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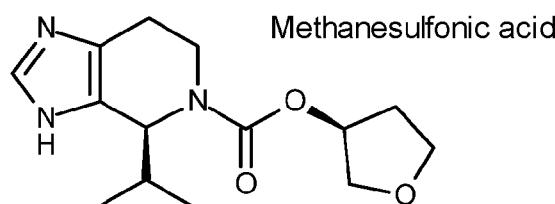
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(54) Title: CRYSTALLINE COMPOUND AS SEMICARBAZIDE-SENSITIVE AMINE OXIDASE (SSAO) ENZYME INHIBITOR



(I)

(57) Abstract: A specific crystalline mesylate salt form of (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate, and the use of the same in medicine. (Formula (I))

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## CRYSTALLINE COMPOUND AS SEMICARBAZIDE-SENSITIVE AMINE OXIDASE (SSAO) ENZYME INHIBITOR

### Field of the Invention

5 This invention relates to the crystalline mesylate salt form of (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate, to methods of production and isolation, to pharmaceutical compositions which include this compound and a pharmaceutically acceptable carrier, and to pharmaceutical methods of treatment.

10

### Background of the Invention

Semicarbazide-sensitive amine oxidase (SSAO) activity is an enzyme activity expressed by Vascular Adhesion Protein-1 (VAP-1) or Amine Oxidase, Copper Containing 3 (AOC3), belongs to the copper-containing amine oxidase 15 family of enzymes (EC.1.4.3.6). Therefore inhibitors of the SSAO enzyme may also modulate the biological functions of the VAP-1 protein.

SSAO activity has been found in a variety of tissues including vascular and non-vascular smooth muscle tissue, endothelium, and adipose tissue [Lewinsohn, *Braz. J. Med. Biol. Res.* **1984**, 17, 223-256; Nakos & Gossrau, *Folia Histochem. Cytobiol.* **1994**, 32, 3-10; Yu et al., *Biochem. Pharmacol.* **1994**, 47, 1055-1059; Castillo et al., *Neurochem. Int.* **1998**, 33, 415-423; Lyles & Pino, *J. Neural. Transm. Suppl.* **1998**, 52, 239-250; Jaakkola et al., *Am. J. Pathol.* **1999**, 155, 1953-1965; Morin et al., *J. Pharmacol. Exp. Ther.* **2001**, 297, 563-572; Salmi & Jalkanen, *Trends Immunol.* **2001**, 22, 211-216]. In addition, SSAO protein is found in blood 25 plasma and this soluble form appears to have similar properties as the tissue-bound form [Yu et al., *Biochem. Pharmacol.* **1994**, 47, 1055-1059; Kurkijärvi et al., *J. Immunol.* **1998**, 161, 1549-1557].

The precise physiological role of this abundant enzyme has yet to be fully determined, but it appears that SSAO and its reaction products may have several 30 functions in cell signalling and regulation. For example, recent findings suggest that SSAO plays a role in both GLUT4-mediated glucose uptake [Enrique-Tarancon et al., *J. Biol. Chem.* **1998**, 273, 8025-8032; Morin et al., *J. Pharmacol. Exp. Ther.* **2001**, 297, 563-572] and adipocyte differentiation [Fontana et al., *Biochem. J.* **2001**,

356, 769-777; Mercier et al., *Biochem. J.* **2001**, 358, 335-342]. In addition, SSAO has been shown to be involved in inflammatory processes where it acts as an adhesion protein for leukocytes [Salmi & Jalkanen, *Trends Immunol.* **2001**, 22, 211-216; Salmi & Jalkanen, in "Adhesion Molecules: Functions and Inhibition" K. Ley (Ed.), **2007**, pp. 237-251], and might also play a role in connective tissue matrix development and maintenance [Langford et al., *Cardiovasc. Toxicol.* **2002**, 2(2), 141-150; Göktürk et al., *Am. J. Pathol.* **2003**, 163(5), 1921-1928]. Moreover, a link between SSAO and angiogenesis has recently been discovered [Noda et al., *FASEB J.* **2008**, 22(8), 2928-2935], and based on this link it is expected that inhibitors of SSAO have an anti-angiogenic effect.

Several studies in humans have demonstrated that SSAO activity in blood plasma is elevated in conditions such as congestive heart failure, diabetes mellitus, Alzheimer's disease, and inflammation [Lewinsohn, *Braz. J. Med. Biol. Res.* **1984**, 17, 223-256; Boomsma et al., *Cardiovasc. Res.* **1997**, 33, 387-391; Ekblom, *Pharmacol. Res.* **1998**, 37, 87-92; Kurkijärvi et al., *J. Immunol.* **1998**, 161, 1549-1557; Boomsma et al., *Diabetologia* **1999**, 42, 233-237; Meszaros et al., *Eur. J. Drug Metab. Pharmacokinet.* **1999**, 24, 299-302; Yu et al., *Biochim. Biophys. Acta* **2003**, 1647(1-2), 193-199; Mátýus et al., *Curr. Med. Chem.* **2004**, 11(10), 1285-1298; O'Sullivan et al., *Neurotoxicology* **2004**, 25(1-2), 303-315; del Mar Hernandez et al., *Neurosci. Lett.* **2005**, 384(1-2), 183-187]. It has been suggested that reactive aldehydes and hydrogen peroxide produced by endogenous amine oxidases contribute to the progression of cardiovascular diseases, diabetic complications and Alzheimer's disease [Callingham et al., *Prog. Brain Res.* **1995**, 106, 305-321; Ekblom, *Pharmacol. Res.* **1998**, 37, 87-92; Yu et al., *Biochim. Biophys. Acta* **2003**, 1647(1-2), 193-199; Jiang et al., *Neuropathol Appl Neurobiol.* **2008**, 34(2), 194-204]. Furthermore, the enzymatic activity of SSAO is involved in the leukocyte extravasation process at sites of inflammation where SSAO has been shown to be strongly expressed on the vascular endothelium [Salmi et al., *Immunity* **2001**, 14(3), 265-276; Salmi & Jalkanen, in "Adhesion Molecules: Functions and Inhibition" K. Ley (Ed.), **2007**, pp. 237-251]. Accordingly, inhibition of SSAO has been suggested to have a therapeutic value in the prevention of diabetic complications and in inflammatory diseases [Ekblom, *Pharmacol. Res.* **1998**, 37, 87-92; Salmi et al.,

*Immunity* **2001**, 14(3), 265-276; Salter-Cid et al., *J. Pharmacol. Exp. Ther.* **2005**, 315(2), 553-562].

WO2007/146188 teaches that blocking SSAO activity inhibits leucocyte recruitment, reduces the inflammatory response, and is expected to be beneficial in 5 prevention and treatment of seizures, for example, in epilepsy.

O'Rourke et al (J Neural Transm. 2007;114(6):845-9) examined the potential of SSAO inhibitors in neurological diseases, having previously demonstrated the efficacy of SSAO inhibition in a rat model of stroke. An SSAO inhibitor is tested on 10 relapsing-remitting experimental autoimmune encephalomyelitis (EAE), a mouse model that shares many characteristics with human multiple sclerosis. The data demonstrates the potential clinical benefit of small molecule anti-SSAO therapy in this model and therefore in treatment of human multiple sclerosis.

