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(71) Applicants *(for all designated States except US)*: ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US). UNIVERSITY OF HAWAII [US/US]; Suite 280, 2800 Woodlawn Drive, Honolulu, HI 96822 (US). WAYNE STATE UNIVERSITY [US/US]; 4012 Faculty Administration Building, Detroit, MI 48202 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: NORMAN, Bryan, H. [US/US]; 8648 Admirals Bay Drive, Indianapolis, IN 46236 (US). SHIH, Chuan [US/US]; 12532 Pebblepoint Pass, Carmel, IN 46033 (US).

(74) Agents: BOUDREAUX, William, R. et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).

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(54) Title: TRIPEPTIDE AND TETRAPEPTIDE PHARMACEUTICAL COMPOUNDS

(57) Abstract

The invention provides novel cryptophycin compounds which can be useful for disrupting the microtubulin system, as antineoplastic agents, and for the treatment of cancer. The invention further provides a formulation for administering the novel cryptophycin compounds.

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Title

TRIPEPTIDE AND TETRAPEPTIDE PHARMACEUTICAL COMPOUNDS

Field Of The Invention

This invention relates to novel cryptophycin compounds useful as anti-microtubule agents.

Background Of The Invention

Neoplastic diseases, characterized by the proliferation of cells not subject to the normal control of cell growth, are a major cause of death in humans and other mammals. Clinical experience in cancer chemotherapy has demonstrated that new and more effective drugs are desirable to treat these diseases. Such clinical experience has also demonstrated that drugs which disrupt the microtubule system of the cytoskeleton can be effective in inhibiting the proliferation of neoplastic cells.

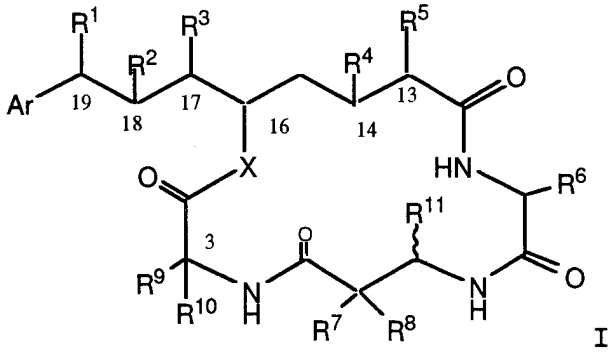
The presently claimed compounds, having an amide substitution in the cryptophycin ring, have surprisingly potent antimicrotuble activity while further having especially desired solubility properties. The compounds claimed herein address the need for compounds having acceptable solubility while retaining the desired antimicrotuble activity.

Further, such agents having the ability to disrupt the microtubule system can be useful for research purposes.

The compounds claimed herein can be prepared using total synthetic methods and are therefore well suited for development as pharmaceutically useful agents.

Summary Of The Invention

The presently claimed invention provides novel cryptophycin compounds of Formula I



I

wherein

Ar is selected from the group consisting of phenyl, any simple unsubstituted aromatic, substituted aromatic, unsubstituted heteroaromatic, and substituted heteroaromatic group;

R^1 is selected from the group consisting of halogen, SH, amino, monoalkylamino, dialkylamino, trialkylammonium, alkylthio, dialkylsulfonium, sulfate, and phosphate;

R^2 is OH or SH; or

R^1 and R^2 may be taken together with C_{18} and C_{19} to form an epoxide ring, an aziridine ring, an episulfide ring, a sulfate ring, or monoalkylphosphate ring; or

R^1 and R^2 may be taken together to form a second bond between C_{18} and C_{19};

R^3 is a lower alkyl group;

R^4 is H or H_2;

R^5 is H or H_2;

R^4 and R^5 may be taken together to form a second bond between C_{13} and C_{14};

R^6 is selected from the group consisting of benzyl, hydroxybenzyl, alkoxybenzyl, halohydroxybenzyl, dihalohydroxybenzyl, haloalkoxybenzyl, and dihaloalkoxybenzyl group;

R^7 is H or a lower alkyl group;

R^8 is H or a lower alkyl group; or
R^7 and R^8 may optionally be taken together to form a cyclopropyl ring;
R^9 is selected from the group consisting of H, a lower alkyl group, (C_1-C_3) alkylaryl, and aryl;
R^{10} is selected from the group consisting of H, a lower alkyl group, (C_1-C_3) alkylaryl, and aryl;
R^{11} is selected from the group consisting of hydrogen, simple alkyl, OH, phenyl, substituted phenyl, benzyl, and substituted benzyl;
X is O, NH or alkylamino; or
a pharmaceutically acceptable salt or solvate thereof.

The present invention provides pharmaceutical formulations, a method for disrupting a microtubulin system using an effective amount of a compound of Formula I, a method for inhibiting the proliferation of mammalian cells comprising administering an effective amount of a compound of Formula I, and a method for treating neoplasia in a mammal comprising administering an effective amount of a compound of Formula I.

Detailed Description of the Invention

As used herein, the term "simple alkyl" shall refer to C_1-C_7 alkyl wherein the alkyl may be saturated, unsaturated, branched, or straight chain. Examples include, but are in no way limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, propenyl, sec-butyl, n-pentyl, isobutyl, tert-butyl, sec-butyl, methylated butyl groups, pentyl, tert pentyl, sec-pentyl, methylated pentyl groups and the like.

As used herein, the term "substituted phenyl" shall refer to a phenyl group with from one to three non-hydrocarbon substituents which may be independently selected from the group consisting of simple alkyl, Cl, Br, F, and I.

As used herein, the term "substituted benzyl" shall refer to a benzyl group with from one to three non-

hydrocarbon substitutents which may be independently selected from the group consisting of simple alkyl, Cl, Br, F, and I.

As used herein "Lower alkoxyl group" means any alkyl group of one to five carbon atoms bonded to an oxygen atom. As used herein "lower alkyl group" means an alkyl group of one to five carbons and includes linear and non-linear hydrocarbon chains, including for example, but not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, sec-butyl, methylated butyl groups, pentyl, tert pentyl, sec-pentyl, and methylated pentyl groups. As used herein "allylically substituted alkene" means any alkene having from one to seven carbon atoms which contains an alkyl substitution on it.

As used herein "epoxide ring" means a three-membered ring whose backbone consists of two carbons and an oxygen atom. As used herein, "aziridine ring" means a three-membered ring whose backbone consists of two carbon atoms and a nitrogen atom. As used herein "sulfide ring" means a three-membered ring whose backbone consists of two carbon atoms and a sulfur atom. As used herein "episulfide ring" menas a three-membered ring whos backbone consists of two carbon and a sulfur atom. As used herein "sulfate group" menas a five membered ring consisting of a carbon-carbon-oxygen-sulfur-oxygen backbone with two additional oxygen atoms connected to the sulfur atom. As used herein, "monalkylphosphate ring" means a five membered ring consisting of a carbon-carbon-oxygen-phosphorous-oxygen backbone with two additional oxygen atoms, one of which bears a lower alkyl group, connected to the phosphorous atom.

As used herein, "simple unsubstituted aromatic group" refers to common aromatic rings having 4n+2 electrons in a moncyclic conjugated system, for example, but not limited to: furyl, pyrrolyl, thienyl, pyridyl and the like, or a bicyclic conjugated system, for example but not limited to indolyl or naphthyl.

As used herein "simple substituted aromatic group" refers to a phenyl group substituted with a single group selected from the group consisting of halogen and lower alkyl group.

As used herein the term "aryl" means an organic radical derived from an aromatic hydrocarbon by the removal of one atom; e.g., phenyl or naphthyl. Most preferably, aryl refers to C_6-C_{10} aryl, wherein the aryl ring system, including any alkyl substitutions, comprises from 6 to 10 carbon atoms.

