(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 September 2014 (18.09.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/145303 A1

(51) International Patent Classification:
A61K 9/107 (2006.01) *A61K 31/57* (2006.01)
A61K 31/34 (2006.01)

(21) International Application Number:
PCT/US2014/030041

(22) International Filing Date:
15 March 2014 (15.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/794,926 15 March 2013 (15.03.2013) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(54) Title: INTRAVENOUS EMULSIONS OF TRIPTOLIDE AS IMMUNOMODULATORS AND ANTICANCER AGENTS I

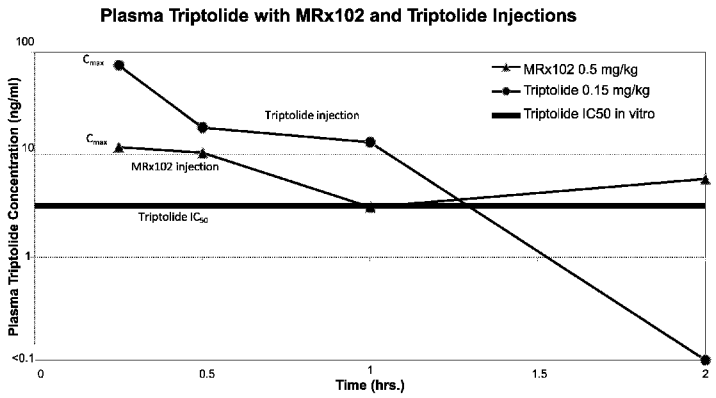

Figure 1

(57) Abstract: Intravenous formulations of triptolide and triptolide derivatives are disclosed for use in immunomodulation and anti-proliferative agents.

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INTRAVENOUS EMULSIONS OF TRIPTOLIDE AS IMMUNOMODULATORS AND ANTICANCER AGENTS

TECHNICAL FIELD

[0001] The present disclosure is directed to formulations of triptolide-derived compounds, useful as immunomodulators, anti-inflammatory and anticancer agents.

BACKGROUND

[0002] Immunosuppressive agents are widely used in the treatment of autoimmune disease and in treating or preventing transplantation rejection, including the treatment of graft-versus-host disease (GVHD), a condition in which transplanted (grafted) cells attack the recipient (host) cells. Common immunosuppressive agents include azathioprine, corticosteroids, cyclophosphamide, methotrexate, 6-mercaptopurine, vincristine, and cyclosporin A. In general, none of these drugs are completely effective, and most are limited by severe toxicity. For example, cyclosporin A, a widely used agent, is significantly toxic to the kidney. In addition, doses needed for effective treatment may increase the patient's susceptibility to infection by a variety of opportunistic invaders.

[0003] The compound triptolide, obtained from the Chinese medicinal plant *Tripterygium wilfordii* (TW), and certain derivatives and prodrugs thereof, have been identified as having significant immunosuppressive activity. Various prodrugs and other analogs of triptolide have also shown such activity. See, for example, U.S. Patent Nos. 4,005,108; 5,294,443; 5,648,376; 5,663,335; 5,759,550; 5,843,452; 5,962,516 and 6,150,539, each of which is incorporated herein by reference in its entirety. Triptolide and certain derivatives / analogs and prodrugs thereof have also been reported to show significant anticancer activity, including reduction of solid tumors *in vivo*; see, for example, Kupchan *et al.*, *J. Am. Chem. Soc.* **94**:7194 (1972), as well as co-owned U.S. Patent No. 6,620,843, also incorporated by reference, herein, in its entirety. Triptolide and its prodrugs and other analogs have also shown significant anticancer activity, including reduction of solid tumors *in vivo*. See, for example, co-owned U.S. Patent No. 6,620,843, which is incorporated herein by reference in its entirety, see, for example, Fidler *et al.*, *Mol. Cancer Ther.* **2**(9):855-62 (2003).

[0004] The analog can be designated a "selectively binding" analog if its binding affinity to a given first target molecule differs from its binding affinity to a second target molecule by a factor of 10 or more.

[0005] Although derivatives and prodrugs of triptolide have provided benefits relative to native triptolide in areas such as pharmacokinetics or biodistribution, *e.g.* by virtue of

differences in lipid or aqueous solubility, or via their activity as prodrugs, the biological activity per se of triptolide derivatives is often significantly less than that of native triptolide.

[0006] The foregoing examples of the related art and limitations related therewith are intended to be illustrative and not exclusive. Other limitations of the related art will become apparent to those of skill in the art upon a reading of the specification and a study of the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] Figure 1: Comparison of plasma triptolide concentrations over time upon injection of the prodrug PG796(MRx102) vs. triptolide

BRIEF SUMMARY

[0008] Examples of the related art and limitations related therewith, as set forth herein, are intended to be illustrative and not exclusive. Other limitations of the related art will become apparent to those of skill in the art upon a reading of the specification and a study of the drawings.

[0009] In one aspect, a composition is provided for intravenous administration of an emulsion comprising triptolide or a triptolide derivative having a clogP of 0.5 or higher, the emulsion comprising (a) 15 to 45 % by weight lipid, (b) 0 to 50 % by weight of a medium chain triglyceride, (c) 0.5 to 3 % by weight phospholipid, (d) 0 to 5 % by weight of glycerin, (e) 0.1 to 0.3% by weight of a sodium cholate, (f) about 50 to 60% by weight water, and (g) about 0.5 to about 3 mg/mL triptolide or a triptolide derivative. In some embodiments, no glycerin is used. In some embodiments, the concentration of triptolide or triptolide derivative is about 0.5 mg/mL to about 3 mg/mL. In some embodiments, the concentration of triptolide or triptolide derivative is about 1 mg/mL to about 2mg/mL.

[0010] In some embodiments, the composition comprises 15 to 45 % by weight lipid, wherein the lipid is selected from the group consisting of soybean oil, castor oil, corn oil, cottonseed oil, olive oil, peanut oil, peppermint oil, safflower oil, sesame oil, coconut oil or palm seed oil.

[0011] In some embodiments, the medium chain triglyceride is 20% by weight and is selected from the group consisting of glyceryl trioctanoate, glyceryl trihexanoate, glyceryl triheptanoate, glyceryl trinonanoate and glyceryl tridecanoate.

[0012] In some embodiments, the phospholipid is selected from the group consisting of hydrogenated soy phosphatidylcholine, distearoylphosphatidylglycerol, L-alpha-dimyristoylphosphatidylcholine and L-alpha-dimyristoylphosphatidylglycerol.

[0013] In some embodiments, the glycerin is selected from the group consisting of polyethylene glycol 300, polyethylene glycol 400, ethanol, propylene glycol, N-methyl-2-pyrrolidone, dimethylacetamide, and dimethylsulfoxide.

[0014] In some embodiments, the sodium cholate is selected from the group consisting of sodium taurocholate, sodium tauro-β-muricholate, sodium taurodeoxycholate, sodium taurochenodeoxycholate, sodium glycocholate, sodium glycodeoxycholate and sodium glycochenodeoxycholate.

[0015] In some embodiments, the composition for intravenous administration of an emulsion comprising triptolide or a triptolide derivative having a clogP of 0.5 or higher, is an emulsion comprising (a) 15 to 45 % by weight lipid, (b) 0 to 95 % by weight of a medium chain triglyceride, (c) 0.5 to 3 % by weight phospholipid, (d) 0 to 5 % by weight of glycerin, (e) 0.1 to 0.3% by weight of a sodium cholate, and (f) about 0.5 to about 3 mg/mL triptolide or a triptolide derivative, and is stored as an anhydrous mixture, and an aqueous solution is added prior to administration.

[0016] In some embodiments, composition for oral administration of an emulsion comprising triptolide or a triptolide derivative having a clogP of 0.5 or higher, is an emulsion comprising (a) 15 to 45 % by weight lipid, (b) 0 to 95 % by weight of a medium chain triglyceride, (c) 0.5 to 3 % by weight phospholipid, (d) 0 to 5 % by weight of glycerin, (e) 0.1 to 0.3% by weight of a sodium cholate, and (f) about 0.5 to about 3 mg/mL triptolide or a triptolide derivative, and is stored as an anhydrous mixture, and an aqueous solution is added prior to administration.

[0017] In one aspect, a composition for oral administration of an emulsion comprising triptolide or a triptolide derivative having a clogP of 0.5 or higher is provided..

[0018] In some embodiments, the composition comprises a triptolide derivative selected from the group consisting of compounds according to structure I. In some embodiments, the composition comprises a triptolide derivative selected from the group consisting of compounds according to structure II. In some embodiments, the composition comprises a triptolide derivative selected from the group consisting of compounds according to structure III. In some embodiments, the composition comprises a triptolide derivative selected from the group consisting of compounds according to structure IV.

[0019] In one aspect, a method is provided for effecting immunosuppression, immunomodulation or inhibiting cell proliferation, wherein the method comprises intravenously administering an emulsion comprising triptolide or a triptolide derivative having a clogP of 0.5 or higher to a subject in need in an amount effective for immunosuppression, immunomodulation or inhibiting cell proliferation.

[0020] In one aspect, a method is provided for inducing apoptosis in a cell, wherein the method comprises intravenously administering an emulsion comprising triptolide or a triptolide derivative having a clogP of 0.5 or higher to a subject in need in an amount effective for inducing apoptosis.

[0021] Additional embodiments of the present methods and compositions, and the like, will be apparent from the following description, drawings, examples, and claims. As can be appreciated from the foregoing and following description, each and every feature described herein, and each and every combination of two or more of such features, is included within the scope of the present disclosure provided that the features included in such a combination are not mutually inconsistent. In addition, any feature or combination of features may be specifically excluded from any embodiment of the present disclosure. Additional aspects and advantages of the present disclosure are set forth in the following description and claims, particularly when considered in conjunction with the accompanying examples and drawings.

DETAILED DESCRIPTION

[0022] Various aspects now will be described more fully hereinafter. Such aspects may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey its scope to those skilled in the art.

I. Definitions

[0023] As used in this specification, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a "polymer" includes a single polymer as well as two or more of the same or different polymers, reference to an "excipient" includes a single excipient as well as two or more of the same or different excipients, and the like.

[0024] Where a range of values is provided, it is intended that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the disclosure. For example, if a range of 1 μm to 8 μm is stated,

it is intended that 2 μm, 3 μm, 4 μm, 5 μm, 6 μm, and 7 μm are also explicitly disclosed, as well as the range of values greater than or equal to 1 μm and the range of values less than or equal to 8 μm. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

[0025] "Alkyl" refers to a saturated acyclic monovalent radical containing carbon and hydrogen, which may be linear or branched. Examples of alkyl groups are methyl, ethyl, n-butyl, t-butyl, n-heptyl, and isopropyl. "Cycloalkyl" refers to a fully saturated cyclic monovalent radical containing carbon and hydrogen, which may be further substituted with alkyl. Examples of cycloalkyl groups are cyclopropyl, methyl cyclopropyl, cyclobutyl, cyclopentyl, ethylcyclopentyl, and cyclohexyl. "Lower alkyl" refers to such a group having one to six carbon atoms, and in some embodiments one to four carbon atoms.

[0026] "Alkenyl" refers to an acyclic monovalent radical containing carbon and hydrogen, which may be linear or branched, and which contains at least one carbon-carbon double bond (C=C). "Alkynyl" refers to an acyclic monovalent radical containing carbon and hydrogen, which may be linear or branched, and which contains at least one carbon-carbon triple bond (C≡C). "Lower alkenyl" or "lower alkynyl" such a group having two to six carbon atoms, and in some embodiments two to four carbon atoms.

[0027] "Acyl" refers to a radical having the form -(C=O)R, where R is alkyl (alkylacyl) or aryl (arylacyl). "Acyloxy" refers to a group having the form -O(C=O)R.

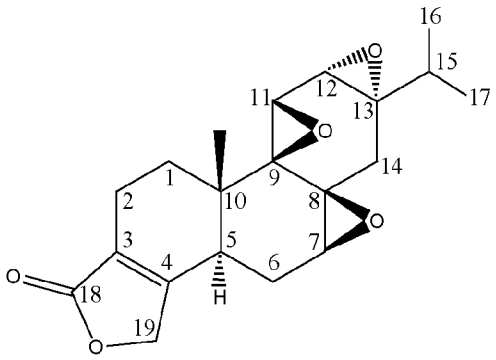
[0028] "Aryl" refers to a monovalent aromatic radical having a single ring (*e.g.*, benzene) or two condensed rings (*e.g.*, naphthyl). As used herein, aryl is a monocyclic and carbocyclic (non-heterocyclic), *e.g.* a benzene (phenyl) ring or substituted benzene ring. By "substituted" is meant that one or more ring hydrogens is replaced with a group such as a halogen (*e.g.* fluorine, chlorine, or bromine), lower alkyl, nitro, amino, lower alkylamino, hydroxy, lower alkoxy, or halo(lower alkyl).

[0029] "Arylalkyl" refers to an alkyl, often lower (C_1-C_4 , or C_1-C_2) alkyl, substituent which is further substituted with an aryl group; examples are benzyl and phenethyl.

[0030] A "heterocycle" refers to a non-aromatic ring, often a 5- to 7-membered ring, whose ring atoms are selected from the group consisting of carbon, nitrogen, oxygen and sulfur. In some embodiments, the ring atoms include 3 to 6 carbon atoms. Such heterocycles include, for example, pyrrolidine, piperidine, piperazine, and morpholine.

[0031] "Halogen" or "halo" refers to fluorine, chlorine, bromine, or iodine.

[0032] For the purposes of the current disclosure, the following numbering scheme is used for triptolide and triptolide derivatives:



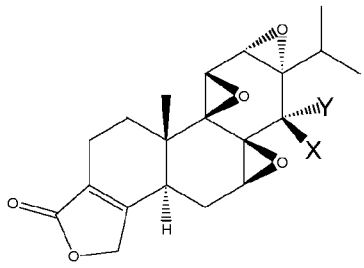
II. Triptolide Analogs

[0033] Triptolide analogs, as the term is used herein, include various structural modifications of the natural product triptolide (designated herein as PG490). They may include naturally occurring analogs, such as 2-hydroxytriptolide or 16-hydroxytriptolide (tripdiolide), although the term generally refers herein to synthetically prepared analogs. As used herein, the term "triptolide-related compounds" refers to triptolide and its analogs, and preferably refers to analogs.

[0034] Structural modifications may include, for example, ring opening of an epoxy or lactone ring of triptolide; conversion of a hydroxyl group (either naturally occurring or produced by such ring opening) to a carboxylic ester, inorganic ester (e.g. sulfonate), carbonate, or carbamate, to an aldehyde or ketone via oxidation, or to a hydrogen atom via subsequent reduction; conversion of a single bond to a double bond, and/or substitution of a hydrogen atom by a halogen, alkyl, alkenyl, hydroxyl, alkoxy, acyl, or amino group. Examples of triptolide analogs have been described in several US patents, including U.S. Patent Nos. 5,663,335, 6,150,539, 6,458,537, and 6,569,893, each of which is hereby incorporated by reference in its entirety. The compounds can be prepared, as described therein, from triptolide, a plant-derived diterpene triepoxide. Triptolide and its analogs have shown beneficial immunosuppressive and cytotoxic activity, as described, for example, in the above-referenced patents.

[0035] Exemplary triptolide analogs include 14-methyltriptolide (designated PG670; see US application pubn. no. 20040152767), triptolide 14-tert-butyl carbonate (designated PG695; see PCT Pubn. No WO 2003/101951), 14-deoxy-14α-fluoro triptolide (designated PG763; see U.S. Provisional Appn. Serial No. 60/449,976), triptolide 14-(α-dimethylamino)acetate (designated PG702; see U.S. Patent No. 5,663,335), 5-α-hydroxy triptolide (designated PG701; see U.S. Provisional Appn. Serial No. 60/532,702), 19-methyl triptolide (designated PG795; see U.S. Provisional Appn. Serial No. 60/549,769), and 18-deoxo-19-dehydro-18-benzoyloxy-19-benzoyl triptolide (designated PG796; see U.S. Provisional Appn. Serial No. 60/549,769). Each of these applications and publications is hereby incorporated by reference in its entirety.

[0036] Many of these compounds are believed to act as prodrugs, by converting *in vivo* to triptolide, as observed for PG490-88, above. Others, such as 14-deoxy-14α-fluoro triptolide (PG763), are not expected to undergo such conversion, but nonetheless exhibit biological activities shown by triptolide (e.g. cytotoxicity in human T cell lymphoma (Jurkat) cells and inhibition of IL-2), as reported in US application serial no. 60/449,976, cited above.



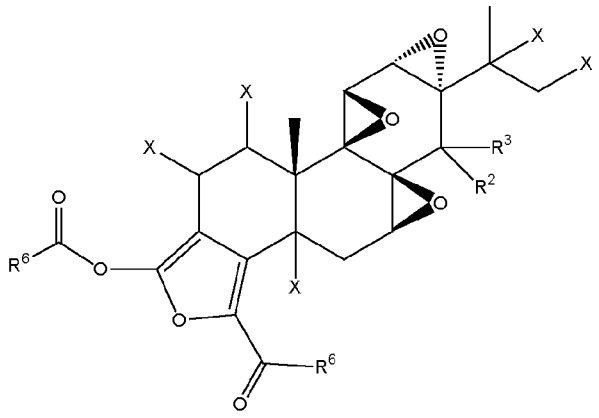
Exemplary Triptolide Derivatives and Prodrugs

Table 1

compound	X	Y
PG490-88	-O(CO)CH_2CH_2COOH	-H
PG670	-OH	-CH_3
PG695	-O(CO)OC(CH_3)$_3$	-H
PG702	-O(CO)CH_2N(CH_3)$_2$	-H
PG673	-H	-F

[0037] Triptolide analogs for screening can be generated by combinatorial chemistry or other type of preparation to generate diversity of chemical structure or substituents.

[0038] The active ingredient in the formulation is triptolide or a derivative of triptolide, as described below. For example, the disclosure provides compounds of structure **I**:



I

where

each R^6 is independently selected from alkyl, alkenyl, alkynyl, or aryl;

CR^2R^3 is CHOH or C=O;

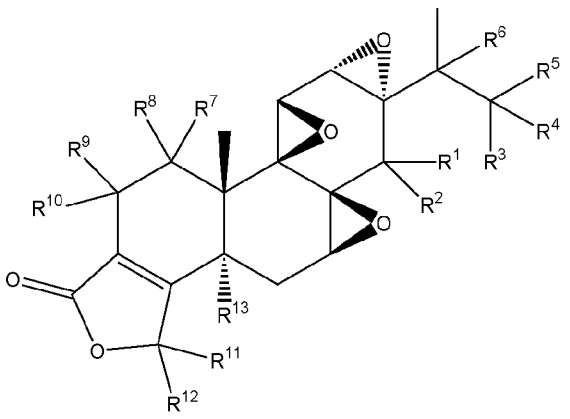
at most one of the groups X is hydroxyl, and the remaining groups X are hydrogen.

[0039] In some embodiments of structure **I**, CR^2R^3 is CHOH, often having the β-hydroxy configuration. In some embodiments, each X is hydrogen; however, in selected embodiments, exactly one of the indicated groups X is hydroxyl. Locations for hydroxyl substitution often include carbons 2 and 16, as shown in the numbering scheme above.

[0040] In some embodiments, each said alkyl, alkenyl, and alkynyl moiety present in a compound of structure **I** includes at most four carbon atoms, and each said aryl moiety is monocyclic and non-heterocyclic; *e.g.* substituted or unsubstituted phenyl.

[0041] In selected embodiments of structure **I**, each R^6 is aryl; often, each R^6 is phenyl. This includes the compound designated herein as PG796, where each R^6 is unsubstituted phenyl.

[0042] The disclosure also provides compounds represented by structure **II**:



II

where:

CR^1R^2 is selected from CHOH, C=O, CHF, CF_2 and $C(CF_3)OH$;

CR^6 and CR^{13} are selected from CH, COH and CF;

CR^7R^8, CR^9R^{10} and $CR^{11}R^{12}$ are selected from CH_2, CHOH, C=O, CHF and CF_2; and

$CR^3R^4R^5$ is selected from CH_3, CH_2OH, C=O, COOH, CH_2F, CHF_2 and CF_3;

such that: at least one of R^1-R^{13} comprises fluorine;

no more than two, and often no more than one, of $CR^3R^4R^5$, CR^6, CR^7R^8, CR^9R^{10}, $CR^{11}R^{12}$, and CR^{13} comprises fluorine or oxygen;

and, when CR^1R^2 is CHOH, $CR^3R^4R^5$ is not CH_2F.

[0043] In some embodiments, the stereochemistry at CR^7R^8 is such that, when CR^7R^8 is CHOH, it has a β-hydroxy configuration, and, when CR^7R^8 is CHF, it has an α-fluoro configuration. Similarly, the stereochemistry at CR^9R^{10} is often such that, when CR^9R^{10} is CHOH, it has a β-hydroxy configuration, and, when CR^9R^{10} is CHF, it has an α-fluoro configuration.

[0044] In some embodiments of structure **II**, CR^1R^2 is CHF, having an α-fluoro configuration.

[0045] Some embodiments also include compounds in which exactly one carbon center selected from CR^1R^2, $CR^3R^4R^5$, CR^6, CR^7R^8, CR^9R^{10}, and $CR^{11}R^{12}$ comprises fluorine. In some embodiments, exactly one of CR^1R^2, CR^6, CR^7R^8, CR^9R^{10}, and $CR^{11}R^{12}$ comprises fluorine.

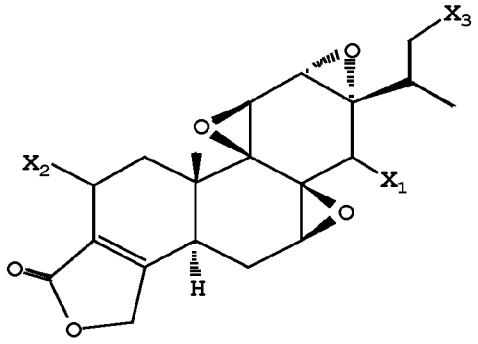
[0046] In selected embodiments, only CR^1R^2 comprises fluorine. Accordingly, in these embodiments, CR^1R^2 is selected from CF_2, CHF, and $C(CF_3)OH$. The stereochemistry at CR^1R^2 is such that, when CR^1R^2 is $C(CF_3)OH$, it has a β-hydroxy configuration, and, when CR^1R^2 is CHF, it has an α-fluoro configuration. In selected embodiments of structure **II**, the compound is PG763.

[0047] In other selected embodiments of structure **II**, either CR^9R^{10} or $CR^3R^4R^5$ comprises fluorine, and CR^1R^2 comprises oxygen; for example, CR^1R^2 is C=O or, in some embodiments, CHOH(β-hydroxy). In these embodiments, for example, CR^9R^{10} is selected from CF_2 and CHF (e.g., α-fluoro), or $CR^3R^4R^5$ is selected from CHF_2 or CF_3.

[0048] In further selected embodiments of structure **II**, either CR^7R^8 or $CR^{11}R^{12}$ comprises fluorine, and CR^1R^2 comprises oxygen; for example, CR^1R^2 is C=O or, in some embodiments,

CHOH(β-hydroxy). In these embodiments, for example, CR^7R^8 is selected from CF_2 and CHF (e.g., α-fluoro), or $CR^{11}R^{12}$ is selected from CF_2 and CHF.

[0049] The disclosure also provides compounds represented by structure **III**.



III

In the structure **III**, the variables are defined as follows:

X^1 is OH or OR^1, and X^2 and X^3 are independently OH, OR^1 or H, with the proviso that at least one of X^1, X^2 and X^3 is OR^1, and at least one of X^2 and X^3 is H; and

OR^1 is O-(C=O)-Z, where Z is selected from the group consisting of: $-OR^2$, $-O-Y-(C=O)-OR^3$, $-O-Y-NR^4R^5$, $-NR^4R^5$, $-NR^3-Y-(C=O)-OR^3$, and $-NR^3-Y-NR^4N^5$;

wherein

Y is a divalent alkyl, alkenyl or alkynyl group having up to six carbon atoms;

R^2 is selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl;

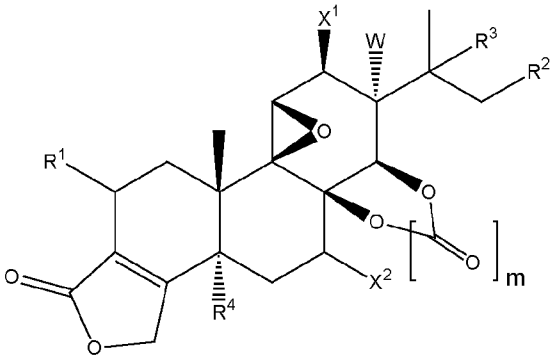
each R^3 is independently selected from hydrogen and R^2; and

R^4 and R^5 are independently selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl, or R^4 and R^5 taken together form a 5- to 7-member heterocyclic ring whose ring atoms are selected from the group consisting of carbon, nitrogen, oxygen and sulfur, wherein the ring atoms include at most 3 heteroatoms.

[0050] The groups defined as R^2, R^3, R^4, and R^5, when selected from alkyl, alkenyl, and alkynyl, can have up to six carbon atoms. When selected from cycloalkyl or cycloalkenyl, they often have 3 to 7, or, for cycloalkenyl, 5 to 7 carbon atoms. When selected from aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl, the alkyl components of these groups often have up to six carbon atoms. In one embodiment, each of these groups is independently selected from alkyl, aryl, aralkyl, and alkoxyalkyl.

