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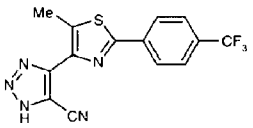
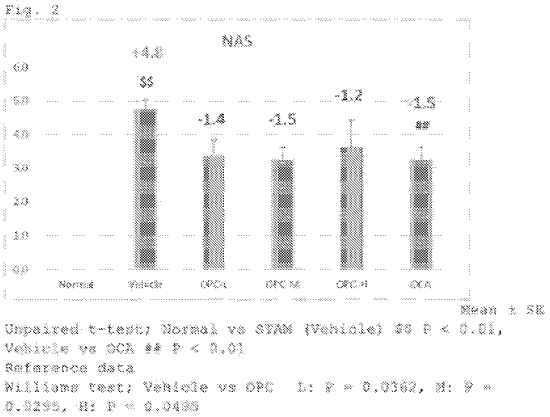
(54) Title: THERAPEUTIC AGENT FOR NONALCHOLIC STETOHEPATITIS



[Fig. 2]



(57) **Abstract:** The present invention relates to a safe and effective therapeutic agent for nonalcoholic steatohepatitis (NASH), more specifically, a therapeutic, preventative and/or diagnostic medicament for NASH, comprising 5-[5-methyl-2-(4-trifluoromethylphenyl)-thiazol-4-yl]-3H-[1,2,3]triazole-4-carbonitrile, represented by the following structural formula or a pharmaceutically acceptable salt thereof.

[Continued on next page]

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Description

Title of Invention: THERAPEUTIC AGENT FOR NON-ALCHOLIC STETOHEPATITIS

Technical Field

[0001] The present invention relates to a therapeutic agent for nonalcholic stetohepatitis (NASH).

Background Art

[0002] Nonalcoholic fatty liver disease (NAFLD) is a liver disease which mainly comprises the fatty liver excluding such causes as alcoholic liver disease, viral liver disease, and drug-induced liver injury. NAFLD is estimated to affect 25% of the adult population worldwide (NPL 1). Nonalcoholic steatohepatitis (NASH), a progressive form of NAFLD, is a disease progressing in the following order: liver metabolic failure, cirrhosis, end-stage liver disease requiring liver transplantation and hepatocellular carcinoma. NASH is a serious medical and public health burden due to the high mortality rate from liver-related and cardiovascular events (NPL 2).

Recently, the terms NAFLD and NASH have been discouraged, and it has been recommended that the terms metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction-associated steatohepatitis (MASH) are used, respectively, almost interchangeably (NPL 3).

[0003] According to the NAFLD/NASH clinical practice guidelines 2020 of the Japanese Society of Gastroenterology and the Japan Society of Hepatology, when treating NASH, the differential diagnosis is required between NASH and simple fatty liver (NAFL), which does not progress further from the fatty liver (NPLs 4, 5). Pathological diagnosis by liver biopsy is considered essential for the differential diagnosis. It is reported that 59% of patients undergoing liver biopsy are diagnosed with NASH. The estimated prevalence of NASH ranges from 1.5% to 6.45% (NPL1).

[0004] NASH is characterized by inflammation with hepatocellular damage caused by intrahepatic fat. Therefore, it is proposed to score steatosis, lobular inflammation, and ballooning of hepatocytes in liver biopsy specimens, and to evaluate the sum of these scores as the NAFLD Activity Score (NAS) as a representative index of pathological diagnostic criteria. NASH is further classified according to the stage of fibrosis, as most cases show fibrosis of the liver. The stage of fibrosis is strongly associated with both total and liver-related mortality, and thus it is important to assess the degree of liver fibrosis (NPLs 4-6).

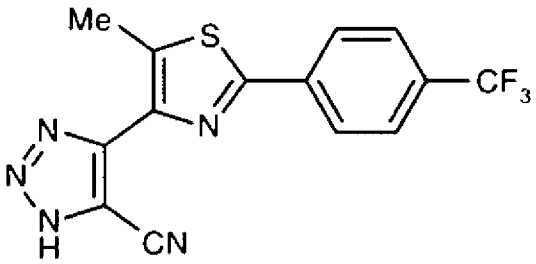
[0005] Most NASH patients are complicated by type 2 diabetes mellitus, obesity, hypertension, dyslipidemia, and others (NPL1). These complications increase cardio-

vascular risks and are also associated with the progression of liver-related pathologies (NPL 7).

[0006] As described above, NASH has significant medical economic impacts on the world. While the FDA approved Rezdiffra (resmetirom) on 14 March 2024 as the first treatment for adult NASH with fibrosis (NPL 8), there remains a significant unmet medical need for the disease.

[0007] PTL 1 discloses 5-[5-methyl-2-(4-trifluoromethyl-phenyl)-thiazol-4-yl]-3H-[1,2,3]triazole-4-carbonitrile, represented by the following structural formula:

[Chem.1]



as an example of a compound that ameliorates hyperglycemia by promoting citric acid cycle activity.

NPL 9 also describes that the above compound ("OPC-163493" in the literature) is a novel mitochondrial uncoupling agent targeting liver, and describes by the experimental results that it showed potent anti-diabetic effects in several animal diabetes models, whether type 2 or type 1 diabetes, that it reduced blood pressure in stroke-prone spontaneously hypertensive rats (SHRSP), delaying the onset of stroke-related symptoms and extending survival, that it also showed an ameliorative effect on renal dysfunction such as albuminuria in SHRSP, and that OPC-163493 exerted the beneficial effects on blood vessels by increasing the bioavailability of nitric oxide in the vascular endothelium.

Furthermore, OPC-163493 is also known to possess a high safety profile (NPL 10).

Citation List

Patent Literature

[0008] PTL 1: WO 2015/008872 A1

Non Patent Literature

[0009] [NPL 1] Younossi, Z. M., et al. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016, 64, 73-84.