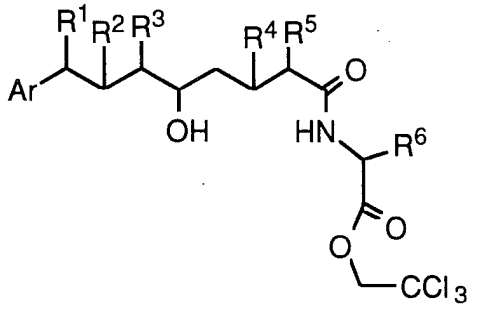
The term "C_1-C_3 alkylaryl" represents an (C_1-C_3) alkylaryl substituent wherein the alkyl group is linear, such as but not limited to, benzyl, phenethyl, 3-phenylpropyl, or phenyl-*t*-butyl; or branched. The alkylaryl moitety is attached to the parent nucleus via the alkyl group.

As used herein, "alkylamino" has its common meaning. Thus, the phrase refers to N-$R^{R'}$ wherein $R^{R'}$ is C_1-C_3 alkyl. When the alkylamino group is contained within the ring as when "X" and/or "Y" is alkylamino then such alkylamino group in the ring can be represented by the group:  .

As used herein, "heteroaromatic group" refers to aromatic rings which contain one or more non-carbon substituent selected from the group consisting of oxygen, nitrogen, and sulfur.

As used herein, "halogen" refers to those members of the group on the periodic table historically known as halogens. Methods of halogenation include, but are not limited to, the addition of hydrogen halides, substitution at high temperature, photohalogenation, etc., and such methods are known to the skilled artisan.

As used herein, the term "crypto A-B-OCH_2CCl_3" or "cryptophycin A-B-trichloroethyl ester" shall mean a group of the formula:



As used herein, the term "mammal" shall refer to the Mammalia class of higher vertebrates. The term "mammal" includes, but is not limited to, a human. The term "treating" as used herein includes prophylaxis of the named condition or amelioration or elimination of the condition once it has been established. The cryptophycin compounds claimed herein can be useful for vetrinary health purposes as well as for the treatment of a human patient.

Some preferred characteristics of this invention are set forth in the following tabular form wherein the features may be independently selected to provide preferred embodiments of this invention. The invention is in no way limited to the features described below:

A) R^8 is ethyl, propyl, isopropyl, butyl, isobutyl or isopentyl;

B) R^7 is ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, or isopentyl;

C) R^7 is H, R^8 is methyl, R^3 is methyl, and X and Y are not both O;

D) R^3 is ethyl, propyl, isopropyl, butyl, isobutyl, pentyl or isopentyl;

E) R^9 is methyl, ethyl, propyl, butyl, isobutyl, pentyl, or isopentyl;

F) R^{10} is methyl, ethyl, propyl, butyl, isobutyl, pentyl, or isopentyl;

G) a cryptophycin compound wherein at least one of the groups selected from the group consisting of C-3, C-6, C-7, C-10, C-16, C-17, and C-18 has *R*

stereochemistry (numbering as set forth in claim 1 *infra.*);

H) a cryptophycin compound wherein at least one of the groups selected from the group consisting of C-3, C-6, C-7, C-10, C-16, C-17, and C-18 has *S* stereochemistry (numbering as set forth in claim 1 *infra.*);

I) Ar is phenyl with a substituent selected from the group consisting of hydrogen, halogen, and simple alkyl;

J) a compound wherein the C-7 substituent is *R* configuration;

K) a compound wherein the C-7 substituent is *S* configuration;

L) R^7, R^8 are each hydrogen;

M) R^7 and R^8 are each selected from hydrogen or OH;

N) R^{11} is simple alkyl;

O) R is selected from the group consisting of methyl, ethyl, n-propyl, and phenyl;

P) R^1 and R^2 form an epoxide ring;

Q) both X and Y are O;

R) R^4 and R^5 form a double bond;

S) R^6 is substituted benzyl wherein one substituent is a halogen and one is an OR^{12} group wherein R^{12} is lower alkyl;

T) a compound of Formula I is used for disruption of a microtubulin system;

U) a compound of Formula I is used as an anti-neoplastic agent;

V) a compound of Formula I is used for the treatment of cancer in a mammal;

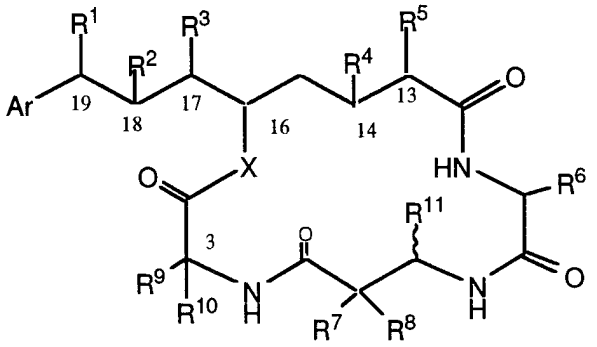
W) a compound wherein Y is selected from the group consisting of O, NH, S, SO and SO_2;

X) a compound wherein Y is C, R^7, R^8, R^9, and R^{10} are each hydrogen; and R^1 and R^2 form an epoxide;

Y) R^{10} is hydrogen; and

Z) R^3 is methyl.

Examples of some preferred compounds of this invention include, but are in no way limited to:



wherein Ar is phenyl, R^1 and R^2 taken together form a three membered epoxide ring, R^3 is methyl, R^4 and R^5 are taken together to form a double bond, R^6 is a chloro-methoxybenzyl, R^{10} is hydrogen, and the remaining variables are as illustrated in the following table:

X	**R^9**	**R^7**	**R^8**	**R^{11}***
O	isobutyl	hydrogen	hydrogen	hydrogen
O	isobutyl	hydrogen	hydrogen	methyl
O	isobutyl	hydrogen	hydrogen	benzyl
O	isobutyl	hydrogen	hydrogen	phenyl
O	isobutyl	hydrogen	hydrogen	p-chloro-phenyl
O	isobutyl	hydrogen	hydrogen	ethyl
O	isobutyl	hydrogen	hydrogen	m-methyl-benzyl
O	isobutyl	hydrogen	hydrogen	p-methyl-phenyl
O	isobutyl	R^7 and R^8 cyclo-propyl	form a	hydrogen
O	isobutyl	R^7 and R^8 cyclo-propyl	form a	methyl
O	benzyl	R^7 and R^8 cyclo-propyl	form a	benzyl
X	**R^9**	**R^7**	**R^8**	**R^{11}***
O	benzyl	hydrogen	hydrogen	hydrogen
O	benzyl	hydrogen	hydrogen	methyl

O	benzyl	hydrogen	hydrogen	benzyl
O	benzyl	hydrogen	hydrogen	phenyl
O	benzyl	hydrogen	hydrogen	p-chloro-phenyl
O	benzyl	hydrogen	hydrogen	ethyl
O	benzyl	hydrogen	hydrogen	m-methyl-benzyl
O	benzyl	hydrogen	hydrogen	p-methyl-phenyl
O	benzyl	R^7 and R^8 cyclo-propyl	form a	hydrogen
O	benzyl	R^7 and R^8 cyclo-propyl	form a	methyl
O	isobutyl	hydrogen	hydrogen	hydrogen
O	isobutyl	hydrogen	hydrogen	methyl
O	isobutyl	hydrogen	hydrogen	benzyl
O	isobutyl	hydrogen	hydrogen	phenyl
O	isobutyl	hydrogen	hydrogen	p-chloro-phenyl
O	isobutyl	hydrogen	hydrogen	ethyl
O	isobutyl	hydrogen	hydrogen	m-methyl-benzyl
O	isobutyl	hydrogen	hydrogen	p-methyl-phenyl
O	isobutyl	R^7 and R^8 cyclo-propyl	form a	hydrogen
O	isobutyl	R^7 and R^8 cyclo-propyl	form a	methyl
NH	isobutyl	hydrogen	hydrogen	hydrogen
NH	isobutyl	hydrogen	hydrogen	methyl
NCH_3	isobutyl	hydrogen	hydrogen	benzyl
NH	isobutyl	hydrogen	hydrogen	phenyl
NH	isobutyl	hydrogen	hydrogen	p-chloro-phenyl
NH	isobutyl	hydrogen	hydrogen	ethyl
NH	isobutyl	hydrogen	hydrogen	m-methyl-benzyl
NH	isobutyl	hydrogen	hydrogen	p-methyl-phenyl
NH	isobutyl	R^7 and R^8 cyclo-propyl	form a	hydrogen

