Department of Technology of Organic Compunds[1], Faculty of Chemical Technology, Univerzity of Pardubice, Zentiva a.s.[2], Praha, the Czech Republic

Pioglitazone impurities

J. Richter[1], J. Jirman[2], J. Havlíček[2], R. Hrdina[1]

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Jindrich Richter, Univerzity of Pardubice, Faculty of Chemical Technology, Department of Technology of Organic Compounds, TP Doubravice, Studentská 95, 532 10, Pardubice, Czech Republic
Jindrich.richter@zentiva.cz josef.jirman@zentiva.cz

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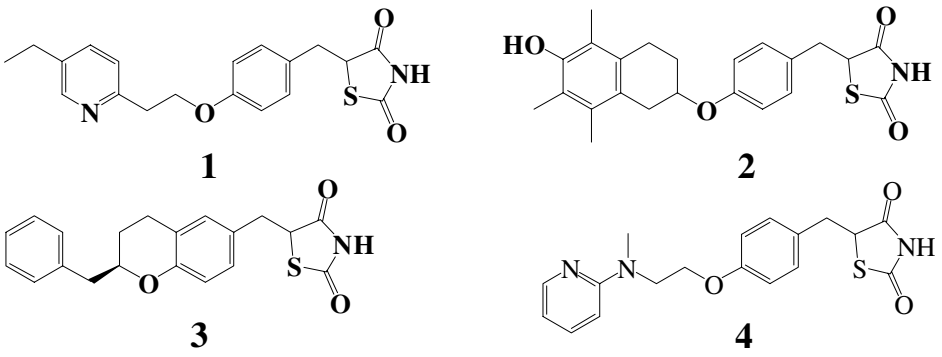
Methods of preparation of API pioglitazone were discussed from the point of view of impurities occurrence. Four real impurities (**I–IV**) of pioglitazone were prepared and characterized by means of NMR spectroscopy.

1. Introduction

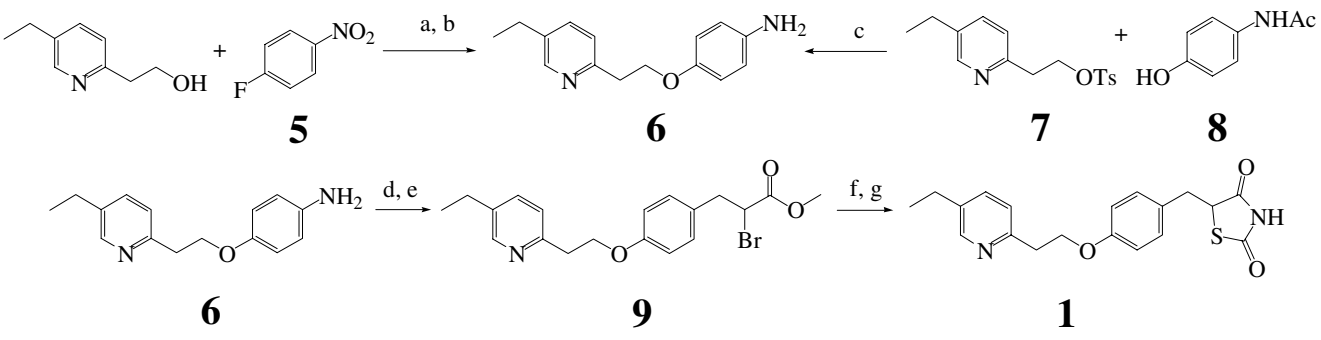
Glitazones, being derivatives of thiazolidine-2,4-dione are used in the treatment of diabetes mellitus type II (non-insulin-dependent diabetes mellitus, NIDDM). They influence insulin resistance and increase body sensitivity to insulin. A large number of glitazone type antidiabetics were developed and tested. Only some of them were used in clinical practice: pioglitazone (**1**) (Sohda et al. 1990) (Takeda Chemical Industries, Inc./Upjohn), troglitazone (**2**) (Sankyo and Parke-Davis), englitazone (**3**) (Pfizer) or rosiglitazone (Oakes et al. 1994) (**4**) (SmithKline Beecham). Most successful on the market are pioglitazone and rosiglitazone. Metabolism of glitazones was studied recently and metabolites of pioglitazone (Tanis et al. 1996) and rosiglitazone (Baldvin et al. 1999; Cox et al. 2000) are described.

