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Use of Parthenolide Derivatives as Antileukemic and Cytotoxic Agents

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The present invention provides compounds of the formula (I)

$$\text{R}_1 \text{R}_2 \text{R}_3 \text{R}_4 \text{R}_5 \text{R}_6 \text{R}_7 \text{R}_8 \text{R}_9 \text{R}_{10}$$

wherein:
- X1, X2, and X3 are heteroatoms;
- R4, R5, R6, R7, R8, and R9 are independently selected from H, halo, or heteroaryl; and
- Z is optionally substituted C1-8 straight-chain or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by R* wherein R* is optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl; or
- optionally comprising 1 or more heteroatoms or a group selected from CO-, SO-, SO2- and PO2-.

The compounds are optionally together with the nitrogen atom form an optionally substituted 5-12 membered ring, said ring optionally comprising 1 or more heteroatoms or a group selected from CO-, SO-, SO2- and PO2- or a pharmaceutically acceptable salt, ester or prodrug thereof.

15 Claims, 12 Drawing Sheets
Related U.S. Application Data

application No. 13/372,178, filed on Feb. 13, 2012, now Pat. No. 8,470,875, which is a division of application No. 12/693,161, filed on Jan. 25, 2010, now Pat. No. 8,124,652, which is a division of application No. 11/031,315, filed on Jan. 7, 2005, now Pat. No. 7,678,904, which is a continuation-in-part of application No. 10/888,274, filed on Jul. 9, 2004, now Pat. No. 7,312,242.

(60) Provisional application No. 60/486,171, filed on Jul. 11, 2003.

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WO WO 02/40017 5/2002
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Ross, JJ et al. (1999) PMID 10193202 “Low concentration of the feverfew component parthenolide inhibit in vitro growth of tumor lines in a cytostatic fashion.”
Wang, et al. (online publication Nov. 28, 2014) (2015) 29(12):1092-1101 Natural Product Research “Parthenolide could become a promising and stable drug with anti-inflammatory effects”.

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FIGURE 8

Graph showing the relationship between cell survival and micromolar concentration for MD-436.

- MD-436
- MEIMP/PT-HCL
- PIPT
- DMAPT

Cell Survival vs. Micromolar Concentration
FIGURE 9

- Linear
- Non-linear bioactive
- Parthenolide
- DMAPT
- ng/mL vs. 3000, 2500, 2000, 1500, 1000, 500, 0
- 4 mg/kg, 20 mg/kg, 40 mg/kg
HT-1376

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UMUC-3

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FIGURE 10
LC-1 μM

0

2

5

+ TRAIL-R2 antibody (10 ng/ml)

FIGURE 11
LC-1 μM

0  2  5

110703.001

2  1  96  1

10^1 10^4

FL1-H

110703.004

2  1  96  1

FL2-H

110703.006

5  4  90  1

FL2-H

110703.007

2  1  96  1

FL1-H

110703.010

13  25  57  5

FL2-H

110703.011

19  40  36  6

FL2-H

+TRAIL (5 ng/ml)

FIGURE 12
USE OF PARTHENOLIDE DERIVATIVES AS ANTILEUKEMIC AND CYTOTOXIC AGENTS


FIELD OF THE INVENTION

The present invention relates to methods for the structural modification of the sesquiterpene lactone, parthenolide, and the use of these parthenolide derivatives in the treatment of carcinomas. More specifically, the invention relates to the methods to prepare structural analogs of the parent compound, parthenolide, in order to obtain new, pharmacologically active chemical entities with improved water solubility characteristics, and to use them in the treatment of leukemias and other parental and multi-drug resistant cancers.

BACKGROUND OF THE INVENTION

Sesquiterpene lactones are a group of secondary plant metabolites consisting of a 15-carbon structure containing an α-methylene-γ-butyrolactone moiety and other additional functional groups. Over the last two to three decades, these terpenoids have received considerable attention due to the broad spectrum of their biological activities, to the plants which produce them, and most importantly, because of their pharmacological effects in humans. About 4,000 of these terpenoids have been isolated and identified, most of them in the Asteraceae (Compositae, sunflower family) (Schmidt, Curr. Org. Chem. 1999, 3, 577-608). Some of these plants have been used for centuries in indigenous medical practices in various cultures worldwide.

Parthenolide (1) is a Germacrane sesquiterpene lactone with a unique structure. It has been isolated from several different species in Asteraceae (Compositae) family, feverfew (Tanacetum parthenium) being one of them.

Feverfew has been used to reduce fever and pain and in the treatment of migraine and rheumatoid arthritis (Heptinstall et al., ACS Symposium Series 1998, 691 (Phytotherapeutics of Europe), 158-175). The active component is parthenolide (1). Recently, it has been revealed that parthenolide (1) can induce tumor apoptosis by the inhibition of NF-κB activities (Cory et al., Anticancer Research 2002, 22, 3805-9; Cory et al., Anticancer Research 2001, 21, 3807-11; Gelfand et al., Blood, 2000, 98, 2508-17; Kang et al., Brit. J. Pharmacol. 2002, 135, 1235-44; Song et al., Asian Nat. Prod. Res. 2001, 3, 285-91).

Parthenolide (1) is a lipophilic, neutral lactone with low polarity, and has a low water-solubility, limiting its development as a therapeutic agent. Thus, a need exists for the development of soluble parthenolide derivatives that retain their anti-cancer activity.

SUMMARY OF THE INVENTION

In accordance with the present invention, a novel class of compounds with antileukemic activity is presented. Accordingly, the present invention provides compounds of formula (I):

![Chemical Structure](image)

wherein:

- $X_1$, $X_2$, and $X_3$ are heteroatoms;
- $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$, and $R_7$ are independently selected from H, halo, —OH, —NO, —CN and optionally substituted aliphatic, cycloalkyl, heterocycloalkyl, aryl or heteroaryl;
- and $Z$ is optionally substituted C, straight-chain or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by R* wherein R* is optionally substituted cycoalkyl, heterocycloalkyl, aryl or heteroaryl; an amino acid residue, H, —CN, —C(O)—, —C(O)(O)—, —C(O)NR—, —N02—, —C(O)NR2—, —C(O)NR—, —NR2—, —O—, —NR2C(O)NR2—, —OC(O)NR—, —NR2CO2—, —O—, —NR2C(O)NR2—, —OC(O)NR2—, —NR2CO2—, —O—, —NR2C(O)NR2—, —OC(O)NR2—, —NR2—, —SR—, —SO2—, —NR2—, —SO2—, —NR2—, or —NR2SO2—, wherein R1 and R2 are independently selected from H and optionally substituted aliphatic, cycloalkyl, heterocycloalkyl, aryl or heteroaryl; or where R* is NR2R2, R1 and R2 optionally together with the nitrogen atom from an optionally substituted 5-12 membered ring, said ring optionally comprising 1 or more heteroatoms and/or a group selected from —CO—, —SO—, —SO2— and —PO—; or a pharmaceutically acceptable salt, ester or prodrug thereof.

The invention also provides a pharmaceutical composition comprising an effective amount of a compound of formula (I), or a pharmaceutically acceptable salt, ester or prodrug thereof, in combination with a pharmaceutically acceptable diluent or carrier.

The invention also provides a method of inhibiting cancer cell growth and metastasis of cancer cells, comprising administering to a mammal afflicted with cancer, an amount of a compound of formula (I), effective to inhibit the growth of said cancer cells.
The invention also provides a method comprising inhibiting cancer cell growth by contacting said cancer cell in vitro or in vivo with an amount of a compound of formula (I), effective to inhibit the growth of said cancer cell.

The invention also provides a compound of formula (I) for use in medical therapy (preferably for use in treating cancer, e.g., solid tumors), as well as the use of such compound for the manufacture of a medicament useful for the treatment of cancer and other diseases/disorders described herein.

The invention further provides methods of treating inflammatory diseases and disorders, including, for example, rheumatoid arthritis, osteoarthritis, allergies (such as asthma), and other inflammatory conditions, such as pain (such as migraine), swelling, fever, psoriasis, inflammatory bowel disease, gastrointestinal ulcers, cardiovascular conditions, including ischemic heart disease and atherosclerosis, partial brain damage caused by stroke, skin conditions (eczema, sunburn, acne), leukotriene-mediated inflammatory diseases of lungs, kidneys, gastrointestinal tract, skin, prostatitis and periodontosis.

The invention further provides methods of treating immune response disorders, whereby the immune response is inappropriate, excessive or lacking. Such disorders include allergic responses, transplant rejection, blood transfusion reaction, and autoimmune disorders such as systemic lupus erythematosus and rheumatoid arthritis.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the effectiveness of parthenolide and derivatives of the present invention against prostate cancer cell line CWR22 in a clonogenic assay.

FIG. 2 shows the effectiveness of parthenolide and derivatives of the present invention against lung cancer cell line A-549 in a cellular proliferation MTS-PMS assay.

FIG. 3 shows the effectiveness of parthenolide and derivatives of the present invention against lung cancer cell line H-522 in a cellular proliferation MTS-PMS assay.

FIG. 4 shows the effectiveness of parthenolide and derivatives of the present invention against lung cancer cell line H-23 in a cellular proliferation MTS-PMS assay.

FIG. 5 shows the effectiveness of parthenolide and derivatives of the present invention against lung cancer cell line H-460 in a cellular proliferation MTS-PMS assay.