X	R^9	R^7	R^8	R^{11*}
NH	isobutyl	R^7 and R^8 cyclo-propyl	form a	methyl
NH	benzyl	hydrogen	hydrogen	hydrogen
O	benzyl	hydrogen	hydrogen	methyl
O	benzyl	hydrogen	hydrogen	benzyl
O	benzyl	hydrogen	hydrogen	phenyl
O	benzyl	hydrogen	hydrogen	p-chloro-phenyl
O	benzyl	hydrogen	hydrogen	ethyl
O	benzyl	hydrogen	hydrogen	m-methyl-benzyl
O	benzyl	hydrogen	hydrogen	p-methyl-phenyl
O	benzyl	R^7 and R^8 cyclo-propyl	form a	hydrogen
O	benzyl	R^7 and R^8 cyclo-propyl	form a	methyl
O	isobutyl	methyl	hydrogen	hydrogen
O	isobutyl	methyl	hydrogen	methyl
O	isobutyl	methyl	hydrogen	benzyl
O	isobutyl	methyl	hydrogen	phenyl
O	isobutyl	methyl	hydrogen	p-chloro-phenyl
O	isobutyl	methyl	hydrogen	ethyl
O	isobutyl	methyl	hydrogen	m-methyl-benzyl
O	isobutyl	hydrogen	methyl	p-methyl-phenyl
O	isobutyl	hydrogen	methyl	hydrogen
O	isobutyl	hydrogen	methyl	methyl
O	benzyl	methyl	hydrogen	benzyl
O	benzyl	methyl	methyl	hydrogen
O	benzyl	methyl	hydrogen	methyl
O	benzyl	hydrogen	methyl	benzyl
O	benzyl	methyl	hydrogen	phenyl
O	benzyl	methyl	hydrogen	p-chloro-phenyl
O	benzyl	methyl	methyl	ethyl
O	benzyl	methyl	hydrogen	m-methyl-benzyl
O	benzyl	hydrogen	methyl	p-methyl-phenyl
O	benzyl	methyl	methyl	hydrogen
O	benzyl	methyl	methyl	methyl

X	R^9	R^7	R^8	R^{11*}
O	isobutyl	methyl	hydrogen	hydrogen
O	isobutyl	methyl	hydrogen	methyl
O	isobutyl	methyl	hydrogen	benzyl
O	isobutyl	hydrogen	methyl	phenyl
O	isobutyl	hydrogen	methyl	p-chloro-phenyl
O	isobutyl	methyl	hydrogen	ethyl
O	isobutyl	hydrogen	methyl	m-methyl-benzyl
O	isobutyl	methyl	hydrogen	p-methyl-phenyl
O	isobutyl	methyl	methyl	hydrogen
O	isobutyl	methyl	methyl	methyl
NH	isobutyl	methyl	hydrogen	hydrogen
NH	isobutyl	hydrogen	methyl	methyl
NCH_3	isobutyl	hydrogen	methyl	benzyl
NH	isobutyl	methyl	hydrogen	phenyl
NH	isobutyl	methyl	hydrogen	p-chloro-phenyl
NH	isobutyl	methyl	hydrogen	ethyl
NH	isobutyl	methyl	hydrogen	m-methyl-benzyl
NH	isobutyl	hydrogen	methyl	p-methyl-phenyl
NH	isobutyl	methyl	methyl	hydrogen
NH	isobutyl	methyl	methyl	methyl
NH	benzyl	methyl	hydrogen	hydrogen
O	benzyl	hydrogen	methyl	methyl
O	benzyl	hydrogen	methyl	benzyl
O	benzyl	methyl	hydrogen	phenyl
O	benzyl	hydrogen	methyl	p-chloro-phenyl
O	benzyl	hydrogen	methyl	ethyl
O	benzyl	hydrogen	methyl	m-methyl-benzyl
O	benzyl	hydrogen	methyl	p-methyl-phenyl
O	benzyl	methyl	methyl	hydrogen
O	benzyl	methyl	methyl	methyl

The present invention provides a method of alleviating a pathological condition caused by hyperproliferating mammalian cells comprising administering to a subject an

effective amount of a pharmaceutical or veterinary composition disclosed herein to inhibit proliferation of the cells. In a preferred embodiment of this invention, the method further comprises administering to the subject at least one additional therapy directed to alleviating the pathological condition. In a preferred embodiment of the present invention, the pathological condition is characterized by the formation of neoplasms. In a further preferred embodiment of the present invention, the neoplasms are selected from the group consisting of mammary, small-cell lung, non-small-cell lung, colorectal, leukemia, melanoma, pancreatic adenocarcinoma, central nervous system (CNS), ovarian, prostate, sarcoma of soft tissue or bone, head and neck, gastric which includes pancreatic and esophageal, stomach, myeloma, bladder, renal, neuroendocrine which includes thyroid and non-Hodgkin's disease and Hodgkin's disease neoplasms.

As used herein "neoplastic" refers to a neoplasm, which is an abnormal growth, such growth occurring because of a proliferation of cells not subject to the usual limitations of growth. As used herein, "anti-neoplastic agent" is any compound, composition, admixture, co-mixture, or blend which inhibits, eleminates, retards, or reverses the neoplastic phenotype of a cell.

Anti-mitotic agents or poisins may be classified into three groups on the basis of their molecular mechanism of action. The first group consists of agents, including colchicine and colcemid, which inhibit the formation of microtubules by sequestering tubulin. The second group consists of agents, including vinblastine and vincristine, which induce the formation of paracrystalline aggregates of tubulin. Vinblastine and vincristine are well known anticancer drugs: their action of disrupting mitotic spindle microtubules preferentially inhibits hyperproliferative cells. The third group consists of agents, including taxol, which promote the polymerization of tubulin and thus stabilizes microtubules.

The exhibition of drug resistance and multiple-drug resistance phenotype by many tumor cells and the clinically proven mode of action of anti-microtubule agents against neoplastic cells necessitates the development of anti-microtubule agents cytotoxic to non-drug resistant neoplastic cells as well as cytotoxic to neoplastic cells with a drug resistant phenotype.

Chemotherapy, surgery, radiation therpy, therapy with biological response modifiers, and immunotherapy are currently used in the treatment of cancer. Each mode of therapy has specific indications which are known to those of ordinary skill in the art, and one or all may be employed in an attempt to achieve total destruction of neoplastic cells. Moreover, combination chemotherapy, chemotherapy utilizing compounds of Formula I in combination with other neoplastic agents, is also provided by the subject invention as combination therapy is generally more effective than the use of a single anti-neoplastic agent. Thus, a further aspect of the present invention provides compositions containing a therapeutically effective amount of at least one compound of Formula I, including the non-toxic addition salts thereof, which serve to provide the above recited benefits. Such compositions can also be provided together with physiologically tolerable liquid, gel, or solid carriers, diluents, adjuvants and excipients. Such carriers, adjuvants, and excipients may be found in the U.S. *Pharmacopeia*, Vol. **XXII** and National Formulary vol **XVII**, U.S. *Pharmacopeia* Convention, Inc. Rockville, MD (1989). Additional modes of treatment are provided in AHFS Drug Information, 1993 e. by the American Hospital Formulary Service, pp. 522-660. Each of these references are well known and readily available to the skilled artisan.

