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Chen et al.

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(54) **PHARMACEUTICAL COMPOSITIONS COMPRISING POH DERIVATIVES**

(71) Applicant: **NeOnc Technologies, Inc.**, Los Angeles, CA (US)

(72) Inventors: **Thomas Chen**, La Canada, CA (US); **Daniel Levin**, La Canada, CA (US); **Satish Puppali**, Rancho Cucamonga, CA (US)

(73) Assignee: **UNIVERSITY OF SOUTHERN CALIFORNIA**, Los Angeles, CA (US)

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A61K 45/06 (2006.01)
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CPC ***A61K 45/06*** (2013.01); ***A61K 9/0043*** (2013.01); ***A61K 31/4015*** (2013.01);
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(58) **Field of Classification Search**
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(Continued)

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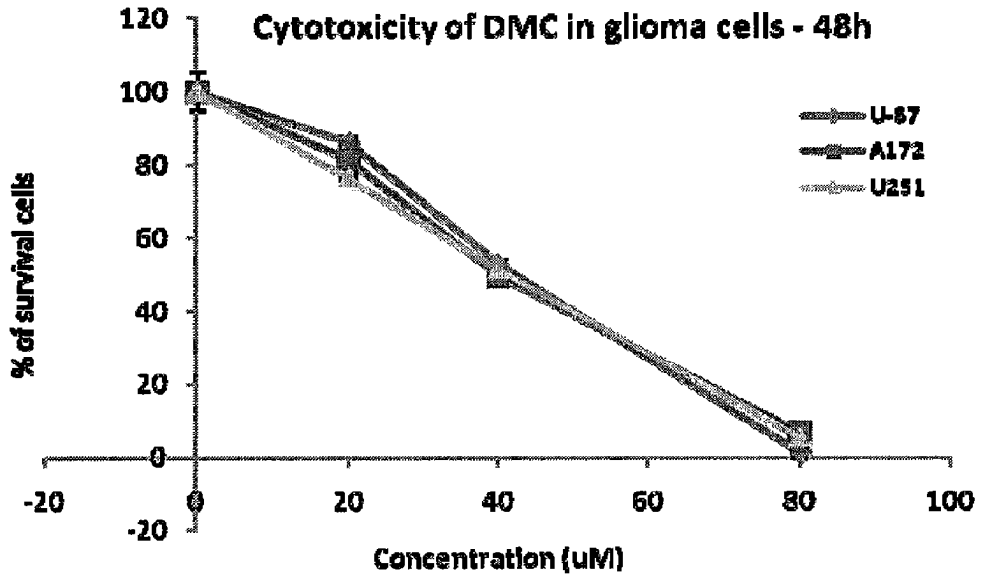
Primary Examiner — Clinton A Brooks
Assistant Examiner — Kyle Nottingham
(74) *Attorney, Agent, or Firm* — LEASON ELLIS LLP

(57) **ABSTRACT**

The present invention provides for a perillyl alcohol (POH) carbamate, such as POH-Rolipram. The present invention also provides for a method of treating a disease such as cancer, by delivering to a patient a therapeutically effective amount of POH-Rolipram.

5 Claims, 27 Drawing Sheets



Related U.S. Application Data

continuation of application No. 14/595,865, filed on Jan. 13, 2015, now abandoned, which is a continuation-in-part of application No. 14/455,293, filed on Aug. 8, 2014, now Pat. No. 9,663,428, which is a continuation of application No. 13/566,731, filed on Aug. 3, 2012, now Pat. No. 8,916,545, which is a continuation of application No. PCT/US2011/049392, filed on Aug. 26, 2011.

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(51) **Int. Cl.**

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(52) **U.S. Cl.**

CPC ***A61K 31/415*** (2013.01); ***A61K 47/54*** (2017.08); ***A61K 47/55*** (2017.08); ***C07D 207/26*** (2013.01); ***C07D 231/12*** (2013.01); ***C07D 487/04*** (2013.01); *A61N 5/10* (2013.01)

(58) **Field of Classification Search**

CPC A61K 47/55; C07D 207/26; C07D 231/12; C07D 487/04; A61N 5/10; A61P 35/00

See application file for complete search history.

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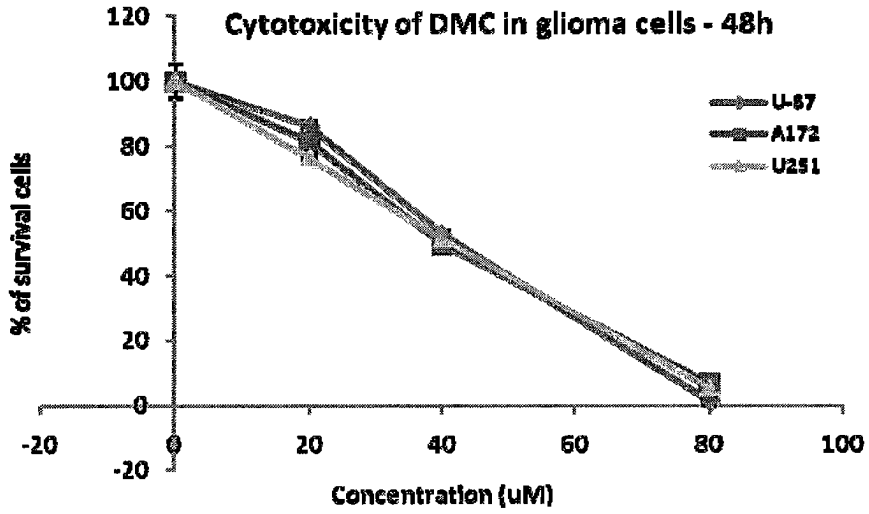
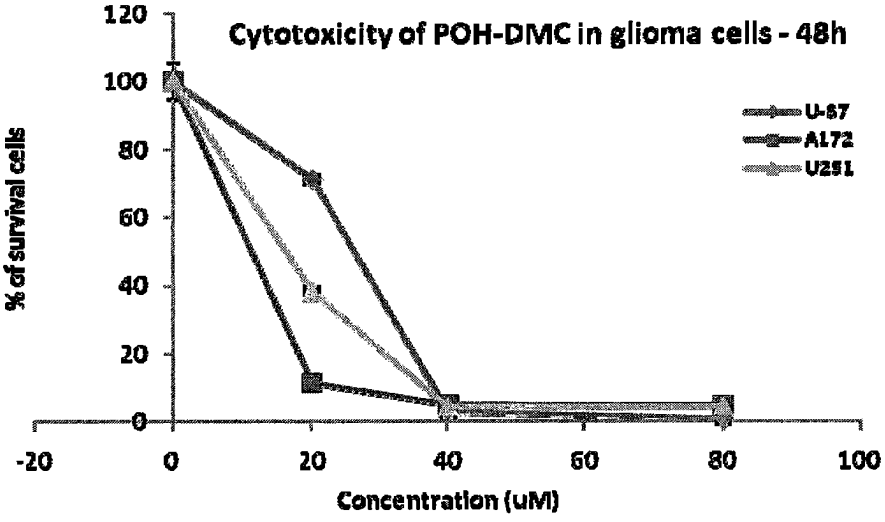


Figure 1



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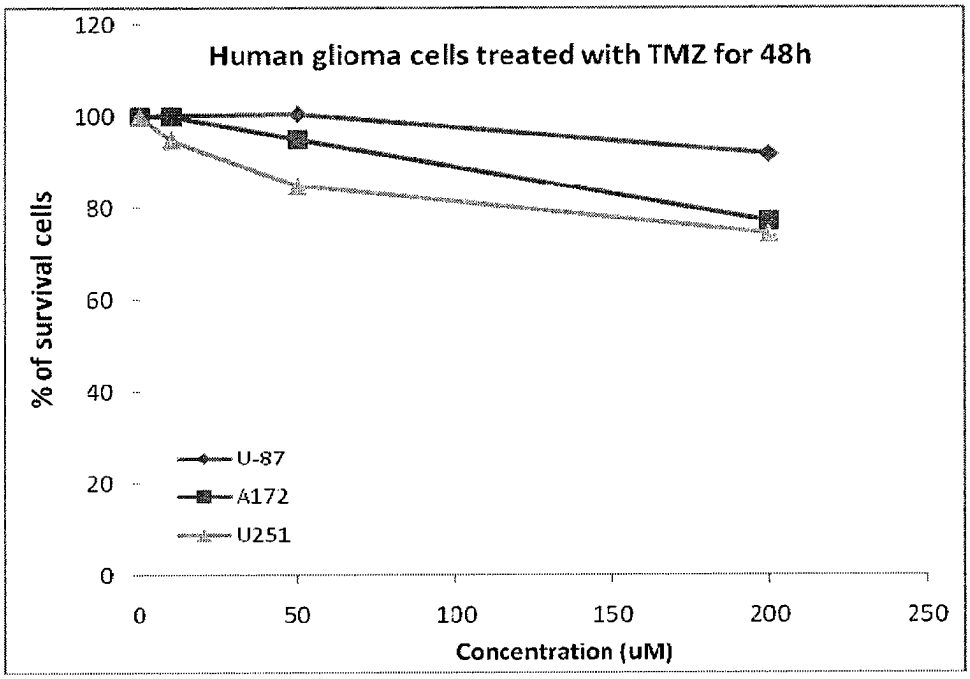


Figure 3

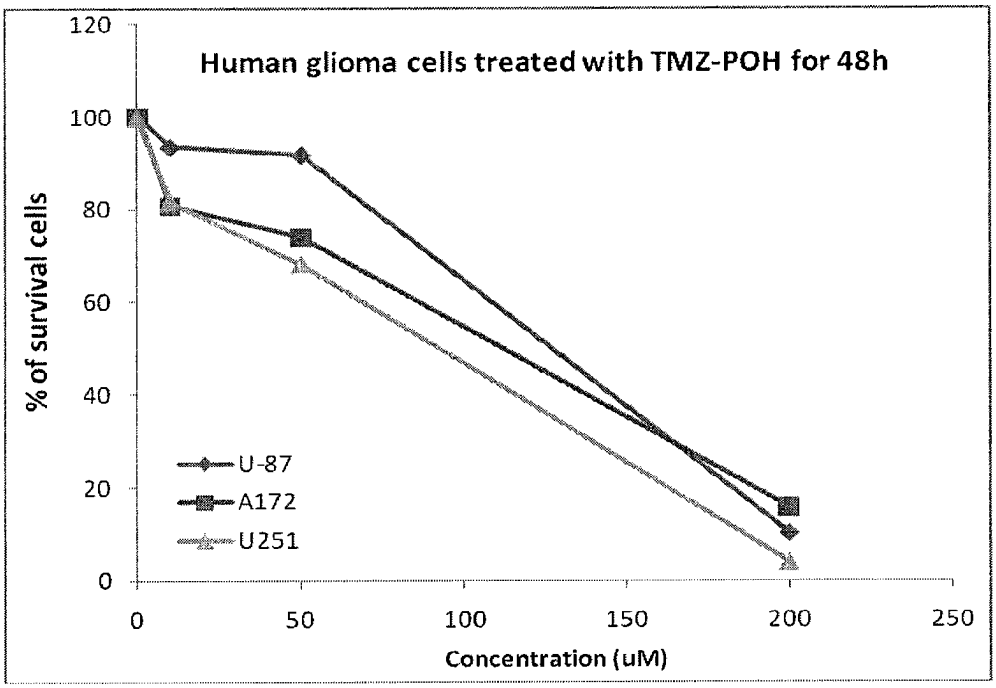


Figure 4

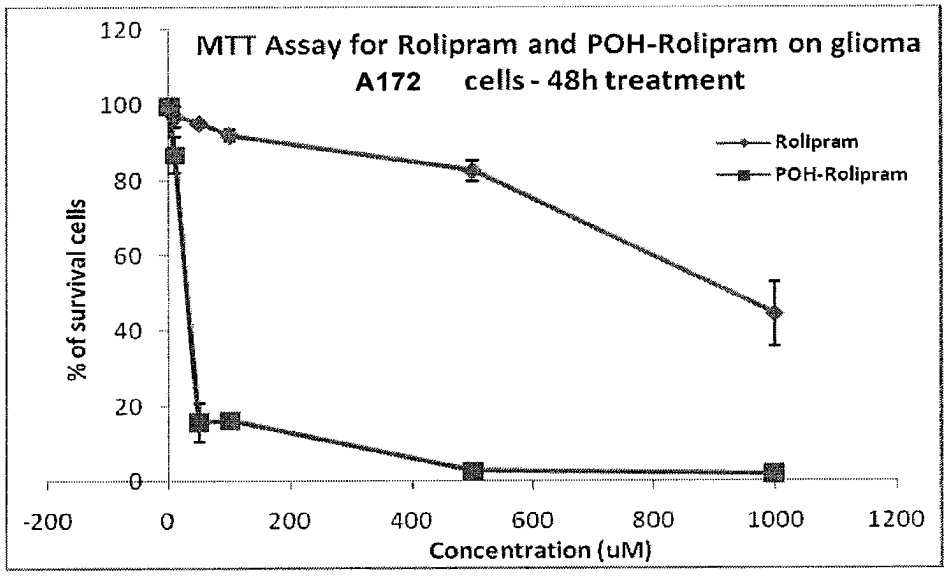


Figure 5

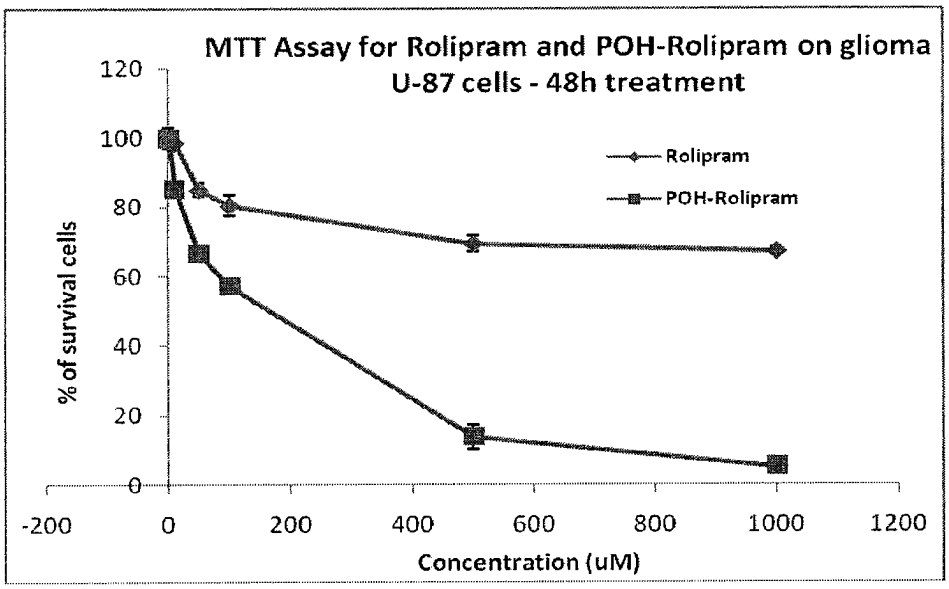


Figure 6

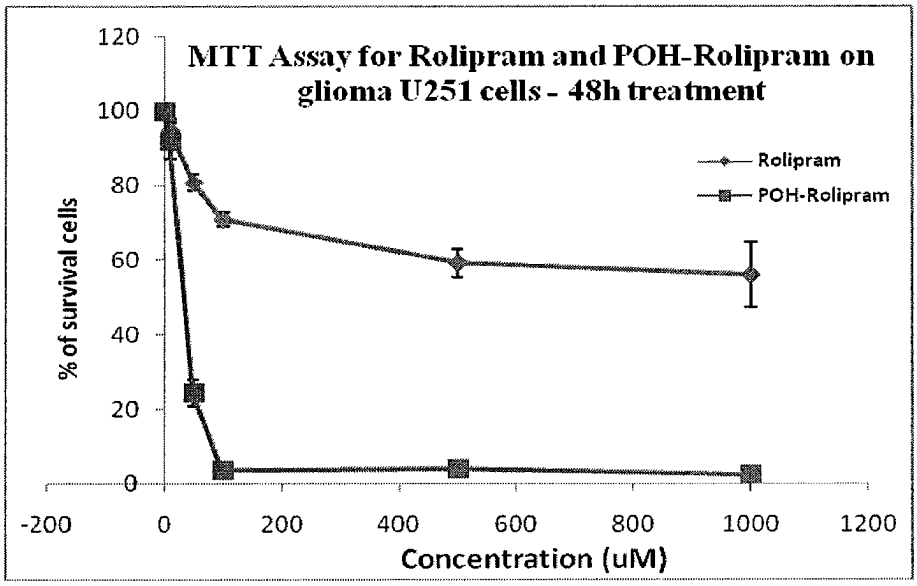


Figure 7

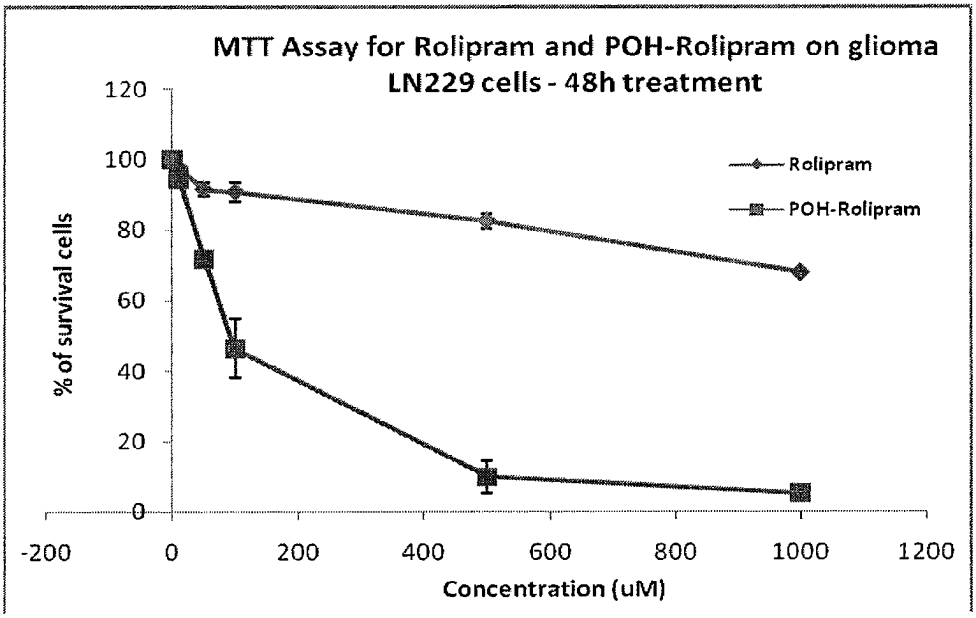


Figure 8

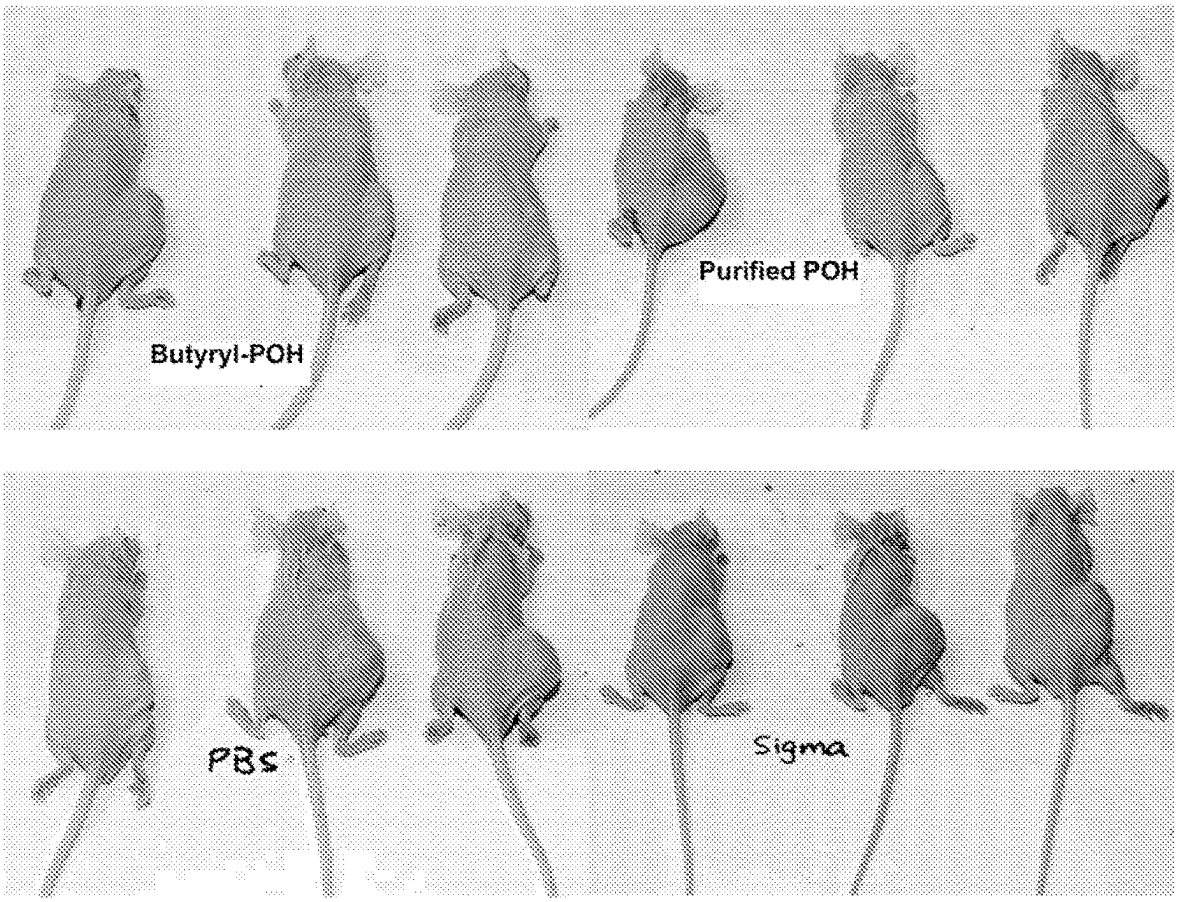


Figure 9A

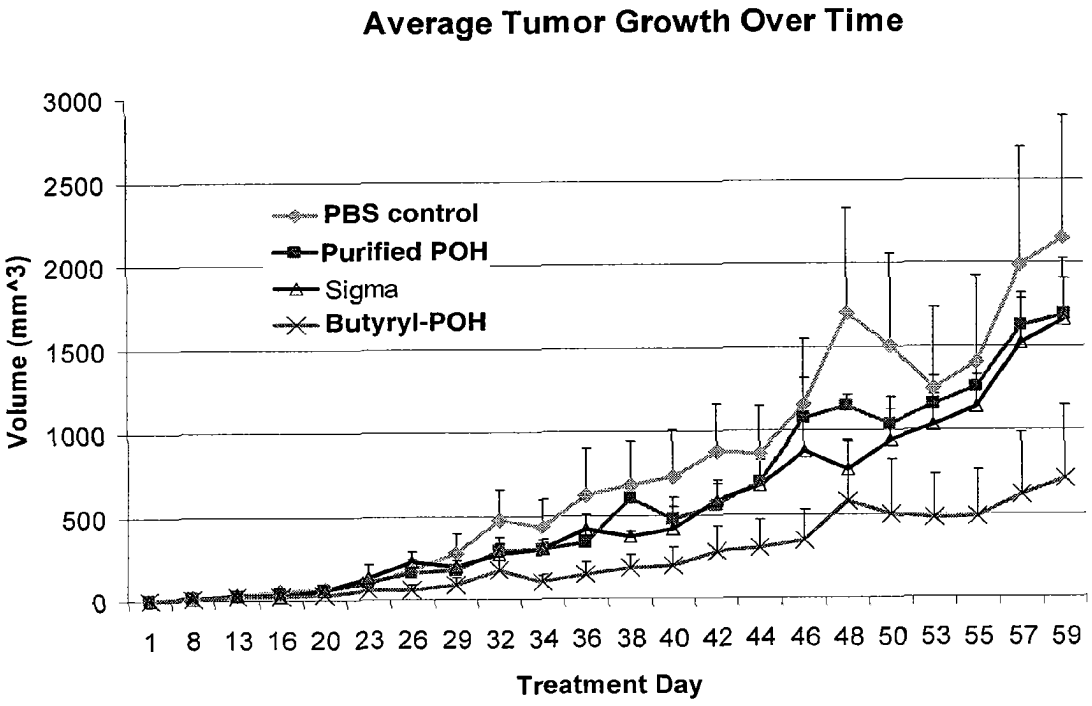

Average Tumor Growth Over Time
Volume (mm^3)
3000
2500
2000
1500
1000
500
0
PBS control
Purified POH
Sigma
Butyryl-POH
1 8 13 16 20 23 26 29 32 34 36 38 40 42 44 46 48 50 53 55 57 59
Treatment Day

