

CRYSTALLINE FORM OF AN AMIDO HETEROAROMATIC COMPOUND

Cross-Reference To Related Patent Application

This specification claims the benefit of priority to U.S. Provisional Patent Application No. 63/599,658 (filed 16 November 2023). The entire text of the above-referenced patent application is

5 incorporated by reference into this specification.

Field

The specification relates to a crystalline form of ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone that inhibits 17 β hydroxy steroid dehydrogenase 13 (17 β HSD13 or HSD17B13), and its use in treating diseases such as liver disease. This specification

10 also relates to pharmaceutical compositions containing the crystalline form.

Background

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of liver disease ranging from simple steatosis (non-alcoholic fatty liver), to non-alcoholic steatohepatitis (NASH) with or without fibrosis, to cirrhosis. Hepatic steatosis is defined as excess fat accumulation in the liver with greater than 5%

15 induced by causes other than alcohol intake. NASH is defined by hepatic steatosis with inflammation and hepatocyte injury, with or without fibrosis. It is estimated that approximately 25% of the global population has NAFLD, and mortality due to NAFLD-related disease is expected to increase significantly through 2030.

To date, there are no approved treatments for NAFLD (such as NASH) and therapeutic interventions

20 focus on addressing co-morbidities that contribute to the pathogenesis of NAFLD, including treating insulin resistance, obesity, type II diabetes mellitus, and dyslipidemia.

Recently, a variant in the 17 β HSD13 gene, was associated in an allele dose-dependent manner with decreased serum aminotransferases levels, as well as a lower risk of liver disease, including alcoholic and non-alcoholic liver disease, cirrhosis and hepatocellular carcinoma (HCC) (Abul-Husn et al, N Engl

25 J Med. 2018, 378(12), 1096-106, Wang et al, Eur Rev Med Pharmacol Sci, 2020, 24(17), 8997-9007).

The 17 β HSD13 splice variant (rs72613567:TA) results in a truncated, unstable and enzymatically inactive protein and has thus been characterized as an 17 β HSD13 Loss of Function (LoF) variant (Ma et al, Hepatology 2019, 69(4), 1504-19). The association between the LoF 17 β HSD13

(rs72613567:TA) and decreased disease severity has been replicated in additional cohorts with

30 histologically proven NAFLD and was also associated with lower plasma transaminases, reduced risk of cirrhosis, HCC and liver related mortality in a study of 111612 individuals from the Danish general

- population (Gellert-Kristensen et al, Hepatology, 2020, 71(1), 56-66). Interestingly, the protective effect of the LoF 17 β HSD13 (rs72613567:TA) variant on plasma transaminases levels appears to be amplified by several key risk factors of liver disease such as obesity, alcohol consumption, as well as established genetic risk factors such as, but not limited to, the (rs738409 C>G) variant in patatin-like phospholipase domain-containing protein 3 (PNPLA3). Further, two additional 17 β HSD13LoF variants (rs62305723) and (rs143404524) were also reported to confer protection from chronic liver disease progression (Kozlitina et al, N Engl J Med, 2018, 379(19), 1876-7). In general, the LoF 17 β HSD13 protective variants has a stronger association with fibrosis and progression to advance liver disease but is not associated with steatosis.
- Based on the genetic validation of 17 β HSD13LoF variants conferring protection against liver disease risk and progression, inhibition of 17 β HSD13 activity with small molecules inhibitors could be an effective therapeutic approach for treating liver diseases such as NAFLD (for example NASH, liver fibrosis, cirrhosis and isolated steatosis), liver inflammation, alcoholic steatohepatitis (ASH), hepatitis C virus (HCV) and hepatocellular carcinoma (HCC), such as in individuals harbouring several key risk factors of liver disease such as obesity, alcohol consumption, as well as established genetic risk factors such as the (rs738409 C>G) variant in PNPLA3.

The compound of the disclosure provide an anti-liver disease effect by, as a minimum, acting as 17 β HSD13 inhibitors. Further, the compound of the disclosure may selectively inhibit 17 β HSD13 over 17 β HSD4 and/or 17 β HSD9.

- Fifteen 17 β HSD (HSD17B) members have been identified in human. The sequence homology among the different members is rather low, but the overall structure seems conserved. 17 β -Hydroxysteroid dehydrogenases are mainly involved in sex hormone metabolism. Some 17 β HSD enzymes also play key roles in cholesterol and fatty acid metabolism (Labrie et al. Journal of Molecular Endocrinology, 2000, 25, 1–16, Wen Su et al. Molecular and Cellular Endocrinology, 2019, 489, 119–125). A clean off-target profile is an advantage for a 17 β HSD13 inhibitor to avoid potential toxicity caused by off-target activity. This includes selectivity to other 17 β HSD members.

- 17 β HSD4/ D-bifunctional protein (DBP) is involved in fatty acid β -oxidation and steroid metabolism. 17 β HSD4 is ubiquitously expressed and play an important role in the inactivation of estrogens in a large series of peripheral tissues. Mutations in 17 β HSD4 are known to cause DBP deficiency, an autosomal-recessive disorder of peroxisomal fatty acid β -oxidation that is generally fatal within the first two years of life. A homozygous missense variant in 17 β HSD4 has been identified in Perrault syndrome, a recessive disorder characterized by ovarian dysgenesis in females, sensorineural

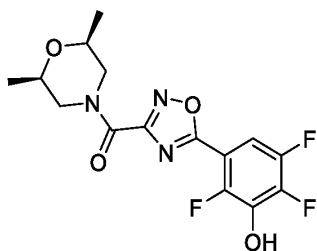
deafness in both males and females, and in some patients, neurological manifestations (Pierce et al. Am. J. Hum. Genet., 2010, 87, 282-8; and Chen et al. BMC Med Genet., 2017, 18, 91).

17 β HSD9/ RDH5 (retinol dehydrogenase 5) is involved in retinoid metabolism. The enzyme is mainly expressed in the retinal pigment epithelium. The RDH5 gene encodes the enzyme that is a part of the visual cycle, the 11-cis retinol dehydrogenase, catalysing the reduction of 11-cis-retinol to 11-cis-retinal. RDH5 gene mutations cause a progressive cone dystrophy or macular dystrophy as well as night blindness. Fundus albipunctatus is a rare, congenital form of night blindness with rod system impairment, characterised by the presence of numerous small, white-yellow retinal lesions. This disorder is caused mostly by mutations in the RDH5 gene (Hotta et al. Am. J. Ophthalmol., 2003, 135, 917-9; and Skorczyk-Werner et al. J. Appl. Genet., 2015, 56, 317-27).

The compound of the specification may also exhibit advantageous physical properties (for example, lower lipophilicity, higher aqueous solubility, higher permeability, lower plasma protein binding, and/or greater chemical stability), and/or favourable toxicity profiles (for example a decreased activity at hERG), and/or favourable metabolic or pharmacokinetic profiles, in comparison with other known 17 β HSD13 inhibitors. Said compound may therefore be especially suitable as therapeutic agents, such as for the treatment of liver disease.

General Description

In one aspect there is provided a crystalline form of ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone



(Compound (III)) that is Form A.

