Compositions and Methods of Use of Phorbol Esters for the Treatment of Stroke

Related Applications

This application is a United States utility patent application which claims priority benefit of United States Provisional Patent Application Serial No. 61/588,167, filed January 18, 2012, which is incorporataed herein in its entirety by reference.

Additional Disclosure

Additional disclosures relating to the instant application may be found in "Compositions And Methods Of Use Of Phorbol Esters" United States Patent Application Serial No. 12/023,753, filed January 31, 2008, to Richard L. Chang, et al, which claims priority benefit of United States Provisional Patent Application Serial No. 60/898,810, filed January 31, 2007, each of which is incorporated herein by reference in its entirety for all purposes.

Technical Field

The present invention relates generally to the medicinal use of phorbol esters in the treatment and prevention of stroke and the effects of stroke.

Background

Plants have historically served many medicinal purposes. The World Health Organization (WHO) estimates that 4 billion people, 80% of the world's population, presently use herbal medicine for some aspect of primary health care. (WHO Fact sheet Fact sheet N°134, December 2008) However, it can be difficult to isolate the specific compound in a plant that has the desired medicinal effect and to reproduce it on a commercial scale. Additionally, while the active compound may be isolated from a plant, the other parts of a plant such as the minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavanoids, and other substances may also be involved in the functioning of the active compound or the medicinal effect for which the plant is known, making the use, purification and commercialization of plant based pharmaceutical agents a challenge.

Phorbol is a natural, plant-derived organic compound of the tigliane family of diterpenes. It was first isolated in 1934 as a hydrolysis product of croton oil derived from the seeds of *Croton tiglium*, a leafy shrub of the Euphorbiaceae family that is native to Southeastern Asia. Various esters of phorbol have important biological properties including the reported ability to mimic diacylglycerols and activate protein

kinase C (PKC); and to modulate downstream cell signaling pathways including the mitogen-activated protein kinase (MAPK) pathways. Phorbol esters are additionally thought to bind to chimaerins, the Ras activator RasGRP, and the vesicle-priming protein Munc-13 (Brose N, Rosenmund C., JCell Sci;115:4399–411 (2002)). Some phorbol esters also induce nuclear factor-kappa B (NF-κB). The most notable physiological property of phorbol esters is their reported capacity to act as tumor promoters. (Blumberg, 1988; Goel, G *et al.*, Int. Journal of Toxicology 26, 279-288 (2007)).

12-O-tetradecanoylphorbol-13-acetate (TPA), also called phorbol-12-myristate-13-acetate (PMA), is a phorbol ester used in models of carcinogenesis as an inducer for differentiation and/or apoptosis in multiple cell lines and primary cells. TPA has also been reported to cause an increase in circulating white blood cells and neutrophils in patients whose bone marrow function has been depressed by chemotherapy (Han Z. T. *et al.* Proc. Natl. Acad. Sci. 95, 5363-5365 (1998)), and to inhibit an HIV- induced cytopathic effect on MT-4 cells. (Mekkawy S. *et al.*, Phytochemistry 53, 47-464 (2000)). However, due to a variety of factors, including caustic reactions when contacted with the skin and concerns for its potential toxicity, TPA has not been shown to be an effective tool for treating, managing, or preventing disease. Indeed, as phorbol esters play a key role in activation of protein kinase C, which triggers various cellular responses resulting in inflammatory responses and tumor development (Goel *et al.*, Int. Journal of Toxicology 26, 279-288 (2007)), phorbol esters would generally be excluded from possible treatment candidates for conditions that involve inflammatory reactions such as stroke.

Heart disease and stroke kill some 17 million people a year, causing almost one-third of all deaths globally. They are predicted to become the leading cause of both death and disability worldwide, with the number of fatalities projected to increase to over 20 million a year by 2020 and to 24 million a year by 2030. (Atlas of Heart Disease and Stroke, World Health Organization 2004).

While there are over 300 risk factors associated with coronary heart disease and stroke (Atlas of Heart Disease and Stroke, World Health Organization 2004), in developed countries, at least 1/3 of all cardiovascular disease is attributable to tobacco use, alcohol use, high blood pressure, high cholesterol and obesity.

Current treatments for the management and prevention of stroke are generally a combination of medications such as ACE inhibitors, aspirin, beta blockers and lipid lowering medications; devices such as pacemakers, implantable defibrillators, coronary stents, prosthetic valves and artificial hearts; and operations such as coronary artery bypass, balloon angioplasty, valve repair and replacement, heart transplants and artificial heart operations. "Increasingly, high-technology procedures are chosen over less expensive, but nevertheless effective , strategies " (Atlas of Heart Disease and Stroke, World Health Organization 2004) adding to the rising costs of health care and leading to marked disparities in the quality of treatment between different groups of individuals.

However, even where advanced technology and facilities are available, 60% of those who suffer a stroke die or become dependent and each stroke significantly increases the risk of further episodes. "Worldwide, treatment of cardiovascular diseases and their risk factors remains inadequate for most patients." (Atlas of Heart Disease and Stroke, World Health Organization 2004). There is therefore clearly a need for new and more effective measures to treat and prevent strokes and to treat or prevent the long term effects caused by stroke.

Summary

The present invention relates to compositions containing and methods of using phorbol esters. The compositions and methods described herein are effective in preventing and treating stroke and in managing the sequelae of stroke including acute ischemic events.

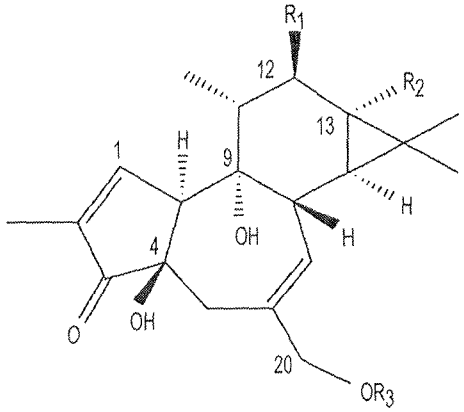
Effects of stroke that may be prevented or treated by using the phorbol esters and derivatives of phorbol esters as described herein include, but are not limited to, paralysis, spatial impairment, impaired judgment, left-sided neglect, memory loss, aphasia, coordination and balance problems, nausea, vomiting, cognitive impairment, perception impairment, orientation impairment, homonymous hemianopsia and impulsivity. In some embodiments, the use of phorbol esters and derivatives of phorbol esters may prevent initial and subsequent strokes from occurring.

Successful treatment will be determined according to conventional methods, such as a reduction in the severity or sequelae of a stroke, a decrease or elimination of

the effects of stroke, a decrease in risk factors that predispose an individual to a stroke, and/or a decrease in the number or severity of strokes including subsequent strokes.

In another embodiment, the phorbol esters and derivatives of phorbol esters as described herein may be used to modulate cell signaling pathways. Such modulation may have a variety of results, for example, in some embodiments, the use of compositions containing phorbol esters and derivatives of phorbol esters may alter the release of Th1 cytokines in mammalian subjects. In a further embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the release of interleukin 2 (IL-2) in mammalian subjects. In an additional embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the release of interferon in mammalian subjects. In yet another embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the rate of ERK phosphorylation.

The invention achieves the foregoing and satisfies additional objects and advantages by providing novel and surprisingly effective methods and compositions for treating and preventing stroke, modulating cell signaling pathways and/or managing, treating and preventing the sequelae of a stroke using compositions containing a phorbol ester of Formula I, below:



FORMULA I

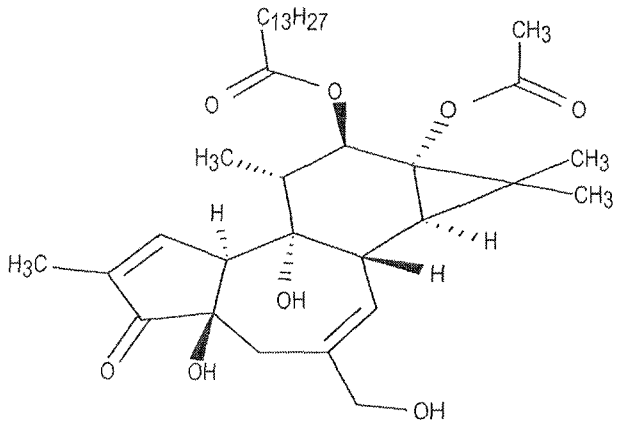
wherein R_1 and R_2 may independently be hydrogen; hydroxyl; —O—C(=O)-alkyl, wherein the alkyl group contains 1 to 15 carbon atoms; —O—C(=O)-lower alkenyl, wherein a lower

alkenyl group contains between 1 to 7 carbon atoms; —O—C(=O)-phenyl; —O—C(=O)-benzyl; and substituted derivatives thereof. R_3 may be independently be hydrogen or —C(=O)-lower alkyl and substituted derivatives thereof. The methods and compositions of the present invention further include any pharmaceutical salts, enantiomers, isomer, polymorphs, prodrugs, hydrates and solvates of the compositions of Formula I.

In some embodiments, at least one of R_1 and R_2 are other than hydrogen and R_3 is hydrogen or —C(=O)-lower alkyl and substituted derivatives thereof. In another embodiment, either R_1 or R_2 is —O—C(=O)—C_1— C_{15} alkyl the remaining R_1 or R_2 is a —O—C(=O)-lower alkyl, wherein a lower alkyl is between 1 and 7 carbons, and R_3 is hydrogen.

The alkyl, alkenyl, phenyl and benzyl groups of the formulas of Formula I herein may be unsubstituted or substituted with halogens, preferably, chlorine, fluorine or bromine; nitro; amino; and/or similar type radicals.

An exemplary phorbol ester composition as used in the methods and compositions as described herein is 12-O-tetradecanoylphorbol-13-acetate (TPA) of Formula II, below:



FORMULA II

Useful phorbol esters of Formula I and related compounds and derivatives within the formulations and methods of the invention include, but are not limited to, other pharmaceutically acceptable active salts of said compounds, as well as active

isomers, enantiomers, polymorphs, glycosylated derivatives, solvates, hydrates, and/or prodrugs of said compounds. Exemplary forms of phorbol esters for use within the compositions and methods of the invention include, but are not limited to, phorbol 13-butyrate; phorbol 12-decanoate; phorbol 13-decanoate; phorbol 12,13-diacetate; phorbol 13,20-diacetate; phorbol 12,13-dibenzoate; phorbol 12,13-dibutyrate; phorbol 12,13-didecanoate; phorbol 12,13-dihexanoate; phorbol 12,13-dipropionate; phorbol 12-myristate; phorbol 13-myristate; phorbol 12-myristate-13-acetate (also known as TPA or PMA); phorbol 12,13,20-triacetate; 12-deoxyphorbol 13-angelate; 12-deoxyphorbol 13-angelate 20-acetate; 12-deoxyphorbol 13-isobutyrate; 12-deoxyphorbol 13-isobutyrate-20-acetate; 12-deoxyphorbol 13-phenylacetate; 12-deoxyphorbol 13-phenylacetate 20-acetate; 12-deoxyphorbol 13-tetradecanoate; phorbol 12-tigliate 13-decanoate; 12-deoxyphorbol 13-acetate; phorbol 12-acetate; and phorbol 13-acetate.

Mammalian subjects amenable to treatment with phorbol esters of Formula I or derivatives of a phorbol ester of the Formula I, particularly TPA, according to the methods of the invention include, but are not limited to, individuals who have suffered or are at risk for a stroke. Subjects amenable to treatment with phorbol esters of Formula I, particularly TPA, additionally include those suffering from the effects of a stroke including, but not limited to, paralysis, spatial impairment, impaired judgment, left-sided neglect, memory loss, aphasia, coordination and balance problems, nausea, vomiting, cognitive impairment, perception impairment, orientation impairment, homonymous hemianopsia and impulsivity.