[NPL 2] Younossi, Z. M., et al. The economic and clinical burden of nonalcoholic

fatty liver disease in the United States and Europe. Hepatology 2016, 64, 1577-1586.
[NPL 3] Rinella ME, et al. A multi-society Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol 2023 June 20; DOI: https://doi.org/10.1016/j.jhep.2023.06.003
[NPL 4] Tokushige, K., et al. Evidence-based clinical practice guidelines for non-alcoholic fatty liver disease/nonalcoholic steatohepatitis 2020. J Gastroenterol 2021, 56, 951-963.
[NPL 5] Tokushige, K., et al. Evidence-based clinical practice guidelines for non-alcoholic fatty liver disease/nonalcoholic steatohepatitis 2020. Hepatol Res 2021, 51, 1013-1025.
[NPL 6] Kleiner, D. E., et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005, 41, 1313-1321.
[NPL 7] Pais, R., et al. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. J Hepatol 2013, 59, 550-6.
[NPL 8] FDA NEWS RELEASE on 14 March 2024. URL: https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-patients-liver-scarring-due-fatty-liver-disease
[NPL 9] Kanemoto, N., et al. Antidiabetic and cardiovascular beneficial effects of a liver-localized mitochondrial uncoupler. Nat Commun 2019, 10, 2172.
[NPL 10] Inoue, Y., et al. Preclinical safety profile of a liver-localized mitochondrial uncoupler: OPC-163493. EXCLI J 2022, 21, 213-235

Summary of Invention

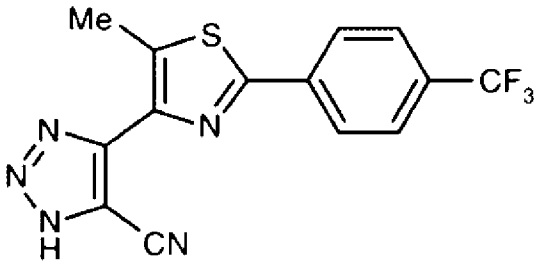
Technical Problem

[0010] The problem of the present invention is to provide a safe and effective therapeutic agent for NASH.

Solution to Problem

[0011] As a result of conducting extensive studies to solve the above-mentioned problems, the inventors of the present invention found that 5-[5-methyl-2-(4-trifluoromethyl-phenyl)-thiazol-4-yl]-3H-[1,2,3]triazole-4-carbonitrile (hereinafter referred to as "OPC-163493"), represented by the following structural formula:

[Chem.2]



has hepatoprotective, fatty liver ameliorative, NASH pathology ameliorative, antifibrotic, and hepatomegaly inhibitory effects in NASH model mice from doses lower than those at which antidiabetic effects are observed, thereby leading to the completion of the present invention.

Namely, the present invention includes the following embodiments.

[1] A therapeutic, preventative and/or diagnostic medicament for NASH, comprising OPC-163493 or a pharmaceutically acceptable salt thereof.

[2] A therapeutic, preventative and/or diagnostic pharmaceutical composition for NASH, comprising OPC-163493 or a pharmaceutically acceptable salt thereof.

[3] OPC-163493 or a pharmaceutically acceptable salt thereof for use in the treatment, prevention and/or diagnosis of NASH.

[4] Use of OPC-163493 or a pharmaceutically acceptable salt thereof in the manufacture of a therapeutic, preventative and/or diagnostic medicament or pharmaceutical composition for NASH.

[5] A method for treating, preventing and/or diagnosing NASH in a subject which comprises administering to the subject an effective amount of OPC-163493 or a pharmaceutically acceptable salt thereof.

Effects of Invention

[0012] OPC-163493 or a pharmaceutically acceptable salt thereof in the present invention has hepatoprotective, fatty liver ameliorative, NASH pathology ameliorative, antifibrotic, and hepatomegaly inhibitory effects from doses lower than those at which antidiabetic effects are observed. Through such effects, the compound can exert a more potent therapeutic effect on NASH than conventional agents. Thus, the present invention can provide a safe and effective therapeutic agent for NASH.

Brief Description of Drawings

[0013] [Fig. 1] Figure 1 shows the concentration of alanine aminotransferase (ALT) in blood after administration of each test compound.

[Fig. 2] Figure 2 shows the NAS (NAFLD activity score) after administration of each test compound.

[Fig. 3] Figure 3a shows steatosis after administration of each test compound, and

Figure 3b shows ballooning after administration of each test compound.

[Fig. 4] Figure 4 shows the fibrosis area after administration of each test compound.

[Fig. 5] Figure 5a shows the liver weight after administration of each test compound, and Figure 5b shows the liver weight/body weight ratio (LW/BW) after administration of each test compound.

Description of Embodiments

[0014] In the present description, "Nonalcoholic steatohepatitis (NASH)" is defined as fat accumulation in more than 5% of hepatocytes, accompanied by hepatocellular damage (ballooning degeneration of hepatocytes) and inflammation. In a classification published by the NASH Clinical Research Network in 2005, a NAFLD activity score (NAS) was proposed, scoring liver specimens by the degree of steatosis (0-3), parenchymatous inflammation (0-2), and the frequency of the degree of ballooned hepatocytes (0-2), with a total score of 2 or less indicating non-NASH, 3-4 indicating borderline, and 5 or more indicating NASH (see NPL 6).

Among these, NASH is particularly one that can be treated, prevented and/or diagnosed by the pharmacological effects (hepatoprotective, fatty liver ameliorative, NASH pathology ameliorative, antifibrotic, and hepatomegaly inhibitory effects) of OPC-163493 or a pharmaceutically acceptable salt thereof, and that is difficult to be treated, etc. with the other compounds. Such NASH can be identified, for example, by confirming that in a subject who is or may be suffering from NASH, the administration of OPC-163493 or a pharmaceutically acceptable salt thereof is effective whereas that of the other NASH-developing compounds (e.g., obeticholic acid) are ineffective.

[0015] In the present description, "treat", "treating" or "treatment" means to treat a disease condition in a subject (patient), including to inhibit (e.g., inhibit or delay progression), alleviate, lighten, ameliorate, cure a disease condition, and the like.

In the present description, "prevent", "preventing" or "prevention" means to substantially prevent or delay the occurrence (onset or expression) of a disease state, including to reduce the risk of occurrence.

In the present description, "diagnose", "diagnosing" or "diagnosis" means to determine (judge) a disease state in a subject (patient), including to determine whether the subject (patient) has a disease, to determine the degree or progression of the disease state, to determine the effectiveness of treatment for the disease, to determine whether there is a risk of recurrence of the disease after treatment, and the like.

