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Structural characterization of impurities in pioglitazone

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In the pioglitazone bulk drug three prominent impurities I-III were detected up to concentrations of 0.1% (ranging from 0.05–0.1%) by reversed phase HPLC. These impurities were isolated from enriched mother liquor samples and characterized as 5-(4-hydroxybenzyl)-1,3-thiazolidine-2,4-dione (I) 5-(4-fluorobenzyl)-1,3-thiazolidine-2,4-dione (II), 2-[2-(4-bromophenoxy) ethyl-5-ethyl pyridine (III) based on their ^{1}H, and ^{13}C NMR, DEPT, Mass and IR spectral data. Structure elucidation and synthesis of these impurities is discussed.

1. Introduction

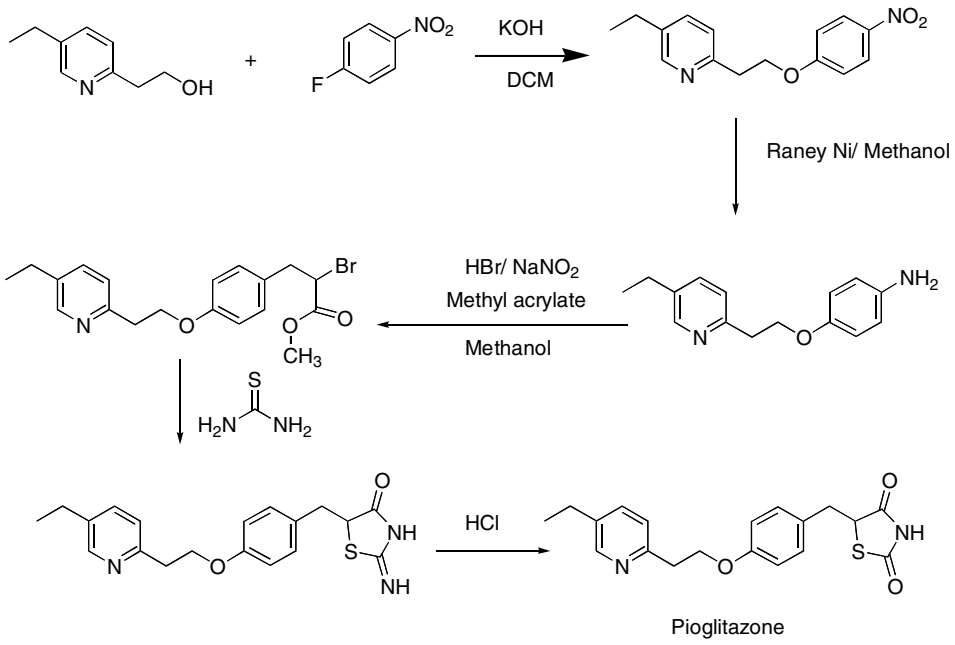
Pioglitazone is a hypoglycemic agent used in the treatment of obese noninsulin-dependent diabetes mellitus (NIDDM). The analysis of pioglitazone bulk drug revealed the presence of three impurities in concentrations up to 0.1%. A HPLC method (Radhakrishna et. al. 2002) was used for this analysis. As per the stringent regulatory requirements the impurity profile study has to be carried out for any final product to identify and characterize all the unknown impurities that are present in level of $> 0.1\%$ (ICH Guideline, 2002). This paper aims the characterization of impurities present in pioglitazone and their synthesis to elucidate their structures.

