

CONJUGATE COMPOUND COMPRISING 9-CIS-RETINOIC ACID AND MONOSACCHARIDE

FIELD OF THE INVENTION

[0001] The present invention relates to a conjugate compound comprising 9-cis-retinoic acid and a monosaccharide, and its pharmaceutically acceptable salts thereof. The present invention also relates to a pharmaceutical composition comprising the compound and a pharmaceutically acceptable carrier.

BACKGROUND OF THE INVENTION

[0002] Retinoic acids including all-trans-retinoic acid (ATRA, also called Vitamin A acid or Tretinoin) and 9-cis-retinoic acid are promising compounds for treatment of various cancers due to their specific effects on cell proliferation, differentiation, and apoptosis, as well as their low toxicity. Biochemists have discovered retinoic acid receptors in human cell nuclei, which are not mutated in cancer cells.

[0003] Several clinical trials are underway to evaluate ATRA as a part of combination therapy. For instance, combining ATRA with different interferons (IFN) have demonstrated enhanced anticancer effects, including growth inhibition and tumor cell death in cell lines. However, to fully harness the therapeutic potential of retinoic acids, studies emphasize the need for a deeper understanding of the mechanisms that block retinoic acid signaling and regulated gene expression in cancers, such as acute myeloid leukemia (AML). These studies suggest that combinatorial therapies targeting multiple gene-silencing mechanisms may be the most effective strategy in reactivating ATRA-sensitive gene expression and differentiation of AML cells, as well as mediating anticancer activities of ATRA in general.

[0004] Currently, identifying classes of proteins that control gene expression via histone and DNA modifications has spurred the development of new therapeutic agents, so-called epigenetic drugs, which alter chromatin structure. However, these epigenetic modifying drugs have proven only partly effective against different cancers when used alone. This limitation highlights the necessity of exploring alternative approaches that can complement or enhance cancer therapies.

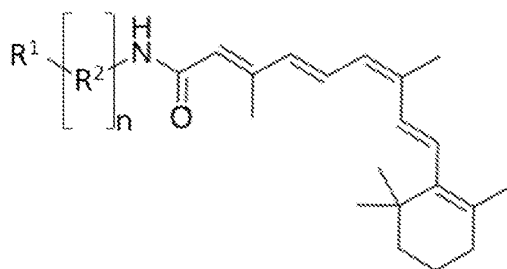
BRIEF DESCRIPTION OF THE DRAWINGS

- [0005] **FIG. 1** illustrates a ¹H NMR spectrum of galactose 9-cis-retinoic acid.
- [0006] **FIG. 2** illustrates a curve diagram of cell viability showing the cytotoxicity effect of galactose 9-cis-retinoic acid on the A549 cancer cell line according to an embodiment of the present invention.
- [0007] **FIG. 3** illustrates a curve diagram of cell viability showing the cytotoxicity effect of galactose 9-cis-retinoic acid on the H460 cancer cell line according to an embodiment of the present invention.
- [0008] **FIG. 4** illustrates a curve diagram of cell viability showing the cytotoxicity effect of galactose 9-cis-retinoic acid on the Huh-7 cancer cell line according to an embodiment of the present invention.
- [0009] **FIG. 5** illustrates a curve diagram of cell viability showing the cytotoxicity effect of galactose 9-cis-retinoic acid on the AsPC-1 cancer cell line according to an embodiment of the present invention.
- [0010] **FIG. 6** illustrates a curve diagram of cell viability showing the cytotoxicity effect of galactose 9-cis-retinoic acid on the MDA-MB-231 cancer cell line according to an embodiment of the present invention.
- [0011] **FIG. 7** illustrates a curve diagram of cell viability showing the cytotoxicity effect of galactose 9-cis-retinoic acid on the SKOV-3 cancer cell line according to an embodiment of the present invention.

DETAILED DESCRIPTION

Conjugate Compound

- [0012] The present invention relates to conjugate compounds comprising 9-cis-retinoic acid and a monosaccharide. The 9-cis-retinoic acid in the conjugate compound provides therapeutic activity, and the monosaccharide in the conjugate compound increases the water solubility of the compound, and significantly reduces the effective dosage of 9-cis-retinoic acid in the compound for treating a disease.
- [0013] In one embodiment, the compound is represented by formula (I):



(I)

wherein R¹ is a substituted or unsubstituted monosaccharide, R² is a substituted or unsubstituted aliphatic group with 1 to 20 carbon atoms, preferably 1 to 10 carbon atoms, and more preferably 1 to 6 carbon atoms, and n is 0 or 1.

[0014] Monosaccharides, also called simple sugars, are the simplest forms of sugar and the most basic units (monomers) from which all carbohydrates are built. Chemically, monosaccharides include polyhydroxy aldehydes with the formula H-[CHOH]_n-CHO or polyhydroxy ketones with the formula H-[CHOH]_m-CO-[CHOH]_n-H with three or more carbon atoms.

[0015] In certain embodiments, the monosaccharide includes, but not limited to, aldehyde sugars such as galactose, glucose, mannose, glyceraldehyde, erythrose, ribose, arabinose, xylose, idose, gulose, talose, allose, and altrose; ketone sugars such as fructose, erythrulose, ribulose, xylulose, psicose, sorbose, tagatose, and deoxy sugars such as deoxyribose, 2-deoxyglucose, fucose, rhamnose, quinovose; and amino sugars such as glucosamine, galactosamine, and mannosamine. Preferred monosaccharides for the present invention include galactose, glucose, mannose, fructose, ribose, deoxyribose and fucose.