These and other subjects are effectively treated prophylactically and/or therapeutically, by administering to the subject an effective amount of a phorbol ester of Formula I or derivatives of a phorbol ester of the Formula I sufficient to modulate NF-κB activity, increase Th1 cytokine activity, prevent or treat paralysis, increase spatial awareness, decrease memory loss, decrease aphasia, increase coordination and balance, prevent or decrease the incidence and severity of a stroke, and improve cognition.

Therapeutically useful methods and formulations of the invention will effectively use a phorbol ester of Formula I or derivative of a phorbol ester of the Formula I in a variety of forms, as noted above, including any active, pharmaceutically acceptable salts of said compounds, as well as active isomers, enantiomers, polymorphs,

solvates, hydrates, prodrugs, and/or combinations thereof. TPA of formula II is employed as an illustrative embodiment of the invention within the examples herein below.

Within additional aspects of the invention, combinatorial formulations and methods are provided which employ an effective amount of a phorbol ester of Formula I or derivative of a phorbol ester of Formula I in combination with one or more secondary or adjunctive active agent(s) that is/are combinatorially formulated or coordinately administered with the phorbol ester compound of Formula I to yield an effective response in the subject.

Combinatorial formulations and coordinate treatment methods in the treatment of stroke employ a phorbol ester compound of Formula I or derivative of the phorbol ester of Formula I in combination with one or more additional, stroke preventing, treating or other indicated, secondary or adjunctive therapeutic agents. The secondary or adjunctive therapeutic agents used in combination with a phorbol ester, e.g., TPA, in these embodiments may possess direct or indirect effects on prevention or recovery from stroke, alone or in combination with the phorbol ester, e.g. TPA; may exhibit other useful adjunctive therapeutic activity in combination with, e.g. TPA (such as anti-clotting, anticholesterolemic, vasodilating, antihypertensive, arteriolar resistance lowering, venous capacity increasing, heart oxygen demand reducing, heart rate decreasing, heart rate stabilizing, or neuroprotecting); or may exhibit adjunctive therapeutic activity useful for treating or preventing stroke or associated symptoms alone or in combination with, e.g. TPA. Such secondary or adjunctive therapeutic agents may be administered prior to, simultaneously, or after administration of a phorbol ester of Formula I or derivative of a phorbol ester of Formula I.

Useful adjunctive or secondary therapeutic agents in these combinatorial formulations and coordinate treatment methods for the prevention or treatment of stroke in a mammalian subject include, but are not limited to, tissue plasminogen activators, anticoagulants, statins, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, anti-platelet agents, beta-blockers, aspirin, fibrates, calcium channel blockers, or diuretics. In addition, adjunctive or secondary therapies may be used such as, but not limited to, surgical intervention including carotid endarterectomy, angioplasty, balloon

angioplasty, valve repair and replacement, coronary artery bypass, stent placement, craniotomy, endovascular coil emobilization, or patent foramen ovale closure.

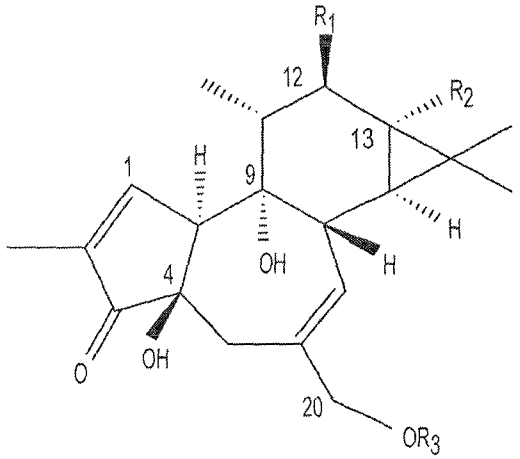
The foregoing and additional objects, features, aspects and advantages of the present invention will become apparent from the following detailed description.

Detailed Description

Novel methods and compositions have been identified for use in preventing and/or treating stroke and the sequelae of stroke in mammalian subjects, including humans.

In various embodiments, the composition and methods as described herein may increase the release of Th1 cytokines, increase ERK phosphorylation, modulate NF-κB activity, prevent or treat paralysis, increase spatial awareness, decrease memory loss, decrease aphasia, increase coordination and balance, improve cognition, improve orientation, decrease the prevalence of subsequent strokes, and decrease impulsivity.

Formulations and methods provided herein employ a phorbol ester of Formula I or derivative compound of a phorbol ester of Formula I as more fully described in U.S. Patent Application No. 12/023,753, filed January 31, 2008, which claims priority benefit of United States Provisional patent application Serial No. 60/898,810, filed January 31, 2007 which is incorporated herein in its entirety by reference.



FORMULA I

wherein R_1 and R_2 may independently be hydrogen; hydroxyl; —O—C(=O)-alkyl, wherein the alkyl group contains 1 to 15 carbon atoms; —O—C(=O)-lower alkenyl, wherein a lower alkenyl group contains between 1 to 7 carbon atoms; —O—C(=O)-phenyl; —O—C(=O)-benzyl; and substituted derivatives thereof. R_3 may be independently be hydrogen or —C(=O)-lower alkyl.

In some embodiments, at least one of R_1 and R_2 are other than hydrogen and R_3 is hydrogen or —C(=O)-lower alkyl and substituted derivatives thereof as novel compositions for use in treating chronic or recurring conditions. In another embodiment, either R_1 or R_2 is —O—C(=O)—C_1— C_{15} alkyl the remaining R_1 or R_2 is a —O—C(=O)-lower alkyl, wherein a lower alkyl is between 1 and 7 carbons, and R_3 is hydrogen.

The alkyl, alkenyl, phenyl and benzyl groups of the formulas herein may be unsubstituted or substituted with halogens, preferably, chlorine, fluorine or bromine; nitro; amino; and/or similar type radicals.

Stroke treating formulations and methods provided herein employ a phorbol ester of Formula I or derivative compound of a phorbol ester of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as anti-stroke agents.

Th1 cytokine increasing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as novel Th1 cytokine increasing agents. A broad range of mammalian subjects, including human subjects, are amenable to treatment using the formulations and methods of the invention. These

subjects include, but are not limited to, individuals who have suffered or are at risk for a stroke.

ERK phosphorylation increasing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as novel ERK phosphorylation increasing agents. A broad range of mammalian subjects, including human subjects, are amenable to treatment using the formulations and methods of the invention. These subjects include, but are not limited to, individuals who have suffered or are at risk for a stroke.

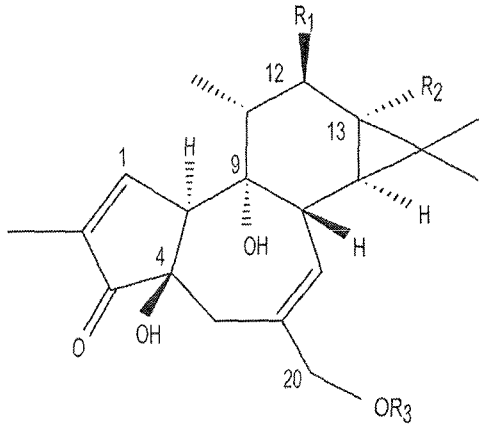
Within the methods and compositions of the invention, one or more phorbol ester compound(s) of Formula I or derivative compounds of a phorbol ester of Formula I as disclosed herein is/are effectively formulated or administered as an agent effective for treating and preventing stroke or the sequelae of stroke. In exemplary embodiments, TPA is demonstrated for illustrative purposes to be an effective agent in pharmaceutical formulations and therapeutic methods, alone or in combination with one or more adjunctive therapeutic agent(s). The present disclosure further provides additional, pharmaceutically acceptable phorbol ester compounds in the form of a native or synthetic compound, including complexes, derivatives, salts, solvates, isomers, enantiomers, polymorphs, and prodrugs of the compounds disclosed herein, and combinations thereof, which are effective as therapeutic agents within the methods and compositions of the invention in the treatment and prevention of stroke and the sequelae of stroke.

Strokes are caused by disruption of the blood supply to the brain. This may result from either blockage (ischaemic stroke) or rupture of a blood vessel (haemorrhagic stroke). The symptoms of a stroke include sudden numbness or weakness, especially on one side of the body; sudden confusion or trouble speaking or understanding speech; sudden trouble seeing in one or both eyes; sudden trouble with walking, dizziness, or loss of balance or coordination; or sudden severe headache with no known cause. Risk factors for stroke include high blood pressure, abnormal blood

lipids, tobacco use, physical inactivity, obesity, stress, diabetes, alcohol use, excess homocystein in the blood, inflammation and abnormal coagulation. There are also non-modifiable risk factors such as age, heredity, gender and ethnicity.

Treatment for stroke has three distinct phases: prevention, therapy immediately after a stroke, and post-stroke rehabilitation. The compositions and methods described herein may be used at any phase of stroke treatment, independently or in conjunction with one or more additional therapies including other pharmaceutical agents, devices or surgical interventions.

Phorbol is a natural, plant-derived polycyclic alcohol of the tigliane family of diterpenes. It was first isolated in 1934 as the hydrolysis product of croton oil derived from the seeds of *Croton tiglium*. It is well soluble in most polar organic solvents and in water. Esters of phorbol have the general structure of Formula I, below:



FORMULA I

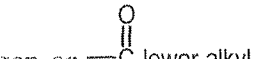
wherein R_1 and R_2 are selected from the group consisting of hydrogen; hydroxyl; —O—C(=O)-alkyl, wherein the alkyl group contains 1 to 15 carbon atoms; —O—C(=O)-lower alkenyl, wherein a lower alkenyl group contains between 1 to 7 carbon atoms; —O—C(=O)-phenyl; —O—C(=O)-benzyl; and substituted derivatives thereof. R_3 may be hydrogen or —C(=O)-lower alkyl and substituted derivatives thereof as well as

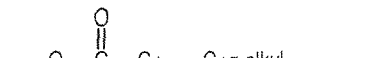
pharmaceutically acceptable salts, enantiomers, polymorphs, prodrugs solvates and hydrates of compounds of Formula I.

The term "lower alkyl" or "lower alkenyl" as used herein means moieties containing 1 to 7 carbon atoms. In the compounds of the Formula I, the alkyl or alkenyl groups may be straight or branched chain. In some embodiments, either or both R_1 or R_2, are a long chain carbon moiety (i.e., Formula I is decanoate or myristate).

The alkyl, alkenyl, phenyl and benzyl groups of the formulas herein may be unsubstituted or substituted with halogens, preferably, chlorine, fluorine or bromine; nitro; amino and similar type radicals.

Organic and synthetic forms of phorbol esters, including any preparations or extracts from herbal sources such as *croton tiglium*, are contemplated as useful compositions comprising phorbol esters (or phorbol ester analogs, related compounds and/or derivatives) for use within the embodiments herein. Useful phorbol esters and/or related compounds for use within the embodiments herein will typically have a structure as illustrated in Formula I, although functionally equivalent analogs, complexes, conjugates, and derivatives of such compounds will also be appreciated by those skilled in the art as within the scope of the invention.

In more detailed embodiments, illustrative structural modifications according to Formula I above will be selected to provide useful candidate compounds for treating and/or preventing strokes, damage due to stroke and/ or managing the effects or sequelae of strokes in mammalian subjects, including humans, wherein: at least one of R_1 and R_2 are other than hydrogen and R_3 is selected from the group consisting of hydrogen or 

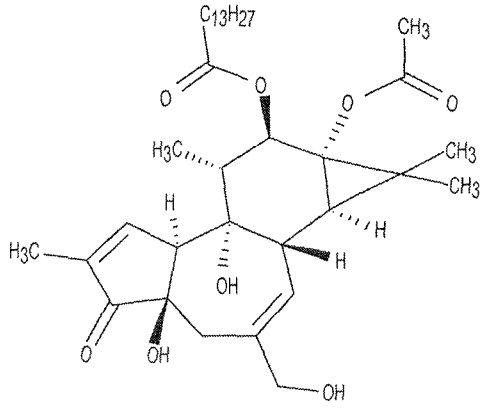
and substituted derivatives thereof. In another embodiment, either R_1 or R_2 is 

the remaining R_1 or R_2 is 

and R_3 is hydrogen.

