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(54) Title: AQUEOUS FORMULATION WITH IMPROVED STABILITY

(57) Abstract: An aqueous formulation with improved storage stability of a biologically active agent is provided. The aqueous formulation includes compounds which can be an organic acid conjugated to a polyalkylene oxide, wherein the organic acid contains one or more cyclic disulfide bond or dithiols, which can form intramolecular cyclic disulfide bond. Preferred compounds included in the aqueous formulation include polyalkylene oxide conjugates of lipoic acid, dihydrolipoic acid, and other 1,3-dithiol or 1,3-disulfide containing organic acids. Additionally, preferred polyalkylene glycols are polyethylene glycol and polypropylene glycol.

AQUEOUS FORMULATION WITH IMPROVED STABILITY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority from U.S. Provisional Patent Application Serial No. 61/373,594 filed August 13, 2010, the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to aqueous formulations with an improved stability. Stabilizing compositions for inclusion thereof are also disclosed.

BACKGROUND OF THE INVENTION

Stable formulations of therapeutic agents are required for administering these agents intact to the subject in need. Many therapeutic agents are known to decompose or change their chemical integrity upon storage due to environmental stresses including oxidation. These changes eventually alter the therapeutic activities of the agents in negative fashion, i.e. reduced potency, etc. Thus, there have been many efforts to protect these therapeutic agents from oxidation.

An antioxidant is a molecule capable of inhibiting the oxidation process. Usually oxidation is a chemical process that removes electron(s) from a substance or reacts with oxygen and degrades. The oxidation process can also generate free radicals which further degrade the substance. Antioxidants inhibit the oxidation of the substance by blocking these processes via various mechanisms, such as by removing oxygen from the environment and thus, anti-oxidants themselves can be oxidized during the process.

Many anti-oxidants are not water soluble and cannot inhibit the oxidation process in aqueous solution effectively and require a non-aqueous solvent in the formulation. Therefore, there is still a need to provide a composition which can be effectively employed in an aqueous formulation or in a lyophilized powder for an aqueous formulation.

SUMMARY OF THE INVENTION

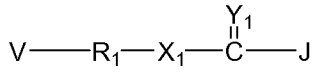
The present invention relates to aqueous formulations with an improved stability, which includes polyalkylene oxide conjugated organic acids, containing disulfide bonds or dithiols.

In one aspect, the present invention more specifically provides an aqueous formulation having improved stability which includes:

a) water or a pharmacologically suitable fluid comprising water;

b) from about 0.01 to about 200 mg/mL of a biologically active agent; and

c) from about 0.2 to about 150 mg/mL of a compound of the formula (I):

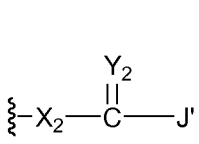


(I)

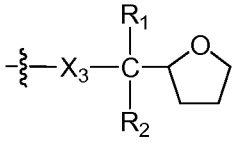
wherein,

R_1 is residue of a polyalkylene oxide;

V is selected from among H, NH_2, OH, CO_2H, C_{1-6} alkoxy C_{1-6} alkyl,



, and



;

wherein,

X_{1-3} are independently O, S or NH;

Y_{1-2} are independently is O, or S;

R_{1-2} are independently selected from among hydrogen,

C_{1-8} alkyl, C_{1-8} branched alkyl, C_{1-8} substituted alkyl, aryl, aralkyl,

C_{1-8} alkylcarbonyl, C_{1-8} branched alkylcarbonyl, C_{1-8} substituted alkylcarbonyl, arylcarbonyl, or aralkylcarbonyl; and

$C(=Y_1)$-J or $C(=Y_2)$-J' is independently a residue of an organic acid containing one or more intramolecular cyclic disulfide bond or dithiol functional groups which can form intramolecular cyclic disulfide bond.

Another aspect of the present invention provides a lyophilized powder for an aqueous formulation. The powder includes:

a) from about 0.01 to about 20 wt% of a biologically active agent; and

b) from about 0.2 to about 15 wt% of a compound of the formula (I) as defined above.

A still further aspect of the present invention provides a method of preparing an aqueous formulation having improved stability from the lyophilized powder. The method includes contacting the lyophilized powder described above with water.

One advantage of the present invention is that unlike most prior art stabilizers, the compounds of Formula (I) are water-soluble. Thus, they can inhibit oxidation of biologically active agents included in the formulations more effectively.

The improved stability properties are also realized after lyophilized powders prepared in accordance with the present invention are reconstituted.

Other and further advantages will be apparent from the following description.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. In the event that there is a plurality of definitions for a term herein, those in this section prevail unless stated otherwise.

For purposes of the present invention, the term "residue" shall be understood to mean that portion of a compound, to which it refers, e.g., sugar, etc. that remains after it has undergone a substitution reaction with another compound.

For purposes of the present invention, the term “alkyl” refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. The term “alkyl” also includes alkyl-thio-alkyl, alkoxyalkyl, cycloalkylalkyl, heterocycloalkyl, and C_{1-6} alkylcarbonylalkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from about 1 to 7 carbons, yet more preferably about 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted, the substituted group(s) preferably include halo, oxy, azido, nitro, cyano, alkyl, alkoxy, alkyl-thio, alkyl-thio-alkyl, alkoxyalkyl, alkylamino, trihalomethyl, hydroxyl, mercapto, hydroxy, cyano, alkylsilyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, alkenyl, alkynyl, C_{1-6} hydrocarbonyl, aryl, and amino groups.

For purposes of the present invention, the term “substituted” refers to adding or replacing one or more atoms contained within a functional group or compound with one of the moieties from the group of halo, oxy, azido, nitro, cyano, alkyl, alkoxy, alkyl-thio, alkyl-thio-alkyl, alkoxyalkyl, alkylamino, trihalomethyl, hydroxyl, mercapto, hydroxy, cyano, alkylsilyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, alkenyl, alkynyl, C_{1-6} alkylcarbonylalkyl, aryl, and amino groups.

For purposes of the present invention, the term “alkenyl” refers to groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has about 2 to 12 carbons. More preferably, it is a lower alkenyl of from about 2 to 7 carbons, yet more preferably about 2 to 4 carbons. The alkenyl group can be substituted or unsubstituted. When substituted the substituted group(s) preferably include halo, oxy, azido, nitro, cyano, alkyl, alkoxy, alkyl-thio, alkyl-thio-alkyl, alkoxyalkyl, alkylamino, trihalomethyl, hydroxyl, mercapto, hydroxy, cyano, alkylsilyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, alkenyl, alkynyl, C_{1-6} hydrocarbonyl, aryl, and amino groups.

For purposes of the present invention, the term “alkynyl” refers to groups containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic

groups. Preferably, the alkynyl group has about 2 to 12 carbons. More preferably, it is a lower alkynyl of from about 2 to 7 carbons, yet more preferably about 2 to 4 carbons. The alkynyl group can be substituted or unsubstituted. When substituted the substituted group(s) preferably include halo, oxy, azido, nitro, cyano, alkyl, alkoxy, alkyl-thio, alkyl-thio-alkyl, alkoxyalkyl, alkylamino, trihalomethyl, hydroxyl, mercapto, hydroxy, cyano, alkylsilyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heteroaryl, alkenyl, alkynyl, C_{1-6} hydrocarbonyl, aryl, and amino groups. Examples of "alkynyl" include propargyl, propyne, and 3-hexyne.

For purposes of the present invention, the term “aryl” refers to an aromatic hydrocarbon ring system containing at least one aromatic ring. The aromatic ring can optionally be fused or otherwise attached to other aromatic hydrocarbon rings or non-aromatic hydrocarbon rings. Examples of aryl groups include, for example, phenyl, naphthyl, 1,2,3,4-tetrahydronaphthalene and biphenyl. Preferred examples of aryl groups include phenyl and naphthyl.

For purposes of the present invention, the term “cycloalkyl” refers to a C_{3-8} cyclic hydrocarbon. Examples of cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

For purposes of the present invention, the term “cycloalkenyl” refers to a C_{3-8} cyclic hydrocarbon containing at least one carbon-carbon double bond. Examples of cycloalkenyl include cyclopentenyl, cyclopentadienyl, cyclohexenyl, 1,3-cyclo-hexadienyl, cycloheptenyl, cycloheptatrienyl, and cyclooctenyl.

For purposes of the present invention, the term “cycloalkylalkyl” refers to an alklyl group substituted with a C_{3-8} cycloalkyl group. Examples of cycloalkylalkyl groups include cyclopropylmethyl and cyclopentylethyl.

For purposes of the present invention, the term “alkoxy” refers to an alkyl group of indicated number of carbon atoms attached to the parent molecular moiety through an oxygen bridge. Examples of alkoxy groups include, for example, methoxy, ethoxy, propoxy and isopropoxy.

For purposes of the present invention, an “alkylaryl” group refers to an aryl group substituted with an alkyl group.

For purposes of the present invention, an “aralkyl” group refers to an alkyl group substituted with an aryl group.

For purposes of the present invention, the term “alkoxyalkyl” group refers to an alkyl group substituted with an alkoxy group.

For purposes of the present invention, the term “alkyl-thio-alkyl” refers to an alkyl-S-alkyl thioether, for example methylthiomethyl or methylthioethyl.

For purposes of the present invention, the term “amino” refers to a nitrogen containing group as is known in the art derived from ammonia by the replacement of one or more hydrogen radicals by organic radicals. For example, the terms “acylamino” and “alkylamino” refer to specific N-substituted organic radicals with acyl and alkyl substituent groups respectively.

For purposes of the present invention, the term “alkylcarbonyl” refers to a carbonyl group substituted with alkyl group.

For purposes of the present invention, the term “heterocycloalkyl” refers to a non-aromatic ring system containing at least one heteroatom selected from nitrogen, oxygen, and sulfur. The heterocycloalkyl ring can be optionally fused to or otherwise attached to other heterocycloalkyl rings and/or non-aromatic hydrocarbon rings. Preferred heterocycloalkyl groups have from 3 to 7 members. Examples of heterocycloalkyl groups include, for example, piperazine, morpholine, piperidine, tetrahydrofuran, pyrrolidine, and pyrazole. Preferred heterocycloalkyl groups include piperidinyl, piperazinyl, morpholinyl, and pyrrolidinyl.

In some embodiments, substituted alkyls include carboxyalkyls, aminoalkyls, dialkylaminos, hydroxyalkyls and mercaptoalkyls; substituted alkenyls include carboxyalkenyls, aminoalkenyls, dialkenylaminos, hydroxyalkenyls and mercaptoalkenyls; substituted alkynyls include carboxyalkynyls, aminoalkynyls, dialkynylaminos, hydroxyalkynyls and mercaptoalkynyls; substituted cycloalkyls include moieties such as 4-chlorocyclohexyl; aryls include moieties such as napthyl; substituted aryls include moieties such as 3-bromo phenyl; aralkyls include moieties such as tolyl; heteroalkyls include moieties such as ethylthiophene; substituted heteroalkyls include moieties such as 3-methoxy-thiophene; alkoxy includes moieties such as methoxy; and phenoxy includes moieties such as 3-nitrophenoxy. Halo shall be understood to include fluoro, chloro, iodo and bromo.

For purposes of the present invention, “positive integer” shall be understood to include an integer equal to or greater than 1 and as will be understood by those of ordinary skill to be within the realm of reasonableness by the artisan of ordinary skill.

For purposes of the present invention, the term “linked” shall be understood to include covalent (preferably) or noncovalent attachment of one group to another, i.e., as a result of a chemical reaction.