SSAO knockout animals are phenotypically overtly normal but exhibit a marked decrease in the inflammatory responses evoked in response to various 15 inflammatory stimuli [Stolen et al., *Immunity* **2005**, 22(1), 105-115]. In addition, antagonism of its function in wild type animals in multiple animal models of human disease (e.g. carrageenan-induced paw inflammation, oxazolone-induced colitis, lipopolysaccharide-induced lung inflammation, collagen-induced arthritis, endotoxin-induced uveitis) by the use of antibodies and/or small molecules has been shown to 20 be protective in decreasing the leukocyte infiltration, reducing the severity of the disease phenotype and reducing levels of inflammatory cytokines and chemokines [Kirton et al., *Eur. J. Immunol.* **2005**, 35(11), 3119-3130; Salter-Cid et al., *J. Pharmacol. Exp. Ther.* **2005**, 315(2), 553-562; McDonald et al., *Annual Reports in Medicinal Chemistry* **2007**, 42, 229-243; Salmi & Jalkanen, in "Adhesion Molecules: 25 Functions and Inhibition" K. Ley (Ed.), **2007**, pp. 237-251; Noda et al., *FASEB J.* **2008** 22(4), 1094-1103; Noda et al., *FASEB J.* **2008**, 22(8), 2928-2935]. This anti-inflammatory protection seems to be afforded across a wide range of inflammatory 30 models all with independent causative mechanisms, rather than being restricted to one particular disease or disease model. This would suggest that SSAO may be a key nodal point for the regulation of the inflammatory response, and it is therefore likely that SSAO inhibitors will be effective anti-inflammatory drugs in a wide range of human and animal diseases. VAP-1 has also been implicated in the progression and maintenance of fibrotic diseases including those of the liver and lung. Weston

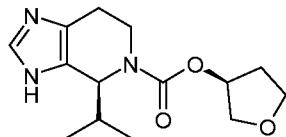
and Adams (J Neural Transm. **2011**, 118(7), 1055-64) have summarised the experimental data implicating VAP-1 in liver fibrosis, and Weston et al (EASL Poster 2010) reported that blockade of VAP-1 accelerated the resolution of carbon tetrachloride induced fibrosis. In addition VAP-1 has been implicated in 5 inflammation of the lung (e.g. Singh et al., **2003**, Virchows Arch 442:491-495) suggesting that VAP-1 blockers would reduce lung inflammation and thus be of benefit to the treatment of cystic fibrosis by treating both the pro-fibrotic and pro-inflammatory aspects of the disease.

SSAO (VAP-1) is up regulated in gastric cancer and has been identified in the 10 tumour vasculature of human melanoma, hepatoma and head and neck tumours (Yoong KF, McNab G, Hubscher SG, Adams DH. (1998), J Immunol **160**, 3978-88.; Irlala H, Salmi M, Alanen K, Grénman R, Jalkanen S (2001), Immunol. **166**, 6937-6943; Forster-Horvath C, Dome B, Paku S, et al. (2004), Melanoma Res. **14**, 135-40.). One report (Marttila-Ichihara F, Castermans K, Auvinen K, Oude Egbrink MG, 15 Jalkanen S, Griffioen AW, Salmi M. (2010), J Immunol. **184**, 3164-3173.) has shown that mice bearing enzymically inactive VAP-1 grow melanomas more slowly, and have reduced tumour blood vessel number and diameter. The reduced growth of these tumours was also reflected in the reduced (by 60-70%) infiltration of myeloid suppressor cells. Encouragingly VAP-1 deficiency had no effect on vessel or lymph 20 formation in normal tissue.

For the above reasons, it is expected that inhibition of SSAO will reduce the 25 levels of pro-inflammatory enzyme products (aldehydes, hydrogen peroxide and ammonia) whilst also decreasing the adhesive capacity of immune cells and correspondingly their activation and final extra-vasation. Diseases where such an activity is expected to be therapeutically beneficial include all diseases where immune cells play a prominent role in the initiation, maintenance or resolution of the pathology, such inflammatory diseases and immune/autoimmune diseases. Examples of such diseases include multiple sclerosis, arthritis and vasculitis.

An unmet medical need exists for new and improved inhibitors of SSAO. 30 WO2010/031789 (the content of which is herein incorporated by reference) discloses a promising class of SSAO inhibitor compounds, especially promising is Example 16, which is the free base of (3*S*)-Tetrahydrofuran-3-yl (4*S*)-4-isopropyl-

1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate, and has the following structure:



5 The free base of (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate is an hygroscopic amorphous glass/gum. There is a need to produce (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate in a pure and crystalline form to enable formulations to meet exacting pharmaceutical requirements and 10 specifications. It is desirable that the active ingredient is produced in a form which is amenable to large-scale production. It is desirable that the product is in a form that is readily filterable and easily dried. It is desirable also that the product is stable for extended periods of time without the need for specialised storage conditions.

15 Summary of the Invention

The applicant has discovered a crystalline form of the mesylate salt of (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate having surprisingly improved properties over the known free base. The improved properties include high thermal stability, ease of filtration, ease 20 of drying, and reduced hygroscopicity.

The mesylate salt (i.e the methanesulphonic acid salt) of (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate is shown to be highly crystalline by X-ray powder diffraction (XRPD) and polarised light microscopy (PLM). The improved thermal stability is 25 demonstrated by the high melting point of 189°C. The reduced hygroscopicity is demonstrated by the stability of the crystalline salt for up to three days storage at a temperature of 40°C in an environment having a relative humidity of 75%. The present invention includes a composition comprising the crystalline mesylate salt of (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate, and one or more pharmaceutically acceptable excipients.

The crystalline mesylate salt of (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate is expected to be useful in the treatment of inflammation, an inflammatory disease, an immune or an autoimmune disorder, or inhibition of tumour growth. In an embodiment the the

5 inflammation or inflammatory disease or immune or autoimmune disorder is arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis and psoriatic arthritis), synovitis, vasculitis, Sjogren's disease, a condition associated with inflammation of the bowel (including Crohn's disease, ulcerative colitis, inflammatory bowel disease and irritable bowel syndrome), atherosclerosis, multiple

10 sclerosis, Alzheimer's disease, vascular dementia, Parkinson's disease, cerebral amyloid angiopathy, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, a pulmonary inflammatory disease (including asthma, chronic obstructive pulmonary disease and acute respiratory distress syndrome), a fibrotic disease (including idiopathic pulmonary fibrosis, cardiac

15 fibrosis, liver fibrosis and systemic sclerosis (scleroderma)), an inflammatory disease of the skin (including contact dermatitis, atopic dermatitis and psoriasis), an inflammatory disease of the eye (including age related macular degeneration, uveitis and diabetic retinopathy), systemic inflammatory response syndrome, sepsis, an inflammatory and/or autoimmune condition of the liver (including autoimmune

20 hepatitis, primary biliary cirrhosis, alcoholic liver disease, sclerosing cholangitis, and autoimmune cholangitis), diabetes (type I or II) and/or the complications thereof, chronic heart failure, congestive heart failure, an ischemic disease (including stroke and ischemia-reperfusion injury) or myocardial infarction and/or the complications thereof, or epilepsy.

25 The present invention includes the use of the crystalline mesylate salt of (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate in the manufacture of a medicament for the treatment or prevention of the above-mentioned conditions and diseases. The invention also includes methods for treatment or prevention of such conditions and diseases,

30 comprising administering to a mammal, including man, in need of such treatment an effective amount of a compound as defined above.

Brief description of the drawings

**Figure 1** shows the XRPD of (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate free base.

5 **Figure 2** shows the XRPD of crystalline of (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate mesylate salt. The XRPD is run in duplicate.

10 Detailed Description of the Invention

Definitions

“Treatment” as used herein includes prophylaxis of the named disorder or condition, or amelioration or elimination of the disorder once it has been established.