An exemplary embodiment of a phorbol ester compound of Formula I useful in the treatment of in treating or preventing strokes, damage from strokes, and/or managing the effects or sequelae of strokes in mammalian subjects, including humans,

is found in phorbol 12-myristate-13-acetate (also known as PMA or 12-O-tetradecanoyl-phorbol-13-acetate (TPA)) shown in Formula II, below,

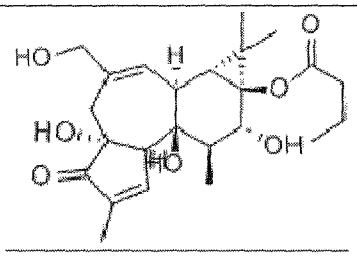
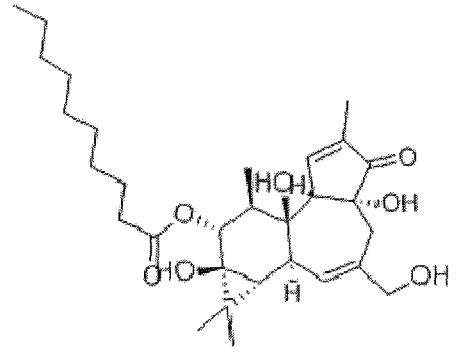
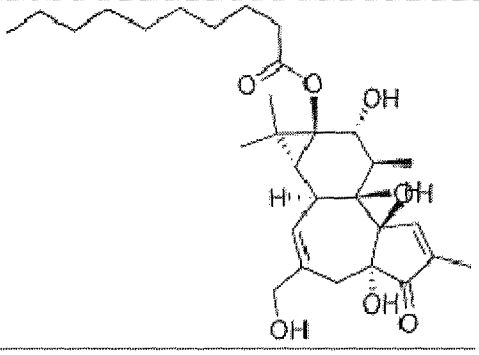
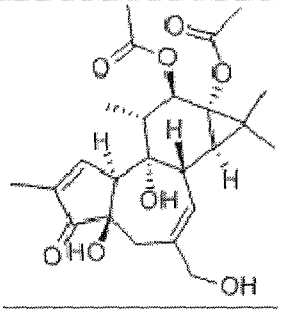
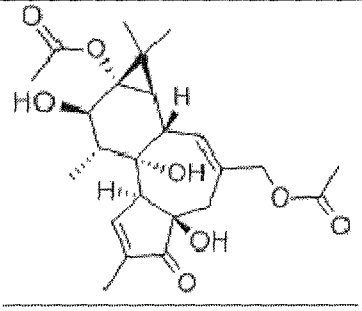


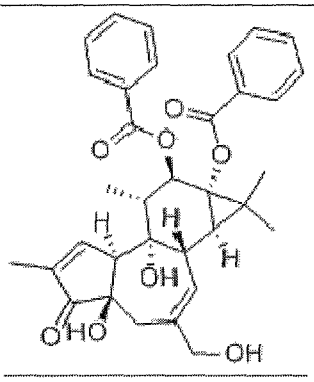
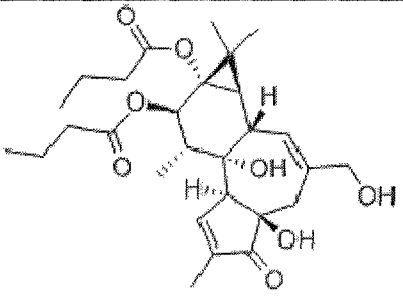
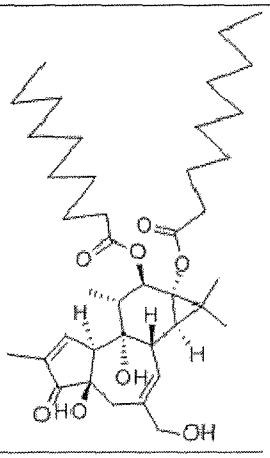
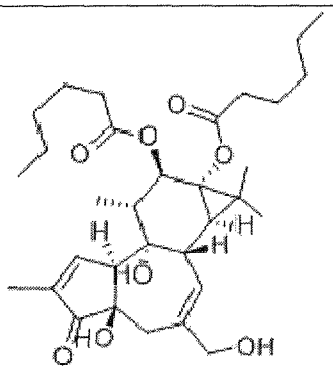
FORMULA II

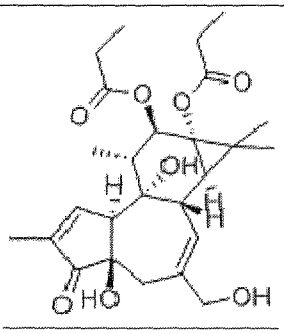
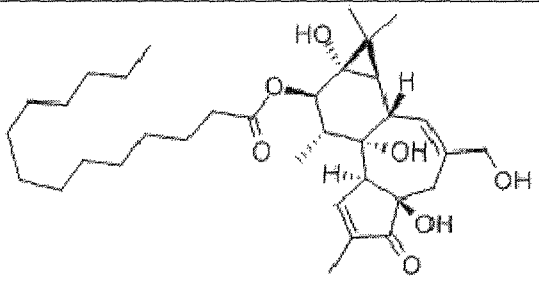
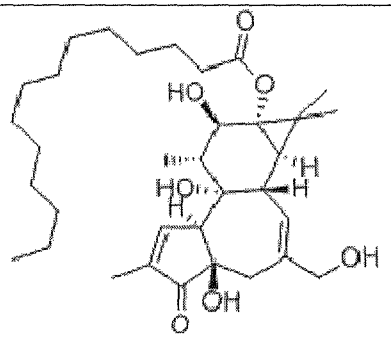
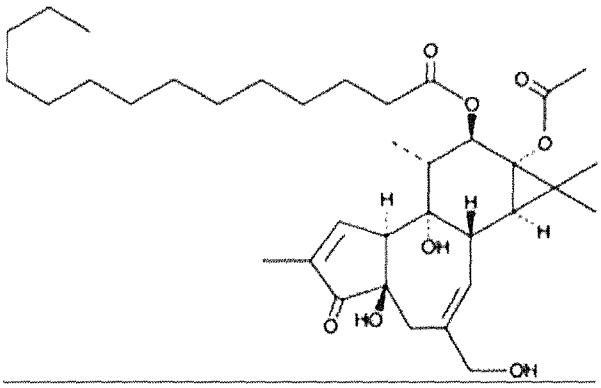
Additional useful phorbol esters and related compounds and derivatives within the formulations and methods of the invention include, but are not limited to, other pharmaceutically acceptable active salts of said compounds, as well as active isomers, enantiomers, polymorphs, glycosylated derivatives, solvates, hydrates, and/or prodrugs of said compounds. Derivatives of phorbol esters of Formula I may or may not be phorbol esters themselves. Further exemplary forms of phorbol esters for use within the compositions and methods of the invention include, but are not limited to, phorbol 13-butyrate; phorbol 12-decanoate; phorbol 13-decanoate; phorbol 12,13-diacetate; phorbol 13,20-diacetate; phorbol 12,13-dibenzoate; phorbol 12,13-dibutyrate; phorbol 12,13-didecanoate; phorbol 12,13-dihexanoate; phorbol 12,13-dipropionate; phorbol 12-myristate; phorbol 13-myristate; phorbol 12,13,20-triacetate; 12-deoxyphorbol 13-angelate; 12-deoxyphorbol 13-angelate 20-acetate; 12-deoxyphorbol 13-isobutyrate; 12-deoxyphorbol 13-isobutyrate-20-acetate; 12-deoxyphorbol 13-phenylacetate; 12-deoxyphorbol 13-phenylacetate 20-acetate; 12-deoxyphorbol 13-tetradecanoate; phorbol 12-tigliate 13-decanoate; 12-deoxyphorbol 13-acetate; phorbol 12-acetate; and phorbol 13-acetate as shown in Table 1.

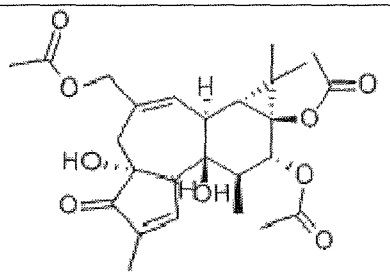
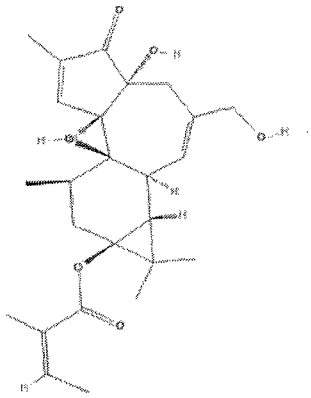
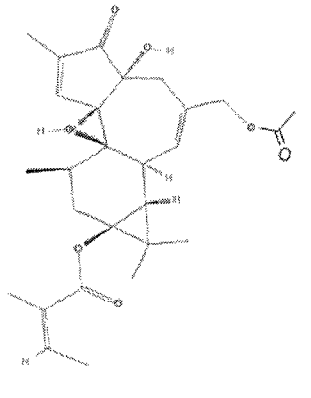
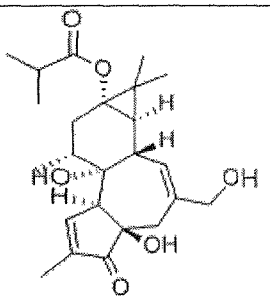
Table 1

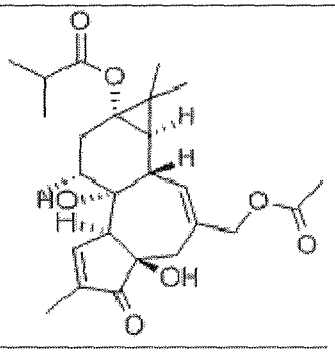
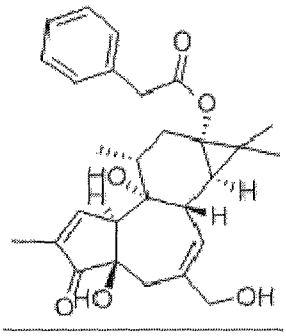
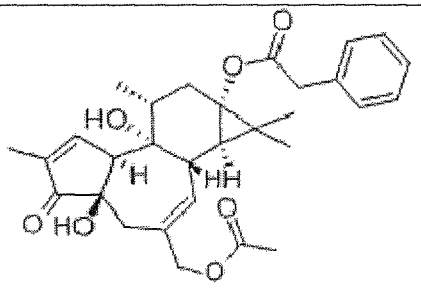
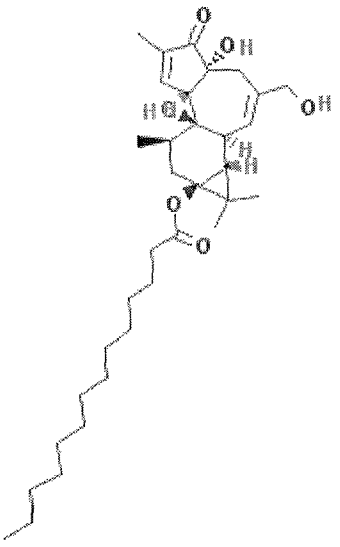
Exemplary Phorbol Esters