2. Investigations and results

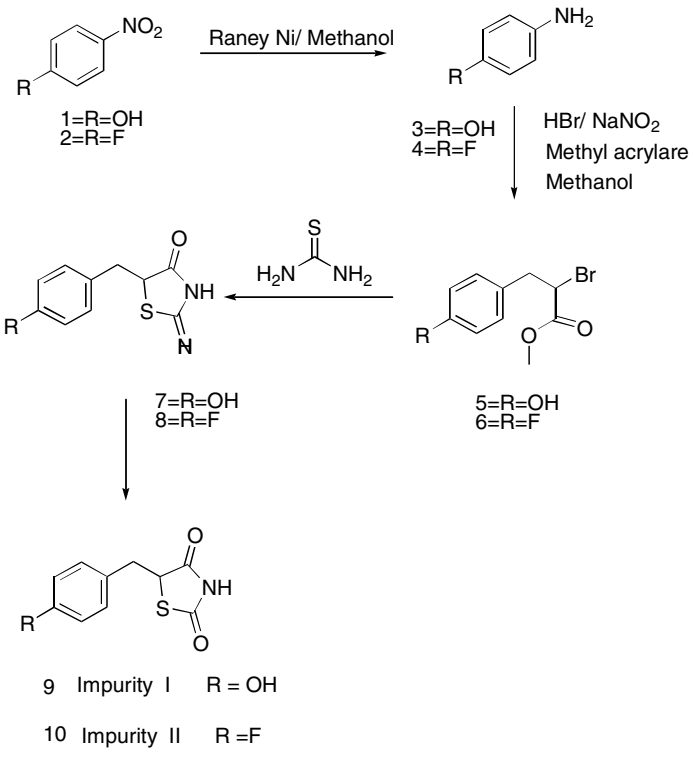
2.1. Detection of impurities I, II and III

Pioglitazone was analyzed by HPLC under the analytical conditions described in section 2.2. The chromatogram displayed three impurities as displayed in the Fig. Retention times in minutes: 3.43 (imp-I), 5.59 (imp-II) 8.70 (pioglitazone) and 18.0 (imp-III). These impurities were

Scheme 1



Scheme 2



isolated by preparative HPLC from mother liquor sampler where these impurities were enriched. The collected impurity fractions were subjected to analytical HPLC and spectroscopic studies. The synthesis and structural elucidation of these impurities is discussed in the following sections.

2.2. Synthesis of pioglitazone

The synthesis of pioglitazone (Prous et. al. 1990) is shown in Scheme 1.

2.3. Synthesis of the impurities

2.3.1. Synthesis of impurity I

The synthesis of impurity I is shown in Scheme 2. Reduction of 4-hydroxy nitrobenzene (**1**) with Raney Nickel followed by diazotization of the resulting amino compound **3** with sodium nitrate and subsequent condensation with methyl acrylate yielded the corresponding ester **5**. This ester was cyclized with thiourea leading to the imino compound **7**, which formed impurity I (**9**) on hydrolysis with HCl.

Table 1: IR and Mass spectral data for pioglitazone and impurities

P	IR: 3082 cm^{-1} (Aromatic C-H stretching) 2964 cm^{-1} (Aliphatic C–H stretching) 1736,1690 cm^{-1} (C=O stretching) 1472,1331 (Aliphatic C–H bending) 1254, 1040 cm^{-1} (Aryl alkyl ether C–O–C stretching) 841,738 (Aromatic C-H bending) MS: m/z, 150,134,121 M^+= 356
I	IR: 3392 cm^{-1} (O–H stretching) 3059 cm^{-1} (Aromatic C–H stretching) 1761,1683 cm^{-1} (C=O stretching) 1242 cm^{-1} (C–O stretching) 831,724 (Aromatic C–H bending) MS: m/z, 107 $M^+ = 223$
II	IR: 3184 cm^{-1}(N-H stretching) 3058 cm^{-1} (Aromatic C–H stretching) 1765,1683 cm^{-1}(C=O stretching) 1228 cm^{-1} (Aromatic C-F stretching) 829,723 (Aromatic C-H bending) MS: m/z, 109 $M^+ = 225$

2.3.2. Synthesis of impurity II

Extension of aforementioned synthetic scheme to 4-fluoronitrobenzene (**2**), one of the precursors for pioglitazone, furnished impurity II (Scheme 2) (**10**).

