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Original article

Design, synthesis, *in silico* molecular docking and biological evaluation of novel oxadiazole based thiazolidine-2,4-diones *bis*-heterocycles as PPAR-γ agonists

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ABSTRACT

A library of novel 1,3,4-oxadiazole and 2-4-thiazolidinedione based *bis*-heterocycles **7 (a–r)** has been synthesized which exhibited significant PPAR-γ transactivation and blood glucose lowering effect comparable with the standard drugs Pioglitazone and Rosiglitazone. Compounds **7m** and **7r** did not cause body weight gain and were found to be free from hepatotoxic and cardiotoxic side effects. Compounds **7m** and **7r** increased PPAR-γ gene expression by **2.10** and **2.00** folds, respectively in comparison to the standard drugs Pioglitazone (**1.5** fold) and Rosiglitazone (**1.0** fold). Therefore the compounds **7m** and **7r** may be considered as potential candidates for development of new antidiabetic agents.

1. Introduction

Type 2 diabetes is a metabolic disorder which due to insulin resistance and impaired insulin secretion leads to hyperglycemia. Patients with type 2 diabetes suffers from several complications such as neuropathy, nephropathy, retinopathy, cardiovascular diseases and atherosclerosis [1,2]. In normal humans, up to 80% of insulin-stimulated glucose disposal occurs in the skeletal muscle, a major site of insulin resistance in type 2 diabetes [3].

2,4-thiazolidinediones (TZDs) are an important class of compounds that enhances insulin action (insulin sensitizers) and promote glucose utilization in peripheral tissues [4]. These are high-affinity ligands of peroxisome proliferator activated receptor-γ [5]. PPAR-γ, a member of a large family of ligand-activated nuclear hormone receptors is an important drug target for regulating glucose metabolism [6–9]. It increases insulin sensitivity in the adipose, muscle and hepatic tissues [10,11]. It leads to the channeling of fatty acids into adipose tissue and reducing their concentration in the plasma and thus alleviating insulin resistance and improving plasma glucose levels effectively [12–14]. 1,3,4-oxadiazoles are another important class of heterocyclic compounds which are known for a number of important pharmacological activities like hypoglycaemic, hypolipidemic, antimicrobial, antiinflammatory, analgesic, antimitotic and anticonvulsive activities [15–22].

Considering the biological importance of 2,4-thiazolidinediones and 1,3,4-oxadiazoles, we have conjugated thiazolidinediones with 1,3,4-oxadiazole under one construct through a methylene linkage to enhance the blood glucose lowering effect and minimize the side

Abbreviations: TZD, thiazolidinediones; STZ, streptozotocin; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; RT, reverse transcription; PCR, polymerase chain reaction.

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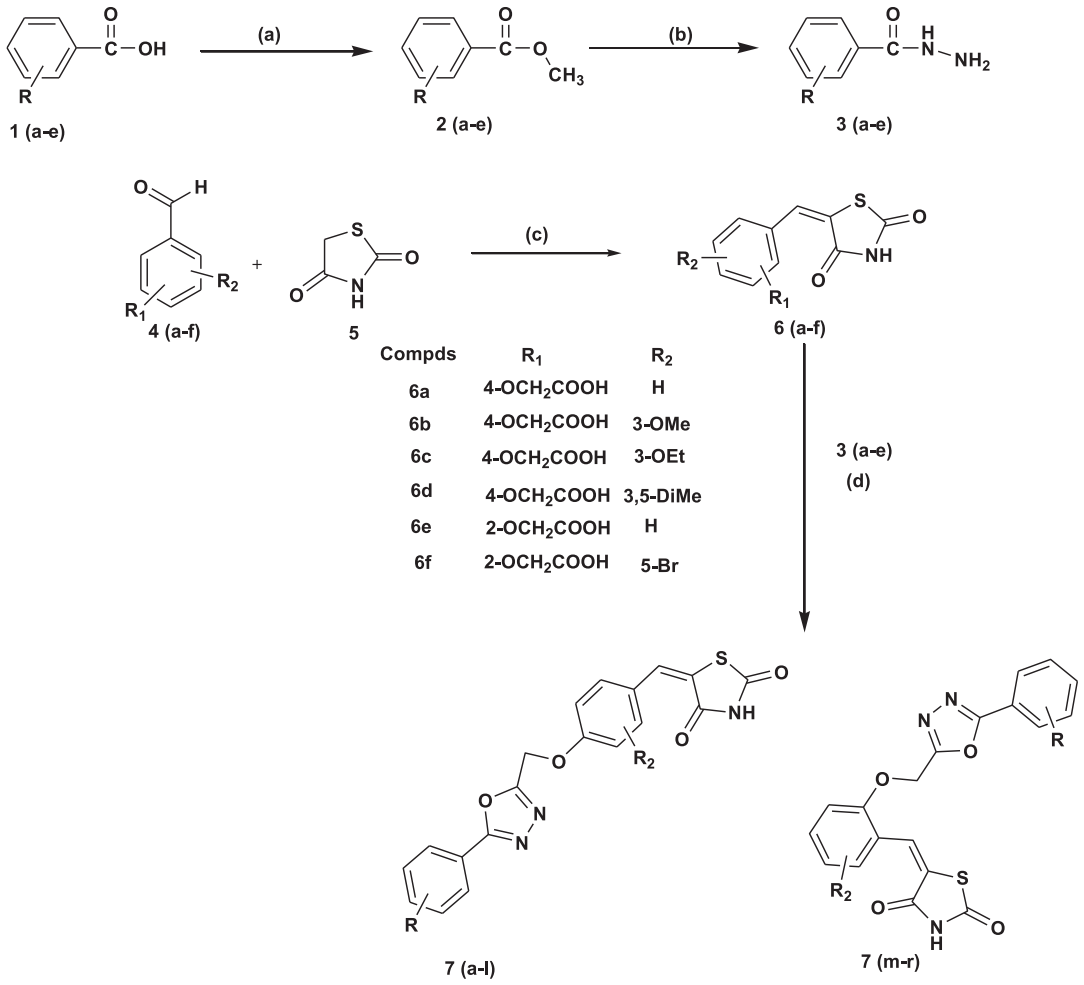

Compds	R_1	R_2
6a	4-OCH_2COOH	H
6b	4-OCH_2COOH	3-OMe
6c	4-OCH_2COOH	3-OEt
6d	4-OCH_2COOH	3,5-DiMe
6e	2-OCH_2COOH	H
6f	2-OCH_2COOH	5-Br

Reagents and conditions: (a) MeOH, H_2SO_4, Reflux, 1-2 h; (b) $NH_2NH_2.H_2O$, Abs. alcohol, 6-8 h; (c)EtOH, NaOH, 0-(-5) °C, 10-12 h; (d) $POCl_3$, RT, 10-12 h.

Compds	R_2	R	Compds	R_2	R
7a	H	H	7j	3-OMe	2-OEt
7b	3-OMe	H	7k	H	Phenyl
7c	3-OEt	H	7l	H	-O-CH_2-2,6-diCl
7d	3,5 DiMe	H	7m	H	H
7e	H	4-Cl	7n	H	4-Cl
7f	3-OMe	4-Cl	7o	H	4-Br
7g	H	4-Br	7p	H	2-OEt
7h	3-OMe	4-Br	7q	-5-Br	H
7i	H	2-OEt	7r	H	-O-CH_2-2,6-diCl

Scheme 1. Synthetic route for novel 1,3,4-oxadiazole-thiazolidine-2,4-diones based *bis*-heterocycles.

effects. We herein report for the first time, the synthesis and *in silico* molecular docking studies of oxadiazole and thiazolidinedione based *bis*-heterocycles **7 (a–r)** against PPAR-γ target. The compounds showing good docking score (>−7.20) were screened for their *in vitro* PPAR-γ transactivation activity. Compounds **7b**, **7h, 7k, 7m** and **7r** showing significant PPAR-γ transactivation activity were further evaluated for their *in vivo* antidiabetic activity and hepatotoxicity. Two compounds **7m** and **7r** showing the most potent *in vitro* and *in vivo* antidiabetic activity were finally evaluated for their cardiotoxic risk evaluation as well as effect on PPAR-γ gene expression.

2. Results and discussion

2.1. Chemistry

Treatment of different aromatic acids **1 (a–e)** with MeOH in presence of a few drops of conc. H_2SO_4 yielded methyl esters of

aromatic acids **2 (a–e)** which on further reaction with hydrazine monohydrate in presence of absolute alcohol afforded aromatic hydrazides **3 (a–e)**. Knoevenagel condensation of different hydroxy benzaldehydes **4 (a–f)** and 2,4-thiazolidinedione **(5)** in presence of absolute alcohol and NaOH at −5 to 0 °C for 10–12 h yielded intermediate phenoxy acetic acid based thiazolidinediones **6 (a–f).** Reaction of different aromatic hydrazides **3 (a–e)** with intermediates **6 (a–f)** in presence of $POCl_3$ gave corresponding 1,3,4-oxadiazole-thiazolidinediones based *bis*-heterocycles **7 (a–r)** in good yields (Scheme 1).

