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(54) **Title:** GUGGULPHOSPHOLIPID METHODS AND COMPOSITIONS

(57) **Abstract:** The present invention relates to the methods for preparing synthetic guggulphospholipids, their fatty acid analogues and other bioactive molecules. The present invention relates to E-guggulsterone and Z-guggulsterone or mixture of E- and Z-guggulsterones. The present invention also provides a novel method for the preparation of E-guggulsterol and Z-guggulsterol or mixture of E- and Z-guggulsterols from a mixture of E- and Z-guggulsterones. The present invention further relates to guggulphospholipids and other bioactive molecules incorporated into complexes such as liposomes, complexes, emulsions, vesicles, micelles, and mixed micelles, which can include other active agents, such as hydrophobic or hydrophilic drugs for use, e.g., in treatment of human and animal diseases.



WO 2008/058156 A3

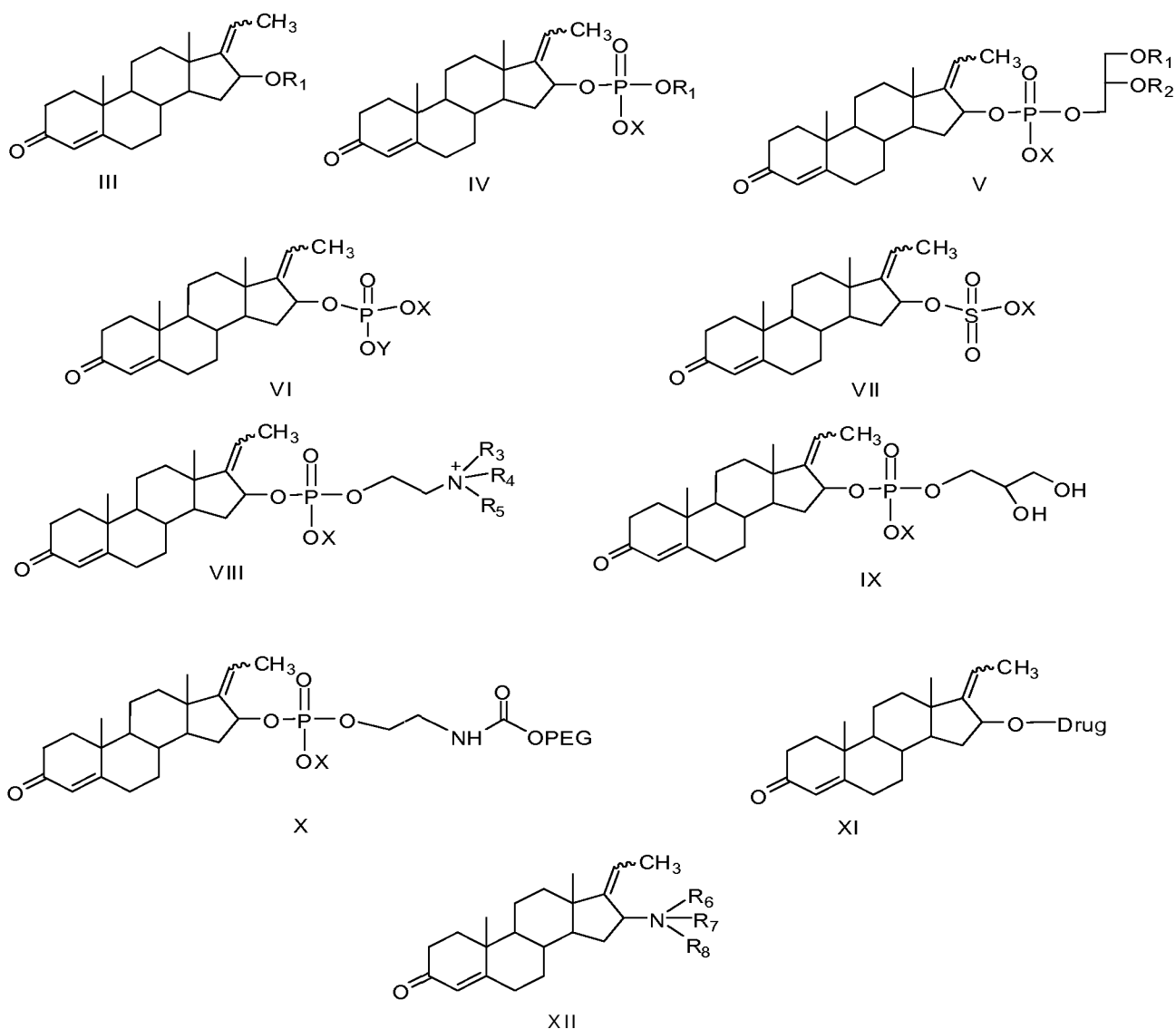
AMENDED CLAIMS

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CLAIMS

What is claimed is:

1. A guggul-based lipid molecule having a general structure selected from the group consisting of structures M-XII.

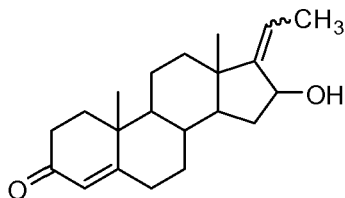


wherein:

- i) in structures III and IV, R_i is a saturated or unsaturated acyl or alkyl groups having between 1 and 34 carbon atoms;

- ii) in structures III and IV, R₁ and R₂ is a saturated alkyl or alkyloxy group optionally substituted with amino or substituted amino group.
- ii) in structure IV, R₁ is a sugar;
- 5 iii) in structure V, R₁ and R₂ are the same or different and at least one of the R₁ or R₂ is a saturated or unsaturated acyl group or alkyl group having between 1 and 34 carbon atoms;
- iv) in structures IV-X, X is hydrogen, methyl, ammonium, sodium, potassium, calcium, barium ion or any non-toxic ion;
- 10 v) in structure VI, X and Y are the same or different and are hydrogen, methyl, hydrogen, ammonium, sodium, potassium, calcium, barium ion or any non-toxic ion;
- vi) in structure VIII, R₃, R₄ and R₅ are the same or different and are hydrogen or methyl group; X is hydrogen or methyl group.
- 15 vii) in structure X, PEG (polyethylene glycol) is a long chain, linear or branched synthetic polymer composed of ethylene oxide units, HO(CH₂CH₂O)_nCH₂CH₂OCH₃, where n is between about 1 and about 1000;
- viii) in structure XI, drug is an active agent; and
- 20 ix) in structure XII, R₆, R₇, and R₈ are the same or different, and are selected from the group consisting of hydrogen, methyl, alkyl, substituted alkyl, alkyoxy, substituted alkyloxy groups, and are optionally hydroxylated, aminated, or polyaminated such that the composition has an overall positive charge;
- 25 and wherein:
- x) structures III-XII are in the form selected from the group consisting of *E*-isomer, *Z*-isomer, or a mixture of *E*- and *Z*- isomers;
- xi) structures III-XII are optically pure, or one or more of structures III-XII are mixtures of optical isomers;
- 30 xii) structure VIII is cationic when X is a methyl group; and
- xiii) structure XII is cationic.

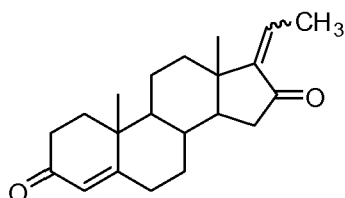
2. A method of preparing a guggul derivative of a formula selected from the group consisting of formula III-XII, comprising reacting a guggulsterol having structure HA



MA

to form a composition comprising a guggul derivative of a formula selected from the group consisting of formula III-XII.

3. A method of preparing a guggul derivative of a formula selected from the group consisting of formula III-XII, comprising reacting a guggulsterone having structure I



I

to form a composition comprising a guggul derivative of a formula selected from the group consisting of formula III-XII.

4. The method of claim 3, wherein said guggulsterone I is an *E*-isomer or *Z*-isomer or a mixture of *E* and *Z* isomers.
5. The method of claim 2, wherein said guggulsterol HA is an *E*-isomer or *Z*-isomer, or a mixture of *E*- and *Z*- isomer.