In the formulation of drug substances, it is beneficial for the drug substance (active compound) to be in a form in which it can be conveniently handled and processed. The chemical stability and the physical stability of the active compound may be important factors in determining the suitability of a solid form for use in the development of pharmaceutical formulations. It is also beneficial for the active compound, and formulations containing it, to be capable of being effectively stored over appreciable periods of time, without exhibiting any significant change in the physico-chemical

characteristics (e.g. chemical composition, density, hygroscopicity and solubility) of the active compound.

In a further aspect there is provided a pharmaceutical composition comprising Compound (III) Form A and a pharmaceutically acceptable excipient.

- 5 In a further aspect there is provided Compound (III) Form A, for use in therapy.

In a further aspect there is provided Compound (III) Form A, for use in the treatment of liver disease.

In a further aspect there is provided the use of Compound (III) Form A, in the manufacture of a medicament.

- 10 In a further aspect there is provided the use of Compound (III) Form A, in the manufacture of a medicament for the treatment of liver disease.

In a further aspect there is provided a method of treating liver disease in a patient comprising administering to the patient an effective amount of Compound (III) Form A.

Definitions

- 15 So that the present specification may be more readily understood, certain terms are explicitly defined below. In addition, definitions are set forth as appropriate throughout the detailed description.

Units, prefixes, and symbols are denoted in their International System of Units (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range.

- 20 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary of Biochemistry and Molecular Biology, Revised, 2000, Oxford University Press,
25 provide one of skill with a general dictionary of many of the terms used in this disclosure.

Description of Figures

Embodiments and experiments illustrating the principles of the disclosure will now be discussed with reference to the accompanying figures in which:

Figure 1A shows a powder X-ray diffraction diagram of ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone Form A.

Figure 1B shows differential scanning calorimetry (DSC) analysis of ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone Form A.

- 5 **Figure 2A** shows thermogravimetric analysis (TGA) analysis of ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone Form A.

Figure 2B Gravimetric Vapor Sorption Analysis of ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone Form A.

Detailed Description

- 10 In a first aspect there is provided ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone Form A, described as Compound (III) Form A herein.

According to the present specification there is provided Compound (III) Form A, which has an X-ray powder diffraction pattern with a specific peak at 2-theta = 10.7° when measured using CuKα radiation, plus or minus 0.2° 2-theta.

- 15 According to the present specification there is provided Compound (III) Form A, which has an X-ray powder diffraction pattern with a specific peak at 2-theta = 19.8° when measured using CuKα radiation, plus or minus 0.2° 2-theta.

In embodiments there is provided Compound (III) Form A, which has an X-ray powder diffraction pattern with specific peaks at 2-theta = 10.7 and 19.8° when measured using CuKα radiation, wherein

- 20 said values may be plus or minus 0.2° 2-theta.

In embodiments there is provided Compound (III) Form A, which has an X-ray powder diffraction pattern with specific peaks at 2-theta = 8.1, 10.7, 19.8, 24.9, and 27.6° when measured using CuKα radiation, wherein said values may be plus or minus 0.2° 2-theta.

- 25 In embodiments there is provided Compound (III) Form A, which has an X-ray powder diffraction pattern with specific peaks at 2-theta = 8.1, 10.7, 13.6, 15.4, 18.9, 19.8, 21.4, 23.1, 24.9, 27.6 and 31.2° when measured using CuKα radiation, wherein said values may be plus or minus 0.2° 2-theta.

According to the present specification there is provided Compound (III) Form A which has an X-ray powder diffraction pattern with a specific peak at about 2-theta = 10.7° when measured using CuKα radiation.

According to the present specification there is provided Compound (III) Form A, which has an X-ray powder diffraction pattern with a specific peak at about 2-theta = 19.8° when measured using CuK α radiation.

5 In embodiments there is provided Compound (III) Form A, which has an X-ray powder diffraction pattern with specific peaks at about 2-theta = 10.7 and 19.8° when measured using CuK α radiation.

In embodiments there is provided Compound (III) Form A, which has an X-ray powder diffraction pattern with specific peaks at about 2-theta = 8.1, 10.7, 19.8, 24.9, and 27.6° when measured using CuK α radiation.

10 In embodiments there is provided Compound (III) Form A, which has an X-ray powder diffraction pattern with specific peaks at about 2-theta = 8.1, 10.7, 13.6, 15.4, 18.9, 19.8, 21.4, 23.1, 24.9, 27.6 and 31.2° when measured using CuK α radiation.

According to the present specification there is provided Compound (III) Form A, which has an X-ray powder diffraction pattern substantially the same as the X-ray powder diffraction pattern shown in Figure 1A when measured using CuK α radiation.

15 When it is stated that the present specification relates to Compound (III) Form A, the degree of crystallinity is greater than about 60%, greater than about 80%, greater than about 90% or greater than about 95%. In embodiments, the degree of crystallinity is greater than about 98%.

In embodiments there is provided Compound (III) Form A, as described herein, wherein the degree of purity is greater than 99%. In embodiments, the degree of purity is greater than 99.5%. In
20 embodiments, the degree of purity is greater than 99.9%.

The Compound (III) Form A provides X-ray powder diffraction patterns substantially the same as the X-ray powder diffraction patterns shown in Figure 1A and has substantially the ten most prominent peaks (angle 2-theta values) shown in Table 2. It will be understood that the 2-theta values of the X-ray powder diffraction pattern may vary slightly from one machine to another or from one sample to
25 another, and so the values quoted are not to be construed as absolute.

It is known that an X-ray powder diffraction pattern may be obtained which has one or more measurement errors depending on measurement conditions (such as equipment or machine used). In particular, it is generally known that intensities in an X-ray powder diffraction pattern may fluctuate depending on measurement conditions. Therefore it should be understood that the Compound (III)
30 Form A of the present specification is not limited to the crystals that provide X-ray powder diffraction patterns identical to the X-ray powder diffraction pattern shown in Figure 1A, and any crystals

providing X-ray powder diffraction patterns substantially the same as those shown in Figure 1A fall within the scope of the present specification. A person skilled in the art of X-ray powder diffraction is able to judge the substantial identity of X-ray powder diffraction patterns.

The present specification is intended to include all isotopes of atoms occurring in the present compounds. Isotopes will be understood to include those atoms having the same atomic number but different mass numbers. For example, isotopes of hydrogen include tritium and deuterium. Isotopes of carbon include ^{13}C and ^{14}C . Isotopes of nitrogen include ^{15}N . Isotopes of fluorine include ^{18}F .

In a further aspect there is provided a pharmaceutical composition comprising Compound (III) Form A, and a pharmaceutically acceptable excipient.

The term "pharmaceutical composition" refers to a preparation which is in such form as to permit the biological activity of the active ingredient, and which contains no additional components which are unacceptably toxic to a patient to which the composition would be administered. Such compositions can be sterile. A pharmaceutical composition according to the present specification will comprise Compound (III) Form A, and a pharmaceutically acceptable excipient.

The pharmaceutical formulations of Compound (III) Form A, described above may conveniently be administered in unit dosage form and may be prepared by any of the methods well-known in the pharmaceutical art, for example as described in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA., (1985).

As a result of their 17BHSD13 inhibitory activity Compound (III) Form A is expected to be useful in therapy, for example in the treatment of diseases or medical conditions mediated at least in part by 17BHSD13, including liver disease, such as NASH.

In one aspect of the present specification there is provided Compound (III) Form A, for use in therapy.