The terms "effective amounts" and “sufficient amounts” for purposes of the present invention shall mean an amount which achieves a desired effect or therapeutic effect as such effect is understood by those of ordinary skill in the art.

It is also to be understood that the terminology employed herein is used for the purpose of describing particular embodiments only and is not intended to be limiting, since the scope of the present invention will be limited by the appended claims and equivalents thereof.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 describes synthesis of bislipoic acid esters of polyethylene glycol (compound **3**) and bisdihydrolipoic acid esters of polyethylene glycol (compound **4**).

FIG. 2 describes synthesis of lipoic acid esters of polyethylene glycol (compound **5**) and dihydrolipoic acid esters of polyethylene glycol (compound **6**).

FIG. 3 describes synthesis of lipoic acid and dihydrolipoic acid esters of polyethylene glycol (compound **9**).

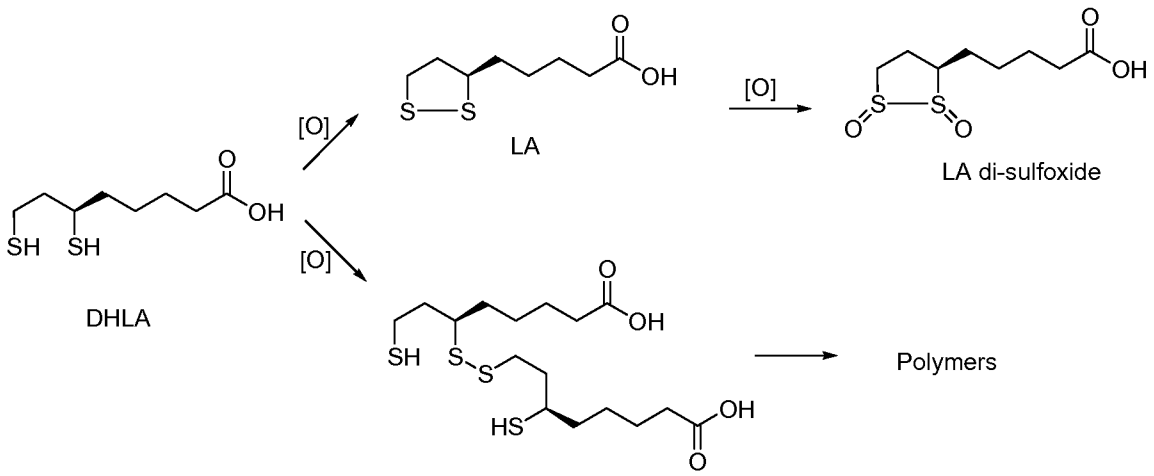
FIG. 4 describes synthesis of lipoic acid esters (compound **11**) and dihydrolipoic acid esters (compound **12**).

DETAILED DESCRIPTION OF THE INVENTION

A. Overview

Many anti-oxidant compounds are reducing agents and include one or more functional groups to be oxidized, e.g. thiols. For example, dihydrolipoic acid (DHLA) is an organosulfur compound and a reduced form of lipoic acid (LA). Even though DHLA or LA can inhibit oxidation processes and can be used as anti-oxidants, their lack of water solubility has limited their utility in liquid pharmaceutical formulations. The present invention addresses this shortcoming by providing water-soluble polyalkylene oxide conjugated anti-oxidants which provide aqueous formulations having improved long-term stability, especially when compared to similar aqueous formulations which do not include a compound of Formula (I) therein.

On a broad aspect, the present invention relates to aqueous formulations containing an antioxidant having dithiol or disulfide moiety and a carboxylic acid moiety which is conjugated to a polyalkylene oxide. The dithiol or disulfide containing organic acids are preferably anti-oxidants or stabilizers containing a disulfide bond which can further oxidized to mono- and di-sulfoxide or dithiol groups which can form disulfide bond upon oxidation which further oxidized to mono- and di- sulfoxides, see, for example, the following diagram for the lipoate system:

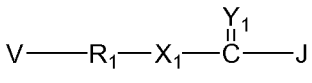


Preferred polyalkylene oxides are polyethylene glycol and polypropylene glycol. Polyvinyl alcohols also can be used as conjugates. In one embodiment, the present invention provides an aqueous formulation (I) having improved stability which includes:

a) water or a pharmacologically suitable fluid including water;

b) from about 0.01 to about 200 mg/mL of a biologically active agent; and

c) from about 0.2 to about 150 mg/mL of a compound of the formula (I):

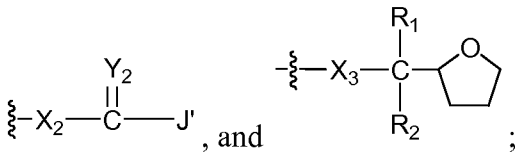


(I)

wherein,

R_1 is residue of a polyalkylene oxide;

V is selected from H, NH_2, OH, CO_2H, C_{1-6} alkoxy C_{1-6} alkyl,



;

wherein,

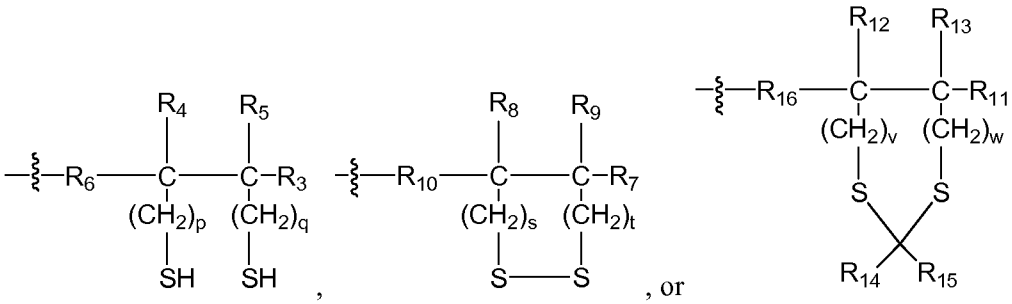
X_{1-3} are independently O, S or NH;

Y_{1-2} are independently is O, or S;

R_{1-2} are independently selected from hydrogen, C_{1-8} alkyl, C_{1-8} branched alkyl, C_{1-8} substituted alkyl, aryl, aralkyl, C_{1-8} alkylcarbonyl, C_{1-8} branched alkylcarbonyl, C_{1-8} substituted alkylcarbonyl, arylcarbonyl, or aralkylcarbonyl; and

$C(=Y_1)$-J or $C(=Y_2)$-J' is independently a residue of an organic acid containing one or more intramolecular cyclic disulfide bond or dithiol functional groups which can form intramolecular cyclic disulfide bond.

The intramolecular cyclic disulfide bond or dithio functional groups of J can be selected from among:



wherein,

R_6, R_{10}, and R_{16} are independently a residue of a bifunctional linking moiety;

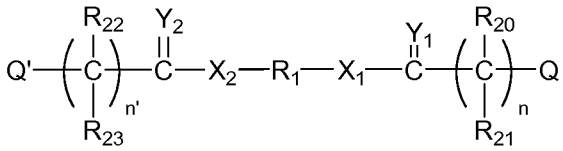
R_{3-5}, R_{7-9}, and R_{11-15} are independently selected from among hydrogen, C_{1-8} alkyls, C_{3-8} branched alkyls, C_{2-8} substituted alkyls, aryls, aralkyls, C_{2-8} alkylcarbonyls, C_{3-8} branched alkylcarbonyls, C_{3-8} substituted alkylcarbonyls, arylcarbonyls, or aralkylcarbonyls; and

(p), (q), (s), (t), (v), and (w) are independently 0, 1, or 2;

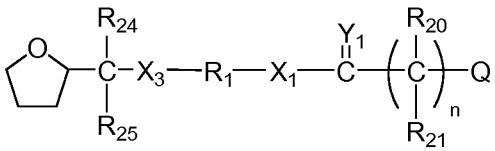
wherein (p)+(q), (s)+(t), and (v)+(w) are independently 1, 2, 3, or 4;

In one embodiment, R_6, R_{10}, and R_{16} are independently a residue of saturated or unsaturated or linear or branched C_{1-10} hydrocarbons; wherein one or more of the carbons is optionally replaced with a heteroatom, which is O, S, or NH.

In one aspect of the invention, the compound of formula (I) is:



(Ia), or



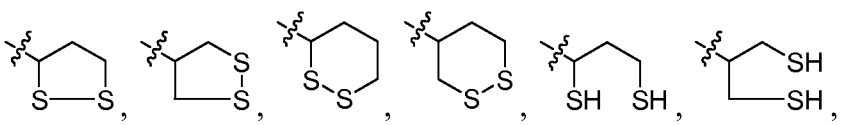
(Ib),

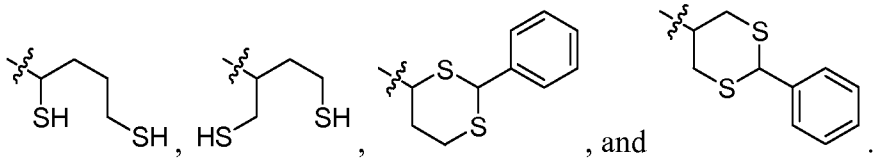
wherein,

R_{20-25} are independently selected from hydrogen, C_{1-8} alkyls, C_{3-8} branched alkyls, C_{2-8} substituted alkyls, aryls, aralkyls, C_{2-8} alkylcarbonyls, C_{3-8} branched alkylcarbonyls, C_{3-8} substituted alkylcarbonyls, arylcarbonyls, or aralkylcarbonyls, preferably C_{1-8} alkyls, and more preferably hydrogen;

(n) or (n') are independently an integer of from about 1 to about 12, preferably 2-10, and more preferably 3-7, and alternatively 4; and

Q or Q' are independently selected from among:





In some embodiments, the aqueous formulations of the present invention contain from about 1.0 to about 100 mg/mL of the compound of Formula (I). More preferably, the aqueous formulations contain from about 2 to about 75 mg/mL of the compound of Formula (I).

In another embodiment, the aqueous formulation of the present invention contains the compound of Formula (I) having from about 1 to about 20 % by weight of sulfur.

In another embodiment, the present invention provides lyophilized powders for an aqueous formulation having improved stability, which includes:

a) from about 0.01 to about 20 wt% of a biologically active agent; and

b) from about 0.2 to about 15 wt% of a compound of the formula (I), as defined above.

B. R_1, Polyalkylene oxide

R_1 is a polyalkylene oxide, more preferably polyethylene oxide or polypropylene oxide. The present invention employs a polyalkylene oxide to provide a water-soluble stabilizer. The polyalkylene oxide can be a linear, branched or multi-armed polyalkylene oxide. Preferred polyalkylene oxide includes polyethylene glycol, methoxy polyethylene glycol, propylene glycol, etc. For purposes of illustration and not limitation, for example, polyethylene glycol portion of R_1 can be represented as polyethylene glycol of the formula, $-O-(CH_2CH_2O)_x-$, wherein, (x) is the degree of polymerization which is an integer from about 1 to about 463, which provides average molecular weight of polyethylene glycol from about 75 daltons to about 20,000 daltons. In an alternate aspects of the invention, the average molecular weight of the polymer range from 75 daltons to 20,000 daltons or preferably from about 400 daltons to about 10,000 daltons or more preferably from about 1,000 daltons to about 6,000 daltons.

