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Synthesis and characterization of some analogues of Febuxostat - An anti-hyperuricemia drug

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ABSTRACT

Febuxostat is indicated use for the treatment of hyperuricemia and gout. During the process development of Febuxostat, ten process related analogues were observed at a level of 0.1-0.15 area percent. Synthesis and characterization of these analogues and investigation of the root cause of their formation is described.

KEYWORDS

Febuxostat;
Febuxostat amide-ester;
Febuxostat diesters;
Synthesis;
Characterization.

INTRODUCTION

Febuxostat is a xanthine oxidase inhibitor used for treating gout[1-3] caused by excessive levels of uric acid in the blood (hyperuricemia)[4]. A study comparing febuxostat to allopurinol found that more individuals treated with febuxostat had decreased levels of uric acid, but there was no difference in the amount of initial gout flares or the surface area of gout tophi[5-7]. Structurally, febuxostat is similar to 4-methyl-1,3-thiazole because it contains 4-methyl-1,3-thiazole moiety that has a 2-alkyl substituent on the thiazole ring. On the other hand, a variety of 2-arylthiazole derivatives and their synthetic intermediate has been proposed to have various physiological activities such as anti-inflammatory[8], analgsic[8] and antimicrobial[9].

Impurity removal is a critical and important task in pharmaceutical process reseach, where the final product meets stringent purity requirements. The presence of impurities in an active pharmaceutical ingredient (API) can have significant impact on the quality and safety of the drug product. International Conference on Harmonization (ICH) guidelines (2006) recommends identifying and characterising all impurities present in APIs at a level of 0.10 % and above. These impurities are required in pure form to understand the impurity profile and development of an accurate analystical method[10-11] during the research and development phase.

An improved and scalable process was developed for febuxostat. At the time of development, ten process related Febuxostat analogus were observed in the reaction mass at a level of 0.1-0.15 area percent. After work up process to isolate the product some of the analogus, febuxostat diester (11-14), feuxostat amide (5) and febuxostat diacid (6) were washed out during isolation and purification of febuxostat stage-2. Process related analogus (5-14) were identified and characterized, however their synthesis and the cause for their formation were not known. Herein, we report the complete impuritiy profile for febuxostat made using im-

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proved process, including indentification, characterization of unknown analogues (5-14) and detailed experimental procedures for the synthesis of analogues.

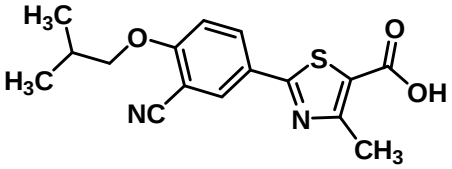


Figure 1 : Structure of febuxostat (1)

MATERIALS AND METHOD

Drugs, chemicals and intruments

The mass spectrum (70 eV) was recorded on HP-5989A LC–MS spectrometer. The purity of compounds was checked by HPLC (Shimadzu LC Solution). Reaction monitoring was checked routinely by TLC (0.5mm thickness) using silica gel-G coated Al-plates (Merck), mobile phase:-Ethylacetate:Hexane (6:4) and spots were visualized by exposing the dry plates in UV light (254 nm) or iodine vapors. The melting points were determined by using the capillary method on POLMON (model MP-96) melting point apparatus and are uncorrected. The solvents and reagents were used without further purification.

NMR spectroscopy

The ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ and DMSO-d_6 using 300 MHz, on a Bruker AVANCE-II 300 MHz FT NMR spectrometer; the chemical shifts (δ_H, δ_C) are reported in δ ppm relative to TMS. (Bruker, Fallanden, Switzerland) equipped with a 5 mm BBO probe and a z-gradient shim system. The ^{1}H spectra were recorded with 1 s pulse repetition time using 30° flip angle, while ^{13}C spectra were recorded with power gated decoupling using 30° flip angle with repetition time of 2 s. Samples were dissolved in $CDCl_3$ or DMSO-d_6.

FT-IR spectroscopy

The FT-IR spectra (υ_{max} in cm^{-1}) were recorded in the solid state as KBr dispersion using Perkin-Elmer 1650 FT-IR spectrophotometer (Thermo Electron Scientific Instruments, Madison, WI, USA) with a DTGBS KBr detector. Data were collected between 400 and 4000 cm^{-1}, with a resolution of 4.0 cm^{-1}. A total of 16 scans were obtained and processed using the OMNIC software version 6.0.

Elemental analysis

The elemental analysis (C, H, N) of compounds was performed on Carlo Erba-1108 elemental analyzer EL III with TCD detector (Elementar Analysensysteme GmbH, Hanau, Germany). Their results were found to be in good agreement with the calculated values. Samples were weighed in a tin boat, to which tungsten oxide was added and neatly packed. The sample in tin boat was loaded in an auto sampler tray and was dropped into the combustion tube automatically at a temperature of 1200°C. Complete combustion of sample was ensured with a special oxygen jet injection.

Experimental and characterization

Procedure for ethyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (4)[12-13]

