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Design, Synthesis, and Biological Evaluation of Thiazolidine-2,4-dione Conjugates as PPAR-γ Agonists

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A library of synthesized conjugates of phenoxy acetic acid and thiazolidinedione **5a–m** showed potent peroxisome proliferator activated receptor-γ (PPAR-γ) transactivation as well as significant blood glucose lowering effect comparable to the standard drugs pioglitazone and rosiglitazone. Most of the compounds showed higher docking scores than the standard drug rosiglitazone in the molecular docking study. Compounds **5l** and **5m** exhibited PPAR-γ transactivation of 54.21 and 55.41%, respectively, in comparison to the standard drugs pioglitazone and rosiglitazone, which showed 65.94 and 82.21% activation, respectively. Compounds **5l** and **5m** significantly lowered the blood glucose level of STZ-induced diabetic rats. Compounds **5l** and **5m** lowered the AST, ALT, and ALP levels more than the standard drug pioglitazone. PPAR-γ gene expression was significantly increased by compound **5m** (2.00-fold) in comparison to the standard drugs pioglitazone (1.5-fold) and rosiglitazone (1.0-fold). Compounds **5l** and **5m** did not cause any damage to the liver and could be considered as promising candidates for the development of new antidiabetic agents.

Keywords: Antidiabetic / Hepatotoxicity / Phenoxy acetic acid / PPAR-γ / 2,4-Thiazolidinedione

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Introduction

2,4-Thiazolidinediones (TZDs) are an important class of compounds that enhance insulin action (insulin sensitizers) and promote glucose utilization in peripheral tissue [1, 2]. The chemistry of TZDs has attracted attention as they have been found to reveal several biological activities [3], such as antihyperglycemic [1], anti-inflammatory [4, 5], antimalarial [6], antioxidant [7], antitumor [8], cytotoxic [9], antimicrobial [10], and antiproliferative [11]. Unlike sulfonylurea and biguanides which lead to severe hypoglycemia, TZDs are not associated with such hypoglycemic incidents.

TZDs are high-affinity ligands of peroxisome proliferator activated receptor-γ (PPAR-γ) [12]. PPAR-γ, a member of a large family of ligand-activated nuclear hormone receptors, is an important drug target for regulating glucose metabolism [13].

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It exists in two forms, mainly PPAR-γ1 found in tissues except muscles and secondly, PPAR-γ2 found in adipose tissues and intestine. These are encoded by *PPAR-γ* gene in humans. It increases insulin sensitivity at adipose, muscle, and hepatic tissues [14, 15]. It leads to the channeling of fatty acids into adipose tissue and reduces their concentration in the plasma. Reducing the fatty acid availability for muscles alleviates insulin resistance, thereby, improving plasma glucose levels effectively [16, 17]. Keeping in view the biological importance of thiazolidinediones as antidiabetic agents, we herein report the synthesis and *in silico* molecular docking study of thiazolidinedione and phenoxy acetic acid conjugates **5a–m**. These conjugates have been evaluated for *in vitro* PPAR-γ transactivation activity, *in vivo* antidiabetic activity with hepatotoxicity risk evaluation, and PPAR-γ gene expression.

Results and discussion

Chemistry

Treatment of different hydroxy benzaldehydes **1a–m** with chloroacetic acid in the presence of diethyl ether and NaOH solution yielded different substituted formyl phenoxy acetic acids **2a–m** [18] which upon Knoevenagel condensation with thiazolidine-2,4-dione **3** in the presence of absolute alcohol and NaOH at −5 to 0°C for 12–14 h yielded intermediates **4a–m** [19]. Reduction of intermediates **4a–m** with sodium borohydride in the presence of $CoCl_2$–DMG complex in DMF for 5–6 h resulted in the hydrogenation of exocyclic double bond between TZD and aromatic ring leading to the formation of target compounds **5a–m** (Scheme 1). A library of 13 new conjugates of phenoxy acetic acid and TZD has been synthesized in good yields.

^{1}H NMR, ^{13}C NMR, and mass spectral data were found to be in agreement with the proposed structures of all the newly synthesized compounds. Formation of substituted formyl phenoxy acetic acids **2a–m** was confirmed by the presence of a singlet at δ 4.62–4.89 for O–CH_2 protons and carboxylic acid protons appeared as a broad singlet at δ 11.02–13.07 in the ^{1}H NMR spectra. Formation of condensed product **4a–m** was confirmed by the absence of aldehydic protons at δ 9.85–10.73 and the presence of exocyclic olefinic proton as a singlet at δ 7.72–7.74 in the ^{1}H NMR spectra. Formation of target compounds **5a–m** was confirmed by the absence of exocyclic olefinic proton singlet and the presence of characteristic three double doublets at δ 4.78–4.98, 3.18–3.63, and 2.94–3.19 due to the newly generated methine and methylene protons in the ^{1}H NMR spectra. In the ^{13}C NMR spectrum of **5a–m**, appearance of peaks at δ 50.06–57.26 for newly generated methine carbon and δ 31.29–38.16 for methylene carbon further confirmed the reduction of exocyclic olefinic bond. Further confirmatory evidence was obtained from the mass spectral data of the compounds.

Molecular docking studies

The above synthesized compounds **5a–m** were docked for *in silico* studies against PPAR-γ target. Molecular docking studies were done to provide insights of molecular binding modes of molecules inside the large pocket of PPAR-γ receptors. The compounds were docked against grid generated by Schrodinger glide software. In order to analyze the binding pattern and energies of new molecules and reference ligand (rosiglitazone), they were docked individually against the generated grid. Initial docking of reference ligand (rosiglitazone) against generated grid helped in validating the grid generated by software. Rosiglitazone is reported to have H-bonding with TYR 473, HIS 449, and CYS 285 [20, 21]. Docking of rosiglitazone against generated grid showed similar docking mode and H-bonds with RMSD value of 2.8 and hence, validated the generated grid. The synthesized molecules docked against the grid showed good binding energies ranging from −59.9 to −31.6 kcal/mol. All the molecules showed good glide score but the most promising molecules were **5b**, **5c**, **5g**, **5h**, and **5j–m** with glide score of −7.15, −6.36, −6.50, −6.54, −6.57, −6.57, −7.04, and −7.03, respectively, whereas glide score of rosiglitazone was −5.77 kcal/mol with respect to the same grid. Compounds **5c** and **5j** showed hydrogen bonding with SERB 342 residue of the protein whereas compounds **5b**, **5l**, and **5m** were found to be aligned perfectly with the hydrophobic pocket of the protein (Fig. 1). The docking score and *in silico* ADME (absorption, distribution, metabolism, and excretion) prediction of the synthesized library are found to be within the acceptable range (Table 1).