FIG. 6 shows the effectiveness of parthenolide and derivatives of the present invention against breast cancer cell line HBL-100 in a clonogenic assay.

FIG. 7 shows the effectiveness of parthenolide and derivatives of the present invention against breast cancer cell line MD-231 in a clonogenic assay.

FIG. 8 shows the effectiveness of parthenolide and derivatives of the present invention against breast cancer cell line MD-468 in a clonogenic assay.

FIG. 9 shows parthenolide and DMAPT plasma concentrations at one hour following oral gavage in mice.

FIG. 10 shows DMAPT dose-dependent inhibition of NF-kB DNA binding in two transitional cell carcinoma cell lines HT-1376 and UMUC-3 in electrophoretic mobility gel shift assay (EMSA).

FIG. 11 shows FSCScan analysis of TRAIL-induced apoptosis assay using MDA-MB-231 breast cancer cells treated first with DMAPT, then TRAIL-R11-activating antibodies.

FIG. 12 shows FSCScan analysis of TRAIL induced apoptosis assay using MDA-MB-231 breast cancer cells treated first with DMAPT, then TRAIL.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the following definitions shall apply unless otherwise indicated.

The phrase “optionally substituted” is used interchangeably with the phrase “substituted or unsubstituted.” Unless otherwise indicated, an optionally substituted group may have a substituent at each substitutable position of the group, and each substitution is independent of any other. Also, combinations of substituents or variables are permissible only if such combinations result in stable compounds. In addition, unless otherwise indicated, functional group radicals are independently selected. Where “optionally substituted” modifies a series of groups separated by commas (e.g., “optionally substituted A, B or C”; or “A, B or C optionally substituted with”), it is intended that each of the groups (e.g., A, B and C) is optionally substituted.

The term “aliphatic” or “aliphatic group” as used herein means a straight-chain or branched C1-C12 hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation, or a monocyclic C5-C8 hydrocarbon or bicyclic C6-C12 hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic (also referred to herein as “carbocyclic” or “cycloalkyl”), that has a single point of attachment to the rest of the molecule wherein any individual ring in said bicyclic ring system has 3-7 members. For example, suitable aliphatic groups include, but are not limited to, linear or branched or alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

The terms “alkyl,” “alkoxy,” “hydroxyalkyl,” “alkoxyalkyl” and “alkoxy-carbonyl,” used alone or as part of a larger moiety include both straight and branched chains containing one to twelve carbon atoms. The terms “alkenyl” and “alkynyl” used alone or as part of a larger moiety shall include both straight and branched chains containing two to twelve carbon atoms.

The terms “haloalkyl,” “haloalkenyl” and “haloalkoxy” means alkyl, alkenyl or alkoxy, as the case may be, substituted with one or more halogen atoms. The term “halo” means F, Cl, Br or I.

The term “heteroatom” means nitrogen, oxygen, or sulfur and includes any oxidized form of nitrogen and sulfur, and the quaternized form of any basic nitrogen. Heteroatom further includes Se, Si and P.

The term “aryl” used alone or in combination with other terms, refers to monocyclic, bicyclic or tricyclic carbocyclic ring systems having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic and wherein each ring in the system contains 3 to 8 ring members. The term “aryl” may be used interchangeably with the term “aryl ring.” The term “alkyl” refers to an alkyl group substituted by an aryl. The term “alkoxy” refers to an alkyl group substituted by an aryl.

The term “heterocycloalkyl,” “heterocycle,” “heterocyclic” or “heterocyclic” as used herein means monocyclic, bicyclic or tricyclic ring systems having five to fourteen ring members in which one or more ring members is a heteroatom, wherein each ring in the system contains 3 to 7 ring members and is non-aromatic.
The term "heteroaryl," used alone or in combination with other terms, refers to monocyclic, bicyclic and tricyclic ring systems having a total of five to fourteen ring members, and wherein: 1) at least one ring in the system is aromatic; 2) at least one ring in the system contains one or more heteroatoms; and 3) each ring in the system contains 3 to 7 ring members. The term "heteroaryl" may be used interchangeably with the term "heteroaryl ring" or the term "heteroaromatic". Examples of heteroaryl rings include 2-furanyl, 3-furanyl, N-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-oxadiazolyl, 5-oxadiazolyl, 2-oxazolyl, 4-oxazolyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 1-pyrazolyl, 2-pyrazolyl, 3-pyrazolyl, 4-pyrazolyl, 2-pyridyl, 3-pyridyl, 4-pyrimidyl, 2-pyrimidyl, 4-pyrimidyl, 5-pyrazinyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 5-tetrazolyl, 2-triazolyl, 3-triazolyl, 2-thiophenyl, 3-thienyl, carbazolyl, benzimidazolyl, benzothiophenyl, benzofuranyl, indolyl, quinolyl, benzotriazolyl, benzothiazolyl, benzoxoxazolyl, benzimidazolyl, benzisoxazolyl, indazolyl, isoindolyl, acridinyl, and benzoisoxazolyl. The term "heteroarylalkyl" refers to an alkyl group substituted by a heteroaryl. The term "heteroaryloxy" refers to an alkoxy group substituted by a heteroaryl. The term "heteroarylsulfonyl" refers to a sulfonyl group substituted by a heteroaryl. The term "heteroarylthio" refers to a thio group substituted by a heteroaryl. The term "heteroaryl ring" includes a heteroaromatic ring in a subject which may be predisposed to the condition accordingly, the treatment constitutes prophylactic treatment, as defined above, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The term "therapeutically effective amount" refers to that amount of a compound of the invention that is sufficient to effect treatment, as defined above, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The term "pharmaceutically acceptable salts" includes, but is not limited to, salts well known to those skilled in the art, for example, mono-salts (e.g. alkali metal and ammonium salts) and poly salts (e.g. di- or tri-salts) of the compounds of the invention. Pharmaceutically acceptable salts of compounds of formulas (I), (II), (III), or (IV) are where, for example, an exchangeable group, such as hydrogen in -OH, -NH-, or -P-(O)(OH)-, is replaced with a pharmaceutically acceptable cation (e.g. a sodium, potassium, or ammonium ion) and can be conveniently be prepared from a corresponding compound of formula (I) by, for example, reaction with a suitable base.
The invention relates to the ability of the a-methylene-γ-butyrolactone moiety in sesquiterpene lactones to be structurally modified by, for example, Michael addition with amines or thiols. Modification of the parthenolide (I) molecule by this methodology using primary and/or secondary amines to form water-soluble amino derivatives, affords amine adducts that can easily be obtained as different inorganic or organic salts to further increase water solubility. Thus, a novel class of more water-soluble parthenolide analogs is described. When compounds in this class were evaluated for antileukemic activity, it was found that these compounds were either equipotent as, or more potent than the parent compound, parthenolide. More importantly, these novel analogs showed greater cytotoxicity towards leukemia cells than towards normal cells. Thus, the present invention provides a new class of parthenolide derivatives with potent and selective anticancer activities.

In accordance with the present invention, there are provided compounds of formula (I):

\[
\begin{align*}
R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9, R_{10} & \text{ are independently selected from } H, \text{ halo, } \text{--COOH, } \text{--NO}_2, \text{--CN and optionally substituted aliphatic, cycloalkyl, heterocycloalkyl, aryl or heteroaryl; and} \\
Z & \text{ is optionally substituted } C_{1-8} \text{ straight-chain or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by } R^* \text{ wherein } R^* \text{ is optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl; and} \\
X_1, X_2, X_3 & \text{ are heteroatoms; } \\
R_a, R_b, R_c, R_d, R_e, & \text{ and } R_f \text{ are independently selected from } H, \text{ halo, } \text{--OH, } \text{--CN and optionally substituted aliphatic, cycloalkyl, heterocycloalkyl, aryl or heteroaryl; and} \\
R_g, R_h, R_i, R_j, R_k & \text{ are independently selected from } H, \text{ halo, } \text{--COOH, } \text{--NO}_2, \text{--CN and optionally substituted aliphatic, cycloalkyl, heterocycloalkyl, aryl or heteroaryl; and} \\
X_4 & \text{ is optionally substituted } C_{1-8} \text{ straight-chain or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by } R^* \text{ wherein } R^* \text{ is optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl; and} \\
X_5 & \text{ is optionally substituted } C_{1-8} \text{ straight-chain or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by } R^* \text{ wherein } R^* \text{ is optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl; and} \\
X_6 & \text{ is optionally substituted } C_{1-8} \text{ straight-chain or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by } R^* \text{ wherein } R^* \text{ is optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl; and} \\
X_7 & \text{ is optionally substituted } C_{1-8} \text{ straight-chain or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by } R^* \text{ wherein } R^* \text{ is optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl; and} \\
X_8 & \text{ is optionally substituted } C_{1-8} \text{ straight-chain or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by } R^* \text{ wherein } R^* \text{ is optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl; and} \\
X_9 & \text{ is optionally substituted } C_{1-8} \text{ straight-chain or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by } R^* \text{ wherein } R^* \text{ is optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl; and} \\
X_10 & \text{ is optionally substituted } C_{1-8} \text{ straight-chain or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by } R^* \text{ wherein } R^* \text{ is optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl; and}
\end{align*}
\]
NR<sup>1</sup>—, NR<sup>2</sup>—, NR<sub>2</sub>—CO—, —S—, —SO—, —SO<sub>2</sub>—, —SO<sub>2</sub>—R<sup>1</sup>—, or —NR<sup>1</sup>SO<sub>2</sub>—, wherein R<sup>1</sup> and R<sup>2</sup> are independently selected from H and optionally substituted aliphatic, cycloalkyl, heterocycloalkyl, aryl or heteroaryl; or where R<sup>1</sup> is NR<sup>2</sup>R<sup>2</sup>—, R<sup>1</sup> and R<sup>2</sup> optionally together with the nitrogen atom form an optionally substituted 5-12 membered ring, said ring optionally comprising 1 or more heteroatoms and/or a group selected from —CO—, —SO—, —SO<sub>2</sub>— and —PO—; or a pharmaceutically acceptable salt, ester or prodrug thereof.