We are focused on the problem of impurities in pioglitazone, because there is a need to know the structure and the quantity of all impurity in pharmaceutical ingredients. Most of synthetic impurities have the basic thiazolidine skeleton and we can suppose their biological activity. The

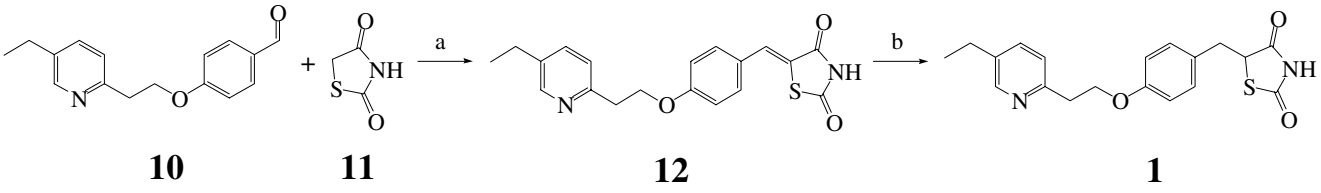
Scheme 1



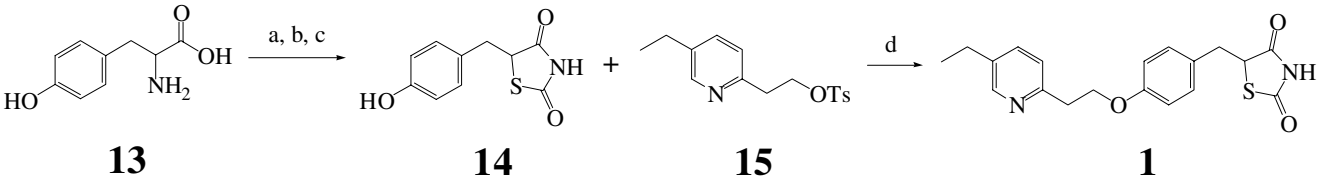
Scheme 2 (a) NaH, DMF; (b) Pd-C, H_2; (c) base; (d) $NaNO_2$; (e) methylacrylate, HBr, Cu_2O; (f) thiourea; (g) HCl



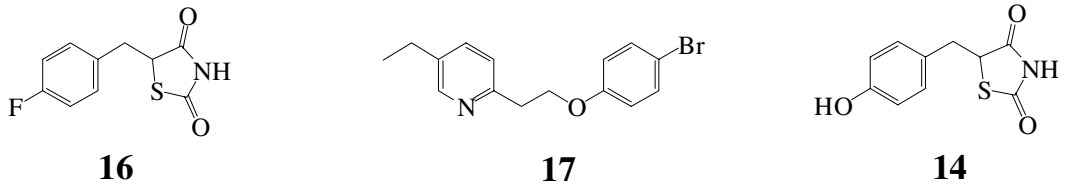
Scheme 3 (a) base; (b) Pd–C, H_2



Scheme 4 (a) $NaNO_2$, HBr; (b) thiourea; (c) HCl; (d) base



Scheme 5



main group of impurities arises from intermediates and products of side reactions during the preparation process. The presence of a particular impurity in the final product is often typical for the method of preparation.

Several methods for the preparation of glitazones are described. Every method has also several modifications, published mainly in the patent literature.

For pioglitazone three methods of preparation are most famous.

The oldest one is based on Meerwein arylation of substited 4-hydroxyaniline (**6**) and followed by closure of the thiazolidindione ring. The key intermediate **6** can be prepared by an older procedure starting from 4-fluoraniline (Meguro and Fujita 1985) (**5**) or starting from the cheaper *N*-(4-hydroxy-phenyl)acetamide (which is used also as an active pharmaceutical ingredient) (Halama et al. 2002) (**8**) (Scheme 2).

The second process is based on Knovenagel condensation of aldehyde **10** with the thiazolidinedione (**11**) and following benzylidene intermediate double bond reduction (**12**) (Momose et al. 1991; Dolitzky 2003) (Scheme 3).

