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(54) Title: COMPOUNDS EFFECTIVE IN TREATING HEPATOTOXICITY AND FATTY LIVER DISEASES AND USES THEREOF

(57) Abstract: The present invention relates to compounds effective in treating hepatotoxicity and fatty liver diseases and uses thereof.

TITLE OF THE INVENTION

COMPOUNDS EFFECTIVE IN TREATING HEPATOTOXICITY AND FATTY LIVER DISEASES AND USES THEREOF

RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application No. 62/222,959, filed on September 24, 2015, U.S. Provisional Application No. 62/257,697, filed on November 19, 2015, and Patent Cooperation Treaty Application No.: PCT/CN2016/078039, filed on March 31, 2016, the content of which is hereby incorporated by reference in its entirety.

TECHNOLOGY FIELD

[0002] The present invention relates to compounds effective in treating hepatotoxicity and fatty liver diseases and uses thereof.

BACKGROUND OF THE INVENTION

[0003] Injuries in organs may be caused by toxic agents such as a therapeutic drug when administered overdose which often leads to injuries in organs especially liver or kidney. Acetaminophen (also known as Panadol) is also called paracetamol or N-acetyl-para-aminophenol (APAP) and is the most widely used pain-relieving and fever-reducing drug on the market. Each year, numerous cases of drug intoxication or suicide are reported due to improper use of APAP, and liver damage caused by APAP is the main cause of severe diseases and death. Alcohols or organic solvents such as carbon tetrachloride (CCl_4) may also cause hepatotoxicity. A number of clinical studies have demonstrated that hepatotoxicity induced by APAP is preventable and early diagnosis along with real-time administration of the antidote N-acetylcysteine (NAC) can prevent the occurrence of hepatotoxicity.

[0004] Early detection of acetaminophen overdose is necessary because the best prognosis can be achieved if the antidote is given within 8 hours after poisoning. The early signs of drug intoxication include discomfort, nausea and vomiting. However, some patients may show no signs of intoxication at the early stage (stage 1) even if their blood concentrations of acetaminophen are at the poisoning levels and their abnormal liver function is apparently abnormal. The signs of hepatotoxicity, such as abdominal pain, persistent vomiting, jaundice, right upper quadrant pain, usually

become apparent 24-48 hours after ingestion of a significant amount of acetaminophen (stage 2). Serum amintransferase usually starts to rise 16 hours after administration with clinical symptoms. Stage 3 usually occurs 3-4 days after administration and the degree of liver damage as well as prognosis can be well predicted at the time. The signs of hepatotoxicity progress from mild symptoms with elevated liver function values (AST > 1,000IU/L) to severe acute fulminant hepatitis accompanied by metabolic acidosis, jaundice, hyperglycemia, AST > 1,000IU/L, abnormal blood clotting and hepatic/brain lesions. Stage 4 will cause oliguria renal failure or death in severe cases.

[0005] Some patients with acetaminophen intoxication show only mild liver damage but with severe renal toxicity which is mainly caused by direct metabolism of APAP in P-450s (cytochrome P450s, CYPs) of the renal tubule. Nonetheless, acute renal failure may also result from hepatorenal syndrome caused by acute liver failure and the fraction excretion of Na (FeNa) can be used for differentiation primary renal damage (FeNa > 1) from hepatorenal syndrome (FeNa > 1). The calculation formula for FeNa is (Sodium urinary ÷ Creatinine urinary) ÷ (Sodium plasma ÷ Creatinine plasma) × 100.

[0006] The peak concentration of acetaminophen in blood is achieved 1-2 hours after oral administration and a significant amount is eliminated by liver, more than 90% is conjugated to glucuronide and sulfate and form non-toxic metabolites and only less than 5% is eliminated by different CYPs, including CYP2E1, CYP1A2 and CYP3A4, and among which CYP2E1 and CYP1A2 are the major enzymes for metabolism. The metabolite produced by these enzymes, N-acetyl-p-benzoquinoneimine (NAPQI) is a very active electrophile. Under normal conditions, NAPQI will react immediately with glutathione in the cell and form non-toxic mercaptide. Overdose of acetaminophen makes the consumption rate of glutathione greater than its synthesis rate and when the glutathione level of the cell is lower than the normal range of 30%, NAPQI will bind to large molecules or nucleic acids containing cysteine and lead to liver damage. From histochemical stains, NAPQI will bind to the thiol group of cysteine and form a covalent bond in centrilobular areas before occurrence of liver cell necrosis.

[0007] Patients with liver disease, alcohol addiction or who are taking drugs which may induce the activity of P450 such as carbamazepine, ethanol, Isoniazid,

Phenobarbital (may be other barbiturates), Phenytoin, Sulfinpyrazone, Sulfonylureas, Rifampin and Primidone are the susceptible groups of developing severe hepatotoxicity caused by APAP and may easily die if the patient also develops complications such as adult respiratory distress syndrome, cerebral edema, uncontrollable bleeding, infection or Multiple organ dysfunction syndrome (MODS). Take alcohol for example, alcohol is mainly eliminated by CYP2E1 of liver and its mechanism of APAP intoxication is divided into three stages: at the first stage alcohol competes the receptors for CYP2E1 with APAP in the liver and the concentration of NAPQI will reduce during the stage, at the second stage alcohol prolongs the half-life of CYP2E1 from 7 hours to 37 hours which increases the level of CYP2E1 in the liver and the concentration of NAPQ1 will slowly increase during this stage, and at the third stage, during alcohol withdrawal, more CYP2E1 is found in the liver to eliminate acetaminophen and consequently the toxic metabolites of acetaminophen increases significantly and lead to liver damage. Recent studies have shown that diallyl sulfide can effectively prevent hepatotoxicity caused by acetaminophen in mice and further demonstrated diallyl sulfide can inhibit the activity of CYP2E1. It is speculated that the protection mechanism of diallyl sulfide against hepatotoxicity induced by acetaminophen is by inhibition of the production of the intermediate NAPQI from acetaminophen. Previous studies have suggested by inhibition the consumption of reduced glutathione in liver cells, oxidation activation, mitochondrial dysfunction and DNA damage caused by NAPQI can be reduced and subsequently minimize liver damage induced by acetaminophen. For example, *Panax notoginseng*, adenosine and its derivatives adenosine monophosphate, adenosine diphosphate and adenosine triphosphate can prevent liver damage induced by acetaminophen through this protection mechanism.

[0008] Fatty liver is considered another factor leading to liver damages. Under normal circumstances, fat accounts for 3% by weight of the liver. Clinically, "fatty liver disease (FLD)" means fat in the liver exceeds 5% by weight of the liver, or more than 10% of the liver cells show vesicular fatty changes in the liver tissue sections. According to the causes of diseases, fatty liver can be divided into alcoholic fatty liver diseases (AFLD), non-alcoholic fatty liver diseases (NAFLD), or other fatty liver diseases derived from other factors, such as drugs. Fatty liver diseases are pathologically characterized by the appearance of fatty metamorphosis or steatosis, steatohepatitis, or the like. By the percentage of liver cells suffering from steatosis,

fatty liver is categorized as mild (<33%), moderate (33-66%) and severe (>66%). Previously, fatty liver was considered a benign and reversible condition, and thus less taken seriously, but recent studies had found that it will lead to severe liver fibrosis and cirrhosis, and even liver cancer. As the population of obese people increases, the prevalence of FLD also increases.

[0009] The main cause of liver diseases in European and American countries is due to chronic excessive drinking, therefore, the vast majority of liver diseases are caused by alcohol lesions. But over the past 15-20 years, NAFLD has become the first cause of diseases to be considered for liver dysfunction in European and American countries. Thaler had ever described NAFLD in 1962. In 1980, Ludwig proposed "Non-alcoholic steatohepatitis (NASH)" from accompanying NAFLD he found in a group of obese female patients with diabetes and hyperlipidemia. Thereafter, in 1986, Schaffner emphasized again that NASH played an important role in the mechanism of fibrosis derivation in the course of NAFLD. Until 1998, Day found that 15-50% of patients with NASH were suffered from different degrees of fibrosis derivation, so clinicians started to pay attention to NAFLD. Today, in addition to AFLD, NASH is not just a stage in the natural progression of NAFLD in clinical practice. Due to the presence of NASH, NAFLD is no longer considered a benign liver disease.

[0010] Regarding the mechanism of NAFLD, Day and James in the United Kingdom proposed Two-hit hypothesis based on a large number of clinical researches and animal experiments. Fatty liver occurs upon the first hit, and steatohepatitis occurs upon the second hit. The first hit is prompted by excessive accumulation of fat in the liver, which is caused by obesity, hyperlipidemia, etc. The second hit is due to oxidative stress and the effect of reactive oxygen species (ROS) in mitochondria, resulting in lipid peroxidation on the liver cell membrane, release of original inflammatory cytokines and free radicals, and fibrosis due to activation of stellate cells, and leading to liver cell necrosis. The mechanism of NASH involves the peroxidation of triglyceride, oxidative stress, ROS response, increased peroxidation of lipids in liver cells, or increase of cytokines and liver enzymes, leading to a series of autoimmune interactions.

[0011] The causes of fatty liver are mostly associated with long-term excessive intake of animal fat, protein, carbohydrates, excess calories transforming into fat accumulated in the body, leading to obesity and fatty liver. Patients with fatty liver

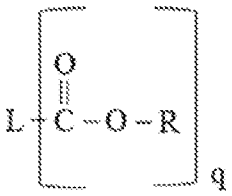
may have normal blood GOT/GPT values. Therefore, a correct diagnosis of fatty liver must use the abdominal ultrasound, which currently provides more than 97% accuracy.

[0012] Currently, there is no ideal drug providing specific therapeutic effects for FLD, the treatment guidelines of which aim at improving the potential risk factors or controlling the progress of chronic diseases by using drugs. It is recommended to apply symptomatic treatments according to the causes of fatty liver. For example, those who suffering from fatty liver caused by overweight should lose weight moderately. Anyone with alcoholic fatty liver needs to quit drinking and eats a balanced diet for improving the conditions. Chemicals or drugs that damage liver and lead to fatty liver diseases through long-term contact shall immediately be stopped using. Fatty liver caused by diseases, such as hepatitis C, high blood fat, etc., shall be treated by treating the original diseases, such as treating hepatitis C or controlling blood lipids. However, if excessive triglycerides are due to personally physical factors, it is hard to ameliorate fatty liver diseases by losing weight.

[0013] However, the current drugs that are commonly used in clinical to lower serum triglycerides and cholesterol are often accompanied with side effects, for example, hepatotoxicity, myopathy such as myalgia, myositis, rhabdomyolysis, and the like. Regarding the lipid-lowering drugs, muscle toxicity is the most notable side effect. Especially, Statins shows the highest occurrence of muscle toxicity, and fibric acid follows. In addition, the lipid-lowering drugs have a "fat driving" effect, which "drives" blood lipids to the liver, where fat accumulation already exists and the influx of lipids is difficult to be processed, leading to excessive accumulation of fat in the liver and making fatty liver worse. It can be seen that the lipid-lowering drugs are not suitable for the treatment of FLD.

BRIEF SUMMARY OF THE INVENTION

[0014] In one aspect, the present invention provides new compounds, the structure of which is represented by Formula (I) as follows



Formula (I),

wherein

L is a saturated or unsaturated aliphatic group;

R is selected from the group consisting of hydrogen, a polyol group and a saccharide group of $(G)_p$ wherein G is a monosaccharide residue and p is an integer from 1 to 100 wherein at least one of the hydroxyl groups in $(G)_p$ is substituted by a halogen atom; and

Q is an integer from 2 to 4, and each of R is the same or different,

or a pharmaceutically acceptable salt thereof.

[0015] In some embodiments, the compounds of the present invention are represented by Formula (II) as follows:

$$R_1\text{-}O\text{-}X\text{-}(CH_2)_m\text{-}X\text{-}O\text{-}R_2 \quad \text{Formula (II)},$$

wherein

X is C=O;

R_1 and R_2 are the same or different, selected from the group consisting of hydrogen, a polyol group and a saccharide group of $(G)_p$ wherein G is a monosaccharide residue and p is an integer from 1 to 100 wherein at least one of the hydroxyl groups in $(G)_p$ is substituted by a halogen atom, wherein when R_1 is hydrogen, then R_2 is not hydrogen; and

m is an integer from 1 to 40.

[0016] In another aspect, the present invention provides a pharmaceutical composition comprising at least one of the compounds as described herein or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier.

[0017] In still another aspect, the present invention provides a treatment method by administering to a subject in need an effective amount of at least one of the compounds as described herein or a pharmaceutically acceptable salt thereof.

[0018] In some embodiments, the method of the present invention is provided to prevent or treat a disease or condition characterized by increased cytochrome P450 activities or increased free radical levels in a subject in need thereof.

[0019] In some embodiments, the method of the present invention is provided to prevent or treat organ injuries in a subject in need.

[0020] In some embodiments, the method of the present invention is provided to prevent or treat hepatotoxicity in a subject in need.

[0021] In some embodiments, the method of the present invention is provided to

prevent or treat fatty liver, protect liver function or ameliorate liver diseases caused by fatty liver or other associated disorders.

[0022] In yet another aspect, the present invention provides use of the compounds as described herein or a pharmaceutically acceptable salt thereof for manufacturing a medicament. In particular, the medicament is useful in preventing or treating (i) a disease or condition characterized by increased cytochrome P450 activities or increased free radical level, (ii) organ injuries, and/or (iii) hepatotoxicity, and/or (iv) preventing or treating fatty liver, protecting liver function or ameliorating liver diseases caused by fatty liver or other associated disorders.

[0023] The details of one or more embodiments of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following detailed description of several embodiments, and also from the appending claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown.

[0025] In the drawings:

[0026] Fig.1 shows the percentage of pro-drug remain or its related metabolites formation in blood (*in vitro*).

[0027] Fig.2 shows the plasma concentration vs. time profile for pro-drug and sucralose after oral administration of pro-drug in SD-rats.

[0028] Fig. 3 shows the plasma concentration vs. time profile for mannitol after oral administration of pro-drug in SD-rats.

[0029] Fig. 4 shows the H&E staining results of liver tissues in animals. (A) the normal control, (B) the control group of APAP-induced liver injuries, (C) the positive control group of treatment with NAC, (D) the experimental group of treatment with mannitol (1.67 mg/kg), (E) the experimental group of treatment with sucralose (1.67 mg/kg), (F) the experimental group of treatment with mannitol (2.51 mg/kg) plus sucralose (2.51 mg/kg), (G) the experimental group of treatment with mannitol

(3.34mg/kg) plus sucralose (3.34 mg/kg), and (H) the experimental group of treatment with NAC and a combination of mannitol (3.34 mg/kg) and sucralose (3.34 mg/kg).

[0030] Fig. 5 shows liver tissue sections taken from mice that were induced fatty liver, and then treated with different test compounds by groups for four weeks.

[0031] Fig. 6 shows a general scheme of synthesis process of the compound of the present invention.

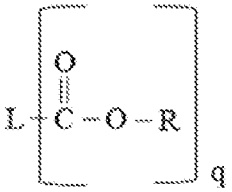
DETAILED DESCRIPTION OF THE INVENTION

[0032] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as is commonly understood by one of skill in the art to which this invention belongs.

[0033] As used herein, the articles "a" and "an" refer to one or more than one (*i.e.*, at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0034] **I. Compounds**

[0035] In one aspect, the present invention provides new compounds, the structure of which is represented by Formula (I) as follows



Formula (I),

wherein

L is a saturated or unsaturated aliphatic group;

R is selected from the group consisting of hydrogen, a polyol group and a saccharide group of $(G)_p$ wherein G is a monosaccharide residue and p is an integer from 1 to 100 wherein at least one of the hydroxyl groups in $(G)_p$ is substituted by a halogen atom; and

Q is an integer from 2 to 4, and each of R is the same or different,

or a pharmaceutically acceptable salt thereof.

[0036] The term "aliphatic" or "aliphatic group", as used herein, denotes a hydrocarbon moiety that may be straight-chain (i.e., unbranched), branched, or cyclic (including fused, bridging, and spiro-fused polycyclic) and may be completely

saturated or may contain one or more units of unsaturation, but which is not aromatic. In general, aliphatic groups contain 1-40 carbon atoms. In some embodiments, aliphatic groups contain 1-20 carbon atoms, or 1-12 carbon atoms, 1-8 carbon atoms, or 1-4 carbon atoms. In some embodiments, aliphatic groups contain 3-20 carbon atoms, or 3-12 carbon atoms, 3-8 carbon atoms, or 3-4 carbon atoms. Suitable aliphatic groups include, but are not limited to, linear or branched, alkyl, alkenyl, and alkynyl groups, and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

[0037] In certain embodiments, the L group in Formula (I) is selected from (a) a straight alkyl group, (b) a branched alkyl group, (c) a straight alkyl group substituted with a benzene ring, (d) a branched alkyl group substituted with a benzene ring, (e) a benzenyl group where the benzene ring contains a straight chain aliphatic group, and (f) a benzenyl group where the benzene ring contains a branch chain of aliphatic group.

[0038] The term "polyol group", as used herein, denotes an alcohol containing multiple hydroxyl groups (two or more hydroxyl groups) per molecule. In particular, the polyol group can be linear or circular, substituted or unsubstituted, or mixtures thereof, so long as the resultant complex is water-soluble and pharmaceutically acceptable.

[0039] In some embodiments, the polyol group is a C3-24 polyol, particularly, a C3-20 polyol, more particularly, a C3-12 polyol, or a C3-12 polyol, containing 2 or more hydroxyl groups.

[0040] In more particular embodiments, the polyol group is represented by -CH-$(CHOH)_n$$CH_2OH$ wherein n is 1-22, 1-18, 1-10, or 1-6. In one certain example, n is 4.

[0041] Preferred polyols are sugar alcohols. Examples of polyols include, but are not limited to, 3-carbon polyols (e.g. glycerol, erythritol and threitol); 5-carbon polyols (e.g. arabitol, xylitol and ribitol); 6-carbon polyols (e.g. mannitol, sorbitol, galactitol, fucitol, iditol and inositol); 12-carbon polyols (e.g. volemitol, isomalt, maltitol and lactitol); 18-carbon polyols (e.g. maltotriitol); and 24-carbon polyols (maltotetraitol).

[0042] In Formula (I), G represents a monosaccharide residue. The monosaccharide as used herein is preferably a 6-carbon monosaccharide having the chemical formula $C_6H_{12}O_6$ (i.e. hexose). The hexose may be in the D configuration,

the L configuration, or a combination thereof. Hexoses are typically classified by functional groups. For example, aldohexoses have an aldehyde at position 1 such as allose, altrose, glucose, mannose, gulose, idose, galactose, and talose; and ketohexoses have a ketone at position 2 such as psicose, fructore, sorbose, and tagatose. A hexose also contains 6 hydroxyl groups and the aldehyde or ketone functional group in the hexose may react with neighbouring hydroxyl functional groups to form intramolecular hemiacetals or hemiketals, respectively. If the resulting cyclic sugar is a 5-membered ring, it is a furanose. If the resulting cyclic sugar is a 6-membered ring, it is a pyranose. The ring spontaneously opens and closes, allowing rotation to occur about the bond between the carbonyl group and the neighbouring carbon atom, yielding two distinct configurations (α and β). The hexose may be in either the S configuration or the R configuration.

[0043] According to the present invention, at least one of the hydroxyl groups in the one or more monosaccharide residues in formula (I) is substituted by a halogen atom. Examples of the halogen atom includes chlorine, bromine and iodine. Specifically, the halogen atom is chlorine.

[0044] As used herein, the term "*S*" or "*R*" is a way to name an optical isomer by its configuration, without involving a reference molecule, which is called the *R/S* system. It labels each chiral center *R* or *S* according to a system by which its ligands are each assigned a priority, according to the Cahn Ingold Prelog priority rules, based on atomic number. This system labels each chiral center in a molecule (and also has an extension to chiral molecules not involving chiral centers). If the compound has two chiral centers, it can be labeled, for example, as an (*S,S*) isomer versus an (*S,R*) isomer.

[0045] As used herein, the term "pharmaceutically acceptable salt" includes acid addition salts. "Pharmaceutically acceptable acid addition salts" refer to those salts which retain the biological effectiveness and properties of the free bases, which are formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, pyruvic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluenesulfonic acid, salicylic acid, trifluoroacetic acid and the like.

[0046] In some embodiments, in Formula (I), q is 2, 3 or 4, at least one of the R

group is different from another one of R.

[0047] In certain embodiments, in Formula (I), q is 2.

[0048] In such embodiments, the compound of the present invention can be represented by Formula (II) as follows:

$$R_1\text{-}O\text{-}X\text{-}(CH_2)_m\text{-}X\text{-}O\text{-}R_2 \quad \text{Formula (II)},$$

wherein

X is C=O;

R_1 and R_2 are the same or different, selected from the group consisting of hydrogen, a polyol group and a saccharide group of $(G)_p$ wherein G is a monosaccharide residue and p is an integer from 1 to 100 wherein at least one of the hydroxyl groups in $(G)_p$ is substituted by a halogen atom, wherein when R_1 is hydrogen, then R_2 is not hydrogen; and

m is an integer from 1 to 40.

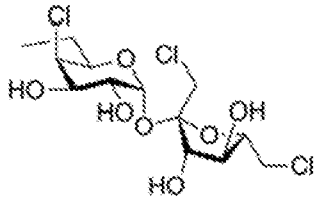
or a pharmaceutically acceptable salt thereof.

[0049] In certain embodiments, in Formula (II), R_1 is the polyol group and R_2 is the saccharide group of $(G)_p$. In such case, the compound of Formula (II) is deemed as a conjugate of the polyol moiety linked to the sugar moiety by a linker via ester bonds. In particular, the linker is represented by $-O\text{-}X\text{-}(CH_2)_m\text{-}X\text{-}O-$ (Formula (L)) wherein X is C=O and m is 1-40, 1-20, 1-12, 1-8 or 1-4, more particular, m is 3-20, 3-12, 3-8 or 3-4. In one certain example, m is 4.

[0050] In some embodiments, p is 2. The saccharide group is represented by $-G_1\text{-}O\text{-}G_2$, wherein G_1 and G_2 are the same or different, selected from the group consisting of an aldohexose and a ketohexose, and at least one of the hydroxyl groups in G_1 or at least one of the hydroxyl groups in G_2 is substituted by a halogen atom.

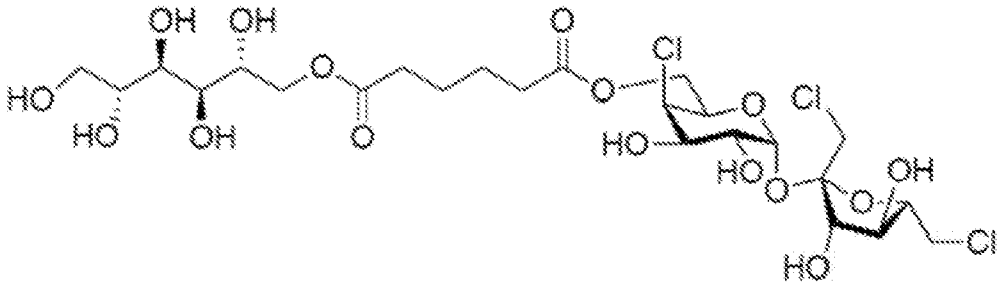
[0051] In some embodiments, G_1 is glucose wherein one of the hydroxyl groups is substituted by chlorine; and G_2 is fructose wherein two of the hydroxyl groups are substituted by chlorine.

[0052] In certain embodiments, the saccharide group is represented by formula (Ia)



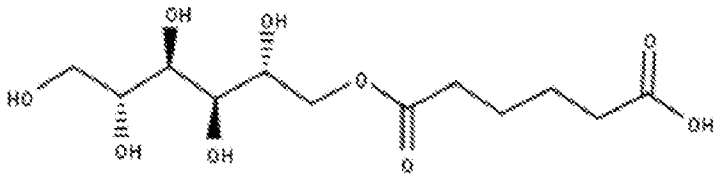
formula (Ia).

[0053] Certain examples of the compound of the present invention are as follows: ((2R,3R,4R,5R,6R)-6-(((2R,5R)-2,5-bis(chloromethyl)-3,4-dihydroxytetrahydro furan-2-yl)oxy)-3-chloro-4,5-dihydroxytetrahydro-2H-pyran-2-yl)methyl ((2R,3R,4R)-2,3,4,5,6-pentahydroxyhexyl) adipate



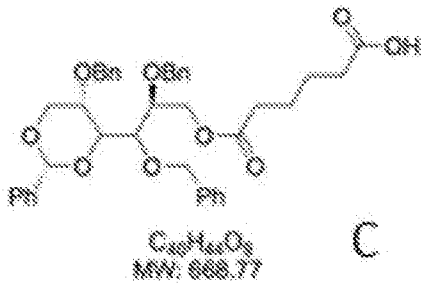
Formula 1, and

C6-mannitol of Formula 2



Formula 2.

[0054] In another aspect, the present invention provides an intermediate of Formula C as follows:



C

wherein Ph is phenyl and Bn is benzyl.

[0055] The compound of Formula (I) can be chemically synthesized for example by a process as shown in the general scheme of Fig. 6.

[0056] In particular, a linker agent that can provide one or more –COOH group to perform esterification with an alcohol is provided. In step 1, the linker agent providing a first –COOH group (others if available are protected) reacts with R having a first free hydroxyl group (others if available are protected) to proceed with the first esterification, producing the compound of Formula (I) where q is 1. In step 2, the

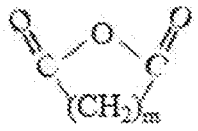
linker agent providing a second –COOH group (others if available are protected) reacts with R having a second free hydroxyl group (others if available are protected) to proceed with the second esterification, producing the compound of Formula (I) where q is 2. In step 3, the linker agent providing a third –COOH group (others if available are protected) reacts with R having a third free hydroxyl group (others if available are protected) to proceed with the third esterification, producing the compound of Formula (I) where q is 3. In step 4, the linker agent providing a fourth –COOH group (others if available are protected) reacts with R having a fourth free hydroxyl group (others if available are protected) to proceed with the third esterification, producing the compound of Formula (I) where q is 4.

[0057] In some embodiments, the linker agent to perform the esterification is represented by Formula (La)

P_1-O-X-$(CH_2)_m$-X-O-P_2 Formula (La)

wherein X and m are as defined above, and P_1 and P_2 are the same or different and are a protecting group or H.

[0058] In some embodiments, the linker agent to perform the esterification is represented by Formula (La)



Formula (Lb).

[0059] As used herein, a "protecting group" is a chemical group that is attached to a functional moiety (for example to the oxygen in a hydroxyl group or the nitrogen in an amino group, replacing the hydrogen) to protect the functional group from reacting in an undesired way. A protecting group includes, for example, t-butyl group, a cycloalkyl group (e.g., cyclohexyl group), an aryl group (e.g., 2,4-dinitrophenyl group), an aralkyl group (e.g., benzyl group, 2,6-dichlorobenzyl group, 3-bromobenzyl group, 2-nitrobenzyl group, 4-dimethylcarbamoylbenzyl group, and triphenylmethyl group), a tetrahydropyranyl group, an acyl group, an alkoxycarbonyl group (e.g., t-butoxycarbonyl group), an aralkyloxycarbonyl group (e.g., benzyloxycabonyl group, 2-bromobenzyloxycarbonyl group), a

dialkylphosphinothioyl group (e.g., dimethylphosphinothioyl group) and a diarylphosphinothioyl group (e.g., diphenylphosphinothioyl group). A preferred protecting group includes an acyl group and the like.

[0060] In one certain example, Scheme 1 is provided in Example 1 showing the particular synthesis process of the compound of the present invention.

[0061] **II. Uses of the compounds of the present invention**

[0062] The compounds of the invention can be used as a medicament for treatment methods. In general, the compound of Formula (I) acts as a prodrug that after administration can turn into metabolites providing therapeutic effects as needed as described herein. In one example, the compound of Formula (I) is compound F, which after administration can turn into mannitol, sucralose and C6-mannitol, all of which can act as P450 inhibitors and provide anti-hepatotoxicity effects, for example. See examples below.

[0063] The present invention provides a treatment method by administering to a subject in need an effective amount of at least one of the compounds as described herein or a pharmaceutically acceptable salt thereof.

[0064] It is found that compounds of the invention are effective as P450 inhibitors, for example.

[0065] In some embodiments, the method of the present invention is provided to prevent or treat a disease or condition characterized by increased cytochrome P450 activities in a subject in need thereof.

[0066] Examples of such diseases or conditions are listed in Table A.

Table A

Diseases	
alcoholic hepatitis	hepatoblastoma
drug-induced hepatitis	Liver, renal chronic disease
alcoholic liver cirrhosis	obesity
liver disease	poisoning
liver cirrhosis	insulin resistance
alcohol abuse	chronic liver disease
isoniazid toxicity	hepatitis chronic
nonalcoholic steatohepatitis	renal disease
tuberculosis	inflammation
Hepatitis	alcohol withdrawal

Fatty liver disease	alcoholic cirrhosis
Hepatocellular carcinoma	liver damage
liver diseases alcoholic	alcoholism
hepatitis halothane	hepatitis toxic
fatty liver alcoholic	
fatty liver	hepatic necrosis
alcohol-related disorders	cirrhosis
cerebrovascular disease	acute alcoholic hepatitis
coronary artery disease	Liver, renal histopathology
Liver, renal cell damage	Ethanol-induced and obesity-induced oxidant stress and liver injury
Liver, renal necrosis	heavy metal poisoning
hepatitis c chronic	liver fibrosis
cardiovascular disease	atherosclerosis

[0067] In some embodiments, the method of the present invention is provided to prevent or treat a disease or condition characterized by increased free radical levels in a subject in need thereof.

[0068] In some embodiments, the method of the present invention is provided to prevent or treat organ injuries in a subject in need.

[0069] In particular examples, the organ injuries are in liver or kidney.

[0070] In particular examples, organ injuries or hepatotoxicity are caused by a therapeutic drug, CCl_4 or lipid accumulation.

[0071] In particular examples, the therapeutic drug is acetaminophen.

[0072] In some embodiments, the method of the present invention is provided to prevent or treat hepatotoxicity in a subject in need.

[0073] In some embodiments, the method of the present invention is provided to prevent or treat fatty liver, protecting liver function or ameliorating liver diseases caused by fatty liver or other associated disorders.

[0074] As used herein, the term "liver fat content" refers to the content of fat that is accumulated in the liver of a subject and includes broadly defined lipids, such as triglyceride (TG) and cholesterol. As used herein, the term "reducing liver fat content" generally refers to the reduction of the content of abnormal liver fat in a subject, i.e. to decrease the content of abnormal liver fat and, more particularly, to lower the content of abnormal liver fat to normal level. For example, under normal

circumstance, fat accounts for 3% by weight of the liver. If fat in the liver exceeds 5% by weight of the liver, it is determined as abnormal fat accumulation (the liver fat content described above is a relative percentage for exemplification, and may vary due to ethnicity and other factors). In a specific aspect, the term "reducing liver fat content" used herein could means that the content of abnormal liver fat in a subject is reduced, for example, from 5% by weight of the liver or more to 3% by weight of the liver. Liver fat content can be assessed by standard analytical methods, including but not limited to ultrasound analysis, magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), computed tomography (CT), and liver biopsy.

[0075] As used herein, the term "liver function" refers to one or more physiological functions performed by the liver. Liver function can be analyzed by a lot of conventional assays, such as alanine aminotransferase (ALT) analysis or aspartate transaminase (AST) analysis. According to the present invention, the compound described herein can be used to maintain the liver function, including improvement of the liver function and preventing the liver from damage.

[0076] As used herein, the term "liver diseases" refers to liver cell injury or damage caused by certain factors, which then potentially lead to liver dysfunction. According to the present invention, the compound proposed herein can be used to ameliorate liver diseases caused by fatty liver in some embodiments. More particularly, "liver damage" used herein refers to liver with histological or biochemical dysfunction, as compared with normal liver. In a specific embodiment, "liver damage" refers to liver lesions caused by alcoholic or non-alcoholic factors, such as high fat diet or obesity, or therapeutic drugs or organic solvents. In a specific embodiment, "liver damage" can be liver tissue damage with one or more characteristics selected from steatosis, lobular inflammation, hepatocyte ballooning, and vesicular fat droplets produced by liver cells. In a specific embodiment, "liver damage" can be biochemical dysfunction of liver, which can be determined from the activity of alanine aminotransferase (ALT) or aspartate transaminase (AST). Higher activity of ALT or AST indicates severer dysfunction of liver's biochemical function.

[0077] As used herein, the term "liver antioxidant activity" refers to the activity or ability against oxidative stress. Improvement of liver antioxidant activity of a subject by the compound according to the present invention refers to, includes, but is not limited to reducing oxidative stress or enhancing enzyme activity or content of the members of antioxidant systems. The members of antioxidant systems may be

glutathione peroxidase (GPx), glutathione (GSH), glutathione reductase (GRd), and/or superoxide dismutase (SOD).

[0078] According to the present invention, the compound described herein includes common excipients and bioflavonoids, which may be used to reduce liver fat content and ameliorate associated disorders. The term "associated disorders" described herein includes the disorders caused by abnormal accumulation of liver fat and including, but not limited to fatty liver diseases, acute and chronic alcoholic fatty liver diseases, acute and chronic non-alcoholic fatty liver diseases, acute and chronic alcoholic hepatitis, acute and chronic non-alcoholic steatohepatitis, non-alcoholic cirrhosis and alcoholic cirrhosis (ICD-9-CM Diagnosis Codes: 571.8, 571.0, 571.1, 571.2, 571.3, 571.4, 571.5, 571.9).

[0079] As used herein, the term "preventing" refers to the preventive measures for a disease or the symptoms or conditions of a disease. The preventive measures include, but are not limited to applying or administering one or more active agents to a subject who has not yet been diagnosed as a patient suffering from the disease or the symptoms or conditions of the disease but may be susceptible or prone to the disease. The purpose of the preventive measures is to avoid, prevent, or postpone the occurrence of the disease or the symptoms or conditions of the disease.

[0080] As used herein, the term "treating" refers to the therapeutic measures to a disease or the symptoms or conditions of a disease. The therapeutic measures include, but are not limited to applying or administering one or more active agents to a subject suffering from the disease or the symptoms or conditions of the disease or exacerbation of the disease. The purpose of the therapeutic measures is to treat, cure, mitigate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptoms or conditions of the disease, disability caused by the disease, or exacerbation of the disease.

[0081] As used herein, a "CYP2E1 inhibitor" is any compound, substance or material that can inhibit CYP2E1 activity. A number of assays are available for analysis of the CYP2E1 activity such as a human or rat liver microsome assay.

[0082] As used herein, a subject in need of the treatment according to the invention includes human and non-human mammals. Non-human mammals include, but are not limited to, companion animals such as cats, dogs and the like and farm animals such as cattle, horses, sheep, goats, swine and the like.

[0083] The term "effective amount" or the like refers to that amount of an active

agent sufficient to achieve a desired therapeutic, prophylactic, and/or biological effect in a subject, such as reducing drug-induced side effects, or prohibiting, improving, alleviating, reducing or preventing one or more symptoms or conditions or progression of a disease. The actual effective amount may change depending on various reasons, such as administration route and frequency, body weight and species of the individual receiving said pharmaceutical, and purpose of administration. Persons skilled in the art may determine the dosage in each case based on the disclosure herein, established methods, and their own experience.

[0084] The term "a standard dose" as used herein refers to an effective dose of a therapeutic agent that is recommended by authoritative sources in the pharmaceutical community including the Food and Drug Administration and often used in routine practice. The term "a reduced dose" as used herein refers to a dose that is lower than a standard dose but still retains substantially the same therapeutic effects of the same therapeutic agent. Specifically, according to the invention, a reduced dose of a therapeutic drug is about 90% or less, 80% or less, 70% or less, 60% or less, 50% or less, of standard therapeutic dose of the therapeutic drug.

[0085] In some embodiments, an effective amount of active ingredients as used herein may be formulated with a pharmaceutically acceptable carrier into a pharmaceutical composition of an appropriate form for the purpose of delivery and absorption.

[0086] As used herein, "pharmaceutically acceptable" means that the carrier is compatible with the active ingredient in the composition, and preferably can stabilize said active ingredient and is safe to the individual receiving the treatment. Said carrier may be a diluent, vehicle, excipient, or matrix to the active ingredient. The composition may additionally comprise lubricants; wetting agents; emulsifying and suspending agents; preservatives; sweeteners; and flavoring agents. The composition of the present invention can provide the effect of rapid, continued, or delayed release of the active ingredient after administration to the patient.

[0087] According to the present invention, the form of said composition may be tablets, pills, powder, lozenges, packets, troches, elixers, suspensions, lotions, solutions, syrups, soft and hard gelatin capsules, suppositories, sterilized injection fluid, and packaged powder.

[0088] The composition of the present invention may be delivered via any physiologically acceptable route, such as oral, parenteral (such as intramuscular,

intravenous, subcutaneous, and intraperitoneal), transdermal, suppository, and intranasal methods. Regarding parenteral administration, it is preferably used in the form of a sterile water solution, which may comprise other substances, such as salts or glucose sufficient to make the solution isotonic to blood. Preparation of an appropriate parenteral composition under sterile conditions may be accomplished with standard pharmacological techniques well known to persons skilled in the art, and no extra creative labor is required.

[0089] In certain embodiments, the compound of Formula (I) of the present invention or a pharmaceutically acceptable salt thereof can be used in preventing or treating injuries in organs e.g. in liver or kidney, which may be caused by overdose of therapeutic drugs (e.g. acetaminophen) or exposure of alcohol, a chemical agent, a biomolecule or any substance that may cause toxic effects in these organs.

[0090] Specifically, injuries in liver may include injuries, damages or loss of hepatic cells or tissues, leading to abnormal liver functions or contents of liver proteins. In some embodiments, the liver injuries as described herein are acute liver injuries which mean liver injuries of relatively rapid onset e.g. less than 12 week, particularly less than 6 weeks duration from time of onset of symptoms. In some embodiments, patients with acute liver injuries are with no background of chronic hepatic diseases.

[0091] Specifically, injuries in kidney may include injuries, damages or loss of renal cells or tissues, leading to abnormal renal functions. Such renal injuries may be identified, for example, by a decrease in glomerular filtration rate, a reduction in urine output, an increase in serum creatinine, an increase in serum cystatin C, etc. In some embodiments, the renal injuries as described herein are acute renal injuries, which may mean an abrupt or rapid decline in renal filtration function, for example, within 14 days, preferably within 7 days, more preferably within 72 hours, and still more preferably within 48 hours.

[0092] In one particular embodiment, the compound of Formula (I) of the present invention or a pharmaceutically acceptable salt thereof is capable of preventing or treating an undesired condition caused by NAPQI (N-acetyl-p-benzoquinone imine).

[0093] Therefore, the present invention provides use of the compound of Formula (I) of the present invention or a pharmaceutically acceptable salt thereof for manufacturing a medicament for preventing or treating an undesired condition caused by NAPQI (N-acetyl-p-benzoquinone imine) in a subject. The present invention also

provides a method for preventing or treating an undesired condition caused by NAPQI (N-acetyl-p-benzoquinone imine) in a subject in need, comprising administering to the subject the compound of Formula (I) of the present invention or a pharmaceutically acceptable salt thereof in an amount effective to prevent or treat the undesired condition.

[0094] III. Combined use of compound of the present invention with other active agent

[0095] The compound of the present invention and/or its metabolites can be administered in combination with one or more additional active agents, particularly those acting as P450 inhibitors and/or providing anti-hepatotoxicity activities and/or those with anti-fatty liver activities, so as to provide a synergistic effect, for example.

[0096] Some active agents acting as P450 inhibitors (named "a first active agent(s)") are described in PCT/CN2013/087049 (USSN 14/441,317, the content of which is hereby incorporated by reference in its entirety). Particular examples of such P450 inhibitors include but are not limited to polyethylene glycol sorbitan monolaurate (Tween 20), microcrystalline cellulose, dicalcium phosphate dihydrate, Brij 35, saccharin, mannitol, Cremophor RH40, sucralose, crospovidone, sodium starch glycolate, Eudragit S100, croscarmellose sodium, Pluronic F68, menthol, low-substituted hydroxypropyl cellulose, pregelatinized starch, Dextrates NF hydrated, citric acid, Cremophor EL, Aerosil 200, Myrj 52, sorbic acid, lemon oil, hydroxypropyl cellulose, Sorbitol, acesulfame potassium, hydroxypropyl methylcellulose, lactose monohydrate, maltodextrin, Brij 58, Brij 76, Tween 80, Tween 40, PEG 400, PEG 4000, PEG 8000, Span 60, sodium benzoate, hydroxy ethylmethylcellulose, methylcellulose, Span 80, sodium cyclamate, glyceryl behenate, oxide red, glycerin monostearate, Copovidone K28, starch acetate, magnesium stearate, sodium lauryl sulfate, Providone K30, PEG 2000, and N-acetylcysteine (NAC) and any combination thereof.

[0097] In certain embodiments, the one or more first active agents to be used in combination with the compound of Formula (I) of the present invention are selected from the group consisting of dicalcium phosphate dehydrate, menthol, mannitol, sucralose, N-acetylcysteine (NAC) and any combination thereof.

[0098] Some active agents with anti-fatty liver activities (named "a second active agent") are described in PCT/CN2016/078039, the content of which is hereby incorporated by reference in its entirety. Particular examples of active agents with

anti-fatty liver activities include but are not limited (ii) a second active agent selected from the group consisting of: sodium lauryl sulfate, menthol, sucralose, mannitol, sorbitol, saccharin, glycerin, sodium benzoate, oxide red, pregelatinized starch, sodium cyclamate, sorbic acid, lemon oil, citric acid, butylated hydroxyanisole, poncirin, isovitexin, eriodictyol, ergosterol, β-myrcene, hyperoside, (+)-catechin, galangin, morin, sciadopitysin, didymin, gossypin, luteolin-7-glucoside, (+)-taxifolin, trans-cinnamic acid, diosmin, linarin, xylitol, luteolin, swertiamarin, puerarin, phloridzin, sinensetin, (-)-epigallocatechin, kaempferol, ursolic acid, silymarin, (+)-limonene, hesperidin, (-)-epicatechin-3-gallate, silybin, formononetin, myristic acid ethyl ester, eicosapentaenoic acid (EPA), wongonin, povidone K-30, protocatechuic acid, umbelliferone, hesperitin, nordihydroguaiaretic acid, neohesperidin, naringin, (-)-epicatechin, glycyrrhizin, baicalin, quercitrin, baicalein and any combinations thereof.

