**ABSTRACT**

Provided herein are compounds, compositions and methods for the treatment of liver disorders, including liver cancer and metabolic diseases, such as diabetes, hyperlipidemia, atherosclerosis, and obesity. Specifically, compounds and compositions of nucleoside derivatives are disclosed, which can be administered either alone or in combination with other anticancer agents.

**Metabolic Stability of B184, (Ex. 3)**

**Monkey Liver S9 (cytosol+microsomes) Fraction**

![Graph showing metabolic stability](image)
Metabolic Stability of B184, (Ex. 3)

Monkey Liver S9 (cytosol+microsomes) Fraction

% Parent Remaining

Incubation Time (min)

FIG. 1
Metabolic Stability of
(B102 (Ex. 2))

Monkey Liver S9 (cytosol+microsomes) Fraction

% Parent Remaining vs Incubation Time (min)

FIG. 2
COMPOUNDS AND PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF LIVER DISORDERS

1. CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims the benefit of priority under 35 U.S.C. § 119 to (1) U.S. Provisional Appl. No. 60/877,944, filed Dec. 28, 2006; (2) U.S. Provisional Appl. No. 60/956,290, filed Jun. 18, 2007; and (3) U.S. Provisional Application No. 60/985,891, filed Nov. 6, 2007. The disclosures of the above referenced applications are incorporated by reference in their entirety herein.

2. FIELD

[0002] The present invention relates to compounds, methods and pharmaceutical compositions, for use in treatment and prevention of disorders of the liver, including cancer.

3. BACKGROUND

[0003] Drug induced toxicities and pharmacological side effects are often associated with interactions by the drug or drug metabolite in tissues not associated with the pharmacological benefits of the drug therapy. In other cases, the desired pharmacological effect is poorly achieved either because of dose-limiting toxicities or inadequate drug levels in the target tissues. Thus, there is a need to deliver drugs to specific tissues or organs. High organ specificity can be achieved by a variety of mechanisms including local administration to the target organ and drug-protein conjugates. Local administration to the target organ is an invasive procedure. Drug-protein conjugates exhibit poor oral bioavailability, limitations in carrier manufacturing and drug loading, a potential for diminished liver uptake due to down regulation of the receptor in diseased tissue, and a high incidence of antibody induction. A third approach entails use of produgs that are activated by enzymes highly enriched in the target organ.

[0004] There is a particular need to deliver drugs to the liver to treat diseases such as cancer and metabolic disorders. Many therapies for these conditions have narrow therapeutic indices and many therapeutic indications could be benefited by selective delivery of the therapeutic agent to the liver.

4. SUMMARY

[0005] Phosphoramide and phosphonoamidate compounds of a variety of therapeutic agents are provided, as well as methods for their manufacture and use in the treatment of a variety of disorders including liver cancer, inflammation, fibrosis and metabolic disorders. In one embodiment, the compound is a S-pivaloyl-2-thioethyl phosphoramide, S-pivaloyl-2-thioethyl phosphonoamidate, S-hydroxypivaloyl-2-thioethyl phosphoramide or S-hydroxypivaloyl-2-thioethyl phosphonoamidate. As used herein, a “phosphoramide or phosphonoamidate compound of a therapeutic agent” includes a therapeutic agent derivatized to include a phosphoramide or phosphonoamidate group. The therapeutic agent is, for example, an anti-cancer agent that includes, or has been derivatized to include, a reactive group, such as a hydroxyl, for attachment of the phosphoramide or phosphonoamidate moiety. Such therapeutic agents include, but are not limited to nucleosides and nucleoside analogs including acyclic nucleosides. In some embodiments, phosphoramide or phosphonoamidate compounds of nucleotides and nucleotide analogs, such as 2'-branched and 4'-branched nucleosides are provided. Such compounds can be administered in an effective amount for the treatment of liver disorders, including cancer.

[0006] In certain embodiments, while not being limited to any theory, it is possible that the parent drug is obtained from selective metabolism of the phosphoramide or phosphonoamide compound in the liver and thus the parent drug provided herein is capable of accumulating in the liver. Accordingly, provided are methods of directing phosphoramide or phosphonoamide compounds disclosed herein to the liver.

[0007] In certain embodiments, phosphoramide or phosphonoamide compounds of pharmaceutical agents for the treatment of a liver disorder can be made and used therapeutically as described herein. A variety of phosphoramide or phosphonoamide compounds can be used in the treatment of liver disorders. In particular, therapeutic agents for the treatment of liver cancer can be derivatized to form a phosphoramide or phosphonoamide compound as described herein, and used for the treatment of liver cancers. Liver cancers that can be treated include benign tumors, malignant tumors, hemangiomas, hepatic adenomas, focal nodular hyperplasia, hepatocellular carcinoma, fibrolamellar carcinoma, cholangiocarcinomas, bile duct cancers, and other primary and metastatic cancers of the liver.

[0008] Phosphoramide and phosphonoamidate compounds of a variety of therapeutic agents are provided. The compounds can be formed using methods available in the art and those disclosed herein. Such compounds can be used in some embodiments to enhance delivery of the drug to the liver. In one embodiment, the compound comprises an S-acyl-2-thioethyl phosphoramide or an S-acyl-2-thioethyl phosphonoamide derivative, e.g., a S-pivaloyl-2-thioethyl phosphoramide or a S-hydroxypivaloyl-2-thioethyl phosphonoamide derivative.

[0009] In some embodiments, the phosphoramide or phosphonoamide compounds, as well as salts thereof, and compositions comprising the compounds, provided herein are useful for treatment of disorders of the liver, including cancer. In other embodiments, the phosphoramide or phosphonoamide compounds, as well as salts thereof, and compositions comprising the compounds, provided herein are useful for treatment of liver fibrosis and inflammation.