15 “An effective amount” refers to an amount of a compound that confers a therapeutic effect on the treated subject. The therapeutic effect may be objective (i.e., measurable by some test or marker) or subjective (i.e., subject gives an indication of or feels an effect).

20 “Pharmaceutically acceptable” means being useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable and includes being useful for veterinary use as well as human pharmaceutical use.

25 Unless stated to the contrary, the term “(3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate” as used in connection with the crystalline salt form described herein includes a mixture of the (3S,4S) and (3R,4R) enantiomers. In an embodiment (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate, and salts thereof, has an absolute purity of >95%, preferably >99%, more preferably >99.5%. In an embodiment (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate means the (3S,4S) enantiomer having an enantiomeric purity of >95%, preferably >99%, more preferably >99.5%. In an embodiment (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-

imidazo[4,5-c]pyridine-5-carboxylate has a diastereoisomeric purity of >95%, preferably >99%, more preferably >99.5%.

### Compositions

5 For clinical use, the crystalline compound of the invention is formulated into pharmaceutical formulations for various modes of administration. It will be appreciated that the compound of the invention may be administered together with a physiologically acceptable carrier, excipient, or diluent. The pharmaceutical compositions of the invention may be administered by any suitable route, preferably 10 by oral, rectal, nasal, topical (including buccal and sublingual), sublingual, transdermal, intrathecal, transmucosal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration.

Other formulations may conveniently be presented in unit dosage form, e.g., tablets and sustained release capsules, and in liposomes, and may be prepared by 15 any methods well known in the art of pharmacy. Pharmaceutical formulations are usually prepared by mixing the active substance, or a pharmaceutically acceptable salt thereof, with conventional pharmaceutically acceptable carriers, diluents or excipients. Examples of excipients are water, gelatin, gum arabicum, lactose, microcrystalline cellulose, starch, sodium starch glycolate, calcium hydrogen 20 phosphate, magnesium stearate, talcum, colloidal silicon dioxide, and the like. Such formulations may also contain other pharmacologically active agents, and conventional additives, such as stabilizers, wetting agents, emulsifiers, flavouring agents, buffers, and the like. Usually, the amount of active compounds is between 0.1-95% by weight of the preparation, preferably between 0.2-20% by weight in 25 preparations for parenteral use and more preferably between 1-50% by weight in preparations for oral administration.

The formulations can be further prepared by known methods such as granulation, compression, microencapsulation, spray coating, etc. The formulations may be prepared by conventional methods in the dosage form of tablets, capsules, 30 granules, powders, syrups, suspensions, suppositories or injections. Liquid formulations may be prepared by dissolving or suspending the active substance in water or other suitable vehicles. Tablets and granules may be coated in a conventional manner. To maintain therapeutically effective plasma concentrations

for extended periods of time, the compound of the invention may be incorporated into slow release formulations.

The dose level and frequency of dosage of the specific compound will vary depending on a variety of factors including the potency of the specific compound  
5 employed, the metabolic stability and length of action of that compound, the patient's age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the condition to be treated, and the patient undergoing therapy. The daily dosage may, for example, range from about 0.001 mg to about 100 mg per kilo of body weight, administered  
10 singly or multiply in doses, e.g. from about 0.01 mg to about 25 mg each. A typical total daily dosage for a human is 1 to 2000mg/day, preferably from 200 to 2000mg/day, more preferably from 500 to 2000mg/day. Normally, such a dosage is given orally but parenteral administration may also be chosen.

15 Experimental Methods

*Analytical Methods*

*X-Ray Powder Diffraction (XRPD)*

X-Ray Powder Diffraction patterns were collected on a Bruker AXS C2 GADDS  
20 diffractometer using Cu K $\alpha$  radiation (40kV, 40mA), automated XYZ stage, laser video microscope for auto-sample positioning and a HiStar 2-dimensional area detector. X-ray optics consisted of a single Göbel multilayer mirror coupled with a pinhole collimator of 0.3mm. A weekly performance check is carried out using a certified standard NIST 1976 Corundum (flat plate). The beam divergence, i.e.  
25 the effective size of the X-ray beam on the sample, was approximately 4mm. A 0-0 continuous scan mode was employed with a sample to detector distance of 20cm which gives an effective 2 $\theta$  range of 3.2° - 29.7°. Typically the sample would be exposed to the X-ray beam for 120 seconds. The software used for data collection was GADDS for WNT 4.1.16 and the data were analysed and  
30 presented using Diffrac Plus EVA v 9.0.0.2 or v 13.0.0.2. Samples run under ambient conditions were prepared as flat plate specimens using powder as received without grinding. Approximately 1-2 mg of the sample was lightly pressed on a glass slide to obtain a flat surface. Samples run under non-

ambient conditions were mounted on a silicon wafer with heat conducting compound. The sample was then heated to the appropriate temperature at ca 10°C/min and subsequently held isothermally for ca 1min before data collection was initiated.

5 Alternatively, X-Ray Powder Diffraction patterns were collected on a Bruker D8 diffractometer using Cu K $\alpha$  radiation (40kV, 40mA),  $\theta$ -2 $\theta$  goniometer, and divergence of V4 and receiving slits, a Ge monochromator and a Lynxeye detector. The instrument is performance checked using a certified Corundum standard (NIST 1976). The software used for data collection was Diffrac Plus

10 XRD Commander v2.5.0 and the data were analysed and presented using Diffrac *Plus* EVA v 11.0.0.2 or v 13.0.0.2. Samples were run under ambient conditions as flat plate specimens using powder as received. Approximately 20mg of the sample was gently packed into a cavity cut into polished, zerobackground (510) silicon wafer. The sample was rotated in its own plane

15 during analysis. The details of the data collection are - angular range: 2 to 42° 2 $\theta$ ; step size: 0.05° 2 $\theta$ ; collection time: 0.5s/step.

#### *Nuclear Magnetic Resonance (NMR)*

<sup>1</sup>H NMR spectra were collected on a Bruker 400MHz instrument equipped with

20 an auto sampler and controlled by a DRX400 console. Automated experiments were acquired using ICONNMR v4.0.4 (build 1) running with Topspin v 1.3 (patch level 10) using the standard Bruker loaded experiments. For non-routine spectroscopy, data were acquired through the use of Topspin alone. Samples were prepared in *d*6-DMSO, unless otherwise stated. Off-line analysis was

25 carried out using ACD SpecManager v 12.00 (build 29094). Alternatively, <sup>1</sup>H NMR spectra were collected on a Bruker Avance III 400MHz QNP Ultrashield Plus Cryo.

#### *Liquid Chromatography-Mass Spectrometry (LCMS)*

30 Analytical LCMS was performed on an Agilent 1100 HPLC system with a Waters ZQ mass spectrometer using a Phenomenex Synergi column (RP-Hydro, 150 x

4.6mm, 4um, 1.5mL/min, 30°C, gradient 5-100% MeCN (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 0.5min, 200-300nm).

*Differential Scanning Calorimetry (DSC)*

5 DSC data were collected on a TA Instruments Q2000 equipped with a 50 position autosampler. The calibration for thermal capacity was carried out using sapphire and the calibration for energy and temperature was carried out using certified indium. Typically 0.5-3mg of each sample, in a pin-holed aluminium pan, was heated at 10°C/min from 25°C to 350°C. A purge of dry nitrogen at 50  
10 mL/min was maintained over the sample. Modulated temperature DSC was carried out using an underlying heating rate of 2°C/min and temperature modulation parameters of ± 1.27°C/min and 60 seconds. The instrument control software was Advantage for Q Series v2.8.0.392 and Thermal Advantage v4.8.3 and the data were analysed using Universal Analysis v4.3A.