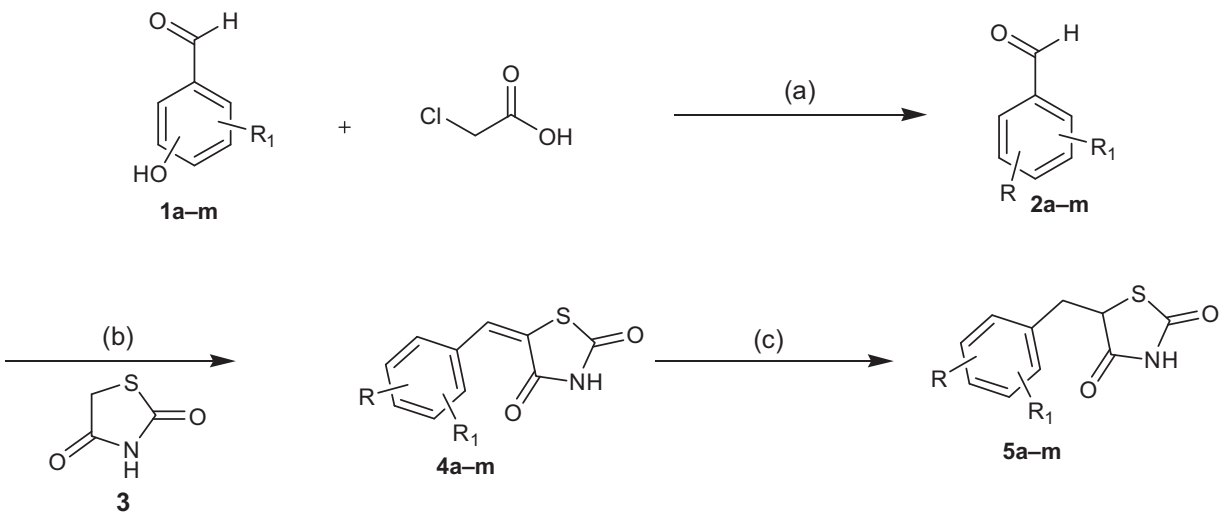
Biological activities

In vitro PPAR-γ transactivation assay

The compounds showing good glide scores (>−6.50) in molecular docking study were screened for *in vitro* PPAR-γ transactivation activity in order to confirm their mode of action. It was observed from Fig. 2 that compounds **5b**, **5g**, **5h**, and **5j–m** were found to be PPAR-γ active. Compounds **5b**, **5l**, and **5m** exhibited PPAR-γ transactivation of 48.35, 54.21, and 55.41%, respectively, in comparison to standard drugs pioglitazone and rosiglitazone which showed 65.94 and 82.21% activation, respectively. Compounds **5g**, **5h**, **5j**, and **5k** showed moderate *in vitro* PPAR-γ transactivation activity. The most active compound **5m** was further tested for *in vitro* PPAR-α and PPAR-δ assay. It was observed that compound **5m** was found to be inactive PPAR-α and PPAR-δ (Table 2). Compound **5m** showed PPAR-γ of EC_{50} of 0.77 in comparison to standard drug rosiglitazone which showed EC_{50} of 0.058.

In vivo blood glucose lowering effect

These compounds (**5b**, **5g**, **5h**, and **5j–m**) were then evaluated for the blood glucose lowering effect in STZ-induced (streptozotocin) diabetic rats. STZ-induced diabetic model was used for the *in vivo* blood glucose lowering effect as it is a more preferred diabetic model than other standard rodent diabetes models, due to its advantages such as longer half-life time and hyperglycaemia duration and better established diabetic complications with lower possibility of ketosis or mortality incidents [22]. The synthesized



Compds	R	R_1	Compds	R	R_1
5a	4-CH_2COOH	H	**5h**	2-CH_2COOH	H
5b	4-CH_2COOH	3-OMe	**5i**	2-CH_2COOH	5-NO_2
5c	4-CH_2COOH	3-OEt	**5j**	2-CH_2COOH	3-OEt
5d	4-CH_2COOH	3,5-DiOMe	**5k**	2-CH_2COOH	5-Cl
5e	4-CH_2COOH	5-Me	**5l**	2-CH_2COOH	5-Br
5f	4-CH_2COOH	3,5-DiMe	**5m**	2-CH_2COOH	5-Br, 3-OMe
5g	4-CH_2COOH	3-Br			

Scheme 1. Synthesis of compounds **5a**–**m**.

compounds **5a**–**m** were administered a single dose on the first day and the blood glucose level was measured on the 1st, 7th, and 15th day of the experiment as per standard protocols. As observed from the data of Fig. 3, it was found that these compounds significantly lowered the blood glucose level in diabetic rats. Compounds **5b**, **5l**, and **5m** significantly lowered blood glucose level to 157.5 ± 5.01, 158.8 ± 7.75, and 159.2 ± 5.50, respectively, comparable to standard drugs pioglitazone (134.2 ± 4.52) and rosiglitazone (142.2 ± 6.12). Compounds **5j** and **5k** showed moderate blood glucose lowering whereas compounds **5g** and **5h** did not show promising lowering in blood glucose level. It was found that the results of *in vitro* study were consistent with *in vivo* results indicating that these compounds have exerted the blood glucose lowering effect by activating PPAR-γ receptors.

As the PPAR-γ agonists are associated with body weight gain, **5m** was also tested for body weight gain study. Compound **5m** treated normal rats did not show any significant change in body weight as compared to normal control rats. However, oral administration of **5m** to diabetic rats for 15 days caused a significant improvement in body weight of diabetic rats (Fig. 4).

Hepatotoxicity studies

TZDs are reported to cause hepatotoxicity which is the major drawback encountered with this class of drug [23]. The active compounds (**5b**, **5j**–**m**) have, therefore, been assayed for aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) levels and histopathological study of the liver. It has been reported that the levels of these

