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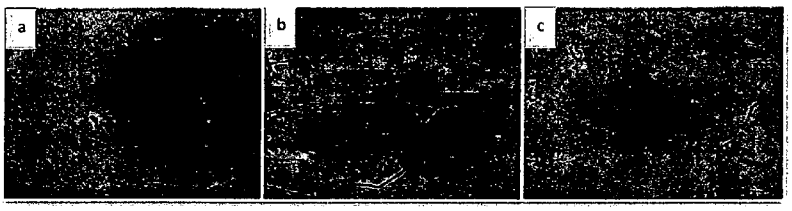
(54) **Title**: COMPOSITIONS AND METHODS FOR PREVENTION AND TREATMENT OF CORONARY HEART DISEASES

FIG. 3A-C



(57) **Abstract**: Disclosed herein are compounds and plant extracts, and methods for preventing or treating heart diseases, including coronary heart diseases. The compounds provided herein can be formulated into pharmaceutical compositions and medicaments that are useful in the disclosed methods. Also provided are the use of the compounds and extracts in preparing pharmaceutical formulations and medicaments.

WO 2010/143062 A1

COMPOSITIONS AND METHODS FOR PREVENTION AND TREATMENT OF CORONARY HEART DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Application No. 61/186,709, filed June 12, 2009, and U.S. Provisional Application No. 61/187,905, filed June 17, 2009, the entire contents of which are hereby incorporated by reference in their entirety.

BACKGROUND

[0002] The following description is provided to assist the understanding of the reader. None of the information provided or references cited is admitted to be prior art to the present invention.

[0003] Coronary heart diseases (CHD) result from occlusion of coronary arteries that in turn cause significantly reduced or interrupted blood supply to a part of the heart, leading to damage or death of heart muscle cells. Despite significant therapeutic advances, CHD remains the leading cause of morbidity and mortality in the Western countries. CHD is the leading cause of death in the US and about 15 million Americans suffer from and 500,000 die from CHD each year. Some developed regions of Asia also see a similar trend (the 2nd most common cause of death in Singapore, the 3rd in Hong Kong and China).

[0004] The root pathology of CHD is due to the narrowing or complete blockage of some of the coronary arteries, which gives rise to ischemia of the supplied myocardium or heart infarction. Although current therapeutic approaches can slow down the pace of CHD, effective therapeutic modalities towards substantial or curative treatment of CHD are not known. In CHD, naturally occurring neoangiogenesis to the ischemic or infarcted regions in the affected hearts is too insignificant to keep up with the demands of oxygen and nutrients required for contractile compensation of the viable heart tissues and is insufficient to support the greater demands of the hypertrophied myocardium, especially the cardiomyocytes along the border zone of the infarct that are at risk. The relative lack of oxygen and nutrients to the hypertrophied myocytes might be an important etiological contributing factor to the death of otherwise viable myocardium, which in turn leads to

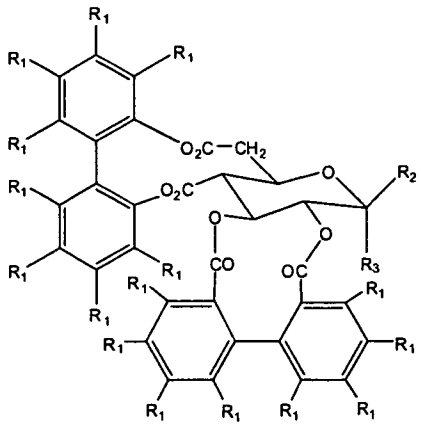
progressive infarct extension and fibrous replacement. Therefore, the most direct way to rescue the cardiac myocytes at risk is to establish a new blood supply at an early stage that would allow circulating stem cells, nutrients and growth factors, in addition to oxygenation, to be delivered to the ischemic or infarcted zone. Restoration of coronary blood flow by rapid angiogenesis should therefore offer a direct and effective therapeutic modality to intractable CHD.

[0005] Although therapeutic angiogenesis by growth factors, such as VEGF, aFGF, bFGF or PDGF, has been studied extensively as an alternative treatment for ischemic vascular diseases, these growth factors take weeks to act and their ability to grow new collateral vessels to the ischemic region is doubtful. However, coronary occlusion induced myocardial necrosis occurs very rapidly within a matter of hours. The consequence is the rapid growth of fibrous tissue replacing the infarcted heart tissues and leaving little room for any newly regenerated myocyte replacement. To date, no drug and therapeutic method that can promote early reconstitution of the damaged coronary vasculature with newly formed vessels is available

SUMMARY

[0006] In various aspects, the disclosure relates to a compounds, compositions and methods for treating coronary heart diseases. In accordance with one aspect, the present technology relates to compounds, compositions and methods for treating or preventing coronary heart diseases. In another aspect, the disclosure relates to compounds, compositions and methods for stimulating the growth of new collateral blood vessels in ischemic hearts that supply oxygen and nutrients to ischemic/infarcted heart tissues throughout the entire ischemic/infarct zone so that it can be used for substantial treatment of coronary heart diseases.

[0007] In one embodiment, the present disclosure provides a method for treating or preventing coronary heart disease in a mammalian subject comprising administering to the subject in need thereof an effective amount of a compound of formula I or formula II



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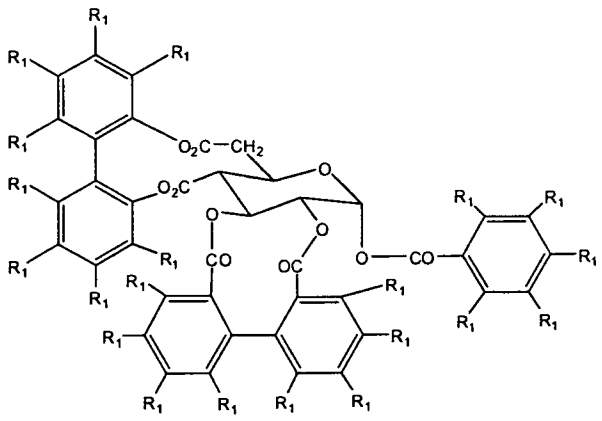


(II)

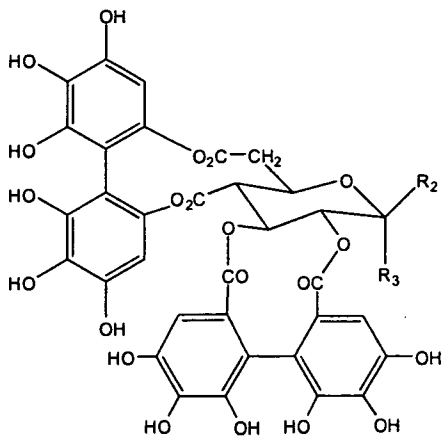
mixture thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

each R_1 is independently selected from H, halogen, hydroxy, carboxy, nitro, amino, nitrile, or a substituted or unsubstituted C_1–C_6 alkyl, alkoxy, hydroxyalkyl, cycloalkyl, cycloalkylalkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, haloalkyl, haloalkoxy, alkanoyl, and alkanoyloxy, groups; and

R_2 and R_3 are each H or O-R_1, such that when R_2 is H, R_3 is O-R1 and when R_2 is O-R_1, R_3 is H.

[0008] In one embodiment, the compound of formula I has the formula IA

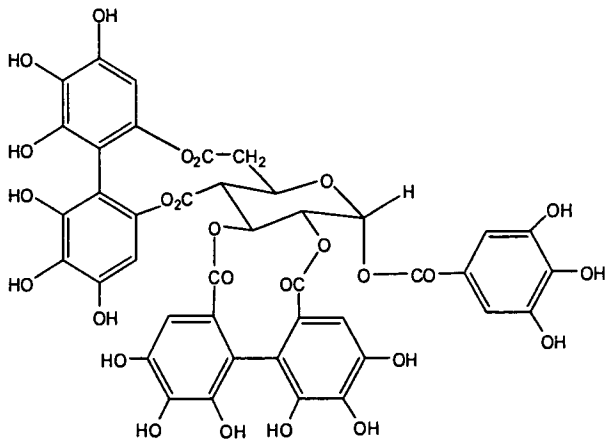


(IA)

wherein

R_2 and R_3 are each H or O-R_1, such that when R_2 is H, R_3 is O-R1 and when R_2 is O-R_1, R_3 is H.

[0009] In another embodiment, the compound of formula II has the formula IIA



(IIA)

[0010] In one embodiment, the present disclosure provides a method for treating or preventing coronary heart diseases in a mammalian subject comprising administering to the subject in need thereof an effective amount of a composition selected from the group consisting of: (i) an organic extract from the plant *Fructus Rosae Laevigatae*; (ii) an active fraction of an organic extract from the plant *Fructus Rosae Laevigatae*; and (iii) compound of formula (I), (IA), (II), or (IIA), mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof.

[0011] In some embodiments, the method comprises administering to the subject in need thereof an effective amount of a one or more of the compounds of formula (I), (IA), (II), and (IIA), mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof.

[0012] In another aspect, the present technology provides a method for promoting revascularization in dead or damaged heart tissues of a mammalian subject caused by an ischemic heart disease, the method comprising administering to the subject an effective amount of a compound of formula I or formula II, mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof.

[0013] In yet another aspect, the present technology provides a method for up-regulating the expression of angiogenic factors to stimulate growth of new coronary collateral vessels in ischemic or infarcted myocardium, in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of formula I or formula II, mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof, wherein the angiogenic factors are one or more of VEGF, VEGFR, EGF, and FGF.

[0014] In still another aspect, the present technology provides a method for treating ischemic heart diseases or ischemic limbs in a mammalian subject, comprising administering to the subject an effective amount of the compound of formula (I) or formula II, mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof.

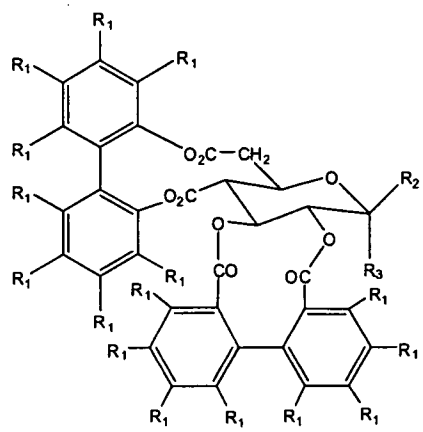
[0015] In some embodiments, the subject can be any mammal in need of angiogenic treatment such as *e.g.,* a human, dogs, cats, cows, sheep, pigs, horses monkey, rats, mice, rabbits and guinea pigs. In some embodiments, the mammalian subject is a human.

[0016] Administration of the compound or composition can be carried out by any suitable route, including orally, intranasally, parenterally, intravenously, intramuscularly, intraperitoneally, subcutaneously, rectally or topically. In some embodiments, the compound or composition is administered intravenously. In other embodiments, the compound or composition is administered orally.

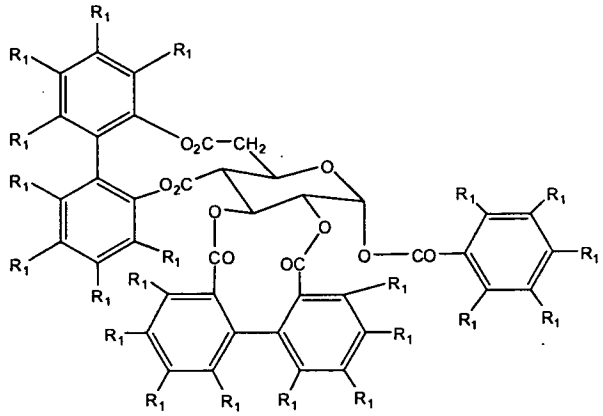
[0017] The compounds and compositions are administered in an amount required to produce the desired effect. In some embodiments, the effective amount includes amounts and dosages that are capable of preventing or treating coronary heart diseases, promoting revascularization in dead or damaged heart tissues and/or up-regulating the expression of angiogenic factors in a mammalian subject. In some embodiments, the compound or composition is administered in an amount of from 0.01 mg/kg/day to 2000 mg/kg/day. In some embodiments, the effective amount of the compound or composition is in the form of a pharmaceutical formulation comprising the compound or composition and a pharmaceutically acceptable carrier.

[0018] In one aspect, the present technology provides pharmaceutical composition, for use in treating or preventing coronary heart diseases, comprising a pharmaceutically acceptable

excipient and an effective amount of a compound selected from formula I or formula II.



(I)



(II)

mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof

wherein

each R_1 is independently selected from H, halogen, hydroxy, carboxy, nitro, amino, nitrile, or a substituted or unsubstituted C_1–C_6 alkyl, alkoxy, hydroxyalkyl, cycloalkyl, cycloalkylalkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, haloalkyl, haloalkoxy, alkanoyl, and alkanoyloxy, groups; and

R_2 and R_3 are each H or O-R_1, such that when R_2 is H, R_3 is O-R1 and when R_2 is O-R_1, R_3 is H.

[0019] In some embodiments, the compound of formula I in the pharmaceutical composition, has the structure IA. In other embodiments, the compound of formula II in the pharmaceutical composition, has the structure IIA. In some embodiments, the pharmaceutical composition may further comprise an anti-arrhythmia agent or a cardiovascular agent.

BRIEF DESCRIPTION OF THE FIGURES

[0020] FIG. 1. is a flow-chart illustrating a method for isolating angioenhancin compounds of formula I and II from the *Fructus Rosae Laevigatae* plant.

[0021] FIG. 2. illustrates a series of micrographs showing differentiation of vessel endothelial cells (HUVEC) induced by angioenhancin compounds of formula I and II. Panels a & b represent the vehicle treated cells showing proliferation but no sign of differentiation. Panel c shows angioenhancin-I (Compound of formula I, 20 μg/ml) treatment enhanced differentiation showing thin and elongated cells. Panel d shows angioenhancin-I (40 μg/ml) treated cells displaying thin and elongated phenotype forming tube-like structures. Panel e shows angioenhancin-II (Compound of formula II, 10 μg/ml) treated cells showing refractive and elongated cells. Panel f shows angioenhancin-II (20 μg/ml) treated cells displaying thin and elongated phenotype with the typical characteristic of differentiation of vessel endothelial cells. Angioenhancin-II (40 μg/ml) treated cells displaying thin, elongated and circled phenotype forming tube-like structures is seen in Panel g.

[0022] FIG. 3. demonstrates data showing angioenhancin enhanced capillary-like tube formation. Panel a shows control human aorta endothelial cells (HAEC), passage 7, cultured for 22 hours formed less capillary-like tubes. Panel b shows Angioenhancin-I (Compound of formula I, 5 μg/ml) enhanced growth of capillary-like tube structures much more than that in non-treated control. Panel c shows Angioenhancin-II (Compound of formula II) enhanced growth of capillary-like tubes more than that in non-treated control, but less than that in angioenhancin-I treated. D. is a graph showing the total lengths of the capillary-like tubes after treatment with various concentrations angioenhancin compounds of formula I or formula II.

[0023] FIG. 4. shows that angioenhancin-treatment induced angiogenesis in ischemic myocardium. C, The infarcted region in vehicle-treated heart, 2 weeks post LAD ligation, showing mainly fibrosis. A-I, The infarcted region in angioenhancin-I (Compound of formula I) treated heart, 2 weeks post LAD ligation. Many newly formed collateral vessels filled with red blood cells were found. A-II, The infarcted region in angioenhancin-II (Compound of formula II) treated heart, 2 weeks post LAD ligation with many newly formed vessels filled with red blood cells observed. C1, The chronic ischemic heart 2 weeks post LAD partial ligation in non-treated control hearts. A1-I, The chronic ischemic heart, 2 weeks post LAD partial ligation, and angioenhancin-I treatment showing more blood vessels compared vehicle-treated hearts (C1). A1-II, The chronic ischemic heart, 2 weeks post LAD partial ligation treated with angioenhancin-II for 2 weeks' showing more blood

vessels than thoses in vehicle-treated heart (C1). Vascular density in angioenhancin-I or II treated ischemic hearts averaged higher than that in the vehicle-treated hearts.

[0024] FIG. 5. illustrates echocardiography evaluation of heart functions in ischemic experimental animals. A: Panel as, shows a representative image of the end-systolic dimension of angioenhancin treated CHD heart. Panel ad, shows a representative echocardiography image of the end-diastolic dimension of the same animal. Panel cs, shows a representative image of the end-systolic dimension of vehicle-treated CHD heart. Panel cd, shows a representative echocardiography image of the end-diastolic dimension of the same animal. B is a graph illustrating the level of LVEF in angioenhancin-I or II treated and vehicle-treated hearts ($p<0.001$). C is a graph showing the level of LVFS in angioenhancin-I or II treated and vehicle-treated hearts ($p<0.001$).

DETAILED DESCRIPTION

[0025] In various aspects, the present disclosure provides compounds, extracts, and methods for preventing or treating coronary heart diseases. The compounds provided herein can be formulated into pharmaceutical compositions and medicaments that are useful in the disclosed methods. Also provided are the use of the compounds and extracts in preparing pharmaceutical formulations and medicaments.

[0026] It is to be appreciated that certain aspects, modes, embodiments, variations and features of the technology are described below in various levels of detail in order to provide a substantial understanding of the present technology. The following terms are used throughout as described below, unless context clearly indicates otherwise.

[0027] Generally, reference to a certain element such as hydrogen or H is meant to include all isotopes of that element. For example, if an R group is defined to include hydrogen or H, it also includes deuterium and tritium. Compounds comprising radioisotopes such as tritium, ^{14}C, ^{32}P and ^{35}S are thus within the scope of the technology. Procedures for inserting such labels into the compounds of the technology will be readily apparent to those skilled in the art based on the disclosure herein.

