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(54) Benævnelse: **HIDTIL UKENDTE SALTE AF DPP-IV-INHIBITOR**

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EP-A1- 2 019 113
WO-A1-03/004498
WO-A1-2005/072530
WO-A1-2013/001457
WO-A2-2009/085990
WO-A2-2010/004231
WO-A2-2010/012781
WO-A2-2010/117738
"SITAGLIPTIN SALTS, PROCESSES FOR THEIR PREPARATION AND THEIR USE FOR THE PREPARATION OF PHARMACEUTICAL DOSAGE FORMS", IP.COM JOURNAL, IP.COM INC., WEST HENRIETTA, NY, US, 21

Fortsættes ...

October 2010 (2010-10-21), XP013143473, ISSN: 1533-0001
KIM B H ET AL: "Pharmacodynamic (Hemodynamic) and pharmacokinetic comparisons of S-amlodipine gentisate and racemate amlodipine besylate in healthy Korean male volunteers: Two double-blind, randomized, two-period, two-treatment, two-sequence, double-dummy, single-dose crossover studies", CLINICAL THERAPEUTICS, EXCERPTA MEDICA, PRINCETON, NJ, US, vol. 32, no. 1, 1 January 2010 (2010-01-01), pages 193-205, XP026908537, ISSN: 0149-2918, DOI: 10.1016/J.CLINTHERA.2010.01.017 [retrieved on 2010-02-08]
LEE H Y ET AL: "Clinic blood pressure responses to two amlodipine salt formulations, adipate and besylate, in adult Korean patients with mild to moderate hypertension: A multicenter, randomized, double-blind, parallel-group, 8-week comparison", CLINICAL THERAPEUTICS, EXCERPTA MEDICA, PRINCETON, NJ, US, vol. 27, no. 6, 1 June 2005 (2005-06-01), pages 728-739, XP027641221, ISSN: 0149-2918 [retrieved on 2005-06-01]

DESCRIPTION

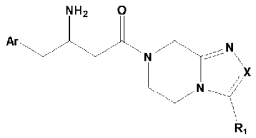
FIELD OF INVENTION

[0001] The present invention discloses certain salts of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine as well as their processes of preparation.

BACKGROUND OF THE ART

[0002] The invention is related to a novel crystalline form of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine besylate .

[0003] WO 03/004498 and US patent No. 6,699,871 both assigned to Merck and Co., describes a class of beta-amino tetrahydrotriazolo[4,3-a]pyrazines, which are inhibitors of DPP-IV. Disclosed therein are compounds, whose general formula is,



[0004] Specifically disclosed in WO 03/004498 is (2R)-4-oxo-4-[3-(trifluoromethyl)-5, 6-dihydro [1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine. WO 03/004498 is silent as to the preparation of and the nature of specific crystal forms of the salts. WO 2005/003135 describes dihydrogenphosphate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine and crystalline hydrates thereof, in particular a crystalline monohydrate.

[0005] In WO 2005/003135 it is said that (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine dihydrogenphosphate salt and crystalline hydrates have advantages in the preparation of pharmaceutical compositions, such as ease of processing, handling, and dosing. In particular, they exhibit improved physical and chemical stability, such as stability to stress, high temperatures and humidity, as well as improved physicochemical properties, such as solubility and rate of dissolution. WO2005/020920 describes the crystalline anhydrate Form I, Form II and Form III as well as solvates of the (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine dihydrogenphosphate salt.

[0006] WO2005/030127 describes novel crystalline anhydrate Form IV of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine dihydrogenphosphate salt.

[0007] WO2006/033848 describes the amorphous form of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine dihydrogenphosphate salt.

[0008] WO2005/072530 describes crystalline hydrochloric acid, benzenesulfonic acid, p- toluenesulfonic acid, 10-camphorsulfonic acid, and tartaric acid salts of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine and hydrates thereof.

[0009] WO2007/035198 describes (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine dodecylsulfate salt, in particular, a crystalline anhydrate form thereof.

[0010] In addition, the hemifumarate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine has been described by D. Kim et al. in J. Med. Chem. 2005, 48, 141-151. Furthermore, International Patent Publication No. WO 2008/000418 discloses the preparation of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine hydrochloride in amorphous form.

[0011] In addition, International Patent Publication No. WO 2009/085990 describes other acid addition salts of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, including salts of di-p-

tolyl-L-tartaric acid, phosphoric acid, sulfuric acid, hydrobromic acid, methanesulfonic acid, acetic acid, benzoic acid, oxalic acid, succinic acid, mandelic acid, fumaric acid, and lactic acid.

[0012] Patent Publication No. WO 2010/00469 describes different crystalline form of salts of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine such as hydrochloride, succinate, lactate, maleate, citrate and mesylate.

[0013] International Patent Publication No. WO 2010/092090 describes other acid addition salts of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, i.e. salts of D-glucuronic acid, L-glucuronic acid, glutaric acid, lactic acid, L-mandelic acid, D-mandelic acid and sulfuric acid.

[0014] International Patent Publication No. WO 2010/012781 describes other acid addition salts of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, *i.e,*. salts of ethanedisulfonic acid, galactaric acid, thiocynic acid, and glutaric acid.

[0015] International Patent Publication No. WO 2010/117738 disclosed different crystalline forms of acetate, oxalate and fumarate of compound of formula I. International Patent Publication No. WO 2012/007455 disclosed orotate salt of compound of formula I.

[0016] Different salts and new crystalline forms of the same pharmaceutically active moiety differ in their physical properties such as melting point, solubility, etc. These properties may appreciably influence pharmaceutical properties such as dissolution rate and bioavailability which in turn may have an impact on their efficacy. Thus there is a continuing need to obtain new forms of salts of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine having improved physical and/or chemical properties. The present invention satisfies this need.

[0017] In view of the foregoing, it would be desirable to provide new crystalline forms of salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

[0018] We herein disclose new crystalline salt forms of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine besylate which have not been disclosed in prior art.