Presently preferred compounds include compounds of formula (I) wherein R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>9</sub> and R<sub>10</sub> are independently selected from H, halo, —OH, —NO<sub>2</sub>, —CN, —CH<sub>3</sub>, —CF<sub>3</sub>, —CH<sub>2</sub>CF<sub>3</sub>, —CH<sub>2</sub>Cl, —CH<sub>2</sub>OH, —CHOH, —CH<sub>2</sub>CH<sub>2</sub>OH and —CH<sub>2</sub>NH<sub>2</sub>. Further preferred embodiments include compounds where R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>9</sub> and R<sub>10</sub> are each H.

Other preferred embodiments of the present invention include compounds where R<sub>4</sub> and R<sub>8</sub> are independently selected from optionally substituted C<sub>1</sub>—C<sub>4</sub> alkyl. In one preferred embodiment, R<sub>4</sub> is —CH<sub>3</sub>, and in another, R<sub>4</sub> and R<sub>8</sub> are each —CH<sub>3</sub>. In yet another embodiment, R<sub>1</sub> and R<sub>2</sub> are —CH<sub>2</sub>(CH<sub>2</sub>)<sub>m</sub>-S(O)<sub>n</sub>-R<sub>1</sub> where m is an integer from 0 to 4, n is an integer from 0 to 4, and where preferably, R<sub>1</sub>, R<sub>2</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>9</sub> and R<sub>10</sub> are each H; and in one particular embodiment, m is 1. In another embodiment n is 0. In other embodiments, R<sup>1</sup> is independently selected from hydrogen, —CN, optionally substituted C<sub>1</sub>—C<sub>4</sub> alkyl or aryl. In particular embodiments, R<sup>1</sup> is selected from —CO—, —SO—, —SO<sub>2</sub>— and —PO—.

Exemplary compounds of the present invention include: 11,13-Dihydro,13-(dimethylaminoparthenolide (DAMPT); 11,13-Dihydro,13-diethylaminoparthenolide; 11,13-Dihydro,13-(tert-butyloxy)parthenolide; 11,13-Dihydro,13-(pyrrolidin-1-yl)parthenolide; 11,13-Dihydro,13-(piperidin-1-yl)parthenolide (PIPT); 11,13-Dihydro,13-(morpholin-1-yl)parthenolide; 11,13-Dihydro,13-(4-methylpiperidin-1-yl)parthenolide (4MEPT); 11,13-Dihydro,13-(4-methylpiperazin-1-yl)parthenolide; 11,13-Dihydro,13-(homopiperidin-1-yl)parthenolide; 11,13-Dihydro,13-(heptamethyleneimino-1-yl)parthenolide; 11,13-Dihydro,13-(azetidin-1-yl)parthenolide; 11,13-Dihydro,13-methylbutylaminoparthenolide; 11,13-Dihydro,13-methylpentylaminoparthenolide; 11,13-Dihydro,13-ethylaminoparthenolide; 11,13-Dihydro,13-methylaminoparthenolide; 11,13-Dihydro,13-cyclopropylaminoparthenolide; 11,13-Dihydro,13-propargylaminoparthenolide; 11,13-Dihydro,13-(N-benzyl-N-ethylamine)parthenolide; 11,13-Dihydro,13-(N-propyl)parthenolide; 11,13-Dihydro,13-(S-thiophenolyl)parthenolide; 11,13-Dihydro,13-(N,N-diethanolamine)parthenolide; 11,13-Dihydro,13-(thiomorpholin-4-yl)parthenolide; 11,13-Dihydro,13-(4-hydroxypiperidin-1-yl)parthenolide; 11,13-Dihydro,13-(1-methylhomopiperizin-4-yl)parthenolide; 11,13-Dihydro,13-(S-mercaptoacetoyl)parthenolide; 11,13-Dihydro,13-(4'-(2'-hydroxyethyl)piperidin-1-yl)parthenolide; 11,13-Dihydro,13-(piperazin-1-yl-4-carboxaldehyde)parthenolide; 11,13-Dihydro,13-(4-benzyl)piperidin-1-yl)parthenolide; 11,13-Dihydro,13-(piperidin-1-yl-4-carboxaldehyde)parthenolide; 11,13-Dihydro,13-(azetidin-1-yl-3-carboxylic acid)parthenolide; 11,13-Dihydro,13-(S-cysteinyl)parthenolide; 11,13-Dihydro,13-(4-piperidin-1-yl)piperidin-1-yl)parthenolide; and 11,13-Dihydro,13-diallylaminoparthenolide.

Those of skill in the art will recognize that the invention comprises compounds that may contain one or more chiral centers on, for example, the parthenolide C-11 and thus can be substantially stereochemically pure or optically pure materials. Suitable stereoselective synthetic procedures for pro-
ducing stereochemically pure or optically pure materials are well known in the art, as are procedures for resolving racemic mixtures into their pure optical enantiomers.

The present invention further provides for compounds having formula (I), or a pharmaceutically acceptable salt thereof, wherein Z is a group that is converted to =CH₂ under physiological conditions during or after administration to a mammalian patient, thereby yielding a methylene group. In particular embodiments X₁, X₂, and X₃ are O; R₄, R₅, and R₁₀ are H; and R₆ and R₈ are —CH₃, and Z is optionally substituted C₁₋₈ straight-chain or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by R*, wherein R* is optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl; an amino acid residue, H, —CN, —C(O)—, —C(O)O—, —C(O)NR₁—, —C(O)ONR₁R₂—, —C(O)NR₁R₂—, or —SO₂—, wherein R₁ and R₂ are independently selected from H and optionally substituted aliphatic, cycloalkyl, heterocycloalkyl, aryl or heteroaryl. In preferred embodiments X₁, X₂, and X₃ are 0; R₆, R₇, R₉, and R₁₀ are H; and R₄ and R₈ are —CH₃.

The present invention further provides for compounds having formula (I), or a pharmaceutically acceptable salt thereof, wherein Z is CH₂N(CH₃)₂ which under physiological conditions during or after administration to a mammalian patient, undergoes mono- or di-demethylation; conversion to =CH₂, or cysteine or protein conjugation. In particular embodiments, X₁, X₂, and X₃ are 0; R₆, R₇, R₉, and R₁₀ are H; and R₄ and R₈ are —CH₃.

The present invention further provides compounds wherein the parthenolide based derivatives of formula (I) form dimers or duplexes with another molecule of formula (I) or with basic nitrogen-containing synergistic anticancer drug molecules such as 5-fluorouracil, cytarabine, mitomycin C, Doxorubicin and Daunorubicin. Accordingly, the present invention provides compounds of the general formula (II):
In the above scheme, the solvent is selected from a low alkyl alcohol, such as methanol, ethanol, propanol, isopropanol, n-butanol, tert-butanol, and chloroform, methylene chloride, benzene, toluene, tetrahydrofuran, dioxane, 1,2-dimethoxyethane, pyridine, carbon tetrachloride, diethyl ether, tert-butyl methyl ether and/or the mixture of two or more of the solvents listed above. The base is selected from a low trialkylamine, such as trimethylamine, triethylamine, tripropylamine, and tributylamine, and pyridine, 2-, 3-, and 4-picolines, 2-, 3-, and 4-dimethylaminopyridines. The temperature is selected from -20° C. to 130° C. The reaction time required to effect the desired coupling reaction can vary widely, typically falling in the range of 30 min to 24 hours. Purification can be achieved by a variety of techniques, such as, liquid chromatography through neutral or basic silica gel, bonded silica gel phases such as octadecysilica, octylsilica and the like, cellulose or alumina with the solvent such as, for example, the mixture of chloroform and methanol or ethanol, the mixture of methylene chloride and methanol or ethanol, the mixture of hexane and acetone or acetonitrile or methanol or ethanol or isopropanol, the mixture of diethyl ether and acetone or acetonitrile or methanol or ethanol or isopropanol; and recrystallization using normal organic solvent or solvent mixture, such as methanol, ethanol, propanol, isopropanol, tert-butanol, acetonitrile, diethyl ether, tert-butyl methyl ether and/or the mixture of two or more solvents listed above. The purity of the invention compounds prepared is assessed by mass spectrometry, nuclear magnetic resonance spectrometry (NMR) and elemental combustion analysis.

Furthermore, in accordance with still another embodiment of the present invention, the methods for the preparation of the invention salts are disclosed in Schemes III and IV.