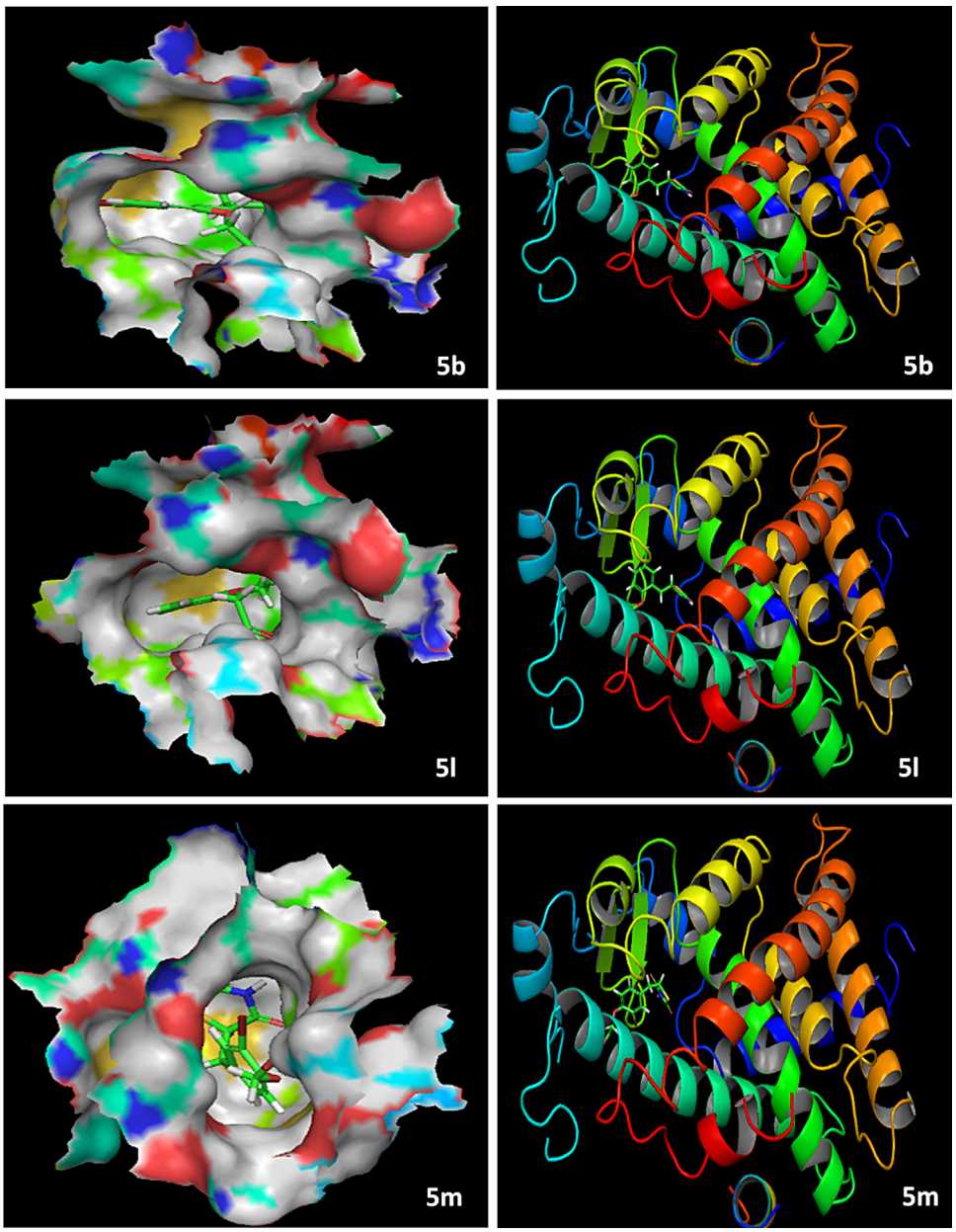


Figure 1. Molecular docking of the compounds against the PPAR-γ target.

enzymes are significantly increased in STZ rats indicating the toxic effect of STZ on liver [24]. The elevation of these enzymes might be due to increased protein catabolism followed by gluconeogenesis as ALT and AST are directly involved in the amino acid conversion to ketoacids. It was observed that the levels of serum AST, ALT, and ALP significantly increased in STZ treated rats were significantly decreased to near normal level after treatment with the compounds **5b** and **5j–m.** Compounds **5l** and **5m** were found to be more potent in lowering the AST, ALT, and ALP levels than the standard drug pioglitazone. Compounds **5b**, **5j**, and **5k** were equally potent to standard drug pioglitazone in lowering the AST, ALT, and ALP levels to normal level, thereby, suggesting that these compounds have protective effect on liver (Fig. 5).

Histopathological study of the liver of the treated animals also showed that the compounds **5b**, **5j**, **5l**, and **5m** did not cause any damage to the liver whereas compound **5k** and standard drug pioglitazone caused mild dilation in sinusoidal space (Fig. 6).

Table 1. Docking score and ADME prediction of the synthesized compounds 5a–m.

No.	Compound	G-Score	G-Energy	Log Po/w	PSA	Log S
1	**5a**	−5.30	−40.60	1.10	130.11	−2.33
2	**5b**	**−7.15**	−59.96	1.58	137.25	−2.93
3	**5c**	**−6.36**	−42.90	1.61	137.48	−2.95
4	**5d**	−4.25	−37.54	1.46	143.65	−2.48
5	**5e**	−5.68	−31.78	1.37	128.76	−2.57
6	**5f**	−5.42	−31.67	1.81	123.66	−3.05
7	**5g**	**−6.50**	−45.97	1.62	132.207	−3.00
8	**5h**	**−6.54**	−37.95	1.27	128.13	−2.51
9	**5i**	−5.81	−41.56	0.52	176.27	−2.75
10	**5j**	**−6.57**	−57.66	1.28	131.10	−2.70
11	**5k**	**−6.57**	−47.33	1.56	130.64	−2.72
12	**5l**	**−7.04**	−43.91	1.91	125.88	−3.3
13	**5m**	**−7.03**	−42.38	1.71	138.83	−3.17
14	Rosi	−5.77	−71.55	3.47	94.37	−4.49

The bold values signify the glide score of these compounds are higher than that of the standard drug Rosiglitazone.

PPAR-γ gene expression activity

Since compound **5m** was found to be most PPAR-γ active, it was further subjected to PPAR-γ gene expression study. Compound **5m** (10 μM) and pioglitazone (10 μM) and rosiglitazone (10 μM) were used for the gene expression study. The PPAR-γ gene expression study was done to know the impact of compound **5m** on PPAR-γ gene. It was observed that the PPAR-γ expression was significantly increased in presence of compound **5m** (2.00-fold) in comparison to the standard drugs rosiglitazone (1.0-fold) and pioglitazone (1.5-fold) (Fig. 7). The increase in PPAR-γ gene expression supports the results of *in vitro* PPAR-γ transactivation study. It is thus clear that the *in vitro* PPAR-γ transactivation and *in vivo* blood glucose lowering activity of compound may be due to increase in the PPAR-γ gene expression. It has been reported that TZDs improve insulin action by effects on gene transcription in the fat cell that lead to diminished plasma levels of free fatty acids (FFAs) and an increase in the level of adiponectin, an adipokine that activates AMPK [25–27]. The increase in gene expression by compound **5m** might be due to the activation of AMPK. The overexpression of PPAR-γ in mature 3T3-L1 adipocytes increases the amount of the mRNA for the ubiquitous GLUT1, whose expression is reported to be downregulated during adipocyte differentiation [28]. The reduction of insulin-stimulated glucose transport in 3T3-L1 adipocytes overexpressing PPAR-γ may be due to the reduced expression of IR, IRS1, IRS2, and GLUT4. Thus, compound **5m** increases the gene expression by maintaining insulin sensitivity in mature 3T3-L1 adipocytes by regulating the expression of genes that encode components of the insulin signaling pathway as well as by increasing the expression levels of GLUT1 and GLUT4 in these cells.

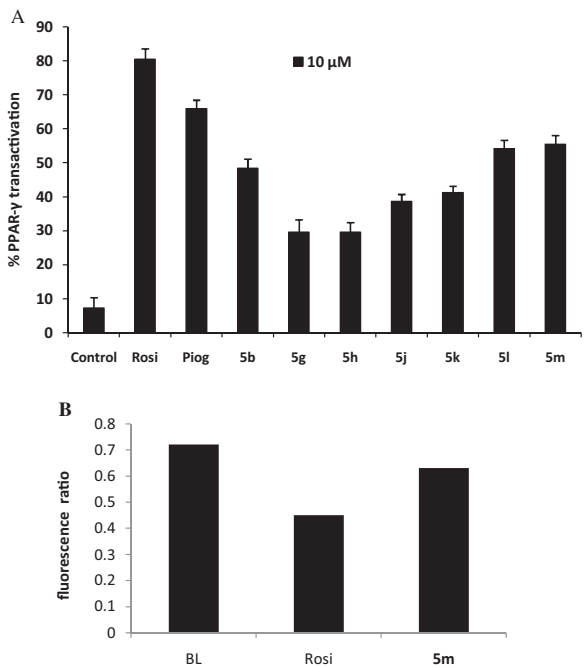


Figure 2. (A) *In vitro* PPAR-γ transactivation assay of the compounds. Values are expressed as mean ± SE from three experiments conducted in triplicate at 10 μM. (B) PPAR-γ binding data of compound **5m**. Activity was measured using Lantha Screen TM TR-FRET PPAR-γ competitive binding assay. BL (blank control, without PPAR-γ LBD), Rosi (rosiglitazone). Activity was assayed at 100 μM. The fluorescence ratio at 520/495 nm is shown.

From the biological data, the structure–activity relationship (SAR) can be drawn as follows. Compounds having *o*-substituted phenoxy acetic acid were more active in lowering the blood glucose level than *p*-substituted compounds (**5h** > **5a**). Compounds containing OCH_3 and halogens (Cl, Br) on the aromatic ring showed promising activity (**5b**, **5l**, **5m**) whereas compound with NO_2 group decreased the activity (**5i**). Increase in number of methoxy groups on the aromatic ring decreased the activity (**5d**). Increase in number of alkyl group decreased the activity (**5f**).