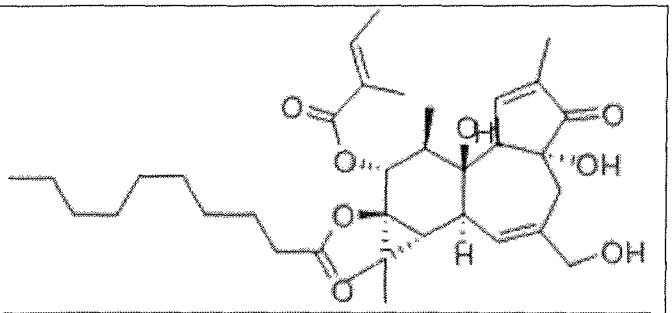
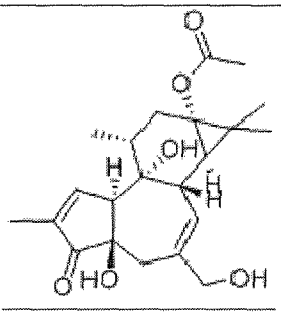
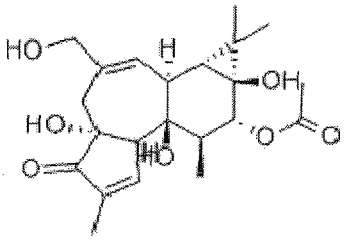
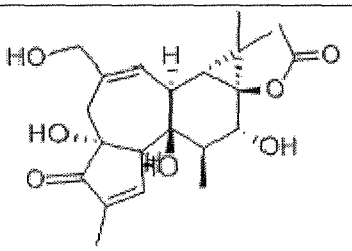
Phorbol 13-Butyrate	
Phorbol 12-Decanoate	
Phorbol 13-Decanoate	
Phorbol 12,13-Diacetate	
Phorbol 13,20-Diacetate	

Phorbol 12,13-Dibenzoate	
Phorbol 12,13-Dibutyrate	
Phorbol 12,13-Didecanoate	
Phorbol 12,13-Dihexanoate	

Phorbol 12,13-Dipropionate	
Phorbol 12-Myristate	
Phorbol 13-Myristate	
Phorbol 12-Myristate-13-Acetate (also known as TPA or PMA)	
Phorbol 12,13,20-Triacetate	

	
12-Deoxyphorbol 13-Angelate	
12-Deoxyphorbol 13-Angelate 20-Acetate	
12-Deoxyphorbol 13-Isobutyrate	

12-Deoxyphorbol 13-Isobutyrate-20-Acetate	
12-Deoxyphorbol 13-Phenylacetate	
12-Deoxyphorbol 13-Phenylacetate 20-Acetate	
12-Deoxyphorbol 13-Tetradecanoate	

Phorbol 12-Tigliate 13-Decanoate	
12-Deoxyphorbol 13-Acetate	
Phorbol 12-Acetate	
Phorbol 13-Acetate	

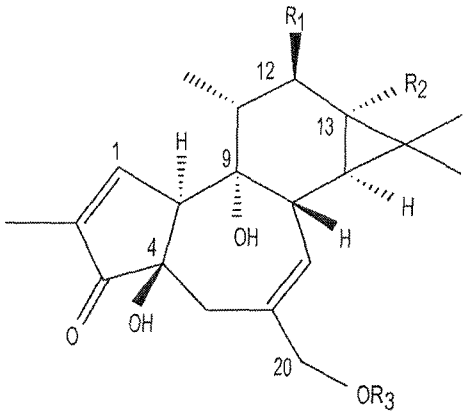
Compositions as described herein comprise stroke treating compositions comprising a stroke damage alleviating or preventing effective amount of a phorbol ester compound of Formula I or derivative compound of a phorbol esters of Formula I, which is effective for prophylaxis and/or treatment of stroke or stroke related symptoms or sequelae in a mammalian subject. A "stroke treating," "anti-clotting," "anticholesterolemic," "vasodilating," "antihypertensive," "arteriolar resistance lowering," " venous capacity increasing," "heart oxygen demand reducing," "heart rate decreasing," "heart rate stabilizing," or "neuroprotective" effective amount of the active compound is therapeutically effective, in a single or multiple unit dosage form, over a specified period of therapeutic intervention, to measurably alleviate one or more

symptoms or sequelae of stroke in the subject. Within exemplary embodiments, the compositions of the invention are effective in treatment methods to prevent or alleviate symptoms of stroke or sequelae of stroke in human and other mammalian subjects vulnerable to or who have suffered a stroke.

Phorbol ester treating compositions of the invention typically comprise an effective amount or unit dosage of a phorbol ester compound of Formula I or derivative compound of a phorbol ester of Formula I, which may be formulated with one or more pharmaceutically acceptable carriers, excipients, vehicles, emulsifiers, stabilizers, preservatives, buffers, and/or other additives that may enhance stability, delivery, absorption, half-life, efficacy, pharmacokinetics, and/or pharmacodynamics, reduce adverse side effects, or provide other advantages for pharmaceutical use. Effective amounts of a phorbol ester compound or related or derivative compound of Formula I (e.g., a unit dose comprising an effective concentration/amount of TPA, or of a selected pharmaceutically acceptable salt, isomer, enantiomer, solvate, polymorph and/or prodrug of TPA) will be readily determined by those of ordinary skill in the art, depending on

CLAIMS

1. A method for preventing or treating one or more of the effects of stroke in a mammalian subject comprising administering an effective amount of phorbol ester of Formula I, or a pharmaceutically-acceptable salt, isomer, or enantiomer, thereof to said subject



FORMULA I

wherein R_1 and R_2 are selected from the group consisting of hydrogen, hydroxyl, —O—C(=O)-alkyl, —O—C(=O)-lower alkenyl, —O—C(=O)-phenyl, —O—C(=O)-benzyl, and substituted derivatives thereof; and R_3 is hydrogen, —O—C(=O)-lower alkyl and substituted derivatives thereof.

2. The method of claim 1, wherein R_1 or R_2 is —O—C(=O)—C_1— C_{15} alkyl the remaining R_1 or R_2 is —O—C(=O)-lower alkyl and R_3 is hydrogen.

3. The method of claim 1, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-

didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

4. The method of claim 1, further comprising administering at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester of Formula I to treat or prevent effects of stroke in said subject.

5. The method of claim 4, wherein the at least one secondary or adjunctive therapeutic agent is administered to said subject in a coordinate administration protocol, simultaneously with, prior to, or after, administration of said phorbol ester to said subject.

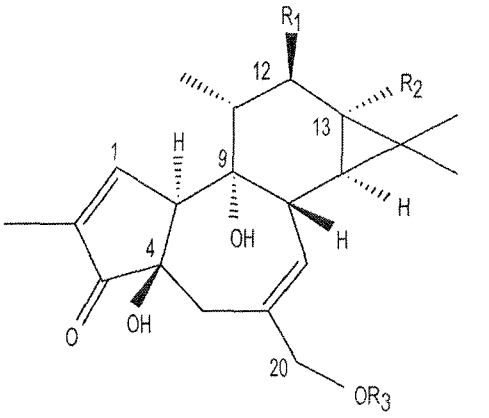
6. The method of claim 4, wherein the at least one secondary or adjunctive therapeutic agent is tissue plasminogen activator, an anticoagulant, a statin, fibrate, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitor, beta-blocker, anti-platelet agent, calcium channel blocker, or diuretic.

7. The method of claim 1, further comprising surgical intervention in combination with phorbol ester of Formula I to treat or prevent effects of stroke in said subject.

8. The method of claim 7, wherein the surgical intervention is a carotid endarterectomy, angioplasty, stent placement, craniotomy, insertion of a pacemaker, implantation of a defibrillator, replacement of valves, coronary artery bypass, heart transplantation, endovascular coil emobilization, or patent foramen ovale closure.

9. The method of claim 1, wherein the one or more effects of stroke are paralysis, spatial impairment, impaired judgment, left-sided neglect, memory loss, aphasia, coordination and balance problems, nausea, vomiting, cognitive impairment, perception impairment, orientation impairment, homonymous hemianopsia, or impulsivity.

10. A composition for the prevention or treatment of one or more effects of stroke in a mammalian subject comprising an effective amount of a phorbol ester of Formula I, or a pharmaceutically-acceptable salt, isomer, or enantiomer thereof



FORMULA I

wherein R_1 and R_2 are selected from the group consisting of hydrogen, hydroxyl, —O—C(=O)-alkyl, —O—C(=O)-lower alkenyl, —O—C(=O)-phenyl, —O—C(=O)-benzyl, and substituted derivatives thereof; and R_3 is hydrogen, —O—C(=O)-lower alkyl and substituted derivatives thereof.

11. The composition of claim 10, wherein R_1 or R_2 is —O—C(=O)—C_1— C_{15} alkyl and the remaining R_1 or R_2 is —O—C(=O)-lower alkyl and R_3 is hydrogen.

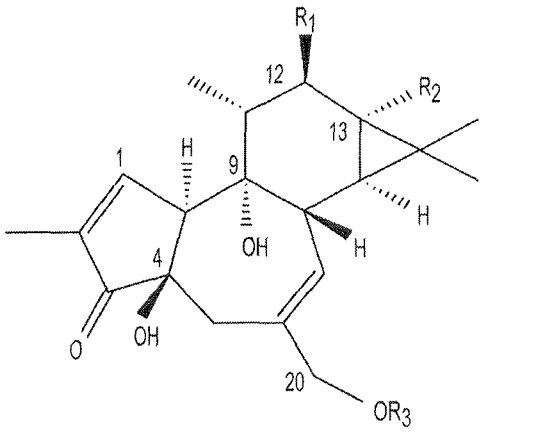
12. The composition of claim 10, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

13. The composition of claim 10, further comprising at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation with said phorbol ester of Formula I to treat or prevent an effect of stroke in said subject.

14. The composition of claim 13, wherein the at least one secondary or adjunctive therapeutic agent is tissue plasminogen activator, an anticoagulant, a statin, fibrate, anti-platelet agent, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitor, beta-blocker, calcium channel blocker, or diuretic.

15. The composition of claim 10, wherein the one or more side effects of stroke is paralysis, spatial impairment, impaired judgment, left-sided neglect, memory loss, aphasia, coordination and balance problems, nausea, vomiting, cognitive impairment, perception impairment, orientation impairment, homonymous hemianopsia, or impulsivity.

16. A method for preventing a stroke in a mammalian subject at risk for a stroke comprising administering an effective amount of phorbol ester of Formula I, or a pharmaceutically-acceptable salt, isomer, or enantiomer, thereof to said subject



FORMULA I

wherein R_1 and R_2 are selected from the group consisting of hydrogen, hydroxyl, —O—C(=O)-alkyl, —O—C(=O)-lower alkenyl, —O—C(=O)-phenyl, —O—C(=O)-benzyl, and substituted derivatives thereof; and R_3 is hydrogen, —O—C(=O)-lower alkyl and substituted derivatives thereof.

17. The method of claim 16, wherein R_1 or R_2 is —O—C(=O)—C_1— C_{15} alkyl the remaining R_1 or R_2 is —O—C(=O)-lower alkyl and R_3 is hydrogen.

18. The method of claim 16, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol

12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

19. The method of claim 16, further comprising administering at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester of Formula I to treat or prevent effects of stroke in said subject, wherein the secondary or adjunctive therapeutic agent is administered simultaneously with, prior to, or after, administration of said phorbol ester of Formula I.

20. The method of claim 19, wherein the at least one secondary or adjunctive therapeutic agent is an anticoagulant, a statin, fibrate, angiotensin II receptor blockers, anti-platelet agent, angiotensin-converting enzyme inhibitor, beta-blocker, calcium channel blocker, or diuretic.

Compositions and Methods of Use of Phorbol Esters for the Treatment of Stroke

Related Applications

[001] This application is a United States utility patent application which claims priority benefit of United States Provisional Patent Application Serial No. 61/588,167, filed January 18, 2012, which is incorporataed herein in its entirety be reference.

Additional Disclosure

[002] Additional disclosures relating to the instant application may be found in "Compositions And Methods Of Use Of Phorbol Esters" United States Patent Application Serial No. 12/023,753, filed January 31, 2008, to Richard L. Chang, et al, which claims priority benefit of United States Provisional Patent Application Serial No. 60/898,810, filed January 31, 2007, each of which is incorporated herein by reference in its entirety for all purposes.