C. Disulfide Bond or Dithiol Containing Moiety

The disulfide or dithiol containing moiety of the present invention benefits the aqueous formulation of the invention by inhibiting oxidation of the biologically active agent in the formulation. During the process, the thiol functional groups will be oxidized to form disulfide bond. Among many thiol containing compounds, those having dithiols which can form intramolecular cyclic disulfide bond, as provided in the present invention, are preferred for stabilizing aqueous formulation due to their fast reaction forming disulfide bond upon

oxidation, and to inhibit oxidation of the biologically active agent in the formulation effectively and such properties are not negatively affected when conjugated to polyalkylene oxides.

Dihydrolipoic acid (DHLA) is an organosulfur compound and a reduced form of lipoic acid (LA). Especially, DHLA or LA contains intramolecular cyclic disulfide bond or dithiols which can form cyclic disulfide bond intramolecularly. DHLA contains 1,3-dithiols, -SH, which easily form five-membered cyclic moiety to form disulfide bond upon oxidation. Even though a supply of an anti-oxidant, such as DHLA or LA, can inhibit oxidation process and be used as anti-oxidant in formulations, many anti-oxidants including LA or DHLA are not water-soluble and thus, have limited or reduced capacity as an anti-oxidant. The present invention overcomes this shortcoming and provides a water-soluble polyalkylene oxide conjugated anti-oxidant which can be employed in an aqueous formulation or in a lyophilized powder to be reconstituted as an aqueous formulation.

D. Synthesis of the Compounds of Formula (I)

Generally, the water-soluble polyalkylene oxide conjugated compound of Formula (I) of the present invention can be made, for example, by attaching a 1,3- or 1,4-dithiol or disulfide containing organic acid to a polyalkylene oxide.

In one aspect of the invention, a polyalkylene oxide, such as polyethylene glycol, is reacted with -COOH groups of dithiol or disulfide bond containing organic acid, such as lipoic acid (LA) or dihydrolipoic acid (DHLA), in the presence of a coupling agent.

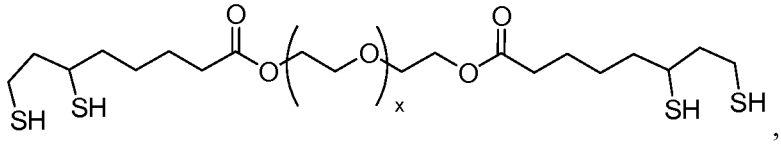
A non-limiting list of suitable coupling agents includes 1,3-diisopropylcarbodiimide (DIPC), any suitable dialkyl carbodiimides, 2-halo-1-alkyl-pyridinium halides (Mukaiyama reagents), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (EDC), propane phosphonic acid cyclic anhydride (PPACA) and phenyl dichlorophosphates, etc. which are available, for example from commercial sources such as Sigma-Aldrich Chemical, or synthesized using known techniques.

Preferably, the reactions are carried out in an inert solvent such as methylene chloride, chloroform, DMF or mixtures thereof. The reactions can be preferably conducted in the presence of a base, such as dimethylaminopyridine (DMAP), diisopropylethylamine, pyridine, triethylamine, etc. to neutralize any acids generated. The reactions can be carried out at a temperature from about 0 ^{o}C up to about 22 ^{o}C (room temperature).

In another aspect, the thiols of the anti-oxidants can be masked with a protecting group such as benzylic acetal, such as thioketal or thioacetal, especially protecting groups which can be deprotected by a mild reaction condition such as weak acid or base, after the conjugation.

Details concerning some preferred aspects of this embodiment are provided in the Examples section and some particular embodiments prepared by the methods described herein are provided below.

In one preferred embodiment, the compound of the formula (I) is selected from among:



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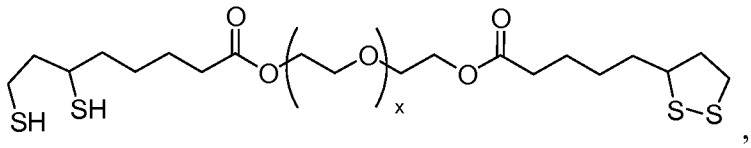

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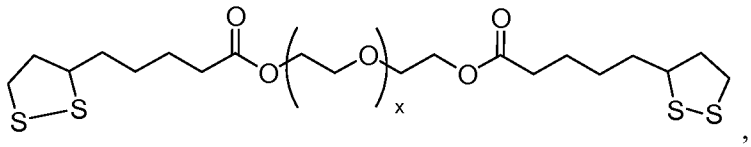

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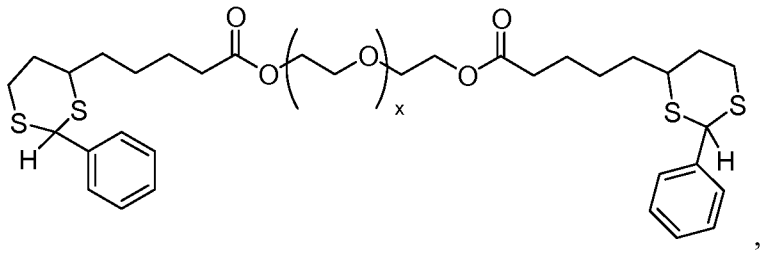

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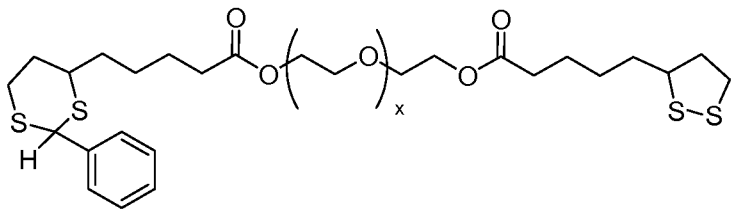

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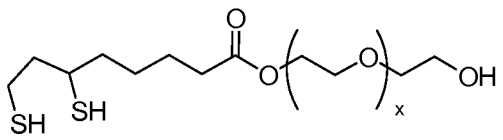

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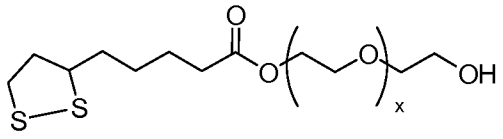

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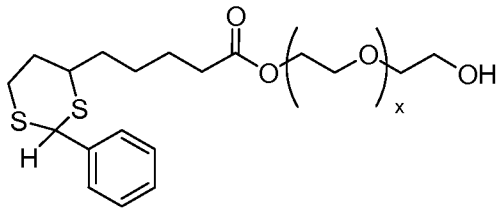

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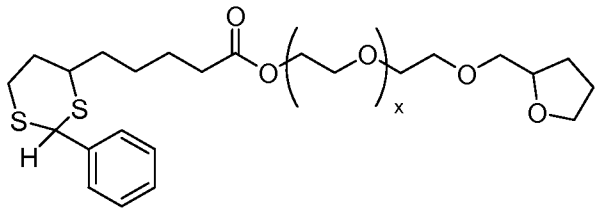
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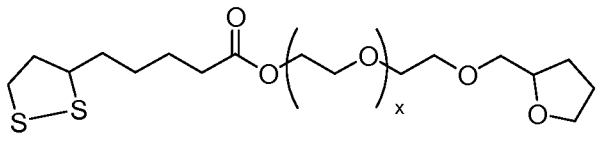
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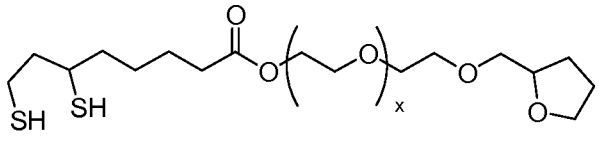
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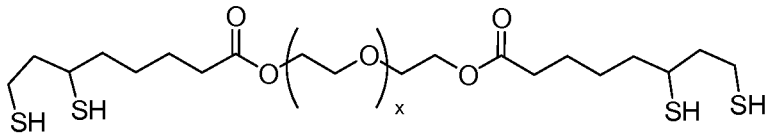
wherein, (x) is an integer from about 1 to about 463.

In another preferred embodiment of the present invention, the compound of Formula (I) is selected from among: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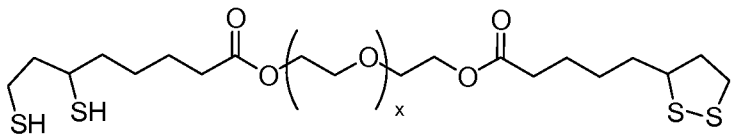
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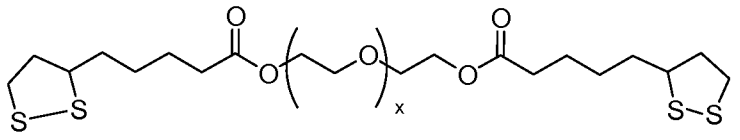
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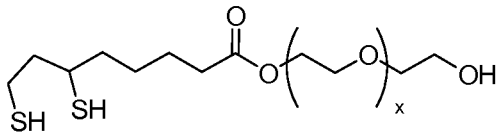
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wherein, (x) is an integer from about 1 to about 463.

E. Biologically Active Agents

The aqueous formulation or the lyophilized powder of the present invention can provide a formulation having an improved stability for many biologically active agents. Many biologically active agents are labile to degradation such as oxidation and can benefit from the formulation provided by the present invention.

In one embodiment of the present invention, the biologically active agent is selected from among antibacterial, antiviral, antiproliferative, anticancer, anti-inflammatory, analgesic, anesthetic, antipyretic, antiseptic, and antimicrobial compounds.

In another embodiment of the present invention, the biologically active agent is selected from among acyclovir, amphotericin B, 3-amino-4-hydroxybutyric acid, argatroban, atorvastatin, bendamustine, carbenicillin, carboplatin, cephalosporin C, cyclosporine, docetaxel, epirubicin, epothilones, etoposide, fluvastatin, fulvestrant, idarubicin, melphalan, mitomycin C, mitoxantrone, nitrogen mustard, paclitaxel, pemetrexed, podophyllotoxin, roxithromycin, sirolimus, taxanes, temsirolimus, tacrolimus, teniposide, raltitrexed, and derivatives of thereof. Preferably, the biologically active agent is selected from among bendamustine, epothilones, fulvestrant, taxanes, pemetrexed, argatroban, nitrogen mustard, paclitaxel, and derivatives of each of the foregoing such as docetaxel, etc.

In some aspects, the amount of the biologically active agent is from about 5 to about 50 mg/mL. In other aspects, the amount of the biologically active agent is from about 10 to about 30 mg/mL.

F. Preparation of an Aqueous Formulation

In most embodiments, the compound of Formula (I) is mixed with the biological agents and other pharmaceutically acceptable excipients in water or pharmaceutically acceptable water-based solution, such as buffer solution.

In some embodiments, the aqueous formulations of the present invention contain from about 0.01 to about 200 mg/mL of a biologically active agent. More preferably, the aqueous formulations contain from about 2 to about 100 mg/mL of a biologically active agent.

In other embodiments, the aqueous formulations of the present invention contain from about 1.0 to about 100 mg/mL of the compound of Formula (I). More preferably, the aqueous formulations contain from about 2 to about 75 mg/mL of the compound of Formula (I).

G. Preparation of Lyophilized Powder for an Aqueous Formulation

In one embodiment, biologically active agents, and other pharmaceutically acceptable excipients are mixed into a solution containing a compound of Formula (I) in a proper buffer fluid, to provide the aqueous formulation. The formulation is filtered through a membrane such as 0.22 μm membrane, followed by removal of the solvent by using a standard lyophilization technique, which as with nitrogen flush, etc. to prepare the lyophilized powder for the aqueous formulation of the present invention. The lyophilized mixture is reconstituted with water or

other pharmaceutically acceptable solution such as phosphate buffer to prepare an aqueous formulation of the present invention.