Table 2. ***In vitro*** **data of compound 5m.**

Compound	PPAR-α (10 μM)	PPAR-δ (10 μM)	PPAR-γ	
			EC_{50} (μM)	%max
5m	IA	IA	0.77	65
WY14643	4.3	ND	ND	ND
Rosiglitazone	ND	ND	0.058	100
GW-501516	ND	5.2	ND	ND

IA, inactive; ND, not determined.

Conclusion

A library of 13 novel conjugates of phenoxy acetic acid and thiazolidinedione have been synthesized, out of which two compounds, **5l** and **5m**, exhibited significant *in vitro* PPAR-γ transactivation and *in vivo* blood glucose lowering effect, comparable to standard drugs rosiglitazone and pioglitazone. Compounds **5l** and **5m** recovered the activity of serum AST, ALT, and ALP levels and did not cause any damage to the liver. Compound **5m** increases the PPAR-γ expression by 2.0-fold in comparison to standard drug rosiglitazone (1.0-fold) and pioglitazone (1.5-fold). Compounds **5l** and **5m** may be considered as promising candidate for development of new antidiabetic agents.

Experimental

Chemistry

All chemicals (reagent grade) used were commercially available. Melting points were measured on a VEEGO-VMP-DS melting point apparatus and are uncorrected. ^{1}H NMR was recorded on Bruker DPX 400, 300 instruments in $CDCl_3$/DMSO-d_6 using TMS as internal standard. Chemical shifts and coupling constants *J* are given in parts per million and hertz, respectively. Mass spectra were recorded on a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV. Mass-spectrometric (MS) data are reported in *m/z*. Elemental analysis was carried out using Elemental Vario EL III elemental analyzer. Elemental analysis data are reported in percent standard.

Synthesis of compounds ***2a–m***

To a solution of substituted hydroxy benzaldehyde (0.01 M) in 100 mL of ether, 25 g of sodium hydroxide dissolved in 50 mL of water was added slowly while the solution was cooled and stirred. To this mixture, a solution of chloroacetic acid (0.01 M) in 30 mL of water was added in the same way. The ether was removed and the mixture was refluxed for 2 h at 120–125°C. The resulting solution was cooled, diluted with water, acidified with concentrated HCl to Congo red, and allowed to stand for 2–3 h at room temperature. The precipitate so formed was filtered, dissolved in sodium bicarbonate

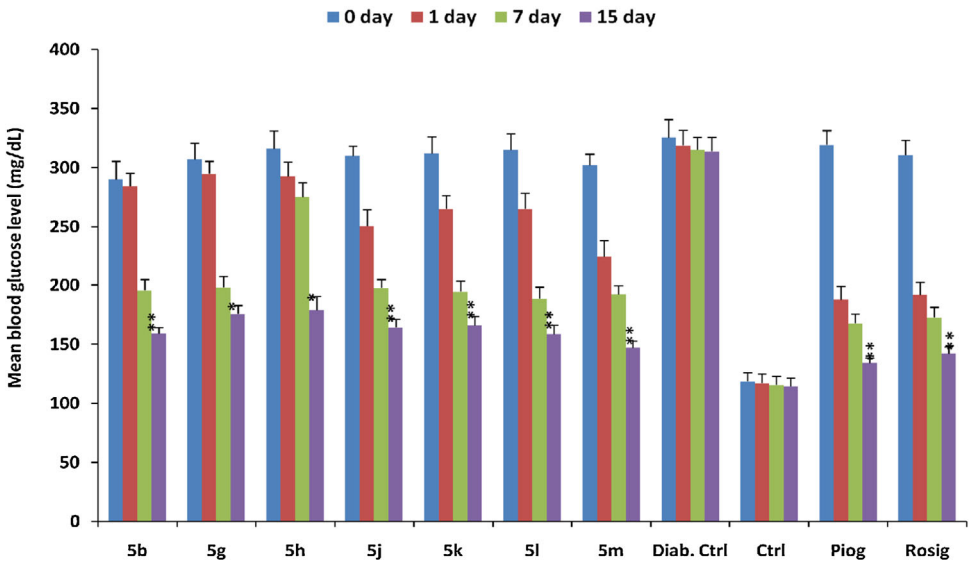


Figure 3. Antidiabetic activity of the compounds in STZ-induced diabetic rats. Data are analyzed by one-way ANOVA followed by Dunnett's *t*-test and expressed as mean ± SEM from five observations; $^{**}p < 0.01$ and $^{*}p < 0.05$ versus diabetic control, Diab. Ctrl, diabetic control; Ctrl, normal control; Piog, pioglitazone; Rosig, rosiglitazone.

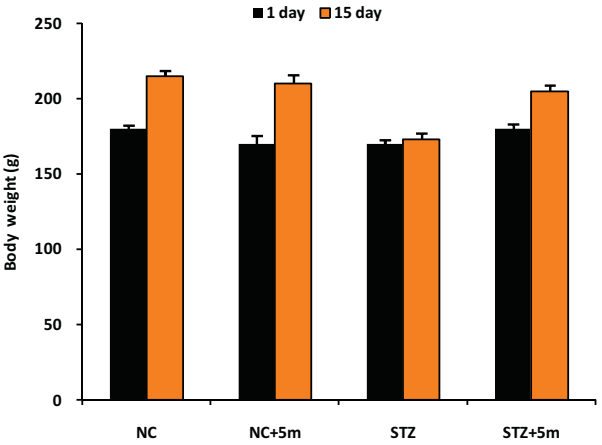


Figure 4. Effect of compound **5m** on body weight *in albino* Wistar rats. Data are analyzed by one-way ANOVA followed by Dunnett's *t*-test and expressed as mean ± SEM from five observations; $*p < 0.01$ versus diabetic control. NC, Normal control; NC + **5m**, normal control + **5m**; STZ, diabetic control; STZ + **5m**, **5m** treated diabetic rats.

solution, and extracted with ether. The aqueous layer was acidified with concentrated HCl to Congo red and left for 2–3 h at 5–10°C. The precipitate so formed was filtered and crystallized from methanol. The experimental data of **2a–m** were compared with the reported literature data [18].

*Synthesis of chalcones **4a–m** [19]*

To a stirred solution of the substituted phenoxy acetic acid **2a–m** (0.01 M) and the appropriate aldehyde (0.01 M) in ethanol (30 mL), 40% aqueous sodium hydroxide (20 mL) was added portionwise at room temperature for 10 min. The mixture was kept overnight at room temperature and then it was poured into crushed ice and acidified with concentrated HCl. The solid separated was filtered and crystallized from ethanol. The experimental data of **4a–m** were compared with the reported literature data [29].

*Synthesis of target compounds **5a–m***

In a 100 mL 3-neck RBF equipped with a thermometer and mechanical stirrer, compounds **4a–m** (0.01 mol) were charged. Then 3 mL of H_2O, 2 mL of MeOH, 3 mL of 1N NaOH were added to the reaction mixture with constant stirring for 15 min. After 15 min, freshly prepared 1.5 mL $CoCl_2$–DMG complex solution (prepared by dissolving 42 mg $CoCl_2 \cdot 6H_2O$ + 250 mg DMG in 5 mL DMF) was added dropwise to the reaction mixture and the stirring was continued for 15 min at 25°C. Freshly prepared sodium borohydride solution (eight equivalents) was added at once to the reaction mixture and temperature was increased to 35°C. On addition of sodium borohydride solution, the color of the reaction mixture changed to deep violet for seconds and then to gray. The reaction was continued for 3 h at 35°C with constant stirring. After the completion of the reaction, monitored by TLC, the reaction mixture was cooled and filtered. The filtrate so obtained was acidified with 1N HCl to bring the pH 3 and stirred for 30 min. The solid so obtained was filtered and dried to yield the pure target compounds **5a–m**. The target compounds were crystallized in MeOH.