[0010] In one embodiment, the compound provided herein is a compound of Formula I:

```
  R1
 /   \
R2----O----W
     /   \
   R3----X
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or a pharmaceutically acceptable salt, solvate, a stereoisomeric, tautomeric or polymorphic form thereof, wherein
[0011] X is

\[ \begin{array}{c}
\text{C--R'} \quad \text{or} \\
\text{S--R'} \\
\end{array} \]

or

\[ \begin{array}{c}
\text{C--R'} \quad \text{or} \\
\text{S--R'} \\
\end{array} \]

[0012] Z is O or S; 
[0013] each W is independently O or S; 
[0014] R' and R'' each independently represent alkyl, alkenyl, alkynyl, aryl, alkyl alky, cycloalkyl, cycloalkenyl, amino, aminoalkyl, alkoxy, heterocyclyl, or heteroaryl, all optionally substituted; 
[0015] R' and R'' are selected as follows: 
[0016] i) R' and R'' are each independently hydrogen, alkyl, carboxyalkyl, hydroxyalkyl, hydroxycyalkyl, acryloxyalkyl, aminoaryalkyl, alkoxyaryalkyl, aminoaryalkyl, aryl, aryl alkyl, cycloalkyl, heteroaryl or heterocyclyl, all optionally substituted; or 
[0017] ii) R' and R'' together with the nitrogen atom on which they are substituted form a 3-7 membered heterocyclic or heteroaryl ring; 
[0018] n is 0-3; n2 is 1-4; and 
[0019] R' is a moiety derivable by removal of a hydrogen from a group, such as a hydroxy group, of a therapeutic agent such as an anti-cancer drug. 
[0020] In another embodiment, 

\[ X' \text{ is } \begin{array}{c}
\text{C--R'} \quad \text{or} \\
\text{S--R'} \\
\end{array} \]

midate compound, e.g. in a formula, those of skill in the art will recognize that the compound comprises a derivative, e.g. a radical of the anti-cancer drug. Those derivatives can for example be prepared by elimination of a hydrogen radical from a hydroxy group of the drug, for instance in a dehydration reaction. 

[0030] In certain embodiments of Formula I, R' is a nucleoside comprising a cyclic or acyclic sugar or an analog thereof. 

[0031] In certain embodiments, R' is an anti-cancer drug selected from cladribine, decitabine, daunorubicin, dihydro-5-azacytidine, doxorubicin, epirubicin, estramusine, etoposide, fludarabine, 7-hydroxychlorpromazine, neplanocin A, podophyllotoxin, tezactibine, troxactibine, vinblastin, vincristin, vinbeside, etoposide, teniposide, NK-111, camptothecin, irinotecan, 9-aminoacaptothecin, OG-211, topotecan, pacitaxel, Azatostin, coformycin, pirurubicin, nelarabine and losoxantrone. 

[0032] In one embodiment, R' is an immunosuppressant, such as pentostatin, combretastatin A-4, mycophenolic acid or mitoxantrone. 

[0033] In certain embodiments according to formula I, R' is substituted alkyl, e.g. hydroxyalkyl or aminoalkyl; and R' and R'' are independently hydrogen, alkyl, substituted alkyl, benzyl or substituted benzyl, for instance hydroxy- or amino-substituted alkyl or benzyl. 

[0034] In another embodiment, R' is --OR', --C(R') or --NHR' where each R' is independently alkyl, substituted alkyl, aryl or substituted aryl, for instance hydroxy- or amino-substituted alkyl or aryl; and R' and R'' are independently hydrogen, alkyl, substituted alkyl, benzyl or substituted benzy, for instance hydroxy- or amino-substituted alkyl or benzyl. 

[0035] In a further embodiment, R' and R'' are independently benzyl or substituted alkyl. In a further embodiment, R' is selected from the group consisting of alkyl and hydrox Ayl. In certain embodiments, R' is --C(CH3)xCH3OH. 

[0036] In certain embodiments, the compounds provided herein are selected such that R' is not 3'-azido-2',3'-dideoxythymidine. 

[0037] In another embodiment, the compound provided herein is a compound of Formula IIa or IIb:

\[ \text{IIa} \]

or a pharmaceutically acceptable salt, solvate, a stereoisomeric, tautomeric or polymorphic form thereof, wherein 

[0038] R' is alkyl, alkenyl, alkynyl, aryl, aryl alkyl, cycloalkyl, cycloalkenyl, amino, aminoalkyl, heteroaryl or heteroaryl, all optionally substituted; 

[0039] R' and R'' are selected as follows: 

i) R' and R'' are each independently hydrogen, alkyl, carboxyalkyl, hydroxyalkyl, hydroxycyalkyl, acryloxyalkyl, aminoaryalkyl, alkoxyaryalkyl, aminoaryalkyl, aryl, aryl alkyl, cycloalkyl, heteroaryl or heterocyclyl, all optionally substituted; or 

ii) R' and R'' together with the nitrogen atom on which they are substituted form a 3-7 membered heterocyclic or heteroaryl ring; 

n is 0-3; n2 is 1-4; and 

R' is a moiety derivable by removal of a hydrogen from a group, such as a hydroxy group, of a therapeutic agent such as an anti-cancer drug. 

Those of skill in the art will recognize that compounds of Formula I can be designed or prepared by reaction, e.g., at a hydroxy group of said drug, for example via condensation or dehydration. For convenience, in the description herein when exemplary substituents, such as R' groups are identified as a drug in a phosphoroamidate or phosphonoa-
aminocarbonylalkyl, alkoxy carbonylalkyl, aryl, aryl alkyl cycloalkyl, heteroaryl or heterocyclyl, all optionally substituted; or

(0041) ii) R" and R" together with the nitrogen atom on which they are substituted form a 3-7 membered heterocyclic or heteroaryl ring; and

(0042) R' is a drug such as an anti-cancer drug.

(0043) In certain embodiments according to Formula IIA or IIB, R' is substituted alkyl, e.g., hydroxyalkyl or aminooalkyl; and R" and R" are each independently hydrogen, alkyl, substituted alkyl, benzyl or substituted benzyl, for instance hydroxy- or amino-substituted alkyl or benzyl. In another embodiment, R' is —OR', —C(R')₃, or —NHR' where each R' is independently alkyl, substituted alkyl, aryl or substituted aryl, for instance hydroxy- or amino-substituted alkyl or aryl; and R" and R" are independently hydrogen, alkyl, substituted alkyl, benzyl or substituted benzyl, for instance hydroxy- or amino-substituted alkyl or benzyl. In a further embodiment, R" and R" are each independently benzyl or substituted alkyl. In a further embodiment, R' is selected from the group consisting of alkyl and hydroxyalkyl. In certain embodiments, R' is —C(CH₃)₃CH₂OH.

(0044) In some embodiments, provided herein are:

(0045) (a) compounds as described herein, e.g., of Formula I, IIA or IIB, and pharmaceutically acceptable salts and compositions thereof;

(0046) (b) compounds as described herein, e.g., of Formula I, IIA or IIB, and pharmaceutically acceptable salts and compositions thereof for use in the treatment and/or prophylaxis of a liver disorder;

(0047) (c) processes for the preparation of compounds as described herein, e.g., of Formula I, IIA or IIB, as described in more detail below;

(0048) (d) pharmaceutical formulations comprising a compound as described herein, e.g., of Formula I, IIA or IIB, or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent; and

(0049) (e) pharmaceutical formulations comprising a compound as described herein, e.g., of Formula I, IIA or IIB, or a pharmaceutically acceptable salt thereof together with one or more other effective anti-cancer agents, optionally in a pharmaceutically acceptable carrier or diluent.

(0050) In certain embodiments, the following phosphorouamide and phosphoronic acid formulas and compounds are provided, which optionally act as thyroid hormone receptor effectors:
wherein

[0051] each R, if present, is independently alkyl, halogen or hydroxy;

[0052] X, if present, is CH₂, O or S;

[0053] R', if present, is optionally substituted alkyl, wherein the substituted alkyl is optionally hydroxyalkyl or aminooalkyl, e.g., —(CH₂)$_n$CH$_2$OH; and

[0054] R$^a$ and R$^b$, if present, are independently hydrogen; unsubstituted alkyl; or alkyl substituted with aryl, amino, amido, hydroxy, alkoxy, aminooalkyl, hydroxyalkyl, aryl, or heteroaryl, each optionally substituted; wherein, in one embodiment, R$^a$ and R$^b$ are independently H or a benzyl that is optionally substituted, for example, with hydroxy or amino.

[0055] In certain embodiments according to formula IIIa or b, IVa or b, Vla or b, VIa or b, VIIa or b, VIII a or b, R$^a$ is hydrogen, R$^b$ is —CH$_2$—C$_6$H$_5$ and R$^c$ is —C(CH$_3$)$_2$CH$_2$OH.

[0056] In certain embodiments, the thyroid hormone receptor effector compound provided herein has a formula selected from:

wherein

[0057] R$^a$ and R$^b$ are each independently hydrogen or alkyl;

[0058] R$^c$ is alkyl;

[0059] R$^d$ is alkyl, alkenyl, alkynyl, aryl, aryl alkyl, cycloalkyl, cycloalkenyl, amino, aminooalkyl, heterocyclyl or heteroaryl, all optionally substituted;

[0060] R$^z$ and R$^u$ are selected as follows:

[0061] i) R$^z$ and R$^u$ are each independently hydrogen, alkyl, carboxyalkyl, hydroxyalkyl, hydroxycarylalkyl, acyloxyalkyl, aminocarbonylalkyl, alkoxy carbonylalkyl, aryl, aryl alkyl, cycloalkyl, heteroaryl or heterocyclyl, all optionally substituted; or

[0062] ii) R$^z$ and R$^u$ together with the nitrogen atom on which they are substituted form a 3-7 membered heterocyclic or heteroaryl ring.
[0063] In certain embodiments, the thyroid hormone receptor effector provided herein is selected from:

\( X_{a} \)

\( X_{b} \)

\( X_{la} \)

\( X_{lb} \)

\( X_{ia} \)

\( X_{ib} \)

\( X_{la} \)

\( X_{lb} \)

\( X_{ia} \)

\( X_{ib} \)

\( X_{la} \)

\( X_{lb} \)
In certain embodiments, R is hydrogen, R is —CH, and R is —(CH2)2CH2OH.

In certain embodiments, the compounds do not affect thyroid function, thyroid production of circulating iodinated thyronines such as T3 and T4, and/or the ratio of T3 to T4.

Also provided are pharmaceutical compositions comprising the compounds, e.g., in a dosage unit suitable for administration, e.g., oral administration.

5. BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 depicts depletion of B184 (NM108 hydroxysulfate phosphoramidate) after incubation with and without NADPH in monkey liver S9.

FIG. 2 depicts depletion of B102 (NM107 hydroxysulfate phosphoramidate) after incubation with and without NADPH in monkey liver S9.

6. DESCRIPTION OF EXEMPLARY EMBODIMENTS

Provided herein are compounds, compositions and methods useful for treating liver disorders, such as cancer, or metabolic diseases, such as diabetes, hyperlipidemia, atherosclerosis, and obesity. Further provided are dosage forms useful for such methods.

6.1 Definitions

When referring to the compounds provided herein, the following terms have the following meanings unless indicated otherwise.

The term “alkyl”, as used herein, unless otherwise specified, includes a saturated straight, branched, or cyclic primary, secondary, or tertiary hydrocarbon of typically C1 to C10, and specifically includes methyl, CF3, Cl, CFCl2, CF2Cl, CF2Cl2, ethyl, CH2CF2, CF3CF2, propyl, isopropyl, cyclopropyl, butyl, isobutyl, secbutyl, t-butyl, pentyl, cyclopentyl, isopentyl, neopentyl, hexyl, isohexyl, cyclohexyl, cyclohexymethyl, 3-methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl. The term includes both substituted and unsubstituted alkyl groups, and particularly includes halogenated alkyl groups, and even more particularly fluorinated alkyl groups. Non-limiting examples of moieties with which the alkyl group can be substituted are selected from the group consisting of halogen (fluoro, chloro, bromo or iodo), hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, et al., Protective Groups in Organic Synthesis, John Wiley and Sons, Second Edition, 1991, hereby incorporated by reference.

The term “lower alkyl”, as used herein, and unless otherwise specified, includes a C to C4 saturated straight, branched, or if appropriate, a cyclic (for example, cyclopropyl) alkyl group, including both substituted and unsubstituted moieties.

“Alkylene” includes divalent saturated aliphatic hydrocarbon groups particularly having up to about 11 carbon atoms and more particularly 1 to 6 carbon atoms which can be straight-chained or branched. This term is exemplified by groups such as methylene (—CH2—), ethylene (—CH2CH2—), the propylene isomers (e.g., —CH2CH2CH2— and —CH2CH(CH3)CH2—) and the like.

“Alkenyl” includes monovalent olefinically unsaturated hydrocarbon groups, in certain embodiment, having...
up to about 11 carbon atoms, from 2 to 8 carbon atoms, or from 2 to 6 carbon atoms, which can be straight-chained or branched and having at least 1 or from 1 to 2 sites of olefinic unsaturation. Exemplary alkyl groups include ethenyl (\(-\text{CH} = \text{CH}_2\)), n-propenyl (\(-\text{CH}_2\text{CH} = \text{CH}_2\)), isopropenyl (\(-\text{C}(\text{CH}_3) = \text{CH}_2\)), vinyl and substituted vinyl, and the like.

[0092] “Alkenylene” includes divalent olefinically unsaturated hydrocarbon groups, in certain embodiments, having up to about 11 carbon atoms or from 2 to 6 carbon atoms which can be straight-chained or branched and having at least 1 or from 1 to 2 sites of olefinic unsaturation. This term is exemplified by groups such as ethenylene (\(-\text{CH} = \text{CH}\)-), the propylene isomers (e.g., \(-\text{CH} = \text{CHCH}_3\)- and \(-\text{CH}(\text{CH}_3) = \text{CH}\)- and \(-\text{CH} = \text{C}(\text{CH}_3)\)-) and the like.

[0093] “Alkynyl” includes acetylenically unsaturated hydrocarbon groups, in certain embodiments, having up to about 11 carbon atoms or from 2 to 6 carbon atoms which can be straight-chained or branched and having at least 1 or from 1 to 2 sites of alkynyl unsaturation. Non-limiting examples of alkynyl groups include acetylenic, ethynyl (\(-\text{C} = \text{CH}\)), propargyl (\(-\text{CH}_2\text{C} = \text{CH}\)-), and the like.

[0094] The term “aryl”, as used herein, and unless otherwise specified, includes phenyl, biphenyl, or naphthyl, and preferably phenyl. The term includes both substituted and unsubstituted moieties. The aryl group can be substituted with any described moiety, including, but not limited to, one or more moieties selected from the group consisting of halogen (fluoro, chloro, bromo or iodo), alkyl, hydroxyl, amino, alkylation, aralkyloxy, allyl, arlyoxy, nitro, cyano, sulfonyl acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, et al., Protective Groups in Organic Synthesis, John Wiley and Sons, Second Edition, 1991.

[0095] “Alkoxy” includes the group —OR where R is alkyl. Particular alkyl groups include, by way of example, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, 1,2-dimethoxy, and the like.

[0096] “Alkoxyalkyl” includes a radical —C(\text{O})-alkoxy where alkyl is as defined herein.

[0097] “Amino” includes the radical —NH₂.

[0098] “Carboxyl” includes the radical —CO\text{OH}.

[0099] The term “allylamino” or “arylamino” includes an amino group that has one or two alkyl or aryl substituents, respectively. Unless otherwise specifically stated in this application, when alkyl is a suitable moiety, lower alkyl is preferred. Similarly, when alkyl or lower alkyl is a suitable moiety, unsubstituted alkyl or lower alkyl is preferred.

[0100] “Halogen” or “halo” includes chloro, bromo, fluoro or iodo.

[0101] “Monoalkylamino” includes the group alkyl-NR—, wherein R is selected from hydrogen and alkyl.

[0102] “Thioalkoxy” includes the group —SR where R is alkyl.

[0103] The term “protected” as used herein and unless otherwise defined refers to a group that is added to an oxygen, nitrogen, or phosphorus atom to prevent its further reaction or for other purposes. A variety of oxygen and nitrogen protecting groups are known to those skilled in the art of organic synthesis.

[0104] “Pharmaceutically acceptable salt” includes any salt of a compound provided herein which retains its biological properties and which is not toxic or otherwise undesirable for pharmaceutical use. Such salts may be derived from a variety of organic and inorganic counter-ions well known in the art. Such salts include: (1) acid addition salts formed with organic or inorganic acids such as hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, sulfamic, acetic, trifluoroacetic, trichloroacetic, propionic, hexanoic, cyclopentylpropionic, glycolic, glutaric, pyruvic, lactic, malonic, seuccinic, sorbic, ascorbic, malic, maleic, fumaric, tartaric, citric, benzoic, 3-(4-hydroxybenzoyl)benzoic, picric, cinnamic, mandelic, phthalic, lauric, methanesulfonic, ethanesulfonic, 1,2-ethane-disulfonic, 2-hydroxyethanesulfonic, benzenesulfonic, 4-chlorobenzenesulfonic, 2-naphthalenesulfonic, 4-toluenesulfonic, camphoric, camphorsulfonic, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic, glucoheptonic, 1,3-propenylcricic, trimethylacetic, tert-butylacetic, lauryl sulfonic, gluconic, benzoic, glutamic, hydroxyapatic, salicylic, stearic, cyclohexylsulfonic, quinic, monacinc and the like acids, or (2) salts formed when an acidic proton present in the parent compound either (a) is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion or an aluminum ion, or (b) is used in a metal or alkaline earth metal hydroxides, such as sodium, potassium, calcium, magnesium, aluminum, lithium, zinc, and barium hydroxide, ammonium or (b) coordinates with an organic base, such as aliphatic, alicyclic, or aromatic organic amines, such as ammonia, methylamine, dimethylamine, diethylamine, diethylamine, picoline, ethanamine, diethanolamine, triethanolamine, ethylenediamine, lysine, arginine, ornithine, choline, N,N,N'-dibenzylethylene-diamine, chloroprocaine, diethanolamine, procaine, N-benzylhexethyamine, N-methylglucamine pipеразин, трис(гидроксими-этил)-аминометан, тетраметиламмоний хлорид, and the like.

[0105] Salts further include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium and the like, and when the compound contains a basic functionality, salts of non-toxic organic or inorganic acids, such as hydrohalides, e.g. hydrochloride and hydrobromide, sulfate, phosphate, sulfamate, nitrate, acetate, trifluoroacetate, trichloroacetate, propionate, hexanoate, cyclopentylpropionate, glycolate, glutarate, pyruvate, lactate, malonate, succinate, sorbate, ascorbate, maleate, maleate, fumarate, tartarate, citrate, benzoate, 3-(4-hydroxybenzoyl) benzoate, picrate, cyanurate, mandelate, phthalate, laurate, ethanesulfonate, 2-hydroxyethanesulfonic, benzenesulfonate (besylate), 4-chlorobenzenesulfonic, 2-naphthalenesulfonic, 4-toluenesulfonic, camphor, camphorsulfonic, 4-methylbicycle[2.2.2]-oct-2-ene-1-carboxylate, glucoheptonic, 3-phenylpropionate, trimethylacetate, tert-butylacetate, lauryl sulfate, gluconate, benzoate, glutamate, hydroxyapatic, salicylate, stearate, cyclohexylsulfamate, quinate, mucate and the like.

[0106] The term “alkyl” or “alkylaryl” includes an aryl group with an alkyl substituent. The term aralkyl or arylalkyl includes an alkyl group with an aryl substituent.

[0107] The term “purine” or “pyrimidine” base includes, but is not limited to, adenine, N⁹-alkylpurines, N⁹-acylpyrimidines (wherein acyl is C(O)alkyl, aryl, alkylaryl, or arylalkyl), N⁹-benzylpurine, N⁹-halopurine, N⁹-vinylpyrimidine, N⁹-acetylenic purine, N⁹-acyl purine, N⁹-hydroxyalkyl purine, N⁹-alkylaminopurine, N⁹-thioalkyl purine, N⁹-alkylpyrimidines, N⁹-alkyl-6-thiopurines, thymine, cytosine, 5-fluorocytosine, 5-methylcytosine, 6-azacytosine, 2- and/or 4-mercaptothymidine, uracil, 5-ha-
louracil, including 5-fluorouracil, C₅-alkylpyrimidines, C₅-benzylpyrimidines, C₅-halopyrimidines, C₅-vinylpyrimidines, C₅-acetylenic pyrimidines, C₅-acyl pyrimidines, C₅-hydroxalkyl purine, C₅-amidopyrimidines, C₅-acyanopyrimidines, C₅-iodopyrimidines, C₅-iodo-pyrimidines, C₅-Br-vinyl pyrimidine, C₅-Cr-vinyl pyrimidine, C₅-nitropyrimidines, C₅-amino-pyrimidine, N₂-alkylpurines, N₂-alkyl-6-thiopurines, 5-azacytidinyl, 5-azauracilyl, triazolopyridinyl, imidazolopyridinyl, pyrrolopyrimidinyl, and pyrazoloopyrimidinyl. Purine bases include, but are not limited to, guanine, adenine, hypoxanthine, 7-deazaguanine, 7-deazadenine, 2,6-diaminopurine, and 6-chloropurine. Functional oxygen and nitrogen groups on the base can be protected as necessary or desired. Suitable protecting groups are well known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, t-butyldimethylsilyl, and t-butyldiphenylsilyl, triyl, alkyl groups, and acyl groups such as acetyl and propionyl, methanesulfonyl, and p-toluenesulfonyl.

0108] The term “acyl” or “O-linked ester” includes a group of the formula C(O)R’, wherein R’ is an straight, branched, or cyclic alkyl (including lower alkyl), carboxylate residue of amino acid, aryl including phenyl, arylalkyl including benzyl, alkoxyalkyl including methoxyalkyl, arylalkoxycarbonyl such as phenoxymethyl; or substituted alkyl (including lower alkyl), aryl including phenyl optionally substituted with chloro, bromo, fluoro, iodo, C₁ to C₆ alkyl or C₁ to C₆ alkoxy, sulfonate esters such as alkyl or arylsulfonyl phenoxy including methanesulfonyl, the mono, di or triphosphate ester, triyl or monomethoxy-triyl, substituted benzyl, arylalkyl including benzyl, alkylalkoxycarbonyl including methoxymethyl, arylalkoxycarbonyl such as phenoxymethyl. Aryl groups in the esters optimally comprise a phenyl group. In particular, acyl groups include acetyl, trifluoracetyl, methylacyl, cyclopropylacetyl, propionyl, butyryl, hexanoyl, heptanoyl, octanoyl, neo-heptanoyl, phenylacetyl, 2-acetoxy-2-phenylacetyl, diphenylacetyl, α-methoxy-α-trifluromethylphenylacetyl, bromoacetyl, 2-nitro-benzoate, 4-chlorobenzoate, 2-chloro-2,2-diphenylacetyl, 2-chloro-2-phenylacyl, trimethylacetyl, chlorodifluoracetyl, perfluoracetyl, fluoroacetoxymethyl, methylacyl, 2-phenethoxyacetyl, chlorosulfonylacetate, 3-methoxyphenylacetate, fluoroacetoxymethyl, tert-butylacetyl, trichloroacetyl, monochloroacetocarbonyl, dichloroacetyl, 7H-dodecafluoroheptanoyl, perfluoro-heptanoyl, 7H-dodecafluoroheptanoyl, 7H-dodecafluorohexanoyl, 7H-dodecafluorohexanoyl, 7H-dodecafluoroheptanoyl, 7H-dodecafluoroheptanoyl, 7H-dodecafluoroheptanoyl, nonadecafluoro-3,6-dioxo-heptanoyl, nonadecafluoro-3,6-dioxo-heptanoyl, perfluoroheptanoyl, methoxybenzoyl, methyl-3-amino-5-phenylthiophen-2-carboxyl, 3,6-dichloro-2-methoxy-benzoyl, 4(1,1,2,2-tetrafluoro-ethoxy)-benzoyl, 2-bromo-propionyl, omega-aminocaproly, deamnoyl, n-pentadecanoyl, stearyl, 3-cyclohexyl-propionyl, 1-benzene-carboxyl, O-acetylmandelyl, pivaloyl acetyl, 1-adamantane-carboxyl, cyclohexane-carboxyl, 2,6-pyridinedicarboxyl, cyclopropane-carboxyl, cylobutane-carboxyl, perfluorocyclohexyl carboxyl, 4-methylbenzoyl, chloromethyl isoxazolyl carbonyl, perfluorocyclohexyl carbonyl, crotonyl, 1-methyl-1H-indazole-3-carboxyl, 2-propenyl, isovaleryl, 1-pyrroldinecarbonyl, 4-phenylbenzoyl.

0109] The term “amino acid” includes naturally occurring and synthetic α, γ, or β or ω amino acids, and includes but is not limited to, amino acids found in proteins, i.e. glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartate, glutamate, lysine, arginine and histidine. In a preferred embodiment, the amino acid is in the L-configuration. Alternatively, the amino acid can be a derivative of alanyl, valinal, leucinal, isoleucinal, prolinyl, phenylalaninal, tryptophanlyl, methionyl, glycyl, serinyl, threonyl, cysteinyl, tyrosyl, asparagynyl, glutaminyl, aspartoyl, glutaryl, lysyl, arginyl, histidinal, β-alanyl, β-valinal, β-leucinal, β-isoleucinal, β-prolinyl, β-phenylalaninal, β-tryptophanlyl, β-methioninal, β-glycinal, β-serinyl, β-threonyl, β-cysteinyl, β-tyrosinal, β-asparagynyl, β-glutaminyl, β-aspartoyl, β-glutaryl, β-lysinyl, β-argininal or β-histidinal.

0110] As used herein, the term “substantially free of” or “substantially in the absence of” with respect to a nucleoside composition includes a nucleoside composition that includes at least 85% or 90% by weight, preferably 95%, 98%, 99% or 100% by weight, of the designated enantiomer of that nucleoside. In a preferred embodiment, in the methods and compounds of this invention, the compounds are substantially free of enantiomers.

0111] Similarly, the term “isolated” with respect to a nucleoside composition includes a nucleoside composition that includes at least 85, 90%, 95%, 98%, 99% to 100% by weight, of the nucleoside, the remainder comprising other chemical species or enantiomers.

0112] “Solvate” includes a compound provided herein or a salt thereof, that further includes a stoichiometric or non-stoichiometric amount of solvent bound by non-covalent intermolecular forces. Where the solvent is water, the solvate is a hydrate.

0113] As used herein, the terms “subject” and “patient” are used interchangeably herein. The terms “subject” and “subjects” refer to an animal, such as a mammal including a non-primate (e.g., a cow, pig, horse, cat, dog, rat, and mouse) and a primate (e.g., a monkey such as a cynomolgus monkey, a chimpanzee and a human), and for example, a human. In one embodiment, the subject is refractory or non-responsive to current treatments for cancer. In another embodiment, the subject is a farm animal (e.g., a horse, a cow, a pig, etc.) or a pet (e.g., a dog or a cat). In one embodiment, the subject is a human.

0114] As used herein, the terms “therapeutic agent” and “therapeutic agents” refer to any agent(s) which can be used in the treatment or prevention of a disorder or one or more symptoms thereof. In certain embodiments, the term “therapeutic agent” includes a compound provided herein. In one embodiment, a therapeutic agent is an agent which is known to be useful for, or has been or is currently being used for the treatment or prevention of a disorder or one or more symptoms thereof.