[0016] In certain embodiments, the compound of formula (I), or the pharmaceutically acceptable salt thereof, can be prepared in dimethyl sulfoxide (DMSO), ethanol, buffer, saline or water for administration. The dosage and the frequency of administration thereof may vary depending on the following factors: the severity of the illness (*e.g.*, cancer) to be treated and the weight, age, physical condition and response of the subject to be treated. The daily dosage of the aforesaid treating agents may be administered in a single dose or in several doses.

[0017] In certain embodiments, the compound of formula (I), or the pharmaceutically acceptable salt thereof, is administered by an oral, intravenous, intramuscular, subcutaneous, intraperitoneal, intranasal, aerosol or topical route.

Pharmaceutical Compositions

[0018] The present invention provides pharmaceutical compositions including one or more pharmaceutically acceptable carriers and the compound of formula (I).

[0019] In one embodiment, the compound is incorporated into any acceptable carrier, including creams, gels, lotions or other types of suspensions that can stabilize the compound and deliver it to the affected area by topical applications. In another embodiment, the pharmaceutical composition can be in a dosage form such as tablets, capsules, granules, fine granules, powders, syrups, suppositories, injectable solutions, patches, or the like. The above pharmaceutical composition can be prepared by conventional methods.

[0020] Pharmaceutically acceptable carriers, which are inactive ingredients, can be selected by those skilled in the art using conventional criteria. Pharmaceutically acceptable carriers include, but are not limited to, non-aqueous based solutions, suspensions, emulsions, microemulsions, micellar solutions, gels, and ointments. The pharmaceutically acceptable carriers may also contain ingredients that include, but are not limited to, saline and aqueous electrolyte solutions; ionic and nonionic osmotic agents such as sodium chloride, potassium chloride, glycerol, and dextrose; pH adjusters and buffers such as salts of hydroxide, phosphate, citrate, acetate, borate; and trolamine; antioxidants such as salts, acids and/or bases of bisulfite, sulfite, metabisulfite, thiosulfite, ascorbic acid, acetyl cysteine, cysteine, glutathione, butylated hydroxyanisole, butylated hydroxytoluene, tocopherols, and ascorbyl palmitate; surfactants such as lecithin, phospholipids, including but not limited to phosphatidylcholine, phosphatidylethanolamine and phosphatidyl inositol; poloxamers and poloxamines, polysorbates such as polysorbate 80, polysorbate 60, and polysorbate 20, polyethers such as polyethylene glycols and polypropylene glycols; polyvinyls such as polyvinyl alcohol and povidone; cellulose derivatives such as methylcellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, carboxymethyl cellulose and hydroxypropyl methylcellulose and their salts; petroleum derivatives such as mineral oil and white petrolatum; fats such as lanolin, peanut oil, palm oil, soybean oil; mono-, di-, and triglycerides; polymers of acrylic acid such as carboxypolymethylene gel, and hydrophobically modified cross-linked acrylate copolymer; polysaccharides such as dextrans and glycosaminoglycans such as sodium hyaluronate. Other pharmaceutically acceptable carriers include xanthan gum, carrageenan, Avicel RC-591 (a combination of microcrystalline cellulose and), and polyethylene glycol. Alternately, the active compound may be dissolved or suspended

in a pharmaceutically acceptable lipid formulation such as those described by Kalepu et al (*Acta Pharmaceutica Sinica B*, 3: 361-372, 2013), for example, vegetable oil, coconut oil, castor oil, etc.

[0021] Such pharmaceutically acceptable carriers may be preserved against bacterial contamination using well-known preservatives, these include, but are not limited to, benzalkonium chloride, ethylenediaminetetraacetic acid and its salts, benzethonium chloride, chlorhexidine, chlorobutanol, methylparaben, thimerosal, and phenylethyl alcohol, or may be formulated as a non-preserved formulation for either single or multiple use.

[0022] For example, a tablet formulation or a capsule formulation of the compound may contain other excipients that have no bioactivity and no reaction with the compound. Excipients of a tablet or a capsule may include fillers, binders, lubricants and glidants, disintegrators, wetting agents, and release rate modifiers. Binders promote the adhesion of particles of the formulation and are important for a tablet formulation. Examples of excipients of a tablet or a capsule include, but not limited to, carboxymethylcellulose, cellulose, ethylcellulose, hydroxypropylmethylcellulose, methylcellulose, karaya gum, starch, tragacanth gum, gelatin, magnesium stearate, titanium dioxide, poly(acrylic acid), and polyvinylpyrrolidone. For example, a tablet formulation may contain inactive ingredients such as colloidal silicon dioxide, crospovidone, hypromellose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, sodium starch glycolate, and/or titanium dioxide. A capsule formulation may contain inactive ingredients such as gelatin, magnesium stearate, and/or titanium dioxide.

[0023] For example, a patch formulation of the compound may include some inactive ingredients such as 1,3-butylene glycol, dihydroxyaluminum aminoacetate, disodium edetate, D-sorbitol, gelatin, kaolin, methylparaben, polysorbate 80, povidone, propylene glycol, propylparaben, sodium carboxymethylcellulose, sodium polyacrylate, tartaric acid, titanium dioxide, and purified water. A patch formulation may also contain skin permeability enhancer such as lactate esters or diethylene glycol monoethyl ether.

[0024] Topical formulations including the compound can be in a form of gel, cream, lotion, liquid, emulsion, ointment, spray, solution, and suspension. The inactive ingredients in the topical formulations for example include, but not limited to, (emollient/permeation enhancer), diethylene glycol monoethyl ether (emollient/permeation enhancer), DMSO (solubility enhancer), silicone elastomer (rheology/texture modifier), caprylic/capric triglyceride, (emollient), octisalate,

(emollient/UV filter), silicone fluid (emollient/diluent), squalene (emollient), sunflower oil (emollient), and silicone dioxide (thickening agent).