Figure 9B

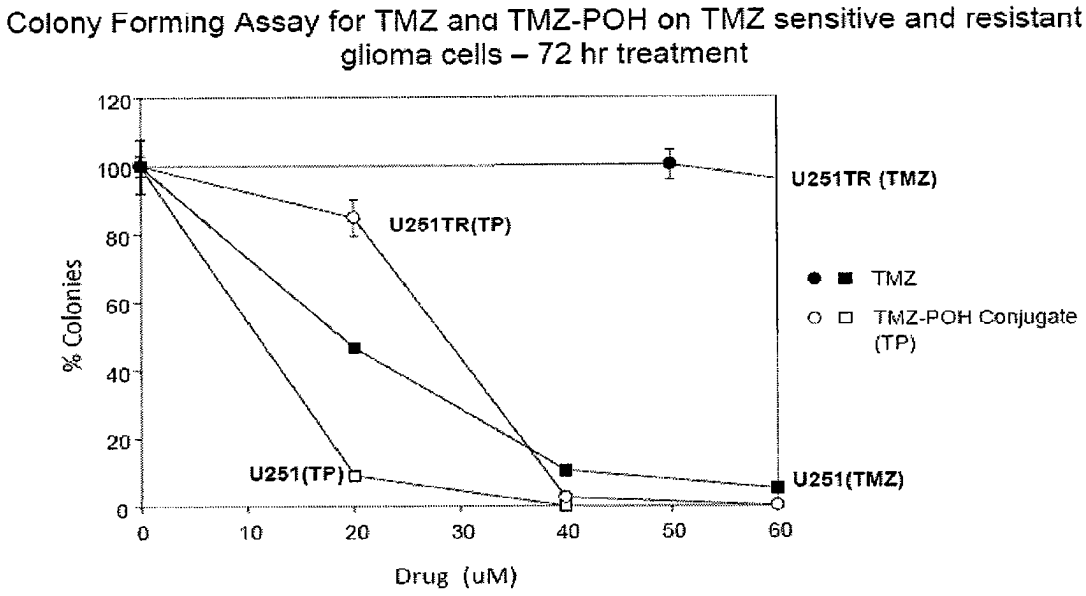


Figure 10

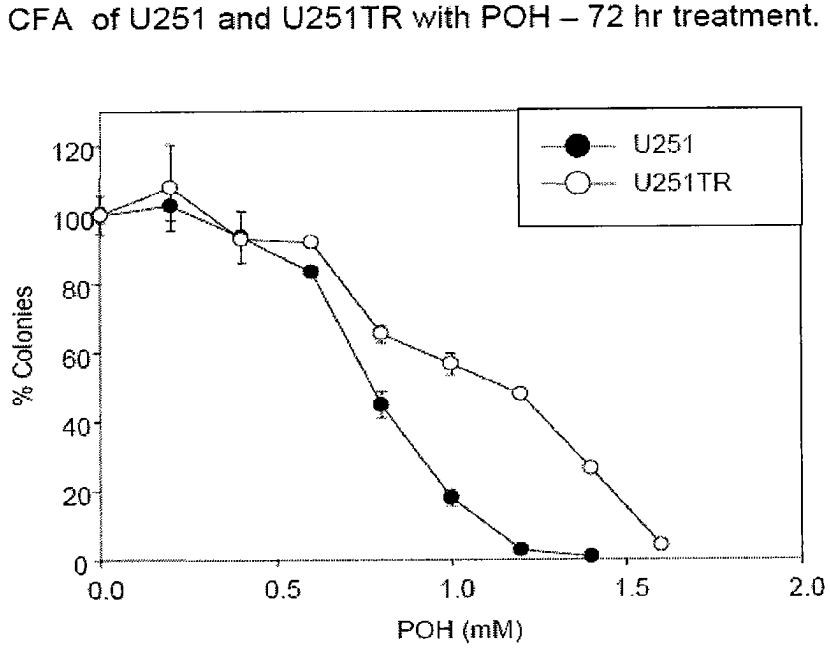


Figure 11

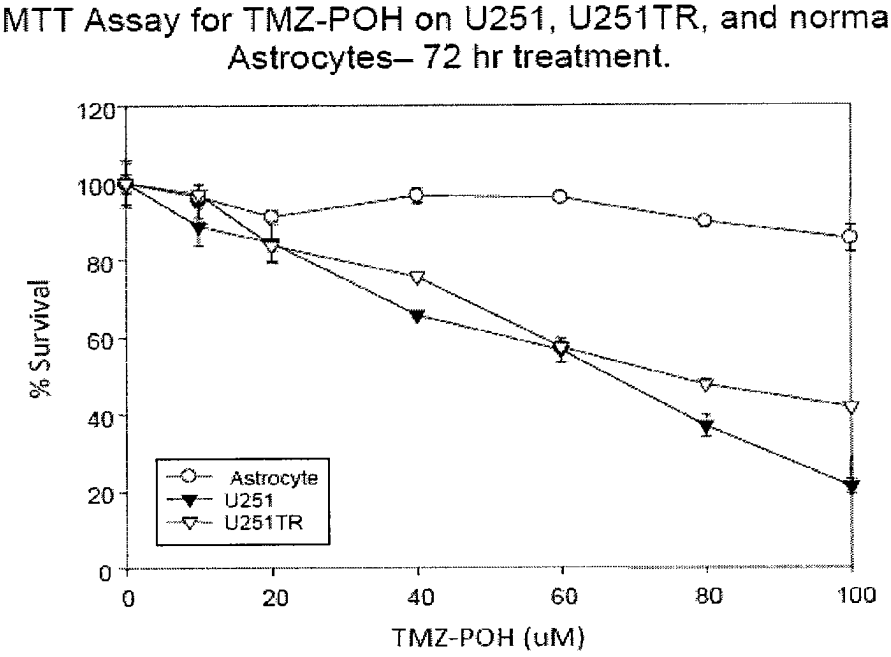


Figure 12

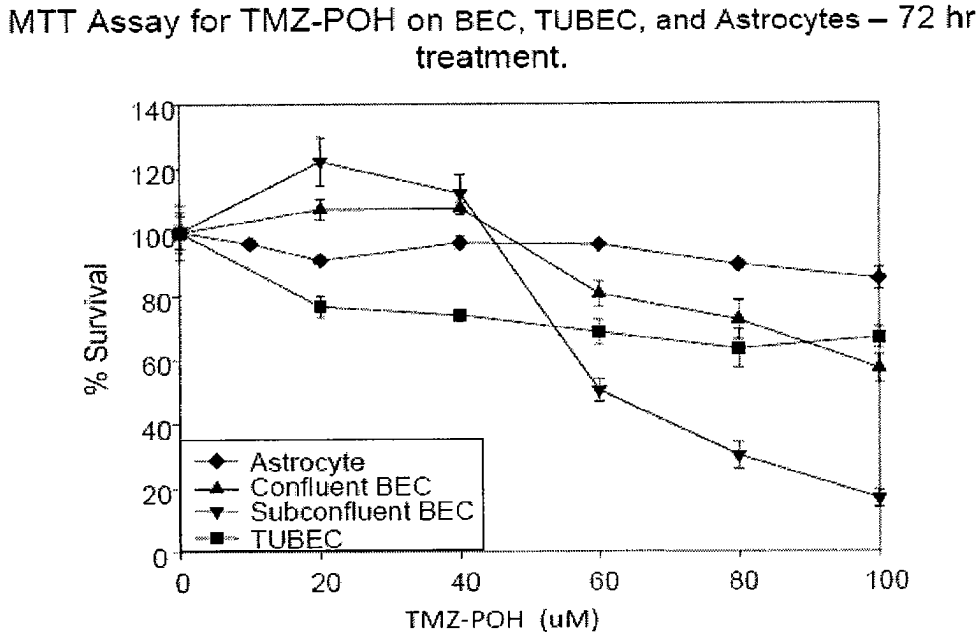


Figure 13

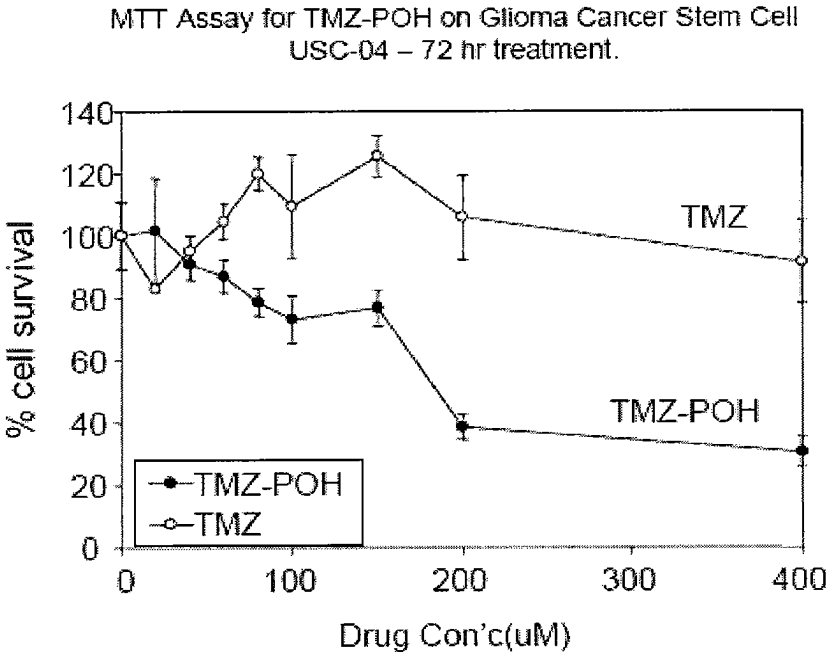


Figure 14

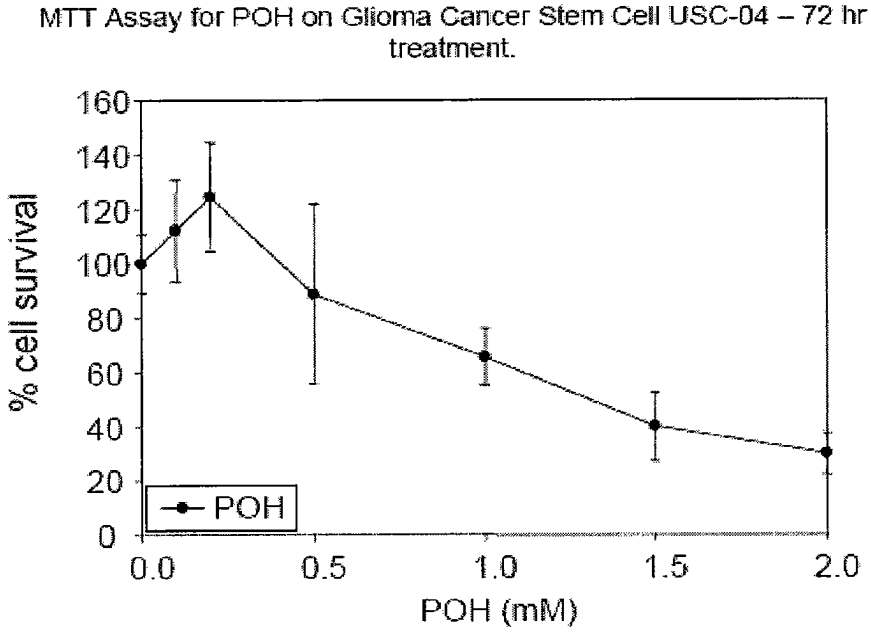


Figure 15

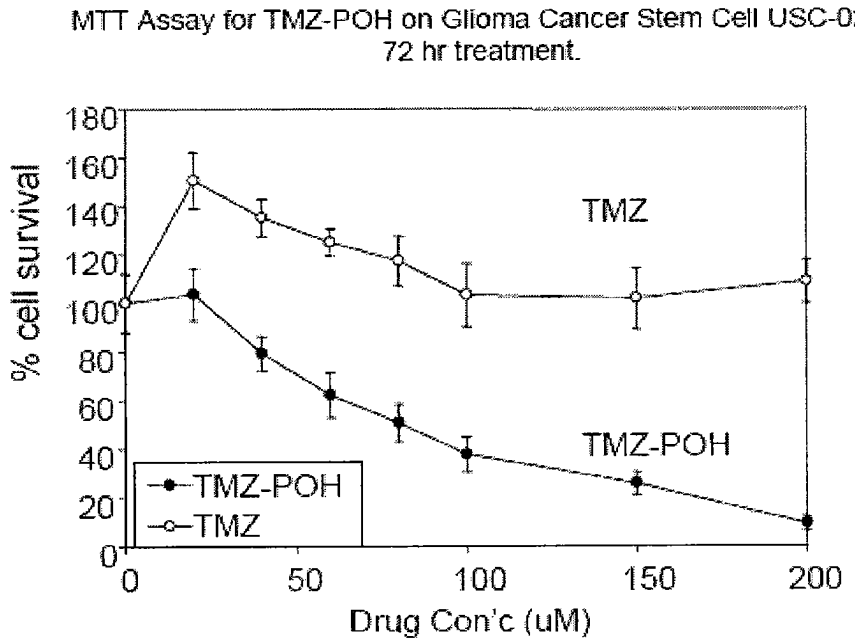


Figure 16

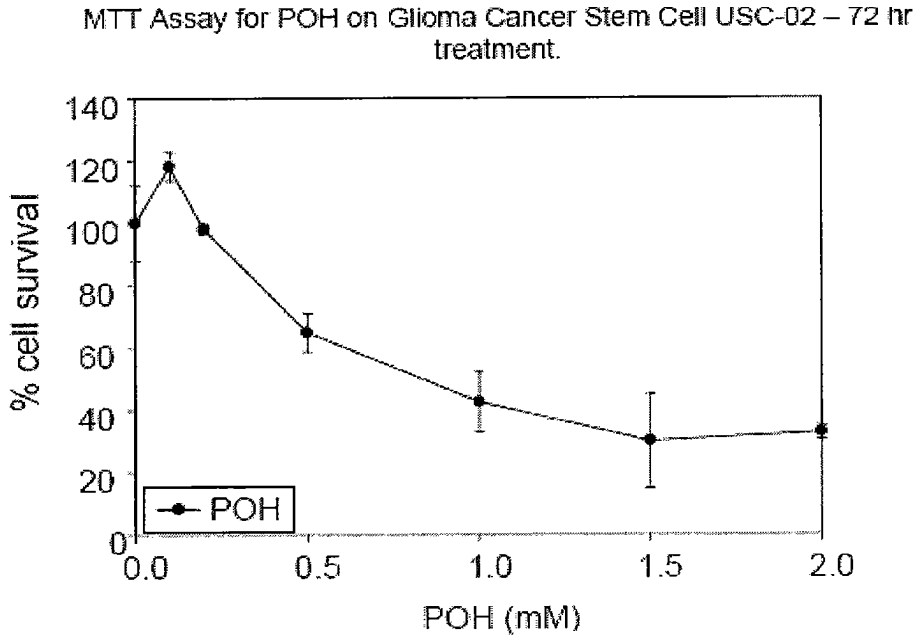


Figure 17

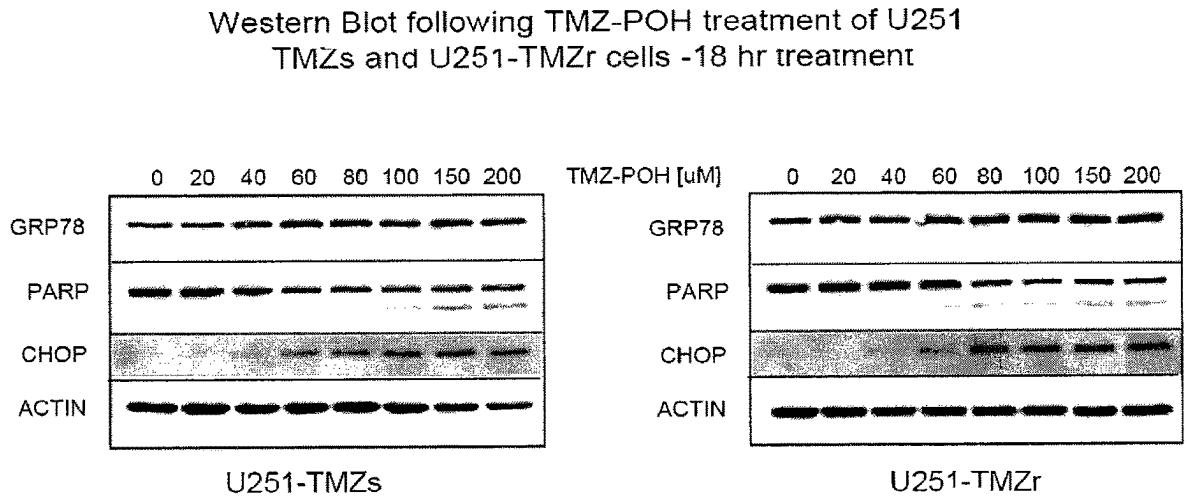

Western Blot following TMZ-POH treatment of U251 TMZs and U251-TMZr cells -18 hr treatment
0 20 40 60 80 100 150 200
TMZ-POH [uM]
0 20 40 60 80 100 150 200
GRP78
PARP
CHOP
ACTIN
GRP78
PARP
CHOP
ACTIN
U251-TMZs
U251-TMZr

Figure 18

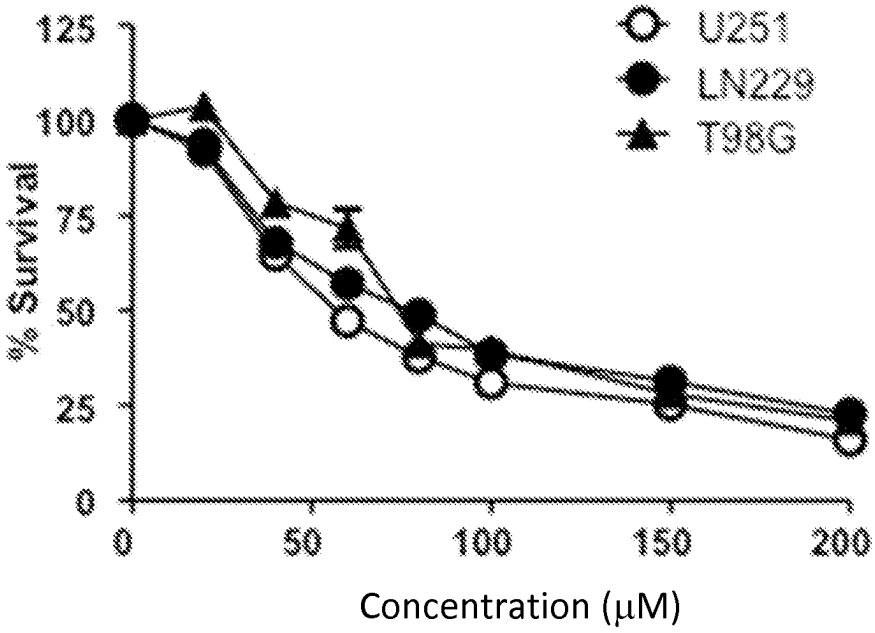

U251
LN229
T98G
% Survival
125
100
75
50
25
0
0
50
100
150
200
Concentration (μM)

FIG. 19

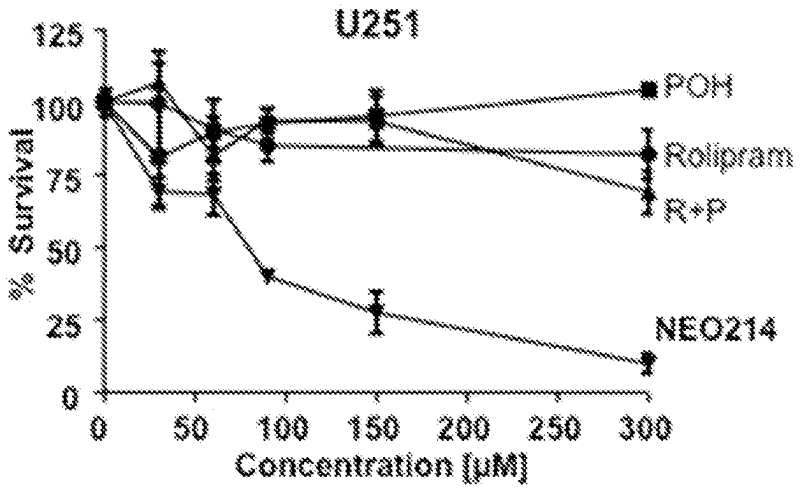


FIG. 20A

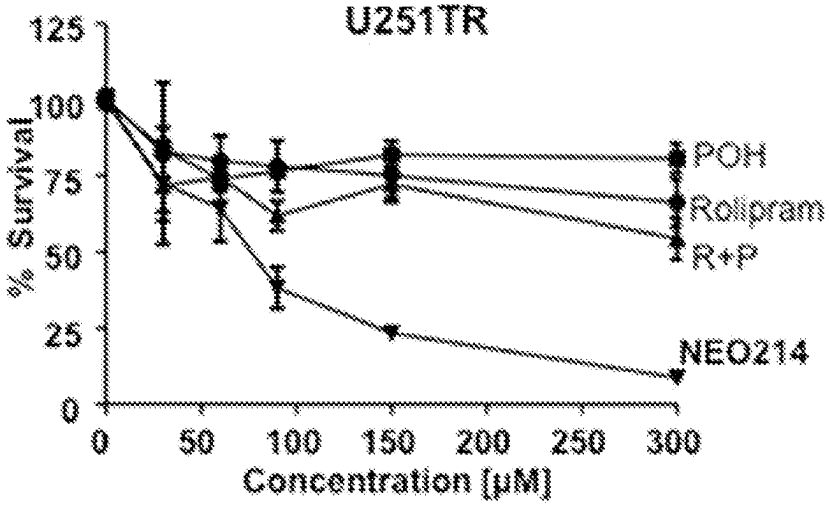


FIG. 20B

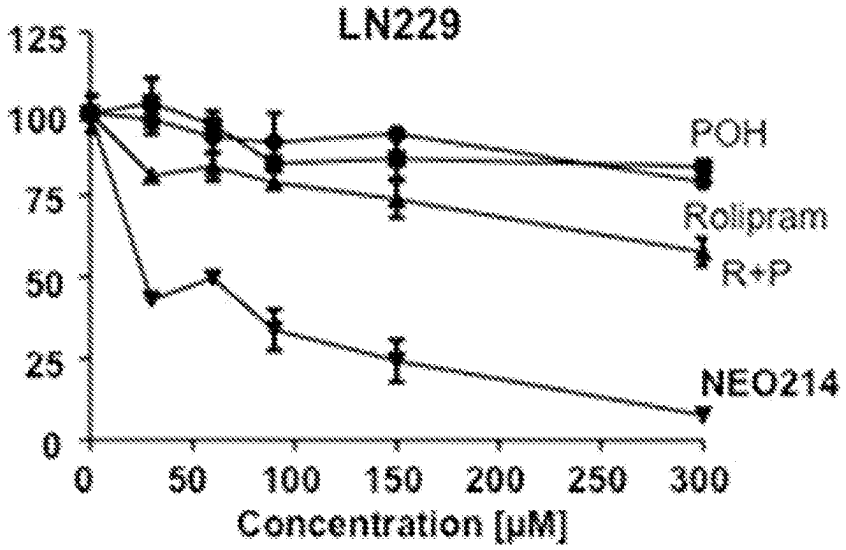


FIG. 20C

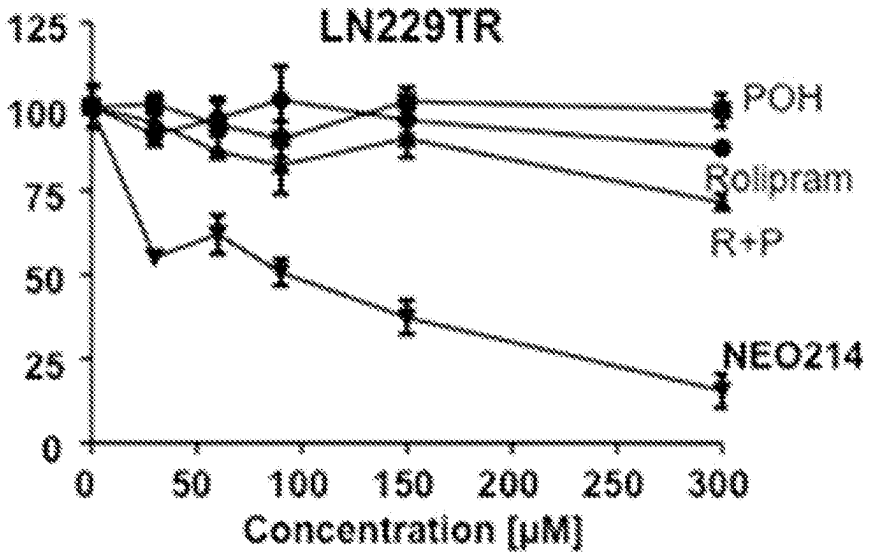


FIG. 20D

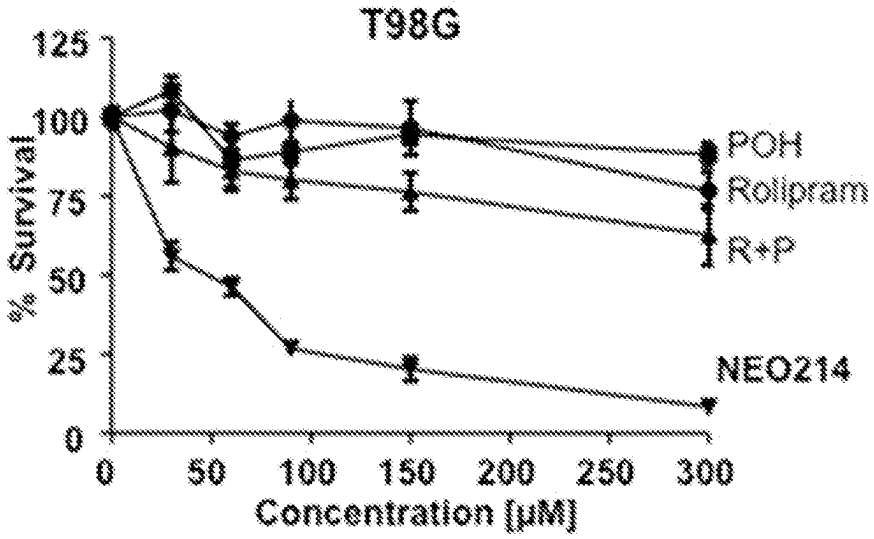

T98G
% Survival
125
100
75
50
25
0
0
50
100
150
200
250
300
Concentration [μM]
POH
Rolipram
R+P
NEO214