^{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 aromatic hydrazides **3 (a–e)** was confirmed by the presence of signals in a range of δ 9.7–10.12 (−CONH−, s) and δ 4.33–4.52 (−NH_2−, s) in the ^{1}H NMR spectra. The absence of methoxy signals in a range of 3.32–3.91 in **3 (a–e)** as seen in the ^{1}H NMR spectra of **2 (a–e)** further confirmed their formation. Formation of intermediates **6 (a–f)** was confirmed by the presence of the singlets in a range of δ 4.62–4.89, δ 7.72–7.74 and δ 11.02–13.07 for O–CH_2, exocyclic olefinic and carboxylic protons, respectively in their ^{1}H NMR spectra. This data was supported by their ^{13}C NMR spectral data. The formation of target compounds **7 (a–r)** was confirmed by the presence of additional aromatic protons in a range of δ 7.02–8.35 and O–CH_2 protons in a range of δ 4.87–5.67 in their ^{1}H NMR spectra. The absence of carboxylic proton signals as seen in the ^{1}H NMR spectra of **6 (a–f)** further confirmed their formation. Further structural confirmation of the target compounds **7 (a–r)** was provided by the presence of absorption bands in a range of 1575–1596 cm^{-1} for C=N stretching of oxadiazole ring in the IR spectra and the signals in a range of δ 161.80–165.89 for C=N carbons in the ^{13}C NMR spectra of these compounds. Further structural confirmation was done by their mass spectral data.

2.2. Molecular docking studies

The 3D structures of **7 (a–r)** were generated using ligand preparation module of Schrodinger suite and molecular docking was carried out using the Glide software. 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 the grid generated by Schrodinger glide software. Rosiglitazone has been reported to show H-bonding with TYR 473, HIS 449 & CYS 285. Docking of Rosiglitazone against the generated grid showed similar docking mode and hydrogen bonds with RMSD value of 2.8 and hence the generated grid was validated. In order to analyse the binding pattern and energies of synthesized compounds, they were docked individually against the generated grid. All the synthesized molecules docked showed good binding energies ranging from −89.4 to −44.5 kcal/mol. All the molecules except **7d** showed good glide score higher than the standard drug Rosiglitazone (−**5.77**) Compound **7c** was found to form hydrogen bonding with ALA 292, **7l** with SER 342, **7r** with LYS 261, **7p** with ARG 288 and**7i** and **7q** with the water molecule of the protein residue. Whereas the compounds **7b**, **7f, 7h, 7j, 7k** and **7m** were found to be aligned perfectly with the hydrophobic pocket of the protein. The *in silico* ADME (Absorption, Distribution, Metabolism and Excretion) prediction of the **7(a–r)** were found to be within the acceptable range. The calculated glide score, binding energies and predicted ADME of all the synthesized molecules are represented in Table 1 and Fig. 1.

2.3. Biological activities

The compounds showing good glide scores (>−7.20) 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 **7b**, **7m** and **7r** exhibited significant PPAR-γ transactivation of **59.81%, 63.78%** and **64.67%** respectively in comparison to standard drugs Pioglitazone and Rosiglitazone which showed **71.94%** and **85.27%** activation respectively. Compounds **7h (52.32%), 7i (49.78%), 7k (52.96%)** and **7q (52.41%)** showed moderate *in vitro* PPAR-γ transactivation activity whereas compound **7c**, **7l** and **7p** did not show good activity. The active compounds (**7b, 7h, 7k, 7m, 7r**) were then evaluated for the blood glucose lowering effect in STZ induced diabetic rats. As observed from the data of Fig. 3, compounds **7b (139.7 ± 6.12)**, **7h (135.2 ± 6.16)**, **7k (142.2 ± 7.18)**, **7m (139.6 ± 6.40)** and **7r (134.0 ± 5.09)** also caused significant lowering in blood glucose level comparable to standard drugs Pioglitazone **(132.0 ± 5.20)** and Rosiglitazone **(144.2 ± 6.12)**. It was observed that the compounds **7b**, **7h, 7k**, **7m** and **7r** exerted much lower PPAR-γ transactivation activity than Pioglitazone and Rosiglitazone but similar or lower blood glucose levels than Pioglitazone and Rosiglitazone. The absence of linear correlation between *in vitro* PPAR-γ transactivation assay and *in vivo* pharmacological profile in albino rats may be attributed to several reasons. For example, the test compounds **7b**, **7h, 7k**, **7m** and **7r** are administered orally and therefore absorption, metabolism, excretion, etc. of the test compounds might have contributed in exerting the significant lowering in blood glucose level [23]. Also, compounds **7b**, **7h, 7k**, **7m** and **7r** might be exhibiting their antihyperglycemic activity through other mechanisms, in addition to binding to PPAR-γ. Similar kind of results have been reported by other groups working with TZDs, e.g., Reddy et al. (1999) have synthesized TZDs which show superior euglycemic and hypolipidemic profiles in db/db mice but not significant *in vitro* PPAR-γ transactivation activity than the standard drug troglitazone [23].

The most active compound **7r** from the *in vivo* study was further tested for oral glucose tolerance as well as for insulin tolerance test (Fig. 4). Oral glucose tolerance test of compound **7r** showed that the administration of compound **7r** causes significant decrease in the blood glucose levels of diabetic rats at 120 min when compared to diabetic control rats indicating that glucose tolerance were

Table 1
Docking score and predicted ADME of the synthesized compounds **7** (**a–r**).

S.No.	Compd	s-Score	e-Energy	Log PO/W	PSA	Log S
1	**7a**	**−6.79**	−50.37	2.94	116.31	−4.62
2	**7b**	**−7.43**	−73.83	2.98	122.21	−4.91
3	**7c**	**−7.72**	−53.76	3.48	124.61	−5.35
4	7d	−5.70	−46.35	3.54	115.69	−5.45
5	**7e**	**−6.92**	−76.51	3.54	116.93	−5.28
6	**7f**	**−7.12**	−73.69	3.48	122.13	−5.65
7	**7g**	**−6.65**	−49.26	3.45	116.90	−5.35
8	**7h**	**−7.75**	−77.95	3.56	121.74	−5.78
9	**7i**	**−7.74**	−52.27	3.69	114.90	−5.40
10	**7j**	**−7.07**	−75.60	3.88	117.32	−5.43
11	**7k**	**−7.20**	−89.45	4.95	121.67	−6.84
12	**7l**	**−7.26**	−60.13	3.81	126.44	−5.46
13	**7m**	**−8.29**	−54.49	3.09	114.76	−5.01
14	**7n**	**−6.80**	−46.68	3.57	115.26	−5.81
15	**7o**	**−6.89**	−49.79	3.64	114.14	−5.90
16	**7p**	**−7.31**	−44.54	3.55	121.02	−5.27
17	**7q**	**−7.70**	−69.34	3.35	119.39	−5.36
18	**7r**	**−8.42**	−60.76	3.91	118.85	−5.81
19	Rosi	**−5.77**	−71.55	3.47	94.37	−4.49