[0025] **Method of Use**

[0026] This claimed compound is useful for treating cancers such as pancreatic cancer, lung cancer, breast cancer, liver cancer, and ovarian cancer. The method includes the step of administering to a cancer patient the claimed compound, in an amount effective to treat cancer. "An effective amount," as used herein, is the amount effective to treat a disease by ameliorating the pathological condition or reducing the symptoms of the disease.

[0027] The claimed compound is also useful for treating viral infections such as respiratory syncytial virus (RSV). The method includes the step of administering to a subject in need thereof a therapeutically effective amount of the claimed compound, wherein the effective amount is sufficient to inhibit viral replication, reduce viral load, or alleviate symptoms associated with the infection. "An effective amount," as used herein, refers to the amount effective to mitigate the pathological condition caused by the viral infection or to improve the clinical symptoms of the infected subject.

[0028] The pharmaceutical composition of the present invention can be applied by systemic administration or local administration and. Local administration includes topical administration and inhalation. Systemic administration includes oral, parenteral (such as intravenous, intramuscular, subcutaneous or rectal), and other systemic routes of administration. In systemic administration, the active compound first reaches plasma and then distributes into target tissues. Intravenous administration is a preferred route of administration for the present invention.

[0029] Dosing of the composition can vary based on the extent of the injury and each patient's individual response. For systemic administration, plasma concentrations of the active compound delivered can vary; but are generally 1×10^{-10} - 1×10^{-4} moles/liter, and preferably 1×10^{-8} - 1×10^{-5} moles/liter.

[0030] In one embodiment, the pharmaceutical composition is administered intravenously to the subject. The dosage for intravenous bolus injection or intravenous infusion is generally 0.01 to 100 mg/kg/day and preferably 0.03 to 30 mg/kg/day.

[0031] In one embodiment, the pharmaceutical composition is orally administered to the subject.

[0032] Those of skill in the art will recognize that a wide variety of delivery mechanisms are also suitable for the present invention.

[0033] The present method is useful in treating a mammal subject, such as humans, horses, and dogs. The present invention is particularly useful in treating humans.

[0034] It is to be understood that the foregoing describes preferred embodiments of the present invention and that modifications may be made therein without departing from the scope of the present invention as set forth in the claims.

[0035] The present invention at least provides the following advantages:

1. The claimed compound and the pharmaceutical composition thereof can enhance the ability to inhibit cancer cells and thus effectively treat various cancers, such as lung cancer, ovarian cancer, breast cancer, pancreatic cancer, and liver cancer.
2. The claimed compound and the pharmaceutical composition thereof can anti-virus activity, particularly Respiratory Syncytial Virus (RSV).
3. The claimed compound has a better water solubility and thus reduces an effective dosage of retinoic acid and minimizes side effect. Such modifications may increase the efficiency and concentration of retinoic acid in acrossing the cell membrane and enter the cell for therapy.

