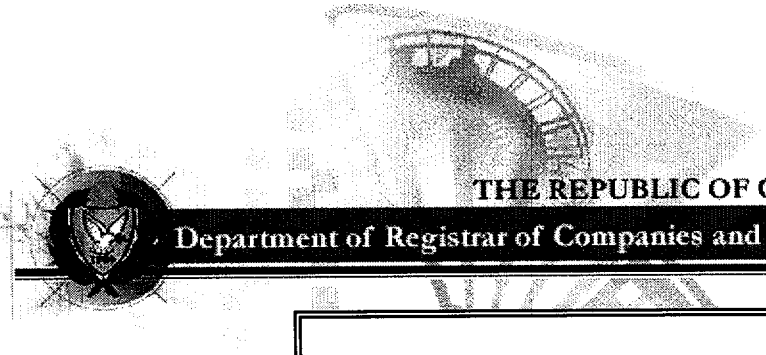

ΚΥΠΡΙΑΚΟ ΓΡΑΦΕΙΟ ΔΙΠΛΩΜΑΤΩΝ ΕΥΡΕΣΙΤΕΧΝΙΑΣ

THE PATENT OFFICE OF CYPRUS

ΑΡΙΘΜΟΣ ΔΗΜΟΣΙΕΥΣΗΣ **CY1625**
PUBLICATION NUMBER

ΑΡΙΘΜΟΣ ΔΗΜΟΣΙΕΥΣΗΣ
ΓΡΑΦΕΙΟΥ ΔΙΠΛΩΜΑΤΩΝ ΕΥΡΕΣΙΤΕΧΝΙΑΣ
ΗΝΩΜΕΝΟΥ ΒΑΣΙΛΕΙΟΥ
UK PATENT OFFICE
PUBLICATION NUMBER GB2207674

Το έγγραφο που παρουσιάζεται πιο κάτω καταχωρήθηκε στο «Γραφείο Διπλωμάτων Ευρεσιτεχνίας» στην Αγγλία σύμφωνα με το Νόμο Κεφ. 266 πριν την 1η Απριλίου 1998. Δημοσίευση έγινε μετέπειτα από το Γραφείο Διπλωμάτων Ευρεσιτεχνίας του Ηνωμένου Βασιλείου μόνο στην Αγγλική γλώσσα.

The document provided hereafter was filed at "The Patent Office" in England under the law CAP.266 before the 1st of April 1998. It was published afterwards by the UK patent office only in English.

(12) UK Patent Application (19) GB (11) 2 207 674 (13) A

(43) Application published 8 Feb 1989

(21) Application No 8818462

(22) Date of filing 3 Aug 1988

(30) Priority data
(31) 81493 (32) 4 Aug 1987 (33) US
199731 27 May 1988

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(51) INT CL⁴
C07H 17/04 A61K 31/70

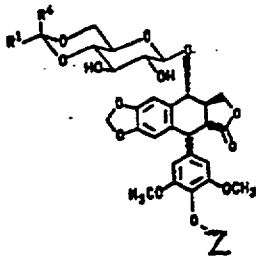
(52) Domestic classification (Edition J):
C2P 1L1 1L2 2E13 2E15A 2E18A 2E18B 2E19C 2E19D 2E19E 2E24 2E25A 2E25B 2E26A 2E26B 2E26C 2E26D 2L13 2L18A 2L18B 2L19C 2L19F 2L25A 2L25B 2L26D 2L30B 2L30C 3B13 3B15A 3B16 3B18B 3B18C 3B18D 3B19C 3B19D 3B19E 3C13 3C19C 3C19F 3C28 3C29A 5B 7 9 A1 A U1S 1313 C2P

(56) Documents cited
US 3524844 US 3408441

(58) Field of search
C2P

(54) Podophyllotoxin derivatives

(57) Phosphorus-containing derivatives of 4'-demethylpipodophyllotoxin glucosides, some of which are *antitumor agents* and offer the pharmaceutical advantage of high water solubility, have the formula:-



wherein
R^6 is H and R^1 is (C_{1-10}) alkyl; (C_{2-10}) alkenyl; (C_{5-6}) cycloalkyl; 2-furyl; 2-thienyl; (C_{6-10}) aryl; (C_{7-14}) aralkyl; or (C_{8-14}) aralkenyl wherein each of the aromatic rings may be unsubstituted or substituted with one or more groups selected from halo, (C_{1-8}) alkyl, (C_{1-8}) alkoxy, hydroxy, nitro, and amino; or R^1 and R^6 are each (C_{1-8}) alkyl; or R^1 and R^6 and the carbon to which they are attached join to form a (C_{5-6}) cycloalkyl group; and Z is a group linked via phosphorus.

GB 2 207 674 A

DESCRIPTION

2207674

PODOPHYLLOTOXIN DERIVATIVES

BACKGROUND OF THE INVENTION

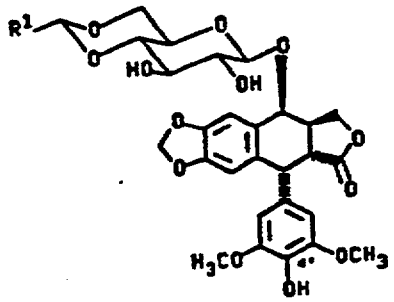
I. Field of The Invention

The present invention relates to 4'-phosphate derivatives of epipodophyllotoxin glucosides, to their antitumor use, and to pharmaceutical compositions containing these new agents.

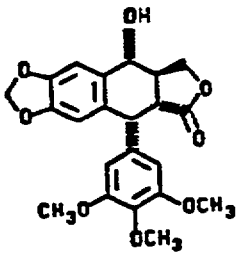
II. Description of the Prior Art

Etoposide (VP-16, I) and teniposide (VM-26, II) are clinically useful anticancer agents derived from the naturally occurring lignan, podophyllotoxin (III); the class of compounds including etoposide and teniposide is sometimes referred to as 4'-demethylepipodophyllotoxin glucosides. Etoposide and teniposide are active in the treatment of a variety of cancers including testicular, small cell lung, ovarian, breast, thyroid, bladder, brain, non-lymphocytic leukemia, and Hodgkin's disease.

Compounds I and II, and the method for producing them are disclosed in U.S. Patent 3,408,441 to Wartburg et al and U.S. Patent 3,524,844 to Keller-Juslen et al. The compounds disclosed therein, in particular etoposide and teniposide, serve as starting material for our preparation of epipodophyllotoxin glucoside 4'-phosphate derivatives of the present invention.



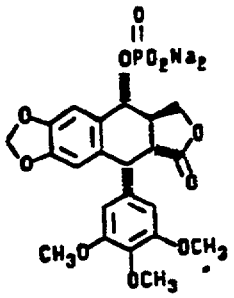
I R^1=CH_3
II R^1=2-thienyl



III

Phosphorylation of therapeutic agents containing a hydroxyl group has been used as a means for drug latentiation; the phosphorylated derivatives may then be cleaved <u>in vivo</u> by a phosphatase to liberate the active parent molecule. A brief discussion of phosphates as potential prodrugs is included in the review article entitled "Rational for Design of Biologically Reversible Drug Derivatives: Prodrugs" (Sinkula and Yalkowsky, J. Pharm. Sci., 1975, 64:181-210 at 189-191). Examples of phosphates of known antitumor agents include camptothecin (Japan Kokai 21-95,394 and 21-95,393, Derwent Abst. No. 87-281016 and 87-281015, respectively) and daurorubicin (U.S. Patent 4,185,111).

Podophyllotoxin phosphate disodium salt <u>IV</u> was prepared by Seligman <u>et al</u>. However, the phosphate was not hydrolyzed by prostatic acid phosphatase and did not show reduced toxicity over the parent podophyllotoxin (Cancer Chemotherapy Reports Part I, 1975, 59:233-242).

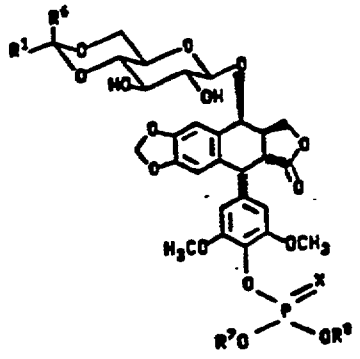


IV

The present invention provides phosphate esters of 4'-demethylepipodophyllotoxin glucosides which are active antitumor agents. In particular, the dihydrogen phosphate of 4'-demethylepipodophyllotoxin glucosides and salts thereof are highly water-soluble thus providing a superior pharmaceutical advantage over the current therapeutic agents of this class, etoposide and teniposide, which have minimal water solubility.