15

*Thermo-Gravimetric Analysis (TGA)*

TGA data were collected on a TA Instruments Q500 TGA, equipped with a 16 position autosampler. The instrument was temperature calibrated using certified Alumel and Nickel. Typically 5-30mg of each sample was loaded onto a prepared platinum crucible and aluminium DSC pan, and was heated at 10°C/min from ambient temperature to 350°C. A nitrogen purge at 60mL/min was maintained over the sample. The instrument control software was Advantage for Q Series v2.8.0.392 and Thermal Advantage v4.8.3  
20

25 *Polarised Light Microscopy (PLM)*

Samples were studied on a Leica LM/DM polarised light microscope with a digital video camera for image capture. A small amount of each sample was placed on a glass slide, mounted in immersion oil and covered with a glass slip, the individual particles being separated as well as possible. The sample was viewed with appropriate magnification and partially polarised light, coupled to a lambda false-colour filter.  
30

*Hot Stage Microscopy (HSM) [melting point]*

Hot Stage Microscopy was carried out using a Leica LM/DM polarised light microscope combined with a Mettler-Toledo MTFP82HT hot-stage and a digital video camera for image capture. A small amount of each sample was placed 5 onto a glass slide with individual particles separated as well as possible. The sample was viewed with appropriate magnification and partially polarised light, coupled to a lambda false-colour filter, whilst being heated from ambient temperature typically at 10-20°C/min.

10 *Chemical Purity Determination by HPLC*

Purity analysis was performed on an Agilent HP1100 series system equipped with a diode array detector and using ChemStation software vB.02.01-SR1 using the method detailed below (Table 1).

Sample Preparation	0.5 mg/ml in acetonitrile : water 1:1		
Column	Supelco Ascentis Express C18, 100 x 4.6mm, 2.7µm		
Column Temperature (°C)	25		
Injection (µl)	5		
Wavelength, Bandwidth (nm)	255, 90 nm		
Flow Rate (ml·min <sup>-1</sup> )	2.0		
Phase A	0.1% TFA in water		
Phase B	0.085% TFA in acetonitrile		
	Time (min)	% Phase A	% Phase B
Timetable:	0	95	5
	6	5	95
	6.2	95	5
	8	95	5

15 **Table 1**

*Chiral Purity Determination by Chiral HPLC*

Chiral HPLC was performed on an Agilent 1200 system using an Astec Chirobiotic T 100 x 4.6mm 5µm column, polar reverse phase, 150 x 4.6mm, 20 5µm, isocratic 85% MeOH 15% 20mM ammonium acetate over 10min, 1.0mL/min, 220nm.

*Water Determination by Karl Fischer Titration (KF)*

The water content of each sample was measured on a Mettler Toledo DL39 Coulometer using Hydranal Coulomat AG reagent and an argon purge. Weighed solid samples were introduced into the vessel on a platinum TGA pan which was connected to a subseal to avoid water ingress. Approx 10mg of sample was 5 used per titration and duplicate determinations were made.

#### *Gravimetric Vapour Sorption (GVS)*

Sorption isotherms were obtained using a SMS DVS Intrinsic moisture sorption analyser, controlled by DVS Intrinsic Control software v1.0.0.30. The sample 10 temperature was maintained at 25°C by the instrument controls. The humidity was controlled by mixing streams of dry and wet nitrogen, with a total flow rate of 200mL/min. The relative humidity was measured by a calibrated Rotronic probe (dynamic range of 1.0-100 %RH), located near the sample. The weight change, (mass relaxation) of the sample as a function of %RH was constantly 15 monitored by the microbalance (accuracy  $\pm 0.005$ mg). Typically 5-20mg of sample was placed in a tared mesh stainless steel basket under ambient conditions. The sample was loaded and unloaded at 40%RH and 25°C (typical room conditions). A moisture sorption isotherm was performed as outlined below (2 scans giving 1 complete cycle). The standard isotherm was performed at 20 25°C at 10%RH intervals over a 0.5-90 %RH range. Data analysis was undertaken in Microsoft Excel using DVS Analysis Suite v6.0.0.7. Method parameters for SMS DVS intrinsic experiments: adsorption scan 1 40-90; desorption/adsorption scan 2 90-0, 0-40; intervals (%RH) 10; number of scans 4; flow rate (mL/min) 200; temperature (°C) 25; stability (°C/min) 0.2; sorption 25 time (h) 6h time out. The sample was recovered after completion of the isotherm and re-analysed by XRPD.

#### *Ion Chromatography (IC)*

Data were collected on a Metrohm 761 Compact IC (for cations) and a Metrohm 30 861 Advanced Compact IC (for anions) using IC Net software v2.3. Accurately weighed samples were prepared as stock solutions in an appropriate dissolving solution and diluted 1:9 prior to testing. Quantification was achieved by

comparison with standard solutions of known concentration of the ion being analysed. IC Method Parameters for Anion Chromatography: type of method - anion exchange; column - Metrosep A Supp 5-250 (4.0x250mm); column temperature (°C) ambient; injection (µl) 20; detection - conductivity detector; 5 flow rate (mL/min) 0.7; eluent 3.2mM sodium carbonate, 1.0mM sodium hydrogen carbonate in 5% aqueous acetone.

*Results*

Crystalline mesylate salt was isolated and characterised using some or all of XRPD, <sup>1</sup>H NMR, DSC, TGA, GVS, IC, PLM, HSM, HPLC and KF (see Table 2).

5 **Summary properties of (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate free base and crystalline mesylate salt**

Sample	Form	MP	Hygroscopicity
Free base	Amorphous gum	-	Deliquesced at 25°C/60%RH in <24h
Crystalline mesylate salt	Crystalline solid	189°C	Deliquesced at 40°C/75%RH after 3 days

Table 2

10 *Stability / hygroscopicity during long term storage*

Crystalline (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate mesylate was also assessed for stability and hygroscopicity when dispensed in 3 g aliquots in double LDPE liners, sealed with cable tie and placed, with a desiccant pouch into a foil bag, which was 15 subsequently heat-sealed. The foil bag was then placed into an HDPE keg fitted with an HDPE lid. These conditions mirror typical GMP level storage conditions. Stability was assessed by HPLC and hygroscopicity was assessed by Karl Fisher (KF) titration. Crystalline (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate mesylate degraded by only 20 0.1% with no water uptake over 3 years at 25°C/60%RH, and by only 0.1% with only 0.1% water uptake after 6 months at 40°C/75%RH.

*Single Crystal X-Ray Diffraction (SXRPD)*

Data were collected on an Oxford Diffraction Supernova Dual Source, Cu at 25 zero, Atlas CCD diffractometer equipped with an Oxford Cryosystems Cobra cooling device. The data was collected using CuK $\alpha$  radiation. Structures were

typically solved using either SHELXS or SHELXD programs and refined with the SHELXL program as part of the Bruker AXS SHELXTL suite. Unless otherwise stated, hydrogen atoms attached to carbon were placed geometrically and allowed to refine with a riding isotropic displacement parameter. Hydrogen atoms attached to a heteroatom were located in a difference Fourier synthesis and were allowed to refine freely with an isotropic displacement factor.

*Single Crystal Structure Determination*

10 A single crystal of (3S)-tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate mesylate was grown by slow evaporation from ethyl acetate / methanol.

The structure solution was obtained by direct methods, full-matrix least-squares refinement on  $F^2$  with weighting  $w^1 = \sigma^2(F_o^2) + (0.0490P)^2 + (0.3000P)$ , where  $P = (F_o^2 + 2F_c^2)/3$ , anisotropic displacement parameters. Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.