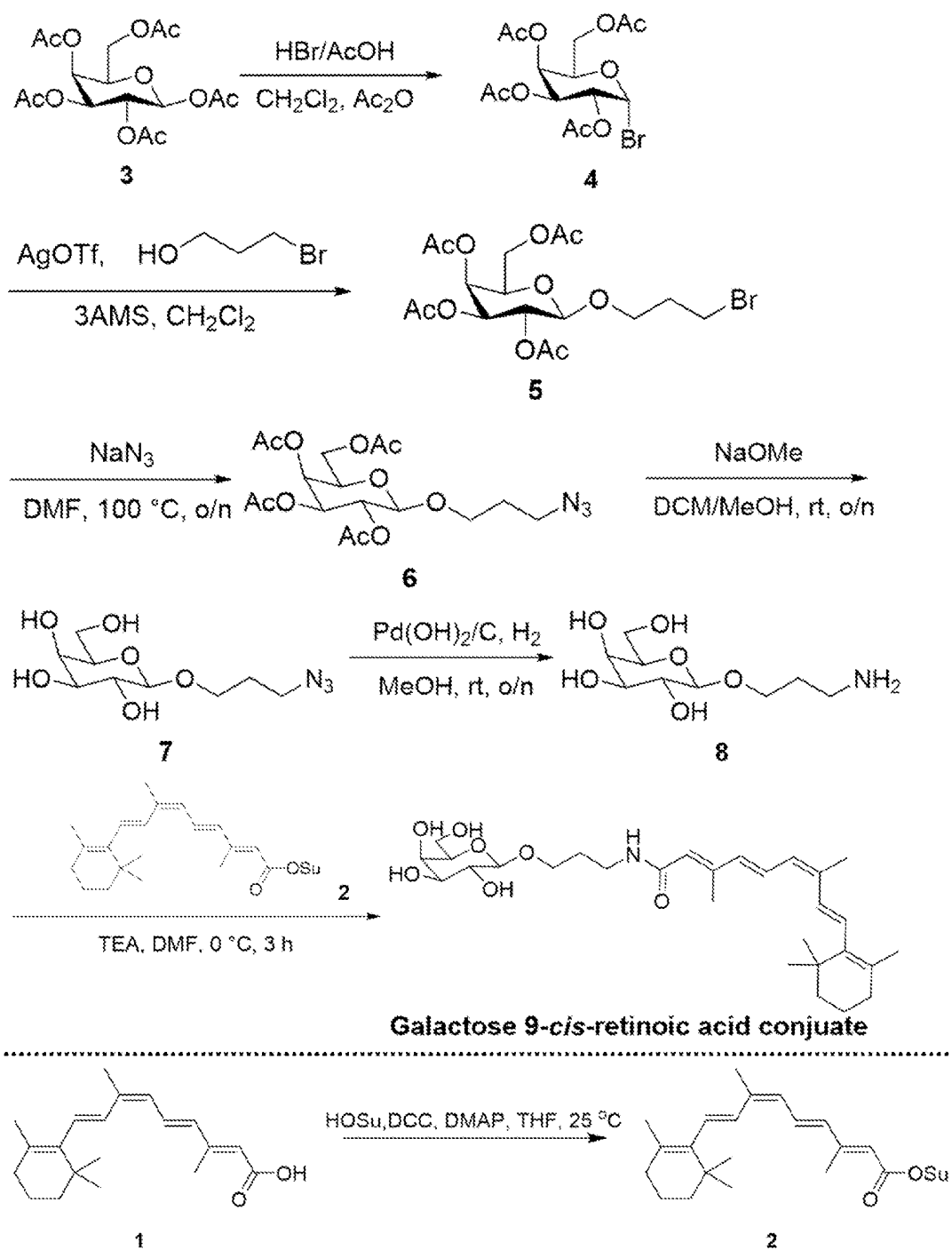
EXAMPLES

[0036] The present invention will be further described by way of the following examples. However, it should be understood that the following examples are intended solely for the purpose of illustration and should not be construed as limiting the present invention in practice.

[0037] **Example 1. Synthesis of Galactose 9-cis-retinoic acid**

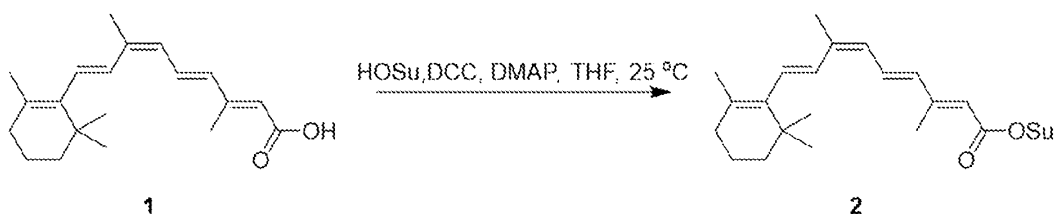
[0038] **Materials and methods**

[0039] Galactose 9-cis-retinoic acid, a conjugate of Galactose linked to 9-cis-retinoic acid is synthesized through the Scheme I below:



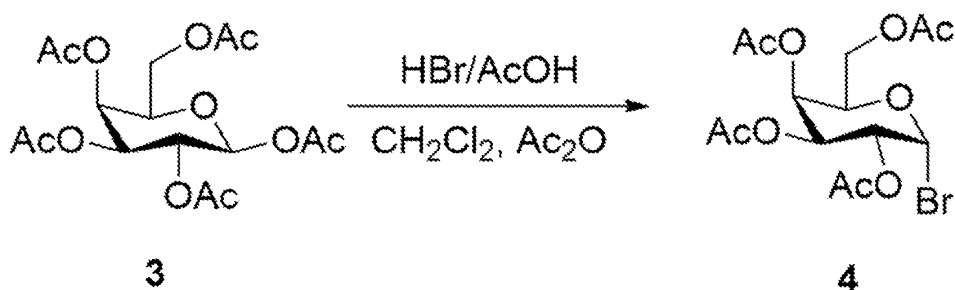
Scheme I

[0040] *Step 1 : Synthesis of the compound 2: 2,5-dioxopyrrolidin-1-yl (2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenoate*



[0041] To a solution of 9-*cis*-retinoic acid (50 mg, 0.17 mmol) in anhydrous DMF (2 mL) was added HOSu (29 mg, 0.25 mmol), DCC (69 mg, 0.34 mmol) and 4-DMAP (21 mg, 0.17 mmol). The reaction mixture was stirred at 25°C for 16 h. The reaction mixture was treated with H₂O and extracted with EtOAc. The combined organic phases were washed with brine twice, dried over Na₂SO₄ and concentrated *in vacuo* to afford the title compound (60 mg, 0.12 mmol, 73% yield) as yellow solid, which was used directly without further purification.

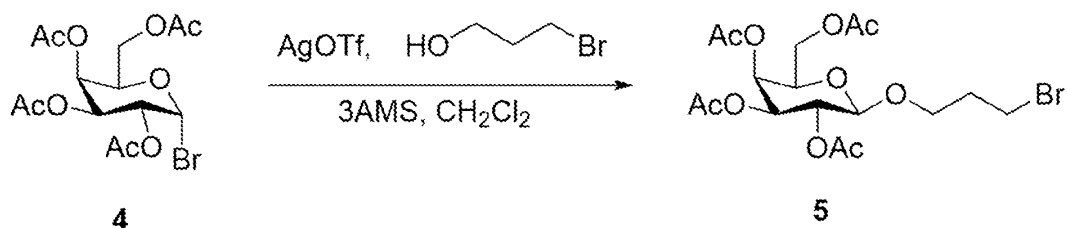
[0042] *Step 2 : Synthesis of the compound 4: (2R,3S,4S,5R,6R)-2-(acetoxymethyl)-6-bromotetrahydro-2H-pyran-3,4,5-triyl triacetate*



[0043] A solution of (2S,3R,4S,5S,6R)-6-(acetoxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl tetraacetate (5 g, 12.8 mmol) in DCM (30 mL) was added 33% of HBr in AcOH solution (8 mL) and acetic anhydride (1.5 mL). The reaction was stirred at 25°C for 6 h. The reaction mixture was poured into ice water and extracted with DCM. The combined organic layers were dried over MgSO₄, filtered, and concentrated. The residue was purified by flash chromatography column (eluent EtOAc in PE = 0-60%) to give the title compound (5.5 g, 12 mmol, 93% yield) as colorless oil.