0115] “Therapeutically effective amount” includes an amount of a compound or composition that, when administered to a subject for treating a disease, is sufficient to effect such treatment for the disease. A “therapeutically effective amount” can vary depending on, inter alia, the compound, the disease and its severity, and the age, weight, etc., of the subject to be treated.

0116] “Treating” or “treatment” of any disease or disorder refers, in one embodiment, to ameliorating a disease or disorder that exists in a subject. In another embodiment, “treating” or “treatment” includes ameliorating at least one physiological parameter, which may be indiscernible by the subject. In yet another embodiment, “treating” or “treatment” includes
modulating the disease or disorder, either physically (e.g., stabilization of a discernible symptom) or physiologically (e.g., stabilization of a physical parameter) or both. In yet another embodiment, “treating” or “treatment” includes delaying the onset of the disease or disorder.

As used herein, the terms “prophylactic agent” and “prophylactic agents” as used refer to any agent(s) which can be used in the prevention of a disorder or one or more symptoms thereof. In certain embodiments, the term “prophylactic agent” includes a compound provided herein. In certain other embodiments, the term “prophylactic agent” does not refer to a compound provided herein. For example, a prophylactic agent is an agent which is known to be useful for, or has been or is currently being used to prevent or impede the onset, development, progression and/or severity of a disorder.

As used herein, the phrase “prophylactically effective amount” includes the amount of a therapy (e.g., prophylactic agent) which is sufficient to result in the prevention or reduction of the development, recurrence or onset of one or more symptoms associated with a disorder (or to enhance or improve the prophylactic effect(s) of another therapy (e.g., another prophylactic agent).

6.2 Exemplary Embodiments

Phosphoroamidate and phosphonoamidate compounds of a variety of therapeutic agents can be formed using methods available in the art and those disclosed herein. Such compounds can be used in some embodiments to enhance delivery of the drug to the liver. In one embodiment, the compound is an S-acetyl-2-thioethyl phosphoroamidate or an S-acetyl-2-thioethyl phosphonoamidate derivative, e.g., a S-pivaloyl-2-thioethyl phosphoroamidate or a S-hydroxypivaloyl-2-thioethyl phosphonoamidate. Therapeutic agents that can be derivatized to compound form include an anti-cancer agent that includes, or has been derivatized to include a reactive group for attachment of the phosphoroamidate or phosphonoamidate moiety, including but not limited to nucleosides and nucleoside analogues including acyclic nucleosides.

Phosphoroamidate or phosphonoamidate compound forms of a variety of nucleosides can be formed from nucleosides disclosed herein and available in the art. In particular, anti-cancer nucleosides can be derivatized to form a phosphoroamidate or phosphonoamidate compound that can enhance delivery to the liver.

In one embodiment, the phosphoroamidate or phosphonoamidate compound provided herein is a compound of formula Ila or IIb:

or a pharmaceutically acceptable salt, solvate, a stereoisomeric, tautomeric or polymorphic form thereof, wherein;

R' is alkyl, alkenyl, alkynyl, aryl, aryalkyl, cy cloalkyl, cycloalkenyl, amino, heterocyclic or heteroaryl, all optionally substituted;

R' and R' are selected as follows:

i) R' and R' are each independently hydrogen, alkyl, carboxyalkyl, hydroxyalkyl, hydroxyaryalkyl, acyloxyalkyl, aminocarboxyalkyl, alkoxycarbonylalkyl, aryl, aryalkyl, cy cloalkyl, heteroaryl or heterocyclic, all optionally substituted; or

ii) R' and R' together with the nitrogen atom on which they are substituted form a 3-7 membered heterocyclic or heteroaryl ring; and

R' is a drug such as an anti-cancer drug.

In certain embodiments, the compound of formula Ila or IIb is selected with a proviso that when R' is tert-butyl or hydroxy-tert-butyl, then R' is not 3'-azido-2',3'-dideoxythymidine.

In certain embodiments, R', R', R' and R' are optionally substituted with one or more substituents as defined herein, e.g., in the definitions.

In certain embodiments, the compounds are of Formula Ila or IIb, wherein R' is alkyl, alkenyl, alkynyl, aryl, aryalkyl, cycloalkyl, cycloalkenyl, amino, heterocyclic or heteroaryl;

R' and R' are each independently hydrogen, alkyl, carboxyalkyl, hydroxyalkyl, hydroxyaryalkyl, acyloxyalkyl, aminocarboxyalkyl, alkoxycarbonylalkyl, aryl, aryalkyl, cycloalkyl, heteroaryl or heterocyclic, all optionally substituted; and

R' is an anti-cancer drug.

In one embodiment, R' or R'—CH₂— is a nucleoside comprising a cyclic or acyclic sugar or analog thereof, including any nucleoside or analogue thereof described herein or known in the art.

In certain embodiments of Formula Ila or IIb, R' is substituted alkyl, e.g. hydroxyalkyl or aminoalkyl; and R' and R' are independently hydrogen, alkyl, substituted alkyl, benzyl or substituted benzyl, for instance hydroxy- or amino-substituted alkyl or benzyl. In another embodiment, R' is —OR', —C(R')₂, or —NHR' where each R' is independently alkyl, substituted alkyl, aryl or substituted aryl, for instance hydroxy- or amino-substituted alkyl or aryl; and R' and R' are independently hydrogen, alkyl, substituted alkyl, benzyl or substituted benzyl, for instance hydroxy- or amino-substituted alkyl or benzyl. In a further embodiment, R' and R' are independently benzyl or substituted alkyl. In a further embodiment, R' is selected from the group consisting of alkyl and hydroxyalkyl. In certain embodiments, R' is —C(CH₃)₂CH₂OH. In certain embodiments according to this paragraph, R' and R' are each hydrogen, R' is hydrogen, R' is —CH₂—C₃H₇ and R' is —C(CH₃)₂CH₂OH.
In one embodiment, \( R' \) is alkyl or hydroxyalkyl. In one embodiment, \( R' \) is methyl, tert-butyl, hydroxy-tert-butyl or hydroxyethyl. In certain embodiments, \( R' \) is \( -\text{C(H}_3\text{)}\text{-CH}_2\text{OH} \).

In one embodiment, \( R' \) and \( R'' \) are each independently hydrogen, alkyl, carboxyalkyl, hydroxycarboxyalkyl, acyl, acyloxyalkyl, amine, aminecarbonylalkyl, alkoxycarbonylalkyl, aryl or aryalkyl, wherein the alkyl groups can be further substituted with one or more substituents. In one embodiment, at least one of \( R' \) or \( R'' \) is other than hydrogen. In one embodiment, \( R' \) and \( R'' \) are each independently hydrogen, methyl or benzyl.

In certain embodiments, \( R' \) is \( -\text{C(H}_3\text{)}\text{)}\text{2CH}_2\text{OH} \) and \( R' \) and \( R'' \) are each independently hydrogen, methyl or benzyl. In certain embodiments, \( R' \) is \( -\text{C(H}_3\text{)}\text{)}\text{3CH}_2\text{OH} \) and \( R' \) is hydrogen and \( R'' \) is benzyl.

In another embodiment, the compound provided herein is a compound of formula:

\[
\begin{align*}
XV \quad & \quad \begin{array}{c}
\text{O} \\
\text{S} \\
\text{O} \\
\text{R'} \\
\text{R''}
\end{array} \\
\text{R'} \quad & \quad \begin{array}{c}
\text{O} \\
\text{S} \\
\text{O} \\
\text{R'} \\
\text{R''}
\end{array}
\end{align*}
\]

wherein \( R' \) and \( R'' \) are as defined in formula Ila or Illa. In one embodiment, \( R' \) is alkyl or hydroxyalkyl. In one embodiment, \( R' \) is methyl, tert-butyl, hydroxy-tert-butyl or hydroxyethyl. In one embodiment, \( R' \) is \( -\text{C(H}_3\text{)}\text{)}\text{-CH}_2\text{OH} \).

In certain embodiments according to formula XVa or XVb, \( R' \) is substituted alkyl, e.g. hydroxyalkyl or aminoalkyl. In another embodiment, \( R' \) is \( -\text{OR}'' \), \( -\text{C}(R'')_n \) or \( -\text{NH}R'' \) where each \( R' \) is independently alkyl, substituted alkyl, aryl or substituted aryl, for instance hydroxy- or amino-substituted alkyl or aryl. In a further embodiment, \( R' \) is selected from the group consisting of alkyl and hydroxyalkyl. In certain embodiments, \( R' \) is \( -\text{C(H}_3\text{)}\text{)}\text{2CH}_2\text{OH} \).

Wherein:

\( R^1 \) is an anti-cancer drug, such as a nucleoside or nucleoside derivative; and

\( R' \) and \( R'' \) are each independently hydrogen, alkyl, carboxyalkyl, hydroxycarboxyalkyl, acyloxyalkyl, aminecarbonylalkyl, alkoxycarbonylalkyl, aryl, aryalkyl, cycloalkyl, heteroaryl or heterocyclyl, all optionally substituted; and

wherein in one embodiment, one of \( R' \) and \( R'' \) is \( H \) and the other is alkyl optionally substituted with aryl, benzyl, or heteroaryl, each optionally substituted.

In certain embodiments according to formula XVIa or XVIb, \( R' \) and \( R'' \) are independently hydrogen, alkyl, substituted alkyl, benzyl or substituted benzyl, for instance hydroxy- or amino-substituted alkyl or benzyl. In another embodiment, \( R' \) and \( R'' \) are independently hydrogen, alkyl, substituted alkyl, benzyl or substituted benzyl, for instance hydroxy- or amino-substituted alkyl or benzyl. In a further embodiment, \( R' \) and \( R'' \) are independently benzyl or substituted alkyl.

In another embodiment, the compound provided herein is a compound of formula:

\[
\begin{align*}
\text{O} \\
\text{S} \\
\text{O} \\
\text{R'} \\
\text{R''}
\end{align*}
\]

wherein \( R' \) is a drug such as an anti-cancer drug.
Exemplary anti-cancer drugs (R’s) that can be derivatized as described herein, for example via a free hydroxyl group, or after adding a hydroxylated linker, are:

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aclarubicin</td>
<td><img src="image" alt="Aclarubicin Structure" /></td>
</tr>
<tr>
<td>Decitabine</td>
<td><img src="image" alt="Decitabine Structure" /></td>
</tr>
<tr>
<td>Daunorubicin</td>
<td><img src="image" alt="Daunorubicin Structure" /></td>
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</tbody>
</table>
-continued

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-azacytidine</td>
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</tr>
<tr>
<td>Doxorubicin</td>
<td><img src="image2.png" alt="Structure" /></td>
</tr>
<tr>
<td>Epirubicin</td>
<td><img src="image3.png" alt="Structure" /></td>
</tr>
<tr>
<td>Estramustine</td>
<td><img src="image4.png" alt="Structure" /></td>
</tr>
<tr>
<td>Etoposide</td>
<td><img src="image5.png" alt="Structure" /></td>
</tr>
<tr>
<td>Name</td>
<td>Structure</td>
</tr>
<tr>
<td>--------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Fludarabine</td>
<td><img src="image" alt="Fludarabine Structure" /></td>
</tr>
<tr>
<td>Neplanocin A</td>
<td><img src="image" alt="Neplanocin A Structure" /></td>
</tr>
<tr>
<td>Tezacitabine (E)-2'-deoxy-2'-&lt;br&gt;(fluoromethylene)cytidine (FMdC))</td>
<td><img src="image" alt="Tezacitabine Structure" /></td>
</tr>
<tr>
<td>Troxacitabine (C)-2'-Deoxy-3'-oxacytidine)</td>
<td><img src="image" alt="Troxacitabine Structure" /></td>
</tr>
<tr>
<td>Vinblastin</td>
<td><img src="image" alt="Vinblastin Structure" /></td>
</tr>
<tr>
<td>Name</td>
<td>Structure</td>
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<tr>
<td>----------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Vincristin</td>
<td><img src="image" alt="Vincristin Structure" /></td>
</tr>
<tr>
<td>Vindesin</td>
<td><img src="image" alt="Vindesin Structure" /></td>
</tr>
<tr>
<td>Teniposide</td>
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</tr>
<tr>
<td>NK-611</td>
<td><img src="image" alt="NK-611 Structure" /></td>
</tr>
<tr>
<td>Name</td>
<td>Structure</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Camptothecin</td>
<td><img src="image" alt="Camptothecin Structure" /></td>
</tr>
<tr>
<td>Irinotecan</td>
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<tr>
<td>9-Aminocamptothecin</td>
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<tr>
<td>Topotecan</td>
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<td>Name</td>
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<td>------------</td>
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</tr>
<tr>
<td>Paclitaxel</td>
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<tr>
<td>Azatoxin</td>
<td><img src="image" alt="Azatoxin Structure" /></td>
</tr>
<tr>
<td>Coformycin</td>
<td><img src="image" alt="Coformycin Structure" /></td>
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<tr>
<td>Pirarubicin</td>
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<td>Name</td>
<td>Structure</td>
</tr>
<tr>
<td>------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Nelarabine</td>
<td><img src="image1" alt="Nelarabine Structure" /></td>
</tr>
<tr>
<td>Losoxantrone</td>
<td><img src="image2" alt="Losoxantrone Structure" /></td>
</tr>
<tr>
<td>Fluororidine</td>
<td><img src="image3" alt="Fluororidine Structure" /></td>
</tr>
<tr>
<td>Mitomycin C</td>
<td><img src="image4" alt="Mitomycin C Structure" /></td>
</tr>
</tbody>
</table>
Exemplary immunosuppressant drugs that can be derivatized as described herein are:

[0147]  

Mitoxantrone

Thalidomide

Erlotinib (Tarceva®)

Combretastatin A-4

Mitoxantrone

2-(2,6-dichloro-3-piperidinyl-1H-isimidole-1,3(2H)-dione) thalidomide

Mycophenolic acid

Pentostatin
In certain embodiments, R' is an anti-cancer drug such as aclacinomicin, decitabine, daunorubicin, dihydro-5-aza-cytidine, doxorubicin, epirubicin, estramustine, etoposide, flavanone, 7-hydroxycoumarin, neplanocin A, podophyllotoxin, tezacinabine, trovacinabine, vinblastine, vincristine, vinodesine, etoposide, teniposide, NK-1611, camptothecin, irinotecan, 9-aminoacanthomycin, GG-211, topotecan, paclitaxel, azotocin, coformycin, pirarubicin and losoxantrone. In a particular embodiment, the anti-cancer drug is camptothecin or azotocin.

In another embodiment, R' is a purine nucleoside analog (see, e.g., Robak et al., Curr. Med. Chem. 2006, 13, 3165-3189). R' is, for example, a cytotoxic agent such as fludarabine (9-β-D-arabinofuranosyl-2-fluoradene), cladribine (2-chloro-2'-deoxyadenosine, ClctA), pentostatin (2'-deoxycoformycin, DCF), clofarabine (CAFAdA), nelarabine, immucillin H (BCX-1777, foreodesine) or 8-chloroadenosine (8-Cl-Ado).

The anti-cancer drug also can be 2(S)-2'-deoxy-2'- C-methylcytidine (SMDC), 1-(2-deoxy-2-methylene-β-D- erythro-pentofuranosyl)cytosine (DMDC), 1-(2-C-cyano-2'- deoxy-1-β-D-arabinofuranosyl)cytosine (CNDAC) or 1-(3-C-ethylβ-D-ribo-pentofuranosyl)cytosine (ECyd). See, e.g., Matsuda et al., Cancer Sci., 2004, 95:105-111.

In one embodiment, R' is an immunosuppressant, such as combretastatin A-4, mycophenolic acid, pentostatin or mitoxantrone.

The anti-cancer drug can be derivatized to include the phosphoramidate or phosphonamidate at, e.g., a free OH or free carboxy group.

In another embodiment, R' is a 2'-O-acetoxy-3'-oxacytidine (BCH-4556, Troxactabinabine):

In another embodiment, R' is tezacinabine (2'-fluoromethylene-2'-deoxyacytidine). In another embodiment, R' is a 5'-aza-pyrimidine, such as 5'-aza-cytidine, 5'-azadecyctidine (decyctbine), or fazarabine.

In certain embodiments, R' is a 2'-deoxy-2'-methylidenepyrimidine nucleoside compound, disclosed, e.g., in U.S. Pat. No. 5,401,726, such as 2'-deoxy-2'-methylidene-5-fluorocytidine, 2'-deoxy-2'-methylidene-5-chlorocytidine, 2'-deoxy-2'-methylidene-5-bromocytidine, 2'-deoxy-2'-methylidene-5-iodocytidine, 2'-deoxy-2'-methylidene-5-methylyctidine, 2'-deoxy-2'-methylidene-5-ethylcytidine, 2'-deoxy-2'-methylidene-5-ethyluridine, 2'-deoxy-2'-methylidene-5-ethyluracil, 2'-deoxy-2'-methylidene-5-ethynyluracil or 2'-deoxy-2'-methylidene-5-fluorocytidine-5'-phosphate acid. In one embodiment, R' is 5-fluorouracil.
Exemplary NSAID drugs suitable for use herein are provided below:
Sodium salicylate Salicylsalicylic acid

Sodium salicylate

Salicylsalicylic acid
In certain embodiments of the compounds of Formula IIa below:

[0163] the moiety:

[0164] is derived from a drug that is an acyclic nucleoside phosphonate, i.e.:

[0165] Thus, compounds of Formula IIa, in one embodiment, are phosphonoamidates of an acyclic nucleoside phosphonate that have potential anti-cancer activity, such as (S)-9-{3-hydroxy-2-(phosphonomethoxy)-propyl}cytosine (HPMPC, cidofovir), (S)-9-{3-hydroxy-2-(phosphonomethoxy)-propyl}adenine (IS)-HPMPA), phosphonomethoxyethylguanine (PMEG), phosphonomethoxyethyladenine (PMEA, adefovir), phosphonomethoxypropyladenine (PMPA, tenofovir), acyclovir, ganciclovir or penciclovir. See e.g., WO/2006/125166 and De Clercq et al., Antiviral Research, Volume 75, Issue 1, July 2007, Pages 1-13.

Embodiments for Delivery of Thyroid Hormone Receptor Effectors

[0166] In certain embodiments, the following phosphoroamidate and phosphonoamidate formulas and compounds are provided, which optionally act as thyroid hormone receptor effectors:

[0162] In certain embodiments of the compounds of Formula IIa below:
wherein

[0167] each R, if present, is independently alkyl, halogen or hydroxyl;

[0168] X, if present, is CH₃, O or S;

[0169] R' is alkyl, alkenyl, alkynyl, aryl, aryl alkyl, cycloalkyl, cycloalkenyl, amino, aminoalkyl, heterocyclyl or heteroaryl, all optionally substituted;

[0170] R⁺ and R⁻ are selected as follows:

[0171] i) R⁺ and R⁻ are each independently hydrogen, alkyl, carboxyalkyl, hydroxyalkyl, hydroxycarboxyalkyl, acetoxyalkyl, aminocarboxyalkyl, alkoxyalkyl, alkyl, aryl alkyl, cycloalkyl, heteroaryl or heterocyclyl, all optionally substituted; or

[0172] ii) R⁺ and R⁻ together with the nitrogen atom on which they are substituted form a 3-7 membered heterocyclic or heteroaryl ring.
In certain embodiments, the compound provided herein has formula selected from \( \text{IIa, IIb, IVa, IVb, Va, Vb, VIa, VIIa, VIIb, VIIIa or VIIIb} \), wherein each \( R \), if present, is independently alkyl, halogen or hydroxyl;

\( X \), if present, is \( \text{CH}_2, \text{O} \) or \( \text{S} \);

\( R^0 \), if present, is optionally substituted alkyl, wherein the substituted alkyl is optionally hydroxyalkyl or aminoalkyl, e.g., \(-\text{C(CH}_3)_2\text{CH}_2\text{OH}\); and

\( R^x \) and \( R^y \), if present, are independently hydrogen; unsubstituted alkyl; or alkyl substituted with aryl, amino, amido, hydroxyl, alkoxy, aminoalkyl, hydroxyalkyl, aryl, or heteroaryl, each optionally substituted; wherein, in one embodiment, \( R^x \) and \( R^y \) are independently H or a benzyl that is optionally substituted, for example, with hydroxy or amino; and

wherein, in another embodiment, if present, \( R^z \) is hydrogen, \( R^x \) is \(-\text{CH}_2\text{C}_n\text{H}_3\), and \( R^y \) is \(-\text{C(CH}_3)_2\text{CH}_2\text{OH}\).

In certain embodiments, the compound provided herein has a formula selected from:

wherein

\( R^3 \) and \( R^4 \) are each independently hydrogen or alkyl;

\( R^5 \) is alkyl;

\( X' \) is \( \text{O} \) or \( \text{S} \);

\( R^0 \) is optionally substituted alkyl, wherein the substituents when present are selected from hydroxy and amino;

\( R^x \) and \( R^y \) are each independently hydrogen or optionally substituted alkyl; where the substituents when present are selected from one or more, in one embodiment, one, two or three groups selected from aryl, amino, amido, hydroxyl, alkoxy, aryl and heteroaryl, each optionally substituted with hydroxy or amino.
In certain embodiments, R and R' are each hydrogen. In certain embodiments, R" is alkyl. In certain embodiments, R" is isopropyl. In certain embodiments, R' is optionally substituted alkyl, wherein the substituents when present are selected from hydroxy and amino. In certain embodiments, R' is C(CH₃)₂CH₂OH. In certain embodiments, R" and R" are each independently hydrogen or optionally substituted alkyl, where the substituents when present are selected from one or more, in one embodiment, one, two or three groups selected from aryl, amino, amido, hydroxyl, alkoxy, aryl and heteroaryl, each optionally substituted with hydroxy or amino. In certain embodiments, R" is hydrogen and R" is benzyl.

In certain embodiments, R" is hydrogen, R' is C₃H₇ and R' is C(CH₃)₂CH₂OH.

In one embodiment, the thyroid receptor effector compound has formula:

In certain embodiments, the compound or Formula selected from IIIa or b, IVa or b, Va or b, Va or b, VIIa or b, VIII a or b is derived from a phosphonate compound useful for inhibiting gluconeogenesis, optionally by inhibiting the enzyme fructose 1,6-bisphosphatase (FBPase).

In certain embodiments, the compound or Formula selected from IXa or b, X a or b, XIa or b, XIIa or b, XIIIa or b, XIVa or b and XVIIIa or b is derived from a compound useful for inhibiting gluconeogenesis, optionally by inhibiting the enzyme fructose 1,6-bisphosphatase (FBPase).

In certain embodiments, the compound or Formula selected from IIIa or b, IVa or b, Va or b, Va or b, VIIa or b, VIII a or b is a phosphonic acid-containing compound that binds to a thyroid receptor in the liver, and is optionally an agonist, antagonist, partial agonist or partial antagonist of T3. Inhibition of gluconeogenesis can result in blood glucose lowering in diabetic subjects. Such compounds can exhibit enhanced pharmacokinetics including oral bioavailability and liver drug levels.

In certain embodiments, provided is a method of treatment of a subject in need thereof, the method comprising administering to the subject a phosphoramidate and phosphonamidate compound or formula selected from IIIa or b, IVa or b, Va or b, Va or b, VIIa or b, VIIa or b, XIa or b, IXa or b, X a or b, XIa or b, XIIa or b, XIIIa or b, XIVa or b and
increasing levels of genes associated with gluconeogenesis;

- reducing triglyceride levels, or increasing the ratio of HDL to LDL;

- treating hyperlipidemia or hypercholesterolemia;

- treating obesity, reducing fat content, treating fatty liver, reducing weight or preventing weight gain;

- treating atherosclerosis, coronary heart disease, heart failure, nephrotic syndrome, or chronic renal failure;

- lowering blood glucose levels, treating diabetes, impaired glucose tolerance, metabolic syndrome x, insulin resistance or hyperinsulinemia;

- increasing levels of genes associated with gluconeogenesis;

- decreasing hepatic glycogen levels or maintaining or improving glycemic control;

- amelioration of hyperinsulinemia and/or decrease of glucose levels in diabetic subjects at doses that optionally do not affect cardiac function, e.g., heart rate, force of systolic contraction, duration of diastolic relaxation, vascular tone, or heart weight;

- treating thyroid disease, thyroid cancer, depression, glaucoma, cardiac arrhythmias, heart failure, or osteoporosis;

- increasing mitochondrial biogenesis, or increasing expression of PGC-1, AMP activated protein kinase or nuclear respiratory factor;

- inhibiting hepatic gluconeogenesis; or

- modulating expression of certain genes in the liver resulting in effects on lipids (e.g., cholesterol), glucose, lipoproteins, and triglycerides, or modulation of T3-responsive genes.

- In certain embodiments, the compounds do not affect thyroid function, thyroid production of circulating iodinated thyronines such as T3 and T4, and/or the ratio of T3 to T4.

- In certain embodiments, provided herein is a method for treatment of liver fibrosis or inflammation by administering a compound provided herein.

- Also provided are pharmaceutical compositions comprising the compounds, e.g., in a dosage unit suitable for administration, e.g., oral administration.

- Optically Active Compounds

- It is appreciated that compounds provided herein have several chiral centers and may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that any racemic, optically-active, diastereomeric, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound provided herein, which possess the useful properties described herein is within the scope of the invention. Techniques known in the art can be used to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase).

- Examples of methods to obtain optically active materials are known in the art, and include at least the following:

- i) physical separation of crystals—a technique whereby macroscopic crystals of the individual enantiomers are manually separated. This technique can be used if crystals of the separate enantiomers exist, i.e., the material is a conglomerate, and the crystals are visually distinct;

- ii) simultaneous crystallization—a technique whereby the individual enantiomers are separately crystallized from a solution of the racemate, possible only if the latter is a conglomerate in the solid state;

- iii) enzymatic resolutions—a technique whereby partial or complete separation of a racemate by virtue of differing rates of reaction for the enantiomers with an enzyme;

- iv) enzymatic asymmetric synthesis—a synthetic technique whereby at least one step of the synthesis uses an enzymatic reaction to obtain an enantiomerically pure or enriched synthetic precursor of the desired enantiomer;