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

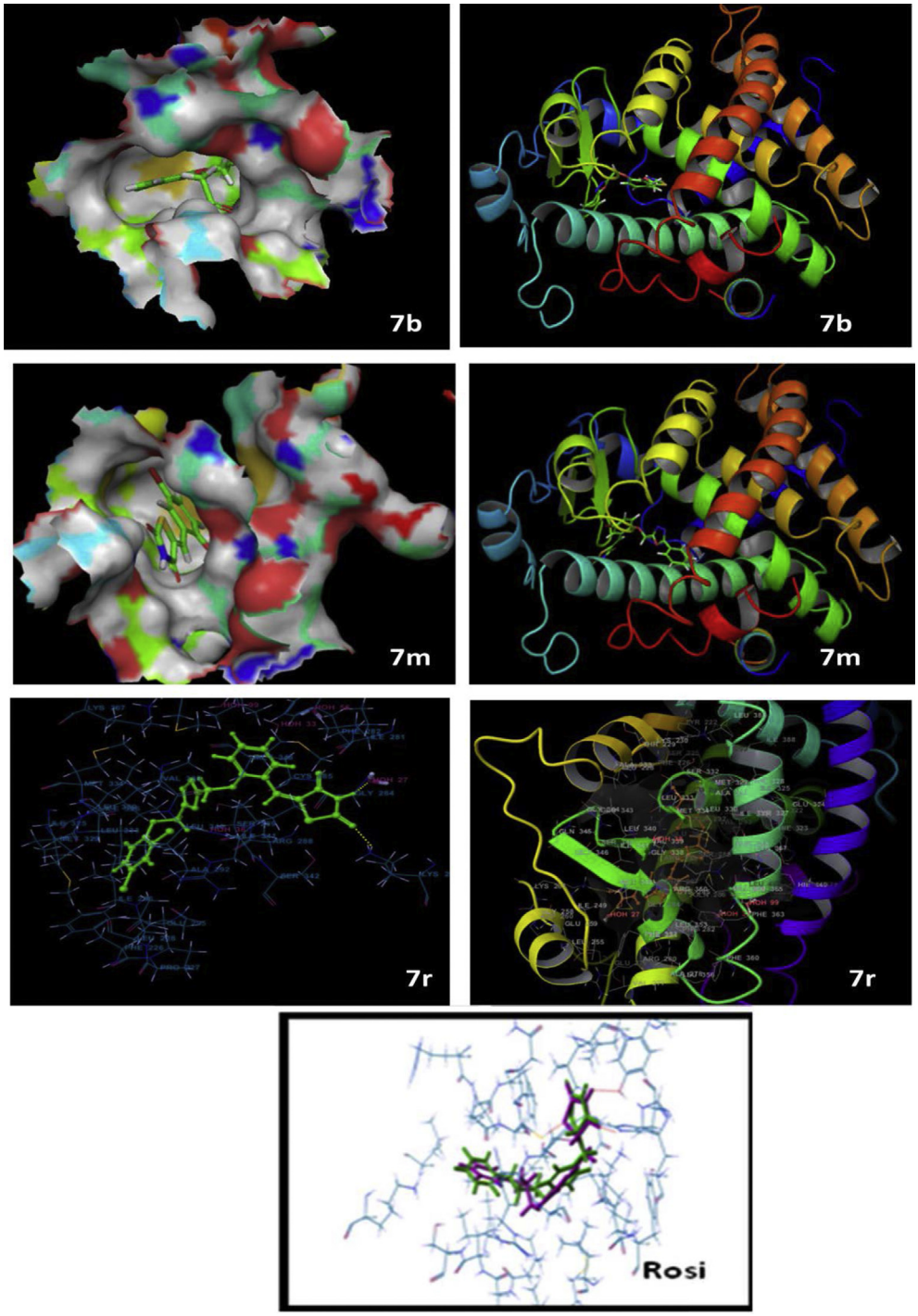

Fig. 1. Molecular docking of compounds against the PPAR-γ target.

improved by administration of compound **7r.** Insulin tolerance test of compound **7r** showed that the blood glucose levels of compound **7r** treated diabetic rats were significantly lowered after 90 min of insulin administration as compared to diabetic rats indicating insulin resistance was improved by compound **7r**. These results suggest that the antihyperglycemic activity of **7r** may result from enhanced insulin and glucose resistance.

As the PPAR-γ agonists are associated with side effect of body weight gain [24], **7r** was tested for body weight gain study. Compound **7r** treated normal rats did not show any significant increase in the body weight as compared to normal control rats. However, oral administration of **7r** to diabetic rats for 15 days caused a significant improvement in body weight of diabetic rats Fig. 5.

From the above results, the structure activity relationship (SAR) can be drawn as follows. Compounds having oxadiazole ring attached to *o*-position of TZD attached aromatic ring (**7m**) were more active in lowering the blood glucose level than the compounds having oxadiazole ring attached to *p*-position (**7m**>**7a**). Methoxy substitution on the TZD attached aromatic ring (**7b**) increased the activity. Compounds with halogen on oxadiazole

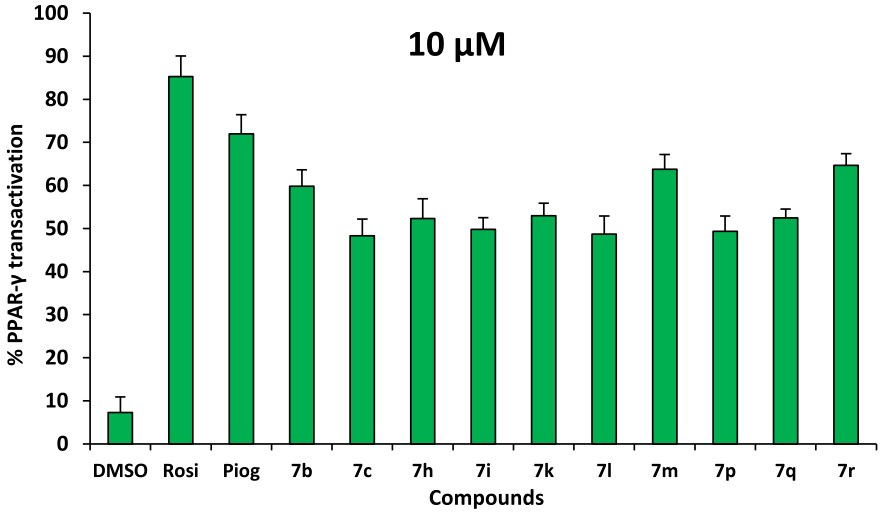

Fig. 2. *In vitro* PPAR-γ transactivation assay of compounds. Values are expressed as mean ± SE from three experiments conducted in triplicate at 10 µM.

attached aromatic ring (**7h**) exhibited promising activity. Presence of phenyl group on oxadiazole attached aromatic ring (**7k**) increased the activity. Dichloro substituted compounds (**7r**) resulted in enhanced activity.

PPAR-γ agonists are also reported to cause hepatotoxicity which is the other major drawback encountered with this class of drug [25,26]. Compounds **7b**, **7h, 7k, 7m** and **7r** have therefore been assayed for AST, ALT and ALP levels in liver. It has been reported that the levels of these enzymes are significantly increased in STZ rats indicating the toxic effect of STZ on liver [27]. 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 keto acids. It was observed that the levels of serum AST, ALT and ALP increased in STZ treated rats were significantly decreased to near normal level after treatment with the active compounds **7b**, **7h, 7k, 7m** and **7r** (Fig. 6). Compounds **7m** and **7r** were found to be more potent in lowering the AST, ALT and ALP level more than the standard drug Pioglitazone. Remaining compounds were as potent as the standard drug Pioglitazone in lowering the AST, ALT and ALP levels to normal level. Therefore, these compounds exhibited a protective effect on liver.

Histopathological study of the liver of the treated animals also showed that the compounds **7b**, **7h, 7m** and **7r** did not cause any damage to the liver. Compound **7k** caused insignificant inflammation in portal vein whereas Pioglitazone caused significant damage i.e. significant dilation in sinusoidal space and inflammation in centrizonal vein of the liver (Table 3, Supplementary data).

TZDs are associated with cardiovascular risks [16] and are the leading cause for the withdrawal of these drugs from the market. We therefore tested compound **7m** and **7r** for hERG inhibition. It has been reported that compounds with an IC_{50} > 10 µM do not inhibit hERG significantly and hence have no cardiotoxicity. Since compound **7m** and **7r** were found to have an **IC_{50} of 41 and 29 µM**, respectively, indicating that compound **7m** and **7r** would not be associated with cardiotoxicity.

Since compounds **7m** and **7r** were found to be the most PPAR-γ active, they were further subjected to PPAR-γ gene expression study. The PPAR-γ gene expression study was done to know the impact of compound **7m** and **7r** on the expression of the PPAR-γ gene. It was observed that the PPAR-γ expression was significantly increased in presence of compound **7m** (**2.10** fold) and **7r** (**2.00** fold) in comparison to the standard drugs Pioglitazone (**1.5** fold) and Rosiglitazone (**1.0** fold). 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 [28–30]. The increase in gene expression by compounds **7m** and **7r** 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 [31]. 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, compounds **7m** and **7r** increase 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.

3. Conclusion

A library of eighteen novel conjugates of oxadiazoles and thiazolidinediones have been synthesized. Three compounds, **7b**, **7m** and **7r** exhibited significant *in vitro* PPAR-γ transactivation activity and *in vivo* blood glucose lowering effect. Compounds **7b, 7m** and **7r** recovered the activity of serum AST, ALT and ALP and did not cause any damage to the liver. Compounds **7m** and **7r** were also found to be free from cardiotoxicity. Compounds **7m** and **7r** increased the PPAR-γ gene expression by 2.10 and 2.0 fold, respectively in comparison to standard drugs Rosiglitazone (1.0 fold) and Pioglitazone (1.5 fold). Compounds **7b, 7m** and **7r** may be considered as potential candidates for the development of new antidiabetic agents.

4. Experimental protocols

4.1. Materials and methods

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 a Bruker

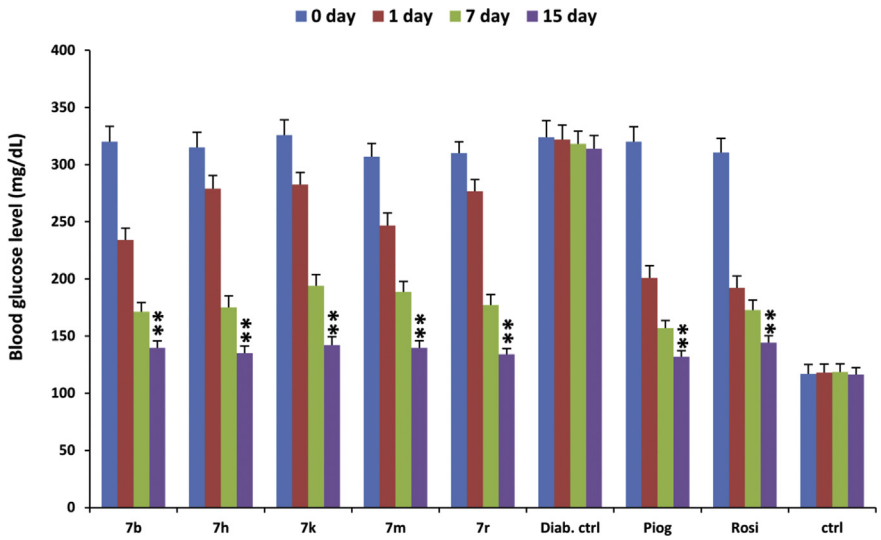

Fig. 3. *In vivo* antidiabetic activity of the active compounds in STZ induced diabetic rats. Data is analysedby one way ANOVA followed by Dunnett's 't' test and expressed as mean ± SEM from five observations; ** indicates $p < 0.01$ vs diabetic control. Piog: Pioglitazone; Rosi: Rosiglitazone; ctrl: normal control; Diab. ctrl: diabetic control.