[0051] In selected embodiments of structure **III**, $X^2 = X^3 = H$, and Y is $-CH_2-$ or $-CH_2CH_2-$. In further embodiments, OR^1 is selected from the group consisting of $O-(C=O)-OR^2$, $O-(C=O)-O-Y-(C=O)-OR^3$, and $O-(C=O)-O-Y-NR^4R^5$ (carbonate derivatives). In other embodiments, OR^1 is -selected from the group consisting of $O-(C=O)-NR^4R^5$, $O-(C=O)-NR^3-Y-(C=O)-OR^3$, and $O-(C=O)-NR^3-Y-NR^4N^5$ (carbamate derivatives). In selected embodiments of structure **III**, the compound is PG695.

[0052] The disclosure also provides compounds represented by structure **IV**.



IV

where

each of R^1, R^2, R^3, and R^4 is independently selected from hydrogen, hydroxyl, $-O(CO)_nX$, $-O(CO)_nOR^5$, and $-O(CO)_nN(R^5)_2$, where X is halogen, R^5 is hydrogen or lower alkyl, and n is 1-2,

with the proviso that at least three of R^1, R^2, R^3, and R^4 are hydrogen;

m is 1-2;

X^2 is halogen, such as F or Cl; and

X^1 is halogen, often Cl, and W is hydroxyl; or X^1 and W together form an epoxy group.

[0053] When any of each of R^1, R^2, R^3, and R^4 is selected from $-O(CO)_nX$, $-O(CO)_nOR^5$, or $-O(CO)_nN(R^5)_2$, the variable n is often 1.

[0054] In selected embodiments of structure **IV**, each of R^1, R^2, R^3, and R^4 is hydrogen. In further selected embodiments, m = 1. In selected embodiments of structure IV, the compound is PG762.

III. Biological Activity

[0055] The cytotoxic activity of a compound according to structure **I**, 18-deoxo-19-dehydro-18-benzoyloxy-19-benzoyl triptolide (designated PG796), can be evaluated using a standard MTT assay, as described in Example 3 and the immunosuppressive activity of these

compounds was evaluated in a standard IL-2 inhibition assay, as described in Example 4. PG796 showed a higher level of activity in both assays than the known prodrug, triptolide 14-succinate (designated PG490-88). In particular, triptolide 14-succinate incubated in human serum was much less active in these assays than triptolide 14-succinate incubated in mouse serum, while PG796 showed high, and essentially equivalent, activity in both cases. (Incubation is expected to convert triptolide 14-succinate to triptolide and PG796 to the monoderivatized compound, 19-benzoyl triptolide, shown in the above synthetic scheme.)

[0056] The cytotoxic activity of three compounds of structure **IV**, designated PG757, PG762 and PG830, and one additional compound designated PG782, can be evaluated using a standard MTT assay as described in Example 2. The immunosuppressive activity of these compounds was evaluated in a standard IL-2 inhibition assay as described in Example 3.

[0057] The compound PG757 incubated in serum was significantly more cytotoxic in the MTT assay than triptolide; see Table 2 below. (The data for test compounds in Table 2 is for compounds incubated in serum for 24 hrs.) Incubated PG782 was also more potent than triptolide, and incubated PG762 was of comparable potency. Several test compounds, when incubated in serum, were comparable to triptolide in suppression of IL-2.

Table 2

Compound	Viability/Cytotoxicity MTT (ED_{50})	Immunosuppression IL-2 (IC_{50})
PG490 (triptolide)	60 nM	4 nM
PG757	32 nM	9 nM
PG762	60 nM	9 nM
PG782	53 nM	2 nM

[0058] Incubation in serum converts prodrug compounds to triptolide, and this has been shown to happen within about 5 minutes for PG757 and PG762.

IV. Therapeutic Compositions

[0059] Formulations containing the triptolide derivatives of the disclosure may take the form of solid, semi-solid, lyophilized powder, or liquid dosage forms, such as tablets, capsules, powders, sustained-release formulations, solutions, suspensions, emulsions, ointments, lotions, or aerosols, and in some embodiments in unit dosage forms suitable for simple administration of precise dosages. The compositions typically include a conventional

pharmaceutical carrier or excipient and may additionally include other medicinal agents, carriers, or adjuvants.

[0060] In some embodiments, the composition will be about 0.5% to 75% by weight of a compound or compounds of the disclosure, with the remainder consisting of suitable pharmaceutical excipients. For oral administration, such excipients include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatin, sucrose, magnesium carbonate, and the like. If desired, the composition may also contain minor amounts of non-toxic auxiliary substances such as wetting agents, emulsifying agents, or buffers.

[0061] The composition may be administered to a subject orally, transdermally or parenterally, *e.g.*, by intravenous, subcutaneous, intraperitoneal, or intramuscular injection. For use in oral liquid preparation, the composition may be prepared as a solution, suspension, emulsion, or syrup, being supplied either in liquid form or a dried form suitable for hydration in water or normal saline. For parenteral administration, an injectable composition for parenteral administration will typically contain the triptolide derivative in a suitable intravenous solution, such as sterile physiological salt solution.

[0062] Liquid compositions can be prepared by dissolving or dispersing the triptolide derivative (about 0.5% to about 20%) and optional pharmaceutical adjuvants in a pharmaceutically acceptable carrier, such as, for example, aqueous saline, aqueous dextrose, glycerol, or ethanol, to form a solution or suspension.

[0063] The compound may also be administered by inhalation, in the form of aerosol particles, either solid or liquid, often of respirable size. Such particles are sufficiently small to pass through the mouth and larynx upon inhalation and into the bronchi and alveoli of the lungs. In general, particles ranging from about 1 to 10 microns in size, and often less than about 5 microns in size, are respirable. Liquid compositions for inhalation comprise the active agent dispersed in an aqueous carrier, such as sterile pyrogen free saline solution or sterile pyrogen free water. If desired, the composition may be mixed with a propellant to assist in spraying the composition and forming an aerosol.

[0064] Methods for preparing such dosage forms are known or will be apparent to those skilled in the art; for example, see Remington's Pharmaceutical Sciences (19th Ed., Williams & Wilkins, 1995). The composition to be administered will contain a quantity of the selected compound in an effective amount for effecting immunosuppression in a subject or apoptosis in a targeted cell.

[0065] As described, for example, in Panchagnula *et al.* (2000), the partition coefficient or logP of a pharmaceutical agent can affect its suitability for various routes of administration, including oral bioavailability. The compounds described herein, by virtue of substitution of fluorine for one or more hydroxyl groups, are expected to have higher calculated logP values than the parent compound, triptolide, making them better candidates for oral availability.

[0066] The lipid formulations disclosed herein are useful for intravenous administration, as well as for oral administration. Lipid and surfactant based formulations are well recognized as a feasible approach to improve oral bioavailability of poorly soluble compounds. Several drug products utilizing lipid and surfactant based formulations and intended for oral administration are commercially available. For example, Sandimmune® and Sandimmune, Neoral® (cyclosporin A, Novartis), Norvir® (ritonavir), and Fortovase® (saquinavir) have been formulated in self-emulsifying drug delivery systems. Indeed, a recent review summarizes published pharmacokinetic studies of orally administered lipid based formulations of poorly aqueous soluble drugs in human subjects. (Fatouros, *et al.*, (2007) Therapeutics and Clinical Risk Management 3(4):591-604).

V. Immunomodulating and Antiinflammatory Treatment

[0067] A compound according to structure **I**, 18-deoxo-19-dehydro-18-benzoyloxy-19-benzoyl triptolide (designated PG796), inhibited IL-2 production in Jurkat cells (see Example 3) in a dose-dependent manner. The disclosure thus includes the use of the formulations containing an active ingredient(s) as immunosuppressive agents, *e.g.* as an adjunct to transplant procedures or in treatment of autoimmune disease.

[0068] One utility envisioned for this disclosure is for the treatment of human diseases of the immune system regulatory abnormalities. Immunoregulatory abnormalities have been shown to exist in a wide variety of autoimmune and chronic inflammatory diseases, including systemic lupus erythematosis, chronic rheumatoid arthritis, type I and II diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, pemphigus, bullous pemphigoid, sarcoidosis, psoriasis, ichthyosis, Graves ophthalmopathy, Grave's disease and asthma. Although the underlying pathogenesis of each of these conditions may be quite different, they have in common the appearance of a variety of autoantibodies and self-reactive lymphocytes. Such self-reactivity may be due, in part, to a loss of the homeostatic controls under which the normal immune system operates.

[0069] Similarly, following a bone-marrow transplant or other transplant of hematopoietic stem cells from a donor tissue source containing mature lymphocytes, the transferred lymphocytes recognize the host tissue antigens as foreign. These cells become activated and mount an attack upon the host (a graft-versus-host response) that can be life-threatening. Moreover, following an organ transplant, the host lymphocytes recognize the foreign tissue antigens of the organ graft and mount cellular and antibody-mediated immune responses (a host-versus-graft response) that lead to graft damage and rejection.

[0070] One result of an autoimmune or a rejection reaction is tissue destruction caused by inflammatory cells and the mediators they release. Anti-inflammatory agents such as NSAIDs act principally by blocking the effect or secretion of these mediators but do nothing to modify the immunologic basis of the disease. On the other hand, cytotoxic agents, such as cyclophosphamide, act in such a nonspecific fashion that both the normal and autoimmune responses are shut off. Indeed, patients treated with such nonspecific immunosuppressive agents are as likely to succumb from infection as they are from their autoimmune disease.

[0071] The compositions of the present disclosure are useful in applications for which triptolide and its prodrugs and other derivatives have proven effective, *e.g.* in immunosuppression therapy, as in treating an autoimmune disease, preventing transplantation rejection, or treating or preventing graft-versus-host disease (GVHD). See, for example, co-owned U.S. Patent No. 6,150,539, which is incorporated herein by reference. Triptolide and the present derivatives are also useful for treatment of other inflammatory conditions, such as traumatic inflammation, and in reducing male fertility.

[0072] The compositions are useful for inhibiting rejection of a solid organ transplant, tissue graft, or cellular transplant from an incompatible human donor, thus prolonging survival and function of the transplant, and survival of the recipient. This use would include, but not be limited to, solid organ transplants (such as heart, lung, pancreas, limb, muscle, nerve, kidney and liver), tissue grafts (such as skin, corneal, intestinal, gonadal, bone, and cartilage), and cellular transplants (*e.g.*, cells from pancreas such as pancreatic–islet cells, brain and nervous tissue, muscle, skin, bone, cartilage and liver) including xenotransplants, etc.

[0073] The compositions are also useful for inhibiting xenograft (interspecies) rejection; *i.e.* in preventing the rejection of a solid organ transplant, tissue graft, or cellular transplant from a non-human animal, whether natural in constitution or bioengineered (genetically manipulated) to express human genes, RNA, proteins, peptides or other non-native, xenogeneic molecules, or bioengineered to lack expression of the animal's natural genes, RNA, proteins, peptides or other normally expressed molecules. The disclosure also includes

the use of a composition as described above to prolong the survival of such a solid organ transplant, tissue graft, or cellular transplant from a non-human animal.

[0074] Also included are methods of treatment of autoimmune diseases or diseases having autoimmune manifestations, such as Addison's disease, autoimmune hemolytic anemia, autoimmune thyroiditis, Crohn's disease, diabetes (Type I, juvenile-onset or recent-onset diabetes mellitus), Graves' disease, Guillain-Barre syndrome, systemic lupus erythematosis (SLE), lupus nephritis, multiple sclerosis, myasthenia gravis, psoriasis, primary biliary cirrhosis, rheumatoid arthritis, uveitis, asthma, atherosclerosis, Hashimoto's thyroiditis, allergic encephalomyelitis, glomerulonephritis, and various allergies.

[0075] Further uses may include the treatment and prophylaxis of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically mediated illnesses, such as psoriasis, atopic dermatitis, pemphigus, urticaria, cutaneous eosinophilias, acne, and alopecia areata; various eye diseases such as conjunctivitis, uveitis, keratitis, and sarcoidosis; inflammation of mucous and blood vessels such as gastric ulcers, vascular damage caused by ischemic diseases and thrombosis, ischemic bowel diseases, inflammatory bowel diseases, and necrotizing enterocolitis; intestinal inflammations/allergies such as Coeliac diseases, Crohn's disease and ulcerative colitis; renal diseases such as interstitial nephritis, Good-pasture's syndrome, hemolytic-uremic syndrome and diabetic nephropathy; hematopoietic diseases such as idiopathic thrombocytopenia purpura and autoimmune hemolytic anemia; skin diseases such as dermatomyositis and cutaneous T cell lymphoma; circulatory diseases such as arteriosclerosis and atherosclerosis; nephrotic syndrome such as glomerulonephritis; renal diseases such as ischemic acute renal insufficiency and chronic renal insufficiency; and Behcet's disease.

[0076] The compositions and method of the disclosure are also useful for the treatment of inflammatory conditions such as asthma, both intrinsic and extrinsic manifestations, for example, bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma, particularly chronic or inveterate asthma (for example, late asthma and airway hyperresponsiveness), or other lung diseases including allergies and reversible obstructive airway disease, including bronchitis and the like. The composition and method may also be used for treatment of other inflammatory conditions, including traumatic inflammation, inflammation in Lyme disease, chronic bronchitis (chronic infective lung disease), chronic sinusitis, sepsis associated acute respiratory distress syndrome, and pulmonary sarcoidosis. For treatment of respiratory conditions such as asthma, the composition is often administered via inhalation, but any conventional route of administration may be useful.

[0077] In treating an autoimmune condition, the patient is given the composition on a periodic basis, *e.g.*, 1-2 times per week, at a dosage level sufficient to reduce symptoms and improve patient comfort. For treating rheumatoid arthritis, in particular, the composition may be administered by intravenous injection or by direct injection into the affected joint. The patient may be treated at repeated intervals of at least 24 hours, over a several week period following the onset of symptoms of the disease in the patient. The dose that is administered is often in the range of 1-25 mg/kg patient body weight per day, often in lower amounts for parenteral administration, and higher amounts for oral administration. Optimum dosages can be determined by routine experimentation according to methods known in the art.

[0078] For therapy in transplantation rejection, the method is intended particularly for the treatment of rejection of heart, kidney, liver, cellular, and bone marrow transplants, and may also be used in the treatment of GVHD. The treatment is typically initiated perioperatively, either soon before or soon after the surgical transplantation procedure, and is continued on a daily dosing regimen, for a period of at least several weeks, for treatment of acute transplantation rejection. During the treatment period, the patient may be tested periodically for immunosuppression level, *e.g.*, by a mixed lymphocyte reaction involving allogeneic lymphocytes, or by taking a biopsy of the transplanted tissue.

[0079] In addition, the composition may be administered chronically to prevent graft rejection, or in treating acute episodes of late graft rejection. As above, the dose administered is often 1-25 mg/kg patient body weight per day, with lower amounts for parenteral administration, and higher amounts for oral administration. The dose may be increased or decreased appropriately, depending on the response of the patient, and over the period of treatment, the ability of the patient to resist infection.

[0080] In treatment or prevention of graft-versus-host disease, resulting from transplantation into a recipient of matched or mismatched bone marrow, spleen cells, fetal tissue, cord blood, or mobilized or otherwise harvested stem cells, the dose is often in the range 0.25-2 mg/kg body weight/day, often 0.5-1 mg/kg/day, given orally or parenterally.

[0081] Also within the scope of the disclosure is a combination therapy comprising a compound of this disclosure and one or more conventional immunosuppressive agents. These immunosuppressant agents within the scope of this disclosure include, but are not limited to, Imurek® (azathioprine sodium), brequinar sodium, Spanidin™ (gusperimus trihydrochloride, also known as deoxyspergualin), mizoribine (also known as bredinin), Cellcept® (mycophenolate mofetil), Neoral® (Cyclosporin A; also marketed as a different formulation

under the trademark Sandimmune®), Prograf™ (tacrolimus, also known as FK-506), Rapimmune® (sirolimus, also known as rapamycin), leflunomide (also known as HWA-486), Zenapax®, glucocortcoids, such as prednisolone and its derivatives, antibodies such as orthoclone (OKT3), and antithymyocyte globulins, such as thymoglobulins. The compounds are useful as potentiators when administered concurrently with another immunosuppressive drug for immunosuppressive treatments as discussed above. A conventional immunosuppressant drug, such as those above, may thus be administered in an amount substantially less (*e.g.* 20% to 50% of the standard dose) than when the compound is administered alone. Alternatively, the disclosed formulation is administered in amounts such that the resultant immunosuppression is greater than what would be expected or obtained from the sum of the effects obtained with the drug and disclosed compound used alone. Typically, the immunosuppressive drug and potentiator are administered at regular intervals over a time period of at least 2 weeks.

[0082] The compositions of the disclosure may also be administered in combination with a conventional anti-inflammatory drug (or drugs), where the drug or amount of drug administered is, by itself, ineffective to induce the appropriate suppression or inhibition of inflammation.

[0083] Immunosuppressive activity of compounds *in vivo* can be evaluated by the use of established animal models known in the art. Such assays may be used to evaluate the relative effectiveness of immunosuppressive compounds and to estimate appropriate dosages for immunosuppressive treatment. These assays include, for example, a well-characterized rat model system for allografts, described by Ono and Lindsey (1969), in which a transplanted heart is attached to the abdominal great vessels of an allogeneic recipient animal, and the viability of the transplanted heart is gauged by the heart's ability to beat in the recipient animal. A xenograft model, in which the recipient animals are of a different species, is described by Wang (1991) and Murase (1993). A model for evaluating effectiveness against GVHD involves injection of normal F1 mice with parental spleen cells; the mice develop a GVHD syndrome characterized by splenomegaly and immunosuppression (Korngold, 1978; Gleichmann, 1984). Single cell suspensions are prepared from individual spleens, and microwell cultures are established in the presence and absence of concanavalin A to assess the extent of mitogenic responsiveness.

VI. Anticancer Treatment

[0084] The following disease states have been shown to be amenable to treatment with triptolide and its prodrugs and other analogs. Such disease states are target areas for treatment with second-generation triptolide analogs. Triptolide analogs and/or prodrug compounds also may be used in combination with conventional therapeutic agents.

[0085] As used herein, "cancer" refers to all types of cancer or neoplasm or malignant tumors found in mammals especially humans, including leukemias, sarcomas, carcinomas and melanoma. Examples of cancers are cancer of the brain, breast, cervix, colon, head and neck, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus and medulloblastoma. The term "leukemia" refers broadly to progressive, malignant diseases of the blood-forming organs and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. The term "sarcoma" generally refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. The term "melanoma" is taken to mean a tumor arising from the melanocytic system of the skin and other organs. The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases.

[0086] Included, for example, are cancers involving cells derived from reproductive tissue (such as Sertoli cells, germ cells, developing or more mature spermatogonia, spermatids or spermatocytes and nurse cells, germ cells and other cells of the ovary), the lymphoid or immune systems (such as Hodgkin's disease and non-Hodgkin's lymphomas), the hematopoietic system, and epithelium (such as skin, including malignant melanoma, and gastrointestinal tract), solid organs, the nervous system, *e.g.* glioma (see Y.X. Zhou *et al.*, 2002), and musculoskeletal tissue. The compounds may be used for treatment of various cancers, including, but not limited to, cancers of the brain, head and neck, lung, thyroid, breast, colon, ovary, cervix, uterus, testicle, bladder, prostate, liver, kidney, pancreas, esophagus and/or stomach. Treatment of breast, colon, lung, and prostate tumors is particularly contemplated. Treatment is targeted to slowing the growth of tumors, preventing tumor growth, inducing partial regression of tumors, and inducing complete regression of tumors, to the point of complete disappearance, as well as preventing the outgrowth of metastases derived from solid tumors. Additional cancers which can be treated with compounds according to the disclosure include, for example, multiple myeloma, medulloblastoma, lymphoma, neuroblastoma, melanoma, premalignant skin lesions,

rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, non-small cell lung, large cell lung, primary brain tumors, endometrial cancer, malignant pancreatic insulinoma, malignant carcinoid, malignant hypercalcemia, and adrenal cortical cancer.

[0087] The compositions may be administered to a patient afflicted with cancer and/or leukemia by any conventional route of administration, as discussed above. The method is useful to slow the growth of tumors, prevent tumor growth, induce partial regression of tumors, and induce complete regression of tumors, to the point of complete disappearance. The method is also useful in preventing the outgrowth of metastases derived from solid tumors.

[0088] The compositions of the disclosure may be administered as sole therapy or with other supportive or therapeutic treatments not designed to have anti-cancer effects in the subject. The method also includes administering the disclosure compositions in combination with one or more conventional anti-cancer drugs or biologic protein agents, where the amount of drug(s) or agent(s) is, by itself, ineffective to induce the appropriate suppression of cancer growth, in an amount effective to have the desired anti-cancer effects in the subject. Such anti-cancer drugs include actinomycin D, camptothecin, carboplatin, cisplatin, cyclophosphamide, cytosine arabinoside, daunorubicin, doxorubicin, etoposide, fludarabine, 5-fluorouracil, hydroxyurea, gemcitabine, irinotecan, methotrexate, mitomycin C, mitoxantrone, paclitaxel, taxotere, teniposide, topotecan, vinblastine, vincristine, vindesine, and vinorelbine. Anti-cancer biologic protein agents include tumor necrosis factor (TNF), TNF-related apoptosis inducing ligand (TRAIL), other TNF-related or TRAIL-related ligands and factors, interferon, interleukin-2, other interleukins, other cytokines, chemokines, and factors, antibodies to tumor-related molecules or receptors (such as anti-HER2 antibody), and agents that react with or bind to these agents (such as members of the TNF super family of receptors, other receptors, receptor antagonists, and antibodies with specificity for these agents).

[0089] Antitumor activity *in vivo* of a particular composition can be evaluated by the use of established animal models, as described, for example, in Fidler *et al.*, U.S. Patent No. 6,620,843. Clinical doses and regimens are determined in accordance with methods known to clinicians, based on factors such as severity of disease and overall condition of the patient.

[0090] A compound of structure **I**, 18-deoxo-19-dehydro-18-benzoyloxy-19-benzoyl triptolide (designated PG796), was cytotoxic to Jurkat cells (according to Example 2) in a

dose-dependent manner. Thus, the present disclosure includes the use of the disclosed compounds as cytotoxic agents, particularly to treat cancers.

VII. Other Indications

[0091] The compounds of the present disclosure may also be used in the treatment of certain CNS diseases. Glutamate fulfills numerous physiological functions, but also plays an important role in the pathophysiology of different neurological and psychiatric diseases. Glutamate excitotoxicity and neurotoxicity have been implicated in hypoxia, ischemia and trauma, as well as in chronic neurodegenerative or neurometabolic diseases, Alzheimer's dementia, Huntington's disease and Parkinson's disease. In view of the reported neuroprotective effects of triptolide, particularly protection from glutamate-induced cell death (Q. He *et al.*, 2003; X. Wang *et al.*, 2003), compounds of the disclosure are envisioned to antagonize the neurotoxic action of glutamates and thus may be a novel therapy for such diseases.

[0092] Recent evidence from MS patients in relapse suggests an altered glutamate homeostasis in the brain of patients with MS. Neurotoxic events occur in MS, and they can be responsible for oligodendrocyte and neuronal cell death in patients with this demyelinating disease. Antagonizing glutamate receptor-mediated excitotoxicity by treatment with compounds of this disclosure may have therapeutic implications in MS patients. Other nervous system diseases such as, Guillain-Barre syndrome, Meniere's disease, polyneuritis, multiple neuritis, mononeuritis and radiculopathy may be treated with the compounds of the present disclosure.

[0093] The compounds of the present disclosure may also be used in the treatment of organ fibrosis, including certain lung diseases. Idiopathic pulmonary fibrosis (PF) is a progressive interstitial lung disease with no known etiology. PF is characterized by excessive deposition of intracellular matrix and collagen in the lung interstitium and gradual replacement of the alveoli by scar tissue as a result of inflammation and fibrosis. As the disease progresses, the increase in scar tissue interferes with the ability to transfer oxygen from the lungs to the bloodstream. A 14-succinimide ester of triptolide has been reported to block bleomycin-induced PF (G. Krishna *et al.*, 2001). Accordingly, the compounds and formulations of the present disclosure may be useful for treatment of PF. Treatment of other respiratory diseases, such as sarcoidosis, fibroid lung, and idiopathic interstitial pneumonia is also considered.

[0094] Other diseases involving the lung and envisioned to be treatable by compounds of this disclosure include Severe Acute Respiratory Syndrome (SARS) and acute respiratory distress syndrome (ARDS). In particular, with respect to SARS, the reduction of virus content (SARS-CoV) before the peak of the disease process and the usefulness of corticosteroid treatment, as noted below, suggest that the development of the most severe, life-threatening effects of SARS may result from the exaggerated response of the body to the infection (immune hyperactivity) rather than effects of the virus itself. (See also copending and co-owned US provisional application Serial No. 60/483,335, incorporated herein by reference.) Corticosteroid treatment has been used in SARS patients to suppress the massive release of cytokines that may characterize the immune hyperactive phase, in the hope that it will stop the progression of pulmonary disease in the next phase. Corticosteroid treatment has produced good clinical results in reduction of some of the major symptoms of SARS. However, there are several treatment-related side effects, and there is a clear need for more selective immunosuppressive and/or antiinflammatory agents.

