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Use of structurally enhanced fatty acids containing sulphur for preventing and/or treating non-alcoholic steatohepatitis

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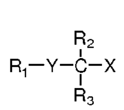
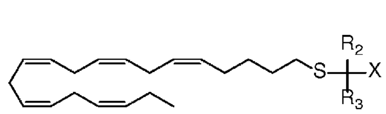
(54) Title: USE OF STRUCTURALLY ENHANCED FATTY ACIDS CONTAINING SULPHUR FOR PREVENTING AND/OR TREATING NON-ALCOHOLIC STEATOHEPATITIS



(II)



(I)

(57) Abstract: The present disclosure relates to a method of preventing and/or treating non-alcoholic steatohepatitis in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of a compound of Formula (II): wherein R_1, R2, R3, X and Y are as defined in the specification; or a pharmaceutically acceptable salt, solvate, or solvate of such a salt. More particularly, the present disclosure relates to a method of preventing and/or treating non- alcoholic steatohepatitis in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of a compound of Formula (I): wherein R2, R_3, and X, are as defined in the specification; or a pharmaceutically acceptable salt, solvate, or solvate of such a salt. Further, the present invention relates to a compound of Formula (I) for preventing and/or treating non-alcoholic steatohepatitis, wherein R_2, R_3 and X are as defined in the specification; or a pharmaceutically acceptable salt, solvate, or solvate of such a salt.

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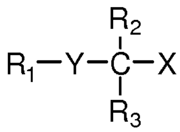
USE OF STRUCTURALLY ENHANCED FATTY ACIDS CONTAINING SULPHUR FOR PREVENTING AND/OR TREATING NON-ALCOHOLIC STEATOHEPATITIS

Field of the invention

The present disclosure relates to a method of preventing and/or treating non-alcoholic steatohepatitis in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of a compound of Formula (II):



(II)

wherein

R_1 is selected from a C_{10}-C_{22} alkenyl having 3-6 double bonds;

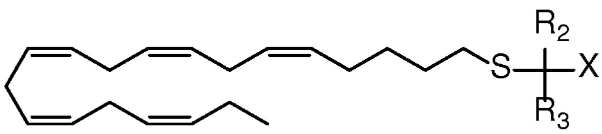
R_2 and R_3 are the same or different and may be selected from a group of substituents consisting of a hydrogen atom, a hydroxy group, an alkyl group, a halogen atom, an alkoxy group, an acyloxy group, an acyl group, an alkenyl group, an alkynyl group, an aryl group, an alkylthio group, an alkoxycarbonyl group, a carboxy group, an alkylsulfinyl group, an alkylsulfonyl group, an amino group, and an alkylamino group, provided that R_2 and R_3 cannot both be a hydrogen atom; or R_2 and R_3 can be connected in order to form a cycloalkane like cyclopropane, cyclobutane, cyclopentane or cyclohexane;

Y is selected from sulphur, sulfoxide, and sulfone;

X represents a carboxylic acid or a derivative thereof, wherein the derivative is a carboxylic ester, a glyceride, a carboxamide or a phospholipid;

or a pharmaceutically acceptable salt, solvate, or solvate of such a salt.

The present disclosure relates to a method of preventing and/or treating non-alcoholic steatohepatitis in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of a compound of Formula (I):



(I)

wherein R_2 and R_3 are independently chosen from the group of a hydrogen atom and linear, branched, and/or cyclic C_1-C_6 alkyl groups, with the proviso that R_2 and R_3 are not both hydrogen; X represents a carboxylic acid or a derivative thereof, wherein the derivative is a carboxylic ester, a glyceride, a carboxamide or a phospholipid; or a pharmaceutically acceptable salt, solvate, or solvate of such a salt.

Further, the present invention discloses compounds of the formulas (I) and (II) for therapeutic and/or prophylactic treatment of non-alcoholic steatohepatitis.

Background of the invention

Dietary polyunsaturated fatty acids (PUFAs), including omega-3 fatty acids, have effects on diverse physiological processes impacting normal health and chronic diseases, such as the regulation of plasma lipid levels, cardiovascular and immune functions, insulin action, neuronal development, and visual function.

Omega-3 fatty acids, e.g. (5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoic acid (EPA) and (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid (DHA), regulate plasma lipid levels, cardiovascular and immune functions, insulin action, and neuronal development, and visual function. Omega-3 fatty acids have been shown to have beneficial effects on the risk factors for cardiovascular diseases, for example hypertension and hypertriglyceridemia (HTG).

The use of omega-3 compounds such as EPA and DHA to treat non-alcoholic steatohepatitis (NASH) have been suggested in the prior art. By way of example, WO 2014/057522 of Mochida relates to compositions comprising ethyl icosapentate for use in treatment or alleviation of symptoms of NASH.

Dignity Science LTD (WO2014/118097) have suggested the use of modified omega-3 compounds, such as 15-hydroxy eicosapentaenoic acid (15-OHEPA), to treat fatty liver disorders, such as non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). Krisani Biosciences (WO2014/045293) have also proposed the use of modified omega-3 compounds for treating different diseases including non-alcoholic steatohepatitis.

Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are frequently used interchangeably despite the fact that NAFLD encompasses a much broader spectrum of liver disease including simple hepatosteatosis (> 5% of hepatocytes histologically). Hepatosteatosis is most likely a relatively benign disorder when not accompanied by an inflammatory response and cellular damage. However, a subgroup of NAFLD patients have liver cell injury and inflammation in addition to hepatosteatosis, a condition known as nonalcoholic steatohepatitis (NASH). NASH is virtually indistinguishable histologically from alcoholic steatohepatitis (ASH). While the simple steatosis seen in NAFLD does not correlate with increased short-term morbidity or mortality, NASH dramatically increases the risks of cirrhosis, liver failure, and hepatocellular carcinoma (HCC). Cirrhosis due to NASH is an increasingly frequent reason for liver transplantation. While the morbidity and mortality from liver causes are greatly increased in patients with NASH, they correlate even more strongly with the morbidity and mortality from cardiovascular disease.

Uniform criteria for diagnosing and staging NASH are still debated (see details in later sections). Key histologic components of NASH are steatosis, hepatocellular ballooning, and lobular inflammation; fibrosis is not part of the histologic definition of NASH. However, the degree of fibrosis on liver biopsy (stage) is predictive of the prognosis, whereas the degree of inflammation and necrosis on liver biopsy (grade) are not.

With respect to the various histological components, treatment with omega-3 fatty acids have been shown to effectively reduce hepatosteatosis in patients with NAFLD (Scorletti E, et al., Effects of purified eicosapentaenoic and docosahexanoic acids in non-alcoholic fatty liver disease: Results from the *WELCOME study, Hepatology. 2014 Jul 4 1) and, if treatment is established at an early stage of the disease, may conceivably slow progression to the latter more severe stages of disease. However, it is questionable whether omega-3 fatty acids are sufficiently potent to treat and/or reverse NASH where pronounced histological/inflammatory changes have developed (Sanyal AJ, et al; EPE-A Study Group, Gastroenterology. 2014 Aug; 147(2):377-84.e1. doi: 10.1053/j.gastro.2014.04.046. Epub 2014 May 9).

The moderate efficacy of omega-3 fatty acids in the treatment of NASH may be secondary to their mild effects upon other pathways that underlie the pathogenesis of NASH. Research in both humans and animal models of NASH have convincingly demonstrated that there are multiple factors involved in the development of steatohepatitis as opposed to isolated hepatosteatosis. These include insulin resistance, oxidative stress, inflammation, gut-derived endotoxin and excessive hepatic cholesterol and bile acids. All these factors have been shown to play important contributing factors in genetically susceptible individuals and drugs targeting these pathways are being developed for the treatment of NASH.

The efficacy of synthetic farnesoid X receptor (FXR) agonists, such as obeticholic acid, in the treatment of established NASH suggest pathways involving cholesterol/bile acid production and clearance play of pivotal role in the pathogenesis of the disease. However, as FXR agonists inhibit the major pathway by which the liver excretes excess cholesterol (conversion to bile acids and biliary excretion), adverse effects upon plasma cholesterol are observed.

Increased hepatocellular cholesterol concentrations can also lead to cholesterol crystal accumulation and cell-death with resultant foam cell formation. Increased oxidized cholesterol (plasma derived or formed in situ) can also incite a hepatic inflammatory reaction and the development of NASH.

Treatments aimed at reducing hepatic cholesterol levels are an attractive target in the prevention and treatment of NASH by both limiting the substrate for both crystal formation and oxidation, but also by decreasing substrate availability for hepatic bile acid synthesis. The advantage of this upstream approach is in addition to tackling key inflammatory inducing components associated with NASH, beneficial effects should also be seen upon atherogenic plasma lipids that frequently accompany the hepatic disease.

WO2010/008299 discloses that structurally enhanced fatty acids including 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid and its derivatives favorably influences lipid profiles, i.a. by lowering plasma triglycerides, plasma cholesterol, plasma insulinetc. Those results demonstrate that 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid and its derivatives may be useful in prevention or treatment of various conditions.

It has surprisingly been found that 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid and its derivatives are useful for preventing and/or treating non-alcoholic steatohepatitis.

Brief Summary of the invention

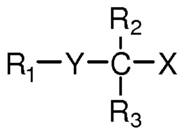
The present disclosure relates to a method of preventing and/or treating non-alcoholic steatohepatitis in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of a compound of Formula (II):

$$\begin{array}{c} \quad\quad\quad R_2 \\ \quad\quad\quad | \\ R_1-Y-C-X \\ \quad\quad\quad | \\ \quad\quad\quad R_3 \end{array} \quad \text{(II)}$$

wherein R_1 is selected from a C_{10}-C_{22} alkenyl having 3-6 double bonds;
R_2 and R_3 are the same or different and may be selected from a group of substituents consisting of a hydrogen atom, a hydroxy group, an alkyl group, a halogen atom, an alkoxy group, an acyloxy group, an acyl group, an alkenyl group, an alkynyl group, an aryl group, an alkylthio group, an alkoxycarbonyl group, a carboxy group, an alkylsulfinyl group, an alkylsulfonyl group, an amino group, and an alkylamino group, provided that R_2 and R_3 cannot both be a hydrogen

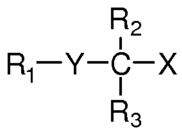
atom; or R_2 and R_3 can be connected in order to form a cycloalkane like cyclopropane, cyclobutane, cyclopentane or cyclohexane;
Y is selected from sulphur, sulfoxide, and sulfone;
X is a carboxylic acid or a derivative thereof, wherein the derivative is a carboxylic ester, a glyceride, a carboxamide or a phospholipid; or a pharmaceutically acceptable salt, solvate, or solvate of such a salt.

