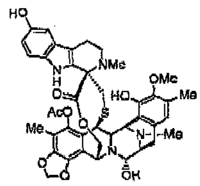
4. Composé selon la revendication 1 de formule (31) :



31

5. Composé selon la revendication 1 de formule (33) :



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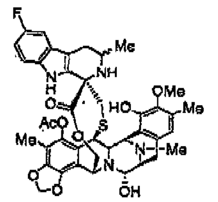
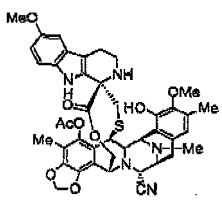
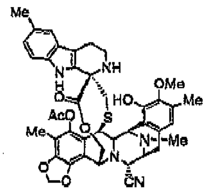
6. Composé selon la revendication 1 de formule :



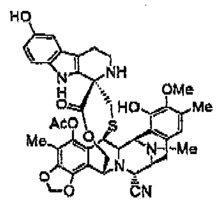
7. Composé selon la revendication 1 de formule (2) :



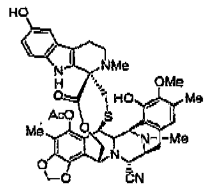
8. Composé selon la revendication 1 de formule (5) :



9. Composé selon la revendication 1 de formule (6) :



10. Composé selon la revendication 1 de formule (8) :



11. Composition pharmaceutique contenant un composé selon l'une quelconque des revendications précédentes avec un diluant pharmaceutiquement acceptable.

12. Utilisation d'un composé selon l'une quelconque des revendications 1 à 10 dans la préparation d'un médicament pour le traitement du cancer.

REFERENCES CITED IN THE DESCRIPTION

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