[0095] Triptolide-related compounds may also be used in the treatment of certain CNS diseases. Glutamate fulfills numerous physiological functions, including an important role in the pathophysiology of various neurological and psychiatric diseases. Glutamate excitotoxicity and neurotoxicity have been implicated in hypoxia, ischemia and trauma, as well as in chronic neurodegenerative or neurometabolic diseases, Alzheimer's disease (AD), Huntington's disease and Parkinson's disease. In view of the reported neuroprotective effects of triptolide, particularly protection from glutamate-induced cell death (He *et al.*, 2003; Wang *et al.*, 2002a), compounds of the disclosure are envisioned to antagonize the neurotoxic action of glutamates and thus may be a novel therapy for such diseases.

[0096] Cerebral amyloid angiopathy is one of the pathological features of AD, and PC12 cells are extremely sensitive to induction of neurotoxicity by mutant β-amyloid protein aggregates. PC12 cells treated with β-amyloid exhibit increased accumulation of intracellular ROS and undergo apoptotic death (Gu *et al.*, 2004). Beta-amyloid treatment induces NF-κB activation in PC12 cells, and increases the intracellular Ca^{2+} level. Triptolide has been shown to markedly inhibit β-amyloid-induced apoptosis to inhibit the increase of intracellular Ca^{2+} concentration induced by β-amyloid. Accordingly, triptolide-related compounds may be effective to prevent the apoptosis cascade induced by β-amyloid and preserve neuronal survival in AD patients.

[0097] Triptolide exerts a powerful inhibitory influence over lipopolysaccharide (LPS)-activated microglial activity by reducing nitrite accumulation, TNF-α and IL-1β release, and induction of mRNA expression of these inflammatory factors (Zhou *et al.*, 2003). Triptolide also attenuates the LPS-induced decrease in ^{3}H-dopamine uptake and loss of tyrosine hydroxylase-positive neurons in primary mesencephalic neuron/glia mixed culture (Li *et al.*, 2004). Triptolide appeared to exert a neurotrophic effect without LPS. Triptolide also blocked LPS-induced activation of microglia and excessive production of TNF-α and nitrite. Triptolide may protect dopaminergic neurons from LPS-induced injury by inhibiting microglia activation, which is relevant to Parkinson 's disease, further illustrating the neuroprotective potential of triptolide-related compounds.

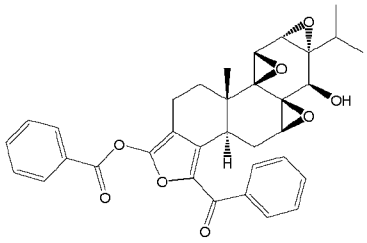
[0098] Tripchlorolide, which has been shown to be a prodrug of triptolide, promotes dopaminergic neuron axonal elongation in primary cultured rat mesencephalic neurons and protects dopaminergic neurons from a neurotoxic lesion induced by 1-methyl-4-phenylpyridinium ion (Li *et al.*, 2003). Tripchlorolide stimulates brain-derived neurotrophic factor mRNA expression as revealed by *in situ* hybridization. Furthermore, in an *in vivo* rat model of PD in which FK506 shows neurotrophic activity, administration of tripchlorolide at 0.5-1 μg/kg improves recovery of rats undergoing neurosurgery, produces significant sparing of SN neurons and preservation of the dendritic processes surrounding tyrosine hydroxylase positive neurons, attenuates dopamine depletion, increases the survival of dopaminergic neurons and attenuates the elevation of TNF-α and IL-2 levels in the brain (Cheng *et al.*, 2002). Moreover, tripchlorolide demonstrates neurotrophic activity at a concentration lower than needed for neuroprotective and immunosuppressive activity.

[0099] Recent evidence from MS patients in relapse suggests an altered glutamate homeostasis in the brain. Neurotoxic events occurring in MS patients can be responsible for oligodendrocyte and neuronal cell death. Antagonizing glutamate receptor-mediated excitotoxicity by treatment with triptolide-related compounds may have therapeutic implications in MS patients. Other CNS diseases such as Guillain-Barre syndrome, Meniere's disease, polyneuritis, multiple neuritis, mononeuritis and radiculopathy may also be treated with triptolide-related compounds.

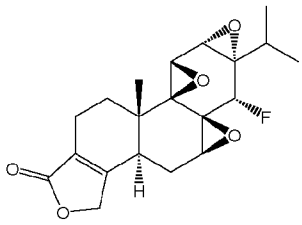
VIII. Active formulations

[0100] The active ingredient can be PG796, PG763, PG762 or PG695, related structures, or any triptolide derivative with a clogP of greater than 0.5 (See Table 3, below).

[0101] The chemical structures of exemplary triptolide analogs are shown below:



PG796



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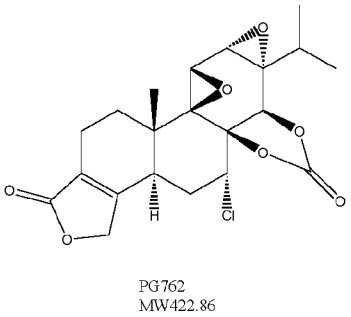
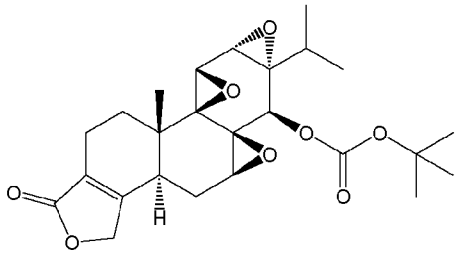


PG762



PG695

[0102] As is known to skilled artisans in the chemical and pharmaceutical sciences, a partition-coefficient or distribution-coefficient is the ratio of concentrations of a compound in a mixture of two immiscible phases at equilibrium. These coefficients are a measure of the difference in solubility of the compound in these two phases. Typically, one of the solvents in the mixture is water while the second is hydrophobic such as octanol. Thus, the partition-coefficient is a measure of how hydrophilic ("water-loving") or hydrophobic ("water-fearing") a chemical substance is. In medical practice, partition coefficients are useful for example in estimating distribution of drugs within the body. Hydrophobic drugs with high octanol/water partition coefficients are preferentially distributed to hydrophobic compartments such as lipid bilayers of cells while hydrophilic drugs (low octanol/water partition coefficients) preferentially are found in hydrophilic compartments such as blood serum. Thus, a formulation can be characterized by its solubility in both water and fat, as an orally administered drug needs to pass through the intestinal lining after it is consumed, carried in aqueous blood and penetrate the lipid cellular membrane to reach the inside of a cell. A model compound for the lipophilic cellular membrane is octanol (a lipophilic hydrocarbon), so the logarithm of the octanol/water partition coefficient, known as “LogP,” is used to predict the solubility of a potential oral drug. This coefficient can be experimentally measured or predicted computationally, in which case it is sometimes called a "calculated partition coefficient” or “cLogP."

[0103] Table 3 cLogP of triptolide and triptolide analogs/derivatives

		cLogP	
Compound	**Chemical Class**	**Method A**	**Method B**
triptolide		-0.08	0.27
PG796 (MRx102)	lactone	3.68	4.11
PG763 (MRx103)	halogens	0.63	0.87
PG762 (MRx104)	c-ring	1.60	1.89
PG490-88 (MRx108)	esters	-0.18	0.19
PG695 (MRx109)	carbonates	1.61	1.85

Method A - Crippen's fragmentation: J.Chem.Inf.Comput.Sci.,27,21(1987)
Method B - Viswanadhan's fragmentation: J.Chem.Inf.Comput.Sci.,29,163(1989)

[0104] From a survey of the literature, it is possible to obtain some general guidelines about the optimum Log P values for certain classes of drugs. (*See* A guide to Log P and pKa measurements and their use by Mark Earll www.raell.demon.co.uk/chem/logp/logppka.html). In general, assuming passive absorption,

- Optimum CNS penetration approximately Log P = 2 +/- 0.7 (Hansch)
- Optimum Oral absorption approximately Log P = 1.8
- Optimum Intestinal absorption Log P =1.35
- Optimum Colonic absorption LogP = 1.32
- Optimum Sublingual absorption Log P = 5.5
- Optimum Percutaneous Log P = 2.6 (& low mw)

[0105] Formulation and dosing forms:

- Low Log P (below 0) Injectable
- Medium (0-3) Oral
- High (3-4) Transdermal
- Very High (4-7) Toxic build up in fatty tissues

[0106] Overall, triptolide compounds having a cLogP of 0.5 or higher are believed not to be amenable to formulations meant for injection. For example, of the compounds in Table 3, compounds PG796, PG763, PG762 or PG695 were generally predicted by skilled artisans to not have a workable cLogP for injectable intravenous administration. Unexpectedly, however, an effective injectible formulation for compounds having a cLogP of 0.5 or higher (such as, for example, PG796, PG763, PG762 or PG695) has been designed and is identified hereinbelow.

Examples

[0107] The following examples are illustrative in nature and are in no way intended to be limiting.

EXAMPLE 1

Emulsion Preparation

[0108] Emulsion components include glyceryl trioctanoate (g) 20%; Soybean oil (g) 20%; Phospholipids ([60%] L-α-phosphatidylcholine, L-lecithin, Sigma 61755) (g) 2%; Sodium cholate (g) 0.2%; Glycerin (g) 2.5%; Water (ml) 55%

Emulsion preparation with PG796(MRx102)

1. Weigh glyceryl trioctanoate, soybean oil, and phospholipids (L-lecithin) into a 15 mL conical plastic centrifuge tube or a suitable test tube (*e.g.*, plastic to avoid breakage).
2. Place the tube over the bottom of the sonicator probe such that the sonicator tip is about 5 mm from the bottom of the tube and the probe is not in contact with the sides of the tube. Clamp it in place. Do not use a cold water bath at this stage.
3. Set the sonicator to a power level a little below the microtip limit and at a duty cycle of 50%. Turn the sonicator on for 20 seconds.
4. Feel the tube to assess its temperature and observe the contents carefully to determine whether the phospholipids are dispersing. Sonicators are very efficient at generating shear energy and cavitation, but are not efficient mixers, so it might be necessary to unclamp the tube and use the probe as a stirrer to break up the phospholipids.
5. In order to disperse the phospholipids, the fluid should be allowed to warm to 40°C - 50°C. Continue sonicating for short intervals until the fluid is warm, but not hot to the touch. Once the fluid has warmed up, suspend the tube in a beaker of warm water and continue sonicating for five minutes or until full dispersion of the phospholipids has been obtained, whichever is longer.
6. Weigh and add PG796(MRx102) in the fluid that is about 20°C - 25°C. Sonicate the solution for short intervals (each about 20 seconds) until the dissolution of the PG796(MRx102) has been obtained. After each interval sonicating, suspend the tube in a beaker of water (about 15°C - 20°C) to cool down the temperature to make sure the temperature is lower than to 40 - 45°C. It may take about 10 interval sonicatings to dissolve PG796(MRx102) completely.
7. Measure/weigh the water and sodium cholate into a beaker and dissolve the sodium cholate. Add and dissolve the glycerin into the sodium cholate solution.
8. Suspend the phospholipid/oil/PG796(MRx102) tube in a cold water bath and add about 1/3 of the water/sodium cholate/glycerin mixture, sonicate for 1 minute with the tube in the cold water bath by adjusting the sonicator to a power level a little below the microtip limit (about 4.9).
9. Add the second third of the water/sodium cholate/glycerin mixture and repeat sonication for 1 minute. Add the last of the water/sodium cholate/glycerin mixture and sonicate for another 1 minute. Sonicate further if the water/sodium cholate/glycerin mixture is not completely dissolved in the emulsion.
10. Remove the tube from the sonication probe and check the pH (around 7.6 for this formulation). Carefully adjust the pH to be in the range of 7.5 to 8.5 using 0.1N sodium hydroxide if necessary. A pH closer to 7.5 is suitable physiologically for dosing animals.
11. Place the tube back on the sonication probe in the cold water bath and sonicate for 8 minutes continuously.
12. Note that the emulsion should be opaque white, similar to thick cream.
13. Filter the emulsion through a 0.45 μm membrane filter (Polyethersulfone 0.45 μm Pore Size filter, such as Millipore Millex-HP Syringe Filter Unit SLHPM33RS, Radio-Sterilized). The emulsion preferably appears unchanged.
14. Introduce the emulsion containing PG796(MRx102) into test subject for appropriate studies.

[0109] Components for preparation of 5 ml of emulsion with PG796(MRx102)

Components with PG796(MRx102)	Amount
Glyceryl trioctanoate (g)	1
Soybean oil (g)	1
Phospholipids (g)	0.1
Glycerin (g)	0.125
Sodium cholate (g)	0.01
PG796(MRx102) (mg)	5
Water (ml)	2.77

[0110] Component (Excipient) Range

Components↓ Formulation →	Range	E-0212-4
Glyceryl trioctanoate	0%-50%	20%
Soybean oil	0%- 45%	20%
Phospholipids	1%-3%	2%
Glycerin	1%-5%	3%
Sodium cholate	0.1%-0.3%	0.2%
Water	50%-60%	55%

[0111] Alternative Components (Excipients)

Alternative components or excipients are indicated below.

1. Glyceryl trioctanoate include
    a. glyceryl trihexanoate
    b. glyceryl triheptanoate,
    c. glyceryl trinonanoate,
    d. glyceryl tridecanoate
2. Soybean oil
    a. castor oil,
    b. corn oil,
    c. cottonseed oil,
    d. olive oil,
    e. peanut oil,
    f. peppermint oil,
    g. safflower oil,
    h. sesame oil,
    i. hydrogenated vegetable oils,
    j. hydrogenated soybean oil, and
    k. medium-chain triglycerides of coconut oil
    l. medium-chain triglycerides palm seed oil
3. Phospholipids
    a. hydrogenated soy phosphatidylcholine,
    b. distearoylphosphatidylglycerol,
    c. L-alpha-dimyristoylphosphatidylcholine,

d. L-alpha-dimyristoylphosphatidylglycerol

4. Glycerin
    a. polyethylene glycol 300,
    b. polyethylene glycol 400,
    c. ethanol,
    d. propylene glycol,
    e. N-methyl-2-pyrrolidone,
    f. dimethylacetamide, and
    g. dimethylsulfoxide
5. Sodium cholate
    a. sodium taurocholate,
    b. sodium tauro-β-muricholate,
    c. sodium taurodeoxycholate,
    d. sodium taurochenodeoxycholate,
    e. sodium glycocholate,
    f. sodium glycodeoxycholate and
    g. sodium glycochenodeoxycholate

[0112] Alternatively, the protocol above may be performed through the first part of step 8, above, whereby PG796(MRx102) is suspended/dissolved in the phospholipid/oil mixture, and the suspension/solution can then be stored as a drug product. Accordingly, the composition is anhydrous, minimizing the potential for hydrolysis of the triptolide or triptolide analog, the shelf life can be prolonged, and the water/sodium cholate/glycerin mixture can then be added according to step 8 and the remainder of the protocol can be carried out, continuing through step 14 above, at the time of administration to a subject.

[0113] Similarly, to aid in stability, dispersion and filtration, the composition can be sterilized (e.g., filtration, autoclaving), and/or other excipients may be added to favor globules of a desired size.

[0114] Preliminary Emulsion Evaluation

[0115] Pharmaceutical emulsions intended for administration by injection or infusion typically consist of a triglyceride such as soybean oil (SBO) with naturally derived phospholipids (egg yolk or soy) emulsified with use of a high pressure homogenizer. Nonionic surfactants such as Tweens (polysorbates), Solutol®, and Kolliphor (Cremophor®), are generally not used in formulations for injection or infusion, because they undergo phase inversions with heating, and injectable emulsions are usually heat sterilized. Nonetheless, some preliminary investigations were initiated with nonionic surfactants.

[0116] Various ratios of the nonionic surfactants polysorbate 80 (a.k.a. Tween 80) and Span 80 were explored, and a formulation was prepared and tested as follows. Glyceryl trioctanoate (GTO) was used as the triglyceride oil, as PG796(MRx102) had been shown to be about 3.4 fold more soluble in GTO than in SBO. The formulation and results are shown in

Table 4. The results of this preliminary experiment were encouraging in that a reasonably high solubility was obtained in a formulation containing almost 70% water.

[0117] Table 4. Preliminary emulsion formulation and solubility.

GTO	Span 80	Tween 80	water	PG796(MRx102) Solubility (μg/ml)
29.4%	1.65%	0.31%	68.6%	681

[0118] Due to the lack of availability of a co-solvent/surfactant formulation with an acceptable side effect profile when injected intravenously into rats, emulsions were considered. The following characteristics were selected as desirable for an emulsion formulation:

- As a vehicle alone, poses no overt side effects in vivo (rodents),
- Has > 2 mg/ml PG796(MRx102) stable concentration,
- Retains 95% PG796(MRx102) concentration after filtration,
- Possesses 7 days of acceptable stability, and
- Is compatible with MRx100.

[0119] Emulsion formulations were prepared using a probe sonicator to disperse the oil phase in the aqueous phase to form a creamy opaque suspension.

[0120] Range-Finding Formulations

[0121] Typical emulsion formulations consist of 10-30% triglyceride, most commonly SBO, dispersed with 0.5-2% phospholipids in an aqueous phase, which contains glycerin as a tonicity agent. However because of the low solubility observed for PG796(MRx102), initial formulations were prepared with 40% of GTO, a medium chain triglyceride in which PG796(MRx102) was found to have higher solubility. Additionally, PEG-400 and ethanol were incorporated into some of the formulations to decrease the polarity of the aqueous phase to enhance solubility. Sodium cholate was included in some formulations as a co-surfactant. The formulations, along with visual assessments and PG796(MRx102) solubility values are shown in Table 5. Solubility of at least 1 mg/mL was obtained in all of the formulations. In each case some loss of potency was observed after eight days of storage, but the majority of the original potency was maintained. PEG-400 and ethanol were only marginally beneficial in improving solubility, and one of the formulations containing PEG-400 failed to form a homogenous emulsion.

[0122] Table 5. First round emulsion formulations and solubilities

Formulation #→ Components↓	E-1	E-2	E-3	E-4	E-5
Glyceryl trioctanoate	40%	40%	40%	40%	40%

Phospholipids		2%	2%	2%	2%	2%
PEG-400		10%	--	--	10%	--
Ethanol		--	10%	--	--	10%
Sodium cholate		--	--	0.2%	0.2%	0.2%
Water		48%	48%	58%	48%	48%
PG796(MRx102)		----------2 mg/mL----------				
Visual assessment		2 layers	homogenous	homogenous	homogenous	homogenous
PG796(MRx102) Solubility (μg/mL)	0 hr	1560	1913	1529	1787	1673
	1 hr	1677	1879	1514	1795	1680
	24 hr	1484	1939	1353	1762	1654
	8 days	---	1353	1176	1470	1329

[0123] Effect of pH on Stability

[0124] Pharmaceutical emulsions are typically prepared at neutral to slightly alkaline pH since they are stabilized by electrostatic repulsion between droplets imparted by pH-sensitive anionic surfactants, such as phosphatidyl ethanolamine, free fatty acid salts, and cholate. However, it was possible that this pH range could be suboptimal for the chemical stability of PG796(MRx102). To test this, emulsions were prepared at different pH values ranging from 4 to 8. Buffers were included to control the pH, and the non-pH sensitive surfactant, sodium dodecyl sulfate was used in place of sodium cholate to assure a negative charge even in the low pH emulsions. Formulations and results are shown in Table 6. All of the formulations were reasonably stable for up to 2 weeks at room temperature. Although there was some variation in potency and purity, there was no trend with pH, indicating that the stability of PG796(MRx102) in the emulsion is not pH-dependent within this range.

[0125] **Table 6 Effect of pH on stability of PG796(MRx102) in emulsions.**

Target pH → **Components ↓**		**4.0**	**5.0**	**6.0**	**7.0**	**8.0**
Glyceryl trioctanoate		40%	40%	40%	40%	40%
Phospholipids		2%	2%	2%	2%	2%
Ethanol		10%	10%	10%	10%	10%
0.1% SDS in buffer		48%	48%	48%	48%	48%
Buffer (10 mM)		acetate	acetate	histidine	phosphate	Tris
PG796(MRx102)		----------1 mg/mL----------				
Measured pH		4.06	4.97	5.98	7.03	8.05
PG796(MRx102) Solubility (μg/mL)	0 hr	870	1006	1093	996	929
	24 hr	917	922	890	849	929
	1 wk	1001	972	948	760	1041
	2 wk	848	910	850	822	930
PG796(MRx102) Purity peak area %	0 hr	98.9	99.2	99.2	99.4	98.7
	24 hr	99.3	99.4	99.2	99.1	99.2
	1 wk	98.9	99.1	98.9	98.8	99.1
	2 wk	97.2	98.3	98.8	91.0	98.3

[0126] Second Round Emulsion Formulations

[0127] To modify the 40% glyceryl trioctanoate vehicles, formulations were prepared using a lower level of triglyceride and/or partial or complete substitution of soybean oil for glyceryl trioctanoate. These formulations and solubility data obtained with them are shown in Table 7. When two values are listed, these are for duplicate analyses. The formulations were heat sterilized for 8 minutes at 121°C. A placebo version of formulation E-0212-4 was also prepared and sterilized to determine the level of placebo component co-elution in HPLC analysis, and this was found to be 1.23%.

[0128] As expected, reducing the triglyceride content and replacing some or all of the glyceryl trioctanoate with soybean oil led to some drop in drug solubility. However, only in formulation E-0212-1, in which the triglyceride content was dropped from 40% to 30% and all of the GTO was replaced with soybean oil, was PG796(MRx102) solubility much less than 1 mg/mL.

[0129] Table 7. Second Round Emulsion Formulations

Formulation #→ / Components↓		E-0212-1	E-0212-2	E-0212-3	E-0212-4	E-0212-5
Glyceryl trioctanoate		--	15%	30%	20%	--
Soybean oil		30%	15%	--	20%	40%
Phospholipids		2%	2%	2%	2%	2%
Glycerin		3%	3%	3%	3%	3%
Sodium cholate		0.2%	0.2%	0.2%	0.2%	0.2%
Water		65%	65%	65%	55%	55%
PG796(MRx102)		----------1 mg/mL----------				
PG796(MRx102) Solubility (μg/mL)	initial	682	929, 928	968	1090, 991	934, 867
	sterilized	621	771, 847	913	1046, 905	913, 867
PG796(MRx102) Purity (peak area %)	initial	96.2%	96.5, 96.6%	97.9%	98.1, 97.3%	95.5, 96.1%
	sterilized	94.6%	95.6, 96.3%	97.2%	97.4, 96.7%	99.6, 95.9%

[0130] Toxicological Observations with Emulsions

[0131] Rats were administered an intravenous bolus of 5 mL/kg of formulation E-3 (40% GTO, 2% phospholipids, 0.2% sodium cholate). The animals appeared normal immediately after injection but became lethargic and were then recumbent with labored breathing within 5 – 10 minutes. The rats recovered and appeared to be normal within 60-90 minutes. A second dose administered the following day appeared to cause more severe symptoms. Injections given the next 2 days produced similar responses. A second cohort of rats was administered an intravenous bolus of 5 mL/kg of formulation E-5 (the same formulation as E-3 but with

addition of 10% ethanol). All of the animals were recumbent and immobile after 10 minutes and died after about 45 minutes.

[0132] Formulation E-3 was tested at the higher concentration of 2 mg/mL PG796(MRx102), which was found to be soluble. The higher concentration would allow dosing at a commensurately lower volume. Accordingly, a cohort of rats was administered a reduced dose of 1.5 mL/kg of formulation E-3. The animals appeared normal for 8-10 minutes after injection, and were then recumbent for 8-10 minutes. Thus the adverse events were less severe, and the period of recumbency and the recovery times were shorter with this dose. The three experiments are summarized in Table 8.

[0133] Table 8. Initial rat studies with emulsion formulations.

	Experiment 1	**Experiment 2**	**Experiment 3**
Components↓ Formulation →	**E-3**	**E-5**	**E-3**
Glyceryl trioctanoate	40%	40%	40%
Phospholipids	2%	2%	2%
Ethanol	--	10%	--
Sodium cholate	0.2%	0.2%	0.2%
Water	58%	48%	58%
Volume injected i.v.	5 ml/kg	5 ml/kg	1.5 ml/kg
Deaths	**0** of 4	**4** of 4	**0** of 4
Recovery Time	60 – 90 min	N/A	15 - 17 min

[0134] In a comparison of emulsions with only soybean oil (40%, emulsion E0212-4) and an equal mixture of glyceryl trioctanoate and soybean oil (20% of each, emulsion E0212-5), rats were administered an intravenous bolus of 3 mL/kg daily for 4 days. On the first day of injection, the animals that were given E0212-4 became slightly lethargic at 7 min, and were fully recovered by 40 min. The E0212-5 rats were slightly lethargic at 8 min, and they had recovered fully by 35 min. Previous tests had shown rats to be recumbent for a protracted period after the intravenous injection of various emulsion formulations, more severe symptoms. The result is improved with these two newest emulsion formulations when injected intravenous into rats. The side effects for the emulsion injections given to rats on days 2-4 were very similar to those observed on Day 1. The use of 40% SBO did not completely eliminate side effects seen with 20% GTO / 20% SBO. Side effects observed after the first injection were less severe than those of Formulation #3 at 5 ml/kg and even at 1.5 ml/kg. There was no labored breathing, and there was only slight lethargy in contrast to the earlier studies showing labored breathing and lethargy.