SUMMARY OF THE INVENTION

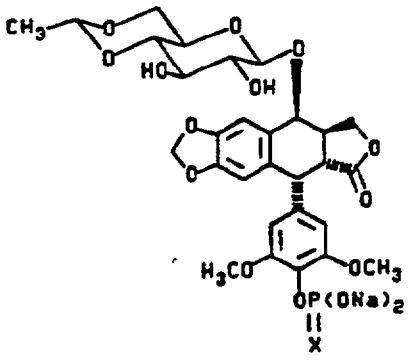
The present invention provides 4'-phosphate derivatives of 4'-demethylepipodophyllotoxin glucosides of general formula V, and pharmaceutically acceptable salts thereof



V

wherein R^6 is H and R^1 is selected from the group consisting of (C_{1-10})alkyl; (C_{2-10})alkenyl; (C_{5-6})cycloalkyl; 2-furyl; 2-thienyl; (C_{6-10})aryl; (C_{7-14})aralkyl; and (C_{8-14})aralkenyl wherein each of the aromatic rings may be unsubstituted or substituted with one or more groups selected from halo, (C_{1-8})alkyl, (C_{1-8})alkoxy, hydroxy, nitro, and amino; or R^1 and R^6 are each (C_{1-8})alkyl; or R^1 and R^6 and the carbon to which they are attached join to form a (C_{5-6}) cycloalkyl group; X is oxygen or sulfur; R^7 and R^8 are independently selected from the group consisting of H, (C_{1-5}) alkyl, A-substituted (C_{1-5})alkyl, (C_{3-6})cycloalkyl, A-substituted (C_{3-6})cycloalkyl, (C_{6-10})aryl, A-substituted aryl, alkyl-substituted aryl, (C_{7-14})aralkyl, A-substituted aralkyl, and alkyl-subsitituted aralkyl; wherein said A-substituents are one or more groups selected from hydroxy, alkoxy, alkanoyloxy, cyano, amino, alkylamino, dialkylamino, carboxy, alkylthio, mercapto, mercaptothio, nitropyridyl disulfide, alkanoylamino, alkanoyl, carbamoyl, nitro, and halo.

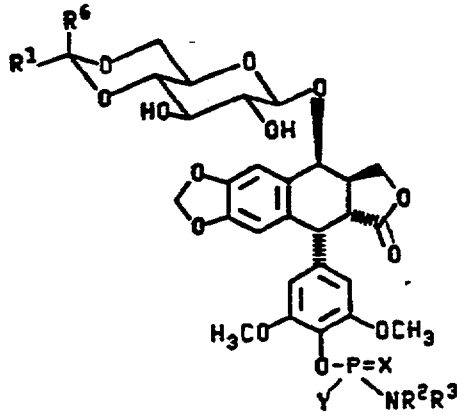
The salts of compound V include both the monoanionic and the dianionic salts. The cation may be a metal ion such as one from the alkali metal or alkaline earth metal groups or other common metal ions; or an organic nitrogen-containing group such as ammonium, mono-, di-, or trialkylammonium, or pyridinium. The cation is preferably selected from the group consisting of sodium, potassium, lithium, cesium, magnesium, calcium, aluminum, ammonium and mono-,di-, and trialkylammonium. A preferred embodiment provides compounds of formula V wherein R^7 and R^8 are both H, and pharmaceutically acceptable salts thereof. A most preferred embodiment provides etoposide 4'-dihydrogen phosphate and thiophosphate, and their respective disodium salts VIa and VIb. A further preferred embodiment provides



VI a: X = O
b: X = S

compounds of formula V wherein R^7 and R^8 are the same and are selected from the group consisting of 2,2,2-trihaloethyl, 2-cyanoethyl, (C_{1-5})alkyl, phenyl, and phenylalkyl, wherein the phenyl ring is optionally substituted with alkyl, halogen, or nitro.

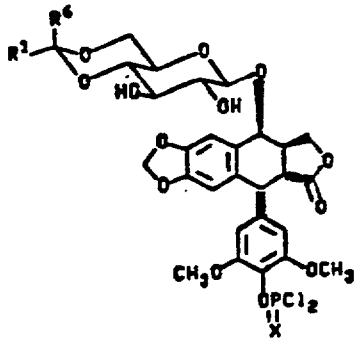
A further aspect of this invention provides antitumor phosphoroamidate derivatives of formula VII and pharmaceutically acceptable salts thereof,



VII

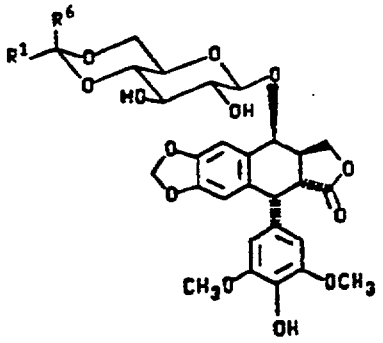
wherein R^1, R^6, and X are as previously defined; Y is Cl, OH, or NR^4R^5; R^2, R^3, R^4, and R^5 are each independently selected from the group consisting of H, (C_{1-5}) alkyl, (C_{2-5}) alkenyl, (C_{3-6}) cycloalkyl, A-substituted (C_{1-5}) alkyl, A-substituted (C_{2-5}) alkenyl, A-substituted (C_{3-6}) cycloalkyl; or R^2, R^3, and the nitrogen to which they are attached together represent a 3- to 6-membered ring; or R^4, R^5, and the nitrogen to which they are attached together represent a 3- to 6-membered ring; wherein said A-substituents are as previously defined.

Another aspect of the present invention provides dichlorophosphate intermediates of formula VIII wherein R^1, R^6 and X are as previously defined; these agents are useful in the preparation of compounds of formula V.



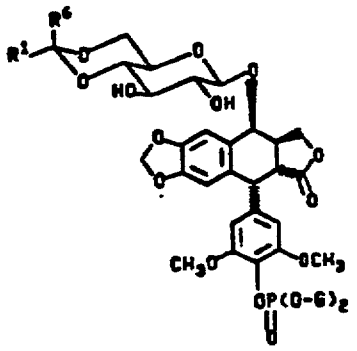
VIII

Yet a further aspect of the present invention provides a process for preparing a compound of formula V wherein R^7 and R^8 are both H and its pharmaceutically acceptable salts, which comprises the steps of (a) converting a compound of formula IX



IX

into a compound of formula X wherein R^1, R^6, and X are as previously defined and G is a phosphate protecting group; (b) removing the phosphate protecting group; and (c)



X

optionally converting the product of step (b) to a pharmaceutically acceptable salt. Phosphate protecting groups include, but are not limited to, those within the definition of R^7 given above except H.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, unless otherwise specified, the term "alkyl" means straight or branched carbon chains; "halo" includes bromo, chloro, fluoro, and iodo; "etopofos" is the compound etoposide 4'-phosphate disodium salt [i.e. compound VIa].

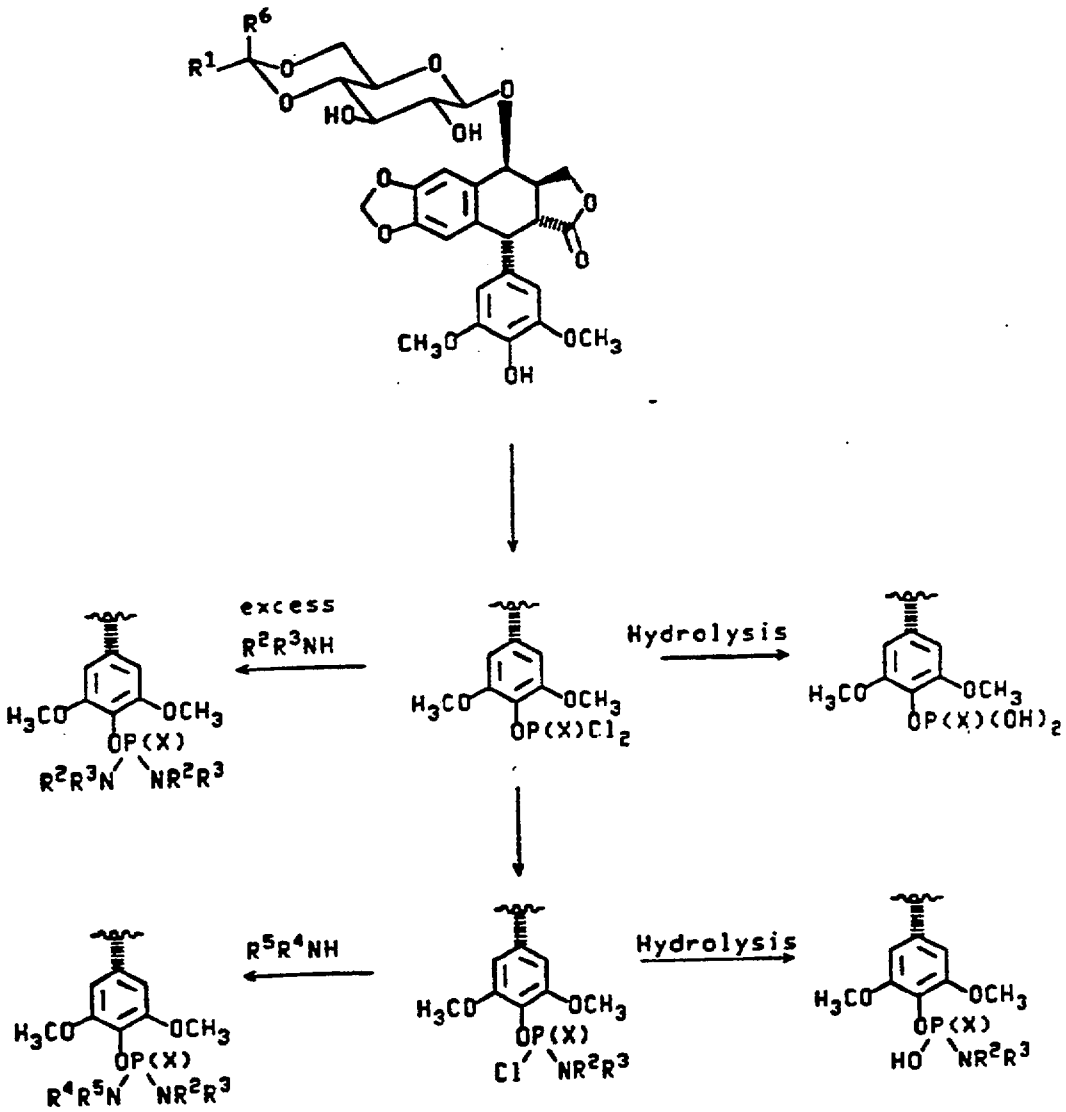
The phenol group of 4'-demethylepipodophyllotoxin glucosides may be phosphorylated with phosphorous oxychloride and thiophosphoryl chloride to give the corresponding dichlorophosphate and dichlorothiophosphate, respectively (formula VIII). The phosphorylation reaction is performed in a suitable anhydrous organic solvent, for example acetonitrile, and preferably in the presence of a tertiary amine base, for example N,N-diisopropylethylamine.