[0016] In the present description, "subject" refers to a human or non-human mammal (e.g., monkey, chimpanzee, muntjac, dog, cat, pig, cow, sheep, goat, mouse, rat, guinea pig, etc.).

[0017] In the present description, "OPC-163493 or a pharmaceutically acceptable salt

thereof" can be synthesized, for example, by the process described in WO 2015/008872 A1.

[0018] OPC-163493 comprises three tautomers in the triazole moiety. The structural formula above shows one tautomer of OPC-163493, and OPC-163493 is not limited to, but also includes the other two tautomers.

[0019] OPC-163493 can readily form a pharmaceutically acceptable salt with common pharmaceutically acceptable acids because of its basic group. Examples of such "acids" include inorganic acids (e.g., hydrochloric acid, hydrobromic acid, nitric acid, sulfuric acid, phosphoric acid, etc.); and organic acids (e.g., methanesulfonic acid, p-toluenesulfonic acid, acetic acid, citric acid, tartaric acid, maleic acid, fumaric acid, malic acid, lactic acid, etc.); and the like.

OPC-163493 can readily form a pharmaceutically acceptable salt by reacting with pharmaceutically acceptable basic compounds because of its acidic group. Examples of such "basic compounds" include inorganic bases (e.g., sodium hydroxide, potassium hydroxide, calcium hydroxide, sodium carbonate, potassium carbonate, sodium hydrogen carbonate, potassium hydrogen carbonate, etc.); and organic bases (e.g., methylamine, diethylamine, trimethylamine, triethylamine, ethanolamine, diethanolamine, triethanolamine, ethylenediamine, tris(hydroxymethyl)methylamine, dicyclohexylamine, N,N'-dibenzylethylenediamine, guanidine, pyridine, picoline, choline, etc.); and ammonium salts; and the like.

Furthermore, OPC-163493 may form a salt with amino acids, for example, lysine, arginine, aspartic acid, glutamic acid, and the like.

[0020] OPC-163493 and a pharmaceutically acceptable salt thereof also encompass each of its various solvates (e.g., hydrates, ethanolates, etc.) and crystalline polymorphs.

[0021] OPC-163493 and a pharmaceutically acceptable salt thereof may be a co-crystal or a co-crystal salt. A "co-crystal" or "co-crystal" salt herein means a crystalline substance composed of two or more molecules, unique solids at room temperature, each having different physical properties (e.g., structure, melting point, heat of melting, etc.). Co-crystal and co-crystal salts can be produced by applying known co-crystallization methods.

[0022] OPC-163493 also encompasses isotope-labeled compounds that are identical to OPC-163493 except those one or more atoms are replaced by one or more atoms having a specific atomic mass or mass number. Examples of the isotope that can be incorporated into OPC-163493 encompass hydrogen, carbon, nitrogen, oxygen, sulfur, and fluorine isotopes such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, and ^{18}F, respectively. Certain isotope-labeled OPC-163493, containing the above isotopes and/or other isotopes of other atoms (e.g., the compounds incorporating radioisotopes such as ^{3}H and ^{14}C), is useful in drug tissue distribution assay and/or substrate tissue distribution

assay. Tritiated (i.e., ^{3}H) and carbon-14 (i.e., ^{14}C) isotopes are particularly preferred due to the ease of their preparation and detectability. In addition, substitution by heavier isotopes, such as deuterium (i.e., ^{2}H), can be expected to provide certain therapeutic benefits due to improvements in metabolic stability (e.g., increases in in vivo half-life or decreases in dose requirements). An isotopically labeled compound of OPC-163493 can be prepared by replacing a non-isotopically labeled reagent with a readily available isotopically labeled reagent in the OPC-163493 production process.

[0023] The following is a description of pharmaceutical compositions comprising OPC-163493 or a pharmaceutically acceptable salt thereof (hereinafter referred to as "the compound" unless otherwise noted) as active ingredient.

[0024] The above pharmaceutical compositions are formulations of the compound in the form of ordinary pharmaceutical compositions, and are prepared using commonly used carriers, diluents and/or excipients such as fillers, bulking agents, binders, humectants, disintegrants, surface active agents and lubricants (also together referred to as "pharmaceutically acceptable carriers" in the description).

[0025] Such pharmaceutical compositions can be selected from a variety of forms according to the therapeutic purpose, and representative examples include tablets, pills, powder, liquid, suspension, emulsion, granules, capsules, suppositories, injections (liquid, suspension, etc.), and the like.

[0026] A wide range of known carriers can be used when molding into tablet form, and examples include excipients such as lactose, white sugar, sodium chloride, dextrose, urea, starch, calcium carbonate, kaolin, crystalline cellulose; binding agents such as water, ethanol, propanol, single syrup, dextrose solution, starch solution, gelatin solution, carboxymethylcellulose, shellac, methylcellulose, potassium phosphate, polyvinylpyrrolidone; disintegrants such as dried starch, sodium alginate, agar powder, laminaran powder, sodium bicarbonate, calcium carbonate, polyoxyethylene sorbitan fatty acid esters, sodium lauryl sulfate, monoglycerides of stearic acid, starch, lactose; disintegration inhibitors such as white sugar, stearic acid, cocoa butter, hydrogenated oil; absorption enhancers such as quaternary ammonium base, sodium lauryl sulfate; moisturizers such as glycerin, starch; adsorbents such as starch, lactose, kaolin, bentonite, colloidal silicate; lubricants such as refined talc, stearate, boric acid powder, polyethylene glycol; and the like.

[0027] Furthermore, the tablets can be coated with ordinary coating materials as needed, for example, sugar-coated, gelatin-coated, enteric-coated, film-coated, or double- or multilayered tablets.

[0028] A wide range of known carriers can be used when molding into pill form, and examples include excipients such as glucose, lactose, starch, cocoa fat, hardened vegetable oil, kaolin, talc; binding agents such as gum arabic powder, tragacanth

powder, gelatin, ethanol; disintegrants such as laminaran, agar; and the like.

[0029] A wide range of known carriers can be used when molding into suppository forms, and examples include polyethylene glycol, cocoa fat, higher alcohol, esters of higher alcohol, gelatin, semi-synthetic glyceride, and the like.

