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(54) **Title:** LIVER-TARGETED PRODRUG COMPOSITIONS AND METHODS OF USING THE SAME

(57) **Abstract:** Disclosed are compositions and methods of delivering a drug to a liver cell or liver tissue in a subject. In some forms, the composition is a prodrug of a drug where the drug, a hydrophilic linker, and a liver-targeted glycol ligand are conjugated together. The hydrophilic linker contains or is coupled to the drug via a cleavable bond such that the cleavable bond is cleaved and the drug released after delivery into a target cell.



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LIVER-TARGETED PRODRUG COMPOSITIONS AND METHODS OF USING THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application No.
5 62/261,069, filed November 30, 2015, the contents of which are incorporated
by reference herein in their entirety.

FIELD OF THE INVENTION

The disclosed invention is generally in the field of drug delivery and
disease treatment, and specifically in the area of drug delivery for and
10 treatment of liver disease, such as hepatocellular carcinoma (HCC).

BACKGROUND OF THE INVENTION

Liver cancer, primarily hepatocellular carcinoma (HCC), is the third
leading cause of death from cancer worldwide and the ninth leading cause of
cancer deaths in the United States (Altekruse et al., *J. Clin. Oncol.* 2009, 27,
15 1485-1491; Parkin et al., *CA Cancer J. Clin.* 2005, 55, 74-108). Relative to
other cancers, the death rate associated with liver cancer has been growing in
the US in recent decades. Approximately, 90% of liver cancers are HCC
(derived from hepatocytes, which are the main functional cells of the liver
and constitute 60 ~ 80% of the mass of the liver mass) and is also considered
20 to be the most lethal among cancers (Cervello et al., *Oncotarget.* 2012, 3,
236-260). To date, surgical resection and liver transplantation are considered
to be main curative treatment options for HCC (El-Serag et al., *J. Hepatol.*
2006, 44, 158-166). However, HCC in the majority (~75%) of patients
present has detected at with advanced tumor stage with exceptionally poor
25 liver function, rendering them ineligible for surgical interventions.

Until the FDA approval of multikinase inhibitor sorafenib (Wilhelm
et al., *Nat. Rev. Drug Discov.* 2006, 5, 835-844, Furuse, *Biologics.* 2008, 2,
779- 788), for treatment of HCC with unresectable disease, there was no
standard systemic therapies. The classical cell killing drugs (administered as
30 mono or in combination) were the only option but with poor response rates
and survival benefit. Although used at a high rate, response rates to sorafenib
are low with overall benefits being modest, and toxicity profile of sorafenib

limits its use in many patients (Zhu, *Semin. Oncol.* 2012, 39, 493-502, Frenette and Gish, *World J. Gastroenterol.* 2012, 18, 498-506, Boudou-Rouquette et al., *PLoS. One.* 2012, 7, e42875). Poor aqueous solubility, high plasma protein binding and sub-optimal systemic absorption at the gastrointestinal tract after oral administration are significant obstacles keeping sorafenib from optimal performance (Di et al., *Clin. Pharmacokinet.* 2011, 50, 551-603, Blanchet et al., *J. Pharm. Biomed. Anal.* 2009, 49, 1109-1114). Even though nanotechnology based methods has been applied to improve solubility and absorption of sorafenib (Wang et al., *Int. J. Pharm.* 2011, 419, 339-346, Carty et al., PCT/US2008/0213374 A1), poor specificity, long intracellular residence time resulting in low therapeutic index and dose limiting systemic toxicity limits its use (Oerlemans et al., *Pharm. Res.* 2010, 27, 2569-2589, Sinha et al., *Mol. Cancer Ther.* 2006, 5, 1909-1917). Thus, there is a need for improved therapies for HCC and for improved delivery and effectiveness of sorafenib.

It is therefore an object of the present invention to provide compositions and methods for more effective treatment of HCC.

It is also an object of the present invention to provide compositions more effectively targeted and delivered into liver tissue and hepatic cells.

It is also an object of the present invention to provide compositions delivered into cells via asialoglycoprotein receptors.

It is also an object of the present invention to provide compositions that more effectively transport and deliver hydrophobic and insoluble compounds and compositions to and into liver tissue and hepatic cells.

It is also an object of the present invention to provide prodrugs cleavable after delivery into cells.

It is also an object of the present invention to provide compositions of sorafenib that more effectively transport and deliver sorafenib to and into liver tissue and hepatic cells.

BRIEF SUMMARY OF THE INVENTION

Disclosed are compositions and methods of delivering a drug to a liver cell or liver tissue in a subject. In some forms, the composition is a prodrug of a drug where the drug, a hydrophilic linker, and a liver-targeted

glycol ligand are conjugated together. The hydrophilic linker contains or is coupled to the drug via a cleavable bond such that the cleavable bond is cleaved and the drug released after delivery into a target cell.

Disclosed are prodrugs comprising a drug moiety and a transport moiety covalently coupled to the drug moiety. The transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer. The hydrophilic spacer comprises a cleavable bond, where cleavage of the cleavable bond separates the drug moiety from the transport moiety. The liver-targeted glycol ligand can be one or more β -D-galactose (Gal) moieties, N-acetylgalactosamine (GalNAc) moieties, or combinations thereof.

In some forms, the hydrophilic spacer can be selected from the group consisting of: a substituted branched or unbranched saturated or unsaturated alkyl chain, a substituted or unsubstituted branched or unbranched saturated or unsaturated alkoxy chain, a substituted or unsubstituted branched or unbranched saturated or unsaturated alkylamino chain, and a substituted or unsubstituted branched or unbranched saturated or unsaturated alkylthio chain. In some forms, the substituted saturated alkyl chain or the substituted unsaturated alkyl chain can comprise an atom or atom group selected from the group consisting of S, O, N, and C=O. In some forms, the hydrophilic spacer can comprise ethylene glycol. In some forms, the hydrophilic spacer has of a length of 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4 or 3 atoms.

In some forms, the drug moiety is covalently coupled to the hydrophilic spacer of the transport moiety via the cleavable bond. In some forms, the cleavable bond is a bond selected from the group consisting of: an ester, an amide, and a carbonate. In some forms, the cleavable bond is an internal bond in the hydrophilic spacer. In some forms, the cleavable bond is hydrolyzable.

In some forms, the drug moiety is a cancer therapeutic. In some forms, the cancer therapeutic is sorafenib. In some forms, the drug moiety is selected from the group consisting of temozolomide, sorafenib, erlotinib, gefitinib, imatinib, pazopanib, rapamycin, raloxifene, lasofoxifene,

basedoxifene, resveratrol, curcumin, etoposide, camptothecin, CPT-11, topotecan, irinotecan, exatecan, lurtecan, DB67, BNP1350, ST1481, CKD602, paclitaxel, docetaxel, vincristine, vinblastine, fingolimod, raltegravir, elvitegravir, MK-2408, lersivirine, daunorubicin, doxorubicin, 5 epirubicin, and idarubicin. In some forms, the drug moiety is selected from the group consisting of taxanes, anthracyclines, and camptothecin analogues.

Disclosed are methods of delivering a drug to a liver cell or liver tissue in a subject, comprising the step of providing to the cell or tissue the disclosed prodrug. Also disclosed are method of treating a subject in need 10 thereof, comprising the step of administering to the subject the disclosed prodrug. In some forms, the prodrug is taken into the cell or tissue by an asialo-glycoprotein receptor (ASGP-R). In some forms, the subject suffers from hepatocellular carcinoma (HCC).

15 Additional advantages of the disclosed method and compositions will be set forth in part in the description which follows, and in part will be understood from the description, or may be learned by practice of the disclosed method and compositions. The advantages of the disclosed method and compositions will be realized and attained by means of the elements and 20 combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

25 The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the disclosed method and compositions and together with the description, serve to explain the principles of the disclosed method and compositions.

Figures 1A and 1B are diagrams of an example of a liver-targeted 30 prodrug of sorafenib showing the prodrug design strategy (A) and ASGP-R mediated drug uptake (B). (A) Design strategy for liver specific prodrug sorafenib analogs. (B) Schematic of ASGP-R mediated drug uptake: (1) Selective binding of terminal sugar ligands to ASGP-R; (2) Internalization of

prodrug analogs through receptor mediate endocytosis (RME); (3) prodrug within the liver cell; (4) Drug released from glyco-conjugate prodrug via cleavable disulfide linker; (5) Recycling ASGP-R; (6) Non-toxic water soluble gal-spacer undergoing lysosomal degradation.

5 Figure 2 is a graph showing in vivo liver specific targeting of a reporter gene by synthetic cationic glycolipids 3 & 6. Synthetic-glycolipids (3 & 6) were prepared by covalently linked single galactose (both cyclic-and open) with varying spacer units to quarternized nitrogen. Mice were injected intravenously with glycolipid: DNA complex at 4:1 charge ratio and
10 luciferase activities were estimated 8 h post-injection using the methods described in Mukthavaram et al., *Biomaterials* 30:2369-2383 (2009).

 Figures 3A, 3B, and 3C are diagrams showing examples of prodrug design strategy. (A) Chemical structure of sorafenib. (B) Interaction of sorafenib with targeted protein B Raf. (C) Chemical structures of glyco-
15 conjugated sorafenib analogs (1-6).

 Figure 4 is a diagram showing Scheme 1: Synthesis of Prodrug Sorafenib analogs 1 & 2.

 Figure 5 is a diagram showing Scheme 1b: Synthesis of building block 10.

20 Figure 6 is a diagram showing: Scheme 2: Synthesis of glycoconjugated sorafenib analogs 3 & 4

 Figure 7 is a diagram showing Scheme 2b: Synthesis of building block 14.

25 Figure 8 is a diagram showing Scheme 3: Synthesis of glycoconjugated sorafenib analogs 5 & 6.

 Figure 9 is a diagram of sorafenib release mechanism of an example of an activatable prodrug from conjugates via a pH sensitive cleavable bond.

 Figure 10 is a diagram of sorafenib release mechanism of an example of an activatable prodrug from conjugates via a disulfide cleavable bond.

30 Figure 11 is a diagram of an example of an ASGP-R drug uptake study.

DETAILED DESCRIPTION OF THE INVENTION

The disclosed method and compositions may be understood more readily by reference to the following detailed description of particular embodiments and the Example included therein and to the Figures and their
5 previous and following description.

Disclosed are compositions and methods of delivering a drug to a liver cell or liver tissue in a subject. In some forms, the composition is a prodrug of a drug where the drug, a hydrophilic linker, and a liver-targeted glycol ligand are conjugated together. The hydrophilic linker contains or is
10 coupled to the drug via a cleavable bond such that the cleavable bond is cleaved and the drug released after delivery into a target cell.

Some forms of the disclosed compositions simplify production of deliverable prodrugs by minimizing the components used and the assembly and stability of the compositions. In some forms, the disclosed compositions
15 use a single cell- or tissue-targeting glycol ligand, such as a single galactose moiety, which is sufficient for high cell- or tissue-specificity (liver specificity in the case of the galactose moiety). In some forms, the disclosed compositions provide improved pharmacokinetics by use of a water soluble spacer or linker, such as an ethylene glycol spacer. By using a bond in the
20 prodrug composition that is cleavable inside the target cells (such as a disulfide bond), a high drug concentration can be delivered at the target cells or tissue. In some forms of the prodrug compositions, a liver-specific Gal/GalNAc additive prodrug with the hydrophilic spacer including a disulfide bond cleavable via GSH results in improved delivery of a drug. For
25 example, such an approach provides an improved version of glycoconjugated sorafenib analogs. Benefits of such prodrug compositions can include (1) enhanced delivery to liver cancer cells through ASGP-R mediated endocytosis, (2) improved pharmacokinetics by modifying its water solubility and bioavailability, (3) reduced unwanted toxicities by liver
30 specific receptors, or combinations thereof.

The disclosed compositions improve results over polymeric nanoscale-based technologies. For example, the single compound form (that is, using a compound with each component of the prodrug covalent coupled

together) eliminates the need for checking of multiple components before encapsulating the drug as is needed for many polymeric nanoscale technologies (e.g., process of liposome preparation with concerns of size and stability). As another example, in some forms, the disclosed compositions improve specificity and reduce intracellular residence time of the delivery components, which can increase the therapeutic index and reduce dose-limiting systemic toxicity of the drug composition. In another example, in some forms, the use of a single form of cell- or tissue-targeting glycol ligand improves the kinetics of drug exposure at the target organ, tissue, or cells (in contrast to more complex glycol and carbohydrate moieties and compounds used for targeting in other compositions).

A. Definitions

“Sugar additive prodrug approach” and similar phrases refer to adding or including one or more sugar moieties to a prodrug composition or compound to facilitate sugar-based binding (and uptake) of the prodrug composition or compound to a sugar receptor. For example, a Gal/GalNAc additive prodrug approach includes a Gal and/or GalNAc moiety to a prodrug composition or compound to facilitate Gal-based binding and uptake of the prodrug composition or compound via asialo-glycoprotein receptor (ASGP-R).

Reference to components (such as a drug moiety and a transport moiety) as being “covalently coupled” means that the components are connected via covalent bonds (for example, that the drug moiety and the transport moiety are connected via covalent bonds). That is, there is a continuous chain of covalent bonds between, for example, the drug moiety and the transport moiety. Components can be covalently coupled either directly or indirectly. Direct covalent coupling refers to the presence of a covalent bond between atoms of each of the components. Indirect covalent coupling refers to the absence of a covalent bond between atoms of each of the components. That is, some other atom or atoms not belonging to either of the coupled components intervenes between atoms of the components. Both direct and indirect covalent coupling involve a continuous chain of covalent bonds.

“Separates the drug moiety from the transport moiety” refers to the drug moiety no longer being covalently coupled to the glycol ligand. Some part of the transport moiety can remain attached to the drug moiety, such as if the cleavable bond is an internal bond of the hydrophobic spacer.

5 “Internal bond” of a compound, group, moiety, or the like refers to a bond between two atoms that are both atoms belonging to the compound, group, moiety, or the like.

“Hydrolysable bond” refers to a bond that can be cleaved in a reaction involving the addition of water.

10 The term “hit” refers to a test compound that shows desired properties in an assay. The term “test compound” refers to a chemical to be tested by one or more screening method(s) as a putative modulator. A test compound can be any chemical, such as an inorganic chemical, an organic chemical, a protein, a peptide, a carbohydrate, a lipid, or a combination
15 thereof. Usually, various predetermined concentrations of test compounds are used for screening, such as 0.01 micromolar, 1 micromolar and 10 micromolar. Test compound controls can include the measurement of a signal in the absence of the test compound or comparison to a compound known to modulate the target.

20 The terms “high,” “higher,” “increases,” “elevates,” or “elevation” refer to increases above basal levels, e.g., as compared to a control. The terms “low,” “lower,” “reduces,” or “reduction” refer to decreases below basal levels, e.g., as compared to a control.