An equal aspect of the disclosure relates to use of a pharmaceutically effective amount of a compound of Formula (II):


(II)

wherein R_1 is selected from a C_{10}-C_{22} alkenyl having 3-6 double bonds;
R_2 and R_3 are the same or different and may be selected from a group of substituents consisting of a hydrogen atom, a hydroxy group, an alkyl group, a halogen atom, an alkoxy group, an acyloxy group, an acyl group, an alkenyl group, an alkynyl group, an aryl group, an alkylthio group, an alkoxycarbonyl group, a carboxy group, an alkylsulfinyl group, an alkylsulfonyl group, an amino group, and an alkylamino group, provided that R_2 and R_3 cannot both be a hydrogen atom; or R_2 and R_3 can be connected in order to form a cycloalkane like cyclopropane, cyclobutane, cyclopentane or cyclohexane;
Y is selected from sulphur, sulfoxide, and sulfone;
X is a carboxylic acid or a derivative thereof, wherein the derivative is a carboxylic ester, a glyceride, a carboxamide, or a phospholipid; or a pharmaceutically acceptable salt, solvate, or solvate of such a salt, in the manufacture of a medicament for preventing and/or treating non-alcoholic steatohepatitis in a subject in need thereof.

An equal aspect of the disclosure relates to a compound of Formula (II)


(II)

wherein R_1 is selected from a C_{10}-C_{22} alkenyl having 3-6 double bonds;
R_2 and R_3 are the same or different and may be selected from a group of substituents consisting of a hydrogen atom, a hydroxy group, an alkyl group, a halogen atom, an alkoxy group, an acyloxy group, an acyl group, an alkenyl group, an alkynyl group, an aryl group, an alkylthio

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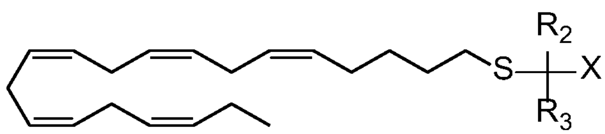
group, an alkoxycarbonyl group, a carboxy group, an alkylsulfinyl group, an alkylsulfonyl group, an amino group, and an alkylamino group, provided that R_2 and R_3 cannot both be a hydrogen atom; or R_2 and R_3 can be connected in order to form a cycloalkane like cyclopropane, cyclobutane, cyclopentane or cyclohexane;

Y is selected from sulphur, sulfoxide, and sulfone;

X represents a carboxylic acid or a derivative thereof, wherein the derivative is a carboxylic ester, a glyceride, a carboxamide, or a phospholipid; or a pharmaceutically acceptable salt, solvate, or solvate of such a salt, for theraputic and/or prophylactic treatment of non-alcoholic steatohepatitis.

In at least one embodiment, R_2 and R_3 are the same or different and may be selected from a group of substituents consisting of a hydrogen atom, an alkyl group, an alkoxy group, an alkenyl group; or R_2 and R_3 can be connected in order to form a cycloalkane like cyclopropane, cyclobutane, cyclopentane or cyclohexane; Y is selected from sulphur; X represents a carboxylic acid or a derivative thereof, wherein the derivative is a carboxylic ester, a glyceride or a phospholipid; or a pharmaceutically acceptable salt, solvate, or solvate of such a salt.

More particularly, in one aspect the present disclosure relates to a method of preventing and/or treating non-alcoholic steatohepatitis in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of a compound of Formula (I):

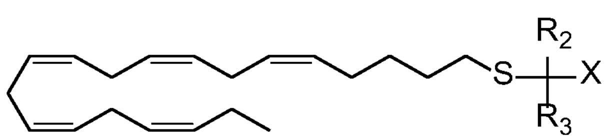


Formula (I)

wherein R_2 and R_3 and X are defined as for Formula (II) and preferably wherein

R_2 and R_3 are independently chosen from the group of a hydrogen atom and linear or branched C_1-C_6 alkyl groups, with the proviso that R_2 and R_3 are not both hydrogen, or R_2 and R_3 are connected to form cyclobutane; and X is a carboxylic acid or a C_1-C_6 alkyl ester, or a pharmaceutically acceptable salt, solvate, or solvate of such a salt.

Likewise, in another aspect the present disclosure relates to use of a pharmaceutically effective amount of a compound of Formula (I):



Formula (I)

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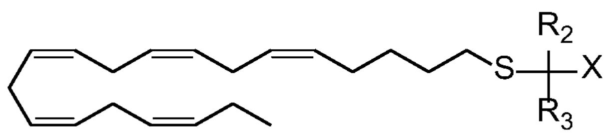
wherein R_2 and R_3 and X are defined as for Formula (II) and preferably wherein R_2 and R_3 are independently chosen from the group of a hydrogen atom and linear or branched C_1-C_6 alkyl groups, with the proviso that R_2 and R_3 are not both hydrogen, or R_2 and R_3 are connected to form cyclobutane; and X is a carboxylic acid or a C_1-C_6 alkyl ester; or a pharmaceutically acceptable salt, solvate, or solvate of such salt thereof, in the manufacture of a medicament for preventing and/or treating non-alcoholic steatohepatitis in a subject in need thereof.

Likewise, in another aspect the present disclosure relates to a compound of Formula (I):

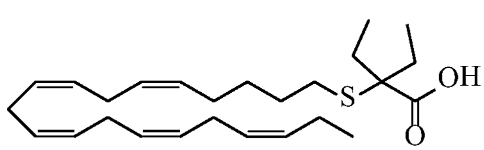


Formula (I)

wherein R_2 and R_3 and X are defined as for Formula (II) and preferably wherein R_2 and R_3 are independently chosen from the group of a hydrogen atom and linear, branched, and/or cyclic C_1-C_6 alkyl groups, with the proviso that R_2 and R_3 are not both hydrogen; X is a carboxylic acid or a derivative thereof, wherein the derivative is a carboxylic ester, a glyceride or a phospholipid; or a pharmaceutically acceptable salt, solvate, or solvate of such a salt, for preventing and/or treating non-alcoholic steatohepatitis.

For Formula (I), when X is a glyceride, this may be chosen from the group of a triglyceride, a 1,2-diglyceride, a 1,3-diglyceride, a 1-monoglyceride and a 2-monoglyceride.

The present disclosure also includes a method of treating and/or preventing non-alcoholic steatohepatitis in a subject in need thereof, the method comprising administering to the subject a pharmaceutically effective amount of 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid:



(Compound N)

or a pharmaceutically acceptable salt or ester thereof.

Likewise, the present disclosure also includes use of a pharmaceutically effective amount of 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid (compound N) or a pharmaceutically acceptable salt or ester thereof in the manufacture of a medicament for treating and/or preventing non-alcoholic steatohepatitis in a subject in need thereof.

The present disclosure also relates to 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid (Compound N) or a pharmaceutically acceptable salt or ester thereof, for preventing and/or treating non-alcoholic steatohepatitis.

Brief description of the drawings

Figure 1 discloses the effects of Compound N on hepatic cholesterol.

Figure 2 discloses the effects of Compound N on hepatic inflammation.

Figure 3 discloses the effects of Compound N on macrovesicular steatosis.

Figure 4 discloses the effects of Compound N on fecal bile acid content.

Figure 5 discloses the effects of Compound N on hepatic fibrosis (collagen content).

Figure 6 discloses the effects of Compound N on total plasma cholesterol.

Detailed description

It should be noted that embodiments and features described in the context of one aspect of the present disclosure also apply to the other aspects of the invention. Particularly, the embodiments applying to the method of preventing and/or treating non-alcoholic steatohepatitis according to the present disclosure also apply to the use of a pharmaceutical effective amount of a compound in the manufacture of a medicament for preventing and/or treating non-alcoholic steatohepatitis and likewise to the aspect of a compound for preventing and/or treating non-alcoholic steatohepatitis, all according to the present disclosure.

Particular aspects of the disclosure are described in greater detail below. The terms and definitions as used in the present application and as clarified herein are intended to represent the meaning within the present disclosure.

The singular forms "a," "an," and "the" include plural reference unless the context dictates otherwise.

The terms "approximately" and "about" mean to be nearly the same as a referenced number or value. As used herein, the terms "approximately" and "about" should be generally understood to encompass ± 5% of a specified amount, frequency, or value.

The terms "treat," "treating," and "treatment" include any therapeutic application that can benefit a human or non-human mammal. Both human and veterinary treatments are within the scope of the present disclosure. Treatment may be responsive to an existing condition or it may be prophylactic, i.e., preventative.

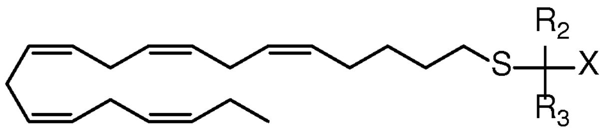
The terms "administer," "administration," and "administering" as used herein refer to (1) providing, giving, dosing and/or prescribing by either a health practitioner or his authorized agent or under his direction a compound or composition according to the present disclosure, and (2) putting into, taking or consuming by the human patient or person himself or herself, or non-human mammal a compound or composition according to the present disclosure. The terms “preventing and/or treating” and “therapeutic and/or prophylactic treatment of” may interchangeably be used. Typically the compounds of Formula (I) will be used for treating, i.e. therapeutic treatment of, NASH. However, it is also foreseen that in some cases the compound of Formula (I) will be used for preventing or for prophylactic treatment of NASH, for example in cases where a patient have a family history of developing NASH.

The term "pharmaceutically effective amount" means an amount sufficient to achieve the desired pharmacological and/or therapeutic effects, i.e., an amount of the disclosed compound that is effective for its intended purpose. While individual subject/patient needs may vary, the determination of optimal ranges for effective amounts of the disclosed compound is within the skill of the art. Generally, the dosage regimen for treating a disease and/or condition with the compounds presently disclosed may be determined according to a variety of factors such as the type, age, weight, sex, diet, and/or medical condition of the subject/patient.

The term "pharmaceutical composition" means a compound according to the present disclosure in any form suitable for medical use.

The compounds of Formula (I) and (II) may exist in various stereoisomeric forms, including enantiomers, diastereomers, or mixtures thereof. It will be understood that the invention encompasses all optical isomers of the compounds of Formula (I) and (II) as well as mixtures thereof. Hence, compounds of Formula (I) and (II) that exist as diastereomers, racemates, and/or enantiomers are within the scope of the present disclosure.

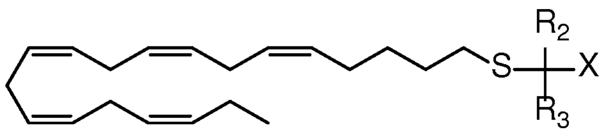
The present disclosure relates to a method of preventing and/or treating non-alcoholic steatohepatitis in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of a compound of Formula (I):



Formula (I)

wherein R_2 and R_3 are independently chosen from the group of a hydrogen atom and linear, branched, and/or cyclic C_1-C_6 alkyl groups, with the proviso that R_2 and R_3 are not both hydrogen; X represents a carboxylic acid or a carboxylic ester; or a pharmaceutically acceptable salt, solvate, solvate of such a salt.