[0044] ^1H NMR (400 MHz, CDCl_3) δ 6.70 (d, $J = 4.0$ Hz, 1H), 5.52 (d, $J = 3.2$ Hz, 1H), 5.41 (dd, $J = 10.8, 3.2$ Hz, 1H), 5.05 (dd, $J = 10.4, 4.0$ Hz, 1H), 4.49 (t, $J = 6.4$ Hz, 1H), 4.14 – 4.07 (m, 2H), 2.15 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H).

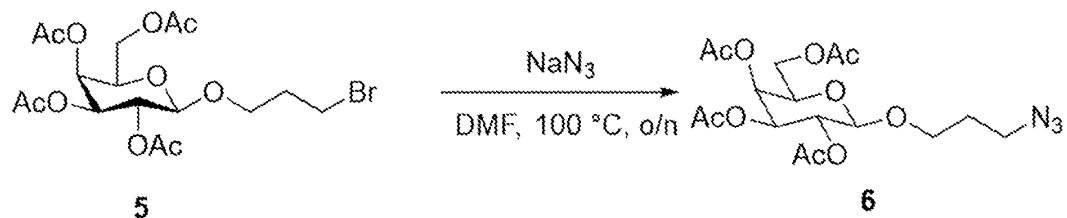
[0045] *Step 3 : Synthesis of the compound 5: [(3S,4S,6R)-3,4,5-tris(acetyloxy)-6-(3-bromopropoxy)oxan-2-yl]methyl acetate*



[0046] To a solution of compound 4 (5 g, 12.2 mmol) and powder 3Å MS (5 g) in dichloromethane (50 mL) was added 3-bromopropan-1-ol (1.7 g, 12.2 mmol) at 0 °C. The mixture was checked by TLC showed no starting material. The mixture was added AgOTf (6.27 g, 24.4 mmol) at 0°C. The mixture was stirred at 25°C for 16 h. The residue was diluted with water and extracted with DCM. The combined organic layers were dried over Na_2SO_4 , filtered, and concentrated, the residue was purified by flash chromatography column (eluent EtOAc in PE = 0–80%) to give the title compound (1.5 g, 2.9 mmol, 24% yield) as a yellow oil.

[0047] ^1H NMR (400 MHz, CDCl_3) δ 5.40 (d, $J = 3.2$ Hz, 1H), 5.20 (dd, $J = 10.4, 8.0$ Hz, 1H), 5.03 (dd, $J = 10.4, 3.2$ Hz, 1H), 4.48 (d, $J = 8.0$ Hz, 1H), 4.22 – 4.10 (m, 4H), 4.05 – 3.98 (m, 1H), 3.92 (t, $J = 6.4$ Hz, 1H), 3.72 – 3.66 (m, 1H), 3.50 – 3.46 (m, 2H), 2.15 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H).

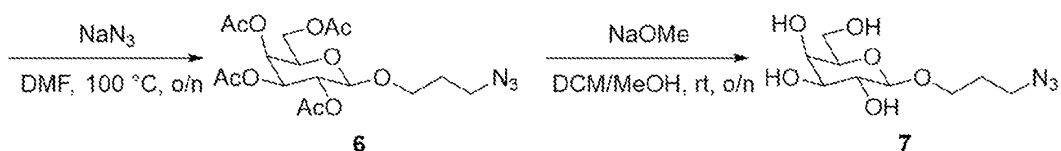
[0048] *Step 4 : Synthesis of compound 6: [(3S,4S,6R)-3,4,5-tris(acetyloxy)-6-(3-azidopropoxy)oxan-2-yl]methyl acetate*



[0049] To a solution of compound 5 (1.5 g, 3.2 mmol) in anhydrous DMF (15 mL) was added NaN_3 (0.42 g, 6.4 mmol) and stirred at 100°C for 16 h. The reaction *MIXTURE* was treated with H_2O and extracted with EtOAc. The combined organic phases were washed with brine, dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography column (eluent EtOAc in PE = 0-90%) to afford the title compound (0.64 g, 1.3 mmol, 41% yield) as a yellow oil.

[0050] ^1H NMR (400 MHz, CDCl_3) δ 5.39 (d, $J = 2.8$ Hz, 1H), 5.20 (dd, $J = 10.4, 8.0$ Hz, 1H), 5.02 (dd, $J = 10.4, 3.2$ Hz, 1H), 4.47 (d, $J = 8.0$ Hz, 1H), 4.22 – 4.13 (m, 2H), 4.02 – 3.88 (m, 2H), 3.64 – 3.58 (m, 1H), 3.41 – 3.34 (m, 2H), 2.16 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.91 – 1.81 (m, 2H).