EXAMPLES

The present invention is further defined in the following Examples. It should be understood that these examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

In the examples, all synthesis reactions are run under an atmosphere of dry nitrogen or argon. All chemicals were used as purchased unless otherwise specified.

^{1}H spectra were obtained using deuteriochloroform as solvent unless specified. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) and coupling constants (J values) are given in hertz (Hz).

Following abbreviations are used throughout the examples: DCM (dichloromethane), DHLA (dihydrolipoic acid), DIEA (diisopropylethylamine), DMAP (4-*N,N*-dimethylamino-pyridine), DMF (N,N'-dimethylformamide), EDCI (1-(3-dimethylaminopropyl)-3-ethyl carbodiimide), EtOAc (ethylacetate), HOBT (1-hydroxybenzotriazole), IPE (diisopropyl ether), and LA (lipoic acid).

Example 1. Preparation of bislipoic acid-PEG 400 ester (compound 3a)

To a solution of lipoic acid (**1**, 25.0 g, 121.2 mmol) in 70 mL of Dichloromethane (DCM) is added a solution of EDCI HCl (55.7 g, 290.7 mmol, 1.2 eq.) in 560 mL of DCM and DMAP (2.95 g, 24.23 mmol, 0.1 eq.), under N_2 atmosphere during 0.5-1 hour and the mixture is stirred for another 1 hour. Then a solution of PEG-400 (**2**, Mol.Wt. 380-400, 97.14 g, 242.3 mmol, 1.05 eq.) in 200 ml of DCM is added to the above stirred mixture drop wise over 0.5 hour, and the resulting mixture is stirred for further 24 hours at room temperature. The mixture is concentrated under reduced pressure and the remaining organic layer is washed with water (2×50 mL). The organic layer is dried over anhydrous sodium sulphate, filtered and evaporated, purified by silica-gel column chromatography using EtOAc and 0-8% MeOH in DCM as eluents to obtain the product.

Example 2. Preparation of bislipoic acid-PEG 1000 ester (compound 3b-e)

Compounds **3b-e** are prepared separately, by using the conditions as described in Example 1 using PEG-1,000, PEG-4,000, PEG-6,000, and PEG-20,000, respectively.

Example 3. Preparation of lipoic acid-PEG 400 ester (compound 5a)

To a solution of Lipoic acid (**1**, 50.0 g, 242.3 mmol) in 140 mL of DCM was added a solution of EDCI.HCl (55.7 g, 290.7 mmol, 1.2 eq.) in 560 mL of DCM and DMAP (2.95 g, 24.23 mmol, 0.1 eq.), under N_2 atmosphere during 0.5-1h and stirred for another 1 h. Then a solution of PEG-400 (**2**, Mol.Wt. 380-400, 97.14 g, 242.3 mmol, 1.05 eq.) in 200 ml of DCM was added to the above stirred mixture drop wise over 0.5 hour, and stirred for further 24 hours at room temperature. The mixture is concentrated under reduced pressure and the remaining organic layer is washed with water (2×50 mL). The organic layer is dried over anhydrous sodium sulphate, filtered and evaporated, purified by silica-gel column chromatography using EtOAc and 0-8% MeOH in DCM as eluents to obtain the product in 27.0 g quantity (18.9% yield): ^{1}H NMR (300 MHz) δ 4.2-4.3 (t, 2H), 3.5-3.8 (m, PEG-CH_2, ~33 H), 3.05-3.25 (m, 2H), 2.40-2.55 (m, 1H), 2.3-2.4(t, 2H), 2.13 (bs, 2H), 1.8-2.0 (m, 1H), 1.6-1.8 (m, 4H), 1.4-1.6 (m, 2H).

Example 4. Preparation of bislipoic acid-PEG esters (compound 5b-e)

Compound **5b**: Compound **5b** was prepared by using the conditions as described in Example 1 using PEG-1,000 in 11 % yield.

Compound **5c**: Compound **5c** was prepared by using the conditions as described in Example 1 using PEG-4,000 in 74 % yield.

Compound **5d**: Compound **5d** was prepared by using the conditions as described in Example 1 using PEG-6,000 in 80 % yield.

Compound **5e**: Compound **5e** was prepared by using the conditions as described in Example 1 using PEG-20,000 in 85% yield.

Example 5. Preparation of bisdihydrolipoic acid-PEG 1,000 ester (compound 6b)

Sodium borohydride (1.59 g, 42.06 mmol, 2 eq.) was added portion wise to a solution of compound **5b** (25 g, 21.03 mmol, 1 eq.) in ethanol (135 mL) at 0 °C under nitrogen atmosphere. After being stirred for 0.5 hour at this temperature the reaction mixture was brought to room temperature and stirred for another 3 hour, acidified to pH ~2 with aqueous HCl (2N). Then ethanol was evaporated under reduced pressure and the obtained aqueous layer was extracted

with DCM (2×500 mL). The organic layer was washed with brine (50 mL) and dried over anhydrous sodium sulphate, filtered and evaporated to obtain crude product, which was purified by silica-gel column chromatography using 5-10% MeOH in DCM as eluent to give product as a pale-yellow waxy material in 18.7 g quantity (75% yield).

Example 6. Preparation of bisdihydrolipoic acid-PEG 1,000 ester (compound 6a)

Compound **6a** is prepared by using the conditions as described in Example 5 using PEG-400.

Example 7. Preparation of bisdihydrolipoic acid-PEG ester (compounds 6c-e)

Compound **6c**: Compound **6c** was prepared by using the conditions as described in Example 1 using compound **5c** in 77 % yield.

Compound **6d**: Compound **6d** was prepared by using the conditions as described in Example 1 using compound **5d** in 85 % yield.

Compound **6e**: Compound **6e** was prepared by using the conditions as described in Example 1 using compound **5e** in 77% yield.

Example 8. Preparation of protected dihydrolipoic acid (compound 7)

To a solution of Dihydrolipoic acid (**1a**, 10 g, 48 mmol) in 60 mL of dry DCM was added benzaldehyde (5.36 ml, 52.8 mmol, 1.1 eq.) under N_2 atmosphere. After being stirred for 10 minutes at room temperature was added Boron trifluoride etherate (6.5 mL, 52.8 mmol, 1.1eq.) and the mixture was stirred overnight. The reaction progress was monitored by TLC using 15% EtOAc in Hexane as the mobile phase. Then the reaction was quenched by adding water (150 mL) and the two layers were separated. The aqueous layer was extracted with DCM (2×25 ml) and the combined organic layers were dried over anhydrous sodium sulphate, filtered and evaporated to obtain crude product. The crude product was purified by column chromatography using 20-30% EtOAc/hexane to get pure title compound **7** in 13.0 g quantity (91.5% yield) as an off white solid: ^{1}H NMR (300 MHz) δ 7.4-7.5 (d, 2H), 7.25-7.35 (m, 3H), 5.1-5.2 (s, 1H), 2.9-3.1 (m, 3H), 1.4-1.8 (m, 7H), 1.1-1.25 (dd,1H).

Example 9. Preparation of lipoic acid-PEG 400-protected dihydrolipoic acid ester (compound 8a)

To a solution of protected Dihydrolipoic acid (**7**, 13.0 g, 43.91 mmol) in 40 mL of Dichloromethane (DCM) was added a solution of EDCI HCl (10.1 g, 52.7 mmol, 1.2 eq.) in 40

mL of DCM and DMAP (0.535 g, 4.39 mmol, 0.1 eq.), under N_2 atmosphere during 0.5 hour and stirred for another 1 hour. A solution of LA-PEG-400 (**5a**, 25.818 g, 43.91 mmol, 1.0 eq.) in 20 mL of DCM was added to the above stirred mixture drop wise over 0.5-1 hour, and the resulting mixture was stirred for further 24 hours at room temperature. The reaction mixture was diluted with 100 mL of DCM and washed with water (2×50 mL) to remove unwanted impurities/side-products. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated to obtain crude product which was purified by silica-gel column chromatography using EtOAc and 0-5% MeOH in DCM as an eluent to obtain pure coupled product Lipoic acid-PEG-400-Protected DHLA ester derivative **8a** as a yellow oil in 15.0 g quantity (39.17% yield): ^{1}H NMR (300 MHz) δ 7.4-7.5 (d,2H), 7.25-7.35 (m, 3H), 5.15 (s, 1H), 4.2-4.3 (m, 4H), 3.50-3.75 (m, PEG-CH2, ~33H), 3.60-3.65 (m, 2H), 2.85-3.30 (m, 2H), 2.6-2.8 (m, 4H), 2.3-2.4 (t, 4H), 1.8-2.0 (m, 2H), 1.7-1.8 (m, 2H), 1.4-1.8 (m, 6H).

Example 10. Preparation of lipoic acid-PEG-protected dihydrolipoic acid ester (compounds 8b-e)

Compounds **8b-e** are prepared separately, by using the conditions as described in Example 9 using **5b-e**, respectively.

Example 11. Preparation of lipoic acid-PEG-dihydrolipoic acid ester (compounds 9a)

To a solution of Potassium iodide (2.57 g, 15.58 mmol, 1.5 eq.) in 45 mL of MeOH, Ferric chloride (2.52 g, 15.58 mmol, 1.5 eq.) was added at room temperature under N_2 atmosphere. After being stirred the mixture for 0.5h was added a solution of LA-PEG-400-Protected DHLA **8a** (9.0 g, 10.39 mmol, 1.0 eq.) in 45 mL of methanol slowly at room temperature and the mixture was stirred for 0.5 hour at room temperature and then heated to reflux for 1-2 hours. The reaction mixture was cooled to room temperature and quenched with water (90 mL) slowly, extracted with EtOAc (90 mL), The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated to obtain crude product as a reddish orange viscous liquid, which was purified by column chromatography using EtOAc (to remove un-reacted LA/DHLA) and 0-5% MeOH in DCM as an eluent to obtain pure product Lipoic acid-PEG-400-DHLA ester derivative **9a** as an orange yellow oil in 2.7 g quantity (33.1% yield): ^{1}H NMR (300 MHz) δ 4.2-4.3 (m, 4H), 3.6-3.85 (m, PEG-CH_2, ~33 H), 3.5–3.6 (m,3H), 3.05-3.25 (m, 3H), 2.40-2.55 (m, 2H), 2.3-2.4(t, 3H), 1.8-2.0 (m, 2H), 1.6-1.85 (m, 4H), 1.40-1.60 (m, 2H), 1.25-1.38 (-SH protons).

Example 12. Preparation of lipoic acid-PEG-dihydrolipoic acid ester (compounds 9b-e)

Compounds **9b-e** are prepared separately, by using the conditions as described in Example 11 using **8b-e**, respectively.