[0028] In general, "substituted" refers to an organic group as defined below (*e.g.*, an alkyl group) in which one or more bonds to a hydrogen atom contained therein are replaced by a bond to non-hydrogen or non-carbon atoms. Substituted groups also include groups in

which one or more bonds to a carbon(s) or hydrogen(s) atom are replaced by one or more bonds, including double or triple bonds, to a heteroatom. Thus, a substituted group is substituted with one or more substituents, unless otherwise specified. In some embodiments, a substituted group is substituted with 1, 2, 3, 4, 5, or 6 substituents. Examples of substituent groups include: halogens (*i.e.*, F, Cl, Br, and I); hydroxyls; alkoxy, alkenoxy, aryloxy, aralkyloxy, heterocyclyloxy, and heterocyclylalkoxy groups; carbonyls (oxo); carboxyls; esters; urethanes; oximes; hydroxylamines; alkoxyamines; aralkoxyamines; thiols; sulfides; sulfoxides; sulfones; sulfonyls; sulfonamides; amines; N-oxides; hydrazines; hydrazides; hydrazones; azides; amides; ureas; amidines; guanidines; enamines; imides; isocyanates; isothiocyanates; cyanates; thiocyanates; imines; nitro groups; nitriles (*i.e.*, CN); and the like. Substituted also includes multiple substitution *e.g.*, disubstituted groups such as dialkyl, diaryl etc.

[0029] Substituted ring groups such as substituted cycloalkyl, aryl, heterocyclyl and heteroaryl groups also include rings and fused ring systems in which a bond to a hydrogen atom is replaced with a bond to a carbon atom. Therefore, substituted cycloalkyl, aryl, heterocyclyl and heteroaryl groups may also be substituted with substituted or unsubstituted alkyl, alkenyl, and alkynyl groups as defined below.

[0030] Alkyl groups include straight chain and branched chain alkyl groups having from 1 to 12 carbon atoms, and typically from 1 to 10 carbons or, in some embodiments, from 1 to 8, 1 to 6, or 1 to 4 carbon atoms. Examples of straight chain alkyl groups include groups such as methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, and n-octyl groups. Examples of branched alkyl groups include, but are not limited to, isopropyl, iso-butyl, sec-butyl, tert-butyl, neopentyl, isopentyl, and 2,2-dimethylpropyl groups. Representative substituted alkyl groups may be substituted one or more times with substituents such as those listed above, and include without limitation haloalkyl (*e.g.*, trifluromethyl), hydroxyalkyl, thioalkyl, aminoalkyl, carboxyalkyl, and the like.

[0031] Cycloalkyl groups include mono-, bi- or tricyclic alkyl groups having from 3 to 14 carbon atoms in the ring(s), or, in some embodiments, 3 to 12, 3 to 10, 3 to 8, or 3, 4, 5, or 6 carbon atoms. Illustrative monocyclic cycloalkyl groups include, but not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl groups. In some embodiments, the cycloalkyl group has 3 to 8 ring members, whereas in other embodiments the number of ring carbon atoms range from 3 to 5, 3 to 6, or 3 to 7. Bi- and

tricyclic ring systems include both bridged cycloalkyl groups such as, but not limited to, adamantyl, and fused rings, such as, but not limited to, decalinyl, and the like. Substituted cycloalkyl groups may be substituted one or more times with, non-hydrogen and non-carbon groups as defined above. However, substituted cycloalkyl groups also include rings that are substituted with straight or branched chain alkyl groups as defined above. Representative substituted cycloalkyl groups may be mono-substituted or substituted more than once, such as, but not limited to, 2,2-, 2,3-, 2,4- 2,5- or 2,6-disubstituted cyclohexyl groups, which may be substituted with substituents such as those listed above.

[0032] Cycloalkylalkyl groups are alkyl groups as defined above in which a hydrogen or carbon bond of an alkyl group is replaced with a bond to a cycloalkyl group as defined above. In some embodiments, cycloalkylalkyl groups have from 4 to 16 carbon atoms, 4 to 12 carbon atoms, and typically 4 to 10 carbon atoms. Substituted cycloalkylalkyl groups may be substituted at the alkyl, the cycloalkyl or both the alkyl and cycloalkyl portions of the group. Representative substituted cycloalkylalkyl groups may be mono-substituted or substituted more than once, such as, but not limited to, mono-, di- or tri-substituted with substituents such as those listed above.

[0033] Alkenyl groups include straight and branched chain alkyl groups as defined above, except that at least one double bond exists between two carbon atoms. Thus, alkenyl groups have from 2 to 12 carbon atoms, and typically from 2 to 10 carbons or, in some embodiments, from 2 to 8, 2 to 6, or 2 to 4 carbon atoms. Examples include, but are not limited to vinyl, allyl,
, $-CH=CH(CH_3)$, $-CH=C(CH_3)_2$, $-C(CH_3)=CH_2$, $-C(CH_3)=CH(CH_3)$, $-C(CH_2CH_3)=CH_2$, among others. Representative substituted alkenyl groups may be mono-substituted or substituted more than once, such as, but not limited to, mono-, di- or tri-substituted with substituents such as those listed above.

[0034] Aryl groups are cyclic aromatic hydrocarbons that do not contain heteroatoms. Aryl groups herein include monocyclic, bicyclic and tricyclic ring systems. Thus, aryl groups include, but are not limited to, phenyl, azulenyl, heptalenyl, biphenyl, fluorenyl, phenanthrenyl, anthracenyl, indenyl, indanyl, pentalenyl, and naphthyl groups. In some embodiments, aryl groups contain 6-14 carbons, and in others from 6 to 12 or even 6-10 carbon atoms in the ring portions of the groups. In some embodiments, the aryl groups are phenyl or naphthyl. Although the phrase "aryl groups" includes groups containing fused

rings, such as fused aromatic-aliphatic ring systems (*e.g.*, indanyl, tetrahydronaphthyl, and the like), it does not include aryl groups that have other groups, such as alkyl or halo groups, bonded to one of the ring members. Rather, groups such as tolyl are referred to as substituted aryl groups. Representative substituted aryl groups may be mono-substituted or substituted more than once. For example, monosubstituted aryl groups include, but are not limited to, 2-, 3-, 4-, 5-, or 6-substituted phenyl or naphthyl groups, that may be substituted with substituents such as those listed above.

[0035] Aralkyl groups are alkyl groups as defined above in which a hydrogen or carbon bond of an alkyl group is replaced with a bond to an aryl group as defined above. In some embodiments, aralkyl groups contain 7 to 16 carbon atoms, 7 to 14 carbon atoms, or 7 to 12 carbon atoms. Substituted aralkyl groups may be substituted at the alkyl, the aryl or both the alkyl and aryl portions of the group. Representative aralkyl groups include but are not limited to benzyl and phenethyl groups and fused (cycloalkylaryl)alkyl groups such as 4-indanylethyl. Representative substituted aralkyl groups may be substituted one or more times with substituents such as those listed above.

[0036] Heterocyclyl groups include non-aromatic ring compounds containing 3 or more ring members, of which one or more is a heteroatom such as, but not limited to, N, O, and S. In some embodiments, the heterocyclyl group contains 1, 2, 3 or 4 heteroatoms. In some embodiments, heterocyclyl groups include mono-, bi- and tricyclic rings having 3 to 16 ring members, whereas other such groups have 3 to 6, 3 to 10, 3 to 12, or 3 to 14 ring members. Heterocyclyl groups encompass partially unsaturated and saturated ring systems, such as, for example, imidazolinyl and imidazolidinyl groups. The phrase "heterocyclyl group" includes fused ring species, including for example, hexahydropyrrolizine. The phrase also includes bridged polycyclic ring systems containing a heteroatom such as, but not limited to, quinuclidyl. However, the phrase does not include heterocyclyl groups that have other groups, such as alkyl, oxo or halo groups, bonded to one of the ring members. Rather, these are referred to as "substituted heterocyclyl groups". Heterocyclyl groups include, but are not limited to, aziridinyl, azetidinyl, pyrrolidinyl, imidazolidinyl, pyrazolidinyl, thiazolidinyl, tetrahydrothiophenyl, tetrahydrofuranyl, dioxolyl, pyrrolinyl, imidazolyl, imidazolinyl, pyrazolinyl, thiazolinyl, piperidyl, piperazinyl, morpholinyl, thiomorpholinyl, tetrahydropyranyl, tetrahydrothiopyranyl, oxathiane, dithianyl, pyranyl, dihydropyridyl, dihydrodithiinyl, dihydrodithionyl, homopiperazinyl, quinuclidyl, indolinyl, indolizinyl,

benzoxazinyl, benzodithiinyl, benzoxathiinyl, benzothiazinyl, benzoxazolyl, benzothiazolyl, benzothiadiazolyl, benzo[1,3]dioxolyl, quinolizinyl, quinoxalinyl, quinazolinyl, cinnolinyl, dihydrobenzothiazinyl, dihydrobenzofuranyl, dihydroindolyl, dihydrobenzodioxinyl, tetrahydroindolyl, tetrahydroindazolyl, tetrahydrobenzimidazolyl, tetrahydrobenzotriazolyl, tetrahydropyrrolopyridyl, tetrahydropyrazolopyridyl, tetrahydroimidazopyridyl, tetrahydrotriazolopyridyl, and tetrahydroquinolinyl groups. Representative substituted heterocyclyl groups may be mono-substituted or substituted more than once, such as, but not limited to, morpholinyl groups, which are 2-, 3-, 4-, 5-, or 6-substituted, or disubstituted with various substituents such as those listed above.

[0037] Heteroaryl groups are aromatic ring compounds containing 5 or more ring members, of which, one or more is a heteroatom such as, but not limited to, N, O, and S. Heteroaryl groups include, but are not limited to, groups such as pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, thiazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, thiophenyl, benzothiophenyl, furanyl, benzofuranyl, indolyl, azaindolyl (pyrrolopyridinyl), indazolyl, benzimidazolyl, imidazopyridinyl (azabenzimidazolyl), pyrazolopyridinyl, triazolopyridinyl, benzotriazolyl, benzoxazolyl, benzothiazolyl, benzothiadiazolyl, imidazopyridinyl, isoxazolopyridinyl, thianaphthyl, purinyl, xanthinyl, adeninyl, guaninyl, quinolinyl, isoquinolinyl, tetrahydroquinolinyl, quinoxalinyl, and quinazolinyl groups. Heteroaryl groups include fused ring compounds in which all rings are aromatic such as indolyl groups and include fused ring compounds in which only one of the rings is aromatic, such as 2,3-dihydro indolyl groups. Although the phrase "heteroaryl groups" includes fused ring compounds, the phrase does not include heteroaryl groups that have other groups bonded to one of the ring members, such as alkyl groups. Rather, heteroaryl groups with such substitution are referred to as "substituted heteroaryl groups." Representative substituted heteroaryl groups may be substituted one or more times with various substituents such as those listed above.

[0038] Heterocyclylalkyl groups are alkyl groups as defined above in which a hydrogen or carbon bond of an alkyl group is replaced with a bond to a heterocyclyl group as defined above. Substituted heterocyclylalkyl groups may be substituted at the alkyl, the heterocyclyl or both the alkyl and heterocyclyl portions of the group. Representative heterocyclyl alkyl groups include, but are not limited to, morpholin-4-yl-ethyl, piperazin-1-yl-methyl, tetrahydrofuran-2-yl-ethyl, and piperidinyl-propyl. Representative substituted

heterocyclylalkyl groups may be substituted one or more times with substituents such as those listed above.

[0039] Heteroaralkyl groups are alkyl groups as defined above in which a hydrogen or carbon bond of an alkyl group is replaced with a bond to a heteroaryl group as defined above. Substituted heteroaralkyl groups may be substituted at the alkyl, the heteroaryl or both the alkyl and heteroaryl portions of the group. Representative substituted heteroaralkyl groups may be substituted one or more times with substituents such as those listed above.

[0040] Groups described herein having two or more points of attachment (*i.e.*, divalent, trivalent, or polyvalent) within the compound of the technology are designated by use of the suffix, "ene." For example, divalent alkyl groups are alkylene groups, divalent aryl groups are arylene groups, divalent heteroaryl groups are divalent heteroarylene groups, and so forth. Substituted groups having a single point of attachment to the compound of the technology are not referred to using the "ene" designation. Thus, *e.g.*, chloroethyl is not referred to herein as chloroethylene.

[0041] Alkoxy and cycloalkoxy groups are hydroxyl groups (-OH) in which the bond to the hydrogen atom is replaced by a bond to a carbon atom of a substituted or unsubstituted alkyl group as defined above. Examples of linear alkoxy groups include but are not limited to methoxy, ethoxy, propoxy, butoxy, pentoxy, hexoxy, and the like. Examples of branched alkoxy groups include but are not limited to isopropoxy, sec-butoxy, tert-butoxy, isopentoxy, isohexoxy, and the like. Examples of cycloalkoxy groups include but are not limited to cyclopropyloxy, cyclobutyloxy, cyclopentyloxy, cyclohexyloxy, and the like. Representative substituted alkoxy groups may be substituted one or more times with substituents such as those listed above.

[0042] The terms "aryloxy" and "arylalkoxy" refer to, respectively, a substituted or unsubstituted aryl group bonded to an oxygen atom and a substituted or unsubstituted aralkyl group bonded to the oxygen atom at the alkyl. Examples include but are not limited to phenoxy, naphthyloxy, and benzyloxy. Representative substituted aryloxy and arylalkoxy groups may be substituted one or more times with substituents such as those listed above.

[0043] The terms "carboxyl" and "carboxy" as used herein refers to a -COOH group.

[0044] The term "ester" as used herein refers to $-COOR^{30}$ groups. R^{30} is a substituted or unsubstituted alkyl, cycloalkyl, alkenyl, alkynyl, aryl, aralkyl, heterocyclylalkyl or heterocyclyl group as defined herein.

[0045] The term "amide" (or "amido") includes C- and N-amide groups, *i.e.*, $-C(O)NR^{31}R^{32}$, and $-NR^{31}C(O)R^{32}$ groups, respectively. R^{31} and R^{32} are independently hydrogen, or a substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heterocyclylalkyl or heterocyclyl group as defined herein. Amido groups therefore include but are not limited to carbamoyl groups ($-C(O)NH_2$) and formamide groups (-NHC(O)H).

[0046] Urethane groups include N- and O-urethane groups, *i.e.*, $-NR^{33}C(O)OR^{34}$ and $-OC(O)NR^{33}R^{34}$ groups, respectively. R^{33} and R^{34} are independently a substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heterocyclylalkyl, or heterocyclyl group as defined herein. R^{33} may also be -H.

[0047] The term "amine" (or "amino") as used herein refers to $-NHR^{35}$ and $-NR^{36}R^{37}$ groups, wherein R^{35}, R^{36} and R^{37} are independently hydrogen, or a substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heterocyclylalkyl or heterocyclyl group as defined herein. In some embodiments, the amine is NH_2, methylamino, dimethylamino, ethylamino, diethylamino, propylamino, isopropylamino, phenylamino, or benzylamino.

[0048] The term "sulfonamido" includes S- and N-sulfonamide groups, *i.e.*, $-SO_2NR^{38}R^{39}$ and $-NR^{38}SO_2R^{39}$ groups, respectively. R^{38} and R^{39} are independently hydrogen, or a substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heterocyclylalkyl, or heterocyclyl group as defined herein. Sulfonamido groups therefore include but are not limited to sulfamoyl groups ($-SO_2NH_2$).

[0049] The term "thiol" refers to –SH groups, while sulfides include $-SR^{40}$ groups, sulfoxides include $-S(O)R^{41}$ groups, sulfones include $-SO_2R^{42}$ groups, and sulfonyls include $-SO_2OR^{43}$. R^{40}, R^{41}, R^{42}, and R^{43} are each independently a substituted or unsubstituted alkyl, cycloalkyl, alkenyl, alkynyl, aryl aralkyl, heterocyclyl or heterocyclylalkyl group as defined herein.

[0050] The term "urea" refers to $-NR^{44}-C(O)-NR^{45}R^{46}$ groups. R^{44}, R^{45}, and R^{46} groups are independently hydrogen, or a substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heterocyclyl, or heterocyclylalkyl group as defined herein.

[0051] The term "amidine" refers to $-C(NR^{47})NR^{48}R^{49}$ and $-NR^{47}C(NR^{48})R^{49}$, wherein R^{47}, R^{48}, and R^{49} are each independently hydrogen, or a substituted or unsubstituted alkyl, cycloalkyl, alkenyl, alkynyl, aryl aralkyl, heterocyclyl or heterocyclylalkyl group as defined herein.

[0052] The term "guanidine" refers to $-NR^{50}C(NR^{51})NR^{52}R^{53}$, wherein R^{50}, R^{51}, R^{52} and R^{53} are each independently hydrogen, or a substituted or unsubstituted alkyl, cycloalkyl, alkenyl, alkynyl, aryl aralkyl, heterocyclyl or heterocyclylalkyl group as defined herein.

[0053] The term "enamine" refers to $-C(R^{54})=C(R^{55})NR^{56}R^{57}$ and $-NR^{54}C(R^{55})=C(R^{56})R^{57}$, wherein R^{54}, R^{55}, R^{56} and R^{57} are each independently hydrogen, a substituted or unsubstituted alkyl, cycloalkyl, alkenyl, alkynyl, aryl aralkyl, heterocyclyl or heterocyclylalkyl group as defined herein.

[0054] The term "imide" refers to $-C(O)NR^{58}C(O)R^{59}$, wherein R^{58} and R^{59} are each independently hydrogen, or a substituted or unsubstituted alkyl, cycloalkyl, alkenyl, alkynyl, aryl aralkyl, heterocyclyl or heterocyclylalkyl group as defined herein.