The third published process is starting from L-tyrosine (**13**) (Halama et al. 2002) (Scheme 4).

Other processes for pioglitazone or glitazones preparation have been patented. For example the process starting from phenyllactic acid or process based on Darzens condensation of aromatic aldehyde and halogenacetic acid (Thijs and Zhu 2005).

Three pioglitazone impurities **16**, **17**, **14** were described besides of metabolites and their preparation was also published (Kumar et al. 2004).

These compounds are typical for the oldest process of pioglitazone preparation starting from 4-fluoronitrobenzene (**5**) (Scheme 2). Compound **14** in this case is not related to the process of preparation but is a decomposition product of pioglitazone.

Benzylidene intermediate **12** and its determination in bulk and pharmaceutical formulations was described earlier (Radhakrishna et al. 2002). Benzylidene intermediate **12** is present in the commercial pioglitazone prepared by Knovenagel condensation (Scheme 3).

2. Investigations, results and discussion

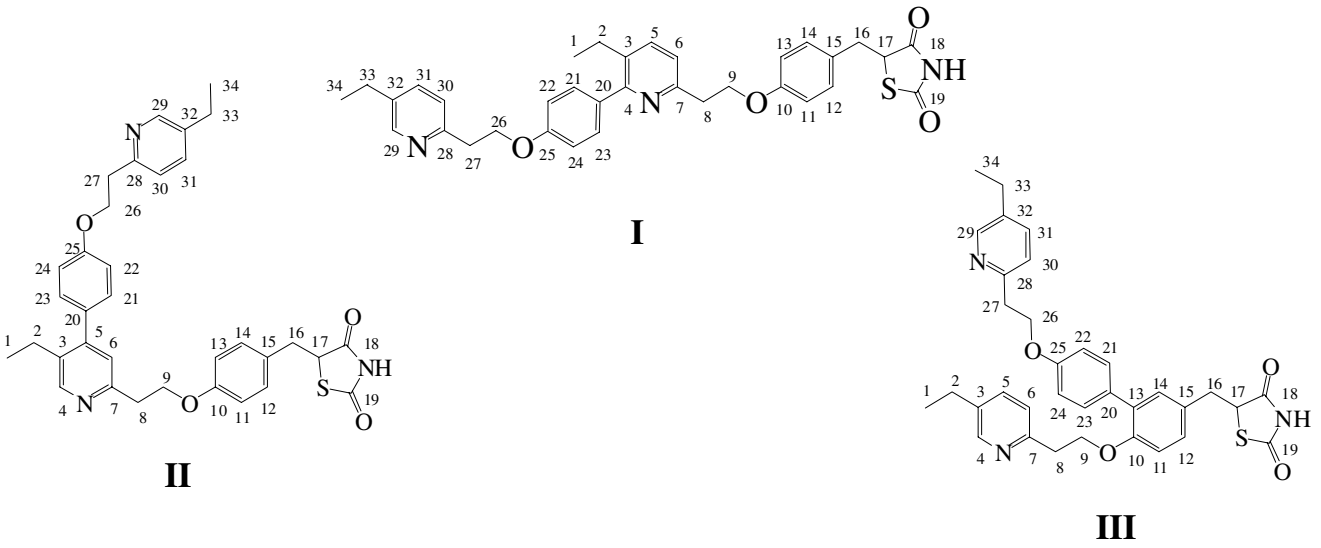
The content and nature of impurities in pioglitazone depends on the method of preparation. In the pioglitazone prepared by Meerwein arylation (Scheme 2) we can detect and identify besides impurity **17** also set of impurities with m/e 581. These compounds have practically the same fragmentation in the MS spectra so they cannot be distinguished from each other. Therefore these impurities were separated by preparative chromatography and structures were determined by assigning of NMR signals (see later). These isomeric impurities have structures **I**, **II** and **III**.

The remaining two possible isomers **18** and **19** were not detected in the product. Also the presence of compound **20** was not confirmed. Standard of compound **20** has been prepared from the disubstituted urea (**21**) (Scheme 8).