[0099] In certain embodiments, the one or more second active agents to be used in combination with the compound of Formula (I) of the present invention are selected from the group consisting of sodium lauryl sulfate, menthol, sucralose, mannitol, sorbitol, saccharin, glycerin, sodium benzoate, oxide red, pregelatinized starch, sodium cyclamate, sorbic acid, lemon oil, citric acid, butylated hydroxyanisole, poncirin, isovitexin, eriodictyol, ergosterol, β-myrcene, hyperoside, (+)-catechin, galangin, morin, sciadopitysin, didymin, gossypin, luteolin-7-glucoside, (+)-taxifolin, trans-cinnamic acid, diosmin, linarin, xylitol, luteolin, swertiamarin, and any combinations thereof.

[00100] In certain embodiments, the one or more second active agents to be used in combination with the compound of Formula (I) of the present invention are selected from the group consisting of puerarin, phloridzin, sinensetin, (-)-epigallocatechin, kaempferol, ursolic acid, silymarin, (+)-limonene, hesperidin, (-)-epicatechin-3-gallate, silybin, formononetin, myristic acid ethyl ester, eicosapentaenoic acid (EPA), wongonin, povidone K-30, protocatechuic acid, umbelliferone, hesperitin, nordihydroguaiaretic acid, neohesperidin, naringin, (-)-epicatechin, glycyrrhizin, baicalin, quercitrin, baicalein and any combinations thereof

[00101] In certain embodiments, the one or more second active agents to be used in combination with the compound of Formula (I) of the present invention are selected from the group consisting of eriodictyol, mannitol, menthol, sucralose, saccharin, and any combinations thereof.

[00102] In certain embodiments, the one or more second active agents to be used in combination with the compound of Formula (I) of the present invention are selected from the group consisting of (1) a combination of saccharin and mannitol, (2) a combination of menthol and mannitol, (3) a combination of sucralose and mannitol, (4) a combination of eriodictyol and mannitol, (5) a combination of eriodictyol and sucralose, (6) a combination of menthol, mannitol, and eriodictyol, and (7) a combination of sucralose, mannitol, and eriodictyol.

[00103] Specifically, the compound of Formula (I) or a pharmaceutically acceptable salt thereof and the one or more additional agents can be administered simultaneously or sequentially.

[00104] In the present invention, it is further provided that the compound of Formula (I) of the present invention or a pharmaceutically acceptable salt thereof is capable of preventing or treating an undesired condition caused by NAPQI (N-acetyl-p-benzoquinone imine).

[00105] As a particular embodiments, the present invention provides a combination of the compound of Formula (I) and/or its metabolites with N-acetylcysteine (NAC). The prevent invention also provides a method for administering N-acetylcysteine (NAC) in a subject in need, comprising administering to the subject NAC in combination with the compound of Formula (I) and/or its metabolites. In one embodiment, the combination or the method of the present invention is effective in preventing or treating a disease or disorder for which NAC is effective. In some embodiments, the disease or disorder to be treated or prevented by NAC is selected from the group consisting of Myoclonus Epilepsy, acute respiratory distress syndrome, heavy metal poisoning, influenza infection, heart disease, Sjogren's syndrome, chronic bronchitis, epilepsy (Unverricht-Lundborg type) and HIV infection.

[00106] The present invention is further illustrated by the following examples, which are provided for the purpose of demonstration rather than limitation.

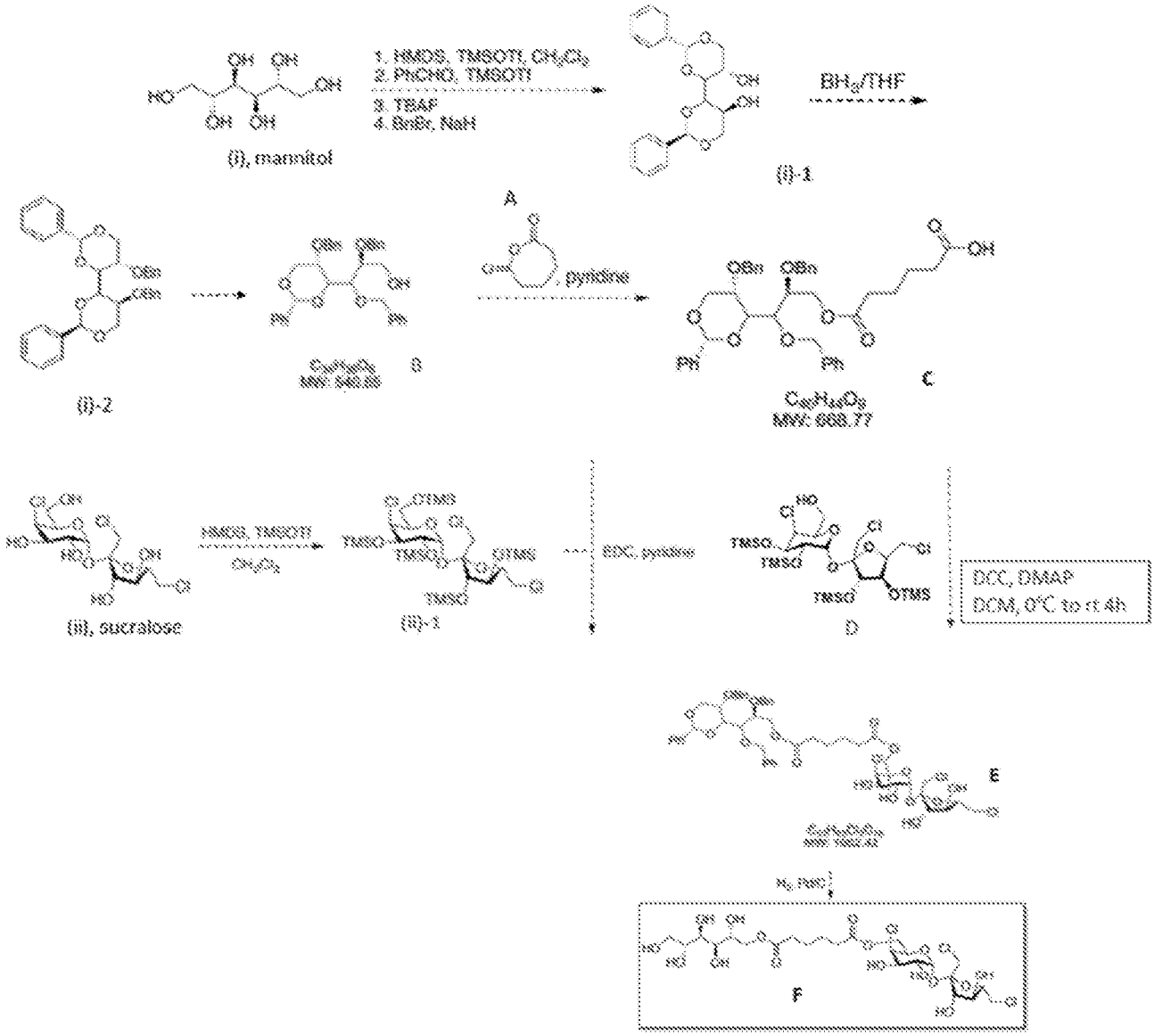
[00107] Examples

[00108] Example 1: Synthesis of Compound of Formula 1 (compound F) of the present invention

[00109] Synthetization of ((2R,3R,4R,5R,6R)-6-(((2R,5R)-2,5-bis(chloromethyl)-3,4-dihydroxytetrahydrofuran-2-yl)oxy)-3-chloro-4,5-dihydroxytetrahydro-2H-pyran-2-yl)methyl ((2R,3R,4R)-2,3,4,5,6-pentahydroxyhexyl) adipate (Formula 1) (compound F)

[00110] The synthetic strategy for the synthesis of Formula 1 (Compound F) is shown in Scheme 1.

[00111] Scheme 1



HMDS = hexamethyldisilazane

TMSOTf = Trimethylsilyl trifluoromethanesulfonate

TBAF = Tetrabutylammonium fluoride

THF = tetrahydrofuran

TMS = trimethylsilyl

DCC = dicyclohexylcarbodiimid

DMAP = 4-Dimethylaminopyridine

DCM = dichloromethane

DMF = N,N'-dimethylformamide

DIBAL = Diisobutylaluminum

Bn = Benzyl ether

[00112] General methods

[00113] All chemicals were obtained from commercial sources and used as received unless otherwise stated.

[00114] The chromatographic purity of products was assessed in a condition as follows:

Mobile phase composition A: Methanol: H_2O=5/95(v/v), Contain 0.05% NH_4OH
B: Methanol : H_2O=95/5(v/v), Contain 0.05% NH_4OH

Chromatography system:

Time	Pump B Conc
0	15
1	15
5	80
5.1	15
10	15

Column type Waters® Acquity UPLC $HSST_3$, 1.8 μm, 100 × 2.1mm

Autosampler temperature	4℃
Column oven temperature	45℃
Flow rate	0.35 mL/min
Analysis time	10 min
Injection volume	5 μL
Retention time	4.8 min

[00115] The MS analysis was conducted in a condition as follows:

Mass spectrometer settings:

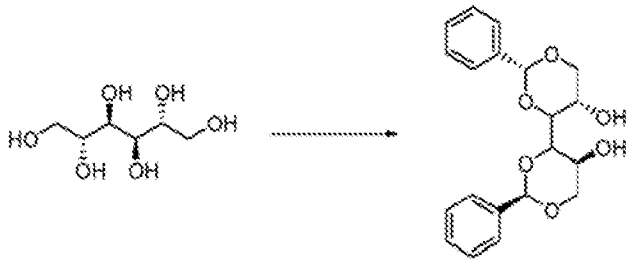
Mass spectrometer	Triple Quadrupole MS (API Qtrap5500) Applied Biosystem, Inc.
Detection	MRM negative mode
Pro-drug	: m/z 688.9 → m/z 180.9

[00116] Bruker AMX-500 NMR spectrometer in MeOH-d_4 (δ_H 3.30, δ_C 49.0) or $CDCl_3$ (δ_H 7.24, δ_C 77.0) using Bruker's standard pulse program; in the HMQC and HMBC experiments, Δ = 1 s and J = 140, 8 Hz, respectively, the correlation maps

consisted of 512 × 1 K data points per spectrum, each composed of 16 to 64 transients.

[00117] 1.1 mannitol (compound (i)) to compound (B)

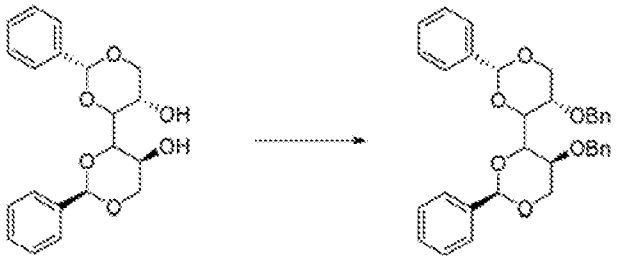
[00118] 1.1.1 mannitol (compound (i)) to compound (i)-1



mannitol, compound (i) compound (i)-1

[00119] To a solution of D-mannitol (25g, 0.137 mol) in DMF (250 mL) was added benzaldehyde (30 mL, 0.345 mmol) at r.t. under Ar. To the mixture was added concentrated sulfuric acid (10 mL) dropwise at 0 °C. After being allowed to warm up gradually to the r.t., the mixture was stirred for 3 day. Then the mixture was poured into ice water (250 mL) and n-hexane (200 mL) under vigorous stirring. After the mixture was warm up to r.t., the precipitate was filtered and washed with n-hexane. The precipitate was suspended in chloroform and heated under reflux for 15 min under vigorous stirring. When the mixture reached r.t., the undissolved precipitate was collected and Recrystallization from EtOH gave desired product as white solid (9.86 g, 20%).R_f = 0.45 (EA/Hex = 1/1).

[00120] 1.1.2 compound (i)-1 to compound (i)-2

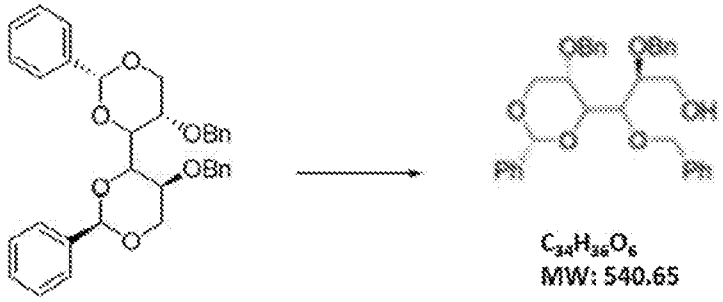


compound (i)-1 compound (i)-2

[00121] To a solution of 1,3,4,6-dibenzylidene (10g, 27.9 mmol) in DMF (100 mL) was added benzyl bromide (7.96 mL, 66.96 mmmol) at r.t. under Ar. The mixture was cooled to 0 °C then 60% NaH (2.68 g, 66.96 mmol) was added in few time. After being allowed to warm up gradually to the r.t., the mixture was stirred for overnight.

Then the reaction was quenched by water(dropwise) and extracted with $NaHCO_{3(aq)}$ and dichloromethane. The organic layer was dried with $MgSO_4$, concentrated in vacuum. The residue was purified by column chromatography on silica gel to afford **desired product**. (10.39 g, 69%). R_f = 0.2 (EA/Hex = 1/6).

[00122] 1.1.3 compound (i)-2 to compound (B)

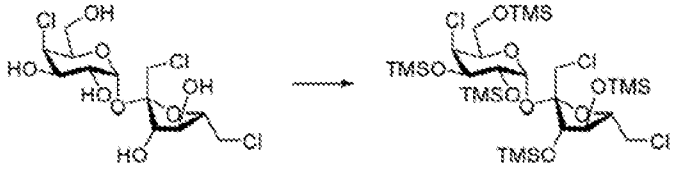


compound (i)-2 compound (B)

[00123] To a solution of 2,5-dibenzyl-1,3,4,6-dibenzylidene (1.5g, 2.78 mmol) in toluene (12.5 mL) was cooled to -18 °C (ice-salt bath). 1.2 M DIBAL was added (18.5 mL, 22.3 mmol) dropwise and warmed to r.t. After 1.5 h, the reaction was cooled to 0 °C then quenched by MeOH and 15% $KOH_{(aq)}$. The mixture was extracted with DCM, organic layer was dry with $MgSO_4$ and concentrated in vacuum. The residue was purify by column chromatography on silica gel to afford desired product. (709 mg, 47%). R_f = 0.1 (EA/HEX = 1/5).

[00124] 1.2 Sucralose (compound (ii)) to compound (D)

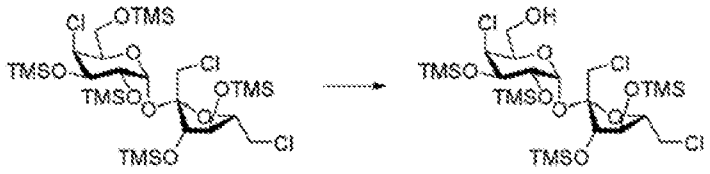
[00125] 1.2.1 compound (ii) to compound (ii)-1



sucralose, compound (ii) compound (ii)-1

[00126] To a solution of sucralose (1 g, 2.5 mmol) in DCM (10 mL) was added HMDS (2.6 mL, 12.57 mmol) and TMSOTf (45 µL, 0.25 mmol). The reaction was stirred for overnight in r.t. The reaction was concentrated in vacuum and pass through the cotton, wash by hexane. The filtrate was concentrated again in vaccum to get the product in quant. (1.9 g, quant.). R_f = 0.9 (EA/HEX= 1/8).

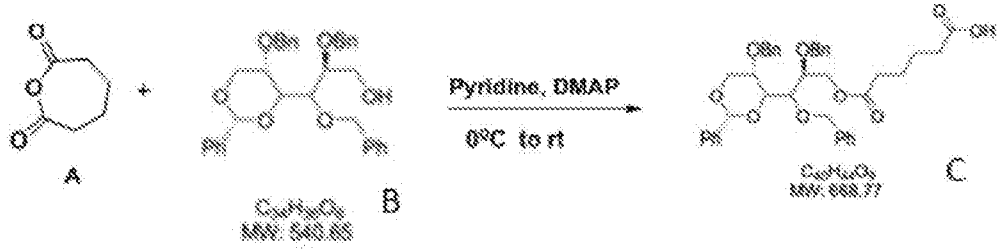
[00127] 1.2.2 compound (ii)-1 to compound (D)



compound (ii)-1 compound (D)

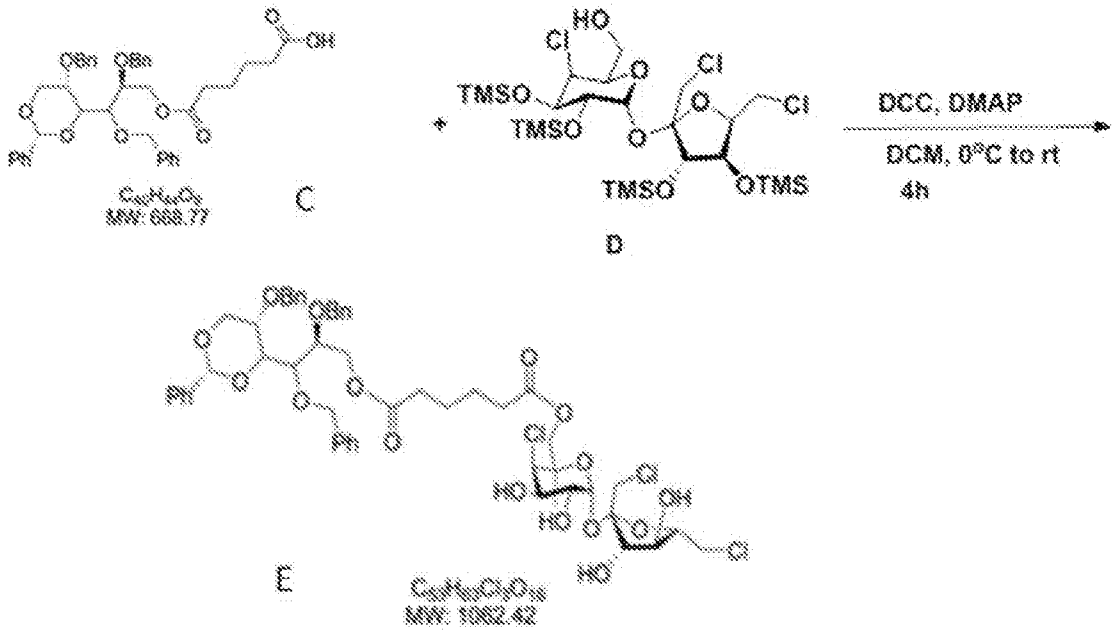
[00128] To a solution of penta-TMS sucralose (5g, 6.6 mmol) in pyridine (150 mL) was added 0.1 M pyridine-TsCl solution (6.6 mL) and stirred for 3 days with open flask. The reaction was concentrate in vacuum and purified by column chromatography on silica gel to afford **desired product** (1.4 g 30%). R_f = 0.5 (EA/HEX = 1/8).

[00129] 1.3 Synthesis of 6-oxo-6-((2R,3R,4R)-2,3,4-tris(benzyloxy)-4-(2-phenyl-1,3-dioxolan-4-yl)butoxy) hexanoic acid (compound (C))



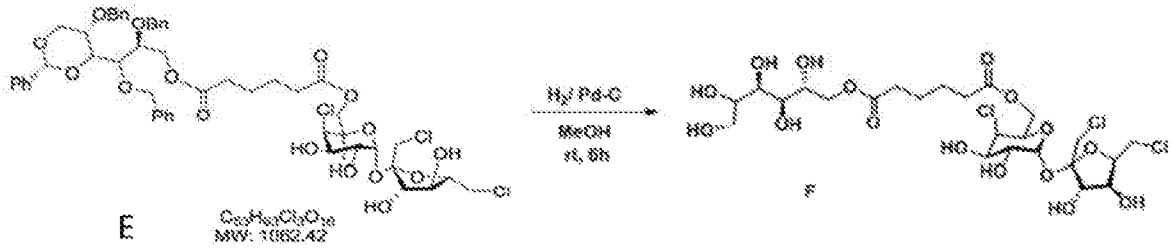
[00130] In a flame dry R.B. flask compound **A** (165 mg, 1 eq.) was dissolved in DCM (5 mL) at 0°C, then to this was added pyridine (0.2 mL) and DMAP (50 mg). Reaction mixture was then stirred for 10 min, followed by Comp. **B** (59 mg, 1.5 eq.) was added. Reaction mixture was then stirred at room temperature for 5 h. TLC confirmed the completion of reaction. Reaction mixture was evaporated to dryness of rotavapour under reduced pressure. The crude compound was further purified by column chromatography to afford the desired compound as a colorless oil (136 mg, 67%).

[00131] 1.4 Synthesis of ((2R,3S,4R,5R,6R)-6-(((2R,5R)-2,5-bis(chloromethyl)-3,4-bis((trimethylsilyl) oxy)tetrahydrofuran-2-yl)oxy)-3-chloro-4,5-bis((trimethylsilyl)oxy)tetrahydro-2 H-pyran-2-yl)methyl ((2R,3R,4R)-2,3,4-tris(benzyloxy)-4-(2-phenyl-1,3-dioxolan-4-yl)butyl) adipate



[00132] To ice cold solution of compound **C** (100 mg, 1.0eq.) in DCM was added DCC (35mg, 1.15 eq.) and stirred for 10 min. Then to this Compound **D** (112 mg, 1.2 eq.) and DMAP (5 mg, 0.25 eq. catalytic) was added. Reaction mixture was allowed to warm to rt and stirred for 4 hours. TLC confirmed the completion of reaction. Reaction mixture was evaporated to dryness on rotavapour under reduced pressure. The crude compound was then purified by column chromatography using neutral silica gel and 5 to 15 % ethyl acetate in Hexane with 1% Triethyl amine as an eluent to afford desired compound **E** as a colourless oil (84 mg, 42%).

[00133] 1.5 Synthesis of ((2R,3R,4R,5R,6R)-6-(((2R,5R)-2,5-bis(chloromethyl)-3,4-dihydroxytetrahydrofuran-2-yl)oxy)-3-chloro-4,5-dihydroxytetrahydro-2H-pyran-2-yl)methyl ((2R,3R,4R)-2,3,4,5,6-pentahydroxyhexyl) adipate (compound F)



[00134] In a flame dry Single neck R.B. flask compound **E** (500 mg, 1 eq.) was dissolved in dry MeOH (20 mL), solution was then degassed by nitrogen gas (Nitrogen gas syringe was deep inside the solution and Nitrogen was purge for 15

min.). Then 10% Pd-C (200 mg, 33% w/w) was added cautiously to reaction mixture. Finally, reaction mixture was stirred under hydrogen balloon pressure for 6 hours. TLC confirmed the completion of reaction. Reaction mixture was then filtered through celite bed and the bed was washed with dry methanol. The filtrate was evaporated to dryness of rotavapour under reduced pressure. Final compound was then kept under high vacuum to afford desired final compound **F** as colorless semisolid or white solid (190 mg, 73%). The structure of compound **F** were identified by high-resolution mass spectrophotometry and ^{13}C NMR.

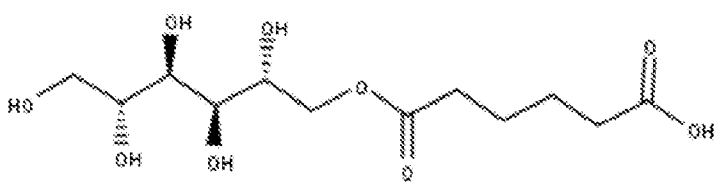
[00135] Example 2: Compound F as a prodrug, generating metabolites when incubated with blood (*in vitro*)

[00136] 2.1 Materials and Methods

[00137] Fresh human whole- blood were used for drug hydrolysis studies. Drug (10mg, compound F) was dissolved in 1mL solution (20% methanol). Drug hydrolysis (n=3) was performed in 20mL of fresh whole-blood aliquots containing 1.0mg of drug in a 50-mL flask thermostat at 37℃ in a shaking water bath. At time 0, the drug was added, and after various times of incubation, the blood samples were collected at 0.25, 0.5, 0.33, 0.75, 1, 2, 4, 6, 12 and 24hrs. Blood sample were used 1 mL acetonitrile to quench the enzymatic hydrolysis of the drug as samples were obtained. Pro-drug and its related metabolites, such as C6-mannitol, mannitol and sucralose in blood were determined by An API QTrap5500 triple-quadrupole mass spectrometer equipped with an ion-spray (ESI) source. The ESI interface was used in the negative-ion mode.

[00138] 2.2 Results

[00139] The pro-drug was monitored at a transition of m/z 688.9→180.9, Sucralose was monitored at a transition of m/z 395 →359, mannitol was monitored at a transition of m/z 452.3 →273.3; C6-mannitol was monitored at a transition of m/z 309 →101.1. All the compounds were identified by high-resolution mass spectrophotometry and ^{13}C NMR. The structure of C6-mannitol (formula (2)) is as follows:



(2)

[00140] The hydrolysis of pro-drug in blood was expressed by plotting the percentage of Pro-drug remaining and the percentage of sucralose, mannitol and C6-mannitol increasing versus time after incubation of the pro-drug in blood (Fig. 1). The results shows that compound F acts as a pro-drug which turns into its metabolites including sucralose, mannitol and C6-mannitol after incubated with blood *in vitro.*

[00141] **Example 3: Pharmacokinetics study in SD (Sprague Dawley)-rats (*in vivo*)**

[00142] **3.1 Materials and Methods**

[00143] SD-rats were orally administered pro-drug at a dose of 3.67 mg/kg BW. Blood samples were collected into heparinized micro centrifuge tubes at intervals of 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h. Plasma samples were immediately obtained by centrifuging the blood samples at 8,000 rpm for 10 min. The plasma samples were then stored at -80°C until use. The plasma samples were analyzed for pro-drug and its related metabolites, such as mannitol and sucralose by API QTrap5500 triple-quadrupole mass spectrometer equipped with an ion-spray (ESI) source. The ESI interface was used in the negative-ion mode.

[00144] **3.2 Results**

[00145] The pro-drug was monitored at a transition of m/z 688.9→180.9, Sucralose was monitored at a transition of m/z 395 →359; mannitol was monitored at a transition of m/z 452.3 →273.3; C6-mannitol was monitored at a transition of m/z 309 →101.1.

[00146] Fig. 2 and Fig. 3 shows the plasma concentration time curves of pro-drug and its related metabolites, such as sucralose and mannitol in SD-rats with single oral dosing of 3.67 *mg/kg* pro-drug, respectively. The results shows that compound F acts as a pro-drug which converts into its metabolites including sucralose, mannitol and C6-mannitol after administration in animals in vivo

[00147] **Example 4: CYP2E1 inhibitory activity assays**

[00148] **4.1 Materials and Methods**

[00149] This example is preparation of microsomes from human liver for in vitro screening of CYP450 isozyme inhibitors. Effective human hepatic CYP450 isozyme inhibitors were tested and the principle for testing the CYP450 isozyme inhibitors is based on the reaction of microsomal CYP450 isozyme prepared from the liver of different origin and its specific substrate Chlorzoxazone (CZX). After addition of the test sample, the amount of CYP450 isozyme metabolite standard 6-OH-CZX

(6-Hydroxy-Chlorzoxazone) is specific used for calculation of the CYP450 isozyme (CYP2E1) inhibition ratio of the test sample by using the amount of 6-OH-CZX of the control group as the baseline.

[00150] All samples were tested in triplicate. To determine the percentage inhibition, each test compound was dissolved in 1, 2, 4 μg/mL to three different concentrations. The CYP2E1 activity levels in the presence of the test compounds were compared with the control incubations. The 500-μL reaction mixture, containing 0.5 mg of microsomal protein, was incubated with 320 μM CZX in the presence of 5 mM $MgCl_2$ and 1 mM NADPH in 50 mM phosphate buffer with pH 7.4 at 37°C for 30 min. The reaction was terminated by ice-cold acetonitrile, and then 4-hydroxyl tolbutamide was added as an internal standard. The organic phase was evaporated to dryness and reconstituted into the mobile phase (methanol: water = 1:1) prior to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. An API 3000 triple-quadrupole mass spectrometer equipped with an ion-spray (ESI) source was used to determine 6-OH-CZX in the human liver microsomes. The ESI interface was used in the positive-ion mode. The 6-OH-CZX was monitored at a transition of m/z 284.5→185.9.

[00151] Analysis of the results: convert the detected signal values obtained from LC/MS/MS into the amount (pmol) of CYP450 isozyme metabolite standard 6-Hydroxy-Chlorzoxazone using the control group as the baseline, *i.e.* the CYP450 isozyme inhibition ratio of the control group is 0%. The CYP450 isozyme activity levels in the presence of the test compounds were compared with the control incubations.

[00152] 4.2 Results

[00153] Diethyldithiocarbamic acid (DDTC) is a well-known inhibitor of CYP2E1. At a concentration of 100 μM, DDTC treatment resulted in 90.9% inhibition of CYP2E1 in human liver microsomes (measured using CZX as a CYP2E1 substrate). On the basis of the observed inhibitory activity of DDTC, we tested the new compound (pro-drug) and its related metabolites for CYP2E1 inhibition at concentrations of 4, 2 and 1 *μg/mL*. The results as summarized in Table 1.

Table 1. The inhibition ratios of CYP2E1 inhibitors from in-vitro screening of human liver microsomes

Test compound	CYP 2E1 inhibition ratio (%)		
Test concentration	4 *μg/mL*	2 *μg/mL*	1 *μg/mL*

Test compound	CYP 2E1 inhibition ratio (%)		
Test concentration	4 μg/mL	2 μg/mL	1 μg/mL
Control group	0	0	0
Positive control (DDTC)	(100 μM) 90.9 ± 0.8	(50 μM) 51.2 ± 3.2	(10 μM) 11.2 ± 2.4
Pro-drug	45.7 ± 2.5	33.3 ± 4.1	17.7 ± 0.7
Metabloite_1 (mannitol)	40.3 ± 1.6	34.1 ± 4.1	30.1 ± 2.4
Metabolite_2 (sucralose)	32.9 ± 4.6	30.2 ± 2.8	25.1 ± 1.4
Intermediate metabolite (C6-mannitol with protecting groups, Formula C)	70.3 ± 2.8	56.5 ± 1.7	40.5 ± 2.3

[00154] The CYP 2E1 inhibition ratios of the test compound detected in the human liver microsomes are shown in Table 1. From the results, test compounds, including the pro-drug (compound F) and its metabolites i.e. mannitol, sucralose and C6-mannitol with protecting group (Formula C), have been demonstrated to be effective as P450 2E1 inhibitors, among which 4 μg/mL intermediate metabolite of pro-drug (i.e. C6-mannitol with protecting groups, Formula C) showed the best inhibition effect (70.3 ± 2.8%).

[00155] **Example 5: Assays of liver injuries induced by acetaminophen (APAP) and CCl_4**

[00156] **5.1 Materials and Methods**

[00157] **5.1.1 Reagents**

[00158] All organic solvents are HPLC grade and are purchased from Tedia (Fairfield, OH, USA). APAP is purchased from Sigma (St. Louis, MO USA), galactose injectable solution is manufactured by Southern Photochemical Co. and is prepared by dissolving 400 g of galactose (Sigma) in 1 L of buffer solution containing isotonic salts for injections.

[00159] **5.1.2 Animals**

[00160] Male SD (Sprague-Dawley) rats weighing 175-280 g were purchased from the National Laboratory Animal Center (NLAC), Taiwan. The study was conducted in accordance with the Guidelines for Conducting Animal Studies of the National Health Research Institute and all rats were placed in the air/humidity controlled environment under the 12 hours of day/12 hours of night cycle and with unlimited water and food supply. During the course of the study, the weights of rats were monitored continuously with normal water supply.

[00161] 5.1.3 Treatments

[00162] 5.1.3.1 liver injuries induced by APAP

[00163] Mannitol and sucralose were used to perform the animal test (rat) in view of liver injuries induced by APAP.

[00164] In the normal control (Group 1), animals were not fed with APAP. In the control group of APAP-induced liver injuries (Group 2), animals were fed with a single dose of APAP in the amount of 2,000 mg per kilogram of body weight to induce hepatotoxicity. In the positive control group of treatment with NAC (Group 3), animals were fed with a single dose of APAP in the amount of 2,000 mg per kilogram of body weight to induce hepatotoxicity, and 4 hours later, a 24-hour treatment period by tube feeding was started, including first administration of 140 mg of NAC (per kilogram of body weight) and later administration of 70 mg of NAC (per kilogram of body weight) every 4 hours for five times. In the experimental group (Group 4), animals were fed with a single dose of APAP in the amount of 2,000 mg per kilogram of body weight to induce hepatotoxicity, and 4 hours later, a 24-hour treatment period by tube feeding was started, including six dosing with the ingredients of the present invention every 4 hours, as follows:

(a) (Group 4.1): administration of mannitol at a dose less than or equivalent to 100 mg per person every 4 hours for 24 hours,

(b) (Group 4.2): administration of double dose of mannitol as in Group 4.1 every 4 hours for 24 hours,

(c) (Group 4.3): administration of sucralose at a dose less than or equivalent to 100 mg per person every 4 hours for 24 hours,

(d) (Group 4.4): administration of double dose of sucralose of Group 4.3 every 4 hours for 24 hours,

(e) (Group 4.5): administration of a combination of 0.5 times the dose of mannitol as in Group 4.1 and 0.5 times the dose of sucralose as in Group 4..3 per kilogram of body weight every 4 hours for 24 hours,

(f) (Group 4.6): administration of a combination of the dose of mannitol as in Group 4.1 and the dose of sucralose as in Group 4.3 every 4 hours for 24 hours,

(g) (Group 4.7): administration of a combination of 1.5 times the dose of mannitol as in Group 4.1 and 1.5 times the dose of sucralose as in Group 4.3 every 4 hours for 24 hours,

(h) (Group 4.8): administration of a combination of double dose of mannitol as in

Group 4.1 and double dose of sucralose as in Group 4.3 every 4 hours for 24 hours, and

(i) (Group 4.9): first administration of 140 mg of NAC per kilogram of body weight and later administration of a combination of 70 mg of NAC plus double dose of mannitol as in Group 4.1 and double dose of sucralose as in Group 4.3 every 4 hours for five times.

[00165] After the 24-hour treatment period, blood was collected from the tail artery of the rats for AST/SLT assays. Subsequently, rats were subjected to GSP tests. Finally, rats were sacrificed and histological analysis was performed.

[00166] **5.1.3.2 liver injuries induced by CCl_4**

[00167] Mannitol and sucralose were chosen from the active ingredients as described herein to perform the animal test (mice) in view of liver injuries induced by CCl_4.

[00168] In the normal control, animals were administered with normal saline by intraperitoneal injection. In the control group of CCl_4 induced liver injuries, animals were intraperitoneally injected with 10 ml/kg CCl_4 (40% in corn oil) to induce hepatotoxicity. In the experimental group, animals were intraperitoneally injected with 10 ml/kg CCl_4 (40% in corn oil) to induce hepatotoxicity, and 4 hours later, different ingredients of the present invention were administered by tube feeding. Blood was collected from the mice before administration with the ingredients of the present invention or at 24 hours after administration with the ingredients of the present invention for AST/ALT assays. Finally, animals were sacrificed at day 2 and blood were collected for AST/ALT assay and histological analysis was performed.

[00169] On the other hand, other experimental groups of mice were fed with the ingredients of the present invention for 12 weeks and the mice were subjected to GSP tests.

[00170] **5.1.4 Blood samples**

[00171] After completion of the treatments, rats were sacrificed under ether anesthesia, and blood was collected from the tail artery of the rats and placed in a test tube containing EDTA. The plasma was centrifuged at 13,000 at 4°C for 15 minutes and the isolated plasma was transferred to Eppendorf tubes in aliquots and stored at -80°C.

[00172] **5.1.5 Biochemical analysis**

[00173] Liver damage is quantified by measuring plasma AST and ALT activity.

AST and ALT are common indicators of hepatotoxicity and are measured by using the Synchron LXi 725 system (Beckman Instruments, U.S.).

[00174] 5.1.6 Optic microscope

[00175] Following scarification of the rats, histological analysis was performed. Liver samples were fixed with 10% phosphate-buffered formalin, dehydrated an embedded in paraffin, Sections were prepared in 5 μm thickness and then stained with hematoxylin and eosin and subjected to Periodic acid Schiff stain (PAS). The stained sections were observed under the optic microscope.

[00176] 5.1.7 Quantitative tests of liver function

[00177] After the study was completed, all rats were subjected to GSP test. Rats were i.v. injected with 0.4 g/ml BW galactose solution 0.5 g/kg within 30 seconds and one blood sample was collected at 5, 10, 15, 30, 45 and 60 minutes post injection from the tail vein. Colorimetric galactose dehydrogenase is used to quantify the concentration of galactose and the test concentration ranges from 50 to 1,000 μg/ml. The within-day variation of each concentration is calculated using standard deviation and coefficient of variation (CV) and the maximum allowable coefficient of variation is 10% CV, whereas day-to-day variation is examined by comparing the slope and intercept of calibration curves. The GSP is the blood galactose concentration obtained 60 seconds after stopping the 30-second injection.

[00178] 5.1.8 Statistical analysis

[00179] All data are represented in mean ± standard deviation (SD) and the results are calculated using ANOVA to determine the significance. Statistical Package of the Social Science program (Version 13, SPSS Inc.) is used for calculations followed by post hoc test to examine the least significant difference for multiple comparisons so as to confirm the significant differences between groups and the average difference between groups was significant $p < 0.05$.

[00180] 5.2 Results

[00181] 5.2.1 Mannitol and sucralose and other ingredients are effective in treating liver injuries induced by APAP

[00182] The results are shown in Table 2.

[00183] Table 2

Liver function parameters	GSP (mg/L)	AST (IU/L)	ALT (IU/L)	Total HAI score	Survival (Day14, n/n)

Group 1: Normal control (NC, n=6)	220 ± 24	186 ± 16	65 ± 16	0.0 ± 0.0	3/3
Group 2: APAP control (2,000 *mg/kg*, n=12)	1017 ± 170	1151 ± 310	746 ± 143	8.6 ± 0.5	2/12
Group 3: NAC (140mg/kg of NAC followed by 5 × 70mg/kg NAC at 4h intervals, n=6)	393 ± 68***	428 ± 74***	221 ± 69***	4.2 ± 0.8***	3/6
Group 4.1 (n=3) (Mannitol at a dose less than or equivalent to 100 mg per person) x6	565 ± 177***	455 ± 78***	209 ± 16***	4.0 ± 0.0***	1/3
Group 4.2 (n=3) (Double dose of Group 4.1 (mannitol)) x6	354 ± 56***	300 ± 40***	166 ± 15***	4.0 ± 1.0***	3/3
Group 4.3 (n=3) (Sucralose at a dose less than or equivalent to 100 mg per person) x6	332 ± 42***	331 ± 41***	154 ± 49***	4.0 ± 1.0***	3/3
Group 4.4 (n=3) (Double dose of Group 4.3 (sucralose)) x6	309 ± 54***	277 ± 78***	136 ± 48***	3.0 ± 1.0***	3/3
Group 4.5 (n=3) (0.5 times the dose of Group 4.1 (mannitol) + 0.5 times the dose of Group 4.3 (sucralose)) x6	332 ± 61***	360 ± 81***	149 ± 19***	2.0 ± 1.0***	3/3
Group 4.6 (n=3) (the dose of Group 4.1 (mannitol) + the dose of Group 4.3 (sucralose)) x6	271 ± 52***	193 ± 34***	81 ± 18***	1.5 ± 1.0***	6/6
Group 4.7 (n=3) (1.5 times the dose of Group 4.1 (mannitol) + 1.5 times the dose of Group 4.3 (sucralose)) x6	265 ± 53***	203 ± 24***	83 ± 25***	1.0 ± 1.0***	3/3

Group 4.8 (n=3) (double dose of Group 4.1 (mannitol) + double dose of Group 4.3 (sucralose)) x6	227 ± 25***	159 ± 21***	69 ± 10***	0.5 ± 0.5***	6/6
Group 4.9 (n=3) 140 mg/kg NAC + 5 x (70 mg NAC + double dose of Group 4.1 (mannitol) + double dose of Group 4.3 (sucralose))	233 ± 41***	171 ± 25***	58 ± 9***	0.3 ± 0.5***	6/6
Group 5 (n=6) (Aerosil 200 at a dose less than or equivalent to 100 mg per person)	280 ± 98***	247 ± 43***	66 ± 18***	2.8 ± 1.0***	6/6
Group 6 (n=6) (Sodium starch glycolate at a dose less than or equivalent to 100mg per person)	294 ± 30***	248 ± 37***	81 ± 27***	2.7 ± 1.2***	6/6
Group 7 (n=6) (Crospovidone at a dose less than or equivalent to 100mg per person)	372 ± 90***	323 ± 40***	175 ± 61***	2.8 ± 1.5***	6/6
Group 8 (n=6) (Microcrystalline cellulose at a dose less than or equivalent to 100mg per person)	259 ± 36***	217 ± 28***	72 ± 21***	2.2 ± 1.0***	6/6
Group 9 (n=6) (Povidone K-30 at a dose less than or equivalent to 100mg per person)	287 ± 38***	220 ± 53***	71 ± 26***	2.5 ± 1.0***	6/6

*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$: comparison of the experimental groups with APAP control

[00184] The results show that liver injuries has occurred in the APAP hepatotoxicity group. In contrast, such liver injuries and survival rate can be improved by use of mannitol and/or sucralose, in a dose dependent manner. Especially, a combination of mannitol and sucralose achieves a synergistic effect; the results are similar to those of normal control and even better than the positive control of standard treatment with

NAC. In addition, other ingredients including Aerosil 200, Sodium starch glycolate, Crospovidone, Microcrystalline cellulose and Povidone K-30 are found effective in treating the liver injuries, also better than the positive control of standard treatment with NAC.

[00185] The improved results are also reflected in the corresponding liver tissues.