The term “modulate” as used herein refers to the ability of a
25 compound to change an activity in some measurable way as compared to an appropriate control. As a result of the presence of compounds in the assays, activities can increase or decrease as compared to controls in the absence of these compounds. Preferably, an increase in activity is at least 25%, more preferably at least 50%, most preferably at least 100% compared to the level
30 of activity in the absence of the compound. Similarly, a decrease in activity is preferably at least 25%, more preferably at least 50%, most preferably at least 100% compared to the level of activity in the absence of the compound.

A compound that increases a known activity is an “agonist”. One that decreases, or prevents, a known activity is an “antagonist”.

The term “inhibit” means to reduce or decrease in activity or expression. This can be a complete inhibition or activity or expression, or a partial inhibition. Inhibition can be compared to a control or to a standard level. Inhibition can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%.

The term “monitoring” as used herein refers to any method in the art by which an activity can be measured.

The term “providing” as used herein refers to any means of adding a compound or molecule to something known in the art. Examples of providing can include the use of pipettes, pipetmen, syringes, needles, tubing, guns, etc. This can be manual or automated. It can include transfection by any mean or any other means of providing nucleic acids to dishes, cells, tissue, cell-free systems and can be *in vitro* or *in vivo*.

The term “preventing” as used herein refers to administering a compound prior to the onset of clinical symptoms of a disease or conditions so as to prevent a physical manifestation of aberrations associated with the disease or condition.

The term “in need of treatment” as used herein refers to a judgment made by a caregiver (e.g. physician, nurse, nurse practitioner, or individual in the case of humans; veterinarian in the case of animals, including non-human mammals) that a subject requires or will benefit from treatment. This judgment is made based on a variety of factors that are in the realm of a caregiver's expertise, but that include the knowledge that the subject is ill, or will be ill, as the result of a condition that is treatable by the compounds of the invention.

As used herein, “subject” includes, but is not limited to, animals, plants, bacteria, viruses, parasites and any other organism or entity. The

subject can be a vertebrate, more specifically a mammal (e.g., a human, horse, pig, rabbit, dog, sheep, goat, non-human primate, cow, cat, guinea pig or rodent), a fish, a bird or a reptile or an amphibian. The subject can be an invertebrate, more specifically an arthropod (e.g., insects and crustaceans).

- 5 The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered. A patient refers to a subject afflicted with a disease or disorder. The term "patient" includes human and veterinary subjects.

- 10 By "treatment" and "treating" is meant the medical management of a subject with the intent to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder. This term includes active treatment, that is, treatment directed specifically toward the improvement of a disease, pathological condition, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, or disorder.
- 15 In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, or disorder. It is understood that treatment, while intended to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder, need not actually result in the cure,
- 20 ameliorization, stabilization or prevention. The effects of treatment can be measured or assessed as described herein and as known in the art as is suitable for the disease, pathological condition, or disorder involved. Such measurements and assessments can be made in qualitative and/or quantitative terms. Thus, for example, characteristics or features of a disease, pathological condition, or disorder and/or symptoms of a disease, pathological condition, or disorder can be reduced to any effect or to any amount.
- 25
30

A cell can be *in vitro*. Alternatively, a cell can be *in vivo* and can be found in a subject. A “cell” can be a cell from any organism including, but not limited to, a bacterium.

In one aspect, the compounds described herein can be administered to
5 a subject comprising a human or an animal including, but not limited to, a mouse, dog, cat, horse, bovine or ovine and the like, that is in need of alleviation or amelioration from a recognized medical condition.

By the term “effective amount” of a compound as provided herein is meant a nontoxic but sufficient amount of the compound to provide the
10 desired result. As will be pointed out below, the exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease that is being treated, the particular compound used, its mode of administration, and the like. Thus, it is not possible to specify an exact “effective amount.” However, an
15 appropriate effective amount can be determined by one of ordinary skill in the art using only routine experimentation.

It will be recognized, of course, that “biocompatibility” is a relative term, and some degree of immune response is to be expected even for polymers that are highly compatible with living tissue. However, as used
20 herein, “biocompatibility” refers to the acute rejection of material by at least a portion of the immune system, *i.e.*, a non-biocompatible material implanted into a subject provokes an immune response in the subject that is severe enough such that the rejection of the material by the immune system cannot be adequately controlled, and often is of a degree such that the material must
25 be removed from the subject. One simple test to determine biocompatibility is to expose a polymer to cells *in vitro*; biocompatible polymers are polymers that typically will not result in significant cell death at moderate concentrations, *e.g.*, at concentrations of 50 micrograms/10⁶ cells. For instance, a biocompatible polymer may cause less than about 20% cell death
30 when exposed to cells such as fibroblasts or epithelial cells, even if phagocytosed or otherwise uptaken by such cells.

References in the specification and concluding claims to parts by weight, of a particular element or component in a composition or article,

denotes the weight relationship between the element or component and any other elements or components in the composition or article for which a part by weight is expressed. Thus, in a compound containing 2 parts by weight of component X and 5 parts by weight component Y, X and Y are present at a weight ratio of 2:5, and are present in such ratio regardless of whether additional components are contained in the compound.

A weight percent of a component, unless specifically stated to the contrary, is based on the total weight of the formulation or composition in which the component is included.

A residue of a chemical species, as used in the specification and concluding claims, refers to the moiety that is the resulting product of the chemical species in a particular reaction scheme or subsequent formulation or chemical product, regardless of whether the moiety is actually obtained from the chemical species. Thus, an ethylene glycol residue in a spacer or linker refers to one or more $\text{-OCH}_2\text{CH}_2\text{O-}$ units in the spacer or linker, regardless of whether ethylene glycol was used to prepare the spacer or linker. Similarly, a sebacic acid residue in a spacer or linker refers to one or more $\text{-CO(CH}_2)_8\text{CO-}$ moieties in the linker or spacer, regardless of whether the residue is obtained by reacting sebacic acid or an ester thereof to obtain the linker or spacer.

The term “alkyl group” as used herein is a branched or unbranched saturated hydrocarbon group of 1 to 24 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *t*-butyl, pentyl, hexyl, heptyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl and the like. A “lower alkyl” group is an alkyl group containing from one to six carbon atoms.

The term “alkoxy” as used herein is an alkyl group bound through a single, terminal ether linkage; that is, an “alkoxy” group may be defined as -OR where R is alkyl as defined above. A “lower alkoxy” group is an alkoxy group containing from one to six carbon atoms.

The term “alkenyl group” as used herein is a hydrocarbon group of from 2 to 24 carbon atoms and structural formula containing at least one carbon-carbon double bond. Asymmetric structures such as $(\text{AB})\text{C}=\text{C}(\text{CD})$ are intended to include both the *E* and *Z* isomers. This may be presumed in

structural formulae herein wherein an asymmetric alkene is present, or it may be explicitly indicated by the bond symbol C.

The term “alkynyl group” as used herein is a hydrocarbon group of 2 to 24 carbon atoms and a structural formula containing at least one carbon-carbon triple bond.

The term “aryl group” as used herein is any carbon-based aromatic group including, but not limited to, benzene, naphthalene, etc. The term “aromatic” also includes “heteroaryl group,” which is defined as an aromatic group that has at least one heteroatom incorporated within the ring of the aromatic group. Examples of heteroatoms include, but are not limited to, nitrogen, oxygen, sulfur, and phosphorus. The aryl group can be substituted or unsubstituted. The aryl group can be substituted with one or more groups including, but not limited to, alkyl, alkynyl, alkenyl, aryl, halide, nitro, amino, ester, ketone, aldehyde, hydroxy, carboxylic acid, or alkoxy.

The term “cycloalkyl group” as used herein is a non-aromatic carbon-based ring composed of at least three carbon atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, etc. The term “heterocycloalkyl group” is a cycloalkyl group as defined above where at least one of the carbon atoms of the ring is substituted with a heteroatom such as, but not limited to, nitrogen, oxygen, sulphur, or phosphorus.

The term “aralkyl” as used herein is an aryl group having an alkyl, alkynyl, or alkenyl group as defined above attached to the aromatic group. An example of an aralkyl group is a benzyl group.

The term “hydroxyalkyl group” as used herein is an alkyl, alkenyl, alkynyl, aryl, aralkyl, cycloalkyl, halogenated alkyl, or heterocycloalkyl group described above that has at least one hydrogen atom substituted with a hydroxyl group.

The term “alkoxyalkyl group” is defined as an alkyl, alkenyl, alkynyl, aryl, aralkyl, cycloalkyl, halogenated alkyl, or heterocycloalkyl group described above that has at least one hydrogen atom substituted with an alkoxy group described above.

The term “ester” as used herein is represented by the formula —
C(O)OA, where A can be an alkyl, halogenated alkyl, alkenyl, alkynyl, aryl,
heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl
group described above.

5 The term “carbonate group” as used herein is represented by the
formula -OC(O)OR, where R can be hydrogen, an alkyl, alkenyl, alkynyl,
aryl, aralkyl, cycloalkyl, halogenated alkyl, or heterocycloalkyl group
described above.

10 The term “carboxylic acid” as used herein is represented by the
formula -C(O)OH.

The term “aldehyde” as used herein is represented by the formula -
C(O)H.

15 The term “keto group” as used herein is represented by the formula -
C(O)R, where R is an alkyl, alkenyl, alkynyl, aryl, aralkyl, cycloalkyl,
halogenated alkyl, or heterocycloalkyl group described above.

The term “carbonyl group” as used herein is represented by the
formula C=O.

20 The term “ether” as used herein is represented by the formula AOA¹,
where A and A¹ can be, independently, an alkyl, halogenated alkyl, alkenyl,
alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or
heterocycloalkenyl group described above.

25 The term “urethane” as used herein is represented by the formula
-OC(O)NRR', where R and R' can be, independently, hydrogen, an alkyl,
alkenyl, alkynyl, aryl, aralkyl, cycloalkyl, halogenated alkyl, or
heterocycloalkyl group described above.

The term “sulfo-oxo group” as used herein is represented by the
formulas -S(O)₂R, -OS(O)₂R, or , -OS(O)₂OR, where R can be hydrogen, an
alkyl, alkenyl, alkynyl, aryl, aralkyl, cycloalkyl, halogenated alkyl, or
heterocycloalkyl group described above.

30 The disclosed prodrug compositions and components thereof, such as
the hydrophilic spacer, can, independently, possess two or more of the
groups listed above. For example, if the hydrophilic spacer is a straight chain
alkyl group, one of the hydrogen atoms of the alkyl group can be substituted

with a hydroxyl group, an alkoxy group, etc. Depending upon the groups that are selected, a first group can be incorporated within second group or, alternatively, the first group can be pendant (*i.e.*, attached) to the second group. For example, with the phrase “an alkyl group comprising an ester group,” the ester group can be incorporated within the backbone of the alkyl group. Alternatively, the ester can be attached to the backbone of the alkyl group. The nature of the group(s) that is (are) selected will determine if the first group is embedded or attached to the second group.

As used herein, the term “activity” refers to a biological activity.

As used herein, the term “pharmacological activity” refers to the inherent physical properties of a peptide or polypeptide. These properties include but are not limited to half-life, solubility, and stability and other pharmacokinetic properties.

The term “modified” is often used herein to describe oligomers and polymers and means that a particular monomeric unit that would typically make up the pure oligomer or polymer has been replaced by another monomeric unit that shares a common polymerization capacity with the replaced monomeric unit. Thus, for example, it is possible to substitute diol residues for glycol in poly(ethylene glycol), in which case the poly(ethylene glycol) will be “modified” with the diol. If the poly(ethylene glycol) is modified with a mole percentage of the diol, then such a mole percentage is based upon the total number of moles of glycol that would be present in the pure polymer but for the modification. Thus, in a poly(ethylene glycol) that has been modified by 50 mole % with a diol, the diol and glycol residues are present in equimolar amounts.

It is to be understood that the disclosed method and compositions are not limited to specific synthetic methods, specific analytical techniques, or to particular reagents unless otherwise specified, and, as such, may 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.

Materials

Disclosed are compositions and methods of delivering a drug to a liver cell or liver tissue in a subject. In some forms, the composition is a

prodrug of a drug where the drug, a hydrophilic linker, and a liver-targeted glycol ligand are conjugated together. The hydrophilic linker contains or is coupled to the drug via a cleavable bond such that the cleavable bond is cleaved and the drug released after delivery into a target cell.

5 Disclosed are prodrugs comprising a drug moiety and a transport moiety covalently coupled to the drug moiety. The transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer. The hydrophilic spacer comprises a cleavable bond, where cleavage
10 of the cleavable bond separates the drug moiety from the transport moiety. The liver-targeted glycol ligand can be one or more β -D-galactose (Gal) moieties, N-acetylgalactosamine (GalNAc) moieties, or combinations thereof.

 In some forms, the hydrophilic spacer can be selected from the group
15 consisting of: a substituted branched or unbranched saturated or unsaturated alkyl chain, a substituted or unsubstituted branched or unbranched saturated or unsaturated alkoxy chain, a substituted or unsubstituted branched or unbranched saturated or unsaturated alkylamino chain, and a substituted or unsubstituted branched or unbranched saturated or unsaturated alkylthio
20 chain. In some forms, the substituted saturated alkyl chain or the substituted unsaturated alkyl chain can comprise an atom or atom group selected from the group consisting of S, O, N, and C=O. In some forms, the hydrophilic spacer can comprise ethylene glycol. In some forms, the hydrophilic spacer has of a length of 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4 or 3 atoms.

25 In some forms, the drug moiety is covalently coupled to the hydrophilic spacer of the transport moiety via the cleavable bond. In some forms, the cleavable bond is a bond selected from the group consisting of: an ester, an amide, and a carbonate. In some forms, the cleavable bond is an internal bond in the hydrophilic spacer. In some forms, the cleavable bond is
30 hydrolyzable.

 In some forms, the drug moiety is a cancer therapeutic. In some forms, the cancer therapeutic is sorafenib. In some forms, the drug moiety is selected from the group consisting of temozolomide, sorafenib, erlotinib,

gefitinib, imatinib, pazopanib, rapamycin, raloxifene, lasofoxifene, basedoxifene, resveratrol, curcumin, etoposide, camptothecin, CPT-11, topotecan, irinotecan, exatecan, lurtecan, DB67, BNP1350, ST1481, CKD602, paclitaxel, docetaxel, vincristine, vinblastine, fingolimod, 5 raltegravir, elvitegravir, MK-2408, lersivirine, daunorubicin, doxorubicin, epirubicin, and idarubicin. In some forms, the drug moiety is selected from the group consisting of taxanes, anthracyclines, and camptothecin analogues.

The disclosed prodrug compositions generally include a drug moiety and a transport moiety covalently coupled to the drug moiety. The transport 10 moiety generally includes a hydrophilic spacer and at least one glycol ligand of a receptor. The hydrophilic spacer is generally covalently coupled to the drug moiety and to the glycol ligand. The hydrophilic spacer generally includes a cleavable bond, where cleavage of the cleavable bond separates the drug moiety from the transport moiety. Components other the drug 15 moiety, hydrophilic spacer, glycol moiety, and cleavable bond can be part of the transport moiety. Generally, such other components should not eliminate, and preferably do not diminish, the properties and functions useful to the function of the prodrug composition.