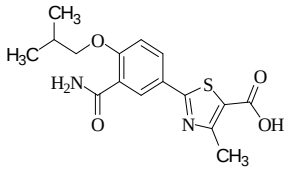
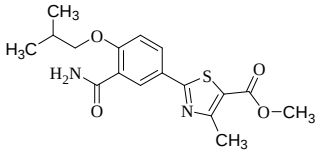
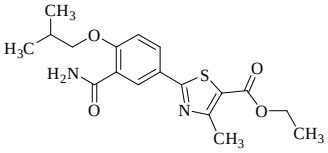
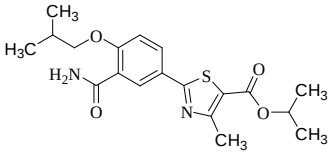
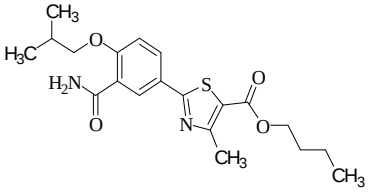
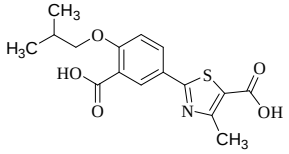
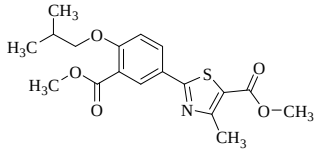
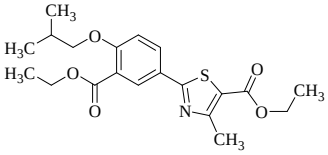
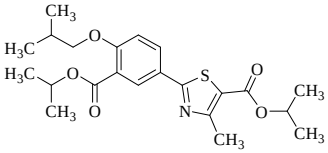
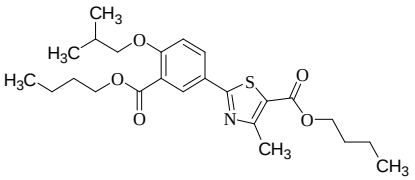
To a stirred solution of 3-cyano-4-isobutoxybenzothioamide (2, 5.0 g, 0.021 mol.) and ethyl-2-chloro-3-oxobutanoate (3, 4.42 mL, 0.032 mol.) in isopropanol (25.0 mL) at 25-30°C. Heat the reaction mass for 3-4 hrs at 75-80°C. The mixture was allowed to cool up to 25-30°C. A pale yellowish solid form, filtrated, washed with isopropanol (10.0 mL) and dried into vacuum tray drier at 50-55°C under vacuum to give ethyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (4).

Procedure for 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (febuxostat tech)[14]

To a stirred solution of ethyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (4, 5.0 g, 0.014 mol.) in n-butanol (50.0 mL) at 25-30°C, are added NaOH (1.74 g, 0.043 mol.) into reaction mass at 25-30°C. Heat the reaction mass for 1-2 hrs at 35-40°C. The mixture was allowed to cool up to 25-30°C. Adjust pH 1-2 of reaction mass, using con. HCl (5.0 mL) at 25-30°C. A white solid form, filtrated, washed with n-butanol:water (1:1) (10.0 mL) and dried in vacuum tray drier at 50-55°C under vacuum to give febuxostat tech.

Procedure for 2-(3-cyano-4-isobutoxyphenyl)-4-

TABLE 1 : List of analogues of febuxostat

Sr.No.	Name of impurity	IUPAC Name	Structure
1.	Febuxostat amide (5)	2-(3-Carbamoyl-4-isobutoxy phenyl)-4-methylthiazole-5-carboxylic acid	
2.	Febuxostat amide-methyl ester (7)	Methyl-2-(3-carbamoyl-4-sobutoxyphenyl)-4-methylthiazole-5-carboxylate	
3.	Febuxostat amide-ethyl ester (8)	Ethyl-2-(3-carbamoyl-4-isobutoxy phenyl)-4-methylthiazole-5-carboxylate	
4.	Febuxostat amide-isopropyl ester (9)	Isopropyl-2-(3-carbamoyl-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate	
5.	Febuxostat amide-butyl ester impurity (10)	Butyl-2-(3-carbamoyl-4-isobutoxy phenyl)-4-methylthiazole-5-carboxylate	
6.	Febuxostat diacid (6)	2-(3-Carboxy-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid	
7.	Febuxostat dimethyl ester (11)	Methyl-2-(4-isobutoxy-3-(methoxycarbonyl)phenyl)-4-methylthiazole-5-carboxylate	
8.	Febuxostat diethyl ester (12)	Ethyl-2-(3-(ethoxycarbonyl)-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate	
9.	Febuxostat diisopropyl ester (13)	Isopropyl-2-(4-isobutoxy-3-(isopropoxycarbonyl)phenyl)-4-methylthiazole-5-carboxylate	
10.	Febuxostat dibutyl ester (14)	Butyl-2-(3-(butoxycarbonyl)-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate	

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methylthiazole-5-carboxylic acid (1)[15]

To a stirred solution of 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (*febuxostat tech*, 5.0 g, 0.015 mol.) in methanol (50.0 mL) at 25-30°C, Heat the reaction mass at 60-65°C up to clear solution obtained. Added water (50.0 mL) drop wise into reaction mass with in 30.0 min. at 60-65°C. A white crystalline solid form, filtrated, washed with water (10.0 mL) and dried in vacuum tray drier at 50-55°C under vacuum to give 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (febuxostat crystal A) (1).

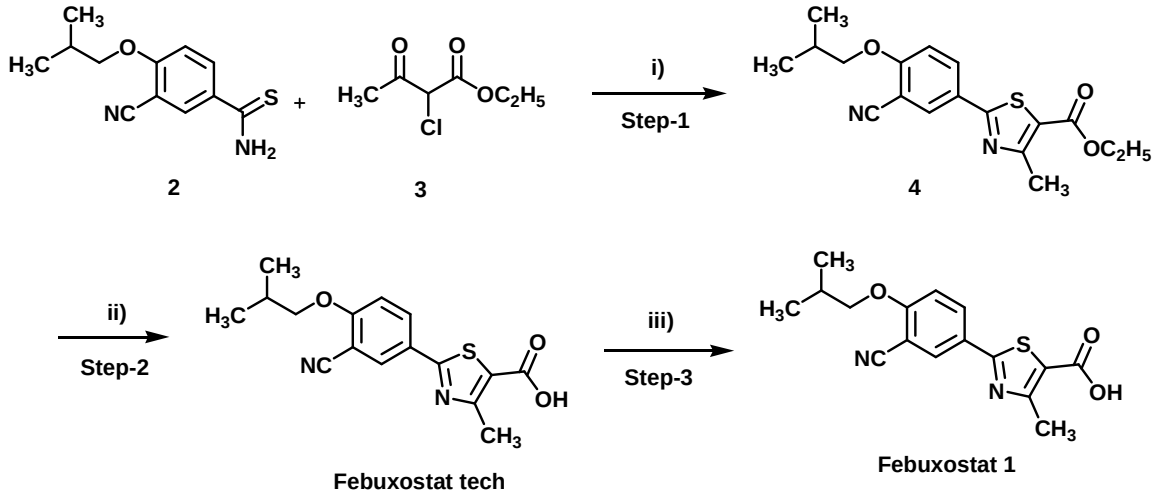
Procedure for 2-(3-carbamoyl-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (5)