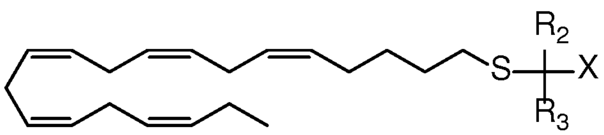
In at least one aspect, the present disclosure relates to use of a pharmaceutically effective amount of a compound of Formula (I):



Formula (I)

wherein R_2 and R_3 are independently chosen from the group of a hydrogen atom and linear, branched, and/or cyclic C_1-C_6 alkyl groups, with the proviso that R_2 and R_3 are not both hydrogen; X represents a carboxylic acid or a carboxylic ester; or a pharmaceutically acceptable salt, solvate, solvate of such a salt, for preventing and/or treating non-alcoholic steatohepatitis in a subject in need thereof.

In at least one aspect, the present disclosure relates to a compound of Formula (I):



Formula (I)

wherein R_2 and R_3 are independently chosen from the group of a hydrogen atom and linear, branched, and/or cyclic C_1-C_6 alkyl groups, with the proviso that R_2 and R_3 are not both hydrogen; X is a carboxylic acid or a carboxylic ester; or a pharmaceutically acceptable salt, solvate, or solvate of such a salt, for preventing and/or treating non-alcoholic steatohepatitis.

For the compounds of Formula (I), the use of these, and for the method of administering these, the following items of disclosure are included:

In those cases were R_2 and R_3 are different, the compounds of Formula (I) are capable of existing in stereoisomeric forms. It will be understood that the invention encompasses all optical isomers of the compounds of Formula (I) and mixtures thereof.

In at least one embodiment, R_2 and R_3 are independently chosen from a hydrogen atom, a methyl group, an ethyl group, a n-propyl group, and an isopropyl group.

In at least one embodiment, R_2 and R_3 are chosen from a hydrogen atom, a methyl group, and an ethyl group.

In at least one embodiment, one of R_2 and R_3 is a hydrogen atom and the other one of R_2 and R_3 is chosen from a C_1-C_3 alkyl group. In one embodiment, one of R_2 and R_3 is a hydrogen atom and the other one of R_2 and R_3 is chosen from a methyl group or an ethyl group.

In at least one embodiment R_2 and R_3 are independently C_1-C_6 alkyl groups. In one embodiment both R_2 and R_3 are C_1-C_3 alkyl groups. In one embodiment R_2 and R_3 are the same or different and each are independently chosen from a methyl group, an ethyl group, an n-propyl group, or an isopropyl group. In one embodiment R_2 and R3 are the same and are selected from a pair of methyl groups, a pair of ethyl groups, a pair of n-propyl groups and a pair of isopropyl groups. In at least one preferred embodiment R2 and R3 are ethyl groups. In one embodiment, one of R2 and R3 is a methyl group and the other one is an ethyl group. In one embodiment, one of R2 and R3 is an ethyl group and the other one is a n-propyl group.

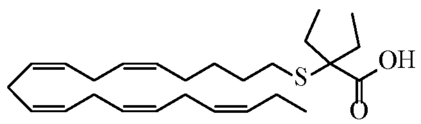
In at least one embodiment, X is a carboxylic acid. In one embodiment, wherein X is a carboxylic ester, this is a C1-C6 alkyl ester. This may be chosen from a methyl ester, an ethyl ester, an isopropyl ester, a n-butyl ester and a tert-butyl ester. Preferably, the ester is selected from a methyl ester and an ethyl ester.

In at least one embodiment, the compound may be present in its various stereoisomeric forms, such as an enantiomer (R or S), diastereomer, or mixtures thereof.

In at least one embodiment, the compound is present in racemic form. In one embodiment, the compound is present in its R form. In another embodiment, the compound is present in its S form.

In cases, where the compound according to Formula (I) is a salt of a counter-ion with at least one stereogenic center, or ester of an alcohol with at least one stereogenic center, the compound may have multiple stereocenters. In those situations, the compounds of the present disclosure may exist as diastereomers. Thus, in at least one embodiment, the compounds of the present disclosure are present as at least one diastereomer.

In at least one embodiment, the compound of the present disclosure is 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid:



(Compound N).

Given the established adverse effects upon hepatic cholesterol upon NASH from both animal studies and human tissue samples, the described examples clearly suggest that compounds of Formula (I) such as Compound N may have beneficial effects in the prevention and/or treatment of NASH.

It was previously shown that both Compound N and Reference A (2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenyloxy)butanoic acid prepared according to Example 2 of WO2010/128401) could significantly decrease plasma cholesterol in APOE*3Leiden.CETP double transgenic mice. The absolute reductions achieved by both compounds were comparable and the proposed mechanism/s by which these reductions are achieved appear similar (SREPB-2 activation and increased hepatic LDL-R expression). It was therefore surprising to find the clear differences observed between the compounds in a number of key components of NASH as described in the examples. These differences may be related to varying effects upon hepatic cholesterol biosynthesis as the differences in hepatic cholesterol ester levels cannot be explained by increased hepatic cholesterol uptake (rate of uptake same for both compounds, data not shown).

Although only fecal bile acid content was measured in this model, the reduction in bile acid excretion along with the decreased hepatic cholesterol ester content suggest that less cellular cholesterol may be available for bile acid synthesis. As evidenced by the beneficial effects of FXR agonists (although there are additional mechanisms by which these compounds work), a reduction in bile acid synthesis should have both anti-inflammatory and anti-fibrotic effects in human NASH. A reduced availability of hepatic cholesterol should also reduce cholesterol crystal formation and oxidised cholesterol content, both potentially important mediators of NASH in humans. The significantly reduced plasma residence time of cholesterol demonstrated in earlier studies (data not shown) may also reduce oxidised cholesterol uptake.

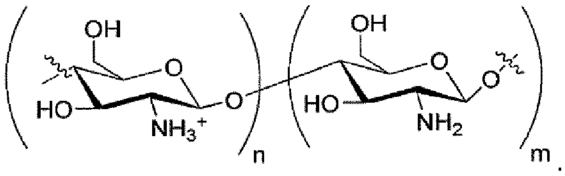
Although reducing hepatic cholesterol has anti-inflammatory effects in animal models of NASH, whether the difference in hepatic cholesterol can explain the superior effects of Compound N versus Reference A upon hepatic inflammation (as shown in examples) is uncertain. The mechanism by which Compound N, and not Reference A, reduces macrovesicular steatosis is also uncertain, but does not appear to be mediated by increased beta-oxidation of fatty acids (data not shown).

As previously described, multiple independent and interdependent metabolic, inflammatory and ultimately fibrotic components converge in the development of human NASH. It is likely that any successful treatment will need to address all aspects of NASH, preferably via upstream metabolic/inflammatory targets. The unique, broad spectrum of metabolic, inflammatory and histological effects as described in the enclosed examples provide justification for the testing of the efficacy of Compound N in human subjects with NASH.

Compounds of Formula (I) can be prepared as described, for example, in PCT Application WO 2010/008299 filed July 13, 2009, and according to Examples below.

Examples 1-13 are exemplary and one skilled in the art would understand how to apply these general methods to arrive at other compounds within the scope of Formula (I). Compounds of the present disclosure may be in the form of a pharmaceutically acceptable salt or ester. For example, the compounds of Formula (I) may be in the form of esters, such as a phospholipid, a glyceride or a C_1-C_6-alkyl ester. In at least one embodiment, the ester is chosen from a glyceride or a C_1-C_6-alkyl ester. In at least one embodiment, the ester is chosen from a triglyceride, a 1,2-diglyceride, a 1,3-diglyceride, a 1-monoglyceride, a 2-monoglyceride, a methyl ester, an ethyl ester, a propyl ester, a isopropyl ester, a *n*-butyl ester and a *tert*-butyl ester. In at least one embodiment, the compound of Formula (I) is present as a methyl ester, an ethyl ester, an isopropyl ester, a *n*-butyl ester or a *tert*-butyl ester, for example as a methyl ester or an ethyl ester. Typically, esters represented by Formula (I) (e.g., ethyl esters) will be hydrolyzed in the gastrointestinal tract.

Salts suitable for the present disclosure include, but are not limited to, salts of NH^{4+}; metal ions such as Li^+, Na^+, K^+, Mg^{2+}, or Ca^{2+}; a protonated primary amine such as tert-butyl ammonium, (3S,5S,7S)-adamantan-1-ammonium, 1,3-dihydroxy-2-(hydroxymethyl)propan-2-ammonium, a protonated aminopyridine (e.g., pyridine-2-ammonium); a protonated secondary amine such as diethylammonium, 2,3,4,5,6-pentahydroxy-N-methylhexan-1-ammonium, N-ethylnaphthalen-1-ammonium, a protonated tertiary amine such as 4-methylmorpholin-4-ium, a protonated quaternary amine such as 2-hydroxy-N,N,N-trimethylethan-1-aminium and a protonated guanidine such as amino((4-amino-4-carboxybutyl)amino)methaniminium or a protonated heterocycle such as 1H-imidazol-3-ium. Additional examples of suitable salts include salts of a diprotonated diamine such as ethane-1,2-diammonium or piperazine-1,4-diium. Other salts according to the present disclosure may comprise protonated Chitosan:



In at least embodiment, the salts are chosen from a sodium salt, a calcium salt, and a choline salt. In one embodiment the salt is a sodium salt or a calcium salt.

The present disclosure provides for a method of preventing or treating NASH in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of a compound of Formula (I). The subject may be a human or a non-human mammal. The compounds presently disclosed may be administered as a medicament, such as in a pharmaceutical composition. Hence, another aspect of the invention is a composition, such as a pharmaceutical composition, comprising a compound of Formula (I) for preventing and/or treating non-alcoholic steatohepatitis.

The composition presently disclosed may comprise at least one compound of Formula (I) and optionally at least one non-active pharmaceutical ingredient, i.e., excipient. Non-active ingredients may solubilize, suspend, thicken, dilute, emulsify, stabilize, preserve, protect, color, flavor, and/or fashion active ingredients into an applicable and efficacious preparation, such that it may be safe, convenient, and/or otherwise acceptable for use. Examples of excipients include, but are not limited to, solvents, carriers, diluents, binders, fillers, sweeteners, aromas, pH modifiers, viscosity modifiers, antioxidants, extenders, humectants, disintegrating agents, solution-retarding agents, absorption accelerators, wetting agents, absorbents, lubricants, coloring agents, dispersing agents, and preservatives. Excipients may have more than one role or function, or may be classified in more than one group; classifications are descriptive only and are not intended to be limiting. In some embodiments, for example, the at least one excipient may be chosen from corn starch, lactose, glucose, microcrystalline cellulose, magnesium stearate, polyvinylpyrrolidone, citric acid, tartaric acid, water, ethanol, glycerol, sorbitol, polyethylene glycol, propylene glycol, cetylstearyl alcohol, carboxymethylcellulose, and fatty substances such as hard fat or suitable mixtures thereof. In some embodiments, the compositions presently disclosed comprise at least one compound of Formula (I) and at least one pharmaceutically acceptable antioxidant, e.g., tocopherol such as *alpha*-tocopherol, betatocopherol, *gamma*-tocopherol, and *delta*-tocopherol, or mixtures thereof, BHA such as 2-*tert*-butyl-4-hydroxyanisole and 3-*tert*-butyl-4-hydroxyanisole, or mixtures thereof and BHT (3,5-di-*tert*-butyl-4-hydroxytoluene), or mixtures thereof.