In one aspect of the present specification there is provided Compound (III) Form A, for use in the treatment of liver disease. In embodiments, the liver disease is selected from alcoholic liver disease, non-alcoholic liver disease, NAFLD (such as NASH, liver fibrosis, cirrhosis, and isolated steatosis), liver inflammation, alcoholic steatohepatitis (ASH), hepatitis C virus (HCV) and hepatocellular carcinoma (HCC).

The term "therapy" is intended to have its normal meaning of dealing with a disease in order to entirely or partially relieve one, some or all of its symptoms, or to correct or compensate for the

underlying pathology. The term "therapy" also includes "prophylaxis" unless there are specific indications to the contrary. The terms "therapeutic" and "therapeutically" should be interpreted in a corresponding manner.

5 The term "prophylaxis" is intended to have its normal meaning and includes primary prophylaxis to prevent the development of the disease and secondary prophylaxis whereby the disease has already developed and the patient is temporarily or permanently protected against exacerbation or worsening of the disease or the development of new symptoms associated with the disease.

The term "treatment" is used synonymously with "therapy". Similarly the term "treat" can be regarded as "applying therapy" where "therapy" is as defined herein.

10 In embodiments, there is provided Compound (III) Form A, for use in providing an inhibitory effect on 17 β HSD13.

In embodiments, there is provided Compound (III) Form A, for use in the treatment of a disease mediated by 17 β HSD13, such as liver disease (e.g. NASH).

15 In embodiments, there is provided Compound (III) Form A, for use in the treatment of fatty liver disease.

In embodiments, there is provided Compound (III) Form A, for use in the treatment of nonalcoholic Fatty Liver Disease (NAFLD), such as isolated steatosis, Nonalcoholic Steatohepatitis (NASH), liver fibrosis or cirrhosis. In further embodiments, the liver disease is end stage liver disease.

20 In embodiments, there is provided Compound (III) Form A, for use in the treatment of liver disease, such as NASH, wherein the patient is also suffering from or susceptible to one or more conditions selected from the group consisting of obesity, dyslipidemia, insulin resistance, Type 2 diabetes, and renal insufficiency.

25 In embodiments, there is provided Compound (III) Form A, for use in the treatment of liver disease, such as NASH, wherein the patient has a body mass index (BMI) of 27 kg/m² to 40 kg/m². In further embodiments, the subject has a BMI of 30 kg/m² to 39.9 kg/m². In further embodiments, the patient has a BMI of at least 40 kg/m². In further embodiments, the patient is overweight. In further embodiments, the patient is obese.

In embodiments, there is provided Compound (III) Form A, for use in the treatment of liver disease, such as NASH, wherein the patient is also suffering from or susceptible to dyslipidemia.

30 In embodiments, there is provided Compound (III) Form A, for use in the treatment of liver disease, such as NASH, wherein the patient is also suffering from or susceptible to insulin resistance.

In embodiments, there is provided Compound (III) Form A, for use in the treatment of liver disease, such as NASH, wherein the patient is also suffering from or susceptible to Type 2 diabetes.

In embodiments, there is provided Compound (III) Form A, for use in the treatment of liver disease, such as NASH, wherein the patient is also suffering from or susceptible to renal insufficiency.

5 In embodiments, there is provided Compound (III) Form A, for use in the treatment of liver disease, such as NASH, wherein the patient is also suffering from or susceptible to liver fibrosis. In further embodiments, the patient is (i) suffering from or susceptible to liver fibrosis, and (ii) suffering from or susceptible to one or more conditions selected from the group consisting of obesity, dyslipidemia, insulin resistance, Type 2 diabetes, and renal insufficiency.

10 In embodiments, there is provided Compound (III) Form A, for use in the treatment of liver disease, such as NASH, wherein the patient is also suffering from or susceptible to cirrhosis. In further embodiments, the patient is (i) suffering from or susceptible to cirrhosis, and (ii) suffering from or susceptible to one or more conditions selected from the group consisting of obesity, dyslipidemia, insulin resistance, Type 2 diabetes, and renal insufficiency.

15 In embodiments, there is provided Compound (III) Form A, for use in the treatment of NAFLD. In further embodiments, the NAFLD is Stage 1 NAFLD. In further embodiments, the NAFLD is Stage 2 NAFLD. In further embodiments, the NAFLD is Stage 3 NAFLD. In further embodiments, the NAFLD is Stage 4 NAFLD. See, e.g., "The Diagnosis and Management of Nonalcoholic Fatty Liver Disease: Practice Guidance From the American Association for the Study of Liver Diseases," Hepatology, Vol.
20 67, No. 1, 2018.

In embodiments, there is provided Compound (III) Form A, for use in the treatment of NAFLD, such as NASH. In further embodiments, the patient is obese. In further embodiments, the patient has alcoholic liver disease. In further embodiments, the patient has a genetic risk factor for liver disease, such as the (rs738409 C>G) variant in PNPLA3.

25 In embodiments, there is provided Compound (III) Form A, for use in the treatment of NASH. In further embodiments, the NASH is Stage 1 NASH. In further embodiments, the NASH is Stage 2 NASH. In further embodiments, the NASH is Stage 3 NASH. In further embodiments, the NASH is Stage 4 NASH. In further embodiments, the patient is also suffering from or susceptible to one or more conditions selected from obesity, dyslipidemia, insulin resistance, Type 2 diabetes, and renal
30 insufficiency.

In embodiments, there is provided Compound (III) Form A, for use in the treatment of liver fibrosis. In further embodiments, the liver fibrosis is Stage 3 liver fibrosis. In further embodiments, the

patient is also suffering from or susceptible to one or more conditions selected from obesity, dyslipidemia, insulin resistance, Type 2 diabetes, and renal insufficiency.

In embodiments, there is provided Compound (III) Form A, for use in the treatment of cirrhosis. In further embodiments, the cirrhosis is stage F4 cirrhosis. In further embodiments, the patient is also
5 suffering from or susceptible to one or more conditions selected from obesity, dyslipidemia, insulin resistance, Type 2 diabetes, and renal insufficiency.

In embodiments, there is provided Compound (III) Form A, for use in the treatment of liver inflammation. In further embodiments, the inflammation is chronic inflammation. In further embodiments, the chronic inflammation is selected from the group consisting of rheumatoid
10 arthritis, osteoarthritis, and Crohn's disease. In further embodiments, the chronic inflammation is rheumatoid arthritis.

In embodiments, there is provided Compound (III) Form A, for use in the treatment of hepatocellular carcinoma (HCC).

In embodiments, there is provided Compound (III) Form A, for use in the treatment of alcoholic
15 steatohepatitis (ASH).

In embodiments, there is provided Compound (III) Form A, for use in the treatment of hepatitis C virus (HCV).

In one aspect of the present specification there is provided the use of Compound (III) Form A, as described herein, in the manufacture of a medicament, such as a medicament for the treatment of
20 disease (e.g. NASH).

In one aspect of the present specification there is provided a method of treating disease, such as NASH, in a patient comprising administering to the patient an effective amount of Compound (III) Form A.

Terms such as "treating" or "treatment" refer to both (1) therapeutic measures that cure, slow
25 down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and (2) prophylactic or preventative measures that prevent and/or slow the development of a targeted pathologic condition or disorder. Thus, those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented.