FIG. 21

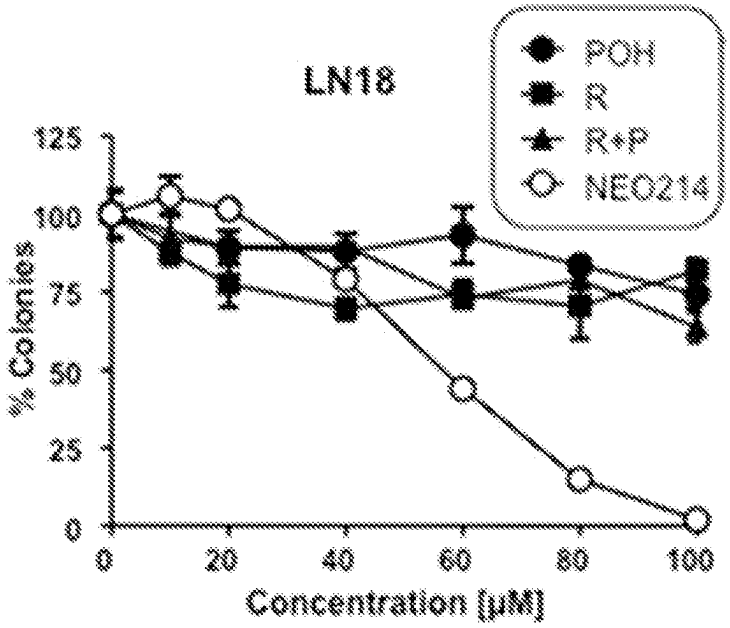


FIG. 22A

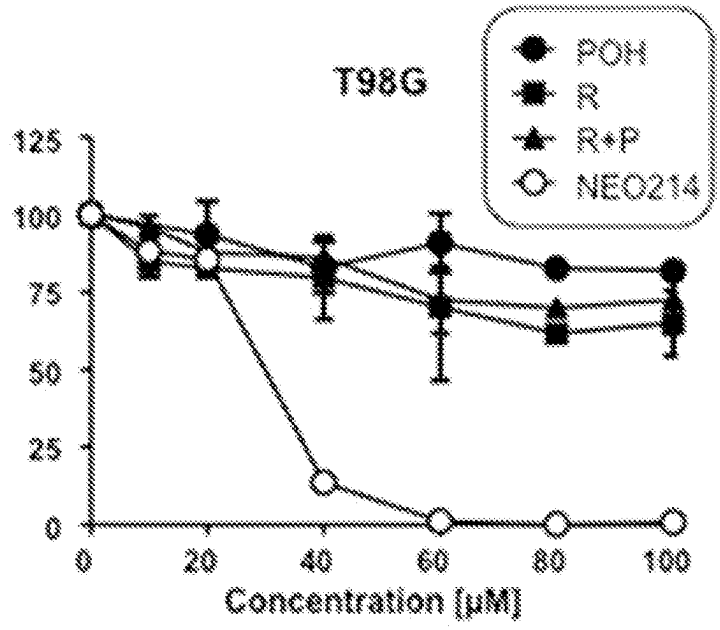


FIG. 22B

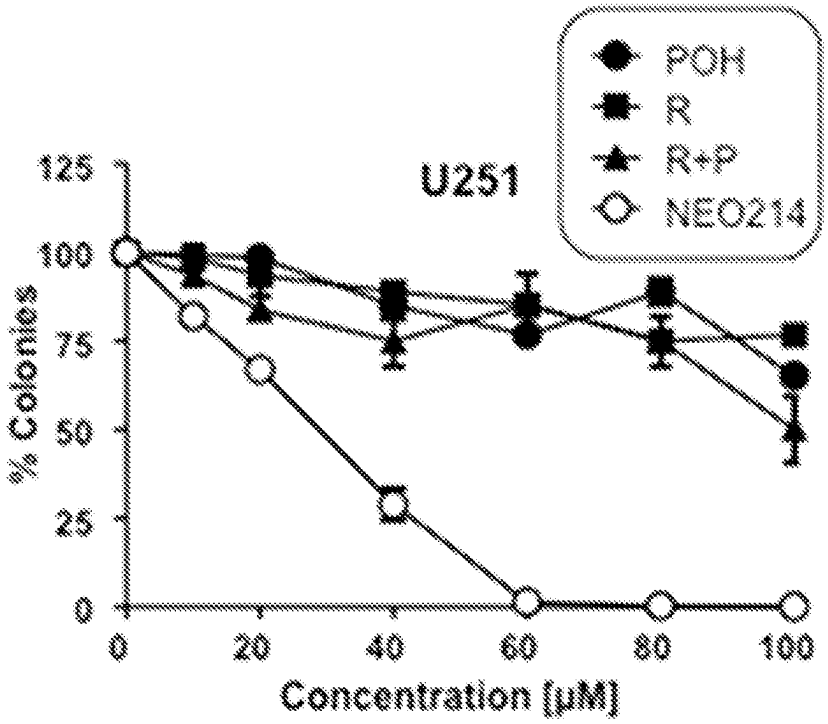


FIG. 23A

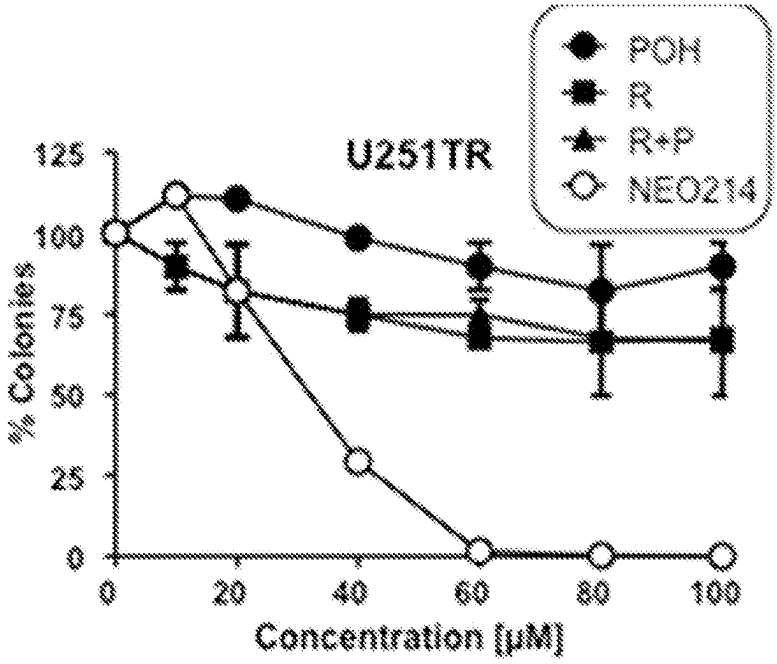


FIG. 23B

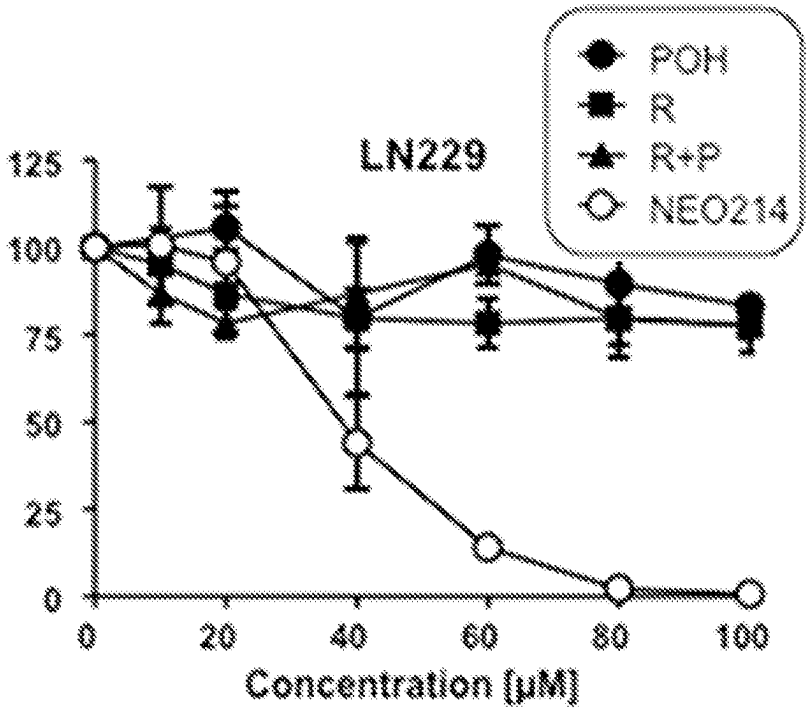


FIG. 23C

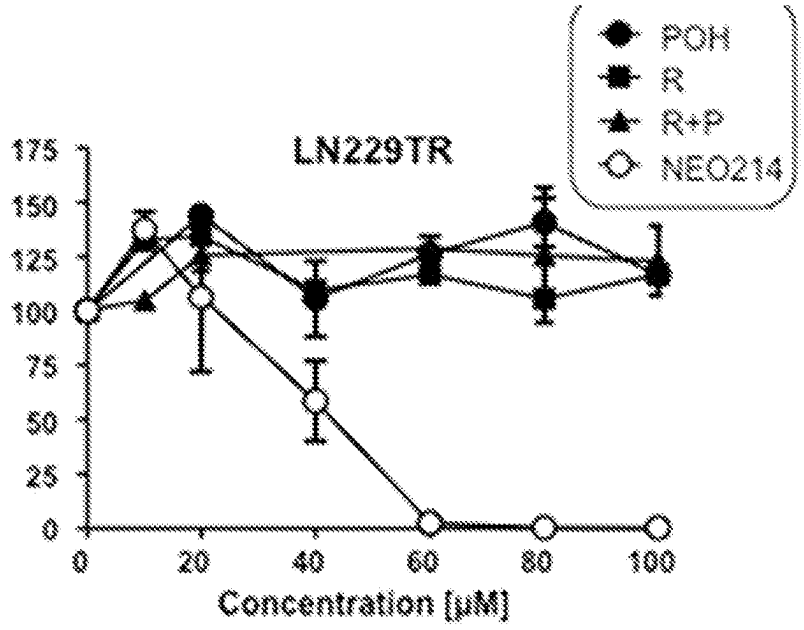


FIG. 23D

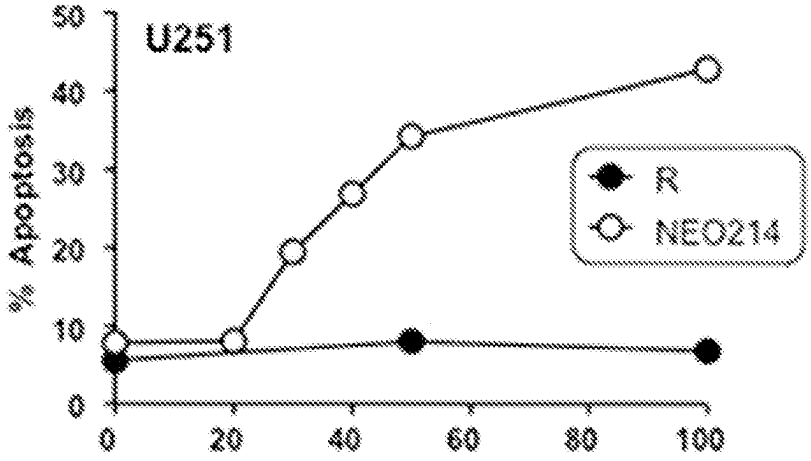


FIG. 24A

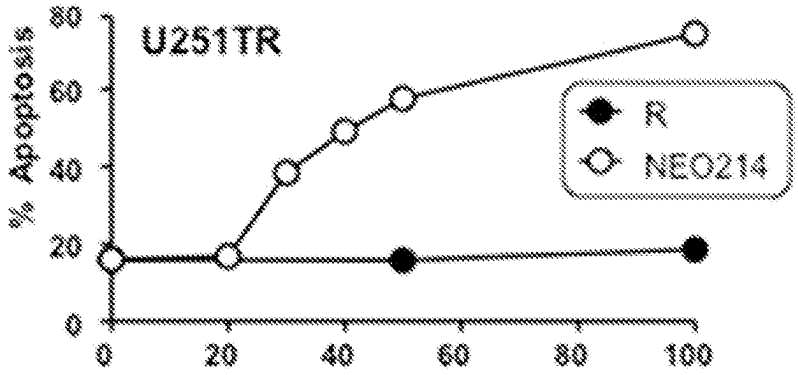


FIG. 24B

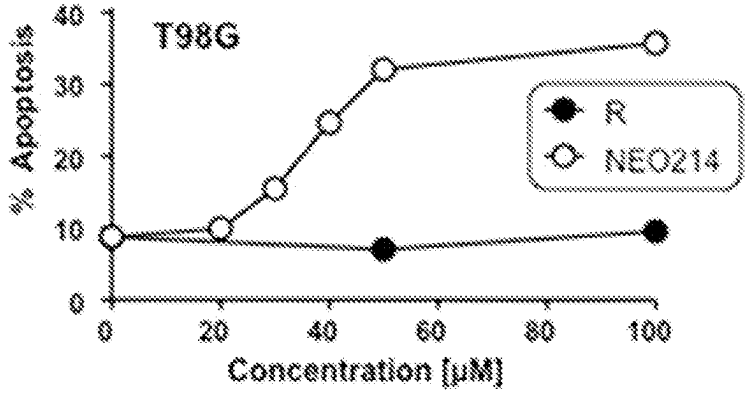


FIG. 24C

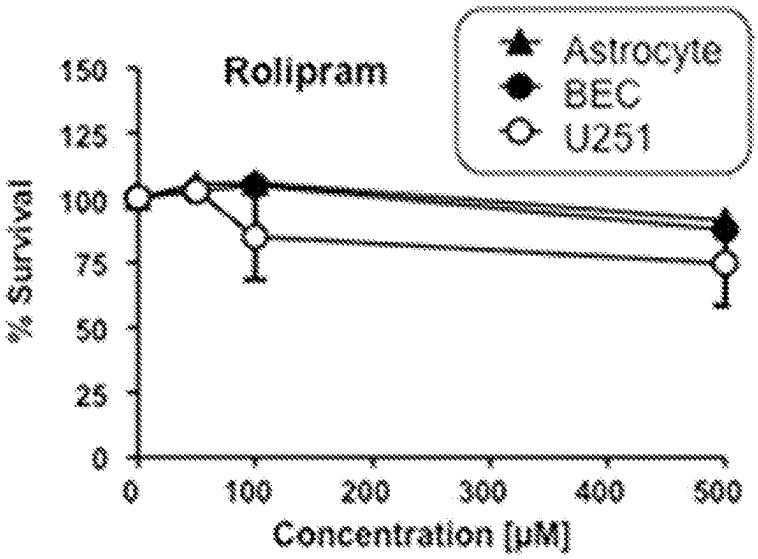


FIG. 25A

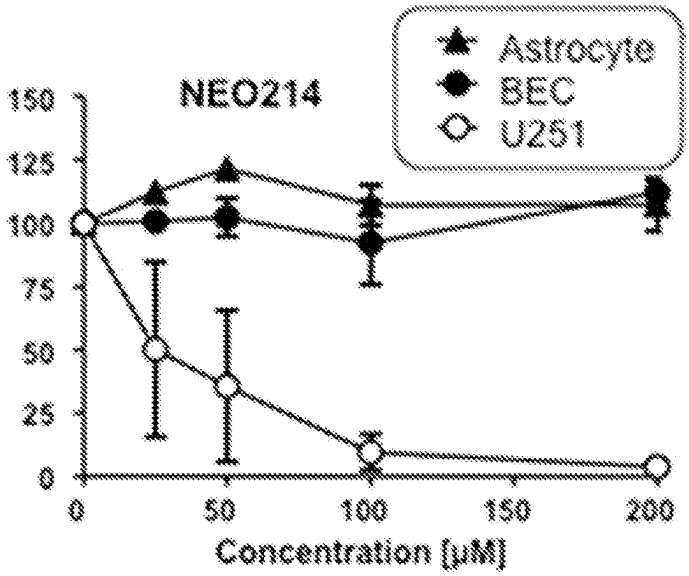


FIG. 25B

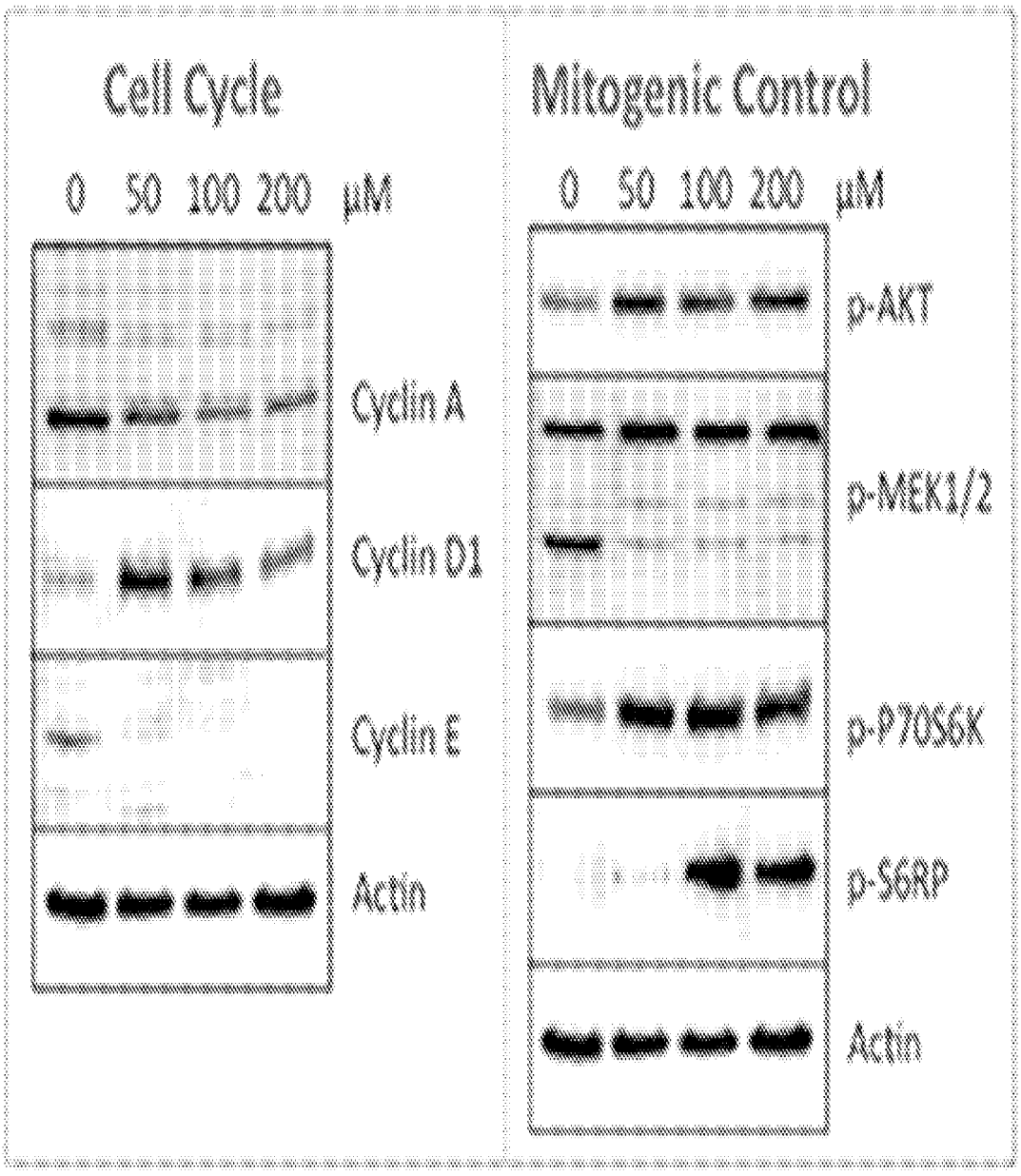

Cell Cycle
0 50 100 200 μM
Cyclin A
Cyclin D1
Cyclin E
Actin
Mitogenic Control
0 50 100 200 μM
p-AKT
p-MEK1/2
p-P70S6K
p-S6RP
Actin

FIG. 26

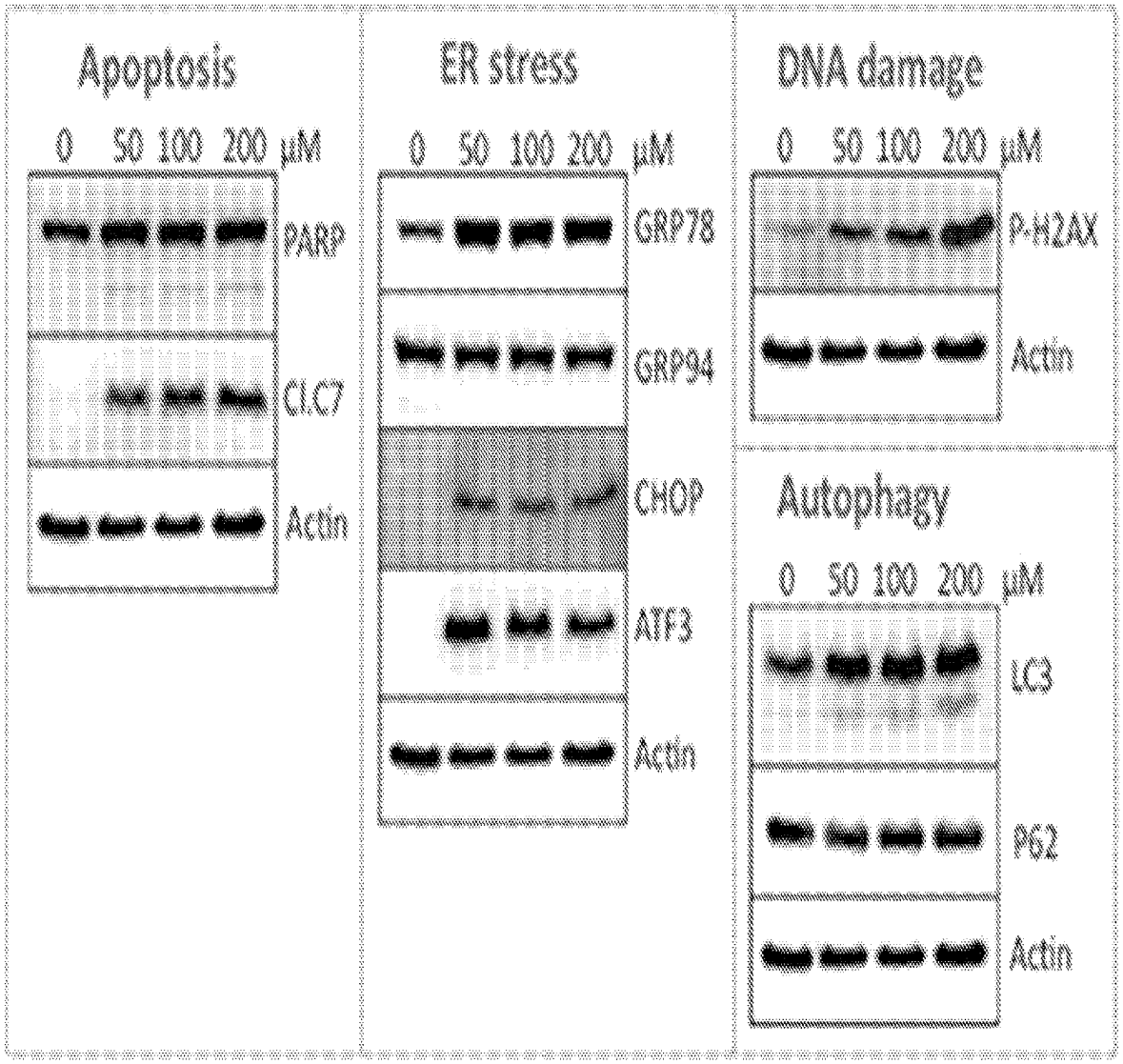

Apoptosis
0 50 100 200 μM
PARP
Cl.C7
Actin
ER stress
0 50 100 200 μM
GRP78
GRP94
CHOP
ATF3
Actin
DNA damage
0 50 100 200 μM
P-H2AX
Actin
Autophagy
0 50 100 200 μM
LC3
P62
Actin