6. The method of claim 2, wherein said guggulsterol is optically pure, and wherein said guggulsterol is a mixture of optical isomers.
7. A method of preparing guggulsterol HA, comprising treating guggulsterone I
5 in one of more steps to produce preparing guggulsterol HA.
8. A method of preparing a compound of formula IV, V, VI, VIII, IX or X of Claim 1, 2, or 3, wherein at least one step comprises the use of a phosphoramidite reagent or a phosphorylating agent.
10
9. The method of claim 8, wherein said phosphoramidite reagent is selected from the group consisting of *N,N*-diisopropylmethylphosphoramidic chloride, (benzyloxy)(*N,N*-diisopropylamino)chlorophosphine, benzyloxybis (diisopropylamino) phosphine, 2-cyanoethyl-*N,N,N,N*-
15 tetraisopropylphosphoramidite, (2-cyanoethyl)(*N,N,N,N*-diisopropylamino)chlorophosphine, difluorenyl diisopropylphosphoramidite, methyl-*N,N,N,N* tetraisopropylphosphorodiamidite, dimethyl *N,N*-diisopropylphosphoramidite, dibenzyl diisopropylphosphoramidite, *di-tert*-butyl-*N,N*-diisopropylphosphoramidite, 2-(diphenylmethylsilyl)ethyl-
20 *N,N,N,N*-tetraisopropylphosphoramidite, (*N*-trifluoroacetylamino) butyl and (*N*-trifluoroacetylamino) pentyl-*N,N,N,N*-tetraisopropylphosphoramidites.
10. The method of claim 8, wherein said phosphorylating agent is selected from the group consisting of 2-bromoethyldichlorophosphate, trimethylsilylethyl
25 dichlorophosphate, methyl dichlorophosphate, 2-chloro-2-oxo-1, 3, 2-dioxaphospholane, 2-chlorophenyl dichlorophosphate, and phosphorus oxychloride.
11. A method of producing a composition of claim 1, wherein compound XI is
30 prepared by conjugating guggulsterol with a drug directly or through a linker.
12. The method of claim 11, wherein compound XI is prepared by conjugating guggulsterol through a linker, wherein the linker is an alkyl group optionally substituted with a functional group selected from the groups consisting of

carbonyl, carboxyl, carbonate, amino, amide, ester, thioester groups, succinate, glutarate, carbamate, ether, phosphate, phosphonate, diphosphate, and pyrophosphate.

- 5 13. A method comprising complexing a guggul-based lipid molecule of claim 1 into a composition comprising a liposome or lipid complex.
14. The method of claim 13, wherein said complex is selected from a group consisting of micelles, vesicles, and emulsions, wherein if said composition
10 comprises a plurality of micelles, said micelles are in the form of monomeric, dimeric, polymeric or mixed micelles.
15. A method of retaining drug in a liposome or complex comprising providing one or more guggul derivatives of claim 1 or preparing one or more guggul
15 derivatives by a method of claims 2-11, and complexing said one or more guggul derivatives and a drug in a composition comprising a liposome or complex.
16. The methods of claims 13-15, wherein said composition further comprises at
20 least one of a phospholipid, a pegylated phospholipid, polyethylene glycol (PEG), a fatty acid, a sterol, cholic acid, or α -tocopherol.
17. The method of claim 16, wherein said composition comprises at least one phospholipid, wherein said phospholipid comprises a phosphatidylcholine, a
25 phosphatidylethanolamine, a phosphatidylglycerol, a phosphatidylserine, a phosphatidylinositol, or a phosphatidic acid.
18. The method of claim 16, wherein said composition comprises at least one polyethylene glycol (PEG), wherein said polyethylene glycol (PEG) has an
30 average molecular weight of 200-20,000.
19. The method of claim 16, wherein said composition comprises at least one fatty acid, wherein said fatty acids is selected from a group consisting of fatty acids having chain length of C_4 - C_{34} .

20. The method of claim 19, wherein said fatty acid is saturated or unsaturated, and wherein said fatty acid is in acidic form, or in salt form.
- 5 21. The method of claim 16, wherein said composition comprises at least one pegylated phospholipid selected from the group consisting of a pegylated derivative of disteoylphosphatidylglycerol, dimyristoylphosphatidylglycerol, or dioleoylphosphatidylglycerol.
- 10 22. The method of claim 16, wherein said cholic acid is sodium deoxycholate.
23. The method of claim 16, wherein said composition further comprises a sterol selected from a group consisting of cholesterol, derivatives of cholesterol, cholesteryl sulfate, cholesterol succinate, cholesterol hemisuccinate, 15 cholesterol oleate, Cortisol, corticosterone, hydrocortisone, cholesterol-PEG, coprostanol, cholestanol, cholestane, β -sitosterol, lanosterol, campesterol, lathosterol, stigmasterol, stigmasterol, calciferol, guggulsterols, derivatives of guggulsterols, and mixtures thereof.
- 20 24. The method of claims 15, wherein said drug comprises a therapeutically active agent.
- 25 25. The method of claim 24, wherein said therapeutically active agent comprises an anticancer drug, an antiviral drug, antibacterial drug an antifungal drug, anti-inflammation drug, cholesterol lowering drug, and wherein at least one of said therapeutically agent is to treat cardiovascular disease, to treat neoplasia, to improve memory lose, to treat skin infection or skin disease, to treat hyperglycemia, or to enhance cognitive function.
- 30 26. A method wherein at least one cationic guggul derivative of formulas VIII and XII of claim 1 is used to facilitate intercellular delivery of a polynucleotide.
27. A method of transfecting a cell with a polynucleotide, comprising (a) preparing a composition comprising a cationic guggul derivative of claim 1

and said polynucleotide, and (b) contacting said cell with said composition whereby said polynucleotide is taken up by the cell.