The present invention further provides that the pharmaceutical composition used to treat neoplastic disease contains at least one compound of Formula I and at least one additional anti-neoplastic agent. Anti-neoplastic agents which may be utilized in combination with Formula I or

Formula III compounds include those provided in the Merck Index **11**, pp 16-17, Merck & Co., Inc. (1989). The Merck Index is widely recognized and readily available to the skilled artisan.

In a further embodiment of this invention, antineoplastic agents may be antimetabolites which may include but are in no way limited to those selected from the group consisting of methotrexate, 5-fluorouracil, 6-mercaptopurine, cytosine, arabinoside, hydroxyurea, and 2-chlorodeoxyadenosine. In another embodiment of the present invention, the anti-neoplastic agents contemplated are alkylating agents which may include but are in no way limited to those selected from the group consisting of cyclophosphamide, mephalan, busulfan, paraplatin, chlorambucil, and nitrogen mustard. In a further embodiment, the anti-neoplastic agents are plant alkaloids which may include but are in no way limited to those selected from the group consisting of vincristine, vinblastine, taxol, and etoposide. In a further embodiment, the anti-neoplastic agents contemplated are antibiotics which may include, but are in no way limited to those selected from the group consisting of doxorubicin, daunorubicin, mitomycin C, and bleomycin. In a further embodiment, the anti-neoplastic agents contemplated are hormones which may include, but are in no way limited to those selected from the group consisting of calusterone, diomostavolone, propionate, epitiostanol, mepitiostane, testolactone, tamoxifen, polyestradiol phosphate, megesterol acetate, flutamide, nilutamide, and trilotane.

In a further embodiment, the anti-neoplastic agents contemplated include enzymes which may include, but are in no way limited to those selected from the group consisting of L-Asparginase and aminoacridine derivatives such as, but not limited to, amsacrine. Additional anti-neoplastic agents include those provided by Skeel, Roland T., "Antineoplastic Drugs and Biologic Response Modifier: Classification, Use and Toxicity of Clinically Useful

Agents" Handbook of Cancer Chemotherapy (3rd ed.), Little Brown & Co. (1991).

These compounds and compositions can be administered to mammals for veterinary use. For example, domestic animals can be treated in much the same way as a human clinical patient. In general, the dosage required for therapeutic effect will vary according to the type of use, mode of administration, as well as the particularized requirements of the individual hosts. Typically, dosages will range from about 0.001 to 1000 mg/kg, and more usually 0.01 to 10 mg/kg of the host body weight. Alternatively, dosages within these ranges can be administered by constant infusion over an extended period of time, usually exceeding 24 hours, until the desired therapeutic benefits are obtained. Indeed, drug dosage, as well as route of administration, must be selected on the basis of relative effectiveness, relative toxicity, growth characteristics of tumor and effect of Formula I or Formula III compound on cell cycle, drug pharmacokinetics, age, sex, physical condition of the patient and prior treatment.

The compound of Formula I or Formula III, with or without additional anti-neoplastic agents, may be formulated into therapeutic compositions as natural or salt forms. Pharmaceutically acceptable non-toxic salts include base addition salts which may be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like. Such salts may also be formed as acid addition salts with any free cationic groups and will generally be formed with inorganic acids such as for example, hydrochloric or phosphoric acids or organic acids such as acetic, oxalic, tartaric, mandelic, and the like. Additional excipients which further the invention are provided to the skilled artisan for example in the U.S. *Pharmacopeia*.

The suitability of particular carriers for inclusion in a given therapeutic composition depends on the preferred route of administration. For example, anti-neoplastic compositions may be formulated for oral administration. Such compositions are typically prepared as liquid solution or suspensions or in solid forms. Oral formulation usually include such additives as binders, fillers, carriers, preservatives, stabilizing agents, emulsifiers, buffers, mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like. These compositions may take the form of solutions, suspensions, tablets, pills, capsules, sustained relsease formulations, or powders, and typically contain 1% to 95% of active ingedient. More preferably, the composition contains from about 2% to about 70% active ingredient.

Compositions of the present invention may be prepared as injectables, either as liquid solutions, suspensions, or emulsions; solid forms suitable for solution in or suspension in liquid prior to injection. Such injectables may be administered subcutaneously, intravenously, intraperitoneally, intramuscularly, intrathecally, or intrapleurally. The active ingredient or ingredients are often mixed with diluents, carriers, or excipients which are physiologically tolerable and compatible with the active ingredient(s). Suitable diluents and excipients are for example, water, saline, dextrose, glycerol, or the like and combinations thereof. In addition, if desired, the compositions may contain minor amounts of auxilary substances such as wetting or emulsifying agents, stabilizing or pH buffering agents.

The invention further provides methods for using Formula I compounds to inhibit the proliferation of mammalian cells by contacting these cells with a Formula I compound in an amount sufficient to inhibit the proliferation of the mammalian cell. A preferred embodiment is a method to inhibit the proliferation of

hyperproliferative mammalian cells. For purposes of this invention "hyperproliferative mammalian cells" are mammalian cells which are not subject to the characteristic limitations of growth (programmed cell death for example). A further preferred embodiment is when the mammalian cell is human. The invention further provides contacting the mammalian cell with at least one Formula I or Formula III compound and at least one anti-neoplastic agent. The types of anti-neoplastic agents contemplated are discussed *supra*.

The invention further provides methods for using a compound of Formula I to inhibit the proliferation of hyperproliferative cells with drug-resistant phenotypes, including those with multiple drug-resistant phenotypes, by contacting said cell with a compound of Formula I in an amount sufficient to inhibit the proliferation of a hyperproliferative mammalian cell. A preferred embodiment is when the mammalian cell is human. The invention further provides contacting a Formula I compound and at least one additional anti-neoplastic agent, discussed *supra*.

The invention provides a method for alleviating pathological conditions caused by hyperproliferating mammalian cells for example, neoplasia, by administering to a subject an effective amount of a pharmaceutical composition containing Formula I or Formula III compound to inhibit the proliferation of the hyperproliferating cells. As used herein "pathological condition" refers to any pathology arising from the proliferation of mammalian cells that are not subject to the normal limitations of growth. Such proliferation of cells may be due to neoplasms as discussed *supra*.

In a further preferred embodiment the neoplastic cells are human. The present invention provides methods of alleviating such pathological conditions utilizing a compound of Formula I in combination with other therapies, as well as other anti-neoplastic agents.

The effectiveness of the claimed compounds can be assessed using standard methods known to the skilled artisan.

The compounds are screened for minimum inhibitory concentrations against KB, a human nasopharyngeal carcinoma cell line, LoVo, a human colorectal adenocarcinoma cell line. The Corbett assay, see Corbett, T.H. et al. Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development, pp 35-87, Kluwer Academic Publishers: Norwell, 1992. see also, Valeriote, et al. Discovery and Development of Anticancer Agents; Kluwer Academic Publishers, Norwell, 1993.

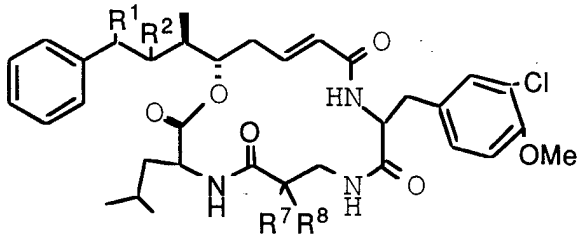
The most active compounds are further evaluated for cytotoxicity against four different cell types, for example a murine leukemia, a murine solid tumor, a human solid tumor, and a low malignancy fibroblast using the Corbett assay.