The course of the reaction may be monitored by thin layer chromatography (TLC) by which the optimum reaction time may be judged by the appearance of product or the disappearance of the starting material, or both. In our experience, the reaction period may take from about 4 hours to about 72 hours. The length of reaction time required appears to be related to the quality of the phosphorous reagent used.

The 4'-dichlorophosphates of formula VIII are versatile intermediates which may subsequently react with nucleophiles to provide a variety of phosphate and thiophosphate derivatives. Thus the intermediates may be hydrolyzed to provide the phosphates, and in the presence of a base the phosphate salts are obtained. For example, VIII treated with an excess of aqueous sodium bicarbonate solution provides the corresponding 4'-phosphate disodium and 4'-thiophosphate disodium salts; bicarbonates of other cations such as potassium and ammonium may also be used to provide the respective salts. The dichlorophosphate intermediate VIII may react with amines to afford either the corresponding phosphorodiamidate or the chlorophosphoromonoamidate. Examples of suitable amines include, but are not limited to, ammonia, primary amines such as ethylamine, chloroethylamine, allylamine, dimethylaminopropylamine, hydroxyethylamine, cyclohexylamine, and aminocyclohexanol; and secondary amines such as diethylamine, piperidine, ethylmethylamine, methylaminoethanol, ethylbutylamine, and the like. The amount of the amine used relative to that of the epidpodophyllotoxin dichlorophosphate may be adjusted so as to favor one or the other reaction product. For example, when a large excess of the amine relative to the -

epipodophyllotoxin is used, the symmetrical phosphorodiamidate is obtained, i.e. compounds of formula VII wherein Y is the same as NR^2R^3; the chlorophosphoromonoamidate, i.e. compounds of formula VII wherein Y is Cl, may be prepared when a more controlled amount of the amine is used. The chlorophosphoromonoamidate may be hydrolyzed to provide compounds of formula VII wherein Y is OH or its salts, or it may react further with a second amine to provide the unsymmetrical phosphorodiamidate, i.e. compounds of formula VII wherein Y is NR^4R^5 and is different from NR^2R^3.

The above-described procedure is illustrated in the following reaction scheme.



Phosphate triesters are compounds of formula V wherein R^7 and R^8 are not H, and they may be prepared by treating a 4'-demethylepipodophyllotoxin glucoside with a halophosphate diester, [i.e. Hal-P(X)(OR^7)(OR^8)]. It has been found that this reaction is most efficiently performed in acetonitrile in the presence of an organic trialkylamine base; the preferred base is diisopropylethylamine. At least one equivalent of the halophosphate and the amine base is used, but both reagents are preferably employed in molar equivalents in slight excess relative to that of the epipodophyllotoxin glucoside reactant. The reaction may be carried out at any temperature conducive to product formation; however, slightly elevated temperatures, e.g. 30-40°C appear to facilitate the reaction which may take up to several days to go to completion. Symmetrical halophosphate diesters [i.e. $R^7=R^8$] may be conventionally prepared from the alcohol and e.g. phosphoryl chloride, and unsymmetrical ones [i.e. $R^7 \neq R^8$] may be prepared from the alcohol and dihalophosphate ester. It is also possible to prepare phosphate triesters by other routes, for example by first converting the phenol into a phosphite ester, e.g. by reacting with a reagent such as $(PhCH_2O)_2PN(i\text{-}pr)_2$, and subsequently oxidizing the phosphate to the phosphate ester using e.g. m-chloro perbenzoic acid.

Phosphate triesters may additionally serve as intermediates in the preparation of compounds of formula V and salts thereof. Thus, for example, the dihydroxy phosphate (V, $R^7=R^8=H$) is obtained when the diphenyl ester (V, $R^7=R^8$=phenyl) is subjected to catalytic hydrogenation. Other suitable phosphate protecting groups include but are not limited to, 2,2,2-trichloroethyl, benzyl, cyanoethyl,

p-nitro substituted phenyl, benzyl, phenethyl, and p-bromophenyl. The dihydroxy phosphate (V, R^7=R^8=H) are converted to base salts by reacting with the appropriate base, e.g. sodium bicarbonate, ammonium bicarbonate or organic amines. Alternatively, the salts may also be

generated by eluting the dihydroxy phosphate through a column of an exchange resin containing the desired cation.

Although the present invention utilizes phosphorous oxychloride, halophosphate diesters, and their respective sulfur analogs as the phosphorylating reagent, it is to be understood that other phosphorous reagents capable of phosphorylating phenols may also be used, and appropriate reaction conditions and medium may be chosen according to the phosphorylating agent selected. The review article entitled "Current Methods of Phosphorylation of Biological Molecules" (Synthesis, 1977, 737-52) contains further examples of phosphorylating agents and is hereby incorporated by reference.

BIOLOGICAL PROPERTIES

Representative compounds of the present invention were evaluated for antitumor activity against transplantable murine P388 leukemia. In all experiments female CDF_1 mice implanted with a tumor inoculum of 10^6 ascites cells of P388 murine leukemia were used. In experiments using etoposide 4'-phosphate, its disodium salt, and etoposide 4'-thiophosphate disodium salt, tumor implantation and drug treatment were both via the iv route. In all other experiments tumor implant and drug treatment were via the ip route. In all cases, however, the positive control, etoposide, was administered ip. The experiments lasted 28 to 46 days at the end of which time the number of surviviors was noted. Antitumor activity is expressed as % T/C which is the ratio of the median survival time (MST) of drug-treated group to the MST of saline-treated control

group. A compound having % T/C value of 125 or greater is generally considered to have significant antitumor activity in the P388 test. Table I presents the results of the above-described evaluation; the maximum % T/C values and doses giving that effect are reported.

Table I. Antitumor Activity Against Murine P388 Leukemia.

Compound of	Dose* (mg/kg/inj)	Route	MST(d)	%T/C
TUMOR CELLS IMPLANTED INTRAVENOUSLY				
Example 1	140	IV	29.0	363
(Etoposide)	50	IP	20.5	256
Example 4	200	IP	18.0	225
(Etoposide)	100	IP	21.5	269
Example 8	125	IV	24.5	306
(Etoposide)	100	IP	29.5	369
TUMOR CELLS IMPLANTED INTRAPERITONEALLY				
Example 2	240	IP	16.5	165
(Etoposide)	60	IP	25.0	250
Example 3	200	IP	15.5	155
(Etoposide)	100	IP	27.0	270

Compound of	(mg/kg/inj)	Route	MST(d)	Dose* %T/C
Example 7	240	IP	25.0	250
(Etoposide)	100	IP	26.0	260
Example 9	150	IP	19.5	217
(Etoposide)	100	IP	24.0	267

*Drugs were administered on day 5 and 8 unless otherwise specified (day 1 being the day of tumor implantation).

The antitumor compounds of the present invention have been demonstrated to be active against transplanted tumors in experimental animals. Specifically, the compound represented by formula VIa ("etopofos") shows significantly higher antitumor activity than etoposide in the P388 test. This selective agent represents a highly water soluble pro-drug of etoposide which has reduced antitumor activity in-vitro and is rapidly cleaved by alkaline phosphatase resulting in the release of etoposide. The etoposide that is released exhibits identical cytotoxicity to the parent drug.

Accordingly, the present invention provides a method for inhibiting mammalian tumors which comprises administering an effective tumor-inhibiting dose of an antitumor compound of formula V or VII to a tumor bearing host. For this purpose, the drug may be administered by conventional routes including, but not limited to,

intravenous, intramuscular, intratumoral, intraarterial, intralymphatic, and oral.

A further aspect of the present invention provides a pharmaceutical composition which comprises a compound of formula V or VII and a pharmaceutically acceptable carrier. The antitumor composition may be made up of any pharmaceutical form appropriate for the desired route of administration. Examples of such compositions include solid compositions for oral administration such as tablets, capsules, pills, powders and granules, liquid compositions for oral administration such as solutions, suspensions, syrups or elixirs and preprations for parenteral administration such as sterile solutions, suspensions or emulsions. They may also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, physiological saline or some other sterile injectable medium immediately before use.