- v) chemical asymmetric synthesis—a synthetic technique whereby the desired enantiomer is synthesized from an achiral precursor under conditions that produce asymmetry (i.e., chirality) in the product, which may be achieved using chiral catalysts or chiral auxiliaries;

- vi) diastereomer separations—a technique whereby a racemic compound is reacted with an enantiomerically pure reagent (the chiral auxiliary) that converts the individual enantiomers to diastereomers. The resulting diastereomers are then separated by chromatography or crystallization by virtue of their now more distinct structural differences and the chiral auxiliary later removed to obtain the desired enantiomer;

- vii) first- and second-order asymmetric transformations—a technique whereby diastereomers from the racemate equilibrate to yield a preponderance in solution of the diastereomer from the desired enantiomer or where preferential crystallization of the diastereomer from the desired enantiomer perturbs the equilibrium such that eventually in principle all the material is converted to the crystalline diastereomer from the desired enantiomer. The desired enantiomer is then released from the diastereomer;

- viii) kinetic resolutions—this technique refers to the achievement of partial or complete resolution of a racemate (or of a further resolution of a partially resolved compound) by virtue of unequal reaction rates of the enantiomers with a chiral, non-racemic reagent or catalyst under kinetic conditions;

- ix) enantiospecific synthesis from non-racemic precursors—a synthetic technique whereby the desired enantiomer is obtained from non-chiral starting materials and where the stereochemical integrity is not or is only minimally compromised over the course of the synthesis;

- x) chiral liquid chromatography—a technique whereby the enantiomers of a racemate are separated in a liquid mobile phase by virtue of their differing interactions with a stationary phase. The stationary phase can be made of chiral material or the mobile phase can contain an additional chiral material to provoke the differing interactions;

- xi) chiral gas chromatography—a technique whereby the racemate is volatilized and enantiomers are separated by virtue of their differing interactions in the gaseous mobile phase with a column containing a fixed non-racemic chiral absorbent phase;
[0223] xi) extraction with chiral solvents—a technique whereby the enantiomers are separated by virtue of preferential dissolution of one enantiomer into a particular chiral solvent;

[0224] xiii) transport across chiral membranes—a technique whereby a racemate is placed in contact with a thin membrane barrier. The barrier typically separates two miscible fluids, one containing the racemate, and a driving force such as concentration or pressure differential causes preferential transport across the membrane barrier. Separation occurs as a result of the non-racemic chiral nature of the membrane which allows only one enantiomer of the racemate to pass through.

Preparation of Compounds

[0225] The compounds provided herein can be prepared, isolated or obtained by any method apparent to those of skill in the art. Exemplary methods of preparation are described in detail in the examples below.

[0226] In certain embodiments, compounds provided herein can be prepared by coupling alcohols and H-phosphonate monoesters as illustrated in the reaction scheme below:

Scheme A1:

\[ \text{Scheme A1:} \]

\[ \text{R}^7 \text{S} - \text{O} - \text{OH} + \text{O} - \text{P} - \text{O} - \text{R} \quad \text{Coupling reagent} \]

\[ \text{R}^7 \text{S} - \text{O} - \text{O} - + \text{HO} - \text{R} \quad \text{Coupling reagent} \]

\[ \text{R}^7 \text{S} - \text{O} - \text{P} - \text{O} - \text{R} \]

Scheme A2:

\[ \text{Scheme A2:} \]

\[ \text{R}^7 \text{S} - \text{O} - \text{OH} + \text{O} - \text{P} - \text{O} - \text{R}^8 \quad \text{Coupling reagent} \]

\[ \text{R}^7 \text{S} - \text{O} - \text{O} - + \text{HO} - \text{R} \quad \text{Coupling reagent} \]

\[ \text{R}^7 \text{S} - \text{O} - \text{P} - \text{O} - \text{R} \]

\[ \text{R}^7 \text{S} - \text{O} - \text{O} - + \text{HO} - \text{R} \quad \text{Coupling reagent} \]

\[ \text{R}^7 \text{S} - \text{O} - \text{P} - \text{O} - \text{R} \]

\[ \text{R}^7 \text{S} - \text{O} - \text{O} - + \text{HO} - \text{R} \quad \text{Coupling reagent} \]

\[ \text{R}^7 \text{S} - \text{O} - \text{P} - \text{O} - \text{R} \]
wherein R^7, R^8, R^9, R^10 are each independently hydrogen, hydroxy, alkyl or alkoxy. Any reactive function on R^7, R^8, R^9, R^10 on the base should be protected during the coupling reaction. Any coupling agent known to one of skill in the art can be used. Exemplary coupling agents for use in the reaction include, but are not limited to HOBt (N-Hydroxybenzotriazole), HBTU (2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylinum hexafluorophosphate), DCC (N,N'-dicyclohexylcarbodiimide), BOP (Benzotriazole-1-yl-oxytris(dimethylamino)-phosphoniumhexafluorophosphate), PyBOP (1H-benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) and others known to one of skill in the art.

[0227] A general scheme for the synthesis of hydroxytBu-SATE N-benzylphosphoramidate nucleoside derivatives represented by B is provided in Schemes B1-B3 below, in which modifications of nucleosides are made by way of example, but the methodology may be used for other active agents as well.

Scheme B1: Synthesis of the H-phosphonate monoester reagent

Scheme B2: Synthesis of the protected nucleosides (R = DMTr and/or R^3/R^5 = isopropylidene)

where R = H, Tr, MMT or DMTr in case of reactive amine; R^1, R^2, R^4, R^6 = H, alkyl or halo and R^3/R^5 are both H or isopropylidene.
In addition, certain nucleosides and analogs thereof and prodrugs thereof can be prepared according to methods known to one of skill in the art. Exemplary nucleosides and analogs are described in International Publication No. WO 06/125166, contents of which are hereby incorporated by reference in their entirety.

The compounds of formula IIIa or b, IVa or b, Va or b, VIa or b or b, VIIa or b and VIII a or b can be prepared by methods described herein and methods known to one of skill in the art, for example, see, Erion et al., Proc. Natl. Acad. Sci., 2007, 104, 15490-15495.

The compounds of formula IXa or b, X a or b, Xla or b, XIIa or b, XIIIa or b, XIVa or b and XVIIa or b can be prepared by methods described herein and methods known to one of skill in the art, for example, see, Dang et al., Discovery of Potent and Specific Fructose-1,6-Bisphosphatase Inhibitors and a Series of orally-Bioavailable Phosphorimidase-Sensitive Prodrugs for the Treatment of Type 2 Diabetes, J. Am. Chem. Soc., 2007, Vol. 129, No. 50, pp. 15491-502.

Assays for other activities, including anti-cancer activity can be done as described in the art. Suitable in vitro assays can be used to preliminarily evaluate the efficacy of a compound in inhibiting growth of cancer cells. The compound can further be evaluated for its efficacy in treating cancer in vivo assays known to those of skill in the art. For example, it can be administered to an animal (e.g., a mouse model) having cancer and its therapeutic effect can then be assessed. Based on the results, an appropriate dosage range and administration route can also be determined. Exemplary assays are described in the paragraphs below.

Anti-Cancer Activity

Compounds provided herein can be shown to inhibit tumor cell proliferation, cell transformation and tumorigenesis in vitro. In vivo, a variety of assays known in the art, or described herein. Such assays can use cells of a cancer cell line, or cells from a patient. Many assays well-known in the art can be used to assess such survival and/or growth, for example, cell proliferation can be assayed by measuring (3H)-
thymidine incorporation, by direct cell count, by detecting changes in transcription, translation or activity of known genes such as proto-oncogenes (e.g., fos, myc) or cell cycle markers (Rb, cdc2, cyclin A, D1, D2, D3, E, etc.). The levels of such protein and mRNA and activity can be determined by any method well known in the art. For example, protein can be quantitated by known immunodiagnostic methods such as Western blotting or immunoprecipitation using commercially available antibodies (for example, many cell cycle marker antibodies are available from Santa Cruz Biototechnology, Inc., Santa Cruz, Calif.). mRNA can be quantitated by methods that are well known and routine in the art, for example, by Northern analysis, RNase protection, and the polymerase chain reaction in connection with the reverse transcription. Cell viability can be assessed by using trypan-blue staining or other cell death or viability markers known in the art. Differentiation can be assessed, for example, visually based on changes in morphology, etc.

[0237] Cell proliferation analysis can be performed using a variety of techniques known in the art, including but not limited to the following.

[0238] As one example, bromodeoxyuridine (BrdU) incorporation may be used as an assay to identify proliferating cells. The BrDU assay identifies a cell population undergoing DNA synthesis by incorporation of BrDU into newly synthesized DNA. Newly synthesized DNA can then be detected using an anti-BrdU antibody (see Hoshino et al., 1986, Int. J. Cancer 38, 369; Campana et al., 1988, J. Immunol. Meth. 107, 97).

[0239] Cell proliferation can also be examined using (3H)-thymidine incorporation (see e.g., Chen, J., 1996, Oncogene 13:1395-403; Jeang, J., 1995, J. Biol. Chem. 270:18367-73). This assay allows for quantitative characterization of S-phase DNA synthesis. In this assay, cells synthesizing DNA incorporate (3H)-thymidine into newly synthesized DNA. Incorporation can then be measured using standard techniques in the art such as by counting of radioisotope in a Scintillation counter (e.g., Beckman LS 3800 Liquid Scintillation Counter).

[0240] Detection of proliferating cell nuclear antigen (PCNA) can also be used to measure cell proliferation. PCNA is a 36 kilodalton protein whose expression is elevated in proliferating cells, particularly in early G1 and S phases of the cell cycle and therefore can serve as a marker for proliferating cells. Positive cells are identified by immunostaining using an anti-PCNA antibody (see Li et al., 1996, Curr. Biol. 6:189-199; Vassilev et al., 1995, J. Cell Sci. 108:1205-15).

[0241] Cell proliferation can be measured by counting samples of a cell population over time (e.g. daily cell counts). Cells may be counted using a hemacytometer and light microscopy (e.g. HuyLite hemacytometer, Hauser Scientific). Cell number may be plotted against time in order to obtain a growth curve for the population of interest. In a preferred embodiment, cells counted by this method are first mixed with the dye Trypan-blue, such that living cells exclude the dye, and are counted as viable members of the population.

[0242] DNA content and/or mitotic index of the cells can be measured, for example, based on the DNA ploidy value of the cell. For example, cells in the G1 phase of the cell cycle generally contain a 2N DNA ploidy value. Cells in which DNA has been replicated but have not progressed through mitosis (e.g. cells in S-phase) exhibit a ploidy value higher than 2N and up to 4N DNA content. Ploidy value and cell cycle kinetics can be further measured using propidium iodide assay (see e.g. Turner, L., et al., 1998, Prostate 34:175-81). Alternatively, the DNA ploidy can be determined by quantitation of DNA Feulgen staining (which binds to DNA in a stoichiometric manner) on a computerized microdensitometry staining system (see e.g., Bacus, S., 1989, Am. J. Pathol. 135:783-92). In an another embodiment, DNA content can be analyzed by preparation of a chromosomal spread (Zabaleau, S., 1994, Hereditas. 120:127-40; Pardue, 1994, Meth. Cell Biol. 44:333-351).

[0243] The expression of cell-cycle proteins (e.g., CycA, CycB, CycC, CycD, cdc2, Cdk4/6, Rb, p21, p27, etc.) provide information relating to the proliferative state of a cell or population of cells. For example, identification in an anti-proliferation signaling pathway can be indicated by the induction of p21. Increased levels of p21 expression in cells results in delayed entry into G1 of the cell cycle (Harper et al., 1993, Cell 75:805-816; Li et al., 1996, Curr. Biol. 6:189-199). p21 induction can be identified by immunostaining using a specific anti-p21 antibody available commercially (e.g., Santa Cruz Biototechnology, Inc., Santa Cruz, Calif.). Similarly, cell-cycle proteins may be examined by Western blot analysis using commercially available antibodies. In another embodiment, cell populations are synchronized prior to detection of a cell cycle protein. Cell cycle proteins can also be detected by FACS (fluorescence-activated cell sorter) analysis using antibodies against the protein of interest.

[0244] Detection of changes in length of the cell cycle or speed of cell cycle can also be used to measure inhibition of cell proliferation by the compounds provided herein. In one embodiment, the length of the cell cycle is determined by the doubling time of a population of cells (e.g., using cells contacted or not contacted with one or more compounds identified using the pharmacophores of the present invention). In another embodiment, FACS analysis is used to analyze the phase of cell cycle progression, or purify G1, S, and G2/M fractions (see e.g., Delia, D. et al., 1997, Oncogene 14:2137-47).

[0245] The compounds useful in the methods of the present invention can also be demonstrated to inhibit cell transformation (or progression to malignant phenotype) in vitro. In this embodiment, cells with a transformed cell phenotype are contacted with one or more compounds of the present invention, and examined for change in characteristics associated with a transformed phenotype (a set of in vitro characteristics associated with a tumorigenic ability in vivo), for example, but not limited to, colony formation in soft agar, a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar transport, decreased serum requirement, or expression of fetal antigens, etc. (see Luria et al., 1978, General Virology, 3d Ed., John Wiley & Sons, New York, pp. 436-446).

[0246] Loss of invasiveness or decreased adhesion may also be used to demonstrate the anti-cancer effects of the compounds useful in the methods of the present invention. For example, a critical aspect of the formation of a metastatic cancer is the ability of a precancerous or cancerous cell to detach from primary site of disease and establish a novel colony of growth at a secondary site. The ability of a cell to invade peripheral sites is reflective of a potential for a cancerous state. Loss of invasiveness may be measured by a variety of techniques known in the art including, for example, induction of E-cadherin-mediated cell-cell adhesion. Such E-cadherin-mediated adhesion can result in phenotypic reversion and loss of invasiveness (Hordijk et al., 1997, Science 278:1464-66).

[0247] Loss of invasiveness may further be examined by inhibition of cell migration. A variety of 2-dimensional and 3-dimensional cellular matrices are commercially available (Calbiochem-Novabiochem Corp, San Diego, Calif.).
migration across or into a matrix may be examined by microscopy, time-lapse photography or videography, or by any method in the art allowing measurement of cellular migration. In a related embodiment, loss of invasiveness is examined by response to hepatocyte growth factor (HGF). HGF-induced cell scattering is correlated with invasiveness of cells such as Madin-Darby canine kidney (MDCK) cells. This assay identifies a cell population that has lost cell scattering activity in response to HGF (Hordijk et al., 1997, Science 278:1464-66).

[0248] Alternatively, loss of invasiveness may be measured by cell migration through a chemotaxis chamber (Neuroprobe/Precision Biochemicals Inc., Vancouver, BC). In such an assay, a chemotactic agent is incubated on one side of the chamber (e.g., the bottom chamber) and cells are plated on a filter located on the opposite side (e.g., the top chamber). In order for cells to pass from the top chamber to the bottom chamber, the cells must actively migrate through small pores in the filter. Checkerboard analysis of the number of cells that have migrated may then be correlated with invasiveness (see e.g., Ohnishi, T., 1993, Biochem. Biophys. Res. Commun. 193:518-25).

[0249] The compounds provided herein can also be demonstrated to inhibit tumor formation in vivo. A number of animal models of hyperproliferative disorders, including tumors and metastatic spread, are known in the art (see Table 317-1, Chapter 317, 'Principals of Neoplasia,' in Harrison's Principles of Internal Medicine, 13th Edition, Isselbacher et al., eds., McGraw-Hill, New York, p. 1814, and Lovejoy et al., 1997, J. Pathol. 181:130-135).

[0250] For example, a compound provided herein can be administered to a test animal, preferably a test animal predisposed to develop a type of tumor, and the test animal subsequently examined for decreased incidence of tumor formation in comparison with controls not administered the compound identified using the pharmacophores of the present invention. Alternatively, a compound useful in the methods of the present invention can be administered to test animals having tumors (e.g., animals in which tumors have been induced by introduction of malignant, neoplastic, or transformed cells, or by administration of a carcinogen) and subsequently examining the tumors in the test animals for tumor regression in comparison to controls that were not administered the compound.

[0251] Thyroid Receptor Binding Activity

[0252] Thyroid receptor binding activity that can serve as a mechanism for treatment of diseases sensitive thereto can be tested using assays available in the art. Thyroid hormones (TH) are synthesized in the thyroid in response to thyroid stimulating hormone (TSH), which is secreted by the pituitary gland in response to various stimulants. Thyroid hormones are iodinated O-aryl tyrosine analogues excreted into the circulation primarily as 3,3',5,5'-tetraiodothyronine (T4). T4 is rapidly deiodinated in local tissues by thyroid 5'-deiodinase to 3,3',5'-triiodothyronine (T3), which is the most potent TH. Most of the circulating T4 and T3 is eliminated through the liver.

[0253] THs have profound physiological effects in animals and humans. Hyper-thyroidism is associated with increased body temperature, general nervousness, weight loss despite increased appetite, muscle weakness and fatigue, increased bone resorption and enhanced calcification, and a variety of cardiovascular changes, including increased heart rate, increased stroke volume, increased cardiac index, cardiac hypertrophy, decreased peripheral vascular resistance, and increased pulse pressure. Hypothyroidism is generally associated with the opposite effects.

[0254] The biological activity of THs is mediated largely through thyroid hormone receptors (TRs). TRs belong to the nuclear receptor superfamily, which, along with its common partner, the retinoid X receptor, form heterodimers that act as ligand-inducible transcription factors.


[0256] THs also stimulate metabolism of cholesterol to bile acids. Hyperthyroidism leads to decreased plasma cholesterol levels, which is likely due to increased hepatic LDL receptor expression. Hypothyroidism is a well-established cause of hypercholesterolemia and elevated serum LDL. L-T3 is known to lower plasma cholesterol levels. In addition, THs are known to affect levels of other lipoproteins linked to atherosclerosis. THs stimulate apo AI and the secretion of apo AI in HDL while reducing apo B100. Accordingly, one would expect T3 and T3 mimetics to inhibit the atherosclerotic process in the cholesterol fed animal.

[0257] THs simultaneously increase de novo fatty acid synthesis and oxidation through effects on enzymes such as ACC, FAS, and spot-14. THs increase circulating free fatty acids (FFA) levels in part by increasing production of FFAs from adipose tissue via THs-induced lipolysis. In addition, THs increase mitochondrial enzyme levels involved in FFA oxidation, e.g., carnitine palmitoyltransferase 1 (CPT-1) and enzymes involved in energy storage and consumption.

[0258] The liver represents a major target organ of THs. Microarray analysis of hepatic gene expression from livers of hypothyroid mice and mice treated with T3 showed changes in mRNA levels for 55 genes (14 positively regulated and 41 negatively regulated) (Feng et al., Mol. EndocrinoL 14(7): 947-55 (2000)). Others have estimated that approximately 8% of the hepatic genes are regulated by T3. Many of these genes are important to both fatty acid and cholesterol synthesis and metabolism. T3 is also known to have other effects in liver, including effects on carbohydrates through increased glycogenolysis and gluconeogenesis and decreased insulin action.

[0259] TH has been used as an antiobesity drug for over 50 years. In the 1940s TH was used alone, whereas in the 1950s it was used in combination with diuretics and in the 1960s in combination with amphetamines. Treating hypothyroidism patients with T3 leads to a decrease in body weight for most patients. T3 and T3 mimetics are thought to inhibit atherosclerosis by modulating the levels of certain lipoproteins known to be independent risk factors or potential risk factors of atherosclerosis, including low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, apoAI, which is a major apoprotein constituent of high density lipoprotein (HDL) particles and lipoprotein (a) or Lp(a).

[0260] Hyperthyroidism worsens glycemic control in type 2 diabetics. TH therapy is reported to stimulate hepatic gluconeogenesis. Enzymes specific to gluconeogenesis and important for controlling the pathway and its physiological role of producing glucose are known to be influenced by TH therapy. Phosphoenolpyruvate carboxykinase (PEPCK) is upregulated by TH (Park et al., J. Biol. Chem. 274:211 (1999)) whereas others have found that glucose 6-phosphatase is upregulated (Feng et al., Mol. Endocrinol. 14:947 (2000)). TH therapy is also associated with reduced glycogen levels. TH therapy results in improved non insulin stimulated and insulin stimulated glucose utilization and decreased insulin resistance in the muscle of ob/ob mice. (Oh et al., J. Nutr. 125:125 (1995)).
Thus, thyromimetics potentially can be used to modulate cholesterol levels, to treat obesity, and other metabolic disorders especially with reduced undesirable effects.

Studies can be used to determine the affinity of T3 and various thyromimetics for human thyroid hormone receptors TRα1 and TRβ1, and their resulting efficacy in related disorders. Binding of compounds to either the TRα1 or TRβ1 receptors can be performed by means of scintillation proximity assays (SPA). The SPA assay, a common method used for the quantitation of receptor-ligand equilibria, makes use of special beads coated with a scintillant and a capture molecule, copper, which binds to the histidine-tagged α or β receptor. When labeled T3 is mixed with receptor and the SPA beads, radioactive counts are observed only when the complex of protein and radiolabeled ligand is captured on the surface of the bead. Displacement curves are generated with labeled T3 and increasing concentrations of unlabeled thyromimetics of interest. Subacute studies can be used in ZDF Rats (Charles River Laboratory) to demonstrate an improved therapeutic index for T3 Mimetics.

Subacute studies also can be conducted in cholesterol-fed rats. The cholesterol-fed rat is an animal model of hypercholesterolemia generated by feeding the animals a diet with high cholesterol content. The purpose of these studies is to evaluate the effects of compounds on serum cholesterol (an efficacy parameter) and on heart weight and heart mGSH activity (potential toxicity parameters). Compounds can be administered, e.g., IP, e.g., once-a-day for seven days.

Microsome/primary hepatocyte stability studies can be conducted using methods available in the art. Prodrug activation in rat liver microsomes can be conducted to determine the kinetics of activation of prodrugs of thyromimetics in microsomal preparations. Microsomes may contain P450 enzyme that may activate a prodrug. The Km, Vmax, and intrinsic clearance values determined are measures of prodrug affinity for the microsomal enzymes, the rate at which the prodrug is activated, and the catalytic efficiency with which the prodrug is activated, respectively. Prodrugs also can be tested for conversion to their respective parent compounds by human liver S9. The S9 fraction is a fraction that contains both cytosolic and microsomal protein. Uptake and activation of prodrug in isolated rat hepatocytes also can be conducted using methods known in the art. Oral bioavailability and liver distribution following oral administration also can be measured using methods available in the art.