The compounds are further evaluated against a broad spectrum of murine and human tumors implanted in mice, including drug resistant tumors.

Tumor burden (T/C) (mean tumor burden in treated animals verses mena tumor burden in untreated animals) are used as a further assessment. T/C values that are less than 42% are considered to be active by National Cancer Institute Standards; T/C values less than 10% are considered to have excellent activity and potential clinical activity by National Cancer Institute standards.

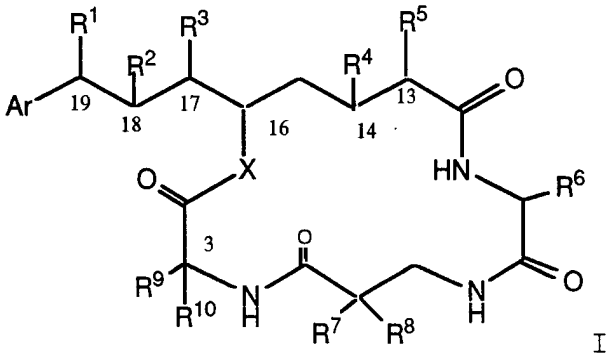
Evaluation of compounds of Formula I suggest that the compounds can be useful in the treatment methods claimed herein. Further, the compounds will be useful for disrupting the microtubule system.

The results of testing compounds of this invention using the above-described assays are as follows:



R^1	R^2	R^7	R^8	IC_{50} nM
R^1 and R^2 Together form a double bond		CH_3	CH_3	3.0
R^1 and R^2 Together form a double bond		H	H	0.77
R^1 and R^2 Together form an epoxide		CH_3	CH_3	0.014 (CCRF-CEM)
R^1 and R^2 Together form an epoxide		H	H	0.16 (CCRF-CEM)

Compounds of the Formula III may be preferred:



III

wherein

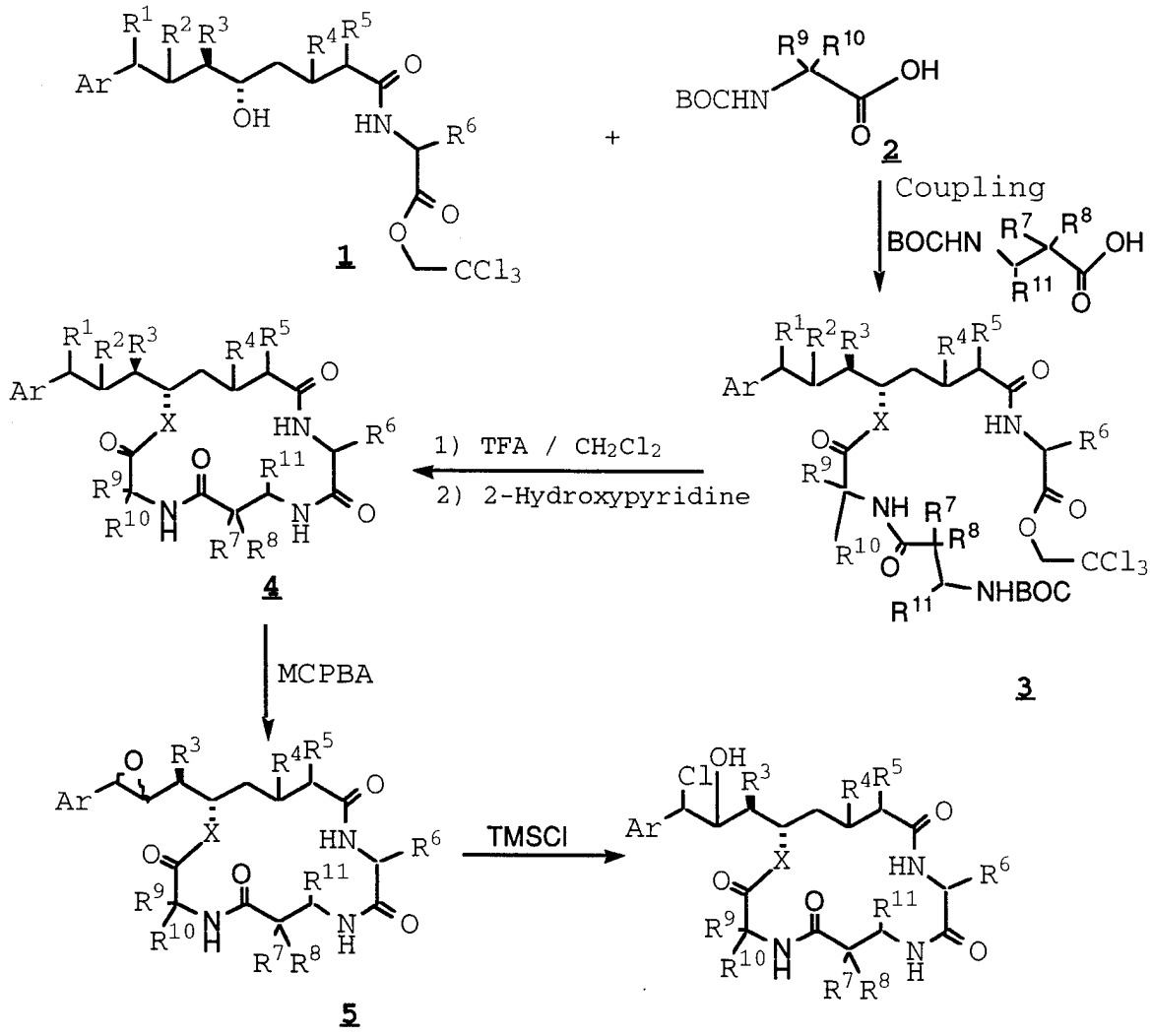
Ar is selected from the group consisting of phenyl, any simple unsubstituted aromatic, substituted aromatic, unsubstituted heteroaromatic, and substituted heteroaromatic group;

R^1 is selected from the group consisting of halogen, SH, amino, monoalkylamino, dialkylamino, trialkylammonium, alkylthio, dialkylsulfonium, sulfate, and phosphate;
R^2 is OH or SH; or
R^1 and R^2 may be taken together with C_{18} and C_{19} to form an epoxide ring, an aziridine ring, an episulfide ring, a sulfate ring, or monoalkylphosphate ring; or
R^1 and R^2 may be taken together to form a second bond between C_{18} and C_{19};
R^3 is a lower alkyl group;
R^4 is H or H_2;
R^5 is H or H_2;
R^4 and R^5 may be taken together to form a second bond between C_{13} and C_{14};
R^6 is selected from the group consisting of benzyl, hydroxybenzyl, alkoxybenzyl, halohydroxybenzyl, dihalohydroxybenzyl, haloalkoxybenzyl, and dihaloalkoxybenzyl group;
R^7 is H or a lower alkyl group;
R^8 is H or a lower alkyl group; or
R^7 and R^8 may optionally be taken together to form a cyclopropyl ring;
R^9 is selected from the group consisting of H, a lower alkyl group, (C_1-C_3) alkylaryl, and aryl;
R^{10} is selected from the group consisting of H, a lower alkyl group, (C_1-C_3) alkylaryl, and aryl;
X is O, NH or alkylamino; or
a pharmaceutically acceptable salt or solvate thereof.