Technical Field

[003] The present invention relates generally to the medicinal use of phorbol esters in the treatment and prevention of stroke and the effects of stroke.

Background

[004] Plants have historically served many medicinal purposes. The World Health Organization (WHO) estimates that 4 billion people, 80% of the world's population, presently use herbal medicine for some aspect of primary health care. (WHO Fact sheet Fact sheet N°134, December 2008) However, it can be difficult to isolate the specific compound in a plant that has the desired medicinal effect and to reproduce it on a commercial scale. Additionally, while the active compound may be isolated from a plant, the other parts of a plant such as the minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavanoids, and other substances may also be involved in the functioning of the active compound or the medicinal effect for which the plant is known, making the use, purification and commercialization of plant based pharmaceutical agents a challenge.

[005] Phorbol is a natural, plant-derived organic compound of the tigliane family of diterpenes. It was first isolated in 1934 as a hydrolysis product of croton oil derived from

the seeds of *Croton tiglium*, a leafy shrub of the Euphorbiaceae family that is native to Southeastern Asia. Various esters of phorbol have important biological properties including the reported ability to mimic diacylglycerols and activate protein kinase C (PKC); and to modulate downstream cell signaling pathways including the mitogen-activated protein kinase (MAPK) pathways. Phorbol esters are additionally thought to bind to chimaerins, the Ras activator RasGRP, and the vesicle-priming protein Munc-13 (Brose N, Rosenmund C., JCell Sci;115:4399–411 (2002)). Some phorbol esters also induce nuclear factor-kappa B (NF-κB). The most notable physiological property of phorbol esters is their reported capacity to act as tumor promoters. (Blumberg, 1988; Goel, G *et al.,* Int, Journal of Toxicology 26, 279-288 (2007)).

[006] 12-O-tetradecanoylphorbol-13-acetate (TPA), also called phorbol-12-myristate-13-acetate (PMA), is a phorbol ester used in models of carcinogenesis as an inducer for differentiation and/or apoptosis in multiple cell lines and primary cells. TPA has also been reported to cause an increase in circulating white blood cells and neutrophils in patients whose bone marrow function has been depressed by chemotherapy (Han Z. T. *et al.* Proc. Natl. Acad. Sci. 95, 5363-5365 (1998)), and to inhibit an HIV- induced cytopathic effect on MT-4 cells. (Mekkawy S. *et al.*, Phytochemistry 53, 47-464 (2000)). However, due to a variety of factors, including caustic reactions when contacted with the skin and concerns for its potential toxicity, TPA has not been shown to be an effective tool for treating, managing, or preventing disease. Indeed, as phorbol esters play a key role in activation of protein kinase C, which triggers various cellular responses resulting in inflammatory responses and tumor development (Goel *et al.,* Int, Journal of Toxicology 26, 279-288 (2007)), phorbol esters would generally be excluded from possible treatment candidates for conditions that involve inflammatory reactions such as stroke.

[007] Heart disease and stroke kill some 17 million people a year, causing almost one-third of all deaths globally. They are predicted to become the leading cause of both death and disability worldwide, with the number of fatalities projected to increase to over 20 million a year by 2020 and to 24 million a year by 2030. (Atlas of Heart Disease and Stroke, World Health Organization 2004).

[008] While there are over 300 risk factors associated with coronary heart disease and stroke (Atlas of Heart Disease and Stroke, World Health Organization 2004), in developed countries, at least 1/3 of all cardiovascular disease is attributable to tobacco use, alcohol use, high blood pressure, high cholesterol and obesity.

[009] Current treatments for the management and prevention of stroke are generally a combination of medications such as ACE inhibitors, aspirin, beta blockers and lipid lowering medications; devices such as pacemakers, implantable defibrillators, coronary stents, prosthetic valves and artificial hearts; and operations such as coronary artery bypass, balloon angioplasty, valve repair and replacement, heart transplants and artificial heart operations. "Increasingly, high-technology procedures are chosen over less expensive, but nevertheless effective , strategies " (Atlas of Heart Disease and Stroke, World Health Organization 2004) adding to the rising costs of health care and leading to marked disparities in the quality of treatment between different groups of individuals.

[010] However, even where advanced technology and facilities are available, 60% of those who suffer a stroke die or become dependent and each stroke significantly increases the risk of further episodes. "Worldwide, treatment of cardiovascular diseases and their risk factors remains inadequate for most patients." (Atlas of Heart Disease and Stroke, World Health Organization 2004). There is therefore clearly a need for new and more effective measures to treat and prevent strokes and to treat or prevent the long term effects caused by stroke.

Summary

[011] The present invention relates to compositions containing and methods of using phorbol esters. The compositions and methods described herein are effective in preventing and treating stroke and in managing the sequelae of stroke including acute ischemic events.

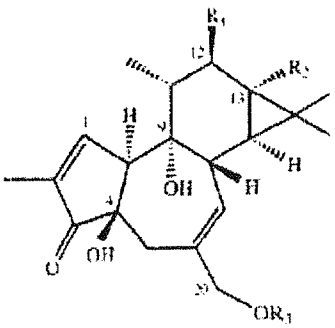
[012] Effects of stroke that may be prevented or treated by using the phorbol esters and derivatives of phorbol esters as described herein include, but are not limited to, paralysis, spatial impairment, impaired judgment, left-sided neglect, memory loss, aphasia, coordination and balance problems, nausea, vomiting, cognitive impairment, perception impairment, orientation impairment, homonymous hemianopsia and impulsivity. In some

embodiments, the use of phorbol esters and derivatives of phorbol esters may prevent initial and subsequent strokes from occurring.

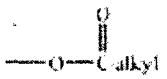
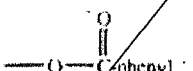
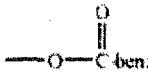
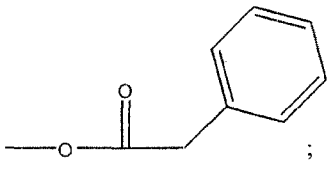
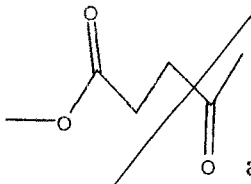
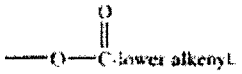
[013] Successful treatment will be determined according to conventional methods, such as a reduction in the severity or sequelae of a stroke, a decrease or elimination of the effects of stroke, a decrease in risk factors that predispose an individual to a stroke, and/or a decrease in the number or severity of strokes including subsequent strokes.

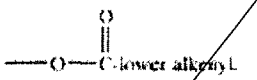
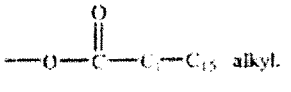
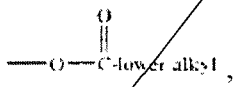
[014] In another embodiment, the phorbol esters and derivatives of phorbol esters as described herein may be used to modulate cell signaling pathways. Such modulation may have a variety of results, for example, in some embodiments, the use of compositions containing phorbol esters and derivatives of phorbol esters may alter the release of Th1 cytokines in mammalian subjects. In a further embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the release of interleukin 2 (IL-2) in mammalian subjects. In an additional embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the release of interferon in mammalian subjects. In yet another embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the rate of ERK phosphorylation.

[015] The invention achieves the foregoing and satisfies additional objects and advantages by providing novel and surprisingly effective methods and compositions for treating and preventing stroke, modulating cell signaling pathways and/or managing, treating and preventing the sequelae of a stroke using compositions containing a phorbol ester of Formula I, below:



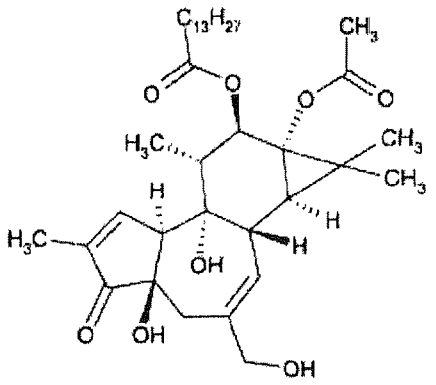
Formula I

wherein R_1 and R_2 may independently be hydrogen; hydroxyl; , , wherein the alkyl group contains 1 to 15 carbon atoms; , wherein a lower alkenyl group contains between 1 to 7 carbon atoms; ; ; ;  and substituted  derivatives thereof. R_3 may be independently be hydrogen or and substituted derivatives thereof. The methods and compositions of the present invention further include any pharmaceutical salts, enantiomers, isomer, polymorphs, prodrugs, hydrates and solvates of the compositions of Formula I.

[016] In some embodiments, at least one of R_1 and R_2 are other than hydrogen and R_3 is hydrogen or  and substituted derivatives thereof. In another embodiment, either R_1 or R_2 is  the remaining R_1 or R_2 is a , wherein a lower alkyl is between 1 and 7 carbons, and R_3 is hydrogen.

[017] The alkyl, alkenyl, phenyl and benzyl groups of the formulas of Formula I herein may be unsubstituted or substituted with halogens, preferably, chlorine, fluorine or bromine; nitro; amino; and/or similar type radicals.

[018] An exemplary phorbol ester composition as used in the methods and compositions as described herein is 12-O-tetradecanoylphorbol-13-acetate (TPA) of Formula II, below:



Formula II

[019] Useful phorbol esters of Formula I and related compounds and derivatives within the formulations and methods of the invention include, but are not limited to, other pharmaceutically acceptable active salts of said compounds, as well as active isomers, enantiomers, polymorphs, glycosylated derivatives, solvates, hydrates, and/or prodrugs of said compounds. Exemplary forms of phorbol esters for use within the compositions and methods of the invention include, but are not limited to, phorbol 13-butyrate; phorbol 12-decanoate; phorbol 13-decanoate; phorbol 12,13-diacetate; phorbol 13,20-diacetate; phorbol 12,13-dibenzoate; phorbol 12,13-dibutyrate; phorbol 12,13-didecanoate; phorbol 12,13-dihexanoate; phorbol 12,13-dipropionate; phorbol 12-myristate; phorbol 13-myristate; phorbol 12-myristate-13-acetate (also known as TPA or PMA); phorbol 12,13,20-triacetate; 12-deoxyphorbol 13-angelate; 12-deoxyphorbol 13-angelate 20-acetate; 12-deoxyphorbol 13-isobutyrate; 12-deoxyphorbol 13-isobutyrate-20-acetate; 12-deoxyphorbol 13-phenylacetate; 12-deoxyphorbol 13-phenylacetate 20-acetate; 12-deoxyphorbol 13-tetradecanoate; phorbol 12-tigliate 13-decanoate; 12-deoxyphorbol 13-acetate; phorbol 12-acetate; and phorbol 13-acetate.

[020] Mammalian subjects amenable to treatment with phorbol esters of Formula I or derivatives of a phorbol ester of the Formula I, particularly TPA, according to the methods of the invention include, but are not limited to, individuals who have suffered or are at risk for a stroke. Subjects amenable to treatment with phorbol esters of Formula I, particularly TPA, additionally include those suffering from the effects of a stroke including, but not limited to, paralysis, spatial impairment, impaired judgment, left-sided neglect, memory loss, aphasia, coordination and balance problems, nausea, vomiting,

cognitive impairment, perception impairment, orientation impairment, homonymous hemianopsia and impulsivity.

[021] These and other subjects are effectively treated prophylactically and/or therapeutically, by administering to the subject an effective amount of a phorbol ester of Formula I or derivatives of a phorbol ester of the Formula I sufficient to modulate NF-κB activity, increase Th1 cytokine activity, prevent or treat paralysis, increase spatial awareness, decrease memory loss, decrease aphasia, increase coordination and balance, prevent or decrease the incidence and severity of a stroke, and improve cognition.