[00186] Fig. 4 shows the results of the histological analysis. The liver tissue sections from the rats in the APAP hepatotoxicity group showed that hepatocytes surrounding the central vein are broken with visible vacuolization and reduced number of nucleuses, some hepatocytes even showed the signs of necrosis and liver damage is more severe when compared with the hepatocytes from rats in the normal control group (Fig. 4B). On the contrary, liver structure of rats in the control group are normal, the hepatocytes are intact and arranged in order with no vacuolization (Fig .4A). As for the liver sections from the experimental groups with treatment by mannitol and/or sucralose, the hepatocytes are relatively intact with visible nucleus and less vacuolization (Fig. 4D, E, F, G, H). Especially, a combination of mannitol and sucralose achieves the best protective effect (Fig. 4G); the results are even better than the positive control of standard treatment with NAC (Fig. 4C).

[00187] **5.2.2 Mannitol is effective in treating liver injuries induced by CCl_4**

[00188] The results are shown in Table 3.

[00189] Table 3

	Liver function parameters			
Groups	GSP (mg/L)	AST (IU/L)	ALT (IU/L)	Total HAI score
Normal control (n=10)	315 ± 48	88 ± 20	57 ± 17	0.0 ± 0.0
CCl_4 control group (n=10)	914 ± 205***	815 ± 216***	770 ± 274***	6.2 ± 2.1***
Dose of kaempfrol less than or equivalent to 100mg per person (n=10)	**456 ± 101*****	**198 ± 105*****	**128 ± 40*****	**4.3 ± 1.3***
Dose of epigallocetechin-3-gallate less than or equivalent to 100 mg per person (n=10)	**312 ± 140*****	**144 ± 49*****	**95 ± 36*****	**1.7 ± 0.9*****

Dose of quercetin less than or equivalent to 100 mg per person (n=10)	**286 ± 70*** **	**115 ± 40*** **	**93 ± 26*** **	**1.1 ± 0.7*** **
Dose of **mannitol** less than or equivalent to 100mg per person (n=10)	**290 ± 78*** **	**91 ± 28*** **	77 ± 22*** **	**0.8 ± 0.5*** **

Statistic analysis: Anova and LSD tests.
***$p < 0.005$, **$p < 0.01$, *$p < 0.05$, comparison of the experimental groups with CCl_4 control group.

[00190] The results show that liver injuries has occurred in the CCl_4 control group. In contrast, such liver injuries can be improved by use of mannitol.

[00191] **Example 6: Assays of Fatty Liver**

[00192] **6.1 Materials and Methods**

[00193] **6.1.1 Cell lines and cell culture media**

[00194] The activity of the various ingredients as described herein, including mannitol and sucralose and others, in reduction of fat content was analyzed by using human hepatoma cell line Hep G2.

[00195] Dulbecco's Modified Eagle's Medium (DMEM) was used to prepare DMEM culture Nos. A-F listed in Table 4 for carrying out subsequent experiments.

[00196] Table 4: Preparations of DMEM culture media Nos. A-F

DMEM cultures	Preparation methods
No. A	DMEM was dissolved in 1,400 mL of water with stirring, and then 2 g of 4- (2-hydroxyethyl) -1-piperazine-ethanesulfonic acid (HEPES) was added to form a solution, to which a sodium bicarbonate solution (4 g of sodium bicarbonate powder dissolved in 400 mL of water by stirring) was added, and the volume was made up to 2,000 mL with water. The pH of the resulting solution was adjusted to 7.3 ± 0.05 by adding 5N HCl. After being filtered through a 0.2 μm sterile membrane, the final solution was dispensed into sterile serum vials and stored at 4°C.
No. B	50 mL of deactivated fetal bovine serum (FBS), 5 mL of sodium pyruvate (100 mM), 5 mL of penicillin (100 U/mL) and streptomycin (100 U/mL), and 5 mL of MEM non-essential amino acid solution(100X) were added into 450 mL of DMEM culture No. A.
No. C	5 mL of sodium pyruvate (100 mM), 5 mL of penicillin (100 U/mL) and streptomycin (100 U/mL), and 5 mL of MEM non-essential amino acid solution(100X) were added into 450 mL

	of DMEM culture No. A.
No. D	DMEM culture No. B was added into the oleate/albumin complex. The oleate/albumin complex was prepared according to the method presented by Van Harken et al. in 1969 (J Biol Chem. 1969 May 10; 244(9):2278-85). The method included taking 25 mL of DMEM culture No. A, into which 5 g of bovine serum albumin (BSA) was added, and then 5 N sodium hydroxide solution was added to adjust the pH to 7.4 to form a mixture. The mixture was then placed in an ice bath at 0°C to form the BSA solution. The oleic acid was dissolved in 50 ml of alcohol (95%) and then titrated to the phenolphthalein titration endpoint with 1N sodium hydroxide solution. The alcohol was blown away by flowing helium. The resulting sodium oleate was dissolved in DMEM culture No. A at 37°C to form a sodium oleate solution. At last, the BSA solution was added dropwise into the sodium oleate solution with stirring to form the oleate/albumin complex solution.
No. E	Various amounts of silymarin were dissolved in DMEM culture No. C.
No. F	Various amounts of the test compounds of the present invention were dissolved in DMEM culture No. C.

[00197] The DMEM cultures Nos. A-F were preserved at 2-8°C, and warmed up in a water bath at 37°C before the experiments.

[00198] **6.1.2 cell counts and survivability test**

[00199] Dead cells would take up 0.4% trypan blue and then had a color; whereas live cells exclude certain dyes due to the intact cell membranes and had a clear color. 100 μl of cell suspension and equal volume of 0.4% trypan blue were mixed uniformly to form a mixture. Some of the mixture (about 20 μl) was added into the groove above the chamber of the hemocytometer, which was then covered with a cover slip for observing under the optical microscope. Live cells were not stained, and dead cells were blue.

[00200] **6.1.3 Oleic acid-induced formation of fatty liver cells from HepG2 cell lines**

[00201] HepG2 cell lines (15×10^6 cells) were cultured in DMEM culture No. B, incubated in an incubator with 5% CO_2 at 37°C for 24 hours, cultured in DMEM culture No. C (serum-free medium) for 24 hours, and finally cultured in DMEM culture No. D (containing oleate/albumin complex) for another 48 hours to induce HepG2 cell lines to form fatty liver cells.

[00202] **6.1.4 Treatments for each group of fatty liver cells**

[00203] HepG2 cell lines were divided into six groups, including: (1) Blank: no

treatment; (2) DMSO group: cells from Blank were treated with dimethyl sulfoxide (DMSO); (3) Control: induction with oleic acid to form fatty liver cells; (4) Vehicle group: fatty liver cells formed by induction with oleic acid were treated with DMSO; (5) Positive control: fatty liver cells were treated with silymarin; and (6) Test Group: fatty liver cells were treated with various compounds of the present invention.

[00204] 6.1.5 Determination of triglyceride (TG) in cells

[00205] After incubation for 72 hours, the treated cells from each group were successively washed twice in PBS, and then incubated with 0.5 ml of trypsin/EDTA for 3 minutes. Afterwards, the cells were scraped with 2 ml of PBS and then transferred to the centrifuge tube to be shattered by ultrasonic. A volume of 20 μl cell extracts was taken to determine the content of protein. TG determination was performed using commercially available combination of agents (Randox). The TG content obtained above was divided by the protein content to get a ratio, which represented the relative content of TG in cells.

[00206] 6.1.6 Animals for experiments

[00207] B6 mice recommended in the specification "Method for evaluating the liver protection and health care efficacies of health food" announced by the Department of Health of Taiwan were chosen for animal testing. More than four mice were used in each group of the pre-test, while more than twelve mice were used in each group of the confirmatory test. Male mice bred at 23±2°C in an animal room with 55 ± 15% relative humidity under normal light/dark cycle (7:00 AM-7:00 PM lights on/7:00 PM-7:00 AM lights off) and weighing 18-23 g were purchased from BioLASCO (Taipei) and housed at Laboratory Animal Center in National Defense Medical Center. The animal test was carried out according to the guideline for animal experiment of National Health Research Institutes. Mice were fed with normal feed at 3-5 g/day and unlimited supply of water for 1-2 weeks and investigated for health condition. The weight of mice was recorded once a week.

[00208] 6.1.7 Animal grouping

[00209] The tested animals were grouped randomly into Blank, High Fat Diet control (HFD), Positive Control (PS), and Test group. The animals of Blank were fed with normal feed. The animals of HFD were fed with high fat feed. The animals of PS were fed with high fat feed, and additionally fed with silymarin (5 mg/kg/day) by a tube. The animals of Test group were fed with high fat feed, and additionally fed with test compounds by a tube.

[00210] **6.1.8 Test methods**

[00211] The animals of Blank were fed casually with normal feed for 12 weeks, while the animals of HFD, PS, and Test group were fed casually with high fat feed for 12 weeks. After 8 weeks of feeding, the animals of Blank and HFD were fed with deionized water by a tube once a day; the animals of PS were fed with silymarin by a tube once a day; and the animals of Test Group were fed with test compounds by a tube once a day for a duration of 4 or 8 weeks.

[00212] Before testing and in the eighth, twelfth, and sixteenth week after testing, blood was collected from the cheek or the heart. At the end of testing, all mice were weighted and then sacrificed, and blood was collected from the cheek or the heart simultaneously. The blood specimens of mice rested at room temperature for one hour to clot, and then the serum was separated by centrifugation in a refrigeration centrifuge at 15,700 x g at 4°C for 5 minutes. Afterwards, biochemical indices of liver function, including aspartate transaminase (AST), alanine aminotransferase (ALT), triglyceride (TG), total cholesterol (TCHO/TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), were detected by the automatic blood biochemistry analyzer.

[00213] In addition, abdominal fat and liver specimens were taken from the abdomens of sacrificed mice and weighted to compare the weight of fat and liver and obtain the ratio of liver weight to body weight. Two tissue blocks with a volume of approximately 1 cm^3 were cut from the largest right lobe of liver, fixed in 10% neutral formalin solution, and then embedded with paraffin for sectioning. The cut sections proceeded with H&E staining for histopathological observation. Moreover, the rest of the liver was frozen for preservation and detection of the contents of triglyceride and total cholesterol in the liver. Furthermore, the liver function of animals of each group were analyzed by Galactose Single Point Method, which was recognized and recommended for quantification of remaining liver function in clinical use by U.S. FDA and Ministry of Health and Welfare, Taiwan. At the end of the tests, 0.5 g of galactose (G.S.P.® 0.4 g/mL) per kg of animal was administered via intravenous. One hour after the administration, about 0.5 ml of whole blood was taken by using a filter paper to evaluate liver function of mice. The higher the value of GSP was, the worse the remaining liver function would be. (FDA: "Guidance for Industry: Pharmacokinetics in Patients with Impaired. Hepatic Function-Study Design, Data Analysis and Impact on Dosing and. Labeling. 2003.

[00214] 6.1.9 Histopathological tissue sectioning:

[00215] At the end of the test, all mice were sacrificed. One tissue block with a volume of approximately 1 cm^3 was cut from the largest right lobe of liver, fixed in 10% neutral formalin, and then dehydrated and hyalinized in various concentrations of ethanol (30、50、70、95、99.5%) and xylene. Afterwards, xylene was replaced with hot paraffin solution. At last, the tissue was embedded with paraffin solution. The finished paraffin specimen was cut into 5 μm-thickness paraffin sections by the microtome. The sections were pasted on clean slides, dried at 37°C, and then stained by H&E staining.

[00216] 6.1.10 hematoxylin and eosin staining (H&E)

[00217] Liver tissue sections were deparaffinized in xylene for 30 minutes, and then successively rehydrated twice in 99.5%, 95%, 70%, 50%, and 30 % aqueous ethanol for 30 minutes respectively. After being soaked in distilled water for 10 minutes, the sections could be stained. The sections were first immersed in hematoxylin for 30 seconds to stain cell nuclei, then washed with distilled water for a few minutes, stained with eosin for 2-5 minutes, and washed with distilled water for a few minutes again. After staining process was finished, the sections were dehydrated successively in 50%, 70%, 95%, and 100% aqueous ethanol twice for 30 seconds respectively, hyalinized twice in xylene, and finally sealed and stored with mounting media.

[00218] 6.1.11 Histopathological Observation

[00219] In order to observe the changes of lesion, fat accumulation, necrosis, or fibrosis in liver cells when there was an ongoing liver damage, liver tissues were H&E stained to evaluate the degree of liver fat accumulation. All the histopathological sections were cut from the same position on the largest right lobe of liver for eliminating bias in subjective observation, and then subject to pathological staining. As for the assessment of semi-quantitative analysis in pathology, it had to be confirmed by a physician or a veterinary pathologist who conducted a double-blind analysis to score (NAS score) and compare all the sections without knowing the test design. At last, the differential analysis of each group was performed by statistical methods.

[00220] 6.1.12 Analysis of liver antioxidant capacity

[00221] About 0.1 g of liver tissue was taken from the sacrificed animal and

homogenized by centrifuge with a biomasher for 10 minutes. A 9-fold weight (w/w) of buffer (pH 7.4, 50 mmol/L Tris-HCl, 180 mmol/L KCl) was added to the homogenized tissue, which was then mixed well by a Vortex mixer for use. The resulting homogenization solution samples of liver tissue was used to analyze the various members of liver antioxidant systems, including glutathione peroxidase (GPx), glutathione (GSH), glutathione reductase (Grd), and superoxide dismutase (SOD). Methods of related analysis can be found in the known literatures, for example, the draft of "Method for evaluating the liver protection and health care efficacies of health food" announced by the Ministry of Health and Welfare, Taiwan.

[00222] 6.1.13 Statistical Analysis

[00223] All data were expressed as means ± standard deviation (SD). Statistically significant difference of the test results was determined by calculation of one-way ANOVA using Statistical Package of the Social Science program, Version 13, SPSS Inc. Thereafter, multiple comparisons were carried out by using least significant difference method in post hoc test to confirm the significant difference between groups. The average difference between groups is judged to be significant when $p < 0.05$.

[00224] 6.2 Results

[00225] 6.2.1 Cell Experiments

[00226] In cell experiments, the results of TG content reduction in HepG2 cells determined in Positive Control (silymarin) were listed in Table 5.

[00227] Table 5: Efficacy of silymarin in reduction of TG content in HepG2 fat cells of Positive Control

Silymarin concentration (μM)	TG content in cells (μg/mg protein)	Reduction rate of TG (%)
0 (Control)	59.43 ± 4.60	-
1.0	44.17 ± 2.41	29 ± 8
5.0	44.59 ± 11.53	28 ± 10
10	26.38 ± 9.12	63 ± 11
100	20.48 ± 4.76	78 ± 5

[00228] The results of TG content reduction in HepG2 fat cells determined using constant concentration of test compounds were shown in Table 6. It can be seen from the results that the test compounds exhibited different degrees of TG content reduction effects in fatty liver cells formed from induced HepG2 cells under the condition of constant test concentration, as compared with Control. The equation for

calculating reduction rate (%) of TG was as follows: [1 - (TG content of Test Group - TG content of Blank) / (TG content of Oleic acid induction Group - TG content of Blank)] x 100 %.

[00229] Table 6: TG content in fatty liver cells reduced by test compounds

Tested substances (1.0 μM)	TG reduction rate (%)
Silymarin Control	35.33 ± 1.96
Puerarin	49.91 ± 7.73
Phloridzin	42.35 ± 6.05
Daidzein	42.3 ± 5.34
Sodium lauryl sulfate	38.73 ± 4.65
Poncirin	38.12 ± 7.22
Sinensetin	36.97 ± 4.84
(-)-Epigallocatechin	36.78 ± 6.67
Kaempferol	36.51 ± 4.78
Isovitexin	35.93 ± 3.35
Ursolic Acid	35.86 ± 8.92
Eriodictyol	35.11 ± 0.87
(+)-Limonene	35.02 ± 10.04
Hesperidin	34.81 ± 5.25
Ergosterol	34.19 ± 3.69
β-myrcene	33.97 ± 11.22
(-)-Epicatechin-3-gallate	32.7 ± 4.33
Hyperoside	30.51 ± 2.8
Silybin	30.26 ± 3.24
(+)-Catechin	29.57 ± 4.02
Formononetin	29.55 ± 1.44
Myristic acid ethyl ester	28.88 ± 3.91
Galangin	28.11 ± 8.62
Sucralose	26.68 ± 2.93
Eicosapentaenoic acid (EPA)	26.15 ± 6.14
Morin	25.84 ± 10.65
Mannitol	22.35 ± 5.74

Sciadopitysin	21.83	±	5.04
Wongonin	20.78	±	1.12
Didymin	20.37	±	12.69
Gossypin	20.25	±	4.63
Sorbitol	20.06	±	2.57
Luteolin-7-glucoside	19.33	±	4.59
Povidone K-30	18.93	±	5.13
Protocatechuic acid	18.57	±	7.6
(+)-Taxifolin	17.91	±	8.35
Saccharin	17.53	±	6.96
Umbelliferone	17.4	±	2.57
Glycerin	16.23	±	4.25
Hesperitin	16.08	±	5.55
Nordihydroguaiaretic acid	15.92	±	2.3
Trans-Cinnamic Acid	15.85	±	0.82
Sodium benzoate	14.35	±	4.86
Oxide red	13.59	±	2.08
Neohesperidin	13.29	±	7.21
Naringin	12.69	±	3.72
Diosmin	11.86	±	3.73
(-)-Epicatechin	10.76	±	8.92
Glycyrrhizin	10.55	±	7.4
Linarin	9.24	±	12.34
Baicalin	9.21	±	6.21
Quercitrin	9.15	±	9.24
Xylitol	7.36	±	6.34
Baicalein	7.09	±	10.88
Luteolin	6.95	±	15.23
Swertiamarin	6.72	±	11.04
Butylated hydroxyanisole	6.21	±	3.8
Sodium cyclamate	4.77	±	4.49
Menthol	66.24	±	1.87

Citric acid	2.55	±	4.43
Lemon oil	0.56	±	1.07
Pregelatinized starch	7.18	±	13.41
Sorbic acid	2.03	±	1.96

[00230] Table 6-1: A portion of test compounds from Table 6 that reduced TG content in fatty liver cells

Tested substances (1.0 uM)	TG reduction rate (%)		
Puerarin	49.91	±	7.73
Phloridzin	42.35	±	6.05
Daidzein	42.3	±	5.34
Sinensetin	36.97	±	4.84
(-)-Epigallocatechin	36.78	±	6.67
Kaempferol	36.51	±	4.78
Ursolic Acid	35.86	±	8.92
Silymarin of Control	35.33	±	1.96
(+)-Limonene	35.02	±	10.04
Hesperidin	34.81	±	5.25
(-)-Epicatechin-3-gallate	32.7	±	4.33
Silybin	30.26	±	3.24
Formononetin	29.55	±	1.44
Myristic acid ethyl ester	28.88	±	3.91
Eicosapentaenoic acid (EPA)	26.15	±	6.14
Wongonin	20.78	±	1.12
Povidone K-30	18.93	±	5.13
Protocatechuic acid	18.57	±	7.6
Umbelliferone	17.4	±	2.57
Hesperitin	16.08	±	5.55
Nordihydroguaiaretic acid	15.92	±	2.3
Neohesperidin	13.29	±	7.21
Naringin	12.69	±	3.72
(-)-Epicatechin	10.76	±	8.92

Glycyrrhizin	10.55	±	7.4
Baicalin	9.21	±	6.21
Quercitrin	9.15	±	9.24
Baicalein	7.09	±	10.88

[00231] Table 6-2: A portion of test compounds (Bioflavonoids) from Table 6 that reduced TG content in fatty liver cells

Tested substances (1.0 uM)	TG reduction rate (%)
Poncirin	38.12 ± 7.22
Isovitexin	35.93 ± 3.35
Eriodictyol	35.11 ± 0.87
Ergosterol	34.19 ± 3.69
β-myrcene	33.97 ± 11.22
Hyperoside	30.51 ± 2.8
(+)-Catechin	29.57 ± 4.02
Galangin	28.11 ± 8.62
Morin	25.84 ± 10.65
Sciadopitysin	21.83 ± 5.04
Didymin	20.37 ± 12.69
Gossypin	20.25 ± 4.63
Luteolin-7-glucoside	19.33 ± 4.59
(+)-Taxifolin	17.91 ± 8.35
Trans-Cinnamic Acid	15.85 ± 0.82
Diosmin	11.86 ± 3.73
Linarin	9.24 ± 12.34
Xylitol	7.36 ± 6.34
Luteolin	6.95 ± 15.23
Swertiamarin	6.72 ± 11.04

[00232] Table 6-3: A portion of test compounds (excipients) from Table 6 that reduced TG content in fatty liver cells

Tested substances (1.0 uM)	TG reduction rate (%)

Sodium lauryl sulfate	38.73	± 4.65
Sucralose	26.68	± 2.93
Mannitol	22.35	± 5.74
Sorbitol	20.06	± 2.57
Saccharin	17.53	± 6.96
Glycerin	16.23	± 4.25
Sodium benzoate	14.35	± 4.86
Oxide red	13.59	± 2.08
Butylated hydroxyanisole	6.21	± 3.8
Sodium cyclamate	4.77	± 4.49
Menthol	66.24	± 1.87
Citric acid	2.55	± 4.43
Lemon oil	0.56	± 1.07
Pregelatinized starch	7.18	± 13.41
Sorbic acid	2.03	± 1.96

[00233] 6.2.2 Animal Experiments

[00234] In the animal experiments, all the animals were treated to induce fatty liver, except the animals of Blank that were fed with normal feed. After eight weeks, the animals of each group were given different treatment for four or eight weeks in addition to the original feed. The animals of Blank and HFD were fed with deionized water; the animals of PS were fed with silymarin; and the animals of Test Group were fed with different test compounds, including puerarin, phloridzin, eriodictyol, sucralose, mannitol, saccharin, hesperitin, menthol, and combinations thereof.

[00235] 6.2.2.1 The effects on body weight, liver weight, and weight of body fat of animals and safety evaluation of test compounds

[00236] From the results of animal experiments, the liver weight, weight of body fat, and increase of body weight of animals of each group were listed in Table 7-1 and 7-2.

[00237] Table 7-1: The analysis results of liver weight and weight of body fat due to test compounds

Items	**Abdominal fat weight**	**Liver weight**

Unit	g			g		
Blank (n=13)	**0.6**	**± 0.2**	***	1.6	± 0.2	0.6
HFD (n=12)	2.8	± 0.4		1.6	± 0.4	2.8
Positive Control						
Silymarin 5.0 mg/kg (n=6)	**2.0**	**± 0.4**	***	**1.2**	**± 0.3**	***
Silymarin 1.5 mg/kg (n=6)	**2.3**	**± 0.5**	*	**1.5**	**± 0.1**	
Single test compound						
Phloridzin 2.5 mg/kg (n=6)	**2.3**	**± 0.6**	*	**1.3**	**± 0.1**	*
Eriodictyol 2.5 mg/kg (n=6)	2.7	± 0.6		**1.3**	**± 0.1**	**
Sucralose 7.5 mg/kg (n=6)	2.4	± 0.3		1.4	± 0.1	
Sucralose 1.5 mg/kg (n=6)	**2.1**	**± 0.6**	**	1.5	± 0.2	
Menthol 1.5 mg/kg (n=6)	**2.3**	**± 0.6**	*	1.6	± 0.2	
Mannitol 7.5 mg/kg (n=6)	2.4	± 0.3		1.4	± 0.1	
Mannitol 4.5 mg/kg (n=6)	2.7	± 0.3		1.4	± 0.2	
Mannitol 1.5 mg/kg (n=6)	**2.0**	**± 0.3**	***	1.4	± 0.2	
Saccharin 1.5 mg/kg (n=3)	2.3	± 0.5		1.5	± 0.1	
Puerarin 2.5 mg/kg (n=6)	2.8	± 0.3		1.4	± 0.2	
Hesperitin 2.5 mg/kg (n=6)	3.0	± 0.5		1.5	± 0.1	
Combinations of two test compounds						
Saccharin + Mannitol 1.5 mg/kg +1.5 mg/kg (n=6)	2.7	± 0.4		1.4	± 0.2	2.7
Menthol + Mannitol 4.5 mg/kg + 4.5 mg/kg (n=6)	3.0	± 0.5		1.6	± 0.3	3.0
Menthol + Mannitol 1.5 mg/kg + 1.5 mg/kg (n=6)	2.3	± 0.6		1.5	± 0.3	2.3
Combinations of three test compounds						
Menthol + Mannitol+ Eriodictyol .5 mg/kg + .5 mg/kg + .8 mg/kg(n=6)	2.6	± 0.6		1.4	± 0.2	2.6

Data were expressed as means ±SD. Statistical difference resulted from ANOVA and LSD was denoted by words. *p<0.05, **p<0.01, ***p<0.005, as compared with HFD.

Hesperitin
Puerarin
TG: triglyceride
TC: total cholesterol

Items	**Abdominal fat weight**	**Liver weight**
Unit	g	g
Eriodictyol		
Phloridzin		
Mannitol		
Menthol		
Sucralose		
Saccharin		

[00238] Table 7-2: The analysis results of increase of body weight due to test compounds

Items	**Increase of body weight**	
Unit	g	
Blank (n=13)	15.6 ± 7.9	
HFD (n=12)	14.0 ± 8.4	
Positive Control		
Silymarin 5.0 mg/kg (n=6)	10.2 ± 12.7	
Silymarin 1.5 mg/kg (n=6)	10.9 ± 4.3	
Single test compound		
Phloridzin 2.5 mg/kg（n=6）	13.7 ± 10.7	
Eriodictyol 2.5 mg/kg（n=6）	8.3 ± 6.7	
Sucralose 7.5 mg/kg（n=6）	8.3 ± 5.4	
Sucralose 1.5 mg/kg（n=6）	17.0 ± 5.6	
Menthol 1.5 mg/kg（n=6）	19.6 ± 5.0	
Mannitol 7.5 mg/kg（n=6）	10.3 ± 8.5	
Mannitol 4.5 mg/kg（n=6）	11.1 ± 7.7	
Mannitol 1.5 mg/kg（n=6）	10.9 ± 7.4	
Saccharin 1.5 mg/kg（n=3）	**27.7 ± 12.7**	**
Puerarin 2.5 mg/kg（n=6）	**21.7 ± 3.1**	*
Hesperitin 2.5 mg/kg（n=6）	14.5 ± 8.3	

Items	**Increase of body weight**
Unit	g
Combinations of two test compounds	
Saccharin + Mannitol 1.5 mg/kg +1.5 mg/kg（n=6）	16.6 ± 6.4
Menthol + Mannitol 4.5 mg/kg + 4.5 mg/kg（n=6）	15.6 ± 5.0
Menthol + Mannitol 1.5 mg/kg + 1.5 mg/kg（n=6）	14.9 ± 6.3
Combinations of three test compounds	
Menthol + Mannitol+ Eriodictyol .5 mg/kg + .5 mg/kg + .8 mg/kg（n=6）	**21.7 ± 3.9 ***
Data were expressed as means ±SD. Statistical difference resulted from ANOVA and LSD was denoted by words. *p<0.05, **p<0.01, ***p<0.005, as compared with HFD.	
	TG: triglyceride TC: total cholesterol
Hesperitin	
Puerarin	
Eriodictyol	
Phloridzin	
Mannitol	
Menthol	
Sucralose	
Saccharin	

[00239] It was shown from the results that the weight of abdominal fat increased in animals induced with fatty liver. Among the test compounds administered separately, mannitol, menthol, and sucralose could reduce the weight of abdominal fat in animals significantly.

[00240] In addition, no abnormal condition was observed in animals of Test Group after the test compounds were administered. No animal died during the test. Occurrence of diseases or clinical symptoms caused by the test compounds was not observed from necropsy studies of sacrificial animals after the tests. Therefore, the test compounds were safe.

[00241] **6.2.2.2 The test compounds are effective in reducing lipid in liver**

[00242] Fig. 5 showed the mice that were induced to exhibit fatty liver whose liver cells near hepatic portal area (including the bile duct, portal vein, hepatic artery) were covered with many large vesicular fat droplets and hepatocellular ballooning appeared, indicating that the animal model of fatty liver was successfully established by induction.

[00243] The results of animal experiments showed that a plurality of test compounds exhibited the effects of lipid reduction in animal livers after administration for a period of 4 or 8 weeks. The results were shown in Tables 8-1 and 8-2.

[00244] Table 8-1: Test compounds could reduce liver lipids in animals (administration period of 4 weeks)

Items	**TG in liver**			**TC in liver**		
Unit	mg/g liver			mg/g liver		
Blank (n=13)	**25.0**	**± 9.2**	***	**2.5**	**± 0.4**	***
HFD (n=12)	132.0	± 69.2		6.6	± 3.5	
Positive Control						
Silymarin 5.0 mg/kg (n=6)	**46.8**	**± 14.4**	***	**3.0**	**± 0.9**	***
Silymarin 1.5 mg/kg (n=6)	**69.9**	**± 32.3**	**	**3.7**	**± 0.4**	**
Single test compound						
Phloridzin 2.5 mg/kg (n=6)	**48.9**	**± 14.1**	***	**2.9**	**± 0.5**	***
Eriodictyol 5.0 mg/kg (n=6)	**54.2**	**± 15.0**	***	**3.0**	**± 0.9**	***
Eriodictyol 2.5 mg/kg (n=6)	**43.1**	**± 13.1**	***	**3.8**	**± 1.1**	**
Sucralose 7.5 mg/kg (n=6)	**56.8**	**± 20.0**	***	5.0	± 0.9	
Sucralose 1.5 mg/kg (n=6)	**68.9**	**± 37.5**	**	**3.0**	**± 0.9**	***
Menthol 1.5 mg/kg (n=6)	**87.3**	**± 72.3**	*	**4.4**	**± 3.5**	*
Mannitol 7.5 mg/kg (n=6)	**53.8**	**± 24.4**	***	4.7	± 1.2	
Mannitol 4.5 mg/kg (n=6)	**71.5**	**± 45.5**	***	7.2	± 2.8	
Mannitol 1.5 mg/kg (n=6)	**61.8**	**± 32.6**	***	**3.4**	**± 0.6**	***
Saccharin 1.5 mg/kg (n=3)	84.0	± 41.4		**2.8**	**± 1.5**	**
Puerarin 2.5 mg/kg (n=6)	**89.4**	**± 49.1**	*	6.7	± 2.7	
Hesperitin 2.5 mg/kg (n=6)	**67.8**	**± 16.6**	***	**3.7**	**± 0.7**	**
Combinations of two test compounds						

Items	**TG in liver**			**TC in liver**	
Unit	mg/g liver			mg/g liver	
Saccharin + Mannitol 1.5 mg/kg +1.5 mg/kg (n=6)	**71.6**	**± 32.0**	***	8.5	± 2.5
Menthol + Mannitol 4.5 mg/kg + 4.5 mg/kg (n=6)	**54.3**	**± 11.8**	***		
Menthol + Mannitol 1.5 mg/kg + 1.5 mg/kg (n=6)	**31.0**	**± 11.2**	***	6.9	± 1.7
Menthol + Mannitol .5 mg/kg + .5 mg/kg (n=6)	96.6	± 77.4		5.9	± 1.7
Combinations of three test compounds					
Menthol + Mannitol+ Eriodictyol .5 mg/kg + .5 mg/kg + .8 mg/kg (n=6)	**83.1**	**± 50.9**	*	6.0	± 2.3
Data were expressed as means ±SD. Statistical difference resulted from ANOVA and LSD was denoted by words. *p<0.05, **p<0.01, ***p<0.005, as compared with HFD.					
Hesperitin Puerarin Eriodictyol Phloridzin Mannitol Menthol Sucralose Saccharin	TG: triglyceride TC: total cholesterol				

[00245] Table 8-2: Test compounds could reduce liver lipids in animals (administration period of 8 weeks)

Items	**TG in liver**			**TC in liver**		
Unit	mg/g liver			mg/g liver		
Blank (n=7)	**22.6**	**± 3.8**	***	**3.8**	**± 0.4**	***
HFD (n=8)	187.3	± 91.2		12.1	± 7.3	
Combinations of two test compounds						
Sucralose + Mannitol 7.5 mg/kg + 7.5 mg/kg (n=5)	**115.3**	**± 36.2**	*	**6.0**	**± 3.0**	**

Items	TG in liver	TC in liver
Unit	mg/g liver	mg/g liver
Sucralose + Mannitol 1.5 mg/kg + 1.5 mg/kg (n=5)	144.4 ± 59.9	**6.0 ± 1.2** *
Eriodictyol + Mannitol 5.0 mg/kg + 7.5mg/kg (n=4)	**64.5 ± 35.7** ***	**3.6 ± 1.1** ***
Eriodictyol + Sucralose 5.0 mg/kg + 7.5 mg/kg (n=6)	**41.1 ± 28.1** ***	**2.8 ± 1.0** ***
Combinations of three test compounds		
Sucralose + Mannitol + Eriodictyol 7.5 mg/kg + 7.5 mg/kg + 2.5 mg/kg (n=6)	**39.7 ± 21.5** *	**4.6 ± 0.6** ***
Data were expressed as means ±SD. Statistical difference resulted from ANOVA and LSD was denoted by words. *p<0.05, **p<0.01, ***p<0.005, as compared with HFD.		
Eriodictyol Mannitol Sucralose	TG: triglyceride TC: total cholesterol	

[00246] The results showed that TG and TC increased in liver of mice induced with fatty liver. Among the test compounds administered separately, hesperitin, puerarin, eriodictyol, phloridzin, mannitol, menthol, and sucralose could reduce TG in liver significantly. In particular, an excellent effect of about 67% reduction in liver TG content ($p<0.005$) was achieved after 4-week treatment of eriodictyol. In addition, hesperitin, eriodictyol, phloridzin, mannitol, menthol, sucralose, and saccharin could reduce TC in liver significantly. Specifically, an excellent effect of about 56% reduction in liver TC content ($p<0.005$) was achieved after 4-week treatment of saccharin.

[00247] When the combination of two test compounds was administered, the combination of saccharin and mannitol, the combination of menthol and mannitol, the combination of sucralose and mannitol, the combination of eriodictyol and mannitol, or the combination of eriodictyol and sucralose could reduce liver TG significantly. In particular, an excellent effect of about 77% reduction in liver TG content ($p<0.005$) could be achieved after 4-week treatment of the combination of menthol and mannitol; and an excellent effect of about 78% reduction in liver TG content ($p<0.005$) could be achieved after 8-week treatment of the combination of eriodictyol and sucralose. In addition, the combination of sucralose and mannitol, the combination of eriodictyol and mannitol, or the combination of eriodictyol and sucralose could reduce liver TC

content significantly, in which an excellent effect of about 77% reduction in liver TC content ($p<0.005$) could be achieved after 8-week treatment of the combination of eriodictyol and sucralose.

[00248] When the combination of three test compounds was administered, the combination of menthol, mannitol, and eriodictyol or the combination of sucralose, mannitol, and eriodictyol could reduce liver TG significantly. In particular, an excellent effect of about 79% reduction in liver TG content ($p<0.005$) could be achieved after 8-week treatment of the combination of sucralose, mannitol, and eriodictyol. In addition, the combination of sucralose, mannitol, and eriodictyol could reduce liver TC significantly.

[00249] **6.2.2.3 The test compounds are effective in reducing liver damage**

[00250] **6.2.2.3.1 Effects of reduction in liver fat and liver damage of liver tissue**

[00251] The results of animal experiments showed that a plurality of test compounds exhibited the efficacies of liver fat and liver tissue damage reduction during the test period of 4 weeks. Fig. 5 showed liver tissue damage of animals having fatty liver. The liver tissue damage included many large vesicular fat droplets covering liver cells near hepatic portal area (including the bile duct, portal vein, hepatic artery) and hepatocellular ballooning. By comparison, after being treated by silymarin, menthol, eriodictyol, or mannitol for 4 weeks, large vesicular fat droplets within liver cells in liver tissue section were significantly reduced. A portion of small broken droplets was still observed in mice treated with silymarin, but the liver tissue type of mice treated with menthol, eriodictyol, or mannitol was close to that of animals in Blank group, indicating mild fatty liver diseases. Furthermore, the result of NAS scoring was shown in Table 9.

[00252] Table 9: The test compounds could reduce the condition of liver damage in animals

Items	**NAS**		
Unit	mg/g liver		
Blank (n=13)	**0.7**	**± 0.5**	***
HFD (n=12)	3.3	± 1.7	
Positive Control			
Silymarin 5.0 mg/kg (n=6)	**0.8**	**± 0.4**	***

Items	NAS		
Unit	mg/g liver		
Silymarin 1.5 mg/kg (n=6)	**1.5**	**± 0.8**	*
Single test compound			
Phloridzin 2.5 mg/kg (n=6)	1.8	± 1.0	
Eriodictyol 5.0 mg/kg (n=6)			
Eriodictyol 2.5 mg/kg (n=6)	**1.5**	**± 0.8**	*
Eriodictyol 7.5 mg/kg (n=6)	1.8	± 1.1	
Eriodictyol 1.5 mg/kg (n=6)	1.8	± 2.0	
Menthol 1.5 mg/kg (n=6)	1.8	± 1.6	
Mannitol 7.5 mg/kg (n=6)	**1.7**	**± 0.8**	*
Mannitol 4.5 mg/kg (n=6)	2.7	± 1.9	
Mannitol 1.5 mg/kg (n=6)	**1.3**	**± 0.8**	*
Saccharin 1.5 mg/kg (n=3)			
Puerarin 2.5 mg/kg (n=6)			
Hesperitin 2.5 mg/kg (n=6)	1.7	± 0.5	
Combinations of two test compounds			
Saccharin + Mannitol 1.5 mg/kg +1.5 mg/kg (n=6)			
Menthol + Mannitol 4.5 mg/kg + 4.5 mg/kg (n=6)	2.2	± 1.5	
Menthol + Mannitol **1.5 mg/kg + 1.5 mg/kg** (n=6)	**0.7**	**± 0.5**	***
Menthol + Mannitol .5 mg/kg + .5 mg/kg (n=6)	2.5	± 1.8	
Combinations of three test compounds			
Menthol + Mannitol+ Eriodictyol **.5 mg/kg + .5 mg/kg + .8 mg/kg** (n=6)	**2.0**	**± 1.4**	

Data were expressed as means ±SD. Statistical difference resulted from ANOVA and LSD was denoted by words. *p<0.05, **p<0.01, ***p<0.005, as compared with HFD.

Hesperitin

Puerarin

Eriodictyol

Items	NAS
Unit	mg/g liver
Phloridzin	
Mannitol	
Menthol	
Sucralose	
Saccharin	

[00253] NAS (Nonalcoholic Fatty Liver Disease Activity Score) indicated the activity score of non-alcoholic fatty liver diseases [Hepatology. 2005 Jun;41(6):1313-21], and comprehensively evaluated the degree of steatosis, lobular inflammation, and hepatocyte ballooning. The score sheet was shown in Table 10. Higher score indicated severer liver damage.

Table 10: NAS Evaluation Project

Items	Score	Degree	Definition and Description
Steatosis	0	<5%	Refers to amount of surface area involved by steatosis as evaluated on low to medium power examination; minimal steatosis (,5%) receives a score of 0 to avoid giving excess weight to biopsies with very little fatty change
	1	5-33%	
	2	>33-66%	
	3	>66%	
Lobular inflammation	0	No foci	Acidophil bodies are not included in this assessment, nor is portal inflammation
	1	<2 foci /200x	
	2	2-4 foci /200x	
	3	>4 foci /200x	
Hepatocyte ballooning	0	None	
	1	few balloon cells	The term "few" means rare but definite ballooned hepatocytes as well as cases that are diagnostically borderline.
	2	Many cells /prominent ballooning	Most cases with prominent ballooning also had mallory's hyaline, but Mallory's hyaline is not scored separately for the NAS.

[00254] The results showed that liver tissue damage occurred in mice induced with fatty liver (NAS increasing). Among the test compounds administered separately,

eriodictyol and mannitol could reduce liver damage significantly. It is notable that when the combination of two compounds was administered, the combination of menthol and mannitol achieved an excellent effect. There was hardly any liver damage appearing. The NAS was the same with that of the Blank.

[00255] 6.2.2.3.2 Effects of reduction in liver dysfunction

[00256] The results of animal experiments showed that a plurality of test compounds exhibited the efficacies of liver dysfunction reduction in animals during administration period of 4 or 8 weeks. The results were showed in Table 11-1 and Table 11-2.

[00257] Table 11-1: Test compounds could reduce liver dysfunction in animals (administration period of 4 weeks)

Items	**ALT**			**AST**		
Unit	U/L			U/L		
Blank (n=13)	**32.6**	**± 16.1**	***	**112.2**	**± 53.9**	***
HFD (n=12)	70.1	± 45.2		156.8	± 100.8	
Positive Control						
Silymarin 5.0 mg/kg (n=6)	**33.9**	**± 9.3**	***	**168.1**	**± 42.6**	
Silymarin 1.5 mg/kg (n=6)	**43.8**	**± 18.7**	*	**153.6**	**± 62.5**	
Single test compound						
Mannitol 7.5 mg/kg (n=6)	**25.0**	**± 10.8**	***	**63.3**	± 7.7	***
Mannitol 4.5 mg/kg (n=6)	**44.5**	**± 15.9**	*	107.6	± 54.3	
Mannitol 1.5 mg/kg (n=6)	**40.8**	**± 11.4**	*	187.2	± 142.1	
Sucralose 7.5 mg/kg (n=6)	**32.3**	**± 10.1**	**	**74.3**	**± 18.6**	**
Sucralose 1.5 mg/kg (n=6)	**30.9**	**± 16.8**	***	127.0	± 31.2	
Eriodictyol 5.0 mg/kg (n=5)	**41.4**	**± 6.3**	*	161.4	± 42.3	
Eriodictyol 2.5 mg/kg (n=6)	**33.7**	**± 18.5**	***	100.9	± 42.0	
Puerarin 2.5 mg/kg (n=6)	**34.4**	**± 14.7**	***	**66.9**	**± 8.5**	***
Phloridzin 2.5 mg/kg (n=6)	**35.7**	**± 9.1**	***	161.9	± 96.2	
Hesperitin 2.5 mg/kg (n=6)	**36.8**	**± 22.1**	**	**72.4**	**± 11.2**	***
Menthol 1.5 mg/kg (n=6)	**41.5**	**± 13.7**	*	129.9	± 37.1	
Saccharin 1.5 mg/kg (n=3)	50.7	± 29.7		170.4	± 28.6	

Items	ALT			AST		
Unit	U/L			U/L		
Combinations of two test compounds						
Menthol + Mannitol .5 mg/kg + .5 mg/kg (n=6)	**23.9**	**± 17.8**	***	**60.4**	**± 8.2**	***
Menthol + Mannitol 1.5 mg/kg + 1.5 mg/kg (n=6)	**16.7**	**± 4.3**	***	**59.8**	± 7.5	***
Sucralose + Mannitol 7.5 mg/kg +7.5 mg/kg (n=6)	**45.5**	**± 15.2**		**91.4**	**± 21.8**	*
Sucralose + Mannitol 1.5 mg/kg +1.5 mg/kg (n=6)	52.4	± 34.0		**92.1**	**± 23.0**	*
Eriodictyol + Mannitol 5.0mg/kg + 7.5mg/kg (n=4)	43.4	± 10.5		151.0	± 54.2	
Eriodictyol + Sucralose 5.0mg/kg + 7.5mg/kg (n=4)	**38.2**	**± 10.9**	*	143.8	± 67.6	
Saccharin + Mannitol 1.5 mg/kg +1.5 mg/kg (n=6)	51.7	± 54.2		**70.0**	**± 27.6**	***
Combinations of three test compounds						
Menthol + Mannitol + Eriodictyol .5 mg/kg + .5 mg/kg + .8 mg/kg (n=6)	**21.2**	**± 8.7**	***	**54.8**	**± 13.2**	***

Data were expressed as means ±SD. Statistical difference resulted from ANOVA and LSD was denoted by words. *p<0.05, **p<0.01, ***p<0.005, as compared with HFD.