[0019] These new crystalline salt forms can show certain superior pharmaceutical properties compared to (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020]

FIG. 1 is a powder X-ray diffraction (XRPD) pattern of crystalline gentisate salt of 2(R) 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

FIG. 2 is a powder X-ray diffraction (XRPD) pattern of amorphous gentisate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

FIG. 3 is a powder X-ray diffraction (XRPD) pattern of crystalline adipate salt of 2(R) 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

FIG. 4 is a powder X-ray diffraction (XRPD) pattern of crystalline hydrochloride salt of 2(R) 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

FIG. 5 is a powder X-ray diffraction (XRPD) pattern of crystalline besylate salt (I)of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

FIG. 6 is a powder X-ray diffraction (XRPD) pattern of crystalline trifluoroacetic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

FIG. 7 is a powder X-ray diffraction (XRPD) pattern of crystalline besylate salt(II) of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine according to the present invention.

FIG. 8 is a powder X-ray diffraction (XRPD) pattern of amorphous besylate salt of 2(R) 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

FIG. 9- Effect of single dose oral administration of ZY compounds at 10 mg/kg, PO on OGTT in male C57 mice.

FIG. 10- Effect of single dose oral administration of ZY compounds at 10mg/kg, PO on OGTT in male C57 mice.

OBJECT OF THE INVENTION

[0021] The present invention provides a new crystalline besylate salt(II) of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, which can be characterized by its powder X-ray diffraction (PXRD) pattern and other characteristic properties as described hereinafter and process for preparation thereof.

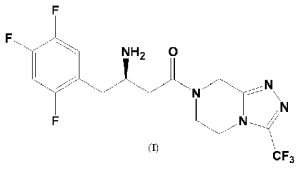
DETAILED DESCRIPTION OF THE INVENTION

[0022] As used herein, the term "reflux temperature" refers to the boiling point of the solvent used.

[0023] As used herein, the term "PXRD" refers to powder X-ray diffraction.

[0024] As used herein, the term " (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine refers to Sitagliptin.

[0025] As used herein, the term "THF" refers to tetrahydrofuran, the term "DCM" refers to dichloro methane, the term "DIPE" refers to di-isopropyl ether, the term "MTBE" refers to methyl t-butyl ether, the term "IPA" refers to isopropyl alcohol, the term "IPAc" refers to isopropyl acetatel, the term "phosphate" refers to dihydrogen phosphate salt, the term "besylate" refers to benzenesulphonic acid salt (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine is having structural formula (I).



(I)

[0026] In an embodiment is provided the crystalline form II of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine besylate having characteristic powder X-ray diffraction pattern having peaks expressed as 2θ at 5.69, 7.45, 10.08, 11.41, 13.09, 18.34, 22.52 and 22.97 ± 0.2 degree two theta.

[0027] Anhydrous (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine required for the preparation of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine besylate salt(II), can be synthesized by processes as mentioned in Example 18 and 19.

[0028] Another aspect of the invention relates to a process for preparing the crystalline form II of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine besylate as above comprising

1. (a) reaction of the free base (formula I) under suitable reaction conditions with the benzenesulfonic acid in suitable solvent selected from a mixture of esters and water, mixture of esters, ethers and water, mixture of esters, alcohols and water, mixture of ester, hydrocarbons and water, mixture of esters, ketones and water;
2. (b) suitably removing the solvent to obtain the crystalline form II of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine besylate.

[0029] The suitable alcohols used is selected from methanol, ethanol, isopropanol, butanol, 1,2-dimethoxy ethanol, 2-methoxy ethanol, 2-ethoxy ethanol, ethylene glycol, hexanol, heptanol, octanol, decanol and the like; esters used is selected from ethyl acetate, isopropyl acetate and the like; chlorinated solvents used is selected from chloroform, dicholomethane and the like; hydrocarbons used is selected from hexane, heptanes, cyclohexane, toluene, xylene, cholobenzene and the like; ketones used is selected from acetone and the like; ethers used is selected from diethyl ether, 1,4-dioxane, DIPE, MTBE, THF and the like.

[0030] The crystalline form II is further characterised by an additional XPRD peaks at about 16.63, 20.82, 21.95, 23.75, 24.49, 25.02, 26.43, 27.66 and 30.19 ± 0.2° degrees 2θ. Fig. 7 illustrate the XRD of crystalline form II of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine besylate salt.

[0031] Anhydrous (2R)-4-oxo-[3-(trifluoromethyl)-5,6-dihydro[1,2,3]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine required for the preparation of amorphous form of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine besylate salt can be synthesised by processes as mentioned in examples 18 and 19.

[0032] The above classes of solvents known to a person skilled in the art are all contemplated without limitation. The organic solvent acceptable for the practice of the process described herein preferably provide sufficient solubility for the active substance, and do not cause any undesirable chemical reactions with the besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, or (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, such as degradation or side reaction, under the conditions of processing.

[0033] The recovering step b) may involve removing the solvent by distillation or by filteration.

[0034] It is generally preferred that rapid drying is utilized to provide the amorphous form of besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine with desired stability, moisture content and residual solvent characteristics. The resultant product may be dried using any methods of drying including spray drying, rotational evaporation (such as using a Buchi Rotavapor), agitated thin film drying, spin-flash drying, fluid-bed drying, lyophilization, or other techniques known in the art.

[0035] The process may also include further drying of the product obtained from the solution by vacuum drying over a desiccant, such as phosphorous pentoxide (P_2O_5). The product can also be obtained with other suitable drying agents such as potassium carbonate (K_2CO_3), sodium carbonate (Na_2CO_3), silica gel and the like, as will be apparent to the skilled artisan.

[0036] Fig. 8 illustrates the XRD of an amorphous form of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine besylate salt.