[0055] The term "imine" refers to $-CR^{60}(NR^{61})$ and $-N(CR^{60}R^{61})$ groups, wherein R^{60} and R^{61} are each independently hydrogen or a substituted or unsubstituted alkyl, cycloalkyl, alkenyl, alkynyl, aryl aralkyl, heterocyclyl or heterocyclylalkyl group as defined herein, with the proviso that R^{60} and R^{61} are not both simultaneously hydrogen.

[0056] The term "leaving group" refers to an atom or group of atoms which may be replaced by another atom or group of atoms (*e.g.*, a nucleophile, such as an amine, thiol, carbanion, and the like) during a chemical reaction. Illustrative leaving groups are well known in the art and include, but are not limited to halogen groups (*e.g.*, I, Br, F, Cl), sulfonate groups (*e.g.*, mesylate, tosylate, triflate), substituted alkylsulfonate groups (*e.g.*, haloalkylsulfonate); C_6-aryloxy or subsituted C_6-aryloxy groups; acyloxy groups and the like.

[0057] The term "protected" with respect to hydroxyl groups, amine groups, carboxy groups, and thiol groups refers to forms of these functionalities that are protected from undesirable reaction by means of protecting groups. Protecting groups such as hydroxyl, amino, carboxy, and thiol protecting groups, are known to those skilled in the art and can be added or removed using well-known procedures such as those set forth in Protective Groups in Organic Synthesis, Greene, T.W.; Wuts, P. G. M., John Wiley & Sons, New York, NY, (3rd Edition, 1999). Examples of protected hydroxyl groups include, but are not limited to, silyl ethers such as those obtained by reaction of a hydroxyl group with a reagent such as, but not limited to, t-butyldimethyl-chlorosilane, trimethylchlorosilane, triisopropylchlorosilane, triethylchlorosilane; substituted methyl and ethyl ethers such as, but not limited to methoxymethyl ether, methythiomethyl ether, benzyloxymethyl ether, t-butoxymethyl ether, 2-methoxyethoxymethyl ether, tetrahydropyranyl ethers, 1-ethoxyethyl ether, t-butyl ether, allyl ether, benzyl ether; esters such as, but not limited to, benzoyl, formate, acetate, trichloroacetate, and trifluoracetate.

[0058] Amino-Protecting groups (also known as N-protecting groups) comprise acyl groups such as formyl, acetyl, propionyl, pivaloyl, t-butylacetyl, 2-chloroacetyl, 2-bromoacetyl, trifluoroacetyl, trichloroacetyl, phthalyl, o-nitrophenoxyacetyl, a-chlorobutyryl, benzoyl, 4-chlorobenzoyl, 4-bromobenzoyl, 4-nitrobenzoyl, and the like; sulfonyl groups such as benzenesulfonyl, p-toluenesulfonyl and the like; carbamate forming groups such as benzyloxycarbonyl, p-chlorobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 3,5-dimethoxybenzyloxycarbonyl, 2,4-dimethoxybenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 2-nitro-4,5-dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, 1-(p-biphenylyl)-1-methylethoxycarbonyl, α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl, benzhydryloxycarbonyl, t-butyloxycarbonyl, diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl, methoxycarbonyl, allyloxycarbonyl, 2,2,2,-trichloroethoxycarbonyl, phenoxycarbonyl, 4-nitrophenoxycarbonyl, fluorenyl-9-methoxycarbonyl, cyclopentyloxycarbonyl, adamantyloxycarbonyl, cyclohexyloxycarbonyl, phenylthiocarbonyl and the like; alkyl groups such as benzyl, triphenylmethyl, benzyloxymethyl and the like; and silyl groups such as trimethylsilyl and the like. Typical amino-protecting groups include formyl, acetyl,

benzoyl, pivaloyl, t-butylacetyl, phenylsulfonyl, benzyl, 9-fluorenylmethyloxycarbonyl (Fmoc), t-butyloxycarbonyl (Boc) and benzyloxycarbonyl (Cbz).

[0059] Examples of protected thiol groups include, but are not limited to, thioethers such as S-benzyl thioether, S-t-butylthioether, and S-4-picolyl thioether; substituted S-methyl derivatives such as hemithio, dithio and aminothio acetals; and others.

[0060] Representative carboxy protecting groups are C_1 to C_8 alkyl (*e.g.*, methyl, ethyl or tertiary butyl and the like); haloalkyl, such as trichloryphtye and the like; alkenyl, such as allyl and the like; cycloalkyl and substituted derivatives thereof such as cyclohexyl, cyclopentyl and the like; cycloalkylalkyl and substituted derivatives thereof such as cyclohexylmethyl, cyclopentylmethyl and the like; arylalkyl, for example, phenethyl or benzyl and substituted derivatives thereof such as alkoxybenzyl or nitrobenzyl groups and the like; arylalkenyl, for example, phenylethenyl and the like; aryl and substituted derivatives thereof, for example, 5-indanyl and the like; dialkylaminoalkyl (*e.g.*, dimethylaminoethyl and the like); alkanoyloxyalkyl groups such as acetoxymethyl, butyryloxymethyl, valeryloxymethyl, isobutyryloxymethyl, isovaleryloxymethyl, 1-(propionyloxy)-1-ethyl, 1-(pivaloyloxyl)-1-ethyl, 1-methyl-1-(propionyloxy)-1-ethyl, pivaloyloxymethyl, propionyloxymethyl and the like; cycloalkanoyloxyalkyl groups such as cyclopropylcarbonyloxymethyl, cyclobutylcarbonyloxymethyl, cyclopentylcarbonyloxymethyl, cyclohexylcarbonyloxymethyl and the like; aroyloxyalkyl, such as benzoyloxymethyl, benzoyloxyethyl and the like; arylalkylcarbonyloxyalkyl, such as benzylcarbonyloxymethyl, 2-benzylcarbonyloxyethyl and the like; alkoxycarbonylalkyl, such as methoxycarbonylmethyl, cyclohexyloxycarbonylmethyl, 1-methoxycarbonyl-1-ethyl, and the like; alkoxycarbonyloxyalkyl, such as methoxycarbonyloxymethyl, t-butyloxycarbonyloxymethyl, 1-ethoxycarbonyloxy-1-ethyl, 1-cyclohexyloxycarbonyloxy-1-ethyl and the like; alkoxycarbonylaminoalkyl, such as t-butyloxycarbonylaminomethyl and the like; alkylaminocarbonylaminoalkyl, such as methylaminocarbonylaminomethyl and the like; alkanoylaminoalkyl, such as acetylaminomethyl and the like; heterocyclylcarbonyloxyalkyl, such as 4-methylpiperazinylcarbonyloxymethyl and the like; dialkylaminocarbonylalkyl, such as dimethylaminocarbonylmethyl, diethylaminocarbonylmethyl and the like; (5-(alkyl)-2-oxo-1,3-dioxolen-4-yl)alkyl, such as (5-t-butyl-2-oxo-1,3-dioxolen-4-yl)methyl and the like; and (5-phenyl-2-oxo-1,3-dioxolen-4-yl)alkyl, such as (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methyl and the like.

[0061] Those of skill in the art will appreciate that compounds of the technology may exhibit the phenomena of tautomerism, conformational isomerism, geometric isomerism and/or stereoisomerism. As the formula drawings within the specification and claims can represent only one of the possible tautomeric, conformational isomeric, stereoisomeric or geometric isomeric forms, it should be understood that the technology encompasses any tautomeric, conformational isomeric, stereoisomeric and/or geometric isomeric forms of the compounds having one or more of the utilities described herein, as well as mixtures of these various different forms.

[0062] The term "Tautomers" refers to isomeric forms of a compound that are in equilibrium with each other. The presence and concentrations of the isomeric forms will depend on the environment the compound is found in and may be different depending upon, for example, whether the compound is a solid or is in an organic or aqueous solution.

[0063] As readily understood by one skilled in the art, a wide variety of functional groups and other structures may exhibit tautomerism, and all tautomers of compounds as described herein are within the scope of the present technology.

[0064] Stereoisomers of compounds (also known as optical isomers) include all chiral, diastereomeric, and racemic forms of a structure, unless the specific stereochemistry is expressly indicated. Thus, compounds used in the present technology include enriched or resolved optical isomers at any or all asymmetric atoms as are apparent from the depictions. Both racemic and diastereomeric mixtures, as well as the individual optical isomers can be isolated or synthesized so as to be substantially free of their enantiomeric or diastereomeric partners, and these stereoisomers are all within the scope of the technology.

[0065] The compounds of the technology may exist as prodrugs and bioisosteres. "Prodrug" as used herein refers to an inactive form of the compound due to the attachment of one or more specialized protective groups used in a transient manner to alter or to eliminate undesirable properties in the parent molecule, which is metabolized or converted into the active compound inside the body (in vivo) once administered. The term "Bioisostere" as used herein refers to a compound resulting from the exchange of an atom or of a group of atoms with another, broadly similar, atom or group of atoms. The objective of a bioisosteric replacement is to create a new compound with similar biological properties

to the parent compound. The bioisosteric replacement may be physicochemically or topologically based.

[0066] The compounds of the technology may exist as solvates, especially hydrates. Hydrates may form during manufacture of the compounds or compositions comprising the compounds, or hydrates may form over time due to the hygroscopic nature of the compounds. Compounds of the technology may exist as organic solvates as well, including DMF, ether, and alcohol solvates among others. The identification and preparation of any particular solvate is within the skill of the ordinary artisan of synthetic organic or medicinal chemistry.

[0067] As used in the present application, the term "functional derivative" means a prodrug, bioisostere, N-oxide, pharmaceutically acceptable salt or various isomers of the compounds of present technology, which may be advantageous in one or more aspects compared with the parent compound. Methods of making functional derivatives are well known in the art. Various high-throughput chemical synthetic methods for making functional derivatives are known in the art. For example, combinatorial chemistry has resulted in a rapid expansion of compound libraries, which when coupled with various highly efficient bio-screening technologies can lead to efficient discovering and isolating useful functional derivatives.

[0068] As used herein, the "administration" of an agent or drug to a subject or subject includes any route of introducing or delivering to a subject a compound to perform its intended function. Administration can be carried out by any suitable route, including orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, or topically. Administration includes self-administration and the administration by another. It is also to be appreciated that the various modes of treatment or prevention of medical conditions as described are intended to mean "substantial", which includes total but also less than total treatment or prevention, and wherein some biologically or medically relevant result is achieved.

[0069] As used herein, the term "effective amount" or "pharmaceutically effective amount" or "therapeutically effective amount" of a composition, is a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, *e.g.*, an amount which results in the prevention of, or a decrease in, the symptoms associated with a disease that is being treated.

One example of an effective amount includes amounts or dosages that yield acceptable toxicity and bioavailability levels for therapeutic (pharmaceutical) use including, but not limited to, the treatment or prevention of heart disease including coronary heart disease, and promoting revascularization in dead or damaged heart tissues caused by ischemic heart disease. Another example of an effective amount includes amounts or dosages that are capable of up-regulating the expression of angiogenic factors including VEGF, VEGFR, EGF and FGF.

[0070] The amount of a composition of the technology administered to the subject will depend on the type and severity of the disease and on the characteristics of the individual, such as general health, age, sex, body weight and tolerance to drugs. It will also depend on the degree, severity and type of disease. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. The compositions of the present technology can also be administered in combination with one or more additional therapeutic compounds. A person of ordinary skill in the art, would have no difficulty determining the appropriate timing, sequence and dosages of administration for particular drugs and compositions of the present technology.

[0071] As used herein, the term "disease" or "medical condition" are used interchangeably and includes, but is not limited to, any condition or disease manifested as one or more physical and/or psychological symptoms for which treatment and/or prevention is desirable, and includes previously and newly identified diseases and other disorders. For example, a medical condition may be coronary heart disease.

[0072] As used herein, the term "subject" or "patient" is a mammal. Typically, the subject is a human, but can also be an animal, *e.g.*, domestic animals (*e.g.*, dogs, cats and the like), farm animals (*e.g.*, cows, sheep, pigs, horses and the like) and laboratory animals (*e.g.*, monkey, rats, mice, rabbits, guinea pigs and the like).

[0073] As used herein, the terms "treating" or "treatment" or "alleviation" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. A subject is successfully "treated" for a disorder if, after receiving a therapeutic agent according to the methods of the present technology, the subject shows observable and/or measurable reduction in or absence of one or more signs and symptoms of a particular disease or condition.

[0074] As used herein, "Pharmaceutically-acceptable salts and esters" refers to salts and esters that are pharmaceutically-acceptable and have the desired pharmacological properties. Such salts include salts that can be formed where acidic protons present in the agent are capable of reacting with inorganic or organic bases. Suitable inorganic salts include those formed with the alkali metals, *e.g.,* sodium and potassium, magnesium, calcium, and aluminum. Suitable organic salts include those formed with organic bases such as the amine bases, *e.g.,* ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. Such salts also include acid addition salts formed with inorganic acids (*e.g.,* hydrochloric and hydrobromic acids) and organic acids (*e.g.,* acetic acid, citric acid, maleic acid, and the alkane- and arene-sulfonic acids such as methanesulfonic acid and benzenesulfonic acid). Pharmaceutically-acceptable esters include esters formed from carboxy, sulfonyloxy, and phosphonoxy groups present in the agent, *e.g.,* C_{1-6} alkyl esters. When there are two acidic groups present, a pharmaceutically-acceptable salt or ester can be a mono-acid-mono-salt or ester or a di-salt or ester; and similarly where there are more than two acidic groups present, some or all of such groups can be salified or esterified. The agent named in this technology can be present in unsalified or unesterified form, or in salified and/or esterified form, and the naming of such agent is intended to include both the original (unsalified and unesterified) compound and its pharmaceutically-acceptable salts and esters.

[0075] As used herein, the term "pharmaceutically-acceptable carrier" or "pharmaceutically-acceptable excepient"is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal compounds, isotonic and absorption delaying compounds, and the like, compatible with pharmaceutical administration.

[0076] Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and compounds for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or compound is incompatible with the agent, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Methods and Compositions for the Prevention or Treatment of Heart Disease

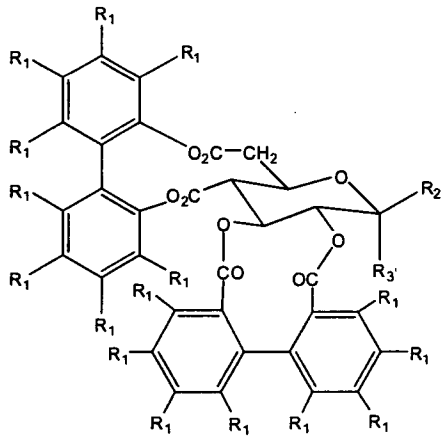
[0077] Coronary heart diseases (CHD) remain the most prevalent cause of premature death. Reconstitution of the damaged coronary vessels with newly formed functional coronary collaterals to functionally bypass the narrowed/occluded arteries would provide curative treatment for CHD.

[0078] The present technology provides methods of treating or preventing a variety of diseases or medical conditions with agents and/or extracts and compounds, and derivatives of such compounds from a variety of plants. In accordance with one aspect, the technology provides methods of treating or preventing heart diseases, including CHD, in a subject in need thereof, which comprises administering to the subject an effective amount of a compound, composition, active fractions, or extract described herein. In another aspect, the present technology provides methods and compositions for stimulating the growth of new collateral blood vessels that supply oxygen and nutrients to ischemic/ infarcted heart tissues throughout the entire ischemic/infarct zone, wherein the method comprises administering to a subject in need thereof an effective amount of a compound, composition, active fractions, or extract described herein. The effects of CHD or symptoms thereof to be improved can be one or more of chest pain, shortness of breath, weakness, fainting spells, alterations in consciousness, extremity pain, paroxysmal nocturnal dyspnea, orthophea, transient ischemic attacks and other such phenomena experienced by the patient. Clinical signs in CHD also include such findings as ECG or echocardiography abnormalities, altered peripheral pulses, arterial bruits, abnormal heart sounds, rates, jugular venous distention, neurological alterations and other such findings discerned by the clinician.

[0079] The compositions used in the present methods may include suitable agents which are capable of treating or preventing heart diseases, including CHD. In some embodiments, the agent is an extract, *e.g.,* an organic extract, of the plant *Fructus Rosae Laevigatae.* In an illustrative embodiment, the agent is a methanol/ethanol extract of *Fructus Rosae Laevigatae* or an active fraction thereof. In one embodiment, the active fraction is FRL-B. In some embodiments, the active fraction is FRL-B-1. In other embodiments, the active fraction is FRL-B-1-1. In yet other embodiments, the active fraction is FRL-B-1-1-2. . In one embodiment, the active fraction is FRL-E. In some embodiments, the active fraction is FRL-E-3. In certain embodiments, the agent is a tannin, including hydrolysable tannins such as pendunculagin, potentillin or derivatives thereof. In one embodiment, the agents

include substantially pure compounds having formula (I), (II), (IA), (IIA), (III), (IIIA), (IIIB), (IIIC), (IIID), (IIIE), (IIIF), (IIIG), (IV), (IVA), (V), (VI), (VII), (VIII), (IX), (X), (XI) or (XII) as shown herein or mixtures thereof, as well as stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof.

[0080] In one embodiment, the agent is a compound of formula (I) and has the following structure



(I)

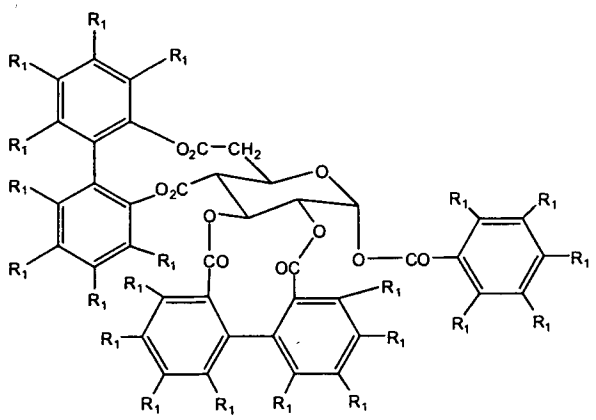
stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

each R_1 is independently selected from H, halogen, hydroxy, carboxy, nitro, amino, nitrile, or a substituted or unsubstituted C_1–C_6 alkyl, alkoxy, hydroxyalkyl, cycloalkyl, cycloalkylalkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, haloalkyl, haloalkoxy, alkanoyl, and alkanoyloxy, groups; and

R_2 and R_3 are each H or O-R_1, such that when R_2 is H, R_3 is O-R1 and when R_2 is O-R_1, R_3 is H.