FIG. 27

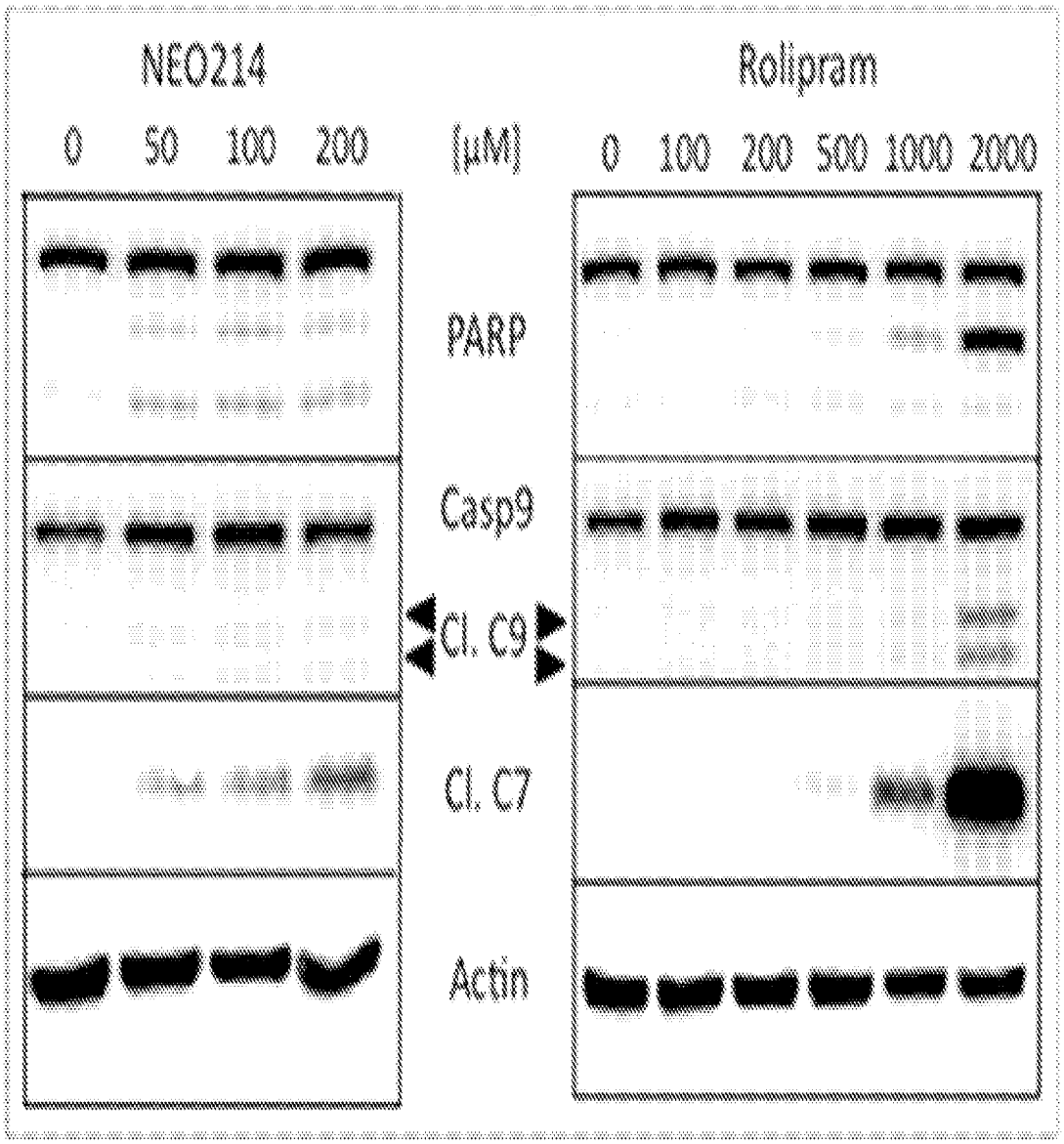

NEO214
0 50 100 200 (μM)
Rolipram
0 100 200 500 1000 2000
PARP
Casp9
Cl. C9
Cl. C7
Actin

FIG. 28

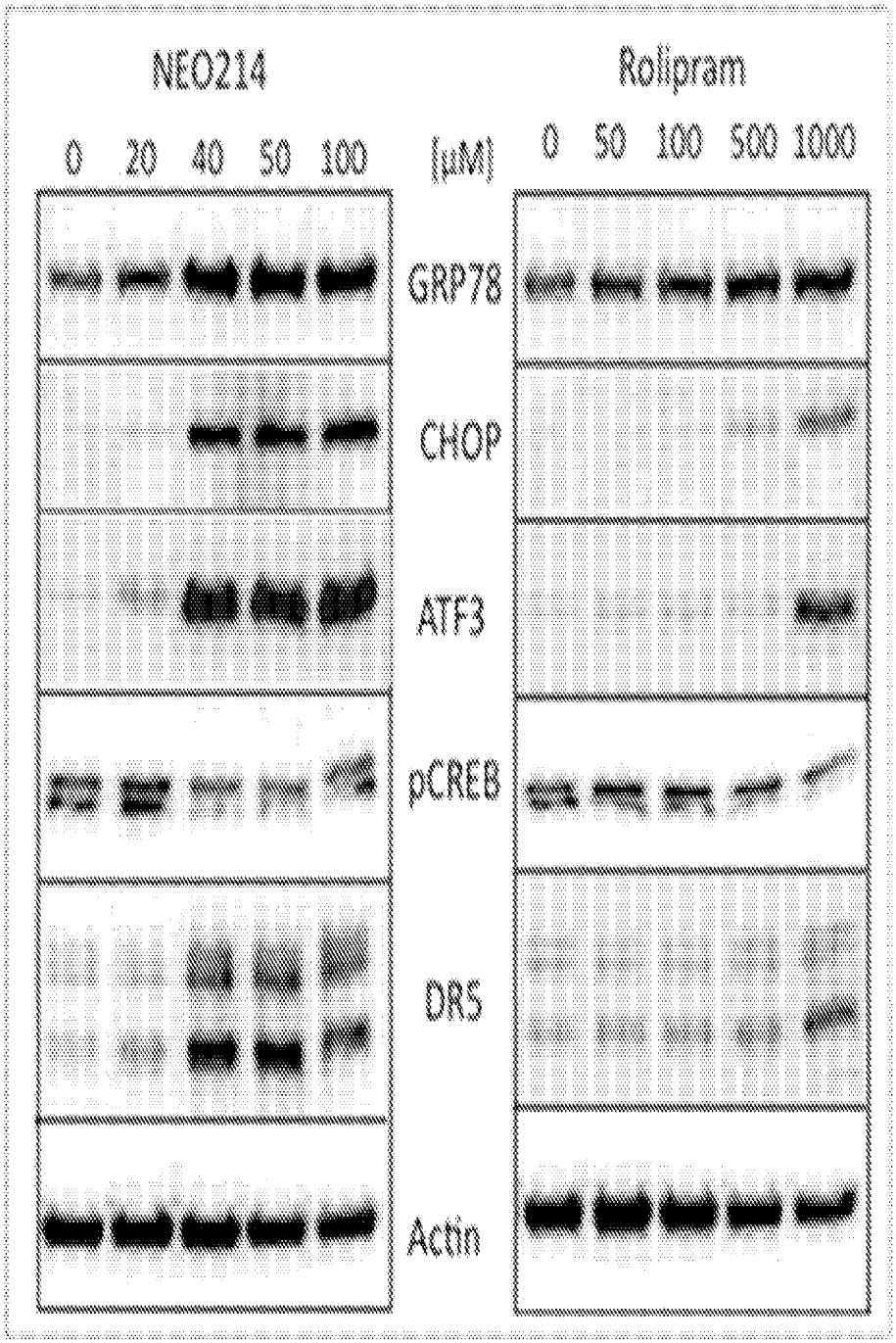

NEO214
0 20 40 50 100
(μM)
Rolipram
0 50 100 500 1000
GRP78
CHOP
ATF3
pCREB
DR5
Actin

FIG. 29

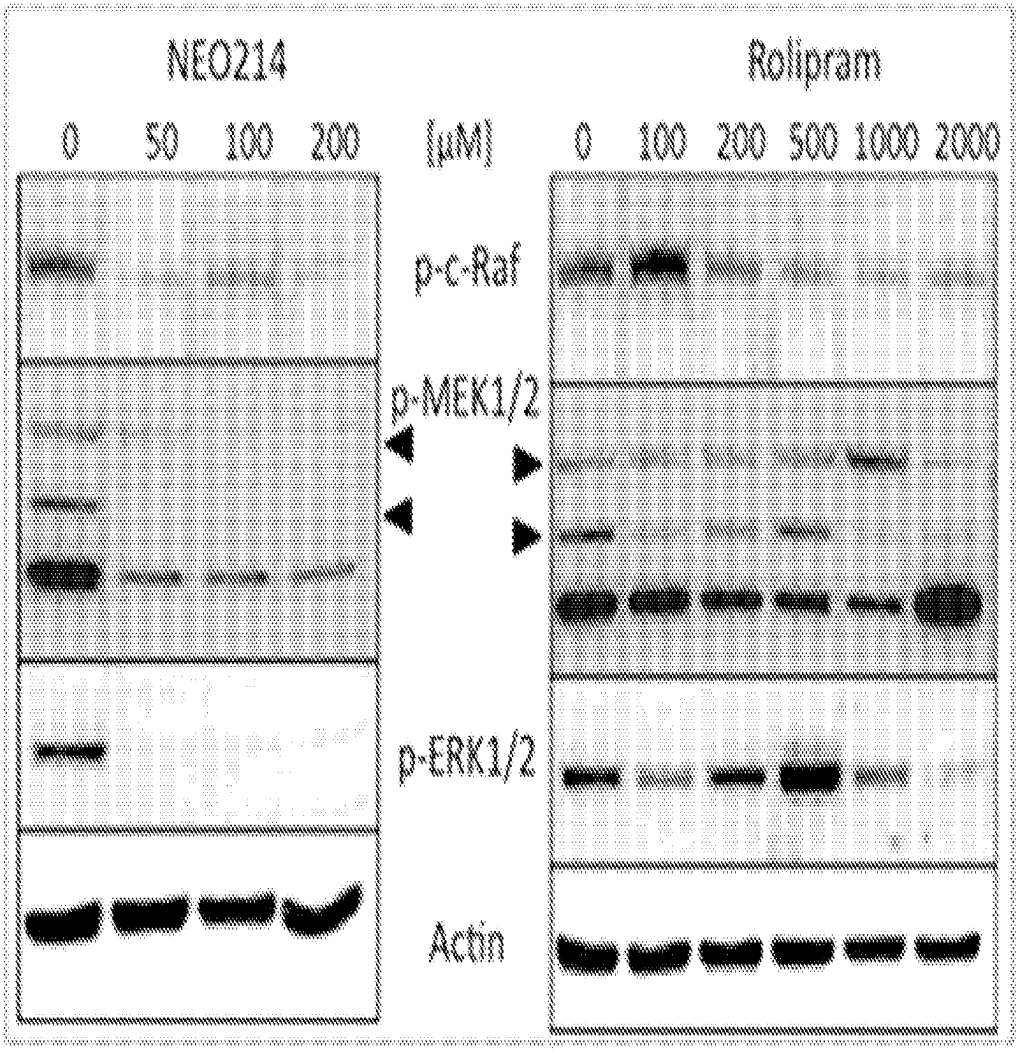

NEO214
0 50 100 200 [μM]
Rolipram
0 100 200 500 1000 2000
p-c-Raf
p-MEK1/2
p-ERK1/2
Actin

FIG. 30

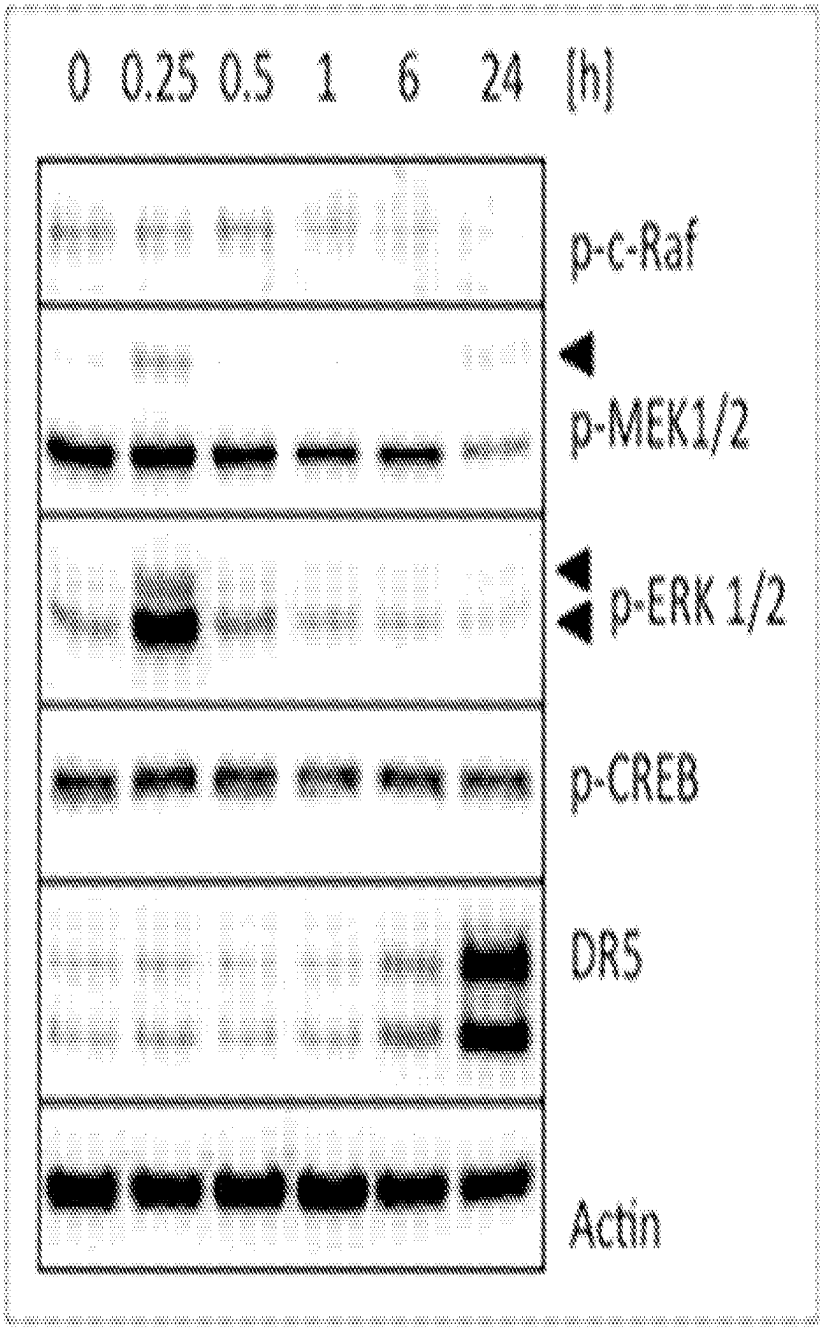


FIG. 31

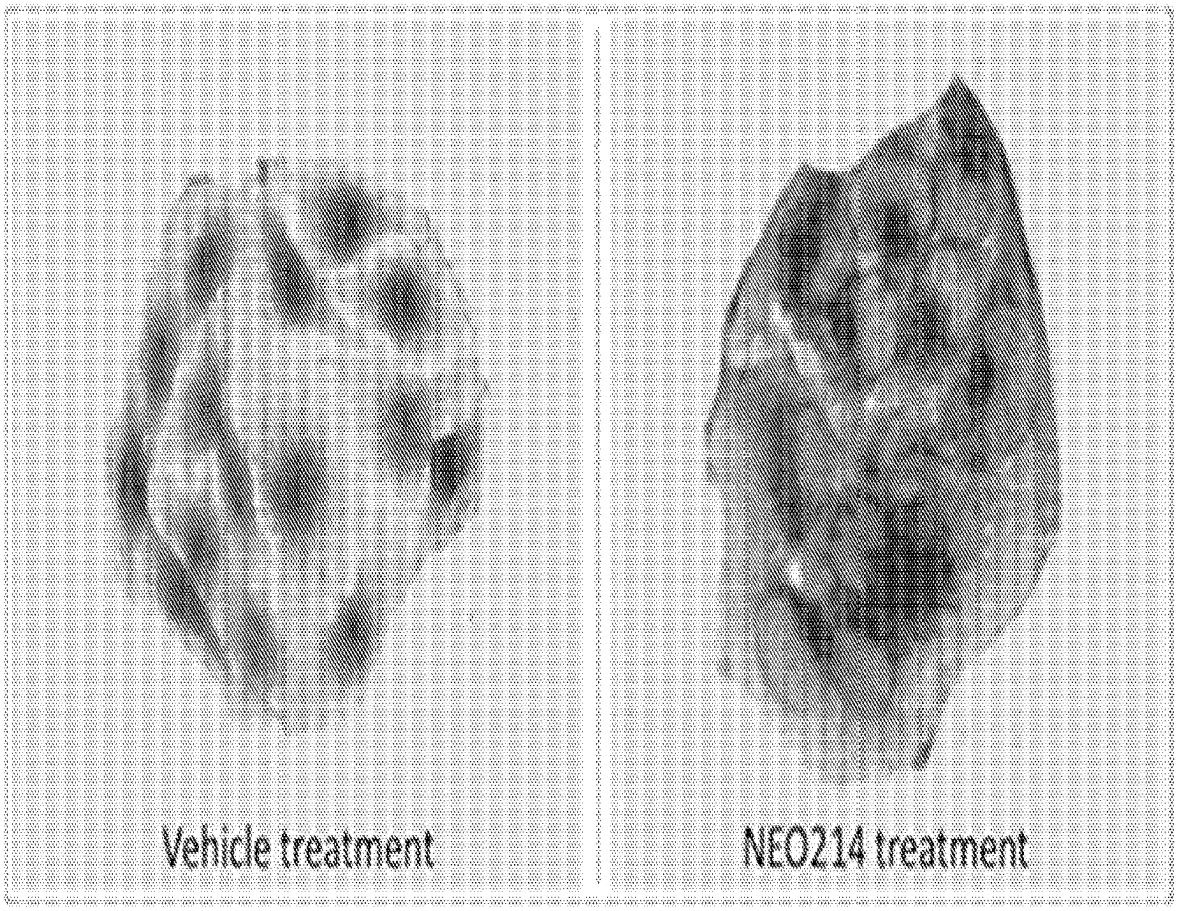


FIG. 32

PHARMACEUTICAL COMPOSITIONS COMPRISING POH DERIVATIVES

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. application Ser. No. 14/455,293, filed Aug. 8, 2014, which is a continuation of U.S. application Ser. No. 13/566,731 filed Aug. 3, 2012, now U.S. Pat. No. 8,916,545, issued Dec. 23, 2014, which is a continuation of International Application No. PCT/US2011/049392 filed Aug. 26, 2011, which claims priority to U.S. Provisional Application Nos. 61/377,747 (filed Aug. 27, 2010) and 61/471,402 (filed Apr. 4, 2011).

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under CA217551 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to POH derivatives. The present invention further relates to methods of using POH derivatives such as POH carbamates to treat a disease, such as cancer.

BACKGROUND OF THE INVENTION

Malignant gliomas, the most common form of central nervous system (CNS) cancers, is currently considered essentially incurable. Among the various malignant gliomas, anaplastic astrocytomas (Grade III) and glioblastoma multiforme (GBM; Grade IV) have an especially poor prognosis due to their aggressive growth and resistance to currently available therapies. The present standard of care for malignant gliomas consists of surgery, ionizing radiation, and chemotherapy. Despite recent advances in medicine, the past 50 years have not seen any significant improvement in prognosis for malignant gliomas. Wen et al. Malignant gliomas in adults. *New England J Med.* 359:492-507, 2008. Stupp et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *New England J Med.* 352: 987-996, 2005. Additionally, acquired resistance of initially well-responding tumors and unwanted side effects are other problems that frequently thwart long-term treatment using chemotherapeutic agents. Hence, various analogues of chemotherapeutic agents have been prepared in an effort to overcome these problems. The analogues include novel therapeutic agents which are hybrid molecules of at least two existing therapeutic agents. For example, cisplatin has been conjugated with Pt-(II) complexes with cytotoxic codrugs, or conjugated with bioactive shuttle components such as porphyrins, bile acids, hormones, or modulators that expedite the transmembrane transport or the drug accumulation within the cell. (6-Aminomethylnicotinate)dichloroplatinum (II) complexes esterified with terpene alcohols were tested on a panel of human tumor cell lines. The terpenyl moieties in these complexes appeared to fulfill a transmembrane shuttle function and increased the rate and extent of the uptake of these conjugates into various tumor cell lines. Schobert et al. Monoterpenes as Drug Shuttles: Cytotoxic (6-minomethylnicotinate)dichloroplatinum (II) Complexes with Potential To Overcome Cisplatin Resistance. *J. Med. Chem.* 2007, 50, 1288-1293.

Perillyl alcohol (POH), a naturally occurring monoterpene, has been suggested to be an effective agent against a variety of cancers, including CNS cancer, breast cancer, pancreatic cancer, lung cancer, melanomas and colon cancer. Gould, M. Cancer chemoprevention and therapy by monoterpenes. *Environ Health Perspect.* 1997 June; 105 (Suppl 4): 977-979. Hybrid molecules containing both perillyl alcohol and retinoids were prepared to increase apoptosis-inducing activity. Das et al. Design and synthesis of potential new apoptosis agents: hybrid compounds containing perillyl alcohol and new constrained retinoids. *Tetrahedron Letters* 2010, 51, 1462-1466.

There is still a need to prepare perillyl alcohol derivatives including perillyl alcohol conjugated with other therapeutic agents, and use this material in the treatment of cancers such as malignant gliomas, as well as other brain disorders such as Parkinson's and Alzheimer's disease. Perillyl alcohol derivatives may be administered alone or in combination with other treatment methods including radiation, standard chemotherapy, and surgery. The administration can also be through various routes including intranasal, oral, oral-tracheal for pulmonary delivery, and transdermal.

SUMMARY OF THE INVENTION

The present invention provides for a pharmaceutical composition comprising a perillyl alcohol carbamate. The perillyl alcohol carbamate may be perillyl alcohol conjugated with a therapeutic agent, such as a chemotherapeutic agent. The chemotherapeutic agent may be rolipram. In one embodiment, the perillyl alcohol carbamate May 4-(3-cyclopentyloxy-4-methoxy phenyl)-2-oxo-pyrrolidine-1-carboxylic acid 4-isopropenyl cyclohex-1-enylmethyl ester (POH-Rolipram).

The pharmaceutical compositions of the present invention may be administered before, during or after radiation. The pharmaceutical compositions may be administered before, during or after the administration of a chemotherapeutic agent. The routes of administration of the pharmaceutical compositions include inhalation, intranasal, oral, intravenous, subcutaneous or intramuscular administration.

The invention further provides for a method for treating a disease in a mammal, comprising the step of delivering to the mammal a therapeutically effective amount of a perillyl alcohol carbamate. The method may further comprise the step of treating the mammal with radiation, and/or further comprise the step of delivering to the mammal a chemotherapeutic agent. The diseases treated may be cancer, including a tumor of the nervous system, such as a glioblastoma. The routes of administration of the perillyl alcohol carbamate include inhalation, intranasal, oral, intravenous, subcutaneous or intramuscular administration.

The present invention also provides for a process for making a POH carbamate, comprising the step of reacting a first reactant of perillyl chloroformate with a second reactant, which may be rolipram. The reaction may be carried out in the presence of tetrahydrofuran and a catalyst of n-butyl lithium. The perillyl chloroformate may be prepared by reacting perillyl alcohol with phosgene.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of dimethyl celecoxib (DMC) in killing U87, A172 and U251 human glioma cells.

FIG. **2** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of the POH-DMC conjugate in killing U87, A172 and U251 human glioma cells according to the present invention.

FIG. **3** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of temozolomide (TMZ) in killing U87, A172 and U251 human glioma cells.

FIG. **4** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of the POH-TMZ conjugate in killing U87, A172, and U251 human glioma cells according to the present invention.

FIG. **5** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of the POH-Rolipram conjugate and Rolipram in killing A172 human glioma cells.

FIG. **6** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of the POH-Rolipram conjugate and Rolipram in killing U87 human glioma cells.

FIG. **7** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of the POH-Rolipram conjugate and Rolipram in killing U251 human glioma cells.

FIG. **8** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of the POH-Rolipram conjugate and Rolipram in killing L229 human glioma cells.

FIGS. **9**A and **9**B show the inhibition of tumor growth by butyryl-POH in mouse models. FIG. **9**A shows the images of subcutaneous U-87 gliomas in nude mice treated with butyryl-POH, purified(S)-perillyl alcohol having a purity greater than 98.5% ("Purified POH"), POH purchased from Sigma chemicals ("Sigma"), or phosphate buffered saline ("PBS"; negative control). FIG. **9**B shows average tumor growth over time (total time period of 60 days).

FIG. **10** shows the results of a Colony forming Assay (CFA) demonstrating the cytotoxic effect of TMZ and TMZ-POH on TMZ sensitive (U251) and TMZ resistant (U251TR) U251 cells.

FIG. **11** shows the results of a Colony forming Assay (CFA) demonstrating the cytotoxic effect of POH on TMZ sensitive (U251) and TMZ resistant (U251TR) U251 cells.

FIG. **12** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of the POH-TMZ conjugate in killing U251 cells, U251TR cells, and normal astrocytes.

FIG. **13** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of the POH-TMZ conjugate in killing normal astrocytes, brain endothelial cells (BEC; confluent and subconfluent), and tumor brain endothelial cells (TuBEC).

FIG. **14** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of TMZ and the POH-TMZ conjugate in killing USC-04 glioma cancer stem cells.

FIG. **15** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of POH in killing USC-04 glioma cancer stem cells.

FIG. **16** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of TMZ and the POH-TMZ conjugate in killing USC-02 glioma cancer stem cells.

FIG. **17** shows the results of the MTT cytotoxicity assays demonstrating the efficacy of POH in killing USC-02 glioma cancer stem cells.

FIG. **18** shows a western blot demonstrating that TMZ-POH induces ER stress (ERS) in TMZ sensitive ("U251-TMZs") and resistant ("U251-TMZr") U251 glioma cells.