[0030] When prepared as injection form, the liquid, emulsion and suspension are sterilized and preferably isotonic to blood. A wide range of known carriers can be used when molding into such liquid, emulsion and suspension forms, and examples include water, ethanol, propylene glycol, ethoxylated isostearyl alcohol, polyoxylated isostearyl alcohol, polyoxyethylene sorbitan fatty acid esters, and the like. In this case, a sufficient amount of salt, glucose or glycerin may be included in the pharmaceutical formulation to prepare an isotonic solution, and the usual dissolution aid, buffer, painless agent, etc., and if necessary, coloring, preservative, flavoring, flavoring, sweetening agent, etc., and/or another pharmaceutical may be included.

[0031] The amount of the compound contained in a pharmaceutical composition is not particularly limited and can be selected from a wide range, but it is usually preferable to include 1 to 70% by weight of the compound in a pharmaceutical composition.

[0032] There is no particular limitation on the method of administration of the above pharmaceutical compositions, and they are administered in various dose forms and/or in a method appropriate to the age, sex, disease state, and other conditions of a subject (especially human). For example, in tablet, pill, liquid, suspension, emulsion, granule, and capsule forms, they are administered orally. In injection forms, they can be administered intravenously alone or in combination with a normal supplement such as glucose or amino acid, or even administered intramuscularly, intradermally, subcutaneously, or intraperitoneally, as needed. In suppository form, they are administered intrarectally.

[0033] The dose of the above pharmaceutical compositions may be selected according to their direction and/or the age, sex, extent of disease, and other conditions of the subject (especially humans), and is typically about 0.001 to 100 mg, preferably about 0.001 to 50 mg per 1 kg of body weight per day, divided into one to several doses.

[0034] The above dose varies according to various conditions, and therefore, in some cases, a dose lower than the above range may be sufficient, while in other cases, a dose higher than the above range may be necessary.

[0035] The present compound possesses excellent hepatoprotective, fatty liver ameliorative, NASH pathology ameliorative, antifibrotic, and hepatomegaly inhibitory effects, as shown in the examples below.

The above "hepatoprotective effects" herein include the effect of decreasing the concentration of ALT in the blood.

The above "NASH pathology ameliorative effects" include the effect of reducing

NAS.

The above "fatty liver ameliorative effects" include the effect of decreasing steatosis.

The above "antifibrotic effects" include the effect of inhibiting the expansion of fibrosis area.

The above "hepatomegaly inhibitory effects" include the effect of decreasing liver weight.

The compound has these effects from doses lower than those at which antidiabetic effects are observed.

Through such effects, the compound can exert a more potent therapeutic effect on NASH than conventional agents. For example, the dose of this compound (OPC) to achieve an effect equivalent to that achieved with a given dose of obeticholic acid (OCA), which has been proven to ameliorate NASH, is significantly lower than the dose of OCA.

Furthermore, the compound has an excellent safety profile (NPL 9).

Therefore, the compound can be applied to such as a safe and effective therapeutic agent for NASH.

[0036] Thus, in one embodiment, the present invention provides a medicament for the treatment, prevention and/or diagnosis of NASH, comprising the compound as active ingredient.

[0037] In one embodiment, the present invention also provides a hepatoprotective agent comprising the compound as active ingredient.

[0038] In one embodiment, the present invention also provides fatty liver ameliorative agent comprising the compound as active ingredient.

[0039] In one embodiment, the present invention also provides a NASH pathology ameliorative agent comprising the compound as active ingredient.

[0040] In one embodiment, the present invention also provides antifibrotic agent comprising the compound as active ingredient.

[0041] In one embodiment, the present invention also provides a hepatomegaly inhibitory agent comprising the compound as active ingredient.

[0042] In one embodiment, the present invention also provides a therapeutic, preventative and/or diagnostic pharmaceutical composition for NASH, comprising the compound as active ingredient and a pharmaceutically acceptable carrier or excipient.

[0043] In one embodiment, the present invention also provides the compound for use in the treatment, prevention and/or diagnosis of NASH.

[0044] In one embodiment, the present invention also provides use of the compound in the manufacture of a therapeutic, preventative and/or diagnostic medicament or pharmaceutical composition for NASH.

[0045] In one embodiment, the present invention also provides a method for treating,

preventing and/or diagnosing NASH, which comprises administering to the subject an effective amount of the compound.

[0046] Disclosures of all patent and non-patent literature cited in the present description are incorporated in the present description in their entirety by reference.

Examples

[0047] The present invention is described in detail in the following by referring to examples, which do not limit the invention.

[0048] Example 1

(Test compound)

The present compound OPC-163493 (OPC) was synthesized by the process described in WO 2015/008872 A1. The control compound obeticholic acid (6-ethyl-kenodeoxycholic acid, OCA) was purchased from Fujifilm Wako Pure Chemicals Co. Each test compound was prepared in 5% gum arabic solution to 10 mL/kg for each dose.

[0049] (NASH model animal STAM mouse)

The STAM mouse model used to evaluate the test compounds is an animal model that exhibits disease progression similar to that of human NASH, with a background of late-stage type 2 diabetes and the disease progressing in the following order: fatty liver, NASH, fibrosis, cirrhosis, and liver cancer. It has characteristics such as (i) ballooned degenerative cell, a characteristic pathology of human NASH; (ii) the phenomenon of burnout NASH, in which fat droplets diminish as fibrosis progresses; (iii) fibrosis develops from the central venous periphery; (iv) mild elevation of ALT, a marker of liver damage; and an effect of the test compound can be evaluated by NAS and fibrosis degree, which are the clinical endpoints of NASH (Fujii, M., et al. A murine model for non-alcoholic steatohepatitis showing evidence of association between diabetes and hepatocellular carcinoma. Med Mol Morphol 2013, 46, 141-52.).

More than 600 studies have been conducted using STAM mice to evaluate drug efficacy, which have been published in papers and at conferences, and the 15 or more test compounds evaluated by SMC Laboratories, Inc. have advanced to the clinical stage (As of May 2021, SMC Laboratories website: https://www.smccro-lab.com/jp/cell/).