A. Hydrophilic Spacer

20 The hydrophilic spacer serves as a bridge connecting a glycol ligand to a drug moiety. In the disclosed prodrug compositions, the hydrophilic spacer also includes or is flanked by a cleavable bond and provides favorable solution and stability characteristics. For example, a hydrophilic spacer of sufficient length and solubility can increase the solubility of the prodrug 25 composition, the half-life or stability of the prodrug composition in the subject, or a combination thereof. The hydrophilic spacer can be any chemical composition that accomplishes one or more of these functions or features.

Preferred hydrophilic spacers are oligomers of hydrophilic and 30 biocompatible monomers. For example, ethylene glycol oligomers, lactide-co-glycolide oligomers, anhydride oligomers, hydroxyacid oligomers, lactic acid oligomers, glycolic acid oligomers, ester oligomers, amide oligomers, orthoester oligomers, phosphazene oligomers, ethylene oxide oligomers,

vinyl alcohol oligomers, hydroxyethyl methacrylate oligomers, acrylamide oligomers, dioxanone oligomers, hydroxyalkanoate oligomers, hydroxybutyrate oligomers, glycerol sebacate oligomers, glycolide oligomers, lactide oligomers, caprolactone oligomers, alkylene glycol oligomers, alkylene oxide oligomers, lysine oligomers, ethylene imine oligomers, acrylic acid oligomers, urethane oligomers, trimethylene carbonate oligomers, acrylic acid oligomers, beta amino esters oligomers, oligomeric derivatives or synthetic forms of natural polymers such as hyaluronic acid, chondroitin sulfate, carboxymethylcellulose, and starch, and co-oligomers of monomers or dimers making up such oligomers are useful as the hydrophilic spacer. The monomers, dimers, and other components of the hydrophilic spacer can be modified or unmodified forms of monomers, dimers, and other components.

B. Glycol Ligand

The glycol ligand is the targeting and/or internalization moiety of the disclosed prodrug compositions. Sugar residues and carbohydrates, principally as the glycol modifications of glycoproteins, are recognition and binding ligands of numerous cell surface receptors. As such, glycol ligands can mediate binding to their cognate receptors and internalization of the glycol ligand and attached moieties into the cell bearing the receptor. Where glycol ligand-specific receptors appear on cells, tissues, and organs of interest, the glycol ligands can facilitate targeting and internalization to and into the cells, tissue, and organs of interest.

Generally, it is preferred that fewer sugar residues be used in the disclosed prodrug compositions. Glycol groups can include a number of sugar residues of heterogeneous structure. Such complex glycol groups can have a number of different receptor targets based on individual sugar residues of parts of the glycol group. Reducing the number of sugar residues used in the prodrug compositions both simplifies the structure and synthesis of the prodrug compositions, but can make the targeting more specific. It has been discovered single sugar residues can support efficient and specific targeting and internalization of compositions bearing the sugar residues. For

example, a single galactose residue can effectively target a sorafenib prodrug composition to cells bearing the ASGP receptor.

By “single sugar residue,” “single glycol ligand,” and the like is meant that a single type of sugar moiety or glycol ligand is included on the prodrug composition, unless the context clearly indicates otherwise.

Although it is preferred that a given prodrug molecule have one single molecule or moiety of sugar residue or glycol ligand (which can be referred to as a single instance of the sugar residue or glycol ligand), the disclosed prodrug compositions can have multiple molecules or moieties of sugar residue or glycol ligand. In the latter case, reference to a “single sugar residue,” “single glycol ligand,” or the like means that each of the multiple molecules or moieties of the sugar residue or glycol ligand will be the same (for example, they will all be galactose moieties).

The glycol ligand can be coupled to any part of the prodrug composition. It is preferred if the glycol ligand is coupled to the hydrophilic spacer. It is also preferred that the glycol ligand be coupled such that it is available for binding to its cognate receptor. It is also preferred for the glycol ligand to be separated from the drug moiety when the cleavable bond is cleaved. Those of skill in the art can easily configure the prodrug composition and the glycol ligand to satisfy some or all of those preferences and other design options.

C. Transport Moiety

The transport moiety includes both the hydrophilic spacer and the glycol moiety. The transport moiety excludes the drug moiety. The transport moiety, by virtue of the location of the cleavable bond flanking or in the hydrophilic spacer, also includes or is flanked by the cleavable bond. Components other the hydrophilic spacer, glycol moiety, and cleavable bond can be part of the transport moiety. Generally, such other components should not eliminate, and preferably do not diminish, the properties and functions useful to the function of the prodrug composition.

D. Drug Moiety

The drug moiety can be any compound to be delivered to cells, tissues, or organs. For example, the drug moiety can be a therapeutic agent.

As used herein, the term “therapeutic agent” means a molecule which has one or more biological activities in a normal or pathologic tissue. A variety of therapeutic agents can be used as a drug moiety.

In some embodiments, the therapeutic agent can be a cancer
5 chemotherapeutic agent. As used herein, a “cancer chemotherapeutic agent” is a chemical agent that inhibits the proliferation, growth, life-span or metastatic activity of cancer cells. Such a cancer chemotherapeutic agent can be, without limitation, sorafenib; a taxane such as docetaxel; an anthracyclin such as doxorubicin; an alkylating agent; a vinca alkaloid; an anti-
10 metabolite; a platinum agent such as cisplatin or carboplatin; a steroid such as methotrexate; an antibiotic such as adriamycin; a isofamide; or a selective estrogen receptor modulator; an antibody such as trastuzumab.

Taxanes are chemotherapeutic agents useful in homing compositions. Useful taxanes include, without limitation, docetaxel (Taxotere; Aventis
15 Pharmaceuticals, Inc.; Parsippany, N.J.) and paclitaxel (Taxol; Bristol-Myers Squibb; Princeton, N.J.). See, for example, Chan et al., *J. Clin. Oncol.* 17:2341-2354 (1999), and Paridaens et al., *J. Clin. Oncol.* 18:724 (2000). In particular, the drug moiety can be a paclitaxel-loaded albumin nanoparticle such as abraxane.

20 A cancer chemotherapeutic agent useful in a homing composition also can be an anthracyclin such as doxorubicin, idarubicin or daunorubicin. Doxorubicin is a commonly used cancer chemotherapeutic agent and can be useful, for example, for treating breast cancer (Stewart and Ratain, In: “Cancer: Principles and practice of oncology” 5th ed., chap. 19 (eds. DeVita, Jr., et al.; J. P. Lippincott 1997); Harris et al., In “Cancer: Principles and
25 practice of oncology,” supra, 1997). In addition, doxorubicin has anti-angiogenic activity (Folkman, *Nature Biotechnology* 15:510 (1997); Steiner, In “Angiogenesis: Key principles-Science, technology and medicine,” pp. 449-454 (eds. Steiner et al.; Birkhauser Verlag, 1992)), which can contribute
30 to its effectiveness in treating cancer.

An alkylating agent such as melphalan or chlorambucil also can be a useful cancer chemotherapeutic agent. Similarly, a vinca alkaloid such as vindesine, vinblastine or vinorelbine; or an antimetabolite such as 5-

fluorouracil, 5-fluorouridine or a derivative thereof can be a useful cancer chemotherapeutic agent.

A platinum agent also can be a useful cancer chemotherapeutic agent. Such a platinum agent can be, for example, cisplatin or carboplatin as described, for example, in Crown, *Seminars in Oncol.* 28:28-37 (2001). Other useful cancer chemotherapeutic agents include, without limitation, methotrexate, mitomycin-C, adriamycin, ifosfamide and ansamycins.

A cancer chemotherapeutic agent useful for treatment of breast cancer and other hormonally-dependent cancers also can be an agent that antagonizes the effect of estrogen, such as a selective estrogen receptor modulator or an anti-estrogen. The selective estrogen receptor modulator, tamoxifen, is a cancer chemotherapeutic agent that can be used in a conjugate for treatment of breast cancer (Fisher et al., *J. Natl. Cancer Instit.* 90:1371-1388 (1998)).

The therapeutic agent can be an antibody such as a humanized monoclonal antibody. As an example, the anti-epidermal growth factor receptor 2 (HER2) antibody, trastuzumab (Herceptin; Genentech, South San Francisco, Calif.) can be a therapeutic agent useful for treating HER2/neu overexpressing breast cancers (White et al., *Annu. Rev. Med.* 52:125-141 (2001)).

Useful therapeutic agents also can be a cytotoxic agent, which, as used herein, can be any molecule that directly or indirectly promotes cell death. Useful cytotoxic agents include, without limitation, small molecules, polypeptides, peptides, peptidomimetics, nucleic acid-molecules, cells and viruses. As non-limiting examples, useful cytotoxic agents include cytotoxic small molecules such as doxorubicin, docetaxel or trastuzumab; antimicrobial peptides such as those described further below; pro-apoptotic polypeptides such as caspases and toxins, for example, caspase-8; diphtheria toxin A chain, *Pseudomonas* exotoxin A, cholera toxin, ligand fusion toxins such as DAB389EGF, ricinus communis toxin (ricin); and cytotoxic cells such as cytotoxic T cells. See, for example, Martin et al., *Cancer Res.* 60:3218-3224 (2000); Kreitman and Pastan, *Blood* 90:252-259 (1997); Allam et al., *Cancer Res.* 57:2615-2618 (1997); and Osborne and Coronado-

Heinsohn, Cancer J. Sci. Am. 2:175 (1996). One skilled in the art understands that these and additional cytotoxic agents described herein or known in the art can be useful in the disclosed conjugates and methods.

In one embodiment, a therapeutic agent can be a therapeutic polypeptide. As used herein, a therapeutic polypeptide can be any polypeptide with a biologically useful function. Useful therapeutic polypeptides encompass, without limitation, cytokines, antibodies, cytotoxic polypeptides; pro-apoptotic polypeptides; and anti-angiogenic polypeptides. As non-limiting examples, useful therapeutic polypeptides can be a cytokine such as tumor necrosis factor- α (TNF- α), tumor necrosis factor- β (TNF- β), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), interferon .alpha. (IFN- α); interferon .gamma. (IFN- γ), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-10 (IL-10), interleukin-12 (IL-12), lymphotactin (LTN) or dendritic cell chemokine 1 (DC-CK1); an anti-HER2 antibody or fragment thereof; a cytotoxic polypeptide including a toxin or caspase, for example, diphtheria toxin A chain, Pseudomonas exotoxin A, cholera toxin, a ligand fusion toxin such as DAB389EGF or ricin; or an anti-angiogenic polypeptide such as angiostatin, endostatin, thrombospondin, platelet factor 4; anastellin; or one of those described further herein or known in the art (see below). It is understood that these and other polypeptides with biological activity can be a “therapeutic polypeptide.”

A therapeutic agent can also be an anti-angiogenic agent. As used herein, the term “anti-angiogenic agent” means a molecule that reduces or prevents angiogenesis, which is the growth and development of blood vessels. A variety of anti-angiogenic agents can be prepared by routine methods. Such anti-angiogenic agents include, without limitation, small molecules; proteins such as dominant negative forms of angiogenic factors, transcription factors and antibodies; peptides; and nucleic acid molecules including ribozymes, antisense oligonucleotides, and nucleic acid molecules encoding, for example, dominant negative forms of angiogenic factors and receptors, transcription factors, and antibodies and antigen-binding fragments

thereof. See, for example, Hagedorn and Bikfalvi, Crit. Rev. Oncol. Hematol. 34:89-110 (2000), and Kirsch et al., J. Neurooncol. 50:149-163 (2000).

Vascular endothelial growth factor (VEGF) has been shown to be
5 important for angiogenesis in many types of cancer, including breast cancer
angiogenesis *in vivo* (Borgstrom et al., Anticancer Res. 19:4213-4214
(1999)). The biological effects of VEGF include stimulation of endothelial
cell proliferation, survival, migration and tube formation, and regulation of
vascular permeability. An anti-angiogenic agent can be, for example, an
10 inhibitor or neutralizing antibody that reduces the expression or signaling of
VEGF or another angiogenic factor, for example, an anti-VEGF neutralizing
monoclonal antibody (Borgstrom et al., supra, 1999). An anti-angiogenic
agent also can inhibit another angiogenic factor such as a member of the
fibroblast growth factor family such as FGF-1 (acidic), FGF-2 (basic), FGF-
15 4 or FGF-5 (Slavin et al., Cell Biol. Int. 19:431-444 (1995); Folkman and
Shing, J. Biol. Chem. 267:10931-10934 (1992)) or an angiogenic factor such
as angiopoietin-1, a factor that signals through the endothelial cell-specific
Tie2 receptor tyrosine kinase (Davis et al., Cell 87:1161-1169 (1996); and
Suri et al., Cell 87:1171-1180 (1996)), or the receptor of one of these
20 angiogenic factors. It is understood that a variety of mechanisms can act to
inhibit activity of an angiogenic factor including, without limitation, direct
inhibition of receptor binding, indirect inhibition by reducing secretion of the
angiogenic factor into the extracellular space, or inhibition of expression,
function or signaling of the angiogenic factor.

25 A variety of other molecules also can function as anti-angiogenic
agents including, without limitation, angiostatin; a kringle peptide of
angiostatin; endostatin; anastellin, heparin-binding fragments of fibronectin;
modified forms of antithrombin; collagenase inhibitors; basement membrane
turnover inhibitors; angiostatic steroids; platelet factor 4 and fragments and
30 peptides thereof; thrombospondin and fragments and peptides thereof; and
doxorubicin (O'Reilly et al., Cell 79:315-328 (1994)); O'Reilly et al., Cell
88:277-285 (1997); Homandberg et al., Am. J. Path. 120:327-332 (1985);
Homandberg et al., Biochim. Biophys. Acta 874:61-71 (1986); and O'Reilly

et al., Science 285:1926-1928 (1999)). Commercially available anti-angiogenic agents include, for example, angiostatin, endostatin, metastatin and 2ME2 (EntreMed; Rockville, Md.); anti-VEGF antibodies such as Avastin (Genentech; South San Francisco, Calif.); and VEGFR-2 inhibitors
5 such as SU5416, a small molecule inhibitor of VEGFR-2 (SUGEN; South San Francisco, Calif.) and SU6668 (SUGEN), a small molecule inhibitor of VEGFR-2, platelet derived growth factor and fibroblast growth factor I receptor. It is understood that these and other anti-angiogenic agents can be prepared by routine methods and are encompassed by the term “anti-
10 angiogenic agent” as used herein.

The homing compositions disclosed herein can also be used to site of inflammation. Moieties useful for this purpose can include therapeutic agents belonging to several basic groups including anti-inflammatory agents which prevent inflammation, restenosis preventing drugs which prevent tissue
15 growth, anti-thrombogenic drugs which inhibit or control formation of thrombus or thrombolytics, and bioactive agents which regulate tissue growth and enhance healing of the tissue. Examples of useful therapeutic agents include but are not limited to steroids, fibronectin, anti-clotting drugs, anti-platelet function drugs, drugs which prevent smooth muscle cell growth
20 on inner surface wall of vessel, heparin, heparin fragments, aspirin, coumadin, tissue plasminogen activator (TPA), urokinase, hirudin, streptokinase, antiproliferatives (methotrexate, cisplatin, fluorouracil, Adriamycin), antioxidants (ascorbic acid, beta carotene, vitamin E), antimetabolites, thromboxane inhibitors, non-steroidal and steroidal anti-
25 inflammatory drugs, beta and calcium channel blockers, genetic materials including DNA and RNA fragments, complete expression genes, antibodies, lymphokines, growth factors, prostaglandins, leukotrienes, laminin, elastin, collagen, and integrins.