FIG. **19** shows Alarmar blue assay results of three different glioblastoma cell lines after 48 hours of treating the cell lines by different concentrations of POH-Rolipram.

FIGS. **20**A-**20**D show MTT assay results of different glioblastoma cell lines (U251, U251TR, LN229, and LN229TR) demonstrating the effective killing of drug-resistant glioblastoma cells by POH-Rolipram.

FIG. **21** shows MTT assay results of T98G glioblastoma cells glioblastoma cells demonstrating the effective killing of a drug-resistant glioblastoma cell line by POH-Rolipram.

FIGS. **22**A-**22**B show colony-formation assay results of T98G and LN18 glioblastoma cell lines demonstrating long term effectiveness of POH-Rolipram in killing these drug-resistant glioblastoma cells.

FIGS. **23**A-**23**D show colony-formation assay results of different glioblastoma cell lines (U251, LN229, U251TR and LN229TR) demonstrating the long term effectiveness of POH-Rolipram in killing glioblastoma cells.

FIGS. **24**A-**24**C show ELISA assay results demonstrating the effectiveness of POH-Rolipram in inducing cell death in U251 glioblastoma cells as well as chemo-resistant glioblastoma lines U251TR and T98G.

FIGS. **25**A-**25**B show Alamar blue assay results demonstrating the tolerability of POH-Rolipram.

FIG. **26** shows Western blot assay results demonstrating the impact of POH-Rolipram on certain intracellular signaling pathways of cancer cells.

FIG. **27** shows Western blot assay results demonstrating the impact of POH-Rolipram on certain intracellular signaling pathways of cancer cells.

FIGS. **28**-**30** show Western blot assay results comparing of the effects of POH-Rolipram and rolipram on intracellular processes.

FIG. **31** shows Western blot assay results depicting the time course of effects of POH-Rolipram on intracellular processes.

FIG. **32** depicts cell membrane localization of death receptor 5 (DR5) after treatment of cells with POH-Rolipram (immuno-cyto-chemistry).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for a derivative of monoterpene or sesquiterpene, such as a perillyl alcohol derivative. The present invention also provides for a pharmaceutical composition comprising a derivative of monoterpene or sesquiterpene, such as a perillyl alcohol derivative. For example, the perillyl alcohol derivative may be a perillyl alcohol carbamate. The perillyl alcohol derivative may be perillyl alcohol conjugated with a therapeutic agent such as a chemotherapeutic agent. The monoterpene (or sesquiterpene) derivative may be formulated into a pharmaceutical composition, where the monoterpene (or sesquiterpene) derivative is present in amounts ranging from about 0.01% (w/w) to about 100% (w/w), from about 0.1% (w/w) to about 80% (w/w), from about 1% (w/w) to about 70% (w/w), from about 10% (w/w) to about 60% (w/w), or from about 0.1% (w/w) to about 20% (w/w). The present compositions can be administered alone, or may be co-administered together with radiation or another agent (e.g., a chemotherapeutic agent), to treat a disease such as cancer. Treatments may be sequential, with the monoterpene (or sesquiterpene) derivative being administered before or after the administration of other agents. For example, a perillyl alcohol carbamate may be used to sensitize a cancer patient to radiation or chemotherapy. Alternatively, agents may be administered concurrently. The route of administration may vary, and can include, inhalation, intranasal, oral, transdermal, intravenous, subcutaneous or intramuscular injection. The present invention also provides for a method of treating a disease such as cancer, comprising the step of delivering to a patient

a therapeutically effective amount of a derivative of monoterpene (or sesquiterpene).

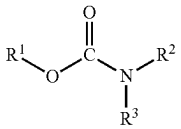
The compositions of the present invention may contain one or more types of derivatives of monoterpene (or sesquiterpene). Monoterpenes include terpenes that consist of two isoprene units. Monoterpenes may be linear (acyclic) or contain rings. Derivatives of monoterpenoids are also encompassed by the present invention. Monoterpenoids may be produced by biochemical modifications such as oxidation or rearrangement of monoterpenes. Examples of monoterpenes and monoterpenoids include, perillyl alcohol (S (−)) and (R (+)), ocimene, myrcene, geraniol, citral, citronellol, citronellal, linalool, pinene, terpineol, terpinen, limonene, terpinenes, phellandrenes, terpinolene, terpinen-4-ol (or tea tree oil), pinene, terpineol, terpinen; the terpenoids such as p-cymene which is derived from monocyclic terpenes such as menthol, thymol and carvacrol; bicyclic monoterpenoids such as camphor, borneol and eucalyptol.

Monoterpenes may be distinguished by the structure of a carbon skeleton and may be grouped into acyclic monoterpenes (e.g., myrcene, (Z)- and (E)-ocimene, linalool, geraniol, nerol, citronellol, myrcenol, geranial, citral a, neral, citral b, citronellal, etc.), monocyclic monoterpenes (e.g., limonene, terpinene, phellandrene, terpinolene, menthol, carveol, etc.), bicyclic monoterpenes (e.g., pinene, myrtenol, myrtenal, verbenol, verbanon, pinocarveol, carene, sabinene, camphene, thujene, etc.) and tricyclic monoterpenes (e.g. tricyclene). See *Encyclopedia of Chemical Technology*, Fourth Edition, Volume 23, page 834-835.

Sesquiterpenes of the present invention include terpenes that consist of three isoprene units. Sesquiterpenes may be linear (acyclic) or contain rings. Derivatives of sesquiterpenoids are also encompassed by the present invention. Sesquiterpenoids may be produced by biochemical modifications such as oxidation or rearrangement of sesquiterpenes. Examples of sesquiterpenes include farnesol, farnesol, farnesylic acid and nerolidol.

The derivatives of monoterpene (or sesquiterpene) include, but are not limited to, carbamates, esters, ethers, alcohols and aldehydes of the monoterpene (or sesquiterpene). Monoterpene (or sesquiterpene) alcohols may be derivatized to carbamates, esters, ethers, aldehydes or acids.

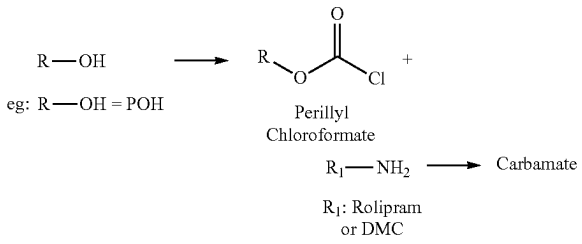
Carbamate refers to a class of chemical compounds sharing the functional group



based on a carbonyl group flanked by an oxygen and a nitrogen. R^1, R^2 and R^3 can be a group such as alkyl, aryl, etc., which can be substituted. The R groups on the nitrogen and the oxygen may form a ring. R^1—OH may be a monoterpene, e.g., POH. The R^2—N—R^3 moiety may be a therapeutic agent.

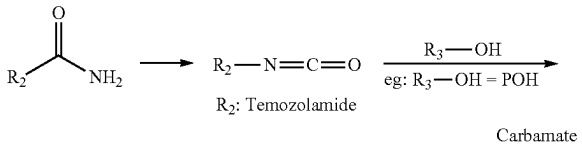
Carbamates may be synthesized by reacting isocyanate and alcohol, or by reacting chloroformate with amine. Carbamates may be synthesized by reactions making use of phosgene or phosgene equivalents. For example, carbamates may be synthesized by reacting phosgene gas, diphosgene or a solid phosgene precursor such as triphosgene with two amines or an amine and an alcohol. Carbamates (also known as urethanes) can also be made from reaction of a urea intermediate with an alcohol. Dimethyl carbonate and diphenyl carbonate are also used for making carbamates. Alternatively, carbamates may be synthesized through the reaction of alcohol and/or amine precursors with an ester-substituted diaryl carbonate, such as bismethylsalicylcarbonate (BMSC). U.S. Patent Publication No. 20100113819.

Carbamates may be synthesized by the following approach:



Suitable reaction solvents include, but are not limited to, tetrahydrofuran, dichloromethane, dichloroethane, acetone, and diisopropyl ether. The reaction may be performed at a temperature ranging from about −70° C. to about 80° C., or from about −65° C. to about 50° C. The molar ratio of perillyl chloroformate to the substrate R—NH_2 may range from about 1:1 to about 2:1, from about 1:1 to about 1.5:1, from about 2:1 to about 1:1, or from about 1.05:1 to about 1.1:1. Suitable bases include, but are not limited to, organic bases, such as triethylamine, potassium carbonate, N,N'-diisopropylethylamine, butyl lithium, and potassium-t-butoxide.

Alternatively, carbamates may be synthesized by the following approach:



Suitable reaction solvents include, but are not limited to, dichloromethane, dichloroethane, toluene, diisopropyl ether, and tetrahydrofuran. The reaction may be performed at a temperature ranging from about 25° C. to about 110° C., or from about 30° C. to about 80° C., or about 50° C. The molar ratio of perillyl alcohol to the substrate R—N=C=O may range from about 1:1 to about 2:1, from about 1:1 to about 1.5:1, from about 2:1 to about 1:1, or from about 1.05:1 to about 1.1:1.

Esters of the monoterpene (or sesquiterpene) alcohols of the present invention can be derived from an inorganic acid or an organic acid. Inorganic acids include, but are not limited to, phosphoric acid, sulfuric acid, and nitric acid. Organic acids include, but are not limited to, carboxylic acid such as benzoic acid, fatty acid, acetic acid and propionic acid, and any therapeutic agent bearing at least one carboxylic acid functional group Examples of esters of monoterpene (or sesquiterpene) alcohols include, but are not limited to, carboxylic acid esters (such as benzoate esters, fatty acid esters (e.g., palmitate ester, linoleate ester, stearate ester, butyryl ester and oleate ester), acetates, propionates (or

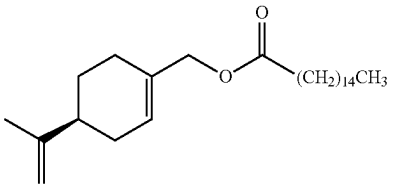
propionates), and formates), phosphates, sulfates, and carbamates (e.g., N,N-dimethylaminocarbonyl).

A specific example of a monoterpene that may be used in the present invention is perillyl alcohol (commonly abbreviated as POH). The derivatives of perillyl alcohol include, perillyl alcohol carbamates, perillyl alcohol esters, perillic aldehydes, dehydroperillic acid, perillic acid, perillic aldehyde derivatives, dehydroperillic acid esters and perillic acid esters. The derivatives of perillyl alcohol may also include its oxidative and nucleophilic/electrophilic addition derivatives. U.S. Patent Publication No. 20090031455. U.S. Pat. Nos. 6,133,324 and 3,957,856. Many examples of derivatives of perillyl alcohol are reported in the chemistry literature (see Appendix A: CAS Scifinder search output file, retrieved Jan. 25, 2010).

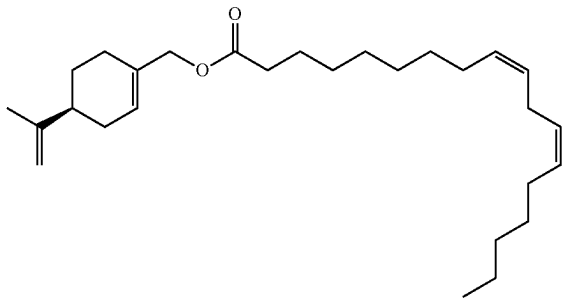
In certain embodiments, a POH carbamate is synthesized by a process comprising the step of reacting a first reactant of perillyl chloroformate with a second reactant such as dimethyl celecoxib (DMC), temozolomide (TMZ) and rolipram. The reaction may be carried out in the presence of tetrahydrofuran and a base such as n-butyl lithium. Perillyl chloroformate may be made by reacting POH with phosgene. For example, POH conjugated with temozolomide through a carbamate bond may be synthesized by reacting temozolomide with oxalyl chloride followed by reaction with perillyl alcohol. The reaction may be carried out in the presence of 1,2-dichloroethane.

POH carbamates encompassed by the present invention include, but not limited to, 4-(bis-N,N'-4-isopropenyl cyclohex-1-enylmethyloxy carbonyl[5-(2,5-dimethyl phenyl)-3-trifluoromethyl pyrazol-1-yl]benzenesulfonamide, 4-(3-cyclopentyloxy-4-methoxy phenyl)-2-oxo-pyrrolidine-1-carboxylic acid 4-isopropenyl cyclohex-1-enylmethyl ester, and (3-methyl 4-oxo-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carbonyl) carbamic acid-4-isopropenyl cyclohex-1-enylmethyl ester. The details of the chemical reactions generating these compounds are described in the Examples below.

In certain embodiments, perillyl alcohol derivatives may be perillyl alcohol fatty acid esters, such as palmitoyl ester of POH and linoleoyl ester of POH, the chemical structures of which are shown below.



Hexadecanoic Acid
4-isopropenyl-cyclohex-1-enylmethyl Ester
(Palmitoyl Ester of POH)



Octadeca-9,12-dienoic Acid
4-isopropenyl-cyclohex-1-enylmethyl Ester
(Linoleoyl Ester of POH)

The monoterpene (or sesquiterpene) derivative may be a monoterpene (or sesquiterpene) conjugated with a therapeutic agent. A monoterpene (or sesquiterpene) conjugate encompassed by the present invention is a molecule having a monoterpene (or sesquiterpene) covalently bound via a chemical linking group to a therapeutic agent. The molar ratio of the monoterpene (or sesquiterpene) to the therapeutic agent in the monoterpene (or sesquiterpene) conjugate may be 1:1, 1:2, 1:3, 1:4, 2:1, 3:1, 4:1, or any other suitable molar ratios. The monoterpene (or sesquiterpene) and the therapeutic agent may be covalently linked through carbamate, ester, ether bonds, or any other suitable chemical functional groups. When the monoterpene (or sesquiterpene) and the therapeutic agent are conjugated through a carbamate bond, the therapeutic agent may be any agent bearing at least one carboxylic acid functional group, or any agent bearing at least one amine functional group. In a specific example, a perillyl alcohol conjugate is perillyl alcohol covalently bound via a chemical linking group to a chemotherapeutic agent.

According to the present invention, the therapeutic agents that may be conjugated with monoterpene (or sesquiterpene) include, but are not limited to, chemotherapeutic agents, therapeutic agents for treatment of CNS disorders (including, without limitation, primary degenerative neurological disorders such as Alzheimer's, Parkinson's, multiple sclerosis, Attention-Deficit Hyperactivity Disorder or ADHD, psychological disorders, psychosis and depression), immunotherapeutic agents, angiogenesis inhibitors, and anti-hypertensive agents. Anti-cancer agents that may be conjugated with monoterpene or sesquiterpene can have one or more of the following effects on cancer cells or the subject: cell death; decreased cell proliferation; decreased numbers of cells; inhibition of cell growth; apoptosis; necrosis; mitotic catastrophe; cell cycle arrest; decreased cell size; decreased cell division; decreased cell survival; decreased cell metabolism; markers of cell damage or cytotoxicity; indirect indicators of cell damage or cytotoxicity such as tumor shrinkage; improved survival of a subject; or disappearance of markers associated with undesirable, unwanted, or aberrant cell proliferation. U.S. Patent Publication No. 20080275057.

Also encompassed by the present invention is admixtures and/or coformulations of a monoterpene (or sesquiterpene) and at least one therapeutic agent.

Chemotherapeutic agents include, but are not limited to, DNA alkylating agents, topoisomerase inhibitors, endoplasmic reticulum stress inducing agents, a platinum compound, an antimetabolite, vincalkaloids, taxanes, epothilones, enzyme inhibitors, receptor antagonists, tyrosine kinase inhibitors, boron radiosensitizers (i.e. velcade), and chemotherapeutic combination therapies.

Non-limiting examples of DNA alkylating agents are nitrogen mustards, such as Cyclophosphamide (Ifosfamide, Trofosfamide), Chlorambucil (Melphalan, Prednimustine), Bendamustine, Uramustine and Estramustine; nitrosoureas, such as Carmustine (BCNU), Lomustine (Semustine), Fotemustine, Nimustine, Ranimustine and Streptozocin; alkyl sulfonates, such as Busulfan (Mannosulfan, Treosulfan); Aziridines, such as Carboquone, Triaziquone, Triethylenemelamine; Hydrazines (Procarbazine); Triazenes such as Dacarbazine and Temozolomide (TMZ); Altretamine and Mitobronitol.

Non-limiting examples of Topoisomerase I inhibitors include Camptothecin derivatives including SN-38, APC, NPC, camptothecin, topotecan, exatecan mesylate, 9-nitrocamptothecin, 9-aminocamptothecin, lurtotecan, rubitecan, silatecan, gimatecan, diflomotecan, exatecan, BN-80927, DX-8951f, and MAG-CPT as described in Pommier Y. (2006) *Nat. Rev. Cancer* 6(10):789-802 and U.S. Patent Publication No. 200510250854; Protoberberine alkaloids and derivatives thereof including berberrubine and coralyne as described in Li et al. (2000) *Biochemistry* 39(24):7107-7116 and Gatto et al. (1996) *Cancer Res.* 15(12):2795-2800; Phenanthroline derivatives including Benzo[i]phenanthridine, Nitidine, and fagaronine as described in Makhey et al. (2003) *Bioorg. Med. Chem.* 11 (8): 1809-1820; Terbenzimidazole and derivatives thereof as described in Xu (1998) *Biochemistry* 37 (10): 3558-3566; and Anthracycline derivatives including Doxorubicin, Daunorubicin, and Mitoxantrone as described in Foglesong et al. (1992) *Cancer Chemother. Pharmacol.* 30 (2): 123-125, Crow et al. (1994) J. *Med. Chem.* 37 (19): 31913194, and Crespi et al. (1986) *Biochem. Biophys. Res. Commun.* 136(2):521-8. Topoisomerase II inhibitors include, but are not limited to Etoposide and Teniposide. Dual topoisomerase I and II inhibitors include, but are not limited to, Saintopin and other Naphthecenediones, DACA and other Acridine-4-Carboxamindes, Intoplicine and other Benzopyridoindoles, TAS-103 and other 7H-indeno[2,1-c]Quinoline-7-ones, Pyrazoloacridine, XR 11576 and other Benzophenazines, XR 5944 and other Dimeric compounds, 7-oxo-7H-dibenz[f,ij]Isoquinolines and 7-oxo-7H-benzo[e]pyrimidines, and Anthracenyl-amino Acid Conjugates as described in Denny and Baguley (2003) *Curr. Top. Med. Chem.* 3(3):339-353. Some agents inhibit Topoisomerase II and have DNA intercalation activity such as, but not limited to, Anthracyclines (Aclarubicin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Amrubicin, Pirarubicin, Valrubicin, Zorubicin) and Anthracenediones (Mitoxantrone and Pixantrone).

Examples of endoplasmic reticulum stress inducing agents include, but are not limited to, dimethyl-celecoxib (DMC), nelfinavir, celecoxib, and boron radiosensitizers (i.e. velcade (Bortezomib)).

Platinum based compounds are a subclass of DNA alkylating agents. Non-limiting examples of such agents include Cisplatin, Nedaplatin, Oxaliplatin, Triplatin tetranitrate, Satraplatin, Aroplatin, Lobaplatin, and JM-216. (see Mckeage et al. (1997) *J. Clin. Oncol.* 201:1232-1237 and in general, CHEMOTHERAPY FOR GYNECOLOGICAL NEOPLASM, CURRENT THERAPY AND NOVEL APPROACHES, in the Series Basic and Clinical Oncology, Angioli et al. Eds., 2004).

"FOLFOX" is an abbreviation for a type of combination therapy that is used to treat colorectal cancer. It includes 5-FU, oxaliplatin and leucovorin. Information regarding this treatment is available on the National Cancer Institute's web site, cancer.gov, last accessed on Jan. 16, 2008.

"FOLFOX/BV" is an abbreviation for a type of combination therapy that is used to treat colorectal cancer. This therapy includes 5-FU, oxaliplatin, leucovorin and Bevacizumab. Furthermore, "XELOX/BV" is another combination therapy used to treat colorectal cancer, which includes the prodrug to 5-FU, known as Capecitabine (Xeloda) in combination with oxaliplatin and bevacizumab. Information regarding these treatments are available on the National Cancer Institute's web site, cancer.gov or from 23 the National Comprehensive Cancer Network's web site, nccn.org, last accessed on May 27, 2008.

Non-limiting examples of antimetabolite agents include Folic acid based, i.e. dihydrofolate reductase inhibitors, such as Aminopterin, Methotrexate and Pemetrexed; thymidylate synthase inhibitors, such as Raltitrexed, Pemetrexed; Purine based, i.e. an adenosine deaminase inhibitor, such as Pentostatin, a thiopurine, such as Thioguanine and Mercaptopurine, a halogenated/ribonucleotide reductase inhibitor, such as Cladribine, Clofarabine, Fludarabine, or a guanine/guanosine: thiopurine, such as Thioguanine; or Pyrimidine based, i.e. cytosine/cytidine: hypomethylating agent, such as Azacitidine and Decitabine, a DNA polymerase inhibitor, such as Cytarabine, a ribonucleotide reductase inhibitor, such as Gemcitabine, or a thymine/thymidine: thymidylate synthase inhibitor, such as a Fluorouracil (5-FU). Equivalents to 5-FU include prodrugs, analogs and derivative thereof such as 5'-deoxy-5-fluorouridine (doxifluridine), 1-tetrahydrofuranyl-5-fluorouracil (ftorafur), Capecitabine (Xeloda), S—I (MBMS-247616, consisting of tegafur and two modulators, a 5-chloro-2,4-dihydroxypyridine and potassium oxonate), raltitrexed (tomudex), nolatrexed (Thymitaq, AG337), LY231514 and ZD9331, as described for example in Papamicheal (1999) The Oncologist 4:478-487.

Examples of vincalkaloids, include, but are not limited to Vinblastine, Vincristine, Vinflunine, Vindesine and Vinorelbine.

Examples of taxanes include, but are not limited to docetaxel, Larotaxel, Ortataxel, Paclitaxel and Tesetaxel. An example of an epothilone is ixabepilone.

Examples of enzyme inhibitors include, but are not limited to farnesyltransferase inhibitors (Tipifarnib); CDK inhibitor (Alvocidib, Seliciclib); proteasome inhibitor (Bortezomib); phosphodiesterase inhibitor (Anagrelide; rolipram); IMP dehydrogenase inhibitor (Tiazofurin); and lipoxygenase inhibitor (Masoprocol). Examples of receptor antagonists include, but are not limited to ERA (Atrasentan); retinoid X receptor (Bexarotene); and a sex steroid (Testolactone).

Examples of tyrosine kinase inhibitors include, but are not limited to inhibitors to ErbB: HER1/EGFR (Erlotinib, Gefitinib, Lapatinib, Vandetanib, Sunitinib, Neratinib); HER2/neu (Lapatinib, Neratinib); RTK class III: C-kit (Axitinib, Sunitinib, Sorafenib), FLT3 (Lestaurtinib), PDGFR (Axitinib, Sunitinib, Sorafenib); and VEGFR (Vandetanib, Semaxanib, Cediranib, Axitinib, Sorafenib); bcr-abl (Imatinib, Nilotinib, Dasatinib); Src (Bosutinib) and Janus kinase 2 (Lestaurtinib).

"Lapatinib" (Tykerb®) is an dual EGFR and erbB-2 inhibitor. Lapatinib has been investigated as an anticancer monotherapy, as well as in combination with trastuzumab, capecitabine, letrozole, paclitaxel and FOLFIRI (irinotecan, 5-fluorouracil and leucovorin), in a number of clinical trials. It is currently in phase III testing for the oral treatment of metastatic breast, head and neck, lung, gastric, renal and bladder cancer.

A chemical equivalent of lapatinib is a small molecule or compound that is a tyrosine kinase inhibitor (TKI) or alternatively a HER-1 inhibitor or a HER-2 inhibitor. Several TKIs have been found to have effective antitumor activity and have been approved or are in clinical trials. Examples of such include, but are not limited to, Zactima (ZD6474), Iressa (gefitinib), imatinib mesylate (STI571; Gleevec), erlotinib (OSI-1774; Tarceva), canertinib (CI 1033), semaxanib (SU5416), vatalanib (PTK787/ZK222584), sorafenib (BAY 43-9006), sutent (SUI 1248) and lefltmomide (SU101).

PTK/ZK is a tyrosine kinase inhibitor with broad specificity that targets all VEGF receptors (VEGFR), the platelet-derived growth factor (PDGF) receptor, c-KIT and c-Fms. Drevs (2003) Idrugs 6 (8): 787-794. PTK/ZK is a targeted drug that blocks angiogenesis and lymphangiogenesis by inhibiting the activity of all known receptors that bind VEGF including VEGFR-I (Flt-1), VEGFR-2 (KDR/Flk-1) and VEGFR-3 (Flt-4). The chemical names of PTK/ZK are 1-[4-Chloroanilino]-4-[4-pyridylmethyl]phthalazine Succinate or 1-Phthalazinamine, N-(4-chlorophenyl)-4-(4-pyridinylmethyl)-butanedioate (1:1). Synonyms and analogs of PTK/TK are known as Vatalanib, CGP79787D, PTK787/ZK 222584, CGP-79787, DE-00268, PTK-787, PTK787A, VEGFR-TK inhibitor, ZK 222584 and ZK.

Chemotherapeutic agents that can be conjugated with monoterpene or sesquiterpene may also include amsacrine, Trabectedin, retinoids (Alitretinoin, Tretinoin), Arsenic trioxide, asparagine depleter Asparaginase/Pegaspargase), Celecoxib, Demecolcine, Elesclomol, Elsamitrucin, Etoglucid, Lonidamine, Lucanthone, Mitoguazone, Mitotane, Oblimersen, Temsirolimus, and Vorinostat.