*5-(4-Carboxymethoxybenzyl)-2,4-thiazolidinedione (**5a**)*

White crystals; yield: 70%; m.p. 115–117°C; IR (KBr): ν (cm^{-1}) 3421, 3325, 1699, 1684, 1541, 1145; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.05 (dd, 1H, $J = 8.8, 14.1$ Hz), 3.30 (dd, 1H, $J = 4.2, 14.1$ Hz), 4.64 (s, 2H O–CH_2), 4.87 (dd, 1H, $J = 4.2, 9.1$ Hz), 6.84 (d, 2H, $J = 8.5$ Hz, Ar-H), 7.15 (d, 2H, $J = 8.6$ Hz, Ar-H), 12.46 (s, 2H, NH, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 36.33, 53.08, 69.45, 114.36, 128.52, 130.44, 157.80, 170.34, 171.83, 175.82; MS FAB (+ve): 282 (M+1). Anal. calcd. for $C_{12}H_{11}NO_5S$: C, 51.24; H, 3.94; N, 4.98; S, 11.40. Found: C, 51.20; H, 3.89; N, 4.94; S, 11.42%.

*5-(4-Carboxymethoxy-3-methoxybenzyl)-2,4-thiazolidinedione (**5b**)*

White crystals; yield: 80%; m.p. 161–162°C; IR (KBr): ν (cm^{-1}) 3446, 3181, 1649, 1518, 1149; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.03 (dd, 1H, $J = 8.0, 12.0$ Hz), 3.33 (dd, 1H, $J = 4.1, 14.0$ Hz), 3.77 (s, 3H, O–CH_3), 4.63 (2H, s, O–CH_2), 4.91 (dd, 1H, $J = 4.0, 12.0$ Hz), 6.73 (d, 1H, $J = 8.0$ Hz, Ar-H), 6.79 (d, 1H, $J = 8.0$ Hz, Ar-H), 6.91 (s, 1H, Ar-H), 12.10 (s, 2H, NH, OH); ^{13}C NMR (75 MHz, DMSO-d_6): 37.88, 53.49, 55.93, 65.36, 113.33, 113.61, 121.42, 130.44, 146.65, 149.03, 170.72, 172.24, 176.2; MS FAB (+ve): 312 (M+1). Anal. calcd. for $C_{13}H_{13}NO_6S$: C, 50.16; H, 4.21; N, 4.50; S, 10.30. Found C, 50.10; H, 4.18; N, 4.47; S, 10.33%.

*5-(4-Carboxymethoxy-3-ethoxybenzyl)-2,4-thiazolidinedione (**5c**)*

Yellow crystals; yield: 65%; m.p. 158–159°C; IR (KBr): ν (cm^{-1}) 3440, 3241, 1715, 1659, 1524, 1157; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.70 (t, 3H, $J = 7.2$ Hz), 3.01 (dd, 1H, $J = 8.2, 12.1$ Hz), 3.32 (dd, 1H, $J = 4.6, 14.1$ Hz), 4.07 (q, 2H, $J = 6.6$ Hz, O–CH_2), 4.34 (2H, s, O–CH_2), 4.78 (dd, 1H, $J = 4.5, 12.2$ Hz), 6.48 (d, 1H, $J = 8.8$ Hz, Ar-H), 6.54 (d, 1H, $J = 8.4$ Hz, Ar-H), 6.65 (s, 1H, Ar-H), 12.10 (s, 2H, NH, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 15.10, 36.25, 53.52, 64.28, 66.15, 113.66, 114.94, 121.61, 130.10, 147.17, 148.18, 170.58, 171.28, 175.33; MS FAB (+ve): 326 (M+1). Anal. calcd. for $C_{14}H_{15}NO_6S$: C, 51.68; H, 4.65; N, 4.31; S, 9.86. Found: C, 51.64; H, 4.63; N, 4.28; S, 9.81%.

*5-(4-Carboxymethoxy-3,5-dimethoxybenzyl)-2,4-thiazolidinedione (**5d**)*

White crystals; yield: 70%; m.p. 168–169°C; IR (KBr): ν (cm^{-1}) 3452, 3179, 3067, 1673, 1692, 1124, 1031; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.10 (dd, 1H, $J = 8.8, 14.08$ Hz), 3.35 (dd, 1H, $J = 4.32, 14.2$ Hz), 3.75 (6H, s, O–CH_3), 4.43 (2H, s, O–CH_2), 4.89 (dd, 1H, $J = 4.36, 8.68$ Hz), 6.58 (s, 2H, Ar-H), 12.10 (s, 1H, NH), 12.72 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 38.16, 53.27, 56.46, 69.18, 106.94, 133.36, 135.06, 152.66, 170.60, 172.23, 176.24; MS FAB (+ve): 342 (M+1). Anal. calcd. for $C_{14}H_{15}NO_7S$: C, 49.26; H, 4.43; N, 4.10; S, 9.39. Found: C, 49.24; H, 4.40; N, 4.14; S, 9.42%.