Optimal dosages and regimens for a given mammalian host can be readily ascertained by those skilled in the art. It will, of course, be appreciated that the actual dose used will vary according to the particular composition formulated, the particular compound used, the mode of application and the particular site, host and disease being treated. Many factors that modify the action of the drug will be taken into account including age, weight, sex, diet, time of administration, route of administration, rate of excretion, condition of the patient, drug combinations, reaction sensitivities and severity of the disease.

The following examples are for illustrative purposes only and should not be construed as limiting the scope of

the invention which is defined solely by the Claims appended to this application.

In the following examples, proton and carbon nuclear magnetic resonance (NMR) spectra (using $CDCl_3$ or D_2O as an internal reference) and phosphorous NMR spectra (using 85% aqueous H_3PO_4 as an external reference) were recorded on a Bruker WM360 spectrometer. Infrared spectra (IR) were determined on a Perkin-Elmer 1800 Fourier Transform Infrared Spectrophotometer. "Flash chromatography" refers to the method described by Still (Still, W. C.; Kahn, M.; Mitra, A.; J. Org. Chem., 1978 43, 2923) and was carried out using E. Merck silica gel (230-400 mesh). Reverse phase chromatography was carried out under a positive nitrogen pressure using C18 (Octadecylsilane) bonded to silica gel (40 - µm diameter, J. T. Baker supplier).

Example 1
Etoposide 4'-Phosphate Disodium Salt (Compound VIa)

A magnetically stirred suspension of etoposide (2.30 g, 3.91 mmol) in dry acetonitrile (210 ml) was warmed to give a nearly complete solution. The solution was allowed to cool to room temperature, and N,N-diisopropylethylamine (2.36 ml, 13.5 mmol) was added. The mixture was then cooled to 0°C and $POCl_3$ (666 mg, 4.34 mmol) was added via syringe over 30 seconds. The mixture was allowed to slowly come to room temperature over 2-3 hours and stirring continued at room temperature for 63 hours. At the end of this period 20% by volume was removed and treated with diethylamine as described in Example 2. The remainder was treated with a solution of sodium bicarbonate (6.0 g, 71.4 mmol) in

deionized H_2O (110 ml), the mixture was stirred at room temperature for 80 minutes, and then partitioned with saturated aqueous sodium bicarbonate (20 ml) deionized H_2O (125 ml), and ethyl acetate (350 ml). The organic layer was further extracted with deionized H_2O (1 x 50 ml) and the combined aqueous layers were washed with ethyl acetate (250 ml) and then subjected to a vacuum of 0.5 mm at room temperature for 1 hour to remove dissolved solvents. The aqueous portion was then applied to a 4 cm diameter column containing 15 cm of octadecylsilane bonded to silica gel which had been packed in methanol and equilibrated with H_2O. After all of the aqueous portion was applied, the column was eluted with H_2O (175 ml) to remove inorganic salts and then 4:1 H_2O: CH_3OH eluted the product. Concentration of the solvent at 0.5 torr provided 744 mg (36%) of the pure title compound as a colorless solid. Alternatively lyophilization provides the pure title compound as a very fluffy low density solid.

IR (KBr) 3426, 1775, 1593, 1505, 1486, 1337, 1239, 1191, 1122, 1078, 1034, 983, 927, 888, 876, 851, 840, 697, 684, 664, 547 cm^{-1}.

360 MHz ^1H NMR (D_2O) δ 6.93 (s,1H), 6.59 (s,1H), 6.27 (s,2H), 5.93 (d,2H), 5.09 (d,1H,J=2.8Hz), 4.83 (q,1H,J=5.0Hz), 4.68 (d,1H,J=7.9Hz), 4.62 (d,1H,J=5.7Hz), 4.47-4.35 (m,2H), 4.24 (dd,1H,J=4.4 and 10.4Hz), 3.64 (s,6H), 3.68-3.52 (m,3H), 3.44-3.30 (m,3H), 3.17-3.07 (m,1H), 1.31 (d,3H,J=5.0Hz).

90 MHz ^{13}C NMR (D_2O) δ 178.5, 151.8, 148.1, 146.1, 135.0, 132.6, 130.9, 127.4, 109.9, 109.5, 107.4, 101.3, 100.4, 99.6, 79.2, 73.7, 72.7, 72.2, 69.1, 67.1, 65.4, 55.6,

42.8, 40.3, 37.5, 18.8.

146 MHz ^{31}P NMR (D_2O) δ 3.79.

Mass spectrum (FAB), m/e; 713 ($M^+ + H$). $C_{29}H_{31}Na_2O_{16}P$ requires M^+, 712.

Anal. Calcd. for $C_{29}H_{31}Na_2O_{16}P$: C,48.89; H,4.39; Na,6.45.

Found*: C,48.72; H,4.56; Na,6.56.

*Adjusted for 8.16% H_2O determined by Karl Fischer analysis.

Example 2

Etoposide 4'-(Bis-[N,N-diethyl]phosphonamide) (VII, X=O, R^1=methyl, R^6=H, Y=$N(Et)_2$, R^2=R^3=Et)

As indicated in Example 1, 20% by volume of the reaction product mixture of etoposide and $POCl_3$ was added to diethylamine (4 mL) and stirred at room temperature for 3 hours. The solvent was evaporated in vacuo and the light orange residue purified by flash chromatography on silica gel. Elution with 4% methanol in methylene chloride provided 271.3 mg (46.9%) of the pure title compound as a light yellow solid.

IR (KBr) 3408, 2974, 2936, 2877, 1774, 1598, 1508, 1486, 1467, 1421, 1383, 1339, 1234, 1191, 1162, 1130, 1098, 1079, 1037, 902, 858, 795, 713, 700, 544 cm^{-1}.

360 MHz ^1H NMR ($CDCl_3$) δ 6.79, (s,1H), 6.50 (s,1H), 6.20 (s,2H), 5.96 (ABq,2H), 4.87 (d,1H,J=3.2Hz), 4.71 (q,1H,J=5.1Hz), 4.61 (d,1H,J=7.6Hz), 4.57 (d,1H,J=5.2Hz), 4.39 (dd,1H,J=9.1 and 10.2Hz), 4.22-4.13 (m,2H), 3.74 (m,1H), 3.65 (s,6H), 3.55 (m,1H), 3.40 (m,1H), 3.32-3.10 (m,11H), 2.94-2.83 (m,1H), 1.37 (d,3H,J=5.1Hz), 1.10 (m,12H).

146 MHz ^{31}P NMR ($CDCl_3$) δ 16.49.

Mass spectrum (FAB), m/e, 779 (M^+ + H), 573 (M^+ - sugar). $C_{37}H_{51}N_2O_{14}P$ requires M^+, 778.

Example 3
Etoposide 4'-(N,N-[2-chloroethyl]phosphoryl chloride) (VII, R^1=methyl, R^6=H, X=O, Y=Cl, R^2=R^3=CH_2CH_2Cl)

A magnetically stirred suspension of etoposide (2.00 g, 3.40 mmol) in dry acetonitrile (220 mL), was warmed to give a nearly complete solution. The mixture was cooled to room temperature and treated with N,N-diisopropylethylamine (2.05 mL, 11.8 mmol). The mixture was then cooled to 0°C under N_2 and phosphorous oxychloride (624 mg, 4.07 mmol) added by syringe over 30 seconds. The mixture was magnetically stirred at 0°C for 2.5 hours and then at room temperature for an additional 1.5 hours. Bis-(2-chlorethylamine) hydrochloride (1.82 g, 10.2 mmol) was then rapidly added followed immediately by additional N,N-diisopropylethylamine (2.10 mL, 12.0 mmol). The mixture was stirred at room temperature for 85 minutes, concentrated in vacuo to a volume of about 5 mL, and dissolved in ethyl acetate (400 mL) and methanol (5 mL). The resulting solution was washed

with pH 5 buffer (2 x 200 mL), water (150 mL), and brine (150 mL) and dried over $Na_2SO4/MgSO4$. Evaporation of the solvent gave a yellow orange solid which was purified by flash chromatography on silica gel with 3-4% methanol in methylene chloride to provide 1.25 g (45.4%) of the pure title compound as a colorless solid.

360 MHz ^1H NMR ($CDCl_3$) δ 6.82 (s,1H), 6.52 (s,1H), 6.27 (s,2H), 5.99 (d,2H), 4.90 (d,1H,J=3.4Hz), 4.73 (q,1H,J=5.0Hz), 4.65-4.60 (m,2H), 4.41 (m,1H), 4.25-4.15 (m,2H), 3.75-3.65 (m,5H), 3.72 (s,6H), 3.60-3.23 (m,9H), 2.91-2.80 (m,1H), 1.38 (d,3H,J=5.0Hz).

146 MHz ^{31}P NMR ($CDCl_3$) δ 11.16 and 10.96 (two peaks due to chiral phosphorous).

Mass spectrum (FAB), m/e, 812, 810, 808.
$C_{33}H_{39}Cl_3NO_{14}P$ requires M^+ (^{35}Cl) 809.