Oxygen consumption studies can be conducted. Thermogenesis is a measurement of energy consumption. Compounds that increase thermogenesis are likely to increase caloric expenditure and thereby cause body weight loss and its associated benefits to metabolic status (e.g., insulin sensitivity). Thermogenesis is assessed in subcellular fractions of various tissues, isolated cells, whole tissues, or in whole animals using changes in oxygen consumption as the endpoint. Oxygen is used up when calories are burned by various metabolic processes.

Mitochondrial thermogenesis is measured polarographically with a Clark-type oxygen electrode using mitochondrial isolated from various tissues, including liver. Mitochondria are isolated by differential centrifugation. State 3 respiration or cytochrome c oxidase activity are measured in isolated mitochondria. (Issa, S., FEBS Letters, 544: 133-7 (2003)). Oxygen consumption rates are measured in isolated hepatocytes using a portable Clark-type oxygen electrode placed in the hepatocyte medium. Hepatocytes are isolated from liver using a two-step collagenase perfusion (Berry, M. N., Friend, D. S., J. Cell Biol 43: 506-520 (1969)) as modified by Groen (Groen, A. K. et al., Eur J Biochem 122: 87-93 (1982)). Non-parenchymal cells are removed using a Percoll gradient and the cells are resuspended in tissue culture medium in a spinner flask. The oxygen consumption of the cells is measured over time once the system is sealed.

Oxygen consumption also can be measured in isolated perfused liver (Fernandez, V., Toxicol Lett, 69:205-10 (1995)). Liver is perfused in situ and oxygen consumption is calculated by measuring the difference between the oxygen saturation of the inflow buffer and the outflow buffer maintained at a constant flow. Whole animal oxygen consumption can be measured using an indirect calorimeter (Oxymax, Columbus Instruments, Columbus, Ohio). Animals are removed from their cages and placed in the chambers. The resting oxygen consumption is measured in animals during periods of inactivity as measured by activity monitors. The oxygen consumption is calculated based on the flow through the chamber and the difference in oxygen partial pressures at the inflow and outlet ports. Carbon dioxide efflux is also measured in parallel using a CO2 electrode.

Tissue distribution and the pharmacokinetics of compounds can be assessed following IP or oral administration to normal rats.

Studies can be conducted to evaluate the effects of a T3 mimetic on serum cholesterol and TSH levels, hepatic and cardiac gene expression and enzyme activities, heart weight, and clinical chemistry parameters using methods available in the art. In one embodiment, rats are made hypercholesterolemic by maintaining a diet containing 1.5% cholesterol and 0.5% cholic acid for at least 2 weeks prior to initiation of treatment. Plasma cholesterol values are assessed prior to and following treatment and the effects of compound are expressed as a percentage change from the pre-dose cholesterol levels. Total cholesterol is analyzed using a commercially available enzymatic kit (Sigma Diagnostics, St. Louis, Mo.).

Effects of T3 mimetic compounds (and prodrugs thereof) in vivo on glucose can be measured in ZDF rats. T3 and T3 mimetic mediated myosin heavy chain gene transcription in the heart can be measured. An RT-PCR assay as disclosed in: Sara Danzi, Kaie Ojamaa, and Irwin Klein Am J Physiol Heart Circ Physiol 284: H2255-H2262, 2003 is used to study both the time course and the mechanism for the triiodothyronine (T3)-induced transcription of the α- and β-myosin heavy chain (MHC) genes in vivo on the basis of the quantity of specific heterogeneous nuclear RNA (hnRNA). The temporal relationship of changes in transcriptional activity to the amount of α-MHC mRNA and the coordinated regulation of transcription of more than one gene in response to T3 and T3 mimetics are demonstrated. Analysis of a time course of T3 and T3 mimetics that are not liver specific show mediated induction of α-MHC hnRNA and repression of β-MHCUn mRNA, whereas no significant effect is observed with compounds at doses that are therapeutically useful.

The effect of T3 on cardiovascular function (heart rate, inotropic state, and aortic pressure) can be studied in the Sprague Dawley (SD) rat model using assay methods known in the art.

Thus, various assays known in the art can be used to assay for thyroid hormone agonist and its accompanying therapeutic activity and to establish appropriate dosages.

Methods of Use

The phosphoromimidate and phosphonoumidate compounds of a variety of therapeutic agents can be formed using methods available in the art and those disclosed herein. Such compounds can be used in some embodiments to enhance delivery of the drug to the liver. In one embodiment,
the compound comprises a S-acyl-2-thioethyl phosphoromoi-
date or S-acyl-2-thioethyl phosphonoamide, e.g., a S-piv-
alkyl-2-thioethyl phosphoroamidate or S-hydroxypropyl-2-
thioethyl phosphonoiminate derivative. Therapeutic agents
that can be derivatized to phosphoroamidate or phosphonoa-
midate compound form include a therapeutic agent such as an
anti-cancer agent or anti-diabetic agent that includes, or has
been derivatized to include a reactive group for attachment of
the phosphoroamidate or phosphonoamide moiety.

[0275] Cancer Treatment Methods

[0276] In one embodiment, therapeutic agents for the treat-
ment of liver cancer can be derivatized to form a phospho-
raimidate or phosphonoamide compound as described herein,
and used for the treatment of liver cancers. Liver cancers
that can be treated include benign tumors, malignant tumors,
hemangioma, hepatic adenomas, focal nodular hyperplasia,
hepato-cellular carcinoma, fibrolamellar carcinoma, cholangi-
ocarcinomas, bile duct cancers, and other primary and meta-
static cancers of the liver.

[0277] Exemplary therapeutic agents include anti-cancer
agents having one or more hydroxy groups that can be deriv-
ated as compounds described herein by removal of a hydro-
gen from one of the hydroxy groups. Exemplary anti-cancer
agents include, but are not limited to aclacinomycin, decitabine,
doxorubicin, dihydro-5-aza-uridine, doxorubicin, epirubi-
cin, estramustine, etoposide, fludarabine, 7-hydroxyclopro-
mazine, neplanocin A, podophyllotoxin, tezacinabine, troxac-
itabine, vinblastine, vincristin, vindesin, etoposide, teniposide,
NK-611, camptothecin, irinotecan, 9-ami-
ocamptothecin, GC-211, topotecan, paclitaxel, azatxin,
colormycin, piranubicin, nelarabine and loxozaxone. Anti-
cancer agents known in the art and described herein can be
derivatized to form a phosphoroamidate or phosphonoamide
compound as described herein. Immunosuppressants, such as
combretastatin A-4, mycophenolic, pentostatin, or mitox-
axone, also can be derivatized to form a phosphoroamidate
or phosphonoamide compound as described herein.

[0278] Such compounds can optionally be used in combi-
nation with another anti-cancer agent that is optionally in
prodrug form.

[0279] Methods of Treating Metabolic Diseases

[0280] In certain embodiments, the compounds provided
herein are useful in methods for inhibiting gluconeogenesis,
optionally by inhibiting the enzyme fructose 1,6-bisphos-
phatase (FBPase).

[0281] In certain embodiments, the compounds provided
herein are useful in methods for inhibiting gluconeogenesis.

[0282] In certain embodiments, the compounds provided
herein are useful in methods for treatment of metabolic dis-
eases. In certain embodiments, the compounds provided
herein are useful in methods for:

[0283] reducing plasma lipid levels, lowering cholesterol
levels, reducing triglyceride levels, or increasing the ratio of
HDL to LDL;

[0284] lowering blood glucose levels;

[0285] treating hyperlipidemia or hypercholesterolemia;

[0286] treating obesity, reducing fat content, treating fatty
liver, reducing weight or preventing weight gain;

[0287] treating atherosclerosis, coronary heart disease,
heart failure, nephrotic syndrome, or chronic renal failure;

[0288] lowering blood glucose levels, treating diabetes,
impaired glucose tolerance, metabolic syndrome x, insulin
resistance or hyperinsulinemia;

[0289] increasing levels of genes associated with glucone-
genesis;

[0290] decreasing levels of genes associated with glucone-
genesis;

[0291] amelioration of hyperinsulinemia and/or decrease of
blood levels in diabetic subjects at doses that optionally
do not affect cardiac function, e.g., heart rate, force of systolic
contraction, duration of diastolic relaxation, vascular tone, or
heart weight;

[0292] treating thyroid disease, thyroid cancer, depression,
glaucoma, cardiac arrhythmias, heart failure, or osteoporosis;

[0293] increasing mitochondrial biogenesis, or increasing
expression of PGC-1, AMP activated protein kinase or
nuclear respiratory factor;

[0294] inhibiting hepatic gluconeogenesis; or

[0295] modulating expression of certain genes in the liver
resulting in effects on lipids (e.g., cholesterol), glucose, li-
proteins, and triglycerides, or modulation of T3-responsive
genes.

[0296] In certain embodiments, the compounds provided
herein are useful in methods for lowering blood glucose lev-
els, treating diabetes, impaired glucose tolerance, metabolic
syndrome x, insulin resistance or hyperinsulinemia.

[0297] Second Agents Useful in the Methods

[0298] In certain embodiments, the compounds and com-
positions provided herein are useful in methods of treatment
of a liver disorder, that comprises further administration of a
second agent effective for the treatment of the disorder, such
as liver cancer in a subject in need thereof. The second agent
can be any agent known to those of skill in the art to be
effective for the treatment of the disorder, including those
currently approved by the FDA.

[0299] In certain embodiments, a compound provided
herein is administered in combination with one second agent.
In further embodiments, a second agent is administered in
combination with two second agents. In still further embodi-
ments, a second agent is administered in combination with
two or more second agents.

[0300] As used herein, the term “in combination” includes
the use of more than one therapy (e.g., one or more prophy-
lactic and/or therapeutic agents). The use of the term “in
combination” does not restrict the order in which therapies
(e.g., prophylactic and/or therapeutic agents) are adminis-
tered to a subject with a disorder. A first therapy (e.g., a
prophylactic or therapeutic agent such as a compound pro-
duced herein) can be administered prior to (e.g., 5 minutes,
15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours,
6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours,
1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks,
or 12 weeks before), concomitantly with, or subsequent to
(e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour,
2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72
hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks,
6 weeks, 8 weeks, or 12 weeks after) the administration of a
second therapy (e.g., a prophylactic or therapeutic agent) to
a subject with a disorder.

[0301] As used herein, the term “syner-
getic” includes a combination of a compound provided herein and another
therapy (e.g., a prophylactic or therapeutic agent) which has been or is
currently being used to prevent, manage or treat a disorder, which is more
effective than the additive effects of the therapies. A synergistic effect of a combination of ther-
apiies (e.g., a combination of prophylactic or therapeutic agents) permits the use of lower dosages of one or more of the therapies and/or less frequent administration of said therapies to a subject with a disorder. The ability to utilize lower dos-
ages of a therapy (e.g., a prophylactic or therapeutic agent) and/or to administer said therapy less frequently reduces the toxicity associated with the administration of said therapy to a subject without reducing the efficacy of said therapy in the prevention or treatment of a disorder). In addition, a syner-
gistic effect can result in improved efficacy of agents in the prevention or treatment of a disorder. Finally, a synergistic effect of a combination of therapies (e.g., a combination of prophylactic or therapeutic agents) may avoid or reduce adverse or unwanted side effects associated with the use of either therapy alone.

[0302] In certain embodiments, the active compounds provided herein can be administered in combination or alternation with another therapeutic agent, for example an anti-cancer agent. In certain embodiments, the active compounds provided herein can be administered in combination or alternation with second agents useful in treating metabolic disorders such as diabetes, obesity, atherosclerosis, heart disease, metabolic syndrome X, nephrotic syndrome, thyroid disease, and symptoms associated therewith. In combination therapy, effective dosages of two or more agents are administered together, whereas in alternation or sequential-step therapy, an effective dosage of each agent is administered serially or sequentially. The dosages given will depend on absorption, inactivation and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens and schedules should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

[0303] The second agent can be one of the agents disclosed herein. In certain embodiments, contemplated additional pharmacologically active substances include drugs commonly used as chemotherapy for treatment of cancer and immune modulator substances. For example, chemotherapeutic agents include anti-metabolites (e.g., Pentostatin®), DNA polymerase inhibitors (e.g., Gemzar®), RNA polymerase inhibitors (e.g., ECydi®, platinum derivatives (e.g., Paraplatin®), anti-estrogens (e.g., Nolvadex®), Taxanes (e.g., Taxotere®), GnRH analogs (e.g., Lupron®), DNA polymerase inhibitors (e.g., Gemzar®), topoisomerase inhibitors (e.g., Hycamtin®), biphosphonates (e.g., Areduc®), somatoxostatins (e.g., Sandostatin®), nucleoside analogs (e.g., Ribavirin®), and IMPDH-inhibitors (e.g., Tiazofurin®). Contemplated immunomodulatory substances include cytokines (e.g., interferon α and γ, IL-2, IL-4, IL-6, IL-8, IL-10, and IL-12), cytokinins (e.g., kinetin), and chemokines (e.g., MIP-1)...

[0304] In certain embodiments, the second agents for use in combination with the compounds provided herein include other agents useful in the treatment, prevention, suppression or amelioration of the diseases or conditions for which compounds provided herein are useful, such as treating metabolic diseases, including diabetes, obesity, atherosclerosis, heart disease, metabolic syndrome X, nephrotic syndrome, thyroid disease, and symptoms associated therewith. Such second agents include, but are not limited to: sulfonlyureas, for example, glibenclamide (DAONIL®), glimepiride (AMAR억L®), glipizide (GLUCOTROL or MINODIAB), glyburide (MICRONASE®), tolbutamide (ORINASE®), acetohexamide (DIABETES®), tolazamide (TOLINSE®) and chlorpropamide (DIABASE®); insulin and insulin mimetics; biguanides such as metformin (GLUCOPHAGE®); α-glucosidase inhibitors including acarbose (PRECOSE®) and miglitol (GLYSET®); meglitinides, for example, nateglinide (STARLIX®) and repaglinide (PARDIN®); thiazolidinediones, for example, euglitazone, englitazone, rosiglitazone (AVANDIA®), pioglitazone (ACTOS®) and troglitazone (REZULIN®); incretin mimetics such as exenatide (BYETTA™); cholesterol lowering agents such as HMGC-CoA reductase inhibitors (e.g., lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin and other statins), bile acid sequestrants (e.g., cholestyramine and colestipol), vitamin B₃ (also known as nicotinic acid, or niacin), vitamin B₂ (pyridoxine), vitamin B₁₂ (cyanocobalamin), fibric acid derivatives (e.g., gemfibrozil, clofibrate, fenofibrate and bezafibrate), probucol, and inhibitors of cholesterol absorption (e.g., beta-sitosterol and acyclovir-CoA-cholesterol acyltransferase (ACAT) inhibitors such as malinamide), HMG-CoA synthase inhibitors, squalene epoxidase inhibitors and squalene synthase inhibitors; antithrombotic agents, such as thrombolytic agents (e.g., streptokinase, alteplase, anistreplase and reteplase), heparin, hirudin and warfarin derivatives, β-blockers (e.g., atenolol), β-adrenergic agonists (e.g., isoproterenol) and ACE inhibitors and vasodilators (e.g., sodium nitroprusside, nicardipine hydrochloride, nitroglycerin and enalaprilat).

[0305] Pharmaceutical Compositions and Methods of Administration

[0306] Phosphoramidate and phosphonoformate compounds of a variety of therapeutic agents can be formulated into pharmaceutical compositions using methods available in the art and those disclosed herein. Such compounds can be used in some embodiments to enhance delivery of the drug to the liver. In one embodiment, the compound comprises a S-acetyl-2-thioethyl phosphoramidate or S-acetyl-2-thioethyl phosphonoformate, e.g., a S-pivaloyl-2-thioethyl phosphoramidate or S-hydroxyvinyl-2-thioethyl phosphonoformate derivative. In certain embodiments, therapeutic agents that can be derivatized to phosphoramidate or phosphonoformate compound form include any anti-cancer agent that includes, or has been derivatized to include a reactive group for attachment of the phosphoramidate or phosphonoformate moiety, including but not limited to nucleosides and nucleoside analogues including acyclic nucleosides. In certain embodiments, therapeutic agents that can be derivatized to phosphoramidate or phosphonoformate compound form include any thyroid hormone receptor effector that includes, or has been derivatized to include a reactive group for attachment of the phosphoramidate or phosphonoformate moiety. Any of the phosphoramidate or phosphonoformate compounds disclosed herein can be provided in the appropriate pharmaceutical composition and be administered by a suitable route of administration.

[0307] The methods provided herein encompass administering pharmaceutical compositions containing at least one compound as described herein, including a compound of general formula I, Ia, Iib, IIIa, IVa, IXb or IXb if appropriate in the salt form, either used alone or in the form of a combination with one or more compatible and pharmaceutically acceptable carriers, such as diluents or adjuvants, or with other therapeutic agents, such as another anti-cancer or anti-diabetic agent.

[0308] In certain embodiments, the second agent can be formulated or packaged with the compound provided herein. Of course, the second agent will only be formulated with the compound provided herein when, according to the judgment of those of skill in the art, such co-formulation should not interfere with the activity of either agent or the method of administration. In certain embodiments, the compound provided herein and the second agent are formulated separately. They can be packaged together, or packaged separately, for the convenience of the practitioner of skill in the art.

[0309] In clinical practice the active agents provided herein may be administered by any conventional route, in particular orally, parenterally, rectally or by inhalation (e.g. in the form of aerosols). In certain embodiments, the compound provided herein is administered orally.
Use may be made, as solid compositions for oral administration, of tablets, pills, hard gelatin capsules, powders or granules. In these compositions, the active product is mixed with one or more inert diluents or adjuvants, such as sucrose, lactose or starch.

These compositions can comprise substances other than diluents, for example a lubricant, such as magnesium stearate, or a coating intended for controlled release.

Use may be made, as liquid compositions for oral administration, of solutions which are pharmaceutically acceptable, suspensions, emulsions, syrups and elixirs containing inert diluents, such as water or liquid paraffin. These compositions can also comprise substances other than diluents, for example wetting, sweetening or flavoring products.

The compositions for parenteral administration can be emulsions or sterile solutions. Use may be made, as solvents or vehicle, of propylene glycol, a polyethylene glycol, vegetable oils, in particular olive oil, or injectable organic esters, for example ethyl oleate. These compositions can also contain adjuvants, in particular wetting, isotonizing, emulsifying, dispersing and stabilizing agents. Sterilization can be carried out in several ways, for example using a bacteriological filter, by radiation or by heating. They can also be prepared in the form of sterile solid compositions which can be dissolved at the time of use in sterile water or any other injectable sterile medium.

The compositions for rectal administration are suppositories or rectal capsules which contain, in addition to the active principle, excipients such as cocoa butter, semi-synthetic glycerides or polyethylene glycols.

The compositions can also be aerosols. For use in the form of liquid aerosols, the compositions can be sterile solutions or solid compositions dissolved at the time of use in apyrogenic sterile water, in saline or any other pharmaceutically acceptable vehicle. For use in the form of dry aerosols intended to be directly inhaled, the active principle is finely divided and combined with a water-soluble solid diluent or vehicle, for example dextran, mannitol or lactose.

In one embodiment, a composition provided herein is a pharmaceutical composition or a single unit dosage form. Pharmaceutical compositions and single unit dosage forms provided herein comprise a prophylactically or therapeutically effective amount of one or more prophylactic or therapeutic agents (e.g., a compound provided herein, or other prophylactic or therapeutic agent), and a typically one or more pharmaceutically acceptable carriers or excipients. In a specific embodiment and in this context, the term “pharmaceutically acceptable” includes approval by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “carrier” includes a diluent, adjuvant (e.g., Freund’s adjuvant (complete and incomplete)), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water can be used as a carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Examples of suitable pharmaceutical carriers are described in “Remington’s Pharmaceutical Sciences” by E. W. Martin.

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well-known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a subject and the specific active ingredients in the dosage form. The composition or single unit dosage form, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

Lactose free compositions provided herein can comprise excipients that are well known in the art and are listed, for example, in the U.S. Pharmacopeia (USP) SP (XXI): NF (XVI). In general, lactose free compositions comprise an active ingredient, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Exemplary lactose free dosage forms comprise an active ingredient, microcrystalline cellulose, pregelatinized starch, and magnesium stearate.

Further encompassed herein are anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long term storage in order to determine characteristics such as shelf life or the stability of formulations over time. See, e.g., Jens T. Carstensen, Drug Stability: Principles & Practice, 2d Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms provided herein can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine can be anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions can be packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

Further provided are pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as “stabilizers,” include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

The pharmaceutical compositions and single unit dosage forms can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Such compositions and dosage forms will contain a prophylactically or therapeuti-
ally effective amount of a prophylactic or therapeutic agent, in certain embodiments, in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. The formulation should suit the mode of administration. In a certain embodiment, the pharmaceutical composition or single unit dosage forms are sterile and in a suitable form for administration to a subject, for example, an animal subject, such as a mammalian subject, for example, a human subject.

0324] A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, intramuscular, subcutaneous, oral, buccal, sublingual, inhalational, intranasal, transdermal, topical, transmucosal, intratumoral, intravaginal, and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal or topical administration to human beings. In an embodiment, a pharmaceutical composition is formulated in accordance with routine procedures for subcutaneous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