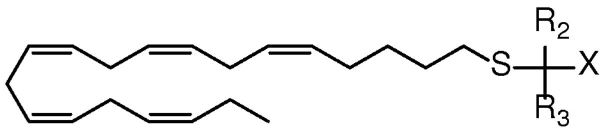
The compositions presently disclosed may be formulated in oral administration forms, e.g., tablets or gelatin soft or hard capsules. The dosage form can be of any shape suitable for oral administration, such as spherical, oval, ellipsoidal, cube-shaped, regular, and/or irregular shaped. Conventional formulation techniques known in the art may be used to formulate the compounds according to the present disclosure. In some embodiments, the composition may be in the form of a gelatin capsule or a tablet.

A suitable daily dosage of a compound of Formula (I) may range from about 5 mg to about 2 g. For example, in some embodiments, the daily dose ranges from about 10 mg to about 1.5 g, from about 50 mg to about 1 g, from about 100 mg to about 1 g, from about 150 mg to about 900 mg, from about 50 mg to about 800 mg, from about 100 mg to about 800 mg, from about 100 mg to about 600 mg, from about 150 to about 550 mg, or from about 200 to about 500 mg. In at least one embodiment, the daily dose ranges from about 200 mg to about 600 mg. In at least one embodiment, the daily dose is about 50 mg, about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, or about 900 mg. The compound(s) may be administered, for example, once, twice, or three times per day. In at least one embodiment, the compound of Formula (I) is administered in an amount ranging from about 200 mg to about 800 mg per dose. In at least one embodiment, the compound of Formula (I) is administered once per day.

The present inventors have found that compounds of Formula (I), such as 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid, have remarkably good pharmaceutical activity. Surprisingly, the compounds of Formula (I) presently disclosed exhibit improved biological activity compared to naturally occurring omega-3 fatty acids, such as EPA and DHA for preventing and/or treating NASH.

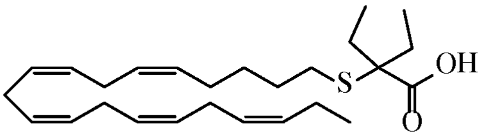
Some specific embodiments of the invention is listed below:

A method of preventing and/or treating non-alcoholic steatohepatitis in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of a compound of Formula (I):



Formula (I)

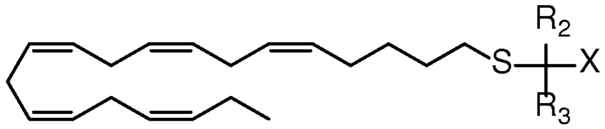
wherein R_2 and R_3 are independently selected from the group of a hydrogen atom and linear, branched, and/or cyclic C_1-C_6 alkyl groups, with the proviso that R_2 and R_3 are not both hydrogen; X is a carboxylic acid or a derivative thereof, wherein the derivative is a carboxylic ester, a glyceride, or a phospholipid; or a pharmaceutically acceptable salt, solvate, or solvate of such a salt. More specifically R_2 and R_3 are independently chosen from a hydrogen atom, a methyl group, an ethyl group, a n-propyl group, and an isopropyl group. More specifically R_2 and R_3 are idependently C_1-C_6 alkyl groups, such as R_2 and R_3 are the same or different and each are independently chosen from a methyl group, an ethyl group, an n-propyl group, or an isopropyl group. Preferably R_2 and R_3 are both ethyl groups. In one embodiment X is a carboxylic acid. In another embodiment, X is a carboxylic ester, such as a C_1-C_6 alkyl ester, such as chosen from a methyl ester, an ethyl ester, an isopropyl ester, a *n*-butyl ester, and a *tert*-butyl ester, such as chosen from methyl ester and an ethyl ester. The method above, wherein the glyceride is chosen from a triglyceride, a 1,2-diglyceride, a 1,3-diglyceride, a 1-monoglyceride, and 2-monoglyceride. In one embodiment the compound is present in the form of an enantiomer, diastereomer, or mixture thereof. The method above wherein the compound is present in its *R* form. In one embodiment the compound is present in its *S* form. In another embodiment, the compound is present in racemic form. In a preferred embodiment, the invention provides a method as above, wherein R_2 and R_3 are ethyl groups and X is a carboxylic acid.The pharmaceutically effective amount of the compound of Formula (I) ranges from about 5 mg to about 2 g per dose, such as from about 200 mg to about 800 mg per dose, such as about 600 mg. In one embodiment of the method the subject is a human. The compound is preferably administered daily, such as once daily. The method as disclosed wherein the compound is formulated as a pharmaceutical composition for oral administration, such as in the form of a gelatin capsule or a tablet. The pharmaceutical composition may further comprise at least one binder, excipient, diluent, or any combinations thereof. The pharmaceutical composition further comprises an antioxidant, such as chosen from tocopherol, BHA, and BHT, or a mixture thereof. A method of treating and/or preventing non-alcoholic steatohepatitis in a subject in need thereof, the method comprising administering to the subject a pharmaceutically effective amount of 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid:



(Compound N)

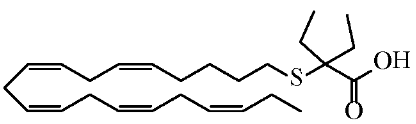
or a pharmaceutically acceptable salt or ester thereof.

Use of a pharmaceutically effective amount of a compound of Formula (I)



Formula (I)

wherein R_2 and R_3 are independently selected from the group of a hydrogen atom and linear, branched, and/or cyclic C_1-C_6 alkyl groups, with the proviso that R_2 and R_3 are not both hydrogen; X is a carboxylic acid or a derivative thereof, wherein the derivative is a carboxylic ester, a glyceride or a phospholipid; or a pharmaceutically acceptable salt, solvate, or solvate of such a salt, in the manufacture of a medicament for preventing and/or treating non-alcoholic steatohepatitis in a subject in need thereof. Use of a pharmaceutically effective amount of 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid:



or a pharmaceutically acceptable salt or ester thereof in the manufacure of a medicament for treating and/or preventing non-alcoholic steatohepatitis in a subject in need thereof.
The use above, wherein the pharmaceutically-effective amount ranges from about 200 mg to about 800 mg per dose. The use above wherein 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid is administered once daily.

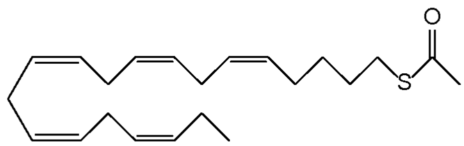
Examples

The present disclosure may be further described by the following non-limiting examples, in which standard techniques known to the skilled chemist and techniques analogous to those described in these examples may be used where appropriate. It is understood that the skilled artisan will envision additional embodiments consistent with the disclosure provided herein.

Unless otherwise stated, reactions were carried out at room temperature, typically in the range between 18-25°C with solvents of HPLC grade under anhydrous conditions. Evaporations were carried out by rotary evaporation in vacuo. Column chromatography was performed by the flash procedure on silica gel 40-63 µm (Merck) or by an Armen Spotflash using the pre-packed silica gel columns “MiniVarioFlash”, “SuperVarioFlash”, “SuperVarioPrep” or “EasyVarioPrep” (Merck). Nuclear magnetic resonance (NMR) shift values were recorded on a Bruker Avance DPX 200 or 300 instrument with peak multiplicities described as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; p, pentet; m, multiplett; br, broad. The mass spectra were recorded with a LC/MS spectrometer. Separation was performed using a Agilent 1100 series module on a Eclipse XDB-C18 2.1 x 150 mm column with gradient elution. As eluent were used a gradient of 5-95% acetonitrile in buffers containing 0.01% trifluoroacetic acid or 0.005% sodium formate. The mass spectra were recorded with a GI956A mass spectrometer (electrospray, 3000 V) switching positive and negative ionization mode. Reported yields are illustrative and do not necessarily represent the maximum yield attainable.

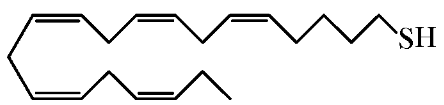
<u>Preparation of intermediates:</u>

Example 1: Preparation of S-(5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenyl ethanethioate



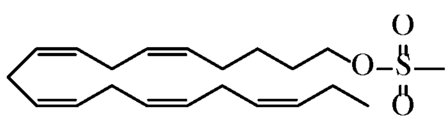
Triphenylphosphine (21.0 g, 80 mmol) was dissolved in dry THF (170 mL) at 0°C under inert atmosphere and added DIAD (15.8 mL, 80 mmol) dropwise. After 40 minutes at 0°C the white suspension was added dropwise to a solution of (5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaen-1-ol (11.5 g, 40 mmol) and thioacetic acid (5.7 mL, 80 mmol) in dry THF (50 mL) during 15 minutes. The resulting turbid mixture was stirred at 0°C for 30 minutes, followed by ambient temperature for 1.5 hour. Heptane was added (200 mL), the mixture was stirred for ten minutes and the precipitated white solid removed by filtration and rinsed with heptane (150 mL). The residue was concentrated to remove most of the THF and stirred at ambient for 18 hours. The mixture was filtered, concentrated and added heptane (200 mL). The resulting mixture was stirred for 2 hours, filtered and evaporated. The residue was purified by flash chromatography on silica gel, using EtOAc: Heptane (2:98), followed by EtOAc: Heptane (4:96) and finally EtOAc: Heptane (5:95). Concentration of the appropriate fractions provided 11.0 g (79% yield) of the title compound as oil. 1H-NMR (300 MHz, CDCl3): δ 0.95 (t, 3H, J=7.5 Hz), 1.40 (m, 2H), 1.58 (m, 2H), 2.06 (m, 4H), 2.29 (s, 3H), 2.77 – 2.87 (m, 10H), 5.25 – 5.42 (m, 10H); MS (CI (CH4)): 387 [M+C3H5]+, 375 [M+C2H5]+, 347 [M+H]+, 333 [M-CH2]+, 305 [R–SH]+.