[0135] The 20% GT / 20% SBO emulsion formulation (E-0212-4) showed an acceptable chemical solubility/stability profile, was non-lethal in tests of the vehicle alone in rat studies,

and caused minimal side effects (less than other emulsion formulation preparations), it was selected as the revised vehicle formulation for use in the Escalating Dose/7-Day Repeat Dose Comparison Study of PG796(MRx102) and MRx100 in rats, and the Escalating Dose/7-Day Repeat Dose Study of PG796(MRx102) in dogs.

[0136] Table 9. Side effect rat studies comparing emulsion formulations

Components↓ Formulation →	**E-0212-4**	**E-0212-5**
Glyceryl trioctanoate	20%	---
Soybean oil	20%	40%
Phospholipids	2%	2%
Glycerin	3%	3%
Sodium cholate	0.2%	0.2%
Water	55%	55%
Volume injected i.v.	3 ml/kg	3 ml/kg
Deaths	**0** of 4	**0** of 4
Recovery Time	40 min	35 min

[0137] Pharmacokinetic/Toxicokinetic Considerations

[0138] Triptolide's molecular mechanism of action has remained elusive, but triptolide was reported to covalently bind to human XPB (also known as ERCC3), a subunit of the transcription factor TFIIH, and to inhibit its DNA-dependent ATPase activity, leading to inhibition of RNA polymerase II-mediated transcription and likely nucleotide excision repair. The identification of XPB as the target of triptolide accounts for the many of the known biological activities of triptolide. For example, triptolide binding to XPB lead to the down regulation of a number of growth and survival promoters including NF kappa B (NF-κB) and the anti-apoptotic factors Mcl-1 and XIAP. (Titov, *et al.*, Nat. Chem. Biol. (2011) 7(3):182-8). Subsequently, the triptolide derivative MRx102 was also found to have these effects, *i.e.*, reduced mRNA levels, reduced NF-κB and reduced Mcl-1 and XIAP. At low nanomolar concentrations, MRx102 also induced apoptosis in bulk, CD34(+) progenitor, and more importantly, CD34(+)CD38(-) stem/progenitor cells from AML patients, even when they were protected by coculture with bone marrow derived mesenchymal stromal cells. In vivo, MRx102 greatly decreased leukemia burden and increased survival time in non-obese diabetic/severe combined immunodeficiency mice harboring Ba/F3-ITD cells. Thus, MRx102 has potent antileukemic activity both *in vitro* and *in vivo*, has the potential to eliminate AML stem/progenitor cells and overcome microenvironmental protection of leukemic cells, and warrants clinical investigation. (Carter, *et al.*, (2012) Leukemia 26:443-50). Furthermore, triptolide and triptolide derivatives can serve as a new molecular probe for studying

transcription and, potentially, as a new type of anticancer agent through inhibition of the ATPase activity of XPB.

[0139] Another consequence of XPB binding is the inhibition of nucleotide excision repair. This activity in blocking DNA repair should enhance the activities of those drugs that have DNA as their target, including cisplatin and topoisomerase 1 inhibitors for solid tumors; both have been shown to act in a synergistic fashion with triptolide. The potential synergy between MRx102 and two drugs used in AML, cytarabine and idarubicin was investigated using MV4-11 cells *in vitro* and synergy was demonstrated between MRx102 and both of these drugs used in AML.

[0140] One concern regarding triptolide and triptolide derivatives is their epoxide structure, viewed as potentially toxic; however, proteosome inhibitor anti-cancer drug, carfilzomib (Kyprolis) is a tetrapeptide epoxyketone containing an epoxide, and was recently FDA approved. Furthermore, triptolide, even though it is a triepoxide, was shown by Titov, *et al.*, (*supra*) to be exquisitely selective, and not promiscuous, in its binding characteristics. Nonetheless, triptolide's reported safety issues in a number of animal studies as well as clinically, have resulted in an "image problem" and potential safety challenges; accordingly, triptolide has not been deemed appropriate for clinical use and has not been commercially developed.

[0141] Triptolide prodrugs are generally believed to be safer than triptolide. In an initial rodent toxicology study, PG796(MRx102) demonstrated no gross or histopathologic toxic effects at intravenous doses up to 1.5 mg/kg/day for seven days. Triptolide prodrugs as an emulsion formulation are believed to have a toxicokinetic profile characterized by a flat AUC with a minimized Cmax. [In conjunction, it was postulated that a sustained inhibition of RNA polymerase is needed for optimum efficacy which in turn requires a pharmcokinetic profile of constant exposure to drug]. **Figure 1** shows a side-by-side comparative toxicology study of PG796(MRx102) and triptolide in which both drugs were administered intravenously to rodents using the novel emulsion formulation disclosed herein demonstrated that PG796(MRx102) was at least 20 times less toxic than triptolide based on both gross and histopathologic criteria. The no effect dose ("NOAEL") of PG796(MRx102) again exceeded 1.5 mg/kg/day intravenously for seven days in rodents confirming the initial results. It is interesting to ask why a prodrug of triptolide would be safer than the natural product itself; while not wishing to be bound by theory, perhaps the answer lies in the pharmacokinetic profile of triptolide administered either directly or released from its carrier, PG796(MRx102). When triptolide is provided alone (*see* line connecting circles in **Fig. 1**) it had a very high

Cmax as well as a rapid decline such that by two hours post-dose none remained in circulation. However, when the prodrug PG796(MRx102) was administered, the triptolide Cmax was approximately one-tenth that noted when triptolide was administered directly (*see* line connecting triangles in **Fig. 1**) and the triptolide blood levels remained relatively constant and demonstrate a longer AUC ("area under the curve") as seen at the two-hour time point. It also remained above the therapeutic levels (shown as a thick line without symbols). The difference in the Cmax/AUC profile of PG796(MRx102) vs. triptolide is believed to be due to the physiochemical properties of the lipid prodrug/emulsion formulation combination. In general, triptolide prodrugs having a cLogP greater than 0.5 are more lipid-soluble than water soluble and are expected to take longer to convert to the drug form; such characteristics may yield a flatter conversion profile and less of a drug-release Cmax spike.

[0142] PG490-88 given intravenously, entered clinical trials and showed promising activity in patients with AML. (Xia Zhi Lin and Zhen You Lan, Haematologica, 93:14 (2008)). However, as a prodrug, it was incompletely and erratically converted to the active entity, triptolide, and, as such, may provide areason it produced toxicity. However, PG490-88 did have an optimized AUC, relatively flat over time with no intense Cmax. The finding that PG796(MRx102) was rapidly and completely converted to triptolide using human serum (as well as seen in vivo in rats and dogs) while PG490-88Na was incompletely converted to triptolide in human serum argues that the conversion of PG796(MRx102) is not dependent on variations in species enzymatic (esterase) activities but is dependent on the physiochemical properties of the lipid prodrug/emulsion formulation.

[0143] Lipid emulsions have been studied as drug delivery systems for some time. (*See* Hippalgaonkar, *et al.*, (2010) AAPS Pharm. Sci. Tech. 11(4):1526–1540; Stevens, *et al.*, (2003) Business Briefing: Pharmatech 2003, p. 1-4). Solid lipid nanoparticle (SLN) delivery systems may have advantages over conventional formulations of bioactive plant extracts, such as enhancing solubility and bioavailability, offering protection from toxicity, and enhancing pharmacological activity. A tripterygium glycoside (TG) solid lipid nanoparticle (TG-SLN) delivery system was reported to have a protective effect against TG-induced male reproductive toxicity. Triptolide (TP) was used as a model drug in a comparative study of the toxicokinetic and tissue distribution of TP-SLN and free TP in rats. A fast and sensitive HPLC-APCI-MS/MS method was developed for the determination of triptolide in rat plasma. Fourteen rats were divided randomly into two groups of 7 rats each for toxicokinetic analysis, with one group receiving free TP (450μg/kg) and the other receiving the TP-SLN formulation (450μg/kg). Blood was obtained before dosing and 0.083, 0.17, 0.25, 0.33, 0.5, 0.75, 1, 1.5, 2,

3 and 4h after drug administration. Thirty-six rats were divided randomly into six equal groups for a tissue-distribution study. Half of the rats received intragastric administration of TP (450μg/kg) and the other half received TP-SLN (450μg/kg). At 15, 45, and 90min after dosing, samples of blood, liver, kidney, spleen, lung, and testicular tissue were taken. TP concentration in the samples was determined by LC-APCI-MS-MS. The toxicokinetic results for the nanoformulation showed a significant increase the area under the curve (AUC) ($P<0.05$), significantly longer T(max) and mean retention times (MRTs) (0-t) ($P<0.05$), significantly decreased C(max) ($P<0.05$). The nanoformulation promoted absorption with a slow release character, indicating that toxicokinetic changes may be the most important mechanism for the enhanced efficacy of nanoformulations. Tissue-distribution results suggest a tendency for TP concentrations in the lung and spleen to increase, while TP concentrations in plasma, liver, kidney, and testes tended to decrease in the TP-SLN group. At multiple time points, testicular tissue TP concentrations were lower in the TP-SLN group than in free TP group. This provides an important clue for the decreased reproductive toxicity observed with TP-SLN. Overall, an orally administered lipid nanoparticle formulation of triptolide promoted absorption with a slow release character. (Xue, *et al.*, (2012) Eur. J. Pharm. Sci., 47(4):713-7). The toxicokinetic results for the nanoformulation showed a significant increase in AUC, and a decreased Cmax. These results indicate that toxicokinetic change are a consideration for enhanced safety.

[0144] Pharmacokinetic Data

[0145] TK comparison of triptolide levels in Calvert and SRI studies - Males and Females

[0146] Plasma Triptolide Concentration (ng/ml)

	Plasma Triptolide Concentration (ng/ml)					
Time (hrs)>	0	0.25	0.5	1	2	24
PG796(MRx102) 0.5 mg/kg (emulsion)	0	11.8	10.5	3.1	5.8	0
Triptolide 0.15 mg/kg (emulsion)	0	74.6	18.4	13.2	0	0
PG796(MRx102) 1.5 mg/kg (DMSO/PEG400/PBS)	0	36.4	29.1	16.7	4.11	0

MRx102 0.5 mg/kg and Triptolide 0.15 mg/kg are from Calvert study; results are from females
MRx102 1.5 mg/kg is from SRI study; results are from males
SRI study - 3,4,8 hrs. triptolide concentration = 0 ng/ml

[0147] TK comparison of triptolide levels in Calvert and SRI studies – Males Only

	Plasma Triptolide Concentration (ng/ml)					
Time (hrs)>	0	0.25	0.5	1	2	24

PG796(MRx102) 0.5 mg/kg (emulsion)	0	32.5	10.9	0.7	1.0	0
Triptolide 0.15 mg/kg (emulsion)	0	59.0	14.9	3.6	0	0
PG796(MRx102) 1.5 mg/kg (DMSO/PEG400/PBS)	0	36.4	29.1	16.7	4.1	0

MRx102 0.5 mg/kg and Triptolide 0.15 mg/kg are from Calvert study; results are from males
MRx102 1.5 mg/kg is from SRI study; results are from males
SRI study - 3,4,8 hrs. triptolide concentration = 0 ng/ml

[0148] Routes of Administration

[0149] Although in some embodiments the route of administration is intravenous, other routes include: epicutaneous or topical, intradermal, subcutaneous, nasal, intraarterial, intramuscular, intracardiac, intraosseous infusion, intrathecal, intraperitoneal, intravesical, intravitreal intracavernous injection, intravaginal, and intrauterine.

EXAMPLE 2

Cytotoxicity (MTT) Assay

[0150] Test compounds may be dissolved in DMSO at a concentration of 20 mM. Further dilutions may be done in RPMI1640 medium (GIBCO, Rockville, MD) supplemented with 10% Fetal Calf Serum (HyClone Laboratories, Logan, UT).

Cytotoxicity of the compounds is determined in a standard MTT assay using Cell Proliferation Kit I (#1 465 007, Roche Diagnostics, Mannheim, Germany). Briefly, human T cell lymphoma (Jurkat) cells (4 x 10^5 per well) aere cultured for 24h, in 96-well tissue culture plates, in the presence of serial three-fold dilutions of test compounds or medium containing the same concentration of DMSO as in the test samples at each dilution point. The cultures are then supplemented with 10 μl/well MTT reagent for 4h and then with 0.1 ml/well solubilizing reagent for an additional 16h. Optical density at 570 nm (OD_{570}) is measured on a ThermoScan microplate reader (Molecular Devices, Menlo Park, CA).

EXAMPLE 3

IL-2 Production Assay

[0151] Test samples can be diluted to 1 mM in complete tissue culture medium. Aliquots are placed in microculture plates coated with anti-CD3 antibody (used to stimulate the production of IL-2 by Jurkat cells), and serial dilutions are prepared so that the final concentration encompass the range of 0.001 to 10,000 nM in log increments. Cells from an exponentially expanding culture of Jurkat human T cell line (#TIB-152 obtained from

American Type Culture Collection, Manassas, VA) are harvested, washed once by centrifugation, re-suspended in complete tissue culture medium, and diluted to a concentration of 2 x 10^6 cells/ml. A volume of 50 µl of Jurkat cells (1 x 10^5 cells) is added to wells containing 100 µl of the diluted compounds, 50 µl of PMA (10 ng/ml) is added to each well, and the plates are incubated at 37°C in a 5% CO_2 incubator. After 24 hours, the plates are centrifuged to pellet the cells, 150 µl of supernatant is removed from each well, and the samples are stored at -20 °C. The stored supernatants are analyzed for human IL-2 concentration using the Luminex 100 (Luminex Corporation, Austin, TX), Luminex microspheres coupled with anti-IL-2 capture antibody, and fluorochrome-coupled anti-IL-2 detection antibody. The data are expressed as pg/ml of IL-2.

[0152] While a number of exemplary aspects and embodiments have been discussed above, those of skill in the art will recognize certain modifications, permutations, additions and sub-combinations thereof. It is therefore intended that the following appended claims and claims hereafter introduced are interpreted to include all such modifications, permutations, additions and sub-combinations as are within their true spirit and scope.

WHAT IS CLAIMED IS:

1. A composition for intravenous administration of an emulsion comprising triptolide or a triptolide derivative having a clogP of 0.5 or higher, the emulsion comprising (a) 15 to 45 % by weight lipid, (b) 0 to 50 % by weight of a medium chain triglyceride, (c) 0.5 to 3 % by weight phospholipid, (d) 0 to 5 % by weight of glycerin, (e) 0.1 to 0.3% by weight of a sodium cholate, (f) about 50 to 60% by weight water, and (g) about 0.5 to about 3 mg/mL triptolide or a triptolide derivative.

2. The composition of claim 1, wherein the 15 to 45 % by weight lipid is a lipid selected from the group consisting of soybean oil, castor oil, corn oil, cottonseed oil, olive oil, peanut oil, peppermint oil, safflower oil, sesame oil, coconut oil or palm seed oil.

3. The composition of claim 1, wherein the medium chain triglyceride is 20% by weight and is selected from the group consisting of glyceryl trioctanoate, glyceryl trihexanoate, glyceryl triheptanoate, glyceryl trinonanoate and glyceryl tridecanoate.

4. The composition of claim 1, wherein the phospholipid is selected from the group consisting of hydrogenated soy phosphatidylcholine, distearoylphosphatidylglycerol, L-alpha-dimyristoylphosphatidylcholine and L-alpha-dimyristoylphosphatidylglycerol.

5. The composition of claim 1, wherein the glycerin is selected from the group consisting of polyethylene glycol 300, polyethylene glycol 400, ethanol, propylene glycol, N-methyl-2-pyrrolidone, dimethylacetamide, and dimethylsulfoxide.

6. The composition of claim 1, wherein the sodium cholate is selected from the group consisting of sodium taurocholate, sodium tauro-β-muricholate, sodium taurodeoxycholate, sodium taurochenodeoxycholate, sodium glycocholate, sodium glycodeoxycholate and sodium glycochenodeoxycholate.

7. The composition of claim 1, comprising a triptolide derivative selected from the group consisting of compounds according to structure I.

8. The composition of claim 1, comprising a triptolide derivative selected from the group consisting of compounds according to structure II.

9. The composition of claim 1, comprising a triptolide derivative selected from the group consisting of compounds according to structure III.

10. The composition of claim 1, comprising a triptolide derivative selected from the group consisting of compounds according to structure IV.

11. A method of effecting immunosuppression, immunomodulation or inhibiting cell proliferation comprising administering to a subject in need of such treatment an effective amount of a composition of claim 1.

12. A method of inducing apoptosis in a cell, comprising contacting said cell with an effective amount of a composition of claim 1.

13. A composition for oral administration of an emulsion comprising triptolide or a triptolide derivative having a clogP of 0.5 or higher, the emulsion comprising (a) 15 to 45 % by weight lipid, (b) 0 to 50 % by weight of a medium chain triglyceride, (c) 0.5 to 3 % by weight phospholipid, (d) 0 to 5 % by weight of glycerin, (e) 0.1 to 0.3% by weight of a sodium cholate, (f) about 50 to 60% by weight water, and (g) about 0.5 to about 3 mg/mL triptolide or a triptolide derivative.

14. A composition for intravenous administration of an emulsion comprising triptolide or a triptolide derivative having a clogP of 0.5 or higher, the emulsion comprising (a) 15 to 45 % by weight lipid, (b) 0 to 95 % by weight of a medium chain triglyceride, (c) 0.5 to 3 % by weight phospholipid, (d) 0 to 5 % by weight of glycerin, (e) 0.1 to 0.3% by weight of a sodium cholate, and (f) about 0.5 to about 3 mg/mL triptolide or a triptolide derivative; wherein an aqueous solution is added prior to administration.

15. A composition for oral administration of an emulsion comprising triptolide or a triptolide derivative having a clogP of 0.5 or higher, the emulsion comprising (a) 15 to 45 % by weight lipid, (b) 0 to 95 % by weight of a medium chain triglyceride, (c) 0.5 to 3 % by weight phospholipid, (d) 0 to 5 % by weight of glycerin, (e) 0.1 to 0.3% by weight of a sodium cholate, and (f) about 0.5 to about 3 mg/mL triptolide or a triptolide derivative; wherein an aqueous solution is added prior to administration.

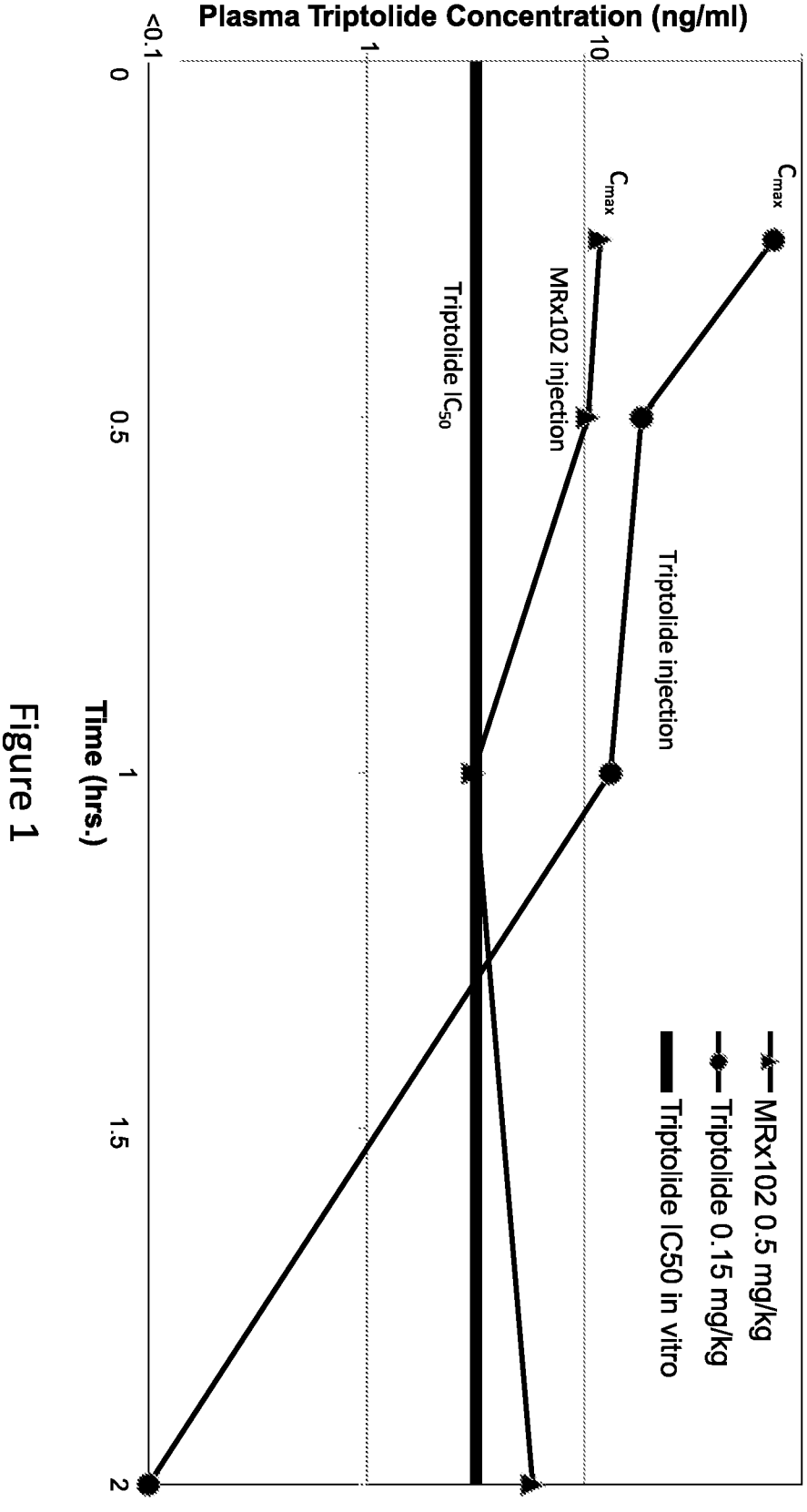

Plasma Triptolide with MRx102 and Triptolide Injections
Plasma Triptolide Concentration (ng/ml)
100
10
1
<0.1
0
0.5
1
1.5
2
Time (hrs.)
Cmax
Cmax
MRx102 injection
Triptolide injection
Triptolide IC50
MRx102 0.5 mg/kg
Triptolide 0.15 mg/kg
Triptolide IC50 in vitro

Figure 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/30041

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 9/107, 31/34, 31/57 (2014.01)
USPC - 514/177, 468; 549/297
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 9/107, 31/34, 31/36, 31/57, 31/343, 31/365, 31/665; A61P 25/00; C07D 307/77, 307/93; C07J 3/00 (2014.01)
UPPC - 424/400; 514/177, 180, 468; 549/297, 298

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google Scholar; PubMed; IP.com; Proquest; Surechem; triptolide, lipid, intravenous, IV, medium chain triglyceride, phospholipid, sodium cholate, cLogP castor oil, peanut oil, glyceryl trioctanoate, blyceryl trihexanoate, glyceryl triheptonoate, hydrogenated soy phosphatidylcholine, emulsion, disteraoylphosphatidylglycerol, polyethylene glycol 300 or 400, sodium taurocholate, immunosuppression, pharmacokinetics

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 7,098,348 B2 (DAI, D et al.) August 29, 2006; column 9, lines 60-65; column 10, lines 3-63	1-15
A	US 2011/0262494 A1 (ACHLEITNER, G et al.) October 27, 2011; paragraphs [0019]-[0022]	1-15
A	PYKA, A et al. A Comparison of Theoretical Methods of Calculation of Partition Coefficients for Selected Drugs. Acta Poloniae Pharmaceutica – Drug Research. Vol. 63, No. 3, pages 159-167. 2006; page 163	1-15
A	WO 1996/008262 A1 (WIEDMANN, TWT et al.) March 21, 1996; page 6, lines 30-34; page 7, lines 1-2; page 14, lines 31-34	1-15
A	US 7,019,151 B2 (DAI, D et al.) March 28, 2006; column 9, lines 65-67; column 10, lines 11-62	1-15
A	US 2011/0064794 A1 (DENG, Y et al.) March 17, 2011; paragraphs [0015], [0022], [0033], [0046], [0051], [0055]	1-15

☐ Further documents are listed in the continuation of Box C. ☐

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

Date of the actual completion of the international search	Date of mailing of the international search report
30 June 2014 (30.06.2014)	24 JUL 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 105263475 A
(43)申请公布日 2016.01.20

(21)申请号 201480015321.7
(22)申请日 2014.03.15
(30)优先权数据
61/794,926 2013.03.15 US
(85)PCT国际申请进入国家阶段日
2015.09.15
(86)PCT国际申请的申请数据
PCT/US2014/030041 2014.03.15
(87)PCT国际申请的公布数据
WO2014/145303 EN 2014.09.18
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(51)Int.Cl.
A61K 9/107(2006.01)
A61K 31/34(2006.01)
A61K 31/57(2006.01)