Scheme III

```plaintext
Acid HX
solvent

NR^1R^2

O

R^1

O

NR^1R^2HX
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Furthermore, in accordance with still another embodiment of the present invention, the methods for the preparation of the invention salts are disclosed in Schemes III and IV.
In these schemes, HX is selected from hydrochloride, hydrobromide, hydroiodide, perchlorate, sulfate, hemisulfate, mesylate, toluenesulfonate, benzenesulfonate, succinate, hemisuccinate, fumarate, tartarate, ascorbate, acetate, hemifumarate, maleate, citrate, oxalate, malonate, malic, propionate and benzoate; Y⁻ is selected from halide (fluoride, chloride, bromide, iodide), methylsulfonate, toluenesulfonate, benzenesulfonate and sulfate; and the solvent is selected from a low alkyl alcohol, such as diethyl ether, methanol, ethanol, propanol, isopropanol, n-butanol, tert-butanol, and chloroform, methylene chloride, benzene, toluene, tetrahydrofuran, dioxane, 1,2-dimethoxyethane, pyridine, carbon tetra chloride, tert-butyl methyl ether, acetone and/or the mixture of two or more of the solvents listed above. The temperature is selected from -20°C to 50°C. Purification can be achieved by recrystallization using normal organic solvent or solvent mixture, such as methanol, ethanol, acetone, propanol, isopropanol, t-butanol, acetonitrile, diethyl ether, chloroform, methylene chloride and the mixture of two or more solvents listed above.
The present invention further provides analogues of compounds of formula (1). Examples are described below and include costunolide, dehydrocostuslactone, alantolactone, isosalantolactone, amino-3-oxo-isosalantolactone, helenalin, 11,13-dihydrohelenalin, aminocyanaropicrin, aminodesacylcyanaropicrin, (+)-aminoreynosin, aminosantamarin, aminosoulangianolide and aminoisotelekin. In addition, the present invention provides compounds of the analogues below, amino-3-oxo-isosalantolactone, aminocyanaropicrin, aminodesacylcyanaropicrin, (+)-aminoreynosin, aminosantamarin, aminosoulangianolide and aminoisotelekin wherein (—CH₂—NR₂) is replaced by Z, where Z is as defined herein.

The invention relates to the ability of the α-methylene-γ-butyrolactone moiety in all the above-mentioned sesquiterpene lactones to be structurally modified by, for example, Michael addition with the following chemical entities to form more water-soluble derivatives than the corresponding parent sesquiterpene, and with improved properties conducive for drug development, such as, chemical stability, reduced toxicity and oral bioavailability.
The present invention further provides compounds of the parthenolide-analog dehydrocostuslactone based derivatives of formula (IV):

![Formula Image]

Presently preferred compounds include compounds of formula (IV) wherein R₃, R₅, R₆, R₉ and R₁₀ are independently selected from H, halo, —OH, —NO₂, —CN, —CH₃, —CF₃, —CH₂CH₃, —CH₂CF₃, —CH₂Cl, —CH₂OH, —CH₂CH₂OH and —CH₂NH₂. Further preferred embodiments include compounds where R₂, R₃, R₆, R₉ and R₁₀ are each H.

Other preferred embodiments of the present invention include compounds where R₄ and R₈ are independently selected from optionally substituted C₃-C₄ alkyl. In one preferred embodiment, R₄ and R₈ are each =CH₂.

In one embodiment, X₁ and X₂ are heteroatoms independently selected from O, N and S, and in one particular embodiment, X₁ and X₂ are each O.

According to a further embodiment of the invention, Z is  dés-(CH₂)₄-NR₁R₂ where m is an integer from 0 to 4, where preferably, R₁, R₂, R₉ and R₁₀ are H; and R₄ and R₈ are each =CH₂. In one particular embodiment, m is 1. In other embodiments, R¹ and R² are independently selected from hydrogen, —CN or optionally substituted C₃-C₆ alkyl. In particular embodiments, R¹ and R² are independently selected from —NO₂, —CN, —CH₃, —CF₃, —CH₂CH₃, —CH₂CF₃, —CH₂Cl, —CH₂OH, —CH₂CH₂OH and —CH₂NH₂.

In yet a further embodiment, R¹ and R² together with N form an optionally substituted ring. The ring is a monocyclic, bicyclic or tricyclic aliphatic or aryl ring system, where the ring system is optionally substituted and optionally comprises one or more heteroatoms or a group selected from —CO—, —SO—, —SO₂— and —PO—. In one particular embodiment, R¹ and R² are —CH₃(CH₂)mCH₂Y—, where Y is a heteroatom or a group selected from —CO—, —SO—, —SO₂— and —PO—; n is an integer 0 to 5; and together with N form an optionally substituted ring, which may be additionally fused to a cycloalkyl or aryl group to form a bicyclic or tricyclic ring system.

Examples of ring systems include an optionally substituted uracil ring or a derivative thereof. Other examples include optionally substitute pyrrole, imidazole, purine and pyrazole and derivative thereof. Examples of fused ring systems include optionally substituted aziridin-1-yl, azetidin-1-yl, pyrrolidin-1-yl, piperidin-1-yl, homopiperidin-1-yl and heptamethylenemin-1-yl.

With respect to formulas (I) and (IV), in other embodiments, Z is hydroxyamine, a hydroxyalkylamino compound, a thioalkylamino compound, a diaminoalkane. Examples include ethylenediamine, piperazine, triminoalkanes, polyamines, polylysine, putrescine, spermine, spermidine, aminouguanides and agmatine. In other embodiments, Z is an amino acid. For example glycine, serine, hydroxyproline, β-alanine, cysteine, homocysteine, arginine, lysine, glutamic acid, ornithine, aspartic acid, γ-aminobutyric acid, or taurine. In other embodiments, Z is an amino sugar; for example glucosamine. In other embodiments, Z is a polyoxethylene glycol of various molecular weights, each of which terminate in an amino functionality that will form an adduct with the appropriate sesquiterpene.

Modification of the sesquiterpene molecules by these methodologies, affords adducts that can easily be obtained as different inorganic or organic salts to further increase water solubility.

The compounds described herein are useful for treating cancer. Cancers treatable by the present therapy include the solid and hematological tumors, such as prostate cancer, ovarian cancer, breast cancer, brain cancer and hepatic cancer, comprising administering to a mammal afflicted with said cancer an amount of parthenolide derivative effective to inhibit the viability of cancer cells of said mammal. The parthenolide derivative may be administered as primary therapy, or as adjunct therapy, either following local intervention (surgery, radiation, local chemotherapy) or in conjunction with at least one other chemotherapeutic agent discussed hereinabove, as well as the solid tumors disclosed in U.S. Pat. No. 5,514,555. Hematological cancers, such as the leukemias are disclosed in the Mayo Clinic Family Health Book, D. E. Larson, ed., William Morrow, N.Y. (1990) and include CLL, ALL, CML, and the like. Compounds of the present invention may be used in bone marrow transplant procedure to treat bone marrow prior to reintroduction to the patient. In addition, the compounds of the present invention may be used as chemotherapy sensitizers or radiation therapy sensitizers. Accordingly, a patient, or cells, or tissues, derived from a cancer patient, are pretreated with the compounds prior to standard chemotherapy or radiation therapy. The present invention contemplates that parthenolide may also be used in such methods.

Within another aspect of the present invention, methods are provided for inhibiting angiogenesis in patients with non-tumorigenic, angiogenesis-dependent diseases, comprising administering therapeutically effective amount of a composition comprising parthenolide derivative to a patient with a non-tumorigenic angiogenesis-dependent disease, such that the formation of new blood vessels is inhibited. Within other aspects, methods are provided for inhibit reactive proliferation of endothelial cells or capillary formation in non-tumorigenic, angiogenesis-dependent diseases, such that the blood vessel is effectively occluded. Within one embodiment, the anti-angiogenic composition comprising parthenolide derivative is delivered to a blood vessel which is actively proliferating and nourishing a tumor.

In addition to tumors, numerous other non-tumorigenic angiogenesis-dependent diseases, which are characterized by the abnormal growth of blood vessels, may also be treated with the anti-angiogenic parthenolide derivative compositions, or anti-angiogenic factors of the present
Anti-angiogenic parthenolide derivative compositions of the present invention can block the stimulatory effects of angiogenesis promoters, reducing endothelial cell division, decreasing endothelial cell migration, and impairing the activity of the proteolytic enzymes secreted by the endothelium. Representative examples of such non-tumorogenic angiogenesis-dependent diseases include corneal neovascularization, hypertrophic scars and keloids, proliferative diabetic retinopathy, arteriovenous malformations, atherosclerotic plaques, delayed wound healing, hemorrhoidal joints, nonunion fractures, Osler-Weber syndrome, psoriasis, pyogenic granuloma, scleroderma, trachoma, menorrhagia, retrolental fibroplasia and vascular adhesions. The pathology and treatment of these conditions is disclosed in detail in published PCT application PCT/CA94/00373 (WO 95/03036), at pages 26-36. Topical or directed local administration of the present compositions is often the preferred mode of administration of therapeutically effective amounts of parthenolide derivative, i.e., in depot or other controlled release forms.