Table 2: NMR assignments of pioglitazone

Position*	^1H	δ (ppm)	J(Hz)**	^{13}C	DEPT
2	–	–	–	151.03	–
3	1 H	8.05	d, 8.0	127.31	CH
4	1 H	8.45	dd, 2.0, 8.0	145.80	CH
5	–	–	–	141.43	–
6	1 H	8.77	d, 1.6	139.86	CH
7	2 H	2.78	q, 7.8	24.55	CH_2
8	3 H	1.22	t, 7.8	14.52	CH_3
9	2 H	3.45	t, 6.4	32.29	CH_2
10	2 H	4.36	t, 6.4	65.47	CH_2
12	–	–	–	156.92	–
13, 17	2 H	6.85	d, 8.6	114.43	CH
14, 16	2 H	7.13	d, 8.6	130.39	CH
15	–	–	–	129.01	–
18	Ha	3.27	dd, 4.4, 14.4	36.19	CH_2
	Hb	3.04	dd, 8.8, 14.4	–	
19	1 H	4.85	d, d, 4.4, 8.8	52.94	CH
20	–	–	–	175.58	–
21	NH[3]	12.0	s	–	–
22	–	–	–	171.58	–

* Refer the structural formula in Scheme 1–3

** This corresponds to the ^1H-^1H multiplicity and coupling constants s-singlet, d-doublet, t-triplet, dd-doublet of doublet, br-broad

Table 3: NMR assignments of impurities I and II

Position*	Impurity I					Impurity II			
	1 H	δ (ppm)	J(Hz)**	^{13}C	DEPT	1 H	δ (ppm)	J(Hz)**	^{13}C
2	–	–	–	171.9	–	–	–	–	171.6
3	NH	11.45	br, s	–	–	NH	11.40	br,s	–
4	–	–	–	175.9	–	–	–	–	175.7
5	1 H	4.42	dd, 4.0, 9.8	53.4	CH	1 H	4.50	dd, 4.0, 9.8	52.7
6	Ha	3.40	dd, 4.0, 14.2	36.6	CH_2	Ha	3.45	dd, 4.0, 14.2	36.3
	Hb	3.03	dd, 9.8, 14.2			Hb	3.20	dd, 9.8, 14.2	
7	–	–	–	126.9	–	–	–	–	132.9
8	1 H	7.03	d, 8.2	130.4	CH	1 H	7.23	dd, 4.0,6.6	131.3 d, 8.3***
9	1 H	6.77	d, 8.2	115.4	CH	1 H	7.01	dd, 4.0,6.6	115.2 d, 20***
10	–	–	–	156.5	–	–	–		162.0 d, 242***
11	1 H	6.77	d, 8.2	115.4	CH	1 H	7.01	dd, 4.0,6.6	115.2 d, 20***
12	1 H	7.03	d, 8.2	130.4	CH	1 H	7.23	dd, 4.0,6.6	131.3 d, 8.3***
13	OH	9.0	br	–	–	–	–	–	–

* Refer the structural formula in Scheme 1–3
** This corresponds to the ^{1}H-^{1}H multiplicity and coupling constants
*** This corresponds to the ^{13}C-^{19}F multiplicity and coupling constants s-singlet,d-doublet,t-triplet,dd-doublet of doublet,br-broad

Table 4: NMR assignments of impurity III

Position*	1 H	δ (ppm)	J(Hz)**	13C	DEPT
1	–	–	–	157.7	–
2	1 H	7.28	d, 7.2	131.9	CH
3	1 H	6.79	d, 7.2	116.9	CH
4	–	–	–	157.7	–
5	1 H	6.79	d, 7.2	116.9	CH
6	1 H	8.39	d, 7.2	131.9	CH
7	2 H	4.30	t, 6.6	67.0	CH_2
8	2 H	3.22	t, 6.6	36.6	CH_2
9	–	–	–	155.1	–
10	1 H	7.48	d, 8.6	129.3	CH
11	1 H	7.20	d, 8.6	148.4	CH
12	–	–	–	136.5	–
13	1 H	7.30	s	135.5	CH
14	2 H	2.63	q, 7.4	24.9	CH_2
15	3 H	1.24	t, 7.4	15.2	CH_3