DPX 400, 300 instruments in $CDCl_3$/DMSO-d_6 using TMS as internal standard. Chemical shifts and coupling constants *J* are given in ppm and Hz 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 is reported in *m/z*. Elemental analysis was carried out using Elemental Vario EL III elemental analyser. Elemental analysis data is reported in % standard.

*4.2. General procedures for the synthesis of oxadiazole based 2,4-thiazolidinediones **7(a–r)***

To 10–15 ml $POCl_3$, 2 mmol of aromatic hydrazides **3 (a–e)** and 2 mmol of different aromatic acids **6 (a–f)** were added, the reaction mixture was kept for stirring at 60–70 °C for 8–14 h. After completion of reaction, monitored by TLC, the reaction mixture was concentrated under reduced pressure, poured on crushed ice and neutralized by sodium bicarbonate. The precipitate so obtained was filtered, washed with cold water, dried and purified by column chromatography using *n*-hexane:EtOAc as an eluent.

*4.2.1. 5-[4-{(5-Phenyl-1,3,4-oxadiazol-2-yl)methoxy}benzylidene] thiazolidine-2,4-dione **(7a)***

Yellow crystals; yield: 60%; m.p. 206–207 °C; IR (KBr): ν (cm^{-1}) 3100, 1715, 1683, 1573, 1141; ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.60 (s, 2H, O–CH_2), 7.28 (d, 2H, *J* = 8.4 Hz, Ar–H), 7.60–7.66 (m, 5H, Ar–H), 7.77 (s, 1H, C=C–H), 8.02 (d, 2H, *J* = 7.2 Hz, Ar–H), 12.62 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 60.22, 116.23, 121.87, 123.42, 127.16, 127.22, 130.01, 131.79, 132.51, 132.81, 159.27, 162.80, 165.30, 168.13, 168.52; MS ES (+ve): 380 $(M+1)^+$; Anal. Calcd. for $C_{19}H_{13}N_3O_4S$: C, 60.15; H, 3.45; N, 11.08; S, 8.45. Found: C, 60.11; H, 3.42; N, 11.10; S, 8.47%.

*4.2.2. 5-[4-{(5-Phenyl-1,3,4-oxadiazol-2-yl)methoxy}-3-methoxybenzylidene]thiazolidine-2,4-dione **(7b)***

White crystals; yield: 60%; m.p. 214–216 °C; IR (KBr): ν (cm^{-1}) 3044, 1715, 1681, 1590, 1139; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.83 (s, 3H, O–CH_3), 5.54 (s, 2H, O–CH_2), 7.18–8.15 (m, 8H, Ar–H), 7.75 (s, 1H, C=C–H), 12.60 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 56.19, 60.88, 114.42, 115.27, 122.30, 123.45, 123.60, 127.16, 128.03, 130.00, 132.09, 132.78, 148.82, 149.92, 162.85, 165.33, 168.05, 168.47; MS ES (+ve): 410 $(M+1)^+$, 411 $(M+2)^+$; Anal. Calcd. for $C_{20}H_{15}N_3O_5S$: C, 58.67; H, 3.69; N, 10.26; S, 7.83. Found: C, 58.63; H, 3.3.71; N, 10.23; S, 7.81%.

*4.2.3. 5-[4-{(5-Phenyl-1,3,4-oxadiazol-2-yl)methoxy}-3-ethoxybenzylidene]thiazolidine-2,4-dione **(7c)***

White crystals; yield: 65%; m.p. 202–203 °C; IR (KBr): ν (cm^{-1}) 3040, 1715, 1620, 1541, 1092; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.90 (t, 3H, *J* = 7.1 Hz, CH_3), 4.08 (q, 2H, *J* = 6.4 Hz, O–CH_2), 5.55 (s, 2H, O–CH_2), 7.06–8.32 (m, 8H, Ar–H), 7.69 (s, 1H, C=C–H), 12.01 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.97, 59.21, 64.78, 113.28, 115.89, 122.12, 123.23, 123.64, 127.12, 128.28, 130.02, 132.14, 143.71, 148.52, 162.75, 165.50, 168.12, 168.64; MS ES (+ve): 423 $(M)^+$, 424 $(M+1)^+$; Anal. Calcd. for $C_{21}H_{17}N_3O_5S$: C, 59.57; H, 4.05; N, 9.92; S, 7.57. Found: C, 59.59; H, 4.02; N, 9.90; S, 7.58%.

*4.2.4. 5-[4-{(5-Phenyl-1,3,4-oxadiazol-2-yl)methoxy}-3,5-dimethylbenzylidene]thiazolidine-2,4-dione **(7d)***

Yellow crystals; yield: 70%; m.p. 195–197 °C; IR (KBr): ν (cm^{-1}) 3043, 1700, 1636, 1556, 1141; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.12 (s, 6H, 2 × CH_3), 4.87 (s, 2H, O–CH_2), 6.92 (s, 2H, Ar–H), 7.28 (d, 2H, *J* = 8.4 Hz, Ar–H), 7.24–7.81 (m, 3H, Ar–H), 7.38 (s, 1H, C=C–H), 11.87 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 15.73, 62.69, 122.26, 122.63, 126.23, 128.52, 129.24, 130.35, 131.11, 131.19, 131.50, 155.86, 161.56, 164.97, 167.07, 167.56; MS ES (+ve): 408 $(M+1)^+$; Anal. Calcd. for $C_{21}H_{17}N_3O_4S$: C, 61.90; H, 4.21; N, 10.31; S, 7.87. Found: C, 61.91; H, 4.19; N, 10.33; S, 7.89%.

*4.2.5. 5-[4-[{5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl}methoxy] benzylidene]thiazolidine-2,4-dione **(7e)***

White crystals; yield: 60%; m.p. 225–226 °C; IR (KBr): ν (cm^{-1}) 3042, 1734, 1684, 1595, 1144; ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.59 (s, 2H, O–CH_2), 7.28 (d, 2H, *J* = 8.7 Hz, Ar–H), 7.61 (d, 2H, *J* = 9.0 Hz, Ar–H), 7.70 (d, 2H, *J* = 8.4 Hz, Ar–H), 7.77 (s, 1H, C=C–H), 8.03 (d, 2H, *J* = 8.7 Hz, Ar–H), 12.51 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 60.18, 114.21, 122.36, 122.54, 126.41, 127.32, 129.18, 132.15, 132.89, 133.14, 159.18, 160.25, 163.98, 168.24, 168.40; MS ES (+ve): 413 $(M)^+$, 415 $(M+2)^+$; Anal. Calcd. for $C_{19}H_{12}ClN_3O_4S$: C, 55.14; H, 2.92; N, 10.15; S, 7.75. Found: C, 55.15; H, 2.94; N, 10.12; S, 7.77%.

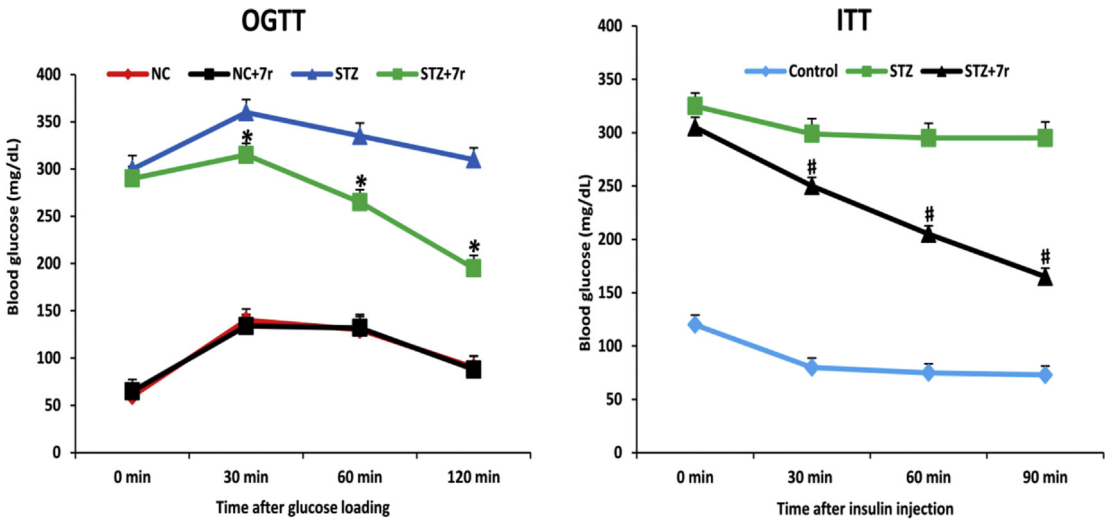

Fig. 4. Effect of compound **7r** on OGTT and ITT. Data is analysed by one way ANOVA followed by Dunnett's 't' test and expressed as mean ± SEM from five observations; * indicates $p < 0.001$ vs diabetic control. # indicates $p < 0.05$ vs diabetic control. NC: normal control; NC+7r: normal control + 7r; STZ: diabetic control; STZ+7r: 7r treated diabetic rats.