Example 4
Etoposide 4'-Thiophosphate Disodium Salt (Compound VIb)

A magnetically stirred suspension of etoposide (2.04 g, 3.47 mmol) in dry acetonitrile (175 mL) was warmed to give a nearly complete solution. The solution was allowed to cool to room temperature and N,N-diisopropylethylamine (2.00 mL, 11.5 mmol) was then added thereto. The mixture was then cooled to 0°C and thiophosphoryl chloride (0.720 g, 4.17 mmol) was added via syringe over a 30 second period. The mixture was allowed to slowly warm to room temperature over 2-3 hours and stirring continued at room temperature for 16 hours. The mixture was then warmed to 30-35°C and kept at

that temperature for an additional 4 hours. A major new spot of higher Rf than etoposide was observed by TLC (5% CH_3OH in CH_2Cl_2). The reaction mixture was treated with solid sodium bicarbonate (7.4 g) and then deionized H_2O (100 mL) was added. The mixture was stirred at 28-25°C for 1.5 hours and at room temperature for 1.5 hours. The mixture was partitioned with deionized H_2O (200 mL), saturated aqueous sodium bicarbonate (30 mL) and ethyl acetate (300 mL). Further workup and reverse phase chromatography was performed according to the procedure delineated in Example 1 to provide 1.03 g (40.8%) of the pure title compound as a colorless solid.

360 MHz ^1H NMR (D_2O) δ 6.93 (s,1H), 6.60 (s,1H), 6.27 (s,2H), 5.93 (d,2H), 5.09 (d,1H,J=2.8Hz), 4.83 (q,1H,J=5.0Hz), 4.68 (d,1H,J=7.8Hz), 4.63 (d,1H,J=5.7Hz), 4.47-4.35 (m,2H), 4.24 (dd,1H,J=4.3 and 10.5Hz), 3.64 (s,6H), 3.67-3.52 (m,3H), 3.47-3.29 (m,3H), 3.17-3.07 (m,1H), 1.31 (d,3H,J=5.0Hz).

Mass spectrum (FAB), m/e 728 (M^+), 706 ($M^+ + H - Na$). $C_{29}H_{31}Na_2O_{15}PS$ requires M^+, 728.

Example 5

Etoposide 4'-[[N,N-bis(2-chloroethyl)amino]-[N-(3-hydroxypropyl)amino]]phosphate (VII, X=O, R^1=methyl, R^6=H, R^2=R^3=2-chloroethyl, Y=-$NH(CH_2)_3OH$

A magnetically stirred solution of the compound of Example 3 (280 mg, 0.346 mmol) in CH_2Cl_2 (3 ml) was treated with a solution of 3-amino-1-propanol (33.5 mg, 0.446 mmol) in CH_2Cl_2 (1 ml). After 5 minutes additional

3-amino-1-propanol (31.0 mg, 0.413 mmol) in absolute methanol (0.5 ml) was added. The reaction mixture was purified by direct application to 4 preparative TLC plates (1 mm, E. Merck silica gel) which were developed using 5-8 % CH_3OH in CH_2Cl_2. Elution of the desired product band using 5% CH_3OH in ethyl acetate followed by evaporation in vacuo and then further drying at 0.1 torr provided 185 mg (63%) of the pure title compound as a colorless solid (mixture of diastereomers at phosphorus).

360 MHz ^1H NMR ($CDCl_3$) δ 7.20 (br s, 1H), 6.80 (s, 1H), 6.50 and 6.48 (2s, 1H), 6.26 and 6.25 (2s, 2H), 5.97 (d, 2H), 4.88 (m, 1H), 4.73 (q, 1H), 4.64-4.57 (m, 2H), 4.40 (m, 1H), 4.21-4.13 (m, 2H), 3.71, 3.70 (2s, 6H), 3.71-3.06 (m, 18H), 2.90-2.80 (m, 1H), 1.37 (d, 3H).

Mass Spectrum (FAB), m/e, 849, 851 ($M^+ + H$, ^{35}Cl, ^{37}Cl). $C_{36}H_{47}Cl_2N_2O_{15}P$ requires M^+ 848 (^{35}Cl) and 850 (^{37}Cl).

Example 6

Etoposide 4'-[[N,N-bis(2-chloroethyl)amino]-[N-[2-[(3-nitro-pyridyl-2--yl)disulfide]ethyl]]amino]phosphate (VII. X=O, R^1=methyl, R^6=H, R^2=R^3=2-chloroethyl, Y=$NH(CH_2)_2$-SS-(3-nitropyridyl-2-yl).

A mixture of the compound of Example 3 (248 mg, 0.306 mmol) and 2-(3-nitropyridyl)-1-(2-aminoethyl) disulfide hydrochloride (105 mg, 0.393 mmol) was treated with CH_2Cl_2 (7 ml) followed by the addition of diisopropylethylamine

(100 µl, 0.570 mmol) and dry methanol (0.5 ml). The resulting solution was stirred at room temperature for 1.5 hours and then purified by direct applicaiton to four preparative TLC plates (1 mm, E. Merck silica gel) which were developed using 4-5% CH_3OH in ethyl acetate. Elution of the desired product band using 5% CH_3OH in ethyl acetate followed by evaporation in vacuo and then further drying at 0.1 torr provided 231.7 mg (75.3%) of the pure title compound as a yellow-brown solid (mixture of diastereomers at phosphorous).

IR (KBr) 1774, 1598, 1584, 1559, 1509, 1486, 1456, 1421, 1397, 1342, 1236, 1160, 1128, 1096, 1038, 1004, 926, 857, 747, 699 cm^{-1}.

360 MHz ^1H NMR ($CDCl_3$) δ 8.81 and 8.77 (2m, 1H), 8.48 (m, 1H), 7.33 (m, 1H), 6.81 (s, 1H), 6.51 and 6.50 (2s, 1H), 6.26 (br s, 2H), 5.97 (d, 2H), 4.89 (m, 1H), 4.73 (q, 1H), 4.65-4.52 (m, 3H), 4.41 (m, 1H), 4.24-4.14 (m, 2H), 3.71, 3.70 (2s, 6H), 3.71-2.85 (m, 19 H), 2.68 (br s, 1H, OH), 2.37 (br s, 1H, OH), 1.37 (d, 3H).

Mass Spectrum (FAB), m/e, 1005, 1007 ($M^+ + H$, ^{35}Cl, ^{37}Cl). $C_{40}H_{47}Cl_2N_4O_{16}PS_2$ requires M^+, 1004 (^{35}Cl) and 1006 (^{37}Cl).

Example 7

Etoposide
4'-diphenyl phosphate ($R^1=CH_3$, $R^6=H$, $R^7=R^8=$phenyl)

A magnetically stirred suspension of etoposide (10.50 g, 17.84 mmol, dried over P_2O_5 at 80°C/0.5 torr) in dry

acetonitrile (450 ml) was treated with diisopropylethylamine (4.20 ml, 24.1 mmol) and then diphenyl chlorophosphate (2.00 ml, 9.65 mmol) was added neat via syringe. The mixture was stirred under N_2 for two hours at 50°C at which point all of the etoposide had dissolved. Additional diphenyl chlorophosphate (1.80 ml, 8.68 mmol) was added and the reaction mixture was held at 45°C for 72 hours. After more of the amine base (0.75 ml, 4.3 mmol) and diphenyl chlorophosphate (0.80 ml, 3.86 mmol) were added, the mixture was stirred at 40-45°C for 27 hours, treated with more diphenyl chlorophosphate (0.40 ml, 1.93 mmol), and maintained at 40-45°C for 22 hours. Isopropanol (20 ml) was then added, the solvent was evaporated <u>in vacuo</u>, and the solid residue was dissolved in CH_2Cl_2 (500 ml), and partitioned with H_2O (400 ml). The aqueous layer was further extracted with CH_2Cl_2 (100 ml) and the combined organic extracts were washed with brine (250 ml) and dried ($Na_2SO_4/MgSO_4$). Rotary evaporation followed by flash chromatography on silica gel using 2-3% CH_3OH in CH_2Cl_2 provided 12.50 g (85%) of the pure title compound as a colorless solid.

FAB MS m/e (relative intensity) 820 $(M+H)^+$.

IR (KBr) 3460, 2925, 1775, 1601, 1490 cm^{-1}.

^1H NMR ($CDCl_3$) δ 7.28 (m,8H), 7.15 (m,2H), 6.78 (s,1H), 6.47 (s,1H), 5.95 (m,2H), 4.85 (d,J=3.5Hz,1H), 4.71 (m,1H), 4.60 (d,J=7.6Hz,1H), 4.56 (d,J=5.1Hz,1H), 4.38 (m,1H), 4.22-4.13 (m,2H), 3.72-3.60 (m,1H), 3.48 (s,6H), 3.54-3.28

(m,3H), 3.23 (dd,J=14.2,5.3Hz,1H), 2.78 m,1H), 1.35 (d,J=5.1Hz,3H).

Anal. Calcd. for $C_{41}H_{41}O_{16}P$: C,60.00; H,5.04. Found: C,60.20; H,5.16.