Analytical methods:

[0037] The complete x-ray powder spectrum, which was recorded with a Rigaku multifelx 2.0 Kilowatt X-ray powder diffractometer model using copper radiation. The X-ray diffraction pattern was recorded by keeping the instrument parameters listed below:

i) X-ray: Cu/40kv/30mA, Diverging slit: 1^o, Scattering slit: 1^o, Receiving slit: 0.15mm, Monochromator RS: 0.8 mm, Counter: Scintillation counters;

Scan mode: Continuous, Scan speed : 4.000 deg./min., Sampling width : 0.010^o, Scan axes : 2 theta vs CPS, Scan range : 4^o to 40.0^o, Theta offset: 0.000.

ii) Differential scanning calorimetric analysis was carried out in a DSC-60 model from Shimadzu (S/W: TA-60WS Aquisition version 2.1.0.0) by keeping following parameters,
Sample Size: Approx. 1-2mg, Sample Pans: Hermetic/Crimping Pans,
Start Temperature: 50°C, End Temperature: 300°C, Rate of Heating: 10 °C/min., Purge Gas: Nitrogen, Flow rate: 20 ml/min

iii) The infrared (IR) spectrum has been recorded on a Shimadzu FTIR-8400 model spectrophotometer, between 450 cm^{-1} and 4000 cm^{-1}, with a resolution of 4 cm^{-1} in a KBr pellet.

iv) (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine and its salts were analyzed for purity by analytical HPLC at λmax 210nm using column YMC-C8, 250 mm x 4.6mm x 4 mm or its equivalent on AGILENT 1100 series under the following conditions, Detector: UV absorption photometer Wave length: 210 nm Column temp. : 25 °C Flow rate: 1.0 mL/min. Injection Vol.: 10 µL
Mobile Phase: 10mM KH_2PO_4 (pH-6.8): Acetonitrile (55:45)

(2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine and its salts were analyzed for chiral purity by HPLC at λmax 268 nm using column Chiral-Cel OJ-H, 250mm x 4.6mm x 5µ or its equivalent on Shimadzu LCVP model under the following conditions, Detector: UV absorption photometer Wave length: 268 nm Column temp. : 35 °C Flow rate: 0.8 mL/min. Injection Vol.: 10 µL
Mobile Phase: 0.1 % diethyl amine in [n-Hexane: Ethanol (90:10)]

v) Melting points were taken on VEEGO make model VMP-D melting point apparatus and are uncorrected.

[0038] Crystalline form II of besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine may also be useful as a therapeutic agent for treatment of certain disorders.

[0039] Crystalline form II of besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine prepared according to the present invention has chemical and polymorphic stability on storage at 25 °C ± 2 °C / 60% ± 5% RH for 3 months. Results of the stability of crystalline form II of besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine are shown in table 1.

Table-1: STABILITY STUDY ON STORAGE (25 °C ± 2 °C / 60%± 5%RH)

Salt	**Test**		**Initial**	**After 3 Month**
besylate crystalline(II)	Description		white colored powder	Off white colored powder
	Purity (By HPLC)		99.87 %	99.97 %
	Water (by KFR)		2.77 %	2.90 %
	Polymorphism	XRD	Crystalline pattern	No Change

[0040] It is to be noted that WO03004498 does not provide any biological activity data of the (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine. The crystalline form II of besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine of the present invention is derived from (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, and therefore, we are providing comparison of the crystalline form II of besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine with crystalline monohydrate form of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine phosphate salt and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

[0041] Comparative efficacy study of (i) (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine gentisate (ii) crystalline form of the besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (iiii) amorphous besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine and vs. crystalline monohydrate form of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine phosphate salt and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (SB):

Test material particulars:

[0042]

1. (i) (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine gentisate (SG).
2. (ii) crystalline besylate salt(II) of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-

trifluorophenyl)butan-2-amine (SB_{II}).

3. (iii) amorphous besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (SBa).
4. (iv) Crystalline monohydrate form of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine phosphate salt (SP).
5. (v) (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin 7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (SB):

Study Protocol:

[0043] Eight to ten weeks old male C57BLKS/J mice obtained from Animal Research Facility of Zydus Research Centre were used. The mice were allowed ad libitum access to chow and water. Animals were on a 12-hour light, 12-hour dark cycle. Protocol for use of animals for conducting this study has been reviewed and approved by Institutional Animal Ethics Committee (IAEC).

[0044] Oral glucose tolerance test (OGTT) was performed on these animals. The mice were deprived of food for 18 h and then drug or vehicles were administered with a glucose load (3 g/kg) by oral route. Blood (200 µL) samples were collected at 0, 30, 60, 120 and 240 min. Serum glucose concentrations were determined using commercially available kits purchased from RFCL (New Delhi, India) using spectrophotometric assay. The glucose values were then analyzed using GraphPad Prism version 5.04 for Windows; GraphPad Software, San Diego, CA, USA. The area under the curve for 120 min was calculated, based on which the percentage inhibition was obtained for each treatment.

Results:

[0045]

Group	% change in AUC glucose
Vehicle control	-
(SB_{II}) (10mg/kg)	-45.7±1.5
(SB) (10mg/kg)	-36.5±1.6
(SG) (10mg/kg)	-39.4 ±1.8
(SBa) (10mg/kg)	-43.3±1.6
(SP) (10mg/kg)	-31.9±1.8

[0046] The results indicate that crystalline besylate salt(II) of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (SB_{II}) and amorphous besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (SBa) are better than crystalline monohydrate form of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine phosphate salt (SP) when tested in the acute oral glucose tolerance test in C57 mice.

[0047] Therefore, the present crystalline form II of besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine have potentially significant therapeutic advantages compared to the (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (SB) from which it is derived and also better than marketed crystalline monohydrate form of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine phosphate salt (SP).

[0048] The invention is further exemplified by the following non-limiting examples, which are illustrative representing the preferred modes of carrying out the invention. The invention's scope is not limited to these specific embodiments only but is defined by the claims.

Reference Example 1

Preparation of adipic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine

[0049] In a 25 mL three neck flask ethanol (10.0 mL) and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (2.0 g) were taken. It was heated to 68-70 °C and adipic acid (0.717 g) was added. The reaction mixture was stirred for 2 hrs at 78-80 °C, gradually cooled to 25-30 °C and again stirred for 2 to 3 hrs. Solid salt precipitated out. The salt was filtered and washed with ethanol which provided the adipic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine was obtained (Wt.: 1.54 g, % Yield: 56.8 %, Purity by HPLC: 99.29 %, % chiral purity by HPLC ofR-isomer 100.0 %), m.p.: 138-142 °C, XRD: crystalline-Fig (3).

Reference Example 2

Preparation of gentisic acid salt (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-triftuorophenyl)butan-2-amine and its amorphous form.