[0081] In one embodiment, the agent is a compound of formula (II) and has the following structure



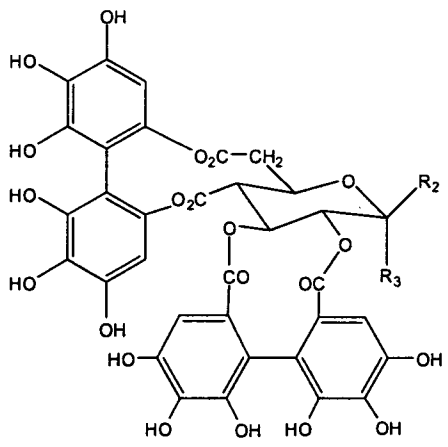
(II)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

each R_1 is independently selected from H, halogen, hydroxy, carboxy, nitro, amino, nitrile, or a substituted or unsubstituted C_1–C_6 alkyl, alkoxy, hydroxyalkyl, cycloalkyl, cycloalkylalkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, haloalkyl, haloalkoxy, alkanoyl, and alkanoyloxy, groups.

[0082] In one embodiment, the agent is a compound of formula (IA) and has the following structure



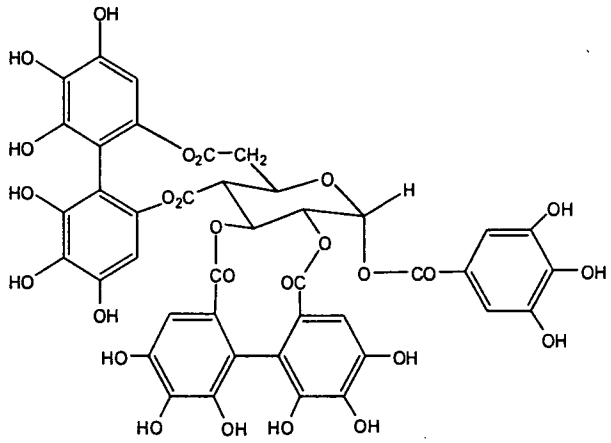
(IA)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

R_2 and R_3 are each H or O-R_1, such that when R_2 is H, R_3 is O-R1 and when R_2 is O-R_1, R_3 is H.

[0083] In one embodiment, the agent is a compound of formula (IIA) and has the following structure



(IIA)

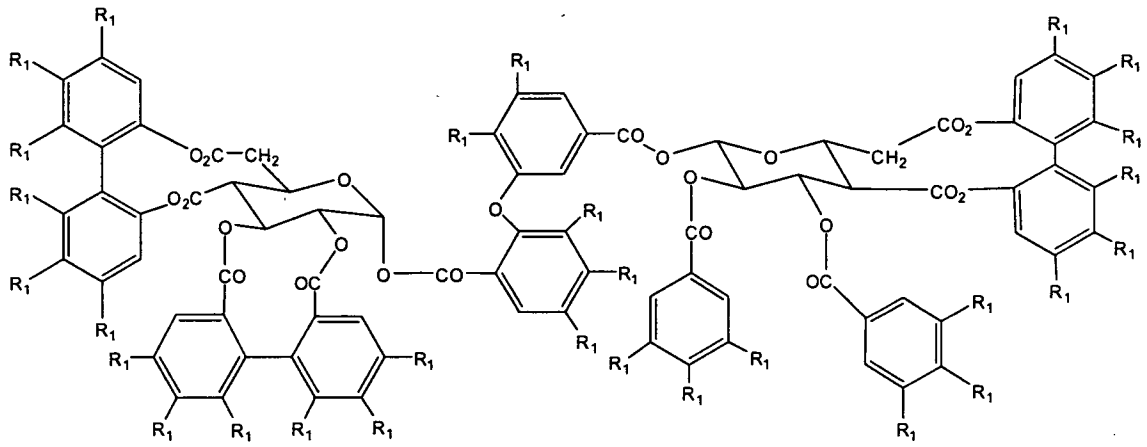
stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

Compound of formula IA is commonly known as Peunculagin and compound of formula IIA is commonly known as Potentillin or Galloyl-pedunculagin. Compounds of formula I, II, IA and IIA are referred to as "angioenhancin" hereinafter.

[0084] One of ordinary skill in the art would appreciate that the backbone compounds of formula I and II can have substituents at various positions, as described above, and yet retain their biological activity. The compounds of formula I and II therefore encompass the backbone compound itself and its substituted variants having similar biological activities. In some embodiments, compounds of formula I, II, IA and IIA may include stereoisomers, tautomers, solvates, pharmaceutically acceptable salts and other functional derivatives as described herein. The technology may also include derivatives, prodrugs and bioisosteres of compounds of formula I and formula II. The tannins, to which the disclosed angioenhancin compounds belong, are conventionally reviewed as non-active ingredients and in the process of identifying the active ingredients in herbal medicines researchers routinely discard the tannins as debris. Angioenhancins, such as compounds of formula I, II, IA or IIA may be isolated from natural resources, particularly from plants or they may be

synthesized using synthetic techniques known in the art, and can be obtained though total or semi-chemical syntheses. These compounds have shown potent beneficial therapeutic effects in treating coronary heart diseases by inducing cappilary-like tube structure formation of vessel endothelial cells *in vitro*, and stimulating the growth of new coronary collateral vessels in ischemic/infarcted myocardia and prevention of further ischemic death of the cardiomyocytes. These compounds are therefore useful in treating CHD including chronic coronary heart disease and heart infarction.

[0085] In one embodiment, the agent is a compound of formula (III) and has the following structure



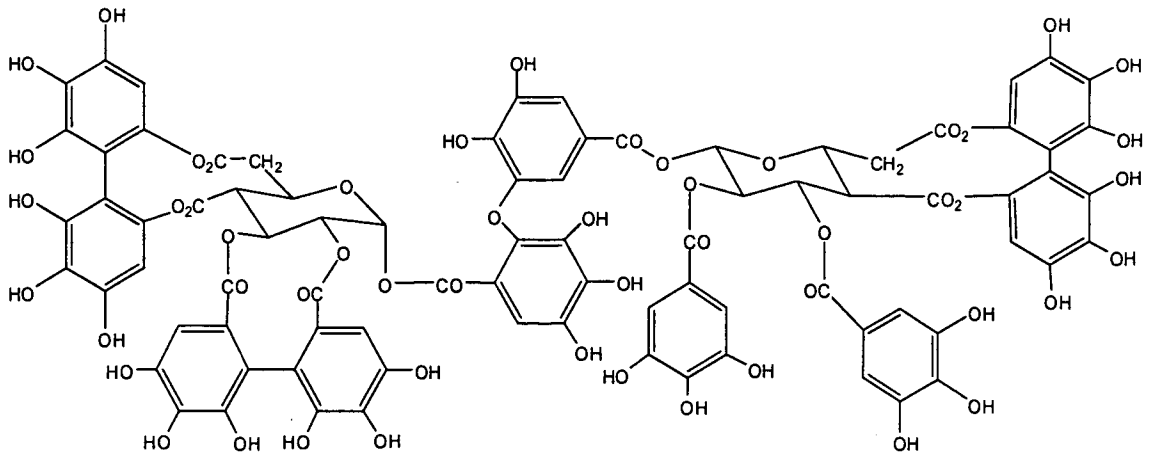
(III)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

each R_1 is independently selected from H, halogen, hydroxy, carboxy, nitro, amino, nitrile, or a substituted or unsubstituted C_1–C_6 alkyl, alkoxy, hydroxyalkyl, cycloalkyl, cycloalkylalkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, haloalkyl, haloalkoxy, alkanoyl, and alkanoyloxy, groups.

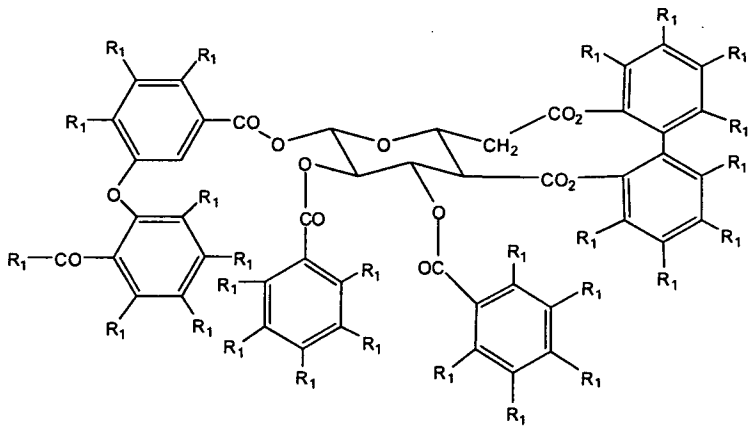
[0086] In one embodiment, the agent is a compound of formula (IIIA) and has the following structure



(IIIA)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

[0087] In one embodiment, the agent is a compound of formula (IIIB) and has the following structure



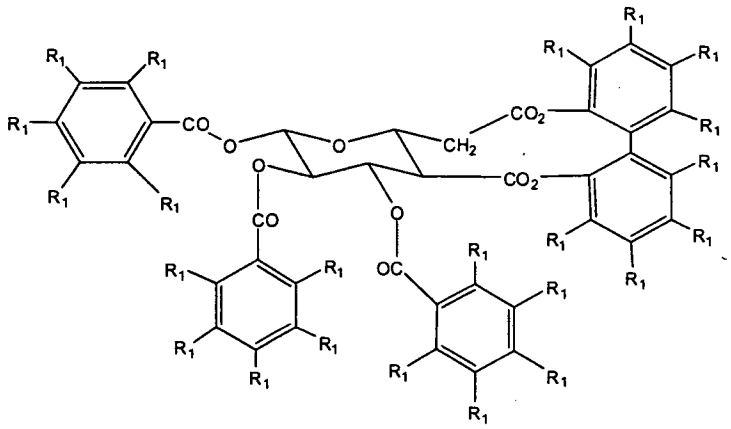
(IIIB)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

each R_1 is independently selected from H, halogen, hydroxy, carboxy, nitro, amino, nitrile, or a substituted or unsubstituted C_1–C_6 alkyl, alkoxy, hydroxyalkyl, cycloalkyl, cycloalkylalkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, haloalkyl, haloalkoxy, alkanoyl, and alkanoyloxy, groups.

[0088] In one embodiment, the agent is a compound of formula (IIIC) and has the following structure



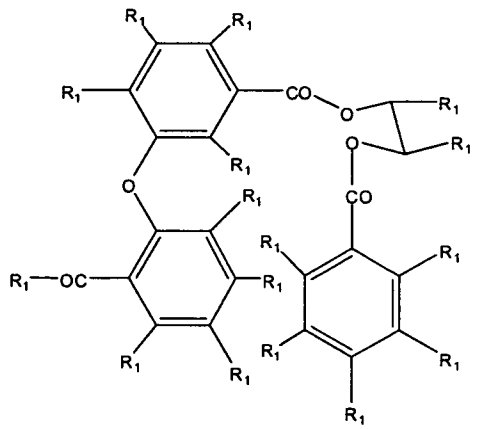
(IIIC)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

each R_1 is independently selected from H, halogen, hydroxy, carboxy, nitro, amino, nitrile, or a substituted or unsubstituted C_1–C_6 alkyl, alkoxy, hydroxyalkyl, cycloalkyl, cycloalkylalkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, haloalkyl, haloalkoxy, alkanoyl, and alkanoyloxy, groups.

[0089] In one embodiment, the agent is a compound of formula (IIID) and has the following structure



(IIID)

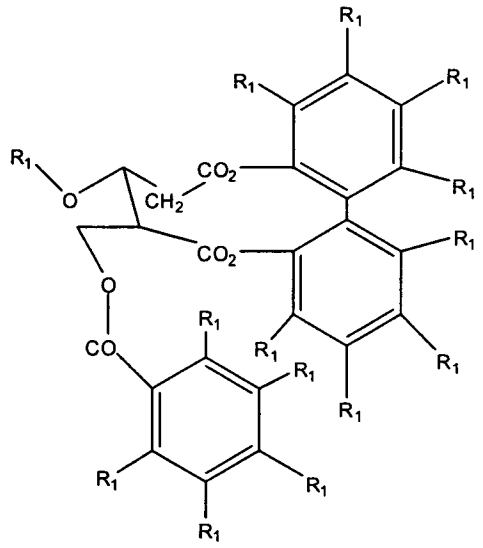
stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

each R_1 is independently selected from H, halogen, hydroxy, carboxy, nitro, amino, nitrile, or a substituted or unsubstituted C_1–C_6 alkyl, alkoxy, hydroxyalkyl, cycloalkyl, cycloalkylalkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, haloalkyl, haloalkoxy, alkanoyl, and alkanoyloxy, groups.

[0090] In one embodiment, the agent is a compound of formula (IIIE) and has the following structure

[0091]



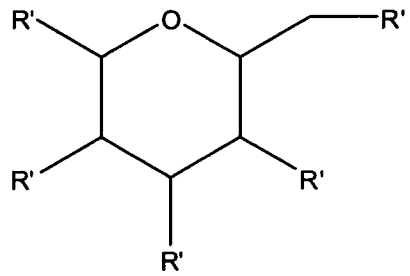
(IIIE)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

each R_1 is independently selected from H, halogen, hydroxy, carboxy, nitro, amino, nitrile, or a substituted or unsubstituted C_1–C_6 alkyl, alkoxy, hydroxyalkyl, cycloalkyl, cycloalkylalkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, haloalkyl, haloalkoxy, alkanoyl, and alkanoyloxy, groups.

[0092] In one embodiment, the agent is a compound of formula (IIIF) and has the following structure



(IIIF)

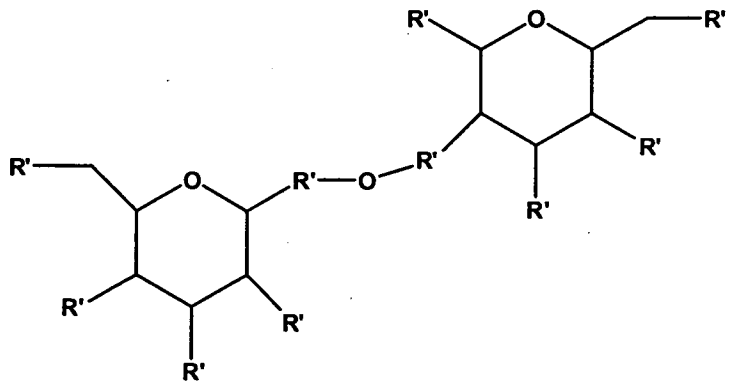
stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

each R' is independently selected from –O-CO-R^a or –COO-R^a

each R^a is independently a substituted or unsubstituted aryl or heteroaryl group which can optionally bond covalently with the adjacent R^a to form substituted or unsubstituted biphenyl group.

[0093] In some embodiments, the agent comprises repetitive units of compound of formula IIIF. For e.g., in one embodiment, the agent comprises a compound of formula IIIG which consists of two units of compound of formula IIIF connected via a –O- bond and has the following structure



(IIIG)

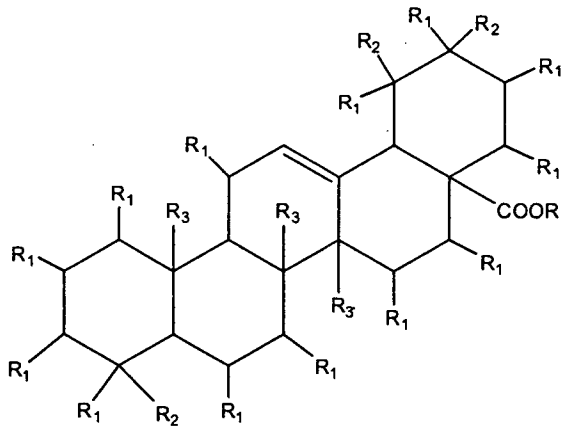
stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

each R' is independently selected from –O-CO-R^a or –COO-R^a

each R^a is independently a substituted or unsubstituted aryl or heteroaryl group which can optionally bond covalently with the adjacent R^a to form substituted or unsubstituted biphenyl group.

[0094] In some embodiments, the agent is a compound of formula (IV) and has the following structure



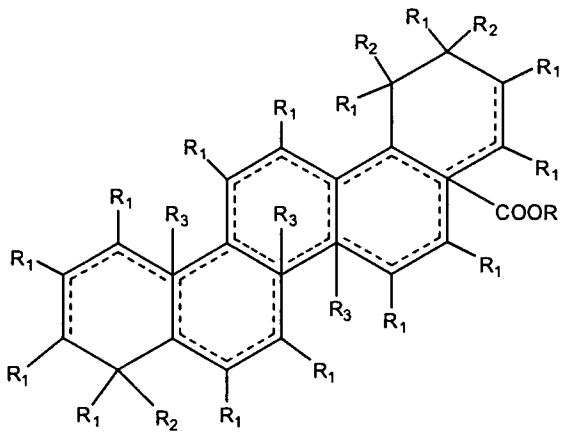
(IV)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

each R, R_1 R_2, R_3 and R_4 is independently selected (independently, collectively, or in any combination) from H, halogen, hydroxy, carboxy, nitro, amino, nitrile, or a substituted or unsubstituted C_1–C_6 alkyl, alkoxy, hydroxyalkyl, cycloalkyl, cycloalkylalkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, haloalkyl, haloalkoxy, alkanoyl, and alkanoyloxy groups.