The monoterpene or sesquiterpene derivative may be conjugated with angiogenesis inhibitors. Examples of angiogenesis inhibitors include, but are not limited to, angiostatin, angiozyme, antithrombin III, AG3340, VEGF inhibitors, batimastat, bevacizumab (avastin), BMS-275291, CAI, 2C3, HuMV833 Canstatin, Captopril, carboxyamidotriazole, cartilage derived inhibitor (CDI), CC-5013, 6-O-(chloroacetyl-carbonyl)-fumagillol, COL-3, combretastatin, combretastatin A4 Phosphate, Dalteparin, EMD 121974 (Cilengitide), endostatin, erlotinib, gefitinib (Iressa), genistein, halofuginone hydrobromide, Id1, Id3, IM862, imatinib mesylate, IMC-IC11 Inducible protein 10, interferon-alpha, interleukin 12, lavendustin A, LY317615 or AE-941, marimastat, mspin, medroxiprogesterone acetate, Meth-1, Meth-2, 2-methoxyestradiol (2-ME), neovastat, osteopontin cleaved product, PEX, pigment epithelium growth factor (PEGF), platelet factor 4, prolactin fragment, proliferin-related protein (PRP), PTK787/ZK 222584, ZD6474, recombinant human platelet factor 4 (rPF4), restin, squalamine, SU5416, SU6668, SU11248 suramin, Taxol, Tecogalan, thalidomide, thrombospondin, TNP-470, troponin-1, vasostatin, VEG1, VEGF-Trap, and ZD6474.

Non-limiting examples of angiogenesis inhibitors also include, tyrosine kinase inhibitors, such as inhibitors of the tyrosine kinase receptors Flt-1 (VEGFR1) and Flk-1/KDR (VEGFR2), inhibitors of epidermal-derived, fibroblast-derived, or platelet derived growth factors, MMP (matrix metalloprotease) inhibitors, integrin blockers, pentosan polysulfate, angiotensin II antagonists, cyclooxygenase inhibitors (including non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen, as well as selective cyclooxygenase-2 inhibitors such as celecoxib and rofecoxib), and steroidal anti-inflammatories (such as corticosteroids, mineralocorticoids, dexamethasone, prednisone, prednisolone, methylpred, betamethasone).

Other therapeutic agents that modulate or inhibit angiogenesis and may also be conjugated with monoterpene or sesquiterpene include agents that modulate or inhibit the coagulation and fibrinolysis systems, including, but not limited to, heparin, low molecular weight heparins and carboxypeptidase U inhibitors (also known as inhibitors of active thrombin activatable fibrinolysis inhibitor[TAFIa]). U.S. Patent Publication No. 20090328239. U.S. Pat. No. 7,638,549.

Non-limiting examples of the anti-hypertensive agents include angiotensin converting enzyme inhibitors (e.g., captopril, enalapril, delapril etc.), angiotensin II antagonists (e.g., candesartan cilexetil, candesartan, losartan (or Cozaar), losartan potassium, eprosartan, valsartan (or Diovan), telmisartan, irbesartan, tasosartan, olmesartan, olmesartan medoxomil etc.), calcium antagonists (e.g., manidipine, nifedipine, amlodipine (or Amlodin), efonidipine, nicardipine etc.), diuretics, renin inhibitor (e.g., aliskiren etc.), aldosterone antagonists (e.g., spironolactone, eplerenone etc.), beta-blockers (e.g., metoprolol (or Toporol), atenolol, propranolol, carvedilol, pindolol etc.), vasodilators (e.g., nitrate, soluble guanylate cyclase stimulator or activator, prostacycline etc.), angiotensin vaccine, clonidine and the like. U.S. Patent Publication No. 20100113780.

Other therapeutic agents that may be conjugated with monoterpene (or sesquiterpene) include, but are not limited to, Sertraline (Zoloft), Topiramate (Topamax), Duloxetine (Cymbalta), Sumatriptan (Imitrex), Pregabalin (Lyrica), Lamotrigine (Lamictal), Valaciclovir (Valtrex), Tamsulosin (Flomax), Zidovudine (Combivir), Lamivudine (Combivir), Efavirenz (Sustiva), Abacavir (Epzicom), Lopinavir (Kaletra), Pioglitazone (Actos), Desloratidine (Clarinex), Cetirizine (Zyrtec), Pantoprazole (Protonix), Lansoprazole (Prevacid), Rabeprazole (Aciphex), Moxifloxacin (Avelox), Meloxicam (Mobic), Dorzolamide (Truspot), Diclofenac (Voltaren), Enlapril (Vasotec), Montelukast (Singulair), Sildenafil (Viagra), Carvedilol (Coreg), Ramipril (Delix).

Table 1 lists pharmaceutical agents that can be conjugated with monoterpene (or sesquiterpene), including structure of the pharmaceutical agent and the preferred derivative for conjugation.

TABLE 1

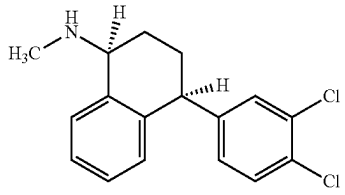
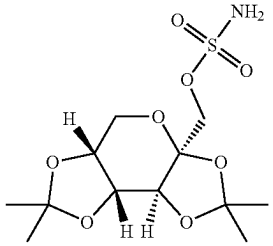
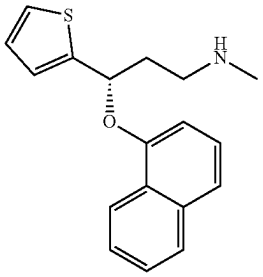
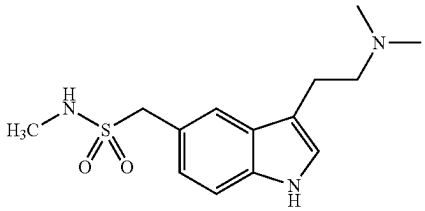
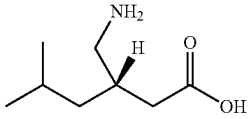
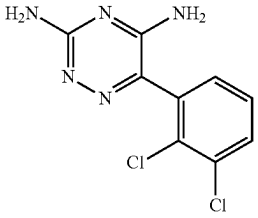
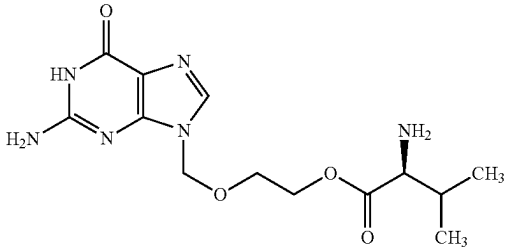
Brand Name	Generic Name	Activity	Structure	Preferred Derivative
Zoloft	Sertraline	Depression		Carbamate
Topamax	Topiramate	Seizures		Carbamate
Cymbalta	Duloxetine	Depression		Carbamate
Imitrex	Sumatriptan	Migraine		Carbamate
Lyrica	Pregabalin	Neuropathic pain		Carbamate or Ester
Lamictal	Lamotrigine	Seizures		Carbamate
Valtrex	Valaciclovir	Herpes		Carbamate

TABLE 1-continued

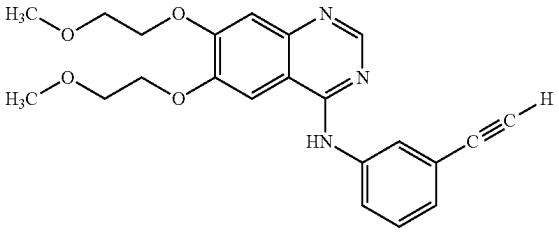
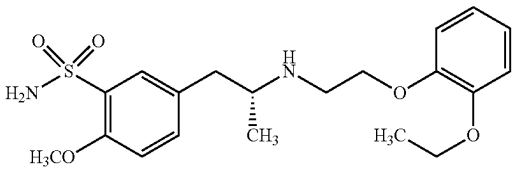
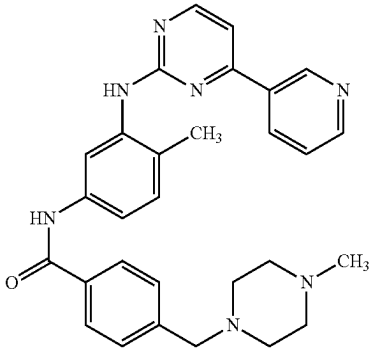
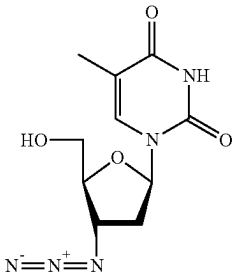
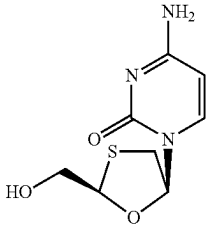
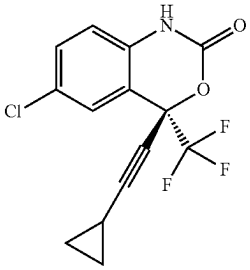
Brand Name	Generic Name	Activity	Structure	Preferred Derivative
Tarceva	Erlotinib	Non-small cell lung cancer		Carbamate
Flomax	Tamsulosin	Benign prostatic Cancer		Carbamate
Gleevec	Imatinib	Leukemia		Carbamate
Combivir	Zidovudine	HIV infection		Carbamate
Combivir	Lamivudine	HIV infection		Carbonate
Sustiva	Efavirenz	HIV infection		Carbamate

TABLE 1-continued

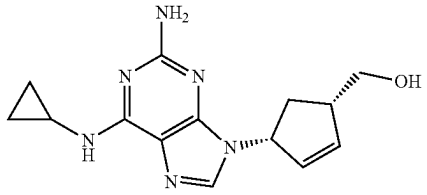
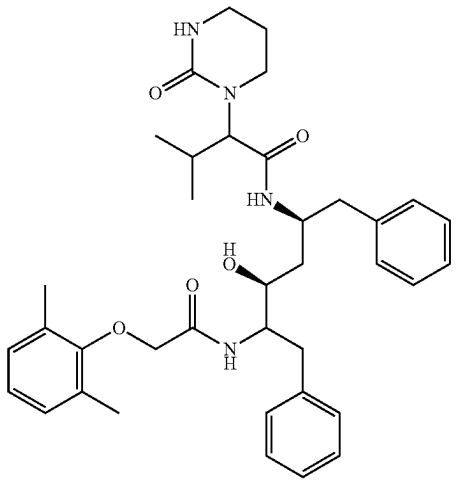
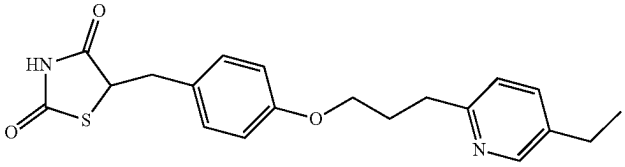
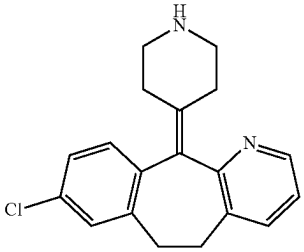
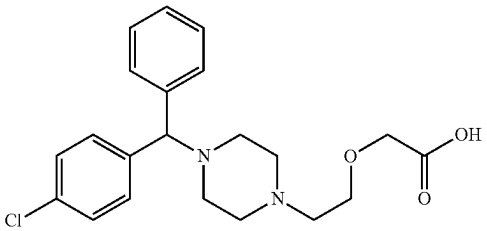
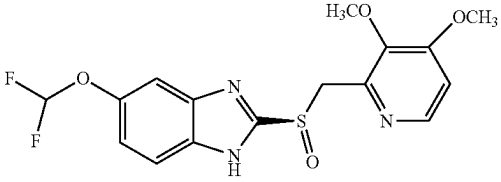
Brand Name	Generic Name	Activity	Structure	Preferred Derivative
Epzicom	Abacavir	HIV infection		Carbamate
Kaletra	Lopinavir	HIV infection		Carbamate
Actos	Pioglitazone	Type-2 diabetes		Carbamate
Clarinex	Desloratidine	Allergic rhinitis		Carbamate
Zyrtec	Cetirizine	Allergic		Ester
Protonix	Pentoprazole	Gastrointestinal		Carbamate

TABLE 1-continued

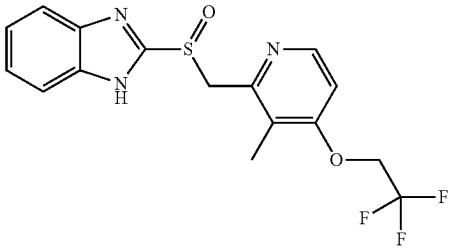
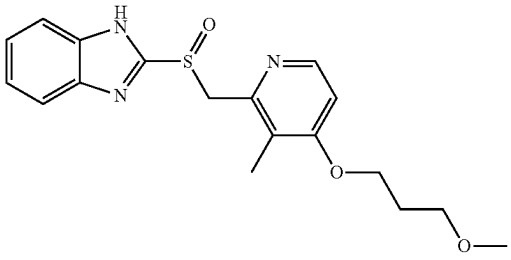
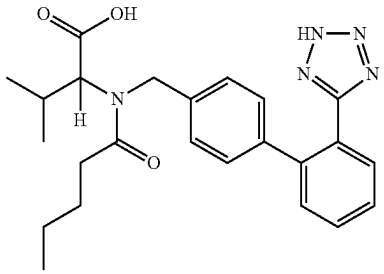
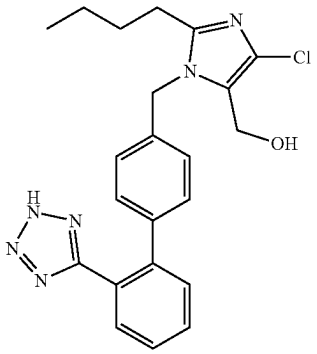
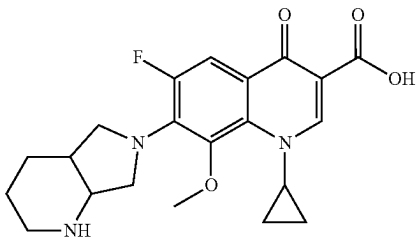
Brand Name	Generic Name	Activity	Structure	Preferred Derivative
Prevacid	Lansoprazole	Gastrointestinal		Carbamate
Aciphex	Rebeprazole	Gastrointestinal		Carbamate
Diovan	Valsartan	Hypertension		Carbamate
Cozaar	Losartan	Hypertension		Carbamate
Avelox	Moxifloxacin	Bacterial infection		Carbamate or Ester

TABLE 1-continued

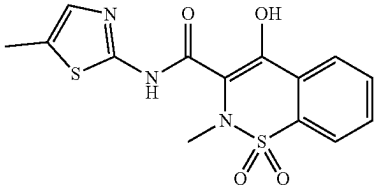
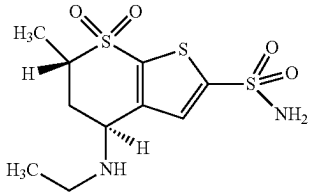
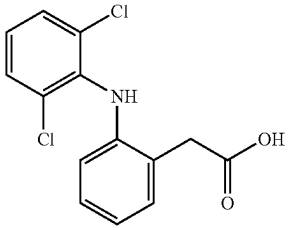
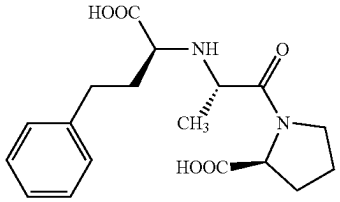
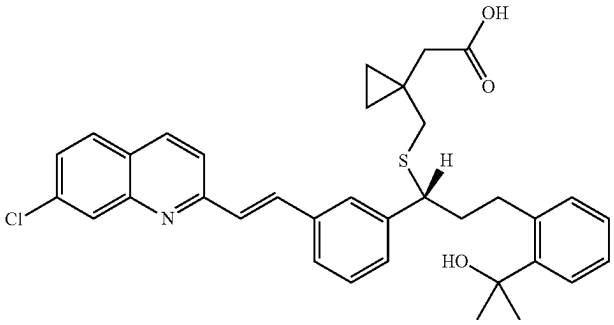
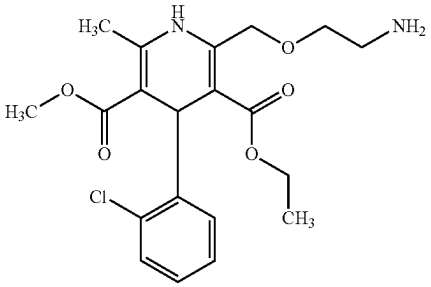
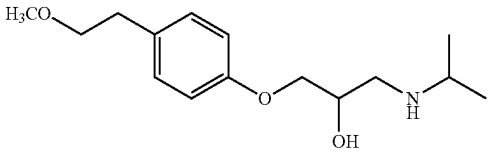
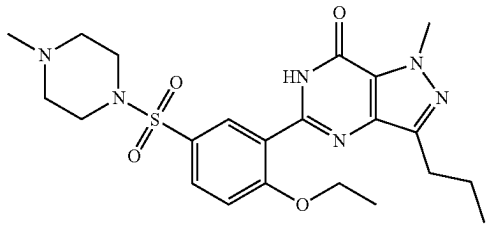
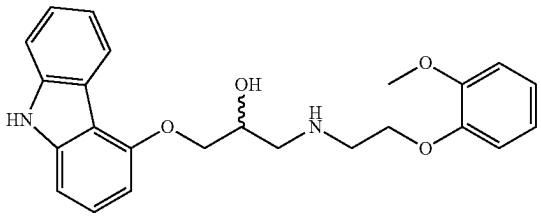
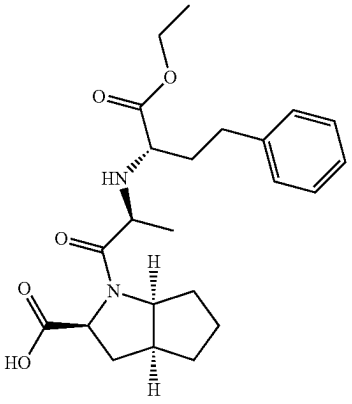
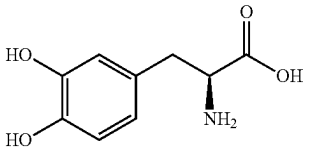
Brand Name	Generic Name	Activity	Structure	Preferred Derivative
Mobic	Meloxicam	Osteoarthritis		Carbamate
Truspot	Dorzolamide	Intraocular pressure		Carbamate
Voltaren	Diclofenac	Osteoarthritis & rheumatoid arthritis		Carbamate or Ester
Vasotec	Enlapril	Hypertension		Carbamate or Ester
Singulair	Montelukast	Asthma		Ester
Amlodin	Amlodipine	Hypertension		Carbamate

TABLE 1-continued

Brand Name	Generic Name	Activity	Structure	Preferred Derivative
Toporol	Metoprolol	Hypertension		Carbamate
Viagra	Sildenafil	Erectile dysfunction		Carbamate
Coreg	Carvedilol	Hypertension		Carbamate
Delix	Ramipril	Hypertension		Carbamate or Ester
Sinemet (Parcopa, Atamet)	L-DOPA	Neurological disorders		Carbamate or Ester

The purity of the monoterpene (or sesquiterpene) derivatives may be assayed by gas chromatography (GC) or high pressure liquid chromatography (HPLC). Other techniques for assaying the purity of monoterpene (or sesquiterpene) derivatives and for determining the presence of impurities include, but are not limited to, nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), GC-MS, infrared spectroscopy (IR), and thin layer chromatography (TLC). Chiral purity can be assessed by chiral GC or measurement of optical rotation.

The monoterpene (or sesquiterpene) derivatives may be purified by methods such as crystallization, or by separating the monoterpene (or sesquiterpene) derivative from impurities according to the unique physicochemical properties (e.g., solubility or polarity) of the derivative. Accordingly, the monoterpene (or sesquiterpene) derivative can be separated from the monoterpene (or sesquiterpene) by suitable separation techniques known in the art, such as preparative chromatography, (fractional) distillation, or (fractional) crystallization.

The invention also provides for methods of using monoterpenes (or sesquiterpenes) derivatives to treat a disease, such as cancer or other nervous system disorders. A monoterpenes (or sesquiterpenes) derivative may be administered alone, or in combination with radiation, surgery or chemotherapeutic agents. A monoterpene or sesquiterpene derivative may also be co-administered with antiviral agents, anti-inflammatory agents or antibiotics. The agents

may be administered concurrently or sequentially. A monoterpenes (or sesquiterpenes) derivative can be administered before, during or after the administration of the other active agent(s).

The monoterpene or sesquiterpene derivative may be used in combination with radiation therapy. In one embodiment, the present invention provides for a method of treating tumor cells, such as malignant glioma cells, with radiation, where the cells are treated with an effective amount of a monoterpene derivative, such as a perillyl alcohol carbamate, and then exposed to radiation. Monoterpene derivative treatment may be before, during and/or after radiation. For example, the monoterpene or sesquiterpene derivative may be administered continuously beginning one week prior to the initiation of radiotherapy and continued for two weeks after the completion of radiotherapy. U.S. Pat. Nos. 5,587,402 and 5,602,184.

In one embodiment, the present invention provides for a method of treating tumor cells, such as malignant glioma cells, with chemotherapy, where the cells are treated with an effective amount of a monoterpene derivative, such as a perillyl alcohol carbamate, and then exposed to chemotherapy. Monoterpene derivative treatment may be before, during and/or after chemotherapy.

Monoterpene (or sesquiterpene) derivatives may be used for the treatment of nervous system cancers, such as a malignant glioma (e.g., astrocytoma, anaplastic astrocytoma, glioblastoma multiforme), retinoblastoma, pilocytic astrocytomas (grade I), meningiomas, metastatic brain tumors, neuroblastoma, pituitary adenomas, skull base meningiomas, and skull base cancer. As used herein, the term "nervous system tumors" refers to a condition in which a subject has a malignant proliferation of nervous system cells.

Cancers that can be treated by the present monoterpene (or sesquiterpene) derivatives include, but are not limited to, lung cancer, ear, nose and throat cancer, leukemia, colon cancer, melanoma, pancreatic cancer, mammary cancer, prostate cancer, breast cancer, hematopoietic cancer, ovarian cancer, basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; breast cancer; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer; intra-epithelial neoplasm; kidney cancer; larynx cancer; leukemia including acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymphoid leukemia; liver cancer; lymphoma including Hodgkin's and Non-Hodgkin's lymphoma; myeloma; fibroma, neuroblastoma; oral cavity cancer (e.g., lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; renal cancer; cancer of the respiratory system; sarcoma; skin cancer; stomach cancer; testicular cancer; thyroid cancer; uterine cancer; cancer of the urinary system, as well as other carcinomas and sarcomas. U.S. Pat. No. 7,601,355.

The present invention also provides methods of treating CNS disorders, including, without limitation, primary degenerative neurological disorders such as Alzheimer's, Parkinson's, psychological disorders, psychosis and depression. Treatment may consist of the use of a monoterpene or sesquiterpene derivative alone or in combination with current medications used in the treatment of Parkinson's, Alzheimer's, or psychological disorders.

The present invention also provides a method of improving immunomodulatory therapy responses comprising the steps of exposing cells to an effective amount of a monoterpene or sesquiterpene derivative, such as a perillyl alcohol carbamate, before or during immunomodulatory treatment. Preferred immunomodulatory agents are cytokines, such interleukins, lymphokines, monokines, interferons and chemokines.

The present composition may be administered by any method known in the art, including, without limitation, intranasal, oral, transdermal, ocular, intraperitoneal, inhalation, intravenous, ICV, intracisternal injection or infusion, subcutaneous, implant, vaginal, sublingual, urethral (e.g., urethral suppository), subcutaneous, intramuscular, intravenous, rectal, sub-lingual, mucosal, ophthalmic, spinal, intrathecal, intra-articular, intra-arterial, sub-arachnoid, bronchial and lymphatic administration. Topical formulation may be in the form of gel, ointment, cream, aerosol, etc; intranasal formulation can be delivered as a spray or in a drop; transdermal formulation may be administered via a transdermal patch or iontophoresis; inhalation formulation can be delivered using a nebulizer or similar device. Compositions can also take the form of tablets, pills, capsules, semisolids, powders, sustained release formulations, solutions, suspensions, elixirs, aerosols, or any other appropriate compositions.

To prepare such pharmaceutical compositions, one or more of monoterpene (or sesquiterpene) derivatives may be mixed with a pharmaceutical acceptable carrier, adjuvant and/or excipient, according to conventional pharmaceutical compounding techniques. Pharmaceutically acceptable carriers that can be used in the present compositions encompass any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions can additionally contain solid pharmaceutical excipients such as starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk and the like. Liquid and semisolid excipients may be selected from glycerol, propylene glycol, water, ethanol and various oils, including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, etc. Liquid carriers, particularly for injectable solutions, include water, saline, aqueous dextrose, and glycols. For examples of carriers, stabilizers and adjuvants, see *Remington's Pharmaceutical Sciences*, edited by E. W. Martin (Mack Publishing Company, 18th ed., 1990). The compositions also can include stabilizers and preservatives.

As used herein, the term "therapeutically effective amount" is an amount sufficient to treat a specified disorder or disease or alternatively to obtain a pharmacological response treating a disorder or disease. Methods of determining the most effective means and dosage of administration can vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Treatment dosages generally may be titrated to optimize safety and efficacy. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. Suitable dosage formulations and methods of administering the agents can be readily determined by those of skill in the art. For example, the composition are administered at about 0.01 mg/kg to about 200 mg/kg, about 0.1 mg/kg to about 100 mg/kg, or about 0.5 mg/kg to about 50 mg/kg. When the compounds described herein are co-administered with another agent or therapy, the effective amount may be less than when the agent is used alone.