[0050] In a 25 mL round bottom flask water (10 mL) and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (2.0 g) were taken. The reaction mixture was heated up to 98-100 °C to obtain clear solution. To this clear solution gentisic acid (0.756 g) was added and stirred at 98-100 °C for 1 to 2 hrs. The solvent was distilled out at reduced pressure to obtain a solid mass. (Wt.: 2.7 g).

[0051] The solid was taken into DIPE (20 mL) and stirred for 1 hr then solvent decanted. Solid was then dissolved into IPA. The solvent was distilled out to obtain the gentisic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine as an amorphous solid. (Wt.: 1.91g, % purity: 93.47 %, % water: 3.99% w/w, XRD=amorphous Fig (2).

Reference Example 3

Preparation of an amorphous form of gentisic acid salt (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine

[0052] In a dry, 50 mL round bottom flask methanol (20 mL) and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (4.0 g) were taken. To this clear solution gentisic acid (1.51 g) was added The reaction mixture was heated up to 65-67 °C and stirred for 2 hrs. The solvent was distilled out at reduced pressure to obtain a solid mass. (Wt.: 5.5 g).
% water: 0.82, XRD: Amorphous Fig (2). % Purity-99.34 %, TGA-4.4 %, DSC-87.3 and 213.2.

Reference Example 4

Preparation of an amorphous form of gentisic acid salt (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine

[0053] The gentisic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine was formed by mixing gentisic acid (0.189 g) and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (0.500 g) in methanol (2.5 mL), followed by stirring at reflux temperature for 2 h. The solvent was distilled out at reduced pressure to obtain solid gentisate salt (Wt.: 0.680 g)

[0054] The salt (0.600 g) was dissolved into IPA (1.2 mL). To the solution DIPE (2.4 mL) was added. It was stirred for 1 h at 25-30 °C, solid was precipitated then solvent decanted. The residual solvent was distilled out under reduced pressure to obtain the gentisic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine as an amorphous solid. (Wt.: 0.260 g, % purity: 99 %, % water: 1.99% w/w, XRD=amorphous Fig (2).

Reference Example 5

Preparation of an amorphous form of gentisic acid salt (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine

[0055] The gentisic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine was formed by mixing gentisic acid (0.113 g), (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (0.300 g) in a mixture of isopropylalcohol (0.6 mL) and chloroform (1.5 mL), followed by stirring at reflux temperature for 2 h. The solvent was distilled out at reduced pressure to obtain solid gentisate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine as an amorphous solid. [Wt.: 0.410 g, % purity: 98.9 %, XRD=amorphous Fig (2)].

Reference Example 6

Preparation of an amorphous form of gentisic acid salt (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine

[0056] The gentisic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine was formed by mixing gentisic acid (0.113 g), (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (0.300 g) in a mixture of isopropylalcohol (0.6 mL) and acetonitrile (1.5 mL), followed by stirring at reflux temperature for 2 h. The solvent was distilled out at reduced pressure to obtain solid gentisate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine as an amorphous solid. [Wt.: 0.410 g, % purity: 98.9 %, XRD=amorphous Fig (2)].

Reference Example 7

Preparation of crystalline form of gentisic acid salt (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine

[0057] In a dry, 25 mL round bottom flask methanol (10 mL) and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (2.0 g) were taken. To this clear solution gentisic acid (0.756 g) was added The reaction mixture was heated up to 65-67 °C and stirred for 2 hrs. The solvent was distilled out at reduced pressure to obtain the gentisic acid salt. The gentisic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (450 mg) was dissolved in ethanol (4.5 mL) and toluene (4.5 mL) was added subsequently, stirred for 2 to 3 hrs. Solid salt was precipitated out, filtered and washed with toluene. Subsequently, solid was dried to obtain the gentisic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine. (Wt.: 257 mg, XRD: crystalline-Fig (1))

Reference Example 8

Preparation of hydrochloride salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

[0058] In a dry, 50 mL three neck flask methanol (2.5 mL), IPA (21.3mL) and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (2.0 g) were taken. The reaction mixture was heated up to 40-45 °C to obtain clear solution. To this clear solution IPA-HCl was added upto acidic pH and then (2.5mL) diethyl ether was added into the reaction mixture. It was stirred for 1 hr at 25-30 °C. Solid salt was precipitated out. It was then heated upto 55 °C and then cooled to 25-30 °C. The solid salt was filtered and washed with IPA. The hydrochloride salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine was obtained (Wt. 1.1g, % Purity by HPLC: 99.98 %, % chiral purity by HPLC: >99 %, m.p. = 168 -170 °C, XRD=crystalline (Fig-4).

Reference Example 9

Preparation of besylate salt(I) of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

[0059] In a dry, 50 mL round bottom flask isopropyl acetate (25 mL) and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (0.5 g) were taken. The reaction mixture was heated up to 45-50 °C and a solution of benzenesulfonic acid (0.194 g) in (2.3mL) IPAc was added. Upon complete addition, solid salt was precipitated out. It was then cooled to 25-30 °C. The salt was filtered and washed with hexane. The besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine was obtained (Wt. 0.63g, % Purity by HPLC: 99.76 %, % chiral purity by HPLC: >99 %), m.p. = 171-174 °C, XRD=crystalline (Fig.5).

Example 10

Preparation of hydrated besylate salt(II) of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

[0060] In a dry, 50 mL round bottom flask isopropyl acetate (250 mL) and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (5.0 g) were taken. The reaction mixture was heated up to 45-50 °C and a solution of benzenesulfonic acid (1.93 g) in a mixture of water (0.5 mL) and isopropyl acetate (23mL) was slowly added. It was stirred for 2 h at 50 °C. It was then cooled to 25-30 °C. The salt was filtered and washed with IPA and Hexane. The besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine was obtained. It was dried. (Wt. 6.7 g, % Water- 2.96 %, % Purity by HPLC: > 99.0 %, % chiral purity by HPLC: > 99 %), m.p. = 172-174 °C, XRD=crystalline (Fig. 7).