Useful therapeutic agents also can be antimicrobial peptides. This can
30 be particularly useful to target a wound or other infected sites. Thus, for example, also disclosed are homing compositions comprising an antimicrobial peptide, where the homing composition is selectively internalized and exhibits a high toxicity to the targeted area. Useful

antimicrobial peptides can have low mammalian cell toxicity when not incorporated into the homing composition. As used herein, the term “antimicrobial peptide” means a naturally occurring or synthetic peptide having antimicrobial activity, which is the ability to kill or slow the growth of one or more microbes. An antimicrobial peptide can, for example, kill or slow the growth of one or more strains of bacteria including a Gram-positive or Gram-negative bacteria, or a fungi or protozoa. Thus, an antimicrobial peptide can have, for example, bacteriostatic or bacteriocidal activity against, for example, one or more strains of *Escherichia coli*, *Pseudomonas aeruginosa* or *Staphylococcus aureus*. While not wishing to be bound by the following, an antimicrobial peptide can have biological activity due to the ability to form ion channels through membrane bilayers as a consequence of self-aggregation.

An antimicrobial peptide is typically highly basic and can have a linear or cyclic structure. As discussed further below, an antimicrobial peptide can have an amphipathic α -helical structure (see U.S. Pat. No. 5,789,542; Javadpour et al., J. Med. Chem. 39:3107-3113 (1996); and Blondelle and Houghten, Biochem. 31: 12688-12694 (1992)). An antimicrobial peptide also can be, for example, a β -strand/sheet-forming peptide as described in Mancheno et al., J. Peptide Res. 51:142-148 (1998).

An antimicrobial peptide can be a naturally occurring or synthetic peptide. Naturally occurring antimicrobial peptides have been isolated from biological sources such as bacteria, insects, amphibians, and mammals and are thought to represent inducible defense proteins that can protect the host organism from bacterial infection. Naturally occurring antimicrobial peptides include the gramicidins, magainins, mellitins, defensins and cecropins (see, for example, Maloy and Kari, Biopolymers 37:105-122 (1995); Alvarez-Bravo et al., Biochem. J. 302:535-538 (1994); Bessalle et al., FEBS 274:-151-155 (1990.); and Blondelle and Houghten in Bristol (Ed.), Annual Reports in Medicinal Chemistry pages 159-168 Academic Press, San Diego). An antimicrobial peptide also can be an analog of a natural peptide, especially one that retains or enhances amphipathicity (see below).

An antimicrobial peptide incorporated into a homing composition can have low mammalian cell toxicity linked to Lyp-1. Mammalian cell toxicity readily can be assessed using routine assays. As an example, mammalian cell toxicity can be assayed by lysis of human erythrocytes *in vitro* as described
5 in Javadpour et al., supra, 1996. An antimicrobial peptide having low mammalian cell toxicity is not lytic to human erythrocytes or requires concentrations of greater than 100 μ M for lytic activity, preferably concentrations greater than 200, 300, 500 or 1000 μ M.

In one embodiment, disclosed are homing compositions in which the
10 antimicrobial peptide portion promotes disruption of mitochondrial membranes when internalized by eukaryotic cells. In particular, such an antimicrobial peptide preferentially disrupts mitochondrial membranes as compared to eukaryotic membranes. Mitochondrial membranes, like
15 bacterial membranes but in contrast to eukaryotic plasma membranes, have a high content of negatively charged phospholipids. An antimicrobial peptide can be assayed for activity in disrupting mitochondrial membranes using, for example, an assay for mitochondrial swelling or another assay well known in the art.

An antimicrobial peptide that induces significant mitochondrial
20 swelling at, for example, 50 μ M, 40 μ M, 30 μ M, 20 μ M, 10 μ M, or less, is considered a peptide that promotes disruption of mitochondrial membranes.

Antimicrobial peptides generally have random coil conformations in dilute aqueous solutions, yet high levels of helicity can be induced by helix-promoting solvents and amphipathic media such as micelles, synthetic
25 bilayers or cell membranes. α -Helical structures are well known in the art, with an ideal α -helix characterized by having 3.6 residues per turn and a translation of 1.5 Å per residue (5.4 Å per turn; see Creighton, *Proteins: Structures and Molecular Properties* W. H Freeman, New York (1984)). In an amphipathic α -helical structure, polar and non-polar amino acid residues
30 are aligned into an amphipathic helix, which is an α -helix in which the hydrophobic amino acid residues are predominantly on one face, with

hydrophilic residues predominantly on the opposite face when the peptide is viewed along the helical axis.

Antimicrobial peptides of widely varying sequence have been isolated, sharing an amphipathic α -helical structure as a common feature
5 (Saberwal et al., *Biochim. Biophys. Acta* 1197:109-131 (1994)). Analogs of native peptides with amino acid substitutions predicted to enhance amphipathicity and helicity typically have increased antimicrobial activity. In general, analogs with increased antimicrobial activity also have increased cytotoxicity against mammalian cells (Maloy et al., *Biopolymers* 37:105-122
10 (1995)).

As used herein in reference to an antimicrobial peptide, the term “amphipathic α -helical structure” means an α -helix with a hydrophilic face containing several polar residues at physiological pH and a hydrophobic face containing nonpolar residues. A polar residue can be, for example, a lysine or
15 arginine residue, while a nonpolar residue can be, for example, a leucine or alanine residue. An antimicrobial peptide having an amphipathic α -helical structure generally has an equivalent number of polar and nonpolar residues within the amphipathic domain and a sufficient number of basic residues to give the peptide an overall positive charge at neutral pH
20 (Saberwal et al., *Biochim. Biophys. Acta* 1197:109-131 (1994)). One skilled in the art understands that helix-promoting amino acids such as leucine and alanine can be advantageously included in an antimicrobial peptide (see, for example, Creighton, *supra*, 1984). Synthetic, antimicrobial peptides having an amphipathic α -helical structure are known in the art, for example, as
25 described in U.S. Pat. No. 5,789,542 to McLaughlin and Becker.

It is understood by one skilled in the art of medicinal oncology that these and other agents are useful therapeutic agents, which can be used separately or together in the disclosed compositions and methods. Thus, it is understood that a homing composition can contain one or more of such
30 therapeutic agents and that additional components can be included as part of the composition, if desired. As a non-limiting example, it can be desirable in some cases to utilize an oligopeptide spacer between Lyp-1 and the

therapeutic agent (Fitzpatrick and Garnett, *Anticancer Drug Des.* 10:1-9 (1995)).

Other useful agents include thrombolytics, aspirin, anticoagulants, painkillers and tranquilizers, beta-blockers, ace-inhibitors, nitrates, rhythm-stabilizing drugs, and diuretics. Agents that limit damage to the heart work best if given within a few hours of the heart attack. Thrombolytic agents that break up blood clots and enable oxygen-rich blood to flow through the blocked artery increase the patient's chance of survival if given as soon as possible after the heart attack. Thrombolytics given within a few hours after a heart attack are the most effective. Injected intravenously, these include anisoylated plasminogen streptokinase activator complex (APSAC) or anistreplase, recombinant tissue-type plasminogen activator (r-tPA), and streptokinase. The disclosed homing compositions can use any of these or similar agents.

In some forms, the drug moiety is a cancer therapeutic. In some forms, the cancer therapeutic is sorafenib. In some forms, the drug moiety is selected from the group consisting of temozolomide, sorafenib, erlotinib, gefitinib, imatinib, pazopanib, rapamycin, raloxifene, lasofoxifene, basedoxifene, resveratrol, curcumin, etoposide, camptothecin, CPT-11, topotecan, irinotecan, exatecan, lurtecan, DB67, BNP1350, ST1481, CKD602, paclitaxel, docetaxel, vincristine, vinblastine, fingolimod, raltegravir, elvitegravir, MK-2408, lersivirine, daunorubicin, doxorubicin, epirubicin, and idarubicin. In some forms, the drug moiety is selected from the group consisting of taxanes, anthracyclines, and camptothecin analogues.

E. Cleavable Bond

Cleavable bonds are included in the prodrug compositions to facilitate separation of the drug moiety from the transport moiety. In some forms, the drug moiety is covalently coupled to the hydrophilic spacer of the transport moiety via the cleavable bond. In some forms, the cleavable bond is a bond selected from the group consisting of: an ester, an amide, and a carbonate. In some forms, the cleavable bond is an internal bond in the hydrophilic spacer. In some forms, the cleavable bond is hydrolyzable.

A cleavable bond can be useful for freeing the drug moiety at the site of targeting, for example. The cleavable bond can be cleaved in any suitable way. For example, the cleavable bond can be cleaved enzymatically or non-enzymatically. For enzymatic cleavage, the cleaving enzyme can be supplied or can be present at a site where the composition is delivered, homes, travels or accumulates. For example, the enzyme can be present inside or in proximity to a cell to which the composition is delivered, homes, travels, or accumulates. For non-enzymatic cleavage, the composition can be brought into contact with a cleaving agent, can be placed in cleaving conditions, or both. A cleaving agent is any substance that can mediate or stimulate cleavage of the cleavable bond. A non-enzymatic cleaving agent is any cleaving agent except enzymes. Cleaving conditions can be any solution or environmental conditions that can mediate or stimulate cleavage of the cleavable bond. For example, some labile bonds can be cleaved in acid conditions, alkaline conditions, in the presence of a reactive group, under reducing conditions, etc. Non-enzymatic cleaving conditions are any cleaving conditions except the presence of enzymes. Non-agent cleaving conditions are any cleaving conditions except the presence of cleaving agents.

A "protease-cleavable bond" refers to a cleavable bond that can be cleaved by a protease. Useful proteases include proteases that may be present at the location where the disclosed compositions are delivered, target, home, etc. Examples of useful proteases include, for example, serine proteases (including, for example, plasmin and pasminogen activators), proprotein convertases (see, for example, Duckert et al., Prediction of proprotein convertase cleavage sites Protein engineering Design and Selection 17(1):107-112 (2004)), furins, and carboxypeptidases. Serine proteases are particularly useful for compositions targeted to cancer cells and tumors. Examples of proteases are also described in Hook, Proteolytic and cellular mechanisms in prohormone and proprotein processing, RG Landes Company, Austin, Texas, USA (1998); Hooper et al., Biochem. J. 321: 265-279 (1997); Werb, Cell 91: 439-442 (1997); Wolfsberg et al., J. Cell Biol. 131: 275-278 (1995); Murakami and Etlinger, Biochem. Biophys. Res.

Comm. 146: 1249-1259 (1987); Berg et al., Biochem. J. 307: 313-326 (1995); Smyth and Trapani, Immunology Today 16: 202-206 (1995); Talanian et al., J. Biol. Chem. 272: 9677-9682 (1997); and Thornberry et al., J. Biol. Chem. 272: 17907-17911 (1997).

5 An "esterase-cleavable bond" refers to a cleavable bond that can be cleaved by a protease. Useful esterases include esterases that may be present at the location where the disclosed compositions are delivered, target, home, etc.

10 Disclosed are materials, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed method and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and
15 permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a hydrophilic spacer is disclosed and discussed and a number of modifications that can be made to a number of molecules including the hydrophilic spacer are discussed, each and every combination and permutation of hydrophilic
20 spacer and the modifications that are possible are specifically contemplated unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited, each is individually and collectively contemplated.
25 Thus, in this example, each of the combinations A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. Likewise, any subset or combination of these is also specifically contemplated and disclosed. Thus, for example, the sub-group of
30 A-E, B-F, and C-E are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. Further, each of the materials, compositions, components, etc. contemplated and disclosed as above can also be specifically and

independently included or excluded from any group, subgroup, list, set, etc. of such materials. These concepts apply to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that
5 can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods, and that each such combination is specifically contemplated and should be considered disclosed.

Methods

10 Disclosed are methods of delivering a drug to a cell, tissue, or organ in a subject by providing to the cell, tissue, or organ a prodrug as disclosed herein. Also disclosed are methods of treating a subject in need thereof by administering to the subject a prodrug as disclosed herein. In some forms, the prodrug is taken into the cell, tissue, or organ by a receptor on the cell, tissue,
15 or organ. For this purpose, the glycol ligand of the prodrug compositions can be chosen based on a receptor present on the cell, tissue, or organ. The general purpose of these method is to deliver the drug moiety into the on the cell, tissue, or organ so that the drug moiety can have an effect on the on the cell, tissue, or organ.

20 Some form of the methods deliver a drug to a liver cell or liver tissue in a subject by providing to the cell or tissue a prodrug having a glycol ligand that can bind a receptor on liver cells or tissue. Also disclosed are methods of treating a subject in need thereof by administering to the subject a prodrug having a glycol ligand that can bind a receptor on liver cells or
25 tissue. In some forms, the prodrug is taken into the cell or tissue by an asialoglycoprotein receptor (ASGP-R). In some forms, the subject suffers from hepatocellular carcinoma (HCC).

The disclosed compositions and methods can be made and tested using techniques suitable to the drug, condition or disease, and glycol ligand
30 used. This can be illustrated with examples of the disclosed prodrug compositions. In particular, the example prodrug compositions are useful to improve both liver specificity and favorable pharmacokinetics of sorafenib. A pro-drug strategy was devised to attach liver-targeting glyco-ligands at the

terminal end of a water-soluble chemical linker and finally attach this linker covalently to sorafenib via pH sensitive hydrazone or disulfide bonds (Figure 1A). β -D- galactose (Gal) or N-acetylgalactosamine (GalNAc) are used as sugar-ligands to target the asialo-glycoprotein (ASGP) receptors on

5 hepatoma cells (Baenziger and Maynard, *J. Biol. Chem.* 1980, 255, 4607-4613, Kim et al., *Nucl. Med. Biol.* 2006, 33, 529-534, Lee et al., *J. Biol. Chem.* 1983, 258, 199-202, Lee et al., *Biochemistry* 1984, 23, 4255-4261, Meier et al., *J. Mol. Biol.* 2000, 300, 857-865, Spiess, *Biochemistry* 1990, 29, 10009-10018). These asialo-glycoprotein receptors (ASGP-R) are high

10 capacity c-type lectin receptors which are exclusively expressed on mammalian hepatocytes compared to other tissues (Zijderhand-Bleekemolen et al., *J. Cell Biol.* 1987, 104, 1647-1654). Furthermore, ASGP-R is expressed in plasma membrane in 80% of well-differentiated HCC samples analyzed by immuno-labeling in a cohort of 60 HCC patients (Trere et al.,

15 *Br. J. Cancer* 1999, 81, 404- 408). Aided by their high hepatocyte expression, specificity in recognition of Gal-terminal ligand, and role in receptor-mediated endocytosis, ASGP receptors can be used as hepatotropic targets for liver uptake of a variety of drugs encapsulated with sugar-decorated nanoparticles, such as liposomes, cyclodextrins and polymer

20 linked oligonucleotides (Slidregt et al., *J. Med. Chem.* 1999, 42, 609-618, Wang et al., *AAPS. PharmSciTech.* 2010, 11, 870-877, Wu and Wu, *Adv. Drug Deliv. Rev.* 1998, 29, 243-248, Wu et al., *Front Biosci.* 2002, 7, d717-d725, Managit et al., *Int. J. Pharm.* 2005, 301, 255-261, Kim et al., *J. Control Release* 2005, 108, 557-567). Unfortunately, such approaches are

25 used primarily in combination of complex polymeric functional groups and multiple sugar residues, which have difficulties in controlling the kinetics of drug exposure at the target tissue. It was discovered that a single galactose ligand is sufficient both for ASGP-R recognition on hepatocytes. This was demonstrated by systemic delivery of a reporter gene to liver using

30 glycolipids containing single galactose head group (Mukthavaram et al., *Biomaterials* 2009, 30, 2369-2384; Lee et al., *J. Am. Chem. Soc.* 2012, 134, 1316-1322).