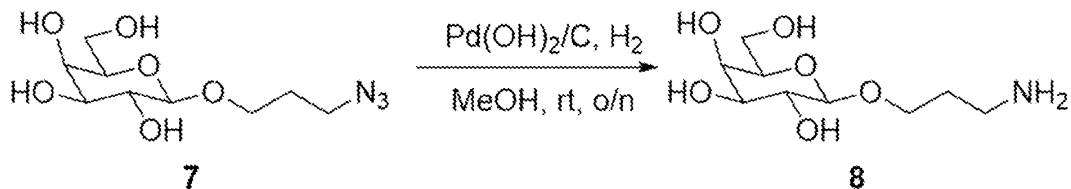
[0051] *Step 5: Synthesis of the compound 7: (2R,4S,5R)-2-(3-azidopropoxy)-6-(hydroxymethyl)oxane-3,4,5-triol*



[0052] To a solution of compound 6 (640 mg, 1.48 mmol) in MeOH (8 mL) and DCM (8 mL) was added NaOMe (401 mg, 7.4 mmol). The reaction mixture was stirred at 25°C for 16 h. The reaction mixture was acidified pH value to 7 by Dowex[®] 50W X8. The mixture was filtered and concentrated *in vacuo*. The residue was purified by flash chromatography column (eluent MeOH in DCM = 0-20%) to afford the title compound (350 mg, 1.19 mmol, 80% yield) as a yellow oil.

[0053] ^1H NMR (400 MHz, CD_3OD) δ 4.21 (d, $J = 7.2$ Hz, 1H), 3.99 – 3.93 (m, 1H), 3.83 (d, $J = 3.2$ Hz, 1H), 3.75 – 3.71 (m, 2H), 3.66 – 3.61 (m, 1H), 3.53 – 3.43 (m, 5H), 1.90 – 1.83 (m, 2H).

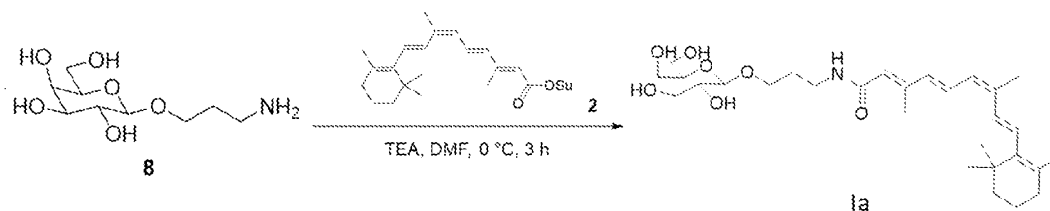
[0054] *Step 6: Synthesis of the compound 8: (2R,4S,5R)-2-(3-aminopropoxy)-6-(hydroxymethyl)oxane-3,4,5-triol*



[0055] To a solution of compound 7 (350 mg, 1.33 mmol) in MeOH (5 mL) was added Pd(OH)₂/C (94 mg, 20 wt.%). The mixture was stirred at 25°C for 16 h. The reaction mixture was filtered by a celite pad and washed with MeOH. The filtrate was concentrated *in vacuo* to afford the title compound (250 mg, 0.84 mmol, 64% yield) as a colorless oil, which was used for next step without further purification.

[0056] ¹H NMR (400 MHz, CD₃OD) δ 4.21 (d, *J* = 7.2 Hz, 1H), 4.03 – 3.94 (m, 1H), 3.82 (d, *J* = 3.2 Hz, 1H), 3.76 – 3.70 (m, 2H), 3.67 – 3.63 (m, 1H), 3.52 – 3.43 (m, 3H), 2.81 – 2.76 (m, 2H), 1.80 – 1.74 (m, 2H).

[0057] *Step 7: Synthesis of the target compound Ia: (2E,4E,6Z,8E)-3,7-dimethyl-N-(3-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]propyl)-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenamide*



[0058] To a solution of compound 8 (40 mg, 0.17 mmol) in anhydrous DMF (3 mL) was added 2,5-dioxopyrrolidin-1-yl(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenoate (60 mg, 0.15 mmol) and triethylamine (52 mg, 0.51 mmol) at 0°C. The reaction mixture was stirred in ice bath for 3 h. The mixture was treated with H₂O and extracted with EtOAc. The residue was purified by flash chromatography column (eluent MeOH in DCM = 0-20%) to afford the title compound (20 mg, 0.04 mmol, 22% yield) as a yellow solid.

[0059] MS (ESI): mass calcd. for C₂₉H₄₅NO₇ 519.32, *m/z* found 542.35 [M+Na]⁺.

[0060] (FIG. 1) ¹H NMR (400 MHz, CD₃OD-*d*₄) δ 7.06 – 6.99 (m, 1H), 6.68 (d, *J* = 16.0 Hz, 1H), 6.29 – 6.23 (m, 2H), 6.08 (d, *J* = 11.6 Hz, 1H), 5.84 (s, 1H), 4.22 (d, *J* = 7.6 Hz, 1H), 3.96 – 3.90 (m, 1H), 3.82 (d, *J* = 3.2 Hz, 1H), 3.78 – 3.70 (m, 2H), 3.66 – 3.60 (m, 1H), 3.54 – 3.44 (m, 3H), 3.41 – 3.32 (m, 2H), 2.27 (s, 3H), 2.06 (t, *J* = 6.4 Hz, 2H), 1.98 (s, 3H), 1.84 – 1.78 (m, 2H), 1.74 (s, 3H), 1.69 – 1.62 (m, 2H), 1.53 – 1.48 (m, 2H), 1.04 (s, 6H); ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.89 (t, *J* = 5.2 Hz, 1H), 7.01 – 6.90 (m, 1H), 6.70 (d, *J* = 16.0 Hz, 1H), 6.26 – 6.20 (m, 2H), 6.12 (d, *J* = 11.2 Hz, 1H), 5.82 (s, 1H), 4.86 (d, *J* = 2.8 Hz, 1H), 4.69 (d, *J* = 3.6 Hz, 1H), 4.58 (t, *J* = 5.6 Hz, 1H), 4.35 (d, *J* = 4.4 Hz, 1H), 4.07 (d, *J* = 6.0 Hz, 1H), 3.79 – 3.73 (m, 1H), 3.62 (s, 1H), 3.55 – 3.45 (m, 3H), 3.28 – 3.23 (m, 3H), 3.21 – 3.11 (m, 2H), 2.24 (s, 3H), 2.05 – 2.00 (m, 2H), 1.95 (s, 3H), 1.72 (s, 3H), 1.69 – 1.64 (m, 2H), 1.63 – 1.55 (m, 2H), 1.47 – 1.42 (m, 2H), 1.02 (s, 6H).