Example 8

Etoposide 4'-phosphate (V; R^1=CH_3; R^6=H, R^7=R^8=H)

Platinum oxide (0.198 g, 0.87 mmol) from a freshly opened bottle (Aldrich Chemical Co.) was added to a soulution of etoposide 4'-diphenyl phosphate (product of Example 7; 0.79 g, 0.962 mmol) in 95 mL of absolute ethanol. The solution was hydrogenated on a Parr apparatus under 45-50 PSI for 4 h at room temperature. The reaction mixture was filtered through a pad of celite using ethanol as eluent. Concentration in vacuo and drying over P_2O_5 for 14 h in vacuo provided the desired product as a white solid (0.627,94%):

FAB MS m/e (relative intensity) 669 $(M+H)^+$

IR (KBr) 3440, 2930, 1778, 1604, 1498 cm^{-1}.

^1H NMR (DMSO-d_6) δ 6.93 (s,1H), 6.46 (s,1H), 6.12 (s,2H), 5.94 (m,2H), 5.17 (bs,1H), 4.86 (d,J=3.93Hz,1H), 4.64 (q,J=7.5,5.8Hz,1H), 4.51-4.42 (m,2H), 4.20 (d,J=10.7Hz,1H), 4.01 (dd,J=12.1,5.3Hz,1H), 3.51 (s,6H), 3.51-2.75 (m,7H), 2.83 (m,1H), 1.16 (d,J=5.1Hz,3H).

^{13}C NMR (DMSO-d_6) δ 174.5, 151.2, 151.1, 147.7, 146.2, 126.1, 132.3, 128.8, 109.8, 109.7, 107.9, 101.5, 101.2, 98.5, 80.0, 74.3, 72.7, 71.7, 67.6, 67.2, 65.7, 55.8, 43.0, 37.1, 20.2, 18.5.

Anal. Calcd. for $C_{29}H_{33}O_{16}P$. 0.85% H_2O: C,50.95; H, 5.11. Found: C,51.42; H,4.97.

Example 9

Etoposide 4'-bis(2,2,2-trichlorethyl)phosphate (VIII; $R^6=CH_3$, $R^1=H$, $R^7=R^8=CH_2CCl_3$)

The procedure described in Example 7 was repeated using bis(2,2,2-trichloroethyl)chlorophosphate to provide the title compound in 100% yield as a colorless solid following flash chromatography on silica gel.

IR (KBr) 1780, 1610, 1490, 1415, 1345, 1240, 1040, 960, 725 cm^{-1}.

300 MHz ^1H NMR ($CDCl_3$) δ 6.81 (s,1H), 6.49 (s,1H), 6.27 (s,2H), 5.98 (dd,2H), 4.88 (d,1H,J=3.4Hz), 4.82-4.70 (m,5H), 4.64 (d,1H,J=7.6Hz), 4.61 (d,1H,J=5.3Hz), 4.41 (dd,1H), 4.25-4.13 (m,2H), 3.75 (m,1H), 3.73 (s,6H), 3.56 (m,1H), 3.43 (dd,1H), 3.34-3.24 (m,3H), 2.91-2.82 (m,1H), 1.38 (d,3H,J=4.9Hz).

mass spectrum (FAB), m/e = 928.9848 (M^++H). $C_{33}H_{36}Cl_6O_{16}P$ requires 928.9872.

Example 10

Etoposide 4'-phosphate disodium salt from etoposide 4'-phosphate

Method A

Commercial Dowex 50 x 8-100 cation exchange resin in the hydrogen form (20 g, Aldrich Chemical Co.) was treated with excess 1N NaOH. The resulting resin in Na^+ form was packed into a 2 cm column and equilibrated with water. Etoposide 4'-phosphate (product of Example 8, 1.25 g, 1.87 mmol) dissolved in 25 ml of deionized water was applied to the top of the packed column and the column was eluted with water. Fractions containing the title compound were pooled, filtered, and lyophilized to yield 1.15 g of the title compound as a white and fluffy material.

Method B

To 2.90 g (4.34 mmol) of crude etoposide 4'-phosphate (product of Example 8) was added deionized water (50 ml) and sodium bicarbonate (3.00 g, 35.7 mmol). The mixture was stirred at room temperature for 30 minutes during which time CO_2 evolution ceased. The mixture was then chromatographed as described in Example 1. Elution with deionized water (300 ml) and then 4:1 H_2O/CH_3OH provided 1.90 g (61%) of pure title compound as a fluffy white solid following lyophilization.

Example 11

The general procedure described in Example 2 is repeated with the exception that the diethylamine used therein is replaced by the amines listed below to provide the corresponding etoposide 4'-phosphorodiamidates.

Amine	Compound VII (X=O, R^1=methyl, R^6=H, Y=NR^2R^3)	
	R^2	R^3
propylamine	H	$CH_2CH_2CH_3$
ethanolamine	H	CH_2CH_2OH
methoxyethylamine	H	$CH_2CH_2OCH_3$
N-acetylethylenediamine	H	$CH_2CHNC(O)CH_3$
2-methylallylamine	H	$CH_2CH(CH_3)=CH_2$
allylamine	H	$CH_2CH=CH_2$
dimethylaminopropylamine	H	$(CH_2)N(CH_3)_2$
N-methylethylenediamine	H	$CH_2CH_2NCH_3$
trifluoroethylamine	H	CH_2CF_3
2-aminoethanethiol	H	CH_2CH_2SH
cyclohexylamine	H	cyclohexyl
2-amino-1-methoxypropane	H	$CH(CH_3)CH_2OCH_3$
2-(ethylthio)-ethylamine	H	$CH_2CH_2SCH_2CH_3$
chloroethylamine	H	CH_2CH_2Cl
4-aminocyclohexanol	H	

Amine	Compound VII (X=O, R^1=methyl, R^6=H, Y=NR^2R^3)	
	R^2	R^3
ethylmethylamine	CH_3	CH_2CH_3
ethylbutylamine	CH_2CH_3	$(CH_2)_3CH_3$
methylaminoethanol	CH_3	CH_2CH_2OH
bis(2-chloroethyl)amine	CH_2CH_2Cl	CH_2CH_2Cl
2-propylaminoethanol	$CH_2CH_2CH_3$	CH_2CH_2OH
3-methylaminopropionitrile	CH_3	CH_2CH_2CN
piperidine	R^2+R^3 =	$-(CH_2)_5-$

Example 12

The general procedure described in Example 3 is repeated with the exception that the bis(2-chloroethyl)amine used there is replaced by the amines listed below to provide the corresponding etoposide chlorophoroamidates.

Amine	Compound VII (X=O, R^1=methyl, R^6=H, Y=Cl)	
	R^2	R^3
propylamine	H	$CH_2CH_2CH_3$
ethanolamine	H	CH_2CH_2OH
methoxyethylamine	H	$CH_2CH_2OCH_3$
N-acetylethylenediamine	H	$CH_2CHNC(O)CH_3$
2-methylallylamine	H	$CH_2CH(CH_3)=CH_2$
allylamine	H	$CH_2CH=CH_2$
dimethylaminopropylamine	H	$(CH_2)N(CH_3)_2$

Amine	Compound VII (X=O, R^1=methyl, R^6=H, Y=Cl)	
	R^2	R^3
N-methylethylenediamine	H	$CH_2CH_2NCH_3$
Trifluoroethylamine	H	CH_2CF_3
2-aminoethanethiol	H	CH_2CH_2SH
cyclohexylamine	H	cyclohexyl
2-amino-1-methoxypropane	H	$CH(CH_3)CH_2OCH_3$
2-(ethylthio)-ethylamine	H	$CH_2CH_2SCH_2CH_3$
chloroethylamine	H	CH_2CH_2Cl
4-aminocyclohexanol	H	4-OH cyclohexyl
ethylmethylamine	CH_3	CH_2CH_3
ethylbutylamine	CH_2CH_3	$(CH_2)_3CH_3$
methylaminoethanol	CH_3	CH_2CH_2OH
diethylamine	CH_2CH_3	CH_2CH_3
2-propylaminoethanol	$CH_2CH_2CH_3$	CH_2CH_2OH
3-methylaminopropionitrile	CH_3	CH_2CH_2CN
piperidine	R^2+R^3 =	$-(CH_2)_5-$

Example 13

The general procedure in Example 5 is repeated with the exception that the 3-aminopropanol used therein is replaced by the following amines to provide the corresponding unsymmetrical etoposide phosphorodiamidates.