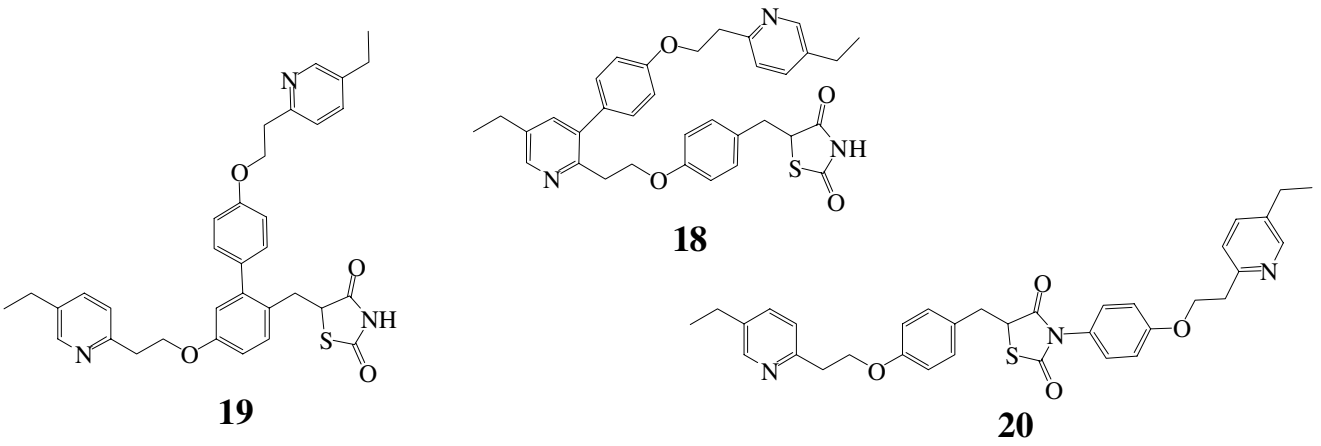
Pioglitazone prepared by the method starting from tyrosine (Scheme 4) contains the impurities **14** and **IV**. Compound **IV** is formed by the consecutive alkylation of pioglitazone (**1**) by the alkylating agent **15** (Scheme 9).

All ^1H and ^{13}C NMR signals of compound **I** were assigned on the basis of 1D and 2D experiments, mainly homonuclear g-COSY and heteronuclear ^1H-^{13}C g-HSQC and ^1H-^{13}C g-HMBC experiments. These heteronuclear 2D experiments are inversion experiments. The resolution in F1 domain in such NMR experiments is worse than