Anti-angiogenic compositions of the present invention may also be utilized in a variety of other manners. For example, they may be incorporated into surgical sutures in order to prevent stitch granuloma, implanted in the uterus (in the same manner as an IUD) for the treatment of menorrhagia or as a form of female birth control, administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis, attached to a monoclonal antibody directed against activated endothelial cells as a form of systemic chemotherapy, or utilized in diagnostic imaging when attached to a radioactively labelled monoclonal antibody which recognizes active endothelial cells. The magnitude of a prophylactic or therapeutic dose of parthenolide derivative, an analog thereof or a combination thereof, in the acute or chronic management of cancer, i.e., prostate or breast cancer, will vary with the stage of the cancer, such as the solid tumor to be treated, the chemotherapeutic agent(s) or other anti-cancer therapy used, and the route of administration. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, and response of the individual patient. In general, the total daily dose range for parthenolide derivative and its analogs, for the conditions described herein, is from about 0.5 mg to about 2500 mg, in single or divided doses. Preferably, a daily dose range should be about 1 mg to about 100 mg, in single or divided doses, most preferably about 5-50 mg per day. In managing the patient, the therapy should be initiated at a lower dose and increased depending on the patient’s global response. It is further recommended that, for example, bolus injection or continuous infusion) and may be administered by parenteral or intravenous injection (e.g., by injection, bolus injection or continuous infusion) and may be presented in discrete unit dosage forms and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, shaping the product into the desired delivery system.

Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives. The compounds according to the invention may also be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dose form in ampules, pre-filled syringes, small volume infusion containers or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulation agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

For topical administration to the epidermis, the compounds may be formulated as ointments, creams or lotions, or as the active ingredient of a transdermal patch. Suitable transdermal delivery systems are disclosed, for example, in A. Fisher et al. (U.S. Pat. No. 4,788,603), or R. Bawa et al. (U.S. Pat. Nos. 4,931,279; 4,668,506 and 4,713,224). Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening agents. The compounds may be administered as the pure chemicals, as by inhalation of a fine powder via an insufflator, or it is preferable to present the active ingredient as a pharmaceutical formulation. The invention thus further provides a pharmaceutical formulation comprising parthenolide derivative or an analog thereof, together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be ‘acceptable’ in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof, such as a human patient or domestic animal.
and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents.

Formulations suitable for topical administration in the mouth include unit dosage forms such as lozenges comprising active ingredient in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; mucosaadherent gels, and mouthwashes comprising the active ingredient in a suitable liquid carrier.

When desired, the above-described formulations can be adapted to give sustained release of the active ingredient employed, e.g., by combination with certain hydrophilic polymer matrices, e.g., comprising natural gels, synthetic polymer gels or mixtures thereof. The polymer matrix can be coated onto, or used to form, a medical prosthesis, such as a stent, valve, shunt, graft, or the like.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the active compound with the softened or melted carrier(s) followed by chilling and shaping in molds. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, dichlorodichloromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compounds according to the invention may take the form of a dry powder composition, for example, a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges or, e.g., gelatin or blister packs from which the compound may be administered with the aid of an inhalator or insufflator.

For intra-nasal administration, the compounds according to the invention may be administered via a liquid spray, such as via an aerosol insufflator, nebulator or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

For administration by inhalation, the compounds according to the invention may be administered into a liquid spray. Such sprays may be prepared by dissolving the compound in anhydrous ethanol and spraying into a chamber maintained at a specific temperature ranging from ambient temperature to the temperature of the refrigerating device (20°C to 4°C) overnight. Ethanol, triethylamine and/or the appropriate volatile amine were then evaporated under vacuum in a rotary evaporator. The resulting residue was subjected to silica gel column chromatographic purification using chloroform-methanol or methylene chloride-methanol mixed solvent as the mobile phase. Derivatives were utilized to assure the identity and purity of the synthetic compounds.

Example 2

Parthenolide (100 mg, 0.4 mmol), diethylamine (2M in methanol, 1 mL), triethylamine (2 mL), ethanol (30 mL) were refluxed overnight. After column purification, 109 mg of pale yellow 11,13-dihydro, 13-dimethylaminoparthenolide was obtained (Yield: 93%). Melting point: 143-144°C. 1H-NMR (300 MHz, CDCl3): 6 5.22 (1H, d), 3.85 (1H, t), 7.25 (2H, m), 2.65 (1H, d), 2.5-2.3 (3H, m), 2.25 (6H, s), 2.5-2.0 (5H, m), 1.7 (3H, s), 1.3 (3H, s), 1.3-1.15 (1H, m). C-NMR (300 MHz, CDCl3): 6 176.1, 134.4, 124.8, 81.9, 66.4, 61.3, 57.6, 47.8, 46.4, 46.1, 41.0, 36.6, 29.9, 24.0, 17.2, 16.9. Mass Spec (GC-MS): 293 (M+) Retention time: 12.56 minutes. Ultra-violet (Methanol): λmax at 214 nm. Infra-Red (Nujol): 1757.9, 16.9. Mass Spec (GC-MS): 293 (M+) Retention time: 12.56 minutes. Ultra-violet (Methanol): λmax at 214 nm. Infra-Red (Nujol): 1757.9, 1460, 1377 cm⁻¹ . X-ray crystallographic analysis using a Nonius KappaCCD diffractometer DMAPT (11S,11,13-dihydro,13-dimethylaminoparthenolide) has the S-configuration at C-11.

Example 3

Parthenolide (100 mg, 0.4 mmol), diethylamine (2M in methanol, 1 mL), triethylamine (2 mL), ethanol (30 mL) were refluxed overnight. After column purification, 114 mg of yellow 11S,13-dihydro,13-diethylaminoparthenolide was obtained (Yield: 88%).

Example 4

Preparation of Salts of 11S,11,13-Dihydro,13-aminoparthenolide Derivatives

The aminoparthenolide derivative was dissolved in anhydrous ether and to this solution was added the corresponding acid in ether or ethanol. The mixture was kept in the refrigerator (4°C) overnight. The crystals formed were filtered and dried under vacuum, or submitted to further recrystallization, if needed.

Example 5

Preparation of 11S,11,13-dihydro,13-(piperidin-1-yl)parthenolide hydrochloride

A mixture of parthenolide (Sigma P 0667, 100 mg, 0.4 mmol), the appropriate primary amine or secondary amine (2 mmol), and triethylamine (1 to 2 mL) in 30 mL of anhydrous ethanol was stirred at a specific temperature ranging from ambient temperature to the temperature of the refrigerating device (~20°C to 4°C) overnight for 24 hours. Ethanol, triethylamine and/or the appropriate volatile amine were then evaporated under vacuum in a rotary evaporator. The resulting residue was subjected to silica gel column chromatographic purification using chloroform-methanol or methylene chloride-methanol mixed solvent as the mobile phase. NMR (Varian, 300 MHz and 400 MHz) and GC/MS (Agilent, 6890GC and 5973 MSD) analysis methodologies were utilized to assure the identity and purity of the synthetic compounds.

EXAMPLES

Example 1

General Synthetic Procedure for the Preparation of 11S,11,13-Dihydro,13-Substituted Aminoparthenolides

A mixture of parthenolide (Sigma P 0667, 100 mg, 0.4 mmol), the appropriate primary amine or secondary amine
ether (1M, 0.015 mL) was added to the ether solution until the solution became cloudy; then more ether was added and the mixture was heated to obtain a clear solution. The mixture was left in refrigerator (4°C) for more than 24 hours. The white crystals that formed were filtered through filter paper, and dried under vacuum overnight (Yield: 18%).

**Example 6**

Preparation of 11S,11,13-dihydro,13-dimethylaminoparthenolide maleate

To 11S,11,13-dihydro,13-dimethylaminoparthenolide (30 mg, 0.1 mmol) in anhydrous ethanol (5 mL) was added maleic acid (12 mg, 0.1 mmol) in 3 mL of anhydrous ethanol. The solution was shaken well and filtered through a regular filter paper. The clear solution was left in the refrigerator for a week. The white crystals formed were obtained by filtration, dried in a desiccator under vacuum, over anhydrous CaCl₂. Recrystallization from acetone-ether afforded pale yellow crystals (Yield: 55%).

**Example 7**

Preparation of 11S,11,13-dihydro,13-Dimethylaminoparthenolide methiodide

To 11S,11,13-dihydro,13-dimethylaminoparthenolide (30 mg, 0.1 mmol) in anhydrous methanol (5 mL) was added iodomethane (90 mg, 0.6 mmol) in methanol (1 mL). The clear solution was shaken and stored at room temperature. After three days, the methanol was evaporated, the pale yellow residue was dried in a desiccator under vacuum, over anhydrous CaCl₂. Recrystallization from acetone-ether afforded pale yellow crystals (Yield: 86%).

**Example 8**

11S,11,13-dihydro,13-(4-Methylpiperidin-1-yl)parthenolide (35 mg, 0.1 mmol) in anhydrous methanol (5 mL) was added iodomethane (90 mg, 0.6 mmol) in methanol (1 mL). The clear solution was shaken and stored at room temperature. After three days, the methanol was evaporated, the pale yellow residue was dried in a desiccator under vacuum, over anhydrous CaCl₂. Recrystallization from acetone-ether afforded pale yellow crystals (Yield: 79%).

**Example 9**

Assay for Antileukemic Activity