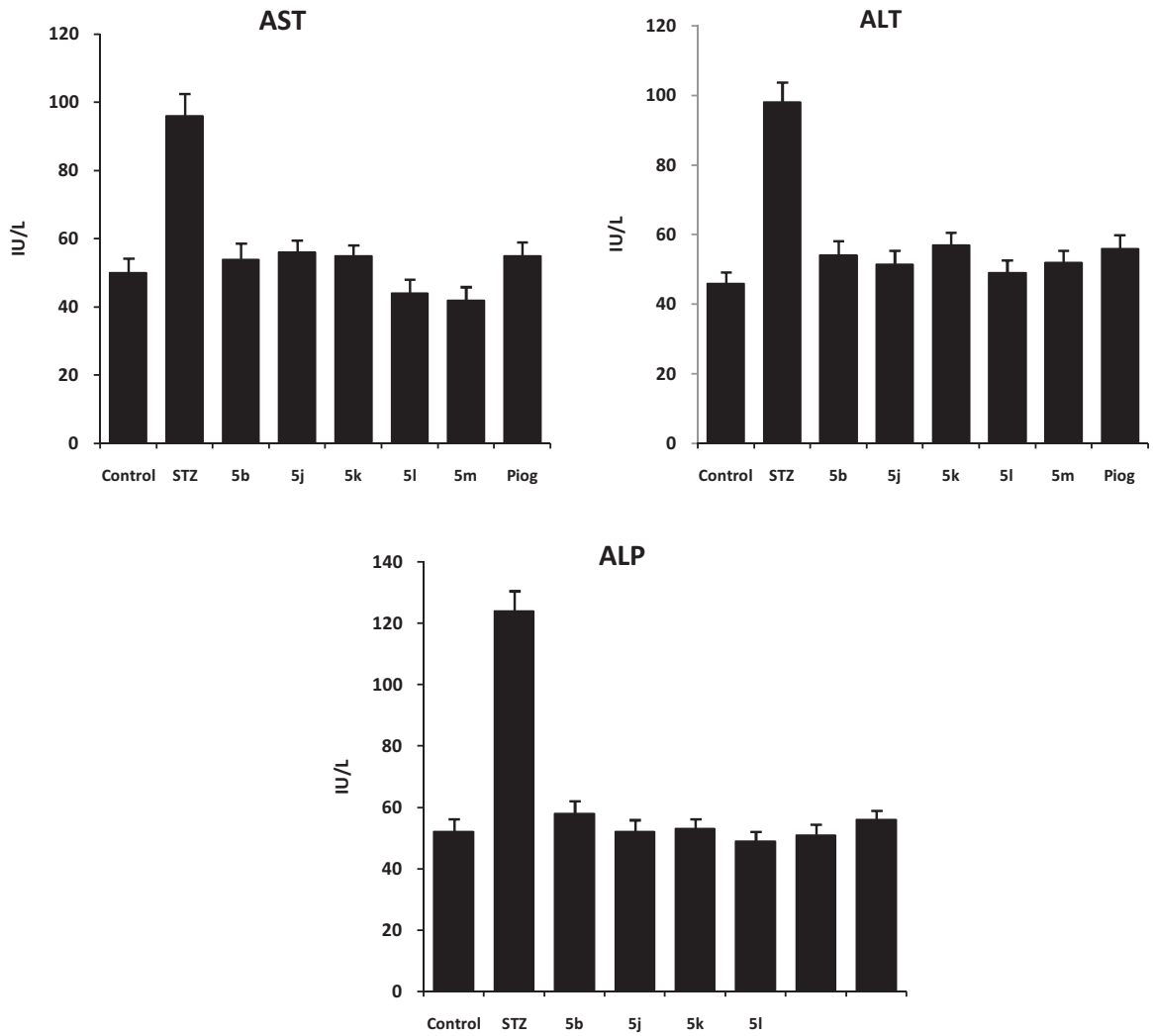


Figure 5. Effect of compounds on serum AST, ALT, and ALP activities. Values are given as mean ± SD.

*5-(4-Carboxymethoxy-3-methylbenzyl)-2,4-thiazolidinedione (**5e**)*
White crystals; yield: 75%; m.p. 162–163°C; IR (KBr): ν (cm^{-1}) 3445, 3177, 3043, 1725, 1676, 1520, 1073; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.12 (s, 3H), 3.02 (dd, 1H, J = 8.2, 12.4 Hz), 3.31 (dd, 1H, J = 4.8, 14.0 Hz), 4.46 (2H, s, O–CH_2), 4.82 (dd, 1H, J = 4.5, 12.3 Hz), 6.72 (d, 1H, J = 8.4 Hz, Ar-H), 6.78 (d, 1H, J = 8.8 Hz, Ar-H), 6.92 (s, 1H, Ar-H), 12.12 (s, 2H, NH, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 15.10, 36.73, 56.63, 67.96, 114.63, 128.56, 129.64, 130.33, 132.51, 155.36, 170.33, 171.78, 175.63; MS FAB (+ve): 296 (M+1). Anal. calcd. for $C_{13}H_{13}NO_5S$: C, 52.87; H, 4.44; N, 4.74; S, 10.86. Found: C, 52.83; H, 4.46; N, 4.72; S, 10.83%.

*5-(4-Carboxymethoxy-3,5-dimethylbenzyl)-2,4-thiazolidinedione (**5f**)*
White crystals; yield: 72%; m.p. 172–174°C; IR (KBr): ν (cm^{-1}) 3450, 3179, 1699, 1682, 1521, 1145, 1031. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.19 (s, 6H), 2.95 (dd, 1H, J = 9.9, 14.1 Hz), 3.29 (dd, 1H, J = 3.9, 13.8 Hz), 4.34 (2H, s, O–CH_2), 4.85 (dd, 1H, J = 4.2, 9.9 Hz), 6.86 (s, 2H, Ar-H), 12.18 (s, 2H, NH, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 16.12, 36.77, 52.89, 68.77, 129.47, 130.36, 132.57, 154.25, 170.31, 171.77, 175.79; MS ES (−ve): 309 $(M)^+$. Anal. calcd. for $C_{14}H_{15}NO_5S$: C, 54.36; H, 4.89; N, 4.53; S, 10.37. Found: C, 54.30; H, 4.91; N, 4.55; S, 10.33%.

*5-(4-Carboxymethoxy-3-bromobenzyl)-2,4-thiazolidinedione (**5g**)*
White crystals; yield: 70%; m.p. 188–190°C; IR (KBr): ν (cm^{-1}) 3450, 3176, 1705, 1680, 1230, 1110; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.96 (dd, 1H, J = 8.4, 14.2 Hz), 3.34 (dd, 1H, J = 4.5, 14.8 Hz), 4.77 (2H, s, O–CH_2), 4.92 (dd, 1H, J = 4.8, 12.5 Hz), 6.72 (d, 1H, J = 8.4 Hz, Ar-H), 6.85 (d, 1H, J = 8.8 Hz, Ar-H), 6.96 (s, 1H, Ar-H), 12.10 (s, 2H, NH, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 36.34, 57.26, 67.16, 114.78, 122.28, 129.56, 130.58,

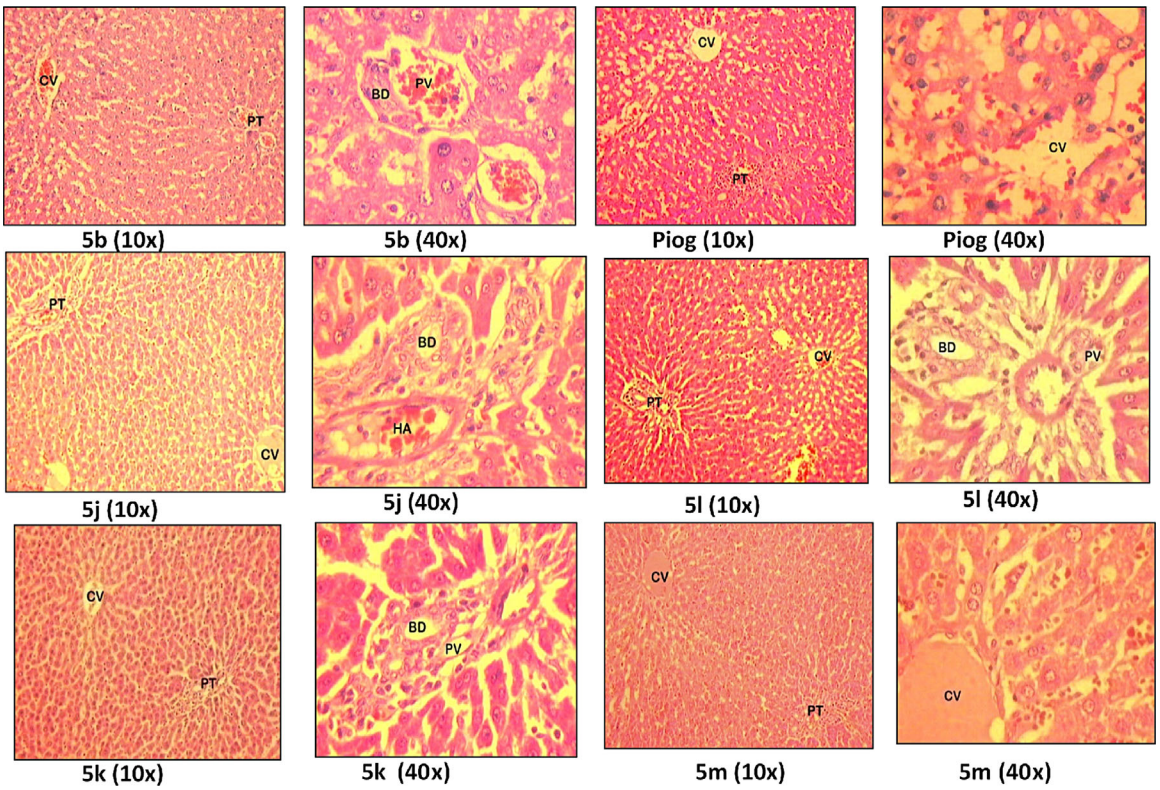


Figure 6. Hematoxylin and eosin immunohistochemical staining of liver after administration of synthesized drugs. Histopathology report of rat liver. As illustrated in the above figure, low and high power photomicrograph of liver from animal treated groups **5b**, **5j**–**5m**, and standard (10×). Low power photomicrograph of liver from corresponding animal **5b**, **5j**–**5m**, and standard treated groups showing arrangement of cells in the liver lobule. PT = portal triad and CV = central vein (40×). High power photomicrograph of liver from animal treated groups **5b**, **5j**–**5m**, and standard showing arrangement of hepatocytes in the centrizonal area. CV = central vein. Compounds **5b**, **5j**, **5l**, and **5m** treated groups showing normal arrangement of cells in the liver lobule and normal arrangement of hepatocytes in the centrizonal area. **5k** and pioglitazone treated groups showing a mild dilatation of sinusoidal spaces around the central vein and mild inflammation in portal vein.