To a stirred solution of 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (1, 5.0 g, 0.016 mol.) in dimethylsulfoxide (50.0 mL), cooled in an ice-water bath (0-5°C), added powder anhydrous K_2CO_3 (5.0 g) and drop wise 30.0 % H_2O_2 (35.0 mL) within 30.0 min. at 0-5°C. The mixture was allowed to warm up to 25-30°C (exotherm was observed). The reaction mass maintained for 24.0 hrs under stirring at 25-30°C. Water (100.0 mL) was added into reaction mass at 25-30°C. Adjust pH 1-2 of the reaction mass using con. HCl (15.0 mL) at 25-30°C (exotherm observed) to obtain white solid which was purified in acetone (50.0 mL) at 25-30°C, filtrated, washed with acetone (10.0 mL) and dried in vacuum tray drier at 50-55°C under vacuum to give febuxostat amide 5.

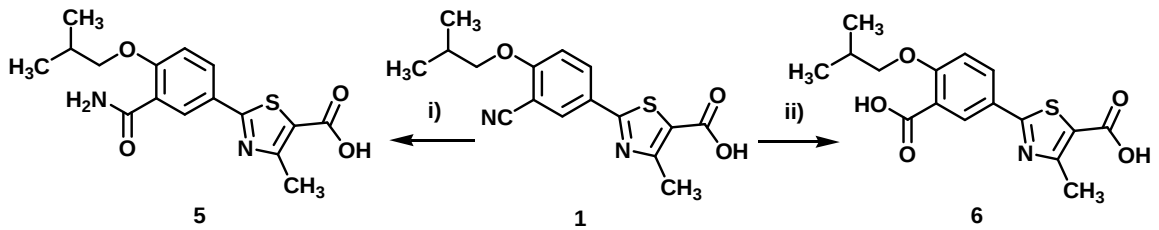
Procedure for 2-(3-carboxy-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (6)

To a stirred solution of 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (1, 5.0 g, 0.016 mol.) in n-butanol (85.0 mL) and water (85.0 mL) at 25-30°C, added NaOH (6.32 g, 0.16 mol.) into reaction mass at 25-30°C. Heat the reaction mass for 48 hrs at 100-110°C. The mixture was allowed to cool up to 25-30°C. Adjust pH 1-2 of the reaction mass, using con. HCl (25.0 mL) at 25-30°C. A white solid was filtrated, washed with n-butanol: water (1:1) (10.0 mL) and dried in vacuum tray drier at 50-55°C under vacuum to give febuxostat diacid 6.

General procedure for substituted alkyl-2-(3-car-



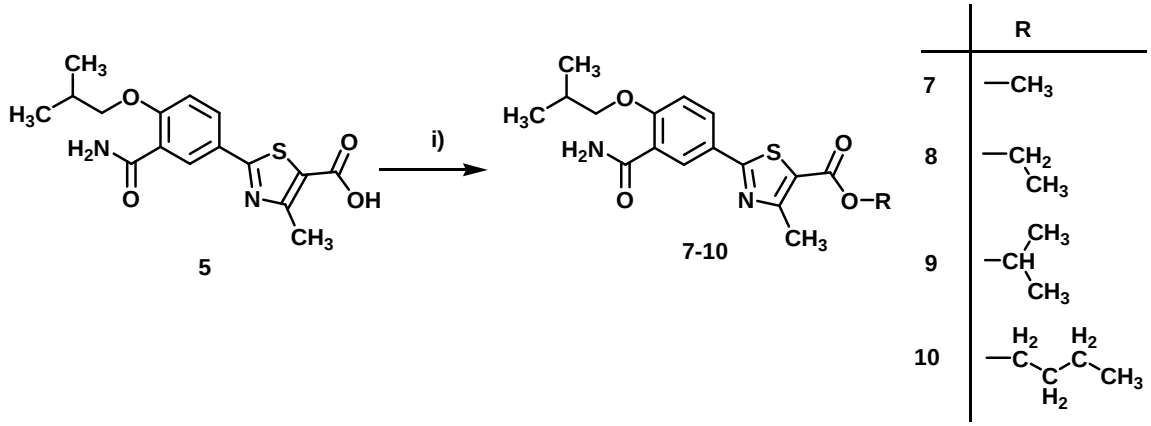
Scheme 1 : Synthesis of febuxostat 1: i) Thioamide compound 2, halo diketo compound 3, isopropyl alcohol, reflux (75-80°C); ii) NaOH, n-butanol, 35-40°C; iii) Methanol, water, reflux (60-65°C)



Scheme 2 : Synthesis of febuxostat amide 5 and febuxostat diacid 6: i) H_2O_2 (30.0 %), DMSO, powder anhydrous K_2CO_3; ii) n-butanol, NaOH, water, reflux (100-110°C)

bamoyl-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (febuxostat amide-esters 7-10)

To a stirred solution of 2-(3-carbamoyl-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (5, 5.0 g, 0.016 mol.), con. H_2SO_4 (3.0-4.0 mL) in alcoholic solvents (50.0 mL) at 25-30°C. Heat the reaction mass for 10-12 hrs at reflux temperature respectively based on alcoholic solvents boiling point. The mixture was allowed to cool up to 25-30°C. Reaction mass was completely distilled out at 50-55°C under vacuum to obtain oily residue. The oily residue dissolve into methylene dichloride (100.0 mL) at 25-30°C and



Scheme 3 : Synthesis of febuxostat amide-esters: (i) Esterification reaction: Con. H_2SO_4, alcoholic solvents [methanol for 7, ethanol for 8, isopropanol for 9 and n-butanol for 10]