30 The term "effective amount" means an amount of an active ingredient which is sufficient enough to significantly and positively modify the symptoms and/or conditions to be treated (e.g., provide a

positive clinical response). The effective amount of an active ingredient for use in a pharmaceutical composition will vary with the particular condition being treated, the severity of the condition, the duration of the treatment, the nature of concurrent therapy, the particular active ingredient(s) being employed, the particular pharmaceutically-acceptable excipient(s)/carrier(s) utilized, and like factors
5 within the knowledge and expertise of the attending physician.

The term "patient" refers to any animal (e.g., a mammal), including, but not limited to humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment.

Typically, the term "patient" refers to a human subject.

In embodiments, there is provided a method of treating disease in a patient comprising
10 administering to the patient an effective amount of Compound (III) Form A, wherein the disease is selected from isolated steatosis, NASH, liver fibrosis and cirrhosis.

In embodiments, there is provided a method of treating a 17 β HSD13 mediated disease in a patient comprising administering to the patient an effective amount of Compound (III) Form A, such as NASH.

15 The compounds of the present disclosure may be used in the methods described above as either as single pharmacological agents or in combination with other pharmacological agents or techniques. Such combination therapies may be achieved by way of the simultaneous, sequential or separate dosing of the individual components of the treatment. These combination therapies (and corresponding combination products) employ the compounds of the present disclosure and the
20 other pharmacological agent(s).

In embodiments, there is provided a combination for use in the treatment of liver disease, such as NASH, comprising Compound (III) Form A, and a sodium-glucose transport protein 2 (SGLT2) inhibitor. In further embodiments, the SGLT2 inhibitor is selected from canagliflozin, dapagliflozin, empagliflozin, ertugliflozin, ipragliflozin, luseogliflozin, and remogliflozin.

25 In embodiments, there is provided a combination for use in the treatment of liver disease, such as NASH, comprising Compound (III) Form A, and metformin, or a pharmaceutically acceptable salt thereof.

In embodiments, there is provided a combination for use in the treatment of liver disease, such as NASH, comprising Compound (III) Form A, and a glucagon-like peptide-1 receptor (GLP1) agonist. In
30 further embodiments, the GLP1 agonist is selected from exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, and semaglutide.

In embodiments, there is provided a combination for use in the treatment of liver disease, such as NASH, comprising Compound (III) Form A, and a dipeptidyl peptidase 4 (DPP4) inhibitor. In further embodiments, the DPP4 inhibitor is selected sitagliptin, vildagliptin, saxagliptin, linagliptin, gemigliptin, anagliptin, teneligliptin, alogliptin, trelagliptin, omarigliptin, evogliptin, gosogliptin, and dutogliptin.

In embodiments, there is provided a combination for use in the treatment of liver disease, such as NASH, comprising Compound (III) Form A, and a PPAR agonist. In further embodiments, the PPAR agonist is a PPAR α agonist. In further embodiments, the PPAR agonist is a PPAR γ agonist. In further embodiments, the PPAR agonist is a PPAR α/γ agonist. In further embodiments, the PPAR agonist is selected from clofibrate, gemfibrozil, ciprofibrate, bezafibrate, and fenofibrate. In further embodiments, the PPAR agonist is a thiazolidinedione. In further embodiments, the thiazolidinedione is selected from pioglitazone, rosiglitazone, lobeglitazone, and rivoglitazone. In further embodiments, the PPAR agonist stimulates liver expression of FGF21. PPAR refers to Peroxisome Proliferator-Activated Receptor.

In embodiments, there is provided a combination for use in the treatment of liver disease, such as NASH, comprising Compound (III) Form A, and a Pan-PPAR agonist. In further embodiments, the Pan-PPAR agonist is lanifibranor.

In embodiments, there is provided a combination for use in the treatment of liver disease, such as NASH, comprising Compound (III) Form A, and a ThrB agonist. In further embodiments, the ThrB agonist is resmetirom.

In embodiments, there is provided a combination for use in the treatment of liver disease, such as NASH, comprising Compound (III) Form A, and a FXR agonist. In further embodiments, the FXR agonist is obeticholic acid.

Although Compound (III) Form A are primarily of value as therapeutic agents for use in patients, they are also useful whenever it is required to inhibit 17 β HSD13. Thus, they are useful as pharmacological standards for use in the development of new biological tests and in the search for new pharmacological agents.

Examples

The specification will now be illustrated by the following non-limiting Examples in which, generally:

- (i) operations were carried out at room temperature (rt), *i.e.* in the range 17 to 28°C and where needed under an atmosphere of an inert gas such as N₂;
- (ii) where reactions refer to being degassed or purged, this can be performed for example by purging the reaction solvent with a constant flow of nitrogen for a suitable period of time (for example 5 to 10 min) or by repeatedly evacuating the vessel and backfill with appropriate inert atmosphere (for example nitrogen (g) or argon (g));
- (iii) where reactions refer to the use of a microwave reactor, one of the following microwave reactors were used: Biotage Initiator, Personal Chemistry Emrys Optimizer, Personal Chemistry Smith Creator or CEM Explorer;
- (iv) in general, the course of reactions was followed by thin layer chromatography (TLC) and/or analytical high performance liquid chromatography (HPLC or UPLC) which was usually coupled to a mass spectrometer (LCMS).
- (v) when necessary, organic solutions were dried over anhydrous MgSO₄ or Na₂SO₄, or by using ISOLUTE Phase Separator, and workup procedures were carried out using traditional phase separating techniques. When a drying agent such as e.g. MgSO₄ or Na₂SO₄ is used for drying an organic layer, it is understood that said organic layer is filtered before concentration of said layer.
- (vi), evaporations were carried out either by rotary evaporation *in vacuo* or in a Genevac HT-4 / EZ-2 or Biotage V10;
- (vii) unless otherwise stated, flash column chromatography was performed on straight phase silica, using either Merck Silica Gel (Art. 9385) or prep-packed cartridges such as BIOTAGE SNAP cartridges (40-63 µm silica, 4-330 g), BIOTAGE Sfär Silica HC D cartridges (20 µm, 10-100 g), INTERCHIM PURIFLASH cartridges (25 µm, 4-120 g), INTERCHIM PURIFLASH cartridges (50 µm, 25-330 g), GRACE GRACERESOLVE Silica Flash Cartridges (4-120 g) or Agela Flash Colum Silica-CS cartridges (80-330 g), or on reversed phase silica using Agela Technologies C-18, spherical cartridges (20-35 µm, 100 A, 80-330 g), manually or automated using a Grace REVELERIS X2 Flash system or similar system;
- (viii) preparative TLC was performed on glass-backed silica plates (20×20 cm) covered with a 1 mm thick silica gel (particle size of 10-40 µm), in a glass chamber, using the appropriate solvent or solvent mixtures as eluant as stated in the experimental description;
- (ix) preparative reverse phase HPLC and preparative reverse phase SFC were performed using standard HPLC and SFC instruments, respectively, equipped with either a MS and/or UV triggered fraction collecting instrument, using either isocratic or a gradient of the mobile phase as described in

the experimental section and using the following method: **PrepMethod F:** The compound was purified by preparative HPLC on a Kromasil C8 column (10 μ m, 250×50 mm ID) using a gradient of MeCN in H₂O/MeCN/FA (95/5/0.2) as mobile phase.

5 In some instances the compound may be dissolved in a solvent e.g. DMSO and filtered through a syringe filter prior to purification on preparative HPLC.