0325] Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suspensions; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; pastes; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a subject, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil in water emulsions, or a water in oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a subject; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a subject.

0326] The composition, shape, and type of dosage forms provided herein will typically vary depending on their use. For example, a dosage form used in the initial treatment of liver cancer may contain larger amounts of one or more of the active ingredients than a dosage form used in the maintenance treatment of the same liver cancer. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients than an oral dosage form used to treat the same disease or disorder. These and other ways in which specific dosage forms encompassed herein will vary from one another and will be readily apparent to those skilled in the art. See, e.g., Remington’s Pharmaceutical Sciences, 20th ed., Mack Publishing, Easton Pa. (2000).

0327] Generally, the ingredients of compositions are supplied either separately or mixed together in unit dosage form, as for example, as a dry lyophilized powder or water (free concentrate) in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

0328] Typical dosage forms comprise a compound provided herein, or a pharmaceutically acceptable salt, solvate or hydrate thereof lie within the range of from about 0.1 mg to about 1000 mg per day, given as a single once-a-day dose in the morning or as divided doses throughout the day taken with food. Particular dosage forms can have about 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 2.5, 5.0, 10.0, 20.0, 25.0, 50.0, 100, 200, 250, 500 or 1000 mg of the active compound.

0329] Oral Dosage Forms

0330] Pharmaceutical compositions that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, Remington’s Pharmaceutical Sciences, 20th ed., Mack Publishing, Easton Pa. (2000).

0331] In certain embodiments, the oral dosage forms are solid and prepared under anhydrous conditions with anhydrous ingredients, as described in detail in the sections above. However, the scope of the compositions provided herein extends beyond anhydrous, solid oral dosage forms. As such, further forms are described herein.

0332] Typical oral dosage forms are prepared by combining the active ingredient(s) in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

0333] Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or non-aqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

0334] For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

0335] Examples of excipients that can be used in oral dosage forms include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose, calcium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

0336] Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein
include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

[0337] Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL PH 101, AVICEL PH 103, AVICEL RC 581, AVICEL PH 105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. An specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC 581. Suitable anhydrous or low moisture excipients or additives include AVICEL PH 101, and Starch 1500 L.M.

[0338] Disintegrants are used in the compositions to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, specifically from about 1 to about 5 weight percent of disintegrant.

[0339] Disintegrants that can be used in pharmaceutical compositions and dosage forms include, but are not limited to, agar agar, algic acid, calcium carbonate, microcrystalline cellulose, crosscarmellose sodium, crospovidone, polacrill potassium, sodium starch glycolate, potato or tapioca starch, pre gelatinized starch, other starches, clays, other alginates, other celluloses, gums, and mixtures thereof.

[0340] Lubricants that can be used in pharmaceutical compositions and dosage forms include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zine stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syndiotactic silicone gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosil of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB O SII (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

[0341] Delayed Release Dosage Forms

[0342] Active ingredients such as the compounds provided herein can be administered by controlled release means or by delivering other celluloses that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; 5,639,480; 5,733,566; 5,739,108; 5,891,474; 5,922,356; 5,972,891; 5,980,945; 5,993,855; 6,045,830; 6,087,324; 6,113,943; 6,197,350; 6,248,363; 6,264,970; 6,267,981; 6,376,401; 6,419,961; 6,589,548; 6,613,558; 6,699,500 of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microencapsules, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients provided herein. Thus encompassed herein are single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled release.

[0343] All controlled release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non controlled counterparts. Ideally, the use of an optimally designed controlled release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled release formulations include extended activity of the drug, reduced dosage frequency, and increased subject compliance. In addition, controlled release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

[0344] Most controlled release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

[0345] In certain embodiments, the drug may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used (see, Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in a subject at an appropriate site determined by a practitioner of skill, i.e., thus requiring only a fraction of the systemic dose (see, e.g., Goodson, Medical Applications of Controlled Release, vol. 2, pp. 115-138 (1984)). Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)). The active ingredient can be dispersed in a solid inner matrix, e.g., polymethylmethacrylate, polybutylmethacrylate, plasticized or unplasticized polyvinylchloride, plasticized nylon, plasticized polyethylene/epichlorohydrin, natural rubber, to those of ordinary skill in the art, and mixtures. Such polymers include, but are not limited to, polyethylene, ethylene-vinylacetate copolymers, silicone rubbers, polydimethylsiloxanes, silicone carbonate copolymers, hydrophilic polymers such as hydrogels of esters of acrylic and methacrylic acid, collagen, cross-linked polyvinylalcohol and cross-linked partially hydrolyzed polyvinyl acetate, that is surrounded by an outer polymeric membrane, e.g., polyethylene, polypropylene, ethylene/propylene copolymers, ethylene/ethyl acrylate copolymers, ethylene/ vinylacetate copolymers, silicone rubbers, polydimethyl...
siloxanes, neoprene rubber, chlorinated polyethylene, polyvinylchloride, vinylchloride copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubber epichlorohydrin rubbers, ethylene/vinyl alcohol copolymer, ethylene/vinyl acetate/vinyl alcohol terpolymer, and ethylene/vinylacroxyethanol copolymer, which is insoluble in body fluids. The active ingredient then diffuses through the outer polymeric membrane in a release rate controlling step. The percentage of active ingredient in such parental compositions is highly dependent on the specific nature thereof, as well as the needs of the subject.

[0346] Parenteral Dosage Forms

[0347] In one embodiment, provided are parenteral dosage forms. Parenteral dosage forms can be administered to subjects by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intrarterial. Because their administration typically bypasses subjects’ natural defenses against contaminants, parenteral dosage forms are typically, sterile or capable of being sterilized prior to administration to a subject. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

[0348] Suitable vehicles that can be used to provide parenteral dosage forms are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer’s Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer’s Injection; water miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

[0349] Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms.

[0350] Transdermal, Topical & Mucosal Dosage Forms

[0351] Also provided are transdermal, topical, and mucosal dosage forms. Transdermal, topical, and mucosal dosage forms include, but are not limited to, ophthalmic solutions, sprays, aerosols, creams, lotions, ointments, gels, solutions, emulsions, suspensions, or other forms known to those skilled in the art. See, e.g., Remington’s Pharmaceutical Sciences, 16th, 18th and 20th eds., Mack Publishing, Easton Pa. (1980, 1990 & 2000); and Introduction to Pharmaceutical Dosage Forms, 4th ed., Leu & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels. Further, transdermal dosage forms include “reservoir type” or “matrix type” patches, which can be applied to the skin and worn for a specific period of time to permit the penetration of a desired amount of active ingredients.

[0352] Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide transdermal, topical, and mucosal dosage forms encompassed herein are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane 1.3 diol, isopropylmyristate, isopropyl palmitate, mineral oil, and mixtures thereof to form lotions, tinctures, creams, emulsions, gels or ointments, which are non toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., Remington’s Pharmaceutical Sciences, 16th, 18th and 20th eds., Mack Publishing, Easton Pa. (1980, 1990 & 2000).

[0353] Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients provided. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, oleyl, and tetrahydrofurfuryl; alkyl sulfonates such as dimethyl sulfonate; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvinone); urea; and various water soluble or insoluble sugar esters such as Tween 80 (polysorbate 80) and Spau 60 (sorbitan monooleate).

[0354] The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or toxicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery enhancing or penetration enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

[0355] Dosage and Unit Dosage Forms

[0356] In human therapeutics, the doctor will determine the posology which he considers most appropriate according to a preventive or curative treatment and according to the age, weight, stage of the disease, for example, cancer and other factors specific to the subject to be treated. In certain embodiments, doses are from about 1 to about 1000 mg per day for an adult, or from about 5 to about 250 mg per day or from about 10 to 50 mg per day for an adult. In certain embodiments, doses are from about 5 to about 400 mg per day or 25 to 200 mg per day per adult. In certain embodiments, dose rates of from about 50 to about 500 mg per day are also contemplated.

[0357] In further aspects, provided are methods of treating or preventing liver cancer in a subject by administering, to a subject in need thereof, an effective amount of a compound provided herein, or a pharmaceutically acceptable salt thereof. In other aspects, provided are methods of treating or preventing metabolic diseases in a subject by administering, to a Subject in need thereof, an effective amount of a compound provided herein, or a pharmaceutically acceptable salt thereof. The amount of the compound or composition which will be effective in the prevention or treatment of a disorder or one or more symptoms thereof will vary with the nature and severity of the disease or condition, and the route by which the active ingredient is administered. The frequency and dosage will also vary according to factors specific for each subject depending on the specific therapy (e.g., therapeutic or prophylactic agents) administered, the severity of the disorder, disease, or condition, the route of administration, as well as age, body, weight, response, and the past medical history of the subject. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0358] In certain embodiments, exemplary doses of a composition include milligram or microgram amounts of the
active compound per kilogram of subject or sample weight (e.g., about 10 micrograms per kilogram to about 50 milligrams per kilogram, about 100 micrograms per kilogram to about 25 milligrams per kilogram, or about 100 microgram per kilogram to about 10 milligrams per kilogram). For compositions provided herein, in certain embodiments, the dosage administered to a subject is 0.140 mg/kg to 3 mg/kg of the subject's body weight, based on weight of the active compound. In certain embodiments, the dosage administered to a subject is between 0.20 mg/kg and 2.00 mg/kg, or between 0.30 mg/kg and 1.50 mg/kg of the subject's body weight.

[0359] In certain embodiments, the recommended daily dose range of a composition provided herein for the conditions described herein lie within the range of from about 0.1 mg to about 1000 mg per day, given as a single once-a-day dose or as divided doses throughout a day. In one embodiment, the daily dose is administered twice daily in equally divided doses. In certain embodiments, a daily dose range is from about 10 mg to about 200 mg per day, in other embodiments, between about 10 mg and about 150 mg per day, in further embodiments, between about 25 and about 100 mg per day. It may be necessary to use dosages of the active ingredient outside the ranges disclosed herein in some cases, as will be apparent to those of ordinary skill in the art. Furthermore, it is noted that the clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with subject response.

[0360] Different therapeutically effective amounts may be applicable for different diseases and conditions, as will be readily known by those of ordinary skill in the art. Similarly, amounts sufficient to prevent, manage, treat, or ameliorate such disorders, but insufficient to cause, or sufficient to reduce, adverse effects associated with the composition provided herein are also encompassed by the above described dosage amounts and dose frequency schedules. Further, when a subject is treated with a single dosage form, not all of the dosages need be the same. For example, the dosage administered to the subject may be increased to improve the prophylactic or therapeutic effect of the composition or it may be decreased to reduce one or more side effects that a particular subject is experiencing.

[0361] In certain embodiment, the dosage of the composition provided herein, based on weight of the active compound, administered to prevent, treat, manage, or ameliorate a disorder, or one or more symptoms thereof in a subject is 0.1 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 10 mg/kg, or 15 mg/kg or more of a subject's body weight. In another embodiment, the dosage of the composition or a composition provided herein administered to prevent, treat, manage, or ameliorate a disorder, or one or more symptoms thereof in a subject is a unit dose of 0.1 mg to 200 mg, 0.1 mg to 100 mg, 0.1 mg to 50 mg, 0.1 mg to 25 mg, 0.1 mg to 20 mg, 0.1 mg to 15 mg, 0.1 mg to 10 mg, 0.1 mg to 7.5 mg, 0.1 mg to 5 mg, 0.1 to 2.5 mg, 0.25 to 20 mg, 0.25 to 15 mg, 0.25 to 12 mg, 0.25 to 10 mg, 0.25 to 7.5 mg, 0.25 mg to 5 mg, 0.5 mg to 2.5 mg, 1 mg to 20 mg, 1 mg to 15 mg, 1 mg to 12 mg, 1 mg to 10 mg, 1 mg to 7.5 mg, 1 mg to 5 mg, or 1 mg to 2.5 mg.

[0362] In certain embodiments, treatment or prevention can be initiated with one or more loading doses of a compound or composition provided herein followed by one or more maintenance doses. In such embodiments, the loading dose can be, for instance, about 60 to about 400 mg per day, or about 100 to about 200 mg per day for one day to five weeks. The loading dose can be followed by one or more maintenance doses. In certain embodiments, each maintenance dose is, independently, about from about 10 mg to about 200 mg per day, between about 25 mg and about 150 mg per day, or between about 25 and about 80 mg per day. Maintenance doses can be administered daily and can be administered as single doses, or as divided doses.

[0363] In certain embodiments, a dose of a compound or composition provided herein can be administered to achieve a steady-state concentration of the active ingredient in blood or serum of the subject. The steady-state concentration can be determined by measurement according to techniques available to those of skill or can be based on the physical characteristics of the subject such as height, weight and age. In certain embodiments, a sufficient amount of a compound or composition provided herein is administered to achieve a steady-state concentration in blood or serum of the subject of from about 300 to about 4000 ng/mL, from about 400 to about 1600 ng/mL, or from about 600 to about 1200 ng/mL. In some embodiments, loading doses can be administered to achieve steady-state blood or serum concentrations of about 1200 to about 8000 ng/mL, or about 2000 to about 4000 ng/mL for one to five days. In certain embodiments, maintenance doses can be administered to achieve a steady-state concentration in blood or serum of the subject of from about 300 to about 4000 ng/mL, from about 400 to about 1600 ng/mL, or from about 600 to about 1200 ng/mL.

[0364] In certain embodiments, administration of the same composition may be repeated and the administrations may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or 6 months. In other embodiments, administration of the same prophylactic or therapeutic agent may be repeated and the administration may be separated by at least at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or 6 months.

[0365] In certain aspects, provided herein are unit dosages comprising a compound, or a pharmacologically acceptable salt thereof, in a form suitable for administration. Such forms are described in detail above. In certain embodiments, the unit dosage comprises 1 to 1000 mg, 5 to 250 mg or 10 to 50 mg active ingredient. In particular embodiments, the unit dosages comprise 1, 5, 10, 25, 50, 100, 125, 250, 500 or 1000 mg active ingredient. Such unit dosages can be prepared according to techniques familiar to those of skill in the art.

[0366] The dosages of the second agents are to be used in the combination therapies provided herein. In certain embodiments, dosages lower than those which have been or are currently being used to prevent or treat the diseases described herein, for example, liver cancer and diabetes, are used in the combination therapies provided herein. The recommended dosages of second agents can be obtained from the knowledge of those of skill. For those second agents that are approved for clinical use, recommended dosages are described in, for example, Hardman et al., eds., 1996, Goodman & Gilman's The Pharmacological Basis Of Therapeutics 9th Ed, McGraw-Hill, New York; Physician's Desk Reference (PDR) 57th Ed., 2003, Medical Economics Co., Inc., Montvale, N.J., which are incorporated herein by reference in their entirety.

[0367] In various embodiments, the therapies (e.g., a compound provided herein and the second agent) are administered less than 5 minutes apart, less than 30 minutes apart, less than 1 hour apart, 1 hour apart, at about 1 hour apart, at about 1 to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to
about 11 hours apart, at about 11 hours to about 12 hours apart, at about 12 hours to 18 hours apart, 18 hours to 24 hours apart, 24 hours to 36 hours apart, 36 hours to 48 hours apart, 48 hours to 52 hours apart, 52 hours to 60 hours apart, 60 hours to 72 hours apart, 72 hours to 84 hours apart, 84 hours to 96 hours apart, or 96 hours to 120 hours part. In various embodiments, the therapies are administered no more than 24 hours apart or no more than 48 hours apart. In certain embodiments, two or more therapies are administered within the same patient visit. In other embodiments, the compound provided herein and the second agent are administered concurrently.

In certain embodiments, a compound provided herein and a second agent are administered to a patient, for example, a mammal, such as a human, in a sequence and within a time interval such that the compound provided herein can act together with the other agent to provide an increased benefit than if they were administered otherwise. For example, the second active agent can be administered at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. In one embodiment, the compound provided herein and the second active agent exert their effect at times which overlap. Each second active agent can be administered separately, in any appropriate form and by any suitable route. In other embodiments, the compound provided herein is administered before, concurrently or after administration of the second active agent.

In other embodiments, the compound provided herein and the second agent are administered at about 2 to 4 days apart, at about 4 to 6 days apart, at about 1 week apart, at about 1 to 2 weeks apart, or more than 2 weeks apart.

In certain embodiments, the compound provided herein and the second agent are cyclically administered to a patient. Cycling therapy involves the administration of a first agent (e.g., a first prophylactic or therapeutic agent) for a period of time, followed by the administration of a second agent and/or third agent (e.g., a second and/or third prophylactic or therapeutic agents) for a period of time and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improve the efficacy of the treatment.

In certain embodiments, the compound provided herein and the second active agent are administered in a cycle of less than about 3 weeks, about once every two weeks, about once every 10 days or about once every week. One cycle can comprise the administration of a compound provided herein and the second agent by infusion over about 90 minutes every cycle, about 1 hour every cycle, about 45 minutes every cycle. Each cycle can comprise at least 1 week of rest, at least 2 weeks of rest, at least 3 weeks of rest. The number of cycles administered is from about 1 to about 12 cycles, more typically from about 2 to about 10 cycles, and more typically from about 2 to about 8 cycles.

In other embodiments, courses of treatment are administered concurrently to a patient, i.e., individual doses of the second agent are administered separately yet within a time interval such that the compound provided herein can work together with the second active agent. For example, one component can be administered once per week in combination with the other components that can be administered once every two weeks or once every three weeks. In other words, the dosing regimens are carried out concurrently even if the therapeutics are not administered simultaneously or during the same day.

The second agent can act additively or synergistically with the compound provided herein. In one embodiment, the compound provided herein is administered concurrently with one or more second agents in the same pharmaceutical composition. In another embodiment, a compound provided herein is administered concurrently with one or more second agents in separate pharmaceutical compositions. In still another embodiment, a compound provided herein is administered prior to or subsequent to administration of a second agent. Also contemplated are administration of a compound provided herein and a second agent by the same or different routes of administration, e.g., oral and parenteral. In certain embodiments, when the compound provided herein is administered concurrently with a second agent that potentially produces adverse side effects including, but not limited to, toxicity, the second active agent can advantageously be administered at a dose that falls below the threshold that the adverse side effect is elicited.

Kits

Also provided are kits for use in methods of treatment of a liver disorder such as cancer or metabolic diseases, such as diabetes, hyperlipidemia, atherosclerosis, and obesity. The kits can include a compound or composition provided herein, a second agent or composition, and instructions providing information to a health care provider regarding usage for treating the disorder. Instructions may be provided in printed form or in the form of an electronic medium such as a floppy disc, CD, or DVD, or in the form of a website address where such instructions may be obtained. A unit dose of a compound or composition provided herein, or a second agent or composition, can include a dosage such that when administered to a subject, a therapeutically or prophylactically effective plasma level of the compound or composition can be maintained in the subject for at least 1 days. In some embodiments, a compound or composition can be included as a sterile aqueous pharmaceutical composition or dry powder (e.g., lyophilized) composition.