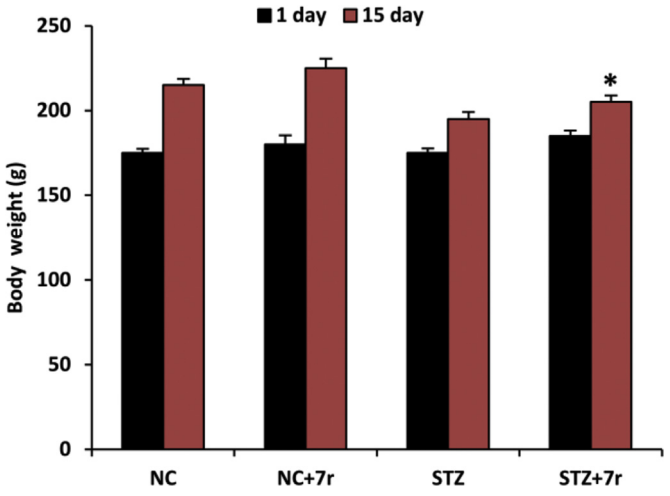

Fig. 5. Effect of compound **7r** on body weight in albino wistar rats. Data is analyzed by one way ANOVA followed by Dunnett's 't' test and expressed as mean ± SEM from five observations; * indicates $p < 0.01$ vs diabetic control. NC: Normal control; NC+7r: normal control + 7r; STZ: diabetic control; STZ+7r: 7r treated diabetic rats.

4.2.6. 5-[4-[{5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl}methoxy]-3-methoxybenzylidene]thiazolidin e–2,4-dione ***(7f)***

White crystals; yield: 60%; m.p. 212–213 °C; IR (KBr): ν (cm^{-1}) 3048, 1716, 1685, 1595, 1176; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.86 (s, 3H, O–CH_3), 5.54 (s, 2H, O–CH_2), 7.19–7.40 (m, 3H, Ar–H), 7.72 (s, 1H, C=C–H), 8.04 (d, 2H, J = 6.9 Hz, Ar–H), 8.15 (d, 2H, J = 9.2 Hz, Ar–H), 12.31 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.32, 60.16, 114.24, 121.36, 121.54, 127.11, 127.72, 129.17, 132.72, 132.89, 133.44, 152.60, 153.21, 160.28, 164.78, 168.44, 168.47; MS ES (+ve): 443 $(M)^+$, 445 $(M+2)^+$; Anal. Calcd. for $C_{20}H_{14}ClN_3O_5S$: C, 54.12; H, 3.18; N, 9.47; S, 7.22. Found: C, 54.14; H, 3.20; N, 9.45; S, 7.24%.

4.2.7. 5-[4-[{5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl}methoxy] benzylidene]thiazolidine-2,4-dione ***(7g)***

White crystals; yield: 72%; m.p. 228–229 °C; IR (KBr): ν (cm^{-1}) 3044, 1715, 1685, 1575, 1152; ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.59 (s, 2H, O–CH_2), 7.27 (d, 2H, J = 9.0 Hz, Ar–H), 7.60 (d, 2H, J = 8.4 Hz, Ar–H), 7.76 (s, 1H, C=C–H), 7.83 (d, 2H, J = 8.4 Hz, Ar–H), 7.95 (d, 2H, J = 8.4 Hz, Ar–H), 12.09 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 60.20, 116.23, 121.82, 122.65, 126.45, 127.22, 129.10, 131.84, 132.50, 133.10, 159.26, 162.98, 164.69, 168.29, 168.47; MS ES (+ve): 457.75 $(M)^+$, 459.91 $(M+2)^+$; Anal. Calcd. for $C_{19}H_{12}BrN_3O_4S$: C, 49.80; H, 2.64; N, 9.17; S, 7.00. Found: C, 49.82; H, 2.62; N, 9.19; S, 7.02%.

4.2.8. 5-[4-[{5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl}methoxy]-3-methoxybenzylidene] thiazolidine –2,4-dione ***(7h)***

White crystals; yield: 68%; m.p. 199–200 °C; IR (KBr): ν (cm^{-1}) 3098, 1720, 1675, 1583, 1151; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.82 (s, 3H, O–CH_3), 5.59 (s, 2H, O–CH_2), 6.98–7.41 (m, 3H, Ar–H), 7.76 (s, 1H, C=C–H), 8.02 (d, 2H, J = 7.5 Hz, Ar–H), 8.13 (d, 2H, J = 8.4 Hz, Ar–H), 12.52 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.35, 61.52, 113.21, 120.32, 122.18, 128.26, 128.95, 129.18, 133.62, 134.43, 148.26, 149.81, 161.28, 165.88, 168.92, 169.43; MS ES (+ve): 486 $(M)^+$, 488 $(M+2)^+$; Anal. Calcd. for $C_{20}H_{14}BrN_3O_5S$: C, 49.19; H, 2.89; N, 8.61; S, 6.57. Found: C, 49.21; H, 2.87; N, 8.63; S, 6.55%.

4.2.9. 5-[4-[{5-(2-Ethoxyphenyl)-1,3,4-oxadiazol-2-yl}methoxy] benzylidene]thiazolidine-2,4-dione ***(7i)***

Yellow crystals; yield: 62%; m.p. 175–177 °C; IR (KBr): ν (cm^{-1}) 3041, 1734, 1682, 1557, 1112; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.47 (t, 3H, J = 6.0 Hz, CH_3), 4.12 (q, 2H, J = 6.5 Hz, O–CH_2), 5.37 (s, 2H, O–CH_2), 7.15 (d, 2H, J = 8.1 Hz, Ar–H), 7.48 (d, 2H, J = 7.8 Hz, Ar–H), 7.73 (s, 1H, C=C–H), 7.89–8.0 (m, 4H, Ar–H), 11.87 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.44, 59.64, 63.56, 114.77, 115.28, 115.73, 122.47, 122.82, 125.40, 125.72, 128.68, 128.96, 132.18, 156.27, 158.58, 162.82, 164.78, 167.41, 168.54; MS ES (+ve): 424 $(M+1)^+$, 425 $(M+2)^+$; Anal. Calcd. for $C_{21}H_{17}N_3O_5S$: C, 59.57; H, 4.05; N, 9.92; S, 7.57. Found: C, 59.59; H, 4.07; N, 9.90; S, 7.57%.

4.2.10. 5-[4-[{5-(2-Ethoxyphenyl)-1,3,4-oxadiazol-2-yl}methoxy]-3-methoxybenzylidene]thiazolidi ne-2,4-dione ***(7j)***

White crystals; yield: 70%; m.p. 178–179 °C; IR (KBr): ν (cm^{-1}) 3040, 1735, 1685, 1575, 1152; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.36 (t, 3H, J = 6.0 Hz, CH_3), 3.82 (s, 3H, O–CH_3), 4.13 (q, 2H, J = 6.9 Hz, O–CH_2), 5.50 (s, 2H, O–CH_2), 7.12–7.33 (m, 7H, Ar–H), 7.71 (s, 1H, C=C–H), 12.51 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.11, 55.24, 60.62, 63.64, 113.25, 114.89, 114.96, 123.77, 125.48, 126.71, 128.90, 133.69, 148.27, 149.12, 155.24, 163.86, 164.76, 167.42, 169.36;