Example 11

Preparation of hydrated besylate salt(II) of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

[0061] In a dry, 50 mL round bottom flask chloroform (5 mL) and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (0.5 g) and water (0.5 mL) were taken. The mixture was stirred at 25-30 °C for 5-10 min. and solid benzenesulfonic acid (0.193 g) was added. It was heated to reflux temperature and stirred for 2 h. It was then cooled to 25-30 °C and stirred for 68 h at 25-30 °C. The salt was filtered and washed with chloroform. The besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine was obtained. It was dried. (Wt. 0.660 g, % Water- 1.93 %, % Purity by HPLC: > 99.0 %, % chiral purity by HPLC: > 99 %).

Example 12

Preparation of hydrated besylate salt(II) of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

[0062] To the 25 mL round bottom flask isopropyl acetate (5 mL), tetrahydrofuran (0.25 mL), water (0.5 mL) and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (0.5 g) were added. The mixture was stirred at 25-30 °C for 5-10 min. and solid benzenesulfonic acid (0.194 g) was added. It was heated to reflux temperature and stirred for 2 h. It was then cooled to 25-30 °C and stirred for 15-30 min. at 25-30 °C. The salt was filtered and washed with isopropyl acetate. The besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine was obtained. It was dried. (Wt. 0.680 g, % Water- 2.2 %, % Purity by HPLC: > 99.0 %, % chiral purity by HPLC: > 99 %).

Example 13

Preparation of hydrated besylate salt(II) of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

[0063] To the 25 mL round bottom flask isopropyl acetate (5 mL), toluene (0.25 mL), water (0.5 mL) and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (0.5 g) were added. The mixture was stirred at 25-30 °C for 5-10 min. and solid benzenesulfonic acid (0.194 g) was added. It was heated to reflux temperature and stirred for 2 h. It was then cooled to 25-30 °C and stirred for 15-30 min. at 25-30 °C. The salt was filtered and washed with isopropyl acetate. The besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine was obtained. It was dried. (Wt. 0.660 g, % Water- 1.61 %, % Purity by HPLC: > 99.0 %, % chiral purity by HPLC: > 99 %).

Reference Example 14

Preparation of Trifluoroacetic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

[0064] In a dry, 25 mL round bottom flask isopropanol (15 mL) and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (1.0 g,) were taken. The reaction mixture was heated up to 78-80 °C and trifluoroacetic acid (0.28 g) was added. It was stirred for 1.5 h at 80-82 °C. It was then cooled to 25-30 °C. Then the solvent was evaporated. The salt was dissolved into IPA and precipitated with DIPE. The trifluoroacetic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine was filtered and washed with DIPE. It was dried. (Wt. 0.89g, % Purity by HPLC: > 98 %, % chiral purity by HPLC: > 99 %), XRD=crystalline (Fig. 6).

Reference Example 15

Preparation of Trifluoroacetic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

[0065] The trifluoroacetic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (6.5 g) was stirred with isopropylacetate (90 mL) for 2 hrs at 25-30 °C. The salt was filtered and washed with isopropylacetate. It was dried (Wt. 5.4 g, % Purity by HPLC: > 98 %, % chiral purity by HPLC: > 99 %, % water-3.14 %).

XRD=crystalline (Fig. 6).

Reference Example 16

Preparation of amorphous benzenesulfonic acid salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

[0066] In a dry, 25 mL round bottom flask methanol (2 mL) and (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (1.0 g,) were taken. Then benzenesulfonic acid (0.38 g) was added. It was stirred for 3h at 28 °C. Further, dichloromethane (10 mL) was added and the reaction mixture was stirred for 1h at 28 °C. Then the solvent was distilled out at 25-30 °C under reduced pressure. The amorphous besylate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine was obtained (Wt. 1.2g, % Purity by HPLC: >98 %, % chiral purity by HPLC: > 99 %), XRD=amorphous (Fig. 8).

Reference Example 17

Preparation of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine from Monohydrate phosphate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

[0067] Monohydrate phosphate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (2 g) and water (10 mL) were taken in a 50 ml three neck flask. To the solution sodium chloride (3.4 g), sodium bicarbonate (0.33 g) in small lots and ethyl acetate (20 mL) were added at 25-30 °C. It was stirred for 15-30 min. at 40-42°C. It was transferred into a separating funnel; organic layer was collected. The aqueous layer was extracted twice with ethylacetate (20 mL);organic layer was collected. All the organic layers were combined. The solvent was distilled out at reduced pressure to obtain (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (Wt. 1.8 g, % Water- 1.18 %, % Purity by HPLC: > 99.0 %).

Reference Example 18

Preparation of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine from Monohydrate

phosphate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

[0068] Monohydrate phosphate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (2 g) and water (10 mL) were taken in a 50 ml three neck flask. To the solution sodium chloride (3.4 g), 10 % aq. sodium hydroxide (3 mL) and ethyl acetate (20 mL) were added at 25-30 °C. It was stirred for 15-30 min. at 25-30 °C. It was transferred into a separating funnel; organic layer was collected. The aqueous layer was saturated with sodium chloride (3.4 g) and extracted twice with ethylacetate (20 mL). All the organic layers were combined and dried over anhydrous sodium sulfate. The solvent was distilled out at reduced pressure to obtain (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (Wt. 1.6 g, % Water- 0.39 %, % Purity by HPLC: > 99.0 %).

Reference Example 19

Preparation of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine

[0069] The (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (21.0 g, Chiral Purity- 96.4 %, prepared as per method disclosed in patent WO 2010/032264) was dissolved in isopropylalcohol (42.0 mL) at 68-70 °C. Then it was cooled to 45-48 °C and n-heptane (168 mL) was added drop by drop over a period of 30-45 min. The solid was precipitated. It was cooled to 25-30 °C and stirred for 1 h. The solid was filtered and washed with a mixture of isopropylalcohol and n-heptane [63 mL (1:4)]. It was dried at reduced pressure to obtain (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (17.1 g, Purity- 99.5 %, Chiral Purity- 99.5 %, % Water - 0.16 %.