[022] Therapeutically useful methods and formulations of the invention will effectively use a phorbol ester of Formula I or derivative of a phorbol ester of the Formula I in a variety of forms, as noted above, including any active, pharmaceutically acceptable salts of said compounds, as well as active isomers, enantiomers, polymorphs, solvates, hydrates, prodrugs, and/or combinations thereof. TPA of formula II is employed as an illustrative embodiment of the invention within the examples herein below.

[023] Within additional aspects of the invention, combinatorial formulations and methods are provided which employ an effective amount of a phorbol ester of Formula I or derivative of a phorbol ester of Formula I in combination with one or more secondary or adjunctive active agent(s) that is/are combinatorially formulated or coordinately administered with the phorbol ester compound of Formula I to yield an effective response in the subject.

[024] Combinatorial formulations and coordinate treatment methods in the treatment of stroke employ a phorbol ester compound of Formula I or derivative of the phorbol ester of Formula I in combination with one or more additional, stroke preventing, treating or other indicated, secondary or adjunctive therapeutic agents. The secondary or adjunctive therapeutic agents used in combination with a phorbol ester, e.g., TPA, in these embodiments may possess direct or indirect effects on prevention or recovery from stroke, alone or in combination with the phorbol ester, e.g. TPA; may exhibit other useful adjunctive therapeutic activity in combination with, e.g. TPA (such as anti-clotting, anticholesterolemic, vasodilating, antihypertensive, arteriolar resistance lowering, venous capacity increasing, heart oxygen demand reducing, heart rate decreasing, heart rate stabilizing, or neuroprotecting); or may exhibit adjunctive therapeutic activity useful for

treating or preventing stroke or associated symptoms alone or in combination with, e.g. TPA. Such secondary or adjunctive therapeutic agents may be administered prior to, simultaneously, or after administration of a phorbol ester of Formula I or derivative of a phorbol ester of Formula I.

[025] Useful adjunctive or secondary therapeutic agents in these combinatorial formulations and coordinate treatment methods for the prevention or treatment of stroke in a mammalian subject include, but are not limited to, tissue plasminogen activators, anticoagulants, statins, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, anti-platelet agents, beta-blockers, aspirin, fibrates, calcium channel blockers, or diuretics. In addition, adjunctive or secondary therapies may be used such as, but not limited to, surgical intervention including carotid endarterectomy, angioplasty, balloon angioplasty, valve repair and replacement, coronary artery bypass, stent placement, craniotomy, endovascular coil emobilization, or patent foramen ovale closure.

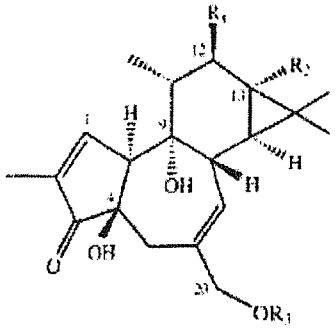
[026] The foregoing and additional objects, features, aspects and advantages of the present invention will become apparent from the following detailed description.

Detailed Description

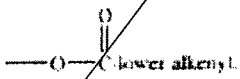
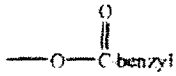
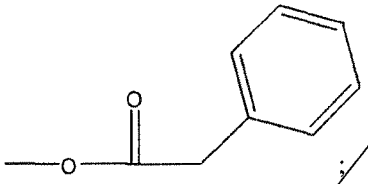
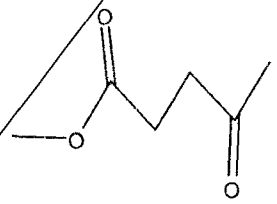
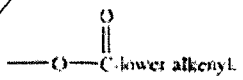
[027] Novel methods and compositions have been identified for use in preventing and/or treating stroke and the sequelae of stroke in mammalian subjects, including humans.

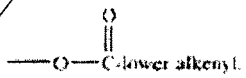
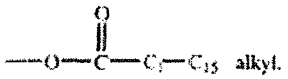
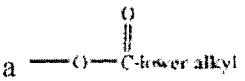
[028] In various embodiments, the composition and methods as described herein may increase the release of Th1 cytokines, increase ERK phosphorylation, modulate NF-κB activity, prevent or treat paralysis, increase spatial awareness, decrease memory loss, decrease aphasia, increase coordination and balance, improve cognition, improve orientation, decrease the prevalence of subsequent strokes, and decrease impulsivity.

[029] Formulations and methods provided herein employ a phorbol ester of Formula I or derivative compound of a phorbol ester of Formula I as more fully described in U.S. Patent Application No. 12/023,753, filed January 31, 2008, which claims priority benefit of United States Provisional patent application Serial No. 60/898,810, filed January 31, 2007, and U.S. Provisional Patent Application No. _____filed _____each of which is incorporated herein in its entirety by reference,



Formula I

wherein R_1 and R_2 may be hydrogen; hydroxyl;  , wherein the alkyl group contains 1 to 15 carbon atoms; ;  , wherein a lower alkenyl group contains between 1 to 7 carbon atoms;  ;  ;  ;  and substituted derivatives thereof. R_3 may be hydrogen or  .

[030] In some embodiments, at least one of R_1 and R_2 are other than hydrogen and R_3 is hydrogen or  and substituted derivatives thereof as novel compositions for use in treating chronic or recurring conditions. In another embodiment, either R_1 or R_2 is  the remaining R_1 or R_2 is a  , wherein a lower alkyl is between 1 and 7 carbons, and R_3 is hydrogen.

[031] The alkyl, alkenyl, phenyl and benzyl groups of the formulas herein may be unsubstituted or substituted with halogens, preferably, chlorine, fluorine or bromine; nitro; amino; and/or similar type radicals.

[032] Stroke treating formulations and methods provided herein employ a phorbol ester of Formula I or derivative compound of a phorbol ester of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as anti-stroke agents.

[033] Th1 cytokine increasing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as novel Th1 cytokine increasing agents. A broad range of mammalian subjects, including human subjects, are amenable to treatment using the formulations and methods of the invention. These subjects include, but are not limited to, individuals who have suffered or are at risk for a stroke.

[034] ERK phosphorylation increasing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as novel ERK phosphorylation increasing agents. A broad range of mammalian subjects, including human subjects, are amenable to treatment using the formulations and methods of the invention. These subjects include, but are not limited to, individuals who have suffered or are at risk for a stroke.

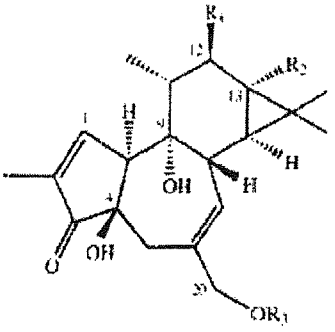
[035] Within the methods and compositions of the invention, one or more phorbol ester compound(s) of Formula I or derivative compounds of a phorbol ester of Formula I as disclosed herein is/are effectively formulated or administered as an agent effective for treating and preventing stroke or the sequelae of stroke. In exemplary embodiments, TPA is demonstrated for illustrative purposes to be an effective agent in pharmaceutical

formulations and therapeutic methods, alone or in combination with one or more adjunctive therapeutic agent(s). The present disclosure further provides additional, pharmaceutically acceptable phorbol ester compounds in the form of a native or synthetic compound, including complexes, derivatives, salts, solvates, isomers, enantiomers, polymorphs, and prodrugs of the compounds disclosed herein, and combinations thereof, which are effective as therapeutic agents within the methods and compositions of the invention in the treatment and prevention of stroke and the sequelae of stroke.

[036] Strokes are caused by disruption of the blood supply to the brain. This may result from either blockage (ischaemic stroke) or rupture of a blood vessel (haemorrhagic stroke). The symptoms of a stroke include sudden numbness or weakness, especially on one side of the body; sudden confusion or trouble speaking or understanding speech; sudden trouble seeing in one or both eyes; sudden trouble with walking, dizziness, or loss of balance or coordination; or sudden severe headache with no known cause. Risk factors for stroke include high blood pressure, abnormal blood lipids, tobacco use, physical inactivity, obesity, stress, diabetes, alcohol use, excess homocystein in the blood, inflammation and abnormal coagulation. There are also non-modifiable risk factors such as age, heredity, gender and ethnicity.

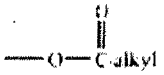
[037] Treatment for stroke has three distinct phases: prevention, therapy immediately after a stroke, and post-stroke rehabilitation. The compositions and methods described herein may be used at any phase of stroke treatment, independently or in conjunction with one or more additional therapies including other pharmaceutical agents, devices or surgical interventions.

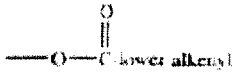
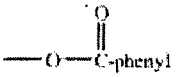
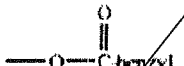
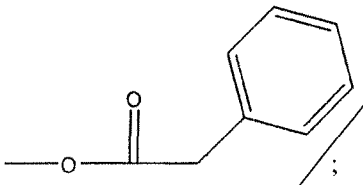
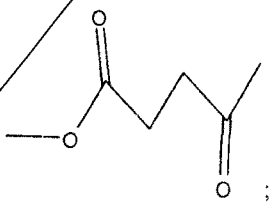
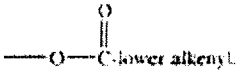
[038] Phorbol is a natural, plant-derived polycyclic alcohol of the tigliane family of diterpenes. It was first isolated in 1934 as the hydrolysis product of croton oil derived from the seeds of *Croton tiglium*. It is well soluble in most polar organic solvents and in water. Esters of phorbol have the general structure of Formula I, below:



Formula I

wherein R_1 and R_2 are selected from the group consisting of hydrogen; hydroxyl;

, wherein the alkyl group contains 1 to 15 carbon atoms;

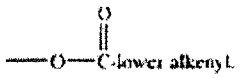
, wherein a lower alkenyl group contains between 1 to 7 carbon atoms; ; ; ; ; and substituted derivatives thereof and R_3 may be hydrogen,  or substituted derivatives thereof as well as pharmaceutically acceptable salts, enantiomers, polymorphs, prodrugs solvates and hydrates of compounds of Formula I and substituted derivatives thereof.

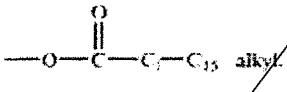
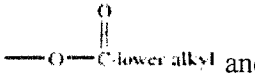
[039] The term "lower alkyl" or "lower alkenyl" as used herein means moieties containing 1 to 7 carbon atoms. In the compounds of the Formula I, the alkyl or alkenyl groups may be straight or branched chain. In some embodiments, either or both R_1 or R_2, are a long chain carbon moiety (i.e., Formula I is decanoate or myristate).

[040] The alkyl, alkenyl, phenyl and benzyl groups of the formulas herein may be unsubstituted or substituted with halogens, preferably, chlorine, fluorine or bromine; nitro; amino and similar type radicals.

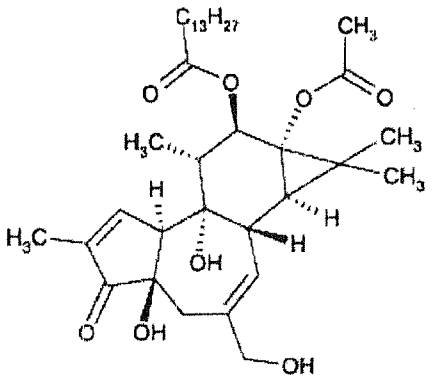
[041] Organic and synthetic forms of phorbol esters, including any preparations or extracts from herbal sources such as *croton tiglium*, are contemplated as useful compositions comprising phorbol esters (or phorbol ester analogs, related compounds and/or derivatives) for use within the embodiments herein. Useful phorbol esters and/or related compounds for use within the embodiments herein will typically have a structure as illustrated in Formula I, although functionally equivalent analogs, complexes, conjugates, and derivatives of such compounds will also be appreciated by those skilled in the art as within the scope of the invention.