In some embodiments, packaging is provided. As used herein, “packaging” includes a solid matrix or material customarily used in a system and capable of holding within fixed limits a compound provided herein and/or a second agent suitable for administration to a subject. Such materials include glass and plastic (e.g., polyethylene, polypropylene, and polycarbonate) bottles, vials, paper, plastic, and plastic-foil laminated envelopes and the like. If e-beam sterilization techniques are employed, the packaging should have sufficiently low density to permit sterilization of the contents.

The following Examples illustrate the synthesis of representative compounds provided herein. These examples are not intended, nor are they to be construed, as limiting the scope of the claimed subject matter. It will be clear that the scope of claimed subject matter may be practiced otherwise than as particularly described herein. Numerous modifications and variations of the subject matter are possible in view of the teachings herein and, therefore, are within the scope of the claimed subject matter.
EXAMPLES
Example 1
Preparation of Hydroxy-4BuSATE N-benzylphosphoamidate derivative A550 of L-2',3'-dideoxycytidine L-ddA

[0378]

Synthetic Scheme:

[0379]
Synthesis of Carboxylic Acid 2:

2,2-Dimethyl-3-hydroxypropanoic acid methyl ester (965 μL, 7.57 mmol) was added dropwise to a stirring solution of 4,4′-dimethoxytrityl chloride (2.82 g, 8.33 mmol) in anhydrous pyridine (7.6 mL) at room temperature. The reaction mixture turned to a red color quickly, then to an orange suspension (ca. 30 min), and this was left stirring overnight. The mixture was poured carefully over saturated aqueous NaHCO₃ solution (30 mL) and the product was extracted with Et₂O (3×20 mL). The combined organic extracts were washed with brine (20 mL), dried (Na₂SO₄) and the volatiles were removed under reduced pressure. The resulting oil was co-evaporated with toluene and the residue was quickly purified by flash column chromatography (SiO₂, 0–4 cm, H–20 cm) eluting with 5→10→20→30% Et₂O in petroleum ether (40–60). Evaporation of the fractions (Rf=0.25, 30% Et₂O in petroleum ether (40–60)) afforded ether 1 as a yellow oil (3.11 g, 95%). This compound (3.00 g, 6.91 mmol) was dissolved in THF (35 mL) and an aqueous solution of NaOH (10%, 3.5 g in 35 mL H₂O) was then added at room temperature. The solution turned instantly dark orange and this was stirred for 2 days. The medium was then carefully neutralized by dropwise addition of HCl (1M). The product was extracted with Et₂O (4×50 mL) and the combined organic extracts were washed with brine (50 mL), dried (Na₂SO₄) and the volatiles were removed under reduced pressure. The crude yellow oil was quickly purified by flash column chromatography (SiO₂, 0–2 cm, H–10 cm) eluting with 50% Et₂O in petroleum ether (40–60). Evaporation of the fractions afforded carboxylic acid 2 as a white foam (2.23 g, 77%). Rf=0.50 (50% Et₂O in petroleum ether (40–60)).

1H-NMR (300 MHz, CDCl₃) 1.10 (s, 6H, 2×CH₃), 3.06 (s, 2H, CH₂O), 3.65 (s, 6H, 2×OCH₃), 6.62-6.79 (m, 4H, PhCH), 7.02-7.46 (stack, 8H, PhCH); 13C-NMR (75 MHz, CDCl₃) 22.6 (2×CH₃), 43.5 (C(CH₃)₂), 55.1 (2×OCH₃), 85.9 (CPh₃), 125.3, 126.7, 127.7, 128.2, 129.1, 130.0, 136.0, 144.9, 158.4 (Ph), some overlap, 182.2 (C=O).

Synthesis of Thioester 3:

1.1′-carbonyldiimidazole (830 mg, 5.12 mmol) was added to a stirring solution of carboxylic acid 2 in anhydrous PhMe/DMF (2/1, v/v, 2.7 mL) at room temperature and the reaction mixture turned turbid instantly. After 30 min, the medium was diluted by adding anhydrous PhMe/DMF (93/7, v/v, 17 mL) and cooled to −10°C. 2-Mercaptoethanol (359 μL, 5.12 mmol) was then added dropwise and the solution was stirred for 1 h at this temperature. The reaction mixture was diluted with H₂O (60 mL) and the product was extracted with Et₂O (3×15 mL). The combined organic extracts were washed with brine (15 mL), dried (Na₂SO₄) and the volatiles were removed under reduced pressure (bath temperature not exceeding 20°C). The residue was purified by flash column chromatography (SiO₂, 0–4 cm, H–15 cm, 1% Et₃N eluting with 60–70% Et₂O in petroleum ether (40–60)). Evaporation of the fractions afforded thioester 3 as a white syrup (1.74 g, 92%) that solidified upon storage at 4°C. Rf=0.35 (70% Et₂O in petroleum ether (40–60)).

1H-NMR (300 MHz, CDCl₃) 1.16 (s, 6H, 2×CH₃), 3.02 (t, J 6.0, 2H, CH₃S), 3.09 (s, 2H, CH₂O), 3.66 (t, J 6.0, 2H, CH₂OH), 3.72 (s, 6H, 2×OCH₃), 6.74-6.78 (m, 4H, PhCH), 7.09-7.36 (stack, 8H, PhCH); 13C-NMR (75 MHz, CDCl₃) 22.9 (CH₃S, 2×CH₂), 31.7 (CH₃S), 51.0 (quat. C, C(CH₃)₂), 55.2 (CH₃S, 2×OCH₃), 61.9 (CH₂S, CH₂OH), 70.0 (CH₂S, CH₂O), 85.8 (quat. C, CPh₃), 113.0 (C=O, CH₂), 126.7 (C=O, Ph), 127.7 (CH, Ph), 128.2 (CH, Ph), 130.1 (CH, Ph), some overlap, 135.9 (quat. C, Ph), 144.8 (quat. C, Ph), 158.4 (quat. C, Ph), some overlap, 205.0 (quat. C, C=O).
Synthesis of H-Phosphonate Monoester 4:

β-L-ddA (1.00 g, 4.25 mmol) was co-evaporated with anhydrous pyridine (3x10 mL) and then dissolved in anhydrous pyridine/DMF (1/1, v/v, 21 mL). Diphosphoryl phosphite (5.76 mL, 29.8 mmol) was then added dropwise to this solution at room temperature. The reaction mixture was stirred for 20 min upon which a mixture of Et3N/H2O (1/1, v/v, 8.5 mL) was added dropwise, and stirring was continued for an additional 20 min. The reaction mixture was concentrated under reduced pressure to approximately 15-20 mL and this residue was directly purified by flash column chromatography (SiO2, O=4 cm, H=15 cm, 1% Et3N) eluting slowly with CH2Cl2 (150 mL) then 5% (200 mL) 10% (200 mL) 15% (300 mL) MeOH in CH2Cl2. Evaporation of the fractions afforded H-phosphonate monoester 4 as a white foam (1.36 g, 80%) that could be kept for several weeks at 4°C. Rf=0.10 (Et3N/MeOH/CH2Cl2, 1:1:1, Jan. 10, 1989). 1H-NMR (300 MHz, CDCl3) 1.21 (t, J 7.4, 9H, 3xNCH2CH3), 1.92-2.50 (stack, 4H, 2x2'2-H, 2x3'2-H), 3.02 (q, J 7.4, 6H, 3xNCH2CH3), 3.96-4.03 and 4.18-4.30 (stacks, 3H, 4'H, 2x5'2-H), 6.28 (m, 1'H), 6.91 (d, J 6,23, 1H, P=H), 7.05 (br s, 2H, NH2), 8.21 (s, 1H), 8.54 (br s, 1H, Off), 8.57 (s, 1H).

Synthesis of Phosphoroamidate Diester 5:

H-Phosphonate monoester 4 (1.03 g, 2.57 mmol) and alcohol 3 (1.66 g, 3.45 mmol) were co-evaporated with anhydrous pyridine (3x5 mL) and then dissolved in anhydrous pyridine (5 mL). PyBOP (H-benzotriazol-1-ylxoytrityl phosphophonium hexafluorophosphate, 1.60 g, 3.08 mmol) was then added in one portion and the reaction mixture was stirred for 15 min at room temperature. The solution was poured over saturated aqueous NaHCO3 solution (30 mL) and the product was extracted with CH2Cl2 (4x15 mL). The combined organic extracts were washed with brine (10 mL), dried (Na2SO4) and concentrated under reduced pressure to leave the corresponding H-phosphonate diester as a slightly yellow oil (1.84 g, assuming 2.41 mmol). This was co-evaporated with anhydrous pyridine (3x5 mL; note: do not evaporate to dryness in order to help further solubilization), and the residue was dissolved in anhydrous CCl4 (24 mL). Benzylamine (79 µL, 7.25 mmol) was added dropwise and the reaction mixture turned cloudy instantly (slight heat development was observed). The milky solution was stirred for 1 h at room temperature and poured over saturated aqueous NaHCO3 solution (30 mL) and the product was extracted with CH2Cl2 (4x15 mL). The combined organic extracts were washed with brine (15 mL), dried (Na2SO4) and concentrated under reduced pressure to afford phosphoroamidate diester 5 as a yellow oil (2.00 g, assuming 2.31 mmol). This was used in the next step without any further purification. Rf=0.29 (4% MeOH in CH2Cl2); 1H-NMR (300 MHz, CDCl3) 1.11 (s, 6H, 2xCH3), 1.91-2.05 (m, 2H), 2.31-2.59 (m, 2H), 3.06 (m, 2H, CH2S), 3.08 (s, 2H, CH2NMeTr), 3.69 (s, 6H, 2xOCH3), 3.83-4.28 (stacks, 7H, CH3O), NCH3Ph, 4'-H, 2x5'-H), 5.71 (br s, 1H, NH), 6.18 (m, 1H, 1'-H), 6.69-6.80 (m, 4H, PhCH), 7.02-7.31 (stack, 13H, PhCH), 7.90 (s, 1H), 8.01 (s, 1H), 8.23 (s, 2H, NH2); 31P-NMR (61 MHz, CDCl3) 8.82, 8.99.
Synthesis of Hydroxy-tBuSATE N-benzylphosphoroamidate Derivative of L-ddA:

Crude phosphoroamidate diester 5 (2.00 g, assuming 2.31 mmol) was dissolved in dioxane/AcOH/H2O (25/17/25, v/v/v, 462 mL) and the solution was stirred for 3 d at room temperature. Evaporation of the volatiles under reduced pressure left a residue that was purified by flash column chromatography (SiO2, 0=3 cm, H=15 cm) eluting with CH2Cl2 (100 mL) then 2% (100 mL)→4% (100 mL)→6% (100 mL)→8% (150 mL) MeOH in CH2Cl2. Evaporation of the fractions left NM 204 as a white foam that was dissolved in MeCN (5 mL). Upon addition of H2O (5 mL), the solution turned turbid and required sonication before lyophilization. The resulting white powder was dried at room temperature (using P2O5 as a desiccant) under vacuum for 1 d. The title compound was obtained as a highly hygroscopic white powder (1:1 mixture of diastereoisomers as judged by 31P-NMR; 499 mg, 35% over 3 steps). [α]20 D +4.2° (c 1.0, CHCl3); Rf=0.29 (4% MeOH in CH2Cl2); 1H-NMR (300 MHz, DMSO-d6) 1.10 (s, 6H, 2xCH3), 2.70-2.84 (m, 2H, CHS), 3.40 (t, J 6.4, 2H, CH2S), 3.43 (d, J 5.0, 2H, CH2O), 3.57-4.07 and 4.18-4.29 (stacks, 7H, CHO, NCHPh, 4'-H, 2x5'-H), 5.02 (t, J 5.0, 1H, OH), 5.62 (m, 1H, NH), 6.25 (s, J 5.1, 1H, 1'-H), 7.16-7.36 (stack, 7H, PhH, NH2), 8.14 (s, 1H), 8.26 (s, 1H); 13C-NMR (75 MHz, DMSO-d6) 21.8 (2xCH3), 25.9 and 26.0 (CH2, 3'-C), 28.2 and 28.3 (CH2, CH2S), 30.9 and 31.0 (CH2, 2'-C), 44.2 (CH2, NCH2Ph), 51.7 (quat. C, C(CH3)2), 63.7 and 63.8 (CH2, CH2O), 66.8 (CH2, m, 5'-C), 68.3 (CH2, CH2OH), 78.9 (CH, m, 4'-C), 84.2 (CH, 1'-C), 118.9 (quat. C), [126.5 (CH, Ph), 127.2 (CH, Ph), 128.1 (CH, Ph), some overlap], 138.8 and 138.9 (CH), 140.5 and 140.6 (quat. C), 148.9 (quat. C), 152.3 (CH), 155.0 (quat. C), 204.0 (quat. C, C==O), 17P-NMR (61 MHz, DMSO-d6) 9.86, 9.95; m/z (FAB+) 563 (2), 306 (76), 153 (100); HRMS 565.2034 ([M+H]+, C21H23O5N2PS requires 565.1998); HPLC tR=3.52 min (20% TEAC 20 mM in MeCN); UV (EtOH 95%) λmax=259 (εmax 15900), λmin=224 (εmin 7200).
Example 2
Preparation of Hydroxy-iBuSATE N-Benzylphosphoroamidate Derivative of 2'-C-methylcytidine

Synthesis of H-Phosphonate Monoester 5

Synthesis of Carboxylic Acid 3:

To a stirred solution of 2,2-dimethyl-3-hydroxypropanoic acid methyl ester (1, 1.5 ml, 117.6 mmol) in an anhydrous mixture of toluene and dimethylformamide (2/1, v/v, 4.5 ml) at room temperature, and the reaction mixture turned turbid instantly. After 30 min, the reaction mixture was diluted with a mixture of toluene and dimethylformamide (93/7, v/v, 28 ml), cooled to –10°C, and 2-mercaptoethanol (1.3 eq, 500 μl) was added. The solution was stirred for 3 h at this temperature. The volatiles were removed under reduced pressure (bath temperature not exceeding 25°C). The residue was dissolved in methylene chloride and washed with water. The organic phases were combined, dried over sodium sulphate (Na₂SO₄), filtered and evaporated to dryness to give compound 4 as a yellow oil. This compound will be coevaporated with anhydrous pyridine and used for the next step without further purification. Rf=0.71 (70%Et₂O in petroleum ether); ¹H-NMR (400 MHz, CDCl₃) 1.20 (s, 6H, 2×CH₃), 3.05 (t, J=6.4 Hz, 2H, CH₃), 3.15 (s, 2H, CH₂OTr), 3.69 (t, J=6.4 Hz, 2H, CH₂OTr), 7.3-7.9 (m, 15H, C₆H₅).
[0394] Phosphorus acid (10 eq, 4.1 g) was coevaporated two times with anhydrous pyridine, dissolved in that solvent (25 ml) and added to crude 4. The reaction mixture was stirred at room temperature and a white precipitate appeared after few minutes. The reaction mixture was cooled down to 0°C and pivaloyl chloride (5.5 eq, 3.4 ml) was added. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. The reaction was stopped by addition of a solution of triethylammonium bicarbonate (TEAB 1M, 10 ml) and diluted with ethyl acetate (EtOAc). After extraction with EtOAc and TEAB 0.5M, the organic phases were combined, dried over sodium sulphate, filtered and evaporated to dryness (bath temperature not exceeding 30°C). The residue was purified by flash column chromatography eluting with 10% of methanol in methylene chloride+1% triethylamine. Evaporation of the fractions afforded the H-phosphonate monoester 5 as a white syrup (90%). \( R_\text{f} = 0.25 \) (70% EtO in petroleum ether); \( ^1\text{H-NMR} (400 \text{ MHz}, \text{CDCl}_3) 1.17 \) (m, 2\( \times \text{CH}_3\), excess (\( \text{CH}_3\text{CH}_2\text{Cl} \)), 2.9 (m, excess (\( \text{CH}_3\text{CH}_2\text{N} \)), 3.12 (t, \( j = 6.8 \text{ Hz} \), 2\( \times \text{H} \), \( \text{CH}_2\text{Cl} \)), 3.37 (s, 2\( \times \text{H} \), \( \text{CH}_3\text{CH} \)), 3.90 (m, 2\( \times \text{H} \), \( \text{CH}_2\text{O} \)), 7.2-7.6 (m, 1\( \times \text{H} \), \( \text{C}_6\text{H}_5 \)), 9.9 (m, excess (\( \text{CH}_3\text{CH}_2\text{NH} \)). \( ^3\text{P-NMR} (161 \text{ MHz}, \text{CDCl}_3) 3.85 \) (s).

Synthesis of Hydroxy-tBuSATE N-benzylphosphoroamidate derivative of 2′C-methylcytidine: The following two strategies were used for the synthesis:

**Strategy A**

Synthesis of the Protected Nucleoside 7

[0395] A mixture of 2′C-methylcytidine (NM107) (10 g, 39.0 mmol), triethyl orthoformate (8.3 eq, 54 ml) and p-toluenesulfonic acid monohydrate (1 eq, 7.4 ml) in anhydrous acetone (650 ml), was refluxed overnight under nitrogen atmosphere. The reaction mixture was neutralized with an aqueous ammonia solution (28%) and the precipitate filtered. The filtrate was evaporated under reduced pressure and coevaporated with ethanol. Purification of the crude mixture on silica gel column chromatography (eluant: stepwise gradient [0-10%] of methanol in methylene chloride) led to compound 6 as a pale-yellow solid (86%). \( R_\text{f} = 0.30 \) (20% MeOH in methylene chloride), \( ^1\text{H-NMR} (400 \text{ MHz}, \text{DMSO-}d_6) 1.06 \) (s, 3\( \times \text{H} \), \( \text{CH}_3 \)), 1.33 (s, 3\( \times \text{H} \), \( \text{CH}_3 \)), 1.47 (s, 3\( \times \text{H} \), \( \text{CH}_3 \)), 3.6 (m, 2\( \times \text{H} ,\text{ H-5}, \text{H-5} \)), 4.1 (m, 1\( \times \text{H} ,\text{ H-4}, \text{H-4} \)), 4.41 (d, 1\( \times \text{H} ,\text{ H-3}, \text{J=3.2 Hz} \)), 5.16 (t, 1\( \times \text{H} ,\text{ OH-5}, \text{J=4.0 Hz} \), \( \text{D}_2\text{O} \) exchangeable), 5.69 (d, 1\( \times \text{H} ,\text{ H-5}, \text{J=8.0 Hz} \)), 6.04 (s, 1\( \times \text{H} ,\text{ H-1} \)), 7.14-7.19 (bd, 2\( \times \text{H} ,\text{ NH}_2 \), \( \text{D}_2\text{O} \) exchangeable), 7.74 (d, 1\( \times \text{H} ,\text{ H-6}, \text{J=8.0 Hz} \)); LC/MS Scan ES− 296 (M-H)), Scan ES+: 298 (M+H)+, \( \lambda_{\text{max}} = 280.7 \text{ nm} \).

[0397] Compound 6 (4.4 g, 14.8 mmol) was dissolved in anhydrous pyridine (74 ml) and chlorotrimethylsilane (3 eq, 5.4 ml) was added. The reaction mixture was stirred at room temperature under nitrogen atmosphere for 2 h, then 4,4′-dimethoxytrityl chloride (1.5 eq, 7.5 g) and 4-dimethylaminopyridine (0.5 eq, 900 mg) were successively added. The reaction mixture was stirred overnight at room temperature, then quenched with a saturated aqueous NaHCO₃ solution. The crude product was extracted with methylene chloride, washed with saturated aq NaHCO₃ solution, and water. The combined organic phases were concentrated under reduced pressure, then dissolved in a mixture of dioxan (160 ml) and aqueous ammonia (28%, 29 ml). The solution was heated at 70°C for 3 h and evaporated to dryness. The crude mixture was purified on silica gel column chromatography (eluant: stepwise gradient of methanol [1-5%] in methylene chloride) to give protected nucleoside 7 as a yellow solid (81%). \( R_\text{f} = 0.16 \) (30% EtOAc in \( \text{CH}_2\text{Cl}_2 \)), \( ^1\text{H-NMR} (400 \text{ MHz}, \text{DMSO-}d_6) 1.03 \) (s, 3\( \times \text{H} \), \( \text{CH}_3 \)), 1.30 (s, 3\( \times \text{H} \), \( \text{CH}_3 \)), 1.42 (s, 3\( \times \text{H} \), \( \text{CH}_3 \)), 3.5 (m, 2\( \times \text{H} ,\text{ H-5}, \text{H-5} \)), 3.71 (s, 6\( \times \text{H} \), 2\( \times \text{CH}_2\text{O} \)), 4.0 (d, 1\( \times \text{H} ,\text{ H-4}, \text{J=3.2 Hz} \)), 4.36 (d, 1\( \times \text{H} ,\text{ H-3}, \text{J=2.8 Hz} \)), 5.1 (m, 1\( \times \text{H} ,\text{ OH-5}, \text{D}_2\text{O} \) exchangeable), 5.90 (s, 1\( \times \text{H} ,\text{ H-1} \)), 6.2 (m, 1\( \times \text{H} ,\text{ H-5} \)), 6.8-7.2 (m, 13\( \times \text{H} ,\text{ DMTr} \)), 7.6 (m, 1\( \times \text{H} ,\text{ H-6} \), 8.32 (s, 1\( \times \text{H} ,\text{ NH} \), \( \text{D}_2\text{O} \) exchangeable); LC/MS Scan ES− 598 (M-H), Scan ES+: 600 (M+H)+, \( \lambda_{\text{max}} = 231.7 \text{ nm} \), \( \lambda_{\text{max}} = 283.7 \text{ nm} \).
Synthesis of the Pronucleotide 10

[0398]

![Chemical Structure](image)

Compounds 7 (2.0 g, 3.34 mmol) and 5 (2.2 eq, 4.3 g) were coevaporated together with anhydrous pyridine and dissolved in this solvent (50 ml). Pivaloyl chloride (2.5 eq, 1 ml) was added dropwise and the solution stirred at room temperature for 2h30. The reaction mixture was diluted with methylene chloride and neutralized with an aqueous solution of ammonium chloride (NH₄Cl 0.5M). After extraction with methylene chloride/aq NH₄Cl 0.5M, the organic phases were combined, evaporated under reduced pressure (bath temperature not exceeding 30°C) and coevaporated with toluene. The crude mixture was purified on silica gel column chromatography (eluant: stepwise gradient 0-5% of methanol in methylene chloride±2% acetic acid) to afford the desired product 8 which was coevaporated with toluene to give a beige foam (94%). Rf 0.63 (5% MeOH in CH₂Cl₂).

H-NMR (400 MHz, CDCl₃) 1.21 (m, 9H, 3 CH₃), 1.42 (s, 3H, CH₂), 1.60 (s, 3H, CH₃), 3.12 (m, 2H, CH₂S), 3.17 (m, 2H, CH₂OTr), 3.79 (s, 6H, 2xOCH₃), 4.1 (m, 2H, CH₂OP), 4.2-4.3 (m, 3H, H-5', H-5'', H-4'), 5.09 (d, 1H, H-3', J=7.6 Hz), 5.89 (d, 1H, H-5, J=5.6 Hz), 6.0 (m, 1H, H-1'), 6.8-7.7 (m, 29H, Tr, DMTr, H-6); ¹³C-NMR (161 MHz, CDCl₃) 7.92, 8.55; LC/MS Scan ES+ 1066 (M+H)⁺, Scan ES− 1064 (M−H)⁻.

[0400] To a solution of compound 8 (3.4 g, 3.15 mmol) in anhydrous carbon tetrachloride (30 ml) was added dropwise benzylamine (10 eq, 3.4 ml). The reaction mixture was stirred at room temperature for 1h30. A white precipitate appeared. The solution was diluted with methylene chloride and neutralized with an aqueous solution of hydrogen chloride (HCl 1M). After successive extractions with CH₂Cl₂/HCl 1M and
CH\textsubscript{2}Cl\textsubscript{2}/aq NaHCO\textsubscript{3}, the organic phases were combined, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and evaporated to dryness. The crude mixture was purified on silica gel column chromatography (eluant: stepwise gradient [0-50%] of methanol in methylene chloride) to give 2 as a yellow foam (87%). R\textsubscript{f}=0.35 (5% MeOH in methylene chloride); \textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}) 1.1-1.2 (m, 9H, 3 CH\textsubscript{3}), 1.40 (s, 3H, CH\textsubscript{3}), 1.59 (s, 3H, CH\textsubscript{3}), 2.9-3.2 (m, 4H, CH\textsubscript{2}O), 3.76 (s, 6H, 2CH\textsubscript{3}CH\textsubscript{2}O), 3.9-4.4 (m, 8H, CH\textsubscript{2}OP, CH\textsubscript{2}N, H-3', H-4', H-5', H-5''), 5.0 (m, 1H, H-5), 6.0 (2s, 1H, H-1'), 6.7-7.7 (m, 3H, Ar, DMTyr. C\textsubscript{6}H\textsubscript{4}CH\textsubscript{3}), H-6'); \textsuperscript{13}P-NMR (161 MHz, CDCl\textsubscript{3}) 8.40, 8.8.68; LC/MS Scan ES+ 1171 (M+H)\textsuperscript{+}. [0401]

Finally, compound 9 (2.39 g, 2.04 mmol) was dissolved in a mixture of methylene chloride (10 ml) and an aqueous solution of trifluoroacetic acid (90%, 10 ml). The reaction mixture was stirred at 35-40°C for 2 h, then diluted with ethanol (140 ml). The volatiles were evaporated under reduced pressure and coevaporated with ethanol. The crude mixture was purified by silica gel column chromatography (eluant: stepwise gradient of methanol [0-30%] in methylene chloride), followed by a purification on reverse phase chromatography (eluant: stepwise gradient of acetonitrile [0-50%] in water), to give the desired product 10 (B102) (1:1 mixture of diastereomers as judged by \textsuperscript{31}P-NMR, 36%) which was lyophilized from a mixture of dioxan/water. R\textsubscript{f}=0.34 (15% MeOH in methylene chloride); \textsuperscript{1}H-NMR (400 MHz, DMSO-d\textsubscript{6}) 0.92 (s, 3H, CH\textsubscript{3}), 1.10 (s, 6H, 2CH\textsubscript{3}), 3.0 (m, 2H, CH\textsubscript{2}S), 3.33 (m, 1H, H-3'), 3.56 (s, 2H, CH\textsubscript{2}OH), 3.8-4.0 and 4.05-4.25 (stcks, 7H, CH\textsubscript{2}OP, CH\textsubscript{2}N, H-4', H-5' and H-5''), 4.9 (m, 1H, OH-3'), 5.4-5.5 Hz, D\textsubscript{2}O exchangeable), 5.07 (s, 1H, OH-2', D\textsubscript{2}O exchangeable), 5.5 (m, 1H, CH\textsubscript{2}OH, D\textsubscript{2}O exchangeable), 5.6-5.7 (m, 2H, H-5 and NH, D\textsubscript{2}O exchangeable), 5.91 (s, 1H, H-1'), 7.3-7.4 (stck, 7H, PH\textsubscript{2}, NH\textsubscript{2}, D\textsubscript{2}O exchangeable), 7.6 (m, 1H, H-6); \textsuperscript{13}P-NMR (161 MHz, DMSO-d\textsubscript{6}) 9.71, 9.91; HPLC \textsubscript{R}t=4.67 min (0-100% acetonitrile over a period of 8 min), \textit{\lambda}_{max,\textsubscript{UV}}=274.9; LC/MS Scan ES+ 587 (M+H)\textsuperscript{+}.

Strategy B [0402]

Synthesis of Protected Nucleoside 11

\[
\begin{align*}
\text{NH}_2 & \\
\text{HO} & \\
\text{CH} & \\
\text{OH} & \\
\text{OH} & \\
\text{OH} & \\
\text{OH} & \\
\text{NHDMTr} & \\
\end{align*}
\]

1) TMSOTf, pyridine
2) DMTyr/DMAP
3) TBAF 1M in THF

[0403] NM107 (10 g, 38.87 mmol) was dissolved in anhydrous pyridine (194 ml) and chlorotrimethylsilane (4.5 eq, 21.6 ml) was added. The reaction mixture was stirred at room temperature under nitrogen atmosphere for 2h30, then 4,4'-dimethoxytrityl chloride (1.5 eq, 19.8 g) and 4-dimethylaminoypyridine (0.5 eq, 2.37 g) were successively added. The reaction mixture was stirred overnight at room temperature, then quenched with a saturated aqueous NaHCO\textsubscript{3} solution. The crude product was extracted with methylene chloride, washed with saturated aq NaHCO\textsubscript{3} solution, and water. The combined organic phases were concentrated under reduced pressure, then dissolved in tetrahydrofuran (110 ml). To that solution was added tetrabutylammonium fluoride 1M in THF (1 eq, 38.87 ml) and the reaction mixture was stirred for 30 min at room temperature. After extraction with EtOAc and water, the organic phases were collected and evaporated to dryness. The crude mixture was purified on silica gel column chromatography (eluant: stepwise gradient of methanol [0-10%] in methylene chloride) to give protected nucleoside 11 as a white solid (93%). R\textsubscript{f}=0.32 (10% MeOH in CH\textsubscript{2}Cl\textsubscript{2}); \textsuperscript{1}H-NMR (400 MHz, DMSO-d\textsubscript{6}) 0.79 (s, 3H, CH\textsubscript{3}), 3.56 (m, 2H, H-5', H-5''), 3.71 (s, 7H, 2CH\textsubscript{2}OH, H-4'), 5.0 (m, 4H, H-3', OH-2', OH-3', OH-5', D\textsubscript{2}O exchangeable), 5.72 (s, 1H, H-1'), 6.16 (m, 1H, H-5), 6.8-7.2 (m, 13H, DMTyr), 7.82 (m, 1H, H-6), 8.24 (m, 1H, NH\textsubscript{2}D\textsubscript{2}O exchangeable); LC/MS Scan ES- 560 (M+H)-, ES- 558 (M-H)-, \textit{\lambda}_{max,\textsubscript{UV}}=284.7 nm.

Synthesis of Protected Phosphoroamidate Pronucleotide 13, Precursor of 10

[0404]
Compound 11 (7 g, 12.5 mmol) and 5 (1.5 eq, 11.0 g) were coevaporated together with anhydrous pyridine and dissolved in this solvent (187 ml). Pivaloyl chloride (2.0 eq, 3.08 ml) was added dropwise at -15° C. and the solution stirred at this temperature for 1 h. The reaction mixture was diluted with methylene chloride and neutralized with an aqueous solution of ammonium chloride (NH₄Cl 0.5M). After extraction with methylene chloride/aq NH₄Cl 0.5M, the organic phases were combined, evaporated under reduced pressure (bath temperature not exceeding 30° C.) and coevaporated with toluene. The crude mixture was purified on silica gel column chromatography (eluant: stepwise gradient [0-5%] of methanol in methylene chloride-acetic acid) to afford the desired product 12 which was coevaporated with toluene to give a white foam (3.5 g, 27%). Rf 0.44 (5% MeOH in CH₂Cl₂); 1H-NMR (400 MHz, DMSO) δ 0.8 (m, 3H, CH₃), 1.14 and 1.06 (2s, 6H, 2CH₂), 3.06 (m, 2H, CH₂S), 3.16 (m, 2H, CH₂OTr), 3.5 (m, 1H, H-3'), 3.7 (m, 6H, 2OCH₃), 3.90 (m, 1H, H-4'), 4.03 (m, 2H, CH₂OP), 4.24 (m, 2H, H-5', H-5''), 5.30 and 5.04 (2s, 2H, OH-2' and OH-3', D₂O exchangeable), 5.78 (m, 1H, H-1'), 5.98 (m, 1H, P-H), 6.22 (m, 1H, H-5), 7.0-7.5 (m, 16H, Tr), 8.32 (m, 1H, H-6); 31P-NMR (161 MHz, DMSO) δ 9.17, 9.65; LC/MS Scan ES+: 1026 (M+H)⁺, λₑₓₘₐₓ = 282.7 nm.

To a solution of compound 12 (500 mg, 0.49 mmol) in anhydrous carbon tetrachloride (4.9 ml) was added dropwise benzylamine (5 eq, 0.266 ml). The reaction mixture was stirred at room temperature for 3 h and the solvent removed under reduced pressure. The crude mixture was purified on silica gel column chromatography (eluant: stepwise gradient [0-5%] of methanol in methylene chloride) to afford compound 13 as a foam (75%). Rf 0.25 (3% MeOH in methylene chloride); 1H-NMR (400 MHz, DMSO) δ 0.79 (s, 3H, CH₃), 1.13 and 1.06 (2s, 6H, 2CH₂), 3.05 (m, 4H, CH₂OTr, CH₂OS), 3.51 (m, 1H, H-3'), 3.69 (s, 6H, 2xOCH₃), 3.87 (m, 3H, CH₂OP, CH₂N, H-3'), 4.08 (m, 2H, H-5', H-5''), 5.19 and 5.0 (2m, 2H, OH-2' and OH-3', D₂O exchangeable), 5.67 (m, 1H, NH, D₂O exchangeable), 5.75 (2s, 1H, H-1'), 6.21 (m, 1H, H-5), 6.7-7.5 (m, 34H, Tr, DMTr, C₆H₅CH₂, H-6); 31P-NMR (161 MHz, DMSO) δ 9.84, 9.69; LC/MS Scan ES+: 1132 (M+H)⁺.