[0050] (Test Method)

Two-day-old C57BL/6J male mice were subcutaneously administered 200 μg/body of streptozotocin. At 4 weeks of age, mice were fed high-fat diet (57% fat, HFD32; Nippon Crea Co., Ltd.) replaced with normal diet to generate NASH-induced STAM mice. At 6 weeks of age, they were divided into groups based on body weight as an indicator. The groups consisted of the following 6 groups: 8 normal mice without

streptozotocin (Normal), 8 NASH-induced STAM mice/vehicle (Vehicle; 5% gum arabic solution), 8 STAM mice/low dose of OPC (OPC-L; 0.6 mg/kg/day), 8 STAM mice/OPC medium dose (OPC-M; 2 mg/kg/day), 8 STAM mice/OPC high dose (OPC-H; 6 mg/kg/day), and 8 STAM mice/OCA (20 mg/kg/day). For 21 days, from 6 weeks to 9 weeks of age, the test compound was administered orally twice a day. Each dose per time was 0.3 mg/kg for the low OPC dose, 1 mg/kg for the medium OPC dose, 3 mg/kg for the high OPC dose, and 10 mg/kg for the OCA dose, respectively. During the administration period, their body weights were measured daily before the first dose and were reflected in the daily doses. No individual was found to drop out of the study in health state observation during the administration period, and autopsies were performed on all cases the day after the last dose. At necropsy, the animals were killed by exsanguination after blood collection by cardiac puncture under isoflurane anesthesia. The collected whole blood was transferred into a heparin (Novoheparin; Mochida Pharmaceutical Co., Ltd.) treated polypropylene tube and centrifuged at 1000 g for 15 min at 4°C, then plasma was obtained. The plasma was stored at -80°C until ALT measurement. After the death from exsanguination, the liver was harvested, washed with cold saline, and weighed. Two pieces of liver tissue were cut from the left lateral lobe for pathology specimens.

ALT in plasma was measured using a FUJI DRI-CHEM 7000 (Fujifilm).

The sections of liver tissue were fixed in Bouin's solution for 24 hours and blocked by paraffin embedding to prepare section slides. The slides were stained with HE-stain (Lilly-Meyer hematoxylin; Mutoh Chemical Co., Ltd., Eosin; Fujifilm Wako Pure Chemicals Co., Ltd.) and Sirius Red-stain (Pichrosirius red; Fujifilm Wako Pure Chemicals Co., Ltd.), respectively. After those slides were blinded, HE-stained slide was used for NAS evaluation and Sirius Red-stained slide was used for fibrosis area measurement.

In NAS evaluation, the three parameters (steatosis, intralobular inflammation, and ballooning of hepatocytes) were scored according to Kleiner's criteria (Kleiner, D. E., et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005, 41, 1313-21.), and the sum of these three parameters was defined as NAS.

Fibrosis area was measured by capturing images of a Sirius Red-stained section at 200x magnification in bright field with a digital camera (DFC295; Leica) and quantifying the positive area in 5 fields per section using ImageJ software (National Institute of Health).

[0051] (Result)

As shown in Figure 1, there was a significant increase in ALT in the STAM mouse group treated with vehicle (Vehicle) compared to normal mice (Normal), while there

was a significant decrease in ALT in the OPC treated group (hepatoprotective effect).
As shown in Figure 2, the STAM mouse group treated with vehicle (Vehicle) showed a 4.8 increase in NAS compared to normal mice (Normal), while the OPC-treated group showed a decrease in NAS from the low dose to the same level as the OCA-treated group (NASH pathology ameliorative effect).
As shown in Figure 3a, steatosis was significantly suppressed in the OPC-treated group from the low dose to the same level as in the OCA-treated group (fatty liver ameliorative effect). As shown in Figure 3b, the OPC-treated group showed the same trend of decreased ballooning as the OCA-treated group.
As shown in Figure 4, the STAM mouse group treated with vehicle (Vehicle) showed a 0.84% increase in fibrosis area from normal mice (Normal), while the OPC-treated group showed a significant fibrosis inhibition effect from the low dose to the same level as the OCA-treated group (antifibrotic effect).
As shown in Figures 5a and 5b, the STAM mouse group treated with vehicle (Vehicle) showed significant liver enlargement from normal mice (Normal), while the OPC-treated group showed a significant reduction in liver weight to the same extent as the OCA-treated group (hepatomegaly inhibitory effect).

[0052] From the above results, OPC showed NASH ameliorative effect from the dose of 0.6 mg/kg/day (minimum effective dose, MED), which was equivalent to that of OCA 20 mg/kg/day, which has been proven to ameliorate NASH in human clinical studies (Randomized Global Phase 3 Study to Evaluate the Impact on NASH With Fibrosis of Obeticholic Acid Treatment (REGENERATE), ClinicalTrials.gov: NCT02548351)(OCA/OPC dose ratio was 33-fold). These results demonstrate the potent NASH therapeutic effect of OPC, and provide hope for its therapeutic effect in human clinical practice.

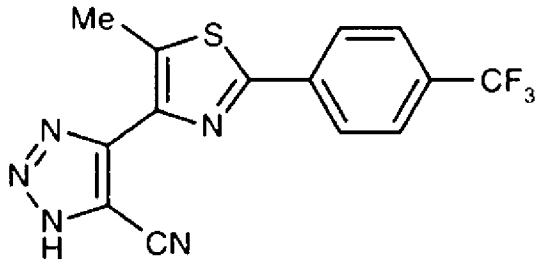
The above results and the fact that the MED of OPC for the diabetes model was 2 mg/kg/day as shown in NPL 7 led us to show that OPC are pharmacodynamically superior as a NASH therapeutic agent (diabetes MED/NASH MED dose ratio of 3.3-fold).

[0053] Thus, the present invention enables to provide OPC-163493 as a safe and effective therapeutic agent for NASH, a disease that is still a serious medical and public health burden with low treatment satisfaction and no therapeutic agent.