- 5 28. The method of claim 26 or 27, wherein said polynucleotide is selected from a group consisting of an oligonucleotide comprising DNA, RNA or DNA and RNA, antisense oligonucleotides, small interfering RNA (siRNA), microRNA (miRNA), aptamers, and ribozymes, and wherein said polynucleotide is a single-stranded or double-stranded.
- 10 29. The method of any of claims 14-28, wherein said composition comprises unilamellar vesicles, multilamellar vesicles, or mixtures thereof.
- 15 30. The method of any of claims 13-29 wherein said liposome or complex has an overall charge selected from a negative charge, a positive charge, or a neutral charge.
31. The method of any of claims 13-30, wherein the liposome or complex has a mean diameter of about 20 micron to less than 1 micron.
- 20 32. The method of any of claims 13-30, wherein the liposome or complex has a mean diameter of about 500 nm to less than 100 nm.
33. The method of any of claims 13-32, wherein said composition is in lyophilized form.
- 25 34. The method claim 33, wherein said composition in lyophilized form comprises a cryoprotectant, wherein said cryoprotectant comprises one or more sugars are selected from a group consisting of trehalose, maltose, lactose, sucrose, and dextran.
- 30 35. The method of any of claims 13-33, wherein said liposome or complex is in a powder form or in a solution form, in a suspension form, in an emulsion form, and wherein said liposome or complex is in a gel form, in micelle form, or in a paste form.

36. The method of any of claims 13-35, wherein said composition is a tablet or is filled in a capsule.

5 37. A method of treating a cell, comprising preparing composition according to any of the claims 1, or 13-36, and exposing said cell to said composition.

38. The method of claim 37, wherein said cell is *in vivo* in a subject.

10 39. A method of treating a human or animal disease, comprising administering a therapeutically effective amount of the composition prepared according to a method of claim 1, 13-36 and exposing the composition to a human or animal in need thereof such that the composition is delivered to the human or animal patient.

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40. A kit of transfecting cells, said kit comprising a cationic guggul derivative of claim 1 and one or more elements selected from the group consisting of a polynucleotide, instructions for formulating the cationic guggul derivative and polynucleotide into a preparation, instructions for transfecting cells using the cationic guggul derivative, reagents for facilitating transfection, containers for storing the cationic guggul derivative, containers for storing the polynucleotide, containers for storing the reagents, containers for storing a preparation including the cationic guggul derivative and polynucleotide, or containers for preparing the preparation, and materials to facilitate transfection.

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41. A composition produced according to a method of any of claims 2-25 or 27-36.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Before the United States Receiving Office
For the Patent Cooperation Treaty

In re Application of	)	
	)	
JINA PHARMACEUTICALS INC.	)	
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Intl. App. No.: PCT/US2007/083832	)	LETTER AND STATEMENT ACCOMPANYING AMENDMENT OF THE CLAIMS UNDER PCT ARTICLE 19
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Intl. Filing Date: 06 November 2007	)	
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For: GUGGULPHOSPHOLIPID METHODS AND COMPOSITIONS	)	
	)	

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Madam/Sir:

Responsive to the International Search Report dated April 9, 2008, Applicant has hereby amended the claims and submits substitute pages for the international application. Claims 1-11 and 13-41 are unchanged. Claim 12 is amended. Replacement sheets for pages 59-66 are enclosed.

STATEMENT UNDER ARTICLE 19(1)

The amendment of Claim 12 is made in order provide the text shown underlined below, which failed to print on the top of Page 63 during PDF printing of the application.

12. The method of claim 11, wherein compound XI is prepared by conjugating guggulsterol through a linker, wherein the linker is an alkyl group optionally substituted with a functional group selected from the groups consisting of carbonyl, carboxyl, carbonate, amino, amide, ester, thioester groups, succinate, glutarate, carbamate, ether, phosphate, phosphonate, diphosphate, and pyrophosphate.

The same text is included in the application as filed at paragraph [0024]. The amendments do not go beyond the disclosure in the international application as filed.

The attached replacement sheets are intended to replace Sheets 59-66 as originally filed on 06 November 2007.

Respectfully submitted,

CASIMIR JONES, S.C.

Dated: 09 June 2008

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