Transdermal formulations may be prepared by incorporating the active agent in a thixotropic or gelatinous carrier such as a cellulosic medium, e.g., methyl cellulose or hydroxyethyl cellulose, with the resulting formulation then being packed in a transdermal device adapted to be secured in dermal contact with the skin of a wearer. If the composition is in the form of a gel, the composition may be rubbed onto a membrane of the patient, for example, the skin, preferably intact, clean, and dry skin, of the shoulder or upper arm and or the upper torso, and maintained thereon for a period of time sufficient for delivery of the monoterpene (or sesquiterpene) derivative to the blood serum of the patient. The composition of the present invention in gel form may be contained in a tube, a sachet, or a metered pump. Such a tube or sachet may contain one unit dose, or more than one unit dose, of the composition. A metered pump may be capable of dispensing one metered dose of the composition.

This invention also provides the compositions as described above for intranasal administration. As such, the compositions can further comprise a permeation enhancer. Southall et al. *Developments in Nasal Drug Delivery,* 2000. The monoterpene (or sesquiterpene) derivative may be administered intranasally in a liquid form such as a solution, an emulsion, a suspension, drops, or in a solid form such as a powder, gel, or ointment. Devices to deliver intranasal medications are well known in the art. Nasal drug delivery can be carried out using devices including, but not limited to, intranasal inhalers, intranasal spray devices, atomizers, nasal spray bottles, unit dose containers, pumps, droppers, squeeze bottles, nebulizers, metered dose inhalers (MDI), pressurized dose inhalers, insufflators, and bi-directional devices. The nasal delivery device can be metered to administer an accurate effective dosage amount to the nasal cavity. The nasal delivery device can be for single unit delivery or multiple unit delivery. In a specific example, the ViaNase Electronic Atomizer from Kurve Technology (Bethell, Washington) can be used in this invention (http://www.kurvetech.com). The compounds of the present invention may also be delivered through a tube, a catheter, a syringe, a packtail, a pledget, a nasal tampon or by submucosal infusion. U.S. Patent Publication Nos. 20090326275, 20090291894, 20090281522 and 20090317377.

The monoterpene (or sesquiterpene) derivative can be formulated as aerosols using standard procedures. The monoterpene (or sesquiterpene) derivative may be formulated with or without solvents, and formulated with or without carriers. The formulation may be a solution, or may be an aqueous emulsion with one or more surfactants. For example, an aerosol spray may be generated from pressurized container with a suitable propellant such as, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, hydrocarbons, compressed air, nitrogen, carbon dioxide, or other suitable gas. The dosage unit can be determined by providing a valve to deliver a metered amount. Pump spray dispensers can dispense a metered dose or a dose having a specific particle or droplet size. As used herein, the term "aerosol" refers to a suspension of fine solid particles or liquid solution droplets in a gas. Specifically, aerosol includes a gas-borne suspension of droplets of a monoterpene (or sesquiterpene), as may be produced in any suitable device, such as an MDI, a nebulizer, or a mist sprayer. Aerosol also includes a dry powder composition of the composition of the instant invention suspended in air or other carrier gas. Gonda (1990) *Critical Reviews in Therapeutic Drug Carrier Systems* 6:273-313. Raeburn et al., (1992) *Pharmacol. Toxicol. Methods* 27:143-159.

The monoterpene (or sesquiterpene) derivative may be delivered to the nasal cavity as a powder in a form such as microspheres delivered by a nasal insufflator. The monoterpene (or sesquiterpene) derivative may be absorbed to a solid surface, for example, a carrier. The powder or microspheres may be administered in a dry, air-dispensable form. The powder or microspheres may be stored in a container of the insufflator. Alternatively the powder or microspheres may be filled into a capsule, such as a gelatin capsule, or other single dose unit adapted for nasal administration.

The pharmaceutical composition can be delivered to the nasal cavity by direct placement of the composition in the nasal cavity, for example, in the form of a gel, an ointment, a nasal emulsion, a lotion, a cream, a nasal tampon, a dropper, or a bioadhesive strip. In certain embodiments, it can be desirable to prolong the residence time of the pharmaceutical composition in the nasal cavity, for example, to enhance absorption. Thus, the pharmaceutical composition can optionally be formulated with a bioadhesive polymer, a gum (e.g., xanthan gum), chitosan (e.g., highly purified cationic polysaccharide), pectin (or any carbohydrate that thickens like a gel or emulsifies when applied to nasal mucosa), a microsphere (e.g., starch, albumin, dextran, cyclodextrin), gelatin, a liposome, carbomer, polyvinyl alcohol, alginate, acacia, chitosans and/or cellulose (e.g., methyl or propyl; hydroxyl or carboxy; carboxymethyl or hydroxylpropyl).

The composition containing the purified monoterpene (or sesquiterpene) can be administered by oral inhalation into the respiratory tract, i.e., the lungs.

Typical delivery systems for inhalable agents include nebulizer inhalers, dry powder inhalers (DPI), and metered-dose inhalers (MDI).

Nebulizer devices produce a stream of high velocity air that causes a therapeutic agent in the form of liquid to spray as a mist. The therapeutic agent is formulated in a liquid form such as a solution or a suspension of particles of suitable size. In one embodiment, the particles are micronized. The term "micronized" is defined as having about 90% or more of the particles with a diameter of less than about 10 μm. Suitable nebulizer devices are provided commercially, for example, by PARI GmbH (Starnberg, Germany). Other nebulizer devices include Respimat (Boehringer Ingelheim) and those disclosed in, for example, U.S. Pat. Nos. 7,568,480 and 6,123,068, and WO 97/12687. The monoterpenes (or sesquiterpenes) can be formulated for use in a nebulizer device as an aqueous solution or as a liquid suspension.

DPI devices typically administer a therapeutic agent in the form of a free flowing powder that can be dispersed in a patient's air-stream during inspiration. DPI devices which use an external energy source may also be used in the present invention. In order to achieve a free flowing powder, the therapeutic agent can be formulated with a suitable excipient (e.g., lactose). A dry powder formulation can be made, for example, by combining dry lactose having a particle size between about 1 μm and 100 μm with micronized particles of the monoterpenes (or sesquiterpenes) and dry blending. Alternatively, the monoterpene can be formulated without excipients. The formulation is loaded into a dry powder dispenser, or into inhalation cartridges or capsules for use with a dry powder delivery device. Examples of DPI devices provided commercially include Diskhaler (GlaxoSmithKline, Research Triangle Park, N.C.) (see, e.g., U.S. Pat. No. 5,035,237); Diskus (GlaxoSmithKline) (see, e.g., U.S. Pat. No. 6,378,519; Turbuhaler (AstraZeneca, Wilmington, Del.) (see, e.g., U.S. Pat. No. 4,524,769); and Rotahaler (GlaxoSmithKline) (see, e.g., U.S. Pat. No. 4,353,

365). Further examples of suitable DPI devices are described in U.S. Pat. Nos. 5,415,162, 5,239,993, and 5,715,810 and references therein.

MDI devices typically discharge a measured amount of therapeutic agent using compressed propellant gas. Formulations for MDI administration include a solution or suspension of active ingredient in a liquefied propellant. Examples of propellants include hydrofluoroalkanes (HFA), such as 1,1,1,2-tetrafluoroethane (HFA 134a) and 1,1,1,2,3,3,3-heptafluoro-n-propane, (HFA 227), and chlorofluorocarbons, such as CCl_3F. Additional components of HFA formulations for MDI administration include co-solvents, such as ethanol, pentane, water; and surfactants, such as sorbitan trioleate, oleic acid, lecithin, and glycerin. (See, for example, U.S. Pat. No. 5,225,183, EP 0717987, and WO 92/22286). The formulation is loaded into an aerosol canister, which forms a portion of an MDI device. Examples of MDI devices developed specifically for use with HFA propellants are provided in U.S. Pat. Nos. 6,006,745 and 6,143,227. For examples of processes of preparing suitable formulations and devices suitable for inhalation dosing see U.S. Pat. Nos. 6,268,533, 5,983,956, 5,874,063, and 6,221,398, and WO 99/53901, WO 00/61108, WO 99/55319 and WO 00/30614.

The monoterpene (or sesquiterpene) derivative may be encapsulated in liposomes or microcapsules for delivery via inhalation. A liposome is a vesicle composed of a lipid bilayer membrane and an aqueous interior. The lipid membrane may be made of phospholipids, examples of which include phosphatidylcholine such as lecithin and lysolecithin; acidic phospholipids such as phosphatidylserine and phosphatidylglycerol; and sphingophospholipids such as phosphatidylethanolamine and sphingomyelin. Alternatively, cholesterol may be added. A microcapsule is a particle coated with a coating material. For example, the coating material may consist of a mixture of a film-forming polymer, a hydrophobic plasticizer, a surface activating agent or/and a lubricant nitrogen-containing polymer. U.S. Pat. Nos. 6,313,176 and 7,563,768.

The monoterpene (or sesquiterpene) derivative may also be used alone or in combination with other chemotherapeutic agents via topical application for the treatment of localized cancers such as breast cancer or melanomas. The monoterpene (or sesquiterpene) derivative may also be used in combination with narcotics or analgesics for transdermal delivery of pain medication.

This invention also provides the compositions as described above for ocular administration. As such, the compositions can further comprise a permeation enhancer. For ocular administration, the compositions described herein can be formulated as a solution, emulsion, suspension, etc. A variety of vehicles suitable for administering compounds to the eye are known in the art. Specific non-limiting examples are described in U.S. Pat. Nos. 6,261,547; 6,197,934; 6,056,950; 5,800,807; 5,776,445; 5,698,219; 5,521,222; 5,403,841; 5,077,033; 4,882,150; and 4,738,851.

The monoterpene (or sesquiterpene) derivative can be given alone or in combination with other drugs for the treatment of the above diseases for a short or prolonged period of time. The present compositions can be administered to a mammal, preferably a human. Mammals include, but are not limited to, murines, rats, rabbit, simians, bovines, ovine, porcine, canines, feline, farm animals, sport animals, pets, equine, and primates.

The invention also provides a method for inhibiting the growth of a cell in vitro, ex vivo or in vivo, where a cell, such as a cancer cell, is contacted with an effective amount of the monoterpene (or sesquiterpene) derivative as described herein.

Pathological cells or tissue such as hyperproliferative cells or tissue may be treated by contacting the cells or tissue with an effective amount of a composition of this invention. The cells, such as cancer cells, can be primary cancer cells or can be cultured cells available from tissue banks such as the American Type Culture Collection (ATCC). The pathological cells can be cells of a systemic cancer, gliomas, meningiomas, pituitary adenomas, or a CNS metastasis from a systemic cancer, lung cancer, prostate cancer, breast cancer, hematopoietic cancer or ovarian cancer. The cells can be from a vertebrate, preferably a mammal, more preferably a human. U.S. Patent Publication No. 2004/0087651. Balassiano et al. (2002) *Intern. J. Mol. Med.* 10:785-788. Thorne, et al. (2004) *Neuroscience* 127:481-496. Fernandes, et al. (2005) *Oncology Reports* 13:943-947. Da Fonseca, et al. (2008) *Surgical Neurology* 70:259267. Da Fonseca, et al. (2008) *Arch. Immunol. Ther. Exp.* 56:267-276. Hashizume, et al. (2008) *Neuroncology* 10:112-120.

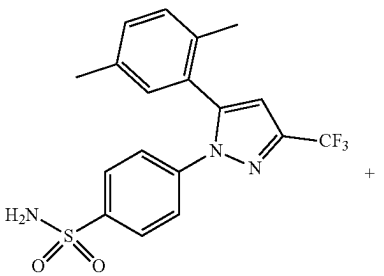
In vitro efficacy of the present composition can be determined using methods well known in the art. For example, the cytoxicity of the present monoterpene (or sesquiterpene) and/or the therapeutic agents may be studied by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]cytotoxicity assay. MTT assay is based on the principle of uptake of MTT, a tetrazolium salt, by metabolically active cells where it is metabolized into a blue colored formazan product, which can be read spectrometrically. *J. of Immunological Methods* 65:55 63, 1983. The cytoxicity of the present monoterpene (or sesquiterpene) derivative and/or the therapeutic agents may be studied by colony formation assay. Functional assays for inhibition of VEGF secretion and IL-8 secretion may be performed via ELISA. Cell cycle block by the present monoterpene (or sesquiterpene) derivative and/or the therapeutic agents may be studied by standard propidium iodide (PI) staining and flow cytometry. Invasion inhibition may be studied by Boyden chambers. In this assay a layer of reconstituted basement membrane, Matrigel, is coated onto chemotaxis filters and acts as a barrier to the migration of cells in the Boyden chambers. Only cells with invasive capacity can cross the Matrigel barrier. Other assays include, but are not limited to cell viability assays, apoptosis assays, and morphological assays.

The following are examples of the present invention and are not to be construed as limiting.

EXAMPLES

Example 1: Synthesis of Dimethyl Celecoxib bis-POH Carbamate (4-(bis-N,N'-4-isopropenyl cyclohex-1-enylmethyloxy carbonyl[5-(2,5-dimethyl phenyl)-3-trifluoromethyl pyrazol-1-yl] benzenesulfonamide)

The reaction scheme is the following:



Dimethyl celecoxib

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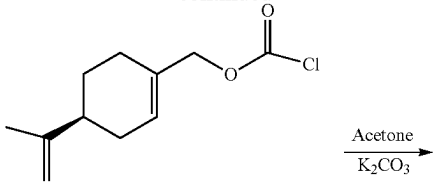
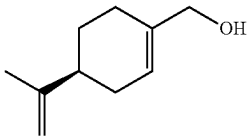
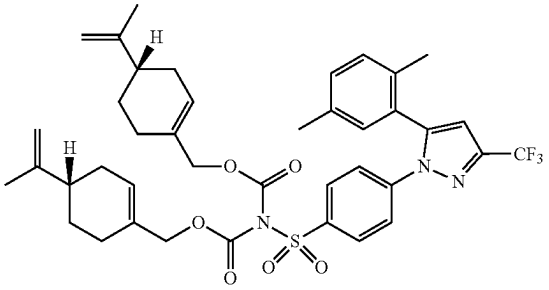


Perillyl chloroformate

Phosgene
Toluene



Perillyl alcohol



DMC-POH

Phosgene (20% in toluene, 13 ml, 26.2 mmol) was added to a mixture of perillyl alcohol (2.0 grams, 13.1 mmol) and potassium carbonate (5.4 grams, 39.1 mmol) in dry toluene (30 mL) over a period of 30 minutes while maintaining the temperature between 10° C. to 15° C. The reaction mixture was allowed to warm to room temperature and stirred for 8.0 hours under N_2. The reaction mixture was quenched with water (30 mL) and the organic layer was separated. The aqueous layer was extracted with toluene (20 mL) and the combined organic layer was washed with water (50 mL×2), brine (15%, 30 mL) and dried over sodium sulfate (20 grams). The filtered organic layer was concentrated under vacuum to give perillyl chloroformate as an oil. Weight: 2.5 grams; Yield: 89%. ^{1}H-NMR (400 MHZ, $CDCl_3$): δ 1.5 (m, 1H), 1.7 (s, 3H), 1.8 (m, 1H), 2.0 (m, 1H), 2.2 (m, 4H), 4.7 (dd, 4H); 5.87 (m, 1H).

Perillyl chloroformate (0.11 grams, 0.55 mmol) was added slowly to a mixture of dimethyl celecoxib (0.2 grams, 0.50 mmol) and potassium carbonate (0.13 grams, 1.0 mmol) in dry acetone (10 mL) over a period of 5 minutes under N_2. The reaction mixture was heated to reflux and maintained for 3 hours. Since TLC analysis indicated the presence of dimethyl celecoxib (>60%), another 1.0 equivalent of perillyl chloroformate was added and refluxed for an additional 5 hours. The reaction mixture was cooled and acetone was concentrated under vacuum to give a residue.

The resulting residue was suspended in water (15 mL) and extracted with ethyl acetate (3×15 mL). The combined organic layer was washed with water (20 mL) followed by brine (15%, 20 mL) and dried over sodium sulfate. The filtered organic layer was concentrated under vacuum to give a residue which was purified by column chromatography [column dimensions: diameter: 1.5 cm, height: 10 cm, silica: 230-400 mesh] and eluted with hexanes (100 mL) followed by a mixture of hexanes/ethyl acetate (95:5, 100 mL). The hexane/ethyl acetate fractions were combined and concentrated under vacuum to give a gummy mass.

The product POH carbamate exhibited a weight of 120 mg and a yield of 31%. ^{1}H-NMR (400 MHZ, $CDCl_3$): δ 0.9 (m, 2H), 1.4 (m, 2H), 1.7 (m, 7H*), 1.95 (m, 8H*), 2.1 (m, 4H), 2.3 (s, 3H), 4.4 (d, 2H), 4.7 (dd, 2H), 5.6 (br d, 2H), 6.6 (s, 1H), 7.0 (br s, 1H), 7.12 (d, 1H), 7.19 (d, 1H), 7.4 (d, 2H), 7.85 (d, 2H); MS, m/e: 751.8 (M 3%), 574.3 (100%), 530.5 (45%), 396 (6%). *N.B. further 2H overlapping from presumed impurity discounted in NMR integration.

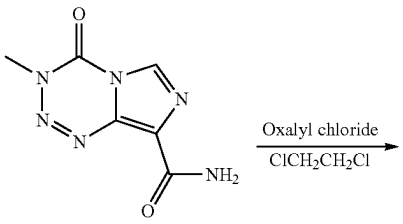
Example 2: In Vitro Cytotoxicity Studies of Dimethyl Celecoxib bisPOH Carbamate (POH-DMC)

First cytotoxicity assays were carried out after cells were treated with dimethyl-celecoxib (DMC) alone. FIG. **1** shows the results of the MTT cytotoxicity assays performed on human malignant glioma cells U87, A172 and U251 with DMC alone.

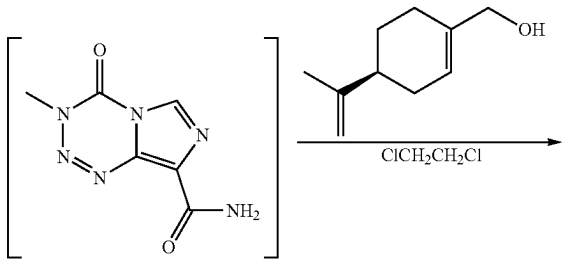
Then U87, A172 and U251 cells were treated with dimethyl celecoxib bisPOH carbamate (POH-DMC) (e.g., synthesized by the method in Example 1), and the MTT cytotoxicity assays performed (FIG. **2**). The results suggest that POH carbamate POH-DMC exhibited much better cytotoxicity than DMC alone.

Example 3: Synthesis of Temozolomide POH Carbamate (3-methyl 4-oxo-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8-carbonyl)-carbamic acid-4-isopropenyl cyclohex-1-enylmethyl Ester)

The reaction scheme is the following:



Temozolamide



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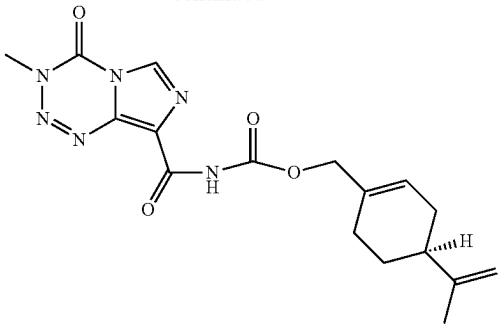


TMZ-POH

Oxalyl chloride (0.13 grams, 1.0 mmol) was added slowly to a mixture of temozolomide (OChem Incorporation, 0.1 grams, 0.5 mmol) in 1,2-dichloroethane (10 mL) over a period of 2 minutes while maintaining the temperature at 10° C. under N_2. The reaction mixture was allowed to warm to room temperature and then heated to reflux for 3 hours. The excess of oxalyl chloride and 1,2-dichloroethane were removed by concentration under vacuum. The resulting residue was re-dissolved in 1,2-dichlorethane (15 mL) and the reaction mixture was cooled to 10° C. under N_2. A solution of perillyl alcohol (0.086 grams, 0.56 mmol) in 1,2-dichloroethane (3 mL) was added over a period of 5 minutes. The reaction mixture was allowed to warm to room temperature and stirred for 14 hours. 1,2-dichloroethane was concentrated under vacuum to give a residue, which was triturated with hexanes. The resulting yellow solid was filtered and washed with hexanes. Weight: 170 mg; Yield: 89%. ^{1}H-NMR (400 MHZ, $CDCl_3$): δ 1.4-2.2 (m, 10H), 4.06 (s, 3H), 4.6-4.8 (m, 4H), 5.88 (br s, 1H), 8.42 (s, 1H), 9.31 (br s, 1H); MS, no molecular ion peak was observed. m/e: 314 (100%), 286.5 (17%), 136 (12%).

Alternatively, temozolomide POH carbamate was synthesized according to the following procedure. Oxalyl chloride (0.13 grams, 1.0 mmol) was added slowly to a mixture of temozolomide (OChem Incorporation, 0.1 grams, 0.5 mmol) in 1,2-dichloroethane (10 mL) over a period of 2 minutes while maintaining the temperature at 10° C. under N_2. The reaction mixture was allowed to warm to room temperature and then heated to reflux for 3 hours. The excess of oxalyl chloride and 1,2-dichloroethane were removed by concentration under vacuum. The resulting residue was re-dissolved in 1,2-dichlorethane (15 mL) and the reaction mixture was cooled to 10° C. under N_2. A solution of perillyl alcohol (0.086 grams, 0.56 mmol) in 1,2-dichloroethane (3 mL) was added over a period of 5 minutes. The reaction mixture was allowed to warm to room temperature and stirred for 14 hours. 1,2-Dichloroethane was concentrated under vacuum to give a residue, which was purified by a short silica-plug column (column dimensions: diameter: 2 cm, height: 3 cm, silica: 230-400 mesh) and eluted with a mixture of hexanes/ethyl acetate (1:1, 100 mL). The hexane/ethyl acetate fractions were combined and concentrated under vacuum to give a white solid residue which was triturated with heptanes and filtered to obtain a white solid. Weight: 170 mg; Yield: 89%. ^{1}H-NMR (400 MHZ, CDCl3): 1.4-2.2 (m, 10H), 4.06 (s, 3H), 4.6-4.8 (m, 4H), 5.88 (br s, 1H), 8.42 (s, 1H), 9.31 (br s, 1H); MS, no molecular ion peak was observed, m/e: 314 (100%), 286.5 (17%), 136 (12%).

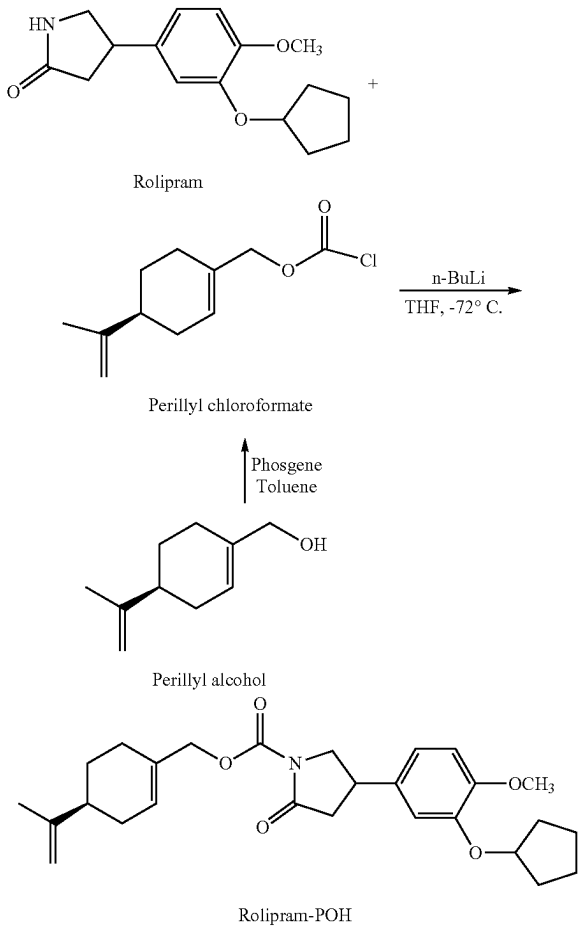
Example 4: In Vitro Cytotoxicity Studies of Temozolomide POH Carbamate (POH-TMZ)

First cytotoxicity assays were carried out after cells were treated with temozolomide (TMZ) alone, the standard alkylating agent used in the treatment of malignant gliomas. FIG. **3** shows the results of the MTT cytotoxicity assays performed on human malignant glioma cells U87, A172 and U251 with TMZ alone. Increasing concentrations of TMZ had minimal cytotoxicity towards the cell lines tested.

Then TMZ-resistant glioma cell lines U87, A172 and U251 cells were treated with temozolomide POH carbamate (POH-TMZ) (e.g., synthesized by the method in Example 3). The MTT assay results (FIG. **4**) showed that POH carbamate POH-TMZ exhibited substantially higher kill rates of the various human glioma cells compared to TMZ alone.