Hesperitin
Puerarin
Hesperitin
Puerarin
Eriodictyol
Phloridzin
Mannitol
Menthol
Sucralose
Saccharin

ALT: alanine aminotransferase
AST: aspartate transaminase

[00258] Table 11-2: Test compounds could reduce liver dysfunction in animals (administration period of 8 weeks)

Items	ALT			AST		
Unit	U/L			U/L		
Blank (n=7)	**65.1**	**± 21.5**	***	**22.6**	**± 4.3**	***
HFD (n=8)	**111.0**	**± 26.2**		**109.4**	**± 46.4**	
Combinations of two test compounds						
Sucralose + Mannitol **7.5 mg/kg +7.5 mg/kg** (n=5)	**92.4**	**± 16.5**		**49.5**	**± 14.4**	***
Sucralose+ Mannitol 1.5 mg/kg +1.5 mg/kg (n=4)	112.5	± 23.8		93.0	± 26.0	
Combinations of three test compounds						
Sucralose + Mannitol + Eriodictyol 7.5 mg/kg + 7.5 mg/kg + 2.5 mg/kg (n=6)				**40.0**	**± 12.2**	***
Data were expressed as means ±SD. Statistical difference resulted from ANOVA and LSD was denoted by words. *p<0.05, **p<0.01, ***p<0.005, as compared with HFD.						
Mannitol Sucralose	ALT: alanine aminotransferase AST: aspartate transaminase					

[00259] ALT and AST are most commonly used as enzyme indicators to reflect the biochemical dysfunction of liver. Under normal circumstances, these enzymes present in liver cells. However, when liver cells are damaged, they will leak. Increases of serum ALT and AST values generally reflect liver inflammation and liver dysfunction.

[00260] The results showed that animals induced with fatty liver (ALT and AST values increasing) suffered from liver dysfunction. Among the test compounds administered separately, all the hesperitin, puerarin, eriodictyol, phloridzin, mannitol, menthol, sucralose, and saccharin could reduce ALT and AST values significantly. In particular, excellent effects of about 64% reduction in ALT value ($p<0.005$) and about 60% reduction in AST value ($p<0.005$) could be achieved after 4-week treatment of mannitol.

[00261] When the combination of two test compounds was administered, both the combination of menthol and mannitol, and the combination of eriodictyol and

sucralose could reduce ALT value significantly. Also, the combination of menthol and mannitol, the combination of sucralose and mannitol, or the combination of saccharin and mannitol could reduce AST value significantly. In particular, excellent effects of about 76% reduction in ALT value ($p<0.005$) and about 62% reduction in AST value ($p<0.005$) could be achieved after 4-week treatment of the combination of menthol and mannitol.

[00262] When the combination of three test compounds was administered, the combination of sucralose, mannitol, and eriodictyol could reduce ALT value significantly ($p<0.005$).

[00263] **6.2.2.4 The test compounds can improve liver antioxidant activity**

[00264] The results of animal experiments showed that a plurality of test compounds exhibited the efficacies of liver antioxidant activity improvement in animals during the test period of 4 weeks. The results were showed in Table 12-1 and Table 12-2.

[00265] Table 12-1: Test compounds could improve liver antioxidant activity in animals (Gpx and GSH)

Items	**Gpx**		**GSH**	
Unit	U/L		U/L	
Blank (n=10)	**2588.0 ± 524.5**		**1224.1 ± 95.5**	
HFD (n=8)	2252.5 ± 395.2		1193.0 ± 203.8	
Positive Control				
Silymarin 5.0 mg/kg (n=6)	**3358.3 ± 1205.3**	***	**1398.8 ± 396.5**	
Single test compound				
Mannitol 7.5 mg/kg (n=6)	**3738.3 ± 665.1**	***	**2147.7 ± 459.1**	***
Mannitol 4.5 mg/kg (n=6)	**3423.3 ± 547.8**	***	**1605.1 ± 305.9**	**
Mannitol 1.5 mg/kg (n=6)	2580.0 ± 555.2		**1502.5 ± 276.9**	*
Puerarin 2.5 mg/kg (n=6)	**3581.7 ± 1056.7**	***	**1498.1 ± 150.0**	*
Sucralose 7.5 mg/kg (n=6)	**3334.0 ± 377.7**	**	**1609.1 ± 201.1**	**
Sucralose 1.5 mg/kg (n=6)	**2995.0 ± 651.1**	*	1448.0 ± 281.5	
Phloridzin 2.5 mg/kg (n=6)	**3234.0 ± 505.1**	**	1387.7 ± 168.2	
Hesperitin 2.5 mg/kg (n=6)	**3133.3 ± 376.9**	*	**1742.6 ± 241.5**	***
Eriodictyol 2.5 mg/kg (n=6)	**3083.3 ± 378.9**	**	1302.0 ± 241.1	

Items	**Gpx**	**GSH**
Unit	U/L	U/L
Menthol 1.5 mg/kg（n=6）	2921.7 ± 640.2	1432.7 ± 104.0
Data were expressed as means ±SD. Statistical difference resulted from ANOVA and LSD was denoted by words. *p<0.05, **p<0.01, ***p<0.005, as compared with HFD.		
Hesperitin		
Puerarin	Gpx: glutathione peroxidase	
Hesperitin	GSH: glutathione	
Puerarin		
Eriodictyol		
Phloridzin		
Mannitol		
Menthol		
Sucralose		

[00266] Table 12-2: Test compounds could improve liver antioxidant activity in animals (Grd and SOD)

Items	Grd		SOD	
Unit	U/L		U/L	
Blank (n=10)	**123.5**	**± 30.9**	**380.3**	**± 38.8**
HFD (n=8)	**82.1**	**± 21.7**	**371.7**	**± 49.3**
Positive Control				
Silymarin 5.0 mg/kg（n=6）	**88.9**	**± 29.2**	**435.9**	**± 59.2 ***
Single test compound				
Mannitol 7.5 mg/kg（n=6）	**117.6**	**± 32.0 ****	**462.8**	**± 52.8**
Mannitol 4.5 mg/kg（n=6）	**110.1**	**± 18.4 ***	**429.2**	**± 85.2**
Mannitol 1.5 mg/kg（n=6）	**95.3**	**± 22.1**	**367.3**	**± 35.6**
Puerarin 2.5 mg/kg（n=6）	**99.0**	**± 17.2**	**434.5**	**± 59.8**
Sucralose 7.5 mg/kg（n=6）	**90.4**	**± 17.2**	**399.0**	**± 34.5**
Sucralose 1.5 mg/kg（n=6）	**100.0**	**± 18.6**	**373.0**	**± 50.4**
Phloridzin 2.5 mg/kg（n=6）	**82.2**	**± 33.6**	**411.5**	**± 87.5**

Items	Grd		SOD	
Unit	U/L		U/L	
Hesperitin 2.5 mg/kg (n=6)	**102.5**	**± 28.3**	**408.3**	**± 66.7**
Eriodictyol 2.5 mg/kg (n=6)	**86.9**	**± 15.7**	**385.9**	**± 34.0**
Menthol 1.5 mg/kg (n=6)	**95.2**	**± 16.2**	**427.9**	**± 41.9**

Data were expressed as means ±SD. Statistical difference resulted from ANOVA and LSD was denoted by words. *p<0.05, **p<0.01, ***p<0.005, as compared with HFD.

Hesperitin
Puerarin
Hesperitin
Puerarin
Eriodictyol
Phloridzin
Mannitol
Menthol
Sucralose

Grd: Glutathione reductase
SOD: Superoxide dismutase

[00267] Gpx, GSH, Grd and SOD are common members of liver antioxidant systems that can reduce oxidative stress in the liver and prevent liver from damage caused by oxidative stress. Increases of Gpx, GSH, Grd and SOD values indicate liver maintaining better antioxidant activity.

[00268] The results showed that the antioxidant activity of mice induced with fatty liver was reduced. Among the test compounds administered separately, all the hesperitin, puerarin, eriodictyol, phloridzin, mannitol, and sucralose could improve antioxidant activity significantly. In particular, excellent effects of substantial increases in Gpx, GSH, Grd, and SOD levels ($p<0.005$) were achieved after 4-week treatment of mannitol.

[00269] In summary, the compounds as tested including mannitol and sucralose and others can reduce fat content in the liver, reduce liver damage, and improve liver antioxidant activity. These compounds had been confirmed safe through animal experiments and found having potential to be developed into health food or drugs for reducing liver fat and ameliorating associated disorders, such as fatty liver diseases, acute and chronic alcoholic fatty liver diseases, acute and chronic non-alcoholic fatty

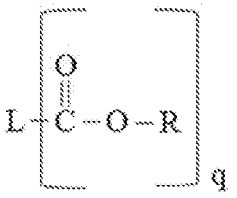
liver diseases (NAFLD), acute and chronic alcoholic hepatitis, acute and chronic non-alcoholic steatohepatitis, non-alcoholic cirrhosis, and alcoholic cirrhosis (ICD-9-CM diagnosis Codes: 571.8, 571.0, 571.1, 571.2, 571.3, 571.4, 571.5, 571.9).

5

CLAIMS

What is claimed is:

1. A compound which is represented by Formula (I),



Formula (I),

wherein

L is a saturated or unsaturated aliphatic group;

R is selected from the group consisting of hydrogen, a polyol group and a saccharide group of $(G)_p$ wherein G is a monosaccharide residue and p is an integer from 1 to 100 wherein at least one of the hydroxyl groups in $(G)_p$ is substituted by a halogen atom; and

q is an integer from 2 to 4, and each of R is the same or different,

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein L is an alkyl group having 1 to 40 carbon atoms.

3. The compound of claim 1, wherein L is selected form the group consisting of a branched-chain alkyl group, a straight-chained alkyl group substituted with a benzene ring, a branched-chain alkyl group substituted with a benzene ring, a benzenyl group substituted with a straight-chained aliphatic group, and a benzenyl group substituted with a branched-chain aliphatic group.

4. The compound of claim 1, wherein the polyol group is linear or circular, substituted or unsubstituted.

5. The compound of claim 1, wherein the monosaccharide residue is a hexose.

6. A compound which is represented by Formula (II),

$$R_1\text{-}O\text{-}X\text{-}(CH_2)_m\text{-}X\text{-}O\text{-}R_2 \quad \text{Formula (II),}$$

wherein

X is C=O;

R_1 and R_2 are the same or different, selected from the group consisting of hydrogen, a polyol group and a saccharide group of $(G)_p$, wherein G is a monosaccharide residue and p is an integer from 1 to 100 wherein at least one of the hydroxyl groups in $(G)_p$ is substituted by a halogen atom, wherein when R_1 is hydrogen, then R_2 is not hydrogen; and

m is an integer from 1 to 40.

or a pharmaceutically acceptable salt thereof.

7. The compound of claim 6, wherein the polyol group is $-CH(CHOH)_nCH_2OH$, wherein n is an integer from 1 to 18.

8. The compound of claim 6, wherein two or more of the hydroxyl groups in $(G)_p$ are substituted by halogen atoms.

9. The compound of claim 6, wherein the monosaccharide residue is a hexose.

10. The compound of claim 9, wherein the hexose is selected from the group consisting of an aldohexose and a ketohexose.

11. The compound of claim 6, wherein the saccharide group R_1 or R_2 is represented by $-G_1-O-G_2$, wherein G_1 and G_2 are the same or different, selected from the group consisting of an aldohexose and a ketohexose, and at least one of the hydroxyl groups in G_1 or at least one of the hydroxyl groups in G_2 is substituted by a halogen atom.

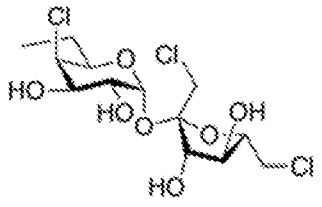
12. The compound of claim 11, the halogen atom is selected from the group consisting of chlorine, bromine and iodine.

13. The compound of claim 12 wherein the halogen atom is chlorine.

14. The compound of claim 11, wherein G_1 is glucose wherein one of the hydroxyl groups is substituted by chlorine; and G_2 is fructose wherein two of the

hydroxyl groups are substituted by chlorine.

15. The compound of claim 11, wherein R_1 or R_2 is represented by formula (Ia)

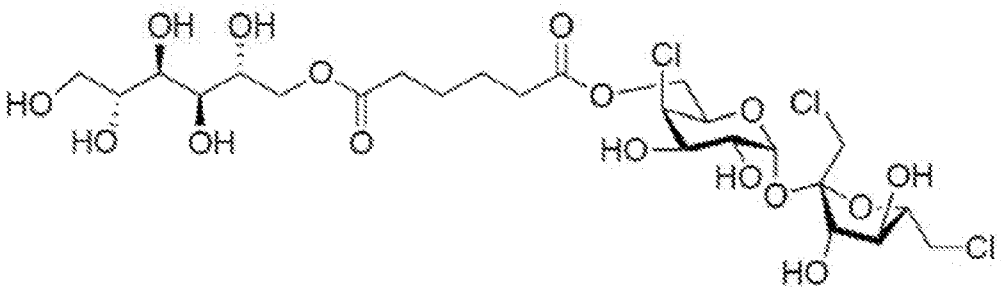


formula (Ia).

16. The compound of claim 7, wherein m and n are 4.

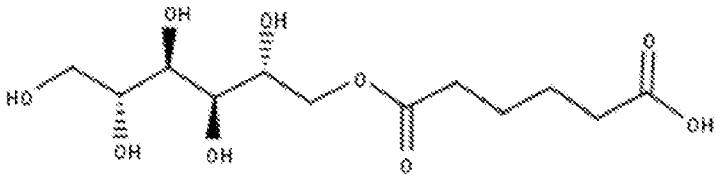
17. The compound of claim 1, which is selected from the group consisting of:

((2R,3R,4R,5R,6R)-6-(((2R,5R)-2,5-bis(chloromethyl)-3,4-dihydroxytetrahydrofuran-2-yl)oxy)-3-chloro-4,5-dihydroxytetrahydro-2H-pyran-2-yl)methyl ((2R,3R,4R)-2,3,4,5,6-pentahydroxyhexyl) adipate of Formula 1



Formula 1.

18. The compound of claim 1, which is C6-mannitol of Formula 2



Formula 2.

19. A compound which is represented by Formula C,

Formula C

wherein Ph is phenyl and Bn is benzyl.

20. A pharmaceutical composition comprising a compound of claim 1 or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier.

21. A method of for **preventing or treating a disease or condition** in a subject in need thereof, comprising administering to the subject an effective amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof.

22. The method of claim 21, wherein the compound of claim 1 or a pharmaceutically acceptable salt thereof is administered in combination with one or more additional agents selected from the group consisting of:

(i) a first active agent selected from the group consisting of: polyethylene glycol sorbitan monolaurate (Tween 20), microcrystalline cellulose, dicalcium phosphate dihydrate, Brij 35, saccharin, mannitol, Cremophor RH40, sucralose, crospovidone, sodium starch glycolate, Eudragit S100, croscarmellose sodium, Pluronic F68, menthol, low-substituted hydroxypropyl cellulose, pregelatinized starch, Dextrates NF hydrated, citric acid, Cremophor EL, Aerosil 200, Myrj 52, sorbic acid, lemon oil, hydroxypropyl cellulose, Sorbitol, acesulfame potassium, hydroxypropyl methylcellulose, lactose monohydrate, maltodextrin, Brij 58, Brij 76, Tween 80, Tween 40, PEG 400, PEG 4000, PEG 8000, Span 60, sodium benzoate, hydroxy ethylmethylcellulose, methylcellulose, Span 80, sodium cyclamate, glyceryl behenate, oxide red, glycerin monostearate, Copovidone K28, starch acetate, magnesium stearate, sodium lauryl sulfate, Providone K30, PEG 2000, and N-acetylcysteine (NAC) and any combination thereof;

(ii) a second active agent selected from the group consisting of: sodium lauryl sulfate, menthol, sucralose, mannitol, sorbitol, saccharin, glycerin, sodium benzoate, oxide red, pregelatinized starch, sodium cyclamate, sorbic acid, lemon oil, citric acid, butylated hydroxyanisole, poncirin, isovitexin, eriodictyol, ergosterol, β-myrcene,

hyperoside, (+)-catechin, galangin, morin, sciadopitysin, didymin, gossypin, luteolin-7-glucoside, (+)-taxifolin, trans-cinnamic acid, diosmin, linarin, xylitol, luteolin, swertiamarin, puerarin, phloridzin, sinensetin, (-)-epigallocatechin, kaempferol, ursolic acid, silymarin, (+)-limonene, hesperidin, (-)-epicatechin-3-gallate, silybin, formononetin, myristic acid ethyl ester, eicosapentaenoic acid (EPA), wongonin, povidone K-30, protocatechuic acid, umbelliferone, hesperitin, nordihydroguaiaretic acid, neohesperidin, naringin, (-)-epicatechin, glycyrrhizin, baicalin, quercitrin, baicalein and any combinations thereof; and
any combination of (i) and (ii).

23. The method of claim 22, wherein the one or more additional agents are selected from the group consisting of dicalcium phosphate dehydrate, menthol, mannitol, sucralose, N-acetylcysteine (NAC) and any combination thereof.

24. The method of claim 22, wherein the one or more additional agents are selected from the group consisting of (1) a combination of saccharin and mannitol, (2) a combination of menthol and mannitol, (3) a combination of sucralose and mannitol, (4) a combination of eriodictyol and mannitol, (5) a combination of eriodictyol and sucralose, (6) a combination of menthol, mannitol, and eriodictyol, and (7) a combination of sucralose, mannitol, and eriodictyol.

25. The method of claim 22, wherein the compound of claim 1 or a pharmaceutically acceptable salt thereof and the one or more additional agents are administered simultaneously or sequentially.

26. A method of for preventing or treating a disease or condition characterized by increased cytochrome P450 activities or increased free radical level in a subject in need thereof, comprising administering to the subject an effective amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof.

27. The method of claim 26, wherein the compound of claim 1 or a pharmaceutically acceptable salt thereof is administered in combination with one or more of the additional agent as defined in claim 20.

28. A method for preventing or treating organ injuries in a subject in need, comprising administering to the subject an effective amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof.

29. The method of claim 28, wherein the organ injuries are in liver or kidney.

30. The method of claim 28 wherein the organ injuries are caused by a therapeutic drug, CCl_4 or lipid.

31. The method of claim 30, wherein the therapeutic drug is acetaminophen.

32. The method of claim 28, wherein the compound of claim 1 or a pharmaceutically acceptable salt thereof is administered in combination with one or more of the additional agent as defined in claim 20.

33. A method for preventing or treating hepatotoxicity in a subject in need, comprising administering to the subject an effective amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof.

34. The method of claim 33, wherein the hepatotoxicity is caused by a therapeutic drug, CCl_4 or lipid.

35. The method of claim 33, wherein the therapeutic drug is acetaminophen.

36. The method of claim 33, wherein the compound of claim 1 or a pharmaceutically acceptable salt thereof is administered in combination with one or more of the additional agent as defined in claim 20.

37. A method for preventing or treating fatty liver, protecting liver function or ameliorating liver diseases caused by fatty liver or other associated disorders, comprising administering to the subject an effective amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof.

38. The method of claim 37, wherein the compound of claim 1 or a

pharmaceutically acceptable salt thereof is administered in combination with one or more of the additional agent as defined in claim 20.

39. Use of a compound of claim 1 or a pharmaceutically acceptable salt thereof for manufacturing a medicament for preventing or treating a disease or condition characterized by increased cytochrome P450 activities or increased free radical level.

40. Use of a compound of claim 1 or a pharmaceutically acceptable salt thereof for manufacturing a medicament for preventing or treating organ injuries.

41. Use of a compound of claim 1 or a pharmaceutically acceptable salt thereof for manufacturing a medicament for preventing or treating hepatotoxicity.

42. Use of a compound of claim 1 or a pharmaceutically acceptable salt thereof for manufacturing a medicament for preventing or treating fatty liver, protecting liver function or ameliorating liver diseases caused by fatty liver or other associated disorders.

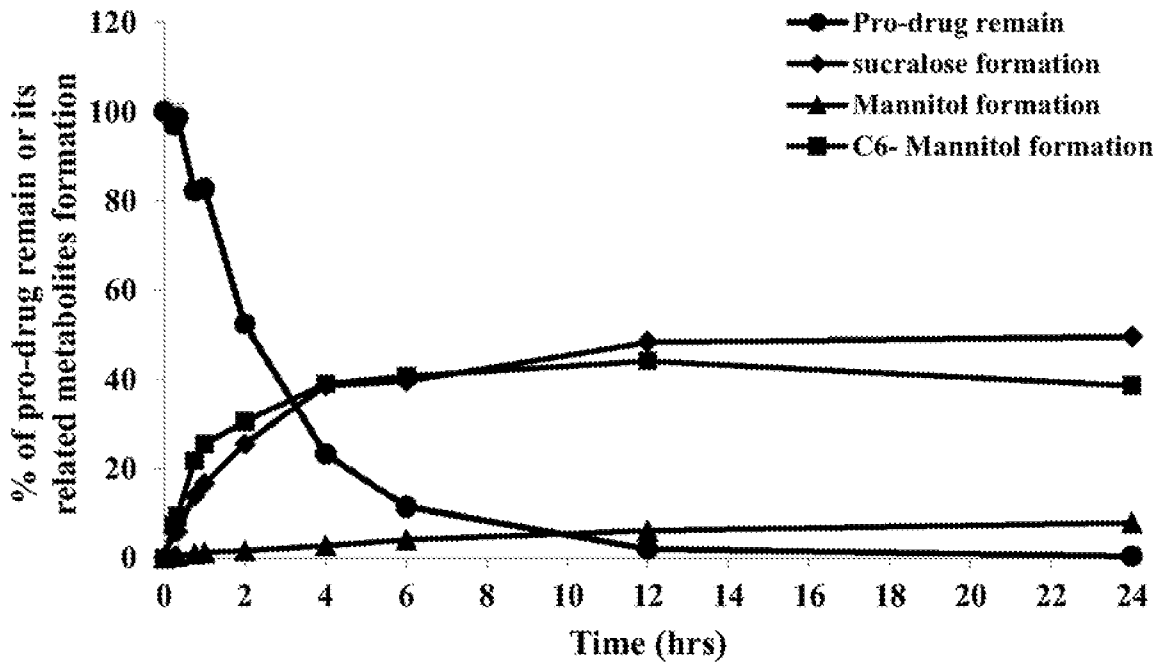

Pro-drug remain
sucralose formation
Mannitol formation
C6- Mannitol formation
% of pro-drug remain or its related metabolites formation
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (hrs)

Fig. 1

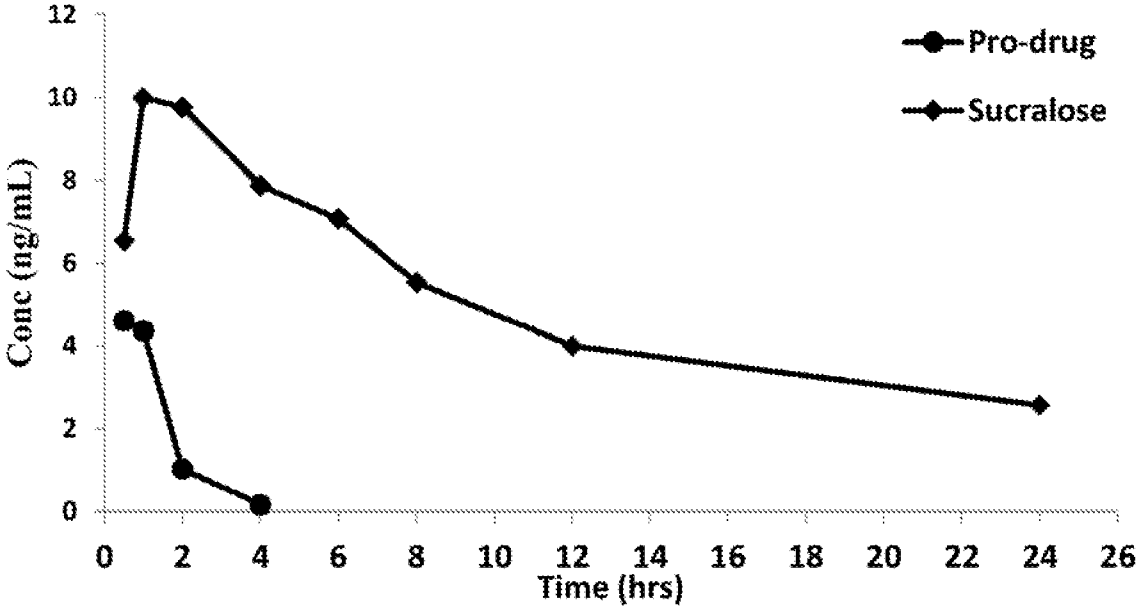

Pro-drug
Sucralose
Conc (ng/mL)
Time (hrs)
0
2
4
6
8
10
12
14
16
18
20
22
24
26

Fig. 2

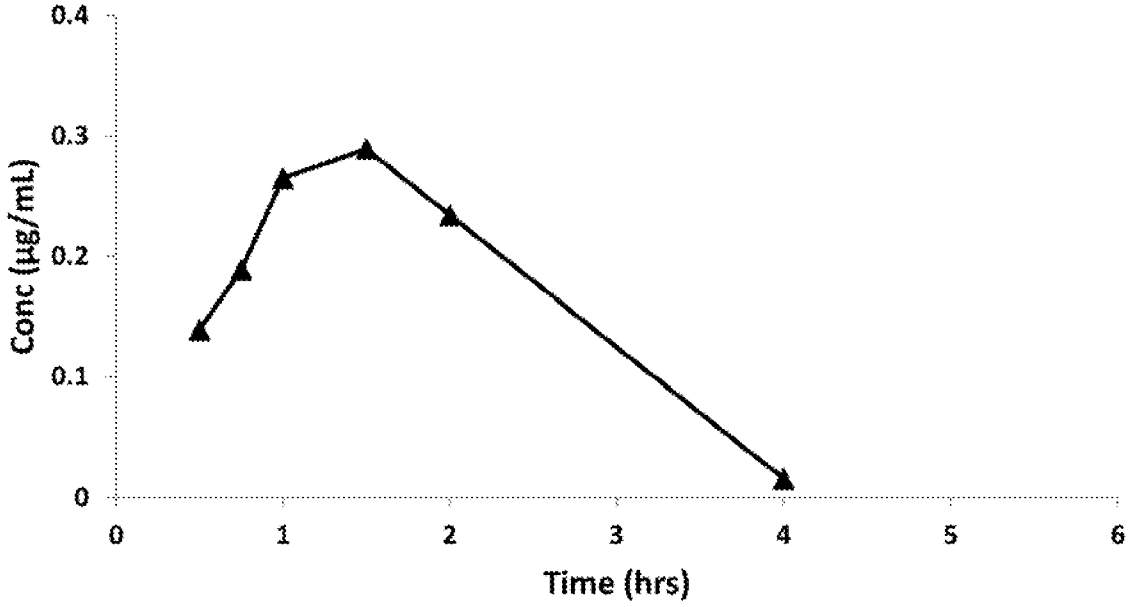


Fig. 3

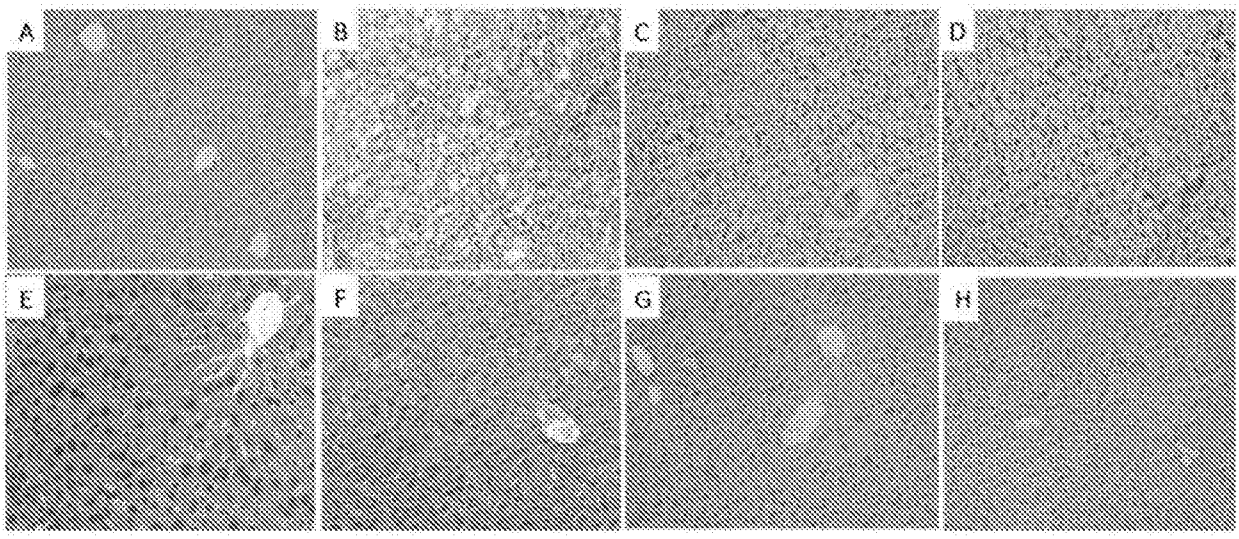


Fig. 4

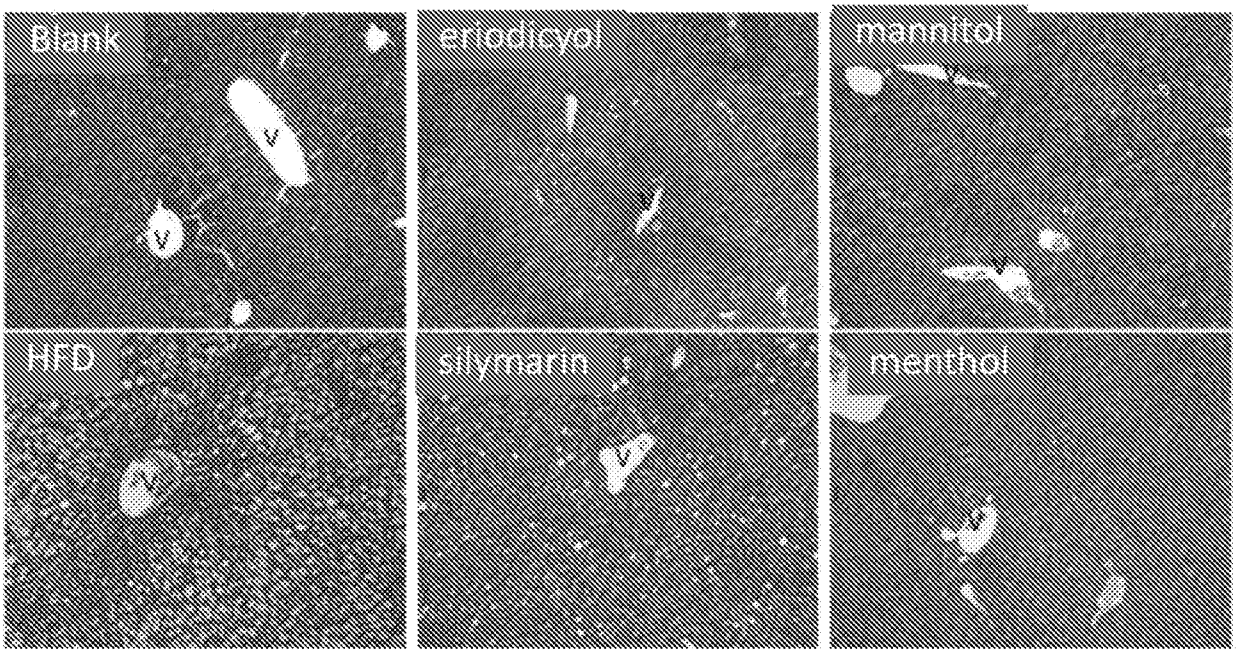


Fig. 5

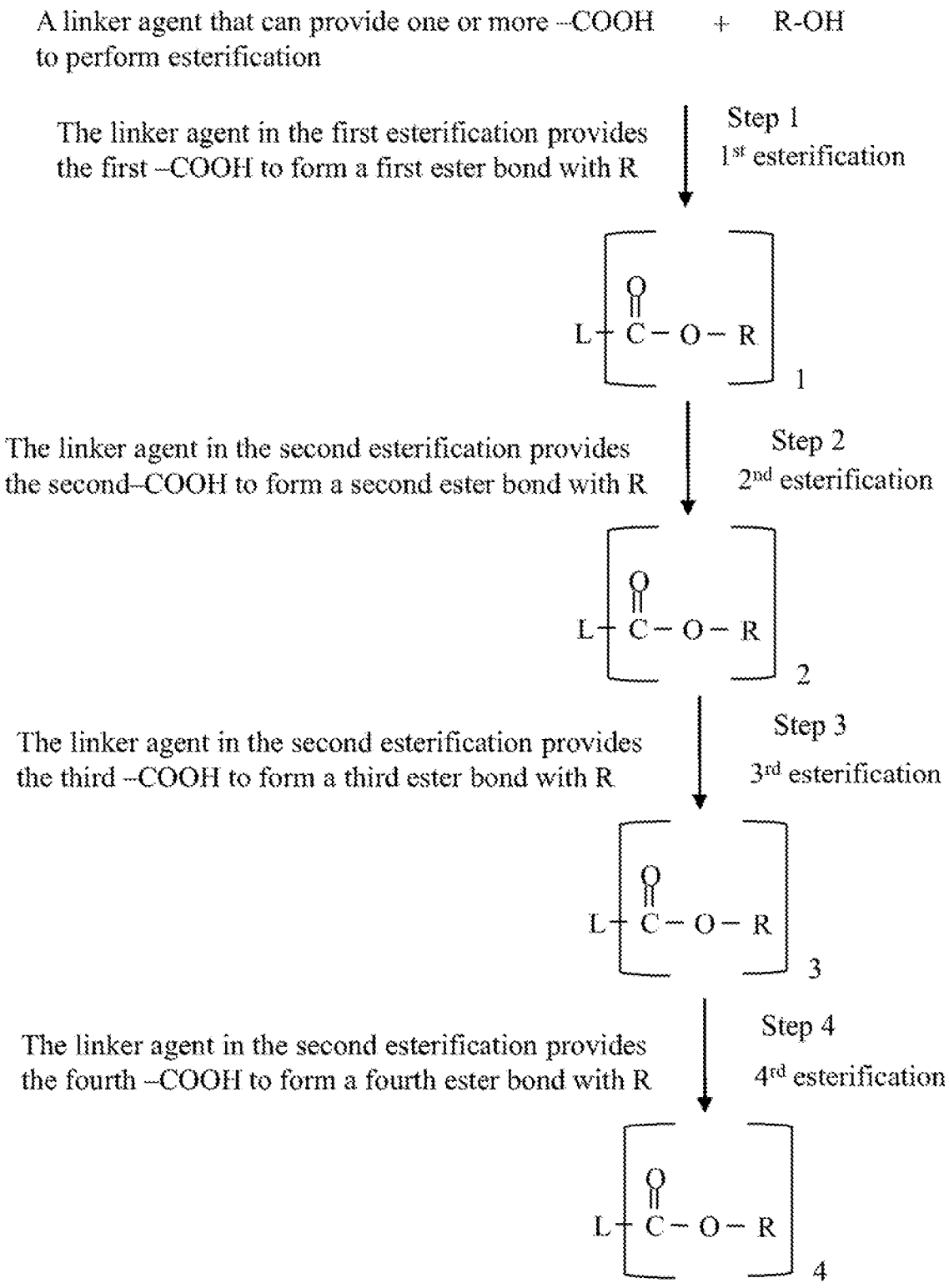

A linker agent that can provide one or more –COOH to perform esterification
+ R-OH
The linker agent in the first esterification provides the first –COOH to form a first ester bond with R
Step 1
1st esterification
L–C(=O)–O–R
1
The linker agent in the second esterification provides the second–COOH to form a second ester bond with R
Step 2
2nd esterification
L–C(=O)–O–R
2
The linker agent in the second esterification provides the third –COOH to form a third ester bond with R
Step 3
3rd esterification
L–C(=O)–O–R
3
The linker agent in the second esterification provides the fourth –COOH to form a fourth ester bond with R
Step 4
4rd esterification
L–C(=O)–O–R
4

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/100187

A. CLASSIFICATION OF SUBJECT MATTER

C07C 51/215(2006.01)i; C07D 319/06(2006.01)i; C07D 407/12(2006.01)i; A61K 31/191(2006.01)i; A61K 31/357(2006.01)i; A61K 31/351(2006.01)i; A61P 1/16(2006.01)i; A61P 13/12(2006.01)i; A61P 39/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07C51; C07D319; C07D407; A61K31; A61P1; A61P13; A61P39

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DWPI, SIPOABS, CNABS, CPRSABS, CNKI, REGISTRY, CAPLUS (STN), hepatotoxicity, liver, kindney, polyol, saccharide, hexose, structure searching according to the compounds in claim 1, 6, 17, 18 and 19

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Mahcamov, R. R.et al. "Synthesis of hydroxyl-containing esters of hexylenesuccinic acid and their effect on surface tension at a mercury-solution interface" *Uzbekskii Khimicheskii Zhurnal,* Vol. 3-4, 31 December 1992 (1992-12-31), pages 27-30	1-14
X	Rohrdanz, D. et al. "Structure of esters of sorbitol with succinic acid" *Deutsche Lebensmittel-Rundschau,* Vol. 79, No. 9, 31 December 1983 (1983-12-31), pages 285-289	1、2、4-14
A	Mahcamov, R. R.et al. "Synthesis of hydroxyl-containing esters of hexylenesuccinic acid and their effect on surface tension at a mercury-solution interface" *Uzbekskii Khimicheskii Zhurnal,* Vol. 3-4, 31 December 1992 (1992-12-31), pages 27-30	15-20, 39-42
A	Rohrdanz, D. et al. "Structure of esters of sorbitol with succinic acid" *Deutsche Lebensmittel-Rundschau,* Vol. 79, No. 9, 31 December 1983 (1983-12-31), pages 285-289	3, 15-20, 39-42
A	CN 1939929 A (BAIYAO GROUP CO LTD) 04 April 2007 (2007-04-04) the whole document	1-20、39-42

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 December 2016	**03 January 2017**

Name and mailing address of the ISA/CN	Authorized officer
STATE INTELLECTUAL PROPERTY OFFICE OF THE P.R.CHINA **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China**	**CHANG,Xiaoyu**
Facsimile No. **(86-10)62019451**	Telephone No. **(86-10)62084213**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/100187

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2006010083 A2 (ERATHODIYIL N ET AL.) 26 January 2006 (2006-01-26) the whole document	1-20、39-42

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/100187

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

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

1. ☑ Claims Nos.: **21-38**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Claims 21-38 relate to the methods of diseases prevention or treatment, which do not comply with the requirement of PCT rule 39.