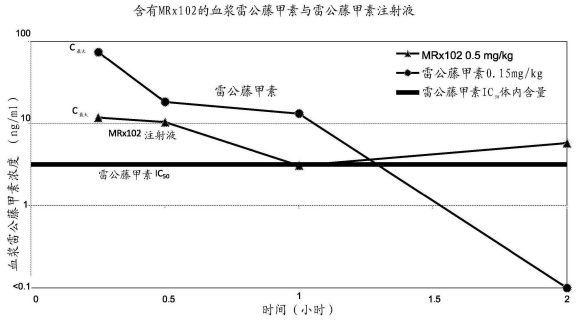
权利要求书2页 说明书28页 附图1页

(54)发明名称

雷公藤甲素静脉注射乳剂用作免疫调制剂和抗癌药

(57)摘要

一种雷公藤甲素和雷公藤甲素衍生物的静脉注射配方用于免疫调节和抗增殖剂。



CN 105263475 A

1.一种用于静脉注射乳剂成分，含有雷公藤甲素或雷公藤甲素衍生物，疏水性系数为0.5或更高，所述乳剂含有(a)重量百分比为15-45％的脂质，(b)重量百分比为0-50％的中链甘油三酯，(c)重量百分比为0.5-3％的磷脂，(d)重量百分比为0-5％的甘油，(e)重量百分比为0.1-0.3％的胆酸钠，(f)重量百分比约为50-60％的水，以及(g)约0.5到约3mg/mL的雷公藤甲素或一种雷公藤甲素衍生物。

2.根据权利要求1中的成分，其中重量百分比为15-45％的脂质是由大豆油，蓖麻油，玉米油，棉花籽油，橄榄油，花生油，薄荷油，红花油，芝麻油，椰子油或棕榈仁油组成的这组物质中选出的一种脂质。

3.根据权利要求1中的成分，其中的中链甘油三脂重量百分比为20％，而且是从由三辛酸甘油酯，三己酸甘油酯，三庚酸甘油酯，三壬酸甘油酯和癸酸丙三醇酯组成的这组物质中选出的。

4.根据权利要求1中的成分，其中的磷脂是从由氢化大豆磷脂酰胆碱，二硬脂酰磷脂酰甘油酯，L-α-二肉豆蔻酰磷脂酰胆碱和L-α-二肉豆蔻酰甘油磷脂组成的这组物质中选出的。

5.根据权利要求1中的成分，其中的甘油是从由聚乙二醇300，聚乙二醇400，乙醇，丙二醇，N甲基-2-吡咯烷酮，二甲基乙酰胺和二甲亚砜组成的这组物质中选出的。

6.根据权利要求1中的成分，其中的胆酸钠是从由牛磺胆酸钠，牛磺-β-鼠胆酸钠，牛磺脱氧胆酸钠，牛磺鹅去氧胆酸钠，甘氨胆酸钠，甘氨脱氧胆酸钠和甘氨鹅脱氧胆酸钠组成的这组物质中选出的。

7.根据权利要求1中的成分，含有从由符合结构I的化合物组成的这组物质中选出的一种雷公藤甲素衍生物。

8.根据权利要求1中的成分，含有从由符合结构II的化合物组成的这组物质中选出的一种雷公藤甲素衍生物。

9.根据权利要求1中的成分，含有从由符合结构III的化合物组成的这组物质中选出的一种雷公藤甲素衍生物。

10.根据权利要求1中的成分，含有从由符合结构IV的化合物组成的这组物质中选出的一种雷公藤甲素衍生物。

11.一种影响免疫抑制、免疫调节或抑制细胞增殖的方法，包含针对需要此类治疗的研究对象的给药，提供有效数量的权利要求1中的成分。

12.一种引发细胞凋亡的方法，包含用有效数量的权利要求1中的成分接触所述细胞。

13.一种用于口服乳剂的成分，含有雷公藤甲素或雷公藤甲素衍生物，疏水性系数为0.5或更高，所述乳剂含有(a)重量百分比为15-45％的脂质，(b)重量百分比为0-50％的中链甘油三酯，(c)重量百分比为0.5-3％的磷脂，(d)重量百分比为0-5％的甘油，(e)重量百分比为0.1-0.3％的胆酸钠，(f)重量百分比约为50-60％的水，以及(g)约0.5到约3mg/mL的雷公藤甲素或一种雷公藤甲素衍生物。

14.根据权利要求，所述乳剂含有(a)重量百分比为15-45％的脂质，(b)重量百分比为0-95％的中链甘油三酯，(c)重量百分比为0.5-3％的磷脂，(d)重量百分比为0-5％的甘油，(e)重量百分比为0.1-0.3％的胆酸钠，以及(f)约0.5到约3mg/mL的雷公藤甲素或一种雷公藤甲素衍生物；其中在给药之前加入水溶液。

15.一种用于口服乳剂的成分，含有雷公藤甲素或雷公藤甲素衍生物，疏水性系数为0.5或更高，所述乳剂含有(a)重量百分比为15-45％的脂质，(b)重量百分比为0-95％的中链甘油三酯，(c)重量百分比为0.5-3％的磷脂，(d)重量百分比为0-5％的甘油，(e)重量百分比为0.1-0.3％的胆酸钠，以及(f)约0.5到约3mg/mL的雷公藤甲素或一种雷公藤甲素衍生物；其中在给药之前加入水溶液。

雷公藤甲素静脉注射乳剂用作免疫调制剂和抗癌药

技术领域

[0001] 本发明公开了一种针对雷公藤甲素衍生化合物的配制，用作免疫调制剂、消炎药和抗癌药。

背景技术

[0002] 免疫抑制剂被广泛用于自身免疫疾病的治疗，并用于治疗或预防移植排斥反应，包括移植物抗宿主疾病 (GVHD) 的治疗，在这种疾病中移植细胞会攻击受体（宿主）细胞。常见的免疫抑制剂包括咪唑硫嘌呤、皮质类固醇、环磷酰胺、甲氨蝶呤、6- 巯基嘌呤、长春新碱以及环孢霉素 A。一般而言，这些药物都不是完全有效的，而且大多数都受限于严重的毒副作用。举例来说，环孢霉素 A，一种广泛使用的药剂，对肾脏毒性作用显著。此外，有效治疗需要的剂量可能增加患者对各种机会感染原的易感染性。

[0003] 化合物雷公藤甲素取自于中国药用植物雷公藤 (TW)，及其某些衍生物和前体药物，已经被确认具有显著的免疫抑制作用。雷公藤甲素的各种前体药物和其他类似物也表现出这类作用。例如，参见美国专利 4,005,108 ;5,294,443 ;5,648,376 ;5,663,335 ;5,759,550 ;5,843,452 ;5,962,516 和 6,150,539，这些专利的每一项都被完整引用纳入本文。据相关报告，雷公藤甲素及其某些衍生物 / 类似物和前体药物还具有显著的抗癌作用，包括减小体内的实体瘤 ;例如，参见 Kupchan 等人在美国化学会志 94:7194(1972) 上的报告，以及共有美国专利编号 6,620,843，同样被完整引用纳入本文。雷公藤甲素及其前体药物以及其他类似物也表现出了显著的抗癌作用，包括减小体内的实体瘤 ;例如，参见共有美国专利编号 6,620,843，同样被完整引用纳入本文。例如，参见 Fidler 等人的分子癌症疗法 2(9):855-62(2003)。

[0004] 如果类似物对给定的第一目标分子的结合亲和力与它对第二目标分子的结合亲和力的差异在 10 倍或以上，则该类似物可以被命名为“选择性结合”的类似物。

[0005] 尽管雷公藤甲素的衍生物和前体药物在药物动力学或生物分布等方面相对优于天然的雷公藤甲素，例如凭借在脂质或水溶性方面的差异，或通过其作为前体药物的作用，雷公藤甲素衍生物本身的生物作用往往显著低于天然的雷公藤甲素。

[0006] 相关领域的上述实例及其相关限制只用于说明，并非专有。相关领域的其他限制对该领域的技术人员来说，在阅读说明和研究图纸后将是显而易见的。

附图说明

[0007] 图 1 :注射前体药物 PG796(MRx102) 后血浆中雷公藤甲素浓度随时间变化与注射雷公藤甲素的对比。

发明内容

[0008] 相关领域的实例及其相关限制，如上文所述，仅用于说明，并非专有。相关领域的其他限制对于该领域的技术人员来说，在阅读说明和研究图纸后将是显而易见的。

[0009] 一方面，专利提供了一种成分，用于含有雷公藤甲素或雷公藤甲素衍生物，疏水性系数为 0.5 或更高的乳剂的静脉注射，该乳剂含有 (a) 重量百分比为 15-45％的脂质，(b) 重量百分比为 0-50％的中链甘油三酯，(c) 重量百分比为 0.5-3％的磷脂，(d) 重量百分比为0-5％的甘油，(e) 重量百分比为0.1-0.3％的胆酸钠，(f) 重量百分比约为50-60％的水，以及 (g) 约 0.5 到约 3mg/mL 的雷公藤甲素或一种雷公藤甲素衍生物。在一些实施例中，未使用甘油。在一些实施例中，雷公藤甲素或雷公藤甲素衍生物的浓度为大约 0.5mg/mL 到大约 3mg/mL。在一些实施例中，雷公藤甲素或雷公藤甲素衍生物为约 1mg/mL 到约 2mg/mL。

[0010] 在一些实施例中，成分含有重量百分比为 15-45％的脂质，其中脂质是从由大豆油、蓖麻油、玉米油、棉花籽油、橄榄油、花生油、薄荷油、红花油、芝麻油、椰子油或棕榈仁油组成的群组中选出的。

[0011] 在一些实施例中，中链甘油三脂的重量百分比为 20％，是从由三辛酸甘油酯、三己酸甘油酯、三庚酸甘油酯、三壬酸甘油酯和癸酸丙三醇酯组成的群组中选出的。

[0012] 在一些实施例中，磷脂是从由氢化大豆磷脂酰胆碱、二硬脂酰磷脂酰甘油酯、L-α-二肉豆蔻酰磷脂酰胆碱和 L-α-二肉豆蔻酰甘油磷脂组成的群组中选出的。

[0013] 在一些实施例中，甘油是从由聚乙二醇 300、聚乙二醇 400、乙醇、丙二醇、N- 甲基 -2- 吡咯烷酮、二甲基乙酰胺和二甲亚砜组成的群组中选出的。

[0014] 在一些实施例中，胆酸钠是从由牛磺胆酸钠、牛磺-β-鼠胆酸钠、牛磺脱氧胆酸钠、牛磺鹅去氧胆酸钠、甘氨胆酸钠、甘氨脱氧胆酸钠和甘氨鹅脱氧胆酸钠组成的群组中选出的。

[0015] 在一些实施例中，用于含有雷公藤甲素或雷公藤甲素衍生物，疏水性系数为 0.5 或更高的乳剂的静脉内给药的成分，乳剂中含有 (a) 重量百分比为 15-45％的脂质，(b) 重量百分比为 0-95％的中链甘油三酯，(c) 重量百分比为 0.5-3％的磷脂，(d) 重量百分比为 0-5％的甘油，(e) 重量百分比为 0.1-0.3％的胆酸钠，以及 (f) 约 0.5 到约 3mg/mL 的雷公藤甲素或一种雷公藤甲素衍生物，作为无水混合物储存，在给药之前加入水溶液。

[0016] 在一些实施例中，用于含有雷公藤甲素或雷公藤甲素衍生物，疏水性系数为 0.5 或更高的乳剂的口服给药的成分，乳剂中含有 (a) 重量百分比为 15-45％的脂质，(b) 重量百分比为 0-95％的中链甘油三酯，(c) 重量百分比为 0.5-3％的磷脂，(d) 重量百分比为 0-5％的甘油，(e) 重量百分比为 0.1-0.3％的胆酸钠，以及 (f) 约 0.5 到约 3mg/mL 的雷公藤甲素或一种雷公藤甲素衍生物，作为无水混合物储存，在给药之前加入水溶液。

[0017] 一方面，提供了用于含有雷公藤甲素或雷公藤甲素衍生物，疏水性系数为 0.5 或更高的乳剂的口服药的成分。

[0018] 在一些实施例中，成分中含有从由符合构造 I 的化合物组成的群组中选出的雷公藤甲素衍生物。在一些实施例中，成分中含有从由符合构造 II 的化合物组成的群组中选出的雷公藤甲素衍生物。在一些实施例中，成分中含有从由符合构造 III 的化合物组成的群组中选出的雷公藤甲素衍生物。在一些实施例中，成分中含有从由符合构造 IV 的化合物组成的群组中选出的雷公藤甲素衍生物。

[0019] 一方面，提供了影响免疫抑制、免疫调节或抑制细胞增殖的一种方法，其中该方法包括给需要对象以有效剂量静脉注射一种含有疏水性系数为 0.5 或更高的雷公藤甲素或雷公藤甲素衍生物的乳剂，用于免疫抑制、免疫调节或抑制细胞增殖。

[0020] 一方面，提供了一种细胞凋亡诱发方法，其中该方法包含给需要对象以有效剂量静脉注射一种含有疏水性系数为0.5或更高的雷公藤甲素或雷公藤甲素衍生物的乳剂用于诱发凋亡。

[0021] 本方法和成分的其他实施例以及类似方法和成分通过下列说明、图纸、实例和权利要求将是显而易见的。根据上述和下文的说明可以了解到，在假设这种组合中所包含的特性并不存在彼此不一致的情况下，本文所描述的每种和每项特性，以及两种或更多此类特性的每种和每项组合，都包含在本专利公开的范围内。此外，特性的任何特性或组合可能被特别排除在本专利公开的任何实施例范围以外。本公开发明的其他方面和优点列举在以下说明和权利要求中，尤其是在结合随附的实例和图纸考虑的情况下。

具体实施方式

[0022] 现在将在下文中更加全面地描述各个方面。但这些方面可能体现在多张不同的表格中，并且不应被理解为受限于本文中所列举的实施例；相反地，这些实施例的提供是为了让本专利公开周密和完整，并向该领域的技术人员完全了解其范围。

I.定义

[0023] 正如在本说明书中的用法那样，单数形式的“一种”、“一项”和“那种”包含复数指示物，除非上下文另行明确说明。因此，举例来说，一种“聚合物”的说法包含单种聚合物，也包含两种或多种相同或不同的聚合物，一种“赋形剂”的说法包含单种赋形剂以及两种或多种相同或不同的赋形剂以及类似物。

[0024] 在提供一个数值范围的情况下，计划每个中间值近似到下限单位的十分之一，除非上下文另行明确说明，该范围的上下限与指定范围内任何其他设定值或中间值之间被包含在该专利公开的范围内。举例来说，如果指定了1m到8m的范围，那么预计2m、3m、4m、5m、6m和7m也得到了明确公开，同样公开的还有大于或等于1m的范围以及小于或等于8m的数值范围。本专利公开包含在设定范围内的任何设定值或中间值与该设定范围内的任何其他设定值或中间值之间的每个较小的范围。这些较小范围的上限和下限都可能被该范围单独包括或排除，而对于两个限值中有任意一个、均不或均包含在较小范围内的每个范围也包括在本专利公开范围内，具体情况取决于该设定范围内任何特别排除的限值。在设定范围包括一个或两个限值的情况下，排除任意一个或同时排除两个所包含的限值的范围也包括在本专利公开范围内。

[0025] “烷基”是指含有碳和氢的饱和非环状单价基，可能为直链或有支链。烷基基团的例子包括甲基、乙基、正丁基、叔丁基、正庚基和异丙基。“环烷基”是指含有碳和氢的完全饱和的环状单价基，可能进一步被烷基所取代。环烷基基团的例子包括环丙基、甲基环丙基、环丁基、环戊基、乙基环戊基和环己基。“低级烷基”是指含有一到六个碳原子的此类基团，而在一些实施例中含有一到四个碳原子。

[0026] “烯基”是指含有碳和氢的非环状单价基，可能为直链或有支链，至少含有一个碳-碳双键(C＝C)。“炔基”是指含有碳和氢的非环状单价基，可能为直链或有支链，至少含有一个碳-碳三键(C＝C)。“低级烯基”或“低级炔基”此类基团含有二到六个碳原子，而在一些实施例中含有二到四个碳原子。

[0027] ″酰基″是指含有-(C＝O)R结构的基团，其中R为烷基（烷基酰基）或芳基（芳

基芳基）。“酰氧基”是指含有 -O(C ＝ O)R 结构的基团。

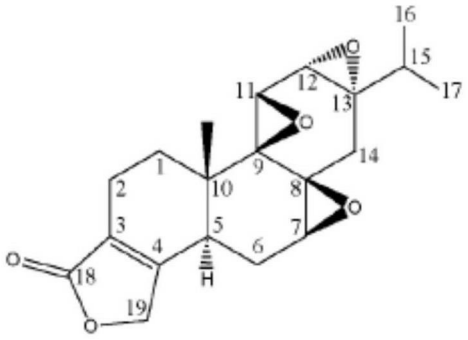
[0028] ″芳基″是指含有一个环（如，苯）或两个稠环（如萘基）的单价芳香基。正如本文中的用法那样，芳基是单环和碳环（非杂环）的，如苯（苯基）环或取代苯环。“取代”的含义是用卤素（如氟、氯或溴）、低级烷基、硝基、氨基、低级烷氨基、羟基、低级烷氧基或卤代基团（低级烷基）等基团取代一个或多个环上的氢。

[0029] ″芳基烷基″是指一种烷基，通常是低级（C_1-C_4，或 C_1-C_2）烷基，取代基进一步被芳基基团所取代；例子有苯甲基和苯乙基。

[0030] “杂环”是指非芳基环，通常为五元到七元环，环上的原子选自于由碳、氮、氧和硫组成的基团。在一些实施例中，环上的原子包括 3 到 6 个碳原子。举例来说，这种杂环包括吡咯烷、哌啶、哌嗪和吗啉。

[0031] “卤素”或“卤代基团”是指氟、氯、溴或碘。

[0032] 针对当前的专利公开，以下编号方案被用于雷公藤甲素和雷公藤甲素衍生物：



II. 雷公藤甲素类似物

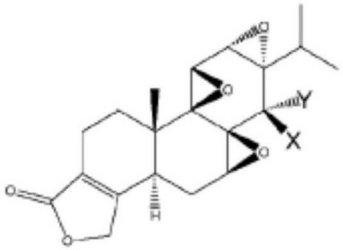
[0033] 雷公藤甲素类似物，作为本文中所使用的术语，包括天然产品雷公藤甲素（本文中命名为 PG490）的各种结构变形。其中可能包括天然产生的类似物，如 2- 羟基雷公藤甲素或 16- 羟基雷公藤甲素（雷公藤乙素），尽管在本文中该术语通常是指合成制备的类似物。正如本文中的用法那样，“雷公藤甲素的相关化合物”是指雷公藤甲素及其类似物，优先指代类似物。

[0034] 举例来说，结构变形可能包括雷公藤甲素环氧环或内酯环的开环；羟基基团（天然产生或通过此类开环产生的）转化为羧酸酯、无机酯（如磺酸盐）、碳酸盐或氨基甲酸酯，通过氧化转化为醛或酮，或通过后续还原转化为氢原子；单键转化为双键，以及 / 或者氢原子被卤素、烷基、烯基、羟基、烷氧基、酰基或氨基基团所取代。雷公藤甲素类似物的例子在多项美国专利中已经进行了说明，包括美国专利号 5,663,335、6,150,539、6,458,537 以及 6,569,893，其中每项专利都通过完整引用而纳入本文。如这些专利中所述，这些化合物可以从雷公藤甲素，一种源于植物的二萜三环氧化物中制得。如上文所述，雷公藤甲素及其类似物已经表现出了有益的免疫抑制和细胞毒性作用，例如在上面所引用的专利中。

[0035] 雷公藤甲素类似物的例子包括 14- 甲基雷公藤甲素（命名为 PG670，参见美国应用公开号 20040152767），雷公藤甲素 14- 叔丁基碳酸盐（命名为 PG695，参见 PCT 公开号：WO 2003/101951），14- 脱氧 -14α- 氟代雷公藤甲素（命名为 PG763；参见美国临时申请序号 60/449,976），雷公藤甲素 14-（α- 二甲基氨基）乙酸盐（命名为 PG702；参见美国专利号 5,663,335），5-α- 羟基雷公藤甲素（命名为 PG701；参见美国临时申请序号 60/532,702），19- 甲基雷公藤甲素（命名为 PG795；参见美国临时申请序号 60/549,769），以及 18- 去

氧 -19- 脱氢 -18- 苯甲酸基 -19- 苯甲酰基雷公藤甲素（命名为 PG796；参见美国临时申请序号 60/549,769）。这些应用和发布中的每一项都通过完整引用纳入本文。

[0036] 许多这类化合物都被认为会通过在体内转化为雷公藤甲素而成为前体药物，正如在上述的 PG490-88 中所观察到的。其他化合物，如 14- 脱氧 -14α- 氟代雷公藤甲素 (PG763)，预期不会进行这种转化，然而仍展示出了雷公藤甲素所表现出的生物活性（如在人类 T 细胞淋巴瘤（淋巴瘤）细胞的细胞毒性以及 IL-2 的抑制），正如上文所引用的美国应用序号 60/449,976 所报告的那样。



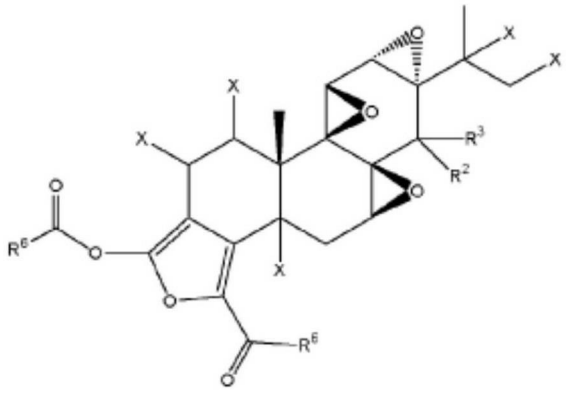
雷公藤甲素衍生物及前体药物示例

表 1

化合物	X	Y
PG490-88	-O（CO）CH_2CH_2COOH	-H
PG670	-OH	$-CH_3$
PG695	-O（CO）OC（CH_3）$_3$	-H
PG702	-O（CO）CH_2N（CH_3）$_2$	-H
PG673	-H	-F

[0037] 通过组合化学或其他类型的制备方法可以产生用于筛选的雷公藤甲素类似物，生成各种化学结构或取代基。

[0038] 配方中的活性成分是雷公藤甲素或雷公藤甲素衍生物，如下文所述。举例来说，该专利公开提供了结构 I 的化合物：



I

其中

每个 R^6 单独选自烷基、烯基、炔基或芳基；

CR^2R^3为 CHOH 或 C ＝ O；

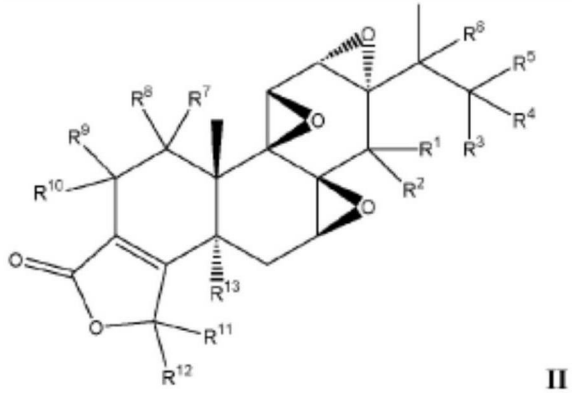
至多 X 基团中有一个为羟基，其余的 X 基团为氢。

[0039] 在结构 I 的一些实施例中，CR^2R^3为 CHOH，通常含有对羟基结构。在一些实施例中，每个 X 为氢；但在选定的实施例中，所表示的基团 X 中恰有一个为羟基。羟基取代的位置通常包括碳 2 和碳 16，如上文中的编号方案所示。

[0040] 在一些实施例中，存在于结构 I 的化合物中的每个所述的烷基、烯基和炔基部分包括最多四个碳原子，而每个所述的芳基部分为单环非杂环；如被取代或未被取代的苯基。

[0041] 在结构 I 的选定实施例中，每个 R^6为芳基；通常，每个 R^6为苯基。其中包括本文中被命名为 PG796 的化合物，其中每个 R^6为被取代或未被取代的苯基。

[0042] 本专利公开还提供了表示为结构 II 的化合物：



II

其中：

CR^1R^2选自于 CHOH、C ＝ O、CHF、CF_2和 $C(CF_3)OH$；

CR^6和 CR^{13}选自于 CH、COH 和 CF；

CR^7R^8、CR^9R^{10}和 $CR^{11}R^{12}$选自于 CH_2、CHOH、C ＝ O、CHF 和 CF_2；以及

$CR^3R^4R^5$选自于 CH_3、CH_2OH、C ＝ O、COOH、CH_2F、CHF_2和 CF_3；

使得：至少一个 R^1-R^{13}含氟；

不超过两个，而且往往不超过一个 $CR^3R^4R^5$、CR^6、CR^7R^8、CR^9R^{10}、$CR^{11}R^{12}$和 CR^{13}含有氟或氧；

而且，当 CR^1R^2为 CHOH 时，$CR^3R^4R^5$不是 CH_2F。

[0043] 在一些实施例中，CR^7R^8处的立体化学使得当 CR^7R^8为 CHOH 时，它包含一个 β－羟基结构，而当 CR^7R^8为 CHF 时，它包含一个 α－氟基结构。相似地，CR^9R^{10}处的立体化学往往使得当 CR^9R^{10}为 CHOH 时，它包含一个 β－羟基结构，而当 CR^9R^{10}为 CHF 时，它包含一个 α－氟基结构。