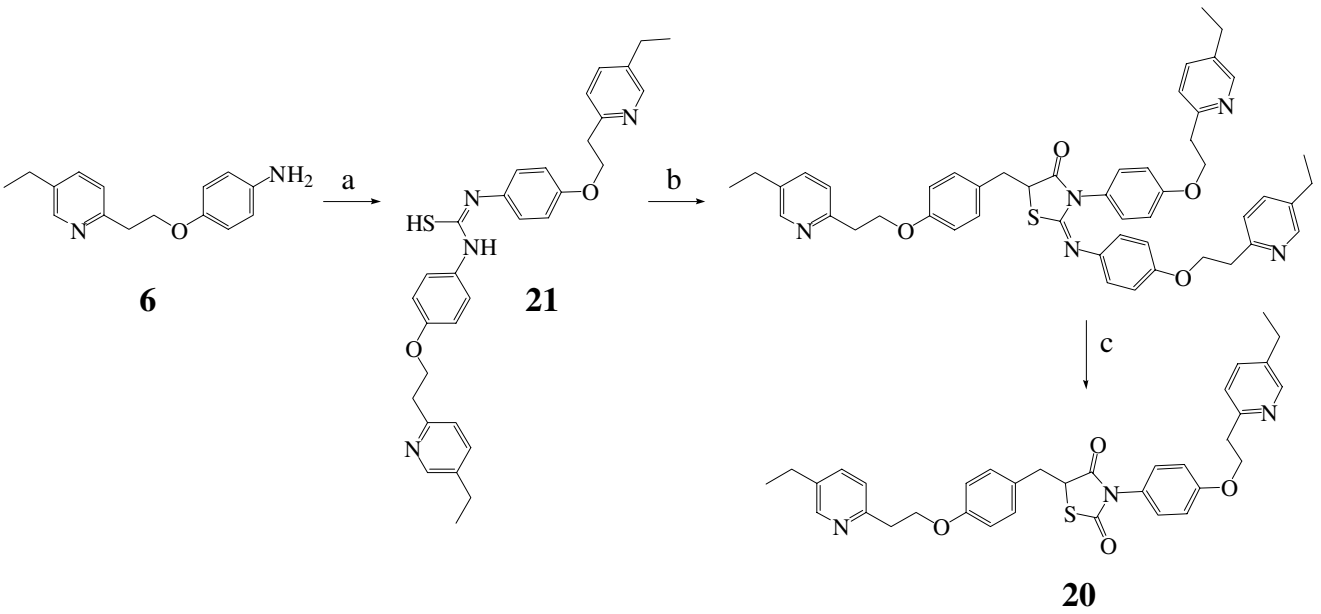
Scheme 6



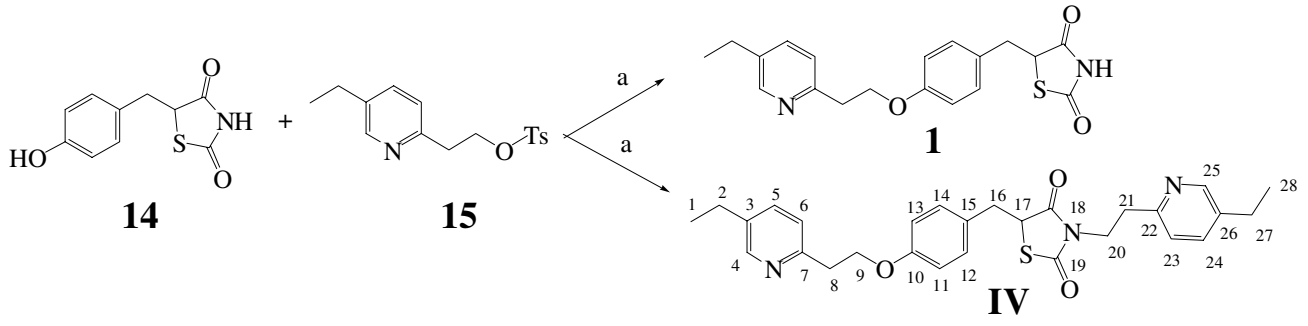
Scheme 7



Scheme 8 (a) CS_2, EtOH; (b) compound (**9**), EtOH; (c) aqueous HCl



Scheme 9 (a) KOH, EtOH



that direct detection experiments like ^{1}H-^{13}C HETCOR or ^{1}H-^{13}C COLOC. Nevertheless resolution in F1 domain was so adequate to distinguish and assign even the ^{13}C NMR signals of quaternary carbons C-10 and C-25 differs each other only 0.2 ppm. Signals of methyl groups C-1 and C-34 were also unambiguously assigned on the basis of combination of HSQC and HMBC optimized for three-bond coupling constant $^{3}J(C, H) = 8$ Hz.

The identification of pioglitazone impurities, when pioglitazone was prepared by Meerwein arylation, gives the opportunity for decreasing their content in the active pharmaceutical ingredient and consequently to fulfil the drug authorities requirements for drug production.

3. Experimental

3.1. Chemicals and apparatus

Nuclear magnetic resonance was measured in $CDCl_3$ solutions on BRUKER AVANCE 500 spectrometer, (Bruker Analytische Messtechnik GmbH) at 500.13 MHz (^{1}H) and 125.77 MHz (^{13}C) respectively. 1D the experiments were carried out using a 5 mm TXO probe. For the 2D experiments the inverse TBI probe was used. All experiments were performed in the solvent $CDCl_3$. For the assignment the ^{1}H-^{13}C g-HSQC and ^{1}H-^{13}C g-HMBC from Bruker library were used. The ^{1}H-^{13}C g-HSQC dataset consisting of 2K and 256 points in F_2 and F_1 dimensions, respectively was chosen. The number of scans was 8 for each t_1 increment. The time domain data was zero-filled to 1024 and 2K data points in F_1 and F_2 dimensions, respectively, and processed with sinusoidal squared sine-bell window function in both the dimensions. The gradient-selected ^{1}H-^{13}C HMBC was performed using 4K and 512 points in F_2 and F_1 dimensions, respectively. The number of scans was 32 for each t_1 increment. The acquired data was zero-filled to 1024 and 2K data points in F_1 and F_2 dimensions, respectively, and processed with sinusoidal squared sine-bell window function in both the dimensions.