The disclosed prodrugs use single sugar residues for targeting to glycol receptors. Changing the targeting moiety from complex oligosaccharides containing multiple sugar residues to single sugar moieties reduces the complexity of targeted drug-delivery compositions and makes design and use of prodrugs more streamlined and efficient. As a demonstration, galactose moieties are used as a glycol ligand targeting liver cells via ASGP-R. The combination of this simple liver-targeted glycol ligand with a useful (but low solubility) drug, sorafenib, in a simple solubility-increasing conjugate is an example of a site specific sugar additive prodrug approach to sorafenib to produce more favorable pharmacokinetics of sorafenib (such as, water solubility, reduced toxicity, and improved tumor bioavailability) by specific targeting to liver cancer cells. As shown in Figure 1B, the modified version of the glyco-conjugated sorafenib can enhance liver targeting through ASGP-R mediated endocytosis and thus release higher drug concentration within the liver cancer cells compared to other normal cells via sensitive chemical linkers. This approach to a prodrug conjugate can thus enhance the therapeutic potential of sorafenib. Regarding solubility, the hydrophilic spacer (short chain ethylene glycol in this illustration) can improve the pharmacokinetics of sorafenib.

Unfavorable pharmacokinetics and toxicity profile of sorafenib hampers its optimal performance in treating liver cancer. It was realized the therapeutic potential of sorafenib could be improved by using a site specific sugar additive prodrug approach involving single Gal/GalNAc ligand attached to the terminal end of a short chain PEG-sorafenib conjugate. The resulting glycoconjugated sorafenib analog has (1) enhanced delivery to liver cancer cells through ASGP-R mediated endocytosis, and (2) improved pharmacokinetics by modifying its water solubility, tumor bioavailability and reduced unwanted toxicities. No prior attempt has been made to improve therapeutic potential of sorafenib by taking advantage of liver cell specific expression of sugar receptors (ASGP-R).

A number of synthetic-galactose terminal ligands for attachment to a drug carrier have previously been developed to make use of hepatocyte targeting. Most of these drug carriers contain complex polymeric functional

groups and multiple sugar residues. It was discovered that specific liver targeting of a reporter luciferase can be achieved by using cationic lipid conjugated to a single galactose head group. We have designed and synthesized Based on this discovery, a series of cationic glycolipids conjugated to single sugar (both cyclic-and open sugar head) were designed and synthesized (Mukthavaram et al., *Biomaterials* 2009, 30, 2369-2384). The number of spacer units between sugar and the quarternized nitrogen atom were varied in these conjugates. As shown in Figure 2, glycolipids 3 & 6 (having five and one spacer units, respectively) were found to be equally effective in selective targeting of reporter luciferase construct to mouse livers. It was also discovered that this observed enhanced liver specific reporter gene delivery was mediated via liver specific ASGP-R. The disclosed liver-targeted prodrug compositions a designed to make use of these discoveries and this delivery path. As a first, specific example of such prodrug compositions and drug delivery, a strategy for targeting sorafenib specifically to liver by conjugating single sugar residue to sorafenib was developed. The disclosed sorafenib prodrug strategy, an example of which is shown in Figure 1A, can improve the therapeutic efficacy of sorafenib by changing its pharmacokinetics and liver specificity.

The strategy overcomes limitations associated with sorafenib and can improve the therapeutic effectiveness of other drugs, especially hydrophobic drugs and those with toxicity problems. In the case of the disclosed sorafenib prodrugs, the strategy modifies the pharmacokinetics and liver specificity of sorafenib. In some forms, a pH sensitive hydrazine linker (that could be further linked water soluble liver specific sugar ligands) was covalently attached at specific position of sorafenib (Figure 3B). This location is consistent with the crystal structure of wild type B-Raf-sorafenib complex (PDB: 1UWH; Wan et al., *Cell* 2004, 116, 855-867). Figure 3C shows the example glyco-conjugated sorafenib analogs 1-6. The number of glycoconjugates is double because for each of the six glycoconjugates one form uses galactose (Gal) as the liver-specific ligand and one form uses N-acetyl-galactosamine (GalNAc). For glyconjuates 1-4 a pH sensitive hydrazone linker is used that can covalently couple the transport moiety

(sugar ligand and hydrophilic linker). Glycoconjugates 1 & 2 illustrate an ethylene glycol hydrophobic linker while glycoconjugates 3 & 4 illustrate a hydrophilic spacer made up of ethylene glycol and an acyl spacer. Prodrug designs like that of glycoconjugates 3 & 4 with an acyl component can be used to increase the lifetime of the prodrug under physiological conditions allowing for their efficient accumulation in the target tissues. In glycoconjugates 5 & 6, a disulfide linker is used as a useful alternative to the hydrazone linker. Because the concentration of thiol-disulfide exchange reactions with intracellular reducing molecules, especially glutathione (GSH), in tumor cells (2-8 mM) is much higher than that in blood plasma (1-2 μ M), using a disulfide bond as the cleavable bond allows for selective separation of the drug in tumor cells (Balendiran et al., *Cell Biochem. Funct.* 2004, 22, 343-352). Unlike delivery methods using polymeric nano-scale carriers, the disclosed prodrug compositions, illustrated by glycoconjugates 1-6, can efficiently deliver sorafenib to liver cells through ASGP-R receptor mediated endocytosis, thus increasing therapeutic potential of the drug in treating liver cancers (Figure 1B).

Synthesis of glycoconjugated-sorafenib analogs with hydrazone and disulfide linkers. As examples of prodrug compositions, reliable cleavable bonds, such as hydrazine and disulfide bonds can be used. Such bonds have been widely studied in conventional prodrug cancer therapeutics to improve efficacy and reducing side effects of conventional chemotherapeutic drugs (Yuan et al., *Langmuir* 2013, 29, 734-743, Rodrigues et al., *Bioorg. Med. Chem.* 1999, 7, 2517-2524, Patil et al., *Int. J. Mol. Sci.* 2012, 13, 11681-11693). In fact, hydrazone has thus far made it into clinical application in an FDA-approved drug to ketone containing antibody (Hamann et al., *Bioconjug. Chem.* 2002, 13, 47-58, Senter, *Curr. Opin. Chem. Biol.* 2009, 13, 235-244).

The example hepatocyte-targeting sorafenib analogs (glycoconjugates 1-6) can be prepared in accord with the synthetic route outlined in schemes 1-3 (Figures 4-8). The hydrazone linker glycoconjugates 1-4 can be synthesized as outlined in schemes 1 & 2 (Figures 4-7). The intermediate compounds 7, 8 and 9 can be synthesized by adapting procedures reported

previously (Babic et al., *Molecules*. 2012, 17, 1124-1137). Briefly, the starting material picolinic acid is first converted to the acid chloride using the thionyl chloride method to yield the desired product 7, amidation of the acid chloride 7 with methyl amine and triethyl amine under room temperature will yields amide of 8, Then 8 will couple with 4-aminophenol to yield ether of 9 using potassium tert-butoxide in the presence of potassium carbonate. The intermediate compound 9 is conjugated to pre-synthesized hydrazide linker 10 (as shown in scheme 1b (Figure 5): briefly, the O-glycosylation of 10 was performed through oxazoline or acetal intermediate as the glycosyl donor, to favour the stereo selective formation of the β -anomer, then coupled to *tert*-Butyl carbazate in the presence of reacted triphosgene, followed by removal of t-Bu group with TFA in the presence of activated molecular sieves in the dark forming stable hydrazone bond 11, which was readily reacted with isocyanate followed by deprotection acetyl groups with NaOCH₃ in methanol to yield final glycoconjugates 1 & 2 (scheme 1; Figure 4).

Similar reaction conditions can be applied for synthesis of glycoconjugates 3 & 4 (scheme 2; Figure 6) except the different hydrazide linker 14 would be used, as shown in the scheme 2b (Figure 7). The acyl hydrazide linkers 18 can be synthesized by adopting the procedures reported previously (Kale and Torchilin, *Bioconjug. Chem.* 2007, 18, 363-370). Briefly, the O-glycosylation thiol intermediate 17 can be mixed with excess of acyl hydrazide linker 18 in anhydrous methanol containing triethyl amine to get the desired hydrazide linker 14.

The disulfide linker glycoconjugates 5 & 6 can be synthesized as outlined in scheme 3 (Figure 8). Briefly, the intermediate 8 can be treated with triphosgene, followed by treatment with 2,2'-dithioethanol, to afford 19, which can then be reacted with O-glycosyl terminated chlorine 16 in the presence of NaH to yield intermediate 20, which can be coupled with 4-aminophenol to yield the ether of 21 using potassium tert-butoxide in the presence of potassium carbonate. The intermediate 21 can be readily reacted with isocyanate followed by deprotection acetyl groups with NaOCH₃ in methanol to yield final the glycoconjugates 5 & 6.

In general, for the production of the disclosed prodrug compositions, the intermediates and final compounds can be characterized with standard analytical techniques (NMR, Mass spectrometry). The final purity of the compounds can also be confirmed by CHN elemental analysis. Other
5 standard or useful characterization techniques can also be used. The disclosed prodrugs can be made using suitable synthetic techniques. Alternative synthetic techniques to those illustrated for the example glycoconjugates can be used.

The efficiency and kinetics of drug separation from the prodrug
10 composition can be assessed using suitable techniques, which can be tailored to the particular drug, cleavable bond, and cell or tissue target. In general, one aspect of therapeutic effectiveness is based on the amount of the drug released from the prodrug composition. For example, the cleavage kinetics of the cleavable bonds (e.g., hydrazone and disulfide bonds) used between the
15 drug and the transport moiety can be assessed and optimized for the specific environmental conditions within cancer cells. For example, free drug release from the prodrug compositions can be assessed by using in vitro conditions similar to the expected in vivo conditions.

The hydrazone linker in glycoconjugates 1-4 is considered an acidic
20 pH sensitive group, which is known to be activated and cleaved at pH 5 at mature endosome (Figure 9) and ideally should not be released at physiological pH 7.4 during systemic delivery (Rodrigues et al., *Bioorg. Med. Chem.* 1999, 7, 2517-2524, Patil et al., *Int. J. Mol. Sci.* 2012, 13, 11681-11693). The disulfide bonds in glycoconjugates 5 & 6 are stable in the
25 mildly oxidizing extracellular milieu, may be prone to rapid cleavage through thiol-disulfide exchange reactions with intracellular reducing molecules, especially with glutathione (GSH) (Raina and Missiakas, *Annu. Rev. Microbiol.* 1997, 51, 179-202, Gilbert, *Methods Enzymol.* 1995, 251, 8-28). Assessment of the kinetics of the cleavable bonds used can be using any
30 suitable techniques. Examples include the dialysis method of Opanasopit et al. (*J. Control Release* 2005, 104, 313-321) as well as HPLC or LC-MS. Released drug can be measured by isolating the free drug after reaction and measure the percentage of free drug by using, for example, HPLC-UV or

LC-MS. Examples of such procedures are described by Blanchet et al. (*J. Pharm. Biomed. Anal.* 2009, 49, 1109-1114) and Bobin-Dubigeon et al. (*Ther. Drug Monit.* 2011, 33, 705-710). Similarly, the pH dependence of the thiol-mediated disulfide bond cleavage can be assessed, for example, in the presence of 5 mM of GSH at different time as per the procedure of Lee et al. (*J. Am. Chem. Soc.* 2012, 134, 1316-1322).

The disclosed prodrug compositions can improve one or more of the components of the absorption, distribution, metabolism, and excretion (ADME) profile for the drug involved. Assessment of ADME is well known and can be applied to the disclosed prodrug compositions. For example, solubility, Caco2 permeability, plasma protein binding, and hERG toxicity can be assessed for the prodrug compositions.

The effectiveness of the particular drug on its target can be assessed using materials and techniques appropriate to the drug, disease or condition, and the cells or tissue affected. For example, the molecular mechanisms of sorafenib mediated cytotoxic/anti-proliferative activity can be assessed using sorafenib prodrug compositions and, for example, human HCC cell lines (such as HepG2 and Huh7). Useful control cells for this include HeLa cell line (null for ASGP-R expression) and a normal mouse myoblast cell line (such as C2C12). Techniques for use of these cell lines are known (see, e.g., Mukthavaram et al., *Biomaterials* 2009, 30, 2369-2384, Mahidhar et al., *J. Med. Chem.* 2004, 47, 3938- 3948, and Mahidhar et al., *J. Med. Chem.* 2004, 47, 5721-5728).

Another measure of the effectiveness of the disclosed prodrug conjugates is the kinetics of uptake by target cells. For example, ASGP-R endocytosis (and the receptor bound drug thereof) can be assessed for prodrug compositions that use galactose as the glycol ligand using cell lines without or with ASGP-R expression. For example, a prodrug composition to be tested can be labeled, such as with fluorescent label such as dansyl. This fluorescence is useful in tracking the prodrug compositions inside or outside the cells in a bound or unbound form with ASGP-R (Figure 11B). The disclosed prodrugs as well as the parent drug can be dansylated. For example, dansylation of sorafenib prodrug by amination reaction between

dansyl ethylene di-amine and glyco-conjugate in the presence of base is shown in Figure 11A. ASGP-R endocytosis can be followed via fluorescent labeling of ASGP-R, such as via CY5-conjugated secondary antibodies (excitation/emission:649/666nm) bound to anti-ASGP-R primary antibodies.

- 5 The endocytosis, co-localization of fluorescence from the labeled prodrug compositions and from ASGP-R immunofluorescence can be tracked, for example, by video lapse microscopy on a real time basis. ASGP-R positive HCC cell lines can be used for these studies involving prodrugs for HCC, with HeLa (null for ASGP-R) as negative control. A higher endocytosis, co-
10 localization of fluorescence should be observed from prodrug compositions than from the parent drug.