[0044] 在结构 II 的一些实施例中，CR^1R^2为 CHF，包含一个 α－氟代结构。

[0045] 一些实施例还包括仅有一个选自 CR^1R^2、$CR^3R^4R^5$、CR^6、CR^7R^8、CR^9R^{10}和 $CR^{11}R^{12}$的碳中心含氟的化合物。在一些实施例中，CR^1R^2、CR^6、CR^7R^8、CR^9R^{10}和 $CR^{11}R^{12}$中仅有一个含氟。

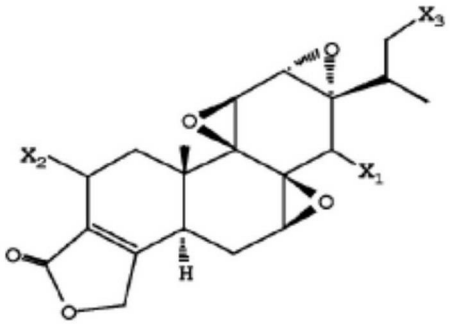
[0046] 在所选的实施例中，只有 CR^1R^2含氟。因此，在这些实施例中，CR^1R^2选自于 CF_2、CHF 和 $C(CF_3)OH$。CR^1R^2处的立体化学使得当 CR^1R^2为 $C(CF_3)OH$ 时，它包含一个 β－羟基结构，而当 CR^1R^2为 CHF 时，它包含一个 α－氟基结构。在所选的结构 II 实施例中，该化合物为 PG763。

[0047] 在结构 II 其他所选的实施例中，CR^9R^{10}或 $CR^3R^4R^5$含氟，而 CR^1R^2含氧；举例来说，

CR^1R^2为 C ＝ O，或者，在一些实施例中，为 CHOH（β－羟基）。在这些实施例中，举例来说，CR^9R^{10}选自于 CF_2和 CHF（例如，α－氟代），或 $CR^3R^4R^5$选自于 CHF_2或 CF_3。

[0048] 在进一步选择的结构 II 的实施例中，CR^7R^8或 $CR^{11}R^{12}$含氟，而 CR^1R^2含氧；举例来说，CR^1R^2为 C ＝ O，或者在一些实施例中，为 CHOH（β－羟基）。在这些实施例中，举例来说，CR^7R^8选自于 CF_2和 CHF（例如 α－氟代），或 $CR^{11}R^{12}$选自于 CF_2和 CHF。

[0049] 本专利公开还给出了用结构 III 表示的化合物。



III

在结构 III 中，各变量定义如下：

X^1为 OH 或 OR^1，而 X^2和 X^3分别为 OH、OR^1或 H，规定 X^1，X^2和 X^3中至少一个为 OR^1，而 X^2和 X^3中至少一个为 H；而

OR^1为 O-(C ＝ O)-Z，其中 Z 选自于由 $-OR^2$、$-O-Y-(C=O)-OR^3$、$-O-Y-NR^4R^5$、$-NR^4R^5$、$-NR^3-Y-(C=O)-OR^3$和 $-NR^3-Y-NR^4N^5$构成的基团；

其中

Y 为含有多达六个碳原子的二价烷基、烯基或炔基基团；

R^2选自于烷基、烯基、炔基、环烷基，环烯基、芳基、芳烷基、羟基烷基、烷氧基烷基、芳氧基烷基和酰氧基烷基；

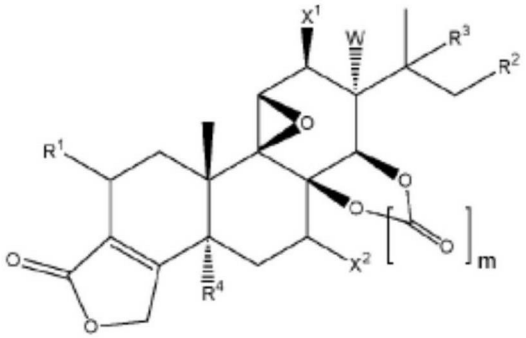
每个 R^3分别选自氢和 R^2；而

R^4和 R^5分别选自于氢、烷基、烯基、炔基、环烷基、环烯基、芳基、芳烷基、羟基烷基、烷氧基烷基、芳氧基烷基和酰氧基烷基，或 R^4和 R^5共同形成 5- 到 7- 元杂环，其环上的原子选自于由碳、氮、氧和硫组成的基团，其中环上的原子包含最多三个杂环原子。

[0050] 定义为 R^2、R^3、R^4和 R^5的基团，当选自于烷基、烯基和炔基时，可能含有多达六个碳原子。当选自于环烷基或环烯基时，它们通常含有 3 到 7 个，或对于环烯基来说，含有 5 到 7 个碳原子。当选自于芳烷基、羟基烷基、烷氧基烷基、芳氧基烷基和酰氧基烷基时，这些基团的烷基成分通常含有多达六个碳原子。在一个实施例中，每个此类基团分别选自于烷基、芳基、芳烷基和烷氧基烷基。

[0051] 在所选的结构 III 的实施例中，X^2＝ X^3＝ H，而 Y 为 $-CH_2-$ 或 $-CH_2CH_2-$。在进一步的实施例中，OR^1选自于由 $O-(C=O)-OR^2$、$O-(C=O)-O-Y-(C=O)-OR^3$和 $O-(C=O)-O-Y-NR^4R^5$（碳酸盐衍生物）组成的基团。在其他实施例中，OR^1选自于由 $O-(C=O)-NR^4R^5$、$O-(C=O)-NR^3-Y-(C=O)-OR^3$和 $O-(C=O)-NR^3-Y-NR^4N^5$（氨基甲酸酯衍生物）组成的基团。在所选的结构 III 的实施例中，该化合物为 PG695。

[0052] 本专利公开还给出了用结构 IV 表示的化合物。



IV

其中

每个 R^1、R^2、R^3和 R^4分别选自于氢、羟基、$-O(CO)_nX$、$-O(CO)_nOR^5$和 $-O(CO)_nN(R^5)_2$，其中 X 为卤素，R^5为氢或低级烷基，而 n 为 1-2，

并规定至少有三个 R^1、R^2、R^3和 R^4为氢；

m 为 1-2；

X^2为卤素，如 F 或 Cl；而

X^1为卤素，通常为 Cl，而 W 为羟基；或 X^1和 W 共同形成一个环氧基。

[0053] 当任何一个 R^1、R^2、R^3和 R^4选自于 $-O(CO)_nX$、$-O(CO)_nOR^5$或 $-O(CO)_nN(R^5)_2$时，变量 n 通常为 1。

[0054] 在所选的结构 IV 的实施例中，每个 R^1、R^2、R^3和 R^4均为氢。

在进一步选择的实施例中，m ＝ 1。在所选的结构 IV 的实施例中，化合物为 PG762。

III. 生物活性

[0055] 基于结构 I，18- 脱氧 -19- 脱氢 -18- 苯甲酸基 -19- 苯酰雷公藤甲素（命名为 PG796），可以采用标准 MTT 比色法对化合物的细胞毒性进行评估，如例 3 所述，而这类化合物的免疫抑制作用通过标准 IL-2 抑制试验进行评估，如例 4 所述。PG796 在两项试验中都表现出了高于已知的前体药物，雷公藤甲素 14- 琥珀酸盐（指定为 PG490-88）的活性。特别值得一提的是，人体血清中培养的雷公藤甲素 14- 琥珀酸盐在这些试验中的活性要比在小鼠血清中培养的雷公藤甲素 14- 琥珀酸盐低得多，而 PG796 在两种情况下表现出了基本相当的高活性。（预期通过培养会将雷公藤甲素 14- 琥珀酸盐转化为雷公藤甲素，将 PG796 转化为单衍生化合物，19- 苯甲酰基雷公藤甲素，参见上文中的合成方案。）

[0056] 可以按照例 2 的描述，采用标准 MTT 比色法对命名为 PG757、PG762 和 PG830 的结构 IV 三种化合物以及另外一种命名为 PG782 的化合物的细胞毒性作用进行评估。可以按照例 3 的描述，在标准 IL-2 抑制试验中对这些化合物的免疫抑制作用进行评估。

[0057] 在 MTT 比色法中血清培养的化合物 PG757 的细胞毒性明显强于雷公藤甲素将；参见下表 2。（表 2 中检测化合物的数据是针对血清培养 24 小时的化合物的。）培养的 PG782 还比雷公藤甲素更有效，而培养的 PG762 具有类似的效力。多种检测化合物在进行血清培养时，在对 IL-2 的抑制方面相当于雷公藤甲素。

表 2

化合物	生存能力/细胞毒性 MTT（ED_{50}）	免疫抑制 IL-2 （IC_{50}）
PG490（雷公藤甲素）	60 nM	4 nM
PG757	32 nM	9 nM
PG762	60 nM	9 nM
PG782	53 nM	2 nM

[0058] 血清培养能将前体药物转化为雷公藤甲素，而研究表明对于PG757和PG762来说，这会在大约5分钟内发生。

IV.治疗成分

[0059] 含有本专利公开的雷公藤甲素衍生物的配方可能采用固体、半固体、冻干粉或液体剂型，比如片剂、胶囊、粉末、缓释配方、溶液、悬浮液、乳剂、药膏、乳液或气雾剂，而在一些实施例中为单位剂型适合于精确剂量的简单给药。成分通常包括传统的药物载体或赋形剂，可能额外包含其他药用制剂、载体或助剂。

[0060] 在一些实施例中，该成分约占本专利公开的一种化合物或多种化合物重量的大约0.5％到75％，其余部分由合适的药用赋形剂构成。对于口服来说，这类赋形剂包括药用级别的甘露醇、乳糖、淀粉、硬脂酸镁、糖精钠、滑石、纤维素、葡萄糖、明胶、蔗糖、碳酸镁及类似物。如果需要，成分中还可能含有少量的无毒助剂，如润湿剂、乳化剂或缓冲液。

[0061] 该成分可能通过口服、经皮或不经肠道，如通过静脉、皮下、腹膜或肌肉注射实现对象给药。用于口服液体制剂时，该成分可以制成溶液、悬浮液、乳剂或糖浆，以液体形式或适合在水或生理盐水中水化的干燥形式提供。对于不经肠道给药，不经肠道给药的可注射成分通常在合适的静脉注射液，如无菌的生理盐水溶液中含有雷公藤甲素衍生物。

[0062] 液体成分的制备方法是将雷公藤甲素衍生物（约0.5％到约20％）和可选的药用辅料溶解或分散在药物学上可以接受的载体中，例如盐水、葡萄糖水溶液、甘油或乙醇，以形成溶液或悬浮液。

[0063] 该化合物的给药方式可能还包括气溶胶颗粒形式的吸入，可为固态或液态，通常为可吸入粒度。此类颗粒小得足以在吸入后通过口腔和咽喉，并进入肺部支气管和肺泡。一般来说，颗粒粒径为大约1-10微米，通常粒径小于大约5微米，是可呼吸的。吸入的液体成分包含分散在无菌无热原盐水溶液或无菌无热原水等水性载体中的活性药剂。如果需要，该成分可以与推进剂混合，促进该成分的喷洒和气雾剂的形成。

[0064] 此类剂型的制备方法是该领域的技术人员所了解或对他们来说将是显而易见的；举例来说，参见雷明顿药物科学（第19版，Williams&Wilkins,1995）。给药成分将含有一定量的选定化合物，有效剂量足以影响研究对象的免疫抑制或目标细胞的凋亡。

[0065] 如本文所述，举例来说，在Panchagnula等(2000)的研究中，药物制剂的分配系数或logP可能影响各种给药途径的适用性，包括口服生物利用度。本文中所描述的化合物，凭借氟对一个或多个羟基基团的取代，预期具有比母体化合物，雷公藤甲素，更高的logP

计算值，这使得它们在口服利用度方面成为了更好的候选药剂。

[0066] 本文公开的脂质配方能有效用于静脉注射，以及口服给药。基于脂质和表面活性剂的配方作为提高溶解性差的化合物的口服生物利用度的可行方法而广受认可。利用基于脂质和表面活性剂的配方的多种口服药品在市场上均有销售。例如，Sandimmune®和Sandimmune、Neoral®（环孢霉素A，诺华公司）、Norvir®（利托那伟），和Fortovase®（沙奎那韦）已经配制成了自乳化药物释放系统。实际上，最近的一份评论对水溶性差的药物的口服脂质基配方的已发布的针对人体研究对象药物动力学研究进行了总结。(Fatouros 等 (2007) 疗法与临床风险管理 3(4):591-604)。

V. 免疫调节和抗炎治疗

[0067] 依据结构 I 的化合物，18- 脱氧 -19- 脱氢 -18- 苯甲酰氧基 -19- 苯甲酰基雷公藤甲素（命名为 PG796），以剂量依赖的方式，抑制了白血病细胞中 IL-2 的产生（参见例 3）。本专利公开因此包括了含有作为免疫抑制剂的活性成分的配方的使用，例如作为移植程序的一种助剂或用于自体免疫疾病的治疗。

[0068] 本专利公开所预期的一种功效是用于治疗免疫系统调节异常的人体疾病的治疗。研究已经表明免疫调节异常存在于各类自体免疫和慢性炎症性疾病中，包括全身性红斑狼疮、慢性类风湿性关节炎、I 型和 II 型糖尿病、炎症性肠病、胆汁性肝硬化、葡萄膜炎、多发性硬化症以及其他失调症状，比如克罗恩氏病、溃疡性结肠炎、天疱疮、大疱性类天疱疮、结节病、牛皮癣、鱼鳞癣、格雷夫斯眼病、格雷夫瓦病和哮喘。虽然上述每种病症潜在的发病机制可能差异巨大，但它们共同拥有各种自身抗体和自反应性淋巴细胞的外观。这种自反应性可能部分是由于丧失了正常免疫系统运行的稳态控制。

[0069] 同样地，在完成来自于含有成熟淋巴细胞的供体组织来源的骨髓移植或造血干细胞的其他移植之后，所转移的淋巴细胞会将宿主组织的抗原视为异质。这些细胞被激活，并攻击宿主（移植物抗宿主反应），可能威胁生命。而且，在器官移植之后，宿主淋巴细胞会识别出器官移植的外来组织抗原，并增加细胞和抗体介导的免疫反应（宿主对抗移植的反应），导致移植受损和排斥。

[0070] 自体免疫或排斥反应的一个结果是炎症细胞以及它们所释放的中介体造成的组织破坏。像 NSAID 这样的消炎药的主要作用方式是阻断这种中介体的作用或分泌，但它并不会更改这种疾病的免疫学基础。另一方面，类似环磷酰胺的细胞毒性药物的作用是非特异性的，使得正常反应和自体免疫反应被同时关闭。实际上，用这种非特异性免疫抑制剂治疗的病人死于感染的可能性与他们死于其自体免疫疾病的可能性是一样高的。

[0071] 本专利公开的成分能有效应用于雷公藤甲素及其前体药物和其他衍生物被证明有效的用途，例如，用于免疫抑制的治疗，正如治疗自体免疫疾病，避免移植排斥反应，或者治疗或预防移植物抗宿主疾病 (GVHD)。例如，参见共有的美国专利号 6,150,539，通过引用纳入本文。雷公藤甲素与本衍生物同样能有效用于治疗其他炎症性疾病，如创伤性炎症，以及降低雄性生育力。

[0072] 这类成分能有效抑制来自不相容的人类供体的实体器官移植、组织移植或细胞移植的排斥反应，从而延长移植器官的存活和功能以及受体的存活。这种用途可能包括，但不限于，实体器官移植（如心脏、肺部、胰腺、四肢、肌肉、神经、肾脏和肝脏），组织移植（如皮肤、角膜、肠道、性腺、骨骼和软骨），以及细胞移植（例如胰腺细胞，比如胰岛细胞、大脑和

神经组织、肌肉、皮肤、骨骼、软骨和肝脏）包括异种移植等。

[0073] 这类成分还能有效用于抑制异种移植（种间）排斥反应；即避免来自非人类动物的实体器官移植、组织移植或细胞移植的排斥反应，不论是天然构造还是经过生物工程改造（转基因）来表达人类基因、RNA、蛋白质、肽或其他非原生异基因分子，或经过生物工程改造去除动物天然基因的、RNA、蛋白质、肽或其他正常表达分子的表达。本专利公开还包括使用上述成分来延长来自非人类动物的此类实体器官移植、组织移植或细胞移植的存活。

[0074] 同时包括自体免疫疾病或具有自体免疫性表现的疾病的治疗方法，比如爱迪生氏病、自体免疫性溶血性贫血、自体免疫性甲状腺炎、克罗恩氏病、糖尿病（I 型，青少年型或最近发生的糖尿病）、格雷夫斯氏病、格林－巴利综合征、全身性红斑狼疮（SLE）、狼疮性肾炎、多发性硬化症、重症肌无力、牛皮癣、原发性胆汁性肝硬化、风湿性关节炎、葡萄膜炎、哮喘、动脉硬化、桥本氏甲状腺炎、过敏性脑脊髓炎、肾小球性肾炎以及各种过敏症。

[0075] 进一步的用途可能包括炎症性和快速增生性皮肤病的治疗与预防以及免疫介导疾病的临床表现，比如牛皮癣、异位性皮炎、天疱疮、荨麻疹、皮肤嗜酸性粒细胞增多症、痤疮和斑秃；各种眼部疾病，比如结膜炎、葡萄膜炎、角膜炎和结节病；粘膜和血管的炎症，比如胃溃疡、缺血性疾病和血栓症造成的血管损伤、缺血性肠道疾病、炎症性肠道疾病，以及坏死性小肠结肠炎；肠道炎症／过敏症，比如乳糜泄、克罗恩氏病和溃疡性结肠炎；肾脏疾病，比如间质性肾炎、古德帕斯彻综合征、溶血性尿毒症综合征和糖尿病肾病；造血系统疾病，比如血小板减少性紫癜和自身免疫性溶血性贫血；皮肤疾病，比如皮肌炎和皮肤 T 细胞淋巴瘤；循环系统疾病，比如动脉硬化和动脉粥样硬化；肾病综合症，比如肾小球性肾炎；肾脏疾病，比如缺血性急性肾功能不全和慢性肾功能不全；以及白塞病。

[0076] 本专利公开的成分和方法同样能有效用于炎症性疾病的治疗，比如哮喘，同时包括内在和外在表现，例如支气管哮喘、过敏性哮喘、内源性哮喘、外源性哮喘以及粉尘性哮喘，特别是慢性或长期哮喘（例如晚发型哮喘和气道高反应），或者包括过敏症和可逆阻塞性气道疾病在内的其他肺部疾病，包括支气管炎等等。该成分和方法还可以用于治疗其他炎症性病症，包括创伤性炎症、莱姆病中的炎症、慢性支气管炎（慢性感染性肺病）、慢性鼻窦炎、败血症相关的急性呼吸窘迫综合征以及肺结节病。对于哮喘此类呼吸疾病的治疗，该成分通常通过吸入给药，但所有传统的给药途径可能都有效。

[0077] 在自体免疫性病症的治疗中，向病人定期提供该成分，例如每周 1-2 次，剂量水平足以减少症状并提高病人的舒适度。尤其是对于风湿性关节炎的治疗，该成分可以通过静脉注射或直接注射进入受影响的关节来进行给药。在病人开始出现疾病症状之后的几周时间内，病人可以按至少 24 小时的重复周期进行治疗。给药剂量通常在每千克病人体重每天 1-25mg/kg 的范围内，通常肠胃外给药剂量较低，而口服剂量较高。最佳剂量可以按照该技术领域的已知方法通过常规试验确定。

[0078] 对于移植排斥的治疗，本方法特别用于心脏、肾脏、肝脏、细胞以及骨髓移植排斥反应的治疗，还可以用于 GVHD 的治疗。治疗通常在手术前后开始，可以在外科移植程序之前或之后不久，并继续每天给药，持续至少数周时间，用于治疗急性移植排斥反应的治疗。在治疗过程中，病人可以通过例如使用包含同种异体淋巴细胞的混合淋巴细胞反应或进行移植组织的活检，定期检查免疫抑制水平。

[0079] 此外，该成分可以长期给药来预防移植排斥，或用于治疗晚期移植排斥的急性发

作。如上所述，给药剂量通常是每千克病人体重每天 1-25mg/kg，肠胃外给药剂量较低，而口服剂量较高。根据病人的响应情况以及治疗期内病人的抗感染能力，剂量可以适当增减。

[0080] 在由于受体接受匹配或不匹配的骨髓、脾脏细胞、胎儿组织、脐带血，或者动员或采集干细胞的移植所造成的移植物抗宿主病的治疗和预防中，在口服或非胃肠给药的情况下，剂量范围通常是每千克体重每天 0.25-2mg/kg，通常为 0.5-1mg/kg/ 天。

[0081] 同时在本专利公开的范围内是含有本专利公开的一种化合物以及一种或多种传统的免疫抑制剂的联合治疗。在本专利公开的范围内的这些免疫抑制剂包括，但不限于，Imurek®（咪唑硫嘌呤钠盐）、布喹那钠、Spanidin™（三盐酸胍立莫司，也被称为脱氧精胍菌素）、咪唑立宾（也被称为布雷青霉素）、Cellcept®（霉酚酸酯）、Neoral®（环孢霉素 A；也以 Sandimmune® 的商标销售不同的配方）、Prograf™（他克莫司，也被称为 FK-506）、Rapimmune®（西罗莫司，也被称为雷帕霉素）、来氟米特（也被称为 HWA-486）、Zenapax®、像泼尼松龙及其衍生物这样的糖皮质激素、像奥素健体 (OKT3) 这样的抗体，以及像胸腺细胞球蛋白这样的抗胸腺细胞球蛋白。如上所述，这些化合物可以有效用作增效剂与另一种免疫抑制药同时给药用于免疫抑制治疗。因此，类似上述的传统免疫抑制药的给药量要远远低于（例如，标准剂量的 20％到 50％）该化合物单独给药的情况。或者，所公开配方的给药量可以使得所产生的免疫抑制作用大于单独使用该药物和所公开的化合物所获得的效果总和的预期或成果。通常来说，免疫抑制药和增效剂在至少 2 周的时间内按有规律的间隔给药。

[0082] 本专利公开的化合物也可能结合一种传统的抗炎药（多种药物）进行给药，其中药物或给药量本身对于适当抑制炎症不起作用。

[0083] 化合物在体内的免疫抑制作用可以使用该领域内已知的已经建立的动物模型进行评估。这种试验可以用于评估免疫抑制性化合物的相对效力，以及估计用于免疫抑制治疗的合适剂量。举例来说，这类试验包括针对同种异体移植的清楚定性的大鼠模型系统，由 Ono 和 Lindsey(1969) 说明，其中移植的心脏连接在同种异体受体动物的腹部大血管上，根据受体动物心脏的搏动能力来判定移植心脏的生存能力。Wang(1991) 和 Murase(1993) 描述了受体动物属于不同种类的一个异种移植模型。评估抗 GVHD 效力的模型包括给正常的 F1 小鼠注射亲本脾脏细胞；小鼠出现以脾脏肿大和免疫抑制为特点的 GVHD 综合症 (Korngold,1978；Gleichmann,1984)。从单个脾脏制备单细胞悬浮液，并在存在和不存在伴刀豆球蛋白 A 的情况下建立微孔基质来评估促有丝分裂的响应性的程度。

VI. 抗癌治疗

[0084] 研究表明下列疾病状态可以接受雷公藤甲素及其前体药物和其他类似药物的治疗。这类疾病状态是第二代雷公藤甲素类似物治疗的目标领域。雷公藤甲素类似物以及 / 或者前体药物化合物还可以与传统治疗药物结合使用。

[0085] 正如本文中的用法那样，“癌症”是指哺乳动物，尤其是人类体内发现的各类癌症或肿瘤或恶性肿瘤，包括白血病、肉瘤、癌和黑素瘤。癌症的例子包括脑癌、乳腺癌、子宫颈癌、结肠癌、头颈癌、肾肿瘤、肺癌、非小细胞肺癌、黑素瘤、间皮瘤、卵巢癌、肉瘤、胃癌、子宫癌和成神经管细胞瘤。“白血病”一词广义上是指造血器官的进展性恶性疾病，一般特点是血液和骨髓中白血球及其前体的扭曲性增殖和发展。“肉瘤”一般是指由胚性结缔组织一类

的物质构成的肿瘤，通常由嵌入纤维或均质物质的密集细胞组成。“黑素瘤”的含义是肿瘤源自于皮肤和其他器官的黑色素细胞系统。“癌症”一词是指由易于渗入周边组织并引发转移的上皮细胞构成的恶性增生。

[0086] 举例来说，癌症可能涉及源于生殖组织的细胞（如睾丸支持细胞、生殖细胞、正在形成或更加成熟的精原细胞、精细胞或精母细胞以及营养细胞、生殖细胞以及卵巢的其他细胞），淋巴或免疫系统（如霍奇金病和非霍奇金淋巴瘤），造血系统，以及上皮细胞（如皮肤，包括恶性黑素瘤，以及胃肠道）、实质性器官、神经系统，例如神经胶质瘤（参见Y.X.Zhou 等，2002），以及肌肉骨骼组织。此类化合物可以用于治疗各种癌症，包括，但不限于，脑癌、头颈癌、肺癌、甲状腺癌、乳腺癌、结肠癌、卵巢癌、子宫颈癌、子宫癌、睾丸癌、膀胱癌、前列腺癌、肝癌、肾肿瘤、胰腺癌、食道癌以及／或者胃癌。其中特别考虑乳腺癌、结肠癌、肺癌和前列腺癌的治疗。治疗的目标是减缓肿瘤的生长、预防肿瘤的生长、诱发肿瘤的部分消退以及诱发肿瘤的完全消退，直至完全消失，以及预防实体瘤造成的转移结果。可以用本专利公开的化合物进行治疗的其他癌症包括，例如，多发性骨髓瘤、成神经管细胞瘤、淋巴瘤、成神经细胞瘤、黑素瘤、前恶性皮肤损伤、横纹肌肉瘤、原发性血小板增多症、原发性巨球蛋白血症、小细胞肺癌、大细胞肺癌、原发性脑瘤、子宫内膜癌、恶性胰岛素瘤、恶性类癌综合征、恶性高钙血症和肾上腺皮质癌。