[0095] In some embodiments, the agent is a compound of formula (IVA) and has the following structure



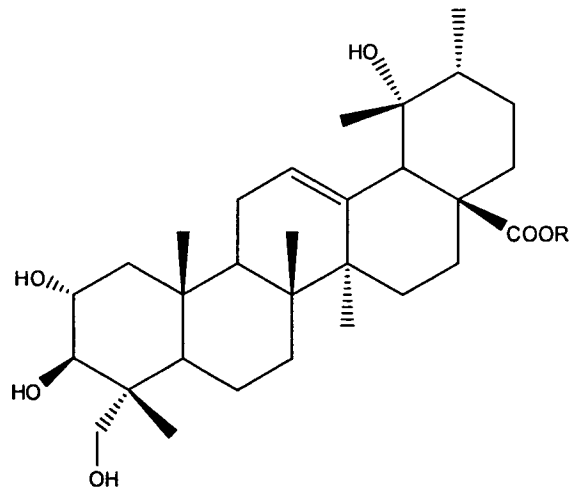
(IVA)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

each R, R_1 R_2, R_3 and R_4 is independently selected (independently, collectively, or in any combination) from H, halogen, hydroxy, carboxy, nitro, amino, nitrile, or a substituted or unsubstituted C_1–C_6 alkyl, alkoxy, hydroxyalkyl, cycloalkyl, cycloalkylalkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, haloalkyl, haloalkoxy, alkanoyl, and alkanoyloxy groups; and the dotted lines indicate optional unsaturation.

[0096] In some embodiments, the agent is a compound of formula (V) and has the following structure

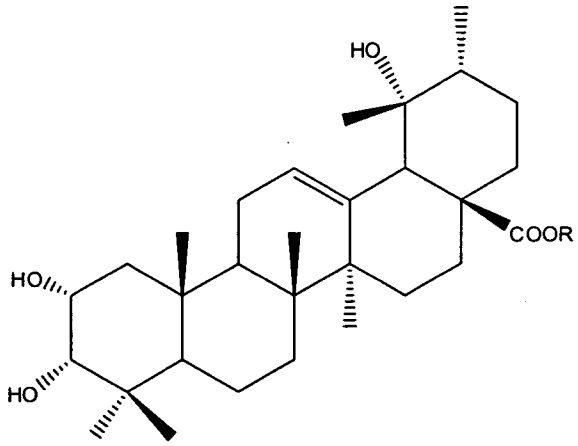


(V)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein R is either a hydrogen or a glucoside.

[0097] In some embodiments, the agent is a compound of formula (VI) and has the following structure

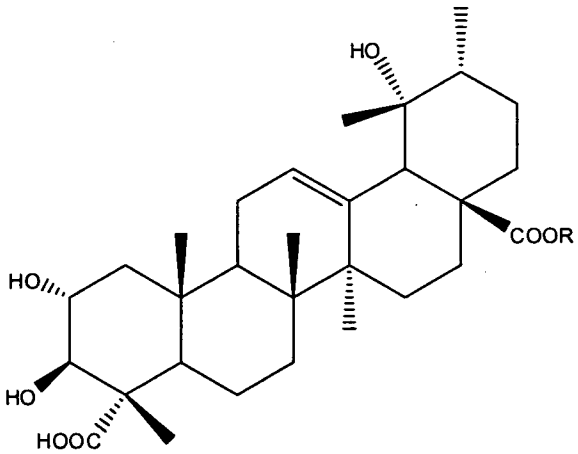


(VI)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein R is either a hydrogen or a glucoside.

[0098] In some embodiments, the agent is a compound of formula (VII) and has the following structure

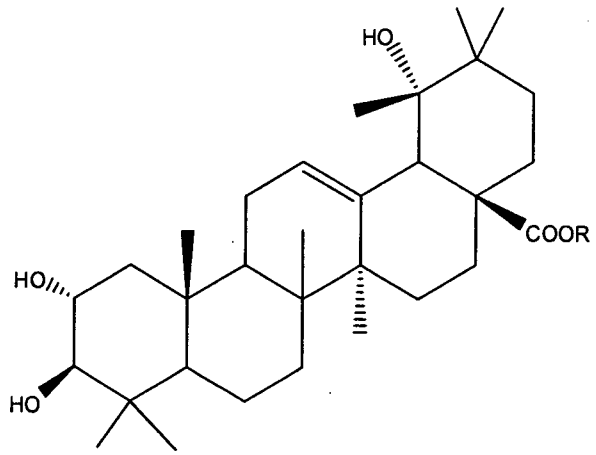


(VII)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein R is either a hydrogen or a glucoside.

[0099] In some embodiments, the agent is a compound of formula (VIII) and has the following structure

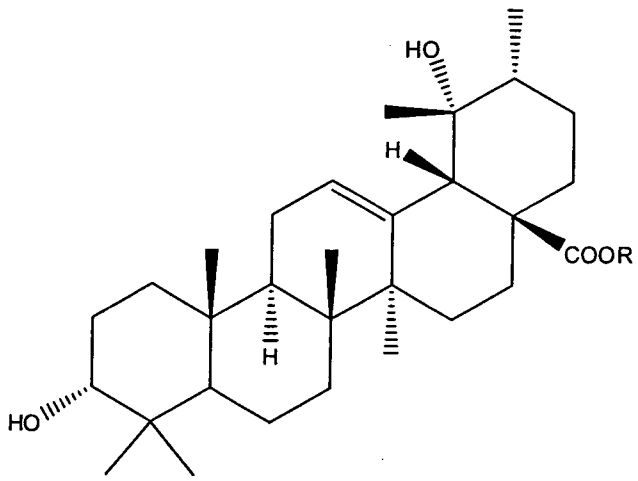


(VIII)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein R is either a hydrogen or a glucoside.

[0100] In some embodiments, the agent is a compound of formula (IX) and has the following structure

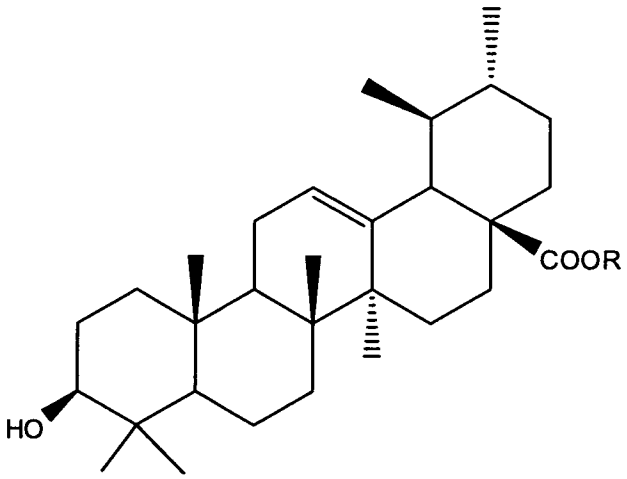


(IX)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein R is either a hydrogen or a glucoside.

[0101] In some embodiments, the agent is a compound of formula (X) and has the following structure

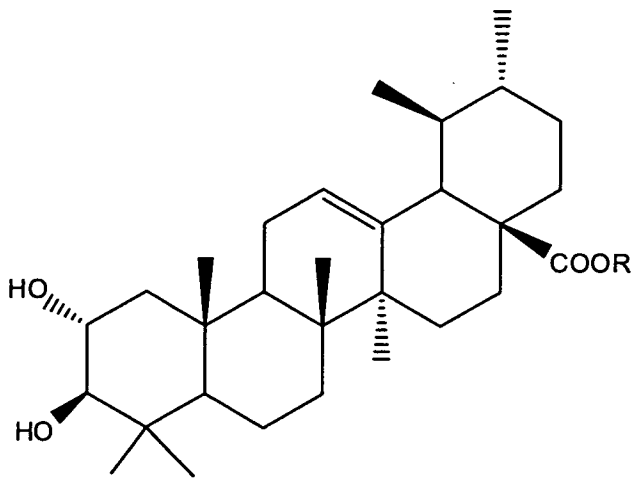


(X)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein R is either a hydrogen or a glucoside.

[0102] In some embodiments, the agent is a compound of formula (XI) and has the following structure

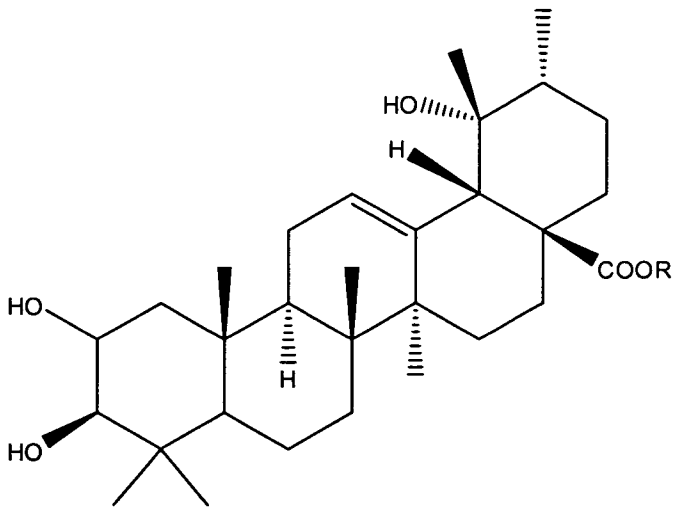


(XI)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein R is either a hydrogen or a glucoside.

[0103] In some embodiments, the agent is a compound of formula (XII) and has the following structure



(XII)

stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein R is either a hydrogen or an O-glucoside.

[0104] The backbone of compounds of formula I-XII can have substituents at various positions and retain similar biological activities as the backbone compound. The substitution can be achieved by means known in the field of organic chemistry. As used herein, the term "a compound of formula I-XII" includes the backbone compound as represented by that formula I-XII as well as its substituted variants with similar biological activities.

[0105] In one aspect, the methods for the prevention or treatment of heart diseases, such as CHD, include administering to a mammal in need thereof agents, fractions, and/or extracts and compounds, and derivatives of such compounds from a variety of plants including *Geum japonicum, Fructus Rosae Laevigatae, R. imperialis, Vochysiapacifica, Rubus coreanus, Rubus allegheniensis, Rubus parviforlius L,. Agrimonia pilosa, Potentilla kleiniana, Coriaria japonica*, and grape seeds. In some embodiments, the extract is an organic extract obtained from the plant *Fructus Rosae Laevigatae.* In certain embodiments, the agent is selected from the group comprising a compound of formula (I), (II), (IA) and (IIA) as shown herein or mixtures thereof. In some embodiments, the agent is selected from the group comprising a compound of formula (III), (IIIA), (IIIB), (IIIC), (IIID), (IIIE),

(IIIF), (IIIG), (IV), (IVA), (V), (VI), (VII), (VIII), (IX), (X), (XI) or (XII) as shown herein or mixtures thereof. Exemplary agents include active fractions FRL-B, FRL-E and tannins, including hydrolysable tannins such as pendunculagin, potentillin or derivatives thereof.

[0106] In accordance with one aspect, the technology provides methods of treating or preventing Coronary Heart Disease (CHD) in a subject in need thereof, which comprises administering to the subject an effective amount of a compound, composition, active fractions, or extract described herein. The effects of CHD or symptoms thereof to be improved can be one or more of chest pain, shortness of breath, weakness, fainting spells, alterations in consciousness, extremity pain, paroxysmal nocturnal dyspnea, orthophea, transient ischemic attacks and other such phenomena experienced by the patient. Clinical signs in CHD also include such findings as EKG abnormalities, altered peripheral pulses, arterial bruits, abnormal heart sounds, rates, jugular venous distention, neurological alterations and other such findings discerned by the clinician.

[0107] In one embodiment, the present disclosure provides a method for treating or preventing coronary heart disease in a mammalian subject comprising administering to the subject in need thereof an effective amount of a compound of formula I or formula II, mixture thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof. In one embodiment, the compound of formula I has the formula IA. In another embodiment, the compound of formula II has the formula IIA.

[0108] In one embodiment, the present disclosure provides a method for treating or preventing coronary heart disease in a mammalian subject comprising administering to the subject in need thereof an effective amount of a composition selected from the group consisting of: (i) an organic extract from the plant *Fructus Rosae Laevigatae*; (ii) an active fraction of an organic extract from the plant *Fructus Rosae Laevigatae*; and (iii) compound of formula (I), (IA), (II), or (IIA), mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof. In some embodiments, the method comprises administering to the subject in need thereof an effective amount of a one or more of the compounds of formula (I), (IA), (II), and (IIA), mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof.

[0109] In another aspect, the present technology provides a method for promoting revascularization in dead or damaged heart tissues of a mammalian subject caused by an ischemic heart disease, the method comprising administering to the subject an effective amount of a compound of formula I or formula II, mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof.

[0110] In yet another aspect, the present technology provides a method for up-regulating the expression of angiogenic factors to stimulate growth of new coronary collateral vessels in ischemic or infarcted myocardium, in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of formula I or formula II, mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof, wherein the angiogenic factors are one or more of VEGF, VEGFR, EGF, and FGF.

[0111] In still another aspect, the present technology provides a method for treating ischemic heart diseases or ischemic limbs in a mammalian subject, comprising administering to the subject an effective amount of the compound of formula (I) or formula II, mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof.

[0112] In still another aspect, the present technology provides a method for treating, or ameliorating a pathological condition in a mammal, where the pathological condition, as judged by people skilled in medicine, can be treated or alleviated by up-regulating the expressions of angiogenic factors, such as VEGF, VEGFR, EGF, and FGF, that would stimulate growth of new coronary collateral vessels in ischemic or infarcted myocardium. The method comprises a step of administering an effective amount of a compound of formula I or II, or a combination thereof, or its functional derivative to the mammal.

[0113] In another aspect, an agent for the treatment or prevention of heart diseases, including CHD, is part of a pharmaceutical composition containing one or more excipients, carriers, or fillers. In one embodiment, the pharmaceutical composition is packaged in unit dosage form. The unit dosage form is effective in improving chest pain, shortness of breath, weakness, fainting spells, alterations in consciousness, extremity pain, paroxysmal nocturnal dyspnea, orthophea, transient ischemic attacks and other such phenomena experienced by the patient.

[0114] In various embodiments of the technology, suitable *in vitro* or *in vivo* assays are performed to determine the effect of an agent (extracts, fractions, and compounds) of the technology and whether its administration is indicated for the treatment or prevention of CHD in a subject. In some embodiments, *in vivo* models of CHD are used to assess the effects of an agent on a subject. Suitable in vivo models include myocardial infarction by complete occlusion of coronary arteries in rats, chronic coronary heart disease by incomplete ligation of coronary artery in rats, and a toxic cardiomyopathy model, which includes the administration of doxorubicin or anthracycline to an animal subject. The effects of the agent in mediating the myocardial infarction and chronic coronary heart disease in animal subjects are investigated and compared to suitable controls.

In some embodiments, plants, extracts, active fractions, and/or compounds of the present technology may be administered as part of a combination therapeutic with an anti-arrhythmia agent. Anti-arrhythmia agents are often organized into four main groups according to their mechanism of action: type I, sodium channel blockade; type II, beta-adrenergic blockade; type III, repolarization prolongation; and type IV, calcium channel blockade. Type I anti-arrhythmic agents include lidocaine, moricizine, mexiletine, tocainide, procainamide, encainide, flecanide, tocainide, phenytoin, propafenone, quinidine, disopyramide, and flecainide. Type II anti-arrhythmic agents include propranolol and esmolol. Type III includes agents that act by prolonging the duration of the action potential, such as amiodarone, artilide, bretylium, clofilium, isobutilide, sotalol, azimilide, dofetilide, dronedarone, ersentilide, ibutilide, tedisamil, and trecetilide. Type IV anti-arrhythmic agents include verapamil, diltaizem, digitalis, adenosine, nickel chloride, and magnesium ions.

[0115] In one embodiment, plants, extracts, active fractions, and/or compounds of the present technology may be administered as part of a combination therapeutic with another cardiovascular agent including, for example, an anti-arrhythmic agent, an antihypertensive agent, a calcium channel blocker, a cardioplegic solution, a cardiotonic agent, a fibrinolytic agent, a sclerosing solution, a vasoconstrictor agent, a vasodilator agent, a nitric oxide donor, a potassium channel blocker, a sodium channel blocker, statins, or a naturiuretic agent.

[0116] Examples of cardiovascular agents include vasodilators, for example, hydralazine; angiotensin converting enzyme inhibitors, for example, captopril; anti-anginal agents, for

example, isosorbide nitrate, glyceryl trinitrate and pentaerythritol tetranitrate; anti-arrhythmic agents, for example, quinidine, procainaltide and lignocaine; cardioglycosides, for example, digoxin and digitoxin; calcium antagonists, for example, verapamil and nifedipine; diuretics, such as thiazides and related compounds, for example, bendrofluazide, chlorothiazide, chlorothalidone, hydrochlorothiazide and other diuretics, for example, fursemide and triamterene, and sedatives, for example, nitrazepam, flurazepam and diazepam.

[0117] The methods include treatment of a mammalian subject. In some embodiments, the subject can be any mammal in need of angiogenic treatment such as *e.g.,* a human, dogs, cats, cows, sheep, pigs, horses monkey, rats, mice, rabbits and guinea pigs. In some embodiments, the mammalian subject is a human. Typically the subject is a human suspected of having a heart disease such as CHD or ischemic heart diseases.

Compounds and Plants Sources

[0118] In one aspect, the disclosure provides compounds, extracts and compositions for use in treatment of heart diseases. The compounds, extracts and compositions may be used in the methods and treatment of heart diseases as described herein.