Mass spectra were recorded on API 3000 LC MS/MS apparatus equipped with E-spray ionization, (PE SCIEX).

Preparative column chromatography was made on silica gel 60 (Fluka) and reverse phase of silica Lichroprep RP-18e (Merck).

Formate buffer was prepared by dissolving of 6.3 g of ammonium formate in 1 L of distilled water. The pH was adjusted to 6 by the addition of diluted (10%) formic acid.

Thin layer chromatography was made on FPKG60 F254 plates (Merck) in the mixture $CHCl_3$-methanol-NH_4OH (25%) (30 : 15 : 0.5).

HPLC was made on Merck Hitachi LaChrom apparatus, endecaped (5 μm) Purospher®STAR RP-18 column, detection Diode Array Detector L-7450A.

3.2. 5-{4-[2-(5-Ethyl-6-{4-[2-(5-ethylpyridin-2-yl)ethoxy]fenyl}pyrid-2-yl)-ethoxy]benzyl}-1,3-thiazolidine-2,4-dione (**I**)*; 5-{4-[2-(5-ethyl-4-{4-[2-(5-ethylpyridin-2-yl)ethoxy]fenyl}pyrid-2-yl)-ethoxy]benzyl}-1,3-thiazolidine-2,4-dione* (**II**)*; 5-{6,4′-bis-[2-(5-ethyl-pyridin-2-yl)ethoxy]biphenyl-3-ylmethyl}-1,3-thiazolidine-2,4-dione* (**III**)

Pioglitazone prepared by the procedure of Meerwein arylation was crystallized from DMF/H_2O (5/1). Mother liquor was concentrated and the residue was extracted by toluene. The rest of pioglitazone was removed from the mixture by concentration of toluene extract and filtration of solid. Evaporation of toluene filtrate finally yielded the solid material containing ca 50% of compound **I**, ca 10% of compound **II** and ca 6% of compound **III**. The rest comprised pioglitazone and several small and unknown impurities. This material was chromatographed through column with reverse phase silica gel in the mixture acetonitrile/formate buffer (55 : 45). Fraction purity was monitored by TLC. Pioglitazone had $R_f = 0.74$, comp. **I** had $R_f = 0.51$, comp. **II** had $R_f = 0.41$ and comp. **III** had $R_f = 0.36$. Fractions containing compound **I** were combined, concentrated and residue extracted with dichloromethane. The product received by dichloromethane evaporating was recrystallized from methanol. White compound **I** was yielded. Melting point = 122.5–124 °C. MS m/e: 581. Anal. Calcd. for $C_{34}H_{35}N_3O_4S$: C, 70.20; H, 6.06; N, 7.22; O, 11.00; S, 5.51 found: C, 69.85; H, 5.85; N, 6.95.

Other compounds **II** and **III** were received from other chromatographic fractions by the similar manner. Compounds **I**, **II** and **III** have on TLC different retention factors. The difference in retention factors of compounds **II** and **III** is not suitable for quite a full separation on column chromatography described in this communication. We have got only the compound **II** a little contaminated by compound **III** for NMR determination and the small amount of compound **III** contaminated by compound **II**. For compound **I** all 1D and 2D techniques for unambiguous assignment of all NMR signals were used. For compound **II** only 1D ^{1}H and ^{13}C and H,H-COSY techniques were used, so some signals are assigned tentatively and assignment could be interchanged. For compound **III** only 1D and 2D ^{1}H NMR spectra were measured (Table 1). Nevertheless the structures of compounds **II** and **III** were proven.