[042] In more detailed embodiments, illustrative structural modifications according to Formula I above will be selected to provide useful candidate compounds for treating and/or preventing strokes, damage due to stroke and/ or managing the effects or sequelae of strokes in mammalian subjects, including humans, wherein: at least one of R_1 and R_2 are other than hydrogen and R_3 is selected from the group consisting of hydrogen



and substituted derivatives thereof. In another embodiment, either R_1 or R_2 is  the remaining R_1 or R_2 is  and R_3 is hydrogen.

[043] An exemplary embodiment of a phorbol ester compound of Formula I useful in the treatment of in treating or preventing strokes, damage from strokes, and/or managing the effects or sequelae of strokes in mammalian subjects, including humans, is found in phorbol 12-myristate-13-acetate (also known as PMA or 12-O-tetradecanoyl-phorbol-13-acetate (TPA)) shown in Formula II, below.

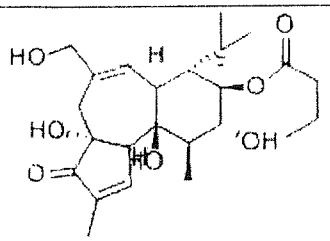
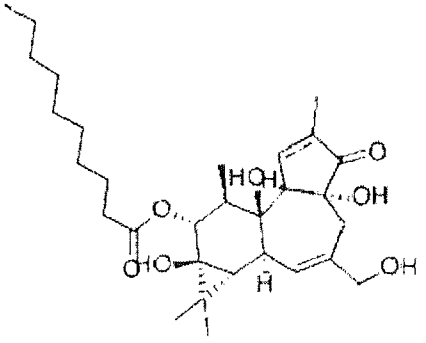


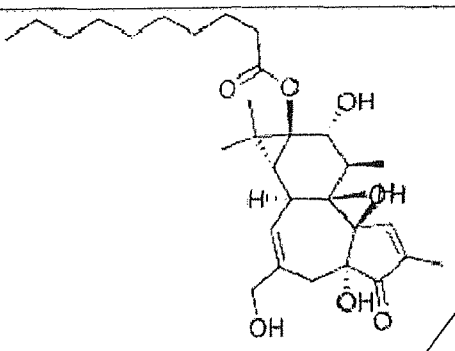
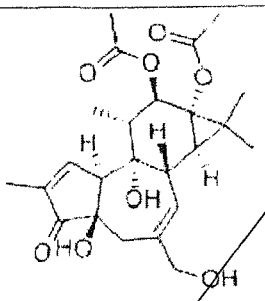
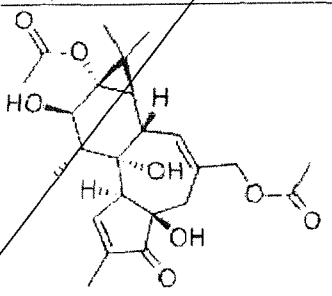
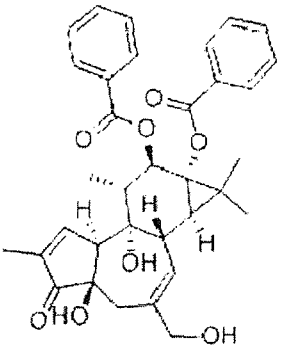
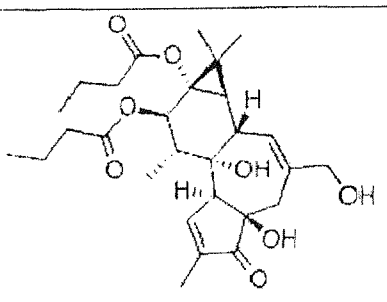
Formula II

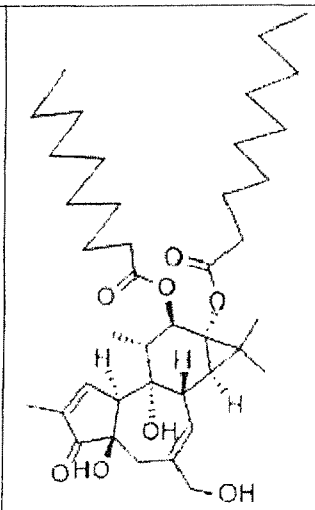
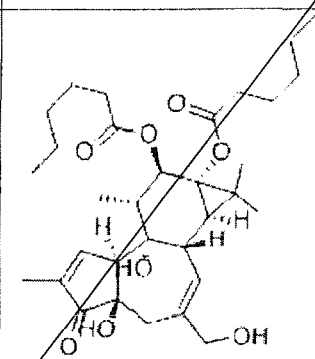
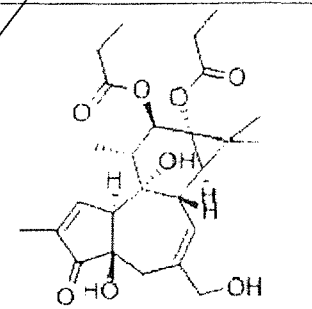
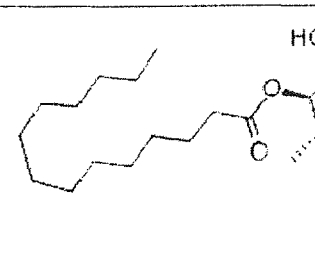
[044] Additional useful phorbol esters and related compounds and derivatives within the formulations and methods of the invention include, but are not limited to, other pharmaceutically acceptable active salts of said compounds, as well as active isomers, enantiomers, polymorphs, glycosylated derivatives, solvates, hydrates, and/or prodrugs of said compounds. Derivatives of phorbol esters of Formula I may or may not be phorbol esters themselves. Further exemplary forms of phorbol esters for use within the compositions and methods of the invention include, but are not limited to, phorbol 13-butyrate; phorbol 12-decanoate; phorbol 13-decanoate; phorbol 12,13-diacetate; phorbol 13,20-diacetate; phorbol 12,13-dibenzoate; phorbol 12,13-dibutyrate; phorbol 12,13-didecanoate; phorbol 12,13-dihexanoate; phorbol 12,13-dipropionate; phorbol 12-myristate; phorbol 13-myristate; phorbol 12,13,20-triacetate; 12-deoxyphorbol 13-angelate; 12-deoxyphorbol 13-angelate 20-acetate; 12-deoxyphorbol 13-isobutyrate; 12-deoxyphorbol 13-isobutyrate-20-acetate; 12-deoxyphorbol 13-phenylacetate; 12-deoxyphorbol 13-phenylacetate 20-acetate; 12-deoxyphorbol 13-tetradecanoate; phorbol 12-tigliate 13-decanoate; 12-deoxyphorbol 13-acetate; phorbol 12-acetate; and phorbol 13-acetate as shown in Table 1.

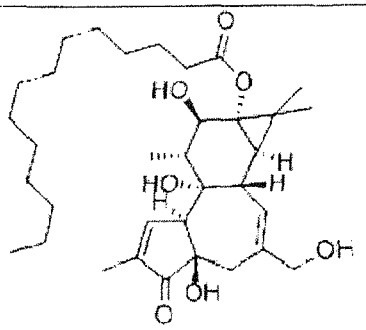
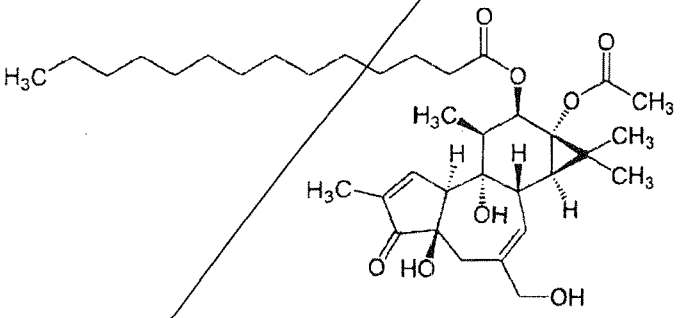
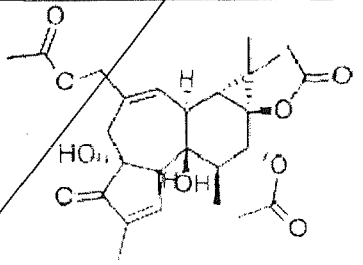
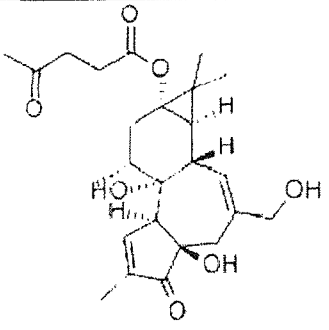
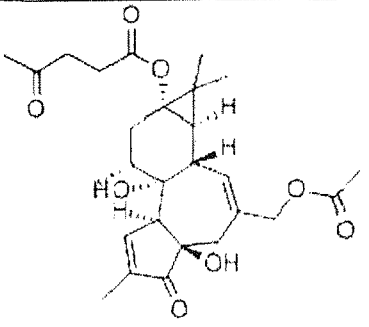
Table 1

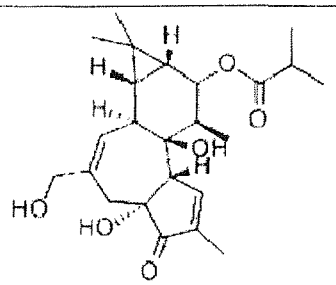
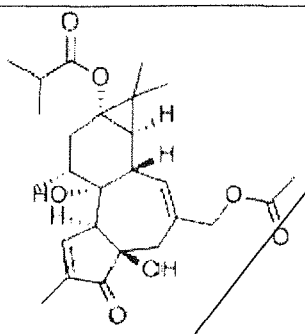
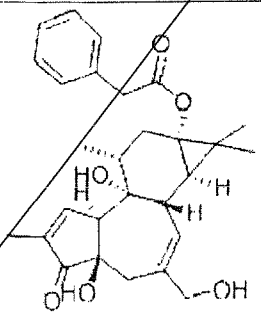
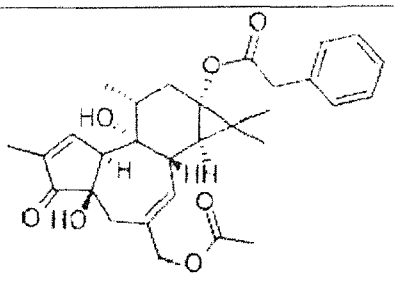
Exemplary Phorbol Esters

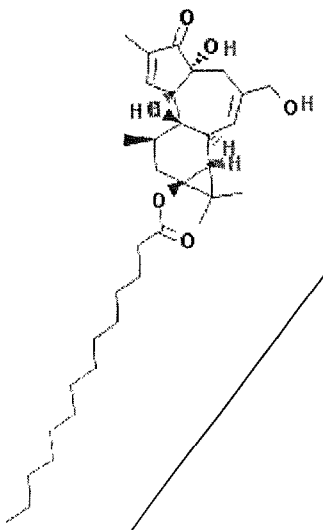
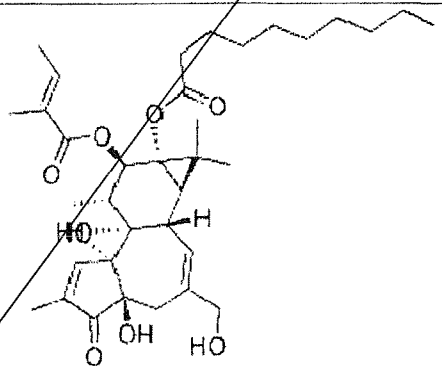
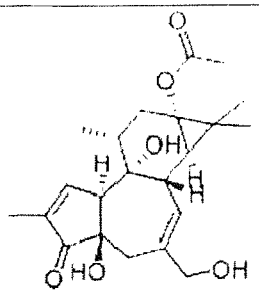
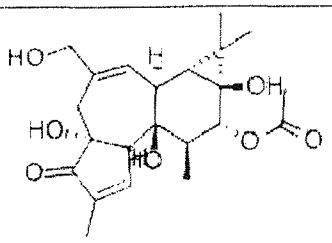
Phorbol 13-Butyrate	
Phorbol 12-Decanoate	