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

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

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2016/100187

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
CN 1939929 A	04 April 2007	None	
WO 2006010083 A2	26 January 2006	US 2007009441 A1	11 January 2007
		WO 2006010083 A3	30 April 2009

(19)中华人民共和国国家知识产权局

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(43)申请公布日 2018.01.19

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PCT/CN2016/078039 2016.03.31 CN

(85)PCT国际申请进入国家阶段日
2017.09.30

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(87)PCT国际申请的公布数据
WO2017/050298 EN 2017.03.30

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(74)专利代理机构 北京戈程知识产权代理有限公司 11314
代理人 程伟

(51)Int.Cl.
C07C 51/215(2006.01)
C07D 319/06(2006.01)
C07D 407/12(2006.01)
A61K 31/191(2006.01)
A61K 31/357(2006.01)
A61K 31/351(2006.01)
A61P 1/16(2006.01)
A61P 13/12(2006.01)
A61P 39/00(2006.01)

权利要求书4页 说明书47页 附图4页

(54)发明名称

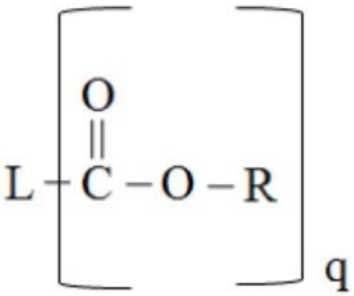
有效于治疗肝毒性及脂肪肝疾病的化合物及其用途

(57)摘要

本发明涉及有效于治疗肝毒性及脂肪肝疾病的化合物及其用途。

CN 107614475 A

1.一种由式(I)表示的化合物，



式(I)，

其中

L为饱和或不饱和脂肪族基团；

R是选自于下列所组成的群组：氢、多元醇基团和$(G)_p$的糖基团，其中G为单醣残基，且p为1至100的整数，其中$(G)_p$中的至少一个羟基被卤素原子取代；以及

q为2至4的整数，且每个R为相同或不同，

或其医药上可接受的盐类。

2.如权利要求1所述的化合物，其中该L为具有1至40个碳原子的烷基。

3.如权利要求1所述的化合物，其中该L是选自于下列所组成的群组：支链烷基、以苯环取代的直链烷基、以苯环取代的支链烷基、以直链脂肪族基团取代的苯基，以及以支链脂肪族基团取代的苯基。

4.如权利要求1所述的化合物，其中该多元醇基团是直链或环状的，经取代或未经取代的。

5.如权利要求1所述的化合物，其中该单醣残基为己糖。

6.一种由式(II)表示的化合物，

R_1-O-X-$(CH_2)_m$-X-O-R_2式(II)，

其中

X为C＝O；

R_1和R_2为相同或不同，是选自于下列所组成的群组：氢、多元醇基团和$(G)_p$的糖基团，其中G为单醣残基且p为1至100的整数，其中在$(G)_p$中的至少一个羟基被卤素原子取代，其中当R_1为氢时，则R_2不为氢；以及

m为1至40的整数，

或其医药上可接受的盐类。

7.如权利要求6所述的化合物，其中该多元醇基团为-CH(CHOH)$_n$$CH_2OH$，其中n为1至18的整数。

8.如权利要求6所述的化合物，其中在$(G)_p$中的二个或更多个羟基被卤素原子取代。

9.如权利要求6所述的化合物，其中该单醣残基为己糖。

10.如权利要求9所述的化合物，其中该己糖是选自于由己醛糖和酮己糖所组成的群组。

11.如权利要求6所述的化合物，其中该糖基R_1或R_2是由-G_1-O-G_2表示，其中G_1和G_2为相同或不同，是选自于由己醛糖和酮己糖所组成的群组，且在G_1中的至少一个羟基或在G_2中的

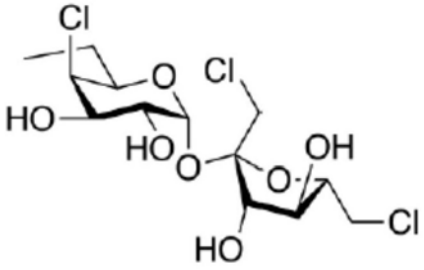
至少一个羟基被卤素原子取代。

12.如权利要求11所述的化合物，其中该卤素原子是选自于由氯、溴和碘所组成的群组。

13.如权利要求12所述的化合物，其中该卤素原子为氯。

14.如权利要求11所述的化合物，其中G_1为葡萄糖，其中一个羟基被氯取代；以及G_2为果糖，其中二个羟基被氯取代。

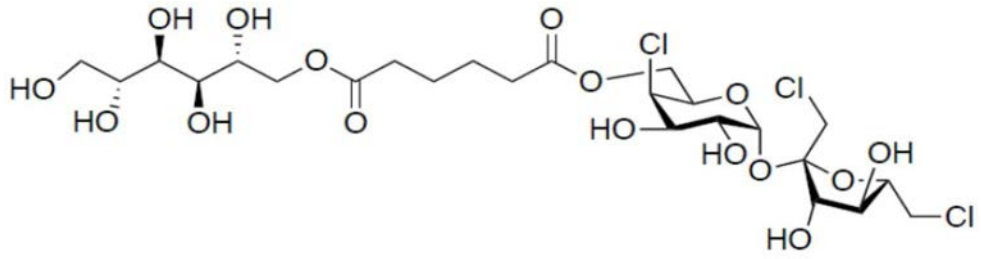
15.如权利要求11所述的化合物，其中R_1或R_2是由式(Ia)表示



式(Ia)。

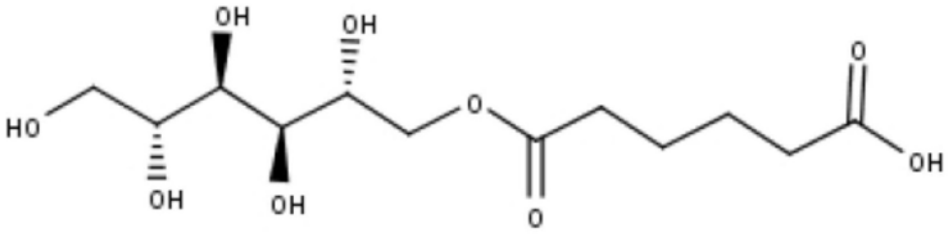
16.如权利要求7所述的化合物，其中m和n为4。

17.如权利要求1所述的化合物，其是选自于下列所组成的群组：式1的((2R,3R,4R,5R,6R)-6-(((2R,5R)-2,5-双(氯甲基)-3,4-二羟基四氢呋喃-2-基)氧基)-3-氯-4,5-二羟基四氢-2H-吡喃-2-基)甲基((2R,3R,4R)-2,3,4,5,6-五羟基己基)己二酸酯



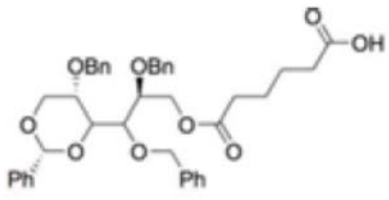
式 1。

18.如权利要求1所述的化合物，其系式2的C6-甘露醇



式 2。

19.一种由式C表示的化合物，



式 C

其中Ph为苯基且Bn为苄基。

20.一种医药组合物，包含如权利要求1所述的化合物或其医药上可接受的盐类以及一

医药上可接受之载体。

21.一种用于在有需要的个体中预防或治疗疾病或病症的方法，包含向该个体施用有一效量的如权利要求1所述的化合物或其医药上可接受的盐类。

22.如权利要求21所述的方法，其中该如权利要求1所述的化合物或其医药上可接受的盐类与选自于下列所组成的群组的一个或多个额外的试剂结合施用：

(i)第一活性剂，是选自于下列所组成的群组：聚乙二醇脱水山梨糖醇单月桂酸酯(Tween 20)、微晶纤维素、磷酸二钙二水合物、Brij 35、糖精、甘露醇，Cremophor RH40、三氯蔗糖、交联聚维酮、淀粉羟乙酸钠、Eudragit S100、交联羧甲基纤维素钠、Pluronic F68、薄荷醇、低取代羟丙基纤维素、预胶化淀粉、Dextrates NF水合物、柠檬酸、Cremophor EL、Aerosil 200、Myrj 52、山梨酸、柠檬油、羟丙基纤维素、山梨醇、乙酰磺胺酸钾、羟丙基甲基纤维素、乳糖单水合物、麦芽糖糊精、Brij 58、Brij 76、Tween 80、Tween 40、PEG 400、PEG 4000、PEG 8000、Span 60、苯甲酸钠、羟乙基甲基纤维素、甲基纤维素、Span 80、环己烷胺基磺酸钠、山嵛酸甘油酯、氧化红、甘油单硬脂酸酯、共聚维酮K28、乙酸淀粉、硬脂酸镁、月桂基硫酸钠、聚维酮K30、PEG2000，以及N-乙酰半胱胺酸(NAC)及其任何组合；

(ii)第二活性剂，是选自于下列所组成的群组：十二烷基硫酸钠、薄荷醇、三氯蔗糖、甘露醇、山梨醇、糖精、甘油、苯甲酸钠、氧化红、预胶化淀粉、环己烷胺基磺酸钠、山梨酸、柠檬油、柠檬酸、丁基化羟基茴香醚、枸杞子、异牡荆素、圣草酚、麦角固醇、β-月桂烯、高胆固醇、(+)-儿茶素、高良姜精、桑色素、金松双黄酮、香蜂草苷、棉纤维素、木犀草素-7-葡萄糖苷、(+)-紫杉叶素、反式肉桂酸、月见草内含物(Diosmin)、蒙花苷、木糖醇、木犀草素、獐牙菜苦苷、葛根素、根皮苷、甜橙黄酮、(-)-表没食子儿茶素、山奈酚、熊果酸、水飞蓟素、(+)-苎烯、橙皮苷、(-)-表儿茶素-3-没食子酸酯、水飞蓟宾、芒柄花素、肉豆蔻酸乙酯、二十碳五烯酸(EPA)、汉黄芩素、聚维酮K-30、原儿茶酸、伞形酮、橙皮素、去甲二氢愈创木酸、新橙皮苷、柚皮苷、(-)-表儿茶素、甘草甜素、黄芩苷、槲皮苷、黄芩素，以及其任何组合；以及

(i)和(ii)的任何组合。

23.如权利要求22所述的方法，其中该一个或多个额外的试剂是选自于下列所组成的群组：脱水磷酸二钙、薄荷醇、甘露醇、三氯蔗糖、N-乙酰半胱胺酸(NAC)及其任何组合。

24.如权利要求22所述的方法，其中该一个或多个额外的试剂是选自于下列所组成的群组：(1)糖精和甘露醇的组合、(2)薄荷醇和甘露醇的组合、(3)三氯蔗糖和甘露醇的组合、(4)圣草酚和甘露醇的组合、(5)圣草酚和三氯蔗糖的组合、(6)薄荷醇、甘露醇和圣草酚的组合，以及(7)三氯蔗糖、甘露醇和圣草酚的组合。

25.如权利要求22所述的方法，其中该如权利要求1所述的化合物或其医药上可接受的盐类与该一个或多个额外的试剂是同时或依序施用。

26.一种用于预防或治疗有需要的个体中的特征为增加的细胞色素P450的活性或增加的自由基含量的疾病或病症的方法，包含向该个体施用有效量的如权利要求1所述的化合物或其医药上可接受的盐类。

27.如权利要求26所述的方法，其中该如权利要求1所述的化合物或其医药上可接受的盐类是与一个或多个如权利要求20所定义的额外的试剂联合施用。

28.一种用于预防或治疗有需要的个体中的器官损伤的方法，包含向该个体施用有效量的如权利要求1所述的化合物或其医药上可接受的盐类。

29.如权利要求28所述的方法，其中该器官损伤是在肝脏或肾脏。

30.如权利要求28所述的方法，其中该器官损伤是由治疗药物、CCl_4或脂质所引起。

31.如权利要求30所述的方法，其中该治疗药物为对乙酰胺基酚。

32.如权利要求28所述的方法，其中该如权利要求1所述的化合物或其医药上可接受的盐类是与一个或多个如权利要求20所定义是额外的试剂联合施用。

33.一种用于预防或治疗有需要的个体中的肝毒性的方法，包含向该个体施用有效量的如请求项1所述的化合物或其医药上可接受的盐类。

34.如权利要求33所述的方法，其中该肝毒性是由治疗药物、CCl_4或脂质所引起。

35.如权利要求33所述的方法，其中该治疗药物为对乙酰胺基酚。

36.如权利要求33所述的方法，其中该如权利要求1所述的化合物或其医药上可接受的盐类是与一个或多个如权利要求20所定义的额外的试剂联合施用。

37.一种用于预防或治疗脂肪肝、保护肝功能或改善由脂肪肝或其它相关疾病引起的肝脏疾病的方法，包含向该个体施用一有效量的如权利要求1所述的化合物或其医药上可接受的盐类。

38.如权利要求37的方法，其中该如权利要求1所述的化合物或其医药上可接受的盐类是与一个或多个如权利要求20所定义的额外的试剂联合施用。

39.一种如权利要求1所述的化合物或其医药上可接受的盐类的用途，其是用于制备预防或治疗特征在于增加的细胞色素P450的活性或增加的自由基含量的疾病或病症的药物。

40.一种如权利要求1所述的化合物或其医药上可接受的盐类的用途，其是用于制备预防或治疗器官损伤的药物。

41.一种如权利要求1所述的化合物或其医药上可接受的盐类的用途，其是用于制备预防或治疗肝毒性的药物。

42.一种如权利要求1所述的化合物或其医药上可接受的盐类的用途，其是用于制备预防或治疗脂肪肝、保护肝功能或改善由脂肪肝或其它相关疾病引起的肝脏疾病的药物。

有效于治疗肝毒性及脂肪肝疾病的化合物及其用途

[0001] 相关申请案

[0002] 本申请案主张于2015年9月24日申请的第62/222,959号美国临时申请案、于2015年11月19日申请的第62/257,697号美国临时申请案,以及于2016年3月31日申请的第PCT/CN2016/078039号专利合作条约申请案的优先权,其内容通过引用方式整体并入本文。

技术领域

[0003] 本发明涉及有效治疗肝毒性和脂肪肝疾病的化合物及其用途。

背景技术

[0004] 器官中的损伤可能由有毒试剂所引起,例如,治疗用药物,当施用过量时,通常会导致器官特别是肝脏或肾脏的损伤。对乙酰胺基酚(亦为已知的Panadol)也被称为对乙酰胺基酚(paracetamol)或N-乙酰基-对-胺基苯酚(N-acetyl-para-aminophenol,APAP),是市面上使用最广泛的缓解疼痛和减少发烧症状的药物。每年都有许多因为不当使用APAP而造成药物中毒或自杀的案例,而且由APAP引起的肝损伤是严重疾病和死亡的主要原因。醇类或有机溶剂如四氯化碳(CCl_4)也可能引起肝毒性。许多临床研究已证实,由APAP诱导的肝毒性是可预防的,而且早期诊断以及实时投用解毒剂N-乙酰半胱胺酸(N-acetylcysteine,NAC)可防止肝毒性的发生。

[0005] 过量使用对乙酰胺基酚的早期检测是必要的,因为如果在中毒后8小时内投与解毒剂,可达到最佳预后效果。药物中毒的早期症状包括不适、恶心和呕吐。然而,有些患者即使其血液中的对乙酰胺基酚的浓度达到中毒的程度而且其异常肝功能呈现明显异常,他们在早期(阶段1)可能没有显现出中毒的迹象。肝毒性的迹象,例如腹痛、持续性呕吐、黄疸、右上腹疼痛,通常在摄入大量的对乙酰胺基酚后24-48小时(阶段2)变得明显。血清转胺酶通常在给药后16小时开始提升,并伴随出现临床症状。阶段3通常在施用后3-4天发生,而且此时很容易预测肝损伤程度以及预后程度。肝毒性的征兆从肝功能指数升高(AST>1,000IU/L)的轻微症状,到伴随代谢性酸中毒、黄疸、高血糖、AST>1,000IU/L、异常凝血和肝/脑病变的严重急性暴发性肝炎。在严重的情况下,阶段4将会引起少尿肾衰竭或死亡。

[0006] 有些对乙酰胺基酚中毒的患者仅显示出轻度肝损伤,但具有严重的肾毒性,这主要是因为在肾小管的P-450s(细胞色素P450s,CYPs)中的APAP的直接代谢所引起。然而,急性肾衰竭也可能由急性肝衰竭所引起的肝肾综合征而引起,且钠(FeNa)的排泄分率(fraction excretion)可用于区别原发性肾损伤(FeNa>1)与肝肾综合征(FeNa>1)。FeNa的计算式为(尿中的钠浓度÷尿中的肌酐浓度)÷(血浆中的钠浓度÷血浆中的肌酸酐浓度)×100。

[0007] 口服给药后1-2小时达到血液中对乙酰胺基酚浓度的高峰,而且肝脏会消除掉大量的对乙酰胺基酚,超过90%的对乙酰胺基酚与葡萄糖苷酸(glutathione)和硫酸盐结合,形成无毒代谢物,只有小于5%的对乙酰胺基酚被不同的CYPs消除,包括CYP2E1、CYP1A2和CYP3A4,其中CYP2E1和CYP1A2是代谢的主要酵素。这些酵素产生的代谢物N-乙酰基对苯醌

亚胺(N-acetyl-p-benzoquinoneimine,NAPQI)是一种非常活跃的亲电体。在正常条件下，NAPQI将立即与细胞中的谷胱甘肽反应并形成无毒的硫醇盐。过量的对乙酰胺基酚使谷胱甘肽的消耗速率大于其合成速率，且当细胞中的谷胱甘肽含量低于正常范围的30％时，NAPQI将结合大分子或含有半胱胺酸的核酸而导致肝损伤。由组织化学染色结果显示，NAPQI将结合到半胱胺酸的巯基，并在肝细胞坏死发生前在中心小叶区域形成共价键。

[0008] 患有肝病、酒精成瘾或正在服用可能诱导P450活性的药物，如卡巴马平(carbamazepine)、乙醇、异烟酸酐(Isoniazid)、乙苯基丙二酰脲(Phenobarbital)(可能是其他巴比妥类(barbiturates)药物)、二苯乙内酰脲(Phenytoin)、苯磺唑酮(Sulfinpyrazone)、磺酰脲类(Sulfonylureas)、立泛霉素(Rifampin)和普瑞米顿(Primidone)，的患者是发展出由APAP造成的严重肝毒性的好发族群，而且若该患者同时产生并发症，例如成人呼吸窘迫综合征、脑水肿、无法控制的出血、感染或多器官功能障碍综合征(Multiple organ dysfunction syndrome,MODS)，则可能很容易死亡。以乙醇为例，乙醇主要由肝脏的CYP2E1消除，其APAP中毒的机制分为三个阶段：第一阶段，乙醇在肝脏中与APAP竞争CYP2E1的受体，且该阶段中NAPQI的浓度降低，在第二阶段，乙醇将CYP2E1的半衰期从7小时延长至37小时，这增加了肝脏中CYP2E1的含量，而且在此阶段NAPQ1的浓度将缓慢增加，而在第三阶段，在酒精戒断期间，肝脏中会出现更多的CYP2E1以消除对乙酰胺基酚，因此对乙酰胺基酚的毒性代谢物显着增加并导致肝脏损伤。最近的研究表明，二烯丙基硫醚可有效预防小鼠中由对乙酰胺基酚所引起的肝毒性，进一步证明二烯丙基硫醚可抑制CYP2E1的活性。据推测，二烯丙基硫醚对乙酰胺基酚诱导的肝毒性的保护机制是通过抑制对乙酰胺基酚产生中间体NAPQI来达成的。以前的研究显示，通过抑制作用，可以减少肝细胞中还原型谷胱甘肽的消耗、由NAPQI引起的氧化活化、线粒体功能障碍，以及DNA损伤，进而将由对乙酰胺基酚诱导的肝损伤降到最低。例如，人蔘属植物三七(Panax notoginseng)、腺苷及其衍生物单磷酸腺苷、二磷酸腺苷和三磷酸腺苷可以通过这种保护机制预防由对乙酰胺基酚诱导的肝损伤。

[0009] 脂肪肝被认为是导致肝损伤的另一个因素。在正常情况下，脂肪占肝脏重量的3％。临床上，“脂肪肝疾病(fatty liver disease,FLD)”是指肝脏中的脂肪超过肝脏重量的5％，或超过10％的肝脏细胞在肝脏组织切片中显现出囊泡脂肪变化。根据疾病的原因，脂肪肝可分为酒精性脂肪肝疾病(alcoholic fatty liver diseases,AFLD)、非酒精性脂肪肝疾病(non-alcoholic fatty liver diseases,NAFLD)或由其他因素，如药物，引起的其他脂肪肝疾病。脂肪肝疾病的病理特征在于出现脂肪变态(metamorphosis)或脂肪变性(steatosis)、脂肪性肝炎(steatohepatitis)或其类似物。基于脂肪变性的肝细胞的百分比，脂肪肝被分类为轻度(<33％)、中度(33-66％)和重度(>66％)。以前，脂肪肝被认为是良性的而且是可逆的情况，因此较少被认真看待，但最近的研究发现它会导致严重的肝纤维化和肝硬化，甚至是肝癌。随着肥胖人群的增加，FLD的罹患率也在增加。

[0010] 欧洲和美洲国家发生肝病的主要原因是慢性过度饮酒，因此，绝大多数肝病是由酒精损伤所引起的。但在过去的15-20年，NAFLD已成为欧洲和美洲国家的肝脏功能障碍的首要疾病。Thaler曾在1962年描述过NAFLD。而在1980年，Ludwig提出了来自伴随的NAFLD之“非酒精性脂肪性肝炎(Non-alcoholic steatohepatitis,NASH)”，他在一群患有糖尿病和高脂血症的肥胖女性患者中发现该病症。此后，在1986年，Schaffner再次强调，NASH在发生

NAFLD过程中诱导纤维化的机制内扮演重要角色。直到1998年,Day发现15-50％的NASH患者具有不同程度的纤维化,因此临床医师们开始注意到NAFLD。到了今日,除了AFLD,NASH不仅只是临床上NAFLD自然进展的一个阶段。由于NASH的存在,NAFLD不再被认为是良性肝病。

[0011] 关于NAFLD的机制,Day和James在英国提出了基于大量临床研究和动物实验的二次打击假说(Two-hit hypothesis)。第一次打击后出现脂肪肝,以及第二次打击后出现脂肪性肝炎。第一次打击是由肝脏中因为肥胖、高脂血症等造成的过度积累的脂肪所引起的。第二次打击是由于氧化压力和活性氧(reactive oxygen species,ROS)在线粒体中的作用,导致肝细胞膜上的脂质过氧化、原炎症细胞激素和自由基的释放,以及由星状细胞活化引起的纤维化,并导致肝细胞坏死。NASH的机制涉及三酸甘油酯的过氧化、氧化压力、ROS反应、肝细胞中脂质过氧化的增加,或细胞激素和肝酵素的增加,导致一系列自体免疫相互作用。

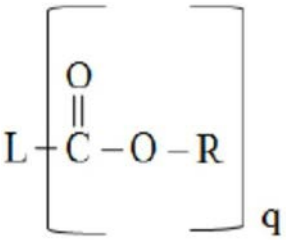
[0012] 脂肪肝发生的原因主要是与长期过度摄入动物脂肪、蛋白质、碳水化合物有关,过量卡路里转化为脂肪积累在体内,导致肥胖和脂肪肝。脂肪肝患者的血液可能具有正常的GOT/GPT值。因此,脂肪肝的正确诊断必须使用腹部超音波,目前腹部超音波可提供超过97％的准确度。

[0013] 目前,尚无理想药物可提供针对FLD的特异性治疗效果,其治疗基准目标为通过使用药物来改善潜在的危险因素或控制慢性疾病的发展。一般建议根据造成脂肪肝的原因对症治疗。例如,那些因为超重引起的脂肪肝的患者应该适度地减肥。任何带有酒精性脂肪肝的患者都需要戒酒,并采取均衡的饮食来改善状况。会损伤肝脏且由于长期接触而导致脂肪肝疾病的化学物或药物应立即停止使用。由于疾病,例如C型肝炎、高血脂等,引起的脂肪肝应通过治疗原发疾病,例如治疗C型肝炎或控制血脂,来治疗。然而,如果过多的三酸甘油酯是由于个人体质的因素所造成,则很难通过减肥来改善脂肪肝疾病。

[0014] 然而,目前临床上常用于降低血清三酸甘油酯和胆固醇的现有药物通常伴随着副作用,例如肝毒性、肌病如肌痛、肌炎、横纹肌溶解症等。关于降脂药物,肌肉毒性是最显着的副作用。特别是,他汀类药物(Statins)显现出最高的肌肉毒性发生率,并且伴随产生纤维酸。此外,降脂药具有“趋脂(fat driving)”效应,其将血脂“驱动(drives)”到肝脏,而肝脏中早已蓄积脂肪,脂质的流入难以处理,导致肝脏中累积过多的脂肪,而使脂肪肝的情况更糟。可以看出,降脂药物并不适合治疗FLD。

发明内容

[0015] 于一方面,本发明提供一种新的化合物,其结构由式(I)表示如下

[0016] 

式(I),

[0017] 其中,

[0018] L为饱和或不饱和脂肪族基团;

[0019] R是选自于下列所组成的群组:氢、多元醇基团(a polyol group)和$(G)_p$的糖基团,其中G为单醣残基,且p为1至100的整数,其中$(G)_p$中的至少一个羟基被卤素原子取代;以及

[0020] Q为2至4的整数,且每个R为相同或不同,

[0021] 或其医药上可接受的盐类。

[0022] 于某些具体实施例中,本发明之化合物由式(II)表示如下:

[0023] R_1-O-X-$(CH_2)_m$-X-O-R_2 式(II),

[0024] 其中,

[0025] X为C=O;

[0026] R_1和R_2为相同或不同,是选自于下列所组成的群组:氢、多元醇基团和$(G)_p$的糖基团,其中G为单醣残基且p为1至100的整数,其中在$(G)_p$中的至少一个羟基被卤素原子取代,其中当R_1为氢时,则R_2不为氢;以及m为1至40的整数。

[0027] 于另一方面,本发明提供了一种医药组合物,其包含至少一种如本文所述之化合物或其医药上可接受的盐类以及一医药上可接受之载体。

[0028] 于另一方面,本发明提供了一种治疗方法,其通过向有需要的个体施用一有效量的至少一种如本文所述之化合物或其医药上可接受的盐类。

[0029] 于某些具体实施例中,提供本发明之方法以预防或治疗在有需要的个体中的特征为增加的细胞色素P450的活性或增加的自由基含量的疾病或病症。

[0030] 于某些具体实施例中,提供本发明之方法以预防或治疗在有需要的个体中的器官损伤。

[0031] 于某些具体实施例中,提供本发明的方法以预防或治疗在有需要的个体中的肝毒性。

[0032] 于某些具体实施例中,提供本发明的方法以预防或治疗脂肪肝、保护肝功能或改善由脂肪肝或其它相关病症引起的肝脏疾病。

[0033] 于另一方面,本发明提供如本文所述之化合物或其医药上可接受的盐类用于制备药物之用途。具体而言,该药物可用于预防或治疗(i)特征在于增加的细胞色素P450的活性或增加的自由基含量的疾病或病症,(ii)器官损伤,及/或(iii)肝毒性,及/或(iv)预防或治疗脂肪肝、保护肝功能或改善由脂肪肝或其它相关疾病引起的肝脏疾病。

[0034] 在下面的描述中阐述了本发明一个或多具体个实施例的细节。从以下几个具体实施例的详细描述以及从所附之申请专利范围中,本发明的其它特征或优点将会显而易见。

附图说明

[0035] 当结合附图阅读时,将更好地理解前述发明内容以及本发明以下之详细描述。为了说明本发明之目的,在图式中展示出目前较佳之具体实施例。然而,应当理解的是,本发明不限于所展示之精确排列及手段。

[0036] 在图式中:

[0037] 图1所示为在血液中(活体外)前药残留或其相关代谢物形成的百分比。

[0038] 图2所示为在SD大鼠中口服前药之后,前药和三氯蔗糖的血浆中浓度对时间的曲线图。

[0039] 图3所示为在SD大鼠中口服前药之后，甘露醇的血浆中浓度对时间的曲线图。

[0040] 图4所示为动物肝组织的H&E染色结果。(A)正常对照组，(B)APAP诱导的肝损伤之对照组，(C)以NAC治疗的阳性对照组，(D)以甘露醇(1.67mg/kg)治疗的实验组，(E)以三氯蔗糖(1.67mg/kg)治疗的实验组，(F)以甘露醇(2.51mg/kg)加三氯蔗糖(2.51mg/kg)治疗的实验组，(G)以甘露醇(3.34mg/kg)加三氯蔗糖(3.34mg/kg)治疗的实验组，以及(H)以NAC和甘露醇(3.34mg/kg)和三氯蔗糖(3.34mg/kg)的组合治疗的实验组。

[0041] 图5所示为取自小鼠的肝组织切片，该小鼠系被诱导产生脂肪肝，然后以不同的试验化合物分组治疗四周。

[0042] 图6所示为本发明之化合物的合成方法的一般方式。

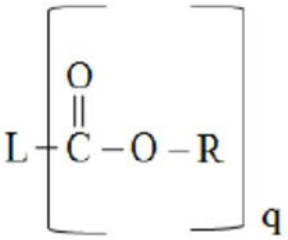
具体实施方式

[0043] 除非另有定义，本文使用的所有技术和科学术语具有与本发明所属领域之技术人员通常理解的相同含义。

[0044] 如本文所用，冠词“一”和“一个”是指该冠词的语法对象的一个或多于一个(即，至少一个)。举例而言，“一个组件”是指一个组件或多于一个组件。

[0045] I.化合物

[0046] 于一方面，本发明提供新的化合物，其结构由如下之式(I)所表示

[0047] 

式(I)，

[0048] 其中

[0049] L为饱和或不饱和脂肪族基团；

[0050] R是选自于下列所组成的群组：氢、多元醇基团(a polyol group)和$(G)-_p$的糖基团，其中G为单醣残基，且p为1至100的整数，其中$(G)_p$中的至少一个羟基被卤素原子取代；以及

[0051] Q为2至4的整数，且每个R为相同或不同，

[0052] 或其医药上可接受的盐类。

[0053] 如本文所用，“脂肪族的(aliphatic)”或“脂肪族基团(aliphatic group)”等词表示为一种可为直链(即，无支链)、支链或环状(包括融合的、桥接的和螺合的多环)的烃部分体(hydrocarbon moiety)，且可为完全饱和的或可含有一个或多个不饱和单元，但并非芳香族的。通常，脂肪族基团含有1-40个碳原子。于某些具体实施例中，脂肪族基团含有1-20个碳原子，或1-12个碳原子、1-8个碳原子或1-4个碳原子。于某些具体实施例中，脂肪族基团含有3-20个碳原子，或3-12个碳原子、3-8个碳原子或3-4个碳原子。合适的脂肪族基团包括，但不限于，直链或支链的烷基、烯基和炔基及其杂合物(hybrids)，例如(环烷基)烷基、(环烯基)烷基或(环烷基)烯基。

[0054] 在某些具体实施例中，式(I)中的L基团是选自(a)直链烷基，(b)支链烷基，(c)由

苯环取代的直链烷基，(d)由苯环取代的支链烷基，(e)苯基，其中该苯环含有直链脂肪族基团，以及(f)苯基，其中该苯环含有脂肪族支链。

[0055] 如本文所用，“多元醇基团(polyol group)”乙词表示每分子含有多个羟基(两个或更多个羟基)的醇类。具体而言，该多元醇基团可为直链或环状的，经取代的或未经取代的，或其混合物，只要所得之复合物为水溶性的且为医药上可接受的。

[0056] 于某些具体实施例中，该多元醇基团为C3-24多元醇，特别是C3-20多元醇，更特别是C3-12多元醇或C3-12多元醇，含有两个或更多个羟基。

[0057] 在更具体的具体实施例中，该多元醇基团由-CH-(CHOH)$_n$CH$_2$OH表示，其中n为1-22、1-18、1-10，或1-6。在一个特定的实例中，n为4。

[0058] 较佳的多元醇为糖醇。多元醇的实例包括，但不限于，3-碳多元醇(例如，甘油、赤藓糖醇和苏糖醇)；5碳多元醇(例如，阿糖醇、木糖醇和核糖醇)；6碳多元醇(例如甘露醇、山梨醇、半乳糖醇、岩藻糖醇、艾杜糖醇和肌醇)；12-碳多元醇(例如，油醇、异麦芽酮糖醇、麦芽糖醇和乳糖醇)；18-碳多元醇(例如，麦芽三糖醇)；和24-碳多元醇(麦芽四糖醇)。

[0059] 在式(I)中，G表示单醣残基。本文所用之单醣较佳为具有化学式C$_6$H$_{12}$O$_6$(即，己糖)的6-碳单醣。该己糖可为D构型、L构型或其组合。己糖通常根据官能团分类。举例而言，醛己糖在位置1具有醛，例如，阿洛糖、阿卓糖、葡萄糖、甘露糖、古洛糖、艾杜糖、半乳糖和塔罗糖；而酮己糖则在位置2具有酮，例如，阿洛酮糖、果糖、山梨糖和塔格糖。己糖还含有6个羟基，己糖中的醛或酮官能基可与相邻的羟基官能基反应，分别形成分子内的半缩醛或半缩酮。若所得之环状糖为5元环，则其为呋喃糖。若所得之环状糖为6元环，则其为吡喃糖。环自发地打开和关闭，允许羰基和相邻碳原子之间的键发生旋转，产生二种不同的构型(α和β)。己糖可为S构型或R构型。

[0060] 根据本发明，式(I)中的一个或多个单醣残基中的至少一个羟基被卤素原子取代。该卤素原子的实例包括氯、溴和碘。特定来说，该卤素原子为氯。

[0061] 如本文所用的，“S”或“R”等词系通过其构型而不涉及参考分子的命名光学异构体的方式，其被称为R/S系统。根据普利洛优先法则(Cahn Ingold Prelog priority rules)，基于原子序数，根据其配体各自被指定优先级的系统标记每个手性中心R或S。该系统标记分子中的每个手性中心(还具有不涉及手性中心的手性分子的延伸)。若化合物具有两个手性中心，则可将其标记为例如(S,S)异构体对(S,R)异构体。

[0062] 如本文所用，“医药上可接受的盐类”乙词包括酸加成盐类。“医药上可接受之酸加成盐类”系指保留生物学有效性和游离碱性质的那些盐类，游离碱系与无机酸如，盐酸、氢溴酸、硫酸、硝酸、磷酸及其类似物，以及有机酸如乙酸、丙酸、丙酮酸、马来酸、丙二酸、琥珀酸、富马酸、酒石酸、柠檬酸、苯甲酸、扁桃酸、甲磺酸、乙磺酸、对甲苯磺酸、水杨酸、三氟乙酸及其类似物形成。

[0063] 于某些具体实施例中，在式(I)中，q为2、3或4，R基团中的至少一个不同于R中的另一个。

[0064] 在某些具体实施例中，在式(I)中，q为2。

[0065] 在这些具体实施例中，本发明之化合物可由式(II)表示如下：

[0066] R_1-O-X-(CH$_2$)$_m$-X-O-R_2 式(II)，

[0067] 其中

[0068] X为C＝O；

[0069] R_1和R_2为相同或不同，是选自于下列所组成的群组：氢、多元醇基团和$(G)_p$的糖基团，其中G为单醣残基且p为1至100的整数，其中在$(G)_p$中的至少一个羟基被卤素原子取代，其中当R_1为氢时，则R_2不为氢；以及

[0070] m为1至40的整数，

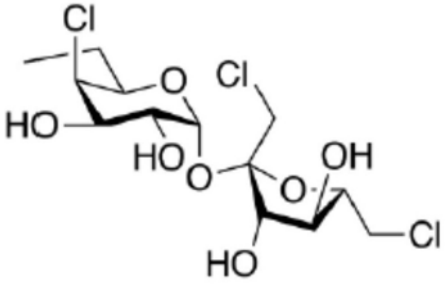
[0071] 或其医药上可接受的盐类。

[0072] 在某些具体实施例中，在式(II)中，R_1为多元醇基团，R_2为$(G)_p$的糖基团。在这种情况下，式(II)的化合物被认为是透过酯键藉由连接符将多元醇部分体与该糖部分体连接的缀合物(conjugate)。具体而言，该连接符由-O-X-$(CH_2)_m$-X-O-(式(L))表示，其中X为C＝O，m为1-40、1-20、1-12、1-8或1-4，更具体而言，m为3-20、3-12、3-8或3-4。在一个特定实例中，m为4。

[0073] 于一些具体实施例中，p为2。该糖基团由$-G_1-O-G_2$表示，其中G_1和G_2相同或不同，是选自于由己醛糖和酮己糖所组成的群组，且在G_1中的至少一个羟基或在G_2中的至少一个羟基被卤素原子取代。

[0074] 于一些具体实施例中，G_1为葡萄糖，其中一个羟基被氯取代；以及G_2为果糖，其中二个羟基被氯取代。

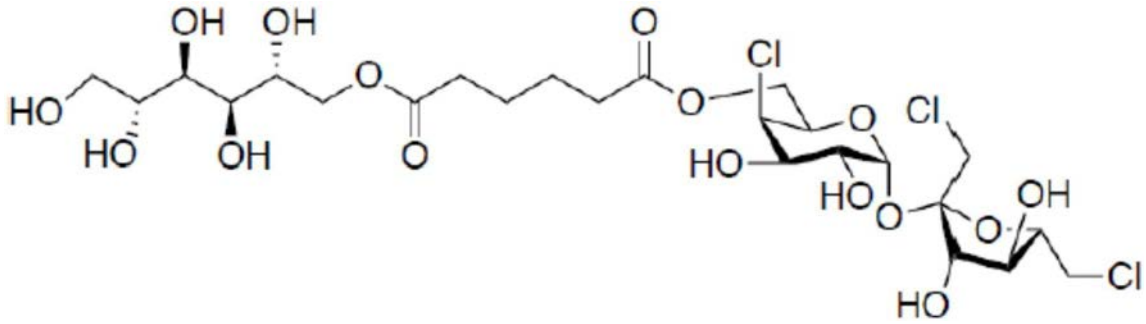
[0075] 在某些具体实施例中，糖基团由式(Ia)表示，

[0076] 

式(Ia)。

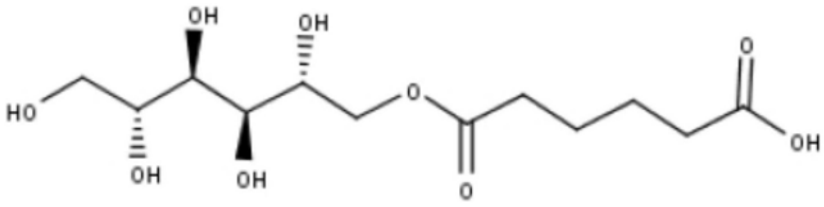
[0077] 本发明化合物的某些实例如下：

[0078] 式1的((2R,3R,4R,5R,6R)-6-(((2R,5R)-2,5-双(氯甲基)-3,4-二羟基四氢呋喃-2-基)氧基)-3-氯-4,5-二羟基四氢-2H-吡喃-2-基)甲基((2R,3R,4R)-2,3,4,5,6-五羟基己基)己二酸酯(((2R,3R,4R,5R,6R)-6-(((2R,5R)-2,5-bis(chloromethyl)-3,4-dihydroxytetrahydro furan-2-yl)oxy)-3-chloro-4,5-dihydroxytetrahydro-2H-pyran-2-yl)methyl((2R,3R,4R)-2,3,4,5,6-pentahydroxyhexyl)adipate)

[0079] 

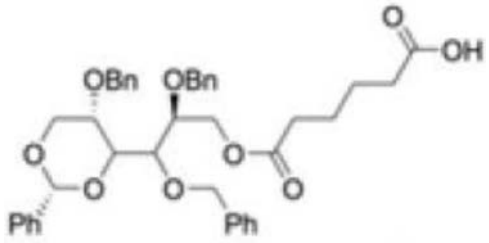
[0080] 式1，以及

[0081] 式2的C6-甘露醇

[0082] 

式2。

[0083] 于另一方面，本发明提供如下式C的中间体：

[0084] 

C

[0085] 其中Ph为苯基且Bn为苄基。

[0086] 式(I)的化合物可以化学合成，例如通过如图6的一般方案中所示的方法进行。

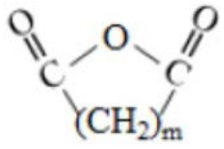
[0087] 具体而言，本发明提供了可以提供一个或多个-COOH基团以与醇类进行酯化作用的连接剂。在步骤1中，该提供第一-COOH基团(其他如果有的话，是被保护的)的连接剂与具有第一游离羟基(其他如果有的话，是被保护的)的R反应，以进行第一酯化作用，产生式(I)的化合物，其中q为1。在步骤2中，提供第二-COOH基团(其他如果有的话，是被保护的)的连接剂与具有第二游离羟基(其他如果有的话，是被保护的)的R反应以进行第二酯化作用，产生式(I)的化合物，其中q为2。在步骤3中，提供第三-COOH基团(其他如果有的话，是被保护的)的连接剂与具有第三游离羟基(其他如果有的话，是被保护的)的R反应以进行第三酯化作用，产生式(I)的化合物，其中q为3。在步骤4中，提供第四-COOH基团(其他如果有的话，是被保护的)的连接剂与具有第四游离羟基(其他如果有的话，是被保护的)的R反应以进行第四酯化作用，产生式(I)的化合物，其中q为4。

[0088] 于一些具体实施例中，进行酯化作用的连接剂由式(La)表示，

[0089] P_1-O-X-$(CH_2)_m$-X-O-P_2 式(La)

[0090] 其中X与m如上所定义，且P_1和P_2相同或不同，为保护基或H。

[0091] 于一些具体实施例中，进行酯化作用的连接剂由式(Lb)表示

[0092] 

式(Lb)。

[0093] 如本文所用，“保护基”为连接到官能部分体(例如，羟基中的氧或胺基中的氮，代替氢)的化学基团，以保护官能基免于以不期望的方式进行反应。保护基包括，例如，叔丁基、环烷基(例如，环己基)、芳基(例如，2,4-二硝基苯基)、芳烷基(例如，苄基、2,6-二氯苄基、3-溴苄基、2-硝基苄基、4-二甲基胺基甲酰基苄基和三苯基甲基)、四氢吡喃基、酰基、烷氧基羰基(例如，叔丁氧基羰基)、芳烷氧基羰基(例如，苄氧羰基、2-溴苄氧羰基)、二烷基硫

代膦酰基(例如,二甲基硫代膦酰基)和二芳基硫代膦酰基(例如,二苯基硫代膦酰基)。较佳的保护基包括酰基及其类似物。

[0094] 在一个特定实例中,在实施例1中提供了方案1,显示本发明之化合物的具体合成方法。

[0095] II.本发明之化合物的用途

[0096] 本发明之化合物可作为用于治疗方法的药物。通常,式(I)的化合物作为前药,在给药后可变为代谢物,提供如本文所述之需要的治疗效果。在一个实例中,式(I)的化合物为化合物F,其在施用后可变为甘露醇、三氯蔗糖和C6-甘露醇,这些都可作为P450抑制剂,并提供例如抗肝毒性的作用。参见以下实例。

[0097] 本发明提供一种治疗方法,其通过向有需要的个体施用一有效量的至少一种如本文所述之化合物或其医药上可接受的盐类。

[0098] 本发明之化合物被发现作为例如P450抑制剂是有效的。

[0099] 于一些具体实施例中,提供本发明之方法以预防或治疗在有需要的个体中的特征为增加的细胞色素P450的活性的疾病或病症。

[0100] 这些疾病或病症的实例系列于表A中。

[0101] 表A

[0102]

疾病	
酒精性肝炎	肝母细胞瘤
药物诱发的肝炎	肝肾慢性疾病
酒精性肝硬化	肥胖
肝病	中毒
肝硬化	胰岛素抗性
滥用酒精	慢性肝病

[0103]