Absolute structure parameter = 0.002(13). Final  $wR^2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]^{1/2}\} = 0.0657$  for all data, conventional  $R_1 = 0.024$  on  $F$  values of 2651 reflections with  $F_o > 4\sigma(F_o)$ ,  $S = 1.007$  for all data and 234 parameters. Final  $\Delta/\sigma(\max) 0.000$ ,  $\Delta/\sigma(\text{mean}) 0.000$ .

Final difference map between +0.24 and -0.288 e Å<sup>-3</sup>.

25 The solved crystal structure parameters were as follows (Table 3):

Molecular formula	$C_{15}H_{25}N_3O_6S$			
Molecular weight	375.44			
Crystal system	Orthorhombic			
Space group	$P2(1)2(1)2(1)$	$a$	8.75780(10) Å	$\alpha$ 90°,
		$b$	9.70830(10) Å	$\beta$ 90°,
		$c$	20.6736(2) Å	$\gamma$ 90°
$V$	$1757.74(3) \text{ Å}^3$			
$Z$	4			
$D_c$	$1.419 \text{ g.cm}^{-3}$			
$\mu$	$1.975 \text{ mm}^{-1}$			
Source, $\lambda$	Cu-K $\alpha$ , 1.5418 Å			
$F(000)$	800			
$T$	100(2) K			
Crystal	Colourless block, 0.09 x 0.05 x 0.02 mm			
Data truncated to	0.80 Å			
$\theta_{\max}$	62.35°			
Completeness	99.2%			
Reflections	13291			
Unique reflections	2736			
$R_{\text{int}}$	0.0256			

**Table 3**

The atomic co-ordinates were as follows:

		x	y	z
5	S1	-0.953688	-0.131935	-0.071370
	O1	-0.020659	-0.149398	-0.484602
	O2	-0.234768	-0.155566	-0.365860
	O3	-0.474652	-0.066100	-0.365092
	O4	-0.912055	-0.134194	-0.140238
10	O5	-1.110792	-0.173740	-0.060477
	O6	-0.844708	-0.208722	-0.032208
	N1	-0.412770	-0.253710	-0.303870
	N2	-0.617297	-0.224842	-0.147335
	H2A	-0.722228	-0.196131	-0.146105
15	N3	-0.392648	-0.259773	-0.107928
	H3A	-0.324241	-0.261543	-0.081273
	C1	0.073273	-0.055487	-0.449929
	H1A	0.096656	0.026304	-0.476759
	H1B	0.170449	-0.099983	-0.437205
20	C2	-0.017615	-0.014548	-0.390676
	H2B	-0.004410	0.084643	-0.381074
	H2C	0.014557	-0.068772	-0.352479
	C3	-0.183226	-0.046385	-0.408629
	H3B	-0.249376	0.037232	-0.405075

	C4	-0.171565	-0.096071	-0.477738
	H4B	-0.248192	-0.168773	-0.486334
	H4C	-0.188739	-0.019057	-0.508271
	C5	-0.383218	-0.150898	-0.346637
5	C6	-0.560166	-0.254872	-0.270030
	H6B	-0.616936	-0.169106	-0.281832
	C7	-0.521333	-0.248342	-0.198878
	C8	-0.536619	-0.232888	-0.092882
10	H8A	-0.575742	-0.221293	-0.050370
	C9	-0.379700	-0.269963	-0.174418
	C10	-0.240769	-0.305362	-0.212417
	H10A	-0.184330	-0.381585	-0.191434
	H10B	-0.172218	-0.224586	-0.215739
15	C11	-0.296381	-0.348962	-0.279498
	H11A	-0.208892	-0.350915	-0.309809
	H11B	-0.339647	-0.443040	-0.277151
	C12	-0.659963	-0.380274	-0.288449
	H12A	-0.612652	-0.464685	-0.269297
20	C13	-0.664766	-0.397753	-0.361667
	H13A	-0.560447	-0.406134	-0.378360
	H13B	-0.714279	-0.317374	-0.381222
	H13C	-0.722663	-0.480993	-0.372510
	C14	-0.821261	-0.366359	-0.261740
25	H14A	-0.816850	-0.355260	-0.214661
	H14B	-0.880046	-0.449226	-0.272353
	H14C	-0.870697	-0.285637	-0.281016
	C15	-0.939731	0.041434	-0.046714
	H15A	-0.966523	0.048834	-0.000821
	H15C	-1.009980	0.097844	-0.072401
30	H15B	-0.834933	0.074003	-0.053214

#### X-Ray Powder Diffraction

The powder X-ray diffraction pattern of the mesylate salt of (3*S*)-Tetrahydrofuran-3-yl (4*S*)-4-isopropyl-1,4,6,7-tetrahydro-5*H*-imidazo[4,5-*c*]pyridine-5-carboxylate had peaks at 8.549, 9.851, 10.899, 12.295, 13.198, 13.393, 14.083, 15.897, 16.280, 17.097, 17.744, 18.289, 19.694, 20.180, 20.443, 20.597, 20.886, 21.948, 22.112, 22.444, 23.194, 23.653, 24.144, 24.714, 25.292, 25.590, 25.810, 26.526, 26.765, 27.084, 27.283, 27.662, 28.159, 28.996, 29.135, 29.912 and 30.868 degrees  $\theta\theta$ . To take into account normal experimental variation, the peaks identified above should be regarded as having an accuracy of up to  $\pm$  0.2 degrees  $\theta\theta$ , such as  $\pm$  0.1,  $\pm$  0.05,  $\pm$  0.01,  $\pm$  0.005, and  $\pm$  0.001. The relative intensities of these peaks were as follows (**Table 4**):

Angle (2-Theta °)	Intensity (%)	Angle (2-Theta °)	Intensity (%)
8.549	3.7	22.112	22.5
9.851	24.2	22.444	4.5
10.899	7.2	23.184	14.6
12.295	2.3	23.653	21.9
13.198	6.9	24.144	11.8
13.393	4.9	24.714	3.3
14.083	4.2	25.292	4.1
15.897	3.2	25.590	5.3
16.260	23.7	25.810	4.3
17.097	13.7	26.526	4.0
17.744	47.0	26.765	4.0
18.289	7.4	27.084	17.2
19.694	21.6	27.283	10.5
20.160	4.2	27.662	3.4
20.443	18.1	28.159	9.8
20.597	7.3	28.936	5.3
20.886	42.8	29.136	4.1
21.948	100.0	29.912	10.5
		30.866	5.0

**Table 4****Synthesis**

5 The following abbreviations have been used:

Aq	Aqueous
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
ee	Enantiomeric excess
10 ES <sup>+</sup>	Electrospray
EtOAc	Ethyl acetate
h	Hour(s)
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
15 LCMS	Liquid chromatography mass spectrometry
M	Molar
MeOH	Methanol
[MH <sup>+</sup> ]	Protonated molecular ion
min	Minutes
20 RP	Reverse phase
MS	Mass spectrometry

R <sub>T</sub>	Retention time
sat	Saturated
THF	Tetrahydrofuran
TFA	Trifluoroacetic acid

5

### *Experimental Methods*

All reagents were commercial grade and were used as received without further purification, unless otherwise specified. Reagent grade solvents were used in all cases.