[0087] 如上文所述，本类化合物可以通过任何传统的给药途径提供给遭受癌症和／或白血病折磨的病人。这种方法能有效减缓肿瘤的生长，预防肿瘤的生长，诱发肿瘤的部分消退以及诱发肿瘤的完全消退，直至完全消失。这种方法还能有效预防实体瘤造成的转移结果。

[0088] 本专利公开的成分可以作为唯一的疗法或配合其他对研究对象不具有抗癌作用的支持性或治疗性用药提供。这种方法还包括本专利公开的成分与一种或多种传统抗癌药物或生物蛋白剂的组合给药，其中药剂本身用量对于适当抑制肿瘤生长不起作用，其药量能为研究对象有效提供预期的抗癌作用。此类抗癌药物包括放线菌素 D、喜树碱、卡铂、顺铂、环磷酰胺、阿糖胞苷、道诺霉素、阿霉素、依托泊苷、氟达拉滨、5- 氟尿嘧啶、羟基脲、吉西他滨、伊立替康、甲氨蝶呤、丝裂霉素 C、米托蒽醌、紫杉醇、泰索帝、替尼泊苷、拓扑替康、长春花碱、长春新碱、长春地辛和长春瑞滨。抗癌生物蛋白剂包括肿瘤坏死因子 (TNF)、肿瘤坏死因子相关凋亡诱导配体 (TRAIL)、其他 TNF 相关或 TRAIL 相关配体和因子、干扰素、白细胞介素 -2、其他白介素、其他细胞因子、趋化激素和因子，以及肿瘤相关分子或受体的抗体（如抗 HER2 抗体）、以及与这类药剂反应或结合的药剂（如受体 TNF 超家族的成员、其他受体、受体拮抗剂以及对这些药剂有特异性的抗体）。

[0089] 如相关研究所述，例如 Fidler 等人的美国专利号 6,620,843，一种特定成分的体内抗肿瘤活性可以采用已经建立的动物模型进行评估。临床剂量和方案可以按照临床医生已知的方法，根据疾病严重程度和病人的整体状况确定。

[0090] 结构 I 的一种化合物，18- 脱氧 -19- 脱氢 -18- 苯甲酸基 -19- 苯甲酰基雷公藤甲素（命名为 PG796）根据剂量，对白血病细胞具有细胞毒性（依据例 2）。因此本专利公开包括使用所公开的化合物作为细胞毒素剂，特别用于治疗癌症。

VII. 其他适应症

[0091] 本专利公开的化合物还可以用于治疗某些 CNS 疾病。谷氨酸酯实现了多种生理机能，但在不同的神经系统和精神疾病的病理生理学中也发挥了重要作用。谷氨酸酯的兴奋

毒性和神经毒性与缺氧、局部缺血和创伤，以及慢性神经退行性疾病或神经代谢疾病、阿尔茨海默氏痴呆、亨廷顿舞蹈病和帕金森病有关。鉴于雷公藤甲素所报道的神经保护作用，尤其是针对谷氨酸酯引发的细胞死亡的保护 (Q.He 等，2003 ;X.Wang 等，2003)，本专利公开的化合物设想用于对抗谷氨酸酯的神经毒性作用，从而成为此类疾病的一种创新疗法。

[0092] 最近有关于处于复发期的 MS 患者的证据表明，MS 患者的大脑中出现了改变了的谷氨酸体内平衡。神经毒性状况出现在 MS 中，它们可能是造成患有脱髓鞘病的病人体内少突细胞和神经细胞死亡的原因。通过采用本专利公开的化合物进行治疗来对抗谷氨酸酯受体介导的兴奋毒性，可能对 MS 病人具有治疗作用。诸如格林－巴利综合征、美尼尔氏病、多神经炎、单神经炎和神经根病之类的其他神经系统疾病可以用本专利公开的化合物进行治疗。

[0093] 本专利公开的化合物还可以用于治疗器官纤维化，包括某些肺病。特发性肺纤维化 (PF) 是一种进展性间质性肺病，病因不明。PF 的特点是肺间质中细胞内基质和胶原的过度沉积以及由于炎症和纤维化，肺泡被瘢痕组织逐渐取代。随着疾病的进展，瘢痕组织的增加干扰了肺部向血流输送氧气的能力。根据相关报告，雷公藤甲素的一种 14- 琥珀酰亚胺酯能够阻断博来霉素引发的 PF(G.Krishna 等，2001)。因此，本专利披露的化合物和配方可以有效治疗 PF。同时考虑了类肉瘤病、纤维化肺和特发性间质性肺炎等其他呼吸道疾病的治疗。

[0094] 其他设想可以采用本专利公开的化合物进行治疗的肺部相关疾病包括严重急性呼吸综合症 (SARS) 和急性呼吸窘迫综合征 (ARDS)。尤其是对于 SARS 来说，如下文所述，在疾病过程达到峰值之前病毒含量的减少 (SARS-CoV) 和皮质类固醇治疗的有效性表明 SARS 最严重的危及生命的影响的形成可能是由于身体对感染的多度反应 (免疫超敏反应)，而不是病毒本身的作用。(同样参见正在共同审理中和共同拥有的美国临时申请序号 60/483,335，通过引用纳入本文。) 皮质类固醇治疗已经被用于 SARS 病人来抑制可能描绘免疫超敏反应特点的细胞因子的大量释放，希望它会在下一阶段终止肺病的发展。皮质类固醇治疗在减少 SARS 的部分主要症状方面已经产生了良好的临床效果。尽管如此，也存在多种与治疗相关的副作用，而且存在对选择性更高的免疫抑制剂以及 / 或者抗炎药的明显需求。

[0095] 雷公藤甲素的相关化合物还可以用于治疗某些 CNS 疾病。谷氨酸酯实现了多种生理机能，包括在各种神经系统和精神疾病的病理生理学中也发挥了重要作用。谷氨酸酯的兴奋毒性和神经毒性与缺氧、局部缺血和创伤，以及慢性神经退行性疾病或神经代谢疾病、阿尔茨海默症 (AD)、亨廷顿舞蹈病和帕金森病有关。鉴于雷公藤甲素所报道的神经保护作用，尤其是针对谷氨酸酯引发的细胞死亡的保护 (He 等，2003 ;Wang 等，2002a)，本专利公开的化合物设想用于对抗谷氨酸酯的神经毒性作用，从而可能成为此类疾病的一种创新疗法。

[0096] 大脑淀粉样血管病是 AD 的病理特征之一，而 PC12 细胞对突变型 β 淀粉样蛋白质聚集体所引发的神经毒性极其敏感。采用 β 淀粉样蛋白治疗的 PC12 细胞表现出了细胞内活性氧累计量的增加，并出现凋亡样死亡 (Gu 等，2004)。β 淀粉体的治疗引发了 PC12 细胞中 NF-κB 的激活，并提高了细胞内的 Ca^{2+} 含量。研究表明雷公藤甲素能显著抑制 β 淀粉样蛋白诱发的凋亡，从而抑制由于 β 淀粉体引发的细胞内 Ca^{2+} 浓度的增加。因此，雷公藤

甲素的相关化合物对于预防 β 淀粉样蛋白引发的细胞凋亡级联以及保护 AD 病人的神经元存活可能是有效的。

[0097] 雷公藤甲素凭借减少亚硝酸盐积累、TNF-α 和 IL-1β 的释放，以及这些炎症因素的 mRNA 诱导表达，对脂多糖 (LPS) 激活小胶质作用表现出了强大的抑制影响。(Zhou 等，2003)。雷公藤甲素还减弱了主要中脑神经元 / 神经胶质混合培养基中 LPS- 诱发的 ^{3}H- 多巴胺摄取量的减少以及酪氨酸羟化酶阳性神经元的损耗 (Li 等，2004)。不含 LPS 的雷公藤甲素表现出了神经营养性效应。雷公藤甲素还阻断了 LPS 诱发的小神经胶质的激活以及 TNF-α 和亚硝酸盐的过度产生。雷公藤甲素可以通过抑制小神经胶质的激活，保护多巴胺能神经元免受 LPS 诱发的伤害，这与帕金森病相关，进一步说明了雷公藤甲素的相关化合物的神经保护潜力。

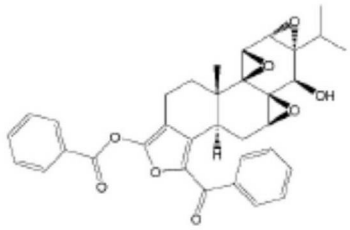
[0098] 雷公藤氯内酯醇已经证明是雷公藤甲素的一种前体药物，促进了在初代培养的大鼠中脑神经元中的多巴胺能神经元轴突伸长，并保护多巴胺能神经元免遭由 1- 甲基 -4- 苯基吡啶嗡离子引发的神经毒性损伤 (Li 等，2003)。原位杂交显示雷公藤氯内酯醇刺激了源于大脑的神经营养因子 mRNA 的表达。而且，在 PD 的一个体内大鼠模型中，FK506 表现出了神经营养活性，以 0.5-1μg/kg 的剂量服用的雷公藤氯内酯醇促进了接受神经外科治疗的大鼠的复原，产生了 SN 神经元的显著保留并保护了酪氨酸羟化酶阳性神经元周围的树突过程，减弱了多巴胺耗竭，增加了多巴胺能神经元的存活，并减弱了大脑中 TNF-α 和 IL-2 水平的提高 (Cheng 等，2002)。而且，雷公藤氯内酯醇在低于神经保护和免疫抑制活性所需的浓度下表现出了神经营养活性。

[0099] 近期有来自处于复发期的 MS 病人的证据表明大脑中存在改变了的谷氨酸体内平衡。神经毒性状况出现在 MS 病人中，它们可能是造成少突细胞和神经细胞死亡的原因。通过采用雷公藤甲素的相关化合物进行治疗来对抗谷氨酸酯受体介导的兴奋毒性，可能对 MS 病人具有治疗作用。诸如格林 - 巴利综合征、美尼尔氏病、多发性神经炎、多神经炎、单神经炎和神经根病之类的其他神经系统疾病可以用雷公藤甲素的相关化合物进行治疗。

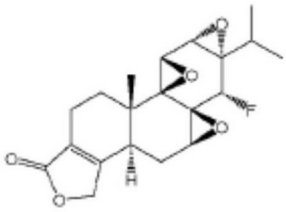
VIII. 活性配方

[0100] 活性成分可能是 PG796、PG763、PG762 或 PG695，相关结构，或疏水性系数大于 0.5 的任何雷公藤甲素衍生物 (参见下面的表 3)。

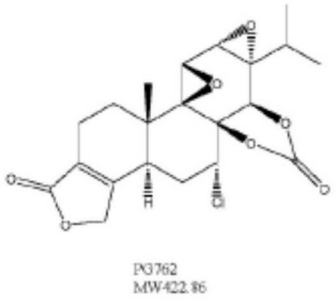
[0101] 示例性雷公藤甲素类似物的化学结构如下所示 ：



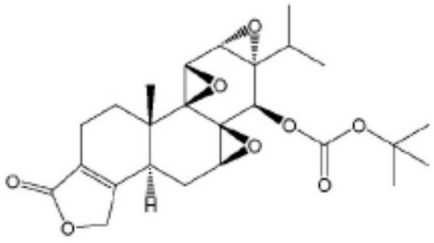
PG796



PG763



PG762



PG695

[0102] 正如化学和制药科学领域的技术人员所了解的那样，分配系数或分布系数是一种化合物在处于平衡的两种非混相的混合物中的浓度比值。这种系数是化合物在这两种相中溶解度差异的一种度量。一般来说，混合物中的一种溶剂是水，而第二种是辛醇这样疏水性的。因此，分配系数是一种化学物质亲水性（“爱水的”）或疏水性（“恐水的”）程度的一种度量。在医疗实践中，举例来说，分配系数可以有效估计药物在体内的分布情况。辛醇/水分配系数高的疏水性药物优先分配给疏水性间室，比如细胞的脂质二重层，而亲水性药物（辛醇/水分配系数低）在血清这样的亲水性间室中优先发现。因此可以根据在水和脂肪中的溶解度分析一种配方的特点，这是因为口服药物需要在服用后通过肠道内层，在血液中携带，并渗透脂质细胞膜到达细胞的内侧。亲脂性细胞膜的一种模型化合物是辛醇（一种亲脂烃），因此辛醇/水分配系数的对数，被称为“LogP”，被用于预测潜在的口服药物的溶解性。该系数可以通过实验测定或进行预测计算，在这种情况下它有时被称为“计算分配系数”或“疏水性系数″。

[0103] 表3雷公藤甲素和雷公藤甲素类似物/衍生物的疏水性系数

		疏水性系数	
化合物	化学类别	方法A	方法B
雷公藤甲素		-0.08	0.27
PG796 （MRx102）	内酯	3.68	4.11
PG763 （MRx103）	卤素	0.63	0.87
PG762 （MRx104）	c-环	1.60	1.89
PG490-88 （MRx108）	酯类	-0.18	0.19
PG695 （MRx109）	碳酸盐	1.61	1.85

方法A－Crippen片段化法：J.Chem.Inf.Comput.Sci.,27,21(1987)

方法 B - Viswanadhan 片段化法 :J.Chem.Inf.Comput.Sci.,29,163(1989)

[0104] 通过文献调查,可以获得关于某些药物类别的最佳 LogP 值的一些一般准则。(参见 LogP 和 pKa 的测定及其使用指南,作者 :Mark Earll www.rael1.demon.co.uk/chem/logp/logppka.html)。

一般来说,假设为被动吸收,

- 最佳 CNS 渗透,约为 LogP = 2+/-0.7(Hansch)
- 最佳口服吸收,约为 LogP = 1.8
- 最佳肠道吸收 LogP = 1.35
- 最佳结肠吸收 LogP = 1.32
- 最佳舌下吸收 LogP = 5.5
- 最佳经皮 LogP = 2.6(和低 mw)

[0105] 配方及剂型 :

- 低 LogP(小于 0) 可注射
- 中 (0-3) 口服
- 高 (3-4) 经皮
- 非常高 (4-7) 在脂肪组织中发生毒性累积

[0106] 总体来说,认为疏水性系数为 0.5 或更高的雷公藤甲素化合物不适合于用于注射的配方。举例来说,在表 3 的化合物中,熟练的技术人员通常预测化合物 PG796、PG763、PG762 或 PG695 不具备适于静脉注射给药的可操作疏水性系数。然而,出人意料的是疏水性系数为 0.5 或更高的化合物的有效可注射配方 (举例来说,比如 PG796、PG763、PG762 或 PG695) 设计且标识如下。

示例

[0107] 以下示例实质上是说明性的,而且不可能打算构成限制。

例 1

乳剂制备

[0108] 乳剂组分包括三辛酸甘油酯 (g)20% ;大豆油 (g)20% ;磷脂 ([60%]L-α - 卵磷脂、L- 卵磷脂、σ 61755)(g)2% ;胆酸钠 (g)0.2% ;甘油 (g)2.5% ;水 (ml)55%

含有 PG796(MRx102) 的乳剂制剂

1. 称量三辛酸甘油酯、大豆油和磷脂 (L- 卵磷脂),放入 15mL 锥形塑料离心管或合适的试管 (如,采用塑料试管以便破损) 中。

2. 将试管放在超声波仪探针的底部上方,使得超声波仪的尖端距离试管底部大约 5mm,而且探针不接触试管的侧面。将其夹好。本阶段不要使用冷水浴。

3. 将超生发生器的功率等级设定为略低于微探头限值,占空比为 50%。开启超声波仪 20 秒。

4. 触摸试管评估其温度,并仔细观察其内容物,以判定磷脂是否分散。超声波仪能非常高效地产生剪切能和气穴现象,但不是高效的混合器,因此可能需要松开管夹,用探针作为搅拌棒来打碎磷脂。

5. 为了分散磷脂,应让液体加热到 40℃ -50℃。继续短时间进行超声波降解直至液体变温,但不要烫手。一旦液体完成升温,将试管悬放在装有温水的烧杯中,并继续进行超声

波降解 5 分钟或直至磷脂达到完全分散，取时间长的一种。

6. 称量 PG796(MRx 102) 并将其加入约为 20℃ -25℃的液体中。对溶液进行短时间的超声波降解（每次大约 20 秒）直至 PG796(MRx102) 溶解。每次超声波降解处理后，将试管悬放在装有水（大约 15℃ -20℃）的烧杯中冷却到确保温度低于 40-45℃。可能大约要进行 10 次超声波降解使 PG796(MRx102) 完全溶解。

7. 测定 / 称量水和胆酸钠，放入烧杯，使胆酸钠溶解。加入甘油并使其溶解在胆酸钠溶液中。

8. 将装有磷脂 / 油 /PG796(MRx102) 的试管悬放在冷水浴中，加入大约 1/3 的水 / 胆酸钠 / 甘油混合物，调节超生发生器的功率等级，使其略低于微探头的限值（约为 4.9），让试管在冷水浴中进行超声波降解 1 分钟。

9. 再加入 2/3 的水 / 胆酸钠 / 甘油混合物，重复超声波降解 1 分钟。加入最后一份水 / 胆酸钠 / 甘油混合物，再进行超声波降解 1 分钟。如果水 / 胆酸钠 / 甘油混合物在乳剂中未能完全溶解，则进一步进行超声波降解。

10. 从超声探针上取下试管，检查 pH 值（本配方约为 7.6）。根据需要，使用 0.1N 氢氧化钠小心将 pH 值调节到 7.5 到 8.5 的范围内。更接近 7.5 的 pH 值在生理学上适合于动物给药。

11. 将试管放回冷水浴中的超声探针上，连续进行 8 分钟的超声波降解。

12. 注意乳剂应为白色不透明，类似浓奶油。

13. 用 0.45μm 的过滤膜（聚醚砜 0.45μm 孔径的过滤膜，如 Millipore Millex-HP 注射器式过滤器 SLHPM33RS，放射消毒）。乳剂最好看来未发生改变。

14. 将含有乳剂的 PG796(MRx102) 用于测试对象开展适当的研究。

[0109] 用于制备 5ml 含有 PG796(MRx102) 的乳剂的成分

配合 PG796（MRx102）的成分	**数量**
三辛酸甘油酯 （g）	1
大豆油 （g）	1
磷脂（g）	0.1
甘油 （g）	0.125
胆酸钠 （g）	0.01
PG796（MRx102）（mg）	5
水 （ml）	2.77

[0110] 成分（赋形剂）浓度范围

成分↓配方→	范围	E-0212-4
三辛酸甘油酯	0％ -50％	20％

大豆油	0% -45%	20%
磷脂	1% -3%	2%
甘油	1% -5%	3%
胆酸钠	0.1% -0.3%	0.2%
水	50% -60%	55%

[0111] 可选成分（赋形剂）

可选成分或赋形剂如下所示。

1. 三辛酸甘油酯包括

a. 三己酸甘油酯

b. 三庚酸甘油酯，

c. 三壬酸甘油酯，

d. 癸酸丙三醇酯

2. 大豆油

a. 蓖麻油，

b. 玉米油，

c. 棉花籽油，

d. 橄榄油，

e. 花生油，

f. 薄荷油，

g. 红花油，

h. 芝麻油，

i. 氢化植物油，

j. 氢化大豆油，以及

k. 椰子油的中链甘油三酯

l. 中链甘油三酯棕榈仁油

3. 磷脂

a. 氢化大豆磷脂酰胆碱，

b. 二硬脂酰磷脂酰甘油酯，

c. L-α-二肉豆蔻酰磷脂酰胆碱，

d. L-α-二肉豆蔻酰甘油磷脂

4. 甘油

a. 聚乙二醇 300,

b. 聚乙二醇 400,

c. 乙醇，

d. 丙二醇，

e. N- 甲基 -2- 吡咯烷酮，

f. 二甲基乙酰胺，以及

g. 二甲亚砜

5. 胆酸钠

a. 牛磺胆酸钠，

b. 牛磺－β－鼠胆酸钠，

c. 牛磺脱氧胆酸钠，

d. 牛磺鹅去氧胆酸钠，

e. 甘氨胆酸钠，

f. 甘氨脱氧胆酸钠，以及

g. 甘氨鹅脱氧胆酸钠

[0112] 或者上述规程可以通过步骤 8 的第一部分执行，其中那个 PG796(MRx102) 悬浮 / 溶解在磷脂 / 油混合物中，而悬浮液 / 溶液随后可以作为药品储存。因此，本成分无水，最大程度地降低了雷公藤甲素或雷公藤甲素类似物的水解可能性，延长了保存期限，然后可以按照步骤 8 加入水 / 胆酸钠 / 甘油混合物，并可以执行剩余程序，继续进行到上述的步骤 14，到向研究对象给药为止。

[0113] 同样地，为了促进稳定性、分散和过滤，可以对该成分进行消毒（如过滤、高压蒸汽灭菌），以及 / 或者可以加入其他赋形剂以促成所需大小的药丸。

[0114] 初步的乳剂评估

[0115] 打算注射或输液给药药用乳剂通常包含甘油三酯，如含有天然磷脂（蛋黄或大豆）的大豆油 (SBO)，采用高压均质机乳化。非离子型表面活性剂，如吐温类（聚山梨醇酯）、Solutol®和 Kolliphor(Cremophor®)，一般不用于注射或输液配方，因为它们受热会发生相转化，而可注射的乳剂通常是经过高温消毒的。但一些初步调研是从非离子型表面活性剂着手的。

[0116] 探究了非离子型表面活性剂聚山梨醇酯 80（又名吐温 80）与 Span 80 的各种比值，制备并检测了以下配方。将三辛酸甘油酯 (GTO) 作为甘油三酯油，因为研究表明 PG796(MRx102) 在 GTO 中的溶解度比在 SBO 中大大约 3.4 倍。配方及结果如表 4 所示。这项初步实验的结果是鼓舞人心的，因为含有近 70％水的配方达到了相当高的溶解度。

[0117] 表 4 :初步乳剂配方及溶解度

GTO	Span 80	吐温 80	水	PG796（MRx102） 的溶解度 （μg/ml）
29.4%	1.65%	0.31%	68.6%	681

[0118] 由于大鼠静脉注射时缺少副作用可以接受的共溶剂 / 表面活性剂配方，因此考虑了乳剂。为乳剂配方选择了下列预期的特性 :

- 单独作为媒介，在体内（对啮齿动物）不具备明显副作用。
- PG796(MRx102) 的稳定浓度 >2mg/ml，
- 过滤后保留了 95％的 PG796(MRx102) 浓度，
- 可在 7 天内保持可以接受的稳定度，以及
- 与 MRx 100 相容。

[0119] 使用探头超声波仪在水相中分散油相，形成奶油状不透明悬浮液，来配制乳剂配方。

[0120] 范围确定配方

[0121] 典型的乳剂配方含有 10-30％的甘油三酯，最常见的是 SBO，用 0.5-2％的磷脂分散在水相中，其中含有甘油作为张度剂。然而，由于 PG796(MRx102) 观察到的低溶解度，初始配方采用 40％的 GTO，一种中链甘油三脂，配制，研究发现其中的 PG796(MRx102) 溶解度较高。此外，部分配方加入了 PEG-400 和乙醇以降低水相的极性，以提高溶解度。一些配方包含胆酸钠作为助表面活性剂。这些配方，加上目测评估和 PG796(MRx102) 的溶解度值参见表 5。所有配方都达到了至少为 1mg/mL 的溶解度。在每种情况下，储存八天后发现效力有所损失，但保持了大部分的原始效力。PEG-400 和乙醇对于提高溶解度只是略微有益，其中一种含有 PEG-400 的配方未能形成均质的乳剂。

[0122] 表 5 :第一轮乳剂配方及溶解度

配方号→ / **组分↓**		**E-1**	**E-2**	**E-3**	**E-4**	**E-5**
三辛酸甘油酯		40%	40%	40%	40%	40%
磷脂		2%	2%	2%	2%	2%
PEG-400		10%	--	--	10%	--
乙醇		--	10%	--	--	10%
胆酸钠		--	--	0.2%	0.2%	0.2%
水		48%	48%	58%	48%	48%
PG796（MRx102）		----------2mg/mL ----------				
目测评估		2 层	均质	均质	均质	均质
PG796（MRx102）的溶解度（μg/mL）	0 小时	1560	1913	1529	1787	1673
	1 小时	1677	1879	1514	1795	1680
	24 小时	1484	1939	1353	1762	1654
	8 天	--	1353	1176	1470	1329