Claims

[Claim 1] A therapeutic, preventative and/or diagnostic medicament for non-alcoholic steatohepatitis (NASH), comprising 5-[5-methyl-2-(4-trifluoromethyl-phenyl)-thiazol-4-yl]-3H-[1,2,3]triazole-4-carbonitrile, represented by the following structural formula:

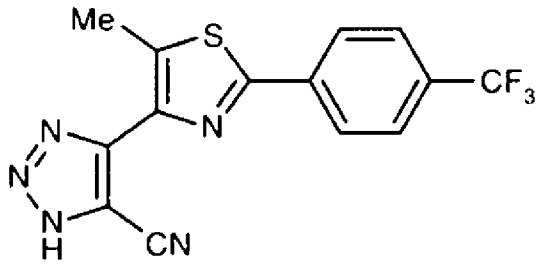
[Chem.1]



or a pharmaceutically acceptable salt thereof.

[Claim 2] A therapeutic, preventative and/or diagnostic pharmaceutical composition for nonalcoholic steatohepatitis (NASH), comprising 5-[5-methyl-2-(4-trifluoromethyl-phenyl)-thiazol-4-yl]-3H-[1,2,3]triazole-4-carbonitrile, represented by the following structural formula:

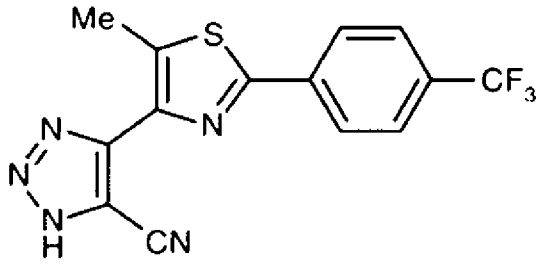
[Chem.2]



or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier or excipient.

[Claim 3] 5-[5-methyl-2-(4-trifluoromethyl-phenyl)-thiazol-4-yl]-3H-[1,2,3]triazole-4-carbonitrile, represented by the following structural formula:

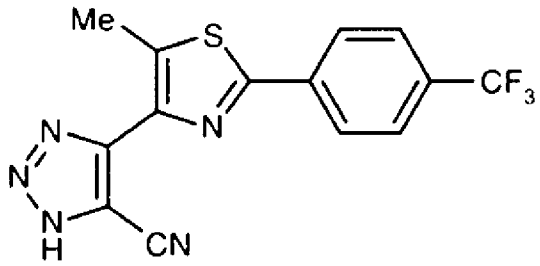
[Chem.3]



or a pharmaceutically acceptable salt thereof for use in the treatment, prevention and/or diagnosis of nonalcoholic steatohepatitis (NASH).

[Claim 4] Use of 5-[5-methyl-2-(4-trifluoromethyl-phenyl)-thiazol-4-yl]-3H-[1,2,3]triazole-4-carbonitrile, represented by the following structural formula:

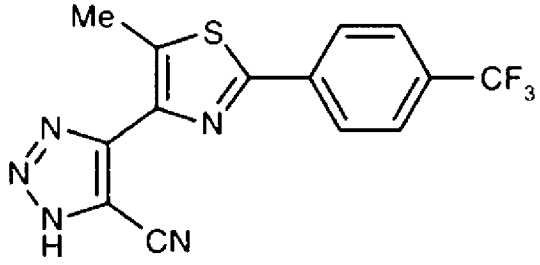
[Chem.4]



or a pharmaceutically acceptable salt thereof in the manufacture of a therapeutic, preventative and/or diagnostic medicament or pharmaceutical composition for nonalcoholic steatohepatitis (NASH).

[Claim 5] A method for treating, preventing and/or diagnosing nonalcoholic steatohepatitis (NASH) in a subject, which comprises administering to the subject an effective amount of 5-[5-methyl-2-(4-trifluoromethyl-phenyl)-thiazol-4-yl]-3H-[1,2,3]triazole-4-carbonitrile, represented by the following structural formula:

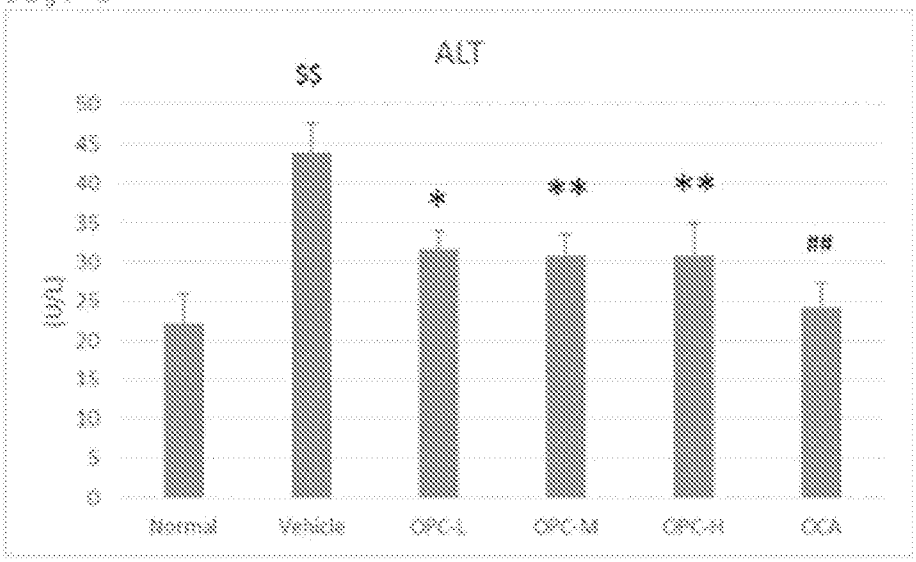
[Chem.5]



or a pharmaceutically acceptable salt thereof.