* Refer the structural formula in Scheme 1–3
** This corresponds to the ^{1}H-^{1}H multiplicity and coupling constants s-singlet, d-doublet, t-triplet, q-quartet

2.3.3. Synthesis of impurity III

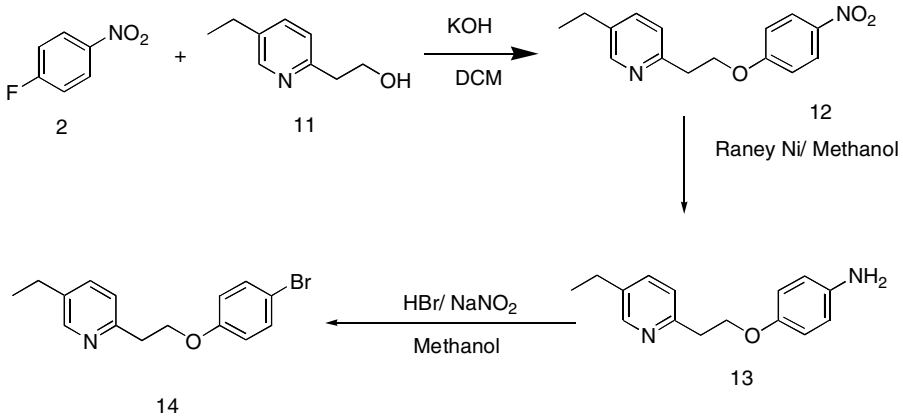
The synthesis of impurity III is shown in Scheme 3. 5-Ethyl-pyridine-2-ethanol (**11**) on condensation with 4-fluoro-nitrobenzene (**2**) in the presence of KOH yielded aryl ether **12**. Reduction of **12** by Raney Nickel to yield the amino compound **13** and subsequent diazotization using sodium nitrate and HBr in the presence of cuprous oxide led to the formation of an aromatic substituted bromo compound, which is impurity III (**14**).

The FT-IR and MS data assignments for pioglitazone and the impurities I, II, III summarized in Table 1. The ^{1}H, ^{13}C NMR and DEPT spectral assignments for pioglitazone and the impurities are listed in Table 2 and Table 3 and Table 4 respectively. Impurities I, II, and III isolated from bulk drug were found to be identical (by co injection in HPLC) with the authentic samples, thus confirming their assigned structures.

3. Discussion

The CI mass spectrum of impurity I displayed the protonated molecular ion at m/z 224. The abundance of M+2 up to 4.2% indicated the presence of one sulphur atom, which in turn indicated the intactness of thiazolidine dione ring in the impurity I structure. Presence of carbonyl and hydroxy functions in impurity I was evidenced by the appearance of corresponding absorptions at 1675 cm^{-1} and 3394 cm^{-1}, respectively in the IR spectrum. A broad deuterium exchangeable signal at 9.0 ppm in the ^{1}H NMR spectrum was attributed to OH proton. The presence of quartenary carbon at 156.5 ppm in ^{13}C NMR further supported the OH group presence.

Scheme 3



Based on the above spectral data observations the structure of impurity I was characterized as 5-(4-hydroxybenzyl)-1,3-thiazolidine-2, 4-dione (**9**).

The CI mass spectrum of impurity II displayed the protonated molecular ion at m/z 226. The abundance of M + 2 up to 4.2% indicated the possibility of sulphur presence, which in turn indicated the presence of thiazolidine dione ring intact in the impurity II also. Unlike in the case of impurity II, the ^{1}H NMR spectrum showed the presence of only one exchangeable proton (NH). The splitting pattern distortion in aromatic region was also distinctly different from that impurity I.