[0119] In some embodiments, the compound is a whole plant or an extract, *e.g.,* an organic extract, of *Fructus Rosae Laevigatae, Geum japonicum,* and Xian he cao, *Agrimonia pilosa* Ledeb. (Rosaceae). In some embodiments, the compound is an extract of these plants in a suitable solvent. In some embodiments, the compound is a methanol/ethanol extract of *Fructus Rosae Laevigatae* or an active fraction thereof. In some embodiments, the compound is a fraction of an extract of *Fructus Rosae Laevigatae.*

Preparation of Organic Extract of Plants

[0120] In one embodiment, the extracts, fractions, and compounds of the technology are obtained by extraction, using water and/or of an organic solvent, from crude plant material comprises the following stages:

1. Extraction by addition to the plant material, of water and/or of organic solvent(s), by subjecting the whole to a treatment such as maceration/lixiviation, ultrasonics or microwaves;

2. Delipidation before or after the extraction stage using a solvent of petroleum ether, hexane or chloroform type;

3. Optionally, additional extraction of the extract recovered by an organic solvent of ethyl acetate or ethyl ether type,

4. Optionally, concentration of the crude extract obtained, and, if desired, its lyophilization.

[0121] According one aspect, considering the enrichment that it allows to be attained, the crude extract may be subjected to a purification stage by chromatography. In one embodiment, centrifugal partition chromatography (CPC) is used. This technique is in particular described by A.P. FOUCAULT, Ed., Centrifugal Partition Chromatography, Chromatographic Science Series, Marcel Dekker Inc., 1995, 68, or W.D. CONWAY, Ed., Countercurrent Chromatography apparatus theory and applications, VCH Publishers Inc., 1990. CPC is based on the partition of the solutes between two non-miscible liquid phases prepared by the mixture of two or more solvents or solutions. One of the two phases is kept stationary by a centrifugal force. The solvents, their proportions and the flow rate chosen closely depend both on the stability of the stationary phase within the CPC column and the actual pressure.

[0122] A person skilled in the art will therefore choose the most appropriate solvent or solvents depending on the nature of the purified extract desired. Thus, crude extracts and enriched fractions are therefore available containing, as majority constituents, any of the compounds of formulas I-X. These different extracts, namely crude or enriched also fall within the scope of the technology. The implementation of additional separation stages allows isolation of these extracts enriched with one or more compounds. These separations can be carried out on fractions enriched from a crude extract or on the crude extract itself by using mixtures of appropriate solvents according to the proportions which are suitable for the sought separation.

[0123] In one aspect, a method for preparing an organic extract from the plant of *Fructus Rosae Laevigatae*. This method comprises the step of (a) extracting the plant of *Fructus Rosae Laevigatae* with an alcohol selected from the group consisting of C1-C4 alcohols. This step maybe repeated several times, generally about 3-6 times, typically about 5 times, at a suitable temperature, typically room temperature. Before performing step (a), the plant material may be chopped into small pieces or even powdered. The C1-C4 alcohols include methanol, ethanol, n-propanol, iso-propanol, n-butanol, iso-butanol, and ter-butanol. Typically, alcohol is added in 1-50 times, typically 1-10 times by weight of the amount of the *Fructus Rosae Laevigatae* to be extracted.

[0124] The methods may further comprise the step of (b) drying the extract obtained from the step of (a) into a dried powder; and (c) suspending the powder obtained from the step of (b) in a suitable solvent, such as water and successively extracting the powder with a solvent or mixture of solvents including chlorinated solvents, esters, and an alcohol. The alcohol can be selected from the group consisting of C1-C4 alcohols. The chlorinated solvents include, for example, chloroform, dichloromethane, chloromethane, carbon tetrachloride, etc. The C1-C4 alcohols include methanol, ethanol, n-propanol, iso-propanol, n-butanol, iso-butanol, and ter-butanol. Esters include ethyl acetate, propyl acetate and butyl acetate etc. The amount of organic solvent to be used is typically 1-10 times by weight of the amount of the powders to be further extracted.

[0125] The method as recited above may also include filtering the extract to remove any insoluble impurities therein. A drying step may be completed under reduced pressure at a temperature higher than room temperature, for example, at 30°C, 35°C, 40°C or 50°C.

[0126] To purify the extracts, the method may further comprise the steps of passing the extracts through a chromatographic column; and eluting the column with a suitable solvent. For example, a Sephadex or reverse phase column may be used. Suitable eluting solvents include an aqueous solution with increasing concentration of an alcohol selected from the group consisting of C1-C4 alcohols or alcohols in combination with acetone. The alcohol used may be any one selected from the group consisting of methanol, ethanol, n-propanol, iso-propanol, n-butanol, iso-butanol, and ter-butanol. Other eluting solvents such as alcohols, ketones, esters, ethers or combinations thereof may also be used. The structure of the isolated active compound can be determined by Mass and NMR analysis. The angioenhancin compounds disclosed herein may be obtained from other known plant sources following suitable extraction methods as described above.

Pharmaceutical Compositions

[0127] In one aspect, the present technology provides pharmaceutical composition, for use in treating or preventing coronary heart disease, comprising a pharmaceutically acceptable excipient and an effective amount of a compound selected from formula I or formula II. In some embodiments, the compound of formula I in the pharmaceutical composition, has the structure IA. In other embodiments, the compound of formula II in the pharmaceutical composition, has the structure IIA. In some embodiments, the pharmaceutical composition

may further comprise a second agent. In some embodiments, the pharmaceutical composition may further comprise an anti-arrhythmia agent or a cardiovascular agent.

[0128] In another aspect, an agent of the technology is part of a pharmaceutical composition containing one or more excipients, carriers, or fillers. In one embodiment, the pharmaceutical composition is packaged in unit dosage form. The unit dosage form is effective in improving various diseases or medical conditions when administered to a subject in need thereof.

[0129] The pharmaceutical composition may be formulated by conventional means known to people skilled in the pharmaceutical industry into a suitable dosage form, such as tablet, capsules, injection, solution, suspension, powder, syrup, etc, and be administered to a mammalian subject suffering MI in a suitable manner. The formulation techniques are not part of the present technology and thus are not limitations to the scope of the present technology.

[0130] The compounds and compositions are administered in an amount required to produce the desired effect. In some embodiments, the effective amount includes amounts and dosages that are capable of preventing or treating coronary heart diseases, promoting revascularization in dead or damaged heart tissues and/or up-regulating the expression of angiogenic factors in a mammalian subject. In some embodiments, the effective amount of the compound or composition is in the form of a pharmaceutical formulation comprising the compound or composition and a pharmaceutically suitable carrier or excipient. One example of an effective amount includes amounts or dosages that yield acceptable toxicity and bioavailability levels for therapeutic (pharmaceutical) purposes in the treatment of heart diseases.

[0131] Typically, an effective amount of the compositions of the present technology, sufficient for achieving a therapeutic or prophylactic effect, range from about 0.000001 mg per kilogram body weight per day to about 10,000 mg per kilogram body weight per day. Suitably, the dosage ranges are from about 0.0001 mg per kilogram body weight per day to about 1000 mg per kilogram body weight per day. For administration of an agent, the dosage ranges may be from about 0.0001 to 1000 mg/kg, and more usually 0.01 to 500 mg/kg every week, every two weeks or every three weeks, of the host body weight. An exemplary treatment regime entails administration once per every two weeks or once a

month or once every 3 to 6 months. In some embodiments, the compound or composition is administered in an amount of from 0.001 mg/kg/day to 10000 mg/kg/day. In some embodiments, the compound or composition is administered in an amount of from 0.01 mg/kg/day to 1000 mg/kg/day. The agent usually administered on multiple occasions. Intervals between single dosages can be daily, weekly, monthly or yearly. Alternatively, the agents can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the agent in the subject. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some subjects continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the subject shows partial or complete amelioration of symptoms of disease. Thereafter, the patent can be administered a prophylactic regime. Specific dosages may be adjusted depending on conditions of disease, the age, body weight, general health conditions, sex, and diet of the subject, dose intervals, administration routes, excretion rate, and combinations of drugs. Any of the above dosage forms containing effective amounts are well within the bounds of routine experimentation and therefore, well within the scope of the instant invention.

[0132] *Toxicity.* Suitably, an effective amount (*e.g.*, dose) of an agent described herein will provide therapeutic benefit without causing substantial toxicity to the subject. Toxicity of the agent described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, by determining the LD_{50} (the dose lethal to 50% of the population) or the LD_{100} (the dose lethal to 100% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic for use in human. The dosage of the agent described herein lies preferably within a range of circulating concentrations that include the effective dose with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the subject's condition. See, *e.g.*, Fingl *et al.*, In: *The Pharmacological Basis of Therapeutics*, Ch. 1 (1975).

[0133] According to the methods of the present technology, the agents can be incorporated into pharmaceutical compositions suitable for administration. In some embodiments, the pharmaceutical compositions may comprise purified or substantially purified extracts of *Geum japonicum* and a pharmaceutically-acceptable carrier in a form suitable for administration to a subject. In other embodiments, the pharmaceutical compositions may comprise Pharmaceutically-acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions for administering the compositions (see, *e.g.*, *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA 18th ed., 1990). The pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

[0134] A pharmaceutical composition of the technology is formulated to be compatible with its intended route of administration. The compositions of the present technology can be administered by parenteral, topical, intravenous, oral, subcutaneous, intraarterial, intradermal, transdermal, rectal, intracranial, intraperitoneal, intranasal; intramuscular route or as inhalants. The agent can optionally be administered in combination with other agents that are at least partly effective in treating various diseases.

[0135] Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial compounds such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating compounds such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and compounds for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0136] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration,

suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, *e.g.*, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, *e.g.*, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal compounds, *e.g.*, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic compounds, *e.g.*, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition a compound which delays absorption, *e.g.*, aluminum monostearate and gelatin.

[0137] Sterile injectable solutions can be prepared by incorporating the agents in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the binding agent into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The agents of this technology can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient.

[0138] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the binding agent can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied

orally and swished and expectorated or swallowed. Pharmaceutically compatible binding compounds, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating compound such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening compound such as sucrose or saccharin; or a flavoring compound such as peppermint, methyl salicylate, or orange flavoring.

[0139] In one embodiment, the agents are prepared with carriers that will protect the agent against rapid elimination from the body or against being degraded by the acid in the stomach, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically-acceptable carriers. These can be prepared according to methods known to those skilled in the art, *e.g.*, as described in U.S. Pat. No. 4,522,811.

[0140] In various embodiments of the technology, suitable *in vitro* or *in vivo* assays are performed to determine the effect of an agent (extracts, fractions and compounds) of the technology and whether its administration is indicated for treatment of the affected disease or medical condition in a subject. Examples of these assays are described above in connection with a specific disease or medical treatment.

Kits

[0141] In yet another aspect, a kit for treating or preventing coronary heart disease in a mammalian subject is provided. In some embodiments, the may include a therapeutic agent described herein and a second agent. In some embodiments, the second agent may be an anti-arrhythmia agent or a cardiovascular agent. In some embodiments, the kit may contain a pharmaceutically acceptable excipient

[0142] The disclosure also provides kits for use in the other methods such as those described above. Exemplary kits comprise: one or more containers, wherein a first container contains compound of formula I or formula II or mixtures thereof, ; and a second container containing the second agent. In some embodiments, the second container may be absent. These kits may further comprise one or more additional containers containing one or more supplements selected from the group consisting of pharmaceutically acceptable carriers, excipient and the like.

EXAMPLES

[0143] The present technology is further illustrated by the following examples, which should not be construed as limiting in any way.

Example 1 - Isolation of Angioenhancins from Fructus Rosae Laevigatae.

[0144] Angioenhancins, such as compounds of formula I and formula II, were isolated from the plant of *Fructus Rosae Laevigatae*. As shown in flow-chart in FIG.1, the plant, collected from Guizhou Province of China in October, was dried (10kg) and percolated with 70% ethanol (60L) at room temperature once daily for three days. The extracts were combined and electro-spray-dried to yield a solid residue. The solid residue was suspended in water (H_2O) (10 L) and successively partitioned with petroleum ether (10 L), ethyl acetate (10 L), followed by n-butanol (10 L), three times respectively, to produce the corresponding fractions. The n-butanol fraction (FRL-B) and ethyl acetate soluble fractions were filtered and evaporated under reduced pressure at 38°C. It was observed that both n-butanol and ethyl acetate soluble fractions could enhance the capillary-like tube formation of human aorta endothelial cells (HAEC) and stimulate angiogenesis in ischemic/infarct zone of hearts in experimental animal models. The n-butanol soluble fraction was applied on a column of Sephadex LH-20 equilibrated with 10% methanol (MeOH) and eluted with increasing concentration of methanol in water, resolving 7 fractions. Fraction 1, eluted with approximately 20% methanol, showed biological activity by stimulating angiogenesis in ischemic myocardium. The compounds contained in fraction 1 were further isolated and tested in capillary-like tube formation assay system. Fraction 1 dissolved in 20% MeOH was filtered, evaporated and further separated using Sephadex LH-20 column chromatography, eluted with MeOH and 50% acetone resulting in the isolation of an angiogenic active fraction (FRL-B-1-1-2). This active fraction was further isolated using

Toyopearl HW-40F chromatography, eluting with H_2O-MeOH resulting in the isolation of the angiogenic compound. The structure of the isolated active compound was determined by Mass and NMR analyses demonstrating its identity as compound of formula IA (pedunculagin) having a molecular weight of 784 and molecular formula: $C_{34}H_{24}O_{22}$. A second angiogenic compound was also isolated from FRL-E fraction using ODS (C18) chromatography, eluting with H_2O-MeOH. The structural analysis demonstrated its identity as compound of formula IIA (Potentillin or Galloyl-pedunculagin) having a molecular weight of 936 and molecular formula: $C_{41}H_{28}O_{26}$. Since these angioenhancins are naturally occurring compounds, they may be obtained from other known plant sources following suitable extraction methods as described herein.

Example 2 - Angioenhancin enhanced capillary-like tube formation of vessel endothelial cells.

[0145] In order to demonstrate whether angioenhancin compounds of formula I or formula II could enhance differentiation of endothelial cells, human umbilical vein endothelial cells (HUVEC) were cultured and angioenhancin induced differentiation of HUVEC were assessed. It was found that both angiogenin compounds of formula I or formula II, enhanced the differentiation of HUVEC in a dose dependent manner as shown in FIG. 2. It was observed that the vehicle treated cells showed proliferation, but no sign of differentiation. By contrast, both angioenhancin compound of formula I and formula II treatment enhanced differentiation of HUVEC in a dose dependent manner. As seen in FIG. 2, the HUVEC, which showed small and triangle phenotype prior to angioenhancin treatment, differentiated into an elongated and thin phenotype with typical characteristics of differentiation of vessel endothelial cells in the presence of either angioenhancin compound of formula I or formula II. When the concentration of angioenhancin in the culture was increased (40 μg/ml), the cells significantly elongated and formed several tube-like structures.

[0146] To further test the angiogenesis promoting effect of the angioenhancin compounds, human aorta endothelial cells (HAECs) were grown to confluence in Ham's F-12 medium supplemented with 15% FBS, 500 U/ml penicillin, 50 μg/ml streptomycin, and 100 μg/ml heparin and 100 μg/ml endothelial cell growth supplement. Cells were incubated at 37°C and equilibrated in 95% air-5% CO_2. Fibrin matrices were prepared by polymerization of fibrinogen solution (5mg fibrinogen/ml serum-free medium) using low concentrations of α-

thrombin (2.5 U/ml). After polymerization, gels were soaked in culture medium containing 10% FBS for 2 h at 37°C to inactivate the thrombin. HAEC were seeded on the surface of the three-dimensional matrix in 96-well plates in Ham's F-12 medium and cultured for 22 hours with different concentrations of angioenhancin compounds of formula I and formula II in a growth medium. Non-treated control was added with equivalent amount of growth medium. Images were captured and the angiogenic properties were determined by calculating the total number of tubes formed.

[0147] It was found that the cells treated with angioenhancin compounds of formula I and formula II formed more capillary-like networks as compared to vehicle-treated cells (Fig. 3A). The total length of the formed capillary-like tubes was significantly augmented with increasing concentrations of angioenhanicn compounds. Among all the concentration tested, the greatest tube length was achieved at an angioenhancin concentration of 5 μg/ml ($p<0.05$) (Fig. 3B, Table 1).

Table 1.

Group	Ctrl	Formula I (5 μg/ml)	Formula I (10 μg/ml)	Formula I (20 μg/ml)	Formula II (5 μg/ml)	Formula II (10 μg/ml)	Formula II (20 μg/ml)
Approx. No. of Tubes	9	45	57	68	67	46	45

Example 3 - Subclinical heart infarction animal model, treatment protocol and assessment

[0148] Myocardial infarction (MI) animal model was induced in male Sprague-Dawley rats (300-350 g) by permanent ligation of the left anterior descending (LAD) coronary artery. The experimental rats were anesthetized with intraperitoneal pentobarbital (50 mg/kg), intubated, and mechanically ventilated with room air. The angioenhancin compound of formula I or formula II (2 mg/kg), dissolved in water (0.5 ml), was orally administrated to MI rats (n=6) for one day post ligation, daily for two weeks with equivalent amount of water to the vehicle-treated rats (n=6). For sham-operated rats (n =5), thoracotomy was performed without ligation. Animals were sacrificed after final echocardiography measurements.