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO03004498A [0003] [0004] [0004] [0040]
- US6699871B [0003]
- WO2005003135A [0004] [0005]
- WO2005020920A [0005]
- WO2005030127A [0006]
- WO2006033848A [0007]
- WO2005072530A [0008]
- WO2007035198A [0009]
- WO2008000418A [0010]
- WO2009085990A [0011]
- WO2010000469A [0012]
- WO2010092090A [0013]
- WO2010012781A [0014]
- WO2010117738A [0015]
- WO2012007455A [0015]
- WO2010032264A [0069]

Non-patent literature cited in the description

- **D. KIM et al.** J. Med. Chem., 2005, vol. 48, 141-151 [0010]

Patentkrav

1. Den krystallinske form II af (2R)-4-oxo-4-[3-(trifluormethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorphenyl)butan-2-aminbesylat med et karakteristik røntgenpulverdiffraktionsmønster med peaks udtrykt som 2θ ved 5,69, 7,45, 10,08, 11,41, 13,09, 18,34, 22,52 og 22,97 ± 0,2 grader 2-theta.

2. Fremgangsmåde til fremstilling af den krystallinske form II af (2R)-4-oxo-4-[3-(trifluormethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorphenyl)butan-2-aminbesylat ifølge krav 1, hvilken fremgangsmåde omfatter

(a) omsætning af den frie base (formel I) under egnede reaktionsbetingelser med benzensulfonsyren i egnet opløsningsmiddel udvalgt fra en blanding af estere og vand, en blanding af estere, ethere og vand, en blanding af estere, alkoholer og vand, en blanding af ester, carbonhydrider og vand, en blanding af estere, ketoner og vand;

(b) egnet fjernelse af opløsningsmidlet for at opnå den krystallinske form II af (2R)-4-oxo-4-[3-(trifluormethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5- trifluorphenyl)butan-2-aminbesylat.

DRAWINGS

Fig. 1

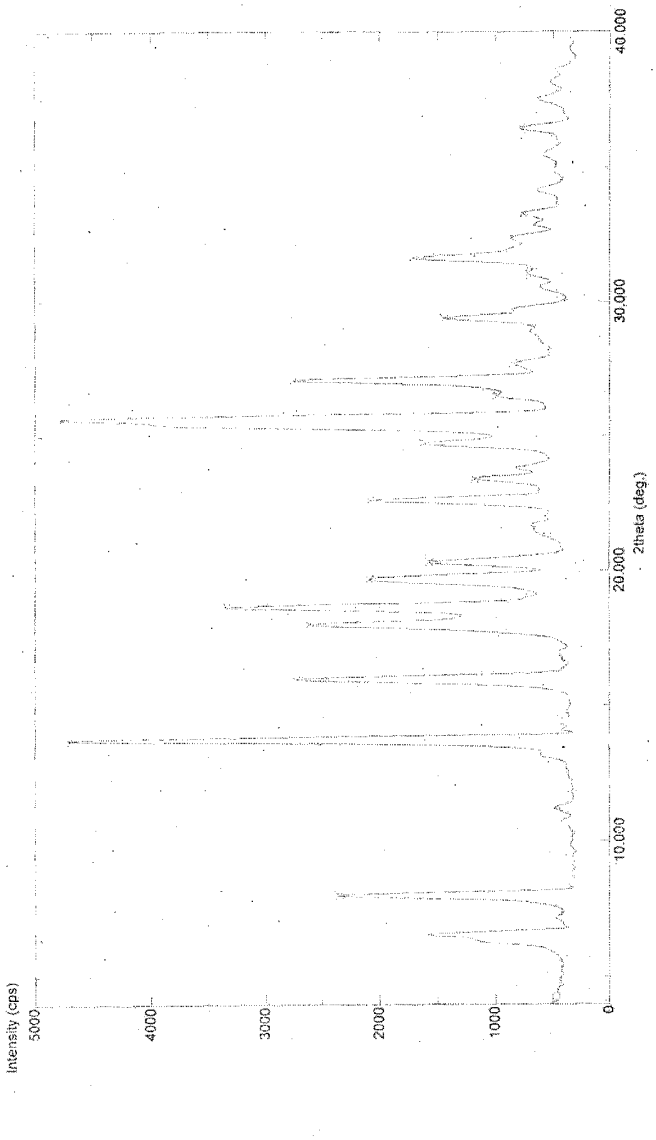

Fig.2

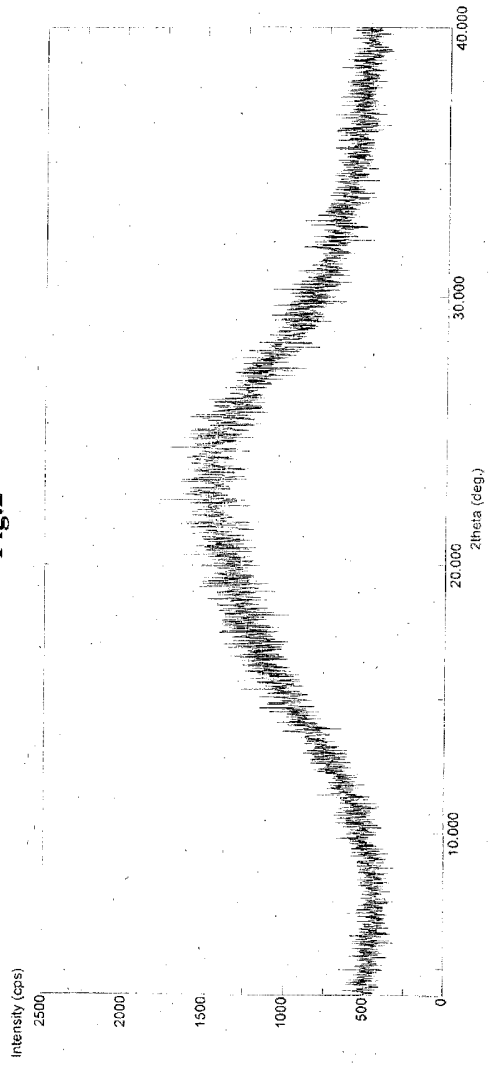
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Fig. 3