[0061] Monosaccharides share several structural similarities, including multiple hydroxyl (-OH) groups attached to their carbon backbone. The primary differences lie in the number and orientation of these hydroxyl groups. As a result, the synthesis scheme for other monosaccharides 9-cis-retinoic acid, follows a similar approach to that outlined in Example 1.

[0062] **Example 2. Evaluation for *in vitro* cytotoxicity effect of galactose 9-cis-retinoic acid on different cancer cell lines**

[0063] In the present example, seven cell lines were used, and are respectively A549, NCI-H460, Huh-7-Ab, AsPC-1-Ab, MDA-MB-231-Ab and cancer cell lines. Each cell line was cultured in indicated growth medium with 10% FBS and were maintained in humidified incubator at 37 °C containing 5% CO₂.

[0064] One day before treatment, cells at log-phase of growth were harvested, counted, and seeded in a 96-well plate at a density of 1×10⁴ cells/100 μL/well. After cultivation overnight, culture media of each well were removed gently and fresh media were added (200 μL/well) subsequently.

[0065] The test article (TA) was the novel retinoic acid compound (abbreviated as “New compound” in the present example) was freshly prepared on the day of treatment with 100% DMSO into a 48 mM stock solution. New compound was serially diluted 2-fold using 100% DMSO to obtain different concentrations of the new compound from 0.375 mM to 48 mM. Treat cells by adding 2.02 μL of the indicated concentrations of new compound to each well to give a

final concentration range of new compound from 3.75 μM to 480 μM and keep all wells containing 1% DMSO, including the DMSO control. All components were gently mixed and incubated for 24 hr.

[0066] At the day of cell viability, culture medium of each well was replaced by fresh-prepared Alamar blue dye (10% v/v) and incubated at 37 °C for 2 to 3 hours. Spectrophotometric absorbances were recorded at wavelengths of 570 nm and 600 nm.

[0067] The percent of cell viability were calculated using the following formula:

$$\% \text{ viability} = ([A_{570}/A_{600} \text{ ratio}] \text{ of TA} / [A_{570}/A_{600} \text{ ratio}] \text{ of DMSO}) * 100\%$$

where TA = test article

A_{570} = absorbance at 570 nm

A_{600} = absorbance at 600 nm

[0068] **Results:** referring to **FIGs. 2-7**, they show New Compound can significantly reduce the cell viability of the all six cancer cell lines, revealing that the novel retinoic acid compound, galactose-modified 9-cis-retinoic acid, may be a potential anticancer drug for effectively treating various cancers, such as lung cancer, ovarian cancer, breast cancer, pancreatic cancer, and liver cancer. Showed the IC_{50} in **Table 1**.

[0069] **Table 1**

Tumor type	Cell line	IC_{50} (μM)
Lung cancer (non-small cell lung cancer, NSCLC)	A549	183.3 ± 11.2
Lung cancer (non-small cell lung cancer, NSCLC)	NCI-H460	141.4 ± 15.4
Liver cancer (hepatocellular carcinoma)	Huh-7	95.4 ± 8.4
Pancreatic cancer	AsPC-1	98.1 ± 1.9
Breast cancer	MDA-MB-231	139.3 ± 8.9
Ovarian cancer	SK-OV-3	113.6 ± 24.3

[0070] **Example 3. Evaluation for *in vitro* cytotoxicity effect of galactose 9-cis-retinoic acid on exhibits anti-virus activity.**

[0071] The anti-virus efficacy of galactose-9-cis retinoic acid were tested against respiratory syncytial virus (RSV), B1 strain, in HEp2 cells.

[0072] Briefly, galactose-9-cis retinoic acid were compared for their anti-viral activities. The stock solutions of galactose-9-cis retinoic acid were prepared with DMSO (Sigma, D2650) at a concentration of 96 mM.

[0073] First, for virus infection, HEp2 cells were seeded into a 12-well plate at a density of 1.5×10^5 cells/well. Then, the cells were cultured in an incubator kept at 37°C and 5% CO₂ for one day. After a wash with Dulbecco's Phosphate-Buffered Saline (DPBS), RSV B1 with multiplicities of infection (MOI) = 0.1 along with 50 µM or 100 µM of galactose-9-cis retinoic acid, or DMSO (vehicle) were added to the cells. The cells were cultured at 37°C and 5% CO₂ incubator for another hour and followed by a wash with DPBS. Then, 1 mL 10% fetal bovine serum (FBS)/ Dulbecco's Modified Eagle Medium (DMEM) was added with 50 µM or 100 µM of galactose-9-cis retinoic acid, or DMSO (vehicle). The cells were cultured at 37°C and 5% CO₂ incubator for 24, 48, and 72 hours. At the end of assay, the culture medium was removed, and the cells were subjected to another DPBS wash. Total RNA of the cultured cells was collected by adding 500 µL RNazol, and the lysate was collected. The collected lysate was stored at -80°C.