Amine	Compound VII (X=O, R^1=methyl, R^6=H, Y=NR^4R^5, R^2=R^3=CH_2CH_2Cl)	
	R^4	R^5
propylamine	H	$CH_2CH_2CH_3$
ethanolamine	H	CH_2CH_2OH
methoxyethylamine	H	$CH_2CH_2OCH_3$
N-acetylethylenediamine	H	$CH_2CHNC(O)CH_3$
2-methylallylamine	H	$CH_2CH(CH_3)=CH_2$
allylamine	H	$CH_2CH=CH_2$
dimethylaminopropylamine	H	$(CH_2)N(CH_3)_2$
N-methylethylenediamine	H	$CH_2CH_2NCH_3$
trifluoroethylamine	H	CH_2CF_3
2-aminoethanethiol	H	CH_2CH_2SH
cyclohexylamine	H	cyclohexyl
2-amino-1-methoxypropane	H	$CH(CH_3)CH_2OCH_3$
2-(ethylthio)-ethylamine	H	$CH_2CH_2SCH_2CH_3$
chloroethylamine	H	CH_2CH_2Cl
4-aminocyclohexanol	H	4-OH cyclohexyl
ethylmethylamine	CH_3	CH_2CH_3
ethylbutylamine	CH_2CH_3	$(CH_2)_3CH_3$
methylaminoethanol	CH_3	CH_2CH_2OH
bis(2-chloroethyl)amine	CH_2CH_2Cl	CH_2CH_2Cl
2-propylaminoethanol	$CH_2CH_2CH_3$	CH_2CH_2OH
3-methylaminopropionitrile	CH_3	CH_2CH_2CN
piperidine	R^2+R^3 =	$-(CH_2)_5-$

Example 14

The general procedure described in Example 7 is repeated with the exception that the diphenyl chlorophosphate used

therein is replaced with the chlorophosphates listed below to provide the corresponding etoposide 4'-phosphate diesters (X=O, R^1=methyl, R^6=H, R^7=R^8=R described below).

chlorophosphates [$(RO)_2P(O)Cl$]

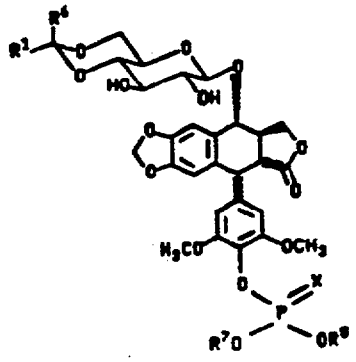
R = methyl
ethyl
benzyl
p-nitrobenzyl
p-nitrophenyl
p-bromobenzyl
p-nitrophenethyl
cyanoethyl
o-(t-butyl)phenyl

Example 15

The general procedures described in Examples 1 to 16 are repeated with the exception that the etoposide starting materials used therein are replaced with the corresponding teniposide compounds to provide the corresponding teniposide products.

CLAIMS

1. A compound having the formula



V

wherein
R^6 is H and R^1 is selected from the group consisting of (C_{1-10})alkyl; (C_{2-10})alkenyl; (C_{5-6})cycloalkyl; 2-furyl; 2-thienyl; (C_{6-10})aryl; (C_{7-14})aralkyl; and (C_{8-14})aralkenyl wherein each of the aromatic rings may be unsubstituted or substituted with one or more groups selected from halo, (C_{1-8})alkyl, (C_{1-8})alkoxy, hydroxy, nitro, and amino; or R^1 and R^6 are each (C_{1-8})alkyl; or R^1 and R^6 and the carbon to which they are attached join to form a (C_{5-6}) cycloalkyl group;

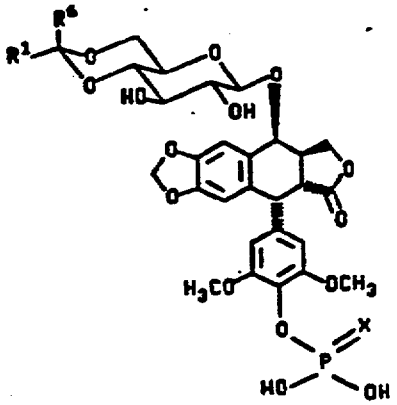
X is oxygen or sulfur;

R^7 and R^8 are independently selected from the group consisting of H, (C_{1-5}) alkyl, A-substituted (C_{1-5})alkyl, (C_{3-6})cycloalkyl, A-substituted (C_{3-6})cycloalkyl, (C_{6-10})aryl, A-substituted aryl, alkyl-substituted aryl, (C_{7-14})aralkyl, A-substituted aralkyl, and alkyl-subsitituted

aralkyl; wherein said A-substituents are one or more groups selected from hydroxy, alkoxy, alkanoyloxy, cyano, amino, alkylamino, dialkylamino, carboxy, alkylthio, mercapto, mercaptothio, nitropyridyl disulfide, alkanoylamino, alkanoyl, carbamoyl, nitro, and halo;

or a pharmaceutically acceptable salt thereof.

2. The compound of Claim 1 having the formula



wherein R^1, R^6 and X are as previously defined; or a pharmaceutically acceptable salt thereof.

3. The compound of Claim 1 wherein R^6 is H and R^1 is methyl or 2-thienyl.

4. The compound of Claim 2 wherein R^6 is H and R^1 is methyl or 2-thienyl.

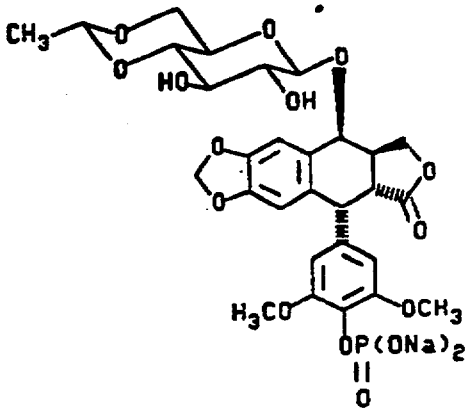
5. The compound of Claim 2 wherein R^6 is H and R^1 is methyl.

6. The compound of Claim 5 wherein X is oxygen.

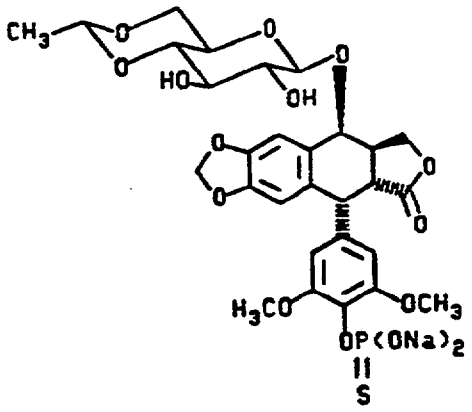
7. The compound of Claim 5 wherein X is sulfur.

8. The compound of any preceding claim wherein the said salt is the sodium salt.

9. The compound etoposide 4'-phosphate disodium salt.



10. The compound etoposide 4'-thiophosphate disodium salt.



11. The compound of claim 1 wherein R7 is selected from the group consisting of (C_{1-5})alkyl; A-substituted (C_{1-5})alkyl; (C_{3-6}) cycloalkyl; A-substituted (C_{3-6}) cycloalkyl; (C_{6-10})aryl; A-substituted aryl; alkyl-substituted aryl; (C_{1-14})aralkyl; A-substitued aralkyl; and alkyl-substituted aralkyl and R^8 is H or a group within the definition of R^7; wherein the A substituents are as previously defined; or a pharmaceutically acceptable salt thereof.

12. The compound of Claim 11 wherein R^6 is H and R^1 is methyl or 2-thienyl.

13. The compound of Claim 12 wherein R^7 and R^8 are independently selected from (C_{1-5})alkyl; halo-substituted (C_{1-5})alkyl; cyano-substituted (C_{1-5})alkyl; (C_{6-10})aryl; and (C_{7-14})aralkyl; wherein the ring portion of said aryl and arylakyl groups is optionally substituted with a group selected from alkyl, halo, and nitro.

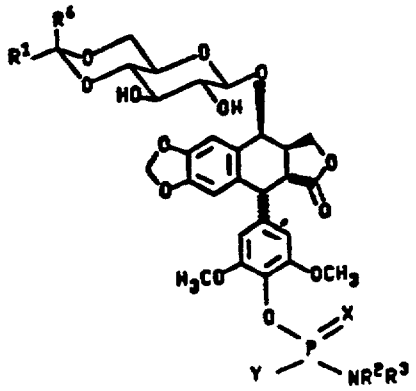
14. The compound of Claim 13 wherein R^1 is methyl.

15. The compound of Claim 14 wherein X is oxygen.

16. The compound of Claim 15 wherein R^7 and R^8 are each phenyl.

17. The compound of Claim 15 wherein R^7 and R^8 are each 2,2,2-trichloroethyl.

18. A compound having the formula



wherein R^1, R^6, and X are as previously defined; Y is Cl, OH, or NR^4R^5; R^2, R^3, R^4, and R^5 are each independently selected from the group consisting of H, (C_{1-5}) alkyl, (C_{2-5}) alkenyl, (C_{3-6}) cycloalkyl, A-substituted (C_{1-5}) alkyl, A-substituted (C_{2-5}) alkenyl, A-substituted (C_{3-6}) cycloalkyl; or R^2, R^3, and the nitrogen to which they are attached together represent a 3 to 6 membered ring; or R^4, R^5, and the nitrogen to which they are attached together represent a 3 to 6 membered ring; wherein said A-substituents are as previously defined; or a pharmaceuticallly acceptable salt thereof.

19. The compound of Claim 18 wherein R^6 is H; R^1 is methyl or 2-thienyl; Y is Cl or NR^4R^5; X is oxygen or sulfur; and R^2, R^3, R^4 and R^5 are independently selected from the group
consisting of H, (C_{1-5}) alkyl, halo substituted (C_{1-5}) alkyl, hydroxy substituted (C_{1-5}) alkyl, and nitropyridyl disulfide substituted (C_{1-5}) alkyl.