Example 2: Preparation of (5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaene-1-thiol



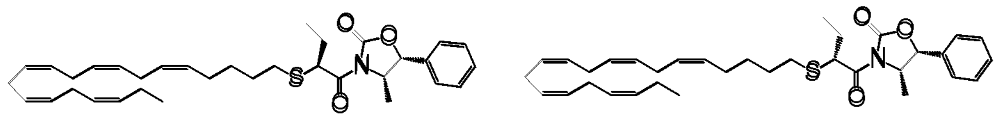
S-(5Z,8Z,11Z,14Z,17Z)-Icosa-5,8,11,14,17-pentaenyl ethanethioate (7.00 g, 20.2 mmol) was dissolved in MeOH (100 mL) by stirring 10 minutes until the droplets of oil dissolved, before anhydrous potassium carbonate, K_2CO_3 (2.79 g, 20.2 mmol) was added in one portion. The mixture was stirred for 1 hour and 20 minutes at ambient temperature and quenched by addition of 1 M HCl (50 mL) and water (150 mL). The white cloudy mixture was added Et_2O (250 mL) and the phases were separated. The water phase was extracted with Et_2O (2×250 mL). The combined organic phases were washed with brine (250 mL) and dried ($MgSO_4$). Filtration and evaporation gave the title compound as oil (5.99 g, 97% yield), which was used without further purification. 1H-NMR (300 MHz, CDCl3): δ 0.96 (t, 3H, J=7.5 Hz), 1.31 (t, 1H, J=7.8 Hz), 1.44 (m, 2H), 1.61 (m, 2H), 2.06 (m, 4H), 2.51 (m, 2H), 2.77-2.85 (m, 8H), 5.28-5.41 (m, 10H); MS (CI (CH4)): 345 [M+C3H5]+, 333 [M+C2H5]+, 305 [M+H]+, 271 [M-SH]+.

Example 3: Preparation of (5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenyl methanesulfonate



Et3N (1.50 mL, 10.8 mmol) and methanesulfonyl chloride (402 µL, 5.20 mmol) was added to a solution of (5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaen-1-ol (1.15 g, 4.0 mmol) in CH_2Cl_2 (40 mL) held at 0°C under nitrogen. The mixture was stirred at 0°C for one hour, and poured into ice-water (100 g) and the water phase extracted with Et_2O (50 mL). The combined organic extracts were added 0.5 M H_2SO_4 (35 mL), the organic phase washed with $NaHCO_3$ (sat. aq.) (25 mL), before dried (Mg_2SO_4, 10 gram). Filtration and concentration in vacuo afforded 1.24 gram of crude oil. Purification on Armen, SVP D26 column packed with 30 gram of 15-40 µm Merck silica, flow 20 mL/min, UV 210 nm and collecting 15 mL fraction, was performed using gradient elution: (starting heptane: EtOAc (100:0) and increasing during 10 min. to 10% EtOAc, then increasing 5 min. to 20% EtOAc (hold 10 min.), then increasing in 5 min. to 40% EtOAc (hold 0 min.). Fractions 6-14 afforded 1.16g (79% yield) of the title compound as oil. 1H-NMR (300 MHz, CDCl3): δ 0.97 (t, 3H), 1.50 (m, 2H), 1.75 (m, 2H), 2.03-2.15 (m, 4H), 2.76-2.86 (m, 8H), 2.99 (s, 3H), 4.22 (t, 2H), 5.27-5.40 (m, 10H); MS (electrospray): 389.2 [M+Na]+.

Example 4: Preparation of (4S,5R)-3-((S)-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoyl)-4-methyl-5-phenyloxazolidin-2-one and (4S,5R)-3-((R)-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoyl)-4-methyl-5-phenyloxazolidin-2-one

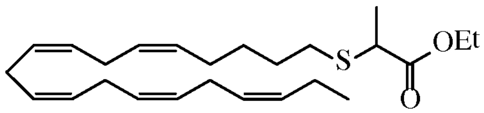


A mixture of 2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid (3.0 g, 7.9 mmol) in dry dichloromethane (40 mL) held at 0°C under nitrogen was added DMAP (1.0 g, 9.5 mmol) and 1,3-dicyclohexylcarbodiimide (DCC) (1.8 g, 8.7 mmol). The resulting mixture was stirred at 0°C for 20 minutes, (4S,5R)-4-methyl-5-phenyl-2-oxazolidinone (1.7 g, 9.5 mmol) was added and the resulting turbid mixture was stirred at ambient temperature for 24 hours. The mixture was filtrated and concentrated under reduced pressure to give a crude product containing the desired product as a mixture of two diastereomers. The residue was purified by flash chromatography on Armen Spotflash instrument on silica gel using 2% ethyl acetate in heptane as eluent. The two diastereomers were separated and the appropriate fractions were concentrated. (4S,5R)-3-((R)-2-((5Z,8Z,11Z,14Z,17Z)-Icosa-5,8,11,14,17-pentaenylthio)butanoyl)-4-methyl-5-phenyloxazolidin-2-one eluted first and was obtained in 0.95 g (47% yield) as an oil. 1.47 g (67% yield) of (4S,5R)-3-((S)-2-

((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoyl)-4-methyl-5-phenyloxazolidin-2-one was obtained as an oil. (4S,5R)-3-((R)-2-((5Z,8Z,11Z,14Z,17Z)-Icosa-5,8,11,14,17-pentaenylthio)butanoyl)-4-methyl-5-phenyloxazolidin-2-one (E1):1H-NMR (300 MHz, CDCl3): δ 0.93-1.06 (m, 9H), 1.45-1.60 (m, 4H), 1.75-1.85 (m, 1H), 2.05-2.15 (m, 5H), 2.55-2.70 (m, 2H), 2.87 (m, 8H), 4.69 (t, 1H), 4.79 (p, 1H), 5.30-5.45 (m, 10H), 5.72 (d, 1H), 7.32 (m, 2H), 7.43 (m, 3H). (4S,5R)-3-((S)-2-((5Z,8Z,11Z,14Z,17Z)-Icosa-5,8,11,14,17-pentaenylthio)butanoyl)-4-methyl-5-phenyloxazolidin-2-one:1H-NMR (300 MHz, CDCl3): δ 0.93 (d, 3H), 0.99 (t, 3H), 1.05 (t, 3H), 1.40-1.56 (m, 4H), 1.50-1.75 (m, 1H), 2.00-2.15 (m, 5H), 2.47-2.65 (m, 2H), 2.83 (m, 8H), 4.62 (t, 1H), 4.85 (p, 1H), 5.25-5.45 (m, 10H), 5.70 (d, 1H), 7.32 (m, 2H), 7.43 (m, 3H).

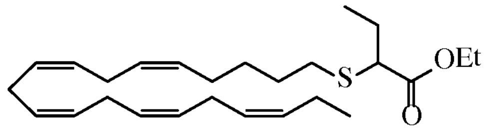
Preparation of target molecules:

Example 5: Preparation of ethyl 2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)propanoate



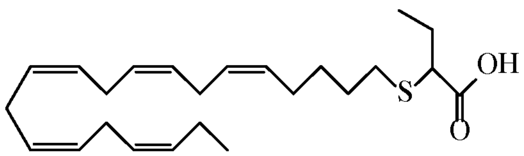
(5Z,8Z,11Z,14Z,17Z)-Icosa-5,8,11,14,17-pentaene-1-thiol (305 mg, 1.00 mmol) was added to a solution of NaH (60% in mineral oil, 44 mg, 1.10 mmol) in dry DMF (10 mL) held at 0°C under inert atmosphere. After ten minutes, ethyl bromopropionate (136 µL, 1.05 mmol) was added and the mixture was stirred for 1.5 hour at 0°C. The reaction mixture was added sat. aq. NH_4Cl (20 mL) and heptane (50 mL). The phases were separated and the water phase extracted with heptane (2×25 mL). The combined organics were washed with brine (25 mL), dried ($MgSO_4$), filtered and evaporated to give 376 mg of title compound as crude oil. Purification by flash chromatography on silica gel using gradient elution (starting pure heptane and increasing stepwise to heptane:EtOAc 95:5) afforded 318 mg (79% yield) of the title compound as oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H), 1.25 (t, 3H), 1.41 (d, 3H), 1.44 (m, 2H), 1.58 (m, 2H), 2.06 (m, 4H), 2.60 (m, 2H), 2.71 – 2.85 (m, 8H), 3.36 (d, 1H), 4.17 (m, 2H), 5.25 – 5.40 (m, 10H); MS (CI (CH_4)): 445 $[M+C_3H_5]^+$, 433 $[M+C_2H_5]^+$, 405 $[M+H]^+$, 359 $[M-OEt]^+$, 331 $[M-CO_2Et]^+$, 303 $[R–S]^{\bullet+}$.

Example 6: Preparation of ethyl 2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoate



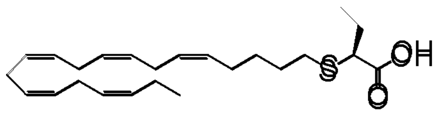
To a solution of (5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaene-1-thiol (305 mg, 1.00 mmol) in dry DMF (10 mL) at 0°C under inert atmosphere was added NaH (60% in mineral oil, 44 mg, 1.1 mmol). After fifteen minutes, ethyl bromobutyrate (154 µL, 1.05 mmol) was added. The mixture was stirred for 1 hour at 0°C. Sat. aq. NH_4Cl (20 mL), water (20 mL) and heptane (50 mL) were added. The phases were separated and the water phase was extracted with heptane (2×25 mL). The combined organics were washed with water (25 mL) and brine (25 mL), dried ($MgSO_4$), filtered and evaporated to give 379 mg of the title compound as a crude oil. Purification by flash chromatography on silica gel using gradient elution (starting pure heptane and increasing stepwise to heptane:EtOAc 95:5) afforded 345 mg (82% yield) of the title compound as oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.93 – 1.00 (m, 6H), 1.25 (t, 3H), 1.44 (m, 2H), 1.59 (m, 2H), 1,68 (m, 1H), 1.87 (m, 1H), 2.07 (m, 4H), 2.57 (m, 2H), 2.73 – 2.88 (m, 8H), 3.12 (m, 1H), 4.17 (m, 2H), 5.27 – 5.46 (m, 10H); MS (CI (CH_4)): 459 $[M+C_3H_5]^+$, 447 $[M+C_2H_5]^+$, 419 $[M+H]^+$, 373 $[M\text{-}OEt]^+$, 345 $[M\text{-}CO_2Et]^+$, 303 $[R–S]^{\bullet+}$.

Example 7: Preparation of 2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid



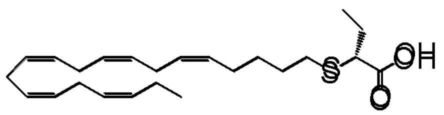
Ethyl 2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoate (209 mg, 0.50 mmol) was dissolved in ethanol (2.5 mL) and added to a solution of LiOH × H_2O (168 mg, 4.0 mmol) in water (2.5 mL). The resulting turbid solution was stirred at 70°C under inert atmosphere for 2 hours, cooled and added water (10 mL) and 1 M HCl (5 mL) to pH = 1–2. The mixture was extracted with heptane (2 × 20 mL) and diethyl ether (20 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give 154 mg of the title compound as crude oil. Purification by flash chromatography on silica gel using gradient elution (starting with pure heptane and increasing stepwise to heptane:EtOAc (with 5% HOAc) 80:20) afforded 151 mg (77% yield) of the title compound as oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H), 1.02 (t, 3H), 1.46 (m, 2H), 1.52 – 1.78 (m, 3H), 1.90 (m, 1H), 2.05 (m, 4H), 2.63 (m, 2H), 2.75 – 2.90 (m, 8H), 3.14 (t, 1H) (m, 1H), 4.17 (m, 2H), 5.27 – 5.46 (m, 10H).