TABLE 2 : Physical and elemental analysis data of febuxostat analogues

Febuxostat analogues	Description	m. p. (°C)	Yield %	Mol. formula	Anal. Calcd (%) (Found)		
					C	H	N
1	White crystalline solid	239	95.0	$C_{16}H_{16}N_2O_3S$	60.74 (60.70	5.10 5.11	8.85 8.87)
5	White solid	258	90.0	$C_{16}H_{18}N_2O_4S$	57.47 (57.50	5.43 5.40	8.38 8.35)
6	White crystal	268	95.0	$C_{16}H_{17}NO_5S$	57.30 (57.33	5.11 5.08	4.18 4.20)
7	Off white crystal	180	82.0	$C_{17}H_{20}N_2O_4S$	58.60 (58.63	5.79 5.82	8.04 8.05)
8	White solid	149	85.0	$C_{18}H_{22}N_2O_4S$	59.65 (59.61	6.12 6.08	7.73 7.75)
9	White solid	168	80.0	$C_{19}H_{24}N_2O_4S$	60.62 (60.58	6.43 6.40	7.44 7.48)
10	White solid	186	88.0	$C_{20}H_{26}N_2O_4S$	61.52 (61.55	6.71 6.64	7.17 7.20)
11	White crystal	232	82.0	$C_{18}H_{21}NO_5S$	59.49 (59.53	5.82 5.78	3.85 3.86)
12	Off white solid	240	85.0	$C_{20}H_{25}NO_5S$	61.36 (61.33	6.44 6.48	3.58 3.60)
13	Pale yellow solid	211	80.0	$C_{22}H_{29}NO_5S$	62.98 (62.95	6.97 6.92	3.34 3.37)
14	White semi solid	---	88.0	$C_{24}H_{33}NO_5S$	64.40 (64.45	7.43 7.46	3.13 3.16)

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TABLE 3 : IR and mass spectral data of febuxostat analogues

Febuxostat analogues	IR (KBr) υ_{max} in cm^{-1}	MS (+ESI) *m/z* (%)
1	3834.61, 3742.03, 3680.30, 3556.85, 3456.55, 2962.76, 2877.89, 2661.85, 2546.12, 2353.23, 2229.79, 2168.06, 2029.18, 1921.16, 1790.00, 1674.27, 1604.83, 1512.24, 1427.37, 1381.08, 1280.78, 1172.76, 1118.75, 1010.73, 918.15, 833.28, 771.55, 725.26, 648.10, 524.66, 462.93.	317.0 (100.0) [M+1], 318.0 (16.0) [M+2], 403.0 (63.0), 512.0 (47.0), 482.0 (46.0), 405.0 (27.0), 468.0 (25.0), 570.0 (24.0).
5	3402.54, 3290.67, 3186.51, 3066.92, 2970.48, 2928.04, 2874.03, 2820.02, 2654.14, 2619.42, 2542.26, 2368.66, 2337.80, 2276.08, 2106.34, 2033.04, 1863.30, 1755.28, 1678.13, 1647.26, 1523.82, 1469.81, 1377.22, 1292.35, 1273.06, 1226.77, 1161.19, 1099.46, 925.86, 721.40, 705.97, 613.38, 570.95, 528.51, 466.79, 443.64, 401.21.	335.0 (100.0) [M+1], 336.0 (25.0) [M+2], 472.0 (13.0), 431.0 (9.0), 493.0 (8.0).
6	3537.57, 3514.42, 3367.82, 3182.65, 3128.64, 3082.35, 3047.63, 2955.04, 2928.04, 2874.03, 2638.71, 2526.83, 2376.38, 2337.80, 1928.88, 1909.59, 1855.58, 1820.86, 1689.70, 1600.97, 1577.82, 1508.38, 1469.81, 1423.51, 1377.22, 1330.93, 1292.35, 1253.77, 1219.05, 1168.90, 1091.75, 1049.31, 1022.31, 956.72, 852.56, 825.56, 798.56, 756.12, 732.97, 678.97, 613.38, 574.81, 528.51, 474.50.	336.0 (100.0) [M+1], 337.0 (11.0) [M+2], 472.0 (8.0), 380.0 (6.0).
7	3402.54, 3290.67, 3194.23, 2966.62, 2928.04, 2874.03, 2777.59, 2519.12, 2357.09, 2326.23, 1712.85, 1697.41, 1647.26, 1597.11, 1508.38, 1438.94, 1415.80, 1373.36, 1330.93, 1192.05, 1161.19, 1018.45, 918.15, 813.99, 763.84, 709.83, 644.25, 613.38, 574.81, 528.51, 486.08.	349.0 (100.0) M+1], 350.0 (21.0) [M+2], 351.0 (6.0) [M+3].
8	3394.83, 3294.53, 3190.37, 3070.78, 2970.48, 2874.03, 2781.44, 2526.83, 2106.34, 1863.30, 1708.99, 1647.26, 1597.11, 1531.53, 1504.53, 1469.81, 1431.23, 1373.36, 1323.21, 1273.06, 1230.63, 1161.19, 1018.45, 960.58, 810.13, 759.98, 705.97, 648.10, 621.10, 574.81, 528.51.	363.0 (100.0) [M+1], 364.0 (22.0) [M+2], 365.0 (4.0) [M+3], 392.0 (26.0).
10	3911.77, 3850.04, 3699.59, 3410.26, 3194.23, 2966.62, 2931.90, 2874.03, 2573.13, 2411.10, 2233.64, 1932.74, 1813.15, 1759.14, 1693.56, 1643.41, 1600.97, 1508.38, 1446.66, 1369.50, 1327.07, 1269.20, 1165.04, 1099.46, 1014.59, 968.30, 918.15, 840.99, 821.70, 763.84, 736.83, 709.83, 640.39, 613.38, 570.95, 497.65, 435.93, 408.92.	391.0 (100.0) [M+1], 392.0 (25.0) [M+2], 294.0 (4.0) [M+4].
11	3911.77, 3834.61, 3765.17, 3664.87, 3649.44, 3518.28, 3433.41, 3371.68, 3286.81, 3086.21, 2955.04, 2885.60, 2715.86, 2646.42, 2530.69, 2476.68, 2414.96, 2384.10, 2245.22, 2121.77, 2021.47, 1936.60, 1697.41, 1612.54, 1512.24, 1512.24, 1442.80, 1334.78, 1211.34, 1157.33, 1087.89, 910.43, 794.70, 748.41, 648.10, 594.10, 524.66.	364.0 (100.0) [M+1], 365.0 (18.0) [M+2], 366.0 (5.0) [M+3], 380.0 (3.0).
12	3390.97, 3228.95, 3124.79, 3078.49, 2962.76, 2935.76, 2908.75, 2877.89, 2630.99, 2849.98, 2549.98, 2484.40, 2357.09, 2330.09, 2090.91, 1708.99, 1604.83, 1577.82, 1508.38, 1431.23, 1369.50, 1315.50, 1276.92, 1242.20, 1207.48, 1168.90, 1076.32, 1049.31, 1014.59, 960.58, 925.86, 825.56, 756.12, 725.26, 694.40, 636.53, 597.95.	392.0 (100.0) [M+1], 393.0 (15.0) [M+2], 394.0 (5.0) [M+3], 396.0 (2.0) [M+5], 472.0 (8.0).
13	ND	420.0 (100.0) [M+1], 421.0 (26.0), 422.0 (9.0) [M+3].
14	3896.34, 3850.04, 3734.31, 3672.59, 3672.59, 3603.15, 3387.11, 3348.54, 3078.49, 2955.04, 2877.89, 2731.29, 2623.28, 2530.69, 2414.96, 2345.52, 2191.21, 2083.19, 2036.90, 1928.88, 1890.30, 1697.41, 1612.54, 1519.96, 1458.23, 1388.79, 1265.35, 1157.33, 1095.60, 1026.16, 964.44, 825.56, 732.97, 648.10, 594.10, 509.22, 432.07.	448.0 (100.0) [M+1], 449.0 (25.0) [M+2], 450.0 (7.0) [M+3], 471.0 (3.0).