20. The compound of Claim 19 wherein X is oxygen.

21. The compound of Claim 20 wherein R^1 is methyl.

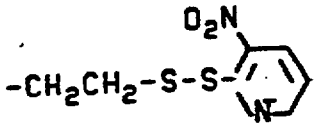
22. The compound of Claim 21 wherein R^2 and R^3 are each 2-chloroethyl; and Y is Cl.

23. The compound of Claim 21 wherein Y is NR^4R^5.

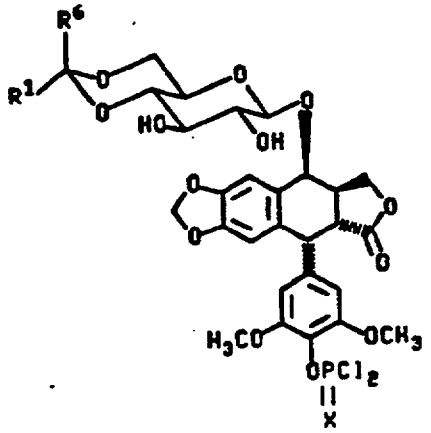
24. The compound of Claim 23 wherein R^2, R^3, R^4, and R^5 are each ethyl.

25. The compound of Claim 23 wherein R^2 and R^3 are each 2-chloroethyl; R^4 is H; and R^5 is 3-hydroxypropyl.

26. The compound of Claim 23 wherein R^2 and R^3 are each 2-chloroethyl; R^4 is H; and R^5 is



27. An intermediate having the formula



wherein R^1, R^6, and X are as previously defined.

28. The compound of Claim 27 wherein R^6 is H; R^1 is methyl; and X is oxygen.

29. The compound of Claim 27 wherein R^6 is H; R^1 is methyl; and X is sulfur.

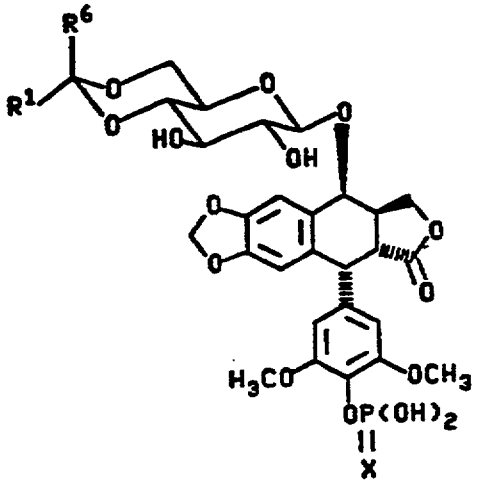
30. A pharmaceutical composition which comprises an antitumor effective amount of a compound of any of claims 1-26 and a pharmaceutically acceptable carrier.

31. A composition according to Claim 30 wherein said compound is etoposide 4'-phosphate disodium salt.

32. A method for inhibiting mammalian tumor which comprises administering to a tumor bearing host an effective antitumor amount of a compound of any of claims 1-26.

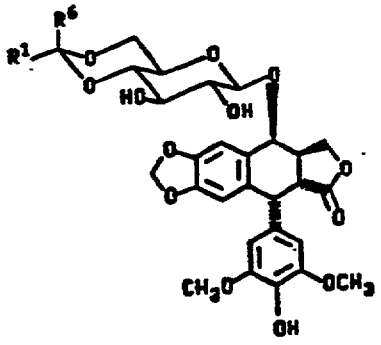
33. A method according to Claim 32 wherein said compound is etoposide 4'-phosphate disodium salt.

34. A process for preparing a compound of the formula



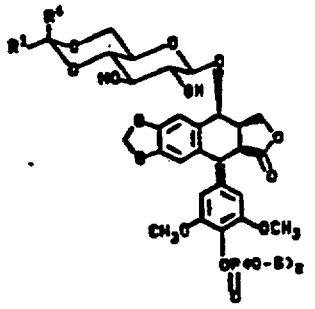
wherein R^1, R^6, and X are as previously defined or a pharmaceutically acceptable salt thereof which comprises the steps of:

(a) converting a compound of formula IX



IX

into a compound of formula X



X

wherein R^1, R^6, and X are as previously defined, and G is a phosphate protecting group.

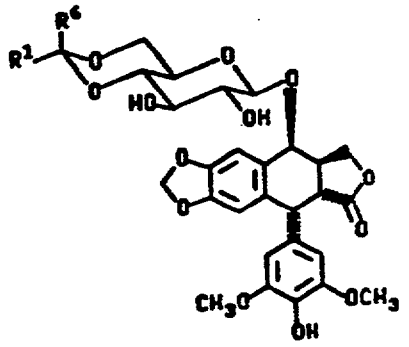
(b) removing the phosphate protecting group; and

(c) optionally converting the product of step (b) into a pharmaceutically acceptable salt.

35. The process of Claim 34 wherein said converting step comprises reacting a compound of formula IX with a compound of the formula Hal-P(X)(O-G)$_2$, wherein Hal is a halogen, and X and G are as previously defined, in acetonitrile or (C_{2-5})CN, and in the presence of a trialkylamine.

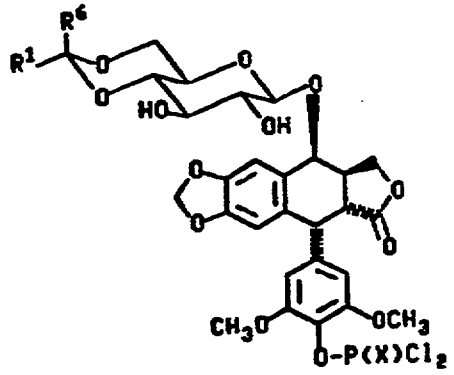
36. A process for preparing a compound of Claim 1 which comprises the steps of:

(a) reacting a compound of formula (1)



(1)

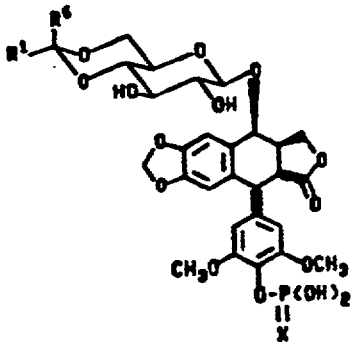
with a compound of formula $P(X)Cl_3$ wherein R^1, R^6, and X are as defined in Claim 1, and in the presence of a hydrogen acceptor to form an intermediate of formula (2)



(2)

and

(b) hydrolyzing a compound of formula (2) optionally in the presence of a base to form a compound of formula (3) or a pharmaceutically acceptable salt thereof



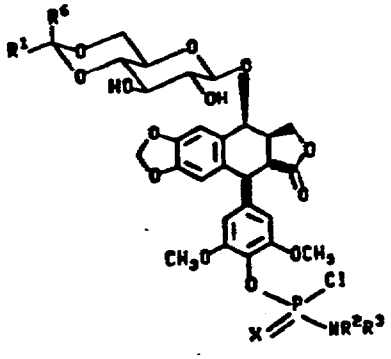
(3)

or

(c) reacting a compound of formula (2) with at least one equivalent each of R^7OH and R^8OH, wherein R^7 and R^8 are as defined in Claim 1 with the proviso that R^7 and ^{8} are not both H, and in the presence of a hydrogen acceptor; and wherein one of R^7 or R^8 is H, optionally converting the compound into a pharmaceutically acceptable salt by treatment with a base.

37. A process for preparing a compound of Claim 18 which comprises the steps of:

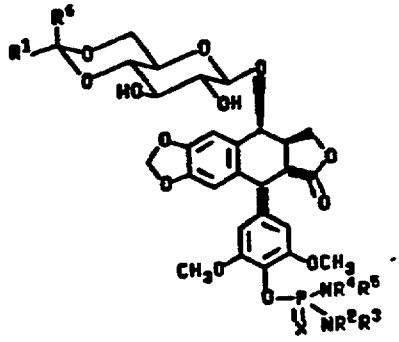
(a) reacting an intermediate of formula (2) with an amine of formula HNR^2R^3 wherein R^2 and R^3 are as defined in Claim 18, or an acid addition salt thereof to form a compound of formula (4)



(4)

and

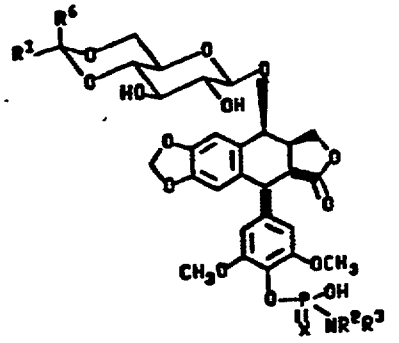
(b) optionally reacting a compound of formula (4) with a second amine of formula HNR^4R^5 wherein R^4 and R^5 are as defined in Claim 18, or an acid addition salt thereof, to form a compound of formula (5)



(5)

or

(c) hydrolyzing a compound of formula (4) optionally in the presence of a base to form a compound of formula (6) or a pharmaceutically acceptable salt thereof



(6)

—

38. A process as claimed in claim 34, 36 or 37, substantially as described in any of the foregoing Examples.

39. A compound of the respective formula specified for the product, prepared by a process as claimed in claim 34, 35, 36, 37 or 38.

40. A pharmaceutical composition which comprises an antitumor effective amount of a compound as claimed in claim 39, and a pharmaceutically acceptable carrier.

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