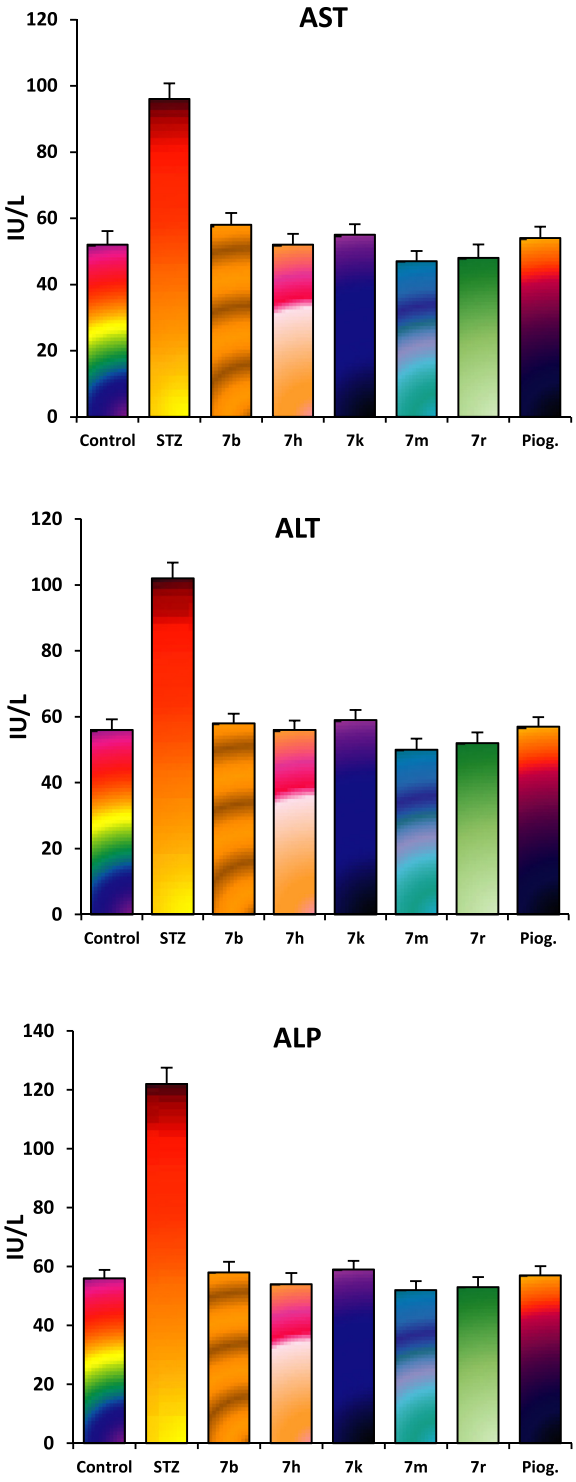

Fig. 6. Effect of compounds on serum AST, ALT and ALP activities. Values are given as mean ± S.D.

MS ES (+ve): 453 $(M)^+$; Anal. Calcd. for $C_{22}H_{19}N_3O_6S$: C, 58.27; H, 4.22; N, 9.27; S, 7.07. Found: C, 58.29; H, 4.23; N, 9.25; S, 7.09%.

4.2.11. 5-[2-[[5-{(4-Phenylphenoxy)methyl}-1,3,4-oxadiazol-2-yl] methoxy]benzylidene]thiazolidine-2,4-dione ***(7k)***

White crystals; yield: 70%; m.p. 231–233 °C; IR (KBr): ν (cm^{-1}) 3162, 3057, 1718, 1623, 1156, 761; ^1H NMR (300 MHz, DMSO-d_6): δ 5.36 (s, 2H, O–CH_2), 5.42 (s, 2H, O–CH_2), 7.06–7.55 (m, 13H, Ar–H), 7.70 (s, 1H, C=C–H), 12.16 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 60.22, 62.53, 116.22, 122.57, 123.22, 128.15, 128.23, 130.12, 131.53, 132.55, 132.98, 158.64, 159.34, 162.84, 165.15, 168.26, 168.46; MS ES (+ve): 486 $(M+1)^+$; Anal. Calcd. for $C_{26}H_{19}N_3O_5S$: C, 64.32; H, 3.94; N, 8.65; S, 6.60. Found: C, 64.34; H, 3.96; N, 8.64; S, 6.62%.

4.2.12. 5-[4-[[5-{(2,4-Dichlorophenoxy)methyl}-1,3,4-oxadiazol-2-yl]methoxy]benzylidene]thiazolidi ne-2,4-dione ***(7l)***

Yellow crystals; yield: 60%; m.p. 238–240 °C; IR (KBr): ν (cm^{-1}) 3041, 1732, 1684, 1572, 1150; ^1H NMR (300 MHz, DMSO-d_6): δ 5.57 (s, 2H, O–CH_2), 5.65 (s, 2H, O–CH_2), 7.17–7.60 (m, 7H, Ar–H), 7.93 (s, 1H, C=C–H), 12.62 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 60.46, 61.88, 114.21, 121.25, 122.62, 129.11, 129.73, 130.42, 131.83, 132.58, 133.19, 149.29, 158.24, 163.52, 164.57, 168.86, 169.36; MS ES (+ve): 477 $(M)^+$, 479 $(M+2)^+$; Anal. Calcd. for $C_{20}H_{13}Cl_2N_3O_5S$: C, 50.22; H, 2.74; N, 8.79; S, 6.70. Found: C, 50.24; H, 2.76; N, 8.81; S, 6.67%.

4.2.13. 5-[2-{(5-Phenyl-1,3,4-oxadiazol-2-yl)methoxy}benzylidene] thiazolidine-2,4-dione ***(7m)***

White crystals; yield: 65%; m.p. 192–193 °C; IR (KBr): ν (cm^{-1}) 3014, 1733, 1650, 1588, 1157; ^1H NMR (300 MHz, DMSO-d_6): δ 5.66 (s, 2H, O–CH_2), 7.17–8.02 (m, 9H, Ar–H), 7.66 (s, 1H, C=C–H), 12.60 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 61.33, 114.62, 122.97, 123.41, 127.13, 127.28, 129.20, 129.98, 132.58, 132.88, 159.25, 164.63, 165.89, 169.86, 169.91; MS ES (+ve): 380 $(M+1)^+$; Anal. Calcd. for $C_{19}H_{13}N_3O_4S$: C, 60.15; H, 3.45; N, 11.08; S, 8.45. Found: C, 60.12; H, 3.47; N, 11.06; S, 8.47%.

4.2.14. 5-[2-[{5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl}methoxy] benzylidene]thiazolidine-2,4-dione ***(7n)***

White crystals; yield: 70%; m.p. 207–208 °C; IR (KBr): ν (cm^{-1}) 3038, 1715, 1699, 1574, 1152; ^1H NMR (300 MHz, DMSO-d_6): δ 5.65 (s, 2H, O–CH_2), 7.16–7.49 (m, 4H, Ar–H), 7.57 (s, 1H, C=C–H), 7.65 (d, 2H, J = 8.0 Hz, Ar–H), 8.01 (d, 2H, J = 8.2 Hz, Ar–H), 11.94 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 61.13, 114.45, 116.78, 122.31, 122.95, 128.94, 130.13, 131.18, 132.36, 134.78, 137.52, 156.53, 163.06, 164.48; 169.89, 169.92; MS ES (+ve): 413.74 $(M)^+$, 415.58 $(M+2)^+$; Anal. Calcd. for $C_{19}H_{12}ClN_3O_4S$: C, 55.14; H, 2.92; N, 10.15; S, 7.75. Found: C, 55.16; H, 2.95; N, 10.13; S, 7.77%.

4.2.15. 5-[2-[{5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl}methoxy] benzylidene]thiazolidine-2,4-dione ***(7o)***

White crystals; yield: 70%; m.p. 182–183 °C; IR (KBr): ν (cm^{-1}) 3100, 1716, 1685, 1574, 1152; ^1H NMR (300 MHz, DMSO-d_6): δ 5.65 (s, 2H, O–CH_2), 7.16–7.69 (m, 4H, Ar–H), 7.36 (s, 1H, C=C–H), 7.82 (d, 2H, J = 8.7 Hz, Ar–H), 7.96 (d, 2H, J = 8.4 Hz, Ar–H), 12.58 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 60.05, 113.90, 122.21, 122.46, 123.20, 123.56, 126.01, 128.42, 128.63, 131.58, 132.64, 156.01, 162.68, 164.17, 168.81, 169.71; MS ES (+ve): 457.80 $(M)^+$, 459.76 $(M+2)^+$; Anal. Calcd. for $C_{19}H_{12}BrN_3O_4S$: C, 49.80; H, 2.64; N, 9.17; S, 7.00. Found: C, 49.83; H, 2.62; N, 9.19; S, 7.02%.

4.2.16. 5-(2-((5-(2-Ethoxyphenyl)-1,3,4-oxadiazol-2-yl)methoxy) benzylidene)thiazolidine-2,4-dione ***(7p)***

White crystals; yield: 55%; m.p. 169–171 °C; IR (KBr): ν (cm^{-1}) 3042, 1715, 1682, 1557, 1154; ^1H NMR (300 MHz, DMSO-d_6): δ 1.36 (t, 3H, J = 6.9 Hz, CH_3), 4.12 (q, 2H, J = 6.9 Hz, O–CH_2), 5.63 (s, 2H, O–CH_2), 7.02–7.97 (m, 8H, Ar–H), 8.02 (s, 1H, C=C–H), 12.62 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.51, 60.65, 63.61, 114.04, 115.10, 115.33, 122.49, 122.52, 124.40, 125.82, 128.56, 128.60, 132.28, 156.17, 161.58, 161.80, 164.77, 167.44, 168.04; MS ES (+ve): 424

$(M+1)^+$; Anal. Calcd. for $C_{21}H_{17}N_3O_5S$: C, 59.57; H, 4.05; N, 9.92; S, 7.57. Found: C, 59.59; H, 4.08; N, 9.94; S, 7.59%.