3.3. 1,3-Bis-{4-[2-(5-ethyl-pyridin-2-yl)-ethoxy]-phenyl}-isothiourea (**21**)

4-[2-(5-Ethylpyridin-2-yl)ethoxy]aniline (**6**) (40 g, 0.165 mol) and CS_2 (25.2 g, 0.332 mol) were dissolved in absolute ethanol (65 ml). The reaction mixture was maintained at 50 °C for 2 h. After finishing the reaction all residual solvents were removed. The residue was dissolved in dichloromethane. Dichloromethane solution was washed with 1 M solution of HCl acid and dried over sodium sulfate. A slightly yellowish crude solid compound was yielded. The crude product was recrystallized from ethanol to give 17.8 g (42%) of pure compound (**21**). M.p. 84.5–86 °C; ^{1}H NMR ($CDCl_3$, 25 °C) δ 9.44 (s, 2), 8.38 (s, 2), 7.60 (dd, J = 7.7, 1.6 Hz, 2), 7.31 (m, 6), 6.91 (d, J = 7.5 Hz, 4), 4.33 (t, J = 6.3 Hz, 4), 3.15 (t, J = 6.3 Hz, 4), 2.52–2.65 (m, 4), 1.19 (t, J = 7.5 Hz, 6); ^{13}C NMR ($CDCl_3$, 25 °C) δ 180.3, 155.9, 155.7, 148.7, 136.9, 135.9, 132.5, 126.2, 123.3, 114.4, 67.1, 37.0, 25.2, 15.6.

3.4. 5-{4-[2-(5-Ethyl-pyridin-2-yl)ethoxy]benzyl}-3-{4-[2-(5-ethyl-pyridin-2-yl)ethoxy] phenyl}-1,3-thiazolidine-2,4-dione (**20**)

Disubstituted thiourea **21** (11.5 g, 0.02 mol) and bromoester **9** (7.9 g, 0.02 mol) were dissolved in ethanol (125 ml). Reaction mixture was heated for 7 h under reflux and then evaporated to dryness. The rest was extracted three times with 40 ml of ethylacetate. Ethylacetate solution was evaporated to dryness and the rest was heated for 5 h in 100 ml of 1 M HCl. Then reaction mixture was cooled to the room temperature and extracted three times with 50 ml of dichloromethane. Dichloromethane extract was washed with a saturated aqueous solution of $NaHCO_3$ (10%), dried over sodium sulfate and concentrated. 6 g of brown solid was obtained after evaporation of the solvent. A white product (**20**) was yielded by recrystallization from cyclohexane: M.p. 82.5–83.5 °C; ^{1}H NMR ($CDCl_3$, 25 °C) δ 8.39 (s, 2), 7.45 (dd, J = 7.7, 1.6 Hz, 2), 7.14–7.26 (m, 8), 6.86 (d, J = 7.5 Hz, 2), 4.54 (dd, J = 8.3, 3.9 Hz, 1), 4.31–4.38 (m, 4), 3.23 and 3.42 (overlapped and dd, J = 13.9, 3.9 Hz, 1), 3.23–3.28 (m, 4), 2.58–2.67 (m, 4), 1.24 (t, J = 7.5 Hz, 6); ^{13}C NMR ($CDCl_3$, 25 °C) δ 173.4, 170.5, 159.3, 158.4, 155.6, 155.4, 149.0, 149.0, 137.7, 137.1, 137.0, 135.7, 132.1, 130.6, 130.6, 128.3, 127.2, 125.2, 115.3, 114.8, 67.5, 67.3, 51.3, 37.8, 37.6, 37.4, 25.7, 15.3.