[0149] *Evaluation of neovascularization in infarcted hearts.* Echocardiography was recorded on day 7 and 14 post operations. The hearts of the sacrificed rats were removed and washed with PBS. For histological studies, the left ventricles of the experimental rats

were removed and cut from apex-to-base in 3 transverse slices and embedded in paraffin. To quantify the density of capillaries in infarct zones, the vessels were counted and averaged in 6 randomly selected high power view fields (HPF, 40X) on each section. Blinded capillary counts were performed on 6 hearts per group by two investigators. The results were expressed as mean±SD capillaries per HPF. The result showed that although the area (2–4 mm in diameter) distal to the ligation site appeared pure white and thin due to ischemic necrosis in vehicle-treated hearts 2 weeks post infarction, the corresponding area in angioenhancin-treated hearts appeared red-gray and the thickness of the ventricle walls were thicker than the vehicle treated. Histological examination revealed that the capillary density in the infarct area of experimental hearts was on an average 11.78 ± 6.67 per HPF (Fig. 4), whereas only 6.67 ± 4.32 vessels per HPF were in the infarct zone of hearts in vehicle treated hearts. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis indicated that the angioenhancin compounds of formula I and formula II up-regulated expressions of angiogenesis-associated genes, such as VEGF, VEGFR, EGF and FGF in angioenhancin treated heart tissues and endothelial cells.

Example 4 - Subclinical chronic coronary heart disease (cCHD) rat model and treatment protocol.

[0150] The subclinical cCHD rat model was produced as described. Briefly, male Sprague-Dawley (SD) rats, weighing 150-200 g, were used. Following proper anesthesia, left thoracotomy was performed on the animals. The pericardium was opened and a probe or surgical suture thread (11-0) was placed onto the epicardium along the LAD. The LAD together with the probe was then ligated 1-2mm from its origin using an 8-0 silk suture followed by gentle removal of the probe, which resulted in an average reduction in luminal diameter by 70-85%. During the LAD ligation, ST segment of the ECG was transiently elevated and significantly lowered after removal of the probe implying ischemia of the ventricle. The experimental rats with ST segment constantly depressed above 0.1mV were selected and randomly divided into two groups. The rats (n=6) in test group were treated with angioenhancin compounds of formula I or formula II dissolved in H_2O at 5mg/kg through intragastric administration for 2 weeks. The rats in vehicle-treated group (n=6) were treated with equivalent period and amount of H_2O. For the sham operation group (n=6), left thoracotomy was performed and the pericardium was opened but with no LAD ligation.

[0151] *Measurement of neovascularization in the ischemic zone.* Left ventricles from the rats sacrificed on day 14 post-ligation were removed and sliced from apex to base in 3 transverse slices. The slices were fixed in formalin and embedded in paraffin. Vascular density was determined on the histology section samples by counting the number of vessels within the ischemic zone using a light microscope under a high power field (HPF) (40x). Eight random and non-overlapping HPFs within the ischemic field were used for counting all the vessels in each section. The number of vessels in each HPF was averaged and expressed as the number of vessels per HPF. Vascular counts were performed by two investigators in a blind fashion.

[0152] Referring to FIG. 4, histology studies revealed that many newly formed vessels filled with blood cells were observed throughout the entire ischemic zone on day 14 post ligation. The capillary density in the ischemic zone of the angioenhancin treated myocardium was on average 12 capillaries (12 ±3.8) filled with blood cells per HPF, calculated from 8 randomly selected view fields on each of the 3 slides from 6 angioenhancin treated hearts on day 14. In contrast, fewer blood vessels (8 ±2.1 per HPF) with an inflammatory cell infiltration were observed in the ischemic zone in the vehicle treated myocardium on day 14 post ligation.

[0153] *Echocardiography Assessment of Heart Function.* In all, 12 SD rats received baseline echocardiography before any experimental procedures. Echocardiography was recorded under controlled anesthesia using a Toshiba Aplio XG Echocardiography with PLT-1202S linear array transducer S10-MHz phased-array transducer. M-mode tracing and 2-dimensional (2D) echocardiography images were recorded from the parasternal long- and short-axis views. Short axis view was at the papillary muscles level. Left ventricular end-systolic and end-diastolic dimensions, as well as systolic and diastolic wall thickness, were measured from the M-mode tracings by using the leading-edge convention of the American Society of Echocardiography. For each M-mode measurement, at least three consecutive cardiac cycles were sampled. All rats in both groups received echocardiography measurements on day 2, 7 and 14 post ligation.

[0154] To demonstrate that the significant neoangiogenesis in ischemic hearts induced by angioenhancin treatment was accompanied by progressively restored functional performance, echocardiography was used to determine the functional performance of the experimental rats. It was found that the lowered left ventricle ejection fraction (LVEF) and

left ventricle fraction shortening (LVFS) due to myocardial ischemia were progressively restored with time in hearts treated with angioenhancin compounds of formula I and II (Fig. 5). By comparison, both LVEF and LVFS were progressively lowered in vehicle treated rats (FIG. 5).

* * * *

[0155] While certain embodiments have been illustrated and described, it should be understood that changes and modifications can be made therein in accordance with ordinary skill in the art without departing from the technology in its broader aspects as defined in the following claims.

[0156] The present disclosure is not to be limited in terms of the particular embodiments described in this application. Many modifications and variations can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the disclosure, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the appended claims. The present disclosure is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled. It is to be understood that this disclosure is not limited to particular methods, reagents, compounds compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0157] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0158] As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower

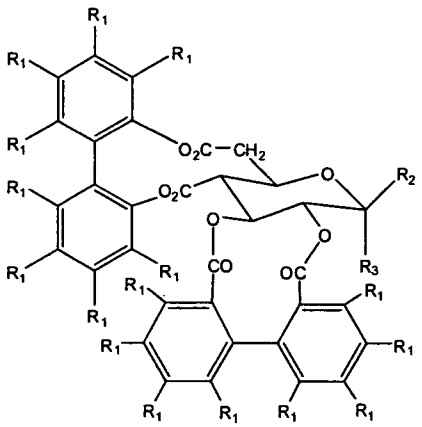
third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 atoms refers to groups having 1, 2, or 3 atoms. Similarly, a group having 1-5 atoms refers to groups having 1, 2, 3, 4, or 5 atoms, and so forth.

[0159] While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

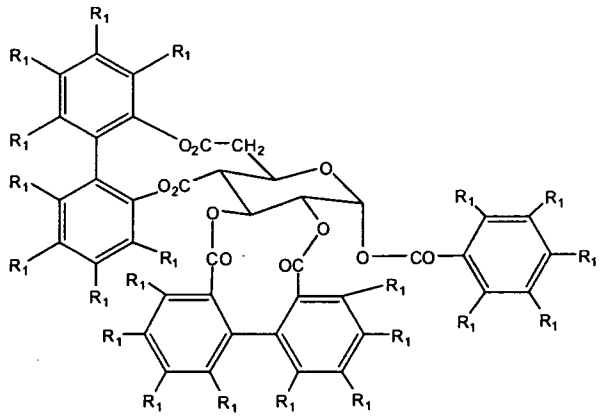
CLAIMS

What is claimed is:

1. A method for treating or preventing coronary heart disease in a mammalian subject comprising administering to the subject in need thereof an effective amount of a compound of formula I or formula II,



(I)



(II)

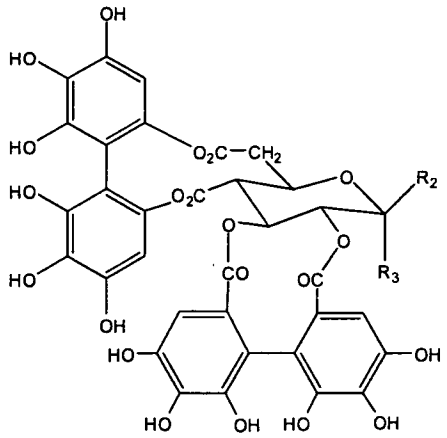
mixture thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof,

wherein

each R_1 is independently selected from H, halogen, hydroxy, carboxy, nitro, amino, nitrile, or a substituted or unsubstituted C_1–C_6 alkyl, alkoxy, hydroxyalkyl, cycloalkyl, cycloalkylalkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, haloalkyl, haloalkoxy, alkanoyl, and alkanoyloxy, groups; and

R_2 and R_3 are each H or O-R_1, such that when R_2 is H, R_3 is O-R1 and when R_2 is O-R_1, R_3 is H.

2. The method of claim 1, wherein the compound of formula I has the formula IA

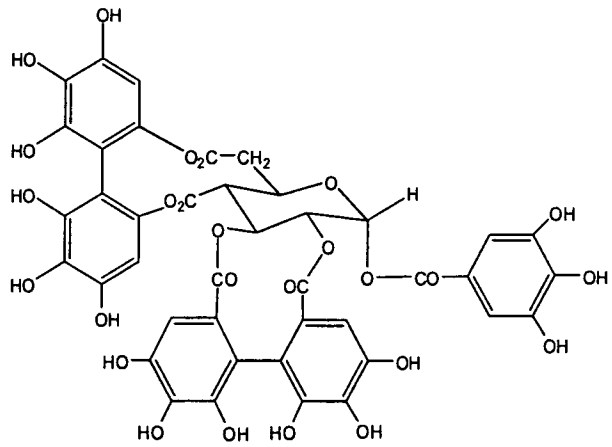


(IA)

wherein

R_2 and R_3 are each H or O-R_1, such that when R_2 is H, R_3 is O-R1 and when R_2 is O-R_1, R_3 is H.

3. The method of claim 1, wherein the compound of formula II has the formula IIA



(IIA)

4. A method for treating or preventing coronary heart disease in a mammalian subject comprising administering to the subject in need thereof an effective amount of a composition selected from the group consisting of: (i) an organic extract from the plant *Fructus Rosae Laevigatae*; (ii) an active fraction of an organic extract from the plant *Fructus Rosae Laevigatae*; and (iii) compound of formula (I), (IA), (II), or

(IIA), mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof.

5. The method of claim 4 comprising administering to the subject in need thereof an effective amount of a one or more of the compounds of formula (I), (IA), (II), and (IIA), mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof.

6. A method for promoting revascularization in dead or damaged heart tissues of a mammalian subject caused by an ischemic heart disease, the method comprising administering to the subject an effective amount of a compound of formula I or formula II, mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof.

7. A method for up-regulating the expression of angiogenic factors to stimulate growth of new coronary collateral vessels in ischemic or infarcted myocardium, in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of formula I or formula II, mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof, wherein the angiogenic factors are one or more of VEGF, VEGFR, EGF, and FGF.

8. A method for treating ischemic heart diseases or ischemic limbs in a mammalian subject, comprising administering to the subject an effective amount of the compound of formula (I) or formula II, mixtures thereof, stereoisomers thereof, tautomers thereof, solvates thereof, and pharmaceutically acceptable salts thereof.

9. The method of any one of claims 1-8, wherein the mammalian subject is a human.

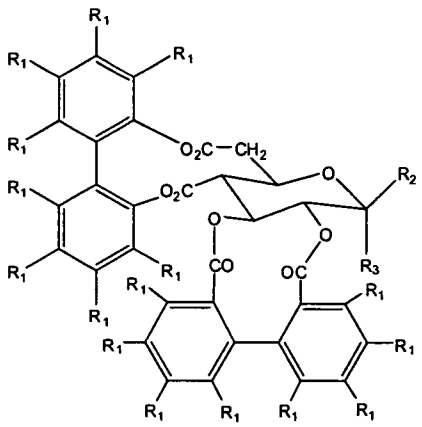
10. The method of any one of claims 1-9, wherein the compound or composition is administered intravenously.

11. The method of any one of claims 1-9, wherein the compound or composition is administered orally.

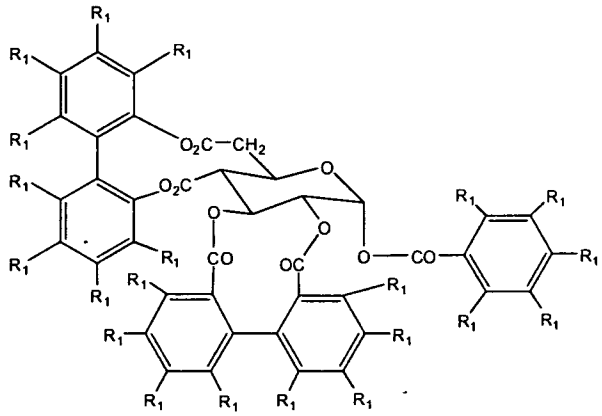
12. The method of any one of claims 1-9, wherein the compound or composition is administered in an amount of from 0.01 mg/kg/day to 1000 mg/kg/day.

13. The method of any one of claims 1-9, wherein the effective amount of the compound or composition is in the form of a pharmaceutical formulation comprising the compound or composition and a pharmaceutically suitable carrier or excipient.

14. A pharmaceutical composition, for use in treating or preventing coronary heart disease, comprising a pharmaceutically acceptable excipient and an effective amount of a compound selected from formula I or formula II.



(I)



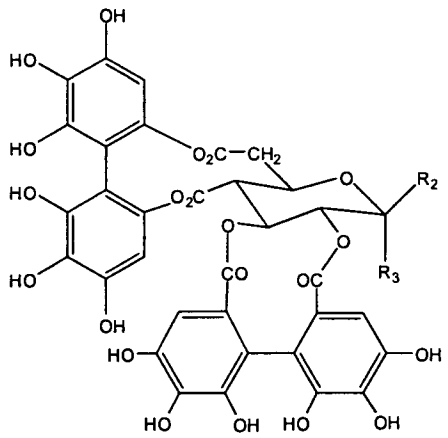
(II)

wherein

each R_1 is independently selected from H, halogen, hydroxy, carboxy, nitro, amino, nitrile, or a substituted or unsubstituted C_1–C_6 alkyl, alkoxy, hydroxyalkyl, cycloalkyl, cycloalkylalkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carboxyalkyl, alkoxyalkyl, aminoalkyl, haloalkyl, haloalkoxy, alkanoyl, and alkanoyloxy, groups; and

R_2 and R_3 are each H or O-R_1, such that when R_2 is H, R_3 is O-R1 and when R_2 is O-R_1, R_3 is H.

15. The composition of claim 14, wherein the compound of formula I has the structure IA



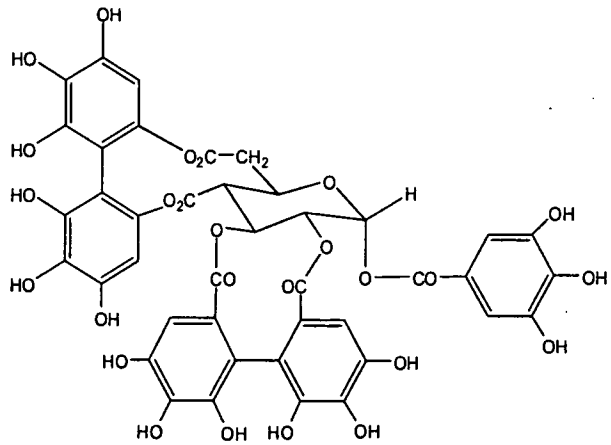
(IA)

wherein

R_2 and R_3 are each H or O-R_1, such that when R_2 is H, R_3 is O-R1 and when R_2 is O-R_1, R_3 is

H.

16. The composition of claim 14, wherein the compound of formula II has the structure IIA



(IIA)

17. The composition of claim 14 further comprising an anti-arrhythmia agent or a cardiovascular agent.

FIG. 1

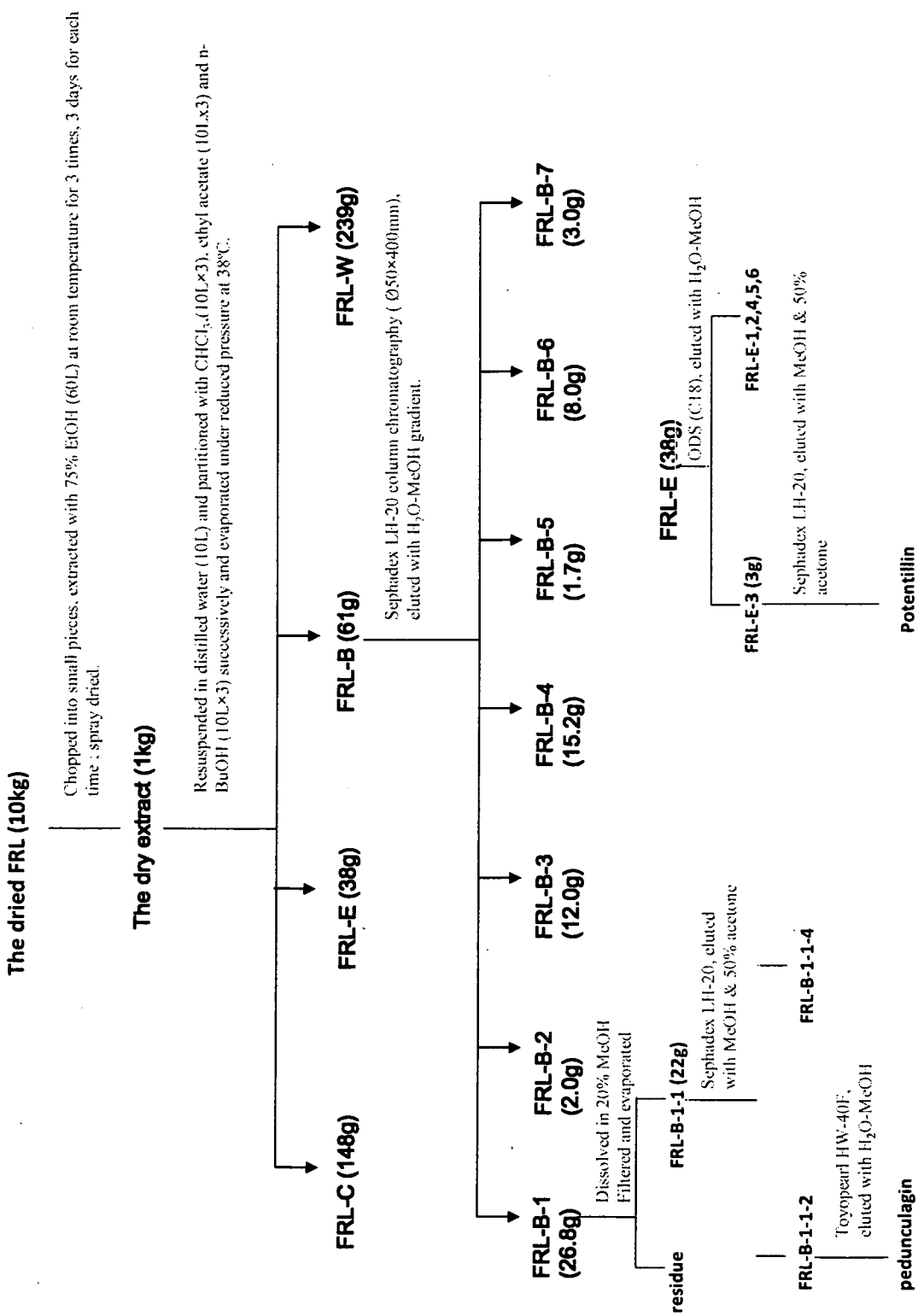

The dried FRL (10kg)
Chopped into small pieces, extracted with 75% EtOH (60L) at room temperature for 3 times, 3 days for each time ; spray dried.
The dry extract (1kg)
Resuspended in distilled water (10L) and partitioned with $CHCl_3$ (10L×3), ethyl acetate (10L×3) and n-BuOH (10L×3) successively and evaporated under reduced pressure at 38°C.
FRL-C (148g)
FRL-E (38g)
FRL-B (61g)
FRL-W (239g)
Sephadex LH-20 column chromatography (Ø50×400mm), eluted with H_2O-MeOH gradient.
FRL-B-1 (26.8g)
FRL-B-2 (2.0g)
FRL-B-3 (12.0g)
FRL-B-4 (15.2g)
FRL-B-5 (1.7g)
FRL-B-6 (8.0g)
FRL-B-7 (3.0g)
Dissolved in 20% MeOH Filtered and evaporated
residue
FRL-B-1-1 (22g)
Sephadex LH-20, eluted with MeOH & 50% acetone
FRL-B-1-1-2
FRL-B-1-1-4
Toyopearl HW-40F, eluted with H_2O-MeOH
pedunculagin
FRL-E (38g)
ODS (C18), eluted with H_2O-MeOH
FRL-E-1,2,4,5,6
FRL-E-3 (3g)
Sephadex LH-20, eluted with MeOH & 50% acetone
Potentillin