Example 13. Preparation of lipoic acid-tetraglycol 75 ester (compounds 11a)

To a solution of Lipoic acid (**1**, 10.0 g, 48.4 mmol) in 30 mL of Dichloromethane (DCM) was added a solution of EDCI HCl (11.15 g, 50.1 mmol, 1.2 eq.) in 100 mL of DCM and DMAP (1.77 g, 1.4 mmol, 0.3 eq.), under N_2 atmosphere during 0.5-1 hour and stirred for another 1 hour. A solution of tetraglycol-75 (**10**, 11.95 g, 62.92 mmol, 1.3 eq.) in 40 ml of DCM was added to the above stirred mixture drop wise over 0.5-1 hour. The mixture was stirred for further 24 hour at room temperature. The reaction mixture was washed with brine (3×5 mL) to remove unwanted impurities/side-products. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated to obtain crude product as a yellow oil, which was purified by silica-gel column chromatography using 0-50% EtOAc in Hexane as eluents to obtain pure product, Lipoic acid-Tetraglycol ester derivative **11a** as a yellow oil in 3.5 g quantity (17.15% yield): ^{1}H NMR (300 MHz) δ 4.2-4.3 (t, 2H), 4.0-4.1(m,1H), 3.8-3.9 (q,1H), 3.6-3.8 (m, CH_2, ~7 H), 3.4-3.6 (m,3H), 3.1-3.3 (m, 1H), 2.40-2.55 (m, 1H), 2.3-2.4 (t, 2H), 1.8-2.0 (m, 4H), 1.5-1.8 (m, 7H), 1.4-1.5 (m, 2H). Mass: 379.3 [M^++H], 396.2 [M^++H_2O] & 401.2 [M^++Na].

Example 14. Preparation of compounds 11b-f

Compounds **11b-f** are prepared separately, by using the conditions as described in Example 13 using PEG-400, PEG-1,000, PEG-4,000, PEG-6,000, and PEG-20,000, respectively.

Example 15. Preparation of dihydrolipoic acid-tetraglycol 75 ester (compounds 12a)

Sodium borohydride (633 mg, 16.67 mmol, 1.8 eq.) was added portion wise to a solution of coupled product **11a** (3.5 g, 9.26 mmol, 1 eq.) in ethanol (35 mL) at 0 °C under nitrogen atmosphere. After 3 hours, the reaction mixture was acidified to pH ~2 with aqueous HCl (2N). Then ethanol was evaporated under reduced pressure and the obtained aqueous layer was extracted with DCM (3×50 mL). The organic layer was washed with brine (5mL) and dried over anhydrous sodium sulphate, filtered and evaporated to obtain crude reduced product **12a**, which was purified by silica-gel column chromatography using 30% EtOAc in Hexane as an eluent to give pure Dihydrolipoic acid-Tetraglycol ester **12a** as a greenish-yellow oil in 1.4 g

quantity (40% yield): ^{1}H NMR (300 MHz) δ 4.2-4.3 (t, 2H), 4.0-4.1(m, 1H),3.8-3.95 (q, 1H), 3.6-3.8 (m, CH_2, ~7 H), 3.4-3.5 (m, 3H), 2.8-2.9 (m, 1H), 2.6-2.8 (m, 2H), 2.30-2.40 (t, 2H), 1.8-2.0 (m, 4H), 1.4-1.8 (m, 8H), 1.3-1.4 (-2 SH protons & 2H). Mass: 381.3 [M^++H] & 398.3 [M^++H_2O].

Example 16. Preparation of compounds 12b-f

Compounds **12b-f** are prepared separately, by using the conditions as described in Example 15 using compounds **11b-f**, respectively.

Example 17. Preparation of an aqueous formulation of pemetrexed containing PEG-lipoic acid

An aqueous formulation of pemetrexed (PMT) containing PEG 6,000-lipoic acid (**5d**) was prepared by adding PMT disodium salt (25 mg/mL) to a solution of compound **5d** (72.2 mg/mL) in 0.05 M sodium citrate tribasic buffer solution. The materials were stirred, filtered through 0.2 micron filter paper and 1mL aliquots of the resultant formulation were transferred into vials and sealed.

Example 18. Preparation of an aqueous formulation of pemetrexed containing PEG-dihydrolipoic acid

An aqueous formulation of pemetrexed (PMT) containing PEG 6,000-dihydrolipoic acid (**6d**) was prepared by adding PMT disodium salt (25 mg/mL) to a solution of compound **6d** (72.2 mg/mL) in 0.05 M sodium citrate tribasic buffer solution. The materials were stirred, filtered through 0.2 micron filter paper and 1mL aliquots of the resultant formulation were transferred into vials and sealed.

Example 19. Preparation of aqueous formulations of bendamustine or nitrogen mustard containing PEG-lipoic acid or PEG-dihydrolipoic acid

The process of Examples 17 and 18 are repeated using 25 mg/mL of bendamustine or nitrogen mustard instead of pemetrexed to provide aqueous formulation of bendamustine containing PEG-lipoic acid or dihydrolipoic acid.

Example 20. Preparation of other aqueous formulations of pemetrexed containing PEG-lipoic acid or PEG-dihydrolipoic acid

Several aqueous formulations using different aqueous fluid or different pH were prepared and the description is provided in Table 1. All formulations were prepared under nitrogen atmosphere and compounds **5d** (PEG 6000-lipoic acid) or **6d** (PEG 6000-dihydrolipoic acid) are employed for Samples 100-118.

TABLE 1. Formulation of Pemetrexed Prepared

Sample No.	Formulation	Sample No.	Formulation	Sample No.	Formulation	Sample No.	Formulation
100	PMT - 5mg/mL Water qs to 1mL pH - 7.00 with 0.01N NaOH	104	PMT - 5mg/mL Water qs to 1mL pH - 8.00 with 0.01N NaOH	108	PMT - 5mg/mL Water qs to 1mL pH - 9.00 with 0.01N NaOH	112	PMT - 5mg/mL Water qs to 1mL pH - 10.00 with 0.01N NaOH
101	PMT - 5mg/mL Na2HPO4 - 0.01M Water qs to 1mL pH - 7.00 with 0.1N NaOH	105	PMT - 5mg/mL TRIS buffer - 0.01M Water qs to 1mL pH - 8.00 with 0.1N NaOH	109	PMT - 5mg/mL Glycine - 0.01M Water qs to 1mL pH - 9.00 with 0.1N NaOH	113	PMT - 5mg/mL Glycine - 0.01M Water qs to 1mL pH - 10.00 with 0.1N NaOH
102	PMT - 5mg/mL Na2HPO4 - 0.01M DHLA-PEG 6000 - 5mg/mL Water qs to 1mL pH - 7.00 with 0.1N NaOH	106	PMT - 5mg/mL TRIS buffer - 0.01M DHLA-PEG 6000 - 5mg/mL Water qs to 1mL pH - 8.00 with 0.1N NaOH	110	PMT - 5mg/mL Glycine - 0.01M DHLA-PEG 6000 - 5mg/mL Water qs to 1mL pH - 9.00 with 0.1N NaOH	114	PMT - 5mg/mL Glycine - 0.01M DHLA-PEG 6000 - 5mg/mL Water qs to 1mL pH - 10.00 with 0.1N NaOH
103	PMT - 5mg/mL Na2HPO4 - 0.01M LA-PEG 6000 - 5mg/mL Water qs to 1mL pH - 7.00 with 0.1N NaOH	107	PMT - 5mg/mL TRIS buffer - 0.01M LA-PEG 6000 - 5mg/mL Water qs to 1mL pH - 8.00 with 0.1N NaOH	111	PMT - 5mg/mL Glycine - 0.01M LA-PEG 6000 - 5mg/mL Water qs to 1mL pH - 9.00 with 0.1N NaOH	115	PMT - 5mg/mL Glycine - 0.01M LA-PEG 6000 - 5mg/mL Water qs to 1mL pH - 10.00 with 0.1N NaOH
116	PMT - 25mg/mL Water qs to 1mL pH - 8.50 with 0.01N NaOH	117	PMT - 25mg/mL DHLA PEG 6000 - 71.5mg/mL Water qs to 1mL pH - 8.50 with 0.1N NaOH (N2 Sparging)	118	PMT - 25mg/mL LA PEG 6000 - 72.2mg/mL Water qs to 1mL pH - 8.50 with 0.1N NaOH (N2 Sparging)		

Example 21. Measurement of stability of the aqueous formulations of pemetrexed (PMT) containing PEG-lipoic acid (5d) or PEG-dihydrolipoic acid (6d)

The products of Examples 17-19 and reported in Table 1 were subjected to accelerated stability testing and compared the total amount of degradants and loss of potency. All samples

were maintained at 40 °C and analyzed periodically for drug content and impurity profile. The results obtained are presented in Tables 2-4.

TABLE 2. Stability of Pemetrexed solutions at pH 8.0

Sample No.	Temp.	Time Period	Conc. (mg/mL)	% of Initial	Degradants					% of Total	pH
					I	II	III	IV	V		Sample
104	Initial		4.29	100	BDL	BDL	BDL	BDL	0.19	0.24	7.65
	40°C	15 d	4.15	96.7	2.60	0.08	BDL	0.26	1.48	4.53	7.21
		1 M	4.04	97.3	5.73	0.08	BDL	0.37	2.21	8.52	7.18
105	Initial		4.39	100	BDL	BDL	BDL	BDL	0.16	0.21	8.33
	40°C	15 d	4.15	94.5	0.55	0.13	BDL	0.36	2.17	3.38	8.43
		1 M	4.06	92.5	2.13	0.08	BDL	0.48	3.20	5.94	8.36
106	Initial		4.68	100	BDL	BDL	BDL	BDL	0.20	0.25	7.98
	40°C	15 d	4.60	98.3	0.42	0.10	BDL	0.34	2.17	3.34	7.36
		1 M	4.33	92.5	1.65	0.09	BDL	0.53	3.56	6.22	7.92
107	Initial		4.59	100	BDL	BDL	BDL	BDL	0.18	0.18	8.12
	40°C	15 d	4.34	94.6	0.15	BDL	0.11	0.15	1.39	1.90	8.03
		1 M	4.35	94.8	0.90	0.09	BDL	0.45	2.90	4.48	7.80

BDL: Below detectable levels

TABLE 3. Stability of Pemetrexed solutions at pH 9.0

Sample No.	Temp.	Time Period	Conc. (mg/mL)	% of Initial	Degradants					% of Total	pH
					I	II	III	IV	V		Sample
108	Initial		4.39	100	BDL	BDL	BDL	BDL	0.16	0.21	8.46
	40°C	15 d	4.15	94.5	1.05	0.09	BDL	0.27	1.56	3.14	6.80
		1 M	93.8	NA	2.93	0.07	BDL	0.39	2.32	6.15	7.03
			0.14		96.0	0.12	BDL	0.37	2.54	99.9	
109	Initial		4.31	100	BDL	BDL	BDL	BDL	0.16	0.21	9.40
	40°C	15 d	4.14	96.1	BDL	0.17	0.17	0.38	2.55	3.46	9.40
		1 M	4.08	98.6	BDL	0.24	0.18	0.55	4.09	5.30	9.32
110	Initial		4.72	100	BDL	BDL	BDL	BDL	0.14	0.20	9.21
	40°C	15 d	4.41	93.4	BDL	0.11	0.08	0.14	1.41	1.85	8.37
		1 M	4.39	93.0	1.22	0.17	0.09	0.40	2.60	4.63	8.19
111	Initial		4.53	100	BDL	0.07	BDL	BDL	0.16	0.28	9.13
	40°C	15 d	4.34	95.8	BDL	BDL	0.17	0.15	1.41	1.83	8.82
		1 M	4.27	94.3	BDL	0.17	0.06	0.44	2.59	3.44	8.58

TABLE 4. Stability of Pemetrexed solutions having different concentration of antioxidants, at pH 8.5

Sample No.	Temp.	Time Period	Content (mg/mL)	% of Initial	Area % of Degradants											% of Total	pH value
					0.40	0.46	0.48	0.51	0.57	0.62	0.72	0.80	1.38	1.98	2.64		Sample
116	Initial		23.4	100	0.12	BDL	BDL	BDL	BDL	0.20	BDL	0.07	BDL	BDL	BDL	0.39	7.88
	40°C	1 M	15.8	67.5	2.72	4.38	0.91	BDL	BDL	17.94	0.15	0.73	BDL	BDL	BDL	26.83	6.54
117 (PEG-DHLA 6000, compound **6d**)	Initial		23.1	100	BDL	BDL	0.05	BDL	BDL	0.35	BDL	0.05	BDL	BDL	BDL	0.45	8.57
	40°C	1 M	22.9	99.1	0.06	0.20	0.10	BDL	BDL	1.02	0.08	0.12	BDL	BDL	BDL	1.58	6.89
118 (PEG-LA 6000, compound **5d**)	Initial		22.8	100	0.12	BDL	BDL	BDL	BDL	0.24	BDL	0.05	BDL	BDL	BDL	0.41	8.32
	40°C	1 M	22.6	99.1	0.13	0.05	BDL	BDL	BDL	0.53	0.08	0.09	BDL	BDL	BDL	0.88	6.84

As shown in the Tables 2-4, formulations of pemetrexed containing 5 mg/mL of PEG-lipoic acid (**5d**) or dihydrolipoic acid(**6d**) showed improved stability in the pH region of 8-10 till 1 month compared to samples without the PEG-lipoic acid or dihydrolipoic acid. Water-soluble antioxidizing agents, PEG conjugates of dihydrolipoic acid as well as lipoic acid, appear to stabilize the pemetrexed solution compared to the formulation containing no antioxidants. See, for example, Sample Nos. 117 and 118, wherein about 3 times higher concentration of the inventive compounds are used, the formulation appear to be intact over 1 month of time at 40 °C by retaining 99.1% of potency while Sample No. 116, showed about 33% loss of potency over the same time period.