Relevant fractions were collected, combined and freeze-dried or evaporated to give the purified compound or relevant fractions were collected, combined and concentrated at reduced pressure, extracted with DCM or EtOAc, and the organic phase was dried either over Na₂SO₄ or by using a phase-separator, and then concentrated at reduced pressure to give the purified compound.

10 (x) preparative chromatography was carried out using HPLC or SFC on a standard HPLC or SFC instruments, respectively, and using either isocratic or gradient run with mobile phase as described in the experimental section;

(xi) yields, where present, are not necessarily the maximum attainable, and when necessary, reactions were repeated if a larger amount of the reaction product was required;

15 (xii) where certain compounds were obtained as an acid-addition salt, for example a mono-hydrochloride salt or a di-hydrochloride salt, the stoichiometry of the salt was based on the number and nature of the basic groups in the compound, the exact stoichiometry of the salt was generally not determined, for example by means of elemental analysis data;

(xiii) in general, the structures of the end-products were confirmed by nuclear magnetic resonance (NMR) and/or mass spectral techniques; proton NMR chemical shift values were measured on the delta scale using Bruker Avance III 300, 400, 500 and 600 spectrometers, operating at ¹H frequencies of 300, 400, 500 and 600 MHz, respectively. The experiments were typically recorded at 25°C.

Chemical shifts are given in ppm with the solvent as internal standard. Protons on heteroatoms such as NH and OH protons are only reported when detected in NMR and can therefore be missing. In 25 certain instances, protons can be masked or partially masked by solvent peaks and will therefore either be missing and not reported or reported as multiplets overlapping with solvent. The following abbreviations have been used (and derivatives thereof, e.g. dd, doublet of doublets, etc.): s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; qn, quintet; p, pentet. In some cases, the structures of the end-products might appear as rotamers in the NMR-spectrum, in which instances 30 only peaks of the major rotamer are reported. In certain instances, the structures of the intermediates and/or the end-products might appear as rotamers in the NMR-spectrum in a more equal relationship, in such instances the peaks of such rotamers are either reported as multiplets, if

the signals of said rotamers are partially overlapping, or as individual peaks, if the signals of said rotamers are well separated and only the total number of protons are reported. The ratio of major vs minor rotamer is reported if known.

- (xiv) Electrospray mass spectral data were obtained using a Waters Acquity UPLC coupled to a Waters single quadrupole mass spectrometer or similar equipment, acquiring both positive and negative ion data, and generally, only ions relating to the parent structure are reported; high resolution electrospray mass spectral data were obtained using a Waters XEVO qToF mass spectrometer or similar equipment, coupled to a Waters Acquity UPLC, acquiring either positive and negative ion data, and generally, only ions relating to the parent structure are reported
- (xv) intermediates were not necessarily fully purified but their structures and purity were assessed by TLC, analytical HPLC/UPLC, and/or NMR analysis and/or mass spectrometry;
- (xvi) unless stated otherwise compounds containing an asymmetric carbon and/or sulfur atom were not resolved;
- (xvii) in general Examples and Intermediate compounds are named using ChemDraw Professional version 22.2.0 from PerkinElmer. ChemDraw Professional version 22.2.0 generates the names of chemical structures using the Cahn-Ingold-Prelog (CIP) rules for stereochemistry and follows IUPAC rules as closely as possible when generating chemical names. Stereoisomers are differentiated from each other by stereodescriptors cited in names and assigned in accordance with the CIP rules.
- ChemDraw is optionally using labels in the graphical representation of stereocenters such as '&' and 'or' to describe the configuration of the stereochemical centers present in the structure. A number following the '&' and 'or' flag is assigned to each stereocenter present in the structure. The numbers are incremented automatically to indicate that stereocenters may vary independently to each other.
- In general, for chemical structures of Examples and Intermediates where more than one stereocenter is present and said stereocenters have a fixed relative configuration, the same number is used after the label '&' and 'or' to indicate that said stereocenters forms a group. A third stereocenter present in the same chemical structure, that varies independently to the former stereocenters, is designated with a unique new number following the label '&' and 'or'.
- In general chemical structures of Examples and Intermediates containing the label '&' at a stereocenter, means the configuration of such Example or Intermediate at that stereocenter is a mixture of both (*R*) and (*S*); and a label 'or' means the configuration of such Example or Intermediate at that stereocenter is either (*S*) or (*R*). Absolute, unspecified, '&', and 'or' stereocenters can all be present in a single structure.

In general, for chemical structures of Examples and Intermediates where only one stereocenter is present and said stereocenter is racemic, no flag is designated to the stereocenter and the structure is drawn with a straight bond at said stereocenter.

In general, for chemical structures of Examples and Intermediates where more than one
5 stereocenter is present and said stereocenters have a fixed relative configuration, the same number is used after the label '&' and 'or' to indicate that said stereocenters forms a group. A third stereocenter present in the same chemical structure, that varies independently to the former stereocenters, is designated with a unique new number following the label '&' and 'or'.

In general for structures of Examples and Intermediates where all of the stereocenters are
10 designated as '&', the structure is named with a "rac-" prefix. For structures of Examples and Intermediates where all of the stereocenters are designated as 'or', the structure is named with a "rel-" prefix.

(xviii) in addition to the ones mentioned above, the following abbreviations and units have been used:

15	Art.	Article
	Aq	Aqueous
	Calcd	Calculated
	DMF	<i>N,N</i> -dimethylformamide
	DMSO	Dimethyl sulfoxide
20	EDC	3-(((ethylimino)methylene)amino)- <i>N,N</i> -dimethylpropan-1-amine
	e.g.	for example
	ESI	Electrospray ionization
	etc.	et cetera
	EtOAc	Ethyl acetate
25	FA	Formic acid
	(g)	gas
	HOBt	1-hydroxybenzotriazole;hydrate
	HPLC	High performance liquid chromatography
	HRMS	High resolution mass spectrometry
30	ID	inner diameter
	i.e.	id est
	LCMS	Liquid Chromatography Mass Spectrometry
	Me ₃ Al	Trimethyl aluminum

	MeCN	Acetonitrile
	MS	Mass spectrometry
	m/z	mass spectrometry peak(s)
	NMR	Nuclear magnetic resonance
5	rt	Room temperature
	SFC	Supercritical fluid chromatography
	TLC	Thin layer chromatography
	UPLC	ultra performance liquid chromatography
	UV	ultraviolet
10	vs	versus

Units

	A	Angström
	C	celsius
15	cm	centimeter(s)
	g	gram
	h	hour(s)
	M	mole per liter
	mg	milligram
20	MHz	megahertz
	min	minute(s)
	mL	milliliter
	mm	millimeter
	mM	millimole per liter
25	mmol	millimole(s)
	μm	micrometer
	μM	micromole per litre
	μL	microlitre
	ms	milliseconds
30	nM	nanomole per litre
	ppm	parts per million