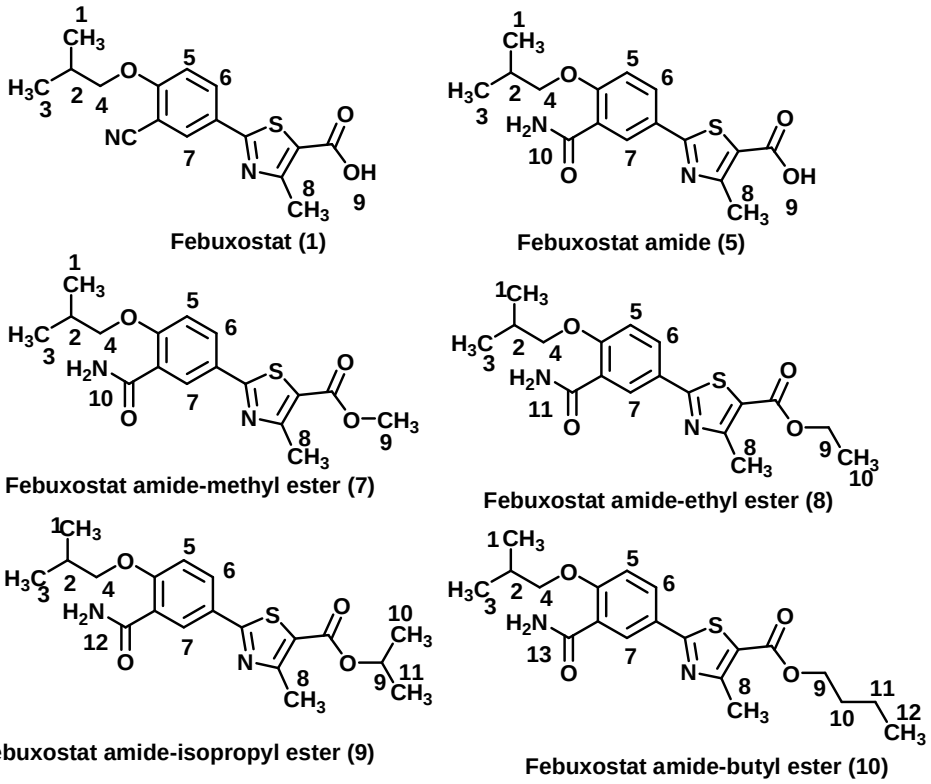
***ND-Not done**

washed with 5.0 % $NaHCO_3$ solution (3 X 20.0 mL). Separated methylene dichloride layer was dried over Na_2SO_4 (2.0 g), filtered, mother liquer was distilled completely at 40-45°C under vacuum to obtain white solid, which was purified in acetone (50.0 mL). A white solid form, filtrated, washed with acetone (10.0 mL) and dried in vacuum tray drier at 50-55°C under vacuum to give febuxostat amide-esters (7-10). [Alcoholic sol-

TABLE 4 : [1]H NMR assignments for febuxostat analogues

Position[a]	Febuxostat δ_H ppm multiplicity	Impurity 5 δ_H ppm multiplicity	Impurity 7 δ_H ppm multiplicity	Impurity 8 δ_H ppm multiplicity	Impurity 9 δ_H ppm multiplicity	Impurity 10 δ_H ppm multiplicity
1.	1.00-1.02 d	0.99-1.01 d	0.99-1.02 d	0.99-1.01 d	1.00-1.02 d	1.00-1.03 d
2.	2.01-2.15 m	2.06-2.19 m	2.06-2.19 m	2.06-2.19 m	1.97-2.10 m	2.05-2.49 m
3.	1.00-1.02 d	0.99-1.01 d	0.99-1.02 d	0.99-1.01 d	1.00-1.02 d	1.00-1.03 d
4.	3.97-3.99 d	3.96-3.98 d	3.97-3.99 d	3.97-3.99 d	3.88-3.90 d	3.97-4.02 d
5.	7.33-7.36 d	7.25-7.28 d	7.26-7.29 d	7.25-7.28 d	7.21-7.24 d	7.37-7.39 d
6.	8.17-8.21 dd	8.01-8.05 dd	8.03-8.07 dd	8.03-8.07 dd	8.06-8.10 dd	8.04-8.08 dd
7.	8.25-8.258d	8.33-8.34 d	8.35-8.36 d	8.35-8.36 d	8.18-8.19 d	8.24-8.25 d
8.	2.64 s	2.66 s	2.68 s	2.68 s	2.67 s	2.68 s
9.	13.39 s	13.36 s	3.83 s	4.25-4.33 q	5.06-5.19 m	4.24-4.28 t
10.	-	7.60-7.77 s	7.59-7.76 s	1.28-1.32 t	1.29-1.30 d	1.62-1.71 m
11.	-	-	-	7.59-7.76 s	1.29-1.30 d	1.35-1.47 m
12.	-	-	-	-	5.06-5.19 m	0.90-0.95 t
13.	-	-	-	-	1.32-1.31 d	7.59-7.77 s
14.	-	-	-	-	1.32-1.31 d	-