4.2.17. 5-[2-{(5-Phenyl-1,3,4-oxadiazol-2-yl)methoxy}-5-bromobenzylidene]thiazolidine-2,4-dione **(7q)**

White crystals; yield: 70%; m.p. 216–218 °C; IR (KBr): ν (cm^{-1}) 3040, 1700, 1636, 1585, 1152; ^1H NMR (300 MHz, DMSO-d_6): δ 5.68 (s, 2H, O–CH_2), 7.41–8.03 (m, 8H, Ar–H), 7.89 (s, 1H, C=C–H), 12.71 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 61.34, 114.31, 116.80, 123.40, 124.91, 125.29, 126.84, 127.14, 130.01, 131.16, 132.84, 134.82, 155.64, 162.60, 165.27, 167.69, 168.03; MS ES (+ve): 457.80 $(M)^+$, 459.76 $(M+2)^+$; Anal. Calcd. for $C_{19}H_{12}$ BrN_3O_4S: C, 49.80; H, 2.64; N, 9.17; S, 7.00. Found: C, 49.82; H, 2.67; N, 9.19; S, 7.02%.

4.2.18. 5-[2-[[5-{(2,4-Dichlorophenoxy)methyl}-1,3,4-oxadiazol-2-yl]methoxy]benzylidene]thiazoli dine-2,4-dione **(7r)**

Yellow crystals; yield: 65%; m.p. 222–223 °C; IR (KBr): ν (cm^{-1}) 3048, 1715, 1675, 1570, 1124; ^1H NMR (300 MHz, DMSO-d_6): δ 5.48 (s, 2H, O–CH_2), 5.23 (s, 2H, O–CH_2), 7.08–7.68 (m, 7H, Ar–H), 7.67 (s, 1H, C=C–H), 12.60 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 60.42, 61.75, 114.25, 121.78, 123.24, 129.15, 129.63, 130.72, 131.42, 132.65, 133.43, 156.23, 158.15, 163.71, 164.49, 168.17, 169.23; MS ES (+ve): 477.63 $(M)^+$, 479.54 $(M+2)^+$; Anal. Calcd. for $C_{20}H_{13}Cl_2N_3O_5S$: C, 50.22; H, 2.74; N, 8.79; S, 6.70. Found: C, 50.24; H, 2.77; N, 8.81; S, 6.73%.

4.3. Molecular docking

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

4.3.1. Protein selection & 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, minimized by removing unwanted molecules and other defects reported by the software. PPAR-γ receptor is a dimer which has two monomers chains (A & 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.

4.3.2. 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.

4.3.3. 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 3-D structure for each input structure. During this step chiralities were maintained.

4.3.4. Docking studies

Docking studies was carried using Glide software. It was carried using Extra precision and write XP descriptor information. This generates favourable 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.

4.4. Pharmacology

4.4.1. 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 6-well plates the day before transfection to give a confluence of 70–80% at transfection. Cells grown in DMEM were inoculated in 96-well plate containing 60,000 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 using the luciferase assay kit according to the manufacturer's instructions. Rosiglitazone and Pioglitazone were used as standard drugs. The results are summarized in Fig. 2.

4.4.2. Antidiabetic activity

The antidiabetic activity was performed according to the reported method [32] using streptozotocin 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 the 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 streptozotocin (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 3rd day after STZ injection. The rats were divided into five groups comprising of 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 (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 day by glucose oxidase method [33]. The results are summarized in Fig. 3.

4.4.3. Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)

OGTT and ITT were performed as reported previously [34]. The results are summarized in Fig. 4.

4.4.4. Biochemical parameters

Serum AST and ALT were assayed according to the reported method of Reitman and Frankel method [35] and ALP assay was done according to method of Walter and Schult [36] using p-nitrophenyl phosphate as the substrate. The results are summarized in Fig. 6.

4.4.5. Hepatotoxicity studies

The hepatotoxicity was performed according to the reported method [37]. For the study, rats were sacrificed under light anaesthesia after 5 h of the administration of the tested drugs (3 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 Haematoxylin and

eosin staining to analyze histological changes and examined under microscope. The results are summarized in Fig. 7.

4.4.6. hERG inhibition assay

The activity was performed according to the reported method of Cheng et al., 2002 [38].

4.5. PPAR-γ gene expression study

4.5.1. Cell culture experiments

3T3-L1 cells (ATCC) were seeded in 24 well plate 24 h before treatment in DMEM containing 10% calf serum (Invitrogen). After 24 h cells were treated with compound **7m** and **7r** (10 μM) and standard drugs, Pioglitazone and Rosiglitazone (10 μM) as positive control and DMSO as negative control, followed by 24 h of incubation of cells in CO_2 incubator at 37 °C and 5% CO_2.

4.5.2. RNA extraction, reverse transcription and gene expression analysis

After 24 h cells were scrapped and collected in 1.5 ml micro centrifuge tubes. The total RNA was isolated by TRI Reagent® (Molecular Research Centre). 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)–PCR (Biological Industries). Primers for real-time PCR were designed for *PPAR-γ* and *β-actin* using the Pearl Primer software and are listed in Table 2 (Supplementary data). 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. The results are summarized in Fig. 8.

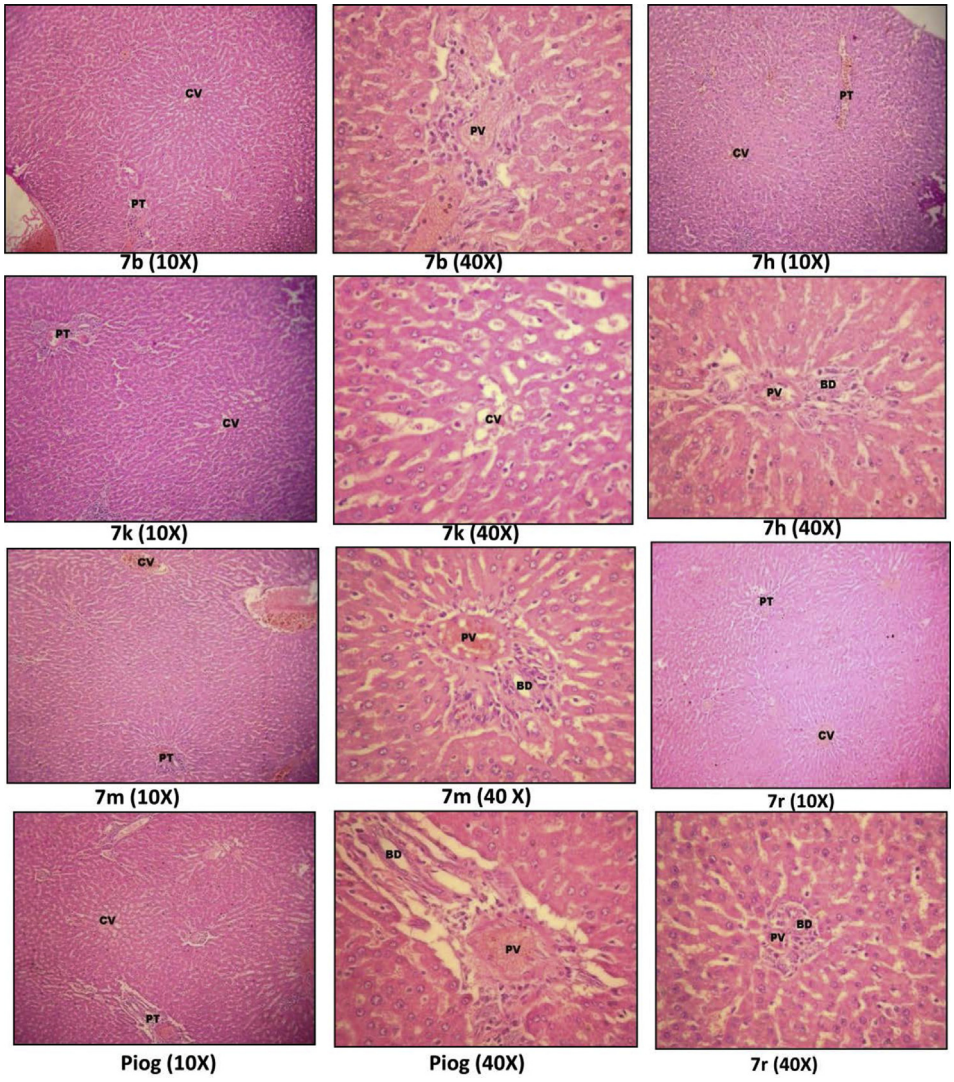

Fig. 7. Haematoxylin and eosin immunohistochemical staining of liver after administration of synthesized drugs. Histopathology report of rat liver. As illustrated in above figure, Low and high power photomicrograph of liver from animal treated groups **7b, 7h, 7k, 7m, 7r** and **standard**. 10×. Low power photomicrograph of liver from corresponding animal **7b**, treated groups showing normal arrangement of cells in the liver lobule. PT = portal triad and CV = central vein. 40×. Compound **7b, 7h, 7m** and **7r** treated groups showing normal arrangement of cells in the liver lobule and normal arrangement of hepatocytes in the centrizonal area. **7k** treated group showing insignificant inflammation in portal vein whereas Pioglitazone treated groups showing a mild dilatation of sinusoidal spaces and mild inflammation in centrizonal vein.