Compound 13 can be converted into the phosphoramidate prodrug 10 (B102) following experimental conditions described for the last step of NM108- and NM105-OH-SATE phosphoramidate synthesis, in Examples 3 and 4, respectively.

Example 3
Preparation of Hydroxy-t-BuSATE-N-Benzylphosphoramidate Derivative of 2'-C-Methylguanosine
Synthetic Strategy:

Strategy A

NM108

1) TMSCl, pyridine
2) DMTrCl/DMAP
3) NH4OH 28%/dioxan

Strategy B

5

HO

OH

NH

NH2

OH

OMe

HO

OH

NH

NH2

Tr

Tr

TeA 99%

DCM

HO

S

O

P

O

H

NH

NH

5

TrCl, pyridine

PivCl, pyridine
[0410] 2’-C-methylguanidine (NM108) (3 g, 10.10 mmol) and compound 5 (6.48 g, 11.10 mmol) were coevaporated together with anhydrous pyridine and dissolved in this solvent (152 ml). Pivaloyl chloride (2.48 ml, 20.18 mmol) was added dropwise at -15°C and the solution was stirred at the same temperature for 2 h. The reaction mixture was diluted with methylene chloride and neutralized with an aqueous solution of ammonium chloride (NH₄Cl 0.5M). After extraction with methylene chloride/aq NH₄Cl 0.5M, the organic phases were combined, dried over Na₂SO₄, evaporated under reduce pressure (bath temperature not exceeding 30°C) and coevaporated twice with toluene. The crude mixture was purified on silica gel flash column chromatography (eluant: stepwise gradient [0-10%] of methanol in methylene chloride). To the purified product 6 (2.5 g, 32%). R₆0.34 (15% MeOH in CH₂Cl₂); ¹H-NMR (400 MHz, DMSO-d₆) 8.00 (s, 3H, CH₃), 1.13 (s, 6H, 2xCH₃), 3.04 (m, 2H, CH₂OTf), 3.14 (m, 2H, CH₂S), 3.97-4.08 (m, 4H, H-3’, H-4’, CH₂OP), 4.28-4.38 (m, 2H, H-5’, H-5”), 5.10-5.35 (m, 2H, OH-2’, OH-3’, D₂O exchangeable), 5.77 (s, 1H, H-1’), 6.62 (bs, 2H, NH, D₂O exchangeable), 7.11-7.42 (m, 15H, Tr), 7.15 (s, 1H, H-8), 10.67 (bs, 1H, NH, D₂O exchangeable); ³¹P-NMR (161 MHz, DMSO-d₆) 9.47, 9.20; LC/MS Scan ES+ 764 (M+H)+, Scan ES− 762 (M−H)−.

[0411] A solution of compound 6 (2.5 g, 3.27 mmol) in anhydrous carbon tetrachloride (33 ml) was added dropwise benzyalmine (5 eq, 1.79 mL). The reaction mixture was stirred at room temperature for 1 h and evaporated under reduced pressure (bath temperature not exceeding 30°C). The crude mixture was purified on silica gel flash column chromatography (eluant: stepwise gradient [0-10%] of methanol in methylene chloride) to give compound 7 (1.379 g, 80% yield). R₆0.27 (10% MeOH in methylene chloride); ¹H-NMR (400 MHz, DMSO-d₆) 0.81 (s, 3H, CH₃), 1.10 (s, 6H, 2xCH₃), 2.99-3.08 (m, 4H, CH₂OTf, CH₂S), 3.87-4.30 (m, 8H, H-3’, H-4’, H-5’, H-5”), CH₂OP, NCHPh), 5.66 (m, 1H, NH, D₂O exchangeable), 5.76 (s, 1H, H-1’), 6.60 (bs, 2H, NH, D₂O exchangeable), 7.17-7.39 (m, 20H, Tr, C₆H₄), 7.77 (s, 1H, H-8); ³¹P-NMR (161 MHz, DMSO-d₆) 9.35, 9.78; LC/MS Scan ES+ 869 (M+H)+, Scan ES− 867 (M−H)−.

[0412] Compound 7 (2.84 g, 3.27 mmol) was dissolved in a mixture of trifluoroacetic acid (1.1 ml) and methylene chloride (11.4 ml). The reaction mixture was stirred 0.5 h at room temperature. The solution was diluted with ethanol, evaporated under reduce pressure (bath temperature not exceeding 30°C) and coevaporated twice with toluene. The crude mixture was purified on silica gel flash column chromatography (eluant: stepwise gradient [0-30%] of methanol in methylene chloride) and then, on reverse phase column chromatography (eluant: stepwise gradient 0-100% of acetonitrile in water) to give the desired product 8 (B184) (1:1 mixture of diastereoisomers according to ³¹P-NMR, 800 mg, 39%) which was lyophilized from a mixture of dioxan/water. R₆0.57 (20% MeOH in methylene chloride); ¹H-NMR (400 MHz, DMSO-d₆) 0.82 (s, 3H, CH₃), 1.09 (s, 6H, 2xCH₃), 3.01 (m, 2H, CH₂S), 3.42 (d, 2H, CH₂OH, J=8.0 Hz), 3.81-4.00 (m, 6H, H-3’, H-4’, CH₂OP, NCHPh), 4.11-4.27 (m, 2H, H-5’, H-5”), 4.92 (t, 1H, CH₂OH, J=8.0 Hz, D₂O exchangeable), 5.16 (s, 1H, OH-2’, D₂O exchangeable), 5.40 (m, 1H, OH-3’, D₂O exchangeable), 5.64 (m, 1H, NH, D₂O exchangeable), 5.75 (s, 1H, H-1’), 6.50 (bs, 2H, NH, D₂O exchangeable), 7.19-7.32 (m, 5H, PhH), 7.77 (s, 1H, H-8), 10.61 (bs, 1H, NH, D₂O exchangeable); ³¹P-NMR (161 MHz, DMSO-d₆) 9.31, 9.78; HPLC tₑₘₐₓ=3.67 min (0-100% acetonitrile over a period of 8 min), λₑₘₐₓ=251.3; LC/MS Scan ES+ 627 (M+H)+, Scan ES− 625 (M−H)−.

Example 4

[0413] A anti-cancer drug, R—OH, such as an antiviral nucleoside, having a free OH group, is derivatized to form a phosphoramidate compound according to the following scheme. Reactive groups on the molecule, such as other hydroxyl groups, are protected using methods known in the art.
Example 5

A phosphoroamidate of 5-azacytidine is prepared as follows:

1. 5-azacytidine is treated with TMSCl and pyridine to form a phosphoroamidate.
2. The resulting phosphoroamidate is then treated with TFA 90% DCM.
3. The final product is obtained by reaction with benzylamine.

Chemical structures are shown for each step of the reaction process.
Examples 6-10 illustrate by way of example the effect of the phosphoroamidate group on an antiviral compound to promote liver specific delivery of an active agent to liver cells.

Example 6

Preparation of Calibration Curve

Measurements of the concentration of 2'-3'-dideoxyadenosine-5'-triphosphate (ddATP) (the triphosphate nucleotide of 2'-3'-dideoxyadenosine (ddA) are performed by liquid chromatography tandem mass spectrometry (LC/MS/MS), e.g., of methanolic extracts of hepatocytes.

The concentration of ddATP is measured by comparison to a standard curve. Working stock solutions of TP-ddA are prepared from a 100 μmol/μL stock solution in de-ionized water of ddATP (tetrasodium salt of >91% purity) purchased from Sigma Chemical Co as follows: ddATP Working Stock Solutions and Preparation of Standard Curve for ddATP.

<table>
<thead>
<tr>
<th>Stock conc pmol/μL</th>
<th>Vol taken μL</th>
<th>D2H2O vol μL</th>
<th>Total vol μL</th>
<th>Conc pmol/μL</th>
<th>mol per 10 μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP-ddA</td>
<td>100</td>
<td>2000</td>
<td>2000</td>
<td>4000</td>
<td>50.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test article</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP-ddA</td>
</tr>
<tr>
<td>TP-ddA</td>
</tr>
<tr>
<td>TP-ddA</td>
</tr>
<tr>
<td>TP-ddA</td>
</tr>
<tr>
<td>TP-ddA</td>
</tr>
</tbody>
</table>

Internal standard (ISTD) working stock are prepared from a 0.50 mg/mL stock solution of 2-deoxyadenosine 5-triphosphate purchased from Sigma Chemical Co.
In some embodiments, calibration standards are prepared as follows using liver samples:

### Preparation of cal stds:

<table>
<thead>
<tr>
<th>cal std#</th>
<th>working stock con pmol/µL</th>
<th>working stock vol uL</th>
<th>ISTD vol uL</th>
<th>MeOH vol uL</th>
<th>total vol uL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blk</td>
<td>50.0</td>
<td>0.1</td>
<td>0</td>
<td>50</td>
<td>940</td>
</tr>
<tr>
<td>†1</td>
<td>0.1</td>
<td>#5</td>
<td>5.0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>†2</td>
<td>125.0</td>
<td>#4</td>
<td>12.5</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>†3</td>
<td>250.0</td>
<td>#3</td>
<td>25.0</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>†4</td>
<td>500.0</td>
<td>#2</td>
<td>50.0</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>†5</td>
<td>1000.0</td>
<td>#1</td>
<td>100.0</td>
<td>10</td>
<td>50</td>
</tr>
</tbody>
</table>

HPLC is conducted on Phenomenex Luna Amino 3 µm 100 A, 30x2 mm column, with a mobile phase: A: 70% 10 mM NH₄OAc 30% ACN pH 6.0; and B: 70% 1 mM NH₄OAc 30% ACN pH 10.5 as follows:

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (min)</th>
<th>Flow (µl/min)</th>
<th>A (%)</th>
<th>B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>400</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>1</td>
<td>1.1</td>
<td>400</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>1.11</td>
<td>400</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>2.11</td>
<td>400</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>2.6</td>
<td>400</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>3.1</td>
<td>400</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>5.5</td>
<td>400</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>5.51</td>
<td>400</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>400</td>
<td>60</td>
<td>40</td>
</tr>
</tbody>
</table>

These control samples were then processed and analyzed as described for test samples.

### LddA-TP formation in hepatocytes

<table>
<thead>
<tr>
<th>ASSO (Ex 1)</th>
<th>Time (hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µM</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>159.5</td>
</tr>
<tr>
<td>Monkey</td>
<td>384.0</td>
</tr>
<tr>
<td>Human</td>
<td>422.0</td>
</tr>
</tbody>
</table>

### In Vitro Phosphorylation in Hepatocytes

In some embodiments, calibration standards are prepared as follows using liver samples:

### Example 7

In Vivo Studies in Rat

### Distribution of A550 (NM-204) (the compound of Example 1 (Hydroxy-tBuSATE N-benzylphosphoramide

As indicated from the data, significant levels of L-ddATP were detected in the hepatocytes. In monkeys, the levels appear to reach a maximum level at 4 hours followed by a rapid decline. In contrast, levels in both rat and human hepatocyte appear to level off after 8 hours.

### Example 8
derivative of L-ddA) in the rat liver was evaluated following a single intravenous (I.V.) or oral administration of A550 (NM-204) at a dose of 20 (oral) or 10 (I.V.) mg/kg body weight. The dose solutions were prepared on the same day prior to dose administration.

[0426] At the specified time point (1 and 3 hours for IV animals or 1, 3 and 8 hours for oral animals), each animal was euthanized by CO2 gas followed by exsanguination via the abdominal vein. Livers were collected immediately after sacrifice, flash frozen in liquid nitrogen, placed on dry ice, and later stored at -70°C, before being analyzed.

Preparation of Calibration Standards from Control Liver Extracts:

[0427] Control rat liver samples were taken from whole frozen livers (Bioreclamation, Inc. Hicksville, N.Y.) with the aid of a tissue coring utensil (Harris Unicorn, 8.0 mm, VWR). Each ~0.1 g sample was placed in individual 2 mL poly vials with 0.940 mL of 80% MeOH/20% DIH2O and homogenates were prepared using a mechanical tissue disruptor (Tissue Master, Omni-International, Inc, Marietta Ga.). The vials received a 10 μL aliquot of a working stock solution and a 50 μL aliquot of the ISTD before vortexing for ~30 sec. The mixtures were stored overnight at -20°C. and the next day were removed for 10 min of centrifugation in a benchtop centrifuge. Each supernatant was transferred to individual centrifugation filtration units (0.45 μm) and the resulting filtrates were transferred to HPLC vials for the LC/MS/MS analysis. The final concentrations of ddATP in the calibration standards was 1000, 500, 250, 125, 50, and 0 pmol/mL. Each calibration standard was directly injected in a 50 μL volume onto the ion-exchange column for analysis. Standard curve analysis of calibration standards from control liver extracts was conducted.

[0428] Analysis of ddATP was done by an ion-exchange chromatography method with on-line positive ionization ESI-MS/MS detection in multiple reaction monitoring (MRM) detection mode. The peak areas obtained for 4 of the 5 calibrants allowed for construction of a standard curve that demonstrated good linearity (R2=0.9996) over a 50-1000 μmol/mL concentration range. This is equivalent to a range of 5-100 μmol per gram liver by the sample preparation employed. The HPLC MS MS conditions described in Example 5 were utilized. The lower limit of quantitation demonstrated by the LC/MS/MS method is e.g., ~0.2 pmol/mL for hepatocyte cellular extracts which contain much less salt.

[0429] The results showing intracellular levels of A550 (NM204) (showing the compound entered the liver cells) and LddATP (showing cleaving of the phosphoroamidate moiety and triphosphorylation of the ddA to the active triphosphate in the liver) are shown below:

| A550 (Ex 1) and LddATP measured in livers of male rats dosed IV or O with A550 (Ex 1) | Concentration | Concentration.  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Number</td>
<td>Compound</td>
<td>Timepoint (hrs)</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>IV dose (10 mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2M1</td>
<td>28.3</td>
<td>1</td>
</tr>
<tr>
<td>2M2</td>
<td>26.0</td>
<td>1</td>
</tr>
<tr>
<td>2M3</td>
<td>29.3</td>
<td>1</td>
</tr>
<tr>
<td>Mean</td>
<td>27.9</td>
<td>1</td>
</tr>
</tbody>
</table>

[0430] Thus, these results show that the compound can be used to enhance concentration of the drug in the liver. These results also show the enhanced concentration of the active triphosphate which is formed in the liver cells.

Example 9

Determination of Total Metabolism in Liver Subcellular Fractions

Depletion of Parent

[0431] NADPH Incubations. Microsomal or S9 incubations were conducted in a final volume of 0.5 mL. Pooled liver microsomal or S9 protein (1.0 mg/mL), suspended in incubation buffer (100 mM potassium phosphate, pH 7.4, 5 mM MgCl2 and 0.1 mM EDTA) was preincubated for 5 min at 37°C, with 10-50 μM OHSATE phosphoroamidate compound from a stock solution in DMSO (final DMSO concentration was 0.1%); the reaction was initiated by the addition of NADPH (3 mM final concentration). Incubations with no NADPH served as controls. At specific times (0-120 min), 0.1 mL samples were taken and the reaction terminated by the addition of 1 volume of stop solution (acetoniitre). The samples were vortex for 30 sec and then centrifuged at 1500 g for 10 min. The supernatant was transferred to HPLC glass vials and analyzed without further processing by HPLC. FIGS. 1 and 2 depict depletion of NM108 SATE and NM107 SATE, respectively, after incubation with NADPH in monkey liver S9.
HPLC System for Medium Samples-Unchanged Prodrug

[0432]

<table>
<thead>
<tr>
<th>HPLC:</th>
<th>Agilent 1100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column:</td>
<td>Phenomenex Luna C18(2), 20 x 2 mm</td>
</tr>
<tr>
<td>Mobile phase (MP):</td>
<td>MP(A) 10 mM K2HPO4 pH 5, MP(B) ACN</td>
</tr>
<tr>
<td>Gradient elution:</td>
<td>20 to 63% MP(B) run from 0 to 30 min</td>
</tr>
<tr>
<td>Runtime:</td>
<td>20 min</td>
</tr>
<tr>
<td>Flow rate:</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>Injection volume:</td>
<td>10-20 µL</td>
</tr>
<tr>
<td>UV:</td>
<td>252 nm-NM108SATE, 272 nm-NM107SATE</td>
</tr>
</tbody>
</table>

[0433] Thus, without being limited to any theory, since the metabolism is NADPH dependent, it is possible that the phosphorimidate compound is preferentially activated by Cytochrome P450 in the liver.

Example 10

Determination of Triphosphate Levels in Cells

[0434] Preparation of Primary Hepatocyte Cultures

[0435] Freshly isolated cells from animal and human liver were obtained in suspension on ice. Following receipt, cells were pelleted by centrifugation at 500 rpm (rat) or 700 rpm (monkey and human) and resuspended at 0.8 million cells per mL of plating medium (HM). Multi-well collagen-coated plates (12-well) were then seeded by addition of 1 mL of cell suspension (0.8 million cells/mL). The plates were gently shaken to evenly distribute the cells and placed in an incubator at 37°C for approximately 4 to 6 hours to allow cells to attach. Once cells have attached, the plating medium was removed and replaced with hepatocyte culture medium (HCM). Cells were left overnight in an incubator at 37°C to acclimatize to culture and the medium.

[0436] Incubations with Test Article

[0437] Hepatocyte incubations were conducted in a final volume of 1.0 mL HCM/well (0.8 million cells/mL). HCM from overnight incubation of cells was removed and replaced with fresh HCM, pre-warmed to 37°C, containing 10 µM test article from a stock solution in DMSO (final DMSO concentration was 0.1%). At specific times (up to 24 hrs), incubation medium was discarded and the cell monolayers were carefully washed two times with ice-cold PBS. Following the last wash, all PBS was carefully removed and 1 mL of extraction buffer (ice-cold 70% methanol) was added. Each well was sealed with parafilm immediately following addition of methanol. Once the entire plate was processed, additional parafilm was placed on entire plate forming a double seal to prevent evaporation during the extraction process. The cover lid was then placed on the plate and sealed with tape. The plates were then stored at -20°C for a minimum of 24 hrs to allow for extraction of intracellular contents.

[0438] Preparation of Huh7 or HepG2 Cultures

[0439] HepG2s or Huh7 cells were plated at 0.4 x 10⁶ cells/well in collagen-coated 12-well plates. Cells were allowed to attach overnight. Culture medium from overnight incubation of cells was removed and replaced with fresh culture medium, pre-warmed to 37°C, containing 10 µM test article from a stock solution in DMSO (final DMSO concentration was 0.1%). After 24-72 hours, incubation medium was discarded and the cell monolayers were carefully washed two times with ice-cold PBS. Following the last wash, all PBS was carefully removed and 1 mL of extraction buffer (ice-cold 70% methanol) was added. Each well was sealed with parafilm immediately following addition of methanol. Once the entire plate was processed, additional parafilm was placed on entire plate forming a double seal to prevent evaporation during the extraction process. The cover lid was then placed on the plate and sealed with tape. The plates were then stored at -20°C for a minimum of 24 hrs to allow for extraction of intracellular contents.

[0440] Sample Preparation for Analysis

[0441] Cellular extracts were prepared by transferring 0.9 mL of extract into 2 mL microfuge tubes followed by centrifugation for 5 min at 14,000 rpm. Approximately 100 µL of the supernatant was transferred to HPLC vials and triphosphate levels determined by LC/MS/MS as described below.

[0442] HPLC conditions: NM107-triphosphate

| HPLC: | Phenomenex Luna Amino 3 µm 100A, 30 x 2 mm, |
| Mobile phases (MP): | (A) 70% 10 mM NH4OAc 30% ACN pH 6.0, (B) 70% 1 mM NH4OAc 30% ACN pH 10.5 |
| Gradient elution: | Step | Time | Flow | A | B |
| 0 | 0.00 | 400 | 80 | 20 |
| 1 | 0.10 | 400 | 80 | 20 |
| 2 | 0.11 | 400 | 40 | 60 |
| 3 | 0.21 | 400 | 40 | 60 |
| 4 | 2.60 | 400 | 100 | 100 |
| 5 | 2.61 | 400 | 0 | 100 |
| 6 | 5.60 | 400 | 0 | 100 |
| 7 | 5.61 | 400 | 80 | 20 |
| 8 | 9.00 | 400 | 80 | 20 |
| Flow rate to MS: | 0.400 mL/min, no split |
| Injection volume: | 10 µL |
| Compound | Precursor ion | Product ion |
| NM107 triphosphate | 498.0 | 112.0 |

[0443] HPLC conditions: NM108-triphosphate

| HPLC: | Phenomenex Luna Amino 3 µm 100A, 30 x 2 mm, |
| Mobile phases (MP): | (A) 70% 10 mM NH4OAc 30% ACN pH 6.0, (B) 70% 1 mM NH4OAc 30% ACN pH 10.5 |
| Gradient elution: | Step | Time | Flow | A | B |
| 0 | 0.00 | 400 | 60 | 40 |
| 1 | 0.10 | 400 | 60 | 40 |
| 2 | 0.11 | 400 | 40 | 60 |
| 3 | 0.21 | 400 | 40 | 60 |
| 4 | 2.60 | 400 | 100 | 100 |
| 5 | 2.61 | 400 | 0 | 100 |
| 6 | 5.60 | 400 | 0 | 100 |
| 7 | 5.61 | 400 | 60 | 40 |
| 8 | 9.00 | 400 | 60 | 40 |
| Flow rate to MS: | 0.400 mL/min, no split |
| Injection volume: | 10 µL |
| Compound | Precursor ion | Product ion |
| NM108 triphosphate | 538.0 | 152.0 |
[0444] NM107 triphosphate levels and B102 in cell extracts were observed as follows:

<table>
<thead>
<tr>
<th>Intracellular Triphosphate</th>
<th>Human</th>
<th>Monkey</th>
<th>HepG2*</th>
<th>Huh7*</th>
</tr>
</thead>
<tbody>
<tr>
<td>drug in culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B102 (Ex. 2)</td>
<td>991</td>
<td>1838</td>
<td>1.5</td>
<td>9.2</td>
</tr>
<tr>
<td>NM107</td>
<td>19</td>
<td>10</td>
<td>17</td>
<td>37</td>
</tr>
</tbody>
</table>

24 hr incubation in 10 μM drug
*72 hr incubation in 10 μM drug

[0445] As seen from the data levels of intracellular triphosphate for B102 (Ex. 2) were much higher as compared to those for NM107.

[0446] All publications and patent, applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. While the claimed subject matter has been described in terms of various embodiments, the skilled artisan will

What we claim is:

1. A compound of formula

or a pharmaceutically acceptable salt, solvate, a stereoisomeric, tautomeric or polymorphic form thereof, wherein:

R<sup>1</sup> is optionally substituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, cycloalkyl, cycloalkenyl, amino, heterocyclic or heteroaryl;

R<sup>1</sup> and R<sup>2</sup> are selected as follows:

i) R<sup>1</sup> and R<sup>2</sup> are each independently hydrogen or optionally substituted alkyl, carboxyalkyl, hydroxyalkyl, hydroxyarylalkyl, acyloxalkyl, aminocarboxyalkyl, alkoxyacylalkyl, aryl, arylalkyl, cycloalkyl, heteroaryl or heterocyclic; or

ii) R<sup>1</sup> and R<sup>2</sup> together with the nitrogen atom on which they are substituted form a 3-7 membered heterocyclic or heteroarylic ring; and

R<sup>1</sup> is a moiety derivable by removal of a hydrogen from a hydroxy group of an anti-cancer drug.

2. The compound of claim 1, having the formula:

or a pharmaceutically acceptable salt, solvate, a stereoisomeric, tautomeric or polymorphic form thereof.

3. The compound of claim 1 having the formula:

or a pharmaceutically acceptable salt, solvate, a stereoisomeric, tautomeric or polymorphic form thereof.

4. The compound of claim 1 having the formula:

or a pharmaceutically acceptable salt, solvate, a stereoisomeric, tautomeric or polymorphic form thereof.

5. The compound of claim 1 where R<sup>1</sup> is optionally substituted alkyl and R<sup>2</sup> and R<sup>3</sup> are each independently hydrogen or optionally substituted benzyl.

6. The compound of claim 5 where R<sup>1</sup> is hydroxyalkyl or aminoalkyl.
7. The compound of claim 1, wherein R" is —C(R')₂ or —NHR where each R' is independently optionally substituted alkyl or optionally substituted aryl; and R" and R' are independently hydrogen, optionally substituted alkyl or optionally substituted arylalkyl.

8. The compound of claim 7, wherein R" and R' are each independently hydrogen or substituted alkyl.

9. The compound of claim 5, wherein R" is selected from the group consisting of alkyl and hydroxyalkyl.

10. The compound of claim 9, wherein R" is —C(CH₃)₃CH₂OH.

11. The compound of claim 9, wherein R" is hydrogen, R' is benzyl and R" is —C(CH₃)₂CH₂OH.


13. A compound of formula:
wherein

each R, if present, is independently alkyl, halogen or hydroxy;
X, if present, is CH₃, O or S;
R' is alkyl, alkenyl, alkynyl, aryl, aryl alkyl, cycloalkyl, cycloalkenyl, amino, aminooalkyl, heteroaryl or heterocyclyl or heteroaryl, all optionally substituted;
R² and R³ are selected as follows:
i) R² and R³ are each independently hydrogen, alkyl, carboxyalkyl, hydroxyalkyl, hydroxyarylalkyl, acyloxyalkyl, aminocarbonylalkyl, alkoxycarbonylalkyl, aryl, aryl alkyl, cycloalkyl, heteroaryl or heterocyclyl, all optionally substituted; or

21. The compound of claim 13, wherein R is hydrogen, R is benzyl and R" is —C(CH)CH₂OH.

22. The compound of claim 13 having the structure:

23. A compound selected from formula:

wherein
R¹ and R² are each independently hydrogen or optionally substituted alkyl;
R⁴ is optionally substituted alkyl; and
X³ is O or S;
R² is alkyl, alkenyl, alkynyl, aryl, arylalkyl, cycloalkyl, heteroaryl or heterocyclyl, all optionally substituted;
R⁴ is hydrogen or substituted alkyl.

22. The compound of claim 13 having the structure:

23. A compound selected from formula:
ii) \( R^a \) and \( R^b \) together with the nitrogen atom on which they are substituted form a 3-7 membered heterocyclic or heteroaryl ring.

24. The compound of claim 23, wherein \( R^a \) is optionally substituted alkyl, wherein the substituents when present are selected from hydroxy and amino.

25. The compound of claim 24, wherein \( R^a \) is \(-C(CH_3)_2\) \( CH_2OH \).

26. The compound of claim 25, wherein \( R^a \) is hydrogen and \( R^b \) is benzyl.

27. The compound of claim 26 having formula selected from:

\[
\begin{align*}
&X_{a} \\
&X_{b} \\
&X_{1a} \\
&X_{1b} \\
&X_{IIa} \\
&X_{IIb} \\
&X_{IIIa} \\
&X_{IIIb} \\
&X_{IVa} \\
&X_{IVb} \\
\end{align*}
\]
28. The compound of claim 27 having formula:


30. A method of lowering plasma lipid levels or lowering blood glucose levels comprising administering a compound of claim 13.

31. A method of lowering blood glucose levels comprising administering a compound of claim 23.

32. A pharmaceutical composition comprising a compound of any of claims 1, 13 or 23 and a pharmaceutically acceptable carrier.

33. The composition of claim 32 that is suitable for oral administration.

34. The composition of claim 33 wherein the composition is in the form of a pill or tablet.