Example 22. Preparation of an aqueous formulation of fulvestrant containing PEG-lipoic acid

An aqueous formulation of fulvestrant containing PEG 400-lipoic acid (**5a**) was prepared by dissolving fulvestrant (250mg) in a solution of compound **5a** (2.35mg) and glycofurol (1.35 mL). The materials were stirred, filtered through 0.2 micron filter paper and 1mL aliquots of the resultant formulation were transferred into vials and sealed.

Example 23. Preparation of an aqueous formulation of fulvestrant containing PEG-lipoic acid

An aqueous formulation of fulvestrant containing PEG 400-lipoic acid (**5a**) was prepared by dissolving fulvestrant (250mg) in a solution of compound **5a** (100mg), glycofurol (1.5 mL) and PG mono caprylate (1.5mL). The materials were stirred, filtered through 0.2 micron filter paper and 1mL aliquots of the resultant formulation were transferred into vials and sealed.

Example 24. Preparation of an aqueous formulation of fulvestrant containing PEG-lipoic acid

An aqueous formulation of fulvestrant containing PEG 400-lipoic acid (**5a**) and PEG 6000-lipoic acid (**5d**) was prepared by dissolving fulvestrant (250mg) in a solution of PG mono caprylate (1.5mL), compound **5a** (1.0mL) and compound **5d** (100mg). The materials were stirred, filtered through 0.2 micron filter paper and 1mL aliquots of the resultant formulation were transferred into vials and sealed.

CLAIMS

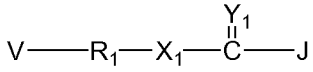
We claim:

1. An aqueous formulation having improved stability comprising:

a) water or a pharmacologically suitable fluid comprising water;

b) from about 0.01 to about 200 mg/mL of a biologically active agent; and

c) from about 0.2 to about 150 mg/mL of a compound of the formula (I):

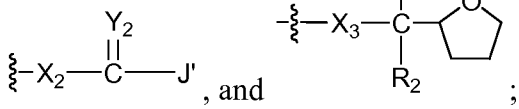


(I)

wherein,

R_1 is residue of a polyalkylene oxide;

V is selected from the group consisting of H, NH_2, OH, CO_2H, C_{1-6} alkoxy C_{1-6} alkyl,



;

wherein,

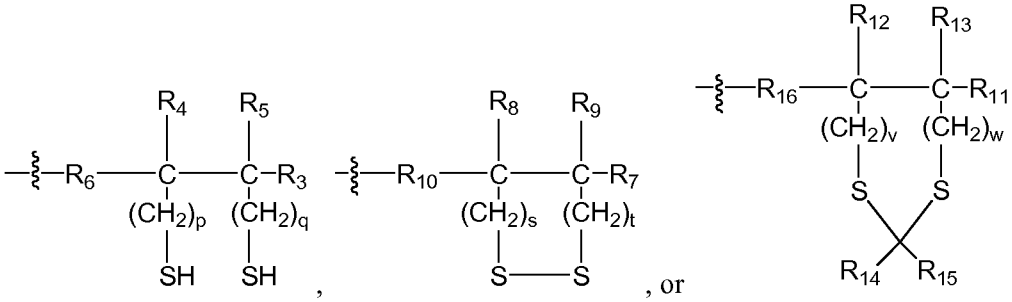
X_{1-3} are independently O, S or NH;

Y_{1-2} are independently is O, or S;

R_{1-2} are independently selected from the group consisting of hydrogen, C_{1-8} alkyl, C_{1-8} branched alkyl, C_{1-8} substituted alkyl, aryl, aralkyl, C_{1-8} alkylcarbonyl, C_{1-8} branched alkylcarbonyl, C_{1-8} substituted alkylcarbonyl, arylcarbonyl, or aralkylcarbonyl; and

$C(=Y_1)$-J or $C(=Y_2)$-J' is independently a residue of an organic acid containing one or more intramolecular cyclic disulfide bond or dithiol functional groups which can form intramolecular cyclic disulfide bond.

2. The aqueous formulation of claim 1, wherein J or J' is:



wherein,

R_6, R_{10}, and R_{16} are independently a residue of a bifunctional linking moiety;

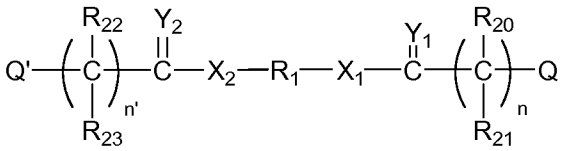
R_{3-5}, R_{7-9}, and R_{11-15} are independently selected from the group consisting of hydrogen, C_{1-8} alkyls, C_{3-8} branched alkyls, C_{2-8} substituted alkyls, aryls, aralkyls, C_{2-8} alkylcarbonyls, C_{3-8} branched alkylcarbonyls, C_{3-8} substituted alkylcarbonyls, arylcarbonyls, or aralkylcarbonyls; and

(p), (q), (s), (t), (v), and (w) are independently 0, 1, or 2;

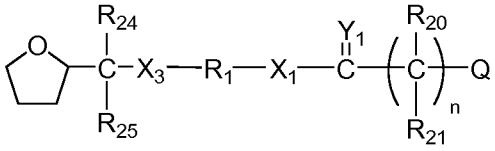
wherein (p)+(q), (s)+(t), and (v)+(w) are independently 1, 2, 3, or 4.

3. The aqueous formulation of claim 2, wherein R_6, R_{10}, and R_{16} are independently a residue of saturated or unsaturated or linear or branched C_{1-10} hydrocarbons; wherein one or more of the carbons is optionally replaced with a heteroatom, which is O, S, or NH.

4. The aqueous formulation of claim 1, wherein the compound of formula (I) is



(Ia), or



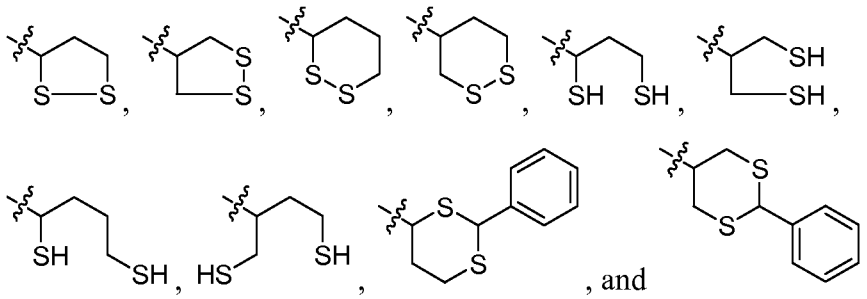
(Ib),

wherein,

R_{20-25} are independently selected from the group consisting of hydrogen, C_{1-8} alkyls, C_{3-8} branched alkyls, C_{2-8} substituted alkyls, aryls, aralkyls, C_{2-8} alkylcarbonyls, C_{3-8} branched alkylcarbonyls, C_{3-8} substituted alkylcarbonyls, arylcarbonyls, or aralkylcarbonyls;

(n) or (n') are independently an integer of from about 1 to about 12; and

Q or Q' are independently selected from the group consisting of:



, and .

5. The aqueous formulation of claim 4, wherein R_{20-25} are independently selected from the group consisting of hydrogen, methyl, ethyl, and isopropyl.

6. The aqueous formulation of claim 1, wherein R_1 is polyethylene glycol or polypropylene glycol.

7. The aqueous formulation of claim 1, wherein R_1 is polyethylene glycol of the formula, -$(CH_2CH_2O)_x$-CH_2CH_2-,

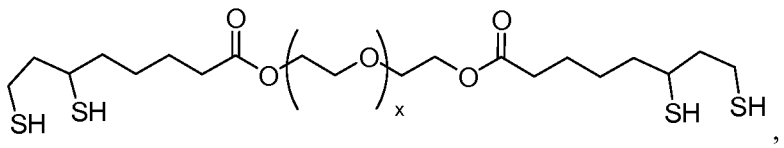
wherein, (x) is an integer from about 1 to about 463.

8. The compound of claim 1, wherein R_1 has an average molecular weight from about 75 to about 20,000 daltons.

9. The compound of claim 1, wherein R_1 has an average molecular weight of from about 400 to about 10,000 daltons.

10. The compound of claim 1, wherein R_1 has an average molecular weight from about 1,000 to about 6,000 daltons.

11. The aqueous formulation of claim 3, wherein the compound of formula (I) is selected from the group consisting of:



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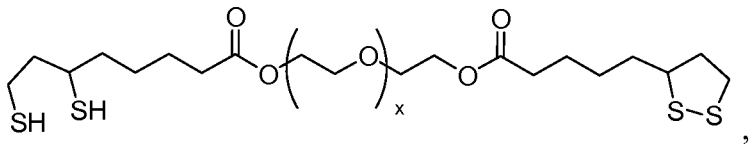

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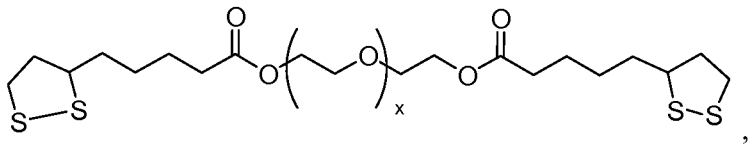

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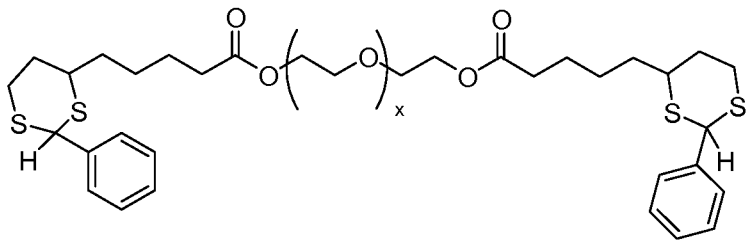