s=singlet; d=doublet; dd=double doublet; t=triplet; q=quartet; m=multiplet; [a]Refer structure formula of febuxostat and its febuxostat amide-esters in Figure 2 for numbering.



[*]Numbering has been assigned for [1]H NMR characterization

Figure 2 : Febuxostat 1, Febuxostat amide 5, Febuxostat amide-methyl ester 7, Febuxostat amide-ethyl ester 8, Febuxostat amide-isopropyl ester 9, Febuxostat amide-butyl ester 10

vents like methanol, ethanol, isopropanol and n-butanol were used respectively to make compounds 7, 8, 9 and 10].

General procedure for substituted alkyl-2-(4-

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isobutoxy-3-(methoxycarbonyl)phenyl)-4-methylthiazole-5-carboxylate (febuxostat diesters 11-14)

To a stirred solution of 2-(3-carboxy-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (6, 5.0 g, 0.016 mol.) and con. H_2SO_4 (3.0-4.0 mL) in alcoholic solvents (100.0 mL) at 25-30°C. Heat the reaction mass for 8-10 hrs at reflux temperature respectively based on alcoholic solvents boiling point. The mixture is allowed to cool up to 25-30°C. Reaction mass is completely distilled out at 50-55°C under vacuum to obtain oily residue. The oily residue dissolved in methylene dichloride (150.0 mL) at 25-30°C and washed with 5.0 % $NaHCO_3$ solution (3 X 10.0 mL). Separated methylene dichloride layer was dried over Na_2SO_4 (2.0 g) and completely distilled out at 40-45°C under vacuum to obtain white solid which was purified in acetone (50.0 mL). A white solid form, which was filtrated, washed with acetone (10.0 mL) and dried in vacuum tray drier at 50-55°C under vacuum to give febuxostat diesters (11-14). [Alcoholic solvents like methanol, ethanol, isopropanol and n-butanol were used respectively to make compounds 11, 12, 13 and 14].

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Reagents

Orthophosphoric acid (HPLC grade), Acetonitrile (HPLC grade), Methanol (HPLC grade), Water (Milli-Q).

Preparation of buffer

Dissolve 0.5 mL of orthophosphoric acid in 1000.0 mL of water.

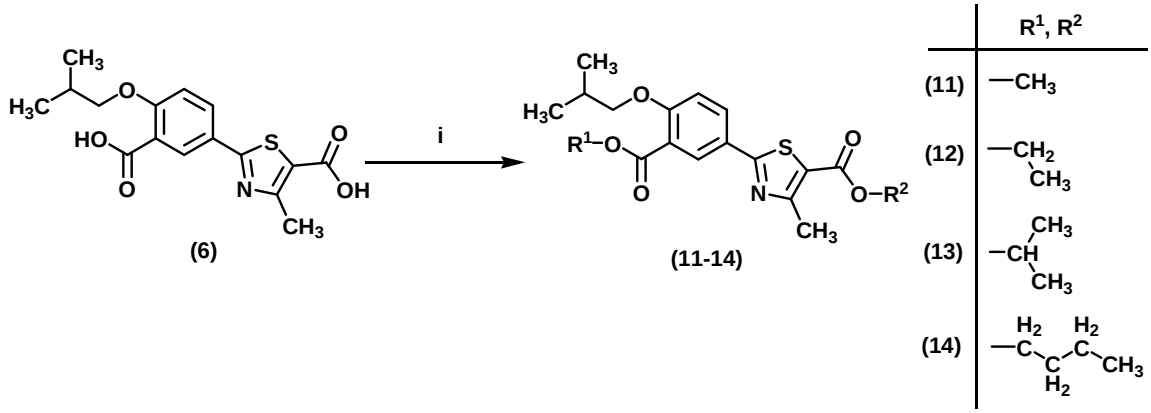
Preparation of mobile phase A

Mix buffer and acetonitrile in the ration of 60:40 v/v.

TABLE 5 : ^{13}C NMR assignments for febuxostat analogues

Position[a]	Febuxostat δ_C ppm	5 δ_C ppm	7 δ_C ppm	8 δ_C ppm	9 δ_C ppm	10 δ_C ppm
1.	18.870	19.204	19.157	19.179	19.012	ND
2.	27.752	27.728	27.686	27.705	27.980	ND
3.	18.870	19.204	19.157	19.179	19.012	ND
4.	75.284	75.195	75.208	75.233	74.819	ND
5.	159.67	159.828	158.987	158.997	159.764	ND
6.	114.017	113.972	114.009	114.035	114.080	ND
7.	133.177	130.595	130.664	130.683	131.571	ND
8.	123.059	124.007	124.096	124.089	121.764	ND
9.	131.664	128.947	129.008	129.032	128.683	ND
10.	101.712	122.299	120.402	120.771	121.219	ND
11.	115.522	163.115	165.774	165.804	161.095	ND
12.	166.334	167.883	168.487	168.422	167.943	ND
13.	125.511	124.944	124.699	124.744	124.289	ND
14.	162.202	158.849	160.620	160.485	160.253	ND
15.	17.187	17.269	17.339	17.383	17.366	ND
16.	162.987	165.853	162.000	161.583	165.184	ND
17.	-	-	52.482	61.317	69.017	ND
18.	-	-	-	14.311	21.807	ND
19.	-	-	-	-	21.807	ND
20.	-	-	-	-	68.526	ND
21.					21.807	ND
22.					21.807	ND

[a]Refer structure formula of febuxostat and its febuxostat amide-esters in Figure 3 for numbering; *ND- Not done.