10 Analytical LCMS was performed on a Waters ZQ mass spectrometer connected to an Agilent 1100 HPLC system. Analytical HPLC was performed on an Agilent 1100 system. High-resolution mass spectra (HRMS) were obtained on an Agilent MSD-TOF connected to an Agilent 1100 HPLC system. During the analyses the calibration was checked by two masses and automatically 15 corrected when needed. Spectra are acquired in positive electrospray mode. The acquired mass range was m/z 100-1100. Profile detection of the mass peaks was used. Flash chromatography was performed on either a CombiFlash Companion system equipped with RediSep silica columns or a Flash Master Personal system equipped with Strata Si-1 silica gigatubes. Reverse Phase 20 HPLC was performed on a Gilson system (Gilson 322 pump with Gilson 321 equilibration pump and Gilson 215 autosampler) equipped with Phenomenex Synergi Hydro RP 150 x 10 mm, YMC ODS-A 100/150 x 20 mm or Chirobiotic T 250 x 10 mm columns. Reverse phase column chromatography was performed on a Gilson system (Gilson 321 pump and Gilson FC204 fraction collector) 25 equipped with Merck LiChroprep® RP-18 (40-63 µm) silica columns. The compounds were automatically named using ACD 6.0. All compounds were dried in a vacuum oven overnight.

Analytical HPLC and LCMS data were obtained with:

30 System A: Phenomenex Synergi Hydro RP (C18, 30 x 4.6 mm, 4 µm), gradient 5-100% CH<sub>3</sub>CN (+0.085% TFA) in water (+0.1% TFA), 1.5 mL/min, with a gradient time of 1.75 min, 200 nm, 30 °C; or

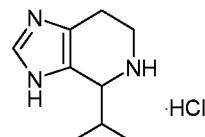
System B: Phenomenex Synergi Hydro RP (C18, 150 x 4.6 mm, 4  $\mu$ m), gradient 5-100% CH<sub>3</sub>CN (+0.085% TFA) in water (+0.1% TFA), 1.5 mL/min with a gradient time of 7 min, 200 nm, 30 °C.

Chiral HPLC data were obtained with:

5 System C: Chirobiotic V polar ionic mode (150 x 4.6 mm), 70% MeOH in 10 mM aq ammonium formate buffer, 1.0 mL/min, over 10 min, 200 nm, 30 °C.

## INTERMEDIATE 1

### 4-Isopropyl-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine hydrochloride



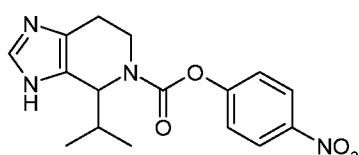
10

Histamine dihydrochloride (61.9 g, 336 mmol) was dissolved in a solution of NaOH (33.6 g, 841 mmol) in water (125 mL) and MeOH (500 mL), and isobutyraldehyde (61.4 mL, 672 mmol) was added. The reaction mixture was heated under reflux at 80 °C for 24 h, cooled to room temperature, the pH was adjusted to 7 with 1 M aq HCl solution (250 mL) and the solvents were removed *in vacuo*. The residue was dissolved in warm MeOH (300 mL), allowed to stand for 1h, filtered and the solvents were removed *in vacuo*. The residue was stirred in MeOH (50 mL) and acetone (400 mL) for 2 h and was cooled to 4 °C for 2 h. The resulting precipitate was filtered and washed with acetone (100 mL) to give 4-isopropyl-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine hydrochloride (33.0 g, 48.7%) as a white solid.

Analytical LCMS: purity >90% (System A, R<sub>T</sub> = 0.51 min), ES<sup>+</sup>: 166.4 [MH]<sup>+</sup>.

## INTERMEDIATE 2

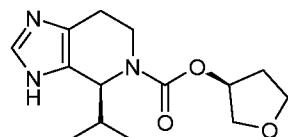
25 **4-Nitrophenyl 4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate**



Intermediate 1 (2.78 g, 8.28 mmol, 60% pure) and DIPEA (5.27 mL, 30.3 mmol) were dissolved in DCM (100 mL). The reaction mixture was cooled to 0 °C and 4-nitrophenyl chloroformate (4.07 g, 20.2 mmol) was added. The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was 5 washed with sat aq NaHCO<sub>3</sub> solution (5 x 100 mL), dried (MgSO<sub>4</sub>) and the solvents were removed *in vacuo* to give 4-nitrophenyl 4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate (5.28 g, crude) as a yellow gum.

Analytical HPLC: purity 41% (System B, R<sub>T</sub> = 4.70 min); Analytical LCMS: purity 10 86% (System A, R<sub>T</sub> = 1.70 min), ES<sup>+</sup>: 331.0 [MH]<sup>+</sup>.

**(3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate**



15 NaH (0.40 g, 10.0 mmol, 60% dispersion in mineral oil) was suspended in anhydrous THF (20 mL), cooled to 0 °C and (S)-3-hydroxytetrahydrofuran (0.88 g, 0.68 mL, 10.0 mmol) was added. The suspension was stirred at 0 °C for 30 min then added to a solution of Intermediate 2 (3.30 g, 10.0 mmol, 70% pure) in THF (60 mL) and the reaction mixture was stirred at room temperature. Two 20 additional such portions of NaH and (S)-3-hydroxytetrahydrofuran in THF were added after 5 and 29 h, respectively. After 2 d the reaction mixture was quenched with water (10 mL) and the solvents were removed *in vacuo*. The residue was dissolved in EtOAc (100 mL), washed with 1 M aq Na<sub>2</sub>CO<sub>3</sub> solution (4 x 100 mL), dried (MgSO<sub>4</sub>) and the solvents were removed *in vacuo*. The 25 residue was purified by column chromatography (normal phase, 20 g, Strata Si-1, silica gigatube, DCM (200 mL) followed by 2%, 4% and 5% MeOH in DCM (200 mL each)) and reverse phase HPLC (YMC ODS-A 100 x 20 mm, 5 µm, 25 mL/min, gradient 30% to 60% (over 7 min) then 100% (3 min) MeOH in 10% MeOH/water) to give (3S)-tetrahydrofuran-3-yl 4-isopropyl-1,4,6,7-tetrahydro-30 5H-imidazo[4,5-c]pyridine-5-carboxylate (34.8 mg, 1.1%) as a white solid.

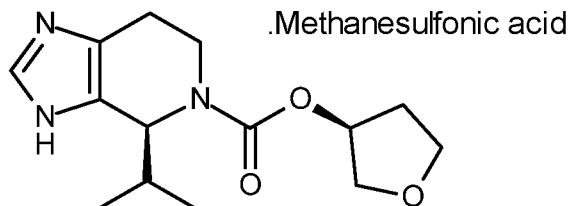
Analytical HPLC: purity 100% (System B,  $R_T$  = 3.63 min); Analytical LCMS: purity 100% (System B,  $R_T$  = 4.01 min),  $ES^+$ : 280.1  $[MH]^+$ .

(3S)-Tetrahydrofuran-3-yl-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate (39.91 mg) was dissolved in 10 mM ammonium formate buffer and MeOH (2 mL, 1:1) and purified twice by reverse phase chiral HPLC (Chirobiotic T 250 x 10 mm, 3 mL/min, isocratic run 70% MeOH in 10 mM ammonium formate buffer (40 min), pH 7.4) to give a single diastereoisomer, (3S)-tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate (6.90 mg, 99% ee).

Analytical HPLC: purity 100% (System B,  $R_T$  = 3.63 min); Chiral HPLC: purity 99.5% (System C,  $R_T$  = 2.22 min); Analytical LCMS: purity 100% (System B,  $R_T$  = 3.90 min),  $ES^+$ : 280.1  $[MH]^+$ ; HRMS calculated for  $C_{14}H_{21}N_3O_3$ : 279.1583, found 279.1571.