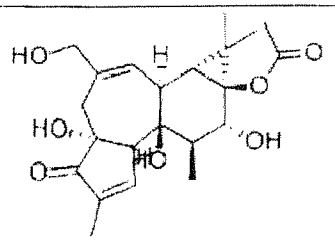
Phorbol 13-Decanoate	
Phorbol 12,13-Diacetate	
Phorbol 13,20-Diacetate	
Phorbol 12,13-Dibenzoate	
Phorbol 12,13-Dibutyrate	

Phorbol 12,13-Didecanoate	
Phorbol 12,13-Dihexanoate	
Phorbol 12,13-Dipropionate	
Phorbol 12-Myristate	

Phorbol 13-Myristate	
Phorbol 12-Myristate-13-Acetate (also known as TPA or PMA)	
Phorbol 12,13,20-Triacetate	
12-Deoxyphorbol 13-Angelate	
12-Deoxyphorbol 13-Angelate 20-Acetate	

12-Deoxyphorbol 13-Isobutyrate	
12-Deoxyphorbol 13-Isobutyrate-20-Acetate	
12-Deoxyphorbol 13-Phenylacetate	
12-Deoxyphorbol 13-Phenylacetate 20-Acetate	

12-Deoxyphorbol 13-Tetradecanoate	
Phorbol 12-Tigliate 13-Decanoate	
12-Deoxyphorbol 13-Acetate	
Phorbol 12-Acetate	

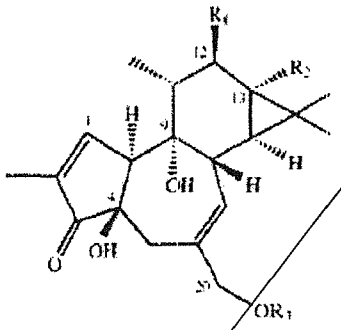
Phorbol 13-Acetate	

[045] Compositions as described herein comprise stroke treating compositions comprising a stroke damage alleviating or preventing effective amount of a phorbol ester compound of Formula I or derivative compound of a phorbol esters of Formula I, which is effective for prophylaxis and/or treatment of stroke or stroke related symptoms or sequelae in a mammalian subject. A "stroke treating," "anti-clotting," "anticholesterolemic," "vasodilating," "antihypertensive," "arteriolar resistance lowering," " venous capacity increasing," "heart oxygen demand reducing," "heart rate decreasing," " heart rate stabilizing," or "neuroprotective" effective amount of the active compound is therapeutically effective, in a single or multiple unit dosage form, over a specified period of therapeutic intervention, to measurably alleviate one or more symptoms or sequelae of stroke in the subject. Within exemplary embodiments, the compositions of the invention are effective in treatment methods to prevent or alleviate symptoms of stroke or sequelae of stroke in human and other mammalian subjects vulnerable to or who have suffered a stroke.

[046] Phorbol ester treating compositions of the invention typically comprise an effective amount or unit dosage of a phorbol ester compound of Formula I or derivative compound of a phorbol ester of Formula I, which may be formulated with one or more pharmaceutically acceptable carriers, excipients, vehicles, emulsifiers, stabilizers, preservatives, buffers, and/or other additives that may enhance stability, delivery, absorption, half-life, efficacy, pharmacokinetics, and/or pharmacodynamics, reduce adverse side effects, or provide other advantages for pharmaceutical use. Effective amounts of a phorbol ester compound or related or derivative compound of Formula I (e.g., a unit dose comprising an effective concentration/amount of TPA, or of a selected pharmaceutically acceptable salt, isomer, enantiomer, solvate, polymorph and/or prodrug of TPA) will be readily determined by those of ordinary skill in the art, depending on

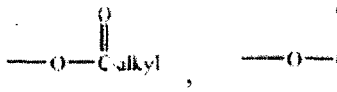
We claim:

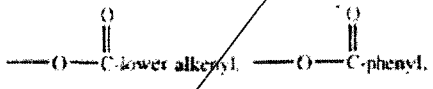
1. A method for preventing or treating one or more of the effects of stroke in a mammalian subject comprising administering an effective amount of phorbol ester of Formula I, or a pharmaceutically-acceptable salt, isomer, or enantiomer, thereof to said subject

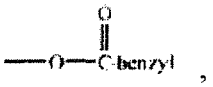


Formula I

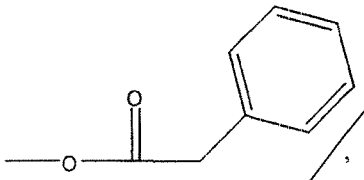
wherein R_1 and R_2 are selected from the group consisting of hydrogen, hydroxyl,

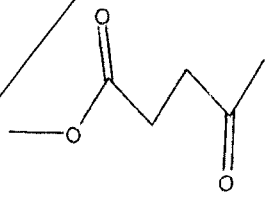


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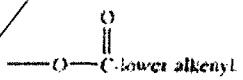


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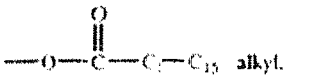


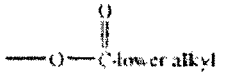
,


, and substituted derivatives thereof;

and R_3 is hydrogen, 

and substituted derivatives thereof.

2. The method of claim 1, wherein R_1 or R_2 is 

the remaining R_1 or R_2 is 

and R_3 is hydrogen.

3. The method of claim 1, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-

myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

4. The method of claim 1, further comprising administering at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester of Formula I to treat or prevent effects of stroke in said subject.

5. The method of claim 4, wherein the at least one secondary or adjunctive therapeutic agent is administered to said subject in a coordinate administration protocol, simultaneously with, prior to, or after, administration of said phorbol ester to said subject.

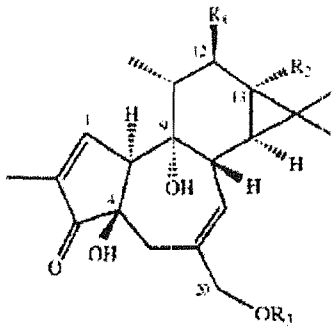
6. The method of claim 4, wherein the at least one secondary or adjunctive therapeutic agent is tissue plasminogen activator, an anticoagulant, a statin, fibrate, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitor, beta-blocker, anti-platelet agent, calcium channel blocker, or diuretic.

7. The method of claim 1, further comprising surgical intervention in combination with phorbol ester of Formula I to treat or prevent effects of stroke in said subject.

8. The method of claim 7, wherein the surgical intervention is a carotid endarterectomy, angioplasty, stent placement, craniotomy, insertion of a pacemaker, implantation of a defibrillator, replacement of valves, coronary artery bypass, heart transplantation, endovascular coil emobilization, or patent foramen ovale closure.

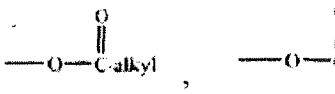
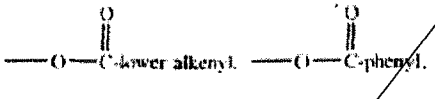
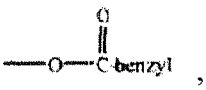
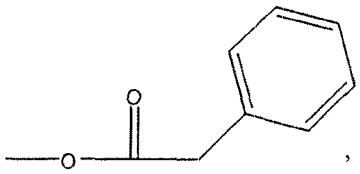
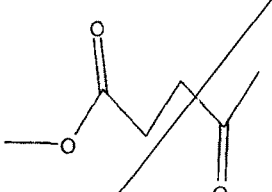
9. The method of claim 1, wherein the one or more effects of stroke are paralysis, spatial impairment, impaired judgment, left-sided neglect, memory loss, aphasia, coordination and balance problems, nausea, vomiting, cognitive impairment, perception impairment, orientation impairment, homonymous hemianopsia, or impulsivity.

10. A composition for the prevention or treatment of one or more effects of stroke in a mammalian subject comprising an effective amount of a phorbol ester of Formula I, or a pharmaceutically-acceptable salt, isomer, or enantiomer thereof

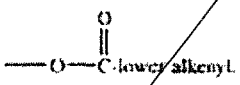


Formula I

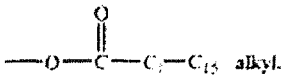
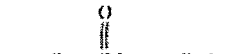
wherein R_1 and R_2 are selected from the group consisting of hydrogen, hydroxyl,

, and substituted derivatives thereof;



and R_3 is hydrogen, and substituted derivatives thereof.

11. The composition of claim 10, wherein R_1 or R_2 is  the remaining R_1 or R_2 is  and R_3 is hydrogen.

12. The composition of claim 10, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol

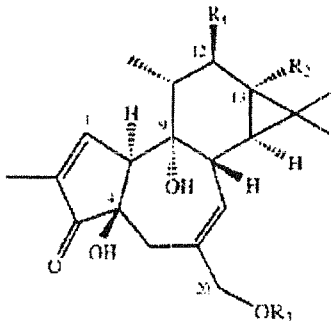
12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

13. The composition of claim 10, further comprising at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation with said phorbol ester of Formula I to treat or prevent an effect of stroke in said subject.

14. The composition of claim 13, wherein the at least one secondary or adjunctive therapeutic agent is tissue plasminogen activator, an anticoagulant, a statin, fibrate, anti-platelet agent, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitor, beta-blocker, calcium channel blocker, or diuretic.

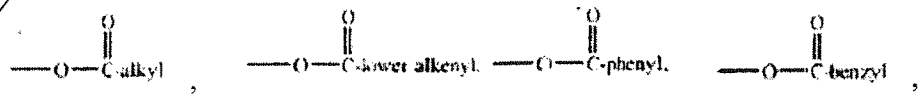
15. The composition of claim 10, wherein the one or more side effects of stroke is paralysis, spatial impairment, impaired judgment, left-sided neglect, memory loss, aphasia, coordination and balance problems, nausea, vomiting, cognitive impairment, perception impairment, orientation impairment, homonymous hemianopsia, or impulsivity.

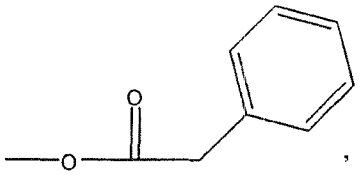
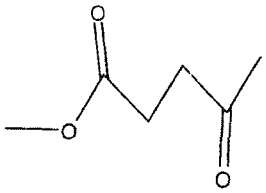
16. A method for preventing a stroke in a mammalian subject at risk for a stroke comprising administering an effective amount of phorbol ester of Formula I, or a pharmaceutically-acceptable salt, isomer, or enantiomer, thereof to said subject

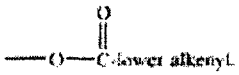


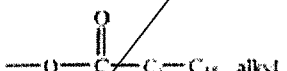
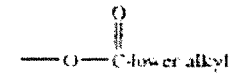
Formula I

wherein R_1 and R_2 are selected from the group consisting of hydrogen, hydroxyl,





, and substituted derivatives thereof;


and R_3 is hydrogen, and substituted derivatives thereof.

17. The method of claim 16, wherein R_1 or R_2 is  the remaining R_1 or R_2 is  and R_3 is hydrogen.

18. The method of claim 16, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

19. The method of claim 16, further comprising administering at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester of Formula I to treat or prevent effects of stroke in said subject, wherein the secondary or adjunctive therapeutic agent is administered simultaneously with, prior to, or after, administration of said phorbol ester of Formula I.

20. The method of claim 19, wherein the at least one secondary or adjunctive therapeutic agent is an anticoagulant, a statin, fibrate, angiotensin II receptor blockers, anti-platelet agent, angiotensin-converting enzyme inhibitor, beta-blocker, calcium channel blocker, or diuretic.