- Uptake by target cells can also be assessed using engineered cells and cell lines. For example, ASGP-R-mediated uptake can be assessed by knocking down ASGP-R protein from positive HCC cell lines and
15 overexpressing ASGP-R protein in negative HeLa cells. A reverse observation of inhibited endocytosis in HCC cells and positive higher endocytosis in HeLa cells of glycoderivatives is expected. Engineering can be accomplished using any suitable method. For example, over and underexpression of protein can be accomplished, for example, using a
20 lentiviral delivery of cDNA and shRNA, respectively. Further confirmation of receptor-mediated uptake can be assessed by using an inhibitor of the receptor. For example, okadaic acid, an inhibitor of ASGP-R endocytosis, can be used for assessing ASGP-R-mediated uptake.

- The biodistribution of the drug in the subject and the concentration of
25 the drug in target cells can be assessed using any suitable method. For example, the effect of a prodrug composition as compared to the parent drug can be assessed by the effect of these on a suitable cell culture. For example, cytotoxicity of selected sorafenib prodrug compositions and parent sorafenib, as reflected by inhibited cell growth, can be determined by WST-1 based
30 viability testing in human hepatoma cell lines.

For sorafenib prodrug compositions, cell viability assays can be performed in HCC cell lines and normal mouse myoblast cell line according to published procedure (Yenugonda et al., *Bioorg. Med. Chem.* 2011, 19,

2714-2725, Yenugonda et al., *Cancer Biol. Ther.* 2012, 13, 925-934).

Briefly, cell survival can be estimated 24 and 48 h after the addition of sorafenib prodrug and sorafenib alone using WST-1 reduction assay. The IC50 value (the concentration yielding 50% growth inhibition) can be
5 interpolated from the graph of the log of compound concentration versus the fraction of surviving cells.

The effectiveness of the drug can also be assessed. For example, the ability of sorafenib prodrug compositions to inhibit activities of select target proteins of sorafenib can be assessed. Sorafenib is the first oral multi-kinase
10 inhibitor that blocks several receptor tyrosine kinase signaling (such as VEGFR, PDGFR, c-Kit and RET) and inhibits downstream (Ras/Raf/MEK/ERK) signaling cascade activity to prevent tumor growth by exerting its anti-angiogenic, antiproliferative and or pro-apoptotic effects (Cervello et al., *Cell Cycle* 2012, 11, 2843-2855, Wilhelm et al., *Mol. Cancer*
15 *Ther.* 2008, 7, 3129-3140). Measurement of activities of these signaling proteins can be used as a direct measure of the activity of the sorafenib prodrug compositions. As an example such measurements can be made by comparing the activities of mentioned signaling proteins in lysates developed from cells treated with control parent sorafenib or sorafenib prodrug
20 compositions using activity specific phospho-antibodies in immunoblot will be compared head to head for. Suitable phosphor-antibodies are commercially available from Cell Signaling Technology, Inc. A higher magnitude of inhibition of these signaling proteins by glyco-conjugate is expected.

25 Assays can be set up in multiplet (n values noted for each experiment, minimum n=3) and the results can be expressed, for example, as means $\pm$ SEM. Statistical analysis and P value determination can be done by two tailed paired t-test with a confidence interval of 95% for determination of the significance differences between treatment groups and are calculated using
30 Sigma Plot software. $P < 0.05$ is considered to be significant.

A. Administration

The disclosed prodrug compositions can be formulated and administered in any suitable manner. Generally, the formulation will depend on the route of administration.

5 The dosages or amounts of the prodrug compositions described herein are large enough to produce the desired effect in the method by which delivery occurs. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent
10 of the disease in the subject and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician based on the clinical condition of the subject involved. The dose, schedule of doses and route of administration can be varied.

 The efficacy of administration of a particular dose of the prodrug
15 compositions according to the methods described herein can be determined by evaluating the particular aspects of the medical history, signs, symptoms, and objective laboratory tests that are known to be useful in evaluating the status of a subject for the treatment of HCC or other diseases and/or conditions. These signs, symptoms, and objective laboratory tests will vary,
20 depending upon the particular disease or condition being treated or prevented, as will be known to any clinician who treats such patients or a researcher conducting experimentation in this field. For example, if, based on a comparison with an appropriate control group and/or knowledge of the normal progression of the disease in the general population or the particular
25 individual: (1) a subject's physical condition is shown to be improved (e.g., a tumor has partially or fully regressed), (2) the progression of the disease or condition is shown to be stabilized, or slowed, or reversed, or (3) the need for other medications for treating the disease or condition is lessened or obviated, then a particular treatment regimen will be considered efficacious.

30 By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material can be administered to a subject along with the selected drug moiety, compound, or prodrug composition without causing any undesirable biological effects or interacting

in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

Any of the prodrug compositions can be used therapeutically in combination with a pharmaceutically acceptable carrier. The prodrug compositions described herein can be conveniently formulated into pharmaceutical compositions composed of one or more of the prodrug compositions in association with a pharmaceutically acceptable carrier. See, e.g., *Remington's Pharmaceutical Sciences*, latest edition, by E.W. Martin Mack Pub. Co., Easton, PA, which discloses typical carriers and conventional methods of preparing pharmaceutical compositions that can be used in conjunction with the preparation of formulations of the prodrug compositions described herein and which is incorporated by reference herein. These most typically would be standard carriers for administration of compositions to humans. In one aspect, humans and non-humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH. Other prodrug compositions will be administered according to standard procedures used by those skilled in the art.

The pharmaceutical compositions described herein can include, but are not limited to, carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions can also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

The prodrug compositions and pharmaceutical compositions described herein can be administered to the subject in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Thus, for example, a prodrug composition or pharmaceutical composition described herein can be administered as an ophthalmic solution and/or ointment to the surface of the eye. Moreover, a prodrug composition or pharmaceutical composition can be administered to a subject vaginally, rectally, intranasally, orally, by inhalation, or parenterally, for example, by intradermal, subcutaneous, intramuscular, intraperitoneal, intrarectal, intraarterial, intralymphatic, intravenous, intrathecal and intratracheal routes.

Parenteral administration, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions which can also contain buffers, diluents and other suitable additives. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives can also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Formulations for topical administration can include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable.

Compositions for oral administration can include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders can be desirable.

It is understood that the disclosed method and compositions are not limited to the particular methodology, protocols, and reagents described as these may 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 limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a ", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a prodrug composition" includes a plurality of such prodrug compositions, reference to "the prodrug composition" is a reference to one or more prodrug compositions and equivalents thereof known to those skilled in the art, and so forth.

Throughout the description and claims of this specification, the word "comprise" and variations of the word, such as "comprising" and "comprises," means "including but not limited to," and is not intended to exclude, for example, other additives, components, integers or steps.

"Optional" or "optionally" means that the subsequently described event, circumstance, or material may or may not occur or be present, and that the description includes instances where the event, circumstance, or material occurs or is present and instances where it does not occur or is not present.

Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, also specifically contemplated and considered disclosed is the range from the one particular value and/or to the other particular value unless the context specifically indicates otherwise. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another, specifically contemplated embodiment that should be considered disclosed unless the context specifically indicates otherwise. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint unless the context specifically indicates otherwise. Finally, it should be understood that all of the individual values and sub-ranges of values contained within an explicitly disclosed range are also specifically contemplated and should be considered disclosed unless the context specifically indicates otherwise. The foregoing

applies regardless of whether in particular cases some or all of these embodiments are explicitly disclosed.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed method and compositions belong. Although
5 any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present method and compositions, the particularly useful methods, devices, and materials are as described. Publications cited herein and the material for which they are cited
10 are hereby specifically incorporated by reference. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such disclosure by virtue of prior invention. No admission is made that any reference constitutes prior art. The discussion of references states what their authors assert, and applicants reserve the right to challenge the
15 accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of publications are referred to herein, such reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

Although the description of materials, compositions, components, steps, techniques, etc. may include numerous options and alternatives, this
20 should not be construed as, and is not an admission that, such options and alternatives are equivalent to each other or, in particular, are obvious alternatives. Thus, for example, a list of different drug moieties does not indicate that the listed drug moieties are obvious one to the other, nor is it an
25 admission of equivalence or obviousness.

Every prodrug composition disclosed herein is intended to be and should be considered to be specifically disclosed herein. Further, every component or part that can be identified within this disclosure is intended to be and should be considered to be specifically disclosed herein. As a result, it
30 is specifically contemplated that any prodrug composition, or component of prodrug compositions can be either specifically included for or excluded from use or included in or excluded from a list of prodrug compositions.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the method and compositions described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

We claim:

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one β -D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety.
2. The prodrug of claim 1, wherein the hydrophilic spacer is selected from the group consisting of: a substituted branched or unbranched saturated or unsaturated alkyl chain, a substituted or unsubstituted branched or unbranched saturated or unsaturated alkoxy chain, a substituted or unsubstituted branched or unbranched saturated or unsaturated alkylamino chain, and a substituted or unsubstituted branched or unbranched saturated or unsaturated alkylthio chain.
3. The prodrug of claim 2, wherein the substituted saturated alkyl chain or the substituted unsaturated alkyl chain comprises an atom or atom group selected from the group consisting of S, O, N, and C=O.
4. The prodrug of any one of claims 1-3, wherein the hydrophilic spacer comprises ethylene glycol.
5. The prodrug of any one of claims 1-4, wherein the drug moiety is covalently coupled to the hydrophilic spacer of the transport moiety via the cleavable bond, wherein the cleavable bond is a bond selected from the group consisting of: an ester, an amide, and a carbonate.
6. The prodrug of any one of claims 1-4, wherein the cleavable bond is an internal bond in the hydrophilic spacer.
7. The prodrug of any one of claims 1-6, wherein the cleavable bond is hydrolyzable.

8. The prodrug of any one of claims 1-7, wherein the hydrophilic spacer is of a length selected from the group consisting of 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4 or 3 atoms.

9. The prodrug of any one of claims 1-8, wherein the drug moiety is a cancer therapeutic.

10. The prodrug of claim 9, wherein the cancer therapeutic is sorafenib.

11. The prodrug of any one of claims 1-8, wherein the drug moiety is selected from the group consisting of temozolomide, sorafenib, erlotinib, gefitinib, imatinib, pazopanib, rapamycin, raloxifene, lasofoxifene, basedoxifene, resveratrol, curcumin, etoposide, camptothecin, CPT-11, topotecan, irinotecan, exatecan, lurtecan, DB67, BNP1350, ST1481, CKD602, paclitaxel, docetaxel, vincristine, vinblastine, fingolimod, raltegravir, elvitegravir, MK-2408, lersivirine, daunorubicin, doxorubicin, epirubicin, and idarubicin.

12. The prodrug of any one of claims 1-9 wherein the drug moiety is selected from the group consisting of taxanes, anthracyclines, and camptothecin analogues.

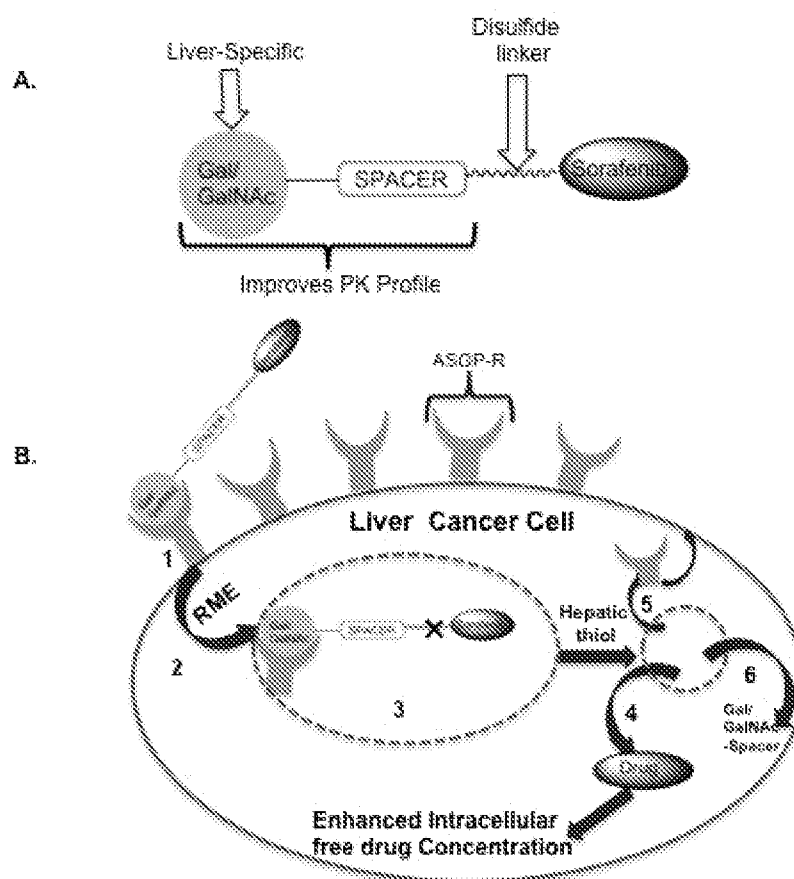
13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.

14. The method of claim 13, wherein the prodrug is taken into the cell or tissue by a asialo-glycoprotein receptor (ASGP-R).