Example 5: Synthesis of Rolipram POH Carbamate (4-(3-cyclopentyloxy-4-methoxy phenyl)-2-oxo-pyrrolidine-1-carboxylic acid 4-isopropenyl cyclo-hex-1-enylmethyl Ester)

The reaction scheme is the following:



Phosgene (20% in toluene, 13 ml, 26.2 mmol) was added to a mixture of perillyl alcohol (2.0 grams, 13.1 mmol) and potassium carbonate (5.4 grams, 39.1 mmol) in dry toluene (30 mL) over a period of 30 minutes while maintaining the temperature between 10° C. to 15° C. The reaction mixture was allowed to warm to room temperature and stirred for 8.0 hours under N_2. The reaction mixture was quenched with water (30 mL) and the organic layer separated. The aqueous layer was extracted with toluene (20 mL) and the combined

organic layer washed with water (50 mL×2), brine (15%, 30 mL) and dried over sodium sulfate (20 grams). The filtered organic layer was concentrated under vacuum to give perillyl chloroformate as an oil. Weight: 2.5 grams; Yield: 89%. ^{1}H-NMR (400 MHZ, $CDCl_3$): δ 1.5 (m, 1H), 1.7 (s, 3H), 1.8 (m, 1H), 2.0 (m, 1H), 2.2 (m, 4H), 4.7 (dd, 4H); 5.87 (m, 1H).

Butyl lithium (2.5 M, 0.18 mL, 0.45 mmol) was added to a solution of rolipram (GL synthesis, Inc., 0.1 grams, 0.36 mmol) in dry THF at −72° C. over a period of 5 minutes under N_2. After the reaction mixture was stirred for 1.0 hours at −72° C., perillyl chloroformate (dissolved in 4 mL THF) was added over a period of 15 minutes while maintaining the temperature at −72° C. The reaction mixture was stirred for 2.5 hours and quenched with saturated ammonium chloride (5 mL). The reaction mixture was allowed to warm to room temperature and extracted with ethyl acetate (2×15 mL). The combined organic layer was washed with water (15 mL), brine (15%, 15 mL), and then dried over sodium sulfate. The filtered organic layer was concentrated to give an oil which was purified by column chromatography [column dimensions: diameter: 1.5 cm, height: 10 cm, silica: 230-400 mesh] and eluted with a mixture of 8% ethyl acetate/hexanes (100 mL) followed by 12% ethyl acetate/hexanes (100 mL). The 12% ethyl acetate/hexanes fractions were combined and concentrated under vacuum to yield a gummy solid. Weight: 142 mg; Yield: 86%. ^{1}H-NMR (400 MHZ, $CDCl_3$): δ 1.5 (m, 1H), 1.6 (m, 2H), 1.7 (s, 3H), 1.9 (m, 6H), 2.2 (m, 5H), 2.7 (m, 1H), 2.9 (m, 1H), 3.5 (m, 1H), 3.7 (m, 1H), 3.8 (s, 3H), 4.2 (m, 1H), 4.7 (m, 6H), 5.8 (br s, 1H), 6.8 (m, 3H); MS, m/e: 452.1 (M^{+1} 1, 53%), 274.1 (100%), 206.0 (55%).

Example 6: In Vitro Cytotoxicity Studies of Rolipram POH Carbamate (POH-Rolipram)

To compare the cytotoxicity of Rolipram POH Carbamate (POH-Rolipram) (e.g., synthesized by the method in Example 5) with rolipram, a type IV phosphodiesterase inducing differentiation and apoptosis in glioma cells, A172, U87, U251 and LN229 human glioma cells were treated with either POH-Rolipram or rolipram for 48 hours. The MTT assay results are shown in FIGS. **5** to **8**. POH-Rolipram exhibited substantially higher kill rates compared to rolipram alone for each of the several different human glioma cell types. FIG. **5** shows the MTT assay for increasing concentrations of rolipram and POH-rolipram for A-172 cells. Rolipram alone demonstrates an IC50 of approximately 1000 uM (1 mM). In the presence of POH-rolipram, IC50 is achieved at concentrations as low as 50 uM. FIG. **6** shows the MTT assay for increasing concentrations of rolipram with U-87 cells. IC50 is not met at 1000 uM. On the other hand, IC50 is achieved at 180 uM with POH-rolipram. FIG. **7** shows that IC50 for rolipram alone for U251 cells is achieved at 170 uM; plateau cytotoxicity is reached at 60%. POH-rolipram achieves IC50 at 50 uM, with almost 100% cytoxicity at 100 uM. FIG. **8** shows that IC50 for rolipram alone for LN229 cells is not achieved even at 100 uM. On the other hand, IC50 for POH-rolipram is achieved at 100 uM, with almost 100% cytotoxicity at 10 uM.

Example 7: In Vivo Tumor Growth Inhibition by POH Fatty Acid Derivatives

Inhibition of tumor growth by butyryl-POH was studied in a nude mouse subcutaneous glioma model. Mice were injected with U-87 glioma cells (500,000 cells/injection) and allowed to form a palpable nodule over two weeks. Once palpable nodule was formed, the mice were treated with local application of various compounds as indicated in FIGS. **9**A and **9**B via a Q-tip (1 cc/application/day) over a period of 8 weeks. FIG. **9**A shows the images of subcutaneous U-87 gliomas in nude mice treated with butyryl-POH, purified(S)-perillyl alcohol having a purity greater than 98.5% ("purified POH"), POH purchased from Sigma chemicals, or phosphate buffered saline (PBS; negative control). FIG. **9**B shows average tumor growth over time (total time period of 60 days). Butyryl-POH demonstrated the greatest inhibition of tumor growth, followed by purified POH and Sigma POH.

Example 8: In Vitro Cytotoxicity Studies of Temozolomide (TMZ) and Temozolomide POH Carbamate (POH-TMZ) on TMZ Sensitive and Resistant Glioma Cells

Colony forming assays were carried out after cells were treated with TMZ alone, POH alone, and the TMZ-POH conjugate. The colony forming assays were carried out as described in Chen T C, et al. Green tea epigallocatechin gallate enhances therapeutic efficacy of temozolomide in orthotopic mouse glioblastoma models. *Cancer Lett.* 2011 Mar. 28; 302(2):100-8. FIG. **10** shows the results of the colony forming assays performed on TMZ sensitive (U251) and TMZ resistant (U251TR) U251 cells with TMZ or TMZ-POH. TMZ demonstrated cytotoxicity towards TMZ sensitive U251 cells, but had minimal cytotoxicity towards TMZ resistant U251 cells. TMZ-POH demonstrated cytotoxicity towards both TMZ sensitive and TMZ resistant U251 cells.

FIG. **11** shows the results of the colony forming assays performed on TMZ sensitive (U251) and TMZ resistant (U251TR) U251 cells with POH. POH demonstrated cytotoxicity towards both TMZ sensitive and TMZ resistant U251 cells. POH-TMZ (FIG. **10**) exhibited substantially greater potency compared to POH alone (FIG. **11**) in the colony forming assays.

Example 9: In Vitro Cytotoxicity Studies of Temozolomide POH Carbamate (POH-TMZ) on U251 Cells, U251TR Cells, and Normal Astrocytes

MTT cytotoxicity assays were carried out after cells were treated with the TMZ-POH conjugate. The MTT cytotoxicity assays were carried out as described in Chen T C, et al. Green tea epigallocatechin gallate enhances therapeutic efficacy of temozolomide in orthotopic mouse glioblastoma models. *Cancer Lett.* 2011 Mar. 28; 302 (2): 100-8. FIG. **12** shows the results of the MTT cytotoxicity assays performed on TMZ sensitive cells (U251), TMZ resistant cells (U251TR) and normal astrocytes. TMZ-POH demonstrated cytotoxicity towards both TMZ sensitive and TMZ resistant U251 cells, but not towards normal astrocytes.

Example 10: In Vitro Cytotoxicity Studies of Temozolomide POH Carbamate (POH-TMZ) on BEC, TuBEC, and Normal Astrocytes

MTT cytotoxicity assays were carried out after cells were treated with the TMZ-POH conjugate. The MTT cytotoxicity assays were carried out as described in Chen T C, et al. Green tea epigallocatechin gallate enhances therapeutic efficacy of temozolomide in orthotopic mouse glioblastoma models. *Cancer Lett.* 2011 Mar. 28; 302(2):100-8. FIG. **13**

shows the results of the MTT cytotoxicity assays performed on normal astrocytes, brain endothelial cells (BEC; confluent and subconfluent), and tumor brain endothelial cells (TuBEC). TMZ-POH did not induce significant cytotoxicity on normal astrocytes, confluent BEC, or TuBEC. Mild to moderate cytotoxicity was demonstrated in subconfluent BEC at high concentrations of TMZ-POH.

Example 11: In Vitro Cytotoxicity Studies of Temozolomide (TMZ) and Temozolomide POH Carbamate (POH-TMZ) on USC-04 Glioma Cancer Stem Cells

MTT cytotoxicity assays were carried out after cells were treated with the TMZ alone, POH alone, or the TMZ-POH conjugate. The MTT cytotoxicity assays were carried out as described in Chen T C, et al. Green tea epigallocatechin gallate enhances therapeutic efficacy of temozolomide in orthotopic mouse glioblastoma models. *Cancer Lett.* 2011 Mar. 28; 302(2):100-8. FIG. **14** shows the results of the MTT cytotoxicity assays performed on USC-04 glioma cancer stem cells. TMZ did not induce significant cytotoxicity with increasing concentrations (0-400 uM). TMZ-POH demonstrated evidence of cytotoxicity with IC50 at 150 uM. FIG. **15** shows the results of the MTT cytotoxicity assays performed on USC-04 glioma cancer stem cells treated with POH. POH demonstrated cytotoxicity on USC-04 with increasing concentrations (0-2 mM).

Example 12: In Vitro Cytotoxicity Studies of Temozolomide (TMZ) and Temozolomide POH Carbamate (POH-TMZ) on USC-02 Glioma Cancer Stem Cells

MTT cytotoxicity assays were carried out after cells were treated with the TMZ alone, POH alone, or the TMZ-POH conjugate. The MTT cytotoxicity assays were carried out as described in Chen T C, et al. Green tea epigallocatechin gallate enhances therapeutic efficacy of temozolomide in orthotopic mouse glioblastoma models. *Cancer Lett.* 2011 Mar. 28; 302(2):100-8. FIG. **16** shows the results of the MTT cytotoxicity assays performed on USC-02 glioma cancer stem cells. TMZ did not induce significant cytotoxicity with increasing concentrations (0-400 uM). TMZ-POH demonstrated evidence of cytotoxicity with IC50 at 60 uM. FIG. **17** shows the results of the MTT cytotoxicity assays performed on USC-02 glioma cancer stem cells treated with POH. POH demonstrated cytotoxicity on USC-02 with increasing concentrations (0-2 mM).

Example 13: In Vitro Studies of ER Stress by Temozolomide POH Carbamate (POH-TMZ) on TMZ Sensitive and Resistant Glioma Cells

Western blots were performed after TMZ sensitive and resistant glioma cells were treated with the TMZ-POH conjugate for 18 hr. FIG. **18** shows a western blot demonstrating that TMZ-POH induces ER stress (ERS) in TMZ sensitive and resistant U251 glioma cells. Activation of the proapoptic protein CHOP was shown at concentrations as low as 60 uM of TMZ-POH.

Example 14: In Vitro and In Vivo Studies POH-Rolipram

In this example, POH-Rolipram is also referred to as NEO214.

Three different established glioblastoma cell lines were exposed to this NEO214, and then the viability of these cells were measured 48 hours later. As shown in FIG. **19**, increasing concentrations of NEO214 exerted strong cytotoxic activity against all three cell lines, with an IC50 (i.e., concentration that kills 50% of cells) in the range of 40-60 micromolar. NEO214 was similarly potent against all three cell lines, which is noteworthy in light of the fact that one of these cell lines, T98G, is known to be highly chemoresistant against the commonly used brain cancer drug, temozolomide, due to the overexpression of a DNA repair protein called MGMT (O6-methylguanine DNA methyltransferase).

The observed tumor-cell killing activity of NEO214 was then compared to the activity of its individual components. Different glioblastoma cells were treated with increasing concentrations of NEO214, rolipram, POH, or rolipram mixed with POH (or rolipram plus POH, noted as R+P). After 48 hours, cell viability was measured via MTT assay. As shown in FIGS. **20**A-**20**D, NEO214 was by far the most cytotoxic treatment and killed these cancer cells much more effectively than either rolipram, or POH, or a mix of the two individual agents.

Intriguingly, NEO214 was equally effective in chemoresistant cells: U251TR cells (FIG. **20**B) and LN229TR cells (FIG. **20**D) are sublines derived from U251 cells (FIG. **20**A) and LN229 cells (FIG. **20**C) that are highly resistant against the chemotherapeutic effect of temozolomide, the standard treatment for malignant glioma. Yet, as shown, NEO214 was very effective against these cells as well.

T98G glioblastoma cells, which are well-characterized temozolomide-resistant cells, were treated with increasing concentrations of NEO214, or with POH alone, rolipram alone, or a mix of rolipram plus POH (R+P). After 48 hours, cell viability was measured via MTT assay. As shown in FIG. **21**, NEO214 was highly effective in these cells, despite their drug resistance status. As well, NEO214 was substantially more effective than its individual components (rolipram alone or POH alone) or a mix of its components (rolipram plus POH).

The results shown in FIGS. **19**-**21** were derived from short-term experiments, where the tumoricidal effects of NEO214 were investigated within a 48-hour time frame. The long-term effects of treatment with NEO214 were also performed by performing experiments to determine whether tumor cells could withstand the toxic effects of this compound and possible recover at a later time, after NEO214 was removed from the cell culture medium. Towards this goal, colony-formation assays were performed to investigate whether NEO214-treated cells could recover within two weeks after an initial 48-hour treatment with this agent. T98G and LN18 are glioblastoma cells that are resistant against treatment with temozolomide, the current chemotherapeutic standard of care of patients with glioblastoma. U251 and LN229 are glioblastoma cell lines; U251TR and LN229TR are sublines that are highly resistant against temozolomide. All the cell lines were treated with increasing concentrations of NEO214, or with POH alone, rolipram alone, or a mix of rolipram plus POH (R+P). After 48 hours, the drugs were removed and the cells received fresh medium without any drugs added. The cells were left undisturbed for 12 days, after which time the number of newly formed colonies was determined. The number of colonies from those cultures that had not received any drugs, or had received vehicle only, was set at 100%. As shown in FIGS. **22**A-**22**B and **23**A-**23**D, NEO214 was highly effective in all of these cell lines, despite their drug resistance status. As

well, NEO214 was substantially more effective than its individual components (rolipram alone or POH alone) or a mix of its components (rolipram plus POH). FIGS. **22**A-B and **23**A-**23**D also show that, in fact, none of the tumor cells was able to recover from NEO214 treatment. The IC50 (i.e., the concentration that kills 50% of the cells) of NEO214 was somewhat lower in this long-term investigation (2 weeks) of drug effects, as compared to the experiments shown in FIGS. **19-21** (48 hours). Here, the IC50 of NEO214 ranged from about 30 to 55 micromolar.

It is noteworthy that in these long-term experiments (2 weeks) shown in FIGS. **22**A-**22**B and **23**A-**23**D, tumor cell survival after treatment with NEO214 at sufficient concentration dropped all the way to zero, meaning that no tumor cells were able to survive treatment with NEO214 in the long term. As well, as observed before in FIGS. **19-21**, highly chemoresistant cells were as effectively killed by NEO214, as were their more chemosensitive counterparts. LN18 and T98G cells (FIGS. **22**A-B) are chemoresistant due to the overexpression of the DNA repair protein MGMT, whereas U251TR and LN229TR cells (FIGS. **23**B, **23**D) are chemoresistant based on other cellular resistance mechanisms. This latter observation indicates that NEO214 may exert potency against tumor cells that have developed different mechanisms of conventional drug resistance.

The potent tumor cell killing ability of NEO214 was further confirmed by cell death ELISA assays, which quantitate the extent of programmed cell death/apoptosis. U251 glioblastoma cells as well as chemo-resistant glioblastoma lines U251TR and T98G were treated with increasing concentrations of NEO214 for 24 hours. Then, cell death ELISA was performed to determine the percentage of dead and dying cells. The results from these assays, as shown in FIG. **24**A-**24**C, validate the strong potency of NEO214 to kill glioblastoma cells, including the chemoresistant lines U215TR and T98G. As well, FIGS. **24**A-**24**C shows that NEO214 is vastly more potent than rolipram, which does not exert any cytotoxic effects at similar concentrations.

An important consideration for the clinical efficacy of any cancer drug is its tolerability, i.e., its ability to kill the intended tumor target, but not healthy normal organs. The specificity of NEO214 for tumor cells were investigated by exposing normal cells derived from the human brain to this compound. In particular, normal human astrocytes and brain endothelial cells (BEC) were exposed to increasing concentrations of NEO214 or rolipram for 72 hours. In parallel, U251 glioblastoma cells were also used. As shown in FIGS. **25**A-**25**B, NEO214 potently killed the U251 tumor cells (with an IC50 of 50 micromolar, and 100 micromolar NEO214 killed >90% of the tumor cells), but had not toxic effect on the normal cells (astrocytes, BEC) even at concentrations as high as 200 micromolar. In comparison, rolipram had no toxic effect on any of these cells, neither the normal cells nor the tumor cells. This result demonstrates that NEO214 is highly selective: it kills tumor cells very effectively, but spares normal cells, such as astrocytes or cells of the tumor vasculature.

Further studies were performed to investigate which molecular components and intracellular pathways of cancer cells are targeted by NEO214. To this end, response of six cellular processes that are critically involved in controlling cell growth, cell death and survival, as well as overall cellular functioning and integrity were studied: (i) Cell cycle, as represented by its key components cyclin A, cyclin D1, and cyclin E; (ii) Mitogenic control, as exerted by Akt/PKB (protein kinase B), MEK (mitogen-activated protein kinase/extracellular signal-regulated protein kinase), P70S6K (S6 protein kinase), and S6RP (S6 ribosomal protein); (iii) Apoptosis, as indicated by PARP (poly ADP-ribose polymerase) and caspase 7 (C7); (iv) ER (endoplasmic reticulum) stress, as revealed by GRP78 (glucose-regulated protein of molecular mass 78), GRP94, CHOP (CCAAT/enhancer-binding protein homologous protein), and ATF3 (activating transcription factor 3); (v) Autophagy, as shown by LC3 (microtubule-associated protein 1A and 1B light chain) and p62 (SQSTM1); and (vi) DNA damage, as indicated by H2AX (histone 2A family member X).

U251 glioblastoma cells were treated with increasing concentrations of NEO214 for 24 hours. Thereafter, cellular lysates were prepared and analyzed by Western blot with specific antibodies. For cell cycle analysis, the following antibodies were used: cyclin A, cyclin D1, and cyclin E. For mitogenic pathways, antibodies that were specific for the phosphorylated form (i.e., the active form of the protein) of Akt, MEK, P70S6K, and S6RP were used. In all cases, an antibody against actin was used as the loading control to confirm equal amounts of protein used in each lane. For apoptosis analysis, the following antibodies were used: PARP (appearance of a second, lower band indicates initiation of cell death) and cleaved caspase 7 (increased intensity indicates that the cells are dying). For ER stress, we used antibodies against GRP78, GRP94, CHOP, and ATF3; the increase in CHOP and ATF3 indicates the presence of pro-apoptotic ER stress. The presence of DNA damage was verified by increased amounts of phosphorylated H2AX (p-H2AX). Autophagy markers analyzed were LC3 and p62; the appearance of a second, lower band of LC3 indicates increased autophagic activity. In all cases, an antibody against actin was used as the loading control to confirm equal amounts of protein used in each lane.

As shown in FIG. **26**, treatment of glioblastoma cells with NEO214 resulted in the down-regulation of cyclin A and cyclin E, and an increase in the expression of cyclin D. This result indicates growth-inhibitory effects of NEO214 and arrest in the G1 phase of the cell cycle. Under the same treatment conditions, there was prominent inhibition of MEK1/2 activity, as indicated by the down-regulation of the phosphorylated (p-MEK, i.e., activated) form of MEK, which reveals blockage of this key mitogenic pathway. There was a weak enhancement of Akt activity (indicated by a slight increase of its phosphorylated, p-Akt, form), and also an increase in the phosphorylation (i.e., activity) of P70S6K and S6RP.

As shown in FIG. **27**, treatment of glioblastoma cells with NEO214 resulted cleavage of PARP (i.e., appearance of a second, faster-migrating signal) and strong induction of cleaved C7, both of which represent established indicators of ongoing cell death/apoptosis. NEO214 also triggered severe endoplasmic reticulum (ER) stress, as indicated by the increased expression of the ER stress markers GRP78, CHOP, and ATF3. There was a weak induction of autophagy, as revealed by the appearance of a faster-migrating second signal for LC3. NEO214 also caused DNA damage, which is indicated by the strong induction of the DNA damage marker p-H2AX. Altogether, these results demonstrate potent impact of NEO214 on several cellular processes that are key for maintaining cell homeostasis.

The potency of NEO214 was compared to the potency of rolipram. U251 glioblastoma cells were treated with increasing concentrations of NEO214 or rolipram for 24 hours. Thereafter, cellular lysates were prepared and analyzed by Western blot with specific antibodies. As summarized in FIGS. **28-30**, rolipram caused some of the same effects as NEO214 on these various markers of cellular homeostasis.

However, NEO214 was strikingly more potent than rolipram. For example, 50 uM NEO214 was sufficient to trigger activation (i.e., cleavage) of the cell death indicator C7, whereas rolipram required more than 10-times higher concentrations for the same effect (FIG. **28**); the stress proteins CHOP and ATF3 were potently induced by NEO214 concentrations as low as 40 uM, whereas rolipram required 500 to 1,000 uM for a similar outcome (FIG. **29**); in the case of the key mitotic stimulatory protein ERK, 50 uM NEO214 caused complete inhibition, whereas 1,000 uM rolipram was unable to mimic this inhibitory effect (FIG. **30**).

U251 glioblastoma cells were treated with NEO214 for different lengths of time up to 24 hours. Thereafter, cellular lysates were prepared and analyzed by Western blot with specific antibodies. As shown in FIG. **31**, the effects of NEO214 became visible as early as 1 hour after the onset of treatment, and increased in strength over the 24-hour time period.

Intriguingly, treatment of glioblastoma cells with NEO214 caused a prominent stimulation of expression of DR5 (death receptor 5), as seen in FIGS. **30** and **31**. This is particularly noteworthy because DR5 belongs to the superfamily of tumor necrosis factor receptors (TNFRs), which trigger extrinsic apoptotic cell death upon binding of ligands, such as TRAIL (TNF-related apoptosis-inducing ligand). DR5, in its active form, is localized at the cell surface, where it receives death-inducing signals, such as TRAIL. U251 glioblastoma cells were treated either with vehicle alone (left) or with 100 uM NEO214 for 24 hours. Thereafter, cells were stained in situ with an antibody against DR5. As shown, NEO214 caused prominent induction of DR5 and presence of DR5 at the surface of the cell. Treatment of glioblastoma cells with NEO214 (U251 glioblastoma cells were treated either with vehicle alone (FIG. **32**, left) or with 100 uM NEO214 for 24 hours (FIG. **32**, right)) showed strongly increased cell surface localization of DR5. Thereafter, cells were stained in situ with an antibody against DR5. indicating the possibility that NEO214 might be able to sensitize tumor cells to much further increased cell death upon combination with the DR5 ligand TRAIL.

In summary, NEO214 exhibited striking in vitro anticancer activity in several different tumor cell lines, including strongly drug-resistant glioblastoma cells, and its tumor cell killing potency is substantially greater than either of its individual components, POH or Rolipram alone. It was also shown that NEO214 was not toxic to normal cells, which suggests that this compound might be well tolerated by patients. Its mechanisms of action appears to involve several key intracellular pathways. These characteristics of NEO214 are desirable for providing effective tumor cell killing by a multipronged, simultaneous attack of the drug on several processes that are crucial for tumor cell homeostasis.

The scope of the present invention is not limited by what has been specifically shown and described hereinabove. Those skilled in the art will recognize that there are suitable alternatives to the depicted examples of materials, configurations, constructions and dimensions. Numerous references, including patents and various publications, are cited and discussed in the description of this invention. The citation and discussion of such references is provided merely to clarify the description of the present invention and is not an admission that any reference is prior art to the invention described herein. All references cited and discussed in this specification are incorporated herein by reference in their entirety. Variations, modifications and other implementations of what is described herein will occur to those of ordinary skill in the art without departing from the spirit and scope of the invention. While certain embodiments of the present invention have been shown and described, it will be obvious to those skilled in the art that changes and modifications may be made without departing from the spirit and scope of the invention. The matter set forth in the foregoing description and accompanying drawings is offered by way of illustration only and not as a limitation.

What is claimed is:

1. A method for treating a cancer in a mammal, comprising administering to the mammal a therapeutically effective amount of perillyl alcohol carbamate using a nasal delivery device, wherein the perillyl alcohol carbamate comprises perillyl alcohol conjugated with rolipram, wherein the cancer is a tumor of the nervous system.

2. The method of claim **1**, wherein the nasal delivery device is selected from the group consisting of an intranasal inhaler, an intranasal spray device, an atomizer, a nebulizer, a metered dose inhaler (MDI), a pressurized dose inhaler, an insufflator, a unit dose container, a pump, a dropper, a nasal spray bottle, a squeeze bottle and a bi-directional device.

3. The method of claim **1**, wherein the tumor is a glioblastoma.

4. The method of claim **1**, further comprising the step of treating the mammal with radiation.

5. The method of claim **1**, further comprising the step of delivering to the mammal a chemotherapeutic agent.

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