***In vitro* 17bHSD13 enzyme assay**

- 10 concentration of compounds (0.2 µl) in DMSO was added to GREINER PP 384 well plate (781280) using ECHO dispensing (BECKMAN COULTER) followed by 20 µl of recombinant 17bHSD13 (N2-K300). The enzyme reaction was initiated by addition, using CERTUS-FLEX dispenser (GYGER), of 20 µl of substrate solution containing NAD (SIGMA, N1511) and Estradiol (SIGMA, E8875). After each
- 5 addition plates were centrifuged for 1 min at 150x g (EPPENDORF, 5810R, A-4-81). Final assay conditions were 80 nM of 17bHSD13, 0.5 mM of NAD, 20 µM Estradiol and various concentrations of compound in buffer (5 mM EDTA (TEKNOVA E0306), 0.01% DDM (AFFYMETRIX D310) in 50mM Tris-Cl, pH 7.4). After 2.5 h the reaction were stopped by addition of 20 µl of 0.6 % Formic acid (MERCK 5.33002) and samples were analyzed using LC-MS/MS.
- 10 SCIEX LC-MS/MS system: Sample was injected with CTC analytical injector, SHIMATZU LC pumps LC20 and analyzed on the SCIEX API 5000 LCMSMS system with the following settings. Samples were chromatographed on a WATERS, SYMMETRY, C8, 3.5 µm, 2.1x 50 mm) column at constant flow rate of 0.5 mL/min. The mobile phases consist of A (water with 0.2% formic acid) and B (acetonitrile with 0.2% formic acid). The LC gradient profile is as follows: 50% B during 0 to 0.5 min, a linear increase to
- 15 100% B during 0.5 to 1 min, hold at 100% B during 1 to 1.6 min then back to 50% B from 1.6 to 2 min. The run time was 2 min with retention times of approximately 0.8 and 1.07 min for Estradiol and Estrone, respectively. Detection was performed on a API 5000 LC/MS/MS system with a triple quadrupole mass spectrometer, a TURBO V ion source, in multiple reaction monitoring (MRM) mode at positive polarity with APCI probe. The MRM pairs were m/z 273.1 to m/z 107.0 and m/z 271.3 to
- 20 107.0. for Estradiol and Estrone, respectively. The dwell times were 100 ms for each transition and a depolarization and collision energy of 100 and 40, respectively. Data from MS signals was using area under curve (AUC). Ratio = Estrone/(Estrone + Estradiol)

***In vitro* 17bHSD13 cell assay**

- Inhibition of 17bHSD13 was measured in a cell-based assay with over expressed HSD17β13 in
- 25 HEK293S cells, measuring estradiol to estrone conversion by LCMS/MS.

- Cells were plated in 384 well plates (GREINER CELL culture plate 384w black/clear Poly-D-Lysine) at 10 K c/w in 30 µl of culture media (DMEM with GLUTAMAX plus 10 % FBS). After the cells were allowed to attach for 6 h, 0.15 µl of 10 concentration of compounds and 0.03 µl of 10 mM Estradiol (SIGMA, E8875) in DMSO, was added using ECHO dispensing (BECKMAN COULTIER). After 18 h of cell
- 30 culturing for 20 µl of media was transferred using BRAVO dispensing robot (AGILENT) to a GREINER PP 384 well plate (781280) and 40 µl of 50 % acetonitrile was added. Samples were analyzed using LC-MS/MS.

SCIEX LC-MS/MS system: Sample was injected with CTC analytical injector, SHIMATZU LC pumps LC20 and analysed on the SCIEX API 5000 LCMSMS system with the following settings. Samples were chromatographed on a WATERS, symmetry, C8, 3.5 μ m, 2.1x 50 mm) column at constant flow rate of 0.5 mL/min. The mobile phases consist of A (water with 0.2% formic acid) and B (acetonitrile with 0.2% formic acid). The LC gradient profile is as follows: 50% B during 0 to 0.5 min, a linear increase to 100% B during 0.5 to 1 min, hold at 100% B during 1 to 1.6 min then back to 50% B from 1.6 to 2 min. The run time was 2 min with retention times of approximately 0.8 and 1.07 min for Estradiol and Estrone, respectively. Detection was performed on a API 5000 LC/MS/MS system with a triple quadrupole mass spectrometer, a TURBO V ion source, in multiple reaction monitoring (MRM) mode at positive polarity with APCI probe. The MRM pairs were m/z 273.1 to m/z 107.0 and m/z 271.3 to 107.0. for Estradiol and Estrone, respectively. The dwell times were 100 ms for each transition and a depolarization and collision energy of 100 and 40, respectively. Data from MS signals was using area under curve (AUC). Ratio = Estrone/(Estrone + Estradiol)

***In vitro* 17 β HSD4 enzyme assay**

10 concentration of compounds (0.2 μ l) in DMSO was added to GREINER FLUOTRAC 200 384 well plate (781076) using ECHO dispensing (BECKMAN COULTER). 80 nl of 10 mM Estradiol (SIGMA, E8875) was added using Echo dispensing. The enzyme reaction was initiated by addition, using MULTIDROP COMBI dispensing (THERMO FISHER), of 40 μ l of a mix containing recombinant 17 β HSD4 (M1-N311) and NAD. Final assay conditions were 40 nM of 17 β HSD4, 0.125 mM of NAD, 15 μ M Estradiol and various concentrations of compound in buffer (5 mM EDTA (TEKNOVA E0306), 0.01% DDM (AFFYMETRIX D310) in 50mM Tris-Cl, pH 7.4). After each addition plates were centrifuged for 1 min at 150x g (EPPENDORF, 5810R, A-4-81). NADH formation was measured by fluorescence intensity (FI) (Ex360/Em460) at time zero (t_0) and at 1.5 h (t_1) in a PHERASTAR FSX (BMG LABTECH). FI for each sample was calculated as FI at t_1 minus FI at t_0 .

***In vitro* 17 β HSD9 cell assay**

Inhibition of 17 β HSD9 was measured in a cell-based assay with over expressed HSD17 β 9 in HEK293S cells, measuring retinol to retinal conversion by LCMS/MS.

Cells were plated in 384 well plates (GREINER CELL culture plate 384w black/clear Poly-D-Lysine) at 10 K c/w in 30 μ l of culture media (DMEM with GLUTAMAX plus 10 % FBS). After the cells were allowed to attach for 6 h, 0.15 μ l of 10 concentration of compounds and 0.015 μ l of 10 mM all-trans-retinol (CAYMAN CHEMICAL, 20241) in DMSO, was added using ECHO dispensing (BECKMAN COULTIER). After 18 h of cell culturing for 20 μ l of media was transferred using BRAVO dispensing

robot (AGILENT) to a GREINER PP 384 well plate (781280) and 40 µl of 50 % acetonitrile was added. Samples were analyzed using LC-MS/MS.

SCIEX LC-MS/MS system: Sample was injected with CTC analytical injector, SHIMATZU LC pumps LC20 and analysed on the SCIEX API 5000 LCMSMS system with the following settings. Samples were chromatographed on a WATERS, symmetry, C8, 3.5 µm, 2.1x 50 mm) column at constant flow rate of 0.5 mL/min. The mobile phases consists of A (water with 0.2% formic acid) and B (acetonitrile with 0.2% formic acid). The LC gradient profile is as follows: 50% B during 0 to 0.1 min, a linear increase to 100% B during 0.1 to 0.8 min, hold at 100% B during 0.8 to 1.5 min then back to 50% B from 1.5 to 1.6 min and hold during run time. The run time was 2 min with retention times of approximately 1.54 and 1.62 min for Retinol and Retinal, respectively. Detection was performed on a API 5000 LC/MS/MS system with a triple quadrupole mass spectrometer, a TURBO V ion source, in multiple reaction monitoring (MRM) mode at positive polarity with ESI probe. The MRM pairs were m/z 269.3 to m/z 93.0 and m/z 285.2 to 161.0. for Retinol and Retinal, respectively. The dwell times were 100 ms for each transition and a depolarization and collision energy of 50 and 25, respectively. Data from MS signals was using area under curve (AUC). Ratio = Retinal/(Retinal+ Retinol).