Scheme 4 : Synthesis of febuxostat diesters: (i) Esterification reaction: Con. H_2SO_4, alcoholic solvents [methanol for 11, ethanol for 12, isopropanol for 13 and n-butanol for 14]

Preparation of mobile phase B

Mix acetonitrile and methanol in the ration of 50:50 v/v.

Diluent

Mix water and acetonitrile in the ration of 10:90 v/v.

Chromatographic conditions

Column: Hypersil BDS C-18, 5µ (150 X 4.6 mm) or equivalent

Pump mode: Gradient

Gradient program

Time (min.)	*Mobile phase-A (% v/v)*	*Mobile phase (% v/v)*
0.001	100.0	0.0
35.0	25.0	75.0
75.0	25.0	75.0
77.0	100.0	0.0
85.0	100.0	0.0
Flow rate :		1.5 mL/min.
Injection volume:		20.0 µL
Column temperature:		20.0 °C
Wave length:		317.0 nm
Run time:		85.0 min.

Preparation of solutions

Reference stock solution

Accurately weight about 2.0 mg of Febuxostat standard into 100.0 mL clean dry volumetric flask, then added 20.0 mL of diluent sonicate to dissolve, make up to volume with diluent. Further dilute 5.0 mL of above solution to 50.0 mL with diluent.

Reference solution

Dilute 10.0 mL of reference stock solution into a 100.0 mL clean dry volumetric flask, make up to volume with diluent.

Sample solution

Accurately weight about 20.0 mg of sample and into a 100.0 mL clean dry volumetric flask, added 20.0 mL of diluent, sonicate to dissolve, make up to volume with diluent.

Evaluation of system suitability

Inject 20.0 µL of blank into the chromatograph and record the chromatogram. Inject 20.0 µL of reference solution (six replicate) into the chromatograph and record the chromatogram. % RSD for six replicate injections of reference solution is not more than 5.0.

Procedure

Inject 20.0 µL of blank into the chromatograph and record the chromatogram. Inject 20.0 µL of sample solution into the chromatograph and record the chromatogram. Examine the blank chromatograms for any peaks due to blank and disregard corresponding peaks observed in the chromatogram of the sample solution.

Retention time of Febuxostat is about 10.0 min

RESULTS AND DISCUSSION

Febuxostat 1 was synthesized by following the known synthetic sequence (Scheme 1). During the process development, ten unknown analogues were detected in crude at significant levels (0.1 - 015 %). On

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TABLE 6 : Chromatographic purity of Febuxostat, febuxostat amide and its febuxostat amide-esters, Febuxostat diacid and febuxostat di esters

Febuxostat, febuxostat amide-ester and febuxostat di-esters.	Purity by HPLC (By % area normalization)	
	Retention time (min.)	Purity (%)
Febuxostat 1	10.20	99.74
Febuxostat amide 5	4.35	99.53
Febuxostat diacid 6	5.18	99.07
Febuxostat amide-methyl ester 7	8.65	93.26
Febuxostat amide-ethyl ester 8	12.37	94.84
Febuxostat amide-isopropyl ester 9	-	ND
Febuxostat amide-butyl ester 10	-	ND
Febuxostat dimethyl ester 11	22.54	98.33
Febuxostat diethyl ester 12	27.35	68.69
Febuxostat diisopropyl ester 13	20.67	86.25
Febuxostat dibutyl ester 14	51.64	96.95

the basis of LC-MS data and chemistry involved in the process, tentative structures were proposed. (TABLE 1). From the synthetic sequence (Scheme 1), Synthesis of febuxostat 1 by using basic hydrolysis (Step 2), febuxostat amide 5 and febuxostat di acid 6 were formed *via* hydrolysis of nitrile group of compound 1. Febuxostat amide 5 is an intermediate during basic hydrolysis of cyno compound 1. Synthesis of 5 & 6 began form compound 1. Basic hydrolysis of compound 4 with NaOH in aqueous n-butanol furnished acid 1. Finally synthesis of febuxostat amide 5 was achieved from compound 1 by sequential $-CONH_2$ bond formation which was further hydrolysis to form febuxostat diacid 6 (Scheme 2). It is expected that febuxostat methyl ester was derived in hantzsch heterocyclization thiazole synthesis (Step 1) of thioamide compound (2) with halo diketo compound (3), methyl-2-chloro-3-oxobutanoate present as a KSM impurity in the starting material halo diketo compound (ethyl-2-chloro-3-oxobutanoate 3), during the basic hydrolysis of febuxostat ethyl ester (4) in aqueous n-butanol, unreacted febuxostat methyl ester was further hydrolysised formed febuxostat amide-methyl ester impurity (7) and 2-isobutoxy-5-(5-(methoxycarbonyl)-4-methylthiazol-2-yl)benzoic acid which is carryover along with acid (1) and further converted in to febuxostat di methyl ester impurity (11) during febuxostat crystat A synthesis (Step 3) of febuxostat (1). Synthesis of this impurity accomplished from febuxostat amide impurity (5) and febuxostat di acid impurity (6) in methanol, by using con. H_2SO_4 as a catalyst in esterification reaction as shown in Scheme 3, 4.