异烟酸酐毒性	慢性肝炎
非酒精性脂肪性肝炎	肾病
肺结核	发炎
肝炎	酒精戒断
脂肪肝疾病	酒精性肝硬化
肝细胞癌	肝损伤
肝病酒精	酗酒
肝炎卤乙烷	肝炎毒性
脂肪肝酒精	
脂肪肝	肝坏死
酒精相关疾病	肝硬化
脑血管疾病	急性酒精性肝炎
冠状动脉疾病	肝肾组织病理学
肝肾细胞损伤	乙醇诱导和肥胖诱导的氧化压力及肝损伤
肝肾坏死	重金属中毒
慢性C型肝炎	肝纤维化
心血管疾病	动脉粥样硬化

[0104] 于一些具体实施例中，提供本发明之方法以预防或治疗在有需要的个体中的特征为增加的自由基含量的疾病或病症。

[0105] 于一些具体实施例中，提供本发明之方法以预防或治疗在有需要的个体中的器官损伤。

[0106] 在具体的实施例中，该器官损伤是在肝脏或肾脏中。

[0107] 在具体的实施例中，器官损伤或肝毒性系由治疗药物、CCl_4或脂质累积所引起。

[0108] 在具体的实施例中，该治疗药物是对乙酰胺基酚。

[0109] 于一些具体实施例中，提供本发明之方法以预防或治疗在有需要的个体中的肝毒性。

[0110] 于一些具体实施例中，提供本发明之方法以预防或治疗脂肪肝，保护肝功能或改善由脂肪肝或其它相关病症引起的肝脏疾病。

[0111] 如本文所用，“肝脏脂肪含量”乙词系指在个体的肝脏中积累之脂肪的含量，并且包括广义的脂质，例如三酸甘油酯(triglyceride，TG)和胆固醇。如本文所用，“降低肝脏脂肪含量”乙词通常指减少个体中异常肝脏脂肪含量，即降低异常肝脏脂肪含量，更具体而言，降低异常肝脏脂肪含量至正常程度。例如，在正常情况下，脂肪占肝脏重量的3％。若肝脏中的脂肪超过肝脏重量的5％，则判断为异常脂肪蓄积(上述肝脏脂肪含量是示例的相对百分比，并且可能由于种族和其他因素而有变化)。于一特定方面，本文所用之“降低肝脏脂肪含量”乙词可表示个体中异常肝脏脂肪的含量，例如从肝脏重量的5％或更高降低至肝脏重量的3％。可以通过标准分析方法评估肝脂肪含量，包括但不限于超音波分析、核磁共振成像(magnetic resonance imaging，MRI)、核磁共振光谱法(magnetic resonance spectroscopy，MRS)、计算机断层扫描(computed tomography，CT)和肝脏活体组织切片检验。

[0112] 如本文所用，“肝功能”乙词系指由肝脏执行的一个或多个生理功能。肝功能可以通过许多常规测定方法，如丙胺酸转胺酶(alanine aminotransferase，ALT)分析或天冬胺酸转胺酶(aspartate transaminase，AST)分析来进行分析。根据本发明，本文所述之化合物可用于维持肝功能，包括改善肝功能和防止肝损伤。

[0113] 如本文所用，“肝脏疾病”乙词系指肝细胞损伤或由某些因素引起之损伤，然后潜在地导致肝功能障碍。根据本发明，于一些具体实施例中，本文提出之化合物可用于改善由脂肪肝所引起的肝脏疾病。更具体而言，本文所用之“肝损伤”系指与正常肝脏相比，具有组织学或生化功能障碍的肝脏。于一特定具体实施例中，“肝损伤”系指由酒精或非酒精因素，例如高脂肪饮食或肥胖，或治疗药物或有机溶剂引起之肝脏损伤。在一特定具体实施例中，“肝损伤”可为具有选自脂肪变性、小叶炎症(lobular inflammation)、肝细胞气球化(hepatocyte balloning)和肝细胞产生的囊泡脂肪滴之一种或多种特征的肝组织损伤。于一特定具体实施例中，“肝损伤”可为肝脏的生化功能障碍，其可由丙胺酸转胺酶(ALT)或天冬胺酸转胺酶(AST)的活性来确定。较高活性的ALT或AST表示肝脏的生化功能具有严重的功能障碍。

[0114] 如本文所用，“肝抗氧化活性”乙词系指针对氧化压力的活性或能力。通过根据本发明之化合物改善个体的肝抗氧化活性是指包括，但不限于，降低氧化压力或增强抗氧化系统成员之酵素活性或含量。抗氧化系统成员可为谷胱甘肽过氧化物酶(glutathione peroxidase，GPx)、谷胱甘肽(glutathione，GSH)、谷胱甘肽还原酶(glutathione reductase，GRd)及/或超氧化物歧化酶(superoxide dismutase，SOD)。

[0115] 根据本发明，本文所述之化合物包括常见的赋形剂和生物类黄酮，其可用于减少肝脂肪含量和改善相关病症。本文所述之“相关病症”乙词包括由肝脏脂肪异常积累引起的病症，包括，但不限于，脂肪肝疾病、急性和慢性酒精性脂肪肝疾病、急性和慢性非酒精性脂肪肝疾病、急性和慢性酒精性肝炎、急性和慢性非酒精性脂肪性肝炎、非酒精性肝硬化和酒精性肝硬化(ICD-9-CM诊断码：571.8、571.0、571.1、571.2、571.3、571.4、571.5、571.9)。

[0116] 如本文所用，“预防”乙词系指针对疾病或疾病的症状或状况的预防措施。预防措施包括，但不限于，对尚未诊断为患有该疾病或该疾病之症状或状况但可能易感染或倾向于感染该疾病之个体，施用或投予一种或多种活性剂。预防措施的目的在于避免、预防或推迟疾病或疾病的症状或病症之发生。

[0117] 如本文所用，“治疗”乙词系指对疾病或疾病的症状或状况的治疗措施。治疗措施包括，但不限于，对患有疾病或疾病的症状或病症或疾病恶化之个体施用或投予一种或多种活性剂。治疗措施之目的在于治疗、治愈、减轻、缓解、改变、补救、改进、改善或影响该疾病、疾病的症状或病症、由疾病引起的残疾或疾病的加重。

[0118] 如本文所用，“CYP2E1抑制剂”为可以抑制CYP2E1活性的任何化合物、物质或材料。许多测定可用于分析CYP2E1活性，例如人或大鼠肝脏微粒体之分析。

[0119] 如本文所用，根据本发明之有治疗需要的个体包括人类和非人类哺乳动物。非人类哺乳动物包括，但不限于，伴侣动物例如猫、狗及其类似物，以及农场动物例如牛、马、绵羊、山羊、猪及其类似物。

[0120] “有效量”乙词或类似用语系指足以在个体中实现期望的治疗、预防及/或生物效应的活性剂的量，例如减少药物诱导的副作用，或者禁止、改善、减轻、减少或预防疾病的一

种或多种疾病之症状或病症或进展。实际有效量可以根据各种原因如施用途径和频率、接受该药物的个体的体重和物种，以及施用目的而改变。本领域技术人员可以基于本文的公开内容，确定的方法，以及他们自己的经验来确定每种情况下的剂量。

[0121] 如本文所用之“标准剂量”乙词系指治疗剂的有效剂量，其由医药社群，包括食品及药物管理局，中的权威来源推荐并常用于常规实践中。如本文所用之“减少的剂量”乙词系指低于标准剂量但仍保持与相同治疗剂基本相同的治疗效果的剂量。具体而言，根据本发明，治疗药物的减少剂量为治疗药物的标准治疗剂量的约90％或更少、80％或更少、70％或更少、60％或更少、50％或更少。

[0122] 于一些具体实施例中，本文所用之有效量的活性成分可以与医药上可接受的载体一起配制为用于递送和吸收的适当形式的医药组合物。

[0123] 如本文所用，“医药上可接受的”系指载体与组合物中的活性成分兼容，并且较佳地可稳定该活性成分，并对于接受治疗的个体是安全的。该载体可为活性成分的稀释剂、载体、赋形剂或基质。组合物可另外包含润滑剂、润湿剂、乳化剂和悬浮剂、防腐剂、甜味剂，和调味剂。本发明之组合物可在投予患者后提供活性成分的快速、持续或延迟释放的效果。

[0124] 根据本发明，该组合物的形式可为片剂、丸剂、粉剂、锭剂、包装、锭剂、酏剂、悬浮液、洗剂、溶液、糖浆、软和硬明胶胶囊、栓剂、灭菌注射液，和包装粉末。

[0125] 本发明之组合物可以通过任何生理学上可接受的途径，例如，口服、肠胃外(例如肌肉内、静脉内、皮下和腹膜内)、经皮输送、栓剂和鼻内途径递送。关于胃肠外给药，较佳为以无菌水溶液的形式使用，其可以包含其它物质，例如足以使溶液与血液等渗透压的盐类或葡萄糖。可使用本领域技术人员熟知的标准药理学技术完成在无菌条件下制备适当的肠胃外组合物，并且不需要额外的创造性劳力。

[0126] 在某些具体实施例中，本发明之式(I)化合物或其医药上可接受的盐类可用于预防或治疗器官中的损伤，例如，在肝脏或肾脏中，其可由过量的治疗药物(例如对乙酰胺基酚)或暴露于醇类、化学剂、生物分子或可在这些器官中引起毒性作用的任何物质所引起。

[0127] 特定而言，肝脏中的损伤可以包括肝细胞或组织的损伤、损害或丧失，导致肝功能异常或肝脏蛋白质含量的异常。于一些具体实施例中，如本文所述之肝损伤为急性肝损伤，其系指相对快速发作的肝损伤，例如，从症状发作起少于12周，具体而言是少于6周的期间。于一些具体实施例中，患有急性肝损伤的患者没有得过慢性肝病。

[0128] 特定而言，肾脏中的损伤可以包括肾细胞或组织的损伤、损害或丧失，导致肾功能异常。这样的肾损伤可以例如通过肾小球滤过率的降低、尿量输出的减少、血清肌酐的增加、血清半胱胺酸蛋白酶抑制剂C的增加等来确定。于一些具体实施例中，本文所述之肾损伤系急性肾损伤，其可表示肾脏过滤功能的突然或快速下降，例如在14天内，较佳在7天内，更佳在72小时内，还更佳的在48小时内。

[0129] 在一个具体实施例中，本发明的式(I)化合物或其医药上可接受的盐类能够预防或治疗由NAPQI(N-乙酰基-对-苯醌亚胺，N-acetyl-p-benzoquinone imine)引起之不希望得到的病症。

[0130] 因此，本发明提供了本发明之式(I)化合物或其医药上可接受的盐类在制备用于预防或治疗在一个体中由NAPQI(N-乙酰基-对-苯醌亚胺)引起之不希望得到的病症的药物之用途。本发明还提供了在有需要的个体中预防或治疗由NAPQI(N-乙酰基-对-苯醌亚胺)

引起之不希望得到的病症之方法，包含施予个体本发明之式(I)的化合物或其医药上可接受的盐类，其量有效预防或治疗该不希望得到的病症。

[0131] III.本发明之化合物与其它活性剂的组合使用

[0132] 本发明之化合物及/或其代谢物可以与一种或多种额外的活性剂组合施用，该活性剂具体而言是作为P450抑制剂及/或提供抗肝毒性活性的活性剂及/或具有抗脂肪肝活性的活性剂，从而例如提供协同效应。

[0133] 作为P450抑制剂(命名为“第一活性剂”)的一些活性剂被描述于PCT/CN2013/087049(USSN 14/441,317，其内容通过引用方式整体并入本文)中。这种P450抑制剂的具体实例包括，但不限于，聚乙二醇脱水山梨糖醇单月桂酸酯(Tween 20)、微晶纤维素、磷酸二钙二水合物、Brij 35、糖精、甘露醇、Cremophor RH40、三氯蔗糖、交联聚维酮、淀粉羟乙酸钠、Eudragit S100、交联羧甲基纤维素钠、Pluronic F68、薄荷醇、低取代羟丙基纤维素、预胶化淀粉、Dextrates NF水合物、柠檬酸、Cremophor EL、Aerosil 200、Myrj 52、山梨酸、柠檬油、羟丙基纤维素、山梨醇、乙酰磺胺酸钾、羟丙基甲基纤维素、乳糖单水合物、麦芽糖糊精、Brij 58、Brij 76、Tween 80、Tween 40、PEG 400、PEG 4000、PEG 8000、Span60、苯甲酸钠、羟乙基甲基纤维素、甲基纤维素、Span 80、环己烷胺基磺酸钠、山嵛酸甘油酯、氧化红、甘油单硬脂酸酯、共聚维酮K28、乙酸淀粉、硬脂酸镁、月桂基硫酸钠、聚维酮K30、PEG2000，以及N-乙酰半胱胺酸(NAC)及其任何组合。

[0134] 在某些具体实施例中，与本发明之式(I)的化合物组合使用的一种或多种第一活性剂是选自于下列所组成的群组：脱水磷酸二钙、薄荷醇、甘露醇、三氯蔗糖、N-乙酰半胱胺酸(NAC)及其任何组合。

[0135] 在PCT/CN2016/078039中描述了一些具有抗脂肪肝活性的活性剂(命名为“第二活性剂”)，该PCT申请案的内容系通过引用方式整体并入本文。具有抗脂肪肝活性的活性剂的具体实例包括，但不限于，(ii)选自以下所组成群组的第二活性剂：十二烷基硫酸钠、薄荷醇、三氯蔗糖、甘露醇、山梨醇、糖精、甘油、苯甲酸钠、氧化红、预胶化淀粉、环己烷胺基磺酸钠、山梨酸、柠檬油、柠檬酸、丁基化羟基茴香醚、枸杞子、异牡荆素、圣草酚、麦角固醇、β-月桂烯、高胆固醇、(+)-儿茶素、高良姜精、桑色素、金松双黄酮、香蜂草苷、棉纤维素、木犀草素-7-葡萄糖苷、(+)-紫杉叶素、反式肉桂酸、月见草内含物(Diosmin)、蒙花苷、木糖醇、木犀草素、獐牙菜苦苷、葛根素、根皮苷、甜橙黄酮、(-)-表没食子儿茶素、山奈酚、熊果酸、水飞蓟素、(+)-苎烯、橙皮苷、(-)-表儿茶素-3-没食子酸酯、水飞蓟宾、芒柄花素、肉豆蔻酸乙酯、二十碳五烯酸(EPA)、汉黄芩素、聚维酮K-30、原儿茶酸、伞形酮、橙皮素、去甲二氢愈创木酸、新橙皮苷、柚皮苷、(-)-表儿茶素、甘草甜素、黄芩苷、槲皮苷、黄芩素，以及其任何组合。

[0136] 在某些具体实施例中，与本发明之式(I)的化合物组合使用的一种或多种第二活性剂是选自于下列所组成的群组：十二烷基硫酸钠、薄荷醇、三氯蔗糖、甘露醇、山梨醇、糖精、甘油、苯甲酸钠、氧化红、预胶化淀粉、环己烷胺基磺酸钠、山梨酸、柠檬油、柠檬酸、丁基化羟基茴香醚、枸杞子、异牡荆素、圣草酚、麦角固醇、β-月桂烯、高胆固醇、(+)-儿茶素、高良姜精、桑色素、金松双黄酮、香蜂草苷、棉纤维素、木犀草素-7-葡萄糖苷、(+)-紫杉叶素、反式肉桂酸、月见草内含物(Diosmin)、蒙花苷、木糖醇、木犀草素、獐牙菜苦苷，以及其任何组合。

[0137] 在某些具体实施例中，与本发明之式(I)的化合物组合使用的一种或多种第二活性剂是选自于下列所组成的群组：葛根素、根皮苷、甜橙黄酮、(-)-表没食子儿茶素、山奈酚、熊果酸、水飞蓟素、(+)-苎烯、橙皮苷、(-)-表儿茶素-3-没食子酸酯、水飞蓟宾、芒柄花素、肉豆蔻酸乙酯、二十碳五烯酸(EPA)、汉黄芩素、聚维酮K-30、原儿茶酸、伞形酮、橙皮素、去甲二氢愈创木酸、新橙皮苷、柚皮苷、(-)-表儿茶素、甘草甜素、黄芩苷、槲皮苷、黄芩素，以及其任何组合。

[0138] 在某些具体实施例中，与本发明之式(I)的化合物组合使用的一种或多种第二活性剂是选自于下列所组成的群组：圣草酚、甘露醇、薄荷醇、三氯蔗糖、糖精，以及其任何组合。

[0139] 在某些具体实施例中，与本发明之式(I)的化合物组合使用的一种或多种第二活性剂是选自于下列所组成的群组：(1)糖精和甘露醇的组合、(2)薄荷醇和甘露醇的组合、(3)三氯蔗糖和甘露醇的组合、(4)圣草酚和甘露醇的组合、(5)圣草酚和三氯蔗糖的组合、(6)薄荷醇、甘露醇和圣草酚的组合，以及(7)三氯蔗糖、甘露醇和圣草酚的组合。

[0140] 特定而言，式(I)化合物或其医药上可接受的盐类和一种或多种额外的试剂可以同时或依序施用。

[0141] 在本发明中，还提供本发明之式(I)的化合物或其医药上可接受的盐类能够预防或治疗由NAPQI(N-乙酰基-对-苯醌亚胺)所引起的不希望得到的病症。

[0142] 作为具体实施例，本发明提供式(I)化合物及/或其代谢物与N-乙酰半胱胺酸(NAC)的组合。本发明还提供了用于在需要的个体中施用N-乙酰半胱胺酸(NAC)的方法，包含对该个体投予NAC与式(I)的化合物及/或其代谢物的组合。在一个具体实施例中，本发明之组合或方法在预防或治疗NAC有效的疾病或病症中是有效的。于一些具体实施例中，由NAC治疗或预防的疾病或病症是选自于下列所组成的群组：肌阵挛性癫痫、急性呼吸窘迫综合征、重金属中毒、流感病毒感染、心脏病、口腔干燥风湿性关节炎症候群、慢性支气管炎、癫痫(Unverricht-Lundborg型)和HIV感染。

[0143] 通过以下实施例进一步说明本发明，该实施例是为了说明而不是限制之目的而提供的。

[0144] 实施例

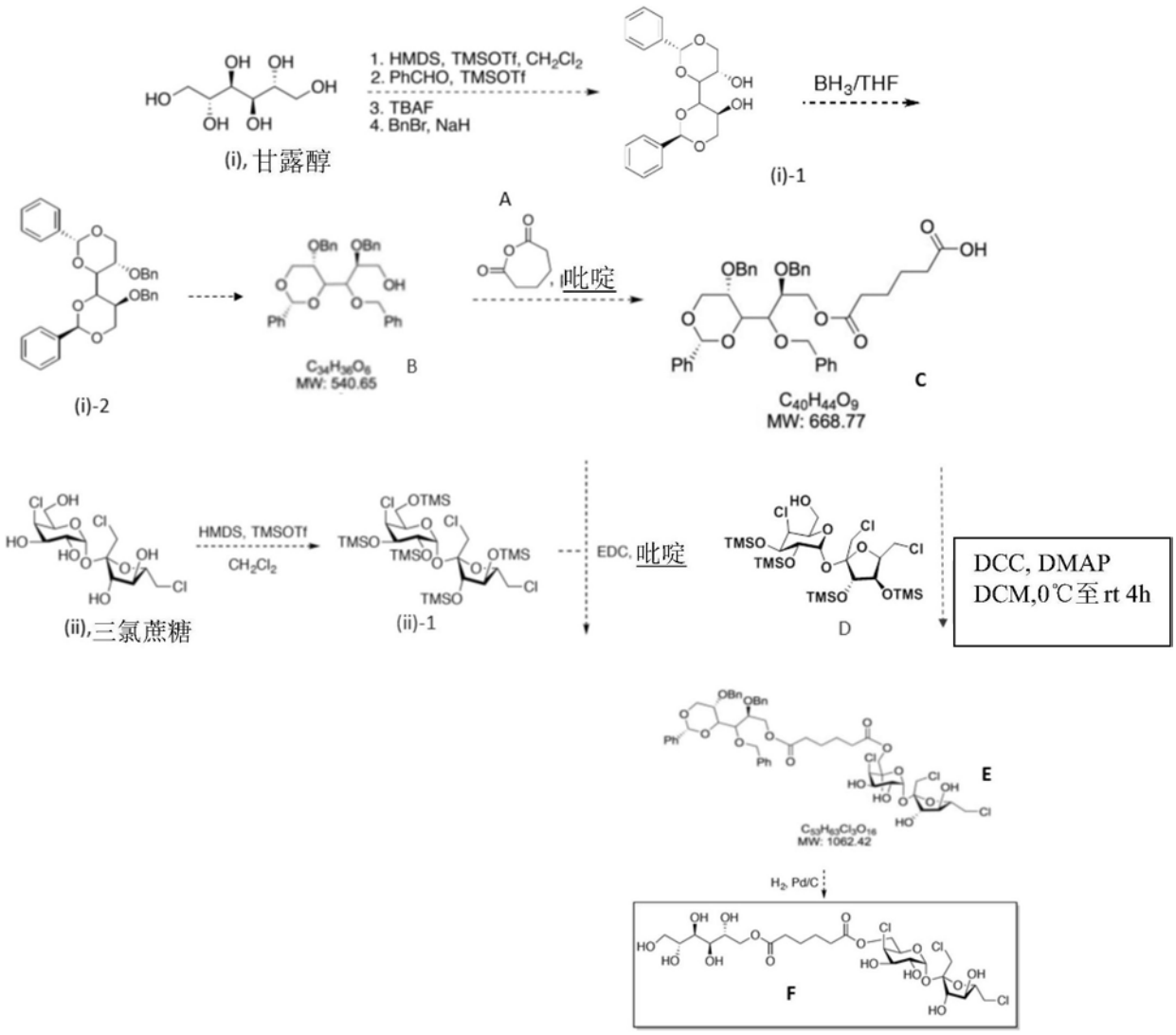
[0145] 实施例1：本发明之式1的化合物(化合物F)之合成

[0146] ((2R,3R,4R,5R,6R)-6-(((2R,5R)-2,5-双(氯甲基)-3,4-二羟基四氢呋喃-2-基)氧基)-3-氯-4,5-二羟基四氢-2H-吡喃-2-基)甲基((2R,3R,4R)-2,3,4,5,6-五羟基己基)己二酸酯(式1)(化合物F)的合成

[0147] 用于式1(化合物F)之合成的合成策略示于方案1中。

[0148] 方案1

[0149]



[0150] HMDS＝六甲基二硅氮烷

[0151] TMSOTf＝三氟甲磺酸三甲基甲硅烷

[0152] TBAF＝氟化四丁铵

[0153] THF＝四氢呋喃

[0154] TMS＝三甲基甲硅烷基

[0155] DCC＝二环已基碳二亚胺

[0156] DMAP＝4-二甲基胺基吡啶

[0157] DCM＝二氯甲烷

[0158] DMF＝N,N’-二甲基甲酰胺

[0159] DIBAL＝二异丁基铝

[0160] Bn＝苄基醚

[0161] 一般方法

[0162] 除非另有说明,所有化学品均来自商业来源并直接使用。

[0163] 在以下之条件下评价产物的色层分析纯度：

[0164] 流动相组成A:甲醇:H_2O＝5/95(v/v),含有0.05％NH_4OH

[0165] B:甲醇:H_2O=95/5(v/v),含有0.05%NH_4OH

[0166] 色层分析系统:

[0167]

时间	帮浦 B 浓度
0	15
1	15
5	80
5.1	15
10	15

[0168]

管柱类型	Waters® Acquity UPLC $HSST_3$, 1.8 μm, 100 × 2.1mm
自动进样器温度	4°C
管柱箱温度	45°C
流速	0.35 mL/分钟
分析时间	10 分钟
注射体积	5 μL
保留时间	4.8 分钟

[0169] MS分析在以下条件下进行:

[0170] 质谱仪设置:

[0171] 质谱仪 三重四极杆质谱(API Qtrap5500)

[0172] 应用生物系统公司

[0173] 检测 MRM阴性模式

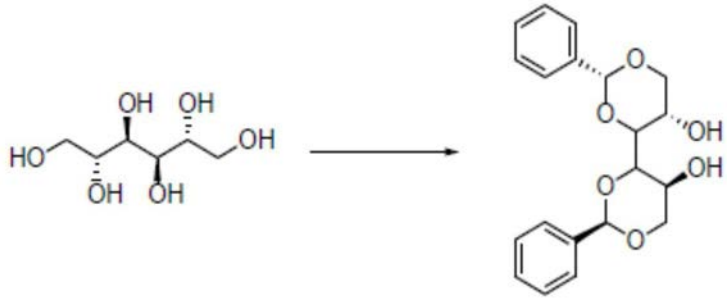
[0174] 前药: m/z 688.9→m/z 180.9

[0175] 使用Bruker标准脉冲程序,在MeOH-d4(δ_H3.30,δ_C49.0)或$CDCl_3$(δ_H 7.24,δ_C77.0)中的Bruker AMX-500NMR光谱;在HMQC和HMBC实验中,Δ=1s且J分别为140,8Hz,相关图分别由每个光谱的512×1K个数据点组成,每个光谱由16到64个瞬间组成。

[0176] 1.1甘露醇(化合物(i))至化合物(B)

[0177] 1.1.1甘露醇(化合物(i))至化合物(i)-1

[0178]

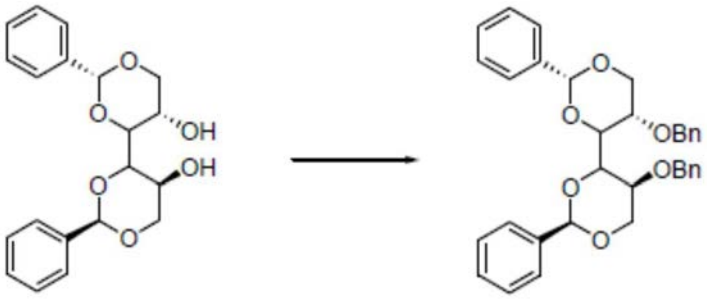


甘露醇，化合物(i) 化合物(i)-1

[0179] 于室温、一大气压下,在D-甘露醇(25g,0.137mol)的DMF(250mL)溶液中加入苯甲醛(30mL,0.345mmol)。在0℃,于混合物中滴加浓硫酸(10mL)。在逐渐升温至室温后,将混合物搅拌3天。然后在剧烈搅拌下将混合物倒入冰水(250mL)和正己烷(200mL)中。将混合物温

热至室温后，过滤沉淀物并用正己烷洗涤。将沉淀物悬浮在氯仿中并在剧烈搅拌下加热回流15分钟。当混合物达到室温时，收集未溶解的沉淀物并从乙醇中重结晶，得到所需产物，为白色固体(9.86g，20％)。Rf＝0.45(EA/Hex＝1/1)。

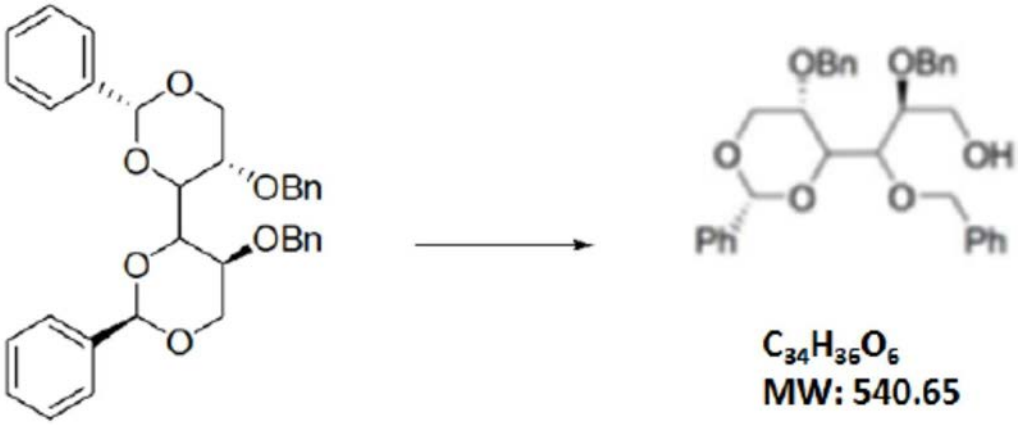
[0180] 1.1.2化合物(i)-1至化合物(i)-2

[0181] 

化合物(i)-1 化合物(i)-2

[0182] 于室温、一大气压下，在1,3,4,6-二亚苄基(10g，27.9mmol)的DMF(100mL)溶液中加入苄基溴(7.96mL，66.96mmol)。将混合物冷却至0℃，然后在几分钟内加入60％NaH(2.68g，66.96mmol)。在使其逐渐升温至室温后，将混合物搅拌整夜。然后将反应以水淬灭(滴加)，以$NaHCO_{3(水溶液)}$和二氯甲烷萃取。将有机层以$MgSO_4$干燥，真空浓缩。将残余物通过硅胶管柱色层分析纯化，得到所需产物。(10.39g，69％)。R_f＝0.2(EA/Hex＝1/6)。

[0183] 1.1.3化合物(i)-2至化合物(B)

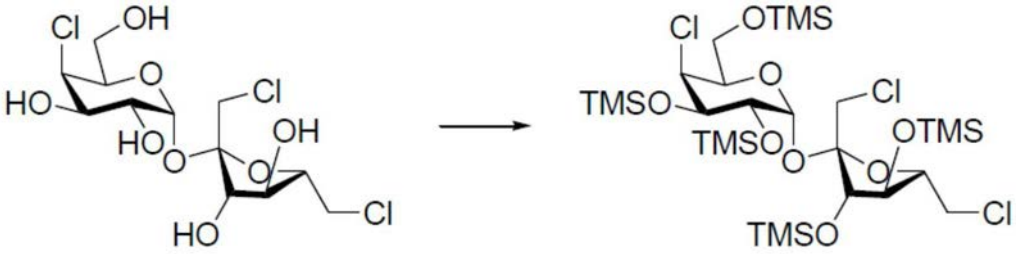
[0184] 

化合物(i)-2 化合物(B)

[0185] 将2,5-二苄基-1,3,4,6-二亚苄基(1.5g，2.78mmol)在甲苯(12.5mL)中的溶液冷却至-18℃(冰盐浴)。滴加1.2M DIBAL(18.5mL，22.3mmol)，升温至室温。1.5小时后，将反应冷却至0℃，然后通过MeOH和15％$KOH_{(水溶液)}$淬灭。将混合物以DCM萃取，将有机层以$MgSO_4$干燥并真空浓缩。将残余物通过硅胶管柱色层分析纯化，得到所需产物。(709mg，47％)。R_f＝0.1(EA/HEX＝1/5)。

[0186] 1.2三氯蔗糖(化合物(ii))至化合物(D)

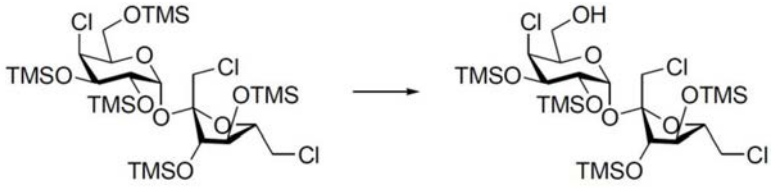
[0187] 1.2.1化合物(ii)至化合物(ii)-1

[0188] 

三氯蔗糖，化合物(ii) 化合物(ii)-1

[0189] 对三氯蔗糖(1g,2.5mmol)在DCM(10mL)中的溶液中加入HMDS(2.6mL,12.57mmol)和TMSOTf(45μL,0.25mmol)。将反应物在室温下搅拌整夜。将反应物在真空中浓缩并通过棉花,以己烷洗涤。将滤液再次真空浓缩,得到产物,定量。(1.9g,定量)。R_f=0.9(EA/HEX=1/8)。

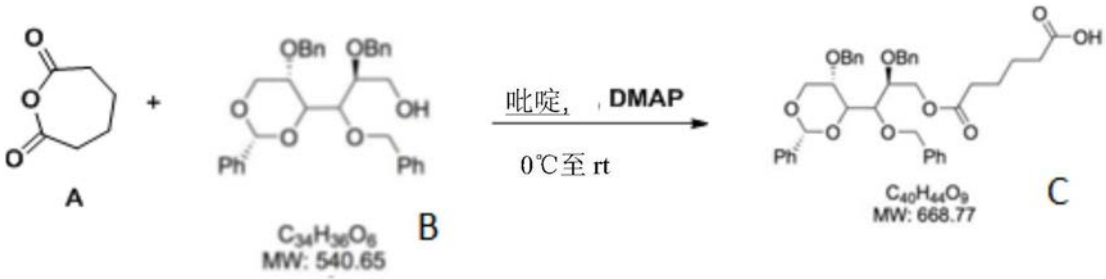
[0190] 1.2.2化合物(ii)-1至化合物(D)

[0191] 

化合物(ii)-1 化合物(D)

[0192] 对五-TMS三氯蔗糖(5g,6.6mmol)在吡啶(150mL)中的溶液中加入0.1M吡啶-TcCl溶液(6.6mL),并于开口烧瓶中搅拌3天。将反应溶液以真空浓缩,通过硅胶管柱色层分析纯化,得到所需产物。(1.4g,30%)。R_f=0.5(EA/HEX=1/8)。

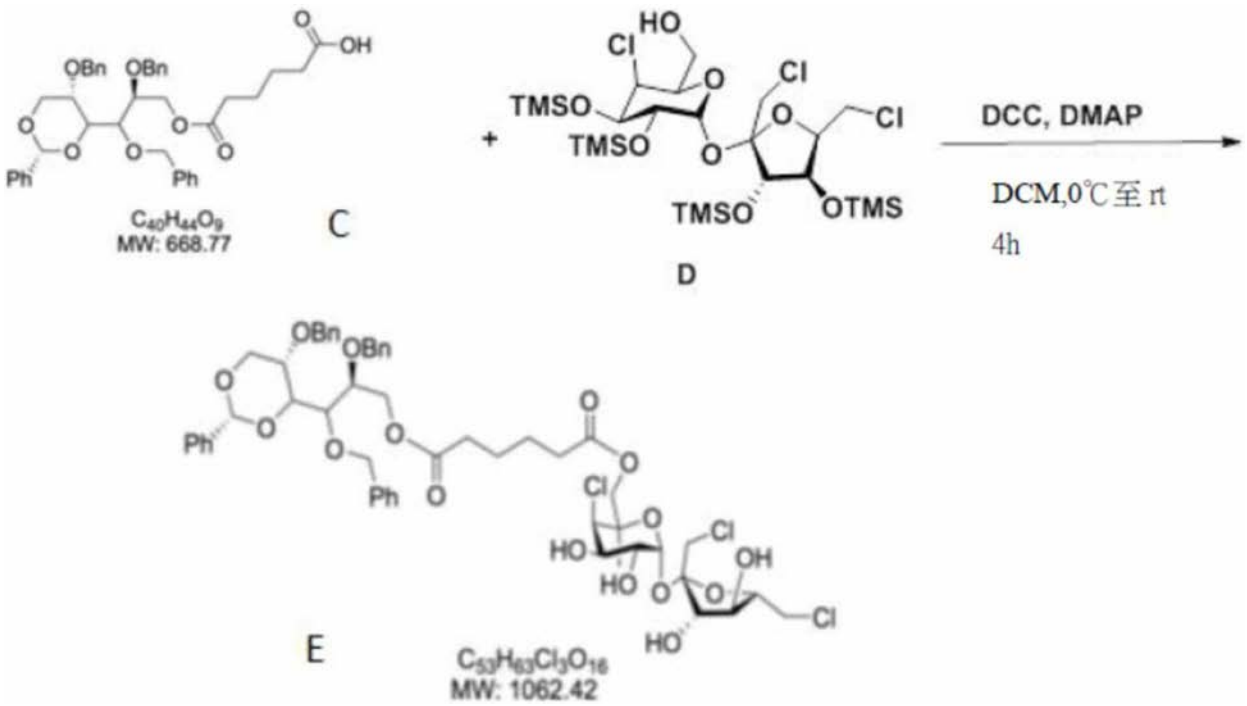
[0193] 1.3 6-氧代-6-((2R,3R,4R)-2,3,4-参(苄氧基)-4-(2-苯基-1,3-二氧戊环-4-基)丁氧基)己酸(化合物(C))的合成

[0194] 

[0195] 在0℃下,在火焰干燥的R.B.烧瓶中,将化合物A(165mg,1当量)溶于DCM(5mL)中,然后向其中加入吡啶(0.2mL)和DMAP(50mg)。然后将反应混合物搅拌10分钟,加入化合物B(59mg,1.5当量)。然后将反应混合物在室温下搅拌5小时。TLC证实反应完成。将反应混合物在减压下在旋转蒸发仪上蒸发至干燥。将粗化合物通过管柱色层分析进一步纯化,得到所需化合物,为无色油状物(136mg,67%)。

[0196] 1.4((2R,3S,4R,5R,6R)-6-(((2R,5R)-2,5-双(氯甲基)-3,4-双((三甲基甲硅烷基)氧基)四氢呋喃-2-基)氧基)-3-氯-4,5-双((三甲基甲硅烷基)氧基)四氢-2H-吡喃-2-基)甲基((2R,3R,4R)-2,3,4-参(苄氧基)-4-(2-苯基-1,3-二氧戊环-4-基)丁基)己二酸酯的合成

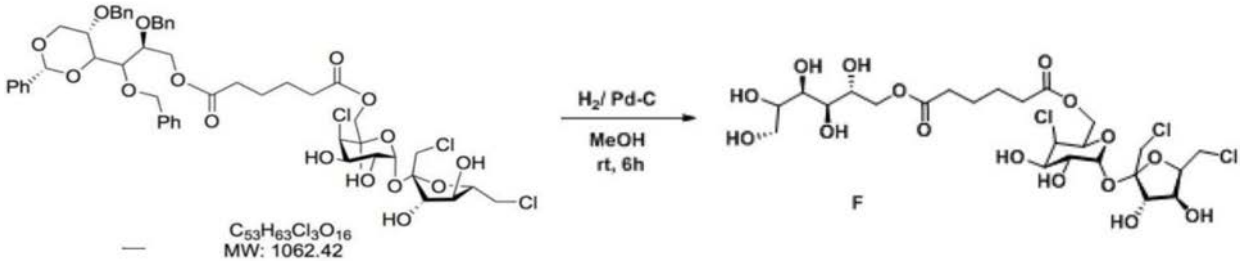
[0197]



[0198] 对化合物C(100mg,1.0当量)在DCM中的冰冷溶液中加入DCC(35mg,1.15当量)并搅拌10分钟。然后向该化合物D(112mg,1.2当量)中加入DMAP(5mg,0.25当量,催化的)。将反应混合物温热至室温并搅拌4小时。TLC证实反应完成。将反应混合物在减压下在旋转蒸发仪上蒸发至干燥。然后通过使用中性硅胶和5至15%乙酸乙酯的己烷溶液,以1%三乙胺作为洗脱剂的管柱色层分析法纯化粗化合物,得到呈无色油状的所需化合物E(84mg,42%)。

[0199] 1.5((2R,3R,4R,5R,6R)-6-(((2R,5R)-2,5-双(氯甲基)-3,4-二羟基四氢呋喃-2-基)氧基)-3-氯-4,5-二羟基四氢-2H-吡喃-2-基)甲基((2R,3R,4R)-2,3,4,5,6-五羟基己基)己二酸酯(化合物F)的合成

[0200]



[0201] 在火焰干燥的单颈R.B.烧瓶中,将化合物E(500mg,1当量)溶解于无水MeOH(20mL)中,然后将溶液通过氮气脱气(氮气注射器在溶液内部深处,氮气吹扫15分钟)。然后小心地向反应混合物中加入10%Pd-C(200mg,33%w/w)。最后,将反应混合物在氢气球压力下搅拌6小时。TLC证实反应完成。然后将反应混合物通过硅藻土床过滤,并以无水甲醇洗涤该硅藻土床。将滤液在减压下在旋转蒸发仪上蒸发至干燥。然后将最终化合物保持在高真空下,得到所需的最终化合物F,为无色半固体或白色固体(190mg,73%)。通过高分辨率质谱分光亮度法和^{13}C NMR鉴定化合物F的结构。

[0202] 实施例2:作为前药的化合物F,当与血液(活体外)培培育时产生代谢物

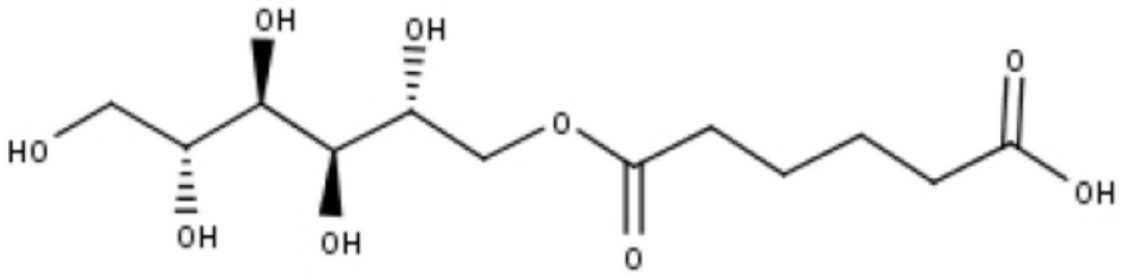
[0203] 2.1材料和方法

[0204] 将新鲜的人类全血用于药物水解作用之研究。将药物(10mg,化合物F)溶于1mL溶液(20％甲醇)中。在含有1.0mg药物的20mL新鲜全血等分试样在50mL烧瓶中,在37℃下在摇动水浴中恒温进行药物水解(n＝3)。在时间为0时,加入药物,并在不同时间的培育后,在0.25、0.5、0.33、0.75、1、2、4、6、12和24小时收集血液样品。血液样品使用1mL乙腈以淬灭药物的酶水解作用,因而获得样品。通过装备有离子喷雾(ion-spray,ESI)源的API QTrap5500三重四极杆质谱仪测定血液中的前药及其相关代谢物,例如C6-甘露醇、甘露醇和三氯蔗糖。ESI接口用于负离子模式。

[0205] 2.2结果

[0206] 在m/z 688.9→180.9的转变处监测前药。在m/z 395→359的转变处监测到三氯蔗糖;在m/z 452.3→273.3的转变处监测到甘露醇;在m/z 309→101.1的转变处监测到C6-甘露醇。所有化合物通过高分辨率质谱分光亮度计和^{13}C NMR鉴定。C6-甘露醇(式(2))的结构如下:

[0207]



(2)