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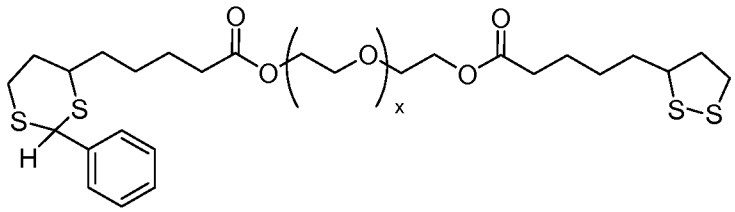
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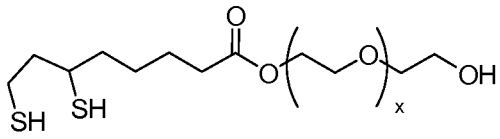
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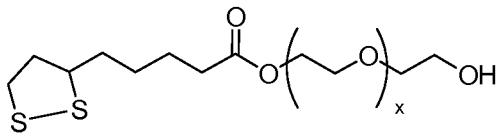
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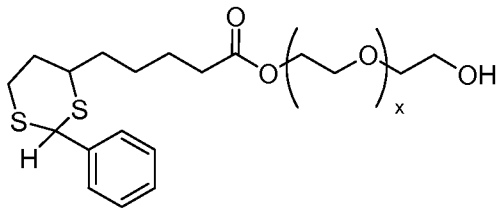
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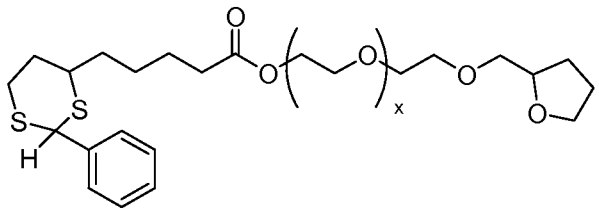
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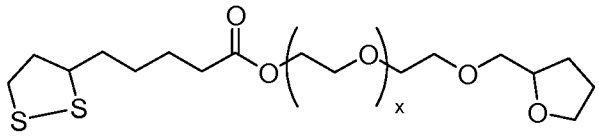
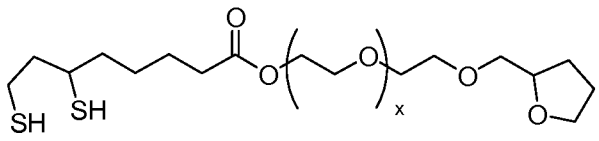
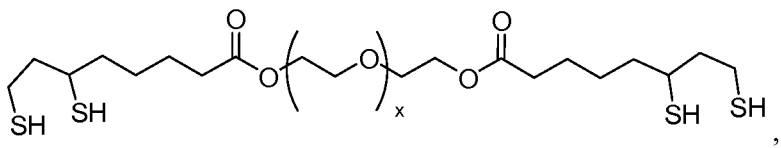

, and


,

wherein, (x) is an integer from about 1 to about 463.

12. The aqueous formulation of claim 3, wherein the compound of formula (I) is selected from the group consisting of: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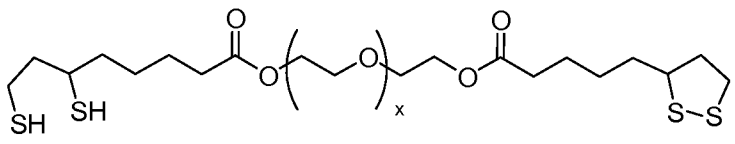
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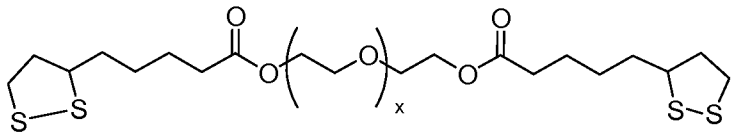
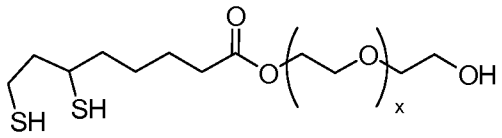


, and



,

wherein, (x) is an integer from about 1 to about 463.

13. The aqueous formulation of claim 1, wherein the concentration of the compound of Formula (I) is from about 1.0 to about 100 mg/mL.

14. The aqueous formulation of claim 1, wherein the concentration of the compound of Formula (I) is from about 2.0 to about 75 mg/mL.

15. The aqueous formulation of claim 1, wherein the compound of Formula (I) contains from about 1 to about 20 % by weight of sulfur.

16. The formulation of claim 1, wherein the biologically active agent is selected from the group consisting of antibacterial, antiviral, antiproliferative, anticancer, anti-inflammatory, analgesic, anesthetic, antipyretic, antiseptic, and antimicrobial compounds.

17. The formulation of claim 1, wherein the biologically active agent is selected from the group consisting of acyclovir, amphotericin B, 3-amino-4-hydroxybutyric acid, argatroban, atorvastatin, bendamustine, carbenicillin, carboplatin, cephalosporin C, cyclosporine, docetaxel, epirubicin, epothilones, etoposide, fluvastatin, fulvestrant, idarubicin, melphalan, mitomycin C, mitoxantrone, nitrogen mustard, paclitaxel, pemetrexed, podophyllotoxin, roxithromycin, sirolimus, taxanes, temsirolimus, tacrolimus, teniposide, raltitrexed, and derivatives of thereof.

18. The formulation of claim 1, wherein the biologically active agent is selected from the ground consisting of epothilones, taxanes, pemetrexed, argatroban, nitrogen mustard, paclitaxel, docetaxel and derivatives of thereof.

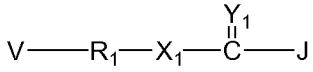
19. The formulation of claim 1, wherein amount of the biologically active agent is from about 5 to about 50 mg/mL.

20. The formulation of claim 1, wherein amount of the biologically active agent is from about 10 to about 30 mg/mL.

21. A lyophilized powder having improved stability comprising:

a) from about 0.01 to about 20 wt% of a biologically active agent; and

b) from about 0.2 to about 15 wt% of a compound of the formula (I):

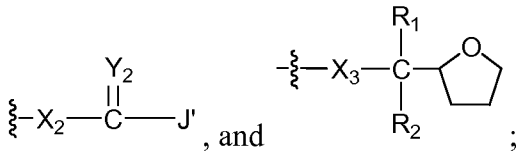


(I)

wherein,

R_1 is residue of a polyalkylene oxide (or water soluble non-antigenic polymer);

V is selected from the group consisting of H, NH_2, OH, CO_2H, C_{1-6} alkoxy C_{1-6} alkyl,



wherein,

X_{1-3} are independently O, S or NH;

Y_{1-2} are independently is O, or S;

R_{1-2} are independently selected from the group consisting of hydrogen, C_{1-8} alkyl, C_{1-8} branched alkyl, C_{1-8} substituted alkyl, aryl, aralkyl, C_{1-8} alkylcarbonyl, C_{1-8} branched alkylcarbonyl, C_{1-8} substituted alkylcarbonyl, arylcarbonyl, or aralkylcarbonyl; and

$C(=Y_1)$-J or $C(=Y_2)$-J' is independently a residue of an organic acid containing one or more intramolecular cyclic disulfide bond or dithiol functional groups which can form intramolecular cyclic disulfide bond.

22. A method of preparing an aqueous formulation having improved stability comprising:

contacting the lyophilized powder of claim 16 with water or a pharmacologically suitable fluid comprising water.

FIG. 1.

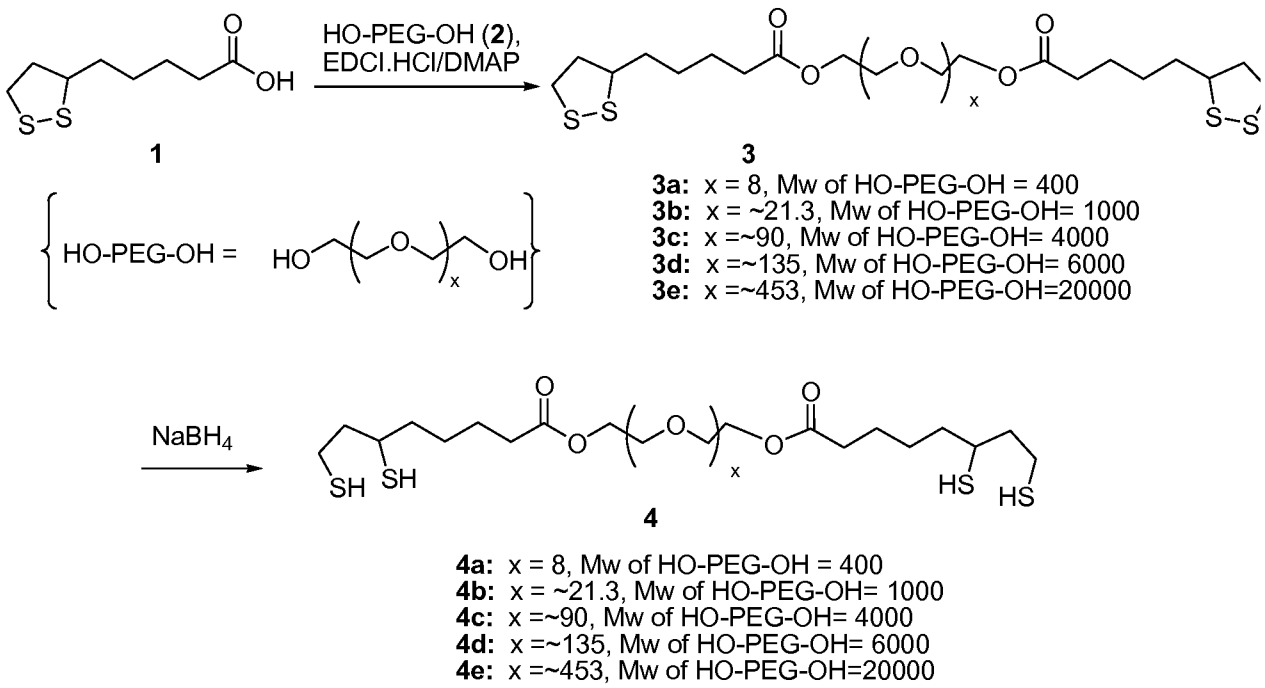

HO-PEG-OH (2),
EDCI.HCl/DMAP
1
3
HO-PEG-OH =
3a: x = 8, Mw of HO-PEG-OH = 400
3b: x = ~21.3, Mw of HO-PEG-OH= 1000
3c: x =~90, Mw of HO-PEG-OH= 4000
3d: x =~135, Mw of HO-PEG-OH= 6000
3e: x =~453, Mw of HO-PEG-OH=20000
NaBH4
4
4a: x = 8, Mw of HO-PEG-OH = 400
4b: x = ~21.3, Mw of HO-PEG-OH= 1000
4c: x =~90, Mw of HO-PEG-OH= 4000
4d: x =~135, Mw of HO-PEG-OH= 6000
4e: x =~453, Mw of HO-PEG-OH=20000

FIG. 2.