[0074] RSV levels in each group of cells were then evaluated. First, RNA isolation was carried out. Cell lysates containing the total RNA were thawed at room temperature and added with 200 µL diethyl pyrocarbonate (DEPC)-H₂O. The mixtures were vortexed and let stand for 15 minutes. Then, the mixtures were centrifuged at 12,000 ×g for 15 min at 4°C. Then, 200 µL 75% EtOH was added to a new Eppendorf, and 500 µL of the supernatant were added. The mixture was let stand for 10 min and centrifuged at 12,000 ×g for 8 min at 4°C. The supernatant was removed, and the precipitate was washed with 500 µL 75% EtOH twice. Then, the RNA was dissolved in 12 µL of DEPC-H₂O, and the RNA was subjected to quantification. Unused RNA was stored at -80°C.

[0075] The isolated RNA was subjected to reverse transcription (RT). First, 4 µg of RNA were diluted to 8 µL with DEPC-H₂O and added with 1 µL of 10× RT reaction buffer and 1 µL of DNase I. The reaction mixture was kept at 37°C for 30 min. Then, 1 µL of 50 mM EDTA was added and then kept at 65°C for another 10 minutes. The reaction was added with 1 µL of random hexamer,

dNTP, and DEPC-H₂O each and kept at 65°C for 5 minutes. Again, 4 µL of 5× first-strand buffer, 1 µL of dithiothreitol (DTT), and GScript RTase were each added. The reaction mixture was kept at 50°C for 50 minutes, followed by 70°C incubation for 15 minutes. The reverse transcription products were stored at -80°C until further analysis. Finally, RSV levels in each treatment cell group were evaluated by qPCR, following the conventional protocol and SYBR with a QuantStudio 6 Flex Real-Time PCR System, using actin as control. The qPCR program was 95°C for 10 sec, followed by 60°C for 20 sec and elongation at 72°C for 1 sec, and repeating for 45 cycles. Primer sequences used for RSV and actin were shown in Table 2 below.

[0076] **Table 2**

Primer Name	SEQ ID NO:	Sequence (5' - 3')
Actin F	1	ACCAACTGGGACGACATGGAGAAA
Actin R	2	TAGCACAGCCTGGATAGCAACGTA
RSVN1248F	3	AAGATGCAAATCATAAATTCACAGGA
RSVN1350R	4	TGATATCCAGCATCTTTAAGTATCTTTATAGTG

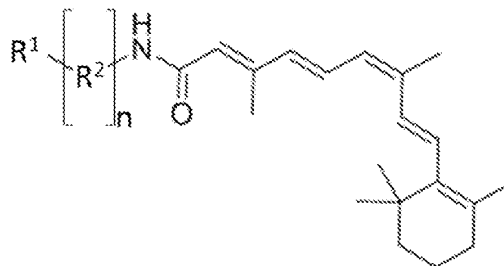
[0077] Results of qPCR with primers for actin and RSV N provide the relative RSV RNA level in different groups of culture cells receiving different treatment. As shown in Table 3 below, cells treated with 50 µM galactose-9-cis retinoic acid in group 2 showed lower RSV RNA level compared to the vehicle group. Treatment with 50 µM galactose-9-cis retinoic acid effectively inhibited RSV replication, providing a 98.4% RSV inhibition after treatment, a 99.0 % RSV inhibition after 48 hours of treatment, and a 94.7% RSV inhibition after 72 hours of treatment. When the compound concentration increased to 100 µM (group 3), galactose-9-cis retinoic acid showed significant virus inhibition rate, greater than 99%.

[0078] **Table 3**

Group Time	24 hr	48 hr	72 hr
1. Vehicle	100%	100%	100%
2. Galactose-9-cis retinoic acid (50 µM)	1.6 %	1.0 %	5.3 %
3. Galactose-9-cis retinoic acid (100 µM)	0.5 %	0.7 %	0.7 %

WHAT IS CLAIMED IS:

1. A compound or a pharmaceutically acceptable salt thereof, wherein the compound is represented by formula (I):



(I)

wherein R¹ is a monosaccharide, R² is a substituted or unsubstituted aliphatic group with 1 to 20 carbon atoms, and n is 0 or 1.

2. The compound or the pharmaceutically acceptable salt thereof according to claim 1, wherein the monosaccharide selected from the group consisting of: galactose, glucose, mannose, glyceraldehyde, erythrose, ribose, arabinose, xylose, idose, gulose, talose, allose, altrose, fructose, erythrulose, ribulose, xylulose, psicose, sorbose, tagatose, deoxyribose, 2-deoxyglucose, fucose, rhamnose, quinovose, glucosamine, galactosamine, and mannosamine.
3. The compound or the pharmaceutically acceptable salt thereof according to claim 1, wherein the monosaccharide is galactose, glucose, mannose, fructose, ribose, deoxyribose, or fucose.
4. A pharmaceutical composition, comprising the compound or the pharmaceutically acceptable salt thereof according to claim 1, 2, or 3, and a pharmaceutically acceptable carrier.
5. A method of treating a cancer in a subject, comprising administering to the subject a therapeutically effective amount of the compound or the pharmaceutically acceptable salt thereof according to claim 1, 2, or 3.
6. The method according to claim 5, wherein the cancer is lung cancer, liver cancer,

pancreatic cancer, breast cancer, or ovarian cancer.

7. A method of treating viral infection in a subject, comprising administering to the subject a therapeutically effective amount of the compound or the pharmaceutically acceptable salt thereof according to claim 1, 2, or 3, to the subject in need thereof.

8. The method according to claim 7, wherein the virus is respiratory syncytial virus.