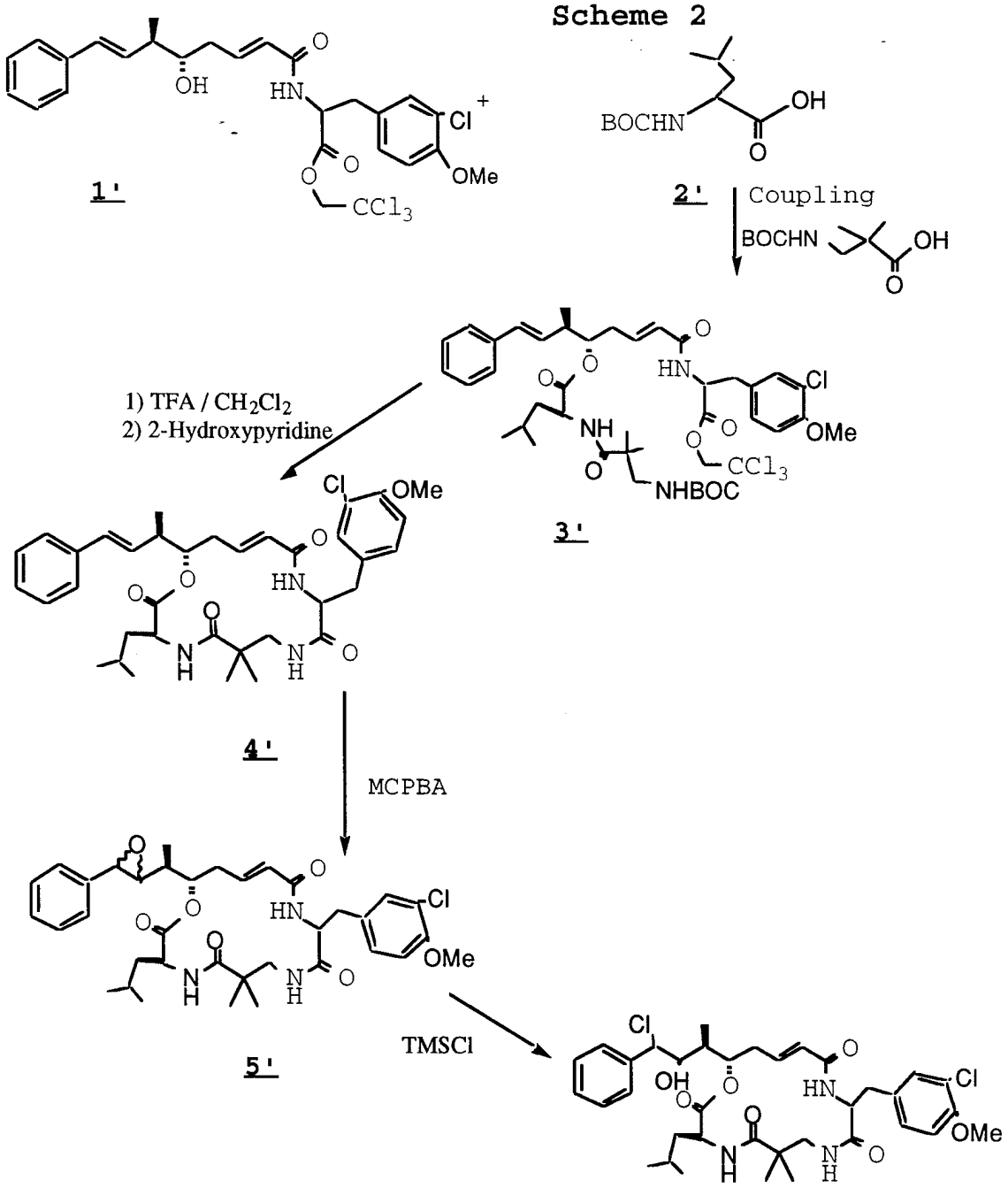
Further preferred compounds can be those wherein R^{11} is C_1-C_2 alkyl or benzyl.

Compounds of this invention can be prepared as illustrated using the following schemes.

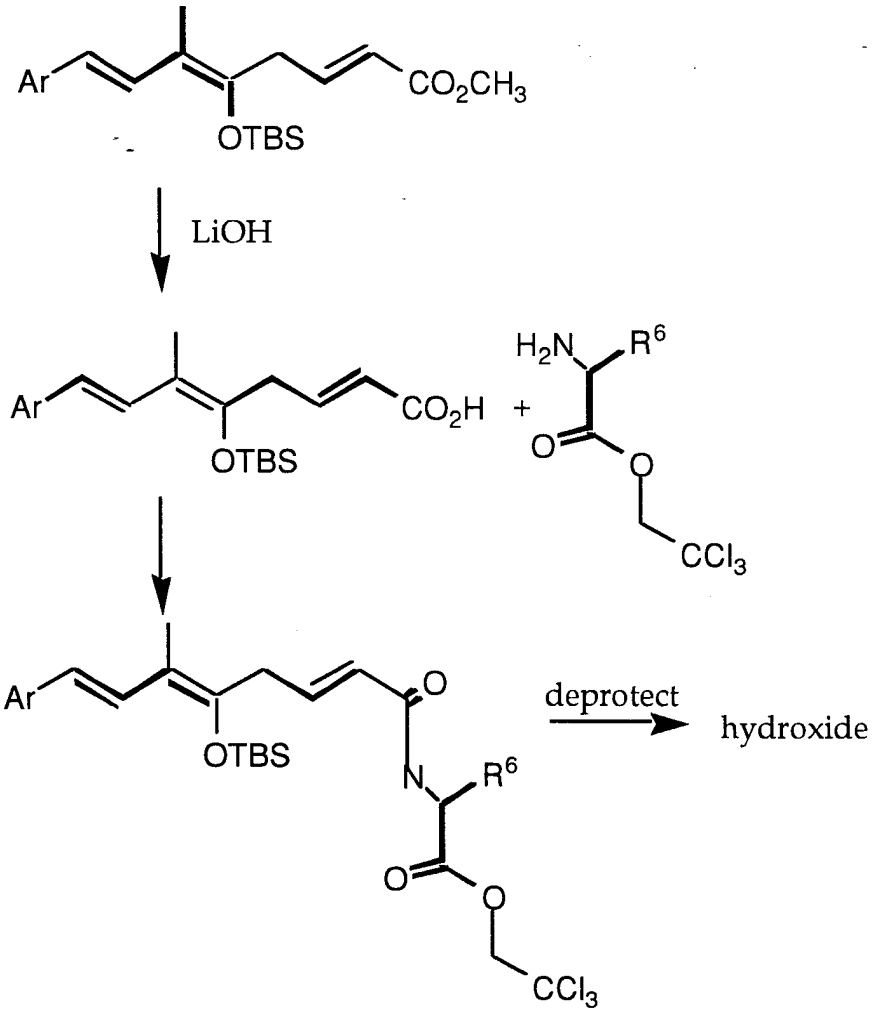
Scheme 1



Scheme 2



The cryptophycin A-B trichloroethyl ester can be prepared using information known in the literature; however, the following method is provided for the convenience of the artisan:

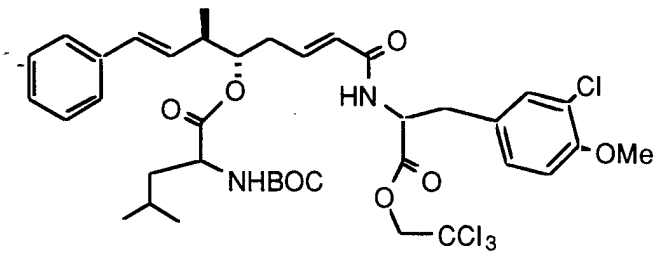


The artisan can utilize appropriate starting materials and reagents to prepare desired compounds using the guidance of the previous schemes and following examples.

The artisan can utilize appropriate starting materials and reagents to prepare desired compounds using the guidance of the previous schemes and following examples.

To further illustrate the invention the following examples are provided. The scope of the invention is in no way to be construed as limited to or by the following examples.