For apoptosis analysis, one million primary acute myelogenous leukemia (AML) cells were washed with cold PBS and resuspended in 200 microliters of Annexin binding buffer (10 mM HEPES/NaOH pH 7.4; 140 mM NaCl; 2.5 mM CaCl₂). Annexin V-FITC (Pharmingen) and 0.25 mg/mL 7-AAD (7-aminoactinomycin D, Molecular Probes, CA) were added and the tubes were incubated at room temperature in the dark for 15 minutes. Cells were then diluted with 200 microliters of Annexin binding buffer and analyzed immediately by flow cytometry. Viable cells were identified as failing to label with Annexin V or 7-AAD. Cells beginning to die label with Annexin V, and as membrane integrity is lost, will also label with 7-AAD. For each parthenolide derivative, the percentage of viable cells was determined after 24 hours of culture at a 10 μM concentration. Data are normalized to untreated control specimens. The data are in Table 1 for aminoparthenolide derivatives and Table 2 for the salts of some aminoparthenolides.

Healthy human bone marrow cells were used in the above assay to test the cytotoxicity of parthenolide. Eighty-five percent of the normal cells survived 10 μM of parthenolide. All the aminoparthenolides evaluated afforded results similar to parthenolide, i.e. the survival rate of healthy human bone marrow cells was over 85% at a concentration of 10 μM.

### Table 1

<table>
<thead>
<tr>
<th>Aminoparthenolides and their antileukemic activity</th>
<th>Reactants and Solvent</th>
<th>Reaction Conditions</th>
<th>Yield Antileukemic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parthenolide</td>
<td>Sigma P0667</td>
<td>Not applicable (N.A.)</td>
<td>N.A. 10 μM, 84%</td>
</tr>
<tr>
<td>11S,11,13-Dihydro,13-dimethylaminoparthenolide</td>
<td>Parthenolide (100 mg), dimethylamine (2M in methanol, 1 mL), triethylamine(2 mL), ethanol (30 mL)</td>
<td>Refluxing overnight</td>
<td>93 5 μM, 31%</td>
</tr>
<tr>
<td>(DMAPT)</td>
<td></td>
<td></td>
<td>10 μM, 90%</td>
</tr>
<tr>
<td>11S,11,13-Dihydro,13-diethylaminoparthenolide</td>
<td>Parthenolide (100 mg), diethylamine (200 mg, 2.7 mmol), triethylamine (2 mL), ethanol (30 mL)</td>
<td>Refluxing overnight</td>
<td>88 10 μM, 60%</td>
</tr>
<tr>
<td>(DEAPT)</td>
<td></td>
<td></td>
<td>20 μM, 95%</td>
</tr>
<tr>
<td>11S,11,13-Dihydro,13-(tert-butylamino)parthenolide</td>
<td>Parthenolide (20 mg), tert-butylamine (0.2 mL), triethylamine (0.4 mL), ethanol (5 mL)</td>
<td>Refluxing 10 hours</td>
<td>39 10 μM, 20%</td>
</tr>
<tr>
<td>Compound</td>
<td>Reactants and Solvent</td>
<td>Reaction Conditions</td>
<td>Yield</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------</td>
<td>---------------------</td>
<td>-------</td>
</tr>
<tr>
<td>11S,11,13-Dihydro,13-(pyrroolidin-1-yl)parthenolide (PyrPT)</td>
<td>Parthenolide (30 mg), pyrroolidine (0.2 mL), triethylamine (0.2 mL), ethanol (5 mL)</td>
<td>Refluxing overnight</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Parthenolide (250 mg), piperidine (1 mL), triethylamine (5 mL), ethanol (100 mL)</td>
<td>Refluxing overnight</td>
<td>5</td>
</tr>
<tr>
<td>11S,11,13-Dihydro,13-(morpholin-1-yl)parthenolide (MorPT)</td>
<td>Parthenolide (100 mg), morpholine (0.5 mL), triethylamine (5 mL), ethanol (30 mL)</td>
<td>Refluxing overnight</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Parthenolide (100 mg), morpholine (0.5 mL), triethylamine (2 mL), ethanol (30 mL)</td>
<td>Refluxing overnight</td>
<td>20</td>
</tr>
<tr>
<td>11S,11,13-Dihydro,13-(4-methylpiperidin-1-yl)parthenolide (4MePipPT)</td>
<td>Parthenolide (30 mg), 4-methylpiperidinlene (0.2 mL), triethylamine (1 mL), ethanol (20 mL)</td>
<td>Refluxing overnight</td>
<td>82</td>
</tr>
<tr>
<td>11S,11,13-Dihydro,13-(heptamethyleneimino-1-yl)parthenolide (HeptaMePipPT)</td>
<td>Parthenolide (100 mg), heptamethyleneimino (500 mg), triethylamine (2 mL), ethanol (30 mL)</td>
<td>Refluxing overnight</td>
<td>74</td>
</tr>
<tr>
<td>11S,11,13-Dihydro,13-(azetidin-1-yl)parthenolide (AzePT)</td>
<td>Parthenolide (100 mg), azetidin (100 mg), triethylamine (2 mL), ethanol (20 mL)</td>
<td>Stirred at room temperature 2 days</td>
<td>93</td>
</tr>
<tr>
<td>11S,11,13-Dihydro,13-diallylamino parthenolide</td>
<td>Parthenolide 25 mg, Methanol, 8 hrs</td>
<td>Room temperature with stirring</td>
<td>88%</td>
</tr>
<tr>
<td>11S,11,13-Dihydro,13-ethylamino parthenolide</td>
<td>Parthenolide 25 mg, Ethylamine 90 mg, Methanol, 5 hrs</td>
<td>Room temperature with stirring</td>
<td>90%</td>
</tr>
<tr>
<td>11S,11,13-Dihydro,13-methylamino parthenolide</td>
<td>Parthenolide 25 mg, Methanol, 5 hrs</td>
<td>Room temperature with stirring</td>
<td>93%</td>
</tr>
<tr>
<td>11S,11,13-Dihydro,13-cyclopropylamino parthenolide</td>
<td>Parthenolide 25 mg, Cyclopropylamine 20 mg Methanol, 6 hrs</td>
<td>Room temperature with stirring</td>
<td>90%</td>
</tr>
<tr>
<td>11S,11,13-Dihydro,13-propargylamino parthenolide</td>
<td>Parthenolide 25 mg, Propargylamine 20 mg Methanol, 6 hrs</td>
<td>Room temperature with stirring</td>
<td>82%</td>
</tr>
</tbody>
</table>
### TABLE 2
Aminoparthenolide salts and their antileukemic activity

| Compound | Reactants and Solvent | Reaction Conditions | Yield Antileukemic activity (%)
|----------|-----------------------|---------------------|------------------
| 11S,11,13-Dihydro,13-dimethylaminoparthenolide hydrochloride | 11S,11,13-Dihydro,13-dimethylaminoparthenolide (10 mg), HCl in ether (1M, 0.03 mL) | Refrigerator, 24 hours | 72 |
| 11S,11,13-Dihydro,13-(pyrrolidin-1-yl)parthenolide hydrochloride | 11S,11,13-Dihydro, 13-(pyrrolidin-1-yl)parthenolide (5 mg), HCl in ether (1M, 0.015 mL) | Refrigerator, 24 hours | 10 |
| 11S,11,13-Dihydro,13-(piperidin-1-yl)parthenolide hydrochloride | 11S,11,13-Dihydro, 13-(piperidin-1-yl)parthenolide (5 mg), HCl in ether (1M, 0.015 mL) | Refrigerator, 24 hours | 18 |
| 11S,11,13-Dihydro,13-(4-methylpiperidin-1-yl)parthenolide hydrochloride | 11S,11,13-Dihydro, 13-(4-methylpiperidin-1-yl)parthenolide (50 mg), HCl in ether (1M, 0.15 mL) | Room temperature for 1 week | 38.3 |
| 11S,11,13-Dihydro,13-dimethylaminoparthenolide maleate | 11S,11,13-Dihydro, 13-dimethylaminoparthenolide (30 mg), maleic acid (12 mg), ethanol (8 mL) | Room temperature for 3 days | 55 |
| 11S,11,13-Dihydro,13-dimethylaminoparthenolide methiodide | 11S,11,13-Dihydro, 13-dimethylaminoparthenolide (30 mg), added iodomethane (90 mg), methanol (6 mL) | Room temperature for 3 days | 86 |
| 11S,11,13-Dihydro,13-(4-methylpiperidin-1-yl)parthenolide methiodide | 11S,11,13-Dihydro, 13-(4-methylpiperidin-1-yl)parthenolide (70 mg), iodomethane (200 mg), methanol (12 mL) | Room temperature for 3 days | 79 |

Example 10

**Analysis of Parthenolide and Dimethylaminoparthenolide (DMAPT) Using Human-Mouse Xenografts**

To assess the effect of parthenolide on primary human stem cell populations, experiments were conducted using transplantation into immune deficient NOD/SCID mice. Successful engraftment of NOD/SCID bone marrow at 6-8 weeks post-transplant has been shown to be a measure of stem cell content for human hematopoietic cell populations (Lapidot et al., *J Mol Med.* 1997; 75: 664-673; Dick, *Curr Opin Hematol.* 1996; 3:405-409). For each experiment, cryopreserved mononuclear cell specimens from normal or AML donors were thawed, and treated in vitro with 7.5 micromolar parthenolide for 12-18 hours. Following culture, 5-10 million cells/animal were injected intravenously into sublethally irradiated (300 Rad) NOD/SCID mice. After 6-8 weeks, animals were sacrificed and bone marrow was analyzed for the presence of human cells using flow cytometry as previously described (Guzman et al., *Proc Natl Acad Sci*).
treated with parthenolide and derivatives PIPT ((11S,11,13-
metric readings were obtained using the MTS/PMS system
tested were from an average of eight wells. Each experiment
was expressed as a percentage of the solvent control and
hormone refractory prostate cancer cell line CWR22Rv1
was treated with increasing concentrations of
parthenolide derivatives DMAPT, PIPT and 4MEPT for
three hours. Cellular proliferation was reduced by up to 80%
at 2 μm in the clonogenic assay (FIG. 1). The breast cancer
clonogenic assay with bcl-100, mdm-231 and 436
cells showed almost complete inhibition of proliferation
with DMAPT at 2 μm concentration in the clonogenic assay
(FIGS. 6-8). Parthenolide also reduced proliferation with similar dosage ranges.

Example 13
cDNA Array Analysis

Total cellular RNA was extracted from the human mono
lytes cell line THP-1 under three conditions 2 hours after
Time 0:
1) Control was added at Time 0
2) Lipopolysaccharide (10 nM) was added at Time plus one
(1) hour.
3) At Time 0, 10 micromoles of DMAPT was added and then at Time=1 LPS (10 nM) was added.
RNA was extracted using RNeasy Min Kit (Qiagen, USA)
according to the manufacturer’s instructions. The Human
Drug Targets for Inflammation and Immunomodulation Q
series GE array kit (HS-048-12) was obtained from Super
Array Bioscience Corporation (Frederick, Md.). The kit
determines expression of 96 genes that are associated with
inflammation. RNA from respective samples was used as a
template to generate biotin labeled cDNA probes using
GEArray Ampolabelling RT kit (SuperArray, Bioscience
Corp., USA). The cDNA probes corresponding to the
mRNA population were then denatured and hybridization
was carried out in GEHyb solution to nylon membranes
spotted with gene specific fragments. Membranes were then
washed in 2xSSC, 1% SDS twice for 15 minutes each,
followed by 0.1 SSC, 0.5% SDS twice for 15 minutes each.
Chromiluminescence was used to visualize the expression
levels of each transcript and the results were quantified with
the GEArray Analyzer. The change in a given gene transcript
was estimated by normalizing the signal intensities with the
signal derived from PPIA and with minimum background
subtraction.

As can be seen in Table 3, transcription of 25 genes was
increased after pre-treatment with LPS. More importantly
pretreatment with DMAPT prevented or blunted the increase
in gene transcription induced by LPS. For example, the
transcription of tumor necrosis factor (TNF), released in
septic shock, is increased by 3 fold (298%) when treated
with LPS. Pretreatment with DMAPT however prevents
transcription of LPS and in fact decreases its production to