Example 8: Preparation of (S)-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid



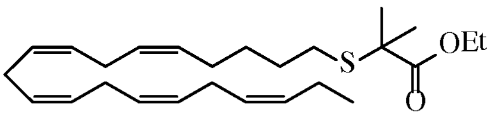
Hydrogen peroxide (30 % in water, 0.71 mL, 6.91 mmol) and lithium hydroxide monohydrate (0.15 g, 3.46 mmol) was added to a solution of (4S,5R)-3-((S)-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoyl)-4-methyl-5-phenyloxazolidin-2-one (0.95 g, 1.73 mmol) in tetrahydrofuran (12 mL) and water (4 mL) held at 0°C under nitrogen. The reaction mixture was stirred at 0°C for 30 minutes. 10% $Na_2SO_{3\,(aq)}$ (30 mL) was added, the pH was adjusted to ~2 with 5M HCl and the mixture was extracted twice with heptane (30 mL). The combined organic extract was dried (Na_2SO_4), filtered and concentrated. The residue was subjected to flash chromatography on silica gel using increasingly polar mixtures of heptane and ethyl acetate (98:8 → 1:1) as eluent. Concentration of the appropriate fractions afforded 0.15 g (17% yield) of the title product as an oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.00 (t, 3H), 1.07 (t, 3H), 1.46 (m, 2H), 1.60-1.75 (m, 3H), 1.85 (m, 1H), 2.10 (m, 4H), 2.66 (m, 2H), 2.80-2.90 (m, 8H), 3.21 (t, 1H), 5.35-5.45 (m, 10H); MS (electrospray): 389.3 $[M-H]^-$; [ ]D -49° (c=0.12, ethanol).

Example 9: Preparation of (R)-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid



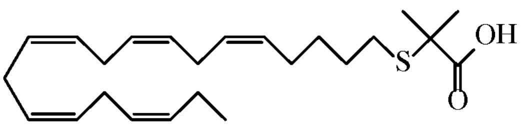
Hydrogen peroxide (30 % in water, 1.04 mL, 10.2 mmol) and lithium hydroxide monohydrate (0.21 g, 5.09 mmol) was added to a solution of (4S,5R)-3-((R)-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoyl)-4-methyl-5-phenyloxazolidin-2-one (1.40 g, 2.55 mmol) in tetrahydrofuran (15 mL) and water (5 mL) held at 0°C under nitrogen. The reaction mixture was stirred at 0°C for 45 minutes. 10% $Na_2SO_{3\,(aq)}$ (35 mL) was added, pH was adjusted to ~2 with 5M HCl and the mixture was extracted twice with heptane (35 mL). The combined organic extract was dried (Na_2SO_4), filtered and concentrated. The residue was subjected to flash chromatography on silica gel using increasingly polar mixtures of heptane and ethyl acetate (98:8 → 1:1) as eluent. Concentration of the appropriate fractions afforded 0.17 g (22 % yield) of the title product as an oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.00 (t, 3H), 1.07 (t, 3H), 1.46 (m, 2H), 1.60-1.75 (m, 3H), 1.85 (m, 1H), 2.10 (m, 4H), 2.66 (m, 2H), 2.80-2.90 (m, 8H), 3.21 (t, 1H), 5.35-5.45 (m, 10H); MS (electrospray): 389.3 $[M-H]^-$; [[illegible]50° (c=0.14, ethanol).

Example 10: Preparation of ethyl 2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)-2-methylpropanoate



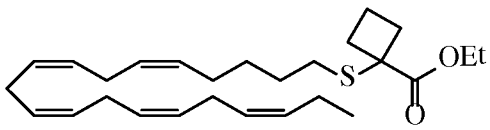
To a solution of (5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaene-1-thiol (305 mg, 1.00 mmol) in dry DMF (10 mL) at 0°C under inert atmosphere was added NaH (60% in mineral oil, 44 mg, 1.1 mmol). After fifteen minutes, ethyl 2-bromo-2-methylbutyrate (154 µL, 1.05 mmol) was added and the mixture was stirred for 1.5 hour at 0°C. The reaction mixture was quenched by addition of sat. aq. NH_4Cl (20 mL). Water (20 mL) and heptane (50 mL) were added and the phases were separated. The water phase was extracted with heptane (2×25 mL). The combined organics were washed with water (25 mL) and brine (2 × 25 mL), dried ($MgSO_4$), filtered and evaporated to give 377 mg of the title compound as a crude oil. Purification by flash chromatography on silica gel using isocratic elution (heptane:EtOAc 98:2) afforded 307 mg (77% yield) of the title compound as oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H), 1.28 (t, 3H), 1.42 (m, 2H), 1.48 (s, 6H), 1.54 (m, 2H), 2.06 (m, 4H), 2.58 (m, 2H), 2.71 – 2.85 (m, 8H), 4.15 (m, 2H), 5.22 – 5.48 (m, 10H); MS (CI (CH_4)): 459 $[M+C_3H_5]^+$, 447 $[M+C_2H_5]^+$, 419 $[M+H]^+$, 373 $[M-OEt]^+$, 345 $[M-CO_2Et]^+$, 303 $[R–S]^{\bullet+}$.

Example 11: Preparation of 2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)-2-methylpropanoic acid



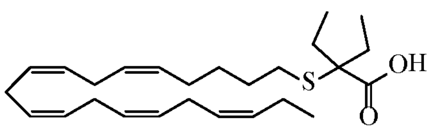
Ethyl 2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)-2-methylpropanoate (209 mg, 0.50 mmol) was dissolved in ethanol (2.5 mL) and added to a solution of LiOH × H_2O (168 mg, 4.0 mmol) in water (2.5 mL). The resulting turbid solution was stirred at 70°C under inert atmosphere for 2 hours, cooled and added water (10 mL) and 1 M HCl (5 mL) to pH = 1–2. The mixture was extracted three times with heptane (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give 101 mg of the title compound as crude oil. Purification by flash chromatography on silica gel using gradient elution (starting with pure heptane and increasing stepwise to heptane:EtOAc (with 5% HOAc) 80 : 20) afforded 78 mg (40%) of the title compound as oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H), 1.35-1.66 (m, 4H), 1.50 (s, 6H), 2.07 (m, 4H), 2.63 (t, 3H), 2.70-2.92 (m, 8H), 5.13- 5.50 (m, 10H).

Example 12: Preparation of ethyl 1-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)cyclobutanecarboxylate



To a solution of (5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaene-1-thiol (305 mg, 1.00 mmol) in dry DMF (10 mL) at 0°C under inert atmosphere was added NaH (60% in mineral oil, 44 mg, 1.1 mmol). After fifteen minutes ethyl 2-bromo-cyclobutane carboxylate (170 µL, 1.05 mmol) was added and the mixture was stirred for 1.5 hour at 0°C. The reaction was quenched by addition of sat. aq. NH_4Cl (20 mL). Heptane (50 mL) was added, and the phases were separated. The water phase was extracted with heptane (2×25 mL). The combined organics were washed with water (25 mL) and brine (25 mL), dried ($MgSO_4$), filtered and evaporated to give 409 mg of the title compound as a crude oil. Purification by flash chromatography on silica gel using isocratic elution (heptane:acetone 98:2) afforded 243 mg (56% yield) of the title compound as oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H), 1.27 (t, 3H), 1.42 (d, 3H), 1.54 (m, 2H), 1.84 (m, 1H), 1.96-2.23 (m, 7H), 2.51 (m, 2H), 2.60 (m, 2H), 2.73-2.90 (m, 8H), 4.18 (m, 2H), 5.23-5.43 (m, 10H); MS (CI (CH_4)): 471 $[M+C_3H_5]^+$, 459 $[M+C_2H_5]^+$, 431 $[M+H]^+$, 385 $[M\text{-}OEt]^+$, 357 $[M\text{-}CO_2Et]^+$, 303 $[R–S]^{\bullet+}$.

Example 13: Preparation of 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid (Compound N).



NaOEt (21 wt.% in EtOH, 0.37 mL, 0.98 mmol) was added dropwise to a solution of 2-mercapto-2-ethyl butyric acid (0.08 g, 0.49 mmol) in dry EtOH (7 mL) held at 0°C under inert atmosphere. The resulting mixture was stirred at 0°C for 30 minutes before a solution of

(5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenyl methanesulfonate (0.15 g, 0.41 mmol) in dry EtOH (3 mL) was added dropwise. The resulting turbid mixture was stirred at ambient temperature for 24 hours, poured into NH4Cl (sat.)(aq.) (15 mL), added 3M HCl to pH ~2 before extracted twice with EtOAc (2x20 mL). The combined organic extracts were washed with brine (10 mL), dried (MgSO4), filtrated and evaporated in vacuo. The residue was purified by flash chromatography on silica gel using a gradient of 10-25% ethyl acetate in heptane as eluent. Concentration of the appropriate fractions afforded 0.12 g (70% yield) of the title compound as oil. 1H-NMR (300 MHz, $CDCl_3$): δ 0.88-1.02 (m, 9H), 1.45-1.58 (2xm, 4H), 1.72 (m, 2H), 1.82 (m, 2H) 2.09 (m, 4H), 2.53 (t, 2H), 2.76-2.86 (m, 8H), 5.29-5.39 (m, 10H. MS (electrospray): 417.3 [M-H]-.

Biological Examples

Evaluation of compound N in a diet induced NAFLD/NASH mouse model (APOE*3Leiden.CETP double transgenic mice)

The APOE*3Leiden.CETP double transgenic mouse is expressing a variant of the human apolipoprotein E3 (APOE3), the APOE*3Leiden, in addition to the human apolipoprotein C1 (APOC1) and CETP. APOE*3Leiden.CETP double transgenic mice exhibit elevated plasma cholesterol and triglyceride levels, mainly confined to the VLDL/LDL sized lipoprotein fraction. By increasing the cholesterol content of the diet in this model, all the characteristics of human NASH develop.

Studies were performed in APOE*3Leiden.CETP mice placed on a high fat diet (24% fat w/w) with varying cholesterol content (0.25-1% cholesterol w/w). In one study (using 1% cholesterol w/w), after a 3 weeks run-in period 17 (20%) low-responder mice were removed from the study and the remaining 65 mice were sub-divided into groups of 12 mice each (+5 in the control group), matched for plasma cholesterol, triglycerides, blood glucose, body weight and age (t=0) and treatment was started. The mice received a daily gavage between 07hr00 and 10hr00 with either Compound N, Reference A or control (corn oil). After 4, 8, 12, 16 and 20 weeks of treatment blood samples were taken after a 5 hour fasting period (also 5 hours after compound or vehicle administration). Plasma cholesterol and triglycerides were measured. After 14 weeks of treatment 5 representative mice from the control group were sacrificed in order to assess the NASH development and to determine the termination of the study. After 20 weeks of treatment mice were sacrificed by CO2 asphyxiation, heparin heart blood was sampled and tissues were collected. Hepatic steatosis, inflammation and collagen content were analyzed.