15

**(3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate, Methanesulfonic acid salt**



(3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate free base (460mg, 1.65mmol) was dissolved in EtOAc (10mL) at room temperature to give a clear colourless solution. Methanesulphonic acid (107uL) was added portion-wise with gentle heating. The solution was allowed to cool to room temperature overnight. The resulting crystals were collected by filtration, washed with EtOAc (2 x 10mL) and dried overnight at 40°C *in vacuo*. (3S)-Tetrahydrofuran-3-yl (4S)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate mesylate salt was obtained with a 99% yield (615mg) as a white crystalline solid. HPLC: Retention time 2.27min, purity 99.5%. Melting point: 189°C. LCMS: Retention time 4.19min,

ES<sup>+</sup> 280.0 [MH]<sup>+</sup>, 100% purity. Chiral HPLC: Retention time 3.70min, >99.5% de. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>): δ<sub>H</sub> 8.72 (1H, m, NHCHNH<sup>+</sup>), 5.29 (1H, m, OCH), 5.05 (0.5H, d, *J* 8.4Hz, CCHN), 4.89 (0.5H, d, *J* 7.6Hz, CCHN), 4.59 (0.5H, m, NCH<sub>A</sub>CH<sub>B</sub>), 4.39 (0.5H, m, NCH<sub>A</sub>CH<sub>B</sub>), 3.97-3.85 (4H, m, CH<sub>2</sub>OCH<sub>2</sub>), 3.20 (1H, m, NCH<sub>A</sub>CH<sub>B</sub>), 2.89 (3H, s, CH<sub>3</sub>SO<sub>3</sub><sup>-</sup>), 2.89-2.72 (2H, m, CCH<sub>2</sub>CH<sub>2</sub>N), 2.23-2.07 (3H, m, CH(CH<sub>3</sub>)<sub>2</sub>, OCH<sub>2</sub>CH<sub>2</sub>), 1.16 (3H, d, *J* 6.4Hz, CH<sub>3</sub>) and 1.06-0.96 (3H, m, CH<sub>3</sub>).

## CLAIMS

1. Crystalline (3*S*)-Tetrahydrofuran-3-yl (4*S*)-4-isopropyl-1,4,6,7-tetrahydro-5*H*-imidazo[4,5-*c*]pyridine-5-carboxylate mesylate salt having the space group 5 *P*2(1)2(1)2(1), and unit cell dimensions substantially as follows:

<i>a</i>	8.75780(10)Å	$\alpha$	90°
<i>b</i>	9.70830(10)Å,	$\beta$	90°,
<i>c</i>	20.6736(2)Å,	$\gamma$	90°,

2. Crystalline (3*S*)-Tetrahydrofuran-3-yl (4*S*)-4-isopropyl-1,4,6,7-tetrahydro-5*H*-imidazo[4,5-*c*]pyridine-5-carboxylate mesylate salt having an XRPD pattern containing the following 2 $\theta$  value measured using CuK $\alpha$  radiation: 21.948.

10

3. The salt according to claim 2 having an XRPD pattern containing the following 2 $\theta$  values measured using CuK $\alpha$  radiation: 17.744 and 21.948.

15

4. The salt according to claim 2 or claim 3 having an XRPD pattern containing the following 2 $\theta$  values measured using CuK $\alpha$  radiation: 17.744, 20.886, 21.948.

5. The salt according to any one of claims 2 to 4 having an XRPD pattern containing the following 2 $\theta$  values measured using CuK $\alpha$  radiation: 17.744, 20.886, 21.948, and 9.851.

20

6. The salt according to any one of claims 2 to 5 having an XRPD pattern containing the following 2 $\theta$  values measured using CuK $\alpha$  radiation: 17.744, 20.886, 21.948, 9.851 and 16.280.

25

7. The salt according to any one of claims 2 to 6 having an XRPD pattern containing the following 2 $\theta$  values measured using CuK $\alpha$  radiation: 9.851, 16.280, 17.097, 17.744, 19.694, 20.443, 20.886, 21.948, 22.112, 23.194, 23.653, 24.144, 27.084, 27.283 and 29.912.

8. The salt according to any one of claims 2 to 7 having an XRPD pattern as set out in Table 4 and/or Figure 2.
9. A salt according to any one of claims 1 to 8 having a purity of greater than 5 95%.
10. A salt according to any one of claims 1 to 8 having a purity of greater than 99%.
- 10 11. A salt according to any one of claims 1 to 8 having a purity of greater than 99.5%.
12. A salt according to any one of claims 1 to 11 having an enantiomeric purity of greater than 95%.  
15
13. A salt according to any one of claims 1 to 11 having an enantiomeric purity of greater than 99%.
14. A salt according to any one of claims 1 to 11 having an enantiomeric purity 20 of greater than 99.5%.
15. A pharmaceutical composition comprising a salt according to an one of claims 1 to 14, and one or more suitable excipients.  
25
16. A salt according to any one of claims 1 to 14 or a pharmaceutical composition according to claim 15 for use in the treatment of, or in the manufacture of a medicament for treatment of, inflammation, an inflammatory disease, an immune or an autoimmune disorder, or inhibition of tumour growth.
- 30 17. A method for the treatment of inflammation, an inflammatory disease, an immune or an autoimmune disorder, or inhibition of tumour growth, which comprises administering to a subject suffering such disease an effective amount

of a salt as claimed in any one of claims 1 to 14 or a pharmaceutical composition as claimed in claim 15.

18. A salt according to claim 16 or a method according to claim 17 wherein

5 the inflammation or inflammatory disease or immune or autoimmune disorder is arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis and psoriatic arthritis), synovitis, vasculitis, Sjogren's disease, a condition associated with inflammation of the bowel (including Crohn's disease, ulcerative colitis, inflammatory bowel disease and irritable bowel syndrome),

10 atherosclerosis, multiple sclerosis, Alzheimer's disease, vascular dementia, Parkinson's disease, cerebral amyloid angiopathy, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, a pulmonary inflammatory disease (including asthma, chronic obstructive pulmonary disease and acute respiratory distress syndrome), a fibrotic disease (including idiopathic

15 pulmonary fibrosis, cardiac fibrosis, liver fibrosis and systemic sclerosis (scleroderma)), an inflammatory disease of the skin (including contact dermatitis, atopic dermatitis and psoriasis), an inflammatory disease of the eye (including age related macular degeneration, uveitis and diabetic retinopathy), systemic inflammatory response syndrome, sepsis, an inflammatory and/or

20 autoimmune condition of the liver (including autoimmune hepatitis, primary biliary cirrhosis, alcoholic liver disease, sclerosing cholangitis, and autoimmune cholangitis), diabetes (type I or II) and/or the complications thereof, chronic heart failure, congestive heart failure, an ischemic disease (including stroke and ischemia-reperfusion injury) or myocardial infarction and/or the complications

25 thereof, or epilepsy.

19. A salt according to claim 16 or method according to claim 17 for treatment of a disease selected from rheumatoid arthritis, osteoarthritis, liver fibrosis, chronic obstructive pulmonary disease, multiple sclerosis, Sjogren's

30 disease, Alzheimer's disease, Parkinson's disease, inflammatory bowel disease, or vascular dementia.