[Fig. 1]

Fig. 1

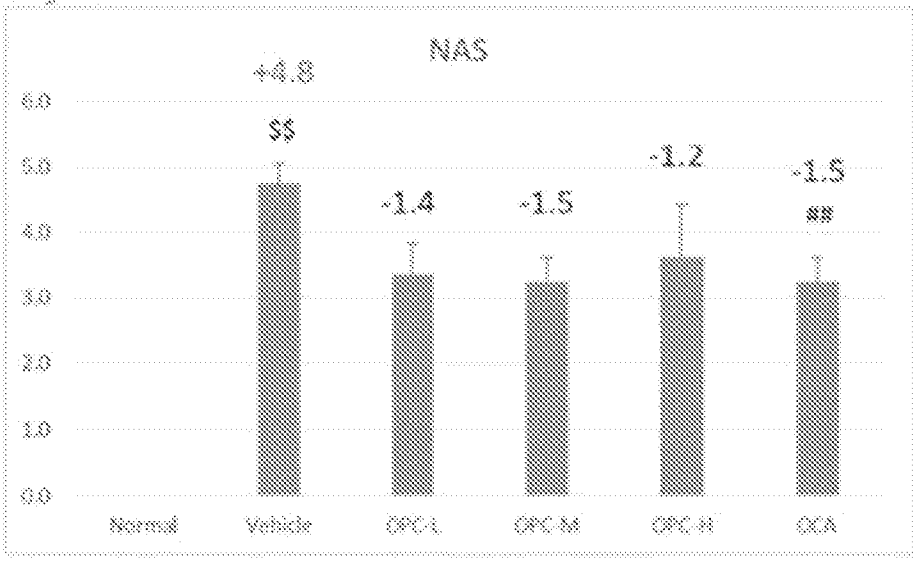


Mean ± SE

Unpaired t-test; Normal vs STAM (Vehicle) $$ P < 0.01,
Vehicle vs OCA ## P < 0.01
Williams test; Vehicle vs OPC * P < 0.025, ** P < 0.005

[Fig. 2]

Fig. 2



Mean ± SE

Unpaired t-test; Normal vs STAM (Vehicle) $$ P < 0.01,
Vehicle vs OCA ## P < 0.01
Reference data
Williams test; Vehicle vs OPC L: P = 0.0362, M: P =
0.0295, H: P = 0.0495

[Fig. 3]

Fig. 3a

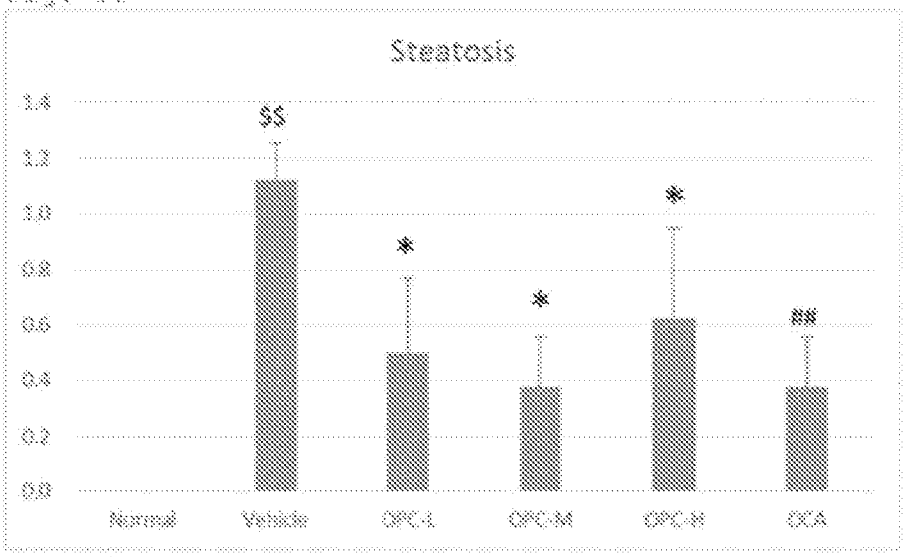
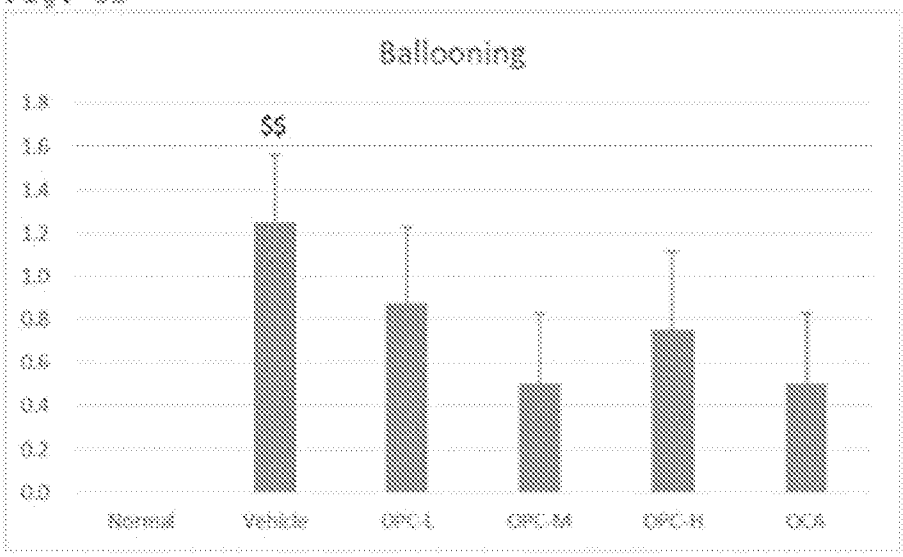


Fig. 3b

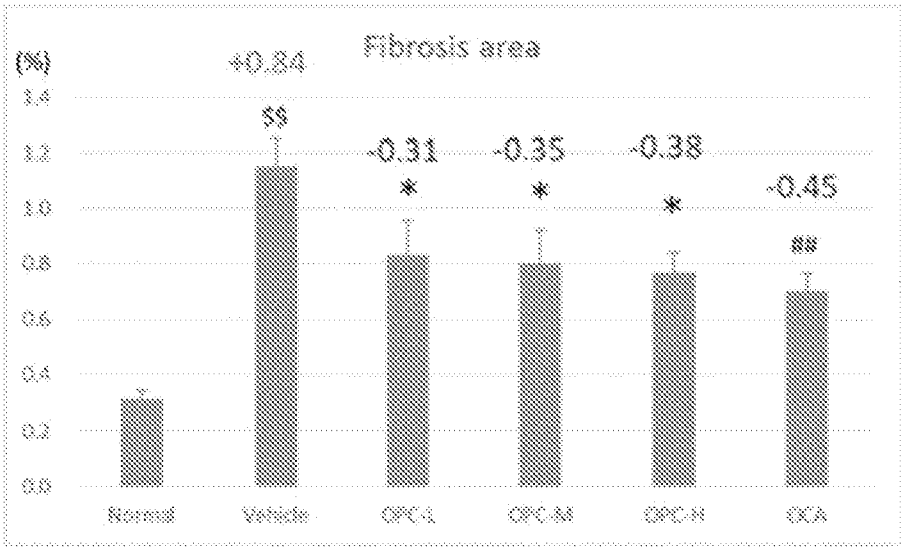


Mean ± SE

Mann-Whitney U-test; Normal vs STAM (Vehicle) $$ P < 0.01,
Vehicle vs OCA ## P < 0.01
Shirley-Williams test; Vehicle vs OPC * P < 0.025