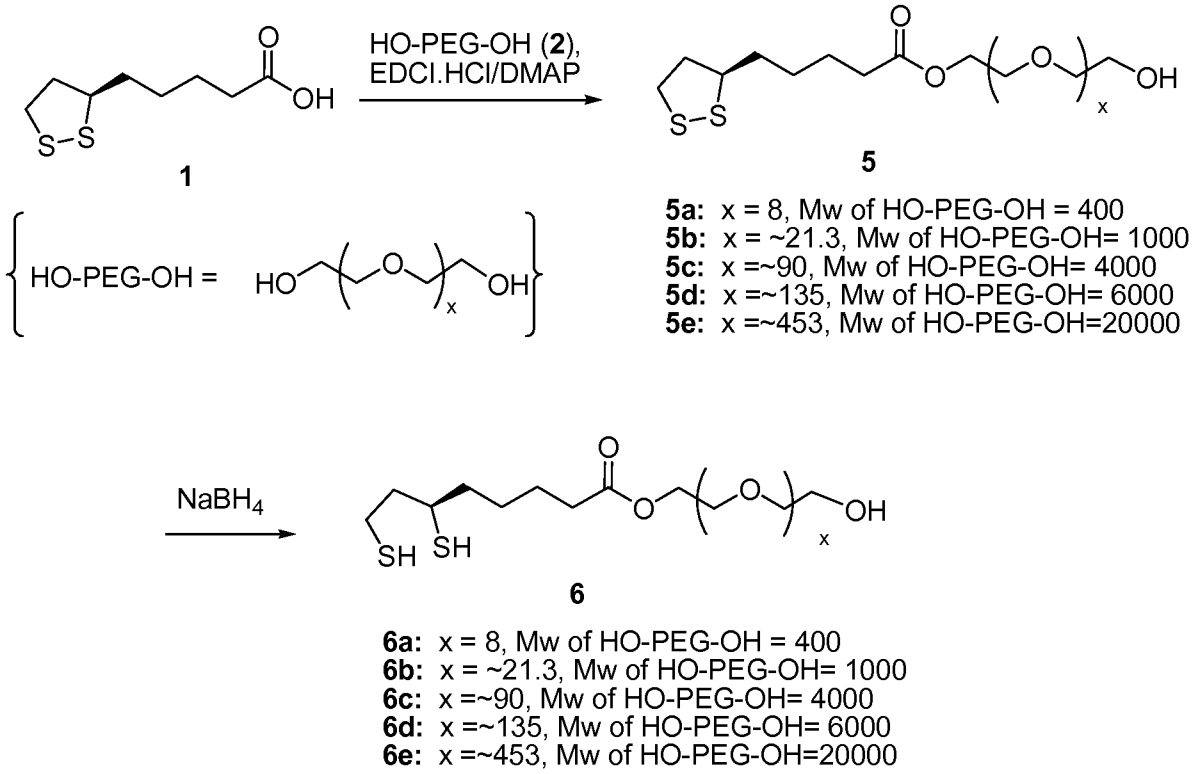

HO-PEG-OH (2),
EDCI.HCl/DMAP
OH
S–S
1
HO-PEG-OH =
HO
O
x
OH
5
5a: x = 8, Mw of HO-PEG-OH = 400
5b: x = ~21.3, Mw of HO-PEG-OH= 1000
5c: x =~90, Mw of HO-PEG-OH= 4000
5d: x =~135, Mw of HO-PEG-OH= 6000
5e: x =~453, Mw of HO-PEG-OH=20000
NaBH4
SH
SH
6
6a: x = 8, Mw of HO-PEG-OH = 400
6b: x = ~21.3, Mw of HO-PEG-OH= 1000
6c: x =~90, Mw of HO-PEG-OH= 4000
6d: x =~135, Mw of HO-PEG-OH= 6000
6e: x =~453, Mw of HO-PEG-OH=20000

FIG. 3.

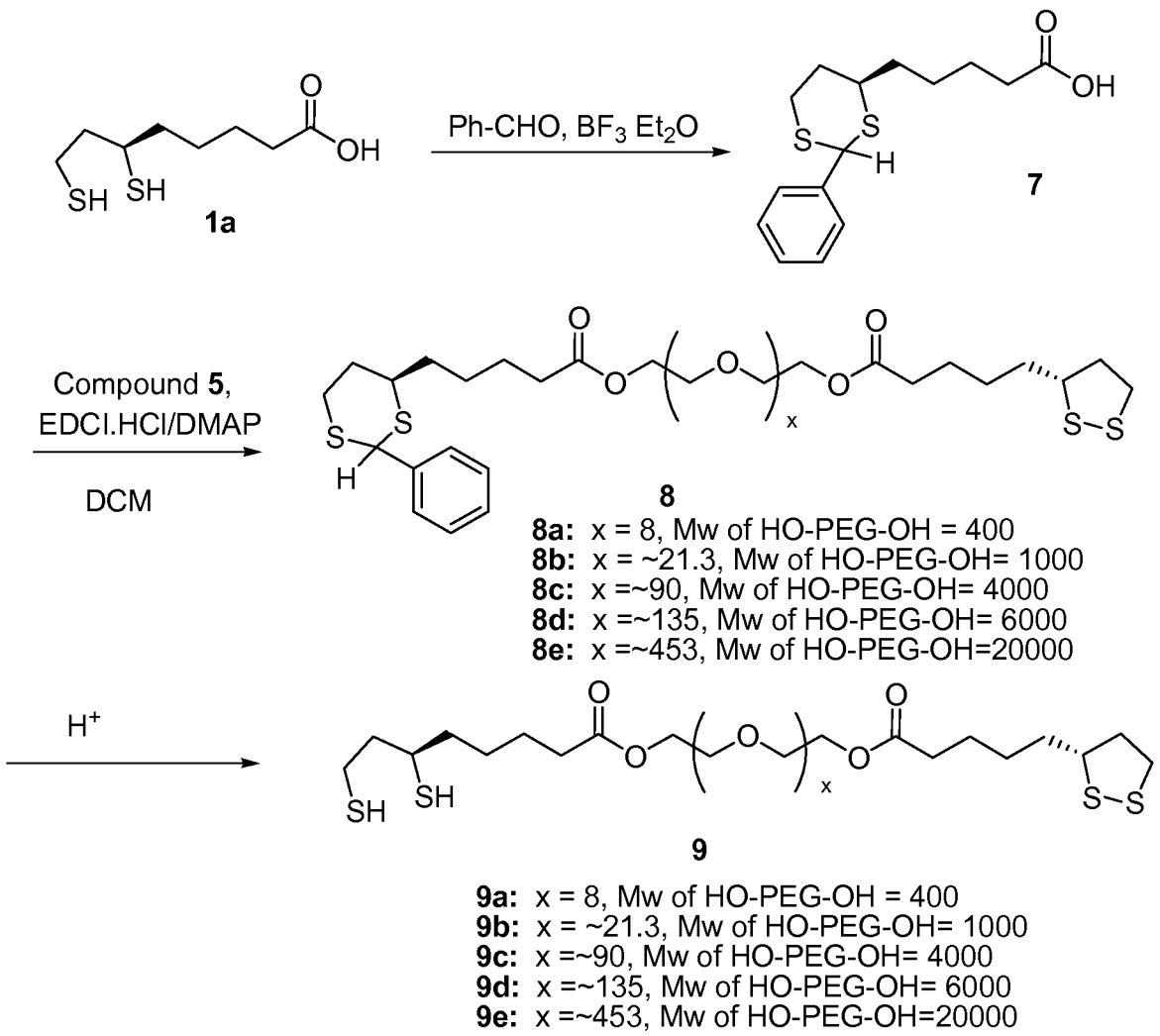
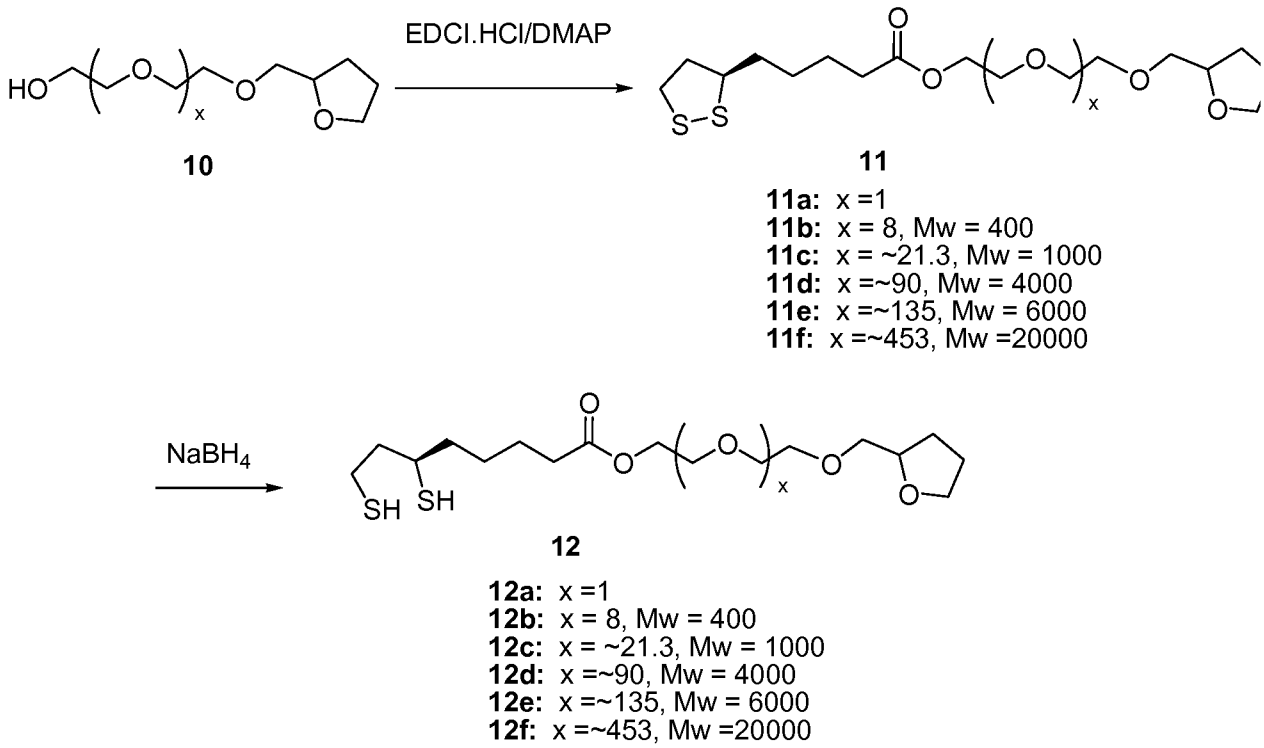

Ph-CHO, BF3 Et2O
1a
7
Compound 5,
EDCI.HCl/DMAP
DCM
8
8a: x = 8, Mw of HO-PEG-OH = 400
8b: x = ~21.3, Mw of HO-PEG-OH= 1000
8c: x =~90, Mw of HO-PEG-OH= 4000
8d: x =~135, Mw of HO-PEG-OH= 6000
8e: x =~453, Mw of HO-PEG-OH=20000
H+
9
9a: x = 8, Mw of HO-PEG-OH = 400
9b: x = ~21.3, Mw of HO-PEG-OH= 1000
9c: x =~90, Mw of HO-PEG-OH= 4000
9d: x =~135, Mw of HO-PEG-OH= 6000
9e: x =~453, Mw of HO-PEG-OH=20000

FIG. 4.


EDCI.HCl/DMAP
HO
O
x
O
O
10
S
S
11
11a: x =1
11b: x = 8, Mw = 400
11c: x = ~21.3, Mw = 1000
11d: x =~90, Mw = 4000
11e: x =~135, Mw = 6000
11f: x =~453, Mw =20000
NaBH4
SH
SH
12
12a: x =1
12b: x = 8, Mw = 400
12c: x = ~21.3, Mw = 1000
12d: x =~90, Mw = 4000
12e: x =~135, Mw = 6000
12f: x =~453, Mw =20000