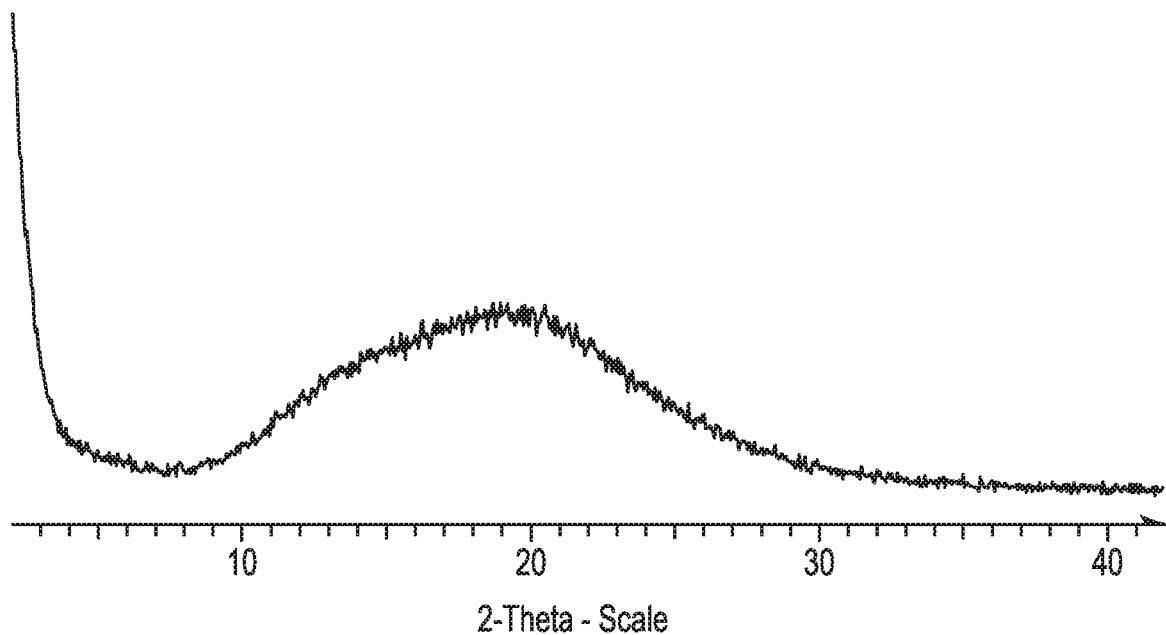
20. A process of making crystalline (3*S*)-Tetrahydrofuran-3-yl (4*S*)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate mesylate salt by forming the mesylate salt from the free base of (3*S*)-Tetrahydrofuran-3-yl (4*S*)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate.

5

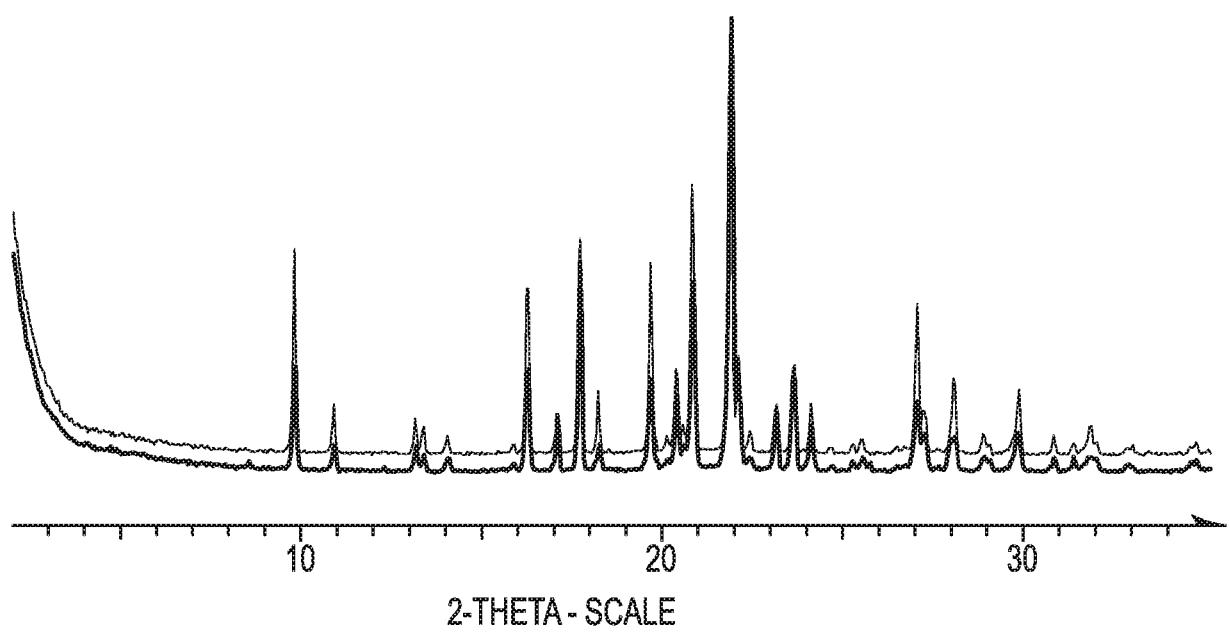
21. A process according to claim 20 wherein the mesylate salt is formed by addition of methanesulphonic acid to the free base.

22. Crystalline (3*S*)-Tetrahydrofuran-3-yl (4*S*)-4-isopropyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5-carboxylate mesylate obtained by the process according to claim 20 or claim 21.

1/1



**Figure 1**



**Figure 2**

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/GB2016/051120

**A. CLASSIFICATION OF SUBJECT MATTER**  
INV. C07D471/04 A61K31/437 A61P29/00 A61P35/00 A61P37/00  
ADD.

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)  
C07D

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

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

EPO-Internal, CHEM ABS Data, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/031789 A1 (BIOVITRUM AB PUBL [SE]; SAVORY EDWARD [GB]; HIGGINBOTTOM MICHAEL [GB];) 25 March 2010 (2010-03-25) cited in the application page 15, line 27 - page 16, line 4; claims 4,6,8,9; example 16 -----	1-22
A	YU L ED - MATTOUSSI HEDI ET AL: "AMORPHOUS PHARMACEUTICAL SOLIDS: PREPARATION, CHARACTERIZATION AND STABILIZATION", ADVANCED DRUG DELIVERY REVIEWS, ELSEVIER, AMSTERDAM, NL, vol. 48, no. 1, 16 May 2001 (2001-05-16), pages 27-42, XP009065056, ISSN: 0169-409X, DOI: 10.1016/S0169-409X(01)00098-9 1. introduction, p.28 ----- -/-	1-14

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search  23 May 2016	Date of mailing of the international search report  03/06/2016
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  Schuemacher, Anne

## INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/051120

## C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CAIRA M R: "CRYSTALLINE POLYMORPHISM OF ORGANIC COMPOUNDS", TOPICS IN CURRENT CHEMISTRY, SPRINGER, BERLIN, DE, vol. 198, 1 January 1998 (1998-01-01), pages 163-208, XP001156954, ISSN: 0340-1022, DOI: 10.1007/3-540-69178-2_5 p.165-166; chapter 3.1 -----	1-14
A	BASTIN R J ET AL: "Salt Selection and Optimisation for Pharmaceutical New Chemical Entities", ORGANIC PROCESS RESEARCH AND DEVELOPMENT, CAMBRIDGE, GB, vol. 4, no. 5, 1 January 2000 (2000-01-01) , pages 427-435, XP008154792, DOI: 10.1021/OP000018U abstract -----	1-14

**INTERNATIONAL SEARCH REPORT**

## Information on patent family members

International application No  
PCT/GB2016/051120

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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			BR PI0918602 A2	15-12-2015
			CA 2737527 A1	25-03-2010
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			EA 201100391 A1	31-10-2011
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			US 2014121219 A1	01-05-2014
			US 2015218161 A1	06-08-2015
			WO 2010031789 A1	25-03-2010

## 摘要

(4S)-4-异丙基-1, 4, 6, 7-四氢-5H-咪唑并[4, 5-c]吡啶-5-羧酸  
(3S)-四氢呋喃-3-基酯的特定结晶甲磺酸盐形式, 及其在医学中的用  
途。 (式(I))