[Fig. 4]

Fig. 4



Mean ± SE

Unpaired t-test; Normal vs STAM (Vehicle $$ P < 0.01,
Vehicle vs OCA ## P < 0.01
Williams test; Vehicle vs OPC * P < 0.025

[Fig. 5]

Fig. 5a

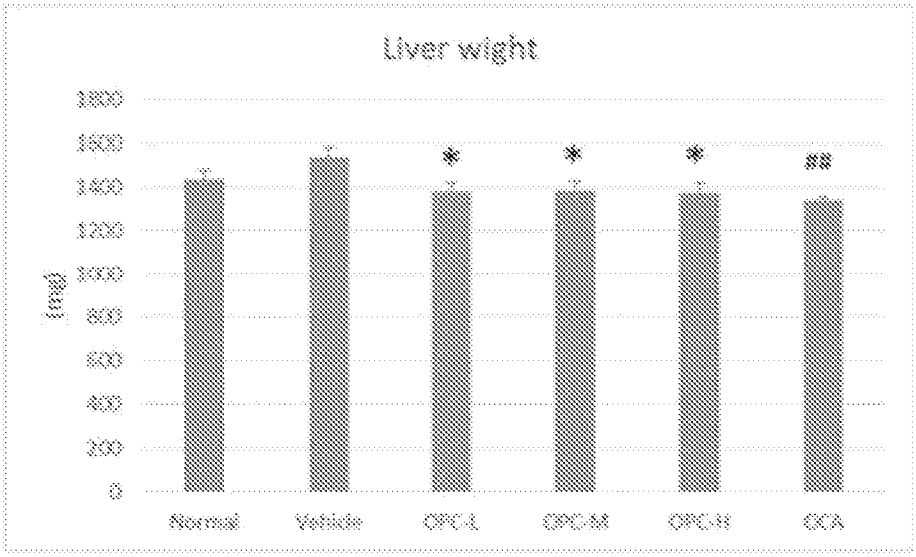
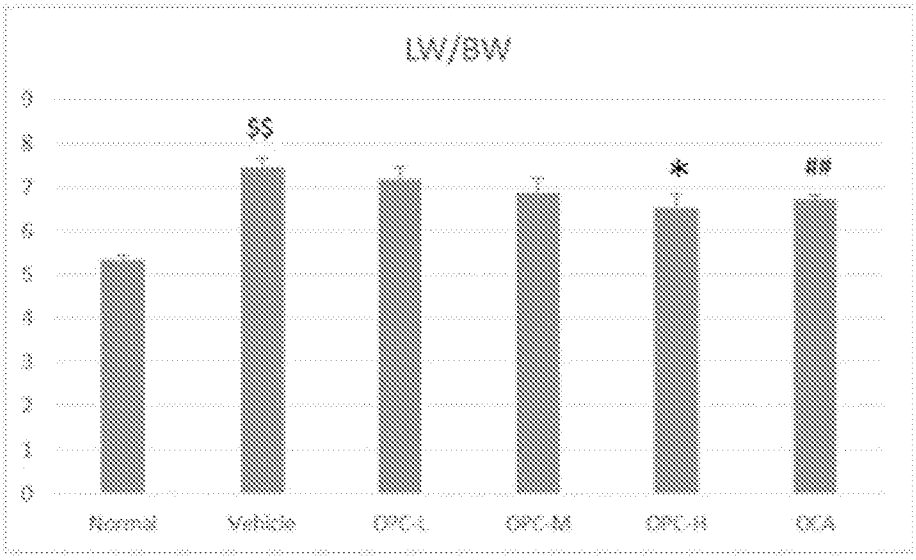


Fig. 5b



Mean ± SE

Unpaired t-test; Normal vs STAM (Vehicle $$ P < 0.01,
Vehicle vs OCA ## P < 0.01
Williams test; Vehicle vs OPC * P < 0.025

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2024/022947

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/427(2006.01)i; ***A61K 9/10***(2006.01)i; ***A61P 1/16***(2006.01)i; ***A61P 3/06***(2006.01)i; ***A61P 29/00***(2006.01)i
FI: A61K31/427; A61P1/16; A61P3/06; A61P29/00; A61K9/10

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)

A61K31/427; A61K9/10; A61P1/16; A61P3/06; A61P29/00

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

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2024
Registered utility model specifications of Japan 1996-2024
Published registered utility model applications of Japan 1994-2024

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

JSTPlus/JMEDPlus/JST7580 (JDreamIII); CAplus/REGISTRY/MEDLINE/EMBASE/BIOSIS (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KANEMOTO, Naohide et al., Antidiabetic and cardiovascular beneficial effects of a liver-localized mitochondrial uncoupler, NATURE COMMUNICATIONS, Article number :2172 (2019), 2019.05.15, https://doi.org/10.1038/s41467-019-09911-6 Especially Fig.1a	3
Y	Especially Title, Abstract, Result	1-2,4-5
X	GOEDEKE, Leigh et al., Therapeutic potential of mitochondrial uncouplers for the treatment of metabolic associated fatty liver disease and NASH, MOLECULAR METABOLISM, Volume 46, Article No. 101178, 2021.02.03, https://doi.org/10.1016/j.molmet.2021.101178 Especially 3.3.3. OPC-163493	3
Y	Especially Title, 3.THERAPEUTIC RELEVANCE OF MITOCONDRIAL, 3.3.Synthetic mitochondrial uncouplers, 3.3.3. OPC-163493	1-2,4-5
A	KATO, Masaki, NAFLD/NASH and diabetes mellitus, Pharma Medica, 37(9), 2019, 35-40	1-5

☐ 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
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"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
30 July 2024	**06 August 2024**

Name and mailing address of the ISA/JP	Authorized officer
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