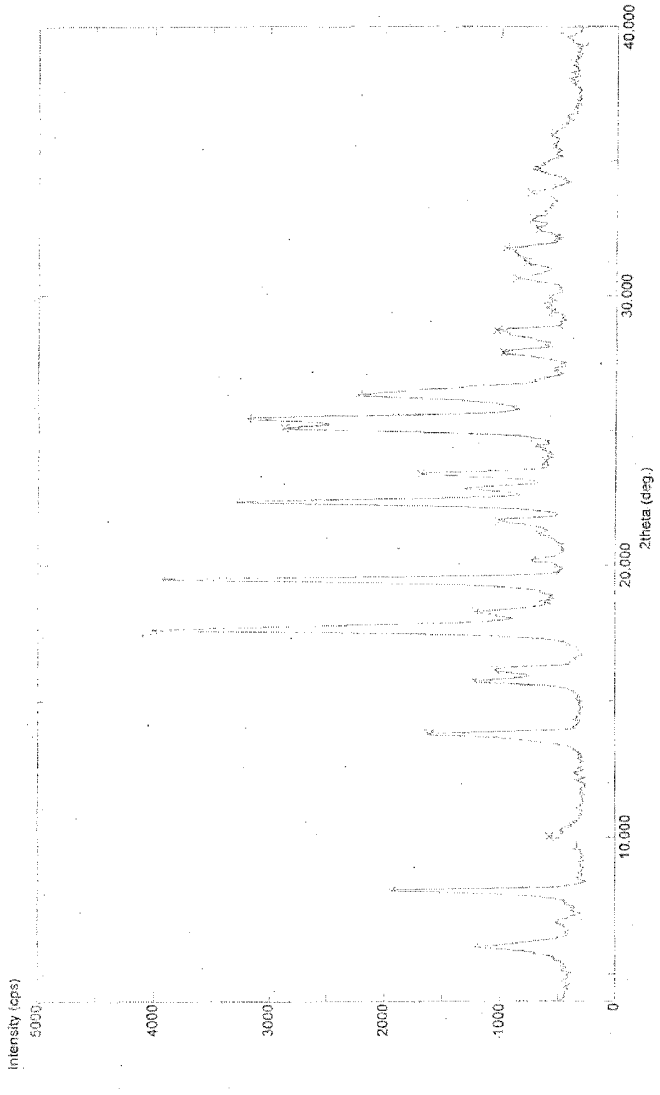
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Fig-4

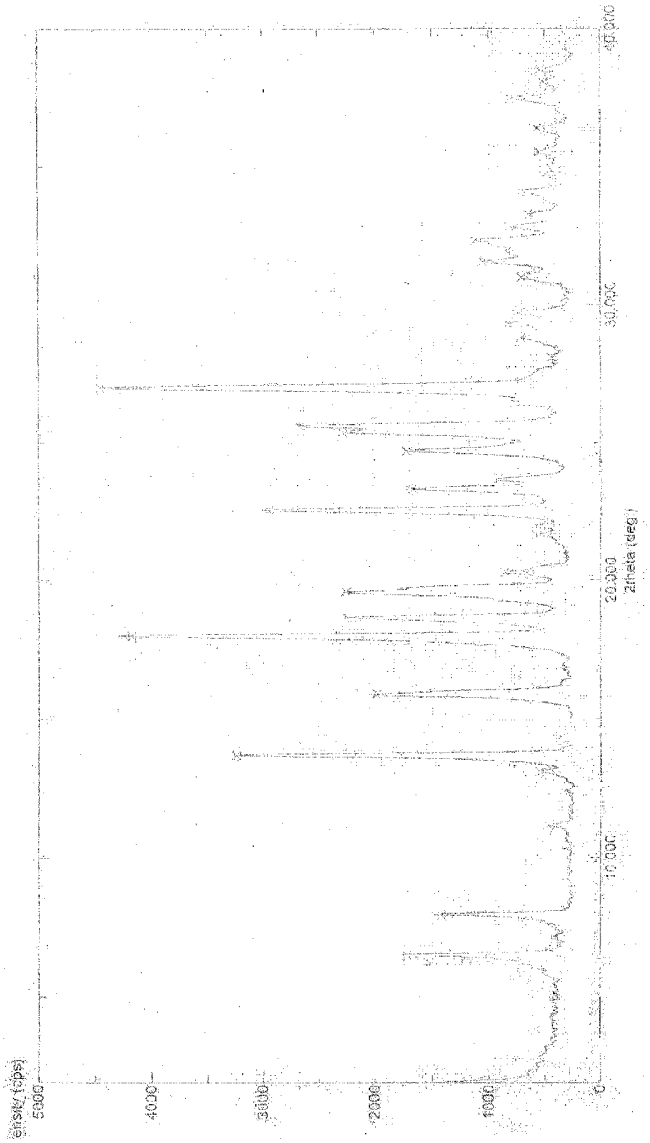
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Fig-5

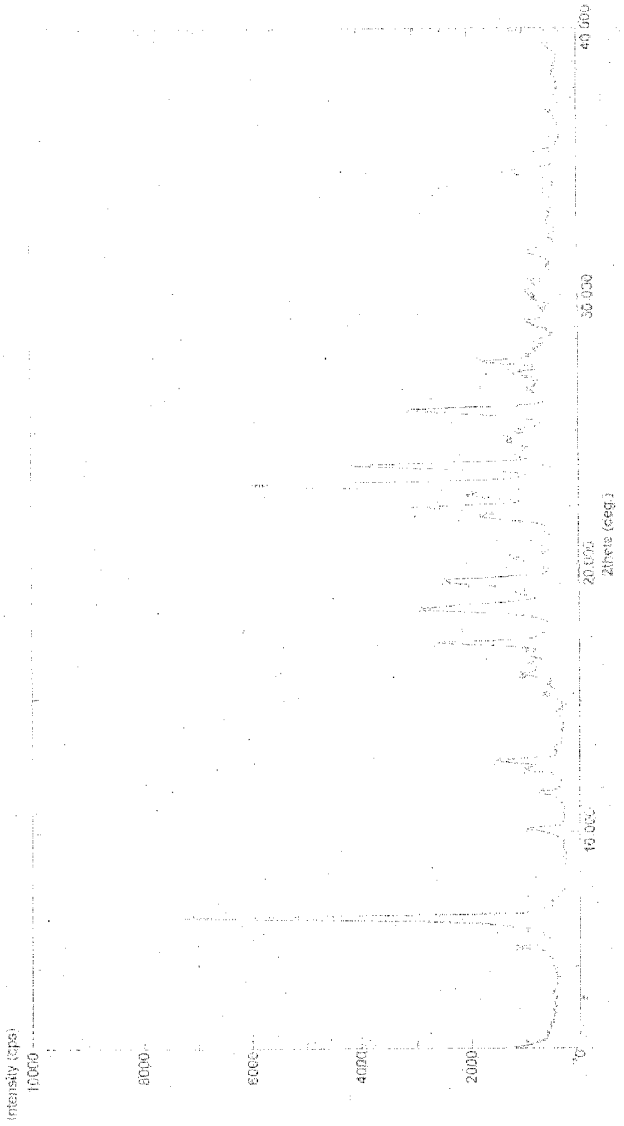
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Fig-6