15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

16. The method of any one of claims 13-15, wherein the subject suffers from hepatocellular carcinoma (HCC).

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FIGS. 1A AND 1B

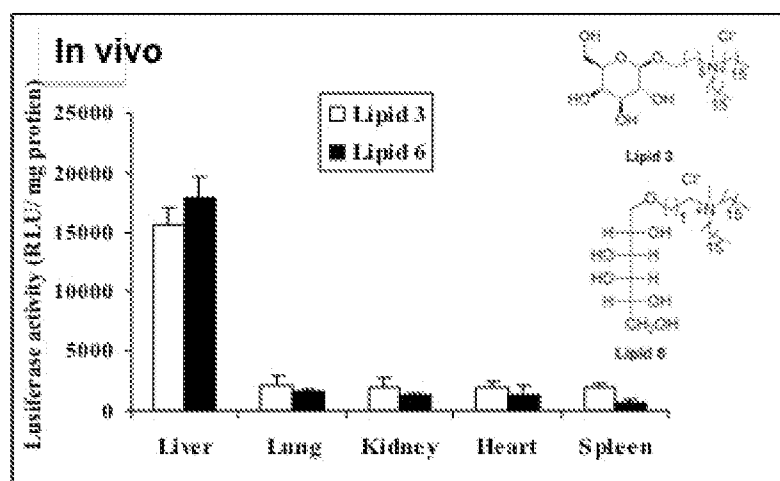
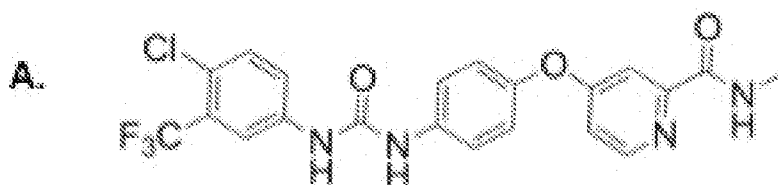


FIG. 2

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Sorafenib

Chemical Formula = $C_{21}H_{16}ClF_3N_4O_3$

Molecular weight = 464.82; log P= 3.8; PSA=92.35

Aqueous Solubility= poorly soluble (free base)

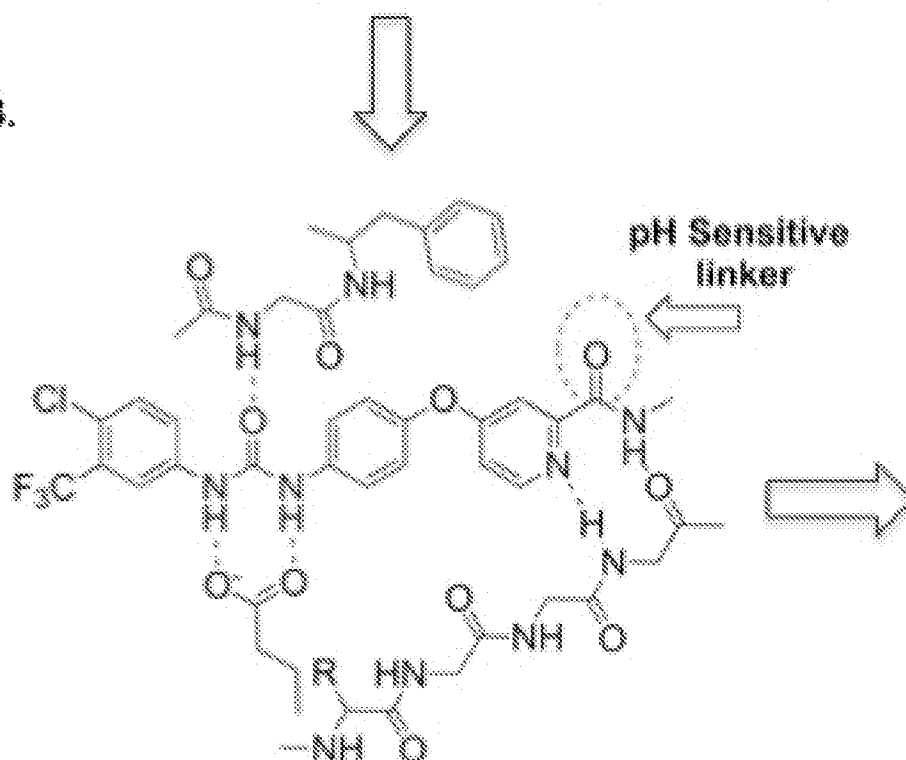
Practically Insoluble (as tosylate salt)

Protein binding = 99.5%

Oral Bioavailability (tablet, Tosylate form) = 38-49%,

Oral Bioavailability (with a high fat meal) = reduced by 29%

B.



FIGS. 3A AND 3B

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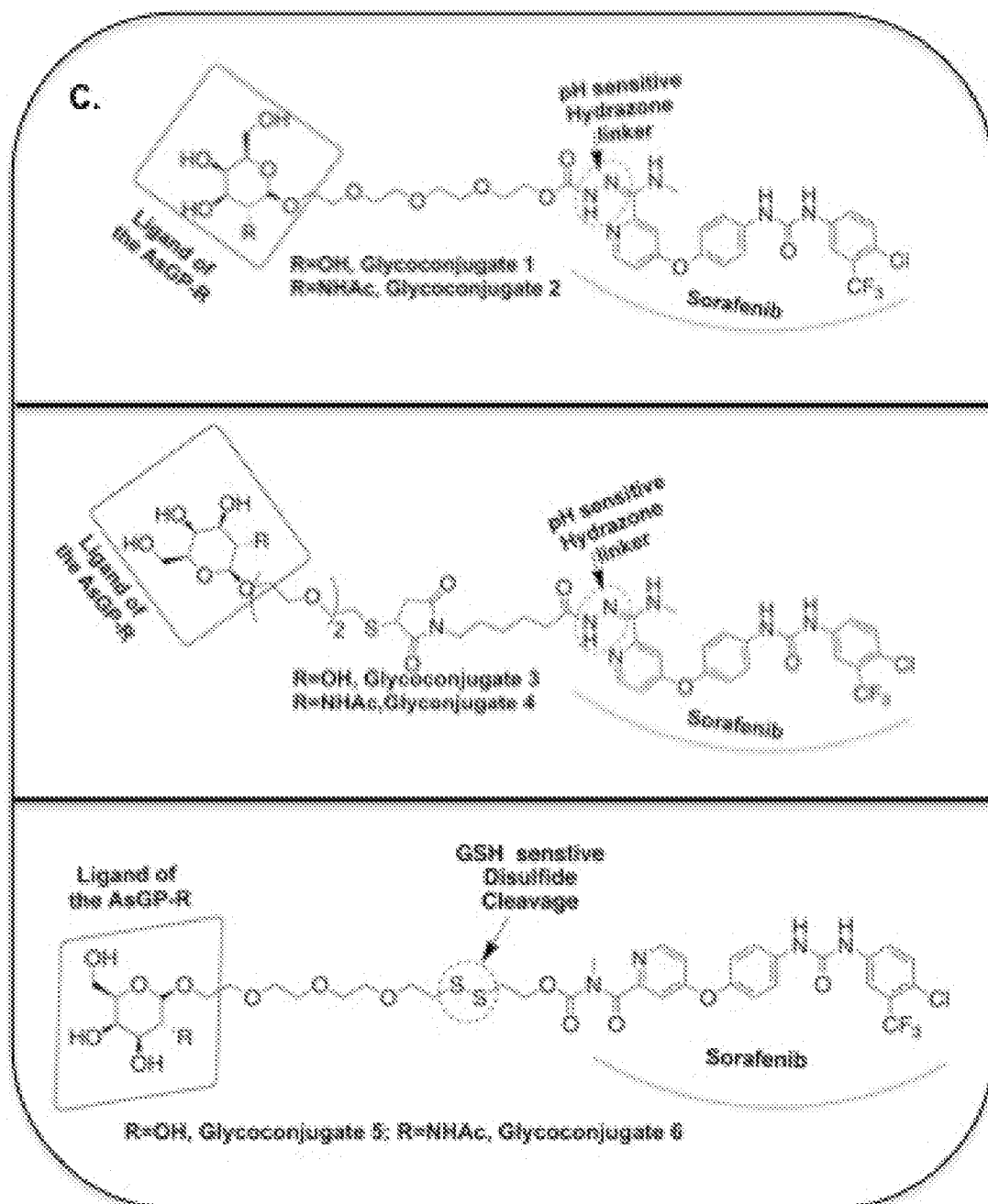


FIG. 3C

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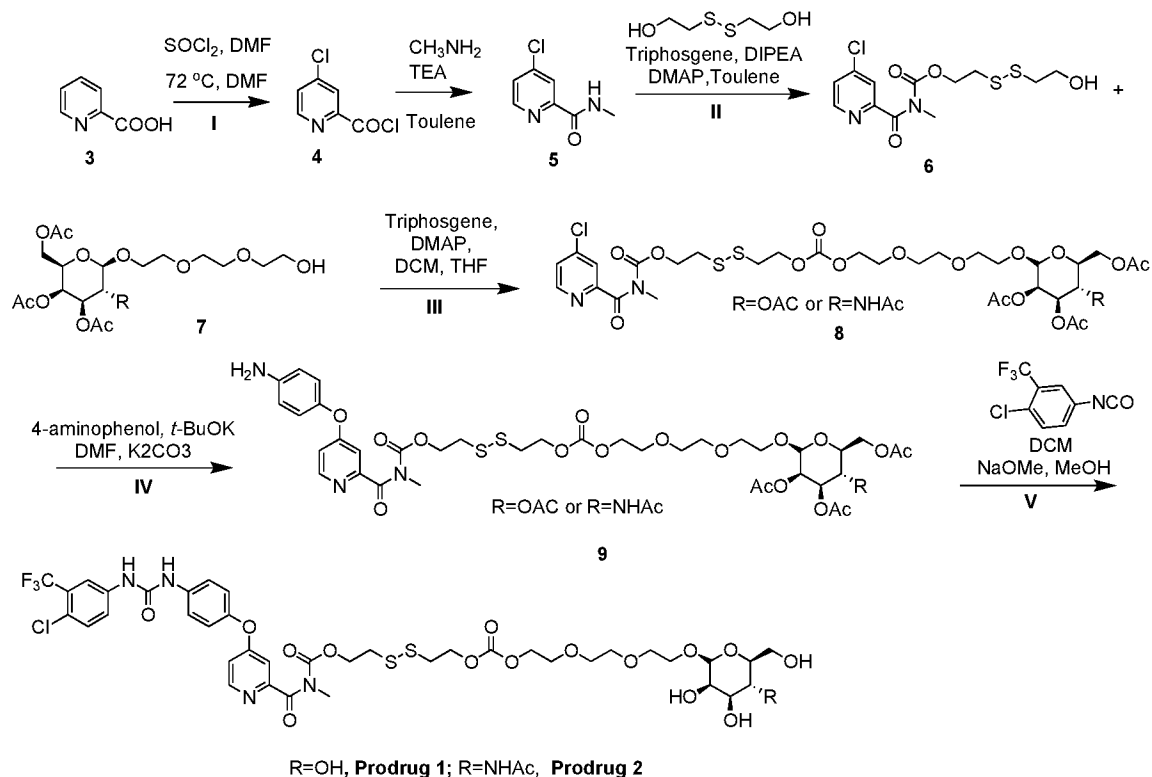


FIG. 4

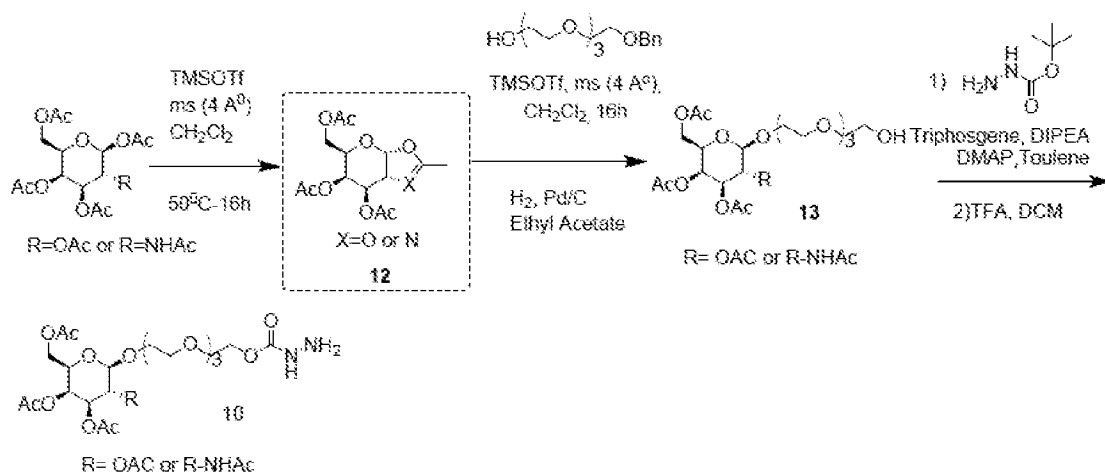


FIG. 5

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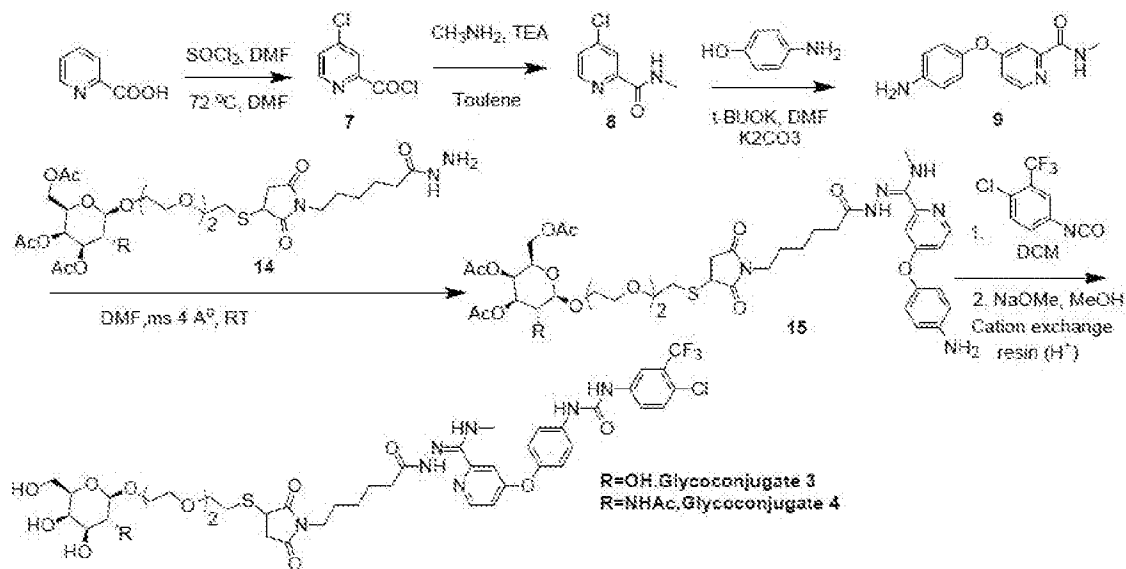