The observation of an additional splitting in para-disubstituted pattern in the aromatic region evidenced the fluorine substitution in place of the hydroxy group. Further the splitting of carbon signals in ^{13}C NMR as doublets (one aromatic quartenary carbon and four aromatic methine carbons) lends support to the fluorine substitution.

Based on the above spectral data the structure of impurity II was characterized as 5-(4-fluorobenzyl)-1,3-thiazolidine-2, 4-dione (**10**).

The CI mass spectrum of Impurity III displayed the protonated molecular ion at m/z 306 with characteristic bromine abundance of $M^+ + 2$, evidencing the presence of bromine in impurity III. The IR spectrum was devoid of carbonyl absorption thus indicating the absence of a thiazolidine dione ring in impurity III. The ^{1}H NMR spectrum displayed one quartet and triplet in upfield region along with one singlet in down field at δ 8.40 ppm, suggesting the presence of an ethyl substituted pyridine moiety like in pioglitazone. The presence of two triplets at δ3.30 and 4.30 ppm further supports the linkage between two aromatic rings like in pioglitazone.

Based on the above spectral data the structure of impurity III was characterized as 2-[2-(4-bromophenoxy) ethyl-5-ethyl pyridine (**14**).

Impurity I can be formed by the degradation of pioglitazone by cleavage at the ether linkage under acidic conditions in hydrolysis stage. Formation of impurity II can be rationalized from the reactions of 4-fluoro nitrobenzene, which will be in trace levels without participating condensation with 5-ethyl pyridine 2-ethanol in pioglitazone synthesis. The impurity III might be formed by diazotization reaction with the possibility of sand mayer reaction.

4. Experimental

4.1. Samples

The investigated samples were obtained from synthetic R & D laboratory of Dr. Reddy's Laboratories Ltd., Bulk Actives -Unit-III, Hyderabad, India. The impurities were synthesized from the same laboratory.

4.2. HPLC (analytical)

A Waters Model Aliance 2690-separation module equipped with a waters 996-photo diode array UV detector was used. The analysis was carried out on a Hichrom-RPB column, 250 × 4.6 mm i.d., 5 µm particle size (Hichrom Ltd. UK) with a mobile phase consisting of 0.01 M KH_2PO_4 (E. Merck) (pH adjusted to 6.0 with 0.1N KOH) and acetonitrile (E. Merck) in the ratio of 50 : 50 (v/v) was used with UV detection at 225 nm at a flow rate of 1.0 ml/min. The data was recorded using Waters Millennium software.

4.3. HPLC (preparative)

A Waters delta 4000 preparative chromatography system equipped with Waters 2487 UV-Vis detector, fraction collector model Waters FCM-II and Rheodyne injector Model 7725I with 1.0 ml loop was used. A 250 × 20 mm i.d column packed with 5 µm Inertsil ODS (GL sciences Inc. Japan) was employed for separation. The mobile phase consisted of 0.01 M CH_3COONH_4 : CH_3CN in the ratio of 40 : 60 (v/v). The flow rate was set at 5.0 ml/min. Detection was carried out at 225 nm.

4.4. NMR spectroscopy

The ^{1}H, ^{13}C and DEPT spectra were recorded on a Varian 200 Gemini spectrometer. The ^{1}H (200 MHz) and ^{13}C NMR (50 MHz) were recorded using TMS and $CDCl_3$ as internal standards respectively.

4.5. Mass spectrometry

Mass spectra (70 eV) were recorded on a HP5989A mass spectrometer. The samples were introduced with a particle beam interface using LC and Reodyne injector. The source manifold and quadrupole temperatures were maintained at 250 and 100 °C respectively. Isobutane was used as reagent gas for chemical ionization (CI) mode.

4.6. FT-IR spectroscopy

FT-IR spectra were recorded on a Perkin-Elmer model Spectrum GX series FT-IR as KBr pellet and neat spectra.

References

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