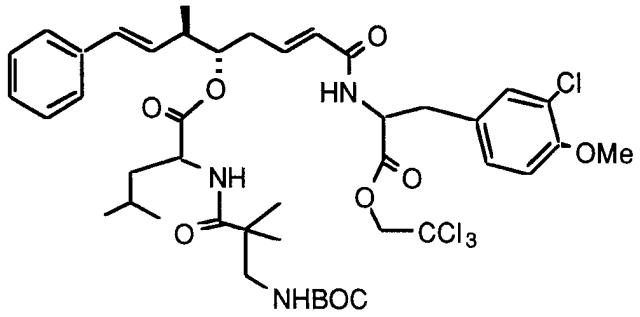
PREPARATION 1



2

To a solution containing 100 mg (0.221 mmol) of Cryptophycin A-B trichloroethyl ester and 82 mg (0.332 mmol) of *N*-BOC-leucine in 5 ml of methylene chloride was added 68 mg (0.332 mmol) of dicyclohexylcarbodiimide (DCC) and 5 mg of *N, N*-4-dimethylaminopyridine (DMAP). The reaction was strirred at 25°C for 30 minutes, then diluted with 50 mL of ethyl acetate. The organic solution was washed with 0.5 N HCl and saturated sodium bicarbonate solution, dried over sodium sulfate and concentrated *in vacuo*. The crude material was purified by flash chromatography using 25% ethyl acetate-hexane as the eluent. The major fraction was concentrated *in vacuo* to give 110 mg (62%) of a white amorphous solid, which was characterized as 2. Mass Spec. (FD^+) m/e 802 (M^+).

PREPARATION 2

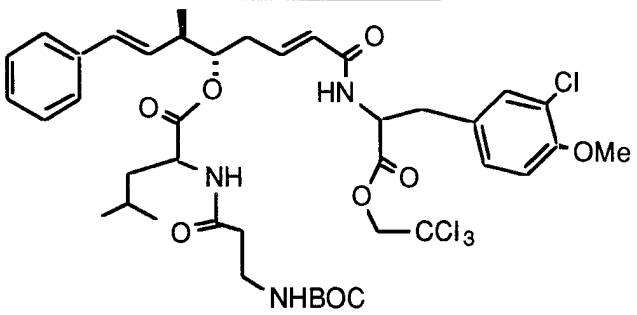


3a

To a solution containing 92 mg (0.115 mmol) of 2 in 2 mL of methylene chloride was added 2 mL of trifluoroacetic

acid (TFA). The reaction was stirred at 25°C for 1 hour and concentrated *in vacuo* to give 100 mg of the corresponding TFA salt, which was used without further purification. To a solution containing 53 mg (0.244 mmol) of *N*-BOC-2,2-dimethyl-b-alanine in 10 mL 50% THF-DMF was added 41 mg (0.305 mmol) of *N*-hydroxybenzotriazole (HOBt) and 52 mg (0.244 mmol) of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). The reaction was stirred at 25°C for 15 min., after which a solution containing the TFA salt and 20 mL (0.183 mmol) of *N*-methylmorpholine (NMM) in 5 mL of DMF was added. The reaction was stirred at 25°C for 15 hours, diluted with 100 mL of ethyl acetate and the organic solution washed twice with 0.5 N HCl, twice with saturated sodium bicarbonate solution and twice with brine. The organic layer was dried over sodium sulfate and concentrated *in vacuo* to give 105 mg (95%) of a white amorphous solid, which was characterized as **3a**. Mass Spec. (FD^+) m/e 901 (M^+).

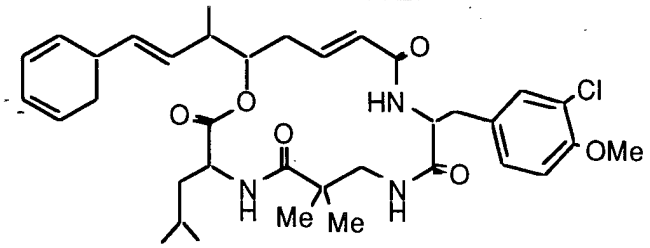
PREPARATION 3



3b

3b was prepared according to the method for the preparation of **3a**. Mass Spec. (FD^+) m/e 873 (M^+).

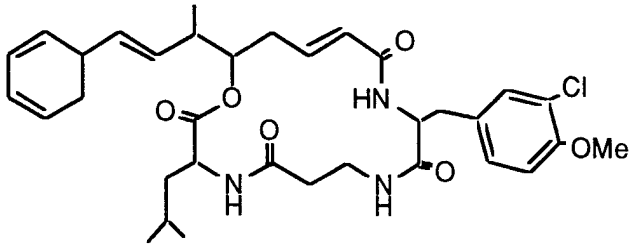
EXAMPLE 1



4a

To a solution of 105 mg (0.116 mmol) of **3a** in 2 mL CH_2Cl_2 was added 2 mL of trifluoroacetic acid (TFA). The reaction was strirred at 25°C for 30 min, after which it was poured into 25 mL of a saturated aqueous sodium bicarbonate solution. An additional 25 mL of CH_2Cl_2 was added and the organic phase was washed with 1N NaOH, dried over sodium sulfate and concentrated *in vacuo*. This crude material was dissolved in 10 mL of toluene and stirred at 25°C as 11 mg (0.116 mmol) of 2-hydroxypyridine was added. The reaction was stirred at 25°C for 18 h. The reaction was diluted with 50 mL of ethyl acetate and the organic phase washed twice with 1N HCl, twice with brine and twice with saturated sodium bicarbonate solution. The organic solution was dried over sodium sulfate and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica gel using 50% ethyl acetate-hexane as the eluent. The major fraction was concentrated *in vacuo* to give 27 mg (36%) of a white amorphous solid, which was characterized as **4a**. Mass Spec. (FD^+) m/e 651 (M^+).

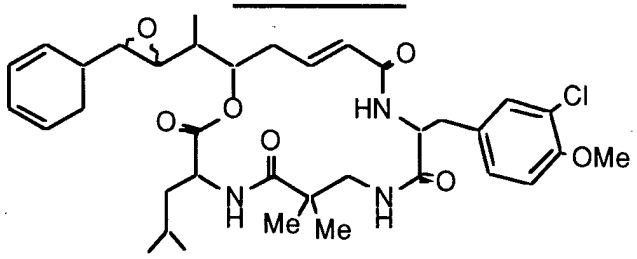
EXAMPLE 2



4b

4b was prepared according to the method for the preparation of **4a**. Mass Spec. (FD^+) m/e 623 (M^+).

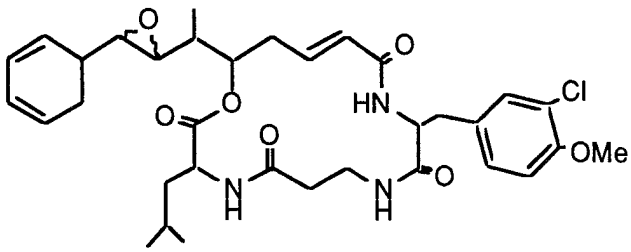
EXAMPLE 3



5a

To a solution containing 20 mg (0.031 mmol) of **4a** in 5 mL of CH_2Cl_2 was added 6.3 mg (0.037 mmol) of *m*-chloroperbenzoic acid (mCPBA). The reaction was strirred at 25°C and monitored by HPLC (reverse phase, C_{18}, 70% acetonitrile-water). After about 5 h, the reaction slowed and an additional 5 mg *m*CPBA was added. The reaction was stirred at 25°C and after another 12 h, was complete. The reaction was diluted with 25 mL CH_2Cl_2 and washed twice with a saturated sodium meta-bisulfite solution and twice with a saturated sodium bicarbonate solution. The organic layer was dried and concentrated *in vacuo* to give 18 mg (85%) of a white amorphous solid, which was charaterized as a 2:1 mixture of diastereomeric epoxides of **5a**. Mass Spec. (FD^+) m/e 667 (M^+).