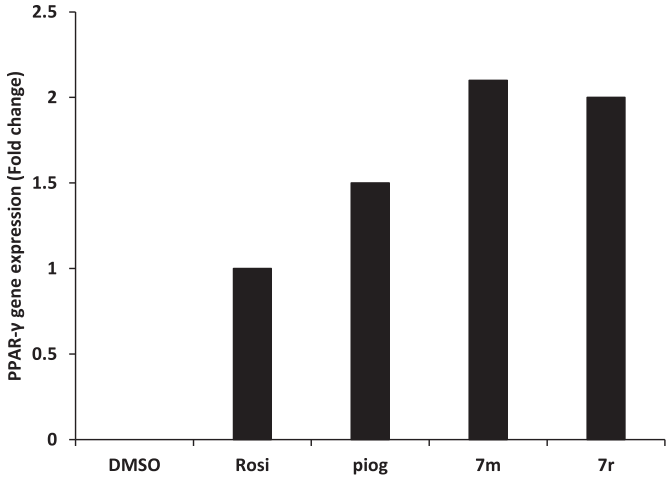

Fig. 8. Effect of compound **7m** and **7r** on PPAR-γ gene expression. The experiments was conducted at 10 μM. Rosi: Rosiglitazone, piog: Pioglitazone. PCR was performed in triplicate and was repeated two times for each gene and each sample. Relative transcript quantities were calculated using the Ct method with *β-actin* as the endogenous reference gene.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.09.010.

References

[1] R. Maccari, R. Ottana, R. Ciurleo, D. Rakowitz, B. Matuszczak, C. Laggner, T. Langer, Bioorg. Med. Chem. 16 (2008) 5840–5852.
[2] G. Viberti, J. Diabet. Complications 19 (2005) 168–170.
[3] D.P. Rotella, J. Med. Chem. 47 (2004) 4111–4112.
[4] E.E. Kershaw, M. Schupp, H. Guan, N.P. Gardner, M.A. Lazar, J.S. Flier, Am. J. Physiol. Endocrinol. Metab. 293 (2007) E1736–E1745.
[5] R. Jeon, S.Y. Park, Arch. Pharm. Res. 27 (2004) 1099–1105.
[6] P. Brun, A. Dean, V.D. Marco, P. Surajit, I. Castagliuolo, D. Carta, M.G. Ferlin, Eur. J. Med. Chem. 62 (2013) 486–487.
[7] A. Carrieri, M. Giudici, M. Parente, M. De Rosas, L. Piemontese, G. Fracchiolla, A. Laghezza, P. Tortorella, G. Carbonara, A. Lavecchia, F. Gilardi, M. Crestani, F. Loiodice, Eur. J. Med. Chem. 63 (2013) 321–332.
[8] S. Raza, S.P. Srivastava, D.S. Srivastava, A.K. Srivastava, W. Haq, S.B. Katti, Eur. J. Med. Chem. 63 (2013) 611–620.
[9] R. Romagnoli, P.G. Baraldi, M.K. Salvador, M.E. Camacho, J. Balzarini, J. Bermejo, F. Estévez, Eur. J. Med. Chem. 63 (2013) 544–557.
[10] K. Rikimaru, T. Wakabayashi, H. Abe, H. Imoto, T. Maekawa, O. Ujikawa, K. Murase, T. Matsuo, M. Matsumoto, C. Nomura, H. Tsuge, N. Arimura, K. Kawakami, J. Sakamoto, M. Funami, C.D. Mol, G.P. Snell, K.A. Bragstad, B.C. Sang, D.R. Dougan, T. Tanaka, N. Katayama, Y. Horiguchi, Y. Momose, Bioorg. Med. Chem. 20 (2012) 714–733.
[11] T.M. Willson, M.H. Lambert, S.A. Kliewer, Annu. Rev. Biochem. 70 (2001) 341–367.
[12] W.L. Hong, B.A. Joong, K.K. Sung, K.A. Soon, C.H. Deok, Org. Process Res. Dev. 11 (2007) 190–199.
[13] A.S.C. Packiavathy, M. Ramalingam, C.A. Devi, Orient. J. Chem. 3 (2013) 7–11.
[14] D.V. Jawale, U.R. Pratap, R.A. Mane, Bioorg. Med. Chem. Lett. 22 (2012) 924–928.
[15] M.M. Ghorab, A.M. El-Sharief, Y.A. Ammolar, S. Mohamed, Phosphorous Sulfur Silicon 173 (2001) 223–233.
[16] W. Zhongyi, S. Haoxin, S. Haijian, J. Heterocycl. Chem. 38 (2001) 355–357.
[17] N.B. Patel, I.H. Khan, S.D. Rajani, Eur. J. Med. Chem. 45 (2010) 4293–4299.
[18] L. Labanauskas, V. Kalcas, E. Udrenaite, P. Gaidelis, A. Brukstus, V. Dauksas, Pharmazie 56 (2001) 617–619.
[19] M.M. Alam, M. Shaharyar, H. Hamid, S. Nazreen, S. Haider, M.S. Alam, Med. Chem. 7 (2011) 663–673.
[20] H.L. Chang, H. In Cho, K.J. Lee, Bull. Korean Chem. Soc. 22 (2001) 1153–1155.
[21] A.Y. Shawa, C. Chang, M. Hsu, P. Lu, C. Yang, H. Chen, C. Lo, C. Shiau, M. Chern, Eur. J. Med. Chem. 45 (2010) 2860–2867.
[22] A.K.M. Iqbal, A.Y. Khan, M.B. Kalashetti, N.S. Belavagi, Y.D. Gong, I.A.M. Khazi, Eur. J. Med. Chem. 53 (2012) 308–315.
[23] K.A. Reddy, B.B. Lohray, V. Bhushan, A.S. Reddy, M.N.V.S. Rao, P.P. Reddy, V. Saibaba, N.J. Reddy, A. Suryaprakash, P. Misra, R.K. Vikramadithyan, R. Rajagopalan, J. Med. Chem. 42 (1999) 3265–3278.
[24] R.W. Nesto, D. Bell, R.O. Bonow, V. Fonseca, S.M. Grundy, E.S. Horton, M.L. Winter, D. Porte, C.F. Semenkovich, S. Smith, L.H. Young, R. Kahn, Circulation 108 (2003) 2941–2948.
[25] G.R. Beecher, J. Nutr. 133 (2003) 3248S–3253S.
[26] M. Chojkier, Hepatology 41 (2005) 237–246.
[27] U.A. Shinde, R.K. Goyal, J. Cell. Mol. Med. 7 (2003) 332–339.
[28] T. Yamauchi, J. Kamon, H. Waki, K. Murakami, K. Motojima, K. Komeda, T. Ide, N. Kubota, Y. Terauchi, K. Tobe, H. Miki, A. Tsuchida, Y. Akanuma, R. Nagai, S. Kimura, T. Kadowaki, J. Biol. Chem. 276 (2001) 41245–41254.
[29] N. Maeda, M. Takahashi, T. Funahashi, S. Kihara, H. Nishizawa, K. Kishida, H. Nagaretani, M. Matsuda, R. Komuro, N. Ouchi, H. Kuriyama, K. Hotta, T. Nakamura, I. Shimomura, Y. Matsuzawa, Diabetes 50 (2001) 2094–2099.
[30] K.L. Nathan, M. Kelly, T. Tsao, S.R. Farmer, A.K. Saha, N.B. Ruderman, E. Tomas, Am. J. Physiol. Endocrinol. Metab. 291 (2006) E175–E181.
[31] Z. Wu, E.D. Rosen, R. Brun, S. Hauser, G. Adelmant, A.E. Troy, C. McKeon, G.J. Darlington, B.M. Spiegelman, Mol. Cell. 3 (1999) 151–158.
[32] R. Murugan, S. Anbazhagan, S.S. Narayanan, Eur. J. Med. Chem. 44 (2009) 3272–3279.
[33] A. Dahlqvist, Biochem. J. 80 (1961) 547–551.
[34] S. Nazreen, M.S. Alam, H. Hamid, M. Shahar Yar, A. Dhulap, P. Alam, M.A.Q. Pasha, S. Bano, M.M. Alam, S. Haider, C. Kharbanda, Y. Ali, K.K. Pillai, Bioorg. Med. Chem. Lett. (2014), http://dx.doi.org/10.1016/j.bmcl.2014.05.034.
[35] S. Reitman, S. Frankel, Am. J. Clin. Pathol. 28 (1957) 56–63.
[36] K. Walter, C. Schult, Bergmeyer (Eds.), Methods of Enzymatic Analysis, 2, Academic Press, New York, 1974, pp. 356–360.
[37] J.D. Lambert, M.J. Kennett, S. Sang, K.R. Reuhl, J. Jihyeung, C.S. Yang, Food Chem. Toxicol. 48 (2010) 409–416.
[38] C. Cheng, D. Alderman, J. Kwash, J. Dessaint, R. Patel, M. Kay, M.K. Lescoe, M.B. Kinrade, W. Yu, Inform 28 (2002) 177–189.