Data analysis

GENEDATA SCREENER was used for curve fitting and calculation of IC₅₀ values.

Compound effect was calculated with the formula below;

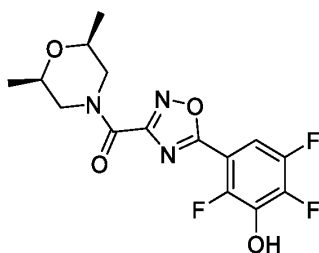
Compound % effect = $-100 \times ((X - \text{min}) / (\text{max} - \text{min}))$

where X represents the effect in the presence of test compound, min is DMSO and max is the maximum inhibition of enzyme using a known inhibitor as control.

Form A ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone

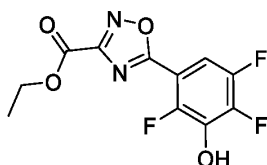
Disclosed here in Form A of ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone.

Synthesis of ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone (Compound (III))



(2*R*,6*S*)-2,6-Dimethylmorpholine (0.520 g, 4.51 mmol) was suspended in dry toluene (12 mL) and Me₃Al in toluene (2 M, 4.34 mL, 8.68 mmol) was added under a N₂(g) atmosphere, and the resulting mixture was stirred at rt for 1 h. The above mixture was added to a stirred slurry of ethyl 5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazole-3-carboxylate **Intermediate A** (1 g, 3.47 mmol) in toluene (12 mL). The resulting solution was heated to 60°C for 20 h. The mixture was cooled to rt, tartaric acid (30%, aq, 100 mL) was added and the mixture was extracted with EtOAc. The organic layer was concentrated, and the residue was purified by preparative HPLC, PrepMethod F (gradient 20-80%) to give the title compound (1.01 g, 81%) as a white solid; HRMS (ESI) *m/z* [M+H]⁺ calcd for C₁₅H₁₅F₃N₃O₄: 358.1008, found: 358.0978; ¹H NMR (500 MHz, CD₃OD) δ 1.14 (3H, d), 1.25 (3H, d), 2.65 (1H, dd), 2.96 (1H, dd), 3.57–3.79 (2H, m), 3.98 (1H, dt), 4.52 (1H, dt), 7.43–7.67 (1H, m).

Intermediate A: Ethyl 5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazole-3-carboxylate



EDC (43.5 g, 227.07 mmol) and HOBt (15.34 g, 113.53 mmol) were added to ethyl (Z)-2-amino-2-(hydroxyimino)acetate (15 g, 113.53 mmol), 2,4,5-trifluoro-3-hydroxybenzoic acid (21.81 g, 113.53 mmol) and NaHCO₃ (28.6 g, 340.60 mmol) in DMF (150 mL) under a N₂(g) atmosphere. The resulting solution was stirred at 100°C for 1 h. The reaction mixture was filtered through CELITE, and the filtrate was concentrated, diluted with DCM (300 mL) and washed with water (300 mL). The organic layer was dried over Na₂SO₄, filtered and evaporated. The crude product was purified by flash chromatography on a C18 column (gradient 50-60% MeCN in water (FA)) to give the title compound (7.0 g, 21%) as a white solid; MS (ESI) *m/z* [M+H]⁺ 289; ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.36 (t, 3H), 4.45 (m, 2H), 7.28 – 7.81 (m, 1H), 11.70 (s, 1H).

Table 1

	17bHSD13 Enzyme assay IC ₅₀ (μM)	17HSD17b4 Enzyme assay IC ₅₀ (μM)	17bHSD1713 Cell assay IC ₅₀ (μM)	17bHSD9 Cell assay IC ₅₀ (μM)
Compound (III)	0.285	>200	0.06	36.3

The data in Table 1 may be from a single experiment or an average of two or more experiments.

1) Cooling crystallisation

Saturated solutions of the isolated sample of ((2R,6S)-2,6-dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone as prepared above were obtained by stirring at 50 °C in one of the following solvents: 1-propanol, 2-butanol, acetone, and ethyl acetate. The solutions were slowly cooled to 5 °C. The precipitates were separated from the liquid phases, dried at ambient or under vacuum (5 mbar and 50 °C) and analysed by XRPD at RT.

2) Evaporative crystallisation

Highly concentrated solutions of the isolated sample of ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone as prepared above were obtained by stirring at room temperature (RT) in one of the following solvents: methanol, ethanol, methyl ethyl ketone (MEK) and acetonitrile (ACN). The liquid phases were allowed to evaporate under ambient conditions and the obtained solids were analysed by XRPD.

Analysis of the respective samples prepared by cooling crystallisation and evaporative crystallisation revealed that the resultant powders were crystalline. Furthermore, XRPD of each powder produced equivalent diffractograms as per that presented as **Figure 1A**. Further analysis as described herein revealed the material to be an anhydrate solid form, Form A, of ((2R,6S)-2,6-dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone (Compound (III)).

Table 2: List of eleven of the most prominent peaks from the X-ray powder diffraction pattern of Compound (III) Form A.

2 θ (°)	Relative Intensity
8.1	m
10.7	vs
13.6	s
15.4	vs
18.9	m
19.8	vs
21.4	s
23.1	vs
24.9	vs
27.6	s
31.2	s

XRPD

The X-ray powder diffraction (XRPD) analysis was performed according to standard methods, which can be found in e.g. Kitaigorodsky, A.I. (1973), Molecular Crystals and Molecules, Academic Press, New York; Bunn, C.W. (1948), Chemical Crystallography, Clarendon Press, London; or Klug, H.P. & Alexander, L.E. (1974), X-ray Diffraction Procedures, John Wiley & Sons, New York.

Persons skilled in the art of X-ray powder diffraction will realize that the relative intensity of peaks can be affected by, for example, grains above 30 microns in size and non-unitary aspect ratios, which may affect analysis of samples. The skilled person will also realize that the position of reflections can be affected by the precise height at which the sample sits in the diffractometer and the zero calibration of the diffractometer. The surface planarity of the sample may also have a small effect. Hence the diffraction pattern data presented are not to be taken as absolute values. (Jenkins, R & Snyder, R.L. 'Introduction to X-Ray Powder Diffractometry' John Wiley & Sons 1996; Bunn, C.W. (1948), Chemical Crystallography, Clarendon Press, London; Klug, H. P. & Alexander, L. E. (1974), X-Ray Diffraction Procedures).

Generally, a measurement error of a diffraction angle in an X-ray powder diffractogram may be approximately plus or minus 0.2° 2-theta, and such a level of measurement error should be taken into account when considering the XRPD pattern in **Figure 1A** and when reading Table 2. Furthermore, peak intensities may fluctuate depending on experimental conditions and sample preparation (preferred orientation).

The XRPD pattern was determined by mounting a sample on a zero-background sample holder with a small depression filled with some of the ground material. A glass slide was used to get an evenly distributed sample with the correct sample height and sample peak position was adjusted with reference to a second sample containing an internal Corundum standard.