A further study was performed in APOE*3Leiden.CETP mice with a 4-week active treatment arm in mice placed on a high fat diet (24% fat and 0.25% cholesterol diet, both w/w) to collect additional metabolic data.

Biological Example 1. Effects of Compound N on hepatic cholesterol content.

Compound N administered to ApoE*3L-CETP mice (0.25% cholesterol diet w/w) induced a significant decrease in hepatic cholesterol ester ($p<0.001$). A mild decrease in cholesterol ester was also observed with Reference A ($p<0.05$). A 43% reduction in total hepatic cholesterol (statistics not performed). The effects of Compound N on hepatic cholesterol are shown in Table 1 and Fig. 1.

Table 1 Liver lipids (µg lipid/mg liver protein), AVG ± SD

Compound	**Free cholesterol** (FC in Fig 1)	**Cholesterol ester** (CE in Fig 1)
Control	12.3 ± 3.0	22.7 ± 5,9
Reference A	9.5 ± 1.3	17.0 ± 3.7
Compound N	9.8 ± 1.2	10.6 ± 2.1

Biological Example 2. Effects of Compound N on hepatic inflammation.

Compound N administered to ApoE*3L-CETP mice induced a significant ($p<0.001$) reduction in the number of inflammatory foci by approximately 85%, leading to a significant reduction in the total inflammation score. Reference A induced a milder reduction in inflammatory foci ($p<0.01$). Compound N has a significantly better effect upon hepatic inflammation than Reference A ($p<0.01$). The effects of Compound N on hepatic i**nflammation** are shown in Table 2 and Fig. 2.

Table 2. Inflammation (number of foci and inflammation score), Mean ± SD.

Compound	Number of foci	Score (0-3)
Control	4.9 ± 2.9	2.7 ± 0.9
Reference A	1.9 ± 1.3	1.9 ± 1.2
Compound N	0.6 ± 0.6	0.6 ± 0.8
Rosiglitazone	1.6 ± 1.6	1.7 ± 1.2

Biological Example 3. Effects of Compound N on macrovesicular steatosis

Compound N administered to ApoE*3L-CETP mice abolished macrovesicular steatosis ($p<0.001$ vs. control). No significant effect upon macrovesicular steatosis was seen with Reference A or Rosiglitazone. Compound N was significantly different from Reference A and Rosiglitazone (both <0.001). The effects of Compound N on macrovesicular steatosis are shown in Table 3 and Fig.

3.

Table 3 Steatosis as % of the liver (Macrovesicular and microvesicular steatosis), Mean ± SD.

Compound	Steatosis as % of the liver	
	Macrovesicular	**Microvesicular**
Control	20.0 ± 15.2	53.6 ± 24.1
Reference A	21.2 ± 15.0	34.6 ± 11.8
Compound N	1.1 ± 1.0	57.9± 25.4
Rosiglitazone	14.3 ± 20.2	52.5 ± 18.4

Biological Example 4. Effects of Compound N on fecal bile acid content

Compound N administered to ApoE*3L-CETP double transgenic mice demonstrated a significant (p=0.006 vs. control) 50% reduction in fecal bile acid excretion. Reference A induced a milder yet significant decrease (p<0.05 vs. control). The Effects of Compound N on fecal bile acid content are shown in Table 4 and Figure 4.

Table 4 Total bile acids (µmol/100 gr mouse/day), AVG ± SD.

Compound	Total bile acids (µmol/100 gr mouse/day)
Control	6.5 ± 1.9
Reference A	4.0 ± 1.2
Compound N	3.2 ± 0.6

Biological Example 5. Effects of Compound N on hepatic fibrosis (collagen content)

Compound N administered to ApoE*3L-CETP double transgenic mice showed a significant (p<0.005) 30% reduction in hepatic collagen content compared to control animals. The effects of Compound N on hepatic fibrosis are shown in Table 5 and Figure 5.

Table 5 Fibrosis (hydroxyprolin/prolin), Mean ± SD.

Compound	Hydroxyprolin/Prolin
Control	0.039 ± 0.010
Compound N	0.027 ± 0.007
Rosiglitazone	0.036 ± 0.007

Biological Example 6. Effects of Compound N on total plasma cholesterol

Compound N administered to ApoE*3L-CETP double transgenic mice demonstrated a 52% reduction in total plasma cholesterol after 4 weeks versus control (p<0.001). Plasma HDL cholesterol values were adoubled (p<0.001, data not shown), underlying the fact that the reduction in plasma cholesterol was specific to the atherogenic apoB particle associated cholesterol fraction. The effects of Compound N on total plasma cholesterol are shown in Table 6 and Figure 6.

Table 6. Total plasma cholesterol (mM) as a function of time in weeks, AVG ± SD.

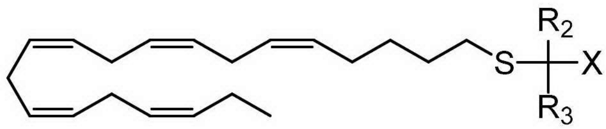
	Cholesterol (mM)		
Compound	**t=0**	**t=2**	**t=4**
Control	6.4 ± 1.5	8.1 ± 1.8	9.7 ± 2.6
Compound N	6.4 ± 1.1	4.2 ± 0.7	4.7 ± 1.3

It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word “comprise” or variations such as “comprises” or “comprising” is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

Claims

1. Use of a compound of Formula (I)



wherein

R_2 and R_3 are independently chosen from the group of a hydrogen atom and linear or branched C_1-C_6 alkyl groups, with the proviso that R_2 and R_3 are not both hydrogen;

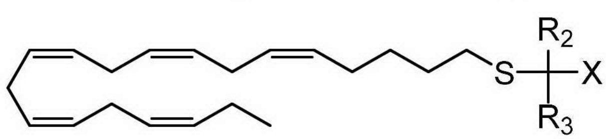
or R_2 and R_3 are connected to form cyclobutane;

X is a carboxylic acid or a C_1-C_6 alkyl ester;

or a pharmaceutically acceptable salt, solvate, or solvate of such salt thereof,

in the manufacture of a medicament for preventing and/or treating non-alcoholic steatohepatitis.

2. A method of preventing and/or treating non-alcoholic steatohepatitis in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of a compound of Formula (I):



Formula (I)

wherein R_2 and R_3 are independently chosen from the group of a hydrogen atom and linear or branched C_1-C_6 alkyl groups,

with the proviso that R_2 and R_3 are not both hydrogen;

or R_2 and R_3 are connected to form cyclobutane;

X is a carboxylic acid or a C_1-C_6 alkyl ester; or a pharmaceutically acceptable salt, solvate, or solvate of such a salt.

3. The use according to claim 1, or the method according to claim 2, wherein R_2 and R_3 are independently chosen from a hydrogen atom, a methyl group, an ethyl group, a n-propyl group and an isopropyl group.

4. The use according to claim 1, or the method according to claim 2, wherein R_2 and R_3 are independently chosen from C_1-C_6 alkyl groups.

5. The use or method according to any one of claims 1-4, wherein R_2 and R_3 are ethyl groups.

6. The use or method according to any one of claims 1-5, wherein X is a carboxylic acid.

7. The use or method according to any one of claims 1-5, wherein X is a C_1-C_6 alkyl ester.

8. The use or method according to any one of claims 1-5 or 7, wherein X is chosen from a methyl ester, an ethyl ester, an isopropyl ester, a *n*-butyl ester and a *tert*-butyl ester.

9. The use or method according to any one of claims 1-5, 7 or 8, wherein X is selected from a methyl ester and an ethyl ester.

10. The use or method according to any one of claims 1 to 9, wherein the compound is present in the form of an enantiomer, a diastereomer or a mixture thereof.

11. The use or method according to claim 10, wherein the compound is present in its R form, in its S form or in racemic form.

12. The use according to claim 1, or the method according to claim 2, wherein R_2 and R_3 are ethyl groups and X is a carboxylic acid.

13. The use or method according to any one of claims 1-12, wherein said compound is administered in a dose ranging from about 5 mg to about 2 g per dose.

14. The use or method according to any one of claims 1-13, wherein said compound is administered in a dose ranging from about 200 mg to about 800 mg per dose.

15. The use or method according to any one of claims 1-14, wherein said compound is administered once daily.

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16.The use or method according to any one of claims 1-15, wherein said compound is formulated as a pharmaceutical composition for oral administration.

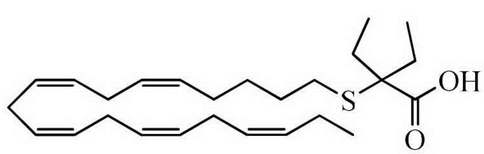
17. The use or method according to claim 16, wherein the pharmaceutical composition is in the form of a gelatin capsule or a tablet.

18. The use or method according to claim 16, wherein the pharmaceutical composition further comprises at least one binder, excipient, diluent, or any combinations thereof.

19. The use or method according to claim 16, wherein the pharmaceutical composition further comprises an antioxidant.

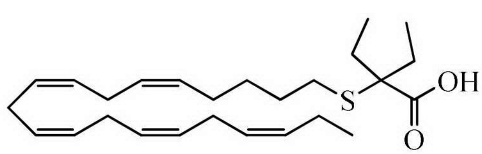
20. The use or method according to claim 19, wherein the antioxidant is chosen from tocopherol, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT), or mixtures thereof.

21. Use of 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid



or a pharmaceutically acceptable salt or ester thereof, in the manufacture of a medicament for preventing and/or treating non-alcoholic steatohepatitis.

22. A method of preventing and/or treating non-alcoholic steatohepatitis in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid



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23. The use according to claim 21, or the method according to claim 22, wherein 2-ethyl-2-((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenylthio)butanoic acid is administered in a dose ranging from about 200 mg to about 800 mg per dose.

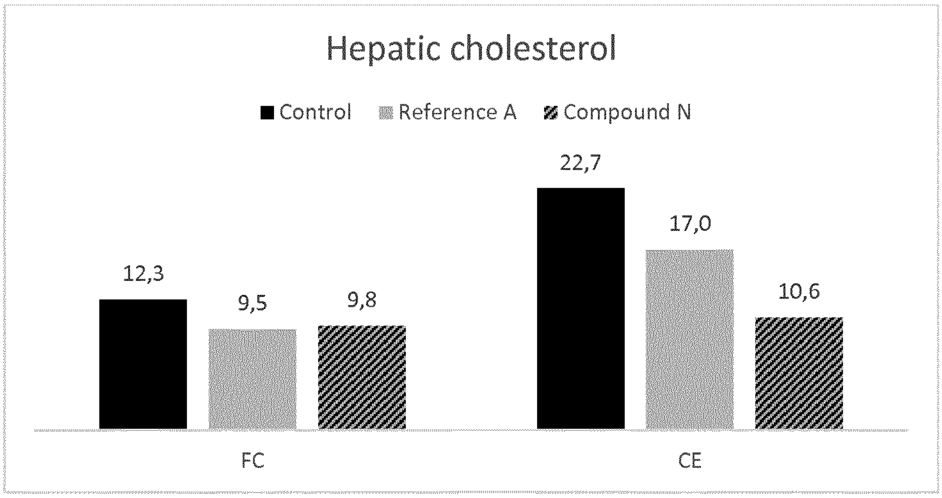

Figure 1.