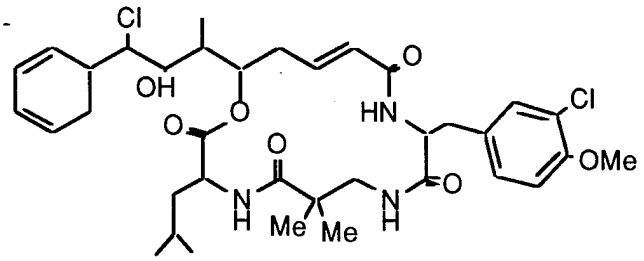
EXAMPLE 4



5b

5b was prepared according to the method for the preparation of **5a**. Mass Spec. (FD^+) m/e 639 (M^+).

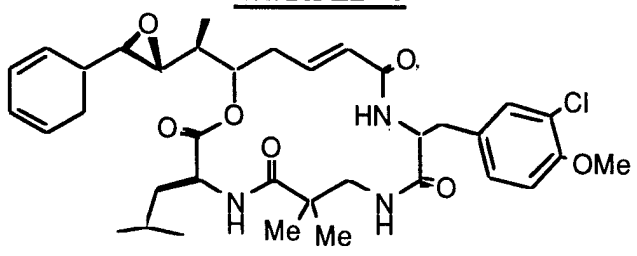
EXAMPLE 5



6

A solution containing 50 mg (0.075 mmol) of **5a** in 2 mL chloroform was cooled to -60°C and 47 mL (0.375 mmol) of chlorotrimethylsilane (TMSCl) was added. The reaction was warmed to 25°C and poured into 20 mL of water. An additional 20 mL of chloroform was added and the organic layer was separated, dried over sodium sulfate and concentrated *in vacuo* to give a mixture of chlorohydrins. The major chlorohydrin was purified by flash chromatography on silica gel using ethyl acetate as the eluent. The major fraction was concentrated *in vacuo* to give 19 mg (54%) of a white amorphous solid, which was characterized as **6**. Mass Spec. (FD^+) m/e 704 (M^+).

EXAMPLE 6



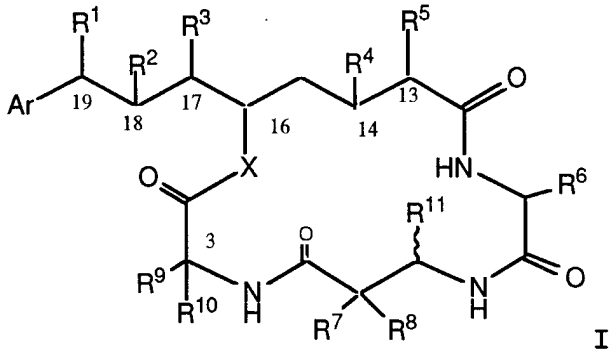
7

To a solution of 5.0 mg (0.0071 mmol) of **6** in 2 mL of 50% acetonitrile:water was added 2 mg of sodium carbonate. The reaction was stirred at 25°C for 1 hour, then diluted with 10 mL ethyl acetate. The organic solution was washed once with water, dried over sodium sulfate and concentrated *in vacuo* to give 4.0 mg (85%) of a white amorphous solid,

which was characterized as pure epoxide 7. Mass Spec. (FD^+) m/e 667 (M^+).

We Claim:

1. A compound of Formula I



I

wherein

Ar is phenyl or any simple unsubstituted or substituted aromatic or heteroaromatic group;

R^1 is halogen, SH, amino, monoalkylamino, dialkylamino, trialkylammonium, alkylthio, dialkylsulfonium, sulfate, or phosphate;

R^2 is OH or SH; or

R^1 and R^2 may be taken together to form a 3 member epoxide ring, an aziridine ring, an episulfide ring, a sulfate ring, or a monoalkylphosphate ring; or

R^1 and R^2 may be taken together to form a second bond between C_{18} and C_{19};

R^3 is a lower alkyl group;

R^4 is H;

R^5 is H;

R^4 and R^5 may be taken together to form a second bond between C_{13} and C_{14};

R^6 is benzyl, hydroxybenzyl, alkoxybenzyl, halohydroxybenzyl, dihalohydroxybenzyl, haloalkoxybenzyl, or dihaloalkoxybenzyl group;

R^7 is H or a lower alkyl group;

R^8 is H or a lower alkyl group; or

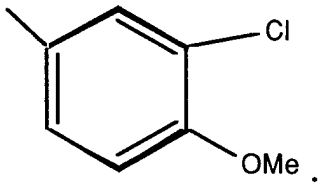
R^7 and R^8 may optionally be taken together to form a cyclopropyl ring;

R^9 is selected from the group consisting of H, a lower alkyl group, (C_1-C_3) alkylaryl, and aryl;
R^{10} is selected from the group consisting of H, a lower alkyl group, (C_1-C_3) alkylaryl, and aryl;
R^{11} is selected from the group consisting of hydrogen, simple alkyl, phenyl, substituted phenyl, benzyl, and substituted benzyl;
X is O, NH or alkylamino; or
a pharmaceutically acceptable salt or solvate thereof.

2. A compound of **Claim 1** wherein R^{11} is hydrogen.

3. A compound of **Claim 2** wherein X is O.

4. A compound of **Claim 3** wherein R^6 is a group of the formula:



5. A compound of **Claim 4** wherein R^8 and R^7 are each methyl.

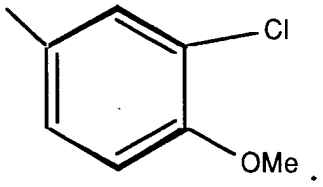
6. A compound of **Claim 5** wherein R^9 is isobutyl and R^{10} is hydrogen.

7. A compound of **Claim 6** wherein R^1 and R^2 form an epoxide group.

8. A compound of **Claim 6** wherein R^1 is Cl and R^2 is OH.

9. A compound of **Claim 1** wherein X is O.

10. A compound of **Claim 9** wherein R^6 is a group of the formula:



11. A compound of **Claim 10** wherein R^8 and R^7 are each methyl.

12. A compound of **Claim 11** wherein R^9 is isobutyl and R^{10} is hydrogen.

13. A compound of **Claim 12** wherein R^{11} is C_1-C_4 alkyl.

14. A compound of **Claim 12** wherein R^{11} is benzyl.

15. A compound of **Claim 14** wherein R^1 and R^2 form an epoxide group.

16. A compound of **Claim 14** wherein R^1 is Cl and R^2 is OH.

17. A method for disrupting microtubule binding in a mammal comprising administering an effective amount of a compound of **Claim 1.**

18. A method for disrupting microtubule binding in vitro comprising administering an effective amount of a compound of **Claim 1.**

19. A method for treating a neoplasm in a mammal comprising administering an effective amount of a compound of **Claim 1** to a patient in need thereof.

20. A formulation comprising a compound of **Claim 1** and one or more pharmaceutically acceptable diluents or carriers therefor.

21. A compound as claimed by Claim 1 or a pharmaceutically acceptable salt thereof, for use as a pharmaceutical.

22. A pharmaceutical formulation comprising as an active ingredient a compound of Claim 1 or a pharmaceutically acceptable salt thereof, associated with one or more excipients or carriers therefor.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/03666

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07D 273/00; A61K 31/395
US CL :540/454, 460; 514/183; 530/330, 331, 332
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 540/454, 460; 514/183; 530/330, 331, 332

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,254,682 A (DHANOA et al.) 19 October 1993, entire document	1-22
A	US 5,225,528 A (BOCK et al.) 06 July 1993, entire document	1-22
A	US 5,194,605 A (GREENLEE et al.) 16 March 1993, entire document	1-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
24 APRIL 1998

Date of mailing of the international search report
03 JUN 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
MATTHEW V. GRUMBLING
Telephone No. (703) 308-1235

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