[0208] 以前药在血液中培育后剩余之前药的百分比,以及三氯蔗糖、甘露醇和C6-甘露醇增加之百分比相对于时间来绘图,以表示血液中前药的水解作用(图1)。结果显示,以化合物F作为前药,在活体外与血液培育后变成其代谢物,包括三氯蔗糖、甘露醇和C6-甘露醇。

[0209] 实施例3:SD(Sprague Dawley)-大鼠(体内)的药物动力学研究

[0210] 3.1材料和方法

[0211] 以3.67mg/kg体重的剂量口服给予SD-大鼠前药。将血液样品以0、0.5、1、2、4、6、8、12和24小时的间隔收集到肝素化微量离心管中。通过以8,000rpm离心血液样品10分钟后立即得到血浆样品。然后将血浆样品储存在-80℃备用。通过配备有离子喷雾(ESI)来源的API QTrap5500三重四极杆质谱仪以分析血浆样品中的前药及其相关代谢物,如甘露醇和三氯蔗糖。ESI接口用于负离子模式。

[0212] 3.2结果

[0213] 在m/z 688.9→180.9的转变处监测到前药。在m/z 395→359的转变处监测到三氯蔗糖;在m/z 452.3→273.3的转变处监测到甘露醇;在m/z 309→101.1的转变处监测C6-甘露醇。

[0214] 图2及图3分别显示在口服给予3.67mg/kg单一剂量前药的SD大鼠中,前药和其相关代谢物,例如三氯蔗糖和甘露醇,的血浆浓度时间曲线。结果显示,化合物F作为前药,给药后在动物体内转化成其代谢物,包括三氯蔗糖、甘露醇和C6-甘露醇。

[0215] 实施例4:CYP2E1抑制活性分析

[0216] 4.1材料和方法

[0217] 本实施例系从人类肝脏中制备微粒体,以用于体外筛选CYP450同工酶抑制剂。基于从不同来源的肝脏制备的微粒体CYP450同工酶与其特异性基质氯唑沙宗(Chlorzoxazone,CZX)的反应,以测试有效的人类肝脏CYP450同工酶抑制剂,并测试CYP450同工酶抑制剂的原理。在添加测试样品后,通过使用对照组的6-OH-CZX的量作为基线,将CYP450同工酶代谢物标准品6-OH-CZX(6-羟基-氯唑酮)的量特定用于计算测试样品的CYP450同工酶(CYP2E1)抑制率。

[0218] 所有样品的测试皆进行三重复。为了测定抑制百分比,将每种试验化合物溶解至1、2、4μg/mL三种不同浓度。将在试验化合物存在下的CYP2E1活性量与对照组的培育物进行比较。将含有0.5mg微粒体蛋白质的500-μL反应混合物与320μM CZX,在含有5mM $MgCl_2$和1mM NADPH且pH 7.4的50mM磷酸盐缓冲液中,于37℃下培育30分钟。以冰冷的乙腈终止反应,然后加入4-羟基甲苯磺酰胺作为内部标准品。在液相色层分析-串联质谱仪(LC-MS/MS)分析之前,将有机相蒸发至干燥并重构到流动相(甲醇:水=1:1)中。使用装备有离子喷雾(ESI)源的API3000三重四极杆质谱仪来测定人类肝脏微粒体中的6-OH-CZX。ESI接口是用于正离子模式。在m/z 284.5→185.9的转变处监测6-OH-CZX。

[0219] 结果分析:使用对照组作为基线,将从LC/MS/MS获得的检测信号值转换为CYP450同工酶代谢物标准品6-羟基-氯唑烷酮的量(pmol),即对照组的CYP450同工酶抑制率为0%。在试验化合物存在下,比较CYP450同功酶活性量与对照组培育物。

[0220] 4.2结果

[0221] 二乙基二硫代胺基甲酸(Diethyldithiocarbamic acid,DDTC)是已知的CYP2E1抑制剂。在100μM的浓度下,处理DDTC导致人类肝脏微粒体中CYP2E1的90.9%抑制率(使用CZX作为CYP2E1的基质来测量)。基于观察到的DDTC的抑制活性,我们测试了在浓度为4、2和1μg/mL时CYP2E1抑制的新化合物(前药)及其相关代谢物。结果总结在表1中。

[0222] 表1.从人类肝脏微粒体的体外筛选而来之CYP2E1抑制剂的抑制率

[0223]

试验化合物	CYP 2E1 抑制率(%)		
测试浓度	4 μg/mL	2 μg/mL	1 μg/mL
对照组	0	0	0
阳性对照组 (DDTC)	(100 μM) 90.9 ± 0.8	(50 μM) 51.2 ± 3.2	(10 μM) 11.2 ± 2.4
前药	45.7 ± 2.5	33.3 ± 4.1	17.7 ± 0.7
代谢物_1 (甘露醇)	40.3 ± 1.6	34.1 ± 4.1	30.1 ± 2.4
代谢物_2 (三氯蔗糖)	32.9 ± 4.6	30.2 ± 2.8	25.1 ± 1.4
中间代谢物 (具有保护基的 C6-甘露醇，式 C)	70.3 ± 2.8	56.5 ± 1.7	40.5 ± 2.3

[0224] 在人类肝脏微粒体中检测的试验化合物的CYP 2E1抑制率如表1所示。从该结果得知,试验化合物,包括前药(化合物F)及其代谢物,即甘露醇、三氯蔗糖与具有保护基的C6-

甘露醇(式C)已经证实作为P450 2E1抑制剂是有效的,其中4μg/mL前药的中间代谢物(即具有保护基的C6-甘露醇,式C)显示出最好的抑制效果(70.3±2.8%)。

[0225] 实施例5:分析以对乙酰胺基酚(APAP)和CCl_4诱导的肝损伤

[0226] 5.1材料和方法

[0227] 5.1.1试剂

[0228] 所有有机溶剂均为HPLC等级,购自Tedia公司(Fairfield,俄亥俄州,美国)。APAP购自Sigma公司(St.Louis,密苏里州,美国),半乳糖可注射溶液由Southern Photochemical Co.公司制造,系通过将400g的半乳糖(Sigma公司)溶解于1L含有注射用的等张盐类的缓冲溶液中制备。

[0229] 5.1.2动物

[0230] 体重为175-280g的雄性SD(Sprague-Dawley)大鼠系购自中国台湾某实验动物机构。该研究系根据中国台湾的卫生研究机构的进行动物研究指南所进行,且所有的大鼠被放置在空气/湿度控制的环境中,在12小时日间/12小时夜间的循环以及无限制供应水及食物的环境下。在研究过程中,在正常供水下连续监测大鼠的体重。

[0231] 5.1.3处理

[0232] 5.1.3.1由APAP诱导的肝损伤

[0233] 基于由APAP诱导的肝损伤,使用甘露醇和三氯蔗糖进行动物试验(大鼠)。

[0234] 在正常对照组(第1组)中,动物没有被喂食APAP。在APAP诱导肝损伤的对照组(第2组)中,动物被喂食单剂量的APAP,其施用量为每公斤体重2,000mg,以诱导肝毒性。在以NAC治疗的阳性对照组(第3组)中,动物被喂食单剂量的APAP,其施用量为每公斤体重2000mg,以诱导肝毒性,4小时后,开始通过试管喂养的24小时治疗期,包括首先施用140mg的NAC(每公斤体重),随后每4小时施用70mg的NAC(每公斤体重)五次。在实验组(第4组)中,动物被喂食单剂量的APAP,其施用量为每公斤体重2,000mg,以诱导肝毒性,4小时后,开始通过试管喂养的24小时治疗期,包括每4小时投与本发明之成分共6次,各小组如下所示:

[0235] (a)(第4.1组):以小于或等于100mg每人每4小时的剂量施用甘露醇,持续24小时,

[0236] (b)(第4.2组):每4小时施用为第4.1组的2倍剂量之甘露醇,持续24小时,

[0237] (c)(第4.3组):以小于或等于100mg每人每4小时的剂量施用三氯蔗糖,持续24小时,

[0238] (d)(第4.4组):每4小时施用为第4.3组的2倍剂量之三氯蔗糖,持续24小时,

[0239] (e)(第4.5组):每4小时施用为第4.1组的0.5倍剂量之甘露醇以及为第4.3组的0.5倍剂量之三氯蔗糖的组合,持续24小时,

[0240] (f)(第4.6组):每4小时施用为第4.1组的剂量之甘露醇以及为第4.3组的剂量之三氯蔗糖的组合,持续24小时,

[0241] (g)(第4.7组):每4小时施用为第4.1组的1.5倍剂量之甘露醇以及为第4.3组的1.5倍剂量之三氯蔗糖的组合,持续24小时,

[0242] (h)(第4.8组):每4小时施用为第4.1组的2倍剂量之甘露醇以及为第4.3组的2倍剂量之三氯蔗糖的组合,持续24小时,以及

[0243] (i)(第4.9组):首先施用140mg的NAC每公斤体重,随后施用70mg的NAC加上为第4.1组的2倍剂量之甘露醇以及为第4.3组的2倍剂量之三氯蔗糖的组合,每4小时一次,共五

次。

[0244] 在24小时治疗期后，从大鼠的尾动脉收集血液，以用于AST/SLT测定。随后，对大鼠进行GSP测试。最后，牺牲大鼠并进行组织学分析。

[0245] 5.1.3.2由CCl_4诱导的肝损伤

[0246] 基于由CCl_4诱导的肝损伤，以选自本文所述之活性成分的甘露醇和三氯蔗糖进行动物试验(小鼠)。

[0247] 在正常对照组中，通过腹腔注射给予动物生理盐水。在CCl_4诱导之肝损伤的对照组中，以腹腔注射给予动物10ml/kg CCl_4(40％在玉米油中)以诱导肝毒性。在实验组中，以腹腔注射给予动物10ml/kg CCl_4(40％在玉米油中)以诱导肝毒性，4小时后，通过试管喂食给予本发明的不同成分。在给予本发明之成分之前或在给予本发明之成分24小时后，自小鼠体内收集血液以进行AST/ALT分析。最后，在第2天牺牲动物，收集血液以进行AST/ALT分析，并进行组织学分析。

[0248] 另一方面，对其他实验组的小鼠喂食本发明之成分12周，并对小鼠进行GSP测试。

[0249] 5.1.4血液样品

[0250] 处理完成后，在乙醚麻醉下牺牲大鼠，从大鼠的尾动脉收集血液，并置于含有EDTA的试管中。将血浆于4℃下以13,000rpm离心15分钟，将分离的血浆等分转移到离心小管中，并储存于-80℃。

[0251] 5.1.5生物化学分析

[0252] 通过测量血浆AST和ALT活性来定量肝损伤。AST和ALT是肝毒性的常见指标，并使用Synchron LXi 725系统(Beckman Instruments公司，美国)进行测量。

[0253] 5.1.6光学显微镜

[0254] 将大鼠牺牲后，进行组织学分析。肝脏样品以含有10％甲醛之磷酸盐缓冲溶液进行固定、脱水、包埋在石蜡中，制备5μm厚度的切片，然后以苏木精和曙红染色，并进行高碘酸希夫染色(Periodic acid Schiff stain，PAS)。在光学显微镜下观察染色切片。

[0255] 5.1.7肝功能的定量测试

[0256] 在研究完成后，对所有大鼠进行GSP测试。以静脉注射在30秒内对大鼠注射0.4g/ml体重的半乳糖溶液0.5g/kg，并于注射后5、10、15、30、45和60分钟时，从大鼠的尾动脉各收集一份血液样品。比色半乳糖脱氢酶用于定量半乳糖的浓度，且该试验浓度范围为50至1,000μg/ml。使用标准偏差和变异系数(CV)计算每个浓度的日内变化，且最大允许变异系数为10％CV，并且通过比较校准曲线的斜率和截距来检查每日之间的变化。GSP为停止该30秒注射后在60秒时获得的血液半乳糖浓度。

[0257] 5.1.8统计分析

[0258] 所有数据以平均值±标准偏差(standard deviation，SD)表示，并且使用ANOVA计算结果以确定显着性。以社会科学统计软件包(版本13，SPSS Inc.公司)进行计算，接着以事后测试来检查多重比较的最小显着差异，以便确认各组之间的显着差异，而且当$p<0.05$时，各组之间的平均差异是显着的。

[0259] 5.2结果

[0260] 5.2.1甘露醇和三氯蔗糖和其它成分能有效治疗由APAP诱导的肝损伤

[0261] 结果如表2所示。

[0262] 表2

[0263]

肝功能参数	GSP (mg/L)	AST (IU/L)	ALT (IU/L)	总 HAI 积分	存活率 (第 14 日，n/n)
第 1 组： 正常对照组	220 ±	186 ±	65 ± 16	0.0 ± 0.0	3/3
(NC, n=6)	24	16			
第 2 组： APAP 对照组 (2,000 *mg/kg*, n=12)	1017 ± 170	1151 ± 310	746 ± 143	8.6 ± 0.5	2/12
第 3 组： NAC (140mg/kg 的 NAC 接着每 4 小时间隔处理一次 5 × 70 mg/kg NAC, n=6)	393 ± 68***	428 ± 74***	221 ± 69***	4.2 ± 0.8***	3/6
第 4.1 组(n=3) (甘露醇的剂量小于或等于每人 100 mg) x6	565 ± 177***	455 ± 78***	209 ± 16***	4.0 ± 0.0***	1/3
第 4.2 组(n=3) (第 4.1 组的 2 倍剂量 (甘露醇)) x6	354 ± 56***	300 ± 40***	166 ± 15***	4.0 ± 1.0***	3/3
第 4.3 组(n=3) (三氯蔗糖的剂量小于或等于每人 100 mg) x6	332 ± 42***	331 ± 41***	154 ± 49***	4.0 ± 1.0***	3/3
第 4.4 组(n=3) (第 4.3 组的 2 倍剂量 (三氯蔗糖)) x6	309 ± 54***	277 ± 78***	136 ± 48***	3.0 ± 1.0***	3/3
第 4.5 组(n=3) (第 4.1 组的 0.5 倍剂量(甘露醇) +第 4.3 组的 0.5 倍剂量(三氯蔗糖)) x6	332 ± 61***	360 ± 81***	149 ± 19***	2.0 ± 1.0***	3/3
第 4.6 组(n=3) (第 4.1 组的剂量(甘露醇) +第 4.3 组的剂量(三氯蔗糖)) x6	271 ± 52***	193 ± 34***	81 ± 18***	1.5 ± 1.0***	6/6
第 4.7 组(n=3) (第 4.1 组的 1.5 倍剂量(甘露醇) +第 4.3 组的 1.5 倍剂量(三氯蔗糖)) x6	265 ± 53***	203 ± 24***	83 ± 25***	1.0 ± 1.0***	3/3
第 4.8 组(n=3) (第 4.1 组的 2 倍剂量(甘露醇) +第 4.3 组的 2 倍剂量(三氯蔗糖))x6	227 ± 25***	159 ± 21***	69 ± 10***	0.5 ± 0.5***	6/6
第 4.9 组(n=3) 140 mg/kg NAC + 5 x (70 mg NAC +第 4.1 组的 2 倍剂量(甘露醇) +第	233 ± 41***	171 ± 25***	58 ± 9***	0.3 ± 0.5***	6/6

[0264]

[0265]

4.3 组的 2 倍剂量(三氯蔗糖))					
第 5 组(n=6)(Aerosil 200 的剂量小于或等于每人 100 mg)	280 ± 98***	247 ± 43***	66 ± 18***	2.8 ± 1.0***	6/6
第 6 组(n=6)(羟基乙酸淀粉钠的剂量小于或等于每人 100 mg)	294 ± 30***	248 ± 37***	81 ± 27***	2.7 ± 1.2***	6/6
第 7 组(n=6)(交聚维酮的剂量小于或等于每人 100 mg)	372 ± 90***	323 ± 40***	175 ± 61***	2.8 ± 1.5***	6/6
第 8 组(n=6)(微晶纤维素的剂量小于或等于每人 100 mg)	259 ± 36***	217 ± 28***	72 ± 21***	2.2 ± 1.0***	6/6
第 9 组(n=6)(聚维酮 K-30 的剂量小于或等于每人 100 mg)	287 ± 38***	220 ± 53***	71 ± 26***	2.5 ± 1.0***	6/6

[0266] *p<0.05,**p<0.01,***p<0.005:各实验组与APAP对照组的比较

[0267] 结果显示,APAP肝毒性组发生肝损伤。相较之下,这种肝损伤和存活率可以通过使用甘露醇及/或三氯蔗糖,以剂量依赖的方式提高。特别是,甘露醇和三氯蔗糖的组合实现协同效应;结果与正常对照组相似,甚至优于以NAC标准治疗的阳性对照组。此外,包括Aerosil 200、羟基乙酸淀粉钠、交聚维酮、微晶纤维素和聚维酮K-30的其它成分也被发现对治疗肝损伤有效,也比使用NAC标准治疗的阳性对照组更好。

[0268] 改善的结果也反映在相应的肝组织中。

[0269] 图4所示为组织学分析的结果。来自APAP肝毒性组的大鼠的肝组织切片显示,围绕中心静脉的肝细胞被破坏,带有可见的空泡化现象以及细胞核数目的减少,一些肝细胞甚至显示出坏死的迹象,而且与来自正常对照组大鼠的肝细胞(图4B)相比,肝损伤更为严重。相反地,对照组大鼠的肝脏结构正常,肝细胞完整,按顺序排列,没有空泡化现象(图4A)。而以甘露醇及/或三氯蔗糖处理的实验组的肝切片,肝细胞相对完整,具有可见的核及较少的空泡化现象(图4D、E、F、G、H)。尤其是,甘露醇和三氯蔗糖的组合实现最佳保护效果(图4G);其结果甚至优于以NAC标准治疗的阳性对照组(图4C)。

[0270] 5.2.2甘露醇可有效治疗由CCl_4诱导的肝损伤

[0271] 结果如表3所示。

[0272] 表3

[0273]

	肝功能参数			
组别	GSP (mg/L)	AST (IU/L)	ALT (IU/L)	总 HAI 积分
正常对照组(n=10)	315 ± 48	88 ± 20	57 ± 17	0.0 ± 0.0
CCl_4 对照组(n=10)	914 ± 205***	815 ± 216***	770 ± 274***	6.2 ± 2.1***
山奈酚的剂量小于或等于每人 100 mg (n=10)	**456 ± 101*** **	**198 ± 105*** **	**128 ± 40*** **	**4.3 ± 1.3***
表没食子儿茶素-3-没食子酸酯的剂量小于或等于每人 100 mg (n=10)	**312 ± 140*** **	**144 ± 49*** **	**95 ± 36*** **	**1.7 ± 0.9*** **
槲皮素的剂量小于或等于每人 100 mg (n=10)	**286 ± 70*** **	**115 ± 40*** **	**93 ± 26*** **	**1.1 ± 0.7*** **
甘露醇的剂量小于或等于每人 100 mg (n=10)	**290 ± 78*** **	**91 ± 28*** **	**77 ± 22*** **	**0.8 ± 0.5*** **

[0274] 统计分析:Anova与LSD测试。

[0275] ***p<0.005,**p<0.01,*p<0.05,实验组与CCl_4对照组比较。

[0276] 结果显示,CCl_4对照组发生肝损伤。相反的,这种肝损伤可以通过使用甘露醇来改善。

[0277] 实施例6:脂肪肝的测定

[0278] 6.1材料和方法

[0279] 6.1.1细胞株和细胞培养基

[0280] 通过使用人类肝癌细胞株Hep G2分析本文所述之各种成分(包括甘露醇和三氯蔗糖以及其它成份)对于减少脂肪含量的活性。

[0281] 使用Dulbecco改良的Eagle培养基(Dulbecco's Modified Eagle's Medium,DMEM)制备表4中列出的用于进行后续实验的DMEM培养基编号A-F。

[0282] 表4:DMEM培养基编号A-F的制备

[0283]

DMEM培养基	制备方法
编号A	将DMEM搅拌溶解于1,400 mL水中，然后加入2 g 4-(2-羟乙基)-1-哌嗪-乙磺酸(HEPES)形成溶液，加入碳酸氢钠溶液(4 g的碳酸氢钠粉末搅拌溶解在400mL水中)，并以水将体积补足至2000mL。以5N HCl将所得溶液的pH调整为7.3 ± 0.05。以0.2 μm无菌膜过滤后，将最终溶液分配到无菌血清瓶，并在4°C下储存。
编号B	50 mL去活性的胎牛血清(FBS)、5mL丙酮酸钠(100 mM)、5 mL青霉素(100 U/mL)和链霉素(100 U/mL)和5 mL MEM非必需胺基酸溶液(100X)加入450 mL DMEM培养基编号A中。

[0284]

编号C	将5mL丙酮酸钠(100 mM)、5mL青霉素(100 U/mL)和链霉素(100 U/mL)和5 mL MEM非必需胺基酸溶液(100X)加入450 mL DMEM 培养基编号A中。
编号D	将DMEM培养基编号B加入到油酸盐/白蛋白复合物中。 根据Van Harken等人在1969年提出的方法制备油酸盐/白蛋白复合物(J Biol Chem. 1969 May 10; 244(9)：2278-85)。该方法包括：取25 mL添加有5 g牛血清白蛋白(BSA)的DMEM培养基编号A，然后加入5N氢氧化钠溶液以将pH调整为7.4以形成混合物。然后将混合物置于0°C的冰浴中以形成BSA溶液。将油酸溶解在50ml乙醇(95%)中，然后以1N氢氧化钠溶液滴定至酚酞滴定终点。通过流动氮气去除乙醇。将所得的油酸钠在37°C下溶解于DMEM培养基编号A中以形成油酸钠溶液。最后，在搅拌下将BSA溶液滴加到油酸钠溶液中以形成油酸盐/白蛋白复合物溶液。
编号E	将各种含量的水飞蓟素溶于DMEM培养基编号C中。
编号F	将各种含量的本发明之试验化合物溶解于DMEM培养基编号C中。

[0285] 将DMEM培养基编号A-F置于2-8℃下保存，并在实验前于37℃水浴中温热。

[0286] 6.1.2细胞计数和存活性试验

[0287] 死细胞会吸收0.4％台盼蓝并显色；而活细胞由于其细胞膜完整而排除某些染料并且呈现透明。将100μl细胞悬浮液和等体积的0.4％台盼蓝均匀混合以形成混合物。将一些混合物(约20μl)加到血球细胞计数器上的凹槽中，然后覆盖盖玻片以在光学显微镜下观察。活细胞没有被染色，死细胞呈现蓝色。

[0288] 6.1.3自HepG2细胞株的细胞以油酸诱导形成脂肪肝细胞

[0289] 将HepG2细胞株(15×10^6个细胞)在DMEM培养基编号B中培养，在37℃、含5％CO_2的培养箱中孵育24小时后，于DMEM培养基编号C(无血清培养基)中培养24小时，最后在DMEM培养基编号D(含有油酸盐/白蛋白复合物)中再培养48小时以诱导HepG2细胞株形成脂肪肝细胞。

[0290] 6.1.4每组脂肪肝细胞的处理

[0291] 将HepG2细胞株分为六组，包括：(1)空白组：无处理；(2)DMSO组：来自空白组的细胞以二甲基亚砜(dimethyl sulfoxide，DMSO)处理；(3)对照组：以油酸诱导形成脂肪肝细胞；(4)载体组：以油酸诱导形成的脂肪肝细胞再以DMSO处理；(5)阳性对照组：以水飞蓟素处理脂肪肝细胞；和(6)测试组：以本发明之各种化合物处理脂肪肝细胞。

[0292] 6.1.5细胞中三酸甘油酯(TG)的测定

[0293] 孵育72小时后，将来自每组处理过后的细胞在PBS中连续洗涤二次，然后与0.5ml胰蛋白酶/EDTA培育3分钟。之后以2ml PBS刮擦细胞，然后转移到离心管中以超音波破碎。取20μl体积的细胞萃取物以测量蛋白质含量。使用市售的试剂组合(Randox)进行TG测定。将上述获得的TG含量除以蛋白质含量，得到之比值表示细胞中TG的相对含量。

[0294] 6.1.6实验动物

[0295] 选择中国台湾的卫生管理机构所公布之规范“评价保健食品的肝保护和保健功效

之方法”中推荐的B6小鼠用于动物试验。在前测的每一组中使用四只以上的小鼠，而在确认试验的每一组中使用十二只以上的小鼠。在23±2℃、具有55±15％相对湿度的动物房中，于正常光/暗循环(上午7:00至下午7:00亮灯/下午7:00至上午7:00关灯)的环境下繁殖的雄性小鼠，重量为18-23g，购自BioLASCO公司(中国台湾，台北)，并置于中国台湾的某医学研究机构的实验动物中心。根据中国台湾的卫生研究机构的动物实验指南进行动物试验。小鼠以3-5g/天喂食正常饲料，无限量供应饮水1-2周，并研究健康状况，小鼠的体重每周记录一次。

[0296] 6.1.7动物分组

[0297] 将测试的动物随机分组为空白组、高脂肪饮食对照组(High Fat Diet，HFD)、阳性对照组(Positive Control，PS)和试验组。对空白组的动物喂食正常饲料。对HFD组的动物喂食高脂肪饲料。对PS组的动物喂食高脂肪饲料，另外并以试管喂食水飞蓟素(5mg/kg/天)。对试验组的动物喂食高脂肪饲料，另外并以试管喂食试验化合物。

[0298] 6.1.8试验方法

[0299] 以正常饲料随意喂食空白组动物12周，而以高脂肪饲料随意喂食HFD组、PS组和试验组的动物12周。喂养8周后，以试管对空白组和HFD组的动物每天加入一次去离子水；每天以试管对PS组动物喂食水飞蓟素一次；而且每天以试管对试验组动物喂食试验化合物一次，持续4或8周的期间。

[0300] 在测试之前以及在测试后的第八、第十二和第十六周，从脸颊或心脏收集血液。在试验结束时，对所有的小鼠称重，然后牺牲小鼠，并同时从脸颊或心脏收集血液。小鼠的血液样品在室温下静置1小时以使其凝结，然后以冷冻离心机中在15,700×g、4℃下离心5分钟以分离血清。随后，以自动血液生化分析仪检测肝功能的生物化学指针，包括天冬胺酸转胺酶(AST)、丙胺酸转胺酶(ALT)、三酸甘油酯(TG)、总胆固醇(TCHO/TC)、低密度脂蛋白胆固醇(LDL-C)和高密度脂蛋白胆固醇(HDL-C)。

[0301] 此外，从牺牲的小鼠腹部取出腹部脂肪和肝脏样品，并称重以比较脂肪和肝脏的重量，并获得肝脏重量与体重的比率。从最大的右肝叶切下体积为约1cm^{3}的二个组织块，固定在10％中性甲醛溶液中，然后以石蜡包埋并进行切片。对切割下的切片进行H&E染色以用于组织病理学观察。此外，将其余肝脏冷冻以保存并检测肝脏中三酸甘油酯和总胆固醇的含量。另外，通过半乳糖单点法分析每组动物的肝功能，该方法被认可并被美国食品药物管理局(FDA)以及中国台湾的卫生管理机构推荐于临床使用上进行剩余肝功能的定量。在试验结束时，以静脉注射给予每只动物0.5g半乳糖G.S.P.®0.4g/mL)/kg动物。给药1小时后，以滤纸取约0.5ml全血，分析小鼠的肝功能。GSP值越高，剩余的肝功能越差。(FDA:“产业指南：受损患者的药物动力学，肝功能研究设计，数据分析及对剂量和标记的影响，2003年”。)

[0302] 6.1.9组织病理学组织切片：

[0303] 在试验结束时，牺牲所有小鼠。从最大的右肝叶切下体积约1cm^{3}的一个组织块，固定在10％中性甲醛中，然后在各种浓度的乙醇(30、50、70、95、99.5％)及二甲苯中脱水和透明化。然后，以热石蜡溶液替换二甲苯。最后，以石蜡溶液包埋组织。以切片机将成品石蜡样品切成5μm厚的石蜡切片。将切片贴在干净的载玻片上，于37℃下干燥，然后以H&E染色法进行染色。

[0304] 6.1.10苏木精和伊红染色(H&E)

[0305] 将肝组织切片在二甲苯中脱蜡30分钟，然后在99.5％、95％、70％、50％和30％含水乙醇中分别连续再水合30分钟。在蒸馏水中浸泡10分钟后，可对切片进行染色。首先将切片浸入苏木精中30秒以对细胞核进行染色，然后以蒸馏水洗涤数分钟后，再以曙红染色2-5分钟，再以蒸馏水再次洗涤数分钟。染色结束后，切片分别在50％、70％、95％和100％乙醇水溶液中脱水两次，每次30秒，在二甲苯中透明化两次，最后密封并用封固剂保存。

[0306] 6.1.11组织病理学观察

[0307] 当存在持续的肝损伤时，为了观察肝细胞中损伤、脂肪蓄积、坏死或纤维化的变化，对肝脏组织进行H&E染色以评估肝脏脂肪蓄积的程度。所有组织病理切片从肝脏最大右叶上的相同位置切除，以消除主观观察中的偏差，然后进行病理染色。针对病理学中的半定量分析评估，必须由医师或兽医病理学家确认，其进行双盲分析以评分(NAS评分)，而且在不知道试验设计的情况下比较所有的切片。最后，以统计学方法进行每组的差异分析。

[0308] 6.1.12肝抗氧化能力的分析

[0309] 从牺牲的动物中取约0.1g肝脏组织，并以生物均质机离心10分钟使其均质化。将9倍重量(w/w)的缓冲液(pH 7.4，50mmol/L Tris-HCl，180mmol/L KCl)加入该均质化的组织中，然后以震荡混合器充分混合以备用。使用所得到的肝组织匀质化溶液样品以分析肝脏抗氧化系统中的各种成员，包括谷胱甘肽过氧化物酶(GPx)、谷胱甘肽(GSH)、谷胱甘肽还原酶(Grd)和超氧化物歧化酶(SOD)。相关分析的方法可以在已知文献中找到，例如，中国台湾的卫生管理机构所公布的“评价保健食品的肝保护和保健功效之方法”的草案。

[0310] 6.1.13统计分析

[0311] 所有数据以平均值±标准偏差(standard deviation，SD)表示。以社会科学统计软件包，版本13，SPSS Inc.公司，计算单因子变异数分析(one-way ANOVA)以决定试验结果的统计学显着性差异。之后，以事后测试中的最小显着差异方法进行多重比较，以确认各组之间的显着差异。当$p<0.05$时，判断组间的平均差异为显着。

[0312] 6.2结果

[0313] 6.2.1细胞实验

[0314] 在细胞实验中，在阳性对照组(水飞蓟)中测量HepG2细胞中TG含量降低的结果如表5所示。

[0315] 表5：水飞蓟素对于在阳性对照组的HepG2脂肪细胞中TG含量降低的功效

[0316]

水飞蓟素浓度(μM)	细胞中 TG 含量(μg/mg 蛋白质)	TG 含量降低率(%)
0 (对照组)	59.43 ± 4.60	-
1.0	44.17 ± 2.41	29 ± 8
5.0	44.59 ± 11.53	28 ± 10
10	26.38 ± 9.12	63 ± 11
100	20.48 ± 4.76	78 ± 5

[0317] 以浓度恒定的试验化合物测量HepG2脂肪细胞中TG含量降低的结果如表6所示。如结果所示，相对于对照组，在浓度恒定的试验条件下，试验化合物在由被诱导的HepG2细胞形成的脂肪肝细胞中表现出不同程度的降低TG含量的效果。计算TG的减少率(％)的方程式如下：[1-(试验组的TG含量-空白组的TG含量)/(油酸诱导组的TG含量-空白组的TG含量)]×100％。

[0318] 表6:以试验化合物减少的脂肪肝细胞中的TG含量

测试物质(1.0 μM)	TG 减少率(%)
水飞蓟素对照组	35.33 ± 1.96
葛根素	49.91 ± 7.73
根皮苷	42.35 ± 6.05
大豆黄素	42.3 ± 5.34
十二烷基硫酸钠	38.73 ± 4.65
枸杞子	38.12 ± 7.22
甜橙黄酮	36.97 ± 4.84
(-)-表没食子儿茶素	36.78 ± 6.67
山奈酚	36.51 ± 4.78
异牡荆素	35.93 ± 3.35
熊果酸	35.86 ± 8.92
圣草酚	35.11 ± 0.87
(+)-苎烯	35.02 ± 10.04
橙皮苷	34.81 ± 5.25
麦角固醇	34.19 ± 3.69
β-月桂烯	33.97 ± 11.22

[0319]

[0320]

(-)-表儿茶素-3-没食子酸酯	32.7	±	4.33
高胆固醇	30.51	±	2.8
水飞蓟宾	30.26	±	3.24
(+)-儿茶素	29.57	±	4.02
芒柄花素	29.55	±	1.44
肉豆蔻酸乙酯	28.88	±	3.91
高良姜精	28.11	±	8.62
三氯蔗糖	26.68	±	2.93
二十碳五烯酸(EPA)	26.15	±	6.14
桑色素	25.84	±	10.65
甘露醇	22.35	±	5.74
金松双黄酮	21.83	±	5.04
汉黄芩素	20.78	±	1.12
香蜂草苷	20.37	±	12.69
棉纤维素	20.25	±	4.63
山梨醇	20.06	±	2.57
木犀草素-7-葡萄糖苷	19.33	±	4.59
聚维酮 K-30	18.93	±	5.13
原儿茶酸	18.57	±	7.6
(+)-紫杉叶素	17.91	±	8.35
糖精	17.53	±	6.96
伞形酮	17.4	±	2.57
甘油	16.23	±	4.25
橙皮素	16.08	±	5.55
去甲二氢愈创木酸	15.92	±	2.3
反式肉桂酸	15.85	±	0.82
苯甲酸钠	14.35	±	4.86
氧化红	13.59	±	2.08
新橙皮苷	13.29	±	7.21
柚皮苷	12.69	±	3.72
月见草内含物	11.86	±	3.73
(-)-表儿茶素	10.76	±	8.92
甘草甜素	10.55	±	7.4

[0321]

蒙花苷	9.24	±	12.34
黄芩苷	9.21	±	6.21
槲皮苷	9.15	±	9.24
木糖醇	7.36	±	6.34
黄芩素	7.09	±	10.88
木犀草素	6.95	±	15.23
獐牙菜苦苷	6.72	±	11.04
丁基化羟基茴香醚	6.21	±	3.8
环己烷胺基磺酸钠	4.77	±	4.49
薄荷醇	66.24	±	1.87
柠檬酸	2.55	±	4.43
柠檬油	0.56	±	1.07
预胶化淀粉	7.18	±	13.41
山梨酸	2.03	±	1.96

[0322] 表6-1:来自表6之部分试验化合物,其可降低脂肪肝细胞中的TG含量

[0323]

测试物质(1.0 μM)	TG 减少率(%)		
葛根素	49.91	±	7.73
根皮苷	42.35	±	6.05
大豆黄素	42.3	±	5.34
甜橙黄酮	36.97	±	4.84
(-)-表没食子儿茶素	36.78	±	6.67
山柰酚	36.51	±	4.78
熊果酸	35.86	±	8.92
对照组的水飞蓟素	35.33	±	1.96
(+)-苎烯	35.02	±	10.04
橙皮苷	34.81	±	5.25
(-)-表儿茶素-3-没食子酸酯	32.7	±	4.33
水飞蓟宾	30.26	±	3.24
芒柄花素	29.55	±	1.44
肉豆蔻酸乙酯	28.88	±	3.91
二十碳五烯酸 (EPA)	26.15	±	6.14
汉黄芩素	20.78	±	1.12

[0324]

聚维酮 K-30	18.93	±	5.13
原儿茶酸	18.57	±	7.6
伞形酮	17.4	±	2.57
橙皮素	16.08	±	5.55
去甲二氢愈创木酸	15.92	±	2.3
新橙皮苷	13.29	±	7.21
柚皮苷	12.69	±	3.72
(-)-表儿茶素	10.76	±	8.92
甘草甜素	10.55	±	7.4
黄芩苷	9.21	±	6.21
槲皮苷	9.15	±	9.24
黄芩素	7.09	±	10.88

[0325] 表6-2:来自表6的部分试验化合物(类黄酮),其降低脂肪肝细胞中的TG含量

[0326]

测试物质(1.0 μM)	TG 减少率(%)	
枸杞子	38.12	± 7.22
异牡荆素	35.93	± 3.35
圣草酚	35.11	± 0.87
麦角固醇	34.19	± 3.69
β-月桂烯	33.97	± 11.22
高胆固醇	30.51	± 2.8
(+)-儿茶素	29.57	± 4.02
高良姜精	28.11	± 8.62
桑色素	25.84	± 10.65
金松双黄酮	21.83	± 5.04
香蜂草苷	20.37	± 12.69
棉纤维素	20.25	± 4.63
木犀草素-7-葡萄糖苷	19.33	± 4.59
(+)-紫杉叶素	17.91	± 8.35
反式肉桂酸	15.85	± 0.82
月见草内含物	11.86	± 3.73
蒙花苷	9.24	± 12.34
木糖醇	7.36	± 6.34

[0327]

木犀草素	6.95	± 15.23
獐牙菜苦苷	6.72	± 11.04

[0328] 表6-3:来自表6的部分试验化合物(赋形剂),其降低脂肪肝细胞中的TG含量

[0329]

测试物质(1.0μM)	TG减少率(%)
十二烷基硫酸钠	38.73±4.65
三氯蔗糖	26.68±2.93
甘露醇	22.35±5.74
山梨醇	20.06±2.57
糖精	17.53±6.96
甘油	16.23±4.25
苯甲酸钠	14.35±4.86
氧化红	13.59±2.08
丁基化羟基茴香醚	6.21±3.8
环己烷胺基磺酸钠	4.77±4.49
薄荷醇	66.24±1.87
柠檬酸	2.55±4.43
柠檬油	0.56±1.07
预胶化淀粉	7.18±13.41
山梨酸	2.03±1.96

[0330] 6.2.2动物实验

[0331] 在动物实验中,除了用正常饲料喂养的空白组动物之外,所有的动物皆经处理以诱导产生脂肪肝。八周后,除了原始饲料之外,给予每组动物不同的治疗四周或八周。对空白组和HFD组的动物喂食去离子水;对PS组的动物喂食水飞蓟素;并且以不同的试验化合物,包括葛根素、根皮苷、圣草酚、三氯蔗糖、甘露醇、糖精、橙皮素、薄荷醇及其组合喂食测试组的动物。

[0332] 6.2.2.1对动物的体重、肝脏重量和体脂肪重量的影响以及试验化合物的安全性评估

[0333] 来自动物实验的结果,每组动物的肝脏重量、体脂肪重量和体重增加如表7-1和表7-2所列。

[0334] 表7-1:因试验化合物所造成之肝脏重量和体脂肪重量的分析结果

[0335]

项目	**腹部脂肪重量**	**肝脏重量**
单位	g	g

[0336]

项目	腹部脂肪重量			肝脏重量		
单位	g			g		
空白组 (n=13)	**0.6**	**± 0.2**	***	1.6	± 0.2	0.6
HFD 组 (n=12)	2.8	± 0.4		1.6	± 0.4	2.8
阳性对照组						
水飞蓟素组 5.0 mg/kg (n=6)	**2.0**	**± 0.4**	***	**1.2**	**± 0.3**	***
水飞蓟素组 1.5 mg/kg (n=6)	**2.3**	**± 0.5**	*	**1.5**	**± 0.1**	
单一试验化合物						
根皮苷 2.5 mg/kg(n=6)	**2.3**	**± 0.6**	*	**1.3**	**± 0.1**	*
圣草酚 2.5 mg/kg(n=6)	2.7	± 0.6		**1.3**	**± 0.1**	**
三氯蔗糖 7.5 mg/kg(n=6)	2.4	± 0.3		1.4	± 0.1	
三氯蔗糖 1.5 mg/kg(n=6)	**2.1**	**± 0.6**	**	1.5	± 0.2	
薄荷醇 1.5 mg/kg(n=6)	**2.3**	**± 0.6**	*	1.6	± 0.2	
甘露醇 7.5 mg/kg(n=6)	2.4	± 0.3		1.4	± 0.1	
甘露醇 4.5 mg/kg(n=6)	2.7	± 0.3		1.4	± 0.2	
甘露醇 1.5 mg/kg(n=6)	**2.0**	**± 0.3**	***	1.4	± 0.2	
糖精 1.5 mg/kg(n=3)	2.3	± 0.5		1.5	± 0.1	
葛根素 2.5 mg/kg(n=6)	2.8	± 0.3		1.4	± 0.2	
橙皮素 2.5 mg/kg(n=6)	3.0	± 0.5		1.5	± 0.1	
二种试验化合物之组合						
糖精 + 甘露醇 1.5 mg/kg +1.5 mg/kg(n=6)	2.7	± 0.4		1.4	± 0.2	2.7
薄荷醇 + 甘露醇 4.5 mg/kg + 4.5 mg/kg(n=6)	3.0	± 0.5		1.6	± 0.3	3.0
薄荷醇 + 甘露醇 1.5 mg/kg + 1.5 mg/kg(n=6)	2.3	± 0.6		1.5	± 0.3	2.3
三种试验化合物之组合						
薄荷醇 + 甘露醇+ 圣草酚 .5 mg/kg + .5 mg/kg + .8 mg/kg(n=6)	2.6	± 0.6		1.4	± 0.2	2.6

数据以平均值±标准偏差(SD)表示。ANOVA 和 LSD 得到的统计学差异以字词表示。与 HFD 组相比，*表示 p <0.05，**表示 p <0.01，***表示 p <0.005。

橙皮素
葛根素
圣草酚
根皮苷

TG：三酸甘油酯
TC：总胆固醇

[0337]

项目	**腹部脂肪重量**	**肝脏重量**
单位	g	g
甘露醇 薄荷醇 三氯蔗糖 糖精		

[0338] 表7-2:因试验化合物所造成之体重增加的分析结果

[0339]