133.57, 156.24, 170.23, 171.47, 175.46; MS FAB (+ve): 359 (M+1), 361 (M+3). Anal. calcd. for $C_{12}H_{10}BrNO_5S$: C, 40.02; H, 2.80; N, 3.89; S, 8.90. Found: C, 40.06; H, 2.82; N, 3.85; S, 8.87%.

*5-(2-Carboxymethoxybenzyl)-2,4-thiazolidinedione (**5h**)*
White crystals; yield: 75%; m.p. 98–99°C; IR (KBr): ν (cm^{-1}) 3446, 3176, 1680, 1651, 1558, 1160, 1074; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.94 (dd, 1H, J = 8.9, 14.10 Hz), 3.59 (dd, 1H, J = 4.5, 14.16 Hz), 4.75 (2H, s, O–CH_2), 4.98 (dd, 1H, J = 4.4, 10.56 Hz), 6.89–7.23 (m, 4H, Ar-H), 12.03 (s, 1H, NH), 12.88 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 36.31, 56.25, 68.85, 114.16, 128.32, 129.32, 131.21, 156.70, 170.14, 170.83, 174.92; MS FAB (+ve): 282 (M+1). Anal. calcd. for $C_{12}H_{11}NO_5S$: C, 51.24; H, 3.94; N, 4.98; S, 11.40. Found: C, 51.20; H, 3.93; N, 4.96; S, 11.38%.

*5-(2-Carboxymethoxy-5-nitrobenzyl)-2,4-thiazolidinedione (**5i**)*
Yellow crystals; yield: 65%; m.p. 172–173°C; IR (KBr): ν (cm^{-1}) 3421, 3307, 1682, 1650, 1520, 1338, 1073; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.99 (dd, 1H, J = 10.5, 13.5 Hz), 3.44 (dd, 1H, J = 7.5, 14.16 Hz), 4.78 (2H, s, O–CH_2), 4.97 (dd, 1H, J = 4.8, 9.9 Hz), 6.90 (d, 1H, J = 8.4 Hz, Ar-H), 7.12–7.57 (m, 2H, Ar-H), 12.05 (s, 1H, NH), 12.35 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 31.29, 50.06, 63.62, 113.28, 113.89, 129.05, 132.19, 133.50, 149.56, 170.32, 172.51, 175.89; MS FAB (+ve): 326 $(M)^+$. Anal. calcd. for $C_{12}H_{10}N_2O_7S$: C, 44.17; H, 3.09; N, 8.59; S, 9.83. Found: C, 44.13; H, 3.08; N, 8.63; S, 9.80%.

*5-(2-Carboxymethoxy-3-ethoxybenzyl)-2,4-thiazolidinedione (**5j**)*
Brown crystals; yield: 70%; m.p. 115–116°C; IR (KBr): ν (cm^{-1}) 3421, 3131, 1702, 1675, 1541, 1457, 1206; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.54 (t, 3H, J = 7.2 Hz), 3.19 (dd, 1H, J = 10.2, 14.1 Hz), 3.63 (dd, 1H, J = 4.8, 13.8 Hz), 4.04 (q, 2H, J = 6.9 Hz, O–CH_2), 4.72 (2H, s, O–CH_2), 4.91 (dd, 1H, J = 4.8, 9.9 Hz), 6.76–7.00 (m, 3H, Ar-H), 12.05 (s, 1H, NH), 12.33 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 15.05, 35.84, 55.52, 63.42, 69.25, 113.38, 122.21, 124.17, 130.85, 145.85, 150.96, 171.19, 172.29, 176.39; MS FAB (+ve): 326 (M+1). Anal. calcd. for $C_{14}H_{15}NO_6S$: C, 51.68; H, 4.65; N, 4.31; S, 9.86. Found: C, 51.65; H, 4.62; N, 4.30; S, 9.89%.

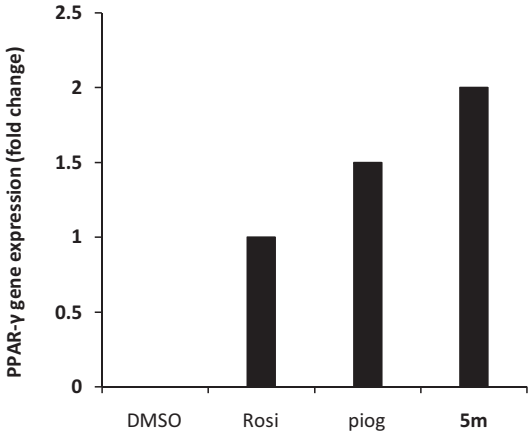


Figure 7. Effect of compound **5m** on PPAR-γ gene expression. The experiments were conducted at 10 μM. Rosi, rosiglitazone; piog, pioglitazone. PCR was performed in triplicate and was repeated two times for each gene and each sample. Relative transcript quantities were calculated using the Ct method with β-actin as the endogenous reference gene.

*5-(2-Carboxymethoxy-5-chlorobenzyl)-2,4-thiazolidinedione (**5k**)*
Yellow crystals; yield: 80%; m.p. 182–183°C; IR (KBr): ν (cm^{-1}) 3420, 3129, 2925, 1699, 1684, 1152, 1070; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.97 (dd, 1H, J = 9.8, 12.5 Hz), 3.18 (dd, 1H, J = 7.3, 13.10 Hz), 4.70 (2H, s, O–CH_2), 4.82 (dd, 1H, J = 5.4, 10.25 Hz), 6.75 (d, 1H, J = 8.8 Hz, Ar-H), 7.22–7.58 (m, 2H, Ar-H), 11.98 (s, 1H, NH), 12.60 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 34.25, 52.76, 66.35, 113.23, 114.42, 123.85, 131.50, 132.27, 148.08, 170.48, 172.09, 176.14; MS FAB (+ve): 315 $(M)^+$, 317 (M+2). Anal. calcd. for $C_{12}H_{10}ClNO_5S$: C, 45.65; H, 3.19; N, 4.44; S, 10.16. Found: C, 45.61; H, 3.16; N, 4.41; S, 10.18%.

*5-(2-Carboxymethoxy-5-bromobenzyl)-2,4-thiazolidinedione (**5l**)*
White crystals; yield: 85%; m.p. 105–106°C; IR (KBr): ν (cm^{-1}) 3342, 3241, 1698, 1675, 1547, 1105, 1029; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.99 (dd, 1H, J = 10.5, 13.5 Hz), 3.44 (dd, 1H, J = 7.5, 14.16 Hz), 4.81 (2H, s, O–CH_2), 4.97 (dd, 1H, J = 4.8, 9.9 Hz), 6.90 (d, 1H, J = 8.4 Hz, Ar-H), 7.12–7.57 (m, 2H, Ar-H), 12.35 (s, 2H, NH, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.35, 51.02, 65.31, 112.28, 114.52, 128.65, 131.08, 133.47, 155.68, 170.42, 172.12, 176.24. MS FAB (+ve): 359 (M+1), 361 (M+3). Anal. calcd. for $C_{12}H_{10}BrNO_5S$: C, 40.02; H, 2.80; N, 3.89; S, 8.90. Found: C, 40.00; H, 2.82; N, 3.86; S, 8.87%.