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Fig-7

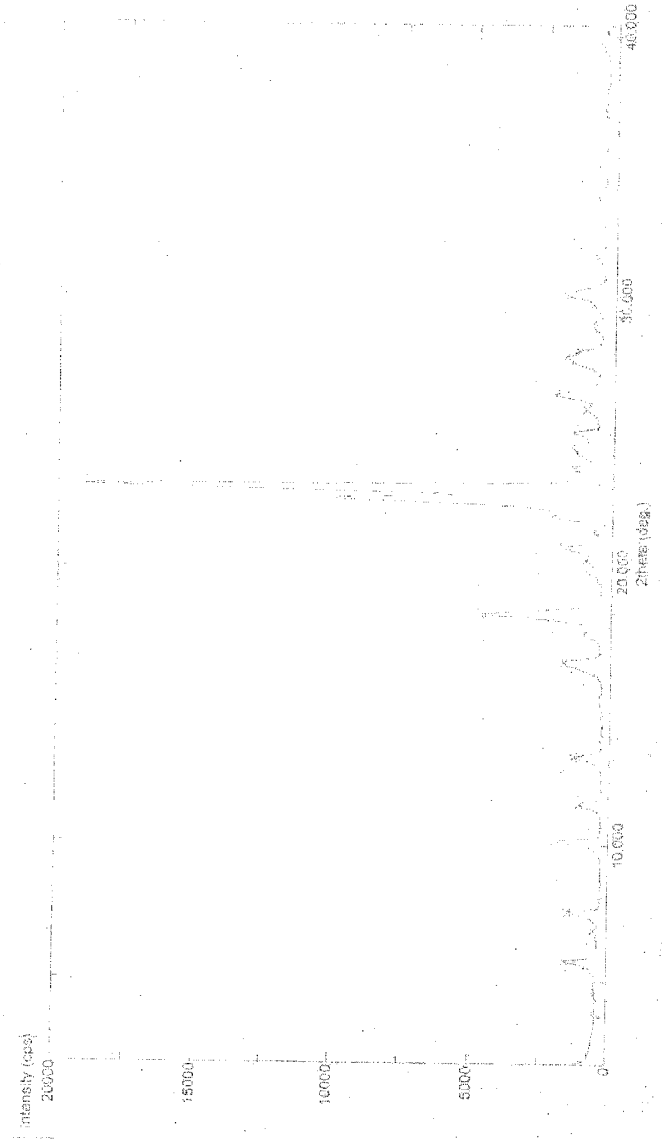

Fig-8

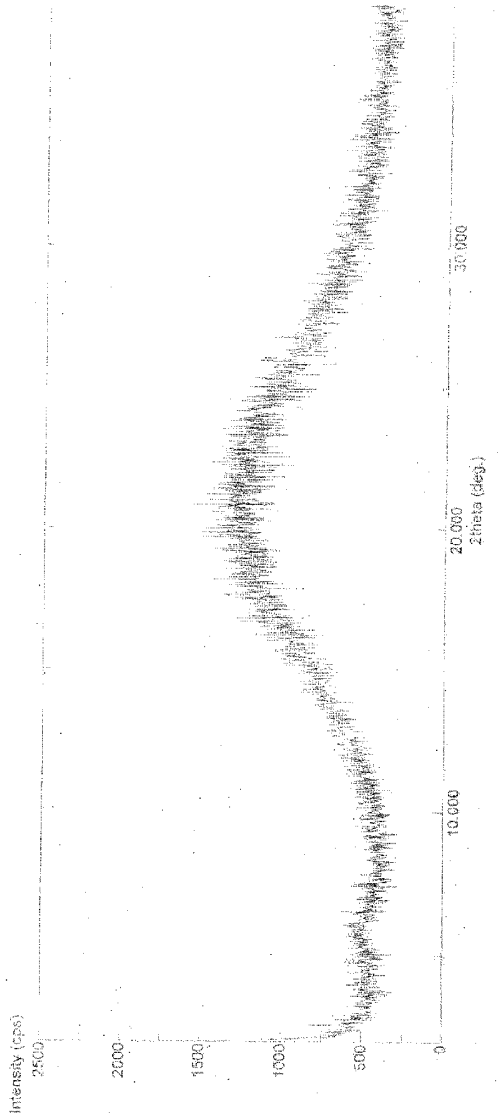
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Figure: 9- Effect of single dose oral administration of ZY compounds at 10mg/kg, PO on OGTT in male C57 mice.

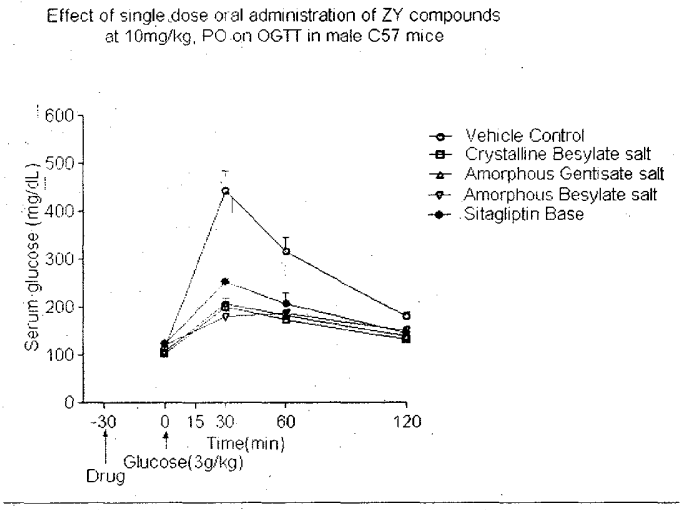

Figure: 10- Effect of single dose oral administration of ZY compounds at 10mg/kg, PO on OGTT in male C57 mice.