项目	**增加的体重**	
单位	G	
空白组 (n=13)	15.6	± 7.9
HFD 组 (n=12)	14.0	± 8.4
阳性对照组		
水飞蓟素组 5.0 mg/kg (n=6)	10.2	± 12.7
水飞蓟素组 1.5 mg/kg (n=6)	10.9	± 4.3
单一试验化合物		
根皮苷 2.5 mg/kg(n=6)	13.7	± 10.7
圣草酚 2.5 mg/kg(n=6)	8.3	± 6.7
三氯蔗糖 7.5 mg/kg(n=6)	8.3	± 5.4
三氯蔗糖 1.5 mg/kg(n=6)	17.0	± 5.6
薄荷醇 1.5 mg/kg(n=6)	19.6	± 5.0
甘露醇 7.5 mg/kg(n=6)	10.3	± 8.5
甘露醇 4.5 mg/kg(n=6)	11.1	± 7.7
甘露醇 1.5 mg/kg(n=6)	10.9	± 7.4
糖精 1.5 mg/kg(n=3)	**27.7**	**± 12.7 ****
葛根素 2.5 mg/kg(n=6)	**21.7**	**± 3.1 ***
橙皮素 2.5 mg/kg(n=6)	14.5	± 8.3
二种试验化合物之组合		
糖精 + 甘露醇 1.5 mg/kg +1.5 mg/kg(n=6)	16.6	± 6.4
薄荷醇 + 甘露醇 4.5 mg/kg + 4.5 mg/kg(n=6)	15.6	± 5.0
薄荷醇 + 甘露醇 1.5 mg/kg + 1.5 mg/kg(n=6)	14.9	± 6.3

[0340]

项目	增加的体重
单位	G
三种试验化合物之组合	
薄荷醇 + 甘露醇+ 圣草酚 .5 mg/kg + .5 mg/kg + .8 mg/kg(n=6)	**21.7 ± 3.9 ***
数据以平均值±标准偏差(SD)表示。ANOVA 和 LSD 得到的统计学差异以字词表示。与 HFD 组相比，*表示 p <0.05，**表示 p <0.01，***表示 p <0.005。	
橙皮素 葛根素 圣草酚 根皮苷 甘露醇 薄荷醇 三氯蔗糖 糖精	TG：三酸甘油酯 TC：总胆固醇

[0341] 该结果显示，以脂肪肝诱导的动物的腹部脂肪重量增加。在各别施用的试验化合物之中，甘露醇、薄荷醇和三氯蔗糖可显着降低动物腹部脂肪的重量。

[0342] 此外，在给予试验化合物后，在测试组的动物中未观察到异常状况。在测试期间没有动物死亡。在测试后对牺牲动物的尸体进行解剖检验，没有观察到由试验化合物引起的疾病或临床症状的出现。因此，试验化合物是安全的。

[0343] 6.2.2.2试验化合物有效降低肝脏中的脂质

[0344] 图5所示为被诱导表现出脂肪肝的小鼠，其肝门区附近(包括胆管、门静脉、肝动脉)的肝细胞覆盖有许多大囊泡脂肪滴，并出现肝细胞肿胀，表示以诱导方式成功建立了脂肪肝的动物模型。

[0345] 动物实验的结果显示，在施用4周或8周时间后，多种试验化合物显现出减少动物肝脏中脂质的效果。结果如表8-1和表8-2所示。

[0346] 表8-1：试验化合物可以减少动物的肝脂质(给药期为4周)

[0347]

项目	**肝脏中的 TG**		**肝脏中的 TC**	
单位	mg/g **肝脏**		mg/g **肝脏**	
空白组 (n=13)	**25.0**	**± 9.2 *** **	**2.5**	**± 0.4 *** **
HFD 组 (n=12)	132.0	± 69.2	6.6	± 3.5
阳性对照组				
水飞蓟素组 5.0 mg/kg (n=6)	**46.8**	**± 14.4 *** **	**3.0**	**± 0.9 *** **
水飞蓟素组 1.5 mg/kg (n=6)	**69.9**	**± 32.3 ** **	**3.7**	**± 0.4 ** **
单一试验化合物				

[0348]

项目	肝脏中的 TG			肝脏中的 TC		
单位	mg/g 肝脏			mg/g 肝脏		
根皮苷 2.5 mg/kg(n=6)	**48.9**	**± 14.1**	***	**2.9**	**± 0.5**	***
圣草酚 5.0 mg/kg(n=6)	**54.2**	**± 15.0**	***	**3.0**	**± 0.9**	***
圣草酚 2.5 mg/kg(n=6)	**43.1**	**± 13.1**	***	**3.8**	**± 1.1**	**
三氯蔗糖 7.5 mg/kg(n=6)	**56.8**	**± 20.0**	***	5.0	± 0.9	
三氯蔗糖 1.5 mg/kg(n=6)	**68.9**	**± 37.5**	**	**3.0**	**± 0.9**	***
薄荷醇 1.5 mg/kg(n=6)	**87.3**	**± 72.3**	*	**4.4**	**± 3.5**	*
甘露醇 7.5 mg/kg(n=6)	**53.8**	**± 24.4**	***	4.7	± 1.2	
甘露醇 4.5 mg/kg(n=6)	**71.5**	**± 45.5**	***	7.2	± 2.8	
甘露醇 1.5 mg/kg(n=6)	**61.8**	**± 32.6**	***	**3.4**	**± 0.6**	***
糖精 1.5 mg/kg(n=3)	84.0	± 41.4		**2.8**	**± 1.5**	**
葛根素 2.5 mg/kg(n=6)	**89.4**	**± 49.1**	*	6.7	± 2.7	
橙皮素 2.5 mg/kg(n=6)	**67.8**	**± 16.6**	***	**3.7**	**± 0.7**	**
二种试验化合物之组合						
糖精 + 甘露醇 1.5 mg/kg +1.5 mg/kg(n=6)	**71.6**	**± 32.0**	***	8.5	± 2.5	
薄荷醇 + 甘露醇 4.5 mg/kg + 4.5 mg/kg(n=6)	**54.3**	**± 11.8**	***			
薄荷醇 + 甘露醇 1.5 mg/kg + 1.5 mg/kg(n=6)	**31.0**	**± 11.2**	***	6.9	± 1.7	
薄荷醇 + 甘露醇 .5 mg/kg + .5 mg/kg(n=6)	96.6	± 77.4		5.9	± 1.7	
三种试验化合物之组合						
薄荷醇 + 甘露醇+ 圣草酚 .5 mg/kg + .5 mg/kg + .8 mg/kg(n=6)	**83.1**	**± 50.9**	*	6.0	± 2.3	
数据以平均值±标准偏差(SD)表示。ANOVA 和 LSD 得到的统计学差异以字词表示。与 HFD 组相比，*表示 p <0.05，**表示 p <0.01，***表示 p <0.005。						
橙皮素 葛根素 圣草酚 根皮苷 甘露醇 薄荷醇 三氯蔗糖 糖精	TG：三酸甘油酯 TC：总胆固醇					

[0349] 表8-2：试验化合物可以减少动物的肝脂质(给药期为8周)

[0350]

项目	肝脏中的 TG	肝脏中的 TC
单位	mg/g 肝脏	mg/g 肝脏
空白组(n=7)	**22.6 ± 3.8 *** **	**3.8 ± 0.4 *** **
HFD 组 (n=8)	187.3 ± 91.2	12.1 ± 7.3
二种试验化合物之组合		
三氯蔗糖 + 甘露醇 7.5 mg/kg + 7.5 mg/kg(n=5)	**115.3 ± 36.2 ***	**6.0 ± 3.0 ** **
三氯蔗糖 + 甘露醇 1.5 mg/kg + 1.5 mg/kg(n=5)	144.4 ± 59.9	**6.0 ± 1.2 ***
圣草酚 + 甘露醇 5.0 mg/kg + 7.5mg/kg(n=4)	**64.5 ± 35.7 *** **	**3.6 ± 1.1 *** **
圣草酚 + 三氯蔗糖 5.0 mg/kg + 7.5 mg/kg(n=6)	**41.1 ± 28.1 *** **	**2.8 ± 1.0 *** **
三种试验化合物之组合		
三氯蔗糖 + 甘露醇 + 圣草酚 7.5 mg/kg + 7.5 mg/kg + 2.5 mg/kg(n=6)	**39.7 ± 21.5 ***	**4.6 ± 0.6 *** **
数据以平均值±标准偏差(SD)表示。ANOVA 和 LSD 得到的统计学差异以字词表示。与 HFD 组相比，*表示 p <0.05，**表示 p <0.01，***表示 p <0.005。		
圣草酚 甘露醇 三氯蔗糖	TG：三酸甘油酯 TC：总胆固醇	

[0351] 该结果显示,脂肪肝诱导的小鼠的肝脏中三酸甘油酯(TG)和总胆固醇(TC)升高。在各别施用的试验化合物中,橙皮苷、葛根素、紫草醇、根皮苷、甘露醇、薄荷醇和三氯蔗糖可显着降低肝脏中的三酸甘油酯(TG)。特别是,以圣草酚治疗4周后,达到肝脏中三酸甘油酯(TG)含量降低约67%(p<0.005)的优异效果。此外,橙皮素、圣草酚、根皮苷、甘露醇、薄荷醇、三氯蔗糖和糖精可以显着减少肝脏中的总胆固醇(TC)。特定而言,在以糖精治疗4周后,达到肝脏中的总胆固醇(TC)含量降低约56%(p<0.005)的优异效果。

[0352] 当施用二种试验化合物的组合时,糖精和甘露醇的组合、薄荷醇和甘露醇的组合、三氯蔗糖和甘露醇的组合、圣草酚和甘露醇的组合,或者圣草酚和三氯蔗糖的组合可以显着地减少肝脏中的三酸甘油酯(TG)。特别是,以薄荷醇和甘露醇的组合治疗4周后,可以达到肝脏中的三酸甘油酯(TG)含量降低约77%(p<0.005)的优异效果;且以圣草酚和三氯蔗糖的组合进行处理8周后,可以达到肝脏中的三酸甘油酯(TG)含量降低约78%(p<0.005)的优异效果。此外,三氯蔗糖和甘露醇的组合,圣草酚和甘露醇的组合,或者圣草酚和三氯蔗糖的组合可以显着降低肝脏中的总胆固醇(TC)含量,其中以圣草酚和三氯蔗糖的组合治疗8周后可以达到肝脏中的总胆固醇(TC)含量降低约77%(p<0.005)的优异效果。

[0353] 当施用三种试验化合物的组合时,薄荷醇、甘露醇和圣草酚的组合或是三氯蔗糖、甘露醇和圣草酚的组合可以显着降低肝脏中的三酸甘油酯(TG)。特别是,以三氯蔗糖、甘露醇和圣草酚的组合治疗8周后,可以达到肝脏中的三酸甘油酯(TG)含量降低约79%(p<

0.005)的优异效果。此外,三氯蔗糖、甘露醇和圣草酚的组合可以显着减少肝脏中的总胆固醇(TC)。

[0354] 6.2.2.3试验化合物有效减少肝损伤

[0355] 6.2.2.3.1减少肝脏组织中肝脏脂肪和肝损伤之效果

[0356] 动物实验的结果显示,多个试验化合物在4周的试验期间显现出减少肝脏脂肪和肝组织损伤的功效。图5显示出具有脂肪肝的动物的肝组织损伤。肝组织损伤包括覆盖肝门区(包括胆管、门静脉、肝动脉)附近肝细胞和肝细胞肿胀的许多大囊泡脂肪滴。相较之下,以水飞蓟素、薄荷醇、圣草酚或甘露醇处理4周后,肝组织切片中肝细胞内的大囊泡脂肪滴显着减少。以水飞蓟素处理的小鼠中仍观察到一部分小的破碎的脂肪滴,但以薄荷醇、圣草酚或甘露醇处理的小鼠的肝组织类型接近空白组中的动物的肝组织类型,这代表轻度脂肪肝疾病。此外,NAS评分的结果如表9所示。

[0357] 表9:试验化合物可以降低动物的肝损伤状况

[0358]

项目	**NAS**		
单位	mg/g 肝脏		
空白组 (n=13)	**0.7**	**± 0.5**	***
HFD 组 (n=12)	3.3	± 1.7	
阳性对照组			
水飞蓟素组 5.0 mg/kg (n=6)	**0.8**	**± 0.4**	***
水飞蓟素组 1.5 mg/kg (n=6)	**1.5**	**± 0.8**	*
单一试验化合物			
根皮苷 2.5 mg/kg(n=6)	1.8	± 1.0	
圣草酚 5.0 mg/kg(n=6)			
圣草酚 2.5 mg/kg(n=6)	**1.5**	**± 0.8**	*
圣草酚 7.5 mg/kg(n=6)	1.8	± 1.1	
圣草酚 1.5 mg/kg(n=6)	1.8	± 2.0	
薄荷醇 1.5 mg/kg(n=6)	1.8	± 1.6	

[0359]

项目	NAS		
单位	mg/g 肝脏		
甘露醇 7.5 mg/kg(n=6)	**1.7**	**± 0.8**	*
甘露醇 4.5 mg/kg(n=6)	2.7	± 1.9	
甘露醇 1.5 mg/kg(n=6)	**1.3**	**± 0.8**	*
糖精 1.5 mg/kg(n=3)			
葛根素 2.5 mg/kg(n=6)			
橙皮素 2.5 mg/kg(n=6)	1.7	± 0.5	
二种试验化合物之组合			
糖精 + 甘露醇 1.5 mg/kg +1.5 mg/kg(n=6)			
薄荷醇 + 甘露醇 4.5 mg/kg + 4.5 mg/kg(n=6)	2.2	± 1.5	
薄荷醇 + 甘露醇 1.5 mg/kg + 1.5 mg/kg(n=6)	**0.7**	**± 0.5**	***
薄荷醇 + 甘露醇 .5 mg/kg + .5 mg/kg(n=6)	2.5	± 1.8	
三种试验化合物之组合			
薄荷醇 + 甘露醇+ 圣草酚 .5 mg/kg + .5 mg/kg + .8 mg/kg(n=6)	**2.0**	**± 1.4**	
数据以平均值±标准偏差(SD)表示。ANOVA 和 LSD 得到的统计学差异以字词表示。与 HFD 组相比，*表示 p <0.05，**表示 p <0.01，***表示 p <0.005。			
橙皮素 葛根素 圣草酚 根皮苷 甘露醇 薄荷醇 三氯蔗糖 糖精			

[0360] NAS(非酒精性脂肪肝活性评分)表示为非酒精性脂肪肝病的活性评分[Hepatology期刊,2005年6月;第41卷第6期:第1313-21页],以及综合评价脂肪变性程度、小叶炎症和肝细胞气球化。评分表如表10所示。高分表示为严重的肝损伤。

[0361]

表 10: NAS 评估项目			
项目	**分数**	**程度**	**定义及描述**

[0362]

脂肪变性	0	<5%	指在低至中功率检查时评估的脂肪变性所涉及的表面积的量；最小脂肪变性(5%)得分为 0，以避免对脂肪变化非常小的活体检验给予过重的评分
	1	5-33%	
	2	>33-66%	
	3	>66%	
小叶炎症	0	无病灶	本评估不包括嗜酸性体及门脉炎症
	1	<2 个病灶/200x	
	2	2-4 个病灶/200x	
	3	>4 个病灶/200x	
肝细胞气球化	0	无	
	1	少数气球细胞	术语“少数”是指罕见但确定的肿胀肝细胞以及在诊断边界的病例。
	2	许多细胞/明显气球化	大多数具有明显气球化的病例也会有马里洛氏玻璃状体，但该 NAS 并未单独对马里洛氏玻璃状体评分。

[0363] 该结果显示，肝组织损伤发生在脂肪肝诱导的小鼠(NAS评分增加)。在各别施用的试验化合物中，圣草酚和甘露醇可以显着降低肝损伤。值得注意的是，当施用二种化合物的组合时，薄荷醇和甘露醇的组合达到了优异的效果。几乎没有任何肝脏损伤出现。其NAS评分与空白组的一样。

[0364] 6.2.2.3.2减少肝功能障碍之效果

[0365] 动物实验的结果显示，在4周或8周的给药期间，多种试验化合物显现出减轻动物肝脏功能障碍的效果。结果如表11-1和表11-2所示。

[0366] 表11-1：试验化合物可以减少动物的肝功能障碍(给药期为4周)

[0367]

项目	ALT			AST		
项目	U/L			U/L		
单位	**32.6**	**± 16.1**	***	**112.2**	**± 53.9**	***
空白组 (n=13)	70.1	± 45.2		156.8	± 100.8	
HFD 组 (n=12)						
阳性对照组	**33.9**	**± 9.3**	***	**168.1**	**± 42.6**	
水飞蓟素组 5.0 mg/kg (n=6)	**43.8**	**± 18.7**	*	**153.6**	**± 62.5**	
水飞蓟素组 1.5 mg/kg (n=6)						
甘露醇 7.5 mg/kg(n=6)	**25.0**	**± 10.8**	***	**63.3**	**± 7.7**	***

[0368]

项目	ALT			AST		
项目	U/L			U/L		
甘露醇 4.5 mg/kg(n=6)	**44.5**	**± 15.9**	*	107.6	± 54.3	
甘露醇 1.5 mg/kg(n=6)	**40.8**	**± 11.4**	*	187.2	± 142.1	
三氯蔗糖 7.5 mg/kg(n=6)	**32.3**	**± 10.1**	**	**74.3**	**± 18.6**	**
三氯蔗糖 1.5 mg/kg(n=6)	**30.9**	**± 16.8**	***	127.0	± 31.2	
圣草酚 5.0 mg/kg(n=5)	**41.4**	**± 6.3**	*	161.4	± 42.3	
圣草酚 2.5 mg/kg(n=6)	**33.7**	**± 18.5**	***	100.9	± 42.0	
葛根素 2.5 mg/kg(n=6)	**34.4**	**± 14.7**	***	**66.9**	**± 8.5**	***
根皮苷 2.5 mg/kg(n=6)	**35.7**	**± 9.1**	***	161.9	± 96.2	
橙皮素 2.5 mg/kg(n=6)	**36.8**	**± 22.1**	**	**72.4**	**± 11.2**	***
薄荷醇 1.5 mg/kg(n=6)	**41.5**	**± 13.7**	*	129.9	± 37.1	
糖精 1.5 mg/kg(n=3)	50.7	± 29.7		170.4	± 28.6	
二种试验化合物之组合						
薄荷醇 + 甘露醇 .5 mg/kg + .5 mg/kg(n=6)	**23.9**	**± 17.8**	***	**60.4**	**± 8.2**	***
薄荷醇 + 甘露醇 1.5 mg/kg + 1.5 mg/kg(n=6)	**16.7**	**± 4.3**	***	**59.8**	**± 7.5**	***
三氯蔗糖 + 甘露醇 7.5 mg/kg +7.5 mg/kg(n=6)	**45.5**	**± 15.2**		**91.4**	**± 21.8**	*
三氯蔗糖 + 甘露醇 1.5 mg/kg +1.5 mg/kg(n=6)	52.4	± 34.0		**92.1**	**± 23.0**	*
圣草酚 + 甘露醇 5.0mg/kg + 7.5mg/kg(n=4)	43.4	± 10.5		151.0	± 54.2	
圣草酚 + 三氯蔗糖 5.0mg/kg + 7.5mg/kg(n=4)	**38.2**	**± 10.9**	*	143.8	± 67.6	
糖精 + 甘露醇 1.5 mg/kg +1.5 mg/kg(n=6)	51.7	± 54.2		**70.0**	**± 27.6**	***
三种试验化合物之组合						
薄荷醇 + 甘露醇 + 圣草酚 .5 mg/kg + .5 mg/kg + .8 mg/kg(n=6)	**21.2**	**± 8.7**	***	**54.8**	**± 13.2**	***

数据以平均值±标准偏差(SD)表示。ANOVA 和 LSD 得到的统计学差异以字词表示。与 HFD 组相比，*表示 p <0.05，**表示 p <0.01，***表示 p <0.005。

橙皮素
葛根素
橙皮素
葛根素
圣草酚

ALT：丙胺酸胺基转移酶
AST：天冬胺酸转胺酶

[0369]

项目	ALT	AST
项目	U/L	U/L
根皮苷		
甘露醇		
薄荷醇		
三氯蔗糖		
糖精		

[0370] 表11-2:试验化合物可以降低动物的肝功能障碍(给药期为8周)

[0371]

项目	ALT			AST		
单位	U/L			U/L		
空白组 (n=7)	**65.1**	**± 21.5**	***	**22.6**	**± 4.3**	***
HFD 组 (n=8)	**111.0**	**± 26.2**		**109.4**	**± 46.4**	
二种试验化合物之组合						
三氯蔗糖 + 甘露醇 **7.5 mg/kg +7.5 mg/kg**(n=5)	**92.4**	**± 16.5**		**49.5**	**± 14.4**	***
三氯蔗糖+ 甘露醇 1.5 mg/kg +1.5 mg/kg(n=4)	112.5	± 23.8		93.0	± 26.0	
三种试验化合物之组合						
三氯蔗糖 + 甘露醇 + 圣草酚 **7.5 mg/kg + 7.5 mg/kg + 2.5 mg/kg**(n=6)				**40.0**	**± 12.2**	***
数据以平均值±标准偏差(SD)表示。ANOVA 和 LSD 得到的统计学差异以字词表示。与 HFD 组相比，*表示 p <0.05，**表示 p <0.01，***表示 p <0.005。						
甘露醇	ALT：丙胺酸胺基转移酶					
三氯蔗糖	AST：天冬胺酸转胺酶					

[0372] 丙胺酸胺基转移酶(ALT)和天冬胺酸转胺酶(AST)最常作为酶指示剂以反映出肝脏的生化功能障碍。在正常情况下,这些酶存在于肝细胞中。然而,当肝细胞损伤时,它们会外泄。血清中的ALT和AST值升高通常代表肝脏发炎和肝功能障碍。

[0373] 该结果显示,以脂肪肝(ALT和AST值增加)诱导的动物患有肝功能障碍。在各别施用的试验化合物中,橙皮素、葛根素、圣草酚、根皮苷、甘露醇、薄荷醇、三氯蔗糖和糖精都可以显着降低ALT和AST值。特别是,以甘露醇处理4周后,可以达到ALT值降低约64%($p<0.005$)且AST值降低约60%($p<0.005$)的优异效果。

[0374] 当施用二种试验化合物的组合时,薄荷醇和甘露醇的组合以及圣草酚和三氯蔗糖的组合可以显着降低ALT值。此外,薄荷醇和甘露醇的组合、三氯蔗糖和甘露醇的组合,或糖精和甘露醇的组合可以显着降低AST值。特别是,以薄荷醇和甘露醇的组合治疗4周后,可以达到ALT值降低约76%($p<0.005$)以及AST值降低约62%($p<0.005$)的优异效果。

[0375] 当施用三种试验化合物的组合时,三氯蔗糖、甘露醇和圣草酚的组合可以显着降低ALT值($p<0.005$)。

[0376] 6.2.2.4试验化合物可以改善肝脏的抗氧化活性

[0377] 动物实验的结果显示，在4周的试验期间，多种试验化合物显现出改善动物肝脏的抗氧化活性之效果。结果如表12-1和表12-2所示。

[0378] 表12-1：试验化合物可以改善动物肝脏的抗氧化活性(Gpx和GSH)

[0379]

项目	Gpx		GSH	
单位	U/L		U/L	
空白组 (n=10)	**2588.0 ± 524.5**		**1224.1 ± 95.5**	
HFD 组 (n=8)	2252.5 ± 395.2		1193.0 ± 203.8	
阳性对照组				
水飞蓟素组 5.0 mg/kg(n=6)	**3358.3 ± 1205.3**	***	**1398.8 ± 396.5**	
单一试验化合物				
甘露醇 7.5 mg/kg(n=6)	**3738.3 ± 665.1**	***	**2147.7 ± 459.1**	***
甘露醇 4.5 mg/kg(n=6)	**3423.3 ± 547.8**	***	**1605.1 ± 305.9**	**
甘露醇 1.5 mg/kg(n=6)	2580.0 ± 555.2		**1502.5 ± 276.9**	*
葛根素 2.5 mg/kg(n=6)	**3581.7 ± 1056.7**	***	**1498.1 ± 150.0**	*
三氯蔗糖 7.5 mg/kg(n=6)	**3334.0 ± 377.7**	**	**1609.1 ± 201.1**	**
三氯蔗糖 1.5 mg/kg(n=6)	**2995.0 ± 651.1**	*	1448.0 ± 281.5	
根皮苷 2.5 mg/kg(n=6)	**3234.0 ± 505.1**	**	1387.7 ± 168.2	
橙皮素 2.5 mg/kg(n=6)	**3133.3 ± 376.9**	*	**1742.6 ± 241.5**	***
圣草酚 2.5 mg/kg(n=6)	**3083.3 ± 378.9**	**	1302.0 ± 241.1	
薄荷醇 1.5 mg/kg(n=6)	2921.7 ± 640.2		1432.7 ± 104.0	

数据以平均值±标准偏差(SD)表示。ANOVA 和 LSD 得到的统计学差异以字词表示。与 HFD 组相比，*表示 $p<0.05$，**表示 $p<0.01$，***表示 $p<0.005$。

橙皮素
葛根素
橙皮素
葛根素
圣草酚
根皮苷

Gpx：谷胱甘肽过氧化物酶
GSH：谷胱甘肽

[0380]

项目	Gpx	GSH
单位	U/L	U/L
甘露醇		
薄荷醇		
三氯蔗糖		

[0381] 表12-2:试验化合物可以改善动物肝脏的抗氧化活性(Grd和SOD)

[0382]

项目	Grd			SOD		
单位	U/L			U/L		
空白组 (n=10)	**123.5**	**± 30.9**		**380.3**	**± 38.8**	
HFD 组 (n=8)	**82.1**	**± 21.7**		**371.7**	**± 49.3**	
阳性对照组						
水飞蓟素组 5.0 mg/kg(n=6)	**88.9**	**± 29.2**		**435.9**	**± 59.2**	*
单一试验化合物						
甘露醇 7.5 mg/kg(n=6)	**117.6**	**± 32.0**	**	**462.8**	**± 52.8**	
甘露醇 4.5 mg/kg(n=6)	**110.1**	**± 18.4**	*	**429.2**	**± 85.2**	
甘露醇 1.5 mg/kg(n=6)	**95.3**	**± 22.1**		**367.3**	**± 35.6**	
葛根素 2.5 mg/kg(n=6)	**99.0**	**± 17.2**		**434.5**	**± 59.8**	
三氯蔗糖 7.5 mg/kg(n=6)	**90.4**	**± 17.2**		**399.0**	**± 34.5**	
三氯蔗糖 1.5 mg/kg(n=6)	**100.0**	**± 18.6**		**373.0**	**± 50.4**	
根皮苷 2.5 mg/kg(n=6)	**82.2**	**± 33.6**		**411.5**	**± 87.5**	
橙皮素 2.5 mg/kg(n=6)	**102.5**	**± 28.3**		**408.3**	**± 66.7**	
圣草酚 2.5 mg/kg(n=6)	**86.9**	**± 15.7**		**385.9**	**± 34.0**	
薄荷醇 1.5 mg/kg(n=6)	**95.2**	**± 16.2**		**427.9**	**± 41.9**	

数据以平均值±标准偏差(SD)表示。ANOVA 和 LSD 得到的统计学差异以字词表示。与 HFD 组相比，*表示 p <0.05，**表示 p <0.01，***表示 p <0.005。

橙皮素
葛根素
橙皮素
葛根素
圣草酚
根皮苷
甘露醇
薄荷醇
三氯蔗糖

Grd：谷胱甘肽还原酶
SOD：超氧化物歧化酶

[0383] Gpx、GSH、Grd和SOD是肝脏抗氧化系统中常见的成员，这些成员可以减少肝脏中的

氧化压力,防止肝脏因为氧化压力而造成损伤。Gpx、GSH、Grd和SOD值的增加表示肝脏保持较佳的抗氧化活性。

[0384] 该结果显示,脂肪肝诱导的小鼠的抗氧化活性降低。在各别施用的试验化合物中,橙皮素、葛根素、圣草酚、根皮苷、甘露醇和三氯蔗糖皆可显着改善抗氧化活性。特别是,以甘露醇治疗4周后达到Gpx、GSH、Grd和SOD含量显着增加($p<0.005$)的优异效果。

[0385] 总之,试验化合物(包括甘露醇和三氯蔗糖等)可以减少肝脏中的脂肪含量、减少肝脏损伤和改善肝脏的抗氧化活性。这些化合物已经通过动物实验被证实是安全的,并且发现有可能发展为保健食品或药物,用以减少肝脏脂肪和改善相关疾病,例如脂肪肝疾病、急性和慢性酒精性脂肪肝疾病、急性和慢性非酒精性脂肪肝疾病(non-alcoholic fatty liver diseases,NAFLD),急性和慢性酒精性肝炎、急性和慢性非酒精性脂肪性肝炎、非酒精性肝硬化和酒精性肝硬化(ICD-9-CM诊断代码:571.8,571.0,571.1,571.2,571.3,571.4,571.5,571.9)。

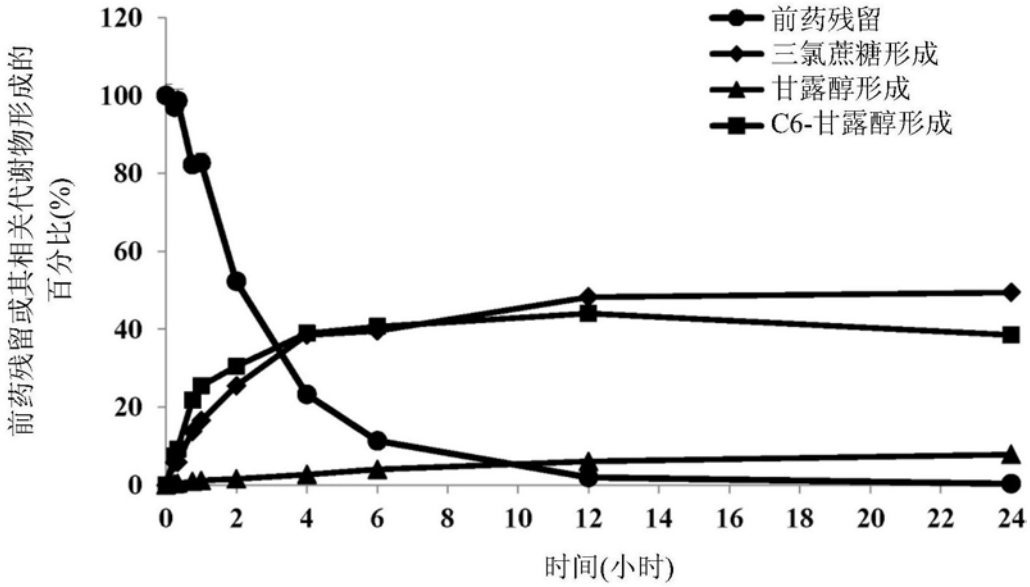

前药残留
三氯蔗糖形成
甘露醇形成
C6-甘露醇形成
前药残留或其相关代谢物形成的百分比(%)
时间(小时)
0
20
40
60
80
100
120
0
2
4
6
8
10
12
14
16
18
20
22
24

图1

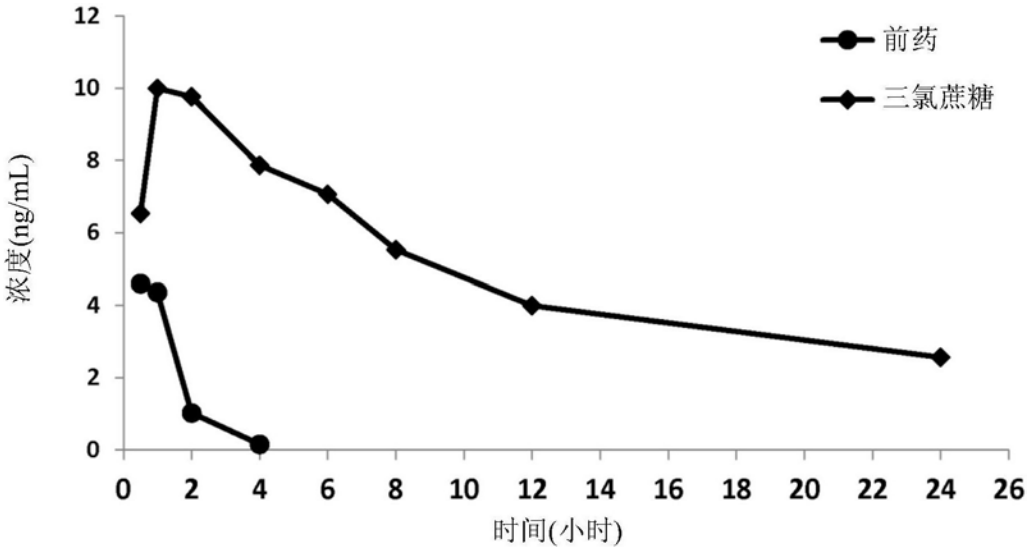

前药
三氯蔗糖
浓度(ng/mL)
时间(小时)
0
2
4
6
8
10
12
0
2
4
6
8
10
12
14
16
18
20
22
24
26

图2

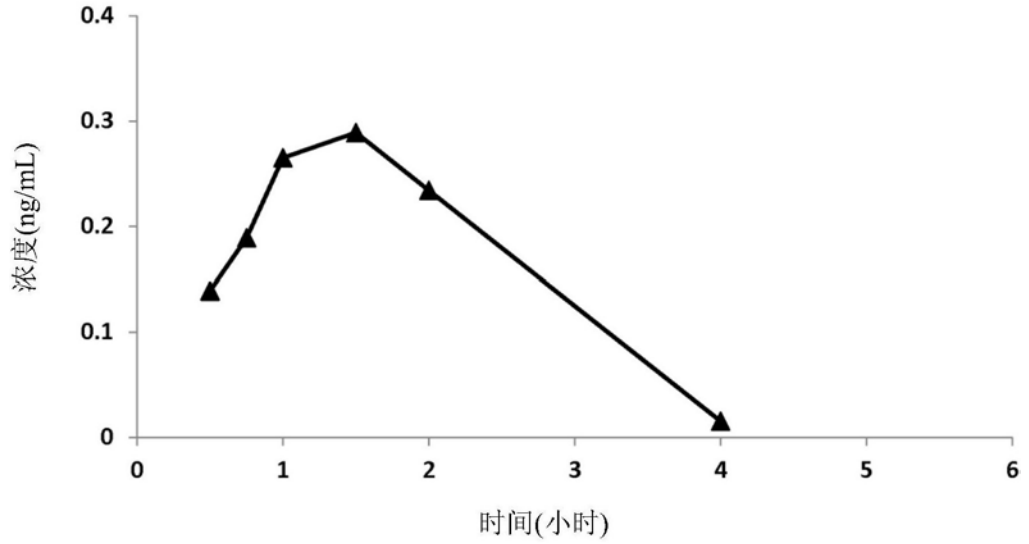


图3

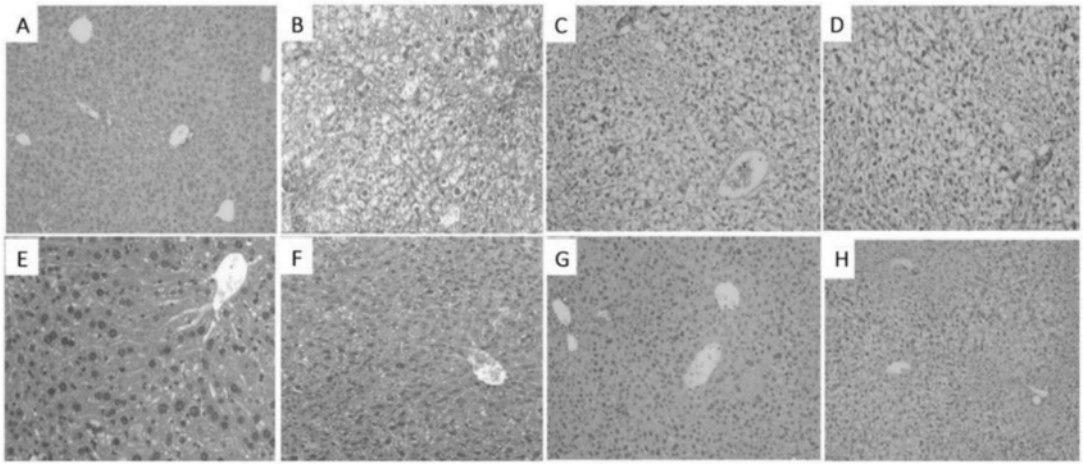


图4

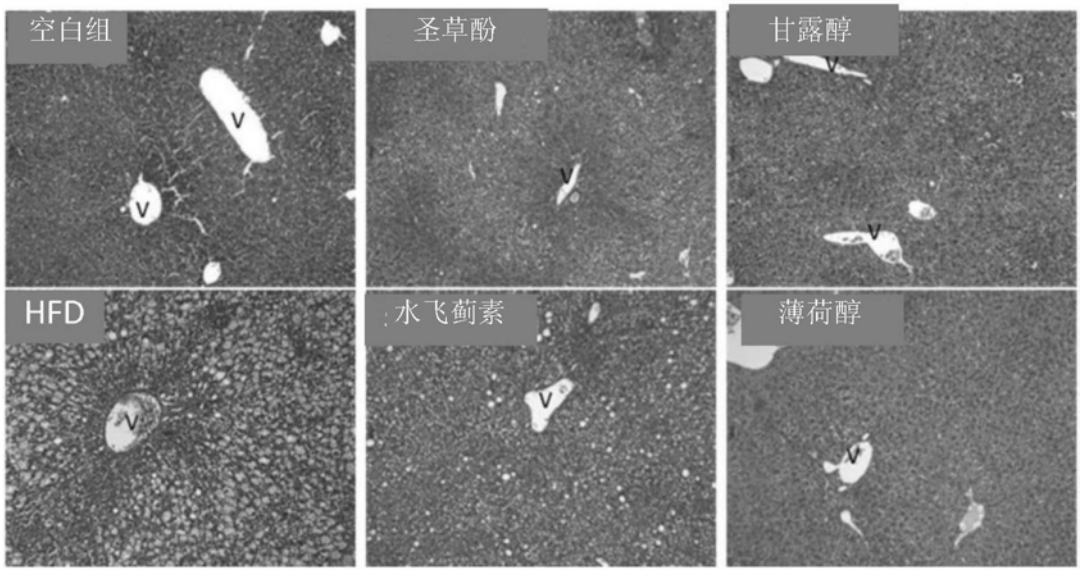


图5

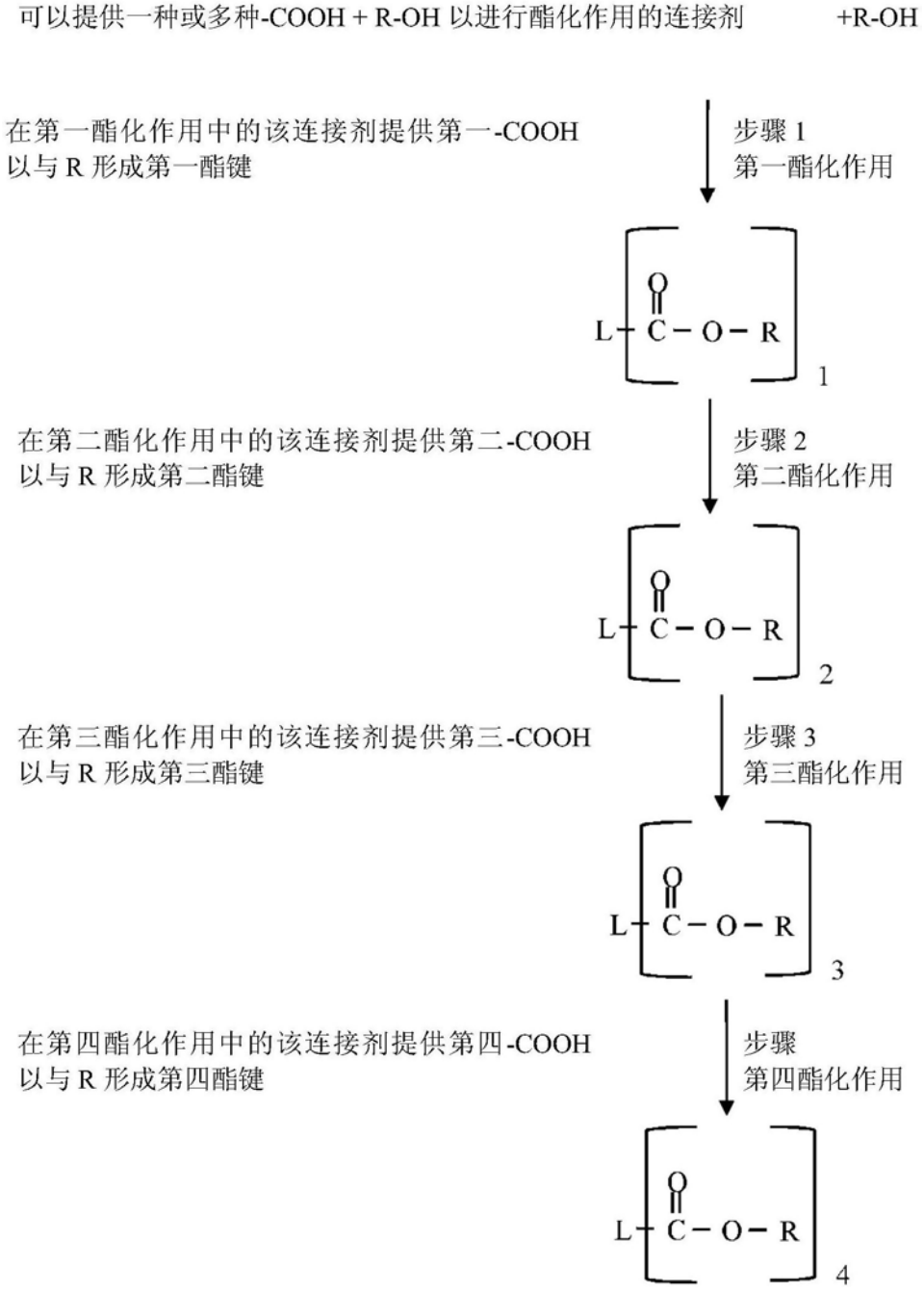

可以提供一种或多种-COOH + R-OH 以进行酯化作用的连接剂
+R-OH
在第一酯化作用中的该连接剂提供第一-COOH
以与 R 形成第一酯键
步骤 1
第一酯化作用
L C O R
1
在第二酯化作用中的该连接剂提供第二-COOH
以与 R 形成第二酯键
步骤 2
第二酯化作用
L C O R
2
在第三酯化作用中的该连接剂提供第三-COOH
以与 R 形成第三酯键
步骤 3
第三酯化作用
L C O R
3
在第四酯化作用中的该连接剂提供第四-COOH
以与 R 形成第四酯键
步骤
第四酯化作用
L C O R
4

图6