- 5 The X-ray powder diffraction was recorded with a theta-two theta scan axis using a Rigaku Miniflex 600 (wavelength of X-rays 1.5418 Å nickel-filtered CuK α radiation, 40 kV, 15 mA) equipped with D/Tex Ultra detector operating in one dimensional mode. Fixed divergence and receiving slits were used together with an automatic variable anti-scatter screen. The samples were rotated at 30 revolutions per minute during measurement. Samples were scanned from 3 - 50° 2-theta (2 θ) using a
- 10 0.01° and 1°/min step width and scan speed respectively.

Definition of relative intensity

% Relative Intensity*	Definition
25 - 100	vs (very strong)
10 - 25	s (strong)
3 - 10	m (medium)
1 - 3	w (weak)

* The relative intensities are derived from diffractograms measured with fixed slits.

Ramp Differential Scanning Calorimetry (DSC)

- The melting point temperature onset (T_m) is determined by Differential Scanning Calorimetry using
- 15 a TA Instruments DSC, Discovery 2500 model. A sample (approximately 2 – 4 mg) is weighed into an aluminium sample pan. The sample is packed to the bottom of a non-hermetic sample pan and a lid is pressed down to ensure good thermal contact. The instrument is purged with nitrogen gas at 50 mL/min and data collected between 0 °C and 220 °C, using a heating rate of 5 °C/minute.

Thermogravimetric Analysis (TGA)

- 20 Thermal gravimetric analysis is performed using a TA Instruments TGA, model TGA5500. A sample (approximately 5-10 mg) is transferred to a tared sample holder. The instrument is purged with nitrogen, oven 25 mL/min and balance 10 mL/min, and data are collected between room temperature and 300 °C, using a heating rate of 5 °C/min.

Gravimetric Vapor Sorption (GVS)

- 25 Gravimetric vapor sorption analysis was performed using a DVS Resolution instrument from Surface Measurement Systems. A sample (approximately 5-10 mg) is transferred to a tared sample holder.

The instrument is initially purged with dry nitrogen, at 25 °C and data are then collected at different relative humidity (%RH) by mixing wet and dry nitrogen flows. Starting at 20% RH and going stepwise up to 80% RH in 10% RH increments and then down stepwise to 0% RH followed by a second cycle going up to 90%RH and back to 0%RH. The equilibrium criteria for moving to the next %RH is reached when the drift criteria (dm/dt) is below 0.002 % for 10 min. The mass at the end of the 0% RH stage has been taken as the reference mass which is used to calculate the change in mass during the experiment.

Hygroscopicity can be assessed, for example, according to the European Pharmacopoeia (EP) classification: non-hygroscopic: < 0.2%; slightly hygroscopic: $\geq 0.2\%$ and < 2%; hygroscopic: $\geq 2\%$ and < 15%; very hygroscopic: $\geq 15\%$; deliquescent: sufficient water is absorbed to form a liquid; all values measured as weight increase at 80% RH and 25 °C).

Results

Figure 1B shows a representative DSC thermogram for ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone Form A recorded on heating from 0 to 220 °C. Exothermic events are plotted in the upward direction. The melting endotherm shown in **Figure 1B** has an onset temperature of about 207 °C and an enthalpy of approximately 133 J/g. The obtained melting temperatures can vary by as much as ± 5 °C depending upon the instrument used, how samples are prepared, and differences between batches. The absence of any thermal events detected in the DSC signal prior to the melting onset temperature indicates that ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone Form A is thermally stable in the temperature range 0 to 200 °C under these experimental conditions. The observed thermal stability range can be considered as advantageous for future formulation development.

Figure 2A shows a representative TGA thermogram for ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone Form A. Form A exhibited minimal weight loss (less than about 0.1 %) upon heating from room temperature (RT), approx. 25 °C, to 200 °C. Form A is thus confirmed as an anhydrous solid form of ((2R,6S)-2,6-dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone.

Figure 2B shows a representative GVS plot for ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone Form A recorded during two sorption/desorption cycles. Form A exhibited a reversible moisture uptake of < 0.05 mass % between 20 % relative humidity and 80 % relative humidity at 25 °C ± 0.1 °C during the first absorption/desorption cycle.

The desorption curve indicates that Form A lost moisture at a similar rate to the moisture gained during sorption, with limited hysteresis. No form change was observed by XRPD after the GVS experiment. According to the European Pharmacopoeia (EP) classification, Form A is therefore identified as a non-hygroscopic (i.e., < 0.2% weight increase) solid form of ((2R,6S)-2,6-

5 Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone.

The above description of illustrative embodiments is intended only to acquaint others skilled in the art with the Applicant's specification, its principles, and its practical application so that others skilled in the art may readily adapt and apply the specification in its numerous forms, as they may be best

10 suited to the requirements of a particular use. This description and its specific examples, while indicating embodiments of this specification, are intended for purposes of illustration only. This specification, therefore, is not limited to the illustrative embodiments described in this specification, and may be variously modified. In addition, it is to be appreciated that various features of the specification that are, for clarity reasons, described in the context of separate embodiments, also

15 may be combined to form a single embodiment. Conversely, various features of the specification that are, for brevity reasons, described in the context of a single embodiment, also may be combined to form sub-combinations thereof.

Claims

1. A crystalline form of ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone (Form A), which has an X-ray powder diffraction pattern with
5 specific peaks at 2-theta = 10.7 and 19.8° when measured using CuK α radiation, wherein said values may be plus or minus 0.2° 2-theta.
2. A crystalline form as claimed in claim 1, which has an X-ray powder diffraction pattern with specific peaks at 2-theta = 8.1, 10.7, 19.8, 24.9, and 27.6° when measured using CuK α radiation,
10 wherein said values may be plus or minus 0.2° 2-theta.
3. A crystalline form as claimed in claim 1 or claim 2, which has an X-ray powder diffraction pattern with specific peaks at 2-theta = 8.1, 10.7, 13.6, 15.4, 18.9, 19.8, 21.4, 23.1, 24.9, 27.6 and 31.2° when measured using CuK α radiation, wherein said values may be plus or minus 0.2° 2-theta.
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4. A crystalline form of ((2R,6S)-2,6-Dimethylmorpholino)(5-(2,4,5-trifluoro-3-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)methanone (Form A), which has an X-ray powder diffraction pattern substantially as shown in Figure 1A, when measured using CuK α radiation.
- 20 5. A pharmaceutical composition comprising a crystalline form as claimed in any one of claims 1 to 4, and a pharmaceutically acceptable excipient.
6. A crystalline form as claimed in any one of claims 1 to 4, for use in therapy.
- 25 7. A crystalline form as claimed in any one of claims 1 to 4, for use in the treatment of liver disease.
8. A crystalline form as claimed in any one of claims 1 to 4, for use in the treatment of a liver disease selected from alcoholic liver disease, non-alcoholic liver disease, NAFLD, NASH, liver fibrosis,

cirrhosis, isolated steatosis, liver inflammation, alcoholic steatohepatitis (ASH), hepatitis C virus (HCV) and hepatocellular carcinoma (HCC).

9. A crystalline form as claimed in any one of claims 1 to 4, for use in the treatment of NASH.

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10. A crystalline form as claimed in any one of claims 1 to 4, for use in the treatment of liver fibrosis.

11. A crystalline form as claimed in any one of claims 1 to 4, for use in the treatment of cirrhosis.

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12. A method of treating liver disease in a patient comprising administering to the patient a crystalline form as claimed in any one of claims 1 to 4.