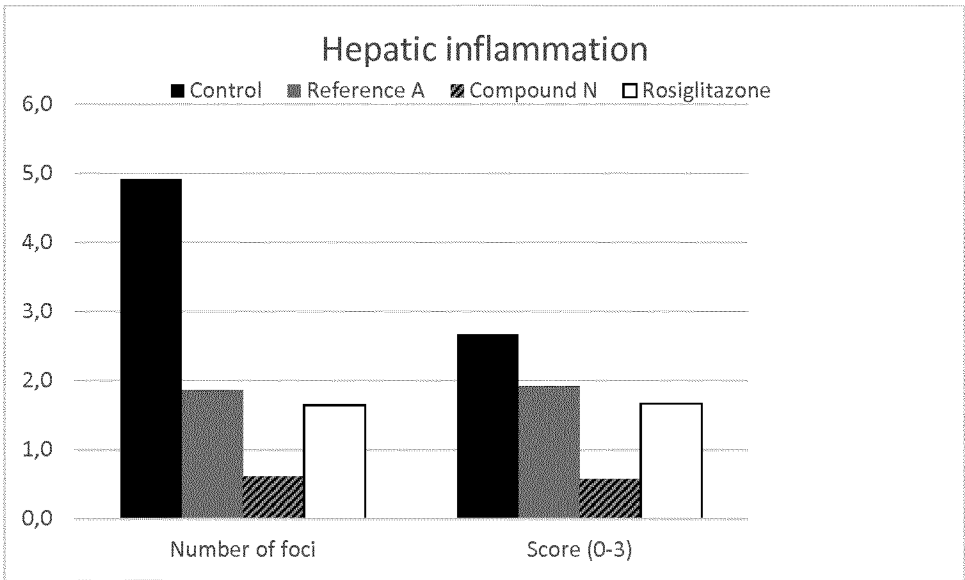

Figure 2.

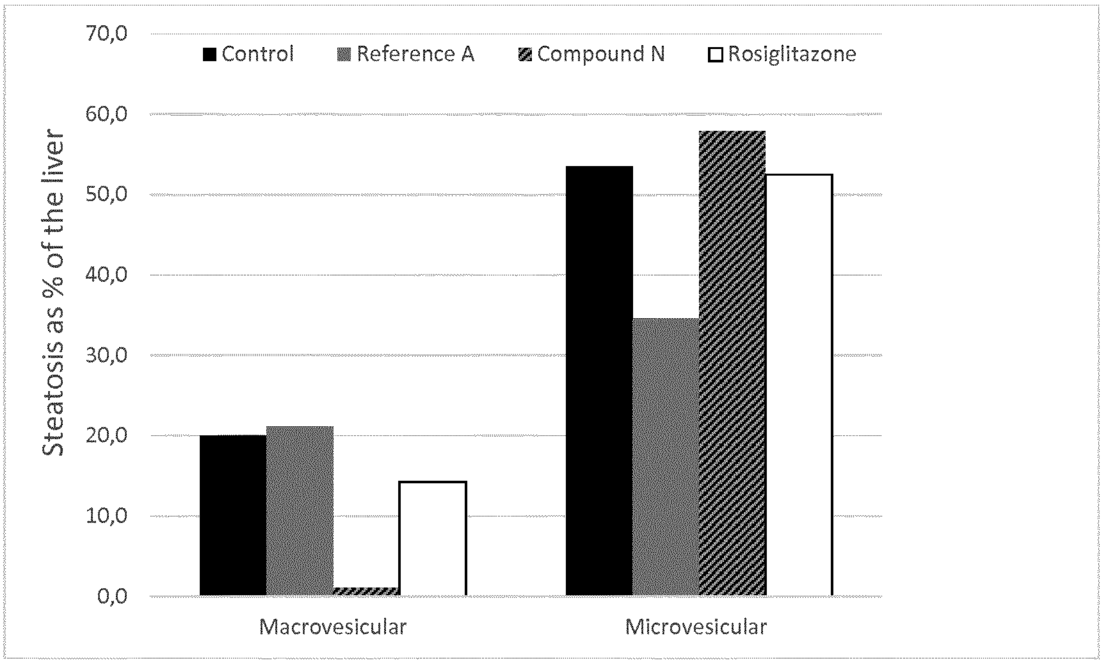

Figure 3.

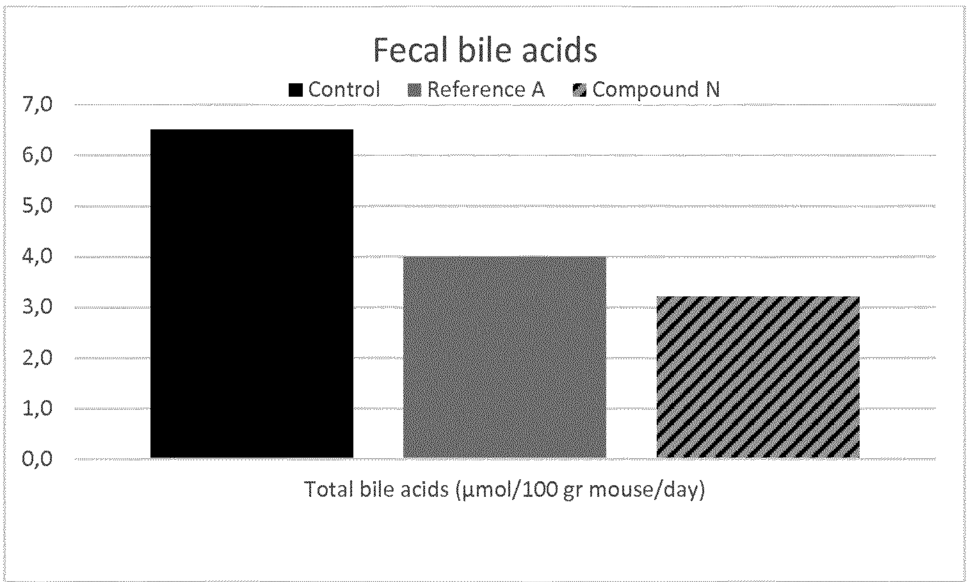

Figure 4.

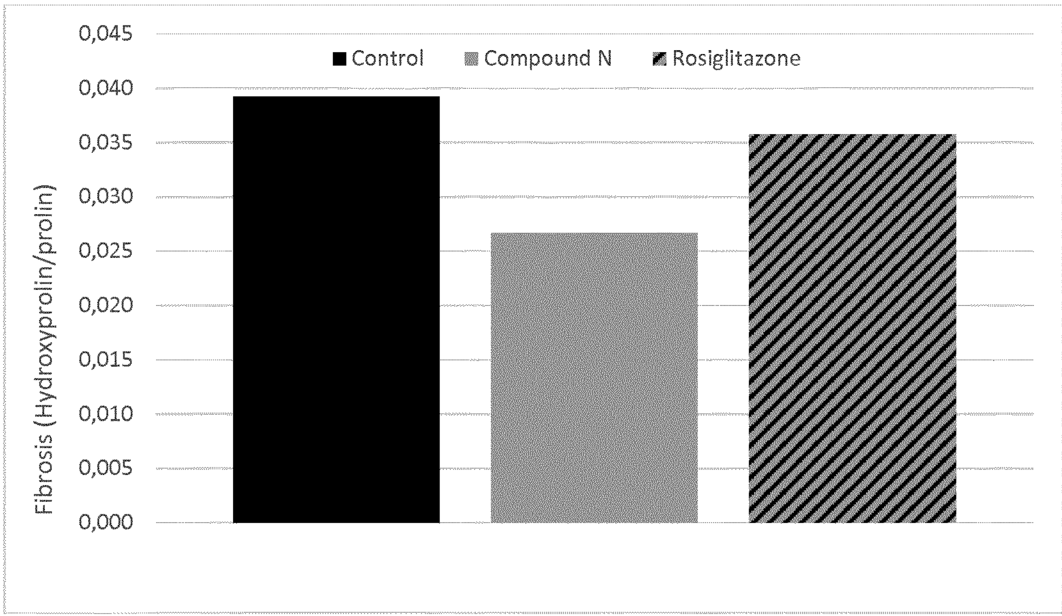

Figure 5.

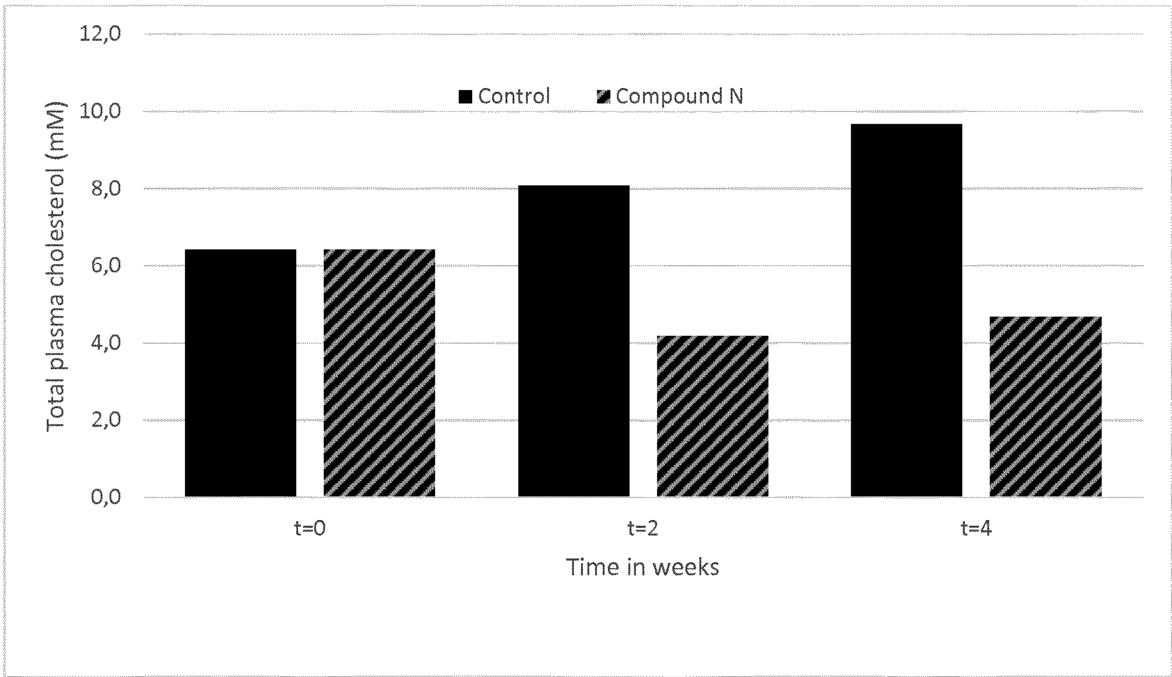

Figure 6.