FIG. 2

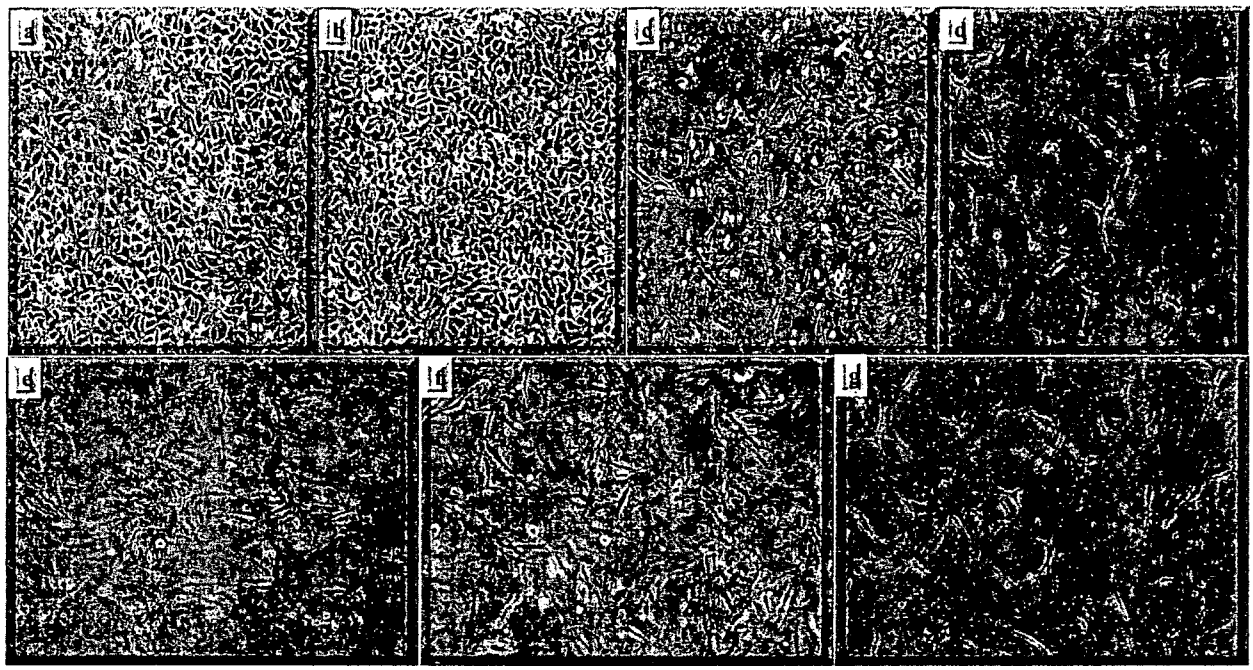


FIG. 3A-C

FIG. 3D

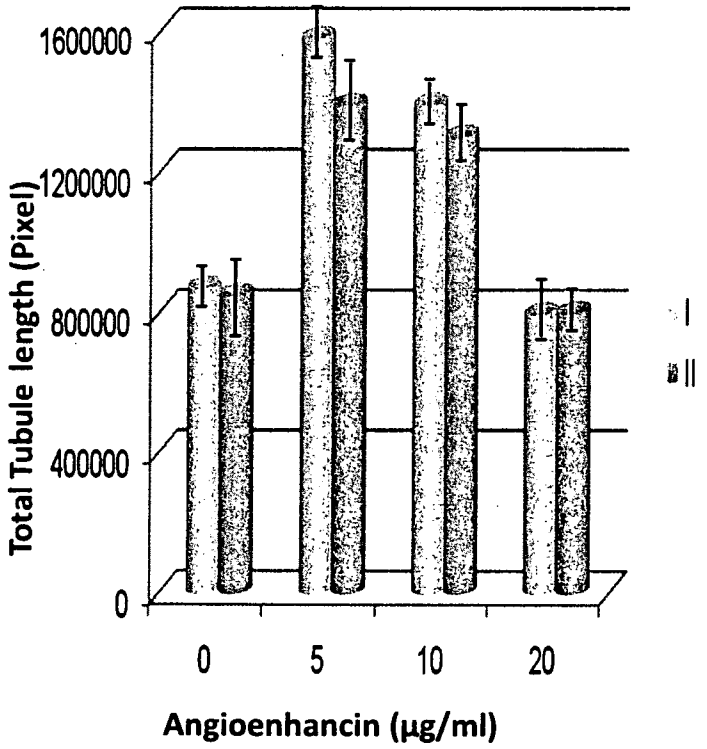


FIG. 4

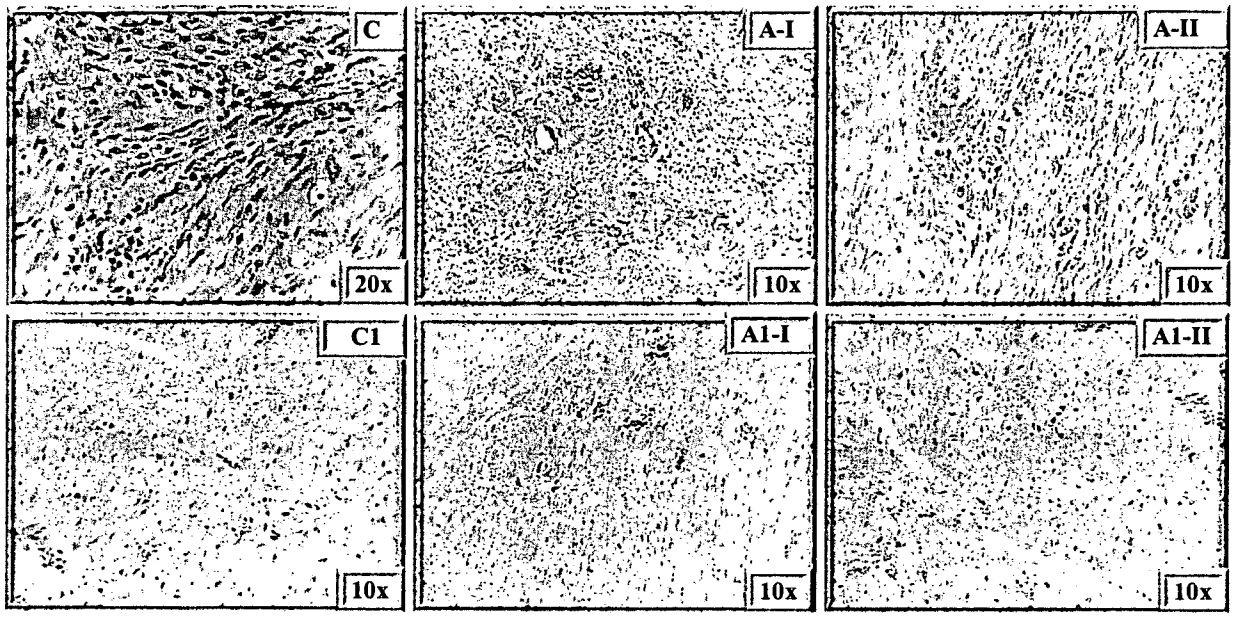


FIG. 5

A

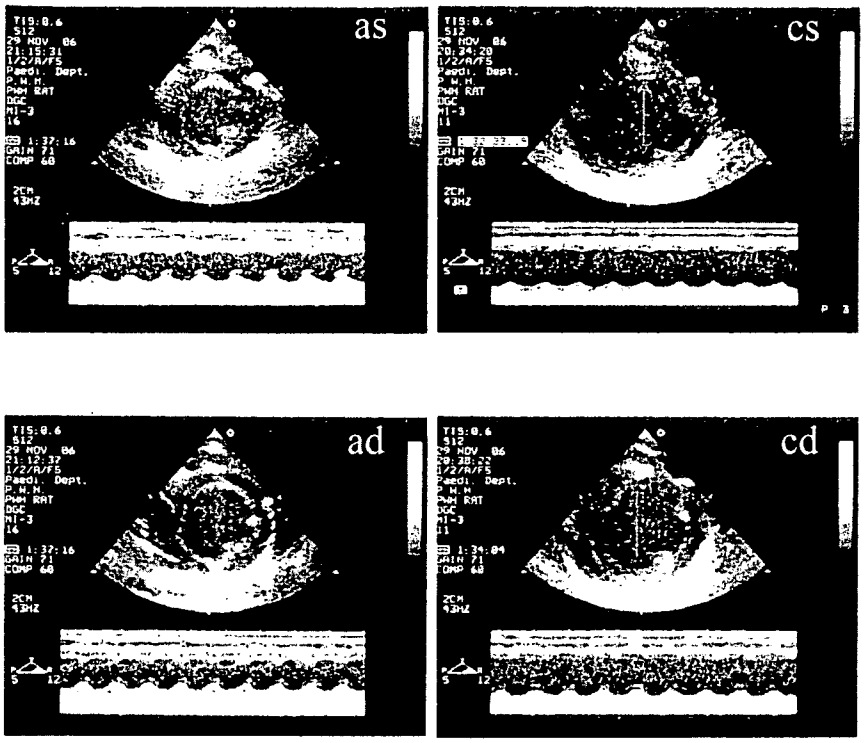
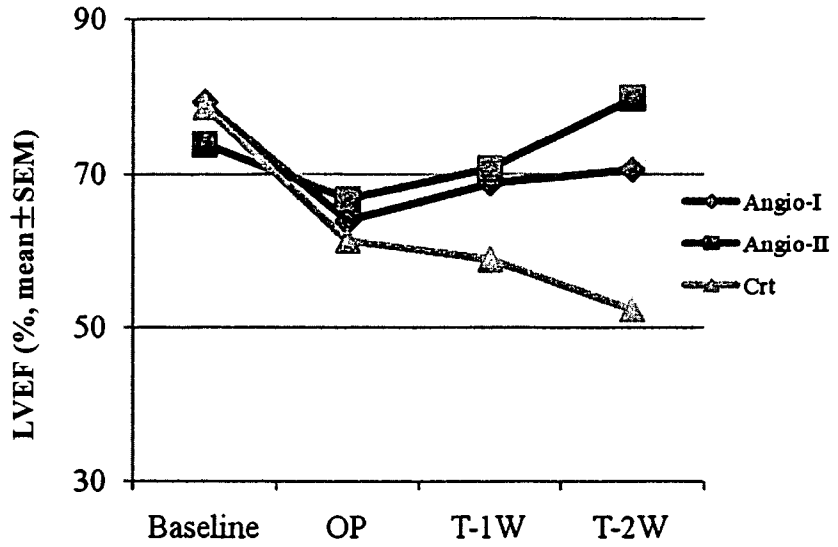
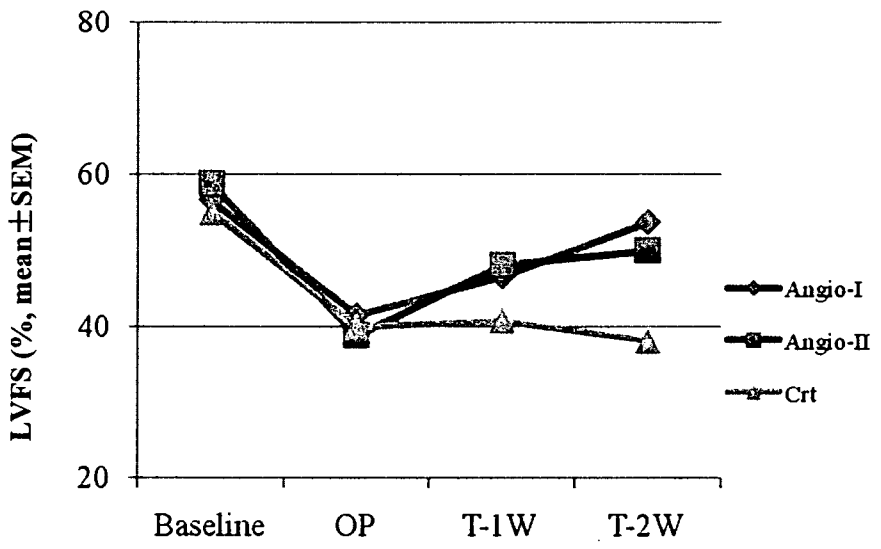


FIG. 5 (CONT.)

B


90
70
50
30
LVEF (%, mean±SEM)
Baseline
OP
T-1W
T-2W
Angio-I
Angio-II
Crt

C


80
60
40
20
LVFS (%, mean±SEM)
Baseline
OP
T-1W
T-2W
Angio-I
Angio-II
Crt

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2010/001416

A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet

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: A61K 31/-; A61K 36/-; A61P 9/-

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)

WPI, EPODOC, CNPAT, CNKI, CA: casuarictin, potentillin, pedunculagin, gemin, coronary, heart, fructus rosae laevigatae, geum japonicum, agrimonia pilosa, extract

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2008-74801 A (ORIZA YUKA KK) 03 Apr. 2008 (03.04.2008) Abstract, paragraphs 0012-0020	14-16
X	YOSHIKI Kashiwada et al. Antitumor agents, 129.1 Tannins and Related Compounds as Selective Cytotoxic Agents. Journal of Natural Products, Aug. 1992, Vol. 55, No. 8, pages 1033-1043	14-16
X	LIU Hongwei et al. Fatty Acid Synthase Inhibitors from Geum Japonicum Thunb. var. Chinense. CHEMISTRY & BIODIVERSITY, 24 Mar. 2009 (24.03.2009) , Vol. 6, Issue 3, pages 402-410	14-16

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* 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
29 Sep. 2010 (29.09.2010)	**11 Nov. 2010 (11.11.2010)**
Name and mailing address of the ISA/CN The State Intellectual Property Office, the P.R.China 6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088 Facsimile No. 86-10-62019451	Authorized officer WANG, Limin Telephone No. (86-10)82245332

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2010/001416

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007048353 A1 (LEAD BILLION LIMITED) 03 May 2007 (03.05.2007) Abstract; page 2, line 17-page 4, line 19	1-17
A	LI, Jiankuan Studies on Bioactive Constituents with Myogenesis and Angiogenesis Activity from Geum Japonicum Thunb. Vax. Chinese F. Bolle. CHINESE Doctoral Dissertation & Master's Theses Full-Text Database (Master), Medicine and Health Sciences, 15 Jan. 2007 (15.01.2007) Abstract, pages 17	1-17
A	WO 03043645 A1 (THE CHINESE UNIVERSITY OF HONG KONG), 30 May 2003 (30.05.2003) Abstract; claims 1-23; example 1	1-17
A	CN 1682788 A (ZHAO, Xiaoang) 19 Oct. 2005 (19.10.2005) Abstract; claim 1; page 4, lines 1-10	1-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2010/001416

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-13
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-13 are directed to methods for treatment of the human or animal body by therapy. This report has been carried out and based on the use of the compound or the composition for preparation of medicine thereof.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2010/001416

A. CLASSIFICATION OF SUBJECT MATTER

A61K31/7024(2006.01)i

A61K31/7028(2006.01)i

A61K31/7032(2006.01)i

A61K31/7034(2006.01)i

A61K31/704(2006.01)i

A61K36/73(2006.01)i

A61P9/10(2006.01)i

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IB2010/001416

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
JP 2008074801 A	03.04.2008	None	
WO 2007048353 A1	03.05.2007	EP 1848445 A1	31.10.2007
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CN 1682788 A	19.10.2005	CN 100509008C	08.07.2009