FIG. 6

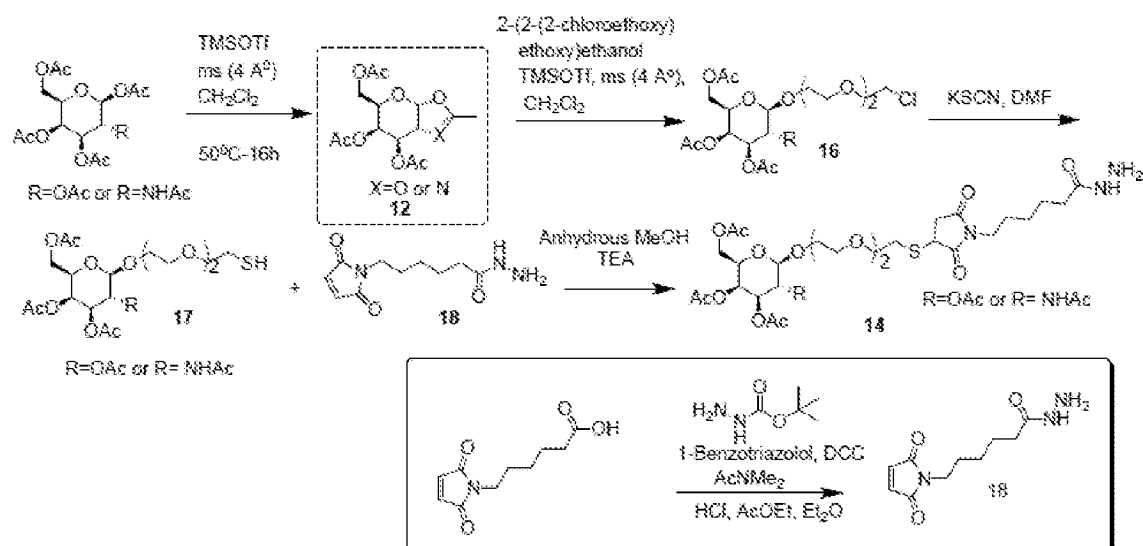


FIG. 7

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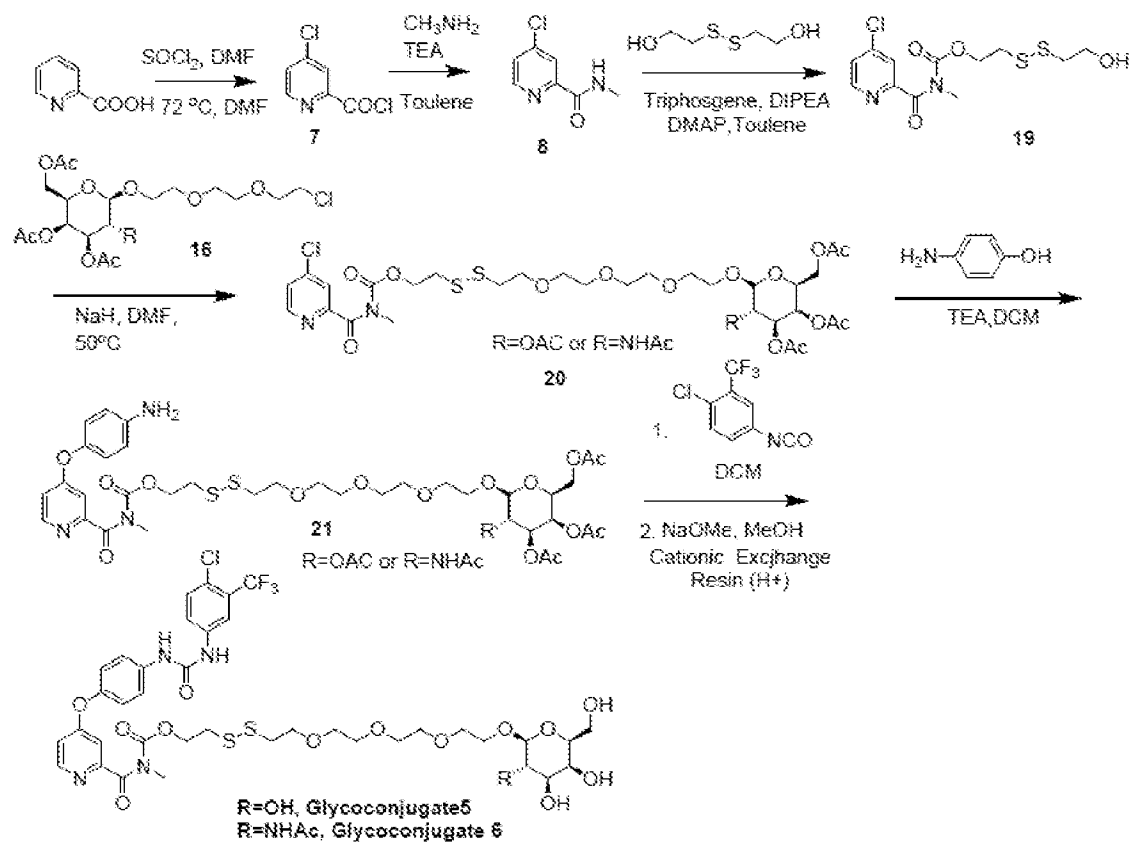


FIG. 8

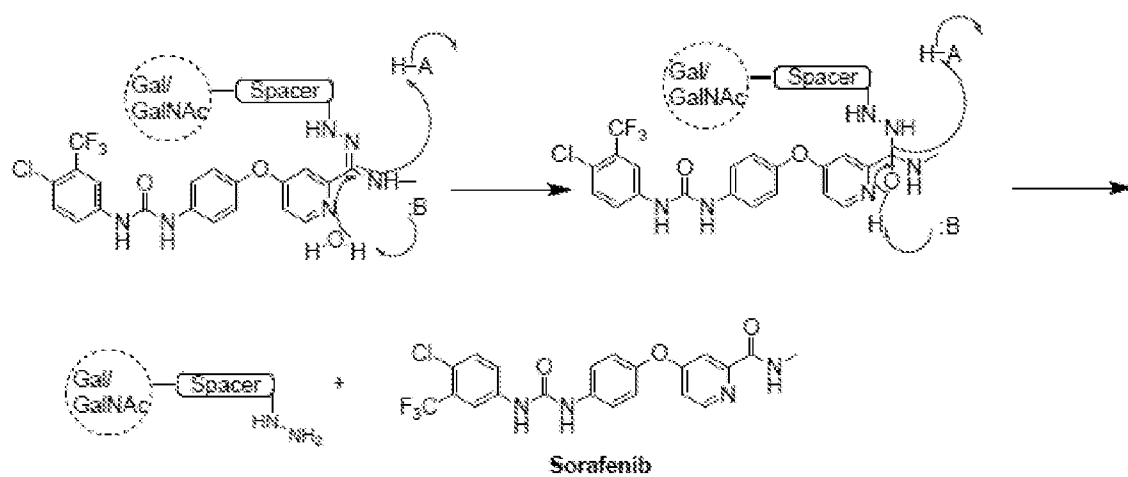


FIG. 9

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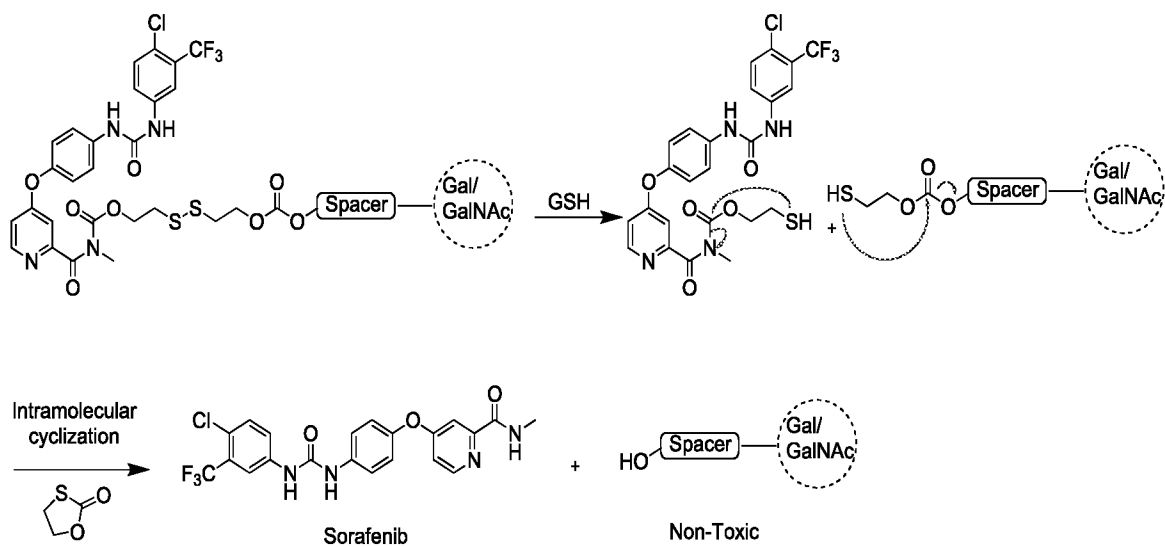


FIG. 10

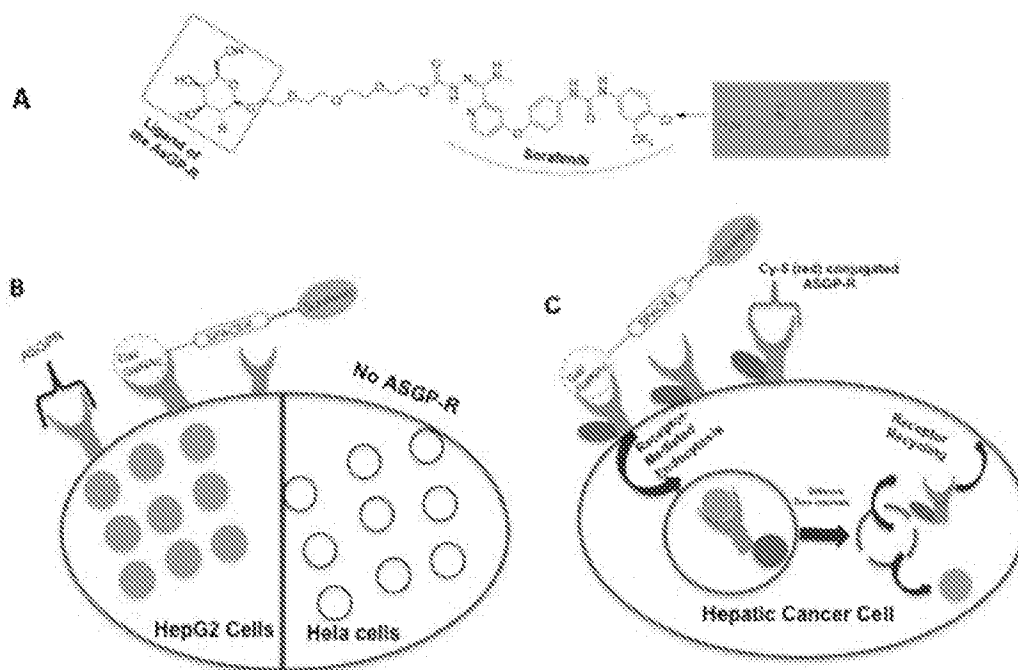


FIG. 11

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/064216

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K47/54 A61P35/00
ADD.

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)

A61K

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)

EPO-Internal, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2015/113922 A1 (ROCHE INNOVATION CT COPENHAGEN AS [DK]) 6 August 2015 (2015-08-06) pages 70-71; claims 3, 7; figure 10; example 14 ----- -/--	1

☒ 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

20 March 2017

Date of mailing of the international search report

23/05/2017

Name and mailing address of the ISA/

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Authorized officer

Langer, Miren

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/064216

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RUI YANG ET AL: "Galactose-Decorated Cross-Linked Biodegradable Poly(ethylene glycol)- b -poly([epsilon]-caprolactone) Block Copolymer Micelles for Enhanced Hepatoma-Targeting Delivery of Paclitaxel", BIOMACROMOLECULES, vol. 12, no. 8, 8 August 2011 (2011-08-08) , pages 3047-3055, XP055349843, US ISSN: 1525-7797, DOI: 10.1021/bm2006856 abstract page 3048; figure 1 page 3047, column 2	1
A	JIN HUANG ET AL: "Liver-targeting doxorubicin-conjugated polymeric prodrug with pH-triggered drug release profile", POLYMER INTERNATIONAL, vol. 59, no. 10, 18 October 2010 (2010-10-18), pages 1390-1396, XP055349693, GB ISSN: 0959-8103, DOI: 10.1002/pi.2880 abstract	1
A	CHRISTIAN K. ADOKOH ET AL: "Synthesis and Evaluation of Glycopolymers Decorated Gold Nanoparticles Functionalized with Gold-Triphenyl Phosphine as Anti-Cancer Agents", BIOMACROMOLECULES, vol. 15, no. 10, 13 October 2014 (2014-10-13), pages 3802-3810, XP055312527, US ISSN: 1525-7797, DOI: 10.1021/bm5010977 page 3803; figure 1	1
X	EMANUELA F. CRAPARO ET AL: "Galactosylated polymeric carriers for liver targeting of sorafenib", INTERNATIONAL JOURNAL OF PHARMACEUTICS, vol. 466, no. 1-2, 4 March 2014 (2014-03-04), pages 172-180, XP055349685, AMSTERDAM, NL ISSN: 0378-5173, DOI: 10.1016/j.ijpharm.2014.02.047 page 176, column 1, paragraph 1	1-11, 13-16
A	WO 2015/038649 A1 (SYNTA PHARMACEUTICALS CORP [US]) 19 March 2015 (2015-03-19) paragraph [1977] - paragraph [1982]	1-11, 13-16
	-/--	

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/064216

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MARIA LUISA BONDÌ ET AL: "Lipid nanocarriers containing sorafenib inhibit colonies formation in human hepatocarcinoma cells", INTERNATIONAL JOURNAL OF PHARMACEUTICS, vol. 493, no. 1-2, 1 September 2015 (2015-09-01), pages 75-85, XP055350212, AMSTERDAM, NL ISSN: 0378-5173, DOI: 10.1016/j.ijpharm.2015.07.055 -----	1-11, 13-16
E	WO 2017/017063 A2 (MIDATECH LTD [GB]) 2 February 2017 (2017-02-02) claims 1, 2, 18 -----	1-11, 13-16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/064216

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.:
because they relate to subject matter not required to be searched by this Authority, namely:
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:

see additional sheet

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 fees.
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.:

10(completely); 1-9, 11, 13-16(partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 10(completely); 1-9, 11, 13-16(partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is sorafenib.
13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.
15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

2. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is temozolomide.
13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.
15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

3. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is erlotinib .
 13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.
 15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

4. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is gefitinib .
 13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.
 15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

5. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is imatinib .
 13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.
 15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

6. claims: 1-9, 11, 13-16(all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is pazopanib .
13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.
15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

7. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is rapamycin .
13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.
15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

8. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is raloxifene, lasofoxifene, basedoxifene.
13. A method of delivering a drug to a liver cell or liver

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.

15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

9. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is resveratrol .

13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.

15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

10. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is curcumin .

13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.

15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

11. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is etoposide.

13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.

15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

12. claims: 1-9, 11-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is camptothecin, CPT-11, topotecan, irinotecan, exatecan, lurtecan, DB67, BNP1350, ST1481, CKD602 or other camptothecin analogues .

13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.

15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

13. claims: 1-9, 11-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is paclitaxel, docetaxel or other taxanes .

13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

14. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is vincristine or vinblastine.

13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.

15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

15. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is fingolimod

13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.

15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

16. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is raltegravir or elvitegravir

13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.

15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

17. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is MK-2408

13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.

15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

18. claims: 1-9, 11, 13-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is lersivirine.

13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.

15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

19. claims: 1-9, 11-16(all partially)

1. A prodrug comprising a drug moiety and a transport moiety covalently coupled to the drug moiety, wherein the transport moiety comprises a hydrophilic spacer covalently coupled to the drug moiety, and at least one liver-targeted glycol ligand covalently coupled to the hydrophilic spacer, wherein the hydrophilic spacer comprises a cleavable bond, wherein cleavage of the cleavable bond separates the drug moiety from the transport moiety, wherein the at least one of the liver-targeted glycol ligand comprises at least one (3-D-galactose (Gal) or N-acetylgalactoseamine (GalNAc) moiety wherein the drug moiety is daunorubicin, doxorubicin, epirubicin, idarubicin or another anthracycline.

13. A method of delivering a drug to a liver cell or liver tissue in a subject, comprising providing to the cell or tissue a prodrug of any one of claim 1-12.

15. A method of treating a subject in need thereof, comprising administering to the subject the prodrug of any one of claims 1-12.

INTERNATIONAL SEARCH REPORT

Information on patent family members

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