2% of control. Similarly, transcription of cyclooxygenase-2,
the target of classical non-steroidal anti-inflammatory
agents, was increased 1.5 fold (150%). In the presence of
DMAPT, the gene expression not only prevented the
increase by LPS but decreased it to 30% (0.7) of solvent
control. DMAPT therefore may act to decrease inflammation
by decreasing cytokines as evidenced by decreased genes in human monocytes

### Table 3

<table>
<thead>
<tr>
<th>Gene Description</th>
<th>cDNA Array Analysis</th>
<th>DMAPT Pre-treatment for LPS Treatment for 1 hour: Treatment of LPS</th>
<th>% Change of Gene</th>
<th>% Change of Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD28 antigen (7p44)</td>
<td>23</td>
<td>0.814</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3G antigen, gamma polypeptide (T3 complex)</td>
<td>14</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Example 11

MTS-PMS Assay

A 96-well U-bottomed plate (Becton Dickinson Labware,
Franklin Lakes, N.J.) at a concentration of 5,000 cells per 50
microliters (mL) of media was incubated in 5%
C. for 24 hours. Varying compound concentrations in 50 mL
of media were added to the media 24 hours later. Colori­
in H522 (FIGS. 1-3).

Example 12

Clonogenic Assay

Initially, 100 cells growing in log phase were plated per
3 mL of media in each well of a six well plate. After 24 hrs
of plating of the cells the test compound was added at
varying concentrations. At 24 and 96 hours after addition of
drug, the media was changed. Hence, the cells were only
exposed to the drug for 24 hrs. When cell colonies appeared
at Day 15 they were stained by Sure Stain Dye and counted.
The hormone refractory prostate cancer cell line CWR22Rv1
was treated with increasing concentrations of

31
USA 2002; 99: 16220-162253). Human specific antibodies
for CD45 were used to assess the level of total engraftment.

In three independent experiments, the level of engraftment
for parthenolide-treated AML cells was dramatically
reduced, which indicates a direct effect on the AML stem
cell compartment. In contrast, no reduction in engraftment
was detected for parthenolide-treated normal specimens,
thus showing the parthenolide does not target normal
hematopoietic stem cells. Similarly, treatment of AML cells
with 7.5 micromolar DMAPT also yielded a strong reduc-
tion in NOD/SCID engraftment while treatment of normal
cells showed no significant effects.
TABLE 3-continued

cDNA Array Analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>LPS Treatment for 1 hour: % Change of Gene</th>
<th>DMAPT Pre-treatment for 2 hours then 1 hour Treatment of LPS: % Change of Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony stimulating factor 2 (granulocyte-macrophage)</td>
<td>26</td>
<td>0.026</td>
</tr>
<tr>
<td>Interleukin 13</td>
<td>93</td>
<td>0.64</td>
</tr>
<tr>
<td>Intercellular adhesion molecule 1</td>
<td>257</td>
<td>58</td>
</tr>
<tr>
<td>Interleukin 1 receptor, type I</td>
<td>10</td>
<td>0.33</td>
</tr>
<tr>
<td>Interleukin 1 receptor, type II</td>
<td>326</td>
<td>0.29</td>
</tr>
<tr>
<td>Nitric oxide synthase 2A (inducible)</td>
<td>226</td>
<td>48</td>
</tr>
<tr>
<td>Phosphodiesterase 4A, cAMP-specific</td>
<td>14</td>
<td>0.46</td>
</tr>
<tr>
<td>Phosphodiesterase 4B, cAMP-specific</td>
<td>220</td>
<td>0.59</td>
</tr>
<tr>
<td>Phospholipase A2, group IB (pancreas)</td>
<td>114</td>
<td>0.57</td>
</tr>
<tr>
<td>Phospholipase A2, group IV</td>
<td>350</td>
<td>0.89</td>
</tr>
<tr>
<td>Phospholipase A2, group VII</td>
<td>129</td>
<td>0.05</td>
</tr>
<tr>
<td>Phospholipase C, gamma 1</td>
<td>342</td>
<td>0.24</td>
</tr>
<tr>
<td>Peroxirane proliferative activated receptor, gamma</td>
<td>49</td>
<td>0.48</td>
</tr>
<tr>
<td>Platelet-activating factor receptor</td>
<td>32</td>
<td>0.002</td>
</tr>
<tr>
<td>Prostaglandin D2 receptor (DP)</td>
<td>35</td>
<td>0.17</td>
</tr>
<tr>
<td>Prostaglandin E receptor (EP)</td>
<td>879</td>
<td>1.46</td>
</tr>
<tr>
<td>Cyclooxygenase 1</td>
<td>176</td>
<td>0.731</td>
</tr>
<tr>
<td>Cyclooxygenase 2</td>
<td>152</td>
<td>0.7</td>
</tr>
<tr>
<td>Thromboxane A synthase 1</td>
<td>283</td>
<td>0.07</td>
</tr>
<tr>
<td>Tumor necrosis factor (TNF superfamily, member 2)</td>
<td>298</td>
<td>0.02</td>
</tr>
<tr>
<td>Tumor necrosis factor (ligand) superfamily, member 13b</td>
<td>217</td>
<td>0.89</td>
</tr>
<tr>
<td>Tumor necrosis factor (ligand) superfamily, member 5</td>
<td>692</td>
<td>23</td>
</tr>
<tr>
<td>Vascular cell adhesion molecule 1</td>
<td>154</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Example 14

Oral Bioavailability in Mice

Preliminary in vivo work was conducted to determine the bioavailability and toxicity of this agent in mice. As shown in FIG. 4 it was demonstrated that whereas 40 mg/kg of oral parthenolide provided a plasma level one hour after oral gavage of only ~200 nM, the same dose of DMAPT provided a plasma level of ~2500 ng/mL (ie 8 nM—average of 5 mice in each group; measured by LC-MS). Given the concern from providing such a high plasma concentration we completed a preliminary toxicity study that showed the mice gained weight and survived three weeks of daily treatment with oral DMAPT at 40 mg/kg with no overt toxicities.

Example 15

Electrophoretic Mobility Gel Shift Assay

Each cancer cell line in exponential growth phase was treated with solvent control or various concentrations of parthenolide derivatives dissolved in 100% ethanol for 3 hours prior to harvesting. Cells were harvested and whole cell extracts were prepared as described previously (Nakshatri et al., Mol Cell Biol, 17: 3629-3639, 1997; Sweeney et al., Clin Cancer Res, 10: 5501-5507, 2004). Extracts were incubated with a radiolabelled NF-κB probe for 30 minutes at room temperature. The oligonucleotide probe binds to the NF-κB DNA binding site in the promoter region of the immunoglobulin gene. Electrophoresis and autoradiography were performed as described previously (Nakshatri et al., 1997) using NF-κB and SP-1 probes (Promega, Madison, Wis.). The specificity of parthenolide and derivative inhibition of NF-κB DNA binding was verified by the use of the SP-1 probe as a control. Identification of the NF-κB subunits binding to DNA and inhibited by DMAPT were identified by gel supershift. Constitutive NF-κB DNA binding activity was determined in the lung cancer cell lines A-549, H-23, H-522, and H-460. All four non-small lung cancer cells were treated with increasing concentrations of DMAPT for three hours, and NF-κB DNA binding was measured by electrophoretic mobility shift assay (EMSA) as described. NF-κB DNA binding activity was highest in A-549 cells, followed by H-23, H-522 and H-460 cells. DMAPT between 2 and 10 micromolar substantially decreased NF-κB DNA binding activity in all lung cancer cell lines tested.

Cancer cell lines HT-1376 and UMUC-3 were treated with increasing concentrations of DMAPT for three hours. Whole cell extracts were prepared as described and DNA binding by NF-κB was analyzed by EMSA with NF-κB and OCT-1 (internal control) probes. DMAPT decreased NF-κB DNA binding in a dose-dependent manner with HT-1376 and UMUC-3 cell lines (FIG. 10). The hormone refractory prostate cancer cell line, CWR22Rv1 was treated with increasing concentrations of DMAPT for three hours. Whole cell extracts were prepared as described and DNA binding by NF-κB was analyzed by EMSA with NF-κB and OCT-1 (internal control) probes. DMAPT decreased NF-κB DNA binding in a dose dependent manner with substantial decreases of NF-κB DNA binding at 10 μM DMAPT.

EMSA results thus showed DMAPT decreased the constitutive NF-κB DNA binding in several cancer cell lines.
Pretreatment of Radiation Sensitive Cell Line A549

The radiation sensitive cell line A549 was pretreated with parthenolide concentrations ranging from 0 to 2.5 micromolar. The cells were then subjected to ionizing radiation doses ranging from 0-6Gy and survival fraction of the cells determined. Results demonstrated that parthenolide induced radiation sensitivity to the cells in a dose-dependent manner with survival fraction at 2.5 micromolar ranging from 10% at 2Gy to less than 1% at 6Gy. Cells not receiving pre-treatment with parthenolide had greater than 50% survival fraction at the highest radiation dose of 6Gy and over 90% survival at 2Gy.

TRAIL Induced Apoptosis Assay

MDA-MB-231 breast cancer cells (2×105 cells in 60 mm plates) were treated first with 2 or 5 μM of DMAPT (LC-1). After two hours, TRAIL (TNF related-apoptosis-inducing-ligand, 5 ng/ml) were added. After 48 hours of TRAIL or TRAIL-RII antibody treatment, cells were harvested and apoptosis was measured using carboxyfluorescein-FLICA assay. Briefly, VAD-FMK and propidium iodide (upper right). Necrotic treatment with parthenolide had greater than activating antibody-induced apoptosis and atypical apoptosis were identified by FACScan analysis. Live cells do not stain radiating sensitivity to the cells in a dose-dependent manner upon pre-treatment with DMAPT.

Example 17

TRAIL Induced Apoptosis Assay

Example 16

Pretreatment of Radiation Sensitive Cell Line A549

The radiation sensitive cell line A549 was pretreated with parthenolide concentrations ranging from 0 to 2.5 micromolar. The cells were then subjected to ionizing radiation doses ranging from 0-6Gy and survival fraction of the cells determined. Results demonstrated that parthenolide induced radiation sensitivity to the cells in a dose-dependent manner with survival fraction at 2.5 micromolar ranging from 10% at 2Gy to less than 1% at 6Gy. Cells not receiving pre-treatment with parthenolide had greater than 50% survival fraction at the highest radiation dose of 6Gy and over 90% survival at 2Gy.

The invention claimed is:

1. A method of inhibiting cancer cell growth comprising administering to a mammal afflicted with a cancer selected from the group consisting of breast cancer, lung cancer and prostate cancer, an effective amount of a compound of formula (I):