[0123] pH 值对稳定性的影响

[0124] 药用乳剂一般是在中性或略碱性 pH 值下配制的，因为它们是通过脑磷脂、游离脂肪酸盐和胆酸盐这类对 pH 值很敏感的阴离子表面活性剂产生的液滴之间的静电排斥实现稳定的。然而，这一 pH 值范围对 PG796(MRx102) 的化学稳定性来说有可能不是最理想的。为了检测这一点，在 4-8 之间的不同 pH 值下配制了多种乳剂。加入了缓冲液来控制 pH 值，并加入了对 pH 值不敏感的表面活性剂，十二烷基硫酸钠来代替胆酸钠，以确保甚至是在低 pH 值乳剂中的负电荷。配方和结果如表 6 所示。所有配方在室温下在长达两周的时间内都相当稳定。尽管效力和纯度有所差异，但相对于 pH 值没有明显趋势，这说明乳剂中 PG796(MRx102) 的稳定性在这一范围内与 pH 值无关。

[0125] 表 6 :pH 值对乳剂中 PG796(MRx102) 稳定性的影响

目标 pH 值 → 组分 ↓		4.0	5.0	6.0	7.0	8.0
三辛酸甘油酯		40%	40%	40%	40%	40%
磷脂		2%	2%	2%	2%	2%
乙醇		10%	10%	10%	10%	10%
缓冲液中 0.1% SDS		48%	48%	48%	48%	48%
缓冲液 （10 mM）		乙酸盐	乙酸盐	组氨酸	磷酸盐	三异丙基乙磺酰
PG796（MRx102）		----------1mg/mL----------				
测得的 pH 值		4.06	4.97	5.98	7.03	8.05
PG796（MRx102）的溶解度（μg/mL）	0 小时	870	1006	1093	996	929
	24 小时	917	922	890	849	929
	1 周	1001	972	948	760	1041
	2 周	848	910	850	822	930
PG796（MRx102）的纯度值面积 %	0 小时	98.9	99.2	99.2	99.4	98.7
	24 小时	99.3	99.4	99.2	99.1	99.2
	1 周	98.9	99.1	98.9	98.8	99.1
	2 周	97.2	98.3	98.8	91.0	98.3

[0126] 第二轮乳剂配方

[0127] 为了改变 40％的三辛酸甘油酯媒介，配方的配制采用含量较低的甘油三酯和 / 或部分或全部取代的大豆油来代替三辛酸甘油酯。这种配方及其溶解度数据如表 7 所示。列出两个值时，针对的是重复分析。这些配方在 121℃下高温消毒 8 分钟。还配制了配方 E-0212-4 的安慰剂版本，并对其进行消毒处理，以测定安慰剂成分在 HPLC 分析中的共出峰水平，试验发现该水平为 1.23％。

[0128] 正如所预期的那样，降低甘油三酯的含量并用大豆油取代部分或全部的三辛酸甘油酯导致药物的溶解度有所降低。但只有在甘油三酯的含量从 40％降到了 30％，所有 GTO 被大豆油所取代的配方 E-0212-1 中，PG796(MRx102) 的溶解度远低于 1mg/mL。

[0129] 表 7 第二轮乳剂配方

配方号 → 组分 ↓	E-0212-1	E-0212-2	E-0212-3	E-0212-4	E-0212-5
三辛酸甘油酯	--	15%	30%	20%	--
大豆油	30%	15%	--	20%	40%
磷脂	2%	2%	2%	2%	2%
甘油	3%	3%	3%	3%	3%
胆酸钠	0.2%	0.2%	0.2%	0.2%	0.2%
水	65%	65%	65%	55%	55%
PG796（MRx102）	----------1mg/mL----------				

PG796（MRx102）的溶解度（μg/mL）	初始	682	929,928	968	1090, 991	934, 867
	消毒后	621	771, 847	913	1046, 905	913,867
PG796（MRx102）的纯度（峰值面积%）	初始	96.2%	96.5, 96.6%	97.9%	98.1,97.3%	95.5, 96.1%
	消毒后	94.6%	95.6, 96.3%	97.2%	97.4, 96.7%	99.6, 95.9%

[0130] 关于乳剂的毒理学发现

[0131] 大鼠静脉注射了5mL/kg的配方E-3(40％GTO,2％磷脂,0.2％胆酸钠)。这些动物注射后短期内表现正常,但变得嗜睡,而且随后在5-10分钟内由于呼吸困难而倒下。大鼠在60-90分钟内复原并表现正常。第二天第二次给药观察到会造成更加严重的症状。接下来两天的注射产生了类似的反应。第二组大鼠静脉注射5mL/kg的配方E-5(与E-3相同的配方,但添加了10％的乙醇)。所有这些动物都在10分钟后躺倒不动,并在大约45分钟后死亡。

[0132] 在2mg/mL PG796(MRx102)的高浓度下对配方E-3进行检测,发现它是可溶的。较高的浓度使剂量响应降低。因此,一组大鼠以1.5mL/kg的配方E-3的降低剂量给药。这些动物注射后8-10分钟内表现正常,随后躺倒8-10分钟。因此不良反应较为不严重,而且这种剂量下躺倒的时间和复原的时间较短。三个实验的结果汇总于表8。

[0133] 表8:采用乳剂配方的初始大鼠研究

	实验1	实验2	实验3
组分↓配方→	E-3	E-5	E-3
三辛酸甘油酯	40％	40％	40％
磷脂	2％	2％	2％
乙醇	- -	10％	- -
胆酸钠	0.2％	0.2％	0.2％
水	58％	48％	58％
注射量i.v.	5ml/kg	5ml/kg	1.5ml/kg
死亡	无	全部	无
复原时间	60-90分钟	不适用	15-17分钟

[0134] 在乳剂与纯大豆油(40％,乳剂E0212-4)和三辛酸甘油酯和大豆油的等量混合物(每种20％,乳剂E0212-5)的比较中,大鼠每天静脉注射3mL/kg,注射4天。在注射的第一

天，这些动物注射的是 E0212-4，注射后在 7 分钟时变得略微嗜睡，到 40 分钟完全复原。注射 E0212-5 的大鼠在 8 分钟时略微嗜睡，到 35 分钟完全复原。之前的测试已经表明大鼠在静脉注射不同的乳剂配方后会躺倒很长一段时间，症状更严重。采用这两种最新的乳剂配方为大鼠进行静脉注射时，结果有所改善。为大鼠进行乳剂注射的副作用在第 2-4 天与第 1 天观察到的情况非常相似。使用 40％的 SBO 不能完全消除使用 20％ GTO/20％ SBO 时观察到的副作用。第一次注射后观察到的副作用不如 5ml/kg，甚至是 1.5ml/kg 的配方 3 严重。没有出现呼吸困难，只有轻微的嗜睡，而较早的研究出现了呼吸困难和嗜睡。

[0135] 20％ GT/20％ SBO 的乳剂配方 (E-0212-4) 表现出了可以接受的化学溶解度 / 稳定性，在大鼠研究的媒介单独检测中是非致死性的，会造成轻微的副作用 (小于其他乳剂配方制剂)，它被选为用于 PG796(MRx102) 和 MRx100 针对大鼠的升级剂量 /7 天重复剂量比较研究，以及 PG796(MRx102) 针对犬类的升级剂量 /7 天重复剂量研究的修改媒介配方。

[0136] 表 9 比较乳剂配方的大鼠副作用研究

组分↓配方→	E-0212-4	E-0212-5
三辛酸甘油酯	20％	- -
大豆油	20％	40％
磷脂	2％	2％
甘油	3％	3％
胆酸钠	0.2％	0.2％
水	55％	55％
注射量 i.v.	3ml/kg	3ml/kg
死亡数	无	无
复原时间	40 分钟	35 分钟

[0137] 药代动力学 / 毒性动力学的考虑

[0138] 雷公藤甲素分子作用机理仍然难以捉摸，但根据有关报告，雷公藤甲素与转录因子 TFIIH 的亚单位，人体 XPB(也被称为 ERCC3) 共价结合，抑制其依赖于 DNA 的 ATP 酶活性，造成对 RNA 聚合酶 II- 介导转录以及可能的核苷酸切除修复的抑制。确认 XPB 为雷公藤甲素的目标解释了雷公藤甲素的许多已知生物作用。例如，雷公藤甲素与 XPB 结合导致包括 NF kappa B(NF-κB) 在内的一些生长和存活促进剂以及抑凋亡因子 Mcl-1 和 XIAP 的下调。(Titov, 等，自然化学生物学 (2011)7(3):182-8)。随后，还发现雷公藤甲素衍生物 MRx102 具有降低 mRNA 水平、降低 NF-κB 以及降低 Mcl-1 和 XIAP 等作用。在纳摩尔级的低浓度下，MRx102 还会引发 AML 患者 CD34(+) 源祖细胞，以及更重要的是 CD34(+)CD38(-) 干细胞 / 源祖细胞的大量凋亡，甚至是在他们受到与源自骨髓的间充质干细胞的共培养保护的情况下。在体内，MRx102 会大大降低白血病的负担，并延长带有 Ba/F3-ITD 细胞的非肥

胖型糖尿病 / 重度联合免疫缺陷小鼠的存活时间。因此,MRx102 在体外和体内都具备有效的抗白血病作用,有可能消除 AML 干细胞 / 源祖细胞,攻克白血病细胞的微环境保护,并保证了临床研究。(Carter 等,(2012) 白血病 26:443-50)。而且,雷公藤甲素和雷公藤甲素衍生物可以作为新型分子探针用于研究转录,还可能通过抑制 XPB 的 ATP 酶活性成为新型抗癌药。

[0139] XPB 结合的另一个结果是抑制核苷酸切除修复。阻断 DNA 修复这种作用会增强以 DNA 为靶标的药物的活性,包括针对实体瘤的顺铂和拓扑异构酶 1 抑制剂 ;研究表明这两种药物都会与雷公藤甲素发生增效作用。采用 MV4-11 细胞在体外对 MRx102 与用于 AML 的两种药物,阿糖孢苷和依达比星,之间可能的增效作用进行了研究,证明 MRx102 与用于 AML 的这两种药物之间存在增效作用。

[0140] 有关雷公藤甲素和雷公藤甲素衍生物的一个担忧是它们的环氧结构,被认为可能有毒 ;但蛋白酶体抑制剂抗癌药卡非佐米 (来那度胺) 是含有环氧化物的一种环氧甲酮四肽,最近获得了 FDA 的核准。而且雷公藤甲素虽然是一种三环氧化物,但 Titov 等人 (见上文) 的研究表明它在结合特性方面具有灵敏的选择性,而不是混杂的。尽管如此,在一些动物研究以及临床中所报告的雷公藤甲素安全问题已经造成了“形象问题”,并构成了可能的安全挑战 ;因此雷公藤甲素仍未被视为适于临床使用,也尚未进行商业开发。

[0141] 通常认为雷公藤甲素前体药物比雷公藤甲素更为安全。在一项最初的啮齿动物毒理学研究中,PG796(MRx102) 在 7 天高达 1.5mg/kg/ 天的静脉注射剂量下未表现出总体的或组织病理学的毒性作用。雷公藤甲素前体药物作为乳剂配方,被认为其毒性动力学曲线的特点是平坦的 AUC 加上最小化的 $C_{最大}$。[两者结合,假定要达到最佳功效需要对 RNA 聚合酶进行持续抑制,则反过来需要长期服用药物的药代动力学曲线]。图 1 给出了 PG796(MRx102) 和雷公藤甲素并行比较毒理学研究,其中两种药物都采用本文所公开的创新型乳剂配方向啮齿动物进行静脉给药,研究证明,基于总体和组织病理学标准,PG796(MRx102) 的毒性至少要比雷公藤甲素低 20 倍。PG796(MRx102) 的无作用剂量 (“NOAEL”) 对于啮齿动物七天静脉注射再次超过 1.5mg/kg/ 天,确认了初始结果。有趣的是询问雷公藤甲素的前体药物为什么会比天然产品本身更安全 ;在不希望被理论所束缚的同时,也许答案就在于直接服用或通过其载体 PG796(MRx102) 释放的雷公藤甲素的药代动力学曲线。单独提供雷公藤甲素时 (参见图 1 中连接圆圈的线条),其 $C_{最大}$ 非常高而且急速下降使得在给药后两个小时,循环中再无残留。尽管如此,施用前体药物 PG796(MRx102) 时,雷公藤甲素的 $C_{最大}$ 约为雷公藤甲素直接给药情况下 (参见图 1 中连接三角形的线条) 的十分之一,而雷公藤甲素的血液浓度仍较为稳定,而且正如在两小时时间点所观察的那样,证明了较长的 AUC(“曲线下面积”)。它也仍高于治疗水平 (用不带符号的粗线表示)。在 PG796(MRx102) 对比雷公藤甲素的 $C_{最大}$ /AUC 曲线图中的差异被认为是由于脂质前体药物 / 乳剂配方组合的理化特性。一般来说,疏水性系数大于 0.5 的雷公藤甲素前体药物相对于水来说,更易溶于油脂,而且预期要花更长的时间来转化药物剂型 ;这些特点可能产生更平的转化曲线和较少的药物释放 $C_{最大}$ 尖峰。

[0142] PG490-88 通过静脉给药,进入临床试验并在 AML 患者身上表现出了前景广阔的作用。(Xia Zhi Lin 和 Zhen You Lan,血液学,93:14(2008))。尽管如此,作为一种前体药物,它被不完全不规律地转化为活性实体,雷公藤甲素,而且,正因为如此,可能成为产生

毒性的原因。但 PG490-88 的确具备得到优化的 AUC，随时间推移相对平坦，没有强烈的 $C_{最大}$。利用人体血清将 PG796(MRx102) 快速而且完全地转化为雷公藤甲素（同样在大鼠和犬类体内观察到），而 PG490-88Na 在人体血清中不完全地被转化为雷公藤甲素的发现论证了 PG796(MRx102) 的转化并不取决于种酶（酯酶）活性的变化，而是取决于脂质前体药物 / 乳剂配方的理化特性。

[0143] 将脂质乳剂作为药物释放体系的研究已经开展了一段时间。(参见Hippalgaonkar 等，(2010)AAPS 制药科技 11(4):1526—1540 ;Stevens 等，(2003) 商业简报 :制药技术 2003,p.1-4)。固体脂质纳米粒 (SLN) 释放体系可能相对于具有生物活性的植物提取物具备优势，比如提高溶解度和生物利用度，提供抗毒保护，以及提高药理活性。根据相关报告，一种雷公藤多苷 (TG) 的固体脂质纳米粒 (TG-SLN) 释放体系具有抗 TG- 引发的雄性生殖毒性的保护作用。在一项针对大鼠的 TP-SLN 和游离 TP 的毒代动力学和组织分布的比较研究中，雷公藤甲素 (TP) 被作为一种模型药物。开发出了一种快速而灵敏的HPLC-APCIMS/MS 方法用于测定大鼠血浆中的雷公藤甲素。十四只大鼠被随机分为 2 组，每组各七只大鼠，每只都进行毒代动力学分析，一组使用游离 TP(450 μg/kg) 而另一组采用 TP-SLN 配方 (450 μg/kg)。给药之前以及给药后 0.083,0.17,0.25,0.33,0.5,0.75,1,1.5,2,3 和 4 小时进行抽血。三十六只大鼠被随机等分成六个组进行组织分布研究。一般的大鼠接受 TP(450 μg/kg) 的灌胃给药，而另一半接受 TP-SLN(450 μg/kg) 给药。在给药后 15,45 和 90 分钟，采取血液、肝脏、肾脏、脾脏、肺部和睾丸组织的样本。采用 LC-APCI-MS-MS 测定样本中的 TP 浓度。纳米配方的毒代动力学结果表现出了曲线下面积 (AUC) 的显著增大 ($P<0.05$)，T（最大）和平均停留时间 (MRTs)(0-t) 的大幅延长 ($P<0.05$)，C（最大）的明显减小 ($P<0.05$)。纳米配方促进了吸收，具有缓释特点，说明毒代动力学的改变对于提高纳米配方的功效来说可能是最重要的机理。组织分布的结果说明了肺部和脾脏中 TP 浓度趋于增加，同时 TP-SLN 组中血浆、肝脏、肾脏和睾丸中的 TP 浓度趋于降低。在多个时间点，TP-SLN 组睾丸组织的 TP 浓度低于游离 TP 组。这为采用 TP-SLN 时所观察到的生殖毒性的降低提供了一条重要线索。总体而言，口服的雷公藤甲素脂质纳米粒配方促进了吸收，具有缓释特点 (Xue 等，(2012) 欧洲制药科学杂志，47(4):713-7)。纳米配方的毒代动力学结果表现出了 AUC 的显著增加和 $C_{最大}$ 的减小。这些结果说明毒代动力学的变化是提高安全性的一个考虑因素。

[0144] 药代动力学数据

[0145] Calvert 和 SRI 研究中雷公藤甲素含量的 TK 比较 - 雄性和雌性

[0146] 血浆雷公藤甲素浓度 (ng/ml)

血浆雷公藤甲素浓度 (ng/ml)

时间 （小时）>	0	0.25	0.5	1	2	24
PG796（MRx102） 0.5 mg/kg （乳剂）	0	11.8	10.5	3.1	5.8	0
雷公藤甲素 0.15 mg/kg （乳剂）	0	74.6	18.4	13.2	0	0
PG796（MRx102） 1.5 mg/kg （DMSO/PEG400/PBS）	0	36.4	29.1	16.7	4.11	0

MRx1020.5mg/kg 和雷公藤甲素 0.15mg/kg 来自于 Calvert 研究；结果来自于雌性。

MRx1021.5mg/kg 来自于 SRI 研究，结果来自于雄性。

SRI 研究 -3,4,8 小时。雷公藤甲素浓度＝ 0ng/ml

[0147] Calvert 和 SRI 研究中雷公藤甲素含量的 TK 比较 - 仅雄性

	血浆雷公藤甲素浓度（ng/ml）					
时间 （小时）>	0	0.25	0.5	1	2	24
PG796（MRx102） 0.5 mg/kg （乳剂）	0	32.5	10.9	0.7	1.0	0
雷公藤甲素 0.15 mg/kg （乳剂）	0	59.0	14.9	3.6	0	0
PG796（MRx102） 1.5 mg/kg （DMSO/PEG400/PBS）	0	36.4	29.1	16.7	4.1	0

MRx1020.5mg/kg 和雷公藤甲素 0.15mg/kg 来自于 Calvert 研究；结果来自于雄性。

MRx1021.5mg/kg 来自于 SRI 研究；结果来自于雄性

SRI 研究 -3,4,8 小时。雷公藤甲素浓度＝ 0ng/ml

[0148] 给药途径

[0149] 虽然在一些实施例中给药途径是静脉注射，但其他途径包括：皮肤表面或局部、皮内、皮下、鼻腔、动脉内、肌肉内、心脏内、骨内输液、囊内、腹膜内、膀胱内、玻璃体内、海绵体内注射、阴道内以及子宫内。

例 2

细胞毒性 (MTT) 试验

[0150] 检测化合物可以以 20mM 的浓度溶解在 DMSO 中。进一步的稀释可以在用 10％的胎牛血清补充的 RPMI1640 媒介 (GIBCO,Rockville,MD) 中完成 (HyClone 实验室,Logan,UT)。

在标准 MTT 试验中使用细胞增殖试剂盒 I(#1465007,罗氏诊断公司，曼海姆，德国）测定化合物的细胞毒性。简单而言，在存在检测化合物连续三倍稀释液或含有和每个稀释点的检测样品浓度相同的 DMSO 的培养基的情况下，将人类 T 细胞淋巴瘤（白血病）细胞（每孔 4x 10^5个）在 96 孔组织培养皿中培养 24 小时。随后培养基用 10μl/ 孔的 MTT 试剂补充 4 小时，然后再加入 0.1ml/ 孔的增溶试剂，再持续 16 小时。在 ThermoScan 微孔板分析仪 (Molecular Devices,Menlo Park,CA) 上测定 570 纳米 (OD_{570}) 处的光密度。

例 3

IL-2 生产试验

[0151] 检测样品在完全组织培养基中可以稀释到 1mM。将等分试样放置在涂有抗 CD3 抗体（用于刺激白血病细胞产生的 IL-2) 的微量细胞培养板中，制备系列稀释液，使最终浓度包含从 0.001 到 10,000nM 按对数增长的范围。采集在白血病人体 T 细胞株的指数膨胀培养基中产生的细胞 (#TIB-152,取自美国标准菌库，马纳萨斯，VA)，离心清洗一次，将其重新悬浮在完全组织培养基中，并稀释到 2x 10^6个细胞 /ml 的浓度。将体积为 50μl 的白血病细胞（1x 10^5个细胞）加入含有 100μl 稀释化合物的孔中，将 50μl PMA(10ng/ml) 加入每个孔，随后将培养板放在 37℃下在 5％ CO2 恒温箱中进行培养。24 小时后，对培养板进行离心

处理以沉淀细胞，从每个孔中取出 150μl 的上清液，将样品储存在 -20℃下。使用 Luminex 100(Luminex 公司，Austin,TX)、Luminex 微球体结合抗 IL-2 捕捉抗体，以及结合荧光的抗 IL-2 检测抗体来分析所储存的上清液的人体 IL-2 浓度。数据用 pg/ml 的 IL-2 来表示。

[0152] 虽然上文探讨了示例的一些方面和实施例，但该领域的那些技术人员将认识到其中的某些修改、置换、添加和重组。因此以下随附的权利要求书和下文介绍的权利要求书预期包含在其真实意志和范围内的所有此类修改、置换、添加和重组。

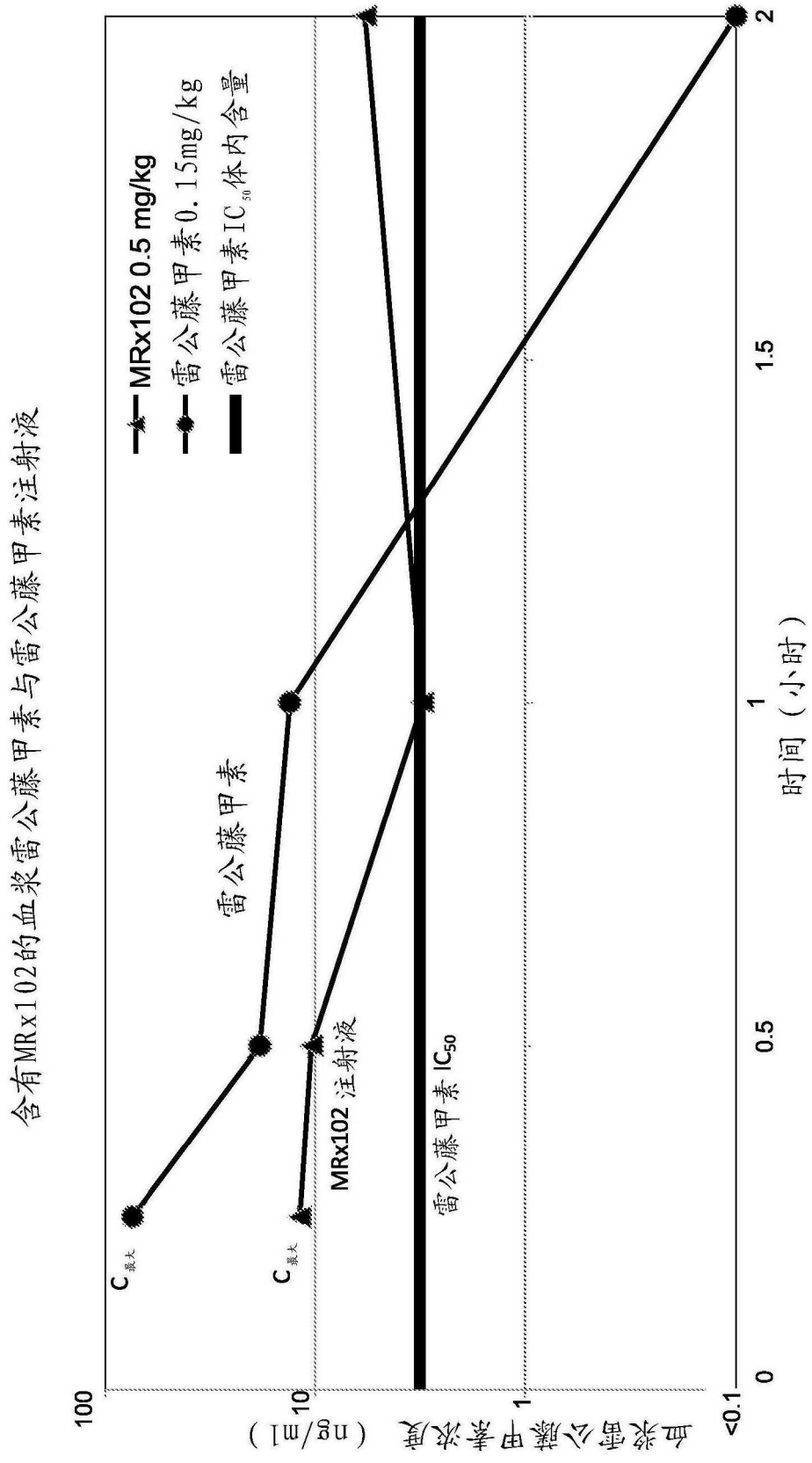

含有MRx102的血浆雷公藤甲素与雷公藤甲素注射液
MRx102 0.5 mg/kg
雷公藤甲素0.15mg/kg
雷公藤甲素IC50体内含量
雷公藤甲素
MRx102 注射液
雷公藤甲素 IC50
C最大
C最大
血浆雷公藤甲素浓度 (ng/ml)
100
10
1
<0.1
0
0.5
1
1.5
2
时间（小时）

图 1