Table: ^{1}H and ^{13}C chemical shifts of the compounds I–IV

C/H	(**I**)	(**II**)	(**III**)	(**IV**)
	δ $^{1}H/^{13}C$ (ppm); (J(H,H) (Hz))	δ $^{1}H/^{13}C$ (ppm); (J(H,H) (Hz))	δ ^{1}H (ppm); (J(H,H) (Hz))	δ $^{1}H/^{13}C$ (ppm); (J(H,H) (Hz))
1	1.11/15.1	1.09/15.3[a]	1.23	1.22/15.2[a]
2	2.62/25.3	2.64/23.2	2.61[a]	2.59[b]/25.7
3	134.7	132.9		137.0
4	157.8	8.44/155.7	8.36 (2.3)	8.37(2.20)/149.0
5	7.57 (7.9)/137.0	155.2	7.37 (7.9, 2.3)	7.42 (8.00, 2.20)/135.7
6	7.17 (7.9)/122.0	7.06 (7.9)/114.7	7.23 (7.9)	7.15 (8.06)/123.2
7	155.1	149.4		155.6
8	3.24/37.6	3.26/37.3	3.12[b]	3.20/37.6
9	4.33/67.5	4.35/67.3[c]	4.30[c]	4.31/67.3
10	158.4	158.6[d]		158.3
11	6.86/115.0	6.85/114.8	6.80 (8.4)	6.83/114.8
12	7.12/130.3	7.13/129.6[b]	7.07 (8.4, 2.3)	7.09/130.3
13	6.86/115.0	6.85/114.8		6.83/114.8
14	7.12/130.3	7.13/129.6[b]	7.10 (2.3)	7.09/130.3
15	127.8	127.8		127.9
16	3.09 (14.1, 9.3), 3.43 (14.2, 4.0)/37.8	3.11 (14.1, 9.3); 3.42 (14.2, 4.0)/37.3	3.09 (14.2, 9.4); 3.42 (14.1, 3.9)	2.95 (overlapped); 3.39 (14.4, 4.0)/38.0
17	4.48 (9.3, 4.0)/53.8	4.48 (9.3, 4.0)/53.7	4.48 (9.3, 3.9)	4.32/51.7
18	174.4	174.6		170.8
19	170.5	170.7		173.6
20	133.3	131.4		3.94/41.4
21	7.37/130.1	7.20/130.2[b]	7.24	2.95/35.0
22	6.96/114.3	6.97/114.5	6.84	155.0
23	7.37/130.1	7.20/130.2[b]	7.24	7.00 (8.00)/122.8
24	6.96/114.3	6.97/114.5	6.84	7.40 (8.00, 2.20)/135.7
25	158.6	158.2[d]		8.36 (2.20)/149.1
26	4.39/67.4	4.41/67.4[c]	4.36[c]	137.2
27	3.27/37.4	3.26/37.7	3.23[b]	2.61[b] 25.7
28	155.7	155.5		1.20/15.3[a]
29	8.41 (2.0)/148.7	8.41 (2.0)/148.7	8.41 (2.2)	
30	7.21 (7.9)/123.5	7.22/123.4	7.00 (7.9)	
31	7.47 (7.8, 2.0)/136.1	7.48/136.0	7.47 (7.9, 2.2)	
32	137.2	137.2		
33	2.64/25.7	2.64/25.7	2.62[a]	
34	1.25/15.3	1.26/15.2[a]	1.23	

a, b, c, d can be interchanged

3.5. 5-{4-[2-(5-Ethyl-pyridin-2-yl)ethoxy]benzyl}-3-[2-(5-ethyl-pyridin-2-yl)ethyl]-1,3-thiazolidine-2,4-dione (**IV**)

Pioglitazone (**1**) (10 g, 28 mmol) was treated with KOH (1.6 g, 28 mmol) in DMF (18 ml) for 30 min at laboratory temperature. To this solution was slowly added dropwise 4-toluenesulfonyl ester of 2-(5-ethyl-pyridin)ethanol (**15**) (8.6 g, 28 mmol) in DMF (10 ml) and the mixture was stirred at 45 °C for 2 h. Separated potassium 4-toluenesulfonate was filtered off after cooling to 15 °C. The filtrate was concentrated on oily residue. This residue was dissolved in toluene and washed with 1 M NaOH. Toluene solution was then dried with sodium sulfate and evaporated to dryness. The received honey like sticky matter was dissolved in boiling cyclohexane, filtered through the bed of carborafine and left to crystallize. 7.3 g (53%) of white product (**IV**) were received. M.p. = 92.6–93.3 °C. MS m/e: 489. Anal. Calcd. for $C_{28}H_{31}N_3O_3S$: C, 68.68; H, 6.38; N, 8.58; O, 9.80; S, 6.55 found: C, 68.12; H, 6.18; N, 7,93.

^{1}H and ^{13}C NMR signals of compound **IV** were assigned the similar way like those of compound **I** (Table 1).

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