```
R1 X1 R2 X2 R3 X3 Z
```

wherein:
- X1, X2, and X3 are O;
- R3, R5, R7, R9, and R10 are H, and R4 and R8 are methyl; and
- Z is —CH3R* wherein R* is an amino acid residue bonded to the Z methylene via a nitrogen or a sulfur atom; or R* is —NR1CO—R2, —NR1(C(O)NR2)—R1, —NR1R2, or —NR1R2R3Y wherein R1 and R2 are independently selected from H, CN, and optionally substituted straight-chained or branched aliphatic optionally containing 1 or more double or triple bonds; wherein optional substituents are selected from one or more of —NH2, —NH(C1-4 aliphatic), —N(C1-4 aliphatic)2, halogen, —OH, —O-(C1-4 aliphatic), —NO2, —CN, —CO2H, —CO2(C1-4 aliphatic), —(halo C1-4 aliphatic), or —halo(C1-4 aliphatic); wherein each C1-4 aliphatic is optionally substituted; or R1 and R2 are independently selected from cycloalkyl, heterocycloalkyl, aryl or heteroaryl; and provided that R1 and R2 are not simultaneously H; or where R* is NR1R2, R1 and R2 optionally together with the nitrogen atom form an optionally substituted 5-12 membered ring, said ring optionally comprising 1 or more heteroatoms or a group selected from —CO—, —SO—, and —SO2—;
- R11 is selected from H or C1-4 aliphatic; and
- Y* is selected from the group consisting of fluoride, chloride, bromide, iodide, sulfate, nitrate, bicarbonate, carbonate, acetate, carbonate, tartarate, succinate, benzoate, ascorbate, alpha-ketoglutarate, alphaglycerophosphate, methylsulfonate, toluenesulfonate, and benzensulfonate; or a pharmaceutically acceptable salt thereof.

2. The method of claim 1 wherein Z is —CH3—NR1R2.

3. The method of claim 2 wherein R1 and R2 are independently selected from hydrogen, —CN or optionally substituted C1-4 alkyl.

4. The method of claim 3 wherein R1 and R2 are independently selected from —NO2, —CN, —CH3, —CF3, —CH2CH3, —CH2CF3, —CH2Cl, —CH2OH, —CH2CH2OH and —CH2NH2.

5. The method of claim 2 wherein R1 and R2 together with N form an optionally substituted ring.

6. The method of claim 5 wherein said ring is a monocyclic, bicyclic or tricyclic alkyl or aryl ring system, said ring system optionally substituted and optionally comprising one or more heteroatoms or a group selected from —CO—, —SO—, and —SO2—.

7. The method of claim 6 wherein R1 and R2 are —CH2(CH3)2—CH2Y—; where Y is a heteroatom or a group selected from —CO—, —SO—, and —SO2--; n is an integer 0 to 5; and together with N form an optionally substituted ring optionally fused to a cycloalkyl or aryl group to form a bicyclic or tricyclic ring system, said system optionally substituted and optionally comprising one or more heteroatoms.

8. The method of claim 6 wherein R1 and R2 are —(CH2)n—Y—(CH2)m—; where Y is a heteroatom or a group selected from —CO—, —SO—, and —SO2--; a is an integer 0 to 5; b is an integer 0 to 5; the sum of a and b being 0 to 5; and together with N form an optionally substituted ring, said ring optionally fused to a cycloalkyl or aryl group to form a bicyclic or tricyclic ring system, said system optionally substituted and optionally comprising one or more heteroatoms.
10. The method of claim 1 wherein the compound of formula (I) is selected from:
11S,11,13-Dihydro,13-dimethylaminoparthenolide;
11S,11,13-Dihydro,13-diethylaminoparthenolide;
11S,11,13-Dihydro,13-(tert-butylamino)parthenolide;
11S,11,13-Dihydro,13-pyridin-1-yl)parthenolide;
11S,11,13-Dihydro,13-morpholin-1-yl)parthenolide;
11S,11,13-Dihydro,13-(4-(2'-hydroxyethyl)piperidin-1-yl)parthenolide;
11S,11,13-Dihydro,13-(piperazin-1-yl-4-carboxylic acid)parthenolide;
11S,11,13-Dihydro,13-(S-cysteinyl)parthenolide;
11S,11,13-Dihydro,13-(N-benzyl-N-ethylamine)parthenolide;
11S,11,13-Dihydro,13-(N-prolyl)parthenolide;
11S,11,13-Dihydro,13-(S-thiophenolyl)parthenolide;
11S,11,13-Dihydro,13-(N,N-diethanolamine)parthenolide;
11S,11,13-Dihydro,13-(thiomorpholin-4-yl)parthenolide;
11S,11,13-Dihydro,13-(4-hydroxypiperidin-1-yl)parthenolide;
11S,11,13-Dihydro,13-(1-methylhomopiperizin-4-yl)parthenolide;
11S,11,13-Dihydro,13-(S-mercaptoacetyl)parthenolide;
11S,11,13-Dihydro,13-(4-(2'-hydroxyethyl)piperidin-1-yl)parthenolide;
11S,11,13-Dihydro,13-(piperazine-1-yl-6-carboxaldehyde)parthenolide;
11S,11,13-Dihydro,13-(piperazine-1-yl-4-carboxaldehyde)parthenolide;
11S,11,13-Dihydro,13-(piperazine-1-yl-3-carboxaldehyde)parthenolide;
11S,11,13-Dihydro,13-(S-cysteinyl)parthenolide;