During the basic hydrolysis of febuxostat ethyl ester (4) in aqueous n-butanol, to form febuxostat amide-ethyl ester (8) and 5-(5-(ethoxycarbonyl)-4-methylthiazol-2-yl)-2-isobutoxybenzoic acid unreacted which is explained by esterification with Step-2 by-product ethanol to formed febuxostat di ethyl ester (12) was carryover along with acid (1). Synthesis of febuxostat di ethyl ester (12) was accomplished from acid (1) in ethanol, by using con. H_2SO_4 as a catalyst in esterification reaction as shown in Scheme 3, 4. The formation of febuxostat isopropyl ester is explained by transesterification of halo di keto compound (3), where in ethyl group was substituted with isopropyl group during heterocylization reaction. Unreacted febuxostat isopropyl ester was further hydrolysised form febuxostat amide-isopropyl ester (9) and 2-isobutoxy-5-(5-(isopropoxycarbonyl)-4-methylthiazol-2-yl)benzoic acid which is carryover along with acid (1) and further converted in to febuxostat di isoproyl ester (13) during febuxostat synthesis (Step 3) of febuxostat (1). Compounds (9, 13) were independently synthesized by esterification of febuxostat amide (5) and 2-isobutoxy-5-(5-(isopropoxycarbonyl)-4-methylthiazol-2-yl)benzoic acid in isopropanol, by using con. sulphuric acid as a catalyst in esterification reaction (Scheme 3,4). Febuxostat butyl ester is a process realated impurity resulting during isolation of febuxostat (1) (acidic pH adjustment) in step 2. During the basic hydrolysis of febuxostat ethyl ester (4) in aqueous n-butanol, to form febuxostat butyl ester which is further hydrolised and converted into febuxostat amide-butyl ester (10) and 5-(5-(butoxycarbonyl)-4-methylthiazol-2-yl)-2-isobutoxy benzoic acid unreacted which is explained by esterification with reaction solvent n-butanol to formed febuxostat di butyl ester (14) was carryover along with acid (1). Synthesis of febuxostat di butyl ester impurity (14) was accomplished febuxostat di acid (6) in n-butanol, by using con. H_2SO_4 as a catalyst in esterification reaction as shown in Scheme 3, 4. These ten impurities were characterized and confirmed by ^1H and ^{13}C NMR, Mass, IR spectroscopic data and elemental analysis (TABLE 3-7). Chromatographic pu-

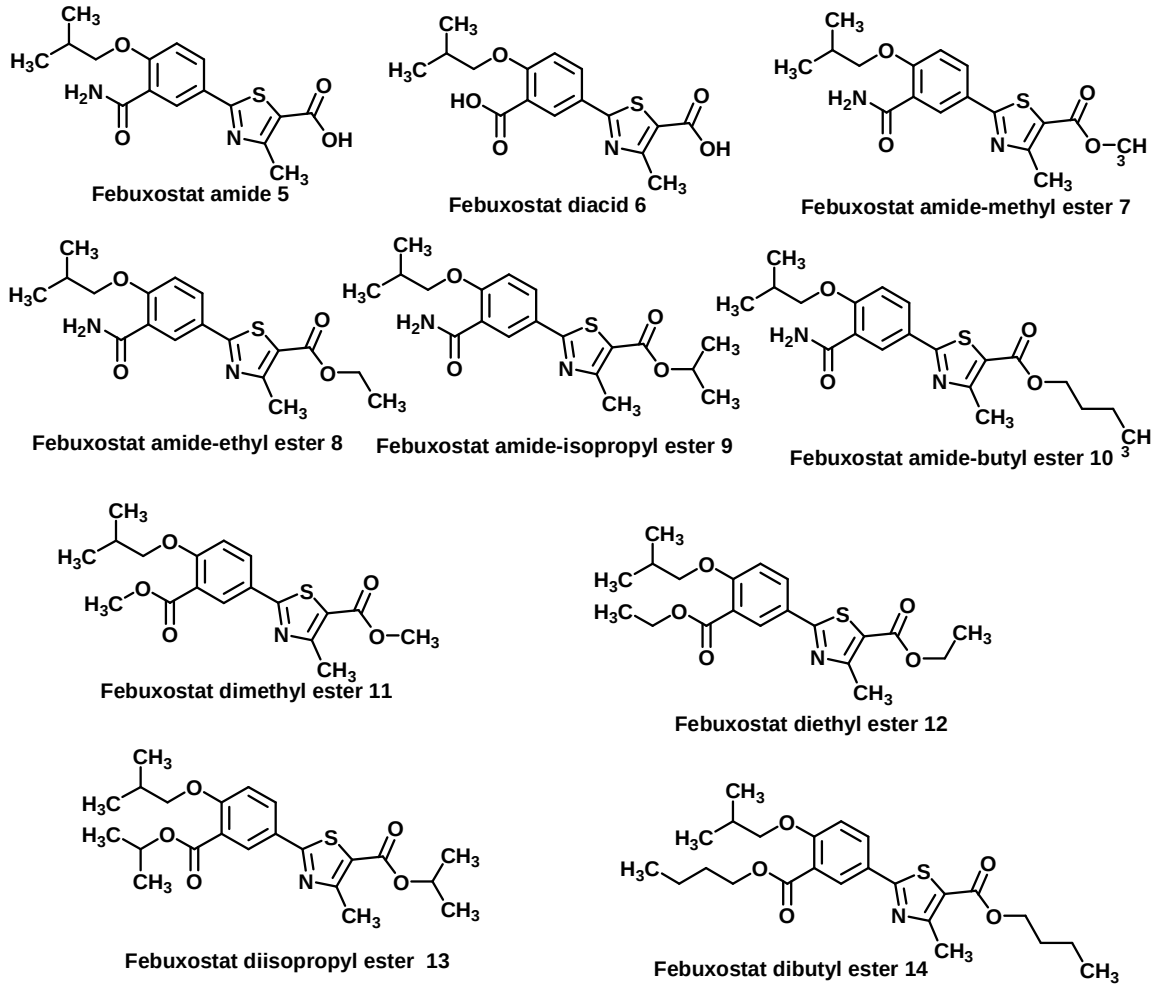

Figure 3 : Structure of critical process related analogues of febuxostat (5-14)

rity of impurities were tabulated in TABLE 6.

CONCLUSIONS

In conclusion, the structures of analogues of fubuxostat and their root cause of formation during the synthesis of the febuxostat were identified. The requirements to synthesize and confirm the structures of proposed analogues of febuxostat have been fulfilled.

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