*5-(2-Carboxymethoxy-5-bromo-3-methoxybenzyl)-2,4-thiazolidinedione (**5m**)*
White crystals; yield: 70%; m.p. 135–136°C; IR (KBr): ν (cm^{-1}) 3450, 3177, 1684, 1521, 1419, 1216, 1151, 1051; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.13 (dd, 1H, J = 9.6, 13.8 Hz), 3.59 (dd, 1H, J = 5.4, 14.10 Hz), 3.86 (s, 3H, O–CH_3), 4.59 (2H, s, O–CH_2), 4.93 (dd, 1H, J = 5.1, 9.6 Hz), 7.16 (s, 1H, Ar-H), 7.01 (s, 1H, Ar-H), 12.05 (s, 1H, NH), 12.78 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 32.48, 51.58, 56.68, 69.11, 115.65, 115.74, 124.78, 132.96, 145.14, 152.74, 170.92, 172.08, 176.20; MS FAB (+ve): 389 (M+1), 391 (M+3). Anal. calcd. for $C_{13}H_{12}BrNO_6S$: C, 40.01; H, 3.10; N, 3.59; S, 8.22. Found: C, 39.98; H, 3.12; N, 3.56; S, 8.20%.

Molecular docking study

Molecular docking studies involve mainly protein selection and preparation, grid generation, ligand preparation, docking, and further analysis of docking studies. Schrodinger software was mainly used for all the above steps.

Protein selection and preparation
Protein with Accession Number 3CS8 was selected and downloaded from Protein Data Bank. This protein is reported to bind with drug rosiglitazone. The protein was imported, optimized, and minimized by removing unwanted molecules and other defects reported by the software. PPAR-γ receptor is a dimer which has two monomers chains (A and B). For the purpose of studies, chain B was deleted and water molecules near the ligands were retained. Finally a low energy minimized protein structure was obtained and used for further docking studies.

Grid generation
Minimized protein was used for grid generation which involves selected ligand as the reference as it signifies the binding sites of drug with respect to the target. The generated grid was used for further docking of new molecules.

Ligand preparation
Molecules drawn in 3D form were refined by LigPrep module. The molecules were subjected to OPLS-2005 force field to generate single low energy 3D structure for each input structure. During this step chiralities were maintained.

Docking studies
Docking studies were carried using Glide software. It was carried using Extra precision and write XP descriptor information. This generates favorable ligand poses which are further screened through filters to examine spatial fit of the ligand in the active site. Ligand poses which pass through initial screening are subjected to evaluation and minimization of grid approximation. Scoring was then carried on energy minimized poses to generate glide score. The results are summarized in Table 1 and Fig. 1.

Biological activities

In vitro PPAR-γ transactivation assay
Human embryonic kidney (HEK) 293 cells were cultured in DMEM with 10% heat inactivated fetal bovine serum in a humidified 5% CO_2 atmosphere at 37°C. Cells were seeded in six-well plates the day before transfection to give a confluence of 70–80% at transfection. Cells grown in DMEM were inoculated in 96-well plates containing 60000 cells/well. Cells were transfected with 2.5 μL of PPRE-Luc, 6.67 μL of

PPAR-γ, 1.0 μL of Renilla, and 20 μL of Lipofectamine. Following 5 h after transfection, cells were treated with compound (10 μM) for 24 h and then collected with cell culture lysis buffer. Luciferase activity was monitored on luminometer (Perkin Elmer, USA) using the luciferase assay kit (Promega) according to the manufacturer's instructions. Rosiglitazone and pioglitazone were used as standard drug. The results are summarized in Fig. 2.

In vivo antidiabetic activity

The antidiabetic activity was performed according to the reported method [30] using STZ-induced diabetic model. Albino Wistar rats of either sex, 150–200 g, were obtained from Central Animal House, Jamia Hamdard University, New Delhi. The rats were kept in cages at room temperature and fed with food and water *ad libitum*. The experiments were performed in accordance with the rules of Institutional Animals Ethics Committee (registration number 173-CPCSEA). The rats were fasted overnight and diabetes was induced by injecting STZ (60 mg/kg body weight) intraperitoneally. STZ was prepared freshly in 0.1 M citrate buffer (pH 4.5). The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. The rats were considered as diabetic if their blood glucose values were above 250 mg/dL on the third day after STZ injection. The rats were divided into five groups comprising six animals in each group. Control rats receiving 0.1 M citrate buffer (Group I), diabetic rats received STZ injection (Group II), diabetic rats orally fed with pioglitazone (as 0.25% carboxymethyl cellulose suspension) at a dose of 36 mg/kg (Group III), diabetic rats orally fed with rosiglitazone (as 0.25% carboxymethyl cellulose suspension) at a dose of 36 mg/kg (Group IV), diabetic rats orally fed with synthesized compounds **5a–m** (as 0.25% carboxymethyl cellulose suspension) at an equimolar dose of the standard drug pioglitazone (Group V). The blood glucose level of each group was checked at 0, 1, 7, and 15 days by glucose oxidase method [31]. The results are summarized in Fig. 3.

Biochemical parameters

Serum AST and ALT were assayed according to the reported method of Reitman and Frankel method [32] and ALP assay was done according to method of Walter and Schult [33] using *p*-nitrophenyl phosphate as the substrate. The results are summarized in Fig. 5.

Hepatotoxicity studies

The hepatotoxicity was performed according to the reported method [34]. For the study, rats were sacrificed under light anesthesia after 5 h of the administration of the tested drugs (three times to the dose used for antidiabetic activity) and their liver specimens were removed and put into 10% formalin solution. Morphological examination was performed with hematoxylin and eosin staining to analyze histological changes and examined under microscope. The results are summarized in Fig. 6.

PPAR-γ gene expression study

Cell culture experiments: 3T3-L1 cells (ATCC) were seeded in 24-well plates 24 h before treatment in DMEM containing 10% calf serum (Invitrogen). After 24 h, cells were treated with compound **5m** (10 μM), and pioglitazone (10 μM), and rosiglitazone (10 μM) as positive control and DMSO as negative control followed by a 24 h of incubation of cells in CO_2 incubator at 37°C and 5% CO_2.

RNA extraction, reverse transcription (RT), and gene expression analysis: After 24 h, cells were scraped off and collected in 1.5 mL microcentrifuge tubes. The total RNA was isolated by TRI Reagent® (Molecular Research Center). RNA quantity and quality were determined on a NanoDrop ND-2000c spectrophotometer and integrity was checked on a 1.5% agarose gel. Total RNA (1 μg) was used to generate cDNA using an EZ-first strand cDNA synthesis kit for RT (reverse transcription)-polymerase chain reaction (PCR) (Biological Industries). Primers for real-time PCR were designed for PPAR-γ and β-actin using the Pearl Primer software. Reactions were run at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Real-time PCR was performed on an ABI Prism 7300 Sequence Detection System (Applied Biosystems) using the SYBR Green PCR Master Mix (Applied Biosystems). PCR was performed in triplicate and was repeated two times for each gene and each sample. Relative transcript quantities were calculated using the Ct method with β-actin as the endogenous reference gene [30]. The results are summarized in Fig. 7.

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